1SB-01 計算機によるタンパク質立体構造のモデリング

**Computational De Novo Design of Protein Structures**

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Protein has an outstanding capability to fold into a unique 3D structure spontaneously based on amino acid sequence. Exploring the principles of protein folding - how do amino acid sequences determine folded structures? - is one of the important challenges for revealing blueprint of life. Here, to shed light on the key issues underlying folding, we computationally designed protein structures completely from scratch with Rosetta software. We focused on 4 alpha+beta folds: Ferredoxin, Rossmann2x2, Flavodoxin, and Rossmann3x3. In folding calculations we found that the probability of folding to a desired target topology was strongly dependent on length of the secondary structure (SS) elements. This led us to develop a principled and systematic approach for rationally designing structures for alpha+beta topologies. Building backbone conformations with SS lengths that maximize foldability, and placing side-chains stabilizing the backbone conformations, we created proteins having supramolecular energy landscape in silico. The designed sequences range in length from 76 to 151 residues. To critically test our approach, we synthesized genes encoding these designs and expressed, purified, and characterized the proteins. Many of the designed proteins are monomeric and highly stable remaining folded even at 100C. The structures were solved by NMR (PDB ID: 2k18, 2kpo, 2ibi, and 2ib2).

1SB-02 計算の基礎としての掌握法の応用

**Identification of an allosteric site from the analysis of protein fluctuation using NMR**


Protein motions are believed to be essential for their functions, but the relationship between motion and function is still obscure. In this work, we characterized the conformational change mechanism of rat heart oxygenase (rHO-1) using NMR, and identified a functionally important site in the region away from the active site, so called “allosteric site”. rHO-1 catalyzes the degradation of heme to biliverdin, CO, and free iron, and is critical for iron homeostasis, defense for oxidative stress. We applied 2D dispersion spectroscopy to free rHO-1, and realized that two distinct regions fluctuates: regions adjacent to the heme-binding site (AB loop to BC loop, and F helix), and distant from the binding site (CD loop). Interestingly, the analysis of the 2D dispersions revealed that the two regions fluctuate cooperatively at a single rate. Since the two regions are involved in a hydrophobic network, we hypothesized that the fluctuation of CD loop plays an important role in the activity through the hydrophobic network. Namely, CD loop might be an allosteric site. To test this hypothesis, we introduced a single point mutation in CD loop. Although the mutation sites are distant from the binding site, the activities were significantly altered. Furthermore, R2 dispersion experiments with one of the mutants, which possesses lower activity, revealed that the same regions fluctuate as the wild type, but the fluctuation rate increased. These results clearly indicate that the CD loops is the allosteric site.

1SB-03 アンフォクタリア観音を計測する方法

**Output systems of cyanobacterial circadian clock**

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The cyanobacterial circadian system is a model for the biological regulatory system that integrates the cellular processes with the endogenous oscillation. The autonomous oscillator is composed of only three clock proteins, KaiA, KaiB, and KaiC. These can be reconstituted in a test tube. This clock outputs the temporal signal to various cellular regulatory machinery for gene expression, metabolism, etc. In cyanobacteria, the pivotal clock protein KaiC both positively and negatively regulates its own gene expression (feedback regulation of KaiC) as well as circadian expression of other genes in response to its amount and molecular states. Molecular genetic analyses revealed that the transcriptional output system is composed of the three major pathways (SiaA-A, LaA-, and CiaA pathways) that independently transmit temporal signals of the Kai protein clock. While SiaA positively regulates the downstream gene expression, the other two acts negatively. Each component participates in cellular physiologic systems other than circadian clock. Those systems appear to be involved in the input system of circadian clock that resets its phase to the cyclic environment. Thus the temporal signal becomes effective on a broad range of cellular regulatory networks in cyanobacteria.

1SB-04 ネットワーク生物学：スイッチ機能における不均一性

**Network Biology: multiple scales and heterogeneities in switching dynamics**


Biological networks have intrinsically heterogeneous features: Networks work across (i) multiple spatial scales ranging from the atomistic protein conformational change to the micrometer chromosome and the millimeter-cell population scales, (ii) multiple temporal scales ranging from the millisecond protein allosteric change to the minute-scale change in gene expression and the hour-day period of cell cycle, and (iii) multiple scales of numbers ranging from the two-state like behaviors of switching to hundreds-thousands of copy numbers of proteins. Understanding heterogeneities in biological network is, therefore, the fundamental problem to explore the design principles of life. In this talk we are going to discuss examples in which heterogeneities play essential roles, in particular on how the temporal heterogeneity works significantly in dynamics of the gene network of embryonic stem (ES) cells. Stochastic fluctuation induced by the existence of heterogeneous dynamics is discussed from the view point of statistical mechanical theory.

1SB-05 ミオニマ細胞の設計原理：比較ゲノムからのアプローチ

**An evaluation of minimal cellular functions to sustain a bacterial cell**

 Motonori Ota (Nagoya Univ.)

Both computational and experimental approaches have been used to determine the minimal gene set required to sustain a bacterial cell. Such studies have provided clues to the minimal cellular-function set needed for life. We evaluate a minimal cellular-function set directly, instead of a gene set. We estimated the essentialities of KEGG pathway maps as the entities of cellular functions, based on comparative genomics and metabolic network analyses. The former examined the evolutionary conservation of each pathway map by homology searches, and detected “conserved pathway maps”. The latter identified “organism-specific pathway maps” that supply compounds required for the conserved pathway maps. Estimating both sets of maps, we finally determined “minimal pathway maps” for E. coli and B. subtilis that could synthesize all of the biomass components, and that was composed of a minimal number of pathway maps. Our analyses of these pathway maps revealed that those functioning in “genetic information processing” are likely to be conserved, but those for catabolism are not, reflecting an evolutionary aspect of cellular functions.


1SC-01 ダイオキサンの降圧電子伝達系

**The respiratory chains of nitrifying bacteria**

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Nitrifying bacteria are chemotrophic bacteria which obtain energy by oxidation of inorganic compounds such as ammonia/nitrite and carbon by the fixation of carbon dioxide. Nitrosomonas oxidizes ammonia to nitrite (NO2-) and Nitrobacter oxidizes nitrite to nitrate (NO3-). Both of these processes are extremely energetically poor leading to very slow growth rates for both types of organisms. However, these bacteria have essential functions in the nitrogen cycle as converters of ammonia to nitrate in the environments, compounds usable by plants. We have studied the respiratory chains of Nitrosomonas europaea and Nitrobacter winogradsky. Respiratory components which participate in the oxidation of ammonia (hydroxylamine) to nitrate in two bacteria have been highly purified and their properties studied in detail. In this symposium, I will review the respiratory chains and discuss the evolutionary features.