

Supporting Information

“Stability and Migration of Metal Ions in G4-wires by Molecular Dynamics Simulations”

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Test: 4-plane G4-quadruplexes with internal Na⁺ cations in different stoichiometries.

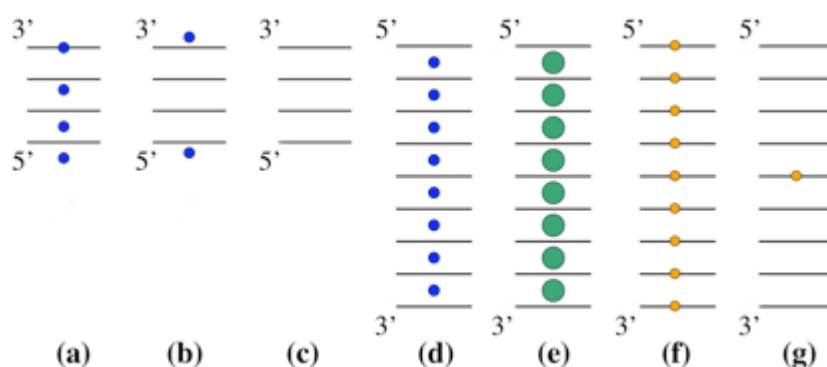


Figure S1. Schematic view of the initial geometries of the simulated guanine quadruplexes. Each horizontal segment represents a G4-tetrad and each dot represents a cation. Na⁺, K⁺ and Li⁺ cations are colored in blue, green and orange, respectively, with the same color code as in Figure 2. The 3' and 5' ends of the G4-quadruplexes are indicated. (a-c) 4-plane G4-quadruplexes used to assess the computational method. (d-f) Fully-coordinated 9-plane G4-quadruplexes. (g) Partially-coordinated 9-plane G4-quadruplex.

We test our method against a reference molecular dynamics simulation of G-quadruplexes¹ (G4w's). We do not repeat exactly the same simulation: in addition to the different computer code, we have an updated force field², a different choice of the number and initial distribution of water molecules and counterions, and other technical differences. Hence, we should not expect precisely equal trajectories: the comparison is based on general qualitative features, mainly the overall stability with respect to deformations.

¹ Spackova, N.; Berger, I.; Sponer, J. *J. Am. Chem. Soc.* **1999**, 121, 5519.

² Wang, J.; Cieplak, P.; Kolmann, P. A. *J. Comput. Chem.* **2000**, 21, 1049. This approach (parm99) uses the simple force-field model parm94 and attempts to add a minimal number of extra Fourier components to the torsional energies, but doing so only when there is a physical justification.

We choose the d(TG2G3G4G5T) molecule of the crystal unit cell as the starting structure (see Figure 1a in the main text). Unlike Spackova *et al.*, we keep both the 3' and 5' terminal thymine residues. Three initial cation dispositions inside the G-channel are considered, as illustrated in Figure S1(a,b,c). The MD production runs are 3 ns long, as in the reference simulations¹.

In the first simulation we retain the four X-ray resolved Na⁺ internal cations in the G-channel using the crystal data³ (see Figure S1(a)). During the system equilibration the sodium ion initially placed below the 5' G4-tetrad (G4t) loses its coordination with the O6 oxygens and the other three cations relocate to reach equidistance with respect to each other and to the O6 oxygens of neighboring G-planes. This disposition is maintained for the whole simulation, as shown in the final snapshot of Figure S2(a). The same behavior was observed by Spackova and coworkers in their simulation¹. Our average value of the root mean square (rms) displacements between the dynamical trajectory and the equilibrated structure is 0.9±0.1 Å, in excellent agreement with the previous result.

In the second simulation, two Na⁺ cations are positioned at the entries of the G-channel, immediately outwards from the 3' and 5' tetrads (Figure S1(b)). During the equilibration phase, they move inwards and are relocated at inter-plane sites between the first and second plane at each terminal (not exactly midway between planes, see Figure S2(b)). The same behavior was observed in the reference simulation¹. Our average rms displacement is 1.2±0.1 Å, exactly the same obtained by Spackova *et al.*¹.

In the third simulation, we remove all the Na⁺ cations from the G-channel. The empty helix is quickly hydrated and remains hydrated throughout the whole dynamics, as shown in Figure S2(c). The structure of the quadruplex is strongly perturbed: in particular, one of its strands experiences a remarkable distortion. Our calculated rms displacements are larger than those of the other two helices, with an average value of 1.7±0.2 Å. Both the observed structural deformation and rms displacements are less pronounced than those reported earlier: in fact, Spackova and coworkers found rms displacements beyond 3.0 Å. The less complete unfolding in our case is probably due to the different environment but may be affected by the short simulation times that are not sufficient to study denaturation.

Based on the above arguments, that prove a satisfactory overall agreement with the previous simulations although not describing the very same trajectories, the adopted computational method is judged suitable to simulate G4w's and therefore adopted in the remainder of the article to describe longer similar quadruplexes, with the aim of comparing the behavior of:

³ (a) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, 273, 171.
(b) Laughlan, G.; Murchie, A. I. H.; Norman, D. G.; Moore, M. H.; Moody, P. C. E.; Lilley, D. M. J.; Luisi, B. *Science* **1994**, 265, 520.

long vs. short quadruplexes; various degrees of filling the G-channel with alkali cations; different metal elements filling the G-channel.

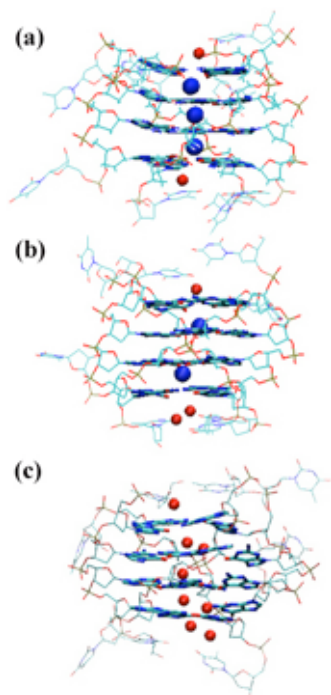


Figure S2. MD simulations of the 4-plane $G4w^3$ with 4 G4t's - snapshots of the MD final structures. Blue spheres represent the internal Na^+ cations: Compare with the initial cation dispositions in Fig. S1(a-c). Red smaller spheres represent the water molecules nearby and inside the G-channel.

Root mean square deviation relative to the equilibrated structures.

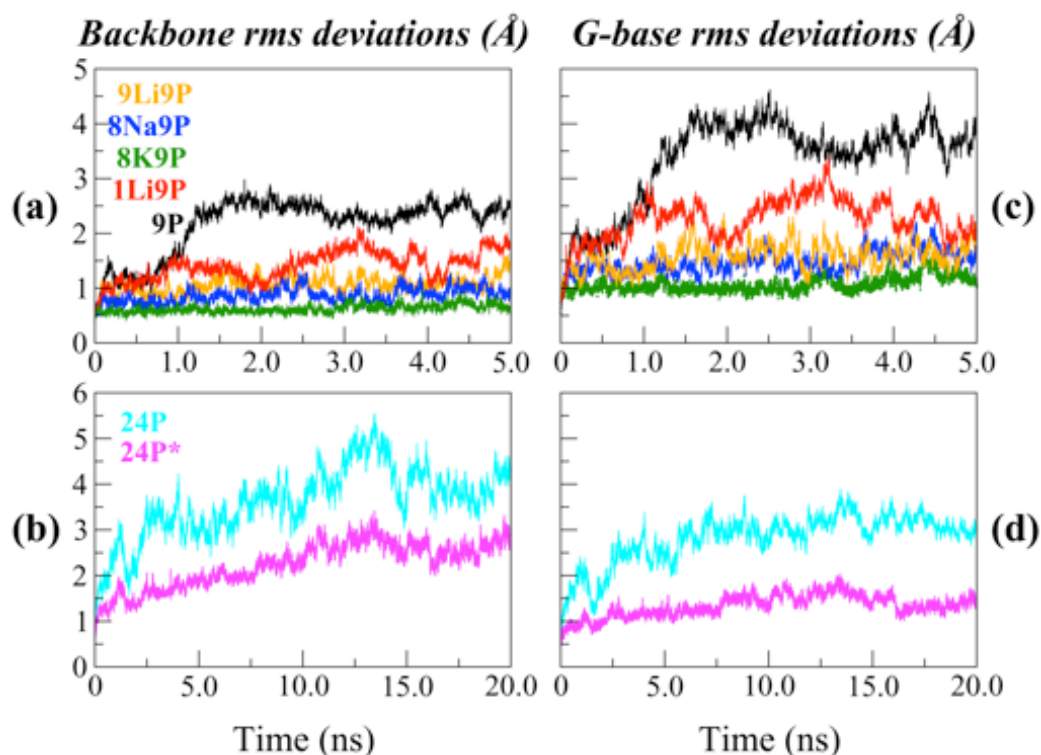


Figure S3. Root mean square displacement profiles during the dynamical runs for 9-plane (top) and 24-plane (bottom) G4-wires. For each system, the signal is evaluated relative to the equilibrated structure. (a,c) Signals for the systems 9Li9P, 8Na9P, 8K9P, 1Li9P and 9P, calculated for the G-base atoms (a) and for the backbone (sugar and phosphate groups) atoms (c). (b,d) G-base (b) and backbone (d) signals for the systems 24P and 24P*. For the statistical analysis presented in the Article, we consider the final 3.5-ns span and the 12-ns span of the simulations of 9-plane (a,c) and 24-plane (b,d) G4-wires, respectively. The plots here show that over these time intervals the structure presents no strong drift with respect to the equilibrated structure (for each system separately), which means that the average configuration is meaningful if the average is computed over such intervals.

Hydration and external cations in the 9-plane G4w's.

Hydration features. We identify four different hydration sites: the phosphate groups, the helical grooves, the G-channel entries, and the very G-channel. Some water molecules cluster around phosphate groups, forming HBs with the phosphate oxygens. The narrowness of the helical grooves leads to highly localized water molecules inside them, as shown in Figure S3a. Water molecules are principally H-bonded with the N2 and N3 nitrogens of the G-bases and, at times, N2 and N3 atoms are connected through water mediated HBs, so that a well-organized hydration network is formed. In the cation-filled G4w's, some water molecules coordinated with the Na^+ and K^+ internal cations are found at the entries of the G-channel (see Figure S3b). The hydration of the empty 9P G-channel is a very fast event, as shown in the MD snapshots of Figure 9. No water molecules gain the G-channel from the grooves in our simulations.

Discussion on hydration. Different hydration sites are found in and around the simulated quadruplexes. Water molecules form HBs with the oxygens of the phosphate groups, clustering around them, and, inside the helix grooves, preferentially with the N2 and N3 nitrogens of the G-bases. Water mediated HBs between the N2 and N3 atoms are also detected. The distribution of water molecules inside the grooves is well ordered, with the formation of strings along the grooves (Figure S3(a)). The groove hydration here observed is in excellent agreement with both theoretical^{1,4} and experimental^{3,5} previous studies. In the absence of cations coordinated with the terminal tetrads, several water molecules readily enter through the free openings and travel along the G-channel. On the other hand, when a cation is coordinated with a terminal G4t, some water molecules cluster around it, preventing the cation to rapidly leave the G-channel. A similar feature was also observed in the X-ray structures³.

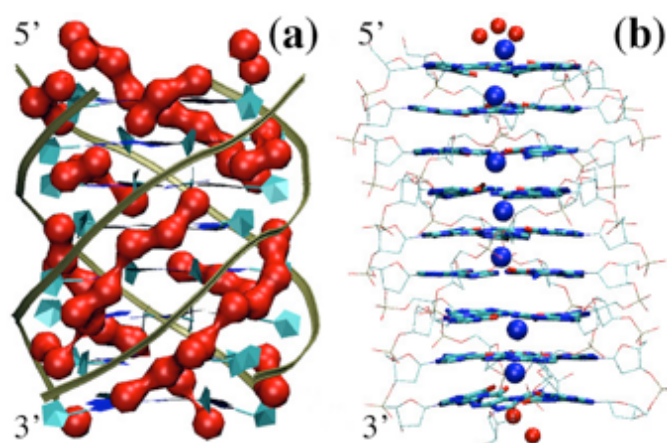


Figure S4. G4w hydration patterns are shown in the 8Na9P quadruplex. Internal Na^+ ions are shown as blue spheres. Water molecules at the openings of the channel are shown as smaller red spheres. (a) Isosurface of water density distribution in the helix grooves. (b) Water molecules at the entries of the G-channel.

⁴ (a) Chowdhury, S.; Bansal, M. *J. Biomol. Struct. Dyn.* **2000**, 18, 11. (b) Chowdhury, S.; Bansal, M. *J. Phys. Chem. B.* **2001**, 105, 7572. (c) Chowdhury, S.; Bansal, M. *J. Biomol. Struct. Dyn.* **2001**, 18, 647.

⁵ Strahan, G. D.; Keniry, M. A.; Shafer, R. H. *Biophys. J.* **1998**, 75, 968.

External cations. Several external Li^+ cations are observed within 3.0 Å of any G4w (guanines and backbone) atom in 9Li9P. The largest part of them is localized near the phosphate groups, the remaining part gains the G4w grooves, in the proximity of the O3', O4' and O5' oxygens of the backbone sugars. A few Na^+ counterions (i.e. 4-5, on average) are also found near the G4w atoms in 8Na9P, especially around the phosphate groups and, at times, near the oxygens of the sugar moieties in the grooves. When the G-channel is devoid of cations (i.e., the 9P system), the number of Na^+ counterions in the vicinity of the G4w increases and some of them are also found near the G-bases, as shown in Figure S4. Only two or three K^+ cations are on average placed near the G4w during the dynamics, but without pushing on to the grooves.

Discussion on external cations. The amount of external cations (i.e. counterions) in the vicinity of the G4w depends on the species and on the presence of internal cations. We observe that the smaller is the cation size (measured through the van der Waals radius), the larger is its ability to settle near the G4w. Several Li^+ counterions bind to the backbone phosphates and sugars in the helix grooves. We also observe a lithium cation entering the G-channel from a groove in the simulation of the 24-tetrad quadruplex. The number of Na^+ cations externally bonded to the G4w is remarkably lower and even smaller is the number of K^+ counterions in the proximity of the wire, especially inside the grooves. Furthermore, when the G-channel is full of cations, the counterions cannot approach the G-bases. On the other hand, if the G-channel is empty, several counterions reach the quadruplex and some of them gain the G-bases through the grooves, because of the absence of the repulsive interaction with the internal cations.

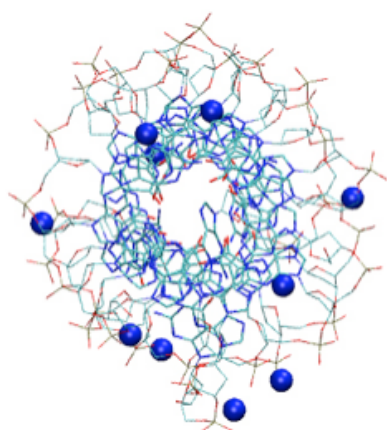


Figure S5. MD snapshot of 9P, top-view from 3'. Blue spheres represent the Na^+ counterions within 3.0 Å of any guanine/backbone atom.

Statistical analysis of the root mean square displacements and of the atomic mean square displacements.

Figures S6 and S7 report the hystograms that describe the distribution of the root mean square displacements (rmsd) and of the atomic mean square displacements (msd), respectively, as described in the text and reported in Figure 4.

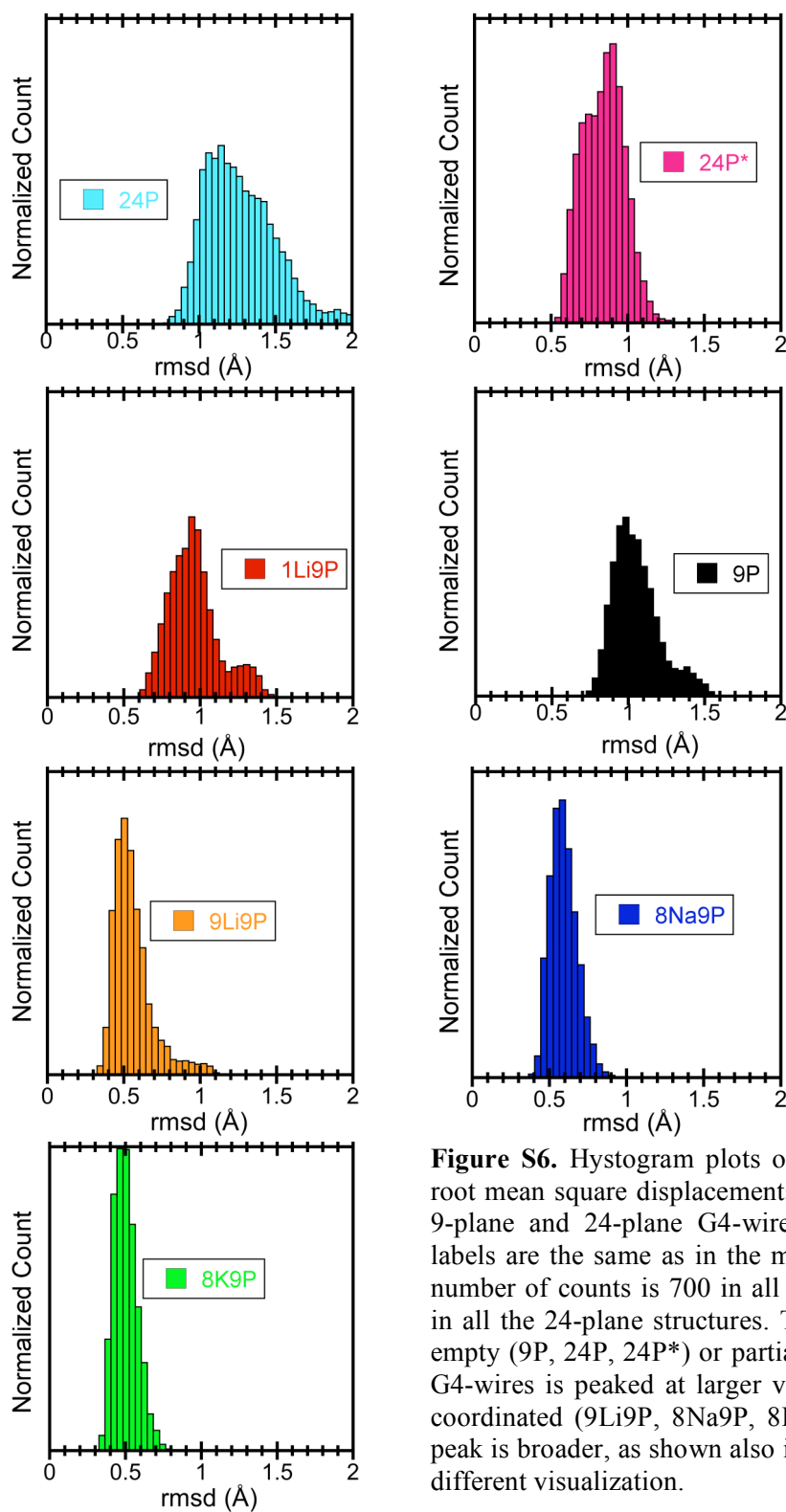


Figure S6. Histogram plots of the distribution of the root mean square displacements for all the investigated 9-plane and 24-plane G4-wires. The color code and labels are the same as in the main text. The maximum number of counts is 700 in all 9-plane structures, 1500 in all the 24-plane structures. The rmsd distribution of empty (9P, 24P, 24P*) or partially-coordinated (1Li9P) G4-wires is peaked at larger values than that of fully-coordinated (9Li9P, 8Na9P, 8K9P) G4-wires, and the peak is broader, as shown also in the text by means of a different visualization.

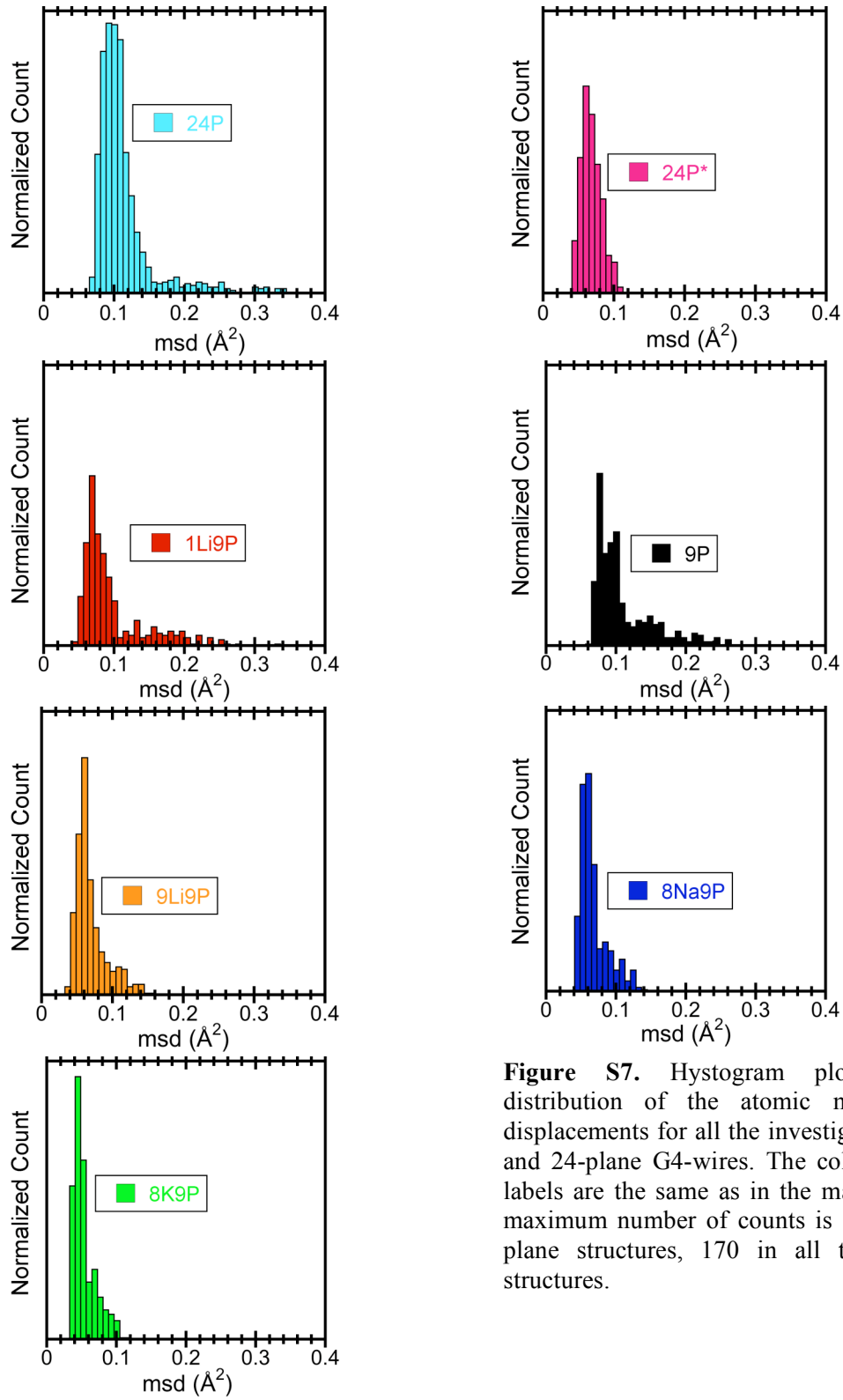


Figure S7. Hystogram plots of the distribution of the atomic mean square displacements for all the investigated 9-plane and 24-plane G4-wires. The color code and labels are the same as in the main text. The maximum number of counts is 130 in all 9-plane structures, 170 in all the 24-plane structures.

χ^2 test on the structure 8K9P for the selection of the reliable block length for the variance statistics.

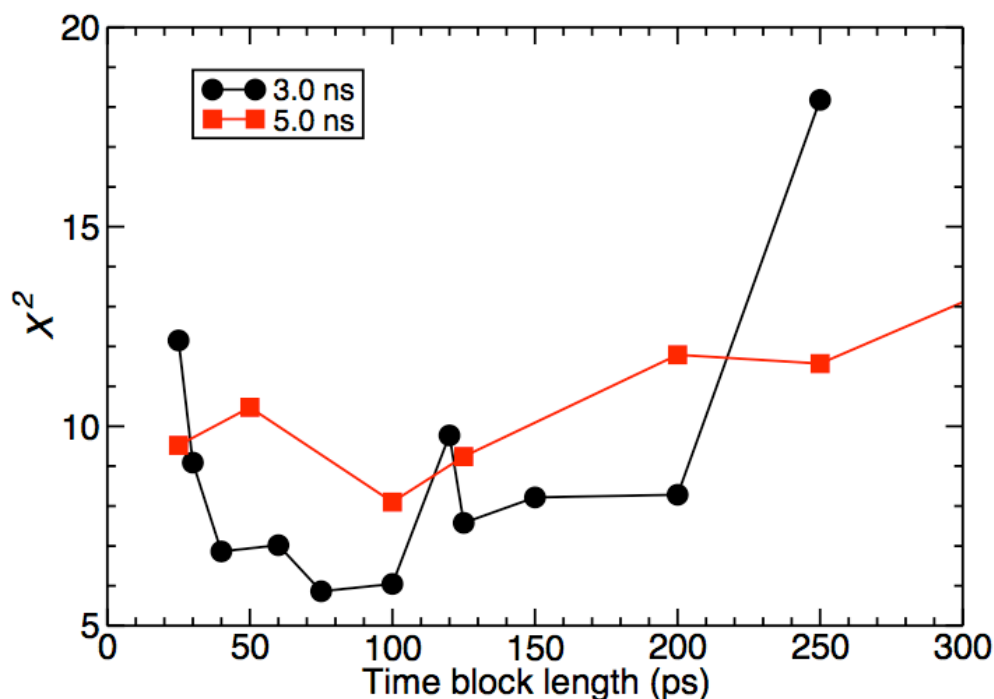


Figure S8. Values of χ^2 variable for the distributions of within-blocks mean square displacements with different block durations, calculated over the whole 5-ns trajectory and over the final 3-ns span, for the 8K9P G4-wire. The stability region can be roughly identified between 60 and 200 ps, and we choose 100 ps as a proper block length for the calculation of atomic mean square displacements within blocks. The χ^2 stability region identifies those block lengths in which range the distributions do not significantly depend on the time block length. It is remarkable that we obtain similar conclusions for different total extents (square and dots).