Supporting information

Proteins

Human serum albumin from Sigma (A3782, fatty acid free, 99% pure) was purified from dimer and contaminating proteins using gel filtration on a 200 x 3.4 cm Sephadex G50 column in ammonium acetate buffer, pH 6.5 (75 mg protein in 20 ml buffer was applied at a time). Fractions containing HSA monomer were pooled, lyophilized and desalted by gel filtration on a G25 Sephadex superfine column in Millipore water. Analytical gel filtration showed that after this procedure more than 99% of the protein was in the monomeric form.

Human calmodulin was expressed from a Pet3a plasmid in *E. coli* Bl21 De3 PlysS Star, and purified using heat treatment, DEAE cellulose anion exchange chromatography, hydrophobic interaction chromatography on a phenyl sepharose column, gel filtration and DEAE sephacel anion exchange chromatography, as described in reference [1].

Human α-lactalbumin was purified from human milk using ammonium sulphate precipitation, hydrophobic interaction chromatography on a phenyl sepharose column, and dialysed, as described in reference [2].

Human carbonic anhydrase II ("pseudo wildtype" with the C206S mutation) was expressed from the pCApwt plasmid (a kind gift from Professor Nalle Jonsson, Linköping University) in *E. coli* Bl21 De3 PlysS Star. The cells were harvested by centrifugation at 6000 g for 5 minutes and the pellet was frozen. The protein was purified using ion exchange chromatography and gel filtration as follows. The cell pellet was suspended in buffer A (10 mM Tris/HCl, pH 7.5 with 1 mM EDTA; total volume 200 ml for pellet from a 5.4 litre culture), sonicated on ice, poured into an equal volume of boiling buffer A, heated to 85 ^{o}C, and then rapidly cooled on ice. Precipitated *E. coli* protein were removed by centrifugation at 15000 rpm for 15 minutes, and the supernatant was pumped onto a (3.4 x 25 cm) DEAE cellulose anion exchange column and eluted using a NaCl gradient from 0 to 0.2 M in buffer A. Carbonic anhydrase II fractions were

lyophlized, dissolved in 25 ml H_2O, and applied to a 3.4 x 180 cm sephadex G50 superfine gel filtration column. The column was packed and operated in 50 mM ammonium acetate buffer, pH 6.5. The pure carbonic anhydrase II fractions were pooled and dialyzed against Millipore water.

Protein G B1 was expressed in *E. coli* Bl21 De3 PlysS Star from a synthetic gene cloned into PetSac plasmid, and purifed using anion exchange chromatography (DEAE cellulose column) and gel filtration (3.4 x 180 cm sephadex G50 superfine column) as described in reference [3].

Lysozyme from chicken egg white was from Sigma and purified using ion exchange chromatography and gel filtration. Ovalbumin and Fibrinogen were purchased from Sigma and used without further purification.

The purity of proteins was confirmed by SDS PAGE and agarose gel electrophoresis, isoelectric focussing and ^{1}H NMR spectroscopy.

Oleic acid was from Sigma (O1008, 99% pure).

Nanoparticles. N-*iso*-propylacrylamide-co-N-*tert*-butylacrylamide (NIPAM:BAM) copolymer particles of diameters from 70-700 nm and with three different ratios of the co-monomers (85:15, 65:35 and 50:50 NIPAM:BAM) were synthesized in SDS micelles as described previously [4] although higher SDS concentrations were used in the present work, resulting in similarly sized particles. The procedure for the synthesis was as follows: 2.8 g monomers (in the appropriate wt/wt ratio), and 0.28 g crosslinker (N,N-methylenebisacrylamide) was dissolved in 190mL MilliQ water with 0.8, 0.575, 0.32, 0.04 and 0.023 g SDS for the 70, 120, 200, 400 and 700 nm particles respectively and degassed by bubbling with N_2 for 30 minutes. Polymerisation was induced by adding 0.095g ammonium persulfate initiator in 10 mL MilliQ water and heating at 70 °C for 4 hours [5]. Particles were extensively dialysed against MilliQ water for several weeks, changing the water daily, until no traces of monomers, crosslinker, initiator or SDS could

be detected by proton NMR (spectra were acquired in D_2O using a 500 MHz Varian Inova spectrometer). Particles were freeze-dried and stored in the fridge until used.

Quantum dots. 16 nm hydrophilic polymer coated quantum dots (7) were provided by W. Parak, Ludwig Maximilians University, München.

Isothermal titration calorimetry. HSA was titrated from a 38 μM stock into nanoparticle solution in 10 mM Hepes/NaOH buffer, pH 7.5 with 150 mM NaCl and 1 mM EDTA at 5 ^{o}C. The reaction cell contained 1mg/ml of the nanoparticles, which corresponds to 9.3, 1.84, 0.4, 0.05 and 0.0093 nM for the 70, 120, 200, 400 and 700 nm particles respectively. The first protein injection was 1 μl followed by 59 injections of 5 μl. The protein concentration in the first HSA stock was determined by amino acid analysis after acid hydrolysis (analysis purchased from BMC Uppsala). The concentration in subsequent stock solutions was determined by UV-absorbance at 280 nm using the extinction coefficient obtained from the first stock.

ITC data were also obtained for titration of proteins into solutions of quantum dots (16 nm diameter) in 10 mM Hepes/NaOH, 0.15 M NaCl, 1 mM EDTA, pH 7.5. HSA (38 μM) was titrated into an 800 nM quantum dot solution and α-lactalbumin (230 μM) was titrated into a 500 nm solution of quantum dots. The first protein injection was 1 μl followed by 39 injections of 5 μl. The stock solution for α-lactalbumin was prepared from Ca^{2+}-depleted protein.

Data analysis. Data were fitted using the software ORIGIN (Microcal, Northhampton, MA), assuming a single set of identical sites binding isotherm with association constant, stoichiometry, and ΔH as variable parameters. The equilibrium association constant, K_A, for adsorption of HSA to a nanoparticle (np) with N equal and independent sites is:

$$K_A = \frac{[nps \bullet HSA]}{[nps][HSA]} \qquad (1)$$

where nps are HSA-binding sites on nanoparticles. The degree of saturation goes from 0-1 for each site and is defined as:

$$f_{sat} = \frac{K_A[HSA]}{1 + K_A[HSA]} \qquad (2)$$

Using that $[HSA]=[HSA]_{tot} - N^* [nps \cdot HSA]$ and $[np]=[np]_{tot} - [nps \cdot HSA]$, f_{sat} can be expressed as:

$$f_{sat} = \frac{1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{NK_A[np]_{tot}}}{2} - \sqrt{\left(1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{K_A N[np]_{tot}}\right)^2 - \frac{4[HSA]_{tot}}{N[np]_{tot}}} \qquad (3)$$

The heat absorbed or released by the adsorption of the protein to the particles in an ITC experiment is:

$$Q = \Delta H^o n_{HSAbound} = \Delta H^o V_{cell} c_{complex} = \Delta H^o V_{cell} N[np]_{tot} f_{sat} \qquad (4)$$

where $n_{HSA\ bound}$ is the number of moles of adsorbed HSA, ΔH^o is the enthalpy change upon binding (J/mol HSA) and V_{cell} is the cell volume. Combining eq. 3 and 4 yields:

$$Q = \frac{V_{cell}[np]_{tot} N\Delta H^o}{2}\left(1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{K_A N[np]_{tot}} - \sqrt{\left(1 + \frac{[HSA]_{tot}}{N[np]_{tot}} + \frac{1}{K_A N[np]_{tot}}\right)^2 - \frac{4[HSA]_{tot}}{N[np]_{tot}}}\right) \qquad (5)$$

Taking into account the displaced volume, the change in heat content from injection i-1 to i is

$$\Delta Q(i) = Q(i) - Q(i-1) + \frac{dV_i}{V_0}\left[\frac{Q(i) + Q(i-1)}{2}\right] \qquad (6)$$

where Q_i is the heat content after injection i, V_0 is the initial cell volume and dV_i is the injected volume.

By an iterative fitting procedure, using standard Marquardt methods, N, K and ΔH^o can be obtained from eq. 5 and 6.

Thiol-linked Nanopartices. 50:50 NIPAM:BAM:acrylic acid copolymer nanoparticles of 70 nm diameter were synthesized as above, with the addition of appropriate amounts of acrylic acid to obtain particles with on average less than one carboxyl group on the particle surface. Acrylic acid was distilled under reduced pressure before use to remove stabilizers. A stock solution of 1 mg/ml acrylic acid was prepared, and 10 µL of this solution was added to the monomer solution (190mL). Reaction proceeded at 70 °C for 4 hours followed by dialysis against MilliQ water for a couple of weeks. The covalent attachment of homocysteine to the acrylic acid groups involves the formation of amide bonds between the primary amino group of the amino acid and carboxylic acid [6]. Briefly, 50 mL of the particle solution (after dialysis) was adjusted to pH 5 by small amounts of 5 M NaOH. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was added to a final concentration of 150 mM to activate the carboxylic acid moieties. After 1 hour of incubation with stirring at 4 °C, 0.4 g homocysteine was added and the pH was readjusted to 5. The reaction mixture was incubated for 5 h at room temperature under stirring, dialysed extensively against MilliQ water to ensure that no residual chemicals remained, and freeze-dried.

Conjugation of nanoparticles to gold surfaces for SPR studies. The SIA Au kit (BIAcore AB, Uppsala) was used for sensor chip preparation. Thiol-linked nanoparticles were dissolved at 0.2 mg/ml in 20 mM sodium phosphate buffer, 100 mM NaCl, pH 7.5) on ice and 120 µl was applied to a 10 x 10 mm gold surface for four hours or over night, before the surface was rinsed with H_2O, dried and assembled in a sensorchip cassette. The change in response units after coupling of the nanoparticles to gold reveals the amount of

immobilized nanoparticles. A densely packed layer of 70 nm particles yields 35 ng/mm^2 and the increase in response obtained (26 ng/mm^2) corresponds to 74 % of this number.

Surface Plasmon Resonance (SPR) experiments. SPR studies of protein associating to the nanoparticles were performed using a BIAcore 3000 instrument (BIAcore AB, Uppsala). The flow buffer contained 10 mM Tris/HCl pH 7.4 with 3 mM EDTA, 150 mM NaCl and 0.005% Tween20, and was filtered (0.2 μm filter) and degassed for 30 minutes. The sensorchip surface with attached particles was washed for 5 hours at a flow rate of 50-100 μl/min and then equilibrated at 10 μl/min for 30 minutes until the baseline was stable. Each protein was dissolved at 5-20 μM concentration in the flow buffer and injected over the sensorchip surface for 30 minutes to study the association kinetics, followed by buffer flow for 5-24 hours at 10 μl/min to obtain a stable baseline before the next injection of protein.

1. Waltersson, Y.; Linse, S.; Brodin, P.; Grundstrom, T., *Biochemistry* **1993,** *32*, (31), 7866-7871.
2. Svensson, M.; Hakansson, A.; Mossberg, A. K.; Linse, S.; Svanborg, C., *Proceedings of the National Academy of Sciences of the United States of America* **2000,** *97*, (8), 4221-4226.
3. Lindman, S.; Xue, W.-F.; Szczepankiewicz, O.; Bauer, M. C.; Nilsson, H.; Linse, S., *Biophys. J.* **2006,** *90*, (8), 2911-2921.
4. Wu, X.; Pelton, R.; Hamielec, A.; Woods, D.; McPhee, W., *Colloid Polym. Sci.* **1994,** *272*, 467.
5. Lynch, I.; Miller, I.; Gallagher, W. M.; Dawson, K. A., *Journal of Physical Chemistry B* **2006,** *110*, (30), 14581-14589.
6. Bernkop-Schnurch, A.; Leitner, V.; Moser, V., *Drug Development and Industrial Pharmacy* **2004,** *30*, (1), 1-8.
7. Liedl, T.; Keller, S.; Simmel, F. C.; Radler, J. O.; Parak, W. J., *Small* **2005,** *1*, (10), 997-1003.