from electron damage. The sample, tagged with organic dyes, is placed on a scintillating cerium-doped yttrium aluminum perovskite (YAP) film. A focused electron beam is then used to generate optical excitations at a specific location on the film opposite the sample, thus forming a 20-nm illumination volume that excites fluorophores. Dye fluorescence is collected within the electron microscope and, as the beam is scanned, an image is formed.
In order to completely block a low-voltage electron beam yet maximize the optical excitation intensity, an extremely thin YAP film is required. We have succeeded in fabricating a 20-nm thick suspended YAP film over an area 30 microns wide via molecular beam epitaxy, pulsed laser deposition, and deep reactive ion etching. Using our ultrathin scintillating films, we demonstrate superresolution imaging of fluorescently labeled particles and polymers. This technique has the potential to revolutionize correlative microscopy by combining a scanned electron probe with fluorescence detection in the same instrument, at the same time. In general, due to its nondestructive nature, it holds great promise as an alternative multiscale fluorescence microscopy, with resolution tunable from tens of nanometers up to microns, especially for damage-sensitive or highly dynamic cellular structures. We are applying this tool toward the study of biologically-inspired nanostructures, thylakoid membrane organization, and molecular motor dynamics.

959-Plat
CCP-SAS - Novel Approaches for the Atomistic Modelling of Small Angle Scattering Data in Biology
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Small angle X-ray and neutron scattering (SAS) experiments and molecular modelling offer powerful tools to understand protein behaviour in solution. SAS experiments have made huge strides in the last two decades due to improved instrumentation and sources. Until recently, using atomistic simulations to interpret SAS experiments has been infrequent. This requires integrating the SAS data with molecular modelling software to reveal how biological structures change in time and space under varying experimental conditions. Recent examples of this approach include new structures for human IgG4 antibody and complement C3b that clarified their function. In order to provide open-source and user-friendly tools for SAS fits based on a web interface, CCP-SAS was set up as a joint UK/USA collaboration. Our project combines existing and novel tools for atomistic modelling trials creation (such as SASSIE) and scattering curve calculation (SCT and SASCalc) with a combined workflow and transparent access to high performance computing resources via the GenApp framework. This novel CCP-SAS approach is being evaluated using complement proteins and antibodies. The combination of crystal structures and atomistic modelling of SAS data for fragments of MASP in the complement lectin pathway using SASSIE and SCT is revealing the solution structure of the intact dimer of MASP, and new insights on its function. The combinations of new glycan modelling of hinge structures in IgA1 antibodies and crystal structures for the IgA1 Fab and Fc regions is revealing its unique structure for its monomer and dimer forms. Details of how these atomistic molecular modelling approaches were applied to these problems will be critically reviewed.

960-Plat
Interpretation of Solution X-Ray Scattering Data by Molecular Dynamics
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Small and wide angle X-ray scattering (SWAXS) and molecular dynamics (MD) simulations are complementary approaches that probe conformational transitions of biomolecules in solution, even in a time-resolved manner. However, the structural interpretation of the scattering signals is challenging, while MD simulations frequently suffer from incomplete sampling or from a force field bias.
To combine the advantages of both techniques, we present a method that incorporates solution scattering data into explicit-solvent MD simulations, termed SWAXS-driven MD, with the aim to direct the simulation into conformations satisfying the experimental data [1]. Because the calculations fully rely on explicit solvent, no fitting parameters associated with the solvation layer or excluded solvent are required, and the calculations remain valid at wide angles [2].
The complementarity of SWAXS and MD is illustrated using three biological examples, namely a periplasmic binding protein, aspartate carbamoyltransferase, and a nuclear exportin. The examples suggest that SWAXS-driven MD is capable of refining structures against SWAXS data without foreknowledge of possible reaction paths. In turn, the SWAXS data accelerates conformational transitions in MD simulations and reduces the force field bias. Extensions towards the interpretation of anisotropic X-ray scattering data are discussed [3].
References:
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[2] Chen & Hub, Validating solution ensembles from molecular dynamics simulation by wide-angle X-ray scattering data, Biophys. J. 107, 435-447 (2014)
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Platform: Ligand-gated Channels

961-Plat
Divergent Roles for M4 in the Gating of Two Prokaryotic Pentameric Ligand-Gated Ion Channels
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Pentameric ligand-gated ion channels (pLGICs) play a fundamental role in synaptic communication and are the sites of action of numerous allosteric modulators. Recently acquired data suggest that the outermost transmembrane helix, M4, is central to the potentiation of gating observed with some allosteric modulators that interact with the transmembrane domain. Specifically, enhanced interactions between M4 and the adjacent transmembrane helices, M1 and M3, promote coupling between the agonist site and transmembrane gate, while weakened interactions inhibit coupling. Here, we explore in detail the functional role of M4 in the gating of two prokaryotic pLGICs, GLIC and ELIC. Ala mutations along the entire length of M4 typically inhibited channel function in GLIC, with the strongest detrimental effects occurring in the C-terminus along the protein-facing surface. In contrast, Ala mutations in ELIC typically led to enhanced channel function with the strongest potentiating effects occurring closer to the N-terminus. Sequential C-terminal deletions of M4 in GLIC led to the abolishment of channel gating and/or trafficking to the cell surface. In contrast, C-terminal M4 deletion mutants in ELIC still gated open in response to agonist, while deletion of eight or more residues led to fast desensitizing channels with gating kinetics similar to those of alpha7 nAChRs. Our results highlight the importance of M4 in channel function, but suggest M4 in ELIC and GLIC have divergent roles in the gating of each prokaryotic pLGIC.

962-Plat
Single Molecule Motion Map of GLIC by Diffracted X-Ray Tracking
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GLIC is a proton-gated bacterial ion channel from Gloeobacter violaceus and is a member of pentameric ligand-gated ion channels. Recently structural information of GLIC in open (acidic pH) and closed state (neutral pH) has become available (1,2), and the gating mechanism is discussed based on the structural information. However the dynamic information of each state in physical point of view is not available. Here we used the diffracted X-ray tracking (DXT) method (3) to detect the motion of the extracellular or transmembrane domain of GLIC. DXT has been considered as a powerful technique in biological science for detecting atomic-scale dynamic motion of the target protein at the single molecular level at several tens of microseconds time resolution. The dynamics of a single protein can be monitored through trajectory of a Laue spot from a nanocrystal which was attached to the target protein immobilized on the substrate surface (4,5).
DXT observation was performed at BL40XU in SPring-8 Japan and showed that tilting motion of the transmembrane domain of GLIC and both tilting and twisting motions of the extracellular domain of GLIC were enhanced in open state (acidic pH). The detailed information on dynamic will be discussed.
[1] N. Bocquet et al., Nature 457:111 (2009)
[2] L. Sauguet et al., PNAS 111:966 (2014)