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The study of the dynamics of a complex system is an important problem that includes large macromolecular complexes, molecular interaction networks, and cell functional modules. Large macromolecular complexes in cellular machinery can be modeled as a connected network, as in the elastic or Gaussian network models as demonstrated by Bahar and colleagues. Here we propose the Perturbation-based Markovian Transmission Model for studying the dynamics of signal transmission in macromolecular machinery. The initial perturbation is transmitted by Markovian processes, where the dynamics of the probability flow is analytically solved using the master equation. Due to the large size of macromolecular complexes, it is very difficult to obtain analytical time-dependent Markovian dynamics of all atoms from the first perturbation until stationary state. To overcome it, we decrease the level of complexity of the transition matrix using Krylov subspace method. This method is equivalent to integrating all eigen modes, and we show it can provide a globally accurate solution to the dynamics problem of signal transmission for very large macromolecular complexes with reasonable computational time. We studied the dynamics of the GroEL-GroES chaperone system by applying uniform perturbation to all residues of the X-ray structure. We predicted a set of pivot, messenger, and effector residues, each with distinct dynamic behavior. We also identified key residues involved in the multiple salt-bridge switches important for the allosteric transition in GroEL. Our predictions are in agreement with published experimental data and with the results of computer simulations that used different methodologies. We also studied a selective perturbation on the surface of ATP binding pocket and predicted the path of maximal probability flow of the signal. It demonstrated clear advantages in studying dynamic behavior of large systems, such as virus capsid, ribosome, or large allosteric proteins.

244-Pos Improvements In Mixing Time And Mixing Uniformity In Devices Designed For Studies Of Protein Folding Kinetics

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Board B76

Using a microfluidic laminar flow mixer designed for studies of protein folding kinetics, we demonstrate a mixing time of 1 ± 1 μs with sample consumption on the order of femtomoles. We recognize two limitations of previously proposed designs:

1. size and shape of the mixing region, which limits mixing uniformity and
2. the formation of Dean vortices at high flow rates, which limits the mixing time.

We address these limitations by using as narrow a shape-optimized nozzle and by reducing the bend of the side-channel streamlines. The final design, which combines both of these features, achieves the best performance. We quantified the mixing performance of the different designs by numerical simulation of coupled Navier-Stokes and convection-diffusion equations, and experiments using fluorescence resonance energy-transfer (FRET)-labeled DNA. These mixers are currently being used for measurements of protein folding kinetics using FRET & tryptophan fluorescence.

244.01-Pos Characterization Of Protein Breathing Using Coordinated WAXS And NSE

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Board B77

In aqueous solution some proteins undergo large-scale rigid body movement of secondary structures, subunits or domains - referred to as protein 'breathing' - that define a native-state ensemble of structures. These motions increase with decreasing protein concentration or increasing temperature. They are sensitive to the nature and concentration of solutes and other proteins and appear to be characteristic of individual proteins. We are using a combination of wide angle x-ray scattering (WAXS), neutron spin echo (NSE) spectroscopy and computational modeling to determine the spatial and temporal scales of these fluctuations including the size of the rigid bodies undergoing these motions; the spatial scale of these motions and the decay rates of the fluctuations.

Concentration-dependent intensity changes in WAXS data have been interpreted in terms of the increase in spatial scale of motion with decreasing concentration. Compared to reference structures at high concentration, hemoglobin in dilute solution undergoes fluctuations in which structural features of characteristic length 10 A exhibit relative motions of ~1.2 A. Myoglobin exhibits similar behavior.

NSE data collected to high Q values from solutions of hemoglobin also suggest relative motions of structural features with characteristic lengths of 10–20 A. Collection of data at lower concentrations will be carried out in order to correlate trends with those seen in the WAXS data.

These experiments are being used to construct a comprehensive description of the spatial and temporal properties of the slow, correlated motions that make up the native state ensemble of a protein in solution.

Physical Chemistry

245-Pos Do Zwitterions Contribute to the Ionic Strength of a Solution?

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Board B78

The question of whether zwitterions contribute to the ionic strength of a solution has been a topic of much confusion over the years. Some authors have assumed that zwitterions do not contribute to ionic strength, while others have assumed the opposite. We have used free solution capillary electrophoresis to answer this question.

The mobility of a 26 base-pair, double-stranded DNA oligomer was measured in cacodylate-buffered solutions containing various concentrations of tetraethylammonium chloride (TEA^+Cl^-) or the zwitterion, $tricine^{+/-}$, present in its charge neutral form. The mobility of the DNA decreased as the square root of ionic strength when TEA^+Cl^- was added to the buffer, as expected by the Debye-Hückel-Onsager theory of electrophoresis. However, the mobility was independent of the concentration of added $tricine^{+/-}$. Hence, zwitterions do not contribute to the ionic strength of a solution.

246-Pos Extreme Accumulation of Nucleotides in Simulated Hydrothermal Pore Systems

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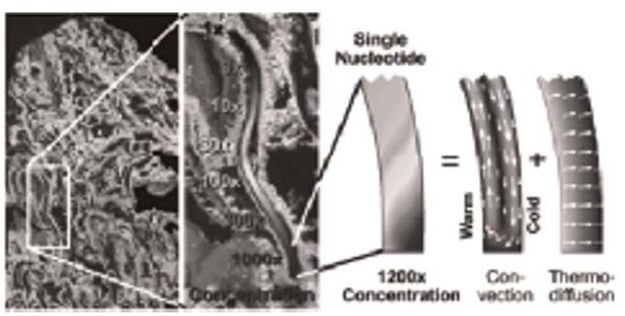
We simulate molecular transport in elongated hydrothermal pore systems influenced by a thermal gradient and find extreme accumulation of molecules in a wide variety of plugged pores. The mechanism is able to provide highly concentrated single nucleotides, suitable for operations of an RNA world at the origin of life. It is driven solely by the thermal gradient across a pore. On the one hand the fluid is shuttled by thermal convection along the pore, whereas on the other hand, the molecules drift across the pore, driven by thermodiffusion. As a result, millimeter-sized pores accumulate even single nucleotides more than 18-fold into micrometer-sized regions. The accumulation is considerably robust with respect to changes in the cleft geometry and the molecular dimensions. This setting also provides a temperature oscillation, shown previously to exponentially replicate DNA in the protein-assisted Polymerase Chain Reaction (PCR). Our results indicate that for life to evolve, complicated active membrane transport is not required for the initial steps. We find that interlinked mineral pores in a thermal gradient provide a compelling high-concentration starting point for the molecular evolution of life.



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247-Pos Cooperative Avalanches in DNA Unzipping

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We present experimental measurements of the mechanical unzipping of double-stranded DNA using dual, counter-propagating optical tweezers. A piece of 2252 base pairs of λ-DNA is unzipped by pulling apart the handles attached to the strands of one end. The two handles are short dsDNA oligos (30 bps) that increase the total stiffness of the system and provide high sensitivity measurements in force, which allows us to deepen in the understanding of the unzipping mechanism. The separation of the double helix is composed of a series of avalanches that open different number of base pairs along the molecule. The location and size of the avalanches are experimentally reproducible and they are correlated with the GC content along the molecule. Experimental data is analyzed taking advantage of polymer models and statistical techniques in order to identify the avalanches. The study shows an experimental limit of 10 base pairs so that smaller avalanches cannot be detected. The avalanche mechanism suggests a connection between the DNA structure and the pauses observed in some molecular motors such as DNA-polymerase that separate the strands of DNA.

248-Pos Optimizing Oligonucleotide Reagents for the Control of Gene Expression

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One focus of our research is to further our understanding of the physico chemical properties of triplexes and their interaction with partially complementary oligonucleotides. These triplexes, and homopyrimidine oligonucleotides, can be used in the control of gene expression. In this work, we used a combination of spectroscopic and calorimetric techniques to investigate:

(a) The unfolding of monomolecular, bimolecular, and trimolecular triplexes with a similar helical stem, TCTCTCT/AGAGAGA/$TC^+TC^+TC^+T$. Triplex stability follows the order: monomolecular > bimolecular > trimolecular.
(b) The unfolding of two bimolecular triplexes, *BiAG6* and *BiAG6*T_{10}; with and without a T_{10} dangling tail, resulting from the 1:1 mixing of d(AGAGAG), or d($AGAGAGT_{10}$), with TCTCTCC_5CTCTCTC, respectively. At low temperatures *BiAG6* and *BiAG6*T_{10} are in the triplex conformation. Each triplex unfolds in monophasic transitions; *BiAG6* has a higher thermal stability than *BiAG6*T_{10}, by ~10 °C, but both unfold with similar enthalpy contributions.
(c) The reaction of a double hairpin intramolecular triplex, d($A_7C_5T_7CT_3CT_7$) (*Triplex*) with its partially complementary

strands, yields the following duplex products: *Duplex-1* (with a nick) *and Duplex-2* (with a dangling end).

We determine exothermic enthalpies for each targeting reaction by ITC and DSC (indirectly); however, the ITC heats were less exothermic, by an average of -21 kcal/mol. This difference is explained in terms of heat capacity effects and a hidden hydration contribution due to an exothermic removal of structural water from *Triplex*.

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249-Pos G-quadruplexes: Energetics and Hydration

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Board B82

Among unusual nucleic acid structures, G-quadruplexes have received considerable attention due to their roles in a variety of cellular functions and as novel targets in cancer therapy. In this work, we used a combination of spectroscopic and calorimetric techniques to determine thermodynamic unfolding profiles for the following G-quadruplexes with sequence: d(GGT_2GG*TGT*GGT_2GG) (*G2*), d(GGGT$_2$GGG*TGT*GGGT$_2$GGG) (*G3*), and other sequences containing base substitutions in the loops.

We use unfolding thermodynamic profiles for pairs of molecules to resolve the following energetic contributions:

(a) Stacking of two G-quartets, which is applied to determine the loop and single strand contributions of several biologically relevant G-quadruplexes;
(b) Removal of the loop methyl groups, by substituting all thymines for uridines in *G2* and *G3*;
(c) Base substitutions of guanine in the *TGT* loop of *G2*;
(d) Role of Cd^{2+} ions on the integrity of a G-quadruplex in terms of the number of G-quartets; and
(e) Targeting G-quadruplexes with partial complementary strands.

The results are summarized as follows:

(a) Favorable formation of a G-quartet stack is accompanied by an enthalpy-entropy compensation, uptake of ions and release of water molecules. The loops and single strands stabilize the G-quadruplex.
(b) Removal of six methyl groups from the thymines in the loops of *G2* and *G3* yielded more stable G-quadruplexes, due to a net release of structural water. However, this pair of molecules yielded a similar thermodynamic profile for the formation of a G-quartet stack.
(c) Base substitutions in *G2* yielded more stable G-quadruplexes, due to increased base-base stacking contributions within the loops.
(d) Cd^{2+} disrupts Cs^{+}-, K^{+}- and Sr^{2+}-*G2*, but is unable to disrupt G-quadruplexes with additional G-quartet stacks, due to their higher thermal stability.
(e) Partial complementary strands are able to disrupt the formation of G-quadruplex by the formation of a duplex with a dangling end.

250-Pos Analyzing The Motion Of Nitroxides Attached To DNA Via Quantitative Spectral Simulation

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Site-directed spin labeling (SDSL) obtains information on a macromolecule via electron paramagnetic resonance (EPR) spectroscopy measurements of site specifically attached nitroxides. The X-band EPR spectral lineshape measures nitroxide motions in the 0.1 – 50 nanosecond regime, which are influenced by the structural and dynamic features at the labeling site. We are exploring spectral simulations for nitroxides attached at various modified phosphorothioate backbone positions of a B-form dodecameric DNA duplex. The goals are to achieve a quantitative description of the nitroxide motion, and use the information to extract information on DNA motion. Simulations were carried out on previously measured spectra of two nitroxide variations (HO368 and HO1820) at three DNA positions. Our calculation of CW EPR spectra are based on the Microspcopic Ordered Macroscopic Disordered (MOMD) model developed by J. Freed and colleagues, which describes the nitroxide motion as diffusion within a confined cone. We found that the HO368 spectra can be simulated by an isotropic motion model, in which the nitroxide motion can be sufficiently described by diffusion rates. The diffusion rate is higher for the nitroxide attached to the DNA terminus, which is consistent with the proposition that DNA fraying contributes to increased nitroxide motion. On the other hand, simulations of the HO1820 spectra require an anisotropic motion model, in which the nitroxide motion is described by both the diffusion rates and the order parameter. Further studies will optimize the simulation procedure, and explore the correlation between the nitroxide motion and DNA location environment.

251-Pos Defining DNA Breathing by 2-Aminopurine Low Energy CD And Fluorescence Spectroscopy

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Thermally induced local fluctuations and distortions affect the stability of a DNA double helix, which predominantly exists in the double-helical Watson-Crick form under physiological conditions. These thermal fluctuations can make the hydrogen bonds of the double helix vulnerable to temporary breakage, resulting in the infrequent event of base-pair opening called DNA 'breathing' or (when it occurs at the end of the double helix or at a primer-template junction) DNA 'fraying'. This breathing provides access to the normally buried bases of the DNA duplex and makes them available for modification and interaction with proteins and is an important step in the unwinding of dsDNA to form the single-stranded DNA

(ssDNA) intermediates that are essential for processes such as DNA replication, repair and recombination. The 'depth' to which this breathing extends into the double-helix and the number of base-pairs (bps) that open simultaneously have not yet been defined, but we have shown that monitoring (as a function of temperature) the spectral properties of site-specifically placed 2-AP residues at varying distances from the end of the DNA duplex can provide systematic insight into these thermal fluctuations. Preliminary experiments using the 2-AP probe with dsDNA alone have shown that breathing extends about two bps into the duplex under typical physiological conditions. We have characterized these breathing events structurally by comparing fluorescence and CD results together with dynamic fluorescence quenching. These breathing properties of DNA in isolation can be used as a platform to study mechanisms of helicase action on duplex DNA and ultimately the coupling of helicases to other components of the DNA replication complex.

252-Pos Folding Transition of a Single Giant Duplex DNA Chain: Weak Interaction Induces an ON/OFF Switch, whereas Strong Interaction Causes Gradual Change

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Large-scale morphological changes in genomic DNA molecules between a compact state and loose state are closely related to gene activation, such as transcription and replication, in living cells. This process often occurs over the range of several tens of kilo base pairs of DNA in an all-or-none manner, concomitant with the cooperative transcription of large numbers of genes. Transcriptional silencing associated with dense methylation also occurs in large-scale genomic DNA in an all-or-none manner, with a dynamic change in the conformation, such as the chromatin structure. Considerable effort has been applied to explain the mechanism of transcriptional action at a distance in terms of key-lock interaction between sequence-specific regulatory proteins and DNA, and have proposed several models. However, it is still unclear whether these mechanisms can explain the ON/OFF switching of a large number of genes that accompanies differentiation, carcinogenesis, etc. In this study, using single-molecule observation of DNA molecules by fluorescence microscopy with the addition of poly-L-lysine with different numbers of monomer units (n = 3, 5, 9 and 92), we found that an ON/OFF discrete transition in the higher-order structure of long duplex DNA is induced by short poly-L-lysine, whereas a continuous gradual change is induced by long poly-L-lysine. On the other hand, polycations with a lower positive charge have less potential to induce DNA compaction. Such drastic difference in the conformational transition of a giant DNA between short and large oligomers is discussed in relation to the mechanisms of gene regulation in a living cell.

253-Pos Investigating Nucleic Acid Counterions using Anomalous Small-Angle X-ray Scattering

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Nucleic acids are highly charged polyanions with -e per nucleotide (-2e per base pair) under physiological conditions. Consequently, positively charged counterions associate with nucleic acid strands and influence their biological behavior. Cations are known to mediate interactions between like polynucleotides (e.g. DNA condensation, RNA folding) and to stabilize nucleic acid structure. Although much theoretical progress has been made towards understanding the role of the counterion cloud around DNA, few experiments target the counterion distribution and its correlation to nucleic acid structural features. We use a tunable synchrotron radiation source and take advantage of energy-dependent, resonant Small-Angle X-ray Scattering (SAXS) of different elements to probe the positive ion atmospheres around short nucleic acid molecules in solution. We discuss how the composition of ionic atmospheres varies with different helical forms of nucleic acid (e.g. B-DNA, Z-DNA) strands.

254-Pos Sliding Of Alkylating Anticancer Drugs Along The Minor Groove Of DNA: New Insights On Sequence Selectivity

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Currently, little is known about the molecular recognition pathways between DNA-alkylating anticancer drugs and their targets, in spite of their pharmacological relevance. In the framework of classical molecular dynamics (MD) simulations, here we use umbrella sampling to map the potential of mean force (PMF) associated with sliding along the DNA minor groove of two of these compounds. These are an indole derivative of duocarmycin (DSI) and the putative reactive form of anthramycin (anhydro-anthramycin, IMI). Twenty-three configurations have been considered for each drug/DNA complex, corresponding to a movement along ~3 base pairs. The alkylation site turns out to be the most favorable for DSI, while a barrier of ~6 kcal/mol separates the reactive configuration of

IMI•DNA from the absolute minimum. An analysis of various contributions to the PMF reveals that solvent effects play an important role for the largest and more flexible drug DSI. Instead, the PMF of IMI•DNA overall correlates with changes in the binding enthalpy. Implications of these results on the sequence-selectivity of the two drugs are discussed.

255-Pos Competitive Exchange Equilibria Under Mechanical Forces: A New Paradigm For Biosensors

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Board B88

We are developing a new class of biosensors that, contrary to existing methods, relies on the breaking of pre-existing biomolecule complexes such as dsDNA or antibody-antigen. In these biosensors, micron-scale superparamagnetic beads are bound to the surface via one or multiple biomolecular tethers consisting of oligonucleotides bound to the surface of the device hybridized with oligonucleotides bound to micro-beads. When oligonucleotides complementary to those on the surface are injected into the system, competitive binding ensues in which the free (analyte) oligonucleotides can replace the bead oligonucleotides in hybridizing with the surface oligonucleotides, breaking the tethers and releasing the beads. The latter functions as the signal transduction. A similar system employing antibody- antigen tethering of the micro-beads can also be envisioned. In the case of nucleic acid tethers, the equilibrium between the analyte molecules and the bead molecules binding with the surface molecules can be changed by controlling the length of the complementary region of both the analyte and micro-bead oligonucleotides. Alternatively, a magnetic force stretching the tethers can be relied on to shift the equilibrium in favor of breaking the tethers. To uniformly apply forces, a magnetic tweezers setup is used. Force/unbinding curves were obtained to characterize the tethers when the number of tethers per bead was varied from one to hundreds per bead. Analyte oligonucleotide injected at low force increased the rate of micro-bead detachment compared to blank buffer injections. The sensitivity of the device scales with the number of tethers attaching individual micro-beads.

256-Pos What Configurational Diffusion Coefficient Characterizes Loop Formation In ss-polynucleotides?

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Board B89

Numerous kinetics measurements on the formation of single-stranded (ss) DNA and RNA hairpin structures with ~4–20 nucleotides (nt) in the loop and ~5–8 base-pairs in the stem, indicate that the time required to form hairpins is ~10–500 microseconds. If ss-polynucleotide chain is treated as an ideal semiflexible polymer with a statistical segment length of ~4 nt, the theoretical estimate for the end-to-end contact time for an ~10-nt long chain is expected to be tens-of-nanoseconds. To explain this discrepancy in time-scale, we proposed that the formation of the nucleating loop, prior to the zipping step, is slowed down as a result of transient trapping in misfolded conformations, with mis-paired base-pairs, non-native hydrogen bonding, or intrastrand stacking interactions in the unfolded state. Experimental measurements of end-to-end contact formation indicate that loop closure times for 4-nt poly(dT) loops are ~400 nanoseconds, and for 4-nt poly(dA) loops are ~8 microseconds, thus confirming that intrachain interactions slow down the configurational diffusion of the chain (Wang and Nau, *J. Am. Chem. Soc*. 2004, ***126***, 808). Interestingly, despite this evidence for intrachain interactions slowing down diffusion, the hairpin closing times for both ssDNA and RNA hairpins are found to scale with the length of the loop as $L^{2.2–2.6}$, in reasonable agreement with the scaling behavior expected for loop-closure of a semiflexible polymer.

Here, we present a kinetic zipper model that explicitly includes all misfolded microstates with non-native contacts, to describe the hairpin closing times. The loop-size dependence is described within the framework of a wormlike chain model, to obtain a characteristic configurational diffusion coefficient that is relevant for formation of the nucleating loop.

Transcription

257-Pos Byophysical Modeling of Transcription Initiation by Bacterial RNA Polymerase

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Board B90

The mechanism by which RNA polymerase (RNAP) forms the open complex (the first step in transcription initiation) is still unknown, despite two decades of intensive experimental research. To distinguish between several qualitative hypotheses, we develop the first quantitative model of the open complex formation by bacterial RNAP [1]. We derive an explicit relationship that connects transcription initiation rate with physical properties of DNA sequence and DNA-RNAP interactions. We compare our model with both biochemical measurements and genomics data and report a very good agreement with the experiments, with no free parameters used in model testing. The agreement strongly supports both the quantitative model that we propose and the qualitative hypothesis on which the model is based. Bioinformatic applications of our model, which allow efficient analysis of kinetic properties of DNA sequences on the whole genome scale [2], will also be addressed.

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