- 1P102 膜タンパク質の糖鎖修飾部位予測のためのアミノ酸配列解析
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- 1aC07 Amino acid sequence analysis of prediction of glycosylation sites in membrane proteins
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Many proteins are modified after translation at ribosome. Glycosylation is the most popular posttranslational modification, it is considered that more than half of all proteins are glycosylated. Its roles are intracellular localization, adhesion between cells and stabilization of proteins and are very important medicinally. Modified sites of proteins are usually discussed with motif sequence. N-linked glycosylation has a short motif sequence as -N-(P)-[ST]-{P}-, but because of its smallness, the prediction of the glycosylation sites is not so accurate. In this work, we aim to find out the common physico-chemical properties and permit accurate prediction of glycosylation sites. First, we searched the differences between overall modified loop regions and unmodified loop regions, we developed a score (Global Score) using the average values of several physico-chemical parameters. Secondly, we also analysed the properties of local segments of 21 amino acids around the glycosylation sites, dividing them to five segments of 4-4-5-4-4 amino acids. Using these average values, we devised a score (Local Score). Finally, we carried out the discriminant analysis, using the two scores. The accuracy of the discrimination of glycosylation sites in membrane proteins was more than 90%.

- 1P104 水-膜分配の測定を目指した水溶性膜貫通ヘリックスのデザイン
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- 1aC09 Design of a soluble transmembrane helix for measurement of water-membrane partitioning
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To understand the folding process of α -helical membrane proteins in a lipid bilayer environment, the thermodynamics of membrane partitioning of a helix should be elucidated. We aimed at determining the thermodynamic parameters (ΔG , ΔH , and ΔS) for transfer of transmembrane helices from water to membranes by modifying the N-terminus of the model peptide (AALALAAALXLAALALAAC(NBD)-NH₂, X = guest residue, NBD = 7-nitro-2-1, 3-benzoxadiazol-4-yl), which was confirmed to form a stable transmembrane helix in membranes. To solubilize the hydrophobic peptide (X=L) in water as a monomer, 10 charged residues (KR)₆ were added at the N-terminus. The modified peptide was soluble in water without aggregation. After addition of large unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, a rapid partitioning of the peptide to membranes was observed, as detected by a blue shift and an increase in NBD fluorescence. Quenching experiments with the membrane impermeable quencher Na₂S₂O₄ showed that most of the peptides (>80%) assumed a transmembrane topology with its C-terminus inside the vesicles. We will determine the thermodynamic parameters for the partitioning.

- 1P103 Asymmetry in membrane protein sequence and structure: Glycine outside rule
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- 1aC08 Asymmetry in membrane protein sequence and structure: Glycine outside rule
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The number of membrane proteins in the protein data bank, although much less than that of soluble ones, is rapidly growing, which makes us possible to perform new statistical survey. Among many statistical surveys on membrane protein structures, amino acid distribution along the normal coordinate is the most popular. It, however, is usually assumed as symmetric ignoring possible difference between outside and inside of the membrane. The recently developed OPM database provides orientation information of membrane protein structures, which motivated us to analyze asymmetry in amino acids distribution of helical transmembrane proteins.

We found that LEU, ILE, LYS, and ARG are distributed more on the inside interface than the outside, whereas GLY distribution is biased to the outside of the membrane. Most interestingly, the bias in GLY distribution is markedly strong. For LYS and ARG, this bias is consistent with the so-called "positive inside rule". Asymmetry in LEU and ILE are modest, yet significant. Bias in GLY in the outside led us to investigate asymmetry in the distribution of secondary structure because GLY is a well-known coil/loop maker. Indeed, we found that the probability of the coil is higher in outside than in inside interface. Yet, it turned out that this bias in the secondary structure alone is not strong enough to explain the stronger bias in GLY distribution. The bias in GLY distribution is at the moment mysterious.

- 1P105 コレステロール含有脂質二分子膜における膜貫通ヘリックス会合の熱力学量の測定
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- 1aC10 Measurement of thermodynamic parameters for self-association of a transmembrane helix in lipid bilayers containing cholesterol
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Self-association of transmembrane helices, which is a crucial thermodynamic step for membrane protein folding, is determined not only by the amino acid sequences but also by lipid compositions. Cholesterol plays an important role in modulation of physical and functional properties of lipid bilayers. For example, it has been known that cholesterol with a planar rigid steroid ring increases bilayer thickness and reduces fluidity. Here, we measured the complete set of thermodynamic parameters (ΔG , ΔH , ΔS , and ΔC_p) for the formation of the antiparallel dimer of the inert hydrophobic model transmembrane helix X(AALALAA)₅Y (X = NBD and Y = NH₂ (I) or X = Ac and Y = DABMI(II)) in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) large unilamellar vesicles containing 30 mol% cholesterol at 15–55°C, based on fluorescence resonance energy transfer from I to II. It was confirmed that the peptide formed a stable transmembrane helix with an orientation angle of about 30° by polarized ATR-FTIR spectroscopy. Stronger dimerization was observed in POPC/cholesterol (7/3) vesicles (ΔG = -20.9 kJ mol⁻¹, ΔH = -65.3 kJ mol⁻¹, - $T\Delta S$ = 44.4 kJ mol⁻¹ and ΔC_p = 1.3 kJ K⁻¹mol⁻¹) than in POPC vesicles (ΔG = -12.8 kJ mol⁻¹, ΔH = -27.9 kJ mol⁻¹ and - $T\Delta S$ = 15.1 kJ mol⁻¹). We will also report the effect of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine, which increases membrane lateral pressure, on thermodynamics for the formation of the antiparallel dimer.