1P193**Application of polarized IR-microscope spectroscopy and solid-state NMR to structural analysis of protein fibers**

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For structural analysis of fibrous proteins, we tried to use the following two methods combined. (1) The polarized IR-microscope spectroscopy, to understand the orientation of components in aligned fibrils, and (2) the ¹³C solid-state NMR, to obtain structural information at atomic resolution. Applying these methods together with CD, AFM, IR, and ¹H-NMR in solution, we perform the structural analysis of the following two model protein fibers. One model is a helical fiber made from α3 peptide [(LETLAKA)₃] and the other is a β-sheet fiber formed from KK peptide [KFIVIFK]. α3 peptide appears to consist of three different constituents: α-helix, random coil, and ₃₁₀ helix or type III turn, judging from IR measurements. Polarized IR-microscope spectra of the single isotope-edited α3 peptide show that the α-helix constituent is the most aligned and others are leaned to some extent. Each terminus of the peptides shows the characteristics of the random coil, whereas the center part shows those of the helical conformation. KK peptide does not form fibril without salts while it remains to hold intramolecular long-range interaction between N- and C-terminal regions in solution. KK peptide with 0.1M salt is known to change into fibrils. In the fibrous state, according to the REDOR NMR experiment of the solid sample, the distance between 2F-6F is shown to be too long for the anti-parallel β-sheet, suggesting that KK peptide forms the parallel β-sheet. We anticipate that these results would suggest versatility of the combined methods.

1P195**Crystal structure of infectious bursal disease virus VP2 subviral particle: Implications in virion assembly and immunogenicity**

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The structural protein VP2 of infectious bursal disease virus (IBDV) spontaneously forms a dodecahedral T = 1 subviral particle (SVP), and is a primary immunogen of the virus. In this study, the structure of IBDV SVP was determined in a cubic crystal and refined to 2.6 Å resolution. It contains 20 independent VP2 subunits in a crystallographic asymmetric unit. Each subunit is folded mainly into a shell domain and a protrusion domain, both with the Swiss-roll topology, plus a small helical base domain. Three VP2 subunits constitute a tight trimer, which is the building block of IBDV (sub)viral particles. The structure revealed a calcium ion bound to three pairs of symmetry-related Asp31 and Asp174 to stabilize the VP2 trimer. Our results of treatment of SVP with EGTA, a Ca²⁺-chelating reagent, indicated that the metal-ion may be important not only in maintaining highly stable quaternary structure but also in regulating the swelling and dissociation of the icosahedral particles. A Ca²⁺-dependent assembly pathway was thus proposed, which involves further interactions between the trimers. The 20 independent subunits showed conformational variations, with the surface loops of the protrusion domain being the most diverse. These loops are targets of the neutralizing antibodies. Several common interactions between the surface loops were clearly observed, suggesting a possible major conformation of the immunogenic epitopes.

1P194**Multiple protein docking guided by low-resolution image of complex using Gaussian mixture model**

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Electron microscopy measurement of single-particles now provides low-resolution 3D images of large bio-molecular complex composed of many proteins. In most of the cases, each protein structures of the complex are solved by X-ray crystallography or NMR. Superimposing these two kinds of data can provide a high-resolution model of large complex. In the calculation, multiple atomic models are assembled to make a complex which overlaps well with the given low-resolution image. The docking of multiple proteins, however, generally required high computational costs. To reduce the cost, we employed the Gaussian mixture model to represent the approximated shape of atomic model. A model with thousands of atom spheres is roughly represented as the sum of several number of 3D Gaussian density functions. Another advantage of Gaussian models is that integrals and derivatives for various terms of Gaussian functions are easily obtained in analytical form. We defined the energy for overlapping an atomic model and an image density using Gaussian function. We also introduced attraction and repulsion potential energy between Gaussian molecules. All these energies, and their forces and torques can be analytically calculated. To find the optimal configuration of protein chains, we implemented the steepest descent method and the simulated annealing Monte Carlo method. Test calculations for simple dimer cases showed that our method efficiently found the reasonably good configurations within a short computational time.

1P196**Purification of the Type III Secretion Machineries of *Vibrio parahaemolyticus***

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Vibrio parahaemolyticus is a world-wide cause of food-borne gastroenteritis. Recent genome sequencing identified two sets of genes for the putative type III secretion systems. 'Type III secretion' is a mechanism by which some pathogenic bacteria inject proteins, called effector proteins, straight into the cytosol of the eukaryotic cells to hijack their intracellular machinery. Recent progress in visualization of the 'injectisomes' of *E. coli*, *Shigella* and *Salmonella* species revealed their needle-like structure (NS) embedded in bacterial membranes. As for *V. parahaemolyticus*, several secreted effector proteins have been identified, and their attribution to pathogenicity is tested and discussed. Since deletion(s) of the component(s) of each TTSS apparatus inhibited the secretion of these effectors, it seems that *V. parahaemolyticus* indeed produces two types of the NS. However, it has not yet been successful in observing the actual apparatuses of either secretion system. The goal of this study is to isolate the 'injectisomes' of either of TTSS for structural observation. So far, we have searched for the optimal condition for each system to differentiate the expression of the two needle structures. Then, the purification strategies are being revised with referring to those of the needles and flagella of other species. At this moment, we have come to observe the ring structures, probably the outer-membrane ring of the NS, since we have chased the structures using the antibody to this ring.