Supporting Information

A small molecule inhibitor of Pot1 binding to telomeric DNA

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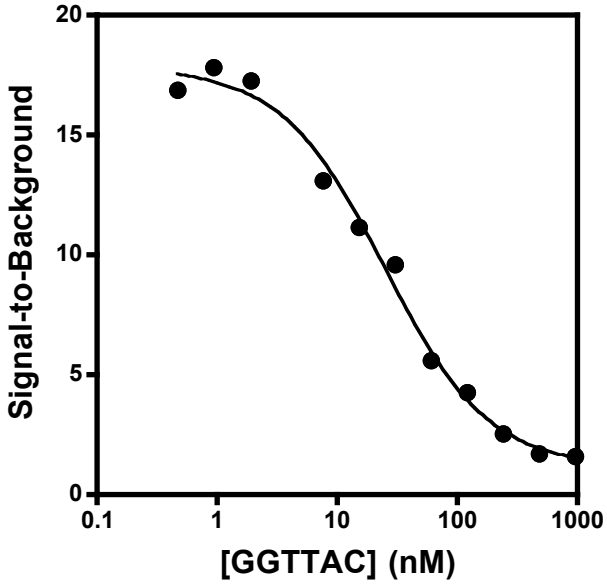

Figure S1. FRET-based competition of unlabeled 6mer against biotin-d(AAGGTTAC) (5 nM) pre-bound to Pot1pN (10 nM) at 25 °C. Signal-to-background is plotted as a function of increasing 6mer concentration. Half maximal signal-to-background was achieved at ~25 nM unlabeled 6mer, which is comparable to the measured Pot1pN/6mer K_D under these conditions (*76*).

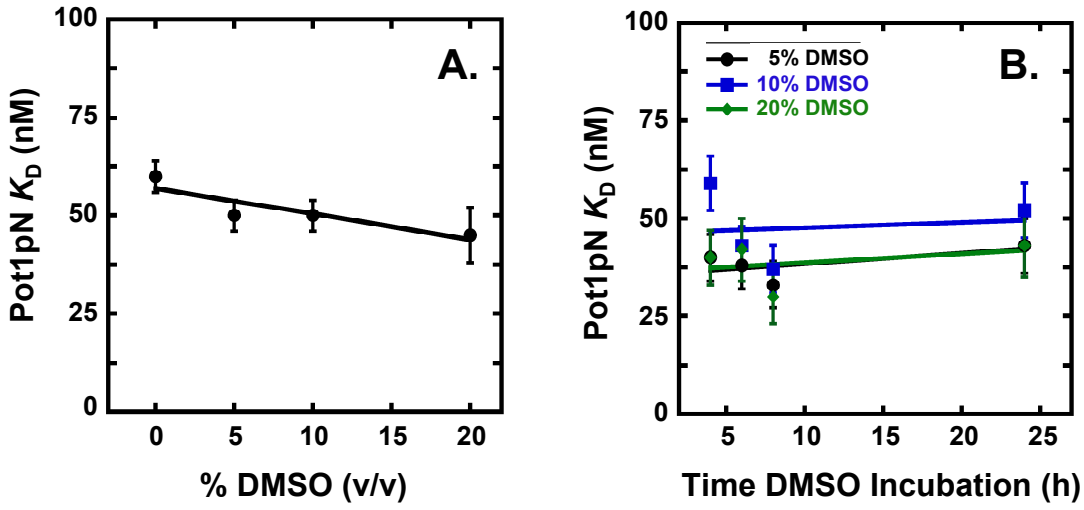

Figure S2. The Pot1pN/d(GGTTAC) complex is stable in up to 20% DMSO for at least 24 hours. (A) K_D was determined in various DMSO concentrations at ambient temperature using a filter binding assay and plotted as Pot1pN K_D (nM) versus % DMSO (v/v). (B) Pot1pN was incubated with the indicated concentrations (v/v) of DMSO for 4, 6, 8 and 24 hours at ambient temperature and subsequently bound to d(GGTTAC). K_D is plotted as function of incubation time for each concentration of DMSO.

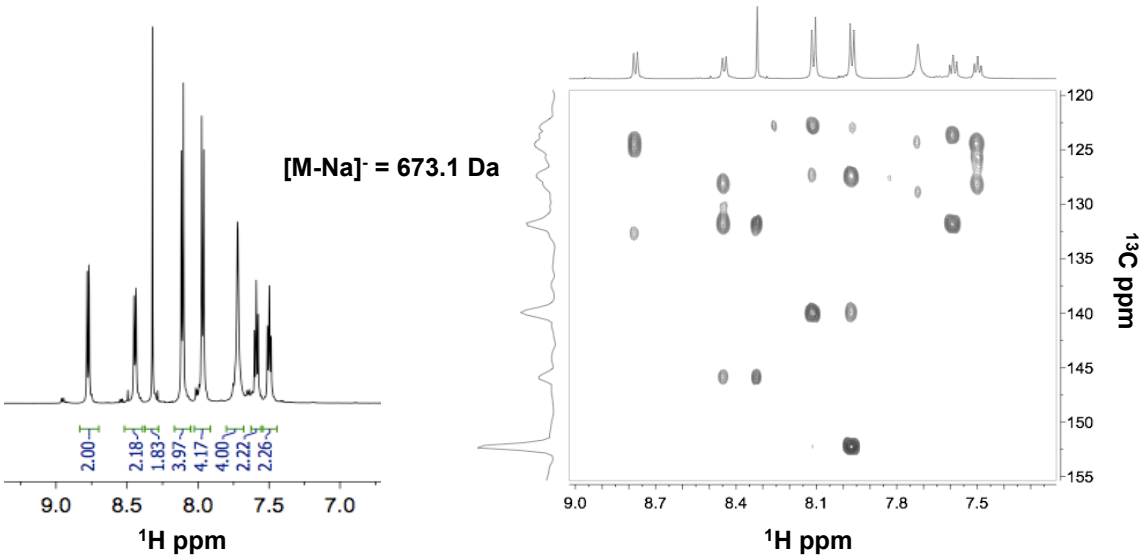

Figure S3. 1D ^{1}H and ^{1}H-^{13}C HMBC NMR spectra for Congo red (ST012888). All peaks are within the aromatic and amide regions. Integration of peaks from the 1D spectrum (left) is consistent with CR structure. ESI mass spectrometry revealed a mass of 673.1 Da for the $[M-Na]^-$ species, consistent with the known mass of 696.7 Da for the CR sodium salt. ^{1}H-^{13}C HMBC NMR spectrum allowed for assignment of small molecule peaks as CR.

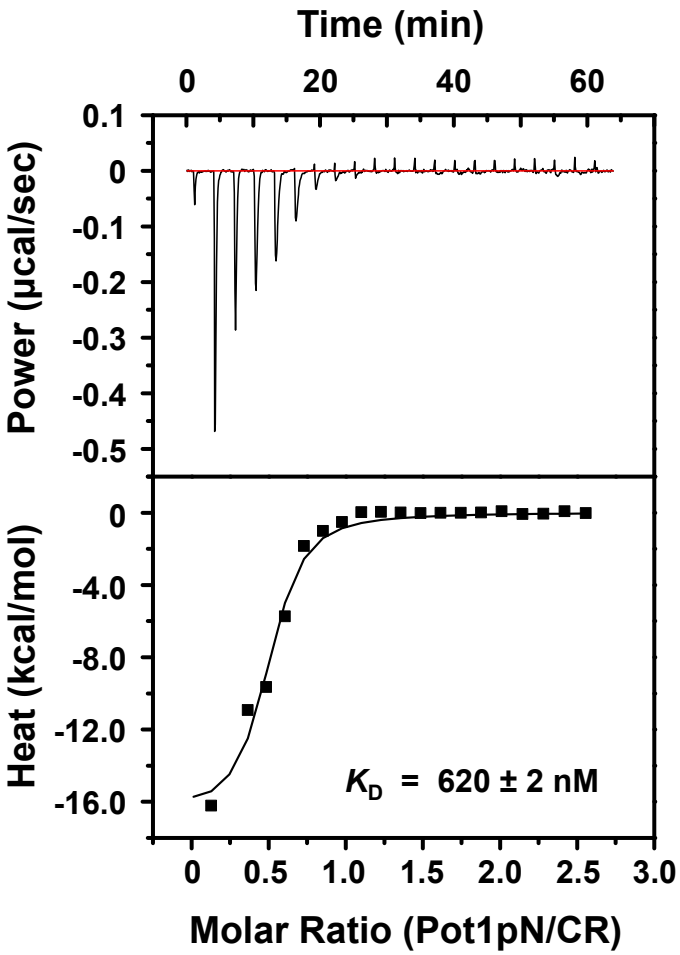

Figure S4. Reverse titration of CR and Pot1pN give same K_D as forward titration. Baseline-corrected raw data is shown in the top panel, and the integrated heat isotherm is shown in the bottom panel. Twenty-one injections of 272 μM Pot1pN were titrated in 22.5 μM CR at 20 °C. The first injection was 0.2 μL, and all subsequent injections were 1.95 μL.

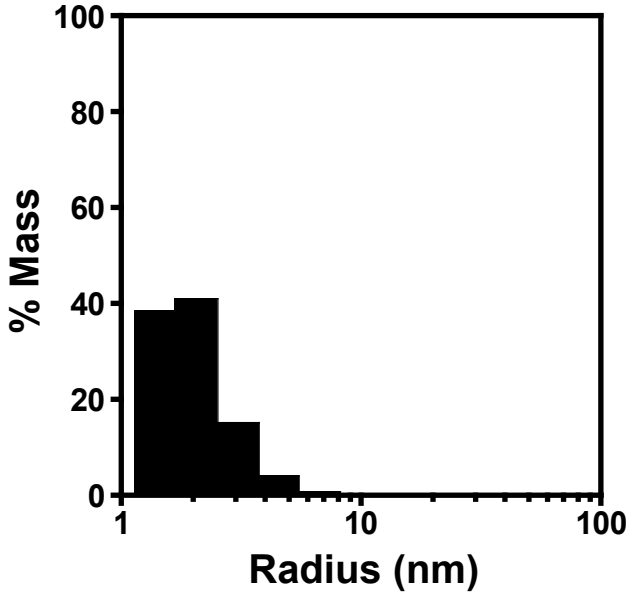

Figure S5. Addition of a 5-fold excess of 6mer resolves the majority of the CR-mediated trimerization. DLS reveals > 80% of the sample mass has a calculated MW of 24.4 kDa, consistent with the size of momomeric Pot1pN. The remaining species appear to be a mixture of dimeric and trimeric Pot1pN complexes. DLS was performed on a DynaPro system (Wyatt Technology Corporation) operating at a wavelength of 633 nm with scattered light detected at 90°. 12 μL samples consisted of 300 μM Pot1pN, 300 μM CR, and 1.5 mM 6mer. Samples were spun down at maximum speed in a microcentrifuge prior to measurement in quartz cuvettes. Data were analyzed using DynaPro Dynamics V 6.3.40 (Wyatt Technology Corporation) to calculate hydrodynamic radius and molecular weight.