Table S1. Cryo-EM collection parameters, analysis, and final resolutions. Related to the STARS Method section.

Data collection	Muddy	Bridgette	Oxtober96	Ziko	Bobi	Adephagia	Che8	Cozz	Ogopogo	Cain
Microscope	Titan Krios 3Gi									
High Tension / kV	300									
Pixel size / Å	1.076	0.6615	0.39915	0.4008	0.83	0.4008	0.83	0.413	0.83	0.4008
Spherical aberration / mm	2.7									
Nominal defocus / µm	1 to 2.5	1 to 3	1 to 3	1 to 3	1 to 3	1 to 2.5	1 to 3	1 to 3	1 to 3	1 to 2.5
Detector (mode)	Falcon III (Counting mode)	Gatan K3 (super resolution)	Gatan K3 (super resolution)	Gatan K3 (super resolution)	Falcon III (Counting mode)	Gatan K3 (super resolution)	Falcon III (Counting mode)	Gatan K3 (super resolution)	Falcon III (Counting mode)	Gatan K3 (super resolution)
Total exposure dose / eÅ-2	50	23	30	26	30	24	30	24	30	25
Number of frames	48	40	32	30	32	50	32	30	32	30
Number of micrographs	1027	4303	10437	5058	1915	5688	1201	2799	2220	4368
Number of particles in final refinement	25244	13926	13198	35950	18969	51783	14972	43611	18736	31878
Extract box size (fourier crop box size)	800 (800)	1600 (800)	2048 (800)	2400 (750)	1024 (686)	2400 (800)	1024 (800)	2400 (800)	1024 (800)	2400 (800)
Final pixel size used in reconstruction	1.076	1.323	1.02182	1.28256	1.24	1.2024	1.0624	1.239	1.0624	1.2024
Ewald sphere correction mask diameter	710	700	650	760	750	760	750	670	750	760
Symmetry	I (I4)	I (I1)	I (I1)	I (I4)	I (I1)	I (I1)	I (I1)	I (I1)	I (I1)	I (I2)
Resolution (FSC 0.143)	2.7	4	2.2	2.6	2.5	2.4	2.5	2.6	2.7	2.9

Table S2. Host and bacteriophage information for each bacteriophage used in this study. Related to the STARS Method section.

Phage name	MCP pham	Cluster/Sub-cluster	Host	Genome length	Growth temp. (°C)
Oxtober96	15199	EA/EA1	*Microbacterium foliorum NRRL B-24224*	41798	32
Bridgette	15199	FA	*Arthrobacter globiformis B-2979*	43113	32
Muddy	15199	AB	*Mycobacterium smegmatis mc²155*	48228	37
Bobi	15199	F/F1	*Mycobacterium smegmatis mc²155*	59179	37
Adephagia	15199	K/K1	*Mycobacterium smegmatis mc²155*	59646	37
Cain	15199	K/K6	*Mycobacterium smegmatis mc²155*	60813	22
Ziko	15199	DP	*Gordonia terrae 3612*	68860	32
Ogopogo	57445	F/F1	*Mycobacterium smegmatis mc²155*	56867	37
Cozz	57445	CT	*Gordonia terrae 3612*	46600	32
Che8	4631	F/F1	*Mycobacterium smegmatis mc²155*	59471	37

Table S3. RMSD values of the Bobi-like (pham 15199) cryo-EM derived major capsid protein models when compared to Bobi. Values are calculated using the Matchmaker command in ChimeraX with default settings. Related to Figure 5.

Bacteriophage	*RMSD (Å) compared to Bobi*
Muddy	1.0
Bridgette	1.2
Oxtober96	1.1
Ziko	1.1
Adephagia	0.6

Table S4. Hydrogen bonds between the A_1 and A_2 loops. Hydrogen bonds and salt bridges specific to the A_1 and A_2 loops were determined in ChimeraX[57]. Related to Figure 6.

Bacteriophage name	*Intermolecular bonds*	*Intramolecular bonds*
Adephagia	3	32
Bobi	5	32
Ziko	6	31
Muddy	8	24
Oxtober96	8	25
Bridgette	4	43

Table S5. Hydrogen bonds in the local 3-fold axis. Hydrogen bonds and salt bridges specific to the P-loop in a single local 3-fold axis were assessed in ChimeraX[57]. The number of bonds between all the major capsid proteins that are involved in the local 3-fold was determined using PDBsum[87]. Related to Figure 6.

Bacteriophage name	*P-loop hydrogen bonds*	*P-loop salt bridges*	*3-fold hydrogen bonds*	*3-fold salt bridges*
Adephagia	41	9	207	73
Bobi	18	3	201	90
Ziko	18	3	236	89
Muddy	24	0	273	65
Oxtober96	16	0	246	108
Bridgette	19	3	198	79

Table S6. Refinement and validation statistics of the structures deposited in the PDB. Calculated with Phenix v1.19. Related to the STARS Method section.

	Adephagia	Bobi	Bridgette	Cain	Che8	Cozz	Muddy	Ogopogo	Oxtober96	Ziko
Protein residues	2763	2709	2453	2754	2448	2205	2208	2772	2128	2898
CC (volume)	0.85	0.84	0.82	0.85	0.84	0.82	0.77	0.83	0.84	0.84
R.M.S. Deviations										
Bond lengths (Angstroms)	0.01	0.015	0.011	0.01	0.01	0.011	0.011	0.011	0.014	0.011
Bond angles (°)	1.914	1.971	1.910	1.888	1.888	1.920	1.964	1.911	1.946	2.008
Molprobity score	0.82	1.03	0.94	0.77	0.75	0.78	0.71	0.78	0.65	0.89
Clash score	0.1	0.23	0.25	0.17	0.22	0.49	0.21	0.34	0.31	0.35
Rotamer outliers (%)	0.19	1.58	0.84	0.43	0	0.93	1.07	0.5	0	1.03
Ramachandran										
Favored regions (%)	95.96	95.76	94.99	96.78	97.04	97.44	97.45	97.13	97.87	96.25
Allowed regions (%)	3.97	4.20	5.01	3.22	2.96	2.46	2.55	2.87	2.13	3.75
Disallowed regions (%)	0.07	0.04	0	0	0	0.09	0	0	0	0

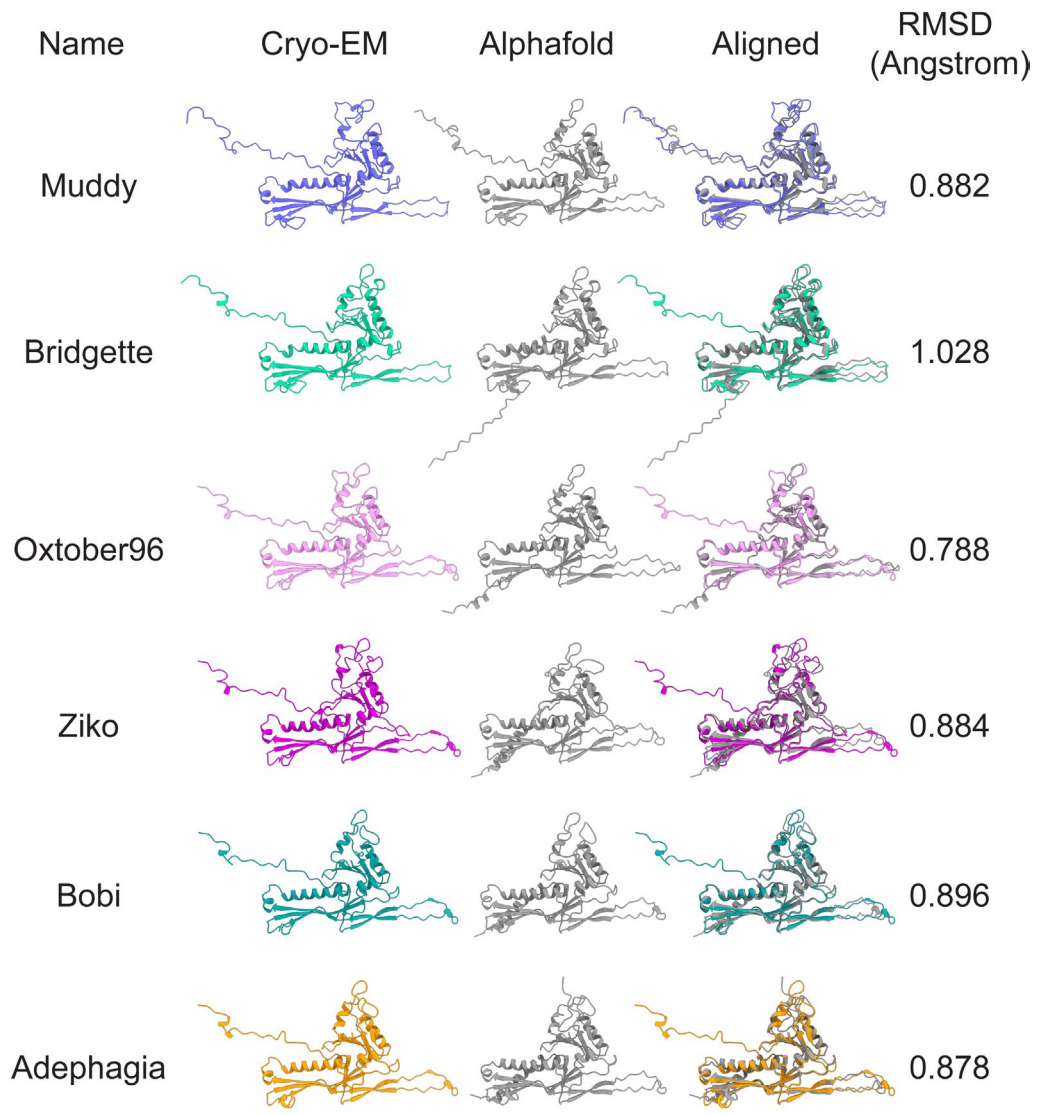


Figure S1. Comparison of cryo-EM derived models and Alphafold predictions of the six major capsid proteins of the Bobi-like phages (pham 15199). Related to Figure 4.

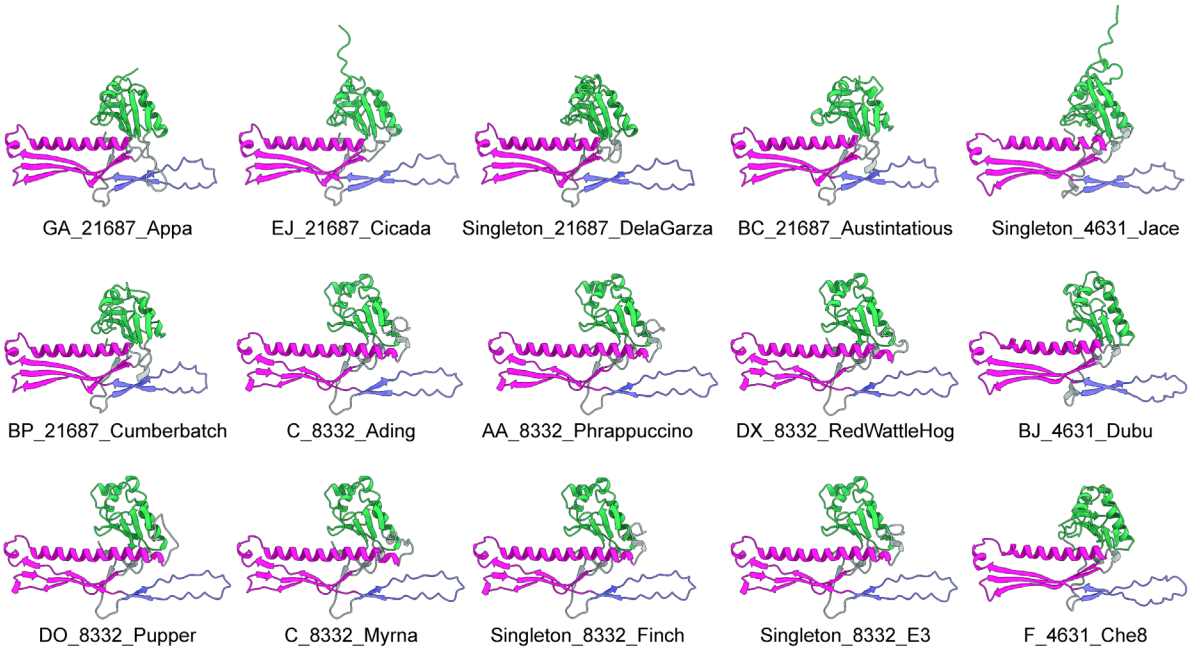


Figure S2. Alphafold predictions of the major capsid proteins from structural Group 1. All major capsid proteins had their N-terminal removed as described in the text. The full-length and truncated PDB files of the predicted structure can be found in Supporting Files. Related to Figure 3.

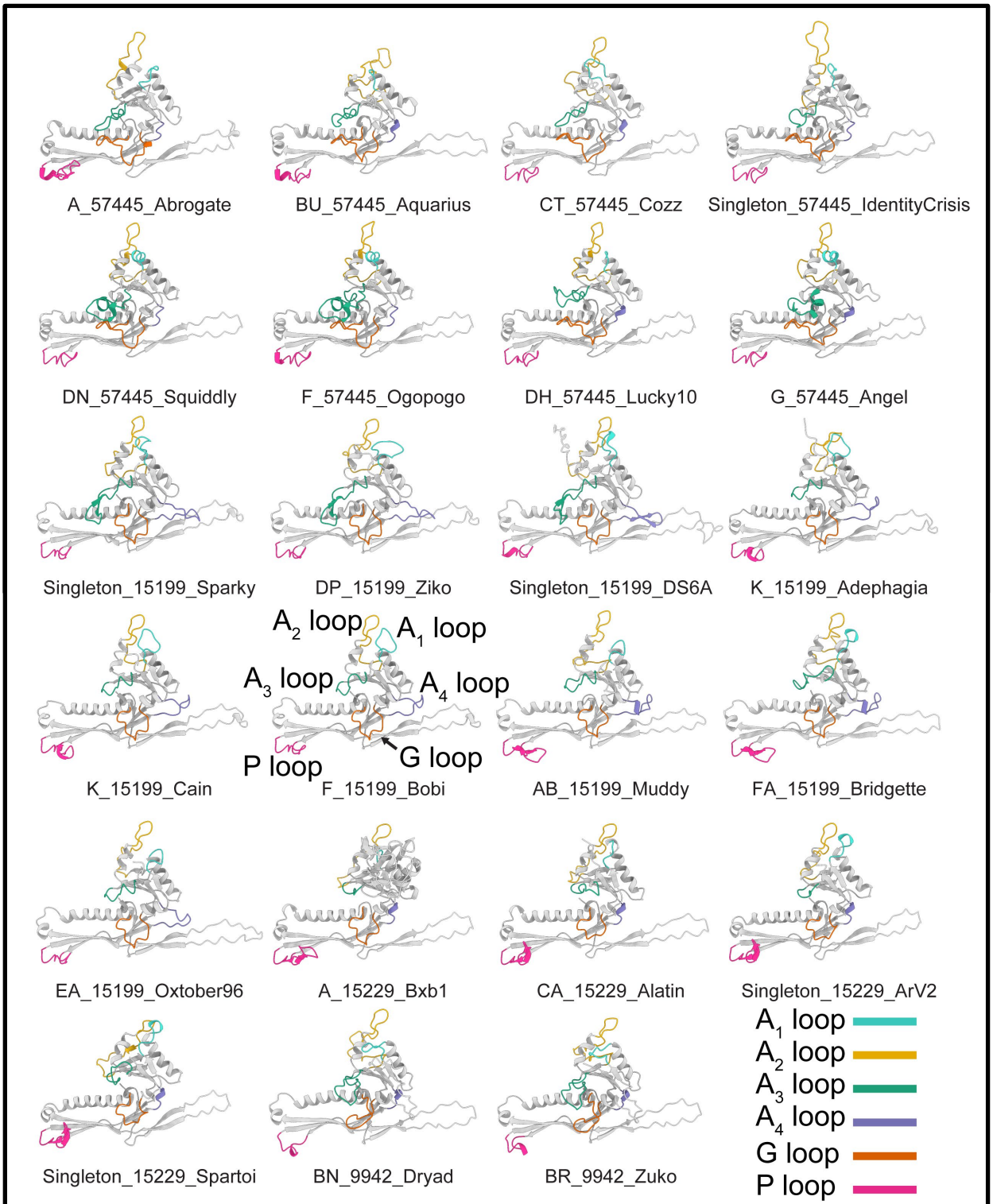


Figure S3. Alphafold predictions of the major capsid proteins from structural Group 2. All major capsid proteins had their N-terminal removed as described in the text. The full-length and truncated PDB files of the predicted structure can be found in Supporting Files. Related to Figure 3.

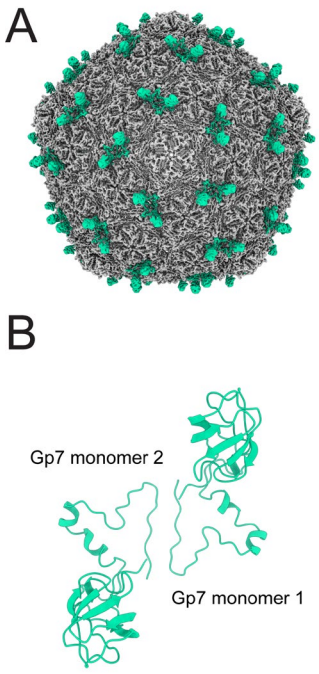


Figure S4. The decoration protein of Bridgette (Gp7). A shows the entire Bridgette capsid with the decoration protein dimers (Gp7) colored. B shows the model of the Gp7 (amino acids 2-125) dimer in the context of the capsid. Related to Figure 4.

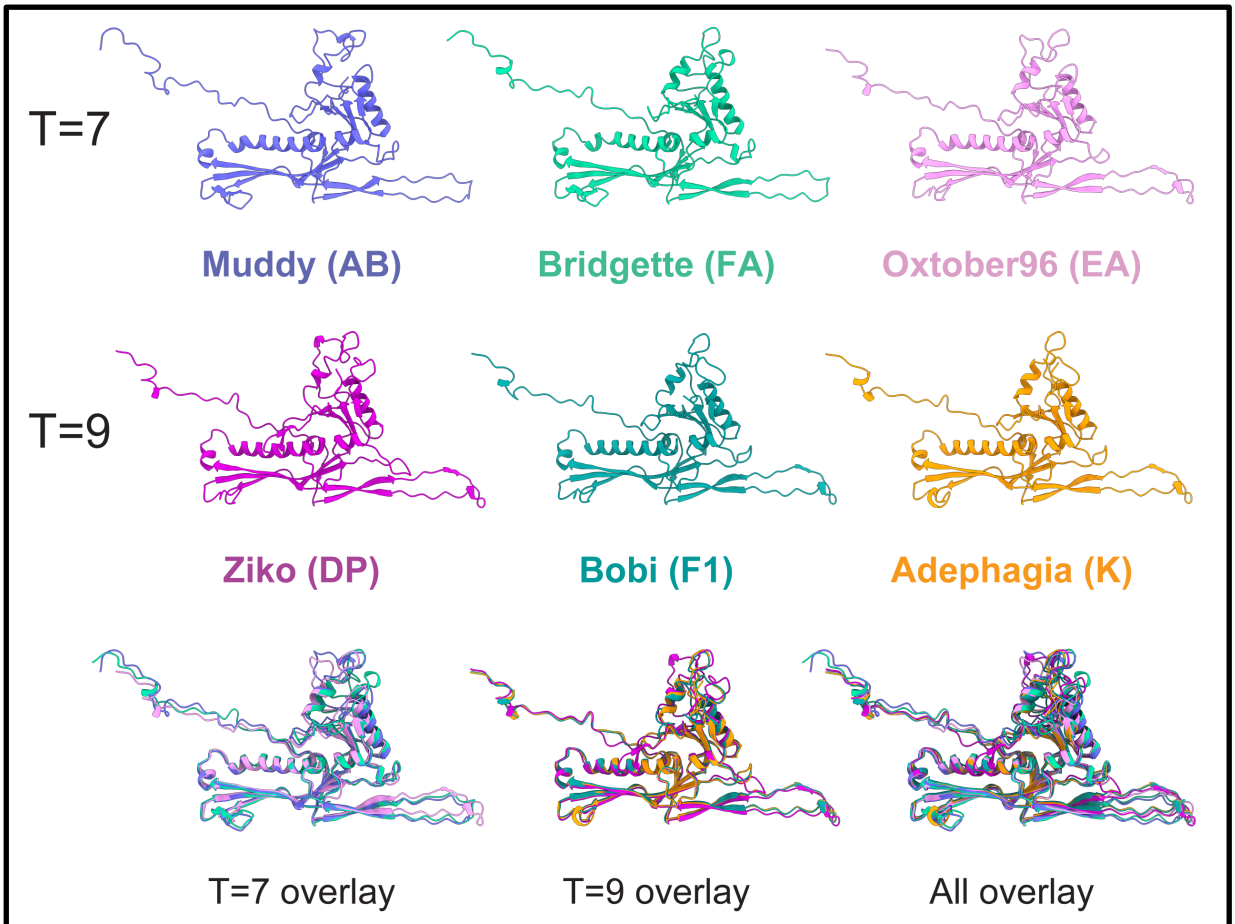


Figure S5. Models of the representative bacteriophages from the Bobi-like (15199) phamily of major capsid proteins. Model of each major capsid protein derived from the cryo-EM maps. Each model is of the hexamer subunit adjacent to the pentamer subunit, so models are directly comparable. Color coding is by phage cluster and is consistent with previous figures. The T=7 forming capsids (Muddy, Bridgette, and Oxtober96) are overlaid, as are the T=9 forming capsids (Ziko, Bobi, and Adephagia). All six models are also overlaid (All overlay). Related to Figure 4.

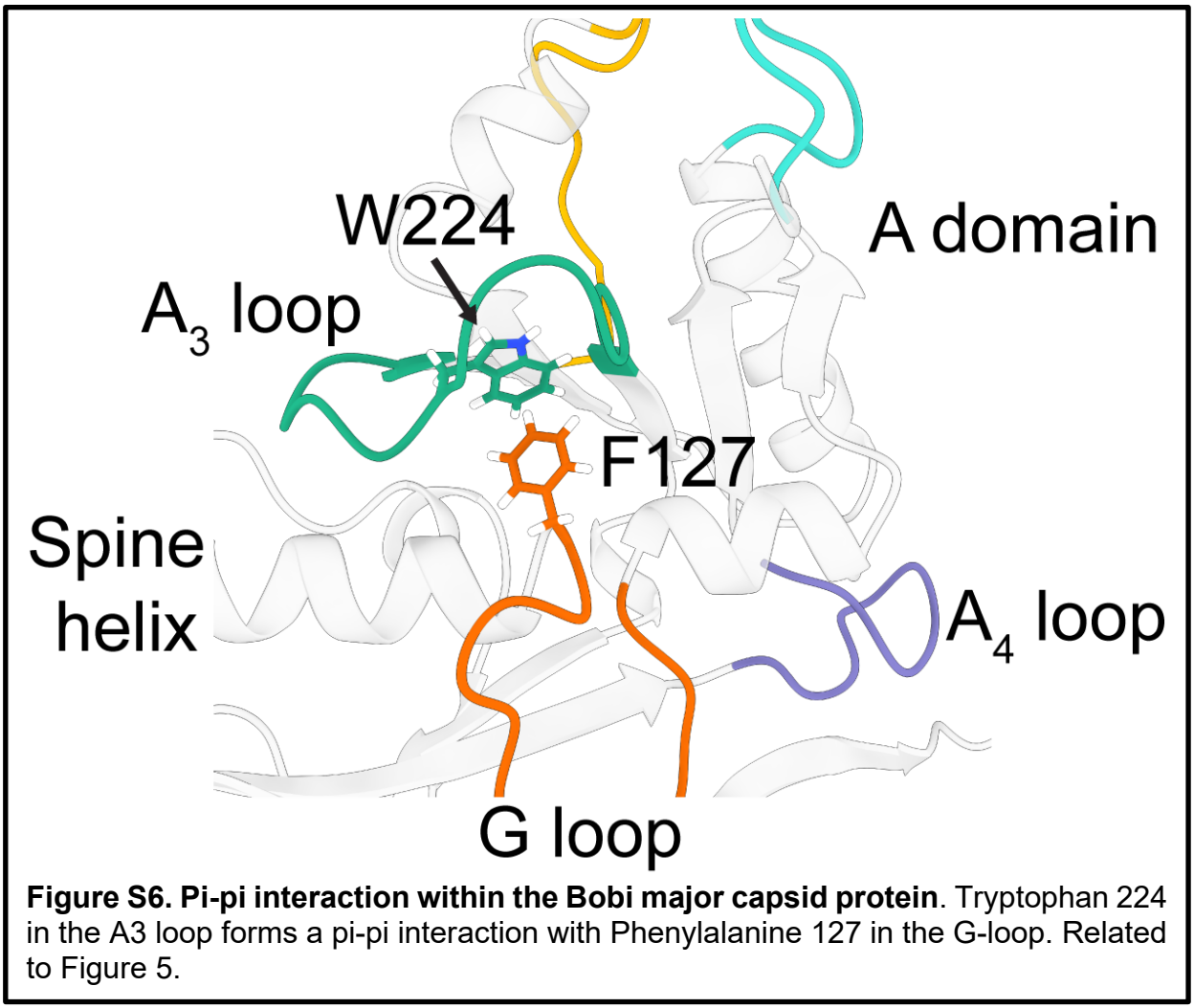


Figure S6. Pi-pi interaction within the Bobi major capsid protein. Tryptophan 224 in the A3 loop forms a pi-pi interaction with Phenylalanine 127 in the G-loop. Related to Figure 5.

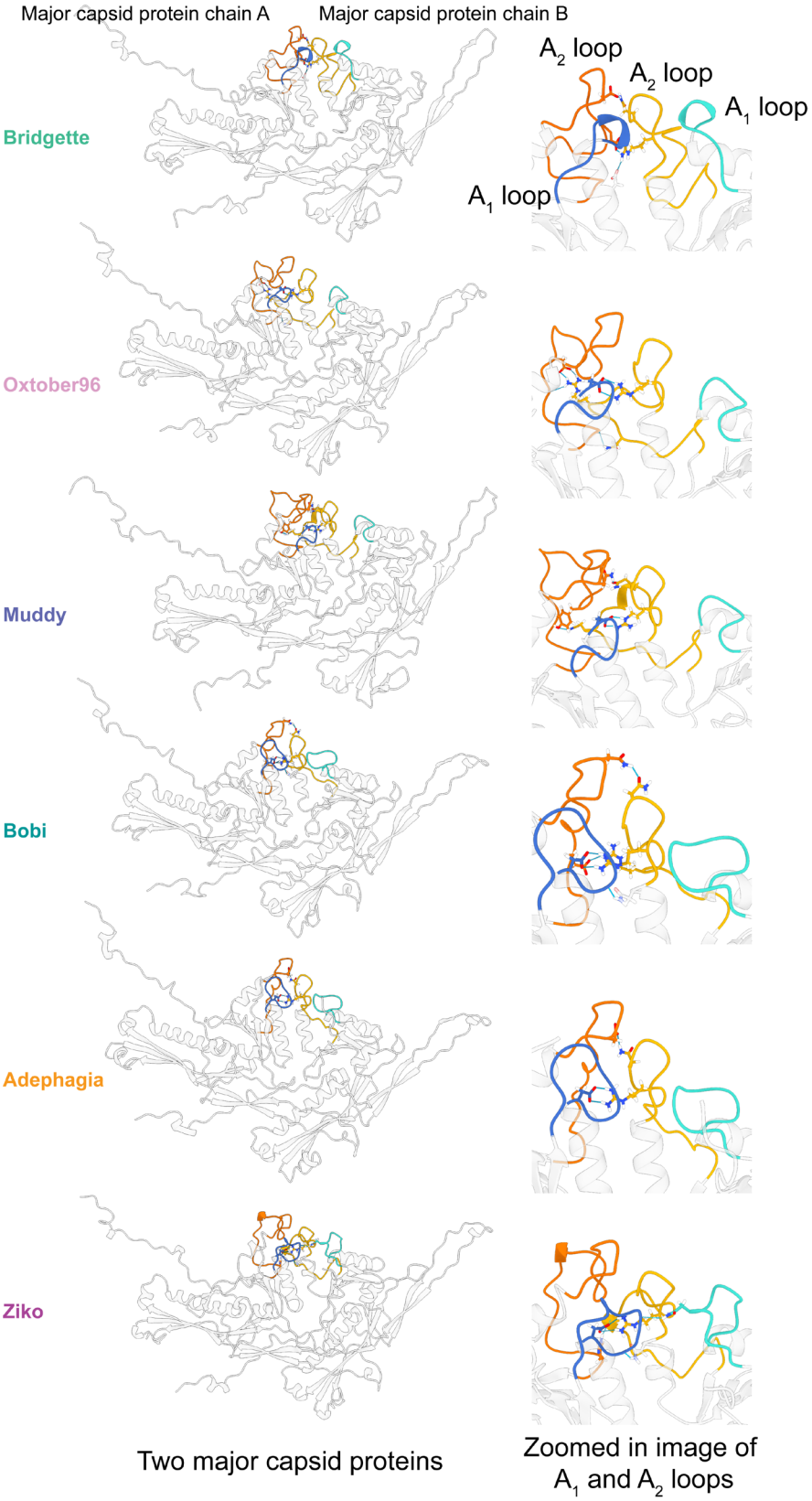
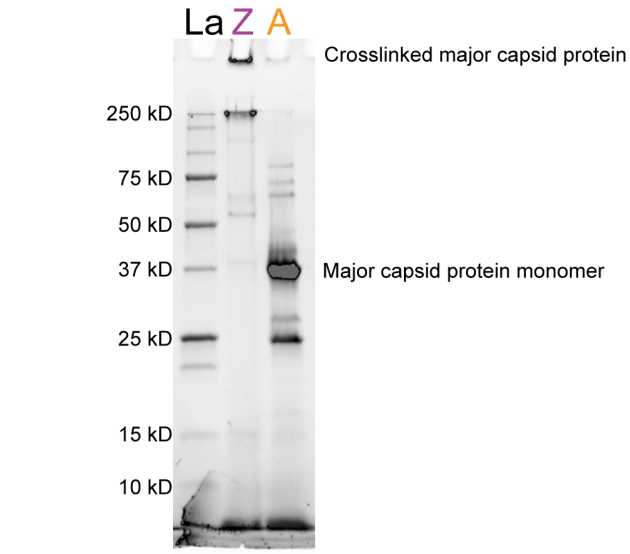


Figure S7. A_1 and A_2 loop interactions of the Bobi-like (15199) bacteriophages. Related to Figure 6.



Predicted molecular weight of Ziko major capsid protein: 34.2 kD

Predicted molecular weight of Adephagia major capsid protein: 32.7 kD

Figure S8. SDS-PAGE of Ziko (Z) and Adephagia (A) bacteriophages. Cesium chloride purified capsids of Ziko and Adephagia were diluted to 1 mg/mL and mixed with Laemmli buffer and boiled for 10 minutes at 95ºC. Boiled samples were loaded onto a 10% SDS-PAGE Bio-Rad stain-free TGX gel and run for 1 hour at 180 V. Protein bands were visualized using the stain-free TGX technology. Ladder (La) was Unstained Precision Plus Protein Standard from Bio-Rad. Related to Figure 7.

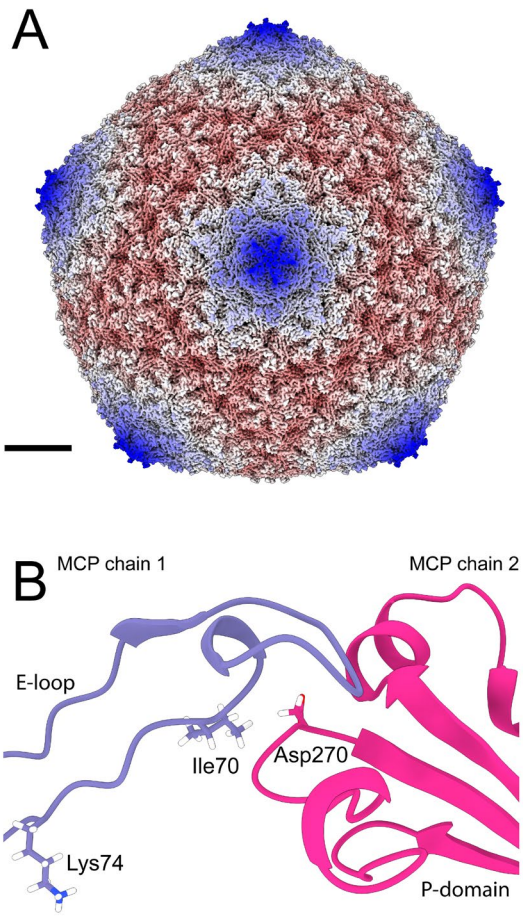


Figure S9. Cain (K cluster, 15199 MCP pham) does not have an isopeptide bond. A) Map of Cain, colored by radius using the same color scheme as in Figure 4. B) Image of two major capsid proteins of Cain around the 3-fold axis highlighting Asp270 and Ile70 that are in equivalent positions of the amino acids in Bobi that forms the isopeptide bond. Lys74 is also highlighted to show it is too distant to be part of an isopeptide bond with the P domain of MCP chain 2. Related to Figure 7.

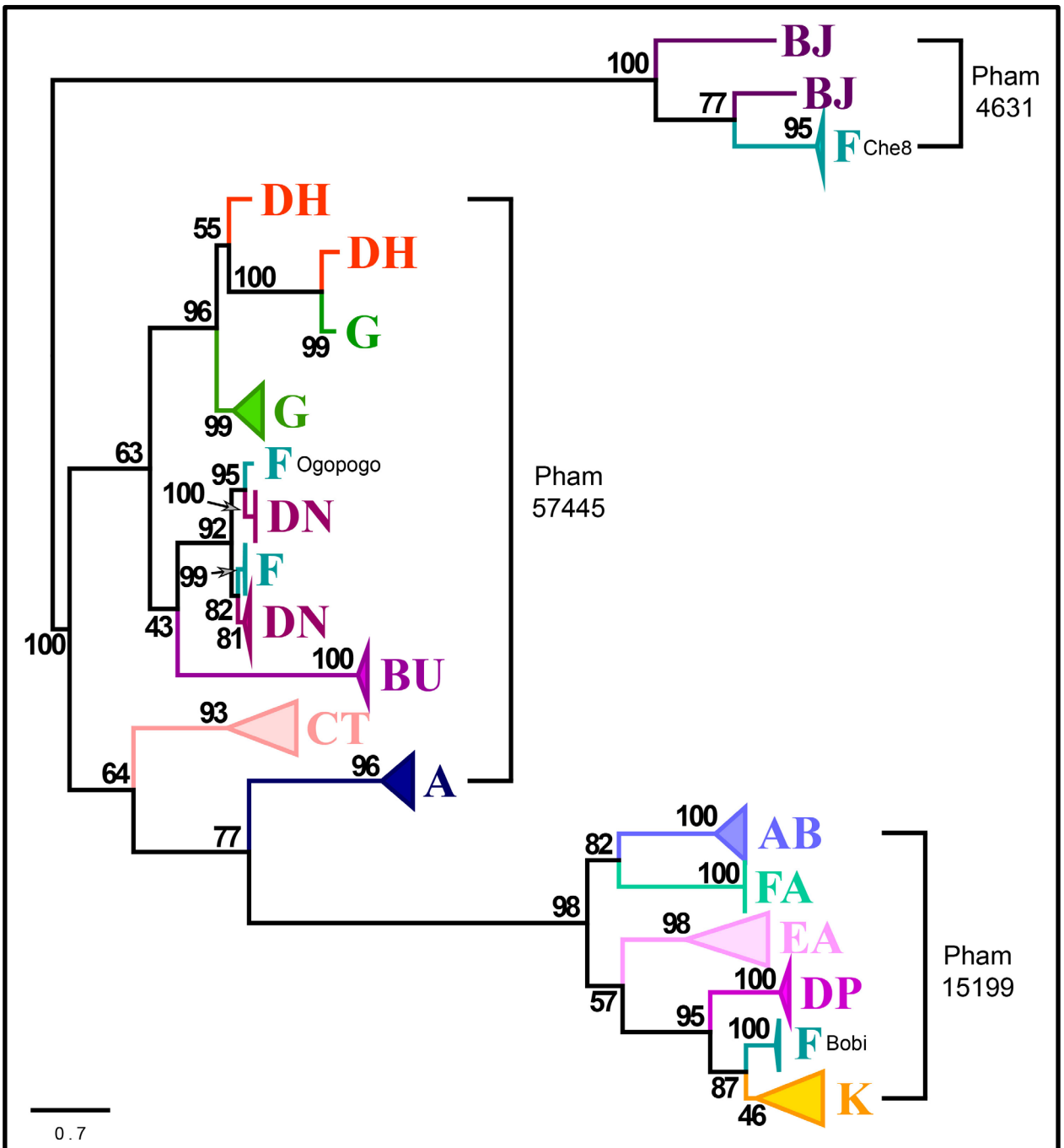


Figure S10. Unrooted phylogenetic tree of the 722 major capsid proteins of the Che8-like bacteriophages (pham 4631); the Ogopogo-like bacteriophages (pham 57445) and the Bobi-like bacteriophages (pham 15199). The tree has been collapsed for clarity. A pdf of the full un-collapsed tree can be found in the Supporting files. Related to Figure 3.

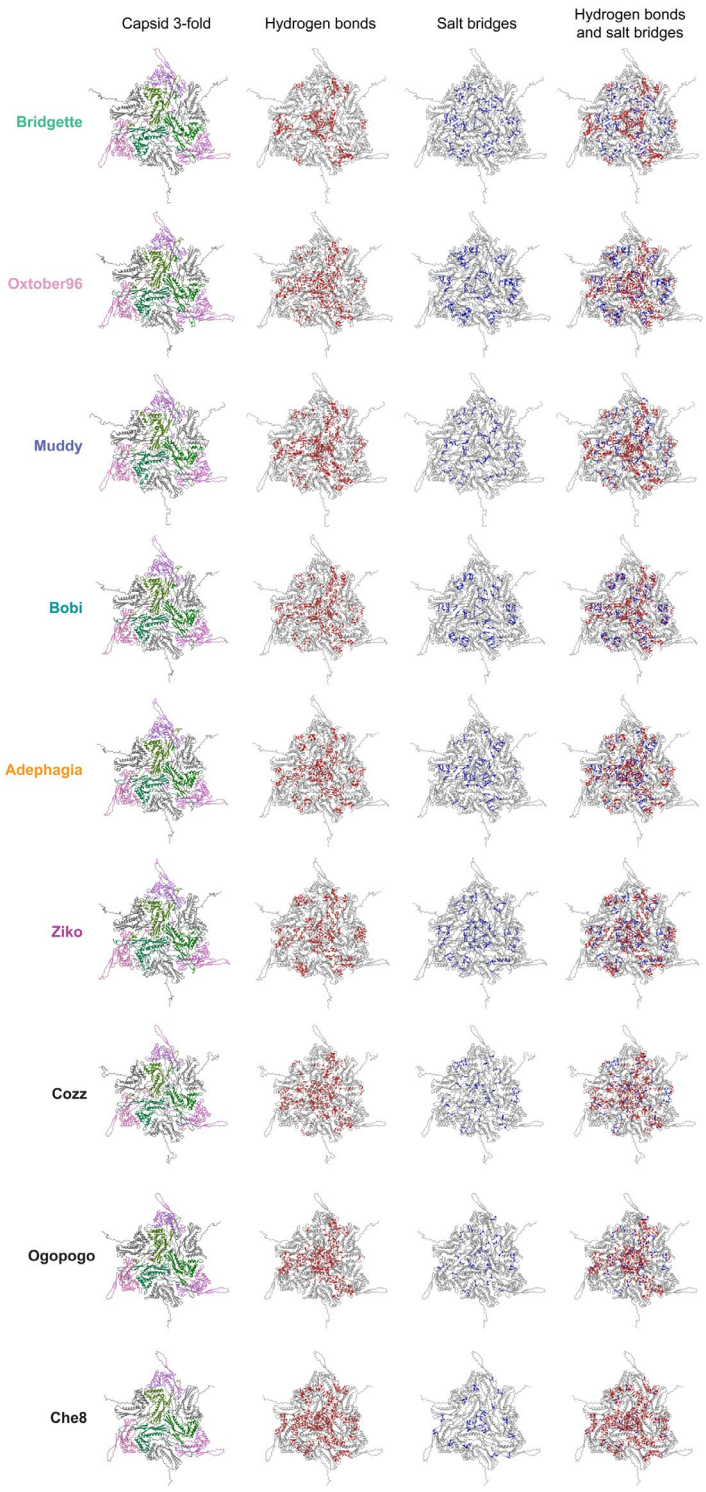


Figure S11. The three-fold axes salt bridges and hydrogen bond distribution around the 3-fold axes of the capsid. The nine major capsid proteins interacting around the three-fold axes are shown for each of the Bobi-like (15199) bacteriophage capsids. The hydrogen bond (red) and salt bridge (blue) networks are shown separately and overlaid. Related to Figure 7.