1P121 新規プリオンアプタマーの探索とその構造研究
Search for novel aptamer against prion protein and its structural study

Tsukasa Mashima¹, Fumiko Nishikawa², Yuji O. Kamatari³, Takashi Nagata^{1,4}, Satoshi Nishikawa², Kazuo Kuwata⁵, Masato Katahira^{1,4} (¹*Inst. of Adv. Energy, Kyoto Univ.*, ²*AIST*, ³*Life Sci. Res. Center, Gifu Univ.*, ⁴*Grad. Sch. of Energy Sci., Kyoto Univ.*, ⁵*Unit. Grad. Sch. of Drug Disc. and Med. Info. Sci., Gifu Univ.*)

We have elucidated the structural basis of anti-prion activity of aptamer against prion protein, r(GGAGGAGGAGGA) (R12). R12 folds into unique quadruplex and its structure is responsible for high affinity to prion protein and anti-prion activity. Here, we have performed screening of the anti-prion activity for the nucleic acids that fold into various kinds of quadruplexes. Then, we have obtained a nucleic acid that reduced the amount of an abnormal prion protein down to 10% of that in the control. We estimated the 50% inhibitory concentration of the newly selected nucleic acid and it was around 100 nM. Analyses of the structure and mechanism by which the newly selected nucleic acid exerts higher anti-prion activity are now in progress.

1P122 剪断流下における二重らせんDNAのダイナミクス
Dynamics of double helix DNA under shear flow

Yosuke Fujita¹, Nobumasa Nakazawa¹, Takako Kato-Minoura¹, Hiroyuki Iwamoto², Shinji Kamimura¹ (¹*Dept. Biol. Sci., Chuo Univ.*, ²*SPRING-8, JASRI*)

The DNA double helix of Watson-Crick model, which is called B-DNA, is composed of base pairs arranged with an approximately 3.4 nm helical pitch. The structure has been believed to be corresponding primarily to that of DNA inside live cells. However, direct evidence to show the structure of native DNA strands in solution has not been enough to get more detailed insights. In the present study, we used a modified rotary rheometer device, which has been successfully applied to align microtubules in solution for the analysis of X-ray fiber diffractions. We observed X-ray diffraction of salmon sperm DNA in solution at BL45XU in SPRING-8. We found dynamic and reversible pitch conversions of DNA between from 3.4 nm to 2.9 nm under shear flow conditions.

1P123 スクレオソームDNA解離の自由エネルギープロファイル
Free Energy Profile of Nucleosomal DNA Unwrapping

Hidetoshi Kono, Shun Sakuraba, Hisashi Ishida (*Molecular Modeling and Simulation, JAEA*)

Eukaryotic genome is compactly stored into a tiny nucleus of cell in the form of protein-DNA complex. This protein-DNA complex is called as nucleosome which is composed of a histone octamer formed by two copies each of the four core histones H3, H4, H2A and H2B and about 150bp of DNA wrapping almost twice around the octamer. This compact form, however, has to be unwrapped in transcription, DNA duplication and DNA repair processes. To study the detailed molecular mechanism, free energy profiles for unwrapping nucleosomal DNA on several nucleosomes were calculated. So far, we estimated that a cost for unwrapping the outer DNA is 0.1 to 0.4 kcal/mol/1bp, consistent with a value experimentally obtained. In the meeting, we will report the cost for unwrapping the inner DNA.

1P124 SecMのN末端側領域による翻訳アレストの安定化
N-terminal region of SecM is essential for its stable translation arrest

Zhuohao Yang, Ryo Iizuka, Takashi Funatsu (*Grad. Sch. of Pharm. Sci., The Univ. of Tokyo*)

SecM, a bacterial secretory protein, contains a specific amino acid sequence at the C-terminus, which interacts with the ribosomal tunnel and arrests its translation. It is accepted that the sequence, called arrest sequence, is sufficient and necessary to induce SecM-mediated translation arrest. However, in cell-free translation assays, we have found that the arrest sequence is necessary but not sufficient to induce translation arrest, when fused to the C-terminus of HaloTag. We have also found that the stability of the translation arrest depends on the protein structure outside the ribosome and the presence of the N-terminal region of SecM increases the stability. We then concluded that the N-terminal region of SecM is essential for its stable translation arrest.

1P125 蛍光タンパク質との融合による光二量体化モジュール (Photodimerizer) の機能評価
Functional evaluation of the light-induced dimerizing module, Photodimerizer, fused with fluorescent proteins

Osamu Hisatomi, Yoichi Nakatani, Yuki Kai (*Grad. Sch. of Sci., Osaka Univ.*)

A synthetic gene encoding the light-induced dimerizing module, Photodimerizer (Pd), was prepared and introduced in *E. coli* cells. Pd protein showed the same properties with a N-terminally truncated mutant (ZLC₂S) of aureochrome-1 [1]. To evaluate the performance of Pd, we prepared YFP-Pd and mCherry-Pd fusion proteins and investigated their oligomeric structures and DNA-bindings. Similar to Pd, both fusion proteins are monomeric in the dark state, and form dimers and increase their affinity for the target DNA sequence in the light state. Our results suggest that Pd is a useful molecular tool for the light-dependent dimerization and DNA-binding of the fusion proteins.

[1] Hisatomi *et al.* (2014) *J. Biol. Chem.* *in press* (published online)

1P126 大腸菌 *E. coli* ゲノム複製開始における DNA 二重らせん開裂機構の分子動力学シミュレーション研究
DNA unwinding mechanism at DNA replication initiation of *E. coli* studied by coarse grained molecular dynamics simulation

Masahiro Shimizu¹, Tsutomu Katayama², Shoji Takada¹ (¹*Dept. of Biophys., School of Sci., Kyoto Univ.*, ²*Dept. of Mol. Biol., Kyusyu Univ.*)

The DNA replication initiation complex is formed on *oriC* region in *E. coli* genome. This complex unwinds the DNA duplex of A/T rich region in *oriC*. But structure of the DNA replication initiation complex, which contains about ten proteins and DNA, is unknown. So the mechanism how this complex unwinds DNA remains elusive. In order to reveal the unwinding mechanism, we conducted coarse-grained molecular dynamics simulation. First, we elucidated the structure of replication initiation complex of *E. coli* including *oriC* left-half region, IHF protein and domain III-IV of DnaA protein. Second we investigated interaction between DnaA protein and A/T rich region in *oriC*. Based on our simulation, we propose the DNA unwinding mechanism in DNA replication initiation complex.