Supporting Information

Exploring host-guest interactions within a 600 kDa DegP protease cage complex using hydrodynamics measurements and methyl-TROSY NMR

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Supplementary Figures

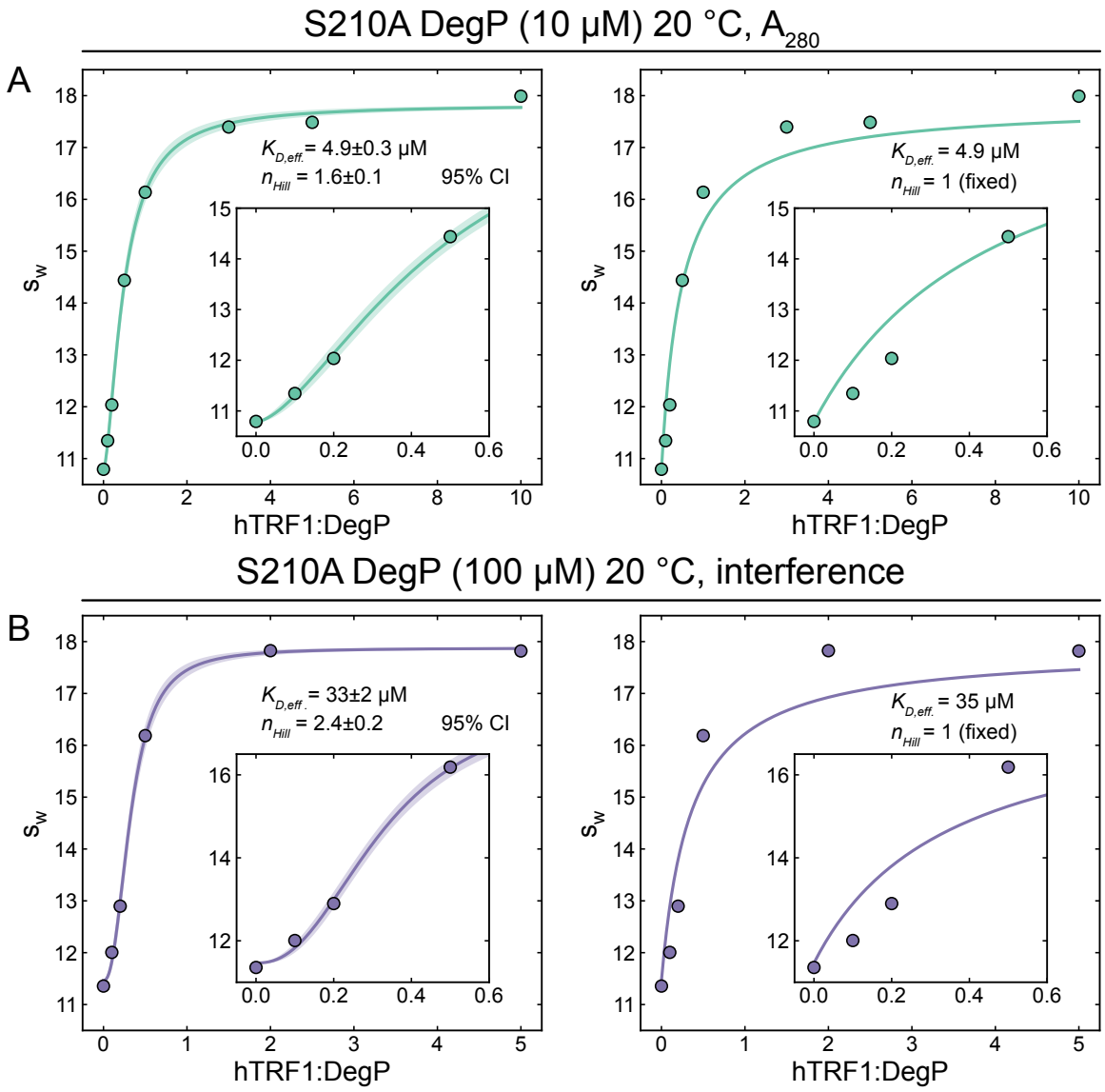

Figure S1. Analysis of weighted s-value (s_w) isotherms for 12mer DegP self-assembly as measured by AUC. (A, B) Isotherms generated by integration over the region 4.5-22S for a titration of DegP (10 µM) with hTRF1 (up to 100 µM) measured by absorbance optics at 280 nm (A_{280}, Figure 1C), or by integration over the region 4.5-25S for a titration of DegP (100 µM) with hTRF1 (up to 500 µM) measured by interference optics (Figure 1D). The isotherms were fit using a modified Hill equation according to $s_w = \Delta s \frac{[hTRF1]^{n_{Hill}}}{K_{D,eff.}{}^{n_{Hill}} + [hTRF1]^{n_{Hill}}} + s$, where Δs and s are scaling and offset

parameters estimated from the minimum and maximum values of the isotherms, and $K_{D,eff.}$ and n_{Hill} are the effective dissociation constant and Hill cooperativity parameter, respectively. Fits were performed where $K_{D,eff.}$ and n_{Hill} were optimized (A, B left), or where $K_{D,eff.}$ was allowed to vary and n_{Hill} was fixed to 1 (*i.e.* non-cooperative assembly, A, B right). Optimal parameters for each fit are shown in the upper right of the plots. The 95% confidence intervals (CI) for the parameter distributions are indicated for the best-fit model in which both $K_{D,eff.}$ and n_{Hill} were varied (A, B left) and correspond to the shaded regions encompassing the fitted curves. Parameter errors were estimated using Monte Carlo simulations. The insets highlight fits to the isotherms over molar ratios of 0-0.6 hTRF1:DegP.

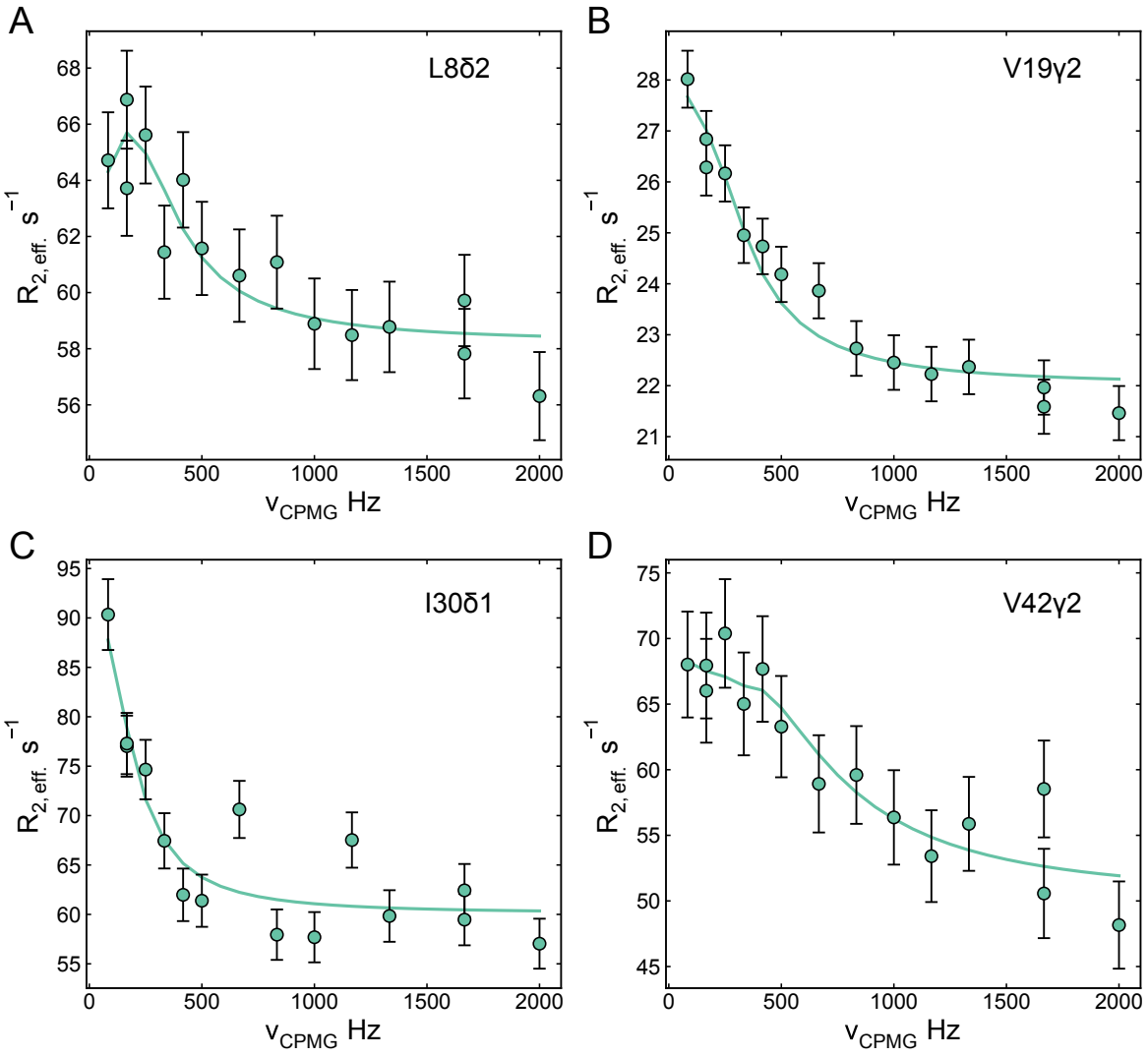

Figure S2. CPMG dispersion profiles for methyl groups in ILVM hTRF1 bound within a U-^{2}H S210A DegP 12mer. Methyl group assignments are given in the top right corner of each plot (A-D). Resonances with the largest dispersions (>5 s^{-1}) are shown. The data were globally fit with a two-state model, yielding an optimal exchange rate (k_{ex}) of 686±142 s^{-1} and a minor state population (p_B) of 7.5±1.1%. The errors in the fitted k_{ex} and p_B were obtained using Monte Carlo simulations. Note that the dispersions do not derive from exchange between DegP-bound and free

hTRF1 as the chemical shift differences obtained in fits of the dispersion profiles are uncorrelated with the shift differences measured for the bound and free hTRF1 states.

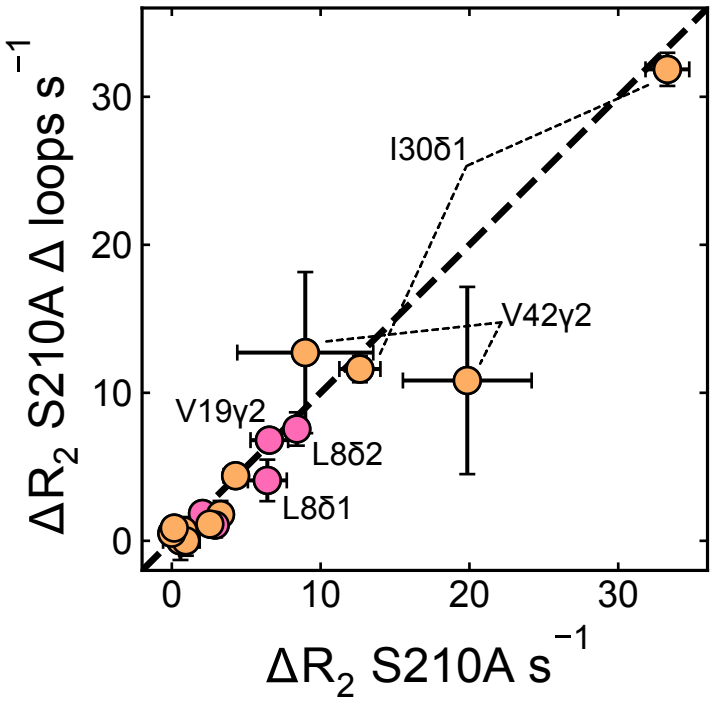

Figure S3. Linear correlation plot of $\Delta R_2 = R_{2,eff}$(83.3 Hz) - $R_{2,eff}$(2000 Hz), the difference in effective transverse relaxation rates measured at the lowest and highest recorded CPMG frequencies, for ILVM-labeled hTRF1 in complex with either U-^{2}H S210A DegP (x-axis) or U-^{2}H S210A Δloops DegP (y-axis). Methyl groups with ΔR_2 values >5 s^{-1} are labeled. The black dashed line corresponds to y=x.

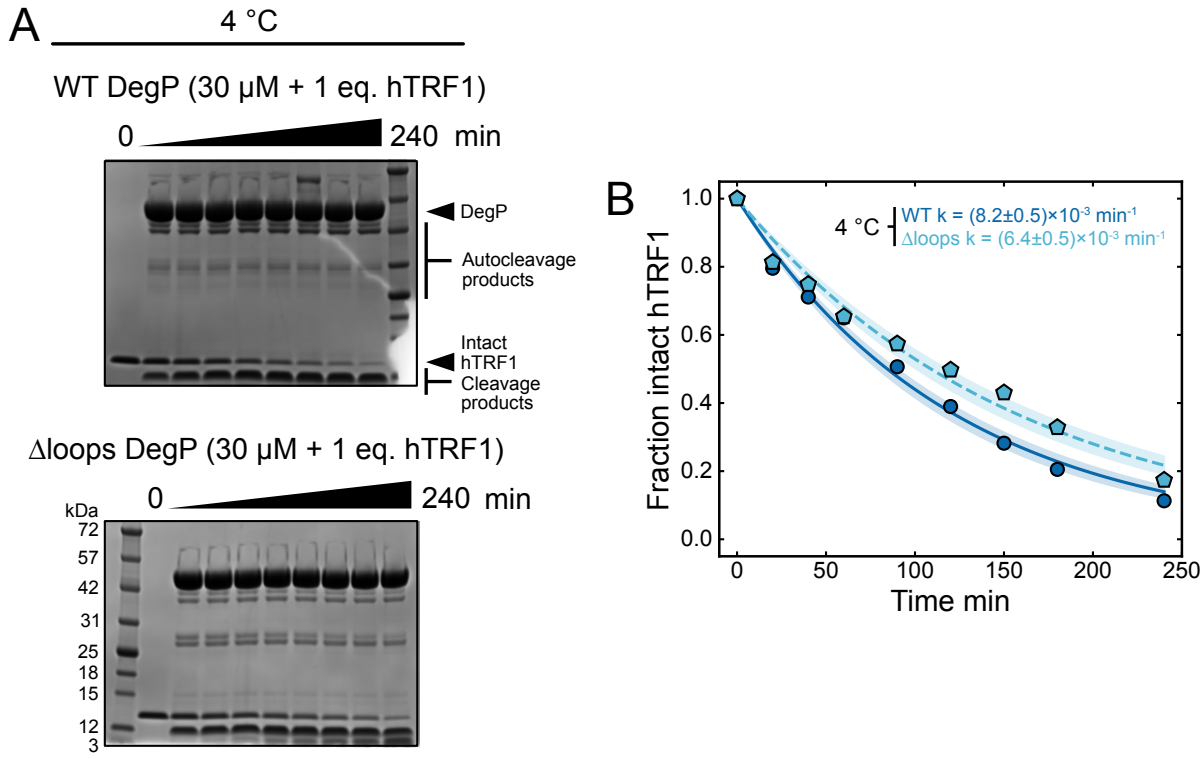

Figure S4. DegP LA loops have little influence on proteolysis at lower temperature and hTRF1 molar ratio. (A) SDS PAGE-based proteolytic activity assays measuring cleavage of hTRF1 by WT DegP (top) and Δloops DegP (bottom) at 4 °C. In each case, 30 μM enzyme and 30 μM hTRF1 were used to explore differences in activity at lower DegP:client ratios (1:1) as opposed to Figure 4B in the main text, where initial concentrations of 10 μM enzyme and 100 μM hTRF1 (1:10) were used in reactions performed at 30 and 37 °C. It was not possible to perform the reaction at 30 and 37 °C by simply decreasing the concentration of hTRF1 to 10 μM to obtain a 1:1 molar ratio, as the reaction is too rapid, and because it is difficult to accurately quantify the hTRF1 band intensities on the gel at this concentration of client. To adequately capture the reaction profile using the decreased hTRF1 concentration, it was necessary to (i) perform the reaction at the lower temperature to slow the decay, and (ii) implement a compensatory increase in the amount of DegP. Note that the bands corresponding to the DegP constructs are much more intense compared to

Figure 4B, owing to the 3-fold increase in enzyme concentration in combination with the 3-fold increase in the gel loading volumes used here (equivalent to a 9-fold increase in DegP band intensity). The larger loading volumes were required for accurate visualization and quantification of the hTRF1 bands at the lower initial concentration used here (30 *vs*. 100 μM in Figure 4B). (B) Plots of the fraction of intact hTRF1 as a function of time for WT (dark blue circles and line) and Δloops DegP (light blue pentagons and dashed line). The solid and dashed lines are the fits of an exponential decay function to each dataset, with the associated decay constants (k) indicated in the top right corner of the plot. The shaded regions encompassing the best-fit curves correspond to the 95% confidence intervals calculated using Monte Carlo simulations.

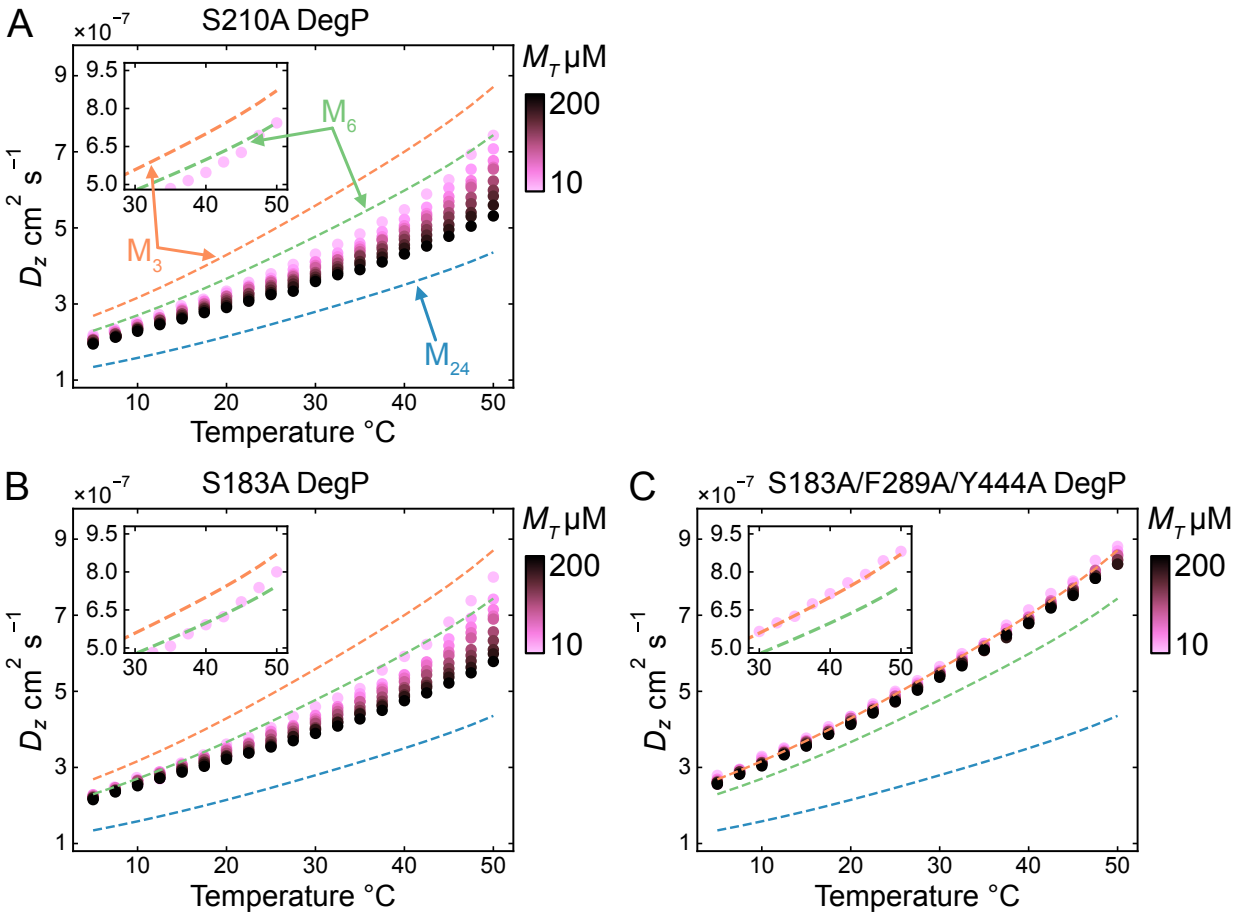

Figure S5. D_z profiles extracted from DLS measurements of (A) S210A, (B) S183A, and (C) S183A/F289A/Y444A DegP constructs as a function of temperature and total monomer concentration M_T in 25 mM HEPES free acid, 25 mM NaCl, 1 mM EDTA, pH 7.0. The colored dashed lines correspond to the temperature-dependent diffusion constants for 3mer, M_{6A}, and 24mer particles calculated as described in the main text. Note that the profiles for S210A DegP are somewhat different to those shown in Figure 5B top due to the lower NaCl concentration used here (25 mM *vs* 200 mM). The lower salt concentration was implemented here for direct comparison with the proteolysis and peptidase assays shown in Figures 4B and 6E, which were performed in the buffer described above.

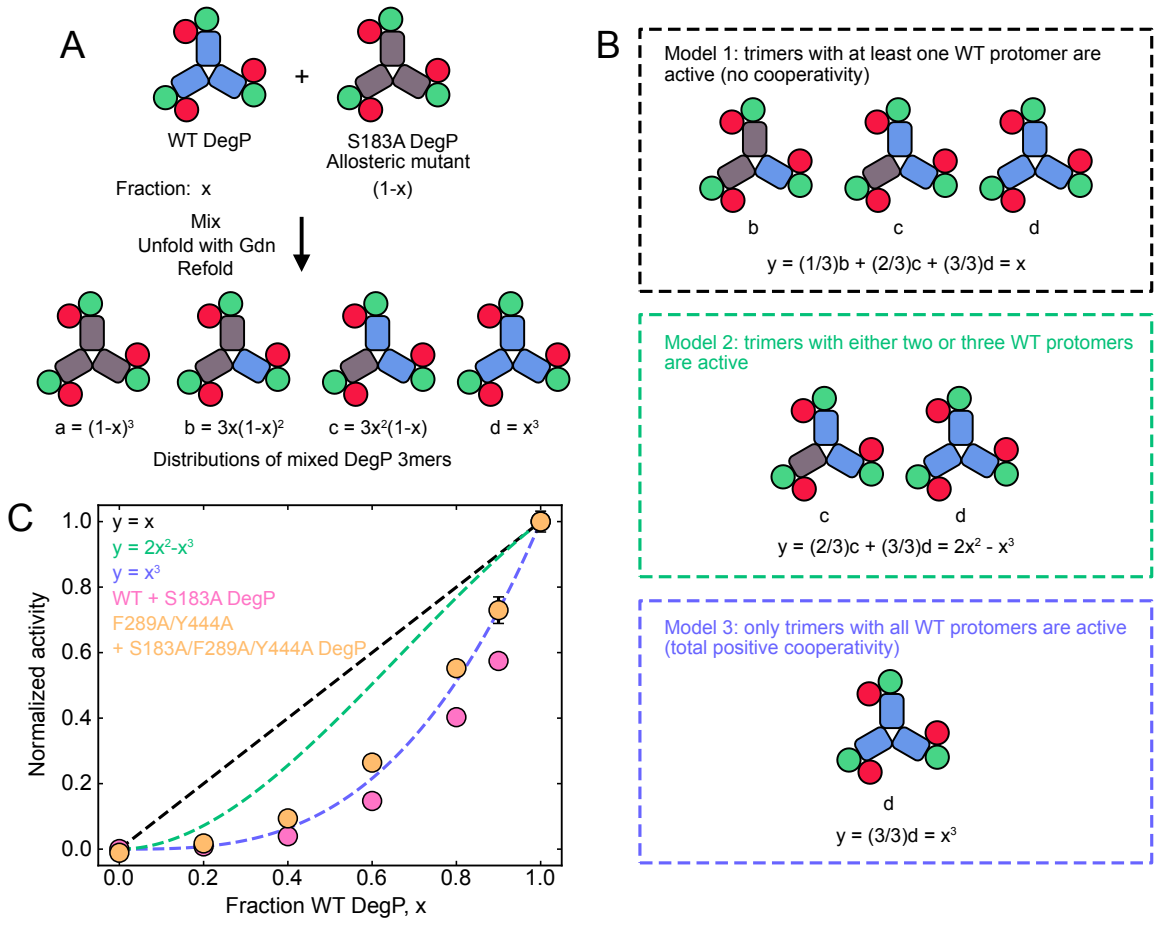

Figure S6. Modeling of DegP activity using mixed protomer samples. (A) Schematic depicting the strategy for preparing mixed protomer DegP samples for fluorescence-based peptidase assays. The WT and S183A allosteric mutant versions of DegP trimers are shown with their protease domains colored blue and brown respectively (top). Mixing of the WT and mutant DegP protomers in ratios of x:(1-x) leads to the formation of four different types of DegP trimers (bottom), with fractional populations as indicated (a-d). Note that only one of three rotationally symmetric conformations of the mixed trimers is displayed. (B) Schematics for three different models of DegP activity. In Model 1 (black), trimers with 1 to 3 WT protomers are active (non-cooperative). In Model 2 (green), trimers with either 2 or 3 WT protomers are active. Finally, in Model 3 (purple),

trimers that have 3 WT protomers are the only active species (total positive cooperativity). Note that the multiplicative factors of (1/3), (2/3), and (3/3) account for the number of WT protomers in each type of trimer. (C) Plot of normalized peptidase activity as a function of the fraction of WT DegP monomers (x). Pink and orange colored circles correspond to activities of mixed samples of WT and S183A (pink) or F289A/Y444A and S183A/F289A/Y444A (orange; the F289A/Y444A double mutant can only form trimers, while WT and S183A can form higher-order oligomers) DegP. The black, green, and dark blue dashed lines, respectively, correspond to the three models of DegP activity in (B). Error bars are one standard deviation based on triplicate measurements.