Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Not All G-Quadruplexes are Created Equal: Investigating the Structural Polymorphism of the *c-Myc* G-Quadruplex-Forming Sequence and its Interaction with the Porphyrin *meso*-Tetra(N-methyl-4-pyridyl)porphine

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Supporting Information

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Methods:

NMR Sample Preparation and Spectroscopy

Fractions of the *c-Myc* quadruplex were separated with a mobile phase consisting of 100 mM KCl and 20 mM K_2HPO_4 (pH=7.0). Fractions were collected at 0.1 ml intervals. The five to seven fractions corresponding to the maxima for the seven major peaks were collected and combined from five separate runs. HPLC fractions were kept frozen at -80°C between runs. This material was concentrated using Microcon spin columns (Millipore). The concentrated material was then diluted to 330 µL total volume with HPLC buffer. 30 µM DSS and 10% D_2O were then added. The un-separated *c-Myc* sample was prepared by adding 10% D_2O and 30 µM DSS to a 1 mM sample. Samples were loaded into 5 mm Shigemi NMR tubes. NMR spectra were recorded using a 5 mm inverse triple resonance (HCN) probe on Varian Inova spectrometer at 14.1 T using a cold probe.

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Figures:

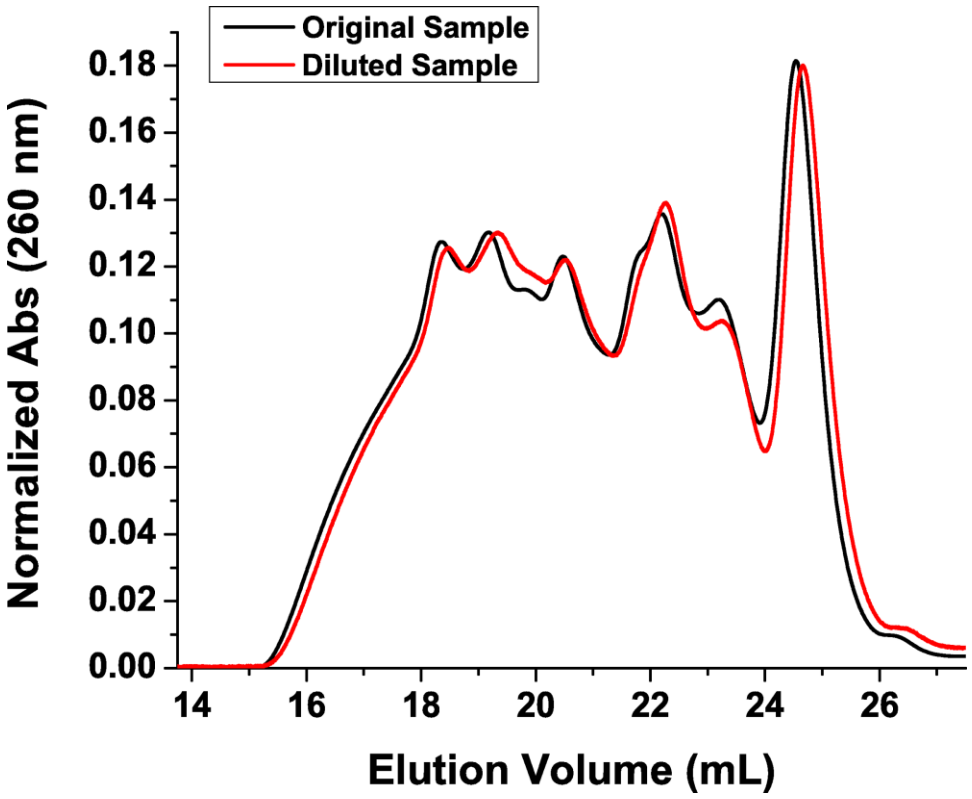

Figure S1. The distribution of Pu27 G-quadruplex species in sample before and after 100 fold dilution was examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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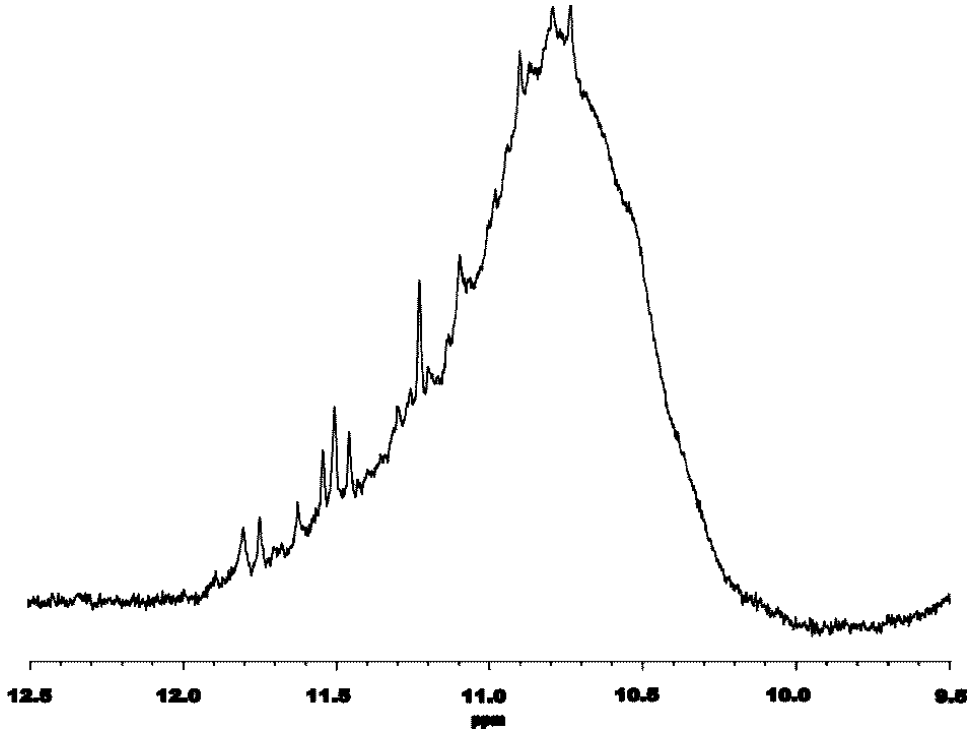

Figure S2. 1D ^{1}H-NMR spectrum of the Pu27 G-quadruplex mixtures prepared under standard conditions. The imino region from the 1D ^{1}H-NMR spectrum of Pu27 G-quadruplex-forming sequence prepared under standard conditions demonstrates the formation of G-quadruplexes *in vitro* and the existence of a complex mixture of monomers and higher-order species.

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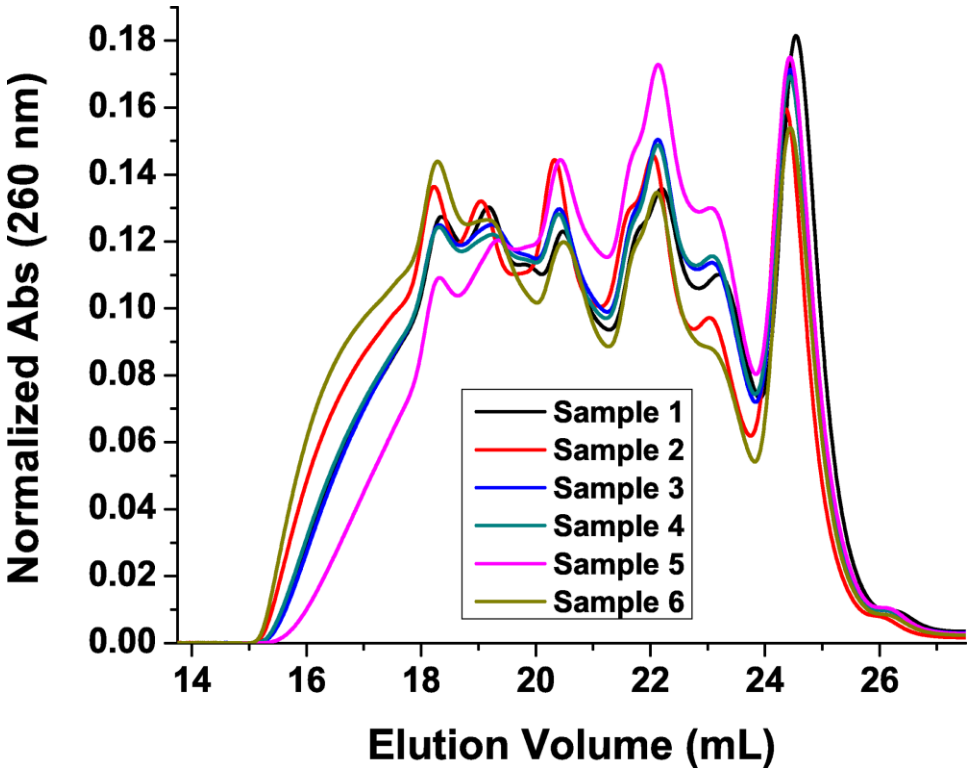

Figure S3. The distribution of G-quadruplex species from six different commercially obtained batches of Pu27 oligonucleotides was examined by SEC. G-quadruplex-forming sequences were prepared under standard conditions. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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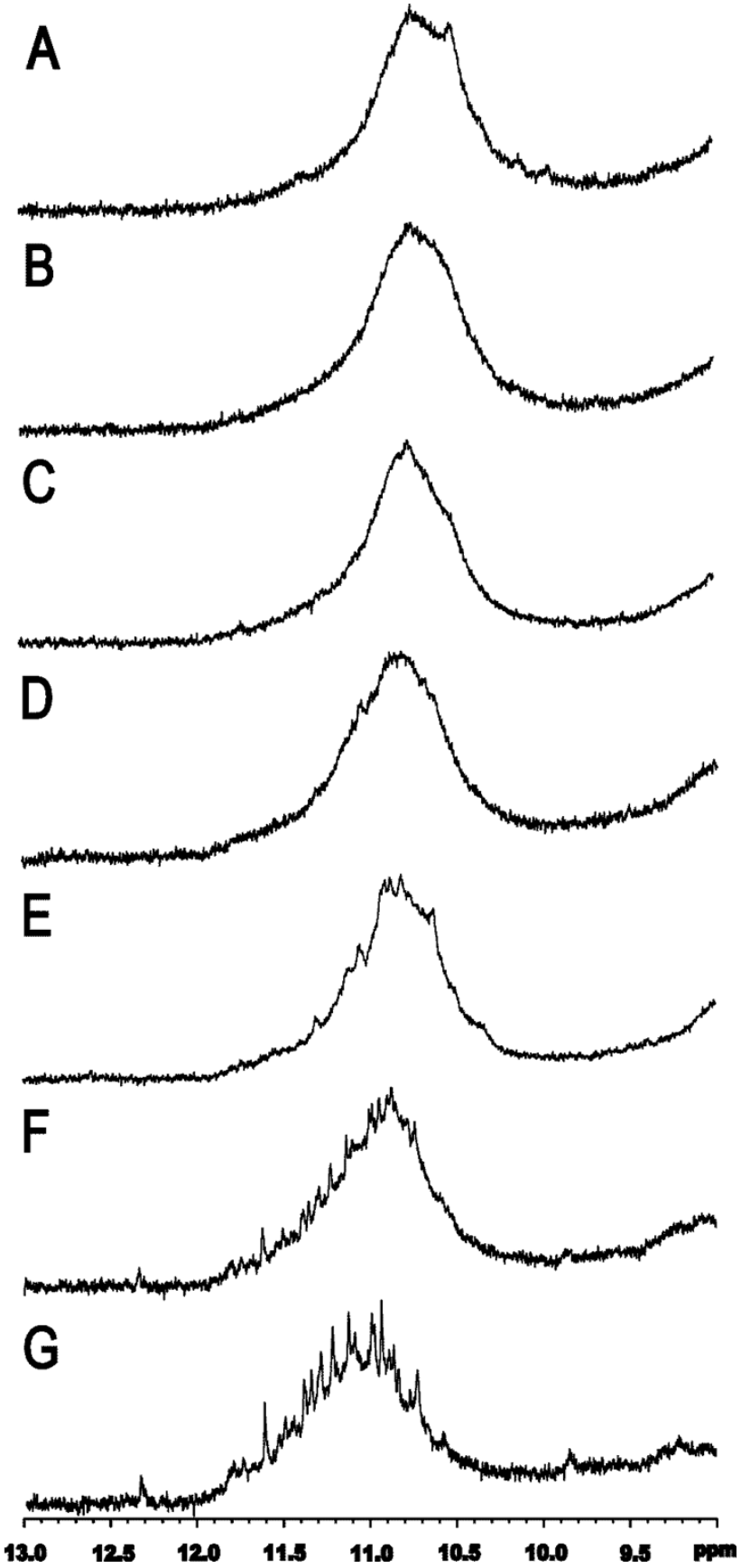

Figure S4. 1D ^{1}H-NMR spectra of the 7 fractions from SEC separation of the Pu27 G-quadruplex mixtures. The imino region from the 1D ^{1}H-NMR spectra of the 7 fractions demonstrated that all 7 fractions contained G-quadruplexes.

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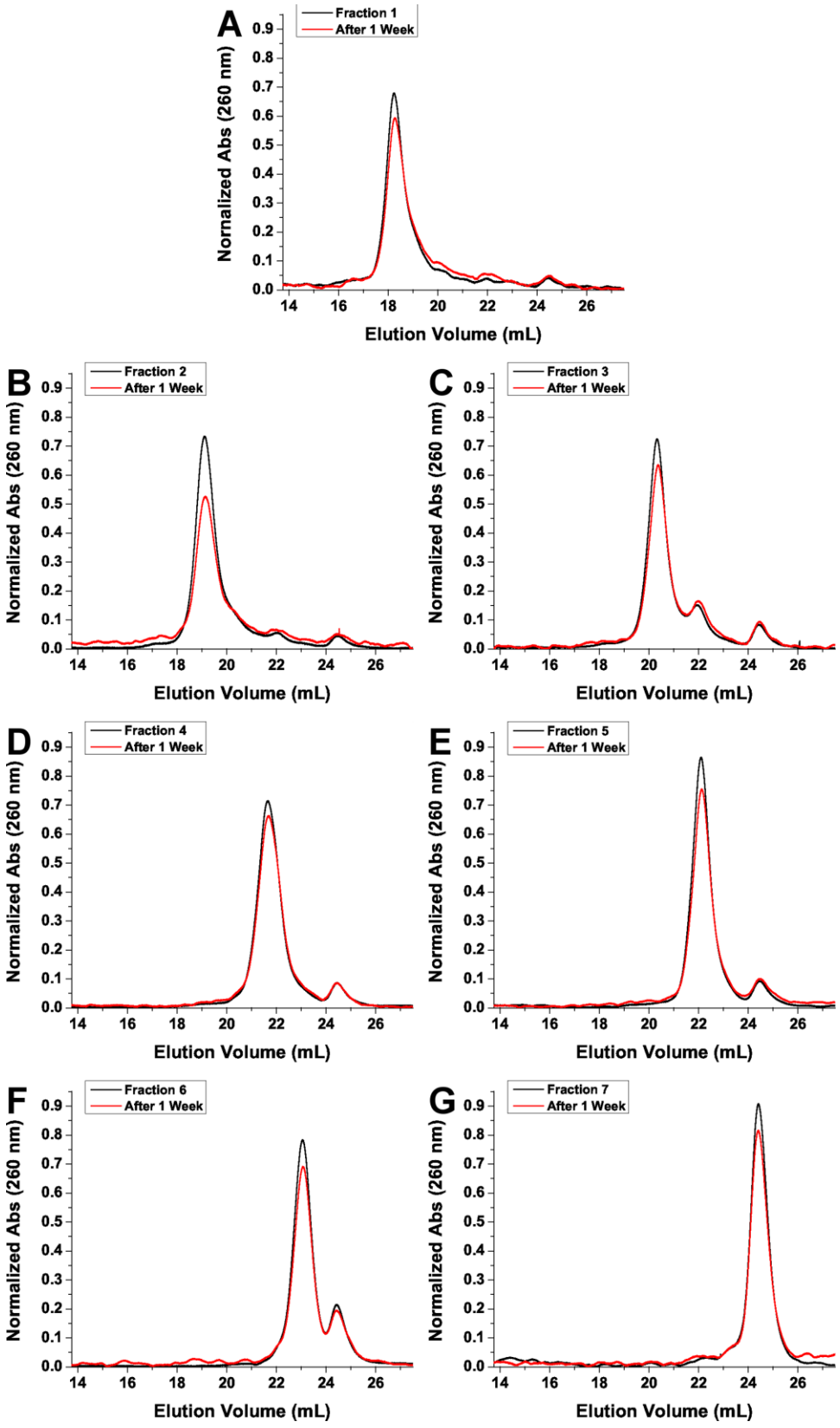

Figure S5. Fractions from SEC separation of Pu27 G-quadruplex mixtures before and one week following AUC analysis were examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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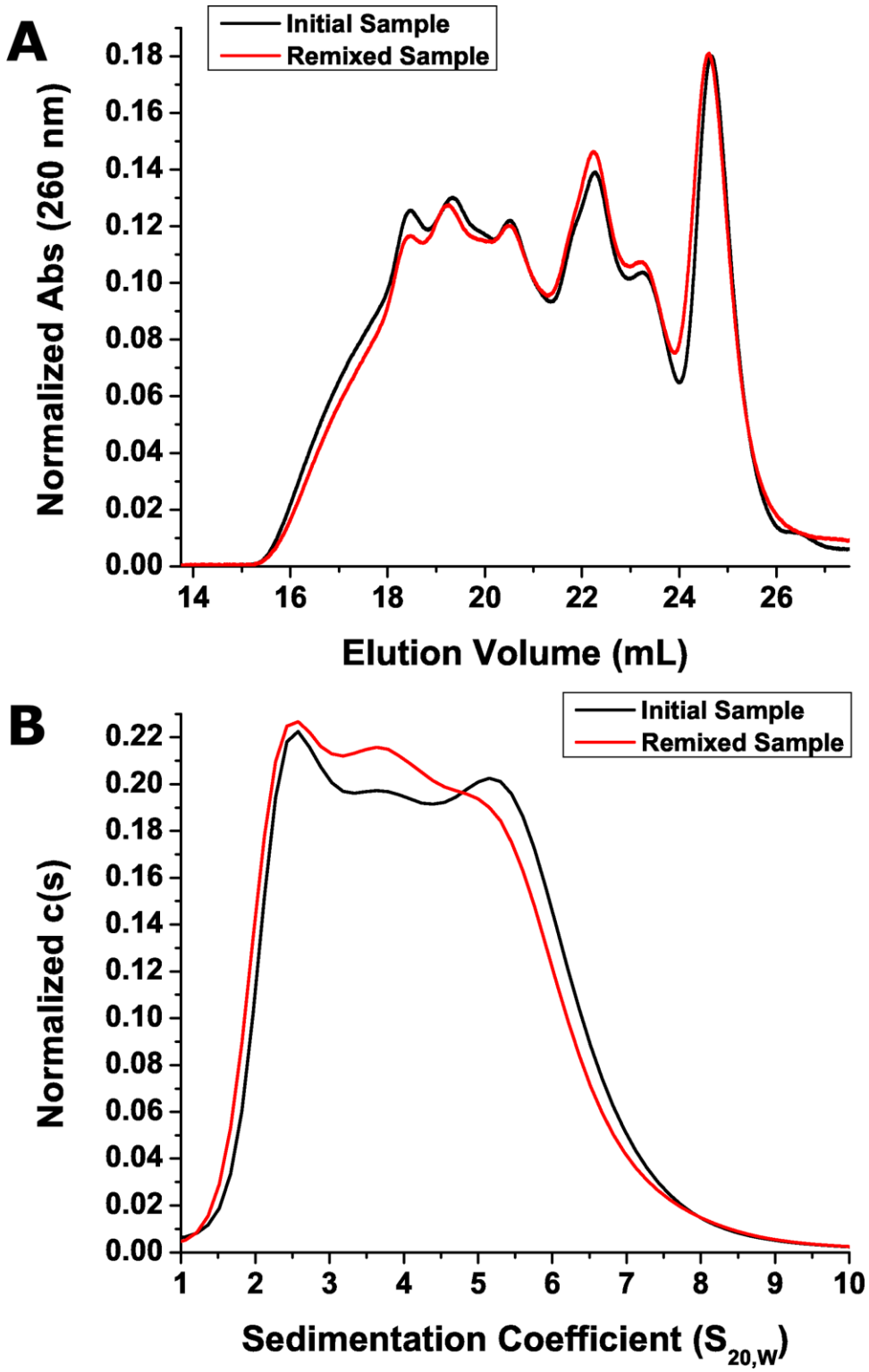

Figure S6. The distribution of Pu27 G-quadruplex species in a sample obtained by remixing SEC separated fractions were examined by SEC (A) and AUC (B). (A) Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve. (B), c(s) is the concentration distribution of sedimenting species based on absorbance at 260 nm and normalized to the area under the curve.

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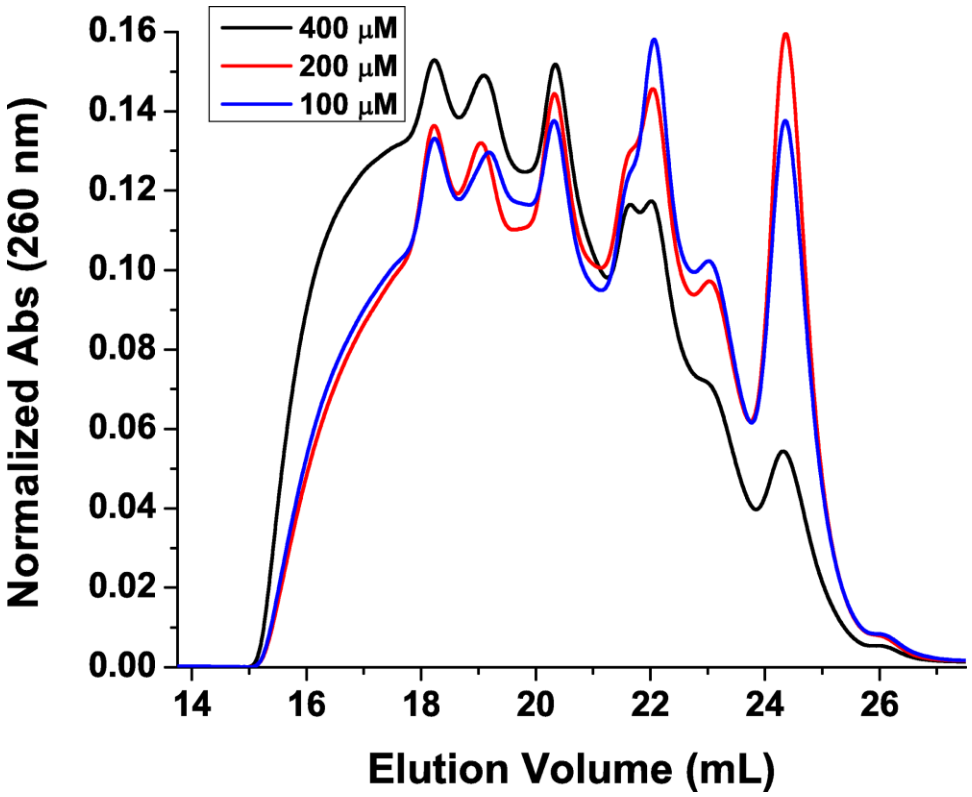

Figure S7. The DNA concentration dependency of G-quadruplex formation was examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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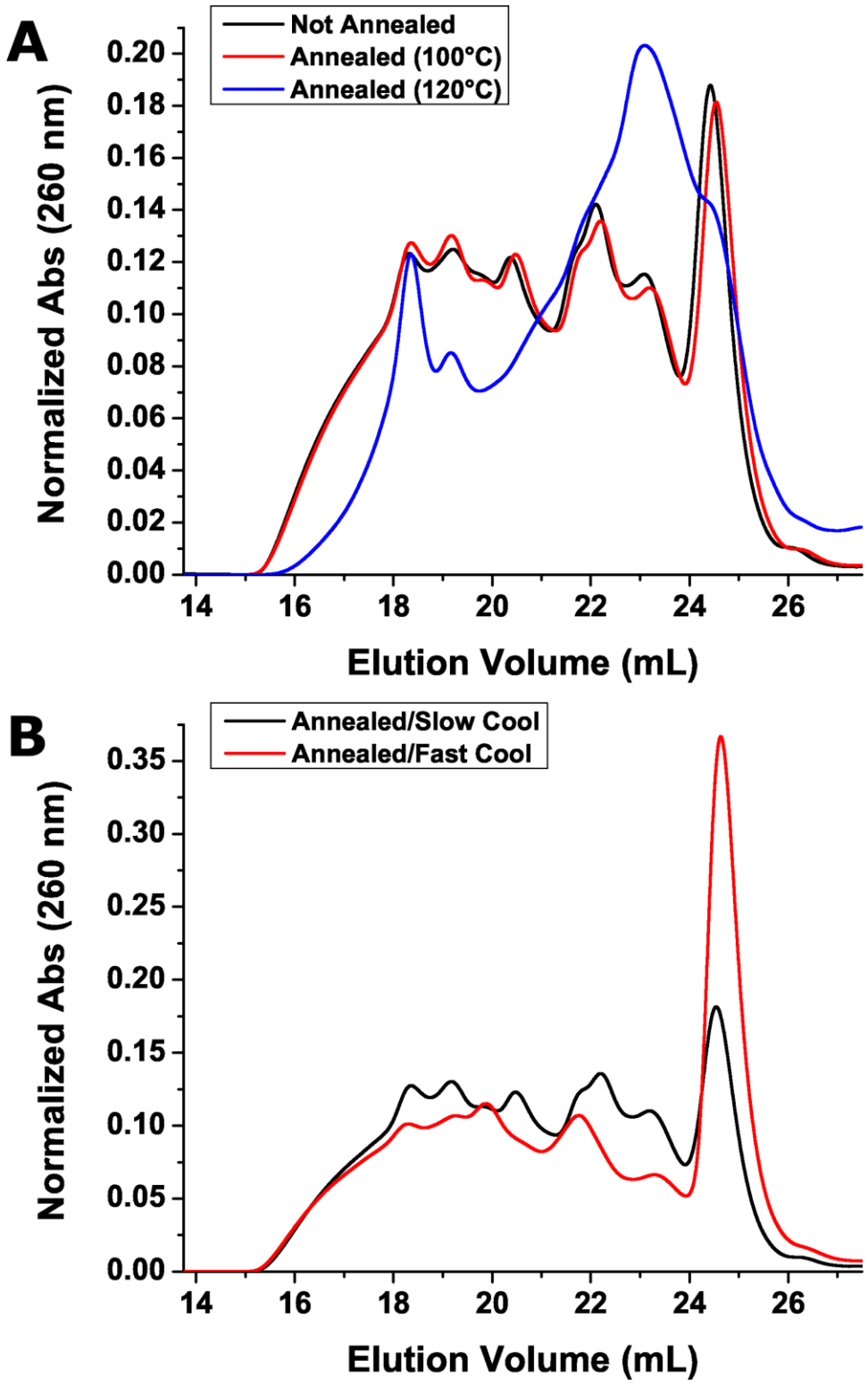

Figure S8. The (A) annealing temperature dependency and (B) quenching protocol dependency of G-quadruplex formation were examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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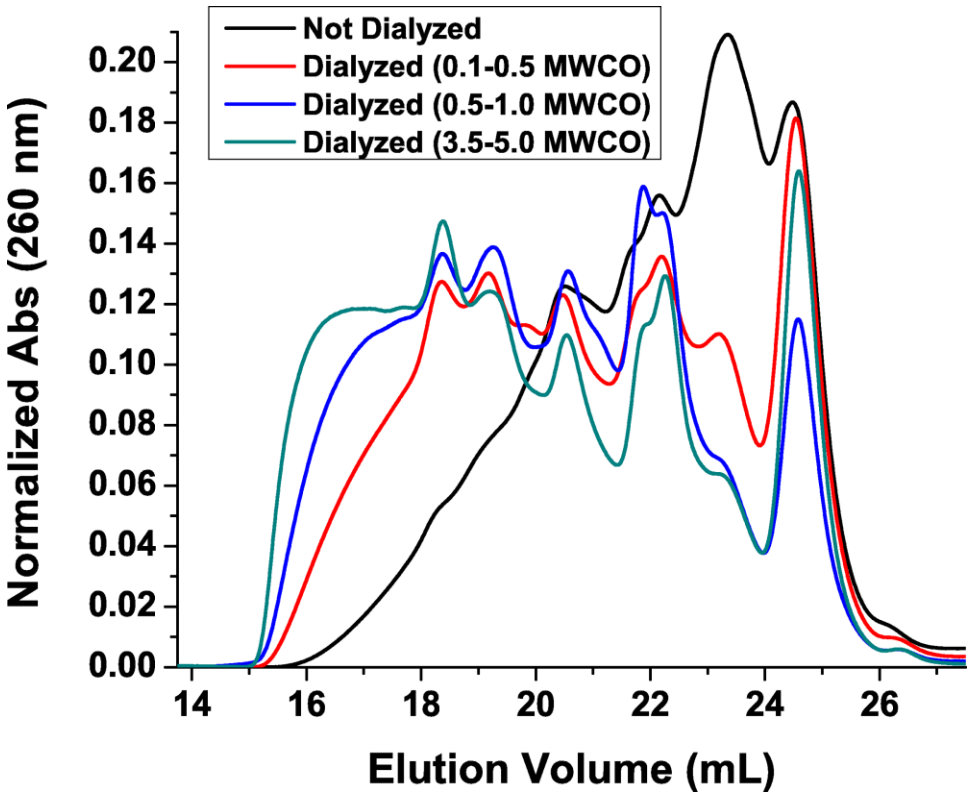

Figure S9. The dialysis dependency of G-quadruplex formation was examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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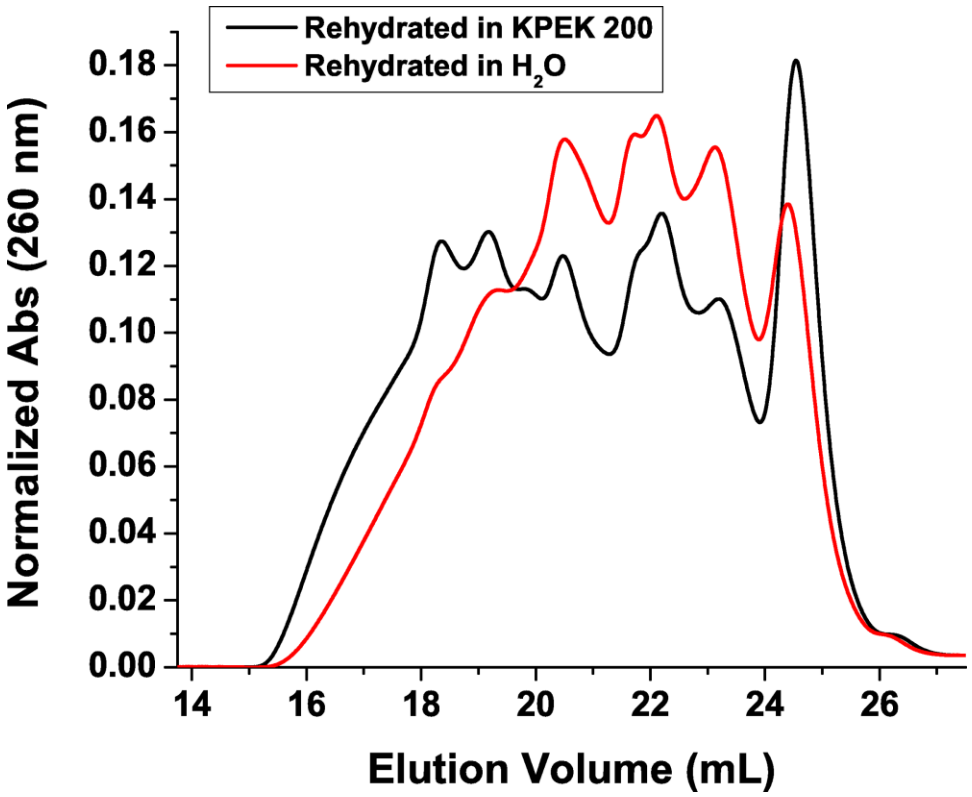

Figure S10. The rehydration of oligonucleotide protocol dependency of G-quadruplex formation was examined by SEC. A greater proportion of monomeric and dimeric G-quadruplex species were produced when the lyophilized DNA was initially dissolved in deionized water instead of KPEK. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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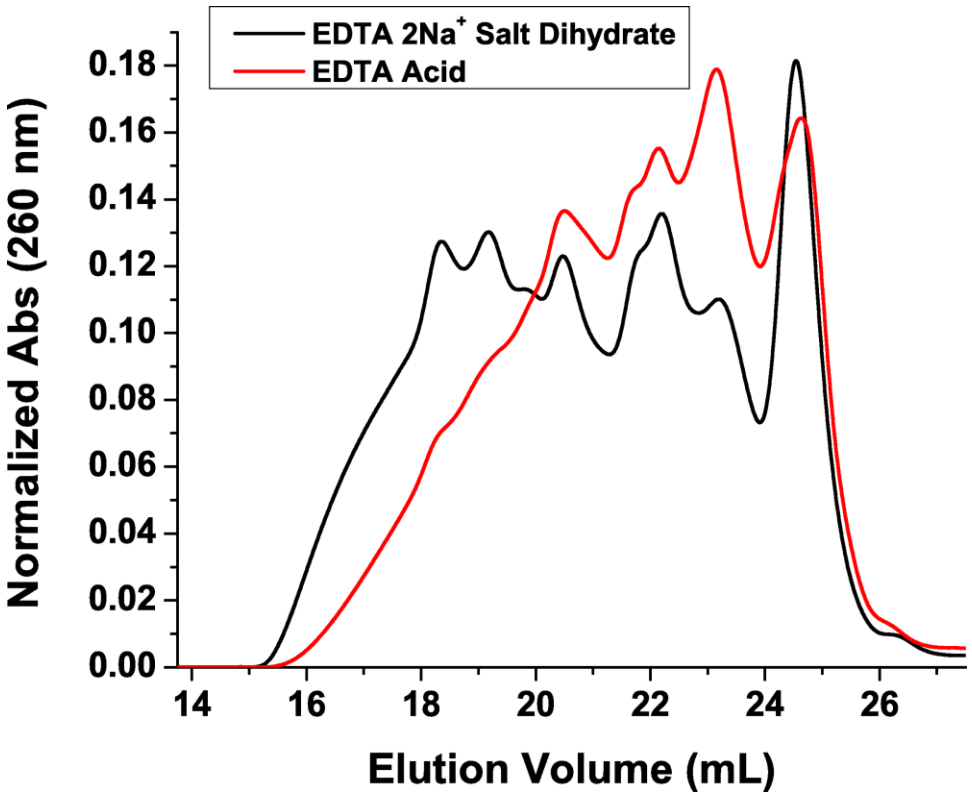

Figure S11. The EDTA dependency of G-quadruplex formation was examined by SEC. When the acid form of EDTA was substituted for the disodium salt dihydrate form, more monomeric species were observed. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve.

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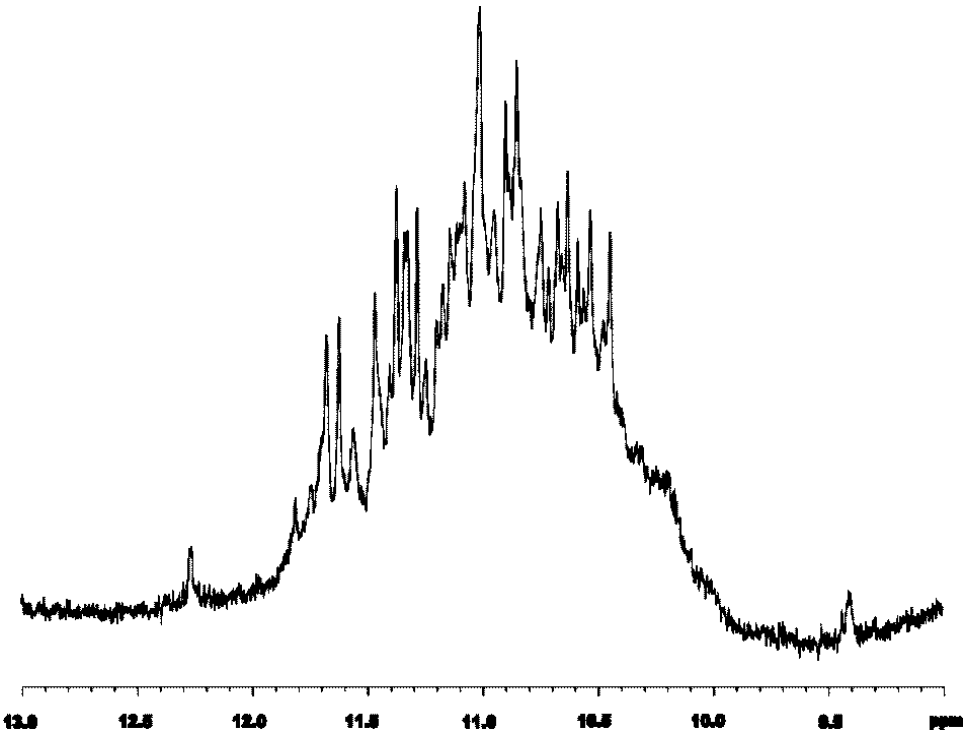

Figure S12. 1D ^{1}H-NMR spectrum of the Pu27 G-quadruplex mixtures prepared under low potassium conditions (25 mM K^{+}). The imino region from the 1D ^{1}H-NMR spectrum of the Pu27 G-quadruplex mixtures in low potassium conditions (25mM) displayed individual resonances indicating a mixture of monomers G-quadruplexes. This NMR spectrum highly resembles the 1D ^{1}H-NMR spectrum of SEC separated fraction 7 (Supporting Information, Figure S5G).

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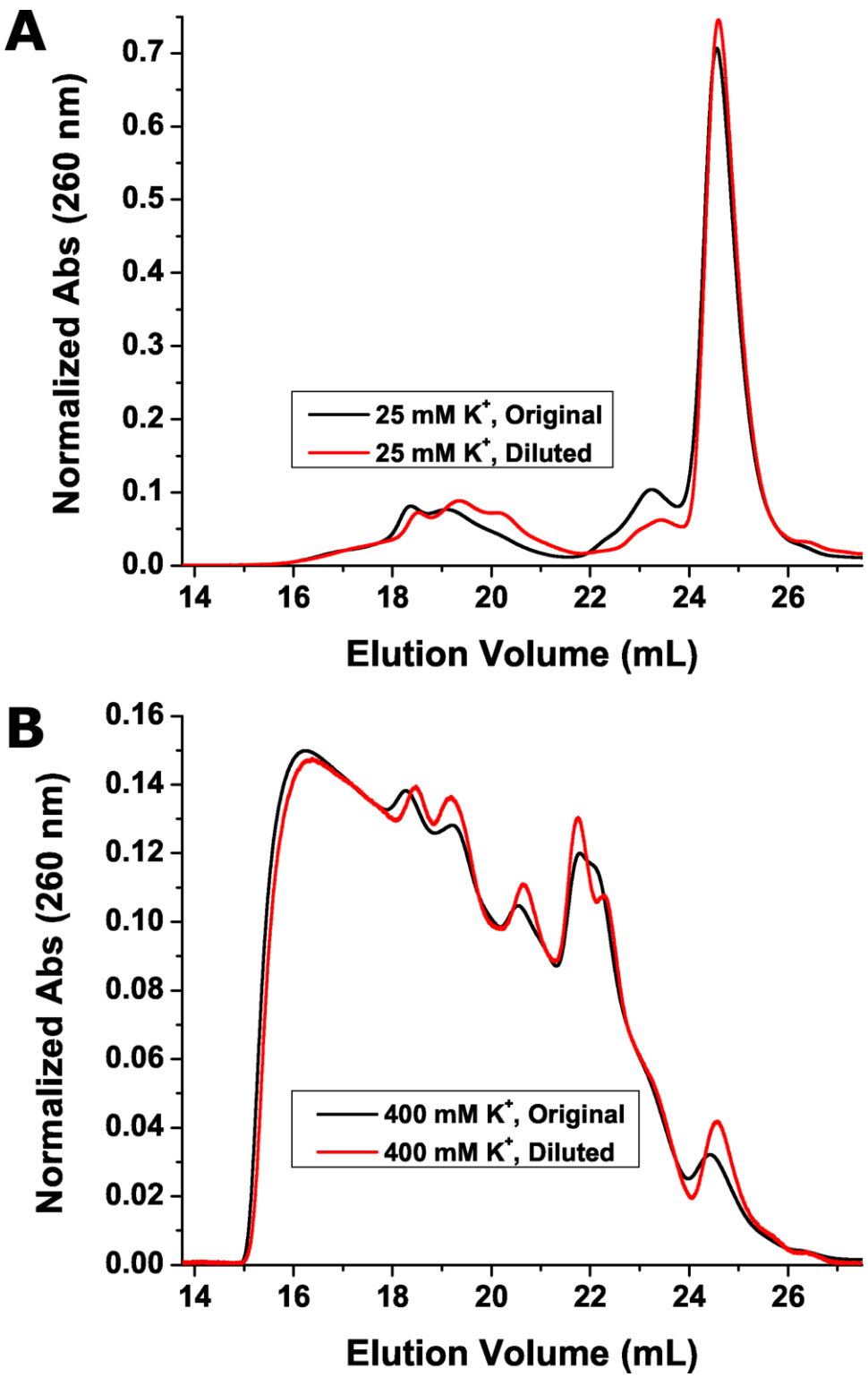

Figure S13. The distribution of Pu27 G-quadruplex species in (A) low potassium conditions (25 mM K^+) and (B) high potassium conditions (400 mM K^+) before and after dilution was examined by SEC. Dilution of the *c-Myc* G-quadruplex-forming sequence did not alter the distribution of G-quadruplex species. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve. The findings here agree with previous observations that dilution after annealing does not alter the distribution of G-quadruplex species even after more than two weeks at room temperature.

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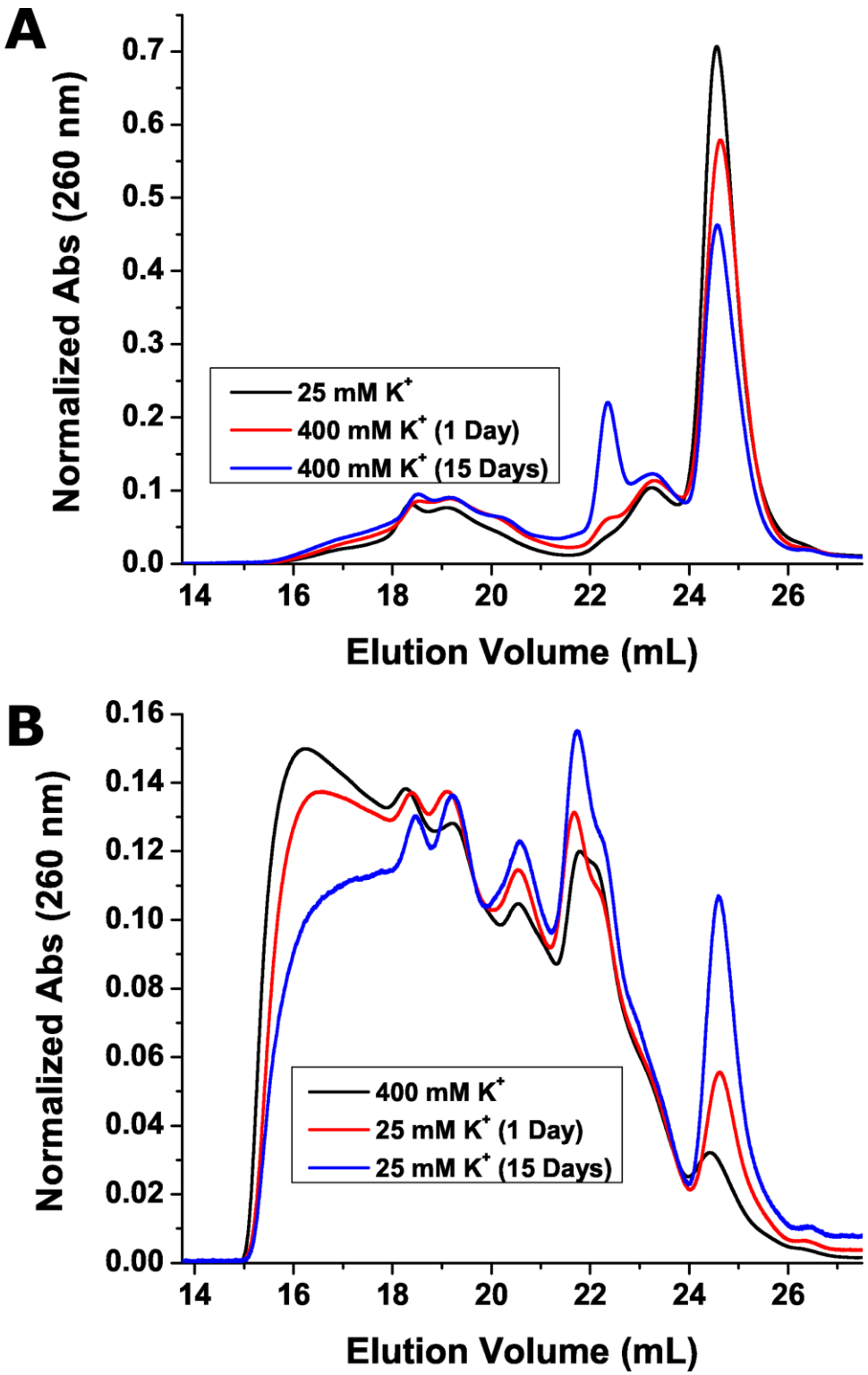

Figure S14. Changes in distribution of Pu27 G-quadruplex species when (A) potassium concentrations were increased from 25 mM to 400 mM and (B) potassium concentrations were decreased from 400 mM to 25 mM were examined by SEC. Absorbance of DNA was monitored at 260 nm and normalized to the area under the curve. It should be noted that significant changes in distribution of Pu27 G-quadruplex species were only observable more than two weeks after potassium concentrations were altered. In addition, it should also be noted that these samples were annealed prior to the changes in potassium concentration. There was no annealing after the potassium concentrations were altered.

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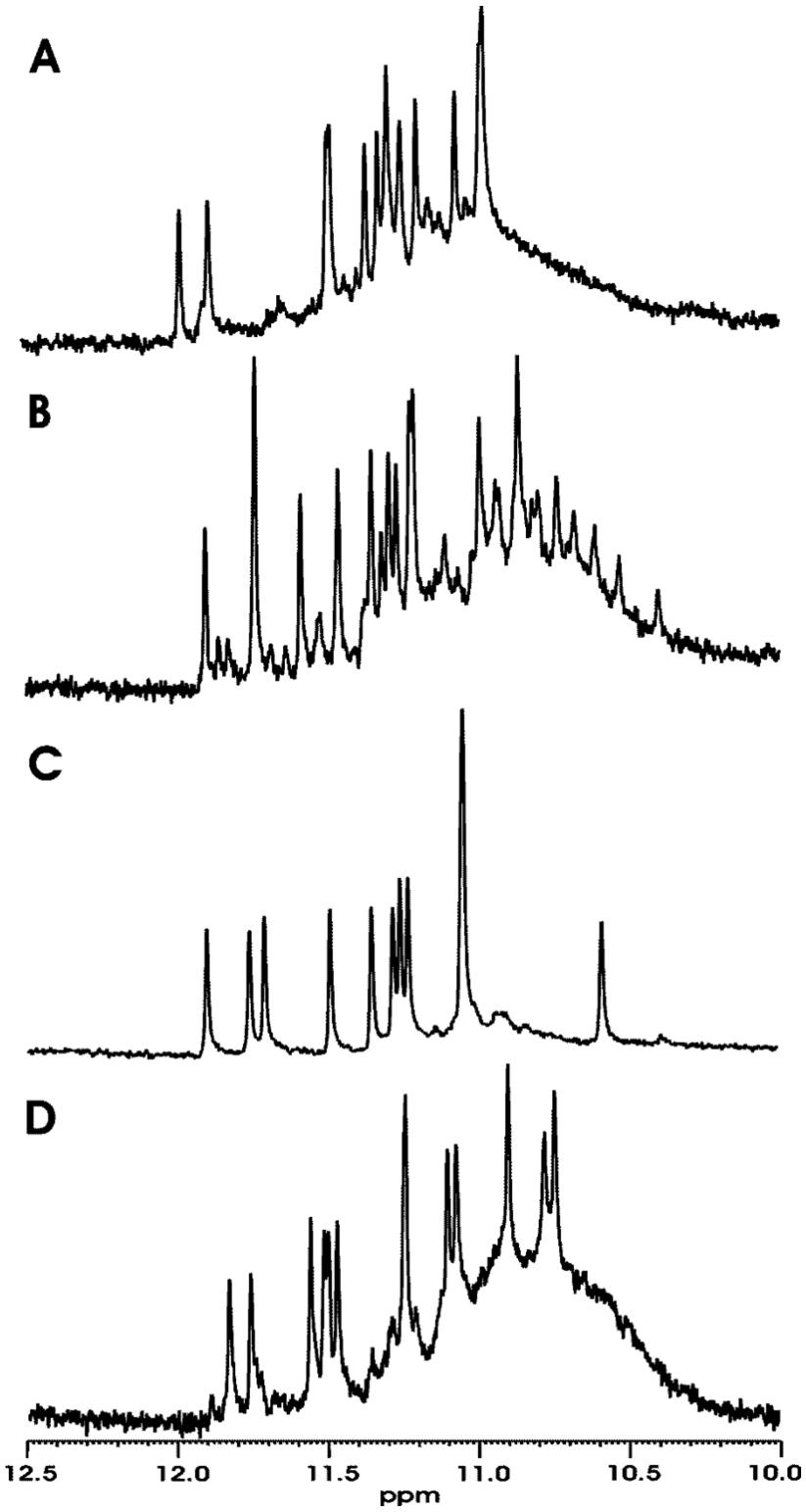

Figure S15. 1D ^{1}H-NMR spectra of G-quadruplexes formed from derivatives of the Pu27 G-quadruplex-forming sequence. The imino region from the 1D ^{1}H-NMR spectra of the modified *c-Myc* G-quadruplex-forming sequence Myc-1245 (A), Myc-2345 (B), MYC22-G14T/G23T (C), and Pu24 (D) shows marked reduction in polymorphism compared to the parent Pu27 sequence (Supporting Information, Figure S1). The spectra here agreed with previously reported spectra for these sequences.