The double-stranded nature of the DNA helix has important implications for the build-up of torque in biological processes that unravel the helix to read out the genetic code. Indeed, excess torque can lead to the formation of supercoils in the DNA which must subsequently be removed.

Using single-molecule techniques, we have studied the physics of supercoil removal to assess the rotational drag of large plectonemic structures. On bare DNA, this rotational drag is found to be quite small, indicating a low resistance to diffusion of supercoils along the DNA and implying that supercoils may be removed via annihilation on a bare circular plasmid (Crut et al., PNAS 2007). However, the rotational drag of DNA inside the nucleus is likely to be significantly larger as a consequence of protein binding, requiring the activity of topoisomerases for supercoil removal. In earlier work, we quantified the mechanism of type 1B topoisomerases and demonstrated that these enzymes use a stochastic process of religation that is torque-dependent (Koster et al., Nature 2005). Recently, we have generalized this torque-dependence of religation to include additional enzymes such as DNA ligases.

We have also examined the effect of chemotherapeutic drugs on the rate of supercoil removal and observed a dramatic reduction in the rate of supercoil removal in the presence of topotecan, a drug in clinical use. This reduction was particularly strong for the removal of positive supercoils. We have consequently investigated whether positive supercoils accumulate in yeast cells and demonstrate such an accumulation in both the G1- and S-phases of the cell cycle. These experiments provide a unique link between single-molecule studies on the one hand, and cellular processes on the other (Koster et al., Nature 2007).

992-Plat DNA Tension Can Regulate Gene Expression

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We have investigated the effect of DNA tension upon the association and dissociation of the T7 RNA polymerase/promoter DNA interaction. Using two optically trapped beads, we suspended a single DNA molecule containing the T7-phi13 promoter above a 3rd surface-immobilized bead that bears active molecules of T7 RNAP. The DNA was brought into contact with the surface bead, while applying a 50 Hz triangle oscillation to the bead upstream of the promoter. Binding of T7 RNAP at the promoter was detected as the decoupling of motion of the two optically trapped beads: oscillations of the downstream bead are reduced or cease altogether upon binding. The DNA tension upon binding is directly proportional to the displacement of the downstream bead from the center of the optical trap. By altering both the mean tension in the DNA - changing trap stiffness or the mean bead-to-bead distance - we have been able to observe promoter binding across the range of force 1-12 pN. From these data we find clear evidence that the mean lifetime of the promoter/T7 RNAP complex decreases with increasing tension as indicated by an increasing dissociation rate constant, k_{off}, from 3.5 s^{-1} at 1.5 pN up to 73 s^{-1} at >8 pN. From these observations, we propose that tension within a DNA molecule is able to regulate gene expression, at least in the case of the bacteriophage T7 enzyme. Based on the recent structural data of the T7 RNAP initiation complex we speculate on the mechanism by which force may affect the dissociation kinetics.

Platform AC: RNA Folding

993-Plat Temperature-Dependent Kinetics of an RNA Tertiary Interaction using Single-Molecule FRET

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The functional diversity of ribozymes lies in RNA's ability to fold into complex structures governed by multiple tertiary interactions. However, little information is available on the kinetic and thermodynamic contributions of individual tertiary motifs to the global RNA fold. In this study, we isolate a single tertiary binding motif, the GAAA tetraloop and its 11 nucleotide tetraloop receptor from the P4–P6 domain of the Tetrahymena ribozyme. By combining

(i) time-correlated single-photon counting,
(ii) confocal microscopy and
(iii) single-molecule FRET, we explore the kinetics and thermodynamics of RNA folding due to the GAAA tetraloop-receptor interaction.

The distance between the tetraloop and receptor in single RNA constructs is interrogated via real-time FRET with undocked and docked states clearly distinguished by low and high FRET efficiencies (E_{FRET}), respectively. Previously, the [Mg^{2+}]-dependence of tetraloop-receptor docking and undocking rate constants (k_{dock} and k_{undock}) was reported [1]. In the present work, we employ precise temperature-control methods to probe the thermodynamics of the tetraloop-receptor interaction at the single-molecule level in both surface-immobilized and freely diffusing RNA, yielding the temperature dependence of k_{dock}, k_{undock}, and K_{dock} at a given [Mg^{2+}]. At 1mM Mg^{2+} the entropy of reaction is -50 ± 1 cal mol^{-1} K^{-1} and the enthalpy of reaction is -17 ± 2 kcal mol^{-1} as determined from both single-molecule trajectories and freely diffusing E_{FRET} population distributions. Preliminary results suggest the tetraloop-receptor RNA folds via a barrierless transition with entropy loss in the docked state dictating the docking rate. Furthermore we explore the effect of temperature on kinetic heterogeneity in the tetraloop-receptor system, i.e. the temperature-dependence of RNA subpopulations with different folding kinetics.

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994-Plat Combining Simulation And Experiment To Investigate The Structural Dynamics Of The S-adenosylmethionine Riboswitch

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Riboswitches control bacterial gene expression by folding into alternate secondary structures. These highly conserved untranslated regions of mRNAs detect the presence of metabolites by forming a specific secondary structure that alters either transcription or translation. We have investigated the dynamics of the S-adenosylmethionine riboswitch using explicit solvent replica exchange molecular dynamics simulations, nucleotide analog interference mapping (NAIM) and x-ray crystallography.

Microsecond-sampling simulations reveal the interplay between entropy and enthalpy during conformational changes of the riboswitch active site. Base 46 is observed to be highly dynamic, consistent with the high resolution x-ray structure. NAIM experiments reveal which hydrogen bonds are critical for S-adenosylmethionine binding.

995-Plat Following the Dynamics of the Hammerhead Ribozyme Reaction by Photoinduced Electron Transfer

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Pyrrolo-cytidine is a fluorescent nucleotide analogue that can report upon changes in its molecular environment. When pyrrolo-C approaches within a few Angstroms of guanosine, its fluorescence is quenched. Our analysis shows that this quenching occurs by transfer of electrons from guanosine to the excited flourophore. This photoinduced electron transfer is dependent upon the oxidation potential of the electron donor, and guanosine is the only normal nucleotide whose oxidation potential enables it to donate electrons effectively. We have incorporated pyrrolo-C into the substrate and ribozyme sequence for the hammerhead ribozyme at positions indicated to be important for catalysis. As control important guanosines in the ribozyme core were substituted with inosine, which fails to quench pyrrolo-C. By probing the fluorescence lifetime on a photon-by-photon basis, we were able to observe the variation of pyrrolo-C17, 3 and 7 and G8, G12 distances over time. We then determined the changes in forces at the cleavage site and used correlation analysis to reveal conformational fluctuation at multiple time scales. This phenomenon suggests the existence of multiple interconverting conformers related to the fluctuating catalytic reactivity.

996-Plat Predicting ribosomal frameshifting

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Many retroviruses use -1 ribosomal frameshifting as part of the mechanism in translational control of viral proteins. Quantitative prediction of -1 frameshifting is crucial for understanding the regulation of viral gene expression. We develop a statistical mechanical model for -1 programmed ribosomal frameshifting by computing the folding thermodynamics of the frameshifting system (codon-anticodon base pairs in the slippery region, downstream messenger RNA structure, and the spacer between the slippery region and the downstream structure). The energetic parameters are calculated from the Vfold model [1, 2]. The results support the tension force-induced mechanism for -1 ribosomal frameshifting. Specifically, the model reveals an intrinsic correlation between the mechanical force-mediated stability of the three-component frameshifting system and the experimentally measured frameshifting efficiency for a range of viral frameshifting systems. We find that both pseudoknot and stem-loop structures of the downstream messenger RNA can possibly induce efficient frameshifting. For pseudoknots, the torsional restraint permits a longer ribosomal pausing time than stem-loops. As a result, a frameshifting pseudoknot can allow for a longer spacer length than a stem-loop structure. In addition, we find that the RNA-DNA handle used in the mechanical unfolding experiments can enhance the thermodynamic cooperativity (with less intermediate states in the unfolding process) for the pseudoknots.

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997-Plat Experimental And Computational Reconstruction Of The Unfolded State Ensemble In Nucleic Acid Folding

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Characterization of the unfolded state of RNAs has been difficult. Experimental approaches — even at the single molecule level — cannot resolve its constituent microstates while theoretical models are of unknown accuracy without experimental validation. In this study we examine the unfolded ensemble of a model system, composed of two DNA duplexes tethered by a polyethylene glycol chain, that is an analog for the base paired regions in an unfolded nucleic acid. The synthesis of theory and small angle x-ray scattering experiments provides a quantitative description of the structural and energetic changes in the unfolded state as ionic conditions are modulated. We find that addition of ions electrostatically relaxes the system, increasing its conformational entropy and stability. Our tests of PB indicate that it is an adequate description of the underlying energies for monovalent ions; however, large deviations are observed for divalent ions.

998-Plat The Contributions Of Electrostatic Screening And Specifc Ion Binding To The Thermodynamics Of RNA Folding

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Nucleic acids are central to the storage, transmission, and control of genetic information. To carry out their biological function, RNA molecules fold into intricate 3-D structures. As the RNA backbone is highly negatively charged, counterions play a crucial role in RNA folding.

Here, we present a comprehensive model of the thermodynamics of RNA folding that explicitly accounts for ion-dependent electrostatic screening and specific ion binding. We apply this framework to two RNA molecules: the P4P6 domain of the group I intron ribozyme, which provides a well characterized model system, and the tandem aptamer of the glycine-riboswitch from V. cholerae[1,2].

Using a combination of SAXS, chemical footprinting, and mutations, we follow the conformational transitions of these two molecules as a function of mono- and divalent ion concentration and identity. Structure reconstructions from SAXS data[3] and a novel fragment assembly of RNA approach[4] permit us to construct intermediate resolution models for the different conformational states.

Our results suggest that for the P4P6 RNA the formation of the tetraloop/tetraloop-receptor interaction does not require specific ion binding and can be quantitatively modeled using Poisson-Boltzmann (PB) theory. In contrast, formation of the metal-ion core requires specific divalent ion binding and we account for the observed trends in a model that combines PB theory with an empirical term for specific binding[5].

Similarly, our experiments indicate that partial folding of the glycine-riboswitch aptamer in the absence of glycine is mediated by electrostatic screening alone, whereas glycine-binding requires the presence of specific metal ions[5].

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999-Plat Effect of Mg2+ Ions on the Stability of a Minimal RNA Kissing Complex

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Stability of RNA tertiary structures, such as kissing complex, depends critically on Mg^{2+} ions. The minimal kissing complex is formed by two GACG loops and consists of merely two GC base pairs. We constructed an intramolecular minimal kissing complex, KC30, in which the two hairpins were linked with 30 As. Using optical tweezers technique, we applied mechanical force to unfold single KC30 RNA molecules. By increasing force, the RNA was unfolded into four different conformations in order: kissing complex, two linked hairpins, one hairpin, and single strand. As force was decreased, the single strand was refolded into the kissing complex in the reverse order. We monitored the disruption and formation of the kissing interaction in real time. The "unkiss" and "kiss" kinetics at each force are computed from lifetimes of the kissing complex and two-linked-hairpin conformations, respectively. We varied concentration of $MgCl_2$ from 0.1 mM to 30 mM. The metal ions, particularly Mg^{2+}, greatly decreased the unkiss rate but had only moderate effect on the kiss kinetics. Addition of 10 mM Mg^{2+} raised the mean unkiss force from 20 pN to 50 pN, whereas the kiss force increased by less than 5 pN. In both unkiss and kiss kinetics, positions of the transition state along the reaction coordinate do not vary with cations; however, heights of the kinetic barrier critically depends on the type and concentration of cation. Mg^{2+} effects on this two-base-pair kissing interaction are saturable, allowing us to quantify Mg^{2+} binding from changes in folding free energy of the kissing complex. Since this minimal kissing complex contains no specific metal ion binding site, it serves as an ideal system to study hydrated, diffusely bound Mg^{2+} ions to RNA tertiary structure.

1000-Plat Simplified Hamiltonians for coarse-grained properties of large single-stranded RNA molecules

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Large single-stranded RNA (ssRNA) molecules with a length of a few thousand to a few tens of thousands of nucleotides are quite common in nature: Examples are some mRNAs in cells and the genomes of many viruses. Due to the large degree of self complementarity, these RNAs generally have a highly branched secondary structure with many short, double-stranded sections. The secondary structure is important for function. For example, a small three-dimensional size of the viral ssRNA genome, which is a consequence of a "small" secondary structure, facilitates its packaging in the protein shell, at least for certain viruses. From a theoretical point of view however, the prediction of the thermally accessible secondary structures of large ssRNAs is complicated, both because of the astronomically high number of structures that are possible, in principle, and the uncertainty in the exact details of the interactions between nucleotides. There are several computer programs available that predict secondary structures of ssRNA. They produce good results for small molecules (hundreds of nucleotides) but are not very reliable for predicting secondary structures of large ones. We are not interested in "high-resolution" structures, however, but in more coarse-grained properties, for example the average three-dimensional size of the molecule. We expect that the available computer programs are useful for determination of these coarse-grained properties but the complicated Hamiltonians they use limit the usefulness of these models for further theoretical investigations. We show that one can simplify these Hamiltonians considerably and still retain important predictive power. The inclusion of stacking energies is crucial but many of the detailed energy rules are not. We define several measures for the size of a secondary structure and we show how these measures are related to each other.