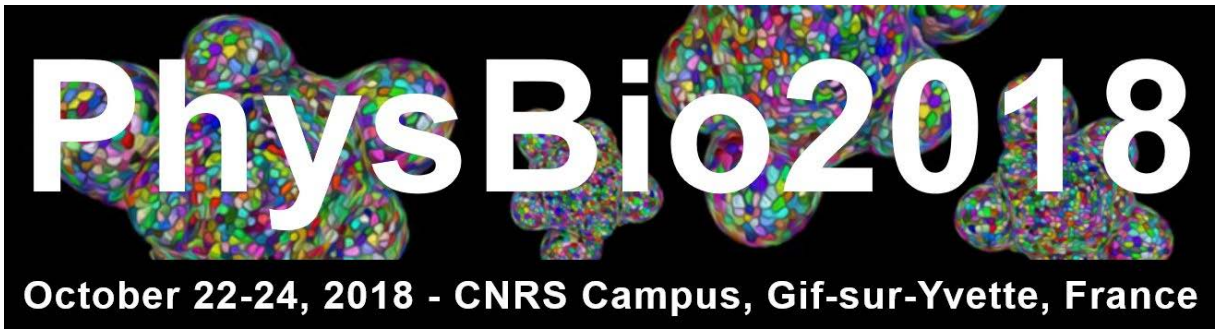

SPEAKER ABSTRACTS

MONDAY 09:00

Biomimetic membranes and layer-by-layer as model systems to study cellular processes

Catherine PICART

Grenoble Institute of Technology, University Grenoble Alpes, France

Understanding how proteins such as growth factors or cytosolic proteins interact with the cellular membranes is of prime importance in order to optimize the cellular responses in cell therapies, in view of regenerative medicine or cancer therapeutics. Biomimetic model systems are an interesting tools since they enable biophysical studies in simplified and well-defined micro-environments. In this presentation, I will present how it is possible to study protein interactions with lipid vesicles of controlled size and supported lipid bilayers containing specific lipids of physiological importance and to get quantitative information about protein/lipid binding [1-3]. In a different context, I will show how layer-by-layer films made of polyelectrolytes can be used to study the interactions between proteins of the growth factor family and cellular receptors [4-6], which are specifically interacting with the growth factors and triggering internal biochemical signaling inside the cell. Engineered materials and surfaces provide a new way to study so far hidden biological phenomena.

[1] Carvalho K, Ramos L, Roy C, Picart C. Biophys J. 2008;95:4348-60.
[2] Maniti O, Carvalho K, Picart C. Biochimie. 2013;95:3-11.
[3] Lubart Q, Vitet H, Dalonneau F, Le Roy A, Kowalski M, Lourdin M, Ebel C, Weidenhaupt M, Picart C. Biophys J. 2018;114:98-112.
[4] Crouzier T, Fourel L, Boudou T, Albiges-Rizo C, Picart C. Adv Mater. 2011;23:H111-8.
[5] Gilde F, Guillot R, Pignot-Paintrand I, Okada T, Fitzpatrick V, Boudou T, Albiges-Rizo C, Picart. C. Acta Biomater. 2016;46:55-67.
[6] Liu XQ, Fourel L, Dalonneau F, Sadir R, Leal S, Lortat-Jacob H, Weidenhaupt M, Albiges-Rizo C, Picart C.. Biomaterials. 2017;127:61-74.

MONDAY 09:45

Is Chromatin Just a Phase?

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Recent work has raised awareness among cell biologists about how biophysical phenomena such as phase separation can arise from networks of molecular interactions within cells. Phase separation can account for 'self-assembly' of functional components that are compartmentalized into bodies without constraining membranes, such as nucleoli, similar to the way mixtures of oil and water ultimately separate into distinct pools. Importantly, these phase systems display 'emergent properties' observed in vivo, e.g., liquid droplet behavior, anomalous diffusion and selective permeability, that likely play important roles in cellular organization and functions.

The possibility that biophysical properties, in particular liquid-liquid phase separation (LLPS), are involved in formation and function of chromatin domains is exciting, because it provides a novel and satisfying mechanism to explain previously enigmatic observations about nuclear structure and function. In particular, LLPS can readily account for formation of 3D chromatin domains and regulation of genome organization. We previously demonstrated that formation of one important chromatin domain, called heterochromatin, depends on phase separation (Strom et al., Nature 2017). I will describe the in vitro and in vivo evidence that supports this conclusion, discuss progress to date on elucidating the critical components responsible for LLPS of heterochromatin, and speculate about how phase separation and associated emergent properties could dramatically impact our understanding of genome organization, dynamics and functions.

MONDAY 11:00

Optoacoustic effect as the basic mechanism for intra-cochlear optical stimulation

N. Kallweit[1,5], P. Baumhoff[2], A. Krueger[1,5], D. Heinemann[1,5], A. Kral[2,5], L. Overmeyer[1,3], A. Heisterkamp[1,4,5], H. Maier[2,5], T. Ripken[1,5]

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Laser stimulation of the cochlea is discussed as a potential treatment for sensorineural hearing loss in recent years. It is contended whether the mechanism of optical stimulation is based on direct neuronal stimulation or on the optoacoustic effect. In previous studies, auditory responses due to laser light could only be detected in hearing animals or animals with residual hearing. In this study, *in vivo* experiments on guinea pigs as well as pressure and temperature measurements inside a model system were performed to analyze the stimulation mechanism, which is important for future applications. Results showed evident similarities between laser induced pressure amplitudes in the model system and *in vivo* measured compound action potentials (CAPs). The signal amplitude depends on the temporal characteristics of the laser pulses. These findings of the *in vivo*, temperature as well as pressure measurements confirm the hypothesis of optoacoustics as the underlying mechanism for optical intra-cochlear stimulation.

MONDAY 11:15

Lipoplex mediated gene therapy at molecular detail

Bart M. H. Bruininks, Paulo Cesar Telles de Souza and Siewert-Jan Marrink*

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The use on non-viral vectors for in vivo gene therapy could drastically increase safety, whilst reducing the cost of preparing the vectors. However, the transfection rate of non-viral vectors are low compared to their viral counterpart. A promising approach to non-viral vectors makes use of DNA/cationic liposome complexes to deliver its genetic material (lipoplexes). For such lipoplexes it is of utmost importance to escape the endosome before their genetic material gets degraded in the lysosome. Even though we know that some of these vectors perform better in this aspect than others, the details on how genetic material escapes the lipoplex and endosome are poorly understood. In this research we show how to build coarse grain molecular dynamics models for lipoplexes using the Martini force field, and use these models to perform fusion experiments at molecular detail. Our fusion experiments show that there are two kinetically distinct methods of fusion: direct and indirect fusion. Direct fusion shows efficient release of genetic material shortly after the initial fusion stalk is formed. Indirect fusion gets trapped at a well defined intermediate state. We also show that target membrane thickness, shape, unsaturation and phase separation, as well as the size and shape of the lipoplex, have a significant effect on the dominant fusion pathway. Since we designed our methods such, that they are easily expandable to other lipoplex formulations, we hope to stimulate a more rational design of these type of vectors. Paving the way to a safe and affordable clinical utilization of gene therapy.

MONDAY 11:30

Probing Molecular Transport in Printed Biomimetic Tissues

Manon Valet, Léa-Laetitia Pontani, Alexis Prevost and Elie Wandersman

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Cell-cell communication in biological tissues depends partly on the passive molecular transport through transmembrane channels. How does such diffusive transport lead to specific patterns in multicellular assemblies? We have recently addressed this question by studying the diffusive properties of fluorophores in biomimetic tissues. They consist of synthetic membranes known as Droplet Interface Bilayers (DIB) [1], connected by passive alpha-hemolysin pores. We have built linear arrays of DIBs using a recently developed droplet-on-demand technique [2]. We have measured the diffusion kinetics and its dependence with the pore concentration in the DIBs. Our results are fully captured by a model based on continuous time random walks and mean first passage times.

[1] – S. Leptihn *et al.*, *Nat. Protocols*, Vol. 8, 1048 (2013).
[2] – M. Valet, L.-L. Pontani, A. M. Prevost, E. Wandesman, *Phys. Rev. Applied,* Vol. 9, 014002 (2018).

MONDAY 11:45

Exploring cell-biology on a molecular level: New methods for live-cell and quantitative localization microscopy

Ulrike Endesfelder

Single-molecule microbiology research group, Department of Systems and Synthetic Microbiology, MPI for Terrestrial Microbiology & SYNMIKRO, Marburg, Germany,
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Light microscopy is one of the highest-impact tools in the life sciences as it underpins our fundamental knowledge on structure and behaviour of living organisms. By making use of the modern sub-diffraction fluorescence imaging methods, researchers today are able to analyse intracellular structures at a near-molecular resolution and to trace individual molecule dynamics [1].

Of these methods, single-molecule localization microscopy (SMLM) has quickly become a widely popular and powerful technique as its simple technical requirements have allowed many laboratories around the world an easy access to super-resolution microscopy. Thus, over the last years, a shift from proof-of-concept SMLM developments to comprehensive studies in biology and medicine took place. Nevertheless, this extensive and widespread use of SMLM has brought novel challenges and requirements to light, and there is still a high demand for robust and broadly applicable SMLM tools.

My group develops and applies advanced SMLM techniques for cell biology - interested in the *in situ* observation of molecular processes in living cells and in a fundamental understanding of how complex interdependencies of single molecules enable life.

Focusing on protein-DNA interactions, we mainly rely on two single-molecule applications - following transient and heterogeneous dynamics of inter-molecular interactions by single-molecule tracking (SMT), and exploiting structural SMLM imaging to precisely map the architecture of large multi-protein complexes.

In this talk, I will introduce some of our recently developed methodological tools alongside with our specific biological questions for two main topics: unraveling the molecular architecture and organization of the kinetochore complex regulating chromosome segregation in *Schizosaccharomyces pombe* and the target detection processes of CRISPR Cas systems:

First, I will present less phototoxic sptPALM imaging by using threonine 69 variants (e.g. Dendra2, mEos3.2-A69T) of the popular green-to-red photoconvertible fluorescent proteins (pcFPs) by primed photoconversion [2, 3]. This advantageously results in a new aberration-free, multi-color imaging scheme together with paFPs (e.g. PAmCherry) which we applied to various targets in fixed and live bacteria, yeast and mammalian cells and combined with correlative imaging methods [3].

I will also introduce a small peptide-tag of only 12 amino acids which is efficiently targeted by a nanobody. It allows for dense fluorophore labeling with minimal linkage errors and results in high-quality SMLM images while not interfering with the examined structures of interest [4].

Finally, I will discuss recent results on how SMLM imaging can be used to quantitatively measure *in vivo* DNA-probing and binding dynamics using our new tracking algorithm *swift* (*unpublished*).

[1] Turkowyd et al. (2016) *From single molecules to life - microscopy at the nanoscale*, Anal Bioanal Chem 408(25).

[2] Turkowyd et al. (2017) *A general mechanism of photoconversion of green-to-red fluorescent proteins based on blue and infrared light reduces phototoxicity in live-cell single-molecule imaging*, Angew Chem 56(38).

[3] Virant et al. (2017) *Combining Primed Photoconversion and UV-Photoactivation for Aberration-Free, Live-Cell Compliant Multi-Color Single-Molecule Localization Microscopy Imaging*, Int J Mol Sci 18(7).

[4] Virant et al. (2018) *A peptide tag-specific nanobody enables high-quality labeling for dSTORM imaging*, Nat Comm 9(1).

MONDAY 14:00

Control of Plant Development by Mechanical Signaling

Elliot Meyerowitz

Howard Hughes Medical Institute and Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, USA

My laboratory works to answer the question of how the stem cells of the Arabidopsis thaliana shoot apical meristem form developmental patterns. These include patterns of cell division, cell expansion, gene expression and floral primordium appearance; each of these originates in part due to cell-cell communication in the meristem. Some of the communication is chemical – for example communication between cells and meristem regions by apoplastic movement of peptides and hormones, and symplastic movement of proteins. Much of the communication, however, is physical, with mechanical interactions between meristem cells serving to control patterns of cell division, cell expansion, tissue shape, phyllotaxis, and also to regulate the flux of some of the chemical signals.

I will discuss two different modes of mechanical interaction. The first is cytoskeletal. The pattern of mechanical stresses in the meristem epidermis results from the shape of the meristem and the balance of turgor pressure and cell wall properties in the tissue. This stress pattern controls the microtubule cytoskeleton such that cortical microtubules align parallel to the maximal tensional stress direction when stress is anisotropic. Alignment of microtubules leads to subsequent alignment of cellulose in the cell wall, causing cells to expand anisotropically, leading to new stress patterns. Overall this mechanism allows cells to know the shape of the tissue in which they reside, and then to change that shape – thus providing a feedback between morphogenesis and cellular behavior.

The second mechanical signal results from local cell expansion, which changes patterns of stress in the shared walls between cells expanding at different rates due to their possession of different concentrations of the plant hormone auxin. This local stress causes asymmetric localization of an auxin transporter, which in turn changes the flow of auxin in the epidermis, and therefore changing the stress pattern. The feedbacks in involved in this mechanism control phyllotactic pattern.

Recent work points toward potential mechanisms for both mechanical feedbacks, and also demonstrates additional mechanical responses of meristem cells.

MONDAY 14:45

Force-controlled manipulation of single cells *in vitro*

Tomaso Zambelli

Laboratory of Biosensors and Bioelectronics, D-ITET, ETH Zurich (Switzerland)

FluidFM is a force-controlled nanopipette, combining AFM technology and microfluidics [1]. A fluidic channel is incorporated directly in a hollow AFM cantilever (Figure 1a). This channel ends in an aperture at the apex of the AFM tip, allowing for local dispensing of soluble molecules in air and in liquid, while retaining the inherent imaging capabilities and force feedback of an AFM system. We have just demonstrated the quantitative and subcompartmental femto-picoliter injection [2] and extraction [3] from single cells in vitro. In particular, we showed the integrity of proteins and transcripts as well as versatility of molecular analyses by high-resolution TEM imaging, minute enzyme assays and qPCR of cytoplasmic and nucleoplasmic extracts from distinct or even the same cell. Finally, an electrode can be implemented in the fluidic circuit enabling simultaneous force and ionic current measurements toward force-controlled patch clamp [4] and scanning ion conductance microscopy [5].

[1] A. Meister, M. Gabi, P. Behr, P. Studer, J. Vörös, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann, T. Zambelli, *Nano Lett,* Vol. 9, 2501-2507 (2009).
[2] O. Guillaume-Gentil, E. Potthoff, D. Ossola, P. Dörig, T. Zambelli, J.A. Vorholt, *Small,* Vol. 9, 1904-1907 (2013).
[3] O. Guillaume-Gentil, R.V. Grindberg, R. Kooger, L. Dorwling-Carter, V. Martinez, D. Ossola, M. Pilhofer, T. Zambelli, J.A. Vorholt, *Cell,* Vol. 166, 1663-1669 (2016).
[4] D. Ossola, M.Y. Amarouch, P. Behr, J. Vörös, H. Abriel, T. Zambelli, *Nano Lett.,* Vol. 15, 1743-1748 (2015).
[5] D. Ossola, L. Dorwling-Carter, H. Dermutz, P. Behr, J. Vörös, T. Zambelli, *Phys. Rev. Lett.,* Vol. 215, 175301 (2015).

MONDAY 16:45

Stress fibers and the cell cortex form a continuous contractile network

Timothée Vignaud[1], Calina Copos[3], Qingzong Tseng[1], Laurent Blanchoin[1,2], Alex Mogilner[3], Manuel Théry[1,2] and Laetita Kurzawa[1,2]

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2 Université Paris Diderot, CEA, INSERM, UMRS1160, Hopital Saint Louis, Institut Universitaire d'Hematologie, CytoMorpho Lab, F-75475, Paris, France.
3 Courant Institute and Department of Biology, New York University, New York, NY, USA.

Mechanical forces are key players in the regulation of cell and tissue morphogenesis. The magnitude and orientation of contractile forces direct cell shape and tissue architecture. Force production is determined by the amount of active myosin and the spatial arrangement of actin filaments. The contraction of the random meshwork forming the cell cortex powers global shape changes during cell migration or division. The alignment of filaments and the accumulation of myosins in stress fibers concentrate most of cellular contractile forces on their anchorage to the extracellular matrix. Whether and how these two networks may compete or synergize in the force generation process is still unknown.

In this study, we combined local ablation of contractile elements and global traction force measurements to investigate the specific contribution of each subcellular structure to the total force production and their mechanical interplay. Our results revealed that stress fibers were not only interconnected but also embedded in the cortical and cytoplasmic meshwork all along their length. The contraction of this surrounding meshwork contributes to a significant part of the total traction force on cell anchorages. Their connection also dissipates and transmits the forces produced along the stress fiber throughout the cell.

Far from being independent, stress fibers and surrounding cortical and cytoplasmic meshworks thus appeared to form a continuous network, exchanging filaments and pulling on each other, thereby enforcing a global integration of contractile forces at the cell level.

MONDAY 17:30

Fluorescence lifetime techniques for surgical imaging, guidance and augmented reality

Laura Marcu

Department of Biomedical Engineering, University of California Davis, USA

This presentation overviews fluorescence lifetime spectroscopy and imaging techniques for label-free *in vivo* characterization of biological tissues. Numerous studies have shown that tissue autofluorescence properties have the potential to assess biochemical features associates with distinct pathologies in tissue and to distinguish various cancers from normal tissues. However, despite these promising reports, autofluorescence techniques have been sparsely adopted in clinics. Moreover, when adopted they were primarily used for pre-operative diagnosis rather than surgery guidance. This presentation overviews clinically-compatible multispectral fluorescence lifetime imaging (FLIM) techniques developed in our laboratory and their ability to operate as stand-alone tools, integrated in a biopsy needle and in conjunction with the da Vinci surgical robot. We present clinical studies in patients undergoing surgery that demonstrate the potential of these techniques for intraoperative delineation of brain tumors and brain radiation necrosis as well as head and neck cancer including image-guided augmented reality during trans-oral robotic surgery (TORS). Challenges and solutions in the clinical implementation of these techniques are discussed.

TUESDAY 09:00

Blistering and buckling in epithelial monolayers

Guillaume Charras[1,2]

[1] *London Centre for Nanotechnology, University College London, UK*
[2]*Department of Cell and Developmental Biology, University College London, UK*

Epithelia are planar tissues, separating the internal environment from the external environment in many organs. Epithelia are subjected to mechanical perturbations that vary greatly in magnitude and timescale during development, normal physiological function and regeneration. The resulting tissue deformations can have a profound impact on epithelial cell biology. Indeed, a reduction in the area of an epithelium attached to a flexible substrate triggers cell cycle arrest, cell extrusion and cell differentiation. However, the relationship between tissue shape, area and mechanical state remains poorly understood. Here, using suspended monolayers, we show that epithelial tissues respond to the rapid application of in-plane compressive strains by buckling followed by flattening over the course of two minutes. Our research shows that resting tension provides epithelia with a mechanism to buffer area changes during physiological function in organs, while the generation of permanent folds may play a role in developmental morphogenesis.

Another major epithelial function is the directed absorption and excretion of nutrients, water, and ions in a process known as vectorial transport. When cultured on impermeable substrates, vectorial transport generates multicellular blisters. We show that blisters arise through the accumulation of fluid into progressively larger dynamic fluid pockets trapped between the cells and the impermeable substrate leading to sub-cellular, cellular, and multi-cellular blisters. By examining the evolution of the average blister size, we show that dome growth belongs to a common class of coarsening phenomena known as Ostwald ripening that underlies the evolution of droplet size in emulsions.

TUESDAY 09:45

Watching individual proteins unfold and refold by 1-μs resolution force spectroscopy

Thomas T. Perkins

JILA, National Institute of Standards and Technology and the University of Colorado, Dpts of Physics and of Molecular,Cellular,and Developmental Biology, University of Colorado, Boulder, USA

Protein folding occurs as a set of transitions between structural states within an energy landscape. An oversimplified view of the folding process emerges when transiently populated states are undetected because of limited instrumental resolution. To achieve state-of-the-art performance, we integrated several recent technical advances that improve the precision, stability, and accuracy of AFM-based single molecule force spectroscopy. Using modified cantilevers optimized for 1-μs resolution, we reexamined the unfolding of individual bacteriorhodopsin (bR) molecules in native lipid bilayers. The experimental data revealed the unfolding pathway in unprecedented detail. Numerous newly detected intermediates—many separated by as few as 2–3 amino acids—exhibited complex dynamics, including frequent refolding and state occupancies of <10 μs. Equilibrium measurements between such states enabled the folding free-energy landscape to be deduced. These results sharpen the picture of the mechanical unfolding of bR. Finally, recent efforts to improve the quantity and quality of AFM studies of diverse biomolecules, including nucleic-acid structures and globular proteins, will be discussed.

TUESDAY 11:00

The Cyphochilus beetle as an inspiration for sustainable white materials

Gianni Jacucci[1], Olimpia D. Onelli[1], Julia Syurik[2], Hendrik Hölscher[2], Olli Ikkala[3], and Silvia Vignolini[1]

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Whiteness arises when light interacts with disordered media, where different wavelengths are scattered with comparable intensity. Such appearance is the result of light undergoing multiple scattering events before exiting the object, i.e. when the object is optically thick. The optical thickness of a material is determined by the ratio between its physical thickness and the transport mean free path, namely the distance that light travels before losing information about its starting propagation direction. Commonly, the transport mean free path in low-refractive index white materials is about tens of micrometres long. Therefore, opacity is achieved for relatively large thicknesses (in the millimetres range) to allow a high enough number of scattering events. **[1]**

Nature provides an invaluable source of inspiration for the study and the manufacturing of thin opaque white materials. The *Cyphochilus* white beetle achieves a high total reflectance (~ 75% over the whole visible range) with

a few micron thick, lightweight, anisotropic network of chitin fibres ($n_c \approx 1.55$). [2,3,4]

Herein, after quantifying the scattering efficiency of the chitin network *via* a coherent backscattering setup, [5] we show an experimental approach to produce bio-inspired, sustainable white materials. [6,7] In particular, we demonstrate that tuning the morphology of a network of polymer fibres strongly affects its optical properties: from transparent, to bright white materials. Notably, our bio-inspired materials achieve high scattering efficiency whilst being only a few micrometres thick (up to 75% reflectance while only 4 μm thick). Our study illustrates the potential of using biopolymers as building blocks to produce next-generation sustainable and biocompatible highly scattering materials. [6,7] In addition, we show that it is possible to manipulate the light transport regime, moving from standard to anomalous diffusion, when a long-tailed distribution of the fibres size is introduced. [7]

[1] S. Caixeiro, M. Peruzzo, O. D. Onelli, S. Vignolini, and R. Sapienza, ACS Applied Materials & Interfaces 2017, 7885.
[2] B. D. Wilts, X. Sheng, M. Holler, A. Diaz, M. Guizar-Sicairos, J. Raabe, R. Hoppe, S.-H. Liu, R. Langford, O. D. Onelli, D. Chen, S. Torquato, U. Steiner, C. G. Schroer, S. Vignolini, A. Sepe, *Adv. Mater.* 2017, 1702057.
[3] M. Burresi, L. Cortese, L. Pattelli, M. Kolle, P. Vukusic, D. S. Wiersma, U. Steiner, S. Vignolini, Sci. Rep. 2014, 4, 6075.
[4] L. Cortese, L. Pattelli, F. Utel, S. Vignolini, M. Burresi, and D.S. Wiersma, Adv. Opt. Mater. 2015, 3: 1337-1341.
[5] O. L. Muskens and A. Lagendijk, Opt. Express 2008 16, 1222.
[6] J. Syurik*, G. Jacucci*, O. D. Onelli, H. Hölscher, S. Vignolini, *Adv. Funct. Mater.* 2018, 1706901.
[7] M. S. Toivonen*, O. D. Onelli*, G. Jacucci, V. Lovikka, O. J. Rojas, O. Ikkala, S. Vignolini, *Adv. Mater.* 2018, 1704050

TUESDAY 11:15

Brillouin microscopy to probe the micromechanics of tissue phantoms

M. Bailey[1], N. Correa[1], M. Alunni Cardinali[2], S. Caponi[3], T. Holsgrove[4], C. P. Winlove[1], D. Fioretto[2] and F. Palombo[1]

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The micromechanical properties of gelatin hydrogels as tissue phantoms to mimic the extracellular matrix have been investigated using Brillouin microscopy and quasi-static compressive testing. The Brillouin response of hydrogels with 0–18% w/w protein concentration was measured and the effect of adding varying concentrations of a cross-linker, formalin, commonly used in histological protocols, was studied. A gradual blue-shift in the Brillouin peak position was observed as both protein and cross-linker concentrations were increased, suggesting an increase in stiffness (longitudinal modulus in the GHz range). Longitudinal and Young's moduli were found to be orders of magnitude apart, indicating that the gels are viscoelastic. The lack of structure in these materials make it possible to characterise their multiscale biomechanics without the interference from hierarchical architectures [1-2].

[1] Edginton RS, Green EM, Winlove CP, Fioretto D, Palombo F. (2018) Dual scale biomechanics of extracellular matrix proteins probed by Brillouin scattering and quasistatic tensile testing, *Proc. SPIE 10504, Biophysics, Biology and Biophotonics III: the Crossroads*, 105040J.
[2] Palombo F, Winlove CP, Edginton RS, Green E, Stone N, Caponi S, Madami M, Fioretto D. (2014) Biomechanics of fibrous proteins of the extracellular matrix studied by Brillouin scattering, *Journal of The Royal Society Interface*, **11**(101):20140739.

TUESDAY 11:30

The effect of twist-bend coupling on DNA mechanics

Stephanos. K. Nomidis[1,2] , E. Skoruppa[1] , M. Caraglio[1] , E. Carlon[1]

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The mechanical properties of DNA play a key role in its function within the cell, such as its interaction with proteins. The standard elastic model describing DNA, the twistable worm-like chain (TWLC), treats the bending and twisting degrees of freedom as independent deformations. However, it has been suggested that the groove asymmetry of DNA calls for a refinement of the TWLC, leading to a coupling between the two degrees of freedom. 1 Here we numerically confirm that prediction, 2 and proceed to investigate the consequences of twist-bend coupling through a combination of analytical calculations, computer simulations and experiments. In particular, we show that at long length scales, i.e. for thousands of base pairs, this interaction leads to a non-trivial modification of the overall mechanical behaviour of DNA. 3 In the other limit of short scales, i.e. for 10-100 base pairs, we consider the distinct cases of DNA minicircles and nucleosome, and show that the coupling leads to some surprising similarities. 4 Finally, we anticipate its relevance in other DNA/protein complexes, as well as in the dynamics of DNA supercoiling.

[1] J. F. Marko, E. D. Siggia, *Macromolecules* , Vol. 27, Issue 4, 981-988 (1994).
[2] E. Skoruppa, M. Laleman, S. K. Nomidis, E. Carlon, *J. Chem.Phys.,* Vol 146, Issue 21, 214902 (2017).
[3] S. K. Nomidis, F. Kriegel, W. Vanderlinden, J. Lipfert, E. Carlon, *Phys. Rev. Lett.* , Vol 118, Issue 21, 217801 (2017).
[4] E. Skoruppa, S. K. Nomidis, J. F. Marko, E. Carlon, *arXiv preprint,* arXiv: 1801.10005 (2018).

TUESDAY 11:45

Isolated Nucleic Acids: Mass Spectrometry, Ion Mobility Spectrometry, and Ion Spectroscopy

Valérie Gabelica

Université de Bordeaux, INSERM & CNRS, Laboratoire Acides Nucléiques : Régulations Naturelle et Artificielle (ARNA, U1212, UMR5320), IECB, F-33600 Pessac, France

Studying nucleic acids biophysics in solution is often complicated because the folding of nucleic acid polyanions is strongly coupled to their interactions with cations, small molecules and proteins. Electrospray ionization sources make it possible to transfer nucleic acid complexes from the solution to the gas phase, where they can be separated according to their mass, and thus stoichiometry. Ion mobility spectrometry and ion spectroscopy can then bring further information on the secondary and tertiary structures of these gas-phase nucleic acid complexes. I will review here the latest advances in this field, from fundamental questions to applications in biophysics.

TUESDAY 14:00

Spatial and temporal variations in cell hydrostatic pressure during plant development

Arezki Boudaoud

Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA

Growth of a plant cell is determined by a delicate balance between the cell turgor pressure and the wall. Considerable attention has been given to cell wall composition and mechanics, whereas variations in turgor pressure have been overlooked. In order to measure pressure during development, we set up an approach combining indentation experiments and mechanical models of cell and tissue mechanics, which we applied to the developing seed and to the shoot apex in the model plant Arabidopsis. First, we demonstrated that the mechanical behaviour of the seed is consistent with the seed coat being in tension generated by endosperm-derived turgor pressure which drives seed expansion. We found that seed growth arrest is associated with a drop of endosperm turgor pressure. Second, we observed heterogeneous hydrostatic pressure in the epidermis of the shoot apex, which, surprisingly, correlates either positively or negatively with cellular growth rate, depending on conditions. We ascribe these spatial variations to local geometry and topology of the tissue, by combining experimental observations with a poroviscoplastoelastic model of tissue growth. Altogether, our results suggest developmental roles for temporal and spatial variations in turgor: growth arrest of the seed and cell size homeostasis for the shoot apex.

TUESDAY 14:45

Charge regulation of complex (bio)colloids

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Charge regulation, implying a variable response of dissociable charge groups on solvent exposed surfaces of macromolecules based on internal non-electrostatic degrees of freedom, is a quintessential feature of polyelectrolytes in biological systems. This applies to bulk protein solutions as well as proteinaceous aggregates such as viral shells and enzymatic nanocontainers, providing scaffolding and compartmentalization for chemical engineering on the nano-scale. Charge regulation alters the behavior of chargeable colloidal systems by fundamentally modifying the standard PB (Poisson-Boltzmann) paradigm. I will describe several of these modifications: (i) alterations of not only in the net charge but also in the direction and strength of all higher multipoles of single proteins; (ii) invalidating the usual assumptions of multipolar expansion in ionic solutions; (iii) emergence of fluctuational Kirkwood-Schumaker interactions; (iv) spontaneous spatial symmetry breaking of electrostatic fields of charge-regulated macromolecules, consequently contradicting one of the fundamental assumptions of the PB theory; (v) charge regulation of complex fluids with mobile macro-ions leads to positional dependence of the effective charge of the macro-ions and a non-monotonic dependence of the effective Debye screening length. These new developments embed the PB paradigm into an entirely new perspective.

[1] Nataša Adžić and Rudolf Podgornik, Phys. Rev. E 91, 022715 (2015).
[2] Anze Losdorfer Bozic and Rudolf Podgornik, Biophys. J., Vol 113, 1454–1465 (2017).
[3] Tomer Markovich, David Andelman and Rudolf Podgornik, Europhys. Letts., Vol. 120 26001 (2017).
[4] Arghya Majee, Markus Bier and Rudolf Podgornik, Soft Matter, Vol. 14, 985 (2018).
[5] Yael Avni, Tomer Markovich, Rudolf Podgornik and David Andelman, Soft Matter, (2018).[6] Anze Losdorfer Bozic and Rudolf Podgornik, J. Chem. Phys., Vol.149, 163307 (2018).

TUESDAY 16:45

Epigenetic dynamics on chromatin in 3D

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One of the most important problems in development is how epigenetic patterns of biochemical histone modifications can first be established, and then stably inherited, within cell lines. To address this question, we propose a polymer model that couples three-dimensional chromatin folding dynamics to a "recoloring" process modelling the writing of epigenetic marks. This baseline model harbours a sharp transition between a swollen and epigenetically disordered phase and a globular epigenetically coherent one. It also explains a possible biophysical basis for the pheomenon of epigenetic memory and recapitulates the ultrasensitive response of epigenetic switches to perturbation. The model can be developed further by adding genomic bookmarking, yielding stable epigenetic patterns which are similar to those seen in vivo in Drosophila. Finally, we show that coupling transcriptional and epigenetic dynamics provides another avenue to create epigenetic domains, associated with 3D structures reminiscent of transcription factories and hubs of active genes.

TUESDAY 17:30

Regulation of chromosome mechanics by a surfactant-like protein

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The reorganization of chromosomes into spatially well-separated bodies is a hallmark of mitosis. We have previously reported that Ki-67, a component of the mitotic chromosome periphery, prevents chromosomes from collapsing into a single chromatin mass after nuclear envelope disassembly. The very large size of the Ki-67 protein, its apparent lack of secondary structure, and high electrical charge suggest that it might function as a steric and electrical barrier, similar to surface-active agents (surfactants) that disperse particles or phase-separated liquid droplets in solvents. Consistent with this idea, dual-color labeling of both protein termini showed that Ki-67 forms densely-grafted polymer brushes as a steric barrier between mitotic chromosomes. In the present study, we hypothesized that this steric barrier facilitates independent chromosome movement during early stages of mitosis, whereas it might be inactivated during mitotic exit to promote merging of all chromosomes into a single nucleus. Indeed, we found that Ki-67 brushes collapse upon exit from mitosis, suggesting that Ki-67's surfactant activity is cell cycle regulated. Coincident with Ki-67 brush collapse, we found that chromosomes coalesced into a single chromatin mass, where they ceased to move independently. The chromosome merge preceded the first interaction with nuclear membranes and might hence shape the surface for nuclear assembly. Our study raises the interesting possibility that upon inactivation of Ki-67 during mitotic exit the surface of mitotic chromosomes changes from repulsive to adhesive, which has potential implications for nuclear assembly.

WEDNESDAY 09:00

Exploring the variability of nucleosome conformation *in vitro* and *in situ* using cryoEM of vitreous sections

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In Eukaryotes, DNA is wound into a left-handed superhelix around the histone octamer forming the basic chromatin unit, the nucleosome. Atomic structures have been obtained from crystallography and single particle cryo electron microscopy of identical engineered particles. But nucleosomes are dynamical entities and native particles have diverse DNA sequence and histone content. Little is known about their conformational variability, especially in the cellular context.

By using cryo electron microscopy and tomography of vitreous sections it is possible to visualize individual nucleosomes within interphase nuclei, at a level of detail sufficient to measure the distance between the DNA gyres of the super helix. Multiple conformations are found, on average more open than the canonical crystallographic structure.

A simple model system, concentrated solutions of isolated nucleosome core particles, let us analyse the influence of several parameters (particle concentration, local order and ionic environment). We demonstrate a salt-dependent transition, with a high salt compact conformation resembling the canonical nucleosome, and an open low salt one, closer to nuclear nucleosomes. Although further particle characterisation and cartography are needed to understand the relationship between this conformational variability and chromatin functional states, this approach opens a route to chromatin exploration *in situ*.

WEDNESDAY 09:45

Isomorphic and Isofunctional Fluorescent Nucleosides, Nucleotides and Oligonucleotides

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Nucleic acids experience a variety of perturbations, which include strand cleavage and ligation, local conformational changes, base damage, modification and flipping, as well as structural and environmental perturbations that are induced upon protein and low MW ligand binding. Additionally, nucleosides and nucleotides are involved in numerous biochemical transformations, as well as signaling and regulatory processes. Isomorphic responsive fluorescent nucleoside analogues, which can serve as faithful surrogates of their native counterparts, are powerful probes for investigating nucleic acids structure, dynamics, recognition and damage as well as metabolic processes involving nucleosides/tides.[1–7] The lecture will present the design, synthesis and photophysical properties of new fluorescent isomorphic nucleoside analogues as well as their utilization for the fabrication of "real-time" fluorescence-based discovery and biophysical assays.

[1] D. Shin, R. W. Sinkeldam, Y. Tor, *J. Am. Chem. Soc.,* Vol. 133, 14912–14915 (2011).
[2] R. W. Sinkeldam, L. S. McCoy, D. Shin, Y. Tor, *Angew. Chem. Int. Ed*., Vol. 52, 14026–14030 (2013).
[3] W. Liu, D. Shin, Y. Tor, B. S. Cooperman, *ACS Chem Biol.,* Vol. 8, 2017–2023 (2013).
[4] A. R. Rovira, A. Fin, Y. Tor, *J. Am. Chem. Soc.,* Vol. 137, 14602–14605 (2015).
[5] A. R. Rovira, A. Fin, Y. Tor, *J. Am. Chem. Soc.***,** Vol. 139*,* 15556–15559 (2017).
[6] Y. Li, A. Fin, L. McCoy, Y. Tor, *Angew. Chem*. *Int. Ed.,* Vol. 56, 1303–1307 (2017).
[7] F. Hallé, A. Fin, A. R. Rovira, Y. Tor, *Angew. Chem. Int. Ed.*, Vol. 57, 1087–1090 (2018).

WEDNESDAY 11:00

Shape and cytoskeleton organization in isolated plant cells

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Despite the significance of cellular morphology as a functional phenotype, it remains challenging to quantitatively relate morphological phenotype to the behavior of subcellular molecules. Molecular studies have identified many components controlling cell morphogenesis, but it is unclear how this information is translated into the physical world. In plant cells, growth requires synthesis of cytoplasmic components as well as expansion of the cell wall. The cell wall is a stiff yet flexible polymeric network that encapsulates cells and counterbalances stress created by turgor pressure inside the cell, thereby controlling cell shape. It is now well established that the cytoskeleton plays a key role in the biogenesis and morphogenesis of the cell wall. While the microtubules guide cellulose synthase complex movement1, the actin network is responsible for global distribution of cellulose synthase complexes 2. It is also suggested that mechanical stresses orient the microtubules along their principal direction 3. Nevertheless, to fully understand how plant cells are shaped and how external mechanical stresses influence this process, a quantitative approach to evaluate the mechano-response in single cells needs to be established.

Here we present a technique to confine single plant protoplasts into molds of defined shapes. The protoplasts are then monitored with a confocal microscope to evaluate changes in cytoskeletal organization and dynamics

during the process of symmetry breaking. These experiments are the basis of assessing quantitatively how different shapes control cytoskeleton organization behavior by regulating the distribution of physical stresses.

1. Paredez, A. R., Somerville, C. R. & Ehrhardt, D. W. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312,** 1491–5 (2006).
2. Gutierrez, R., Lindeboom, J. J., Paredez, A. R., Emons, A. M. C. & Ehrhardt, D. W. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nat. Cell Biol.* **11,** 797–806 (2009).
3. Hamant, O. *et al.* Developmental patterning by mechanical signals in Arabidopsis. *Science* **322,** 1650–5 (2008).

WEDNESDAY 11:15

Direct measure of thickness and dynamic of the cell cortex

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Cell migration is central to many biological and physiological processes and happens in a variety of way. The different actin networks of the cell play different roles in migration either by actively generating forces or by influencing the mechanical properties of the whole cell. In the case of confined migration the cell cortex is often compressed between the outside elements (ECM, other cells…) and the cell nucleus. It has been shown that in constricted microchannels mimicking such environments a perinuclear branched actin network can start polymerizing [1]. Furthermore in vitro experiments on branched actin network have shown a response to confinement in both mechanical properties and polymerization dynamics [2]. Recently the observation of actin waves in 3D [3] also asks questions about the formation and physiological relevance for migration of such complexes structures of branched actin networks.

We developed a new tool to study the behavior of the cortex to understand these different types of activities and the mechanics behind

complexes actin structures. We use super-paramagnetic beads under a controlled magnetic field: in this situation, the beads develop their own dipolar moment and are attracted to each other with a known force [4]. Thanks to the macropinocytosis ability of dendritic cell we can create a system where we have one bead inside the cell and one outside. We can thus confine the membrane and the cortex between these beads and track their position with a precision of few tens of nanometers.

This system allows for different measurements and tests upon the cell cortex. Due to the precision available in the tracking of the beads we can measure the thickness of the cortex at different levels of confinement. By combining our system with fluorescent microscopy we can observe a confined portion of cortex for signs of actin polymerization due to confinement. But we also record what seems to be the passage of actin waves between the beads. We can thus study the cortex and its dynamical features in different cases such as with various compressing forces or with drugs to affect the biochemical composition of the cortex.

[1] H. R. Thiam et al. - Nat Com 2016
[2] P. Bieling et al. – Cell 2016
[3] Fritz-Laylin et al. – bioRxiv preprint 2017
[4] T. Pujol et al. – PNAS 2012

WEDNESDAY 11:30

Mechanical constraints in the morphogenesis of the gastrovascular system of the jellyfish *Aurelia aurita*

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The jellyfish *Aurelia aurita* is equipped with a gastrovascular system which displays a branched pattern spanning the endodermal cells monolayer. It develops by radial centripetal growth from the rim and connection to neighbouring vessels. This network is an interesting model for describing the growth of a network in a growing and contractile environment: it is planar and relatively simple, the animal body grows homogeneously up to 30% per day, and the swimming movement is periodic and easy to describe due to rotational symmetry.

We hypothesize that the canals growth and reconnections are triggered by the repeated mechanical constraints due to the swimming movement, in analogy with cracks propagation. We have acquired timelapse series of the canals growth and films of the swimming movement. We quantified the pattern's geometry and topology on the timelapse series and the local deformations in the films. We showed that the topologies can be very diverse from one individual to the other, and that the swimming movement does not deform all regions of the endoderm homogeneously. This heterogeneity could explain privileged locations for reconnections. This would be an argument in favour of an example of mechanics driven morphogenesis in a basally branching Metazoan.

WEDNESDAY 11:45

Mechanical communication in cardiac cell beating and in the sensory nervous system

Shelly Tzlil

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Cell-cell communication enables cells to coordinate their activity and is essential for growth, development and function. Intercellular communication is discussed almost exclusively as having a chemical or an electrical origin, however; recent experiments demonstrate that cells can communicate mechanically by responding to mechanical deformations generated by their neighbors. The characteristics of mechanical communication, its role and its ability to regulate biochemical processes within the cell are still largely unknown.

In this talk, I will describe the progress made in our lab in understanding the role of mechanical communication in cardiac cell beating and in the sensory organ of the fly.

We have recently shown that an isolated cardiac cell can be trained to beat at a given frequency by mechanically stimulating the underlying substrate using a 'mechanical cell'. The 'mechanical cell' consists of an oscillatory probe that mimics the mechanical aspect of a cell by generating substrate deformations identical to the ones induced by a neighboring beating cell. Cardiac cell beating is stochastic and beating cells can go in and out of phase even if beating at the same average frequency. Therefore, continuing on this work, we study the regulation of beating noise by mechanical coupling and the influence of beating stochasticity on cardiac cell pacing. We measure the mechanical interaction between neighboring cells and show that beat-to-

beat variability decays exponentially with mechanical coupling and that noise reduction by mechanical coupling is sensitive to the stochastic nature of the ‘master’ cell. Both pacing and noise reduction persist long after mechanical stimulation stops, implying that mechanical communication induces changes in the biochemical network kinetics that governs spontaneous beating in cardiac cells. By quantitatively measuring the reduction of noise with mechanical coupling strength, we could identify microtubule integrity, NOX2 and CaMKII as key mediators of mechano-transduction.

We also explore the role of the mechanical properties of the connective tissue on muscle-neuron mechanical coupling using the sensory organ of the fly as a model system. Changes in ECM composition, alter the viscoelastic properties of the sensory organ and consequently interfere with the propagation of mechanical deformations and proper sensing.

WEDNESDAY 14:00

Tuning mechanosensing at the membrane interface by assymmetry of transbilayer pressure profile

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Efficient mechanotransduction processes occur in living organisms via mechanosensors or mechanoreceptors linking external mechanical stimuli at the cell surface to intracellular signaling events and downstream effectors. Mechanosensitive (MS) ion channels are among the fastest primary mechanosensors converting mechanical stimuli into intracellular signals on a submillisecond time scale. Much of our understanding of the biophysical principles that underlie and direct conversion of mechanical force into conformational changes in MS channels comes from studies based on MS channel reconstitution into lipid bilayers. My talk focuses on close interactions between MS channels and the lipid bilayer and discusses the central role that transbilayer pressure profile plays in mechanosensitivity and gating of these fascinating membrane proteins.

WEDNESDAY 14:45

Exploring the molecular landscape of *Chlamydomonas* with *in situ* cryo-electron tomography

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Cells accomplish the biochemical reactions of life by concentrating their proteins into a variety of subcellular compartments called organelles. Our group explores the relationship between the *form* of the organelle and the *function* of its resident macromolecules. How does organelle architecture direct molecular function, and reciprocally, how do macromolecules sculpt and shape organelles? To investigate these questions, we use focused ion beam (FIB) milling of frozen cells followed by cryo-electron tomography to image macromolecules within their native cellular environment. Through a combination of nanometer-precision localization and high-resolution structural analysis, we aim to chart the molecular landscapes of organelles.

Thanks to its superb cryo-EM contrast and textbook organelle architecture, the unicellular green alga *Chlamydomonas* is an ideal specimen for this approach. We have taken a holistic approach to survey the whole integrated "planimal", with *in situ* molecular studies of the nuclear envelope, ER, Golgi, basal body apparatus (centrioles), and chloroplast. In this talk, I will provide an overview of some of these studies, touching on proteasome-rich degradation centers [1], the nuclear pore complex [2], COPI coats [3], IFT

train assembly, centriole structure, and the molecular organization of chloroplast's thylakoid membranes and pyrenoid [4].

[1] S. Albert, M. Schaffer, F. Beck, S. Mosalaganti, S. Asano, H.F. Thomas, J.M. Plitzko, M. Beck, W. Baumeister, B.D. Engel, *Proc Natl Acad Sci U S A,* Vol. 114, 13726-13731 (2017).
[2] S. Mosalaganti, J. Kosinski, S. Albert, M. Schaffer, D. Strenkert, P.A. Salomé, S.S. Merchant, J.M. Plitzko, W. Baumeister, B.D. Engel, M. Beck. *Nature Communications*, Vol. 9, 2361 (2018).
[3] Y.S. Bykov, M. Schaffer, S.O. Dodonova, S. Albert, J.M. Plitzko, W. Baumeister, B.D. Engel, J.A. Briggs. *Elife*, Vol. 6, e32493 (2017).
[4] E.S. Freeman Rosenzweig, B. Xu, L. Kuhn Cuellar, A. Martinez-Sanchez, M. Schaffer, M. Strauss, H.N. Cartwright, P. Ronceray, J.M. Plitzko, F. Förster, N.S. Wingreen, B.D. Engel, L.C.M. Mackinder, M.C. Jonikas. *Cell*, Vol. 171, 148-162.e119 (2017).

WEDNESDAY 16:00

The Two-State World View as Biology's Greatest Model

Rob Phillips

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Only ten years after the discovery of the iconic structure of DNA, new questions were on biologist's minds, namely, how are the macromolecules of the cell regulated so that they do what they are supposed to when and where they are needed? The initial resolution of the challenging question of biological regulation came in the form of the notion of "allostery", an idea that its discoverer Jacques Monod himself referred to as "the second secret of life". We recently celebrated the 50th anniversary of the classic paper of Monod, Changeux and Jacob that introduced this far reaching idea. That important paper was followed shortly thereafter by a second one that revealed their musings on how simple statistical mechanical models can be used to capture how such allosteric transitions work mechanistically. In this talk, I will review the key features of the famed Monod-Wyman-Changeux (MWC) model and then describe its broad reach across many different domains of biology with special reference to the physics of ion channels and how genes are turned on and off. One of the intriguing outcomes of this class of models is a beautiful and predictive scheme for collapsing data from entire libraries of mutants. Once we have considered some of the traditional uses of the MWC model, I will turn to more speculative recent ideas which use the MWC approach to consider the nature of kinetic proofreading.

WEDNESDAY 16:45

Is Extracellular Matrix a pressure sensor?

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Growth of solid tumors occurs in a constrained environment and requires a competition for space. This results in a bidirectional mechanical coupling between the tumor and its close environment, the stroma. The expanding neoplastic tissue compresses the stroma, thus builds up, and stores an internal stress; in parallel, the stroma is contractile and exerts a mechanical stress on the tumor.

To evaluate the effect of such a stress, we grow multicellular aggregates made of cancer cells under a controlled mechanical pressure. We observe that a gentle compression (500 Pa) drastically reduces the growth rate of spheroids made of cancer cells [1]–[3], whereas it has no impact on the same cells, when grown individually.

A major difference between individual cells and multicellular aggregates is the presence of Extracellular Matrix (ECM) in the multicellular context. Contrarily to cells, which are almost incompressible, the ECM is permeable to water and highly compliant. Thus, a gentle compression generates a much larger strain in the ECM than in the cells. Thus, we model and characterize the compressibility and the permeability of composite aggregate, made of cells and ECM.

We find that ECM properties mainly determine the mechanical response of a multicellular aggregate to external stimuli. In addition, we observe that the ECM compression has a large impact on cell proliferation, migration and morphology. We conclude that the ECM play the role of a pressure sensor in a multicellular context.

[1] F. Montel *et al.*, "Stress clamp experiments on multicellular tumor spheroids," *Phys. Rev. Lett.*, vol. 107, no. 18, pp. 1–4, 2011.

[2] M. Delarue, F. Montel, D. Vignjevic, J. Prost, J. F. Joanny, and G. Cappello, "Compressive stress inhibits proliferation in tumor spheroids through a volume limitation," *Biophys. J.*, vol. 107, no. 8, pp. 1821–1828, 2014.

[3] M. E. Dolega, M. Delarue, F. Ingremeau, J. Prost, A. Delon, and G. Cappello, "Cell-like pressure sensors reveal increase of mechanical stress towards the core of multicellular

spheroids under compression," *Nat. Commun.*, vol. 8, no. May 2016, pp. 1–9, 2017.