1P103 **NMR, QCM, MD シミュレーションによる κ -オピオイド受容体細胞外第2ループとダイノルフィンとの細胞膜中での相互作用解析**
Interaction of ECL-II of κ -opioid receptor with dynorphin in membrane environments as revealed by solid state NMR, QCM and MD simulation

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κ -receptor is a member of the opioid receptor family and selectively interacts with dynorphin (Dyn). Interaction of extracellular loop II (ECL-II) of the κ -receptor and N-terminal α -helix of Dyn was investigated. 13C MAS NMR spectra were recorded and the 13C chemical shift perturbation clearly indicated that Dyn interacts with ECL-II at the location of Val10-Ala15. QCM measurements were performed to determine the binding constant of ECL-II with Dyn and indicated that the binding constant between Dyn and ECL-II was 72 times larger than that between Dyn and lipids. The result of the MD simulation indicates that the C-terminus of Dyn interacts with the amino acid residues of the region between Val10-Gln14 of ECL-II.

1P104 **分子動力学計算から明らかにされた細菌機械受容チャネル MscL のゲーティングにおける N 末端領域のメカノセンサーとしての役割**
The N-terminal domain acts as a mechanosensor in the gating of the bacterial mechanosensitive channel MscL: molecular dynamics study

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E-coli mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane (TM) helices, and its 3D structure of the closed state has been resolved. The major issue is to understand the gating mechanism driven by tension in the membrane. We performed MD simulations for the opening of new MscL model with the N-terminal (S1) helices running parallel to the cytoplasmic membrane. Upon membrane stretch, the TM helices are dragged and tilted by lipids at Phe78 at the periplasmic side, leading to the gate expansion. Furthermore, it is found that the cytoplasmic side of the TM helices is expanded more widely than that of the previous MscL model with the bundled S1 helices, suggesting that the S1s of the new model MscL play a role of a mechsnosensor.

1P105 **改良した多リン酸力場を用いた ATP/ADP 結合状態の筋小胞体カルシウムポンプの分子動力学計算**
Molecular dynamics simulations of ATP/ADP bound forms of SR Ca²⁺-ATPase using CHARMM force field with modified polyphosphate parameters

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Sarcoplasmic reticulum (SR) Ca²⁺-ATPase transports two Ca²⁺ from the cytoplasm into the SR lumen utilizing ATP hydrolysis. It is fundamental to know how ATP/ADP can bind stably in the cytoplasmic domain of the ATPase before/after the reaction. We performed all-atom molecular dynamics (MD) simulations of the ATPase in the ATP/ADP bound forms with explicit solvent and a DOPC lipid bilayer. We observed the phosphates structure in the bound ATP changed rapidly into the extended form using the original CHARMM force field. After some modifications of the force field, we could simulate the protein without such changes. We examine the essential interactions stabilizing bound ATP with the ATPase and discuss the relationship between the simulation results and experimental data.

1P106 **膜孔形成蛋白質 FraC の構造変化機構の解析：可溶性構造から膜貫通構造までの変遷**
Structural analysis of the α -helical pore-forming toxin FraC; metamorphosis from a water-soluble to a transmembrane protein

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The pore forming toxins (PFTs) are cytolytic proteins that bind to cellular membranes, where spontaneously metamorphose from a water-soluble form to transmembrane pores. Fragaceatoxin C (FraC) is an α -PFT secreted by the sea anemone *Actinia fragacea*.

In this study we have investigated the structural transition of FraC by determining its crystal structures in the water-soluble and lipid-bound monomeric forms and that of an assembly intermediate. We are also working on the preparation of crystals of the transmembrane pore of FraC. Our results demonstrate multivalent protein-lipid and protein-protein interactions in the context of the membrane trigger the spontaneous conformational change and assembly of a soluble protein into a multitopic membrane protein.

1P107 **性フェロモン生合成活性化神経ペプチド受容体 (PBANR) の発現、リガンド結合、会合状態の解析による結晶化のための T4 リゾチーム置換位置の検討**
Positional optimization of T4L that replaces IL3 of PBANR for crystallization by expression, ligand binding, and size exclusion analyses

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The silkworm pheromone biosynthesis-activating neuropeptide receptor (PBANR), a class-A GPCR, activates the biosynthesis of sex pheromone by binding PBAN. We aim to solve the crystal structure of the PBAN-PBANR complex. For crystallization, we designed several PBANR constructs with truncated N- and C-termini and the third intracellular loop (IL3) mostly replaced by T4 lysozyme (T4L). The best PBAN-PBANR microcrystal so far diffracted X-rays to 12-Å resolution. To obtain better diffracting crystals of the complex, we have optimized the insertion position of T4L out of 21 PBANR constructs by comparing their expression levels, ligand binding ability and elution patterns in FSEC. Based on these data, we have chosen two as the constructs suitable for crystallization.

1P108 **レプリカ交換アンブレラサンプリングシミュレーションでのコレステロール分子との相互作用によるアミロイド前駆体の C99 単量体の構造変化**
Structural change of APP-C99 induced by interactions with cholesterol studied by Replica Exchange Umbrella Sampling (REUS) simulation

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The A β peptide, critical to Alzheimer's Disease (AD), is produced by cleavage of the transmembrane of Amyloid-Precursor-Protein-C99 (C99) by γ -secretase. It has been observed that increased levels of cholesterol are correlated with early onset of AD. It has also been proposed that cholesterol binds specifically to C99 and that interaction with cholesterol induces a structural change in C99, possibly affecting the recognition of C99 by γ -secretase. This research investigated the C99-cholesterol complex structures, including the structural change of C99 (686-726) monomer, by all-atom REUS simulations in an implicit membrane model. Results of REUS simulations were compared with NMR experiments to gain insight into the impact of cholesterol on C99 structure and processing.