

Supporting information

for

Viral capsids as templates for the production of monodisperse Prussian blue nanoparticles.

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General experimental procedures

Unless otherwise stated, all reagents and chemicals were obtained from commercial sources and used without further purification. All FPLC chromatograms were performed using buffers of the following composition: 50 mM sodium acetate or Tris-HCl, 500 mM NaCl, and 1 mM DTT at pH 5.0 (sodium acetate) or pH 7.5 (Tris). The FPLC column used for analytical purposes was superose 6 3/100 (Amersham biosciences) with a 20 μ L injection volume. In some cases, the injection loop was changed to 100 μ L to purify larger amounts of the PB-CCMV biohybrid. Sephadex G-100 columns were run with identical buffers as for the FPLC. The dialysis eppendorfs were obtained from BIO-RAD and had a molecular weight cut off (MWCO) of 3000 Da. All compounds used for the preparation of buffers were of analytical quality. Buffers were prepared from ultrapure (Milli-Q) water. UV-Vis spectra were recorded using a Cary 50 UV spectrophotometer. Transmission Electron Microscopy (TEM) was performed as follows: Formvar-carbon coated grids were hydrophilized in a glow discharge apparatus and 5 μ L of the desired sample was applied onto the grid. After leaving the sample for 1 minute, the excess of liquid was drained using a piece of filter paper. Ammonium molybdate (5 μ L, 5% w/v) or uranyl acetate (5 μ L, 5% w/v) was then applied and the drying procedure was repeated. The size distribution of the particles observed was determined by measuring the diameter of more than 80 PB-CCMV nanoparticles.

Calculation of the concentration of PB building units inside the viral capsid (ICP-OES)

$(\lambda_{emission})$	ppb (μ g/liter)	ppb μ mol/liter		ppb (μ mol/liter)		ppb (μ mol/liter)
Fe (259.9 nm)	659	12.203704	PB building units	6.10185185	PB building units	6.101851852
S (182.0 nm)	80	2.2566996	coat protein	0.75223319	capsid	0.004179073
Ratio (Fe/S)	8.2375	5.4077662	Ratio	8.11164931	Ratio	1460.096875

$$\text{Volume of capsid (L)} = 3.05363\text{E-}21$$

$$\text{PB building units (mol) per capsid} = 2.43349\text{E-}21$$

$$[\text{PB building units}] \text{ (M) in capsid} = \mathbf{0.796919187}$$

S1

Purification by SEC (sephadex G-100 column)

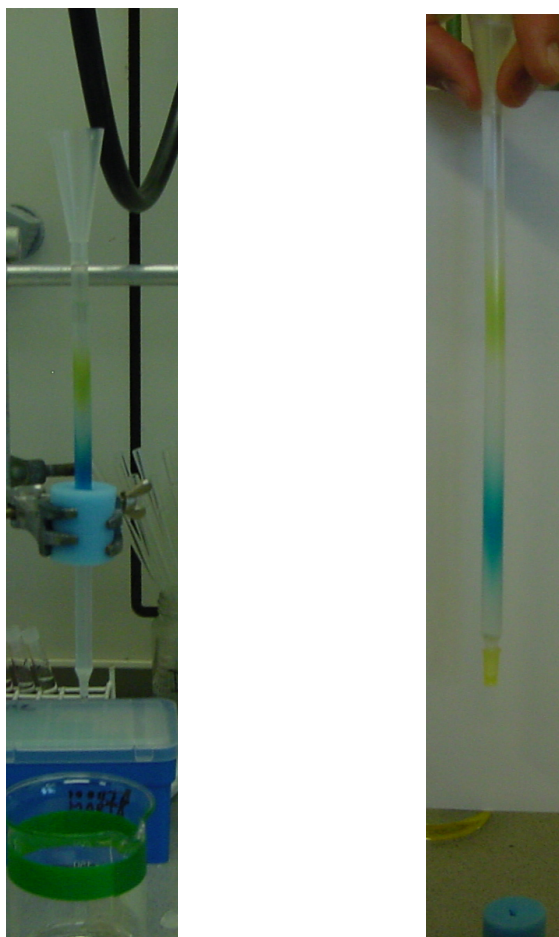


Figure S1 Sephadex G-100 column showing a very efficient separation, shortly after the column was loaded, of PB-CCMV and PB precursors.

S2

UV-Vis spectra of PB-CCMV and control experiments

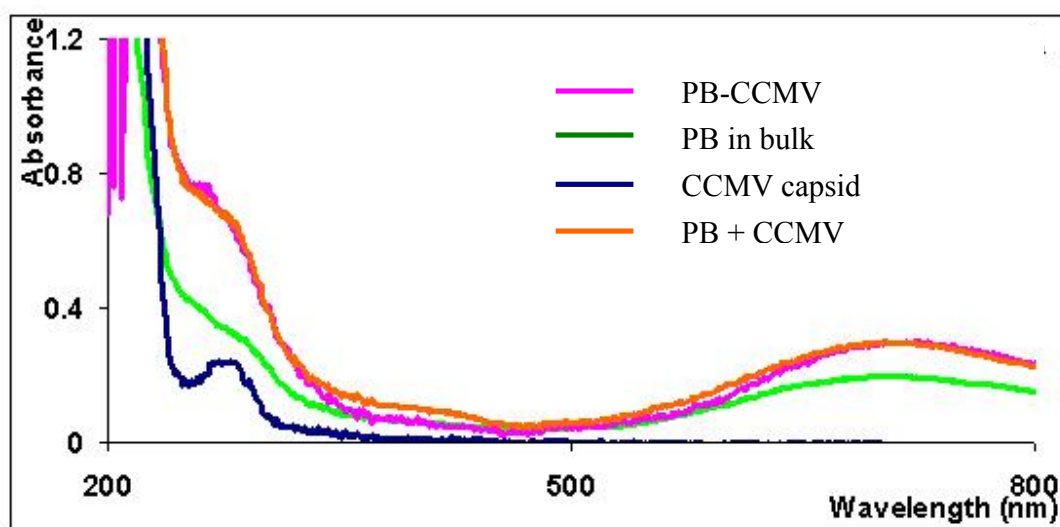


Figure S2 UV-Vis spectra of PB inside the CCMV capsid, PB in bulk, the CCMV capsid, and a linear combination of the spectra of PB and CCMV capsid.

S3

Synthesis of PB in bulk followed by UV-Vis spectroscopy

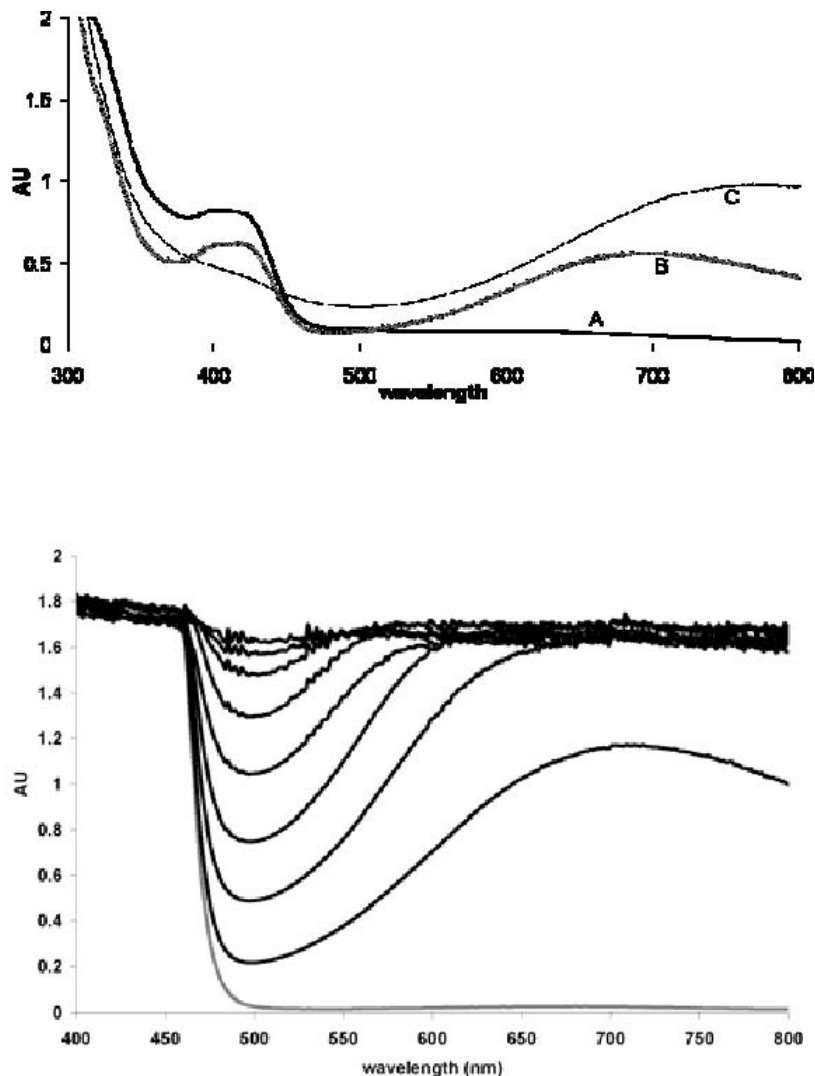


Figure S3 *Top*: several UV-Vis traces of Prussian blue precursors and the product after irradiation. All concentrations are 1 mM. A) Prussian blue precursors; B) 1.0 M solution of Prussian blue, irradiated for >6 hrs (and subsequently diluted and measured); C) 1.0 M sample, irradiated, diluted to 10 mM, and irradiated for a further 6 hrs. *Below*: UV-Vis spectra recorded at different intervals showing the sharp increase of absorption at 720 nm. Even at this high concentration (0.1 M), no absorption at 600 - 800 nm is observed when Prussian blue is not present (grey curve). The increasing height of the curves occurred within 15 min by irradiation of the sample with a 405 nm laser.

S4

TEM images of PB prepared in the absence of capsid

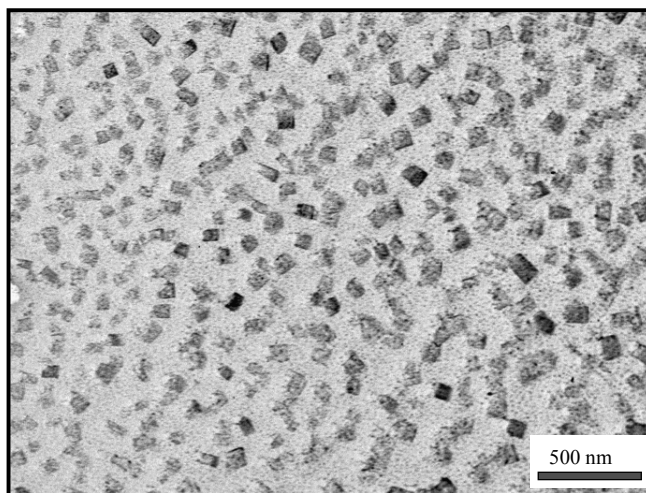
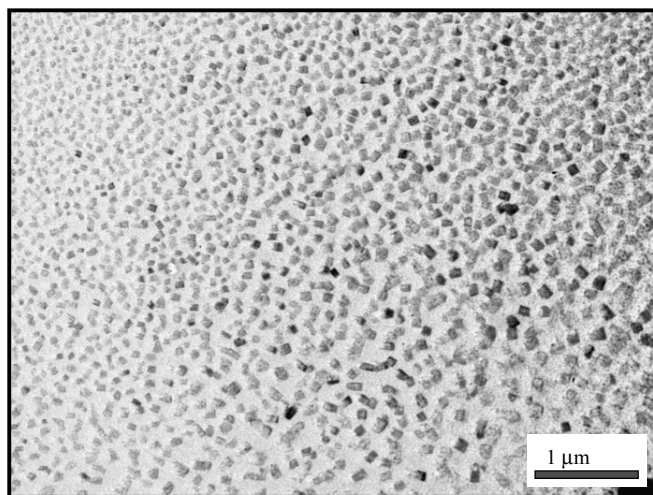
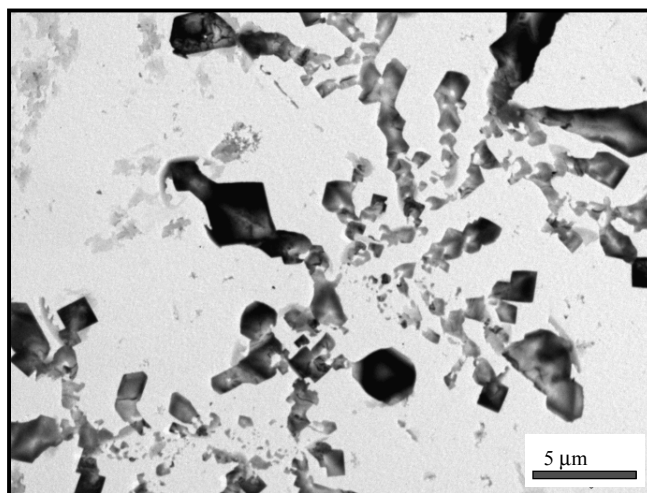


Figure S4 TEM images of bulk Prussian Blue without staining.