Supplementary Information

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SI Materials and Methods.

Cloning, Overexpression, and Purification of gp16 Domains. The *g16* domain sequences of phages T4 and 44RR2 were amplified by PCR using specific primers and phage DNA as templates (the phage DNAs from T4-related phages were a kind gift from Dr. James D. Karam, Tulane University School of Medicine, New Orleans, LA). The N-C domain fusion was constructed using the PCR directed splicing by overlap extension strategy (1, 2). The amplified DNA fragments were concentrated by ammonium acetate/isopropyl alcohol precipitation, digested with appropriate restriction enzymes, purified by agarose gel electrophoresis, and ligated to the linearized pET-28b plasmid DNA. In-frame insertion of these fragments into the vector resulted in the fusion of a hexa-histidine tag to the N terminus of each construct. The ligated DNAs were transformed into *Escherichia coli* XL10 Gold-competent cells (Stratagene) and miniprep plasmid DNAs were prepared by the alkaline lysis procedure (Qiagen). The presence of DNA inserts and their orientation were tested using restriction digestion and/or amplification with insert-specific primers. The accuracy of the cloned DNA was confirmed by DNA sequencing (Davis Sequencing, Inc.). For overexpression of the gp16 domains, the plasmids were transformed into *E. coli* BL21 (DE3) pLysS-competent cells (Stratagene) and induced with IPTG at 30 °C for 2.5–3.5 h. The solubility of the overexpressed proteins was tested with bacterial protein extraction reagent (Pierce). For purification of gp16 domain proteins, cells following overexpression were harvested by centrifugation at 8,200 × *g* for 15 min at 4 °C and were lysed using an Aminco French press (Thermo Fisher Scientific Inc.). The cell lysate was centrifuged at 34,000 × *g* for 20 min at 4 °C and the supernatant containing soluble proteins was purified by successive chromatography on Histrap HP (affinity for the hexa-histidine tag) and Hiload Superdex 200 prep grade (size exclusion) columns using AKTA-PRIME and AKTA-FPLC systems (GE Healthcare), respectively. For some purifications, the samples after Histrap chromatography were purified by Mono Q-5/50 GL ion exchange chromatography (GE Healthcare) followed by Superdex 200 gel filtration.

Crystallization. The full length and a 1–125 amino acid fragment of 44RR2 gp16 were concentrated to 10 mg/mL in 20 mM Tris-Cl (pH 8.0) and 100 mM NaCl. Crystals were grown by vapor diffusion in hanging drops at 20 °C. Two crystal forms were obtained, one from 25% PEG3350, 0.1 M Hepes (pH 7.5) with space group $P2_12_12_1$ and the other from 12.5% PEG3350, 0.1 M NaCl, 0.1 M Hepes (pH 7.5) with space group *R*3.

Structure Determination. Crystals were flash-frozen and data were collected at 100 K at the Advanced Photon Source GM/CACAT 23IDB beamline. Data were processed with the HKL2000 program (3). For the $P2_12_12_1$ space group crystals, Matthews coefficient calculations and self-rotation function calculations using program GLRF (4) indicated that there is an 11-fold symmetric ring in each asymmetric unit. An 11-mer model was generated from the 8-mer structure of SF6 G1P (5) and was used as a molecular replacement search model using the programs Beast (6) and Phaser (7). Top ten solutions were used in phase extension to 6-Å resolution coupled with 11-fold averaging using the program DM (8). Sausage-like densities were observed in one of the resulting maps indicating the presence of helices in the structure. A more precise mask was generated on the basis of the preliminary electron density map. Phases were then further extended to 2.8-Å resolution. The structure was built manually using the program Coot (9) and refined with the program Refmac (10) to 2.8-Å resolution. The structure for the *R*3 space group crystals was determined by molecular replacement using the $P2_12_12_1$ monomer structure as a search model with the program Phaser (7) and refined using the program Refmac (10) to 2.8-Å resolution (Table S1).

The structure for the *R*3 space group crystals was determined by molecular replacement using the $P2_12_12_1$ monomer structure as a search model with the program Phaser (7) and refined using the program Refmac to 1.8-Å resolution (10) (Table S1).

ATPase Assay. A previously developed ATPase assay protocol was used in this study (11). In brief, purified gp17 (0.5 μM), either alone or with 5 μM various gp16 domains (approximately 1∶1 ratio of gp16 oligomer to gp17 monomer), was incubated at 37 °C for 20 min in a 20-μL reaction mixture containing 0.5 mM unlabeled (cold) ATP and 75 nM [γ-^{32}P] ATP (specific activity, 3,000 Ci/mmol; GE Healthcare) in ATPase buffer (50 mM Tris·HCl, pH 7.5, 0.1 M NaCl, 5 mM $MgCl_2$). EDTA was added to a final concentration of 50 mM to stop the reaction, and the ATP hydrolysis products were separated by thin layer chromatography on polyethyleneimine-cellulose plates (Sigma-Aldrich). The plates were air-dried for autoradiography and phosphorimaging (Storm 820 PhosphorImager; GE Healthcare). The radioactive spots were quantified using ImageQuant software (GE Healthcare). Data shown are averages of four independent experiments.

Nuclease Assay. gp17 was incubated either alone or in the presence of gp16 domains with 100 ng of 48.5-kb phage λ DNA (Fermentas) in a 20-μL reaction mixture containing 10 mM Tris·HCl, pH 8, 15 mM NaCl, and 5 mM $MgCl_2$ for 15 min at 37 °C. The reactions were terminated by the addition of EDTA to a final concentration of 50 mM, and the samples were electrophoresed on a 0.8% (wt/vol) agarose gel followed by ethidium bromide staining (12).

DNA Packaging Assay. gp17 (2 μM) were incubated with T4 partial heads purified according to the procedure described previously (13) (10^{10} particles), gp16 domains, and 600 ng of 48.5-kb phage λ DNA in a 20-μL reaction mixture containing 25 mM Tris·HCl, pH 8, 3 mM $MgCl_2$, 1 mM ATP, and 65 mM NaCl for 45 min at room temperature. Unpackaged DNA was degraded by adding DNase I (Sigma-Aldrich) to a final concentration of 0.5 μg/μL and incubated for 30 min at 37 °C. Proteinase K (Fermentas) was added to a final concentration of 0.5 μg/μL in 50 mM EDTA (pH 8) and 0.2% SDS, and the samples were incubated for 30 min at 65 °C to release the packaged (DNase-protected) DNA. The reaction mixtures were loaded on a 0.8% agarose gel for electrophoresis. The ethidium bromide-stained DNA was quantified by the Gel DOC XR imaging system (BioRad) (14).

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Fig. S1. Superposition of 44RR gp16 N-terminal model predicted by Robetta (cyan) with the crystal structure of phage λ small terminase N-terminal domain (orange).

Fig. S2. Polyacrylamide gel showing isolation of the DNA tightly bound to gp16. Purified T4 gp16 (lane 2) was treated with Benzonase (200 U/mg of gp16) and incubated at room temperature overnight to degrade the loosely bound DNA (lane 3). Benzonase was removed by passing the sample through a Hiload Superdex 200 prep grade size exclusion column and the tightly bound DNase-protected DNA was released by treatment with Proteinase K at 65 °C for 30 min (lane 4). The samples were then electrophoresed on a 4–20% gradient polyacrylamide gel in 1× Tris/Borate/EDTA buffer. DNA molecular weight standards were shown in lane 1. The gel was first stained with SYBR Green I to detect DNA and then with Coomassie Blue R-250 to detect protein. The arrow indicates the position of the approximately 200-bp size tightly bound DNA fragments released from T4 gp16 following Proteinase K digestion (lane 4). Note that the top bands before Proteinase K digestion (lanes 2 and 3), but not the approximately 200-bp band (lane 4), stained for both protein and DNA.

Table S1. Data collection and refinement statistics

	11-mer	12-mer
Data collection		
X-ray source	23-ID-B	23-ID-B
Wavelength, Å	1.0332	1.0332
Resolution, Å	2.8	1.8
Space group	$P2_12_12_1$	$R3$
Unit cell, Å	$a = 83.0$, $b = 106.3$, $c = 136.7$	$a = b = 140.6$, $c = 53.6$
Unique reflections	30,487	35,876
Average redundancy	5.5	5.8
I/σ*	19.9 (4.8)	25.0 (5.0)
Completeness, %	99.9 (100)	99.6 (99.9)
R_{merge}, %[†]	7.8 (38.9)	5.5 (39.2)
Refinement		
Resolution, Å	2.8	1.8
$R_{working}$, %[‡]	23.9 (31.0)	18.7 (23.3)
R_{free}, %[§]	29.9 (34.0)	22.0 (27.7)
Average B factor, $Å^2$	58.4	32.4
rmsd bonds, Å	0.005	0.008
rmsd angles, °	0.812	1.022
Ramachandran disallowed	0	1

*Values in parentheses throughout the table correspond to the last shell.
[†]$R_{merge} = \sum |I - \langle I \rangle| / \sum I$, where I is measured intensity for reflections with indices hkl.
[‡]$R_{working} = \sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$.
[§]R_{free} has the same formula as $R_{working}$ except that calculation was made with the structure factors from the test set.

Table S2. Interactions among subunits in the structure of 44RR gp16 (α: α-helix, *t*: turn, *s*: strand)

Interaction partner I			Interaction partner II			
Monomer	Atom	Position	Monomer	Atom	Position	Type of interaction
n	LYS 43 NZ	α1	*n* + 1	ASN 40 OD1	*s*	H-bond side chain
	TYR 50 OH	α1		ASP 45 OD2	α1	H-bond side chain
	ARG 54 NH1	α1		VAL 36 O	*s*	H-bond main chain
	ARG 54 NH1	α1		SER 37 OG	*s*	H-bond side chain
	ARG 54 NE	α1		ASP 49 OD2	α1	salt bridge
	HIS 58 NE2	α1		ASP 49 OD1	α1	pH-dependent salt bridge
	SER 61 OG	α1		ASN 56 ND2	α1	H-bond side chain
	GLN 62 OE1	α1		ARG 33 N	*s*	H-bond main chain
	LYS 76 NZ	α1		ASP 66 OD2	α1	salt bridge
	ASP 79 OD1	*t*		HIS 83 NE2	α2	pH-dependent salt bridge
	MET 91 O	α2		GLN 60 NE2	α1	H-bond main chain
	THR 95 OG1	α2		GLN 60 NE2	α1	H-bond side chain
	THR 95 OG1	α2		THR 97 OG1	α2	H-bond side chain
	HIS 105 ND1	α2		ARG 42 NH1	α1	H-bond side chain
	HIS 105 NE2	α2		ASP 49 OD2	α1	pH-dependent salt bridge
	LYS 106 NZ	α2		GLU 107 OE1	α2	salt bridge
	LYS 106 NZ	α2		ASP 110 OD2	α2	salt bridge
	LYS 109 NZ	α2		ASP 110 O	α2	H-bond main chain
n	GLU 73 OE2	α1	*n* + 2	LEU 30 N	*s*	H-bond main chain
	LYS 76 NZ	α1		ALA 28 O	*s*	H-bond main chain
	ASN 77 OD1	α1		TYR 27 N	*s*	H-bond main chain
n	V53, M63, M67,V86, L90, M108, L111		*n* + 1	A66, L72, A75, V84, F87, A88, M91, M94, M101, L102,		hydrophobic interaction