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1 **Calicivirus VP2 forms a portal-like assembly following**  
2 **receptor engagement.**

3

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22 **Abstract**

23           To initiate infection many viruses enter their host cells by triggering endocytosis  
24 following receptor engagement. The mechanisms by which non-enveloped viruses escape  
25 the endosome are however poorly understood. Here we present near-atomic resolution  
26 cryoEM structures for feline calicivirus (FCV) both undecorated and labelled with a soluble  
27 fragment of its cellular receptor feline junctional adhesion molecule A (fJAM-A). We show  
28 that VP2, a minor capsid protein encoded by all caliciviruses <sup>1,2</sup>, forms a large portal-like  
29 assembly at a unique three-fold symmetry axis following receptor engagement. This feature,  
30 which was not detected in undecorated virions, is formed of twelve copies of VP2 arranged  
31 with their hydrophobic N-termini pointing away from the virion surface. Local  
32 rearrangement at the portal site leads to opening of a pore in the capsid shell. We  
33 hypothesise that the portal-like assembly functions as a channel for delivery of the  
34 calicivirus genome through the endosomal membrane into the cytoplasm of a host cell to  
35 initiate infection. While VP2 was known to be critical for the production of infectious virus <sup>3</sup>,  
36 its structure and function were hitherto undetermined. Our findings therefore represent a  
37 major step forward in our understanding of the *Caliciviridae*.

38

39 **Main Text**

40           The *Caliciviridae* family of viruses includes noroviruses and sapoviruses, which cause  
41 outbreaks of acute gastroenteritis in humans known as winter vomiting disease <sup>4,5</sup>. Feline  
42 calicivirus (FCV), a major cause of respiratory disease in felids <sup>6</sup>, represents an excellent  
43 model system for the study of calicivirus biology as it is readily propagated *in vitro* and is  
44 one of only three caliciviruses to date for which a protein receptor has been identified <sup>7</sup>.

45

46 Caliciviruses have small positive sense RNA genomes (~7.5 kb) that are packaged within a  
47 T=3 icosahedral shell, assembled from 180 copies of the major capsid protein (VP1) in three  
48 quasi-equivalent settings. Termed A, B and C, these VP1 conformers form 90 dimeric  
49 capsomeres. AB capsomeres are arranged around the 5-fold symmetry axes while CC  
50 capsomeres are located at the 2-fold symmetry axes <sup>8</sup>. The mature capsid protein has a  
51 short N-terminal arm (NTA), a shell (S) domain, comprising an eight-stranded antiparallel  $\beta$ -  
52 barrel motif, and a protruding (P) domain. The P domain is divided into the proximal P1 and  
53 distal P2 sub-domains, with P2 incorporating the receptor binding site and major  
54 immunodominant epitopes. In addition to VP1, an essential minor capsid protein VP2 is also  
55 incorporated into the virion at low copy number <sup>1-3</sup>.

56

57           To initiate infection, FCV binds to the tight-junction cell-adhesion molecule feline  
58 junctional adhesion molecule-A (fJAM-A) <sup>7</sup>. JAM-A comprises a short cytoplasmic tail, a  
59 transmembrane domain and two immunoglobulin-like domains (D1 and D2). X-ray  
60 crystallography studies of murine and human JAM-A have shown them to form U-shaped  
61 homodimers via interactions at the membrane distal D1 <sup>9,10</sup>. We have previously shown that  
62 the outer face of FCV P2 binds to fJAM-A D1 causing the capsid spikes to rotate

63 approximately 15° counter-clockwise <sup>11,12</sup>. We hypothesised that these conformational  
64 changes were priming events that would prepare the virion for genome release following  
65 internalisation. FCV enters cells via clathrin-mediated endocytosis. Endosomal acidification  
66 has been shown to be a necessary step in the viral entry process, although the mechanisms  
67 by which the virus escapes the endosome have not been elucidated <sup>13</sup>.

68

69 To construct an atomic resolution model of FCV interacting with its cellular receptor fJAM-A  
70 and resolve the molecular detail of conformation changes induced, we collected high-  
71 resolution cryo-EM images of FCV, both decorated with fJAM-A and undecorated. These  
72 data were processed to calculate icosahedral reconstructions of unlabelled and receptor  
73 bound virions at resolutions of 3 and 3.5 Å respectively (fig. 1a,b, ED 1, Supplemental Video  
74 1). Blurring of the P-domain and receptor density in the FCV-fJAM-A map confirmed our  
75 previous findings that conformational changes following receptor engagement break  
76 icosahedral symmetry and result in incoherent averaging <sup>11,12</sup>. To address this, we first  
77 performed model-based classification of FCV-fJAM-A in pre and post-conformational change  
78 states <sup>12</sup>. We identified a very small subset of particles that were found to be in a pre-  
79 conformational change state, i.e. all of the capsomeres were in the same orientation as the  
80 undecorated virus (ED2). The vast majority of particles however were grouped into classes  
81 in which the P-domains remained poorly resolved, indicating that the receptor induced  
82 conformational change is not coordinated. To calculate 3D reconstructions at sufficiently  
83 high resolution to allow building of atomic models of FCV P-dimers bound to fJAM-A, we  
84 adopted a focussed classification approach <sup>14-16</sup>. This yielded 3D reconstructions of  
85 individual capsomeres with improved resolution, revealing the range and extent of  
86 conformational changes in both AB and CC dimers (ED2, Supplemental Video 2).

87

88 In the course of this analysis three classes emerged in which we saw a previously  
89 unreported structure. Twelve fingers of density protrude from the capsid floor, arranged  
90 about a three-fold symmetry axis (ED 2, Supplemental Video 2). The P-fJAM-A density in  
91 these classes was determined at improved resolution, suggesting that the capsomeres were  
92 constrained by the presence of the novel feature. A second focussed classification  
93 experiment was performed at the three-fold axis confirming the presence of a large  
94 dodecameric portal-like assembly extending approximately 13 nm from the capsid shell (fig.  
95 1c-e, ED 3, Supplemental Video 3). The predominantly alpha-helical protein monomers are  
96 arranged in a circle about the icosahedral three-fold symmetry axis, forming a funnel-  
97 shaped structure that is 7nm in diameter at its base and 5nm in diameter at its tip, where  
98 the density becomes fuzzy and poorly resolved. There is an opening in the capsid shell at the  
99 portal-associated three-fold symmetry axis that is not seen at non-portal axes (fig. 1d, ED  
100 3d, Supplemental Video 3). Our analysis shows that the most populous class of virions  
101 present a single portal at a unique three-fold axis (ED 3e).

102

103 To test whether the portal-like structure was present in undecorated particles, we  
104 performed the same focussed-classification analysis on our undecorated dataset. No such  
105 feature was found, indicating that the assembly emerges from within the virion following  
106 receptor engagement. An analogous structure has been described for the single stranded  
107 DNA containing bacteriophage  $\Phi$ X174<sup>17</sup>. This virus encodes a protein (H) that assembles a  
108 tube at a unique 5-fold axis following host engagement. The 'H-tube' penetrates the  
109 bacterial cell wall to deliver the viral genome.

110

111 Our atomic model of VP1 for the FCV vaccine strain F9 closely matches that of the virulent  
112 systemic (VS) FCV strain 5<sup>18</sup>, with the most significant differences being in the P2 domain  
113 (ED 4 and 5, Supplemental Video 4). Within this region we saw density consistent with the  
114 presence of a metal ion. This has not been reported in any calicivirus VP1 structures to date  
115 and was strongly resolved with density visible at an isosurface threshold 12  $\sigma$  above the  
116 mean intensity. The distances between the putative metal-ion density and surrounding  
117 oxygens ( $\sim 2.8$  Å), along with the preponderance of main-chain carbonyl interactions, lead us  
118 to suggest that potassium is the most likely candidate for these densities<sup>19</sup>. Mutational  
119 analysis and sequence comparisons of VS-FCV and non-VS strains suggest that the metal-  
120 binding site may be important for infectivity and pathogenesis<sup>20</sup> (ED 4).

121

122 Atomic models for FCV VP1, VP2 and fJAM-A were built into the asymmetric FCV-fJAM-A  
123 density map at the C3 portal-vertex (ED 5). The asymmetric unit comprises one AB-dimer  
124 and one CC-dimer. To distinguish chains in the CC dimer, we designated the VP1 protomer  
125 proximal to the portal axis as chain D. Our model provides a detailed view of the virus-  
126 receptor interface (ED 4, SD 2, Supplemental Video 5). The footprint of each fJAM-A  
127 molecule is between 984 Å<sup>2</sup> (fJAM-A<sup>D</sup> – the molecule bound primarily to chain D) and 1068  
128 Å<sup>2</sup> (fJAM-A<sup>A</sup>). Interactions at the interface between virus and receptor are largely  
129 electrostatic. Several hydrogen bonds are predicted to form between the capsid and  
130 receptor fragments, including between residues N444 and D445 in VP1 and Y51 and F54 in  
131 fJAM-A. These are of particular interest as a loop on the outer face of VP1 at residues 436-  
132 448 undergoes significant rearrangements upon fJAM-A binding, forming a cleft into which  
133 VP2 binds. It is also notable that K480 and K481 are identified as interacting with fJAM-A.  
134 These lysine residues lie within the putative metal binding site of VP1.

135

136 Aligning the P domains of the undecorated and fJAM-A-labelled models highlights the  
137 structural rearrangements within the P-domain following receptor engagement. The most  
138 striking structural changes take place in the P2 domains, in the above-mentioned 436-448  
139 loops, which rise 3 Å towards the receptor (fig. 2a,b. Supplemental Video 6). In the S-  
140 domain following receptor engagement significant structural rearrangements at the  
141 icosahedral three-fold axis lead to the opening of a pore in the capsid shell (figure 2b-e).  
142 Consistent with the T=3 structure of the FCV capsid, the three-fold axes exhibit quasi-six-  
143 fold symmetry with six tyrosine residues (Y300) from chains B and C alternating about the  
144 axis. Y300<sub>B</sub> side-chains are tilted towards the capsid interior while those of Y300<sub>C</sub> are tilted  
145 towards the exterior. Upon receptor engagement the loops bearing these residues (293-  
146 307) fold outwards, resulting in the opening of a pore in the capsid shell that is ~1 nm in  
147 diameter (Supplemental Video 7). The presence of six tyrosine residues lining the three-fold  
148 axis is seen in all known vesivirus structures and appears to be conserved across the genus  
149 <sup>21</sup>. Structures of norovirus and rabbit haemorrhagic disease virus have phenylalanine  
150 residues at this site (ED6, SD3) <sup>8,22</sup>. A solvent-excluded surface representation of our atomic  
151 model shows that the counter-clockwise rotation of the capsomeres arranged about the  
152 portal vertex, following receptor binding, leads to a closing together of the P1 domains,  
153 forming a cup around the pore (figure 2d).

154

155 Twelve copies of the minor capsid protein VP2 are arranged about the unique three-fold  
156 axis to form a funnel shaped tube (fig. 3 and Supplemental Video 8). The asymmetric unit  
157 therefore includes four copies of VP2 (designated as chains I-L). The structure of VP2  
158 includes three α-helices. The first and longest α-helix (*α*) makes up the body of the funnel

159 and extends from residue 18 to 62. The N-terminal 17 residues are not resolved and are  
160 distal to the capsid surface. Interestingly this region is characterised as being highly  
161 hydrophobic by Kyte-Doolittle analysis (ED6d). It has been shown previously that the N-  
162 terminal region of VP2 is critically important in FCV. Experiments to recover virus following  
163 mutagenesis of the viral genome identified two mutations, L7D and L7E, that gave rise to  
164 virus that was replication competent but non-infectious<sup>3</sup>. Likewise, transposon mediated  
165 insertional mutagenesis of murine norovirus revealed that the hydrophobic N-terminal  
166 region of VP2 in that virus is also intolerant of mutations (ED6e)<sup>23</sup>. These findings lead us to  
167 suggest that the distal tips of VP2 likely insert into the endosomal membrane.

168

169 VP2 is present in two distinct conformations that present different folds for residues 75-106.  
170 The two conformers alternate about the portal axis (six copies of each conformer). In  
171 conformer one (chains J and L),  $\alpha$ -helices *b* (61-74) and *c* (91-106) bind to the capsid P  
172 domains via electrostatic interactions (ED7, SD4). In the second conformation of VP2  
173 (designated as chains I and K) the C-terminal residues (75-106) do not bind VP1, rather they  
174 fold into the lumen of the portal, such that six symmetry-related C-terminal  $\alpha$ -helices form  
175 an inner ring of fingers pointing away from the capsid surface. The C-terminal 6 residues of  
176 this conformer were not resolved. Conformer two barely contacts VP1 at all, the contact  
177 interface between this conformation and the capsid is  $\sim 150\text{\AA}^2$ . VP2 is instead held within  
178 the portal-like complex primarily by hydrophobic interactions along the interface between  
179 the N-terminal  $\alpha$ -helices (*a*). The C-terminal region of this conformer presents a largely  
180 negatively charged surface to the interior of the portal-like structure.

181

182 As FCV entry is dependent on acidification of the late endosome<sup>13</sup>, we tested for release of  
183 viral genomes at low pH in the presence or absence of fJAM-A. Negative stain transmission  
184 electron microscopy and RNA release assays showed that in the presence of fJAM-A, virions  
185 disassemble at pH 4 and below (ED8, SD5). H<sup>+</sup> concentration in the late endosome is  
186 thought to reach a pH of ~5<sup>24</sup>, thus virion disassembly under very low pH conditions is likely  
187 not physiologically relevant. These methods allowed us to establish the stoichiometry of  
188 receptor binding sufficient to induce structural changes in FCV. Measurements of RNA  
189 release from FCV virions in the presence of varying quantities of the fJAM-A ectodomain and  
190 at low pH showed that a 1:10 ratio of fJAM-A:VP1 is the minimum required to destabilise  
191 virions (ED9, SD6).

192

193 FCV engages fJAM-A such that a single receptor molecule binds to each VP1 monomer. We  
194 used small-angle X-ray scattering (SAXS) to calculate a low-resolution model of the protein  
195 solution structure. This showed that, like its human and murine orthologs, fJAM-A forms  
196 dimers in solution<sup>9,10</sup> (ED10, Supplemental Video 9). The FCV:fJAM-A interface is on the  
197 opposite side of the receptor to the D1:D1 dimerisation site. This site is however occluded  
198 by the D2 domain of symmetry-related fJAM-A molecules when bound to FCV<sup>25</sup>. Indeed, a  
199 significant contact interface arises between the D1 and D2 domains of the two receptor  
200 molecules when bound to the capsid (672 Å<sup>2</sup>) (SD7). It is possible then that D1:D1 fJAM-A  
201 homodimers at the cell surface may be disrupted by binding of FCV. Interestingly,  
202 adenovirus fibre-knob protein has been shown to trigger endocytosis by disruption of  
203 homodimers of the immunoglobulin-like tight-junction protein CAR (coxsackie-adenovirus  
204 receptor)<sup>26</sup>. Thus, disruption of fJAM-A homodimers at the cell surface may be the  
205 mechanism by which FCV triggers endocytosis.

206

207 Taken together our data lead us to propose a mechanistic model of FCV entry and  
208 endosome escape: FCV binding to fJAM-A at the cell surface leads to internalisation by  
209 endocytosis, triggered by fJAM-A homodimer disruption. Enwrapping of the virion within  
210 the endosome leads to further binding of fJAM-A molecules, triggering conformational  
211 changes in the capsid that result in formation of the portal-like assembly at a unique vertex.  
212 The hydrophobic N-termini of VP2 insert into the endosomal membrane forming a channel  
213 through which the genome may be released. In our study viral genomic RNA remained  
214 associated with the capsid following receptor engagement, only being released at low pH as  
215 a consequence of virion disassembly. Further structural changes and possibly the action of  
216 an unknown cofactor may therefore be required to trigger release of the viral genome  
217 through the portal vertex *in vivo*.

218

219 It has long been known that large DNA-containing viruses, such as tailed bacteriophages and  
220 herpesviruses have unique portal-vertices that mediate genome release. Such features have  
221 not however been previously described in small RNA-containing viruses. Our discovery of a  
222 portal-like assembly at a unique vertex in caliciviruses therefore represents a paradigm shift  
223 in our understanding of the structure and biology of this class of viruses.

224

225 **Supplementary information** is linked to the online version of the paper at  
226 [www.nature.com/nature](http://www.nature.com/nature).

227

228 **Author contributions**

229 MJC, IGG and DB conceived the study; MJC, MM and LA performed the experiments; MJC,  
230 OB and DB analysed the data; MG and DB performed validation; DB supervised the project;  
231 MJC and DB wrote the manuscript; all authors reviewed the manuscript.

232

### 233 **Author information**

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235 declare no competing financial interests. Correspondence and requests for materials should  
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237

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249

### 250 **Figure Legends**

251 **Figure 1 Cryo-EM structures of FCV bound to fJAM-A reveal a portal-like assembly.** An  
252 icosahedral three-dimensional reconstruction of the unlabelled virion structure was

253 calculated to an overall resolution of 3 Å. An isosurface thresholded view is shown coloured  
254 and filtered according to local resolution, showing that in the shell of the capsid the  
255 resolution approaches 2.8 Å, while on the outer faces of the protruding capsomeres the  
256 resolution is closer to 3.5 Å (a). Icosahedral reconstruction of receptor decorated FCV virions  
257 resulted in a lower overall resolution of 3.5 Å. The isosurface representation is similarly  
258 coloured and filtered according to local resolution, revealing that in this map the dimeric P-  
259 domain spikes and fJAM-A fragments are resolved at poorer than 4 Å resolution as a  
260 consequence of the receptor-induced conformational changes in this region (b). C3-  
261 symmetric 3D reconstruction of FCV decorated with fJAM-A following focussed classification  
262 analysis revealed the structure of a portal-like assembly at a unique three-fold symmetry  
263 axis. A wall-eyed stereo-pair view of the reconstruction along the icosahedral two-fold axis  
264 is shown coloured to highlight the portal vertex, (c). A close-up view of the portal assembly  
265 along the unique three-fold axis highlights the presence of a pore in the capsid shell at the  
266 centre of this symmetry axis (d). A cut-away view perpendicular to the portal axis (e). In  
267 panels c-e the map is coloured to highlight individual components: VP2 is coloured  
268 orange/red, VP1 dimers arranged about the portal vertex are coloured purple, associated  
269 fJAM-A molecules are coloured blue. The remaining density is radially coloured with a  
270 rainbow palette.

271

272 **Figure 2 Conformational changes in VP1 following receptor engagement.** Ribbon diagrams  
273 of the atomic models for FCV VP1 and fJAM-A following receptor engagement are shown for  
274 the AB-dimer (a) and CC-dimer (b). In panel (a), chain A is coloured pale pink, while chain B  
275 is coloured hot pink, fJAM-A<sup>A</sup> is coloured blue, fJAM-A<sup>B</sup> is coloured green. Overlaid on the  
276 structure are the aligned AB P-domains of the undecorated virus (purple), highlighting the

277 structural rearrangements that occur following fJAM-A binding. The most striking structural  
278 change is the upwards movement of loop 436-448, indicated by black arrows. Panel (b)  
279 shows the structure for the CC dimer, the portal proximal chain (now designated chain D) is  
280 coloured dark purple, while the distal chain C is coloured pale purple, and fJAM-A molecules  
281 are coloured blue. As in panel (a), the structure of VP1 in the absence of receptor is overlaid  
282 (pink) to highlight the structural rearrangements that occur following receptor engagement.  
283 This representation of the CC dimer also highlights the structural rearrangements that occur  
284 in the S-domain leading to the opening of a pore in the capsid shell. Compare the  
285 orientation of the 293-307 loop in chain D (red arrow) with the same loop in chain C.  
286 Solvent-excluded surfaces are presented to show VP1 dimers arranged about the three-fold  
287 symmetry axis in undecorated FCV (c), and fJAM-A labelled FCV at the portal axis (d) –  
288 coloured by radius. A close-up view of the icosahedral three-fold axis in the undecorated  
289 FCV reconstruction viewed from the capsid exterior (e) shows that this axis is surrounded by  
290 six tyrosine (Y300) side chains that point to the centre of the symmetry axis. Three tyrosine  
291 residues are each donated by chains B and C, that alternate about the symmetry axis  
292 following the quasi-six-fold symmetry seen in a T=3 icosahedral structure. Y300<sub>B</sub> points  
293 towards the capsid interior, while Y300<sub>C</sub> points outwards from the capsid surface. Following  
294 receptor engagement, a portal complex assembles at a unique three-fold axis. At the centre  
295 of this axis, a pore opens in the capsid surface by the folding outwards of loop 293-307 (f).  
296 Panels (e) and (f) present both modelled coordinates (ribbon diagram in rainbow colour  
297 scheme, atomic representation in pink) and the reconstructed density map (transparent  
298 grey).

299

300 **Figure 3 The structure of the VP2 portal-like assembly.** A solvent-excluded surface  
301 representation of the portal vertex is presented viewed along the unique three-fold axis (a).  
302 Six dimers of VP1 are shown, AB (pink) and CD dimers (purple) alternate about the  
303 symmetry axis. On the outer surface of the P2 domain, fJAM-A molecules are bound  
304 (blue/green), with one receptor molecule binding to each VP1. The portal assembly,  
305 comprising twelve copies of the VP2 protein is shown as a ribbon diagram (orange/red). The  
306 portal-like assembly is viewed perpendicular to the portal axis and cutaway to show the  
307 interior features (b). The VP2 dodecameric portal structure is shown alone, viewed  
308 perpendicular to the portal axis (c). Two conformers of the molecule are seen in this  
309 assembly, conformer one (orange) and conformer two (red). The front-most VP2 chains K  
310 and L are shown in rainbow colouring, highlighting that the N-terminus of VP2 is distal to  
311 the capsid shell (c-d). VP2 has three  $\alpha$ -helices designated *a-c* (e-f). The C-terminal residues  
312 (75-106) present different folds in the two conformers, with conformer one showing a well-  
313 ordered helix *c* that splays outwards, while the C-terminus of conformer two folds into the  
314 lumen of the portal.

315

316 **Materials and Methods**

317 **Virus culture and purification**

318 Feline calicivirus strain F9 was propagated in Crandell Reese Feline Kidney cells (CrFK) for 16  
319 hours (all cell stocks were regular tested for Mycoplasma contamination). The cell debris  
320 was pelleted from the culture medium by centrifugation (1,500xg, 10 min at 4°C). The virus  
321 particles within the supernatant were pelleted by centrifugation at 20,000rpm for 2 hours at  
322 4°C using a Surespin630 rotor. The pellets were resuspended in phosphate buffered saline  
323 (PBS) and the virus purified by centrifugation through a caesium chloride gradient (1.31-  
324 1.45g/ml) using a SW-41 Ti rotor at 28,000rpm for 8 hours and at 12°C. The purified virus  
325 particles were extracted from the gradient and pelleted by centrifugation at 20,000rpm for  
326 2 hours at 4°C using an SW-41 Ti rotor. The resultant pellet was then resuspended in 100µl  
327 PBS and stored at 4°C until use.

328

329 **Expression and purification of fJAM-A**

330 The expression and purification of the soluble fJAM-A ectodomain was performed as  
331 previously described<sup>11</sup>. RNA was isolated from CrFK cells and the sequence encoding the  
332 signal peptide and ectodomains of fJAM-A was amplified by reverse transcription-PCR (RT-  
333 PCR). The PCR product was used to generate a eukaryotic expression plasmid termed  
334 pDEF:fJAM-A:Fc which contained the extracellular domains of fJAM-A with the Fc domain of  
335 human IgG1 fused at the C terminus. The fJAM-A and Fc domains were separated by a factor  
336 Xa cleavage site to allow downstream removal of the Fc tag. Chinese hamster ovary cells  
337 stably expressing the soluble fJAM-A:Fc fusion protein were generated and soluble fJAM-  
338 A:Fc protein purified from tissue culture supernatant using protein A Sepharose. Monomeric

339 fJAM-A was generated by factor Xa cleavage of fJAM-A:Fc, removal of factor Xa by using  
340 Xarrest agarose (Novagen), and removal of the released Fc domain by protein A dynabeads.

341

#### 342 **Sequencing of FCV VP1 and VP2 ORFs**

343 The sequences for the FCV capsid protein ORFs were determined to facilitate atomic model  
344 building. RT-PCR was used to isolate the relevant DNA for sequencing using the Sanger  
345 method, performed at the MRC Protein Phosphorylation Unit, University of Dundee.

346

347 Primers were designed based on published FCV Strain LLK VP1 and VP2 sequence data  
348 (Genbank U07131<sup>27</sup>) as follows:

349 VP RT primer : 5'-ttaggcgcaggtgcggcagc-3'

350 VP1 PCR1 5'-atgtgctcaacctgcgctaactgtgct-3

351 VP1 PCR2 5'-tcataacttagtcatgggactcctaa-3'

352 VP2 PCR1 5'-atgaattcaatattaggcctgatt-3'

353 VP2 PCR2 5'-aattttaaacaatttttatatga-3'

354

355 RNA was extracted from 140 µl of FCV strain F9 infected cell culture medium using a Qiagen  
356 QiaAMP Viral RNA mini kit according to the manufacturers protocol. The purified RNA was  
357 reverse transcribed using a Superscript IV RT kit (Thermo Fisher) and VP RT primer in a 20 µl  
358 reaction volume according to the manufacturer's protocol. PCR was carried out on 6 µl of  
359 the RT reaction using the Q5 High Fidelity PCR kit (NEB). VP1 was amplified using primers  
360 VP1 PCR1 and VP1 PCR2, and VP2 was amplified using primers VP2 PCR1 and VP2 PCR2. PCR  
361 products were purified using agarose gel electrophoresis and the GeneClean Turbo kit  
362 (MPBio).

363

364 **Negative stain electron microscopy**

365 Purified FCV particles were incubated with the soluble ectodomain of fJAM-A at pH7 or pH3  
366 for 1 h at 4°C. FCV particles, both undecorated and labelled with fJAM-A, were imaged by  
367 negative stain transmission electron microscopy. 5 µl of virus preparation was loaded onto a  
368 freshly glow-discharged continuous carbon TEM grid. The grid was washed in two 20µl  
369 droplets of 2% uranyl acetate, drained and dried at room temperature. The grids were then  
370 imaged in a JEOL 1200 EX II transmission electron microscope equipped with a GATAN Orius  
371 camera.

372

373 **Cryo-electron microscopy**

374 Purified FCV particles were incubated with the soluble ectodomain of fJAM-A for 1 h at 4°C.  
375 The undecorated and decorated virions were then prepared for cryo-electron microscopy by  
376 loading 5µl onto freshly glow-discharged, carbon coated c-flat holey carbon grids (CF-22-4C,  
377 Protochips Inc.) in a Vitrobot vitrification robot (FEI) held at 4°C and 100% humidity. Grids  
378 were blotted for 4 seconds prior to being frozen by plunging into a bath of liquid nitrogen-  
379 cooled liquid ethane.

380

381 Vitrified specimens were imaged at low temperature in a Thermo-Fisher Titan Krios  
382 equipped with a Falcon III detector. The column was operated at 300 keV accelerating  
383 voltage and a nominal magnification of 75,000x resulting in a pixel size of 1.065 Å/pixel.  
384 Each micrograph was recorded as a movie of 50 individual fractions with a total dose of 63  
385 e/Å<sup>2</sup>. Automated data collection was performed using EPU software. 5,198 micrographs of

386 FCV and 13,865 micrographs of FCV-fJAM-A were recorded. Data collection was performed  
387 at the Astbury BioStructure Laboratory, University of Leeds.

388

### 389 **Three-dimensional image reconstruction**

390 Micrograph stack files were corrected for drift using MotionCor2<sup>28</sup> and defocus estimation  
391 performed by Gctf<sup>29</sup> as implemented in Relion 2.1<sup>30</sup>. 59,531 undecorated particles were  
392 automatically picked from 5198 micrographs and 129,884 fJAM-A decorated particles from  
393 13,865 micrographs. Particle images were subjected to both 2D and 3D classification to  
394 exclude false-positives and damaged particles. Final datasets of 41,436 undecorated and  
395 71,671 fJAM-A decorated virions were subjected to 3D refinement to calculate final  
396 icosahedrally averaged three-dimensional reconstructions. These were then subjected to  
397 post-processing and local resolution analysis in Relion 2.1 to determine both final resolution  
398 and optimal sharpening parameters. Maps were visualised using UCSF Chimera<sup>31</sup> and  
399 ChimeraX and coloured according to local resolution, unless otherwise specified.

400

### 401 **Model based classification**

402 To attempt to separate fJAM-A decorated FCV virions into separate classes based on the  
403 extent of conformational change they had undergone, 3D classification was performed in  
404 Relion 2.1 using our previously published structures as starting models<sup>12</sup>.

405

### 406 **Focussed classification**

407 To resolve the structures of individual asymmetric features we employed a focussed  
408 classification approach in Relion 2.1<sup>14-16</sup>. Briefly, following 3D refinement with imposition of  
409 icosahedral symmetry, the symmetry of the data set was expanded such that each particle

410 was assigned 60 orientations corresponding to its icosahedrally redundant views. A  
411 cylindrical mask was prepared to exclude all of the capsid structure except the area of  
412 interest, using SPIDER<sup>32</sup>. 3D classification was then performed without orientation  
413 refinement. Thus, a single capsomere or symmetry axis was reconstructed, and classification  
414 was performed to resolve structural differences.

415

#### 416 **Atomic model building**

417 Atomic models were built using Coot<sup>33</sup>, the CCP-EM<sup>34</sup> suite and Phenix<sup>35</sup>. The structure for  
418 VP1 of FCV in the undecorated virus was built starting from our previously calculated  
419 homology model<sup>11</sup>. The structure was manually edited in Coot to correct the sequence and  
420 improve the correspondence to our EM density map. This was followed by rounds of  
421 iterative real-space refinement in Phenix. To build an atomic model of VP1 in our receptor-  
422 bound models, the P-domains and S-domains were docked independently as rigid bodies  
423 using UCSF Chimera<sup>31</sup>. The models were then manually edited and iteratively refined as  
424 above. Likewise, the structure for fJAM-A was built starting from our previously calculated  
425 homology model and refined using Coot and Phenix. The structure of VP2 was manually  
426 built *ab initio* in Coot and refined using Phenix. Model validation was performed using  
427 MolProbity<sup>36</sup>, as implemented in Phenix. Modelling of metal ions was validated using the  
428 checkmymetal server ([https://csgid.org/csgid/metal\\_sites](https://csgid.org/csgid/metal_sites))<sup>19</sup>. Interactions between fJAM-A  
429 and VP1 were characterised for each of the four VP1 chains in the asymmetric unit using  
430 tools in UCSF Chimera<sup>31</sup> and the 'Protein interfaces, surfaces and assemblies' (PISA) service  
431 at the European Bioinformatics Institute ([http://www.ebi.ac.uk/pdbe/prot\\_int/pistart.html](http://www.ebi.ac.uk/pdbe/prot_int/pistart.html))  
432<sup>37</sup>. Protein sequence alignments were calculated using Clustal Omega  
433 (<https://www.ebi.ac.uk/Tools/msa/clustalo/>)<sup>38</sup>.

434

435 **RNA release assay**

436 Equal concentrations of FCV and fJAM-A ectodomain were combined in Tris buffer of the  
437 appropriate pH. 10 $\mu$ l Syto9 nucleic acid binding dye (ThermoFisher Scientific) was added to  
438 a final volume of 100 $\mu$ l in a black 96-well plate. For positive controls, 550 ng FCV RNA was  
439 added in place of the FCV and fJAM-A samples. Fluorescence readings were collected every  
440 5 minutes over a period of 4 hours using a PHERAstar FS (BMG labtech) plate reader  
441 equipped with a 485/520 filter cube. Data were normalised to the corresponding samples  
442 containing only FCV and Syto9. Plots of normalised fluorescence against time were  
443 evaluated by linear regression to determine whether the slope in each plot was significantly  
444 different from zero, using GraphPad Prism.

445

446 **Small Angle X-ray Scattering (SAXS)**

447 The fJAM-A ectodomain was dialysed into PBS containing 0.01% sodium azide, 1% sucrose  
448 and 10 mM potassium nitrate and samples taken to the Diamond Light Source (Oxfordshire,  
449 UK) where high-pressure liquid chromatography (HPLC) small angle X-ray scattering (SAXS)  
450 data were acquired. The HPLC column (Superdex 200 (GE Healthcare)) was equilibrated with  
451 buffer for 1 hour, the sample loaded onto the column from a 96-well plate and the  
452 diffraction data collected. Eight 60-second frames of data, for which the radius of gyration  
453 ( $R_g$ ) was observed to be constant, were selected for further processing with PRIMUS<sup>39</sup>, part  
454 of the ATSAS program suite for small-angle scattering data analysis from biological  
455 macromolecules<sup>40</sup>. A Guinier region was observed between  $0.26 \leq qR_g \leq 1.29 \text{ \AA}^2$  (where  $q$  is  
456 the momentum transfer ( $4\pi\sin(\Theta)/\lambda$ )) giving  $R_g = 30.7 \text{ \AA}$ . The particle distance distribution  
457 function ( $p(r)$  versus  $r$ , where  $r$  is the real-space distance) was evaluated using GNOM<sup>41</sup> with

458 a maximum dimension of 102.8 Å. An *ab initio* envelope was computed from  $p(r)$  vs  $r$  using  
459 DAMMIF<sup>42</sup>. The dimeric model of human fJAM-A ectodomain (PDB-1NBQ<sup>10</sup>) was fitted into  
460 the SAXS envelope using UCSF Chimera<sup>31</sup>.

461

#### 462 **Data availability statement**

463 The icosahedral reconstruction of undecorated FCV and the C3-symmetrised reconstruction  
464 of FCV-fJAM-A are deposited in the EM databank with accession numbers EMD-0054 and  
465 EMD-0056 respectively. The atomic coordinates for the FCV capsid asymmetric unit (VP1)  
466 are deposited in the protein data bank with accession number PDB-6GSH. The atomic  
467 coordinates for the FCV-fJAM-A portal vertex (VP1, VP2 and fJAM-A) are deposited in the  
468 protein data bank with accession number PDB-6GSI. Motion corrected micrographs of  
469 undecorated and fJAM-A labelled FCV (the raw data) are deposited in the EMPIAR data bank  
470 with accession numbers EMPIAR-10192 and EMPIAR-10193 respectively.

471

#### 472 **Extra Data Legends**

##### 473 **ED 1**

474 *CryoEM of FCV decorated with soluble f-JAM-A.* Cryogenic electron microscopy of feline  
475 calicivirus (FCV) strain F9 virions both unlabelled (a) and decorated with soluble ectodomain  
476 fragments of feline-junctional adhesion molecule A (fJAM-A) (d), scale bar is 100 nm.  
477 Icosahedral three-dimensional reconstructions were calculated. A central section through  
478 the reconstructed density map for the unlabelled virion shows that both the shell (S) and  
479 protruding spike (P) domains were sharply resolved (b). A central slice through the  
480 reconstruction of receptor decorated FCV virions shows that while the capsid shell is sharply  
481 resolved, the P-domains and fJAM-A components are blurred as a consequence of the  
482 receptor-induced conformational changes in this region (e). Gold-standard Fourier shell  
483 correlation plots for the icosahedral reconstructions show a nominal resolution of 3 Å for  
484 unlabelled FCV (c) and 3.5 Å for the receptor decorated structure (f).

485

486 **ED 2**

487 *Conformational changes in FCV following receptor engagement, revealed by focussed*  
488 *classification.* 3D reconstruction of FCV decorated with soluble fJAM-A ectodomain, in the  
489 pre-conformational change state. Model based classification was used to identify a small  
490 subset of particles (493/71,671) that were found not to have undergone the rotation of  
491 capsomeres usually induced by receptor engagement. These data gave a reconstruction  
492 with a nominal resolution of 6 Å. The surface-rendered representation is coloured and  
493 filtered according to local resolution (a). A central section through the map revealed density  
494 that was less blurred than in particles showing capsomere rotation (compare figure ED2b  
495 with figure ED1e). To resolve the range and extent of capsomere movement following  
496 receptor engagement, focussed classification was applied to reconstruct individual  
497 capsomeres at the AB- and CC-dimer positions. Montages of images showing individual  
498 capsomeres reveal the range of conformations present at the AB-dimer (c) and CC-dimer (d)  
499 positions. The first panel of each montage (top-left) shows the unlabelled dimer, the second  
500 panel (top, second from left) shows the class presenting a pre-conformational change state,  
501 remaining panels show the extent of conformational changes at each capsomere. Surface  
502 representations are filtered and coloured according to local resolution (Å – see colour keys).  
503 Red arrows highlight the position of a novel feature that we have shown to be VP2.

504

505 **ED 3**

506 *A portal-like assembly located at a unique three-fold axis.* Sections through the  
507 reconstructed portal-like assembly and virion viewed along the three-fold symmetry axis of  
508 the portal assembly. Viewed through the distal tips of the structure (a), viewed through the  
509 middle of the structure, also showing fJAM-A density (b) and viewed through the base of the  
510 assembly, also showing VP1 P-domain density (c). A transverse section through the virion  
511 along the portal axis shows the extent of the assembly and the presence of blurred density  
512 extending from the distal tip (d). We can also see the clear opening of a pore in the capsid  
513 shell at the portal-vertex (red arrow), which is not present at the opposite non-portal vertex  
514 (white arrow). Histogram to show the number of views contributed to the portal  
515 reconstruction by each particle (e). Focussed classification was used to identify and  
516 reconstruct the unique portal axis. Each of the twenty, three-fold symmetry axes present in  
517 every particle image was, in-turn, sampled such that it was oriented to lie within a 3D

518 cylindrical mask that covered a single three-fold axis of the icosahedral reference structure.  
519 Furthermore, each of the particle's three-fold axes were tested in three possible  
520 orientations, owing to the C3 symmetry of that axis. Thus, 60 views were evaluated for each  
521 particle, corresponding to the redundancy of an icosahedral symmetric object. Our data set  
522 of 71,671 particle images therefore gave rise to 4,300,260 views that were tested. 234,076  
523 particle views were assigned to the class in which the portal assembly was present,  
524 corresponding to 5.44% of the dataset. This is consistent with each particle having a single  
525 portal at a unique three-fold axis, as there are 20 three-fold symmetry axes per virion. The  
526 median number of views for each particle is indeed 3, consistent with the C3 symmetry of  
527 the assembly. Only 58,510 particles contributed to the reconstruction however, suggesting  
528 that ~20% of particles had either not assembled their portals, or the particle orientations  
529 were such that the portal was not discernible. In total 35,714 particles (61%) were found to  
530 contribute three views (i.e. contain a single portal) while 12,974 particles (22%) were found  
531 to contribute six views (two portals). 1,706 particles (2%) contributed nine views (three  
532 portals). We should assume a degree of error in the assignment of particles to each class  
533 however. Those particles that were found to contribute numbers of views that are not  
534 multiples of three are likely not entirely correctly classified. Moreover, particles presenting  
535 six or nine views might also be misclassified. The focussed classification analysis sorts data  
536 according to the presence of small/weak differences that are superimposed onto the  
537 projected density of the entire icosahedral object. Nonetheless, overall these data indicate  
538 that the most populous class of particles present a single portal at a unique three-fold axis.

539

#### 540 **ED 4**

541 *The structure of FCV VP1 showing sites of metal binding and receptor engagement.* Ribbon  
542 diagram to show the atomic model of the FCV strain F9 major capsid protein VP1, calculated  
543 by modelling the protein sequence (SD 1) into the icosahedral reconstruction of the  
544 undecorated virion (a). The AB-dimer is shown. Chain A is coloured pale pink, chain B is  
545 presented in rainbow representation (N-terminus blue, C-terminus red). A side-view of the  
546 dimeric capsomere is labelled to identify the shell (S) and protruding (P1 and P2) domains.  
547 The S domain shows the characteristic 'β-jellyroll' motif seen in all calicivirus structures  
548 solved to date. A top-view of the VP1 dimer is shown with a box to highlight the location of  
549 a putative metal ion (b). A close-up view of this region is presented identifying the

550 coordinating interactions within the metal binding site. Based on the distances measured  
551 for these interactions we suggest that this metal ion may be potassium (c). A recent  
552 mutational analysis of FCV VP1 fJAM-A binding identified several amino-acid residues within  
553 or close to the coordinating sphere of this metal-ion, that were critical to virus infectivity.  
554 Viruses in which these sites were mutated (I482, K480 and H516) were able to both  
555 assemble capsids and bind to fJAM-A but were not infectious. Sequence analysis also  
556 highlighted differences between VS-FCV and non-VS strains within this region, including  
557 residue D479, which in VS-FCV strain 5 is an asparagine residue and is oriented away from  
558 the site of metal binding we see in strain F9 (d - PDB 3M8L). Furthermore, Q474 and K481  
559 are oriented in a manner that is not compatible with metal-binding. The deposited structure  
560 factors for PDB 3M8L were downloaded for VS-FCV strain 5. Close inspection of the density  
561 revealed no evidence of metal binding. The atomic model for VP1 chains A (pale pink) and B  
562 (hot pink) of FCV decorated with fJAM-A<sup>A</sup> (the molecule bound primarily to chain A - blue)  
563 and fJAM-A<sup>B</sup> (the molecule bound primarily to chain B - green) is shown as a solvent  
564 excluded surface (e). The interface is exposed by opening the fJAM-A/VP1 surfaces like a  
565 book. Contact atoms are highlighted in the colour of the molecule they are interacting with  
566 (f). Coulombic surface colouring highlights the charge distribution (g), the contact interface  
567 is indicated by a black outline.

568

#### 569 **ED5**

570 CryoEM form (no legend).

571

#### 572 **ED 6**

573 *Structures of the icosahedral three-fold axes in related caliciviruses and hydrophobicity*  
574 *analysis of VP2.* Ribbon diagrams to show the icosahedral three-fold symmetry axes of other  
575 known calicivirus structures. Similar to FCV, the vesivirus San Miguel Sealion virus (PDB  
576 2GHT) has six tyrosine residues (a). Rabbit haemorrhagic disease virus (b – PDB 2J1P) and  
577 norovirus (c – PDB 1IHM) both have phenylalanines.

578 The N-terminal region of VP2 is highly hydrophobic, leading us to suggest it may insert into  
579 the endosomal membrane. Kyte-Doolittle plots showing the hydrophobicity profile of VP2  
580 for FCV (d) and MNV (e). A positive number indicates a predominantly hydrophobic region  
581 (Figure generated using ExPasy Protscale - <https://web.expasy.org/protscale/>). Mutational

582 studies of VP2 in FCV and MNV have both shown that the hydrophobic N-terminal region is  
583 intolerant of mutagenesis. Analyses of the VP2 sequences for human norovirus (strain  
584 GI/Human/United States/Norwalk/1968) and rabbit haemorrhagic disease virus also show  
585 the presence of a hydrophobic N-terminus indicating that this feature is conserved across  
586 the *Caliciviridae* (data not shown).

587

#### 588 **ED 7**

589 *The structure of FCV VP2 and interactions with the capsid surface.* Wall-eyed stereo pair  
590 images of VP2 to show the folds of the two conformers that are seen to alternate about the  
591 three-fold portal axis and highlight the interactions between conformer one (orange) and  
592 the major capsid protein VP1. A dimer of VP2 showing the two conformations is presented  
593 viewed from the portal interior,  $\alpha$ -helices are labelled. The poorly-ordered C-terminal helix *c*  
594 of conformer two can be seen leaning towards the viewer (a). A solvent-excluded surface  
595 representation of this view, coloured to show surface potential (negative = red, positive =  
596 blue), shows that the C-terminal region of conformer two presents a negatively charged  
597 surface to the portal interior (black arrow – (b)). Panels (c) and (d) show the same VP2 dimer  
598 rotated 180° about the vertical axis, the outward facing C-terminal helix of conformer one is  
599 now leaning towards the viewer. Interestingly, both helix *b* and helix *c* in this conformer  
600 present positively charged surfaces (black and red arrows respectively), which bind to  
601 negatively charged clefts on the capsid surface (e). Two binding sites are present on the  
602 capsid surface, helix *c* binds to the P1 domains of both VP1 molecules in each dimeric  
603 capsomere arranged about the portal axis (AB or CD) (f). The *bc* loops (residues 75-90) wrap  
604 across the surface of the adjacent VP1 molecule laying closest to the portal axis (chains D or  
605 B respectively) and helix *b* binds to a cleft on the adjacent portal proximal VP1 molecule in  
606 the P2 domain (g). This binding site is opened up by the upwards movement of loop 436-448  
607 following fJAM-A binding. Ribbon diagrams of the portal proximal VP1 molecule (in this case  
608 chain D) are shown for both fJAM-A decorated (purple) and unlabelled (blue) VP1, showing  
609 that without the structural rearrangements of loop 436-448 brought about by receptor  
610 binding, the helix and loop (450-460) laying immediately below clashes with helix *b* of VP2  
611 (yellow arrow) (h). Thus, each of the VP2 molecules in the first conformation binds to three  
612 VP1 molecules: VP2<sub>J</sub> is anchored to VP1<sub>AB</sub> in the P1 binding site. The *bc* loop then wraps  
613 across the surface of VP1<sub>D</sub> and helix *b* inserts into the second binding site, in the P2 domain

614 of VP1<sub>D</sub>. Likewise, helix c of VP2<sub>L</sub> binds to VP1<sub>CD</sub> in the P1 cleft. It then folds across the face  
615 of VP1<sub>B</sub> binding into the second interaction site on that protomer.

616

#### 617 **ED 8**

618 *FCV disassembles at low pH in the presence of fJAM-A.* RNA release assay plots showing  
619 fluorescence induced by release of RNA from FCV virions in the presence of fJAM-A and  
620 under varying pH conditions (a). These data show that at pH4 or below, FCV particles  
621 labelled with fJAM-A release their RNA. Measurements were taken from distinct samples  
622 (n=3) and normalised data is shown with error bars representing the standard deviation  
623 (\*\**p*=0.0002, \*\*\*\**p*<0.0001 – supplemental data 5). Negative-stain transmission  
624 electron microscopy of purified FCV virions showed that fJAM-A decorated FCV virions  
625 release their RNA at low pH as a consequence of capsid disassembly. Micrographs are  
626 shown for FCV alone at pH3 (b) and pH7 (d) and FCV decorated with soluble fJAM-A at pH3  
627 (c) or pH 7 (e). Virions can be clearly seen in both experiments performed at neutral pH,  
628 however at pH3 and in the presence of fJAM-A, no virions were seen. FCV was incubated for  
629 1h in the presence or absence of fJAM-A and at neutral or low pH.

630

#### 631 **ED 9**

632 *Stoichiometry analysis of fJAM-A binding to VP1 leading to capsid destabilisation.* RNA  
633 release assay plots showing the RNA released by virions at low pH and in the presence of  
634 varying ratios of fJAM-A to VP1 (a). Clear evidence of RNA release is seen at ratios down to  
635 1:9, while between 1:11 and 1:14 the assay is equivocal. Below 1:16 there is no evidence of  
636 RNA release. Measurements were taken from distinct samples (n=3) and normalised data is  
637 shown with error bars representing the standard deviation (\*\**p*=0.0002, \*\*\*\**p*<0.0001  
638 – supplemental data 6). Negative stain TEM imaging of FCV virions in the presence of  
639 differing ratios of fJAM-A:VP1 and at pH 7 and pH3 (b-i). At a ratio of fJAM-A:FCV-VP1 of 1:9,  
640 intact virions are seen at pH7 (b), while at pH3 no virions were seen (c). Likewise, at a ratio  
641 of 1:10 virions were seen to disassemble at pH3 (pH7 - d, pH3 - e). At a ratio of 1:11 (pH7 - f,  
642 pH3 – g), disrupted or partially disassembled particles were visible at pH3 while at a ratio of  
643 1:12 particles were intact at both neutral and low pH (pH7 - h, pH3 – i). In each case FCV  
644 was incubated for 1h under the relevant experimental conditions. These images show that a  
645 ratio of 1 fJAM-A molecule to 10 capsid proteins is sufficient to destabilise capsids, causing

646 them to completely disassemble at low pH. Imaging disassembled virions revealed the  
647 presence of small dense balls of density, that we interpret as being condensed viral RNA  
648 (white arrows - c, e, g, i). These were sometimes also seen in intact preparations of FCV at  
649 neutral pH (red arrow - h).

650

651 **ED Fig 10**

652 *fJAM-A is a dimer in solution.* Small angle X-ray scattering was used to calculate a low-  
653 resolution envelope for fJAM-A ectodomain fragments in solution (a), this closely matched  
654 the structure of the human JAM-A homodimer (PDB 1NBQ). The atomic model of hJAM-A is  
655 docked and shown as a ribbon diagram (b,c) and solvent-excluding surface (c). The  
656 homodimerisation interface (red, d) is on the opposite face of D1 to the FCV binding site  
657 (pink, e). In fJAM-A molecules bound to the P2 domains of FCV VP1 dimers, the  
658 homodimerisation sites at D1 are occluded by the D2 domains of the symmetry related  
659 bound receptor molecules (f,g). Indeed, an alternate dimerisation interface arises between  
660 D1 and D2 domains of bound molecules.

661

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## 738 **Methods**

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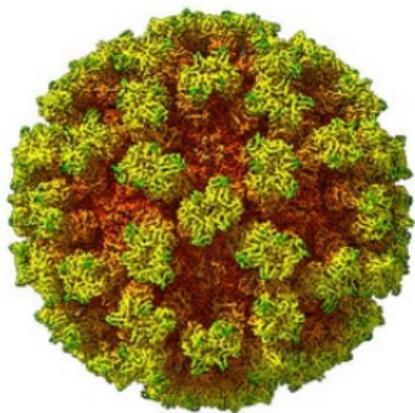
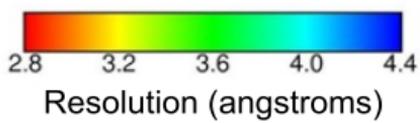
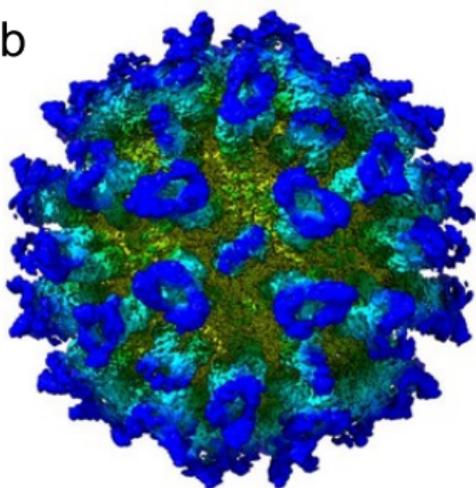
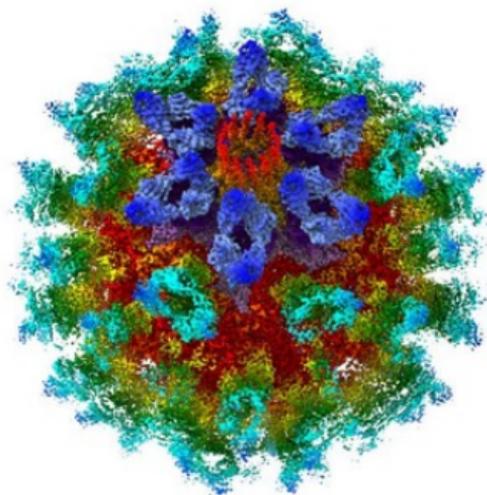
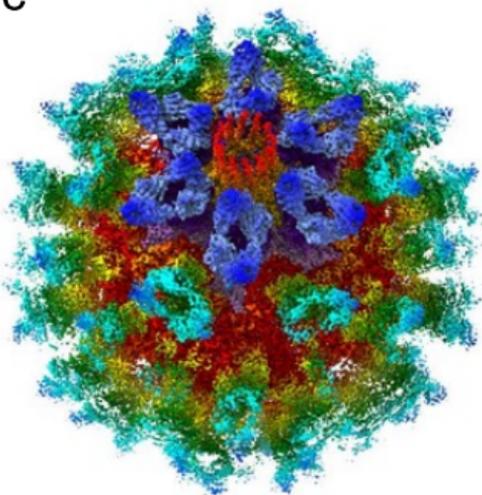
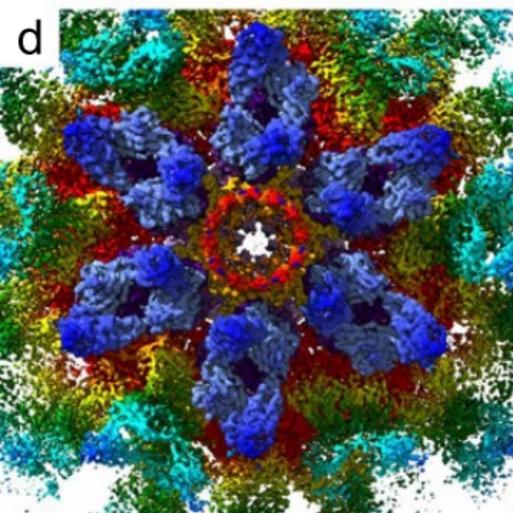
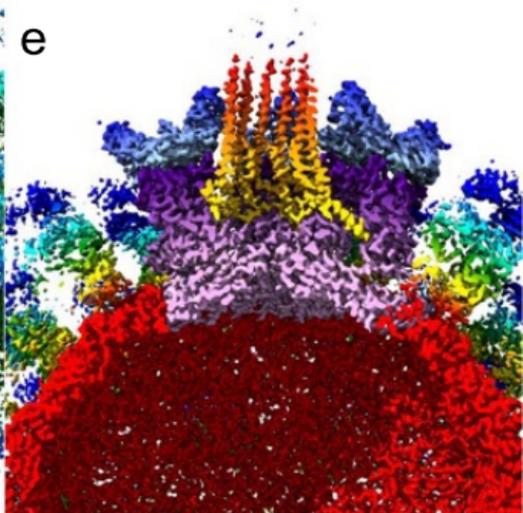
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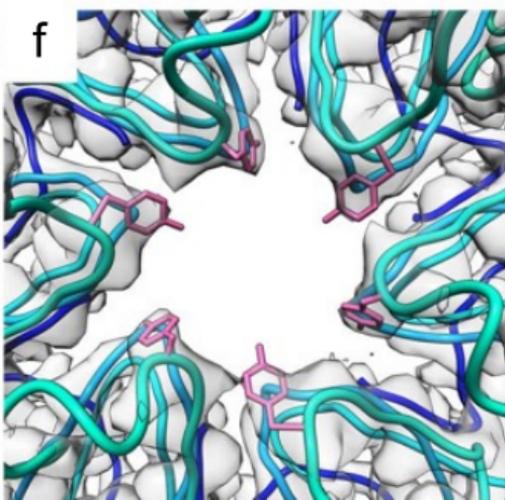
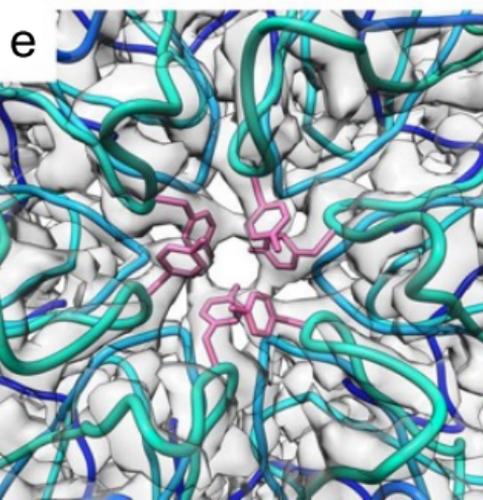
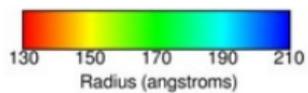
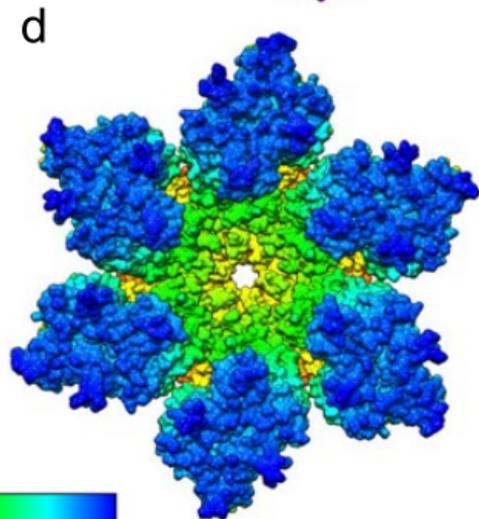
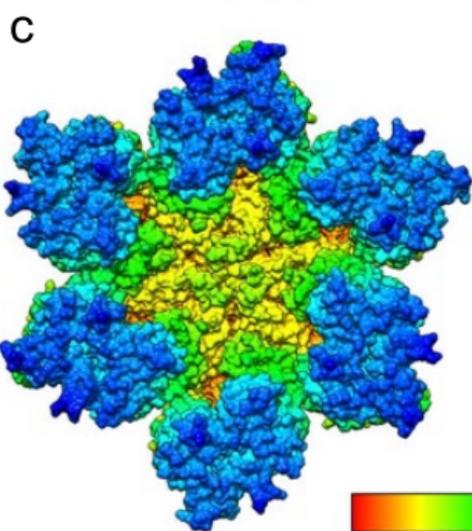
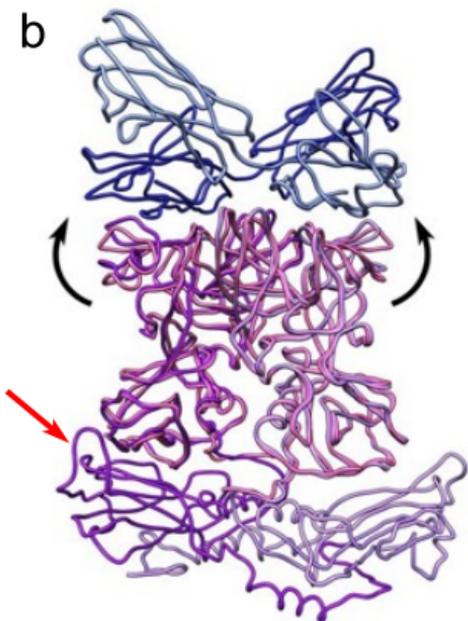
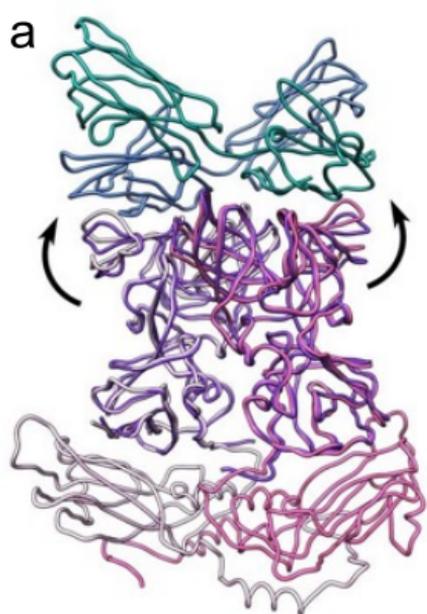
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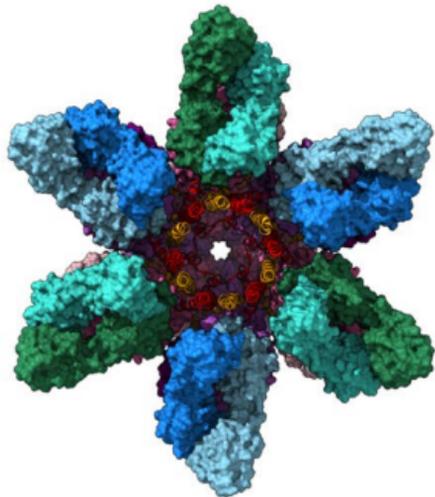
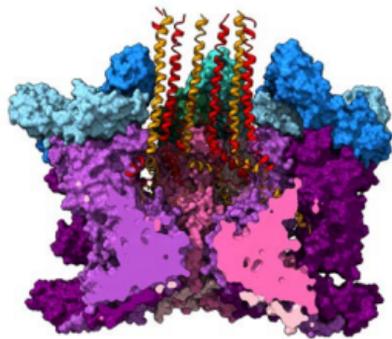
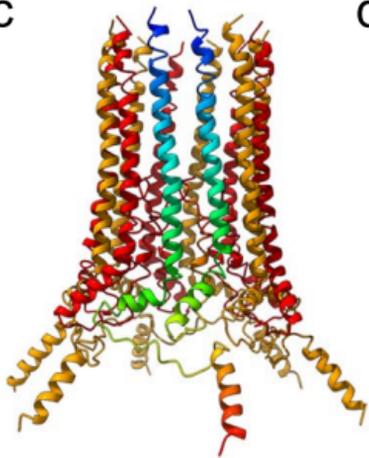
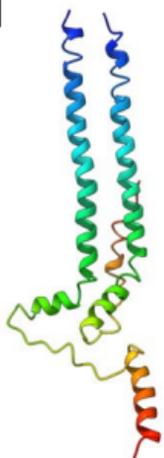
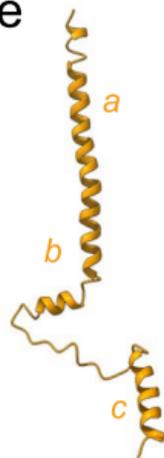
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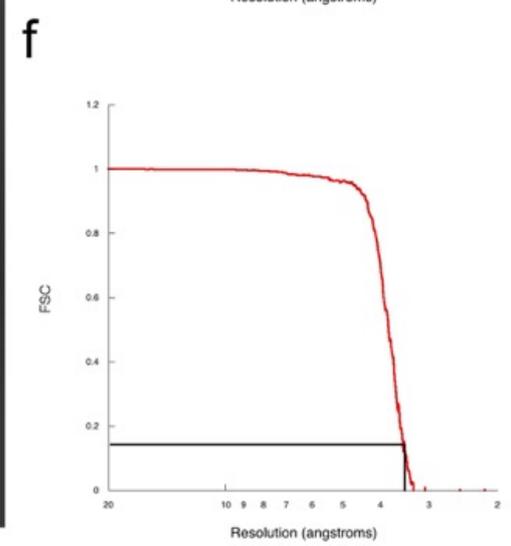
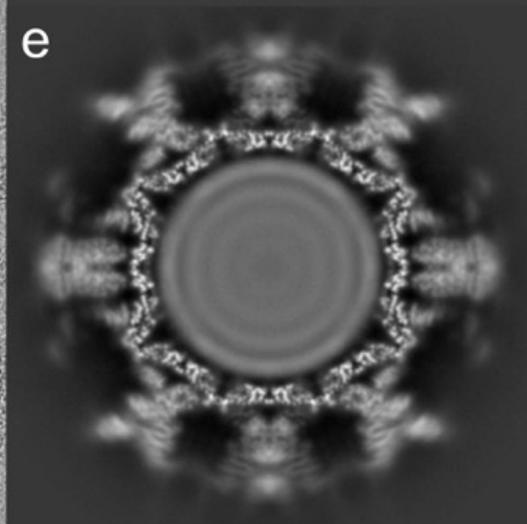
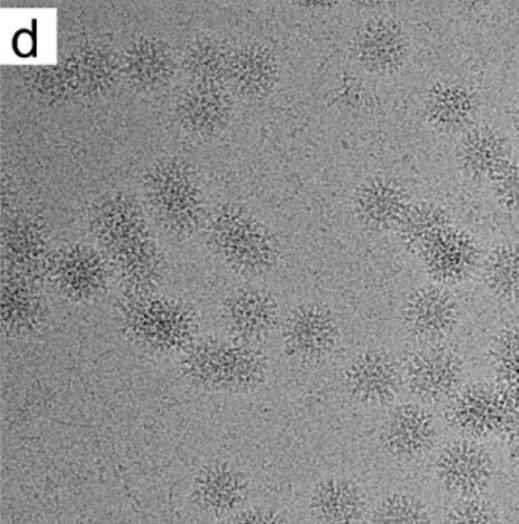
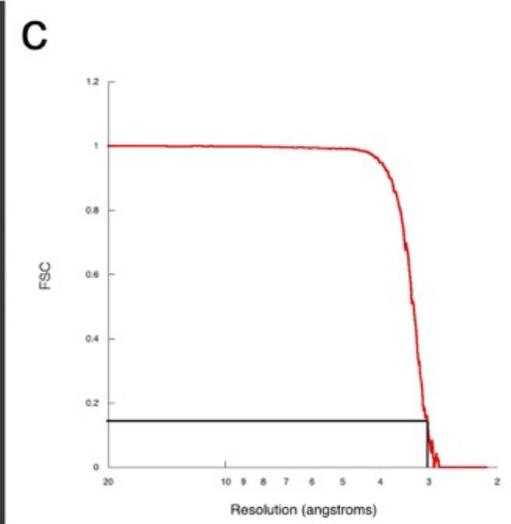
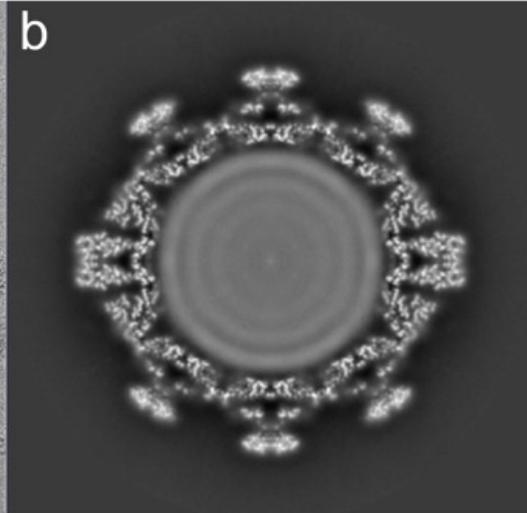
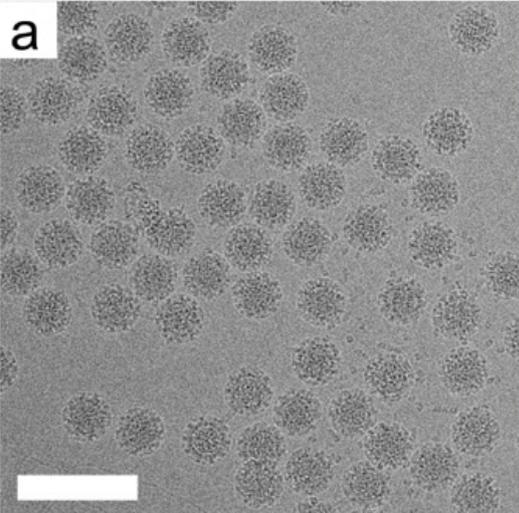
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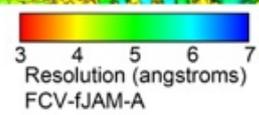
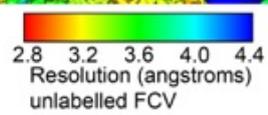
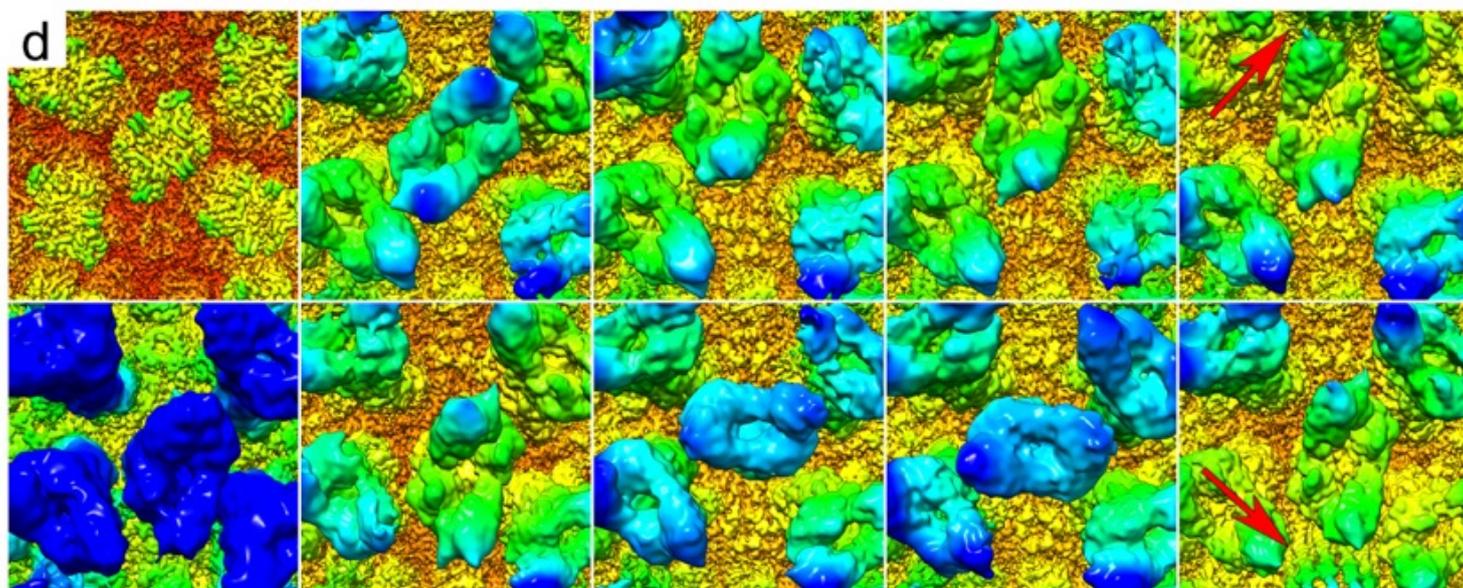
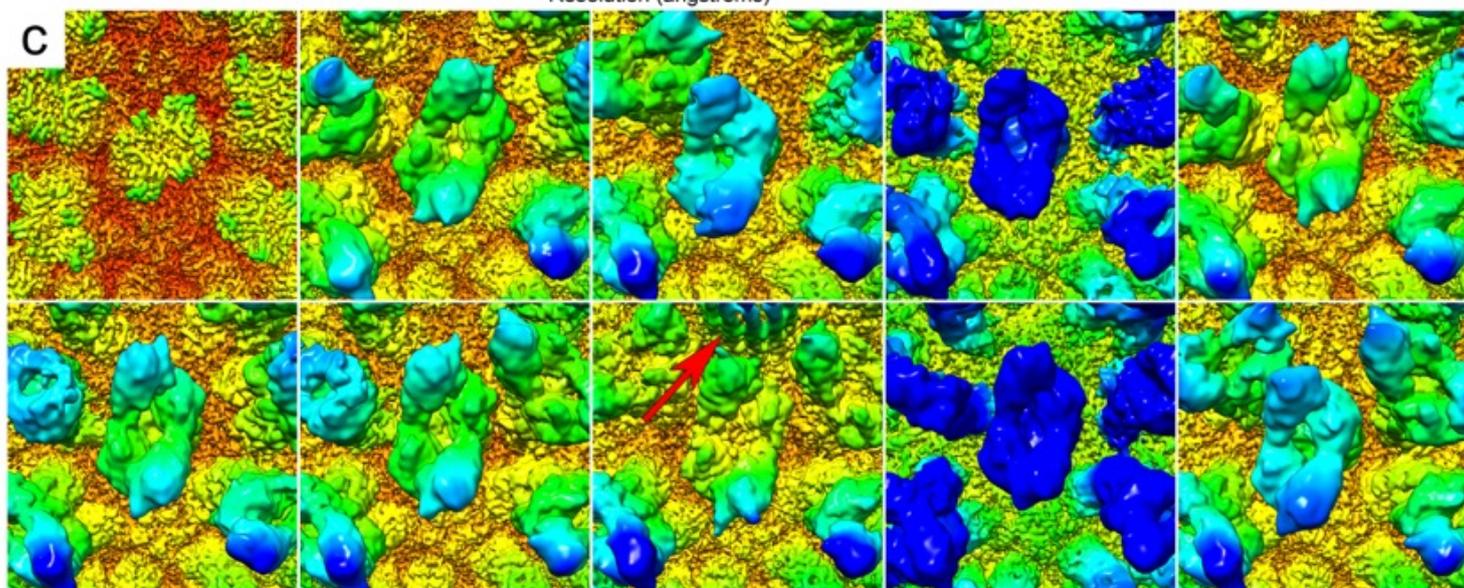
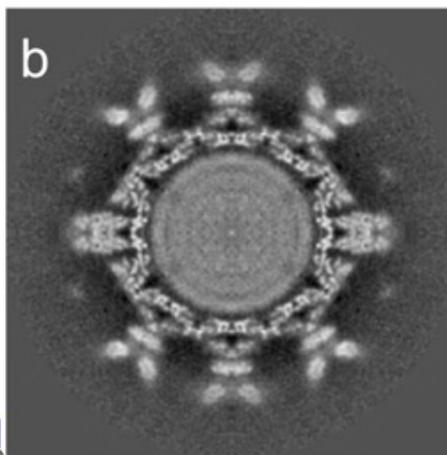
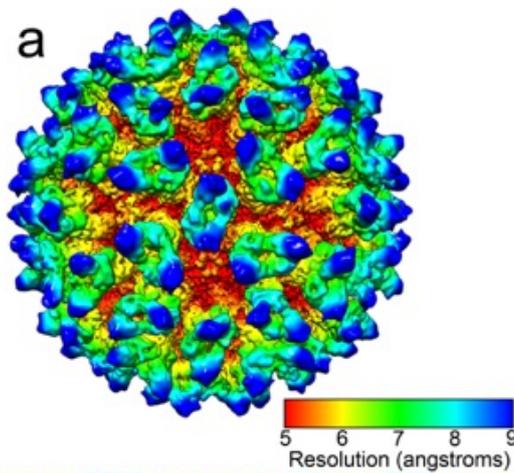
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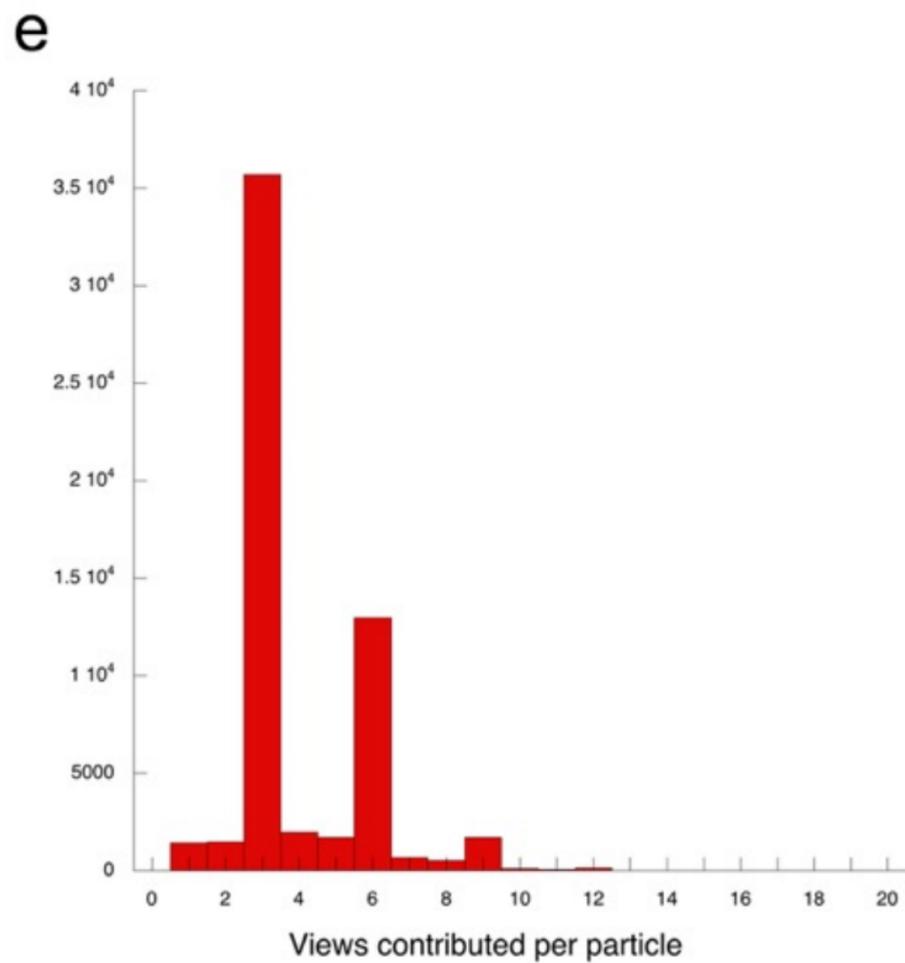
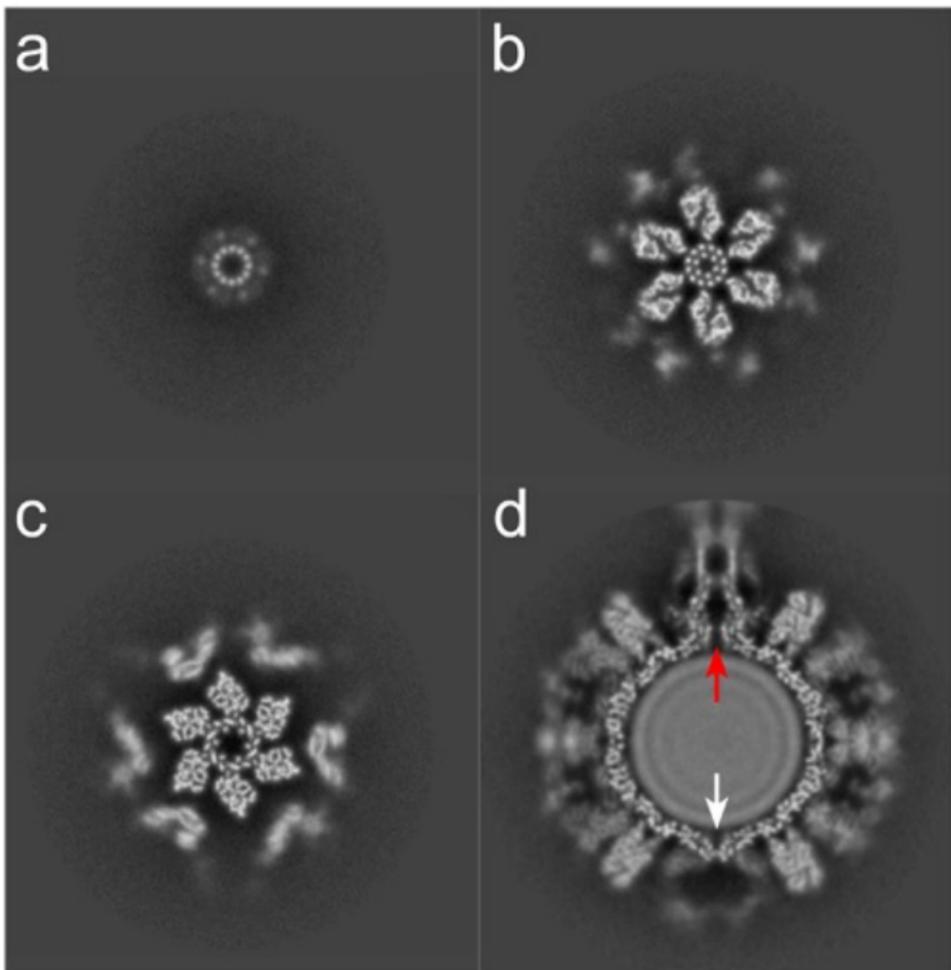
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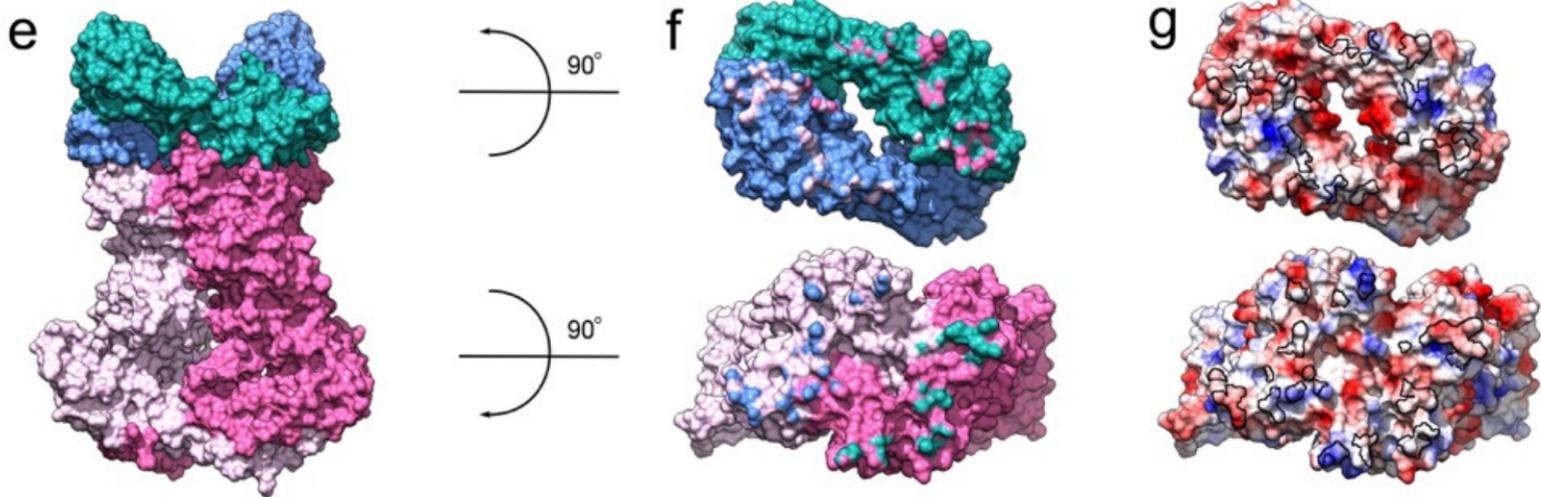
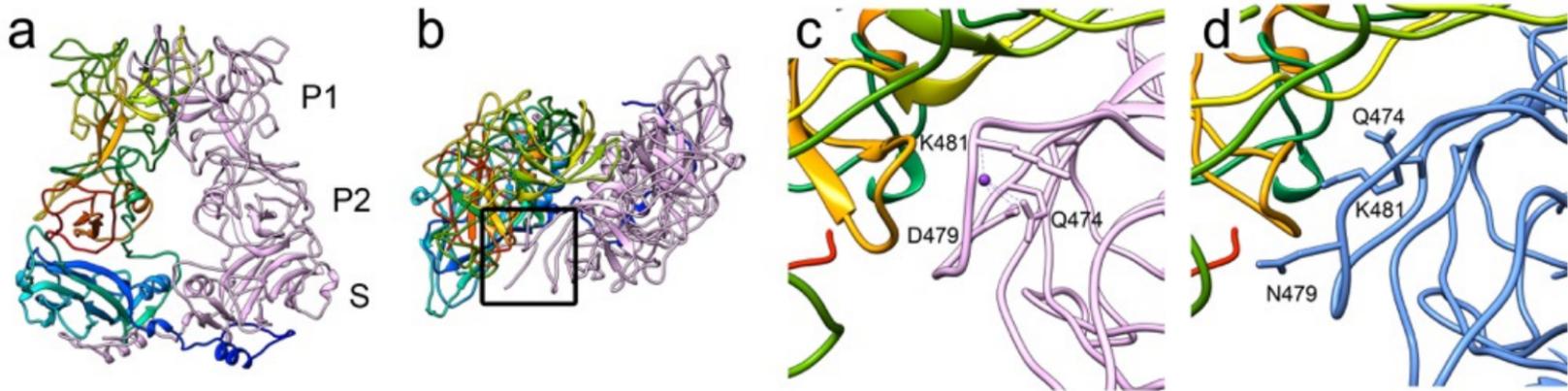


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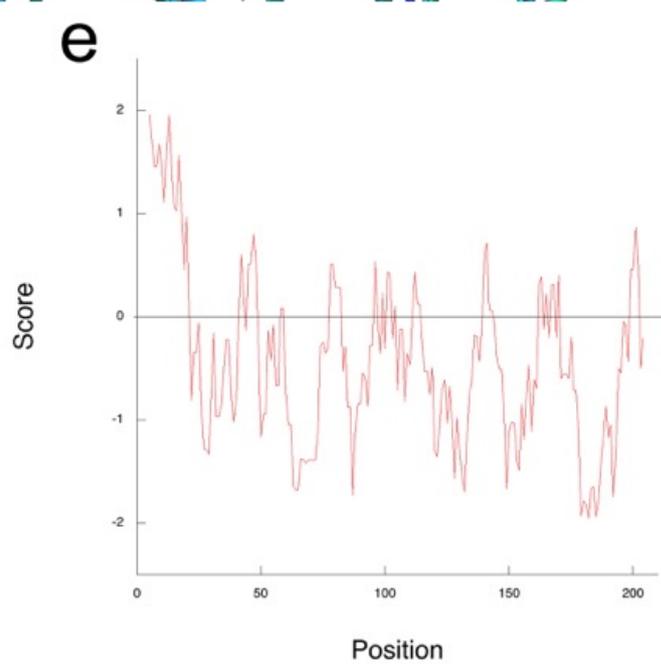
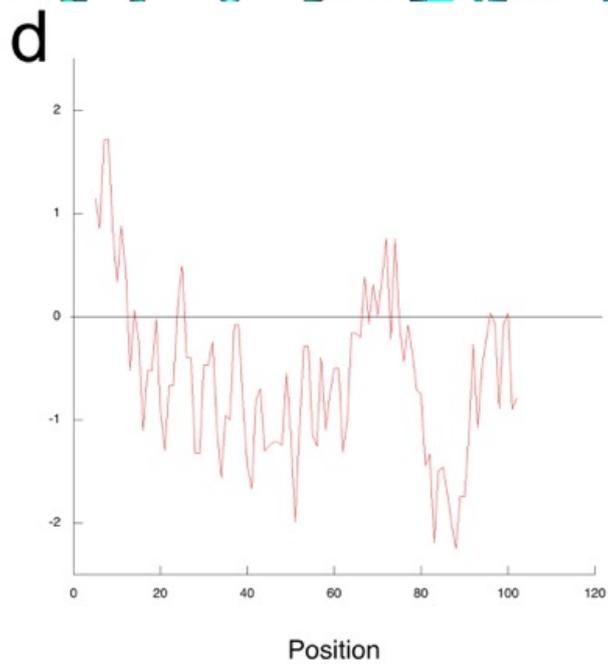
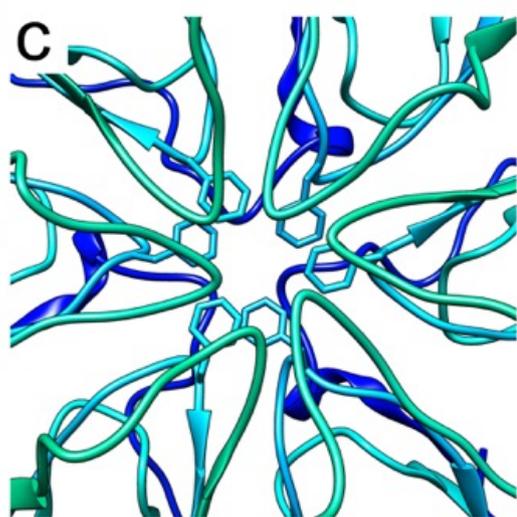
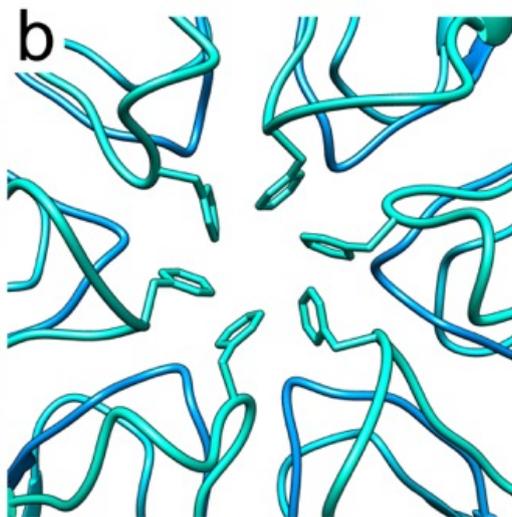
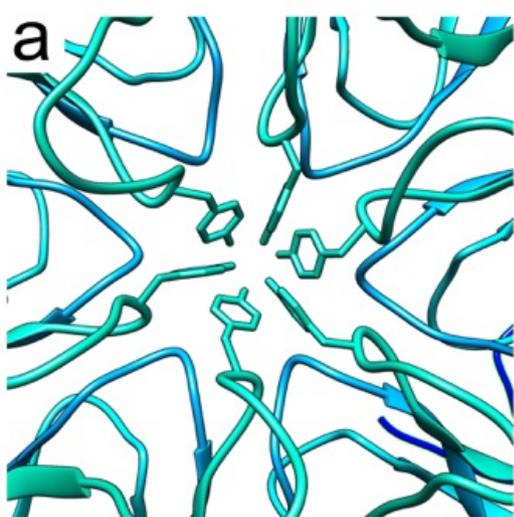


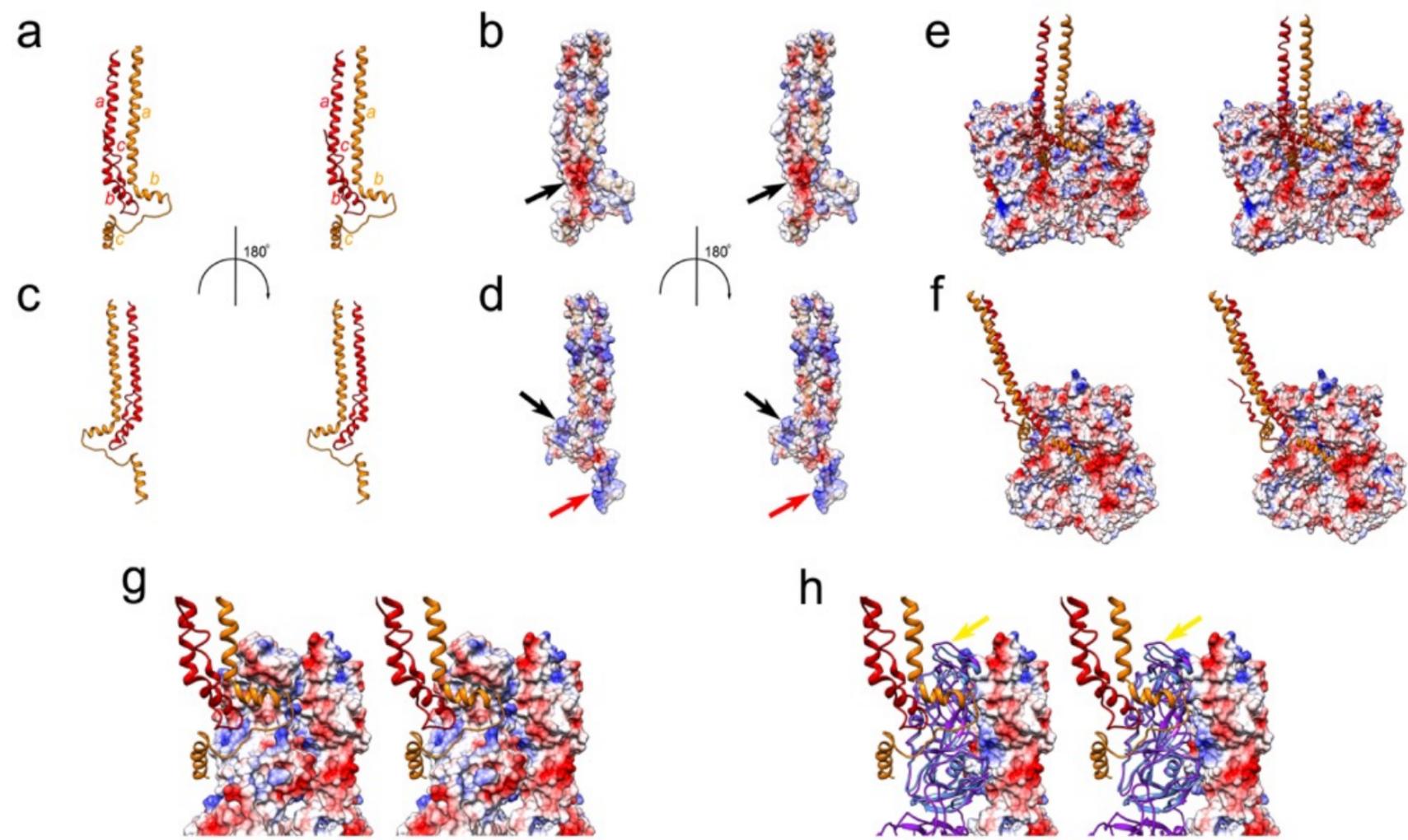


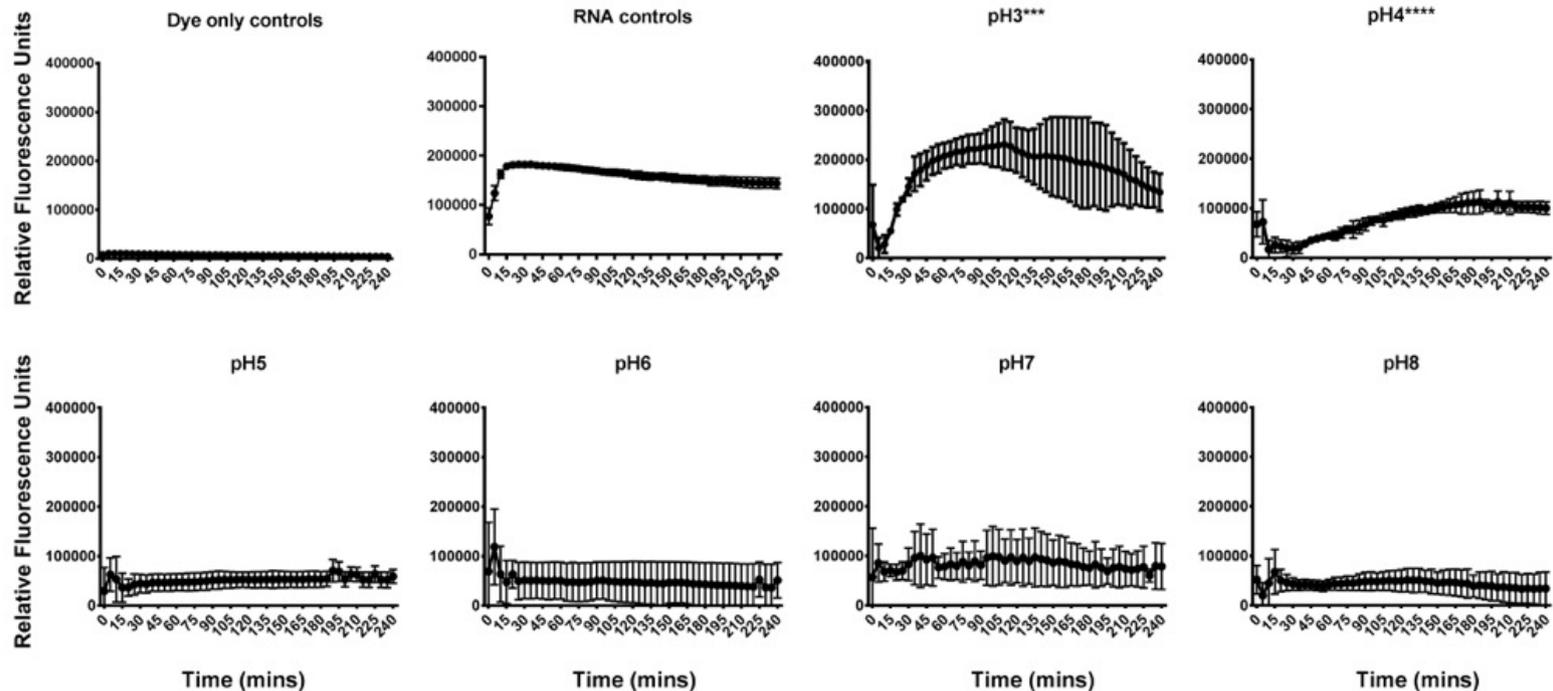
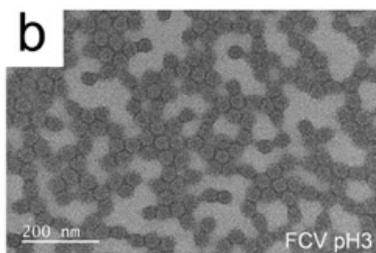
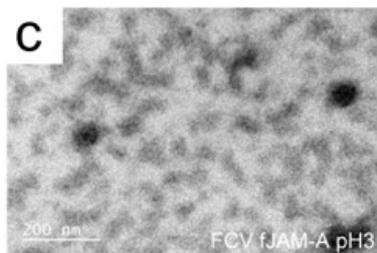
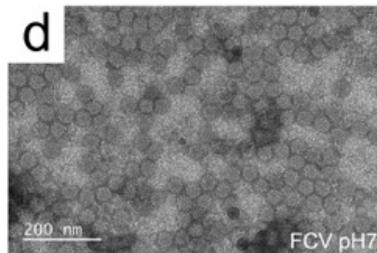
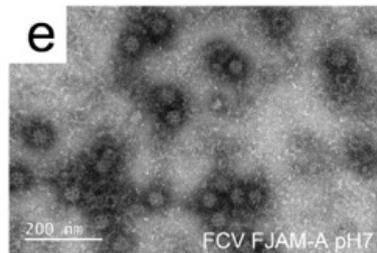


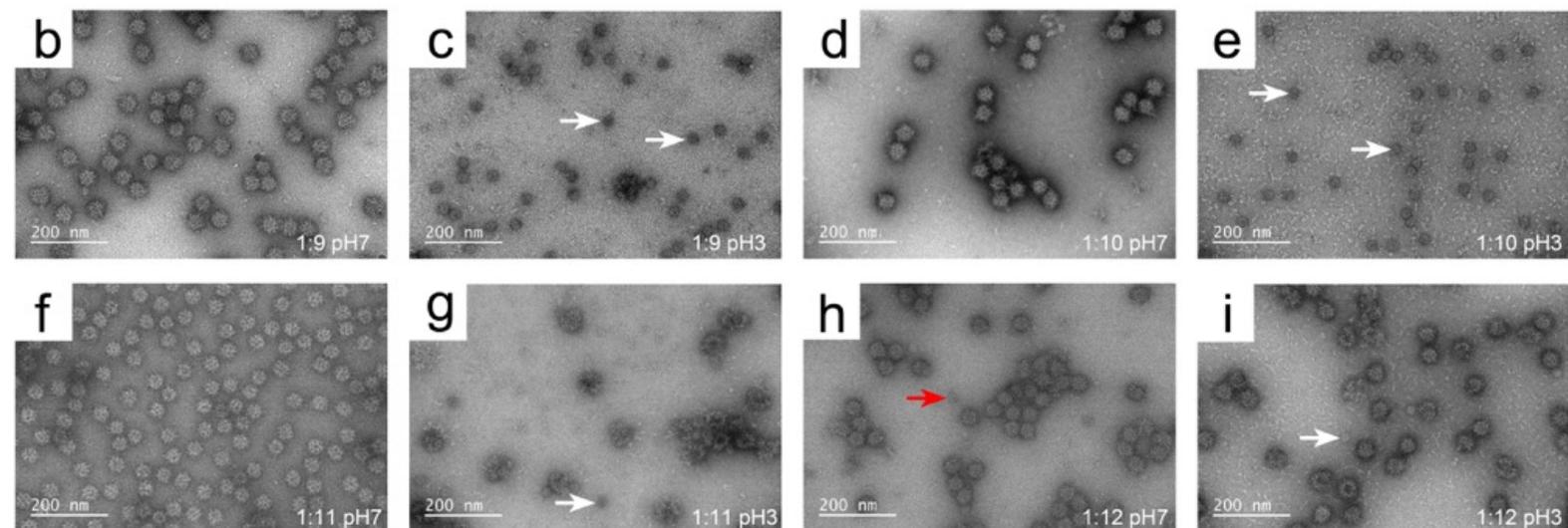
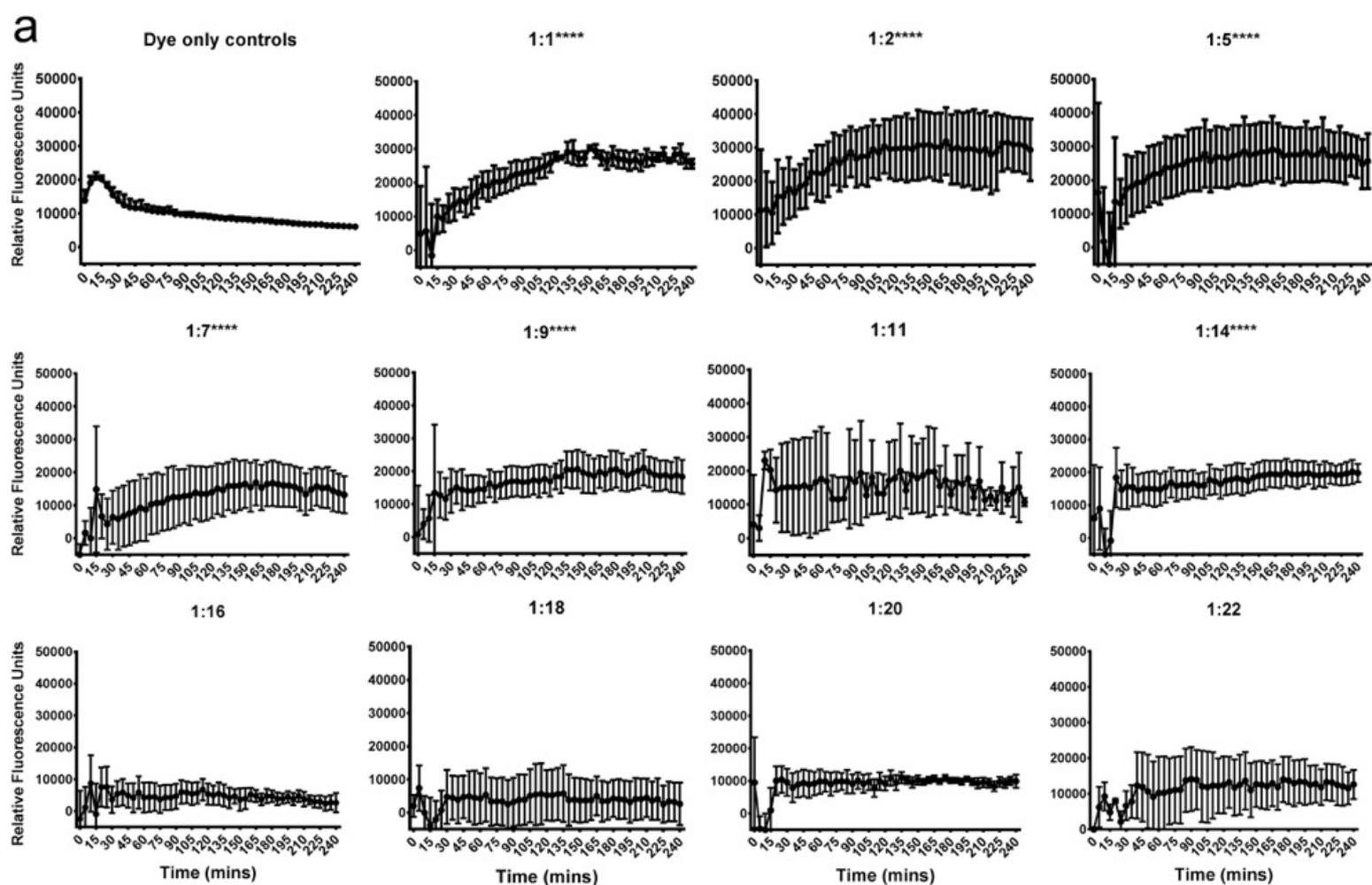
## Cryo-EM data collection, refinement and validation statistics

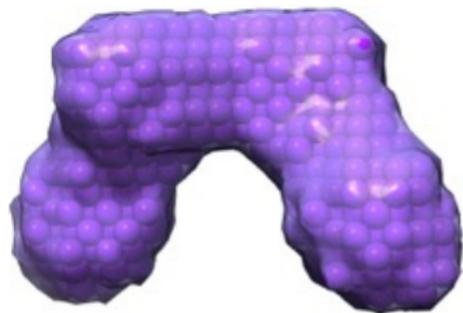
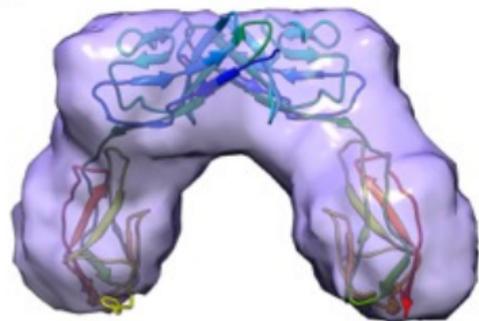
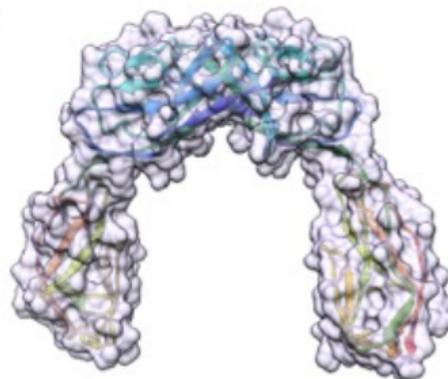
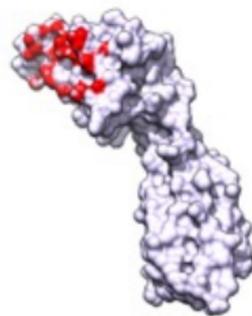
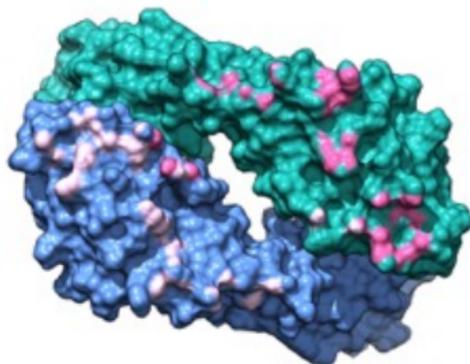
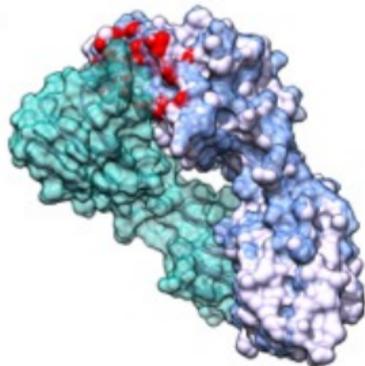
	FCV VP1 (virion) (EMDB-0054) (PDB 6GSH)	FCV VP1, fJAM-A and VP2 (portal) (EMDB-0056) (PDB 6GSI)
<b>Data collection and processing</b>		
Magnification	75,000x	75,000x
Voltage (kV)	300	300
Electron exposure (e-/Å <sup>2</sup> )	63	63
Defocus range (µm)	-1.2 to -3.5	-1.2 to -3.5
Pixel size (Å)	1.065	1.065
Symmetry imposed	I2	C3
Initial particle images (no.)	59,531	129,884
Final particle images (no.)	41,436	71,671
Map resolution (Å)	3	3.5
FSC threshold	0.143	0.143
Map resolution range (Å)	2.8-3.2	3.2-4.4
<b>Refinement</b>		
Initial model used (PDB code)	Undeposited homology model	PDB 6GSH
Model resolution (Å)		
FSC threshold		
Model resolution range (Å)		
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-194	-182
Model composition		
Non-hydrogen atoms	12265	25390
Protein residues	12262	25386
Ligands	3	4
<i>B</i> factors (Å <sup>2</sup> )		
Protein	mean = 32.7	mean = 118.0
Ligand	max = 84.9	max = 205.7
	min = 12.1	min = 67.5
	mean = 30.5	mean = 110.0
	max = 35.0	max = 123.7
	min = 25.6	min = 98.7
R.m.s. deviations		
Bond lengths (Å)	0.0104	0.0109
Bond angles (°)	1.30	1.24
Validation		
MolProbity score	1.66	2.08
Clashscore	3.33	7.47
Poor rotamers (%)	0.44 %	0.53 %
Ramachandran plot		
Favored (%)	90.63 %	84.60 %
Allowed (%)	9.05 %	15.22 %
Disallowed (%)	0.31 %	0.18 %





**a****b****c****d****e**



**a****b****c****d****e****f****g**