1349-Pos Board B241
Internal Friction of a Migrating Holliday Junction
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Friction within biomolecules has recently gained increasing interest. Here we present a method that allows to study the friction that occurs during fast, few nm-sized refolding processes of nucleic acids. Branch migration of a homologous Holliday junction serves as an ideal system where such friction can be investigated. In this four-arm DNA junction the opposing arms possess identical sequences with respect to the junction center. In the absence of external constraints the junction is mobile such that one pair of homologous arms can expand at the expense of the other in single base pair diffusive steps. We measure the dynamics of the branch migration process by stretching a torsionally constrained Holliday junction using magnetic tweezers and measuring the length fluctuations of the arms with high-speed videomicroscopy at ~3 kHz. Since DNA has a helical structure, branch migration causes twisting of the arms with one turn per helical pitch moved. This constrains the movement of the junction within the tweezers to ~10 bp. Single base pair diffusive steps are expected to occur on a sub-millisecond time scale and to be much smaller than the overall DNA length fluctuations. Thus they cannot be directly resolved. However, power-spectral-density analysis of the length fluctuations is able to clearly resolve the overall dynamics of the branch migration process. Theoretical modeling considering the elastic coupling of DNA bending fluctuations and the junction movement allows to quantitatively determine the stepping rate and thus the friction of the branch migration process. We expect that our method is widely applicable to study local-scale molecular friction in biological systems.

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Accurate Distance and Structure Determination of Three Different RNA Three-Way Junctions via High Precision FRET
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RNA three-way junctions are important ribosomal structural motifs. They are also widely used as building blocks and functional components in nanotechnology applications. Förster-Resonance-Energy-Transfer (FRET) restrained high-precision structural modeling in combination with molecular dynamics simulations was used to determine the structure of RNA three-way junction (3WJ) without bulges, RNA three-way junction with a small (two unpaired nucleotides) bulge and RNA three way junction with bigger (5 unpaired nucleotides) bulge. In total 81 Donor-Acceptor pairs were measured using single-molecule multi-parameter fluorescence detection (smMFD). This allows us to observe structural changes of the molecule induced by addition of bulge to the initial structure. Rigid body models for the major conformers were obtained by docking rigid double-stranded A-RNA helices explicitly taking into account dye position distributions. This is done many times (1000 iterations) with random starting conformations, yielding all local minima. Obtained models were then refined by all-atom MD simulations [1,2]. First results indicate the presence of two coaxially stacked helices for 3WJs with additional bulges at the junction, and absence of such stacking in case with no bulge. noteworthy the stacked helices are different for the 3WJs with two and five nucleotides in the bulge.
Our studies showed that high precision FRET measurements are a valuable tool to complement the structural information obtained by X-ray crystallography or NMR spectroscopy as these techniques are limited in detecting minority conformers.
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1351-Pos Board B243
Effect of AU Substitutions at the Stem on the Stability of Kissing Loop Complexes
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Kissing loop complexes form when two RNA hairpins interact through their complementary loops. We have investigated the role of the helical stems adjacent to the loops on the overall stability of a model kissing loop complex.
Our results show that a minimum length of 6 base pairs in the hairpin stems is required to form kissing loop complexes (in the absence of $Mg2^{+}$) and that changing a GC base pair to an AU base pair near the loop facilitates the formation of kissing loop complexes. For instance, the melting temperature (Tm) of a kissing loop complex with a GC → AU substitution near the loop is 6 °C higher than the Tm of the parent complex. To determine if this stabilization is due to a decreased stability of the hairpin stems, we have tested the effect of AU substitutions that are further away from the loop. Our results show that these far away substitutions do not stabilize the kissing loop complex, suggesting that the facilitation observed with AU substitutions near the loop arises from specific effects at the stem-loop interface and not from an overall decrease in the stability of the stems. We are currently investigating whether AU substitutions near the loop eliminate a metal-ion binding site or affect the stacking interactions between the loops. Our overall results contribute to a better understanding of the rules governing the formation of kissing loop complexes, which are important to rationalize the principles of RNA-RNA recognition.

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Impact of Osmolytes on the RNA Hairpin as Studied using Molecular Dynamics Simulations
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Osmolytes such as urea and trimethylamine N-oxide (TMAO) accumulate in response to osmotic stress. Urea is known for its role as a protein destabilizer, while TMAO is known for its role as a protein stabilizer. However, the molecular mechanism by which these two osmolytes destabilize or stabilize RNA is not well understood. We perform multiple molecular dynamics (MD) simulations to determine the effects of urea and TMAO on RNA secondary structure using a RNA hairpin as a model system. From the simulations we are able to examine the molecular mechanism of the osmolytes' influence on the hairpin structure.

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Analysis of L30 mRNA Kink-Turn Conformational Changes by Analytical Ultracentrifugation
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In Saccharomyces cerevisiae, ribosomal protein L30e acts as an autoregulator by inhibiting the splicing and translation of its mRNA. The L30 protein-RNA binding site has been previously studied, revealing a kink-turn motif, which is characterized by a sharp bend in the phosphodiester backbone due to unpaired nucleotides and internal tertiary interactions. The 3D structure of the kink-turn is critical for L30e binding. Preliminary studies utilizing analytical ultracentrifugation (AUC) reveal that kink-turned RNA is more sharply bent in the presence of magnesium ($Mg2^{+}$) than in its absence; no structural change was observed upon addition of $Mg2^{+}$ when the kink-turn was disrupted. This confirms the hypothesis that, in solution, the kink-turn exists in equilibrium between a more open and tightly bent conformation, and that addition of Mg2+ shifts this toward the sharply kinked structure.

1354-Pos Board B246
The Interaction of Zn Metalloporphyrin with Different Forms of tRNA
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The interaction of meso-tetra-(4N-oxyethylpyridyl)porphyrin (TOEPyP4) and its Zn(II), Cu(II), -metallocomplexes with tRNA at low ionic strength we have investigated earlier [1]. In this work we have studied the binding of ZnTOEPyP4 with tRNA which has 2 forms: hairpin structure and spatial reversed "L" structure. The interaction of tRNA from E.Coli with ZnTOEPyP4 is studied by UV/Vis Spectrophotometry and Circular Dichroism methods.
The measurements were performed in 0.1 BPSE and 1BPSE buffers (1 BPSE = 6 mM Na_2HPO_4+ 2 mM NaH_2PO_4 +185 mM NaCl + 1 mM Na_2EDTA), correspondingly μ = 0.02M and μ = 0.2M, pH 6.57. (tRNA has hairpin form at μ = 0.02M and reversed "L" structure, when μ = 0.2M.)
From the spectrophotometric titration datas the Scatchard binding isotherms for porphyrin-tRNA complexes are built and binding parameters are calculated (the number of binding sites per molecule of tRNA, and the binding constant). In both cases of tRNA's structure the induced CD spectra (at 400-470 nm) for complex of ZnTOEPyP4 with tRNA are very unusual. The induced CD spectra of complex change a sign and continue to grow (remaining negative) starting from a certain relative concentration. In case of tRNA's reversed "L" structure the intensity of ICD spectra for the same relative concentration of porphyrin is twice bigger than in case of hairpin structure. It is possible that at high relative concentration of porphyrins the liquid crystal form may exist in the solution.
The binding constants with tRNA in case of hairpin form are an order of magnitude greater than in case of reversed "L" structure. It means that this porphyrin interacts stronger with tRNA when it has hairpin form.
Reference
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