## MS.01.1

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**Structure, oligomerization and mechanism of dynamin superfamily proteins**

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GTPases of the dynamin superfamily remodel cellular membranes in response to nucleotide binding and hydrolysis. The molecular details of membrane interaction and the role of nucleotide-dependent changes for the function of these proteins are just emerging. Here, I present structural data on the dynamin-related proteins EHD2 [1] and MxA [2] which shed light on the mechanism of oligomerisation and the mechano-chemical function of these proteins. EHD2 oligomerizes via two distinct interfaces in the GTPase domain resulting in ring-like oligomers. Using electron paramagnetic resonance studies, we show that not only the tips of the helical domains but also the amino-terminus contribute to lipid binding. Furthermore, the Eps15 homology domains of EHD2 might switch from the top of the GTPase domain, as found in the crystal structure, to the side of the GTPase domain and also participate in membrane binding. Another mode of oligomerisation is found in the antiviral MxA GTPases which oligomerises via the helical stalk region to form ring-like structures. Furthermore, the GTPase domains of MxA might contribute to oligomerisation by connecting neighbouring rings. This assembly mode suggests a mechanism for the mechano-chemical function which is consistent with previous models for dynamin function. Finally, I will show how structural information obtained for MxA can be employed to obtain insights into structure and function of the dynamin GTPase.

[1] O. Daumke, R. Lundmark, Y. Vallis, S. Martens, P.J. Butler, H.M. McMahon, *Nature* **2007**, *449***,** 923-927, [2] S. Gao, A. von der Malsburg, S. Paeschke, J. Behlke, O. Haller, G. Kochs, O. Daumke, *Nature* **2010**, *465*, 502-506.

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**Telomerase structure function**

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Telomerase is a specialized DNA polymerase that extends the 3′ ends of eukaryotic linear chromosomes, a process required for genomic stability and cell viability. We have determined crystal structures of the active *Tribolium castaneum* telomerase catalytic subunit, TERT, alone [1] and in complex with an RNA-DNA hairpin designed to resemble the putative RNA-templating region and telomeric DNA [2]. The structures, together with existing biochemical data, provide novel insights into the basic mechanism of telomere replication and length homeostasis by telomerase. Moreover, this data further enriches our understanding of the mechanism of DNA replication by polymerases in general and it provides a framework to design small molecule modulators of telomerase activity that may be of therapeutic value for cancer and other diseases associated with cellular aging.

[1] A.J. Gillis, A.P. Schuller, E. Skordalakes, *Nature* **2008**, *455*(7213), 633-7. [2] M. Mitchell, et al., *Nature structural & molecular biology* **2010,** *17*(4), 513-8.

**Keywords: telomerase, cancer, aging**

## MS.01.3

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**Structural basis of the anaphase promoting complex**

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The anaphase promoting complex or cyclosome (APC/C) is a multi-subunit cullin-RING E3 ubiquitin ligase that functions to regulate progression through the mitotic phase of the cell cycle and controls entry into S phase [1]. APC/C-mediated coordination of cell cycle progression is achieved through the temporal regulation of APC/C activity and substrate specificity. The APC/C is an unusually large E3 ubiquitin ligase assembled from 13 different proteins, mostly highly conserved and essential for function, generating a macromolecular machine exceeding 1.2 MDa in mass. Information on how its 13 constituent proteins are assembled, and how they interact with co-activators, substrates and regulatory proteins is limited.

We developed a recombinant expression system that allows the reconstitution of holo APC/C and its sub-complexes that, when combined with electron microscopy, mass spectrometry and docking of crystallographic and homology-derived coordinates, provides a precise definition of the organisation and structure of all essential APC/C subunits, resulting in a pseudo-atomic model for 70% of the APC/C. A lattice-like appearance of the APC/C is generated by multiple repeat motifs of most APC/C subunits. Three conserved tetratricopeptide repeat (TPR) subunits share related superhelical homo-dimeric architectures that assemble to generate a quasi-symmetrical structure. I will describe the structure of the APC/C and its complex with co-activator and a destruction box (D-box) substrate which indicates that the D-box binding site is formed from a co-receptor of Cdh1 and Apc10 [2-5].

[1] D. Barford, *Q Rev Biophys* **2011,** *44*, 153-190. [2] P.C. da Fonseca, *et al. Nature* **2011,** *470*, 274-278. [3] A. Schreiber, *et al. Nature* **2011,** *470*, 227-232. [4] Z. Zhang, K. Kulkarni, S.J. Hanrahan, A.J. Thompson, D. Barford, *The EMBO journal* **2010**. [5] Zhang, Z. *et al. J Mol Biol* **2010,** *397*, 1316-1328.

**Keywords: anaphase promoting complex, cell cycle, electron microscopy**

## MS.01.4

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**Complement convertase formation based on the structures of C3b in complex with factors B and D**

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Activation of the complement cascade involves multiple proteolytic reactions mediated by large macromolecular complexes. Upon initiation, the three complement pathways converge into the critical step of the conversion of C3 to its activated form C3b by means of short-lived enzymatic complexes called C3 convertases. These convertases amplify C3b production near target surfaces, resulting in opsonization of target cells, activation of the complement terminal pathway and stimulation of the adaptive immune response. Generation and activation of complement convertases are multi-step processes that require localization of serine protease-containing proenzymes on large protein subunits. The interplay between formation of large multi-domain enzymes, extensive conformational changes and specific proteolytic cleavages highlights the complexity of the complement system.

We investigated the process of convertase formation in the alternative pathway of complement. In this pathway, the C3 convertase complex is generated when the proenzyme factor B (FB) interacts with surface-bound C3b to form the pro-convertase C3bB, which is then specifically cleaved by the soluble, self-inactivated serine protease factor D (FD), yielding the active yet unstable C3 convertase C3bBb.

We present here the crystal structures of the pro-convertase complex C3bB, formed by C3b (12 domains, 160 kDa) with factor B (5 domains, 90 kDa) at 4-Å resolution and the transient complex formed by C3bB with an inactive mutant of factor D (1 domain, 27 kDa) at 3.5-Å resolution. In agreement with previously published electron microscopy data, our structures highlight the equilibrium between an initial (closed/loading) state and a subsequent (open/activation) state of factor B. The structures reveal unexpected conformational changes that create the "open" state which exposes the scissile loop of FB for proteolytic cleavage. In the C3bBD* complex, we show how the open state of FB provides a docking platform for factor D distant from its catalytic site, which has been caught in an activated conformation.

Additional biochemical and biophysical analyses confirm the observed structural features and reveal a highly concerted and specific activation mechanism based on cofactor-dependent and substrate-induced proteolysis, which provides an important "double-safety" catch to restrict complement amplification to C3b-tagged target cells. By adding new frames to the "structural movie" of complement activation, our data also provide new valuable information for potential structure-based drug design of complement inhibitors.

[1] F. Forneris, D. Ricklin, J. Wu, A. Tzekou, R.S. Wallace, J.D. Lambris, P. Gros, *Science* **2010**, *330*, 1816-1820.

**Keywords: complement, immunology, proteolysis**

## MS.01.5

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**Crystal structure of the Usher:Chaperone:Adhesin subunit complex - insights into pilus assembly mechanism**

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Gram-negative bacteria express different classes of adhesive surface organelles that allow them to specifically bind and infect human cells. Among them, the chaperone-usher (CU) fibers constitute the most abundant group of bacterial cell surface appendages and have been the most extensively studied [3]. Type 1 pili are the archetypal representative of this class of adhesive multisubunit fibres. During pilus assembly, subunits dock as chaperone-bound complexes to an usher platform which catalyses their polymerization and mediates pilus translocation across the outer membrane. We report the 2.8 Å crystal structure of the usher (FimD) in its active conformation, bound to the chaperone (FimC) and adhesin subunit (FimH) complex [1]. The structure shows the adhesin subunit inserted inside the usher 24-stranded -barrel translocation channel, held in place through interactions with the two C-terminal periplasmic domains of the usher (CTD1 and CTD2). To accommodate the adhesin subunit, the usher plug domain is displaced from the barrel lumen to the periplasm, concomitant with a dramatic conformational change in the $\beta$-barrel [2]. The displaced plug positions the N-terminal domain of the usher (NTD) in an ideal conformation to catalyse incorporation of the next recruited chaperone: subunit complex. The usher:chaperone:adhesin subunit (FimD: FimC:FimH) structure captures the first view of a membrane protein transporter in the act of secreting its cognate substrate, giving insight into the molecular mechanism of the pilus assembly and certainly will serve as a new basis for drug-design.

[1] G. Phan, H. Remaut, T. Wang, W.J. Allen, K.F. Pirker, A. Lebedev, N.S. Henderson, S. Geibel, E. Volkan, J. Yan, M.B.A. Kunze, J.S. Pinkner, B. Ford, C.W. McKay, H. Li, S. Hultgren, D.G. Thanassi, G. Waksman, *Nature* submitted. [2] H. Remaut, C. Tang, N.S. Henderson, J.S. Pinkner, T. Wang, S.J. Hultgren, D.G. Thanassi, G. Waksman, H. Li, *Cell* **2008**, *16,* 640-652. [3] G. Waksman, S.J. Hultgren, *Nature Review Microbiology* **2009**, *7*, 765-767.

**Keywords: microbial, transporter, pilus**

## MS.02.1

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**Cryo–EM of the ribosome–SecYEG complex in nanodiscs**

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The ubiquitous SecY/Sec61–complex translocates nascent secretory proteins across cellular membranes and integrates membrane proteins into lipid bilayers. Several structures of mostly detergent solubilized Sec–complexes have been reported. Here, we solved a single–particle cryo–electron microscopy structure of the SecYEG complex in a membrane environment at sub–nanometer resolution, bound to a translating ribosome. Using the SecYEG complex reconstituted in a so–called Nanodisc, we could trace the nascent polypeptide chain from the peptidyl transferase center into the membrane. In order to build a near complete molecular model we used existing X-ray structures, homology models and Molecular Dynamics-based models of the nanodisc, and docked them into our