

Manuscript version: Author's Accepted Manuscript

The version presented in WRAP is the author's accepted manuscript and may differ from the published version or Version of Record.

Persistent WRAP URL:

<http://wrap.warwick.ac.uk/130196>

How to cite:

Please refer to published version for the most recent bibliographic citation information. If a published version is known of, the repository item page linked to above, will contain details on accessing it.

Copyright and reuse:

The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions.

Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Publisher's statement:

Please refer to the repository item page, publisher's statement section, for further information.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk.

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: <i>Smith_ED_Fig1.jpg</i>	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.
Extended Data Fig. 1	Restriction activity and surface exposure of SERINC.	Extended_Data_Fig1_VEP.jpeg	<p>a,b, HIV-1 restriction activity of <i>DmSERINC</i> compared with human SERINC5 and SERINC2. Human and <i>DmSERINC</i> proteins with HA tags at their C-termini were expressed in HEK293T cells with two different expression vectors (pcDNA and pBJ6) which provide high and low expression, respectively. Levels of the indicated SERINC proteins were assessed by Western blotting using an anti-HA antibody (uncropped blot images are shown in the Source Data) (a) and by flow cytometry (b) to detect the proteins surface expression (b, top) or total expression (b, bottom) using an anti-FLAG antibody on non-permeabilized and permeabilized cells respectively. c, Effect of SERINC expression on infectivity of HIV-1 produced in HEK293T cells transfected to express the indicated SERINC-iFLAG-HA and Nef-deficient HIV-1_{NL4-3}. (Data shown are mean and s.d. of n=4 technical repeats. Data are provided in the Source Data). d, Insertion of the FLAG epitope into ECL4 does not interfere with the anti-HIV-1 restriction activity of SERINC5. Infectivity of Nef-deficient HIV-1_{NL4-3} produced in HEK293T cells transfected to express unmodified human SERINC5-HA or a variant modified by inserting a FLAG tag within its ECL4 (SERINC5-iFLAG-HA). Two different expression vectors (pcDNA and pBJ6) were used in order to obtain high and low SERINC5 expression as shown</p>

			<p>above (Data shown are mean and s.d. of n=4 technical repeats. Data are provided in the Source Data). e, Effect of ECL5 SERINC5 variants on HIV-1 susceptibility to neutralization. IC₅₀ values derived from fitted sigmoidal curves shown in figure 4, obtained from quadruplicate repeats using antibodies 2F5 and 4E10 on Nef-defective HIV-1_{NL4-3} pseudotyped with the envelope glycoprotein derived from HIV-1_{JR-FL}, produced by transfecting HEK293T cells with the indicated PBJ5-SERINC5-iFLAG-HA variants or the empty vector control (Data shown are mean and 95% confidence interval of n=4 technical repeats. Data are provided in the Source Data).</p>
Extended Data Fig. 2	Human SERINC5 purification and EM	Extended_Data_Fig2_VE P.jpeg	<p>a, Size exclusion chromatography profile. b, Left: SDS-PAGE analysis of resulting fractions; right: cleavage of TwinStrep tag and deglycosylation (uncropped gel images are shown in the Source Data). c, Sample micrograph of negatively stained particles. d, Representative 2D class averages. e, Schematic of image processing and reconstruction of the human SERINC5 cryo-EM structure. Details are given in Extended Methods. f, Left: Gold standard FSC curve for the cryo-EM reconstruction of SERINC5, Right: Euler angle distribution plot for particles included in the final 3D reconstruction; 3DFCS reports a sphericity of 0.976. g, The map colored according to local resolution estimated with blocres.</p>
Extended Data Fig. 3	<i>Dm</i> SERINC purification and EM.	Extended_Data_Fig3_VE P.jpeg	<p>a, Left: chromatography profile of <i>Dm</i>SERINC on a Superdex 200 column; the blue arrow highlights elution of the material, which was re-injected onto the column. Right: elution profile of hexameric <i>Dm</i>SERINC. b, Left: SDS PAGE</p>

			<p>analysis of chromatography fractions; Right: purified hexamer (first 4 lanes) and monomer (last four lanes) uncleaved vs cleaved sample showing higher oligomeric states in hexamer sample shift upon cleavage of the C-terminal TwinStrep tag (uncropped gel images are shown in the Source Data). c, Sample micrograph of negatively stained <i>DmSERINC</i> sample from 9.8-ml peak. d, 2D class averages of negatively stained <i>DmSERINC</i>. e, Schematic of image processing and 3D reconstruction of the <i>DmSERINC</i> hexamer. Volumes are shown at two contour levels, towards the protein level in solid white and the outline of the detergent micelle in transparent grey. Details of the image processing and reconstruction are given in Extended Methods. f, Left: Gold-standard FSC curve for the refined <i>DmSERINC</i> cryo-EM map. f, Right: Euler angle distribution plot for aligned particles contributing to the 3D reconstruction; bar lengths and color (blue low, red high) correspond to numbers of particles in corresponding orientations. g, Cryo-EM map colored according to local resolution estimated with blocres and shown at high (left) and low (right) contour levels. h, Cryo-EM maps of the asymmetrical <i>DmSERINC</i> hexamer (corresponding to 3D classes 3 and 8 in Extended Data Fig. 3e) with fitted model: viewed down 6-fold axis (top) or from side (bottom). The map is contoured to highlight the protein components (right) or the detergent micelle (left).</p>
Extended Data Fig. 4	Structural features of <i>DmSERINC</i>	Extended_Data_Fig4_VE_P.jpeg	<p>a, Transmembrane topology diagram of <i>DmSERINC</i> structure with residues not resolved in the cryo-EM map shaded grey. b,</p>

			<p>Topology diagram of the SERINC protein fold, colored as in Fig. 1b. ECLs and ICLs are labelled along with disulphide bonds and subdomains A and B. c, Scatter plot of top 500 results from analysis using Dali server, showing numbers of aligned residues versus root mean square deviations (Å) of Cα atom positions. d, <i>DmSERINC</i> hexamer colored by conservation; Guillemet indicates the viewpoint on the protomer-protomer interface labeled with asterisk that is shown in the sideview on the right. e, Examples of <i>DmSERINC</i> cryo-EM map with fitted model. f, Two disulphide bonds identified on the extracellular side of <i>DmSERINC</i>. Left: Cryo-EM map showing profile of Cys71-Cys91 disulphide bond within ECL1. Right: Cryo-EM map showing profile of Cys238-Cys299 disulphide bond between ECL3 and ECL4. Thermostability of the <i>DmSERINC</i> hexamer (g), monomer (h), and SERINC5 (i) with the addition of reducing agents (0.5 mM DTT and 0.5 mM TCEP); data shown are mean and s.d. n=3-4 technical repeats, data are provided in source data. j, Molecular dynamic simulations of solvation. Left top: Density analysis of waters (blue surface) around <i>DmSERINC</i> (grey cartoon) in one repeat of atomistic 230-ns simulation. Left bottom: Water density shown as a 2D heatmap slice. Right: <i>DmSERINC</i> residues implicated in controlling water wire highlighted in green.</p>
<p>Extended Data Fig. 5</p>	<p>Lipidomics of <i>DmSERINC</i> structure</p>	<p>Extended_Data_Fig5_VE P.jpeg</p>	<p>a, Cryo-EM map features of <i>DmSERINC</i> displaying similarities with cardiolipin viewed with (right) and without (left) coordinates built, from two angles. b, Positions of the tentative cardiolipin sandwiched between the protomers of the hexamer. c-e, Identification of</p>

			<p>lipids associated with <i>DmSERINC</i> by mass spectrometry: c, Lipidomics LC-MS analysis of hexameric <i>DmSERINC5</i> purified from yeast cells. Ions corresponding to phospholipids (PE, PC, PI) and cardiolipin compositions are indicated. d, Structures within each lipid class are confirmed by MS/MS fragmentation. Neutral loss fragments, such as R1COO⁻ and R2COO⁻ ions, are diagnostic for PE, PC, PI and cardiolipin (CL). e, Native mass spectra of <i>DmSERINC</i> monomers (10⁺ to 15⁺ charge state distribution) isolated from LMNG micelles spiked with PC, PG, PE or CL lipids added at a 1:1 molar ratio. Up to two equivalents of bound CL were observed whereas no distinct binding was detected for PC, PG, or PE.</p>
Extended Data Fig. 6	Lipid screening	Extended_Data_Fig6_VE_P.jpeg	<p>a, Lipid binding groove apparent in <i>DmSERINC</i> structure, top left: Surface representation of <i>DmSERINC</i> monomer revealing a groove formed between TMs 5, 7, 8 and 4. top right: Lipid moiety modeled into the groove, shown in spheres, illustrating complementary size, shape and location for lipid binding. bottom left: Cartoon representation of the same view with helices labelled and colored as Fig. 1b. bottom right: Cartoon representation with lipid shown in stick format. b, Cryo-EM map has lipid-like features in this groove, left: map with PS modelled in, right: map carved to 2.5 Å around the modelled PS to highlight the lipid-like map features. e. View of <i>DmSERINC</i> in a POPC membrane, following 215 ns of atomistic simulation. The protein is shown as blue cartoon and transparent surface, and the POPC lipids as red, orange and grey spheres. Lipids in front of the protein have been removed to reveal how the protein sits in the</p>

			<p>membrane. d, Post 215 ns view of <i>DmSERINC</i> from atomistic MD simulation, showing a POPC lipid bound to the groove between TM 5 and 8. The protein is shown in white cartoon, the lipid in green, red and gold spheres. Note that this lipid remains bound for the full simulation. e-g, Lipid thermostability assay. e, Change in thermostability of <i>DmSERINC</i> hexamer upon the addition of specific lipid. f, Change in thermostability of <i>DmSERINC</i> monomer upon the addition of a specific lipid; g, Change in thermostability of SERINC5 upon the addition of a specific lipid (select sample of lipids). Data shown in e-f are mean and s.d. of 3-6 technical repeats, data are provided in Source Data.</p>
Extended Data Fig. 7	HDX of lipid interactions with <i>DmSERINC</i>	Extended_Data_Fig7_VE_P.jpg	<p>a, Left: Peptide coverage of <i>DmSERINC</i> monomer for HDX. Right: Structure of <i>DmSERINC</i> (with undefined loops modeled in using SWISS MODEL) with coverage highlighted in blue. b-e, HDX profile of purified monomeric <i>DmSERINC</i> in LMNG micelles prior to (b) or after spiking with exogenous DPPS (c), sulfatides (d), or PC (e) Peptide residue numbers are shown on the x-axis. f, Protected regions determined by HDX mapped onto the <i>DmSERINC</i> structure and highlighted in red (with undefined loops modelled in using SWISS MODEL). g, surface representation of <i>DmSERINC</i> structure colored as in Fig. 1b. with protected regions highlighted in red.</p>
Extended Data Fig. 8	Juxtaposition of SERINC5 and the trimeric HIV-1 envelope spike.	Extended_Data_Fig8_VE_P.jpeg	<p>The model of human SERINC5 is shown in grey cartoons with residues important for restriction highlighted in blue and modeled loops in white transparent. The illustrative model of full-length trimeric HIV-1 Env was assembled</p>

			using PDB 6E8W (model 1; pinks) and PDB 5FUU (gp41 browns; gp120 purples), MPER (653-683) is shown in cyans, all structures shown in cartoons; membrane is in cream. a , Side-by-side comparison; b , Models shown in closer proximity, c , 90° rotation and zoom of model in panel b showing the distance between ECL5 and ECL3 is approximately the same distance (~30 Å) as that between MPER α helices in gp41.
Extended Data Fig. 9			
Extended Data Fig. 10			

1

Item	Present?	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	A brief, numerical description of file contents. <i>i.e.: Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.</i>
Supplementary Information	Yes	Supplementary_all_together_VEP.pdf	Supplementary Tables 1-2 and Supplementary Note (Extended Methods).
Reporting Summary	Yes	Nr-reporting-summary1_VEP.pdf	

2

Type	Number If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_Supplementary_Video_1.mov</i>	Legend or Descriptive Caption Describe the contents of the file
Supplementary Video	1	Movie1_structure_of_DmSERINC.mov	Structure of DmSERINC
Supplementary Video	2	Movie2_structure_of_SERINC5	Structure of SERINC5

Choose an item.			

3

Figure	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_SourceData_Fig1.xls</i> , or <i>Smith_Unmodified_Gels_Fig1.pdf</i>	Data description i.e.: Unprocessed Western Blots and/or gels, Statistical Source Data, etc.
Source Data Fig. 1		
Source Data Fig. 2		
Source Data Fig. 3		
Source Data Fig. 4	SourceData_Figure4.xlsx source_data_blot_Fig4C.pdf	Statistical Source Data for Fig 4 a, b, e Unprocessed Western Blots for Fig 4c
Source Data Fig. 5		
Source Data Fig. 6		
Source Data Fig. 7		
Source Data Fig. 8		
Source Data Extended Data Fig. 1	source_data_blot_gels_ExtData_Fig1a.pdf SourceData_ExtData_Fig1.xlsx	Unprocessed Western Blots Statistical Source Data for Extended Data fig 1 c-e
Source Data Extended Data Fig. 2	source_data_gels_ExtData_2b.pdf	Unprocessed SDS PAGE gels
Source Data Extended Data Fig. 3	source_data_gels_ExtData_3b.pdf	Unprocessed SDS PAGE gels
Source Data Extended Data Fig. 4	ExtData_Fig4_thermostability_reducing_agents_source_data.xlsx	Statistical Source Data for Extended Data fig 4 g-i
Source Data Extended Data Fig. 5		

Source Data Extended Data Fig. 6	ExDataFig6_thermostability_lipids_source_data.xlsx	Statistical Source Data for Extended Data fig 6e-g
Source Data Extended Data Fig. 7		
Source Data Extended Data Fig. 8		
Source Data Extended Data Fig. 9		
Source Data Extended Data Fig. 10		

4

5

6 **A bipartite structural organization defines the**

7 **SERINC family of HIV-1 restriction factors**

8

9 Valerie E. Pye¹, Annachiara Rosa^{1&}, Cinzia Bertelli^{2&}, Weston B. Struwe³, Sarah L.
10 Maslen⁴, Robin Corey⁵, Idir Liko³, Mark Hassall⁶, Giada Mattiuzzo⁶, Allison
11 Ballandras-Colas¹, Andrea Nans⁷, Yasuhiro Takeuchi^{6,8}, Phillip J. Stansfeld^{5, 9}, J.
12 Mark Skehel⁴, Carol V. Robinson³, Massimo Pizzato^{2*} & Peter Cherepanov^{1,10*}

13

14 ¹Chromatin Structure and Mobile DNA Laboratory, Francis Crick Institute, 1 Midland
15 Road, London, NW1 1AT, UK; ²University of Trento, Department of Cellular,
16 Computational and Integrative Biology, 38123 Povo, Italy; ³Physical and Theoretical
17 Chemistry Laboratory, South Parks Road, Oxford, OX1 3QZ, UK; ⁴Biological Mass
18 Spectrometry and Proteomics Laboratory, MRC Laboratory of Molecular Biology,
19 Cambridge, CB2 0QH, UK; ⁵Department of Biochemistry, University of Oxford,
20 OX1 3QU, UK; ⁶National Institute for Biological Standards and Control, Blanche
21 Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK; ⁷Structural Biology
22 Science Technology Platform, Francis Crick Institute, NW1 1AT, UK; ⁸UCL Division

23 of Infection and Immunity, The Rayne Building, 5 University Street, London, WC1E
24 6EJ, UK; ⁹School of Life Sciences & Department of Chemistry, University of
25 Warwick, Gibbet Hill Campus, CV4 7AL, UK; ¹⁰Department of Medicine, Imperial
26 College London, St-Mary's Campus, Norfolk Place, London, W2 1PG, UK.

27

28 &Contributed equally

29 *Correspondence:

30 Email: peter.cherepanov@crick.ac.uk (PC); massimo.pizzato@unitn.it (MP)

31

32

33 **The human integral membrane protein SERINC5 potently restricts HIV-1**
34 **infectivity and sensitises the virus to antibody-mediated neutralisation. Here,**
35 **using cryo-electron microscopy, we determined the structures of human**
36 **SERINC5 and its ortholog from *Drosophila melanogaster* at sub-nm and near-**
37 **atomic resolution, respectively. The structures reveal a novel fold comprised of**
38 **ten transmembrane helices organised into two subdomains and bisected by a**
39 **long diagonal helix. A lipid binding groove and clusters of conserved residues**
40 **highlight potential functional sites. A structure-based mutagenesis scan**
41 **identified surface-exposed regions and the interface between the subdomains of**
42 **SERINC5 as critical for HIV-1 restriction activity. The same regions are also**
43 **important for viral sensitisation to neutralising antibodies, directly linking the**
44 **antiviral activity of SERINC5 with remodelling of the HIV-1 envelope**
45 **glycoprotein.**

46

47 Host organisms employ a range of mechanisms to impede replication of pathogens,
48 and the latter evolve countermeasures to circumvent the innate immunity of their
49 hosts¹. One such antagonistic interaction involves the human transmembrane protein
50 SERINC5, which when incorporated into budding HIV-1 virions, can strongly inhibit
51 their subsequent entry into target cells^{2,3}. To negate the effect of SERINC5, HIV-1
52 encodes the endocytic adaptor protein Nef, which redirects this restriction factor,
53 normally present in the plasma membrane, to endosomal compartments, thereby
54 preventing its inclusion into budding viral particles^{2,3}. The ability to ablate plasma
55 membrane-associated SERINC proteins is conserved among retroviruses²⁻⁵,
56 suggesting a strong evolutionary pressure to prevent virion incorporation of these host
57 factors. Exclusive to eukaryotes, SERINC proteins share high amino acid identity among

58 themselves (Supplementary Table 1) but lack homology to any other known protein
59 family. The term SERINC was coined after the proposed role of serine incorporation
60 into membranes during lipid biosynthesis⁶. However, this function has so far not
61 found independent confirmation, and mass spectrometry analyses failed to detect
62 SERINC-dependent changes in cellular or virion lipid composition^{7,8}. The human
63 genome encodes five SERINC paralogs, of which one, SERINC2, lacks the HIV-1
64 restriction activity^{9,10}. Recent studies highlighted associations of *SERINC* genes with
65 autism¹¹, borderline personality disorders¹², alcohol dependence¹³, and
66 cancerogenesis¹⁴⁻¹⁷.

67 SERINC5 inhibits HIV-1 infectivity by specifically interfering with viral
68 entry^{2,3}, although the precise mode of restriction remains enigmatic. HIV-1 particles
69 assemble at lipid rafts within plasma membrane and utilize host endosomal sorting
70 complexes required for transport (ESCRT) machinery to bud from the infected cell
71 (reviewed in ref. 18). Following their maturation, virions infect target cells through
72 fusion of the viral and host cell membranes. This process is orchestrated by the viral
73 envelope glycoprotein (Env), which is a trimer comprised of the surface glycoprotein
74 gp120 and the single-pass transmembrane subunit gp41, (gp41-gp120)₃. A series of
75 conformational changes within HIV-1 Env, triggered by the interaction of gp120 with
76 CD4 and a co-receptor (CCR5 or CXCR4) on surface of a target cell, lead to the
77 insertion of the gp41 fusion peptide into the target cell plasma membrane. The
78 subsequent collapse of the gp41 structure into a 6-helix bundle is believed to initiate
79 the fusion of viral and host cell membranes¹⁹. HIV-1 Env is highly variable, and
80 individual viral isolates display vastly different sensitivities to neutralizing antibodies
81 and restriction by SERINC5. Thus, while SERINC5 can ablate infectivity of the
82 HIV-1 clone NL4-3, it has only a modest effect on the tier 2 and 3 HIV-1 isolates,

83 such as JRFL^{2,3,10,20,21}. Nevertheless, JRFL becomes considerably more sensitive to
84 some neutralizing antibodies and the CCR5 antagonist Maraviroc in the presence of
85 SERINC5, suggesting that the restriction factor exerts a direct effect on the
86 conformation of HIV-1 Env^{10,20}.

87 Here we present the three-dimensional structure of a SERINC family member
88 to near-atomic resolution and show that the observed conformation is consistent with
89 that of human SERINC5, which we resolved at sub-nm resolution. Additionally,
90 using an extensive panel of amino acid substitutions, we identify the regions of
91 SERINC5 that are critical for its antiviral activities.

92

93 **Results**

94 **Structure determination of *Drosophila melanogaster* SERINC.** The fly possesses a
95 single SERINC ortholog (referred to here as *DmSERINC*, and also known as TMS1)
96 that shares 36% amino acid sequence identity with human SERINC5 (Supplementary
97 Table 1). Ectopic expression of *DmSERINC* during production of Nef-deficient HIV-
98 1 virions strongly suppressed their infectivity (Extended Data Fig. 1 a-c), confirming
99 conservation of functional restriction by the insect ortholog. To structurally
100 characterise the SERINC family of proteins, we produced recombinant human
101 SERINC5 and *DmSERINC* in human and yeast cells, respectively. When subjected to
102 chromatography through a 24-ml Superdex-200 sizing column, SERINC5 migrated as
103 a single peak with elution volume of 12 ml. Imaging negatively-stained material from
104 this peak revealed featureless micelles, which failed to produce discrete 2D averages
105 (Extended Data Fig. 2a-d). By contrast, separation of *DmSERINC* resulted in an
106 additional species eluting at 9.8 ml, containing well-defined assemblies with
107 prominent six-fold symmetry (Extended Data Fig. 3a-d). Encouraged by these results,

108 we acquired micrograph movies of frozen-hydrated *DmSERINC* from this fraction.
109 Image processing of single particles resulted in a 3D reconstruction to an overall
110 resolution of 3.3 Å with a local resolution of 2.8-3 Å for the majority of the protein
111 (Extended Data Fig. 3e-g). Atomic coordinates were built *ab initio* into the cryo-EM
112 map revealing the details of the homo-hexameric *DmSERINC* structure (Fig. 1a,b,
113 Table 1 and Supplementary Video 1).

114 Each of the six identical *DmSERINC* subunits consists of ten transmembrane
115 helices (TM) arranged into two subdomains revealing a tertiary fold that is ~35 Å by
116 ~50 Å in the membrane plane and ~50 Å traversing the membrane (Fig. 1b). A 39-
117 residue-long α-helix (TM4) spans the membrane diagonally intersecting subdomain A
118 (TM1, TM2, TM3, TM9) and subdomain B (TM5, TM6, TM7 and TM10). A shorter
119 diagonal α-helix TM8 crosses back from subdomain B to A, forming an asymmetrical
120 cross with TM4 in the centre. Two disulphide bonds are identified on the extracellular
121 side: one within extracellular loop (ECL) 1 (Cys71-Cys95, conserved in most species
122 except plants and some lower eukaryotes) and one between ECL3 and ECL4
123 (Cys238-Cys299, conserved throughout the SERINC family) (Fig. 1b, Extended Data
124 Fig. 4f, Supplementary Table 1 and Supplementary Video 1). Concordantly, exposure
125 of *DmSERINC* and SERINC5 to reducing agents decreased their thermostability
126 (Extended Data Fig. 4g-i). The disulphide bonds and the location of ECL4, the
127 equivalent of which harbours glycosylated Asn294 in SERINC5²², confirms the
128 assigned orientation within the plasma membrane, with both the N- and C-termini of
129 the protein residing in the cytoplasm (Fig. 1b, Supplementary Table 1 and Extended
130 Data Fig. 4a,b).

131 A query of the wwPDB using the Dali server²³ did not identify proteins
132 sharing extensive structural similarity with *DmSERINC* (Extended Data Fig. 4c).

133 Accordingly, the *DmSERINC* structure represents a novel membrane protein fold. We
134 considered if the hexameric arrangement of *DmSERINC* was characteristic of the
135 SERINC family. Protomer interface contacts bury 958 Å², comprising only 5.3% of
136 the total *DmSERINC* monomer surface. Furthermore, the interfaces are devoid of
137 conserved amino acid residues (Extended Data Fig. 4d) and are largely lipid-
138 mediated. Based on the cryo-EM map, we assigned one of the protomer-bridging
139 lipids as cardiolipin and confirmed its presence in our *DmSERINC* preparations by
140 mass spectrometry (Extended Data Fig. 5). Consistent with the structure, we observed
141 binding of up to two molecules of cardiolipin per *DmSERINC* monomer (Extended
142 Data Fig. 5e). We conclude that while the hexameric state of *DmSERINC* made it
143 conducive to high-resolution cryo-EM that afforded *de novo* model building, it may
144 not be conserved throughout the protein family. Accordingly, we readdressed
145 structural characterisation of human SERINC5 (see below).

146

147 **Potential functional features of SERINC.** Exploring the *DmSERINC* structure
148 further, we identified clusters of highly-conserved residues, which may represent
149 functional sites in the fold (Fig. 2). Within subdomain A, lipid-buried Lys143
150 assembles with Tyr42, Gln136, Trp140, Tyr395, and His401 to form a small pocket at
151 the membrane-cytosol interface (Fig. 2, bottom). At the extracellular side of the
152 structure, Gln181, Tyr282, Tyr285, Ser289, Lys438, and Thr414 line a hydrophilic
153 cleft between the two subdomains that is corked by Trp418. This crevice extends half-
154 way through the lipid bilayer, where it is plugged by invariant Phe177 (Fig. 2, top).
155 Molecular dynamics simulations of lipid-embedded *DmSERINC* suggested that
156 solvent molecules can freely enter the space between the subdomains (Extended Data

157 Fig. 4j). Searching structural databases with ProFunc²⁴ did not reveal similarity of
158 either conserved pocket to any of the known functional sites.

159 The surface of subdomain B features an elongated groove formed by TM5, 7,
160 8 and the C-terminal end of TM4, which is an appropriate size, shape, and location to
161 accommodate a lipid moiety, potentially allowing a head group to access the
162 hydrophilic cleft between the subdomains (Extended Data Fig. 6a,b). When we
163 embedded the protein structure in a lipid bilayer *in silico*, the groove became readily
164 occupied by lipids during molecular dynamics (MD) simulations, and the cryo-EM
165 map revealed presence of an acyl chain bound at this site (Extended Data Fig. 6c,d).
166 We conducted a screen of lipids and found that addition of phosphatidylserine,
167 cholesterol or sulfatide increased *DmSERINC* and *SERINC5* protein thermostability,
168 while cardiolipin stabilised *DmSERINC* only (Extended Data Fig. 6e-g). Moreover,
169 hydrogen/deuterium exchange (HDX) experiments with *DmSERINC* revealed that
170 phosphatidylserine affected isotope exchange around the potential lipid binding
171 groove in subdomain B (Extended Data Fig. 7).

172

173 **The structure of human *SERINC5*.** Composed of two subdomains, the *DmSERINC*
174 structure is suggestive of conformational flexibility. To determine if the conformation
175 observed in *DmSERINC* represents that of the *bona fide* HIV-1 restriction factor, we
176 imaged *SERINC5* in detergent micelles doped with phosphatidylserine, which was
177 found to increase thermostability of the protein (Extended Data Fig. 6g). To aid in
178 single particle image alignment of this 51 kDa protein, we acquired cryo-EM data in
179 the presence of the antigen binding fragment (Fab) of a monoclonal antibody that
180 recognizes *SERINC5* ECL4. The resulting 3D reconstruction at a local resolution of
181 6.5-7 Å contains a single molecule of *SERINC5* in a conformation very similar to that

182 of *DmSERINC* protomers within the hexamer (Fig. 3, Extended Data Fig. 2 and
183 Supplementary Video 2), leading us to conclude that the conformation we observe in
184 the *DmSERINC* structure is also adopted by human SERINC5.

185

186 **Mapping SERINC5 regions critical for the antiviral activity.** To identify regions
187 of SERINC5 involved in HIV-1 restriction, we conducted an extensive mutagenesis
188 screen, informed by the structure and amino acid sequence conservation with
189 SERINC2, which lacks the HIV-1 restriction activity^{9,10}. The mutations were
190 introduced into constructs designed to express SERINC5 harbouring a FLAG epitope
191 implanted within ECL4. This modification enabled surface exposure measurements of
192 the modified proteins by flow cytometry without interfering with restriction activity
193 (Extended Data Fig. 1d). In total, ninety-four SERINC5 mutants were tested for the
194 ability to inhibit infectivity of Nef-negative HIV-1 (Fig. 4, Supplementary Table 2).
195 The loss-of-function mutants clustered into two classes. The first class, comprising
196 variants that also failed to localise to the plasma membrane, harboured the majority of
197 mutants at highly conserved or invariant positions across the entire SERINC family,
198 such as substitutions of Lys130, Phe165, Tyr388, His394, Trp411, Lys433, and
199 Tyr444 (Fig. 4a). The second class of restriction-defective mutants retained the ability
200 to localise to the plasma membrane. The majority of these carried substitutions within
201 the ECLs or in proximity to the interface between the subdomains (Fig. 4b, d). This
202 class included variants with substitutions within ECL5, such as a partial swap for the
203 equivalent loop from SERINC2 (the mutant designated ECL5B), and double mutants
204 NY413KP and IE419KM.

205 Next, we tested a representative subset of SERINC5 variants for incorporation
206 into HIV-1 particles. As can be expected, class 1 mutants with severe defects in

207 surface expression (K130A and K433A) were deficient for glycosylation and failed to
208 incorporate into virions (Fig. 4c), explaining their inability to restrict infection. In
209 contrast, class 2 mutants that were competent for plasma membrane localization
210 incorporated into viral particles (Fig. 4c). To test the ability of these mutants to
211 remodel HIV-1 Env, we pseudotyped Nef-negative HIV-1 particles with the tier-2
212 glycoprotein JRFL, which is substantially resistant to restriction by SERINC5³. In
213 agreement with published observations^{10,20,25}, the virus produced in the presence of
214 WT SERINC5 displayed enhanced sensitivity to neutralisation by the monoclonal
215 antibodies 4E10 and 2F5, both of which target the highly-conserved membrane
216 external proximal region (MPER) of HIV-1 Env (Fig. 4e). Remarkably, class 2
217 SERINC5 mutants were substantially compromised in their ability to sensitize the
218 virus to neutralization (Fig. 4e). Thus, despite incorporation into virions, class 2
219 mutants are defective for both of the antiviral activities.

220

221 **Discussion**

222 The conserved cellular functions of SERINC5 remain unclear, underscoring the
223 difficulty in determining structure-function relationships from first principles. Further
224 studies will be required to test the functional significance of phosphatidylserine,
225 sulfatide and/or cholesterol binding in biological functions of SERINC5 proteins.
226 Affinity for cholesterol and/or exposed phosphatidylserine could help explain the
227 association of SERINC5 with lipid rafts^{9,26} and ultimately incorporation of the protein
228 into budding virions.

229 The failure of class 1 SERINC5 mutants to inhibit HIV-1 infectivity strongly
230 argues that the protein must be located at the plasma membrane to exert its antiviral
231 activity. Intriguingly, despite virion incorporation, class 2 mutants were deficient for

232 both restriction and sensitization to neutralizing antibodies. The congruence of these
233 antiviral activities suggests a specific interaction resulting in the conformational
234 remodelling of surface-exposed regions of HIV-1 Env. The involvement of the ECLs
235 and the interface between the subdomains in restriction suggests that the restriction
236 depends on a specific conformation of SERINC5 and strongly indicates that this
237 activity takes place on the external surface of the virion.

238 For illustrative purposes, we modelled the complete HIV-1 Env trimer using
239 recent partial experimental structures^{27,28} (Extended Data Fig. 8a). In contrast to the
240 extended viral spike, SERINC5 is almost entirely membrane embedded, poised to
241 reach only the membrane proximal and/or embedded regions of Env (Extended Data
242 Fig. 8a). Intriguingly, the spacing between ECL3 and ECL5 of SERINC5, both of
243 which are important for the antiviral activities, matches that between the MPER
244 regions within the Env trimer (Extended Data Fig. 8b,c). We speculate that interaction
245 with the neighbouring MPER domains could explain both the inhibition of HIV-1
246 fusion and the sensitization to 2F5 and 4E10 antibodies by SERINC5. This hypothesis
247 is consistent with the genetic studies that mapped the determinants of SERINC5
248 sensitivity to the variable loops in gp120^{20,29}, as amino acid changes in the variable
249 loops can modulate the accessibility of MPER³⁰. Moreover, the interaction with CD4,
250 which triggers an open HIV-1 Env conformation and increases MPER accessibility³¹,
251 rendered tier 2 and 3 viral isolates sensitive to restriction by SERINC5²¹. At present,
252 we cannot exclude other possibilities, such as interactions with viral glycans and/or
253 conformational movements of the spike that would enable direct SERINC5 contacts
254 with gp120. Our structures and extensive functional data provide the initial insights at
255 the molecular level of SERINC proteins and their ability to restrict HIV-1 infection,

256 warranting further research to clarify the molecular basis of the engagement between
257 the host factor and HIV-1 Env.

258

259 **Acknowledgements**

260 We thank the UK Biological Services Division at the National Institute of Biological
261 Standards and Control for their expertise in animal husbandry for the production of
262 the antibody; R. Peat and the Cell Services Platform (Crick Institute) for upscaling of
263 the hybridoma culture and purification of the antibody; J. Diffley for advice on
264 codon-optimisation for protein expression in yeast; J. Frigola and G. Coster for the
265 generous gift of JF1 cells and pGC014; R. Carzaniga for the maintenance of Vitrobot
266 and Tecnai G2 microscope and user training; P. Walker, A. Purkiss and M. Oliveira
267 for computer and software support; M. Silva dos Santos for assistance with lipid
268 preparations; D. Wu (University of Oxford) for lipidomics support; the UK National
269 Institute for Biological Standards and Control, and depositor H. Katinger, for
270 providing anti-HIV-1 4E10, 2F5 and p55/p24 antibodies; A. Engelman (Dana-Farber
271 Cancer Institute) and J. Luban (University of Massachusetts) for comments on the
272 manuscript. PJS and RAC were funded by Wellcome Trust (208361/Z/17/Z).
273 Research in PJS's lab is supported by the MRC (MR/S009213/1) and BBSRC
274 (BB/P01948X/1, BB/R002517/1, BB/S003339/1). This project made use of time on
275 ARCHER and JADE granted via the UK High-End Computing Consortium for
276 Biomolecular Simulation, HECBioSim (<http://hecbiosim.ac.uk>), supported by EPSRC
277 (grant no. EP/R029407/1). This research was funded by US National Institutes of
278 Health grant P50 AI150481 and the Francis Crick Institute, which receives its core
279 funding from Cancer Research UK (FC001061), the UK Medical Research Council
280 (FC001061), and the Wellcome Trust (FC001061).

281

282 **Author Contributions**

283 V.E.P. expressed, purified and characterised *DmSERINC*, built the atomic model,
284 developed and conducted thermostability assays; V.E.P., P.C., and A.B.-C. prepared
285 and screened cryo-EM grids; A.N. collected all cryo-EM data; V.E.P. and P.C. refined
286 the *DmSERINC* structure; A.R. and P.C. generated stable cell line for SERINC5
287 expression, purified and characterised SERINC5 and determined the structure; A.R.
288 conducted thermostability assays on SERINC5 and purified the Fab; P.C. produced
289 mutant SERINC5 constructs; M.P. and C.B. developed and performed assays to
290 measure surface exposure, restriction activity and virion incorporation of SERINC5
291 variants; W.B.S., I.L. and C.V.R. analysed lipid composition of *DmSERINC*
292 preparations; S.L.M. and J.M.S. designed, conducted and analysed HDX/MS
293 experiments; R.C. and P.S. conducted MD simulations on lipid-imbedded
294 *DmSERINC*; M.H., G.M. and Y.T. generated hybridomas for monoclonal anti-
295 SERINC5 antibody; P.C. and M.P. conceived and directed the work; V.E.P., M.P. and
296 P.C. wrote the manuscript with contributions from all authors.

297

298

299 **Ethics declarations**

300 **Competing interests**

301 The authors declare no competing interests.

302 **Figure Legends**

303 **Figure 1 | The structure of *DmSERINC*.** **a**, Cryo-EM map of the hexamer with each
304 protomer individually colored; the map was Gaussian filtered with a standard
305 deviation of 5 Å to represent the detergent micelle, grey (left) and a cartoon
306 representation of the *DmSERINC* hexamer (right). **b**, Detailed representation of an
307 isolated monomer, colored in green to dark blue gradient from N- to C-terminus with
308 transmembrane alpha helices numbered and loops labelled (intracellular loops (ICL)
309 and extracellular loops (ECL)). The position of the lipid groove is indicated by a grey
310 rectangle. Disulphide bonds are labelled and shown in stick format. The outer and
311 inner plasma membrane surfaces are depicted as chocolate and olive dotted planes,
312 respectively.

313

314 **Figure 2 | Potential functional sites identified in *SERINC* structure.** Sequence
315 conservation mapped onto the *DmSERINC* structure, with invariant residues in dark
316 blue and most variable in red; the outer and inner plasma membrane surfaces are
317 depicted as chocolate and olive dotted planes, respectively. The insets show details of
318 the hydrophilic cleft between the subdomains (top) and a highly conserved pocket
319 (bottom).

320

321 **Figure 3 | Structure of human *SERINC5* bound to Fab.** The cryo-EM map is
322 shown as a semi-transparent white surface, with fitted atomic models of *DmSERINC*
323 (cartoons colored as in Fig. 1b) and Fab (purple cartoons). Top view shows
324 transmembrane helices traversing the detergent micelle and bottom view is a
325 perpendicular slice showing *SERINC5* surrounded by the micelle.

326

327 **Figure 4 | SERINC5 residues critical for HIV-1 restriction activity. a and b,**
328 restriction activity and surface expression of human SERINC5 variants relative to
329 SERINC5 wt (data shown are mean and s.d. of n=3 independent experiments). **a,**
330 Class 1 amino acid substitutions interfere with Nef-defective HIV-1_{NL4-3} restriction
331 and surface expression. **b,** Class 2 amino acid substitutions do not affect surface
332 expression but compromise Nef-defective HIV-1 restriction. **c,** SERINC5
333 incorporation into virion particles. Immunoblots of Nef-defective HIV-1 particles and
334 corresponding producer cell lysates expressing the indicated SERINC5 variants. The
335 right-most lane contains a Gag-defective provirus control. Arrowheads and asterisks
336 indicate migration position of glycosylated and non-glycosylated SERINC5,
337 respectively. Note the selective incorporation of the glycosylated form into viral
338 particles²² **d,** Class 1 and 2 residues mapped onto a model of SERINC5, in red and
339 blue, respectively. **e,** Neutralisation of Nef-deficient HIV-1_{NL4-3} carrying the JRFL
340 envelope by 2F5 and 4E10 monoclonal antibodies. Residual infectivity is relative to
341 that of untreated viruses ($n=4$, mean \pm 95% confidence interval, technical repeats),
342 IC₅₀ values are shown in Extended Data Fig. 1e. Uncropped images for panel c and
343 data for graphs a, b and e are available as Source Data.

344

345

346

347

348

349

350

351

352

353

354 Table 1. **Cryo-EM data collection, refinement and validation statistics**

	<i>Dm</i> SERINC (EMD-10279, PDB 6SP2)	SERINC5 (EMD-10277)
Data collection and processing		
Magnification	36,232	128,440
Voltage (kV)	300	300
Electron exposure (e ⁻ /Å ²)	50	33.6
Defocus range (μm)	-1.6 to -4	-1.6 to -4
Pixel size (Å)	1.38	1.09
Symmetry imposed	C6	C1
Initial particle images (no.)	1,857,080	2,502,546
Final particle images (no.)	159,252	270,151
Map resolution (Å)	3.3	7.1
FSC threshold	0.143	0.143
Map resolution range (Å)	2.8-50.0	6.5-50
Refinement		
Model resolution (Å)	3.3	
FSC threshold	0.143	
Model resolution range (Å)	2.8-4.0	
Map sharpening <i>B</i> factor (Å ²)	-186.2	
Model composition		
Nonhydrogen atoms	18,498	
Protein residues	2,190	
Ligands	CDL:6, LMN:12, P5S:6	
<i>B</i> factors (Å ²)		
Protein	150.59	
Ligand	163.13	
R.m.s. deviations		
Bond lengths (Å)	0.004	
Bond angles (°)	0.72	
Validation		
MolProbity score	1.77	
Clashscore	8.22	
Poor rotamers (%)	0.0	
Ramachandran plot		
Favored (%)	95.28	
Allowed (%)	4.72	
Disallowed (%)	0	

355

356

357

358

359

360 **References**

- 361
- 362 1. Duggal, N.K. & Emerman, M. Evolutionary conflicts between viruses and
363 restriction factors shape immunity. *Nat Rev Immunol* **12**, 687-95 (2012).
- 364 2. Rosa, A. et al. HIV-1 Nef promotes infection by excluding SERINC5 from
365 virion incorporation. *Nature* **526**, 212-7 (2015).
- 366 3. Usami, Y., Wu, Y. & Gottlinger, H.G. SERINC3 and SERINC5 restrict HIV-1
367 infectivity and are counteracted by Nef. *Nature* **526**, 218-23 (2015).
- 368 4. Chande, A. et al. S2 from equine infectious anemia virus is an infectivity
369 factor which counteracts the retroviral inhibitors SERINC5 and SERINC3.
370 *Proc Natl Acad Sci U S A* **113**, 13197-13202 (2016).
- 371 5. Ahmad, I. et al. The retroviral accessory proteins S2, Nef, and glycoMA use
372 similar mechanisms for antagonizing the host restriction factor SERINC5. *J*
373 *Biol Chem* **294**, 7013-7024 (2019).
- 374 6. Inuzuka, M., Hayakawa, M. & Ingi, T. Serinc, an activity-regulated protein
375 family, incorporates serine into membrane lipid synthesis. *J Biol Chem* **280**,
376 35776-83 (2005).
- 377 7. Trautz, B. et al. The host-cell restriction factor SERINC5 restricts HIV-1
378 infectivity without altering the lipid composition and organization of viral
379 particles. *J Biol Chem* **292**, 13702-13713 (2017).
- 380 8. Chu, E.P. et al. Disruption of Serinc1, which facilitates serine-derived lipid
381 synthesis, fails to alter macrophage function, lymphocyte proliferation or
382 autoimmune disease susceptibility. *Mol Immunol* **82**, 19-33 (2017).
- 383 9. Schulte, B. et al. Localization to detergent-resistant membranes and HIV-1
384 core entry inhibition correlate with HIV-1 restriction by SERINC5. *Virology*
385 **515**, 52-65 (2018).

- 386 10. Sood, C., Marin, M., Chande, A., Pizzato, M. & Melikyan, G.B. SERINC5
387 protein inhibits HIV-1 fusion pore formation by promoting functional
388 inactivation of envelope glycoproteins. *J Biol Chem* **292**, 6014-6026 (2017).
- 389 11. Hnoonual, A. et al. Chromosomal microarray analysis in a cohort of
390 underrepresented population identifies SERINC2 as a novel candidate gene for
391 autism spectrum disorder. *Sci Rep* **7**, 12096 (2017).
- 392 12. Lubke, G.H. et al. Genome-wide analyses of borderline personality features.
393 *Mol Psychiatry* **19**, 923-9 (2014).
- 394 13. Zuo, L. et al. Rare SERINC2 variants are specific for alcohol dependence in
395 individuals of European descent. *Pharmacogenet Genomics* **23**, 395-402
396 (2013).
- 397 14. Zeng, Y. et al. SERINC2-knockdown inhibits proliferation, migration and
398 invasion in lung adenocarcinoma. *Oncol Lett* **16**, 5916-5922 (2018).
- 399 15. Bossolasco, M., Veillette, F., Bertrand, R. & Mes-Masson, A.M. Human
400 TDE1, a TDE1/TMS family member, inhibits apoptosis in vitro and stimulates
401 in vivo tumorigenesis. *Oncogene* **25**, 4549-58 (2006).
- 402 16. Margue, C. et al. New target genes of MITF-induced microRNA-211
403 contribute to melanoma cell invasion. *PLoS One* **8**, e73473 (2013).
- 404 17. Player, A. et al. Identification of TDE2 gene and its expression in non-small
405 cell lung cancer. *Int J Cancer* **107**, 238-43 (2003).
- 406 18. Hurley, J.H. & Cada, A.K. Inside job: how the ESCRTs release HIV-1 from
407 infected cells. *Biochem Soc Trans* **46**, 1029-1036 (2018).
- 408 19. Chen, B. Molecular Mechanism of HIV-1 Entry. *Trends Microbiol* **27**, 878-
409 891 (2019).

- 410 20. Beitari, S., Ding, S., Pan, Q., Finzi, A. & Liang, C. Effect of HIV-1 Env on
411 SERINC5 Antagonism. *J Virol* **91**(2017).
- 412 21. Zhang, X. et al. CD4 expression and Env conformation are critical for HIV-1
413 restriction by SERINC5. *J Virol* (2019).
- 414 22. Sharma, S., Lewinski, M.K. & Guatelli, J. An N-Glycosylated Form of
415 SERINC5 Is Specifically Incorporated into HIV-1 Virions. *J Virol* **92**(2018).
- 416 23. Holm, L. & Sander, C. Dali: a network tool for protein structure comparison.
417 *Trends Biochem Sci* **20**, 478-80 (1995).
- 418 24. Laskowski, R.A. The ProFunc Function Prediction Server. *Methods Mol Biol*
419 **1611**, 75-95 (2017).
- 420 25. Lai, R.P. et al. Nef decreases HIV-1 sensitivity to neutralizing antibodies that
421 target the membrane-proximal external region of TMgp41. *PLoS Pathog* **7**,
422 e1002442 (2011).
- 423 26. Brugger, B. et al. The HIV lipidome: a raft with an unusual composition. *Proc*
424 *Natl Acad Sci U S A* **103**, 2641-6 (2006).
- 425 27. Fu, Q. et al. Structure of the membrane proximal external region of HIV-1
426 envelope glycoprotein. *Proc Natl Acad Sci U S A* **115**, E8892-E8899 (2018).
- 427 28. Lee, J.H., Ozorowski, G. & Ward, A.B. Cryo-EM structure of a native, fully
428 glycosylated, cleaved HIV-1 envelope trimer. *Science* **351**, 1043-8 (2016).
- 429 29. Usami, Y. & Gottlinger, H. HIV-1 Nef responsiveness is determined by Env
430 variable regions involved in trimer association and correlates with
431 neutralization sensitivity. *Cell Rep* **5**, 802-12 (2013).
- 432 30. Chakrabarti, B.K. et al. Direct antibody access to the HIV-1 membrane-
433 proximal external region positively correlates with neutralization sensitivity. *J*
434 *Virol* **85**, 8217-26 (2011).

435 31. Ivan, B., Sun, Z., Subbaraman, H., Friedrich, N. & Trkola, A. CD4 occupancy
436 triggers sequential pre-fusion conformational states of the HIV-1 envelope
437 trimer with relevance for broadly neutralizing antibody activity. *PLoS Biol* **17**,
438 e3000114 (2019).

439

440

441

442 **Methods**

443 **Protein expression and purification.** *DmSERINC* carrying a C-terminal TwinStrep
444 tag was over-expressed in *S. cerevisiae* and solubilised in a buffer containing 1.5% n-
445 dodecyl- β -D-maltoside (DDM, Anatrace). During affinity purification the detergent
446 was exchanged to 0.05% lauryl maltose neopentyl glycol (LMNG, Anatrace), the
447 concentration of which was reduced to a minimum for size exclusion
448 chromatography. Human SERINC5 protein was purified from a stable cell line
449 adapted to suspension culture following the protocol for *DmSERINC* with the
450 addition of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS; Echelon Biosciences)
451 in the sample for cryo-EM. Detailed descriptions are in Supplementary Note 1.

452

453 **Generation of the monoclonal antibody and the Fab to SERINC5.** A monoclonal
454 antibody EVG_S5.2 was produced in a single mouse immunised with recombinant
455 SERINC5. A hybridoma line producing the antibody was expanded, and the Fab
456 fragment was produced by digestion of the antibody with papain. Detailed
457 descriptions are in Supplementary Note 1, and the monoclonal antibody is available
458 from the Centre for AIDS Reagents (CFAR, www.NIBSC.org) for distribution.

459

460

461 **Negative-stain EM.** Purified *DmSERINC* and *SERINC5* (4 μ l of ~0.01 mg/ml) were
462 applied to glow-discharged carbon-coated 300-mesh copper grids (EM Resolutions)
463 for 1 min before brief blotting and staining with 2 % (w/v) uranyl acetate. Grids were
464 imaged on a Tecnai G2 Spirit LaB6 transmission electron microscope (FEI) operating
465 at 120 keV. Images were collected with an Ultrascan-1000 camera (Gatan) at a
466 nominal magnification of 30,000 \times (corresponding to a pixel size of 3.45 \AA at the
467 specimen level), with an average electron dose of 35 $e/\text{\AA}^2$ and defocus values of -0.5
468 to -2.0 μ m. Particles, picked semi-automatically using EMAN2 Boxer tool³², were
469 subjected to reference-free 2D classification in Relion-2.03³³ or cryoSPARC³⁴.

470

471 **Cryo-EM grid preparation and data collection.** Four μ l of hexameric *DmSERINC*
472 at 0.5 mg/ml, freshly isolated by size exclusion chromatography, was applied onto
473 glow-discharged holey carbon 400-mesh Quantifoil R1.2/1.3 grids (Quantifoil) for 1
474 min, with 100% humidity at 20°C, before blotting for 3-4 sec and plunge freezing into
475 liquid ethane using Vitrobot Mark IV (FEI). Data were collected on a Titan Krios
476 microscope operating at 300 keV and equipped with a Gatan post-column energy
477 filter, selecting a 20-eV window, on a GIF Quantum K2 direct electron detector
478 (Gatan) operating in counting mode. A total of 5,807 movies were recorded using
479 single electron counting mode with a magnified pixel size of 1.38 \AA and a defocus
480 range of -1.6 to -4 μ m. The total electron dose was 50 $e/\text{\AA}^2$, spread over 30 frames
481 (Table 1).

482 Freshly isolated *SERINC5* in LMNG micelles supplemented with DPPS and
483 equimolar amount of Fab was subjected to size exclusion chromatography through a
484 Superdex 200 Increase 3.2/300 column. Four μ l of isolated *SERINC5*-Fab complex

485 was applied onto non-glow discharged C-flat 400-mesh R1.2/1.3 holey carbon grids
486 (EMS) for 30 sec under 100% humidity at 20°C, blotted for 7 sec, and vitrified by
487 plunging into liquid ethane using Vitrobot Mark IV. Data were collected on a Titan
488 Krios microscope at 300 keV equipped with a Falcon 3 detector. A total 7,165 movies
489 were recorded in single electron counting mode with a pixel size of 1.09 Å and a
490 defocus range -1.6 to -4 μm, using total electron doze of 33.6 e⁻/Å² over 30 frames
491 (Table 1).

492

493 **Cryo-EM image processing and 3D reconstruction.** Micrograph movies were
494 aligned with dose weighting applied as implemented in MotionCor2³⁵ and the contrast
495 transfer function (CTF) parameters were estimated from the frame sums using Gctf-
496 v1.06³⁶. Movies exhibiting ice contamination were discarded at this stage, leaving
497 4,238 (*DmSERINC*) and 7,021 (*SERINC5-Fab* complex) movies for further
498 processing. A sub-set of particles semi-automatically picked in EMAN2 Boxer³² were
499 used to generate the starting 2D class averages, which, upon low-pass filtering to 20
500 Å, served as templates for auto-picking of the entire dataset (Extended Data Fig. 3e).

501 The initial *DmSERINC* particle dataset was picked with Relion-2.1³³ resulting
502 in 1,857,080 particles. Reference-free 2D classification was performed in cryoSPARC
503 using 40 online-EM iterations into 200 classes. Particles belonging to well-defined 2D
504 classes exhibiting secondary structure elements (717,011 particles) were selected for
505 further processing. The starting model for 3D classifications was obtained using *ab*
506 *initio* mode of cryoSPARC. 3D classification was carried out using Relion-2.1 into 9
507 classes without applying symmetry; the hexamer class (159,252 particles) was *in*
508 *silico* purified from other states. The final 3D reconstruction was obtained using non-
509 uniform refinement as implemented in cryoSPARC2 with C6 symmetry applied.

510 Classes containing asymmetrical hexamers (classes 3 and 8; Extended Data
511 Fig. 3e) were investigated further, implementing C1 symmetry, to determine any
512 structural rearrangements compared with the hexamer. The asymmetrical hexamers
513 exhibited the exact same secondary structure conformations as the hexamer however
514 were lacking some of the detergent micelle on one side (Extended Data Fig. 3h).
515 Dimers of hexamers were also present in the sample (classes 1, 2, 4, 7 and 9;
516 Extended Data Fig. 3e) and were likely formed by asymmetrical hexamers as a
517 consequence of the limiting concentration of detergent used in sample preparation.

518 2,502,546 particles of SERINC5-Fab complex, autopicked using Gautomatch
519 (<http://www.mrc-lmb.cam.ac.uk/kzhang/>), were subjected to reference-free 2D
520 classification in cryoSPARC. The initial 3D model was obtained using *ab initio*
521 reconstruction in cryoSPARC from a subset of particles belonging to 2D classes
522 displaying clear density attributable to Fab. A total of 1,449,789 particles from well-
523 defined 2D classes were subjected to classification in Relion-3 into eleven 3D classes.
524 The most populated class containing 270,151 particles displayed high-resolution
525 features with strong density corresponding to Fab outside and SERINC5 TMs inside
526 the micelle. These particles were used for 3D auto-refinement in Relion-3 with a soft
527 mask around the entire particle using C1 symmetry, including the detergent micelle
528 resulting in a 3D reconstruction to an overall resolution of 8.2 Å and a local resolution
529 throughout the protein density of 6.5-7 Å (Extended Data Fig. 2e-g). Resolution is
530 reported according to the gold-standard Fourier shell correlation (FSC), using the
531 0.143 criterion³⁷ (Table 1, Extended Data Figs 2f,g and 3f,g). Local resolution was
532 estimated using blocres tool from BSOFTE package³⁸.

533

534 **Model building and refinement.** The *DmSERINC* model was built *ab initio* into the
535 cryo-EM map using Coot³⁹. Initially, poly-Ala α -helices were placed for each of the
536 10 TM regions in one monomer and bulky side-chain features for aromatic residues
537 were used to identify the sequence register. Further residues were added manually to
538 complete one subunit of the hexameric ring, this was then used to generate the
539 remaining five monomers in the hexamer. The hexameric model was then subjected to
540 real space refinement using Phenix^{40,41} with NCS constraints and refining group B-
541 factors (one per residue) and assessed using MolProbity⁴² and EMringer⁴³. The final
542 model contains 365 amino acid residues per subunit, lacking N-terminal peptide
543 (residues 1-29) and portions of ECL1 (residues 75-89), ECL4 (residues 306-321),
544 ICL4 (residues 354-389), and the C-terminal peptide (residues 462-465) as illustrated
545 in the topology diagram in Extended Data Fig. 4a. One cardiolipin, two LMNG, and
546 one PS molecule per monomer were tentatively built into the cryo-EM map and are
547 included in the co-ordinates. Data statistics are given in Table 1. A homology model
548 of SERINC5 was assembled based on *DmSERINC* structure using SWISS-MODEL
549 server⁴⁴. Interface surface areas were calculated using the PISA server⁴⁵, and
550 membrane buried regions were predicted using the PPM server⁴⁶.

551

552 **Molecular dynamics.** The atomic coordinates of *DmSERINC* (protein only) were
553 converted to their Martini CG representation^{47,48}, and built into POPC membranes.
554 Missing loops were modelled using SWISS-MODEL⁴⁴, with the loop between TM1
555 and TM2 modelled as (GlySer)₃ hexapeptide, the loop between TM7 and TM8
556 modelled using the native structure (MFGMMEG), and the loop between TM8 and
557 TM9 modelled as (GlySer)₇. Simulations were run over 100 ns using a 20 fs time-

558 step, in the NPT ensemble at 323 K with the V-rescale thermostat, and 1 bar using
559 semi-isotropic Berendsen pressure coupling.

560 Atomistic simulations were run following conversion of 100 ns CGMD
561 snapshots in a POPC bilayer. Conversions were carried out using the CG2AT
562 protocol⁴⁹, with Charmm36 forcefield⁵⁰ used to describe the system. Electrostatics
563 were handled using the Particle-Mesh-Ewald method, and a force-switch modifier
564 was applied to the Van der Waals forces. Dispersion corrections were turned off.
565 Simulations were run for ~215 ns with Velocity-rescaling temperature coupling at 310
566 K using a time constant of 0.1 ps and Parrinello-Rahman semi-isotropic pressure
567 coupling of 1 Bar with a time constant of 2 ps, using 4 fs time steps with virtual-sites
568 on the protein and lipids⁵¹. All simulations were run in Gromacs 2018⁵²; images were
569 made in VMD⁵³.

570

571 **Infectivity assays.** The effect of SERINC5 mutants on the infectivity of HIV-1 was
572 studied using virions limited to a single round of replication. To this end, 200,000
573 HEK293T cells were transfected with 800 ng of an *env*-defective and *nef*-defective
574 HIV-1_{NL4-3} provirus construct together with 100 ng of PBJ5-HXB2-Env² and a
575 plasmid encoding variants of SERINC5 harbouring an internal FLAG epitope
576 (DYKDDDDKDI, inserted between residues 290 and 291) and a C-terminal HA tag
577 (SERINC5-iFLAG, HA), or an appropriate empty vector control using calcium
578 phosphate. High-level overexpression of SERINC5 results in suppression of viral
579 release, which is not characteristic of the restriction factor at endogenous levels².
580 Therefore, SERINC5 mutants were tested using constructs based on pBJ6 or pBJ5,
581 both of which harbour a weak promoter, resulting in moderate and low expression
582 compared to pcDNA, respectively². All experiments used a matched wild type

583 SERINC5 control and the constructs and amount of SERINC5 expressing plasmid
584 used in specific experiments are described in Supplementary Table 2. Virus-
585 containing culture supernatants were collected 48 h after transfection, clarified by
586 centrifugation at 300g for 5 min and passed through a 0.45- μ m filter. Virus in cell
587 supernatants was quantified using the Sybr Green PCR-enhanced reverse transcriptase
588 assay^{25,54}, and five serial dilutions were inoculated onto HeLa-TZM-GFP reporter
589 cells, which contain an integrated reporter gene encoding GFP with a nuclear
590 localization signal under the transcriptional control of the HIV-1 long terminal
591 repeat². Infections were performed in quadruplicate and the number of cells infected
592 was evaluated using an EnSight plate reader (Perkin Elmer). Infectivity was measured
593 by normalizing numbers of infected cells according to the reverse transcriptase
594 activity in each viral preparation. Only dilutions within a linear range were
595 considered. Reduction of infectivity caused by SERINC5 proteins was then calculated
596 by dividing the infectivity of viruses produced in the absence of SERINC5 with the
597 infectivity of viruses produced when co-expressing SERINC5. The residual restriction
598 activity of each SERINC5 mutant on HIV-1 infectivity was then expressed in relation
599 to the reduction caused by the wild type protein, considered as 100%. Where error
600 bars are present, they represent standard error of the mean obtained from three
601 independent biological triplicates or four technical replicates as indicated.

602

603 **Surface exposure of SERINC5 mutants.** To investigate SERINC5 expression by
604 flow cytometry, 200,000 HEK293T cells were co-transfected with DNA constructs
605 expressing SERINC5-iFLAG-HA variants based on either pcDNA3.1 or pBJ5 vectors
606 as indicated in Supplementary Table 2 along with 300 ng pIRES2-GFP, to allow for
607 selective gating of transfected cells, and 1 μ g pBluescript. Forty-eight h post-

608 transfection, cells were collected, washed with ice-cold PBS and fixed with 2%
609 paraformaldehyde. Each sample was then divided in two, and processed for staining
610 with anti-FLAG antibody for selective detection of SERINC5 on the cell surface in
611 non-permeabilized cells or for detection of total SERINC5 expression after
612 permeabilisation with Wash&Perm solution (Becton Dickinson). SERINC5-iFLAG-
613 HA was detected using mouse M2 anti-FLAG antibody (Sigma) diluted 1:500 and an
614 APC-conjugated anti-mouse IgG (Jackson Immunoresearch) diluted 1:500. Samples
615 were analysed using a FACS Canto (Becton Dickinson). SERINC5 expression was
616 assessed on the live cell population gated on FSC vs SSC, followed by gating the
617 transfected cell population, which express GFP, as illustrated in Supplementary
618 Information Table 2.

619

620 **SERINC5 incorporation into virus particles and immunoblotting.** To detect
621 SERINC5 associated with virus particles, 5 million HEK293T cells were transfected
622 with 18 µg *env*-defective and *nef*-defective HIV-1_{NL4-3} provirus construct together
623 with 2 µg pBJ5-HXB2-Env and 1 µg pBJ5-SERINC5-iFLAG-HA. To verify specific
624 detection of virus-associated SERINC5, a control sample was produced by
625 transfecting a Gag-defective HIV-1_{NL4-3} provirus construct. Cell supernatants from
626 transfected cells were clarified by centrifugation at 300g for 5 min, passed through
627 filters with 0.45-µm pores, overlaid on a 25% sucrose cushion and concentrated by
628 centrifugation for 2 h at 100,000g in a SW41 swingout rotor (Beckman Coulter). The
629 resulting viral pellets were resuspended in Laemmli sample buffer supplemented with
630 50 mM TCEP. Virus producing cells were collected in PBS, pelleted and lysed in an
631 ice-cold buffer containing 100 mM NaCl, 10 mM HEPES pH 7.5, 1% DDM and
632 protease inhibitors. Lysates, clarified by centrifugation and mixed with Laemmli

633 buffer supplemented with 50 mM TCEP, were separated on 12.5% acrylamide Tricine
634 gels and transferred onto Immobilon-FL PVDF membrane (Millipore). Blots were
635 probed with mouse anti-HA (clone 16B12, Covance) diluted 1:1,000, rabbit anti-
636 β actin (Li-COR) diluted 1:1,000 and rabbit anti-HIV-1 p24 (ARP432; National
637 Institute for Biological Standards and Control) antibody. Secondary antibodies were
638 conjugated to IRDye 680 or IRDye 800 (Li-COR), and blots were imaged using an
639 Odyssey infrared imaging System (Li-COR).

640

641 **HIV-1 neutralization assays.** Sensitivity to neutralizing antibodies was measured
642 using virions limited to a single round of replication produced by transfection of
643 HEK293T cells similarly to as described for the infectivity assay, with the only
644 difference that the HIV-1_{NL4-3} Env defective provirus construct was complemented in
645 *trans* with a vector encoding Env derived from HIV-1_{JRFL} (pSV-JRFL), which allows
646 production of virions minimally sensitive to the effect of SERINC5 on infectivity.
647 Viruses were normalized based on reverse transcriptase activity and the inocula were
648 adjusted to produce between 1% and 3% infection of the monolayer. Viruses were
649 incubated with serially diluted neutralizing antibodies for 1 h at room temperature.
650 The complexes were added to TZM-bl GFP cells, seeded onto 96-well tissue culture
651 plates a day prior to neutralization, incubated at 37°C for 2 h, followed by two washes
652 with PBS before being cultured in fresh complete DMEM. Infected cells were
653 incubated at 37°C for 42 h before scoring number of infected cells per each well using
654 an Ensign plate reader.

655 Neutralization was measured by calculating the residual infectivity of treated
656 virus samples considering the infectivity of the untreated sample as 100%. Fitted
657 sigmoidal curves and the IC₅₀ values were obtained using Prism (Graphpad) with the

658 least square variable slope method using the dose-normalized response protocol.
659 Neutralizations were performed in quadruplicate with each combination of virus and
660 antibody to be analyzed and data shown are the average with the error bars
661 representing the 95% confidence interval as calculated by Prism. Neutralizing
662 antibodies 4E10 (ARP3239) and 2F5 (ARP3063) were obtained from the UK
663 National Institute for Biological Standards and Control.

664

665 **Protein thermostability assay.** Melting curves were recorded using 20-95°C
666 1.5°C/min temperature ramps on a Promethius NT.48 instrument with standard
667 capillaries (Nanotemper). Proteins were diluted to 1 mg/ml in 40 mM NaCl, 10 mM
668 HEPES-NaOH, pH 7.5, 0.0003% LMNG; where indicated, samples were spiked with
669 0.1 mM lipid. Lipid stock details are given in Supplementary Note 1.

670

671 **Native protein mass spectrometry.** Purified *DmSERINC* was buffer exchanged into
672 200 mM ammonium acetate, pH7 supplemented with 0.002% LMNG using Bio-Spin
673 6 Columns (BioRad). Samples were analysed at a final concentration of 5 µM using a
674 Q-Exactive mass spectrometer (Thermo Fisher Scientific) modified for high
675 molecular weight ions as previously described⁵⁵. Cardiolipin (Avanti), PI(3,4)P2
676 (Avanti), PI(4,5)P2 (Avanti), POPE (Avanti), POPC (Avanti) and POPG (Avanti)
677 were reconstituted to 3 mM in 200 mM ammonium acetate supplemented with
678 0.017% DDM and diluted into the desired concentration using 200 mM ammonium
679 acetate with 0.002% LMNG. Lipids were added to *DmSERINC* samples and
680 equilibrated for 20 min prior to analysis. Ions were generated by static nano-
681 electrospray using gold-coated capillaries prepared in-house as described
682 previously⁵⁶. Instrument settings were as follows: capillary voltage 1.5 kV, source

683 temperature 40°C, S-lens RF = 200, maximum injection time 150, HCD voltage 200
684 V, desolvation voltage -10 (positive mode), with in-source trapping on. Data were
685 acquired between 2,000-15,000 with 10 microscans, and results were processed using
686 XCalibur 2.2 software (Thermo Fisher Scientific). *DmSERINC* peak assignments
687 were done manually using in-house software as well as with UniDec software⁵⁷.

688

689 **Lipidomics.** Sequencing grade trypsin (Promega) was added to 20 µg *DmSERINC*
690 (5:1 *SERINC* to trypsin) and incubated overnight at 37°C. Samples were dried in a
691 vacuum concentrator and reconstituted (15 min sonication) in 30 µl acetonitrile:water
692 (60:40, v/v), 10 mM ammonium formate, 0.1% formic acid (buffer A). Samples were
693 centrifuged for 3 min at 10,000g and 15 µl was removed for analysis. For each run, 5
694 µl was injected onto a C18 column (Acclaim PepMap 100, C18, 75 mm × 15 cm;
695 Thermo Scientific) on a Dionex UltiMate 3000 RSLC nanoLC System. A 32-99%
696 linear gradient to buffer B (isopropanol:acetonitrile (90:10, v/v), 10 mM ammonium
697 formate, 0.1% formic acid) was used with a flow rate of 300 nl/min over 30 min. Data
698 were acquired using an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific)
699 in negative ion mode with the following data-dependent acquisition settings: full-scan
700 mass range 350-2,000 *m/z*, resolution 60,000, normalized collision energy 38%,
701 activation time 30 msec, automatic gain control target 30,000, capillary voltage 1.8
702 kV, and capillary temperature 180°C. Collision-induced dissociation was performed
703 for the five most intense ions, data were interpreted manually using XCalibur 2.2
704 software (ThermoFisher Scientific).

705

706 **HDX mass spectrometry.** Deuterium exchange reactions of *DmSERINC*
707 (monomeric fraction) in LMNG micelles with or without addition of exogenous DPPS

708 (Echelon Biosciences), sulfatide (Matreya LLC) or PC (Echelon Biosciences), were
709 initiated by diluting the proteins in D₂O (99.8% D₂O, Sigma-Aldrich) in 10 mM
710 HEPES pH 7.5, 40 mM NaCl, 2 mM DTT and 1 mM TCEP to obtain a final D₂O
711 concentration of ~91%. For all experiments, deuterium labelling was carried out at
712 23°C (unless otherwise stated) at four points, 3, 30, 300, and 3,000 sec in triplicate.
713 The exchange was quenched by the addition of chilled 2 M guanidinium
714 hydrochloride in 100 mM phosphate buffer, pH 2.4 (adjusted with formic acid), 15
715 mM TCEP and 0.1% v/v DDM. Samples, snap frozen in liquid nitrogen, were stored
716 at -80°C prior to analysis.

717 The quenched protein samples were rapidly thawed and subjected to
718 proteolytic cleavage with pepsin followed by reversed phase chromatography. Briefly,
719 the protein was passed through a 5- μ m 2.1 \times 30 mm Enzymate BEH immobilized
720 pepsin column, (Waters) at 200 μ l/min for 2 min; the peptic fragments were trapped
721 and desalted on a 1.7- μ m 2.1 \times 5 mm C18 trap column equipped with Acquity BEH
722 C18 Van-guard pre-column (Waters). Trapped peptides were subsequently eluted
723 over 11 min with a 3%–43% gradient of acetonitrile in 0.1% v/v formic acid at 40 μ
724 l/min. Peptides were separated on a reverse phase column (Acquity UPLC BEH C18
725 column 1.7 μ m, 100 mm x 1 mm, Waters) and detected on a SYNAPT G2-Si HDMS
726 mass spectrometer (Waters) over a m/z of 300 to 2000, with the standard electrospray
727 ionization (ESI) source with lock mass calibration using [Glu1]-fibrino peptide B (50
728 fmol/ μ l). The mass spectrometer was operated at a source temperature of 80°C and a
729 spray voltage of 2.6 kV. Spectra were collected in positive ion mode. Peptide
730 identification was performed by MS^{E58} using an identical gradient of increasing
731 acetonitrile in 0.1% v/v formic acid over 11 min.

732 The resulting MS data were analysed using Protein Lynx Global Server 3.0
733 software (Waters) with an MS tolerance of 5 ppm. Mass analysis of the peptide
734 centroids was performed using DynamX 3.0 software (Waters). Only peptides with
735 scores exceeding 6.4 were considered. The first round of analysis and identification
736 was performed automatically by the DynamX 3.0 software, however, all peptides
737 (deuterated and non-deuterated) were manually verified at every time point for the
738 correct charge state, presence of overlapping peptides, and correct retention time.
739 Deuterium incorporation was not corrected for back-exchange and represents relative,
740 rather than absolute changes in deuterium levels. Changes in H/D amide exchange in
741 any peptide may be due to a single amide or a number of amides within that peptide.
742 The DynamX 3.0 software plots the standard deviation for every peptide. The error
743 band shows the standard deviation of the plotted uptake or difference for each
744 peptide. When there are multiple exposures, as in this experiment, for a given peptide,
745 the maximum standard deviation is plotted for each peptide. A sigma multiplier of 1 is
746 applied to the standard deviation to produce the grey error bar plotted in Extended
747 Data Fig. 7b-e.

748

749

750 **Data Availability.** The cryo-EM maps and the refined atomic model of *DmSERINC*
751 were deposited in the EMDB and wwPDB, respectively, with accession codes EMD-
752 10277 and EMD-10279 and PDB 6SP2. Source data for Figures 4a, 4b, 4c, 4e and for
753 Extended Data Figures 1a, 1c, 1d, 1e, 2b, 3b, 4g-i, 6e-g, are available with the paper
754 online.

755

756

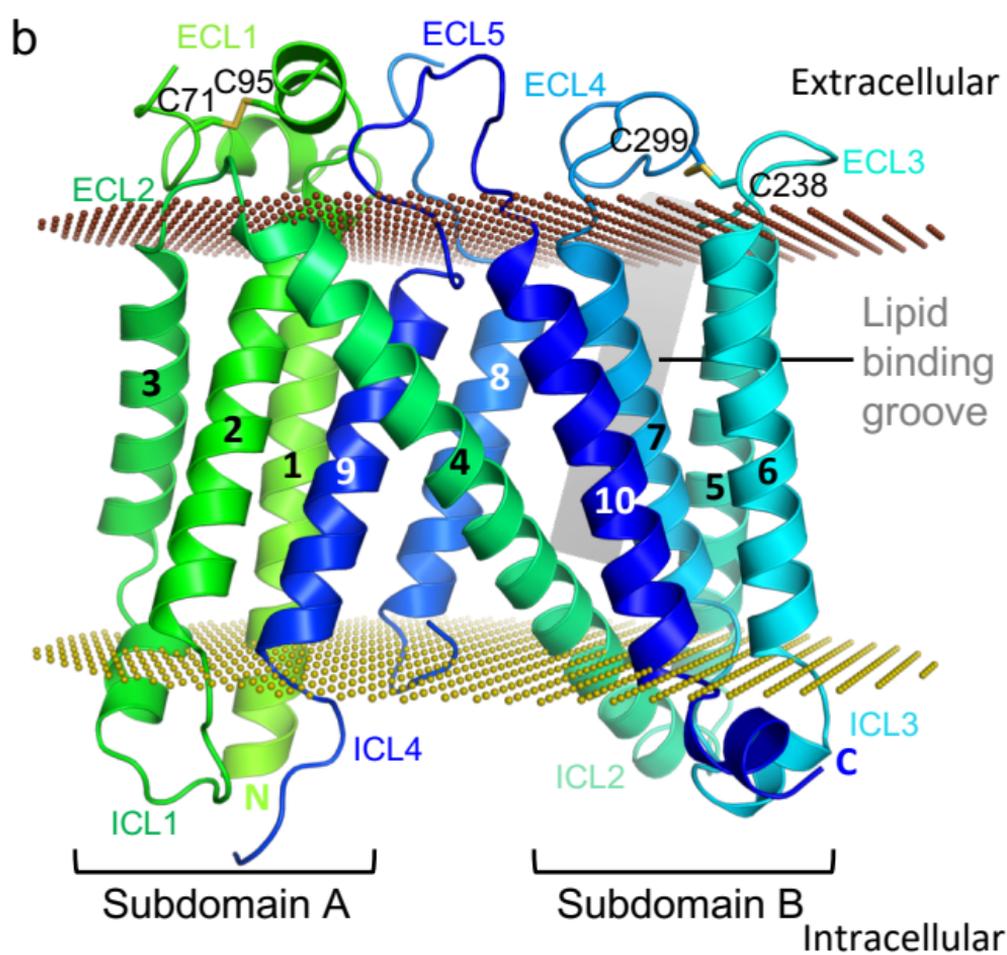
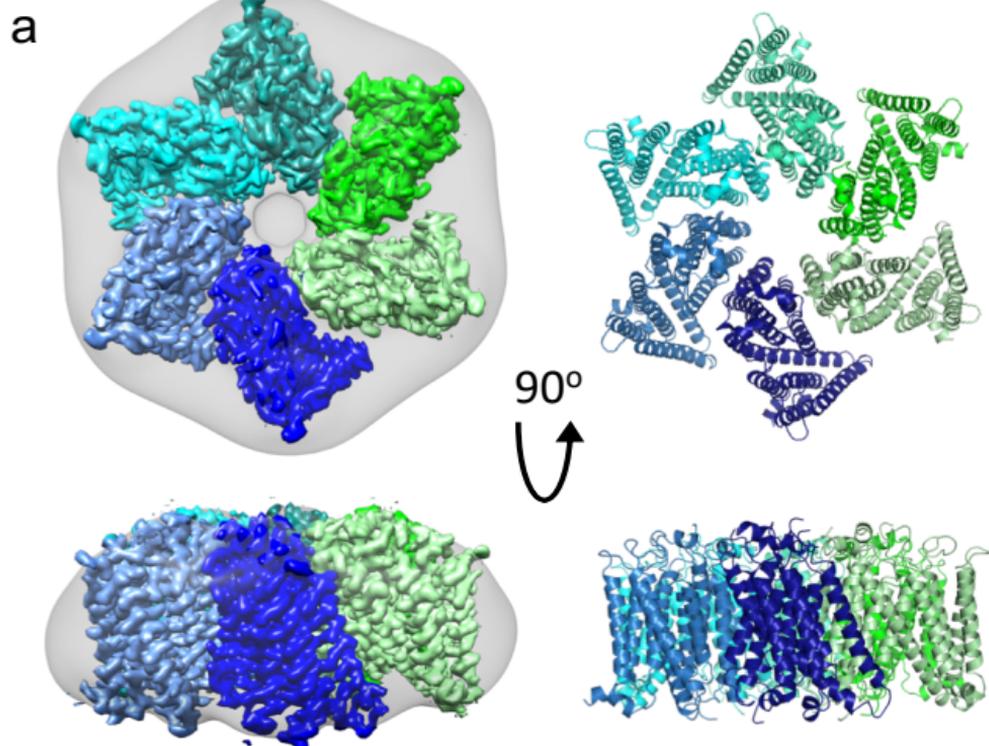
757

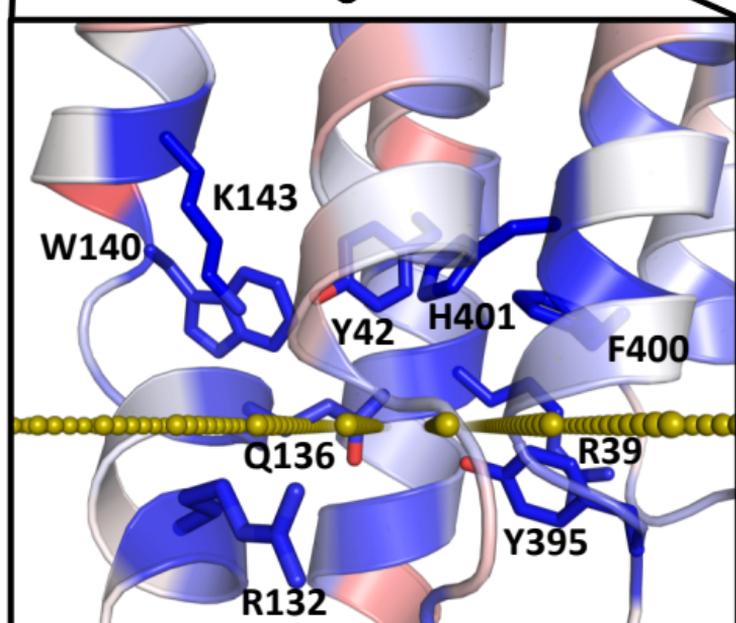
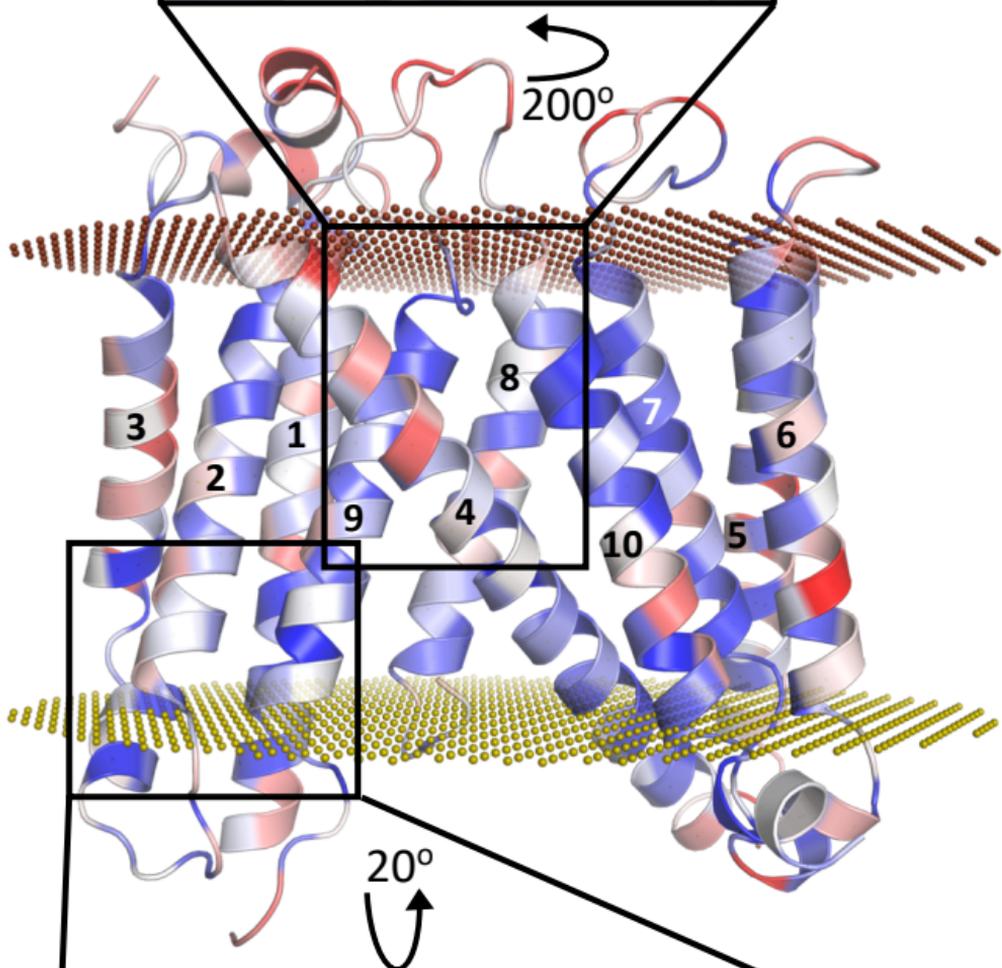
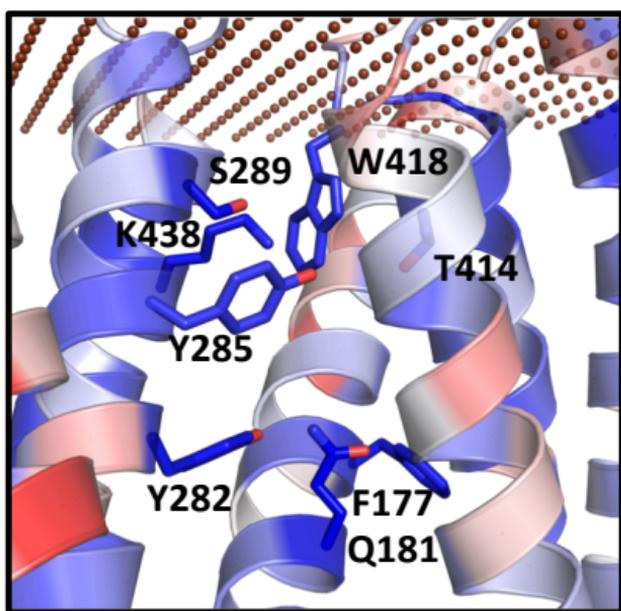
758 **References**

- 759
760
761 32. Bell, J.M., Chen, M., Baldwin, P.R. & Ludtke, S.J. High resolution single
762 particle refinement in EMAN2.1. *Methods* **100**, 25-34 (2016).
- 763 33. Scheres, S.H. RELION: implementation of a Bayesian approach to cryo-EM
764 structure determination. *J Struct Biol* **180**, 519-30 (2012).
- 765 34. Punjani, A., Rubinstein, J.L., Fleet, D.J. & Brubaker, M.A. cryoSPARC:
766 algorithms for rapid unsupervised cryo-EM structure determination. *Nat*
767 *Methods* **14**, 290-296 (2017).
- 768 35. Zheng, S.Q. et al. MotionCor2: anisotropic correction of beam-induced motion
769 for improved cryo-electron microscopy. *Nat Methods* **14**, 331-332 (2017).
- 770 36. Zhang, K. Gctf: Real-time CTF determination and correction. *J Struct Biol*
771 **193**, 1-12 (2016).
- 772 37. Scheres, S.H. & Chen, S. Prevention of overfitting in cryo-EM structure
773 determination. *Nat Methods* **9**, 853-4 (2012).
- 774 38. Heymann, J.B. & Belnap, D.M. Bsoft: image processing and molecular
775 modeling for electron microscopy. *J Struct Biol* **157**, 3-18 (2007).
- 776 39. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics.
777 *Acta Crystallogr D Biol Crystallogr* **60**, 2126-32 (2004).
- 778 40. Afonine, P.V. et al. Real-space refinement in PHENIX for cryo-EM and
779 crystallography. *Acta Crystallogr D Struct Biol* **74**, 531-544 (2018).
- 780 41. Adams, P.D. et al. PHENIX: a comprehensive Python-based system for
781 macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **66**,
782 213-21 (2010).
- 783 42. Chen, V.B. et al. MolProbity: all-atom structure validation for macromolecular
784 crystallography. *Acta Crystallogr D Biol Crystallogr* **66**, 12-21 (2010).

- 785 43. Barad, B.A. et al. EMRinger: side chain-directed model and map validation
786 for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-6 (2015).
- 787 44. Waterhouse, A. et al. SWISS-MODEL: homology modelling of protein
788 structures and complexes. *Nucleic Acids Res* **46**, W296-W303 (2018).
- 789 45. Krissinel, E. & Henrick, K. Inference of macromolecular assemblies from
790 crystalline state. *J Mol Biol* **372**, 774-97 (2007).
- 791 46. Lomize, M.A., Pogozheva, I.D., Joo, H., Mosberg, H.I. & Lomize, A.L. OPM
792 database and PPM web server: resources for positioning of proteins in
793 membranes. *Nucleic Acids Res* **40**, D370-6 (2012).
- 794 47. Marrink, S.J., Risselada, H.J., Yefimov, S., Tieleman, D.P. & de Vries, A.H.
795 The MARTINI force field: coarse grained model for biomolecular
796 simulations. *J Phys Chem B* **111**, 7812-24 (2007).
- 797 48. Monticelli, L. et al. The MARTINI Coarse-Grained Force Field: Extension to
798 Proteins. *J Chem Theory Comput* **4**, 819-34 (2008).
- 799 49. Stansfeld, P.J. & Sansom, M.S. From Coarse Grained to Atomistic: A Serial
800 Multiscale Approach to Membrane Protein Simulations. *J Chem Theory*
801 *Comput* **7**, 1157-66 (2011).
- 802 50. Huang, J. & MacKerell, A.D., Jr. CHARMM36 all-atom additive protein force
803 field: validation based on comparison to NMR data. *J Comput Chem* **34**, 2135-
804 45 (2013).
- 805 51. Olesen, K., Awasthi, N., Bruhn, D.S., Pezeshkian, W. & Khandelia, H. Faster
806 Simulations with a 5 fs Time Step for Lipids in the CHARMM Force Field. *J*
807 *Chem Theory Comput* **14**, 3342-3350 (2018).

- 808 52. Berendsen, H.J.C., van der Spoel, D. & van Drunen, R. GROMACS: A
809 message-passing parallel molecular dynamics implementation. *Comput Phys*
810 *Commun* **91**, 43-56 (1995).
- 811 53. Humphrey, W., Dalke, A. & Schulten, K. VMD: Visual molecular dynamics *J*
812 *Mol Graph* **14**, 33-8 (1996).
- 813 54. Pizzato, M. et al. A one-step SYBR Green I-based product-enhanced reverse
814 transcriptase assay for the quantitation of retroviruses in cell culture
815 supernatants. *J Virol Methods* **156**, 1-7 (2009).
- 816 55. Gault, J. et al. High-resolution mass spectrometry of small molecules bound to
817 membrane proteins. *Nat Methods* **13**, 333-6 (2016).
- 818 56. Hernandez, H. & Robinson, C.V. Determining the stoichiometry and
819 interactions of macromolecular assemblies from mass spectrometry. *Nat*
820 *Protoc* **2**, 715-26 (2007).
- 821 57. Marty, M.T. et al. Bayesian deconvolution of mass and ion mobility spectra:
822 from binary interactions to polydisperse ensembles. *Anal Chem* **87**, 4370-6
823 (2015).
- 824 58. Silva, J.C. et al. Quantitative proteomic analysis by accurate mass retention
825 time pairs. *Anal Chem* **77**, 2187-200 (2005).
- 826 59. Frigola, J., Remus, D., Mehanna, A. & Diffley, J.F. ATPase-dependent quality
827 control of DNA replication origin licensing. *Nature* **495**, 339-43 (2013).
- 828 60. Ulm, J.W., Perron, M., Sodroski, J. & R, C.M. Complex determinants within
829 the Moloney murine leukemia virus capsid modulate susceptibility of the virus
830 to Fv1 and Ref1-mediated restriction. *Virology* **363**, 245-55 (2007).
- 831 61. Robert, X. & Gouet, P. Deciphering key features in protein structures with the
832 new ENDscript server. *Nucleic Acids Res* **42**, W320-4 (2014).

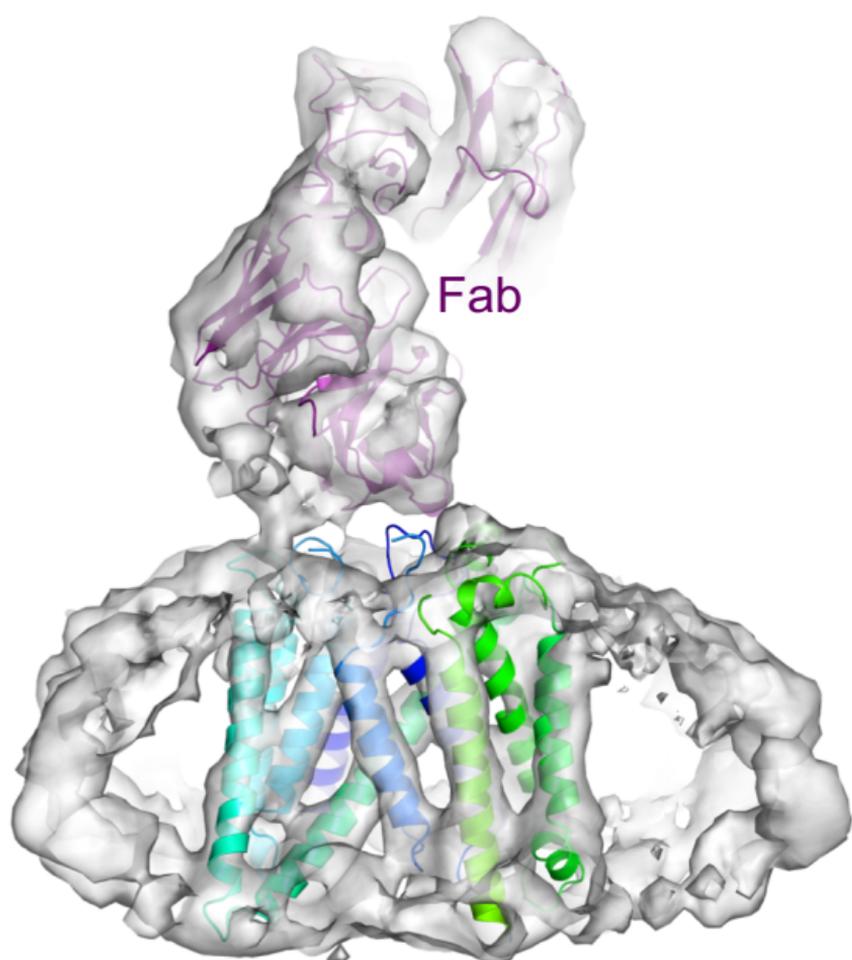




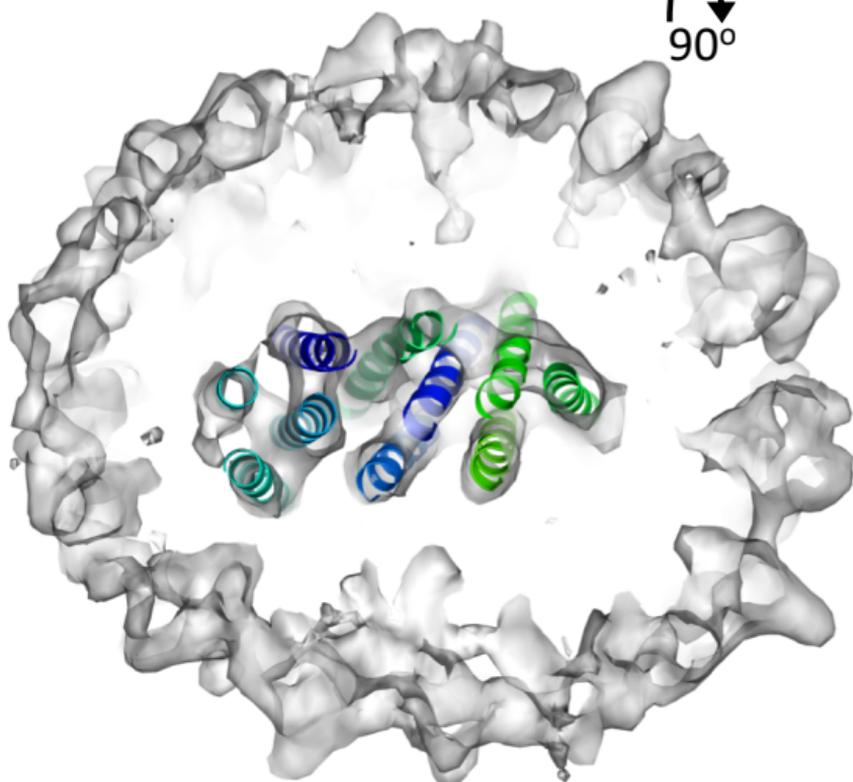
Invariant

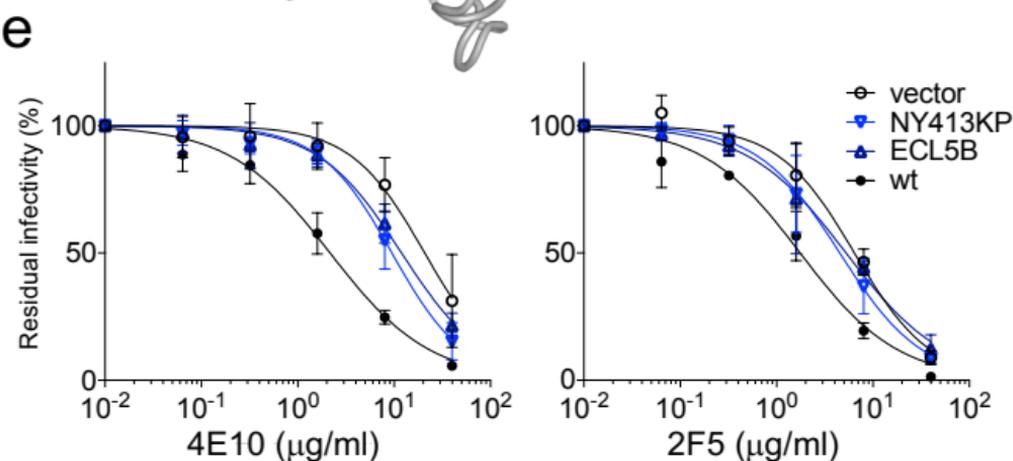
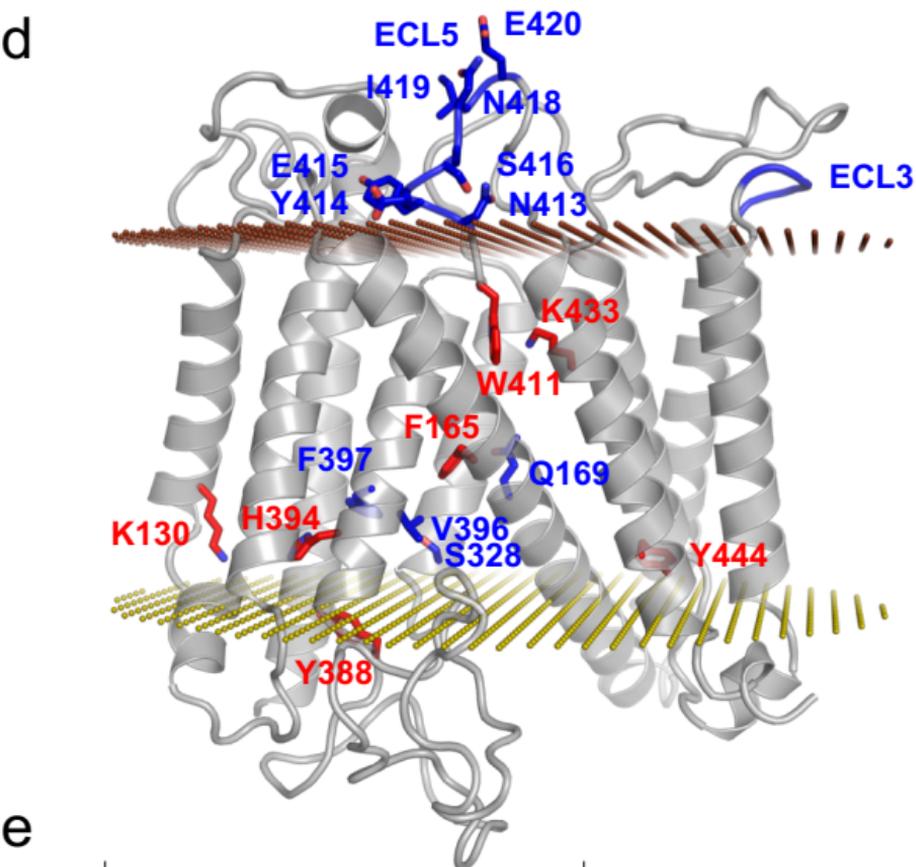
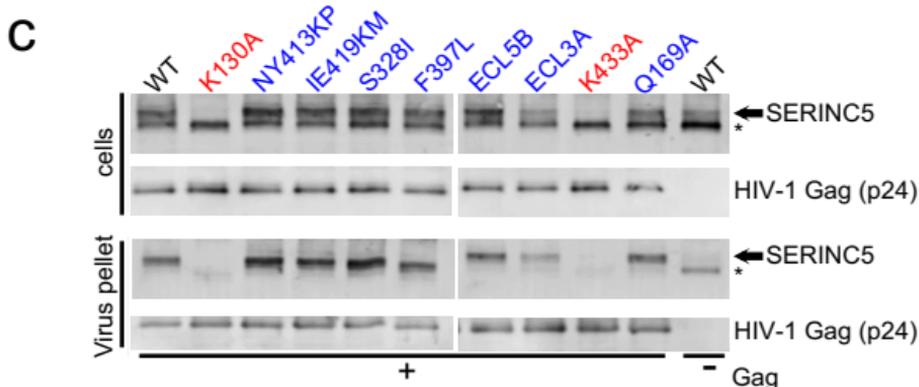
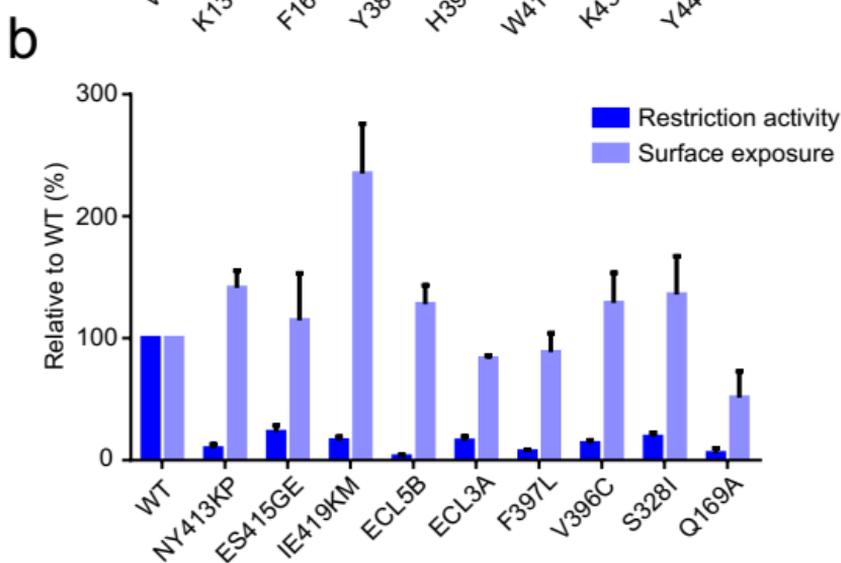
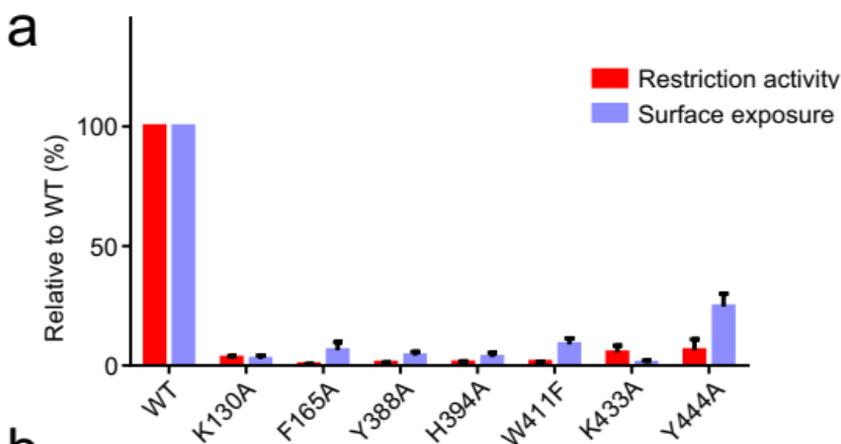


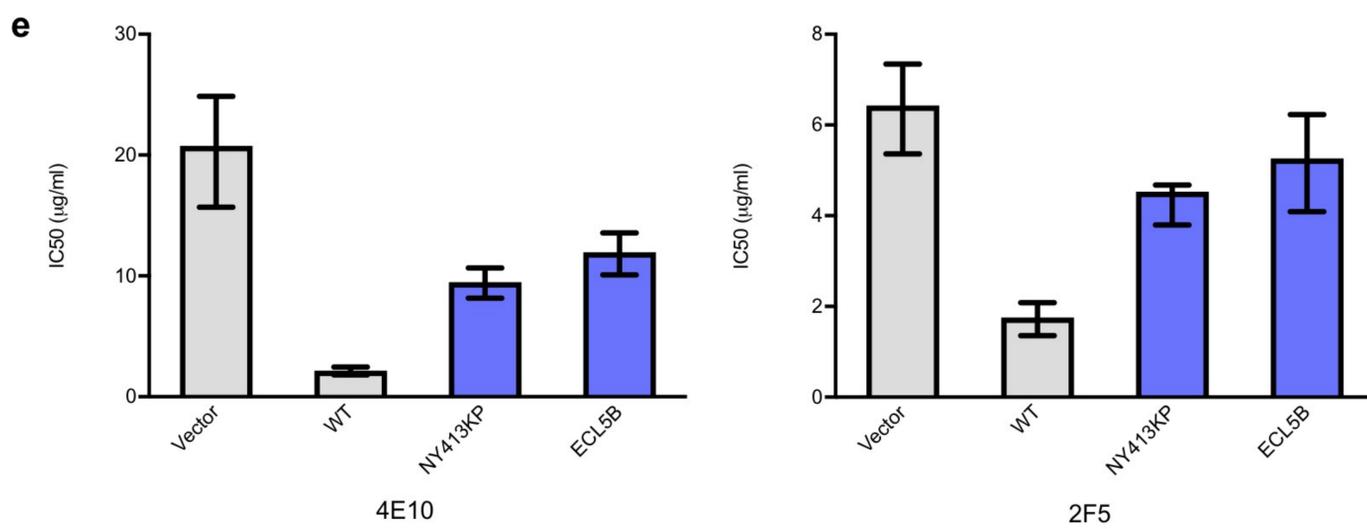
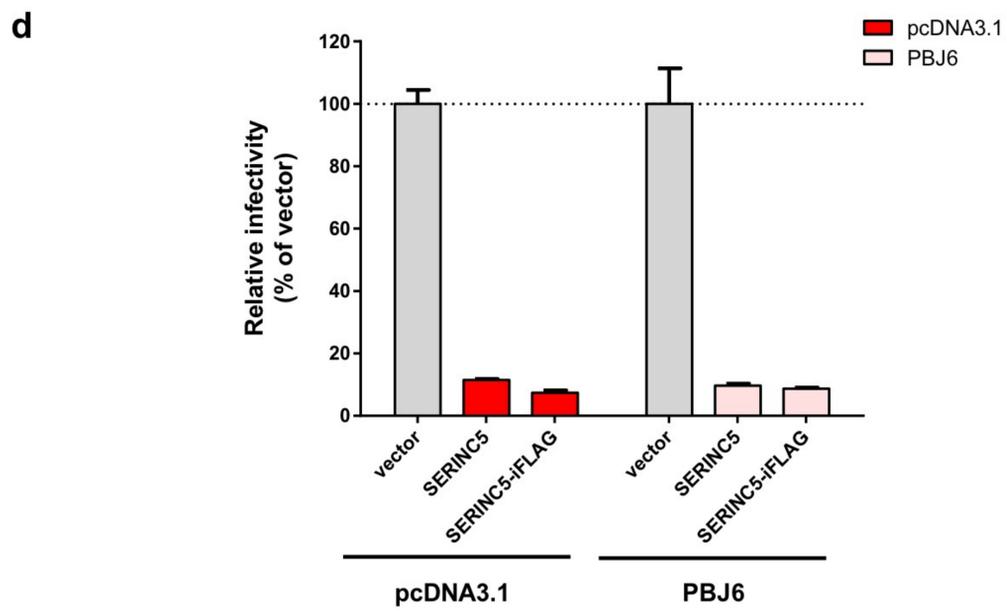
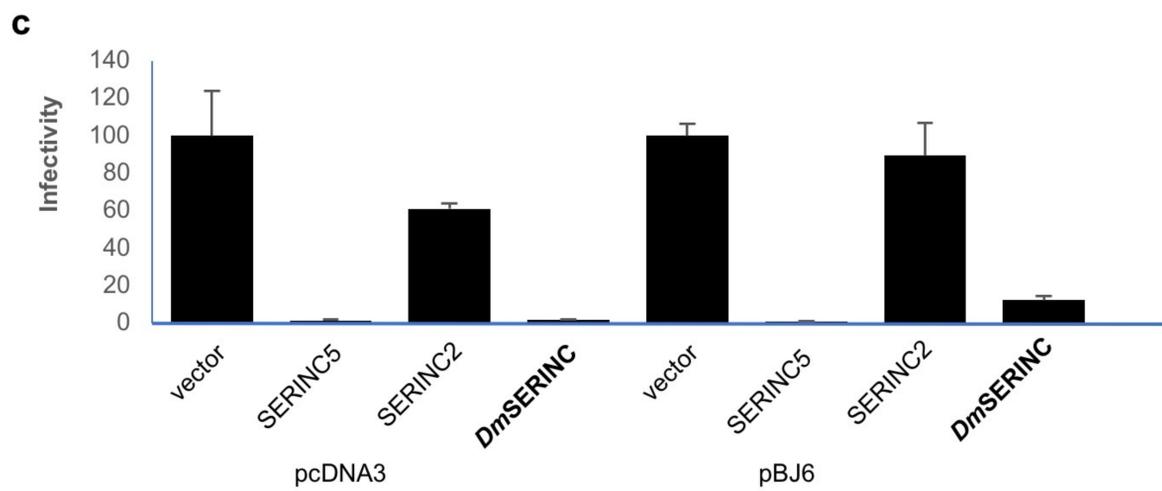
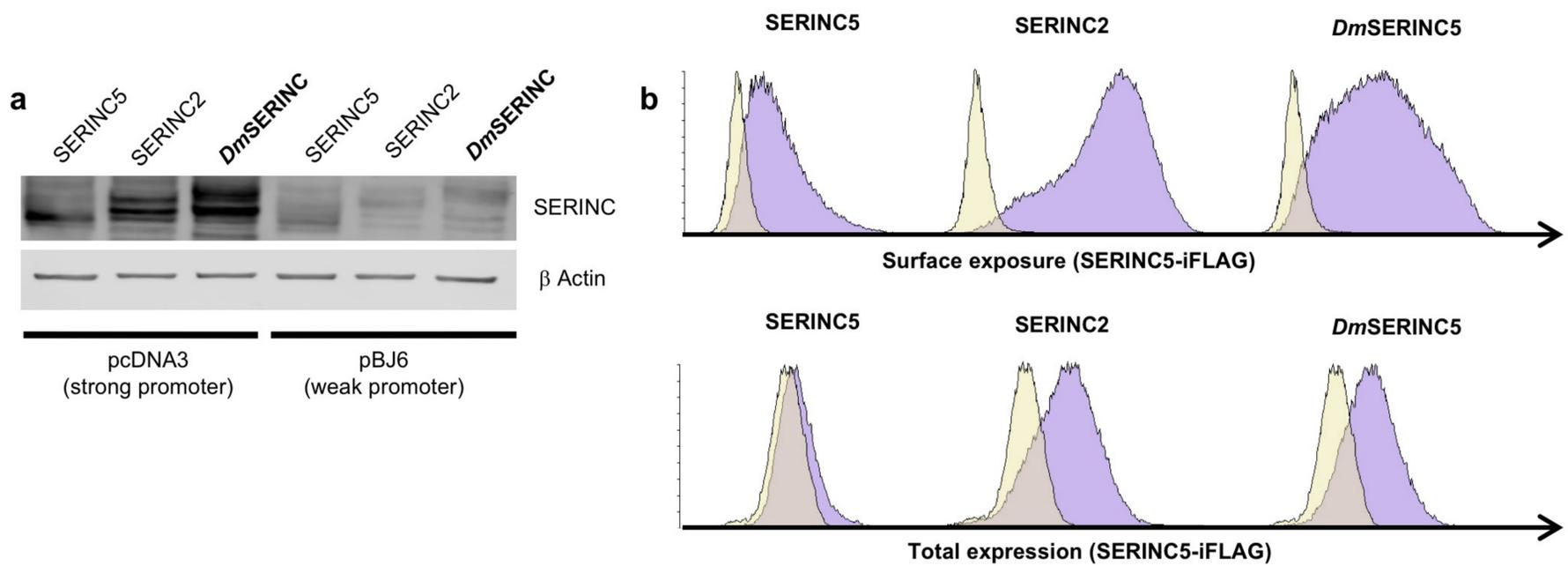
Most Variable

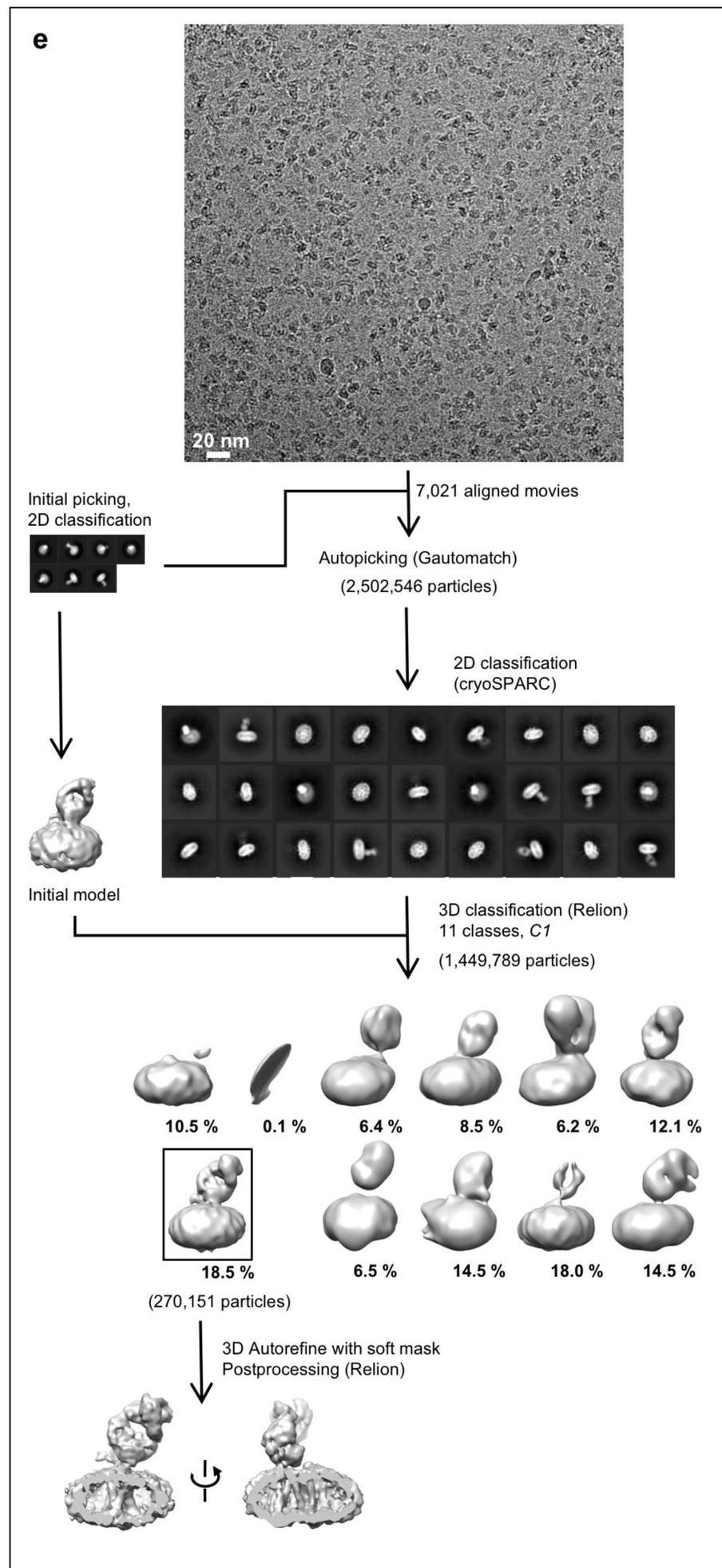
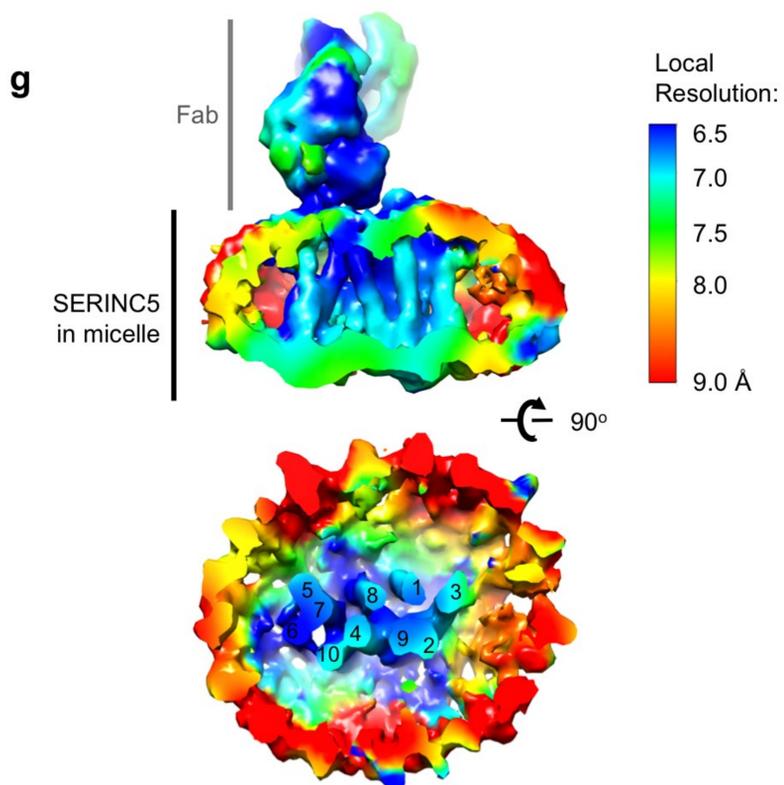
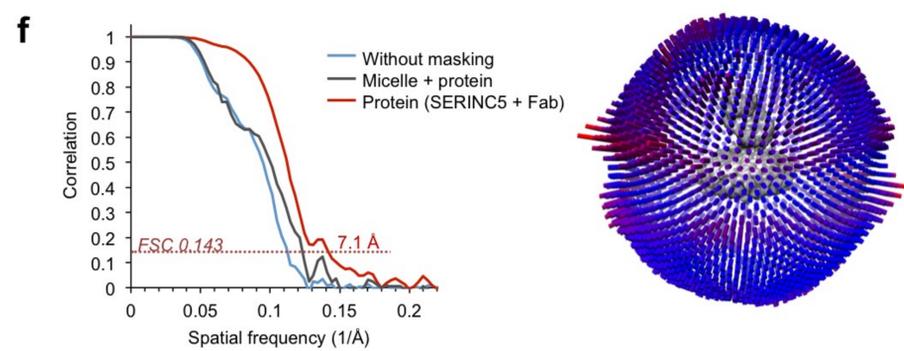
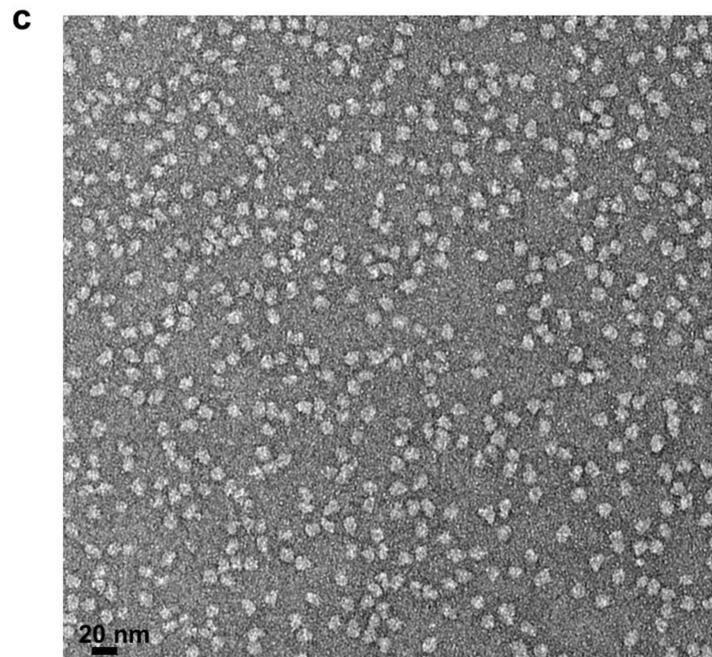
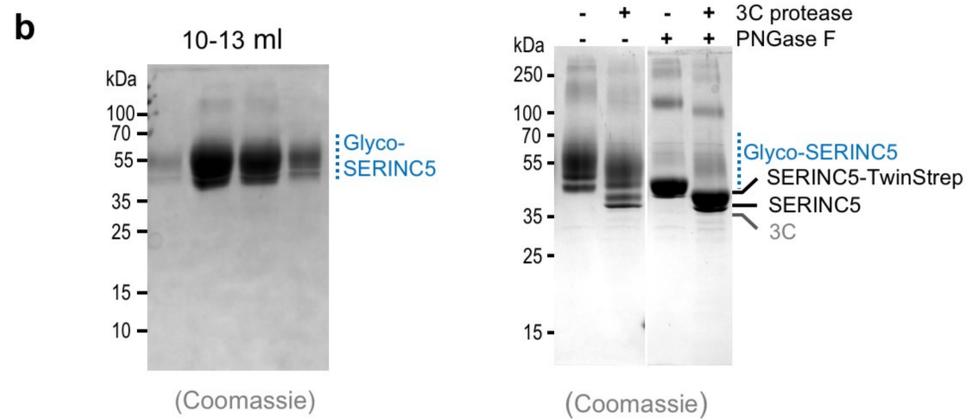
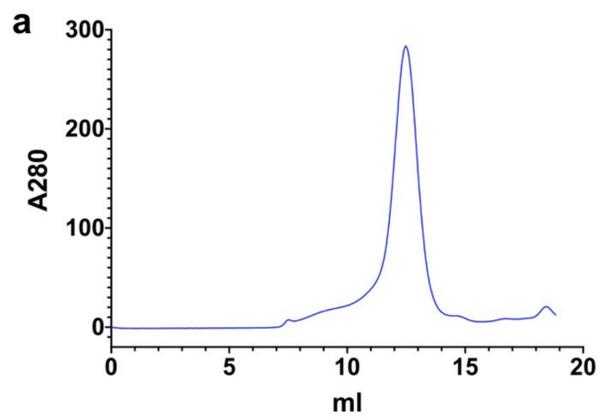


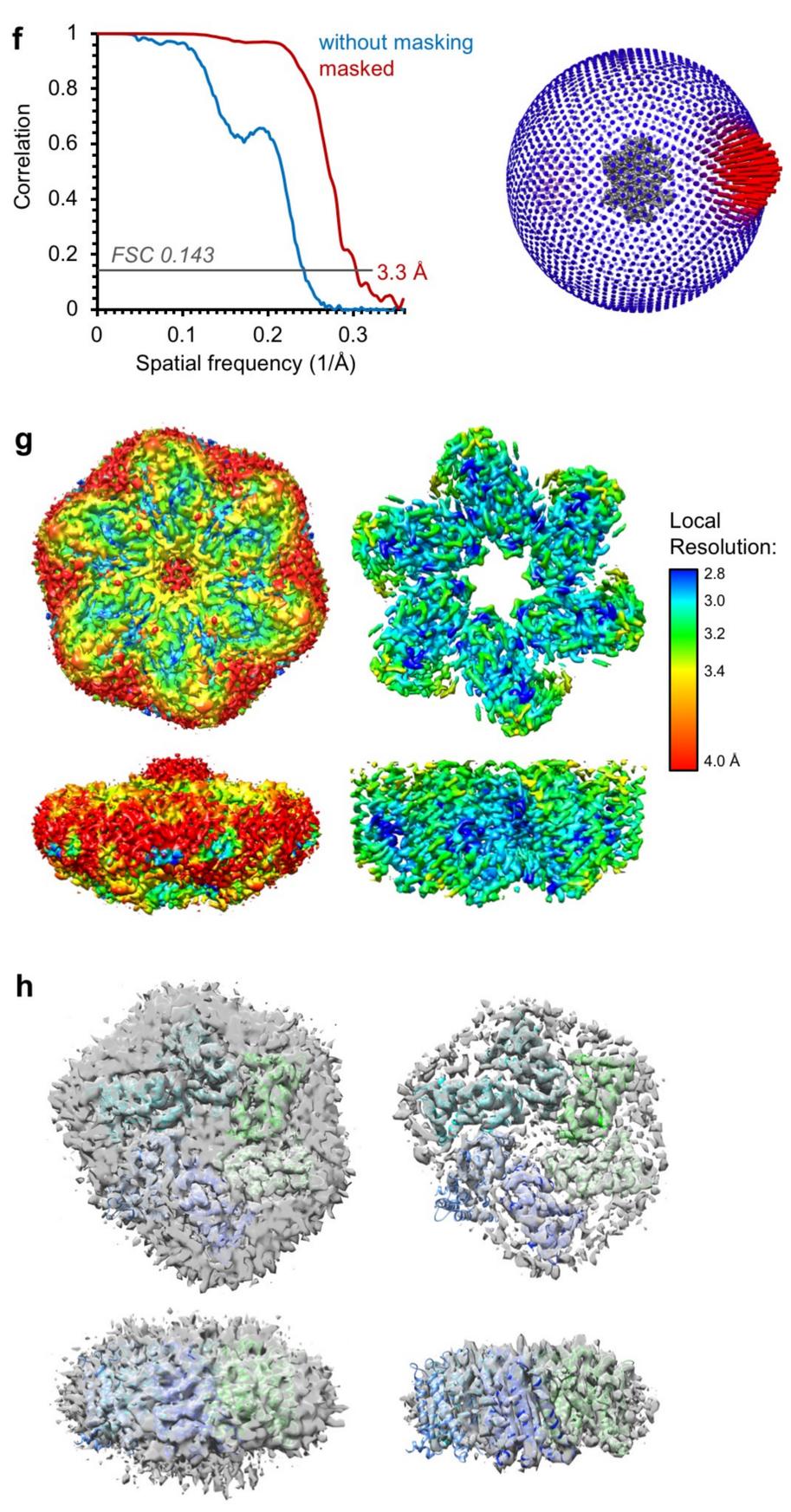
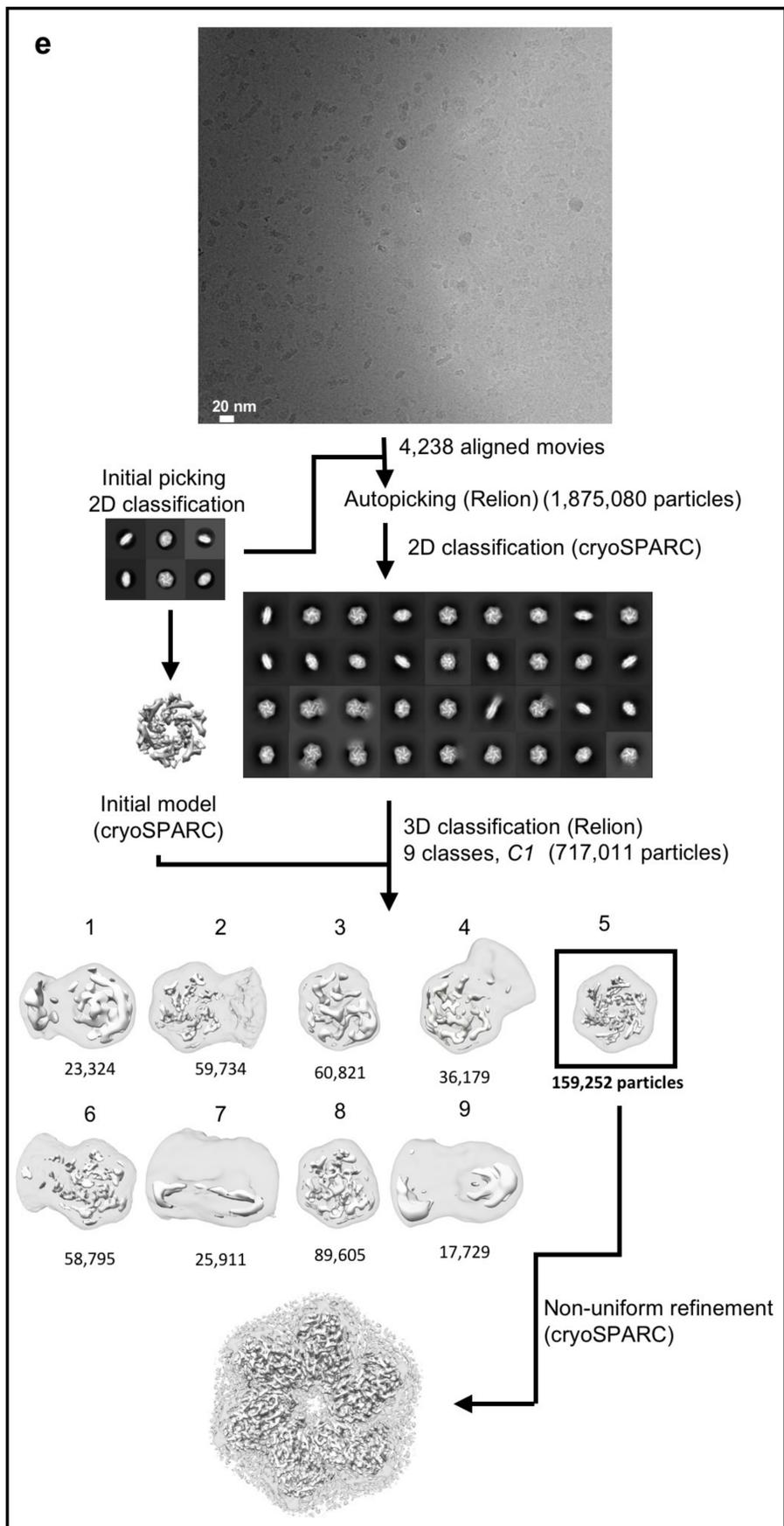
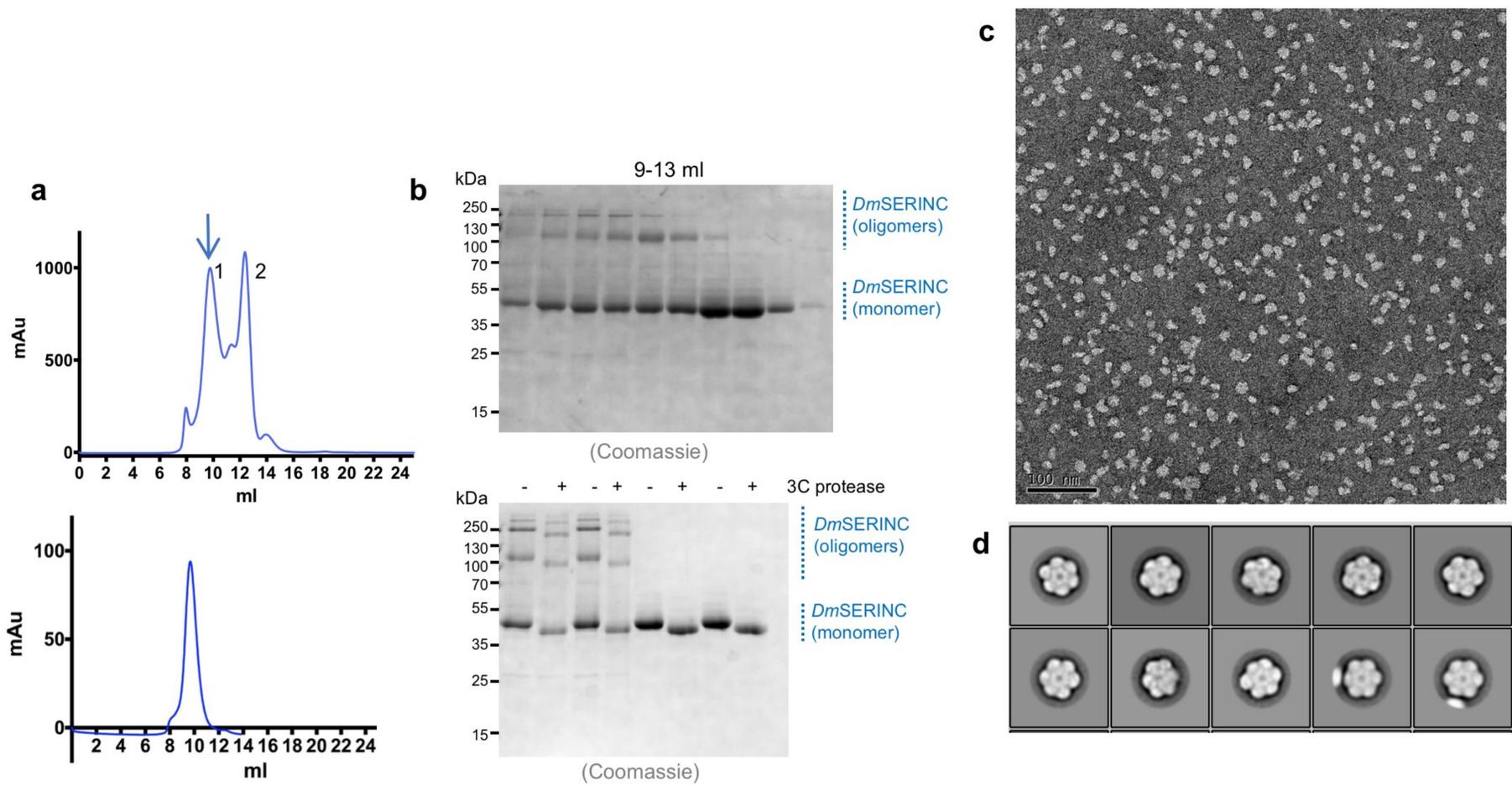
SERINC5 in micelle

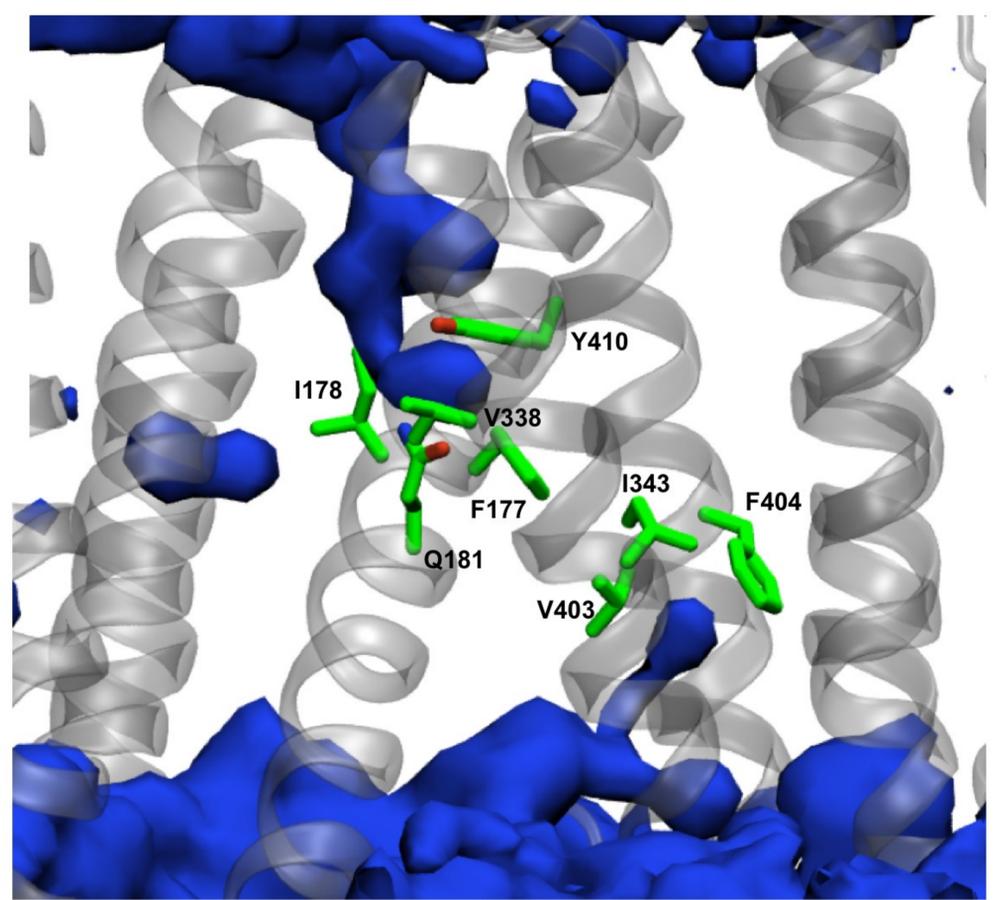
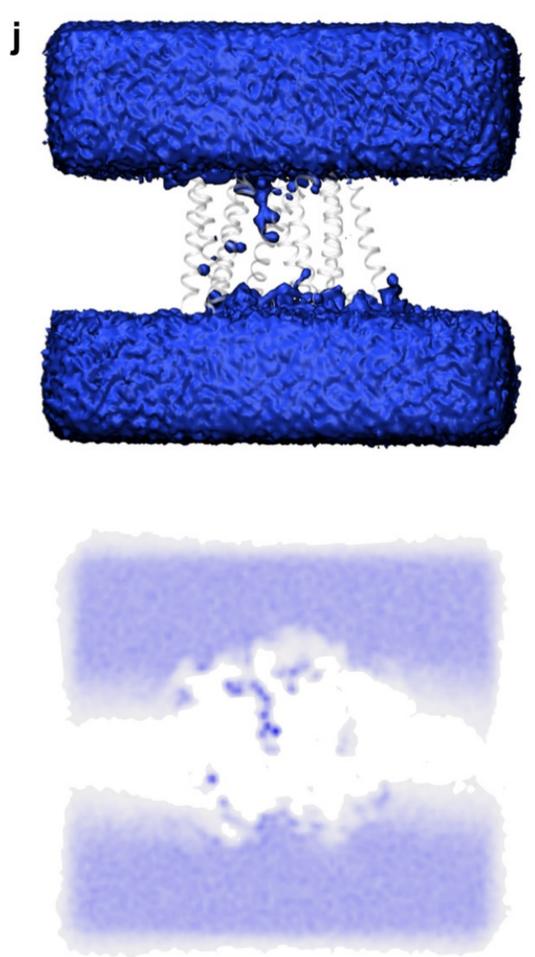
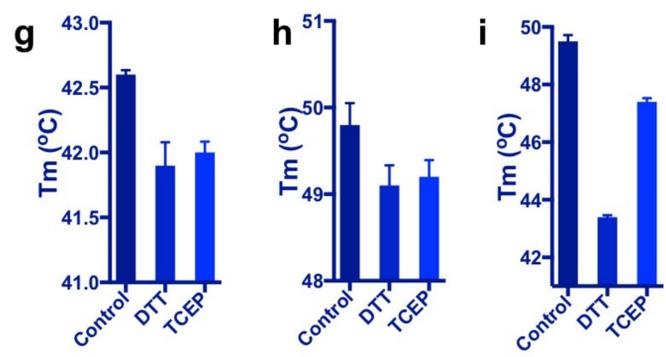
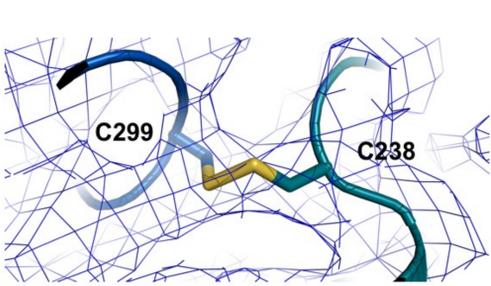
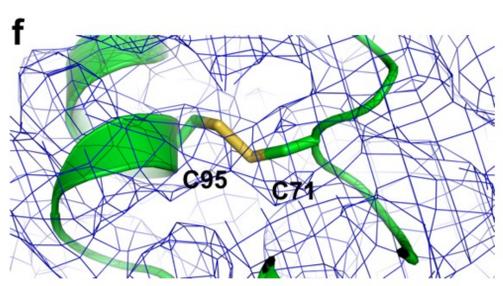
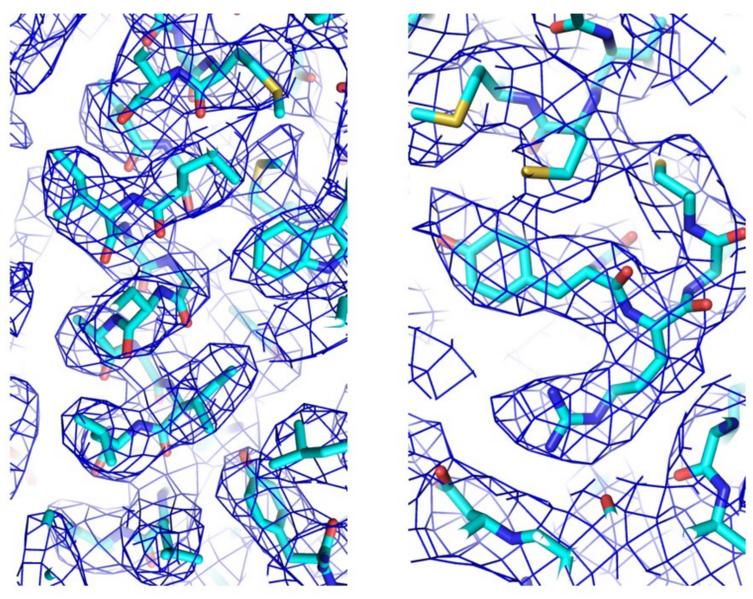
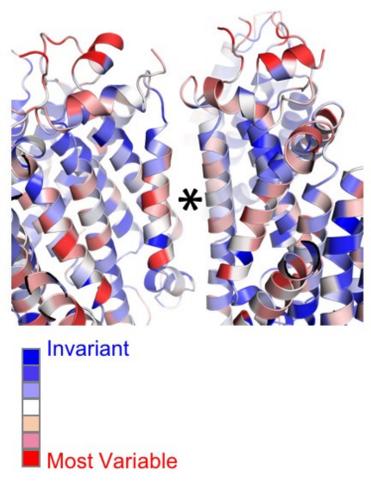
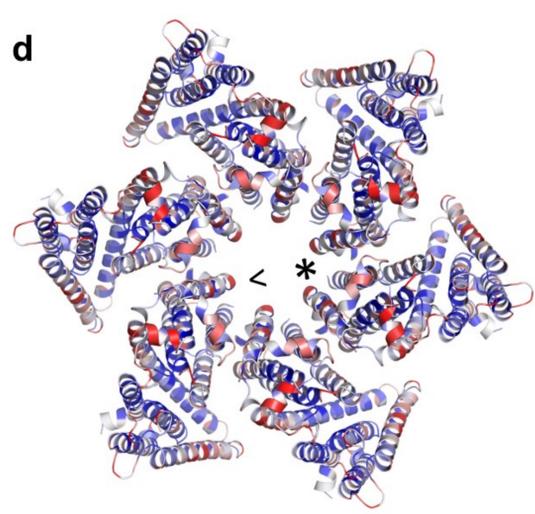
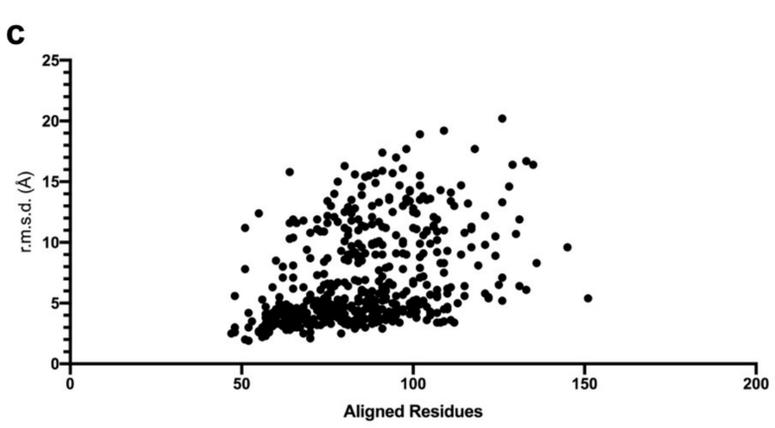
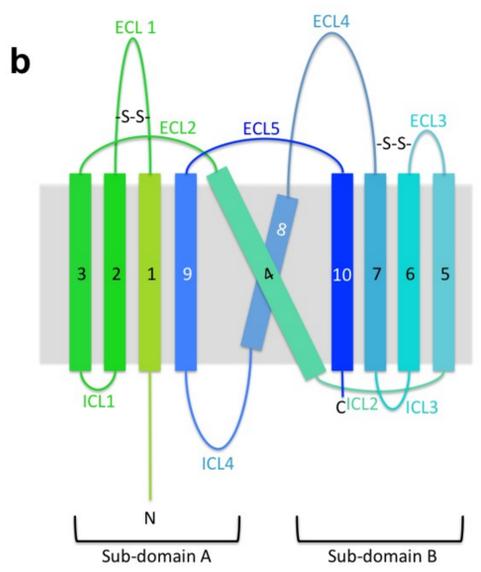
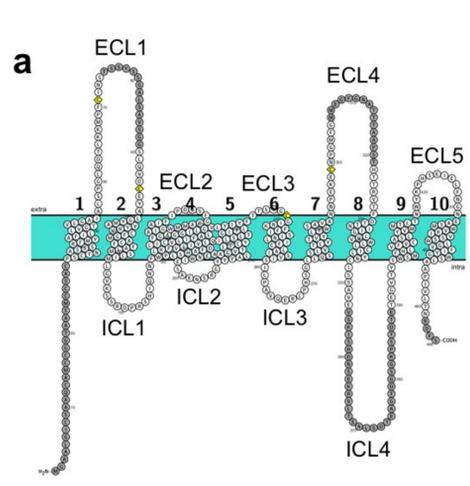


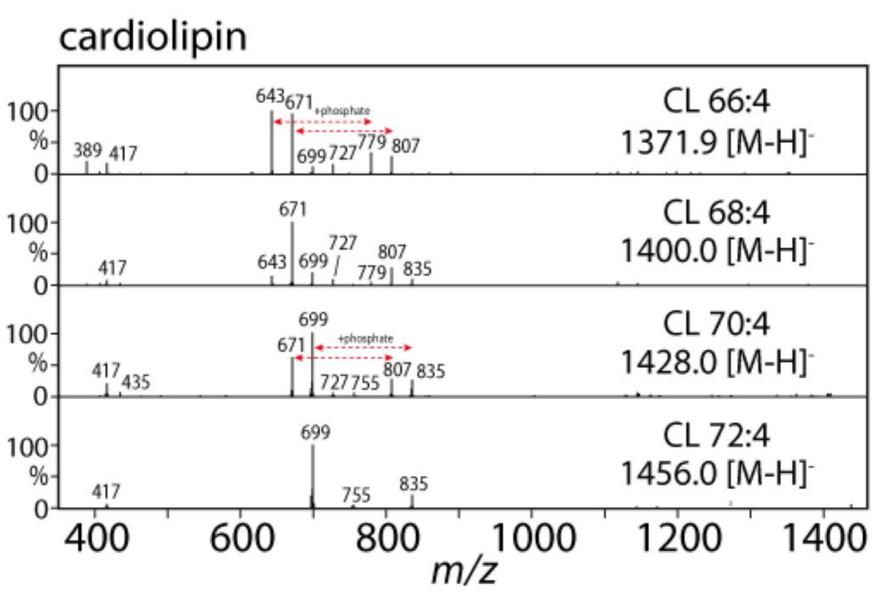
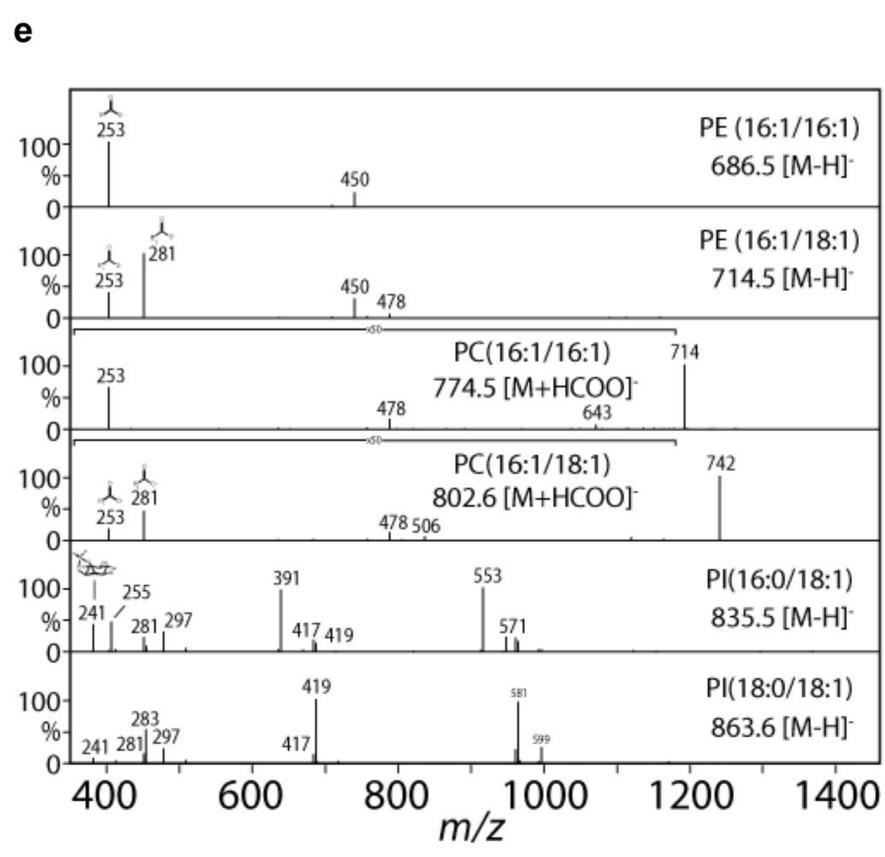
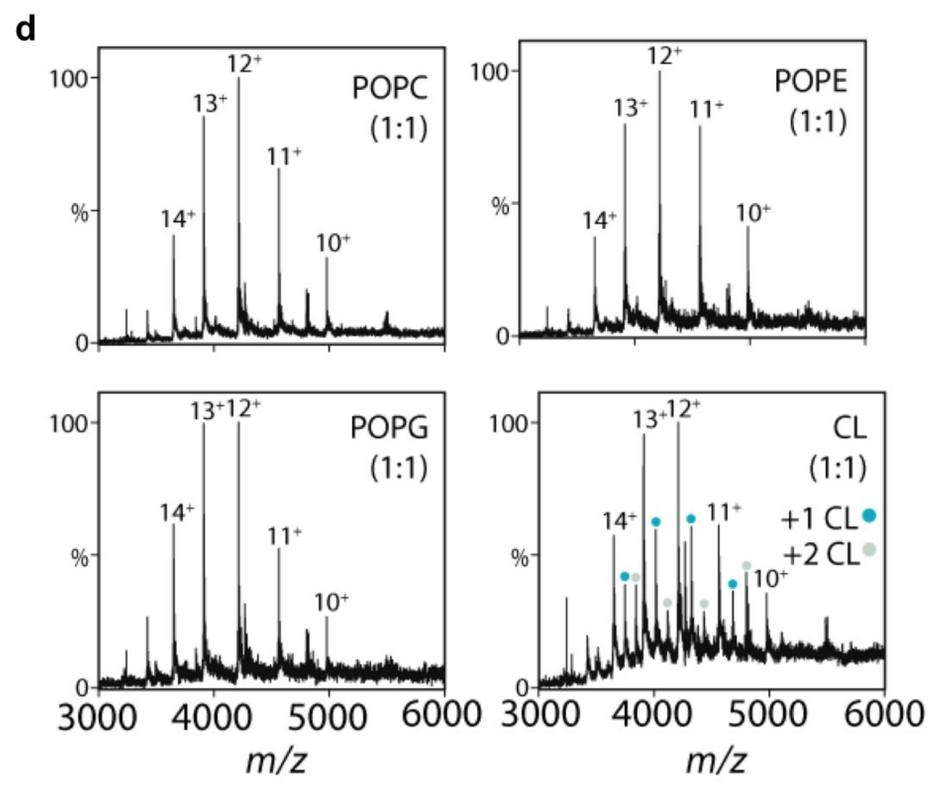
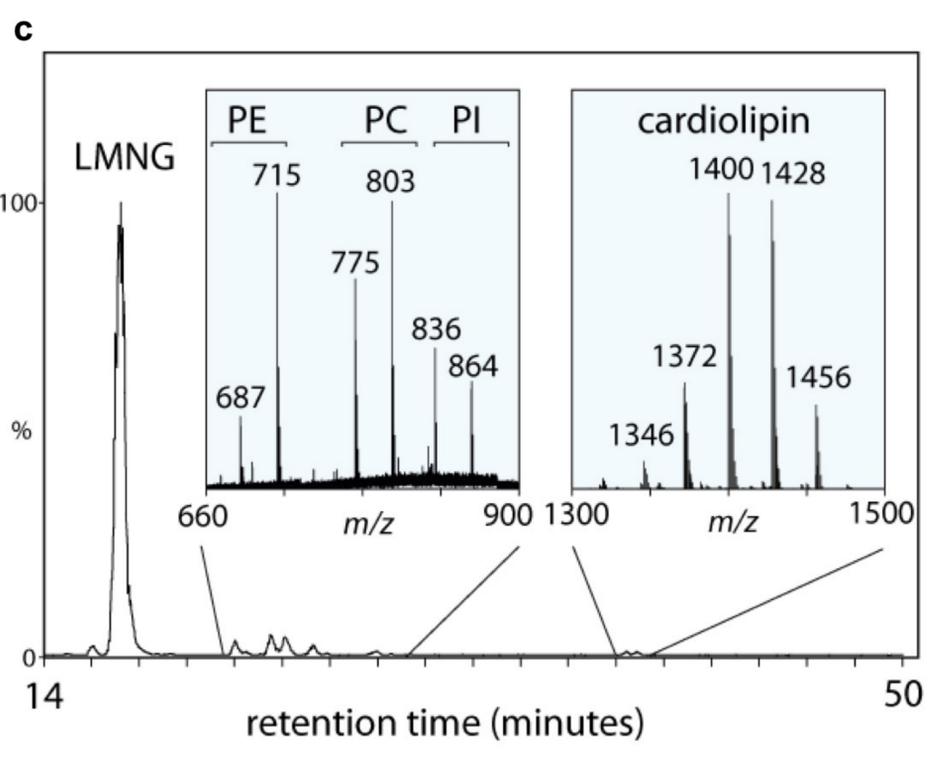
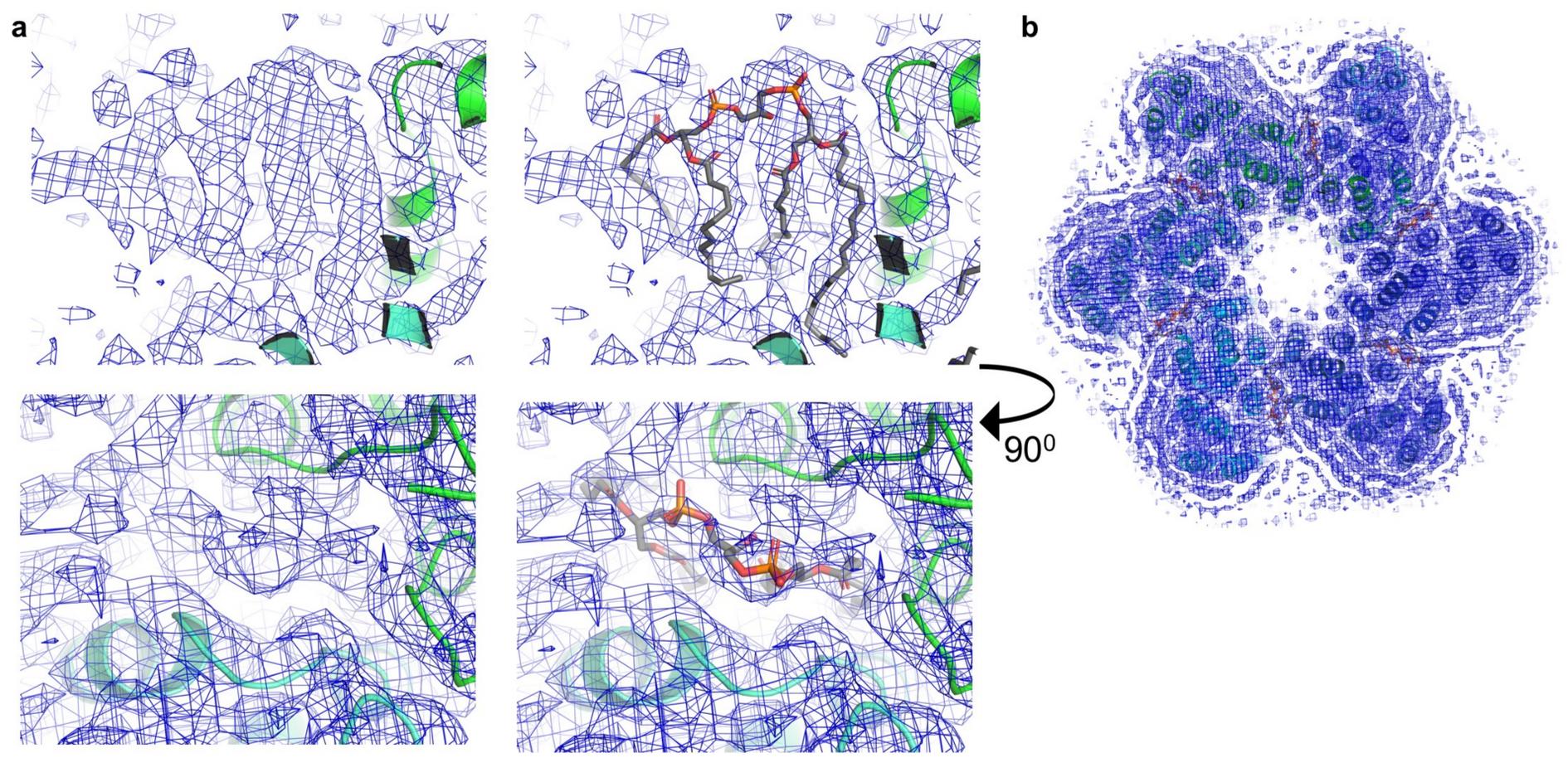


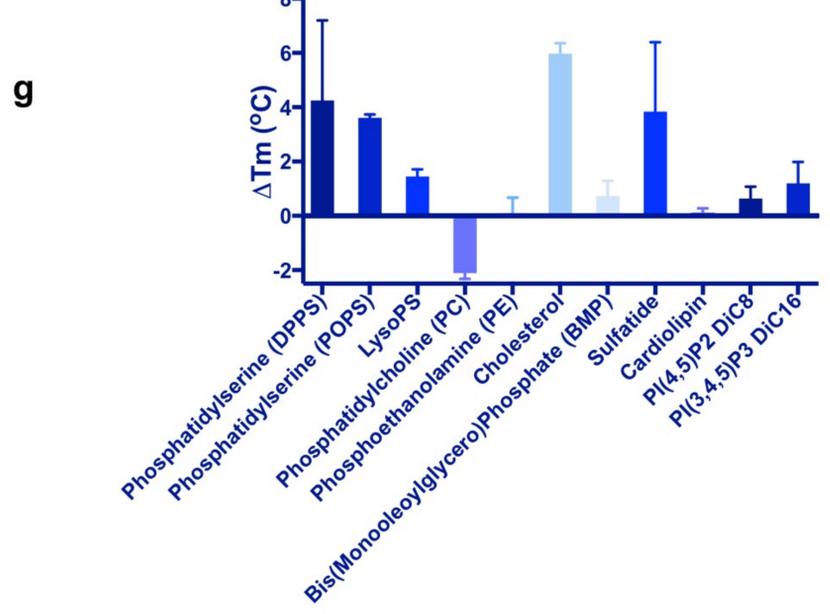
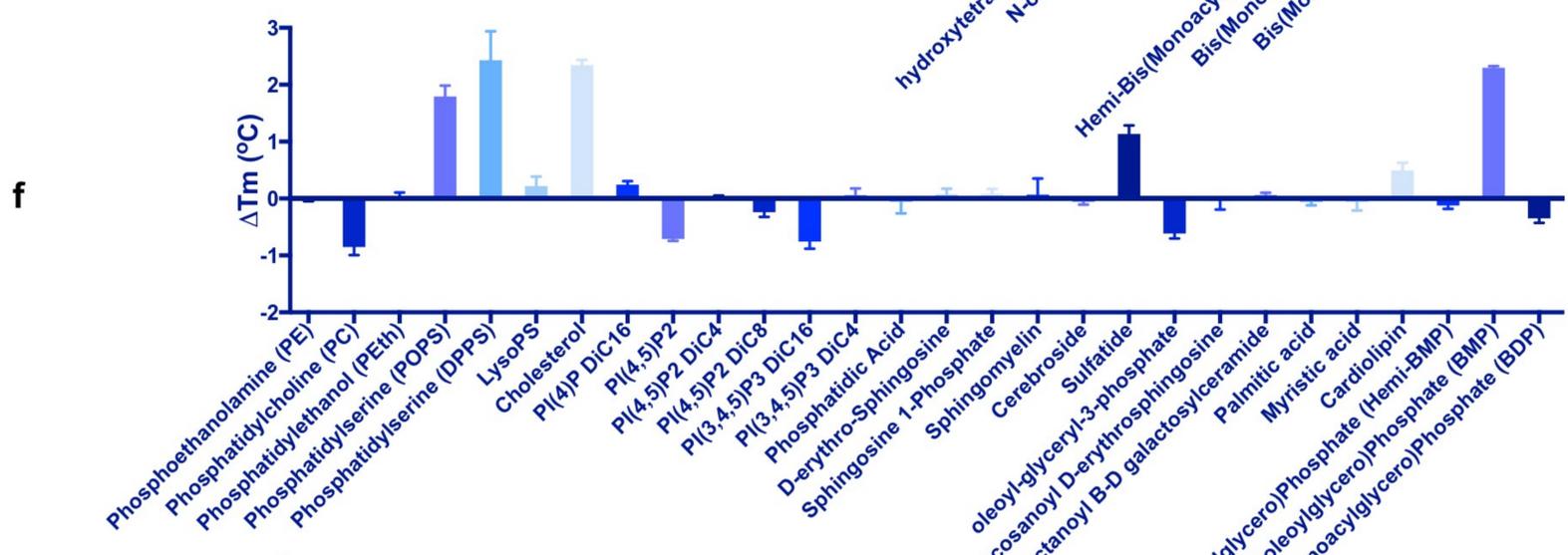
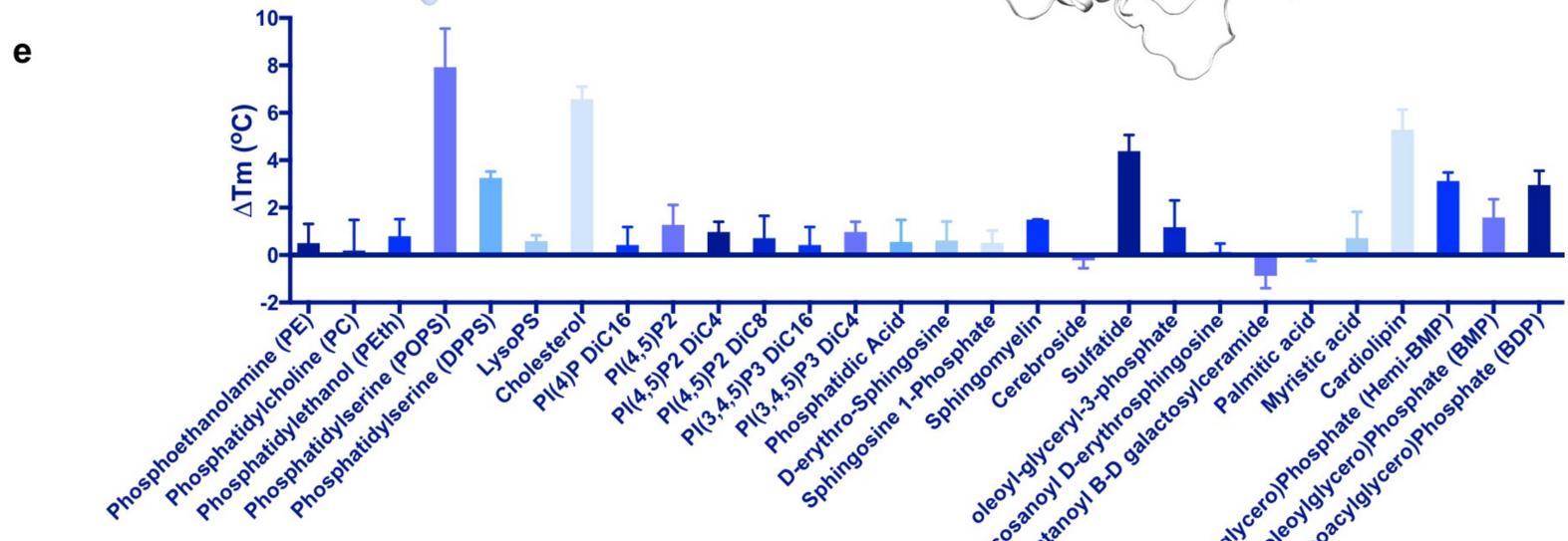
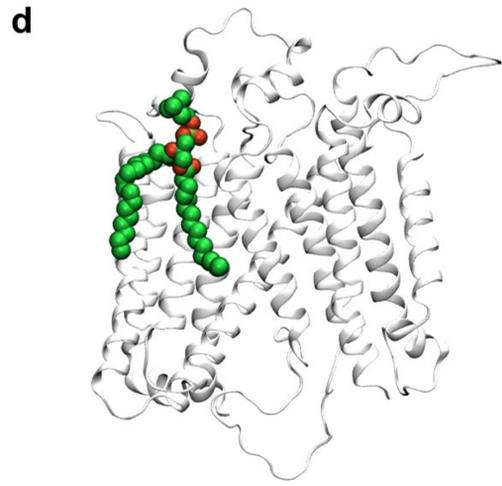
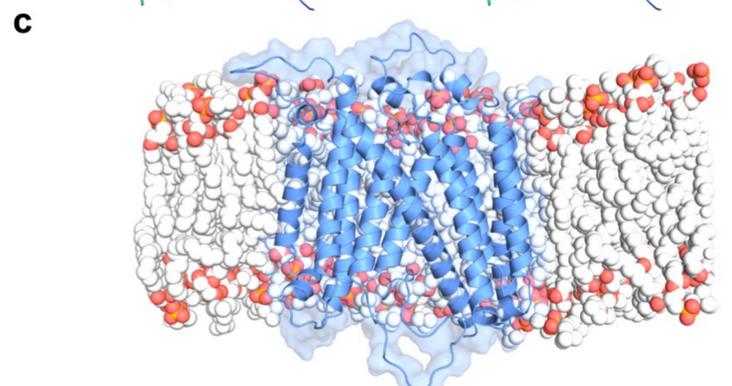
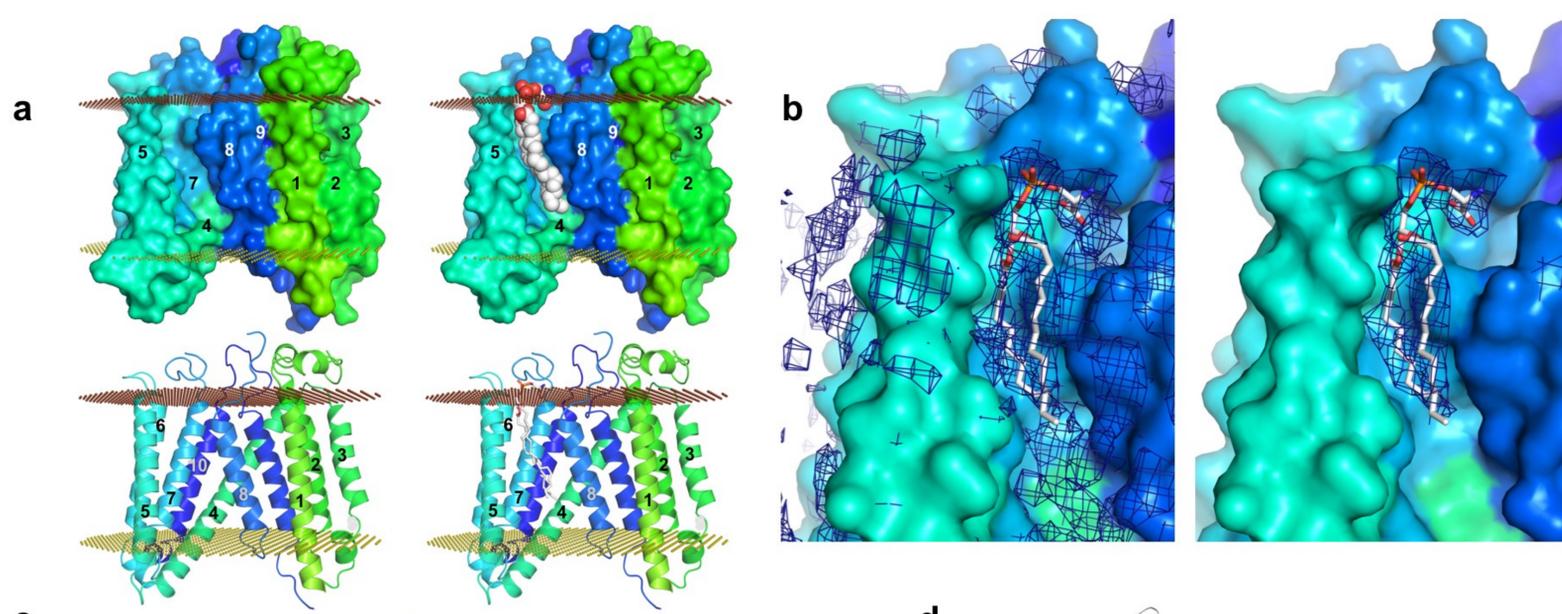


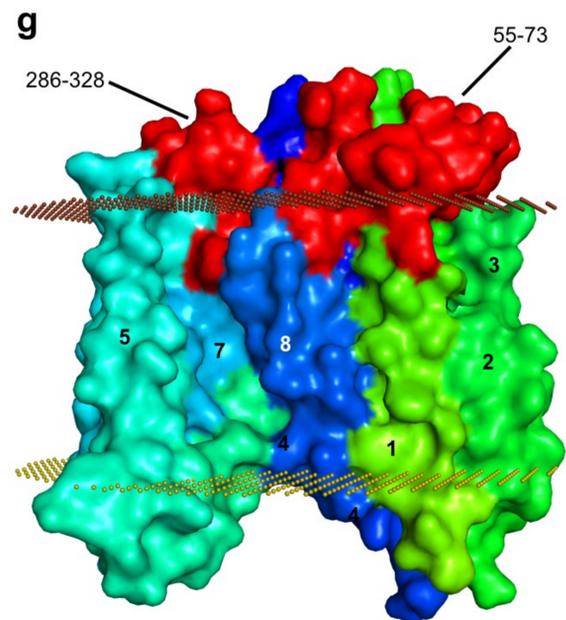
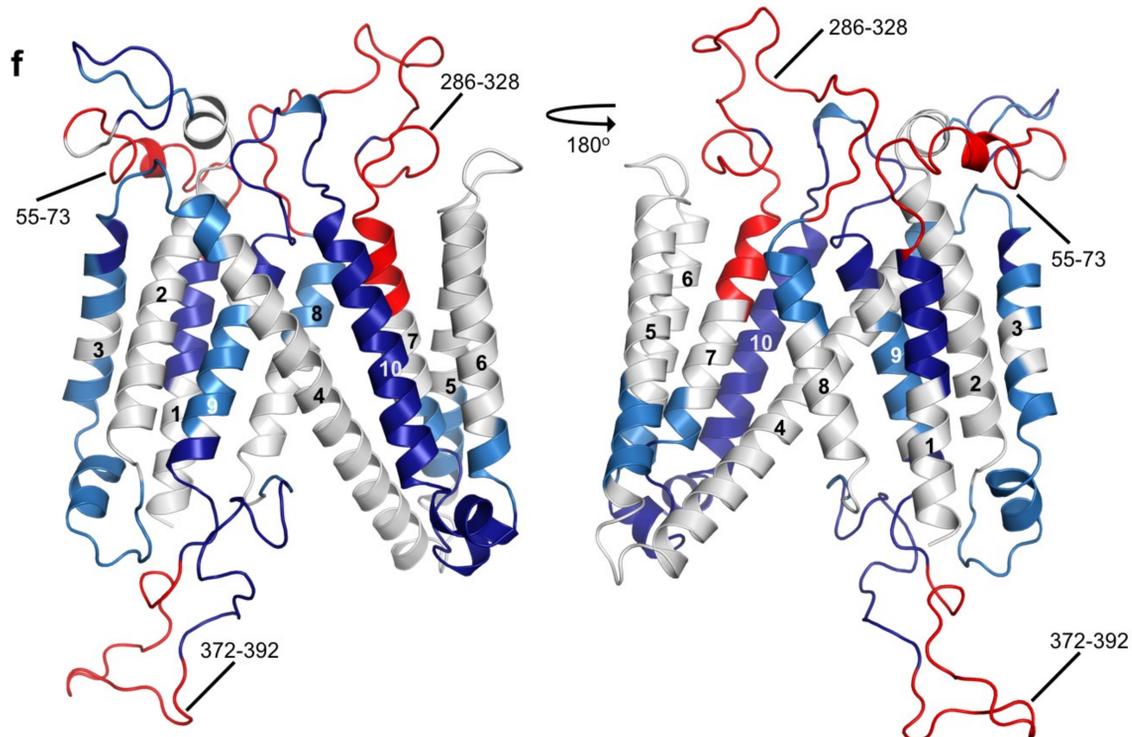
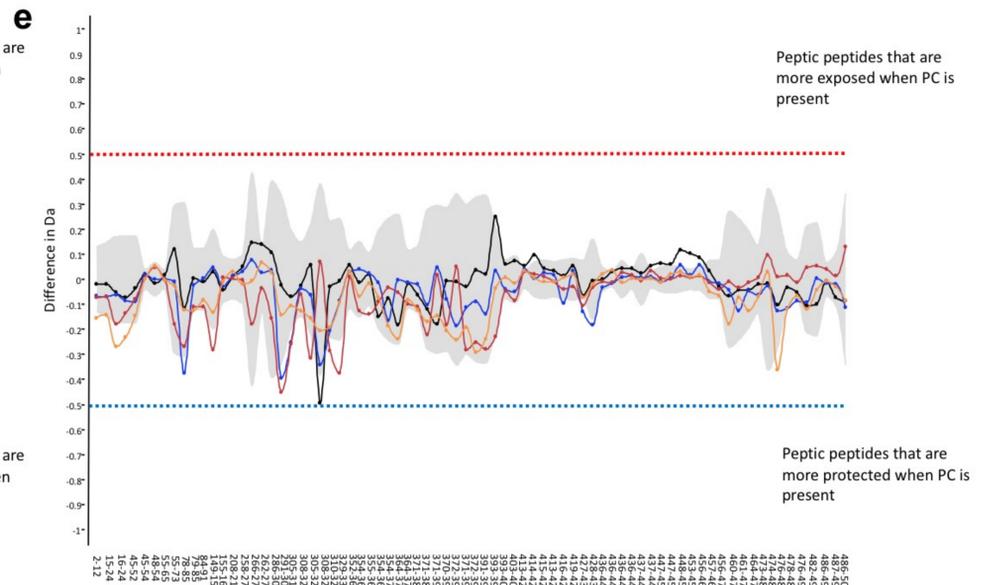
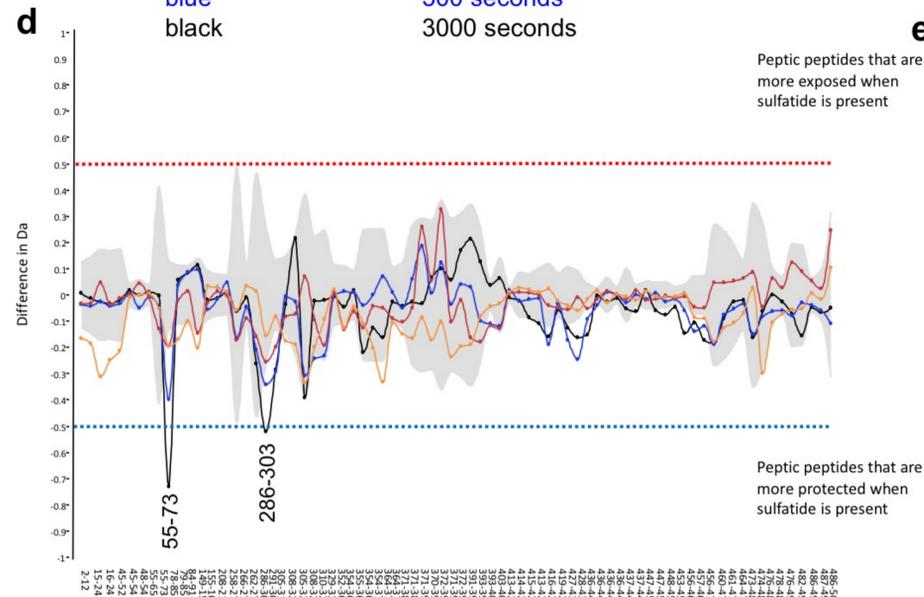
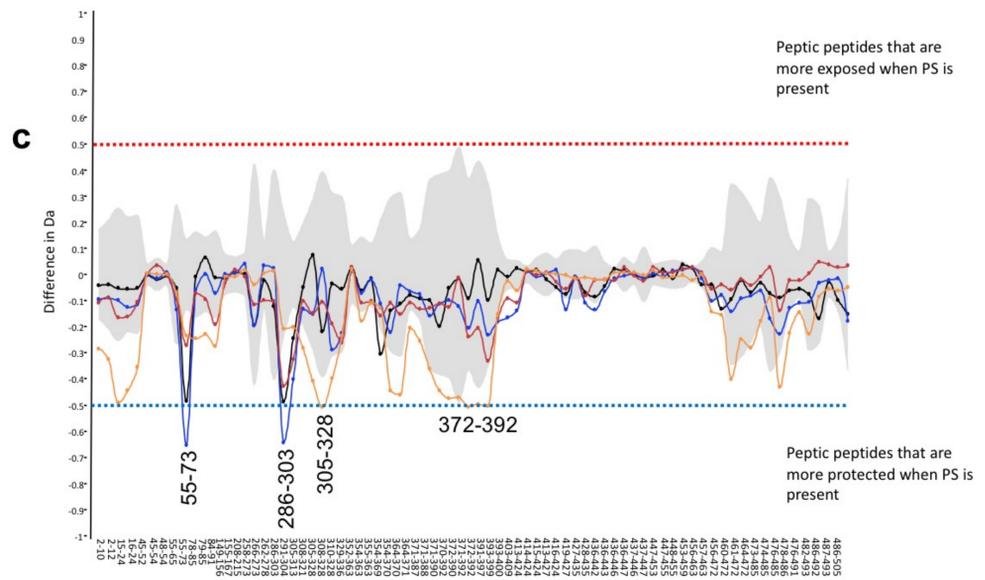
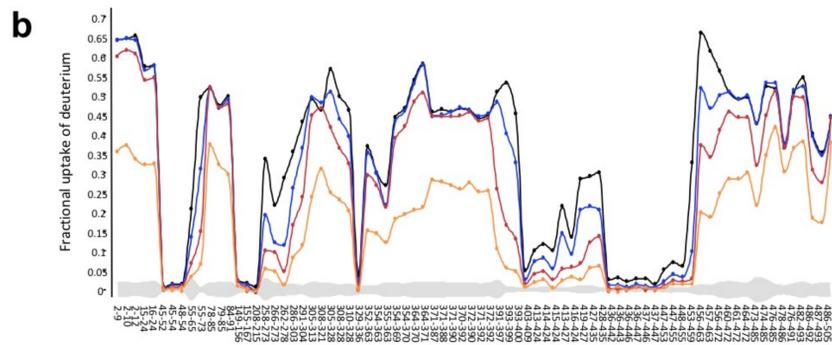
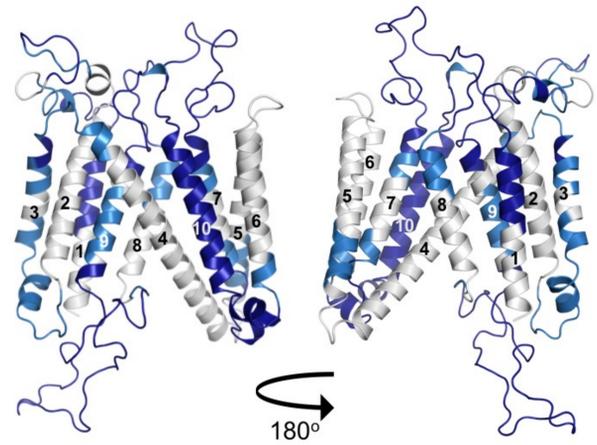
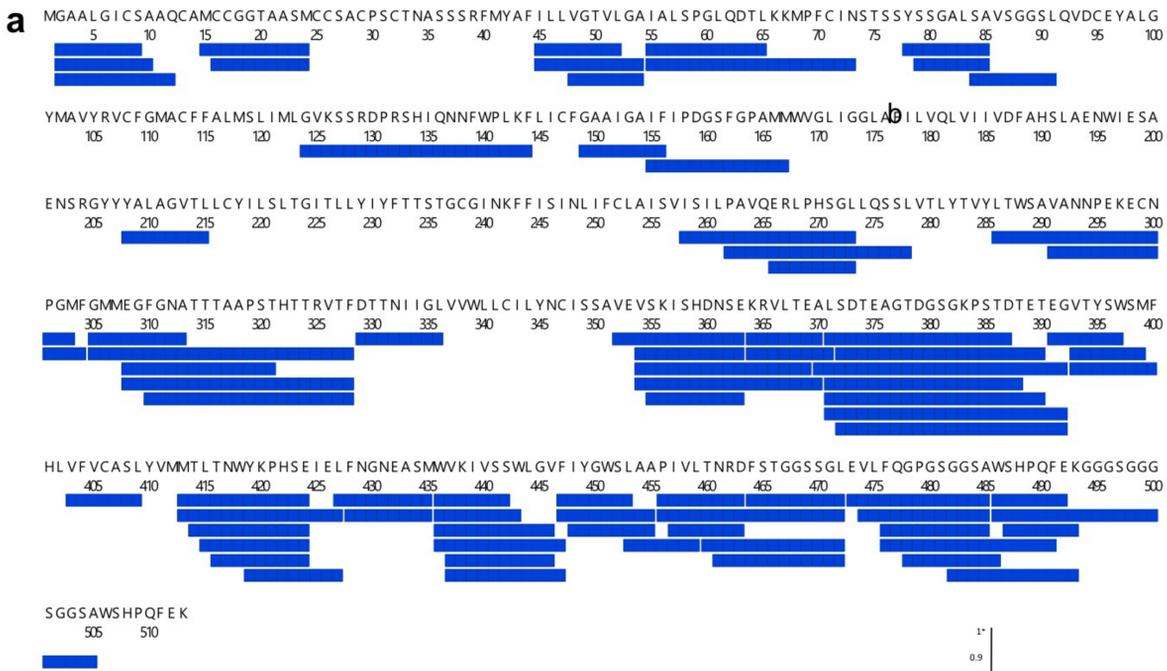


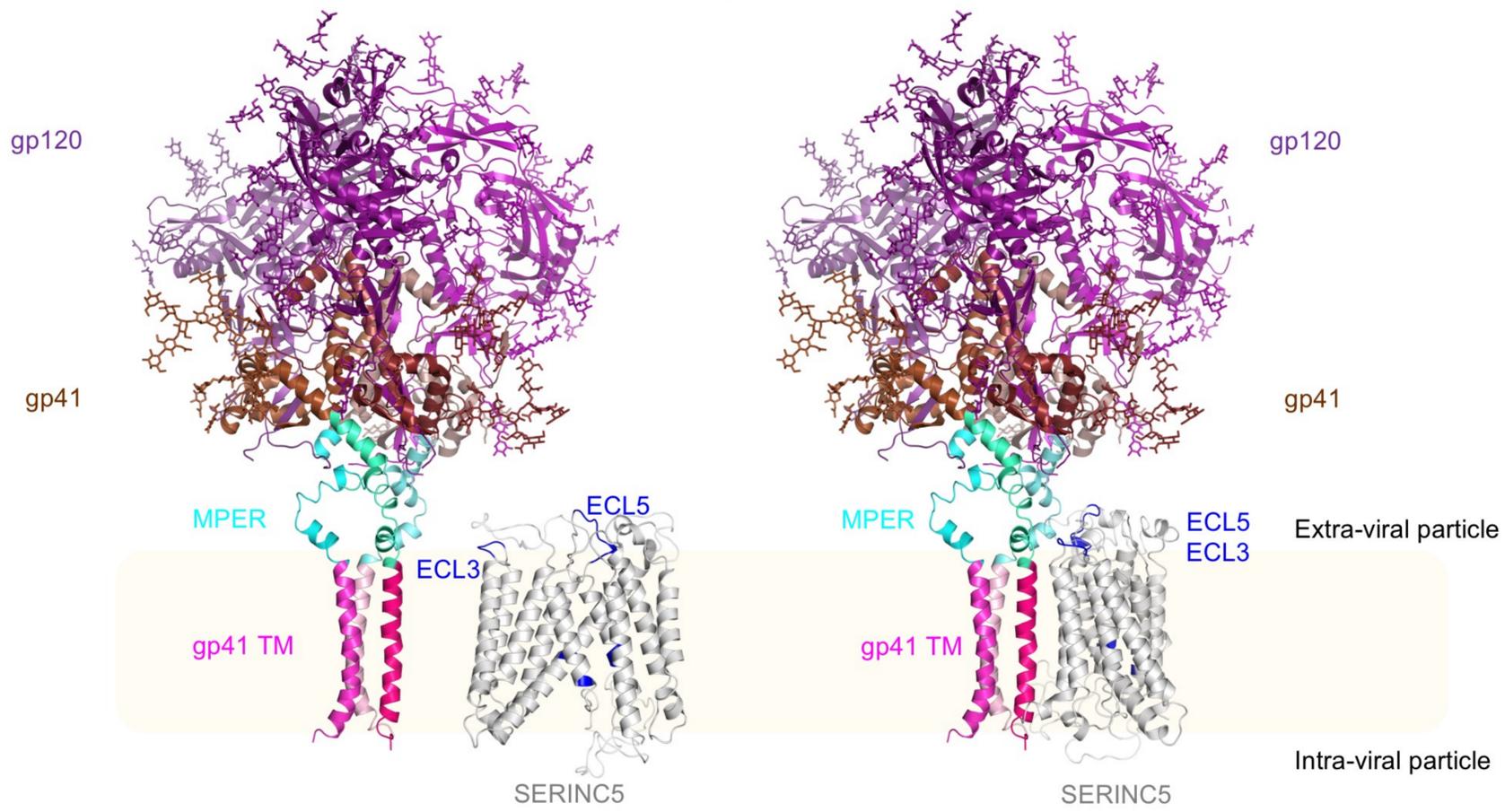










a**c**