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Site-Directed Mutagenesis of the P22 Scaffolding protein using Polymerase Chain Reaction (PCR): The P22 assembler plasmid (pET11a (Novagen)) contains the gene for scaffolding protein (SP) upstream from the gene for coat protein. The SP gene is flanked by the upstream restriction enzyme site NdeI and downstream site BamHI. In order to make P22 virus cages with truncated scaffolding protein (SP_{238}) we used PCR to create a gene for truncated SP flanked by the restriction enzyme sites NdeI and BamHI. We then digested both the PCR product containing the gene for SP_{238} and the assembler plasmid with NdeI and BamHI and ligated the SP_{238} gene into the P22 assembler plasmid. The oligonucleotide primers for the mutagenesis are (Forward: 5'-GGAATTCCATATGGAGCTCCCATGGATGTGTACTCGACTATCCG-3' and Reverse: 5'-AAGGATCCTTATCGGATTCCTTTAAGTTTTGCCTTTAGCTTGCGG-3') along with the template gene SP_{238} (in a background of pET3a (Novagen). These were used to create a SP_{238} gene with the upstream restriction enzyme site NdeI and downstream site BamHI. The SP_{238} gene was successfully cloned into the P22 assembler plasmid, resulting in a plasmid containing both the truncated SP and coat protein, and was confirmed by DNA sequencing. To add the polyanionic peptide (ELEAE) to the N-terminus of the truncated scaffolding protein the mutagenic oligonucleotide primer (5'-CATATGGAGCTCGAAGCTGAGCCATGGCTGGTGCCG-3') and the P22 SP_{238} DNA clone as a template were used. This mutation, called P22 $SP_{Glu\ 238}$, was confirmed by DNA sequencing.

Protein Expression: The P22 viral coat protein and scaffolding protein were heterologously expressed in BL21(DE3) cells. High levels of protein expression were induced with IPTG and yielded assembled viral cages devoid of nucleic acid. The viral cages were purified to homogeneity by lysis (50 mM phosphate, 100 mM NaCl, pH 7.6) followed by centrifugation (12,000xg, 60 minutes) to remove cell debris and ultra-centrifugation (48,000 rpm, 50 minutes) to pellet the virus particles. The resulting virus containing pellet was resuspended in buffer (50 mM phosphate, 25 mM NaCl, pH 7.6) and purified over a Sephacryl S-500 High Resolution column (GE Healthcare).

Iron Oxide Mineralization: The various P22 constructs were suspended in buffer (100 mM MOPS, 37.5 mM Na_2SO_4, pH 6.8) at a concentration of 6 mg/mL and treated with Fe^{2+} with air as the only oxidant. Fe^{2+} was added to the P22 solution at a rate of 166 Fe per viral capsid per minute to a final amount of 20,000 Fe atoms per viral capsid. Mineralized virus samples were purified using a Sephacryl S-500 High Resolution column (GE Healthcare) and the elution was monitored simultaneously at 280 nm (protein) and 350 nm (mineral).

Transmission Electron Microscopy (TEM): TEM data were collected on a Leo 912, with Ω filter, operating at 80 keV. Samples were concentrated using microcon ultrafilters (Microcon YM-100) with 100 kDa Mw cutoff and transferred to carbon coated copper grids. Samples were imaged both stained with uranyl acetate and unstained.

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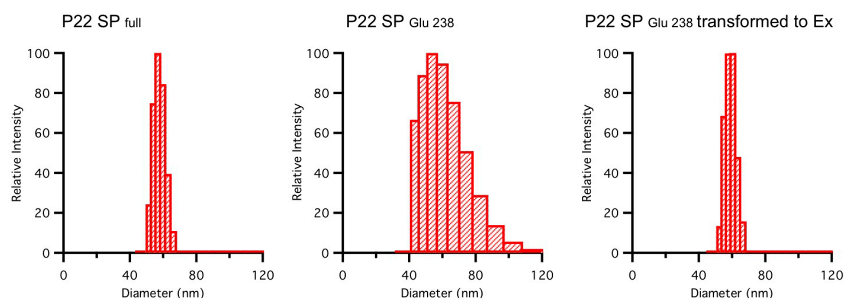
Dynamic Light Scattering (DLS). Dynamic light scattering (DLS) measurements were made on a Brookhaven Instruments ZetaPals (phase analysis light scattering) particle size analyzer. DLS was measured at 90° using a 661 nm diode laser, and the correlation functions were fit using a non-negatively constrained least-squares analysis.



Supporting Figure 1. DLS analysis of various P22 cage constructs utilized in this study.