2P-121 可逆的 DNA 光ライゲーションを用いた DNA コンピューティング

Autonomous DNA Computing Machine Based on Reversible DNA Photoligation

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A major challenge in molecular computing is to construct logic gates that are devices that perform the fundamental logic operations NOT, AND, and OR, as well as their combination including full-adder. In particular, DNA has proven to be highly useful as building blocks for the construction of logic gates in several molecular logic gates because DNA offers immense information-encoding capacity and predictable recognition through well-defined Watson-Crick complementarity. Many types of DNA-based logic gate were reported such as deoxyribozyme, DNA tile, and telomere DNA structure. Although basic logic gates can be easily assembled by the above systems, complicated logic circuits, for example, binary digit addition that is a key operation in information processing, can not, due to the difficulty of constructing an autonomous cascading system using DNA. We report the construction of one-pot autonomous DNA computing machine base on photochemical gate transition and performed binary digit additions using this machine. In our method, photoligation via 5-carboxyvinyldeoxyuridine containing ODN and photo-cleavage via carbazole-modified ODN, were employed. The binary digit additions were autonomously carried out by one-time irradiation at 366 nm in the single test tube. The fluorescence readout by DNA chip was good agreement with correct answer of binary digit additions. We believe that this system is easily applicable to correlation analysis between SNPs as well as the other binary digit processing, such as the subtraction.

2P-122 分子内二次構造による DNA ハイブリダイゼーション反応の阻害

Inhibition of DNA intramolecular secondary structures by intramolecular secondary structures

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The hybridization reaction of nucleic acids is fundamental event in bioengineering system such as RNAi, molecular probe designing, DNA computing, and DNA nanotechnology. In these studies, rates of hybridization reactions must be taken into account in order to work these systems properly and effectively; however, there are not enough data for controlling hybridization rates of DNA/RNA sequence efficiently. In our previous report, method to design sequences which hybridize quickly is shown. The method is based on nucleation ability, which relies on secondary structures at the ends of anti-parallel DNA. However, the method has problems: for example, the method is based on practical results and has not sufficient theoretical support. Purpose of this study is revealing effects of secondary structures on DNA hybridization kinetics and supporting the method theoretically.

To eliminate effect of thermodynamic behaviors on kinetics, we used *OrthoNormal Sequences* DNA, which have uniformed hybridization melting temperature (T_m), Gibbs' free energy. Fluorescence spectrometer equipped by stopped-flow accessory is used for detect time courses of hybridization. Single/Double exponential models are used to analyze time courses. Secondary structures are analyzed by UNAFold system.

2P-123 3'-アミノ-2',4'-BNA 修飾による生理的条件でのピリミジン型 3 本鎖核酸形成の促進

Promotion of Pyrimidine Motif Triplex Formation at Physiological pH by 3'-Amino-2',4'-C-Methylene Bridged Nucleic Acid Modification

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A triplex nucleic acid is formed through the sequence-specific interaction of a single-stranded triplex-forming oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA. In the pyrimidine motif triplex, a homopyrimidine TFO binds parallel to the homopurine strand of the target duplex by Hoogsteen hydrogen bonding. Extreme instability of the pyrimidine motif triplex at physiological pH severely limits its use in an artificial control of gene expression *in vivo*. Stabilization of the pyrimidine motif triplex at physiological pH is crucial in improving its therapeutic potential. In this study, we have examined the thermodynamic effect of our previously reported chemical modification, 3'-amino-2',4'-C-methylene bridged nucleic acid (3'-Amino-2',4'-BNA) modification of TFO, on the pyrimidine motif triplex formation at physiological pH. The thermodynamic analyses indicated that the 3'-Amino-2',4'-BNA modification of TFO increased the binding constant of the pyrimidine motif triplex formation at neutral pH by about 20 times. The consideration of the observed thermodynamic parameters suggested that the increased rigidity of the 3'-Amino-2',4'-BNA-modified TFO in the free state relative to the unmodified TFO may enable the significant increase in the binding constant at neutral pH. Our results certainly support the idea that the 3'-Amino-2',4'-BNA modification of TFO could be a key chemical modification and may eventually lead to progress in therapeutic applications of the antigene strategy *in vivo*.

2P-124 ENA 修飾による生理的条件でのピリミジン型 3 本鎖核酸形成の促

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Promotion of Pyrimidine Motif Triplex Formation at Physiological pH by 2'-O,4'-C-Ethylene Bridged Nucleic Acid (ENA) Modification

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A triplex nucleic acid is formed through the sequence-specific interaction of a single-stranded homopurine or homopyrimidine triplex-forming oligonucleotide (TFO) with the major groove of homopurine-homopyrimidine stretch in duplex DNA. In the pyrimidine motif triplex, a homopyrimidine TFO binds parallel to the homopurine strand of the target duplex by Hoogsteen hydrogen bonding. Extreme instability of the pyrimidine motif triplex at physiological pH severely limits its use in an artificial control of gene expression *in vivo*. Stabilization of the pyrimidine motif triplex at physiological pH is crucial in improving its therapeutic potential. In this study, we have investigated the thermodynamic effect of 2'-O,4'-C-ethylene bridged nucleic acid (ENA) modification of TFO on the pyrimidine motif triplex formation at physiological pH. The thermodynamic analyses by isothermal titration calorimetry indicated that the ENA modification of TFO increased the binding constant of the pyrimidine motif triplex formation at neutral pH by about 20 times. The consideration of the observed thermodynamic parameters suggested that the more rigidity of the ENA-modified TFO in the free state relative to the unmodified TFO may enable the significant increase in the binding constant at neutral pH. This information will present an effective approach for designing chemically modified TFO with higher binding affinity in the triplex formation at physiological pH, which may eventually lead to progress in therapeutic applications of the antigene strategy *in vivo*.

2P-125 DNA の構造分布の非調和性を考慮した DNA・DNA-タンパク質複合体 構造評価ポテンシャルの開発

A new DNA-deformation energy function taking account of the anharmonicity of the conformational distribution

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DNA binding proteins recognize specific DNA sequences and regulate the essential processes in a cell, such as replication, transcription and ligation. The strict recognition is achieved through two mechanisms: direct and indirect readouts. The former represents the direct interactions between amino acid side chains and nucleotide bases, while the latter is caused by deformation of the DNA structure upon binding with the protein. Not only the direct readout, but also the indirect readout depends on the nucleotide sequence: the substitution of the nucleotide that does not interact with protein atoms has been shown to affect the affinity. In the present study, we developed a new DNA-deformation energy function, taking account of the anharmonicity of the distribution of the DNA conformation. We calculated the distributions of the base-pair step parameters in the ensembles generated by molecular dynamics simulations of dodecamer DNAs, each of which has one of 136 unique tetranucleotides at its center, and converted them into free-energy maps. To evaluate the ability of the function to recognize the native sequence, Z-score was calculated for each of the tetranucleotide structures available in PDB from the difference between the energy of the native sequence and the average of the energies of the random sequences. We found that the new energy function tends to give better Z-scores for more tetranucleotides than the previous one that approximates the distribution function with a Gaussian function.

2P-126 ナノ開口基板上でのタンパク質翻訳中における高濃度 tRNA の 1 分子ダイナミクス

Real time imaging of tRNA dynamics with a zero-mode waveguides during translation

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Single molecule fluorescence imaging has been a powerful tool for understanding the dynamics of molecular interaction. However, it is hardly visualize single molecule fluorescence beyond approximately 20nM concentrations of dyes by conventional total internal reflection fluorescence microscope (TIRFM) due to higher background noise, although many biological processes occur at μ M concentrations. Recently Levene et al have developed novel zero-mode waveguides (ZMW) method to overcome this limitation. We have applied this method into the protein synthesis system to investigate translational mechanism at single molecule level. To visualize fluorescence labeled tRNA during translation, the ribosomes were immobilized on the ZMW surface by biotin-avidin bond. tRNA(fMet) and Phe-tRNA(Phe) were labeled with Cy3 and Cy5 respectively. Translation process could be carried out by PURE system, one of cell-free protein synthesis system. Here we could achieved to visualize the dynamics of single molecule fluorescence labeled tRNA at μ M concentrations during translation by immobilized ribosomes on the modified ZMW surface, on which the signal-to-background ratio is twice that obtainable with a conventional ZMW. As