# Probing self-assembly dynamics by high speed-atomic force microscopy

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be inserted into practically any biological RNA of interest. 4. The low off-rate of the fluorogenic ligand also enables native pulldowns of RNA-protein complexes. Together with their high contrast, these fluorogenic aptamers are proving to be robust tools for understanding RNA within living or fixed cells. To be truly useful, two-channel RNA imaging is needed. By connecting rigid Mango and Spinach fluorogenic aptamers to RNA structures, we have demonstrated orientational FRET, which will allow the construction of cellular sensors in the future. In this regard and to complement our Mango aptamer studies, we have developed a new class of fluorogenic aptamers, called ''Peach, which offers the potential for dual-color imaging. Remarkably, these new aptamers can distinguish between extremely similar fluorogenic ligands giving them orthogonal imaging potential. We are currently extending the selectivity of these aptamers using a combination of *in vitro* selection and chemical synthesis. I will discuss how high affinity Mango and Peach type fluorogenic aptamers simplify RNA-protein characterization and cellular RNA imaging.

# Workshop: 3D High-Resolution Cellular Microscopy

**1538-Wkshp**

**Mechanobiological control of T-cell activation**

Huw Colin-York, Liliana Barbieri, Veronika Pfannenstill, Kseniya Korobchevskaya, **Marco Fritzsche**.
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New perspective of mechanobiology is currently emerging across multiple disciplines in the biomedical sciences. In contrast to conventional believes, recent evidence indicates that cells regulate their cell mechanics not downstream of signalling events triggered by ligand-receptor binding, but that cells employ a diversity of feedback mechanisms to dynamically adjust their mechanics in response to external stimuli. Quantifying cellular forces has therefore become an contentious challenge across multiple disciplines at the interface of biophysics, cell-biology, and immunology. Mechanical forces are especially important for the activation of immune T cells. Using a suite of advanced quantitative super-resolution imaging and force probing methodologies to analyse resting and activated T cells, we demonstrate activating T cells sequentially rearrange their nanoscale mechanobiology, creating a previously unreported ramifying actin network above the immunological synapse (IS). We show evidence that the kinetics of the antigen engaging the T-cell receptor controls the nanoscale actin organisation and mechanics of the IS. Using an engineered T-cell system expressing a specific T-cell receptor and stimulated by a range of antigens, force measurements revealed that the peak force experienced by the T- cell receptor during activation was independent of the kinetics of the stimulating antigen. Conversely, quantification of the actin retrograde flow velocity at the IS revealed a striking dependence on the antigen kinetics. Taken together, these findings suggest that the dynamics of the actin cytoskeleton actively adjusted to normalise the force experienced by the T-cell receptor in an antigen specific manner. Consequently, tuning actin dynamics in response to antigen kinetics may thus be a mechanism that allows T cells to adjust the length- and time- scale of T-cell receptor signalling.

**1539-Wkshp**

**In situ structural analysis of virus infection by cellular cryo-tomography and subtomogram averaging**

**Peijun Zhang**.
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Structural biology methods using cryoEM and cryo-electron tomography (cryoET) have become major tools for studying macromolecular complexes that are intrinsically flexible and dynamic, and often function in higher-order assemblies that are difficult to purify. The study of these complexes and assemblies in situ using cryoET and subtomogram averaging at sub-nanometer to near-atomic resolutions, coupled with cryoFIB and correlative and integrative imaging, opens a new frontier in structural cell biology, as exemplified in virus infection in human cells. I will present some of our technology development towards this goal and our recent studies of virus infection processes to demonstrate the power of cryoET and sub-tomogram averaging.

# Workshop: High Speed AFM

**1540-Wkshp**

**Direct molecular level visualization of the structure-activity-relationship of drugs on biological membrane mimics**

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Label-free visualization of the activity of drugs on membranes at the molecular level has recently become a reality using state-of-the-art high-speed atomic force microscopy (hs-afm). Label-free visualization of the activity of drugs is a novel and rich source of data of the Structure-Activity-Relationship (SAR): it provides, among others, the stoichiometry of the drug-oligomers on the membrane, the energy-landscape of the interaction of the drug-oligomers, the visualization of the individual structural transitions of the molecular at the early and late stages of the action of the drugs on the membranes. Moreover, the technique enables that only one or the two membrane leaflets are exposed to the drug. At this communication, we will show how the hs-afm opens the door to non-averaged molecular information for the creation of extensive databases of the SARs of membrane-interaction drugs.

**1541-Wkshp**

**Elucidating mechanisms of protein self-organization on membranes**

**Petra Schwille**.
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The MinDE protein system is a paradigm for molecular self-organization and pattern formation on membranes. In spite of intense research being done on MinDE dynamics *in vivo* and particularly *in vitro*, the exact molecular mechanisms of the nonlinear association/dissociation processes between the proteins and the membrane as a catalytic surface are still unknown. We have in the past years applied high-speed atomic force microscopy, single molecule TIR microscopy, and recently also mass photometry on that system and combined it with rational protein mutagenesis. This allowed us to arrive at stunning new insights into the complex design of this self-organizing machinery, which I will summarize here. These insights may in the future inform us about how to build synthetic pattern-forming systems on membranes from the bottom-up.

**1542-Wkshp**

**Probing self-assembly dynamics by high speed-atomic force microscopy**

**Wouter H. Roos**.
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Cellular life harbours a fascinating variety of complex processes and we are still at the beginning of our understanding of these processes. Atomic-scale reconstructions using crystallography or electron microscopy approaches have unveiled great views on cellular components such as proteins and higher-order proteinaceous assemblies. However, these static techniques do not reveal the dynamics of the studied constructs. Using High Speed-Atomic Force Microscopy (HS-AFM) we are now able to scrutinize the dynamics of molecular processes at the nanometre scale, in real time, in liquid. I will start off with discussing the principles and background of HS-AFM and discuss practicalities such as surface treatment and experimental approach. Next I will dive into the applications. Thereby, I will show how we are using the HS-AFM technique to study the fascinating physics of sub-cellular dynamics and biomimetic assembly processes. This will be illustrated by discussing assembly (and disassembly) of ESCRT-III protein complexes and HS-AFM visualization of the dynamics of supramolecular polymerization of synthetic self-replicators. Furthermore, the formation dynamics of 2D capsid protein lattices of human immunodeficiency virus (HIV) will be discussed, particularly revealing how complex the kinetics of viral self-assembly can be, with multiple assembly pathways and continuously occurring assembly and disassembly events. Finally, studies of nucleus formation and growth of hepatitis B virus (HBV) capsid protein complexes are shown revealing real time binding of capsid proteins and the dynamics of assembly initiation of HBV. Combining the insights from the static atomic-scale reconstructions with the dynamic molecular-scale HS-AFM experiments we are now finally able to provide a comprehensive view on the dynamics of sub-cellular processes.