Quantitative Analysis of Multi-component Spherical Virus Assembly: Scaffolding Protein Contributes to the Global Stability of Phage P22 Procapsids

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Assembly of the hundreds of subunits required to form an icosahedral virus must proceed with exquisite fidelity, and is a paradigm for the self-organization of complex macromolecular structures. However, the mechanism for capsid assembly is not completely understood for any virus. Here we have investigated the in vitro assembly of phage P22 procapsids using a quantitative model specifically developed to analyze assembly of spherical viruses. Phage P22 procapsids are the product of the co-assembly of 420 molecules of coat protein and ~100–300 molecules of scaffolding protein. Scaffolding protein serves as an assembly chaperone and is not part of the final mature capsid, but is essential for proper procapsid assembly. Here we show that scaffolding protein also affects the thermodynamics of assembly, and for the first time this quantitative analysis has been performed on a virus composed of more than one type of protein subunit. Purified coat and scaffolding proteins were mixed in varying ratios in vitro to form procapsids. The reactions were allowed to reach equilibrium and the proportion of the input protein assembled into procapsids or remaining as free subunits was determined by size exclusion chromatography and SDS-PAGE. The results were used to calculate the free energy contributions for individual coat and scaffolding proteins. Each coat protein subunit was found to contribute $-7.2(\pm 0.1)$ kcal/mol and each scaffolding protein $-6.1(\pm 0.2)$ kcal/mol to the stability of the procapsid. Because each protein interacts with two or more neighbors, the pair-wise energies are even less. The weak protein interactions observed in the assembly of procapsids are likely important in the control of nucleation, since an increase in affinity between coat and scaffolding proteins can lead to kinetic traps caused by the formation of too many nuclei. In addition, we find that adjusting the molar ratio of scaffolding to coat protein can alter the assembly product. When the scaffolding protein concentration is low relative to coat protein, there is a correspondingly low yield of proper procapsids. When the relative concentration is very high, too many nuclei form, leading to kinetically trapped assembly intermediates.

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Introduction

The amino acid sequence of capsid subunits must contain information not only about the structure of the subunits but also about the self-assembly process. While some viruses assemble from small preformed oligomers of their coat proteins, other viruses such as phage P22 and herpesvirus assemble from monomeric proteins1–4. The process of subunit assembly is strictly controlled through protein–protein interactions such that icosahedral structures are formed, rather than aberrant non-icosahedral structures. Weak protein–protein interactions through polyvalent subunits have been

Abbreviations used: SEC, size exclusion chromatography; CCMV, cowpea chlorotic mottle virus; HBV, hepatitis B virus.
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suggested to be the driving force for virus assembly. 5–7 These weak subunit associations allow for thermodynamic editing during the assembly process, whereas high affinity interactions are predicted 8–11 and have been shown 12,13 to lead to kinetic trapping of assembly intermediates or off-pathway products. Detailed analysis of the binding affinities of capsid subunits has been done with cowpea chlorotic mottle virus (CCMV) and hepatitis B virus (HBV), both of which have capsids comprised of a single capsid protein that forms a stable dimer. 26,27 Here, we investigate the interactions involved in capsid assembly for the procapsid of the T = 7 Salmonella bacteriophage P22, a capsid comprised of coat and scaffolding proteins, to understand the energetics of the interactions in this more complex macromolecular assembly.

The morphogenic pathway of phage P22 involves the co-assembly of 420 molecules of coat protein (product of gene 5; gp5) with ~100–300 molecules of scaffolding protein (product of gene 8) in a nucleation-limited reaction. Some minor proteins (products of genes 7, 16 and 20, referred to as injection proteins) and the portal protein complex (product of gene 1) are also incorporated during assembly. Scaffolding protein directs the assembly of the procapsid. Without scaffolding protein, coat protein assembles into T = 4 capsids and aberrant spiral structures that have their fivefold and sixfold vertices located inappropriately so that closed procapsid structures do not form. The formation of these misassembled structures occurs only at relatively high coat protein concentrations. 12–18 The number of scaffolding protein molecules in a procapsid varies from about 100 to 300 molecules, depending on the conditions of assembly. 19,20 The minor injection proteins are incorporated early in assembly 21–23 also through interactions with scaffolding protein. 24 The double-stranded (ds) DNA is actively packaged into the procapsid through the unique portal vertex. 25

Concomitant with DNA packaging, scaffolding protein exits from the procapsid to take part in additional rounds of assembly, and the capsid matures. 26,27 The dsDNA is stabilized by the addition of proteins that close the portal vertex, and finally tailspikes, the cell recognition and attachment proteins, are added. In the processes of folding and assembly, none of the proteins are covalently modified or proteolyzed. 28

An important advantage of phage P22 as an assembly model is the simplicity of the system. The proteins needed for assembly of P22 procapsids can all be purified and are active for assembly. 29 In vitro, only coat and scaffolding proteins are required to assemble a procapsid-like particle, which we will refer to as a procapsid. 30 The in vitro assembled procapsid has the same morphology and size as the in vitro generated procapsid. 4 Because of the relative simplicity of the reaction, we can manipulate the outcome; we recently showed that changing the composition of the buffer alters the product of assembly. 12 When the anion concentration in the buffer is low, then too many nuclei form and assembly is trapped in a partially assembled state, which we call partial capsids. We proposed that the affinity between coat and scaffolding proteins is too high in these buffer conditions.

While there is a plethora of general information on the assembly of phage P22, the only rigorous thermodynamic analysis of in vitro assembly was based on a model of assembly of filamentous proteins. 31 For filamentous protein assembly, where there is one nucleus for a very long polymer, the process of slow nucleation followed by growth of the large polymer leads to the observed sigmoidal assembly kinetics. 32 For an icosahedral particle, where there are at most a few hundred subunits, the observed sigmoidal assembly reaction kinetics result from the time it takes to achieve a steady state concentration of assembly intermediates rather than very slow nucleation. 8 Thus, the analysis of capsid assembly reactions must differ from that of filamentous protein assembly. 33

Zlotnick’s group has recently established a generalized, thermodynamically rigorous model to describe the assembly of icosahedral viruses. 9,33,34 These models have been used in the characterization of the assembly of HBV, CCMV 35 and papillomavirus. 36 Essentially, assembly is described in terms of a cascade of reactions. Though the overall reaction can be expressed in terms of a simple mass action law (see equations (1)–(3)), cascades can have distinctly complex behavior. While assembly reactions rapidly reach equilibrium, dissociation of spherical assemblies is impeded by a large, kinetically derived energy barrier. This hysteresis arises from the rate of removing multivalent subunits from such complexes compared to the rate with which a free subunit will bind to a multivalent site. The assembly of HBV, CCMV 35 requires only one protein species and is accurately described by the virus-specific model. Here, we expand the use of this model to the assembly of P22 procapsids and find that each subunit contact is weak but when the many contacts required to form a procapsid are combined, the result is a globally stable structure.

Results

Analysis of procapsid assembly reactions at equilibrium

In this study, we have determined the contributions of coat and scaffolding proteins to the stability of phage P22 procapsids based on a virus-specific thermodynamic–kinetic model. 34 Because the geometry of subunit interactions is not well defined for P22, we can only determine the per subunit association energy. The equilibrium
expression for the formation of a procapsid is:

\[
420 \text{ coat protein} + Z \text{ scaffolding protein} \rightarrow \text{procapsid}
\]  

(1)

Here the \(Z\) indicates that the number of scaffolding proteins incorporated varies with assembly conditions, especially total [scaffolding protein]. Then, the equilibrium constant for procapsid formation (\(K_{PC}\)) is:

\[
K_{PC} = \frac{[PC]}{[\text{coat}]^{420}[\text{scaffolding}]^Z}
\]  

(2)

\[
\log K_{PC} = \log[PC] - 420 \log[\text{coat}]
\]

\[ Z \log[\text{scaffolding}] \]  

(3)

Because of the magnitude of \(K_{PC}\), it is more convenient to work with the logarithmic form of the mass action law. \(\log K_{PC}\) can be calculated because all of the concentrations on the right side of the equation can be determined. Because the number of coat proteins in a procapsid is absolute, it is simple to determine the concentration of procapsids assembled based on the amount of coat protein in the procapsid peak in a size exclusion chromatography (SEC) experiment (see Figure 1(a)). From the concentration of procapsids, the number of scaffolding protein molecules per procapsid particle can be calculated.

A series of assembly reactions were done in 20 mM phosphate with 50 mM NaCl, conditions where complete procapsids are efficiently generated.\(^{12}\) In a given series of reactions, we varied the ratio of scaffolding:coat protein. After equilibrium was reached (>20 h), the unassembled coat and scaffolding proteins were separated from procapsids by SEC (Figure 1). An aliquot of each fraction was run on SDS-polyacrylamide gels and silver stained (Figure 1(a)). Shown in Figure 1(a) is an example of the separation achieved in the column chromatography under conditions with the highest protein concentrations. The amount of coat protein and scaffolding protein in each fraction was calculated from densitometric scans of the gels (Figure 1(b)). Any material observed between the procapsid and monomer peaks (less than ~5% of the total at the highest protein concentration) was simply divided between them; there is no evidence that assembly intermediates persist in reactions at equilibrium under these experimental conditions.\(^{12}\)

From these data, the concentration of coat and scaffolding proteins in procapsids and as soluble subunits were determined. Once the concentration of procapsids was determined, the number of scaffolding proteins per procapsid was calculated.

Isotherms showing that the concentration of coat protein assembled into procapsids varies with the input concentration of coat protein were generated from quantification of the assembly reactions. The concentration of procapsids \textit{versus} input coat protein concentration for each scaffolding:coat protein ratio gives approximately the same slope of 0.00193 ± 0.0003 [procapsid] M/[coat protein] M for all ratios (Figure 2(a)). The addition of 1 mol of coat protein should give 1/420 mol of procapsids or 0.002 mol. Thus, the dependence of procapsid assembly on input coat protein concentration is exactly as anticipated.

At the three representative scaffolding:coat protein ratios shown in Figure 2(a), the data show the expected pseudo-critical concentration, or \(K_{D\text{apparent}}\), of ~5, 6.5 and 10.5 \(\mu\)M (see the arrows in Figure 2(a)). These agree qualitatively with older data where the pseudo-critical concentration for coat protein was determined to be ~6 \(\mu\)M at a single scaffolding protein concentration.\(^{31}\) A pseudo-critical concentration derives from the extremely steep concentration dependence on coat protein for the procapsid assembly reaction, as described by the mass action law (equation (1)). In practical terms, above the \(K_{D\text{apparent}}\) value almost all of the additional coat protein in the initial reaction will go to form procapsids with a near constant amount of coat protein remaining when the reaction reaches equilibrium.

Though it is easily accessible experimentally, \(K_{D\text{apparent}}\) is a rigorously defined function of capsid stability and geometry.\(^{8}\) A true critical concentration specifies that subunits freely equilibrate between two phases; \(K_{D\text{apparent}}\) is defined specifically for spherical oligomers because (i) free subunits will slowly increase with the total concentration of input coat protein, as predicted by equation (3), and (ii) hysteresis observed in the dissociation reaction of spherical oligomers will prevent the re-equilibration required for critical concentration phenomena.\(^{8}\) This expected pseudo-
critical concentration ($K_{D\text{apparent}}$) has been observed not only for bacteriophage P22,31,37 but also HBV5 and CCMV.13 In Figure 2(a) we observe for the first time that $K_{D\text{apparent}}$ is dependent on the proportion of scaffolding protein present in the reaction, which indicates that scaffolding contributes to procapsid stability. The number of scaffolding proteins per procapsid was determined for each set of reactions performed at a particular molar ratio of scaffolding:coat protein. Shown in Figure 2(b), the number of scaffolding proteins per procapsid varied with the input scaffolding protein, as expected.38,39 The data suggest that there is a lower limit to the number of scaffolding proteins needed to support assembly, and an upper limit to the number of protein binding sites within the T=7 capsid. Therefore, the data were fit to a rectangular hyperbola (line in Figure 2(b)), which gave a minimum of about 90 and a maximum of about 350 scaffolding protein subunits per procapsid at the limits of low and high scaffolding:coat protein ratios, respectively. This is in good agreement with previous work.31,40,41

Each coat or scaffolding protein subunit contributes only weakly to the stability of the procapsid

Assembly reactions were done with scaffolding:coat protein molar ratios from 0.28 to 2.8. For each reaction, the log $K_{PC}$ value was determined (equation (3)). In addition, an average log $K_{PC}$ was determined for all the reactions at each ratio. The log $K_{PC}$ is converted to $\Delta G_{PC}$ using:

$$\Delta G_{PC} = -2.303RT \log K_{PC} \quad (4)$$

The contributions of scaffolding and coat proteins to procapsid stability were determined by plotting $\Delta G_{PC}$ versus the number of scaffold proteins per procapsid. These data readily fit to:

$$\Delta G_{PC} = 420\Delta G_{coat} + Z\Delta G_{scaffold} \quad (5)$$

where $Z$ is the number of scaffolding proteins per procapsid. The linearity of the data strongly supports our analysis. This treatment yields an assessment of both $\Delta G_{coat}$ and $\Delta G_{scaffold}$ under the condition of co-assembly while only making the assumption of thermodynamic linkage, i.e. that all proteins contribute to the overall procapsid stability.9,34,42 Note that these are average values for association energy without reference to quasi-equivalence. In Figure 3(a), the $\Delta G_{PC}$ value is plotted against the number of scaffolding proteins per procapsid for each reaction. The fit of the data with equation (5) yielded a $\Delta G_{coat}$ of $-7.2 \pm 0.1$ and a $\Delta G_{scaffold}$ of $-6.1 \pm 0.2$. These energies are equivalent to a dissociation constant of 5 $\mu$M for coat protein and 28 $\mu$M for scaffolding protein, and are consistent with estimates determined from different experiments.6,41 Overall, coat protein contributes about $-3000$ kcal/mol to the stability of a procapsid and if the procapsid contains 300 scaffolding proteins, then scaffolding protein contributes about $-1800$ kcal/mol to procapsid stability.

Figure 3(a) shows the saturation of data on the plot of $\Delta G_{PC}$ versus scaffolding proteins/procapsid that we were able to achieve in these experiments. The $\Delta G_{PC}$ averaged over for an experimental series at a constant scaffolding:coat ratio (Figure 3(b)) gave $\Delta G$ values within the error of the values determined in Figure 3(a) and again show the scaffolding protein dependence on the stability of procapsids. Because the scaffolding and coat protein concentrations are in the range of their respective per subunit dissociation constants, the number of bound scaffolding proteins per capsid varies within an isotherm, contributing to the apparently large error bars in this plot.
There is an optimal range of scaffolding protein concentrations for procapsid assembly

The concentration of procapsids assembled in each reaction was plotted against the concentration of input scaffolding protein and organized by the input scaffolding:coat protein ratio (Figure 4(a)). At low input ratios, where scaffolding protein is limiting for assembly, increased concentrations of subunits directly yielded more procapsids. At higher input ratios, where scaffolding protein is in excess, the yield of the reactions became more dependent on the coat protein concentration, a dependence that is different from that seen when the data were plotted versus the input coat protein concentration (Figure 2(a)). The dependence of the slopes of the lines as a function of molar input ratio is shown in Figure 4(b). This plot shows that the dependence of the yield of procapsids on scaffolding protein decreases as the ratio of scaffolding:coat protein increases. These data suggest that the yield of procapsids should become close to zero at a high input ratio.

A high concentration of scaffolding protein inhibits assembly

Based on our previous work and the data in Figure 4, we are led to the prediction that at very high scaffolding protein ratios, too many nuclei would form and ultimately inhibit assembly of procapsids. To test our prediction, we assembled...
procapsids at increasing scaffolding:coat protein ratios with coat protein held at 10.7 μM. An aliquot of each reaction was run on an agarose gel to visualize the products of assembly (Figure 5(a)). When the ratio was greater than 7.0, the amount of procapsids decreased. In addition, a band of lower mobility, which corresponded to assembly intermediates increased in intensity at higher ratios. This band does not run in the same position as the partial capsids observed when assembly reactions are done in the absence of additional NaCl12 (data not shown). In Figure 5(b)–(e), negative stain electron micrographs of several assembly reactions are shown. Figure 5(b) shows partial capsids generated without NaCl, while Figure 5(c)–(e) show the products of assembly with increasing scaffolding protein, from 0.8 M ratio (Figure 5(c)), which gives normal procapsids, to 27.8 M scaffolding:coat protein ratio, which shows many assembly intermediates that are smaller, more numerous, and distinct in morphology, than the partial capsids shown in Figure 5(b). Electron micrographs were taken of the monomeric subunits and no species that looked like the assembly intermediates, or aggregates, were observed (data not shown). At high ratios, scaffolding protein clearly initiates too
many nuclei causing the formation of distinct assembly intermediates, as we predicted.

Discussion

Weak interactions drive assembly

Previously, Ceres & Zlotnick\(^5\) proposed that capsid assembly could be driven by weak protein–protein interactions. Weak protein–protein interactions were also suggested to be important for thermodynamic editing so that if improper structures are initiated, they could disassemble and allow the subunits to be reused. Here we have shown that each coat protein and scaffolding protein of phage P22 contributes only a small amount of free energy to the procapsid, about \(-7\) kcal/mol per coat protein subunit and about \(-6\) kcal/mol per scaffolding protein subunit. The contributions are in line with those for the subunit interactions of CCMV and HBV, which both are about \(-7\) kcal/mol.\(^{6,13}\) Because there are so many copies of coat and scaffolding protein, the overall stability of the capsid is large even though the contribution of each protein is small.

Scaffolding protein is found within procapsids in two states: approximately 60 tightly bound scaffolding proteins and the rest which are loosely bound.\(^{37,40}\) The \(\Delta G\) value for the tightly bound scaffolding proteins was determined previously to be about \(-9.0\) kcal/mol \((K_d = \sim 100–300\, \text{nM})\), but these investigators could not measure the \(\Delta G\) value for the more loosely bound scaffolding proteins.\(^{41}\) This binding energy is an upper limit for the energy each scaffolding protein can contribute to assembly and would be around \(540\) kcal/mol for a procapsid.

We have measured the average contribution of scaffolding protein to procapsid stability. In vitro, about 150–350 scaffolding proteins are found in procapsids,\(^{19,43,44}\) which is consistent with the numbers found in our in vitro assembly reactions. Thus, the loosely bound scaffolding proteins outnumber the tightly bound scaffolding proteins. Our data suggest that the loose scaffolding proteins contribute significantly to the stability of procapsids, from around \(~ -350\) kcal/mol to \(~ -1560\) kcal/mol procapsid when from 150 to 350 scaffolding proteins are incorporated, explaining why loosely bound scaffolding proteins are included during assembly.

Scaffolding protein concentration is important for assembly

In a normal phage P22 infection in vivo, the concentration of free scaffolding protein is around 27 \(\mu\)M and total concentration is around 55 \(\mu\)M.\(^{45}\) The total intracellular concentration includes scaffolding protein found transiently in procapsids, before DNA packaging occurs. In contrast, the coat protein concentration ultimately reaches over 400–500 \(\mu\)M within the infected cell producing approximately 400 phage/cell. Though coat protein and scaffolding protein are in the same operon, and regulated by only a single promoter, scaffolding protein autogenously modulates its own translation so that the total scaffolding protein concentration is low compared to coat protein.\(^{46}\) Each scaffolding protein is recycled about five to six times during an infection.\(^{44}\) Other viruses proteolyze their scaffolding proteins so there is never a large intracellular concentration.\(^{47,49}\)

Why do viruses so carefully regulate scaffolding protein levels during infection? Under the protein concentrations used for the analysis of \(\Delta G_{\text{PC}}\), scaffolding protein is mostly monomeric based on the work by Parker et al.\(^{50}\) We chose these concentrations to match the physiological conditions described above. Our data indicate that the optimal concentration of scaffolding protein is important for proper procapsid assembly. When scaffolding:coat protein ratios are too low, the yield of assembly products is low. When scaffolding protein levels are too high, where scaffolding protein will be mostly in a higher-order association, too many nuclei form so that coat protein becomes limiting for the reaction. In vitro, these conditions lead to formation of aberrant assembly intermediates and represent a kinetic trap for assembly.\(^{12}\) In vivo, then, it would be crucial for viruses that use a scaffolding protein to regulate the intracellular concentration to achieve a good infection.

The thermodynamic-kinetic model can be used for capsids with more than one protein species

Minimal assumptions are made in the virus-specific thermodynamic-kinetic model.\(^53\) In the model, the reactions are assumed (and have been shown for phage P22) to reach equilibrium.\(^59\) The interactions contributed by capsid proteins are considered equivalent at all times in the reaction; that is for phage P22, all coat protein interactions were treated equally, as were all the interactions for scaffolding protein, yielding an average association energy. The nucleus, which is only critical in kinetic simulations,\(^9\) is assumed to be assembled one subunit at a time, similar to Matsudaira’s observation of actin nucleation.\(^{51}\) The forward microscopic rates are also considered to be equivalent except for the nucleation rates, which can be determined independently.

Here we have shown that this model can be easily applied to a system where two proteins are required for assembly. The predictions made for assembly reactions at equilibrium (e.g. pseudo-critical concentration, weak association energy, and thermodynamic linkage demonstrated by the linear relationship between stability and scaffolding protein content) are demonstrated experimentally in this more complicated system. The model should be readily adaptable to even more complex systems.
to understand the function of other capsid proteins in virus assembly reactions.

Materials and Methods

Chemicals, buffers and proteins

Ultrapure urea was purchased from ICN. All other chemicals were reagent grade and purchased from common sources. Purification of coat protein was done as described. All experiments described below were done in 20 mM sodium phosphate, 50 mM NaCl buffer, made using Na2HPO4, with the pH adjusted to 7.6 with H3PO4.

Refolded coat protein monomers

Coat protein monomers were obtained from urea-denatured empty procapsid shells, as described, using dialysis at 4 °C against 20 mM sodium phosphate buffer (pH 7.6). Coat protein monomers obtained in this way are assembly competent but will not assemble into procapsids until the addition of scaffolding protein. Monomers were centrifuged at 175,000g at 4 °C for 20 min to remove any aggregated or associated structures prior to assembly.

Assembly reactions

To assemble procapsids, refolded coat protein monomers at final concentrations ranging 0.3–0.9 mg/ml (~6.5–19 μM) were mixed with scaffolding protein. The lower limit of the coat protein concentration was set by the pseudo-critical concentration for assembly; that is, below ~6.5 μM coat protein, assembly does not proceed; the upper limit was determined by the solubility of monomeric coat protein (~25 μM) and the required dilution of the proteins. The scaffolding protein was added at concentrations corresponding to molar ratios ranging from 0.28 to 2.8 for a total of 50 reactions, i.e. for the ratio of 2.8, sample one contained coat protein at 0.3 mg/ml (~6.5 μM) and scaffolding protein at 0.6 mg/ml (~18 μM), sample two contained coat protein at 0.4 mg/ml (~8.5 μM) and scaffolding protein at 0.8 mg/ml (~24 μM), etc. Assembly reactions were performed in 20 mM sodium phosphate (pH 7.6), 50 mM NaCl and incubated at 20 °C for >20 h in a total volume of 125 μl.

Analysis of equilibrated assembly reactions

Assembly reactions described above were applied to a 15 ml Sepharose 4B column run at a flow rate of 0.5 ml/min at room temperature in 20 mM sodium phosphate, 50 mM NaCl buffer. Samples of each fraction were then run on SDS-(10%) gels. The gels were silver stained and coat and scaffolding protein bands were visualized using a Kodak EDAS system. The response of the silver stain was linear for the range of protein concentrations used in these experiments (data not shown). Since the yield from the column was greater than 90–95%, the fraction of scaffolding or coat protein was simply calculated from the total of each protein applied to the column. These data were analyzed to determine ΔGRC as described in Results. In Figure 2(b), data were fit with a rectangular hyperbola in the form of:

\[ Y = \frac{\text{min } \# \text{ scaffolding proteins} + (\text{max } \# \text{ scaffolding proteins} \times Z \times \text{molar input ratio})}{1 + (Z \times \text{input ratio})} \]

where Z is a unitary association constant of scaffolding protein for procapsids that scales the ratio of bound to the ratio of input scaffolding protein.

Agarose electrophoresis

Agarose gels were prepared and run as described. In brief, the samples for the native agarose gels were prepared by combining a portion of the assembly reactions with agarose gel sample buffer and loaded onto 1.2% (w/v) Seakem HGT agarose gel. The gels were run at 50 V constant for ~5 h at 4 °C. The agarose gels were stained with Coomassie blue.

Negative stain electron microscopy

A portion of some assembly reactions were used for negative stain electron microscopy (EM). The samples were spun in a microfuge at maximum speed for 5 min to remove any debris. Three microliters of the sample was allowed to absorb to the carbon-coated grid for 1 min. Two to three drops of water were used to wash the grid. The sample was stained with two to three drops of 1% (w/v) aqueous uranyl acetate for 30 s. The excess liquid was wicked off and the grid air-dried. The samples were viewed using a Philips model 300 at 80 kV with magnifications of both 43,400× and 87,400×.

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References

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