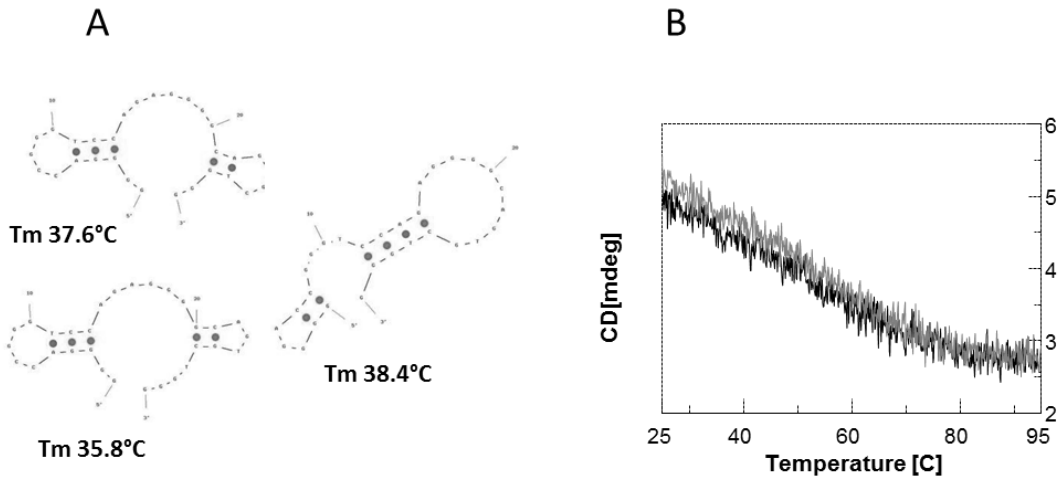
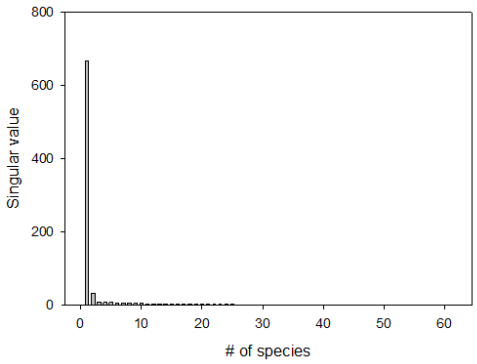


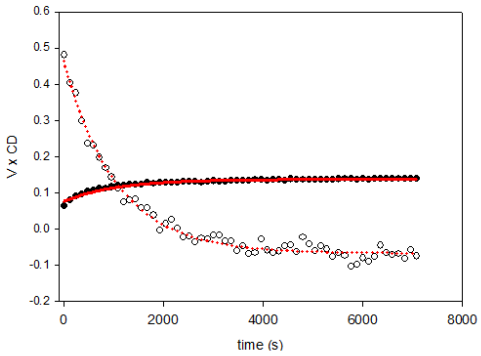
Supplementary Figure S1

Theoretical intramolecular folding of EGFR-272 as derived by IIT-Oligoanalyzer tool (A) and melting profile of 4 μM EGFR-272 recorded at 260 nm in 10 mM Tris, pH 7.5, 25 °C (B). Black and gray lines refer to melting and annealing processes performed at 0.8°C/min.

A



C



B

	#	frequency
V	1	0.9879
	2	0.8628
	3	0.0439

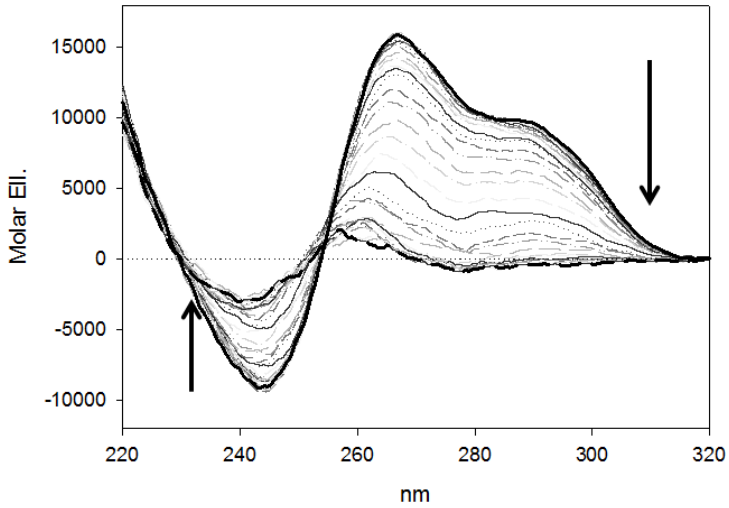
D

Best fitting equation	R^2	Rate constant (s^{-1})
$y(t)=y_0+a*(\exp(-k*t))$	0.989	$(9.54 \pm 0.24) \times 10^{-4}$

Supplementary Figure S2

Data derived for the kinetic rearrangement of 4 µM EGFR-272 upon addition of 200 mM KCl in 10 mM Tris, pH 7.5, 25 °C: singular values (A), V matrix autocorrelation coefficients (B, the not significant coefficient is highlighted in grey), amplitudes of the first three ***V*** vectors (C) and final fitting model (D).

A

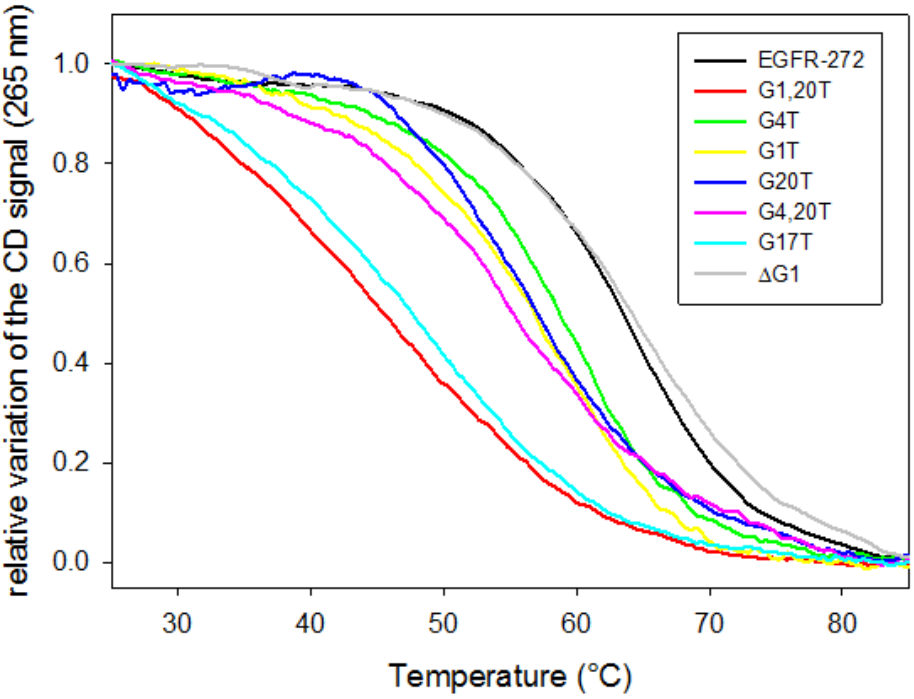


B

	#	frequency
V	1	0.9696
	2	0.9534
	3	0.6542
	4	0.2950

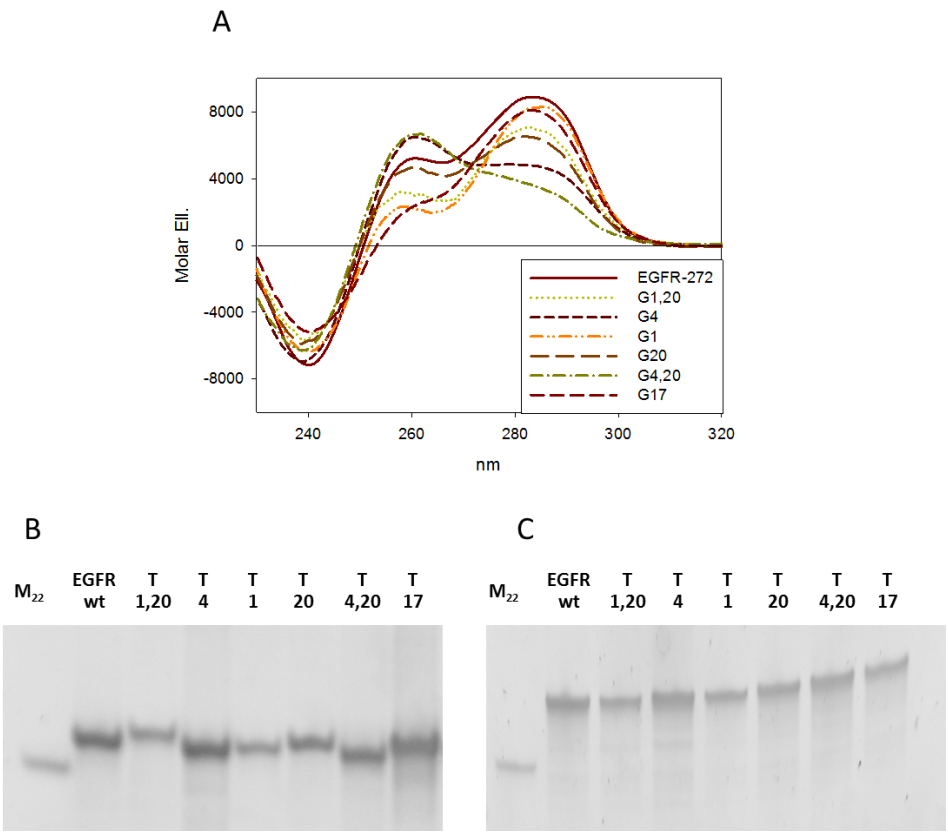
Supplementary Figure S3

Variation of CD spectra of EGFR-272 acquired in in 10 mM Tris, 200 mM KCl, pH 7.5 upon increment of the temperature (A) as showed by the arrows. In (B), V matrix autocorrelation coefficients derived from this data set are reported; the not significant coefficient is highlighted in grey.



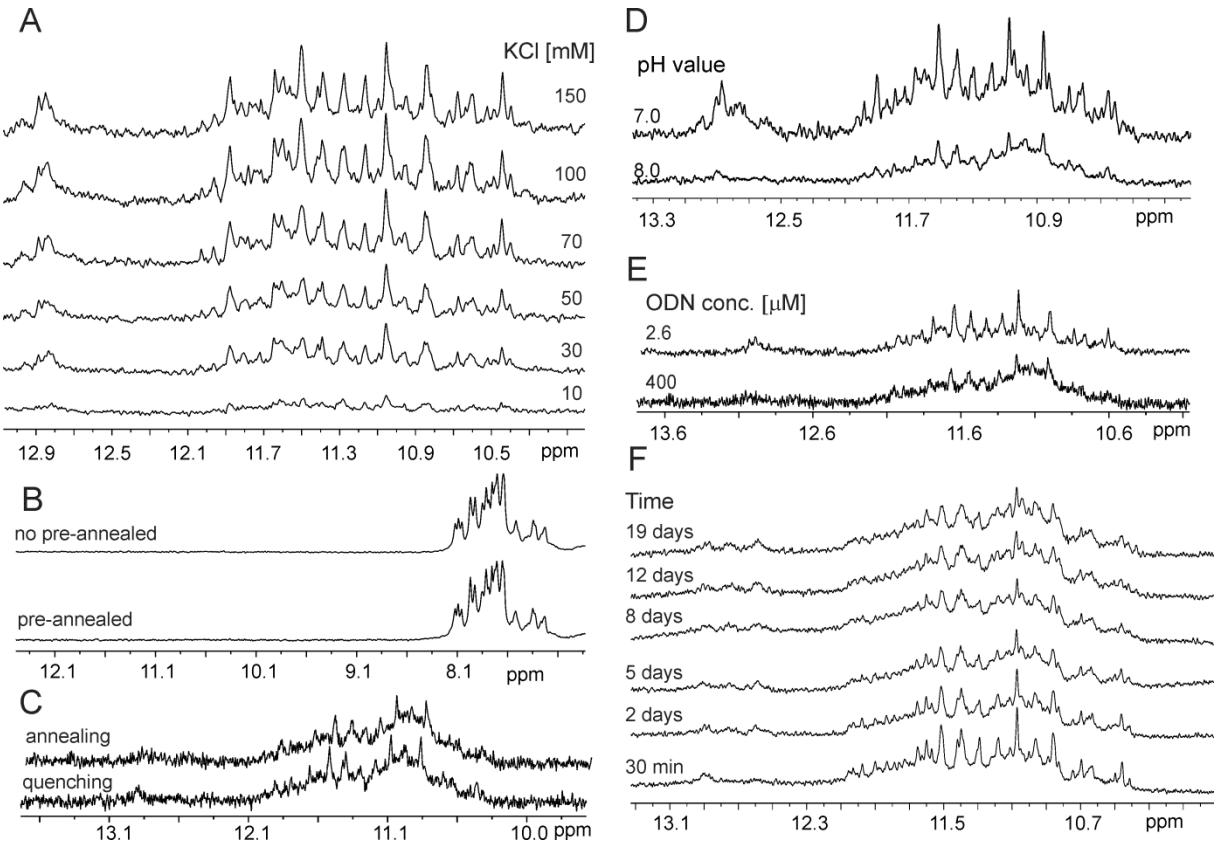
Supplementary Figure S4

Thermal denaturation profiles of 4 μM EGFR-272 and related mutants previously folded in 200 mM KCl acquired by CD spectroscopy at 265 nm by applying a heating rate of 50 °C/h.



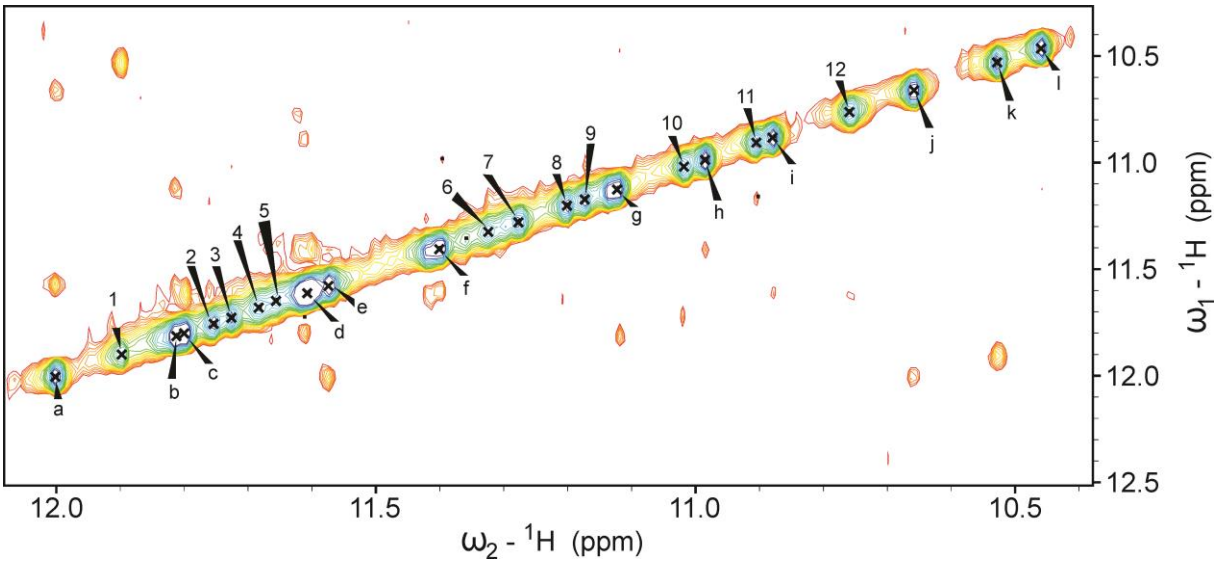
Supplementary Figure S5

CD spectra of 4 μM EGFR-272 and related mutants in 10 mM Tris, pH 7.5, 25 °C (PANEL A) and PAGE resolution of the same samples run under native (PANEL B) or denaturating (PANEL C) conditions.



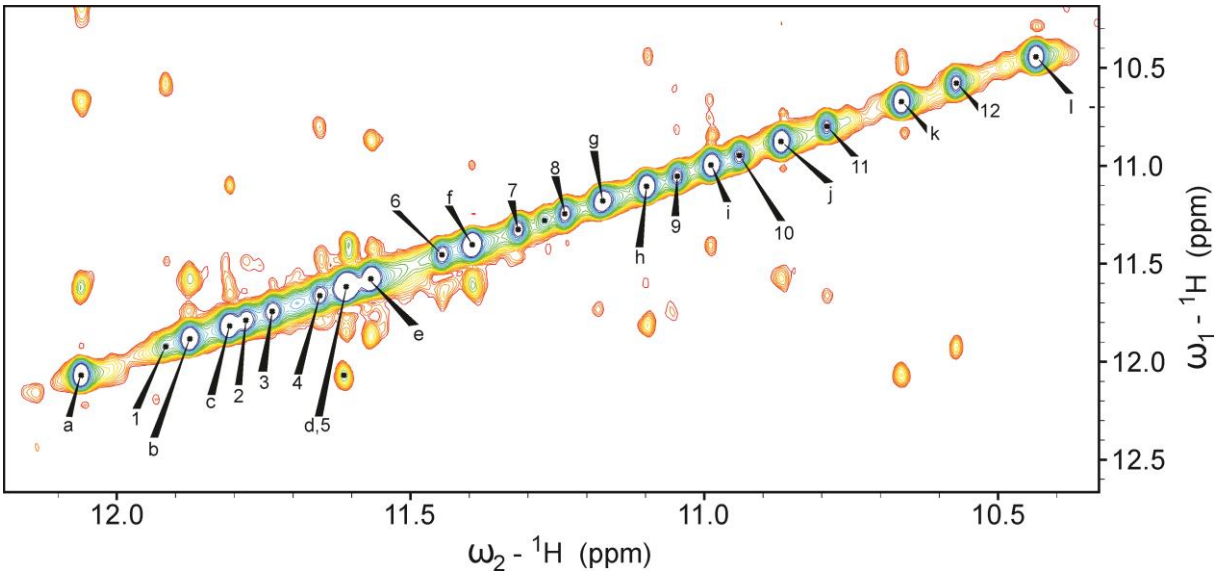
Supplementary Figure S6

Imino and aromatic region of ^{1}H NMR spectra of EGFR-272 recorded at different experimental conditions. All NMR spectra were obtained in H_2O:$^{2}H_2O$=9:1 at 25°C. PANEL A: NMR spectra of EGFR-272 as K^+ ions are titrated into solution (pH 7.0) at 0.1 mM concentration of the oligonucleotide per strand and at 800 MHz. The concentration of K^+ ions is indicated on the right. PANEL B: The NMR spectra of the sample that was not annealed and of the sample that was annealed over night before adding the K^+ ions to solution (pH 6-7) at 0.4 mM concentration of the oligonucleotide per strand and at 300 MHz. PANEL C: NMR spectra obtained after annealing (sample was heated to 95°C and slowly cooled down to room temperature) and quenching (sample was heated to 95°C and quickly cooled down on ice). The spectra were recorded in 10 mM Tris buffer (pH 8.0), at 200 mM concentration of KCl, 0.4 mM concentration of the oligonucleotide per strand and at 600 MHz. PANEL D: The influence of pH value on NMR spectra that were recorded at pH values 7.0 (10 mM potassium phosphate buffer) and 8.0 (10 mM Tris buffer) after overnight annealing, at 200 mM concentration of KCl, 0.4 mM concentration of the oligonucleotide per strand and at 600 MHz. PANEL E: NMR spectra at different concentration of oligonucleotide. The spectra were obtained at 2.6 and 400 μM concentration of the oligonucleotide per strand, after overnight annealing, at 200 mM concentration of KCl in 10 mM Tris buffer (pH 8.0) and at 600 MHz. PANEL F: The influence of time from 30 min to 19 days on folding of EGFR-272 oligonucleotide. The sample was prepared in 10 mM Tris buffer (pH 8.0), 200 mM concentration of KCl, 0.35 mM concentration of the oligonucleotide per strand and recorded on 600 MHz spectrometer. The sample was stored in NMR tube at room temperature.



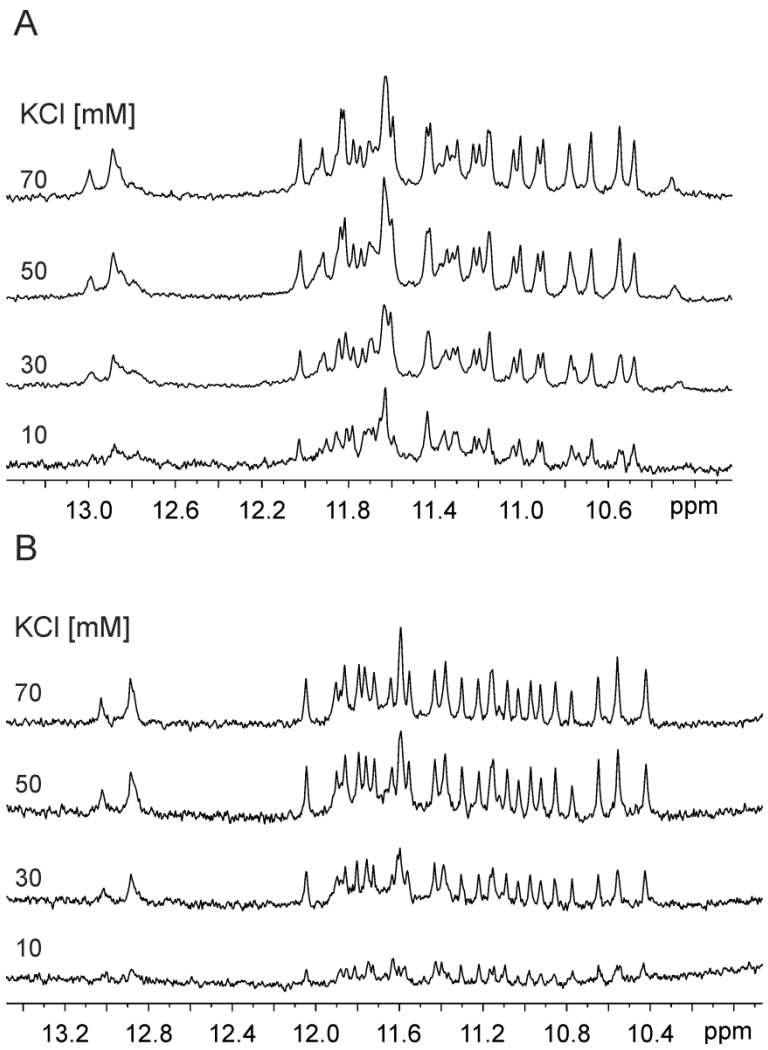
Supplementary Figure S7

Imino-imino region of NOESY spectrum of ΔG1 mutant in the presence of 70 mM KCl at pH 7.0, 25°C in H_2O:^2H_2O=9:1 at 600 MHz. Mixing time was 300 ms. Diagonal cross peaks corresponding to two major forms of G4 structures are labeled with numbers and letters.



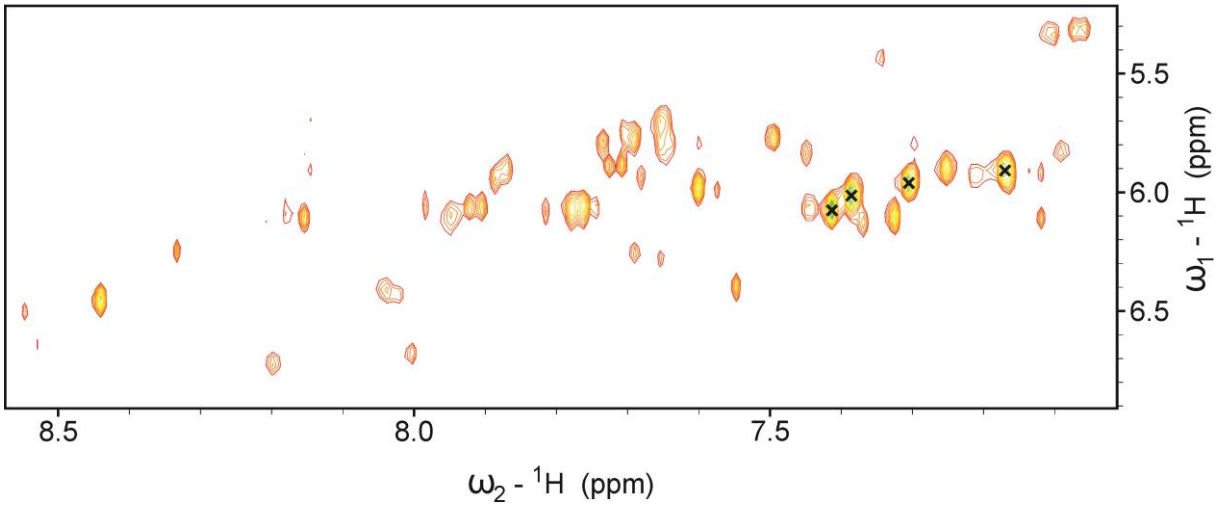
Supplementary Figure S8

Imino-imino region of NOESY spectrum of T4 mutant in the presence of 70 mM KCl, at pH 7.0, 25°C in H_2O:^2H_2O=9:1 at 600 MHz. Mixing time was 300 ms. Diagonal cross peaks corresponding to two major forms of G4 structures are labeled with numbers and letters.



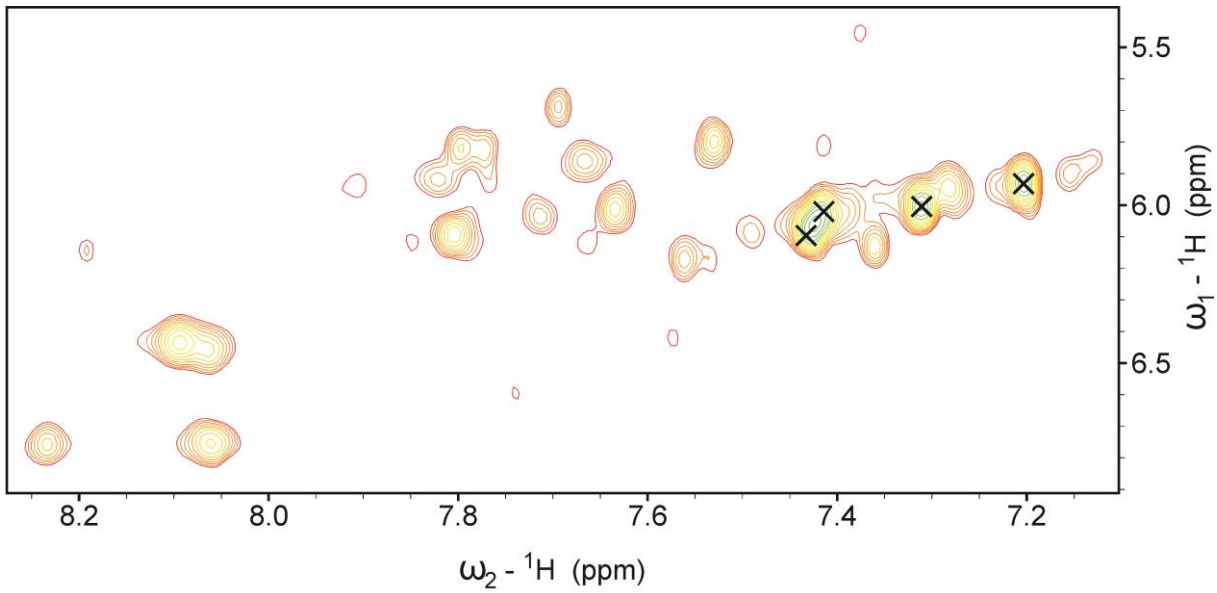
Supplementary Figure S9

Imino region of ^{1}H NMR spectra of ΔG1 (PANEL A) and T4 (PANEL B) mutants at various concentrations of K^+ ions as indicated on left. NMR spectra were recorded in H_2O:$^{2}H_2O$=9:1, at 0.1 mM concentration of the oligonucleotide per strand, pH 7.0, 25°C at 600 MHz.



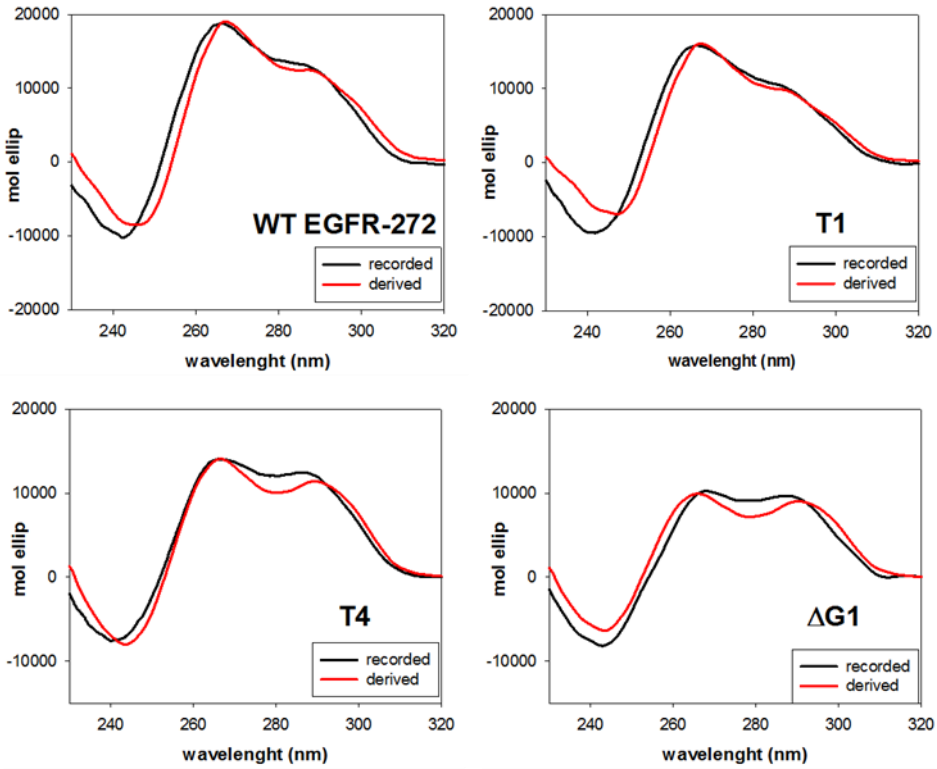
Supplementary Figure S10

Aromatic-anomeric region of NOESY spectrum of ΔG1 mutant in the presence of 70 mM KCl at pH 7.0, 25°C in H_2O:^2H_2O=9:1 at 600 MHz. Mixing time was 150 ms. Very intense intermolecular NOE cross-peaks between H8 and H1' protons are marked with cross.



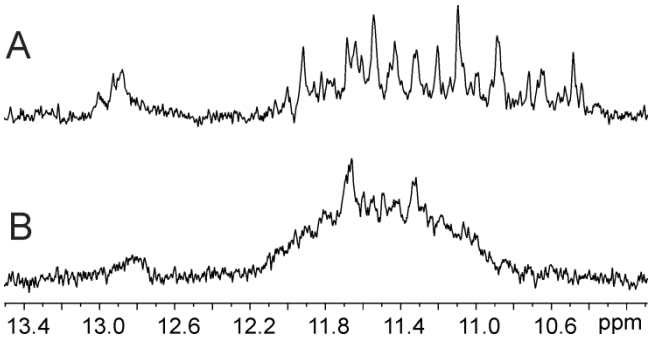
Supplementary Figure S11

Aromatic-anomeric region of NOESY spectrum of T4 mutant in the presence of 70 mM KCl at pH 7.0, 25°C in H_2O:^2H_2O=9:1 at 600 MHz. Mixing time was 80 ms. Very intense intermolecular NOE cross-peaks between H8 and H1' protons are marked with cross.



Supplementary Figure S12

Description of experimentally acquired CD spectra (black lines) from analytical combination of the SVD derived spectra corresponding to folded form1 and form2 reported in Fig. (red lines)



Supplementary Figure S13

Imino region of ^{1}H NMR spectra of EGFR-272 (PANEL A) and C25T-G26T mutant (PANEL B). NMR spectra were recorded in H_2O:$^{2}H_2O$=9:1, at 0.1 mM concentration of the oligonucleotide per strand, at 100 mM KCl (EGFR-272) and 120 mM KCl (C25T-G26T), pH 7.0, 25°C at 600 MHz.