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.