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

Tomoshi Kameda (CBRC, AIST)

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

molecules in the groove on each structure. The variability of the conformation was different depending on the solvation condition. The DNA conformation seemed to be influenced by the localization of the water molecules in the groove.

3PT109 Model for self-assembly of flexible DNA motifs using stacking interactions

Keitel Cervantes-Salguero, Shogo Hamada, Satoshi Murata (*Tohoku University*)

Molecular Clusters (MCs) with variable number of constituents seem to be possible if building units are properly designed. Recently, concentration-dependent self-assembly of MCs was demonstrated by utilizing both flexibility of DNA motifs and inter-molecular binding by sticky-ends (He, Y. et al., *Nature* 2008). Here we propose a cluster of DNA motifs by using DNA blunt-end stacking and flexibility introduced into the motif. First, we study an abstract model of flexible planar monomers (triangle with sticky sides A and B, and shrinkable side C. Angle between A and B is about 60 deg.) that move randomly on a surface colliding against each other. Sides A and B of different monomers can stack, thus forming a flexible dimer. Over time we expect to obtain clusters from dimers up to hexamers. We simulated the dynamics of the cluster formation based on chemical kinetics successfully applied to rigid monomers (Hosokawa, K. et al. *Artificial life* 1995). Our simulation shows that the amount of trimers overcomes the tetramers. However, contrary to the work of Hosokawa et al., dimers and pentamers seem to be equally formed. Second, to realize the concept, we have designed a DNA motif with blunt-end stacking interactions, which were proved to be a potential tool for self-assembling larger DNA structures (Woo, S. et al., *Nature Chemistry* 2011).

3PT110 A Growth mechanism of DNA tile array

Yuki Matsubara, Nao Otani, Miho Tagawa, Akira Suyama (*Univ Tokyo, Dept Life Sci*)

We report a growing mechanism of DXAB (DNA double-crossover AB-staggered) tile array known to form planar structures and grow to micro-scale. DXAB tile array is a kind of DNA tile arrays and can work as a scaffold to control positions of nano-particles. Growth mechanisms of the array are important information to set its properties, such as size, accuracy of position control, yield, or the required time of annealing. Here, we report that the growth of array is a co-operative phenomenon in narrow temperature range. This transition width is shorter than a prediction by model based on lengths of the sticky-ends. This has been visualized by fluorescence optical microscope, because, unlike the tiles' assembly, growth of the tile arrays cannot be observed in melting curve. From this knowledge we show two applications, making big isolated sheets and shortening the annealing time in protocol, as examples. These experimental results demonstrate that such knowledge about growth mechanisms is useful to control properties of DNA nanostructures.

3PT111 DNA ナノ構造を用いた DNA-RNA ポリメラーゼ・ハイブリッド ナノマシンの構築と機能評価

Construction and functional analysis of DNA origami base DNA-RNAP hybrid nanomachine

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DNA nanotechnology has recently developed a new nanoscale device called as DNA origami tile (DNA origami). DNA origami is a method for folding long single strand DNA into arbitrary two or three dimensional structures and can be used as a molecular platform which allows proteins and other molecules to place with precisely controlled patterns at nanometer order. Here, we applied DNA origami technology into transcription system. Transcription system is thought to couple to other system in *E. coli* (e.g. translation and replication). And reconstitution of such sequential reactions *in vitro* is important for science and engineering, for which the DNA origami technology may provide platform. As a first step, we assembled T7 RNA polymerase (T7-RNAP) on DNA origami. Rectangle DNA origami (90 x 60 nm) are prepared as reported (Rothenmund *Nature* 2006), and SNAP-tag protein fused T7-RNAP (T7-RNAP-SNAP) were site-specifically anchored on DNA origami through its specific ligand (benzylguanine (BG)-ligand) included on the DNA origami. With the optimized assembly condition, we succeeded in measuring the activity of T7 assembled DNA origami (T7-Tile). We will check the effect of the number and the layout

of T7-RNAP on the activity of T7-Tile.

3PT112 リボソームへの DNA リングの吸着

Adsorption of DNA ring onto Liposome

Yumiko Kumagai, Shogo Hamada, Shin-ichiro Nomura, Satoshi Murata (*Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Japan*)

The various molecular devices made of biomolecules such as nucleic acid, protein and lipid are reported. We are now able to design each devices with certain complexity, although it is still difficult to combine multiple devices into one functional system. Embedding various molecular devices within a given-size compartment is one of the potential solutions to solve this problem and enable us to achieve programmed behavior of the system. A key component to realize this concept is a channel device integrated into the compartment, which allows materials pass through the compartment.

Here, we adopt a phospholipid as material for micron-sized compartment, and DNA nanostructure for the channel device embedded on the compartment surface. In the design of the DNA channel, we have been inspired by the macrocyclic resorcin channel, which is an artificial mimic of a natural ion channel. We have adopted the T-motif DNA ring that has a similar structure to this. The surface of the DNA ring is hydrophilic, therefore, has affinity to the lipid membrane surface. In order to integrate the ring into the membrane, hydrophobic groups are attached to the DNA rings. We adopt Cholesteryl-TEG as the hydrophobic groups and confirmed the formation of those DNA rings. We also found that DNA structures tend to rest on the lipid membrane surface by giving positive charge to the lipid. Currently, we are working on the optimization of the lipid composition to give an appropriate charge condition.

3PT113 脂質二重膜上での DNA ナノ構造の基板上成長

Substrate-Assisted Self-Assembly of DNA Nanostructure on lipid bilayer

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Substrate-Assisted Self-Assembly (SASA) by Hamada *et al.* enables us to make large 2-D DNA nanostructures that are difficult for conventional self-assembly in free solution. So far, limited materials, e.g., cleaved mica, are known for suitable substrate for the method. It is necessary to extend the method to utilize various materials for the substrate. However, the process of SASA is very complex and not yet thoroughly understood.

To model SASA, we divide it into adsorption and self-assembly processes. The adsorption process is described by DLVO theory. Following Sushko, we can calculate energy of DNA adsorption by DLVO. We also measured minimal length of DNA, or equivalently energy per base pair, to be absorbed on mica substrate. In order to extend SASA to other materials, measured absorption energy between DNA and mica surface roughly indicates the feasible condition for SASA. Based on this consideration, we chose lipid bilayer (DOPG) for the substrate, because the calculated energy between 100 base pairs of DNA and the bilayer is enough to absorb when magnesium ion concentration is 12.5 mM. However, we haven't confirmed DNA adsorption under this condition. Currently, we are working on experiments to search appropriate conditions for SASA.

3PT114 中性膜に結合した LFampinB の膜結合構造と膜親和性の固体 NMR と QCM による解析

Structure and affinity of bovine lactoferrampin bind to neutral model membrane as studied by by solid state NMR and QCM

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Recently antimicrobial peptide is focus attention on a potential alternative for antibiotics. Analysis of membrane bound structure of peptide provides important information on efficiency and side effect of medicine.

Bovine lactoferrampin (LFampinB) is an antimicrobial peptide found in the N1-domain of bovine lactoferrin (268-284) and consists of 17 amino acid residues. Bovine lactoferrin (bLF) is an iron binding glyco protein with innate immunity factor in mammalian secretory fluid for example, tear saliva and milk.

In this study, the structure of LFampinB bound to the neutral membrane was determined using chemical shift oscillation analysis by observing the chemical