**Supplementary Figure 1. The isolated barrel domain (res 603-725) does not form a dodecamer in solution.** (**A**) Size exclusion chromatography analysis of the isolated barrel domain (M.W. ~14 kDa) analyzed on a Superdex 200 16/60 column (GE Healthcare) equilibrated in GF-buffer (20 mM Tris-Cl pH 8.0, 150 mM NaCl, 1 mM MgCl2, 1 mM β-mercaptoethanol). The column was calibrated using cytochrome C (12.4 kDa, elution volume = 96.9 ml), carbonic anhydrase (29 kDa, elution volume = 89.4 ml), albumin (66 kDa, elution volume = 79.8 ml), alcohol dehydrogenase (150 kDa, elution volume = 74.4 ml), β-Amylase (200 kDa, elution volume = 70.6 ml) and blue dextran (2,000 kDa) from the Gel Filtration Molecular Weight Markers Kit (Sigma). Based on calibration markers, the barrel domain has an estimated M.W. ~55 kDa, possibly consistent with a very elongated monomer or a dimer. (**B**) Coomassie blue stained SDS-PAGE of the eluted barrel domain.
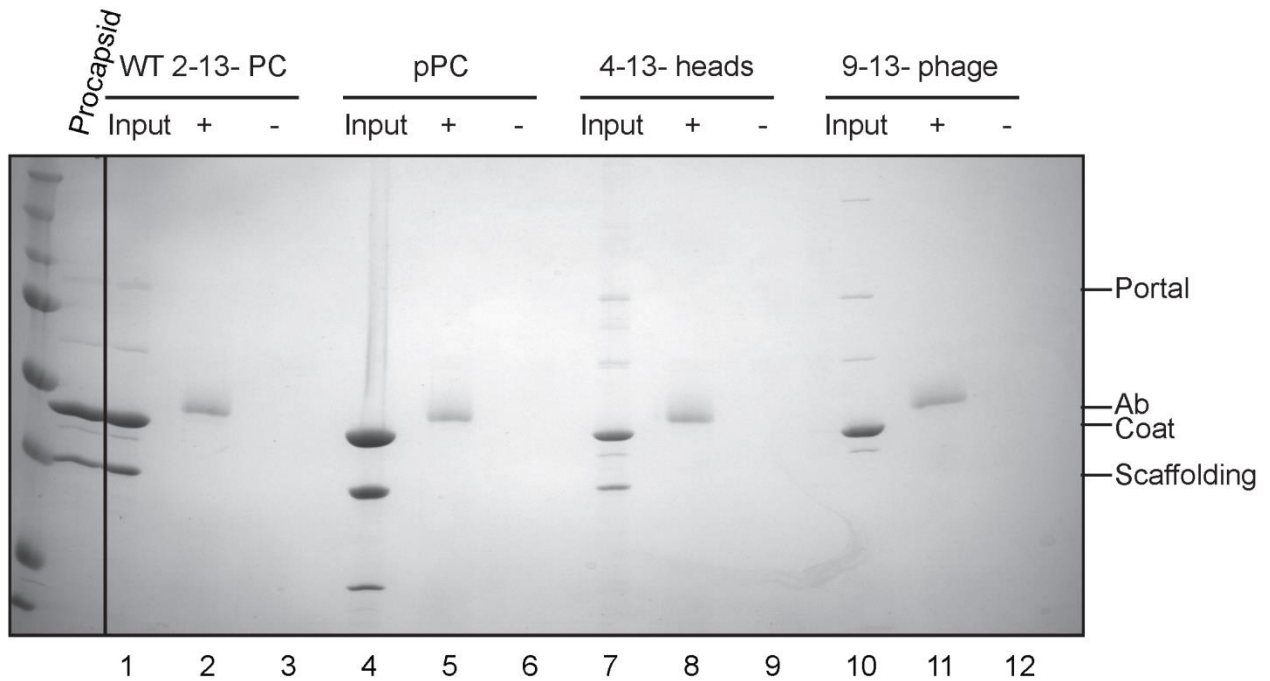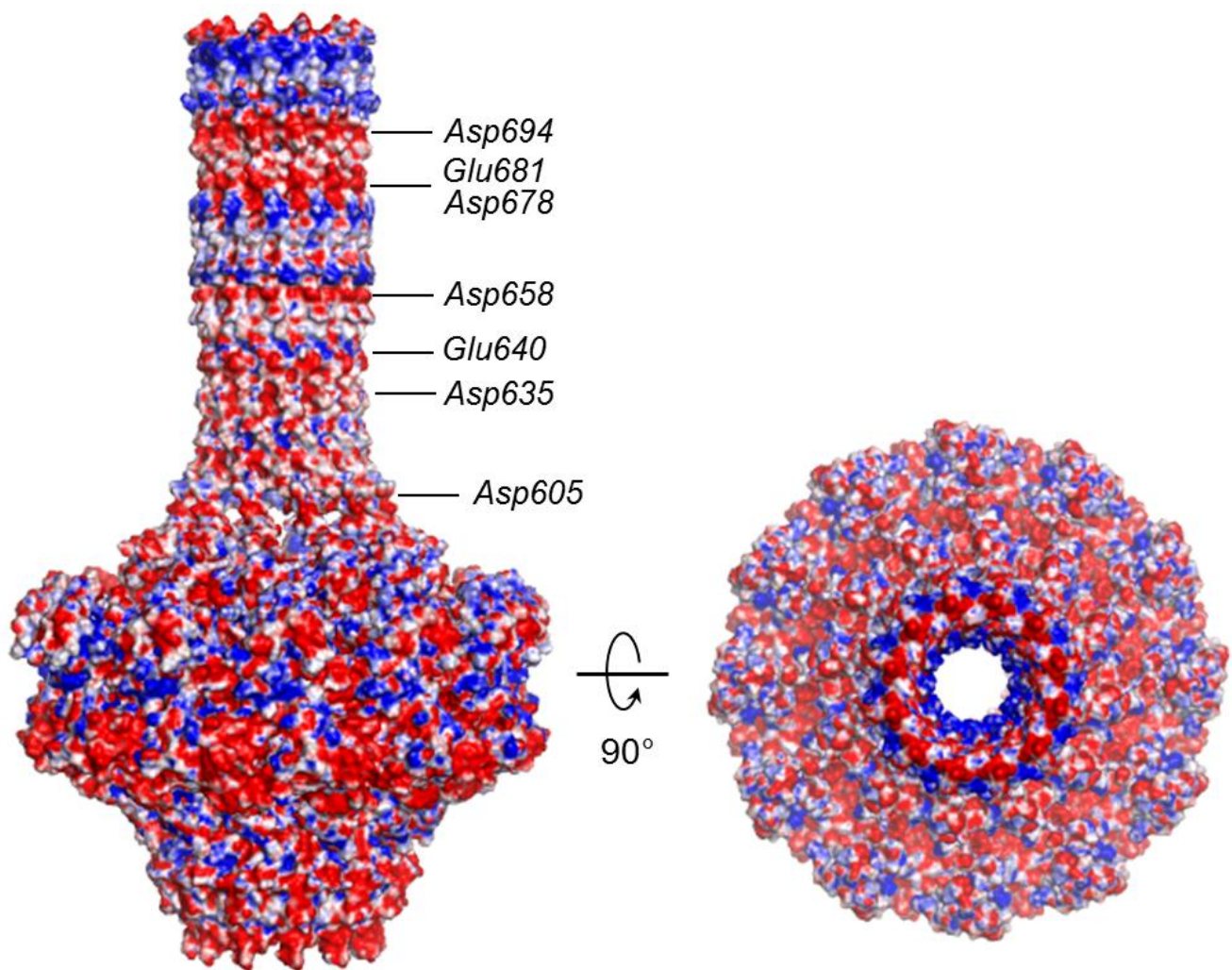
Supplementary Figure 2. Portal protein immunoprecipitation assay. Coomassie blue stained SDS-PAGE of a representative experiment. 'Input': 10 μg of control samples (see Methods); '+': proteins immunoprecipitated by the anti-stalk antibody; '-': proteins incubated with the Protein A agarose beads but without the antibody. 'WT 2⁻13⁻ PC' are genuine P22 procapsids; 'pPC' indicates procapsids assembled *in vivo* without portal; '4⁻13⁻ heads' are empty mature P22 heads; '9⁻13⁻ phages' are tail-less P22 phages. The migration of portal protein, antibody IgG band (Ab), coat protein, and scaffolding protein is indicated on the right.
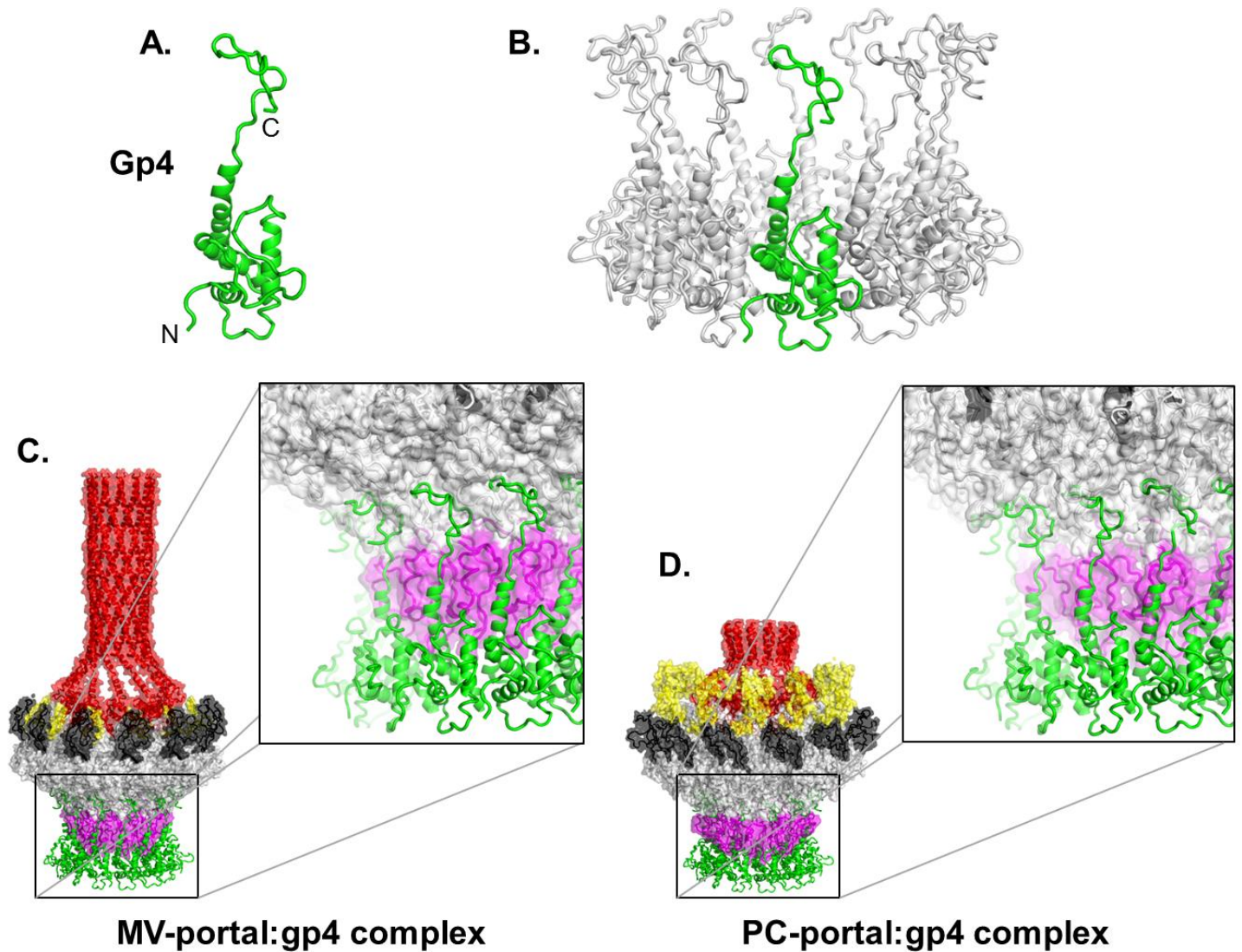
Supplementary Figure 3. Gp4 can bind both PC- and MV-portal protein. Ribbon diagram of (**A**) monomeric and (**B**) dodecameric gp4 (from pdb 3LJ4). Model of (**C**) MV-portal and (**D**) PC-portal protein bound to 12 copies of gp4. In both panels, the zoom-in window shows gp4 lateral binding at the portal protomer:protomer interface.
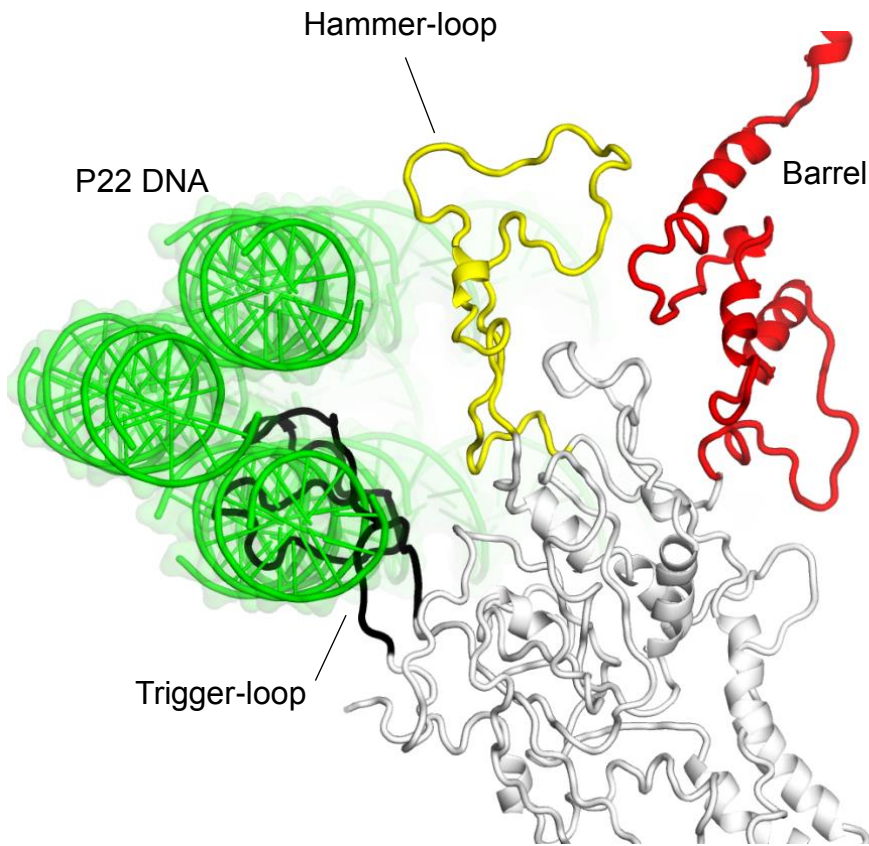
Supplementary Figure 4. Molecular model of a PC-portal protomer surrounded by the three rings of DNA visible in the cryo-EM reconstruction of the P22 mature virion [3,4]. The conformation of the trigger-loop in PC-portal protein is incompatible with packaged DNA filling the capsid.

Supplementary Figure 5. The electrostatic surface charge distribution of MV-portal protein. Surface-exposed acidic residues in the barrel domain are shown by arrows. Overall, the barrel is mainly acidic between residues 605-694. Non-linear Poisson-Boltzmann electrostatic calculations were performed using APBS Tools [5] and surfaces rendered in program Pymol [6].

	PC-portal refined without NCS	PC-portal refined with NCS-restraints *	PC-portal refined with NCS-constraints
R_{work}/R_{free} (%)	29.5 / 31.5	30.5 / 32.7	49.4 / 50.2
RMSD bonds / angles (Å)	0.004 / 1.033	0.010 / 2.219	0.029 / 3.047
Ramachandran (%) favored / allowed / outliers	76.89/ 21.71 / 1.40	73.14 / 19.97 / 6.89	59.19 / 29.09 / 11.72
Romater outliers (%)	0.0	2.2	10.4
C-beta outliers	0	86	130
Clashscore **	18.9	27.3	63.1
MolProbity Score **	2.55	3.01	3.96

* ~42,239 torsion-angle NCS restraints generated from the 12 subunits using a *max_RMSD* cut-off of 5.5 Å, as implemented in *phenix.simple_ncs_from_pdb* [1]
** As defined in reference [2]

Supplementary Table 1. Effect of NCS on the crystallographic refinement of PC-portal protein. The final model was subjected to three macro-cycles of crystallographic refinement using *phenix.refine* [1] without imposing NCS (left column), with torsion-angle NCS restraints (central column) and with 'strict' NCS (e.g. NCS-constraints) that assume all 12 subunits are identical (right column). Each macro-cycle includes cycles of bulk solvent correction, real space, XYZ positional and individual B-factor refinement.

Supplementary References

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