

Title

Antibodies disrupt bacterial adhesion by ligand mimicry and allosteric interference

Authors

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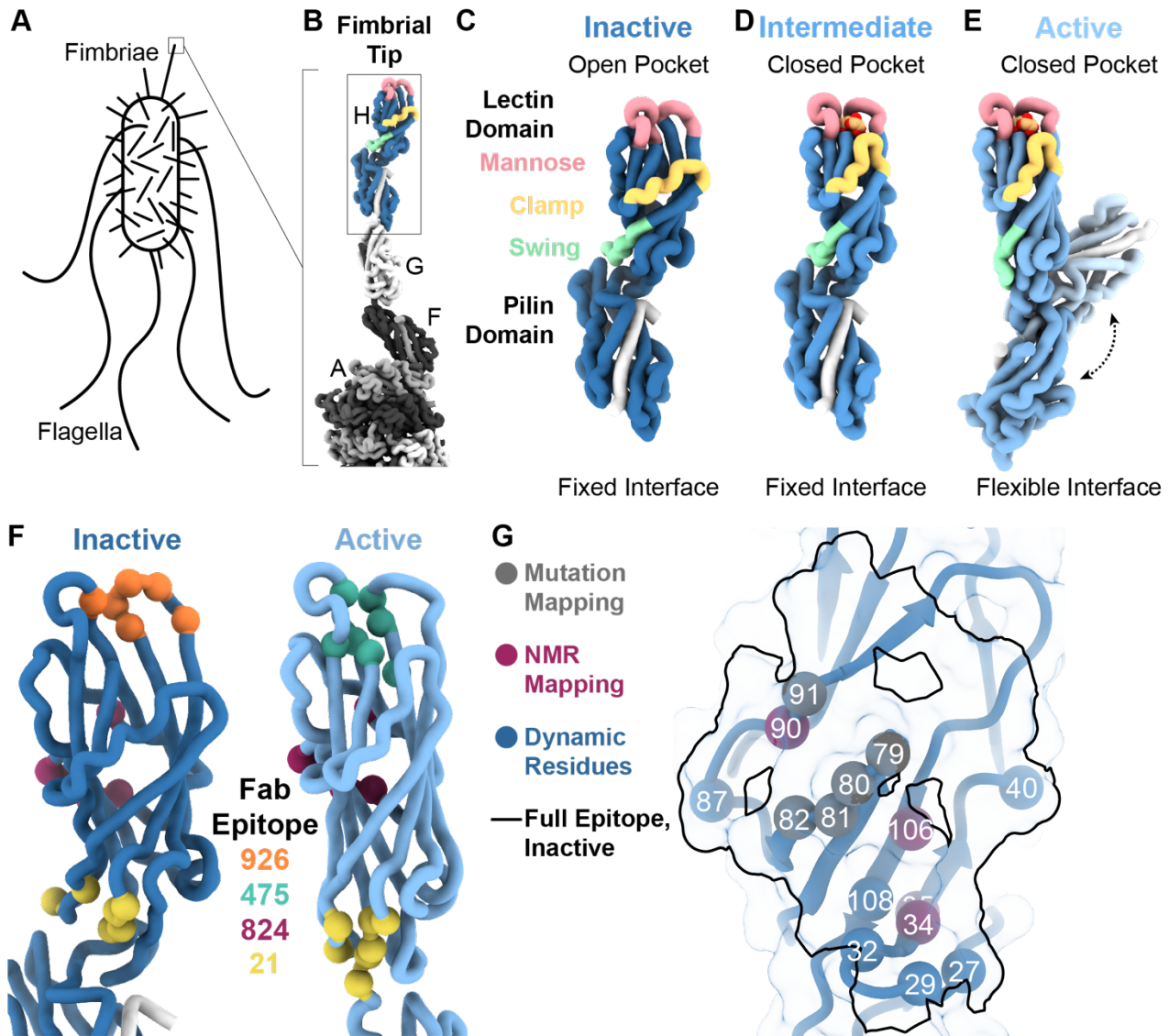
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Supplementary Tables and Figures

Supplementary Figures 1-17



Supplementary Figure 1: FimH conformations and prior knowledge of antibody epitopes. **A.** Cartoon showing flagella and fimbriae of *E. coli*. **B.** Ribbon diagram of the fimbrial tip. FimH in blue, with FimG, F, and A in greys. PDB IDs 6C53, 3JWN. **C-E.** Ribbon diagrams of FimH in the inactive state (**C**, no mannose bound; dark blue; PDB ID 3JWN), an intermediate state with mannose bound (**D**, blue with mannose in orange; PDB ID 4XOE), and the active state (**E**, mannose bound; light blue with mannose in orange; PDB IDs 1KLF, 4XOB, Chain G). FimH undergoes large structural changes in the transition from inactive to active conformations. Loops have been highlighted to demonstrate the changes: the swing loop (pale green) and clamp loop (pale gold) move away from each other and the mannose binding loops (pale red) and the clamp loop encircling the ligand. **F.** C α carbons (spheres) from FimH residues, previously determined by FimH mutagenesis, to interact with bound antibodies mapped onto the inactive (PDB ID 3JWN; dark blue ribbon) and active (PDB ID 1KLF, light blue ribbon) conformations of FimH: orange spheres, Fab926; teal spheres, Fab475; maroon spheres, Fab824; gold spheres, Fab21. **G.** Epitope of Fab824 (black outline) over ribbon representation of FimH + Fab824/926 (transparent surface with blue ribbon). Amino acid C α of previously identified residues shown as spheres: grey, mutation-identified residues; maroon, NMR-identified residues; blue, dynamic FimH residues.

A Sample Preparation

Manual plunging

Data Collection

SerialEM and/or Leginon
0.4215 Å/px

cryoSPARC

Motion Correction

Patch Motion; F-crop 1/2

CTF Estimation

Patch CTF

Particle Picking

Blob Picker - Elliptical

Min Dia: 100 Å; Max Dia: 300 Å; Sep: 0.4

Extract

Box: 640 px; F-crop 160px

2D Classification

2x; 300 classes;

Iter: 50; Batch Size: 400

3D Ab-Initio

C1 symmetry

3D Homogenous Refinement

C1 symmetry

Extract

Box: 640 px; F-crop 480px

3D Homogenous Refinement

C1 symmetry

Window: 0.65

NU Refinement

Window: 0.65; Dyn Mask: 4 Å

Remove Duplicates

NU Refinement + Global CTF Refine

2x; Window: 0.65; Dyn Mask: 4 Å

Fit SA; Tetrafoil; Aniso Mag.

3D Classification

Scale/Init: Input; Force Hard Class;

Aniso Mag Off

Full Particle Solvent Mask

Lectin/Fv Focus Mask

K = 5

NU Refinement

Full Particle Solvent Mask

3D Local Refinement

Static Lectin/Fv Mask

Unsharp.
Half-maps

Unsharp.
Half-maps

Phenix

Density Modification

Model Refinement

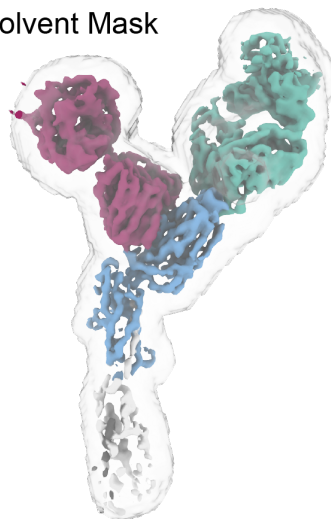
ChimeraX/Isolde

Coot

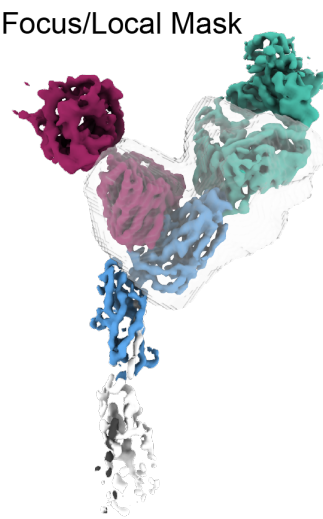
Model Refinement

B

Solvent Mask



Focus/Local Mask



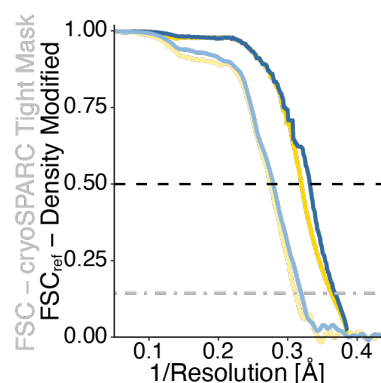
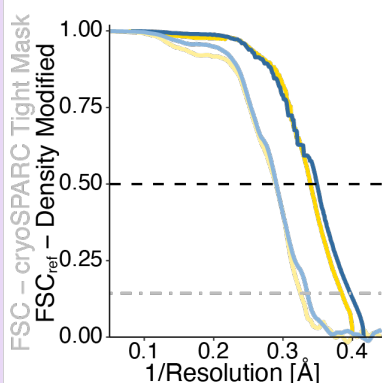
C

FimH + Fabs

Lectin Domain + Fvs

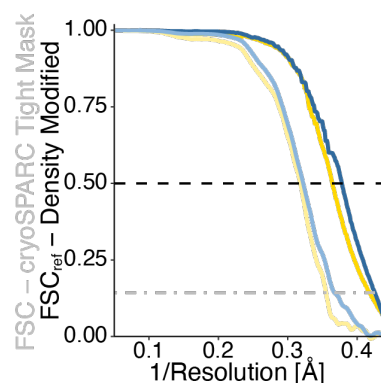
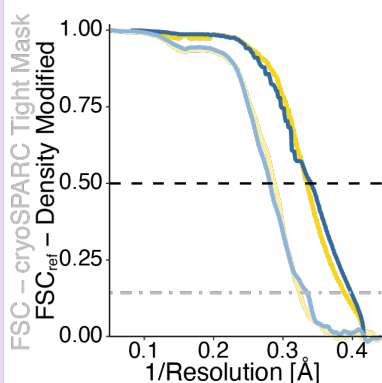
FimH + Fab824/926

FimH + Fab475/824



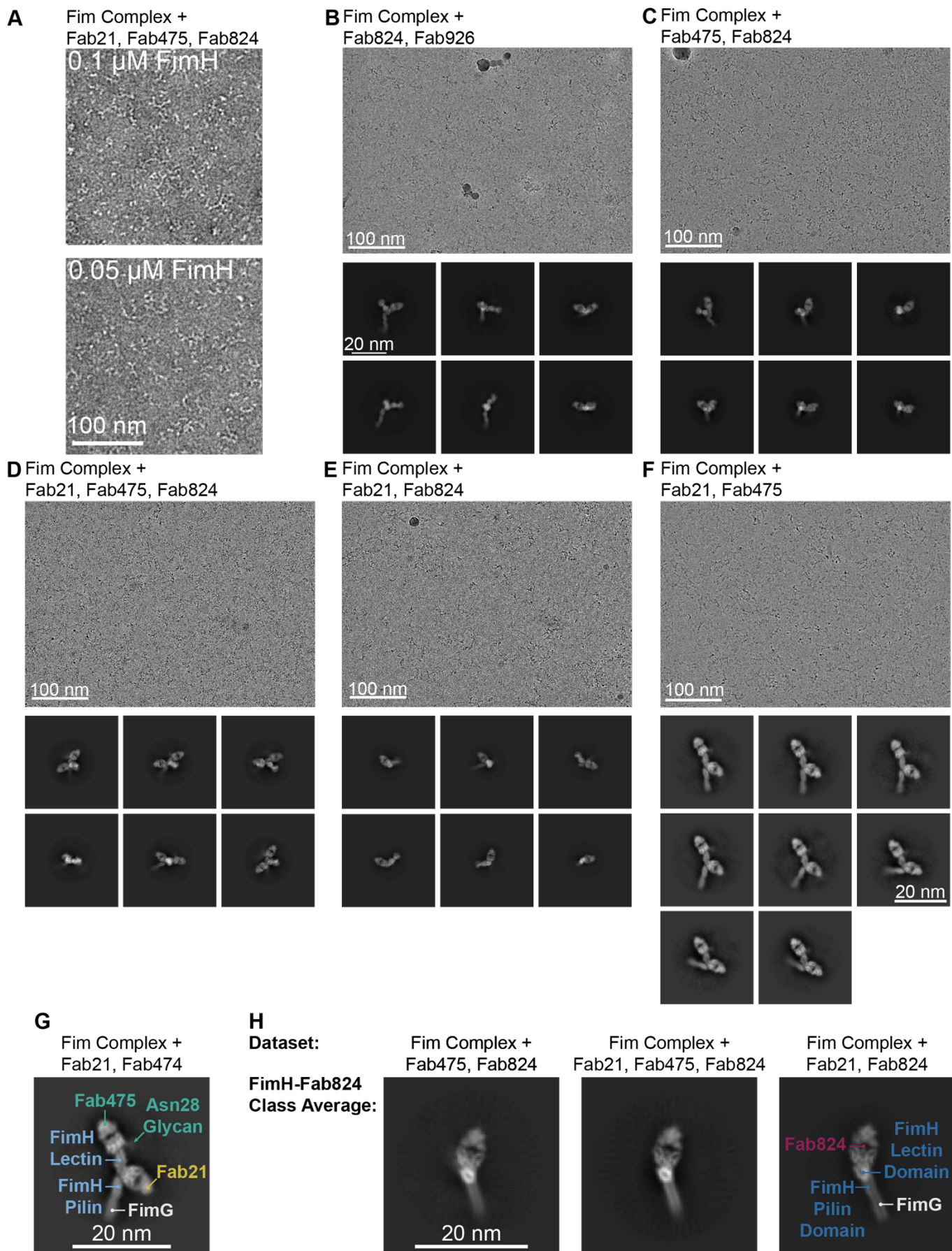
FimH + Fab21/475/824

FimH + Fab21/824



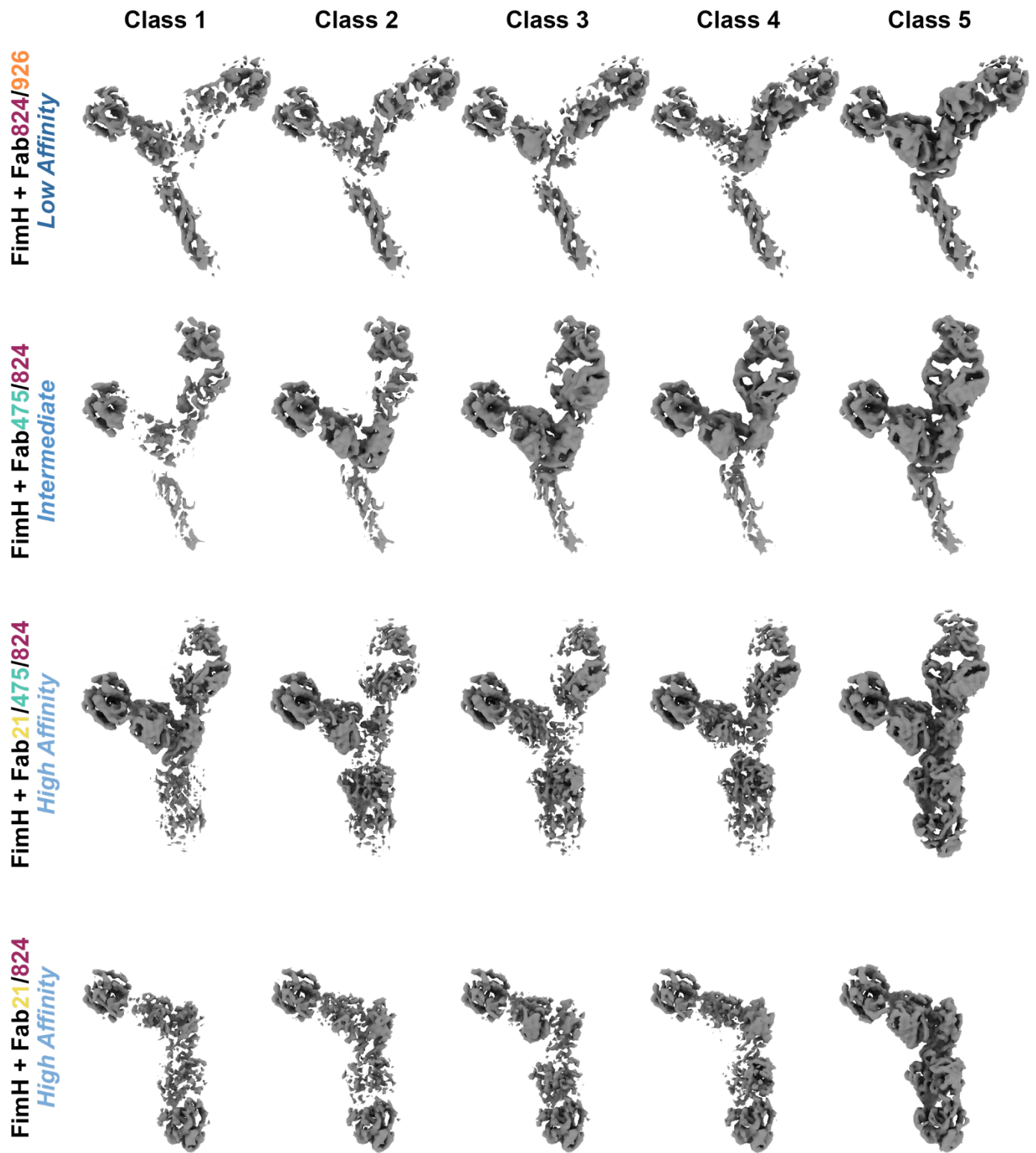
Supplementary Figure 2: Cryo-EM data processing scheme and data quality. **A.** Sample preparation and data collection strategy for all high-resolution datasets (#1-4 in Supplementary Table 2). **B.** Masks (transparent

grey) used for high-resolution structure determination for Fab475 (teal) and Fab824 (maroon) bound to the Fimbrial Tip (FimH, blue; FimG, white). Full particle (left) and lectin/Fv local (right) masks were used for all high-resolution datasets (#1-4 in Supplementary Table 2). **C.** Fourier Shell Correlation curves for the final maps (#1-4 in Supplementary Table 2) generated in cryoSPARC and the density modified maps generated in Phenix. Pale colors represent cryoSPARC gold-standard maps with an FSC cutoff of 0.143 (grey dash-dot line) and dark colors represent density modified maps with an FSC cutoff of 0.5 (black dashed line). Yellow curves represent the full FimH-Fab structures with solvent mask only, while blue curves are reconstructions assembled using the focus mask shown in **B**.

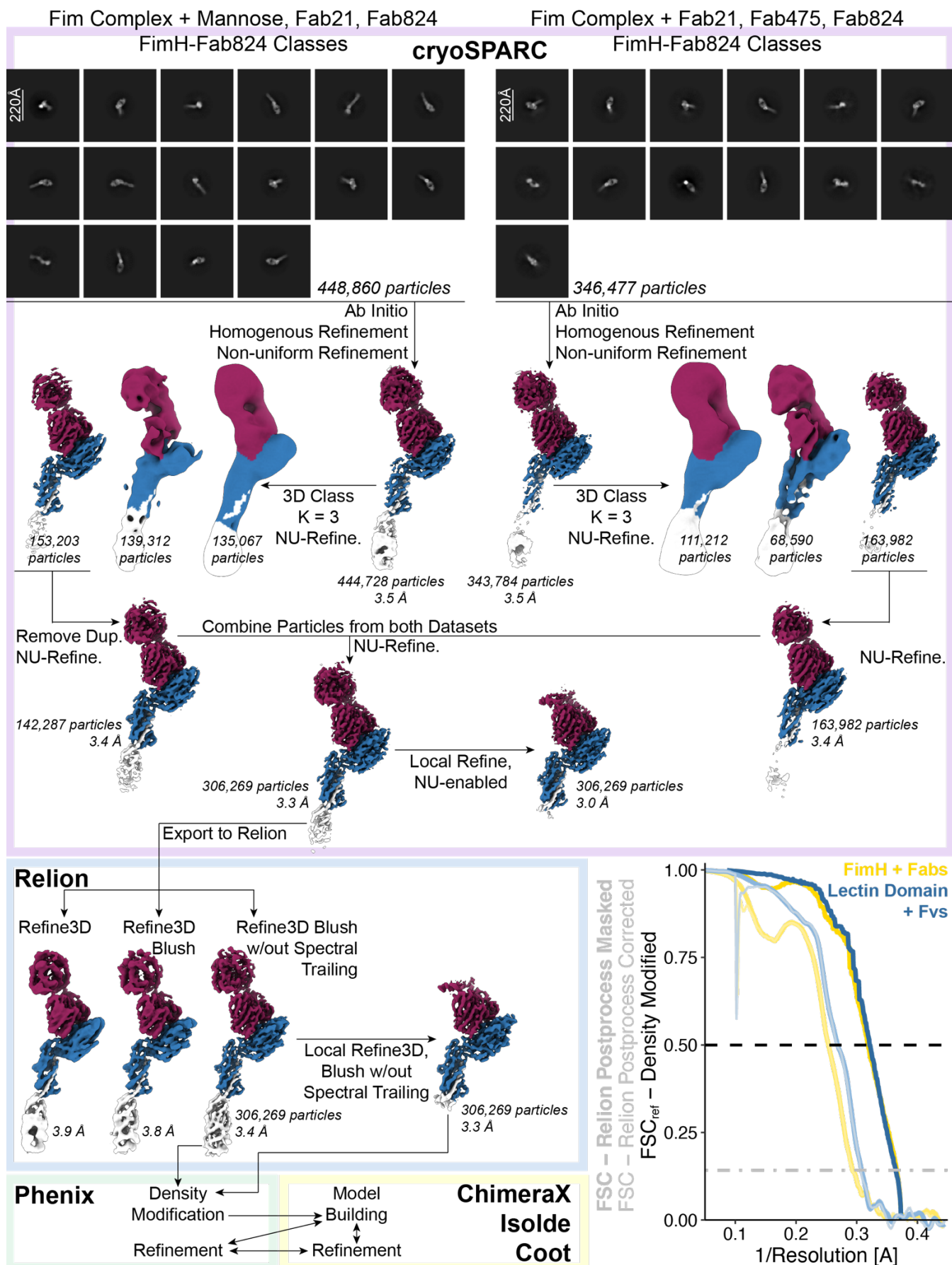


Supplementary Figure 3: Micrographs and two-dimensional class averages from FimH + Fab datasets.
A. Example negative stain micrographs, with a FimH:Each-Fab ratio of 1:1.2, used for concentration range

finding. **B.** Aligned, dose-weighted, and summed micrograph and two-dimensional class averages from the dataset containing FimH + Fab824/926. **C.** Aligned, dose-weighted, and summed micrograph and two-dimensional class averages from the dataset containing FimH + Fab475/824. **D.** Aligned, dose-weighted, and summed micrograph and two-dimensional class averages from the dataset containing FimH + Fab21/475/824. **E.** Aligned, dose-weighted, and summed micrograph and two-dimensional class averages from the dataset containing FimH + Fab21/824. **F.** Aligned, dose-weighted, and summed micrograph and two-dimensional class averages from the dataset containing FimH + Fab21/475. Two-dimensional class averages have been reoriented and cropped for comparison. **G.** Two-dimensional class average of FimH + Fab21/475. The glycan attached to Asn28 is visible as a hazy spot in the average. **H.** Representative class averages from the three datasets listed showing one viewing direction of FimH bound to Fab824 only.

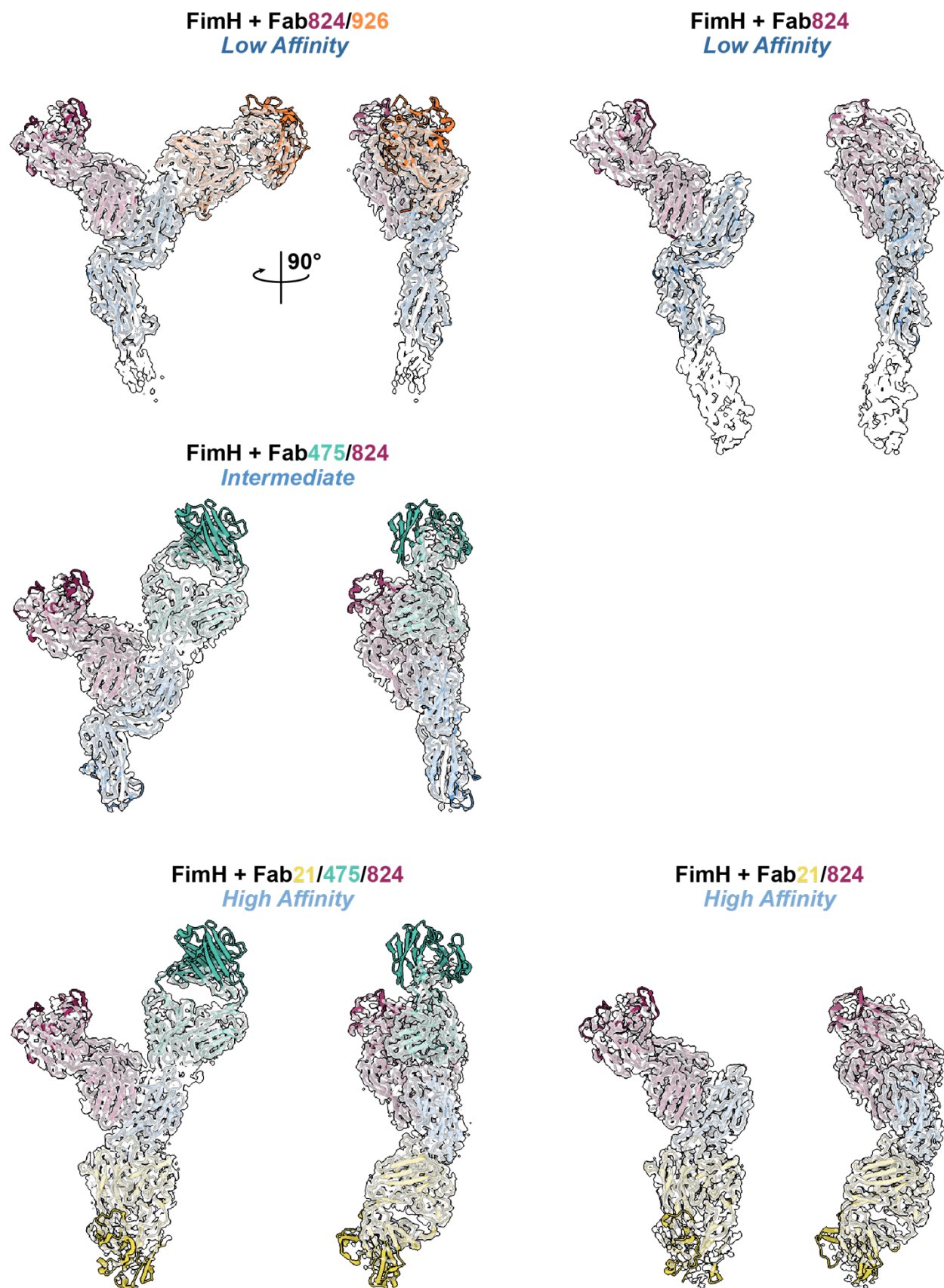


Supplementary Figure 4: Volumes from 3D classification step of cryo-EM data processing pipeline. Volumes (grey) shown for the 3D classification step performed in cryoSPARC, before further refinement. Maps in each dataset are contoured to the same level. The map and particles from Class 5 were used in downstream refinement steps for each dataset.

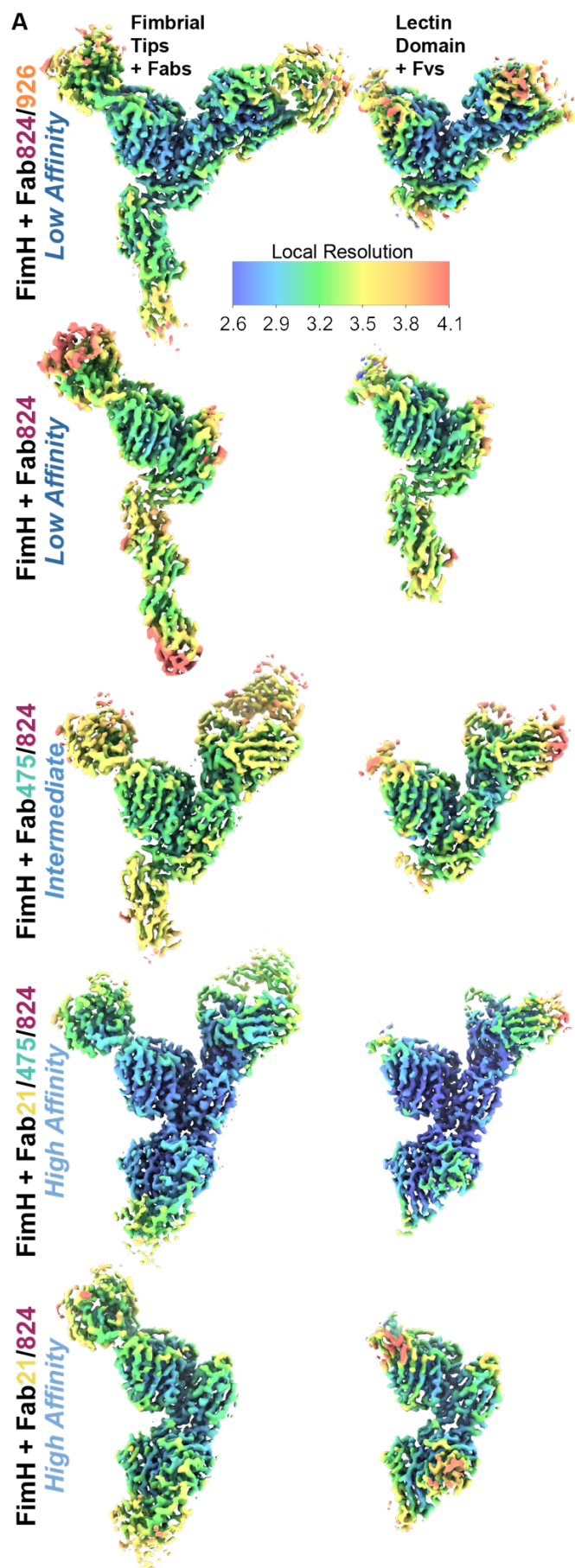


Supplementary Figure 5: Data processing scheme and FSC curves for FimH + Fab824 only. Sample preparation and data collection strategy for FimH-Fab824 complex (FimH, blue; FimG, white; Fab824,

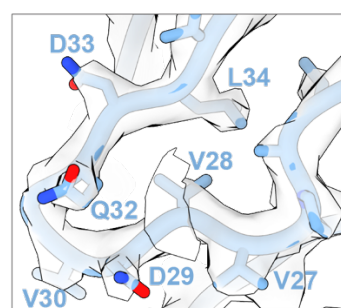
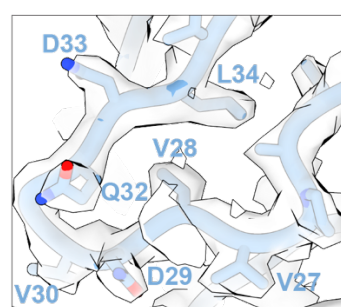
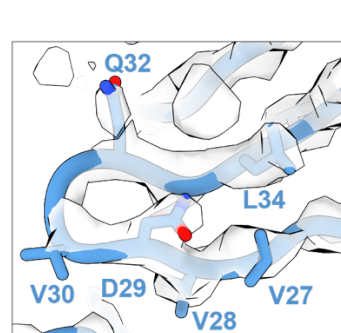
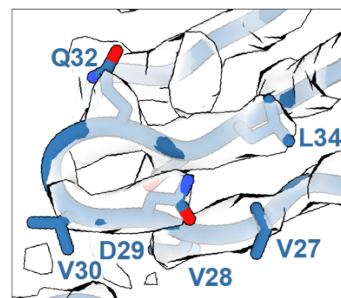
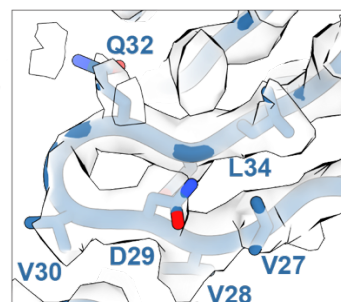
maroon). **Bottom Right:** Fourier Shell Correlation curves for the final maps generated in Relion and the density modified maps generated in Phenix. Pale colors represent Relion gold-standard maps with an FSC cutoff of 0.143 (grey dash-dot line) and dark colors represent density modified maps with an FSC cutoff of 0.5 (black dashed line). Yellow curves represent the full FimH-Fab structures with solvent mask only, while blue curves are reconstructions assembled using the focus mask shown in **Supplementary Fig. 2B**.



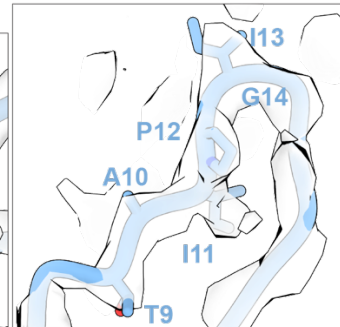
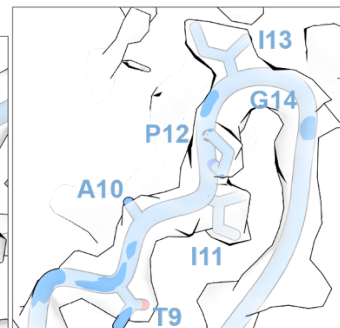
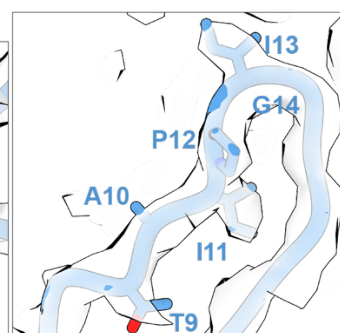
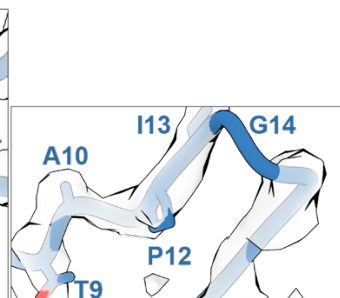
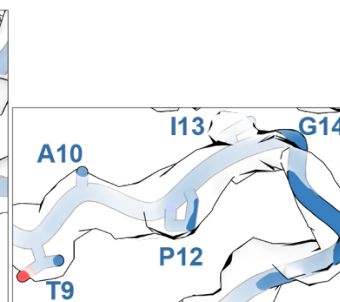
Supplementary Figure 6: Full Volume and Models in two viewing directions. Volume (surface in transparent white) and models (ribbons in: FimH, blue; FimG, grey; Fab21, gold; Fab475, teal; Fab824, maroon; Fab926, orange) for all high-resolution datasets.



B *Swing Loop*

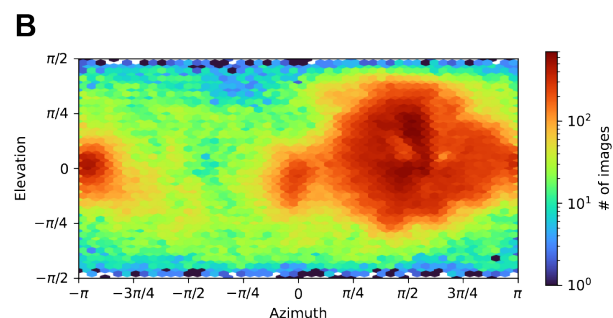
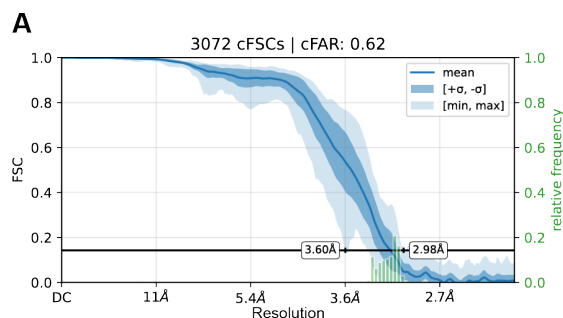


C *Clamp Loop*

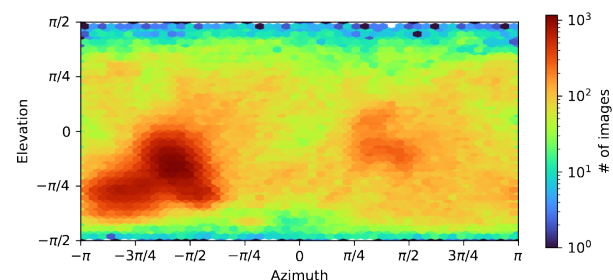
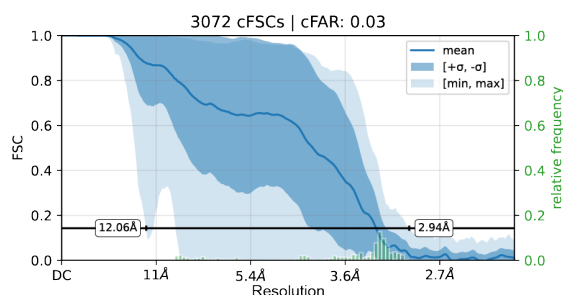


Supplementary Figure 7: Local resolution estimations and representative volume and model sections.
A. Local resolution estimation for all high-resolution structures, calculated in cryoSPARC and mapped onto the density modified volumes; scale in Å shown in top left quadrant applies to all maps. **B.** Volume and model for the swing loop from each of the high-resolution datasets. **C.** Volume and model for the clamp loop from each of the high-resolution datasets. **B-C.** FimH inactive conformation, dark blue; FimH intermediate conformation, blue; FimH active conformation, light blue; volume in transparent white.

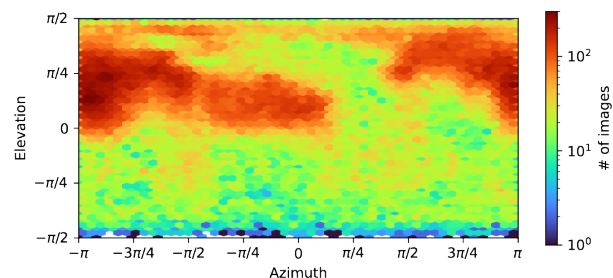
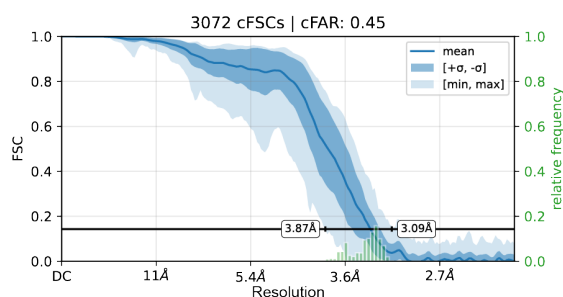
FimH + Fab824/926
Low Affinity



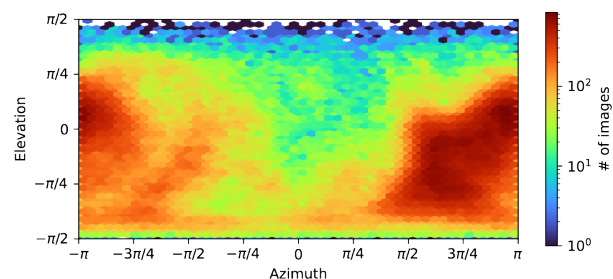
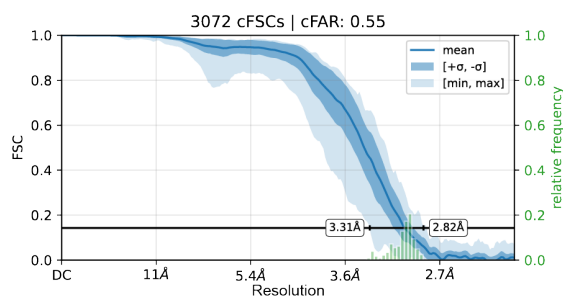
FimH + Fab824
Low Affinity



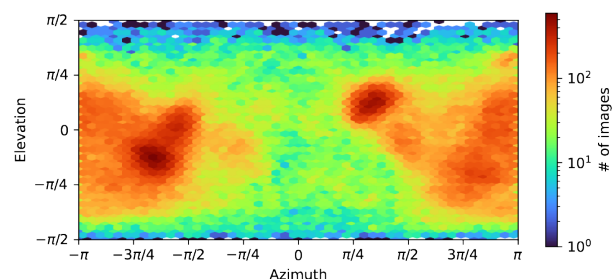
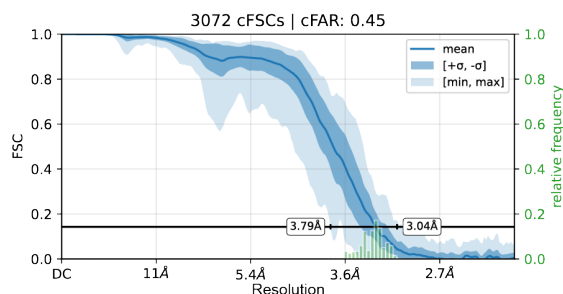
FimH + Fab475/824
Intermediate



FimH + Fab21/475/824
High Affinity

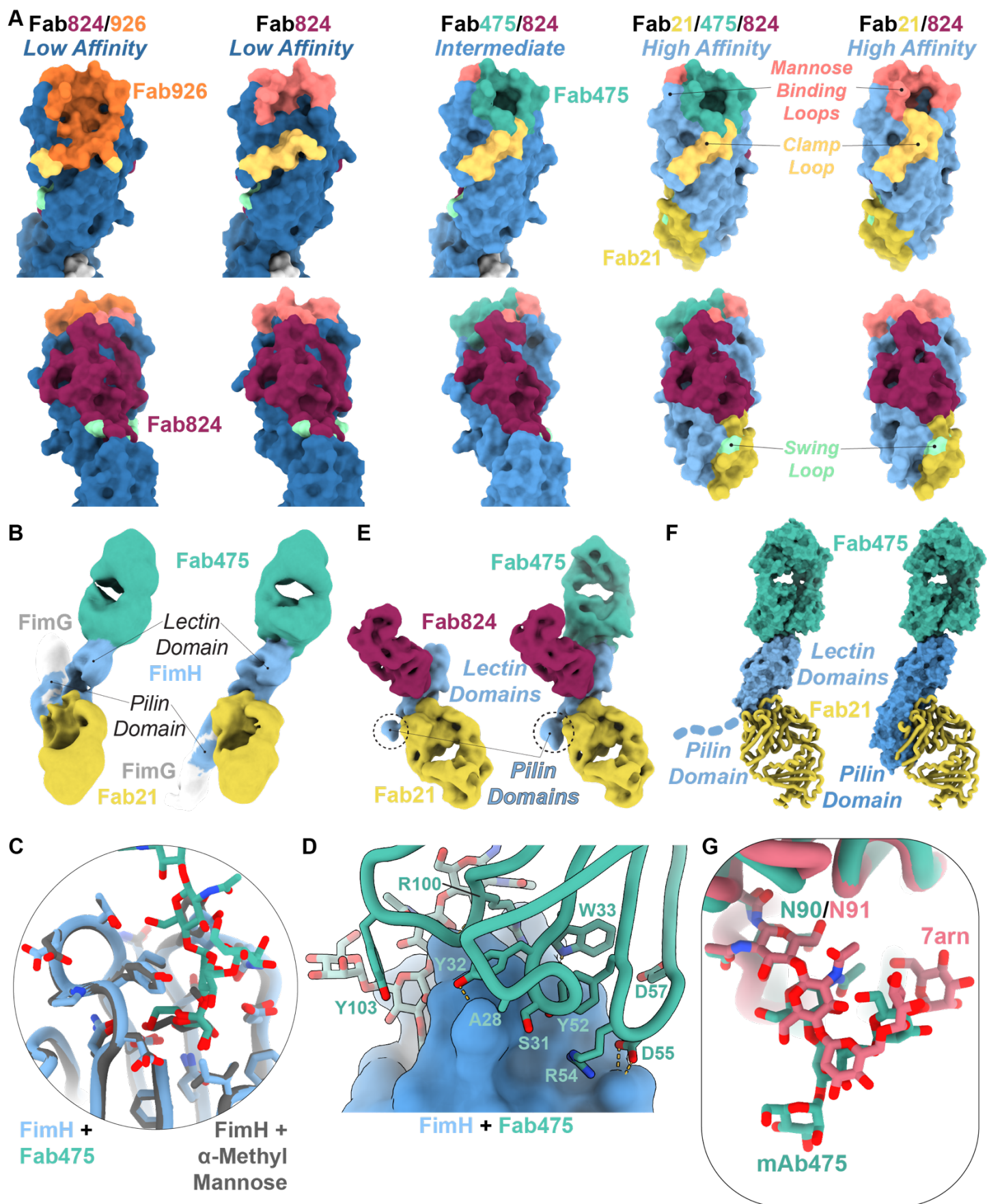


FimH + Fab21/824
High Affinity



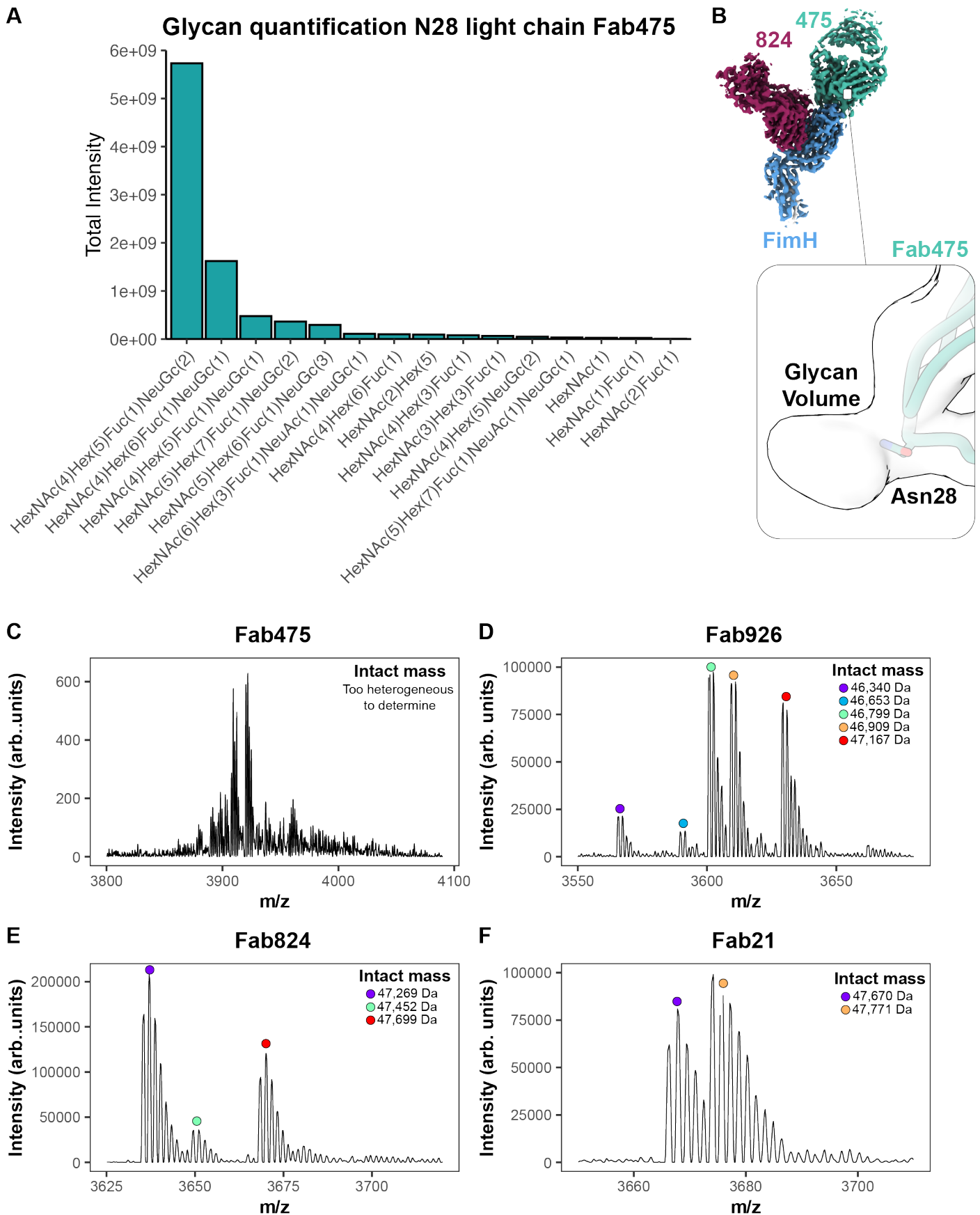
Supplementary Figure 8: Conical FSC plots and viewing direction distribution plots. *Left:* Conical Fourier Shell Correlation (cFSC) plots (showing the range of resolutions produced when the Fourier sphere is split into

cones) for the final full volume determined in cryoSPARC for each high-resolution dataset. Black horizontal line indicates the 0.143 FSC cutoff. *Right:* Viewing direction distribution plots for each high-resolution datasets for the final full volume determined in cryoSPARC for each high-resolution dataset. All plots generated in cryoSPARC.



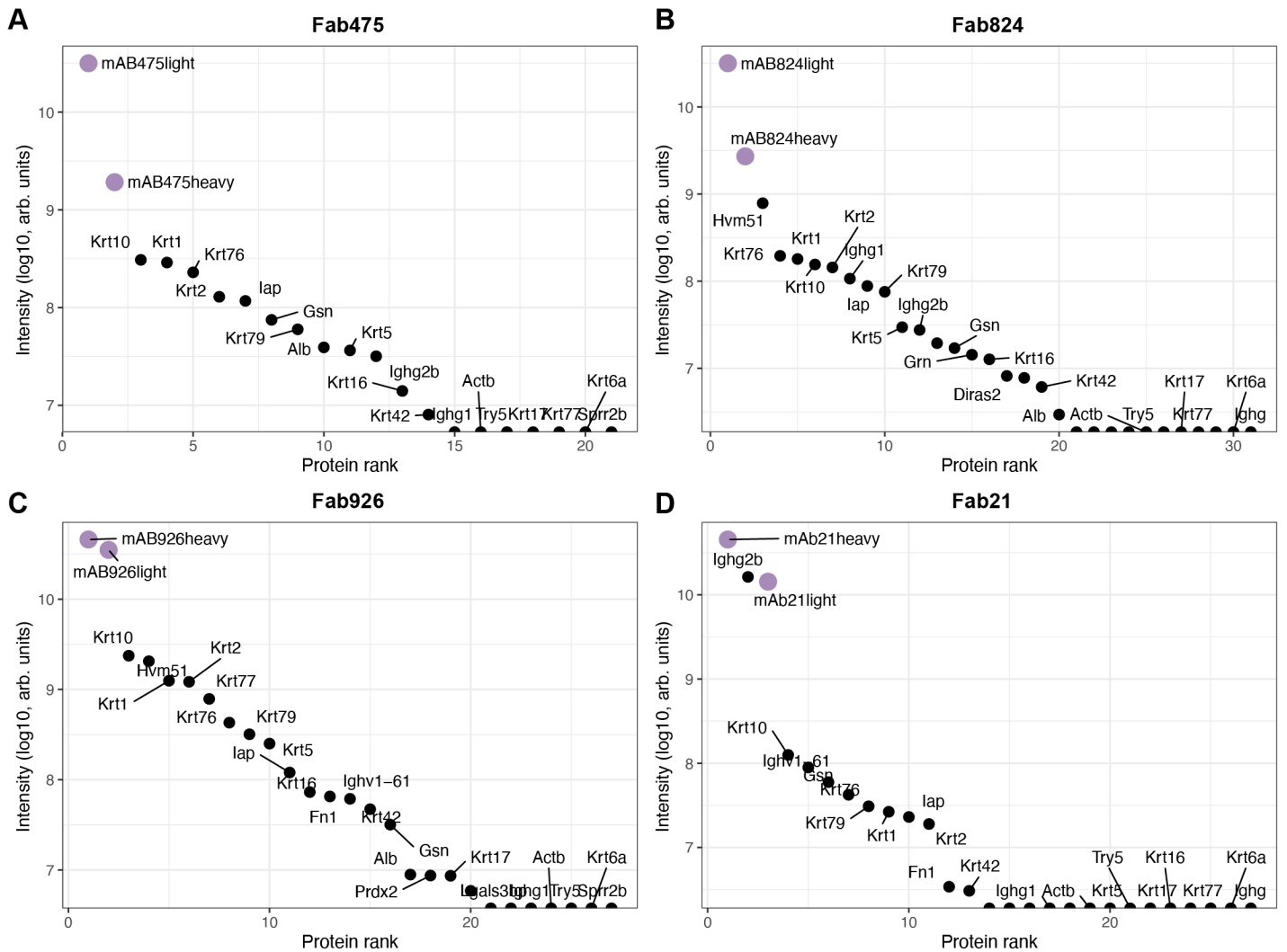
Supplementary Figure 9: FimH binding epitopes and supporting figure panels for FimH in complex with Fab475 and Fab21. A. Surface representation of FimH from each of the high-resolution structures with

epitopes (dark colors) mapped onto the surface. Surfaces are 180° rotated for each model. Fab surfaces: orange, Fab926; teal, Fab475; maroon, Fab824; gold, Fab21. Loops: pink, mannose binding; light yellow, clamp; light green, swing. Quantification of epitope surface area can be found in **Ext. Data Table 1. B**. Reconstruction of FimH bound to Fab21 (gold) and Fab475 (teal) with the pilin domain (FimH, light blue) and FimG (light grey) in two distinct orientations. **C**. Alignment of FimH lectin domains from the FimH-Fab21/475/824 complex (FimH: light blue sticks and ribbon; Fab475: teal sticks and ribbon) and the FimH-Fab21/824 complex (grey sticks and ribbon). **D**. Contact surface between Fab475 (teal sticks) and FimH (blue surface). Fab475 residues within 5 Å of FimH shown. **E**. Volumes of FimH + Fab21/824 (left) or Fab21/475/824 (right) reconstructed with only a large circular mask and Gaussian filtered. Portion of pilin domain present in volumes indicated with dashed circle. FimH (light blue); Fab21 (gold); Fab475 (teal); Fab824 (maroon). **F**. Surface representation of Fab475 (teal) and FimH (blue) demonstrating how Fab21 (gold ribbon) does not clash with the pilin domain in the active conformation (light blue, left) but does clash in the inactive or intermediate conformations (blue, right). **G**. Overlay of glycan from Fab475 (teal) with glycan from a Fab derived from a surface immunoglobulin (PDB ID: 7ARN; rose). Alignment on the Fab light chains, with the panel centered on the glycans.



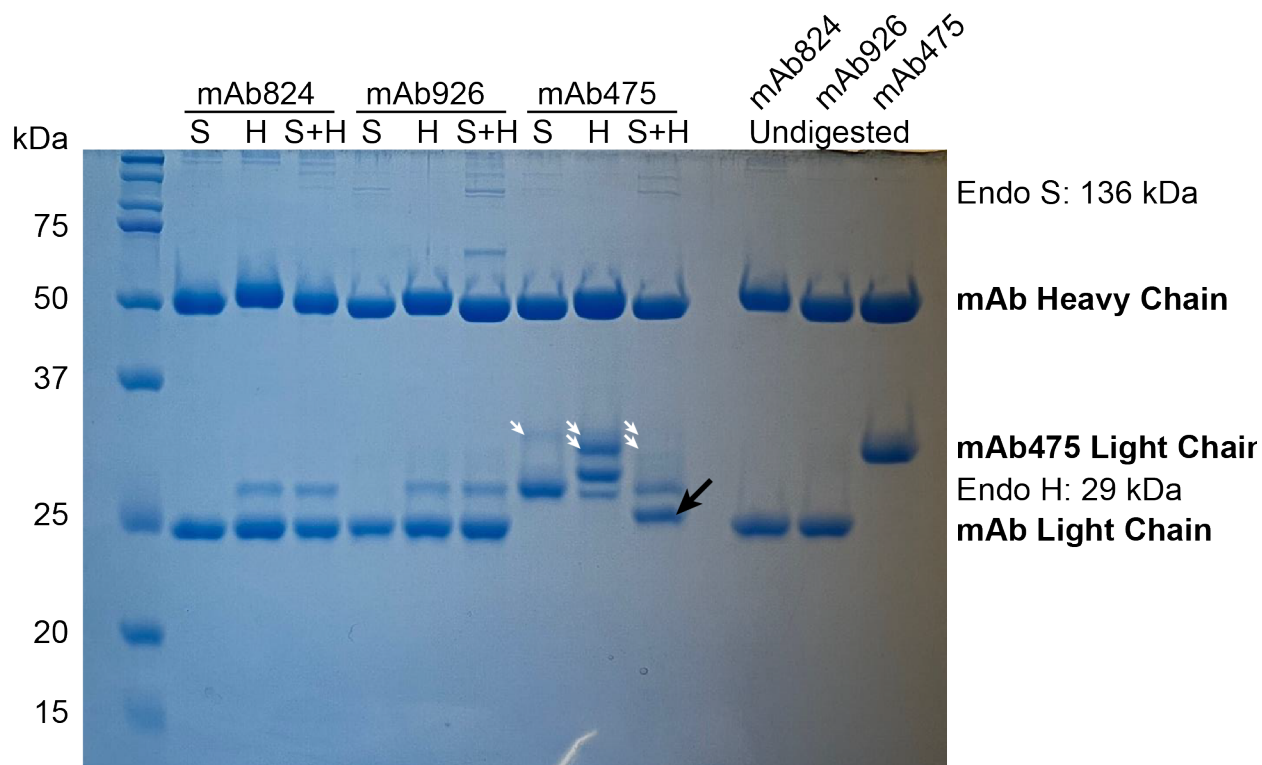
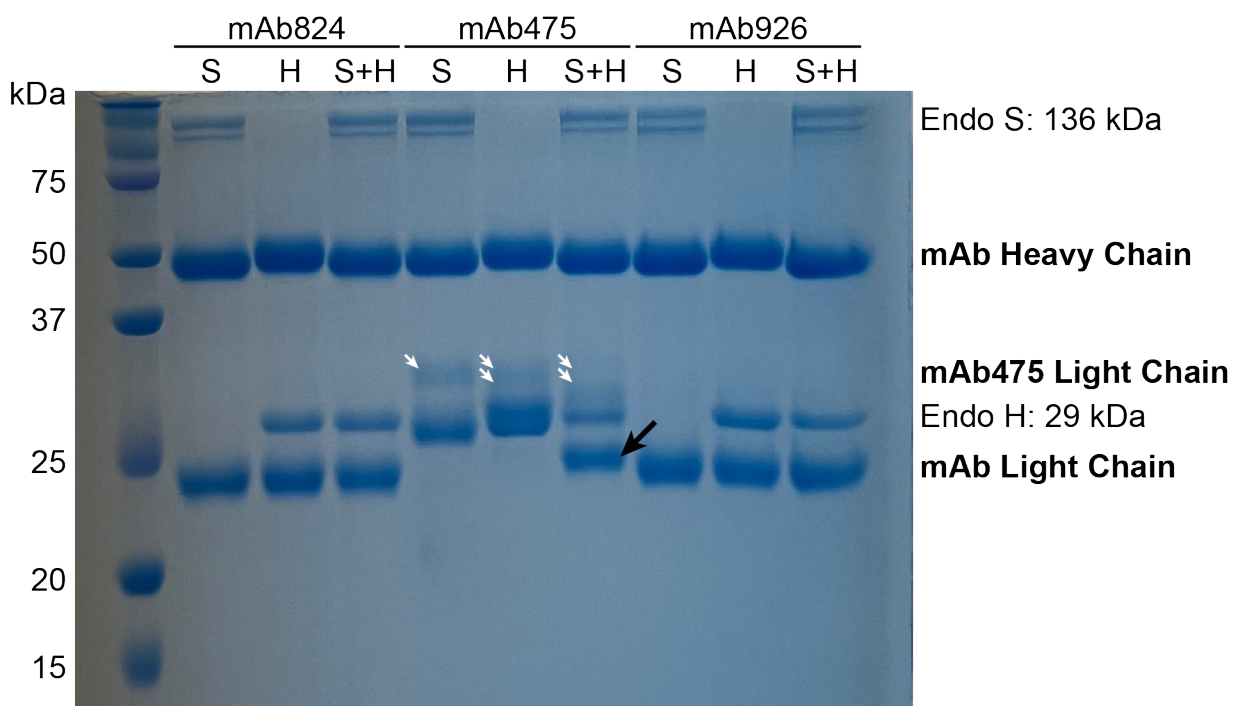
Supplementary Figure 10: Quantification of N-glycosylation 28 on the Fab 475 light chain and intact glycopeptide mass spectrometry analysis of the Fabs. A. Fab molecules were subjected to intact glycopeptide mass spectrometry analysis, and glycan abundances were quantified based on the area under

the curve of the extracted ion chromatogram. **B.** Volume of FimH + Fab475/824 with site of Asn28 shown by white box. *Inset:* Low-pass filtered volume of FimH in complex with Fab475 showing volume for glycosylation at Asn28. FimH (blue); Fab475 (teal); Fab824 (maroon) **C.** Fab475 showed highly heterogeneous spectra preventing intact mass calculations, which is typical for glycoproteins. **D-F.** Fab molecules 926, 824, and 21 were deconvoluted using UniDec¹, and their calculated intact mass is presented.

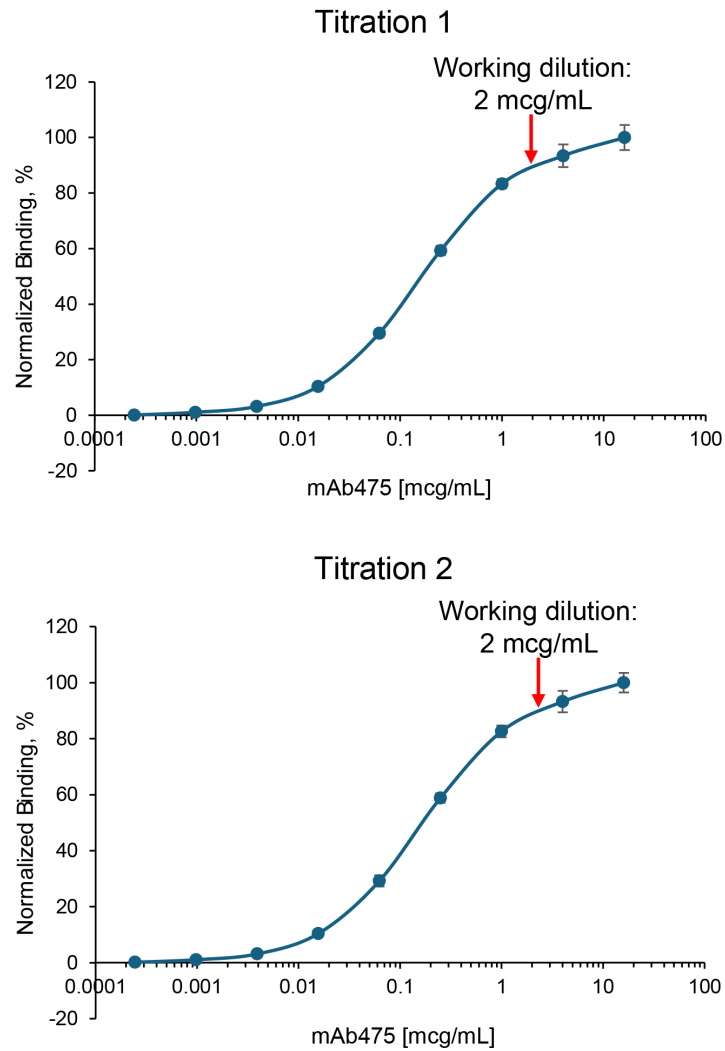


Supplementary Figure 11: Fab purity by Mass Spectrometry. A-D. Search of the bottom-up mass spectrometry data against the whole mouse proteome to confirm the purity of the Fabs. Quantification of the proteins was performed using the unique peptides identified for each protein. Light purple circles indicate target protein. A (Fab475), B (Fab824), and C (Fab926) exhibit high purity and primarily contain keratin as a source of contamination (a common background contaminant in all proteomics experiments). In the Fab21 sample (D), only immunoglobulin heavy constant gamma 2B (Ighg2b) was also identified at a similar abundance.

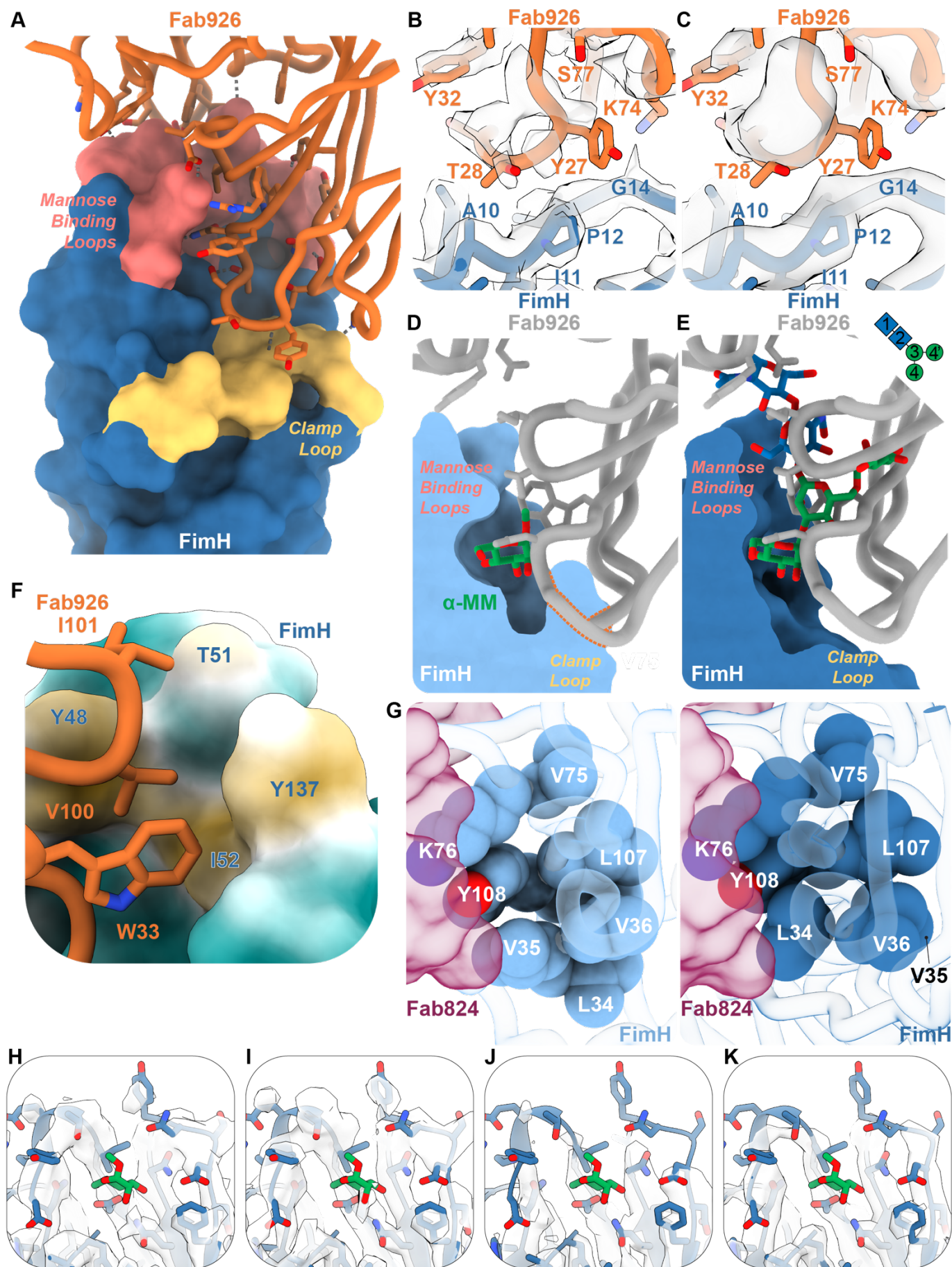
Fab fragments digested with endoglycosidase S, endoglycosidase H, or both endoglycosidases. Full, uncut acrylamide gels can be found in Supplementary Fig. 10. *Bottom*: Normalized ELISA results of antibody after glycosylation treatment listed on the x-axis. mAb475, mAb926: N = 3 biological replicates; mAb824: N = 2 biological replicates; bar chart presented as mean values $\pm$ SD. **B**. Sequence alignment of light chains from mAb475, mAb475-like, and outgroup antibodies. mAb475 shown in teal; teal boxes indicate Asn28 and Asn90 glycosylation site locations. Text in red indicates glycosylation motif present (Nxs/T, x $\neq$ P). Scale bar represents number of substitutions per site. **C**. ELISA results for each antibody in this study against the FimH variant FocH and the mutant FocH-F1L. N = 3 biological replicates; bar chart presented as mean values $\pm$ SEM. **D**. *Left*: Diagram of serum adsorption and FimH ELISA setup for identification of mAb475-like antibodies in the serum pool; normalized results and significances shown in lower left plot. N = 3 biological replicates; bar chart presented as mean values $\pm$ SD. P-values calculated using a Student's t-test for pairwise comparison to evaluate the significance independently. *Right*: ELISA results before normalization in the absence (white) or presence (green) of mannose with serum-adsorbed samples. N = 3 biological replicates; bar chart presented as mean values $\pm$ SD.



Supplementary Figure 13. SDS-PAGE gels of endoglycosidase-treated mAbs. The sugars remaining on Asn90 and Asn28 cause the deglycosylated light chain of mAb475 to migrate more slowly than the light chains of the other mAbs (black arrows). The mAb475 digests (white arrows) contain uncleaved light chain and species not found in the other antibody digests. Sections from lower gel shown in **Supplementary Fig. 11A**.

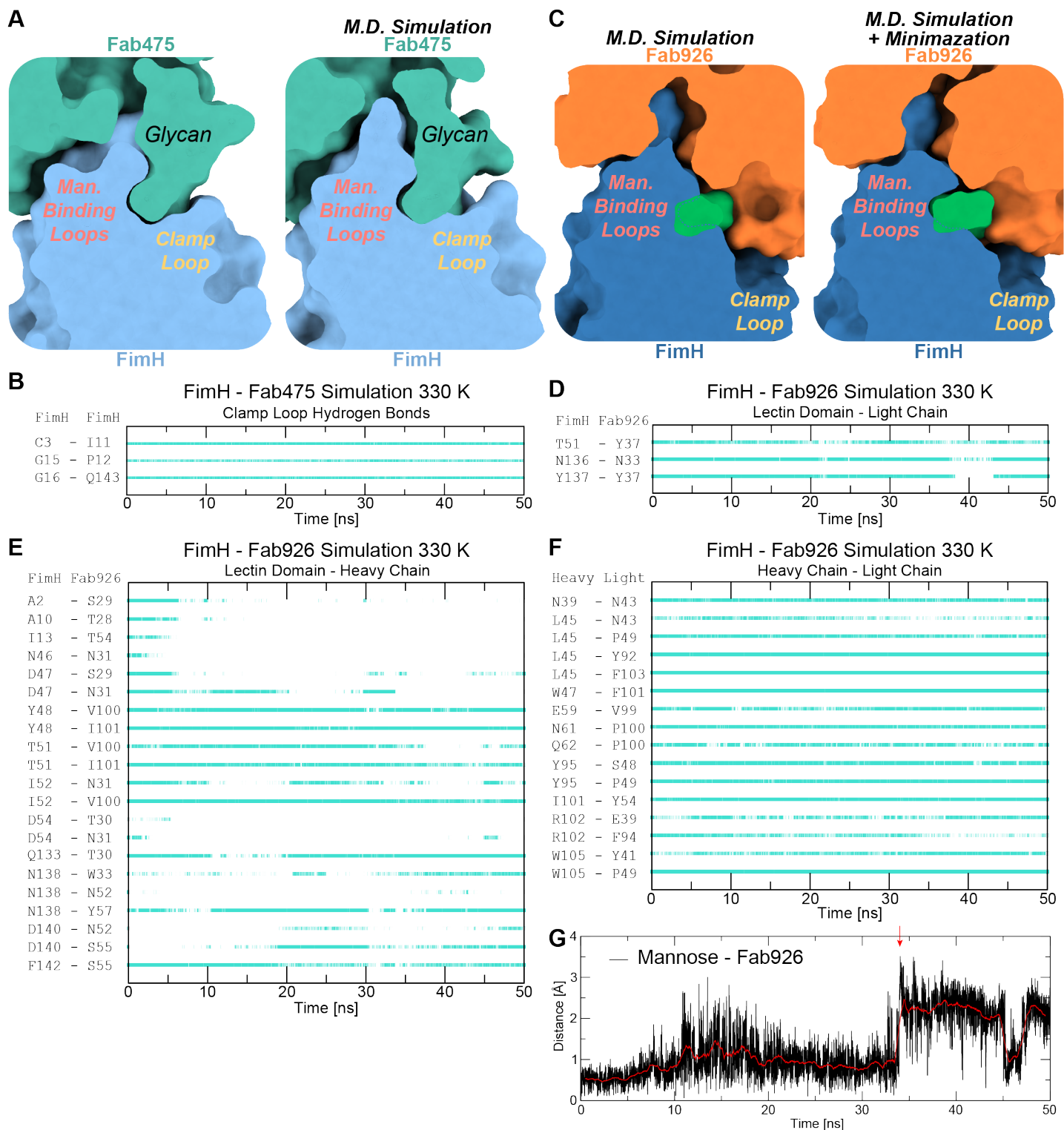


Supplementary Figure 14. Titrations of mAb475 in ELISA assays against FimH-containing pili. Red arrow indicates the concentration used for the ELISA data shown in **Fig. 2C** and **Supplementary Fig. 11A**. N = 4 technical replicates per titration; data presented at mean values $\pm$ S.D.



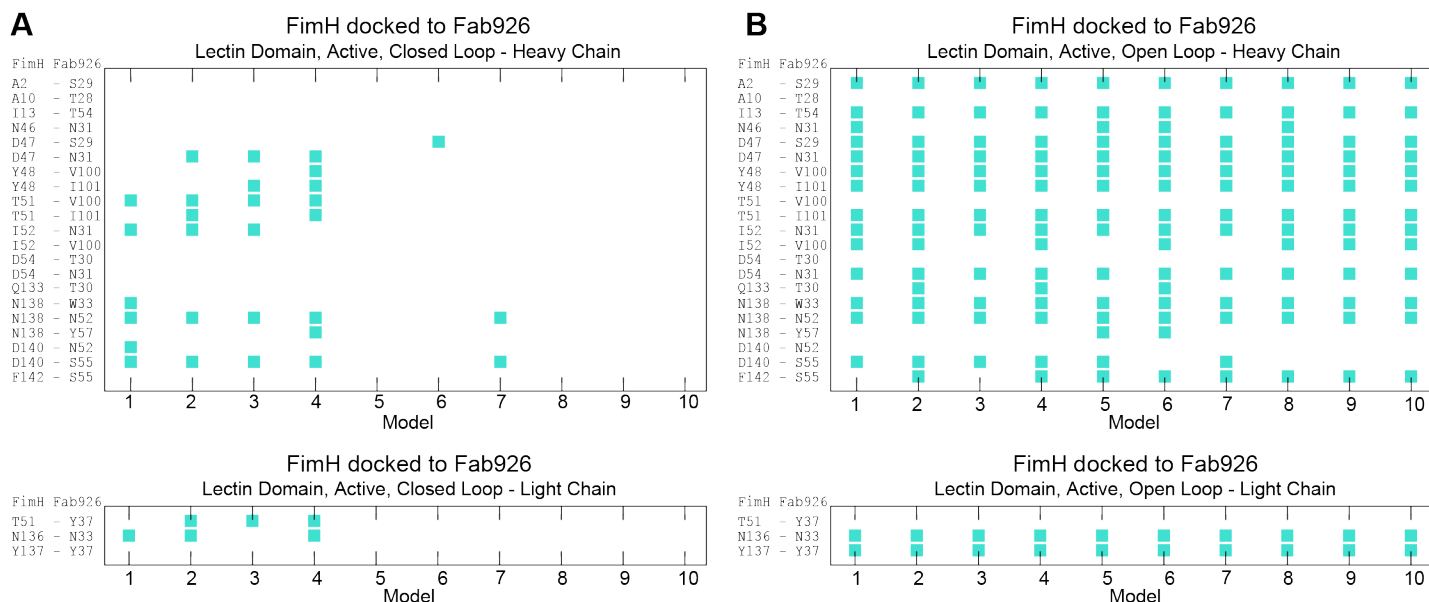
Supplementary Figure 15. Supplementary figure panels for FimH in complex with Fab926 and Fab824.

A. Ribbon and stick diagram of Fab926 showing residues buried in interaction with FimH (dark blue surface). Hydrogen bonds indicated with dashed grey lines. **B-C.** Fab926 (orange) interface with FimH clamp loop (dark blue). *B* shows low-pass filtered volume from *C*. **D.** Alignment of FimH (light blue, surface) in complex with α -methyl mannose (green sticks) with Fab926 (gray sticks and ribbon). Structures were aligned on C α carbons 1, 46-54, and 136-140 of FimH. Orange dotted lines indicate Fab926 loops that clash with the FimH clamp loop. **E.** Alignment of FimH (dark blue, surface) in complex with Fab926 (gray sticks and ribbon) with oligomannose-3 (green/blue; PDB ID 2VCO). Structures were aligned on C α carbons 1, 46-54, and 136-140 of FimH. **F.** FimH surface showing hydrophobic patch (hydrophobicity shown as yellow to teal transition; yellow: more hydrophobic; teal: more hydrophilic). Fab926 residues shown in orange. **G.** Residues in β -strands near the toggle switch residues L34/V35 (blue spheres) in FimH (transparent blue ribbon) pack tightly against each other and Fab824 (transparent maroon surface). **H-K.** Volumes and model of ligand binding pocket of FimH bound to Fab824 only. Mannose position approximated by aligning mannose binding loops of the model containing FimH + Fab21/824/ α -methyl mannose to the model containing FimH + Fab824 only. FimH, blue sticks and ribbon; α -methyl mannose, green sticks. *H.* Final, full, density-modified volume. *I.* Final, locally refined, density-modified volume. *J.* Intermediate volume from FimH + Fab21/475/824 dataset. *K.* Intermediate volume from FimH + Fab21/824/ α -methyl mannose dataset.



Supplementary Figure 16. Molecular dynamics simulations suggest flexibility at the FimH-Fab926 binding interface. **A.** Slice through the cryo-EM structure (left) and the model from the molecular dynamics simulation at 50 ns (right); FimH (light blue surface) bound to Fab475 (teal surface). **B.** Time series of hydrogen bond contacts between FimH clamp loop and the rest of FimH. The frames in which a contact is formed in the simulation are indicated in cyan. **C.** Slice through the molecular dynamics simulation at 50 ns (left) and the molecular dynamics simulation at 50 ns with minimization (right); FimH (dark blue surface) bound to Fab926 (orange surface), with mannose shown in green. Dotted line indicates surface of FimH. **D-E.** Time series of side chain contacts formed between FimH and the variable domains of Fab926. The frames in which a contact is formed in the simulation is indicated in cyan. Simulation was completed with the entire FimH lectin domain (residues 1-160) and Fab926 heavy (2-115) and light chains (2-112), but only those residues that

contact Fab926 are shown in the plots. **D.** FimH-Fab926, light chain contacts. **E.** FimH-Fab926, heavy chain contacts. **F.** Time series of side chain contacts formed between the heavy chain and the light chain of Fab926. The frames in which a contact is formed in the simulation are indicated in cyan. **G.** Heavy atom distances between the docked mannose and Fab926 during the 50 ns simulation. The red line represents the rolling average over a 12-ns window. The gap between FimH and Fab926 widened after 34 ns of simulation time (red vertical arrow).



Supplementary Figure 17. Molecular dynamics simulations suggest Fab926 can displace mannose. A-B. Models and side chain contacts established from rigid body docking of FimH with Fab926 in pyDockWEB. Cyan squares represent side chain contacts formed. **A.** FimH lectin domain in the active conformation with the clamp loop closed docked to Fab926. **B.** FimH lectin domain in the active conformation with the clamp loop open (from²) docked to Fab926.

Supplementary Tables 1-7.

Supplementary Table 1: Protein and buffer concentrations for cryo-EM samples.

	#1	#2	#3	#4	#5†	#6
	Fim. Tips* + Fab824/Fab926	Fim. Tips + Fab475/Fab824	Fim. Tips + Fab21/Fab475/ Fab824	Fim. Tips + Mann./Fab21/ Fab824	Fim. Tips + Fab824	FimH + Fab21/Fab475
Protein	Tip: 0.5 µM Fab824: 0.6 µM Fab926: 0.6 µM	Tip: 0.5 µM Fab475: 0.6 µM Fab824: 0.6 µM	Tip: 0.5 µM Fab21: 0.6 µM Fab475: 0.6 µM Fab 824: 0.6 µM	Tip: 0.5 µM Fab21: 0.6 µM Fab 824: 0.6 µM Mannose: 50 mM	Tip: 0.5 µM Fab 824: 0.6 µM	Tip: 0.5 µM Fab21: 0.6 µM Fab475: 0.6 µM
Buffer	68.5 mM NaCl 1.35 mM KCl 5 mM Na ₂ HPO ₄ 0.9 mM KH ₂ PO ₄ pH to 7.4 with HCl					

*Fimbrial tip purification included FimH, FimG, FimF, and FimC.

†The volume and model presented as #5 is derived from a subset of the data available in datasets #3 and #4

Supplementary Table 2: Cryo-EM Map and Model Statistics.

	#1 Fim. Tips + Fab824/ Fab926	#2 Fim. Tips + Fab475/ Fab824	#3 Fim. Tips + Fab21/Fab475/ Fab824	#4 Fim. Tips + Fab21/Fab824 + Mannose	#5† Fim. Tips + Fab824
Data Collection					
Grid	ANTcryo 2/1 300Au holey NiTi	CF 2/2 400Cu holey carbon	CF 2/1 400Cu holey carbon	Quantifoils 2/1 300Au holey carbon	Quantifoils 2/1 300Au holey carbon
Microscope	Krios	Krios	Krios	Krios	Krios
Camera	K3	K3	K3	K3	K3
Software	Serial EM 4.1 & Legion 3.5	Serial EM 4.1	Serial EM 4.1	Serial EM 4.1	Serial EM 4.1
Micrographs (total)	14339	7832	9176	10383	19559
Nominal magnification	105,000 X	105,000 X	105,000 X	105,000 X	105,000 X
Voltage (kV)	300	300	300	300	300
Electron Fluence (e ⁻ /Å ²)	42/55	42	42	42	42
Pixel Size (Å)	0.842	0.842	0.842	0.842	0.842
Defocus Range (µm)	-0.5 to -1.25	-0.75 to -1.50	-0.75 to -1.50	-0.75 to -1.50	-0.75 to -1.50
Map Statistics*					
Number of particles (final)	253 258	129 751	333 855	163 363	306 269
Symmetry	None	None	None	None	None
Map Resolution (Masked) FSC _{0.143} (Å)	3.1	3.2	2.8	3.1	3.4
B factor (Å ²)	85	102	74	77	73
Density Modified Resol. (Masked) FSC Ref _{0.5} (Å)	2.9	3.1	2.7	3.0	3.1
Model Statistics‡					
Starting Models	3JWN 15C8	3JWN 15C8 2VOC	5JCR 15C8	5JCR 15C8 AlphaFold	9ME5
Composition					
Protein Residues	1126	1124	1371	943	696
Ligands		NAG: 2 MAN: 3	NAG: 2 MAN: 3	MMA: 1	
R.M.S. deviations					
Bond lengths (Å)	0.003	0.005	0.004	0.009	0.004
Bond angles (°)	0.587	0.562	0.604	0.74	0.667
Validation					
Molprobity Score	1.54	1.47	1.81	1.63	1.68
Clash Score	3.59	4.99	4.22	4.04	5.6
Rotamer Outliers (%)	1.65	0.62	3.29	2.33	0.51
B-factors (min/max/mean)					
Protein	2/167/56	5/196/73	1/187/57	4/166/54	17/124/57
Ligand		18/71/31	18/89/37	47/63/53	
Ramachandran Plot					
Favored (%)	96.4	96.5	96.7	97.1	94.5
Allowed (%)	3.6	3.5	3.3	2.9	5.5
Outliers (%)	0	0	0	0	0
EMDB ID	EMD-48184	EMD-48183	EMD-48185	EMD-48186	EMD-71842
PDB ID	9ME5	9ME4	9ME6	9ME7	9PTM

†The volume and model presented as #5 is derived from a subset of the data available in datasets #3 and #4

*Gold standard FSC_{0.143} calculated in cryoSPARC or Relion and Density Modified Resolution FSC_{0.5} calculated in Phenix.

‡Model statistics generated using Phenix Validation Tools.

Supplementary Table 3: Data Collection Parameters and Map Statistics for FimH + Fab21/Fab475.

	#6 FimH + Fab21/Fab475 Conformation 1	#6 FimH + Fab21/Fab475 Conformation 2
Data Collection		
Grid	CF 2/2 400Cu holey carbon	CF 2/2 400Cu holey carbon
Microscope	Glacios	Glacios
Camera	K3	K3
Software	Leginon 3.5	Leginon 3.5
Micrographs (total)	3222	3222
Nominal magnification	45,000 X	45,000 X
Voltage (kV)	200	200
Electron Fluence (e ⁻ /Å ²)	50	50
Pixel Size (Å)	0.89	0.89
Defocus Range (μm)	-0.75 to -1.50	-0.75 to -1.50
Map		
Number of particles (final)	100 853	87 632
Symmetry	None	None
Map resolution (Masked)	5.4	6.7
FSC _{0.143} (Å)		
<i>B</i> factor (Å ²)	-467	-273
EMDB ID	EMD-48187	

Supplementary Table 4: FimH Epitope Residues.

mAB/Fab Designation	FimH residues interacting with Fab light chain*	FimH residues interacting with Fab heavy chain*	Buried Area (Å ²)			Prior Data Source Ref.
			Light	Heavy	Total	
mAB21	23-24, 26-27 , 118, 122, 151- 153, 155	25-26 , 27-28, 29-30 , 32-33, 35, 37, 117, 155-157 , 158	302	516	756	3
mAB475	1 , 13, 46-48 , 51, 52, 54, 133, 135 , 137, 138, 140, 142	51, 52 , 55, 92, 134, 135, 136 , 137, 138, 139	372	384	712	4
mAB824 <i>FimH inactive conformation</i>	55,78, 79-80,82,88-90, 91 , 92, 94	27, 29-30, 32, 34 , 37, 39-40, 74, 76, 78, 79-82 , 83, 87, 88, 101-102, 104, 108	363	676	983	5
mAB824 <i>FimH active conformation</i>	55,78, 79-80,82,88-90, 91 , 92, 94	33, 35 , 37, 39, 74, 76, 78, 79-80 , 81-83, 87-88, 91 , 104, 108	333	537	839	5
mAB926	50, 51, 136 , 137	1, 2, 10-15, 46-48, 51, 52 , 54, 133, 135, 137 , 138 , 139, 140 , 142	282	727	983	4

*Epitope residues were determined with the “measure buriedArea” command in ChimeraX.

Bolded residues were identified in prior mutational analyses or NMR studies cited in the Prior Data column.

Italics indicate conformation where relevant.

Supplementary Table 5. Parameters for molecular dynamics simulations.

FimH Lectin Domain Conformation	Antibody	Box* Dimension [Å]	Number of Atoms	Temperature [K]	Duration [ns]
Inactive (Low Affinity)	mAb926	118	157,158	300	2 x 50
Inactive (Low Affinity)	mAb926	118	157,158	330	50
Active (High Affinity)	mAb475	125	187,370	300	2 x 50
Active (High Affinity)	mAb475	125	187,370	330	50
Inactive (Low Affinity)	mAb824	100	94,455	300	2 x 50
Active (High Affinity)	mAb824	100	94,494	300	2 x 50
Active (High Affinity)	mAb21	116	149,046	300	2 x 50

*Cubic water box used.

Supplementary Table 6: RMSD Calculations for the FimH lectin domain in the absence and presence of each mAb.

FimH Conformation	Antibody	MD Simulation Clamp Loop RMSD $\pm$ SEM [Å]	P-value* (antibody-bound vs. unbound)	MD Simulation Swing Loop RMSD $\pm$ SEM [Å]	P-value* (antibody-bound vs. unbound)	Number of Simulations (N)
Active (High Affinity) + Mannose	None	0.84 $\pm$ 0.01	N/A	0.62 $\pm$ 0.06	N/A	2
Active (High Affinity)	mAb475	0.58 $\pm$ 0.03	0.0114	1.33 $\pm$ 0.10	0.0255	2
Active (High Affinity)	None	1.69 $\pm$ 0.44	N/A	0.55 $\pm$ 0.01	N/A	3
Active (High Affinity)	mAb21	0.96 $\pm$ 0.27	0.3115	0.44 $\pm$ 0.06	0.0997	2
Active (High Affinity)	mAb824	0.90 $\pm$ 0.01	0.2545	0.50 $\pm$ 0.01	0.0403	2
Inactive (Low Affinity)	None	1.46 $\pm$ 0.01	N/A	0.64 $\pm$ 0.08	N/A	2
Inactive (Low Affinity)	mAb824	1.16 $\pm$ 0.03	0.012	0.80 $\pm$ 0.15	0.4255	2
Inactive (Low Affinity)	mAb926	1.09 $\pm$ 0.05	0.0161	0.89 $\pm$ 0.15	0.2747	2

*The P-values were calculated from a two-tailed Student's t-test; P-values < 0.05 highlighted in bold. The full description of the calculations can be found in the methods.

Colors as defined in the table indicate the conformation of FimH and the antibody as shown in Main Text Figure 1.

Supplementary Table 7. Primers used to sequence mAB21.

Chain	Name	Direction	Sequence
Light Chain	VL4	forward	CCAGTTCCGAGCTCCAGATGACCCAGTCTCCA
Light Chain	VL5	forward	CCAGATGTGAGCTCGTGATGACCCAGACTCCA
Light Chain	VL7	forward	CCAGTTCCGAGCTCGTGATGACACAGTCTCCA
Light Chain	VLr	reverse	GCGCCGTCTAGAATTAACACTCATTCTGTTGAA
Heavy Chain	VH1	forward	AGGTCCAGCTGCTCGAGTCTGG
Heavy Chain	VH3	forward	AGGTCCAGCTTCTCGAGTCTGG
Heavy Chain	VH5	forward	AGGTCCAAGTCTCGAGTCAGG
Heavy Chain	VH3_1	reverse	AGGCTTACTAGTACAATCCCTGGGCACAAT
Heavy Chain	VH3_2a	reverse	GTTCTGACTAGTGGGCACTCTGGGCTC

Supplementary References

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