1P134 2 箇の C:C ミスマッチ塩基対と銀(I) イオンとの特異的相互作用の熱力学的特性
○小笹 哲夫¹、小野 晶²、鳥越 秀峰¹
¹東理大・理、²神奈川大・工

1pF11 Thermodynamic properties of the specific interaction between two C:C mismatch base pairs and silver (I) cations
Tetsuo Kozasa (1), Akira Ono (2) and Hidetaka Torigoe(1). (1: Dept. Applied Chemistry, Fac. Science, Tokyo Univ. of Science; 2: Dept. Applied Chemistry, Fac. Engineering, Kanagawa Univ.)

Single nucleotide polymorphisms (SNPs) are the most abundant form of genetic variation in the human genomic DNA, accounting for most of all differences between individuals. Analyses of SNPs can help to identify genes affecting many human phenotype variations, including complex diseases and drug responses. When two kinds of duplex DNA with different SNPs are mixed, heated, and annealed, novel two kinds of mismatch base paired heteroduplex are formed in addition to the initial two kinds of perfectly matched homoduplex. The heteroduplex analysis to detect the newly formed mismatch base pairs is one of useful approaches for SNP genotyping. Our isothermal titration calorimetric (ITC) study has already found that a single silver (I) cation specifically binds to a single C:C mismatch base pair in heteroduplex with a binding constant of 10^6 M^{-1} , a negative enthalpy change, and a positive entropy change.

Here, to examine the thermodynamic properties involving two C:C mismatch base pairs, we analyzed the interaction between silver (I) cations and heteroduplex involving two C:C mismatch base pairs by ITC. Two silver (I) cations bind with two C:C mismatch base pairs. The magnitude of the binding constant for the second silver (I) cation was similar to that for the first silver (I) cation. Unlike the case for the interaction between mercury (II) cations and two T:T mismatch base pairs, binding cooperativity was not observed between silver (I) cations and two C:C mismatch base pairs.

1P136 DNA 塩基と蛋白質アミノ酸の相互作用自由エネルギーランドスケープ
○吉田 智喜^{1,2}、土居 英男^{1,2}、相田 美砂子^{1,2}、河野 秀俊³、Kumar Shaji⁴、Gromiha Michael M.⁵、皿井 明倫⁶
¹広島大・院理、²広島大 QULiS、³原研、⁴Advanced Tech. Inst.、⁵CBRC、⁶九大・情報工

Interaction Free Energy Landscapes between DNA Bases and Protein Side Chains
Tomoki Yoshida (1,2), Hideo Doi (1,2), Misako Aida (1,2), Hidetoshi Kono (3), Shaji Kumar (4), Michael M. Gromiha (5) and Akinori Sarai (6). (1: Dept. of Chem., Grad. Sch. of Sci. Hiroshima Univ.; 2: Center for Quantum Life Sciences (QULiS); 3: Japan Atomic Energy Agency (JAEA); 4: Advanced Tech. Inst.; 5: Comput. Biol. Res. Cen. (CBRC); 6: Dept. of Biosci. and Bioinfo., Kyushu Inst. of Tech.

To understand the origin of the specificity in protein-DNA recognition, we calculated the free energy (ΔG), enthalpy (ΔH), entropy ($T\Delta S$) and minimum interaction energy (ΔE_{min}) for the interactions between an amino-acid side chain and a base pair (AT or GC) by means of the systematic sampling. We presented them as maps on the base pair plane. We also calculated ΔG maps between an amino-acid side chain and 32 kinds of stacked three base pairs. By utilizing thus calculated ΔG maps, we can construct ΔG isosurfaces for any DNA sequences. Some isolated areas with lower ΔG values are found along the major groove of DNA sequences. We demonstrate that the predicted low ΔG value regions are consistent with the regions where amino-acid side chains are involved in direct contacts with DNA bases within protein-DNA complexes in Protein Data Bank (PDB). We also present the comparison between the systematic sampling and the multi-canonical Monte Carlo sampling methods to generate interaction configurations between base pairs and a protein side chains.

1P135 ハイブリダイゼーション速度が速くなるように考慮された DNA 配列の設計手法
○北島 哲郎¹、瀧ノ上 正浩¹、庄田 耕一郎¹、陶山 明¹
¹東大院・総文広域・生命環境系

1pF12 Method of designing DNA sequences which hybridize rapidly
Tetsuro Kitajima(1), Masahiro Takinoue(1), Ko-ichiro Shohda(1), and Akira Suyama(1) (1: The Graduate School of Arts and Sciences, The University of Tokyo.)

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 no data for controlling hybridization rates of DNA/RNA sequence efficiently enough.

There is a little study which suggests that DNA hybridization is slow when the DNA folds self-folding secondary structures. Thus, we tried to reveal the reason to slow down DNA hybridization with measuring DNA hybridization rates systematically on an experimental basis and considering relations between the rates and secondary structures of DNA. Hybridization rates are measured by fluorescence spectrometer with a stopped-flow equipment and picogreen dye which fluoresces in the presence of a DNA duplex. Secondary structures were calculated by mfold system. Researching the relations, the result showed that DNA-DNA hybridization is inhibited when (i) ΔG value of the self-folding secondary structure is under -1 kcal / mol, or (ii) both tail at 5'- and 3'-end of its forward strand cannot be involved in the nucleation.

1P137 アプタマーを用いた可逆的標識による細胞分離技術の開発
○安西 悠¹、寺薗 英之²、安田 賢二²
¹東大院・総合文化、²東京医科歯科大・生材研

Simple repeatable noninvasive cell separation method using aptamer-conjugated magnetic microbeads and nuclease digestion
Yu Anzai (1), Hideyuki Terazono (2) and Kenji Yasuda (2). (1: Department of Life Sciences, Graduate School of Arts and Sciences, Univ of Tokyo; 2: Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University)

Separation of different phenotype cells is so important both for post genome cell based studies and practical applications of cells. Particularly cell separation using magnetic cell sorting becomes more popular because its simple, speedy, and economical procedures, which is based on the affinity between antibody decorated on the magnetic beads and its antigen expressed on the target cells and is accomplished by the following magnetic pulling up the target cells. However the problem of MACS is its irreversible conjugation between antigen on cell and antibody remaining after pulling up as well as other antibody-based labeling method, because the attachment of antibodies can affect on the state of target cells such as activity and interaction efficiency with other cells. We have developed a new cell separation method exploiting sequential procedure of the selective attachment of the aptamer-decorated magnetic microbeads with target cells and following digestion of aptamer with. In this method, the magnetic microbeads were decorated by high affinity nucleotide, aptamer, which can be digested by DNase and can attach to human acute lymphoblastic leukemia, CCRF-CEM, and were used for picking up the cells using a magnet, and finally they were effectively released from the cells by DNase digestion within 30 min. Moreover the DNase treatment had no obvious influence on the growth of the detached cells and the second separation under the same condition. In this meeting, we will report the detailed conditions of our method.