grained simulations

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Nucleosome is a basic unit of chromatin structure in which DNA duplex wraps about 1 and 3/4 times around histone octamer. Poly-nucleosome takes higher order chromatin structure to store the very long genome into compact space. On the other hand, nucleosome is needed to be unstructured dynamically for function; recognized by transcription factor and transcription to mRNA. Recently, many experiments have investigated mono-nucleosome dynamics. Unwrapping of DNA duplex from histone octamer is observed by equilibrium and force unwrapping experiments. We simulate these nucleosome dynamics by using coarse-grained DNA-protein model, in which one amino acid is represented by one bead and one nucleotide is represented by three beads corresponding to phosphate, sugar, and base. Simulation results reproduce partial/full unwrapping of DNA. We also simulate model poly-nucleosome systems to investigate higher-order structure of chromatin.

3PT010 Zero-dipole summation 法を用いた Est1-DNA 複合体の分子動力学シミュレーション

Molecular Dynamics Simulations of Ets1-DNA complexes using Zero-Dipole Summation Method

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For understanding the phenomenon of life, it is important to investigate ingenious transcription mechanisms. Ets1 is a transcription factor, which has a highly conserved DNA binding domain, the Ets domain, and functions in various biological important procedures, such as the regulation of developmental processes in response to extracellular signals. The DNA binding activity of Ets1 is in an autoinhibited state enhanced by phosphorylation. This fact clearly suggests that phosphorylation of transcription factors plays a critical role linking signal transduction to transcriptional regulation. Here, we attempted to unravel how phosphorylation affects protein's DNA-binding ability by applying molecular dynamics (MD) simulations in explicit water molecules with small ions using zero-dipole summation method (Fukuda et al., 2011) to several transcription complexes having Ets1. Zero-dipole summation is a novel idea for calculations of long-ranged Coulombic interactions as a kind of truncation methods, and it achieved good accuracy and high parallelization efficiency in several biological systems even with highly charged DNA double-strands. In addition, this method is formally free from the periodicity assumption, and thus we can avoid the relevant artifacts. The effect of phosphorylation for DNA-binding will be discussed based on the results of long MD simulations.

3PT011 分子シミュレーションによる TFIIIA ジンクフィンガーの DNA 上の拡散

Diffusion of TFIIIA zinc fingers along DNA studied by molecular simulations

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TFIIIA is a transcription factor which recognizes a site-specific sequence of DNA. Generally, transcription factors search on DNA sequence by various diffusion mechanisms. As one of search mechanisms, one-dimensional diffusion along DNA, or sliding, is proposed. Sliding is that a transcription factor moves along DNA sequence by keeping contact with DNA. It has been suggested that one-dimensional diffusion coefficient is independent of ion concentration when a transcription factor slides on DNA. To investigate if this phenomenon is true or not, we performed a series of coarse-grained (CG) simulations. The system of simulations includes one zinc finger motif of TFIIIA and 200-bp DNA. We used Go model for proteins, de Pablo model for double-strand DNA, and electrostatic and excluded-volume interactions for protein-DNA interactions.

According to our simulation, the one-dimensional diffusion coefficient increases when ion concentration increases from 1 mM to about 200 mM. Moreover, the diffusion coefficient is constant when ion concentration is higher than 200 mM. Hence, it is realized that electrostatic interaction between a transcription factor and DNA influences the diffusion coefficient when ion concentration varies from diluted condition to physiological condition. This phenomenon suggests that experimental results often done at dilute solution are not necessarily the same as the results of physiological solution. The same

tendency is observed in each of zinc fingers in TFIIIA, and thus this may be applied to other proteins.

3PT101 溶液環境が決定付ける単一 DNA 分子の凝縮速度

How dose Environmental Solution Conditions Determine the Compaction Velocity of Single DNA Molecules?

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Understanding the mechanisms of DNA compaction is becoming increasingly important for gene therapy and nanotechnology. The kinetics of the compaction velocity of single DNA molecules was studied using two non-protein condensation systems, poly(ethylene glycol) (PEG) with Mg²⁺ as a representative of crowding effect and spermine as a polycationic condensation agent. The compaction velocities of single tandem λ -DNA molecules were measured at various PEG and spermine concentrations with video fluorescent microscopy. Single DNA molecules were observed using a molecular stretching technique under microfluidic flow. The results show that the compaction velocity of a single DNA molecule was proportional to the PEG or spermine concentration to the power of a half. Theoretical considerations indicate that the compaction velocity is related to differences in the free energy of a single DNA molecule between the random coil and compacted states. In the compaction kinetics with PEG, acceleration of the compaction velocity occurred above the overlap concentration while considerable deceleration occurred during the coexistence state of the random coil and the compacted conformation. This study demonstrates quantitative approach to determine the kinetics of DNA compaction by use of environmental conditions as the control parameters. Further study on such kinetics on the change of the higher-order structure in genomic giant DNA molecules would exhibit of biological importance to understand the essential feature of living matter. [Ref] NAR,40(2012)284.

3PT102 新規なフォトクロミック ATP アナログによるキネシン / 微小管系の駆動と光可逆的な運動制御

Driving and Reversible Photo-control of Motility of a Kinesin/Microtubule System by a Novel Photochromic ATP Analogue

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Adenosine triphosphate (ATP) is a biogenic molecule that functions as an energy donor and interacts with various proteins, ATPases. Therefore an ATP analogue having the potential to artificially control the functions of ATPases would be a promising molecule to control various biochemical processes. In this regard, a photo-controllable ATP analogue has the advantage of using an external stimulus, light, with high spatiotemporal resolution.

We designed and synthesized novel nucleotide analogues, ATP-Azos, consist of ATP and photochromic compounds, azobenzene derivatives. It is confirmed with a spectrophotometer that ATP-Azos exhibit *trans*-to-*cis* and reverse *cis*-to-*trans* isomerizations in the azobenzene moieties through alternating irradiation with UV ($\lambda = 365$ nm) and visible ($\lambda = 436$ nm) lights, respectively.

Kinesin, an ATPase bio-nano-motor moving along a microtubule filament, is allowed to be driven by ATP-Azos without ATP molecules confirmed by an *in vitro* motility assay. Furthermore, in the presence of the ATP-Azo possessing *tert*-butyl group in the azobenzene moiety (ATP-tBuAzo), the gliding velocity (at ≤ 300 μ M ATP-tBuAzo condition) and binding manner (1 mM ATP-tBuAzo) of the kinesin-microtubule system in the assay was reversibly photo-controlled by alternate irradiations with UV and visible lights.

We anticipate that ATP-Azos might find wide applicability in nanobiotechnology using bio-motors and other ATPases. Furthermore, replacing ATP with other nucleotide might allow related systems to apply to other nucleotidases.

3PT103 ナノメートルサイズの軸で巻くことにより DNA の曲げ弾性を測定 Bending stiffness of double-stranded DNA measured by winding single-molecule on a nanometer-sized reel

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The bendability of double-stranded DNA (dsDNA) with curvature radius of 2-20 nm is important in many cellular process such as genomic DNA packaging, transcriptional regulation, and also important in fabrication of DNA

nanostructures. Previous measurements were based on the analysis of thermal bending of dsDNA. However, sharp bending to curvature radius of 2-20 nm was rarely occurred thermally. Therefore, methodology that controls the bending curvature of dsDNA is required to explore the micromechanics of sharply bent dsDNA. For mechanically bent dsDNA to nanometer curvatures, we developed a molecular system of a nanometer-sized reel based on F_1 -ATPase, a rotary motor protein. By combination with magnetic tweezers and optical tweezers system, single-molecule dsDNA was wound around the molecular reel. The bending stiffness of dsDNA was estimated based on the winding tension (0.9-6.0 pN) and the diameter of the wound loop (21.4-8.5 nm). Our results were in good agreement with the conventional worm-like chain model and were used to estimate a persistence length of 54 ± 9 nm. This molecular reel system offers a new platform for single-molecule study of micromechanics of sharply bent DNA molecules and is expected to be applicable to the elucidation of the molecular mechanism of DNA-associating proteins on sharply bent DNA strands.

3PT104 1 分子機械的アンジッピングが示すエントロピー駆動的 DNA 構造安定性の GC と AT 塩基対における違い

GC and AT pairs show different entropic stabilization of DNA structures found in single-molecule mechanical unzipping

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Unzipping of double-stranded DNA is involved in several biological processes. We have performed a quasi-static mechanical unzipping of single-molecule DNA using intermolecular force microscopy, modified atomic force microscopy with high resolution. We obtained force-extension (FE) curves of two oligonucleotides with a stem-loop containing six tandem repeats of a 6-bp sequence. To reduce noise, FE curves of the unzipping region were averaged using correlation analysis. FE curves showed a dependence on the ruptures of single hydrogen bonds.

To reveal the molecular mechanism of stabilization of DNA structures by hydrogen bonds, we have carried out the molecular dynamics (MD) simulations of mechanical unzipping of DNA. Cross-correlation analysis of FE curves showed the force-peaks of single hydrogen bonds more clearly than those by the unzipping experiments. Furthermore, we depicted the two-dimensional (2D) energy landscape, trajectory density map to visualize the behavior of DNA unzipping from MD simulations. Trajectories tended to escape the energetic mountain and took heterogeneous pathways on rugged energy landscape. We calculated entropy from trajectory densities and total energy. The entropy of the AT pair was negatively correlated with the number of hydrogen bonds. In contrast, not the entropy but the total energy of GC pair was correlated with the number of hydrogen bonds. The result suggests that the formation of GC pair is driven by mainly energy and that of AT pair is driven by not only energy but also entropy.

3PT105 Controllable adsorption-desorption of double-stranded DNA onto single-walled carbon nanotube functionalized with polyethylene glycol

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Since the discovery of the hybridization of DNA and the single-walled carbon nanotube (SWNT), various researches have been conducted on the mechanism of the interaction between single-stranded DNA (ssDNA) and SWNTs during the hybrid formation. However, the adsorption mechanism of double-stranded DNA (dsDNA) onto SWNTs has not yet been studied as comprehensively. Further, there are few reports on the detachment of DNA molecules from a DNA-SWNT hybrid, although it may be important to understand the desorption technique of ssDNA from the ssDNA-SWNT hybrids in order to employ the hybridization for medical applications such as biodevices or drug delivery. In this study, we employed an SWNT functionalized with polyethylene glycol (PEG SWNT) for controlling ssDNA adsorption and desorption onto the SWNT. Firstly, we achieved the hybridization of dsDNA and the PEG SWNT in aqueous solution for the first time. Secondly, we succeeded in the detachment of dsDNA from the PEG SWNT by annealing at 95°C; the desorption was not observed in the use of usual SWNT. Atomic force microscopy, electrophoresis, and Raman spectroscopy indicated the occurrence of dsDNA adsorption onto and desorption from the PEG SWNT. Our results demonstrated the potential of controlling the interaction between ssDNA and PEG SWNT in aqueous solutions.

3PT106 分子動力学シミュレーションによる dGMP、8-oxo-dGMP のコンフォメーション比較：イオンの影響

Comparison of dGMP and 8-oxo-dGMP conformation by molecular dynamics simulations: effect of ions

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8-oxo-7, 8-dihydroguanine (8-oxo-G) is produced by the oxidation at C8 position of G and is the most abundant species among bases in DNA modified by oxygen radicals. 8-oxo-G causes transversion as well as transition mutation. Human MutT homologue-1 (hMTH1) hydrolyzes oxidized 8-oxo-dGTP to 8-oxo-dGMP, thereby preventing the misincorporation of oxidized nucleotides during replication. Although the “syn” conformation predominates in 8-oxo-dGTP, the crystal structure of hMTH1 indicates that the protein binds the mononucleotide in the “anti” conformation with no direct interaction between hMTH1 and the 8-oxo position of the oxidized base. It is unclear how the protein structurally distinguishes 8-oxo-dGTP from dGTP. To elucidate the specific interaction of 8-oxo-dGTP by hMTH1 on the basis of mononucleotide conformation, molecular dynamics (MD) simulations were carried out for deprotonated/protonated dGMP and 8-oxo-dGMP in explicit water. The effect of ions on the mononucleotide conformation was specifically examined, by including Li^+ , Na^+ , Mg^{2+} , or Ca^{2+} in the system. In a series of MD simulations, dGMP took both the stable “anti” and “syn” conformation. Although 8-oxo-dGMP took the stable “syn” conformation in most cases, 8-oxo-dGMP with Mg^{2+} took the stable “anti” conformation as well as the stable “syn” conformation. We will discuss the “anti” conformational stability of 8-oxo-dGMP with ion in detail.

3PT107 粗視化モデル分子動力学法を用いた RNA 立体構造予測 RNA 3D structure prediction by using coarse-grained molecular dynamics simulation

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During the last two decades, significant developments have showed the importance of RNA in biological functions. To perform their functions, many RNA molecules adopt well defined tertiary structures. Hence, there is an interest in accurate *ab initio* prediction of three-dimensional (3D) structure of RNAs. However, RNA 3D structure prediction is not studied well, compared to protein three dimensional (3D) structure prediction. The one of reasons is that the size of nucleic acid is larger than amino-acid, which needs quite a long time for molecular dynamics (MD) simulation even if we deal with small RNA molecules. Hence, to predict RNA 3D prediction, we developed a simplified, coarse-grained RNA model for molecular dynamics simulation. In our model, one base consists of 3 beads (P; phosphate, S; sugar, B; base). When our model is applied to small RNA (<60bases), it takes only ~5 minutes for the structure prediction using single processor (1 core). The details will be shown in the presentation.

3PT108 分子動力学シミュレーションによる DNA 構造に対する溶媒条件の影響の研究

The influence of solvent condition on DNA structures studied by molecular dynamics simulations

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The conformational properties of DNA play important roles in various biological processes. For example, interactions of proteins with DNA sequences are known to be affected by the conformational properties of DNA. It has also been known that the transitions among various conformational states are induced by the change in environments: Variations in ionic strength and identity, sequence, water activity, and ligand/protein binding all can modulate DNA structure. In this study, we analyzed how the sequence and the environments such as water activity influence the DNA conformations. We have carried out a series of molecular dynamics (MD) simulations to explore the conformational preferences and dynamical behavior of DNA duplex. We prepared the initial structure models with two different sequences in two kinds of conformations; d(CGCGTATACGCG)₂ and d(CGCGTATACGCG)₂ in B- and A-forms. The MD simulations were carried out in four different environments: in only water solvent and in 75, 85 and 95 % (v/v) ethanol / water mixture solvent. We analyzed the differences of the DNA backbone conformation, the base-pair step parameter and the localization of water