Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for

Cooperative Hybridization of γPNA Miniprobes to a Repeating Sequence Motif and Application to Telomere Analysis

Ha H. Pham[†§], Connor T. Murphy[‡§], Gopalsamy Sureshkumar[†§], Danith H. Ly[†§], Patricia L. Opresko[‡§] and Bruce A. Armitage[†§*]

[†] Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213

[§] Center for Nucleic Acids Science and Technology, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213

[‡] Department of Environmental and Occupational Health, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA 15219

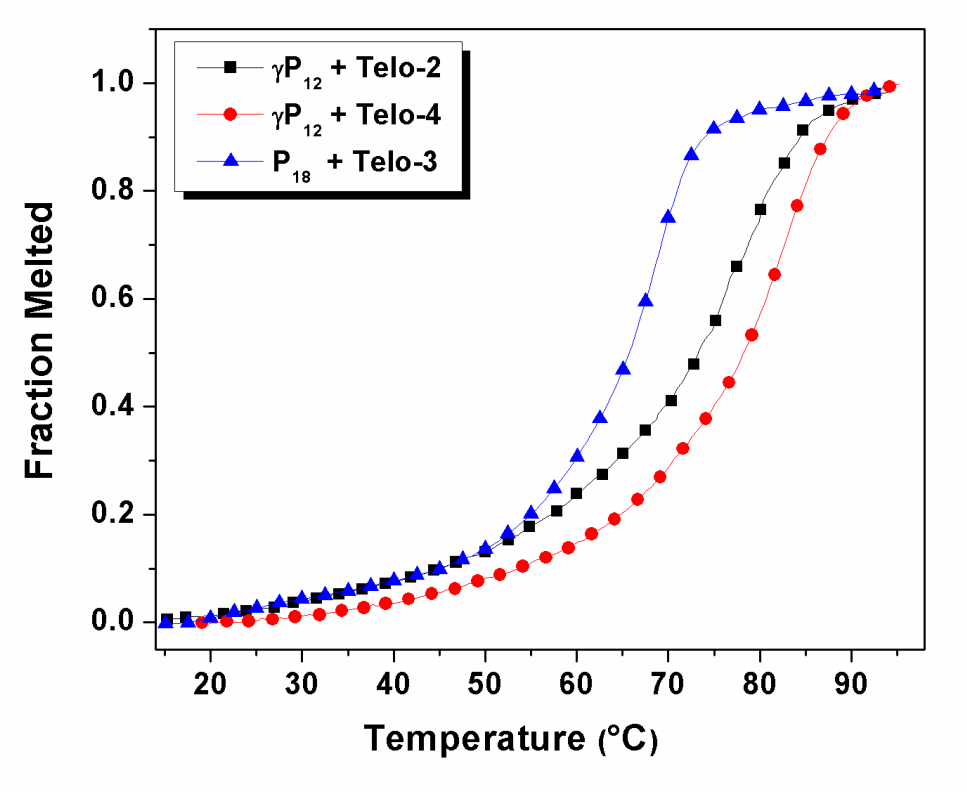

Figure S1. UV melting curves for $\gamma\mathbf{P_{12}}$ with **Telo-2** (black squares) or **Telo-4** (red circles) and $\mathbf{P_{18}}$ with **Telo-3** (blue triangles). Samples contained 2 μM $\gamma\mathbf{P_{12}}$ and either 2 μM **Telo-2** or 1 μM **Telo-4** or 1.3 μM $\mathbf{P_{18}}$ and 1.3 μM **Telo-3**. Buffer contained 10 mM Tris-HCl (pH = 7), 0.1 mM EDTA and 100 mM KCl. Important results include (a) higher melting temperature for $\gamma\mathbf{P_{12}}$ + **Telo-2** versus $\mathbf{P_{18}}$ + **Telo-3**, illustrating higher affinity of γPNA versus PNA, and (b) higher melting temperature for $\gamma\mathbf{P_{12}}$ + **Telo-4** versus $\gamma\mathbf{P_{12}}$ + **Telo-2**, illustrating cooperativity of hybridization of $\gamma\mathbf{P_{12}}$ + **Telo-2** to two adjacent sites on the same DNA complement versus hybridization to two separate DNA oligonucleotides.

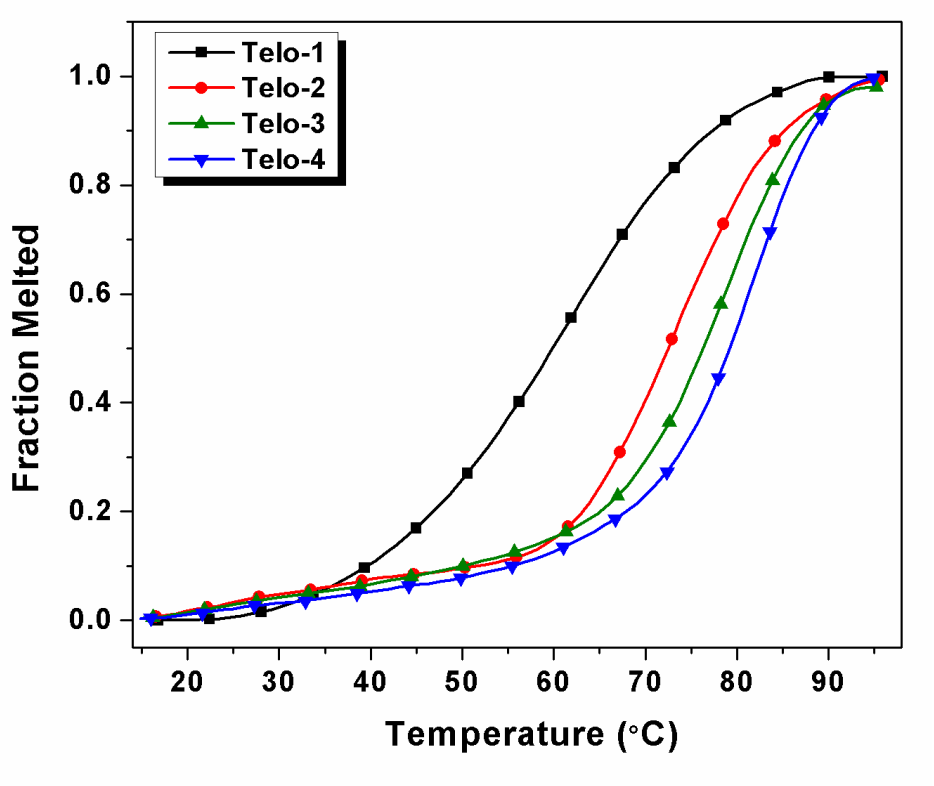

Figure S2. UV melting curves for $\gamma\mathbf{P}_{\mathbf{6\text{-}clamp}}$ with **Telo-1-4**. Samples contained 4 μM $\gamma\mathbf{P}_{\mathbf{6\text{-}clamp}}$ and either 4 μM **Telo-1**, 2 μM **Telo-2**, 1.3 μM **Telo-3** or 1 μM **Telo-4**. Buffer contained 10 mM Tris-HCl (pH = 7), 0.1 mM EDTA and 100 mM KCl. Significant results include the substantial increase in melting temperature as the number of complementary repeats in the DNA oligonucleotide increases, again illustrating the cooperativity of hybridization to adjacent sites on the DNA complement.

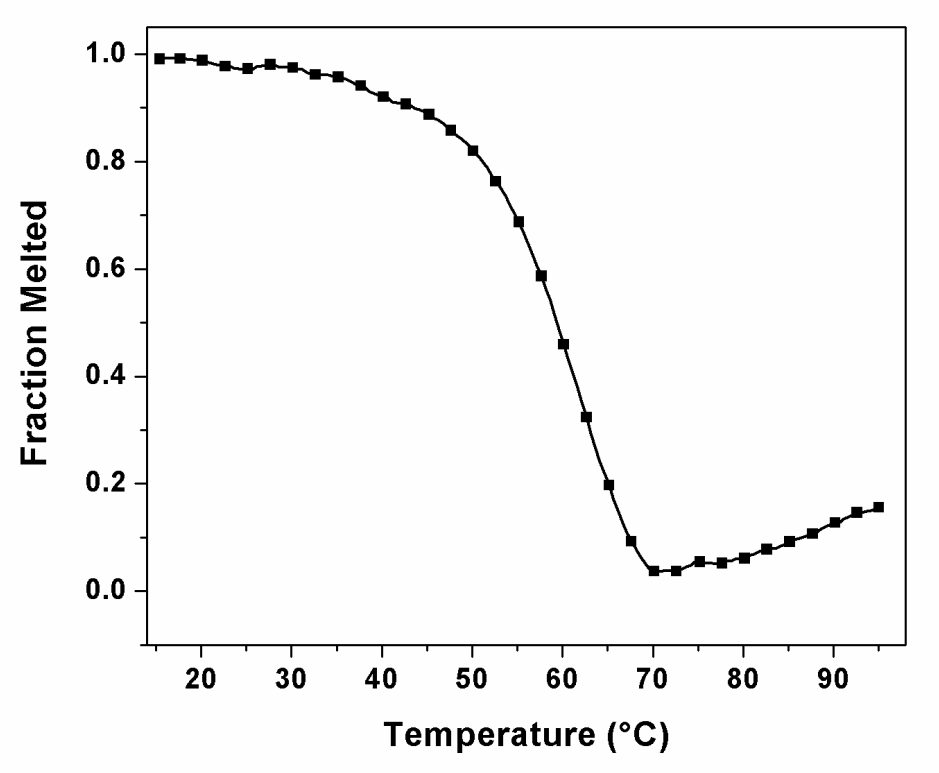

Figure S3. UV melting curve recorded at 295 nM for **Telo-4** in the presence of 100 mM KCl. The hypochromic transition is indicative of a G-quadruplex structure.

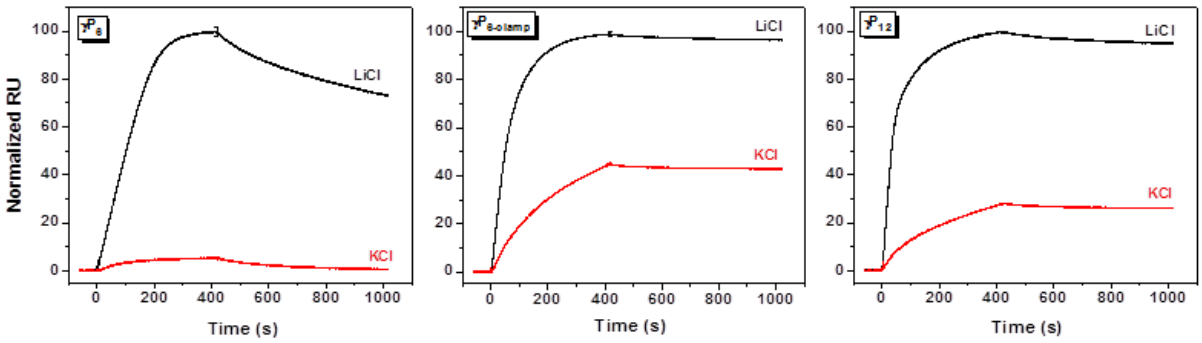

Figure S4. SPR sensorgrams for association/dissociation of γPNA miniprobes with **Telo-4** in the presence of 100 mM KCl (red) or LiCl (black). The large increases in association in the presence of LiCl are consistent with the formation of a DNA quadruplex structure in KCl, which inhibits hybridization of the γPNAs. Raw data for each γPNA were normalized to the maximum response unit (RU) signal observed in LiCl.

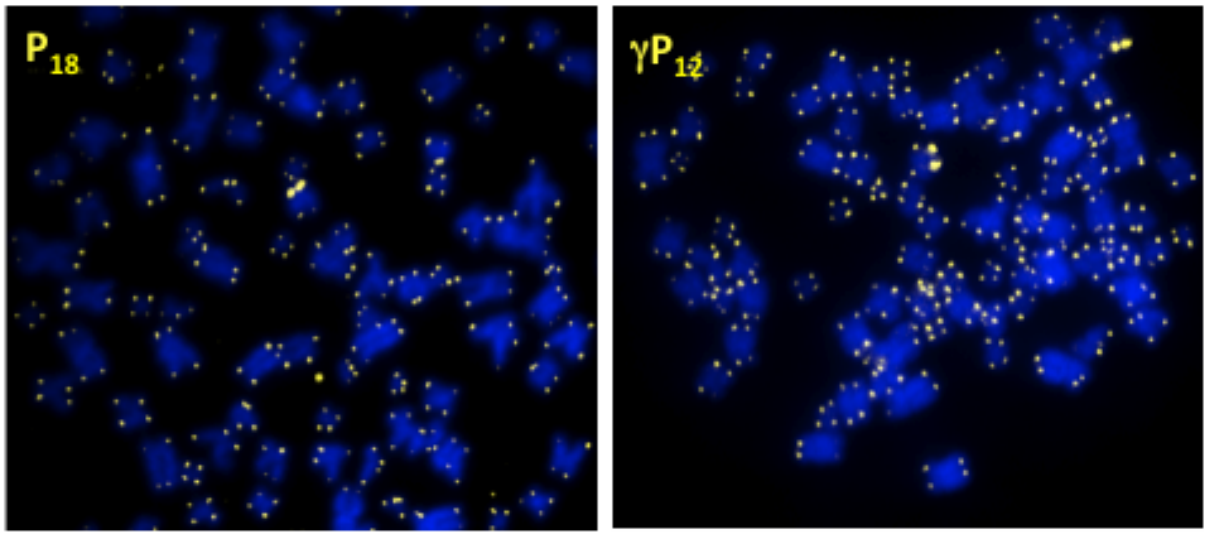

Figure S5. Telomere staining of Jurkat metaphase chromosome spreads using $\mathbf{P_{18}}$ (Left) or $\boldsymbol{\gamma}\mathbf{P_{12}}$ (Right). More telomeres are evident after staining with $\boldsymbol{\gamma}\mathbf{P_{12}}$. See Figure 7A for quantitative analysis.

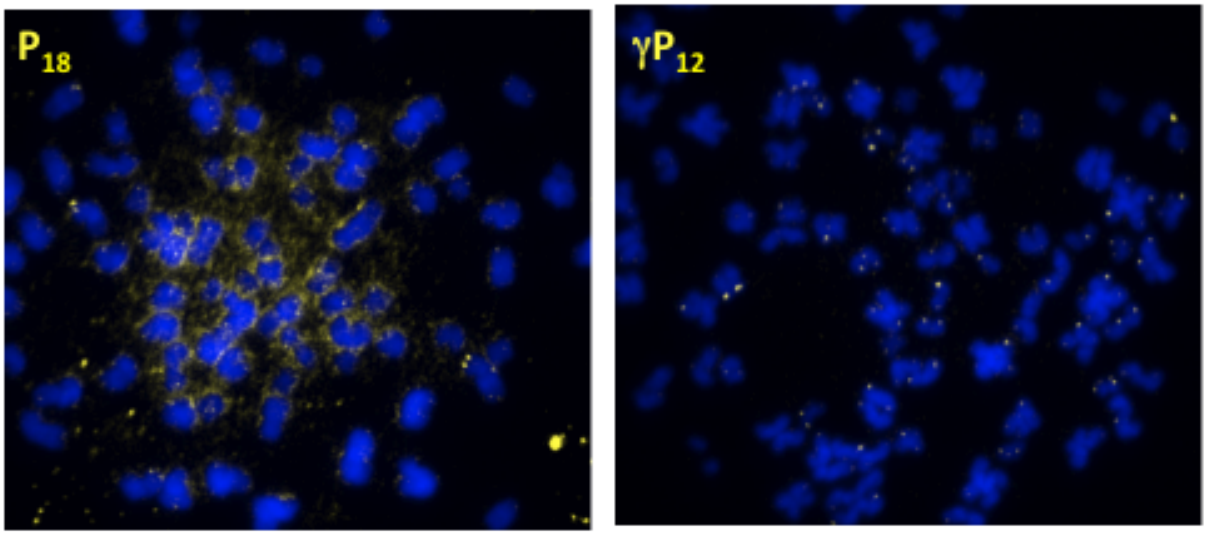

Figure S6. Telomere staining of Jurkat metaphase chromosome spreads using $\mathbf{P_{18}}$ (Left) or $\mathbf{\gamma P_{12}}$ (Right) under nondenaturing conditions. Metaphases were prepared and stained with a modified protocol that omitted formamide from the hybridization buffer and eliminated the heating step. The exposure time for the Cy3 channel was increased to 800ms for these experiments. Quantitative comparison of denaturing and nondenaturing conditions for telomere staining by $\mathbf{\gamma P_{12}}$ is shown in Figure 7.

γP_6 Characterization

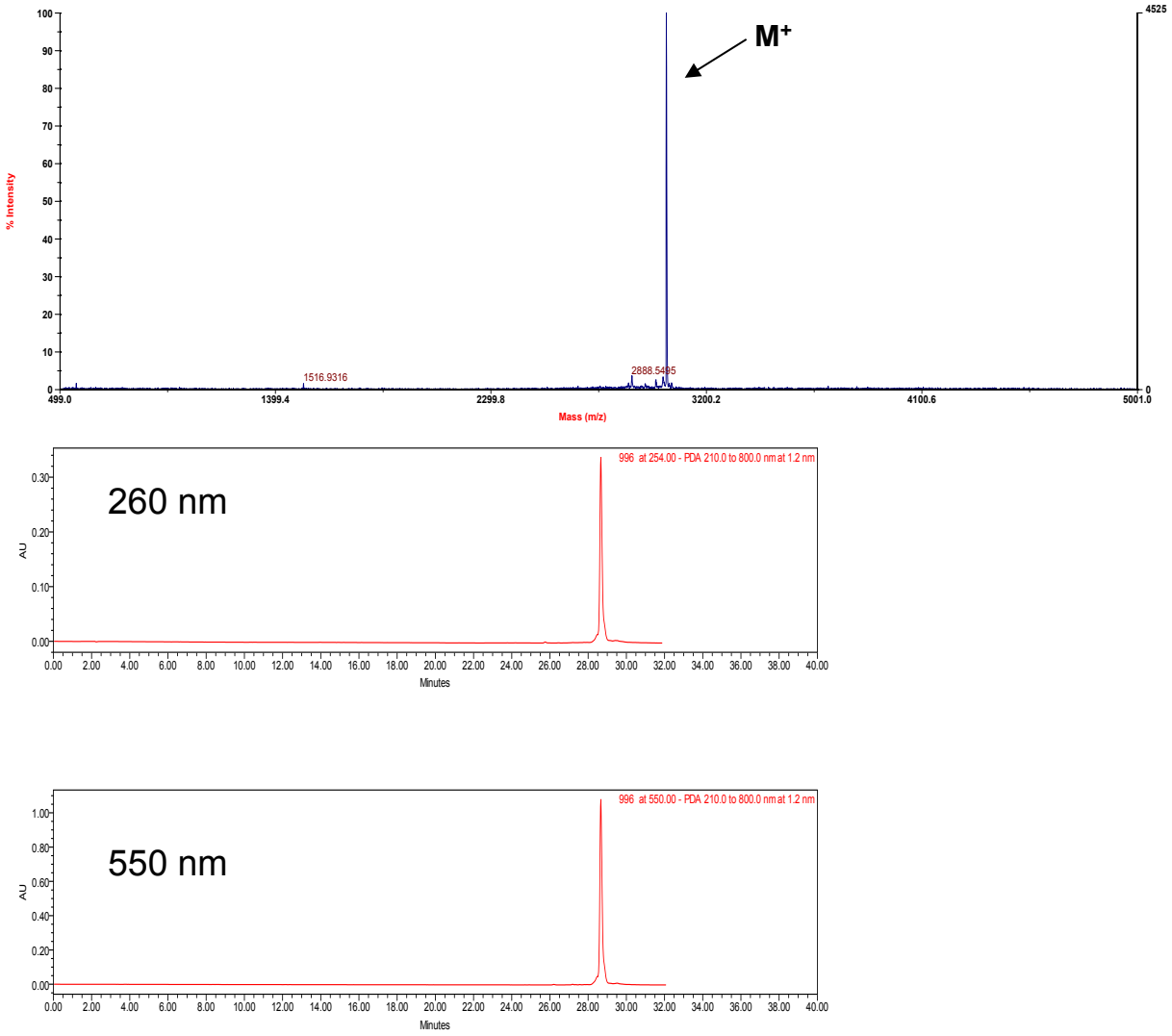

Figure S7. MALDI and HPLC traces of γP_6. m/z = 3032.7 (observed), 3036.2 (calculated).

$\gamma P_{6\text{-Clamp}}$ Characterization

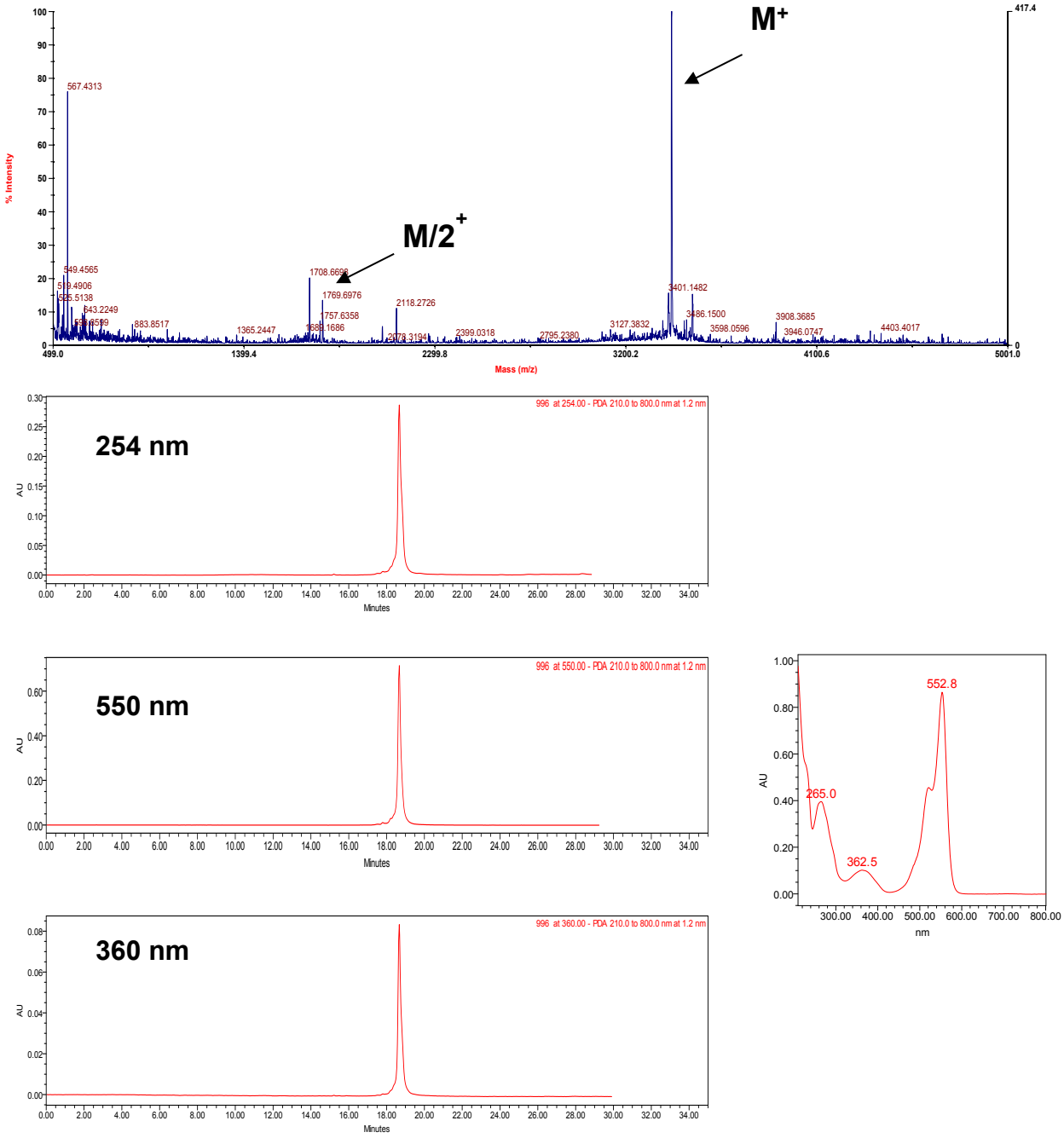

Figure S8. MALDI and HPLC traces of purified $\gamma P_{6\text{-Clamp}}$. m/z = 3416.1 (observed), 3420.6 (calculated).

γP_{12} Characterization

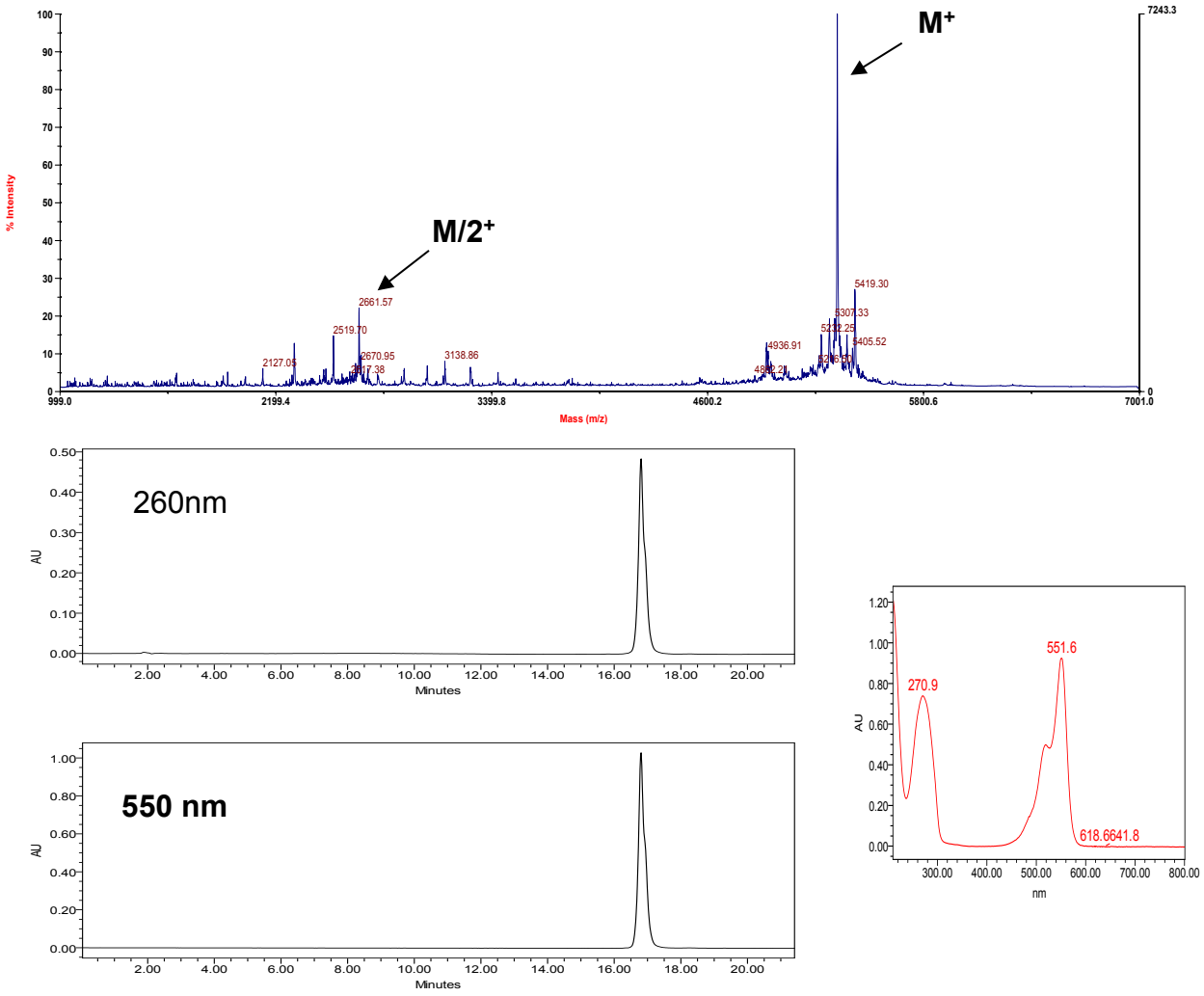

Figure S9. MALDI and HPLC traces of γP_{12}. m/z = 5320.9 (observed), 5315.5 (calculated).