Supporting Information

Guzmán et al. 10.1073/pnas.0905796107

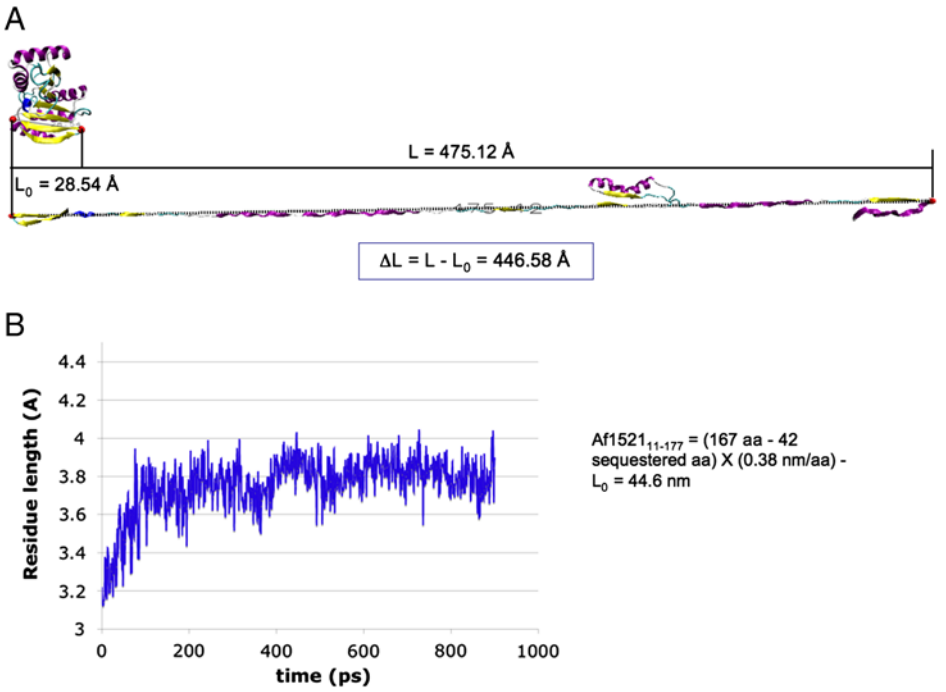

Fig. S1. Calculating contour length of $Af1521_{11–177}$ from simulations. (*A*) Distance between anchoring residues of folded protein L_0 and the length of unfolded protein with intact disulfide C104–C147 (sequestering 42 residues) L; (*B*) plot of the length of a single residue over time shows the average length at 3.8 Å, which was used in calculating the contour length of the protein.

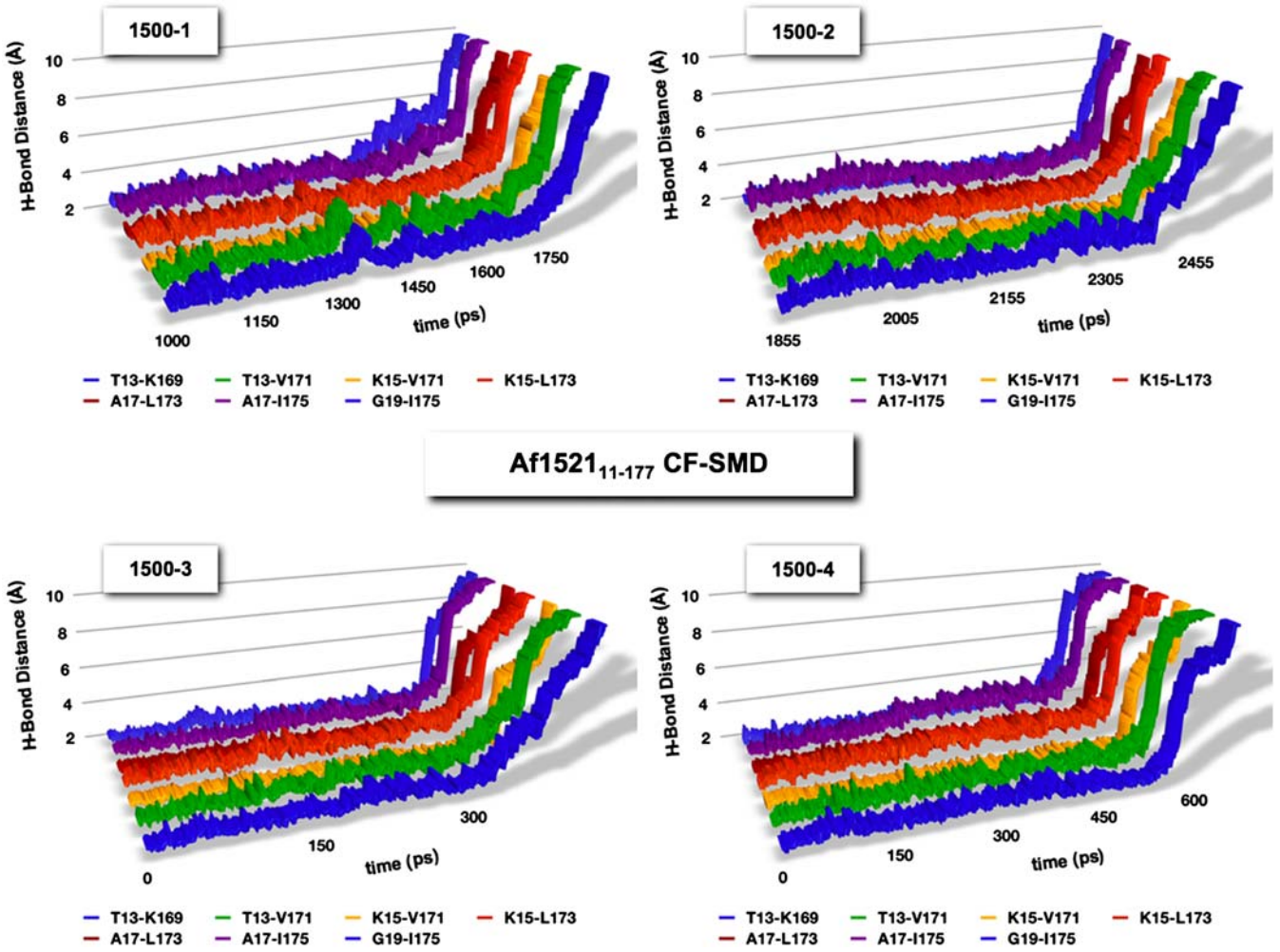

Fig. S2. Af1521$_{11–177}$ distance-time profiles for CF-1500 simulations depict similar unfolding patterns. The seven hydrogen bonds connecting strands 2 and 7 were monitored over time. A distance of 5 Å indicates that the bonds are fully dissociated.

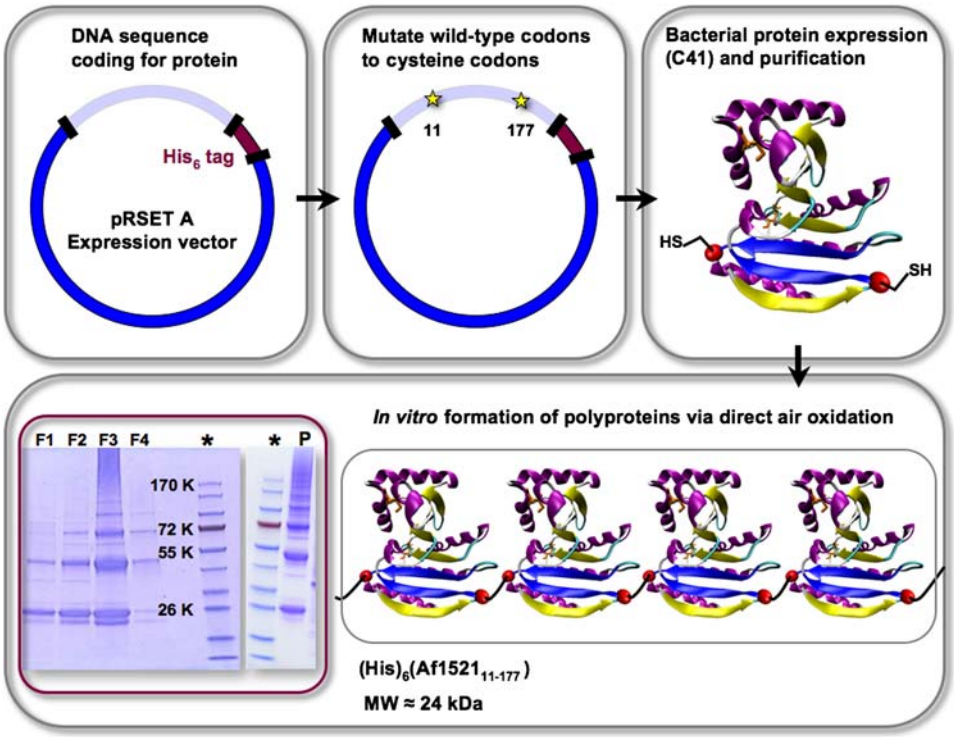

Fig. S3. Cysteine engineering of polyprotein $(Af1521_{11-177})_n$. SDS-PAGE analysis showing fractions (F1, F2, etc.) from nickel-affinity purification, which already exhibits growing chains of the polyprotein. The molecular weight of the protein is ~24 kDa. The asterisks (*) indicate the molecular weight ladder, and P is the polyprotein after ~80 hr of exposure to air at room temperature. Figure redrawn from ref. 1.

1. Dietz H, et al. (2006) Cysteine engineering of polyproteins for single-molecule force spectroscopy. *Nat Protoc* 1:80–84.

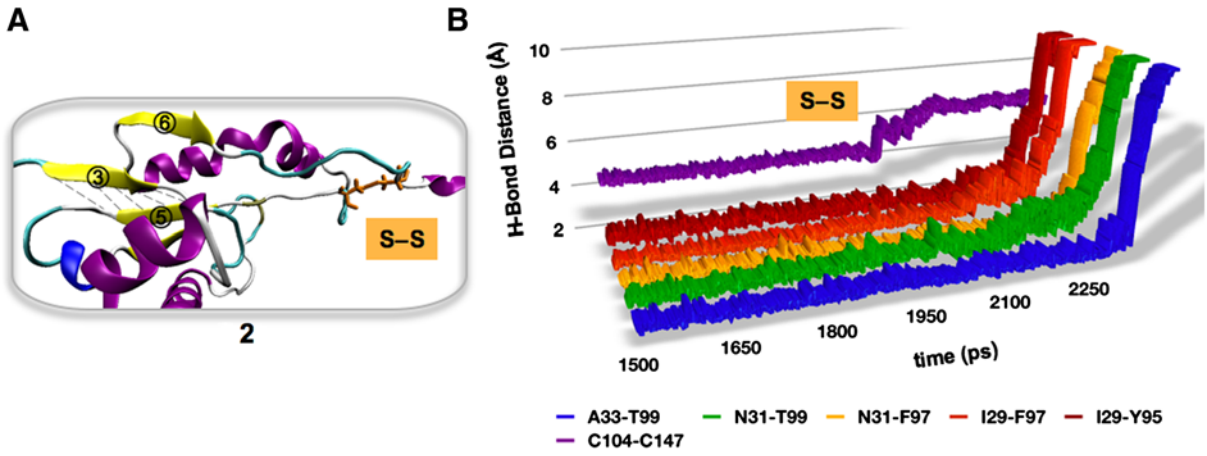

Fig. S4. CF-SMD of $Af1521_{11-177}$ at constant force 1,500 pN. (*A*) Intermediate structure **2** is formed by a fully extended disulfide bond and five sacrificial hydrogen bonds between β-strands 3 and 5. (*B*) Plot of H-bond distance and disulfide bond distance (C104–C147) over time illustrates that the disulfide bond becomes strained prior to rupture of the five hydrogen bonds.

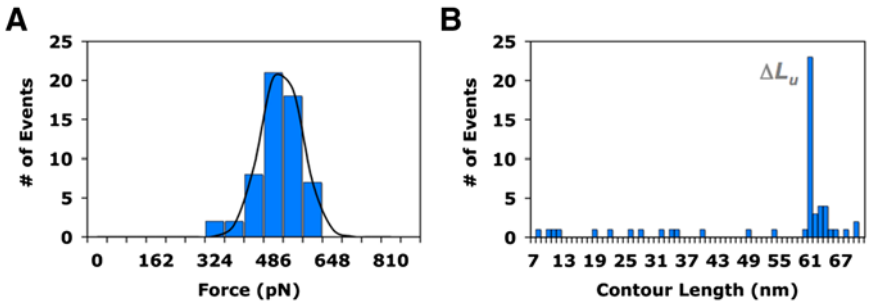

Fig. S5. AFM single-molecule force spectroscopy data for -ssAf1521$_{11–177}$ (a "reduced" version of Af1521$_{11–177}$ in which the internal disulfide C104–C147 is substituted by alanine residues). (*A*) Force distribution is unaffected by the amino acid substitutions, displaying a most probable force similar to that of the domain with intact disulfide at 1, 000 nm s^{-1}. (*B*) Contour length matches the calculated length gain for the unfolded protein without sequestered amino acids [167 aa $\times$ (0.38 nm/aa) $-$ L$_0$ $=$ 61 nm].

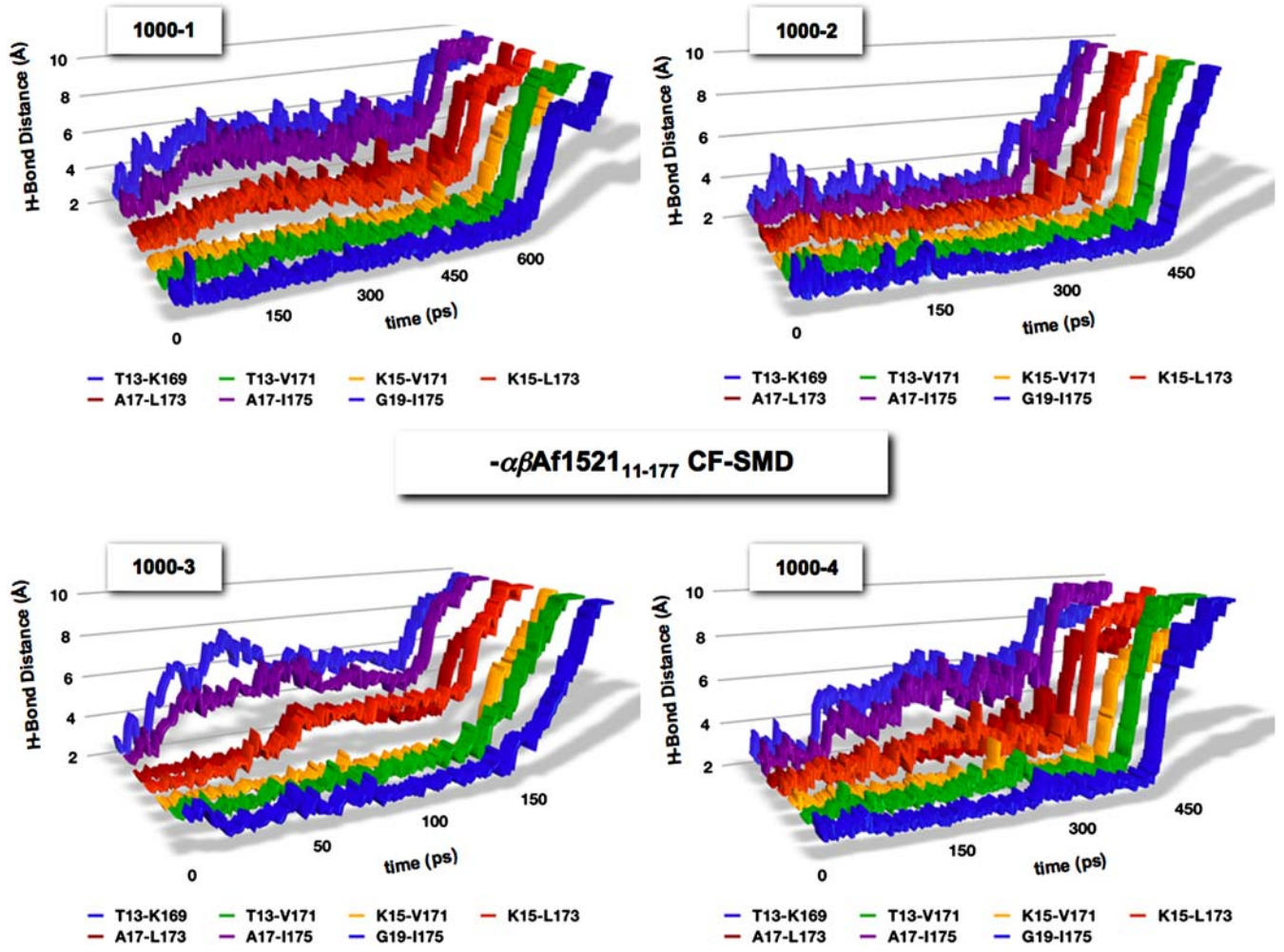

Fig. S6. -$\beta\alpha$Af1521$_{11–177}$ distance-time profiles for CF-1000 simulations depict similar unfolding patterns where bonds G19—I175 and A17—I175 are broken prematurely.

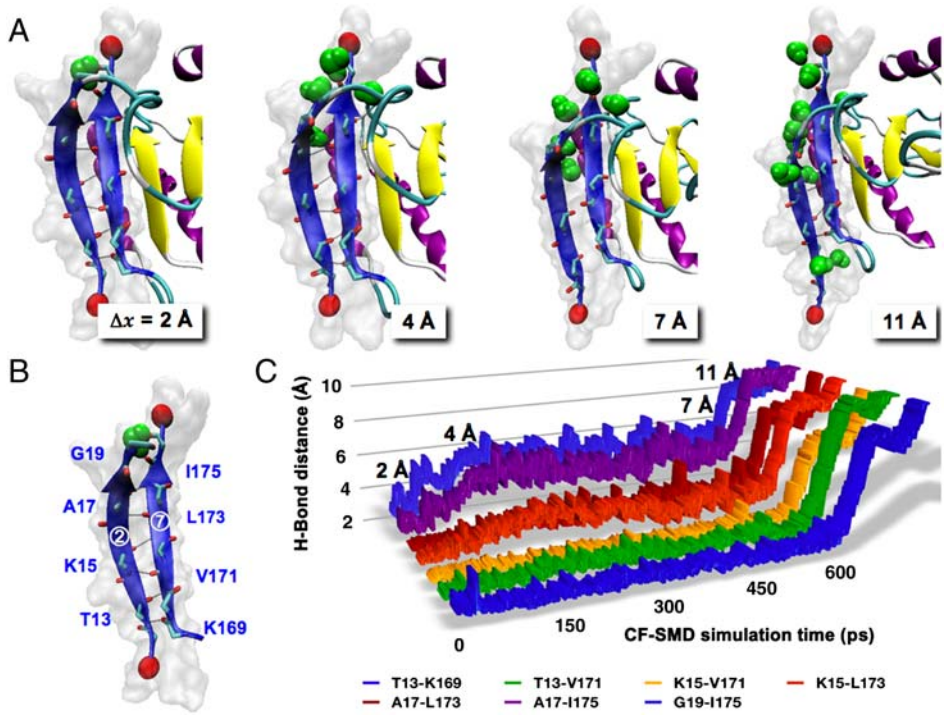

Fig. S7. CF-1000-1 trajectory analysis for -$\beta\alpha$Af1521$_{11–177}$. (*A*) Representative snapshots illustrating water interaction with bond-breaking events between strands 2 and 7 at $\Delta x = 2$–11 Å; water molecules are shown in green; (*B*) load-bearing strands depicting amino acids; and (*C*) distance-time plot of the seven load-bearing H bonds.

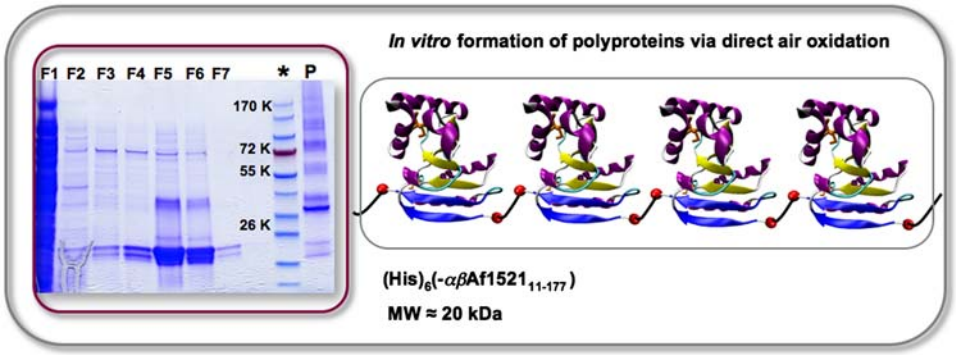

Fig. S8. Cysteine engineering of polyprotein $(\text{-}\beta\alpha\text{Af1521}_{11–177})_n$. SDS-PAGE analysis showing fractions (F1, F2, etc.) from nickel-affinity purification, which already exhibits growing chains of the polyprotein. The molecular weight of the protein is ~20 kDa. The asterisks (*) indicate the molecular weight ladder, and P is the polyprotein after ~80 hr of exposure to air at room temperature.

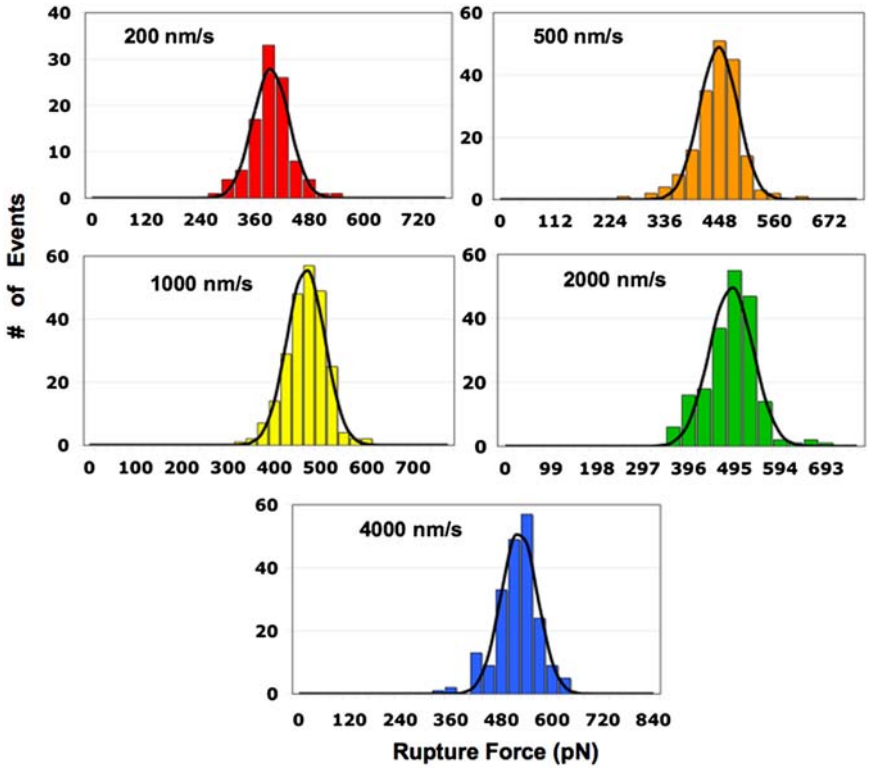

Fig. S9. AFM force distribution histograms for $Af1521_{11–177}$ at various pulling speeds.

Table S1. Structural categories of 134 proteins resulting from bioinformatics screening

(i) β-sandwich*		(ii) β-grasp†		(iii) Neither‡
1tiu_a	1byo_A	1e0z_A	1wmh_A	1pqe_A
1u2h_A	1teg_A	1rfk_A	1p1a_A	1mgr_A
1fhg_A	1tef_A	1e10_A	1t0y_A2	1tq1_A
1x44_A	1ag6_a	1doi_a	1ef5_A	1vaz_A
1wit_a	1oow_A	1iue_A	1m94_A	**2bfr_A (Af1521)**
2ncm_a	1plb_a	1awd_a	1aar_A	2cu6_A
2cqv_A	1byp_A	1j7b_A	2bgf_A	
2imn_a	9pcy_a	1qoa_A	2faz_A	
1efq_A	1suh_a	1j7c_A	2bwf_A	
1ci5_A	1acz_a	1qog_A	1tbe_A	
2imm_a	1kum_a	1j7a_A	1f2r_C	
1xau_A	2c3x_A	1frd_a	1wv8_A	
3lve_a	1cqy_A	1frr_A	1vjk_A	
1lve_a	1ohz_A	1a70_a	1sif_A	
1t6w_A	1lmi_A	1fxa_A	1u4a_A	
1hcv_a	1uwf_A	1qob_A	1c9f_A	
5lve_A	1sp0_A	1qof_A	1rlf_a	
1sjx_A	1tyj_A	1wju_A	1p0r_A	
2rhe_a		1off_A	1wm2_A	
1hkf_A		1wjn_A	1l2n_A	
1i9e_A		1wgd_A	1k52_A	
1neu_a		1wri_A	1k53_A	
1npu_A		1rax_A	1hz5_A	
1o7s_A		2asq_B	1hz6_A	
1py9_A		1lxd_A	1kh0_A	
1od9_A		1km7_A	2igg_a2	
1ehx_A		2rgf_a	1mhx_A	
1pd6_A		1v6e_A	1mi0_A	
1nko_A		1wy8_A	1gb4_a	
1rsf_A		1wxv_A	1fcl_A	
1pko_A		1q1o_A	3gb1_A	
1o7v_A		1l7y_A	1em7_A	
1xt5_A		1iyf_A	1qkz_A	
1cdb_a		1v5t_A	1p7f_A	
1nci_A		1h8c_A	1fcc_C	
1m1s_A		1j0g_A	1igc_A	
1gxe_A		1wh3_A		
1plc_a		1jru_A		

*Similar to β-sandwich using a TM score of 0.50 as cutoff and 1tiu as representative.

†Similar to β-grasp using a TM score of 0.50 as cutoff and 1igc_A as representative.

‡Similar to neither using a TM score of 0.50 as cutoff.