Molecular dynamics simulations reveal the balance of forces governing the formation of a guanine tetrad – a common structural unit of G-quadruplex DNA

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SUPPLEMENTARY DATA

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Methods

Free energy profile for the dissociation of a single guanine residue

To determine the free energy profile for a dissociation of a single guanine from the 3’-terminal G-tetrad of the parallel-stranded G-quadruplex and, furthermore, to decompose it into enthalpic and entropic contributions, we used the adaptive biasing force (ABF) method\(^1,2\) implemented in NAMD 2.8.\(^3\) The system composition and the parameter set was the same as in the simulations of the dissociation of the entire G-tetrad. MD simulations were carried in the NPT ensemble, using Langevin dynamics and the Nose-Hoover Langevin piston pressure control.\(^4\) Periodic boundary conditions were applied, and electrostatic interactions were calculated using the Particle Mesh Ewald method\(^5\) with real-space cutoff of 1.2 nm and Fourier grid spacing of 0.1 nm. A cut-off of 1.2 was used for Lennard-Jones interactions. Bonds in DNA involving hydrogen atoms were constrained using SHAKE algorithm.\(^6\) SETTLE\(^7\) was used to constrain water geometry. The equations of motion were integrated using the velocity verlet algorithm with a 2 fs time step.

To prepare the initial configurations for the free energy simulations, the system was subject to 100-ns-long steered molecular dynamics with the external harmonic potential (with a spring constant of 2092 kcal/(mol·nm\(^2\))) enforcing dissociation of guanine IV (see Fig. 1) from the 3’-terminal G-tetrad in the parallel-stranded G-quadruplex. The potential was applied to the coordinate defined as the distance between the center of mass of all heavy atoms of the 3’-terminal guanine and the center of mass of all heavy atoms of the remaining guanine residues. Further, eight configurations were extracted from the steered MD run to initiate eight 0.3 nm-wide overlapping ABF windows spanning the reaction coordinate from 0.6 nm (for a fully-formed G-tetrad) to 1.8 nm (for a dissociated guanine IV). In each of these windows the system was simulated for 300 ns, while storing the instantaneous values of the force acting along the reaction coordinate in 0.05 Å-wide bins. The free energy profile was determined from the last 280 ns of the production simulations.
by integrating gradients of the conformational coordinate sampled during simulation. Free energy error bars were estimated as standard errors corrected for the autocorrelation of the gradient time series. To be able to decompose free energy into the enthalpic and entropic contributions, we used the same adaptive biasing force protocol to obtain the free energy profiles at three different temperatures: 295, 300 and 305 K (Fig. S9).

Assuming that the specific heat of the system is constant along the reaction coordinate, \( r \), which implies that \( \Delta H \) and \( \Delta S \) do not depend implicitly on temperature, i.e., \( \frac{\partial \Delta H(r,T)}{\partial T} = 0 \) and \( \frac{\partial \Delta S(r,T)}{\partial T} = 0 \), the entropic contribution is simply given by the total derivative of the free energy with respect to temperature, at constant pressure, \( \Delta S(r) = \left( \frac{d \Delta G(r,T)}{dT} \right)_{N,P} \). At each point along the reaction coordinate, this derivative was approximated by the slope of the linear function fitted to the free energy values at three different temperatures.

**Estimation of mean first-passage time**

The mean first-passage times (MFPT) for the dissociation of the first and second guanine from the G-tetrad were estimated according to Zwanzig formula: 8

\[
MFPT = \int_{x_0}^{x_1} dx \exp(\beta G(x)) \cdot \frac{1}{D} \int_a^x dy \exp(-\beta G(y))
\]  

where \( G(x) \) is the free energy profile, and the integration boundaries \( x_0 \) and \( x_1 \) define the initial and final state of the dissociation process (e.g., \( x_0 \) is located at state 4 minimum and \( x_0 \) at state 3 minimum (see Fig. 2) for the MFPT of the first guanine dissociation). \( \beta \) is the inverse temperature \((kBT)^{-1}\). \( a \) is the position of the reflecting barrier placed at \( N_{hb} = 8 \) (for the first guanine dissociation) or \( N_{hb} = 4 \) (for the second guanine dissociation) and \( D \) is the diffusion coefficient along the collective coordinate \( N_{hb} \). The latter was estimated in the global free energy minimum using formula proposed by Hummer: 9

\[
D = \frac{\sigma^2}{\tau_A}
\]
with $\sigma^2$ being the variance of the distribution of number hydrogen bonds and $\tau_A$ the $N_{hb}$ autocorrelation time.

**Free energy profile for the position of K$^+$ in the G-quadruplex central channel**

To determine the free energy profile for the position of the a single K$^+$ ion along the central channel of the parallel-stranded G-quadruplex, we used the well-tempered metadynamics method,$^{10}$ implemented in GROMACS$^{11}$ via the PLUMED plugin.$^{12}$ The system composition, force field parameter set and MD protocol were the same as those used in the simulations of the dissociation of the 3’-terminal G-tetrad (see Methods in the main text). The initial configuration with a single K$^+$ ion bound at position 2 of the G-quadruplex channel (see Fig. 3d) was taken from the equilibrium simulation. As the reaction coordinate, along which a bias potential was deposited, we used the signed distance between the ion and the center of mass of all guanine O6 atoms forming the channel, in the range from $-0.225$ to the $0.225$ nm. The Gaussian functions, with the height and width of $0.05$ kJ/mol and $0.01$ nm, respectively, were deposited every 500 steps and the well-tempered bias factor was set to 6. The system was simulated for 645 ns.

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Fig. S1: Switching function used to define a native hydrogen bonds in the G-tetrad. The hydrogen bond existence is a function of a distance between donor and acceptor forming the bond.
Fig. S2: Four typical geometries of the dissociated state of the G-tetrad showing stacking interactions between guanine and thymines in the TTA loops (in brown), the neighboring dissociated guanines, and the middle G-tetrad (in grey). The tendency of adenine residues to stack onto the 5′-terminal G-tetrad is also clear from the top panel.

Fig. S3: Evolution of the native guanine-guanine hydrogen bonds in additional 4 µs equilibrium MD simulation. After 750 ns, guanine IV of the 3′-terminal G-tetrad spontaneously dissociates resulting in the guanine triplet (see Fig. S4), in which the native hydrogen bonds are observed only occasionally.
Fig. S4: Additional 4 µs equilibrium MD simulation reveals the formation of a guanine triplet in which each guanine residue participates in three hydrogen bonds with either the two other guanine residues.

Fig. S5: Probabilities of finding K$^+$ ion in four ion-binding sites along the G-quadruplex central channel as a function of time in an additional 3 µs MD simulation.
Fig. S6: The free energy profile for the position of a single K$^+$ ion along the G-quadruplex channel calculated every 100 ns of a 645 ns-long metadynamics simulation run. Ion-binding sites 2 and 3 are defined as in Fig. 3d.

Fig. S7: Representative structure of the parallel-stranded telomeric G-quadruplex from the MD simulation showing the tendency of the adenines to stack onto the 5'$\text{'}$-terminal G-tetrad (PLANE3).
Fig. S8: Pairwise contributions to the enthalpy change upon G-tetrad formation due to interactions between guanine nucleotides I–IV, divided into guanine (G), phosphate (P) and deoxyribose (dR), and the rest of the system. The rest of the G-quadruplex was divided into: guanine residues of the middle (PLANE2) and 5′-terminal (PLANE3) G-tetrads, adenine (ADE) and thymine (THY) residues, and the sugar-phosphate backbone (DNA BB).
Fig. S9: Remaining contributions to the enthalpy change upon G-tetrad formation with the same labels as in Fig. 4 and Fig. S7.

Fig. S10: Free energy as a function of the distance between the centers of mass of a single guanine residue (IV in Fig.1b) and the remaining guanine residues of the 3′-terminal G-tetrad, at three temperatures: 295 K, 300 K and 305 K.
Fig. S11: Convergence of the quasi-entropic entropy values as a function of time for 8 successive intervals along the $N_{hb}$ coordinate.
Fig. S12: a) Equilibrium distributions of the dihedral angles defining the geometry of the G-tetrad-forming nucleotides in the native (red) and dissociated (green) states. b) Definition of the dihedral angles. c) Representative arrangement of the 3'-terminal guanine residue with respect to the middle G-tetrad (grey, transparent) in the native (top) and dissociated (bottom) states.