# Structural Energy Bioscience Research Section

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## 1. Introduction

We explore the way how biomolecules such as proteins (involving enzymes) and functional nucleic acids (DNA and RNA) work at atomic resolution based on structural biology with NMR. We determine both static and dynamical structures with the aid of our own development of the new methodology and elucidate the underlying mechanism of functions of these biomolecules. Structural biological approach is also applied to analyze enzymes involved in degradation of wood biomass at atomic resolution. The analysis is useful to develop the way to extract energy and valuable materials that can be used as starting materials of various products from the wood biomass. Thus, we pursue to contribute to the paradigm shift from oil refinery to biorefinery. Followings are main research achievements in the year of 2022.

## 2. The base-pair opening dynamics of nucleic acids in living human cells

DNA and RNA are polymers having not only genetic information but also functions such as regulating gene expression, catalyzing reactions, and so on. The structure, dynamics, and interactions of nucleic acids, which are related to these funations, might be different under in vitro and cellular conditions. A base pair is a fundamental unit of nucleic acids structures. However, studying the base-pair opening dynamics inside living cells had been challenging. In this study, we investigated the base-pair opening kinetics inside living human cells using the in-cell NMR technique. We determined the exchange rate constant ($k_{ex}$) of the imino proton involved in hairpin and G-quadruplex (GQ) structures with the proton of solvent water. It was deduced by the obtained $k_{ex}$ values that at least some G-C base pairs of the hairpin structure and all G-G base-pairs of the GQ structure open more frequently in living human cells than in vitro. In vitro NMR analysis using various crowding agents suggested that interactions with endogenous proteins, especially positively charged ones, could be responsible for the increase in frequency of base-pair opening. This study demonstrated a difference in dynamics of nucleic acids between in-cell and in vitro conditions. Finally, we assume that partially unfolded structures that are supposed to be present inside cells can be targeted by drugs. Our in-cell NMR technique can be applied for the development of these drugs.

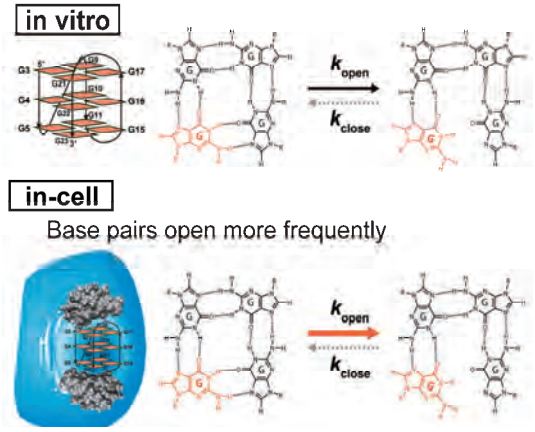


**Figure 1.** All G-G base-pairs in the GQ structure open more frequently in living cells than *in vitro*.

## 3. The first heteronuclear 2D in-cell NMR spectra of nucleic acids in living human cells

To investigate the structure, dynamics and interaction of biomolecules in living cells, in-cell NMR is a powerful tool. In in-cell NMR, living cells containing the biomolecule of interest are put into the NMR magnet and the NMR signal of the biomolecule in living cells are directly recorded. We have been developing the methodology of the in-cell NMR for nucleic acids. This year, we succeeded in recording the first heteronuclear 2D in-cell NMR spectra of RNA aptamer. To obtain milligram quantities of $^{13}C$, $^{15}N$-isotopically labeled RNA cost-effectively, we adopted a tRNA-scaffold system. In this system, the RNA of interest is transcribed in *E. coli* as a chimeric transcript, in which the RNA of interest being inserted into tRNA via two hammerhead (HH) ribozymes. tRNA protects entire RNA from degradation by RNases, while HH ribozymes enable the RNA of interest to be cleaved from the tRNA-scaffold during the purification procedure. The prepared isotopically labeled RNA aptamer for HIV Tat protein was incorporated into living human cells. Then, we successfully recorded the first 2D $^{1}H$–$^{13}C$ and $^{1}H$–$^{15}N$ HMQC in-cell NMR spectra of the RNA aptamer in complex with the ligand. The development of this methodology has the

following advantages. Firstly, the signals of the introduced nucleic acids can be clearly distinguished from those of the intracellular endogenous molecules and/or of the molecules in the medium. Secondly, in the 2D $^{1}H$–$^{15}N$ HMQC and 2D $^{1}H$–$^{13}C$ HMQC in-cell NMR spectra, the resolution of the signals of the introduced nucleic acids are improved as compared in the 1D $^{1}H$ in-cell NMR spectrum. Thirdly, the $^{15}N$ and $^{13}C$ chemical shift values of the introduced nucleic acids could be used as new sources to monitor the state of the nucleic acids, in addition to the $^{1}H$ chemical shift values. Therefore, it is expected that the developed methodology will be widely applied in the field of nucleic acids research.

**4. Structure-function relationship of a feruloyl esterase that targets a variety of substrates**

Woody biomass comprises cellulose, hemicellulose, and lignin. Hemicellulose is a branched heteropolysaccharide consisting of hexoses and pentoses. Hemicellulose is also linked with modification groups and lignin through covalent bonds. Decomposition and isolation of these components are attractive because they are regarded as valuable biorefinery precursors. Feruloyl esterases (FAEs) are the enzymes that hydrolyze ester bonds connecting hemicellulose with the modification groups and lignin. Fungal FAEs belonging to subfamily (SF) 6 are known to catalyze decomposition of woody biomass and produce ferulic acid derivatives. The molecular mechanisms underlying substrate recognition and catalysis by SF6 FAEs are still elusive. Here, we selected SF6 FAE of *Aspergillus sydowii* (*As*FaeE), which was shown previously to exhibit higher activity towards variety of substrates; most of SF6 FAEs are known to target only a certain substrate. We obtained a recombinant *As*FaeE and investigated the specific activity towards a variety of model substrates. We then determined the crystal structure of

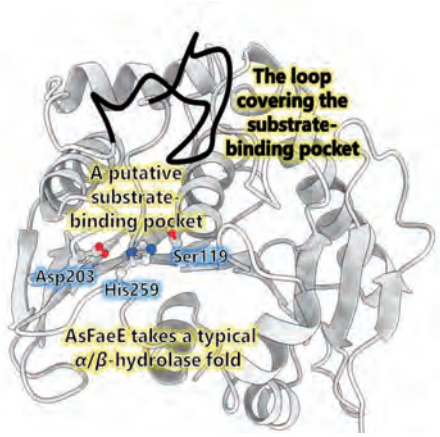


**Figure 2.** The determined crystal structure of *As*FaeE.

*As*FaeE. *As*FaeE took a typical $\alpha/\beta$-hydrolase fold with a canonical serine-histidine-aspartate catalytic triad. *As*FaeE was found to have a loop covering the substrate-binding pocket, which is a unique feature among others; this loop should play a key role in substrate binding and/or recognition. We are currently carrying out structure-based functional mutagenesis and further structural analyses. These studies should give a clue to engineering functionally advanced FAEs in the future.

**5. Structure and interaction analysis of human origin recognition complex subunit 1**

Human origin recognition complex (hORC) initiates the DNA replication from particular loci in genomes called replication origins. Our previous study suggested that hORC recognizes the replication origin in a G-quadruplex (GQ) structure-dependent manner. However, the mechanism how hORC recognizes a GQ-forming DNA is not clear. Here, we analyzed the interaction between the GQ-binding region of the subunit 1 of hORC, hORC1(GQ), and DNA that forms GQ structure (GQ-DNA). Firstly, we prepared the $^{13}C$, $^{15}N$-labeled hORC1(GQ) and assigned most amide proton signals by heteronuclear multidimensional NMR experiments. NMR and CD analyses indicated that hORC1(GQ) is mostly disordered in both free and complex with GQ-DNA. The chemical shift perturbation analysis for hORC1(GQ) upon binding of GQ-DNA indicated that the specific basic amino acids and polar amino acids of hORC1(GQ) are responsible for binding GQ-DNA. The obtained structural insight is helpful for understanding how hORC1 recognizes the position of replication origin.

**6. Dynamics of DNA in the nucleic acid-nucleic acid condensate**

Currently, the condensate of biomolecule, such as a protein and nucleic acid (NA) is an emerging target. Although, there are abundant studies on the protein-protein or protein-nucleic acid condensate, studies on NA-NA condensate is limited. We used one of the reported NA-NA condensate-forming system. In the system, three DNA strands having partially complimentary sequences to each other form Y-shape structure and the base pairs were formed at the ends of Y-shape DNAs. That causes the formation of Y-DNA condensate. We carried out NMR analysis on the dynamics of oligo DNAs forming G-quadruplex structure (GQ-DNA) introduced into the Y-DNA condensate. The translational motion of GQ-DNA inside the Y-DNA condensate was affected by surrounding environment. Interestingly, on the other hand, the rotational motion of GQ-DNA inside the Y-DNA condensate was not affected by the condensate. Our dynamics analysis gives us the spatial information inside the Y-DNA condensate and it is useful to develop the reaction field by NA-NA condensate.

## Collaboration Works

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**2. Others**

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