3P079 抗菌ペプチド cecropin P1 の大腸菌発現系における発現効率に影響を与える要因の解明
Elucidation of influential factor for productivity of the antimicrobial peptide using Escherichia coli


Cecropin P1 (CP1), an antimicrobial peptide found in nematodes from the stomachs of pigs, is 31 amino acids in length. In a membrane-like environment, CP1 forms an α-helical structure and believed to damage microbial membranes. We designed a soluble fusion protein that contains thioredoxin on the N-terminal side of CP1. This fusion construct still showed the cytotoxicity upon the expression in the Escherichia coli cells. However, this toxic behavior was improved when we tried the expression of the derivatives lacking a few C-terminal residues. In this study, we focused on the C-terminal region of CP1 and investigated the contribution of this region to the inhibition of E. coli growth and to the interaction with E. coli membranes using some fluorescent dyes.

3P080 ベイズ推定を用いた NMR 立体構造計算法の開発
A refinement method for NMR protein structure determination based on Bayesian inference

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Conventional NMR structure determination requires to determine a weight factor for balancing experimental data and a physical forcefield, and to estimate calculation constants that convert NOE data into three-dimensional structure information. Thus, it is necessary in the conventional method to empirically fix those parameters despite of different attributes of the experimental data derived from noise and ambiguities. We developed a refinement method for NMR protein structure determination based on Bayesian inference. The new structure calculation method shows that it can provide sufficiently accurate structures even with less NOE peaks. Moreover, it dramatically improves protein structures of the proteins in living E.coli cells.

3P081 複雑分子系の異性化反応ネットワークに埋め込まれた時間階層構造の抽出
An extraction of hierarchical organization of embedded timescales buried in complex reaction network

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Reactions involved in complex molecular systems such as proteins have a variety of timescales from fs to seconds or much longer even in a molecule. To provide theoretical framework for multiple timescales systems, we develop a novel method for constructing a timescale hierarchal diagram of a Markov chain (or “energy landscape”). Since one can derive a Markov chain from a 1st order chemical reaction, our method can capture the hierarchical organization of embedded timescales buried in complex reaction network. As an illustrative example, we apply our method to a reaction network of Claisen rearrangement of allyl vinyl ether, computed quantum-chemically in terms of the global reaction route mapping method and compare the results with the experimental rate constant.

3P082 RI に依存しない高感受度 MGMT 活性測定法の開発と新型マイクロアレイ MMV への適用化
Development of MGMT activity assay methods of high sensitivity and being adaptable to the novel-concept microarray

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For the treatment of a brain cancer glioblastoma, MGMT behaves as a suppressor of anti-cancer drug TMZ (temozolomide) due to the cancellation effect of TMZ-derived methylation. We have devised a novel in vitro selection system based on MMV (well type novel concept microarray), which enables function-based screening of target molecules. For this screening system, RI-nondependent high sensitivity assay system of MGMT was prerequisite and thus we have devised and developed it originally with utilizing fluorescence and PCR technologies and adapting them to the MGMT system. These RI-nondependent approaches were estimated to be the highest sensitivity in measuring the MGMT activity, especially PCR-based one, constructing the function-based in vitro selection system.

3P083 X 線自由電子レーザーを用いたパターンマッチング法による第一原理構造モデリングの検討
Examination of ab initio structural modeling for the pattern matching method using X-ray free electron laser

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Coherent diffractions patterns observed by X-ray free electron laser provide information on the structure and dynamics of biomolecules. New computational approaches are necessary to annotate experimental data. We are exploring new algorithms to model biomolecular structures from a limited amount of experimental data. Such algorithms would rely on conformational sampling methods and similarity detection to experimental data. In our preliminary study, an exhaustive search approach is used, to generate the positions of gold colloids. Simulated diffraction patterns from modeled assemblies of gold particles are compared with the reference diffraction pattern to determine the actual structure of the colloids.

3P084 １分子イメージングによる PI3K の活性制御機構の解析
Analysis of the Regulation Mechanism of PI3K Activity by Live-cell Single-molecule Imaging

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Cell migration depends on pseudopod formation, which is mediated by PI3P that induces actin polymerization and is produced by PI3K. Previous studies proposed that an efficient migration requires a positive feedback loop that activates PI3K at the leading edge via F-actin-dependent membrane localization, although the mechanism is remained unclear. In this study, by single-molecule imaging of PI3K in living Dictyostelium cells, we found that membrane-binding lifetime was prolonged and lateral diffusion became immobile in the presence of functional F-actin. This indicates that F-actin stabilizes membrane binding of PI3K. By including an analysis of activation of PI3K by Ras-binding, we will discuss the essential molecular processes of the positive feedback.