A Unified Computational Framework for G-Quadruplex Drug Discovery: Assessing Stability, Ion Coordination, and Ligand Binding

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G-quadruplexes (G4s) are non-canonical DNA secondary structures whose involvement in the regulation of nucleic acids replication and transcription has attracted increasing attention[1–4]. These distinctive structural motifs represent attractive therapeutic targets in both viral genomes and human regulatory regions. While molecular dynamics (MD) simulations provide valuable insights into G4 conformational dynamics and stability, the reliability of computational investigations strongly depends on the adopted simulation protocol. In this work, we present a systematic computational workflow for the investigation of G4 structural integrity and for the generation of reproducible structural models suitable for structure-based drug design studies.

To demonstrate the applicability of the proposed approach, four distinct G4 architectures were investigated within a unified methodological framework. Specifically, the study includes two experimentally resolved structures, the human telomeric G4 (PDB ID: 1KF1[5]) and the Human Papillomavirus type 52 (HPV-52) G4 (PDB ID: 5O4D[6]), together with two homology models generated for Monkeypox and Zika virus sequences. For all systems, production MD simulations ranging from 200 to 1000 ns were performed using the AMBER software package. Simulations were conducted over an extended temperature range, from 300 K up to 650 K, to accelerate unfolding events and enable comparative stability assessments under increasingly destabilizing conditions[7].

The structural integrity of the investigated targets, evaluated through extensive RMSD-based trajectory analyses, provided insights into the factors governing G4 stability and unfolding. Application of the workflow revealed that thermal resistance is strongly correlated with the number of stacked G-tetrads, highlighting their central role in maintaining structural integrity. Furthermore, the protocol enables the systematic assessment of the modulatory effects of central coordinating ions and candidate ligands through direct comparison with unliganded and ion-substituted reference simulations.

This comparative approach successfully captured the differential impact of ion coordination on conformational dynamics. In particular, K^+ coordination provided superior structural stabilization, preserving the native G4 architecture even at elevated temperatures, whereas Na^+-coordinated systems exhibited pronounced structural disruption under identical conditions. Analogous comparative analyses are currently being extended to the 1KF1 sequence through direct evaluation of K^+- and Na^+-coordinated states. In addition, the methodology effectively detected ligand-induced stabilization effects: porphyrin-based ligands reduced the mobility of tetrad-forming nucleotides and significantly delayed thermal denaturation across the investigated topologies. Overall, this work provides a comprehensive computational framework for the in silico investigation of G-quadruplex architectures, supporting the rational evaluation of novel G4-targeting therapeutics.

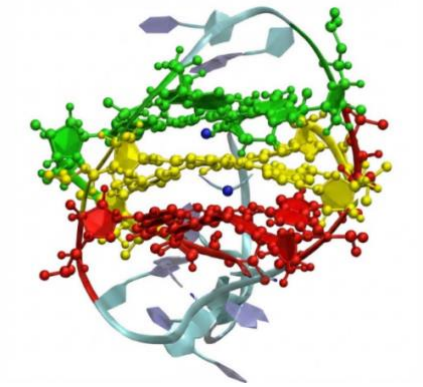

Figure 1. Three-dimensional structure of the Human Papillomavirus type 52 G-quadruplex (PDB ID: 5O4D). Guanine residues involved in G-tetrad formation are highlighted in green, yellow, and red, whereas the remaining nucleotides of the DNA sequence are shown in light blue.

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