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I E C B

Mass Spectrometry Study of G-Quadruplex Nucleic Acids: Folding Pathways and Ligand Binding Modes

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A G-quadruplex (G4) is a non-canonical nucleic acids structure formed by guanine-rich sequences. Some G4s are polymorphic, a given sequence can form G4s of different topologies. G4s are proposed to be biological regulators because they are found in key regions of the genome, for example, in gene promoters or at the telomeres. Stabilizing G4s formed in those regions as compared to the duplex form is a strategy to fight cancer. To do so, specific and affine ligands are used. Ligand design usually implies the optimization of large aromatic planes to π-π stack on external G-quartets. However, if this was the only binding mode, all ligands would bind with similar affinities to all G4s. To characterize which structures should be targeted and how the ligands interact with these structures, we used native mass spectrometry (MS).
First, we developed a MS-compatible sample preparation method in KCl conditions in which G4s are folded with similar topologies as compared to those obtained in biologically relevant conditions. Then, we characterized the K+ binding equilibria and G4s folding pathways. This folding pathway involves the presence of a dead-end constituted by antiparallel G4s with either 1- or 2-K+ cations that are folded first. Finally, our ligand binding studies showed that some of the most affine ligands can influence G4's structures, as probed by the number of K+ ions bound. Ligands Phen-DC3, 360A and PDS are able to shift the equilibria towards the 1-K+ antiparallel G4s. The formation of antiparallel with 2-K+ complexes is induced by the cooperative binding of two Cu-ttpy ligands. Our results demonstrate the importance to characterize ternary complex stoichiometries (G4:ligand:K+) as obtained from native mass spectrometry.