1381-Pos
Single Molecule FRET Characterization of DNA G-Quadruplexes Formed In The Promoter of Human MEF2D and TNNI3 Genes
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DNA G-quadruplexes are enriched near the transcription start site (TSS) of the human genes and have been suggested to be involved in gene transcription and translation. Informations about G-quadruplex conformation and dynamics is crucial to our understanding of the roles of quadruplex in gene regulation as well as to the development of novel therapeutic agents that interact with the quadruplex therefore modulate gene expression. Single molecule fluorescence resonance energy transfer (smFRET) can resolve conformational heterogeneity and dynamic fluctuations in nucleic acids, providing unique insights into the biophysics of quadruplex. We have recently elucidated the conformational heterogeneity and dynamics of the quadruplexes formed in the promoter of human c-myc and c-kit genes by smFRET. Here we present single molecule analysis of DNA quadruplex elements found in the TSS of the promoter of the MEF2D, a member of MEF2 (myocyte enhancer factor-2) family of transcription factors which regulate the response of heart to cardiac stress signals, and also in the chromosome 19 specific minisatellite sequences in the promoter of human cardiac troponin I (TNNI3), a gene encodes constituent protein of the troponin complex on the thin filament of cardiac muscle.

1382-Pos
Structural Diversity of G-Quadruplexes: Potassium Concentration Effect
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G-quadruplex (G4) structure, folded from Guanine-rich sequences, is a well known unique DNA secondary structure through Hoogsteen base pairing in the presence of monovalent cation. The importance of G4 structure is not only in human telomeres for protecting the ends of chromosomes, but also in several gene promoters for regulating gene expression.
Here we have combined gel electrophoresis, circular dichroism, and thermal melting to study the possible coexistence of the intramolecular and intermolecular G-quadruplexes in the presence of various concentrations of potassium cation (K^+). Our results showed that an appreciable amount of intermolecular G-quadruplex structures are detected in c-myc even at 1mM K^+, and increases at high K^+ concentration. Together with the quantification system, the amounts of intramolecular G-quadruplex structures of c-myc and bcl2 decrease as a function of K^+ concentration. However, no discernible intermolecular structures of human telomeric sequences up to 150 mM K^+ solution. In addition, upon late change of K^+ concentration at room temperature, no appreciable exchange between intra- and intermolecular structures of blc2 is observed. Moreover, the change in melting temperature upon altering K^+ concentration indicate that K^+-quadruplex association is faster than the K^+-quadruplex dissociation. Further thermodynamic studies based on differential scanning calorimetry and isothermal titration calorimetry measurements will be discussed.

1383-Pos
Does the Unfolding State of the Human Telomere Exist Upon Ion Exchange?
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The guanine-rich (G-rich) repeats of human telomere, $d(TTAGGG)_n$, can form different G-quadruplex (G4) structures in the presence of sodium and potassium cations. Folding of telomeric DNA into G4 structure could inhibit telomerase activity for cancer cell growth. Understanding the formation of G-quadruplexes and the conformational flexibility is essential not only for revealing their biological role, but also for developing anticancer drugs. Recently, Phan *et al.* found that the anti-parallel G4 structure of $d[(GGGTTA)_3GGGT]$ (NF3) with two G-quartet layers is quite different from the undetermined G4 structure of $d[AGGG(TTAGGG)_3]$ (HT22) with three G-quartet layers in K^+ solution. We found similar spectral conversion of circular dichroism for both sequences in Na^+ solution upon K^+ titration, even in the molecular crowding environment, which is more physiological condition. We further use the FRET efficiency from Cy3-DNA-Cy5 to monitor the time trace upon Na-K exchange. Our results show that the FRET efficiency in K^+ solution is similar to that in Na^+ solution for both HT22 and NF3. However, the time trace shows more different in normal condition than in crowding condition. The conversion rate is slower under molecular crowding environment. In addition, the stopped-flow FRET study shows a fast arising with ~300 ms for HT22 and ~200 ms for NF3 followed by decay without observing significant FRET efficiency drop, implies that within the time resolution of ~10 ms the unfolding intermediate state is unlikely the mechanism for the spectral conversion upon K^+ titration. Moreover, we have applied ITC to measure the formation heat of G4 structure and further confirmed that there are different conformations between H22 and NF3 under potassium stabilized G4 solution. Our results suggest that the spectral conversion of G4 under potassium titration is more likely due to loop rearrangement.

1384-Pos
Metadynamics Study of the Free Energy Surface of a G-Quadruplex DNA Structure
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Molecular Dynamics Simulations of Biomolecules present some limitations as the current accessible time scales which are significantly shorter that the time scale of a majority of biologically interesting conformational changes, and the evaluation of free energy fails due to the problem of trapping in free energy minima. In this work, we studied the conformational transitions of a four stranded nucleic acid structure (G-quadruplex) formed by a guanine-rich strands by means of Metadynamics method which is a technique to enhance sampling of conformational space systems as to built free energy surface in a modest quantity of time. We present one and two dimensional free energy surfaces of G-quadruplex in terms of properly selected collective variables. Our results show that free energy surfaces present two well defined local minima. We associate two different structural conformations to these minima by comparing with experimental data.

1385-Pos
Local Dynamic Studies of Guanine Residues within the Human Telomeric DNA G-Quadruplexed Conformation
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Formation and stabilization of guanine-rich G-quadruplexed DNA conformations can inhibit the abnormal activity of the enzyme telomerase in tumor cells, making it a target for potential cancer therapeutics. To study the effect individual guanine residues have on the folding process and stabilization of the G-quadruplex conformations, the fluorescence of HT_4 oligonucleotides incorporating the fluorescent guanine analog 6-methyl-8-(2-deoxy-D-ribofuranosyl) isoxanthopterin (6MI) into different tetrads of the quadruplex (G1,G4,G5,G9 and G11) were investigated. This guanine probe exhibits changes in fluorescence intensity sensitive to base-stacking and hydrogen-bonding. Fluorescence intensities quench for G4, G5, G9 and G11, and de-quench for G1 when each 6MI-labelled oligonucleotide folds to the G-quadruplex conformation with addition of K^+-ion. This suggests stronger base-stacking interactions with neighboring bases for G4, G5, G9 and G11, compared with G1 located on the 5'-end. Fluorescence intensity peaks observed for G1 and G11 also show significant red wavelength shifts with folding. This suggests these guanine positions may be more exposed to a polar environment within the folded state. Fluorescence lifetime studies of the labeled quadruplex sequences reveal that the observed intensity quenching arises predominantly from fast (sub-nanosecond) quasi-static self-quenching. This self-static quenching apparently arises from the proximity of 6MI to neighboring bases. The ''dark'' component (A_{dark}) dominates the decay behavior for both the folded and unfolded conformations of each 6MI-labeled sequence. With folding, the contribution of A_{dark} increases for all labeled oligonucleotide sequences as the conformation is now more compact. This effect is greatest for those 6MI replacements located in the loop regions. Overall these studies suggest individual guanines play different roles in the stabilization of G-quadruplex structures. This work was supported by NIH SCORE Grant S06 GM 060654.

1386-Pos
Benzo[b]Fluorenone as a Quadruplex Interactive Agent (Qia): Binding Studies and Quadruplex Formation with the Human Telomeric HT4 Sequence
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Many previously investigated agents capable of binding to and stabilizing G-quadruplex DNA conformations possess aromatic, planar chemical structures.

Here, initial investigations on the interaction of benzo[b]fluorenone (BF) with the human telomeric forming oligonucleotide HT4 using circular dichroism (CD), thermal melting and steady-state fluorescence are reported. CD studies suggest that BF exhibits binding selectivity for G-quadruplex over model double-stranded DNA structures. BF binds to hybrid-mixed type quadruplex structures in the presence of physiological concentrations of K^+ ions (100mM). However, even in the absence of salt, BF can induce an anti-parallel type quadruplex conformation. Thermal melting studies on quadruplex HT4 in the presence and absence of salt show BF has little effect on the melting temperature, suggesting that BF may associate with the G-quadruplex structure through a non-intercalating mode. As BF is known to be a solvatochromic fluorophore, steady state fluorescence emission spectra were taken for BF titrated with the HT4 quadruplex formed at 100mM K^+, to explore the ability of BF to act as a potential probe of the environment of its binding site. Based on the red shift of BF emission, BF/quadruplex association appears to involve a relatively polar and hydrogen-bonded environment. This work was supported by NIH SCORE Grant S06 GM 060654.

1387-Pos

Which Comes First, The Deformation or the Binding?

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Which comes first, the deformation or the binding?

High specificity of protein-DNA interaction is often related with specific deformation of the binding site. B-Z transition is the most dramatic structural change induced by protein-DNA interaction, where some segment of DNA abruptly changes from the right-handed B-DNA to the left-handed Z-DNA by the help of specific proteins.

Here, we report single-molecule FRET studies on protein-induced Z-DNA formation. DNA duplexes with six CG-repeats were prepared. To monitor the conformational dynamics of the CG-repeat, we labeled Cy3 and Cy5 at each end of the CG-repeat. Surface-immobilized DNA molecules did not show any structural dynamics in normal physiological conditions. When a Z-DNA inducing protein, Zα, was added to the buffer solution, however, fluorescence intensity increased abruptly without any accompanying FRET change. Abrupt FRET change occurred with time delay (~10 minute at 25°C on average). When the proteins were washed out, molecules didn't recover the original FRET value for more than 3 hours, but molecules without the FRET change readily recovered their original fluorescence intensity. From these result, we conclude that Zα protein weakly interact with B-DNA, but the interaction becomes extremely strong once Z-DNA is formed.

Next, we prepared a DNA duplex with methylated cytosine in the CG repeat. With millimolar Ni^{2+} in the buffer solution, we observed the intrinsic B-Z transition dynamics, and Z-DNA stabilization by Zα proteins. The transition time from B-DNA to Z-DNA, however, was not affected by the presence of Zα proteins, which strongly support that Zα protein induces Z-DNA by passively trapping Z-DNA structure transiently formed by the intrinsic B-Z transition dynamics. Even though we cannot directly observe Z-DNA, Z-DNA's are actually waiting there inside the cell to play their biological roles on time.

1388-Pos

Monitoring Structural Transitions of a DNA Holliday Junction Using an Acoustic Wave Sensor

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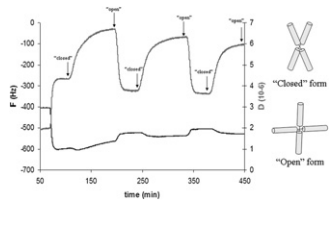
Structural DNA nanotechnology deals with building DNA molecules with particular geometrical features; creating nanoscale shapes and patterns using long DNA scaffolds, as in DNA origami, or changing DNA shape in a controlled way and in response to an external stimulus, as in molecular switches. Such nanomachines can perform computation, actuation and diagnostic tasks.

We took advantage of a biomolecular structure comprising a four-way DNA (Holliday) junction which belongs to a molecular machines group capable of moving between distinct states. This particular molecular switch exists in either an open (extended) or closed (coaxially stacked) conformation and it is proposed to be used as a principle for sequence-specific nucleic acid recognition. In this study we used the quartz crystal microbalance with energy dissipation (QCM-D) to study the applicability of this structure to detect DNA hybridization on a device surface and test its potential to act as a controllable switch.

We present a novel way of monitoring in real time both oligonucleotide binding and the transition from the closed to the open state of the junction. This transition can be reversed and repeated indefinitely in a fully controllable way.



1389-Pos

Single Molecule FRET Measures Structure and Fast Dynamics of DNA and RNA Four-Way Junctions

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Using a confocal fluorescence microscope multiparameter fluorescence detection (MFD) enables us to simultaneously collect all fluorescence information such as intensity, lifetime, anisotropy in several spectral ranges) from picoseconds to seconds. MFD and fluorescence correlation spectroscopy is applied to perform single-molecule FRET studies with an ultimate level of precision in determining separations with FRET of 1% of the Förster radius [J.Phys.Chem.B 110, 6970 (2006), J.Phys.Chem.B 112, 8361 (2008)]. In addition we can unambiguously distinguish between stochastic processes and broadening due to static or dynamic heterogeneity. In this way we measured bends and kinks in dsDNA. The high accuracy allowed us the detection of sequence-dependent DNA bending by 16° [PNAS 105, 18773 (2008)]. Moreover we studied the Mg-dependent structural dynamics of a four-way DNA (Holliday-) junction in order to find out whether the postulated extended square structure accumulates indeed as an intermediate or whether it should be considered more as a very short lived transition state. We found a complex Mg dependent kinetics, which must be described by a four species model with only two distinct FRET and two kinetic levels. The species with the same FRET value differ in their conformational flexibility: one is quasi-static, the other is dynamic. Our FRET data are clearly inconsistent with an accumulation of a single extended square junction structure at very low Mg concentrations. Finally we compare the structure and dynamics of DNA- and RNA four-way junctions. Thereby the structure of RNA four-way junction was characterized by 24 FRET distances, which allowed us to prove the existence of 3 of the 4 possible stacking conformers. These studies show that sm FRET studies are valuable tool to complement the structural and dynamic information obtained by X-ray crystallography or NMR spectroscopy.

Protein-Nucleic Acid Interactions II

1390-Pos

Kinetic Enhancement of NF-kB/DNA Dissociation by IkBα

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The nuclear factor kappa B (NF-kB) family of transcription factors is involved in inter- and intracellular signaling, cellular stress response, growth, survival, and apoptosis. Specific inhibitors of NF-κB transcription including IκBα, IκBβ, and IκBϵ, block the transcriptional activity of p65 and c-Rel-containing NF-κB dimers. DNA binding by NF-kB is inhibited by the ankyrin repeat protein kappa B (IkBα), which sequesters NF-kB to the cytosol. The mechanism and kinetics of DNA binding inhibition by IkBα are still unknown, but we recently demonstrated that NF-kB can be ''stripped'' off DNA by IkBα. We are investigating the effect of IkBα on the association and dissociation rates of NF-kB/DNA complex formation using titration measurements, stop flow fluorescence and ITC. We are using pyrene labeled DNA or IkBα or NF-κB to study the fluorescence changes occurring during the NF-kB ''stripping''. Our results show that IkBα increases the dissociation rate of the DNA from the NF-kB complex in a concentration-dependent manner and with high efficiency. We are studying also IkBβ which appears to stabilize the NF-kB/DNA interaction. This could suggest the formation of a ternary complex DNA/NF-κB/IkBβ.

1391-Pos

Prion Aptamer, Free and Bound States

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Aptamers are short single strands of DNA or RNA that bind to proteins, peptides, and small molecules. They are likely to fold into different structures when free in solution than when they are bound to a molecular target. The free structures are difficult to determine experimentally, though they can be modeled by calculating the minimum thermodynamic states. We test the validity of the thermodynamic models of a prion aptamer using single molecule pair Forster resonance energy transfer (spFRET) as a structural reporter. The FRET states of the unbound aptamers, the hybridized aptamers and the aptamers bound to PrP peptides are characterized. The DNA aptamers to PrP has a pair of thermodynamic states of roughly the same energy at 25 oC. Their presence in solution is characterized by comparing the single stranded aptamers to its hybridized configuration, thereby removing any internal structure of the aptamers. We demonstrate the existence of both unbound thermodynamic states as well as different interactions between the aptamer and each PrP peptide from static data measuring spFRET in solution.