SUPPLEMETARY INFORMATION

Atomic structure of the Epstein-Barr virus portal

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Supplementary Fig. 1. a. Representative cryo-EM micrograph of the EBV portal sample. The scale bar represents 50 nm. **b.** RELION 2D averages used for model building, showing end-on and side-views. **c.** RELION resolution FSC curve. The FSC= 0.143 gold-standard cutoff criterion was used for determining the final resolution of the model (3.5 Å). **d.** RELION 3D reconstruction of EBV portal dodecamer model showing the angular distribution plot of the particles. **e.** Extraction of the density from the 3D reconstruction showing the placement of the side chains in two different areas. **f.** MonoRes local resolution map showing a side view (left) and an end-on view (right). The resolution plot ranges from 3.01-5.0 Å. All panels were generated from the data set collected at NeCEN.



Supplementary Fig. 2. Comparison of EBV portal with bacteriophage portal proteins. Portal proteins in monomeric display with the wing, crown, stem and clip domains colored in sand, blue, light green and gray, respectively. **a.** EBV portal protein. **b.** gp1 P22 portal protein (3LJ5). **c.** gp6 SPP1 portal protein (2JES). **d.** gp10 \u03c629 portal protein (1H5W). **e.** gp8 T7 portal protein (6QX5). **f.** gp20 T4 portal protein (3JA7).

	His-Z-pBBRF1 NeCEN EMD-10010 PDB 6RVR	His-Z-pBBRF1 DLS EMD-10011 PDB 6RVS
Data collection and processing		
Nominal magnification	130,000	130,000
Voltage (kV)	300 kV	300 kV
Electron exposure (e ⁻ /Å ²)	40	40
Defocus range (µm)	- 1.0 to - 3.0	- 1.0 to - 3.0
Pixel size (Å/pix)	1.10	1.06
Symmetry imposed	C12	C12
Initial particle images (no.)	404,914	250,788
Final particle images (no.)	73,395	35,063
Map resolution (Å)	0.143	0.143
FSC threshold	3.5 Å	3.6 Å
Map resolution range	3.5 to 5 Å	3.6 to 5 Å
Refinement		
Initial model used	None (<i>ab-initio</i>)	3.46 Å resolution model
Map sharpening <i>B</i> factor (Å ²) Model composition	-189.91	-165.00
Non-hydrogen atoms	37668	38340
Protein residues	4680	4764
<i>B</i> factors (Å ²)		
Protein	28.20	81.18
R.m.s. deviations		
Bond lengths (Å)	0.01	0.01
Bond angles (°)	1.137	0.971
Validation		
MolProbity score	2.34	1.76
Clashscore	9.44	4.62
Poor rotamers (%)	2.87	0.82
Ramachandran plot		
Favored (%)	91.71	91.00
Outliers (%)	0.26	0.51

Supplementary Table 1. Cryo-EM data collection, refinement and validation statistics.