Materals and Methods

Materials: Restriction enzymes and DNA modifying enzymes were purchased from Promega (Madison, WI) and New England Biolabs (Ipswich, MA). Primers were ordered from Eurofins MWG Operon (Huntsville, AL). *E. coli* strains (XL1) and (BL21(DE3)) were purchased from Agilent Technologies. The plasmid containing the *Pyrococcus furiosus* GalA gene was a gift of Prof John van der Oost (Wageningen, the Netherlands). The plasmid containing the complete sequence for PR8 (H1N1) hemagglutinin protein was purchased from Genescript (Piscataway, NJ). QIAprep miniprep kits and QIA quick gel extraction kit were purchased from the Qiagen (Valencia, CA). All other chemicals were purchased from Fisher Scientific in (Pittsburgh, PA), unless otherwise indicated.

Molecular Biology: The GalA gene was amplified from the plasmid, using primers 5'-GCGCGCCATGGCAAGTCTTTCATGGG-3' and 5'-GCGCGGGATCCATCCATTTTCACTCC-3' and HA_{head} using primers 5'-AAAACAGTCCCATGGCAAAGGGCATCGCACCGCTGCAGCTGGGTAAATGCAATATTGCT-3' and 5'-TTTTGTGCGGATCCACTGGTGATAATGCCCGAACCAAAGCCGCGTGACAGGGCGAA-s'by PCR using OneTaq (NEB). The PCR product was digested for 1 hour using Nco1-HF and BamH1-HF. The pETDuet plasmid containing P22 CP and SP, previously described, was cut using the same restriction enzymes. The vector and insert were then ligated together and transformed into electrocompetent XL1 *E. coli* cells. Colonies were grown on an Amp plate, and screened by restriction enzyme digest (NcoI and BamHI). DNA giving consistent banding pattern was sequenced by Seqwright (Houston, TX) and the correct plasmid DNA was then transformed into BL21(DE3) *E. coli* cells for expression.

Protein Expression and Purification: *E. coli* cells were grown in a 10mL starter culture overnight at 37°C from a glycerol stock of P22-GalA. 1L of Lysogeny Broth was inoculated with 1mL of the starter culture and allowed to grow until OD_{600} reached 0.6-0.8 (about 3 hours) when it was then induced with 0.5mM IPTG. Cells were allowed to grow for another 5 hours until centrifuged down at 3700g for 20 minutes. Cell pellets were resuspended in PBS pH 7.0 buffer and frozen in a -80°C freezer until needed. Cell pellets were thawed in room temperature water, and then incubated with DNAse, RNAse, and lysozyme (Sigma-Aldrich) on ice for 30 minutes.

Cells were further lysed by sonication on ice. Cell debris was removed by centrifugation at 12,000g for 1 hour. The supernatant was loaded onto a 35% sucrose cushion and ultra-centrifuged at 48,000rpm for 50 minutes at 4°C. The resulting pellet was resuspended overnight in PBS pH 7.0 buffer, and spun at 16,000rpm for 20 minutes for lipid removal. The crude P22-GalA PC VLPs were then loaded onto a Biorad FPLC with a Sephacryl S-500 (GE) for further purification.

SDS-PAGE: Samples were mixed with a 4x DTT loading buffer and heated in a boiling water bath for 6 minutes. Samples were spun down on a table-top centrifuge and subsequently run on a gel containing a 5% acrylamide stacking gel and a 12% acrylamide separating gel for 45 minutes at a constant current of 35mA. Gels were stained with Coomasie blue stain for 15 minutes, placed in destain overnight, and then imaged using a UVP MultiDoc-IT Digital Imaging System.

Size Exclusion Chromatography Coupled with MALS and Refractive Index detectors: Samples were separated over a size exclusion column (WTC-0200S by Wyatt Technologies) on an Agilent 1200 HPLC at a flow rate of 0.7mL/min. The column was equilibrated and run with a 100mM sodium chloride, 50mM phosphate, pH 7.2 buffer containing sodium azide at 200ppm. Samples (25µL) were injected onto the column and run for 30 minutes. Sample data was collected using a Wyatt HELEOS multi-angle light scattering laser detector, and a Wyatt Optilad rEX differential refractometer. All calculations for average molecular weights were performed using the Wyatt Astra 5.3.14 software.

Transmission Electron Microscopy: Samples of 10µL at 0.2mg/mL protein were applied to formvar coated grids and incubated for a few seconds when all excess liquid was wicked away with filter paper. Grids were then stained with 5µL of 1% uranyl acetate. TEM Images were recorded on a LEO 912AB transmission electron microscope operating at 100 keV.

GalA Kinetics: Activity Assays were carried out using a Agilent 8453 UV-visible spectrophotometer equipped with an Agilent 8-cell changer. Substrate buffers were preheated for 15 minutes in a hot water bath with lines coupling the bath to the 8-cell sampler that maintained constant temperature in the cuvette holder. The substrate was para-Nitrophenyl-α-D-

galactopyranoside (pNpαGal) from Gold Biotechnologies in St. Louis, MO)and was prepared by dilution series from 3mM-0.05mM in a 50mM phosphate, 100mM sodium chloride, pH 7.0 buffer. 5µL of active P22-GalA was thoroughly mixed with the substrate buffers and the cleavage of pNpαGal was monitored by para-nitrophenol production which increases absorbance in the 405nm region. Activity assays were carried out in triplicates at 60°C and plots of the initial rates of pNpαGal depletion were fit to a Michaelis-Menten kinetic curve using Prism5.

Nucleotide sequences

GalA-SP

ATGAGAGCACTAGTCTTTCATGGGAATTTACAATATGCAGAAATACCAAAGAGCGAAATTCCAAAGGTTATAGAAAA
AGCATACTTCCCCACTATTTCAGAACTCATAAGAAGAGAGATACCCTTTGGCCTTAACATAACGGGATACTCATTAA
GCTTTCTCCCAAAAGATTTGATTGCCCTCATAAAGGAGGGAATAGAGAGTGGCCTCATAGAGATTCTTGGAACAAGT
TATACTCACGCAATTCTCCCACTTCTCCCACTCTCTAGAGTTGAGGCACAAATAAAAAGAGATAGAGAAGTGAAAGA
GAACATATTGGAAGTCTCCCCAGAAGGATTCTGGCTTCCCGAATTGGCATATGACCCAATAATTCCAGCAATTTTGA
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ATGCTGCTGCGAGCAAGGGAGATGTGGAAACCTACCGCAAGCTAAAGGCAAAACTTAAAGGAATCCGATAA

HA_{head}-SP

ATGGCAAAGGGCATCGCACCGCTGCAGCTGGGTAAATGCAATATTGCTGGTTGGCTGCTGGGCAACCCGGAATGTGA
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ATGTCAGCGCAGCAAATAAAGATGCCATTCGTAAACAAATGGATGCTGCTGCGAGCAAGGGAGATGTGGAAACCTAC
CGCAAGCTAAAGGCAAAACTTAAAGGAATCCGATAA

P22 CP

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Amino acid sequences

GalA-SP

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IEILGTSYTHAILPLLPLSRVEAQIKRDREVKENILEVSPEGFWLPELAYDPIIPAILRDNNYEYLFADG
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VSINTAVMLGAGRFPLMNPKKVAKWVKEKDEILLYGTDIEFLGYRDIAGYKITISNLLEIINELEGELGL
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AFMQLVPPAVGADIMRLFPEKSAALMYHLGANPEKARQLLAMDGQSALIELTRLSERLTLKPRGKQISSA
PHADQPITGDVSAANKDAIRKQMDAAASKGDVETYRKLKAKLKGIR

HAhead-SP

MAKGIAPLQLGKCNIAGWLLGNPECDPLLPVRSWSYIVETPNSENGICYPGDFIDYEELREQLSSVSSFE
RFEIFPKESSWPNHNTNGVTAACSHEGKSSFYRNLLWLTEKEGSYPKLKNSYVNKKGKEVLVLWGIHHPP
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P22 CP

MALNEGQIVTLAVDEIIETISAITPMAQKAKKYTPPAASMQRSSNTIWMPVEQESPTQEGWDLTDKATGL
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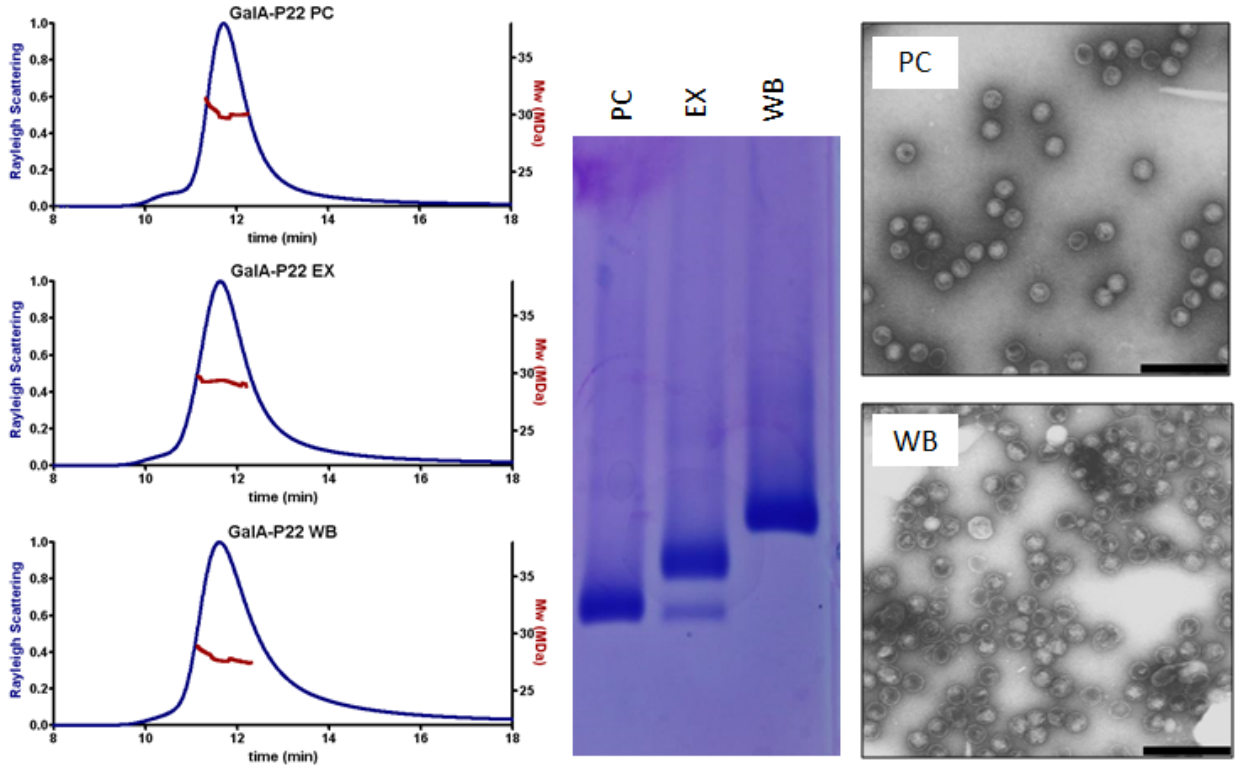
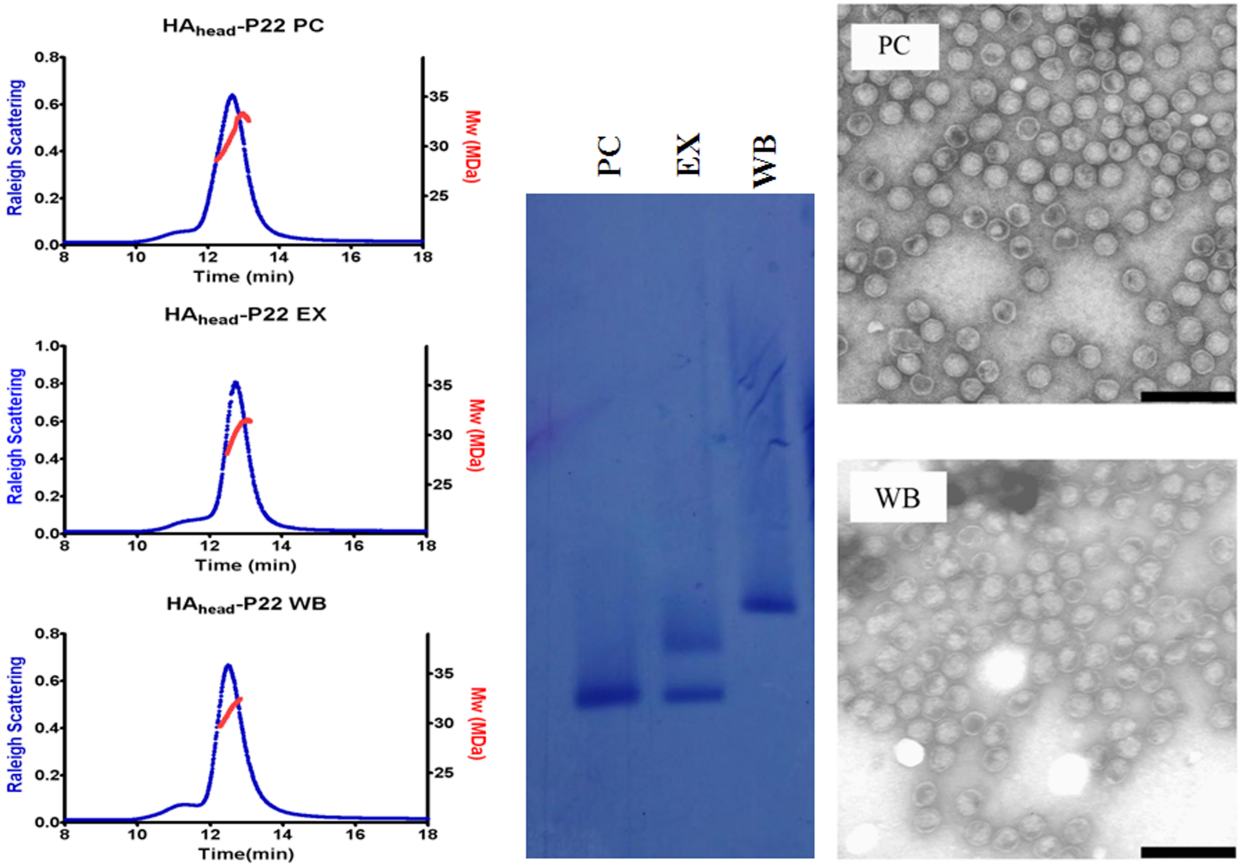
Supplementary Data



Supplementary Figure S1. Characterization of GalA-P22 to undergo morphological changes as exhibited by wt P22 particles. The far left image shows M_w by MALS as 30.13±0.29 MDa for P22-GalA Procapsid (PC), 29.29±0.30 MDa for Expanded (EX), and 28.06±0.30 MDa for Wiffleball (WB). The net loss of 2.1MDa from PC to WB is consistent with the expected mass loss associated with the 12 pentamers that are released upon WB formation. The middle image is an agarose gel shift assay of the P22 forms. Roughly 80% transformation is observed from PC to EX, whereas the WB is a complete transformation. TEM images show P22-GalA particles in the PC and WB form with similar sizes to the wt P22 morphologies. Scale bars are set at 200nm.



Supplementary Figure S2. Characterization of HA_{head}-P22 to undergo morphological changes as exhibited by wt P22 particles. The far left image shows M_w by MALS as 32.38 ± 0.31 MDa for P22-GalA Procapsid (PC), 31.87±0.20 MDa for Expanded (EX), and 31.11±0.19 MDa for Wiffleball (WB). The net loss of 1.3 MDa from PC to WB is consistent with the expected mass loss associated with the 12 pentamers that are released upon WB formation. The middle image is an agarose gel shift assay of the P22 forms. Roughly 50% transformation is observed from PC to EX, whereas the WB is a complete transformation. TEM images show HA_{head}-P22 particles in the PC and WB form with similar sizes to the wt P22 morphologies. Scale bars are set at 200nm.