Biophysical Conference 2019

Tuesday 20 August 2019 - Friday 23 August 2019

Hotel Amfora, Hvar, Croatia

Book of Abstracts

Contents

Poster Session / 23

A Model of the Cellular Resource Allocation Strategy in Bacteria

Anjan Roy[1] ; Rami Pugatch[2]

[1] *ICTP, Italy*

[2] *Ben Gurion University of Negev, Israel and ICTP, Italy*

Corresponding Author(s): aroy1@ictp.it

The central target of the bacterial life is to survive, and one of the central task to achieve this target is for the bacterial cell to grow. The machine which is responsible for performing the bulk of this task is the Ribosome. However, it not only has to produce all the required proteins of the cell but also copies of itself, to be divided among its daughter cells so as to keep this process self sustaining. In doing so, it has to decide on how much it should dedicate itself to produce its copies in comparison to producing the metabolic proteins needed to provide the fuel for this task, apart from other proteins. We present a simple, biologically motivated, mathematical model which incorporates this trade-off and shed some understanding on the nature of the control implemented by the bacteria in taking this decision.

Poster Session / 10

A hydraulic instability regulates cell death and Oocyte size selection in C.elegans germline

Arghyadip Mukherjee[1] ; Nicolas Chartier[2] ; Julia Pfanzelter[3] ; Sebastian Fürthauer[4] ; Frank Julicher[5] ; Stephan Grill[6]

[1] *MPI-PKS*

[2] *TU Dresden*

[3] *BIOTEC*

[4] *Flatiron Institute*

[5] *MPIPKS Dresden*

[6] *MPI-CBG*

Corresponding Author(s): argo@pks.mpg.de, nicolas.chartier@biotec-tu.dresden, stephan.grill@tu-dresden.de

The process of making an oocyte starting from a germline tissue is a fundamental cellular process.Oogenesis demonstrates remarkable mechanical as well as hydrodynamic phenomena across organisms. Dynamic size regulation and mechanical symmetry breaking in germ cell population (within a syncytia) leads to heterogeneous growth leading to cell fate decisions. The roundworm C. elegans has a tubular syncytial (tissue architecture with connected cytoplasm) germline, which achieves germ-cell growth by hydrodynamic flows that range across 400 microns.
By quantitative analysis and theoretical modeling, we discover that germ cells actively generate long-range hydrodynamic flows along the germline, while also locally maintaining their homogenous size. The coupling of cell mechanics and hydrodynamic fields lead to active pressure-tuning, which yields a hydraulic instability setting a critical size for the germ-cells in the absence of active sources. This mechanism ensures selection and growth of germ cells beyond a critical size at the expense of smaller cells and is independent of the apoptotic machinery. We unravel the physical basis of oogenesis and cell elimination by combining cellular mechanics and active hydrodynamics. Our findings elucidate a novel connection of cell fate and mechanics of volume regulation, and proposes a cell death mechanism that is emergent out of cellular competition rather than programmed.

Session 3 / **17**

A single molecule perspective on the role of biomolecular condensation in FUS mediated DNA damage repair

Author(s): Roman Renger[1]

Co-author(s): José Lantero Morin [1] ; Stephan W. Grill [1]

[1] *MPI-CBG*

Corresponding Author(s): morinlan@mpi-cbg.de, renger@mpi-cbg.de, grill@mpi-cbg.de

Biomolecular condensation (BC) has recently been found to govern the spatial and temporal organization of the intracellular space with respect to various processes. FUS (Fused in Sarcoma) is an intrinsically disordered protein that forms condensates at sites of DNA damage in vivo and liquid-like droplets that harden over time in vitro. A detailed picture of the assembly process of FUS based repair compartments at DNA damage sites is, however, still missing. We study the role of BC in the interaction of FUS and DNA on the single molecule level using optical tweezers-based micromanipulation combined with confocal fluorescence imaging.
We found that FUS shows different binding modes with DNA and forms dynamic condensates with free single stranded DNA at DNA nicks. The material properties and composition of these condensates depend on the FUS concentration and the number of incorporated nucleotides.
We propose that dynamic repair compartments formed by BC might not only facilitate recruitment of downstream repair factors, but also prevent disassembly of DNA fragments and promote local concentration of damage sites and thus assist the efficient assembly and functionality of the DNA damage repair machinery.

Poster Session / **14**

Actin(g) on phase separation

Tina Wiegand[1]

[1] *Hyman*

Corresponding Author(s): wiegand@mpi-cbg.de

F-actin networks play a crucial role for cellular integrity and induction of shape changes during development and homeostasis. Actin polymerization is therefore highly regulated by multiple pathways and means such as local monomer concentration, nucleating and sequestering factors. Recently, actin partioning into biomolecular condensates has been reported as additional mechanism to transiently enhance polymerization kinetics. During establishment of the actin cortex in the *C. elegans* oocyte we observe actin droplets from which fibers extrude to form a contractile network. We want to further investigate the proteins driving this condensation of actin into puncta and the resulting implications on the formation of the cortex. Therefore, we reconstitute the main components wsp-1 and the arp2/3 complex in vitro and study their interaction with actin. Furthermore, the partitioning of actin inhibitors into the drops and the resulting polymerization rates will be quantified to test if the polymerization reaction can be relieved from inhibition by differential partitioning.

Session 3 / **21**

Balance of forces and torques in a mean-field approximation in mitotic spindles

Arian Ivec[1] ; Nenad Pavin[2] ; Iva Tolić[3]

[1] *Faculty of Science, University of Zagreb*

[2] *Faculty of science, University of Zagreb*

[3] *Ruđer Bošković Institute, Zagreb, Croatia*

Corresponding Author(s): npavin@phy.hr, ivec.arian@gmail.com

The mitotic spindle is a self-organized micro-machine composed of microtubules and associated proteins, which divides genetic material between its two nascent daughter cells. Forces exist in the spindle throughout mitosis and are crucial for spindle functioning in each phase. In metaphase, the mitotic spindle has a recognizable shape with a characteristic arrangement of microtubules. Microtubules extend from opposite spindle poles and interact with the chromosomes and with each other. Though a significant progress in understanding the mechanics of the spindle has been achieved, the question of force balance in the spindle is still open. We aim to explore the force balance of the entire spindle, based on our previous work on individual microtubule bundles. We describe the force balance of the spindle by introducing a mean-field approach, in which discrete microtubule bundles in a certain region, together with forces and torques exerted by these bundles, are approximated by an averaged bundle. The model provides predictions for forces and torques in the spindle, and consequently it predicts the shape of the entire spindle, including the shapes of inner and outer bundles. The predicted shapes will be compared with shapes observed in our experiments. Based on this information, we provide a mechanical explanation for the shapes of inner and outer bundles, including major differences between them. This approach provides comprehensive insight into forces and torques acting in the entire spindle, which are crucial for proper cell division.

Session 2 / **18**

Bridging microtubules promote centering of the kinetochores by length-dependent pulling forces

Agneza Bosilj[1] ; Mihaela Jagrić[2] ; Jelena Martinčić[2] ; Patrik Risteski[2] ; Iva Tolić[2] ; Nenad Pavin[3]

[1] *Department of Physics, Faculty of Science, University of Zagreb*

[2] *Ruđer Bošković Institute, Zagreb, Croatia*

[3] *Department of Physics, Faculty of Science, Zagreb, Croatia*

Corresponding Author(s): agneza.bosilj@gmail.com

Chromosome positioning to the equatorial plane of the mitotic spindle is necessary to prevent lagging chromosomes and abnormal nuclear envelope reformation [1,2]. It has been proposed that two centering mechanisms play a key role here, microtubule (MT) catastrophe promoted by kinesin-8 motors and pushing forces exerted by chromokinesins. Surprisingly, our experiments suggest that removal of PRC1 molecules from the antiparallel overlaps of the bridging MTs [3] disrupts alignment of the kinetochores. Here we show, by introducing a theoretical model, that kinetochore MTs cross-linked by bridging MTs exert centering pulling forces. Our model also shows that length-dependent catastrophe and rescue regulated by motor proteins and passive cross-linkers are necessary for well defined length of MTs and their antiparallel overlap, respectively [4]. We predict that stable antiparallel overlaps subsequently navigate positioning of the kinetochores in the center of the metaphase plate. These predictions were confirmed in experiments with overexpression of PRC1 proteins and silencing of Kif18A motors.

[1] Fonseca et al. 2019 J Cell Biol [2] Stumpff et al. 2012 Cell [3] Vukušić, Buđa, Bosilj et al. 2017 Dev Cell [4] Klemm, Bosilj et al. 2018 Mol Biol Cell

Session 2 / **3**

Cell lineage dependent chiral flows at the actomyosin cortex drive cellular rearrangement in early development.

Lokesh Pimpale[1] ; Teije Middelkoop[None] ; Stephan Grill[2]

[1] *BIOTEC, TU Dresden*

[2] *MPI-CBG*

Corresponding Author(s): teije.middelkoop@tu-dresden.de, teije@biotec.tu-dresden.de, lokesh.pimpale@tu-dresden.de

Cells need to be positioned correctly during embryogenesis for achieving important processes like body axis formation and organ development. The mechanisms by which cells reposition in the early developing embryo are still not completely understood. Recently, Naganathan et al 2014, showed that the gradient of myosin in the actomyosin cortex generates chiral flows and these flows are important for breaking left-right symmetry in a developing *C. elegans* embryo. We here show that chiral flows arise in the AB lineage only, and that the presence of these flows correlates with cellular repositioning in the embryo. Using reverse genetics approach and temperature sensitive mutants we demonstrate that cellular rearrangements in the AB lineage are driven by chiral actomyosin flows. Thus, we conclude that chiral actomyosin flows drive cellular rearrangement in early development.

34

Chromosome segregation errors and aneuploidy

Patrik Risteski[1] ; Snježana Kodba[1] ; Nenad Pavin[2] ; Iva M. Tolić[1]

[1] *Ruđer Bošković Institute, Zagreb, Croatia*

[2] *Faculty of Science, University of Zagreb, Zagreb, Croatia*

Corresponding Author(s): pristesk@irb.hr, tolic@irb.hr, npavin@phy.hr

Chromosome segregation errors cause aneuploidy, a state of numerical alterations in chromosomes that contributes to tumor initiation and progression. Improper attachments between chromosomes and microtubules are the most common mechanism causing chromosome mis-segregation [1]. However, frequency and identity of error mechanisms have not been studied extensively. Here, we quantitatively assessed errors in chromosome segregation in 2D cultures, both in healthy (RPE1) and cancer cell lines (U2OS and HeLa) and found that cancer cells can enter anaphase with persistent mono-oriented chromosomes. We found this rate to be 3-4% in U2OS and HeLa cells. Prior to anaphase, the frequency of mono-oriented chromosomes was roughly 2x higher in U2OS than in HeLa cells. In U2OS cells, we found a 70 minute-delay in anaphase entry during which most of the mono-oriented chromosomes were resolved. Interestingly, we found multiple mono-oriented chromosomes in 2% of U2OS cells. Our results demonstrate that mono-oriented chromosomes can lead to aneuploidy, presumably due to spindle assembly checkpoint override, and indicate that multiple errors per cell are due to defects in the mitotic machinery.
[1] Compton DA. Curr Opin Cell Biol, 2011.

Poster Session / 16

Decoding dynamic coiled-coils

Marcus Jahnel[1] ; Peter Steinbach[1] ; Stephan W. Grill[1]

[1] *MPI-CBG*

Corresponding Author(s): grill@mpi-cbg.de, jahnel@mpi-cbg.de, steinbac@mpi-cbg.de

How biomolecules reliably generate mechanical forces and torques in a fluctuating environment is an enduring problem of biological physics and molecular biology.

An unusual pair recently found to generate forces are the long membrane tethering protein early endosome antigen 1 (EEA1) — a 220 nm long coiled-coil protein — and the small GTPase Rab5*. Binding of the small GTPase to the end of the coiled-coil triggers long-range conformational changes to modulate the flexibility of EEA1, allowing it to sample conformations with a reduced end-to-end distance. However, what is the molecular basis for EEA1's multistability, and how is energy transmitted from top-to-bottom?

Here, I will discuss how an internal competition between unbalanced hydrophobic residues and electrostatic interactions can give rise to EEA1's dynamic behaviour. I will highlight our recent efforts to combine coarse-grained modelling and atomistic molecular dynamics simulations to explain the force generation mechanism of long coiled-coil proteins.

*Murray, D. and Jahnel, M. et al., An endosomal tether undergoes an entropic collapse to bring vesicles together. Nature (2016)

Session 3 / **31**

Dynamics of microtubule bundle formation during prometaphase

Juraj Simunić[1] ; Martina Manenica[1] ; Jurica Matković[1] ; Iva Tolić[2]

[1] *Ruđer Bošković Institute*

[2] *Ruđer Bošković Institute, Zagreb, Croatia*

Corresponding Author(s): juricamatkovic55@gmail.com, jsimunic@irb.hr, manenica.martina@gmail.com

The spindle during metaphase consists of evenly distributed and well-organized microtubule bundles. It is unknown how this recognizable architecture arises during prometaphase. Here we show that during prometaphase the spindle contains antiparallel PRC1 labelled bundles the number of which increases in time by longitudinal splitting of preexisting bundles and by de novo assembly. By using live cell imaging of HeLa cells expressing PRC1-GFP we observe that in prometaphase, the spindle has a small number (13+/-2 s.e.m.) of PRC1 labelled bundles of uneven size and irregular spatial distribution. The number of these bundles increases up to about 30, and the bundles become distributed evenly throughout the equatorial plane. We also show that siRNA of HAUS6 component of augmin complex causes a smaller number of bundles in metaphase (17+/-1 s.e.m.). The spatial distribution of these bundles is perturbed with more bundles being present around the perimeter of the spindle and fewer in the central part. Our results show how microtubule bundles form in prometaphase and provide evidence that microtubule nucleation by augmin is involved in this process.

Session 3 / **26**

Extreme-Value Statistics of Molecular Motors

Alexandre Guillet[1] ; Edgar Roldan[None] ; Frank Julicher[2]

[1] *ICTP and LOMA*

[2] *MPIPKS Dresden*

Corresponding Author(s): edgar.roldan@fis.ucm.es, alexandre.guillet@u-bordeaux.fr

We derive exact expressions for the finite-time statistics of extrema (maximum and minimum) of the spatial displacement and the fluctuating entropy flow of continuous-time random walks describing the dynamics of molecular motors at the single-molecule level. Our results generalize the infimum law for entropy production and reveal a symmetry of the distribution of maxima and minima of

stochastic entropy production, which are confirmed by numerical simulations of stochastic models of molecular motors. Finally we identify a timescale at which extreme-value distributions become universal, revealing a connection between extrema statistics and the Marcenko-Pastur law of random-matrix theory.

Poster Session / 7

Fluctuating Energetics in Long Range Interacting Systems

Edgar Roldan[1] ; Stefano Ruffo[2] ; Ashwin Gopal[None]

[1] *QLS, ICTP*

[2] *SISSA*

Corresponding Author(s): ashwingop12345@gmail.com, edgar.roldan@fis.ucm.es, ruffo@sissa.it

Long-range interacting systems are characterized by interaction potentials that decay slowly with the separation distance, e.g. with power-law dependencies. Such systems give rise to a plethora of intriguing physical phenomena such as ensemble in-equivalence, ergodicity breaking, synchronization, etc. The dynamics of long-range interacting systems in bounded domains can be quantitatively studied using tools from non-equilibrium statistical mechanics. However, little is known about the thermodynamics, and in particular, the energetics of long-range interacting systems in the presence of noise. Very recently, several fluctuation theorems from the emerging field of stochastic thermodynamics have been verified numerically in multi-particle systems with long-range interactions, using the mean field approximation. Such test opens the possibility of understanding notions like fluctuating fluxes of energy, matter of entropy in this class of systems. Moreover, an important question is how to achieve efficient conversion of heat from thermal fluctuations into useful work in the presence of long-range interactions.

We investigate the thermodynamics of stochastic models with long-range interactions using the framework of stochastic energetics. We perform a thorough numerical and analytical study of a simple one-dimensional model describing a dissipative forced pendulum. We study the transition in the stochastic energetics of the pendulum due to the presence of a separatrix in the motion between oscillation and rotations. These results are put in context with active biological oscillations.

Session 4 / 11

Generosity, selfishness and exploitation as optimal greedy strategies for resource sharing

Andrea Mazzolini[1] ; Antonio Celani[2]

[1] *ICTP, Quantitative Life Science section, Trieste, Italy*

[2] *ICTP, QLS secion, Trieste, Italy*

Corresponding Author(s): andrea.mazzolini.90@gmail.com

There is increasing evidence that fairness and generosity are not exclusive human traits.
Indeed, several experiments on chimpanzees, monkeys and other mammals show an inequity-aversion behavior.
Namely, if some valuable resource, e.g. food, is unevenly divided between two individuals, the one who gets a smaller share may reject the reward, and, in some cases, there can even be an attempt of the animal with the larger share at equalizing the division.
Therefore, animals can deliberately lower their gain in name of an apparent sense of fairness.
Here we show the emergence of generosity in a resource-gathering-and-sharing game inspired by

animal behavior. The players act greedily, that is, they try to individually maximize only their personal income. Nonetheless, the analytical solution of the model shows that three optimal behaviors emerge depending on conditions. Besides the obvious case when players are selfish in their choice of resource division, there are conditions under which both the players are generous. Moreover, we also found a range of situations in which one selfish player exploits another generous individual, for the satisfaction of both players.
Our results show that inequity aversion is favored by three factors: a long time horizon over which the players try to optimize their own game, by the similarity among players in their ability of performing the resource-gathering task, as well as by the availability of resources in the environment. These concurrent requirements lead to identify necessary conditions for the emergence of generosity.

Poster Session / 27

How the size of a cell can determine its fate

Author(s): Julia Pfanzelter[1]

Co-author(s): Frank Julicher [2] ; Sebastian Fürthauer [3] ; Nicolas Chartier [4] ; Arghyadip Mukherjee [5] ; Stephan Grill [6]

[1] *BIOTEC*

[2] *MPIPKS Dresden*

[3] *Flatiron Institute*

[4] *TU Dresden*

[5] *MPI-PKS*

[6] *MPI-CBG*

Corresponding Author(s): nicolas.chartier@biotec-tu.dresden, stephan.grill@tu-dresden.de, argo@pks.mpg.de

Production of viable oocytes is an essential process during sexual reproduction. The C. elegans gonad is composed of roughly 1000 germ cells, half of which develop into oocytes while the rest undergo apoptosis. These dying cells are thought to act as nurse cells donating material to their growing neighbours. Long-range cytoplasmic flows through the connecting tube of this syncytial tissue promote the growth of oocytes, however it remains unknown what triggers cell death in the C. elegans gonad. One hypothesis is that cell size becomes a determining factor that destines smaller cells for apoptosis and larger ones for becoming oocytes. To test this hypothesis I employed FLUCS, a microscopy-based manipulation technique that allows me to create cytoplasmic flows in the germ line. By directing flows into or out of a cell its size can be changed and with that also its fate. While in control cells apoptosis is only triggered in 15% of the cases, inducing flows out of a germ cell leads to cell death in over 50% of the manipulated cells. This indicates that cell size is a critical parameter in the decision between life and death in C. elegans germ cells.
While it has been reported that cell size can influence cell fate decisions also in other systems, it remains unclear how cell size is sensed, especially in a syncytial structure such as the C. elegans gonad.

Session 3 / 38

In vivo perturbation of active forces in the actomyosin cortex of C. elegans embryo

Dora Polic[None] ; Enrico D. Perini[None] ; Stephan Grill[None]

Corresponding Author(s): polic@mpi-cbg.de

Morphogenesis is one of the fundamental processes during development. Critically, it depends on the generation of forces by the actomyosin cortex: a dynamic contractile apparatus that emerges

from the cooperative interaction between actin networks and non-muscle myosin II (Alberts, B. et al., 2008, Philips, R. et al., 2012, Salbreux, G. et al., 2012). The past decades have brought a detailed understanding of how myosin generates force at the molecular level (Howard, J., 2001., Ishii, Y. et al., 2004). However, very little is known about the mechanisms by which myosin force generation leads to larger scale responses in cells and tissues.
To address this question, we genetically modify the myosin responsible for morphogenesis in *C. elegans*, NMY-2, with the aim to perturb the naturally occurring forces that drive the actomyosin cortex. NMY-2 is composed of a "head" domain at its N-terminus and a "tail" domain at its C-terminus. The head is the motor domain that hydrolises ATP and binds actin. The tail is required for dimerization and formation of myofilaments. The region between head and tail, called the "lever arm", is responsible for the conversion of the chemical energy into mechanical action. The length of the lever arm determines the distance myosin makes walking on actin filament per ATP hydrolyses, so called "step size". We hypothesize that altering the length of the lever arm would cause a change in the step size which in turn would alter the "power stroke" of myosin and the generated force.
To test this hypothesis, we have generated mutants of myosin that have a longer lever arm and expressed them in *C. elegans* embryos. Using this method, we can modulate active actomyosin tension and torque generation and assess the influence of myosin force generation on large scale response.

Poster Session / 15

Investigating actin nucleation through active phase separation

Victoria Victoria Yan[None] ; Stephan Grill[None]

Corresponding Author(s): victoria.yan.tjy@gmail.com

In the cytoplasm, proteins observed in liquid-like phases are thought to contribute to compartmentalization of the cytoplasm. However, it is unclear whether demixing of cytoskeletal components could also play a role in partitioning actin nucleators to the membrane during the formation of an actomyosin cortex. Members of the N-Wasp Cdc-42 Arp2/3 nucleation pathway contain intrinsically disordered domains, and have been shown to undergo liquid-liquid phase separation in vitro. However, it is unclear whether the F-actin nucleation module can undergo phase separation in vivo to promote local enrichment of nucleators. During actomyosin cortex formation in the C. elegans oocyte to zygote transition, I observed biphasic cortical F-actin network condensation during Meiosis. In addition to regulated clustering of the N-Wasp pathway components N-wasp and Arp2/3. To investigate the role of F-actin phase separation in vivo, I aim to first characterize and confirm liquid-like behaviour of the actin nucleators in C. elegans embryos. Then I will identify the molecular interactions necessary for cortical clustering of nucleators. Lastly, I will identify the biological role of the nucleation clustering by decoupling localization of function of the nucleators. These findings suggest that cortical dynamics during morphogenesis may be controlled through regulated phase separation of actin nucleators.

Session 4 / 13

Learning to flock with reinforcement learning.

Mihir Durve[1] ; Fernando Peruani[2] ; Antonio Celani[3]

[1] *Department of Physics, University of Trieste*

[2] *University of Nice Sophia Antipolis*

[3] *The Abdus Salam International Centre for Theoretical Physics*

Corresponding Author(s): mihirdurve@gmail.com

In many biological systems the individual agents cluster together in space and exhibit collective behavior [1]. Thousands of starling birds show spectacular collective aerial maneuvers near their

home, migratory birds migrate as a flock, school of fish forage together, thousands of insects march and feast on the crop fields etc are few examples[2-4].
Many simulation models are proposed to understand the fundamentals principles governing the collective behavior in such systems [5-7]. Yet such rules are not well understood.
We study multi-agent systems with machine learning techniques to understand the optimal decision making process by the agents to exhibit collective behavior. One of the widely used machine learning technique, that we implemented, is called the reinforcement learning[8]. The broad scheme of the reinforcement learning technique can be summarized as following. Agent as a decision maker takes action in its environment which is in state (s) and environment provides a reward signal and new state of the environment (s') to the agents as a consequence of the action performed by the agent. The goal of the agent is to discover a policy (by try and error) that maximize the total reward. A policy is a map from states to actions that dictates the best action (a*) to perform in the state (s).
We implement reinforcement learning technique to understand the decision making process by the individual agents in order to form a flock. For that purpose, we set reward scheme that encourage congregation of the agents. We observe that agents with learning algorithm discover multiple policies to maximize the total reward for congregation. While following these policies agents not only congregate but also form highly polar ordered states as observed in real flocks[5]. In highly polar ordered states, all the agents move in the same heading direction. And one of the policies that agents discovered is equivalent to the well known statistical physics model called the Vicsek model[6].
Ref :
[1] J. Parrish and W. Hamner, Animal Groups in Three Dimensions (Cambridge University Press, 1997)
[2] M. Ballerini et al., PNAS 105, 1232 (2008)
[3] T. Pitcher, et al., Behav. Ecol. Socio. Biol. 10, 149 (1982)
[4] J. Buhl et al., Science 312, 1402 (2006)
[5] A. Cavagna et al., J. Stat. Phys. 158, 601 (2005)
[6] T. Vicsek et al., Phys. Rev. Lett. 75, 1226 (1995).
[7] I. Couzin et al., Journal of Theoretical Biology 218, 1 (2002).
[8] R. Sutton and A. Barto, Reinforcement Learning: An Introduction (MIT Press, Cambridge, MA, 1998)

Session 2 / **19**

Mechanical symmetry breaking in C. elegans dorsal-ventral axis establishmen

Masatoshi Nishikawa[None]

Corresponding Author(s): masatoshi.nishikawa.93@hosei.ac.jp

In *Caenorhabditis elegans*, the initial event of spontaneous symmetry breaking that gives rise to embryonic polarity is the midbody remnant in the two-cell embryo being off-centered, which sets the dorsal-ventral axis. This results from the asymmetric ingression of contractile ring in first cleavage, but their underlying mechanisms remain largely unexplored. Here I demonstrate that a hydrodynamic coupling between the cell cortex and cytoplasm facilitates asymmetric ingression of the cytokinetic furrow. I identified two prerequisites for this symmetry breaking: cortical contractility to drive cytoplasmic flow, and the link between the cortex and the mitotic spindle to set long-ranged cytoplasmic flow, suggesting that cytoplasmic flow influences the contractile furrow ingression.

Poster Session / **25**

Mechanism of pioneer transcription factor binding to the DNA

sandeep choubey[None]

Corresponding Author(s): sandeep@pks.mpg.de

The mechanistic details of how pioneer transcription factors exert their functions over a range of specific targets in a timely and coordinated way during early development remains in its infancy. For instance, zygotic genome activation (ZGA) in fruit fly Drosophila melanogaster, is largely controlled by a protein called Zelda, a pioneer factor, binds at the vicinity of multiple developmental and constitutive genes and drives their activation during ZGA. Uncovering the mechanisms through which Zelda binds to its binding sites on the DNA and exerts its effects has been a challenge. In vitro experiments that observe the binding of Zelda to the DNA show that Zelda form spherical 'drop-like' structures at the specific binding sites on the DNA at low concentrations of the proteins in the bulk. As the bulk concentration of the protein is increased, they wet the whole DNA. To understand these observations, we have developed a simple model of 'Pre-wetting transition" following Cahn-Landau formalism. This model can explain the key features observed in the experiments such as the stationary size of Zelda drops on the DNA which remains mostly unaltered irrespective of the bulk concentration. Overall, our experimental framework sheds light onto the binding of pioneer transcription factor to the DNA, and offer experimentally testable predictions.

Poster Session / 28

Microtubule bundle formation

Marcel Prelogović[1] ; Lora Winters[2] ; Ana Milas[3] ; Iva Tolić[3] ; Nenad Pavin[1]

[1] *University of Zagreb, Faculty of science*

[2] *MPI-CBG, Dresden*

[3] *Ruđer Bošković Institute, Zagreb*

Corresponding Author(s): tolic@irb.hr, npavin@phy.hr, mprelogovic@phy.hr

During mitosis, microtubules form a spindle, which is responsible for the segregation of chromosomes. In yeast cells, the spindle has a rod-like structure and is made of microtubules emanating from two poles connected by cross-linking proteins. Microtubules self-organize into parallel or antiparallel bundles, depending on whether they grow from the same or two different poles and our goal here is understanding how such structures form. Our model includes thermally driven angular pivoting of microtubules around the poles and elastic forces between them mediated by cross-linking proteins, which can detach to and detach from microtubules, as well as move along them. The solutions of our model imply that the random motion of the microtubules allows them to find a their pair, while the short-range interactions caused by the cross-linking proteins align them into bundles. Parallel bundling can occur in the presence of either passive crosslinkers or plus-end directed motors, while the formation of antiparallel bundles requires minus-end directed motors. The model predicts the average bundling time, which is in agreement with our experimental measurements. Additionally, for the case of antiparallel bundle formation, the model predicts that the velocity of the microtubules gliding along each other is the same as the velocity at which the motors move along the microtubules, and this was also confirmed experimentally. In conclusion, the main contributors to the formation of microtubule bundles are angular diffusion of microtubules around the poles allowing them to come into contact and short-range forces caused by cross-linking proteins that align them.

Poster Session / 9

Opticals Tweezers: Force Sensors for Unzipping the Coiled-Coil EEA1

Christoph Ehrlich[None]

Corresponding Author(s): cehrlich@mpi-cbg.de

Optical Tweezers are a nowadays common tool to study the single molecule world. As force sensors, they are especially interesting to investigate binding energies and, due to their high spatial

and temporal resolution, protein dynamics (e.g. polymerases). However, careful calibration of an optical tweezers setup is required in order to achieve that resolution. Berg-Sørensen and Flyvbjerg (2003) suggested a calibration method for a single trap that does not require prior knowledge of the experimental settings (viscosity, bead radius etc.). Here we present the extension of this method to dual-trap optical tweezers setups taking into account hydrodynamic interactions of the beads.
Our so calibrated setup is then used to unzip the ~200 nm coiled-coil protein EEA1 and we report the progress of these experiments.

Poster Session / 24

Patterning of Cytokinetic ring

Sourabh Monnappa Kuppanda Jafri[1]

[1] *Biotec, Technische Univeristat Dresden*

Corresponding Author(s): sourabh_monnappa.kuppanda_jafri@mailbox.tu-dresden.de

Cytokinesis is a fundamental step of cell division. This event results in the assembly of circumferential actomyosin-based contractile ring at the equatorial cortex of the cell. The contractile ring is positioned properly by the spatiotemporal cues from the mitotic spindle to the cortex. Experiments have revealed that the localization of myosin in the contractile ring is controlled via the RhoA GTPase, which receives signals from the mitotic spindle through centralspindlin and the chromosomal passenger complex. The general link between a particular spindle morphology and the density of cytokinetic regulators is however poorly understood. Here, we aim to combine single-molecule TIRF imaging of myosin, myosin-regulators and tubulin in C.elegans single-cell embryos with genetic perturbations to investigate the spindle-cortex communication during cytokinesis. To do so, we aim to do image analysis on quantities like microtubule densities, densities of antiparallel microtubules, myosin densities to understand how microtubule distribution and orientation can regulate the actomyosin cortex. This study aims at unraveling the transport rules of cytokinetic regulators during the active ring formation and establish the bridge between the spindle and the cortex communication.

Poster Session / 30

Probing Chemical and Geometrical Properties of Crystal Surfaces with First Passage Times

Muhammad Nawaz Qaisrani[1] ; Roman Belousov[2] ; Narjes Ansari[2] ; Ali Hassanali[2] ; Edgar Rolden[2]

[1] *[1] The International School for Advanced Studies (SISSA), Trieste, Italy [2] The Abdus Salam International Center for Theoretical Physics(ICTP), Trieste, Italy*

[2] *The Abdus Salam International Center for Theoretical Physics(ICTP), Trieste, Italy*

Corresponding Author(s): ahassana@ictp.it, belousov@ictp.it, edgar@ictp.it, mqaisran@ictp.it, nansari@ictp.it

The statistics of first passage times (FPT) is used widely in the field of statistical physics. In this work, we study the FPT distributions of water near three different crystal surfaces of the amino acid glutamine. We show that each surface leaves a unique fingerprint of the structural and geometrical properties of crystal in the FPT distributions. We also establish a theoretical model for the FPT in bulk water using a simple Langevin dynamics, hence providing a framework to understand water fluctuations near proteins.

Push me if you can: role of antiparallel microtubule sliding in anaphase spindle elongation

Author(s): Kruno Vukušić[1] ; Renata Buđa[1] ; Iva Tolić[1]

Co-author(s): Ivana Ponjavić [1] ; Dora Zvjerković [1]

[1] *Ruđer Bošković Institute, Zagreb, Croatia*

Corresponding Author(s): kvukusic@irb.hr, tolic@irb.hr, rbudja@irb.hr, dora.zvj@gmail.com, iponjav@irb.hr

The nature of forces driving chromosome segregation in human mitotic spindle remains one of the most challenging questions in the field. Although different microtubule populations have been well characterized in anaphase, their contribution to segregation remains unclear. Recently, it was shown that antiparallel interpolar microtubules in central spindle are strongly crosslinked with kinetochore fibers and are able to slide apart in order to separate kinetochores and spindle poles [1]. However, despite extensive knowledge on sliding proteins *in vitro* and in spindle formation, it remains unknown what proteins generate and transmit sliding forces in anaphase. Here we show that spindle elongation requires combined action of the antiparallel passive crosslinker PRC1 and kinesin-5 (Eg5) motor. Combined depletion of PRC1 and inhibition of Eg5 by STLC prevents spindle poles to elongate when Eg5 is inhibited in metaphase or stops spindle elongation when inhibited in anaphase in RPE-1 cells. However, poleward movement of kinetochores continues, though at a slower rate, when compared to control cells. Interestingly, we found that depletion/inhibition of neither these proteins individually or other candidate proteins (kinesin-12, kinesin-6 and kinesin-4) caused reduced rates of spindle elongation. Our results indicate that sliding of microtubules in the spindle midzone is controlled by combined action of Eg5 and PRC1 and is essential for spindle pole separation in human cells.

[1] Vukusic K, Buda R et al., *Dev Cell.* 2017.

Poster Session / 32

Quantification of individual myosin minifilaments in the cortex of the C. elegans zygote

Fereshteh Rafieian[None]

Contractile forces generated in the actomyosin cortex of cells drive many different processes during cellular and tissue morphogenesis. In the cortex, Nonmuscle myosin motors assemble into bipolar minifilaments, crosslink actin filaments and exert forces on them. Physical descriptions of the cortex link the myosin activity to the cortical dynamics at the cellular level and singling pathways that regulate myosin activity, have been described. At the mesoscale, however, the mechanism in which the interaction of the minifilaments and actin filaments results in contractile forces is not clearly understood yet. This is mainly due to the limitations in direct observation of such interactions. Identifying the mesoscale properties of single minifilaments that vary in order to regulate levels of contractile forces at the cortex can lead to insights into the mechanism behind force generation. Thus, we sought for such properties. We utilised the cytokinetic ring of the C. elegans zygote, as a system with high spatiotemporal regulation of contractile forces.
We observed that there is a spacial redistribution of myosin at the cortex that manifests as the variation of both the size of minifilaments and their density. At the ring, this is achieved by nucleation of new minifilaments and their growth. By down-regulation of myosin activity, through let-502 RNAi, we observed a significant decay of cortical contractility which is linked with a decay in the density and size of the minifilaments and a delay in redistribution of myosin at the ring.
These results suggest that recruitment of more minifilaments at the cortex and more myosin motors in the minifilaments increases the levels of contractile force at the cortex. This is most probably by strengthening the ability of minifilaments to cross-link actin filaments.

Poster Session / 20

Stochastic model for error propagation over multiple cell generations

Ivana Ban[1] ; Iva Tolic[2] ; Nenad Pavin[1]

[1] *Faculty of science, University of Zagreb*

[2] *Institute Ruder Boskovic*

Corresponding Author(s): npavin@phy.hr, iban@phy.hr, tolic@irb.hr

Chromosome segregation during cell division is carefully choreographed to ensure equal partitioning of the duplicated genetic material. If this process fails to occur accurately, the resulting daughters might have karyotype imbalance, known as aneuploidy. Even though mitotic errors have been studied extensively, the mechanisms generating various errors, their propagation and effects on genome integrity are not well understood. Here we develop a stochastic model that describes error propagation through many cell generations and its effect on cell vitality. For each cell division, the model considers the state of the mother cell, to predict the state of the daughter cells. The state of a cell is determined by the number of chromosomes and following mitotic surveillance mechanisms: attachment error-correction (EC), spindle assembly checkpoint (SAC) and apoptosis. Using this model, we describe the evolution of a cell's state over the generations. This allows us to give predictions about the contribution of surveillance mechanisms in a cell which will lead to a lower error rate to maintain cell viability. Model predictions will be tested experimentally which will help us understand how mitotic errors arise, how they propagate and how they impact on cell populations. Taken together, the model will provide a consistent explanation for aneuploidy in healthy and cancer cells and tissues.

Poster Session / 29

The Empty Space in Liquids and its Role in Hosting Small Polymers

Narjes Ansari[None] ; Edgar Roldan[1] ; Alessandro Laio[2] ; Ali Hassanali[1]

[1] *ICTP*

[2] *SISSA*

Corresponding Author(s): nansari@ictp.it, edgar@ictp.it, ahassana@ictp.it, laio@sissa.it

Here we present insights into the nature of structural heterogeneities in liquid water by characterizing the empty space in the hydrogen bond network. Using molecular dynamics simulations and a battery of data analysis tools, we show that density fluctuations create regions of empty space characterized by a diverse morphology - from spherical to dendritic voids. The environment of the voids allows us for the identification of both low- and high-density water in terms of long-range, collective fluctuations of the water network. We also demonstrate that dendritic voids have shapes that are similar to those of small polymers

Session 4 / 12

The actin nucleator CYK-1/mDia drives chirality of actomyosin flows and facilitates left-right symmetry breaking in early C. elegans embryo's.

Teije Middelkoop[None] ; Porfi Quintero Cadena[None] ; Lokesh Pimpale[None] ; Shahrzad Yazdi[None] ; Stephan Grill[None]

Corresponding Author(s): teije.middelkoop@gmail.com

The emergence of organismal left-right asymmetry has puzzled developmental biologists for decades. In recent years, actomyosin activity has proven to be instrumental in driving the chirality of various cells, tissues and organisms. While several myosin motors and actin nucleators of the Formin family can rotate helical actin filaments in vitro, little is known about how these activities can lead to the chirality of entire cellular actin networks in vivo. Recently, it was shown that the *C. elegans* actomyosin cortex generates active torques that drive chiral cortical flows and organismal left-right symmetry breaking. These chiral flows are dependent on the RhoA GTPase and its target non-muscle myosin II (NMII), but the underlying molecular mechanism remains elusive. By combining the strength of *C. elegans* genetics with quantitative live-imaging, we show that the activity of the Formin CYK-1/mDia is a key determinant for chiral morphogenesis of *C. elegans* embryos. Like NMII, CYK-1/mDia is recruited to active cortical RhoA patches. Moreover, loss of *cyk-1/mDia* prevents proper left-right symmetry breaking and, in sharp contrast to loss of the NMII complex, fully rescues the hyperchiral flow phenotype induced by ectopic RhoA activity. Finally, expression of a constitutively active CYK-1/mDia construct significantly increased the chiral component of the flow. These results imply that RhoA activates NMII to trigger overall actomyosin flow magnitude, while activation of CYK-1/mDia determines the strength of flow chirality. This is consistent with a mechanism in which active tension and torque generation in the actomyosin layer are molecularly distinct and driven by NMII and CYK-1/mDia respectively.

Poster Session / 4

The journey of a pioneer transcription factor towards its target sequence.

Jose Alberto Morin[1] ; Sina Wittmann[1] ; Adam Klosin[1] ; Sandeep Choubey[2] ; Roman Renger[1] ; Anthony Hyman[1] ; Stephan Grill[1]

[1] *MPI-CBG*

[2] *MPI-PKS*

Corresponding Author(s): roman.renger@tu-dresden.de, wittmann@mpi-cbg.de, 007007.sandeep@gmail.com, morinlan@mpi-cbg.de, hyman@mpi-cbg.de, klosin@mpi-cbg.de, stephan.grill@tu-dresden.de

Non-membrane bound compartments, exhibiting material properties ranging from classic liquids to gel-like play a mayor role in cell material compartmentalization. These granules are commonly composed of nucleic acid-binding proteins containing low complexity domains. Using state of the art optical tweezers coupled with fluorescence microscopy we will explore, starting at the single molecule level, the structure and function of model systems exhibiting liquid like properties: How do pioneer transcription factors scan a single DNA molecule to reach a target sequence? Which material properties are required to achieve 3D genome organization?

Poster Session / 37

Tissue-scale flows in the early quail embryo

Arjun Narayanan[1]

[1] *MPI-CBG*

Corresponding Author(s): anarayan@mpi-cbg.de

The coherent tissue-scale motion of cells in early quail embryos include the well-described “polonaise motion” of cells during the formation of the primitive streak and the less well-studied chiral flow of cells at the tip of the streak just before streak retraction. Passive flows ranging in scale from molecular fluids to sand dunes share a common description in terms of the types of forces fluid elements may exert on neighbors. Similarly, both the cortical flows generated by chiral active proteins within a cell and tissue-scale flows of active, dividing, potentially chiral cells within a tissue should

share descriptions. Building on the lab expertise in intra-cellular chiral flows and focusing on the flow of cells at onset of streak retraction at the tissue scale, we will explore the applicability of this analogy. We will ask what can be learnt about cell-cell interactions from the large scale flows and what implications they may have for patterning.

Poster Session / 36

Two Stochastic Resetting applications to Biophysics

Daniel Sanchez-Taltavull[1] ; Ana Lisica[2] ; Edgar Roldan[3] ; Stephan W. Grill[4]

[1] *Visceral Surgery, Department of BioMedical Science, University of Bern, Murtenstrasse 35, 3013 Bern, Switzerland.*

[2] *London Center for Nanotechnology, University College London, London, United Kingdom*

[3] *ICTP*

[4] *MPI-CBG*

Corresponding Author(s): daniel.sanchez@dbmr.unibe.ch, edgar@ictp.it, grill@mpi-cbg.de

Biophysics is a growing field that involves mathematical modelling and data analysis to study biological, medical and ecological processes. In this presentation we show two applications of stochastic resetting to understand drug resistance development and RNA transcription.
Drug resistance development is the process in which the current therapy against an infection is decreased in efficacy, when that occurs a therapy needs to be changed. A change in the therapy can occur either during periodic visits, after a detection of a decrease in the number of healthy cells during another medical procedure or after the appearance of a drug with less secondary effects. We model the mutations of the infection as a random walk in genotype space leading to drug resistant phenotypes and the therapy changes are described as a stochastic resetting that transports the infection resistance phenotype to its initial state.
Transcription is a key process in gene expression, in which RNA polymerases produce a complementary RNA copy from a DNA template. RNA polymerization is frequently interrupted by backtracking, a process in which polymerases perform a random walk along the DNA template. Recovery of polymerases from the transcriptionally inactive backtracked state is determined by a kinetic competition between one-dimensional diffusion and RNA cleavage. Here we describe backtrack recovery as a continuous-time random walk, where the time for a polymerase to recover from a backtrack of a given depth is described as a first-passage time of a random walker to reach an absorbing state. We represent RNA cleavage as a stochastic resetting process and derive exact expressions for the recovery time distributions and mean recovery times from a given initial backtrack depth for both continuous and discrete-lattice descriptions of the random walk. We show that recovery time statistics do not depend on the discreteness of the DNA lattice when the rate of one-dimensional diffusion is large compared to the rate of cleavage.

Poster Session / 22

Uncovering the physical constraints on cell shape for a functional actomyosin ring during cytokinesis

Julia Garcia[1]

[1] *Student*

Corresponding Author(s): julia.garcia_baucells@mailbox.tu-dresden.de

Cytokinesis is a fundamental cellular process, which consists in the physical division of a cell into two, once the genetic material has been replicated. In animal cells, this is achieved by constriction of an actomyosin ring assembled to the equatorial cell cortex. For cytokinesis to take place, the shape of the cell during cell division might be essential. Indeed, most eukaryotic cells round up when

entering mitosis in a process called mitotic cell rounding, which seems to be a general feature of cell division. Mitotic cell rounding is believed to play a role in satisfying the geometric requirements of the mitotic spindle during cell division. However, the mechanism by which cytokinesis is dependent on mitotic rounding is still obscure. We observe that mechanically constricted C. elegans embryos at one-cell stage, which are squeezed to approx. 1/3 of their height, fail to undergo cytokinesis. We find that physically limiting mitotic cell height leads to an inhomogeneous non-muscle myosin distribution along the cytokinetic ring. This finding help to disclose the mechanical requirements on cell shape for a functional cytokinetic ring during cytokinesis.

Session 3 / 8

Understanding how temperature affects the fertility of Caenorhabditis elegans by studying the liquid-like properties of P granules

Author(s): Mark Leaver[None]

Co-author(s): Stephan Grill

Because of ongoing climate change, we urgently need to know what effect temperature has on the fertility of cold blooded organisms. Fertility has a complex response to temperature, for example it effecting development and the reproductive output of *C. elegans*. At extremes of high and low temperatures, the development of the gonad (the organ which produces sperm and eggs) fails and *C. elegans* becomes sterile. There is also an optimum temperature where fertility is maximum. However, as temperature deviates from this optimum the number of offspring drops until animals become completely sterile. Despite data quantifying this effect, the cell biological mechanism is behind this response is currently unknown. A class of non-membrane bound organelles called P granules are intimately associated with the cells destined to become the gonad and act my programing their fate. Mutations in P granule genes causes infertility at higher temperatures, suggesting that there is a link between P granules and the temperature dependence of fertility. Recent it has become clear that P granules have liquid-like properties, leading to a hypothesis about how temperature might affect of *C. elegans*: that temperature effects the material properties of P granules which has a knock-on effect on their dynamics and causes the infertility at extremes of high and low temperatures. I aim to test this hypothesis with a combination of cell biological techniques and a biophysical approach.