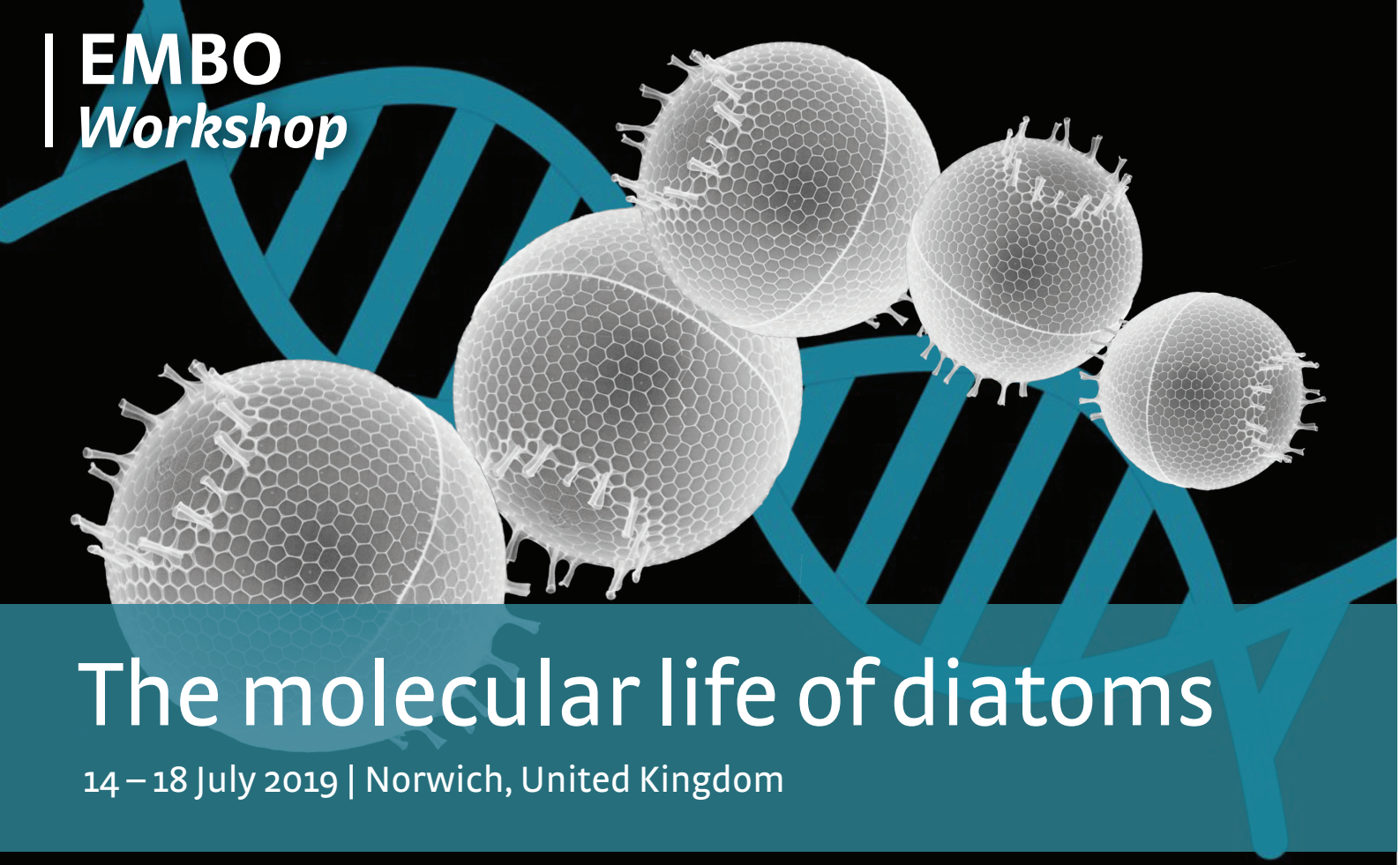
EMBO *Workshop*



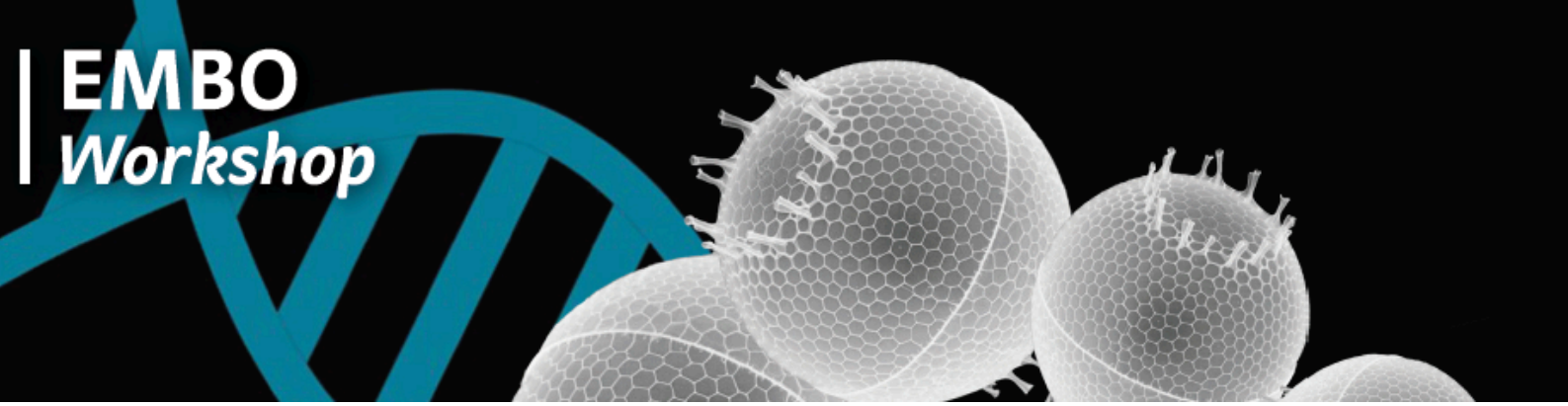
The molecular life of diatoms

14 – 18 July 2019 | Norwich, United Kingdom

Programme and Abstract Book

meetings.embo.org/event/19-diatoms

EMBO excellence in life sciences
UEA University of East Anglia
UEA University of East Anglia | SCHOOL OF ENVIRONMENTAL SCIENCES
EMBO reports
EMBOpress



Dear colleagues,

I am delighted to welcome you to the **5th Molecular Life of Diatoms Conference** in Norwich, United Kingdom. This bi-annual international conference provides a forum for scientists to discuss their latest results in the field of diatom molecular biology from genes to ecosystems. The main conference is accompanied by a satellite meeting on Friday the 19th of July, which will focus on genome editing including methods development, mutant collections, plasmid and data deposition. Diatoms have received increasing attention as more genomes became available and because of the development of genome editing tools. CRISPR/Cas9 technology has made diatoms as genetically tractable as well-established biological model species. At this EMBO workshop, we will bring together over 200 scientists from more than 35 countries for the 5th time to present and to discuss their latest data and developments in our field of research. As our community currently is experiencing a step change in understanding diatoms, it will be critical to allow synergistic discussions and networking between scientists working on diverse aspects of diatom biology and evolution. The aim will be to develop new scientific concepts and experimental approaches based on the application of the latest molecular tools and genomic information to explore the fascinating lifestyle of diatoms. The conference is accompanied by a special issue to be published in '***Biology***' (MDPI).

I am looking forward to seeing and hearing fantastic molecular science with diatoms. All abstracts for talks and posters are available throughout this conference programme.

Thomas Mock, and on behalf of the international steering committee:

Andrew E. Allen (Scripps Oceanography, USA), Atle M. Bones (Norwegian Univ. Science & Tech., Norway), Angela Falciatore (Sorbonne Univ., France), Peter G. Kroth (Univ. of Konstanz, Germany), Nicole Poulsen (Univ. of Dresden, Germany), Kim Thamatrakoln (Rutgers Univ., USA), Jodi Young (Univ. of Washington, USA)

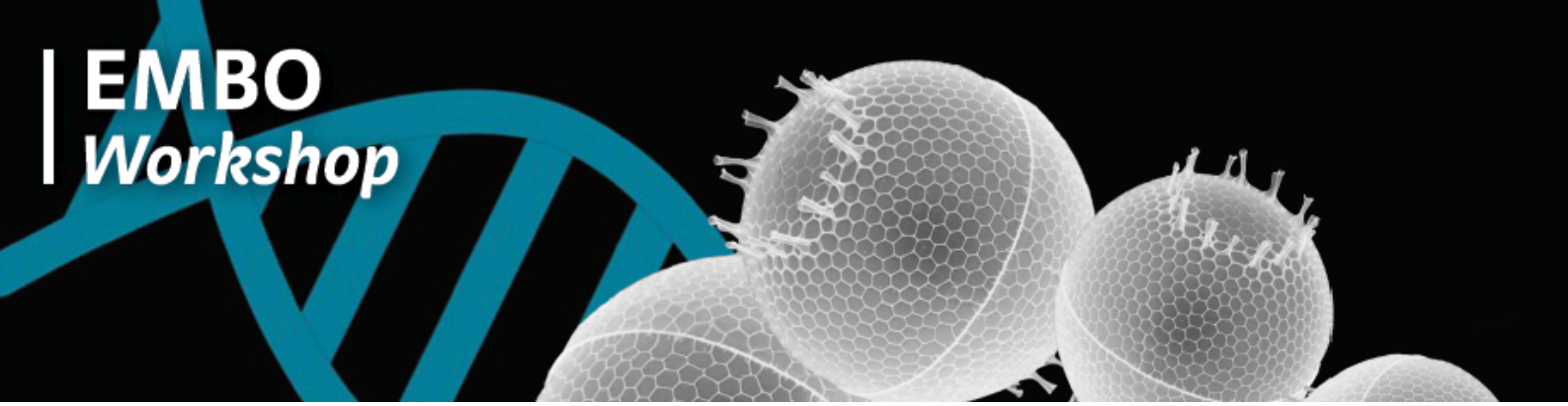


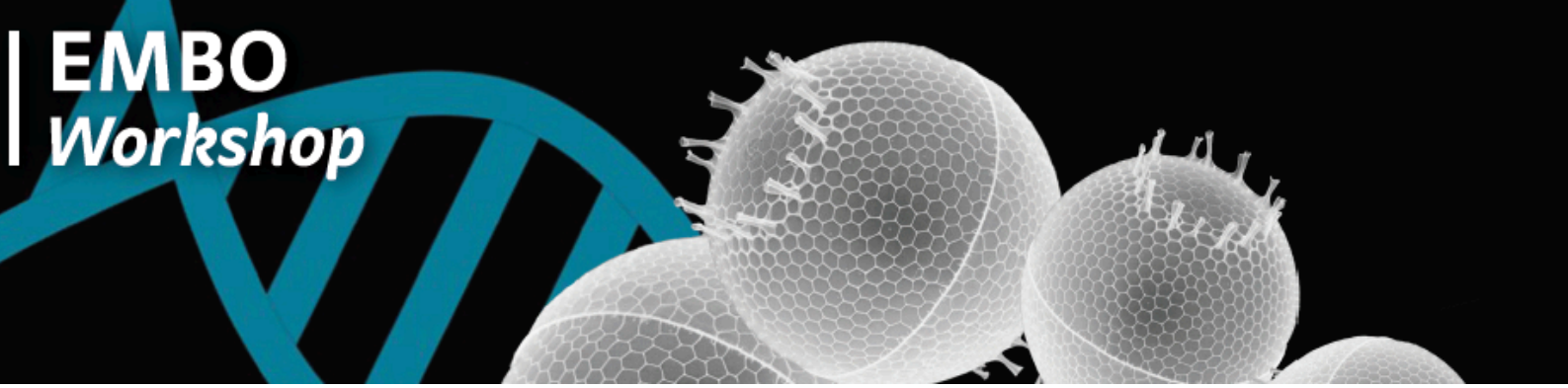
Table of Contents

Page

CAMPUS MAP

Taxi Companies:
ABC Taxis: 01603 666333
Goldstar Taxis: 01603 700700
Happy Cabs: 01603 717273

Busses:
Bus **#25** or **#26** to Norwich City Centre



GENERAL INFORMATION

Abstracts
All abstracts for talks and posters can be found both in this programme book and on the conference website:
http://meetings.embo.org/event/19-diatoms

Badges
Your name badge must be worn at all times during the conference for security reasons.

Certificate of attendance
If you wish to receive a certificate of attendance after the conference, please email your request to embo2019.env@uea.ac.uk.

Dietary requirements
All dietary requirements made during registration have been shared with our catering team and dedicated special dietary catering points will be open during lunchtimes.

Nursing Facilities
Nursing facilities are available at the School of Environmental Sciences at UEA. Please ask at the venue help desk for further information.

Photography and filming
Photography or recording in oral sessions or in poster sessions is allowed.

Posters
There will be seven poster sessions during days 2-4 of the conference. The top 5 posters will receive awards on day 5 in a special session. We request the awardees to give 5 min. flash talks.

Social media
#EMBOdiatoms
facebook.com/events/370996617021353/

Transportation
The venue is easily accessible from Norwich city centre by bus lines 25 and 26. Please refer to the website https://www.firstgroup.com/ for the bus timetables. If you arrive by car, please park in the Main Car Park. For the exact location see the campus map on page 3.

Venue
The Enterprise Centre is one of the UK's most sustainable, low carbon building. It minimizes its carbon footprint through the innovative use of natural and recycled materials, including timber, straw, hemp, clay and stone. Thus, attendees will not be able to charge their electronic devices at the venue as there are **no electrical sockets**. Please keep this in mind and bring your laptops and mobile devices fully charged.

Wi-Fi
Free Wi-fi is available and is provided via 'The Cloud'. To register please select 'The Cloud' in the device's Wi-Fi settings. Open browser and follow the on-screen instructions to register or log on. Eduroam is also available in residences and in campus buildings.

Conference Excursion - Norfolk Broads

WELCOME ABOARD THE 'SOUTHERN COMFORT'

The Southern Comfort is a double-deck paddle boat purpose built for discovering the Norfolk Broads, a National Park with over 125 miles of navigable lock-free waterways set in beautiful countryside and studded with charming and picturesque towns and villages. We have hired this luxury river cruiser for participants of the conference. If you signed up for this memorable river experience, sit back, relax and enjoy the trip from the beautiful village of Horning, along the River Bure through the village and out to Ranworth Broad and back, passing lovely old thatched houses, windmills and Norfolk reed beds. There is the ever-present bustle of river traffic with cruisers, yachts and the occasional wherry.

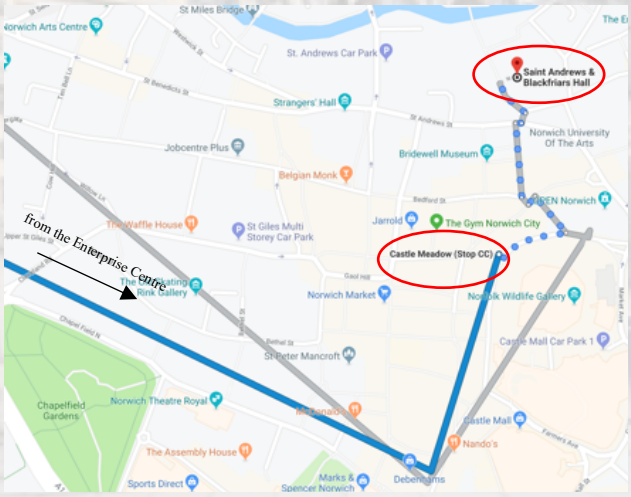
General Information:

- **When:** Wednesday 17th of July
- Coach travel to and from UEA is included. Coaches depart UEA at 12.30hrs allowing you some free time in Horning before boarding the 'Southern Comfort' moored at The Swan Hotel, Horning ready for 15.00hrs. Coaches will depart Horning at 17.00hrs and will be back to campus by 18.00hrs
- Your ticket price includes two alchoholic (or non-alcoholic) drinks from behind the bar. A cash bar selling drinks and snacks will also be available.
- Toilets
- Wheelchair access

Gala Dinner and Conference Party

The Conference gala dinner and party will take place on the evening of the 17th of July at the magnificent St Andrew's Hall in Norwich city centre. St Andrew's Hall has a fascinating history, from origins as a friary church, to surviving the reformation and becoming a 'common hall'.

When: Wednesday 17th of July, 6PM - Midnight
Where: St Andrew's Hall
How to get there: Take bus **26** from UEA Main Bus stop. Get off at **Castle Meadows** and walk about 3 min to St Andrew's Hall (see map)
Dress code: Business Casual
Band playing: Distant Sun

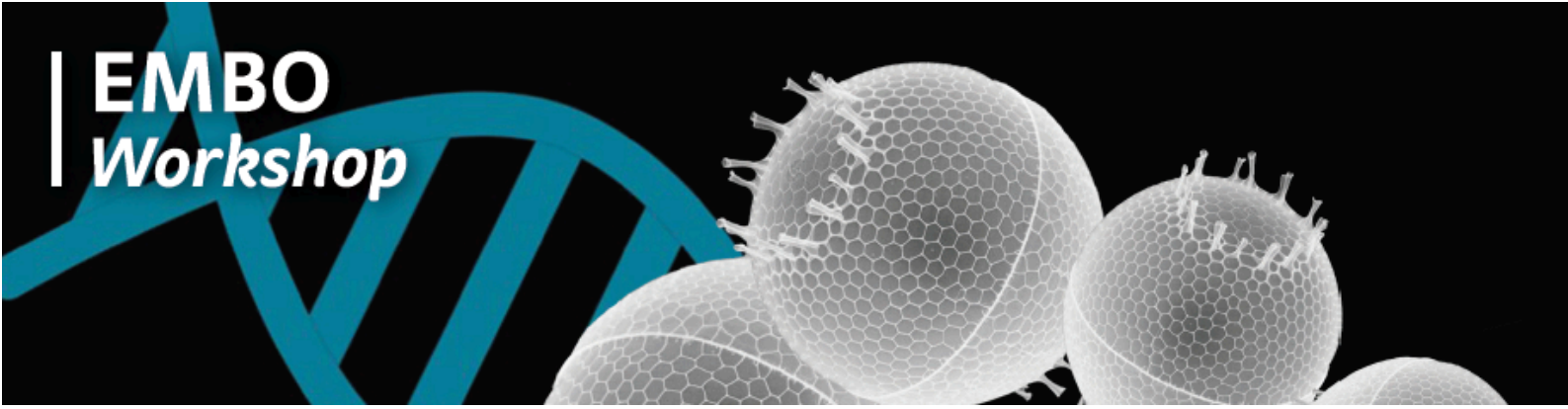


Phaeodactylum Party

On Thursday the 18th of July, Chris Bowler and co-authors will host the **'Phaeodactylum genome 10-year birthday party'** from 7PM in the Blue Bar on the UEA Campus. There will be free food, a bar to order drinks, some cool music and a few surprises....
Entry is free but can only be provided to those who have signed up. For more information contact cbowler@biologie.ens.fr.

When: Thursday 18th of July

Where: Blue Bar at UEA Campus



Satellite Meeting

The molecular life of diatoms

Satellite meeting on diatom genome editing

Friday July 19th: 10:00 - 15:00

Topics

Introduction (Peter Kroth)

Session 1: CRISPR/Cas9
* CRISPR/Cas9 (Amanda Hopes)
* CRISPR Nickase (Hermanus Nawaly)
* RNP genome editing (Manual Serif)

Session 2: Bacterial conjugation & Cloning
* Bacterial conjugation (Mark Moosburner)
* MoClo (Katrin Geisler & Fernán Federici)

Session 3: Deposition of Algae, Sequences, Plasmids and Protocols
* Belgian Diatom collection (Wim Vyerman)
* Addgene (Benoit Giquel)
* protocols.io (Nicole Poulsen)
* Genome sequence databank (Chris Bowler)

Organizers: Peter Kroth, Nicole Poulsen & Thomas Mock
Contact: embo2019.env@uea.ac.uk

EMBO reports EMBOpress UEA University of East Anglia UEA University of East Anglia SCHOOL OF ENVIRONMENTAL SCIENCES

Conference Programme

(Invited speakers in bold font)

Day 1	**Welcome reception & registration**		18:00-21:00	Mixer at Modern Life Café (The Sainsbury Centre for Visual Arts on the UEA campus)	
	Session	Chair	Time	Title	Speaker
Day 2	**Registration**		08:00-		
	Opening of the meeting	**Thomas Mock**	08:45-09:00	Opening introduction	Thomas Mock
	Session 1: Hildebrand memorial session	**Kim Thamatrakoln**	09:00-09:15	In memoriam Mark Hildebrand	Kim Thamatrakoln
			09:15-09:45	The beauty of diatoms through the life's work of the late Mark Hildebrand	**Mark Brzezinski**
			09:45-10:00	An expanded family of diatom-like silicon transporters in haptophytes and other eukaryotes	Glen Wheeler
			10:00-10:15	Dynamic subcellular translocation of V-type H+-ATPase is essential for diatom silica cell wall biomineralization	Daniel Yee
			10:15-10:30	Morphological and molecular characterization of the valve silica deposition vesicle	Christoph Heintze
			10:30-10:45	Characterizing the influence of two actin associated proteins on cell wall formation in *Thalassiosira pseudonana*	Sarah Lerch
			10:45-11:30	Coffee break	
	Session 2: Cell biology	**Nicole Poulsen**	11:30-12:00	Structural bases of phytoplankton acclimation responses	**Giovanni Finazzi**
			12:00-12:15	Excitable diatoms: sophisticated mechanisms for electrical signalling enable environmental sensing in the oceans	Katherine Helliwell
			12:15-12:30	A sticky situation: understanding the molecular mechanism of diatom underwater adhesion	Jirina Zackova Suchanova
			12:30-12:45	Bloom and bust	Krisztina Sárközi
			12:45-13:00	Control of Biosilica Morphology and Mechanical Performance by the Conserved Diatom Gene Silicanin-1	Stefan Görlich
			13:00-14:15	Lunch	
	Session 3: Genomics and genome engineering	**Angela Falciatore**	14:15-14:45	Genome editing in diatoms	**Fayza Daboussi**
			14:45-15:00	What is the origin of high haplotype diversity within clonal diatom cultures?	Petra Bulankova
			15:00-15:15	Diatom genetic transformation with Universal Loop assembly (uLoop): an open, efficient	Fernan Federici

				and species-agnostic DNA fabrication method	
			15:15-15:30	Systematic and functional analysis of horizontal gene transfer events within diatoms	Emmelien Vancaester
			15:30-16:15	Coffee break	
			16:15-16:45	Microalgae epigenomics and interactions with the environment	**Leila Tirichine Delacour**
			16:45-17:00	Re-analysis of the Fragilariopsis cylindrus genome reveals a third subgenome copy, supporting a hypothesis for polyploidy	Kat Hodgkinson
			17:00-17:15	Multiplexed CRISPR-Cas9 mutagenesis development and applications	Mark Moosburner
			17:15-17:30	Insights into the genomic diversity of the marine diatom Chaetoceros muelleri	Raffaela Abbriano
	Poster session 1		17:30-19:00	Posters 1-33	
	Non-Diatom Lecture	**Nicole Poulsen**	19:00-19:30	Whole-cell spatial proteomics of apicomplexans and dinoflagellates: mapping genome divergent cells	**Ross Waller**
	Session	Chair	Time	Title	Speaker
Day 3	**Session 4:** Biotechnology	**Peter Kroth**	09:00-09:30	Triacylglycerol accumulation and catabolism in Phaeodactylum tricornutum	**Hanhua Hu**
			09:30-09:45	Using the diatom Phaeodactylum tricornutum as a chassis for biological degradation of plastics	Daniel Moog
			09:45-10:00	Regioselective immobilization of an enzyme cascade on diatom biosilica	Ekta Kumari
			10:00-10:15	Interrogating the genomes of transgenic Phaeodactylum tricornutum strains for improved genetic engineering strategies in biotechnological applications	Jestin George
			10:15-11:00	Coffee break	
			11:00-11:30	Metabolic engineering of the oleaginous diatom Fistulifera solaris for biofuel application and beyond	**Tsuyoshi Tanaka**
			11:30-11:45	Diatom Cell Walls as Biogenic Alumosilicates – Cultivation, Modification, and Application	Lydia Köhler
			11:45-12:00	Structure and functions of the allopolyploid genome of the oleaginous diatom Fistulifera solaris	Yoshiaki Maeda
			12:00-12:15	Diatom biofilms: towards sustainable closed loop	Thomas Kiran Marella

				through nutrient recycling and biodiesel production	
			12:15-12:30	The lipid droplet proteome of Phaeodactylum tricornutum	Ben Leyland
			12:30-14:00	Lunch	
	Session 5: Ecophysiology I	**Atle M. Bones**	14:00-14:30	The pyrenoid based CO2-concentrating mechanism in marine diatoms	**Yusuke Matsuda**
			14:30-14:45	Exploring domoic acid biosynthesis in vivo: Building model systems	Patrick Brunson
			14:45-15:00	A transcription factor (PtPSR) controlling P acquisition in Phaeodactylum tricornutum	Amit Kumar Sharma
			15:00-15:15	CRISPR/Cas9 mutagenesis and gene expression profiling to sketch phosphorus atlas in a diatom	Senjie Lin
			15:15-16:00	Coffee break	
			16:00-16:30	Different strokes for different diatoms: Mechanisms of iron storage and their ecological significance	**Adrian Marchetti**
			16:30-16:45	Seeing the light with different eyes – photoreceptors in diatoms	Peter Kroth
			16:45-17:00	The bHLH-PAS protein RITMO1 regulates diel biological rhythms in the marine diatom Phaeodactylum tricornutum	Angela Falciatore
			17:00-17:15	Fe limitation decreases transcriptional regulation in the model diatom Thalassiosira pseudonana over the diel cycle	Johanna Goldman
	Poster session 2		17:30-19:30	Posters 34-66	
	Session	Chair	Time	Title	Speaker
Day 4	**Session 6:** Evolution	**Jodi Young**	09:00-09:30	Exploring drivers of diatom species diversification	**Wim Vyverman**
		Anna Godhe memorial talk	09:30-09:50	Controlling sexual reproduction in a marine diatom	Olga Kourtchenko
			09:50-10:05	Molecular characterisation of proteins shaping the diatom chloroplast	Richard Dorrell
			10:05-10:20	Inter- and intra-species analysis of the Seminavis robusta genome offers insights into the complexity, evolution and ecological adaptation of benthic diatoms.	Cristina Maria Osuna-Cruz
			10:20-11:00	Coffee break	
			11:00-11:30	Sex determination in Pseudo-nitzschia multistriata	**Mariella Ferrante**
			11:30-11:45	Evolution and Metabolic Configuration of Nitrogen Flux in a Model Marine Diatom	Sarah Smith

			11:45-12:00	Evolutionary origins of DNA methylation: what can we learn from diatoms?	Antoine Hoguin
			12:00-12:15	Analysis of a subset of Theriot et al. (2015) Seven Gene Data Set Using Multiple Outgroups	Linda Medlin
	Poster session 3		12:30-14:00	Posters 67-99	
				Afternoon free for excursion to Norfolk Broads (Boat trip)	
			18:00	Gala Dinner and Conference Party	

	Session	Chair	Time	Title	Speaker
Day 5	**Session 7:** Ecophysiology II	**Thomas Mock**	10:00-10:30	Metaproteomic investigations into the response of diatom-dominated marine microbial communities to multiple micronutrient stressor	**Erin Bertrand**
			10:30-10:45	Transcriptional investment facilitates temperature-adaptation and shapes the acclimatory response	Yue Liang
			10:45-11:00	Dark metabolism: a molecular insight into how the Antarctic sea-ice diatom Fragilariopsis cylindrus survives long-term darkness	Fraser Kennedy
			11:00-11:45	Coffee break	
	Session 8: Ecology	**Andy Allen**	11:45-12:15	Diatoms - nano-pourous miracles	**Angela Wulff**
			12:15-12:30	A new method to measure photosynthetic activity in algal mixtures highlights allelopathy between diatoms and dinoflagellates	Alexandra Peltekis
			12:30-12:45	Determining iron limited remodeling of chloroplasts in Southern Ocean diatoms through “Meta-Plastid” analyses	Kristofer Gomes
			12:45-13:00	Diatom Virus Investigations: Toward the development of a new ecologically relevant model system	Lisa Zeigler Allen
			13:00-13:15	Silicon limitation facilitates virus infection and mortality of diatoms	Chana Kranzler
			13:30-15:00	Lunch	
			15:00-15:30	Pairing up in the plankton: evolution, ecology and activity of diatom-cyanobacteria symbiosis	**Rachel Foster**
			15:30-15:45	Metabolomics approaches to address microbial complexity in phytoplankton communities	Marine Vallet
			15:45-16:00	Disentangling microbial networks in the sea: sulfonate-based trophic interactions between diatoms and bacteria	Bryndan Durham

			16:00-16:15	Roseobacteria dominate interactions between a cosmopolitan diatom and its microbiome.	Ahmed Shibl
			16:15-16:45	From the micro to the mesoscale: characterizing drivers of diatom physiological ecology in the open ocean	**Sonya Dyhrman**
			16:45-17:30	Coffee break	
	Poster Awards	**Thomas Mock**	17:30-18:00	Poster awards and flash talks (5 min. each) by awardees.	
	Looking back & ahead		18:00-18:30	The Phaeodactylum genome at ten years	**Chris Bowler**
	End of meeting		18:30	Closing remarks and looking ahead	Thomas Mock and Andrew Allen

Invited Speakers

Erin Bertrand — **Dalhousie University, Canada**

Chris Bowler — **Ecole Normale Supérieure, France**

Mark Brzezinski — **University of California, Santa Barbara, USA**

Fayza Daboussi — **National Institute of Applied Sciences, France**

Leila Tirichine Delacour — **CNRS, The National Center for Scientific Research & University of Nantes, France**

Sonya Dyhrman — **Columbia University, USA**

	**Mariella Ferrante**	**Stazione Zoologica Anton Dohrn Napoli, Italy**
	**Giovanni Finazzi**	**Institute of Grenoble, France**
	**Rachel Foster**	**Stockholm University, Sweden**
	**Hanhua Hu**	**Chinese Academy of Science, China**
	**Adrian Marchetti**	**University of North Carolina, USA**
	**Yusuke Matsuda**	**Kwansei Gakuin University, Japan**
	**Tsuyoshi Tanaka**	**Tokyo University of Agriculture & Technology, Japan**

Wim Vyverman — **University of Ghent, Belgium**

Ross Waller — **Cambridge University, UK**

Angela Wulff — **University of Gothenburg, Sweden**

Organizers

Thomas Mock — **University of East Anglia, UK**

Andrew E. Allen — **J. Craig Venter Institute & Scripps Institution of Oceanography, USA**

Atle M. Bones — **Norwegian University of Science & Technology, Norway**

	**Angela Falciatore**	**French National Center for Scientific Research (CNRS)/Sorbonne University, France**
	**Peter G. Kroth**	**University of Konstanz, Germany**
	**Nicole Poulsen**	**Technical University of Dresden, Germany**
	**Kim Thamatrakoln**	**Rutgers University, USA**
	**Jodi Young**	**University of Washington, USA**

ORAL PRESENTATION ABSTRACTS

Session 1: Hildebrand memorial session

In Memorium: Mark Hildebrand

Kim Thamatrakoln

Rutgers University, New Brunswick, United States

Celebrating the life of the late Mark Hildebrand

The Beauty of Diatoms Through the Life's Work of the Late Mark Hildebrand

Mark Brzezinski

University of California, Santa Barbara, United States

This presentation will honor the life's work of our colleague, Mark Hildebrand, who lost his battle with cancer last year at age 59. Mark's love of diatoms began in 1987 as a postdoc with Ben Volcani at Scripps Institution of Oceanography where he pioneered the use of modern molecular tools to investigate diatom silicon metabolism. Highlights will include his pioneering efforts to identify silicon responsive genes by comparing gene expression in *Cylindrotheca fusiformis* synchronized through light- vs Si- limitation. That effort ultimately led to Mark's landmark discovery of silicon transporter proteins (SITS) and his subsequent studies of SIT diversity, SIT regulation and their dual roles as transporters and sensors. Mark was also a key contributor to the annotation of the first diatom genome. He combined this new resource, his novel method of synchronizing diatom cultures and transcriptomics to confirm the vital role of polyamines in frustule morphogenesis in vivo. Mark discovered silicalemma associated proteins (SAPS) by exploiting continuing advances in gene expression and diatom transformation and demonstrated the unique influence of different SAPS on frustule morphology. His leadership was marked by numerous review papers and book chapters (7), authored over 18 years, which often charted the future of diatom silicification research. Mark was also a leader in diatom biofuel research with the Department of Energy designating his laboratory as a top algae biofuel laboratory in the US for his ground breaking success in increasing lipid production in *T. pseudonana* without impacting growth rate. Our field owes much to Mark.

An expanded family of diatom-like silicon transporters in haptophytes and other eukaryotes

Glen Wheeler [1], **Gerald Langer** [1], **Colin Brownlee** [1], **Alison Taylor** [2], **Daniel Richter** [3]

[1] Marine Biological Association, Plymouth, United Kingdom
[2] Department of Biology and Marine Biology, The University of North Carolina, Wilmington, United States
[3] Institut de Biologia Evolutiva (UPF–CSIC), Barcelona, Spain

Diatoms are characterised by their ability to acquire silicon from the surrounding seawater in order to build their ornate silicified frustules. This is achieved by Na+-coupled silicon transporters known as SITs. These transporters possess ten transmembrane domains (10-TM), arranged in two pseudo-repeats of 5 transmembrane domains (5-TM). SITs are present in diatoms and in siliceous choanoflagellates, but their origin and wider distribution are not well understood. In recent years, we have identified SITs in haptophyte algae, including both siliceous and non-siliceous lineages. The discovery of SITs in calcifying coccolithophores is particularly surprising as there is no known requirement for silicon in the calcification process. Coccolithophores also possess a family of transporters related to SITs that only possess 5-TM. This group of SIT-like proteins (SITLs) may resemble the ancestor of SITs prior to gene duplication and fusion. We find that SITLs are widely distributed amongst eukaryotes, most notably in major silicified plankton lineages such as radiolarians, chrysophytes and silicoflagellates. Phylogenetic analyses provide evidence for multiple independent duplication/fusion events in SITLs that have led to distinct groups of SITs. Expression of coccolithophore SITLs in heterologous systems reveals that SITLs can function act as Na+-coupled Si transporters. We find that coccolithophore species that possess SITs or SITLs have a requirement for Si in the calcification process. These studies reveal that the SITs, first discovered in diatoms, belong to a wider family of Si transporters that are broadly distributed in eukaryotes and likely underpin Si acquisition in many other silicified plankton lineages.

Dynamic subcellular translocation of V-type H+-ATPase is essential for diatom silica cell wall biomineralization

Daniel Yee, Mark Hildebrand, Martin Tresguerres

Scripps Institution of Oceanography, La Jolla, United States

Diatom cell walls, called frustules, are the main source of biogenic silica in the ocean and their intricate morphology is an inspiration for nanoengineering. Here we show novel dynamic aspects of frustule morphogenesis involving acidification of the silica deposition vesicle (SDV) by the V-type H+-ATPase (VHA). Transgenic *Thalassiosira pseudonana* expressing the VHA B subunit tagged with eGFP allowed us to visualize subcellular protein localization in live cells. In exponentially growing cultures, VHA was found in diverse subcellular localizations including the cytoplasm, the SDV, and the vacuole. We studied the role of VHA in frustule formation in synchronized cell cultures of *T. pseudonana*, a method that enriches for cells in the process of frustule formation. During the making of new frustules for the daughter cells, VHA first localized in the SDV making the girdle bands, and subsequently in the SDV making the valves. VHA localization in the SDV precluded accumulation of the acidotropic silica biomineralization marker PDMPO. Furthermore, pharmacological VHA inhibition prevented PDMPO accumulation in the SDV, frustule morphogenesis, and cell division, as well as the fusion of the silicalemma-associated protein SAP1 to the SDV. Finally, partial inhibition of VHA activity affected the nanoscale morphology of the distal valve. Altogether, these results indicate VHA activity is essential for frustule morphogenesis by acidifying the SDV and by regulating the insertion of structural proteins into the SDV, and identify VHA as a target protein for frustule nanoengineering.

Morphological and molecular characterization of the valve silica deposition vesicle

Christoph Heintze [1], Petr Formanek [2], Nico Sommerdjik [3], Nils Kröger [1]

[1] B CUBE Center for Molecular Bioengineering, CMCB, TU Dresden, Dresden, Germany
[2] Leibniz-Institut für Polymerforschung Dresden e. V., Dresden, Germany
[3] Department of Chemical Engineering and Chemistry, Laboratory of Materials and Interface Chemistry and Centre for Multiscale Electron Microscopy, Eindhoven University of Technology, Eindhoven, Netherlands

The variety of the cell wall morphologies among the diatom kingdom and the hierarchical patterns inside the silica structures have inspired people since decades. The diatom cell wall is a silica-based, nano-structured bio-mineral, formed in specialized subcellular compartments, called silica deposition vesicles (SDVs). So far, little is known about the molecular organization of the SDVs, due to the limited accessibility of the SDVs in vivo and vitro. Here, we describe an approach to investigate silica morphogenesis inside valve SDVs in vitro, and to analyze their biomolecular composition. After lysis of synchronized *Thalassiosira pseudonana* cells, the formation of dendritic silica structures and hierarchical pore patterns was followed by imaging valve SDVs at various developmental stages using transmission electron microscopy (TEM). Particularly in valve SDVs during early developmental stages, spherical nanoparticles (ca. 20 nm) were identified that were associated with the dendritic silica structures. EELS analysis revealed that the nanoparticles contained silicon, suggesting that they are carriers of the silica material inside the SDVs. EDX analysis demonstrated that the developing silica contained nitrogen, sulfur and phosphorus, which might be indicative of the presence of organic components. Using immunofluorescence microscopy Silicanin-1 was detected in demineralized SDVs, thus revealing the presence of an organic matrix in the lumen of valve SDVs. In TEM analysis the organic matrix showed a dendritic morphology that resemble the structure of the developing valve silica. Our data suggest that morphogenesis of valve biosilica involves a protein-based nanopatterned organic matrix and mineralization through the fusion of silica nanoparticles.

Characterizing the influence of two actin associated proteins on cell wall formation in *Thalassiosira pseudonana*

Sarah Lerch [1], **Daniel Yee** [2], **Mark Hildebrand** [2]

[1] University of Rhode Island, Kingston, United States
[2] Scripps Institution of Oceanography, University San Diego California, San Diego, United States

The question of how diatoms create their intricately patterned silica cell walls has long intrigued the scientific community. Efforts to answer this question have identified multiple organic molecules which catalyze silica polymerization, influencing nanoscale cell wall morphology. Higher order meso- and macroscale structures, such as valve size and morphology, are shaped by the cytoskeletal protein actin. Though actin's role in cell wall formation is well documented, how its dynamics are regulated during this process remains unknown. In this study we investigated two proteins as potential regulators of cell wall associated actin dynamics in *Thalassiosira pseudonana*. Gene knockdown and over-expression techniques were used to alter target expression; transgenic lines were then screened for changes silicification, cell wall morphology and growth rate. Co-knockdown lines were characterized by reduced silicification, decreased valve surface ornamentation and slowed valve formation. Over-expression lines displayed similar changes in valve morphology which were unstable and quickly reverted to wild type morphologies. These phenotypes were accompanied by highly variable changes in target transcript abundance. Based on these findings we have developed a model for how these two proteins regulate the actin dynamics associated with cell wall formation, ultimately influencing valve morphology. This study provides the first investigation into regulation of the actin dynamics associated with cell wall formation in *T. pseudonana* and identifies two potential actin regulatory proteins, making it a valuable addition to our understanding of diatom cell wall formation.

Session 2: Cell biology

Structural bases of phytoplankton acclimation responses

Giovanni Finazzi [1], Serena Flori [1, 2], Denis Falconety [1], Clarisse Uwizeye [1], Pierre-Henri Jouneau [1], Davide Dal Bo [1], Claire Seydoux [1], Florence Courtois [1], Johan Decelle [1], Gilles Curien [1], Guy Schoehn [1], et al.

[1] Institut de Recherche Interdisciplinaire CEA Grenoble, Grenoble, France
[2] Marine Biological Association of the UK, Pluymouth, United Kingdom

Oxygenic photosynthesis is the basis of life on earth thanks to oxygen and reduced carbon production. To function properly, photosynthesis requires a correct balance between light absorption and utilisation to produce energy-rich molecules (ATP and NADPH) needed for CO2 assimilation into organic matter. Central to this balance is the Proton Motive Force (PMF), i.e. a gradient of proton and ions that is generated by photosynthesis itself and regulates several steps of this process: light absorption (via a negative feedback: Non Photochemical Quenching), electron flow and ATP synthesis. While the basic mechanisms of photosynthesis are largely conserved between organism issued from primary and secondary endosymbiosis, the regulation of the PMF is likely different between them. By combining molecular engineering and in vivo spectroscopy, we have pinpointed molecular actors (ion channels, redox transporters) that likely regulate the PMF in the diatom *Phaeodactylum tricornutum*. These elements actively modify the light acclimation responses of this organism in laboratory conditions. Moreover, using single cell tomography we have identified the structural bases of this regulation, showing the existence of subcellular interactions between the energy producing organelles, the chloroplast and mitochondria. A survey of several phytoplankton members suggests a conservation of these interactions and of their role in driving acclimation to the environment. By influencing different lifestyles in the ocean, subcellular interactions likely represent a fundamental regulative process in marine microorganisms.

Excitable diatoms: sophisticated mechanisms for electrical signalling enable environmental sensing in the oceans

Katherine Helliwell [1, 2], Abdul Chrachri [1], Friedrich Kleiner [1], Susan Wharam [1], Julie Koester [3], Alison Taylor [3], Glen Wheeler [1], Colin Brownlee [1]

[1] Marine Biological Association, Citadel Hill, Plymouth PL12PB, UK, Plymouth, United Kingdom
[2] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4QD UK, Exeter, United Kingdom
[3] Department of Biology and Marine Biology The University of North Carolina, Wilmington, NC 28403, USA, Wilmington, United States

Diatoms thrive in dynamic ecosystems where environmental conditions can fluctuate rapidly over diverse spatiotemporal time-scales. Metabolic attributes enabling diatoms to sense and rapidly acclimate to key environmental drivers are therefore critical to their ecological success. An important innovation in eukaryotes for environmental perception has been the evolution of rapid Na+/Ca2+-based action potentials, best characterised in animal neuromuscular cells. Such fast membrane excitability enables eukaryote cells to convert environmental stimuli into membrane depolarisations that govern numerous physiological responses. Diatoms also exhibit fast Na+/Ca2+-based action potentials. However, many species lack the four-domain voltage-gated Cav/Nav (4D-Cavs/Navs) channels that underpin membrane excitability in animals and other eukaryotes. We report the characterisation of a novel class of voltage-gated ion channels (EukCatsAs) found exclusively in diatoms, which resemble prokaryote single-domain voltage-gated Na+ channels. Functional characterisation of diatom EukCatAs indicates that they are voltage-gated Na+- and Ca2+-permeable channels, with rapid kinetics resembling metazoan 4D-Cavs/Navs. Development of single-cell imaging techniques in *Phaeodactylum tricornutum* coupled with gene knockout approaches, has revealed EukCatAs underpin voltage-activated Ca2+-signalling important for membrane excitability, and mutants also exhibit impaired motility. This suggests diatoms possess alternative mechanisms for fast electrical signalling. Building on the tools we have developed we are now characterising the broader roles of Ca2+ signalling in diatom cell biology. In particular, the role of spatiotemporal patterns of intracellular Ca2+ in governing perception of fluctuating osmotic conditions will be described. Together our findings demonstrate diatoms possess a suite of sophisticated signalling mechanisms for environmental sensing in the oceans.

A sticky situation: understanding the molecular mechanism of diatom underwater adhesion

Jirina Zackova Suchanova, Beata Wilgan, Nils Kröger, Nicole Poulsen

Center for Molecular and Cellular Bioengineering, B CUBE, Technische Universität Dresden, Dresden, Germany

Together with bacteria, diatoms dominate marine biofilms in sunlit marine environments. The initial event of underwater adhesion is accomplished through the secretion of carbohydrate-rich extracellular polymeric substances (EPS). In motile pennate diatoms the adhesive EPS strands are secreted through the raphe, and are deposited as trails on the substratum thus providing the traction required for cell motility. So far, the molecular composition of the diatom adhesive trails has remained poorly characterized. Recently, we have developed a method for isolating cell-free diatom adhesive trails, and demonstrated that they contain a complex mixture of carbohydrates and proteins. To gain a deeper understanding of the role of proteins in diatom adhesion we have performed a proteomics analysis of the adhesive trails isolated from the fouling diatom *Craspedostauros australis*. A number of novel proteins were identified that share sequence features similar to those found in extracellular and bacterial cell-wall anchored proteins (Choice-of-Anchor A domain, von Willebrand factor type A domain, PTS-rich domain). The localization of some of these proteins in adhesive trails and biofilms was confirmed by the GFP-tagging and immunolabeling. This work provides insights into the biomacromolecular structure of diatom EPS trails and lays the foundation for unraveling the molecular mechanism of diatom underwater adhesion.

Bloom and bust

Krisztina Sárközi [1], Rachel Hipkin [1], Amy Kirkham [1], Andrew Toseland [1], Amanda Hopes [1], Mohammed Aldholmi [2], A Ganesan [2], Thomas Mock [1]

[1] School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom
[2] School of Pharmacy, University of East Anglia, Norwich, United Kingdom

Diatoms display an opportunistic growth described as a 'bloom and bust' life cycle, and they have a remarkable ability to adapt to extreme and fluctuating environments and move into new ones. The processes that enable the 'bloom and bust' life cycle are mostly unknown. Here we show that a novel conserved DNA binding protein (BIG1) differentially regulates genes involved in the progression through the cell cycle in centric diatoms. Reverse genetics complemented by CRISPR-Cas generated gene knock-outs, and biochemical approaches were combined in a comprehensive study with the aim of elucidating the role of this regulatory protein for bloom formation. Although its precise biochemical mechanism is still enigmatic, we confirmed that it is a regulatory DNA-binding protein influencing the expression of many genes and metabolites responsible for cell division and photosynthesis. Thus, our results provide insights into regulatory processes that govern diatoms' opportunistic growth and therefore will help to understand why this algal group is so successful in many different aquatic environments.

Control of Biosilica Morphology and Mechanical Performance by the Conserved Diatom Gene Silicanin-1

Stefan Görlich, Damian Pawoski, Igor Zlotnikov, Nils Kröger

Technische Universität Dresden, Dresden, Germany

Diatoms represent a large group of unicellular, eukaryotic microalgae that are most well known for their ability to produce cell walls made of nanopatterned porous biosilica. The species-specifically patterned cell walls are paradigms for biological mineral morphogenesis and the evolution of lightweight materials with exceptional mechanical performance. The formation of biomineral building blocks often takes place within specialized intracellular vesicles, which in diatoms are called Silica Deposition Vesicles (SDV). Recently two families of SDV membrane proteins have been identified that are well conserved throughout all diatoms and may therefore play a fundamental role in diatom cell wall formation. One promising candidate is Silicanin-1 (Sin1), which was shown to have moderate silica-formation activity in vitro that is enhanced by the addition of long-chain polyamines. It was therefore speculated that Sin1 may influence the assembly of biosilica-forming biomolecules within the SDV lumen. Here we describe the CRISPR-Cas9 mediated gene knockout of Sin1 in *Thalassiosira pseudonana*. Although the mutants grew normally, the knockout cell lines exhibit a reduced biosilica content and showed pleiotropic effects on silica morphogenesis, which drastically compromised the strength and stiffness of their cell walls. These results identify Sin1 as a key player in the development of specific structural features of the *T. pseudonana* cell wall and as essential for the biogenesis of mechanically robust diatom cell walls, thus providing an explanation for the conservation of this gene throughout the diatom realm.

Session 3: Genomics and genome engineering

Genome editing in diatoms: tools, accomplishments and prospects

Serif Manuel [1], Gwendoline Dubois [1], Anne Laure Finoux [1], Marie-Ange Teste [1], Gilles Defrel [1], Denis Jallet [1], Fayza Daboussi [1, 2], et al.

[1] Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France, Toulouse, France
[2] Toulouse White Biotechnology, France., Toulouse, France

Diatoms are highly prized in industrial biotechnology. Several years of investigation have led to the development of the genome engineering tools for exploring and exploiting the metabolism of these organisms. Thus, custom molecular scissors have recently emerged as useful tools to inactivate single target genes in *Phaeodactylum*, either for functional analysis or to rewire its metabolism. It has been mediated by transforming plasmids encoding a nuclease and an antibiotic resistance cassette into the cells, both then stably integrated at random sites within the nuclear genome. Disadvantages of this approach include: the random integration of all or part of the plasmid DNA into the genome can lead to undesired gene disruptions or uncontrolled effects on gene expression near the integration site(s); the long-term expression of the nuclease can potentially induce off-target cleavage; and the impossibility to eliminate background mutations or integrated transgenes through outcrossing in Phaeodactylum, because this is a diploid organism with no known sexual reproduction. Here, we report a highly efficient multiplex genome-editing method in the diatom *P. tricornutum*, relying on the biolistic delivery of the CRISPR/Cas9 molecular scissor in the protein form (RNPs) coupled with the identification of two endogenous counter-selectable markers, PtUMPS and PtAPT. First, we demonstrate the functionality of RNP delivery by positively selecting the disruption of each of these genes. The power of this methodology was confirmed by creating strains in which three genes were simultaneously inactivated (triple knock-out) without introducing any selection marker or DNA into the cells.

What is the origin of high haplotype diversity within clonal diatom cultures?

Petra Bulankova [1, 2], Ilse Vercauteren [1, 2], Cristina Osuna Cruz [1, 2], Emmelien Vancaester [1, 2], Klaas Vandepoele [1, 2], Koen Sabbe [3], Wim Vyverman [3], Lieven De Veylder [1, 2]

[1] Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium
[2] VIB Center for Plant Systems Biology, Ghent, Belgium
[3] Laboratory for Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium

In contrast to most other algal groups, diatoms are diploid organisms possessing two sets of chromosomes, each inherited from one parental gamete during mating. Each chromosome represents a set of linked single-nucleotide polymorphism alleles called haplotype. In general, chromosomes (and so haplotypes) are propagated faithfully through mitotic divisions. Therefore in a population of diatoms derived from a single founder cell, we would expect to contain a maximum of two haplotypes for most genomic loci. In cultures established from single cells of the pennate diatom Seminavis robusta we repeatedly observed the presence of multiple haplotypes for several loci. We hypothesize that this haplotype variability in clonal populations is caused by enhanced recombination of homologous chromosomes during vegetative proliferation, a process known as mitotic recombination. Although harmful for multicellular organisms, we suppose that enhanced mitotic recombination can lead to rapid fixation of new advantageous mutations in clonally propagating single cellular organisms like diatoms. In general, two main outcomes of mitotic recombination can be observed: accumulation of new haplotypes in clonal populations and a loss of heterozygosity (LOH) at the single cell level. We were able to quantify genome-wide the haplotype diversity for S. robusta and for *P. tricornutum* and confirm it by Sanger sequencing of individual emulsion PCR products from selected loci. Currently, we are following the LOH in diatoms populations started from a single cell. At the meeting, I will present our latest progress on the frequency of mitotic recombination in diatoms.

Diatom genetic transformation with Universal Loop assembly (uLoop): an open, efficient and species-agnostic DNA fabrication method

Bernardo Pollak [2, 6], Tamara Matute [2, 5], Isaac Nuñez [2, 5], Ariel Cerda [1, 2, 5], Valentina Vargas [1, 3], Constanza Lopez [1, 3], Vincent Bielinski [4], Chris Dupont [4], Pater von Dassow [1, 3], Fernan Federici [1, 2, 5]

[1] Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile;, Santiago, Chile
[2] Millennium Institute for Integrative Biology (iBio), Santiago, Chile
[3] Instituto Milenio de Oceanografia de Chile, Santiago, Chile
[4] John Craig Venter Institute, San Diego, United States
[5] Fondo de Desarrollo de Áreas Prioritarias, Center for Genome Regulation, Santiago, Chile
[6] Fundacion Ciencia & Vida, Santiago, Chile

Golden Gate (GG) methods for DNA construction are becoming ubiquitous due to its efficiency. A 'common syntax' has been defined to allow interoperability between GG libraries. However, the vector backbones used for assembly have remained the same as the ones commonly used for the genetic manipulation of each target organism, limiting the assembly performances. In addition, GG assembly systems become laborious beyond the assembly of 4 transcriptional units. Here, we describe "universal Loop" (uLoop) assembly, a simple GG assembly method that enables hierarchical fabrication of large DNA constructs (> 30 Kb) for any organism of choice with high efficiency and fidelity. This system uses a compact library of 8 plasmids (2 sets of 4), enabling the assembly of 4 genetic modules per round. DNA elements are composed in an exponential manner (1, 4, 16…) by alternating between the same two vector sets and the repetitive use of BsaI and SapI enzymes, where the product of one reaction becomes the substrate of the following one. The elements required for transformation/maintenance in target organisms are added to the vector during the assembly reactions, enabling customization of host-specific plasmids without affecting their assembly performance. Thus, uLoop is a species-agnostic method that decouples the optimization of each of these two processes. We show the engineering of conjugating vectors for multi-gene expression in the diatom *Phaeodactylum tricornutum*, and other species. This effort has led to the generation of a growing library of DNA parts for diatoms, available through an OpenMTA for unrestricted sharing and open-access.

Systematic and functional analysis of horizontal gene transfer events within diatoms

Emmelien Vancaester [1, 2], **Thomas Depuydt** [1, 2], **Cristina Maria Osuna-Cruz** [1, 2], **Klaas Vandepoele** [1, 2]

[1] Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium
[2] VIB Center for Plant Systems Biology, Ghent, Belgium

Horizontal gene transfer (HGT) is the transfer of genetic information between species by a route other than from parent to offspring. It has been shown that HGT genes provide novel functionalities which can function to orchestrate survival under environmental stress conditions. Whole-genome sequences of representatives from the polar centrics (*Thalassiosira pseudonana*, Thalassiosira oceanica, Cyclotella cryptica), araphid pennates (Synedra acus) and raphid pennates (*Phaeodactylum tricornutum*, Fistulifera solaris, Fragilariopsis cylindrus, Pseudo-nitzschia multistriata) have become available in recent years, allowing the analysis of the evolutionary history within diatoms. Here, we report the first large-scale analysis which applied a high-throughput gene family phylogenetics-based approach to detect HGT events across all sequenced diatoms. Between 3-5% of their respective gene repertoire was assigned as originating from horizontal descent. Interestingly, we found a complex pattern of HGT influx, with 15-30% of HGT genes which originated at the ancestor of the diatom lineage. Moreover, HGT genes in all diatoms are enriched in several catalytic processes, such as ‘cellular amino acid metabolic process’, ‘methyltransferase activity’ and ‘oxidoreductase activity’, suggesting to act as a way to expand their metabolic capacities. Exploiting large-scale expression data, we observed that the expression specificity decreases with age , both for genes of horizontal and vertical descent . HGT genes under negative purifying selection based on the resequencing data available for *Phaeodactylum tricornutum* and showing a specific expression pattern, will be selected for further investigation. Overall, we show that horizontal gene transfer is a prevalent mechanism within diatoms to aid their adaptive capabilities.

Environmental epigenetics in diatoms

Leila Tirichine

CNRS UMR 6286, UFIP, UFR Sciences et Techniques, Université de Nantes, 2, rue de la Houssinière 44322, Nantes, France

Epigenetic marks including DNA methylation, Post Translational Modifications of histones (PTMs) and non-coding RNAs are known to be important players of genome regulation in response to environmental triggers. These modifications lead to permanent or reversible changes affecting the phenotype, physiology and ecology of species. We have identified in diatoms including the model species *Phaeodactylum tricornutum* a conserved epigenetic machinery and additional features not always present in animals and plants likely contributing to the success of this class of species. Here we discuss ongoing work to understand the role of epigenetic mechanisms in the response of diatoms to their environment.

Re-analysis of the *Fragilariopsis cylindrus* genome reveals a third subgenome copy, supporting a hypothesis for polyploidy

Kat Hodgkinson [1], Jonathan Wright [2], Gonzalo Garcia Accinelli [2], Luis Yanes [2], Darren Heavens [2], Amanda Hopes [1], Thomas Mock [1], Cock van Oosterhout [1], Bernardo Clavijo [2]

[1] University of East Anglia, Norwich, United Kingdom
[2] Earlham Institute, Norwich, United Kingdom

There are currently two assemblies for *F. cylindrus*, but neither are suitable for evolutionary genomics; the diploidised PacBio assembly has low consensus accuracy, and the Sanger assembly is largely fragmented. With the aim of evolutionary analysis, we constructed an assembly with high base-pair accuracy from Illumina sequencing, and combined this with Nanopore long-reads for greater contiguity. We found a strong signature for 3 different copy numbers in the genome: X, 2*X and 3*X. The topology of the graph and the motif frequencies of its components reveal 3 categories of syntenic regions: 1 different and 2 similar copies, 3 very similar copies and 3 very different copies. We used the BSG (Basic Sequence Graph) approach to recover haplotype-representative specific content, confirming the k-mer spectra and topological observations and supporting a working hypothesis of 3 full-genome haplotypes (A, B and C). A large 70% of the genome is represented in regions where A and B are close, but C is diverged, 14% where all the haplotypes are close, and 14% where all the haplotypes are diverged. These results provide an alternative explanation for differential expression between haplotypes as an expected result of diverging sub-genomes in a polyploid organism. Chromosome counts are varied for many diatoms, and polyploidy, an important mechanism in diatom evolution, may be even more prevalent than previously described. We hope future genomic analyses, conducted with consideration for haplotype-specificity and ploidy, may further expand our understanding of diatom evolution and genome composition.

Multiplexed CRISPR-Cas9 mutagenesis development and applications

Mark Moosburner [1, 2], Pardis Gholami [2], James McCarthy [2], Maxine Tan [1, 2], Vince Bielinski [2], Andrew Allen [1, 2]

[1] Scripps Institution of Oceanography, UCSD, La Jolla, United States
[2] J. Craig Venter Institute, La Jolla, United States

Marine diatoms play significant ecological and biogeochemical roles in oceans and have strong potential as organismal platforms for biotechnological and industrial purposes. In order to address both modes of research, sophisticated molecular and genetic tools are required. Presented here are improved methodologies for introducing CRISPR-Cas9 to *Phaeodactylum tricornutum* cells and a streamlined protocol for genotyping mutant cell lines without previously known phenotypes. First, bacterial-conjugation transformation was optimized for the delivery of Cas9 by transcriptionally fusing Cas9 to a selectable marker by the 2A peptide. An episome cloning strategy using both negative and positive selection was developed to streamline CRISPR-episome assembly. Next, genotyping strategies were developed to shorten the time to generate mutants that utilizes manual sequencing curation, TIDE sequencing analysis, and T7 endonuclease assays. Following the new experimental pipeline, both single-gene and two-gene knockout cell lines were generated at mutagenesis efficiencies of 48% and 25%, respectively. Additionally, this CRISPR-Cas9 system has been used for saturation mutagenesis (multiple targets for one gene) and three-gene mutagenesis. The new CRISPR-Cas9 multiplex knockout pipeline is being used to functionally characterized nitrogen metabolism genes in Phaeodactylum. In order to utilize the multiplex capabilities of CRISPR-Cas9, the four GS/GOGAT pathway enzymes were targeted individually and in paired combinations that terminate nitrogen assimilation in the chloroplast and mitochondria. The set of mutant cell lines will be characterized by measuring growth on multiple nitrogen source and will be supported by metabolite profiling.

Insights into the genomic diversity of the marine diatom *Chaetoceros muelleri*

Raffaela Abbriano [1], Tim Kahlke [1], Michele Fabris [1, 2], Peter Ralph [1]

[1] University of Technology Sydney, Climate Change Cluster, Ultimo, Australia
[2] CSIRO Synthetic Biology Future Science Platform, Brisbane, Australia

Chaetoceros is one of the most widespread and diverse diatom genera in the world's oceans. Given its global distribution, it is a well-suited model for exploring genetic adaptations across various environmental niches. In addition, certain *Chaetoceros* species are of interest to industry, including extensive use in aquaculture. However, the genetic basis for desirable industrial traits such as high productivity and lipid content, remain to be identified. Here we present insights into the genome structure and content of *Chaetoceros muelleri* CCMP 1316, a commercially relevant temperate marine diatom, as the first representative genome from the *Chaetoceros* genus. We also demonstrate an application of two cutting-edge and complementary sequencing technologies to capture and reconstruct haplotypes and structural variants within the *C. muelleri* genome. A hybrid strategy was developed using linked reads generated through the 10X Chromium System for de novo assembly, accompanied by long-read nanopore sequencing. The use of these new technologies for sequencing and genome assembly allow for a more comprehensive analysis of the variation inherent in diatom genomes. The final annotated genome will be presented in the context of other sequenced diatom genomes from a regulatory, metabolic, and evolutionary perspective.

Non-diatom lecture

Whole-cell spatial proteomics of apicomplexans and dinoflagellates: mapping genome-level complexity onto these divergent cells.

Ross Waller

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom

Our appreciation of the complexity and evolution of protist cells is highly constrained by our limited knowledge of the locations and functions of most of the cell's proteome. Typically, few, if any, proteins have been located in a given taxon, and even the better studied protists have only a very small fraction of proteins' locations experimentally determined. At best, many protein locations are predicted based on studies of homologues from distant relatives. But, more often, proteins predicted from protist genomes or transcriptomes are 'hypotheticals' unique to a taxon's lineage, stymying even predictions of location or function by comparative biology. To address this deficit in our basic understanding of the compositional organisation of the cell, we have used a spatial proteomics method called hyperLOPIT to simultaneously capture the subcellular association of thousands of proteins in the apicomplexan Toxoplasma. We have resolved almost 4000 proteins to locations including endosymbiotic organelles, secretory compartments related to invasion, cytoskeletal structures, sub-nuclear compartments and large molecular complexes. These protein atlases reveal: protein associations throughout the cell providing testable hypotheses of their function; coordinated transcriptional control of discrete cell compartments; conservation and novelty of compartment proteomes both between apicomplexans, and other eukaryotes; different paces of evolution across the different cell compartments and structures in Toxoplasma; and clear instances of protein relocation from one organelle to a different one over evolutionary time. This new, global view of the cell proteome provides a much more complete framework for understanding the mechanisms of function and evolution of these cells.

Session 4: Biotechnology

Triacylglycerol accumulation and catabolism in *Phaeodactylum tricornutum*

Hanhua Hu

Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

Triacylglycerol (TAG) is synthesized through transferring acyl from acyl-CoA or phospholipid to diacylglycerol, and the two acylation reactions are catalyzed by diacylglycerol acyltransferase (DGAT) and phospholipid : diacylglycerol acyltransferase (PDAT) respectively. There are 6 DGATs and one PDAT-encoding genes in *Phaeodactylum tricornutum*, and 4 of DGATs are located on chloroplast endoplasmic reticulum membrane (cERM), while PDAT and 2 of DGATs are located on the chloroplast inner envelope membrane (IEM). It has been well documented that TAG accumulation becomes noticeable as a result of nutrient stress. However, transcript abundances of DGATs and PDAT are very high when TAG accumulation has not yet begun and lipid droplets are usually not visible in the plastid of *P. tricornutum*. On the other hand, there are 18 TAG lipase (TGL) genes in *P. tricornutum*, and their transcript abundances are not only high during cellular exponential growth but also high under nutrient stress conditions. At least 8 out of the 18 TGLs are located in the plastid with a wide distribution from cERM, cER, periplastidial membrane, outer envelope membrane, IEM, to stroma. TGLs play an important role in the regulation of TAG accumulation, and knockdown of TGLs dramatically decreases TAG degradation but gives rise to lower growth. In addition, redirection and acceleration of carbon flux from branched-chain amino acid degradation toward TCA cycle also contribute to TAG accumulation. I will present the TAG synthesis, catabolism and accumulation in *P. tricornutum*, and accordingly several suitable strategies for engineering microalgae to increase TAG productivity will also be proposed.

Using the diatom *Phaeodactylum tricornutum* as a chassis for biological degradation of plastics

Daniel Moog, et al.

Laboratory for Cell Biology, Philipps University Marburg, Karl-von-Frisch-Str. 8, 35032, Marburg, Germany
Center for Synthetic Microbiology (SYNMIKRO), Hans-Meerwein-Str. 6, 35032, Marburg, Germany

Plastic is an extremely useful material with a wide range of applications and seemingly no longer indispensable for our daily life. However, the tremendous amount of plastic waste that is produced each year has become a major ecological issue on our planet in the last decades, mostly due to inadequate disposal and the high durability of the synthetic material. The consequences of plastic pollution for Earth's ecosystems are so far unforeseeable, but it becomes more and more evident that the plastic accumulating in nature is harmful for life. Although bioremediation would be highly desirable and probably a solution to the problem of continuous plastic pollution, the bulk of plastics produced so far is not biodegradable and thus extremely durable. The reason for this is the artificial nature of plastic and its relatively short existence on Earth. Remarkably, some life-forms have acquired the capability to degrade plastics and can use them as a nutrient for growth. Such organisms might provide promising solutions for bioremediation of plastic waste in future biotechnological applications. *Phaeodactylum tricornutum* is a photosynthetic marine diatom that has developed into an extremely valuable model system for molecular biology and biotechnology. In this project, *P. tricornutum* was modified via synthetic biology using bacterial genes, which encode plastic-hydrolyzing enzymes, to generate a chassis for biological degradation of plastics in a saltwater-based environment. The results demonstrate the potential of the diatom for future applications in biological plastic degradation up to the generation of eco-friendly and comprehensive recycling processes for synthetic plastics.

Regioselective immobilization of an enzyme cascade on diatom biosilica

Ekta Kumari, Nils Kröger

Center for Molecular and Cellular Bioengineering, B CUBE, Technische Universität Dresden, Dresden, Germany

Diatom biosilica is a favorable support material for enzyme immobilization, as its hierarchical mesoporous structure provides a large surface area for the attachment and allows the efficient reactant diffusion. The immobilization of multi-enzyme systems is an emerging technology that enables the rapid multi-step conversion of a substrate into a desired product. Previously it was shown that the activity of immobilized glucose oxidase is strongly influenced by the species-specific diatom biosilica structures. However, it has remained unknown, whether the activity of an enzyme cascade is influenced by its positioning within structurally different regions (i.e. girdle bands vs valve) of the biosilica from the same diatom species. Here we report the engineering of four *Thalassiosira pseudonana* strains each carrying a simple enzyme cascade, consisting of glucose oxidase and horseradish peroxidase. The strains differ by the location of the enzymes giving rise to four different enzyme cascade configurations. This was achieved by genetic fusion to region-specific silica-associated proteins (cingulins, silaffins). The four enzyme cascade configurations exhibited striking differences in specific catalytic activities. Transformants with both enzymes immobilized in the valve exhibited the lowest specific activity, whereas the specific activity of transformants containing both enzymes in the girdle band region was 3- to 6-fold higher. Further investigations into the cause for the activity differences ruled out a significant influence of the silica targeting tag, and differences in the accessibility of the enzymes for the substrate. These results confirm the remarkable, yet little understood, effect of silica structure on enzyme activity.

Interrogating the genomes of transgenic *Phaeodactylum tricornutum* strains for improved genetic engineering strategies in biotechnological applications

Jestin George [1], Michele Fabris [1, 2], Tim Kahlke [1], Raffaela Abbriano [1], Peter Ralph [1]

[1] University of Technology Sydney, Climate Change Cluster, Broadway Campus, Ultimo NSW 2007, Australia, Sydney, Australia
[2] CSIRO Synthetic Biology Future Science Platform, PO Box 2583, Brisbane, Qld, 4001, Brisbane, Australia

Diatoms are biotechnologically important for manufacturing natural and non-native high-value products. Elevating diatom strains to commercially competitive standards often requires genetic engineering for novel traits or improved outputs. This is predominantly achieved by random integration of transgenes into nuclear chromosomal DNA. Unfortunately, this method is beset with transgene expression issues (due to position effect), requiring extensive and costly screening. Recently, a new and efficient transformation technique was demonstrated in a model diatom, *Phaeodactylum tricornutum*, for extrachromosomal transgene expression that bypasses issues related to random chromosomal integration. However, there is little knowledge available regarding the molecular mechanisms of both chromosome-integrated and extrachromosomal expression in diatoms. Therefore, we transformed *P. tricornutum* using both methods to express a heterologous enzyme fused to fluorescent protein, mVenus. We showed that large-scale screening of random chromosome-integrated clones resulted in a small selection of high performing strains. We phenotyped these strains and used Oxford Nanopore's MinION sequencer to interrogate the genomes of superior chromosome-integrated clones, as well as an extrachromosomal-expression clone. Our results revealed a complete analysis of all integration events, genomic locations and transgene arrangements associated with high-performing clones at a nucleotide-scale detail, never before been reported in any diatom. Our findings are important for developing better, more precise gene engineering approaches in *P. tricornutum*, including possible genomic safe harbour locations which support high transgene expression for targeted integration strategies. Furthermore, we have demonstrated that transgene DNA is not integrated inadvertently into the nuclear genome of extrachromosomal-expression clones, an important characterization of this novel transformation method.

Metabolic engineering of the oleaginous diatom *Fistulifera solaris* for biofuel application and beyond

Tsuyoshi Tanaka

Institute of Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan

Marine diatom *Fistulifera solaris* is recognized as a promising host for production of neutral lipids as feedstock of biofuels. Since genetic engineering techniques including CRISPR/Cas9-mediated genome editing were established for *F. solaris*, we have demonstrated the enhanced production of triacylglycerols (TAG) by reinforcing a lipid synthesis pathway or suppressing a lipid degradation pathway. Besides the improvement of TAG quantity, we recently achieved to alter the quality of TAG. More specifically, engineering of fatty acid composition was demonstrated for enhancement of polyunsaturated fatty acids (PUFA) and middle-chain fatty acids by overexpression of desaturase and thioesterase, respectively. These fatty acids are useful for not only biofuels but also various applications including health supplements and detergents. Biosynthesis of pharmaceutical compounds converted from the abundant PUFA was also demonstrated. All of these applications are supported by the superior oleaginous phenotype of *F. solaris*. Mass cultivation of the diatom is an essential technique for stable production of these valuable compounds. However, in outdoor cultivation, zooplanktonic grazers can collapse the mass cultures. To address this issue, we converted chlorophylls into phototoxic compounds namely chlorophyllides by expressing a plant chlorophylase. When the engineered *F. solaris* and zooplanktonic grazers were cultured, we confirmed that the growth of grazers was suppressed. In addition, chromosome-scale assembly of *F. solaris* genome was recently achieved with the aid of long-read nanopore sequencing technology. The new assembly highlighted the allopolyploid genome structure of *F. solaris*. In this presentation, structures and functions of the allopolyploid genome related to the oleaginous phenotype will be discussed.

Diatom Cell Walls as Biogenic Alumosilicates – Cultivation, Modification, and Application

Lydia Köhler [1], Kaitlin K. K. Kammerlander [1], Susanne Machill [1], Anja Werner [2], Carolin Selzer [2], Stefan Kaskel [2], Eike Brunner [1]

[1] Chair of Bioanalytical Chemistry, Technische Universität Dresden, Dresden, Germany
[2] Chair of Inorganic Chemistry I, Technische Universität Dresden, Dresden, Germany

Diatoms exhibit a uniquely patterned silica cell wall, the frustule. It consists not only of SiO_2, but also of foreign elements such as metalloid and metal ions. Aluminum is a particularly interesting constituent because it directly replaces silicon in the silica framework, forming an alumosilicate. The present study aims at characterizing aluminum-enriched diatom cell walls by various methods including spectroscopy and sorption. In addition, the general influence of aluminum on the living cells is evaluated. Cultures were monitored with respect to deformations, changed fluorescence, and decreased growth rates as signs of aluminum toxicity. Several diatom species grown under varying aluminum concentrations were examined via optical, fluorescence, and scanning electron microscopy. Species from diverse habitats were chosen. They differ in terms of hierarchical pore patterns, motility, and cell wall thickness. The selected species include *Thalassiosira pseudonana*, *Stephanopyxis turris*, *Seminavis robusta*, and *Coscinodiscus granii*. Furthermore, growth parameters were evaluated to tailor materials properties of the frustules. Especially the molar ratio of aluminum to silicon is important with respect to future applications. Interestingly for sorptive and especially catalytic purposes, aluminum insertion in the silica framework causes negative charges, which are balanced by additional alkaline and earth alkaline metal ions. The present study demonstrates that these counter ions can be exchanged for protons acting as Brønsted acid sites. The resulting material was shown to be catalytically active in an alkylation of aromatic compounds, monitored by gas chromatography coupled with mass spectrometry.

Structure and functions of the allopolyploid genome of the oleaginous diatom *Fistulifera solaris*

Yoshiaki Maeda, Ryosuke Kobayashi, Tomoko Yoshino, Tsuyoshi Tanaka

Institute of Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan

The marine oleaginous diatom, *Fistulifera solaris*, has been recognized as a promising host for production of biofuels due to its steady growth and superior oleaginous phenotype. The preliminary genome analysis with pyrosequencing had been carried out to analyze the oleaginous phenotype, and revealed a striking genomic feature that *F. solaris* was likely an allopolyploid organism possessing two types of subgenomes. As allopolyploid plants, such as wheat and cotton, exhibit advantageous agricultural phenotype including broad environmental adaptability and high yield due to heterosis effects, the allopolyploid genome of *F. solaris* might be related to its high yields of biomass and lipids. However, the allopolyploid genome structure of *F. solaris* was not completely elucidated because of the limitation of the previously performed pyrosequencing. To further investigate the genomic landscapes of the alloplyploid genome, we carried out whole-genome re-sequencing using a long-read nanopore sequencer. As a result, we obtained the genome assembly with high contiguity, in which the genome sequences obtained with pyrosequencing were consistently included. All of 45 contigs consisting of 22 homologous chromosome pairs had telomeric repeats, and 37 had them at the both ends, suggesting relatively high completeness of our sequencing. Comparison of sequences between two subgenomes revealed multiplexing of a TAG-synthesis gene in one subgenome, which could be related to the high yield of lipids in this diatom. In this presentation, structural features of the allopolyploid genome of *F. solaris* will be introduced, and its functions related to the high yield of lipids will be discussed.

Diatom biofilms: towards sustainable closed loop through nutrient recycling and biodiesel production

Thomas Kiran Marella [1], **Sreenath Dixit** [1], **Archana Tiwari** [2]

[1] International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, India
[2] Diatom Research Laboratory, Department of Biotechnology, Amity University, Noida, India

Algae culture technologies like raceway ponds and photobioreactors are in use for decades but they are expensive and energy intensive due to which net energy recovered from algae oil is high. Method of cultivation plays a key role in how the biomass can be harvested, using traditional bioreactors with suspended cultures the cost and energy consumption for harvesting increases which influence the cost of biodiesel produced. So inexpensive and energy efficient technologies with high biomass productivity with efficient harvesting methods are the demand of the hour worldwide. The aim of this work was to study diatom dominated algal flow way (AFW) to treat urban wastewater and to evaluate biomass productivity, lipid contents and biodiesel production. The results indicated the seasonal average algae productivity of 34.83 g dry weight m2 d-1 with a nutrient removal rate of 2.52 g m2 d-1 N and 1.25 g m2 d-1 P while the lipid content ranged between 14-22% of dry cell weight with the highest lipid productivity of 9.29 g m-2 d-1 during summer. Biodiesel quality was superior during summer with the high centane number and cold filter plugging point values. High eicosapentaenoic acid content was found during winter growth cycles. AFW algae community was dominated by pennate diatoms during all growing seasons. This study is one of its kinds in tropical climates and it provides fundamental information for further optimization and use of AFW to treat domestic wastewater and to produce algae biofuel feedstock.

The lipid droplet proteome of *Phaeodactylum tricornutum*

Ben Leyland, Inna Khozin-Goldberg, Sammy Boussiba

The Landau Family Microalgal Biotechnology Laboratory, The French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sde Boker, Israel

During nitrogen starvation, diatoms arrest photosynthesis, cannibalize nitrogenous biomolecules, and use TCA cycle enzymes to increase the production of acetyl-CoA, the precursor of fatty acid biosynthesis. Fatty acids produced de novo, as well as recycled from other cellular membranes, are assembled into storage lipids, triacylglycerols (TAGs). TAGs are themselves stored in lipid droplets, specialized organelles composed of a spherical core of neutral lipids surrounded by a polar lipid monolayer. Lipid droplets have a diverse array of functions, such as shielding the intracellular environment from the cytotoxic effects of free fatty acid accumulation, prevention of hydrophobic peptide aggregation, energy storage, and assistance in the maintenance of redox homeostasis. Lipid droplets exchange lipids, proteins, and metabolites with other organelles, such as mitochondria and autophagosomes, via vesicular transport as well as by direct contact via membrane bridges. A diverse assemblage of proteins associated with lipid droplets facilitate their complex and dynamic functions. Using novel methods, we have isolated lipid droplets from the pennate diatom *Phaeodactylum tricornutum* strain Pt4, and sequenced their proteome using advanced mass spectroscopic techniques. The lipid droplet proteome of *P. tricornutum* reveals the complex interplay between lipid droplets and other intracellular compartments, mechanistic details into how they function, and conserved features in common with other eukaryotic organisms. This dataset will serve as the jumping off point towards a deeper understanding of diatom lipid metabolism, and facilitate future biotechnological advancements.

Session 5: Ecophysiology I

The pyrenoid based CO_2-concentrating mechanism in marine diatoms

Yusuke Matsuda, Yoshinori Tsuji, et al.

Department of Bioscience, School of Science and Technology, Kwansei Gakuin University, Sanda, Japan

Marine diatom acquire inorganic carbon from seawater and accumulate intracellularly to create an high CO_2 condition at close proximity of the CO_2 fixing enzyme, RubisCO. As a major mechanism of the CO_2 concentrating mechanism (CCM), operation of the biophysical CCM in diatoms is now well established. The biophysical CCM pumps dissolved inorganic carbon (DIC) from seawater across plasmalemma and chloroplast envelope and strategically mobilize accumulated DIC towards the place of fixation, the pyrenoid structure, where most RubisCO in the chloroplast is sequestrated. However, detailed mechanism of this CO_2 mobilization within the chloroplast and the pyrenoid is not known well. In our recent studies, an occurrence of new type theta carbonic anhydrase (CA) is detected at the lumen of the thylakoid membrane which penetrating the pyrenoid of *Phaeodactylum tricornutum*. This CA was shown to be vital for the growth under low CO_2 and the operation of the biophysical CCM, strongly suggesting that CCM is tightly related to the function of the specific thylakoid membrane. Structural feature of the pyrenoid which enables best efficient DIC flow under the operation of CCM is another important issue which should yet to be answered. We employed a photoaffinity labeling of the structure of the pyrenoid and identified several new pyrenoidal components by subsequent mass spectrometry. The newly found factors was also shown to be vital for the operation of the efficient CCMs in both *P. tricornutum* and *T. pseudonana*. DIC flow model within the diatom pyrenoid will be proposed.

Exploring domoic acid biosynthesis in vivo: Building model systems

Patrick Brunson [1, 2], **Shaun McKinnie** [1], **Vincent Bielinski** [3], **Bradley Moore** [1, 5], **Andrew Allen** [2, 4]

[1] Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, La Jolla, United States
[2] Microbial and Environmental Genomics Group, J. Craig Venter Institute, La Jolla, United States
[3] Synthetic Biology and Bioenergy Group, J. Craig Venter Institute, La Jolla, United States
[4] Integrative Oceanography Division, Scripps Institution of Oceanography, La Jolla, United States
[5] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, United States

Harmful Algal Blooms (HABs) pose a serious threat to both the environment and the seafood industry. Many HABs also have the capability to produce small molecule toxins which carry significant human health risks. Although biosynthetic genes are well described for the freshwater cyanobacterial HAB toxins, no such system has been described for HAB marine eukaryotes. By using RNA sequencing approaches, we have identified the biosynthetic genes for domoic acid (DA), a neurotoxic glutamate receptor agonist produced by the marine diatom genus *Pseudo-nitzschia*. As hypothesized, DA biosynthesis implicates novel enzymology to create the characteristic DA pyrrolidine core from L-glutamate and geranyl pyrophosphate (GPP). Furthermore, these genes appear to be limited to diatoms of the *Pseudo-nitzschia* genus and are not present in any other sequenced microalgae. We plan to further investigate DA biosynthesis in vivo, including building a model system for DA expression in *Phaeodactylum tricornutum*. Bioinformatics analysis and preliminary *P. tricornutum* expression suggests a compartmentalized biosynthetic pathway, similar to pathway organization seen in plant terpenoid biosynthesis. Successful heterologous expression of DA biosynthesis in *P. tricornutum* will allow us to answer specific questions about the subcellular compartmentalization of biosynthetic steps, export of DA from the cell, and the effects of DA cproduction on primary metabolism and cellular physiology.

A transcription factor (PtPSR) controlling P acquisition in *Phaeodactylum tricornutum*

Amit Kumar Sharma [1], Alice Mühlroth [1], Ralph Kissen [1], Eric Maréchal [2], Juliette Jouhet [2], Leila Alipanah [1], Tore Brembu [1], Atle M Bones [1], Per Winge [1]

[1] Cell, Molecular biology and Genomics Group, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[2] Laboratoire de Physiologie Cellulaire Végétale, Centre National de la Recherche Scientifique, Commissariat à l'Energie Atomique, Institut National de la Recherche Agronomique, Université Grenoble Alpes, Grenobe, France

Phaeodactylum tricornutum is a unicellular diatom that has gained attention by researchers and industrial biotechnologist for various applications, such as a biofuel and recombinant protein expression due to its high growth rates. Phosphorus (P) is one of the main limiting factors for algal growth in marine environments. Microalgae have evolved to cope with P limiting conditions by remodeling cellular metabolism and mobilize internal phosphate resources, often resulting in accumulation of lipids. Understanding the regulation of phosphorus homeostasis and metabolism is critical to improve algae biomass productivity. Here we describe a transcription factor, PtPSR (*Phaeodactylum tricornutum* Phosphorus Starvation Response), which acts as a key regulator of P adaption responses. Bioinformatics analyses of the promoter region of highly P stress-induced genes revealed an over representation of a small DNA motif, which is related to DNA binding motifs of MYB transcription factors in Arabidopsis thaliana that regulate phosphate responses. Electrophoretic mobility shift assays shows that PtPSR bind to DNA probes containing this motif. CRISPR/Cas9 knock-out mutants of PtPSR showed a reduction in specific cell growth under P limitation. Expression of P stress-induced genes is strongly reduced and alkaline phosphatase activity is almost absent under P limitation. Lipid profiles show reduced phospholipid degradation in PtPSR mutants under P limited condition compared to PtWT. We conclude that PtPSR is one of the key regulators regulating P scavenging, remodelling and cell growth in response to P stress in diatoms. Understanding the regulators like PtPSR may help to optimize algae strains for better utilization of phosphorus.

CRISPR/Cas9 mutagenesis and gene expression profiling to sketch phosphorus atlas in a diatom

Senjie Lin [1, 2], **Kaidian Zhang** [1, 2], **Xin Lin** [1], **Zhi Zhou** [3]

[1] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China
[2] Department of Marine Sciences, University of Connecticut, Connecticut, United States
[3] Key Laboratory of Tropical Biological Resources of Ministry of Education, Hainan University, Haikou, China

As an essential nutrient, phosphorus is a limiting factor for phytoplankton growth and primary productivity in the ocean but its metabolic pathways and regulatory mechanisms is poorly understood. Here, we employed the CRISPR/Cas9 technique to knock out two alkaline phosphatase (AP) genes (PhoA and PhoD) and a putative upstream regulator (SPX). Remarkable decease in AP activity and the ability to utilize dissolved organic phosphate (glycerol-3-phosphate, G3P) was observed in M_PhoA AP mutant. Further analysis revealed PhoA-PhoD compensatory regulation mechanisms. Moreover, SPX knockout indicated that SPX is an upstream regulator of AP. In addition, transcriptomic profiling of the knockout mutants revealed remarkable alteration of metabolic pathways in the cell, and network analysis uncovered potential interactions of some of these pathways. This is the first effort to elucidate the regulatory cascade of phosphorus nutrient and to construct an algal phosphorus metabolic atlas, which is hoped to improve the understanding on how phosphorus limitation may influence metabolism, growth, and reproduction of diatoms and other marine algae.

Different strokes for different diatoms: Mechanisms of iron storage and their ecological significance

Adrian Marchetti

University of North Carolina at Chapel Hill, Department of Marine Sciences, Chapel Hill, United States

Diatom growth is limited by the availability of iron in vast expanses of the ocean. These regions are sporadically pulsed with iron inputs via dust or lateral advection from continental margins, which often result in massive diatom blooms. Learning the strategies diatoms invoke to cope with variable iron concentrations is critical to our understanding of what influences the distribution and abundance of diatoms as well as their impact on ocean biogeochemistry. As iron inputs are often sporadic, diatoms have evolved mechanisms such as the ability to store iron that enable them to bloom when iron is resupplied and then persist when low iron levels are reinstated. Two iron storage mechanisms have been previously described: the protein ferritin and vacuolar storage. Ferritin may be well-suited for less frequent and pulsed iron inputs, although may serve functional roles other than long-term iron storage that are independent of diatom phylogeny. In future oceans, diatom community composition may shift to ferritin-utilizing diatoms in potential scenarios where iron availability is reduced.

Seeing the light with different eyes – photoreceptors in diatoms

Peter Kroth [1], Manuel Serif [1], Marcus Mann [2], Marion Eisenhut [3], Andreas Weber [3], Shvaita Madhuri [1], Soo Hyun Im [1], Bernard Lepetit [1], Christian Wilhelm [2]

[1] Department of Biology, University of Konstanz, Konstanz, Germany
[2] Plant Physiology, University of Leipzig, Leipzig, Germany
[3] Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Algal research to date experiences a strong boost from current genome projects and the development of molecular tools to study algal processes, like large-scale transcription analyses, transformation techniques and gene silencing/knock-out approaches. Aureochromes (AUREOs) are a novel type of blue-light photoreceptors (discovered in 2007). They so far have only been found in Stramenopiles. They have a very peculiar structure: Besides a blue-light sensing flavin-binding LOV-domain, there additionally is a DNA-binding bZIP domain, which is typically found in transcription factors. Therefore, Aureochromes can be considered as light-driven transcription factors. The pennate diatom *Phaeodactylum tricornutum* possesses four different Aureochromes (PtAUREO1a,1b,1c,2), which are expressed differently throughout the day. We used a TALEN-based approach to generate different knockout cell lines of PtAUREOs in *P. tricornutum*, which were confirmed by genetic complementation studies. Highly interestingly, the knockout of PtAureo1a showed a drastic impact on global gene expression of the algae, influencing all major metabolic and physiological pathways. This indicates that PtAUREO1a might function as a master switch for blue light induced responses. Phenotypically, PtAureo1a knockout mutants showed a distinctive phenotype of a low capacity for Non Photochemical Quenching (NPQ), a prominent photoprotection mechanism in diatoms. Here, we will discuss how Aureochromes could be such influential photoreceptors.

The bHLH-PAS protein RITMO1 regulates diel biological rhythms in the marine diatom *Phaeodactylum tricornutum*

Angela Falciatore [1, 2], Rossella Annunziata [1], Andres Ritter [1], Antonio Emidio Fortunato [1], Alessandro Manzotti [1, 2], Soizic Cheminant-Navarro [1, 2], Marie J. J. Huysman [3], Per Winge [4], Atle Bones [4], Jean Pierre Bouly [1, 2]

[1] Sorbonne Université, CNRS, Laboratory of Computational and Quantitative Biology, Paris, France
[2] Institut de Biologie Physico-Chimique, Laboratory of Chloroplast Biology and Light Sensing in Microalgae, Paris, France
[3] Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium
[4] Department of Biology, NTNU Norwegian University of Science and Technology, Trondheim, Norway

Periodic light-dark cycles govern the timing of basic biological processes in organisms inhabiting land as well as the sea, where life evolved. Although prominent marine phytoplanktonic organisms such as diatoms show robust diel rhythms, the mechanisms regulating these processes are still obscure. By characterizing a *Phaeodactylum tricornutum* bHLH-PAS nuclear protein, hereby named RITMO1, we shed light on the regulation of the daily life of diatoms. Alteration of RITMO1 expression timing and level results in lines with deregulated diurnal gene expression profiles compared to the wild-type cells. Reduced gene expression oscillations are also observed in these lines in continuous darkness, showing that the regulation of rhythmicity by RITMO1 is not directly dependent on light inputs. We also describe strong diurnal rhythms of cellular fluorescence in wild-type cells, which persist in continuous light conditions, indicating the existence of an endogenous circadian clock in diatoms. The altered rhythmicity observed in RITMO1 over-expression lines in continuous light supports the involvement of this protein in circadian rhythm regulation. Phylogenetic analysis reveals a wide distribution of RITMO1-like proteins in the genomes of diatoms as well as in other marine algae, which may indicate a common function in these phototrophs. This study adds new elements to our understanding of diatom biology and offers new perspectives to elucidate timekeeping mechanisms in marine organisms belonging to a major, but under-investigated, branch of the tree of life.

Fe limitation decreases transcriptional regulation in the model diatom *Thalassiosira pseudonana* over the diel cycle

Johanna Goldman [1], Megan Schatz [1], Chris Berthiaume [1], Sacha Coesel [1], Monica Orellana [1, 2], E. Virginia Armbrust [1]

[1] University of Washington, Seattle, United States
[2] Institute for Systems Biology, Seattle, United States

Transcriptional regulation in diatoms is influenced by nutrient availability and modulated by the diel cycle. We investigated these interactions dynamics in the diatom *Thalassiosira pseudonana* grown on a day:night cycle, under different CO_2/pH and iron concentrations, that in combination generated available iron (Fe') concentrations of 1160, 233, 58 and 12 pM. The main driver of transcriptional change was the day:night cycle, followed by Fe', with minimal impact of CO_2 changes. We therefore assessed the effect of Fe' changes on gene expression at midday and midnight. Half of the transcribed genes were differentially expressed between day and night at the highest Fe', decreasing to only a quarter at the lowest Fe'. Only a small subset of transcription factors and signaling molecules maintained diel periodicity in transcript abundance regardless of Fe'. A majority of genes within carbon metabolism-related pathways also lost diel periodicity in transcript abundance with reduced Fe', with the notable exception of glycolysis. We evaluated whether glycolysis was regulated via additional mechanisms. We identified a non-canonical splicing of transcripts encoding triose-phosphate isomerase, a key-enzyme of glycolysis, generating transcripts isoforms that would encode proteins with and without an active site. Transcripts that encode an active enzyme maintained a diel pattern at low Fe', while transcripts that encoded the non-active enzyme lost the diel pattern. Considering that future ocean conditions will reduce the availability of Fe in many parts of the oceans, our work identifies some of the regulatory mechanisms that may shape future ecological communities.

Session 6: Evolution

Exploring drivers of diatom species diversification

Wim Vyverman

Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University
Krijgslaan 281 S8, Gent, Belgium

The extraordinary morphological diversity of diatoms has fascinated scientists since their first discovery in the early 1700s, but our understanding of the drivers and mechanism of species diversification remains limited. For multicellular organisms, two main mechanistic macroevolutionary models attempt to explain the generation and maintenance of species diversity. In the Red Queen model, diversification is hypothesized to be driven by interactions within and among species, or their ecology and life-history traits. In contrast, the Court Jester model is largely based on paleontological evidence and considers diversification dynamics to result from historical abiotic forces, including changes in climate or geological events that drive speciation and extinction rates. Ultimately, however, macroevolutionary patterns are generated by microevolutionary processes acting at the population level. A complicating issue when studying these processes and testing macroevolutionary models in diatoms relates to severe undersampling of diversity in phylogenetic analyses, a fragmented and limited fossil record of especially non-marine diatoms, and in particular to difficulties in recognizing species and (meta)populations due to the widespread occurrence of cryptic species diversity. This calls for an increased focus on understudied clades, extensive taxon and population sampling, and the combination of –omics approaches with classical biology.

Controlling sexual reproduction in a marine diatom

Olga Kourtchenko [1], Anna Godhe [1], Marina Montresor [2], Anke Kremp [3], et al.

[1] University of Gothenburg, Gothenburg, Sweden
[2] Stazione Zoologica Anton Dohrn, Napoli, Italy
[3] Leibniz Institute for Baltic Sea Research, Warnemunde, Germany

We have induced sexual reproduction in a common marine diatom *Skeletonema marinoi* in a laboratory environment, using exposure to the conditions of the resting stage development as a cue. The objective was to investigate whether we could cross geographically isolated and genetically distinct (FST >0.1) S. marinoi strains from the Skagerrak-Kattegat region, and the Baltic Sea. The successful first generation hybrids were confirmed by pedigree analyses using microsatellite markers. We re-sequenced chloroplast genomes of both hybrid and parental strains (Illumina). These data were used to investigate the potential existence of consistent mating types among different *S. marinoi strains*, by tracing the origin of the chloroplast genome in a mixed genotype back to the maternal one. Presented study provides new insights into *S. marinoi* reproductive strategies and offers necessary tools for further developing and employing this organism as a genetic and evolutionary model.

Molecular characterisation of proteins shaping the diatom chloroplast

Richard Dorrell [1], Tomomi Nonoyama [1, 2], Elena Kazamia [1], Gillian Gile [3], Raphael Meheust [1, 4, 5], Giselle McCallum [1, 6], Yoshinori Tsuji [7], Yusuke Matsuda [7], Eric Bapteste [4], Tsuyoshi Tanaka [2], Chris Bowler [1], et al.

[1] Institut de biologie de l'Ecole normale supérieure (IBENS), Ecole normale supérieure, CNRS, INSERM, PSL Université, Paris, France
[2] Tokyo University of Agriculture and Technology, Tokyo, Japan
[3] Arizona State University, Tempe, United States
[4] Université Pierre et Marie Curie, Paris, France
[5] University of Berkeley, Berkeley, United States
[6] Concordia University, Montreal, Canada
[7] Kwansei Gakuin University, Nishonimiya, Japan

The most dramatic change in the cellular organisation of eukaryotes since their radiation is the acquisition of chloroplasts through endosymbiosis. This has occurred a multitude of times across the tree of life, including the acquisition of photosynthesis by a stramenopile ancestor of diatoms through the secondary or more complex endosymbiotic uptake of a red algal plastid. Each of these chloroplast lineages is supported by a complex network of nucleus-encoded, and chloroplast-targeted proteins, which are imported into the chloroplast and perform the majority of its biological functions. Changes to this protein complement can dramatically alter chloroplast metabolism, and underpin broader changes in the life strategies and eco-physiological adaptations of extant algae. In this talk, I will explore which chloroplast-targeted metabolism pathways have shaped the origins of diatom algae, and may even underpin their success in the contemporary ocean. I will focus on two questions. First, I will demonstrate how combined phylogenomic and molecular techniques have been successfully leveraged to reveal the deep origins of photosynthesis in the stramenopiles. Through this, I will show that the diatom chloroplast has from its very origins been an evolutionary mosaic, using proteins acquired from a multitude of different evolutionary sources. Next, I will present ongoing work within our group, using phylogenetics, microscopy, and CRISPR-mediated mutagenesis to characterise novel chloroplast proteins involved in nutrient acquisition and photoprotection that may specifically differentiate diatoms from other groups of algae in the ocean.

Inter- and intra-species analysis of the Seminavis robusta genome offers insights into the complexity, evolution and ecological adaptation of benthic diatoms.

Cristina Maria Osuna-Cruz [1, 2], **Members of the Seminavis Genome Consortium** [3]

[1] Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium
[2] VIB Center for Plant Systems Biology, Ghent, Belgium
[3] Ghent University and collaborative institutes, Ghent, Belgium

In aquatic environments, the benthos stores high levels of carbon and nutrients, leading to the formation of numerous microenvironmental niches. Although diatoms are often part of this microbial community, most genome studies have focused on the ecological function of planktonic diatoms. Here we report a reference genome for *Seminavis robusta* in order to gain more insight into the gene functions and adaptation of benthic diatoms. Additionally, we have generated 167 RNA-Seq samples under a large variety of stress conditions and reproductive stages. *S. robusta* is the diatom with the largest number of predicted protein-coding genes (36,254) to date, presenting 63% of genes with protein homology to other species and 88% with expression support. Of the 14,571 genes lacking functional annotation, 68% showed differential expression, paving the way for functional characterization. Intriguingly, 25% of *S. robusta* genes are clustered in expanded gene families of which many are enriched for tandemly duplicated genes. Ninety-two of these expanded gene families are significantly enriched in differentially up-regulated genes across either many different or few specific conditions, indicating a strong link between gene family evolution and Seminavis' environmental response and ecological adaptation. To further assess the functional and evolutionary importance of *S. robusta* genes, re-sequencing of 41 different strains revealed that approximately 24% of the genes in *S. robusta* species complex are likely dispensable. Overall, our results shed light on the inter- and intraspecific complexity of *S. robusta* and offer an invaluable resource to improve our understanding of gene functions and dynamics in diatoms.

Sex determination in *Pseudo-nitzschia multistriata*

Mariella Ferrante [1], Monia Teresa Russo [1], Rossella Annunziata [1], Camilla Borgonuovo [1], Francesco Manfellotto [1], Pina Marotta [1], Laura Vitale [1], Wim Vyverman [2], Remo Sanges [3], Marina Montresor [1]

[1] Stazione Zoologica Anton Dohrn, Naples, Italy
[2] Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium
[3] Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

Pseudo-nitzschia is an important genus of marine pennate diatoms responsible for blooms in coastal and oceanic waters. Our model species, *Pseudo-nitzschia multistriata*, has a well described, typical heterothallic life cycle with a sexual phase triggered by the encounter of two opposite mating types (MT). To explore the process of sexual reproduction, we took advantage of a number of resources generated in the past years, including a fully sequenced genome and protocols for genetic transformation. We combined different approaches to investigate the genetic bases of mating type determination in *P. multistriata*. Using RNA-seq, we compared the expression profile of the two MTs and identified five MT-biased genes, three specific for MT+ and two for MT-. These genes play a role in governing cell behaviour during sexual reproduction, being induced upon sexualization. To identify the MT determining gene, we explored the intergenic regions upstream of the MT-biased genes in the *P. multistriata* genome and found structural differences between the two MTs in the promoter region of MRP3. This gene is expressed exclusively in MT+ strains in a monoallelic manner. MRP3 overexpression in an MT- strain induced sex reversal: the transgenic MT- could mate with another MT- strain and displayed altered regulation of the other MT-biased genes, indicating that they lie downstream. The five MT-biased genes are not conserved outside of the *Pseudo-nitzschia* and *Fragilariopsis* genera. Sex determination systems can vary substantially in eukaryotes, and to what extent the mechanism described for P. multistriata is conserved in other diatoms remains to be determined.

Evolution and Metabolic Configuration of Nitrogen Flux in a Model Marine Diatom

Sarah Smith [1], **Christopher Dupont** [1], **McCarthy James** [1], **Andrew Allen** [1, 2] **et al.**

[1] J. Craig Venter Institute, La Jolla, United States
[2] Scripps Institution of Oceanography, La Jolla, United States

Diatoms dominate phytoplankton communities by outcompeting other groups for nitrate, yet little is known about the mechanisms underpinning this ability. Genome and genome-enabled studies have shown that diatoms possess unique metabolic features compared to other phototrophs, such as mitochondrial glycolysis and the presence of a urea cycle. In diatoms, the cycle is known to be important for recovery from nitrogen limitation, however there are open questions about how the cycle is integrated within the cell-wide metabolic network. To develop a whole-cell level understanding of the impact of nitrogen source and status on *Phaeodactylum tricornutum*, we investigated gene expression and metabolic flux in in experiments aimed at eliciting shifts in nitrogen status over the short term. Using a combination of transcriptomics, proteomics, metabolomics, fluxomics, and flux balance analysis, we have arrived at a systems-level understanding of how nitrogen is assimilated and distributed within *P. tricornutum*. We found a high degree of metabolic network connectivity between the chloroplast and mitochondria of pathways at the critical intersection of carbon and nitrogen metabolism. We characterize the differentiated function of organellar GS-GOGAT cycles and describe aspartate and alanine systems used to exchange amino moieties between organelles. We also describe an arginine biosynthesis pathway that is split across organelles in diatoms, clarifying the role of the urea cycle. We propose that the unique configuration and high degree of metabolic integration between the major energy organelles allows diatoms to efficiently respond to changing nitrogen status, conferring an ecological advantage over other phytoplankton taxa.

Evolutionary origins of DNA methylation : what can we learn from diatoms?

Antoine Hoguin [1], Fabio Rocha Jimenez Vieira [1], Ouardia Ait-Mohamed [1], Catherine Cantrel [1], Chris Bowler [1], Auguste Genovesio [1], Magali Lescot [2], Leila Tirichine [1, 3]

[1] IBENS, Département de Biologie, Ecole Normale Supérieure, CNRS, Inserm, PSL Research University,75005 Paris, France, Ecology and Evolutionary Biology Section, Institut de Biologie de l'École Normale Supérieure (IBENS), CNRS UMR8197 INSERM U1024, 46 rue d', Paris, France
[2] Information Génomique et Structurale, UMR7256, CNRS, Aix-Marseille Université, Institut de Microbiologie de la Méditerranée (FR3479), Parc Scientifique de Luminy, Marseille, France, Marseille, France
[3] CNRS UMR6286, UFIP UFR Sciences et Techniques, Université de Nantes, 2 rue de la Houssinière 44322, Nantes Cedex 03, Nantes, France

DNA methylation occurring at the fifth carbon of cytosine (5mC) is a common epigenetic mark observed in eukaryotes including plants, mammals and fungi. 5mC has been associated with repressive gene expression, X-chromosome inactivation, parental imprinting, DNA repair, and transposable elements repression. Recent studies unveiled the presence of 5mC in early divergent eukaryotes including 4 diatom species, further stemming 5mC to the origin of eukaryotes. Diatoms however encode a divergent set of DNA Methyl Transferases (DNMTs) named DNMT4, 5 and 6; that set and propagate 5mC. In order to get a better understanding of the phylogenetic relationship of those enzymes, we scanned the diatom's transcriptomes of the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) and defined their reference domains and structures. Because micro-eukaryotes could use DNA methylation as an adaptive mechanism, we screened the TARA ocean data base using our newly defined reference sequences. Here, we provide the very first phylogenetic tree of DNMTs based on oceanic samples and expanded our knowledge towards dinoflagellates and haptophytes DNMTs. The in vivo function(s) of the different DNMTs in diatoms still remains elusive. We thus generated a DNMT5 KO in *Phaeodactylum tricornutum* using the CRISPR/Cas9 system. High throughout put RNA sequencing showed that the loss of DNMT5 was associated with a reactivation of transposable elements, highlighting the conservative role of DNA methylation in divergent eukaryotes. Overall, our work opens up the epigenetic field to the micro-algae and set up a strong basis to understand the complexity of diatoms genetic regulatory network.

Analysis of a subset of Theriot et al. (2015) Seven Gene Data Set Using Multiple Outgroups

Linda K. Medlin, Yves Desdevises

MBA The Citadel PL1 2PB, Plymouth, United Kingdom

Multiple outgroups and full SSU genes recover three monophyletic diatom classes with strong BT support (Medlin 2014). Three additional plastid genes and multiple heterokonts recovered the same (Medlin and Desdevises 2013). A 7-gene data set, which omitted the two most variable V4 SSU helices and SSU positions after 1200 with only Bolidomonas (Theriot et al. 2015), recovered grades from radial centrics into polar centrics, into pennates. This structural gradation hypothesis (SGH) contrasts with the Medlin and Kaczmarska's CMB hypothesis (Coscinodiscophyceae, (Mediophyceae, Bacillariophyceae)). Only species with all seven genes plus their full length SSU gene were selected for two ML analyses with sequentially added outgroups: 1) a partitioned model for each gene and codon position (MCP) and 2) no partitioning (NMCP). In MCP, we recovered with each additional outgroup, the same clade arrangement: three clades of radial centrics, a monophyletic Mediophyceae and Bacillariophyceae: BT for the Mediophyceae substantially improved with each outgroup addition. NMCP recovered a monophyletic, strongly supported Coscinodiscophyceae and three clades in Mediophyceae. MCP-based trees, with each sequential outgroup, were constrained by a tree reflecting the CMB hypothesis and tested to determine if different. Results were different depending on outgroup and whether tested within IQ-Tree or PAUP. The coding of morphological data resulted in three monophyletic classes but only in the NMCP, with the MCP analysis a grade of clades was recovered. Our conclusions are that the evolutionary history of the plastid genes is not homologous and likely reflects the differences in meroplasidic or holoplastic inheritance of the genes.

Session 7: Ecophysiology II

Metaproteomic investigations into the response of diatom-dominated marine microbial communities to multiple micronutrient stressors

Erin Bertrand [1], J. Scott McCain [1], Andrew E Allen [2], et al.

[1] Dalhousie University Department of Biology, Halifax, Canada
[2] J. Craig Venter Institute and Scripps Inst. Oceanography, La Jolla, United States

Productivity in the Southern Ocean is profoundly influenced by the availability of trace metals and other micronutrients. Here we investigate the molecular underpinnings of a transition from micronutrient-replete, early growing season communities into a multiple micronutrient-stressed, diatom dominated community. To do this, we've developed a coupled global and targeted metaproteomic approach to assessing diatom community molecular physiology and applied this approach to a time series in the coastal Ross Sea. We leverage extensive laboratory-based culture experiments, nutrient-manipulation field experiments, and a proteomic allocation model to interpret metaproteomic signatures observed in this time series. We document simultaneous expression of protein signatures induced by low manganese, iron and cobalamin availability in diatoms and implicate diatom-bacterial interactions in the maintenance of this multiple micronutrient stress condition. We present evidence for widespread multiple micronutrient co-limitation of diatom growth in the ocean and discuss potential implications for our understanding of diatom ecophysiology.

Transcriptional investment facilitates temperature-adaptation and shapes the acclimatory response

Yue Liang [1], Julie Koester [2], Justin Liefer [3], Zoe Finkel [1], Andrew Irwin [4]

[1] Department of Oceanography, Dalhousie University, Halifax, Canada
[2] Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, United States
[3] Department of Biology/Geography and Environment, Sackville, Canada
[4] Department of Mathematics and Statistics, Dalhousie University, Halifax, Canada

Ocean temperatures are projected to increase over the coming century, with dramatic consequences for the marine biosphere. However, we lack the information necessary to predict how primary producers, such as the diatoms, are likely to acclimate and adapt to changes in temperature anticipated over the next centuries. Differential expression analysis of the transcriptomes of two closely related Chaetoceros sp. revealed molecular mechanisms of temperature acclimation and adaptation. The species and growth conditions were selected to separate the effects of changing temperature and sub-optimal growth conditions; the species had different optimal growth temperatures and were each grown under cooler and warmer conditions relative to this optimum. Our results suggest that evolutionary changes in baseline gene expression, that we term transcriptional investment (elevated baseline expression level) or divestment (lowered baseline expression level), is a key mechanism used by diatoms to adapt to different growth temperatures. In particular, transcriptional regulation of lipid composition of membranes, protein metabolism, metabolic and redox homeostasis appear to be critical processes used by diatoms to adapt to different temperatures. As a result, transcriptional responses to sub-optimal and supra-optimal growth temperatures relative to the optimal growth temperature will be species-specific, shaped by evolutionary history.

Dark metabolism: a molecular insight into how the Antarctic sea-ice diatom *Fragilariopsis cylindrus* survives long-term darkness

Fraser Kennedy [1], Andrew Martin [1], John Bowman [2], Richard Wilson [3], Andrew McMinn [1]

[1] Institute for Marine and Antarctic Studies, Hobart, Australia
[2] Centre for Food Safety and Innovation, Tasmanian Institute of Agriculture, Hobart, Australia
[3] Central Science Laboratory, University of Tasmania, Hobart, Australia

Summary • Light underneath Antarctic sea-ice is below detectable limits for up to four months of the year. The ability of Antarctic sea-ice diatoms to survive this prolonged darkness relies on their metabolic capability. This study is the first to examine the proteome of a prominent sea-ice diatom in response to extended darkness, focusing on the protein-level mechanisms of dark survival. • The Antarctic diatom *Fragilariopsis cylindrus* was grown under continuous light or darkness for 120 days. The whole cell proteome was quantitatively analysed by nano-LC-MS/MS to investigate metabolic changes that occur during sustained darkness and during recovery under illumination. • Enzymes of metabolic pathways, particularly those involved in respiratory processes, tricarboxylic acid cycle, glycolysis, Entner-Doudoroff pathway, urea cycle and the mitochondrial electron transport chain became more abundant in the dark. Within the plastid, carbon fixation halted while the upper sections of the glycolysis, gluconeogenesis and pentose phosphate pathways became less active. • We have discovered how *F. cylindrus* utilises ancient alternative metabolic mechanisms that enable its capacity for long-term dark survival. By sustaining essential metabolic processes in the dark, *F.cylindrus* retains the functionality of the photosynthetic apparatus, ensuring rapid recovery upon re-illumination.

Session 8: Ecology

Diatoms – nano-porous miracles

Angela Wulff

Dept of Biological and Environmental Sciences, University of Gothenburg, Goteborg, Sweden

The diatom cell wall (frustule) is incorporated by silica, ornamented with pores, and ensures mechanical protection, and flux of e.g. nutrients and gases, but it also provides optimization of light harvesting, including protection against harmful radiation (e.g. ultraviolet radiation, UVR, 280-400 nm). Protection from UVR may be one reason for the evolution of frustules. Experimental evidence for harmful effects of UVR is found for many species and conditions, but it is also clear that diatoms can cope with relatively high UVR intensities. There seems to be a difference between centric and pennate diatoms. We have observed high UVR tolerance in pennate diatoms despite that they, in contrast to centrics, contain no or very small amounts of UVR-absorbing compounds. To explore the role of frustules, we carried out optical studies and electromagnetic simulations of simplified geometrical models. We suggest that the redistribution of UVR due to frustules is an important evolutionary cause of the presence and evolution of frustules in diatoms, by decreasing the rate of UVR-induced degradation of DNA inside the cells. The ability of frustules to protect from UVR can be utilized to protect also humans and sensitive materials. The intrinsic structure of the frustules can enhance the efficiency of solar panels by improved light absorptions and redistribution of UVR. The nano-sized structure, allowing flux of gases and chemicals, are being explored in several applications by the Swedish Algae Factory, among others, and include applications such as skin care products, batteries, sensors and drug delivery.

A new method to measure photosynthetic activity in algal mixtures highlights allelopathy between diatoms and dinoflagellates

Alexandra Peltekis [1], Marc Long [2], Adèle Micoulaut [1], Laure Guillou [3], Pierre Cardol [4], Francis-André Wollman [1], Hélène Hegaret [2], Benjamin Bailleul [1]

[1] UMR7141 IBPC CNRS, PARIS, France
[2] UMR 6539, Brest, France
[3] UMR 7144 Station Biologique de Roscoff, ROSCOFF, France
[4] Université de Liège, Liège, Belgium

Little is known about allelopathy in marine microalgae, in spite of the competitive advantage that these interactions could provide to invasive species. We developped a new approach to measure the photosynthetic activity of species in a mixture, which was a methodological bottleneck.The method is based on a physical phenomenon, the electro-chromic shift (ECS) which is a modification of the absorption of photosynthetic pigments when they are subjected to the electric field generated by photosynthesis across the thylakoid. ECS shows a different spectral signature in each photosynthetic clade of microalgae. Therefore, such an “internal voltmeter” allows to work in mixtures and reveals allelopathic interactions targeting photosynthesis. The photosynthetic activity of several diatoms was inhibited when mixed with dinoflagellates, e.g. *Thalassiosira pseudonana*, *Phaeodactylum tricornutum* or diatoms from the field (with the dinoflagellate *Amphidinium carterae*) and *Chaetoceros muelleri* (with *Alexandrium minutum*). All complexes involved in photosynthesis contribute to the generation (PSII, PSI, cytochrome b6f) or consumption (ATPsynthase) of the trans-thylakoidal electric field. With ECS, we can therefore identify the targets of the released secondary metabolites on the photosynthetic apparatus and decipher the mechanisms of inhibition. Both *Amphidinium carterae* and *Alexandrium minutum* suppress the proton motive force in the diatoms thylakoids. *A. minutum* seems to affect the integrity (permeability and polarity) of diatom’s thylakoid membranes and to impair the diffusion of plastoquinols. A. carterae seems to induce an uncoupling effect in the diatom thylakoids, inhibiting photosynthesis and growth of the diatom *T. pseudonana* in co-culture experiments.

Determining iron limited remodeling of chloroplasts in Southern Ocean diatoms through "Meta-Plastid" analyses

Kristofer M. Gomes [1], Laura Z. Holland [1], Kristen N. Buck [3], P. Dreux Chappell [4], Randelle M. Bundy [5], Bethany D. Jenkins [1, 2]

[1] Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI, United States
[2] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, United States
[3] College of Marine Science, University of South Florida, St. Petersburg, FL, United States
[4] Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Norfolk, VA, United States
[5] School of Oceanography, University of Washington, Seattle, WA, United States

Diatoms are important primary producers in the world's oceans, yet their growth is constrained in large regions by low bioavailable iron (Fe). Fe-limitation of primary production is due to requirements for Fe as a co-factor in proteins involved in photosynthesis within the chloroplast. As Fe is introduced into low Fe waters, diatoms bloom and accumulate significant biomass. This bloom response indicates adaptations allowing for survival in Fe-limited waters and rapid growth when Fe becomes readily available. To ascertain impacts of Fe on chloroplast function we conducted proteomic analysis of isolated *Thalassiosira pseudonana* chloroplasts grown under Fe-sufficiency and limitation. These experiments showed Fe-limitation regulates major metabolic pathways in the chloroplast, including expression of Calvin cycle proteins and components of the photosynthetic electron transport chain. This *T. pseudonana* chloroplast reference proteome was then used to build a diatom chloroplast transcriptome database by querying publicly available diatom transcriptomes. In addition, we conducted a series of incubation experiments following diatom response to modulated Fe using metatranscriptomics in the chronically Fe-limited Southern Ocean during austral spring of 2016. We sought to understand the impacts of Fe status on the diatom chloroplast-localized proteome in these experiments. By utilizing our diatom chloroplast transcriptome database, we used a network approach to identify within these metatranscriptome changes in chloroplast functional genes. Within the resulting Southern Ocean "Meta-Plastid", we have identified regulation of chloroplast function in relation to modulated Fe status across over twenty diatom genera, including modulation of light harvesting complex proteins and metabolic pathways such as nitrate reduction.

Diatom Virus Investigations: Toward the development of a new ecologically relevant model system

Lisa Zeigler Allen [1, 2], Sarah Schwenck [1, 2], Hong Zheng [1], Ariel Rabines [1, 2], Shawn Polson [3], John McCrow [1], Nora Reed [4], Laura Bortolin [4]

[1] J Craig Venter Institute, La Jolla, United States
[2] Scripps Institution of Oceanography, University of California San Diego, La Jolla, United States
[3] University of Delaware, Newark, United States
[4] Harvard University, Cambridge, United States

As the most abundant biological entities in aquatic environments, viruses turnover more than a quarter of the photosynthetically-fixed carbon, thereby fueling microbial foodwebs and short-circuiting carbon export to higher trophic levels and the deep sea. We have focused our efforts on the meroplanktonic pennate diatom *Cylindrotheca* due in part to its ease of isolation in local waters, global distribution, capacity for benthic life-style, existence of established transformation protocols and ability to undergo sexual reproduction that can be replicated and manipulated in the laboratory. Currently, we have established a culture collection from single cell isolates of approximately 110 stable *Cylindrotheca* isolates from four Southern California coastal locations. Following infection with local viral consortia +ssRNA viruses infective for CCE *Cylindrotheca*, similar to the Bacillarnaviridae, were isolated and characterized using TEM and RNAseq. Additionally, the development of methods targeting viral RNAs from cellular mRNA was accomplished enabling more directed studies on the viral consortia associated with transcriptionally active cellular microorganisms. To investigate viral/host interactions further, the genome of an environmental isolate of *Cylindrotheca* was completed and single-cell RNAseq (scRNAseq) from environmental and cultured isolates using Dropseq and whole-transcriptome targeting was performed to increase the throughput and replicability, thereby providing more robust measurements of RNA virus physiology and impacts on their host transcriptional activity.

Silicon limitation facilitates virus infection and mortality of diatoms

Chana Kranzler [1], Jeffrey Krause [2, 3], Mark Brzezinski [4], Bethanie Edwards [5], William Biggs [1], Michael Maniscalco [4], John McCrow [6], Benjamin Van Mooy [7], Kay Bidle [1], Andrew Allen [6, 8], Kimberlee Thamatrakoln [1]

[1] Department of Marine and Coastal Sciences, Rutgers University, New Brunswick, United States
[2] Dauphin Island Sea Lab, AL, United States
[3] University of South Alabama, AL, United States
[4] Marine Science Institute, University of California, Santa Barbara, CA, United States
[5] Earth and Planetary Science, University of California, Berkeley, CA, United States
[6] Microbial and Environmental Genomics, J. Craig Venter Institute, La Jolla, CA, United States
[7] Woods Hole Oceanographic Institute, MA, United States
[8] Scripps Institution of Oceanography, University of California, San Diego, CA, United States

Diatoms are one of the most widely distributed and ecologically successful groups of organisms in the modern ocean, and are responsible for 40% of the global marine primary productivity. Given their obligate silicon (Si) requirement for cell wall formation, diatoms effectively couple the Si and carbon (C) cycles, converting dissolved Si into biogenic silica that ballasts substantial vertical flux of C out of the euphotic zone into the mesopelagic and deep ocean. Viruses are key players in ocean biogeochemical cycles, yet little is known about how viruses specifically impact diatom populations. Our findings demonstrate that Si limitation facilitates virus infection and mortality in diatoms in the highly productive coastal waters of the California Current Ecosystem. We diagnosed early, active and lytic stages of viral infection across a gradient of Si stress, using a suite of chemical and biological measurements alongside metatranscriptomic analyses of cell-associated diatom viruses and targeted, quantitative PCR of free, extracellular viruses. In Si-limited cultures of the centric, bloom-forming diatom, *Chaetoceros tenuissimus*, viral-induced mortality was accelerated, with unimpaired viral production. Together, these findings contextualize diatom-infecting viruses within the ecophysiological framework of Si availability and diatom-mediated biogeochemical cycling.

Pairing up in the plankton: evolution, ecology, and activity of diatom-cyanobacteria symbioses

Rachel Foster [1], Mercedes Nieves Morion [1], Andrea Caputo [1], Marcus Stenegren [1], Enrique Flores [2], et al.

[1] Stockholm University, Stockholm, Sweden
[2] Instituto de bioquímica vegetal y fotosintesis, Seville, Spain

Some of the most enigmatic components of the plankton are the microalgal groups, which carry intimately associated cyanobacteria as symbionts. In the sunlit zone of the aquatic ecosystems where dissolved nutrients are largely limiting, cyanobacteria pair up with a diverse group of diatoms. In these planktonic symbioses, the cyanobacterial partners are either heterocyst-forming and in two genera, including a freshwater family of diatoms, the cyanobacteria are unicellular. In all, the cyanobacteria are N_2-fixers, and thus function as a nitrogen (N) source for their respective hosts. The diatom symbioses are broadly distributed in the world's oceans, and contribute to global N and carbon (C) cycles due to high fixation and sinking rates. Despite their ubiquitous distribution and biogeochemical significance, our understanding of the intimate nature between the partners remains understudied. Recently we identified a continuum of symbiont integration in the symbioses, where the symbiont cellular location is tightly coupled to genome size, content, transporter number, and the timing of the partnerships. Time-calibrated trees dated the appearance from 100-50 Mya and was consistent with the symbiont cellular location: recently evolved symbionts are externally located. Internal symbionts have fewer transporters for N substrates, and several C transporters appear to be incomplete, or missing. There is a size and light dependent relationship where larger diatom hosts team up with larger symbionts, and this is also reflected in higher N_2 fixation. Using several methodologies including confocal microscopy, comparative genomics, gene complementation, and secondary ion mass spectrometry, we have identified several intriguing aspects for these planktonic partnerships.

Metabolomics approaches to address microbial complexity in phytoplankton communities

Marine Vallet [1], Tim U. H. Baumeister [1], Filip Kaftan [2], Ales Svatos [2], Georg Pohnert [1, 3]

[1] Research Group Plankton Community Interaction, Max Planck Institute for Chemical Ecology, Jena, Germany
[2] Research Group Mass Spectrometry/Proteomics, Max Planck Institute for Chemical Ecology, jena, Germany
[3] Institute for Inorganic and Analytical Chemistry Bioorganic Analytics, Friedrich-Schiller-Universität Jena, Jena, Germany

Oomycetes are major pathogens of terrestrial plants and humans. These parasites also prevail in marine and freshwater ecosystems infecting fish and algae, thereby causing important economic loss in fisheries and aquaculture. In addition, intracellular parasitic oomycetes are frequently reported during microalgal blooms and manipulated the plankton species succession during short-term epidemics, although the underlying mechanism is unknown. Hence there is a striking lack of understanding of one of fundamental infection processes that shape central marine ecosystems. Here, we developed several analytical approaches using high-resolution mass spectrometry (HR-MS) and untargeted metabolomics to profile the metabolome of algal cells under stress or infected by flagellates parasites. The physiological status and strain-specificity of different microalgae, including diatoms, chlorophytes and dinoflagellates, were determined at the single-cell level and we can now link the observed variability and phenotype of infected cell to chemical explanations. We investigated a pathosystem comprising of the marine oomycete Lagenisma coscinodisci and the bloom-forming diatom *Coscinodiscus granii* to gain mechanistic insights into oomycete pathogenicity. We showed how parasitoid oomycetes rewire the algal metabolome to guarantee infection success by identifying the overabundance alkaloids from the β-carbolines family using a non-contact dual cultivation approach and functional bioassays. Here, we provide the first comprehensive understanding of how the marine oomycete Lagenisma coscinodisci effectively parasitize its diatom host, completing its lifecycle to ensure its dissemination in the algal population.

Disentangling microbial networks in the sea: sulfonate-based trophic interactions between diatoms and bacteria

Bryndan Durham, Angela Boysen, Laura Carlson, Ryan Groussman, Katherine Heal, Kelsy Cain, Rhonda Morales, Sacha Coesel, Robert Morris, Anitra Ingalls, E. Virginia Armbrust

University of Washington, Seattle, United States

In the surface ocean, phytoplankton transform inorganic substrates into organic matter that fuels the activity of heterotrophic microbes, creating intricate metabolic networks that determine the extent of carbon recycling and storage in the ocean. Yet, the diversity of organic molecules and interacting organisms has hindered detection of specific relationships that mediate this large flux of energy and matter. Here we show that a tightly coupled microbial network based on organic sulfur compounds (sulfonates) exists among key lineages of eukaryotic phytoplankton producers and heterotrophic bacterial consumers in the North Pacific. We find that cultured diatoms produce sulfonates, often at millimolar internal concentrations. These same sulfonates support growth requirements of an open-ocean isolate of the SAR11 clade, the most abundant group of marine heterotrophic bacteria. Expression of putative sulfonate biosynthesis genes and sulfonate abundances in natural plankton communities over the diel cycle link sulfonate cycling to light availability and to the changing taxonomic landscape of the subtropical and subpolar gyres in the North Pacific. Contemporaneous expression of sulfonate catabolism genes in heterotrophic bacteria highlights active recycling of sulfonates in situ. Our study provides evidence that sulfonates serve as an ecologically important currency for nutrient and energy exchange between microbial autotrophs and heterotrophs, highlighting the importance of organic sulfur compounds in regulating ecosystem function.

Roseobacteria dominate interactions between a cosmopolitan diatom and its microbiome.

Ahmed A. Shibl [1], Ashley Isaac [1], Michael Ochsenkuehn [1], Cong Fei [1], Anny Cárdenas [2], Chris R. Voolstra [2], Shady A. Amin [1]

[1] Experimental Research Building, New York University Abu Dhabi (NYUAD), Saadiyat Island 129188, Abu Dhabi, United Arab Emirates
[2] Red Sea Research Center, King Abdullah University of Science and Technology (KAUST), 23955-6900, Thuwal, Saudi Arabia

Interactions between diatoms and surrounding microbial communities influence their physiology, food web dynamics and global biogeochemical cycles. Members of these microbiomes often belong to a small number of genera, suggesting their importance to diatoms. However, molecular mechanisms underpinning how specific microbes are recruited by a diatom host are poorly understood. Here, we isolate a cosmopolitan diatom, *Asterionellopsis glacialis*, and acclimate it without its microbiome. Subsequently, we reseed this microbiome back into the bacteria-free (axenic) *A. glacialis* culture and use (meta)genomics, (meta)transcriptomics and metabolomics to shed light on their interactions and responses. Within 0.5 hours of reseeding, the diatom upregulated genes involved in the biosynthesis and export of amino acids, biosynthesis of the polyamines spermine and spermidine, and production of phosphoglycerate and citrulline. Despite increased biosynthesis of these central metabolites, their relative abundance in the exometabolome were dampened relative to axenic controls, suggesting active bacterial uptake. A small subset of Roseobacteria in the microbiome upregulated several transporters for polyamines and amino acids, suggesting assimilation of these metabolites. Cumulatively, these results suggest that Roseobacteria dominate interactions with *A. glacialis* due to their rapid response to excreted metabolites. To better understand this, we assemble four near-complete genomes belonging to the Sulfitobacter and Phaeobacter genera, in addition to potentially novel genera within the Roseobacter-clade. Culturing closely related bacterial isolates from the microbiome suggest they are highly attuned to rapidly utilize diatom-derived organic carbon. Our findings provide a mechanistic understanding of how Roseobacteria access diatom-derived carbon leading to persistence and colonization of the diatom microenvironment.

From the micro to the mesoscale: characterizing drivers of diatom physiological ecology in the open ocean

Sonya Dyhrman

Columbia University, New York, United States

It is well known that diatoms are major contributors to marine primary production, particularly in coastal environments. We are working to better understand what controls the distribution and activity of diatoms across different ocean ecosystems, with a focus on low nutrient open ocean ecosystems. Metatranscriptomic analyses were used to profile patterns of diversity and reconstruct gene expression profiles in a series of studies focused on how resource availability shapes eukaryotic phytoplankton assemblages and how taxa respond to resource changes across different ocean ecosystems with a focus on diatoms. In a recent sample set from the western North Atlantic, we identified shifts in diatom community composition that tracked with shifts in water masses and biogeochemistry along a coastal to open ocean transect. Diatom gene expression patterns also shifted across this gradient, and a correspondence analysis identified phosphate concentration as a significant driver of the expression differences between ocean regions. Consistent with this observation, the relative expression pattern of a diatom phosphate transporter inversely tracked with phosphate concentration across biogeochemical provinces despite shifts in diversity. Collectively, these data suggest that phosphate concentration is an important driver of diatom physiological ecology in the western North Atlantic. Continuing to apply these types of approaches across a wide range of time and space scales will further our understanding of how different ocean ecosystems influence diatom physiological ecology.

The *Phaeodactylum* genome at 10 years old

Chris Bowler

Institut de Biologie de l'Ecole Normale Supérieure (IBENS), Paris, France

Phaeodactylum tricornutum was first described by Bohlin in 1897, from the English Channel (Plymouth) and the Baltic Sea. Since then it has become a model organism for diatoms, due to its extensive use in aquaculture and ease of lab growth. It was one of the very first diatoms to be genetically transformed, in 1996, and was the second diatom, after *Thalassiosira pseudonana*, to have its whole genome sequenced. It is now ten years since the genome paper was published in 2008 so it is a good time to celebrate the achievement, made possible by a large collaborative team of annotation experts, and to take stock of how our understanding of diatom biology has changed since before and after the genome sequence was made available. Since the genome was sequenced, hundreds of transcriptomes have been generated as well, its epigenome has been described, and several ecotypes with differing physiologies have been characterized. Strategies for gene overexpression, fluorescent fusion, gene knockdown and gene knockout have now become routine and both nuclear and chloroplast transformation are possible. To date, the species has been particularly useful to reveal insights into diatom C, N and Fe metabolism, as well as cell cycle, and to explore the potential of diatoms as cell factories for production of high value compounds and biofuels.

POSTER ABSTRACTS

1 Identification of transcription factor binding sites and characterization of promoter architecture in Phaeodactylum tricornutum

Sarah Smith, Andrew allen, et al.

J. Craig Venter Institute, La Jolla, United States

Transcription factors (TFs) regulate gene expression by binding DNA in gene promoters and have an important role in activating the cellular response to shifting environmental conditions. To date, several diatom transcriptome studies have shown that suites of genes are co-expressed in response to shifting conditions (i.e. nutrients or light), identifying putative gene regulons. However, little is known about the roles or binding sites of specific TFs that elicit these transcriptional responses as only a few TFs have been characterized in diatoms. We investigated transcription factor binding sites, using a combination of bioinformatics-based promoter analysis and high-throughput in vitro DNA affinity purification sequencing (DAP-seq). DAP-seq enables genome-wide characterization of transcription factor binding sites. We identify a nitrate-response element enriched in the promoters of nitrate assimilation genes that is similar to the binding site for the human transcription factor ETS. We also characterize the optimal binding sites for different genes in the bZIP transcription factor family from *P. tricornutum*, facilitating description of the genes they regulate. Specific knowledge of promoter architecture is valuable for the development of tools for molecular investigations and genetic engineering of diatoms and is essential in order to understand how reprogramming of gene expression is accomplished to achieve appropriate cellular responses to environmental signals.

2 Genome-enabled phylogenetic and functional reconstruction of an araphid pennate diatom CCMP470, previously assigned as a radial centric diatom, and its bacterial commensal

Shinya Sato [1], Deepak Nanjappa [2], Richard Dorrell [3], Fabio Rocha Jimenez Vieira [3], Elena Kazamia [3], Leila Tirichine [3, 4], Alaguraj Veluchamy [3], Zoltan Fussy [5, 6], David Mann [7, 8], Chris Bowler [3], Adriana Zingone [9]

[1] Fukui University, Fukui, Japan
[2] Reliance Industries Limited, Mumbai, India
[3] Ecole Normale Superieure, Paris, Paris, France
[4] University of Nantes, Nantes, France
[5] University of South Bohemia, Ceske Budejovice, Czech Republic
[6] Charles University, Prague, Czech Republic
[7] Royal Botanic Garden, Edinburgh, United Kingdom
[8] Institute for Food and Agricultural Research and Technology (IRTA), Sant Carles de la Ràpita, Spain
[9] Stazione Zoologica Anton Dohrn, Napoli, Italy

Diatoms are an ecologically fundamental and highly diverse group of algae, dominating marine primary production in both open-water and coastal communities. The diatoms include both the centric species, which may have radial or polar symmetry, and the pennate diatoms, which include raphid and araphid species, and arose within the centric lineage. Here, we use combined microscopic and molecular information to reclassify a diatom strain CCMP470, previously

annotated as a radial centric species related to Leptocylindrus danicus, as an araphid species within the staurosiroid lineage, closely related to the genus Plagiostriata. CCMP470 shares key ultrastructural features with staurosiroid taxa, such as the presence of a sternum with parallel striae, and the absence of a labiate process on its value; and this evolutionary position is robustly supported by multigene phylogenetic analysis. We additionally present a draft genome of CCMP470, which is the first genome available for a staurosiroid lineage. Notably, our genome library contains the genome of a bacterial commensal related to Pelagicola within the Rhodobacterales, a lineage known frequently to associate with algae in culture. We demonstrate the presence of Rhodobacteralean sequences in other published algal genome and transcriptome datasets, and analyse the possible evolutionary and functional interactions between the diatom host and its bacterial commensal.

3 Diatom proteorhodopsins and their potential role in the iron-limitation response

Brian Hopkinson [1], Alecia Septer [2], Susumu Yoshizawa [3], William Sunda [2], Adrian Marchetti [2]

[1] Department of Marine Sciences, University of Georgia, Athens, United States
[2] Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, United States
[3] Genetic Research Section, Center for Earth Surface System Dynamics, Atmosphere and Ocean Research Institute, The University of Tokyo, Tokyo, Japan

Proteorhodopsins (PRs) are retinal-binding membrane proteins that can act as light-driven proton pumps to generate energy for metabolism and growth. PRs were originally discovered in heterotrophic bacteria where their expression offers access to a previously unavailable energy source: light. However, PRs have recently been identified in photosynthetic organisms such as diatoms where their role is less obvious. We have been investigating the role of PR in oceanic diatoms and to date our work suggests PR is used to provide supplemental energy under iron-limited conditions, especially at low-temperatures. We hypothesize that PR is beneficial under cold, iron-limited conditions because it offers a way to generate energy without iron and its reaction rate is insensitive to temperature as its turnover rate is limited by light absorption. Transcriptomic, metatranscriptomic, and protein analysis all show that PR is upregulated under iron-limited conditions in PR-containing diatoms such as Pseudo-nitzschia spp. Furthermore, a survey of the distribution of PR-containing diatoms shows they are most prevalent in cold, iron-limited regions such as the Southern Ocean and sub-Arctic North Pacific. Heterologous expression and functional characterization of PR from Pseudo-nitzschia granii verified that it pumps protons and the reaction rate was unaffected by temperature in the range of 5 to 25 °C. Continuing work aims to functionally characterize additional PRs, compare expression responses in different diatom species, and constrain the quantitative significance of PR energy generation relative to conventional photosynthetic pathways, especially at the cold temperatures of the Southern Ocean (-2 to 10 oC), a vast iron-limited ocean region.

4 Green life in the dark

Nathalie Joli [1], Marcel Babin [2], Ouardia Ait-Mohamed [1], Flavienne Bruyant [2], Fredy Barneche [1], Théo Sciandra [2], Christopher Bowler [1]

[1] Institut de Biologie de l'École Normale Supérieure (IBENS), CNRS et ENS, Paris, France
[2] Département de Biologie de l'Université Laval, CNRS, QUébec, Canada

Although potentially of fundamental importance for many phototrophs on Earth, the physiological mechanisms and molecular underpinnings that allow survival over the long periods of dark that occur in polar regions remain a mystery. We are using diatoms, specifically *Fragilariopsis cylindrus* (polar pennate diatom with a sequenced genome), as an experimental system to characterize the physiological, cellular, genomic, epigenomic, and metabolic state of cells during adaptation to prolonged darkness and the return to light. *F. cylindrus* has been exposed to light transitions from light to dark, and dark to light in specially designed bioreactors under stable cold temperature conditions. Cellular energy flow and allocation have been monitored before, during and after simulated 1-, 3- and 5-month polar nights. In the dark for 1 to 3 months, *F. cylindrus* stops growing but remains viable and slowly consumes lipids. Growth resumes quickly when brought back to light. After five months in the dark, significant mortality was observed, but when re-exposed to light for several days, viable cells resumed growth very quickly. Transcriptomics coupled with mass spectrometry and immunoblotting on extracted chromatin revealed enormous changes in gene expression accompanied by a range of histone post translational modifications. Ongoing investigations aim to determine the chromatin state of the *F. cylindrus* and their functional meaning genome in darkness and during the return to light. A summary of the preliminary results will be shown.

5 Insights into the sterol biosynthesis of diatoms

Ana Jaramillo [1], Justin Ashworth [1], Michele Fabris [1, 2], Peter Ralph [1]

[1] University of Technology Sydney, Sydney, Australia
[2] CSIRO Synthetic Biology Future Science Platform, Brisbane, Australia

Diatoms are a large group of microalgae that have genetically diversified their physiology and metabolism while adapting to numerous environments. Unique metabolic features reflect their peculiar evolutionary history and frequent gene transfer. Diatom metabolism has often unusually different arrangements even in conserved metabolic pathways. These features highlight their unique biology and potential for natural compounds production. Among these products are phytosterols, triterpenoid metabolites under tight regulation in model organisms. Diatoms produce a broad diversity of phytosterols, which raises questions about the arrangement of their little-known sterol biosynthesis. Phytosterols have been studied for health benefits including demonstrated cholesterol-lowering properties. To characterize the sterol metabolic pathway of diatoms, treatment with chemical inhibitors specific to enzymes in the sterol biosynthesis was carried out. We investigated the diversity and response to inhibitors treatment of three diverged diatom metabolisms Chaetoceros muelleri, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* using metabolite profiling, and transcriptomics. Results demonstrate that these three diatoms, which are representative of distinct clades, share a core phytosterol biosynthesis pathway that relies on a terbinafine-insensitive alternative squalene epoxidase and the cyclization of 2,3 into cycloartenol by a conserved oxidosqualene cyclase. Our findings suggest that the formation of different end-point sterol products is likely occurring at the end of the metabolic pathway, in which specialized enzymes catalyze the different decorations in the side chain of the final specie-specific sterols. These findings help to refine metabolic networks in each species studied, as well as provide new information for the engineering of diatoms to produce valuable new natural products.

6 The low CO2-inducible protein, LCIP63, from Thalassiosira pseudonana is a new subclass of carbonic anhydrase

Erik Jensen [1], Romain Clement [1], Artemis Kosta [2], Stephen Maberly [3], Brigitte Gontero [1]

[1] Aix Marseille Univ, CNRS, BIP, UMR 7281, IMM, FR3479, 31 Chemin J. Aiguier, 13 402 Marseille Cedex 20, France, Marseille, France
[2] Microscopy Core Facility, Aix Marseille Univ, CNRS, IMM, FR3479, 31 Chemin J. Aiguier, 13402 Marseille Cedex 20, France, Marseille, France
[3] Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP UK, Lancaster, United Kingdom

Most aquatic photosynthetic microorganisms, including diatoms, rely on CO2-concentrating mechanisms (CCM) in order to fulfil RuBisCO-mediated CO2 fixation. Carbonic anhydrases are important components of CCM as they catalyze the interconversion of CO2 and HCO3-. Some CAs are shown to be highly up-regulated under low CO2 concentrations. Eight different CA- subclasses have been described so far and at least 5 of them are found in diatoms genomes and their location predicted or experimentally confirmed. Recently, a low-CO2 inducible protein (LCIP63) has been found in the marine diatom *Thalassiosira pseudonana*. Here, we show that LCIP63 has a CA and esterase activity and is located near the inner chloroplast envelope. Furthermore, its activity can be inhibited by CA inhibitors and seems to be modulated by the presence of the metal ion Mn2+ instead of the typical CA cofactor Zn2+. In addition, LCIP63 is important for CCM in *T. pseudonana* since an increase in the affinity for dissolve inorganic carbon was observed when LCIP63 was overexpressed. Since LCIP63 has a low sequence identity with other known CA-subclasses, we proposed that it belongs to a new subclass that we designated as ι-CA (iota-CA). LCIP63 homologs are geographically and taxonomically widespread among photosynthetic eukaryotes and prokaryotes, as well as bacteria and archaea, and thus it could play an important role in global carbon cycling (1). (1) Jensen, EL. et al. (2019) A new widespread subclass of carbonic anhydrase in marine phytoplankton. The ISME journal. Published online: 25 April 2019. Doi: 10.1038/s41396-019-0426-8.

7 Chloroplast-targeted repeat proteins in Diatom genomes

Olivier Vallon, Yves Choquet, Angela Falciatore

UMR 7141, IBPC, CNRS/Sorbonne Université, Paris, France

In photosynthetic eukaryotes, the expression of the chloroplast genome is controlled largely by nuclear-encoded factors which are imported into the organelle and bind to specific RNAs and control a variety of molecular functions. These include endo- or exonucleolytic processing, splicing, editing, initiation of translation etc.… In green algae and land plants where these processes have been intensively studied, several families of repeat proteins have expanded which cover a large portion of these functions. The best characterized are the PentatricoPeptide Repeat (PPR), TetratricoPeptide Repeat (TPR) proteins and OctotricoPeptide Repeat (OPR) proteins. They are based on the repetition of a short protein motif forming a pair of antiparallel alpha-helices and exposing an RNA-interaction surface. Sequence specificity of the protein is dictated by that of each repeat, through a code which has thus far only been elucidated for PPRs. In spite of its paramount ecological importance, photosynthesis in Diatoms has received too little attention. In particular the modalities of expression of the chloroplast genome are practically unknown. As a first step towards an experimental characterization of these processes, we will present an overview of these repeat protein families in selected Diatom genomes, and try to correlate it to the structure of the plastome and the biology of the organism.

8 A micro-morphologic approach to the biogeography of freshwater diatom species in Indian waters of Western Ghats (WG), Central Highland (CH), and West Himalaya (WH) in the Indian Subcontinent

Jyoti Verma [1], Prakash Nautiyal [2]

[1] University of Allahabad, Allahabad, India, ALLAHABAD, India
[2] HNB Garhwal University, Srinagar, Uttarakhand, India

Fifty one, fifty and forty three genera were recorded from the West Himalaya (WH), Central Highlands (CH) and Western Ghats (WG), respectively. Thirty six genera are common to these three biogeographic regions of the Indian subcontinent. Currently, the Indo-Gangetic Plains separate the Himalaya and the Central Highlands but are connected by the Gangetic drainage. The WG and the CH are connected like 'elbow' at northern and western extremity, respectively and extend to south and east located perpendicular to each other. These two regions constitute the Deccan Peninsula the oldest part of the Indian subcontinent, a historical reason for high similarity between these two biogeographic regions. However, similarity with the Himalaya (part of the sub continent) created historically by recent upheavals, other geological activities in the Deccan and glaciations and current geography account for this high similarity. Preliminary investigations show presence of fifty eight genera from three biogeographic regions the CH, WH and WG of the Indian subcontinent. Fifty one taxa were recorded from the Himalaya (Garhwal region), fifty from Central Highlands and forty three from Western Ghats. Thirtysix genera were common to these three regions. Historical rather than geographical factors appear to have played a greater role, due to movement of landmasses and upheavals, which not only led to changes in the drainage patterns but also climatic conditions. The recently deglaciated high altitude locations in the Himalaya have not been geologically stable while the CH and the WG in the peninsula has been geologically stable.

9 Use of diatom communities as indicators of conductivity and ionic composition in a small Austral temperate river system.

Tinotenda Mangadze

Rhodes University, Grahamstown, South Africa

The aim of this study was to determine if benthic diatoms can be used as effective and reliable indicators of ionic composition and conductivity in different stream order categories. Samples were collected twice at 22 sampling sites within the Bloukrans River system, Eastern Cape Province, South Africa. The data collected were subjected to multivariate statistical technique i.e. CCA to determine environmental gradients along which the diatom species were distributed as well as to elucidate hypothesized differences in community structure per stream order. Significant differences between the two sampling periods were observed in dissolved oxygen, temperature, Na, B, Ca, Zn, Cu, Cr, K, Fe, phosphate, conductivity, salinity and nitrate, while significant stream order variation was observed for conductivity, salinity, Mg, Ca and sediment nitrates. Study sites were grouped into roughly two broad categories (stream order 1 and 2/3 sites) based on CCA. As pollution increased, low to moderate pollution tolerant species such as Fragilaria tenera, Cyclostephanos dubius and Gyrosigma acumina¬tum were replaced by high pollution tolerant species such as Nitzschia palea, Gomphonema parvulum, Tryblionella apiculata, Diploneis vulgaris and Staurosira elliptica. This shows that diatom assemblages are appropriate indicators of ionic composition/conductivity and hydromorphological characteristics (e.g., stream size) of running waters. The results highlight the importance of creating regional calibration datasets which will make it possible to develop finely tuned models to quantitatively infer conductivity and ion concentrations. Conductivity, ionic composition, diatoms, stream order, water quality

10 Silicon metabolism during the resting stage cells formation in marine diatom Thalassiosira pseudonana

Jun-Rong Liang [1, 2], Shan-Shan Zhuang [1], Qian-Qian Huang [1], Peng-Yu Ji [1], Lu Huang [1], Chang-Ping Chen [1, 2], Ya-Hui Gao [1, 2]

[1] School of Life Sciences, Xiamen University, Xiamen, China
[2] Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen, China

Diatom resting stage (including resting cell and resting spore) must be considered as an effective strategy for maintaining diatom populations in marine ecosystems under adverse conditions. One of outstanding characteristics of resting stage cells is producing more heavily silicified frustules, which increasing the sinking rates of the cells to improve the export of the carbon and silicon into the deeper ocean as biological pump. However, the mechanism of biosilicification during resting stage cell formation in diatoms is still unknown. Here, the resting cells of the modern diatom *Thalassiosira pseudonana* were induced under dark and 4℃ for three months. Cellular response and genes involved in biosilicification during resting cells formation were investigated using transcriptome analysis. A total of 11882 unique genes were identified in six time points (weeks 0, 4、6、8、10、12) and 7428 genes with significant expression levels were selected for further analysis. Especially, several genes related to girdle band and valve formation kept up-regulation in the process of resting cells formation, indicating their roles in heavy silicification. Our findings have provided a new insight into silicon metabolism associated with resting cells formation, which will enhance our understanding of the role of diatom resting cells in biogeochemical cycles of silicon and carbon. This work was supported by the National Natural Science Foundation of China (Grant No. 41576138 and 41276130). diatom, *Thalassiosira pseudonana*, resting cells, biosilicification, silica-forming genes, biogeochemical cycles of silicon and carbon

11 Comparative transcriptomics of three diatom species focused on silica biomineralization

Sayako Iwaki [1], Kiori Obuse [1], Takashi Tamura [1], Kenji Inagaki [1], Shigeki Mayama [2], Michiko Nemoto [1]

[1] Graduate School of Environmental and life Science, Okayama University, Okayama, Japan
[2] Department of Biology, Tokyo Gakugei University, Tokyo, Japan

The silica cell walls of diatoms, which exhibit species-specific micro- and nano- patterned structures are promising candidates for applications in nanotechnology. To-date, molecular biological analyses toward understanding of diatom cell wall formation have been mostly limited to model diatom species and general silica formation process in diatoms is still incompletely understood. In this study, to gain a comprehensive insight into diatom silica biomineralization, transcriptome data of three non-model diatom species, Nitzschia palea, Achnanthes kuwaitensis and Pseudoleyanella lunata, were newly developed and used for a comparative transcriptomic analysis. Furthermore, the transcriptome data were used for proteomic analyses to identify the silica cell wall-associated proteins. The reads obtained from RNA sequencing were assembled into 40,498, 71,930 and 45,400 unique transcripts for N. palea, A. kuwaitensis and P. lunata, respectively. In order to identify the diatom-specific core proteins, three transcriptome data sets developed in this study and the protein-coding gene sets of five sequenced diatoms were compared. The proteins shared only by eight diatom species that are predicted to possess an endoplasmic reticulum (ER)-targeting signal peptide were selected for further analyses. Proteomic analysis identified a number of silica cell wall-associated proteins in N. palea. It includes a novel protein with ER signal peptide and a unique repetitive sequences. The genes encoding a number of proteins identified in this study, which were shown to respond to silicon were suggested to be implicated in silica biomineralization.

12 Expression patterns of predicted viral ORFs of diatom-infecting DNA viruses

Takashi Kadono [1, 2], Masao Adachi [1], Yuji Tomaru [2]

[1] Laboratory of Aquatic Environmental Science, Faculty of Agriculture and Marine Science, Kochi University, Nankoku, Japan
[2] National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Hatsukaichi, Japan

Marine diatom-infecting viruses (DIVs) are of broad interest in the basic and applied research of diatoms. We recently investigated the activity of promoters derived from marine DIVs in the marine diatom, *Phaeodactylum tricornutum*, for the metabolic engineering of diatoms. Among these, the activity of the promoter region of the Chaetoceros lorenzianus-infecting DNA virus replication-associated protein (VP3) gene (ClP1) was significantly higher than the activity of endogenous diatom promoters used for the metabolic engineering of *P. tricornutum* (Kadono et al. Sci. Rep. 2015; 5: 18708). However, the mechanisms controlling viral gene expression in host diatoms remain largely unknown. In silico analysis of DIV genomes has revealed the presence of some putative open reading frames (ORFs): VP3 gene, structural protein (VP2) gene, and two genes of unknown function (VP1 and VP4). In this study, we analyzed the gene expression of the putative viral ORFs of three DIVs in their host diatoms. RT-PCR analysis was performed to assess the gene expression pattern of viral ORFs in each infected host diatom. The timing of expression of the VP1 and VP4 genes in the host diatoms was observed to be earlier than that of the VP2 and VP3 genes after viral infection. In addition, we identified several consensus sequences in the putative promoter regions of the ORFs using consensus motif-finding algorithms. Some consensus sequences were found for the potential promoter regions of the ORFs. These findings may assist in future investigations of the mechanisms of DIV gene expression during viral infection of diatoms.

13 Multi-omics Paradigm to Unveil the Mechanism of Acyl-Lipid Pathway(s) in Oleaginous Microalga Parachlorella kessleri

Pannaga Pavan Jutur

International Centre for Genetic Engineering and Biotechnology, New Delhi, India

Microalgae are capable of converting atmospheric carbon dioxide to substantial biomass and biofuel precursors, utilizing non-agricultural land and waste or marine water, which is a distinct prerequisite for both the food and energy sectors. Understanding the molecular imprints of lipid metabolism is important for explicating the functional mechanisms of lipid accumulation in microalga. Parachlorella kessleri, a marine unicellular green microalga belonging to class Trebouxiophyceae, accumulate large amounts of lipids under nutrient deprivation. In the present study, a new molecular perspective of multi-omics was applied to study the molecular changes in the strain subjected to nutrient deprivation (nitrogen starvation). P. kessleri genome sequenced using dual sequencing techniques i.e. Illumina and Nanopore Oxford technologies were assembled into the size of ~64.9 Mb comprising around 400 scaffolds including 8,371 gene-encoding proteins with a GC content of 54%. A number of significant transcripts were found to be differentially regulated (375) in the transcriptome analysis followed by proteome profiling which showed expression changes in ~166 proteins. Our qualitative metabolomics has identified nearly 85 metabolites, of which significant fold- change was observed in various molecules that seem to play an important role in maintaining the growth profile and lipid content. In conclusion, our understanding of the entire system through multi-omics will lead to the identification of relevant pathways involved in the biosynthesis and degradation of precursor molecules that may have the potential for biofuel production, aiming towards the vision of tomorrow's bioenergy needs.

14 Proteomics of diatoms: discovery of polyamine modifications in biosilica-associated proteins

Alexander Milentyev [1], Christoph Heintze [2], Nicole Poulsen [2], Nils Kröger [2], Matthias Wilm [3], Andrej Shevchenko [1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2] B CUBE - Center for Molecular Bioengineering, TU Dresden, Dresden, Germany
[3] Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

Diatoms are unicellular algae that use highly specialized proteins to produce nano silicified cell walls. These proteins termed silaffins share no homology across diatom species. Silaffins are heavily modified, and their lysine residues bear ε-polyamine chains. However, the structure and biological role of ε-polyamines remain elusive. To this end, we developed a method to identify and quantify ε-polyamine-modified lysines and to map them back to silaffin proteins from three diatom species (*Thalassiosira pseudonana*, T. oceanica, and Cyclotella cryptica). In total, 17 novel modifications were discovered, including acid-resistant phosphoester-containing polyamines. We demonstrated that the pattern of polyamine modifications reflects the phylogenetic proximity of the diatom species. We identified modified lysine residues by polyamine-specific fragments in MS/MS spectra followed by iterative searches and deconvolution of raw MS/MS spectra. Next, we localized 130 polyamine-modified sites in 26 proteins from the three diatom species and revealed three consensus motives common to all three diatoms, although full sequences of the modified proteins were not conserved. Additionally, we developed an approach for the systematic identification of ε-polyamine-modified peptides in total biosilica extracts that relied on the polyamine-specific fragments and applied all-ions fragmentation (AIF) LC-MS/MS. For this purpose, Arcadiate software was customized for a systematic search of marker fragment ions originating from the same peptide precursor by alignment of their XIC traces, thus bypassing recognition and fragmentation of very low abundant peptide precursors by the conventional data-dependent acquisition (DDA) approach.

15 Altering growth conditions to increase plant natural product yield in bioengineered diatom Phaeodactylum tricornutum

Fatima Awwad, Nikunj Sharma, Gabriel Fleurent, Fatma Meddeb, Isabel Desgagné-Penix

Plant Biology Research Group, Université du Québec à Trois-Rivières, 3351 boul. des Forges, Trois-Rivières, QC, G9A 5H7, Canada, Trois-Rivières, Canada

Plant natural products (PNP) are important resources for pharmaceutical and food industry. In the last decades, the market price of several PNP inflated because of the limited amounts produced in plants and the challenges in growing healthy crops. To overcome this problem, our team developed a multitool box of molecular methods to transform marine algae *Phaeodactylum tricornutum*. Bioengineered diatoms are great candidates for heterologous plant gene expression because of the relatively close translation mechanisms to plants' compared to bioengineered bacteria or yeast. We designed and inserted genes encoding enzymes involved in PNP biosynthesis into an episomal vector to avoid nuclear silencing. Positively transformed *P. tricornutum* were selected and grown using different growth conditions and we compared the PNP production yield. We aimed to optimize growth conditions of transformed *P. tricornutum* in order to channel the production of the desired molecules. Growing *P. tricornutum* in different mixotrophic conditions allowed us to obtain a different metabolic profile depending on Carbon, Nitrogen and Phosphorus supplementation and different light cycles. The best set of parameters that allows optimal production of PNP in bioengineered *P. tricornutum* will be presented.

16 Metabolic Engineering of Escherichia coli with Marine Diatom Polyamine Biosynthesis Pathway for the Production of Longer Uncommon Polyamines

Hung-Yun Lin [1, 2], Chun-Ting Lee [1], Chung-Hsiao Leu [1], Man-Jun Xu [1], Han-Jia Lin [1, 2]

[1] Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, Keelung City, Taiwan
[2] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan, Keelung City, Taiwan

Polyamines have been proved to be responsible for maintaining the basic physiological functions of cells. Common polyamines are found in almost all species including putrescine (Put), spermidine (Spd) and spermine (Spm) while uncommon polyamines are only found in special species such as thermophilic microorganisms, sponges and microalgae. Recent studies have found that the application of uncommon polyamines is quite extensive. For example, thermospermine (TSpm) and homocaldopentamine (HCPA) can trigger the ability of higher plant systemic acquired resistance (SAR) to fight against viruses. Diatoms, as an indispensable model species in the global ecosystem, has been found to have the ability to produce a variety of uncommon polyamines. In this study, we first identified the gene involved in the polyamine biosynthetic pathway in *P. tricornutum*. Previously bioinformatics analysis has been reported that up to seven polyamine synthase genes found in *P. tricornutum*. So far, the biochemical experiments confirmed that both PtSDS1 and PtSDS2 are spermidine synthetase. The enzyme substrate selectivity of PtTSMS is quite special. In addition to the ability to convert Spd or NSpd to TSpm or NSpm, it is possible to continue to extend the reaction to synthesize higher order polyamines, like HCPA. These genes are then introduced into Escherichia coli to alter their polyamine metabolites and provide the ability to produce HCPA. In the future, as we gain an increased understanding of the various polyamine synthesis mechanisms, we will be able to develop a variety of diatom-specific polyamine production platforms from a more macroscopic perspective with efficient manner.

17 Identification and charactization of two spermidine synthase in marine diatom Phaeodactylum tricornutum

Chung-Hsiao Liu [1], Hung-Yun Lin [1, 2], Chun-Ting Lee [1], Han-Jia Lin [1, 2]

[1] Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, keelung, Taiwan
[2] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan, keelung, Taiwan

Polyamines are organic compounds that are positively charged in organisms. It's responsible for maintaining basic physiological functions. Polyamines are also common metabolites of diatom. Diatom is an eukaryote which provide global carbon fixation and marine primary productivity. polyamine composition in diatom is more complex than eukaryotic cells. For example, spermidine is one of the main components in the model species *P. tricornutum* with a small amount of nor-Spermine, Putrescine and CPA. Previous reports have indicated that two spermidine/spermine synthase genes are predicted in *P. tricornutum*. This study verified that both genes (PtSDS1 and PtSDS2) are spermidine synthase. We want to identify the role of PtSDS1 and PtSDS2 in the physiology of *P. tricornutum*. In general, the spermidine synthase sequence has three active sites. Sequence align revealed that PtSDS1 retained all active sites, but one active site in PtSDS2 was mutated (Tyr79>Phe79). PtSDS1 and PtSDS2 recombinant proteins were produced using the E. coli expression system and test by enzyme activity assay. The results show that even if PtSDS2 has a mutation at the active site, it can convert putrescine to spermidine with the same specificity as PtSDS1. However, the enzyme activity of PtSDS2 seems to be poor than PtSDS1. These results indicate that *P. tricornutum* can use PtSDS1 and PtSDS2 to synthesize spermidine in cells, but the

physiological functions of the two genes require more research. In the future, as we understand the mechanism of spermidine synthase in diatom, we can explore the role of spermidine in the growth of diatom.

18 Integrated analyses of the consequences of horizontal gene transfer for diatom evolution

Richard Dorrell [1], Guillaume Blanc [2], Adrien Villain [2], Achal Rastogi [1], Giselle McCallum [3], Guillemette Audren de Kerdrel [1], Ouardia ait-Mohamed [1], Kyle Frischkorn [1], Fabio Rocha Jimenez Vieira [1], Leila Tirichine [1, 4], Chris Bowler [1]

[1] Ecole Normale Supérieure, Paris, France
[2] Université Aix-Marseille, Marseille, France
[3] Concordia University, Montréal, Canada
[4] Université Nantes, Nantes, France

Horizontal gene transfer, both from prokaryotic and eukaryotic sources, has had quantifiable impacts on the evolution of eukaryotic algae, although its exact extent and functions remain controversial. Here, we utilise novel and published sequence datasets, and combined bio-informatic and wet techniques, to profile the impacts of different horizontal gene transfers on the evolution of the model diatom Phaeodactylum; and to more globally resolve the consequences of horizontal gene transfer from bacteria throughout the evolutionary history of ochrophyte lineages, including the origins and subsequent radiation of diatoms. We show that diatom genomes are evolutionary mosaics, with previously undetected evidence for horizontal gene transfer events involving other algal lineages with secondary plastids; and identify a continuous flux of horizontal gene transfer from bacterial sources, which may have had particular significance for the early evolution of diatoms. We additionally demonstrate that different horizontal gene transfer events have contributed different functions to diatom biology. We show that bacterial horizontal gene transfer events from earlier time points predominantly affected the biology of ochrophyte chloroplasts and mitochondria, including a number of horizontally acquired genes encoding aminoacyl tRNA synthetases that are dual-targeted to both organelles. In contrast, more recent horizontal gene transfer events appear to predominantly have transformed the diatom secretome, and contain a number of genes with well-coordinated transcriptional dynamics, suggesting common functions.

19 Biosynthesis of longer uncommon polyamines by polyamine synthase from marine diatom

Chun-Ting Lee [1], Hung-Yun Lin [1, 2], Han-Jia Lin [1, 2]

[1] Institute of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung City, Taiwan
[2] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung City, Taiwan

The presence of longer uncommon polyamines (LUPAs) can be found in a few species such as thermophilic microorganisms and diatoms. LUPAs are physiologically related to the heat tolerance of thermophilic microorganisms, and studies have indicated that they can enhance the ability of plants to fight viruses. For the study of LUPAs synthesis pathways, only two LUPA synthases from archaea have been identified, which can synthesize CPA, a type of LUPA, using NSpm as a precursor. However, it is still unclear what LUPA synthases can synthesize HCPA or TPA, which may be based on Spm or TSpm. In this study, a LUPA synthase candidate gene (PID 51460) of *Phaeodactylum tricornutum* was found by bioinformatics analysis. The in vitro reaction of the recombinant protein expressed by E. coli showed that the enzyme could synthesize CPA from NSpm, and it was also possible to synthesize HCPA or TPA from TSpm or Spm, respectively. Since

this enzyme can synthesize various LUPAs, we named it PtLUPAS (longer uncommon polyamine synthase). Next, using the strategy of synthetic biology, we engineered the polyamine synthesis pathway of E. coli. In addition to expressing PtLUPAS in cells, SAMDC was also overexpressed to provide more dcSAM. The results showed that the metabolically engineered E. coli produced HCPA. This study identified the first enzyme that synthesizes HCPA or TPA. It is of help in studying the physiological functions of these LUPAs in the future. This enzyme also can be applied to synthetic biology for producing special polyamines.

20 Genetical conversion of a photoautotrophic Phaeodactylum tricornutum strain to grow mixotrophic and its impact on the lipid content

Liv Celin Krämer, Daniel Wasser, Claudia Büchel

Plant Cell Physiology, Institute of Molecular Biosciences, Goethe University, Frankfurt, Germany

Most known microalgae are photoautotrophic organisms, and only few are naturally obligate or facultative heterotrophs. So far, naturally synthesized products or biomass of microalgae are commercially used mainly from cultures in open ponds or in expensive and quite ineffective bioreactors. There is a huge need for using microalgae as recombinant expression systems, but genetically modified organisms must be cultured in a closed system, and efficient productivity is mostly achieved by heterotrophic growth in fermenters. Thus, having heterotrophic strains of otherwise obligate phototrophs would remove the limitation to a small number of known heterotrophic microalgae. During the last years access to more genome data banks of microalgae became available and new genome engineering methods were established to improve i.a. expression yields. We hereby show how to overcome further obstacles and start using the oleaginous diatom, *Phaeodactylum tricornutum* as recombinant expression system under mixotrophic conditions. In this investigation we expressed different glucose transporter proteins in the alga *P. tricornutum*. Belonging to the Major Facilitator Superfamily (MFS) of membrane transporters, we choose glucose transporter of the taxon Viridiplantae. By using different promoters and optimising codon bias, we try to enhance the expression level of these heterologous genes in diatoms. To study, if changed growth metabolism caused changes in the lipid content, total lipid content was measured, too. With this knowledge we did a further step by establishing a heterotrophic microalgae strain that can improve biotechnology research on microalgae for e.g. biodiesel, pigment or antioxidant production.

21 Genome-scale modeling of metabolism in the cold-adapted diatom Fragilariopsis cylindrus underscores the strong resilience of growth rate to cellular perturbations

Michel Lavoie [1], Blanche Saint-Béat [1], Jan Strauss [2], Antoine Allard [1], Simon Hardy [1], Angela Falciatore [3], Marcel Babin [1], Johann Lavaud [1]

[1] Université Laval, Quebec, Canada
[2] European Molecular Biology Laboratory, Hamburg, Germany
[3] CNRS, Institut de Biologie Paris-Seine, Paris, France

Diatoms are major primary producers in polar environments. They can actively grow in polar environments that are characterized by extremely variable environmental conditions including light and temperature. Integrative modeling using genome-scale metabolic model (GSM) is a powerful approach to decipher the complex interactions between components of diatom metabolism and can provide insights into metabolic mechanisms that explain their evolutionary success in these extreme environments. Here, we developed the first GSM for a cold-adapted diatom, Fragilariopsis cylindrus. The model allowed us to analyze the resilience of growth rates to changes in biochemical composition or model structure. Local and global sensitivity analyses show that the predicted growth rates are most sensitive to variations in carbon uptake and total cellular proteins, lipids or

carbohydrates. Yet, varying individual model parameters by ± 40% for 64 out of 65 parameters affected growth rates of *F. cylindrus* by less than 14%, leaving carbon uptake rate as the most important parameter controlling the growth rate. Sensitivity analyses of intracellular reaction fluxes and reaction deletions on the metabolism also demonstrated the strong resilience of *F. cylindrus* growth. Overall, our results support the general assumption that measured growth rates during ecophysiological studies are robust estimators of cell physiological states, and they underscore the importance of resilience of metabolism in F. cylindrus, a feature that undoubtedly helps to maintain cell homeostasis under extremely variable environmental conditions in polar environments.

22 Polycomb Repressive Complexes in the diatom Phaeodactylum tricornutum

Xue Zhao, Leila Tirichine, et al.

1CNRS UMR6286, UFIP UFR Sciences et Techniques, Université de Nantes, 2, rue de la Houssinière, 44322, Nantes Cedex 03, Nantes, France

Polycomb Repressive Complexes PRC2 and PRC1 are two groups of proteins known to be associated with the repressive histone marks, H3K27me3 and H2AK119Ub respectively. Together with heterochromatin protein HP1, they regulate silencing and transcription in multicellular species. Because of their absence in yeast and strong role in development, they were thought to be absent in unicellular species. However their discovery in recent years in several unicellular algae raises the question of their role in these species. The marine diatom *Phaeodactylum tricornutum* shows a high conservation of HP1 and PcG family (Enhancer of zeste (E(z)), Extra Sex Comb (Esc), Suppressor of zeste 12 (Su(z)12), Nucleosome remodeling factor 55 kDa subunit (Nurf-55), RING finger protein1(RING1) and (PCGFs/Psc) proteins. We used CRISPR cas9 editing to generate knock out of E(z), Esc and PRC1 components in *P. tricornutum* to comprehend the role of these proteins and the recruitment of Polycomb Repressive complexes to their targets. We demonstrated that E(z) is the methyltransferase responsible of di and tri methylation of lysine 27 of histone H3, Esc is also essential to the deposition of H3K27me3. Mutants from both PRC1 and PRC2 in *P. tricornutum* might shed light on the existing cross talk between these two complexes which is not fully understood in multicellular organisms. Furthermore, *P. tricornutum* shows a unique pattern of co-occurrence of repressive marks such as H3K27me3/K9m2/me3 and DNA methylation which are investigated using mutants of the Polycomb complex, other histone methyltransferases and genome wide approaches such as sequential ChIP followed by bisulfite sequencing.

23 Investigating DNA methylation in diatoms

Agnes Groisillier, Carine Pruvost, Leila Tirichine

CNRS UMR 6286, UFIP, UFR Sciences et Techniques, Université de Nantes, Nantes, France

DNA methylation at the 5-carbon position of cytosine (5mC), the most common epigenetic modification plays a major role in the regulation of eukaryotic genomes. It was reported to be essential in a wide range of biological processes including cell differentiation, development and response to stress. Previous studies on DNA methylation have shed light on the highly variable evolution of 5mC across eukaryotic groups and lineages. While absent from the yeast Saccharomyces cerevisiae, the nematode Caenorhabditis elegans, and the brown alga Ectocarpus siliculosus, it is reported to occur in several animals, plants and microalgae including dinoflagellates and diatoms with different patterns, and landscapes reflecting a complex evolutionary history. 5mC is established and maintained by different methyltransferases which were found to be encoded by several diatom genomes. To understand the role of DNA methylases in diatom ecology, their functional diversity and the molecular mechanisms involved in their recruitment to specific regions of the genome, we use *Phaeodactylum tricornutum* and a wide range of molecular tools. Recent progress is presented.

24 Phaeodactylum tricornutum as a biological solution for the Global CO2 Challenge

Deepak Sethi, Seetharaman Vaidyanathan

Department of Chemical and Biological Engineering, University of Sheffield, Mappin Street, Sheffield, S13JD, UK, Sheffield, United Kingdom

Increased CO2 levels are responsible for global warming, drastic changes in the global weather patterns and acidification of oceans. Diatoms found in the oceans, waterways and soil are responsible for the generation of 20% of O2 in our atmosphere and are one of the most ideal biological solutions for the excess CO2. But, for an effective understanding and effective process development, the physiology of the diatom CCM; function of the bicarbonate transporters; location and function of the carbonic anhydrases; the activity and the components of the controversial C4 pathway has to be further studied. Tremendous applications and commercial opportunities can be developed by utilizing diatoms for the biological sequestration of CO2. In a *P. tricornutum* CCM, the major driver of the CCM is a 'chloroplast-pump' that actively transports bicarbonate into the chloroplast. In general diatoms can take up both CO2 and HCO3− from the environment but the preferred one is yet shrouded in mystery. The Rubisco embedded in the pyrenoid, a membrane less organelle that exists in various photosynthetic organisms such as algae, is the location where in most of the global CO2 fixation occurs. The structure, protein composition and dynamics of the pyrenoid in evidently plays a very important role in the CO2 fixation process. The need of the hour is a deep study of the pyrenoid that will aid in the effective utilization of *Phaeodactylum tricornutum* as a carbon sink for the biological solution for the Global CO2 Challenge.

25 Accelerating molecular studies in algae by developing genome-wide mutant collections

Xiaobo Li

Westlake University, Hangzhou, China

Genome-wide mapped mutant libraries are powerful resources for genetic research in any organism. For reverse genetics, they provide mutants for genes of interest; for forward genetic screens, hit genes are immediately identified without the need to go through tedious mutation mapping processes. However, for a long-time, such resources were not available in unicellular photosynthetic organisms. During my postdoc, I led the generation of a mutant library in the green alga Chlamydomonas by combining robotic methods and advanced molecular tools. This library has provided thousands of mutants to hundreds of labs. In addition, a screen using this library has yielded 303 candidate genes required for photosynthesis, including 65 known photosynthesis genes and 238 novel ones (Li et al. 2019 Nature Genetics). After starting a lab at Westlake University in 2018, I have been interested in the biology of diatom chloroplasts, especially how they work differently from the two-membraned chloroplasts in green plants. At this meeting, I hope to discuss potential approaches to develop genome-wide screen tools in diatoms with colleagues.

26 Exploring Si Uptake Mechanism in Thalassiosira pseudonana through CRISPR/Cas9 Gene Editing

Lior Aram [1], Oz Ben Joseph [1], Shifra Ben-Dor [2], Assaf Gal [1]

[1] Plant and Environmental Sciences department, Weizmann Institute of Science, Rehovot, Israel
[2] Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

The formation of the silicified cell wall of diatoms is a tightly regulated cellular process. Understanding the silicification mechanism is crucial, both as it influences the ecophysiology of diatoms, and as a remarkable example for the ability of organisms to control mineral formation. The first step in the silicification process is the transport of silicic acid from the environment, across the plasma membrane and against a concentration gradient, into the cell. Silicic acid transporters (SITs) are thought to mediate this process, but several observations, such as the diffusibility of silicic acid through membranes, render Si uptake mechanisms unclear. The three SIT proteins of the model species *Thalassiosira pseudonana* were extensively studied, suggesting that SIT1 and SIT2 have a role in silicic acid transport, while SIT3 has a regulatory function. Using the recent advancements in the CRISPR\Cas9 gene editing methods, we mutated the sit genes in order to study their functional roles during silicification in vivo. We generated three knockout strains of sit1, sit2, and sit3. Surprisingly, all sit mutant cells are viable, grow similarly to the wt at Si-replete conditions, and show no alteration in their cell-wall morphology. Preliminary results suggest that sit1 and sit2 mutant cells have a reduced ability for Si uptake when grown at Si-deplete conditions. Characterization of the sit mutant cells should allow us to analyze the phenotypic plasticity of the SIT proteins in situ and will give a direct evidence for the regulatory roles of SITs in the silicification process.

27 Comprehensive RNA-seq gene expression profiling of the cold-adapted diatom Fragilariopsis cylindrus across an array of experimental conditions including sea ice formation

Jan Strauss [1], Shazia Aslam [2], Andrew Toseland [3], Pirita Paajanen [4], Cock van Oosterhout [5], Mark McMullan [3], Graham Underwood [6], Thomas Mock [5]

[1] Centre for Structural Systems Biology (CSSB), DESY and European Molecular Biology Laboratory, Hamburg, Germany
[2] Department of Chemistry, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[3] Earlham Institute, Norwich Research Park, Norwich, United Kingdom
[4] Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, United Kingdom
[5] School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, United Kingdom
[6] School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, United Kingdom

Diatoms often dominate polar marine ecosystems, where photosynthetic growth is governed by nutrient availability and seasonal fluctuations in light, temperature and the extent of sea ice. Molecular adaptations enable the cold-adapted diatom *Fragilariopsis cylindrus* to thrive in such ecosystems. Previously, we have highlighted specific adaptations of F. cylindrus, including the differential expression of divergent alleles and metabolic pathways for the production of extracellular polymeric substances (EPS) in response to highly variable environmental conditions. However, it has become apparent that adaptations to extreme conditions are conferred by a collection of synergistic adaptive changes including gene regulation. To provide deeper insights into the individual contribution of gene regulation to molecular adaptions in F. cylindrus, we performed a comprehensive analysis of RNA-seq datasets from an array of 18 experimental

conditions including unpublished datasets from cells grown under low silicate (0.3 µM), blue (480 – 540 nm) and red (550 – 700 nm) light as well as conditions simulating sea ice formation. Analysis of distinct gene sets that were significantly regulated in response to single specific experimental conditions underscored the high plasticity of gene expression in F. cylindrus, which was most pronounced during prolonged darkness affecting 1,848 genes. Furthermore, we identified functional modules, potential gene members of protein complexes and binding partners of key genes (e.g. Fragilariopsis rhodopsin) using weighed gene co-expression network analysis. Altogether, the compiled transcriptome data, together with the available genome sequence of *F. cylindrus* and genomic tools will be a key asset to fully explore gene-environment interactions in the future.

28 Investigating the role of chloroplast Ca2+ signalling in diatoms

Serena Flori, Glen Wheeler, Colin Brownlee

the Marine Biological Association of the UK, Plymouth, United Kingdom

Ca2+ plays an important role in many signaling pathways in eukaryotes. Abiotic and biotic inputs, such as pathogen attacks, osmotic or oxidative stress result in specific Ca2+ elevations within the cytosol that activate a range of downstream signal transduction pathways. Recently, it has become clear that Ca2+ elevations may also occur in organelles, with chloroplast Ca2+ signalling implicated in the regulation of several aspects of photosynthesis in land plants. However, little is known about Ca2+ signaling in diatoms (see Falciatore et al., 2000; Vardi et al., 2008), and the complex evolutionary origins of their plastids via secondary endosymbiosis suggest that they may possess alternative Ca2+ signaling pathways from those identified in plants and green algae. In this project, we investigate the role of Ca2+ signaling in diatom chloroplasts in response to environmental stress. The approach we employed is to use genetically-encoded calcium sensors targeted to different organelles in the model diatom *Phaeodactylum tricornutum*. In this study, we have been able to monitor intracellular Ca2+ signaling in response to oversaturating light, oxidative stress and hyperosmotic shocks. Our preliminary data indicate that organelle-specific Ca2+ transients are present in response to different environmental stimuli. The results demonstrate that chloroplast-localised Ca2+ signaling also occurs in plastids derived by secondary endosymbiosis and suggest that Ca2+ is likely to play a major role in the regulation of photosynthesis in diatoms.

29 Investigating the role of myosins in the diatom adhesion motility complex

Metin Gabriel Davutoglu, Valeria Sabatino, Veikko Geyer, Stefan Diez, Nils Kröger, Nicole Poulsen

Center for Molecular and Cellular Bioengineering, B CUBE, Technische Universität Dresden, Dresden, Germany

Although the unusual gliding motility of diatoms has fascinated biologists for more than a century, the underlying molecular mechanism remains poorly understood. Pennate diatoms are capable of high speed, bi-directional motility up to 25 µm/s, which in eukaryotes is only rivaled by motility mechanisms that utilize external appendages (e.g. flagella). To provide the traction for gliding, pennate diatoms secrete extracellular polymeric substances (EPS) through the raphe slit thereby achieving adhesion to the substratum. The force for diatom motility is hypothesized to be powered by an intracellular actin-myosin complex that translocates the EPS strands in a rearward direction

through the raphe slit thereby propelling the cell forwards. To date, apart from actin, none of the components of the proposed adhesion motility complex have been identified. Previously, we have demonstrated that the myosin inhibitor, 2,3-Butanedione monoxime, is a potent inhibitor of gliding, thereby providing evidence for a role of myosin in motility. To identify myosins involved in diatom gliding, we have performed a phylogenetic analysis of ~250 myosin sequences from motile and non-motile diatoms. This lead to the identification of a class of myosins that are unique to motile species. GFP-tagging of four Craspedostauros australis myosins from this class has enabled us to investigate their roles in vesicle trafficking and gliding using confocal and TIRF microscopy. Furthermore, to enable the knockout of myosin genes in C. australis using CRISPR/Cas9, we have sequenced the genome and small RNAs of this adhesion model diatom species

30 Studying CCM components in Thalassiosira pseudonana using improved gene editing approaches

Irina Grouneva, Luke Mackinder

University of York Department of Biology, York, United Kingdom

We are studying putative components of the CO2 concentrating mechanism (CCM) in diatoms using a dual approach of fluorescent protein tagging and CRISPR/Cas9-mediated knockout. This will provide experimental data on both localisation and function. We have adopted the method of Karas et al. (2015) for transforming *T. pseudonana* via bacterial conjugation. For this purpose we have domesticated and incorporated both plasmid transfer and episome maintenance elements into a level zero plasmid according to the syntax of Golden Gate Molecular Cloning for Plants (Engler et al. 2014, Patron et al. 2015). Bacterial conjugation resulted in high transformation efficiency (more than 50 colonies per plate) and gene editing activity. All screened transformants were positive for the presence of Cas9. In addition, using a single sgRNA resulted in up to five different NHEJ repair events (indels) per colony, proving Cas9 activity. We are also interested in expanding on homology-directed repair (HDR) in *T. pseudonana* (Belshaw et al. 2017) with an aim to achieve seamless knock-in applications. A first attempt at deletion of a complete gene via HDR was successful, albeit with low efficiency.

31 Genomic diversity in an oceanographic context in the pelagic diatom of the Southern Ocean Fragilariopsis kerguelensis

Ute Postel [1, 2], Lena Eggers [1], Barbara Glemser [1], Gernot Glöckner [2], Uwe John [1], Katherine Salazar [1], Klaus Valentin [1], Bánk Beszteri [1]

[1] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[2] Institute for Biochemistry I, Medical Faculty, University Cologne, Joseph-Stelzmann-Strasse 52, D50931 Cologne, Germany

The Southern Ocean is one of the main silica sinking regions of the world and the thick-shelled pennate diatom Fragilariopsis kerguelensis (O'Meara) Hust. one of the most important silica exporting organisms of this region. Its huge geographic distribution area across the Antarctic Circumpolar Current is characterised by a clear biogeographic zonation deriving from dominating ocean currents and considerable meridional environmental gradients crossing them. We hypothesised that this oceanographic setting promotes zonal differentiation detectable in patterns of genomic variation at or below the species level of F. kerguelensis. In this context we performed

morphometric, genomic as well as mating compatibility analyses of isolates from populations along a meridional transect. Additionally phenotypic parameters and gene expression patterns were compared collected under varying growth conditions. Variation in organellar and nuclear genomes congruently revealed three distinct genotypic groups. Whereas the members of one of these groups occurred along the entire sample-transect (gt-SN) and thus in sympatry with both of the other two groups, the other two were observed only in allopatry, limited either to the Southern (gt-S) or to the Northern (gt-N) sampling region respectively. In laboratory crosses members of gt-SN were never observed to mate with gt-S or gt-N group members, while in contrast gt-S and gt-N members were capable of producing viable auxospores. These observations point to a complex interplay between genes and environments and further underline the differences in diversification processes themselves in the open ocean, exemplifying sympatric speciation and allopatric divergence without the emergence of reproductive barriers.

32 A global perspective on diatom metabolism and their symbionts through the Tara Oceans lens

Juan José Pierella Karlusich [1], **Fabio Rocha** [1], **Eric Pelletier** [2], **Tara Oceans Coordinators** - [1], **Rachel Foster** [3], **Chris Bowler** [1]

[1] Institut de Biologie de l'École Normale Supérieure (IBENS), Paris, France
[2] CEA ? Institut François Jacob, Genoscope, Evry, France
[3] Stockholm University, Stockholm, Sweden

Diatoms, considered one of the most diverse and ecologically important phytoplanktonic groups, have a key biogeochemical role in the ocean. They are responsible for 20% of global primary productivity and they are the world's largest contributors to biosilicification due to their silica cell wall. They also have impact in the marine nitrogen cycle as some species carry symbiotic N2-fixing cyanobacteria. With such an influence on oceanic nutrient cycles, it is key to understand how diatom communities respond to environmental variability by modulation of, for example, gene expression and biotic interactions. The Tara Oceans circumnavigation collected samples from a wide range of oceanic regions using a standardized sampling procedure. Here, we have explored the corresponding metatranscriptomic dataset to characterize diatom gene expression patterns from piconano-, nano-, micro-, and meso-planktonic communities from a worldwide range of oceanic regions and depths. We identified the cellular functions at which diatom communities invest the bulk of energy and resources and we were able to disentangle the functional variation with environmental changes. We found quantitative differences in these cell processes among size fractions, suggesting an important role for cell size and aggregation in shaping the most active metabolisms in diatom communities. In addition, we have analysed the imaging and meta-omics datasets to provide a new estimate of the diversity and global view of diatom-diazotroph associations. Overall, these patterns will sharpen our understanding of how and why diatoms are one of the most abundant photosynthetic groups in the global ocean and their impact on nutrient cycles.

33 Exploring the Diatom World by Forward Genetic Screening

Huan Zhang, **Xiaobo Li**

Westlake University, Hangzhou, China

Diatoms, the most prosperous photosynthetic organisms in the ocean, play essential roles in the global ecosystem. In addition, they can also be used as a biological factory for the high-value

production, fuels and other chemicals. Although the genome sequences have been published in several model diatoms, the high transformation cost and low transformation efficiency used to impede molecular manipulations of diatoms, and most of the previous researches were conducted at physiological level. With the development of gene-editing technology and the success of transformation by bacterial conjugation, genome-editing in diatom has gained much research interest in recent years. But it is still difficult to discover novel genes or pathways by large-scale forward genetic screening. In our study, we hope to establish a genome-wide mapped mutant library using the CRISPR-Cas9 genome editing approach. We plan to achieve this by combining large-scale guide RNA template synthesis, bacterial conjugation to deliver the Cas9 gene and guide RNA template, and high-throughput screening for knock-out mutants facilitated by deep sequencing. We anticipate that such a mutant library will accelerate molecular characterization of various processes in diatoms.

34 Influences of ambient ammonium and phosphate concentrations on the expression of nutrient transporter genes in a marine diatom, Skeletonema tropicum

Lee-Kuo Kang

Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung, Taiwan
Bachelor Degree Program in Marine Biotechnology, National Taiwan Ocean University, Keelung, Taiwan

For a better understanding of how phytoplankton respond to the interaction of nitrogen and phosphorus stress, an experiment with full factorial design was performed to study the transcriptional regulation of nutrient transporter genes in an ecologically important diatom, Skeletonema tropicum. Cells were grown in 16 combinations of ammonium concentrations (500, 200, 100, 50 μM) and phosphate concentrations (36.3, 18.1, 9.0, and 2.0 μM). During the 4-day incubation period, the cell density, maximum quantum efficiency of photosynthesis (Fv/Fm), ambient concentrations of ammonium and phosphate, and transcript levels of selected genes were daily measured. The mRNA levels of 3 nutrient transporter genes including nitrate transporter gene (Nrt2), type II phosphate transporter gene (Npt2bl) and type III phosphate transporter genes (Pho) were measured by Q-RT-PCR. The results showed that the transcript level of Nrt2 was significantly regulated by the ambient ammonium concentrations, where it was repressed in the presence of ammonium, and highly expressed when ammonium was depleted in the media. In contrast, Npt2bl mRNA expression increased with incubation time and was significantly regulated by the ambient phosphate concentrations. Interestingly, Pho mRNA levels also increased with incubation time, but declined under nitrogen-depleted conditions. Pho mRNA expression was regulated by both ammonium and phosphate concentrations.

35 About the diatom metabolism: a new perspective for producing biomaterials

Gabriella Leone [1, 2], Stefania Cicco [3], Danilo Vona [1], Roberta Ragni [1], Gianluca Farinola [1], et al.

[1] University of Bari Aldo Moro, Bari, Italy
[2] Center of Nanoscience and Technology-italian institute of technology IIT-CNST, Milan, Italy
[3] CNR_ICCOM, Bari, Italy

After billion years of evolution, Nature has developed efficient and sophisticated structures which are artificially irreproducible, even at high costs. Among all, diatoms are the major group of photosynthetic microalgae and are able to fabricate a glass shell, frustule, whose hierarchical organization of silica layers shows extraordinarily intricate pore patterns. Easy to extract, via acid oxidative treatments, frustules represent a low-cost source of mesoporous biosilica. Due to the high surface area, mechanical resistance, unique optical features and biocompatibility, frustules have been chemically functionalized with several organic molecules tailored for applications ranging from photonic to biomedicine. Beside the traditional surface chemistry, the emerging biotechnological

approaches focus on the possibility to in vivo decorate the biosilica shell, by simply feeding the algae with tailored molecules that can be embedded in the frustule biosilica during the biomineralization pathway. Harnessing the metabolism of living algae is a powerful biotechnological route for producing biosilica-based materials whose peculiar characteristics could be different from those obtained via surface chemistry.

36 Intraspecific diversity in the cold stress response of transposable elements in the diatom Leptocylindrus aporus

Katerina Pargana [1], Francesco Musacchia [1], Remo Sanges [1], Maria Immacolata Ferrante [1], Chris Bowler [2], Adriana Zingone [1]

[1] Stazione Zoologica Anton Dohrn, Napoli, Italy
[2] Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS, INSERM, PSL Université Paris 75005, Paris, France

Diatoms are able to acclimatize and adapt successfully in many environments. Under unfavorable conditions, several stress response mechanisms take place, including the activation of transposable elements (TEs), which have been proposed to contribute to the generation of genetic and phenotypic diversity in diatoms. The transcriptome variations of three strains of the centric diatom Leptocylindrus aporus, a warm water species, exposed to changing temperature conditions and the possible involvement of TEs in their physiological response were explored. The prolonged exposure of L. aporus to low temperature (13 °C) led to overexpression of stress response proteins such as heat shock factors, stress activated kinases, cold shock proteins and finally TE-related transcripts, which were in fact the most significantly enriched among the differentially expressed transcripts. However, the expression levels of the TEs, as well as most of the stress-related proteins, varied significantly among strains, with differences even exceeding in intensity the response of the individual strains to different temperature conditions. The significant intraspecific variation indicates that the response to changing environments is not only species- but also strain-specific, while the distinct reaction of one strain maintained for long periods in culture might have stemmed from in-culture evolution, taking that strain far from its original state in the natural environment. The present study draws attention to the interplay between the high intraspecific variability and the physiological plasticity of diatoms, both contributing to the adaptation of a species to a wide range of conditions in the marine environment.

37 The diatom Phaeodactylum tricornutum utilizes a mitochondria-located b-oxidation cycle for the catabolism of fatty acids released from storage lipids.

Denis Jallet [1, 2], Denghui Xing [1], Alexander Hughes [1], Mark Simmons [1], Mark Moosburner [3, 4], Andrew E. Allen [3, 4], Graham Peers [1]

[1] Department of Biology, Colorado State University, Fort Collins, United States
[2] Toulouse Biotechnology Institute, Toulouse, France
[3] Scripps Institution of Oceanography, UC San Diego, La Jolla, United States
[4] J. Craig Venter Institute, La Jolla, United States

Diatoms store energy collected from sunlight in the carbon-carbon bonds of various biopolymers, including the neutral lipid triacylglycerol (TAG). TAG is synthesized during the day, while photosynthesis operates. At night, cells catabolize part or all of the ensuing stocks to fuel anabolism, but the underlying mechanisms remain poorly understood. We identified a bacterial-type Acyl-CoA Dehydrogenase (PtMACAD1) that localizes to mitochondria in the model diatom *Phaeodactylum tricornutum*. PtMACAD1 was bioinformatically predicted to constitute the entry point to a complete fatty acid b-oxidation pathway within this organelle. PtMACAD1 homologues

were found in the stramenopile and hacrobian lineages of eukaryotes but not in plants, animals or fungi. The PtMACAD1 encoding gene was knocked out employing the CRISPR-Cas9 system in *P. tricornutum*. Noticeably, TAG cellular content did not diminish at night in the mutants, suggesting that PtMACAD1 is required for nighttime TAG catabolism. While *P. tricornutum* has all the genes to perform peroxisomal fatty acid b-oxidation, these genes were not differentially regulated in response to day/night cycles in the wild-type or to the knockout of the PtMACAD1 gene in the mutants. Overall, our data indicate the presence of a mitochondria-based b-oxidation of fatty acids derived from storage lipids in *P. tricornutum*, which is in stark contrast to the peroxisomal pathways observed in plants and green algae.

38 Tackling the role of Two-pore channels in diatoms

Friedrich Kleiner [1, 2], Katherine Helliwell [1], Glen Wheeler [1], Colin Brownlee [1, 2]

[1] Marine Biological Association of the United Kingdom, Plymouth, United Kingdom
[2] National Oceanography Centre Southampton, Southampton, United Kingdom

Calcium signalling is employed ubiquitously in eukaryotes and bacteria to mediate their response to a range of abiotic and biotic stimuli. Upon sensing a stimulus, Ca2+ channels located in cell-membranes open and allow Ca2+ to quickly enter the cytosol. These changes in Ca2+ are detected by diverse Ca2+ binding proteins, triggering a range of downstream responses. Within the Ca2+ signalling toolkit, voltage-gated channel calcium channels are known for their fast opening and closing kinetics, making them a vital part of rapid signalling events such as muscle contraction or neuronal action potentials in vertebrates. In particular, we are interested in the role of voltage-gated two-pore channels (TPC) in marine diatoms. TPC channels are typically found in endomembranes (lysosomes, vacuoles), where they have been implicated in the release of Ca2+ or other cations from internal stores. In diatoms, we identified conventional animal-like TPCs as well as a novel kind of TPC that was entirely restricted to diatoms. The cellular roles of these two types of algal TPCs remain completely unknown. We are currently investigating the role of the TPCs in marine diatoms using *Phaeodactylum tricornutum* as model. Gene knockout technologies are being employed to examine the roles of TPCs in diatom signalling processes. The mutant's general fitness and Ca2+ signalling activity towards a range of stressors will be examined. The results indicate that diatoms possess unique mechanisms for sensing and responding to their environment and more widely provide insight into the evolution of calcium-dependent signalling processes in eukaryotes.

39 Monitoring the localization of three iron starvation induced proteins (ISIPs) in a single transformant of Phaeodactylum tricornutum under a range of iron supplementation regimes.

Elena Kazamia [1], Jan Mach [2], Jeff McQuaid [3], Marie Olsinova [2], Radek Machan [2], Tyler Coale [3], Emmanuel Lesuisse [4], Andrew Allen [3], Robert Sutak [2], Chris Bowler [1]

[1] Institut de Biologie, École Normale Supérieure, 46 rue d'Ulm, Paris 75005, France., Paris, France
[2] Department of Parasitology, Charles University in Prague, Faculty of Science, Vini?ná 7, 128 43 Praha 2, Czech Republic., Prague, Czech Republic
[3] Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, California 92093-0218, United States of America., La Jolla, United States
[4] Université Paris Diderot (Paris 07), Centre National de la Recherche Scientifique, Institut Jacques Monod, F-75013 Paris, France., Paris, France

P. tricornutum is a model species for diatom iron physiology, found to survive chronically low iron concentrations and to respond readily to iron stimulation, similarly to "low–iron quota" diatoms found in iron-deprived regions of the ocean. A transcriptome analysis of *P. tricornutum* found that iron

deprivation leads to the up-regulation of genes encoding proteins designated as “iron starvation–induced proteins” (ISIPs), specifically ISIP1, ISIP2a, and ISIP3 (Allen et al., 2008 PNAS 105, 10438–10443). ISIPs are considered key to the diatom iron response, and have partially resolved cellular functions. Phylogenetic analysis identified ISIP1 as a largely diatom-specific protein, and experimental evidence showed that it is involved in the assimilation/uptake of siderophores via endocytosis (Kazamia et al., 2018, Science Advances 4 (5), eaar4536). ISIP2a is a ‘phytotransferrin’, involved in the nonreductive uptake of ferric iron at the cell surface, also trafficked into the cell interior via endocytosis (Morrissey et al., 2015, Curr. Biol. 25, 364–371; McQuaid et al., 2018 Nature 555, 534–537). The role of ISIP3 remains unknown. In the present study we used a trichromatic line of *P. tricornutum*, with the three ISIP proteins tagged to fluorophores, driven by native promoters (ISIP1::YFP, ISIP2a::RFP,. ISIP3::CFP), to observe the localization of the proteins under different iron supplementation regimes (siderophore or ferric iron addition, or starvation) over a range of timescales (minutes, hours, days). We report a colocalization of ISIP2a and ISIP1 to an ‘iron processing’ compartment adjacent to the chloroplast and for the first time characterize localization of ISIP3.

40 Genome-wide identification of long noncoding natural antisense transcripts and their response to phosphate stress in the model diatom Phaeodactulum tricornutum

Maria Helena Cruz de Carvalho [1, 2, 3], **Hai-Xi Sun** [2], **Chris Bowler** [1], **Nam-Hai Chua** [2]

[1] IBENS, Paris, France
[2] Rockefeller University, New York, United States
[3] UPEC, Paris, France

With the advent of next generation sequencing large amounts of transcripts have been characterized as long non-protein coding RNAs (lncRNAs). These transcripts have been longtime ignored and considered to be mere transcriptional noise but are now gaining momentum as key regulators of gene expression. Based on their relationship with protein coding genes, lncRNAs can be called intergenic (lincRNAs) when they are expressed between coding genes, intronic (incRNAs) when they are expressed from introns, or natural antisense transcripts (NATs) when they are expressed from the opposite strand of their cognate sense genes. In the model pennate diatom, *Phaeodactylum tricornutum*, we have previously reported the occurrence of 1,510 lincRNAs, many of which being specifically regulated by phosphate (Pi) or nitrogen (N) deprivation (Cruz de Carvalho et al., 2016) or by high pCO2 (Huang et al., 2019), thereby suggesting function. Using strand specific RNA-sequencing we now expand the portfolio of *P. tricornutum*’s lncRNAs by identifying 3,280 novel lncNATs. Furthermore we investigated the expression profiles of these new lncNATs and found that amongst the 1,742 that were -Pi stress regulated, 130 formed sense-antisense lncNAT-mRNA pairs (NAT pairs), the majority of which were concordant NAT pairs (105) and only a small fraction were discordant (25). These results suggest a new level of gene regulation whereby lncNAT positively regulates the expression of their cognate sense protein coding genes. Functional studies are now underway to validate the occurrence of such positive regulation.

41 Insights into the photoprotective role of LHCX1 protein in Phaeodactylum tricornutum

Vasco Giovagnetti, Mahendra Shukla, Alexander Ruban

Queen Mary University of London, School of Biological and Chemical Sciences, Department of Biochemistry, London, United Kingdom

Photosynthetic organisms must effectively regulate the absorption of light to prevent photodamage. Among the short-term regulations activated to achieve such a task, secondary red plastid-containing diatoms mainly induce a process measured as non-photochemical quenching of

chlorophyll fluorescence (NPQ). The so-called energy-dependent quenching, qE, represents the major flexible component of NPQ that grants rapid and reversible regulation of light harvesting. qE is feedback-modulated by the acidification of the thylakoid lumen, which triggers the activity of xanthophyll cycle and LHCX proteins in diatoms. The genome of the pennate diatom *Phaeodactylum tricornutum* encodes four LHCX isoforms (LHCX1-4) of which LHCX1 appears to constitutively control qE. Nonetheless, LHCX1 function in the mechanism of flexible quenching remains poorly understood. We grew *P. tricornutum* cells under continuous and intermittent light conditions to modulate the extent of qE in vivo and pinpoint the function of LHCX1 in qE. LHCX1 was isolated, and characterised biochemically and spectroscopically to investigate if it is directly or rather indirectly involved in the formation of the quencher(s) in the photosynthetic antenna of diatoms. Functional comparisons are drawn between LHCX1 and the LHCX orthologous light-harvesting complex stress-related (LHCSR) proteins, as well as the photosystem II subunit S (PsbS) protein, which are essential for qE in green algae and plants, respectively. Molecular and evolutionary implications are discussed in the context of the current understanding of qE mechanism.

42 Manipulating the macromolecular composition of diatoms

Hannah Connabeer [1], Thomas Bibby [1], Raffael Jovine [2]

[1] University of Southampton, Southampton, United Kingdom
[2] FeedAlgae, London, United Kingdom

Diatoms have vast potential for biotechnological research. Their high growth rates, high lipid content, large cell size and diversity of naturally synthesised bioactive compounds make them attractive for a number of applications. Despite this, there are barriers that prevent their biotechnological development from a 'proof-of-concept' model to a commercial-scale production system. Despite impressive recent progress, many diatom species lack the genetic tools required to make organisms viable for industrial applications and issues remain related to GM technology. A key question therefore is whether an organism's physiology and macromolecular composition can be manipulated in a targeted way by using external stimuli. There is convincing evidence that limiting nitrogen causes changes in the diatom's molecular composition, particularly lipid content. Here we identify the plasticity of macromolecular composition of diatoms over a range of different growth conditions including nitrate, phosphate, silicate limitation. We also assess whether these changes can be reproduced in a commercial setting on an economically viable scale. This research will help to clarify how diatoms respond to environmental stress at a molecular level and will increase our understanding of both their environmental and biotechnological roles.

43 Phylogenetic and ecophysiological diversity within the Cylindrotheca closterium species complex

Sien Audoor [1], Willem Stock [1], Darja Belišová [1], Katerina Pargana [1], Bart Vanelslander [1], Sofie D'Hondt [1], Ulf Karsten [2], Koen Sabbe [1], Wim Vyverman [1]

[1] Research Group of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Gent, Belgium
[2] Institute of Biological Sciences, Applied Ecology and Phycology, University of Rostock, Rostock, Germany

Similar to many other diatoms, the cosmopolitan raphid pennate diatom known as Cylindrotheca closterium comprises of a species complex: a group of close but genetically distinct species with similar or identical morphologies. Using a collection of strains isolated from tropical to polar coastal habitats, we show the presence of multiple clades using phylogenetic analyses of 5 different markers: ITS, LSU, psbA, cox1 and rbcL. Clades differ markedly in their ecophysiology and show

distinct growth response patterns in terms of optimum growth temperature and thermal niche width. Strains from the same clade displayed a similar thermal response, suggesting niche conservation between closely-related strains (or species). Furthermore, we show that C. closterium strains harbor different associated bacteria, and differ in their response to specific bacterial taxa as well as in their capability to use organic substrates for growth. This illustrates that even between closely-related strains (or putative species), niches can be remarkably dissimilar. Incomplete reproductive barriers suggest ongoing speciation in this complex.

44 Thylakoid Membrane Architectural Proteins

Adrien Thurotte, Claudia Büchel

Institute of Molecular Biosciences, Goethe University, Frankfurt, Germany

Photosynthesis is performed by complex protein assemblies, which are located in a dedicated lipid membrane - the thylakoid membrane (TM). This specialised membrane has evolved in a primordial cyanobacterium and has been conserved from cyanobacteria to higher plant chloroplasts. However, despite recent progresses the architectural proteins shaping, remodelling and eventually protecting the TMs are poorly known. In particular, in Diatoms the determinants responsible for the organisation of the TMs as three parallel stacks and their remodelling (e.g. multistacks formed upon red light) is totally unknown. I focus on TM architectural proteins in diatoms with a focus on highly conserved proteins that shapes the TM, addressing whether the sequence divergence could explain the different TM architecture. After sequence conservation and checking on expression evidence at the mRNA-level, I selected 4 gene candidates for a topdown approach in *Phaeodactylum tricornutum*. On this conference, I will sum up the reasons for choosing these proteins, present my first results and the next steps potentially leading to the identification of new architectural proteins. Among my proteins of interest are: (1) the TM-associated IM30/VIPP1 protein that possesses membrane-shaping capability (2) A predicted beta-carotene isomerase that has several cysteines, potentially pH-regulated (3) a vesicle-coating protein that possess a GTPase activity (4) the Fluctuating-Light-Acclimation Protein1 potentially involved in the control of proton homeostasis. CURT genes are not on the list since no homologs exist in diatoms.

45 Exploring red and far red light sensing in the oceans: the study of diatom phytochromes

Carole Duchene, Jean-Pierre Bouly, Angela Falciatore, Marianne Jaubert

Sorbonne Université, CNRS, Institut de Biologie Physico-Chimique, IBPC, Paris, France

Light is an essential source of energy and information for photosynthetic organisms. In the marine environment, the light field is structured by depth, as red and far-red light are quickly attenuated while blue and green penetrate deeper in the water column. Accordingly, diatoms possess a wide array of blue and green photodetectors (cryptochromes, aureochromes, rhodopsins). More surprisingly, diatoms also possess red/far-red phytochrome photoreceptors, which regulate gene expression in response to far red light (750nm) in the model diatom *P. tricornutum*. This finding brings up questions about the functions and modes of actions of diatom phytochromes (DPHY) in the oceans. To address these questions, we have generated *P. tricornutum* transgenic reporter lines, expressing YFP under the control of a DPHY-regulated gene promoter. By measuring the YFP signal of cells grown in different conditions (light colors, intensities and red/far-red ratios, cell concentration, etc.) we have been able to follow the DPHY activity in vivo, and to start characterizing its photochemical properties (sensitivity, reversibility), giving hints at the conditions in which it can be active in the marine environment. In parallel, we opened the questions of far-red sensing by studying the occurrence of DPHY genes in the meta-omics Tara oceans data. We showed that although not all diatoms possess phytochromes, phytochrome-containing diatoms are present in the seas all around the world.

46 Sulfur metabolism in the model diatom Phaeodactylum tricornutum

Daniel Pousa Kurpan Nogueira [1], Peter Kroth [2], Mario Giordano [1]

[1] Università Politecnica delle Marche, Ancona, Italy
[2] Konstanz University, Konstanz, Germany

Sulfate reduction is often assumed to be conserved in all photosynthetic organisms. The available information, however, is mostly limited to vascular plants, with insufficient information on other clades. Some peculiarity appears to exist in algal sulfur metabolism. For instance, different modes of regulation have been identified in assimilatory ATP sulfurylase (ATP-S), which in oceanic cyanobacteria and eukaryotic algae (except dinoflagellates) appears to be redox regulated; but the same is not true in plants, freshwater and coastal cyanobacteria and, possibly, in dinoflaglellates. The role of different isoforms of ATP-S is unclear. The level of redundancy of ATP-S or the allocation of specific isoforms to either sulfate reduction (primary S metabolism), or sulfation (secondary S metabolism) needs to be clarified. In our study, we used the marine diatom *Phaeodactylum tricornutum* as a model organism. This model diatom possesses only two isoforms of ATP-S, which simplifies the investigation (other organisms have multiple isoforms). We hypothesize that the plastidial isoform is mainly involved in sulfate reduction, whilst the other (in the cytosol) intervene in the sulfation pathway. The aim of this study is to confirm ATP-S isoforms' localization in *P. tricornutum* using Green Fluorescent Protein (GFP) insertions and generate and characterize ATP-S knockout cell lines for both isoforms separately using the Transcription Activator-Like Effector Nucleases (TALEN) technique. The results are discussed in the frame of the allocation of S, carbon and reducing power within the cell.

47 Identification of novel regulators of diatom photosynthesis by Transcription Factor-based genetic engineering

Alessandro Manzotti [1, 2], Soizic Cheminant-Navarro [1, 2], Antonio Emidio Fortunato [2], Andrés Ritter [2], Alessandra Bellan [3], Tomas Morosinotto [3], Jean-Pierre Bouly [1, 2], Angela Falciatore [1, 2]

[1] Université Sorbonne, CNRS, Institut de Biologie Physico-Chimique (IBPC), UMR 7141, Laboratory of chloroplast biology and light sensing in Microalgae, Paris F75005, France
[2] Sorbonne Université, CNRS, Institut de Biologie Paris-Seine, IBPS, UMR 7238, Laboratory of Computational and Quantitative Biology, Paris F75005, France
[3] Università di Padova, Dipartimento di Biologia, Laboratory of Photosynthesis and plant biotechnology, Padova 35121, Italy

Accounting for more than 40% of marine primary production, diatoms represent one of the most prominent phytoplankton groups and a major actor for global carbon cycle. Despite this ecological relevance, the knowledge on the regulation of diatom photosynthesis and on the genetic control of its plasticity is still very limited. To identify the unknown molecular regulators underlying these processes, we applied a large-scale genetic engineering approach to the diatom model species *Phaeodactylum tricornutum*. We first generated a collection of transcription factor knock-down transgenic lines via by RNA interference. Phenotyping by non-invasive imaging techniques based on chlorophyll a fluorescence was then used to find strains with photophysiological alterations by monitoring photosynthetic responses. An initial selection of 300 clones with deregulation of the bHLH-containing transcription factor family was tested, leading to the identification of the bHLH/PAS protein bHLH2 as a regulator of diatom chloroplast activity. In particular, these lines showed a reduction in non-photochemical quenching and maximal quantum yield of PSII, but unaltered growth, compared to the Wt. bHLH2 is also the first example of a bHLH/PAS transcription factor characterized as a modulator of photosynthesis. This protein will represent an entry point for the dissection of the gene regulatory network controlling the response to light and chloroplast physiology in diatoms. The results obtained prove that this collection of mutants and this integrated

functional genomics approach for non-invasive, large-scale phenotyping can be also exploited to select novel diatom strains showing traits of interest for biotechnological applications.

48 Cylindrotheca closterium - a new pennate model diatom for life cycle studies using forward and reverse genetics

Darja Belišová [1], Sien Audoor [1], Aikaterini Pargana [1], Petra Bulankova [2], Peter Chaerle [1], Nicole Poulsen [3], Lieven De Veylder [2], Wim Vyverman [1]

[1] Research Group Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium
[2] VIB-UGent Center for Plant Systems Biology, Ghent, Belgium
[3] B-CUBE, Technische Universität Dresden, Dresden, Germany

Despite rapid progress in the development of molecular tools to study diatom biology using reverse genetics techniques, the development of forward genetics approaches has been lagging behind. This is largely due to the lack of suitable diatom model species in which sexual reproduction can be controlled. Here we introduce Cylindrotheca closterium as a promising candidate for both, reverse and forward genetics studies. C. closterium is a raphid pennate diatom, globally distributed and frequently abundant in coastal habitats and inland saline waters. It has a well-described heterothallic sexual reproduction comprising two mating types, it is easy to cryopreserve, has a high growth rate and can be routinely cultivated in large volumes. Importantly, we have developed a protocol to experimentally manipulate its cell size and are thus able to shorten sexual generation time from 6 to less than 3 months. Genome assembly is in progress, with the genome size estimated to be 75 Mb. Furthermore, we have successfully established a transformation protocol for C. closterium and were able to cross two sexually compatible C. closterium strains with two different marker genes (YFP/CFP), resulting in double mutant progeny.

49 Function of thylakoidal anion transporters in the marine diatoms

Ryosuke Amano, Kansei Yamagishi, Yoshinori Tsuji, Yusuke Matsuda

Department of Bioscience,Kwansei Gakuin University, Sanda, Japan

Marine diatoms have the biophysical CO2-concentration mechanism (CCM) by which dissolved inorganic carbon is actively taken up and [CO2] at the vicinity of RubisCO is elevated. HCO3- transporters and carbonic anhydrases (CA) were critical in CCM. In the previous study, θ-CA were localized specifically in the lumen of the pyrenoid-penetrating thylakoid. We hypothesized that HCO3- was transported into the thylakoid lumen by unidentified transporter and converted into CO2 by θ-CA, yielding high [CO2] around RubisCO. In addition to CO2 source, the supplied HCO3- could regulate the proton motive force by dissipating $\Delta\Psi$ across the thylakoid membrane. These functions may contradict each other with regard to the formation of ΔpH if they occur in the same space since the former would diminish the ΔpH but the latter enhances it. Thus import of HCO3- into the thylakoid lumen must require fine spatial and conditional regulations. To investigate functional cooperation between CCM and photosystems, we analyzed HCO3- transporter candidates (Ptbest1, 2 and Tpbest1, 2) in *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. GFP tagged Ptbests were localized on the thylakoid membrane throughout the chloroplast and Tpbest2 in the central part of the chloroplast. In *P. tricornutum*, NPQ decreased in overexpressing mutant of Ptbests relative to that of WT. In contrast, NPQ increased in the overexpression mutant of Tpbest2 in *T. pseudonana*. These results suggest that these candidates are tightly related to the function of photosystems but function differs depending on their location relative to the pyrenoid structure.

50 Mapping Phaeodactylum tricornutum's terpenoid metabolism provides new insights into the evolution of steroid biosynthesis in eukaryotes

Jacob Pollier [1, 2], Emmelien Vancaester [1, 2], Unnikrishnan Kuzhiumparambil [3], Claudia Vickers [4, 5], Jestin George [3], Peter Ralph [3], Klaas Vandepoele [1, 2], Alain Goossens [1, 2], Michele Fabris [3, 4]

[1] Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium
[2] VIB Center for Plant Systems Biology, Ghent, Belgium
[3] Climate Change Cluster, University of Technology Sydney, Ultimo, Australia
[4] CSIRO Synthetic Biology Future Science Platform, Brisbane, Australia
[5] Australian Institute for Bioengineering and Nanotechnology, University of Queensland, St Lucia, Australia

Terpenoids are diverse class of metabolites that have primary biological roles and global ecological relevance, as they include essential cellular components, signalling and defence molecules. Many terpene-based compounds have commercial applications as fragrances, biofuels, nutraceuticals and pharmaceuticals, often industrially sourced from engineered microbes. Diatoms are attractive novel candidates for the heterologous production of terpene-based products, with marked differences from current microbial platforms. However, while the metabolism of terpenoids is well characterised in model organisms, this is largely uncharted in diatoms. To explore the suitability of *Phaeodactylum tricornutum* as synthetic biology-based terpenoid production platform, we mapped the main metabolic hubs, such as prenylphosphates and squalene biosynthesis. The former are building blocks of all terpenoids, whereas squalene is the universal precursor of steroids, which serve essential roles as membrane components and signalling molecules. The flavoprotein squalene epoxidase (SQE) catalyses the first oxygenation reaction in steroid biosynthesis and is key in eukaryote evolution. Despite its conservation in animals, plants, and fungi, diatoms lack an SQE-encoding gene. Here, we report the discovery of an alternative SQE (AltSQE), through the screening of a *P. tricornutum* gene library in an SQE-deficient yeast. AltSQE is unrelated to SQE, surprisingly widespread in many eukaryotic lineages with a patchy distribution within monophyletic clades, and mutually exclusive with SQE. Our discovery raises questions on eukaryote metabolic evolution and provides new elements to advance the knowledge on key metabolic hubs of *P. tricornutum*, central to the design of synthetic biology strategies for the heterologous production of high-value terpenoids in diatoms

51 Using synthetic biology and microfluidics to engineer lipid metabolism in marine algae

Katrin Geisler [1], Ziyi Yu [2], Chris Abell [2], Alison G. Smith [1]

[1] Dept of Plant Sciences, University of Cambridge, Cambridge, United Kingdom
[2] Dept of Chemistry, University of Cambridge, Cambridge, United Kingdom

Marine microalgae like the diatom *Phaeodactylum tricornutum* are exciting biotechnological chassis as they can grow to high density on non-potable water sources. The availability of genomic resources and the ability to accumulate naturally large amounts of lipids in form of triacylglycerols (TAGs) make *P. tricornutum* an interesting host to produce novel fatty acids or valuable oils for food and feed. Over the last few years, we and others have developed a range of established and novel DNA parts (constitutive and inducible promoters, 5'UTRs, 3'UTRs and target peptides) for *P. tricornutum* based on the MoClo syntax and Golden Gate assembly. A similar toolkit has been recently established for the green algae Chlamydomonas reinhardtii (Crozet et al., 2018). Using these DNA parts, we have assembled molecular devices to manipulate the lipid metabolism in *P. tricornutum*. We will present data on the overexpression of diacylglycerol acyltransferases (DGATs) and phospholipid: diacylglycerol acyltransferase (PDAT), enzymes involved in TAG synthesis. In addition, we are combining metabolic engineering with state-of-the-art microfluidic devices for the encapsulation and growth of wildtype and transformed microalgae cells in microdroplets, allowing

the analysis of single cells and the screening of them in a high-throughput manner. The sorting technology can be used to screen for highly expressing lines, and to detect strains within a mutagenized population with desirable characteristics. Ref.: Crozet P, et al (2018) Birth of a Photosynthetic Chassis: A MoClo Toolkit Enabling Synthetic Biology in the Microalga Chlamydomonas reinhardtii. ACS Synth Biol 7: 2074–2086

52 Genome-editing targeting θ-type carbonic anhydrase by CRISPR/Cas9 Nickase (D10A) in the marine diatom Thalassiosira pseudonana

Hermanus Nawaly, Yoshinori Tsuji, Yusuke Matsuda

Department of Bioscience, School of Science and Technology, Kwansei Gakuin University, Sanda, Japan

Genome editing technology with CRISPR/Cas9 and TALEN is rapidly progressing in the research of marine diatoms, allowing deeper understandings of diatom physiologies. In our previous studies, it was demonstrated that biophysical CO2 Concentration Mechanism (CCM) in marine diatoms requires bicarbonate transporters and carbonic anhydrases (CAs). Diatoms possess several types of CAs and the newest subclass, θ-type CA has been identified and one them was localized in the pyrenod-penetrating thylakoid lumen of *Phaeodactylum tricornutum*. The function of this new CA was shown to be vital for autotrophic growth and the CCM in *P. tricornutum*, strongly suggesting the functional corporation between the photosystems and the biophysical CCM. Its orthologous factors also occur in *Thalassiosira pseudonana* (TpθCA1, TpθCA2, TpθCA3, and TpθCA4). In this study, the Cas9 nickase (D10A) system was constructed and applied to edit Tpθca3, which was targeted by a pair of single guide RNA to form double nick at both strand of the target DNA sequence in *T. pseudonana*. The first screened nourseothricin resistant (NATR) colonies tend to comprise of various mutant genotypes. By three additional repeat of NATR screening, monoclonal mutant colonies carrying biallelic mutations were obtained. Three selected monoclonal mutant lines possessed a range of deletions of 20-54 bp or insertion sized at 150 bp precisely at the target site. This indicates a first example of a successful application of Cas9 nickase for targetable genome editing in the marine diatom, *T. pseudonana*.

53 Correlative single-molecule localization and electron microscopy of biosilica forming proteins

Adeeba Fathima, Andre Ohara, Nicole Poulsen, Nils Kröger, Michael Schlierf

B CUBE – Center for Molecular Bioengineering, TU Dresden, Dresden, Germany

Diatom cell wall morphogenesis is presumably a self-assembly process by several biomolecules. We pursue advanced microscopy techniques to map distribution of biosilica associated proteins in *Thalassiosira pseudonana*. We show for the first time discernible patterns in both frustule and the organic matrix using correlative single-molecule localization fluorescence and transmission electron microscopy (sCLEM). We dissolve the biosilica cell walls on a carbon-coated TEM grid and subsequently localize the distribution of proteins of the Cingulin-family in the organic matrix using Stochastic Optical Reconstruction Microscopy (STORM). After a complete localization, we obtain a corresponding transmission electron microscopy image which is aligned with fiducial markers. While electron microscopy allows nanometer resolution of the isolated protein matrix, it does not allow protein specific labelling. STORM allows sub-diffraction imaging of a specific fluorescently labelled protein, thereby allowing to localize proteins of interest within the protein matrix. Identifying the patterns formed by different proteins and their correspondence to the physical structure of the organic matrix and biosilica by sCLEM images sheds light on their roles during the self-assembly process and during the formation of different morphological features of the diatom biosilica.

54 Single-cell heterogeneity in response to oxidative stress reveals pathways involved in death and survival in diatoms

Avia Mizrachi [1], Shiri Graff van Creveld [1], Chuan Ku [1, 2], Orr H. Shapiro [3], Shilo Rosenwasser [4], Assaf Vardi [1]

[1] Weizmann Institute of Science, Rehovot, Israel
[2] Academia Sinica, Taipei, Taiwan
[3] Agricultural Research Organization, Rishon LeZion, Israel
[4] The Hebrew University of Jerusalem, Rehovot, Israel

Diatoms are photosynthetic microorganisms of great ecological and biogeochemical importance. Nevertheless, the cellular strategies that underline their ecological success are still underexplored. Current understanding of phytoplankton acclimation to stress is based on population-level analysis, masking cell-to-cell variability. Here we investigated heterogeneity within isogenic diatom populations in response to oxidative stress, which mediates a wide range of environmental stress conditions. We combined flow cytometry and a microfluidics system for live-imaging microscopy to measure redox dynamics at the single-cell level. Using the redox-sensitive sensor roGFP, we measured in vivo oxidation patterns in the model diatom *Phaeodactylum tricornutum*. Chloroplast targeted roGFP exhibited a light-dependent, bi-stable oxidation pattern in response to H2O2 and high light, revealing two distinct subpopulations. The oxidized subpopulation was sensitive to the stress and subsequently died, while the reduced subpopulation survived. Oxidation of the chloroplast glutathione pool preceded commitment to cell death, and was used as a novel predictor of cell fate. Transcriptome analysis of subpopulations sorted based on chloroplast roGFP oxidation revealed genes involved in cell death and survival, which are currently under an ongoing investigation. These findings suggest that light intensity greatly affects the sensitivity of diatoms to other environmental stressors. We propose that phenotypic variability within diatom populations can provide an ecological strategy to cope with rapid environmental fluctuations in the marine ecosystem.

55 The transcriptional environmental stress response of a model diatom

Zhengke Li [1, 3], Andrew Irwin [2, 4], Zoe Finkel [1, 3]

[1] Department of Oceanography, Dalhousie University, Halifax, Canada
[2] Department of Mathematics and Statistics, Dalhousie University, Halifax, Canada
[3] Environmental Science Program, Mount Allison University, Sackville, Canada
[4] Department of Mathematics and Computer Science, Mount Allison University, Sackville, Canada

We investigate transcriptomic changes in a model diatom in search of universal responses to different stresses. Common environmental stress responses have been identified for bacteria and yeast. We grew *T. pseudonana* in ten different environmental conditions (N-, P-, Si-, Fe-, high and low temperature, high and low light, low pH, and H2O2) with four replicates per treatment and took RNA samples after 0, 2, 6, 24, and 72 h. We identify the genes and pathways with common patterns of differential expression across all ten treatments. Many other patterns in gene expression can be identified including (1) subsets of the stress treatments with common, contrasting or specific patterns of gene expression and (2) pathways with similar time courses of expression corresponding to different temporal patterns in the physiological strategies for acclimation and stress response.

56 DNA metabarcoding and morphological analyses of the diatom biofilm associated with loggerhead sea turtles in the Mediterranean Sea

Suncica Bosak [1], Hrvoje Visic [1], Klara Filek [1], Kaethe Robert [2, 3], Bart Van de Vijver [2, 3], Adriana Trotta [4], Aliki Panagopolou [5], Roksana Majewska [6, 7]

[1] Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, Zagreb, Croatia
[2] Research Department, Meise Botanic Garden, Nieuwelaan 38, Meise, Belgium
[3] Department of Biology - ECOBE, University of Antwerp, Universiteitsplein 1, Wilrijk, Belgium
[4] Department of Veterinary Medicine, University of Bari "Aldo Moro", Strada Provinciale per Casamassima km.3, Valenzano, BA, Italy
[5] ARCHELON, the Sea Turtle Protection Society of Greece, Solomou 57, Athens, Greece
[6] Unit for Environmental Sciences and Management, School of Biological Sciences, North-West University, Private Bag X6001, Potchefstroom, South Africa
[7] South African Institute for Aquatic Biodiversity (SAIAB), Private Bag 1015, Grahamstown, South Africa

The external surfaces of large marine vertebrates, including sea turtles, provide hard substrata suitable for the development of rich microbial photoautotrophic biofilms. Among microeukaryotes, diatoms constitute the main biofilm component, with densities sometimes exceeding those known from other marine substrata. While some of the sea turtle-associated diatom taxa seem to be exclusively epizoic, others may also be common in habitats occupied by turtles thriving on both biotic and abiotic hard surfaces of, for instance, seagrass blades or sand grains. Many aspects of sea turtle ecology and behavior remain unknown, and micro-epibionts can be used as indicators of their behavioral patterns or as proxies of the anthropogenic impact on coastal marine habitats where sea turtles reside. Notably, these micro-communities are largely unexplored and there is a need to assess their taxonomic composition. In recent years, a DNA metabarcoding approach using rbcL marker has been applied to characterize diatom communities in different habitats. However, the lack of a reliable DNA reference database represents a serious impediment to the exclusive usage of molecular tools for the taxonomic assessment and they should be complemented with microscopical analyses. In this study, we aimed to use both molecular and morphology-based approaches to provide a detailed description of diatom communities associated with Mediterranean loggerhead sea turtles. We present the results of the analyses of the carapace and skin biofilm samples from ten loggerhead turtles collected from distinct habitats in the Mediterranean Sea including Croatian and Italian coastal areas within the Adriatic Sea and Amvrakikos Gulf in Greece.

57 Insight into diatoms nitrate transporter family (NRT2s and NPFs): molecular evolution and functional characterization

Monia T Russo [1], Carmela Borzacchiello [1], Luigi Caputi [1], Greta Busseni [1], Benoit Lacombe [3], Antonella Longo [4], Maurizio Ribera D'Alcalà [1], Maurizio Chiurazzi [2], Maria I. Ferrante [1], Alessandra Rogato [1, 2]

[1] Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology, Villa Comunale, 80121, Naples, Italy
[2] Institute of Biosciences and BioResources, IBBR-CNR, Via P. Castellino 111, 80131, Naples, Italy
[3] Biochimie et Physiologie Moléculaire des Plantes UMR CNRS/INRA/SUPAGRO/UM Place Viala 34060, Montpellier, France
[4] Biodiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, United States

Diatoms thrive in highly variable environmental conditions suggesting the presence of sophisticated mechanisms to perceive and adapt to them. Among the others, they have to cope with temporal and spatial variability of nitrogen (N) availability, an essential element for their survival and growth.

The molecular mechanisms allowing diatoms to efficiently cope with that variability remain largely unknown. The low and high affinity nitrate family members (NPF/NRT2) are expected to play a key role in this, because NO3− represents the major N source in the ocean. Our analyses showed that the presence of multiple, differentially regulated, NO3− (NRT2s/NPFs) transporter genes, is a conserved feature of diatom species (Rogato et al., 2015) and we provide now a complete phylogenetic assessment (Busseni et al.). *Phaeodactylum tricornutum* genome encodes for two NPFs and six NRT2s genes. Structural alignment to the solved crystal structures of homologous NRT2 and NPF transporters from bacteria and plants allowed us to identify key amino acid residues involved in substrate binding and transport. In order to investigate the functional role of each gene we are taking advantage of recent advances in diatom genomics and genetics, to generate loss of function mutants using the CRISPR/Cas9 system coupled with proteolistic (Serif et al., 2018). To characterize the transport capacities and substrate specificity of the genes we expressed NRT2s/NPFs in Xenopus laevis oocytes. Our preliminary studies provide a springboard to shed light on the evolutionary advantage of these N transporters and on the N metabolism in diatoms.

58 A polyphasic approach for identification of epibiotic diatoms associated with loggerhead sea turtles in the Adriatic Sea

Klara Filek [1], Lucija Kanjer [1], Antonija Matek [1], Adriana Trotta [2], Roksana Majewska [3, 4], Matt P. Ashworth [5], Bart Van de Vijver [6, 7], Sunčica Bosak [1]

[1] Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, Zagreb, Croatia
[2] Department of Veterinary Medicine, University of Bari “Aldo Moro”, Strada Provinciale per Casamassima km.3, Valenzano, BA, Italy
[3] Unit for Environmental Sciences and Management, School of Biological Sciences, North-West University, Private Bag X6001, Potchefstroom, South Africa
[4] South African Institute for Aquatic Biodiversity (SAIAB), Private Bag 1015, Grahamstown, South Africa
[5] UTEX Culture Collection of Algae, University of Texas, Austin, United States
[6] Research Department, Meise Botanic Garden, Nieuwelaan 38, Meise, Belgium
[7] Department of Biology - ECOBE, University of Antwerp, Universiteitsplein 1, Wilrijk, Belgium

The loggerhead sea turtle is widely distributed throughout the world's oceans with a now-thriving population in the Mediterranean Sea. Loggerhead sea turtles seem to harbor a diverse assemblage of macro- and micro-epibionts living on their shells and skin. Recent studies focused on sea turtle-associated diatoms indicate that some of them may be exclusively epizoic or even sea turtle-specific as they have not yet been found on other immersed objects in the sea. New genera (e.g. Medlinella, Chelonicola, Poulinea) and species (e.g. Achnanthes elongata, Labellicula lecohuiana, Tursiocola denysii) have been found and described from several species of sea turtles from various geographical locations mainly through morphological studies whereas molecular information is lacking. Moreover, the exact composition of the diatom community associated with geographically isolated sea turtle populations, its change in time and space, the epibiotic diatom ecological function, and complex relationships between diatoms and other microbes within the sea turtle holobiont remain poorly understood. Here we show the results of implementing morphological and molecular phylogenetic approaches to gain more complete information about the loggerhead-associated diatom communities in the Adriatic Sea. By isolating living cells from loggerhead carapace and skin scrapings and growing them in monocultures we obtained enough material for both morphological (LM and SEM characterization) and molecular analyses (using molecular markers rbcL, psbC, and SSU). The isolated strains include Amphora, Navicula, Parlibellus, and Nitzschia species, possibly growing on sea turtles opportunistically, but also potential exclusively epizoic taxa belonging to genera such as Poulinea, Achnathes, Proschkinia, and Craspedostauros.

59 Knock out of a major lipid droplet protein in diatom by 2A peptide-combined Cas9 expression system

Kohei Yoneda [1], Masaki Yoshida [2], Makoto M. Watanabe [2], Yusuke Matsuda [1], Iwane Suzuki [2]

[1] School of Science and Technology, Kwansei Gakuin University, Sanda, Japan
[2] Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

Diatom *Phaeodactylum tricornutum* accumulates triacylglycerol in lipid droplets (LD). The authors previously identified a novel major LD protein in *P. tricornutum* and named as the Stramenopile-type LD protein; however its function is so far unclear. To characterize the function, we attempted to generate knockout mutant using the CRISPR-Cas9 system. Expressions of the Cas9 nuclease and an antibiotics resistant gene are independent in the general monocistronic vector system, and thus we cannot judge whether or not transformants express Cas9 nuclease through the antibiotics screening. To save screening efforts, we developed 2A peptide-combined bicistronic Cas9 expression vector. 2A peptide is a virus-derived short polypeptide whose C-terminus is automatically cleaved out during continuous ribosomal translation; two polypeptides can occur from a single mRNA. In the present study, we inserted the coding region of the bleomycin resistance gene (ble) combined with the 2A peptide gene sequence at the upstream of the Cas9 gene in the diatom Cas9 expression vector designed by Nymark et al. (2016). This construct was introduced into chromosomal DNA of *P. tricornutum* using electroporation. All 16 cell lines that were transformed using a linearized plasmid harbored the full DNA region of ble::2A::Cas9 and a single guide RNA expression cassettes. We confirmed that 13 out of 16 cell lines showed mosaicism at the sgRNA targeting region, namely the editing efficiency was ca. 81.25%. This 2A-combined Cas9 expression system could be an efficient tool to perform genome editing with a single vector that saves screening efforts with high ratio of positive cell line.

60 Mapping the possible fitness landscapes of marine diatoms

Sinead Collins [1], Jana Hinners [1], Nathan Walworth [2], Phoebe Argyle [3], Martina Doblin [3], Naomi Levine [2]

[1] University of Edinburgh, Edinburgh, United Kingdom
[2] University of Southern California, Los Angeles, United States
[3] University of Technology Sydney, Sydney, Australia

To understand how phytoplankton may adapt to environmental changes, it is important to understand the shape of the potential trait space that they can occupy. This can be visualized using a fitness landscape, in which some trait value combinations lead to a high fitness, and thus a fitness peak, and others lead to a lower fitness, and thus a valley. Experiments with freshwater model systems suggest that the number of trait correlations is limited, such that the directions of trait evolution in multidimensional space are limited. However, how this might translate into fitness landscapes for different phytoplankton functional groups is poorly understood. We aim to map how connected different fitness peaks are for Thalassiosira by using mutation accumulation experiments where we force the diatoms from their current fitness peaks into fitness valleys, and allow them to then evolve and potentially move around the fitness landscape. Since this is an ongoing project, I will mainly present the conceptual framework and preliminary results.

61 Diatom Permease, a Bioindicator for Ocean Acidification

Monica Orellana [1, 2], **Jacob Valenzuela** [2], **Allison Lee** [3], **Nitin Baliga** [2, 4]

[1] University of Washington, Polar Science Center, Seattle, United States
[2] Institute for Systems Biology, Seattle, United States
[3] Scripps Institution of Oceanography, La Jolla, United States
[4] University of Washington, Microbiology, Biology and Molecular Engineering Sciences, Seattle, United States

Rising atmospheric CO2 and the resulting acidification of the surface ocean impacts the structure of marine ecosystems. Diatoms may thrive under high CO2 conditions due to their "ecological resilience," or stability under stress. The centric diatom *Thalassiosira pseudonana* has been used as a model to investigate the effect of ocean acidification in controlled laboratory experiments. Mesocosms represent an intermediate experimental design between laboratory and natural conditions. In this study we used *T. pseudonana* grown in mesocosms to evaluate the physiological and transcriptional profiles of this diatom grown on a day: night cycle, at low (L) and high (H) CO2 (high and low pH respectively), and in transition from L to H-CO2 conditions. Analysis of the resulting genome-wide transcriptional data allowed verification under natural conditions of the reduced requirement of the carbon concentrating mechanisms (CCMs) under H-CO2. CCMs are very quickly downregulated when the cells transitioned from L-CO2 to H-CO2, saving an estimated 3-6% of the energy expended on carbon fixation. The experiments verified an increase of nighttime up-regulated putative permease (262258; discovered previously by Ashworth et al.,2013; Hennon et al., 2015; and Valenzuela et al., 2018) in chloroplasts under H-CO2 conditions. While the function of the permease is still unknown, the dynamics of changes in permease expression in diatoms serves as a bioindicator of elevated CO2 conditions in marine environments.

62 Investigating diatom viruses: extending single-cell laboratory techniques to the environment

Sarah Schwenck [1, 2], **Alice Lévesque** [2], **Michael Landry** [1], **Andrew Allen** [1, 2], **Lisa Zeigler Allen** [1, 2]

[1] Scripps Institution of Oceanography, UC San Diego, La Jolla, CA, United States
[2] J. Craig Venter Institute, La Jolla, CA, United States

Accounting for ~40% of global primary productivity, diatoms are key players in carbon uptake, export, and nutrient cycling. While historically, diatom mortality was thought to be primarily through zooplankton grazers, there is increasing evidence of diatom susceptibility to infection by RNA viruses. As viruses are estimated to be responsible for the turnover of more than a quarter of photosynthetically fixed carbon by cell lysis, diatom infection could have a significant role in the flux of carbon that is passed to higher trophic levels or exported to deeper waters versus carbon that is recycled via remineralization. Through a series of dilution experiments to investigate the role of virus-induced mortality in the California Current, phytoplankton virus influences were found to be strongest within upwelling waters of a coastal filament. In particular, the diatom Chaetoceros had a negative correlation with its virus and when viral predation pressure was reduced it became the predominant phytoplankton based on 18S rRNA. However, how viruses impact cellular response within a single host is not well known. To further investigate, we have developed a model system of the diatom Cylindrotheca and its associated +ssRNA virus. Preliminary experiments using single cell Drop-Seq technology have revealed significant upregulation of processes related to cell signaling and stress in infected cells relative to uninfected cells. These single cell techniques will be applied to environmental samples on the upcoming CCE-LTER process cruise in August to compare uninfected and infected diatoms within the natural environment, with a focus on Chaetoceros within upwelling waters.

63 Photosynthetic strategy of diatoms in the Ross Sea, Antarctica

Thomas Bibby [1], Mark Moore [1], Tommy Ryan-Keogh [2]

[1] University of Southampton Ocean and Earth Science National Oceanography Center, Southampton, Southampton, United Kingdom
[2] Southern Ocean Carbon and Climate Observatory CSIR - Natural Resources and the Environment, Cape Town, South Africa

The bioavailability of iron influences the distribution, biomass and productivity of phytoplankton in the Ross Sea, one of the most productive regions in the Southern Ocean. We mapped the spatial and temporal extent and severity of iron limitation of the native phytoplankton assemblage using long- (>24 h) and short-term (24 h) iron-addition experiments, along with physiological and molecular characterisations during a cruise to the Ross Sea. Phytoplankton increased their photosynthetic efficiency in response to iron addition, suggesting proximal iron limitation throughout most of the Ross Sea during summer. Molecular and physiological data further indicate that as nitrate is removed from the surface ocean the phytoplankton community transitions to one dominated by diatoms, which display an iron-efficient photosynthetic strategy characterised by an increase in the size of photosystem II (PSII) photochemical cross-section (σPSII) and a decrease in the chlorophyll-normalised PSII abundance. These results suggest that diatoms with the ability to reduce their photosynthetic iron requirements are selected as the growing season progresses, which may drive the well-documented progression from Phaeocystis antarctica assemblages to diatom-dominated phytoplankton. Such a shift in the assemblage-level photosynthetic strategy potentially mediates further drawdown of nitrate following the development of iron-deficient conditions in the Ross Sea

64 Lipid droplet degradation mechanism in oleaginous diatom Fistulifera solaris JPCC DA0580 suggested by lipid droplet proteome

Tomomi Nonoyama [1], Daisuke Nojima [1], Masayoshi Noda [1], Yoshiaki Maeda [1], Tomoko Yoshino [1], Mitsufumi Matsumoto [2], Chris Bowler [3], Tsuyoshi Tanaka [1]

[1] Institute of Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan
[2] Electric Power Development CO, Ltd., Kitakyushu, Japan
[3] Institute de Biologie de l'Ecole Normale Superieure, Paris, France

Enhancing lipid content in microalgae is one of the effective strategies to improve biofuel productivity for commercializing microalgal biofuel. Since lipids, mainly TAGs, are stored in the compartment called lipid droplet, elucidating lipid droplet dynamics can give us insights to elevate lipid content. In mammalian cells and higher plants, many regulator proteins have been found from the surface of lipid droplets. These lipid droplet-associated proteins regulate lipid droplet dynamics, i.e. size and timing of degradation. We firstly fractionated lipid droplets from oleaginous diatom Fistulifera solaris JPCC DA0580 for lipid droplet proteome. Next, the lipid droplet fraction was washed by several reagents, and we determined appropriate washing reagent. The washed fraction was analyzed by nanoLC-MS/MS in order to investigate lipid droplet-associated proteins. As a result, some membrane trafficking proteins which were related to autophagy process were detected. Subsequently, the effect of these membrane trafficking proteins on lipid droplet dynamics was investigated based on inhibitor assay. The inhibitors of membrane trafficking proteins suppressed reduction of lipid droplets volume, suggesting these autophagy-related proteins may be involved in lipid droplet degradation. The autophagic lipid droplets degradation has been reported in yeasts, and a vacuole participates in that process. In F. solaris, electron microscopy suggested that lipid droplets locate nearby the vacuoles. Taken together, autophagy process may be related to lipid droplets degradation in F. solaris.

65 Improvement of lipid productivity in oleaginous diatom Fistulifera solaris JPCC DA0580 by knocking-down of triacylglycerol lipase

Kahori Watanabe [1], Daisuke Nojima [1], Yoshiaki Maeda [1], Tomoko Yoshino [1], Mitsufumi Matumoto [2], Tsuyoshi Tanaka [1]

[1] Institute of Engineering Tokyo University of Agriculture and Technology, Tokyo, Japan
[2] Electric Power Development CO, Ltd, Kitakyusyu, Japan

Microalgal biofuel production has been studied as alternative resource to fossil fuels because of their high lipid productivity. We have investigated an oleaginous diatom, Fistulifera solaris JPCC DA0580, which accumulates high content of lipids up to 65%, towards biofuel production. However, further improvements of lipid productivity are necessary for commercialization. Genetic modification is one of the promising methods for the enhancement of lipid productivity. In particular, inhibition of lipid degradation by triacylglycerol (TAG) lipases could be an effective strategy. In this study, we identified the candidate genes of TAG lipases of F. solaris using whole genome and transcriptome data. Interestingly, we found that putative lipase genes termed Tgl1 were highly up-regulated during lipid degradation in F. solaris. Further investigation was carried out by knocking-down of the identified genes with expression of anti-sense fragments. As a result, the knock-down mutants formed lipid droplets with significantly larger volume than those of wild type under a lipolytic condition. Thin layer chromatography revealed that the TAG contents of the mutant was 2.8 times higher than that of wild type at a maximum. These results suggest that Tgl1 is responsible for lipid degradation in F. solaris under a lipolytic condition. Our study provides new insights into lipid degradation mechanism in diatoms, and could be applied for enhanced biofuel production.

66 The Role of NADPH Oxidase in Diatom ROS Signalling

Jack Dickenson [1, 2], Glen Wheeler [1], Colin Brownlee [1, 2]

[1] Marine Biological Association of the UK, Plymouth, United Kingdom
[2] University of Southampton, Southampton, United Kingdom

Tight control of cellular redox state is very important for healthy cell homeostasis. Imbalance in the production or removal of reactive oxygen species (ROS) can cause oxidative stress damage to cells. However, controlled production of ROS can be harnessed by cells for signalling or other aspects of metabolism. NADPH Oxidase (NOX) proteins function primarily to produce extracellular ROS. Intracellular electrons are pumped across the cells plasma membrane and combine with extracellular oxygen to form ROS. ROS production by NOX proteins has a well-characterised role in defence in mammalian cells. However, NOX proteins are diverse in structure, being present in plants, algae, fungi and bacteria, and likely produce extracellular ROS for functions other than defence. Understanding of the roles of NOX proteins outside of mammalian and plant cells remains limited. Our work has focused upon characterising the roles of NOX proteins in diatoms. Diatoms have a greater diversity of NOX proteins than any other group, suggesting an interesting evolution of NOX proteins. We have characterised the role of NOX proteins in the model diatom *Phaeodactylum tricornutum* using a range of physiological and molecular techniques. Inhibition of diatom NOX proteins using diphenyleneiodonium chloride (DPI) influences photosynthetic output and leads to changes in cellular redox status. These findings suggest that diatom NOX proteins are important for maintaining healthy cell physiology in diatoms and that they may perform different roles compared to NOX proteins present in other organisms, adding to the diversity of functions for NOX derived ROS.

67 Suppression of grazers of the marine diatom Fistulifera solaris by enhancement of chlorophyllase activity

Issei Terauchi [1], Yuichiro Kashiyama [2], Yuki Ishizuka [1], Yoshiaki Maeda [1], Tomoko Yoshino [1], Mitsufumi Matsumoto [3], Tsuyoshi Tanaka [1]

[1] Institute of Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan
[2] Graduate School of Engineering, Fukui University of Technology, Fukui, Japan
[3] Electric Power Development CO, Ltd., Kitakyushu, Japan

Commercialization of useful compounds derived from microalgae has been intensively attempted around the world. However, undesired contamination of outdoor cultures of microalgae by zooplanktonic grazers is a serious issue for a stable biomass production. For example, amoebae often contaminated outdoor mass cultures of the marine diatom Fistulifera solaris JPCC DA0580, which is a promising biofuel producer. While conventional approaches against the contamination such as chemicals and/or filtrations are expensive and energy-intensive, an approach based on genetic manipulation on algal producers would be an alternative strategy; thus, we aimed to establish a technique to suppress grazers of F. solaris by genetic manipulation. Recently, it was suggested the chlorophyllase (CLH), which converts chlorophylls into phototoxic chlorophyllides, is responsible for a defensive function in higher plants to avoid the grazers. To mimic this defensive function, a CLH derived from Arabidopsis thaliana was overexpressed in F. solaris, and mutants with elevated CLH activity were obtained. Subsequently, we examined whether the mutants exhibit steady growth by suppressing the grazing activity of an amoeba that was isolated from an outdoor culture of F. solaris. As a result, the growth inhibition on F. solaris in presence of the amoeba was significantly mitigated, suggesting that the overexpression of CLH suppressed grazing by the amoeba. This is the first study on suppression of grazers based on genetic modification in eukaryotic microalgae. Further investigation on phototoxicity of chlorophyllide will provide mechanistic insights into the defense mechanism conferred by the elevated CLH activity, and will contribute to stable outdoor cultivation.

68 Variation in physiological and molecular responses to ocean acidification among strains of a model diatom

Ruiping Huang [1], Jiazhen Sun [1], Chris Bowler [2], Xin Lin [1], Kunshan Gao [1]

[1] State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen, China
[2] Institut de Biologie de l'École Normale Supérieure (IBENS), Département de Biologie, Ecole Normale Supérieure, CNRS UMR8197, Inserm U1024, PSL Research University, Ecology and Evolutionary Biology Section, Paris, France

Diatoms as major primary producers have been well documented on their responses to ocean acidification (OA), however little is known about their strain level responses. Here, we selected 4 strains of the model diatom *Phaeodactylum tricornutum* isolated from different regions of global oceans, representing all genotypes based on ITS2 sequences, to investigate strain-specific responses to OA. Our results showed that responses of some carbon metabolism to HC varied among strains, although no significant differences in responses to HC treatment in terms of the growth rate and pigment contents. The expression of plasma membrane bicarbonate transporters were downregulated in Pt4 in response to HC, reflecting that potential decrease in active HCO3- uptake reduced efficiency of CO2 concentrating mechanisms (CCMs), which was contrasting different from other strains. Reduction of CCMs efficiency was also indicated by the downregulated expression of carbonic anhydrase PtCA1 catalyzing conversion of HCO3- to CO2 for carboxylation in the chloroplast. Whereas PtCA2 and Pt43233 displaying the same roles with PtCA1were

differentially regulated among these strains. On the other hand, C4-like metabolism appeared to dissipate excess energy and re-distribute carbon flux to gluconeogenesis in Pt1 or lipid synthesis in Pt8 and Pt11, rather than participating in net CO2 fixation under HC. These variations in responses to HC were not phylogenetically related, implying that living environment may drive distinct strategies in response to OA. Our study suggested that strain-specific response should be considered to better understand effects of climate change on marine phytoplankton at a global scale.

69 Following Phaeodactylum tricornutum cell fate during nitrogen limitation and light/dark cycles by orchestrated "omics" analysis.

Sarah D'Adamo [1], Ilse Remmers [1], Packo Lamers [1], Rene Wijffels [1, 2], et al.

[1] Bioprocess Engineering, Wageningen University and Research, Wageningen, Netherlands
[2] Biosciences and Aquaculture, Nord University, Bodø, Norway

Phaeodactylum tricornutum is considered as a model diatom for physiology, biochemistry and genomics studies. These microalgae found industrial space for aquaculture and nutraceutical production, and their successful engineering opens novel opportunities for their exploitation as microbial chassis for industrial biotechnology[1,2]. However, more efforts are necessary to understand their metabolism and find key factors for process optimization. In order to obtain a full and reliable picture of cellular metabolism, we used highly-controlled photobioreactors and steady state growth to comprehensively profile the physiology, transcriptome, proteome and metabolome during Light:Dark cycle and both nitrogen (N) limited and replete levels[3]. During N limitation, neutral lipid content increased from 4% to 16% of dry weight and total sugars almost doubled, at the expenses of growth, photosynthetic efficiency, protein and membrane lipid content. Interesting, cells growing in turbidostat-mode showed a clear peak in culture dilution rate, equivalent to specific growth rate, around mid-day (i.e. 6-10h) for both N-replete and limited conditions; this suggests that circadian clock was unaffected by N-limitation. Intriguingly, we found few harvesting associated proteins (LHCR 6-8-10 and LHCX4) upregulated even in the dark period. N-assimilation and central carbon metabolism were also upregulated, with mitochondrial genes for C4 assimilation and TCA cycle being induced. Overall these results suggest that cells are re-cycling existing intracellular components and that mitochondrion is highly engaged in this process.

1. D'Adamo S, et al. Plant Biotechnol J. 2018 doi:10.1111/pbi.12948
2. Kroth PG, et al. Plant Cell Rep. 2018; doi:10.1007/s00299-018-2334-1
3. Remmers IM, et al. Algal Res. 2018; doi:10.1016/j.algal.2018.08.012

70 Phosphate uptake by SLC-type transporters in marine diatoms

Kanako Maeda, Nanae Kimura, Yohei Fukuchi, Toshiki Sugiyama, Kensuke Nakajima, Yoshinori Tsuji, Yusuke Matsuda

Kwansei-Gakuin University Department of Bioscience, Sanda-shi, Japan

Marine diatoms drive global scale elemental cycles as a major oceanic primary producer. Dissolved inorganic P (Pi) in the ocean is assimilated by photoautotrophs such as diatoms, and a part of which is incorporated into ocean food web. Large fraction of fixed Pi sink to the bottom of sea but little can be back to the land spontaneously because of the non-volatile nature of phosphorous. However, part of Pi incorporated into the food web can be transported to the land by the activities of sea birds and anadromous fish. Uptake of Pi by marine photoautotrophs is thus an essential initial step for a counter gravity movement of P. Molecular mechanisms of Pi acquisition in diatoms are yet unclear. Marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* has ten and five genes encoding putative solute carrier (SLC) type Pi transporters, respectively. Of these, transcripts of

PtSLC20, PtSLC34-2, -4, -5, and TpSLC34-2 were increased by Pi limitation. GFP-tagging localization of PtSLC34-2, -5 and TpSLC34-2 showed occurrences at the plasma membrane. Knock down mutants of PtSLC34-2 generated by RNAi showed reduced Pi uptake rate while over expression of PtSLC34-2 resulted in increase of Pi uptake rate under Pi-limiting conditions. Moreover, knock down mutants of TpSLC34-2 generated by RNAi reduced Pi uptake while the transcription level was relatively stable, suggesting that RNAi affected to posttranscriptional processes. These data suggest that Na+-dependent SLC34s are diatom Pi transporters functioning under Pi-limiting conditions.

71 Novel mechanisms for controlling intracellular pH in marine diatoms

Dorothee Kottmeier [1], Katherine Helliwell [1, 2], Abdesslam Chrachri [1], Alison Taylor [3], Glen Wheeler [1], Colin Brownlee [1]

[1] Marine Biological Association, Plymouth, United Kingdom
[2] Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom
[3] Department of Biology and Marine Biology, The University of North Carolina, Wilmington, United States

All organisms must tightly regulate intracellular pH in order to maintain optimal conditions for biochemical reactions. Many metabolic pathways contribute to the net production or consumption of H+, requiring cellular mechanisms for H+ transport and pH homeostasis. The mechanisms of pH homeostasis in marine diatoms have not yet been explored in detail, but are likely to play a major role in cellular metabolism. An examination of the mechanisms of H+ transport in marine diatoms identified the presence of voltage-gated H+ channels (Hv1) in all diatom genomes. Hv1 channels are found in highly specialised mammalian cells where they contribute to rapid H+ efflux across the plasma membrane. Hv1 channels have also been identified in a group of marine phytoplankton, the coccolithophores, where they allow H+ efflux across the plasma membrane to prevent intracellular H+ accumulation during calcification. However, the function of Hv1 channels in diatoms remains unclear. To better understand the process of pH homeostasis in marine diatoms, we have characterised diatom Hv1 channels in heterologous systems and confirmed that they perform as highly-selective voltage-gated H+ channels. Furthermore, expression of Hv1-GFP fusion in *Phaeodactylum tricornutum* revealed that Hv1 localises primarily to the plasma membrane. Together these results suggest that diatom Hv1 channels are involved in H+ efflux across cell plasma membrane. We are therefore examining pH homeostasis in diatoms in greater detail using measurements of intracellular pH and gene editing. The findings are likely to help us understand how diatoms respond to changes in pH, both internally and externally.

72 Ship-Seq: nanopore sequencing of polar microbes onboard research vessels

Emma Langan [1, 2, 3], Thomas Mock [1], Richard Leggett [2], Clara Manno [3], Vincent Moulton [1]

[1] University of East Anglia, Norwich, United Kingdom
[2] Earlham Insitute, Norwich, United Kingdom
[3] British Antarctic Survey, Cambridge, United Kingdom

There is increasing interest in the study of polar phytoplankton such as diatoms, which are the main drivers of polar ocean biogeochemical cycles and primary production. Polar oceans are biodiversity hot-spots which disproportionately contribute to global biogeochemical cycles, and they are under threat due to anthropogenic environmental change. The study of polar phytoplankton is challenging as many of them are cold adapted, which limits our ability to transport them to laboratories for experiments. Long-term maintenance in the laboratory is challenging as these species require

polar-specific environments. We are addressing this challenge by using the Oxford Nanopore Technology MinION for real-time studies on the diversity and function of polar phytoplankton communities. Our aim is to use genome sequencing to generate a real-time assessment of population diversity and to produce genome assemblies of polar phytoplankton. In early 2019, we carried out our first trial onboard the RRS Discovery, using the Nanopore MinION with NanoOK RT software for in situ sequencing and real-time analysis of metagenomic samples. Our results indicate that MinION sequencing is a powerful tool for polar phytoplankton research, although a lack of reference genomes currently limits its power. The results will be validated against previous data from similar locations, alongside sequencing of sample replicates using alternative platforms. For further investigations, alongside the production of more reference genomes, analysis pipelines will be tailored to target specific genes and species that are of interest in terms of their function and ecological role.

73 Efficient CRISPR/Cas-mediated homologous recombination in the model diatom Thalassiosira pseudonana

Nigel Belshaw [1], Reuben Gilbertson [1], Irina Grouneva [1], Lior Aram [2], Assaf Gal [2], Amanda Hopes [1], Thomas Mock [1]

[1] School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom
[2] Department of Plant and Environmental Sciences, Faculty of Biochemistry, Weizmann Institute of Science, Rehovot, Israel

Plants and algae possess unique molecular processes of high interest across many fields from biotechnology to evolutionary biology. The CRISPR/Cas system is a powerful tool enabling targeted genome editing in model plants and algae. Despite wide-ranging applications, efficient gene editing via homologous recombination (HR) has been limited to the haploid stage of photosynthetic organisms. To induce HR, a sequence specific CRISPR/Cas construct and a donor sequence were transformed into the model diatom *Thalassiosira pseudonana* through biolistic particle bombardment. The silacidin gene was disrupted by CRISPR and replaced with the introduced resistance cassette (FCP:NAT) through HR with 85% efficiency. Positive HR events were confirmed by nested PCR and sequencing of the products. As well as markedly increasing the HR efficiency, the role of silacidin as a cell size regulator was confirmed as the mutant lines displayed a significant increase in cell size. By significantly increasing HR efficiency further research can carried out in a variety of fields with *T. pseudonana* to understand the underpinnings of diatom genetics and potential biotechnological applications.

74 Characterization of the light-harvesting complex in the ALB3b knock out lines of the diatom Phaeodacthylum tricornutum

Charlotte Volpe [1], Manuel J. Llansola-Portoles [2], Marianne Nymark [3], Per Winge [3], Bruno Robert [2], Claudia Büchel [4], Olav Vadstein [1]

[1] Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway
[2] Institute for Integrative Biology of the Cell (I2BC), IBITECS, CEA, CNRS, Universite´ Paris-Saclay, Paris, France
[3] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
[4] Department of Biosciences, Institute of Molecular Biosciences, Goethe University, Frankfurt, Germany

The underwater light climate differs considerably from the terrestrial one, with only blue-green light successfully penetrating the water column. Aquatic photosynthetic organisms have therefore

evolved their light-harvesting capabilities in order to adapt to the marine light environment. The high ecological success of diatoms has often been attributed to the ability of the light-harvesting complexes (known as Fucoxanthin (Fx)-chlorophyll (Chl) a/c antenna complex or FCPs) to absorb in the blue-green region of the light spectrum. This is mainly due to the peculiar bathochromatic shift that occurs when the carotenoid Fx binds to FCPs, extending the absorption properties towards the red. FCP proteins are mainly integrated in the thylakoid membrane by the Albino3b integrase. ALB3b knockout mutants in the diatom Phaeodacthylum tricornutum shows a reduced antenna and altered absorption proprieties compared to WT resulting in a green coloration of the cells. In order to investigate the composition of FCPs in the mutant lines, these were isolated and analyzed. The results show that the remaining FCPs in the mutant are still intact and present in a higher oligomeric state compared to WT. Resonance Raman (RR) spectroscopy analysis shows shifts in the pigment-protein binding interactions, which might indicate a functional replacement of the missing FCPs. MS analysis of the mutant FCPs will reveal if and which proteins have been inserted in the attempt to replace the missing light harvesting proteins. Once identified, results from RR coupled with MS might shed some light on the characteristics of other members of the FCP family.

75 Characterization of RNA silencing pathways and physiological roles in the model diatom Phaeodactylum tricornutum

Emilia Grypioti [1, 2], Angela Falciatore [4], Kriton Kalantidis [1, 2], Frederic Verret [1, 2, 3]

[1] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas (IMBB,FORTH), Heraklion, Greece
[2] Department of Biology, University of Crete, Heraklion, Greece
[3] Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Heraklion, Greece
[4] Laboratoire de Biologie Computationnelle et Quantitative, Institut de Biologie Paris-Seine, Sorbonne Université, CNRS, 75005, Paris, France

RNA silencing is a conserved mechanism of regulation of gene expression mediated by small RNAs (sRNAs) and a set of key proteins including Dicer (DCR), Argonaute, and RNA dependant RNA polymerase (RdRP). Gene silencing can occur transcriptionally by RNA-directed DNA methylation (RdDM) or post transcriptionally (PTGS) either by cleavage or translational inhibition of targeted mRNA. Previous studies in *Phaeodactylum tricornutum* have suggested the presence of an endogenous RdDM pathway which may play a role in the acclimatory response to environmental stress conditions. To date, no diatom DCR, AGO and RdRP homologue has been cloned and direct evidence for their respective contribution and mode of action in RNA silencing, as well as for the importance of RNA silencing in diatom ecophysiology, is still lacking. Phylogenetic analysis across a large number of diatom species revealed that changes in DCR/AGO/RdRP gene repertoire coincides with transition in diatom evolution. *P. tricornutum* DCR/AGO/RDR homologues were cloned, YFP-tagged, and ectopically expressed for determination of their subcellular localization. Functional characterization of DCR was first attempted by heterologous expression approach in Saccharomyces cerevisiae and Nicotiana benthamiana. In a second approach DCR-KO lines were generated by CRISPR-Cas9 mutagenesis and their mRNA and sRNA transcriptomes were investigated by high-throughput RNA sequencing. This project is funded by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No 483 RADIO (VF), and MIS 5002670 CMBR (GE, KK and VF).

76 Ribosome Profiling to Reveal the Effects of Temperature and Codon Usage on Protein Synthesis in Diatoms

Monica Pichler[1], Amanda Hopes[1], Annemarie H. Eckes[1, #] and Thomas Mock[1]

[1] School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom
[2] Department of Geography, University of Cambridge, Cambridge, United Kingdom

Translation is a fundamental cellular process; however, little is known about translational regulation in response to changing environmental conditions in diatoms. Recently, we have developed a ribosome profiling method for the model diatom *Thalassiosira pseudonana*, which enables genome-wide analysis of in vivo translation with nucleotide resolution. Successful library preparation and sequencing of ribosome footprints indicates the precise positions and densities of actively translating ribosomes on each transcript. To study how temperature affects translation and cellular fitness in *T. pseudonana*, we will perform ribosome profiling on temperature acclimated (8°C, 22°C, 32°C) cells. Next, we plan to investigate the effects of codon usage on protein synthesis by performing ribosome profiling on codon-modified cell lines. CRISPR/Cas-mediated homologous recombination will be used to knock-out two genes (ribosomal protein L10, Lhcx6) and replace them with codon-optimized/suboptimized versions. In order to reveal the effects of codon usage upon environmental stress, temperature and light experiments will be conducted on the transgenic cell lines. Data from this project will reveal new insights into the translational dynamics in diatoms and thus will have a fundamental impact on diatom research and biotechnology.

77 Multiple gene expression using a polycistronic system in Phaeodactylum tricornutum

Gilles Defrel [1], Fayza Daboussi [1, 2]

[1] LISBP, Centre National de la Recherche Scientifique, INRA, INSA, Université de Toulouse, Toulouse, France
[2] Toulouse White Biotechnology (TWB), Toulouse, France

The genetic engineering of diatoms is booming. It is now possible to specifically inactivate one or more genes via the use of molecular scissors such as TALEN, CRISPR/Cas9... However, the use of microalgae as industrial biofactories able to produce new compounds for the pharmaceutical, cosmetic, green chemistry and energy sectors is still in its infancy. One basic challenge is the difficulty of expressing and integrating an artificial biosynthesis pathway in a heterologous eukaryotic host. As an example the generation of a yeast-producer of hydrocortisone has required more than 10 different genome modifications. To meet this challenge we developed a polycistronic system, using modular cloning and the 2A “self-cleaving” peptides, allowing to express several genes encoding for different products under the control of one promoter. In parallel we optimized the homologous frequency, i.e the ability to integrate an artificial metabolic pathway at a specific locus. Altogether, these new developments will in turn allow a better understanding of gene expression and the creation synthetic gene circuits, which is the basis of synthetic biology.

78 Whole genome analysis of the genus Skeletonema reveals evolution of genes supporting microalgal blooms

Satoshi Nagai [1], Yoko Kawakami [2], Ryuhei Minei [3], Atsushi Ogura [3], Sirje Sildever [1], Nanako Kanno [1], Katsuhiko Mineta [4], Takashi Gojobori [4]

[1] National Research Institute of Fisheries Science., Kanagawa, Japan
[2] AXIOHELIX Co. Ltd, Tokyo, Japan
[3] Nagahama Institute of Bioscience and Technology, Shiga, Japan
[4] King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

In this study, we sequenced the genome of Skeletonema costatum (Sc), one of the most dominant planktonic diatom species in eutrophicated coastal waters all over the world, and also performed RNA-sequencing analysis of the samples which were grown at different temperature, irradiance, and nutrient conditions under laboratory conditions. From the k-mer analysis, genome size of Sc is estimated as 51,364,529 bp and analyses by Platanus (46.9 Mb) and SOAPdenovo2 (52.7 Mb) showed close genome size. To clarify the common genetic background in the diatom and to specify the genetic novelty of Sc from other diatoms, we estimated orthologous gene groups that share the same common ancestral gene and are supposed to have the same functionality. Total number of genes, 16,449 genes can be categorized to 11,577 genes (in Orthologous groups), 4741 (in singlet genes), and 151 genes (from 27 Sc only Orhologous groups). The proportion of conserved genes between Sc and *Thalassiosira pseudonana* (Tp) that are closest to Sc were about 65.4%, calculated by dividing the total of Sc-Tp shared genes by the total number of Sc genes (11,557 + 4,741). The duplicated genes gained by Sc are associated with growth and oxidative stress response facilitating rapid proliferation in suitable environmental conditions and maintenance of bloom. Currently, we are accumulating whole genome data in several diatom species to understand the evolutionary processes of genes that support rapid growth of diatoms.

79 Characteristics of Proteins in soluble Biosilicomes from three Diatom Species

Andre Ohara [1], Alastair Skeffington [2], Alexander Milentyev [3], Christoph Heintze [1], Stefan Görlich [1], Nicole Poulsen [1], Nils Kröger [1]

[1] Center for Molecular Bioengineering B CUBE, Technische Universität Dresden, Dresden, Germany
[2] Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany
[3] Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Diatoms are model organisms for studying biological silica morphogenesis. However, until now little sequence information is available for biosilica associated proteins, thereby limiting insight into their specific roles in silica formation. In this project, we have compared the soluble 'biosilicome' (i.e. biomolecules released by ammonium fluoride extraction of isolated cell walls) from the closely related diatom species *T. pseudonana*, Cyclotella cryptica, and Thalassiosira oceanica. A proteomics analysis of the three soluble biosilicomes identified 26 proteins from *T. pseudonana*, 29 from C. cryptica, and 23 from T. oceanica. In general, the proteins display very low sequence similarity and often contain intrinsically disordered regions. Only one protein was conserved amongst the three species. While *T. pseudonana* and C. cryptica share 14 homologous proteins, T. oceanica did not share any other pairwise conserved proteins with either *T. pseudonana* or C. cryptica. Despite the very low sequence similarities, 51 proteins contain KxxK motifs that identify them as silaffins or silaffins-like proteins. For a significant fraction of these proteins, we have mapped post translationally-modified lysine residues. Other recurring motifs shared by many proteins are domains rich in (i) P and T, (ii) the heptapeptide Cx5C, and (iii) the tripeptide MSM. With this study, we have greatly extended our knowledge about the features of biosilica associated proteins, and laid the foundation for identifying species-specific as well as general proteins of the silica biomineralization machinery in diatoms.

80 Discovering and enhancing bioactives in marine diatoms

Francesco Manfellotto [1], Maria Immacolata ferrante [1], Angela Falciatore [2], et al.

[1] Stazione Zoologica Antonio Dohrn, napoli, Italy
[2] Laboratory of Computational and Quantitative Biology (LCQB), Institut de Biologie Paris-Seine (IBPS), paris, France

Diatoms are a major group of microalgae found in all aquatic ecosystems, adapted to different and sometimes extreme environments. They naturally produce various substances beneficial for human health, food and feed, such as polyunsaturated fatty acids, vitamins, antioxidants, enzymes, polysaccharides and carotenoids, and therefore represent a potential source for commercial and industrial applications. Our research focuses on planktonic species, for which we have developed several resources, including genomes, transcriptomes and tools for functional genomics approaches. Within a large-scale project for the identification of novel marine natural products, we analysed metabolomics profiles of different marine diatom species and ecotypes to discover bioactive compounds. We optimized different algal cultivation conditions in order to enhance the physiologic production of bioactive substances and maximize yield. In addition, we focused on carotenoid and xanthophylls, known for their anti-oxidant activity, anti-metabolic syndrome activities (anti-obesity, anti-diabetes) and beauty-enhancing activities (skin-enhancing, skin-lightening, anti-acne). In order to increase carotenoid synthesis in the marine diatom *Phaeodactylum tricornutum*, we exploited genetic engineering techniques to overexpress genes involved in the carotenoid biosynthesis pathway obtaining transgenic lines with increased carotenoid content.

81 Engineering Phaeodactylum tricornutum for the controlled production of plant secondary metabolites

Patrick Hickland, Alison G Smith

Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom

Microalgae exhibit increasing potential as chassis organisms for the industrial production of pharmaceutically important plant secondary metabolites. This is in part due to an adoption of a synthetic biology approach to metabolic engineering and an expansion of the molecular tools available to researchers working with certain model species as well as a better understanding of culturing techniques required for scaling up production. The marine diatom *Phaeodactylum tricornutum* is an interesting target chassis for the heterologous production of high value terpenoid compounds as it possesses both the chloroplastic MEP and cytosolic MVA pathway required for the supply of terpene precursor compounds, GGPP and FPP, and has a track record of heterologous terpene production. Despite this reported success it is necessary to improve product yield by orders of magnitude if *P. tricornutum* is to rival bacterial and yeast based production systems. I have identified a novel inducible promoter that exhibits both complete “off” and tuneable expression characteristics as well as increases to protein abundance of 13x and 27x compared to the widely used LHCF1 and EfTu promoters respectively. My studies also reveal a promoter independent connection between promoter activity and the growth phase of the culture. I demonstrate the utility of this inducible promoter in the production of a pharmaceutically relevant diterpene, showing production in both the cytosol and the chloroplast. I also present strategies for improving yield to titers in excess of 10x higher than those previously reported for similar compounds in *P. tricornutum*.

82 Expanding the CRISPR toolbox – multiplexing, cloning, homologous recombination and psychrophilic diatoms.

Amanda Hopes [1], Nigel Belshaw [1], Shiri Graff van Creveld [2], Irina Grouneva [1], Krisztina Sarkozi, [1], Monica Pichler [1], Reuben Gilbertson [1], Thomas Mock [1]

[1] School of Environmental Sciences, University of East Anglia, Norwich, United Kingdom
[2] Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

Since the advent of gene editing via CRISPR-Cas, this technique has become a highly valuable tool in molecular biology, rapidly expanding in both applications and species. CRISPR is now available for two model diatoms –*Thalassiosira pseudonana* and Pheodactylum tricornutum. Within the Mock lab we are expanding our CRISPR toolbox. A flexible, reduced Golden-gate system is available for both species, allowing modules to be altered and multiple genes/sites to be simultaneously targeted. CRISPR-mediated homologous recombination appears to be highly efficient in *T. pseudonana*, with applications for knocking-out genes with a selective marker as well as replacing genes with modified versions. Introducing a deletion via two sgRNAs allows easy screening – this method has been tested to remove large fragments of genes, removing the need for frameshift causing mutations or active-site targeting. We are currently adapting CRISPR for psychrophilic diatoms. Whilst the Cas9 system has been successfully used in temperate species, alternatives are needed for polar diatoms such as Fragilariopsis cylindrus. In-vitro temperature assays have revealed an alternative CRISPR nuclease that functions at low temperatures. Assays have also highlighted the need to test gRNAs prior to transfection and show evidence that gRNA arrays can be used for multiplexing. Both expression based and RNP systems are being used. We are currently applying our improved CRISPR methods to a range of projects, including adaptive evolution in F. cylindrus, the effect of codon bias on translation in *T. pseudonana* and the role of silacidins and Big1. Both recent advances and current applications will be discussed.

83 Application of large-scale gene expression profiling in Seminavis robusta reveals new insights about gene functions involved in (a)biotic stress and life cycle progression.

Gust Bilcke [1, 2, 3], Members of the Seminavis genome consortium [4]

[1] Protistology and Aquatic Ecology, Department of Biology, Ghent University, Ghent, Belgium
[2] VIB Center for Plant Systems Biology, Ghent, Belgium
[3] Department of Applied mathematics, computer science and statistics, Ghent University, Ghent, Belgium
[4] Ghent University and collaborative institutes, Ghent, Belgium

We present the Seminavis robusta gene expression atlas, mapping gene expression across the entire life history of this benthic pennate diatom. We explore the transcriptomic profiles in 31 different conditions comprising abiotic stressors, biotic interactions and (a)sexual life cycle events. Gene expression quantification detected 31,839 expressed genes, of which 27,963 were significantly differentially expressed (DE) in at least one condition. Leveraging the expression atlas, we characterized marker genes and gene families that are enriched in DE genes in specific conditions. Genes containing a von Willebrand Factor type D domain are enriched in 9 different conditions, suggesting a pleiotropic role for these genes. Oppositely, MYND-type zinc finger genes are only enriched during stages of the mating process, indicating a specific role during sexual reproduction. Allele specific expression (ASE), an imbalance in the expression levels of two parental alleles, was recently described for the cold-adapted diatom *F. cylindrus* (Mock et al. 2017, Nature). Using the broad range of conditions in the expression atlas, we show that ASE is a common phenomenon in S. robusta: more than 90% of the 1323 loci having 2 alleles show ASE in at least one condition. Moreover, many allele pairs show an asymmetric response to a treatment, suggesting a different regulatory control and functional role for each allele. The potential for different

regulation of alleles adds another level of transcriptional control on top of the large genome size and frequent gene family expansion in S. robusta. We developed a website to explore gene expression and ASE: https://gustbilcke.shinyapps.io/Seminavis_MLD/

84 Polyploidy detection in the "diploid" Fragilariopsis cylindrus

Kat Hodgkinson [1], Jonathan Wright [2], Gonzalo Garcia Accinelli [2], Luis Yanes [2], Darren Heavens [2], Amanda Hopes [1], Thomas Mock [1], Cock van Oosterhout [1], Bernardo Clavijo [2]

[1] University of East Anglia, Norwich, United Kingdom
[2] Earlham Institute, Norwich, United Kingdom

There are currently two assemblies for F. cylindrus, but neither are suitable for evolutionary genomics; the diploidised PacBio assembly has low consensus accuracy, and the Sanger assembly is largely fragmented. With the aim of evolutionary analysis, we constructed an assembly with high base-pair accuracy from Illumina sequencing, and combined this with Nanopore long-reads for greater contiguity. We found a strong signature for 3 different copy numbers in the genome: X, 2*X and 3*X. The topology of the graph and the motif frequencies of its components reveal 3 categories of syntenic regions: 1 different and 2 similar copies, 3 very similar copies and 3 very different copies. We used the BSG (Basic Sequence Graph) approach to recover haplotype-representative specific content, confirming the k-mer spectra and topological observations and supporting a working hypothesis of 3 full-genome haplotypes (A, B and C). A large 70% of the genome is represented in regions where A and B are close, but C is diverged, 14% where all the haplotypes are close, and 14% where all the haplotypes are diverged. These results provide an alternative explanation for differential expression between haplotypes as an expected result of diverging sub-genomes in a polyploid organism. Chromosome counts are varied for many diatoms, and polyploidy, an important mechanism in diatom evolution, may be even more prevalent than previously described. We hope future genomic analyses, conducted with consideration for haplotype-specificity and ploidy, may further expand our understanding of diatom evolution and genome composition.

85 Investigating whether the Phaeodactylum tricornutum thiamine pyrophosphate riboswitch is functional

Marcel Llavero Pasquina, Katrin Geisler, Payam Mehrshahi, Alison Smith

Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom

Thiamine pyrophosphate (TPP), the active form of vitamin B1, is an essential co-factor used by all organisms to catalyse key reactions of their central metabolism. Thiamine biosynthesis is an expensive metabolic process, and the expression of thiamine-related genes is generally highly regulated. The first enzymes in the thiamine anabolic pathway, THIC, THI4 and NMT1, are switched off in the presence of thiamine by TPP riboswitches in bacteria, fungi, plants and green algae. Croft et al. (2007) and McRose et al. (2014) have previously used a bioinformatic approach to predict the presence of riboswitches in the 3'UTR of the THIC and SSSP genes of diatoms *P. tricornutum*, *T. pseudonana* and F. cylindrus. We have investigated whether these predicted TPP riboswitches in diatoms are functional using a range of methods, including determining intracellular levels of thiamine, RT-qPCR of transcript levels, in vivo expression of reporter constructs, and mutagenesis experiments. We demonstrate that sequence-based riboswitch identification without experimental characterisation is not sufficient to confirm riboswitch function. Furthermore, our data suggests that transferring eukaryotic riboswitches across species is not straightforward, because trans-acting factors and processes, such as splicing and translation initiation, vary across species. Our findings open interesting questions on the molecular evolution of the TPP riboswitch in the diatom lineage

and the environmental conditions that influence thiamine biosynthesis regulation in marine ecosystems.
REFERENCES: Croft et al. (2007). PNAS, 104(52), 20770-20775. McRose et al. (2014). The ISME Journal, 8(12), 2517.

86 Molecular regulation of diatom life cycles: insights from sexual reproduction in Pseudo-nitzschia multistriata

Rossella Annunziata [1], Massimiliano Volpe [1], Laura Entrambasaguas [1], Maurizio Ribera d'Alcalà [1], Remo Sanges [2], Daniele Iudicone [1], Marina Montresor [1], Mariella Ferrante [1]

[1] Stazione Zoologica Anton Dohrn, Napoli, Italy
[2] Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

The majority of diatom species alternate a phase of mitotic divisions, causing cell size reduction, and a phase of sexual reproduction, leading to cell size restoration. Despite the increasing interest in diatom biology and ecology, the molecular mechanisms regulating their life cycles are little explored. Our laboratory has recently identified mating type (MT) specific genes and among them, a gene responsible for sex determination in the planktonic heterothallic Pseudo-nitzschia multistriata. This diatom produces the neurotoxin domoic acid, making the understanding of its life cycle regulation highly relevant. To obtain a detailed picture of the molecular regulation of P. multistriata sexual reproduction, we have generated transcriptomic datasets from different phases during a mating event, including early stages (gametes and zygotes) and late stages (auxospores and F1 initial cells). As previously observed, when the two opposite MTs were crossed, all cells arrested their growth although not all of them were engaged in meiosis: in fact only ~20% of cells produced gametes. Transcriptomic analyses revealed significant changes in cell physiology and metabolism during sexual reproduction. In particular, genes related to photosynthesis and nutrient uptake were strongly downregulated, while genes related to DNA-repair and epigenetic modifications were upregulated compared to control conditions. Functional genetics approaches combined with transcriptomic investigations on the individual cell types occurring during sexual reproduction promise to advance our understanding of the strategies that diatoms use to optimize life cycle decisions and their possible effects on the marine ecosystem.

87 Episome-deployed diatom genetic parts registry with associated DNA assembly pipeline

Chris Dupont [1], Fernan Federici [2], Erin Garza [1], Vince Bielinski [1], Bernardo Pollak [1, 2]

[1] J. Craig Venter Institute, La Jolla, United States
[2] Pontificia Universidad Católica de Chile, Santiago, Chile

Diatoms are unicellular photosynthetic protists in the Stramenopile lineage. Evolutionarily derived from serial endosymbiotic events between Eukaryotes, diatom genomes and metabolism are a mosaic unique relative to other model systems. Despite this, the availability of genetic tools and fundamental biochemical or genetic knowledge about diatoms lags behind that of other organisms. Here, we describe the development of genome engineering and synthetic biology tools to enable advances in our understanding of diatom biology. The development of stable episomes has facilitated both targeted and untargeted characterization of genetic parts such as gene promoters and terminators and chromosome centromeres. Conjugative transfer of these episomes is three orders of magnitude more efficient than traditional methods, allowing for efficient forward discovery based approaches. By combining them with reporter proteins, the gene expression profiles for

numerous new promoters and terminators have been characterized in a replicated fashion. These promoters and terminators, along with a variety of genetic tools (affinity tags, fluorescent proteins, Cas proteins), have been domesticated into a living genetic parts registry for community usage. The domestication builds a given genetic part into a level 0 vector of the Loop DNA assembly system, allowing for highly efficient recombination with other level 0 parts and rapid assembly of more complex expression systems. The system has been tested by numerous research groups in two model diatoms and it is anticipated that a living parts registry with associated information will be public in the next six months.

88 Functional analysis of the Silicanin family in diatom cell wall biomineralization

Marthe Hafskjold, Annika Messemer, Per Winge, Olav Vadstein, Tore Brembu

Norwegian University of Science and Technology, Trondheim, Norway

Characteristic for diatoms are their ability to create hierarchically nano- and micropatterned siliceous cell walls, or frustules. Exactly how silica polymerization and nanopatterning takes place inside the silica deposition vesicle (SDV) of diatoms is still largely unknown. Knockdown of proteins associated with the SDV membrane have demonstrated that the structure of the silica frustule can be altered by knocking down a single gene. A previous transcriptome study from our lab revealed a family of previously uncharacterized diatom-specific transmembrane proteins related to Silicanin-1 (Sin1). We hypothesized that the family is associated with the SDV membrane and is likely involved in silica biomineralization of the diatom frustule. To gain a broader understanding of the role of Silicanin proteins, the CRISPR/Cas9 system was used to generate knockout mutants of Silicanin family members in the diatoms *T. pseudonana* and *P. tricornutum*. The localization and dynamics of the Silicanin proteins was investigated by expressing Silicanins fused with a fluorescent protein and observing their expression and position during a full cell cycle. Novel understanding of silica deposition and patterning resulting from these experiments can potentially help generate genetically modified diatoms with modified frustules optimized for specific applications in e.g. nanotechnology.

89 Growth & lipid accumulation in Phaeodactylum tricornutum Bohlin under sub-tropical climatic conditions in India.

Debesh Chandra Bhattacharya

Deapartment of Microbiology, Vidyasagar University, Midnapore 721102, Midnapore, India

Diatoms, its intricate ornamental structure with nanopores and a silicified wall has definitely been a major topic of investigation with the emergence of omics era. Complete genome sequence studies with P.tricornutum has also made this tiny organism a popular one for the study of lipid accumulations. However few attempts have been done to cultivate diatoms under sub-tropical conditions. The present study reports considerable growth advantage and ability to accumulate lipid by P.tricornutum under Indian conditions which was considered almost impossible by the earlier reports. The rate of normal lipid accumulation was found to be in the range of 46.8 to 49.2 mg/lit/day and the specific growth rate was 0.631 day-1 under a saturated PAR of 460 µmol m-2 s-1. The medium used was modified Gf/2 medium and the different treatments yielded for the first time the growth of P.tricornutum at 31°C which itself is unique and not yet reported. In-silico studies were also done on the lipid accumulation with respect to key enzymes under temperature stress as found in Indian climate.

90 Diatoms in heterotrophy: metabolism and potential biotechnological applications of cyclotella cryptica

Adelaide Cupo, Carmela Gallo, Simone Landi, Genoveffa Nuzzo, Angela Sardo, Angelo Fontana, Giuliana d'Ippolito

National Research Council of Italy, Institute of biomolecular chemistry, (CNR-ICB), Pozzuoli, Italy

Diatoms have traditionally been cultivated under photoautotrophic conditions, but a strong interest in heterotrophic systems has recently arisen. In this context, our aim was to assess the potentiality of the diatom Cyclotella cryptica in heterotrophy. Cyclotella cryptica grown in the dark represents a simplified model to study light-dependent metabolic pathways and a powerful tool to give further insight into diatom physiology. For this purpose, we set-up growth culture conditions in heterotrophy on small and medium scale, using glucose as organic source. The “dark phenotype” was biochemically characterized, in terms of biomass productivity and distribution among lipid, protein and carbohydrate pools. Then an integrated approach based on transcriptomic (RNAseq) and lipidomic (1H-NMR, LC-MS and GC-MS) was used to characterize key enzymes involved in adaptation to heterotrophic growth and in the remodeling of lipid biosynthesis. Our results contribute to define the prospective of diatoms growth in heterotrophic condition for several industrial applications, such as production of protein and lipid rich flour as well as ω-3 fatty acids (EPA and DHA). The “dark” biomass large-scale production could overcome the limitations imposed by photosynthesis, such as light and temperature fluctuations, using fermenters technologies. Thus, heterotrophic diatom growth may provide a cost-effective alternative for the cultivation of microalgae species by reducing costs of microalgae-based ingredients functional for nutrition, health and nutraceuticals.

91 Analysis of short-term transcriptome responses to silicon addition in Thalassiosira pseudonana

Tore Brembu [1], Annika Messemer [1], Marthe Hafskjold [1], Per Winge [2], Olav Vadstein [1]

[1] Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway
[2] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

The diatom cell wall, or frustule, is the product of a highly organized biomineralization process. Synthesis of the frustule involves a complex interplay between silicic acid, organic molecules and a number of protein families, most of which are diatom-specific. Several studies have provided a detailed and fairly consistent picture of transcriptional responses to silicon starvation or replenishment in *Thalassiosira pseudonana* on hour resolution level. However, the initial responses to changes in Si availability have not been assessed. We analysed the early transcriptional responses to Si replenishment from a previously published dataset. Based on these results, we performed a new Si replenishment experiment, with higher temporal resolution. We also included cell cycle controls and additional measurements. Results from these experiments will be presented.

92 The Molecular Response of Two Key Southern Ocean Diatoms to Iron and Temperature

Loay Jabre [1], Andrew E. Allen [2, 3], J. Scott McCain [1], David A. Hutchins [4], Erin M. Bertrand [1]

[1] Dalhousie University, Halifax, NS, Canada
[2] J. Craig Venter Institute, La Jolla, CA, United States
[3] Scripps Institution of Oceanography, La Jolla, CA, United States
[4] University of Southern California, Los Angeles, CA, United States

Iron and temperature are important variables influencing the growth and physiology of marine diatoms. Iron is a cofactor in many vital proteins, and can be a limiting nutrient in marine systems, while temperature is a key biological variable and influences cells in a variety of ways. Despite this, we still lack a phenomenological and mechanistic understanding of how iron and temperature interactively influence diatom growth. Here, we performed a bottle incubation bioassay on microbial communities from the Southern Ocean where we incubated samples with and without the addition of iron at three temperatures. We then generated community composition assessments and metatranscriptome profiles to examine the response of two dominant taxa, Fragilariopsis and Pseudo-nitzschia. Pseudo-nitzschia grew more with increased temperature and no added iron when compared to Fragilariopsis, while the latter required iron addition to better benefit from temperature increase. Additional laboratory studies also show that *Fragilariopsis cylindrus* requires iron addition to receive growth rate benefit from increased temperature. The apparent advantage Pseudo-nitzschia shows under elevated temperature and low iron can be explained by upregulation of light harvesting complex (LHC) transcripts, iron transporters (ISIP2A & ISIP3) and the constitutive substitution of iron-containing proteins with non-iron containing counterparts. Conversely, iron addition, and not increased temperature, caused a larger upregulation of LHC and other iron-containing photosynthetic transcripts in Fragilariopsis. This suggests that the two taxa prioritize different metabolic processes under the same environmental conditions to support growth, raising the possibility of an increasingly important role for Pseudo-nitzschia in warming Southern Ocean ecosystems.

93 Mass Culture for the Masses: Targeted Large Scale Biomass Production for Collaborative Research

Matthew Julius

Department of Biological Sciences St. Cloud State University, St. Cloud, United States

The promise of microalgae for the production of commercial biomass is tied to their rapid. Oceanic ecosystems require phytoplankton biomass to double each day to meet the energy demands of marine food webs. Additionally, these disparately related organisms have radiated to fill countless niches in which they successfully confront a variety of environmental selective pressures. This has resulted in species diversity estimated in the millions with tens of thousands of formally described species. Despite this rapid natural growth and species diversity, researchers find it difficult to successfully mass culture species. Additionally, investigations of metabolic processes and controls are limited to a few taxa broadly available from culture facilities. These limitations may be the result of a bottleneck in collaboration and exchange between researchers transdiciplinarily. Systematists are partially to blame for this phenomena, rather than embracing their role to facilitate scientific communication and an understanding of diversity they often choose to obfuscate how species delineation and identification can be done effectively and efficiently. Further compounding the problem is a failure to understand that many species means many niches and a singular method for growing taxa is simply not a logical conclusion. The strategy and implementation of a mass culture facility for >500g biomass quantities is presented. The laboratory utilized multiple media, photobioreactors, and harvesting strategies to produce biomass suitable for tailored research studies. Taxon selection is guided by systematic expertise and the facility is supported by a materials characterization laboratory. Past collaborative success are described and opportunities for collaboration are described.

94 Identification and characterization of genes in Phaeodactylum tricornutum that enable high maximum nutrient uptake rates and storage capabilities in diatoms.

James McCarthy [1], Maxine Tan [1, 2], Mark Moosburner [1, 2], Andrew Allen [1, 2]

[1] J. Craig Venter Institute, La Jolla, United States
[2] Scripps Institution of Oceanography, La Jolla, United States

The capacity of diatoms for rapid uptake and potential storage of NO3− provides a competitive advantage over other species, and when elevated NO3− occurs, they may take up more NO3− than their immediate growth rates demand. Recent identification of 3 putative outer membrane NO3− transporters, and 4 putative vacuolar transporters may provide specifics for this ecologically-advantageous physiological trait. Outer membrane transporters (J26029, J54560 and EG02608) are homologous to high-sensitivity NRT2.1 transporters in Arabidopsis; three of the four diatom vacuolar transporters, EG01952, J28245, J46097 and J52412 also share homology with their Arabidopsis counterparts. Transgenic lines for 2 *P. tricornutum* NRT genes, J26029 and J54560, and 2 vacuolar NO3− transporters, EG01952 and J28245, have been generated by CRISPR-Cas9 cutting and NHEJ. Positive knockout clones for all four genes have been determined by sequence analysis, and TIDE and T7 assays. Phenotypic assays for several clones in each line show a range of attenuated protein activity from slight to pronounced. NRT2.1, J26029 knockout lines when grown in NO3− media, as compared to WT, show diminished grow growth rates until day 4 when rates slowly return to WT levels, suggesting another outer membrane transporter, yet to be identified, rescues the phenotype. Of the two vacuolar transporter knockouts, J28245 clones show a more impaired phenotype, but not a lethal one, when grown on NO3−. Dual gene knockout lines for both the NRT2.1 and the vacuolar transporter protein pairs have been constructed, conjugated and sequenced. Investigations into a phenotypic response to growth on NO3− are underway.

95 Isoprenoid-based advanced biofuel molecules from diatoms: Perspective, challenges and opportunities

Nikhil Kadlag [1], Gandhali Naik [1], Arvind Lali [2], Gunjan Prakash [1]

[1] DBT-ICT Centre For Energy Biosciences Institute of Chemical Technology, Mumbai, Mumbai, India
[2] Department of Chemical Engineering Institute of Chemical Technology, Mumbai, Mumbai, India

Engineered microbial platforms are the cost-effective solution for large-scale production of isoprenoid-based advanced biofuels. E. coli and S. cerevisiae have been extensively engineered for a range of isoprenoid-based biofuel molecules. However, current heterotrophic and phototrophic microbial host chassis are limited in two aspects ie contrary to terrestrial plants they are non-native producers of isoprenoid-based fuels and possess either MEP or MVA branch of isoprenoid pathway. Diatoms are one of the most ecologically successful classes of photosynthetic marine eukaryotes in the contemporary oceans. They contribute up to 40% of organic matter production in world oceanic system. There are sparse evidences of diatoms being the primary species involved in oceanic volatile emissions. This establishes them as the aquatic counterparts of terrestrial plants for volatile emissions. Additionally, Diatom possess both MEP and MVA branch of isoprenoid pathway due to secondary endosymbiosis event leading to higher overall isoprenoid pool for isoprenoid-based fuel production. Modifying a host with both MEP and MVA pathway and natural ability to produce volatiles is expected to have a more realistic impact in comparison to the non-native host chassis. Being microscopic in nature they also offer large-scale cultivation protentional in open or closed photo-bioreactors for industrial applications. They are already being commercially exploited for their high lipid content, essential fatty acid and fucoxanthin. Since photosynthetic isoprenoid-based production represents the most direct conversion of sunlight energy into the next-generation biofuels. Present work will discuss the perspective, challenges and opportunities associated with establishing diatom as host chassis for isoprenoid-based biofuel production.

96 Diatoms are important producers of DMSP?

Ana Bermejo Martinez [1], Andrew Curson [1], Peter Rivera [1], Beth Williams [1], Barbara Lyon [1], Jan Strauss [5], Jean-Baptiste Raina [4], Thomas Mock [2], Jonathan Todd [1]

[1] School of Biological Sciences University of East Anglia, Norwich, United Kingdom
[2] School of Environmental Sciences University of East Anglia, Norwich, United Kingdom
[3] Laboratory of Microbiology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain, Barcelona, Spain
[4] Climate Change Cluster (C3), Faculty of Science, University of Technology, Sydney, New South Wales, Australia, Sydney, Australia
[5] European Molecular Biology Laboratory (EMBL) Hamburg, c/o German Electron Synchrotron (DESY), Notkestraße 85, 22607, Hamburg, Germany, Hamburg, Germany

Billions of tons of the organosulfur osmolyte dimethylsulfoniopropionate (DMSP) is produced each year by marine microorganisms. This DMSP and its gaseous catabolite dimethylsulfide (DMS) are key marine carbon and sulfur sources, info-chemicals and have roles in global sulfur cycling, atmospheric chemistry and potentially climate. It is generally thought that diatoms produce low intracellular levels of DMSP and that they are not major environmental producers of these molecules. We have identified the key methyltransferase gene DSYD for DMSP synthesis in many diatoms, which is different to the DSYB equivalent gene found in many DMSP-producing phytoplankton such as haptophytes, dinoflagellates, and some diatoms. We study the regulation of DMSP synthesis and DSYD expression in model diatoms and localise DMSP and their DSYD enzyme within cells. Functional DSYD genes are conserved in most diatoms species, some macroalgae and, interestingly, some bacteria. Insights gained from marine metatranscriptome data suggests that diatoms are very likely important DMSP producers in marine environments ranging from surface seawater to sediment.

97 Coastal surface sediments for organosulfur cycling driven by diatoms and bacteria

Peter Paolo Rivera, Beth Williams, Andrew Curson, Charles Brearley, Jonathan Todd

School of Biological Sciences, UEA, Norwich, United Kingdom

The osmolyte dimethylsulfoniopropionate (DMSP) and its degradation product dimethylsulfide (DMS) play major roles in biogeochemical cycling of carbon and sulfur, signalling pathways and in atmospheric chemistry. Coastal wetlands are known as global hotspots for the production of these influential organosulfur compounds and this is thought to be a consequence of the cordgrass Spartina which produces high DMSP levels. Our recent work suggests that bacteria and diatoms in coastal surface sediments also produce significant amounts of DMSP and DMS. Microbial community analysis on surface sediment from a local salt marsh showed that ~ 9% of the community were diatoms and that the numerically dominant diatom was from the genus Asterionella (~ 6 %), a member of the Fragilariaceae family. We isolated a strain of chain-forming Asterionella from the sediment and its monoclonal culture produced low intracellular DMSP levels. Here we discuss the effects of varying environmental growth conditions on Asterionella DMSP production. Furthermore, since neither the DSYB nor DSYD DMSP synthesis genes have been identified in the Fragilariaceae family of diatoms we are investigating the mechanism of DMSP production in this model diatom. Given the abundance of DMSP-producing diatoms in saltmarsh surface sediments, they likely play an important role in organosulfur cycling in such environments.

98 Deciphering the role of multiple ferric permeases in the diatom Thalassiosira pseudonana

Adam Kutska[1], Robert Bornhorst[1], Roshan, Shrestha[2], Mark Hildebrand[2,3]

1 Department of Earth and Environmental Sciences, Rutgers University, Newark, NJ USA
2 Scripps Institution of Oceanography, University of California San Diego, San Diego, CA USA
3 Deceased

One model for iron uptake in diatoms requires a ferric reduction step at the cell surface. In yeast, such a reduction step is part of the reductive-oxidative Fe uptake pathway through a ferric reductase protein (FRE). In this pathway reduction is followed by the coordinated action of a ferroxidase-ferric permease (FET3/FTR), complex which concomitantly binds, oxidizes, and transports Fe(III). FET and FTR are co-obligate partners, and the yeast permease cannot access Fe(III) directly from the dissolved milieu. Reductases are nearly ubiquitous among diatoms, whereas several transcriptomes also reveal permeases but no apparent ferroxidases (Groussman et al. 2015). In *Thalassiosira pseudonana*, two FTRs but only one FET3 homologue are present in the genome, and both permeases are well represented in proteomes, opening the possibility of ferroxidase-independent roles for one or both permeases. We investigated the role of the ferric permeases (FTR1, FTR2) using reverse genetics and radiotracer methods. FTR transcriptional knock-down clones grew slower, and achieved lower iron uptake rates, under steady-state iron limiting conditions. It's noteworthy that the antisense-based knockdowns may affect expression of both paralogs. GFP chimeras of both proteins, under native promotion, were localized at the cell surface and upregulated in low-Fe, although FTR1-GFP also exhibited intracellular localization. These differences suggest the two paralogs may not be functionally redundant. Ongoing kinetic experiments with over-expression clones should further reveal the roles of permeases and how they relate to the reductive-oxidative model of iron uptake.

99 Positional significance of silica targeting sequence in designing biosilica-targeted fusion proteins

Nicole R. Ford, Karen Hecht, Thomas C. Squier, Greory L. Rorrer, Guritno Roesijadi

Department of Medicine and Biosciences, Kansas City University, USA

The most commonly used silica-targeting peptide in *Thalassiosira pseudonana* is $Sil3_{T8}$, which consists of a 17 amino acid endoplasmic reticulum (ER)-targeting signal sequence and a 37 amino acid silica targeting fragment. To date, all previous fusion proteins targeted to diatom biosilica have been of the structure: ER signal sequence – targeting peptide – gene(s) of interest. Requiring the silica targeting peptide, however, to be located at the N-terminus of these complex fusion proteins can limit the functional genes of interest that are able to be expressed in diatom biosilica. Yet, no precedents have been set to have the $Sil3_{T8}$ silica targeting function uncoupled from its trafficking signal sequence, nor to locate the targeting peptide at the C-terminus of the chimeric fusion protein. Therefore, as part of optimizing our diatom expression system, we uncoupled the two functions of $Sil3_{T8}$ (targeting and trafficking) and compared the functionality of a fusion protein partner coupled to N-terminal trafficking + targeting domains vs. an N-terminal trafficking domain + C-terminal targeting domain. We found that for one particular protein, having the $Sil3_{T8}$ targeting peptide at the C-terminus of the fusion protein drastically improved its function in diatom biosilica.

POSTER PRESENTERS

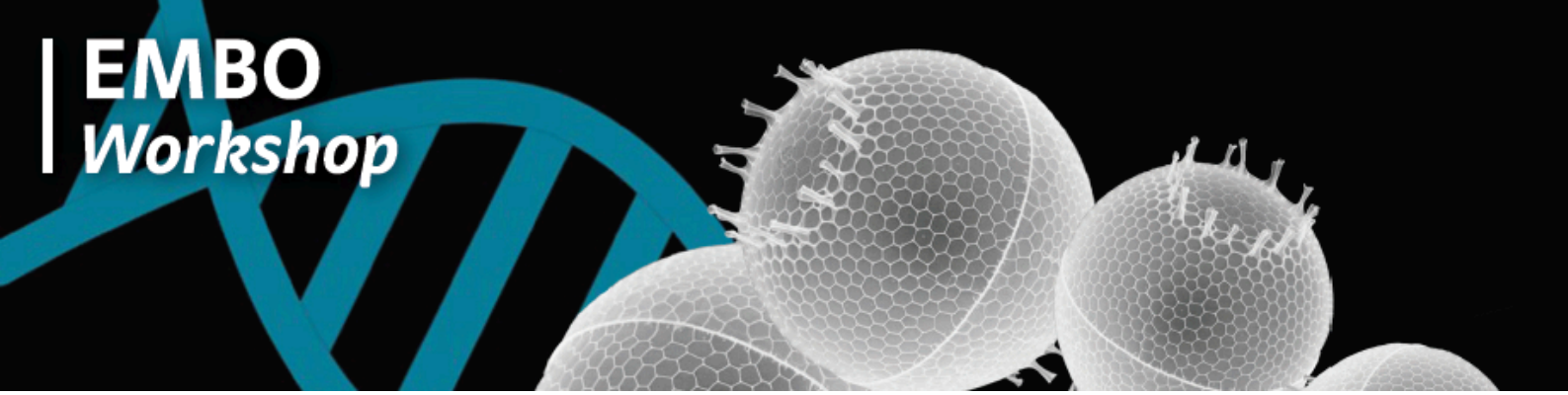
LIST OF PARTICIPANTS

First name	Last name	Email
Raffaela	Abbriano Burke	raffaela.abbriano@uts.edu.au
Joy	Akinyi	joy.akinyi@embo.org
Andrew	Allen	aallen@jcvi.org
Cicero	Alves-Lima	caljr86@gmail.com
Ryosuke	Amano	dqj96681@kwansei.ac.jp
Rossella	Annunziata	rossella.annunziata@szn.it
Lior	Aram	lior.aram@weizmann.ac.il
Ginger	Armbrust	armbrust@uw.edu
Ayesha	Asif	ayesha.asif@embo.org
Sien	Audoor	sien.audoor@ugent.be
Fatima	Awwad	FATIMA.AWWAD@USHERBROOKE.CA
Benjamin	Bailleul	bailleul@ibpc.fr
Zinka	Bartolek	zinkab@uw.edu
Darja	Belisova	darja.stojanovova@ugent.be
Erin	Bertrand	erin.bertrand@dal.ca
Debesh Chandra	Bhattacharya	debu720@gmail.com
Thomas	Bibby	tsb@noc.soton.ac.uk
Gust	Bilcke	gust.bilcke@gmail.com
Atle M.	Bones	atle.m.bones@ntnu.no
Suncica	Bosak	suncica.bosak@biol.pmf.hr
Chris	Bowler	cbowler@biologie.ens.fr
Tore	Brembu	tore.brembu@ntnu.no
Colin	Brownlee	cbr@mba.ac.uk
John	Brunson	jkbrunso@ucsd.edu
Mark	Brzezinski	mark.brzezinski@lifesci.ucsb.edu
Petra	Bulankova	petra.bulankova@psb.vib-ugent.be
Sinead	Collins	s.collins@ed.ac.uk
Hannah	Connabeer	hc9g14@soton.ac.uk
Maria Helena	Cruz de Carvalho	cruz@biologie.ens.fr
Adelaide	Cupo	adelaide.cupo@gmail.com
Sarah	D'Adamo	sarah.dadamo@wur.nl
Fayza	Daboussi	fayza.daboussi@insa-toulouse.fr
Gilles	Defrel	defrel-p@insa-toulouse.fr
Jack	Dickenson	jacdic@mba.ac.uk
Richard	Dorrell	richard.dorrell.algae@gmail.com
Carole	Duchene	carole.duchene@upmc.fr
Chris	Dupont	cdupont@jcvi.org
Bryndan	Durham	bpdurham@uw.edu
Sonya	Dyhrman	sdyhrman@ldeo.columbia.edu
Michele	Fabris	michele.fabris@uts.edu.au
Angela	Falciatore	angela.falciatore@upmc.fr
Fernan	Federici	ffederici@bio.puc.cl
Maria Immacolata	Ferrante	mariella.ferrante@szn.it
Klara	Filek	klara.filek@biol.pmf.hr
Giovanni	Finazzi	giovanni.finazzi@cea.fr
Zoe	Finkel	Zfinkel@dal.ca
Serena	Flori	serflo@mba.ac.uk
Nicole	Ford	niford@kcumb.edu
Rachel	Foster	rachel.foster@su.se

Simin	Gao	simin.gao@uea.ac.uk
Katrin	Geisler	kg404@cam.ac.uk
Jestin	George	jestin.george@student.uts.edu.au
Reuben	Gilberson	r.gilbertson@uea.ac.uk
Vasco	Giovagnetti	v.giovagnetti@qmul.ac.uk
Anna	Godhe	anna.godhe@gu.se
Johanna	Goldman	johannag@uw.edu
Kristofer	Gomes	kristofer_gomes@uri.edu
Stefan	Görlich	stefan.goerlich@tu-dresden.de
Agnes	Groisillier	agnes.groisillier@univ-nantes.fr
Irina	Grouneva	irina.grouneva@york.ac.uk
Aimilia	Grypioti	emilygrip@yahoo.com
Marthe	Hafskjold	marthe.hafskjold@ntnu.no
Christoph	Heintze	christoph.heintze@tu-dresden.de
Katherine	Helliwell	katherine.helliwell@mba.ac.uk
Patrick	Hickland	prh42@cam.ac.uk
Kat	Hodgkinson	a.hodgkinson@uea.ac.uk
Antoine	Hoguin	ahoguin@biologie.ens.fr
Amanda	Hopes	a.hopes@uea.ac.uk
Brian	Hopkinson	bmhopkin@uga.edu
Hanhua	Hu	hanhuahu@ihb.ac.cn
Ruiping	Huang	rphuang@stu.xmu.edu.cn
Andrew	Irwin	A.irwin@dal.ca
Sayako	Iwaki	pl7177db@s.okayama-u.ac.jp
Loay	Jabre	loay.jabre@dal.ca
Denis	Jallet	jallet@insa-toulouse.fr
Ana	Jaramillo	anacristina.jaramillomadrid@student.uts.edu.au
Marianne	Jaubert	marianne.jaubert@sorbonne-universite.fr
Bethany	Jenkins	bjenkins@uri.edu
Erik	Jensen	ejensen@imm.cnrs.fr
Nathalie	Joli	joli@biologie.ens.fr
Igor	Jukic	jukic@embo.org
Matthew	Julius	mljulius@stcloudstate.edu
Pannaga Pavan	Jutur	jppavan@icgeb.res.in
Takashi	Kadono	kadono-takashi@kochi-u.ac.jp
Lee-Kuo	Kang	lkkang@mail.ntou.edu.tw
Elena	Kazamia	kazamia@biologie.ens.fr
Fraser	Kennedy	f.c.kennedy@utas.edu.au
Friedrich	Kleiner	frikle@mba.ac.uk
Lydia	Köhler	lydia.koehler@tu-dresden.de
Dorothee	Kottmeier	dorkot@mba.ac.uk
Olga	Kourtchenko	olga.kourtchenko@marine.gu.se
Liv Celin	Krämer	Kraemer@bio.uni-frankfurt.de
Chana	Kranzler	chana.kranzler@marine.rutgers.edu
Nils	Kröger	nils.kroeger@tu-dresden.de
Peter	Kroth	Peter.Kroth@uni-konstanz.de
Ekta	Kumari	ekta.kumari@tu-dresden.de
Daniel	Kurpan	danielkurpan@gmail.com
Adam	Kustka	kustka@rutgers.edu
Emma	Langan	emma.langan@uea.ac.uk
Michel	Lavoie	michel.lavoie.4@ulaval.ca
Chun-Ting	Lee	0033b207@gmail.com

Gabriella	Leone	gabriellaleone90@gmail.com
Sarah	Lerch	slerch@uri.edu
Ben	Leyland	leyland@post.bgu.ac.il
Xiaobo	Li	lixiaobo@westlake.edu.cn
Zhengke	Li	zkli@dal.ca
Yue	Liang	yliang@mta.ca
Jun-Rong	Liang	sunljr@xmu.edu.cn
HungYun	Lin	hungyun59@gmail.com
Senjie	Lin	senjie.lin@xmu.edu.cn
Chung-Hsiao	Liu	lighmor@gmail.com
Marcel	Llavero Pasquina	ml787@cam.ac.uk
Luke	Mackinder	luke.mackinder@york.ac.uk
Yoshiaki	Maeda	y_maeda@cc.tuat.ac.jp
Kanako	Maeda	a9xx1220@gmail.com
Wilhelm	Mandl	w.mandl@conference-service.com
Francesco	Manfellotto	francesco.manfellotto@szn.it
TInotenda	Mangadze	mangadzetinotenda@gmail.com
Alessandro	Manzotti	alessandro.manzotti.1994@gmail.com
Adrian	Marchetti	amarchetti@unc.edu
Thomas Kiran	Marella	tomccmb@gmail.com
Yusuke	Matsuda	yusuke@kwansei.ac.jp
James	McCarthy	jmccarth@jcvi.org
Linda	Medlin	lkm@mba.ac.uk
Annika	Messemer	annika.messemer@ntnu.no
Alexander	Milentyev	milentyev@mpi-cbg.de
Avia	Mizrachi	avia.mizrachi@weizmann.ac.il
Thomas	Mock	t.mock@uea.ac.uk
Anna-Marie	Moody	a.moody@uea.ac.uk
Daniel	Moog	daniel.moog@biologie.uni-marburg.de
Mark	Moosburner	mmoosbur@jcvi.org
Satoshi	Nagai	snagai@affrc.go.jp
Hermanus	Nawaly	herrynawaly@gmail.com
Michiko	Nemoto	mnemoto@okayama-u.ac.jp
Mai	Nguyen	maingoc303@gmail.com
Tomomi	Nonoyama	t-nonoyama@st.go.tuat.ac.jp
Marianne	Nymark	nymark@bio.ntnu.no
Andre	Ohara	andre.ohara@tu-dresden.de
Monica	Orellana	morellan@uw.edu
Cristina Maria	Osuna Cruz	crosu@psb.vib-ugent.be
Karen	Page	karen.page@ucl.ac.uk
Katerina	Pargana	aikaterini.pargana@ugent.com
Katerina	Pargana	aikaterini.pargana@ugent.be
Alexandra	Peltekis	alexandra_kala@hotmail.fr
Stephen	Pewter	stephen.pewter@embo.org
Monica	Pichler	m.pichler@uea.ac.uk
Juan José	Pierella Karlusich	pierella@biologie.ens.fr
Ute	Postel	ute.postel@awi.de
Nicole	Poulsen	nicole.poulsen@tu-dresden.de
Gunjan	Prakash	gunjaniit@gmail.com
Peter Paolo	Rivera	p.rivera@uea.ac.uk
Deborah	Robertson	debrobertson@clarku.edu
Elizabeth	Robertson	elizabeth.robertson@gu.se

Alessandra	Rogato	alessandra.rogato@ibbr.cnr.it
Monia Teresa	Russo	moniarusso@gmail.com
Valeria	Sabatino	valeria.sabatino@tu-dresden.de
Remo	Sanges	remo.sanges@gmail.com
Krisztina	Sárközi	k.sarkozi@uea.ac.uk
Michael	Schlierf	michael.schlierf@tu-dresden.de
Sarah	Schwenck	sschwenc@ucsd.edu
Manuel	Serif	manuel.serif@ntnu.no
Deepak	Sethi	drsethi1@sheffield.ac.uk
Amit	Sharma	contactamyt@gmail.com
Ahmed A.	Shibl	ahmed.shibl@nyu.edu
Mahendra Kumar	Shukla	m.shukla@qmul.ac.uk
Alison	Smith	as25@cam.ac.uk
Sarah	Smith	sarah.smith@jcvi.org
Mohamamd	Soleimani	m.soleimani@tue.nl
Jan	Strauss	jan.strauss@embl-hamburg.de
Barbara	Szantho	Barbara.szantho@gmail.com
Tsuyoshi	Tanaka	tsuyo@cc.tuat.ac.jp
Issei	Terauchi	s175427w@st.go.tuat.ac.jp
Kim	Thamatrakoln	thamat@marine.rutgers.edu
Adrien	Thurotte	adrienthurotte@gmx.fr
Leila	Tirichine Delacour	tirichine-l@univ-nantes.fr
Jonathan	Todd	jonathan.todd@uea.ac.uk
Andrew	Toseland	a.toseland@uea.ac.uk
Luis	Valente	luis.valente@embo.org
Klaus	Valentin	valentin@awi.de
Adeeba Fathima	Valiya Thodiyil	adeeba_fathima.valiya_thodiyil@tu-dresden.de
Marine	Vallet	mvallet@ice.mpg.de
Olivier	Vallon	ovallon@ibpc.fr
Emmelien	Vancaester	emcae@psb.ugent.be
Jyoti	verma	diatombuster@gmail.com
Frédéric	Verret	fverret@imbb.forth.gr
Charlotte	Volpe	charlotte.volpe@ntnu.no
Wim	Vyverman	wim.vyverman@ugent.be
Ross	Waller	rfw26@cam.ac.uk
Gerlind	Wallon	wallon@embo.org
Pamela	Walsh	pamela.walsh@qub.ac.uk
Kahori	Watanabe	s155298u@st.go.tuat.ac.jp
Glen	Wheeler	glw@mba.ac.uk
Matthias	Windhagauer	matthias.windhagauer@student.uts.edu.au
Per	Winge	per.winge@ntnu.no
Taoyang	Wu	taoyang.wu@uea.ac.uk
Angela	Wulff	angela.wulff@bioenv.gu.se
Daniel	Yee	daniel.p.yee@gmail.com
Kohei	Yoneda	yoneda30311@gmail.com
Jodi	Young	youngjn@uw.edu
Jirina	Zackova Suchanova	Jirina.Zackova_Suchanova@tu-dresden.de
Jonathan	Zehr	jpzehr@gmail.com
Lisa	Zeigler Allen	lzeigler@jcvi.org
Huan	Zhang	zhanghuan@westlake.edu.cn
Xue	Zhao	xue.zhao@univ-nantes.fr



SPONSORS

EMBO reports

EMBOpress

University of East Anglia

University of East Anglia | SCHOOL OF ENVIRONMENTAL SCIENCES

biology
an Open Access Journal by MDPI

SPRINGER NATURE