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Supplemental Information

Bacteriophage P23-77 Capsid Protein Structures Reveal the Archetype of an Ancient Branch from a Major Virus Lineage

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Inventory of Supplemental Information

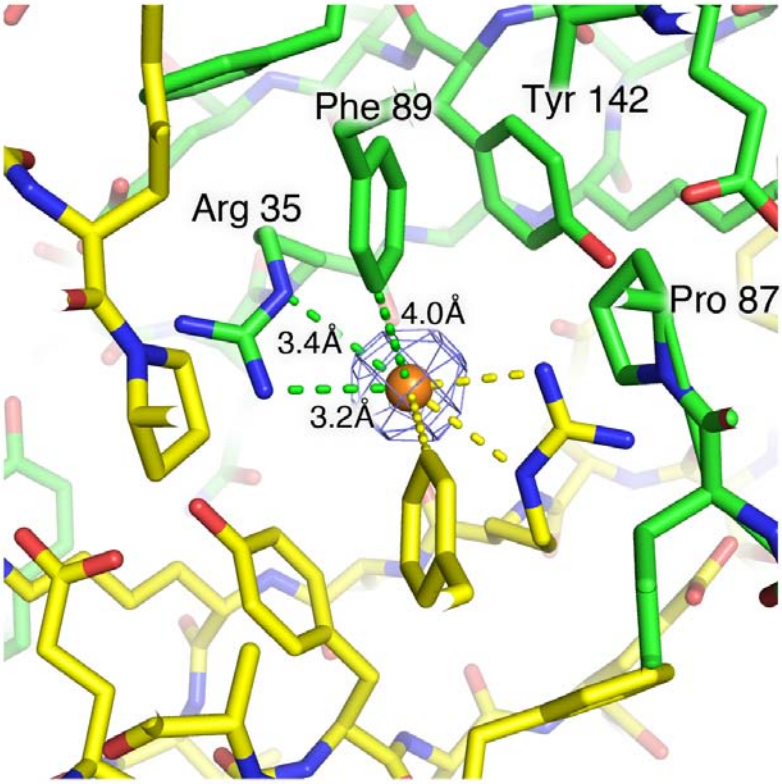

Figure S1. Environment of the Strand-Swap in the VP16 Dimer, Related to Figure 1

Figure illustrates the chloride ion binding site in the VP16 dimer, viewed down the molecular dimer axis. The Fo-Fc difference map (contoured at 5 sigma) showing the putative chloride ion, is drawn as blue chicken wire. The map was calculated using phases derived from the refined model with the chloride ion removed. The subunits of VP16 are drawn in green and yellow, residues from one subunit that form the binding site are labeled, and the atomic distances are highlighted.

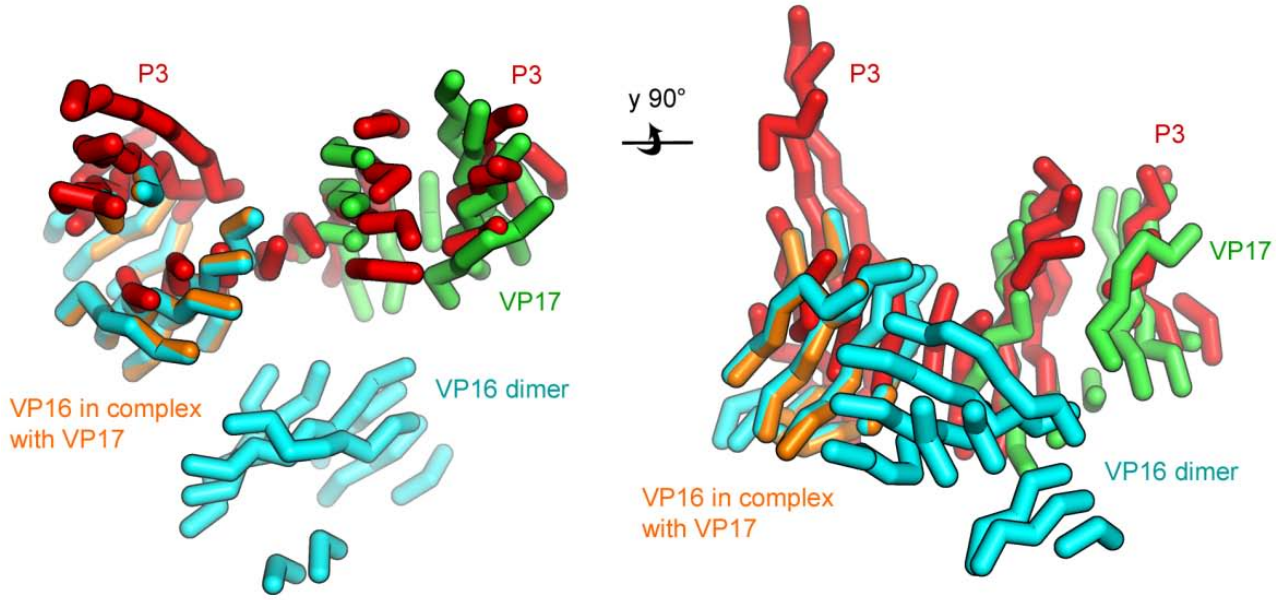

Figure S2. P23-77 Capsid Proteins Superposed on PRD1 P3, Related to Figure 5

Superposition of the VP16 subunit (orange) of the VP16-VP17 complex on the PRD1 major capsid protein P3 (red), places the VP17 subunit (green) on the neighboring P3. Superposition reveals that the β-barrels of each P3 match well in position and orientation to the β-barrels of VP16 and VP17 in complex. VP16 dimer (cyan), superposed on the VP16 subunit of the VP16-VP17 complex, illustrates the rotation of the β-barrels in the dimer. P3 structures are from the PRD1 asymmetric unit, PDB code 1W8X.

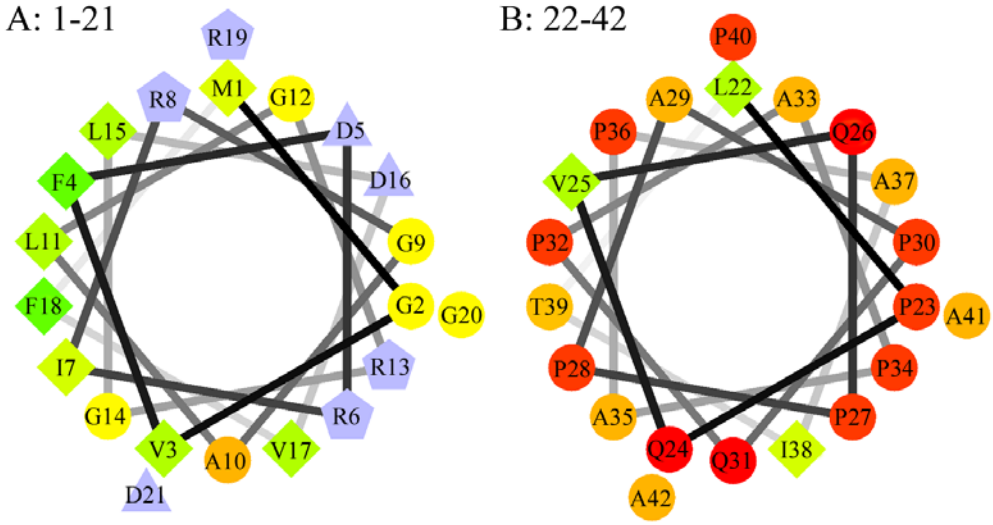

Figure S3. Helical Wheel Analysis of the Undetermined N-terminal Domain of VP17, Related to Figure 3 and Section "Major Capsid Protein Structures Identify P23-77 as a Relative of the Double β-Barrel Lineage."

Part A shows the first 21 N-terminal residues while part B shows the residues 22-42. Color code: red, hydrophilic; green, hydrophobic; yellow-orange, slightly hydrophobic; grey, charged. Figure was done with the wheel.pl application created by Don Armstrong and Raphael Zidovetzki. Version: 0.10 p06 12/14/2001 DLA, modified by Jim Hu.

AD-Fusion	DBD-Fusion	Miller units
AD	**VP16**	**71 ± 19**
VP16	**DBD**	**101 ± 86**
AD	**VP17**	**69 ± 19**
VP17	**DBD**	**52 ± 11**
VP16	**VP16**	**1204 ± 268**
VP17	**VP17**	**62 ± 17**
VP17	**VP16**	**70 ± 23**
VP16	**VP17**	**725 ± 95**

Table S1. Yeast Two-Hybrid Experiment on the Interactions of P23-77 Capsid Proteins, Related to Figure 4 and Section "Capsid Model Provides Insight into P23-77 Virion Architecture and Assembly."

Interactions of major capsid proteins VP16 and VP17 were studied in yeast two-hybrid system. Transcriptional activation of *lacZ*-reporter gene by interaction of yeast DNA activation domain (AD) and DNA binding domain (DBD) fusion proteins was quantified by determination of β-galactosidase activity (Miller, 1972). Values represent averages of two independent transformants, each measured in triplicate. First four rows represent the controls.

Supplemental Experimental Procedures

Protein Oligomeric State and Interaction Studies

Oligomeric states of VP16 and VP17 were studied with analytical gel filtration chromatography. A standard mixture of conalbumin (75 kDa), ovalbumin (44 kDa) and lysozyme (14.3 kDa) was run at 0.2 ml/min flow rate in Superdex 200 10/300 GL column (GE Healthcare) connected to ÄKTAprime plus chromatography system (GE Healthcare). The resulting reference chromatogram showed elution peaks corresponding to standard protein sizes: at 66 min for 75 kDa conalbumin, 75.3 min for 44 kDa ovalbumin and at 132 min for 14.3 kDa lysozyme. Afterwards, samples of purified VP16 and VP17 were run separately with identical parameters to the standard run. Chromatograms showed VP16 to peak at 81.7 min, corresponding roughly to the 40 kDa mass of a dimer, while VP17 peaked at 80.1 min, corresponding more closely to the 32 kDa mass of monomeric protein than to that of an oligomer of at least 64 kDa.

This is in line with yeast two-hybrid studies based on the LexA System (Fashena et al., 2000) that we conducted to measure interactions between the proteins *in vivo*. For the construction of the hybrid plasmids we first used PCR to introduce a sequence coding for a flexible linker of six glycine residues downstream of either the DNA-binding domain (DBD) or the activation domain (AD) encoding sequence of *Escherichia coli*/yeast shuttle vectors pEG202 (lexA-DBD) and pJG4-5 (B42-AD) in order to enhance the possibility of a protein-protein interaction on the N-terminus that might be otherwise blocked by the yeast domain. Then appropriate primers were

designed for amplification of full-length *ORF16* and *ORF17* flanked by restriction sites allowing us to generate in-frame fusions with the DBD/linker and AD/linker sequence. Genes were amplified with P23-77 chromosomal DNA as template. The resulting hybrid plasmids were transformed into yeast reporter strain EGY48(pSH18-34) using the PEG/lithium acetate method as described before (Gietz et al., 1992). DBD or AD linker in-frame fusions were co-transformed with corresponding "empty" plasmids pEG202/linker and pJG4-5/linker in order to analyze the ability of the fusion proteins to self-activate reporter gene expression. Strain EGY48(pSH18-34) carries a chromosomal LEU2 reporter gene and an episomal *lacZ* reporter gene. Transcriptional activation of these reporter genes was analyzed 1) by growth on plates lacking leucine 2) by the formation of blue colonies on plates containing X-Gal and 3) quantitative measurement of β-galactosidase activity (Miller, 1972). None of the VP16 and VP17 fusions showed significant background transcriptional activity (self-activation). A strong activation of the reporter genes was shown for VP16 with itself and – to less extent – between VP16 and VP17 but only in the combination AD-VP16 / DBD-VP17 (Supplemental Table S1). No interaction was detected between VP17 molecules. These results support the formation of VP16 dimers and VP16/VP17 heteromers.

PISA

To analyze the surface contacts of the capsid proteins we used protein interfaces, surfaces and assemblies service PISA at the European Bioinformatics Institute, by E. Krissinel and K. Henrick (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html).

PISA analyses were run for 1) VP16-type-1, 2) a computationally modified VP16-type-1 where the swapped strand has been refolded into the β-barrel and 3) VP16/VP17 complex structure. PISA yielded the interface area of 3151.9 $Å^2$ for the native VP16 dimer, 1090.9 $Å^2$ for the modified VP16 dimer, and 733.0 $Å^2$ for the interface between VP16 and VP17 in complex. This illustrates the importance of the strand swap in the dimer interaction of VP16 and the strength of VP16-VP17 multimerization. Surface areas of the tested combinations correspond to the interaction tendencies observed in yeast two-hybrid experiments and support the assembly hierarchy presented in the main text.

Membrane Interaction by Helical Wheel Analysis

We determined the structure of VP16 nearly completely, VP16-type-1 lacking only the N-terminal methionine. The fact that all the amino acids except the methionine are determined supports the mode of action we have proposed for VP16: it is a strong capsid component not connected to the internal membrane of P23-77. However, the membrane is a likely factor influencing the capsid assembly via VP17. The crystal structure of VP17 has 42 undetermined amino acids at the N-terminus and 18 at the C-terminus, consistent with flexible domains stretching beneath the capsid shell. Helical wheel analysis of the undetermined N-terminal amino acids of VP17 reveals a likely membrane domain consisting of an array of hydrophobic residues after a proline-containing helix. Analysis was done with the wheel.pl application at http://trimer.tamu.edu/cgi-bin/wheel/wheel.pl and the results are presented in Supplemental Figure S3.

Capsid Model Building with VEDA

The Visual Environment for Docking Algorithms (VEDA) is software tailored for refining viral capsid protein structures to cryo-EM maps of corresponding viral capsids (http://mem.ibs.fr/GAEL/index.html). The program requires the capsid electron density map and the asymmetric unit proteins as input from which it will produce the entire symmetrical protein capsid and proceed to refine the protein structure into the capsid density. We used the P23-77 cryo-EM map (14 Å resolution) and fitted in our asymmetric unit of proteins, each as a separate chain. The asymmetric unit is composed of 18 copies of VP16 and 9 copies of VP17. Regardless of the orientations of the proteins, as long as they were approximately inside the density, VEDA refined them to positions corresponding exactly to those observed in the crystal structures; e.g. VP16-VP17 positioning as in the complex and VP16-VP16 as in the dimer. This refinement yielded an R-value of 50.2% and CC-value of 74.1% and confirms our hypothesis that the interactions seen in the crystal structures are replicated in the native capsid.

Barrel Rotation in P23-77

VEDA refinement of the VP16 and VP17 protein structures to the P23-77 capsid electron density shows strong similarity to the typical protein arrangement in the double β-barrel lineage. After refining the proteins to density with VEDA, coordinates were exported and analyzed in Coot in comparison to the PM2 P2 and PRD1 P3. Superimposition of the pseudo-hexameric capsomers shows that the β-barrels of VP16 and VP17 of P23-77 occupy essentially same positions as the barrels of PM2 or PRD1 (see Figure S2).

Supplemental References

Fashena, S.J., Serebriiskii, I.G., and Golemis, E.A. (2000). LexA-based two-hybrid systems. Methods in enzymology *328*, 14-26.

Gietz, D., St Jean, A., Woods, R.A., and Schiestl, R.H. (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res *20*, 1425.

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