Supporting Information for

Direct Observation of Multimer Stabilization in the Mechanical Unfolding Pathway of a Protein Undergoing Oligomerization

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Protein Sequences

Protein sequences of constructs used in this study (mutations from wildtype highlighted in red):

SM_{wt} (wildtype)	AEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWT VAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS
SM_{W120F}	AEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWT VAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAFKSTLVGHDTFTKVKPSAAS
$SM_{W120A,K121A}$	AEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWT VAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAASTLVGHDTFTKVKPSAAS
SM_{dim} (W120K)	AEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGSGTALGWT VAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAKSTLVGHDTFTKVKPSAAS
SM_{mon} (V55T, T76R, L109T, V125R)	AEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYTLTGRYDSAPATDGSGTALGWR VAWKNNYRNAHSATTWSGQYVGGAEARINTQWTLTSGTTEANAWKSTLRGHDTFTKVKPSAAS

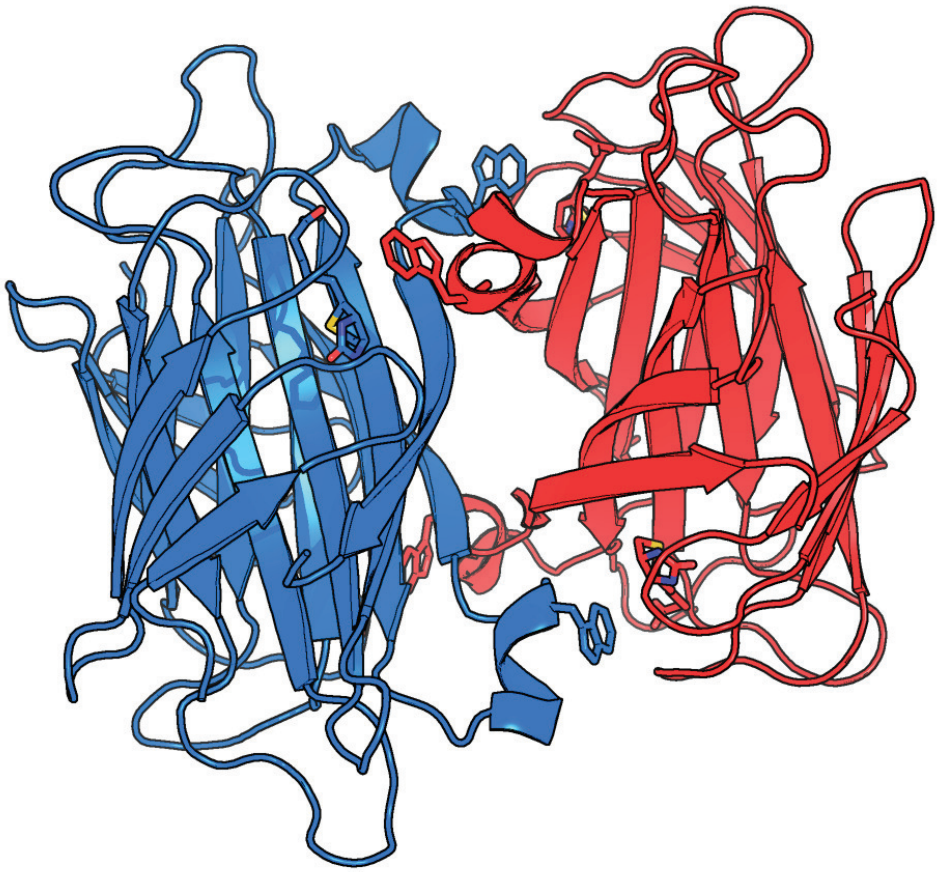

Figure S1: Cartoon of dimer-dimer interaction from PDB 1SWE.[75] Streptavidin first forms a strong dimer (blue or red) which then dimerize together, and this dimerization is stabilized by a long arm (seen easily in blue) that reaches over to the other dimer. This arm contains Trp120 which greatly stabilizes the interaction with biotin.

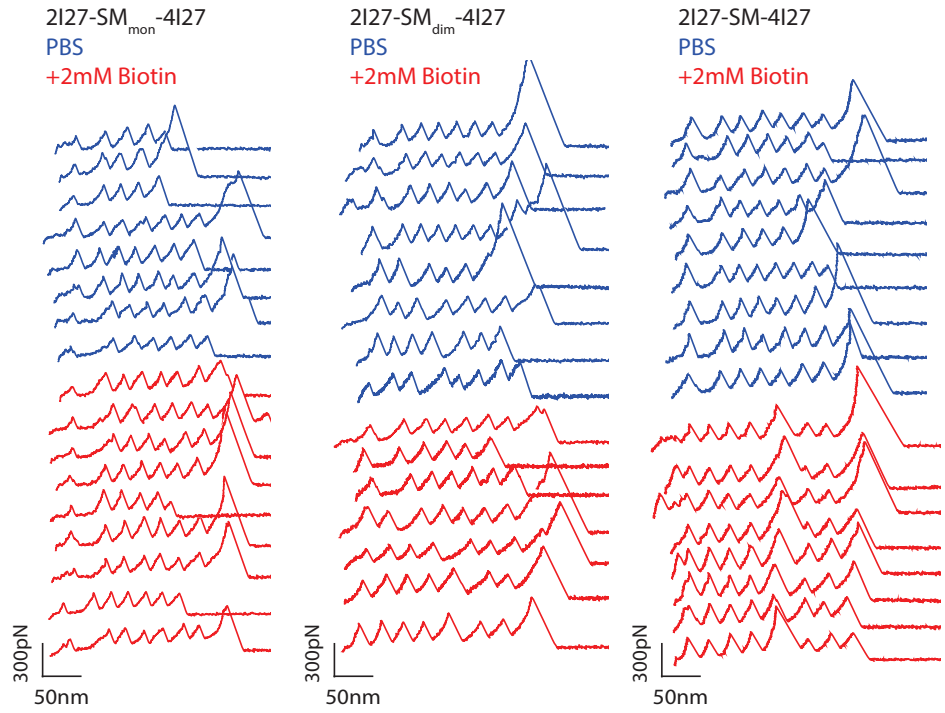

Figure S2: Examples of the force-extension curves for unfolding SM_{mon}, SM_{dim} and SM (wildtype) without biotin (blue) and with biotin (red).

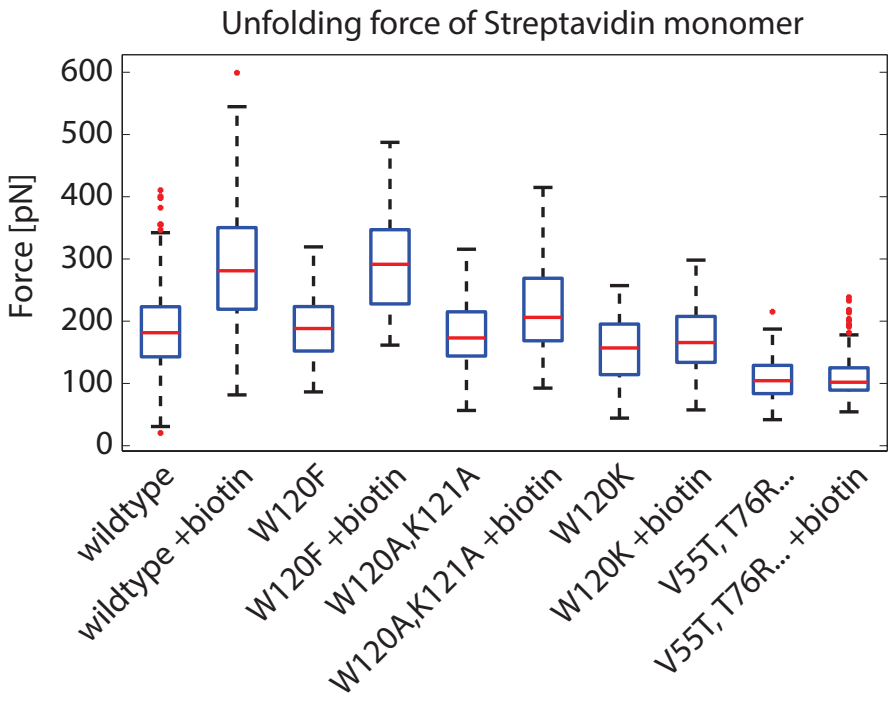

Figure S3: Box and whisker plots for each $I27_2$-SM-$I27_4$ construct is shown. The left side shows unfolding forces measured in PBS only, and the right side shows unfolding forces measured in PBS + 2 mM biotin. The red line indicates the median, the edges of the blue rectangle represent the 25th and 75th percentiles, and the dashed lines represent 99.3% coverage of the data. The red dots represent outliers.

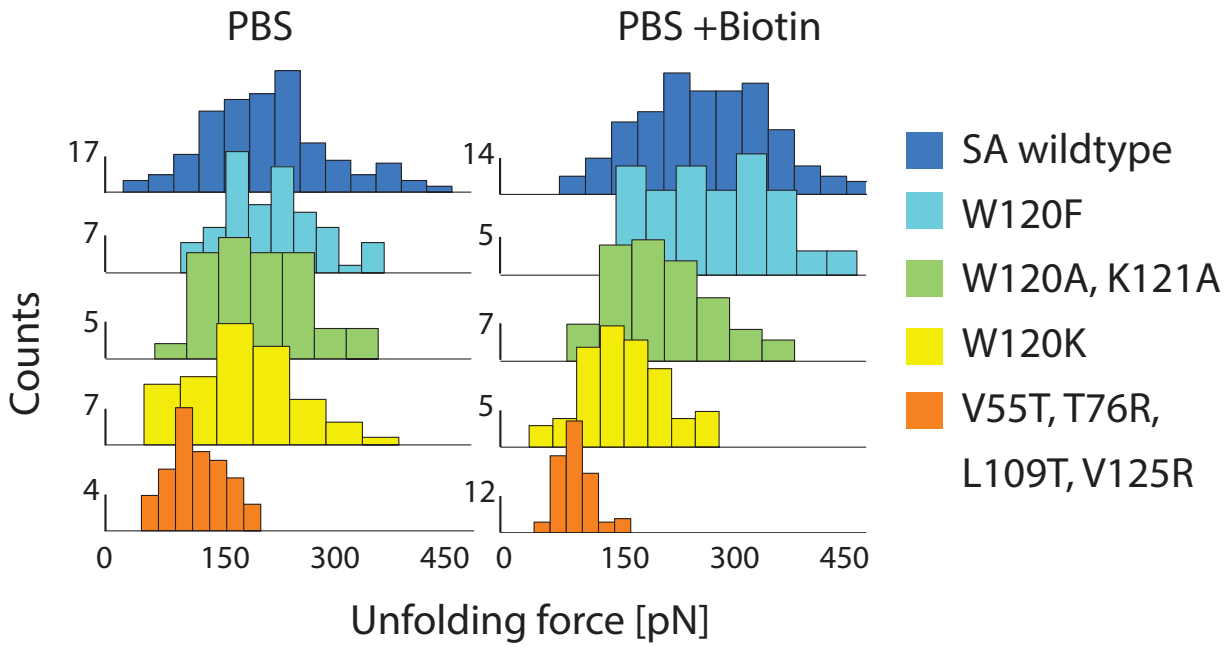

Figure S4: Distributions of unfolding forces for Streptavidin in each of the studied mutants, without and with added biotin.

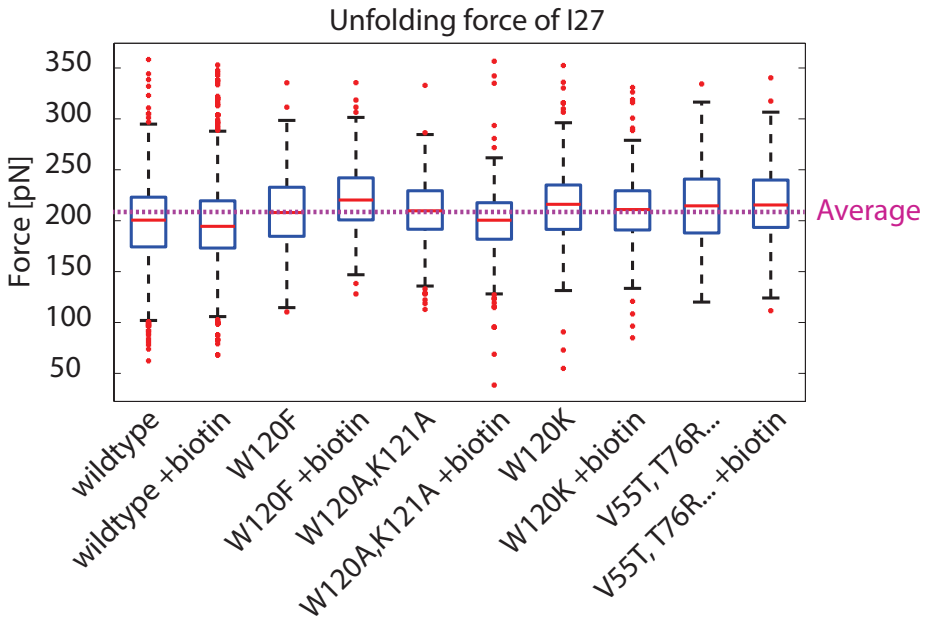

Figure S5: Comparing distributions between different experiments. This shows the mean and standard deviation from unfolding forces of the I27 domains in the experiments with different SA mutants in different conditions (PBS or PBS +2mM Biotin). The mean unfolding force of I27 in all experiments is 204.6 pN. Any mean of a given experiment only differs from this mean by -11.0 to +5.6 pN which is far less than the changes seen in Streptavidin unfolding (main text). This serves as a negative control and shows that unfolding forces of SA can be compared without systematic error between experiments. The biotin has no effect on the unfolding force of I27 (mean difference is 0.4 pN; p=0.7).

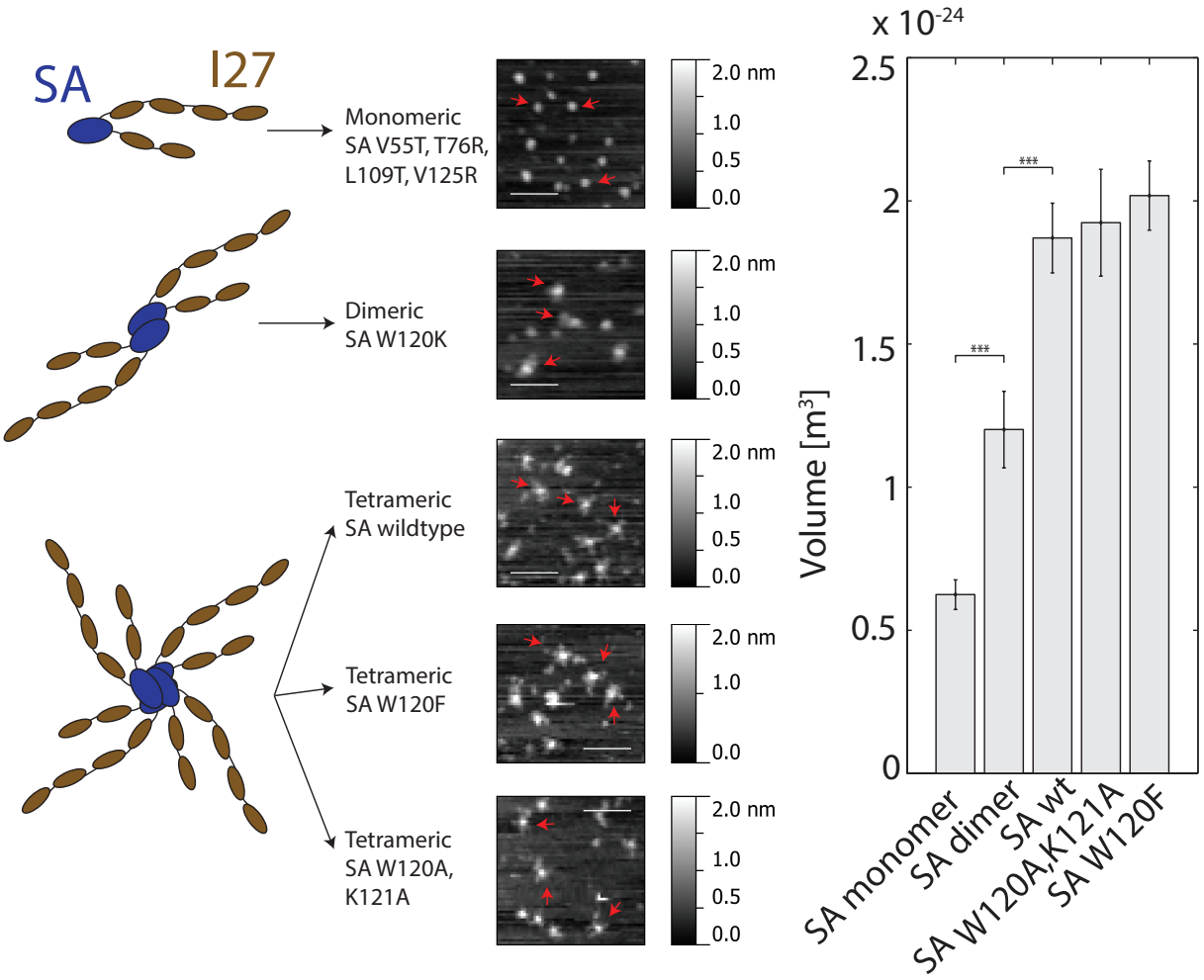

Figure S6: Left: Example of AFM images for each variation of I27-flanked Streptavidin complex used in this study. AFM images were taken and analyzed according to Materials and Methods. Right: The volume mean and 95% confidence intervals calculated from the individual particles from each AFM image. The difference between monomeric and dimeric, as well as between dimeric and tetrameric, are statistically significant (p-value $< 10^{-6}$). Also, as expected, the volumes also increase roughly linearly from monomeric, to dimeric to tetrameric.

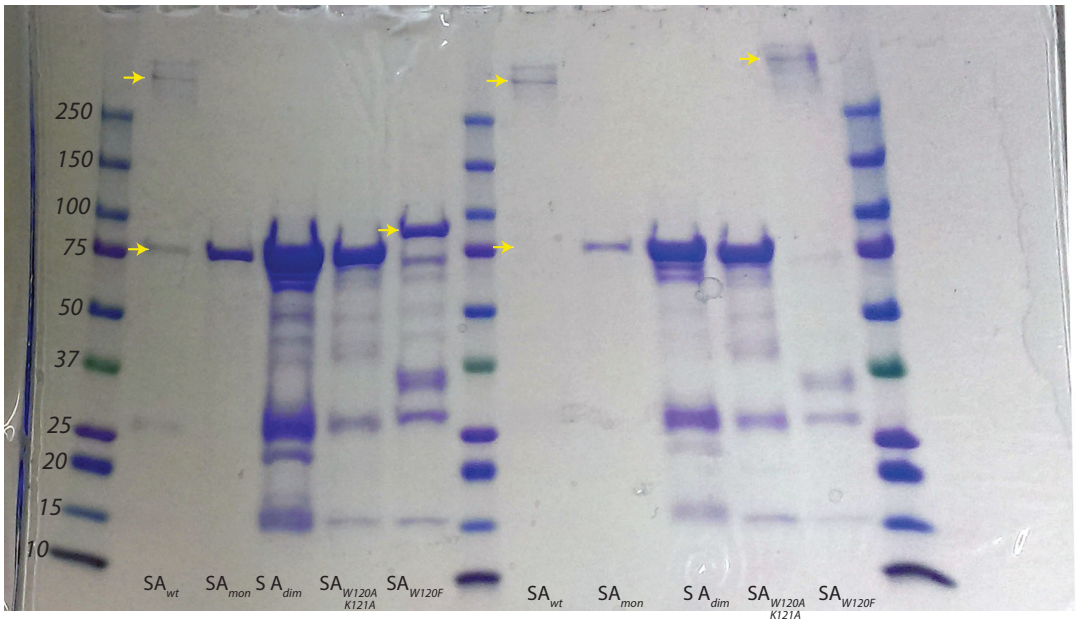

Figure S7: SDS-Gel of the Streptavidin proteins used in this study. Before loading, the proteins were heat-denatured at 70C for 10 minutes. In principle, this should allow all non-covalent interactions to be disrupted and allow each protein to run as a unfolded polypeptide. As can be seen, most proteins have a theoretical molecular weight of $\sim$ 77 kDa (except for SA W120F which contained an additional I27 domain) and the most prominent band corresponds well with their theoretical weight. Unexpectedly, for $I27_2$-SA-$I27_4$ with and without biotin, and $I27_3$-SAW120F-$I27_4$ with biotin, there are also top bands which have an average molecular weight of 310 kDa which corresponds very well to the molecular weight of a tetramer (4 $\times$ molecular weight of monomer). These bands are surprising because it shows that the Streptavidin wildtype tetramerization is a strong enough noncovalent interaction to resist heat and SDS denaturation. The ability for wildtype Streptavidin to still form tetramers indicates that I27 domains do not have a detrimental effect on tetramerization or stability.

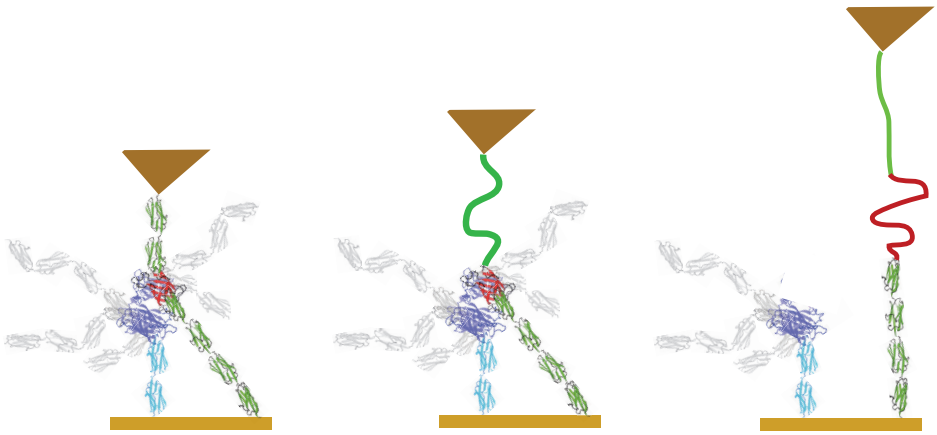

Figure S8: Cartoon of the multiple-attachment that leads to unfolding events after either two I27 events (shown) or after four I27 events. A single molecule (green I27 flanking the red SA monomer) is pulled, but is tethered to a second location through the cyan I27 which are attached to another SA monomer that binds to the first SA noncovalently. Unfolding of the first two I27 domains (green) unfold normally, but the force to the last four I27 domains (green, tethered) is not as strong because of the cyan I27 domains tethering. Once the red SA unfolds, the tetramerization is disrupted and the unfolding proceeds along the single covalent polypeptide chain. Evidence of this was seen through the anisotropy of the SA peaks on either side, S14.

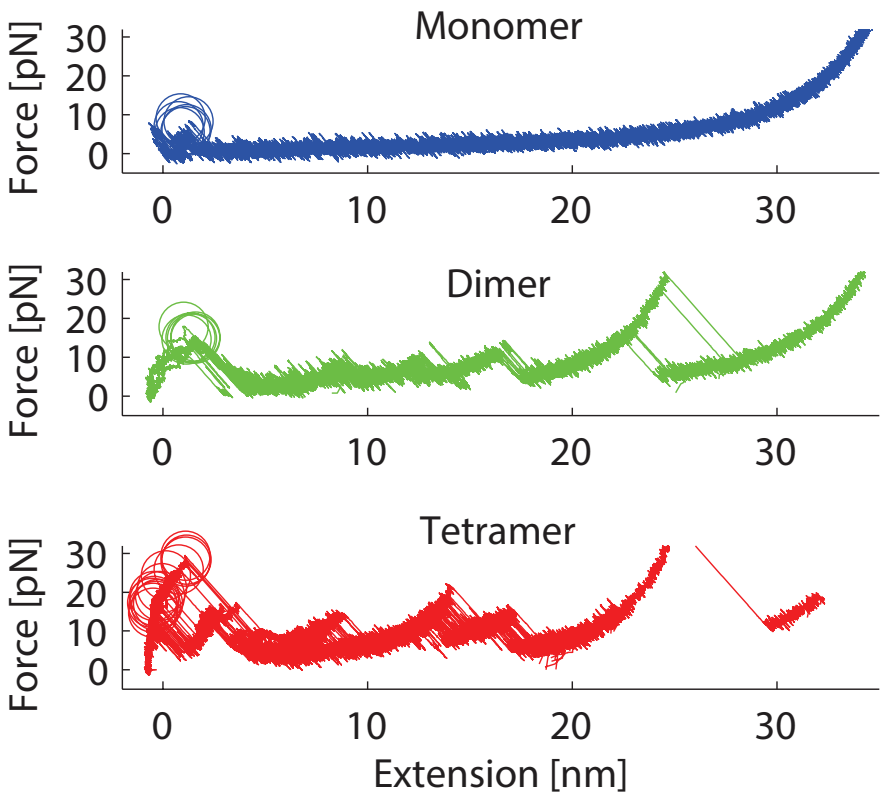

Figure S9: Examples of force-extension curves from coarse-grain simulation of Streptavidin unfolding.

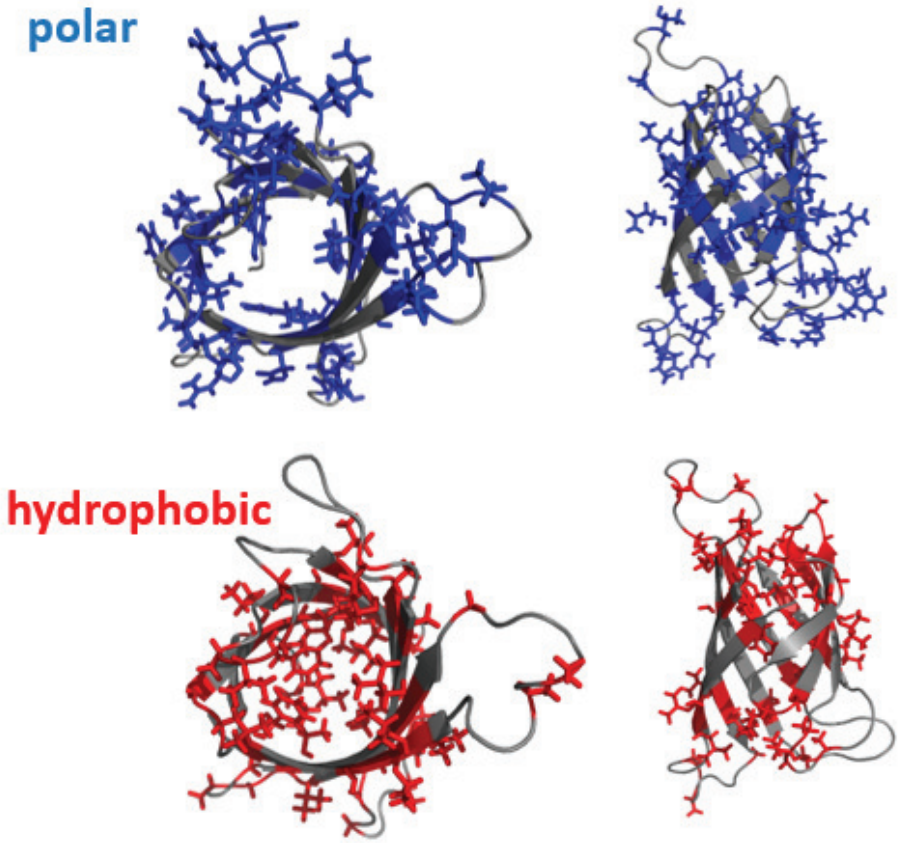

Figure S10: Polar (blue) and hydrophobic residues (red) of Streptavidin (gray) monomer.

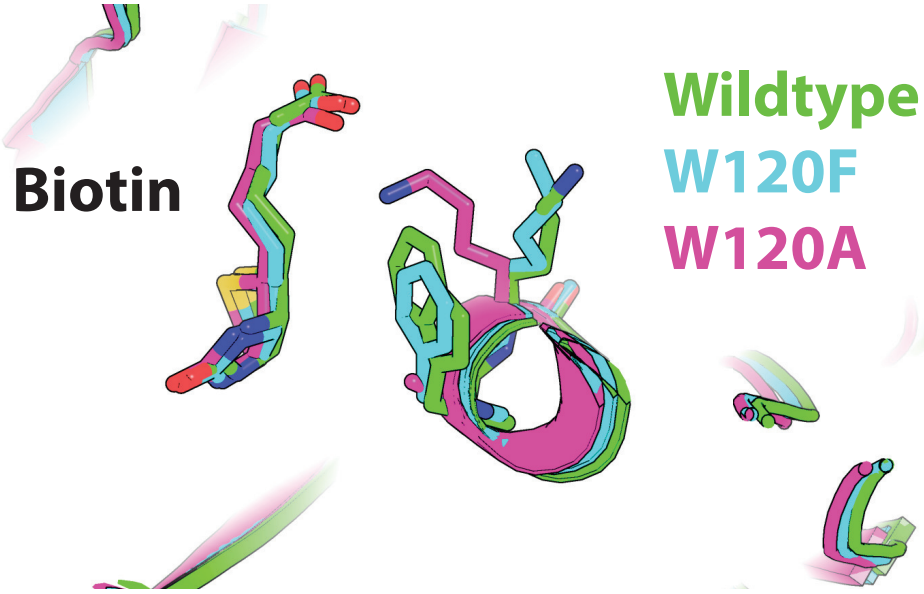

Figure S11: Structure of Streptavidin Mutants Biotin-binding pocket.

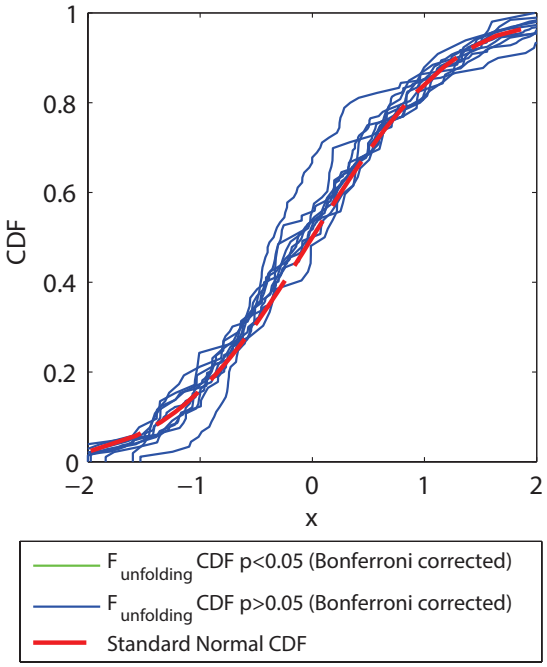

Figure S12: Tests of the standard normal distribution (Kolmogorov-Smirnov test) for each of the unfolding distributions in Fig. S4. All distributions were found to be unable to reject the null hypothesis that they deviate from normality.

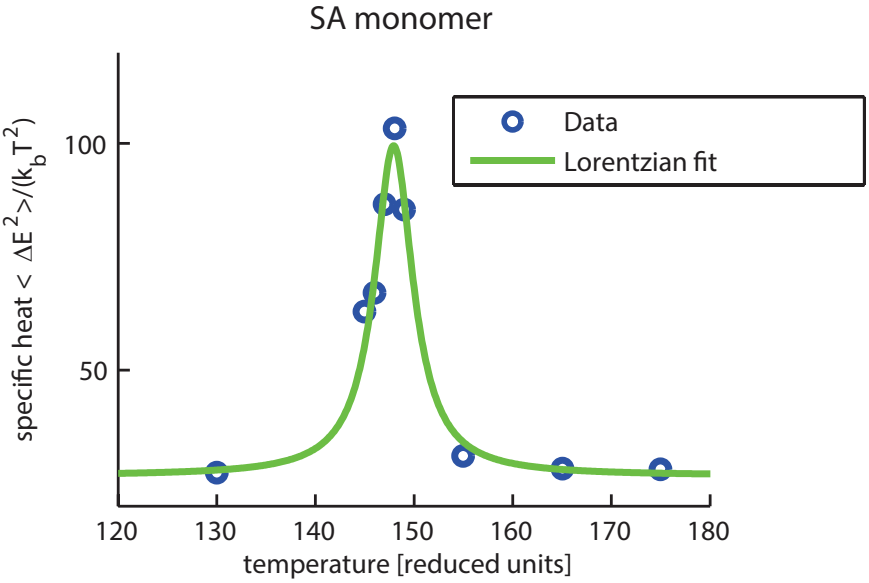

Figure S13: Specific heat calculations for simulations of the coarse-grain model of the Streptavidin monomer had a maximum at T=148.

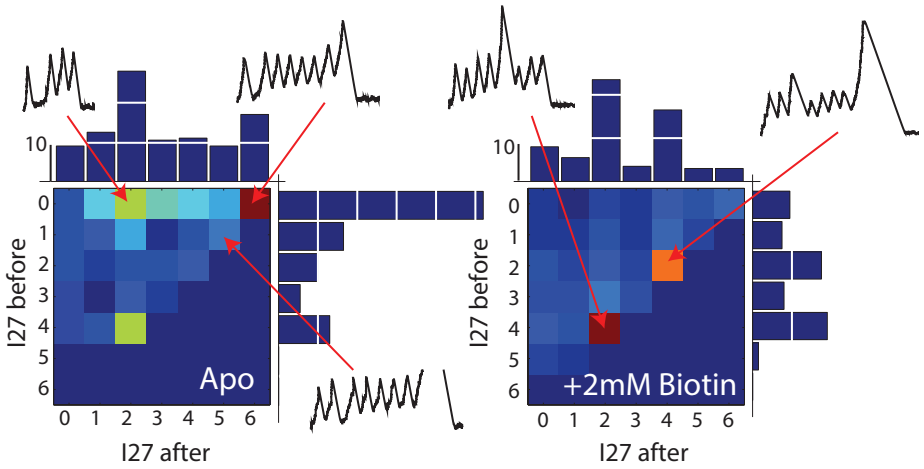

Figure S14: Anisotropy of SA peaks. We found that, with biotin, SA mostly unfolded after either two I27 domains, or after four I27 domains (Fig. S2, left) whereas the order of unfolding should follow the order of mechanical stabilities in a covalently tethered molecule. Since this construct contains SA flanked by two I27 domains on the N-terminal side and four I27 domains on the C-terminal side, this unfolding pattern implies that, with biotin, only half of the protein construct is available for forced unfolding. It seems likely then, the effect is simply due to the stabilized tetramerization which allow other handles to stick nonspecifically to the surface and effectively transmit the force from the tip through the handles instead of the end of the molecule. Once the SA unfolds, the tetramerization is destroyed and force transmits through the other handle (Fig. S8).