Complete dissociation of the HIV-1 gp41 ectodomain and membrane proximal regions upon phospholipid binding

Julien Roche[1], John M. Louis[1], Annie Aniana[1], Rodolfo Ghirlando[2] and Ad Bax[1]

[1]Laboratory of Chemical Physics and [2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

SUPPORTING INFORMATION

SI Materials and Methods

Circular Dichroism. CD spectra were recorded at 310 K in 50 mM sodium acetate buffer at pH 4.0 on a JASCO J-810 spectropolarimeter using a 0.01 cm path length cell. The CD spectra of $Core^{S\ 35\text{-}144}$ and $Core^{S\ 17\text{-}172}$ were recorded at a protein concentration of 15 µM with 10 mM DPC. Alpha-helical content was determined using the CDNN program (Bohm et al. 1992).

Sedimentation velocity. Sedimentation velocity experiments were conducted at 50,000 rpm and 20°C on a Beckman Coulter ProteomeLab XL-I analytical ultracentrifuge following standard protocols (Zhao et al. 2013). Samples of $Core^{S\ 35\text{-}144}$, $Core^{1L\ 35\text{-}144}$ and $Core^{S\ 17\text{-}172}$ in 50 mM sodium acetate, pH 4 with and without DPC (as described in Table S1) were loaded in 2-channel centerpiece cells and scans were collected using both the absorbance (280 nm) and Rayleigh interference (655 nm) optical detection systems. High concentration samples were loaded into 3 mm path-length cells, whereas low concentration samples were loaded into standard 12 mm path-length cells. In all cases a DPC free matching buffer was used as a reference. Sedimentation data were time-corrected (Ghirlando et al. 2013) and analyzed in SEDFIT 14.4f (Schuck 2000) in terms of a continuous c(*s*) distribution of Lamm equation solutions with a resolution of 0.05 S and a maximum entropy regularization confidence level of 0.68. To account for the presence of the DPC detergent, interference data collected in the presence of DPC were analyzed in terms of a single species representing the detergent micelle and a non-overlapping continuous *c(s)* distribution. In all cases, excellent fits were obtained with best-fit absorbance and interference rmsd. values ranging from 0.0034 – 0.0060 A_{280} and 0.0028 – 0.014 fringes. Solution densities ρ and viscosities η were calculated in SEDNTERP (Cole et al. 2008) (http://sednterp.unh.edu/). The protein partial specific volume *v* and absorbance extinction coefficient (ε_{280}) were calculated in SEDNTERP based on the amino acid composition. The partial specific volume was corrected for the ^{2}H, ^{15}N and ^{13}C isotopic composition. The interference signal increment (ε_J) for the protein was calculated in SEDFIT 14.4f based on the amino acid composition (Zhao et al. 2011). A partial specific volume of 0.937 cm^3g^{-1} was used for DPC (le Maire et al. 2000), and the corresponding interference signal increment (ε_J) of 750 fringes $M^{-1}cm^{-1}$ was based on a refractive index increment (*dn/dc*) of 0.1398 cm^3g^{-1} (https://www.anatrace.com/Products/Lipids/FOS-CHOLINE/F308.aspx). Sedimentation coefficients *s* were corrected to $s_{20,w}$ using partial specific volumes based on the complex protein:detergent stoichiometry. The experimental protein:detergent stoichiometry was based on the integrated absorbance (protein alone) and interference (protein and detergent) signal for the major species observed in the *c(s)* distribution (Inagaki et al. 2013). This stoichiometry was used to calculate the partial specific volume of the sedimenting protein-detergent complex and subsequently to derive its molar mass.

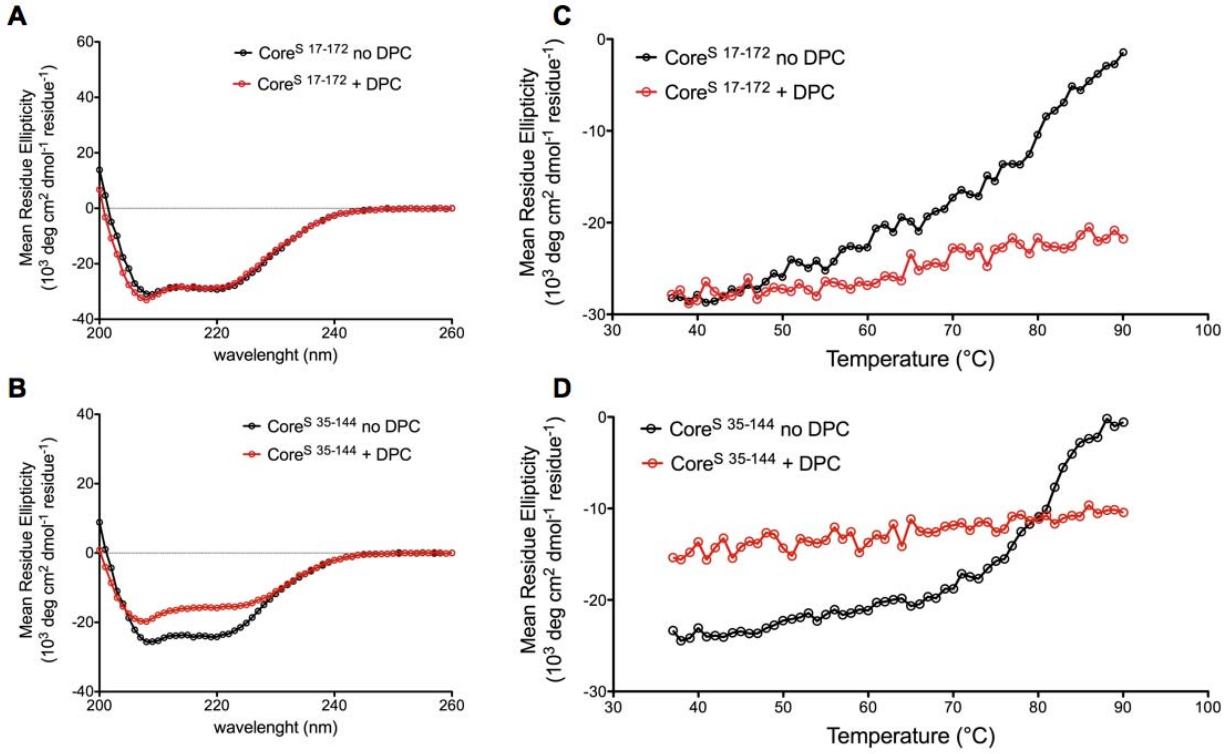

Figure S1: Secondary structure and thermostability of the ectodomain constructs. CD spectra of (A) Core$^{S\ 17-172}$ and (B) Core$^{S\ 35-144}$ recorded at 37°C in the absence of detergent (black) and in the presence of 10 mM DPC (red). Mean residue ellipticity measured at 222 nm between 37°C and 90°C for (C) Core$^{S\ 17-172}$ and (D) Core$^{S\ 35-144}$ in the absence of detergent (black) and in the presence of 10 mM DPC (red).

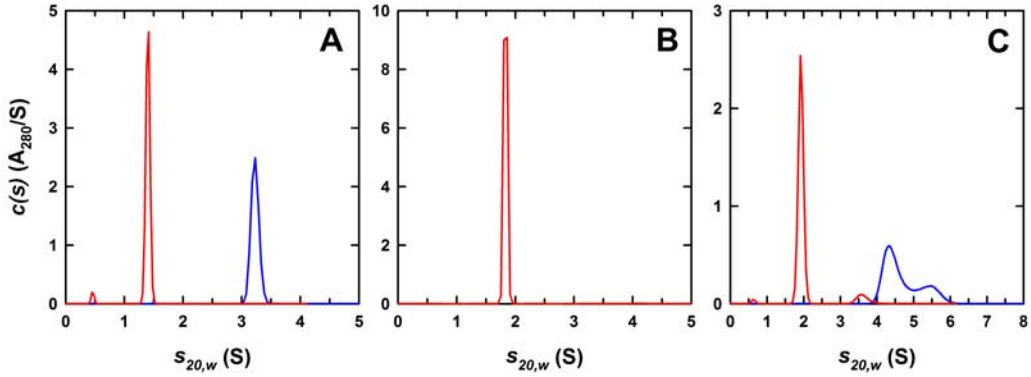

Figure S2: Sedimentation velocity absorbance *c(s)* distributions for (A) Core$^{S\ 35-144}$, (B) Core$^{1L\ 35-144}$ and (C) Core$^{S\ 17-172}$ in the absence of DPC (blue curves) and presence of at least one molar equivalent of DPC micelles (red curve). (A) Distributions for 22 µM Core$^{S\ 35-144}$ (blue) and 22 µM Core$^{S\ 35-144}$ with 10 mM DPC (red), (B) Distribution for 22 µM Core$^{1L\ 35-144}$ with 10 mM DPC (red) and (C) Distributions for 10 µM Core$^{S\ 17-172}$ (blue) and 10 µM Core$^{S\ 17-172}$ with 10 mM DPC (red). Similar profiles were observed using the interference optics, except for the presence of free DPC micelles when detergent was added.

Table S1. Summary of the sedimentation velocity experiments carried out on various constructs

Construct	Protein (μM)	DPC (mM)	protein:micelle ratio	$M_{experimental}$ (kDa) (Sedimentation coefficient (S))	$M_{calculated}$ (kDa)	Major species
$Core^{S\ 35\text{-}144}$	10	-	-	32.1 ± 2.0 (3.22 ± 0.02 S)	9166	trimer
$Core^{S\ 35\text{-}144}$	10	2.15	1:1	31.4 ± 2.9 (1.42 ± 0.03 S)	9166	monomer + 55 DPC
$Core^{S\ 35\text{-}144}$	22	-	-	29.2 ± 0.8 (3.24 ± 0.01 S)	9166	trimer
$Core^{S\ 35\text{-}144}$	110	-	-	28.7 ± 0.1 (3.12 ± 0.01 S)[a]	9166	trimer
$Core^{S\ 35\text{-}144}$	22	10	1:6	30.4 ± 2.4 (1.42 ± 0.02 S)	9166	monomer + 55 DPC
DPC	-	10	-	24.2 (0.55 S)[b]	352	69 DPC
$Core^{IL\ 35\text{-}144}$	22	10	1:6	40.4 ± 1.2 (1.84 ± 0.01 S)	16585	monomer + 63 DPC
$Core^{S\ 17\text{-}172}$	10	-	-	46.5 / 63.0 (4.44 / 5.44 S)[e]	14825	trimer/tetramer
$Core^{S\ 17\text{-}172}$	10	10	1:13	40.5 ± 2.6 (1.94 ± 0.01 S)	14825	monomer + 73 DPC

(a) Due to the higher loading concentration, a slightly smaller sedimentation coefficient is determined reflecting repulsive non-ideality.
(b) A partial specific volume of 0.937 mL/g was used for DPC in these studies.
(c) The *c(s)* distribution showed the presence of two sedimenting species, simply interpreted in terms of a mixture of trimers and tetramers. As data were only collected at a single concentration, a reversible trimer to higher order aggregate self-association cannot be ruled out. In such a case, and in the presence of fast exchange kinetics, the faster sedimenting species would represent a reaction boundary.

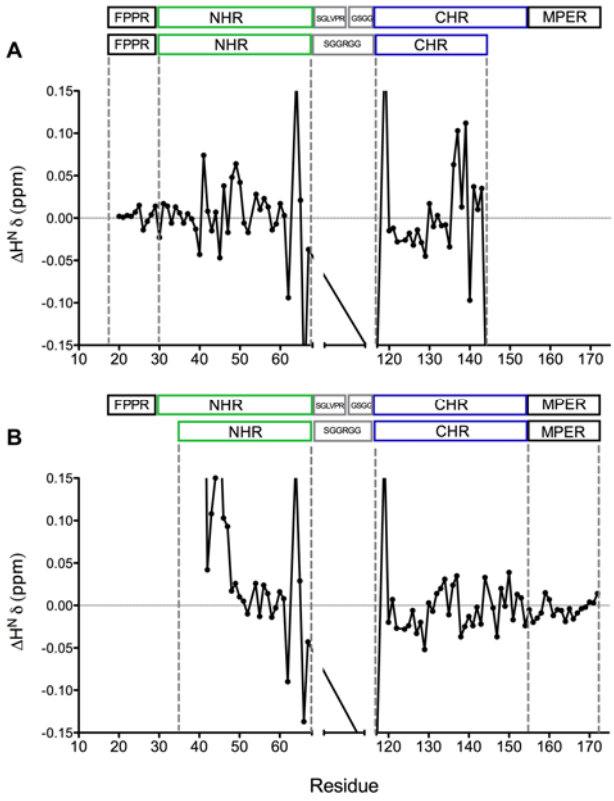

Figure S3: Comparison of the backbone chemical shifts recorded for Core$^{S\ 17\text{-}144}$, Core$^{S\ 35\text{-}172}$ and Core$^{SP\ 17\text{-}172}$ in 25 mM sodium phosphate (pH 6.0) in the presence of 100 mM DPC. The length of each region (FPPR, NHR, CHR and MPER), is depicted at the top of each panel (see Material and Methods for details about the Core$^{SP\ 17\text{-}172}$ construct). (A) Backbone amide proton chemical shifts difference between Core$^{S\ 17\text{-}144}$ and Core$^{SP\ 17\text{-}172}$. (B) Backbone amide proton chemical shifts difference between Core$^{S\ 35\text{-}172}$ and Core$^{SP\ 17\text{-}172}$. Note that the NHR and CHR helices of Core$^{SP\ 17\text{-}172}$ each extend by three additional residues towards IL, and also contain several terminal residues resulting from the thrombin cleavage site (See Fig. 2F, main text).

Table S2: Backbone chemical shifts measured in presence of 100 mM DPC at 310 K, for Core$^{S\ 17\text{-}172}$, Core$^{S\ 17\text{-}144}$ and Core$^{S\ 35\text{-}172}$ in 50 mM sodium acetate pH 4.0, and for Core$^{SP\ 17\text{-}172}$, Core$^{S\ 17\text{-}144}$ and Core$^{S\ 35\text{-}172}$ in 25 mM sodium phosphate pH 6.0. [a]

	17-172 ^{15}N pH 4.0	17-172 H^N pH 4.0	17-172 $^{13}C^\alpha$ pH 4.0	17-144 ^{15}N pH 4.0	17-144 H^N pH 4.0	17-144 $^{13}C^\alpha$ pH 4.0	35-172 ^{15}N pH 4.0	35-172 H^N pH 4.0	17-144 $^{13}C^\alpha$ pH 4.0	17-172SP ^{15}N pH 6.0	17-172SP H^N pH 6.0	17-144 ^{15}N pH 6.0	17-144 H^N pH 6.0	35-172 ^{15}N pH 6.0	17-144 H^N pH 6.0
T529	115.88	8.527	62.15	115.97	8.538	62.13									
M530	123.01	8.444	55.64	123.03	8.460	55.62									
G531	110.42	8.269	45.11	110.45	8.286	45.10				110.19	8.279	110.32	8.281		
A532	124.51	8.099	52.62	124.50	8.116	52.62				124.51	8.113	124.51	8.114		
A533	122.75	8.214	52.69	122.73	8.231	52.69				122.47	8.228	122.56	8.231		
S534	114.49	8.047	58.71	114.49	8.061	58.73				114.30	8.055	114.38	8.057		
M535	122.50	8.133	55.69	122.51	8.142	55.71				122.41	8.112	122.43	8.119		
T536	115.27	7.869	62.26	115.27	7.884	62.31				115.26	7.876	115.28	7.891		
L537	123.29	8.456	56.75	123.20	8.458	56.74				122.98	8.447	123.07	8.433		
T538	112.72	8.019	64.76	112.77	8.019	64.77				112.53	8.018	112.63	8.014		
V539	122.02	7.653	65.67	121.98	7.671	65.70				122.08	7.620	122.17	7.624		
Q540	120.19	8.146	58.47	120.12	8.141	58.49				119.76	8.107	119.73	8.121		
A541	122.30	8.418	54.97	122.35	8.426	54.97				122.20	8.465	122.25	8.442		
R542	117.32	8.100	59.60	117.23	8.111	59.65				117.26	8.093	117.32	8.110		
Q543	118.98	7.980	58.39	119.04	8.025	58.40				119.20	7.998	119.22	8.012		
L544	121.82	7.961	57.49	121.82	7.972	57.51				121.74	7.974	121.75	7.968		
L545	118.23	8.110	57.09	118.25	8.125	57.14				118.14	8.102	118.18	8.115		
S546	113.83	8.044	61.32	113.79	8.061	61.35				113.84	8.049	113.82	8.055		
G547	108.70	7.906	46.36	108.73	7.909	46.40	110.44	8.597	44.83	108.60	7.906	108.57	7.900		
I548	121.16	7.759	63.74	121.22	7.778	63.82	121.15	8.182	61.41	121.06	7.745	121.06	7.750	121.12	8.197
V549	117.74	7.807	64.88	117.68	7.798	64.97	123.79	8.050	62.60	117.69	7.809	117.64	7.808	123.53	8.047
Q550	119.46	7.767	57.40	119.34	7.757	57.45	123.86	8.245	56.04	118.61	7.844	118.50	7.831	123.68	8.250
Q551	119.36	7.713	55.74	119.19	7.687	55.62	121.31	8.230	55.99	117.57	7.756	117.72	7.713	121.20	8.227
Q552	119.59	7.949	57.32	119.70	7.934	57.38	121.22	8.290	56.20	119.63	7.781	119.69	7.855	120.24	8.296
N553	118.58	8.200	55.44	118.43	8.238	55.52	120.08	8.341	54.00	118.38	8.276	118.31	8.284	118.84	8.318
N554	118.76	8.186	55.00	118.73	8.123	54.97	120.54	8.398	54.75	119.61	8.303	119.75	8.288	120.26	8.411
L555	122.67	8.122	57.69	122.65	8.134	57.69	123.22	8.284	57.67	122.26	8.085	122.31	8.092	122.81	8.235
L556	118.75	8.097	57.73	118.73	8.062	57.73	118.84	7.975	57.54	118.54	7.999	118.65	7.952	118.50	8.231
R557	117.63	7.792	58.67	117.51	7.773	58.66	117.90	7.833	58.53	117.72	7.708	117.60	7.746	117.76	7.811
A558	121.98	7.665	54.51	121.89	7.667	54.48	121.97	7.723	54.50	122.22	7.685	122.04	7.668	122.32	7.778
I559	118.04	7.996	64.36	118.11	8.016	64.36	118.06	7.947	64.27	117.48	7.867	117.60	7.915	117.55	7.884
E560	117.59	8.119	57.97	117.43	8.114	57.91	117.77	8.100	57.98	119.52	8.046	119.58	8.110	117.60	8.072
A561	119.84	7.507	52.72	119.72	7.435	52.62	119.89	7.516	52.82	120.07	7.583	120.15	7.625	120.17	7.593
Q562	118.66	7.675	54.85	118.78	7.650	54.82	118.90	7.694	54.97	118.21	7.819	118.17	7.813	118.32	7.824
Q563	120.58	8.002	58.35	120.78	8.027	58.27	120.61	7.985	58.40	120.48	7.991	120.57	7.974	120.52	7.981
H564	117.55	8.656	56.98	117.46	8.697	56.88	117.66	8.651	57.10						
L565	120.46	8.088	57.77	120.43	8.017	57.74	120.47	8.054	57.74	120.31	7.922	120.37	7.950	120.43	7.948
L566	121.98	8.438	58.15	122.00	8.461	58.18	121.96	8.403	58.17	122.30	8.363	122.12	8.373	122.25	8.350
Q567	118.53	8.080	58.90	118.49	8.083	58.87	118.52	8.076	58.85	118.77	8.018	118.86	8.041	118.82	8.042
L568	120.75	8.018	57.65	120.90	8.039	57.63	120.80	8.006	57.65	120.93	7.990	120.83	8.003	120.90	8.004
T569	116.71	8.037	64.90	116.77	8.043	64.92	116.71	8.029	64.88	116.60	8.032	116.62	8.018	116.62	8.018
V570	121.14	8.075	66.79	121.18	8.077	66.80	121.18	8.073	66.80	121.20	8.077	121.21	8.070	121.17	8.074
W571	120.59	7.991	61.43	120.57	7.989	61.41	120.61	7.976	61.43	119.89	7.920	119.80	7.937	119.87	7.936
G572	107.86	8.847	47.26	107.92	8.853	47.26	107.90	8.847	47.27	107.85	8.853	107.94	8.856	107.95	8.861
I573	122.31	8.429	64.90	122.31	8.435	64.86	122.33	8.431	64.87	122.42	8.532	122.28	8.438	122.32	8.442
K574	119.89	7.991	59.26	119.90	7.981	59.25	119.92	7.980	59.25			119.88	7.974	119.84	7.963
Q575	118.70	7.842	57.13	118.89	7.812	57.17	118.80	7.810	57.18	118.54	7.633	118.80	7.800	118.89	7.803
L576	120.17	7.870	57.16	120.23	7.880	57.20	120.27	7.880	57.23	120.16	7.846	120.18	7.867	120.25	7.875
Q577	118.91	8.258	58.24	118.88	8.231	58.25	118.87	8.221	58.29	118.73	8.371	118.81	8.161	118.84	8.234
A578	121.51	7.662	53.39	121.36	7.655	53.45	121.40	7.655	53.48	120.09	7.688	121.41	7.651	121.34	7.645
R579	117.73	7.632	56.29	117.58	7.627	56.25	117.61	7.621	56.28			117.72	7.617	117.71	7.602
loopS1	115.07	7.839	58.86	114.98	7.834	58.92	114.93	7.830	58.94			115.07	7.847	114.99	7.844
loopG2	110.37	8.137	45.32	110.37	8.140	45.37	110.37	8.130	45.38			110.45	8.153	110.43	8.152
loopG3	108.87	8.174	45.00	108.84	8.192	44.99	108.87	8.186	45.00			108.92	8.179	108.92	8.186
loopR4	120.73	8.127	56.03	120.67	8.121	56.09	120.69	8.103	56.09			120.67	8.093	120.63	8.080
loopG5	109.48	8.369	45.11	109.40	8.382	45.15	109.37	8.367	45.14			109.67	8.387	109.59	8.382
loopG6	109.10	8.021	45.09	109.08	7.999	45.12	109.09	7.987	45.12			109.07	8.130	109.04	8.124
W628	121.44	8.231	58.65	121.24	8.179	58.57	121.29	8.172	58.59	122.13	8.371	121.23	8.199	121.27	8.179
M629	118.76	8.172	57.46	118.62	8.120	57.49	118.67	8.145	57.48			118.17	8.019	118.86	8.026
E630	119.71	7.850	57.57	119.60	7.853	57.55	119.70	7.845	57.58	120.33	7.612	119.66	7.822	119.70	7.802
W631	121.00	7.838	59.08	120.90	7.834	58.98	121.02	7.826	59.05	120.60	7.868	120.53	7.853	120.56	7.848
D632	118.22	8.330	56.02	118.20	8.332	56.00	118.27	8.335	56.09	119.94	8.145	119.95	8.133	119.80	8.152
R633	119.62	7.860	58.51	119.54	7.867	58.49	119.57	7.848	58.53	119.39	8.051	119.48	8.023	119.67	8.024
E634	118.60	7.884	57.91	118.47	7.879	57.86	118.55	7.871	57.94	119.67	8.37	119.88	8.061	119.34	8.040
I635	118.89	8.210	64.12	118.81	8.212	64.05	118.86	8.213	64.16	119.61	8.295	119.69	8.269	119.46	8.267
N636	119.66	8.091	55.73	119.60	8.093	55.71	119.64	8.091	55.77	120.15	8.183	120.07	8.165	119.89	8.159
N637	118.85	8.042	55.13	118.65	8.031	55.05	118.87	8.028	55.17	118.88	8.039	118.76	8.007	118.90	8.033
Y638	118.58	8.125	60.56	118.49	8.117	60.44	118.57	8.109	60.59	118.86	8.198	118.74	8.184	118.81	8.165
T639	114.44	8.284	66.24	114.53	8.252	66.21	114.44	8.292	66.26	113.93	8.340	114.03	8.311	114.06	8.320
S640	118.30	8.051	61.34	118.34	8.082	61.30	118.24	8.035	61.33	118.34	8.054	118.23	8.009	118.18	8.002
L641	123.97	7.636	57.72	123.94	7.650	57.70	123.97	7.623	57.74	123.70	7.604	123.70	7.621	123.78	7.607
I642	118.82	8.051	64.50	118.80	8.035	64.49	118.87	8.039	64.50	118.36	8.052	118.33	8.042	118.33	8.045

H643	117.81	8.323	59.11	117.82	8.345	59.07	117.84	8.322	59.14	117.91	8.245	117.86	8.248	117.81	8.259
S644	115.38	8.134	61.65	115.34	8.146	61.59	115.41	8.135	61.66	114.95	8.076	114.90	8.067	115.06	8.096
L645	122.57	7.920	57.35	122.48	7.918	57.26	122.62	7.923	57.40	122.08	7.809	121.93	7.801	122.16	7.840
I646	118.13	7.931	63.94	117.93	7.925	63.80	118.24	7.941	64.02	116.20	7.752	116.34	7.718	116.30	7.741
E647	119.53	7.873	57.46	119.59	7.912	57.49	119.49	7.868	57.55	121.40	7.867	121.37	7.930	121.36	7.891
E648	119.30	8.012	56.95	119.03	8.057	57.03	119.07	7.965	56.96	120.31	8.087	120.40	8.190	120.37	8.122
S649	115.44	7.932	59.55	115.30	7.909	59.57	115.40	7.928	59.61	116.03	7.991	116.06	8.004	116.08	7.954
Q650	120.84	7.953	56.61	120.49	7.843	55.78	120.79	7.944	56.71	120.40	7.981	121.03	8.093	120.49	7.956
N651	118.13	8.025	53.75	119.23	8.080	53.26	118.23	8.047	53.81	119.84	8.157	119.41	8.060	119.73	8.144
Q652	120.61	8.057	56.41	120.79	8.165	55.70	120.46	8.045	56.35	120.63	8.145	121.11	8.182	120.59	8.121
Q653	120.29	8.224	57.05	121.15	8.222	55.61	120.19	8.230	57.14	121.09	8.200	121.46	8.210	121.03	8.198
E654	119.90	8.128	57.03	122.36	8.210	55.62	119.92	8.147	57.05	121.78	8.258	123.13	8.293	121.69	8.236
K655	121.28	8.010	57.30	126.99	7.904	56.74	121.18	7.982	57.23	120.54	7.977	127.89	7.802	120.56	8.010
N656			54.08						54.02						
E657	120.54	8.224	57.92				120.49	8.237	58.00	121.55	8.384			121.44	8.381
Q658	119.25	8.179	58.04				119.11	8.164	58.03	120.12	8.206			120.02	8.169
E659	119.56	8.066	58.02				119.46	8.051	57.99	121.28	8.113			121.22	8.133
L660	119.94	7.890	57.11				119.87	7.882	57.09	119.84	7.935			119.78	7.934
L661	118.75	7.800	56.73				118.65	7.769	56.63	118.29	7.750			118.38	7.789
E662	119.15	7.779	57.41				119.07	7.759	57.27	118.59	7.941			118.65	7.924
L663	120.72	7.971	57.33				120.85	7.978	57.33	121.07	7.961			121.04	7.974
D664	117.16	8.241	55.55				117.19	8.256	55.63	119.09	8.358			118.99	8.367
K665	120.05	7.796	58.01				119.98	7.781	57.96	121.30	7.942			121.37	7.918
W666	120.84	7.865	58.58				120.87	7.845	58.54	120.46	7.771			120.42	7.766
A667	121.26	8.130	54.31				121.29	8.122	54.32	121.34	8.100			121.37	8.080
S668	112.52	7.850	60.30				112.51	7.842	60.29	113.06	7.983			113.08	7.968
L669	122.23	7.625	56.42				122.24	7.610	56.41	123.04	7.716			123.00	7.707
W670	118.86	7.629	57.70				118.82	7.607	57.75	118.51	7.887			118.54	7.902
N671	117.54	7.961	53.84				117.57	7.959	53.85	116.37	7.963			116.48	7.970
W672	121.26	8.056	59.05				121.23	8.024	59.03	121.82	8.108			121.86	8.096
F673	118.27	7.847	58.80				118.19	7.823	58.72	117.97	7.838			118.07	7.833
N674	118.51	7.784	53.58				118.51	7.763	53.49	118.25	7.691			118.33	7.685
I675	119.90	7.910	63.14				119.95	7.912	63.13	119.86	7.947			119.86	7.928
T676	113.79	7.884	65.02				113.90	7.864	65.01	114.69	7.811			114.60	7.807
N677	119.50	7.760	54.85				119.51	7.763	54.72	119.46	7.786			119.44	7.770
W678	119.74	7.724	58.69				119.74	7.714	58.64	119.73	7.709			119.70	7.700
L679	118.33	7.786	56.64				118.33	7.747	56.55	118.26	7.764			118.25	7.760
W680	117.53	7.577	58.69				117.61	7.539	58.61	117.92	7.470			117.89	7.468
Y681	116.73	7.436	59.29				116.82	7.419	59.25	116.70	7.389			116.75	7.393
I682	115.99	7.416	60.93				116.05	7.405	60.86	115.59	7.400			115.65	7.403
K683	123.65	7.404	56.06				124.77	7.389	56.26	127.48	7.296			127.56	7.310

[a] Chemical shifts are in ppm relative to DSS (^{1}H, ^{13}C) and liquid ammonia (^{15}N). Residue numbering follows that of full-length gp41 (see Fig 2, main text). The six residues replacing the immunodominant loop (I69-T116) in the Core^{S} constructs are numbered loopS1-loopG6.

Table S3: ^{15}N relaxation rates R_1 and R_2 and heteronuclear ^{15}N-{^{1}H} NOE measured at 600 MHz in the presence of 100 mM DPC for Core$^{S\ 17\text{-}172}$, Core$^{S\ 17\text{-}144}$ and Core$^{S\ 35\text{-}172}$ in 50 mM sodium acetate pH 4.0, at 310 K.

	17-172 R_1 (s^{-1}) 600 MHz	17-172 R_2 (s^{-1}) 600 MHz	17-172 NOE 600 MHz	17-144 R_1 (s^{-1}) 600 MHz	17-144 R_2 (s^{-1}) 600 MHz	17-144 NOE 600 MHz	35-172 R_1 (s^{-1}) 600 MHz	35-172 R_2 (s^{-1}) 600 MHz	35-172 NOE 600 MHz
T529	0.77 (±0.03)	2.02 (±0.04)	-0.94 (±0.01)	0.78 (±0.04)	1.70 (±0.05)	-0.88 (±0.01)			
M530	0.96 (±0.04)	2.65 (±0.03)	-0.73 (±0.01)	0.98 (±0.03)	1.97 (±0.04)	-0.75 (±0.01)			
G531	1.01 (±0.03)	2.95 (±0.06)	-0.52 (±0.03)	1.03 (±0.03)	2.29 (±0.05)	-0.51 (±0.02)			
A532	1.11 (±0.05)	3.20 (±0.05)	-0.25 (±0.02)	1.13 (±0.04)	2.78 (±0.06)	-0.23 (±0.01)			
A533	1.15 (±0.05)	4.14 (±0.08)	-0.11 (±0.01)	1.18 (±0.05)	3.42 (±0.07)	-0.09 (±0.01)			
S534	1.19 (±0.06)	4.55 (±0.08)	-0.01 (±0.01)	1.24 (±0.07)	3.74 (±0.08)	0.02 (±0.01)			
M535	1.27 (±0.04)	5.45 (±0.10)	0.23 (±0.02)	1.35 (±0.05)	4.99 (±0.09)	0.31 (±0.02)			
T536	1.30 (±0.05)	7.92 (±0.08)	0.37 (±0.01)	1.38 (±0.06)	6.41 (±0.08)	0.40 (±0.01)			
L537	1.22 (±0.06)	8.53 (±0.06)	0.57 (±0.02)	1.37 (±0.08)	7.72 (±0.07)	0.53 (±0.01)			
T538	1.24 (±0.08)	10.39 (±0.04)	0.61 (±0.02)	1.33 (±0.09)	10.00 (±0.05)	0.62 (±0.01)			
V539	1.19 (±0.06)	12.55 (±0.07)	0.68 (±0.03)	1.33 (±0.06)	9.86 (±0.08)	0.69 (±0.02)			
Q540	1.25 (±0.06)	13.38 (±0.08)	0.67 (±0.02)	1.37 (±0.06)	10.40 (±0.08)	0.67 (±0.02)			
A541	1.24 (±0.05)	13.58 (±0.09)	0.73 (±0.01)	1.34 (±0.06)	11.87 (±0.09)	0.75 (±0.01)			
R542	1.19 (±0.07)	12.70 (±0.08)	0.69 (±0.01)	1.33 (±0.07)	11.39 (±0.07)	0.71 (±0.01)			
Q543	1.22 (±0.05)	13.82 (±0.07)	0.69 (±0.02)	1.32 (±0.09)	10.95 (±0.06)	0.70 (±0.01)			
L544	1.22 (±0.06)	13.98 (±0.06)	0.71 (±0.01)	1.33 (±0.08)	10.88 (±0.06)	0.71 (±0.02)			
L545	1.18 (±0.04)	14.33 (±0.09)	0.68 (±0.02)	1.32 (±0.07)	11.12 (±0.10)	0.70 (±0.01)			
S546	1.14 (±0.06)	14.35 (±0.08)	0.68 (±0.01)	1.26 (±0.06)	11.06 (±0.09)	0.68 (±0.01)			
G547	1.21(±0.07)	14.63 (±0.09)	0.65 (±0.01)	1.33 (±0.07)	10.69 (±0.08)	0.64 (±0.02)	0.65 (±0.04)	1.88 (±0.06)	-0.98 (±0.01)
I548	1.16 (±0.07)	14.25 (±0.09)	0.64 (±0.03)	1.28 (±0.05)	10.84 (±0.09)	0.66 (±0.03)	0.98 (±0.03)	2.60 (±0.07)	-0.40 (±0.01)
V549	1.12 (±0.08)	14.55 (±0.07)	0.66 (±0.01)	1.26 (±0.06)	11.55 (±0.08)	0.66 (±0.01)	1.13 (±0.04)	3.93 (±0.06)	-0.36 (±0.02)
Q550	1.22 (±0.09)	15.07 (±0.08)	0.60 (±0.01)	1.33 (±0.09)	10.48 (±0.07)	0.62 (±0.01)	1.20 (±0.06)	4.16 (±0.08)	-0.26 (±0.01)
Q551	1.18 (±0.08)	14.01 (±0.09)	0.63 (±0.02)	1.28 (±0.08)	9.58 (±0.08)	0.58 (±0.01)	1.28 (±0.05)	4.41 (±0.08)	-0.06 (±0.02)
Q552	1.18 (±0.09)	13.73 (±0.10)	0.56 (±0.02)	1.29 (±0.06)	10.01 (±0.10)	0.59 (±0.02)	1.34 (±0.07)	5.08 (±0.09)	0.12 (±0.02)
N553	1.16 (±0.06)	13.68 (±0.08)	0.55 (±0.02)	1.28 (±0.07)	9.69 (±0.09)	0.58 (±0.01)	1.39 (±0.06	6.13 (±0.08)	0.32 (±0.02)
N554	1.14 (±0.07)	13.83 (±0.07)	0.62 (±0.01)	1.26 (±0.06)	10.82 (±0.07)	0.65 (±0.01)	1.37 (±0.07)	7.76 (±0.07)	0.48 (±0.01)
L555	1.12 (±0.06)	15.14 (±0.06)	0.68 (±0.02)	1.22 (±0.06)	11.26 (±0.07)	0.66 (±0.01)	1.32 (±0.06)	10.02 (±0.07)	0.61 (±0.01)
L556	1.11 (±0.09)	15.57 (±0.08)	0.69 (±0.01)	1.26 (±0.07)	11.17 (±0.08)	0.67 (±0.01)	1.29 (±0.07)	10.83 (±0.08)	0.64 (±0.02)
R557	1.15 (±0.06)	14.77 (±0.09)	0.66 (±0.02)	1.28 (±0.06)	11.20 (±0.08)	0.72 (±0.02)	1.29 (±0.07)	11.29 (±0.08)	0.68 (±0.01)
A558	1.12 (±0.10)	15.54 (±0.09)	0.68 (±0.03)	1.28 (±0.09)	11.97 (±0.09)	0.72 (±0.02)	1.34 (±0.09)	10.90 (±0.09)	0.70 (±0.02)
I559	1.10 (±0.08)	16.06 (±0.08)	0.70 (±0.01)	1.24 (±0.08)	11.77 (±0.08)	0.71 (±0.01)	1.28 (±0.08)	10.87 (±0.07)	0.68 (±0.01)
E560	1.11 (±0.09)	17.70 (±0.10)	0.70 (±0.02)	1.28 (±0.08)	12.08 (±0.09)	0.71 (±0.01)	1.29 (±0.08)	12.12 (±0.10)	0.69 (±0.01)
A561	1.14 (±0.07)	17.85 (±0.08)	0.67 (±0.01)	1.25 (±0.06)	11.83 (±0.10)	0.70 (±0.02)	1.30 (±0.07)	11.59 (±0.08)	0.68 (±0.02)
Q562	1.09 (±0.08)	17.57 (±0.09)	0.66 (±0.01)	1.21 (±0.07)	11.10 (±0.08)	0.66 (±0.01)	1.24 (±0.06)	11.02 (±0.08)	0.66 (±0.01)
Q563	1.06 (±0.09)	18.29 (±0.08)	0.68 (±0.01)	1.23 (±0.06)	11.83 (±0.09)	0.71 (±0.01)	1.21 (±0.08)	11.01 (±0.09)	0.72 (±0.02)
H564	1.13 (±0.10)	18.28 (±0.11)	0.67 (±0.01)	1.27 (±0.09)	11.14 (±0.10)	0.70 (±0.01)	1.24 (±0.09)	11.81 (±0.10)	0.71 (±0.01)
L565	1.07 (±0.08)	18.85 (±0.09)	0.68 (±0.02)	1.19 (±0.07)	11.86 (±0.09)	0.70 (±0.01)	1.17 (±0.07)	12.25 (±0.08)	0.74 (±0.01)
L566	1.10 (±0.08)	17.87 (±0.08)	0.75 (±0.01)	1.23 (±0.08)	12.48 (±0.08)	0.74 (±0.02)	1.18 (±0.08)	12.98 (±0.09)	0.77 (±0.01)
Q567	1.11 (±0.09)	17.58 (±0.09)	0.71 (±0.02)	1.17 (±0.08)	12.17 (±0.08)	0.74 (±0.01)	1.15 (±0.07)	12.49 (±0.07)	0.73 (±0.01)
L568	1.02 (±0.06)	18.02 (±0.07)	0.73 (±0.02)	1.22 (±0.06)	11.92 (±0.07)	0.71 (±0.02)	1.15 (±0.05)	13.10 (±0.06)	0.74 (±0.01)
T569	1.04 (±0.07)	17.37 (±0.05)	0.75 (±0.02)	1.21 (±0.07)	12.45 (±0.06)	0.77 (±0.01)	1.14 (±0.06)	13.03 (±0.08)	0.78 (±0.02)
V570	1.01 (±0.08)	17.19 (±0.07)	0.74 (±0.01)	1.22 (±0.09)	12.49 (±0.05)	0.76 (±0.02)	1.17 (±0.07)	13.22 (±0.06)	0.76 (±0.01)
W571	1.06 (±0.09)	16.90 (±0.09)	0.69 (±0.03)	1.23 (±0.08)	12.69 (±0.08)	0.73 (±0.02)	1.18 (±0.08)	12.46 (±0.08)	0.72 (±0.01)
G572	1.05 (±0.08)	17.25 (±0.11)	0.75 (±0.01)	1.23 (±0.06)	12.78 (±0.09)	0.77 (±0.01)	1.17 (±0.06)	13.22 (±0.09)	0.76 (±0.01)
I573	1.09 (±0.07)	17.44 (±0.09)	0.72 (±0.02)	1.28 (±0.06)	11.69 (±0.10)	0.75 (±0.02)	1.19 (±0.06)	13.35 (±0.09)	0.80 (±0.03)
K574	1.10 (±0.06)	18.05 (±0.07)	0.73 (±0.01)	1.24 (±0.07)	12.73 (±0.07)	0.78 (±0.01)	1.18 (±0.08)	12.94 (±0.07)	0.75 (±0.01)
Q575	1.07 (±0.07)	17.20 (±0.08)	0.67 (±0.01)	1.22 (±0.07)	12.26 (±0.08)	0.74 (±0.02)	1.18 (±0.08)	12.85 (±0.08)	0.76 (±0.02)
L576	1.06 (±0.05)	17.21 (±0.08)	0.70 (±0.01)	1.22 (±0.05)	12.10 (±0.08)	0.72 (±0.01)	1.18 (±0.06)	12.84 (±0.09)	0.72 (±0.01)
Q577	1.15 (±0.07)	16.65 (±0.09)	0.62 (±0.02)	1.20 (±0.07)	10.85 (±0.09)	0.70 (±0.03)	1.19 (±0.08)	13.75 (±0.08)	0.72 (±0.02)
A578	1.11 (±0.08)	15.94 (±0.06)	0.65 (±0.01)	1.25 (±0.08)	11.32 (±0.07)	0.68 (±0.01)	1.19 (±0.07)	11.95 (±0.06)	0.69 (±0.01)
R579	1.11 (±0.10)	13.95 (±0.09)	0.56 (±0.02)	1.23 (±0.09)	9.93 (±0.08)	0.61 (±0.01)	1.19 (±0.07)	10.56 (±0.08)	0.62 (±0.02)
loopS1	1.22 (±0.04)	10.39 (±0.05)	0.45 (±0.02)	1.33 (±0.05)	8.01 (±0.06)	0.52 (±0.01)	1.30 (±0.06)	8.66 (±0.05)	0.53 (±0.02)
loopG1	1.33 (±0.04)	7.27 (±0.04)	0.40 (±0.01)	1.40 (±0.04)	6.36 (±0.04)	0.45 (±0.01)	1.39 (±0.05)	6.77 (±0.05)	0.46 (±0.01)
loopG2	1.36 (±0.03)	5.70 (±0.05)	0.33 (±0.01)	1.42 (±0.03)	5.13 (±0.04)	0.37 (±0.01)	1.42 (±0.04)	5.49 (±0.04)	0.39 (±0.01)
loopR4	1.33 (±0.05)	6.02 (±0.06)	0.35 (±0.01)	1.41 (±0.04)	5.49 (±0.05)	0.38 (±0.02)	1.40 (±0.04)	5.88 (±0.04)	0.40 (±0.01)
loopG5	1.36 (±0.04)	5.54 (±0.04)	0.31 (±0.01)	1.42 (±0.05)	5.26 (±0.04)	0.37 (±0.01)	1.42 (±0.03)	5.57 (±0.05)	0.38 (±0.01)
loopG6	1.38 (±0.04)	6.98 (±0.04)	0.42 (±0.01)	1.45 (±0.04)	5.90 (±0.04)	0.44 (±0.02)	1.44 (±0.04)	6.23 (±0.04)	0.46 (±0.02)
W628	1.24 (±0.08)	11.95 (±0.08)	0.52 (±0.01)	1.38 (±0.08)	8.96 (±0.08)	0.59 (±0.02)	1.41 (±0.04)	10.88 (±0.07)	0.59 (±0.01)
M629	1.24 (±0.08)	14.66 (±0.09)	0.62 (±0.02)	1.37 (±0.06)	10.27 (±0.08)	0.65 (±0.01)	1.33 (±0.08)	11.52 (±0.08)	0.63 (±0.02)

E630	1.16 (±0.09)	14.31 (±0.09)	0.65 (±0.01)	1.32 (±0.09)	9.48 (±0.09)	0.65 (±0.01)	1.26 (±0.09)	11.03 (±0.11)	0.68 (±0.01)
W631	1.16 (±0.10)	14.82 (±0.11)	0.65 (±0.01)	1.33 (±0.08)	10.53 (±0.10)	0.66 (±0.02)	1.27 (±0.08)	11.15 (±0.08)	0.67 (±0.02)
D632	1.11 (±0.08)	16.52 (±0.10)	0.69 (±0.02)	1.29 (±0.08)	11.39 (±0.09)	0.72 (±0.01)	1.23 (±0.09)	12.06 (±0.08)	0.69 (±0.01)
R633	1.10 (±0.07)	16.90 (±0.09)	0.64 (±0.01)	1.26 (±0.06)	12.00 (±0.08)	0.65 (±0.01)	1.25 (±0.08)	11.31 (±0.09)	0.68 (±0.01)
E634	1.12 (±0.07)	16.81 (±0.08)	0.63 (±0.01)	1.29 (±0.07)	10.77 (±0.08)	0.66 (±0.01)	1.24 (±0.07)	11.76 (±0.08)	0.69 (±0.01)
I635	1.11 (±0.06)	16.28 (±0.07)	0.66 (±0.01)	1.28 (±0.07)	11.46 (±0.07)	0.71 (±0.01)	1.21 (±0.07)	12.02 (±0.06)	0.72 (±0.02)
N636	1.10 (±0.08)	17.06 (±0.08)	0.67 (±0.01)	1.29 (±0.06)	11.10 (±0.08)	0.70 (±0.02)	1.23 (±0.06)	12.34 (±0.08)	0.70 (±0.01)
N637	1.07 (±0.09)	17.11 (±0.07)	0.68 (±0.01)	1.27 (±0.09)	10.89 (±0.07)	0.67 (±0.01)	1.22 (±0.09)	11.84 (±0.08)	0.70 (±0.02)
Y638	1.11 (±0.06)	16.96 (±0.08)	0.67 (±0.02)	1.31 (±0.07)	11.29 (±0.07)	0.66 (±0.03)	1.24 (±0.08)	11.42 (±0.07)	0.68 (±0.03)
T639	1.05 (±0.05)	17.46 (±0.08)	0.69 (±0.01)	1.26 (±0.06)	11.51 (±0.08)	0.73 (±0.01)	1.18 (±0.06)	12.75 (±0.07)	0.73 (±0.02)
S640	1.09 (±0.07)	16.92 (±0.09)	0.71 (±0.01)	1.28 (±0.07)	11.58 (±0.08)	0.74 (±0.01)	1.17 (±0.06)	12.36 (±0.08)	0.71 (±0.01)
L641	1.07 (±0.08)	18.12 (±0.10)	0.70 (±0.02)	1.29 (±0.06)	11.72 (±0.09)	0.72 (±0.01)	1.20 (±0.07)	12.79 (±0.09)	0.73 (±0.01)
I642	1.13 (±0.09)	16.95 (±0.06)	0.68 (±0.03)	1.33 (±0.09)	10.70 (±0.05)	0.72 (±0.02)	1.18 (±0.08)	12.99 (±0.06)	0.70 (±0.01)
H643	1.10 (±0.09)	17.32 (±0.07)	0.73 (±0.01)	1.33 (±0.08)	11.22 (±0.07)	0.73 (±0.01)	1.23 (±0.09)	12.60 (±0.07)	0.75 (±0.02)
S644	1.06 (±0.06)	17.35 (±0.08)	0.72 (±0.01)	1.29 (±0.05)	11.02 (±0.08)	0.72 (±0.01)	1.20 (±0.05)	12.58 (±0.08)	0.75 (±0.01)
L645	1.09 (±0.08)	17.68 (±0.09)	0.72 (±0.01)	1.34 (±0.07)	11.27 (±0.09)	0.70 (±0.02)	1.22 (±0.06)	12.67 (±0.08)	0.72 (±0.01)
I646	1.10 (±0.09)	18.09 (±0.09)	0.70 (±0.02)	1.35 (±0.08)	10.71 (±0.09)	0.69 (±0.01)	1.22 (±0.07)	13.03 (±0.09)	0.69 (±0.02)
E647	1.13 (±0.10)	16.38 (±0.06)	0.63 (±0.01)	1.35 (±0.09)	10.38 (±0.08)	0.64 (±0.01)	1.24 (±0.06)	12.35 (±0.08)	0.70 (±0.01)
E648	1.13 (±0.06)	15.80 (±0.07)	0.57 (±0.02)	1.38 (±0.06)	8.86 (±0.07)	0.56 (±0.01)	1.22 (±0.08)	10.92 (±0.06)	0.64 (±0.02)
S649	1.17 (±0.06)	14.97 (±0.08)	0.52 (±0.01)	1.39 (±0.06)	7.41 (±0.08)	0.45 (±0.02)	1.27 (±0.08)	10.02 (±0.08)	0.59 (±0.01)
Q650	1.17 (±0.08)	13.13 (±0.09)	0.52 (±0.02)	1.41 (±0.07)	6.28 (±0.09)	0.33 (±0.01)	1.30 (±0.07)	9.90 (±0.09)	0.57 (±0.01)
N651	1.18 (±0.07)	12.87 (±0.08)	0.49 (±0.02)	1.38 (±0.06)	4.36 (±0.08)	0.14 (±0.01)	1.28 (±0.05)	9.08 (±0.08)	0.55 (±0.02)
Q652	1.19 (±0.08)	12.19 (±0.09)	0.46 (±0.02)	1.32 (±0.06)	2.76 (±0.08)	-0.12 (±0.02)	1.32 (±0.06)	7.40 (±0.09)	0.46 (±0.02)
Q653	1.25 (±0.07)	11.80 (±0.06)	0.39 (±0.01)	1.23 (±0.07)	2.09 (±0.06)	-0.38 (±0.01)	1.34 (±0.06)	8.02 (±0.08)	0.46 (±0.01)
E654	1.25 (±0.09)	12.50 (±0.12)	0.43 (±0.03)	1.08 (±0.05)	1.62 (±0.06)	-0.69 (±0.01)	1.34 (±0.07)	8.53 (±0.11)	0.48 (±0.02)
K655	1.23 (±0.05)	12.31 (±0.05)	0.43 (±0.01)	0.84 (±0.04)	1.14 (±0.05)	-1.18 (±0.01)	1.34 (±0.05)	8.65 (±0.06)	0.52 (±0.01)
N656									
E657	1.20 (±0.07)	12.80 (±0.06)	0.51 (±0.01)				1.30 (±0.07)	9.82 (±0.07)	0.56 (±0.01)
Q658	1.18 (±0.08)	13.64 (±0.07)	0.51 (±0.01)				1.29 (±0.08)	9.33 (±0.07)	0.60 (±0.02)
E659	1.18 (±0.09)	14.31 (±0.08)	0.61 (±0.02)				1.29 (±0.08)	10.77 (±0.08)	0.63 (±0.01)
L660	1.18 (±0.10)	15.18 (±0.09)	0.61 (±0.03)				1.30 (±0.09)	11.33 (±0.10)	0.63 (±0.03)
L661	1.15 (±0.07)	15.97 (±0.06)	0.67 (±0.01)				1.26 (±0.07)	10.94 (±0.07)	0.65 (±0.01)
E662	1.17 (±0.07)	14.91 (±0.08)	0.64 (±0.01)				1.27 (±0.06)	11.54 (±0.08)	0.66 (±0.02)
L663	1.14 (±0.09)	15.58 (±0.12)	0.70 (±0.01)				1.29 (±0.08)	11.37 (±0.09)	0.62 (±0.01)
D664	1.13 (±0.06)	15.96 (±0.08)	0.65 (±0.01)				1.25 (±0.05)	11.76 (±0.08)	0.68 (±0.01)
K665	1.14 (±0.08)	15.95 (±0.09)	0.65 (±0.02)				1.27 (±0.06)	11.49 (±0.08)	0.69 (±0.01)
W666	1.14 (±0.09)	15.10 (±0.10)	0.65 (±0.01)				1.25 (±0.08)	11.57 (±0.09)	0.68 (±0.02)
A667	1.13 (±0.07)	16.05 (±0.09)	0.70 (±0.01)				1.24 (±0.07)	12.10 (±0.07)	0.69 (±0.01)
S668	1.09 (±0.06)	16.55 (±0.08)	0.68 (±0.01)				1.21 (±0.06)	12.98 (±0.08)	0.68 (±0.02)
L669	1.18 (±0.06)	15.62 (±0.07)	0.66 (±0.01)				1.28 (±0.06)	13.22 (±0.08)	0.67 (±0.01)
W670	1.14 (±0.07)	16.25 (±0.08)	0.60 (±0.02)				1.26 (±0.07)	13.07 (±0.08)	0.71 (±0.02)
N671	1.13 (±0.08)	17.84 (±0.08)	0.63 (±0.01)				1.25 (±0.08)	14.32 (±0.07)	0.67 (±0.02)
W672	1.12 (±0.08)	17.95 (±0.09)	0.64 (±0.01)				1.24 (±0.06)	14.28 (±0.09)	0.65 (±0.02)
F673	1.17 (±0.06)	17.90 (±0.06)	0.66 (±0.02)				1.28 (±0.07)	14.19 (±0.06)	0.72 (±0.01)
N674	1.19 (±0.07)	17.36 (±0.07)	0.63 (±0.01)				1.31 (±0.06)	13.95 (±0.08)	0.64 (±0.01)
I675	1.17 (±0.05)	16.86 (±0.06)	0.61 (±0.01)				1.27 (±0.05)	12.93 (±0.07)	0.64 (±0.02)
T676	1.21 (±0.06)	14.62 (±0.08)	0.71 (±0.02)				1.32 (±0.06)	12.23 (±0.07)	0.70 (±0.01)
N677	1.20 (±0.08)	13.91 (±0.08)	0.68 (±0.01)				1.31 (±0.08)	11.94 (±0.08)	0.68 (±0.02)
W678	1.20 (±0.06)	13.87 (±0.09)	0.65 (±0.01)				1.32 (±0.07)	10.82 (±0.10)	0.64 (±0.01)
L679	1.22 (±0.07)	14.42 (±0.06)	0.65 (±0.01)				1.34 (±0.07)	9.58 (±0.06)	0.65 (±0.02)
W680	1.25 (±0.06)	13.60 (±0.05)	0.65 (±0.02)				1.38 (±0.06)	10.52 (±0.06)	0.66 (±0.01)
Y681	1.26 (±0.05)	13.94 (±0.06)	0.62 (±0.01)				1.40 (±0.05)	9.84 (±0.05)	0.62 (±0.01)
I682	1.34 (±0.05)	11.67 (±0.06)	0.54 (±0.01)				1.42 (±0.06)	9.71 (±0.05)	0.56 (±0.01)
K683	1.38 (±0.03)	11.98 (±0.04)	0.43 (±0.01)				1.43 (±0.04)	8.02 (±0.06)	0.43 (±0.01)

The six residues replacing the immunodominant loop (I69-T116) in the Core^{S} constructs are numbered loopS1-loopG6

References

Bohm, G, Muhr, R, Jaenicke, R (1992) Quantitative analysis of protein far UV circular dichroism spectra by neural networks. Protein Eng 5: 191-195.

Cole, JL, Lary, JW, Moody, TP, Laue, TM (2008). Analytical ultracentrifugation: Sedimentation velocity and sedimentation equilibrium. Biophysical Tools for Biologists: Vol 1 in Vitro Techniques. **84:** 143-179.

Ghirlando, R, Balbo, A, Piszczek, G, Brown, PH, Lewis, MS, Brautigam, CA, Schuck, P, Zhao, H (2013) Improving the thermal, radial, and temporal accuracy of the analytical ultracentrifuge through external references. Anal Biochem 440: 81-95.

Inagaki, S, Ghirlando, R, Grisshammer, R (2013) Biophysical characterization of membrane proteins in nanodiscs. Methods 59: 287-300.

le Maire, M, Champeil, P, Moller, JV (2000) Interaction of membrane proteins and lipids with solubilizing detergents. Biochimica Et Biophysica Acta-Biomembranes 1508: 86-111.

Schuck, P (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and Lamm equation modeling. Biophys J 78: 1606-1619.

Zhao, H, Brautigam, CA, Ghirlando, R, Schuck, P (2013) Overview of current methods in sedimentation velocity and sedimentation equilibrium analytical ultracentrifugation. Current protocols in protein science / editorial board, John E Coligan [et al] Chapter 20: Unit20.12-Unit20.12.

Zhao, H, Brown, PH, Schuckt, P (2011) On the Distribution of Protein Refractive Index Increments. Biophys J 100: 2309-2317.