Biophysical Journal, Volume *122*

Supplemental information

Template-free prediction of a new monotopic membrane protein fold and assembly by AlphaFold2

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Supporting Information

Template-free prediction of a new monotopic membrane protein fold and oligomeric assembly by Alphafold2

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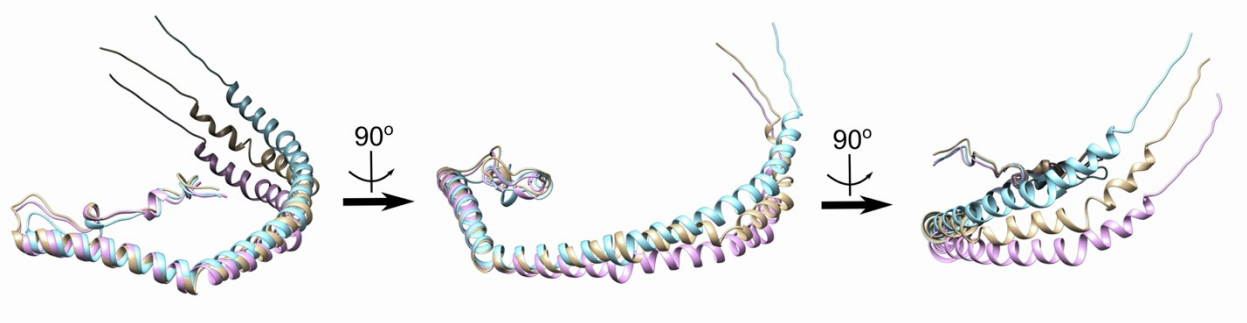
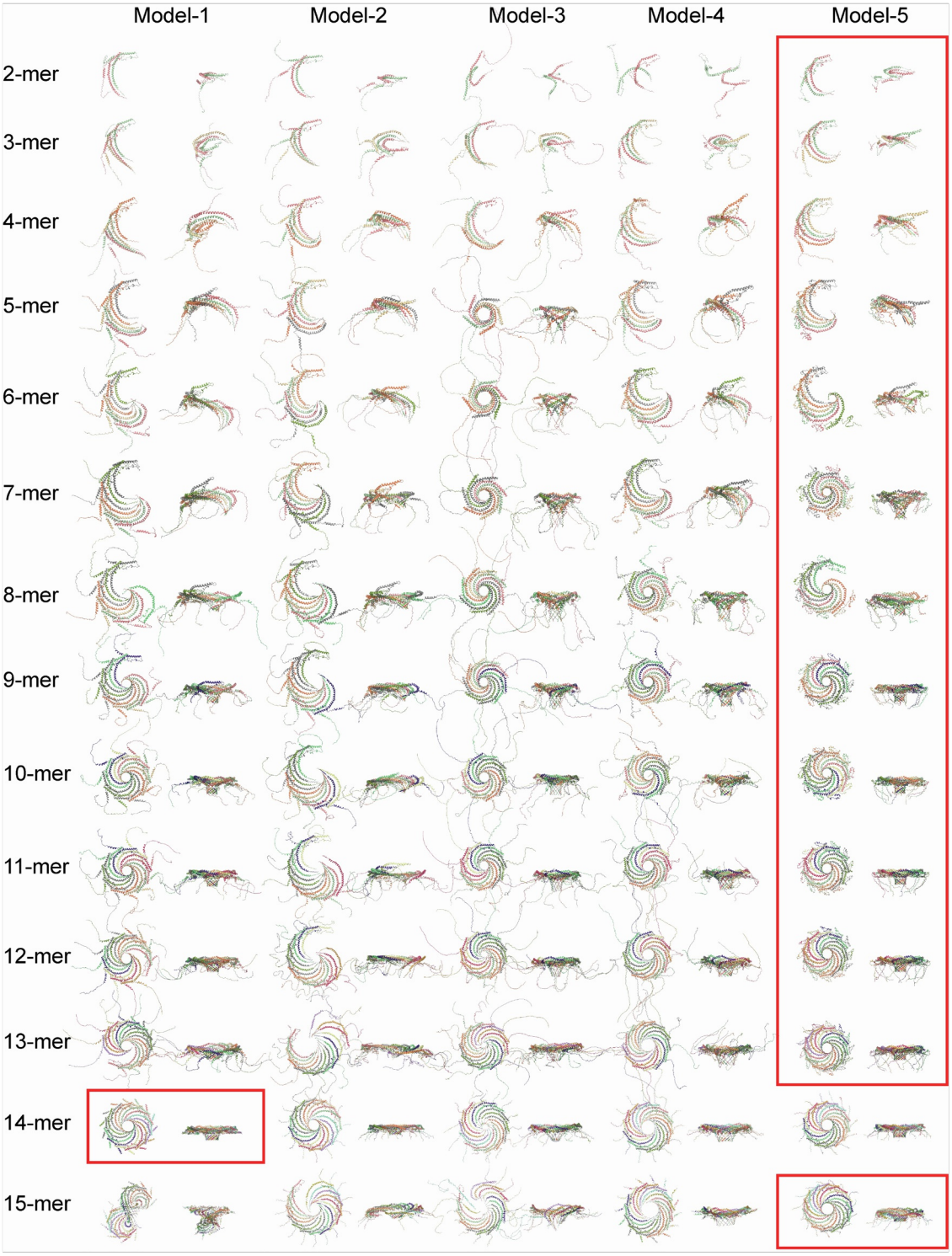
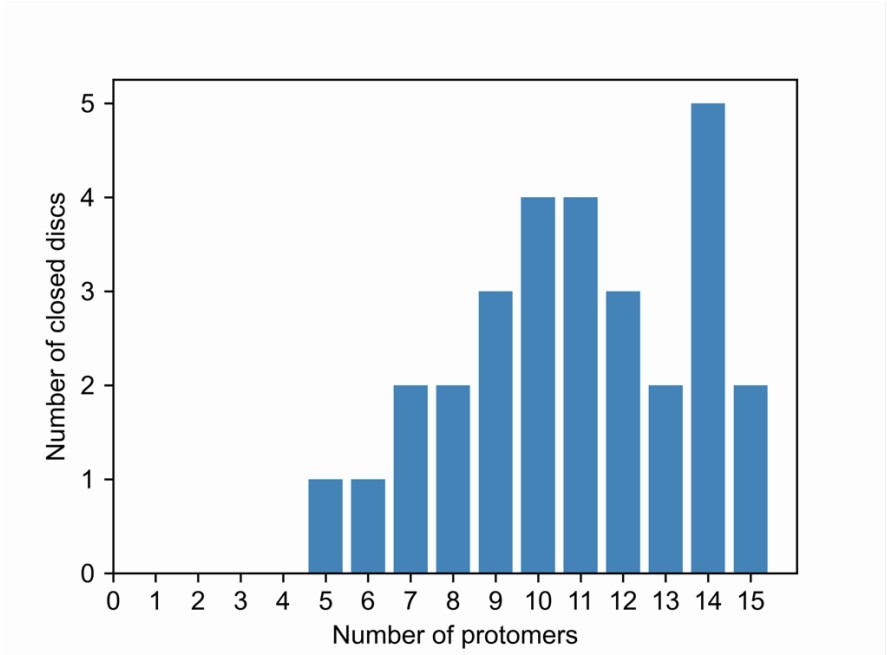


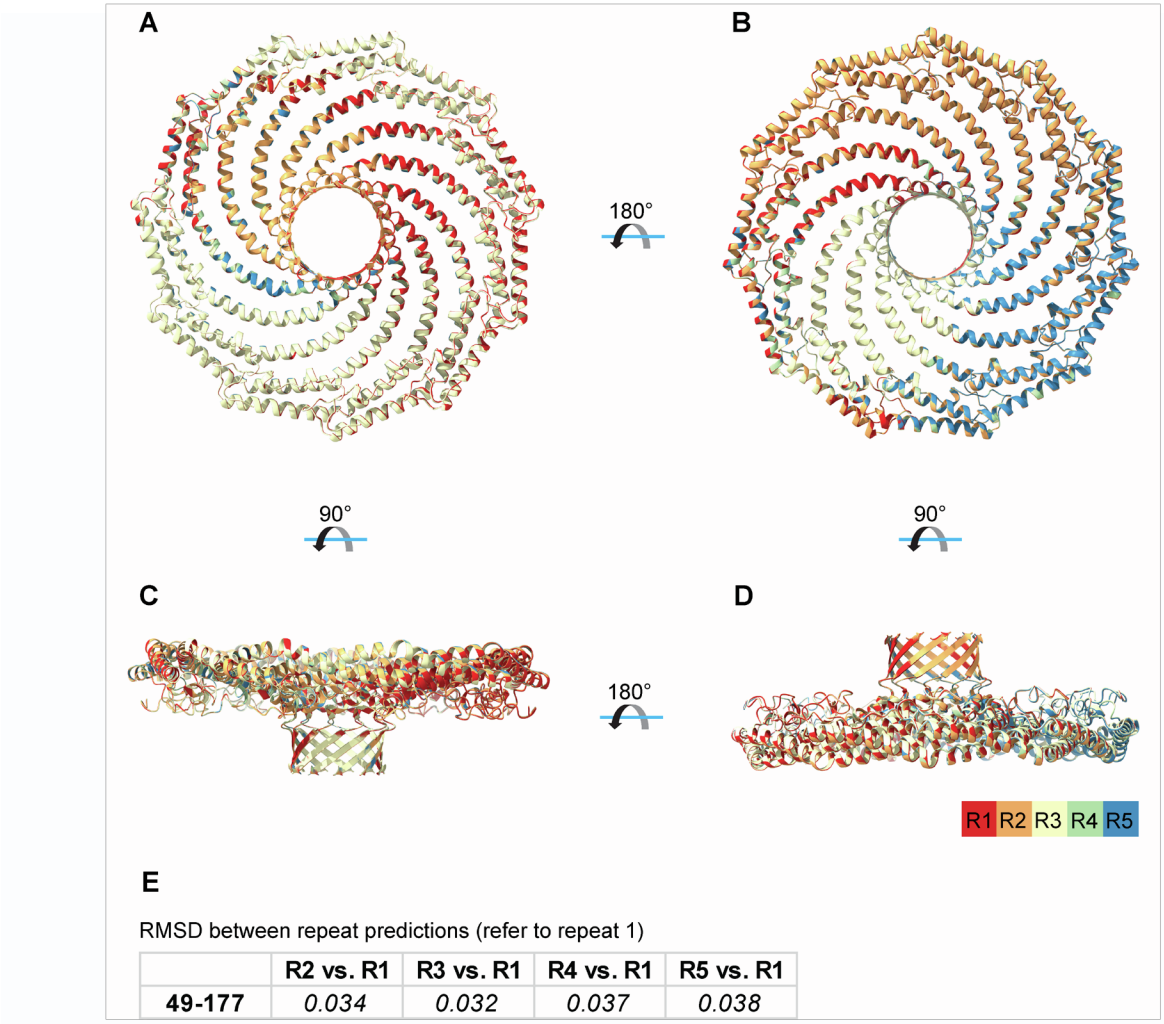
Supplementary Figure 1: **Comparison of experimental and predicted CAV1 protomer and monomer structures.** Alignment of the experimental 11-meric CAV1 protomer (beige), CAV1 AF2 monomer (blue), and CAV1 AF2 protomer from an 11-meric prediction (pink) using helix α1 as the anchor point viewed from three different angles.



Supplementary Figure 2: Top and side views of CAV1 homo-oligomers predicted by AF2.2. Five models are show for each prediction. The best model from each prediction is outlined by a red box. For the 2- to 13-mers, the predictions were based on full length alpha isoform (1-178) of human CAV1. Predictions for the 14 and 15-mers were based on the sequence of the beta isoform (32-178) of human CAV1. Each protomer is indicated using a different color within a given complex.



Supplementary Figure 3: **The probability of forming a closed structure varies across n-mers.** Distribution of the predicted number of closed structures (out of the top 5 predictions) as a function of the number of input CAV1 protomers. A closed structure is defined as an oligomer in which all protomers folded into a closed disc-shaped assembly.

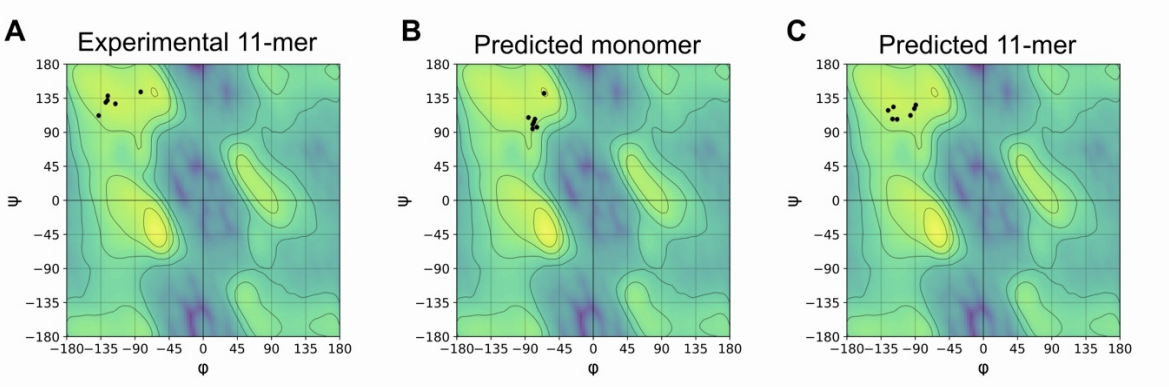


E

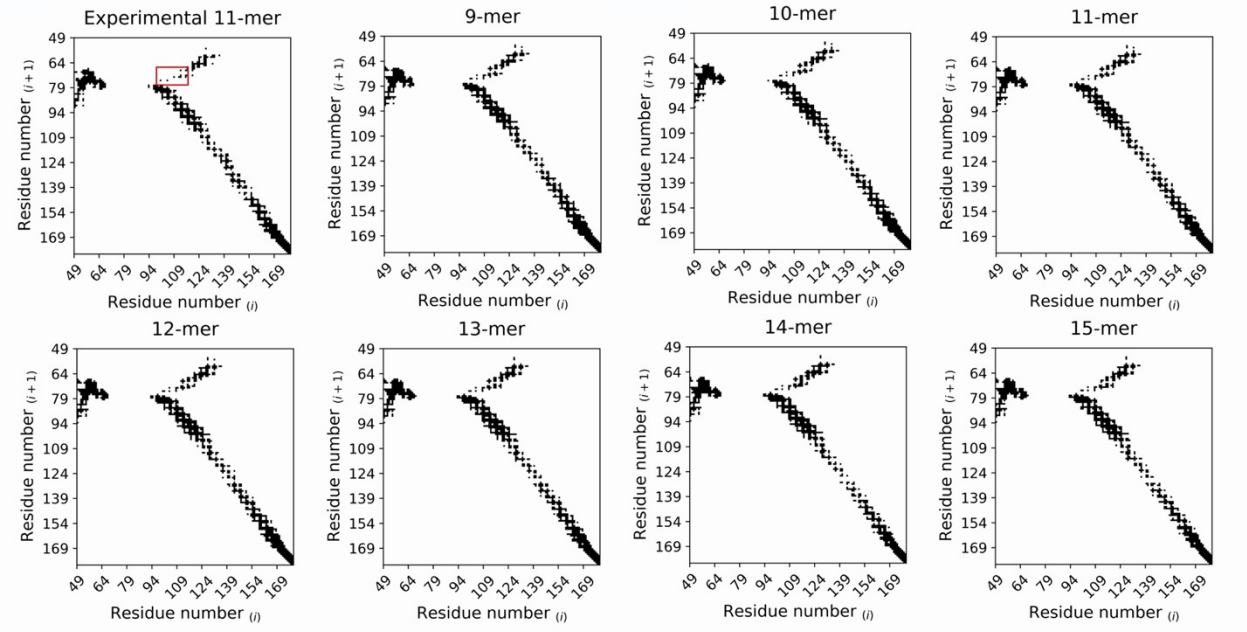
RMSD between repeat predictions (refer to repeat 1)

	R2 vs. R1	R3 vs. R1	R4 vs. R1	R5 vs. R1
49-177	*0.034*	*0.032*	*0.037*	*0.038*

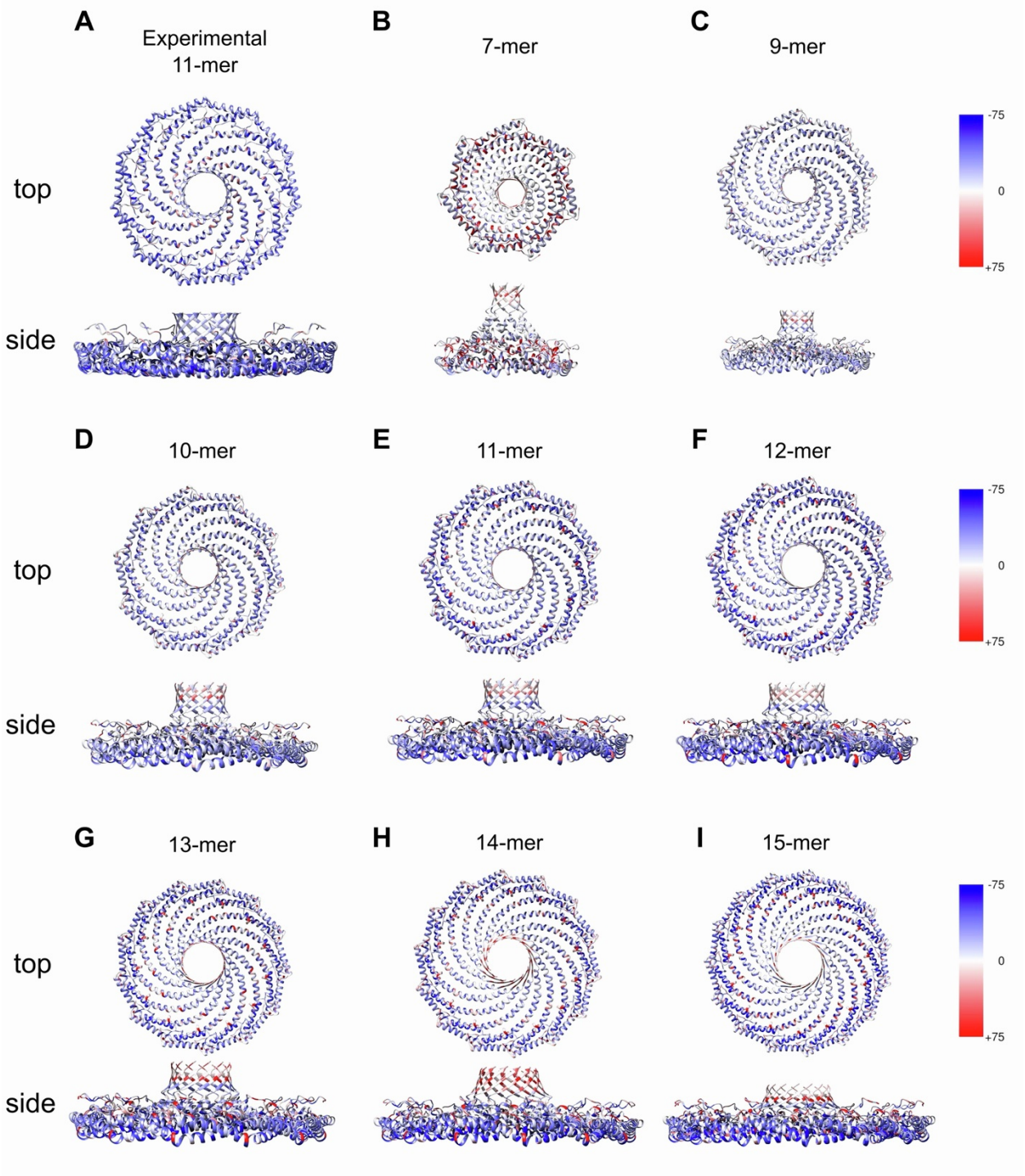
Supplementary Figure 4: AF2.2 predictions for CAV1 11-mers are highly reproducible. (A-D) Overlay of five independent predictions of best fitting AF2.2 models of the CAV1 11-mer (predicted using residues 49-177)**.** The best fitting model for each repeat (R1-R5) is shown in a different color, as indicated in the legend. Four different views of the complex are shown. **(E)** Table of RMSD values compared across independent repeat predictions (repeats 1 through 5). Comparisons were all made with respect to repeat 1 (R1).



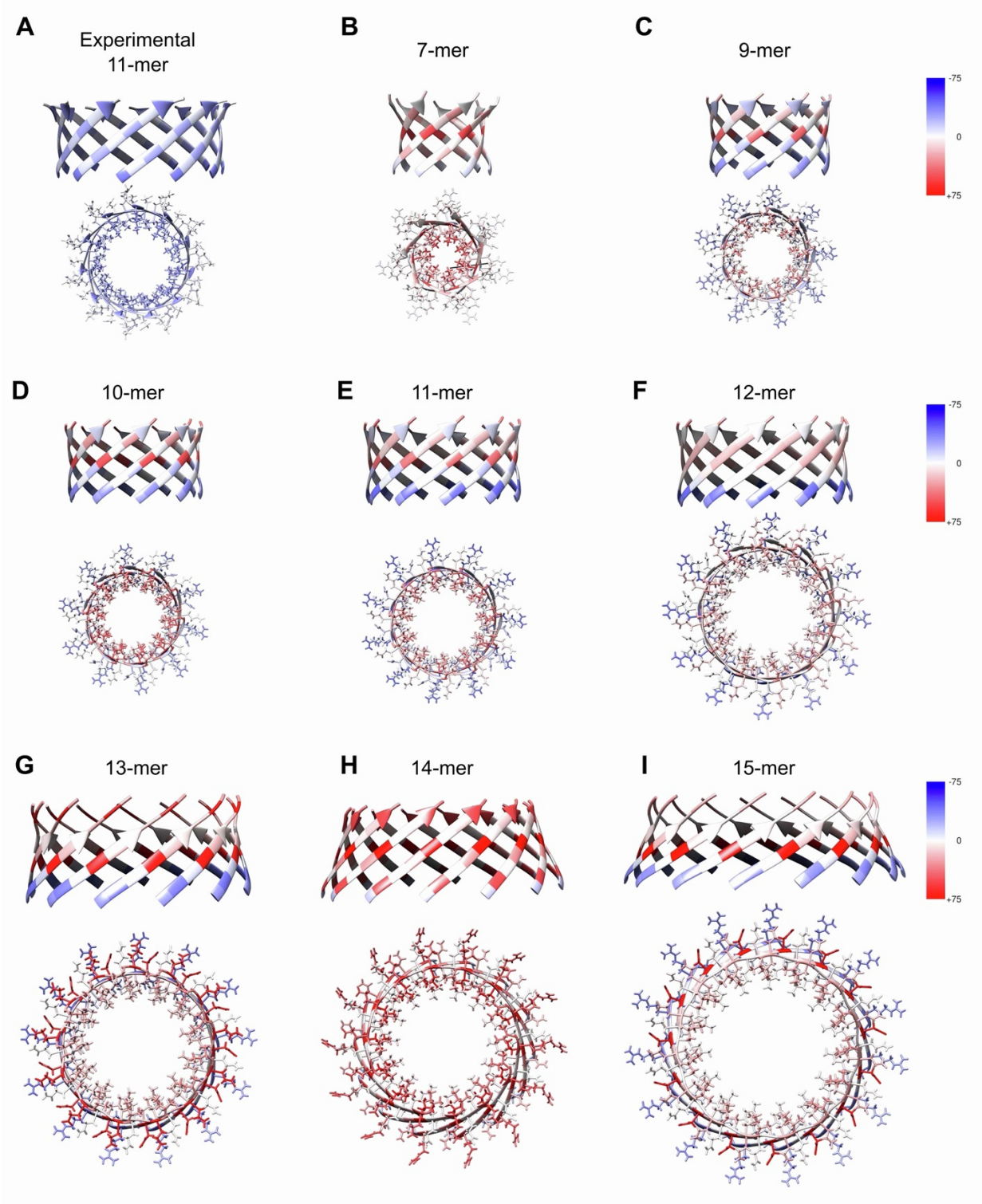
Supplementary Figure 5: **Ramachandran plots for residues within the β-strand region of the experimental CAV1 protomer structure.** (**A**) Protomer from the experimental CAV1 11-mer structure. (**B**) AF2-predicted CAV1 monomer. (**C**) Protomer from the AF2-predicted CAV1 11-mer.



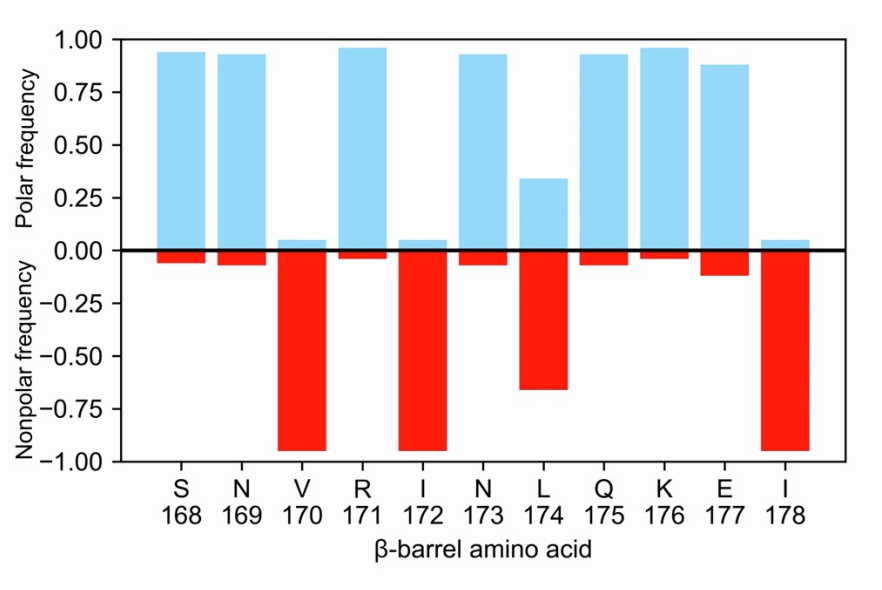
Supplementary Figure 6: Contact maps calculated for different oligomeric assemblies of CAV1 including the 11-meric experimental CAV1 structure and the AF2.2-predicted oligomers consisting of 9 to 15 protomers. A contact is considered as the proximity of any two residues within 12 Å of each other. The x-axis stands for the residue number at any given protomer, and the y-axis stands for the residue numbering at the neighboring protomer in the counter-clockwise direction. The region that showed increased contacts in AF2 models compared to the experimental structure is highlighted with a red box.



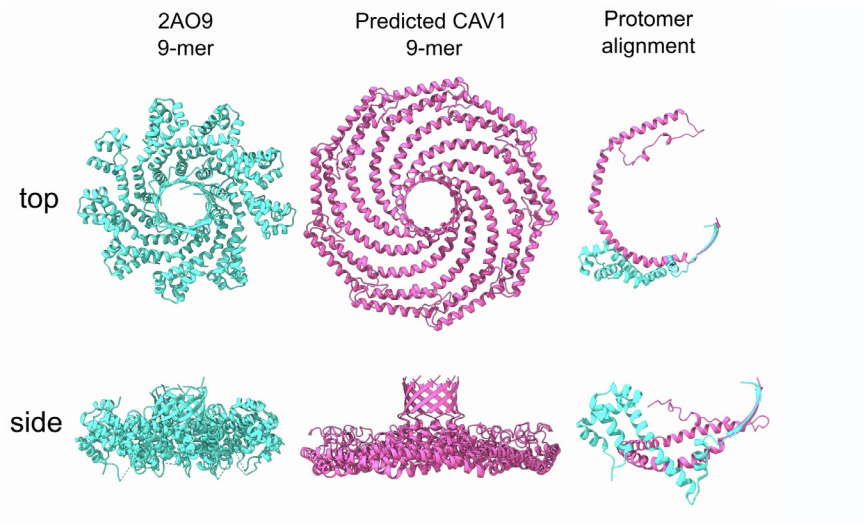
Supplementary Figure 7: Per-residue energies calculated for the experimental 11-meric CAV1 structure and the AF2.2-generated CAV1 structures consisting of seven to 15 protomers. Red color indicates unfavorable energies and blue color indicates favorable energies. All scores are in Rosetta Energy Units (REU). All structures are shown at the same scale.



Supplementary Figure 8: Per-residue energies for CAV1 β-barrels. Shown are per-residue energies calculated using Rosetta for experimental 11-meric CAV1 structure and the AF2-predicted CAV1 oligomers from 7 to 15 protomers. Red color indicates unfavorable energies and blue color indicates favorable energies. All scores are in Rosetta Energy Units (REU). All structures are shown at the same scale.



Supplementary Figure 9: The presence of alternating polar and non-polar residues, together with a lysine near the C-terminus is a conserved motif found in the β-barrel region of CAV1 across multiple species. Polar amino acid frequencies were calculated for the sequences of 315 CAV1 from different species. The x-axis shows the corresponding sequence from human CAV1 (residues 168-178), and the y-axis reports the probability of having a polar residue (blue) or nonpolar residue (red) at the corresponding position among different CAV1 sequences.



Supplementary Figure 10: **Comparison of the 9-meric experimental 2AO9 structure (cyan) and the AF2-predicted 9-meric CAV1 structure (pink) viewed from top (top row) and side (bottom row).** Alignment of a single protomer from each structure based on β-barrel coordinates is shown in the right panel. All structures are shown at the same scale.