process occurs in the native mixture between two dimeric forms, MxM (swapped) and M=M (unswapped), held together by the additional constraints of two inter-chain disulphide bridges. This peculiarity offers the opportunity to study the effects of selected mutations at the O-interface on the M=M / MxM equilibrium, within a substantially invariant quaternary assembly. Two variants, having Pro 19 (P19A) or the whole sequence of the hinge peptide 16-22 replaced by the corresponding residues of RNase A (BS-hinge-A), show equilibrium and kinetic parameters of the swapping similar to those of the parent protein. On the contrary, mutation of L28 (L28Q, P19A/L28Q) significantly affects the swapping processes. The X-ray structures of the MxM forms of P19A and BS-hinge-A, and the MxM and M=M forms of the double mutant P19A/L28Q have been determined. The structural effects of the mutations are discussed on the basis of the MxM/M=M equilibrium data measured in solution. The relative insensitivity of the swapping tendency to the substitutions in the hinge region, and in particular to the replacement P19A, contrasts with the results obtained for other swapped proteins and can be rationalized in terms of the unique features of the seminal enzyme. On the other hand, the substitution in position 28 points to a crucial role of the interface residues in the swapping of BS-RNase.

Keywords: swapping , ribonuclease mutations, structural analysis

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Molecular Mechanisms of Thrombin-Mediated Fibrin Assembly

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Conversion of fibrinogen into fibrin (fibrin assembly) is mediated by thrombin, which cleaves two pairs of fibrinopeptides, fpA and fpB, from fibrinogen Aα and Bβ chains, respectively. Fibrin assembly occurs in two stages starting with the removal of fpA, which triggers formation of two-stranded protofibrils, followed by the removal of fpB, which enhances lateral association of protofibrils into thicker fibers. Non-substrate interaction of thrombin with the central part of fibrin(ogen) seems to play an important role in these processes. To establish the structural basis for the sequential cleavage of fpA and fpB, we crystallized thrombin in complex with a fragment corresponding to the central part of fibrin(ogen), and solved the structure of this complex at 3.65 Å resolution [1]. Next, we modeled possible arrangements of fibrinopeptides-containing NH_2-terminal portions of the Aα and Bβ chains, which were not identified in the electron density map. The resulting structure reveals that thrombin binds fibrinogen in a way that promotes positioning of the Aα chains in its active site cleft. This explains the preferential removal of fpA at the first stage of fibrin assembly. Subsequent modeling of a protofibril by docking the structure of the dimeric fibrin-derived D-D fragment into the complex suggests that the fpB-containing portions in such structure should interact with D-D to restrict their positions to the vicinity of the active site cleft. In agreement, surface plasmon resonance experiments reveal that these portions indeed interact with D-D. These provide the rationale for the accelerated removal of fpB upon the formation of protofibrils.

[1] Pechik I., Madrazo J., Mosesson M.W., Henandez I., Gilliland G.L., Medved L., *Proc. Nat. Acad. Sci. USA*, 2004, **101**, 2718.

Keywords: protein complex structure, fibrinogen, blood coagulation

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The Complex of the Polycomb Group Proteins Ring1B (RNF2) and Bmi1 –A Structure-function-analysis

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The Ring finger proteins Ring1B and Bmi1 belong to the Polycomb group proteins (PcG), which form large multimeric protein complexes. These complexes covalently modify histones leading to the transcriptional repression of specific genes. Mammalian homologs of Ring1B and Bmi1 are part of the PRC1L complex (Polycomb repressive complex 1-like), which acts as an E3 ligase in the ubiquitination of histone H2A [1].

Here we present the crystal structure of the Ring1B-Bmi1 Ring domain dimer at a resolution of 2.0 Å. The structure reveals that the interaction is mainly determined by structural elements that flank the Ring motifs. Details of the structure will be presented.

An *in vitro* ubiquitination assay was used to study E3 ligase activity of the Ring1B-Bmi1 complex. Adding either the full length Ring-Ring complex or the truncated form significantly activated ubiquitination of histone H2A.

[1] Wang H., Wang L., Erdjument-Bromage H., Vidal M., Tempst P., Jones R.S., Zhang Y., *Nature*, 2004, **431**, 873.

Keywords: ubiquitin system, Ring1B (RNF2), Bmi1

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Structure of a Giant Hemoglobin of the Gutless Beard Worm *Oligobrachia mashikoi*

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Pogonophorans and vestimentiferans obtain their nutrition solely from their symbiotic chemoautotrophic sulfur-oxidizing bacteria because they have no mouth or gut. These animals have sulfide-binding 400 kDa and/or 3500 kDa hemoglobin (Hb) which transports oxygen and sulfide simultaneously, and the symbiotic bacteria are supplied with sulfide by these giant Hbs. We have determined for the first time the crystal structure of a 400 kDa vascular Hb from pogonophoran *Oligobrachia mashikoi* at 2.85 Å resolution as an intact oligomeric form. The structure is hollow-spherical, with outer and inner diameters of about 120 Å and 50 Å, respectively, and composed of a total of 24 globins as a dimer of dodecamer. This dodecameric assemblage would be a common unit to both 400 kDa and 3500 kDa Hb. The structure of the mercury derivative used for phasing provides insights into the sulfide-binding mechanism. The mercury compounds bound to all free Cys residues that have been expected as sulfide binding sites. Some of the free Cys residues are surrounded by Phe aromatic rings, and mercury atoms come into contact with these residues in the derivative structure; it is strongly suggested that sulfur atoms bound to these sites could be stabilized by aromatic-electrostatic interactions by the surrounding Phe residues.

Keywords: proteins, hemoglobins, supramolecular assemblies

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Structural Biology of Type IV Secretion System

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Type IV secretion systems (T4SSs) are machineries used for the transport of macromolecules across the bacterial cell envelopes of Gram-negative bacteria. The archetypal T4SS, the VirB/D system was first 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) whereas T4SS in other bacteria can display homologues for only a subset of those proteins. T4SSs are highly versatile and have been found in many bacterial pathogens such as *Helicobacter pylori* (Cag PAI), *Brucella suis* (VirB/D), and *Legionella pneumophila* (Dot, Icm). The secretion machinery spans both bacterial

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