Table S1

***In vivo* coat protein binding properties of scaffolding protein deletion mutants.**

Protein variant	Coat protein binding[2]
WT	+
1-294	+
141-303[1]	+
D281A[1]	+
A284D[1]	+
K286A or E[1]	+
G287A or P[1]	+
D288A or R[1]	+
V289A or D[1]	+
E290A or K[1]	+
T291D[1]	+
Y292D[1]	+
R293A or E[1]	+
K294A[1]	+

1. These substitutions were generated in the 141-303 scaffolding protein fragment with no histidine tag.
2. The truncated scaffolding proteins were expressed from plasmid pET3a (Novagen, Madison, WI) (Studier *et al.*, 1990) that were transformed into *Salmonella enterica* serovar Typhimurium LB5000 (Bullas and Ryu, 1983) cells carrying the T7 RNA polymerase encoding plasmid pGP1-2 (Tabor & Richardson, 1985). Mutant plasmids were generated by QUICKCHANGE® site directed mutagenesis (see MATERIALS AND METHODS of the paper). T7 RNAP expression is repressed by the temperature labile phage lambda repressor, which is expressed constitutively from the plasmid. Expression of scaffolding protein mutants was induced by shifting cells from 30°C to 37°C. After 15 min, cultures were infected at a multiplicity of infection of 5 with P22 *8*$^{-}$amN123, *2*$^{-}$amH202, *13*$^{-}$amH101, *c1*$^{-}$7. This phage is deficient in DNA packaging (*2*$^{-}$) to assure that products of phage assembly could not progress beyond the procapsid stage and was defective in lysis of the host cells (*13*$^{-}$), which allowed the products of phage assembly to be concentrated by pelleting the infected cells. Establishment of lysogeny was averted by the inclusion of a clear mutation (*c1*$^{-}$). After 120 minutes at 37°C, the infected cells were pelleted, resuspended in TM (10 mM Tris pH 7.5, 1 mM $MgCl_2$) and lysed by vortexing with 10% $CHCl_3$. Excess free DNA was digested by addition of DNaseI (Roche Applied Science, Indianapolis, IN) at 0.5 µg/ml to lower the viscosity, and cellular debris was removed by centrifugation at 10,000 RCF for 20 minutes at 4°C. The supernatant was layered onto 20% sucrose-TM and centrifuged at 100K RCFs for 90 minutes. The pellet containing the large molecular weight complexes was resuspended and applied to tubes containing 10-40% sucrose gradient and spun in an SW41 swinging bucket rotor (Beckman-Coulter, Fullerton, CA) at 25K rpm for 120 minutes. Gradients were formed using a Gradient Master (BioComp Instruments, New Brunswick, Canada) and were harvested by fractionation on a Fractionator (BioComp Instruments). Fractions were analyzed by SDS-PAGE. Co-migration of coat and scaffolding through the gradient and the presence of intact shells, as indicated by native agarose gel electrophoresis and/or electron microscopy, demonstrated interaction of coat and scaffolding protein. Control experiments showed that, if the infecting phage was unable to produce coat protein, all the scaffolding protein fragments remained at the top of the gradient.

Table S2

Melting temperatures of scaffolding protein variants

Scaffolding protein variant	Melting point (^{o}C) [a]	ΔT (°C)
WT	49.4	-
WT 6-his	50.4	+1.0
K286A	49.4	0.0
D288A	48.3	-1.1
E290A	49.3	-0.1
R293A	49.6	+0.2
R293E	50.2	+0.6
K294A	48.5	-0.9
K296A	49.5	+0.1
K298A	49.5	+0.1
K300A	50.3	+0.9
R303A	48.1	-1.3
5-Ala	48.3	-1.1
SM1	49.5	-0.9
SM2	46.3	-3.1
SM3	47.4	-2.0
SM4	47.1	-2.3

[a] The melts were done and T_ms determined as described in MATERIALS AND METHODS. The T_m of each protein is the average of the two data points nearest 50% unfolded. Since the folding of scaffolding protein is not well characterized, we chose not to use any particular folding model to fit these data.

Figure S1

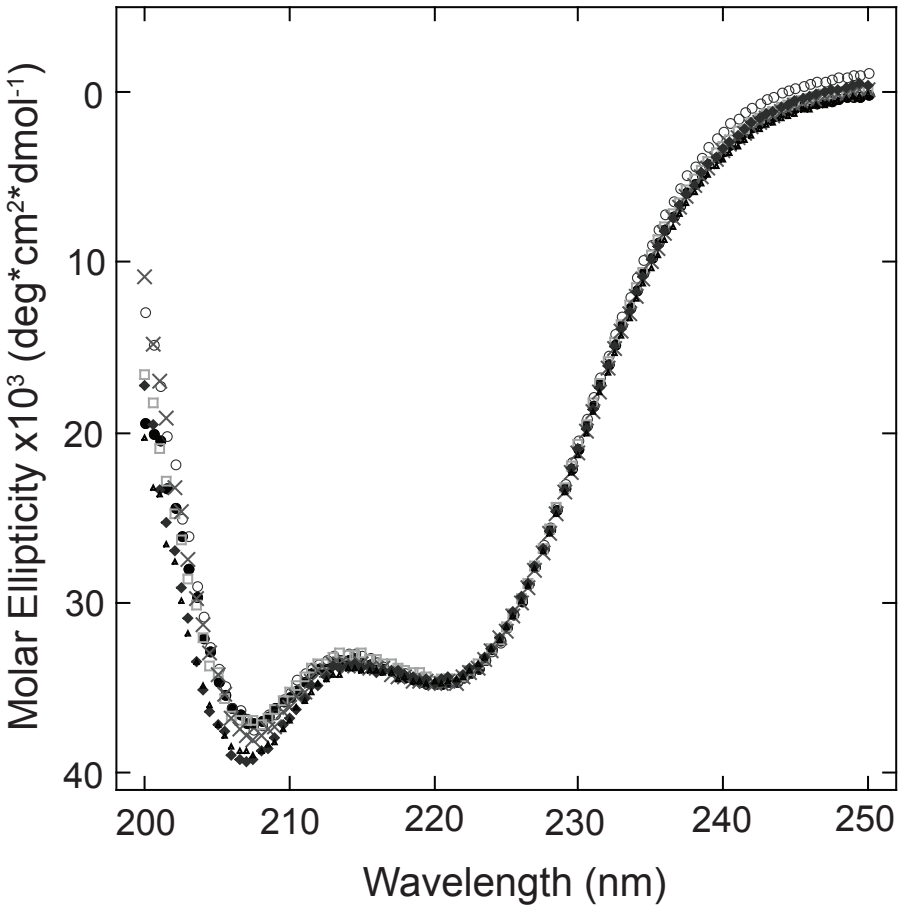

Figure S1. Scaffolding protein circular dichroism spectra. (●) authentic WT scaffolding protein, (○) his-tagged wild-type scaffolding protein, (□) R293A, (×) K296A, (◆) 5-Ala and (△) SM2 scaffolding proteins.

Figure S2

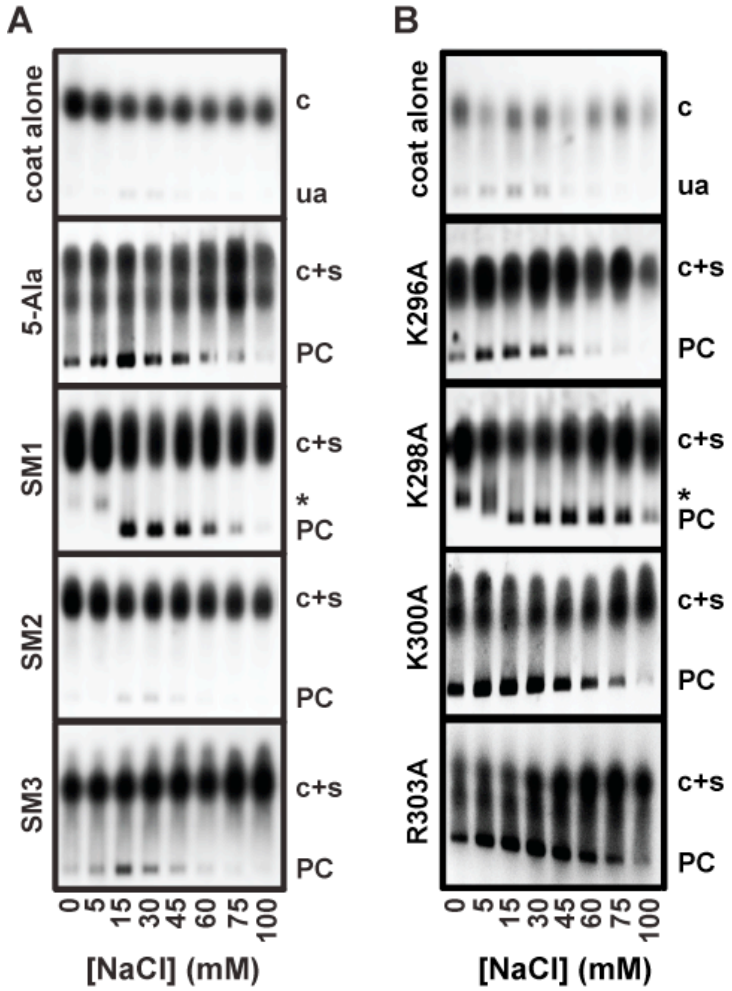

Figure S2. Agarose gel analysis of salt titration *in vitro* procapsid assembly reactions.

Left panel. The indicated scaffolding protein variants were incubated with coat monomers at the final NaCl concentration shown below.

Right panel. K296A, K298A, K300A and R303A scaffolding proteins and coat protein were incubated in the presence of increasing concentrations of NaCl.

Reaction products were analyzed by 1.2% agarose gels, and gel positions are indicated as follows: coat protein uncontrolled assembly, 'ua'; coat monomers, 'c'; coat and scaffolding proteins monomers, labeled 'c+s'; whole procapsids, 'PC'; and 'halves', an asterisk. It is important to note that coat protein and scaffolding protein co-migrated in the agarose gels, therefore, the individual proteins cannot be visually isolated. The salt titrations results are representative of at least 3 distinct reactions.

SUPPLEMENTARY MATERIAL REFERENCES

Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol* **185**, 60-89.

Tabor, S. & Richardson, C. C. (1985). A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc Natl Acad Sci U S A* **82**, 1074-1078.