whose charge-transfer absorption bands are indicative of a weakly covalent interaction. Since the halides interact with the -face of the arene in a similar manner as does water with tryptophan in proteins and deoxyribose with nucleobases in nucleic acids (see figure below), on may ask to which extent charge transfer, evidently operating in the halide-arene complexes, contributes to the binding of the lp- interactions in the biopolymers. Asking more generally: What are the energy components stabilizing lone-pair- interactions and how does their balance depend on the molecular properties of the interacting partners? Do force-field calculations portray such interactions properly?

The talk will attempt to provide the answer.

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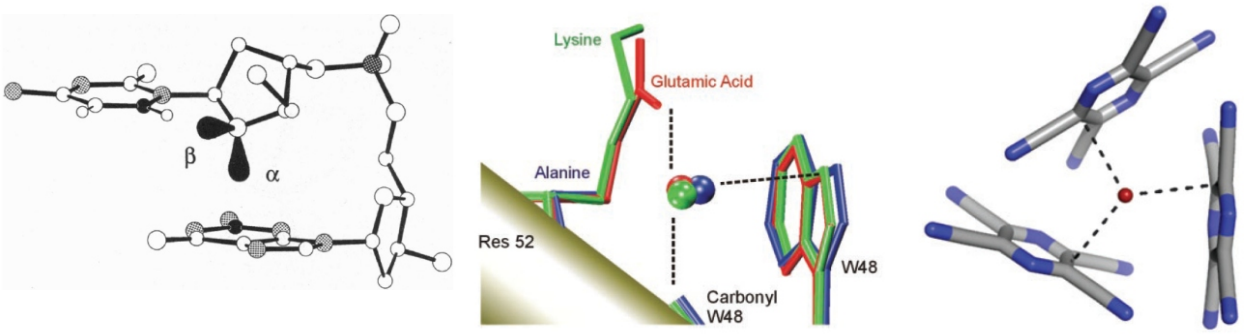

Left: lp- interaction stabilizing Z-DNA [1]. Middle: lp- interaction suggested to operate between a conserved water molecule and tryptophan W48 in the Engrailed homeodomain and its mutants [2]. Right: lp- interaction observed in the X-ray structure of the charge-transfer complex between Br^- and TCP [3].

Friday, March 18, afternoon, Session VI

L23

STRUCTURAL INSIGHTS AND IN VITRO RECONSTITUTION OF MEMBRANE TARGETING AND ACTIVATION OF HUMAN PI4KB BY THE ACBD3 PROTEIN

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Phosphatidyl inositol 4-kinase beta (PI4KB) is one of four human PI4K enzymes that generate phosphatidyl inositol 4-phosphate (PI4P), a minor but essential regulatory lipid found in all eukaryotic cells. To convert their lipid substrates, PI4Ks must be recruited to the correct membrane compartment. PI4KB is critical for the maintenance of the Golgi and trans Golgi network (TGN) PI4P pools, however, the actual targeting mechanism of PI4KB to the Golgi and TGN membranes is unknown. Here, we present an NMR structure of the complex of PI4KB and its interacting partner, Golgi adaptor protein acyl-coenzyme A binding domain containing protein 3 (ACBD3). We show that ACBD3 is capable of recruiting PI4KB to membranes both *in vitro* and *in vivo*, and that membrane recruitment of PI4KB by ACBD3 increases its enzymatic activity and that the ACBD3:PI4KB complex formation is essential for proper function of the Golgi.

L24

THE INS AND OUTS OF MEMBRANE-IMMERSED CATALYSIS: STRUCTURE, MECHANISM AND INHIBITION OF RHOMBOID INTRAMEMBRANE PROTEASES

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Intramembrane proteases control the fate of many integral membrane proteins, regulate a number of signaling processes, are key for maintaining homeostasis, and their dysregulation is associated with pathological states. Despite their biological importance, the mechanism of membrane-immersed catalysis, the basis of their substrate specificity and tools for their selective inhibition are mostly lacking. The main structural and mechanistic models for investigating intramembrane proteolysis are the serine intramembrane proteases of the rhomboid family. Rhomboids are evolutionarily widespread and regulate growth factor secretion, biogenesis of mitochondrial proteins and mitochondrial dynamics, invasion of the malaria parasite and membrane protein quality control. Beyond being model intramembrane proteases, rhomboids are increasingly being explored as potential drug targets, but selective and potent rhomboid inhibitors are not available.

We investigate rhomboid protease mechanism, specificity and substrate-enzyme interactions using enzymology and X-ray crystallography (Zoll *et al* EMBO J 2014) with the aim to understand the catalytic events along the whole reaction coordinate structurally. Based on this, we have developed novel mechanism-based covalent reversible inhibitors of rhomboid proteases that are specific and have high affinity. We demonstrate the mode of binding of these compounds by co-crystal structures with a rhomboid protease, and we provide a general modular platform for the design of isoform-specific inhibitors.

L25

LARGE MOTIONS THE PROTRUDING DOMAIN IN A NATIVE IFLAVIRUS

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Iflaviruses are non-enveloped single-stranded RNA viruses, which infect various insects including economically important agricultural pollinators honeybees and bumblebees. Studies of the life cycle and pathogenesis of iflaviruses are impeded by the lack of a honeybee cell cultures. Here, we present two crystal structures of an iflavirus Slow Bee Paralysis Virus (SBPV) purified from a natural source, solved at 3.4 and 2.6Å resolutions. We found that the C-terminus of a capsid protein VP3 forms a protruding domain (P-domain) located on the virion surface. The P-domain exhibits a novel fold, which is different from that seen in any other viruses characterized to date. P-domains undergo large movements toward icosahedral five-fold axis, where they are found in a crown-like arrangement. Despite sharing the same quasi T=3 symmetry with known insect picorna-like viruses, SBPV exhibits a unique surface topology and differs in the overall organization of the capsid. Our findings provide structural framework for further investigations of infection mechanisms employed by the agriculturally important class of viral pathogens.

L26

STRUCTURE AND GENOME DELIVERY MECHANISM OF *STAPHYLOCOCCUS AUREUS* PHAGE THERAPY AGENT phi812-K1 DETERMINED BY CRYO-ELECTRON MICROSCOPY

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Worldwide occurrence of multidrug-resistant pathogenic bacteria has increased interest in alternative treatments including bacteriophage-based therapy. Bacteriophage phi812 belongs to genus Twort-like virus, subfamily *Spounavirinae* and can infect at least 75% of Methicilin-resitant *S. aureus* strains (MRSA) and 95% of Methicillin-sensitive *S. aureus* strains. We have employed cryo-electron microscopy to determine structure and genome delivery mechanism for polyvalent staphylococcal backteriophage phi812-K1. Phi812-K1 has a 90 nm diameter isometric head and 240 nm long contractile tail ended by a double layered baseplate. The tail and baseplate of the native phage are dynamic. Therefore, a divide-and-conquer strategy was employed to separately determine the cryo-EM reconstructions of the individual phage parts. The structure of the icosahedral head could be refined to 5.0 Å resolution and additional sub-averaging within the T=16 icosahedral asymmetric unit allowed determination of the major capsid protein to 3.8 Å resolution. The structures of the native tail and baseplate were solved to 8 Å and 12 Å resolution, respectively. In order to examine the mechanism of the infection process, we determined the structure of the phage in the contracted state. The phage head is not altered after the DNA ejection. However, both the baseplate and tail undergo large reorganizations documented in their 6 Å and 8 Å resolution structures. Comparison of the tail and baseplate structures in the native and contracted conformation allowed us to determine the changes accompanying cell wall recognition and binding which is then followed by injection of the bacteriophage genome into the host bacteria.

L27

STRUCTURAL STUDY OF TICK-BORNE ENCEPHALITIS VIRUS USING CRYO-ELECTRON MICROSCOPY

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Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. The arrangement of these proteins in the virion is unknown, therefore, detailed structural study of the virus is needed.

We determined structure of mature TBEV virions grown in tissue culture and of complexes of virions with Fab fragments of neutralizing antibodies. Because of fragileness and non-homogeneity of the virions, we used cryo-electron microscopy to determine the structures. The observed particles (~50 nm in diameter) were suitable for reconstruction of the virus envelope. To obtain high-resolution electron density maps, single particle reconstruction techniques were employed, using programs from image-processing packages EMAN2, XMIPP, and RELION. The final reconstructed volume revealed structure in accordance with general structural organization of other flaviviruses including dengue and West Nile viruses. The reconstructions of TBEV particles in complexes with neutralizing antibodies showed attachment of the antibodies to specific sites on the viral surface.

Further improvement of the reconstructions may provide electron density maps of resolutions suitable for *de novo* model building of structural proteins, detailed structural studies of the virus shell and identification of virus residues constituting the binding site of the neutralizing antibodies. These structural studies may help to get better insight into TBEV particle organization as well as to obtain therapeutic anti-TBEV antibodies.