Ebola virus (EBOV) causes a hemorrhagic fever associated with fatality rates up to 90%. The EBOV entry process is complex and incompletely understood. Following attachment to host cells, EBOV is trafficked to late endosomes where its glycoprotein (GP) is processed to a 19-kDa form, allowing the glycoprotein to bind to its intracellular receptor Niemann Pick type C1. We previously showed that the cathepsin protease inhibitor, E64d, blocks infection by pseudovirus particles bearing 19-kDa GP, suggesting that further cathepsin action is needed to trigger fusion. This, however, has not been demonstrated directly. Since 19-kDa Ebola GP fusion occurs in late endosomes, we devised a system in which enriched late endosomes are used to prepare supported planar endosomal membranes (SPEMs) and fusion of fluorescent (pseudo)virus particles is monitored by TIRF microscopy. We validated the system by demonstrating the pH dependencies of influenza virus HA- and Lassa virus GP-mediated fusion. Using SPEMs, we next showed that fusion mediated by 19-kDa Ebola GP is dependent on low pH and enhanced by Ca^{2+}, consistent with other studies. We further showed that addition of cathepsins (somewhat more prominently with cathepsin B than L) augments both hemi- and full fusion. Subsequently we found that SPEMs appear to retain cathepsin activity, and that E64d inhibits both hemi- and full fusion mediated by 19-kDa GP. Hence we provide both gain- and loss-of-function evidence that further cathepsin action enhances the fusion activity of pre-primed 19-kDa Ebola GP. Thus we have provided new evidence for how Ebola GP mediates fusion with endosomes, and we have developed a novel approach employing SPEMs that can now be used for studies of any virus that fuses in endosomes.

1566-Plat

Drug discovery targeting SARS-CoV-2 membrane fusion

Kailu Yang[1], Chuchu Wang[1], Alex J. B. Kreutzberger[2], Ravi Ojha[3], Suvi Kuivanen[3], Sergio Couoh-Cardel[1], Serena Muratcioglu[4], Timothy J. Eisen[4], K. Ian White[1], Richard Pfuetzner[1], John Kuriyan[4], Olli Vapalahti[3], Giuseppe Balistreri[3], Tomas Kirchhausen[2], Axel T. Brunger[1].
[1]Stanford University, Stanford, CA, USA, [2]Harvard University, Boston, MA, USA, [3]University of Helsinki, Helsinki, Finland, [4]University of California Berkeley, Berkeley, CA, USA.

SARS-CoV-2 infect hosts by membrane fusion, a process where the virus membrane and host membrane merge together to allow the viral genetic material to enter the host cell. The viral membrane fusion is catalyzed by the viral spike protein (S). S, after receptor binding and proteolytic activation, dissociates into S1 and S2 subunits, the latter of which then goes through large conformational changes to bring the two membranes together. The heptad repeat 1 (HR1) and 2 (HR2) domains of S, that are far from each other in the primary sequence of S and spatially far apart in the prefusion structure of S, zipper into a 6-helix bundle in the postfusion S, which presumably provides the energy for overcoming the kinetic barrier of fusing membranes. We developed a method to efficiently determine high-resolution bundle structures by molecular scaffolding and cryogenic electron microscopy. We have utilized this method to study the structural conservation of the postfusion bundle, and to guide structure-based design of potent inhibitors. We have discovered an HR2-based peptide, named longHR2_42, that potently inhibits the infection by the wildtype SARS-CoV-2 and several variants with IC_{50} in the range of 1-4 nM.

1567-Plat

Coherent diffractive imaging of lipid vesicles and synaptic vesicles by femtosecond x-ray FEL pulses

Charlotte Neuhaus[1], Jette Alfken[1], Karlo Komorowski[1], Moritz L. Stammer[1], Richard Bean[2], Johan Bielecki[2], Raphael de Wijn[2], Romain Letrun[2], Safi Rafie-Zinedine[3], E. Juncheng[2], Adrian Mancuso[2], Reinhard Jahn[4], Tim Salditt[1].
[1]Institut für Röntgenphysik, Universität Göttingen, Göttingen, Germany, [2]Single Particles, Clusters, and Biomolecules & Serial Femtosecond Crystallography, European X-Ray Free-Electron Laser Facility (XFEL), Hamburg, Germany, [3]European XFEL, Hamburg, Germany, [4]Institute of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

The structure of biological vesicles, in particular synaptic vesicles (SVs), as well as synthetic lipid model systems, in particular small unilamellar lipid vesicles (LVs) has been subject of abiding interest. Given the small size of both LVs and SVs ($R \approx 20$ nm), high spatial resolution is required to identify the distribution of lipids and protein constituents. Cryogenic electron microscopy studies of synaptic vesicles have revealed the outer and inner layer of proteins around the lipid bilayer. However, the samples have to be cryogenically fixed for this technique and throughput is limited. Another standard technique for structural characterization is solution small angle x-ray scattering (SAXS), which enables the measurement of vesicles in a quasi-physiological environment combined with a high spatial resolution. However, due to the average over an extremely large ensemble, SAXS yields information only about the average structure (size and electron density profile). The distribution function of structural parameters is not accessible, and many structural details are lost or screened by polydispersity, as well as by powder averaging. To overcome these limitations, we now have performed coherent diffractive imaging experiments on single vesicles using single femtosecond x-ray free electron laser (XFEL) pulses. For these measurements, single vesicles surrounded by a thin buffer layer are delivered into a nano-focused XFEL beam by an aerosol injector. By the 'diffract-before-destroy' principle, the individual vesicles can be probed without radiation damage. This approach leads to the measurement of thousands of diffraction patterns that can now be analyzed and reconstructed. The (preliminary) results of this analysis will be presented.

1568-Plat

Remodeling and dynamics of membrane-proximal actin in live B cells

Adam M. Decker[1], Jennifer C. Flanagan-Natoli[2], Andrea K. Stoddard[1], Sarah L. Veatch[2].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Biophysics, College of Literature, Science, and the Arts, University of Michigan, Ann Arbor, MI, USA.

The interaction between the actin cytoskeleton and the plasma membrane is suggested to play an important role in membrane organization. We expressed the membrane-anchored actin-binding probe, known as MPAct, in live B cells to quantify these interactions. Our MPAct construct consists of a membrane anchor with a prenylation and polybasic amino acid sequence derived from KRas, the actin-binding domain from f-tractin, and mEos3.2, a photoswitchable fluorophore. Using single-molecule tracking and steady-state correlation analysis, we isolated multiple diffusing populations of MPAct, which indicate binding to membrane-proximal actin. Changes in the relative size of these populations in response to stimulus indicate changes in actin density near the membrane. We measured the effects of the actin perturbing drugs Latrunculin A and Jasplakinolide on MPAct diffusion showing that both drugs slow down actin dynamics. Modifying the membrane anchor of MPAct with a more liquid-ordered preferring anchor suggests a slight disordered domain preference of membrane-proximal actin. Additionally, we have quantified MPAct dynamics upon B cell activation. During B cell activation the membrane and cytoskeleton remodel to support the clustering of B-cell receptor (BCR) signaling domains. We observed that the formation of these domains is accompanied by a decrease in membrane-proximal actin along with a later recovery after 2 minutes. MPAct/BCR cross-correlation analysis shows that membrane-proximal actin co-localizes with BCR during cluster formation. In contrast, we see on average a decrease in the co-localization between *global* actin and BCR upon activation. Future studies will investigate membrane-actin adaptor proteins, like the ERM protein family, to understand how cytoskeletal pinning affects the formation of signaling domains in the plasma membrane. Our study applies a novel single-molecule probe to characterize actin dynamics in live B cells, suggesting a role for actin in the assembly of BCR signaling domains.

1569-Plat

Polyunsaturated lipids promote membrane phase separation and antimicrobial sensitivity

Hugo MacDermott-Opeskin[1], Katie A. Wilson[1], Bart Eijkelkamp[2], **Megan L. O'Mara**[1,3].
[1]Research School of Chemistry, Australian National University, Canberra, Australia, [2]College of Science and Engineering, Flinders University, Adelaide, Australia, [3]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia.

Gram-negative bacteria such as *Acinetobacter baumannii* sequester host lipids from the site of infection for incorporation into lipid synthesis pathways, altering the membrane lipidome. Changes in membrane composition from the incorporation of host-derived polyunsaturated fatty acids (PUFAs) help restore sensitivity to antimicrobials in several species of Gram-negative bacteria. Using coarse-grained simulations based on lipidomic data of *A. baumannii* inner membrane collected under three different growth conditions, we show PUFA-incorporation alters membrane biophysical properties, increasing the phase separation between ordered and disordered lipid domains resulting in thinner, less ordered membranes. We show that