1P025 アカネ科由来抗腫瘍活性ペプチド RA-VII の構造解析
Structure analysis of antitumor peptide RA-VII from *Rubia Cordifolia*

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RA-VII is antitumor cyclic hexapeptide, which is isolated from *Rubia cordifolia* and *Rubia akane*. This peptide is comprised of alanines and modified tyrosines. The peptide has an 18-membered cyclohexapeptide ring and a 14-membered cycloisodityrosine ring in the structure. It is suggested that 14-membered ring and Tyr-3 of 18-membered ring are indispensable for the antitumor activity, and the active conformation of 14-membered ring is maintained by the presence of the 18-membered ring. We study the conformers of RA-VII and its analogs by quantum chemical calculation to explore the relationship between the structure of RA-VII and the antitumor activity.

1P026 分子シミュレーションによるラクトースリプレッサーの転写制御機構のモデル提案
MD and *ab initio* MO simulations on transcriptional mechanism controlled by lactose repressor protein and ligand

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Lactose repressor protein (LacR) controls the transcriptional mechanism of gene information from DNA to mRNA in a ligand-dependent manner. Although the ligand-binding to LacR was found to change the mechanism drastically, the effect of ligand-binding on the conformation of LacR+DNA complex has not been clarified at atomic and electronic levels. We here investigated the change in conformation of LacR-dimer+DNA complex induced by the ligand-binding, using molecular dynamics simulations and *ab initio* fragment molecular orbital calculation. The results elucidate that the binding of an inducer to LacR significantly changes the LacR structure to cause strong interactions between LacR monomers, resulting in weakening the interactions between LacR-dimer and DNA.

1P027 抗体の親和性成熟に着目した抗原 - 抗体結合挙動への洞察
Insight into the antigen binding motion of germline and affinity-matured antibodies

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The main purpose of this study is to elucidate the change of structural dynamics of antibodies during affinity maturation and their antigen binding mechanisms. We expressed single-chain Fvs (scFvs) of anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies in *E. coli*, and purified them. Analysis of antigen binding kinetics using Biacore showed that affinity-matured C6 scFv formed more stable complex than germline N1G9 scFv and mutants, mainly due to the dissociation rates. Analysis of structural dynamics at the single-molecule level using diffracted X-ray tracking showed that the fluctuations of scFvs were decreased upon the antigen binding. In comparison with N1G9, C6 with antigen had less fluctuated motion, which might be correlated with the more stable complex.

1P028 L-Thr と NAD⁺ の結合に伴う *Cupriavidus necator* 由来 L-スレオニン脱水素酵素の構造変化の解析
Analysis of structural change of L-threonine dehydrogenase from *Cupriavidus necator* (CnThrDH) by binding of L-Thr and NAD⁺

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We determined and analyzed the structural changes of CnThrDH: apo (2.25Å), NAD⁺-binding (2.5Å) and L-Thr and NAD⁺ binding (2.5Å) forms. Differences of these three structures indicated that switching of “open” and “closed” states occurs at the regions of 80-87 and 180-186 during the dehydrogenation reaction. MD simulation and kinetic analysis also supported the switching of the states: the closed state changed to open state during the simulation, and the *kcat*/*Km* values of variants which are difficult to form the closed state are much lower than that of wild type. We concluded that the switching is essential to exhibit the high reactivity of CnThrDH [1].

[1] Nakano et al., J. Biol. Chem. 2014, 289: 10445-10454

1P029 アナアオサ由来のプラストシアニンにおける弱い相互作用の役割
The role of weak interaction in a blue copper protein, plastocyanin from *Ulva pertusa*

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Plastocyanin from *Ulva pertusa* (UPc) is a blue copper protein, which performs as an electron carrier between cytochrome b6f complex and P700. The active site copper atom of UPc is coordinated by His37, His87, Cys84, and Met92 with a distorted tetrahedral structure.

Several weak interaction in the protein has been considered to express the unique structure and properties of UPc. The X-ray crystallographic structure analyses of UPc were reinvestigated to know the more details of the correlation between the weak interaction and properties. The EXAFS studies of UPc was also performed to obtain the precise active site structural information. The crystallographic structural data and the bond distances evaluated from EXAFS of UPc demonstrated different structural parameters.

1P030 ブルー銅タンパク質シュウドアズリン Met16His/His6Val 変異体の性質と構造の pH 依存性
pH dependency of the structure and properties of a blue copper protein, Met16His/His6Val pseudoazurin mutant

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The active site of pseudoazurin (PAz) is coordinated by two nitrogen atoms (His40, His81), one thioether S (Met86), and thiol S (Cys78) with distorted tetrahedral structure. Met16 is located at the vicinity of His81. The weak interaction between Met16 and His81 has been explained to be important for regulation of electronic structure of PAz. Met16His/His6Val double mutant was constructed to know the effect of His closed to active site.

The electronic absorption spectra of the Met16His/His6Val mutant protein in the acidic pH range demonstrated the increasing of the axial component, and a pKa of newly introduced His16 was estimated to be 4.62, which is relatively lower pKa value of the normal His imidazole group by the positive charge effect of the active site copper ion.