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Supplementary Materials for

A viral scaffolding protein triggers portal ring oligomerization and incorporation during procapsid assembly

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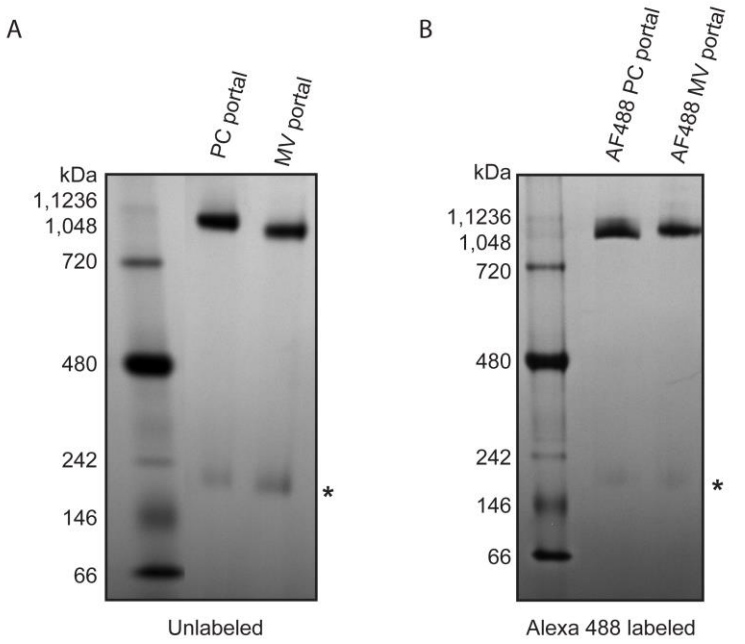
This PDF file includes:

Supplementary Materials



Supplemental Figure 1

fig. S1. Analysis of PC and MV portals by native protein gel electrophoresis. Native gel electrophoresis of unlabeled (**A**) and Alexa Fluor 488 labeled (**B**) PC and MV portal was performed with 4-16% Bis-Tris NativePAGE™ gels. Two micrograms of unlabeled or labeled protein was loaded on the gels. Shown in lane 1 is NativeMark™ standard. The asterisks indicate migration of portal monomers in PC portal or MV portal samples.

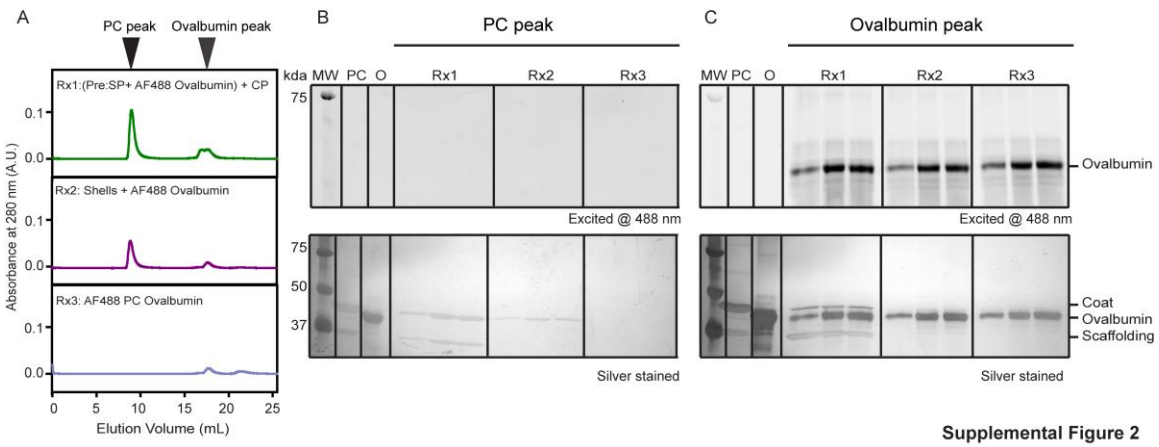
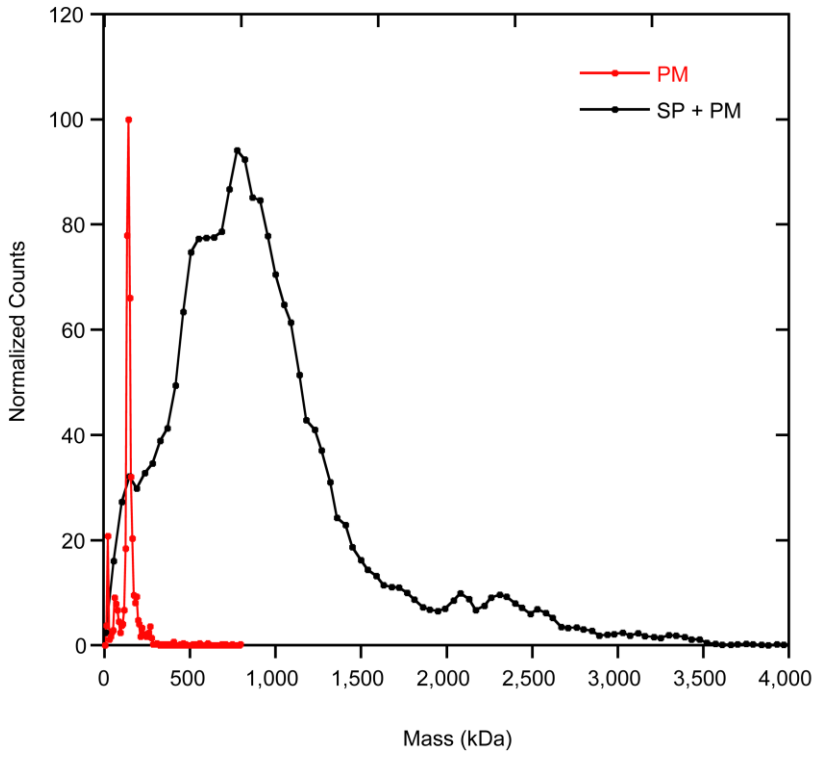


fig. S2. Ovalbumin is not coassembled into PC. (**A**) Superose 6 Increase elution profiles of the assembly reactions of coat (CP) and scaffolding (SP) protein with AF488 ovalbumin (Rx1) is shown along with the elution profiles from the mock reactions with empty procapsid shells (Rx2) and AF488 labeled ovalbumin alone (Rx3). The black arrowheads indicate the elution volume of *in vitro* assembled procapsid (PC) and ovalbumin peaks from the column. The three fractions corresponding to the size exclusion chromatography (SEC) purified PC and ovalbumin peaks from Rxs 1-3 were subjected to SDS-PAGE. Shown also is molecular weight standard (MW, lane 1), PC marker (PC, lane 2) and ovalbumin marker (O, lane 3). The presence of labeled ovalbumin in the PC peak (**B**) and ovalbumin peak (**C**) was visualized using a Pharos Fx Plus Molecular Imager (top) followed by silver staining (bottom) as described in Materials and Methods. During silver staining both the gels were exposed to the developer for the same amount of time.



Supplemental Figure 3

fig. S3. Charge detection mass histogram of SP catalyzed de novo portal rings. The portal monomer (red) shows a well-resolved peak with a mass centered around 84 kDa. P22 Portal ring nucleation reaction (black) shows a large broad peak with a mass centered around 1 MDa with a high mass shoulder out to 3.5 MDa. The portal monomer was collected using a 2,000 m/z mass cutoff, the portal ring nucleation reaction was collected using a 4,000 m/z mass cutoff. The portal ring nucleation reaction was prepared by mixing 0.27 μM SP and 0.059 μM PM. The reaction was left at RT for 4 hours before being buffer exchanged, using SEC, into 100 mM ammonium acetate.