Supplemental Information

EXPERIMENTAL PROCEDURES

Synchronous DNA Packaging Assay. Complex I was formed at room temperature (RT) with the addition of 162 nM terminase protomer to 8.3 nM pCT-λ in 83.3 mM Tris-HCl buffer, pH 8.0, containing 16.6 mM $MgCl_2$, 1.6 mM ATP, 82 nM IHF, 37.5 μM gpF1, 1.25 % glycerol, 12.5 mM NaCl, 88 μM β-ME, and 125 μM EDTA and the reaction was allowed to proceed for five minutes. The DNA packaging reaction was then initiated with the addition of procapsids and gpD to afford a reaction mixture (20 μL) that contained 50 mM Tris-HCl buffer, pH 8.0, 10 mM $MgCl_2$, 2 mM spermidine, 1 mM ATP, 50 nM IHF, 7.5 μM gpF1, 0.75 % glycerol, 7.5 mM NaCl, 52 uM β-ME, and 80 μM EDTA, 100 nM protomer, 5 mM pCT-λ, 5 μM gpD, and 40 nM procapsids, and the reaction was allowed to proceed for 25 minutes at RT. Aliquots were removed at the indicated times and unpackaged DNA was degraded with the addition of one μL 0.2 mg/mL DNase digest and incubation at room temperature for five minutes. Two uL of 10% (w/v) SDS and 23 μL of 1:1 phenol : chloroform was then added to quench the DNase reaction and release the packaged DNA from the nucleocapsids. The samples were thoroughly mixed, centrifuged for two minutes to separate the phases, and the DNA containing upper aqueous layer was removed (20 μL). Agarose gel analysis and quantitation of packaged DNA was performed as previously described (*17, 27*).

Table S1. The data presented in Table 4A were analyzed using a two-tailed T-test.

Statistics	Deg. Freedom	t	P (two tail)
Protomer vs. +pCT-λ	7	10.39	<0.0001
+pCT-λ vs. +pCT-λ +IHF	4	3.28	0.0306
Protomer vs. +pCT-λ + IHF	7	3.32	0.0128
Protomer vs. +ns-274mer	7	4.94	0.0016
Protomer vs.+ cos-274mer	7	0.188	0.856
Protomer vs. +cos-274mer+IHF	7	1.78	0.119
+ns-274mer vs. +cos-274mer	4	6.10	0.0037
+ns-274mer vs. +ns-274mer+IHF	4	3.14	0.0348
+cos-274mer vs. +cos-274mer+IHF	4	2.05	0.1097
+ns-274mer+IHF vs. +cos-274mer+IHF	4	2.47	0.0692

Protomer: n=6

All others: n=3

Figure S1.

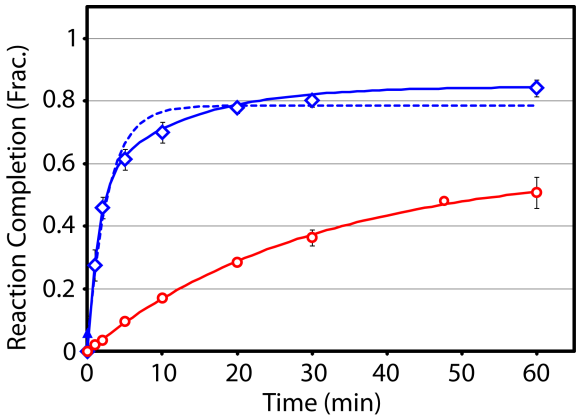

Figure S1. Kinetic Time Course for the *cos* Cleavage (blue) and Strand Separation (red) Reactions. The reaction was performed as described in Experimental Procedures using 20 nM terminase protomer. The *cos*-cleavage data were fit using both single- (dotted line) and double- (solid blue line) exponential kinetic models. The strand separation data were well described by a single- exponential kinetic model (red line).