SUPPLEMENTAL INFORMATION

Supplemental Data

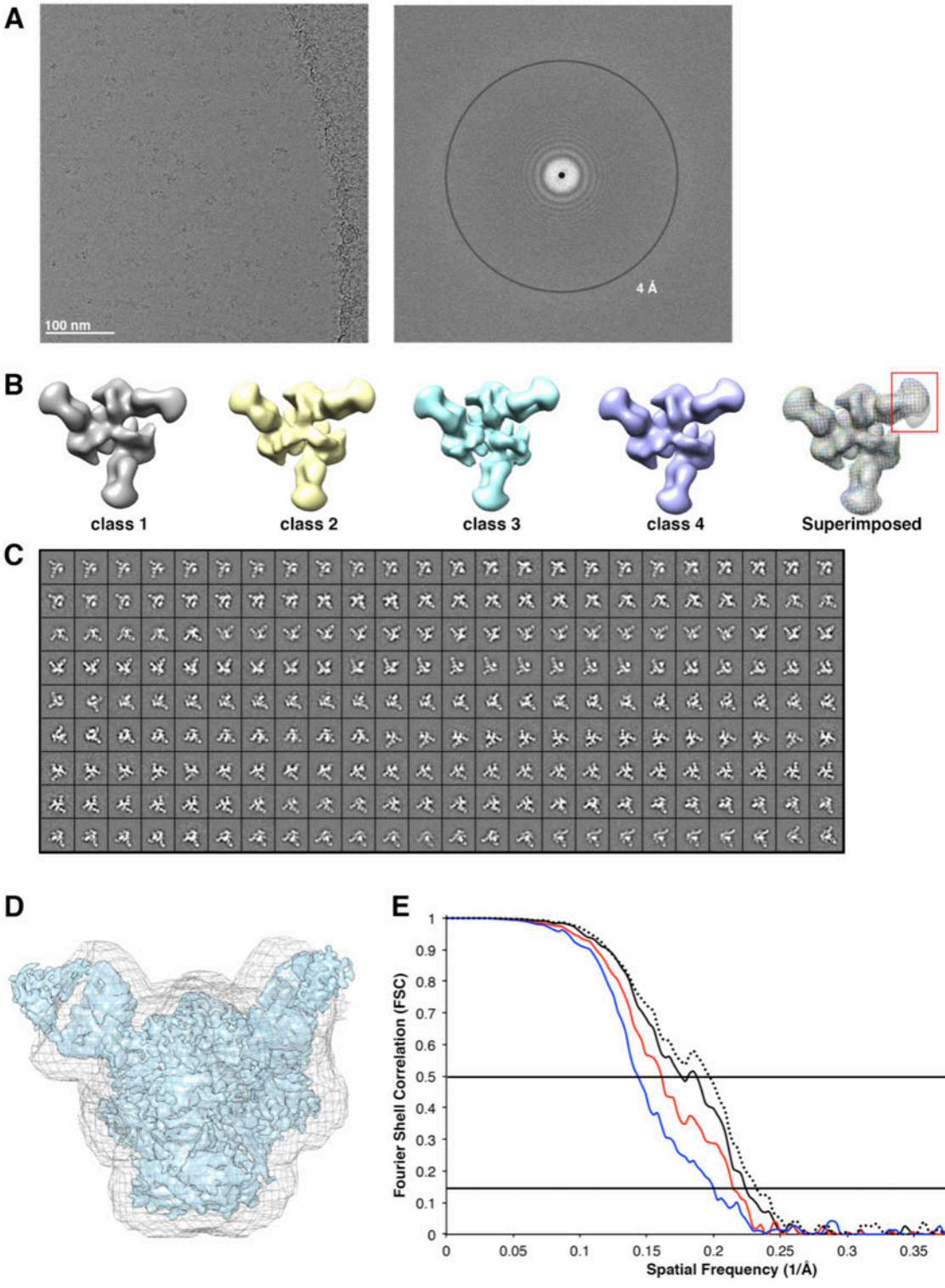


Figure S1, related to Figure 1. Data collection and refinement of BG505 SOSIP.664 trimer in complex with PGT128 Fab.

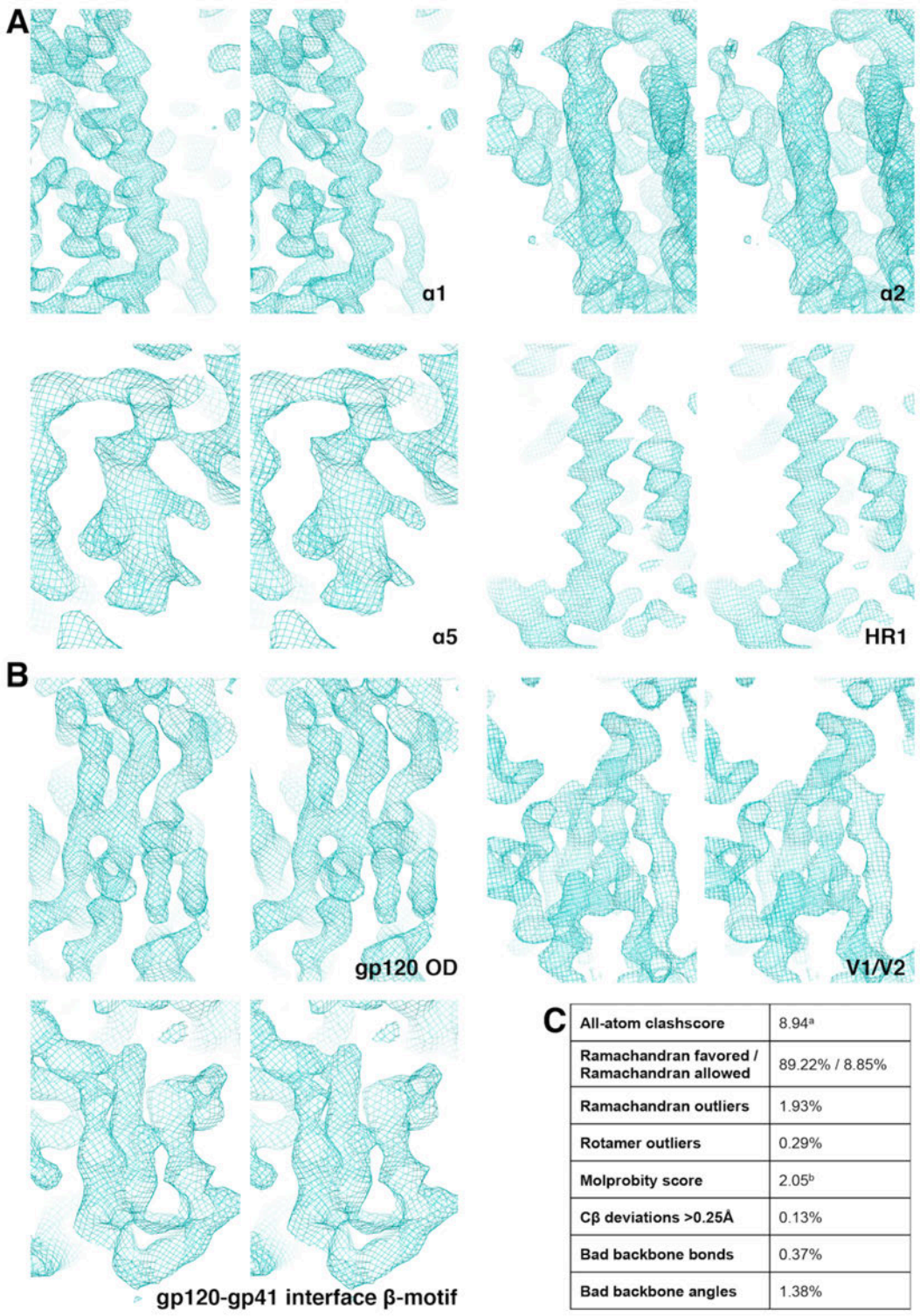
(A) Frame-aligned micrograph (left), and its corresponding FFT (right). The image was shot at a nominal defocus of -2.20 µm, with a total exposure dose of 32.88 $e^{-}/Å^{2}$.

(B) Reconstructions from the second round of 3D classification from data set 1. The classes all represent the BG505 SOSIP.664 trimer in complex with PGT128 in a near-3 fold symmetric manner. However, when the four classes are superimposed, variability in the Fab constant region can be seen. The pronounced variability in the Fab is outlined by the red box in the superimposed image of all four classes.

(C) Reference free 2D class averages generated by an iterative topology alignment method (Ogura et al., 2003).

(D) A mask (mesh) was applied in the final rounds of refinement to exclude the flexible Fab constant region.

(E) Gold standard Fourier shell correlation (FSC) curves for data 1 (red), data 2 (blue), combined (black), and combined and masked (dotted line) refinements. The resolution for the final refinement (dotted line) is 4.36 Å at FSC=0.143.



C

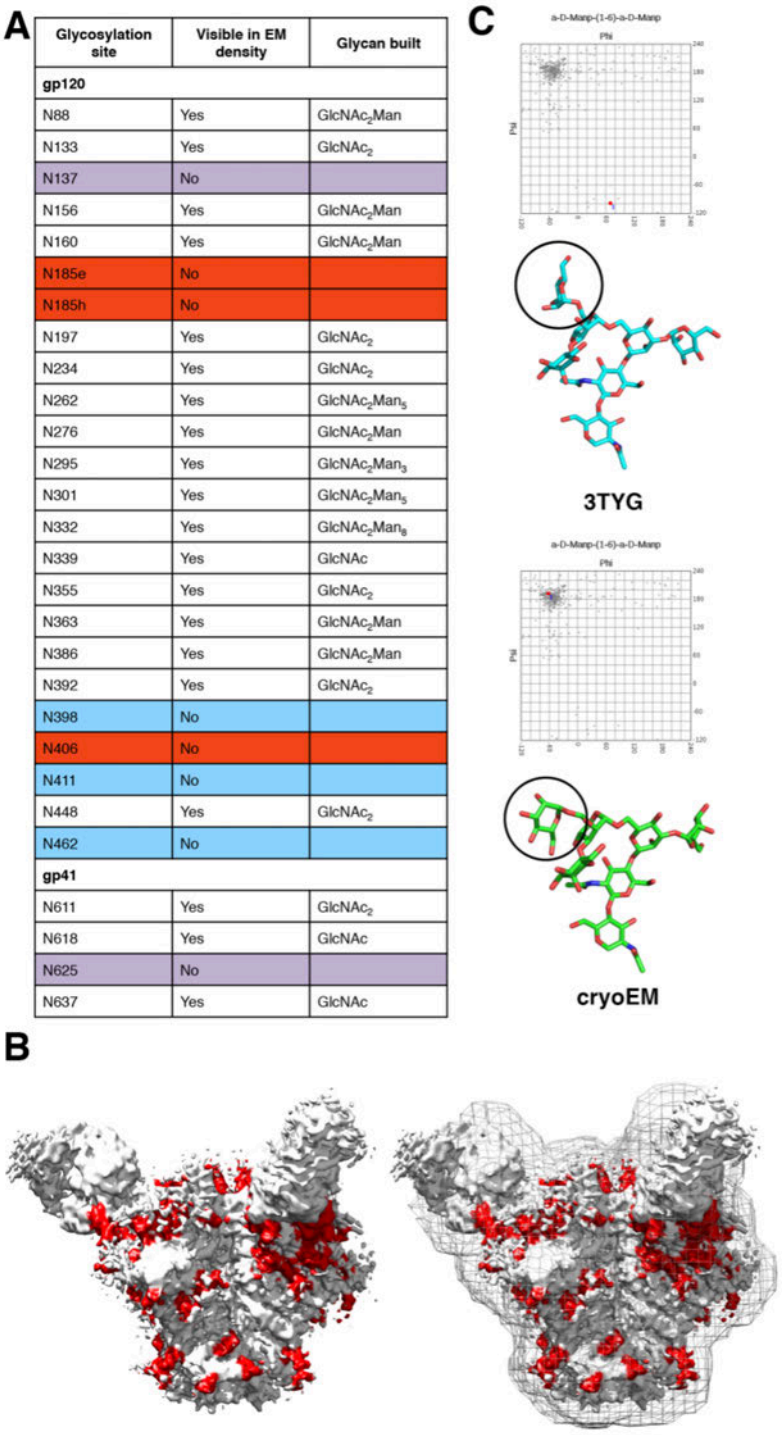
All-atom clashscore	8.94[a]
Ramachandran favored / Ramachandran allowed	89.22% / 8.85%
Ramachandran outliers	1.93%
Rotamer outliers	0.29%
Molprobity score	2.05[b]
Cβ deviations >0.25Å	0.13%
Bad backbone bonds	0.37%
Bad backbone angles	1.38%

Figure S2, related to Figure 2. Refinement of the trimer: Fab complex.

(A) Stereo view of the α-helical densities shown in Figure 2A.

(B) Stereo view of the β-sheet densities shown in Figure 2B.

(C) Geometric parameters for all atoms from Molprobity after refinement. [a]97th percentile among structures >3 Å resolution. [b]100th percentile for structure between 3.25-4.61 Å resolution.



Glycosylation site	Visible in EM density	Glycan built
gp120		
N88	Yes	$GlcNAc_2Man$
N133	Yes	$GlcNAc_2$
N137	No	
N156	Yes	$GlcNAc_2Man$
N160	Yes	$GlcNAc_2Man$
N185e	No	
N185h	No	
N197	Yes	$GlcNAc_2$
N234	Yes	$GlcNAc_2$
N262	Yes	$GlcNAc_2Man_5$
N276	Yes	$GlcNAc_2Man$
N295	Yes	$GlcNAc_2Man_3$
N301	Yes	$GlcNAc_2Man_5$
N332	Yes	$GlcNAc_2Man_8$
N339	Yes	GlcNAc
N355	Yes	$GlcNAc_2$
N363	Yes	$GlcNAc_2Man$
N386	Yes	$GlcNAc_2Man$
N392	Yes	$GlcNAc_2$
N398	No	
N406	No	
N411	No	
N448	Yes	$GlcNAc_2$
N462	No	
gp41		
N611	Yes	$GlcNAc_2$
N618	Yes	GlcNAc
N625	No	
N637	Yes	GlcNAc

Figure S3, related to Figure 4. Glycan density analysis.

(A) The list of all potential glycosylation sites in BG505 SOSIP.664 and indication of whether they are visible in the EM density map. The third column indicates the glycoform built into the atomic model. Eight glycans were not built due to lack of density. The reasons for this are color-coded as follows. Red: Glycan in a region that was not built due to disorder or ambiguous density. Blue: Glycan near missing

density. Purple: Not visible due to reasons unknown, although some glycosylation sites in BG505 may not be fully occupied.

(B) Areas of the EM map corresponding to peptide (white) and ordered glycans (red) are colored differently. The map is contoured to a level where noise starts to become visible. On the left, the mask (mesh) used in the final 3 refinement iterations (Figure S1D) is superimposed onto the EM map on the right. All connected glycan densities are well within the map. Thus, the glycans that are disordered beyond the density that we can see are likely not visible due to intrinsic disorder and not due to a masking artifact.

(C) Glycan torsion analysis of the N301 D3 arm Mannose residue shows that this linkage is a severe torsion outlier (red dot) in the 3TYG structure (left). After the glycan torsion is modified to fit the EM density better, the problematic dihedral angles are resolved.

Collection Parameters	**Data1**	**Data2**	**Combined**
Microscope	FEI Titan Krios		
Detector	Gatan K2 Direct Electron Detector		
Collection Mode	Counting mode		
Acceleration voltage	300 keV		
Magnification	22,500x		
Pixel Size	1.31 Å/pixel		
Data collection duration	3 days	18 hrs	
Number of Micrographs	1,359	752	
Dose	32.88 $e^-/Å^2$	35.07 $e^-/Å^2$	
Frames used	All	All	
Nominal defocus range	1.5-3.5 µm	1.5-3.5 µm	
Processing Parameters			
Particle box size	256x256 pixels	256x256 pixels	256x256 pixels
Particles picked	181,074	154,960	
Particles after 2D classification	70,782	62,572	
Particles after 3D classification	41,173	50,922	
Symmetry Applied	C3		
Initial low-pass filtering of reference model	30 Å		
Number of refinement iterations to convergence	25	24	22 (3)[a]
Number of particles in final reconstruction	41,173	50,922	92,095
Resolution at FSC=0.143	4.66 Å	5.01 Å	4.36[b]
B-factor applied in sharpening			-183.51 $Å^{2\,b}$

Table S1, related to the experimental procedures of EM data collection and refinement.

[a]Additional refinement iterations after applying mask to remove Fab constant region

[b]Values obtained for final model after mask applied refinement.

Supplemental References

Ogura, T., Iwasaki, K., and Sato, C. (2003). Topology representing network enables highly accurate classification of protein images taken by cryo electron-microscope without masking. Journal of structural biology *143*, 185-200.