

Supplemental information

Soluble prefusion-closed HIV-envelope

trimers with glycan-covered bases

Adam S. Olia, Cheng Cheng, Tongqing Zhou, Andrea Biju, Darcy R. Harris, Anita Changela, Hongying Duan, Vera B. Ivleva, Wing-Pui Kong, Li Ou, Reda Rawi, Yaroslav Tsybovsky, David J. Van Wazer, Angela R. Corrigan, Christopher A. Gonelli, Myungjin Lee, Krisha McKee, Sandeep Narpala, Sijy O'Dell, Danealle K. Parchment, Erik-Stephane D. Stancofski, Tyler Stephens, Ivy Tan, I-Ting Teng, Shuishu Wang, Qing Wei, Yongping Yang, Zhengrong Yang, Baoshan Zhang, VRC Production Program, Jan Novak, Matthew B. Renfrow, Nicole A. Doria-Rose, Richard A. Koup, Adrian B. McDermott, Jason G. Gall, Q. Paula Lei, John R. Mascola, and Peter D. Kwong

Table S1. Antigenic screening of base-covered trimer designs, related to Figure 1. (A) Original high-throughput ELISA data. Color scale ranges from white (lowest value) to red (highest value). (B) ELISA raw data values normalized by the reference strain BG505-RnS(7MutV1)-2G-3mut-DS_SOSIP. We consider a trimer to be in the prefusion-closed conformation if either VRC26.25, PG1145, or PGDM1400 binding is greater 0.75.

A

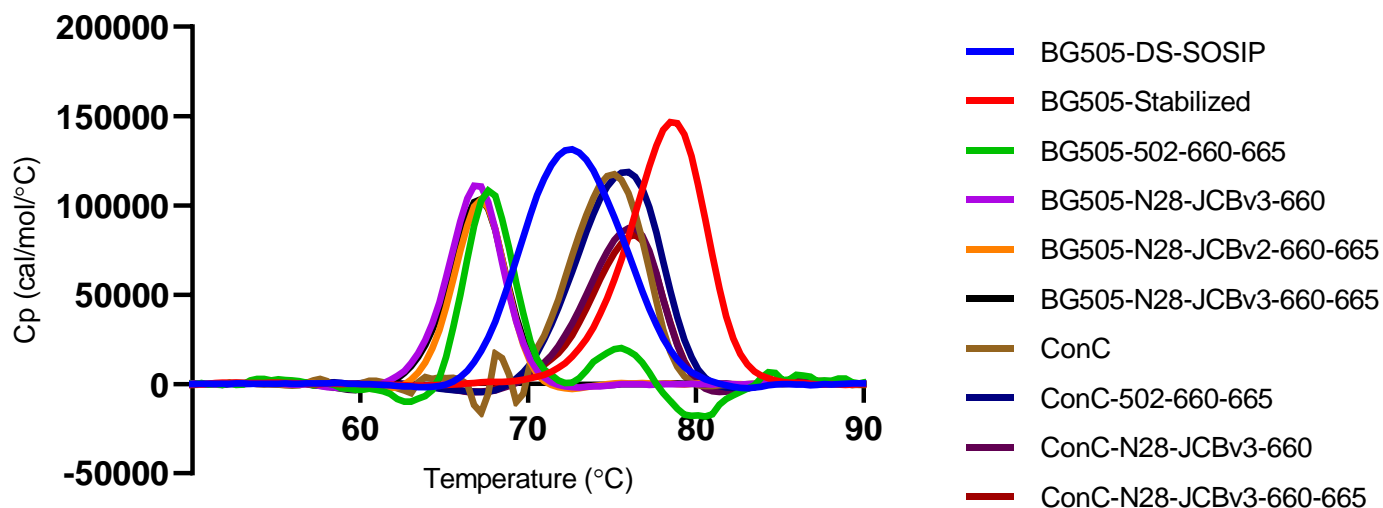
Trimer Construct	VRC26.25	PG1145	PGDM1400	PGT151	35022	VRC34	PGT122	N6	3BNC117	F105	447-52D	17b	17b-CD4	1E6	5H3	3H2	989	RM20A3
BG505-RnS(7MutV1)-2G-3mut-DS_SOSIP-scFc	0.7097	0.6834	0.9852	1.2956	1.0098	0.783	1.0493	0.811	1.0396	1.1344	0.5004	0.0844	0.0858	3.013	2.8426	3.0678	0.1953	2.6452
BG505-RnS(7MutV1)-2G-3mut-DS_SOSIP-502-660-NxT-scFc	0.6698	0.5903	1.1126	0.8404	0.9137	0.5669	1.0211	0.8165	1.0522	1.1949	1.3055	0.2111	0.0719	1.9949	1.3055	0.2111	0.1094	2.0276
BG505-DSSOSIP (non-scFc)	0.2128	0.1514	0.3322	1.1526	0.296	0.4572	0.3242	0.5538	0.6036	1.288	0.464	0.0513	0.052	1.7076	1.6523	1.6086	0.4052	1.4786
BG505-RnS7MutN28-502-660	0.5948	0.393	1.0888	0.5863	0.8204	0.4681	0.8009	0.7076	0.8509	1.0188	0.4727	0.0728	0.0703	1.3954	0.6765	0.143	0.1629	1.156
BG505-RnS7MutN28-JCBv2-660	0.5272	0.508	1.0459	0.9529	0.7687	0.4609	0.8615	0.7172	0.8376	1.0129	0.4661	0.0778	0.0688	0.6948	0.3487	0.123	0.1146	1.0426
BG505-RnS7MutN28-502-660-665	0.5547	0.531	0.9816	0.5018	0.7854	0.4367	0.8638	0.6656	0.8413	1.0162	0.4534	0.0697	0.0668	0.5019	0.2292	0.1062	0.1	0.6078
BG505-RnS7MutN28-JCBv1-660	0.6878	0.521	0.9719	0.784	0.9439	0.7315	0.8651	0.7033	0.9533	1.0119	0.4163	0.0716	0.0699	1.0194	0.6564	0.9006	0.1063	2.688
BG505-RnS7MutN28-JCBv2-660	0.5914	0.3345	1.03	0.7853	0.7394	0.605	0.8946	0.7067	0.9769	1.203	0.4631	0.0828	0.0881	0.3602	0.7243	0.1258	0.1159	1.4536
BG505-RnS7MutN28-JCBv3-660	0.5882	0.2786	0.9981	0.5759	0.7495	0.6638	0.8742	0.6668	0.9391	1.1239	0.4478	0.0695	0.076	0.3509	0.4952	0.1229	0.1204	1.0324
BG505-RnS7MutN28-JCBv1-660	0.7449	0.6327	0.9999	0.6327	0.8412	0.726	0.8993	0.7154	0.9165	1.1108	0.4409	0.0748	0.0707	2.1004	2.6831	0.8311	0.1153	2.7296
BG505-RnS7MutN28-JCBv2-660	0.6286	0.2708	1.0573	0.4196	0.7241	0.6901	0.8906	0.7759	0.9536	1.3105	0.4751	0.1091	0.0929	0.2692	0.3307	0.1107	0.1175	0.6388
BG505-RnS7MutN28-JCBv3-660	0.7543	0.4267	1.0882	0.428	0.7281	0.6479	0.9601	0.7172	1.0124	1.1962	0.505	0.0732	0.0876	0.2594	0.3061	0.1105	0.1459	0.8193
BG505-RnS7MutN28-JCBv1-660-665	0.6711	0.4485	1.0172	0.6906	0.9306	0.747	0.7197	0.7396	0.936	1.1105	0.469	0.0699	0.0882	0.9773	1.8077	0.2431	0.1178	2.0142
BG505-RnS7MutN28-JCBv2-660-665	0.1354	0.1982	0.9484	0.2104	0.2585	0.2135	0.2454	0.6594	0.7344	0.0866	0.4569	0.0679	0.0767	0.0958	0.3004	0.784	0.0846	0.396
BG505-RnS7MutN28-JCBv3-660-665	0.5267	0.2007	1.0799	0.3515	0.6331	0.6907	0.8136	0.8107	0.9648	1.1276	0.477	0.07	0.066	0.1835	0.1102	0.0997	0.1021	0.2524
BG505-RnS7MutN28-JCBv1-660-665	0.7183	1.0318	0.9555	0.9673	0.8729	0.7556	1.0305	0.7257	0.8793	1.0189	0.4342	0.074	0.0668	1.2531	2.4027	3.2513	1.0854	2.7193
BG505-RnS7MutN28-JCBv2-660-665	0.5437	0.276	1.0263	0.3824	0.6279	0.7288	1.005	0.8161	0.9521	1.407	0.4751	0.0715	0.0681	0.1913	0.1187	0.1024	0.1011	0.2581
BG505-RnS7MutN28-JCBv3-660-665	0.564	0.2189	1.0688	0.2939	0.6323	0.6824	0.8744	0.7951	0.9812	1.1567	0.4775	0.0701	0.0659	0.158	0.0949	0.1024	0.0977	0.2012
BG505-DSSOSIP-N332-RnS7Mut-502-660	0.5186	0.3331	0.9985	0.8651	0.8035	0.4597	0.8017	0.6875	0.8468	1.0257	0.4613	0.1153	0.0668	1.6257	0.9672	0.1703	0.1053	1.6221
scFc-3C-6931-502-660	0.7697	0.6993	0.502	0.465	0.8538	0.738	1.3564	0.8889	1.0564	0.311	0.3629	0.0637	0.2196	0.1526	0.0731	0.1129	0.1114	0.0766
scFc-3C-6931-DS-502-660	0.6966	0.5988	0.5125	0.3859	0.7938	0.6726	1.2645	0.7923	0.9698	0.3036	0.3904	0.0641	0.0637	0.1429	0.0842	0.1026	0.1043	0.0755
scFc-3C-6931-28-502-660	0.4774	1.2607	0.3632	0.8113	0.68	0.8345	1.1005	0.9527	0.7035	0.3625	0.2506	0.0723	0.3282	0.2895	0.0943	0.0934	0.1136	0.0848
scFc-3C-6931-28-JCBv1-660	0.116	0.057	0.8343	0.5227	0.2907	0.75	1.4531	0.856	1.1255	0.4913	0.465	0.094	0.3054	0.4816	0.0814	0.085	0.1187	0.088
scFc-3C-6931-28-502-660-665	0.9052	1.7704	0.824	0.5161	0.9075	0.8133	1.5308	0.9494	1.1625	0.106	0.44	0.082	0.3877	0.1418	0.0952	0.1124	0.1381	0.0923
scFc-3C-6931-JCBv4V1-660	0.9524	1.2986	0.7995	0.6959	1.0953	1.0574	1.4368	0.9716	1.1739	0.3626	0.4426	0.0764	0.2371	0.1508	0.0857	0.1262	0.1088	0.831
scFc-3C-6931-JCBv4V2-660	0.8474	0.9306	0.7377	0.9122	0.9467	0.9947	1.4316	0.9781	1.1563	0.4077	0.4549	0.0877	0.3433	0.1525	0.0879	0.1569	0.1143	0.8006
scFc-3C-6931-JCBv4V3-660	0.845	1.0386	0.8166	0.8023	0.9057	0.984	1.4554	1.0396	1.1858	0.4802	0.4681	0.1221	0.3174	0.1743	0.0811	0.1192	0.1022	0.8006
scFc-3C-6931-28-JCBv4V1-660	1.0692	1.5232	0.8676	0.7782	1.0882	1.0422	1.4258	0.9507	1.1363	0.3353	0.3784	0.0787	0.1917	0.1752	0.0883	0.1099	0.1528	0.086
scFc-3C-6931-28-JCBv4V2-660	0.806	0.9723	0.7066	0.7629	0.8735	1.0008	1.4242	0.9792	1.1305	0.3967	0.4054	0.0781	0.3054	0.1585	0.0814	0.085	0.1187	0.0842
scFc-3C-6931-28-JCBv4V3-660	0.855	0.8257	0.7575	0.8527	0.859	0.9714	1.4531	0.9317	1.1679	0.412	0.383	0.0781	0.3959	0.1559	0.0749	0.1116	0.1074	0.0739
scFc-3C-6931-JCBv4V1-660-665	0.908	1.3799	0.8096	1.3173	0.9663	0.9744	1.4047	0.9127	1.1648	0.3975	0.4353	0.1078	0.2654	0.1398	0.0788	0.1096	0.1253	0.0794
scFc-3C-6931-JCBv4V2-660-665	0.6743	0.6982	0.7076	0.7349	0.827	0.8605	1.4582	0.9489	1.1616	0.4747	0.4623	0.0753	0.4504	0.1548	0.0752	0.1129	0.1124	0.0788
scFc-3C-6931-JCBv4V3-660-665	0.6345	0.6578	0.7871	0.5087	0.7383	0.846	1.3991	1.0211	1.2297	0.473	0.4644	0.0743	0.4278	0.1493	0.0752	0.1128	0.1339	0.0923
scFc-3C-6931-28-JCBv4V1-660-665	0.9788	1.7291	0.7754	1.5497	0.9683	0.9497	1.4591	0.919	1.1807	0.32	0.3951	0.0737	0.2384	0.1531	0.0865	0.1253	0.1321	0.0895
scFc-3C-6931-28-JCBv4V2-660-665	0.5881	0.4141	0.4386	0.3994	0.6837	0.7607	1.343	0.9255	1.1841	0.3082	0.3287	0.0938	0.4293	0.1556	0.0723	0.087	0.1145	0.0798
scFc-3C-6931-28-JCBv4V3-660-665	0.5106	0.334	0.5233	0.312	0.6152	0.8282	1.3411	0.9903	1.1907	0.4025	0.3583	0.0871	0.4885	0.1415	0.0732	0.0777	0.1086	0.8971
scFc-3C-6931-DS-28-502-660	0.9518	1.2915	0.8805	0.6124	0.9488	0.8195	1.452	0.9204	1.0959	0.375	0.4862	0.0758	0.0724	0.1606	0.1332	0.1264	0.1313	0.8859
scFc-3C-6931-DS-502-660-665	0.788	0.7999	0.8094	0.4164	0.9312	0.6847	1.3427	0.9238	1.0384	0.3906	0.5016	0.0754	0.0684	0.1449	0.0812	0.1136	0.1118	0.0747
scFc-3C-6931-DS-28-502-660-665	0.8741	0.9377	0.8806	0.4116	0.9158	0.7177	1.3601	0.8761	1.0321	0.337	0.4179	0.0731	0.0699	0.1406	0.0788	0.1033	0.1014	0.0753
scFc-3C-6931-DS-JCBv4V1-660	0.8053	0.6727	0.5861	1.7975	0.9671	0.8951	1.243	0.7811	0.9504	0.2849	0.3701	0.0682	0.0651	0.1881	0.0811	0.1145	0.0968	0.0725
scFc-3C-6931-DS-JCBv4V2-660	0.7453	0.5732	0.4356	0.4585	0.848	0.8958	1.3883	0.9466	1.1421	0.4574	0.4574	0.0719	0.0681	0.1665	0.1152	0.1553	0.1889	0.0858
scFc-3C-6931-DS-JCBv4V3-660	0.6182	0.5086	0.4959	0.3458	0.8476	0.7919	1.699	0.8103	0.9775	0.3042	0.3498	0.0752	0.0827	0.1453	0.0668	0.1053	0.0889	0.0658
scFc-3C-6931-DS-28-JCBv4V1-660	0.9052	1.2647	0.8457	0.6332	0.9222	0.9677	1.4341	0.8805	1.1198	0.3622	0.4287	0.0672	0.0691	0.1464	0.0834	0.1238	0.1514	0.08
scFc-3C-6931-DS-28-JCBv4V2-660	0.9794	1.2584	0.8607	0.5043	0.9002	0.8896	1.4298	0.9181	1.1328	0.4322	0.4611	0.0742	0.0699	0.1649	0.0716	0.1185	0.1162	0.0805
scFc-3C-6931-DS-28-JCBv4V3-660	0.8376	1.0525	0.7265	0.7834	0.9411	0.9287	1.3936	0.867	0.9938	0.4003	0.4349	0.07	0.0592	0.146	0.0685	0.1136	0.1155	0.816
scFc-3C-6931-DS-JCBv4V2-660-665	0.7509	0.9481	0.8203	0.5039	0.8217	0.8436	1.3883	0.9446	1.1421	0.4741	0.5293	0.0719	0.0699	0.1522	0.0843	0.1154	0.1202	0.0788
scFc-3C-6931-DS-JCBv4V3-660-665	0.88578	0.8378	0.8729	0.8356	0.4337	0.7688	1.0767	0.8856	1.149	0.4574	0.4574	0.0719	0.0681	0.139	0.0739	0.1152	0.1553	0.0838
scFc-3C-6931-DS-28-JCBv4V1-660-665	0.945611	0.656277	1.032481	1.3881	0.8803	0.9803	1.0205	0.9484	1.1054	0.4205	0.468	0.0775	0.0711	0.1505				

Table S2. Cryo-EM data collection, processing, and model refinement statistics for the glycan-base ConC structure, related to Figure 4.

Glycan-base ConC	
EMDB ID	EMD-28910
PDB ID	8F7T
<u>Data Collection</u>	
Microscope	FEI Titan Krios
Voltage (kV)	300
Electron dose (e ⁻ /Å ²)	43.54
Detector	Gatan K3 BioQuantum
Pixel Size (Å)	0.415 / 0.830
Defocus Range (µm)	-0.7 / -2.0
Magnification	105,000
<u>Reconstruction</u>	
Software	cryoSPARC v3.3.1
Particles	12,967
Symmetry	C3
Box size (pix)	256
Resolution (Å) (FSC _{0.143})	4.10
<u>Refinement</u>	
Software	Phenix 1.20
Protein residues	1641
Chimera CC	0.77
EMRinger Score	1.86
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.746
<u>Validation</u>	
Molprobit score	2.26
Clash score	14.5
Favored rotamers (%)	99.1
Ramachandran	
Favored regions (%)	89.1
Allowed regions (%)	10.5
Disallowed regions (%)	0.4

Table S4. Background-corrected ELISA data, related to Figures 6 and 7, and Figure S5.

		Mouse 341 (Protein-base BG505 immunized)										Mouse 342 (Glycan-base BG505 immunized)										
Dilution factor		E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	
anti-Protein base BG505	Week 8	100	2.613	2.655	2.590	1.835	1.891	2.917	2.626	2.620	2.353	2.347	0.351	0.342	-0.005	0.004	0.013	0.25	0.68	0.28	-0.05	0.09
		500	2.546	1.672	2.517	2.284	1.681	2.913	2.434	2.238	2.496	2.158	-0.020	-0.052	-0.048	-0.059	-0.061	-0.06	-0.08	-0.07	-0.11	-0.07
		2,500	2.011	2.279	1.292	1.569	1.087	2.712	2.617	1.786	2.072	1.991	-0.052	-0.070	-0.078	-0.071	-0.078	-0.03	-0.12	-0.11	-0.12	-0.12
		12,500	1.203	1.652	0.574	0.313	0.579	2.107	1.729	0.723	0.964	1.087	-0.036	-0.074	-0.077	-0.081	-0.078	-0.06	-0.12	-0.13	-0.13	-0.13
		62,500	0.420	0.256	0.160	0.058	0.137	1.130	0.687	0.259	0.384	0.431	0.001	-0.077	-0.077	-0.079	-0.080	-0.07	-0.12	-0.13	-0.13	-0.13
		312,500	0.105	0.045	0.007	0.002	0.009	0.550	0.245	0.053	0.086	0.083	-0.044	-0.080	-0.082	-0.072	-0.083	-0.05	-0.12	-0.12	-0.13	-0.13
1,562,500	-0.014	0.001	-0.009	-0.027	-0.020	0.352	0.072	-0.016	-0.002	0.004	-0.038	-0.062	-0.071	-0.077	-0.071	-0.01	-0.09	-0.12	-0.11	-0.12		
anti-Glycan base BG505	Week 8	100	0.04	0.13	0.08	0.12	0.04	0.47	0.28	0.07	0.07	0.34	1.08	1.17	1.39	1.36	0.95	1.49	1.24	1.40	1.34	0.98
		500	-0.02	0.02	-0.02	0.00	-0.02	0.09	0.11	0.00	-0.01	0.14	0.76	0.68	0.58	0.77	1.14	0.78	0.86	1.05	0.86	0.80
		2,500	-0.03	-0.03	-0.03	-0.03	-0.03	0.02	0.06	-0.02	-0.02	0.08	0.43	0.24	0.19	0.30	0.59	0.27	0.39	0.43	0.34	0.30
		12,500	-0.04	-0.03	-0.04	-0.04	-0.04	0.02	-0.02	-0.03	-0.03	0.00	0.09	0.02	0.00	0.04	0.18	0.05	0.08	0.10	0.06	0.04
		62,500	-0.04	-0.03	-0.03	-0.03	-0.04	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02	-0.04	-0.03	-0.03	0.01	-0.02	-0.01	0.00	-0.01	-0.01
		312,500	-0.04	-0.04	-0.04	-0.04	-0.04	-0.02	-0.02	-0.03	-0.02	-0.03	-0.05	-0.05	-0.05	-0.05	-0.04	-0.03	-0.03	-0.03	-0.02	-0.02
1,562,500	-0.04	-0.04	-0.04	-0.04	-0.03	-0.02	-0.03	-0.03	-0.03	-0.03	-0.05	-0.05	-0.05	-0.04	-0.05	-0.03	-0.03	-0.03	-0.02	-0.03		
		Mouse 343 (Protein-base ConC immunized)										Mouse 344 (Glycan-base ConC immunized)										
Dilution factor		E21	E22	E23	E24	E25	E26	E27	E28	E29	E30	E31	E32	E33	E34	E35	E36	E37	E38	E39	E40	
anti-Protein base ConC	Week 8	100	1.732	1.772	2.133	1.713	1.985	2.039	1.997	1.989	1.888	1.819	0.831	0.931	0.214	0.640	0.224	0.22	0.17	0.49	0.10	0.12
		500	1.910	1.647	2.150	1.895	2.048	2.065	1.535	2.018	1.956	1.595	0.677	0.288	0.009	0.253	0.003	0.01	0.03	0.11	-0.01	-0.02
		2,500	0.979	1.008	1.864	1.772	1.629	1.838	1.016	1.789	1.416	0.988	0.036	0.097	-0.001	0.077	-0.019	-0.04	-0.03	-0.03	-0.06	-0.06
		12,500	0.341	0.345	1.377	1.282	1.050	1.396	0.344	1.515	0.636	0.309	-0.022	0.001	-0.035	-0.017	-0.038	-0.03	-0.06	-0.06	-0.07	-0.07
		62,500	0.064	0.047	0.548	0.474	0.382	0.476	0.054	0.749	0.144	0.047	0.017	-0.031	-0.031	-0.031	-0.038	-0.05	-0.07	-0.07	-0.07	-0.07
		312,500	0.049	-0.015	0.111	0.095	0.063	0.138	-0.035	0.228	-0.040	-0.039	-0.039	-0.040	-0.036	-0.043	-0.043	-0.05	-0.07	-0.07	-0.07	-0.08
1,562,500	0.001	0.000	0.006	0.002	-0.004	0.006	-0.051	0.058	-0.046	-0.054	-0.001	-0.032	-0.035	-0.039	-0.040	-0.06	-0.07	-0.07	-0.07	-0.07		
anti-Glycan base ConC	Week 8	100	0.27	0.05	0.17	0.04	0.02	0.03	0.74	-0.03	-0.02	0.02	0.95	1.64	1.24	2.45	1.10	1.00	1.22	1.15	1.28	0.74
		500	0.07	-0.01	0.07	0.00	-0.01	-0.04	0.00	-0.05	-0.05	-0.04	0.54	1.17	0.87	1.75	0.63	0.76	1.02	0.87	0.80	0.45
		2,500	0.01	-0.01	0.01	-0.01	-0.01	-0.05	-0.04	-0.05	-0.05	-0.05	0.25	0.56	0.47	0.95	0.20	0.31	0.49	0.35	0.39	0.17
		12,500	-0.01	-0.01	-0.01	-0.01	-0.01	-0.05	-0.05	-0.05	-0.05	-0.05	0.07	0.17	0.15	0.33	0.04	0.10	0.15	0.09	0.12	0.03
		62,500	0.00	-0.01	-0.01	-0.01	-0.01	-0.05	-0.05	-0.05	-0.05	-0.05	0.01	0.04	0.04	0.08	0.00	0.02	0.03	0.02	0.03	0.00
		312,500	-0.01	-0.01	-0.01	-0.02	-0.01	-0.05	-0.05	-0.05	-0.05	-0.05	0.00	0.01	0.00	0.01	-0.01	0.00	0.00	-0.01	0.00	0.00
1,562,500	-0.01	0.00	0.00	0.01	-0.01	-0.04	-0.05	-0.04	-0.04	-0.04	-0.01	-0.01	-0.01	-0.01	0.00	-0.01	-0.01	-0.01	-0.01	-0.01		
		GP2108 (Protein-base BG505 immunized)										GP2109 (Glycan-base BG505 immunized)										
Dilution factor		GP2108-1	GP2108-2	GP2108-3	GP2108-4	GP2108-5	GP2108-6	GP2108-7	GP2108-8	GP2108-9	GP2108-10	GP2109-1	GP2109-2	GP2109-3	GP2109-4	GP2109-5	GP2109-6	GP2109-7	GP2109-8	GP2109-9	GP2109-10	
anti-Protein base BG505	Week 6	1,000	3.295	3.193	1.464	2.021	1.799	3.436	3.406	3.435	3.424	3.359	0.515	0.518	-0.054	0.991	0.033	0.32	0.99	0.49	0.89	0.39
		5,000	3.165	2.822	0.270	0.993	0.523	3.443	3.409	3.352	3.387	3.335	0.142	-0.007	-0.128	0.231	-0.102	0.09	0.24	0.29	0.36	0.08
		25,000	1.988	1.057	0.275	0.311	0.175	2.977	2.413	2.749	2.767	2.238	0.141	-0.103	-0.138	-0.060	-0.117	0.04	0.03	0.01	0.06	0.02
		125,000	0.618	0.106	0.041	-0.012	0.018	1.393	0.789	1.070	1.342	0.699	0.041	-0.128	-0.145	-0.113	-0.141	0.00	-0.03	-0.04	-0.03	-0.02
		625,000	0.144	-0.024	-0.043	-0.039	-0.035	0.516	0.193	0.274	0.422	0.188	-0.137	-0.123	-0.146	-0.136	-0.143	-0.01	0.09	-0.04	-0.05	-0.04
		3,125,000	0.052	-0.043	-0.046	-0.050	-0.047	0.200	0.066	0.075	0.123	0.041	-0.135	-0.112	-0.146	-0.144	-0.139	0.00	-0.04	-0.05	-0.05	-0.05
15,625,000	0.048	-0.036	-0.033	-0.047	-0.040	0.214	-0.016	0.017	0.008	-0.014	-0.106	-0.049	-0.113	-0.135	-0.129	0.06	-0.02	-0.03	-0.04	-0.03		
anti-Glycan base BG505	Week 6	1,000	0.08	0.18	0.30	0.35	0.48	0.43	0.09	0.37	0.28	0.67	2.58	3.04	1.28	2.51	2.54	2.53	3.26	2.59	3.12	3.26
		5,000	-0.07	-0.04	-0.02	-0.04	0.02	0.10	-0.01	0.09	0.07	0.29	1.75	2.83	0.71	2.29	1.23	1.73	2.80	2.43	2.89	2.33
		25,000	-0.07	-0.07	-0.05	-0.03	-0.02	-0.01	-0.04	-0.02	-0.02	0.01	0.55	1.30	0.30	0.83	0.32	0.55	0.98	1.08	1.14	0.64
		125,000	-0.07	-0.06	-0.06	-0.07	-0.04	-0.03	-0.04	-0.04	-0.03	0.00	0.09	0.29	0.02	0.21	0.08	0.08	0.36	0.31	0.30	0.14
		625,000	-0.07	-0.07	-0.07	-0.07	-0.04	-0.04	-0.04	-0.03	-0.03	0.00	-0.03	-0.05	-0.04	-0.02	-0.02	-0.03	0.02	0.06	0.00	0.03
		3,125,000	-0.07	-0.07	-0.06	-0.04	0.00	-0.04	-0.04	-0.04	-0.04	-0.01	-0.06	-0.03	-0.06	-0.02	-0.03	-0.06	-0.05	-0.03	-0.01	-0.03
15,625,000	-0.06	-0.06	-0.05	-0.03	-0.03	-0.03	-0.04	-0.03	-0.02	0.01	-0.05	-0.03	-0.02	-0.02	0.00	-0.06	-0.05	-0.03	0.01	0.00		
		GP2207 (Protein-base ConC immunized)										GP2208 (Glycan-base ConC immunized)										
Dilution factor		GP2207-1	GP2207-2	GP2207-3	GP2207-4	GP2207-5	GP2207-6	GP2207-7	GP2207-8	GP2207-9	GP2207-10	GP2208-1	GP2208-2	GP2208-3	GP2208-4	GP2208-5	GP2208-6	GP2208-7	GP2208-8	GP2208-9	GP2208-10	
anti-Protein base ConC	Week 6	1,000	2.021	2.758	2.634	2.465	2.754	2.513	2.753	2.974	3.089	3.160	-0.015	-0.132	-0.082	-0.120	-0.069	-0.030	-0.056	-0.076	0.022	-0.087
		5,000	1.944	2.355	1.503	1.582	2.336	2.358	2.406	3.067	2.302	2.767	-0.103	-0.158	-0.123	-0.132	-0.118	-0.097	-0.100	-0.087	-0.093	-0.108
		25,000	1.073	1.744	0.839	0.486	1.561	1.000	1.501	2.683	0.943	1.260	-0.144	-0.166	-0.123	-0.163	-0.164	-0.108	-0.074	-0.085	-0.093	-0.066
		125,000	0.291	0.547	0.166	0.047	0.480	0.290	0.429	1.303	0.246	0.442	-0.134	-0.148	-0.141	-0.169	-0.143	-0.122	-0.127	-0.125	-0.119	-0.126
		625,000	0.050	0.117	-0.019	-0.041	0.065	0.079	0.106	0.376	0.050	0.104	-0.143	-0.160	-0.141	-0.144	-0.129	-0.123	-0.125	-0.122	-0.122	-0.125
		3,125,000	-0.020	-0.027	-0.057	-0.062	-0.003	0.030	0.057	0.110	0.007	0.020	-0.156	-0.158	-0.159	-0.170	-0.156	-0.121	-0.129	-0.127	-0.131	-0.131
15,625,000	-0.037	-0.054	-0.066	-0.067	-0.064	0.017	0.001	0.016	-0.004	-0.002	-0.150	-0.167	-0.152	-0.132	-0.158	-0.118	-0.125	-0.113	-0.126	-0.129		
anti-Glycan base ConC	Week 6	1,000	0.019	-0.044	-0.029	-0.044	-0.041	0.027	0.040	0.008	0.073	0.021	2.285	1.935	2.114	1.574	1.546	1.624	1.692	1.379	1.974	1.137
		5,000	-0.055	-0.070	-0.067	-0.064	-0.067	-0.003	0.005	-0.008	0.007	-0.004	1.096	1.089	1.233	0.560	0.898	0.629	0.866	0.402	0.908	0.642
		25,000	-0.069	-0.071	-0.069	-0.065	-0.052	-0.008	-0.008	-0.008	-0.007	-0.007	0.260	0.363	0.411	-0.069	0.271	0.158	0.238	0.074	0.241	0.139
		125,000	-0.060	-0.072	-0.070	-0.070	-0.070	-0.														



Constructs	Melting Temperature (°C)
BG505-DS-SOSIP	72.6
BG505-Stabilized	78.5
BG505-502-660-665	67.7
BG505-N28-JCBv3-660	66.9
BG505-N28-JCBv2-660-665	67.3
BG505-N28-JCBv3-660-665	67.3
ConC	75.2
ConC-502-660-665	76.0
ConC-N28-JCBv3-660	76.0
ConC-N28-JCBv3-660-665	76.5

Figure S1. Differential Scanning Calorimetry for top BG505 and ConC base-covered variants, related to Figure 2. DSC scans of glycan-base candidates for BG505 and ConC as compared to the protein-base templates, with calculated melting temperature (T_m) for each construct.

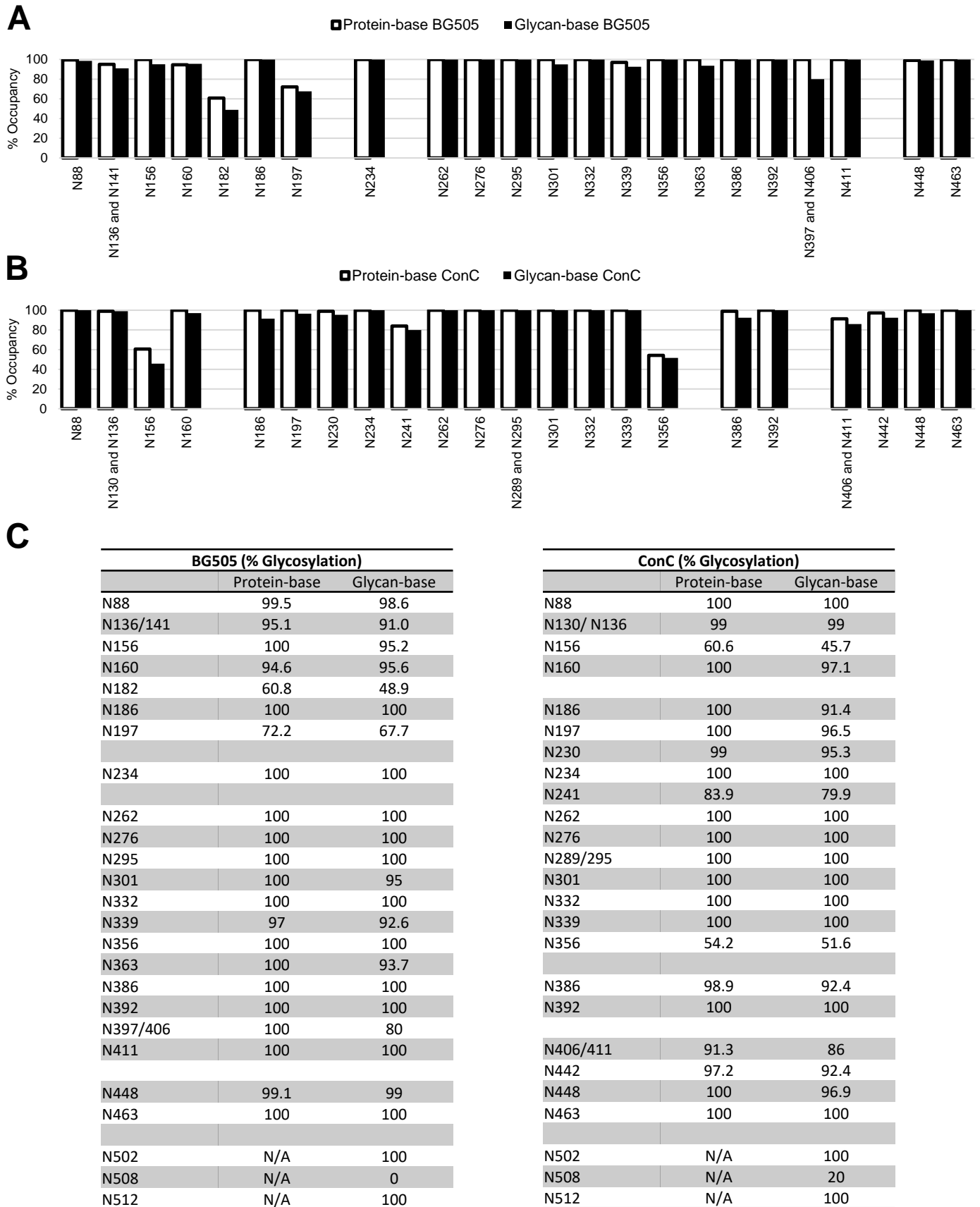


Figure S2. Glycan analysis of gp120 of protein-base and glycan-base trimers, related to Figure 3. (A) Percent glycan occupancy at each glycosylation site in gp120 of either protein-base or glycan base BG505 or (B) ConC. (C) Occupancy values at each site.

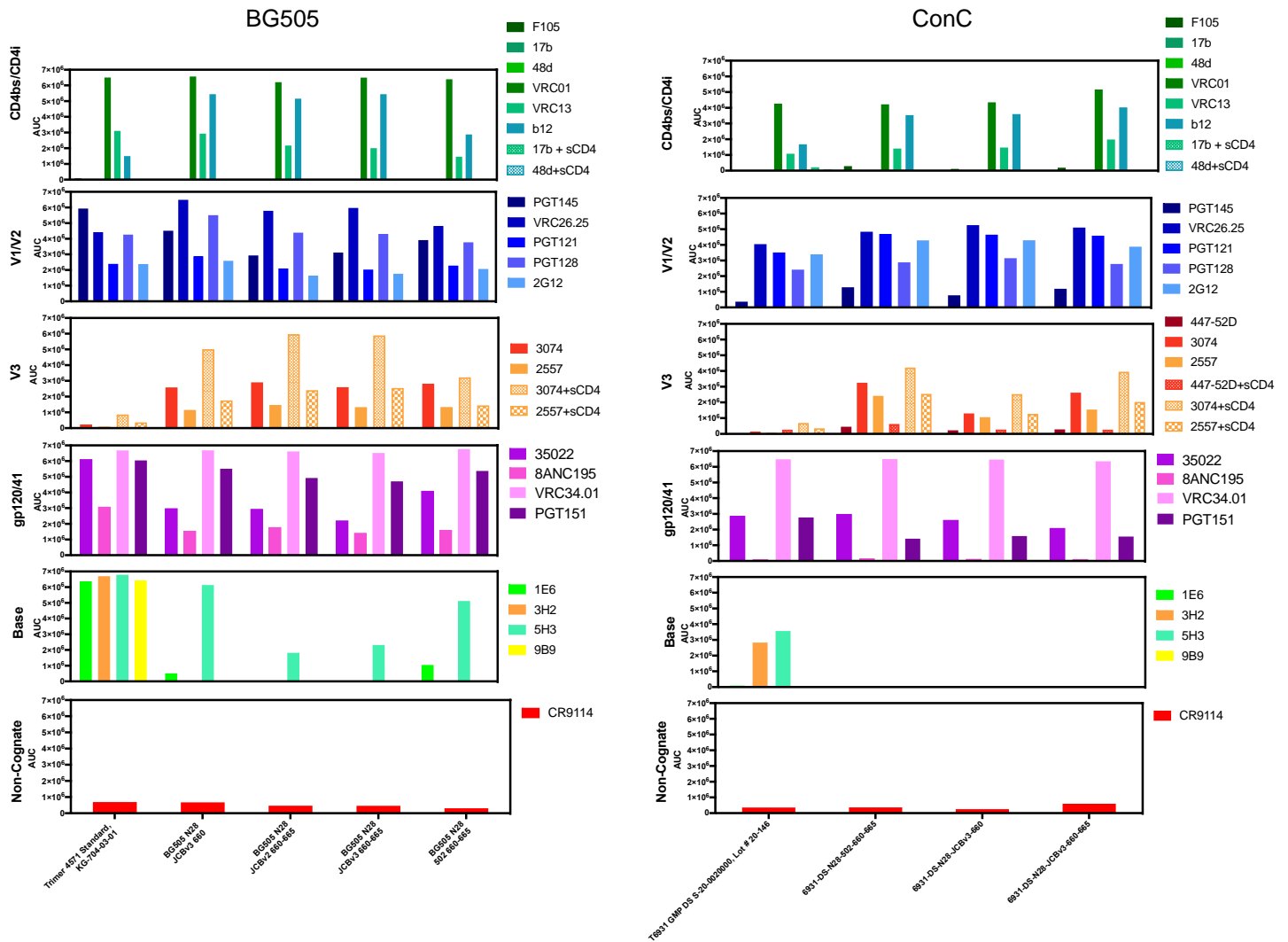


Figure S3. MSD for top BG505 and ConC base-covered variants, related to Figure 3. MSD antigenicity analysis of glycan-base candidates for BG505 and ConC. In both cases, glycan-base constructs show similar binding to antibody panel as the protein-base counterpart, with the exception of slightly high V3 reactivity in most cases.

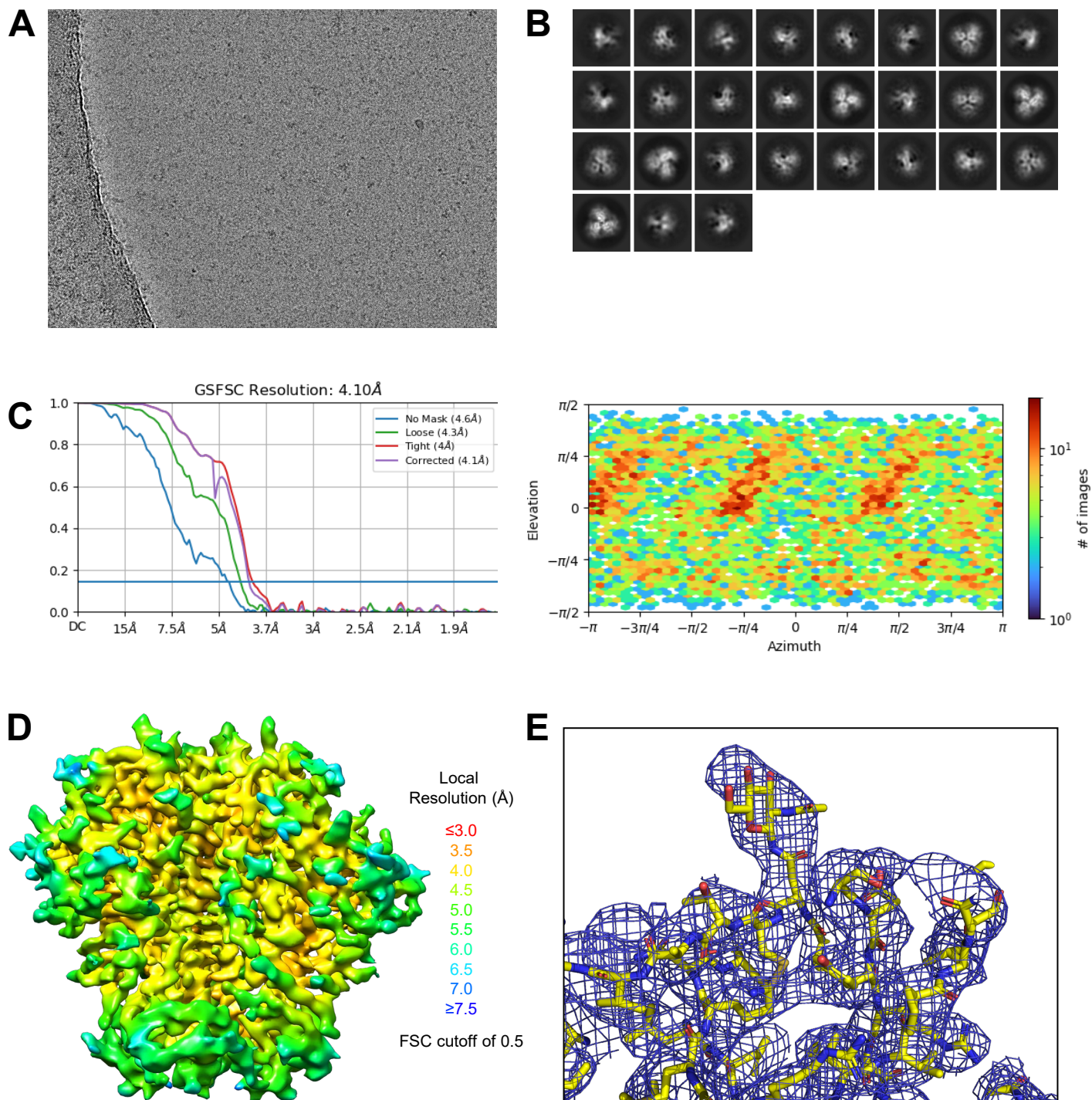


Figure S4. Cryo-EM details of Glycan-base ConC, related to Figure 4. (A) Representative micrograph. (B) Representative 2D class averages. (C) The gold-standard Fourier shell correlation resulted in a resolution of 3.39 Å for the overall map using non-uniform refinement with C3 symmetry (left panel); the orientations of all particles used in the final refinement are shown as a heatmap (right panel). (D) Local resolution of the final reconstruction, shown by color coding from red to blue for high to low resolution. (E) Representative map after sharpening and improvement in Resolve. Map is contoured at 2σ .

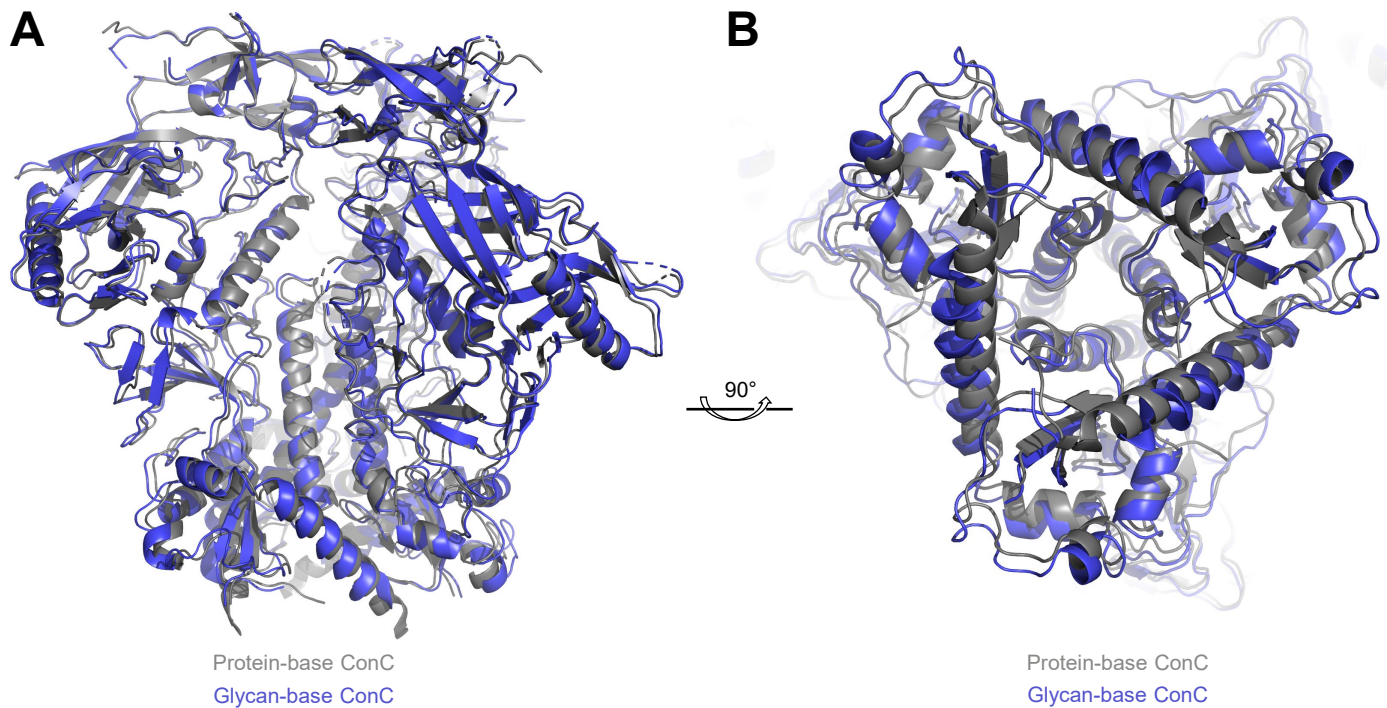


Figure S5. Superposition of protein-base ConC structure onto glycan-base ConC, related to Figure 4. (A) Structural superposition of published protein-base ConC structure (PDB 6CK9) in grey with the glycan-base ConC structure in blue. (B) Bottom view of the superposition showing the movement at the base of the trimer.

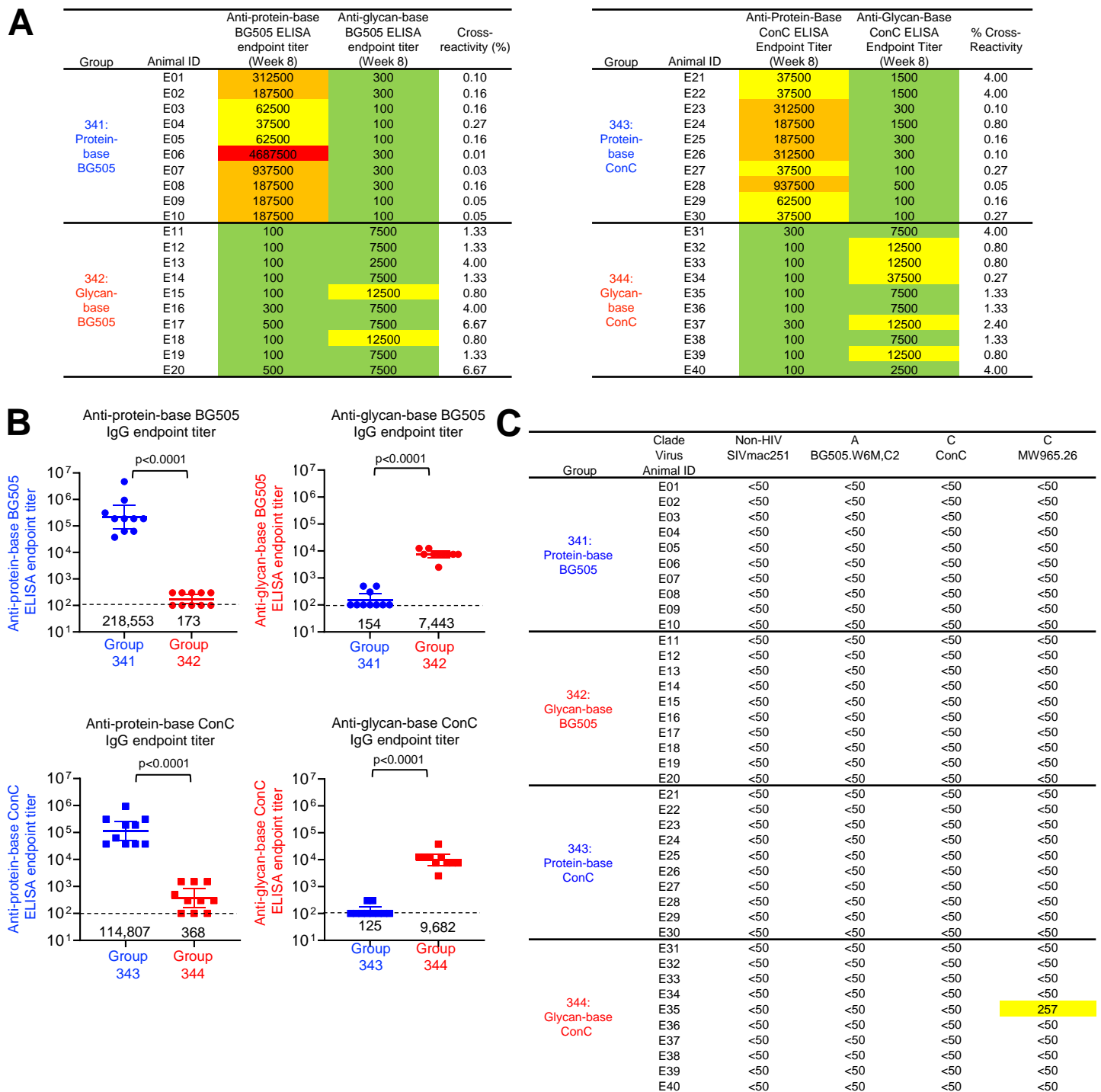


Figure S6. ELISA endpoint titers and serum neutralization (ID_{50}) titers elicited at Week 8 for BG505 or ConC trimer-immunized mice, related to Figure 5. (A) Week 8 ELISA endpoint titers using a starting dilution of 1:100 and cross-reactivity (%) of responses are listed for each animal (B) Group comparison shown for week 8 ELISA endpoint titers against protein-base and glycan-base variants of BG505 trimer (left) and ConC trimer (right) (C) Week 8 neutralization (ID_{50}) titers against wildtype BG505, ConC, MW965.26, and SIVmac251 strains.