Supplementary information:

Inactivation and adsorption of human carbonic anhydrase II by nanoparticles

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Isothermal titration calorimetry

We describe here briefly the model used to obtain the biding equilibrium constant K_a and number of proteins adsorbed on the particle surface, n. We apply a simple model in which the nanoparticles are assumed to have n independent and equal protein binding sites (the number of sites would change with the type of protein since the particle surface is considered homogeneous).

The equilibrium association constant is

$$K_a = \frac{[NPs-HCAII]}{[NPs]_f[HCAII]_f} \quad \text{(S1)}$$

where NPs are the protein binding sites on the particle and can be expressed as function of the total concentration of nanoparticles, $[NP]_t$

$$[NPs]_t = n[NP]_t \quad \text{(S2)}$$

Taking into account eq (S1), (S2) and the mass balances for HCAII and NPs

$$[HCAII]_t = [HCAII]_f + [NPs - HCAII] \quad \text{(S3)}$$

$$[NPs]_t = [NPs]_f + [NPs - HCAII] \quad \text{(S4)}$$

We can obtained an expression for the fraction of sites with bound protein, *f*, as a function of the total protein and nanoparticles concentrations $[HCAII]_t$ and $[NP]_t$

$$f = \frac{1}{2}\left[1 + \frac{[HCAII]_t}{n[NP]_t} + \frac{1}{nK_a[NP]_t} - \sqrt{\left(1 + \frac{[HCAII]_t}{n[NP]_t} + \frac{1}{nK_a[NP]_t}\right)^2 - \frac{4[HCAII]_t}{n[NP]_t}}\right] \text{ (S5)}$$

The amount of heat absorbed or released in each titration Q depends on the amount of protein bound and is given by

$$Q = nf[NP]_t V_{cell}\Delta H \quad \text{(S6)}$$

where V_{cell} is the volume of the ITC cell and ΔH the molar enthalpy change. Combining equations (S5) and (S6):

$$Q = \frac{n[NP]_t V_{cell}\Delta H}{2}\left[1 + \frac{[HCAII]_t}{n[NP]_t} + \frac{1}{nK_a[NP]_t} - \sqrt{\left(1 + \frac{[HCAII]_t}{n[NP]_t} + \frac{1}{nK_a[NP]_t}\right)^2 - \frac{4[HCAII]_t}{n[NP]_t}}\right] \text{ (S7)}$$

The change of heat from injection *i*-1 to 1 is given by

$$\Delta Q_i = Q_i - Q_{i-1} + \frac{dV_i}{V}\left(\frac{Q_i - Q_{i-1}}{2}\right) \text{ (S8)}$$

where Q_i is the heat amount after injection *i*, V is the volume in the cell and dV_i the injected volume. Using equations (S7) and (S8), n, K_a and ΔH can be calculated using an iterative fitting, based on standard Marquant methods. ΔS can be calculated consequently taking into account that:

$$\Delta G = -RTLnK_a = \Delta H - T\Delta S \quad \text{(S9)}$$

The dissociation constant is $K_d = 1/K_a$.

Supplementary figures

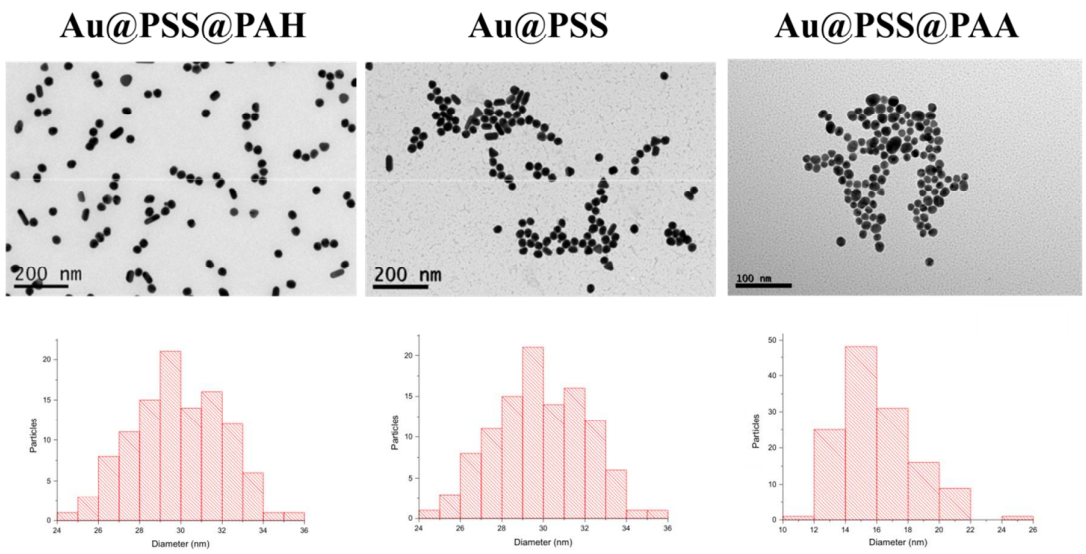

Figure S1: Upper row) TEM images of the polyelectrolytes coated gold nanoparticles and lower row) particle size distribution obtained from the size measurement of 100 nanoparticles.

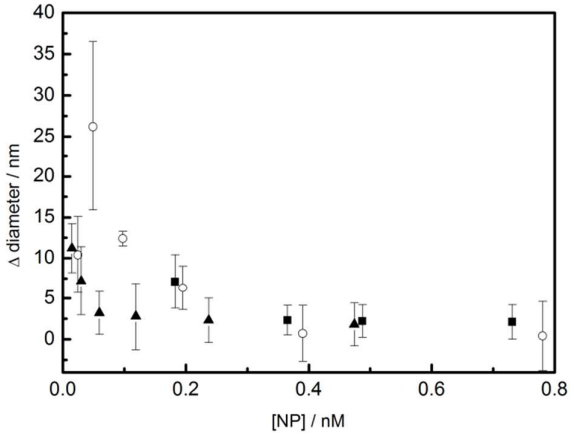

Figure S2: Variation of hydrodynamic diameter of polystyrene nanoparticles when titrated in a solution of 0.36 μM HCAII. ■) PSCOOH, ▲) PS, ○) PSNH2.

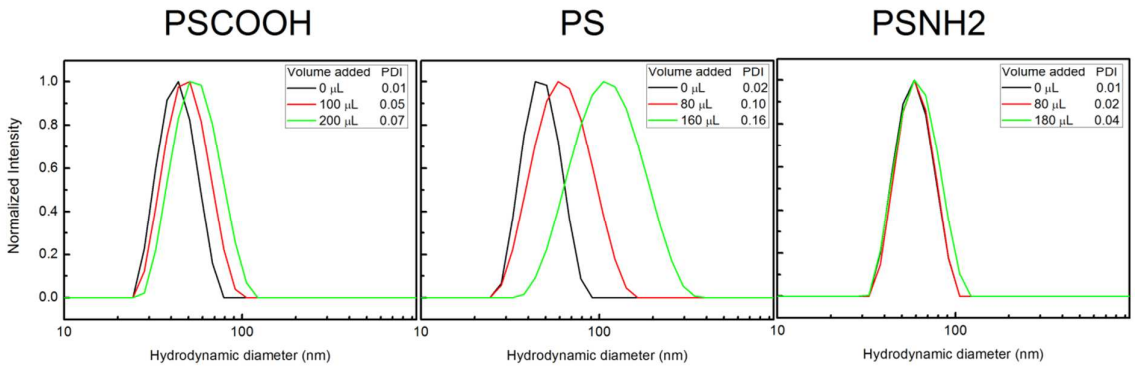

Figure S3: Nanoparticle size distribution by DLS of the different nanoparticles in absence (black) and presence of protein. Legend indicates the added volume of a stock solution of HCA in 5 mM Hepes/NaOH buffer pH 7.4 ([HCA] stock = 12 µM) as well as the polydispersity index (PDI) of the size distribution.

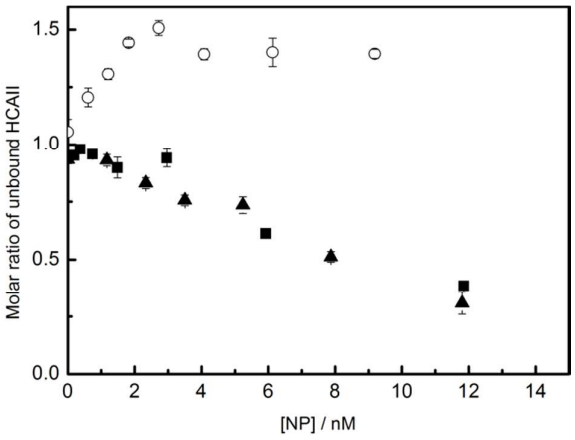

Figure S4. Variation in the molar ratio of unbound HCAII with the concentration of added polystyrene nanoparticles ■) PSCOOH, ▲) PS, ○) PSNH2 in 5 mM Hepes/NaOH buffer pH 7.4 at room temperature. [HCA] = 8µM,

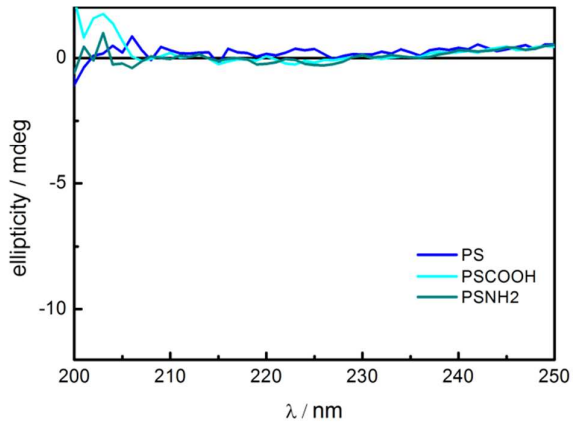

Figure S5: Far UV-CD spectra of polystyrene nanoparticles in buffer. [PS] = 1.8 nM , [PSCOOH] = 0.3 nM and [PSNH2]= 0.3 nM.

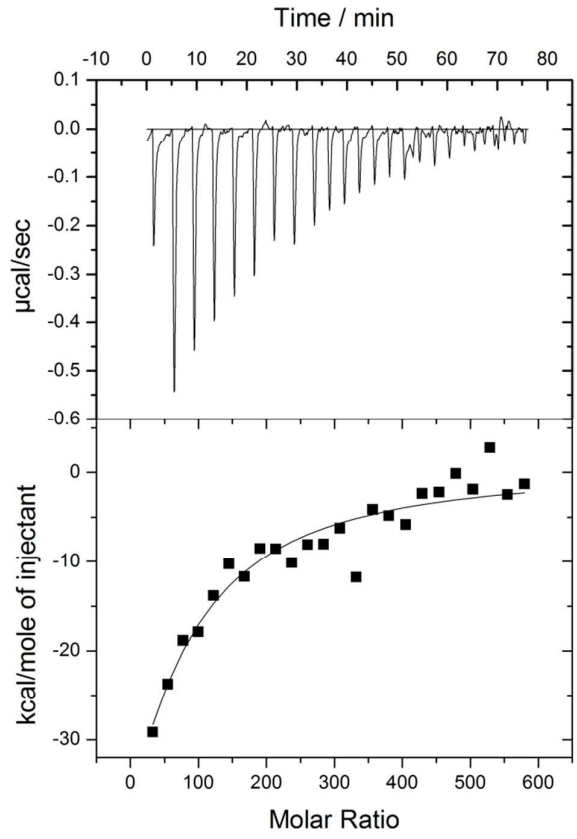

Figure S6: ITC data for the titrations of human serum albumin, HSA, into PSCOOH. [HSA]= 60 µM and [PS-COOH] = 18 nM in 5mM Hepes/NaOH, pH 7.4 at 25°C. The upper panel

show the raw data and the lower panel the integrated heats for each injection (■) and the best fit of a 1:1 binding model (line).