- 1P079** DFTによるテラヘルツ領域におけるアミノ酸とペプチドの低振動モードの帰属
DFT approach for the assignment of low-frequency vibrational modes of amino acids and peptides in the terahertz frequency region

Ohki Kambara (*RIE, Shizuoka Univ.*)

To understand the low-frequency vibrational modes observed in the terahertz (THz) spectroscopic region, not only the experimental study but also the theoretical works are needed. In this paper, the frequency calculation of amino acid including glycine and L-alanine and these short peptides are performed with density functional theory (DFT) package, CRYSTAL09, where the periodic boundary condition (PBC) is implemented. The number of theoretically obtained normal modes in the THz region increase with increasing chain length. This trend continues to the bigger polypeptides, proteins, whose THz spectra are uniformly smoothed and featureless. On the other hand, temperature dependence of calculated outcomes is discussed at the same time.

- 1P082** 一分子力学測定によるポリプロリンヘリックスの高弾性の研究
Single molecule force spectroscopy by AFM indicates highly resilient structure of polyproline helix

Masaru Kawakami (*School of Materials Science, Japan Advanced Institute of Science and Technology*)

The polyproline helix II (PPII) plays an important role in protein-protein interaction for intercellular cohesion or enzyme activation. The PPII structure has also known as a “rigid rod”, and has been used as “spacer” or “molecular ruler” in the fluorescence spectroscopy. We performed single molecule force spectroscopy by AFM to measure the mechanical property of PPII. PPII showed a completely linear response to tensile force, which is quite different from other polypeptide chains. This is indicating that PPII behaves as a rigid spring. Force curve analysis provided a quantitative estimate of the molecular stiffness of PPII, and we found that the stiffness of the PPII and PPI are comparable to those of globular proteins, or much higher than those of helical proteins.

- 1P080** 吸引式反応システムを用いたウェスタンブロットティング法によるペプチドの高感度検出
A new approach to detect small peptides clearly and sensitively by Western blotting using a vacuum-assisted detection method

Satoshi Tomisawa, Chiharu Abe, Masakatsu Kamiya, Takashi Kikukawa, Makoto Demura, Keiichi Kawano, Tomoyasu Aizawa (*Grad. Sch. Life Sci., Hokkaido Univ.*)

Western blotting is a widely used technique for the detection and quantification of proteins and peptides. However, it is difficult to detect small peptides efficiently by the conventional Western blotting method with shaking. In this study, we show that the previously developed vacuum-assisted detection method greatly improves the detection of small peptides without additional protocol modification. The vacuum-assisted method was developed to shorten the time required for all immunodetection steps, and all the Western blotting solutions penetrated the membrane quickly and efficiently by this method. By using this vacuum method, we succeeded in detecting small peptides that were completely undetectable by the conventional Western blotting method.

- 1P083** 赤痢菌ニードル複合体の極低温電子顕微鏡による構造解析
Structural analysis of needle complex from *shigella flexneri* by cryo electron microscopy

Naoko Kajimura^{1,2}, Martin P. Cheung³, Takayuki Kato¹, Ariel J. Blocker³, Keiichi Namba^{1,4} (¹*Grad. Sch. of Frontier Biosci., Osaka Univ.*, ²*JEOL Co., Ltd.*, ³*Sch. of Cell. & Mol. Med., Univ. of Bristol*, ⁴*QBiC., RIKEN*)

Many gram negative pathogenic bacteria possess a Type III Secretion System (T3SS) which plays a key role in injecting virulence proteins into host cells. This huge molecular machinery is believed to be composed of more than 20 different proteins and divided into three domains: an extracellular needle, a transmembranous basal body and cytoplasmic domain. Although, a part of T3SS components have been characterized by structural, biochemical and genetic approach, many questions concerning the arrangement of components and of the regulation of virulence effector protein secretion are remained. We will report the results of structural analysis and characterization of T3SS complex purified from *shigella* using single particle image analysis.

- 1P081** Grb2 結合にともなう EGFR のキネティクスとダイナミクスの 1 分子計測
Single-molecule measurements of kinetics and dynamics of an epidermal growth factor receptor upon Grb2-binding

Kenji Okamoto, Yasushi Sako (*RIKEN*)

Epidermal growth factor receptor (EGFR) is one of the membrane proteins which trigger cellular signal transduction pathways. EGFR has multiple Grb2-binding sites on its intracellular C-tail domain, which is intrinsically disordered (ID). Years ago, Grb2-binding kinetics was found to be nonlinear and it has been suggested that structural dynamics of this ID domain plays an important role for the kinetics. We have prepared the C-tail fragment molecule and investigated the Grb2-binding kinetics and the structural dynamics on it by *in vitro* single-molecule imaging and single-molecule fluorescence resonance energy transfer (FRET) measurement. The results suggest the existence of multiple conformational states induced by Grb2 binding and dynamical transitions between them.

- 1P084** 高分解能構造解析に向けた電子顕微鏡用カメラの評価
Evaluation of cameras for high resolution structural analysis by cryoEM

Takayuki Kato¹, Tomoko Miyata¹, Keiichi Namba^{1,2} (¹*Grad. Sch. Frontier Biosci., Osaka univ.*, ²*QBiC, Riken*)

The CCD and CMOS cameras have been developed as a new recording media substituting for the film, and they are now widely accepted by those in the electron microscopy field. However, the point spread function (PSF) of the CCD/CMOS cameras is worse than that of the film, so nowadays the film is still used for high resolution structural analysis of biological macromolecules. A state of the art camera, direct electron detector, can detect electrons without converting to photons by scintillators and therefore, a PSF as good as that of the film can be achieved. Here, we will compare the PSF and the 3D structure of biological macromolecules reconstructed from the data recorded on commercially available standard cameras and direct electron detecting cameras.