Supporting Information for “A novel stabilization mechanism accommodating genome length variation in evolutionarily related viral capsids”

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Figure S1. Genomes of Patience and Adjutor. A) shows the full genome of Adjutor and Patience. Orange/yellow lines between the two genomes shows genes that have nucleotide sequence identity. The length of the genomes is shown to the right. B) shows the zoomed in area of the structural genes. Certain genes are annotated. The number above each gene is the pham number. The number in brackets is the number of other phages with the same pham protein. For example, the major capsid protein is Pham 8871 with 98 other bacteriophages containing the same Pham protein. Genome cartoon was created with Phamerator[1].

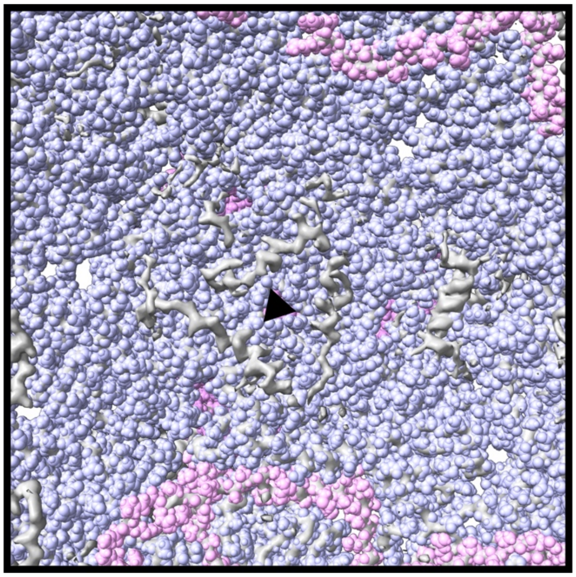


Figure S2. Unmodelled density in Patience. The areas in grey are unmodelled density observed on the underside of the capsid near the local three-fold (black triangle) axis. Blue shows major capsid protein while pink shows gp4. The helix can be observed on the right hand side.

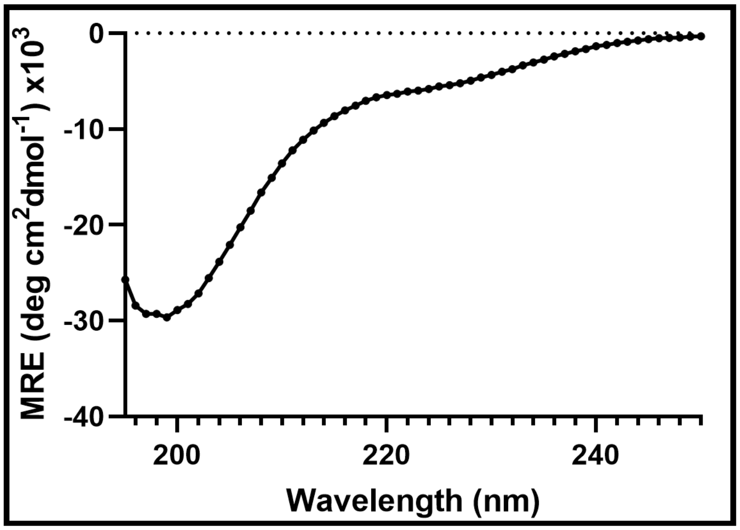


Figure S3. Free Patience gp4 exists as a random coil. A CD spectra of free Patience gp4 at 1 mg/mL.

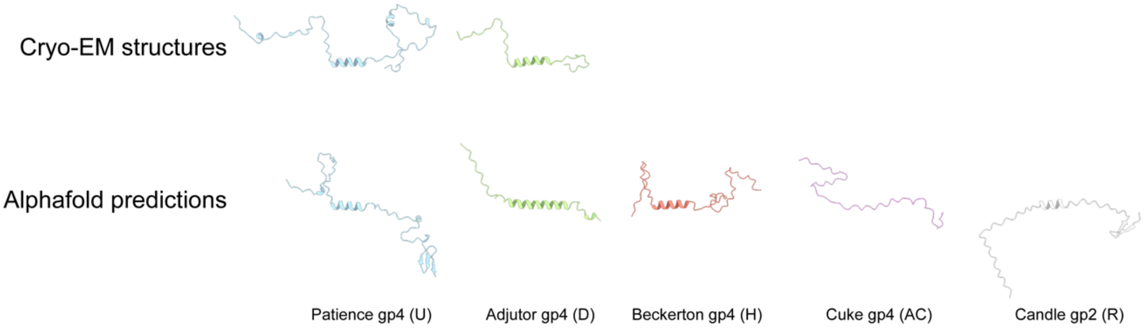


Figure S4. Alphafold predicted structures of putative gp4 homologs. Patience and Adjutor cryo-EM models and their predictions are shown for comparison.

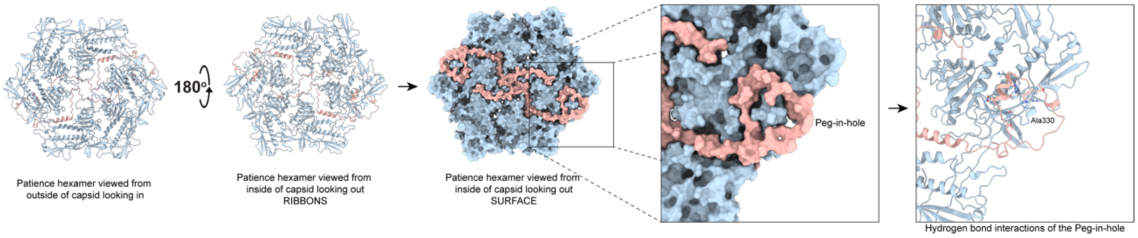
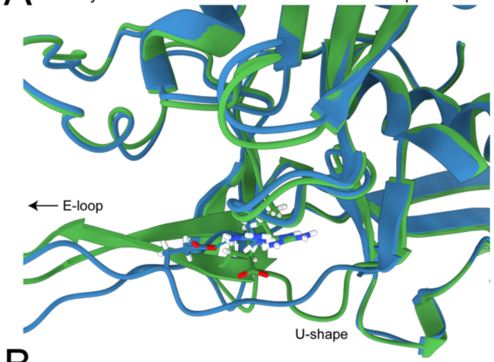


Figure S5. Peg-in-hole interaction of Patience gp4. Shows the ribbon diagram of the Patience hexamer capsomer from the outside looking into the capsid. This is in flipped 180o so that the underside of the ribbon diagram of the hexamer is seen. The surface is then shown and zoomed in to highlight the peg-in-hole interaction. Finally, the ribbon diagram of the peg-in-hole is shown with Ala330 labelled as the center of the major capsid protein peg. The hydrogen bonds and salt bridges are revealed.

A Overlay of chains A and B of Patience hexamer capsomer



B Chain B of Patience hexamer capsomer

C Chain A of Patience hexamer capsomer

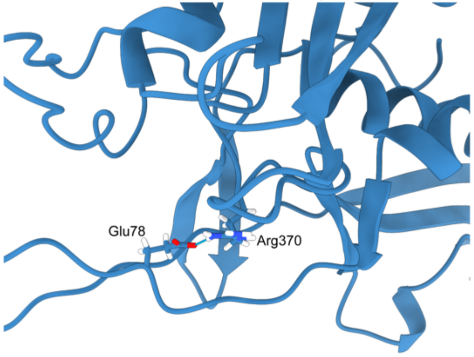


Figure S6. Straightening of the E-loop of Chain A to bridge the 2-fold chasm. Shows the overlay of chains A and B of the hexamer capsomer (A) as well as the two chains separately with Arg370 and Glu78 highlighted (B and C).

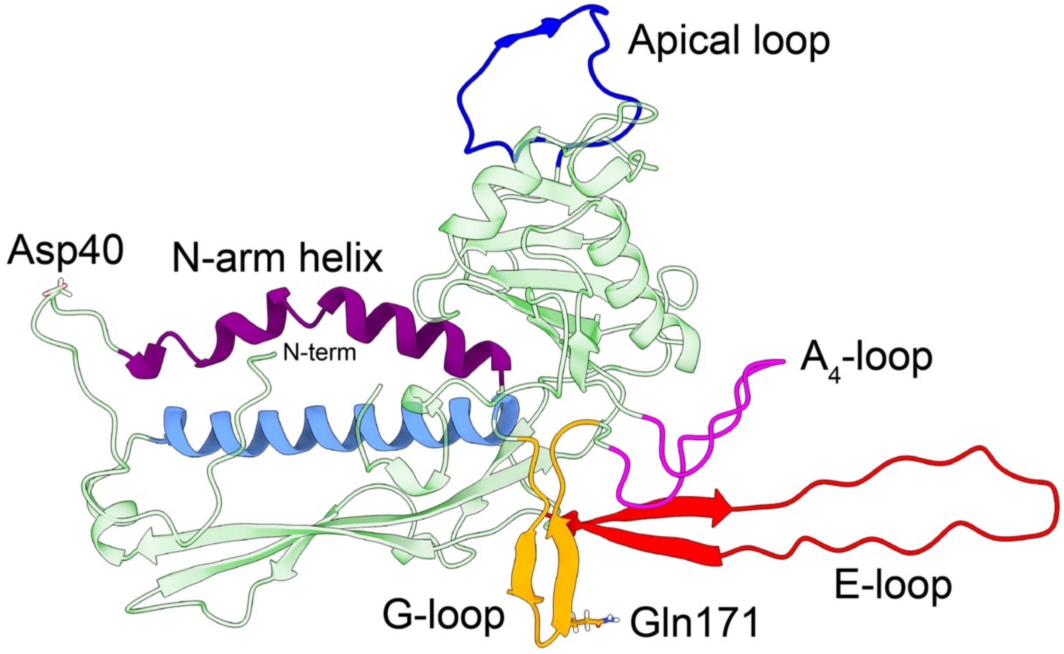
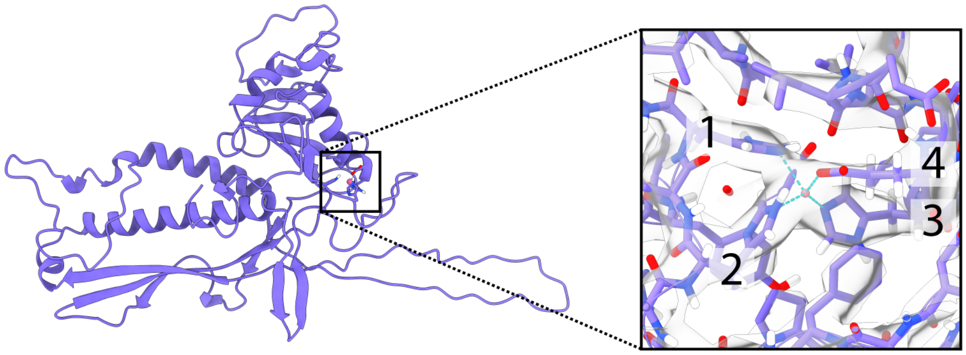
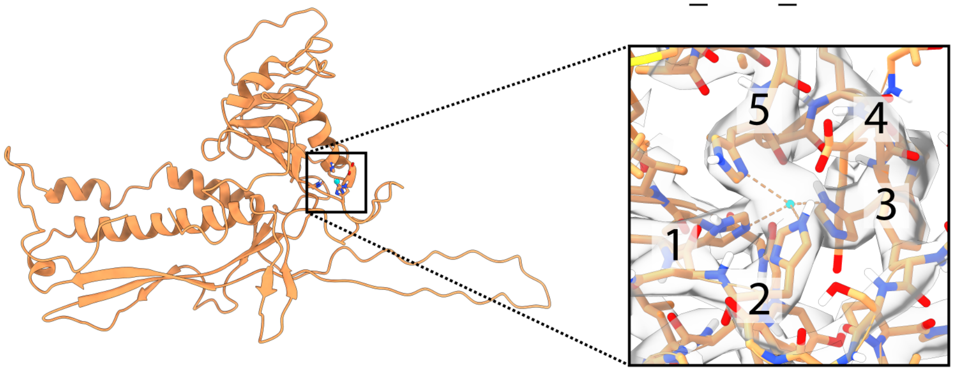


Figure S7. The hexamer major capsid protein.



U_8871_Patience



D_8871_Adjutor

Figure S8. Metal co-ordination in Patience and Adjutor. The cryo-EM derived models are shown on the left with the putative metal binding domain highlighted with a black box. On the right the putative metal binding domain is shown closer up with the cryo-EM map overlaid (grey color). Predicted contacts are represented by dashed lines and residues potentially involved in the metal coordination are numbered.

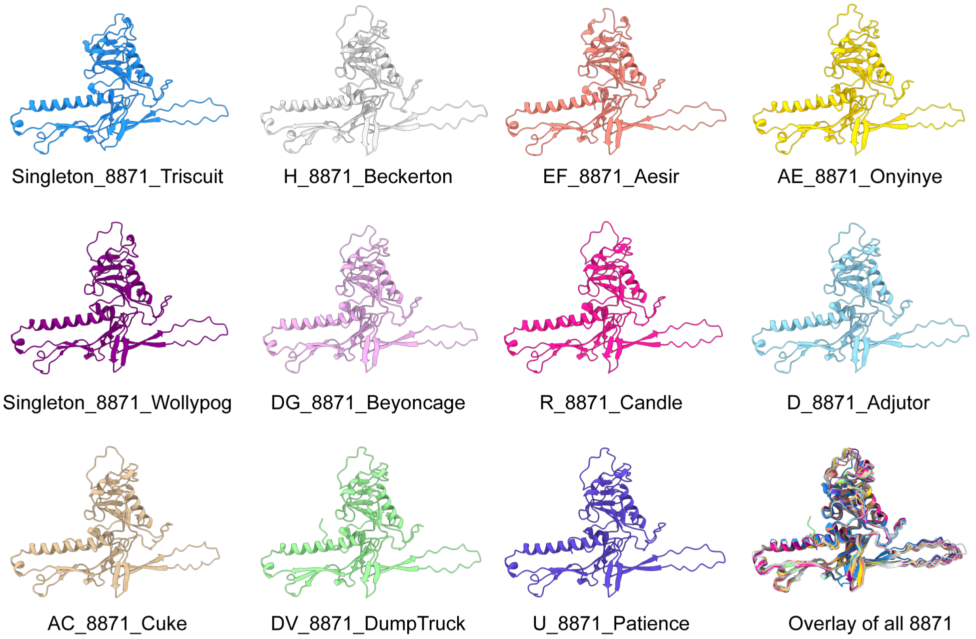
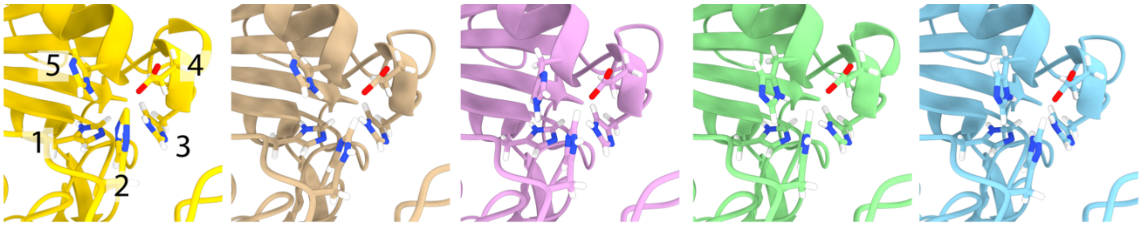
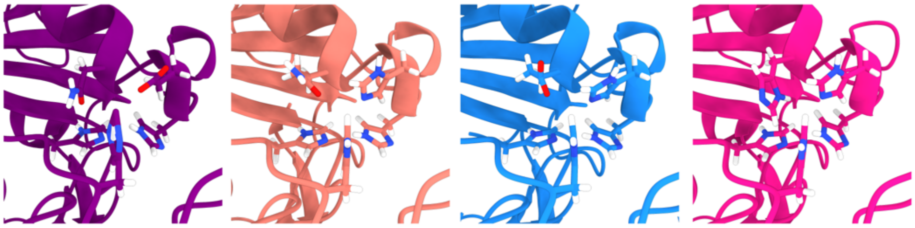


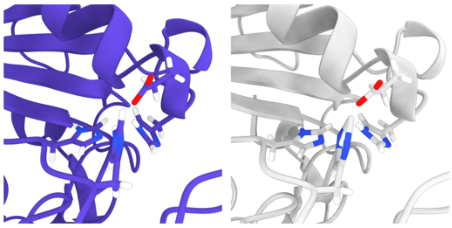
Figure S9. Predicted structures of all the major capsid proteins found in the Patience-like major capsid protein family. All structures were predicted with Alphafold. The N-terminus was truncated to where it crosses behind the spine helix due to the poor prediction.



AE_8871_Onyinye AC_8871_Cuke DG_8871_Beyoncage DV_8871_DumpTruck D_8871_Adjutor



Singleton_8871_Wollypog EF_8871_Aesir Singleton_8871_Triscuit R_8871_Candle



U_8871_Patience H_8871_Beckerton

Figure S10. Metal co-ordination in the Patience-like major capsid protein. All structures shown were predicted with Alphafold. No metal ion has been modeleld in. Every major capsid protein of the Patience-like major capsid protein family shows similar metal co-ordination. In Onyinye, the five amino acids involved in the co-ordination are labelled. The first row all show similar co-ordination and have the same amino acids. The second row highlights the major capsid proteins that have different amino acids in positions 4 and 5. The final row shows Patience and Beckerton that lack the position 5 amino acid.

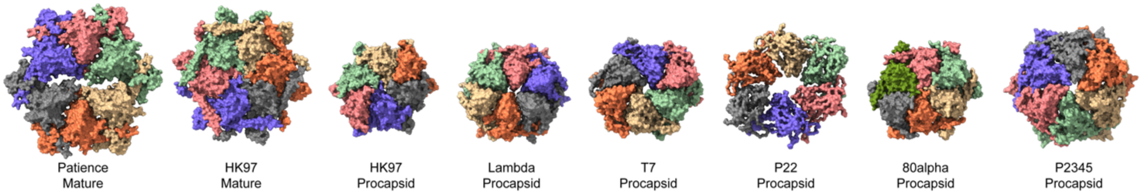


Figure S11. HK97-fold procapsid hexamer structures. Six procapsid hexamer structures with the mature capsid hexamer of Patience and HK97 bacteriophage for reference. Original PDB files are as follows. HK97 mature capsid (1OHG). HK97 procapsid (3E8K). Lambda procapsid (7VI9). P22 procapsid (2XYY). P2345 procapsid (6IBC). T7 procapsid (3J7V).

Table S1. Cryo-EM collection parameters, analysis, and final resolutions.

Data collection	Adjutor	Patience
Microscope	Titan Krios	Titan Krios
Acceleration voltage (keV)	300	300
Spherical aberration (mm)	2.7	2.7
Pixel size / Å	0.40075	0.3915
Nominal defocus / µm	0.8 to 2.1	1 to 3
Detector (mode)	Gatan K3 (super resolution)	Falcon III (counting mode)
Total exposure dose / eÅ-2	30	30
Number of frames	30	30
Number of micrographs	6664	8640
Number of particles in final refinement	44239	61957
Extract box size (fourier crop box size)	2560/800	2400/800
Final pixel size used in reconstruction	1.28	1.19745
Ewald sphere correction mask diameter	760	788
Symmetry	I (I1)	I (I1)
Resolution (FSC 0.143)	2.66	2.39

Table S2. Members of the Patience-like major capsid protein family of Patience and Adjutor. Gp4 homologs are all putative and not confirmed by cryo-EM (apart from Patience and Adjutor that have been identified in this paper).

Cluster	Number of members	Gp4 homolog pham	Average genome length (bp)	Host	Life Cycle
AC	4	18383	70,029	Mycobacterium	Lytic
AE	2	Unknown	71,497	Mycobacterium	Unknown
D	21	6785	64,805	Mycobacterium	Lytic
DG	9	Unknown	66,155	Gordonia	Lytic
DV	17	Unknown	67,413	Gordonia	Unknown
EF	22	Unknown	56,496	Microbacterium	Lytic
H	10	16432	69,108	Mycobacterium	Lytic
R	8	36408	71,339	Mycobacterium	Lytic
U	3	16432	66,864	Mycobacterium	Lytic
Singleton_Triscuit	1	Unknown	67,539	Microbacterium	Unknown
Singleton_Wollypog	1	Unknown	63,364	Arthrobacter	Unknown

Table S3. Interactions between Adjutor gp4 and hexamer major capsid proteins. Interactions predicted with the PDBsum server.

Chain	Number of interface residues	Interface area (A^2)	Number of salt bridges	Number of hydrogen bonds	Number of non-bonded contacts
Gp4:A	27:33	1765:1598	4	17	183
Gp4:B	2:2	97:95	0	1	3
Gp4:C	-	-	-	-	-
Gp4:D	-	-	-	-	-
Gp4:E	4:5	257:233	0	1	9
Gp4:F	26:25	1598:1534	5	20	156

Table S4. Interactions between Patience gp4 and hexamer major capsid proteins. Interactions predicted with the PDBsum server.

Chain	Number of interface residues	Interface area (A^2)	Number of salt bridges	Number of hydrogen bonds	Number of non-bonded contacts
Gp4:A	47:55	2993:2755	1	27	267
Gp4:B	3:3	160:164	0	2	18
Gp4:C	1:1	66:59	0	1	6
Gp4:D	5:9	308:283	3	16	149
Gp4:E	14:22	818:704	0	5	72
Gp4:F	27:28	1481:1484	5	17	142

Table S5. Metal Ion-Binding site prediction scores of the putative metal binding sites in the Patience-like family of major capsid proteins. The Alphafold prediction pdb files were used with the online server.

Metal Ion	**Patience (score/residues involved)**	**Adjutor (score/residues involved)**	**Candle (score/residues involved)**
Ca^{2+}	0/None	0/None	0/None
Cu^{2+}	0.933/185P, 198H, 200H, 224H	0.963/202H,224H,228H	1.035/207H,229H,233H
Fe^{3+}	0.721/198H/200H/223E	0.896/202H,204H,224H	0.943/207H,229H,232H, 233H
Mg^{2+}	0/None	1.054/202H,204H	1.150/207H,209H
Mn^{2+}	0/None	0/None	0/None
Zn^{2+}	1.077/198H,200H,226Y	1.259/202H,204H	1.214/207H,209H,229H
Cd^{2+}	0/None	0/None	0/None
Fe^{2+}	0.873/198H,200H,224H,372R	1.156/202H,204H	0.791/208D,209H,232H, 233H
Ni^{2+}	1.059/198H,200H,223E	0.966/202H,204H, 227E	1.048/209H,229H
Hg^{2+}	0.580/200H/377I	0.739/198F,202H	0.550/209H,387V
Co^{2+}	1.276/198H,200H,223E	1.098/224H,228H	1.177/229H,233H
Cu^{2+}	0.750/200H/220L/224H	0.915/202H,204H,228H	1.35/232H,233H

Table S6. Geometrical analysis of the HK97-fold capsid range. The columns include the triangulation number (T), the hexagonal coordinates separating nearby pentamers (h,k), the class P, the multiplicity f, the generating hexagonal coordinates of the class, (h0,k0), the presence of a hexamer centered on the 3-fold axis (3-fold H), the triangulation number divided by three (T/3), and the hexagonal coordinates of the 3-fold hexamer (h_H, k_H). The capsids containing a hexamer centered on the 3-fold axis are shaded in grey.

T	h	k	P	f	h_0	k_0	3-fold H	T/3	h_H	k_H
1	1	0	1	1	1	0	no	1/3		
3	1	1	3	1	1	1	yes	1	1	0
4	2	0	1	2	1	0	no	4/3		
7	2	1	7	1	2	1	no	7/3		
9	3	0	1	3	1	0	yes	3	1	1
12	2	2	3	2	1	1	yes	4	2	0
13	3	1	13	1	3	1	no	13/3		
16	4	0	1	4	1	0	no	16/3		
19	3	2	19	1	3	2	no	19/3		
21	4	1	21	1	4	1	yes	7	1	2
25	5	0	1	5	1	0	no	25/3		
27	3	3	3	3	1	1	yes	9	3	0
28	4	2	7	2	2	1	no	28/3		
31	5	1	31	1	5	1	no	31/3		
36	6	0	1	6	1	0	yes	12	2	2
37	4	3	37	1	4	3	no	37/3		
39	5	2	39	1	5	2	yes	13	1	3
43	6	1	43	1	6	1	no	43/3		
48	4	4	3	4	1	1	yes	16	4	0
49	5	3	49	1	5	3	no	49/3		
49	7	0	1	7	1	0	no	49/3		
52	6	2	13	2	3	1	no	52/3		