The Biophysics of Motile Cilia - from Structure to Function

830. WE-Heraeus-Seminar

30 March - 02 April 2025

at the Physikzentrum Bad Honnef, Germany

WILHELM UND ELSE
HERAEUS-STIFTUNG

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 830. WE-Heraeus-Seminar:

Motile cilia are fundamental organelles of eukaryotic cells and regulate numerous biological processes. For example, in the respiratory tract or fallopian tube, the entire epithelial surface is covered with a carpet of motile cilia, whose beat is synchronised to transport mucus, pathogens, and dirt particles out of the airways. Motile cilia are not only used for fluid transport, but also for locomotion of unicellular organisms and single cells as well as to perceive environmental cues. A prime example are sperm cells that are propelled by their single motile cilium, named flagellum, which also serves as a rudder and sensory antenna. Unravelling the physiology of these integral cell organelles is an interdisciplinary endeavour at the interface of physics and biology. Integrated research approaches combining basic research on both the fundamental biological and physical principles of ciliary function are required to gain insights into the structure and function of individual cilia as well as the mechanisms orchestrating and synchronizing ciliary beating patterns. Clinical research and treatment of patients with ciliopathies depends on the biophysical knowledge gained by such approaches.

The aim of this seminar is to bring together scientists from different disciplines to address the physical, biological, and clinical aspects of motile cilia.

Introduction

Scientific Organizers:

Prof. Dr. Benjamin M. Friedrich	Physics of Life, TU Dresden Dresden, Germany
Dr. Veikko F. Geyer	B CUBE, TU Dresden Dresden, Germany
Prof. Dr. Timo Strünker	Centre of Reproductive Medicine and Andrology University of Münster Münster, Germany

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Introduction

Venue:

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Registration:

Marion Reisinger (WE Heraeus Foundation)
at the Physikzentrum, reception office
Sunday (14:30 h – 19:30 h) and Monday morning

Program

Program

Sunday, 30 March 2025

14:30 – 19:30	Registration	
16:45-17:00	Benjamin Friedrich, Veikko Geyer, Timo Strünker	**Welcoming Note**
	Ultrastructure of cilia (chair: Timo Strünker)	
17:00-17:45	Gaia Pigino	**Towards a mechanistic understanding of ciliary transport and assembly**
17:45-18:30	Tzviya Zeev Ben Mordehai	**Molecular specialisation of the sperm flagella**
18:30-19:30	*BUFFET SUPPER and informal get-together*	
	Keynote lecture (chair: Benjamin Friedrich)	
19:30-20:30	Manu Prakash	**tba**
20:30-22:00	***Get-together***	

Program

Monday, 31 March 2025

07:45	*BREAKFAST*	
08:45– 09:00	Scientific Organizers	**Video "About the Wilhelm and Else Heraeus Foundation"**
	Motor dynamics and ciliary beat (chair: Veikko Geyer)	
09:00 - 09:45	Alan Brown	**Structural studies of axonemal dyneins**
09:45 – 10:30	Antonio de Simone	**Recent results on the modeling of the flagellar beat**
10:30 –10:35	CONFERENCE PHOTO (in front of the lecture hall)	
10.35-11:00	*COFFEE BREAK*	
11:00-12:30	***Data blitz***	
	Cynthia He	**ARL3 GTPases facilitate ODA16 unloading from IFT in motile cilia**
	Tomer Avidor-Reiss	**The Centriolar Rod Protein POC1B Controls Sperm Tail Beating and Head Kinking**
	Ron Orbach	**Unraveling the Mechanisms of Microtubule Stabilization in Ciliary Axonemes**
	Louis Woodhams	**Internal forces, axoneme distortion, and dynamic instability in multi-filament models of the ciliary axoneme**
	Andrej Vilfan	**Hydrodynamic synchronization of cilia in finite systems**
12:30-14:00	*LUNCH*	

Program

Monday, 31 March 2025

Modeling of motor control (chair: Jonathon Howard)

14:00 - 14:45	Jonathon Howard	**Mechanics of Ciliary motility**
14:45 - 15:30	Hermes Gadelha	**Motor Organization in Axonemal Machines: A Story Far from Over**
15:30 – 16:00	*COFFEE BREAK*	
16:00 -17:30	**Poster blitz** (one-minute oral presentations of poster presentations)	
17:30 – 18:30	**Poster session**	
18:30 – 19:30	*DINNER*	
19:30-21:00	**Poster session**	

Program

Tuesday, 01 April 2025

08:00	*BREAKFAST*	
	3D ciliary waveforms (chair: Gaia Pigino)	
09:00 – 09:45	Kirsty Wan	**Origins of diaplectic metachronal waves**
09:45 – 10:30	Laurence Wilson	**Holographic imaging of the flagellar waveform of Chlamydonomas reinhardtii**
10:30 – 11:00	*COFFEE BREAK*	
	Ciliary synchronization (chair: Nathalie Jurisch-Yaksi)	
11:00 – 11:45	Pietro Cicuta	**Fluid flows within and close to ciliated epithelia**
11:45 – 12:30	Jana Nawroth	**Structure-function relationships of human airway mucociliary clearance**
12:30 – 14:00	*LUNCH*	
	Cilia signalling and navigation (chair: Benjamin Kaupp)	
14:00 – 14:45	Gaspar Jekely	**Multiciliated sensory cells mediate hydrostatic pressure sensation in zooplankton**
14:45 – 15:30	Alan Tsang	**Phototactic responses of ciliary microorganisms**
15:30 – 16:00	*COFFEE BREAK*	
	Cilia mechano-sensing (chair: Daniel Tam)	
16:00 – 16:45	Julien Vermot	**Cilia motility, mechanosensitivity and left-right specification in vertebrate embryos**

Program

Tuesday, 01 April 2025

16:45 – 17:30	Oliver Bäumchen	**Light-Switchable Ciliary Adhesion to Surfaces**
	Keynote lecture (chair: Veikko Geyer)	
17:30 – 18:30	David Mitchell	**Why and how did 9+2 cilia evolve?**
18:30 – 18:45	**Poster Prizes**	
18:45	*HERAEUS DINNER* *(social event with cold & warm buffet with complimentary drinks)*	

Wednesday, 02 April 2025

07:30 – 08:30	*BREAKFAST*	
	Tools to study cilia dynamics (chair: Jean-Ju Chung)	
08:30 – 09:15	Jan Hansen	**Studying dynamics and heterogeneity of cilia**
09:15 -10:00	***Data Blitz***	
	Martin Striegler	**Twist–torsion coupling in beating axonemes**
	Cesar O. Pacherres	**Effects of environmental stress on the cilia beating frequency and vortex formation in corals**
10:00 – 10:30	*COFFEE BREAK*	
10:30 – 11:30	***Data blitz***	
	Emma van Grinsven	**Basal body blueprint: Expansion microscopy of microtubules in multiciliated cells**

Program

Wednesday, 02 April 2025

	Jean-Ju Chung	**CATSPERε extracellular domains are essential for sperm CatSper channel assembly and activity modulation**
	Jane Chui	**Synchronisation of flagella in cellular carpets derived from Volvox carteri**

Ciliopathies (chair: Timo Strünker)

11:30 – 12:15	*Nathalie Jurisch-Yaksi*	*The role of cilia and ependymal cells in cerebrospinal fluid dynamics*
12:15 – 13:00	*Melanie Balbach*	*Soluble adenylate cyclase – Sperm metabolic sensor and contraceptive target?*
13:00 – 14:30	*LUNCH*	

End of the seminar and departure

For participants leaving on Thursday a self-service breakfast will be provided on Thursday morning.

Posters

Poster Session – Monday 31 March 2025, afternoon

Goncalo Antunes	**Corrugated channels can filter ciliated microorganisms based on the metachronal wavelength**
Markus Baer	**Hydrodynamic synchronization of elastic cilia: The impact of surface effects and unsteady viscous flow on the characteristics of metachronal waves**
Rafał Błaszkiewicz	**Ciliary cooperation and transport in unsteady Stokes flows**
Alexander Boggon	**Complexity in the ciliary dynamics of a swimming microorganism**
Christoph Brenker	**Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation**
Bin Cai	**Structure and assembly of A-C linker connecting microtubule triplets in centrioles**
Viridiana Carmona Sosa	**How cilia beating affects the swimming of exogenous bodies in airways?**
Rodrigo E. Catalan	**A flavin-based photoreceptor controls the photoactivation of ciliary adhesion in Chlamydomonas**
Michal Czerepaniak	**Curves and currents: modelling the effects of surface curvature on ciliary flows**
Sophia Fochler	**Axonemal dynein contributions to the symmetric and asymmetric waveforms in Leishmania.**
Meurig Gallagher	**Driving Curvature: The influence of sperm head shape on Flagellar Kinematics**
Sebastian George	**Developments of the invitro ciliary transport assay for the diagnosis of Primary Ciliary Dyskinesia**
Leonie Herrmann	**Chemosensory signaling in human sperm is controlled by Ca^{2+} influx via CatSper and Ca^{2+} clearance via plasma membrane Ca^{2+} ATPases**

Poster Session – Monday 31 March 2025, afternoon

Lara Melanie Hoepfner	**Unwrapping the ciliary coat: high-resolution structure and function of the ciliary glycocalyx in Chlamydomonas reinhardtii**
Yameng Huang	**ARL13 and ARL3 regulate the unloading of ODA16 from IFT in motile cilia**
Meiqin Jiang	**Human IFT-A complex structures provide molecular insights into ciliary transport**
Kei Jokura	**Neuronal coordination of Gravity-Sensing Cilia in the ctenophore apical organ**
Olivia Kendall	**Soluble adenylyl cyclase (sAC) in non-mammalian sperm is directly controlled by pH, not HCO_3- or Ca^{2+}**
Friedrich Kleiner	**Role of mastigonemes in the green alga C. reinhardtii**
Julia Koenig	**A range of 30%-62% of functioning multiciliated airway cells is sufficient to maintain ciliary airway clearance**
Elijah Lee	**The wavelength of the ciliary beat in wild-type and mutant Chlamydomonas reinhardtii saturates at ciliary lengths above 15 µm**
Hakon Leffler	**Galectins and Cilia**
Girish Ram Mali	**Molecular basis for the activation of outer dynein arms in cilia**
Mariana Medina Sanchez	**Cilia & Flagella: Bio-inspired solutions for microrobotics**
Yasmin Magdy Emadeldin Mohamed Abdelghaffar	**Stochastic modeling of a two-component polymer engine**
Adrian Nievergelt	**The energetics of swimming motility in Chlamydomonas**
Xiaoyi Ouyang	**Identification of force-generating dynein states in reactivated Chlamydomonas axonemes**

Poster Session – Monday 31 March 2025, afternoon

Andrea Perna	**Relationship between movement, feeding and the energy budget of the ciliate Tetrahymena pyriformis**
Nunziana Pezzella	**The role of OFD1 in motile cilia**
Emmie Pohjanen	**Subcellular protein architecture of human sperm**
Arun Ravi	**Synchronization driven flows and motility of spherical ciliates**
Xiaomeng Ren	**Fluid flow reconstruction around a free-swimming sperm in 3D**
Lea Rupprecht	**Ciliary Adhesion of Chlamydomonas reinhardtii on Charge-Functionalized Surfaces**
Daniel Tam	**Phase sensitivity of flagellar beating to external forces**
Darren Teo	**Fantastic LCs and what do they do?**
Alina Wilken	**Primary ciliary dyskinesia associated disease-causing variants in CCDC39 and CCDC40 cause axonemal absence of inner dynein arm heavy chains DNAH1, DNAH6, and DNAH7**
Kai Wohlgemuth	**Pathogenic variants in CFAP46, CFAP54, CFAP74 and CFAP221 cause primary ciliary dyskinesia with a defective C1d projection of the central apparatus**
Hengxi Zhang	**In situ arrangement of CatSper Ca^{2+} channels in CATSPER2-deficient human sperm**

Abstracts of Lectures

(in alphabetical order)

The Centriolar Rod Protein POC1B Controls Sperm Tail Beating and Head Kinking

L. Achinger[1], L. Yaghutian[1], K. Turner[1] , G. Jolivet[3], S. Khanal[1], S. Unnikrishnan[2], C-K. Tung[2], and T. Avidor-Reiss[1, 4]

[1] Department of Biological Sciences, College of Natural Sciences and Mathematics, University of Toledo, Toledo, OH, 43606, USA. [2] Department of Physics, North Carolina A&T State University, Greensboro, NC, United States. [3] Biologie du Développement et Reproduction, UMR1198, INRA ENVA, 78352, Jouy-en-Josas, France. [4] Department of Urology, College of Medicine and Life Sciences, University of Toledo, Toledo, OH, United States

Spermatozoa exhibit coordinated tail beating (whip-like, back-and-forth tail bending movement) and head kinking (hinge-like back-and-forth bending) behavior during swimming in snap-frozen bovine specimens[2]. However, the precise mechanism coordinating these two behaviors is unknown. We proposed that the spermatozoa centrosome mediates this coordination. The centrosome includes a splayed (atypical) distal centriole with two unique rod-like structures scaffolding its right and left sides[1]. During sperm swimming, the distal centriole rods slide in correlation with the sperm tail beating and head kinking[2]. Here, we studied head kinking in bovine sperm and the essential function of rod-sliding in swimming by mutating the rabbit rod protein POC1B.

We observed head kinking in live bovine sperm videos, and its periodicity correlated with tail curvature. The period length correlates with head kinking amplitude, indicating these two movements are linked. Modeling the tail as two filaments, based on tail curvature and distal centriole rod positions, suggests that the head position correlates the most with the sperm tail microtubule sliding within the proximal 24 microns.

We used rabbits as a genetic model as, unlike mice, rabbit sperm centrioles resemble those in human sperm. POC1B complete loss results in abnormal sperm morphology, blocking swimming studies. We overcome this by making a subtle mutation, deleting 78 amino acids (Δ78) from POC1B's C-terminus. POC1BΔ78 spermatozoa centrioles lack POC1B, indicating the essential role in its centriolar localization. POC1BΔ78 mutant mails are fertile, and their spermatozoa have normal morphology and are swimmers. However, preliminary data suggests that the mutant sperm have short, static fused centriole rods, indicating a critical POC1B role in rod formation and sliding. POC1BΔ78 has reduced tail beating and head kinking ranges and periodicity compared to the wild type, suggesting that rod fusion limits the axoneme movement. Finally, POC1B Δ78 male fertility was reduced in sperm competition experiments, implying that centriole rod sliding provides a reproductive advantage.

Our preliminary data suggest that sperm tail beating drives head kinking via the sperm centriole and that POC1B and the rods control the amplitude of these processes, which is essential for sperm competition in double-mating experiments. These findings support the hypothesis that the sperm centrosome is a transmission system connecting and coordinating sperm tail beating and head kinking.

References

[1] Fishman, E.L., et al. (2018). Nature Communications 9, 2210.
[2] Khanal, S., et al. (2021). Nat Commun 12, 3808.

Soluble adenylate cyclase – Sperm metabolic sensor and contraceptive target?

Sara Violante[1], Lana Kouatli[2], Lonny R. Levin[3], Jochen Buck[3], Melanie Balbach[2]

[1]Donald B. and Catherine C. Marron Cancer Metabolism Center, Memorial Sloan Kettering Cancer Center, New York City, NY

[2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

[3]Department of Pharmacology, Weill Cornell Medicine, New York City, NY

Mammalian sperm are stored in the epididymis in a dormant state. Upon ejaculation, they must immediately start producing sufficient energy to maintain motility and support maturation in the female genital tract (capacitation). While the increased energy demand during capacitation is well-established, it remains unclear how mammalian sperm modify their metabolism to meet this need. Soluble adenylyl cyclase (sAC) is essential for sperm motility and capacitation. In somatic cells, sAC was identified as a major regulator of metabolic reprogramming. In sperm, the interplay between sAC, sperm maturation, and sperm metabolism remains mostly unexplored.

Previously, we showed that capacitating sperm increase the rates of both glycolysis and TCA cycle/oxidative phosphorylation, demonstrating that sperm actively regulate the flux through central carbon metabolism[1,2]. Using an extracellular flux analyzer, metabolomics, and stable isotope labeling, we compare the metabolism of non-activated and activated sperm in the absence and presence of sAC inhibition *in vitro* and *in vivo*. Using the same pharmacology, we explore the effects of on-demand sAC inhibition *in vivo* on male fertility.

By combining these techniques we identify phosphofructokinase as the main rate-regulating step in sperm glycolysis, observe a uniquely rapid uptake and metabolism of carbohydrates into lactate and pyruvate into citrate, and reveal that during capacitation the activity of glucose-6-phosphate dehydrogenase is downregulated, resulting in reduced carbon flux through the pentose phosphate pathway. Our study suggests that a subset of glycolytic steps and the uptake of pyruvate into the TCA cycle are sAC-dependent. Moreover, we show that a single dose of a safe, acutely-acting sAC inhibitor with long residence time renders male mice temporarily infertile[3].

Our study provides new insights into the energy production during mammalian sperm capacitation and experimental evidence for a link between sAC-regulated signaling pathways and mammalian sperm metabolism. Our studies also define sAC inhibitors as leads for on-demand contraceptives for men, and they provide *in vivo* proof-of-concept for previously untested paradigms in contraception; on-demand contraception after just a single dose and pharmacological contraception for men.

1 Balbach, M. *et al.* Metabolic changes in mouse sperm during capacitation. *Biol Reprod* **103**, 791-801 (2020). https://doi.org/10.1093/biolre/ioaa114

2 Balbach, M. *et al.* Capacitation induces changes in metabolic pathways supporting motility of epididymal and ejaculated sperm. *Front Cell Dev Biol* **11**, 1160154 (2023). https://doi.org/10.3389/fcell.2023.1160154

3 Balbach, M. *et al.* On-demand male contraception via acute inhibition of soluble adenylyl cyclase. *Nat Commun* **14**, 637 (2023). https://doi.org/10.1038/s41467-023-36119-6

Light-Switchable Ciliary Adhesion to Surfaces

O. Bäumchen[1]

[1]*Experimental Physics V, University of Bayreuth, Bayreuth, Germany*

The light-switchable adhesiveness of the cilia of *Chlamydomonas*, a unicellular biciliated microorganism [1], represents an evolutionary advantage of photosynthetic microbes for the optimization of their ability to harvest light. In conjunction with phototaxis, a light-sensitive motility mode, these microbes can identify ideal conditions to thrive in their natural habitats. Since the discovery of the light-controlled on/off-switch of ciliary adhesion to surfaces [2], the identification of the biomolecular mechanism [3], signal transduction and the underlying photoreceptor(s) [4] have played pivotal roles in research.

Recently, we identified two abundant cryptochromes that govern this protein-mediated ciliary adhesion phenotype. We apply single-cell *in vivo* micropipette force spectroscopy [5], a non-invasive technique to measure ciliary adhesion to surfaces with a force resolution of about 10 pN at tailored light conditions, to wild-type cells as well as a series of photoreceptor deletion mutants. Following this experimental approach, we decipher the unique role of the cryptochromes for this unspecific [6] and universal adhesion phenotype of eukaryotic cilia.

References

[1] R. Catalan, A. Fragkopoulos, A. Girot, M. Lorenz, and O. Bäumchen, Nature Protocols, in press (2025).

[2] C.T. Kreis, M. Le Blay, C. Linne, M.M. Makowski, and O. Bäumchen, Nature Physics **14**, 45 (2018).

[3] N. Xu, A. Oltmanns, L. Zhao, A. Girot, M. Karimi, L. Höpfner, S. Kelterborn, M. Scholz, J. Beissel, P. Hegemann, O. Bäumchen, L. Liu, K. Huang, and M. Hippler, eLife **9**, e58805 (2020).

[4] R. Catalan, A. Fragkopoulos, N. von Trott, S. Kelterborn, O. Baidukova, P. Hegemann, and O. Bäumchen, Soft Matter **19**, 306 (2023).

[5] M. Backholm and O. Bäumchen, Nature Protocols **14**, 594 (2019).

[6] C.T. Kreis, A. Grangier, and O. Bäumchen, Soft Matter **15**, 3027 (2019).

Structural diversity of axonemal dynein subtypes

A. Brown[1]

[1]*Harvard Medical School, Boston, USA*

The rhythmic oscillations of cilia and flagella generate forces essential for cell propulsion and fluid dynamics in eukaryotes. The oscillating motions are tailored to specific functions and vary between species and cell types. This diversity of beating patterns occurs despite broadly uniform flagellar structures in which nine parallel and evenly spaced doublet microtubules (DMTs) encircle a central pair (CP) of singlet microtubules, an arrangement known as the 9+2 axoneme. Each DMT is studded by two rows of dynein motors, categorized as the outer dynein arm and inner dynein arm based on their respective positions. The outer dynein arm is formed of 24-nm repeating copies of a large dynein complex (ODA) with two or three heavy chains (HCs), depending on the species. The inner dynein arm is a collection of smaller dynein complexes (IDAs), with each IDA repeating every 96 nm. Seven different IDAs can occupy a single 96-nm DMT segment: one with two HCs (IDA*f*) and six with one HC (IDA*a*-*e* and IDA*g*). Each HC contains an N-terminal “tail” that contributes to docking to the DMT and binds auxiliary subunits and a C-terminal ATPase “head” domain which consumes ATP to drive a dynein step cycle.

In this talk, I will describe single-particle analysis electron cryomicroscopy (SPA cryo-EM) studies of DMT-bound axonemal dyneins from a variety of species including *Chlamydomonas reinhardtii* [1,2], mammals including humans [2,3] and *Leishmania tarentolae*. Structural comparison reveals how axonemal dynein structure, subunit composition and microtubule docking differs between organisms. The relevance of these species-specific differences to ciliary motility will be discussed.

References

[1] T. Walton, H. Wu, A. Brown, Nat. Commun., **21**, 477 (2021)

[2] T. Walton, M. Gui, et al., Nature, **618**, 625–633 (2023)

[3] M. Gui, H. Farley, P. Anujan, J.R. Anderson, et al., Cell, **184**, 5791–5806 (2021)

Synchronisation of flagella in cellular carpets derived from *Volvox carteri*

J. Y. Y. Chui[1] and R. E. Goldstein[1]

[1]University of Cambridge, Cambridge, United Kingdom

From unicellular ciliates to respiratory epithelium, the existence of cilia carpets on these cell surfaces enable important functions, such as travelling through the earth's oceans or debris clearing via fluid flow generation respectively. These cilia are not centrally controlled, but rather work collectively by synchronizing their beating cycles and generating metachronal waveforms. Both theoretical and experimental studies of the mechanisms leading to the emergence of these metachronal waves have been done largely independent of boundary conditions, and so in this study we seek to investigate the validity of this assumption as well as observe the effects of different boundary conditions. Colonial alga *Volvox carteri* are an ideal model organism for this study due to their size and penchant for metachronal waves. We break these spherical colonies into pieces using a homogenizer, and use imaging techniques to observe how changes in the number of cells, shape, and boundary conditions change how the cilia interact and synchronize with each other. The characteristic shape and size of these broken-off pieces will also inform us on residual stresses in the extracellular matrix (ECM) of a volvox colony as it expands over its lifecycle, and contribute to important questions regarding structural integrity and aging as it relates to ECM in general.

CATSPERε extracellular domains are essential for sperm CatSper channel assembly and activity modulation

Jae Yeon Hwang[1,2,5], Huafeng Wang[1,5], Gillian Clouser[1], Jong-Nam Oh[1], Sarah F. Finnegan[1], Niels E. Skakkebaek[3] and Jean-Ju Chung[1,4,*]

[1]Department of Cellular and Molecular Physiology, Yale School of Medicine, CT; [2]Department of Molecular Biology, Pusan National University, Busan, South Korea; [3]Department of Growth & Reproduction and EDMaRC, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [4]Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, CT

[5]Equal contribution

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Abstract

The flagellar-specific Ca^{2+} channel CatSper is a multiprotein complex that is critical for successful fertilization by controlling the sperm Ca^{2+} signaling in space and time. Large extracellular domains (ECDs) of four single-pass transmembrane subunits, CATSPERβ, γ, δ, and ε, form a unique canopy structure over the pore-forming channel. However, the molecular mechanisms of canopy assembly during development and its physiological function in mature sperm remain unknown. Here, using two genetic mouse models and the biochemical isolation of a bioactive CATSPERε fragment, we report that CATSPERε ECDs are essential for assembling the CatSper canopy, and thus the entire channel complex, and for modulating CatSper function for sperm hyperactivation and fertilization. CATSPERε-deficient males are sterile because their sperm fail to develop hyperactivated motility due to the absence of the entire channel. In transgenic mice overexpressing CATSPERε with truncated ECDs in testicular germ cells, truncated CATSPERε is unable to interact with native CatSper subunits and incorporate into the complex, thus failing to rescue the defective sperm hyperactivation and infertility of *Catspere*-null males. However, the addition of a purified Ig-like domain to normal sperm significantly reduced CatSper channel activity during sperm capacitation. These findings provide insight into the underlying molecular and developmental mechanisms of CatSper complex assembly and how CatSper channels can be modulated in physiological settings and by therapeutic intervention.

Fluid flows within and close to ciliated epithelia

Pietro Cicuta

University of Cambridge, Cambridge, UK

The ciliated epithelium of the human respiratory tract is covered by the airway surface liquid (ASL), a protective fluid consisting of two layers: the periciliary layer (PCL), where motile cilia reside and generate fluid flow, and an overlying mucus layer.

The complex structure and stratified nature of the PCL complicate both the prediction and quantification of fluid flow at the scale of individual or small groups of cilia, making it difficult to connect microscopic flows to macroscopic clearance. To tackle this challenge, we developed a novel methodology that involves ‘un-caging’ a fluorescent compound to trace the flow field within the PCL. Fluorescence is activated at micrometric spots within the cilia layer, and displacement vectorsand diffusion are recorded using high-speed video. Our experiments reveal a complex fluid transport pattern, with displacement velocity along the epithelial surface varying due to a non-uniform vertical flow field. Additionally, we observed that cilia expel fluid at their tips, a mechanism likely aimed at preventing pathogen access to the epithelium. Simulations, where cilia are modeled as arrays of rigid rods with length asymmetry, support these findings and offer new insights into the dynamics of fluid transport in the respiratory tract and the critical role of cilia coordination.

The fluid flow away from the beating epithelium is also complex. We measure this by using a set of microscopic colloidal particles, held in weak optical traps, such that the cilia-induced flows cause fluctuations of the particle positions. This data allows us to map the spatial decay of the parallel and perpendicular components of flow, as a function of distance from the beating plane.

Antonio De Simone
The Biorobotics Institute, Scuola Superiore Santanna, Pisa
SISSA-Mathlab, Trieste

Title:
Recent results on the modeling of the flagellar beat

Abstract:
Active matter is a broad field with many potential applications. A common thread underlying many of the current research lines is the study of systems powered by some internal energy source, as in the case of organisms moving thanks to food metabolism. In fact, self-propelling systems need to overcome the resistance of the surrounding medium, drawing the required energy from internal sources. The study of locomotion and self-propulsion in biological and bio-inspired artificial system appears, therefore, as an ideal testing ground to put the concepts and tools of active matter at work.
We will report on recent progress coming from case-studies on the motility of unicellular organisms (flagellates and ciliates) and bio-inspired micro-robots, studied from the point of view of the mechanics of active matter. The study of the microscopic underpinnings of the beat of eukaryotic cilia and flagella will be the focus of the presentation.

Motor Organization in Axonemal Machines: A Story Far from Over

Hermes Bloomfield-Gadelha [1]

[1]*School of Engineering Mathematics and Technology and Bristol Robotics Laboratory, University of Bristol, UK*

This talk presents a few updates from Polymaths Lab on the fascinating topic of self-organized axonemal beating. We explore how molecular motors coordinate rhythmic ciliary motion and how three-dimensionality shapes, and sometimes suppresses, these dynamics.

We will cover three main areas: (i) mathematical modelling of axonemal mechanochemistry in 2D and 3D (J Cass & P Fuchter), (ii) the paradox of planar wave emergence in 3D beating (Fuchter), (iii) characterization of 3D waveforms with the Corkidi and Darszon labs, and what fluid mechanics from experimental 3D waveforms (X Ren) reveals about sperm swimming.

A recent reaction-diffusion model abstraction enables comparisons with bull sperm and *Chlamydomonas reinhardtii* data, showing that sliding-controlled motors can sustain autonomous bending waves independently of the fluid. A 3D multi-physics approach reveals that planarity emerges via self-organized dynein clustering, periodically splitting the axoneme into two halves to generate planar beats. Meanwhile, 3D microscopy exposes counter-rotating vortices in human sperm, revealing fundamental hydrodynamic patterns. We also show that sperm stabilize progressive swimming by spinning like tops, even with asymmetric beats.

In all, we will showcase new empirical and mathematical tools developed at Bristol to study the endless universe of the axoneme. As for every answer found, more questions emerge. Axoneme self-organization seems destined to remain a mystery for a very long time…

References

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TOWARDS SYSTEMS-LEVEL CILIA SCIENCE: DATA-DRIVEN EXPLORATION OF CILIA DYNAMICS AND HETEROGENEITY

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Cilia are tiny, filamentous organelles emanating from the surface of most human cells. Their dysfunction is linked to hereditary ciliopathies with diverse symptoms. Despite their significance, we still have much to learn about the roles, functions, and mechanisms of cilia in different human cell types. Most studies investigate a few individual cells/cilia limiting the accuracy, reproducibility, and applicability of the study or pool millions of cells/cilia for analysis, lacking spatial and single-cilia resolution.

Addressing this gap, we developed innovative techniques to study cilia with higher precision and higher throughput. Traditionally, examining cilia relies heavily on image-based analysis which has limitations when applied on a large scale. Over the past decade, we've advanced this field by developing user-friendly but comprehensive image analysis tools, such as: SpermQ, for analyzing the beat of sperm flagella and motile cilia; FreQ, to study motion in multi-ciliated cells; and CiliaQ, to examine the length, shape, and content of primary cilia.

Currently, our research focuses on developing and applying large-scale imaging-based spatial proteomics approaches to map the protein composition of single cilia with high resolution and relate it to ciliary and cellular states. We created a cilia and flagella section in the Human Protein Atlas (https://www.proteinatlas.org/) [1], featuring 17,000 and 11,000 high-resolution confocal microscopy 3D-stacks of over 100,000 cells with primary cilia and over 200,000 sperm cells, respectively. The atlas reveals the subciliary localization patterns of over 600 proteins across individual cilia or flagella and serves as a template for studying subciliary protein localization, dynamics, and heterogeneity. Analyzing these data sets, we observed that the ciliary proteome in different human cell types is highly cell-type specific and heterogeneous even among cells belonging to the same cell type. Furthermore, we reveal subciliary domains and explore the link between ciliary and cellular states.

In my talk, I will present our work to systematically study human cilia on a large scale and across cell types and organs, and I look forward to presenting the assays and image analysis tools that make it possible.

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Cynthia He

ARL3 GTPases facilitate ODA16 unloading from IFT in motile cilia

Ciliary cargos synthesized in the cytoplasm bind directly or indirectly to the IFT and are transported into the ciliary compartment, where the cargos are released. Cargo adapters expand the range of IFT cargos and aid ciliary transport. While recent structure biology studies have advanced our understanding of IFT complex assembly and provided some hints for potential cargo recognition mechanisms, how IFT cargos and cargo adapters are discharged from IFT remains largely unknown. During our explorations of small GTPases ARL13 and ARL3 in *Trypanosoma brucei*, we found that ODA16, a known IFT cargo adapter present exclusively in motile cilia, is a specific effector of ARL3. ODA16 is required for cell motility and may carry additional axonemal cargos besides outer dynein arm complexes. In the cilia, active ARL3 GTPases bind to ODA16 and dissociate ODA16 from the IFT complex. Depletion of ARL3 GTPases stabilizes ODA16 interaction with the IFT, leading to ODA16 accumulation in cilia and defects in axonemal assembly. The interactions between human ODA16 homolog HsDAW1 and ARL GTPases are conserved, and these interactions are altered in HsDAW1 disease variants. These findings revealed a conserved function of ARL GTPases in IFT transport of motile ciliary components, and a mechanism of cargo unloading from the IFT. Our work also suggests that there are likely additional IFT cargo adapters and cargo release factors yet to be identified, all of which play a crucial role in maintaining the selective and precise transport of cargos within cilia.

Mechanics of Ciliary motility

Joe Howard, Elijah Lee, Timo Ouyang

Yale University

A major open question in ciliary and flagellar motility is how dynein motor activities are coordinated to produce the characteristic sinusoidal beating patterns of these slender organelles. In this lecture, I will discuss: (i) the arrangement of dyneins within the axoneme—the motile structure inside cilia and flagella; (ii) how this arrangement transforms sliding forces into bending moments; (iii) the elastic and viscous forces that resist bending; and finally, (iv) models of how these forces might coordinate the dyneins. To test these models, I present new data from our laboratory concerning how the beat wavelength depends on the length of cilia from mutants of the unicellular alga Chlamydomonas reinhardtii. Our finding that the wavelength saturates at longer lengths constrains these models and provides estimates of the mechanical properties of the axoneme.

Multiciliated sensory cells mediate hydrostatic pressure sensation in zooplankton

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Hydrostatic pressure is a dominant environmental cue for vertically migrating marine organisms but the physiological mechanisms of responding to pressure changes remain unclear. We uncovered the cellular and circuit bases of a barokinetic response in the planktonic larva of the marine annelid Platynereis dumerilii. Increases in pressure induced a rapid, graded and adapting upward swimming response due to faster ciliary beating. By calcium imaging, we found that brain ciliary photoreceptors showed a graded response to pressure changes. The photoreceptors in animals mutant for ciliary opsin-1 had a smaller ciliary compartment and mutant larvae showed diminished pressure responses. The ciliary photoreceptors synaptically connect to the head multiciliary band that propels swimming via serotonergic motoneurons. Genetic inhibition of the serotonergic cells blocked pressure-dependent increases in ciliary beating. We conclude that ciliary photoreceptors function as pressure sensors and activate ciliary beating through serotonergic signalling during barokinesis.

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The role of cilia and ependymal cells in cerebrospinal fluid dynamics

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Ependymal cells are specialized motile ciliated cells lining the surface of the brain ventricles and spinal canal, which generate directional cerebrospinal fluid (CSF) flow. Ependymal cells are highly conserved among vertebrates and associated with a variety of neurological disorders, including hydrocephalus a condition associated with CSF accumulation. To date, the function of motile cilia-mediated fluid flow in CSF movement, solute transport and brain physiology is still poorly understood. To address these questions, we use the zebrafish as a model as it allows us to monitor and manipulate cellular and neurological processes *in vivo* in an intact brain. Using genetics, imaging and histology, we first identified that the zebrafish has an evolutionary conserved ventricular system, consisting of interconnected ventricles lined by ciliated ependymal cells and CSF-producing choroid plexus. Next, we observed that several physiological factors, beside ependymal cilia, regulate CSF dynamics and solute transport in the brain ventricles. These include the cardiac cycle, bodily movements, diffusion and CSF secretion by the choroid plexus. Despite this, we identified that motile cilia are critical for brain physiology through modulation of neural and astroglial networks. We are now studying the underlying molecular mechanisms leading to altered brain activity and maturation of neuronal circuits in motile cilia mutants.

Why and how did 9+2 cilia evolve?

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All known extant branches of eukaryotes include organisms with motile cilia built on 9 + 2 microtubule scaffolds, in which the motile machinery is arrayed in 96 nm repeats; therefore, this architecture was present in the last eukaryotic common ancestor (LECA). Many complex traits of 9+2 organelles likely reflect the relative importance of selective pressures that drove ciliary evolution in single-celled, biflagellate predatory protists, including rapid motility for dispersal into favorable environments, generation of feeding currents to increase sampling volume, and tactic responses to environmental signals based in part on flagellar membrane receptors. Our goal is to clarify the evolutionary steps that led to this complex structure. Required steps include diversification of tubulin isoforms and selection for doublet and triplet microtubule formation, cylindrical axoneme architecture, diversification of axonemal dynein motors, and formation of radial spokes and a central apparatus to counteract compressive forces. We propose that 96 nm periodicity was then generated by superposition of two structural elements, proto-axonemal dyneins that attached every 24 nm (every 3 tubulin dimers), and radial spokes that bound every 32 nm (every 4 dimers). Stabilization of this 96 nm repeat motif then favored addition of regulatory complexes (DRC, MIA, CSC) once every 96 nm. A shift in the position of radial spoke 3 likely coincided with expansion or diversification of single-headed inner row dyneins, providing an additional level of motility regulation. Two final steps may have generated selective advantages that favored survival of the LECA over competing eukaryotic organisms: 1) formation of an asymmetric central apparatus (central pair) to support more complex, planar waveforms and 2) formation of a dikinetid flagellar apparatus (FA), to support unique anterior and posterior flagella. The FA typically supports four unique microtubule rootlets, R1-R4, whose nucleation, biochemical components and physiological rolls remain poorly understood. Together, these structures form a chiral cytoskeletal organizing center that creates anterior/posterior, dorsal/ventral and left/right axes, defining an overall polarity to the last eukaryotic common ancestor. For reasons yet unknown, regulation of bend asymmetry by the central pair split into two radically different mechanisms early after divergence from the LECA; current phylogenies have not shown which (if either) mechanism is ancestral.

Structure-function relationships of human airway mucociliary clearance

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Abstract

Mucociliary clearance is a key mechanical defense mechanism of human airways, and clearance failure is linked to major respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma. While single-cell transcriptomics have unveiled the cellular complexity of the human airway epithelium, our understanding of the mechanics that link epithelial structure to clearance function mainly stem from animal models. This reliance on animal data limits crucial insights into human airway barrier function and hampers the human-relevant in vitro modeling of airway diseases. Our study fills this crucial knowledge gap and for the first time (1) maps the distribution of ciliated and secretory cell types on the mucosal surface along the proximo-distal axis of the rat and human airway tree, (2) identifies species-specific differences in ciliary beat and clearance function, and (3) elucidates structural parameters of airway epithelia that predict clearance function in both native and in vitro tissues alike. Our broad range of experimental approaches and physics-based modeling translate into generalizable parameters to quantitatively benchmark the human-relevancy of mucociliary clearance in experimental models, and to characterize distinct disease states.

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Unraveling the Mechanisms of Microtubule Stabilization in Ciliary Axonemes

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Cilia are fundamental organelles vital for cellular motility, signaling, and development. At the core of their structure lies the axoneme, a complex microtubule-based scaffold stabilized by Microtubule-Inner Proteins (MIPs). Among these, Sperm Acrosome Associated 9 **(**SPACA9) is a conserved and enigmatic MIP predominantly found in sperm and respiratory cilia. While its abundance suggests a critical role in maintaining axonemal integrity, its precise molecular function remains unclear.
In this talk, I will discuss our efforts to characterize the function of human SPACA9 in maintaining microtubule stability and its potential influence on the mechanical properties of the axoneme. Using in vitro reconstitution assays and advanced microscopy, we examine how SPACA9 interacts with microtubules to stabilize their structure and whether it contributes to resilience under mechanical stress. Our findings contribute to a broader understanding of MIP-mediated stabilization mechanisms and their implications for the assembly, maintenance, and mechanical stability of the ciliary axoneme.

Effects of environmental stress on the cilia beating frequency and vortex formation in corals

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Cilia are conserved cellular structures that perform essential functions in sensing, motility and transport in all species throughout the animal kingdom. In the case of corals, which foster symbiotic photosynthetic algae inside their tissue, cilia function has recently been linked to enhanced mass transfer via vortical flows facilitating regulation of oxygen at the tissue surface alleviating extreme values over diel cycles. Environmental stressors, i.e., high ocean temperatures and increased hypoxic events, can induce and exacerbate coral bleaching, but how these stressors affect the function of cilia remains unknown. Using a new *in-vivo* cilia observation chamber, we show that cilia beating frequency (CBF) increases when corals are exposed to increasing temperature until a critical temperature threshold, above which desynchronization occurs followed by a full arrest of cilia movement. Using Particle Image Velocimetry (PIV), we observed that these changes lead to larger and faster moving vortices, potentially improving mixing within the diffusive boundary layer (DBL), thereby improving the metabolite exchange between the ambient water and the coral tissue. In contrast, experiments manipulating oxygen concentrations in the water showed that CBF response is rather species specific, with some corals showing no signs of CBF alterations under hypoxic or anoxic conditions, while CBF in other corals started to desynchronize and stop under oxygen stress. These results scratch the surface on the role of cilia on modulating coral response to climate change.

Towards a mechanistic understanding of ciliary transport and assembly

Gaia Pigino

Recent advances in hardware and computational techniques for cryo-electron microscopy (cryo-EM) have elevated in situ cryo-electron tomography to the forefront, emerging as one of the most potent tools for visualizing and understanding cellular processes at the molecular level.

For our research we adopts a multifaceted approach by integrating structural biology methodologies with cell biology techniques. We aim at gaining mechanistic insights into the molecular processes governing the assembly and function of both motile and primary cilia—microtubule-based organelles essential for the functioning of most eukaryotic cells and crucial for human health.

In this talk, I will present our comprehensive investigation of the ciliary transport system leveraging genome engineering, in situ cryo-electron tomography (cryo-ET), AlphaFold2 protein structure prediction, structural proteomics, correlated light and electron microscopy (CLEM), expansion microscopy, total internal reflection fluorescence (TIRF) microscopy, and in vitro reconstitution assays. Through this integrated approach, we have successfully unveiled the molecular structure, dynamics, and underlying mechanisms of Intraflagellar Transport (IFT). IFT, is the universally conserved bidirectional transport system, required for the assembly of cilia and eukaryotic flagella across all ciliated cells. The understanding of the structural and functional complexity of IFT is of paramount importance, as defects in this system can lead to ciliopathies.

Twist–torsion coupling in beating axonemes

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Motile cilia and flagella produce regular bending waves that enable single-cell navigation due to non-planar waveforms with characteristic torsion. However, it is not known how torsion, a geometric property of the three-dimensional waveform, relates to mechanical twist deformations of the axoneme, the conserved cytoskeletal core of cilia and flagella. Here we show that axoneme twisting and torsion are coupled and that twist waves propagate along the beating axoneme of *Chlamydomonas reinhardtii* algae. We resolve the three-dimensional shapes of the axonemal waveform with nanometre precision at millisecond timescales using defocused dark-field microscopy and beat-cycle averaging, observing regular hetero-chiral torsion waves propagating base to tip. To investigate whether the observed torsion results from axonemal twist, we attach gold nanoparticles to axonemes and measure their cross-section rotation during beating. We find that, locally, the axonemal cross-section co-rotates with the bending plane, evidencing twist–torsion coupling. Our results demonstrate the link between shape and mechanical deformation in beating axonemes and can inform models of the dynamics of motor proteins inside the axoneme responsible for shaping the beat of motile cilia.

Phototactic responses of ciliary microorganisms

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Phototactic swimming microorganisms respond to light stimuli and exhibit versatile phototaxis strategies to navigate their environment and search for optimal light conditions for their survival. How phototactic microswimmers convert light signals into subcellular flagella beat patterns for phototactic responses and how such responses can lead to long-term phototaxis strategies are not well understood. In this talk, I will discuss the phototactic responses of two representative model microswimmers, namely *Euglena gracilis* and *Chlamydomonas reinhardtii.* I will demonstrate how *Euglena* and *Chlamydomonas* use a small number of flagella beat states to trigger a rich variety of phototactic behaviors, including positive and negative phototaxis. I will also introduce a biophysical model that can capture these phototaxis transitions observed in different cells. These findings suggest generalizable biophysical laws for taxis behaviors and navigation of microswimmers, with implications in the design and control of natural and synthetic microswimmers.

Basal body blueprint: Expansion microscopy of microtubules in multiciliated cells

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Basal bodies are centriole-like structures on which cilia form. In multiciliated cells basal bodies also serve as an organizing center for the cytoplasmic microtubule network. Although microtubule organizing centers in cycling cells are extensively studied, their composition and role in differentiated cells is less well understood. In this work, we used Ten-Fold Robust Expansion (TREx) microscopy to explore microtubule networks in polarized human airway multiciliated cells. We were able to resolve the apicobasal microtubule network in 3D as well as the dense apical meshwork located between basal bodies. By studying microtubule recovery after nocodazole-induced microtubule depolymerization, we found that apicobasal microtubules both anchor and nucleate from the part of the basal body known as the basal foot. Using STED microscopy, we could localize to the basal body many known centrosomal proteins. This suggests that basal bodies act as the main microtubule organizing center (MTOC) in multiciliated cells. To explore the composition of this MTOC, we applied a novel averaging tool to map on the basal body the position of multiple proteins involved in microtubule nucleation and anchoring. We found that γ-TuRC and its recruiters NEDD1 and the Augmin/HAUS complex, as well as ninein, a protein involved in microtubule anchoring at the centrosome, localize to the tip of the basal foot. Our data provide insight into the formation of elaborate and dense microtubule arrays supporting the function of multiciliated epithelial cells.

Left-right symmetry breaking using mechanosensitive cilia

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Abstract
Fluid flows generated by motile cilia play a crucial role in establishing left-right asymmetry in the vertebrate left-right organizer (LRO). Although mechanosensation by cilia bending has been proposed as a mechanism for symmetry breaking through the detection of flow direction, its reliability is questioned due to the high spatial variability of the flow. Moreover, the magnitudes of the flow on the left and right peripheries are similar, further complicating the use of mechanosensation for left-right specification. Yet, cilia bending is asymmetric in the LRO. We will discuss our work trying to explain these limitations using biophysical approaches.

Hydrodynamic synchronization of cilia in finite systems

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When many cilia are located on the surface of a microorganism, their beating can synchronise such that their phases form metachronal waves. To understand the process of synchronisation, we study a model where each cilium is represented as a spherical particle, moving along a tilted trajectory with a position-dependent active driving force and a position-dependent internal drag coefficient. The model thus takes into account all the essential broken symmetries of the ciliary beat. We show that taking into account the near-field hydrodynamic interactions, the effective coupling between cilia can become nonreciprocal: the phase of a cilium is more strongly affected by an adjacent cilium on one side than by a cilium at the same distance in the opposite direction. As a result, synchronisation starts from a seed at the edge of a group of cilia and propagates rapidly across the system, leading to a synchronisation time that scales proportionally to the linear dimension of the system. A ciliated surface is thus characterised by three different velocities: the velocity of fluid transport, the phase velocity of metachronal waves and the group velocity of order propagation. Unlike in systems with reciprocal coupling, boundary effects are not detrimental for synchronisation, but rather help to initiate the wave.

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On the origins of diaplectic metachronal waves

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Ciliated structures assume a myriad of configurations, depending on the topology and geometry of the organism. Groups of cilia enable feeding or swimming motility when attached to a cell body, while mucociliary clearance arises from the coordinated activity of multiciliated epithelia [1]. In all these cases, multiple cilia interact to produce different types of local and global coordination patterns, including robust metachronal waves. How metachronal waves emerge in different systems remains mysterious and system dependent. Diaplectic metachrony, where the ciliary power stroke is perpendicular to the wave propagation direction, does not appear to optimise fluid transport and yet is highly prevalent in many naturally occurring multiciliated systems [2,3]. We propose new and emerging model organisms to study the propagation dynamics and emergence of diaplectic metachronal waves in dense arrays of cilia, revealing the importance of non-planar 3D beat patterns in the establishment of spatiotemporal symmetry breaking [4].

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Holographic imaging of the flagellar waveform of *Chlamydonomas reinhardtii*

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The relationship between structure and function in motile cilia and flagella has driven research in the field for decades. A critical step in mapping structure to function is understanding the motion of cilia in their biological context. Advances in instrumentation have allowed ever-finer understanding of the molecular configuration within the axoneme, and cryo-EM tomography has produced exquisite snapshots of the configurations of individual motors. The other side of the problem - capturing the motion of cilia in living cells - presents a significant future challenge to microscopists. We demonstrate the use of high-speed holographic imaging to resolve the flagellar beat of a *Chlamydomonas reinhardtii* cell held on a micropipette, in three dimensions [1] (Fig. 1). We uncover results around the handedness of the waveform at initiation and during wave propagation that are only accessible thanks to the spatial and temporal resolution afforded by our technique. We are excited by the possibility of combining this approach with genetic manipulations to uncover the mechanical role of flagellar constituents. As well as high-precision imaging of the beat, digital holography is also useful for the study of microorganism motion more generally. At the end of the presentation I will introduce a recent example in which holography was used to shed light on the physics governing predation in the carnivorous plant *Genlisea*.

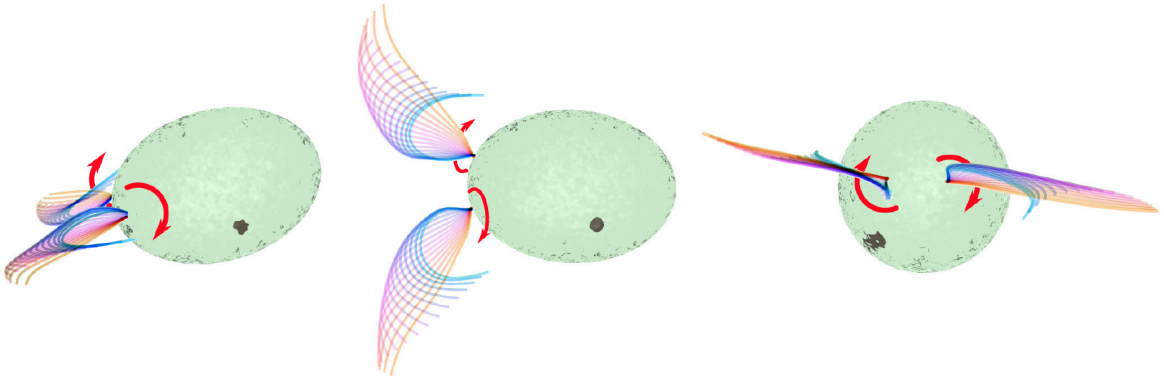

Figure 1: Visualisation of the three-dimensional beat of C. reinhardtii. The arrows indicate the the direction of flagellar motion at the initiation of a flagellar beat, and the dark spot in the cell body indicates the position of the eye spot.

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Internal forces, axoneme distortion, and dynamic instability in multi-filament models of the ciliary axoneme

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While the basic structure of the axoneme has been known for decades, the internal forces, torques, and mechanisms driving oscillatory beating remain incompletely understood. Theories include dynein regulation by curvature, twist, or sliding feedback mechanisms, signal-driven switching or inhibition, and regulation by inter-doublet spacing. Previous work has shown that dynamic regulation of dynein (i.e., temporal variation in dynein activity at the frequency of beating) may not be necessary to produce oscillatory waveforms [1]. An alternative explanation is that oscillations can arise naturally in slender, axially loaded filaments due to re-orientation of follower forces leading to dynamic instability (flutter). Such dynamic instabilities are well known in mechanical and aerospace engineering and are the reason for the fluttering of a flag in steady wind or the oscillation of a runaway fire hose.

In recent work [2, 3] we have built multi-filament models of cilia driven by steady dynein activity and studied the effects of the mechanical properties of internal axonemal components (radial spokes, circumferential links). These models maintain strictly balanced internal forces and torques as the axoneme distorts during beating. We have confirmed that spontaneous oscillations arise over a large space of physically plausible parameter combinations. One notable result is that torsional stiffness in the radial spokes provides a mechanism to prevent the axoneme from excessively twisting when dynein is active between all doublets and promotes flutter in the model. Another is that increasing the axial stiffness of radial spokes does not inhibit flutter as long as circumferential motion of the doublets is permitted. Additionally, we explore the potential effects of dynein motor protein kinematics on ciliary waveforms and frequencies.

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Molecular specialisation of the sperm flagella

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Using cryoelectron microscopy, cryoelectron tomography and proteomics we compared sperm flagella to epithelial motile cilia. We found that sperm axonemal doublet microtubules (DMTs) are the most specialised (Cell 2023, PMID 37327785), with epithelial cilia having only minor differences across tissues. For example, sperm DMT anchors a T-complex protein ring complex (TRiC) chaperone every 96 nm that may contribute to construction or maintenance of the long flagella of mammalian sperm (Nature 2025, PMID 39743588). Furthermore, we resolved the structure of radial spoke 3 revealing the binding sites of kinases associated with regeneration of ATP and regulation of ciliary motility. Finally, we captured axonemal dyneins in their prestroke states, illuminating conformational changes that occur during ciliary movement. Taken together, our results illustrate how elements of chemical and mechanical regulation are embedded within the axoneme.

Abstracts of Posters

(in alphabetical order)

Hydrodynamic synchronization of elastic cilia: The impact of surface effects and unsteady viscous flow on metachronal waves

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We model hydrodynamically interacting cilia by microspheres elastically bound to circular orbits, whose inclinations with respect to a no-slip wall model the ciliary power and recovery stroke, resulting in an anisotropy of the viscous flow [1]. A coupled phase-oscillator description is derived based on the steady Stokes equation by reducing the microsphere dynamics to the slow timescale of synchronization and determine analytical metachronal wave solutions and their stability in a periodic chain setting. The flow near the surface stabilizes metachronal waves with long wavelengths propagating in the direction of the power stroke and metachronal waves with short wavelengths propagating perpendicularly to the power stroke. In open chains of phase oscillators, the dynamics of metachronal waves is fundamentally different. Here the elasticity of the model cilia controls the wave direction and selects a particular wave number: at large elasticity, waves traveling in the direction of the power stroke are stable, whereas at smaller elasticity waves in the opposite direction are stable. For intermediate elasticity both wave directions coexist.

In a second study, we present a model of elastic cilia coupled by transient viscous flow in the bulk fluid obtained by using the unsteady Stokes equation [2]. Therein, vorticity diffusion impacts cilia coordination qualitatively and quantitatively. In particular, metachronal waves occur even in the absence of surface effects for cilia chains larger than the viscous penetration depth.

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Ciliary cooperation and transport in unsteady Stokes flows

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Cilia are essential in a variety of biological systems, serving critical functions such as locomotion in unicellular eukaryotes and nutrient collection or waste removal in marine organisms like corals and larvae. In humans, they play vital roles in clearing mucus from the respiratory system and transporting egg cells through the fallopian tubes. While ciliary flows are typically described as steady activity, the unsteady, inertial flows they produce have received comparatively little attention.

Unsteady Stokes flows occur in biological contexts—such as aiding organisms in nutrient transport and threat evasion. In these cases, the timescale of actuation often becomes small compared to that of viscous momentum diffusion. To explore the dynamics of these flows, we study a model system described by time-dependent, linear Stokes equations, which can be solved using time-dependent Green's functions with analytically defined memory kernels.

We demonstrate that unsteady effects in low Reynolds number flows enhance mixing by breaking time reversibility. In the unsteady regime, vorticity diffusion leads to complex trajectories of advected particles. Our research focuses on the transport properties of flows generated by time-varying forces that mimic the action of individual and collective ciliary motion. As a fundamental building block, we examine the point-like hydrodynamic singularity known as the Pufflet. Through a combination of theoretical analysis, simulations, and experiments, we explore fluid transport driven by such rapid actuation. For multiple actuation sites, we observe new patterns of transport, which we optimize in terms of the spatial and temporal arrangement of the forcing. This model aims to describe biological phenomena as a result of parameter optimization.

The findings highlight the distinctions between steady-state Stokes flows and dynamic unsteady regimes, offering new insights into ciliary transport mechanisms.

Complexity in the ciliary dynamics of a swimming microorganism

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Eukaryotic microorganisms often display a range of motile behaviours driven by multiple modes of ciliary actuation. Yet the techniques used to characterise these often overlook the complexity present by considering a single, typically large, spatio-temporal scale. Here we conduct a multi-scale analysis of the cilium driven dynamics in the marine alga *Pterosperma*. This organism consists of an ellipsoidal cell body approximately $8\mu m$ in diameter and four extremely long cilia ($\sim 70\mu m$). We perform comprehensive low-resolution behavioural tracking to reveal three distinct motility macrostates and show that these result from stereotyped beating of its four cilia, which exhibit multiple discrete oscillatory modes. We discovered a novel quiescent state defined by small amplitude, low frequency oscillations of unbundled cilia. Conversely, bundling of the four cilia into a single, synchronously beating compound cilium produces high amplitude waves with a distribution of frequencies. Ultrafast reorientations are produced via large curvature bends of this compound cilium. Using a novel mathematical framework based on shape decomposition of ciliary waveforms extracted from high-resolution recordings, we reveal underlying structure in the ciliary dynamics of this organism that is not otherwise accessible. This allows us to map the entire spectrum of ciliary dynamics in a living cell and uncover a novel dispersion relation that constrains the cilium shape space. Quantifying the complete spectrum of ciliary oscillations in a single organism is essential for developing an accurate mechanistic picture of how the cilium beats.

Human fertilization in vivo and in vitro requires the CatSper channel to initiate sperm hyperactivation

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Human sperm have to fulfil a series of demanding functions during fertilization: they navigate along various chemical and physical cues to localize the oocyte, and they break through its protective vestments by hyperactivation and acrosomal exocytosis. These processes are controlled by changes in intracellular pH (pH_i), membrane potential (V_m), and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), mediated by a subset of unique ion channels. The sperm-specific ion channels CatSper serves as central signalling node and controls $[Ca^{2+}]_i$. Using a simple motility-based test, we routinely assess the function of the sperm-specific multisubunit CatSper-channel complex in sperm of men undergoing a fertility workup. Thereby, we identified a group of men with normal semen parameters, but defective CatSper function. These men or couples failed to conceive naturally and upon medically assisted reproduction via intrauterine insemination and in vitro fertilization, but required Intracytoplasmic sperm injection (ICSI) to conceive a child. We show that the defective CatSper function was caused by variations in CATSPER genes. Moreover, we unveil that CatSper-deficient human sperm are unable to undergo hyperactive motility and, therefore, fail to penetrate the egg coat. Thus, we provide the first experimental evidence that sperm hyperactivation is required for human fertilization, explaining the infertility of CatSper-deficient men and need of ICSI for medically assisted reproduction. Finally, our study also reveals that this channelopathy represents the most common cause of unexplained male infertility known thus far.

Title:

Structure and assembly of A-C linker connecting microtubule triplets in centrioles

Authors:

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Summary:

Centriole assembly involves the coordination of centriolar modules. One module is the A-C linker, an enigmatic protein assembly connecting the A-microtubule of one microtubule triplet to the C-microtubule of neighboring microtubule triplet. Here, we integrated biochemistry, multi-scale cryo-electron microscopy and AlphaFold modeling to decipher the architecture of the centriole. Using an improved centriole isolation method, we determined the structure of the A-C linker bound to microtubule triplets, which elucidated how the A-C linker crosslinks microtubules and integrates with centriolar modules. We discovered dramatic changes in the structure and composition of the A-C linker that correlate with the presence of other centriolar modules, including the pinhead, cartwheel, and inner scaffold. Our findings show that the A-C linker is a highly integral component of the centriole whose polymorphism may orchestrate the assembly of spatially distinct centriolar modules, and provide a powerful framework for dissecting the biology of centrioles.

Keywords:

Centriole, basal body, A-C linker, microtubule triplet, cryo-EM

How cilia beating affects the swimming of exogenous bodies in airways?

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In the respiratory system, micrometric whip-like structures,known as cilia, work collectively to keep the airways free from pathogens. Mucociliary clearance is the process by which cilia move in a coordinated way to remove the inhaled particles preventing them from reaching the tissue in the lungs. In patients with conditions such as primary ciliary dyskinesia, or chronic obstructive pulmonary disease, dysfunctional cilia fail in generating a coordinated sweeping of the mucus, exacerbating severe infections and chronic inflammatory conditions. With these issues in mind, here we focus on understanding the flow produced by the cilia in the airways from a hydrodynamic point of view, which might help explain how the initial phase of an infection arises. To do so, we use optical tweezers to study the influence of the ciliary movement on a passive agent, which is used as a simplified model of a real infection.

A flavin-based photoreceptor controls the photoactivation of ciliary adhesion in *Chlamydomonas*

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Light-activated proteins or photoreceptors play a crucial role on the behavior and, ultimately, the survival of photoactive microorganisms. The unicellular biciliated microalga *Chlamydomonas reinhardtii* has become a model organism to study light-mediated phenotypes, such as photosynthesis, phototaxis, and the circadian rhythm, among several others. Recently, we discovered that *C. reinhardtii* can reversibly switch on and off the adhesiveness of their cilia in blue and red light, respectively [1,2]. We characterized the *in vivo* action spectrum of this phenotype in wild-type (WT) *C. reinhardtii* cells via single-cell micropipette force measurements and showed that it resembles the spectral sensitivity of a flavin-based photoreceptor. Further comparison of the ciliary adhesion forces between WT and photoreceptor-targeted mutants reveals that the deletion of two flavin-containing photoreceptors, namely animal- and plant cryptochromes, completely disrupts light-switchable adhesion. The elucidation of the molecular mechanisms supporting this phenotype, from stimulus to response, will give insights into understanding signal transduction in light-mediated biological switches and the dynamics of ciliary membrane proteins mediating adhesion.

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Curves and currents: modelling the effects of surface curvature on ciliary flows

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Motile cilia play a pivotal role in fluid transport and locomotion for a wide range of microorganisms. Understanding how the curvature of ciliated surfaces influences the surrounding flow fields is essential for unraveling their mechanisms of feeding and propulsion. In this study, we present a generic model that captures the effects of surface curvature on flow fields generated by ciliated microorganisms. Our approach involves modeling the organism as a spherical cell body combined with a collection of rotlets that represent localized rotational flow sources [1,2]. The dynamics are analyzed under the Stokes flow regime, where viscous forces dominate inertial effects. This framework allows us to systematically study how the spatial distribution and force modulation of actuators shapes the induced vortical flow patterns. The model provides insights into the hydrodynamics of curved ciliated structures, with implications for understanding the movement and feeding, and filtering mechanisms of marine larvae and other aquatic organisms. By offering a simple yet versatile representation of cilia-driven flows, our work may prove useful for exploring diverse applications, from ecological studies of larval transport to engineering bio-inspired microrobotic swimmers. This research highlights the utility of minimalistic biophysical models in capturing the essential features of complex biological systems, bridging the gap between theoretical hydrodynamics and biological functionality.

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Axonemal dynein contributions to the symmetric and asymmetric waveforms in *Leishmania*.

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Axonemal dyneins are molecular motor proteins transducing chemical energy of ATP into microtubule sliding in motile cilia. The outer dynein arms (ODA) are understood to be the main force generators and the inner dynein arms (IDA) the controllers of beat propagation and shape. Several questions remain: does this long-standing dogma apply to all cilia, how do individual dyneins contribute to wave propagation and shape and what coordinates the switch between symmetric and asymmetric waveforms.
Here we address these questions in an early diverging eukaryote, *Leishmania*, using a library of dynein gene deletion mutants and high-speed video microscopy.
Loss of the ODA microtubule-binding domain associated light chain 1 (LC1) was enough to half the beat frequency. However, loss of the entire ODA density, by deletion of the ODA docking complexes, reduced the cell displacement efficiency to levels similar to a paralysed mutant (ΔPF16). Flagella lacking IDAf formed a proximal flagellar curl that hindered movement. Upon loss of single headed dynein IDA4a (IDA d), normal frequency and amplitude were maintained, while loss of other IDAs all changed these waveform parameters.
We also found that the phosphodiesterase inhibitor CpdB induced a wave reversal, which was dependent on cyclic AMP response protein 1 (CARP1). Furthermore, loss of IDA4a caused a reduction in CpdB sensitivity, suggesting IDA4a lies downstream of the cAMP-CARP1 pathway leading to a waveform switch.
Our investigations bring us one step closer to understanding how the coordination of IDA and ODA downstream of second messengers modulate flagellar movement, ultimately resulting in complex biological behaviours.

Driving Curvature: The Influence of Sperm Head Shape on Flagellar Kinematics

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Infertility affects 1 in 6 couples, is emotionally devastating, and requires expensive and invasive treatments. Importantly, we place a significant and unequal burden on women, who often require risk-bearing procedures to address what is caused by, in 50% of cases, a male factor. Sperm assessment routinely comprises counting the number of sperm together with visual assessments of motility (head motion), and morphology (shape) of fixed and stained cells at high magnification, which give some indication of the fertile potential of a sperm sample but lack deep insight into sperm quality.

When it comes to the task of sperm selection (i.e. for intracytoplasmic sperm injection – ICSI – treatment), the detailed aspects of morphology are intractable. Instead, decisions are made on gross features of morphology together with motility. These are not, however, two entirely independent measurements – morphology has an inherent influence on swimming.

In this simulation study, we set out to characterise how variations in sperm head shape impacts the flagellar waveform. Simulations employed an efficient integral formation for electrohydrodynamic modelling[(a)] of the interactions between elastic flagellum, solid prolate spheroidal head, and surrounding fluid, together with an active curvature control model[(b)] for flagellar beat generation. With this approach, we assessed changes in flagellar waveform arising from modification of head-axis lengths while maintaining a fixed volume. We find that changes in head shape impact the propulsive section of the waveform, which is reflected in a change in sperm velocity. The proximal part of the flagellum (close to the head) exhibits little change, indicating that changes in head morphology more strongly impact the flexible region of tail rather than at the neck.

Since the distal end of the flagellum contributes maximally to the propulsive effectiveness of the sperm, changes in head size modulate sperm motility through processes beyond simply changing the drag profile of the head. These observable differences in curvature could be used to gaining insight into subtle differences in head morphology in live swimming cells, essential for improving sperm assessment.

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Developments of the *invitro* ciliary transport assay for the diagnosis of Primary Ciliary Dyskinesia

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Primary Ciliary Dyskinesia (PCD) is a genetically heterogeneous disorder characterized by destructive airway disease with recurrent respiratory tract infections. They are caused by structural defects in motile cilia leading to impaired mucociliary clearance [1]. This transport process is driven by constant beating of motile cilia on ciliated cells. The complexity of PCD prevents yet the availability of a simple diagnostic test confirming or excluding these rare diseases. Here, a recently established ciliary transport assay (CTA) [2] based on Air-Liquid Interface (ALI) cultures of human respiratory epithelial cells (hRECs) promises important assistance in the diagnosis of PCD. After establishing the CTA its capabilities and applications are continuously pushed forward. Recent studies will be presented and discussed.

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Chemosensory signaling in human sperm is controlled by Ca^{2+} influx via CatSper and Ca^{2+} clearance via plasma membrane Ca^{2+} ATPases

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Loss of function of the sperm-specific Ca^{2+} channel CatSper is a common channelopathy that causes male infertility. CatSper controls the intracellular Ca^{2+} concentration and, thereby, the motility of human sperm. Activation of CatSper by oviductal ligands evokes a transient Ca^{2+} increase, which entails changes in the flagellar beat that are required for fertilization. The CatSper-mediated Ca^{2+} influx has been studied extensively, whereas the mechanisms underlying Ca^{2+} clearance and recovery from Ca^{2+} influx have remained ill-defined.

We examined how pharmacological suppression of Ca^{2+} export from the cytosol into the extracellular space or Ca^{2+} uptake into intracellular stores affects the resting Ca^{2+} concentration and CatSper-mediated Ca^{2+} signals in human sperm. We studied sperm of healthy volunteers and infertile men lacking functional CatSper channels, using kinetic Ca^{2+}- and pH-fluorometry as well as patch-clamp recordings.

We show that Ca^{2+} entering human sperm via CatSper is predominantly, if not exclusively, exported by plasma membrane Ca^{2+} ATPases (PMCA). Na^{+}/Ca^{2+} exchange and Ca^{2+} uptake into intracellular stores or mitochondria play no or only a negligible role in Ca^{2+} clearance in human sperm. In summary, Ca^{2+} signalling in human sperm is controlled by the functional interplay of CatSper and PMCA, i.e. the balance between Ca^{2+} influx and Ca^{2+} export that is required for human sperm function and fertilization.

Unwrapping the ciliary coat: high-resolution structure and function of the ciliary glycocalyx in *Chlamydomonas reinhardtii*

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The glycocalyx, a highly heterogeneous glycoprotein layer of cilia regulates adhesion and force transduction and is involved in ciliary signalling. The high-resolution molecular architecture of this layer is currently not understood. We describe the structure of the ciliary coat in the green alga *Chlamydomonas reinhardtii* by cryo-electron tomography and proteomic approaches and present the high-resolution cryoEM structure of the main component, FMG1B. We describe FMG1B as a putative mucin orthologue which lacks the major *O*-glycosylation of mammalian mucins but is *N*-glycosylated. FMG1A, a previously undescribed isoform of FMG1B is likewise expressed in *C. reinhardtii*. By microflow-based adhesion assays we observe increased surface adhesion in the glycocalyx deficient double-mutant *fmg1a-fmg1b*. We find this mutant to be capable of surface-gliding, with neither isoform required for extracellular force transduction by intraflagellar transport. Our results find FMG1 to form a protective layer with adhesion-regulative instead of adhesion-conferring properties and a putative example of an undescribed class of mucins.

ARL13 and ARL3 regulate the unloading of ODA16 from IFT in motile cilia

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ARL13B and ARL3 are ciliary GTPases implicated in human Joubert Syndrome, affecting ciliary membrane and axoneme organization. Although the mechanism of ARL13B as a guanine nucleotide exchange factor (GEF) of ARL3 and the function of ARL13B and ARL3 in ciliary membrane protein transport are well established, their role in axoneme biogenesis is unclear. In *Trypanosoma brucei*, TbARL13 acts as a GEF for two distinct TbARL3 proteins, TbARL3A and TbARL3C. Here, we identified the *T. brucei* homolog of ODA16, a cargo adapter facilitating intraflagellar transport (IFT) of motile ciliary components, as an effector of both TbARL3A and TbARL3C. In the cilia, active ARL3 GTPases bind to ODA16 and dissociate ODA16 from the IFT complex. Depletion of ARL3 GTPases stabilizes ODA16 interaction with the IFT, leading to ODA16 accumulation in cilia and defects in axonemal assembly. Depletion of ARL13 also results in ODA16 accumulation in cilia and colocalizing with IFT. The displacement of ODA16 from IFT by active ARL3 is likely to occur very rapidly once ODA16 enters the cilia where TbARL13 is enriched, which is the reason why ODA16 is normally seen at the ciliary base. The interactions between human ODA16 homolog HsDAW1 and ARL GTPases are conserved, and these interactions are altered in HsDAW1 disease variants. Our findings established a functional link between ARL13B and the IFT pathway via an ARL3 effector ODA16, explaining the essential and diverse roles of ARL13B in ciliary transport of both membrane proteins required for signaling and axonemal cargos important for motility, and providing insights to the disease mechanisms of ARL13B-ARL3 in motile ciliopathies.

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Human IFT-A complex structures provide molecular insights into ciliary transport

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Intraflagellar transport (IFT) complexes, IFT-A and IFT-B, form bidirectional trains that move along the axonemal microtubules and are essential for assembling and maintaining cilia. Mutations in IFT subunits lead to numerous ciliopathies involving multiple tissues. However, how IFT complexes assemble and mediate cargo transport lacks mechanistic understanding due to missing high-resolution structural information of the holo-complexes. Here we report cryo-EM structures of human IFT-A complexes in the presence and absence of TULP3 at overall resolutions of 3.0-3.9 Å. IFT-A adopts a "lariat" shape with interconnected core and peripheral subunits linked by structurally vital zinc-binding domains. TULP3, the cargo adapter, interacts with IFT-A through its N-terminal region, and interface mutations disrupt cargo transport. We also determine the molecular impacts of disease mutations in complex formation and ciliary transport. Our work reveals IFT-A architecture, sheds light on ciliary transport and IFT train formation, and enables the rationalization of disease mutations in ciliopathies.

Neuronal Coordination of Gravity-Sensing Cilia in the Ctenophore Apical Organ

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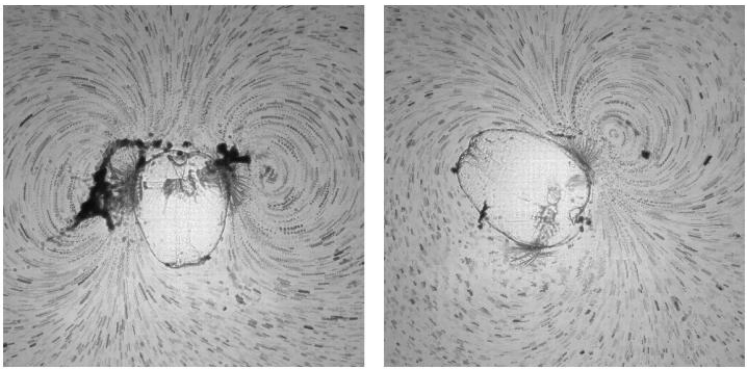
Ctenophores possess eight rows of iridescent compound cilia, known as comb plates, which regulate posture and swimming. The beating of comb plates is paced by balancer cilia in the apical organ. The balancer cilia support the statolith and detect its tilt as a mechanical stimulus. However, instead of converting stimuli into electrical signals, they directly modulate their beating pattern and transmit this motion to the comb plate cilia. Thus, balancer cilia function as a sophisticated hydrodynamic ciliary control system.
To investigate the gravity-dependent regulation of balancer cilia, we analysed changes in water flow generated by comb plates using a 90-degree tilted microscope. Our results showed tilt-dependent changes in the flow pattern (Figure). Since comb plate beating is regulated by balancer cilia, we examined their beat frequency under varying gravitational orientations and found similar tilt-dependent changes. To understand how the four balancer cilia coordinate their movement, we reconstructed the connectome of the apical organ by volume electron microscopy. We identified approximately 900 cells, including monociliated balancer cells forming four clusters of ~30 cells. These cells bundle into compound cilia. Additionally, we identified three syncytial multinucleated neurons and synapsing on the balancer clusters. The analysis of balancer ciliary beat patterns revealed coordinated changes in ciliary responses that were dependent on body tilt, suggesting neuronal coordination of balancer cilia by the syncytial neurons. This study provides new insights into the neural regulation of ciliary sensing and movement in ctenophores.



Soluble adenylyl cyclase (sAC) in non-mammalian sperm is directly controlled by pH, not HCO_3^- or Ca^{2+}.

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Soluble adenylyl cyclase (sAC) is critical for sperm function in both human and sea urchin. Human sAC is activated by bicarbonate (HCO_3^-), and activation relies on two residues (Lys95 or Arg176) that bind HCO_3^-. Either one of these two residues are not conserved in orthologs from 13 phyla, suggesting that HCO_3^- does not bind and activate sAC in these orthologs.

The effect of this substitution was examined in *Arbacia punctulata* sAC (*Ap*sAC), where the arginine is not conserved (Asn198) and the lysine is conserved (Lys117). Here, we show that *Ap*sAC is insensitive to HCO_3^- and is instead activated by alkaline pH. When Lys117 is substituted, the pH sensitivity is greatly decreased; thus, Lys117 plays a key role in the pH-sensing mechanism. The substitution N198R in *Ap*sAC, which mimics the HCO_3^--binding residue of human sAC, does not induce HCO_3^- sensitivity. Two peaks in *Ap*sAC activity are observed over the pH range 6.5 – 8.0; the peaks coincide with two alkalinization and functional events of *Arbacia punctulata* sperm, namely spawning (pH 6.8 - 7.2), which activates motility, and chemoattractant binding (pH > 7.2), which controls chemotactic navigation.

These results indicate that in phyla where the residues are not conserved, the sAC is pH- and not bicarbonate-sensitive. A picture of evolutionary significance emerges: across phyla, sAC regulation by HCO_3^- or pH represents an adaptation to high- or low-HCO_3^- environments, respectively.

Role of mastigonemes in the green alga *C. reinhardtii*

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In various single celled eukaryotes, flagella are decorated with thin, hair-like glycoproteins called mastigonemes [1]. While “stiff” mastigonemes are instrumental in swimming for some eukaryote algae, the “flimsy” mastigonemes of the model green alga *C. reinhardtii* do not assist fluid flow generation exerted by a beating flagellum [2]. The function of “flimsy” mastigonemes is therefore still debated. As mastigonemes in *C. reinhardtii* are linked to the flagella membrane via the putative ion channel PKD2 [1], we decided to investigate their potential role in mechanosensing. Swimming and obstacle-avoidance of mastigoneme- and PKD2 mutant strains was explored with high-speed microscopic imaging. Preliminary data suggest no role of mastigonemes in sensing of obstacles, but a regulative role in the orchestration of beating synchrony in confined environments.

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A range of 30%-62% of functioning multiciliated airway cells is sufficient to maintain ciliary airway clearance

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Primary ciliary dyskinesia (PCD) is a genetic disorder caused by defective motile cilia function, leading to impaired ciliary airway clearance and recurrent airway infections. To determine the minimum number of functional multiciliated airway cells required for effective ciliary clearance, we investigated the molecular defects associated with the X-linked recessive PCD subtype caused by pathogenic variants in *DNAAF6* (*PIH1D3*). This subtype is characterized by immotile cilia in affected males. We conducted a comprehensive analysis of the clinical phenotype and molecular defects using immunofluorescence and transmission electron microscopy, and evaluated ciliary clearance through *in vitro* ciliary transport assay in air-liquid interface cultures and *in vivo* radiolabeled tracer studies. These analyses included respiratory cells from females with heterozygous and males with hemizygous pathogenic *DNAAF6* variants.
Males with hemizygous *DNAAF6* pathogenic variants exhibited entirely immotile cilia, a complete absence of ciliary clearance, and severe PCD symptoms. In contrast, six females with heterozygous pathogenic *DNAAF6* variants displayed random or skewed X-chromosome inactivation, resulting in an average of 54.3%±10 (range 38%-70%) defective multiciliated cells. Despite this, both *in vitro* and *in vivo* assessments revealed normal or slightly impaired ciliary airway clearance. Correspondingly, heterozygous females presented with no or only mild respiratory symptoms.
Our findings suggest that 30%-62% of functional multiciliated respiratory cells are sufficient to achieve normal or slightly reduced ciliary clearance. Given that heterozygous females experienced minimal or no respiratory symptoms, precision medicine targeting the correction of at least 30% of cells could potentially restore ciliary airway clearance and alleviate clinical symptoms in PCD patients.

The wavelength of the ciliary beat in wild-type and mutant *Chlamydomonas reinhardtii* saturates at ciliary lengths above 15 μm

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In short cilia, such as those of *Chlamydomonas reinhardtii*, the wavelength of the ciliary beat is approximately proportional to the length of the cilium. On the other hand, for longer flagella, such as those of sea urchin and mammalian sperm, the wavelength is shorter than the length, so that each flagellum supports multiple wavelengths. Biophysical models suggest that the transition between the short- and long-length behavior depends on the relative magnitudes of the viscous forces (from hydrodynamic and internal friction) and the elastic forces (from the flexural rigidity of the cilium). To gain insight into the roles of these forces in generating the ciliary beat, we took advantage of *Chlamydomonas* mutants in which flagellar length control is altered, leading to cilia that are longer and shorter than wild-type cells. This allowed us to probe the transition between short- and long-length behavior in a single organism, rather than comparing different organisms. To test quantitatively the relationship between flagellar length and beat length, we developed a Fourier-based estimator for the beat wavelength, accurate in the regime where the length is greater than half the wavelength. We confirmed that the wavelength of the dynamic beat increased with flagellar length as previously found. Interestingly we discovered that wavelength stopped increasing beyond a length of 14-15 μm. We use this new finding to test biophysical models of the ciliary beat.

Galectins and Cilia

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Galectins, a family of about 15 small soluble proteins in mammals, are abundant and widespread (1). As carbohydrate-binding proteins (lectins), they bind typically extracellular/intravesicular glycoproteins and glycolipids but are made as cytosolic proteins also binding cytosolic proteins. Detailed roles of galectins have been defined for certain cell surface signaling, endocytosis and intracellular trafficking of glycoproteins, and for detecting disrupted vesicles and linking this to membrane repair and/or autophagy. Other clear but less defined functional implications include regulation of cilia as published by mainly others but also us:

A) Galectins are found at centrosomes and the basal bodies of cilia, in cultured cells and in polycystic kidney disease (2). **B)** Depletion of galectin-3 in cells causes primary cilia to become longer (3). **C)** Knock out of galectin-3 in mice disrupts the order of airway motile cilia, proposed due to mis-recruitment of γ-tubulin, disorganization of microtubule framework with loss of basal-body alignment and cilium orientation, defects in cilium organization and reduced fluid flow in the tracheal lumen (4). **D)** A mutant of galectin-3, with specific defects in tumor promoting activities intracellularly, accumulates selectively at centrosomes (5). **E)** Another role of galectin-8 interacting with lipid rafts to regulate primary cilia (6).

Despite such clear evidence only about 20 publications out of >11000 on galectins, have studied the link with cilia and centrosomes. This presentation is to stimulate more research in this direction. Besides the basic mechanisms there are also clinical aspects. Potent galectin inhibitors have been developed, both as cell biology tools, but also as possible therapeutics in cancer and fibrotic disease, and clinical trials are ongoing. A disruption of ciliary functions in treated subjects would be an important source of possible adverse effects, which might be mitigated by deeper mechanistic understanding.

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Molecular basis for the activation of outer dynein arms in cilia

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Multiciliogenesis requires large-scale biosynthesis of motility-powering axonemal inner and outer dynein arm motors (IDA and ODA) prior to their intraflagellar transport (IFT) into cilia. ODAs are inhibited by the packaging chaperone Shulin during ciliogenesis in *T. thermophila* [1]. How Shulin is released for ODAs to become active inside cilia remains unclear. Here we uncover a molecular mechanism for ODA activation. We establish interactions between DNAAF9 (human Shulin) and mammalian ODA subunits, IFT proteins and the ciliary small GTPase ARL3 using proteomics and *in vitro* reconstitutions. Mutagenesis combined with biochemical and structural studies reveal that DNAAF9 and Shulin preferentially bind active Arl3-GTP. GTP-loaded Arl3 can access, bind and displace Shulin from the packaged ODA-Shulin complex. We propose that once the inhibited ODA complex enters growing cilia, Arl3-GTP displaces Shulin (DNAAF9) and sequesters it away from ODAs promoting activation of their motility specifically inside cilia.

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Cilia & Flagella: Bio-Inspired Solutions for Microrobotics

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Cilia and flagella, essential organelles responsible for cellular motility in nature, have driven significant advances in microrobotics, particularly in the development of biohybrid systems and artificial micro-actuators. This talk highlights the innovative use of sperm cells and artificial cilia in microrobotics, emphasizing their potential in both fundamental research and biomedical applications. Sperm cells, with their natural flagellar propulsion, offer a promising platform for creating bio-hybrid robots capable of navigating intricated environments, making them especially relevant for applications in assisted reproduction, , and targeted drug delivery. Meanwhile, the integration of artificial cilia opens new opportunities for controlling microfluidic devices, enabling the precise manipulation of particles and cells/microorganisms at the microscale. By merging biological systems with advanced engineering techniques, cilia and flagella-based microrobotics present innovative solutions for fundamental science research and novel precision therapies.

Stochastic modeling of a two-component polymer engine

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Long coiled-coil tethering proteins and small GTPases have recently been shown to form a new class of biomolecular motors driven by entropic collapse. The working principle of this motor is a cyclic flexibility transition of its filamentous tether, triggered by the binding of the GTPase unit. While a basic working model was proposed, many fundamental aspects of these two-component molecular motors remain unexplored. Here, we developed a stochastic model as an over-damped two-state semi-flexible polymer to describe the mechanochemical cycle that drives this motor. We expand this model by introducing force-dependent rates in the mechanochemical coupling of our model, we can potentially explain previous discrepancies in the measured hydrolysis rate of GTP between bulk experiments, which occur under no force, and tweezer experiments, where the system is under tension. Using this model, we can predict how efficiency and power of this motor are affected by changes in model parameters such as persistence length. This allows us to determine the physical limits that constrain the operation of this motor and the optimum conditions under which one can extract the maximum power.

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The energetics of swimming motility in *Chlamydomonas*

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Motile cilia dissipate energy during their beat cycle in order to generate a power-stroke. The amount of energy used is an important quantity of cilia biophysics that is necessary to better understand the mechanism of the periodic beating motion at a molecular level. However, how much energy is exactly dissipated over time has been a matter of some discussion for a long time. I will discuss how we use micro isothermal calorimetry [1] to quantitatively dissect the energy use of the cilia of the green alga *Chlamydomonas*. By measuring the heat generated from a well-defined growing population of cells, we can probe the swimming contribution to the total heat by mutagenesis [2], environmental conditions and the use of drugs that influence the swimming behavior. Our results show a significant and complex impact of motility on energy dissipation and life cycle of *Chlamydomonas*. Finally, by introducing light into our microcalorimeter setup, we see circadian effects on motility dependent heat production.

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Identification of force-generating dynein states in reactivated Chlamydomonas axonemes

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Motile cilia generate beating patterns that are crucial for various biological processes, including microorganism locomotion, embryo development, and airway clearance. The outer dynein arm (ODA) with a 24-nm repeating unit is essential for driving cilia beating, as it plays a significant role in modulating the beating frequency. Though earlier studies have shown that motor activity is correlated with waveform shape, the phase relationship between the motor's conformational states and the curvature of the axoneme remains unresolved. Based on previous studies of conformational changes in ODA during the recovery stroke, we aim to link ODA activities with axonemal curvatures from reactivated *Chlamydomonas* cilia using a hybrid data acquisition approach combining single-particle analysis (SPA) and cryo-electron tomography (cryoET). Direct measurements of the phase difference will identify which dynein state is force-generating and may provide insight into the internal damping within the beating axoneme.

Relationship between movement, feeding and the energy budget of the ciliate *Tetrahymena pyriformis*

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Swimming ciliates rely on limited energy that they obtain from feeding to fuel competing processes of growth, reproduction, and movement. We expect that these microorganisms modulate the proportion of energy allocated to movement, as a strategy to cope with different environmental conditions.
In a series of experiments on the ciliate Tetrahymena pyriformis we observed short-term changes of movement speed related to environmental temperature. These short-term changes could entirely be explained by temperature-induced changes of metabolic rate with an unchanged proportion energy allocated to movement. On a longer time scale, movement speed was influenced by both temperature and nutrients available in the environment, indicating that Tetrahymena changed its allocation of metabolic energy to movement as an adaptation to the conditions of the environment. Mechanistically, this modulation of movement speed was largely achieved through changes in cell volume and shape, likely through the effect of these variables on the energetic costs of movement and on the metabolic production of energy.
Experiments like these can help bridge between the biophysics of ciliate movement and the ecological costs and benefits of different movement speeds and strategies.

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The role of OFD1 in motile cilia

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OFD1 is a pleiotropic protein that localizes to centrosome, centriolar satellites and nuclei. It is encode d by a complex 23-exon gene located on the X chromosome, whose mutations have been linked to fo ur ciliopathies: OFD syndromes type I (OFDI), Joubert (JBS10), retinitis pigmentosa (RP23) and prim ary ciliary dyskinesia (PCD). The presence of patients affected by PCD suggests a functional role of OFD1 also in motile cilia, which to date is much less characterized than its role in primary cilia. The e vident clinical and genetic heterogeneity associated with OFD1-related phenotypes, suggests differen t underlying molecular events that we aim to identify. We selected representative variants for the four OFD1-related ciliopathies and generated different lentiviral vectors to subsequently infect KO-OFD1 HK-2 cells, to establish mutant stable cell lines. The characterization of our mutants by super-resoluti on microscopy approaches and OMICs techniques led us to identify several phenotypes, specific for t he disorder, for the mutant expressing the PCD phenotype. Initial characterization with axoneme mar kers indicated that the PCD mutant exhibits a significant hyper-elongation of the cilium; this led us to hypothesize a compromised sensorial function of the cilia, which Transcriptomics analyses confirmed, showing downregulation of genes involved in signaling pathways. Surprisingly, it also revealed down-r egulation of genes involved in mitochondrial function/structures. We performed Mito-stress and TMRE assays that confirmed alteration of mitochondria parameters; this is consistent with the emerging corr elation between cilia and mitochondria. To obtain a more suitable experimental model, we are currentl y generating a stable PCD mutant in multi-ciliated respiratory epithelium cells and repeating our exper iments on patient derived cells. Our study is also supported by a bioinformatics approach that uncove red a recurrent gene pattern in PCD patients with mutations in OFD1.

Subcellular protein architecture of human sperm

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Abstract

From ejaculation to fertilization, the human sperm cell needs to travel a long distance, navigate in a highly-complex environment, and mature to be capable to ultimately penetrate the egg vestments and fertilize the egg. These processes rely on the expression, functionality and localization of a large set of proteins, many of which are unique to sperm and poorly characterized. How proteins are spatially organized in the sperm cell is poorly understood. In this study, we applied antibody-based spatial proteomics coupled to high-resolution confocal microscopy to systematically map the subcellular spatial distribution of a wide range of proteins in single human sperm cells. We identified 654 proteins and annotated their localization to 11 different subcellular structures, including 73 proteins not previously detected in human sperm. We observe that roughly 55% of the sperm proteome varies in expression levels and/or spatial distribution between individual cells. Our spatial map of the human sperm proteome is available through the Human Protein Atlas, making it broadly accessible as a resource for advancing research on the molecular architecture and mechanisms of the sperm cell and male infertility phenotypes.

Synchronization driven flows and motility of spherical ciliates

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Motile cilia are actively driven hair-like membrane-bound appendages in eukaryotic cells. They exert forces on their surrounding fluid medium and detect chemical and mechanical signals from their environment. Collectives of cilia are observed to synchronize their beat patterns through hydrodynamic interactions to increase the efficiency of fluid transport by coordinating into metachronal waves [1].

Although this problem has been extensively studied in flat periodic systems [2][3][4], most ciliated tissues in biology are topologically and geometrically more complex. As an example, most microorganisms are homeomorphic to a 2-sphere and have curved surfaces along which the cilia are distributed. However, the effects of topology and geometry in their self-organized synchronization remain unclear. Here, we present a theoretical study of ciliary synchronization in spherical topologies.

Our approach is to coarse-grain the microscopic physics of a minimal model of non-reciprocally beating cilia into a continuum theory following [4]. In doing so, we arrive at predictions for the synchronization patterns of a spherical swimmer, which will also help us to understand its swimming trajectories. Further, our description generalizes to arbitrary surface geometries and topologies, and thus will allow us to investigate cilia-driven fluid transport in a plethora of living systems.

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Fluid flow reconstruction around a free-swimming sperm in 3D

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We investigate the dynamics and hydrodynamics of a human spermatozoa swimming freely in 3D. We simultaneously track the sperm flagellum and the sperm head orientation in the laboratory frame of reference via high-speed high-resolution 4D (3D+t) microscopy, and extract the flagellar waveform relative to the body frame of reference, as seen from a frame of reference that translates and rotates with the sperm in 3D. Numerical fluid flow reconstructions of sperm motility are performed utilizing the experimental 3D waveforms, with excellent accordance between predicted and observed 3D sperm kinematics. The reconstruction accuracy is validated by directly comparing the three linear and three angular sperm velocities with experimental measurements. Our results reveal that, due to the sperm's streamlined body shape, proximity to a boundary has negligible effects on swimming motility, though it significantly alters the surrounding flow field, inducing rapid flow decay near the substrate. Our microhydrodynamic analysis uncovers a novel fluid flow pattern, characterized by a pair of vortices that circulate in opposition to each other along the sperm cell. We further demonstrate that these counter-rotating vortices are not exclusive to the experimental sperm beat but can also be reproduced by bacterium and idealized waveform models, highlighting a fundamental flow structure shared by free-swimming organisms propelled by a single 3D beating flagellum.

Ciliary Adhesion of *Chlamydomonas reinhardtii* on Charge-Functionalized Surfaces

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Elucidating the physical phenomena underlying the interactions between microorganisms and surfaces is crucial for the development of technologies to control the formation of microbial biofilms. While most studies use bacteria as model organisms, the principles of microbial adhesion remain rather elusive for eukaryotic photosynthetic microorganisms. Recently, it was discovered that the model unicellular microalga *Chlamydomonas reinhardtii* adheres to surfaces by means of its two cilia under blue light [1]. Using *in vivo* single-cell micropipette force spectroscopy [2], we characterized the ciliary adhesion forces of *C. reinhardtii* on functionalized substrates to dissect the influence of surface energy, long-ranged van der Waals and electrostatic interactions [3]. The results suggest that the predominant nature of the protein-mediated cilia-substrate adhesion of *C. reinhardtii* is due to electrostatic interactions. Here we present adhesion force measurements of *C. reinhardtii* on poly-L-lysine- and recombinant spider silk-coated silicon wafers [4], revealing no charge preference for ciliary adhesion. In contrast to prokaryotic microorganisms our results demonstrate that *C. reinhardtii* feature highly versatile cilia to realize microbial adhesion to abiotic surfaces of a broad range of physicochemical properties.

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Phase sensitivity of flagellar beating to external forces

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Eukaryotes swim with coordinated flagellar (ciliary) beating and steer by fine-tuning the coordination. The model organism for studying flagellate motility, Chlamydomonas reinhardtii, employs synchronous, breaststroke-like flagellar beating to swim, and it modulates the beating amplitudes differentially to steer. In this study, we combine experiments, computations, and modeling efforts to elucidate the roles played by each flagellum in synchronous beating. With a non-invasive technique to selectively load each flagellum, we show that the coordinated beating essentially only responds to load exerted on the cis flagellum; and that such asymmetry in response derives from a unilateral coupling between the two flagella. We also investigate the load response of flagella to an impulsive force imposed over a very short time-period. We use nonlinear system identification to determine the phase-sensitivity of cilia to external forces and find that the power stroke is rather insensitive to external forces, whereas the recovery stroke is very sensitive to external forces.

Fantastic LCs and what do they do?

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The outer dynein arm (ODA) complex in *Trypanosoma brucei*, similar to its mammalian and *Chlamydomonas* counterparts, comprises two heavy chains (ODAα and ODAβ), two intermediate chains (IC1 and IC2), and multiple light chains (LCs). During cytoplasmic assembly of the ODA complex, IC1 and IC2 form a stable intermediate complex, which subsequently assembles with the heavy chains to form the complete ODA complex [1]. Both heavy and intermediate chains are essential for the full complex formation and transport of the ODA complex into the flagella. Heavy chain depletion, however, leads to cytosolic accumulation of the IC1-IC2 intermediate complex [2].

In contrast to the well-characterized heavy and intermediate chains, the roles of light chains in ODA assembly and their functions within the flagella remain poorly understood.

A recent proteomic analysis identified 12 putative ODA light chains as interacting partners of cytoplasmic IC1 [3], yet only two have been characterized. To address this gap, I developed a systematic workflow leveraging advanced molecular genetic tools in *T. brucei*. I aim to: (1) determine if a light chain is exclusively associated with the ODA complex in the flagella, (2) assess its effect on the ODA complex in the flagellum, and (3) evaluate its role in ODA preassembly within the cytosol.

This study is the first systematic characterization of ODA light chains in *T. brucei*. The workflow developed here provides a framework for future studies on light chains and their functions in ODA assembly across species. Several differences to LCs in *Chlamydomonas* are also noted.

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Primary Ciliary Dyskinesia Associated Disease-Causing Variants in *CCDC39* and *CCDC40* Cause Axonemal Absence of Inner Dynein Arm Heavy Chains DNAH1, DNAH6, and DNAH7

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Background: Primary ciliary dyskinesia (PCD) is an inherited disorder characterized by impaired ciliary function, causing chronic airway disease. Bi-allelic variants in *CCDC39* and *CCDC40*, encoding components of a molecular ruler complex critical for 96 nm repeats in the ciliary axoneme, are common genetic causes. Loss of this complex disrupts associated structures, including the inner dynein arm (IDA) protein DNALI1 and the nexin-dynein regulatory complex (N-DRC) protein GAS8, leading to axonemal disorganization. Affected individuals exhibit stiff, rapid, and flickering ciliary beating pattern, laterality defects (50%), male infertility, and significantly reduced lung function compared to other PCD genotypes.

Objective: The study aimed to analyze a cohort of 51 individuals with disease-causing *CCDC39* and *CCDC40* variants. In addition to DNALI1 defects, we sought to investigate the localization and involvement of dynein heavy chains (DNAH1, DNAH7 and DNAH6) in the context of molecular ruler defects.

Methods: We identified 51 individuals with *CCDC39* and *CCDC40* variants by next-generation sequencing (NGS). Immunofluorescence analyses with antibodies against DNAH1, DNAH6, and DNAH7 assessed dynein localization. Transmission electron microscopy (TEM) evaluated axonemal ultrastructure defects. Molecular findings were correlated with clinical phenotypes.

Results: Our analysis showed that CCDC39 or CCDC40 deficiency cause a complete loss of the dynein heavy chains DNAH1 and DNAH7, corresponding to IDAd and IDAb/e, respectively. In addition, the inner dynein arm subunit containing CETN2 and heavy chain DNAH6 (IDAg) was absent. TEM confirmed microtubular disorganization consistent with the loss of the ruler proteins CCDC39 and CCDC40. Clinical manifestations include stiff, rapid and flickering ciliary movements, impaired lung function, laterality defects in 50% of individuals, and male infertility due to immotile sperm flagella.

Conclusion: We revealed the crucial role of CCDC39 and CCDC40 in the assembly and function of IDAs in human respiratory cilia. Antibodies targeting DNAH1, DNAH6, and DNAH7 provide valuable diagnostic tools for CCDC39- and CCDC40-related defects. Beyond the diagnostic aspect, these insights enhance patient counseling by facilitating early and appropriate treatment.

Pathogenic variants in *CFAP46*, *CFAP54*, *CFAP74* and *CFAP221* cause primary ciliary dyskinesia with a defective C1d projection of the central apparatus

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Primary ciliary dyskinesia (PCD) is a rare genetic disorder caused by insufficient mucociliary clearance leading to chronic airway infections. According to European Respiratory Society guidelines, diagnosis involves clinical history, nasal nitric oxide (nNO) production rate measurements, high-speed video-microscopy analysis (HSVMA) of the ciliary beating, and transmission electron microscopy (TEM), with genetic testing as a final step. Aim of this study was to characterise PCD due to a defective C1d projection of the central apparatus.

Using a high-throughput sequencing approach of corresponding known and candidate genes in a cohort of PCD-suspected individuals, we identified unreported pathogenic variants in the novel PCD genes *CFAP46* and *CFAP54*, and the known PCD gene *CFAP221*. To fully assess this PCD sub-group, we also reviewed individuals with pathogenic variants in *CFAP74*. To characterise identified variants we examined high-resolution immunofluorescence (IF) microscopy, TEM, western blot analysis and *in-vitro* ciliary transport assays.

Careful assessment revealed that C1d-defective PCD is associated with normal situs composition, normal nNO production rates, normal ciliary ultrastructure in TEM, and normal ciliary beating in HSVMA. In contrast, IF analyses showed abnormal results. Despite chronic respiratory disease, even current predictive tools do not reliably detect this PCD variant. However, we could show by *in-vitro* ciliary transport assays that affected individuals exhibit insufficient ciliary clearance.

Overall, this study extends the spectrum of known PCD genes and highlights that C1d-defective PCD easily escapes current diagnostics. To enable diagnosis of those PCD variants too, genetic testing should be prioritised in future guidelines.

In situ arrangement of CatSper Ca^{2+} channels in CATSPER2-deficient human sperm

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The intracellular Ca^{2+} concentration and, thereby, the motility of human sperm are controlled by the sperm-specific multisubunit CatSper-channel complex [1, 2], which is arranged along the flagellum in quadrilateral zigzag rows of channel dimers [3]. Loss of CatSper function is a common channelopathy that leads to male infertility [4]. Homozygous deletion of *CATSPER2* is the most common cause of CatSper-related infertility [4], but the molecular pathology underlying the loss of CatSper function in these patients remains unclear. Previous studies by our group indicate that the expression of the other channel subunits as well as their quadrilateral arrangement along the flagellum are preserved [5], suggesting that non-functional channel complexes assemble in the absence of *CATSPER2*. To elucidate the molecular pathology leading to loss of CatSper function and the infertility of affected men, we employ state-of-the-art cryo-ET techniques to determine the composition, architecture, and arrangement of the CatSper-complex in human sperm *in situ*. Our preliminary data on CatSper in cryo-FIB-milled human sperm flagella align with that of a previous study using a similar approach [3]. We are currently refining cryo-ET protocols to investigate the structure of the CatSper complex in sperm from control donors versus *CATSPER2*-deficient patients. This will allow structure-function analysis of the channel in different (patho)physiological conditions. Thereby, we will solve the molecular pathomechanism underlying this common sperm channelopathy.

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