Supplementary Results

Stereo pairs showing electron density for the E and F spike tips

Figure S1. Here we show density for the E spike (residues A54 to W102), as viewed from the F subunit. At this contour (1.2 σ), density is present for all amino acid side chains, except E77 at the top of the spike. As with Figure S2, this view is similar to that shown in Figure 2d.

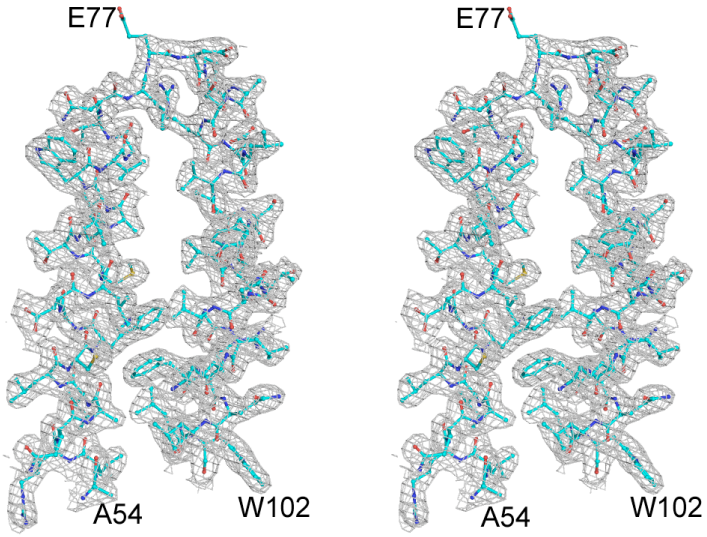
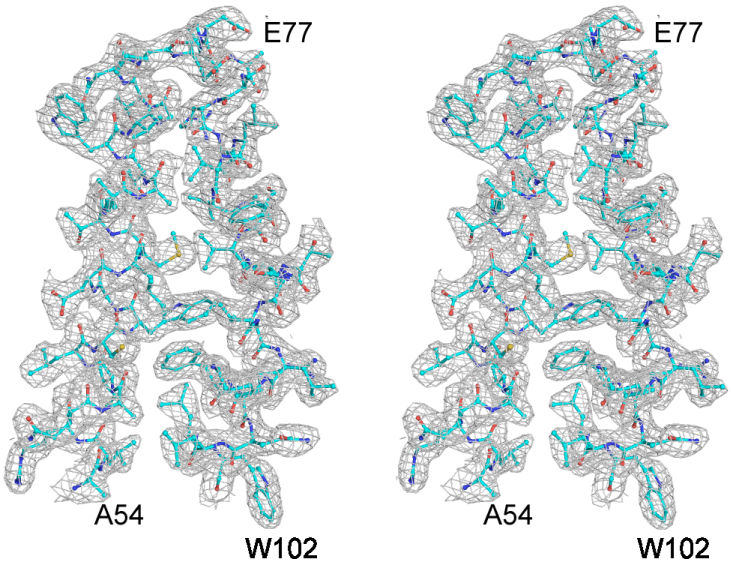


Figure S2. An equivalent view of the F chain (shown below) shows density for all amino acids.



Structural comparisons between dimers

In Figure 2, we compared capsid dimers and Cp149-Y132A "free" dimers. In supplementary table S1 we expand upon that by examining alpha carbon RMSDs for several comparisons. In all comparisons involving the Y132A mutant, alignments are based on aligning the chassis domain of a single subunit, as in the text. If alignments were based on a global minimization of RMSD, all values would be slightly lower (about 0.1 Å).

1. Capsid dimers are symmetrical and very similar.

2. The differences between Y132A dimers can be substantial. Note that the Y132A AB dimer overlays poorly on Y132A BA but overlays closely with CD.

3. The chassis domain between free dimers is structurally even more alike than it is between free dimer and capsid.

Supplementary Table S1.
Structural comparisons

Comparison	RMSD
Capsid (1QGT) dimers	
1QGT AB v BA	0.87
1QGT AB v CD	1.06
Y132A AB with Y132A BA, DC and EF	
Dimer AB v dimer BA	2.67
Dimer AB v dimer DC	1.97
Dimer AB v dimer EF	2.25
Chassis AB with BA, DC and EF (1-10; 25-63 and 93-111)	
Dimer AB v dimer BA	0.41
Dimer AB v dimer DC	0.60
Dimer AB v dimer EF	0.26

Solution Redox studies

In order to further demonstrate conformational changes associated with assembly in support with the structural data from Cp149-Y132A, we focused on the apparently invariant chassis region. Within this region is a conserved disulfide bond at position 61 (Figure 5a). Previous experiments wherein cysteine 61 was mutated to serine or alanine have shown this disulfide to be dispensable for assembly despite its conservation (unpublished data). Examining the effect of the presence or absence of the disulfide in the context of co-assembly of Cp149-Y132A with wild type protein would provide evidence of conformational changes with assembly in the chassis region that is otherwise not apparent from the crystal structure.

To separate the effects of the disulfide in assembly of the mutant from that of the wild type, co-assembly reactions were carried out using a mutant of the wildtype in which cysteine 61 has been mutated to a serine; this mutant displays normal extent and kinetics of assembly. Cp149-Y132A with either allowed to partially air oxidize or was reduced by the addition of 5% βME. Oxidation state was verified by non-reducing SDS-PAGE; oxidized protein was crosslinked by the intradimer disulfide and ran as twice the weight of the monomer (Figure S3).

Assembly studies showed that Cp149-C61S assembled normally in the presence or absence of βME (Figure 5b). When a mixture of approximately 50% oxidized Cp149-Y132A was co-assembled with Cp149-C61S, the increase in the amount of capsid indicated incorporation of the inactive mutant without a change in reaction rate. However, when Cp149-Y132 was co-assembled in the presence of 5% βME, there was a dramatic decrease in the overall amount of capsid produced, as well as an apparent decrease in the rate of reaction. When this reaction was repeated at 37°C to increase the extent of assembly, the co-assembly reaction, while still slowed, appeared to be approaching the same final extent of assembly as the reaction with Cp149-C61S alone (Figure S4)

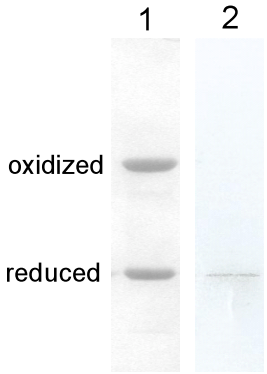


Figure S3. Non-Reducing SDS-PAGE showing redox state of Cp149-Y132A for assembly studies. Reduced and oxidized samples were blocked with excess iodoacetamide then electrophoresed under non-reducing conditions. Lane 1: Partially oxidized Y132A. Lane 2: Y132A after treatment with 5% β-mercaptoethanol.

Figure S4. Comparison of C61S assembly and C61S-reduced Y132A coassembly kinetics over time in the presence at 37°C over 24h. Steady-state rate of formation of capsids correlates to the rate of nucleation. SEC of 20 μM reduced Cp149-Y132A with 5 μM C61S (filled triangles) results shows a slowed rate of nucleation with the extent of reaction approaching that of 5uM C61S alone (filled squares). The inset shows an size exclusion chromatograph of a co-assembly reaction with reduced Cp149-Y132A and Cp149-C61S recorded 24 hours after assembly was initiated.