Sunday, Sept. 17:

02:30-04:00pm **Opening Remarks**: Satyajit Mayor, NCBS, India

Research Lecture 1: Jan Ellenberg, EMBL, Heidelberg

Location: Happus, LH1 (Eastern Laboratory Complex)

04:15-05:30pm **Didactic Lecture 1:** Basics of Microscopy**,** Köhler illumination and Resolution, Diffraction. - Rahul Roy, IISc, India
Location: Happus, LH1 (Eastern Laboratory Complex)

Wednesday, Sept. 20:

09:30-10:30am **Research Lecture 2:** Jennifer Ross, UMass Amherst, USA

Location: Malgova

05:00-06:00pm **Research Lecture 3:** Clare M. Waterman, NIH/NHLBI, USA

Location: Happus (LH1)

Friday, Sept. 22:

09:30- 10:30am **Research Lecture 4:** Sudipta Maiti, TIFR, Mumbai

Location: Happus (LH1)

05:00-06:00pm **Research Lecture 5:** Satyajit Mayor**,** NCBS, India
Location: **Happus** (LH1)

Saturday, Sept. 23:

09:00-10:00am **Research Lecture 6:** Takahiro Fujiwara, Kyoto University, Japan
Location: **Happus (LH1)**

10:00-11:00am **Research Lecture 7:** Rahul Roy, IISc, India
Location: **Happus (LH1)**

Jennifer Ross

Abstract

Weak and Transient Interactions have Big Effects for Intracellular Transport

The cell is a complex machine taking in information, performing computations, and responding to the environment. To enable agile read/write capabilities, much of the molecular biochemistry that performs these computations must be transient and weak, but perhaps numerous and coupled to enable high activity. Traditionally, biochemical interactions that we study in the test tube have examined strongly interacting systems because we perform our experiments in dilute solutions over long times. I will present excerpts from stories where many weak, transient interactions can have strong effects on the overall activity and can, in fact, overpower strongly interacting systems. These studies involve the microtubule cytoskeleton and the transport motor, kinesin-1. These examples will set the stage for proposing to study weak, transient interactions so that we can elucidate their fundamental activities and ultimately learn how to engineer them to create biological or biomimetic devices.

Clare M. Waterman

Abstract

Actin retrograde flow actively aligns and orients ligand-engaged integrins in focal adhesions.

Integrins are transmembrane receptors that, upon activation, bind extracellular ligands and link them to the actin cytoskeleton to mediate cell adhesion and migration. Cytoskeletal forces in migrating cells generated by polymerization or contractility drive "retrograde flow" of actin from the cell leading edge have been hypothesized to mediate integrin activation for ligand binding. This predicts that these forces should align and orient activated, ligand-bound integrins at the leading edge. Here, polarization-sensitive fluorescence microscopy of GFP-αVβ3 integrins in fibroblasts shows that integrins are co-aligned in a specific orientation within focal adhesions (FAs) in a manner dependent on binding immobilized ligand and a talin-mediated linkage to the actin cytoskeleton. These findings, together with Rosetta modelling, suggest that integrins in FA are co-aligned and may be highly tilted by cytoskeletal forces. Thus, the actin cytoskeleton sculpts an anisotropic molecular scaffold in FAs, and this feature may underlie the ability of migrating cells to sense directional extracellular cues.

Sudipta Maiti

Abstract

Nanosecond fluctuations as a route to designing ligands for structure-less proteins

How do we design a drug for an intrinsically disordered protein (IDP), which has no well-defined structure? This an important unsolved challenge in Biophysics, since many incurable diseases are associated with such disordered proteins. Here we suggest that dynamics of spontaneous fluctuations of the disordered protein can be a guide for designing and testing ligands for them. We hypothesize that a molecule which is structurally close to the putative ligand would alter the dynamics, even if binds to the protein only weakly or transiently. We test this hypothesis in Amylin, a peptide associated with type II diabetes. We measure single-molecule fluctuations with ~10ns accuracy using Fluorescence Cross Correlation Spectroscopy (FCCS), by probing changes in Forster Resonance Energy Transfer between the two termini. We find that the distance between the two termini fluctuates with a time scale of about 140 ns. This agrees with the order of magnitude expectation from Statistical Mechanics predictions about such disordered proteins. Interestingly, it falls in the time scale inaccessible to most other techniques, including NMR. We use this fluctuation time scale as a guide for diagnosing interaction of amylin with potential ligands. We propose dynamics-based measurement as a general approach for discovering ligands for IDPs.

Satyajit Mayor

Abstract

Photonics in the pursuit of living cell membrane structure and function

The broad aim of my laboratory is to develop an understanding of how a eukaryotic cell regulates local composition influencing the construction of signaling complexes, and also how it may engage in membrane deformation, thereby influencing global membrane properties, in a regulated fashion. We have taken a multi-disciplinary approach, combining biology with physics and chemistry to address the main theme of our research.

To study the organization of cellular components, we explore the dynamics of fluorescently-tagged molecules in a variety of living cells from the nanometer scale in specialized domains in cell membranes to the micron scale prevalent in mapping endocytic pathways. We have used and developed many microscopy tools suitable for these explorations. Our studies provide a new picture of the cell membrane as an *active composite* of the lipid bilayer and a dynamic cortical actin layer beneath, wherein, dynamic actin filaments help in controlling the local composition of membranes. In addition the sorting properties and endocytic pathways of a variety of molecules, including membrane proteins, lipids and lipid-tethered proteins *in vivo,* have led to new insights about endocytic pathways that function at the cell surface. In this lecture I will discuss how we and others have utilized a variety of photonic tools in the pursuit of understanding the dynamic architecture of the living cell membrane and its deformation.

Takahiro Fujiwara

Abstract

Revealing mesoscopic organization and function of the plasma membrane by developing high-speed single-molecule microscopy

Molecular dynamics in the plasma membrane (PM) is fundamentally important for the molecular functions in the PM, but the mechanisms by which the molecular diffusion in the PM is regulated still remain unresolved. By applying single-particle (40-nm-diameter colloidal gold) tracking at high time resolutions of up to 0.025 ms (a 40-kHz frame rate), we found confined diffusion + hop movements (termed "hop diffusion") for both a transmembrane (TM) protein, transferrin receptor, and a non-raft phospholipid, DOPE, which could be well hidden at observation rates slower than hop rates, and are mistakenly judged as simple Brownian diffusion. It turned out that these two molecules sensed the equal compartment sizes in the PMs of five different cell lines examined, PtK2, NRK, T24, HeLa, and HEPA-OVA (actual sizes were cell-dependent and ranging from 45 to 250 nm; Fujiwara *et al.*, 2002, 2016). Electron tomography identified the actin-based membrane skeleton (MSK) right on the PM cytoplasmic surface, and demonstrated that the MSK mesh size was the same as the compartment size for the PM molecular diffusion (Morone *et al.*, 2006; Fujiwara *et al.*, 2016).

These results support the model of the actin-induced mesoscopic compartmentalization of the PM. TM proteins are temporarily corralled due to the collisions of their cytoplasmic domains with the actin-based MSK mesh bound to the PM inner surface, called the "MSK fence", and various TM proteins, anchored to and aligned along the MSK, called the "anchored TM-protein pickets", create a barrier against the free diffusion of both TM proteins and lipids. Importantly, these pickets not only exert steric hindrance effects but also hydrodynamic friction effects on the surrounding molecules, thus inducing confining effects on membrane molecules.

Recently, we succeeded in developing a new high-sensitive high-speed camera system, which now allows us to image single fluorescent molecules in the PM at a time resolution of 0.1 ms (a 10-kHz frame rate), the fastest ever performed. By employing a much smaller fluorescent probe Cy3, it was found that virtually all TM proteins and lipids undergo hop diffusion in the PM, strongly indicating that our previous data were not affected by the binding of the large colloidal gold particle, but actually reflected the true behavior of TM proteins and lipids in the PM. Thus, we have proposed a paradigm shift for the long-range structure of the PM is required from the fluid mosaic model of Singer and Nicolson to the compartmentalized fluid model. The involvement of the compartmentalized PM in regulating biological functions of the PM, for example, how the membrane domains such as rafts and focal adhesion structures fit into the view of the compartmentalized PM, will be addressed.

References

Fujiwara, T. *et al.* 2002. Phospholipids undergo hop diffusion in compartmentalized cell membrane. J. Cell Biol. 157: 1071-1081.

Fujiwara, T.K. *et al.* 2016. Confined diffusion of transmembrane proteins and lipids induced by the same actin meshwork lining the plasma membrane. Mol. Biol. Cell 27: 1101-1119.

Morone, N. *et al.* 2006. Three-dimensional reconstruction of the membrane skeleton at the plasma membrane interface by electron tomography. J. Cell Biol. 174: 851-862.

Rahul Roy

Abstract

2D and 3D assembly on biomembranes

Biological self-assembly is a critical process in the formation of life. Biomembranes, self-assembled from lipid molecules, act as a platform for the assembly of several 2D and 3D biological structures. I will describe our efforts to understand biological assembly on lipid membranes using bacterial pore forming toxins (PFTs) and filamentous bacteriophages. PFTs, a major class of bacterial toxins are released as water soluble monomers. Upon binding the bilayer membrane, they undergo structural rearrangements and oligomerize to form a stable transmembrane pore. The molecular mechanisms giving rise to selective pore formation on target eukaryotic membranes have been unclear. Using single molecule imaging, we dissect the assembly pathway of ClyA, a representative αPFT and demonstrate how cholesterol is a key component for effective pore formation and a possible means for selective targeting of PFTs to their target cells.

Next, I will discuss the assembly of M13 filamentous phages in bacteria. After infection, phage components use the inner membrane of the bacteria to assemble the coat protein on the phage DNA allowing the phage to be extruded out without rupturing the cell. We visualize M13 assembly on live bacteria using super-resolution microscopy. The assembly kinetics is fast but variable within the bacterial population indicating possible rate limiting steps in phage assembly.