A G-rich sequence within the c-kit oncogene promoter forms a parallel G-quadruplex having asymmetric G-tetrad dynamics

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c-kit2 exhibits very slow folding kinetic

Immediately after the annealing treatment, the imino proton spectrum of wt *c-kit2* exhibits a large number of resonances, some of which are weak and poorly resolved (**Figure S1**, upper left). The imino proton spectrum of G21T, on the other hand, shows much better-defined resonances (**Figure S1**, upper right), however, the number of resolved imino proton signals remains in excess of the 12 that are expected from a G-quadruplex that has three G-tetrads, suggesting the presence of some degree of structural heterogeneity. Following an extended period of sample incubation at 4°C after several months, the imino proton spectrum of wt shows drastic changes in comparison with the initial spectrum such that 12 resolved imino proton could then be identified (**Figure S1**, lower left). Similar spectral changes are observed for G21T but the time required to yield the much more simplified imino proton spectrum is on the timescale of weeks, rather than months (**Figure S1**, lower right). These findings suggest that the folding energy landscape of *c-kit2* into a specific G-quadruplex conformation is highly complex in part due to the presence of four consecutive guanine residues in the last strand that leads to an expansion of the conformational space accessible. The folding complexity is greatly reduced by the single nucleotide replacement, G21T; nonetheless, local conformational rearrangements remain required for G21T to attain low-energy states following the annealing process. From the viewpoint of structure determination of *c-kit2* by NMR spectroscopy, wt and G21T share a common conformation that is highly populated in solution, as judged from the similarities of the corresponding ^1H - ^1H nuclear Overhauser effect (NOE) correlations identified in respective 2D ^1H NOESY spectra recorded immediately after the annealing processes (**Figure S2**). With the advantage of a much faster refolding kinetics, we have hence decided to investigate in detail the structure and dynamics of G21T.

Figure S1. Slow kinetics of *c-kit2* quadruplex formation. 700 MHz ^1H NMR spectra of wt *c-kit2* (left panel) and those of the G21T variant (right panel), recorded at 310K. The initial spectra recorded immediately after overnight annealing are shown on top and those recorded after an extended incubation period are shown below.

Figure S2. Comparison of NOESY spectra of wt and G21T *c-kit2*. Immediately after the samples were prepared, NOESY spectra of wt (red) and G21T (blue) *c-kit2* were recorded at 700 MHz, 310K with a mixing time of 240 ms. Key resonances of the loop residues are labeled accordingly and the chemical shifts of T21 H6 and T21 Me (methyl protons of the thymine base) of G21T are indicated, which are only present in G21T as a result of the G21T replacement. Note that wt exhibits additional cross-peaks, particularly in the H6/H8-H2'/H2'' region (dashed box) that are likely to correspond to the minor conformation adopted through G-tetrad register switching.

Figure S3. Potassium dependent structural polymorphism of *c-kit2*. Imino proton NMR spectra of *c-kit2* in the presence (red) and in the absence (black) of potassium. The later was recorded using a wt *c-kit2* sample buffered in 20 mM Tris buffer, pH 7.4. Both spectra were recorded at 298K using a 700MHz NMR spectrometer, with a jump-and-return water suppression scheme to minimize saturation of labile proton signals. The imino proton resonances with chemical shifts ranging from 10.7 to 12 ppm in the lower panel have been unambiguously attributed to a quadruplex with an all-parallel topology in this work. In the absence of potassium ions, similar resonances are observed and they are boxed together with the assigned resonances in the lower panel. Those that have distinctly different chemical shifts are tentatively attributed to an anti-parallel conformation based on the CD spectrum recorded under the same condition (cf. J. Dash *et al.*, *J. Am. Chem. Soc.* (2008) **130**, 15950-6).

Table S1. Chemical shift assignments of imino protons that are involved in the G-tetrads of various *c-kit2* loop variants at 298K.

	Residue	G21T	G21T/C9T	G21T/G10A	G21T/C11T	G21T/A17T
S1	G2	11.96	11.96	11.97	11.97	11.97
	G3	11.25	11.32	11.22	11.26	11.29
	G4	11.03	11.39	11.36	11.34	11.39
S2	G6	11.66	11.68	11.68	11.70	11.69
	G7	11.58	11.57	11.62	11.65	11.58
	G8	11.20	11.02	10.80	10.83	11.53
S3	G14	11.77	11.78	11.78	11.81	11.80
	G15	11.18	11.24	11.16	11.26	11.27
	G16	10.97	10.97	10.96	10.96	10.93
S4	G18	11.16	11.16	11.26	11.16	11.14
	G19	11.28	11.28	10.86	11.27	11.29
	G20	11.16	11.06	11.06	11.06	11.05

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