Physics and Physiology of Motile Cilia

688. WE-Heraeus-Seminar

27 – 30 January 2019
Physikzentrum Bad Honnef/Germany

WILHELM UND ELSE
HERAEUS-STIFTUNG

Subject to alterations!

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.

Scope of the 688. WE-Heraeus-Seminar:

Across the animal and plant kingdom, motile cilia and flagella serve many important biological functions, including cellular propulsion, fluid transport, and sensory signaling – to name only a few. Unravelling the physiology of these integral cell organelles is an interdisciplinary endeavour at the interface of physics and biology. This research field involves studies of ciliary structure, self-organized dynamics of the ciliary beat, collective dynamics at the cellular and multicellular level, and the pathophysiology of impaired cilia function in ciliopathies. The aim of this seminar is to bring together scientists from different disciplines to address the physical, biological, and medical aspects of motile cilia.

The main topics of the seminar are:

- Ciliopathies
- Structure and assembly of cilia
- Motor control in the axoneme
- Flagellar hydrodynamics
- Navigation of ciliated swimmers
- Flagellar synchronization
- Collective dynamics
- Cilia as signaling centers

Scientific Organizers:

Prof. Dr. Timo Strünker
University of Muenster, GERMANY
Molecular Reproductive Physiology
E-mail timo.struenker@ukmuenster.de

PD Dr. Benjamin M. Friedrich
Technical University of Dresden, GERMANY
cfaed & Institute of Theoretical Physics
E-mail benjamin.m.friedrich@tu-dresden.de

Dr. Veikko F. Geyer
Technical University of Dresden, GERMANY
B CUBE - Center for Molecular Bioengineering
E-mail veikko.geyer@tu-dresden.de

Program

Program

Sunday, 27 January 2019

11:00 – 15:00	Registration	
12:30	*LUNCHTIME SNACKS and WELCOME COFFEE* *Informal get together*	
14:30 – 14:45	Scientific organizers	**Opening and welcome**
Flagellar hydrodynamics I / Chair: Benjamin Friedrich		
14:45 – 15:30	Ramin Golestanian	**A few examples of emergent behaviour in active hydrodynamic systems**
15:30 – 16:15	Jens Elgeti	**Hydrodynamics of sperm cells**
16:15 – 16:45	*COFFEE BREAK*	
Data Blitz I / Chair: Benjamin Friedrich		
16:45 – 17:00	Olivier Thouvenin	**Ciliary motility instructs the formation of the central canal and bidirectionality of cerebrospinal fluid flow to ensure effective transport during embryogenesis**
17:00 – 17:15	Anders Andersen	**Swimming, feeding, and stealth of flagellates**
Key note lecture I / Chair: Timo Strünker		
17:15 – 18:15	Heymut Omran	**The human β-HC paralogues DNAH9 and DNAH11 achieved during evolution specific functional roles for the distinct proximal and distal axonemal compartments of motile respiratory cilia**
18:30	*DINNER*	
Key note lecture II / Chair: Veikko Geyer		
19:45 – 20:45	Raymond Goldstein	**Dynamics of adaptive phototaxis in uni- and multicellular green algae**

Program

Monday, 28 January 2019

08:00	*BREAKFAST*	
Structure and assembly of cilia / Chair: Jonathon Howard		
09:00 – 09:45	Daniela Nicastro	**Structure and function of motile cilia studied by cryo-electron tomography**
09:45 – 10:30	Gaia Pigino	**Ciliary assembly and transport**
10:30 – 11:00	*COFFEE BREAK*	
Data Blitz II / Chair: Veikko Geyer		
11:00 – 11:15	Mohamed Mohamed	**Motor recruitment and activation for intraflagellar transport**
11:15 – 11:30	Thomas Fai	**Length regulation of multiple flagella that self-assemble from a shared pool of components**
Dynamics of molecular motors / Chair: Veikko Geyer		
11:30 – 12:15	Frank Jülicher	**Theory of ciliar dynamics**
12:15	**Conference Photo** (in the foyer of the lecture hall)	
12:30	*LUNCH*	

Program

Monday, 28 January 2019

Motor control in the axoneme / Chair: Frank Jülicher

14:00 – 14:45	Jonathon Howard	**Curvature feedback coordinates axonemal dyneins to drive the flagellar beat in *Chlamydomonas***
14:45 – 15:30	Philip Bayly	**Flagella motion from dynamic instability without dynein regulation**
15:30 – 16:00	*COFFEE BREAK*	

Data Blitz III / Chair: Timo Strünker

16:00 – 16:15	Soheil Mojiri	**Multi-plane phase contrast imaging reveals fast 3D observation of axonemes**
16:15 – 16:30	Hermes Gadelha	**How does the human sperm flagellum beat in 4D?**
16:30 – 17:30	**Poster blitz presentations** (1 min each / 1 slide)	
17:30 – 18:15	**Poster session**	
18:30	*DINNER*	
19:45 – 20:45	**Poster session (continued)**	

Program

Tuesday, 29 January 2019

08:00	*BREAKFAST*	
Cilia as signaling centers I / Chair: Heymut Omran		
09:00 – 09:45	Benjamin Kaupp	**Deconstruction of cellular computations in a flagellum by reverse opto-chemical engineering**
09:45 – 10:30	Markus Delling	**The function of TRPP channels as flow sensors in establishing left right asymmetry**
10:30 – 11:00	*COFFEE BREAK*	
Cilia as signaling centers II / Chair: Heymut Omran		
11:00 – 11:45	Jean-Ju Chung	**A novel pH-dependent Ca2+ sensor for regulating sperm motility and fertility**
11:45 – 12:30	Dagmar Wachten	**Optogenetic analysis of signaling in sperm flagella**
12:30	*LUNCH*	

Program

Tuesday, 29 January 2019

Data Blitz IV / Chair: Veikko Geyer

14:00 – 14:15	Timothy Krüger	**Collective swimming behaviour of a high density flagellate population on semi-solid surfaces**
14:15 – 14:30	Christian Schiffer	**Rotational motion and rheotaxis of sperm do not require CatSper Ca2+ channels**

Navigation of ciliated swimmers I / Chair: Benjamin Kaupp

14:30 – 15:15	Gáspár Jékely	**Neuronal coordination of locomotor cilia in Platynereis larvae**
15:15 – 16:00	Luis Alvarez	**Hacking sperm's navigation program**
15:30 – 16:45	COFFEE BREAK	

Navigation of ciliated swimmers II / Chair: Benjamin Kaupp

16:45 – 17:30	Mariana Medina-Sánchez	**Exploiting sperm power in biomedicine**
17:30 – 18:15	Laurence Wilson	**Adaptive swimmers: Leishmania parasites**
18:15 – 18:30	Stefan Jorda	**About the Wilhelm and Else Heraeus Foundation**
18:30	*HERAEUS DINNER at the Physikzentrum and get together (cold & warm buffet, free beverages)*	

Program

Wednesday, 30 January 2019

08:00	*BREAKFAST*	
Collective dynamics I / Chair: Ramin Golestanian		
08:30 – 09:15	Eberhard Bodenschatz	**Cilia mediated flow and cell polarity of the brain ventricular system**
09:15 – 10:00	Nathalie Delgehyr	**Ependymal cilia beating induces an actin network to protect centrioles against shear stress**
10:00 – 10:30	*COFFEE BREAK*	
Collective dynamics II / Chair: Ramin Golestanian		
10:30 – 11:15	Nathalie Jurisch-Yaksi	**The function of motile-cilia driven flow in the nervous system**
11:15 – 12:00	Benjamin Friedrich	**Synchronization of cilia and flagella**
12:00 – 12:15	Scientific organizers	**Poster awards / summary and closing remarks**
12:15	*LUNCH*	

End of the seminar *and FAREWELL COFFEE* ***/ Departure***

Please note that there will be ***no*** *dinner at the Physikzentrum on Wednesday evening for participants leaving the next morning.*

Posters

Posters

1.	Mohammad Abu Hamed	**Longwave nonlinear theory for chemically active droplet division instability**
2.	Tomer Avidor-Reiss	**The centriole anchoring the sperm flagellum has an atypical structure in mammals**
3.	Thomas Böddeker	**Propulsion force measurements of motile flagella using micropipette force sensors**
4.	Serhii Boryshpolets	**Fertilization in fresh water fish: The role of the flagellum, and evidence of guidance and selection**
5.	Gabriel Corkidi	**Intracellular calcium stores are involved in the correlation between $[Ca2^{+}]i$ oscillations and three-dimensional flagellar beating in human sperm**
6.	Debasish Das	**On the motor torque of Escherichia coli**
7.	Elke Gabriel	**Eyecup containing brain organoids from human iPSCs**
8.	Azam Gholami	**Out-of-plane beating components of active axonemes isolated from *Chlamydomonas reinhardtii***
9.	An Gong	**From 3D beating to 3D swimming: An excursion to the egg**
10.	Jay Gopalakrishnan	**Human brain organoids to model disorders due to primary cilia dysfunctions**
11.	Jon Hall	**A relationship between metachronal wavelength and fluid flow rate**
12.	Jan N. Hansen	**SpermQ - a simple analysis software to comprehensively study flagellar beating and sperm steering**
13.	Myriam Jory	**Cilia coordination and mucus flow on airway epithelia**
14.	Vitaliy Kholodnyy	**Do the rainbow trout ovarian fluid navigate the sperm on its way to the egg?**
15.	Michelina Kierzek	**Simultaneous recording of rapid cellular signaling events**
16.	Veronika Magdanz	**Getting to the egg – the link between motility, metabolism & tail length of sperm**

Posters

17.	Pascal Martin	**Self-organized wave-like beating of actin bundles**
18.	David Mick	**Investigating ciliopathies by primary cilia proteomics**
19.	Anne Oltmanns	**Altered *N*-glycosylation of FMG-1B impairs gliding motility in *Chlamydomonas reinhardtii***
20.	Nicola Pellicciotta	**Synchronisation of mammalian cilia by hydrodynamic forces**
21.	Sebastian Rode	**Sperm motility in modulated microchannels**
22.	Miriam Schmidts	**Engine failure: Biological roles of (cytoplasmic) dyneins and their dysfunction in human disease**
23.	Simon Schoeller	**Linking individual and collective dynamics of sperm in suspension**
24.	Reinhard Seifert	**The solute carrier SLC9C1 is a Na^+/H^+-exchanger gated by an S4-type voltage-sensor and cyclic-nucleotide binding**
25.	Anton Solovev	**Hydrodynamic interactions of beating cilia**
26.	Willi Stepp	**Kinesin-2 stepping reflects its heteromeric nature**
27.	Daniel Tam	**On the validity of Stokes equations to model ciliary flows**
28.	Peter Tennant	**Study of axonemal dynein assembly using endogenous protein tagging and primary ciliary dyskinesia model mice**
29.	Gerhard van der Horst	**Tracking sperm in three and four dimensions from X and Y coordinates and future prospects**
30.	Csaba Verasztó	**Ciliomotor circuitry underlying whole-body coordination of ciliary activity in the *Platynereis* larva**
31.	Yong Wang	**Three-dimensional flow in the ventral third ventricle of the brain**
32.	Christian Westendorf	**Quantitative analysis of cilia mediated flow and cell polarity of the brain ventricular system**
33.	Samuel Young	**Deafness-infertility syndrome: A model to unravel the role of CatSper in human sperm (dys)function**

Abstracts of Lectures

(in alphabetical order)

Hacking Sperm's Navigation Program

A. Gong[1], F. Lavryk[1], H. Hamzeh[1], R. Pascal[1], B. Friedrich[2], B. U.B. Kaupp[1], and L. Alvarez[1]

[1]Center of Advanced European Studies and Research (caesar), Bonn, Germany
[2]Center for Advancing Electronics, Dresden, Germany

Active exploration of sensory fields can be found across many different model organisms, cells, and robots and for sensory modalities as diverse as touch, olfaction, and vision [1-5]. To unveil the underlying cellular program translating sensory input into a motility pattern is a daunting task because sensory information is intimately coupled to motility itself. By moving, a sensory system sets the local sensory landscape of stimulation and concurrently, the stimulation sets up the corresponding motility pattern. Such integral sensory-motor units are subject of research across many disciplines. Sperm capture sensory cues that are processed and translated into a spatio-temporal pattern of the flagellar beat for propulsion and steering [6]. To understand the sensory-motor unit of sperm from sea urchins, we make use of an optochemical approach allowing the precise control of cGMP, the intracellular messenger that is synthesized during chemotaxis [7]. By adjusting the temporal pattern of the second messenger, we can produce a family of geometrical swimming paths that can be explained by a simple theory. Furthermore, we exploit the insights of this theory for remote control of sperm by light, thus, hacking into the sperm navigation program.

References

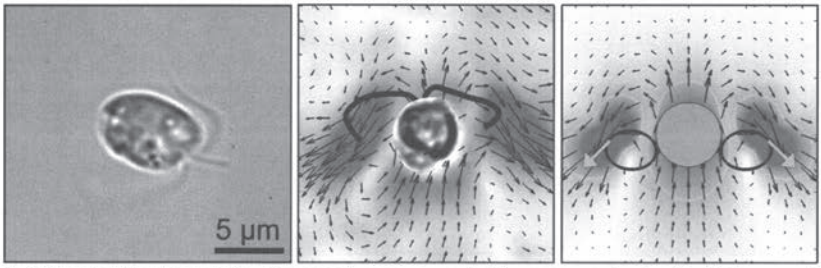
[1] E. E. Fanselow & B. W. Connors. Neuron. **45**, 329
[2] C. Petersen. www.youtube.com/watch?v=l9Dxitis0Kg (2016)
[3] S. J. Huston et al. Neuron. **88**, 403 (2015)
[4] M. Leinweber et al. Neuron. **95**, 1420
[5] R. Der & G. Martius. PNAS. **112**, E6224 (2015)
[6] J. Jikeli et al. Nat Commun. **6**, 7985 (2015)
[7] U.B. Kaupp, & L. Alvarez. Eur. Phys. J. Spec. Top. **225**, 2119 (2016)

Swimming, feeding, and stealth of flagellates

A. Andersen[1], J. Dölger[1], L. T. Nielsen[2], and T. Kiørboe[2]

[1]Department of Physics and Centre for Ocean Life, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark
[2]National Institute of Aquatic Resources and Centre for Ocean Life, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

Flagellates in the aquatic environment use their flagella to propel themselves and to create flows that support their prey capture. However, the created flows also expose the flagellates to flow-sensing predators, and stealthy swimming is therefore advantageous to avoid predation. Ecologically important flagellates display a wide variety of flagellar arrangements, and it remains largely unknown what these are adapted for with respect to swimming, feeding, and stealth. Here, we explore the dependence of swimming kinematics, prey clearance rate, and flow disturbance on flagellar arrangement, and we determine optimal flagellar arrangements and essential trade-offs. To describe near-cell flows around freely swimming flagellates we use a low Reynolds number flow model in which we represent the cell by a no-slip sphere and each flagellum by a point force. We have measured flow fields around individuals of two species of haptophytes with qualitatively different flagellar arrangements [1]. Based on observations and model results we find that equatorial force arrangements are advantageous for fast and stealthy swimming, whereas puller force arrangements support prey capture (figure). The haptophytes are able to perform photosynthesis in addition to prey capture, and we show that this mixotrophic strategy is necessary for survival, since prey capture alone cannot fulfil the energy needs of the organisms.



Individual of the flagellate *Prymnesium parvum* (left), measured velocity field around *P. parvum* (middle), and velocity field in the theoretical model of *P. parvum* (right). The two point forces (orange vectors) model the two beating flagella (middle) [1].

References

[1] Julia Dölger, Lasse Tor Nielsen, Thomas Kiørboe, and Anders Andersen, Scientific Reports **7**, 39892, 10 pages (2017).

Flagella motion from dynamic instability without dynein regulation

P.V. Bayly

Washington University in Saint Louis, Missouri, USA

The cytoskeletal structure of cilia and flagella, the "9+2" axoneme, consists of nine outer doublet microtubules, a central pair, radial spokes and circumferential links. Dynein molecules form an array of cross-bridges between pairs of microtubule doublets and exert forces that cause sliding of one doublet relative to the other. Active shear forces from dynein combine with the forces from passive structural elements (doublets, nexin links and radial spokes) to produce bending.

Since dynein activity on opposite sides of the axoneme produces antagonistic bending forces, it is widely believed that dynein activity must be dynamically regulated (switched on and off, or modulated, in every beat) to produce oscillatory waveforms. A number of theoretical studies have explored the effect of feedback from mechanical deformation to regulate dynein force. Though such feedback models exhibit oscillatory waves, evidence that dynein regulation is required for bending oscillations remains circumstantial. For example, Lin and Nicastro (2018) have shown by cryo-electron microscopy (cryo-EM) that the dominant conformation of dynein arms differs between doublet pairs in bent sections of sea urchin flagella.

Intriguingly, Geyer et al. (2016) have shown show that gradually increasing [ATP] in reactivated axonemes leads both to (1) a steady increase in static mean curvature, and (2) an abrupt transition to oscillatory beating. These data suggest that simply increasing net average force from dynein activity might lead to beating.

We recently showed (Bayly and Dutcher, 2016) that switching or modulation of dynein activity is not required to generate propulsive, oscillatory waveforms. In an elastic structure submerged in viscous fluid, like the axoneme, steady, distributed axial loading of the doublets (i.e., steady dynein activity) leads to a dynamic structural instability comonly known as flutter. This dynamic instability can occur in flagella instead of the familiar static instability, buckling, because dynein forces remain tangent to the doublets as they deform. Oscillations arise because deformation of the structure itself changes the direction of these tangential loads.The idea that steady dynein activity is sufficient to cause flagellar beating is supported by results from three complementary, mathematical methods: (i) stability analysis of the linearized partial differential equations (PDEs) of doublet motion; (ii) simulation of nonlinear equations of doublet motion; and (iii) simulation of 3D finite-element (FE) models of flagella subjected to steady, distributed, axial inter-doublet forces. Each model and analysis approcah predicts oscillatory, propulsive waves propagating from the base to tip of the flagellum, under steady dynein loading.

References

[1] Lin J, Nicastro D. Science 360, 6387 (2018)

[2] Geyer VF, Sartori P, Friedrich BM, Jülicher F, Howard J. Curr Biol 26:1098 (2016)

[3] Bayly PV, Dutcher SK. J R Soc Interface 13:20160523 (2016)

Cilia mediated flow and cell polarity of the brain ventricular system

C. Westendorf[1], S. Kapoor[2], G. Eichele[2], and E. Bodenschatz[1]

[1]Max Planck Institute for Dynamics and Self-Organization, Goettingen, Germany
[2] Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

The brain ventricles, which are filled with cerebrospinal fluid (CSF), are lined with ciliated epithelial cells. The beating of these cilia pushes the CSF and thereby transports its constituents directionally. Within the ventral 3rd ventricle the cilia carpets drive a complex transport network with several distinguishable flow domains [1]. Here we present a review of the phenomena and present results from experiments on the flow domains, the beating direction of the cilia, and the underlying cell polarity of the epithelial layer in a quantitative manner and over the entire extension of the ventricular walls of the ventral 3rd ventricle. We demonstrate that the foundation of the flow domains correlates well the translational and rotational polarities of the cilia bundles. The rotational polarities go hand in hand with the local directionality and the spatial changes of the cilia beating on a single cell level.

References

[1] R. Faubel, C. Westendorf, E. Bodenschatz, and G. Eichele, Science 353 (6295)

A novel pH-dependent Ca^{2+} sensor for regulating sperm motility and fertility

Jae Yeon Hwang[1], Nadja Mannowetz[2], Yongdeng Zhang[3], Robert A. Everley[4], Steven P. Gygi[4], Joerg Bewersdorf[3], Polina V. Lishko[2], and Jean-Ju Chung[1,5,*]

[1]Department of Cellular and Molecular Physiology, [3]Department of Cell Biology, [5]Department of Gynecology and Obstetrics; Yale School of Medicine; New Haven, CT, 06510; USA. [2]Department of Molecular and Cell Biology; University of California; Berkeley, CA, 94720; USA. [4]Department of Cell Biology; Harvard Medical School; Boston, MA, 02115, USA.

Varying pH of luminal fluid alsong the female reproductive tract is a physiological cue that modulates sperm motility. CatSper is a sperm-specific, pH-sensitive calcium channel essential for hyperactivated motility and male fertility. Multi-subunit CatSper channel complexes organize linear Ca^{2+} signaling nanodomains along the sperm tail. Here, we identify EF-hand calcium-binding domain-containing protein 9 (EFCAB9) as a dual function, cytoplasmic machine modulating the channel activity and the domain organization of CatSper. Knockout mice studies demonstrate that EFCAB9, in complex with the CatSper subunit, CATSPERζ, is essential for pH-dependent and Ca^{2+} sensitive activation of the CatSper channel. In the absence of EFCAB9, sperm motility and fertility is compromised and the linear arrangement of the Ca^{2+} signaing domains is disrupted. EFCAB9 interacts directly with CATSPERζ in a Ca^{2+} dependent manner and dissociates at elevated pH. These observations suggest that EFCAB9 is a long-sought, intracellular, pH-dependent Ca^{2+} sensor that triggers changes in sperm motility.

Ependymal cilia beating induces an actin network to protect centrioles against shear stress

A. Mahuzier[1], A. Shihavuddin[1,2], C. Fournier[3,4], P. Lansade[1], M. Faucourt[1], N. Menezes[1], A. Meunier[1], M. Garfa-Traore[5], MF. Carlier[3], R. Voituriez[6,7], A. Genovesio[1], N. Spassky[1] & N. Delgehyr[1]

[1]IBENS, Ecole Normale Supérieure, CNRS UMR8197, INSERM U1024, Paris, France.
[2]Present address : Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kgs. Lyngby, Denmark ;
[3]Biochimie Biophysique et Biologie Structurale, I2BC, CNRS 91198 Gif-sur-Yvette, France.
[4]Present address : Sorbonne Université, UPMC University Paris 06 UM 1127, Inserm U1127, CNRS UMR7225, ICM, Paris, France.
[5]Cell Imaging Platform, INSERM US24 Structure Fédérative de Recherche Necker, Paris Descartes Sorbonne Paris Cité University, Paris, France
[6]Laboratoire de Physique Théorique de la Matière Condensée, CNRS/ UPMC, Paris, France.
[7]Laboratoire Jean Perrin, CNRS/ UPMC, Paris, France.

Multiciliated ependymal cells line all brain cavities. The beating of their motile cilia contributes to the flow of cerebrospinal fluid, which is required for brain homeostasis and functions. Motile cilia, nucleated from centrioles, persist once formed and withstand the forces produced by the external fluid flow and by their own cilia beating. Here, we show that a dense actin network around the centrioles is induced by cilia beating, as shown by the disorganisation of the actin network upon impairment of cilia motility. Moreover, disruption of the actin network, or specifically of the apical actin network, causes motile cilia and their centrioles to detach from the apical surface of ependymal cell. In conclusion, **cilia beating controls the apical actin network around centrioles; the mechanical resistance of this actin network contributes, in turn, to centriole stability.**

The function of TRPP channels as flow sensors in establishing left right asymmetry

Markus Delling

University of California San Francisco
1550 4th street
San Francisco, 94158 CA

A fundamental yet unsolved problem in biology is: How do higher organisms reliably establish asymmetry? Breaking symmetry is an essential event for proper orientation of visceral organs, such as the heart and gut. Essential key players in this left-right (L-R) patterning are motile cilia generated fluid flow, primary cilia, ion channels and the embryonic node. The key sensory organelle in L-R patterning is the primary cilium, a hair-like structure protruding from the plasma membrane of most mammalian cells and believed to function like an antenna. The key organizing structure is the embryonic node, a small cup-shaped structure that consists of only 250 cells. It is highly decorated by motile cilia on pit cells at the bottom of the node. Motile cilia in the pit generate directional flow carrying information that determines the left side. Primary cilia on the perimeter of the node act as flow sensors and do so by using TRPP ion channels. That fluid flow can be exploited to break symmetry is an intriguing, but still poorly understood phenomenon. In this talk I will discuss our approach to understand the function of TRPP channels in primary cilia signaling and L-R patterning.

Hydrodynamics of Sperm Cells

J. Elgeti, S. Rode and G. Gompper

Forschungszentrum Jülich, Germany
Theoretical Soft Matter and Biophysics

Sperm cells swim in fluids. Without a fluid, the cell can not swim. In essence, it is the interaction with the surrounding fluid that allows the sperm cell to fulfill its task. This interaction with, and within the fluid is called hydrodynamics. Evidentially hydrodynamics are essentially important for sperm swimming, however it is not always clear to which extend they need to be considered. For some phenomena, the complex hydrodynamics can be simplified to effective coarse grained models. To first order, the fluid only provides friction, and the beat a propulsive force. The result is the famous active Brownian particle model, that can already explain increased cell densities at interfaces. The next higher order is anisotropic friction: A rod dragged through a fluid experiences a friction dependent on its orientation. In many cases this anisotropic friction is sufficient to explain propulsion and sperm swimming trajectories. Full hydrodynamics in turn are necessary to understand sperm – sperm and sperm – confinement interactions. In this talk I will discuss some examples where these simplifications can be done, and to which detail they are valid.

References

[1] Y. Yang, J. Elgeti, G. Gompper PRE **78** 61903 (2008)
[2] J. Elgeti, G. Gompper, EPL **85** 3802 (2009)
[3] J. Elgeti, G. Gompper, EPL **85** 3802 (2009)
[4] J. Elgeti, UB. Kaupp, G. Gompper, Biophys J **99** 1018 (2010)
[5] J. Elgeti, G. Gompper, EPL **101** 48003 (2013)
[6] J. Elgeti, G. Gompper, EPL **109** 58005 (2015)
[7] J. Elgeti, R. Winkler, G. Gompper, Rep. Prog. Phys. **78** 056601 (2015)
[8] J. Elgeti, G. Gompper, EPJST **225** 2333 (2016)
[9] J. Elgeti, et al. Nat. Commun. **8** 1415 (2017)
[10] S. Rode, J. Elgeti, G. Gompper, NJP (to appear 2018)

Length regulation of multiple flagella that self-assemble from a shared pool of components

T. Fai[1], L. Mohapatra[1], J. Kondev[1], and A. Amir[2]

[1]Brandeis University, Waltham, MA, USA
[2] Harvard University, Cambridge, MA, USA

The single cell green alga *Chlamydomonas reinhardtii* with its two flagella has proved to be a very useful model organism for studies of size control. We consider a model of flagellar length control whose key assumption is that proteins responsible for the intraflagellar transport (IFT) of tubulin are present in limiting amounts. We show that this limiting-pool assumption and simple reasoning based on the law of mass action leads to an inverse relationship between the rate at which a flagellum grows and its length, which has been observed experimentally, and has been shown theoretically to provide a mechanism for length control. We extend our length-control model to two flagella by considering different mechanisms of their coupling. Within our theoretical framework we conclude that, if tubulin and IFT proteins are freely exchanged between flagella, simultaneous length control is not possible if the disassembly rate is constant. However, if disassembly depends on the concentration of IFT proteins at the tip of the flagellum, simultaneous length control can be achieved.

References

[1] W. Marshall, H. Qin, M. Brenni, and J. Rosenbaum, Molecular Biology of the Cell, **16,** 270 (2005).

[2] T. Fai, L, Mohapatra, J. Kondev, and A. Amir, bioRxiv 436360 [**Preprint**], (2018). doi: https://doi.org/10.1101/436360.

Synchronization of cilia and flagella

B.M. Friedrich[1]

[1]*TU Dresden, Dresden, Germany*

I will present a historical overview on self-organized synchronization of cilia and flagella, as observed in the symmetric swimming gaits of flagellated microswimmers, and metachronal waves in cilia carpets.

I will address key experiments in the field and theoretical concepts, with particular focus on the minimal model of rotating spheres of Vilfan and Jülicher, which has been influential in the field and highlights how synchronization depends on broken symmetries [1]. I will compare two physical mechanisms of synchronization, (i) by direct hydrodynamic interactions [2] and (ii) by mechanical self-stabilization as proposed for free-swimming *Chlamydomonas*. Both mechanisms rely on a load response of the flagellar beat, i.e. the fact that flagella reversibly change speed and shape of their beat in response to time-dependent external hydrodynamic forces [3,4].

References

[1] A Vilfan, F Jülicher: Hydrodynamic flow patterns and synchronization of beating cilia. *Phys. Rev. Lett.* **96**, 058102 (2006)

[2] DR Brumley, KY Wan, M Polin, RE Goldstein: Flagellar synchronization through direct hydrodynamic interactions. *eLife* **3**, 5030732 (2014)

[3] BM Friedrich: Hydrodynamic synchronization of flagellar oscillators. *Eur. Phys. J. Spec. Top.* **225**, 2353 (2015)

[4] GS Klindt, C Ruloff, C Wagner, BM Friedrich: Load response of the flagellar beat. *Phys. Rev. Lett.* **117**(25), 258101 (2016)

How does the human sperm flagellum beat in 4D?

H. Gadelha[1], P. Hernández-Herrera[2], F. Montoya[2], A. Darszon[3] and G. Corkidi[2]

[1]Department of Mathematics, University of York, York, UK
[2] Laboratorio de Imágenes y Visión por Computadora, Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Mexico.
[3] Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM, Cuernavaca, Mexico.

The waving motion of human sperm flagella is the archetype of spatiotemporal self-organization in nature and critical for reproduction [1,2]. Flagellar waves result from the combined action of hundreds of molecular motors deeply embeded in the flagellar structure that bend, shear and twist the flagellum in three-dimensions to generate a helical propulsion [1]. Nevertheless, our understanding of the flagellar movement has been limited thus far to microscope planar projections [2]. Here we employ a state-of-the-art, high-speed 3D microscope imaging capture with bespoke mathematical imaging processing capable of resolving the flagellar movement in 4D (3D+time) with high-resolution [1]. This allows novel exploration of the flagellar kinematics in 3D, from simple head trajectories to curvature and torsion with a microscopic resolution. This revealed a novel complex regulatory mechanism coupling bending and torsional travelling waves. We observe for the first time that kinematic torsion is singularly distributed along the flagellum, and capable of inducing a novel flagellar helical 'perversion' phenomenon. The latter is found in helices composed by sections of opposite chirality. Such flagellar perversion is however dynamical, and travels during the beat. As a result, the chirality of the flagellum, that is the handiness of the helical waveform, is not conserved along the flagellum. We further show that this phenomenon may lead to artefacts on the curvature waves if examined with a 2D microscope. The lack of conserved chirality of the flagellar waveform may stabilise the sperm ascension in convoluted flows and geometry within the reproductive tract.

References

[1] Corkidi G, Montoya F, Hernández-Herrera P, Ríos-Herrera WA, Müller MF, Treviño CL, Darszon A. Mol Hum Reprod. **23(9)** (2017)

[2] Gaffney EA, Gadelha H, Smith DJ, Blake JR, Kirkman-Brown JC.. Annual Review of Fluid Mechanics, **43** (2011)

Dynamics of Adaptive Phototaxis in Uni- and Multicellular Green Algae

Raymond E. Goldstein

Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, University of Cambridge, Cambridge CB3 0WA, United Kingdom

One of the most fundamental issues in understanding the earliest stages of the evolution of multicellularity is how the simplest such organisms can exhibit coordinated behavior in the absence of a central nervous system. Green algae belonging to the lineage that spans from *Chlamydomonas* to *Volvox* can serve as model organisms for studying many issues in biological fluid dynamics [1], and are remarkably well-suited model to this issue. In this lecture, I first review our earlier work on phototaxis in *Volvox* [2], which showed by experiment and theory how a rather precise tuning between the timescale of adaptation in the flagellar photoresponse and the organism's rotational period allows for accurate steering. I then discuss extensions of this work to *Chlamydomonas* [3] and the fascinating 16-cell organism *Gonium* [4]. Taken together, these investigations shed light on the evolutionary steps involved in multicellular phototaxis.

References

[1] "Green algae as model organisms for biological fluid dynamics," R.E. Goldstein, *Annu. Rev. Fluid Mech.* **47**, 343-375 (2015).

[2] "Fidelity of adaptive phototaxis," K. Drescher, R.E. Goldstein and I. Tuval, *Proc. Natl. Acad. Sci. USA* **107**, 11171-11176 (2010).

[3] "An adaptive flagellar photoresponse determines the dynamics of accurate phototactic steering in *Chlamydomonas*," K.C. Leptos, M. Chioccioli, S. Furlan, A.I. Pesci, and R.E. Goldstein, preprint (2018).

[4] "Phototaxis in the simplest multicellular alga," F. Moisy, H. de Maleprade, K.C. Leptos, and R.E. Goldstein, preprint (2018).

A few examples of emergent behaviour in active hydrodynamic systems

R. Golestanian[1,2]

[1]*Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany*
[2]*Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, UK*

I will discuss a number of different examples where using actuation of some components that couple to each other through a viscous fluid will lead to emergent properties that are not *a priori* expected. These example include a strategy to control collective dynamical rotational patterns in assemblies of magnetically actuated components in a viscous fluid [1], controlling synchronization in models of hydrodynamically coupled motile cilia [2,3], and a steering mechanism for phototaxis in *Chlamydomonas* [4].

References

[1] D. Matsunaga, J.K. Hamilton, F. Meng, F.Y. Ogrin, J.M. Yeomans, and R. Golestanian, submitted (2019)
[2] A. Maestro, N. Bruot, J. Kotar, N. Uchida, R. Golestanian, and P. Cicuta, Communications Physics **1**, 28 (2018)
[3] N. Uchida, R. Golestanian, R.R. Bennett, J. Phys. Soc. Jpn. **86**, 101007 (2017)
[4] R.R. Bennett and R. Golestanian, J. R. Soc. Interface **12**, 20141164 (2015)

Curvature feedback coordinates axonemal dyneins to drive the flagellar beat in *Chlamydomonas*

Jonathon Howard

Yale University, New Haven, USA

The snake-like beating patterns of sperm tails and the breast-stroke-like swimming strokes of ciliated organisms are driven by the molecular motor dynein. This motor protein generates sliding forces between adjacent microtubule doublets within the axoneme, the motile cytoskeletal structure within cilia and flagella. To create regular, oscillatory beating patterns, the activities of the dyneins must be coordinated, both spatially and temporally. It is thought that coordination is mediated by stresses or strains that build up within the moving axoneme, but it is not known which components of stress or strain are involved, nor how they feed back on the dyneins. To answer this question, we have measured the beating patterns of isolated, reactivate axonemes of the unicellular alga *Chlamydomonas reinhardtii* [1]. We compared the measurements in wild-type and mutant cells with models derived from different feedback mechanisms. We found that regulation by changes in axonemal curvature was the only mechanism that accords with the measurements [2]. We suggest that distortions due to bending of twisted axonemes may provide a mechanism by which the motors sense curvature [3]. T o facilitate modeling studies of axonemal beats, we have published a simplified version of our model [4].

References

[1] Geyer, V. F., Sartori, P., Friedrich, B. M., Jülicher, F., & Howard, J. *Current Biology* **26**, 1098–1103 (2016).

[2] Sartori, P., Geyer, V. F., Scholich, A., Jülicher, F., & Howard, J. *eLife*, **5**, 343 doi.org/10.7554/eLife.13258 (2016)

[3] Sartori, P., Geyer, V. F., Howard, J., & Jülicher, F. *Physical Review E*, **94.042426** (2016)

[4] Geyer, V. F., Sartori, P., Jülicher, F., & Howard, J. In *Dyneins* (1st ed., Vol. 32, pp. 192–212). Academic Press. (2017)

Neuronal coordination of locomotor cilia in Platynereis larvae

Gáspár Jékely[1]

[1]*Living Systems Institute, University of Exeter, Exeter, UK*

In ciliary swimmers, ciliary beating, arrests, and changes in beat frequency are often coordinated across extended or discontinuous surfaces. To understand how such coordination is achieved, we studied the ciliated larvae of Platynereis dumerilii, a marine annelid. Platynereis larvae have segmental multiciliated cells that regularly display spontaneous coordinated ciliary arrests. With whole-body connectomics, activity imaging, transgenesis, and neuron ablation we characterized the entire ciliomotor circuitry of Platynereis. The circuit consists of cholinergic, serotonergic, and catecholaminergic ciliomotor neurons. The synchronous rhythmic activation of cholinergic cells drives the coordinated arrests of all cilia. The serotonergic cells are active when cilia are beating. Serotonin inhibits the cholinergic rhythm, and increases ciliary beat frequency. Based on their connectivity and alternating activity, the catecholaminergic cells may generate the rhythm. The ciliomotor circuitry thus constitutes a stop-and-go pacemaker system for the whole-body coordination of ciliary locomotion. The cholinergic neurons can also be activated upon hydrodynamic stimulation. This response is part of the startle reaction that contributes to predator avoidance.

References

[1] Verasztó et al., eLife **6:e26000** (2017)
[2] Bezares-Calderón et al. biorXiv (2018)

Theory of ciliar dynamics

Frank Jülicher

Max Planck Institute for the Physics of Complex Systems, Dresden,
Nöthnitzer Str. 38, 01187 Dresden, Germany

I will present general theoretical approaches to the dynamics of real and artificial cilia. Starting from specific models, I will outline a general theory of active filaments. This general approach allows us to distinguish different regimes of active filament dynamics. In this general framework we recover previous models as special cases and study new regimes of oscillatory dynamics.

The function of motile-cilia driven flow in the nervous system

Nathalie Jurisch-Yaksi
Kavli Institute for Systems Neuroscience, NTNU, Olav Kyrres Gate 9, 7030 Trondheim, Norway

Coordinated beating of motile cilia leads to a directional fluid flow, which is important for various biological processes from respiration to reproduction. In the nervous system, motile ciliary beating of ependymal cells allows the cerebrospinal fluid (CSF) to flow through the ventricular system. Such flow plays a central role in the nervous system as human patients or animal models with ciliary defects develop neurological features including hydrocephalus and spine curvature. Still, very little is known about how the nervous system generates and regulates specific flow patterns and how flow controls neural activity and animal behavior. Here, I will first describe the mechanisms used by motile cilia to generate specific flow pattern in the zebrafish olfactory epithelium and the function of the flow in olfactory processing. Later, I will discuss how motile cilia and other physiological factors act jointly to regulate cerebrospinal fluid flow dynamics in the brain ventricles. I will also describe the importance of ciliary beating during brain development. Altogether, our long-term goal is to understand the role of this increasingly popular organelle in the brain, which has recently attracted much attention from diverse disciplines of neuroscience.

References

[1] I Reiten et al, Current Biology; 27 (2), p166-174 (2017)
[2] E. W. Olstad et al, Available at SSRN 3199994 (2018)

Deconstruction of cellular computations in a flagellum by reverse opto-chemical engineering

H. Hamzeh, R. Seifert, H.G. Körschen, L. Alvarez, & U.B. Kaupp

Center of Advanced European Studies and Research (caesar), Bonn, Germany

Cells can register a large number of chemical and physical environmental cues that initiate cellular signaling and ultimately lead to a behavioral response. Inside an organism, resident cells are exposed to a rich, rapidly changing chemical milieu, i.e. a spatial or temporal chemical landscape. The complexity is further aggravated in motile cells, because navigation shapes a cell's "perception" of a sensory landscape, a concept called information self-structuring. While moving, cells are exposed to puffs, plumes, ramps, periodic cycles, or voids of diverse stimuli. The relation between a stimulus pattern s(t), the cellular responses $c_i(t)$, and the ensuing locomotion $\boldsymbol{r}(t)$ is not fully understood for any biological system. Our lack of knowledge is largely due to the fact that naturalistic stimulus patterns are spatially complex, highly dynamic, and difficult to emulate in a laboratory. Furthermore, it is technically challenging to simultaneously map in real time the *recursive* interactions between a spatio-temporal sensory landscape, the encoding cellular signaling network, and eventually locomotion behavior.

We will present a new concept, called *reverse opto-chemical engineering* (ROCE), to deconstruct the computational algebra of signaling reactions that encode a stimulus pattern and generate a behavioral response. The concept is reminiscent of reverse genetics, which introduces specific mutations into genes and analyzes the resulting phenotype. By analogy, using light-sensitive signaling molecules, we impose a signaling pattern ("mutation") onto cells by light and record the resulting signaling and behavioral response ("phenotype"). The basic idea is to create intracellularly by light a *virtual* stimulus function $s_{vir}(t)$, which mimics a well-defined external stimulus pattern that emulates a near-naturalistic sensory landscape. We applied the ROCE concept to cGMP-signaling in cell lines and sperm.

Collective swimming behaviour of a high density flagellate population on semi-solid surfaces

Timothy Krüger and Markus Engstler

[1]*Cell and Developmental Biology, University Würzburg, Würzburg, Germany*

The flagellate parasite *Trypanosoma brucei* exhibits a series of interesting swimming behaviours during its life cycle [1]. We have analysed the swimming behaviour of most developmental morphotypes on the single cell level (Fig. 1). The cell forms include several special and unique microswimmer types, which imply specific behaviours depending on various host environments. Of special interest are collective swimming behaviours that occur at high densities in the tsetse fly host [1]. Certain developmental stages also show fascinating patterns of collective migration in artificial environments and cell signalling has been implied to regulate such behaviour [2, Fig. 2]. Both the mechanism of collective migration and the potential signalling mechanisms are unclear. We have developed cell lines and assays in order to analyse cell motility of a specific flagellate type during mass migration and show first quantitative single cell data of collective motility regulation depending on defined states of the hydrogel environments (Fig. 3).

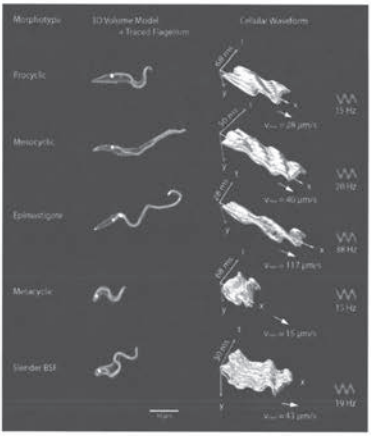


Fig. 1 Lifecycle stages of *T. brucei* (from [3])

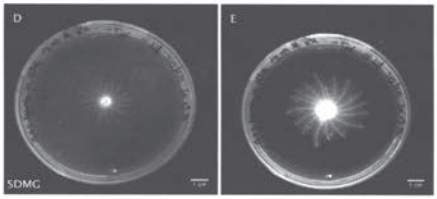


Fig. 2 Collective motility pattern

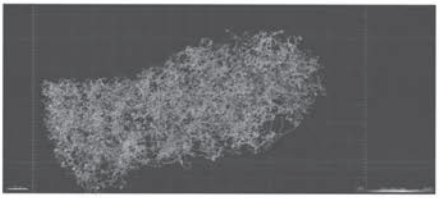


Fig. 3 Single cell tracking in migrating population

References

[1] Schuster S et. al., eLife Sciences. 2017;6: e27656 doi:10.7554/eLife.27656

[2] Eliaz D et al., PLOS Pathogens. 2017;13: e1006245 doi:10.1371/journal.ppat.1006245

[3] Krüger T. and Engstler M. Micromachines. 2018;9: 63. doi:10.3390/mi9020063

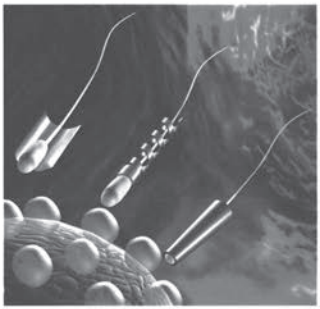
Exploiting Sperm Power in Biomedicine

M. Medina-Sánchez[1], L. Schwarz[1], H. Xu[1], F. Striggow[1], A. Aziz[1], and O.G. Schmidt[1,2]

[1] Institute for Integrative Nanosciences, Leibniz IFW Dresden, Helmholtzstrasse 20, 01069 Dresden, Germany

[2] Center for Materials, Architectures and Integration of Nanomembranes (MAIN), TU Chemnitz, Reichenhainer Strasse 10, 09107 Chemnitz, Germany

Sperms are becoming key components of medical microrobotics due to their intrinsic characteristics such as efficient motility, capability to carry and deliver small molecules, ability to fuse with somatic cells, sensitivity to different natural guidance mechanisms and their biocompatible nature, which make them suitable candidates to carry therapeutics, imaging agents or to perform their natural function even in case of certain infertility problems. In this presentation, we will show our vision of using sperm cells as main components of bio-hybrid microrobotics and review the different biomedical applications that our group has reported so far, including assisted fertilization and targeted drug delivery. We will highlight aspects such as biocompatibility, sperm selection and migration, imaging and control towards in vivo research. The exploitation of such a highly available cell line has recently opened new routes in the biomedical field that will be further broadened and refined in the next years.



References

[1] V. Magdanz, S. Sanchez, O.G. Schmidt, Advanced Materials, **25**(45), 6581-6588 (2013)

[2] M. Medina-Sánchez, L. Schwarz, A.K. Meyer, F. Hebenstreit, O.G Schmidt, Nano Letters, **16**(1), 555-561 (2015)

[3] H. Xu, M. Medina-Sánchez, V. Magdanz, L. Schwarz, F. Hebenstreit, O.G Schmidt, ACS Nano, 12(1), 327-337 (2017)

[4] M. Medina-Sánchez and O.G. Schmidt, Nature, 545(7655), 406-408 (2017)

Motor recruitment and activation for intraflagellar transport

Mohamed A. A. Mohamed[1], Willi L. Stepp[1] and Zeynep Ökten[1,2]

[1]Physik Department E22, Technische Universität München, Munich, Germany
[2] Munich Center for Integrated Protein Science, Munich, Germany

Despite the functional diversity, almost all cilia, with very few exceptions, are built by the highly conserved intraflagellar transport (IFT) machinery[1,2]. The continuous bidirectional transport (IFT), towards the ciliary tip (anterograde) is powered by kinesin-2 motor and towards the ciliary base (retrograde) is powered by dynein-2 motor, of ciliary cargoes and ciliary precursors is crucial for cilia assembly[3,4,5,6]. In C. *elegans* sensory cilia, the anterograde transport is driven by the heterotrimeric kinesin-II (KLP-11,KLP-20,KAP1) and the homodimeric kinesin-2 (OSM-3) motors[5,6]. Here we used the bottom-up approach to in vitro reconstitute the first functional multi-component IFT complex that is deployed in the sensory cilia of C. *elegans*. Our work shows that the DYF-1, an IFT-B complex subunit, is the key component for recruitment of OSM-3 to its physiologically relevant context which in turn allosterically activates the motor for efficient transport. This study also demonstrates the power of the bottom-up approach to unravel molecular mechanisms of highly convoluted processes such as ciliogenesis.

References

[1] Pazour, G. J. & Rosenbaum, J. L. Intraflagellar transport and cilia-dependent diseases. Trends Cell Biol. **12**, 551–555 (2002).

[2] Sharma, N., Berbari, N. F. & Yoder, B. K. Ciliary dysfunction in developmental abnormalities and diseases. Curr. Top. Dev. Biol. **85**, 371–427 (2008).

[3] Pedersen, L. B. & Rosenbaum, J. L. Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. Curr. Top. Dev. Biol. **85**, 23–61 (2008).

[4] Kozminski, K. G., Johnson, K. A., Forscher, P. & Rosenbaum, J. L. A motility in the eukaryotic flagellum unrelated to flagellar beating. Proc. Natl Acad. Sci. USA **90**, 5519–5523 (1993).

[5] Prevo, B., Scholey, J. M. & Peterman, E. J. G. Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. FEBS J. **284**, 2905–2931 (2017).

[6] Ou, G., Blacque, O. E., Snow, J. J., Leroux, M. R. & Scholey, J. M. Functional coordination of intraflagellar transport motors. Nature **436**, 583–587 (2005).

Multi-plane Phase Contrast (MPC) imaging

S. Mojiri, I. Gregor, A. Gholami, J. Enderlein

III. Institute of Physics, Georg August University, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

Max Planck Institute for Dynamics and Self-organization, Am Fassberg 17, 37077 Göttingen, Germany

Abstract: Phase contrast microscopy is a label-free technique, which exploits phase shifts in white-light traversing through a transparent specimen. The core idea is to convert these phase shifts to changes in intensity in the final image. The technique, capable of resolving sub-cellular organelles in living cells, has found significant applications in cell biology. However, most of the volume reconstruction analysis used by three-dimensional imaging methods compromise temporal resolution and are complex in nature. Here, we propose the combination of phase contrast apparatus in illumination and a customized prism in detection path rendering simultaneous acquisition of eight planes through the specimen depth. Using this approach, we recorded 3D images of living cells (axonemes) up to 200 Hz. Most importantly, the method does not demand any iteration, reconstruction, or post-processing analysis.

Key words: multi-plane imaging, phase contrast microscopy.

Structure and function of motile cilia studied by cryo-electron tomography

D. Nicastro

University of Texas Southwestern Medical Center, Dallas, USA

Rapid freezing of cells can provide outstanding structure preservation and time resolution of dynamic cellular processes. Cryo-electron tomography (cryo-ET) of rapidly frozen specimens is a powerful technique for imaging biological structures in their native state and in an unperturbed cellular environment. We integrate high-resolution imaging by either cryo-ET and sub-tomogram averaging, or TYGRESS (Tomography-Guided 3D Reconstruction of Subcellular Structures), with comparative genetics, biochemical methods and EM-visible labeling to deconstruct the in situ 3D structure and functional organization of macromolecular complexes. We study the protein composition, 3D structure, function and regulation of motile cilia and flagella as model system to advance techniques and approaches for high-resolution imaging of complex cellular structures. Cilia and flagella are conserved and ubiquitous eukaryotic organelles that are composed of more than 600 different proteins and have important biological roles in motility and sensation; defects in their assembly or function cause severe human diseases. Our cryo-ET studies visualize the three-dimensional structures of intact wild type and mutant flagella, and dissect the organization of key macromolecular complexes in different functional states. Such information can provide detailed insights into the structural basis and ultimately the function of many cellular processes.

The human β-HC paralogues DNAH9 and DNAH11 achieved during evolution specific functional roles for the distinct proximal and distal axonemal compartments of motile respiratory cilia

Heymut Omran

Department of General Pediatrics, University Children's Hospital Muenster, Albert-Schweitzer-Campus 1, Geb. A1, D-48149 Münster, Germany

Primary ciliary dyskinesia (PCD) is characterized by chronic airway disease and randomization of the left/right body axis caused by defects of motile cilia. Outer dynein arm heavy chains (ODA HCs) are essential for ciliary beat generation. We have shown that human respiratory cilia contain distinct ODA types: The panaxonemally distributed □-HC DNAH5, proximally located □-HC DNAH11 (ODA type 1) and the distally located □-HC DNAH9 (ODA type 2). Loss of axonemal ODAs type 1 and type 2 caused *PIH1D3* and *C11ORF70* mutations result in immotility of respiratory cilia due to defective cytoplasmic assembly of ODAs. PIH1D3 and C11ORF70 interact with the cytoplasmic dynein assembly factor DNAAF2 consistent with a role of both proteins in assembly of ODAs.

We now identified loss-of-function mutations in *DNAH9* which result in laterality defects. *In-situ* hybridization revealed *Dnah9* expression in pit cells at the embryonic node in mice. *DNAH9*-mutant respiratory cilia show distally impaired ciliary bending and lack of other ODA-type-2 components such as DNAH5 from the distal axonemal compartment. This demonstrates an essential role of DNAH9 for axonemal assembly of ODAs type 2. In contrast, in *DNAH11*-mutant cilia proximal bending is altered but other ODA-type-1 components can still be assembled. Interestingly, during ciliogenesis of respiratory cilia first proximally located DNAH11 and then distally located DNAH9 becomes assembled into the axoneme. We propose that the □-HC paralogues DNAH9 and DNAH11 achieved specific functional roles for the distinct axonemal compartments during evolution and human DNAH9 matches that of ancient □-HCs e.g. of the unicellular *Chlamydomonas reinhardtii*.

Ciliary assembly and transport

Gaia Pigino[1]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

Assembly of the cilium requires the rapid bidirectional intraflagellar transport (IFT) of building blocks to and from the site of assembly at its tip [1]. This bidirectional transport is driven by the anterograde motor kinesin-2 and the retrograde motor dynein-1b [2][3]. However, to drive retrograde transport, dynein-1b must first be delivered to the ciliary tip by anterograde IFT trains. In other bidirectional transport processes, the presence of opposing motors leads to periodic stalling and slowing of cargos moving along the microtubule. However, no such braking effect appears to occur in IFT. Here we use the most advanced technologies in cryo-electron tomography and sub-tomogram averaging to reveal the 3D structure of the complex IFT machinery (25 proteins) [4]. We show that a tug-of-war between kinesin-2 and dynein-1b is prevented by loading dynein-1b onto anterograde IFT trains in an inhibited conformation and by positioning it away from the microtubule track to prevent binding. Once at the ciliary tip, anterograde trains disassemble and release dynein-1b in an intermediate "open" conformation, which then transitions into an active form to drive the movement of retrograde trains. These findings show how tightly coordinated structural changes mediate the behavior of complex cellular machines.

References

[1] Kozminski, K. G., Johnson, K. A., Forscher, P. & Rosenbaum, J. L., PNAS 90, 5519–5523 (1993).

[2] Cole, D. G. et al., J. Cell Biol. 141, 993–1008 (1998).

[3] Porter, M. E., Bower, R., Knott, J. A., Byrd, P. & Dentler, W., Mol. Biol. Cell 10, 693–712 (1999).

[4] Jordan, M.A., Diener, R.D., Stepanek, L., Pigino G., Nature Cell Biology 20(11), 1250–1255 (2018).

Rotational motion and rheotaxis of sperm do not require CatSper Ca^{2+} channels

C. Schiffer[1], S. Rieger[2], C. Brenker[1], S. Young[1], H. Hamzeh[3], D. Wachten[4,5], F. Tüttelmann[4], A. Röpke[4], U. B. Kaupp[3], C. Krallmann[1], S. Kliesch[1], C. Fallnich[2,6], T. Strünker[1,6]

[1]Center of Reproductive Medicine and Andrology, University Hospital Münster, University of Münster, Münster, Germany
[2]Department of Optical Technologies, Institute of Applied Physics of the University of Münster, Münster, Germany
[3]Molecular Sensory Systems, Center of Advanced European Studies and Research, Bonn, Germany
[4]Center of Advanced European Studies and Research (caesar), Minerva Max Planck Research Group, Molecular Physiology, Bonn, Germany
[5]Institute of Innate Immunity, University Hospital, University of Bonn, Bonn, Germany
[6]Cells-in-Motion Cluster of Excellence (EXC1003-CiM), Münster, Germany

Rheotaxis, the navigation in flows of fluid, serves as a long-range oviductal guidance mechanism for mammalian sperm. Rheotactic navigation is promoted by the rotation of sperm around their longitudinal axis (rolling). Rolling and rheotaxis reportedly require Ca^{2+} influx via the Ca^{2+} channel CatSper. To scrutinize this model, we studied sperm from healthy donors and from patients that suffer from the deafness-infertility syndrome (DIS). DIS patients lack functional CatSper channels (see poster by Young *et al.*). Dark-field imaging of freely moving human sperm and optical trapping of single sperm show that rolling does not require Ca^{2+} influx via CatSper. Moreover, microfluidic experiments demonstrate that CatSper is dispensable for rheotaxis of human sperm. Finally, we show that also CatSper-deficient mouse sperm display a readily observable longitudinal rolling and navigate by rheotaxis. Altogether, these results refute the model that CatSper is required for sperm rolling and rheotaxis.

Ciliary motility instructs the formation of the central canal and bidirectionality of cerebrospinal fluid flow to ensure effective transport during embryogenesis

Olivier Thouvenin[1,*], Ludovic Keiser[2,*], Yasmine Cantaut Belarif[1], Martin Carbo-Tano, Pierre-Luc Bardet[1], François Gallaire[2,#], Claire Wyart[1,#]

1. Institut du Cerveau et de la Moelle épinière (I.C.M.), Inserm U 1127, CNRS UMR 7225, Sorbonne Université, F-75013, Paris, France

2. Laboratory of Fluid Mechanics and Instabilities, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

** Equal contribution*

 # Correspondance : claire.wyart@icm-institute.org, francois.gallaire@epfl.ch

The circulation of cerebrospinal fluid (CSF) plays pivotal roles for body axis formation, spine organogenesis and brain development. During embryogenesis, we have shown that the CSF contains dense lipidic particles and flows bidirectionally in the central canal. The origins of this bidirectional flow and its impact on transport are unknown. Here, we show that motile cilia are critical for opening the lumen of the central canal. Our experiments and model demonstrate that the dynamics of CSF flow is locally-generated by caudally-polarized motile cilia confined to the ventral central canal. This active flow enables to speed up the long-range bidirectional transport of particles in a size-independent manner. The spontaneous twitches of the embryo increase local flow and thereby boost long-range transport in the CSF. Altogether, our data demonstrate that during embryogenesis cilia motility is the main drive of CSF bidirectional circulation in the central canal, and that bidirectional flow combined with spontaneous muscle contractions boost long range transport in order to convey metabolites throughout the body.

Optogenetic analysis of signaling in sperm flagella

Melanie Balbach[1#], Hussein Hamzeh[1], Jan F. Jikeli[2], Christoph Brenker[3], Jan N. Hansen[2], Wolfgang Bönigk[1], Christian Trötschel[4], Frank Tüttelmann[5], Albrecht Röpke[5], Harvey M. Florman[6], U. Benjamin Kaupp[1], Timo Strünker[3*], Dagmar Wachten[2, 7*]

[1]Center of Advanced European Studies and Research (caesar), Department of Molecular Sensory Systems, Bonn, Germany
[2]Institute of Innate Immunity, University Hospital, University of Bonn, Bonn, Germany
[3]Center of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany
[4]Biochemie der Pflanzen, Ruhr-Universität Bochum, Bochum, Germany
[5]Institute of Human Genetics, University Hospital Münster, Münster, Germany
[6]Department of Cell and Developmental Biology, University of Massachusetts Medical School Worcester, MA, USA
[7]Center of Advanced European Studies and Research (caesar), Minerva Max Planck Research Group, Molecular Physiology, Bonn, Germany

[#]present address:
Department of Pharmacology, Weill Cornell Medical College, New York, NY, USA

**equally contributed*

Mammalian oocytes are enveloped by the *zona pellucida* (ZP), an extracellular matrix of glycoproteins. To fertilize the oocyte, sperm have to penetrate the ZP. To this end, interaction with ZP proteins evokes certain sperm behavioral responses. However, the mechanisms underlying ZP action in sperm are only ill-defined. Here, we investigate the action of native and recombinant ZP proteins in mouse and human sperm. Using a combination of state-of-the-art techniques, e.g. optogenetics, we show that in both sperm species, ZP proteins evoke a rapid intracellular pH_i increase and Ca^{2+} influx via the sperm-specific Ca^{2+} channel CatSper. The mechanisms of CatSper activation and the molecules underlying the pH_i increase are, however, distinctively different in human versus mouse sperm. Our findings reveal several molecular components and species-specific differences underlying ZP action in sperm and support the notion that signaling molecules in mammalian sperm feature species-specific properties.

Adaptive swimmers: Leishmania parasites

R. C. Findlay[1,2], P.B. Walrad[1] and L.G. Wilson[2]

[1] *Department of Biology, University of York, Heslington, York, UK*
[2] *Department of Physics, University of York, Heslington, York, UK*

Single-cell parasites of the genus *Leishmania* cause leishmaniasis, the second-deadliest parasitic disease after malaria. The complicated lifecycle of this parasite is still poorly understood, with rather imprecise morphological distinctions between parasites at different stages. Despite this, it offers an intriguing system for the study of navigation in flagellated microswimmers. The parasite radically changes its physiology as it transitions from replicative procyclic form, to human-infective metacyclic form. The cell body shrinks by a factor ~5 in volume, and the flagellum doubles in length. The cells also generate an extracellular polymer matrix that acts as a 'sieve' in the mouthparts of its insect vector, allowing only the adapted, infective cells to pass through and infect the next host. We have combined state-of-the-art holographic imaging [1] and molecular markers [2] to examine the differences in swimming behavior between thousands of cells across different motile life cycle stages. Their swimming patterns change from slow helical swimming (Fig. 1a) to run-and-tumble motility (Fig. 1b) reminiscent of enteric bacteria such as *E. coli*, albeit with an unusual distribution of run durations. We have gone on to study the chemotactic navigation behavior of these two life cycle stages in the presence of human-derived immune cells (macrophages), across three distinct stages of activation.

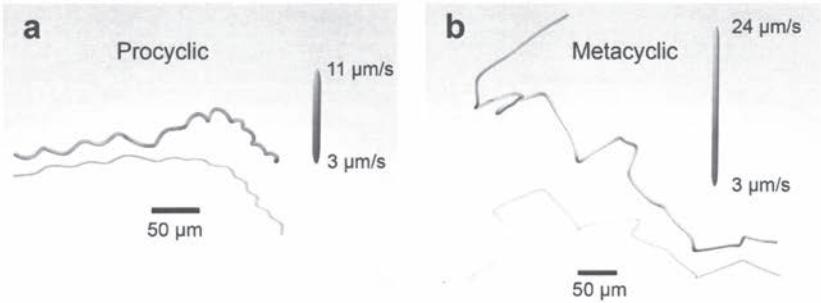


Figure 1: 3D tracks of a replicative procyclic (**a**) and infective metacyclic (**b**) promastigote cells of *L. Mexicana*. The swimming trajectory of a human-infective form shows a distinct swimming behavior and faster speed.

References

[1] Kuhn *et al.*, *Nat. Commun (accepted)* [2] Inbar *et al.*, *mBio* (2017).

Abstracts of Posters

(in alphabetical order)

Longwave nonlinear theory for chemically active droplet division instability

Mohammad Abu Hamed[1,2] **and Alexander A. Nepomnyashchy**[1]

[1] *Department of Mathematics, Technion-Israel Institute of Technology, Haifa 32000, Israel*

[2] *Department of Mathematics, The College of Sakhnin - Academic College for Teacher Education, Sakhnin 30810, Israel*

It has been suggested recently that growth and division of a protocell could be modeled by a chemically active droplet with a simple chemical reactions driven by an external fuel supply. This model is called the continuum model. Indeed it's numerical simulation reveals a shape instability which results in droplet division into two smaller droplets of equal size resembling cell division [1].
In this paper, we investigate the reduced version of the continuum model, which is called the effective model. This model is studied both in the linear and nonlinear regime. First, we perform a linear stability analysis for the flat interface, and then we develop a nonlinear theory using the longwave approach. We find that the interface at the leading order is governed by the modified Kuramoto-Sivashinsky equation. Therefore the interface is subject to a logarithmic blow up after a finite time. In addition, an expression for the interface local velocity is obtained.

References

[1] D. Zwicker, R. Seyboldt, C. A. Weber, A. A. Hyman, and F. Julicher, Nature Physics 13, 408 (2017).

The Centriole Anchoring the Sperm Flagellum Has an Atypical Structure in Mammals

T. Avidor-Reiss[1]

[1]*University of Toledo, Toledo, Ohio, USA*

One of the major unresolved questions during fertilization in human and most other mammals is the precise way in which the centrioles are inherited and function in the spermatozoon. A key reason for this is the lack of accurate tracking of the centriole since many centriolar proteins are transient due to a process referred to as Centrosome Reduction. As a result, the current dogma holds that centrosome reduction leads to structural degeneration and functional inactivation of the centriole that is located at the base of the flagellum (the distal centriole), causing the spermatozoa to have only one intact, functional centriole (the proximal centriole). The idea that the spermatozoon has one centriole and this centriole is not connected to the flagellum adds to the difficulty in understanding centriole inheritance and sperm flagellum movement.

Recently, our studies in insects and mammals suggested that sperm have an atypical centriole that is remodeled during sperm formation [1, 2]. These findings suggest a new hypothesis, Centriole Remodeling, and enables, for the first time, the study of the role of the centriole in sperm movement. The structure of the remodeled distal centriole consists of splayed microtubules that are flanked by two major bars made of centriole luminal proteins at the base of the axoneme, with only a subset of the centriolar proteins typically found in a centriole.

The flagellum is a moving whip-like cellular appendage that propels the sperm toward the egg by beating. Like other flagella and cilia, the sperm tail's beating is generated by sliding of the axoneme microtubules relative to each other with one axoneme end being more stable than the other. For the sliding microtubules to generate a beating, it is thought that the axoneme microtubules must be anchored to the distal centriole that prevents local microtubules sliding. Therefore, it is perplexing that the distal centriole structure suggests that it allows relative movement of its microtubules. We propose the hypothesis that the remodeled distal centriole exhibits bilateral symmetry to enhance the sperm's planar movement that is suitable for navigating a complex environment such as the female reproductive tract [3].

References

[1] A. Khire et al., Curr Biol 26, 3183-3189 (2016).
[2] E. L. Fishman et al., Nat Commun 9, 2210 (2018).
[3] T. Avidor-Reiss, Cells 7, 67 (2018).

Propulsion Force Measurements of Motile Flagella using Micropipette Force Sensors

Thomas Josef Böddeker[1,2], Christian Titus Kreis[1], Quentin Magdelaine[1] and Oliver Bäumchen[1]

[1]Max Planck Institute for Dynamics and Self-Organization (MPIDS), Göttingen, Germany

[2]now at ETH Zürich, Zürich, Switzerland

Although the motility of microbes has received a lot of attention in recent years, methods for direct propulsion force measurements of motile flagella are still limited. We present a novel approach utilizing micropipettes as force sensors [1, 2] to study the propulsion forces of the unicellular, biflagellated microorganism *Chlamydomonas*. We perform high-speed, *in vivo* measurements on single cells held by a micropipette within a liquid cell with full optical access to the sample. A Fourier-signal analysis of the micropipette fluctuations reveals a quantitative signature of the energy output of the microswimmer and provides a measure of the frequency and of the force associated to the oscillatory, breaststroke-type flagella beating. This setup allows for controlling various experimental conditions and parameters. For example, we can control illumination, exposure to chemical inhibitors and proximity of the beating flagella to interfaces. Having established the method in bulk conditions, we demonstrate the versatility of micropipette force sensors by probing hydrodynamic interactions of the beating flagella with a solid interface.

References

[1] M. Backholm and O. Bäumchen, *Nature Protocols*, in press (2018).

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

Fertilization in fresh water fish: the role of the flagellum, and evidence of guidance and selection

S. Boryshpolets[1], V. Kholodnyy[1], H. Gadelha[1,2] and J. Cosson[1] F.

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic
[2] Department of Mathematics, University of York, York, UK.

Externally fertilizing species, especially freshwater fish, reproduce in an environment that is very harsh for gametes. They are under pressure of various external factors (temperature, water flow, pH, ion composition, viscosity, presence of ovarian fluid etc.), thus limiting lifetime of gametes to only several minutes. The gametes have variable responses to these parameters, ranging from the activation of egg or initiation of sperm motility, to changes in speed, duration and direction of motion, contributing to appearance of the wide spectrum of reproduction strategies. Thus, the success of fertilization in these species is directly related to ability of the spermatozoa to reach the egg in an open environment but during a very short period of time, highlighting in this way the critical importance of the flagellar. So the existence of specific mechanisms for guiding sperm to find the egg would be critical under these conditions. To date, however, no demonstration of guidance exists for fresh water fish species. Our recent experiments shed light to different guidance strategies that may be operating for different fresh water fish species. Here we show that factors, such as part of ovarian fluid or chemicals released directly by the egg, significantly affect the behaviour of male gametes by adjusting flagellar beat accordingly. We hypothesise that such coordination may influence the fertilization success in open water. However, the specific mechanisms supporting the selection by externally fertilizing in freshwater females are unclear. Our observations suggest that guidance during fertilization is a critical phenomenon during fresh water fish reproduction.

Acknowledgements: The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA"(No. CZ.1.05/2.1.00/01.0024), "CENAKVA II"(No. LO1205 under the NPU I program), CZ.02.1.01./0.0/0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture and by the Czech Science Foundation (18-12465Y).

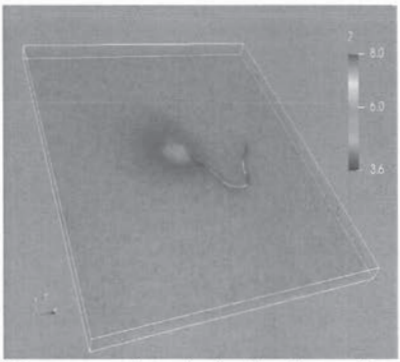
Intracellular calcium stores are involved in the correlation between $[Ca^{2+}]i$ oscillations and three-dimensional flagellar beating in human sperm

Corkidi G., Montoya F., Hernández-Herrera P., Darszon A.

Instituto de Biotecnología, UNAM, P.O. Box 510-3, 62210, Cuernavaca, Mor., México

Fast $[Ca^{2+}]i$ oscillations that correlate to the hamster sperm flagellar beating swimming in a restricted volume were detected twenty eight years ago [1]. Such findings had not been confirmed in any other mammalian species for many years.

Recently, with a new system allowing $[Ca^{2+}]i$ and flagellar beat measurements in 3D [2,3], we had reported that in human spermatozoa there is a statistically significant correlation between $[Ca^{2+}]i$ oscillations and flagellar beating, which were not observable in 2D. We had concluded that these oscillations may arise from intracellular sources and/or Ca^{2+} transporters, as they were insensitive to external Ni^{2+}, a non-specific plasma membrane Ca^{2+} channel blocker.



3D segmentation of the flagellum of a human spermatozoon. The pseudocolor scale indicates the vertical Z component (microns) in a fluorescence image stack.

In the present work we have explored if intracellular sources are involved in this observed correlation. Using thapsigargin and ciclopiazonic acid, two compounds that release Ca^{2+} from internal stores, in Fluo-4 loaded human spermatozoa, we examined the behavior of $[Ca^{2+}]i$ oscillations and flagellar beat characteristics in 3D. We found that the correlation between the Ca^{2+} oscillations and the flagellar beat in 3D were lost upon calcium store emptying. These findings suggest that these stores influence this correlation. Future experiments in 3D will be performed to explore in detail how human sperm flagellar beating properties are regulated by intracellular Ca^{2+} stores and identify them.

References

1. Suarez SS, *Proc Natl Acad Sci,* 90, 4660-4664, 1993.
2. Corkidi G., Molecular Human Reproduction, 23:9, 583–593, 2017.
3. Hernandez-Herrera, P., IEEE Trans on Medical Imaging. 37:10, 2236-2247, 2018.

On the motor torque of Escherichia coli

D. Das[1] **and E. Lauga**[1]

[1]*Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, University of Cambridge, Wilberforce Road, Cambridge CB3 0WA, UK*

The rotary motor of bacteria is a natural nano-technological marvel that enables cell locomotion by powering the rotation of semi-rigid helical flagellar filaments in fluid environments. It is well known that the motor operates essentially at constant torque in counter-clockwise direction but past work have reported a large range of values of this torque. Focusing on Escherichia coli cells that are swimming and cells that are stuck on a glass surface for which all geometrical and environmental parameters are known[1], we use two validated numerical methods to compute the value of the motor torque consistent with experiments. Specifically, we use (and compare) a numerical method based on the boundary integral representation of Stokes flow and also develop a hybrid method combining boundary element and slender body theory to model the cell body and flagellar filament, respectively. Using measured rotation speed of the motor, our computations predict a value of the motor torque in the range 440 pN-nm to 829 pN-nm, depending critically on the distance between the flagellar filaments and the nearby surface.

References

N. C. Darnton et al., J. Bacteriol., 2007, 189, 1756-1764.

Human brain organoids with functional eye cups from human iPSCs

Elke Gabriel, Marco Gottardo, Walid Albanna, Jay Gopalakrishnan

Laboratory for Centrosome & Cytoskeleton Biology, Institute of Human Genetics, University of Düsseldorf, Düsseldorf, Germany

An important branch of regenerative medicine is human induced pluripotent stem cell (iPSC)-based tissue engineering. It opens up new possibilities to generate specific cell types and even tissues from the patient´s own material. Today, novel techniques of cell culture allow the differentiation of homogenous cell monolayers, and also the growing of 3D tissue-like structures with self-assembled heterogeneous stratified cell layers.

Retinitis pigmentosa (RP) is a neuroretinal disorder due to progressive loss of retinal function. Protecting photoreceptors from cell death is one of the therapeutic strategies to maintain the patients´ retinal function as long as possible. For this, it is crucial to shed light on and understand the underlying pathogenic processes in RP disease progression, for example by using animal models or in vitro models of RP.

Here, we developed a new protocol for the generation of human iPS cell derived brain organoids with eyecups, so-called eye organoids. We found that these eye-like structures contain different relevant retinal cell types. RPE cells featured a hexagonal morphology, numerous melanosomes, and a primary cilium. EM serial sections showed photoreceptor connecting cilia with differentiated inner and outer segments. Photoreceptors were Rhodopsin/RAX positive and showed light response.

Eye organoids serve as a tool to study human eye development in health and disease in a complex 3D tissue-like context. In future, we will generate eye organoids from patients with ciliopathies to study how structural defects in connecting cilia affect photoreceptor function and development.

Out-of-plane beating components of active axonemes isolated from *Chlamydomonas reinhardtii*

A. Gholami[1], S. Mojiri[2], J. Enderlein[2] and E. Bodenschatz[1]

[1]MPI for Dynamics and Self-Organization, Göttingen, Germany
[2] Georg-August University, Göttingen, Germany

Cilia and flagella are ubiquitous in the living world. They are essential for micro-scale driven transport of fluids or cells by cilia/flagellar beating. Their slender bodies are composed of a microtubule/molecular motor structure that when taken independently are called an axoneme. Axonemes move by bending waves that emerge from the interplay between internal stresses generated by dynein motor proteins . Here we use the novel multi-plane phase contrast imaging technique to record the three dimensional beating pattern of isolated axonemes from *Chlamydomonas reinhardtii* that beat in the vicinity of a substrate. We measure the torsion of the axoneme along the contour length with high spatiotemporal resolution. High precision information on out-of-plane beating component of axonemes allows us to check the validity of the resistive-force theory.

From 3D beating to 3D swimming: an excursion to the egg

An Gong, Luis Alvarez, and U. Benjamin Kaupp

Department of Molecular Sensory Systems, caesar research center, Bonn, Germany

The tail of motile sperm - called flagellum - is used for propulsion and steering. The action of this appendage is controlled; thus sperm can navigate. Chemical and physical cues gathered by sperm from the environment are translated by molecular machinery into specific flagellar beat waveforms for navigation. Sea urchin sperm represent a prime example for sperm navigation. Here, eggs release a chemical factor that eventually forms a gradient around them. Sperm are able to sense the direction of such chemical gradient and steer their path to the egg.

In the past, we studied sea urchin sperm while navigating in 3D chemoattractant gradients by using holographic microscopy and other photonic techniques. Using numerical simulations and experimental data, we inferred the flagellar beat waveforms used by sperm for navigation. we use new holographic methods to study the flagellar beat of sperm in 3D to test the theoretic predictions. In addition, we aim to reproduce numerically the swimming trajectory of sperm from the reconstructed flagellar beat. I also apply these techniques to study mammalian sperm from mouse and human. For these species, fertilization takes place within the female reproductive tract, where interactions with the walls and hydrodynamics might have evolved different flagellar waveforms during evolution. The differences and commonalities among sperm from different species are described.

Human brain organoids to model disorders due to primary cilia dysfunctions

Elke Gabriel, Gladiola Goranci and **Jay Gopalakrishnan**

Centrosomes and cilia are conserved cellular structures essential for organism development (1). Mutations resulting in defective centrosomes and cilia cause microcephaly, a developmental disorder where brain size is severely reduced (2). Dissecting pathobiological mechanisms of microcephaly was not possible due to the restricted availability of suitable in vitro models that can reliably recapitulate complex human brain tissues. In order to address this question, we have generated iPSC-derived human brain organoids to study brain development and to model microcephaly (2-3). By studying microcephaly brain organoids, we identify a "cilia checkpoint" and explain how a timely cilia disassembly is critical to regulate neural stem cell homeostasis during brain development. In addition, sharing our preliminary data, I shall highlight how we use brain organoids to model glioblastoma multiforme (GBM), in which we identify an unexpected role for cilia regulating glioblastoma stem cell proliferation and differentiation (4). Depending on the availability of time, I shall also share new developments in brain organoids such as generation of brain organoids with functional eyecups, a hybrid organoids that can allow modelling retinal disorders due to defects in connecting cilia.

References:

1. Zheng X, [...] Gopalakrishnan J*, Li H*. Molecular basis for CPAP-tubulin interaction in controlling centriolar and ciliary length. **Nat Commun.** Jun 16;7:11874 **(2016).**

2. Gabriel E, [...] **Gopalakrishnan J***. CPAP promotes timely cilium disassembly to maintain neural progenitor pool. **EMBO Journal**. Apr 15;35(8):803-19. (**2016**).

3. Gabriel E, Ramani A, [...] Utermöhlen O, **Gopalakrishnan J*.** Recent Zika virus isolates induce premature differentiation of neural progenitors in human brain organoids. **Cell Stem Cell** 20, 1-10 **(2017).**

4. Goranci-Buzhala G, [...] Gopalakrishnan J* Losers of Primary Cilia Gain the Benefit of survival. **Cancer Discovery** 12, 1374-1375 **(2017).**

A Relationship Between Metachronal Wavelength and Fluid Flow Rate

Jon Hall and Nigel Clarke

University of Sheffield, Sheffield

Cilia driven flow occurs through a complex coupling between fluid dynamics and solid mechanics that is dependent on several physical characteristics including mucus rheology, cilia density, and beat coordination. We have developed a computational model of an array of beating cilia using a hybrid immersed boundary lattice Boltzmann algorithm, and used this model to study the how the flow behaviour is affected by the coordination of cilia beating. Early observations indicated that there was a relationship between the metachronal wavelength imposed on the system and the resulting fluid flow rate. We theorised that the variation in flow rate is largely due to the geometric properties of the cilia array, and how much the flow caused by each cilium was impeded by the other cilia in the array. Based on this theory we derived an equation for the geometric free space available to each cilium as it beats. We find that the variation of fluid flow rate correlates well with the variation of free space and show that this correlation holds for different cilia spacings.

SpermQ - a simple analysis software to comprehensively study flagellar beating and sperm steering

Jan N. Hansen[1], Sebastian Raßmann[1], Jan F. Jikeli[1]*, and Dagmar Wachten[1,2]*

1 Institute of Innate Immunity, Biophysical Imaging, University Hospital Bonn, University of Bonn, 53127 Bonn, Germany

2 Center of Advanced European Studies and Research (caesar), Molecular Physiology, 53175 Bonn, Germany

Motile cilia, also called flagella, are found across a broad range of species; some cilia propel prokaryotes and eukaryotic cells like sperm, while cilia on epithelial surfaces create complex fluid patterns e.g. in the brain or lung. For sperm, the picture has emerged that the flagellum is not only a motor, but also a sensor that detects stimuli from the environment, computing the beat pattern according to the sensory input. Thereby, the flagellum navigates sperm through the complex environment in the female genital tract. However, we know very little about how environmental signals change the flagellar beat and, thereby, the swimming behavior of sperm. It has been proposed that distinct signaling domains in the flagellum control the flagellar beat. However, a detailed analysis has been mainly hampered by the fact that current comprehensive analysis approaches rely on complex microscopy and analysis systems. Thus, knowledge on sperm signaling regulating the flagellar beat is based on custom quantification approaches that are limited to only a few aspects of the beat pattern, do not resolve the kinetics of the entire flagellum, rely on manual, qualitative descriptions, and are little comparable among each other. Here, we present SpermQ, a ready-to-use and comprehensive analysis software to quantify sperm motility. SpermQ provides a detailed quantification of the flagellar beat based on common time-lapse images acquired by dark-field or epi-fluorescence microscopy, making SpermQ widely applicable. We envision SpermQ becoming a standard tool in flagellar and motile cilia research that allows to readily link studies on individual signaling components in sperm and distinct flagellar beat patterns.

Cilia beating coordination and mucus flow on airway epithelia

M. Jory[1], C. Vernisse[2,3], K. Bellouma[1], D. Donnaruma[1], C. Blanc[1], L. Casanellas[1], A. Petit-Fort[2,3], I. Vachier[2,3], A. Bourdin[2,3], G. Massiera[1]

[1]*Laboratoire Charles Coulomb, Université de Montpellier, CNRS UMR 5221, Montpellier, France*
[2]*PhyMedExp, Université de Montpellier, INSERM U1046, CNRS UMR 9214, Montpellier, France*
[3]*Département de Pneumologie et d'Addictologie, Hôpital Universitaire Arnaud de Villeneuve, Montpellier, France*

The mucociliary function of the bronchial epithelium ensures the continuous clearance of our airways. Mucus is a visco-elastic gel trapping dust and pathogens present in the inhaled air and thus acts as a protective barrier on top of the airway tissue. Its transport is a key element to ensure an efficient clearance of the respiratory system. It relies on two main elements: mucus rheology and cilia beating coordination which generates mucus transport.

Our biophysical approach consists in understanding how mucus flows performing rheology at different length scales, and in understanding how cilia coordinate by analyzing the beating of cilia from videomicroscopy movies. The resulting mucus transport is also analyzed. As an experimental model, we use ALI (Air-liquid interface) cultures of bronchial epithelium: cells from a biopsy are put in culture at an interface between the nutritive liquid and air to obtain differentiation towards an airway epithelium displaying goblet cells producing mucus and ciliated cells.

Cilia beating coordination - We will describe our method to extract the trajectory of each cilia from videomicroscopy recordings of the ALI epithelium. We quantify their coordination degree and the influence of various factors such as density and spatial distribution of the cilia. Maps of beating orientation and frequency can be produced and compared to mucus velocity field. All of these analysis tools allow to quantify and understand how beating coordination occurs within a ciliated cell (tens of cilia) and at the length scale of several ciliated cells. Our experimental results will be compared to theoretical models [1,2] from statistical physics developed to describe cilia synchrony.

References

[1] S. Dey, G. Massiera, E. Pitard, Phys.Rev. E 97, 012403 (2017)
[2] J. Elgeti and G. Gompper, PNAS (2013) 110 (12) 4470-4475

Do the rainbow trout ovarian fluid navigate the sperm on its way to the egg?

Vitaliy Kholodnyy, Jacky Cosson, Serhii Boryshpolets

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic

Fertilization of fresh water fish occurs in an environment affecting negatively the gametes, therefore the fish male gametes should reach their counterpart, the female gamete, as soon as possible because spermatozoa become damaged within minutes (sometimes even less than a minute) due to osmotic shock. Existence of specific mechanism guiding and triggering the encounter of gametes would be highly expedient in these conditions. More than likely that the only source of signaling may come from the egg or ovarian fluid (OF). In this light we aimed to explore how fresh water fish sperm behaviour is affected by OF, using rainbow trout as a model. It was found that presence of OF affects significantly the behavior of rainbow trout spermatozoa, in particular increase their velocity and longevity. There was a change in pattern of motility from tumbling, observed in water, to directed straightforward moving in OF. These changes were provided by shift from asymmetric flagellar wave propagation to symmetric ones. The effect could be associated particularly with osmotic properties of the fluid and with its protein content. Its different molecular weight fractions affect the kinetics and the motility patterns of spermatozoa in a various way. Rainbow trout OF renders a trapping effect on activated male gametes. The most significant trapping effect was rendered by low molecular fraction and the possible chemotactic agent is thermostable. A kind of calcium ion redistribution was observed in the flagella of spermatozoa responding to the trapping effect. Nevertheless the effect of OF on overall outcome of artificial fertilization was not obvious, and at least low sperm to egg ratio was needed to observe it. Collectively, ovarian fluid is an important part of rainbow trout fertilization process, nevertheless the fine machinery of the effect needs to be ascertained.

Acknowledgements: The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA" (No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I program), CZ.02.1.01./0.0/0.0/16_025/0007370 Reproductive and genetic procedures for preserving fish biodiversity and aquaculture, by the Grant Agency of the University of South Bohemia in Ceske Budejovice (013/2018/Z) and by the Czech Science Foundation (18-12465Y).

Simultaneous recording of rapid cellular signaling events

M. Kierzek[1], P.E. Deal[2], U.B. Kaupp[3], T. Strünker[1] and C. Brenker[1]

[1] *Centre of Reproductive Medicine and Andrology, Münster, Germany*
[2] *University of California Berkeley, California, United States*
[2] *Center of Advanced European Studies and Research, Bonn, Germany*

Sperm locate the egg by chemotaxis. The chemosensory signaling pathways triggered by the egg´s chemoattractant involve changes in membrane potential (V_m) and intracellular pH (pH_i), sodium ($[Na^+]_i$), and calcium ($[Ca^{2+}]_i$). These signaling events have been unraveled and investigated using kinetic stopped-flow fluorimetry[1]. Accordingly, sperm loaded with an indicator for either $[Na^+]_i$, $[Ca^{2+}]_i$, pH_i, or V_m are rapidly mixed with the chemoattractant and the ensuing dynamics of the particular signaling modality are monitored. Here, we push the envelope of kinetic fluorimetry to monitor up to three of the signaling events simultaneously. To this end, sperm are loaded with three indicators and each is excited by light modulated at a different frequency. The fluorescence emitted by the indicators bears the respective frequency of the excitation light and is, thereby, selectively amplified by Lock-in amplifiers. This frequency-selective amplification maximizes the signal-to-noise ratio and is largely resistant to cross-talk that might arise from partially overlapping spectra. The frequency-selective multiplexing technique is amenable to various combinations of fluorescent indicators as well as with optochemical tools. Moreover, it is not only suitable for monitoring signaling events in sperm by the stopped-flow technique, but also in live single-cell microscopy. Taken together, we introduce a powerful optical multiplexing technique to delineate signaling events and interrogate signaling interplay.

References

[1] T. Strünker, L. Alvarez & U.B. Kaupp, Current Opinion in Neurobiology **34**, 110-116 (2015).

Getting to the egg – the link between motility, metabolism & tail length of sperm

Veronika Magdanz [1], Sergii Boryshpolets [2], Clara Ridzewski [1], Barbara Eckel, [1] Klaus Reinhardt[1]
[1] Chair of Applied Zoology, TU Dresden, Germany
[2] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Vodnany, Czech Republic

It is still a mystery, why so many sperm cells start off their journey to the egg and only very few make it. Also, what does it take to get through the female reproductive tract? Is it just being lucky, flagella-driven high motility or most efficient swimming? It is thought that sperm cells must manage their energy expenditure very well in order to migrate over such long distance and time. Even though most of a sperm's energy goes towards motility, a clear link between metabolic rates and motility has not been demonstrated. In our study, we mimic sperm migration by the swim-up method, which is a clinical method used to select the most motile sperm in the upper fraction and leaving sperm with low or no motility in the lower fraction. Our study shows that the upper swim-up fraction of bull sperm has high motility, shows higher metabolic rates in two different viscosities than the cells in the lower fraction. We suggest that metabolic assays could serve as an alternative, label-free method to evaluate sperm quality. Because we also found that sperm with longer flagella are selected in the upper fraction, our current research is directed towards differences in flagella beat frequencies. Augmenting kinetic studies on sperm cells with metabolic measurements may reveal differences in metabolic pathways, how these vary depending on ecological parameters, and identify sperm traits that predict successful migration through the different environments of the female reproductive tract.

Self-organized wave-like beating of actin bundles.

Marie Pochitaloff[1], Mathieu Richard[1], Takagi Yasuharu[2], Enrique De La Cruz[3], Jim Sellers[2], Jean-François Joanny[1], Frank Jülicher[4], Laurent Blanchoin[5], Pascal Martin[1].

[1]Institut Curie, Paris, France
[2]NHLBI - NIH, Bethesda, USA
[3]Yale University, New Haven, USA
[4]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany
[5]CEA, Grenoble, France.

The emergent active behaviors of systems comprising large numbers of molecular motors and cytoskeletal filaments remain poorly understood, even though individual molecules have been extensively characterized. Here, we show in vitro that flagellar-like beating can be produced naturally and robustly in polar bundles of filaments. Using surface micro-patterns of a nucleation-promoting factor, we controlled the geometry of actin polymerization to produce thin networks of parallel actin filaments. In the presence of either myosin Va or heavy-mero myosin II motors added in bulk, growing actin filaments self-organized into bundles that displayed periodic wave-like beating resembling those observed in eukaryotic cilia and flagella. The waveform of oscillation was similar for the two types of motors. However, oscillations with myosin II were one order of magnitude faster than with myosin Va. In both cases, a wave of bending deformations propagated at a uniform velocity from the anchored base of the actin bundle towards the freely-moving tip. As polymerization proceeded, the actin bundle elongated at a constant velocity, resulting in a proportional increase of the period of oscillation. Remarkably, the propagation velocity of the bending wave did not vary with the increasing bundle length, indicating that the bundle length set the wavelength. Our work on a minimal acto-myosin system demonstrates that active flagellar-like beating emerges as an intrinsic property of polar bundles of filaments in interaction with molecular motors. Structural control over the self-assembly process provides key information to clarify the underlying physical principles of flagellar-like beating.

Investigating ciliopathies by primary cilia proteomics

David U. Mick[1,2]

[1]*Center of Human and Molecular Biology (ZHMB), Molecular Biology, Saarland University, D-66421 Homburg, Germany*
[2]*Center for Molecular Signaling (PZMS), Saarland University School of Medicine, D-66421 Homburg, Germany*

Primary cilia are solitary, 2-10 µm long, and non-motile but share the basic structural features with motile cilia [1]. Primary cilia function as signaling compartments by dynamically concentrating signaling molecules to transduce external stimuli [2]. In order to fulfill this function they must consist of defined sets of proteins that are adapted dynamically in response to external stimuli. Many ciliopathies are based on defects in cilia integrity as well as protein trafficking components, leading to aberrant protein composition and consequently inability to fulfill the diverse cilia functions.

Here we present our proteomic approaches to assess alterations in the protein composition of mutant cilia, exemplified by models of Bardet-Biedl Syndrome. To this end we employ proximity labeling methods using cilia-targeted ascorbate peroxidase (cilia-APEX) to specifically biotinylate ciliary proteins in living cells, which allows effective isolation and identification by mass spectrometry [3]. Moreover, by controlling the labeling activity in combination with state-of-the-art quantitative mass spectrometry methods using tandem-mass-tags (TMT), we can compare proteomes of cilia not only in different mutants but also at different states, such as varying signaling status. These approaches allow us to describe the proteomic alterations of primary cilia in two Bardet-Biedl Syndrome mutants, revealing common but also mutation-specific changes compared to wild-type cilia. Further, we reveal the proteomic remodeling of the primary cilia signaling environment during Sonic Hedgehog signaling and gain novel mechanistic insight into protein kinase A signaling in cilia.

We aim to combine our proteomic studies to dissect the precise signaling defects in Bardet-Biedl Syndrome and expand our studies to other ciliopathies to reveal their underlying pathomechanisms on a molecular level.

References

[1] M. Mirvis, T. Stearns, W.J. Nelson, Biochem. J. **475**, 2329-2353 (2018)
[2] G. Wheway, L. Nazlamova, J.T. Hancock, Front. Cell. Dev. Biol. **6**, eCollection (2018)
[3] D.U. Mick, R.B. Rodrigues, R.D. Leib, C.M. Adams, A.S. Chien, S.P. Gygi, M.V. Nachury, Dev. Cell **35**, 497-512 (2015)

Altered *N*-glycosylation of FMG-1B impairs gliding motility in *Chlamydomonas reinhardtii*

Anne Oltmanns[1], Nannan Xu[2], Julia Beißel[1], Kaiyao Huang[2], Michael Hippler[1]

[1]*University of Münster, Münster, Germany*
[2]*Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China*

Chlamydomonas reinhardtii (*C. reinhardtii*), a unicellular green alga, is a perfect model organism for studying e.g. photosynthesis, phototaxis and motility. Flagella of *C. reinhardtii* are perfectly suited to study cilia associated malfunctions, since their flagella and mammalian cilia show a high structural and functional homology. Accordingly, intraflagellar transport (IFT) or flagella assembly are in the focus. By contrast, flagella protein membrane dynamics are less investigated, although they provide interesting features to flagella such as cell gliding along surfaces or binding of small objects (e.g. to polystyrene microspheres).

The protein mainly contributing to gliding motility is a 350 kDa, heavily *N*-glycosylated protein denoted as flagella membrane glycoprotein 1B (FMG1-B); a membrane anchored protein with only 2% of the protein located inside the lumen. Recent studies indicate a calcium dependent interaction of FMG1-B with IFT trains inside the flagellum, thus enabling its transport along the membrane required for efficient gliding (Shih et al. 2013). Additionally, studies with a mutant showing an altered *N*-glycan pattern indicate an importance of *N*-glycosylation for gliding (Bloodgood 1989).

In our study, we analyzed microsphere binding to flagella of three insertional mutants affected in specific steps in *N*-glycosylation, resulting in defined *N*-glycan patterns deviating from wildtype (WT) as measured by mass spectrometry (Schulze et al., 2018). In all three mutants, reduced microsphere binding as well as less microsphere movement along flagella was observed. To analyze, what might cause this differential behavior, flagella were isolated and analyzed by mass spectrometry. Our data indicate that FMG1-B is still transported into flagella, whereas its *N*-glycan pattern diverges from WT as described (Schulze et al, 2018). Furthermore, functional IFT is observed. Thus suggesting that differential surface adhesion (caused by an altered *N*-glycan structures) causes reduced microsphere binding in the mutant strains.

Overall, our study provides valuable and unique insights into the importance of *N*-glycosylation on protein function in *C. reinhardtii*. Considering similarities between flagella and human cilia, this might pave the way for a better understanding of certain *N*-glycosylation associated ciliopathies.

References

[1] Shih et al., eLife (2013)
[2] Bloodgood and Salomonsky, Cell Motility and the Cytoskeleton **13**, 1 (1989)
[3] Schulze et al., Plant Physiology **176**, 1952 (2018)

Synchronisation of mammalian cilia by hydrodynamic forces

N. Pellicciotta[1], E. Hamilton[1], J. Kotar[1], M. Faucourt[2], N. Spassky[2], P. Cicuta[1],

[1]Cavendish Laboratory, Cambridge University, UK
[2] Institut de Biologies de l' Ecole Normale Superieure (IBENS), Paris, France

Fluid flow generated by a ciliated epithelium is a fascinating example of collective behaviour in nature: thousands of microscale whip-like structures called motile cilia beat at the same frequency and direction in a coordinated fashion. This dynamics has been reported to have fundamental roles in microorganisms and in many organs of mammals such as fallopian tubes, airway and brain ventricles. Recent hypothesis, supported by simulations [1], experiments with microorganisms [2] and with cilia models [3], proposed that hydrodynamic interaction between cilia could provide physical mechanism for their coordination, whereas other team have proposed a role of the cytoskeletal elastic coupling between cilia [4,5].

We investigated experimentally the coupling between hydrodynamic forces and mammalian cilia using a controlled oscillatory external flow, similarly to ref [5]. Specifically we grew multiciliated cells from mouse brain ventricles cultured in a packed epithelium [6]. We found that synchronisation with external flow strongly depends on the number of cilia per cell. We speculate that this effect comes from the hydrodynamic interaction between the cilia of the same cell. We are currently testing this hypothesis with simulation of model cilia [3]. Our experimental preliminary results suggest that the external hydrodynamic force needed to entrain synchronisation of multiciliated cells with physiologically relevant number of cilia exceed the force that regions of the multiciliated epithelium can apply on each other through the fluid. This means that either the collective system is particularly susceptible to supporting collective states, or that other mechanisms, such as cytoskeleton connections, may be involved in the coordination of cilia motion in mammals.

References

[1] Elgeti J., Gompper G., PNAS, **110** : 4470-4475, (2103).
[2] Brumley B. et al., Physical Review Letters **109**, 268102 (2012)
[3] Kotar J. et al. , PNAS, **107,** 7673, (2010)
[4] Liu Y. et al. , arXiv:1806.06466 (2018)
[5] Quaranta G et al. , Physical Review Letters, **115,** 238101,(2015)
[6] Guirao et al. , Nature Cell Biology , **12**, 341-350 (2010)

Sperm motility in modulated microchannels

S. Rode, J. Elgeti and G. Gompper

Forschungszentrum Jülich, Germany
Theoretical Soft Matter and Biophysics

Sperm cells swim through the fluid by a periodic wave-like beating of their flagellum [1-3]. At low Reynolds numbers and in confinement, the directed motion of sperm is strongly influenced by steric and hydrodynamic surface interactions. We model sperm motility in mesoscale hydrodynamics simulations by imposing a planar traveling bending wave along the flagellum [2]. Thus, the flagellum beats in a periodic pattern within a defined beat plane. Sperm are simulated swimming in curved, straight, shallow and zigzag-shaped microchannels [4]. Changes in the sidewall modulations and the imposed beat pattern allow the identification of a strong dependence of the surface attraction on the beat-shape envelope of the sperm cell. The simulations reveal a strong dependence of the deflection angle on the orientation of the beat plane with respect to the channel sidewall, and thus deepen the understanding of sperm navigation under strong confinement. Detachment of sperm, while swimming along curved walls, is dominated by the change of beat-plane orientation. In particular, we will discuss how strong confinement in shallow channels drastically increases wall attraction. Our simulation results reveal a consistent picture of passive sperm guidance that is dominated by the steric interactions of the beat pattern with the nearby surfaces.

References

[1] J. Elgeti et al., Rep. Prog. Phys. 78, 056601 (2015)
[2] J. Elgeti et al., Biophys. J. 99, 1018 (2010)
[3] G. Saggiorato et al., Nat. Commun. 8, 1415 (2017)
[4] S. Rode, J. Elgeti, G. Gompper, NJP (accepted for publication)

Engine failure: biological roles of (cytoplasmic) dyneins and their dysfunction in human disease

Miriam Schmidts [1,2]

[1] *Genome Research Division, Human Genetics Department, Radboud university medical center and Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, The Netherlands*

[2] *Center for Pediatrics and Adolescent Medicine, University Hospital Freiburg, Germany*

Dyneins are very large, multi-unit cellular motor complexes powering cargo transport along microtubules. Axonemal dyneins enable microtubule movement within the ciliary axoneme and dysfunction results in therefore in impaired cilia (or flagella) motility, often causing Primary Ciliary dyskinesia (PCD) in humans. Cytoplasmic dyneins in contrast are essential for transport along microtubules. In mammals, two types of cytoplasmic dyneins occur: cytoplasmic dynein-1 acting within the cytosol whereas cytoplasmic dynein-2 serves as a motor for retrograde intraflagellar transport (IFT) within the ciliary axoneme. In humans, hypomorphic recessive mutations in cytoplasmic dynein-2 encoding genes (*DYNC2H1*, *WDR60*, *WDR34*, *DYNC2LI1* and *TCTEX1D2*) cause non-motile ciliopathies, Short-rib polydactyly syndrome (SRPS) and Jeune Syndrome (JATD). Cytoplasmic dynein-2 associates with IFT complexes to accomplish retrograde axonemal transport, and likewise, mutations in IFT genes are a frequent cause of SRPS and JATD in humans. However, many structural-, functional- and disease-related characteristics of cytoplasmic dynein-2 and associated IFT molecules remain poorly understood.

Interestingly, dynein-2 dysfunction causes a severe skeletal phenotype in humans without clinically relevant extraskeletal manifestations while IFT-protein dysfunction results in mild skeletal changes but severe renal and retinal disease. This points towards an additional role of IFT proteins outside of IFT itself or may be a result of changed IFT cargo as a result of the mutations.

We have generated several dynein- as well as IFT-defective cell lines using CRISPR/Cas9 technology to identify functions of dynein-2 and IFT proteins in shared and divergent cellular pathways using transcriptomics and cilia specific proteomics analyses.

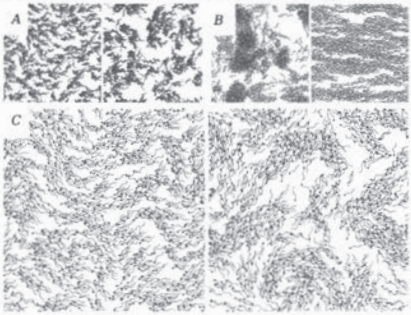
Linking individual and collective dynamics of sperm in suspension

S. F. Schoeller[1] and E. E. Keaveny[1]

[1]Department of Mathematics, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

Suspensions of sperm cells can display a diverse array of collective behaviour ranging from turbulence-like dynamics [1] to coherent motion of tightly packed groups. Collective dynamics of swimming suspensions are often investigated using models that treat cells as point particles or rigid objects that exert steady dipolar forces on the surrounding fluid. While these models can reproduce the turbulence-like state, through their waving flagella, sperm exert time-dependent, beyond-dipolar forces on the fluid. When these details are included, interactions through the fluid lead to attraction and flagellar synchronisation of neighbouring cells.

Using simulations [2] of up to 1000 cells immersed in a Stokes fluid, we investigate how time-varying features at the scale of the individual affect the collective dynamics on a larger scale. Resolving the fluid flow, steric interactions and elasticity of the flagella, we test how hydrodynamic interactions and the variability of the swimmers' undulation frequency affect collective dynamics.



If the frequencies of all swimmers are the same, we find that swimmers will form growing clusters (Fig. A). If there is sufficient variation in the undulation frequencies, however, the suspension develops vortices and swirls (Fig. B) much larger than the size of the individual cells. Excluding hydrodynamics inhibits both swirling and clustering (Fig. C). We also find that the onset of the swirling state depends strongly on the cell density. Further, a quantitative analysis of the swirling collective dynamics reveals that the flows generated by flagellum undulations contribute substantially to the overall energy in the fluid.

References

[1] A. Creppy et al., Phys. Rev. E **92**, 032722 (2015)

[2] S. F. Schoeller and E. E. Keaveny, J. R. Soc. Interface **15** 20170834 (2018)

The solute carrier SLC9C1 is a Na^+/H^+-exchanger gated by an S4-type voltage-sensor and cyclic-nucleotide binding

F. Windler[1], W. Bönigk[1], H. G. Körschen[1], E. Grahn[1], T. Strünker[2], R. Seifert[1] & U. B. Kaupp[1]

[1]Center of Advanced European Studies and Research (caesar), Department Molecular Sensory Systems, Ludwig-Erhard-Allee 2, 53175, Bonn, Germany
[2] University Hospital Münster, Center of Reproductive Medicine and Andrology, Albert-Schweitzer-Campus 1, Geb. D11, 48149, Münster, Germany

Several signaling molecules involved in sperm motility are controlled by intracellular pHi. A sperm-specific subfamily of solute carriers (SLC9C1) has been proposed to control pHi by sodium/proton (Na+/H+) exchange. It features a unique tripartite structure: an exchanger domain, a voltage-sensing domain (VSD), and a cyclic nucleotide-binding domain (Wang et al., 2003). The physiological properties of SLC9C1 are unknown. Here we functionally characterize SLC9C1 from the sea urchin *Strongylocentrotus purpuratus*. We studied Na^+/H^+ exchange and the underlying activation mechanism by single-cell electrophysiology and voltage-clamp fluorimetry. *Sp*SLC9C1 is activated by hyperpolarization that also evokes gating currents. Neutralizing a critical Arg in the S4 motif shifts the voltage dependence. Cyclic AMP modulates both gating currents and Na^+/H^+ exchange. Disabling cAMP binding prevents modulation by cAMP. Thus, SLC9C1 represents a phylogenetic and functional hybrid between ion antiporters and ion channels. Voltage gating of Na^+/H^+ exchange might represent a physiological adaptation to prevent ions to redistribute passively unless triggered by a physiological stimulus. Mouse SLC9C1 lacks key residues in all three functional domains. Because signaling molecules from different sperm species often adopt entirely different properties (Kaupp and Strünker, 2016), mammalian SLC9C1 may serve a different function.

References

[1] Wang, D., King, S. M., Quill, T. A., Doolittle, L. K. & Garbers, D. L. A new sperm-specific Na+/H+ exchanger required for sperm motility and fertility. Nat. Cell Biol. 5, 1117–1122 (2003).

[2] Kaupp, U. B. & Strünker, T. Signaling in sperm: more different than similar. Trends Cell Biol. 27, 101–109 (2017).

Hydrodynamic interactions of beating cilia

A. Solovev[1] and B.M. Friedrich[2]

Center for Advanced Electronics Dresden (cfaed) &
Institute for Theoretical Physics, TU Dresden, Germany
[1]anton.solovev@tu-dresden.de, [2]benjamin.m.friedrich@tu-dresden.de

We perform hydrodynamic simulations of beating cilia using experimental beat patterns. From pairwise hydrodynamic interactions, we deduce synchronization strength as a function of cilia orientation. The aim of this project is to understand how disorder in cilia orientation affects emergent metachronal waves in collections of beating cilia.

We use a previously developed description of the cilia beat as limit-cycle oscillator with known load response to external hydrodynamic forces [1,2], using the framework of generalized forces and a fast boundary-element method to solve the three-dimensional Stokes equation.

References

[1] Klindt et al. Load response of the flagellar beat. *Physical Review Letters 117(25)* (2016)

[2] B.M. Friedrich, Hydrodynamic synchronization of flagellar oscillators. *The European Physical Journal Special Topics 225.11-12* (2016)

Kinesin-2 stepping reflects its heteromeric nature

Willi L Stepp[1] and Zeynep Ökten[2]

[1]Physik Department E22, Technische Universität München, Garching, Germany
[2]Munich Center for Integrated Protein Science, Munich, Germany

Intraflagellar transport (IFT) is central to ciliogenesis in virtually all cells. This transport is driven by specialized kinesin-2 motors that have co-evolved with cilia. It is long-known that these kinesin-2 motors are heteromeric. Two different catalytic subunits combine with a third non-motor subunit to form the heterotrimeric motor. Yet the functional significance of this heteromerization remains largely unknown. We made use of dual-color superresolution microscopy (dcFIONA) to follow the two different heads of the KLP11/20 kinesin-2 motor from C. elegans while walking on microtubules. The heads were labeled with different colors, enabling concurrent step detection of both heads. Following the two heads for the first time for a kinesin, we show that the heads have distinct stepping behaviors. Our preliminary data unexpectedly points towards the C-terminal end of the motor, that appears to directly influence the stepping pattern. This potential allosteric interaction we observe holds the promise to deliver key insights into the specialization of kinesin-2 for IFT.

On the validity of Stokes equations to model ciliary flows

D. Wei, P. Ghoddoosi, M.-E. Aubin-Tam and D. TAM

Technische Universiteit Delft, Mekelweg 2, 2628CD, Delft, Netherlands

The Stokes equations are commonly used to model the hydrodynamic flow around cilia on the micron scale. The validity of this assumption is investigated experimentally with a flow velocimetry approach based on optical tweezers, which allows the measurement of periodic flows with high spatial and temporal resolution. We find that beating cilia generate a flow, which fundamentally differs from the stokeslet field predicted by Stokes equation. In particular, the flow velocity spatially decays at a faster rate and is gradually phase delayed at increasing distances from the cilia. This indicates that the quasi-steady approximation and use of Stokes equations for unsteady ciliary flows is often not justified and the finite timescale for vorticity diffusion cannot be neglected. Our results have significant implications in studies of synchronization and collective dynamics of cilia and microswimmers.

Study of axonemal dynein assembly using endogenous protein tagging and primary ciliary dyskinesia model mice.

Daniel Dodd[1], Girish Mali [1,2], Margaret Keighren[1], Alex von Kreigsheim[1], Peter Tennant[1], Emma Hall[1], Patricia Yeyati[1] and Pleasantine Mill[1]

1 MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, the University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, UK.

2 MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK.

Defective ciliary motility can lead to a severe congenital human ciliopathy called Primary Ciliary Dyskinesia (PCD, OMIM: 242650) characterised by respiratory distress in newborns which can progress to life-threatening complications. A major cause of PCD is a failure in dynein motor function or assembly. The majority of PCD causing mutations are found in genes encoding structural sub-units of the outer dynein arm (ODA) motor complex, the most common of these disease genes is Dynein Axonemal Heavy Chain 5 (*DNAH5*). It is thought that in order to fold into the correct structure the large dynein heavy chains require chaperones, while the precise nature of these chaperones has yet to be discovered it is known that certain Dynein Axonemal Assembly Factors (DNAAFs) are required for the correct localisation and assembly of the axonemal dyneins. We have made a mouse model where absence of one such DNAAF (*Zmynd10*) results in the degradation of several dynein proteins including DNAH5 in ciliated mouse epithelial cells resulting in a PCD phenotype. We have also made a mouse with a SNAP tagged DNAH5, which we will be able to use to score the effectiveness of therapeutic intervention as well as to study the fundamental steps in the formation and regulation of ODAs. We plan to use the tagged protein to pulldown heavy chain interactors during axonemal dynein assembly, giving us a rare insight into the way in which axonemal dynein heavy chains are controlled to form the motors that power ciliary beat. We hope this combination of mouse models will help us to understand the basic biology behind motile cilia as well as give us a way in which to test treatments for motile ciliary disease.

Tracking sperm in three and four dimensions from X and Y coordinates and future prospects

G. van der Horst[1] and E. Sanchez[2]

[1]Department Medical Biosciences, University of the Western Cape, Bellville, South Africa, [2] Microptic SL, Barcelona, Spain

Application of newly developed CASA sperm motility functional tests showed good discrimination between good and poor sperm samples. However, it is based on accurately constructing X and Y coordinates. These are not considered inappropriate but in this investigation we tried to develop models to reconstruct 3D/4D tracks that may provide more information on the nature of sperm patterns/functionality. The most applicable kinematic parameters to formulate the 3D helix construction are ALH (radius of helix), VCL or VSL (distance from the first point to last point of track) and BCF (frequency of VAP crossing VCL). This will describe the "distance between the 2D peaks" and as a first approximation we used VSL/(BCF/2 x ALH). The results obtained from this initial modellling showed that it is possible to construct 3D tracks from 2D CASA tracks. By simply changing any of the kinematic parameters ALH, BCF or VSL we can model spherical helices. One of the current weaknesses of the model is that we assume that sperm swim harmonically but they change velocity and amplitude for example. The next step will be to approximate the helix to the real track. Accordingly, we will need to analyze sperm movement from point to point and calculate the actual track. Furthermore, this may allow the possibility of deriving three new parameters from the helix model and they relate to length as a function of speed, and curvature and torsion as components of the helix. We have furthermore started a project in 2D mathematical modelling with the School of Mathematics group, University of Birmingham using X and Y coordinates but data is currently analyzed.

The above studies have been based on tracking the centroid of the sperm head movement of a wide range of species (vertebrate and invertebrate) and this is open to criticism. While the flagellar wave may behave like the head during very straight line swimming this is not the case during most other swimming patterns. The Birmingham group (Smith, Gallagher and Kirkman-Brown) developed novel software to allow analysis/modelling of the flagellar wave at moderatly high frame rates such as 200fps. Also in this regard a joint research programme has been developed with the Birmingham group and we provide them with 200fps *.avi files for flagellar analysis of a wide range of species and data processing is in progress.

Finally, we have also assessed the possibility of looking at sperm motility pattern recognition in relation to Mandelbrot fractals (several vertebrate species). While our progress is still primodrial in this context, it shows great potential. In conclusion, it appears that our four approaches above may be complimentary to understand sperm motility/function more comprehensively.

Ciliomotor circuitry underlying whole-body coordination of ciliary activity in the *Platynereis* larva

Csaba Verasztó[1], Nobuo Ueda[2], Luis A Bezares-Calderón, Aurora[2] Panzera, Elizabeth A Williams, Réza Shahidi, and Gáspár Jékely

[1]University of Exeter, Living Systems Institute, United Kingdom
[2] Max Planck Institute for Developmental Biology, Germany

Ciliated surfaces harbouring synchronously beating cilia can generate fluid flow or drive locomotion. In ciliary swimmers, ciliary beating, arrests, and changes in beat frequency are often coordinated across extended or discontinuous surfaces. To understand how such coordination is achieved, we studied the ciliated larvae of *Platynereis dumerilii*, a marine annelid. *Platynereis* larvae have segmental multiciliated cells that regularly display spontaneous coordinated ciliary arrests. We used whole-body connectomics, activity imaging, transgenesis, and neuron ablation to characterize the ciliomotor circuitry. We identified cholinergic, serotonergic, and catecholaminergic ciliomotor neurons. The synchronous rhythmic activation of cholinergic cells drives the coordinated arrests of all cilia. The serotonergic cells are active when cilia are beating. Serotonin inhibits the cholinergic rhythm, and increases ciliary beat frequency. Based on their connectivity and alternating activity, the catecholaminergic cells may generate the rhythm. The ciliomotor circuitry thus constitutes a stop-and-go pacemaker system for the whole-body coordination of ciliary locomotion.

Three-dimensional flow in the ventral third ventricle of the brain

Y. Wang[1], S. Kapoor[2], C. Westendorf[1], G. Eichele[2], E. Bodenschatz[1]

[1] *Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany*
[2] *Max Planck Institute for Biophysical Chemistry, Göttingen, Germany*

The walls of the ventricular system of mammalian brain are lined with ependymal cells, each of which sprouts a bundle of cilia that constantly beat and thereby maintain directional cerebrospinal fluid (CSF) flow. A transport network driven by coordinated motile cilia inside the ventral third ventricle (v3V) was reported [1]. Particle tracking showed recently that in mouse brain this flow network locally differs between the two sides of the v3V and changes with age, which implies an age dependent complex delivery system for CSF constituents. We also study numerically the contribution of the flow network to the CSF flow in the overall three-dimensional v3V cavity via the lattice Boltzmann method and immersed boundary method, and uncover likely physiological consequences of the flow pattern.

References

[1] R. Faubel, C. Westendorf, E. Bodenschatz, & G. Eichele. Science **353(6295)**, 176-178 (2016).

Quantitative analysis of cilia mediated flow and cell polarity of the brain ventricular system

C. Westendorf[1], S. Kapoor[2], G. Eichele[2] and E. Bodenschatz[1]

[1]Max Planck Institute for Dynamics and Self-Organization, Goettingen, Germany
[2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

The brain ventricles, which are filled with cerebrospinal fluid (CSF), are lined with cilliated epithelial cells. The beating of these cilia pushes the CSF and thereby transports its constituents through the different cavities. Within the ventral 3rd ventricle the cilia beats create a complex flow network with several distinguishable flow domains [1]. Here we present a set of both experimental and computational methods to further explore these flow domains, the beating direction of the cilia, and the underlying cell polarity of the epithelial layer in a quantitative manner and over the entire extension of the ventricular walls. The methods include: (1) the averaging of bead tracking data, (2) the recording and directionality analysis of large scale DIC movies of beating cilia, and (3) the antibody staining and subsequent image segmentation of confocal micrographs of the epithelial cell layer. With these methods we now demonstrate that the foundation of the flow domains is based on the translational polarities of the cells and the rotational polarities of the cilia. The analysis of the rotational polarities further enables the description of the local directionality and the spatial changes of the cilia beating on a single cell level.

References

[1] R. Faubel, C. Westendorf, E. Bodenschatz and G. Eichele, Science **353** (6295) 176–178.

Deafness-infertility syndrome: a model to unravel the role of CatSper in human sperm (dys)function

S. Young[1], C. Schiffer[1], C. Brenker[1], F. Tüttelmann[2], A. Röpke[2], H. Hamzeh[3], D. Wachten[3,4], U.B. Kaupp[3], S. Kliesch[1] and T. Strünker[1]

[1]Centre of Reproductive Medicine and Andrology, Münster, Germany; [2]Institute of Human Genetics, Münster, Germany; [3]Center of Advanced European Studies and Research, Bonn, Germany; [4]Institute of Innate Immunity, University Hospital, University of Bonn, Bonn, Germany

In human sperm, the sperm-specific Ca^{2+} channel CatSper serves as a polymodal chemosensor that registers ligands released by the oviductal epithelium and cells surrounding the oocyte. Thereby, CatSper translates the chemical code of the oviductal microenvironment into changes of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which controls the flagellar beat and swimming behavior [1]. We identified three infertile patients that suffer from the so called deafness-infertility syndrome (DIS). DIS patients lack the genes encoding for stereocilin (STRC) and CatSper 2; STRC is expressed in cochlear hair cells. The phenotype of the CATSPER2-deficient sperm was thoroughly characterized by standard semen analysis, electrophysiology, Ca^{2+}-fluorimetry, motility analysis, and 3D-STORM. We show that CatSper-mediated Ca^{2+} influx and membrane currents are abolished in sperm from DIS patients, demonstrating that the homozygous deletion of CATSPER2 abrogates the expression of functional CatSper channels [2, 3]. Though, in the absence of CatSper 2, CatSper 3 and CatSper 4 assemble into non-functional protein complexes, whose sub-cellular arrangement is similar to that of the functional CatSper-channel complex. Moreover, according to standard semen analysis, the DIS patients are normozoospermic, indicating that male infertility caused by the lack of functional CatSper channels escapes current andrological methods used to assess sperm function and male fertility. Finally, we demonstrate the utility of CatSper-deficient human sperm as a model to gain insight into the function of CatSper: see poster "Rotational motion and rheotaxis of sperm does not require functional CatSper Ca^{2+} channels" by Schiffer et al.

References

[1] C. Brenker et al., The CatSper channel: a polymodal chemosensor in human sperm, EMBO J **31**, 1654–1665 (2012)

[2] J.F. Smith et al., Disruption of the principal, progesterone-activated sperm Ca^{2+} channel in a CatSper2-deficient infertile patient, PNAS **110**, 6823-6828 (2013)

[3] C. Brenker et al., Synergistic activation of CatSper Ca^{2+} channels in human sperm by oviductal ligands and endocrine disrupting chemicals, Human Reproduction **33**, 1915-1923 (2018)