FA1-MS05-O1

Structure and Assembly of a Bacterial Type IV Secretion Core Complex. Remi Fronzes[a], Gabriel Waksman[a]. [a]*Institute of Structural and Molecular Biology, Birkbeck and UCL, Malet Street, London, WC1E 7HX, United Kingdom.*
E-mail: g.waksman@ucl.ac.uk

Type IV secretion systems (T4SSs) are molecular machines used for the transport of macromolecules across the bacterial cell envelope in Gram-negative bacteria. T4SSs are highly versatile. Conjugative T4SSs translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as *Helicobacter pylori* (Cag PI), *Brucella suis* (VirB/D), or *Legionella pneumophila* (Dot, Icm), T4SSs are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4SS, the VirB/D system, was defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefasciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4). In the past few years, atomic structures of isolated components such as VirD4, VirB11, VirB5, virB8, virB10 and virB9 have become available and have provided seminal insights into the mechanism of T4SS assembly and substrate secretion. However, no structural data was available concerning the assembly of the complex, particularly at the level of both bacterial membranes where the T4SS is supposed to form pores/channels. We here present the cryoEM structure of a 1 MDa complex composed of VirB7, VirB9 and VirB10 homologues from the *E. coli* conjugative plasmid pKM101 T4SS. The molecular characterization of this ternary complex provides key insights into the type IV secretion systems architecture and assembly

[1] Fronzes R., Schaefer E., Saibil H., Orlova E. and Waksman G., *Science*, **2009**, 323, 266.

Keywords: secretion systems; type IV secretion; structure

FA1-MS05-O2

Structure of the Catalytic Subunit of Telomerase; a Major Target for Cancer and Aging Therapies. Andrew Gillis[a], Anthony Schuller[a], Emmanuel Skordalakes[a]. [a]*The Wistar Institute, 3601 Spruce St, Philadelphia, PA 19104, USA.*
E-mail: skorda@wistar.org

A common hallmark of human cancers is the overexpression of telomerase, a ribonulceoprotein complex responsible for maintaining the length and integrity of chromosome ends. Telomere length deregulation and telomerase activation is an early and perhaps necessary step in cancer cell evolution. In fact ~90% of human tumors show high levels of telomerase activity when it is dormant in most somatic cells. Efforts to elucidate in detail the complex mechanism of telomere replication by telomerase as well as attempts to discover cancer and aging therapies that target this enzyme have been hindered to a certain extend by the absence of structural information. We recently determined the high-resolution structure of the catalytic subunit of telomerase from *Tribolium castaneum [1]*. The structure reveals a number of novel and unexpected results that greatly enhance our understanding of telomerase action on telomeres. The protein consists of four highly conserved domains, organized into a ring-like structure that shares common features with retroviral reverse transcriptases, viral RNA polymerases and to a certain extend bacteriophage, B-family DNA polymerases. Domain organization places motifs implicated in substrate binding and catalysis in the interior of the ring, which can accommodate seven-to-eight bases of double stranded nucleic acid. Modeling of an RNA/DNA heteroduplex in the interior of this ring reveals a perfect fit between the protein and the nucleic acid substrate and positions the 3'-end of the DNA primer at the active site of the enzyme providing some evidence for the formation of an active telomerase elongation complex.

[1] Gillis, A.J., A.P. Schuller, and E. Skordalakes, *TERT. Nature*, **2008**. 455(7213): p. 633-7.

Keywords: telomerase; cancer; aging

FA1-MS05-O3

Structural Studies of Candida Albicans Pathogenicity Factors: ALS Adhesins Family. Paula S. Salgado[a], Robert Yan[a], Lois Hoyer[b], Emesto Cota[a]. [a]*Division of Molecular Bioscience, Imperial College London, London, UK.* [b]*Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA.*
Email: p.salgado@imperial.ac.uk

Adherence to host cells is one of the key determinants of pathogenesis, yet limited information has been published on the structure and mechanism of fungal adhesins. C. albicans is a common human commensal that can cause a range of infections, from skin/mucosal 'thrush' to severe systemic candidiasis. The ALS family of surface glycoproteins is sufficient to confer key adhesive properties. These adhesins are able to bind a broad range of targets in host cell surfaces and the extracellular matrix [1, 2]. Moreover, they bind to small peptides and unstructured regions in folded domains in a stable, reversible and specific manner [3]. This seems to confer C. albicans with a unique mode of binding, eliminating the need of specific surface complementarity, as observed in most pathogen/host cell interactions. ALS adhesins consist of 3 domains: ~30kDa N-terminal, central Thr-rich domain with a variable number of 38aa repeats and a STN-rich membrane-anchoring C-terminal of variable length [4]. The N-terminal is proposed to be sufficient for adhesion and is, therefore, a possible target for new therapeutic strategies. Preliminary NMR data from NT-ALS1 reveals an IgG superfamily secondary structure topology, identical to that of the fibrinogen-binding regions

of clumping factor A from S. aureus [5] and sdrG from S. epidermidis [6]. Crystallographic studies of several ALS family members are under way and good native data has been obtained. ALS proteins require expression in specific E. coli strains that allow formation of disulphide bridges in the cytoplasm [7], critical for correct folding of the proteins. Combined with the low number of methionines present (<1/150aa), the need for strains that are not methionine auxotrophs significantly lowers the possibility of success using Se-SAD/MAD phasing methods. As observed with other IgG superfamily proteins, particularly considering the low sequence homology with available models, molecular replacement has proved difficult. However, a combination of careful model selection, iodide-soaks data and NMR structure information might prove successful, as on-going studies seem to indicate. We are currently working on determining structural models of NT-ALS9 in the apo and peptide-bound forms (data collected at BM14, ESRF, France) that will allow a better understanding of these adhesin family and their binding mechanisms. Studies of other family members, including different constructs and binding assays with a wider peptide range are also on-going. The structural information obtained will provide insights into C. albicans adherence to host cells, shedding light into the mechanisms underlying pathogenicity of this otherwise harmless human commensal.

[1] Gaur, N.K. and S.A. Klotz, Infect Immun, **1997**. 65(12): p. 5289-94. [2] Phan, Q.T., et al., PLoS Biol, **2007**. 5(3): p. e64. [3] Gaur, N.K. and S.A. Klotz, Microbiology, **2004**. 150(Pt 2): p. 277-84. [4] Hoyer, L.L., Trends Microbiol, **2001**. 9(4): p. 176-80. [5] Ponnuraj, K., Bowden, M. G., Davis, S., Gurusiddappa, S., Moore, D., Choe, D., Xu, Y., Hook, M. and Narayana, S. V. L. **2003** Cell. 115, 217-228. [6] Deivanayagam, C.C.S.,Wann, E.R., Chen, W., Carson, M., Rajashankar, K.R.,Hook, M. and Narayana, S.V.L. **2002**, EMBO J 21, 6660–6672. [7] Bessette, P.H., Aslund, F., Beckwith, J. and Georgiou, G. **1999** Proc. Natl. Acad. Sci. USA 96,13703–13708.

Keywords: fungal adhesins; crystal structure determination; NMR and crystallography

FA1-MS05-O4

Recognition and Activation of the RTK Met by the Bacterial Invasion Protein InlB.

Hartmut H. Niemann[a], Davide Ferraris[b], Ermanno Gherardi[c], Dirk W. Heinz[b]. [a]*Department of Chemistry, Bielefeld University, Germany.* [b]*HZI, Braunschweig, Germany.* [c]*MRC, Cambridge, UK.*
E-mail: Hartmut.Niemann@uni-bielefeld.de

Listeria monocytogenes targets the human receptor tyrosine kinase Met to induce its own uptake into a variety of normally non-phagocytic cells. We investigate the interaction between Met and its bacterial ligand, the listerial invasion protein InlB, using X-ray crystallography and other biophysical and biochemical methods. The crystal structure of the complex in two different crystal forms defines the details of the interaction revealing two interfaces between InlB and Met. The rigid internalin domain of the leucine-rich repeat protein InlB simultaneously binds to two different domains of Met that are separated by a flexible linker [1]. This two-site interaction probably fixes the otherwise flexible receptor in a signaling competent conformation. However, this postulated conformational change upon ligand binding does not fully explain the mechanism of receptor activation. In general, receptor tyrosine kinases are thought to be activated by ligand induced oligomerization. *In vitro*, we have so far not been able to observe Met dimerization upon binding of InlB. Based on different assemblies present in our crystal structures we discuss the structure of the signaling competent, dimeric activation complex.

[1] Niemann H.H., Jäger V., Butler P.J., van den Heuvel J., Schmidt S., Ferraris D., Gherardi E., Heinz D.W. *Cell*. 2007, 130, 235

Keywords: bacterial pathogenesis; ligand-receptor complexes; tyrosine kinase receptors

FA1-MS05-O5

Structure and Assembly of a Bacterial Pilus with Stabilizing Isopeptide Bonds.

Edward N. Baker[a], Neil Paterson[a], HaeJoo Kang[a]. [a]*School of Biological Sciences, University of Auckland, Auckland, New Zealand.*
E-mail: ted.baker@auckland.ac.nz

Pili are long, hairlike protein assemblies that extend from a bacterial surface and mediate colonization and pathogenesis. The pili of Gram-positive organisms are formed by covalent polymerization of a major pilin subunit that forms the shaft of the assembly. The crystal structure of the major pilin from *Streptococcus pyogenes* [1] revealed another striking feature in the form of internal covalent crosslinks (isopeptide bonds) formed between Lys and Asn side chains. Intramolecular crosslinks of this kind had never been seen before in proteins. We have solved the 1.6 Å resolution structure of SpaA, the major pilin protein that forms the shaft of the pili expressed by *Corynebacterium diphtheriae*. The structure shows that SpaA is folded into 3 tandem Ig-like domains, two of which contain self-generated isopeptide bonds between Lys and Asn residues. These bonds have been confirmed by mass spectrometry, which has also been used to identify the intermolecular isopeptide linkages in the native pili. The crystal packing reveals long columns of pilin molecules, modeling the assembly believed to occur in the pili. This structure, with that of the *S. pyogenes* major pilin, reveals key principles in Gram-positive pilus structure and stability: both intermolecular and intramolecular covalent crosslinks for strength and stability, and a modular construction that allows the incorporation of other Ig-like subunits into the pilus for specialized functions such as cell adhesion.

[1] Kang, H.J., Coulibaly, F., Clow, F., Proft, T., Baker, E.N. Science, **2007**, 318, 1625-1628.

Keywords: microbial pathogenesis; pilus assembly; isopeptide bonding