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Supplementary Materials for

The novel asymmetric entry intermediate of a picornavirus captured with nanodiscs

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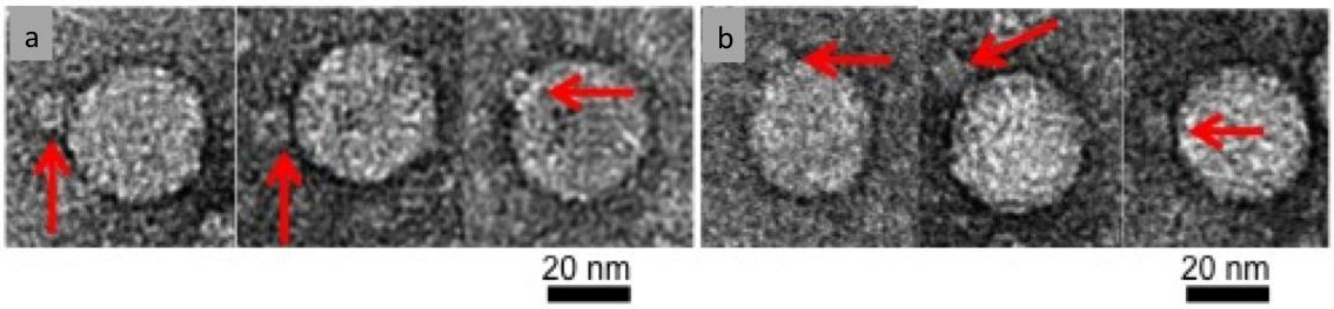

fig. S1. Negative-stain transmission electron microscopy images of CAR-nanodiscs bound to CVB3. **(A)** The CVB3 was incubated with CAR-nanodiscs at 4°C and **(B)** incubated at 37°C for 30 min. TEM images show virus bound to CAR-nanodiscs (red arrows).

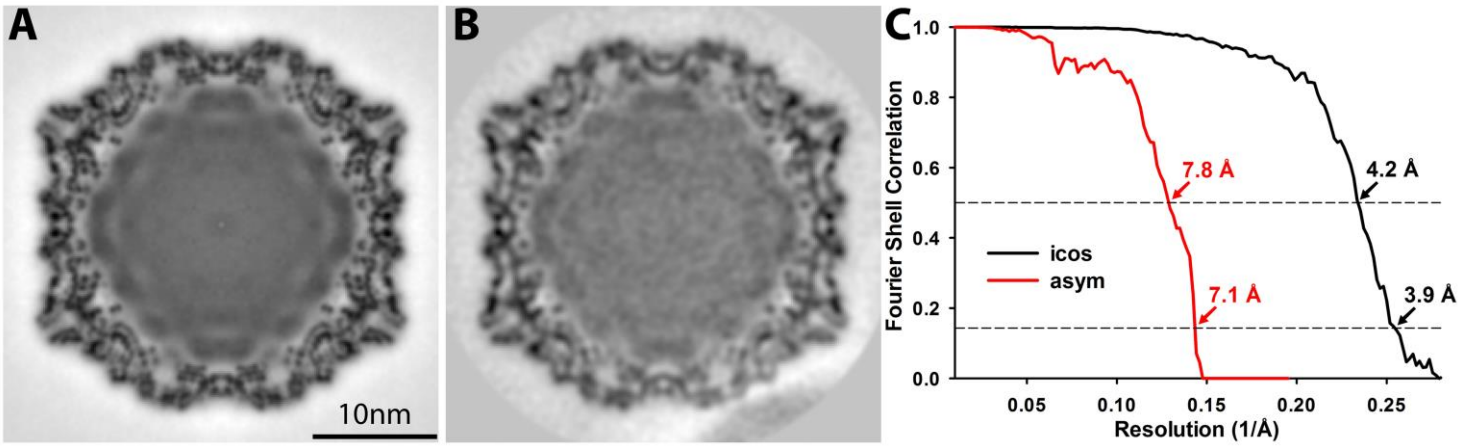

fig. S2. Central sections and FSC curves of the icosahedral and asymmetric 3D maps. (A and **B)** The map central section shows the quality of the reconstruction, with dark densities of visible, traceable protein chains. **(C)** The FSC graph (black line) for the icosahedral reconstruction (icos) indicates a resolution of 3.9 Å where the curve crosses at 0.143. The asymmetric reconstruction (asym) was done with a new protocol (sym_break) in RELION and the resolution is estimated where the FSC curve (red) crosses at 0.5, since the gold standard was not followed.

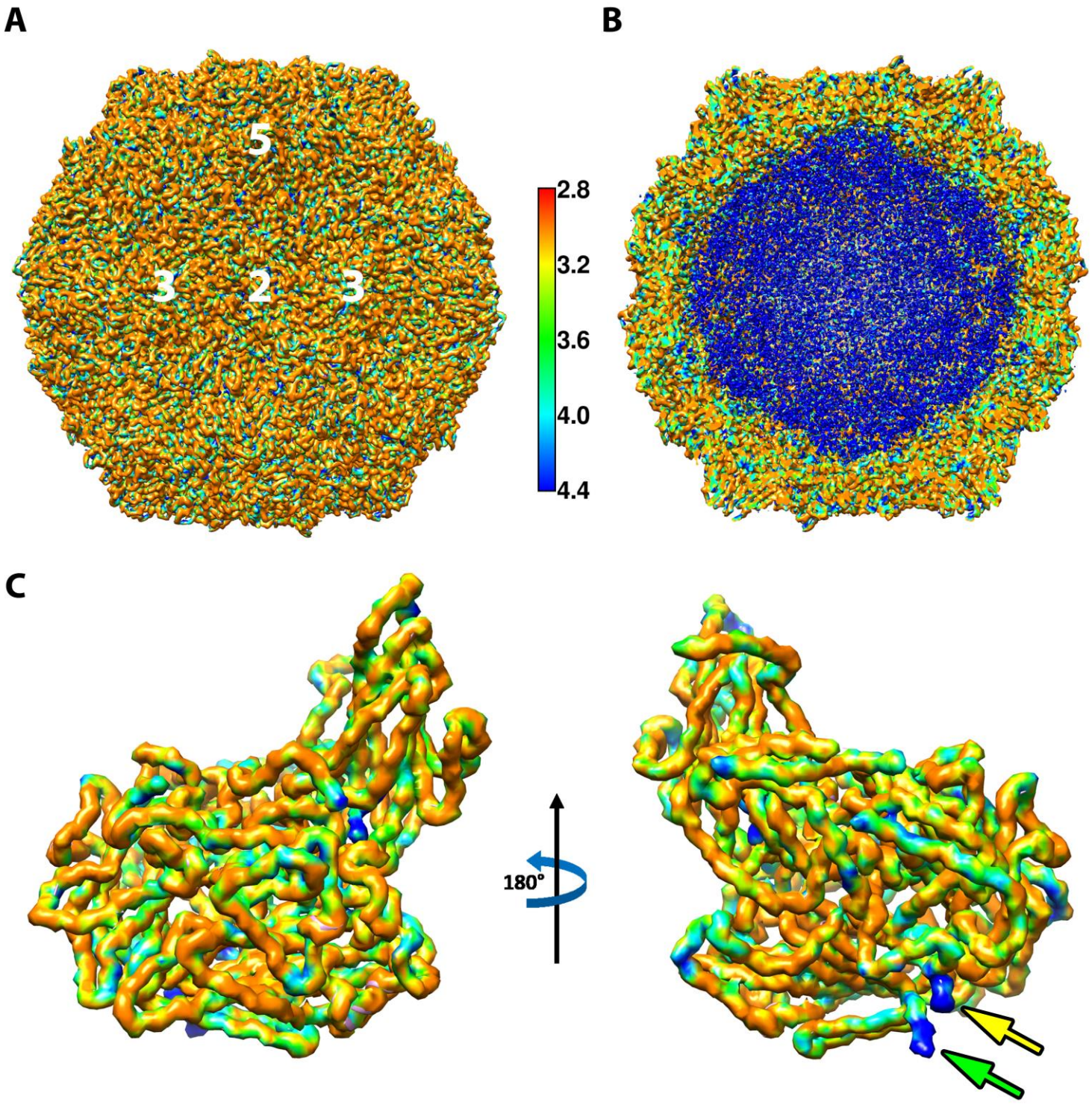

fig. S3. Local resolution of the icosahedral 3D reconstruction. (A) The sharpened icosahedral 3D map is rendered and colored for local resolution according to the scale bar. **(B)** The frontal half of the map is removed to show the local resolution of the capsid interior. **(C)** The 3D density map was generated from the atomic model of the asymmetrically stimulated A-particle and colored according to the same local resolution. The N-terminal residues of VP2 and VP4 (indicated by green and yellow arrows, respectively) are low resolution suggesting flexibility or movement.

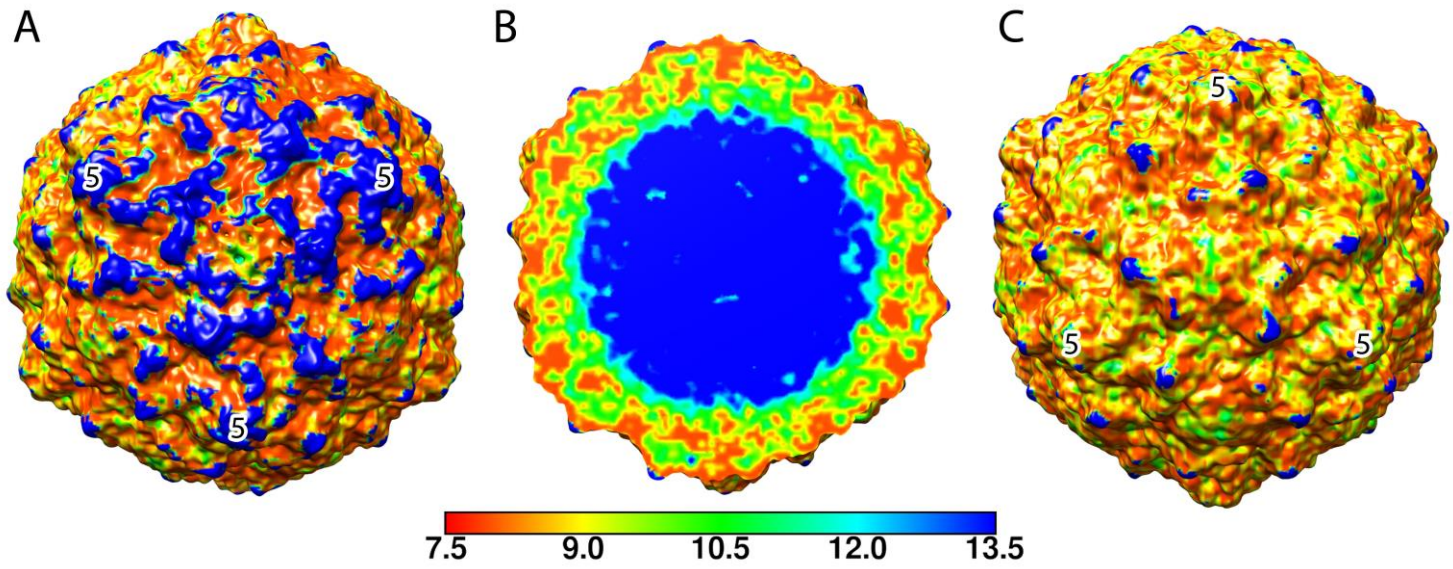

fig. S4. Local resolution of the asymmetric 3D reconstruction. (A) The asymmetric 3D map is rendered and colored for local resolution according to the scale bar with the same view as Fig. 4C to show the region of the unique site. **(B)** The front half of the map is removed to show the local resolution of the capsid interior. **(C)** The map is rotated 180° around vertical axis to show the opposite side, which has higher resolution indicating less flexible structures. Notably, the propeller tip appears lower resolution (blue) suggesting flexibility capsid-wide.

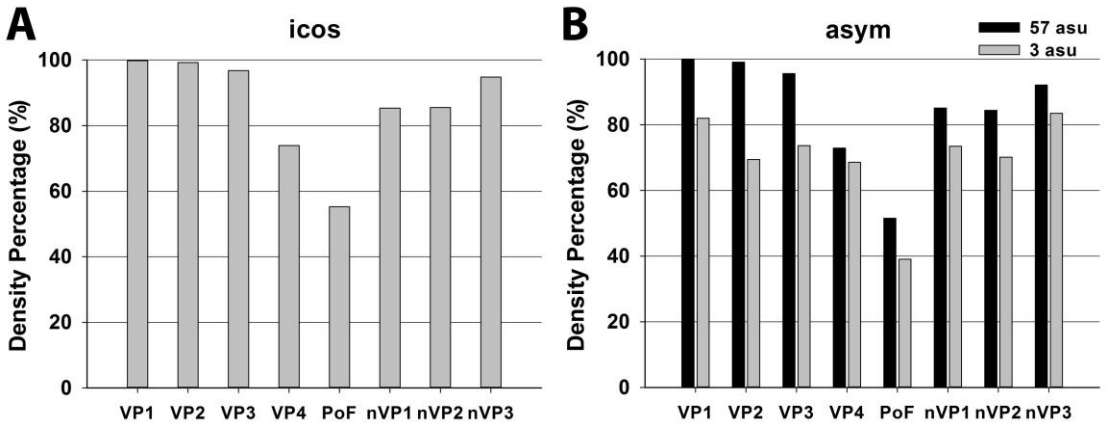

fig. S5. Quantification of density. (A and B) Mean values of the densities corresponding to the capsid components in the icosahedral (A) and asymmetric (B) maps were measured. Each virus protein was measured in entirety, along with pocket factor (PF), and the N-terminal residues for VP1 to VP3 (nVP1 to nVP3) (see Methods). The values were normalized by the mean intensity of the VP1 from the corresponding reconstruction. (B) The three asymmetric unit protomers (3 asu, gray bars) shown in Fig. 4D were compared with the other 57 protomers (57 asu, black bar).

table S1. Superimposing CVB3 (PDB ID: 1COV) with CAR-nanodisc A-particle structure illustrates rigid body movement of the protomer and describes the expansion of about 2 Å.

Protein	Residues	Atom pairs	RMSD (Å)
VP1	13-281	270	1.850
	13-59	47	0.973
	60-281	223	1.986
VP2	8-263	256	1.808
	8-56	49	1.229
	57-263	207	1.919
VP3	1-238	238	1.817
	1-42	42	1.232
	43-238	196	1.919
VP4	2-69	55	0.844

table S2. Classification of the CAR-nanodisc particles.

3D classes			**Particle Number**	**Features**
Total			137,469	
Class 1-3			75,142	Multiple neighbors
Class 4	All		62,327	0-2 neighbors
	Discarded		5,124	virus
	Selected	All	57,203	
		Sub 1	2,492	Density extending from the propeller tips
		Sub 2	3,230	Density extending from the propeller tips & 3F axis
		Sub 3	2,025	Density extending from the threefold axis