Abstracts

– 1. Protein Structure and Function –

P-74

R209H Mutation in Collybistin causes lipid binding defects at inhibitory synapses

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Proper clustering of the scaffold protein Gephyrin and of gamma-aminobutyric acid type A receptors (GABAARs) is a prerequisite for efficient signaling at nerve cells. In specific areas of the mammalian brain this clustering is critically depdendent on the brain-specific guanine nucleotide exchange factor (GEF) Collybistin (Cb). Recently, it was discovered that an R290H missense mutation in the B-cell lymphoma homology (DH) domain of Cb, which carries the GEF activity, leads to epilepsy and intellectual disability in human patients. Based on biochemical and cell biological methods, it was demonstrated that Cb-dependent clustering of Gephyrin in COS7 cells is significantly impaired in the presence of mutant Cb. This can be attributed to a binding defect of the Cb PH domain to phosphatidylinostol-3-phosphate (PI3P) caused by the R/H mutation. Molecular dynamics simulations of wt and R/H mutated Cb show that the R/H mutation affects the strength of intermolecular interactions between the DH and PH domains of Cb. We observe an increased confomational heterogeneity of Cb, specifically concerning the orientation of the PH domain relative to the DH domain, which affects PI3P binding by the PH domain.

P-75

Structure of a 13-fold superhelix (almost) determined from first principles

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Our aim was to co-crystallize the glucocorticoid receptor in complex with a ligand and a short peptide. Quite surprisingly, a 1.8 Å resolution diffraction dataset could not be phased by molecular replacement. HPLC analysis of the crystals revealed that only the peptide was present. The self-rotation function displayed 13-fold symmetry, which initiated an exhaustive but frustratingly unsuccessful molecular-replacement approach using motifs of 13-fold symmetry such as α- and β-barrels in various geometries. The structure was ultimately determined using a single ideal α-helix of less than 4% total scattering mass and the novel software ARCIMBOLDO, which combines PHASER solutions as seeds for density modification and model building in SHELXE. Systematic variation of the helix length revealed upper and lower size limits for successful structure determination. A beautiful but entirely unanticipated structure was obtained forming superhelices with left-handed twist throughout the crystal, stabilized by unusual ligand interactions. Together with the increasing diversity of structural elements in the Protein Data Bank our results confirm the potential of fragment-based molecular replacement to accelerate phasing.

P-76

Type-II NADH: quinone oxidoreductase from *Staphylococcus aureus* has two distinct binding sites

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We explored protein-substrate interaction in Type-II NADH:quinone oxidoreductase (NDH-II) from *Staphylococcus aureus*, a worldwide problem in clinical medicine due to its multiple drug resistant forms. NDHs-II are membrane proteins involved in respiratory chains and recognized as suitable targets for novel antimicrobial therapies, since these provide the only NADH:quinone oxidoreductase activity present in many pathogenic organisms. We obtained the X-ray crystal and solution (SAXS) structures, showing that NDH-II is a dimer in solution. We detected, by stopped-flow analyses, a charge-transfer complex upon NADH reduction, which is dissociated by the quinone. This indicates the catalytic mechanism may involve a ternary complex. We determined that quinone reduction is the rate limiting step and also the only half-reaction affected by the presence of the inhibitor HQNO. We performed titrations of NDH-II with its substrates, monitored by fluorescence and STD-NMR spectroscopies. Our results demonstrate unequivocally the functional presence of distinct binding sites for the two substrates, resolving the controversy whether the substrates in NDH-II bind to the same site. Moreover, quinone binding can be allosterically perturbed.

P-77

Analysis of antigen-antibody binding process using extensive molecular dynamics simulations

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Colon cancer antigen, epiregulin (mature EPR; 46 residues), is a member of the epidermal growth factor family that binds to epidermal growth factor receptor. Our EPR antibody (436 residues for fab form) has a proline at the residue 103 in the third complementarity-determining region (CDR) of the heavy chain, which is in the cis conformation for EPR free antibody and the trans one for the complex of EPR-antibody obtained from X-ray crystallography. In order to clarify the structural changes in the binding process, we have performed extensive molecular dynamics (MD) simulations (964 independent 200 ns simulations) of EPR-antibody systems in which an initial distance between the EPR and the free structure of the antibody is 9 Å. It is found that after 200 ns, EPRs evenly contact with not only to CDRs but also other part such as constant region of the antibody. It is also found that the water molecules sandwiched between EPR and the antibody has important role in the binding process of EPR. Several EPR-contacted structures are similar to the complex one obtained from X-ray crystallography. To see the stability of the structure, we have conducted further ten independent 2 μs MD simulations. Highly similar EPR contacted structures to the complex one are found.