2P115 蛍光相互相関分光法を用いたグルココルチコイドレセプターと DNA 間相互作用の定量化

Determination of the quantitative interaction between glucocorticoid receptor and DNA by fluorescence cross-correlation spectroscopy

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Glucocorticoid receptor (GR) belongs the nuclear receptor superfamily. Upon binding to the ligand, GR regulates the transcription of target genes through direct binding to glucocorticoid response element (GRE) as a dimer. Recently, it was reported that a monomeric GR could regulate the genes which is different from that controlled by dimeric GR. Our purpose is determination of dissociation constant of monomeric and dimeric GR toward GRE to reveal the mechanism as initiation of transcription. For this purpose, human wild type GR and dimerization deficient mutant were purified as fusion protein with EGFP from insect cells. We quantified the interaction between purified EGFP-GR and Alexa647-labeled GRE in vitro by fluorescent cross-correlation spectroscopy.

2P116 一分子蛍光観測法による癌抑制蛋白質 p53 の DNA 探索機構の研究

Investigation of DNA search mechanism of tumor suppressor p53

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Tumor suppressor protein p53 is considered to perform 1D sliding, 3D search and intersegmental transfer to find the target sequence on DNA genome. In this study, we aim to investigate the heterogeneity in the 1D sliding of p53 and the search mechanism of p53 on the collapsed DNA. Firstly, we measured the sliding of p53 along the stretched DNA using a single-molecule fluorescence microscopy, and revealed that the 1D sliding of p53, previously considered as the simple diffusive movement, has multiple sliding modes with different diffusion constants. Secondly, we constructed the novel experimental system based on the rapid cycling of stretch and collapse of DNA, and examined the frequency of 3D search or intersegmental transfer of p53 on the collapsed DNA.

2P117 二種の好熱菌由来ヌクレオチドキナーゼと変異体における高次構造変化とヌクレオチド結合の蛍光分光学的解析

Fluorescence spectroscopic studies on conformational changes and Nucleotide binding of thermophilic nucleotide kinases and their variants

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In previous studies, we reported the application of fluorescence spectroscopic methods for the conformational changes by ANS, and also the binding of aromatic molecule by fluorescence quenching.

In this study, we investigated the conformational changes of two thermophilic nucleotide kinases and their variants by using intrinsic Tyr residues and ANS as fluorescence probe, and their nucleotide binding properties by quenching of Tyr-excited fluorescence. As results, it was elucidated that evaluations for the differences in the conformational changes after heating, and nucleotide binding properties might be possible even in cases of thermophilic nucleotide kinases and their variants by these fluorescence spectroscopic applications.

2P118 哺乳類ヌクレオチド除去修復タンパク質 XPC の DNA 結合モードの 1 分子イメージング

Single-molecule direct visualization of DNA binding modes of the mammalian nucleotide excision repair protein XPC

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XPC-RAD23B protein complex is known to be responsible for damage recognition in mammalian nucleotide excision repair. To address the damage recognition mechanism, we have visualized single-molecule Qdot-labeled XPC-RAD23B protein complexes along λDNA tethered by our developed PEG-based single-molecule imaging platform. On damaged λDNA created by UV-irradiation, we visualized association of XPC-RAD23B with a lesion most of which remained bound at least several hours. On undamaged λDNA, in contrast, XPC-RAD23B dissociated from DNA in several seconds on average and some performed one-dimensional bidirectional diffusion with a variety of diffusion coefficients. We will present the data and discuss relationship between the observed binding modes and damage recognition.

2P119 15N NMR 分光法を用いた C-Ag(I)-C 塩基対の構造解析 Nitrogen-15 NMR spectroscopic studies of Ag (I)-mediated C-C base-pairs

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In DNA duplex, a Ag (I) ion is selectively captured by a cytosine-cytosine mismatch and formed Ag (I)-mediated cytosine-cytosine base pair (C-Ag(I)-C). However, the structure of C-Ag(I)-C in DNA duplex was not fully understood. To determine the chemical structure of C-Ag(I)-C, uniformly 15N-labeled DNA duplex was enzymatically synthesized. In the 1D 15N NMR spectra of the 15N-labeled DNA duplex, the 15N signals of cytosine residues of C-Ag(I)-C base pair were observed. We will discuss determined chemical structure of C-Ag(I)-C base pair.

2P120 スクレオソーム 3 量体の構造ダイナミクスの粗視化分子動力学法と X 線小角散乱による研究

Structural dynamics of tri-nucleosome studied by combination of coarse grained molecular simulation and SAXS

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The dynamic change of chromatin structure is the basis of transcriptional activity control. Thus it is important to know structures of nucleosome-array in a thermal equilibrium state. Since the angle made of contiguous three nucleosomes is especially critical to understand the packing of chromatin, tri-nucleosome can be a nice and minimal system to address folding of chromatin. In this research, the structure dynamics of the tri-nucleosome was investigated by the coarse graining molecular simulation. Using structural ensemble of various simulations, we computed SAXS profiles, which were compared with the experimental SAXS data.