membranes and translocates substrates from the cytoplasm to the external milieu (secretioin) or vice-versa (uptake).

In the past few years, crystal structures of the components VirD4, VirB11 and VirB5 have become available and have provided seminal insights into the mechanism of T4SS assembly and substrate secretion. We here describe and analyse the structures of the perisplamic domains of VirB8 from *B. suis* and Comb10 from *H. pylori* (homologues of VirB8 and VirB10 from *A. tumefaciens*, respectively) which were solved by X-ray crystallography. These structures defines prototypes for their respective families of proteins and, together with other studies, will help define the secretion mechanism and/or machinery assembly of T4SSs.

**Keywords: DNA/protein transport, molecular machinery, bacterial pathogenesis**

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**Crystallization and X-ray Analysis of a Flagellar Hook Capping Protein**

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Many bacteria have flagella for chemotaxis. The bacterial flagellum is a macromolecular structure composed of the filament, hook and basal body. The flagellar hook capping protein FlgD is essential for the assembly of the hook. FlgE, the hook structural protein, is excreted to the cell exterior without polymerization in the absence of FlgD. With the hook cap FlgD, FlgE could fold property and get incorporated into the growing end of the hook. We recently revealed the atomic structure of the core fragment of FlgE and the dynamic mechanism of the hook as a molecular universal joint [1]. For further understanding of the hook assembly, we overproduced and purified FlgD from *S.typhimurium*. Analytical ultracentrifugation and chemical cross-linking experiment showed that FlgD forms pentamer in solution, suggesting that the FlgD pentamer is the capping structure [2]. We succeeded in obtaining single crystals of FlgD by the hanging drop vapor diffusion method and these crystals diffracted to 3.6 - 4.0 Å at SPring8 BL41XU. Currently we are working on improvements of crystal quality for higher resolution structure analysis.

[1] Samatey F.A., Matsunami H., Imada K., Nagashima S., Shaikh T.R., Thomas D.R., Chen J.Z., DeRosier D.J., Kitao A., Namba K., *Nature*, 2004, **431**, 1062-1068. [2] Matsunami H., Furukawa Y., Namba K., *in preparation.*

**Keywords: complexes, bacterial chemotaxis, macromolecular structure**

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**High Symmetry Involved in Cellular Regulation**

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170aa protein product of Ycfc gene crystallized in space group P23 with unit cell axes of 230.6 Å. The expected numbers of methionines in the A.U. (~240) discouraged Se-Met approach. SIRAS data for the Hg-derivative were collected to 4.2 Å. However, NCS could not been identified by standard programs. Derivative crystals had a substantial non-isomorphism with native data. Moreover, native Patterson map indicated translational pseudosymmetry. NCS was found using *ad hoc* software based on guessing the NCS arrangement from space group and packing considerations. The structure was solved by a combination of SAD phasing, NCS averaging and multiple crystal averaging.

Structural analysis revealed 2 identical, 24-meric oligomeric assemblies with 432 symmetry, placed on a diagonal, 3-fold crystallographic axis. At every non-crystallographic four-fold axis two molecules of $Cl^-$ ligand were identified.

Based on crystallographic and sequence conservation analysis, we hypothesize that this complex regulates gene activity, with Cl ions stabilizing a highly symmetrical form, possibly active as a repressor and/or activator of transcription. Binding of 12 Cl ions in a symmetrical assembly potentially creates a very steep response to chloride ion concentration.

Crystallographic and biophysical data will be presented for potential regulation mechanism.

**Keywords: non-crystallographic symmetry, pseudosymmetry, regulation**

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**Nanotubular Structures of Microtubule Complexes with Spermine and Lipid Membrane**

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The self-assembled structures of microtubules (MT) in the presence of spermine and charged membranes were investigated. Small angle X-ray diffraction and electron microscopy revealed several distinct morphologies of assembly. Complexation with spermine induced MT's to transform into a columnar phase of inverted tubules, in which the orientation of the tubulin units was switched from inside out. This rearrangement between two arrays of hierarchically structured nanotubules occurs through a novel phase transition driven by a discrete conformational change in the constituent tubulin subunit. In MT-membrane complexes, two new structures were observed. Depending on conditions, lipid vesicles either adsorb onto the microtubule, forming a 'beads on a rod' structure, or coat the microtubule to form a sheath. Tubulin rings can then coat the external lipid bilayer to form a multi-shell tubular structure with a tubulin-lipid-tubulin radial profile. Kinetic experiments were conducted to shed light on the mechanism of hierarchical complex formation.

**Keywords: microtubule, protein nanotube, small-angle X-ray scattering**

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**Structure of an Inter-ring Allosteric GroEL Mutant (E461K) at 3.3Å Resolution**

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The chaperonin GroEL in complex with its co-chaperonin GroES helps unfolded polypeptides to gain the active conformation through a nucleotide-regulated cyclic reaction. GroEL is an homo-oligomeric double heptameric toroid of 800 KDa with positive intra-ring and negative inter-ring cooperativity in ATP binding and hydrolysis. GroES is a dome-like 70kDa homo-heptamer that binds to the same GroEL ring where the other ligands (non-native polypeptide and nucleotide) are already bound. In this way, the complex GroES-GroEL forms a hydrophobic cavity where the peptide search for the productive structure in and isolated environment within the Anfinsen cage, and afterwards is delivered back to the medium. GroEL inter-ring communication is a temperature dependent interaction and salt-bridges E461-R452 and E434-K105 at the inter-ring interface regulate the 'thermostat' of GroEL. Disruption upon mutation of any of the two ionic contacts allows GroEL mutants to weaken the inter-ring negative cooperativity. Inter-ring communication disappears in wt GroEL at 42ºC whereas for E461K mutant this temperature is 37ºC. In order to understand how the thermostat of GroEL is programmed we have solved the structure of E461K mutant. Here we report the characteristics of this structure and give an explanation for the quaternary structural changes induced by this mutation.

**Keywords: GroEL, E461K mutant, inter-ring communication**