Supplementary information

Size and Geometry Dependent Protein-Nanoparticle Self-Assembly

Mrinmoy De,[a] Oscar R. Miranda,[a] Subinoy Rana[a] and Vincent M. Rotello*[a]

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts, 01003, USA.; Tel: (+1) 413-545-2058; E-mail:rotello@chem.umass.edu.

Materials:

All the reagents, bovine serum albumin (BSA) and acid phosphatase (PhosA, from potato) were purchased from Sigma and used as received. Green fluorescence protein (GFP) was expressed according to the known procedure from the Starter cultures from a glycerol stock of GFP (Enhanced GFP (eGFP) was cloned into the pET21d vector (Novagen) where Hisx6 tag was located at N-terminus) in BL21 (DE3).[1] The nanoparticles are prepared according to our published procedure.[2]

Isothermal Titration Calorimetry:

An isothermal calorimeter purchased from Microcal Inc. (Northampton, MA), was used in all isothermal titration calorimetric (ITC) experiments, operated at 30 °C. Each microcalorimetric titration experiment consisted of 30-45 successive injections of a constant volume (6 μL/injection) of GFP, BSA and phosA solution (100 μM) into the reaction cell (1.4 mL) charged with four different NP solutions (2 μM for GFP and 5 μM for BSA and PhosA) in the same buffer (5 mM sodium phosphate buffer, pH 7.4). The control experiment was also carried out by titrating proteins at same concentration in to the buffer only. The final titration curves were obtained by subtracting the control enthalpies from the enthalpies measured in the titration experiments. The Origin program

supplied by Microcal Inc. was used to calculate the binding constant (K_S), binding ratios (n) and molar enthalpy change (ΔH) from the titration curve. The molar Gibbs free energy changes (ΔG) and entropies (ΔS) of reaction were calculated from the experimentally determined K_S and ΔH values.

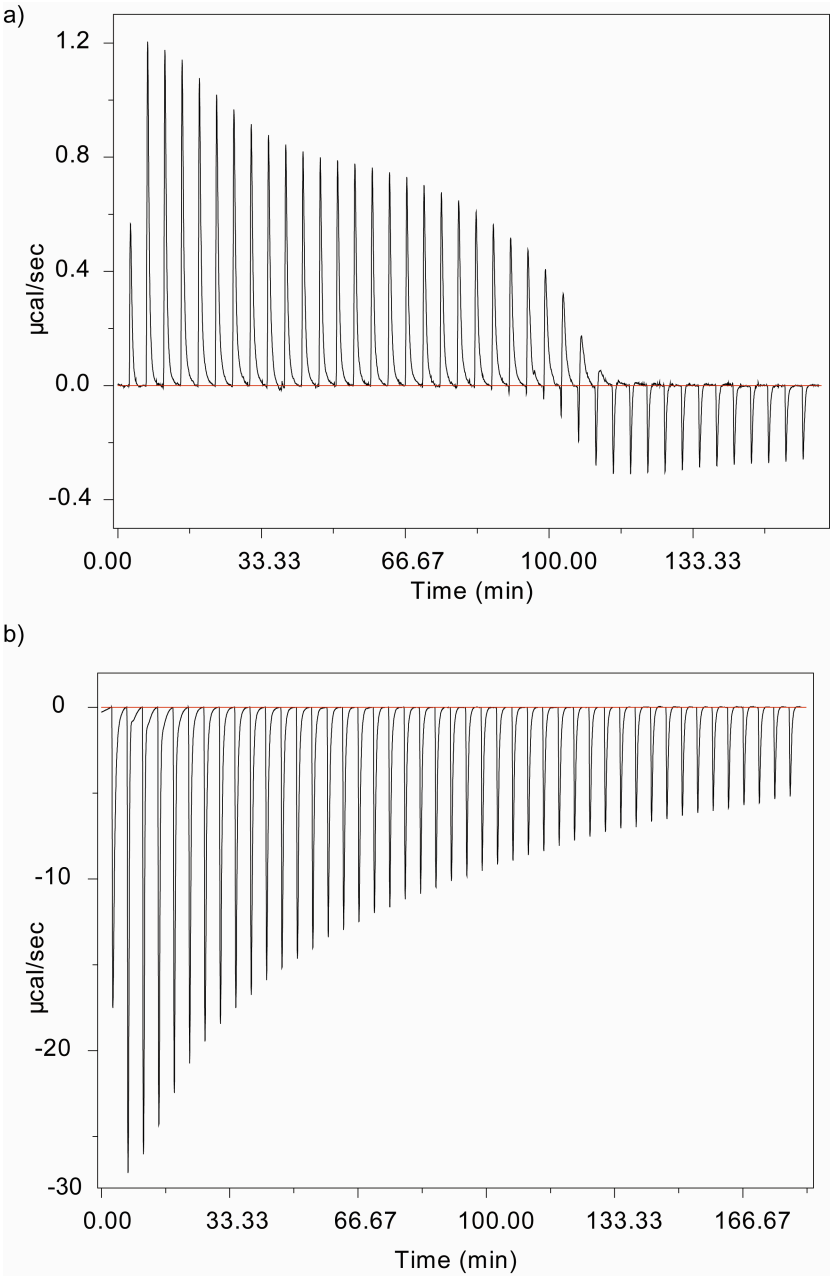


Figure S1. Raw ITC data for the nanoparticle-protein interaction resulting: a) aggregated and precipitated (BSA-**NP3**) and b) soluble (PhosA-**NP2**) complex after the completion of ITC titration.

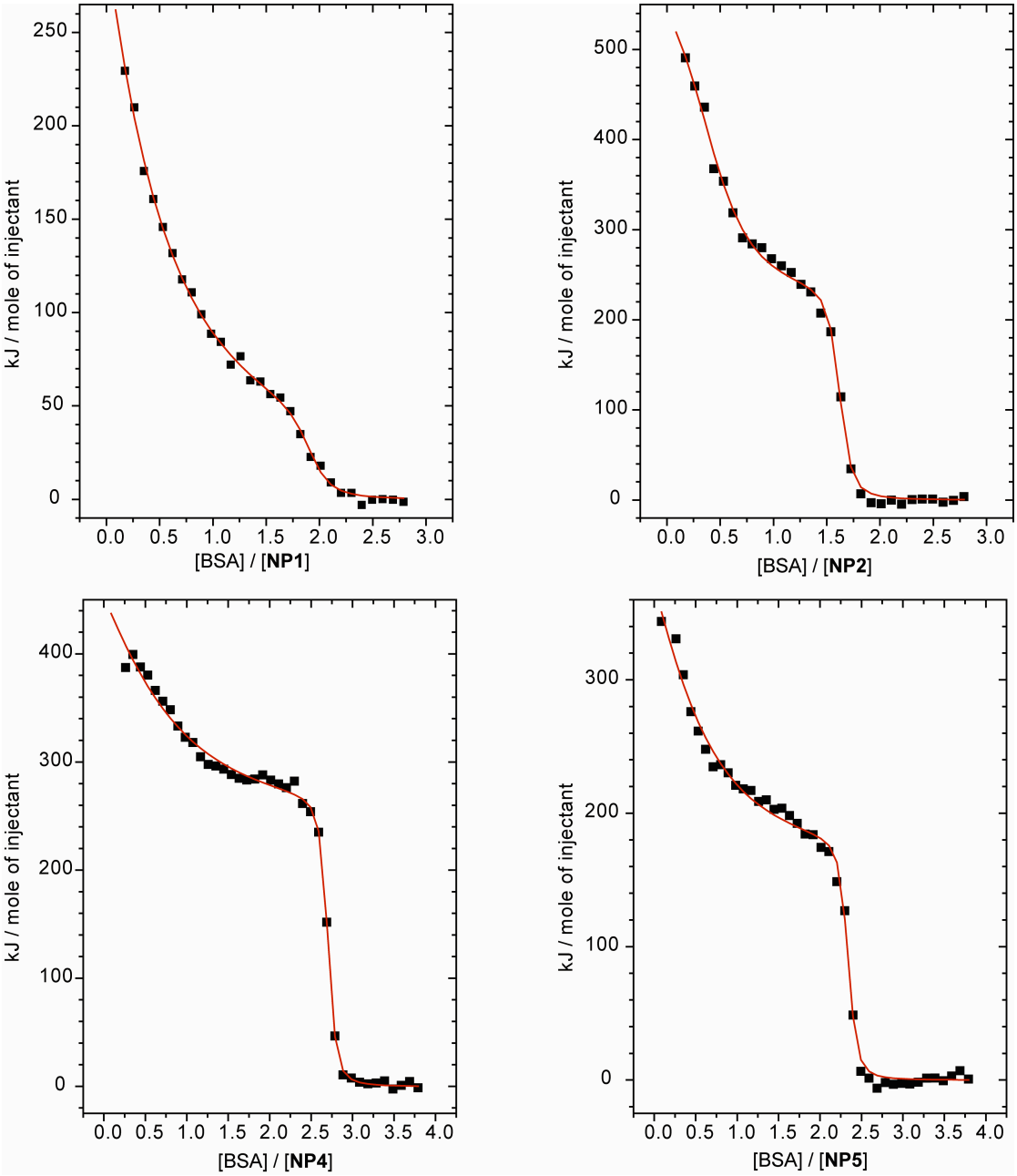


Fig. S2. ITC analysis of the interaction of BSA with cationic nanoparticles in 5 mM sodium phosphate buffer (pH = 7.4). The initial concentrations of nanoparticles and BSA were 5 μM and 100 μM, respectively.

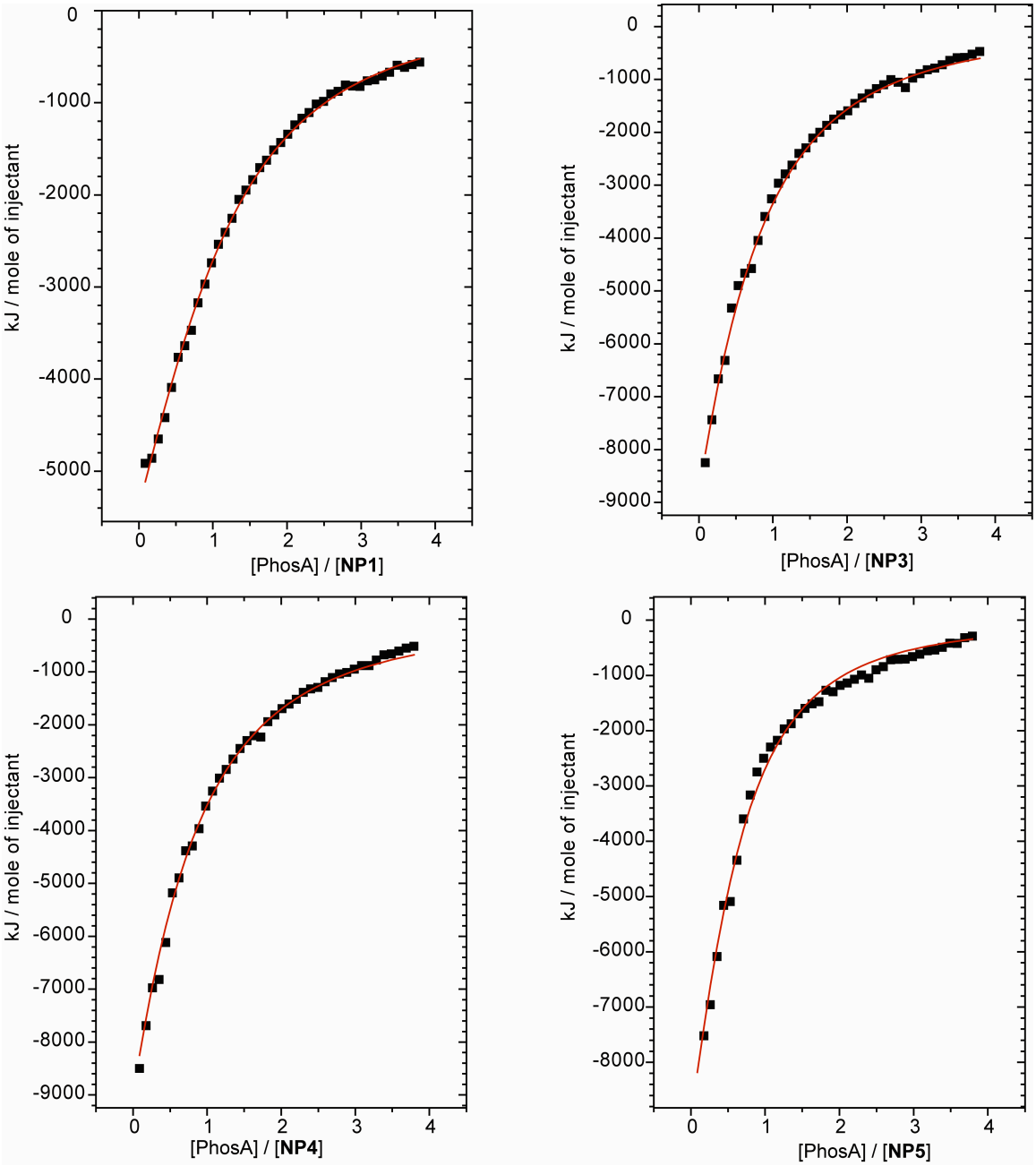
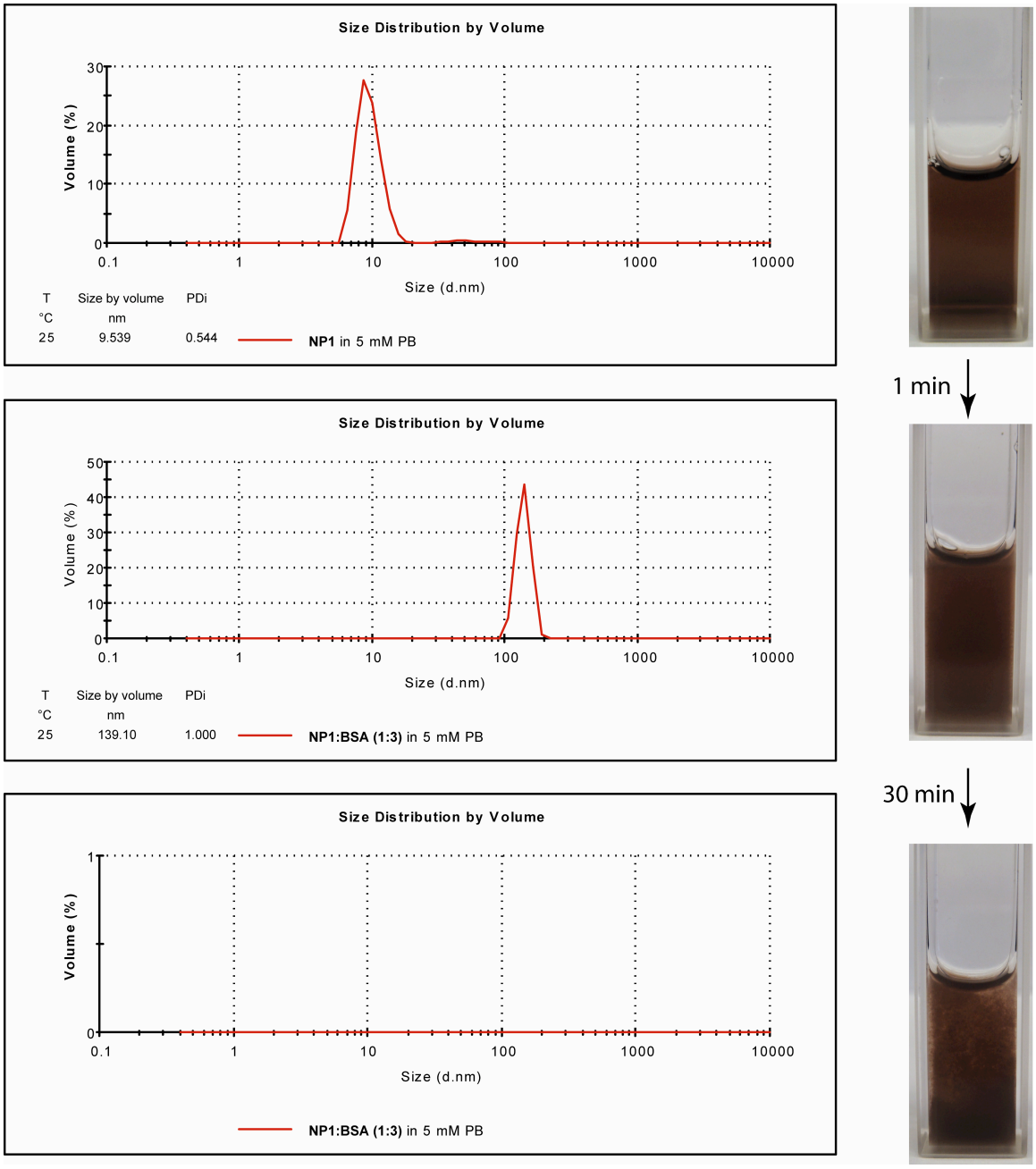


Fig. S3. ITC analysis of the interaction of PhosA with cationic nanoparticles in 5 mM sodium phosphate buffer (pH = 7.4). The initial concentrations of nanoparticles and PhosA were 5 μM and 100 μM, respectively.

Dynamic Light Scattering:

Gold nanoparticles were dissolved in 5 mM sodium phosphate buffer, pH 7.4 to make a 2 μM solution and their size was measured on a MALVERN Zetasizer Nano ZS

instrument. The similar experiment was repeated with the mixture of 2 μM nanoparticle and 6 μM BSA. Just after the addition of BSA the aggregation was started and the average diameter was 139 nm. After 15 minute the solution becomes cloudy and the measurement was not possible any more. After 2 hours we saw the complete precipitation of the protein nanoparticle conjugates.



1 M. De, S. Rana, V. M. Rotello, *Macromol. Biosci.*, 2009, **9**, DOI: 10.1002/mabi.200800289
2 C. C. You, O. R. Miranda, B. Gider, P. S. Ghosh, I. B. Kim, B. Erdogan, S. A. Krovi, U. H. F. Bunz, and V. M. Rotello, *Nature Nanotechnology*, 2007, **2**, 318.