s1.m8.p16 **3D Domain-swapped dimers of human cystatin C and their interactions - implications for amyloidogenesis.** M.Jaskolski, R.Janowski, *Department of Crystallography, A.Mickiewicz University and Center for Biocrystallographic Research, Inst. Bioorg. Chem., Pol. Acad. Sci., Poznan, Poland. E-mail: mariuszj@amu.edu.pl*

**Keywords: 3D domain swapping; Protein aggregation; Amyloid**

Proposed earlier as a possible mechanism of amyloid formation, the phenomenon of 3D domain swapping has been first observed in aggregation of an amyloidogenic protein in the case of human cystatin C (HCC). HCC is a physiological inhibitor of cysteine proteases, present in particularly high concentration in the cerebrospinal fluid, but it is also found in amyloid aggregates formed in the brain arteries at advanced age. This pathological process is greatly accelerated with a naturally occurring L68Q variant, resulting in fatal amyloidosis in early adult life. When proteins are extracted from HCC amyloid deposits, an N-truncated cystatin C variant (THCC) is found, lacking the first 10 amino acids of the native sequence. Human cystatin C has been studied in three crystal forms, one of which corresponds to the N-truncated variant, and in all cases 3D domain-swapped dimers were found. The dimers arise by partial unfolding of the monomeric protein through opening of a β-hairpin loop followed be a reconstruction of the monomeric fold from two protein chains. The domain that is exchanged consists of a long α-helix flanked by two β-strands. In one of the structures, the dimers have crystallographic two-fold symmetry, while in all other cases (involving multiple copies in the asymmetric unit) they are pseudosymmetric. The act of domain swapping is in all the cases the same (conservation of the closed interface) but the dimers are not identical, reflecting the flexibility of the linker region (open interface). This is particularly pronounced in the newly determined tertragonal structure, where the mutual orientation of the two monomer-like domains is totally different from the previous situations. The closed dimers enter into conserved intermolecular interactions suggestive of their possible significance for higher aggregation of HCC. One of the packing modes involves the formation of closed octameric assemblies via convoluted H-bonding interactions between a loop structure and the extended molecular β-sheet. Another pattern involves hydrophobic interactions between the dimer-specific open interface regions. The most intriguing patterns take the form of intermolecular β-sheet bonds, reminiscent of the cross-β interactions believed to form the core of the amyloid structure. In the case of THCC, a dimer β-sheet is extend to another dimer with recruitment of a water molecule. The tetragonal HCC structure suggests how the β-sheet interactions could be propagated infinitely, as would be expected in an amyloid fibril.

s1.m8.p17 **On the Construction of a Model Signaling System with Structural Information: Structural Studies of *Bacillus subtilis* Stress Response Regulators.** Tomonori Kaneko, Hiroyuki Koyama, Nobuo Tanaka and Takashi Kumasaka, *Dept. of Life Science, Tokyo Institute of Technology, Japan. E-mail: tokaneko@bio.titech.ac.jp*

**Keywords: Network; $\sigma^B$; Sporulation**

A large number of biological macromolecules interact with one another to work inside, outside or on the boundary of cells. Integrating such interactions results in a construction of a network. Here, each macromolecule, usually a protein, is a device of the network. The nature of this network is expected to be very different from that of other networks such as the Internet or brain/neural networks. The features of these networks are that they are composed of simple devices, although the output of their resulting networks can be complicated. On the other hand, in a macromolecular network, each device itself is a complex matter. Can we understand such type of networks at the molecular level? Can we predict their outputs as *in vivo* events? We propose here a model system to analyze a signaling pathway based on the structural information of each component. Once a *Bacillus subtilis* cell suffers from a stress, it stimulates signal regulator proteins that finally induce sporulation in the cell. Nutritional stress signal is transmitted from a putative hydrolase RsbQ to a transcription factor $\sigma^B$, through a phosphatase RsbP, an anti-anti-$\sigma^B$ protein RsbV, and an anti-$\sigma^B$ kinase RsbW. As the first stage, we have been trying to solve these structures. The structure of RsbQ has already been solved. This putative hydrolase has been proposed to activate the down-stream phosphatase RsbP by modifying it in an unknown way. Although RsbP has a PAS domain, the function as well as its ligand has yet to be elucidated. Some of our recent results about them will be shown.