Supporting Information

Antibody-like Biorecognition Sites for Proteins from Surface Imprinting on Nanoparticles

Snehasis Bhakta,[†] Mohammad Saiful Islam Seraji,[†] Steven L. Suib,[*,†,§] James F. Rusling[*, †, §, ⊥, ‖]

[†]Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269-3060

[§]Institute of Materials Science, University of Connecticut, Storrs, Connecticut 06269-3136

[⊥]Department of Cell Biology, University of Connecticut Health Center, Farmington, Connecticut 06030

[‖]School of Chemistry, National University of Ireland at Galway, Galway, Ireland

*E-mail: James.Rusling@uconn.edu

*E-mail: steven.suib@uconn.edu

Synthesis of Silica nanoparticles

All chemicals and solvents were equilibrated at room temperature prior to every use. 40 mL of ammonium hydroxide (28-30%) was mixed with 345 mL of ethanol in a 1 L round bottom flask with continuous stirring at 600 rpm at room temperature. After 10 min stirring, 15 mL of Tetraethylorthosilicate, ≥99% (TEOS) were added to the mixture and stirring continued for 20 h at room temperature. The white milky suspension of silica nanoparticles (SiNP) was centrifuged at 3200 rpm for 10 min, and the precipitate was isolated and reconstituted in ethanol. The suspended particles were washed two more times with ethanol with centrifugation at 3200 rpm as before, then washed three more times with water as mentioned. Finally the SiNP precipitate was collected and resuspended in water and the colloidal solution stored at 4 ºC.

Optimization of the organosilane monomer ratios

Four different organosilanes were chosen to feature various amino acids like groups to enhance specific binding interaction with the template protein. The monomers were chosen to provide amine (NH_3^+), hydroxyl (OH), for hydrogen binding interactions and benzyl (C_6H_6), and propyl ($CH_2CH_2CH_3$) groups. Different combinations of silane monomers were used and the final combination gave highest binding strength and was used to obtain all the results in the paper. The same rations were used for both of the AAs due to similarity in number of amino acids such as leucine, threonine, glycine, and phenylalanine in their sequences. E.g., optimum ratios for lysine, phenylalanine, glycine in HSA and GOx are 1.6:1.1:1.6, respectively. At pH 7.3, both the template proteins are negatively charged (see pI values).

Stability of the template protein after rebinding with its artificial antibodies (AA)

The stability of the AA_{HSA}-HSA (HSA = human serum albumin,) bound conjugate was monitored by subsequent washing and measurement of the protein concentration in the supernatant. In brief, a 1:1 ratio of freshly prepared protein solution in 10 mM phosphate buffer (pH 7.3) was mixed with AA_{HSA} and incubated for 2 h in a slow rotation mode. Then the solution was centrifuged at 3200 rpm for 3 minutes and the supernatant solution was analyzed by a Bradford assay to determine the amount of protein bound to the antibodies. The conjugates were washed two more times in 30 min. interval and similarly a Bradford assay was performed on the supernatant to calculate the amount of proteins leached out. It was measured that almost nothing leached out even after three washing steps indicating that proteins were bound very strongly with AA. Figure S3 shows approximately 99 % of proteins remain bound with AA even after 2 h.

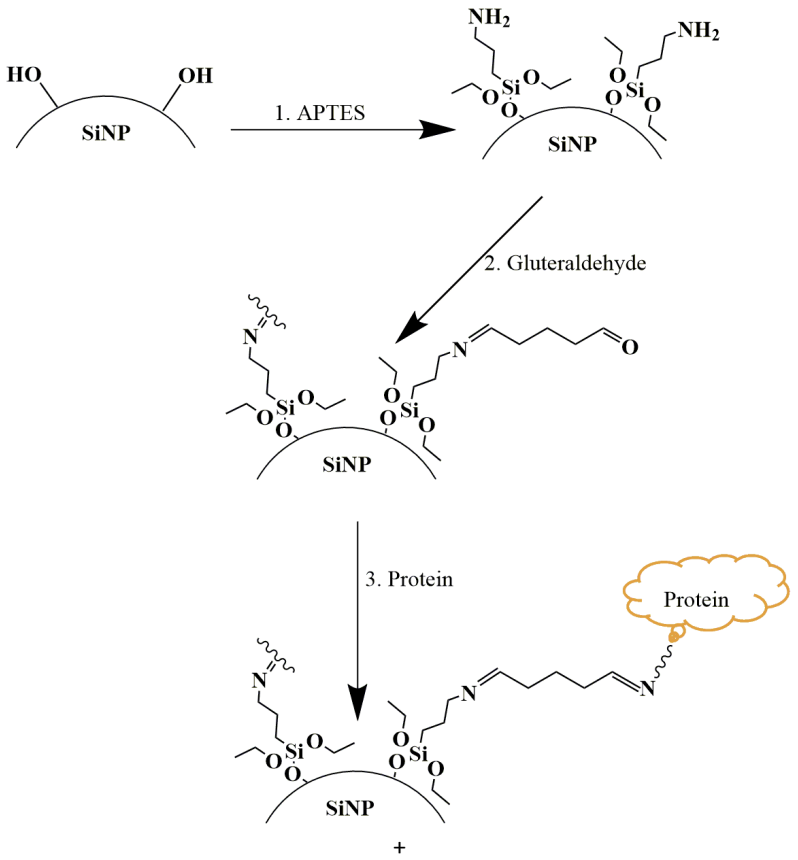

Figure S1. Schematic representation of the chemistry of synthesis of the artificial antibody sites on SiNPs

Control experiments were done by measuring non-specific binding proteins on SiNP by measuring the total protein remaining in solution with Bradford assays (see main article). The Langmuir-Freundlich constant (K_{LF}) was estimated from binding isotherm of human serum albumin (HSA) and glucose oxidase (GOx) on bare SiNPs using non-linear regression analysis. K_{LF} was found using the Langmuir-Freundlich equation:

$$X/X_m = K_{LF}C^{1/n}/(1+ K_{LF}C^{1/n})$$

Where X is the concentration of the protein bound per mg of artificial antibodies, AA, X_m is the maximum concentration of template proteins bound with its corresponding AA, K_{LF} is the Langmuir-Freundlich constant, C is the concentration of analyte and n is an empirical constant which is in between 0.4 and 0.5 for protein adsorption on solid surfaces. By considering X/X_m as X' and $C^{1/n}$ as C', K_{LF} was found by non-linear regression fitting of the isotherm data onto the Langmuir-Freundlich equation.

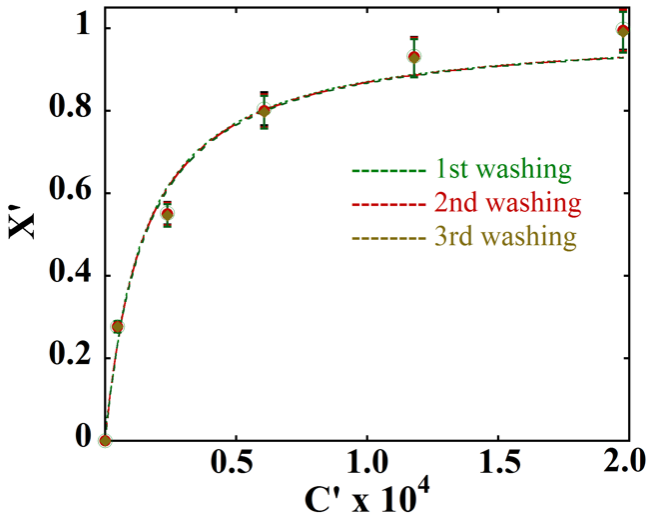

Figure S2. The amount of protein bound after each wash within 30 minutes interval for 2 h was plotted against the concentration of the proteins. Three washings were performed and amount of proteins after each washing in the supernatant was measured using a Bradford assay to find amount of proteins leached out from the AA-proteins conjugate. The protein bound was measured by subtraction method. It was found that ~99 % of bound protein remains conjugated with the AA after 3 washings for 30 minutes interval in 2 h. total time period.

From the fitting K_{LF} were calculated as 0.15 x10^4 for HSA on bare SiNPs, only 2.2% of 6.7x10^4 for AA_{HSA}-HSA and 0.16x10^4 for GOx on bare SiNPs, only 3.4% of the value of 4.7x10^4 for GOx on AA_{GOx} (see main article). The results suggest only non-specific binding of the proteins to bare SiNP.

Apparent percentage specificity was calculated by taking ratio of maximum amount of proteins bound to maximum amount of template proteins bound on AAs (see Figure 3 in main article). The apparent non-specificity were 20% for homologous BSA and 2.7%, 60% and 75% for non-homologous lysozyme, glucose oxidase and hemoglobin respectively for the AA_{HSA}.

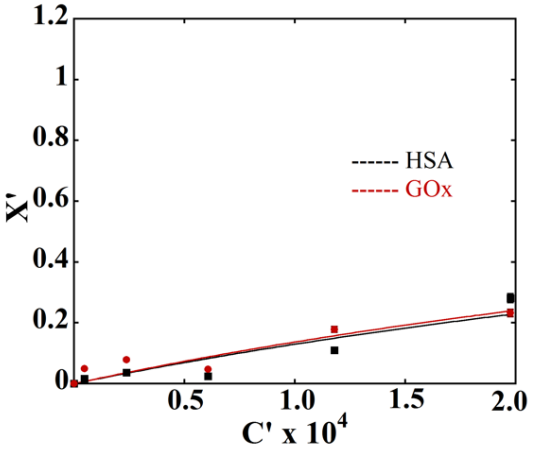

Figure S3. Binding of human serum albumin (black line) and glucose oxidase (red) on bare silica nanoparticles (SiNPs) based on a Bradford assay. Both the lines show lower adsorption on bare SiNPs than the artificial receptor (see main article). X' (X'=X/X_m, where X is the concentration of the protein bound per mg of artificial antibodies, AA, X_m is the maximum concentration per mg of AA of the template proteins bound with its corresponding AA) was plotted against C' (C'=$C^{1/n}$, where C is the concentration of proteins and n is 0.45) and then the data was fitted to the Langmuir-Freundlich equation using non-linear regression method to calculate K_{LF}.

Fluorescent labeling of HSA and binding to AA_{HSA}

HSA was reacted with 10-fold molar excess of fluorescein isothiocyanate (FITC, Thermo Scientific, product number 46425) in 100 mM sodium bicarbonate buffer, pH 9.0. Briefly, 60 µL of 2.5 mM FITC was mixed with 1 mg of HSA and incubated for 5 h in dark under slow rotation in sodium bicarbonate buffer, pH 9.0. Volume was kept to 1 mL in all the reactions. Excess FITC was removed using a 3 kDa molecular cut off centrifugal filter tube (EMD Milipore, cat. No. UFC500396) and FITC-HSA was reconstituted in 10 mM phosphate buffer, pH 7.3

Binding of FITC-HSA to A_{HSA} was evaluated in calf serum. Different concentration of FITC-HSA was placed in 3.0 mg/mL AA_{HSA} for 3 h under slow rotation in 1 mL of 2% calf serum diluted in phosphate buffer, pH 7.3. Then AA_{HSA}-FITC-HSA conjugate was separated by centrifugation, washed 3 times with buffer, pH 7.3 and finally reconstituted the buffer. Fluorescent intensity was measured using 495 nm excitation wavelength and 525 nm emission wavelength. The amount of bound FITC-tagged HSA onto AA_{HSA} was then calculated from the calibration curve (Figure S4). See Table 4 in main paper for results

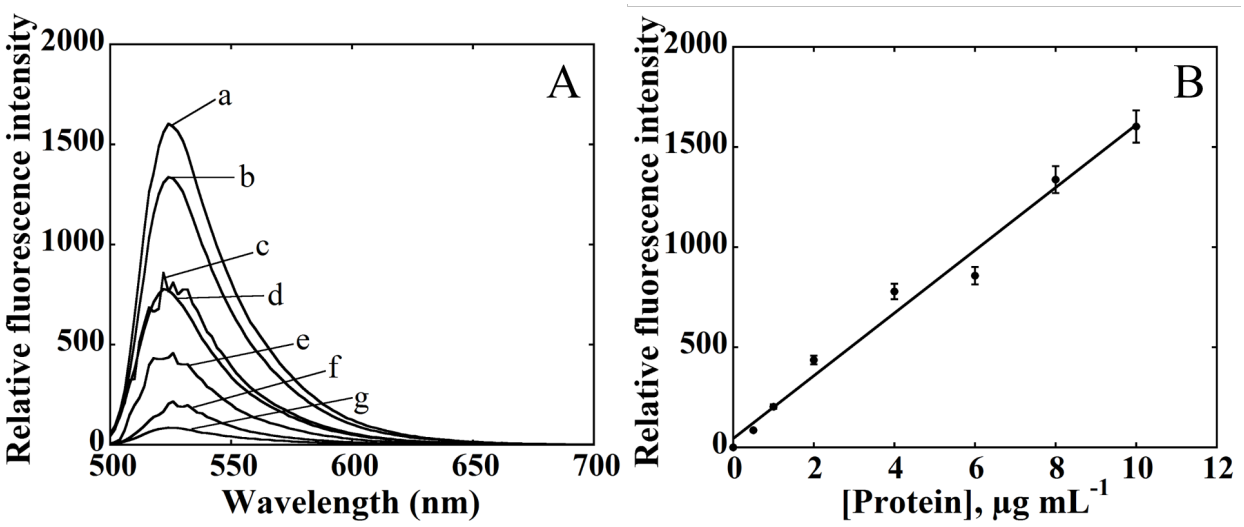

Figure S4: Calibration data for fluorescence spectra of FITC-HSA in 2% calf serum (A) spectra at concentrations in µg/mL a: 10.0, b: 8.0, c: 6.0 d: 4.0, e: 2.0 f: 1.0, g: 0.5 (B) Calibration curve for FITC-HSA.

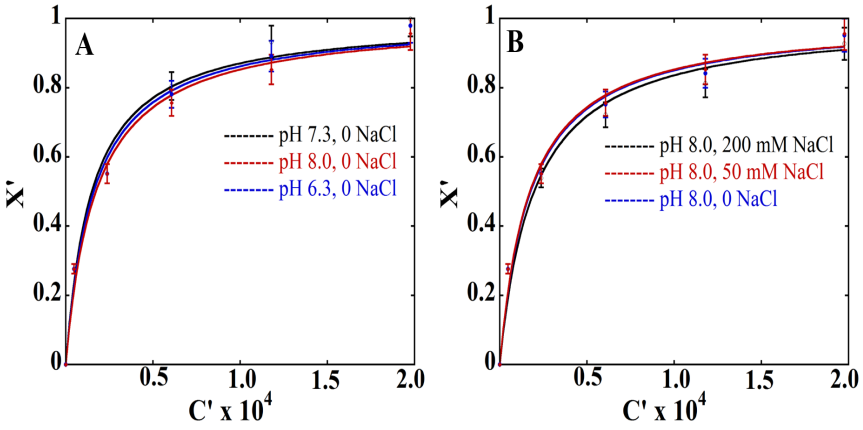

Figure S5: Binding isotherms of HSA to AA_{HSA}: (A) at different pH and (B) different NaCl concentrations pH 8.0. At pH 6.3 and 8.0, the binding efficiencies also decreases with increasing salt.

Influence of pH and salt concentration. The effect of pH and NaCL were analyzed by measuring binding constant K_{LF}-values at different conditions. Briefly, binding experiments were performed at pH 6.3, 7.3 and 8.0 with varying salt concentrations from 0 to 50 mM and 200 mM NaCl in all pH conditions. At pH 6.3 (K_{LF}, 6.2 x10^4) and 8.0 (K_{LF}, 5.8 x 10^4) the binding efficiencies decreased compared to pH 7.3 (K_{LF}, 6.7 x 10^4). With increasing salt concentration at pH 8.0, the binding efficiency decreased (K_{LF} at 50 mM NaCl, 5.6 x 10^4; K_{LF} at 200 mM NaCl, 5.1 x 10^4). Sample isotherms are shown in Figure S5 are results summarized Table S1.

Table S1. K_{LF} values for artificial antibody (AA) protein interaction at different pH and salt concentration

pH	K_{LF} $(mL/mg)^{1/n}$ at different sodium chloride concentrations (mM)		
	0	50	200
6.3	6.2 x10^4	6.0 x 10^4	5.3 x 10^4
7.3	6.7 x 10^4	6.4 x 10^4	5.9 x 10^4
8.0	5.8 x 10^4	5.6 x 10^4	5.1 x 10^4

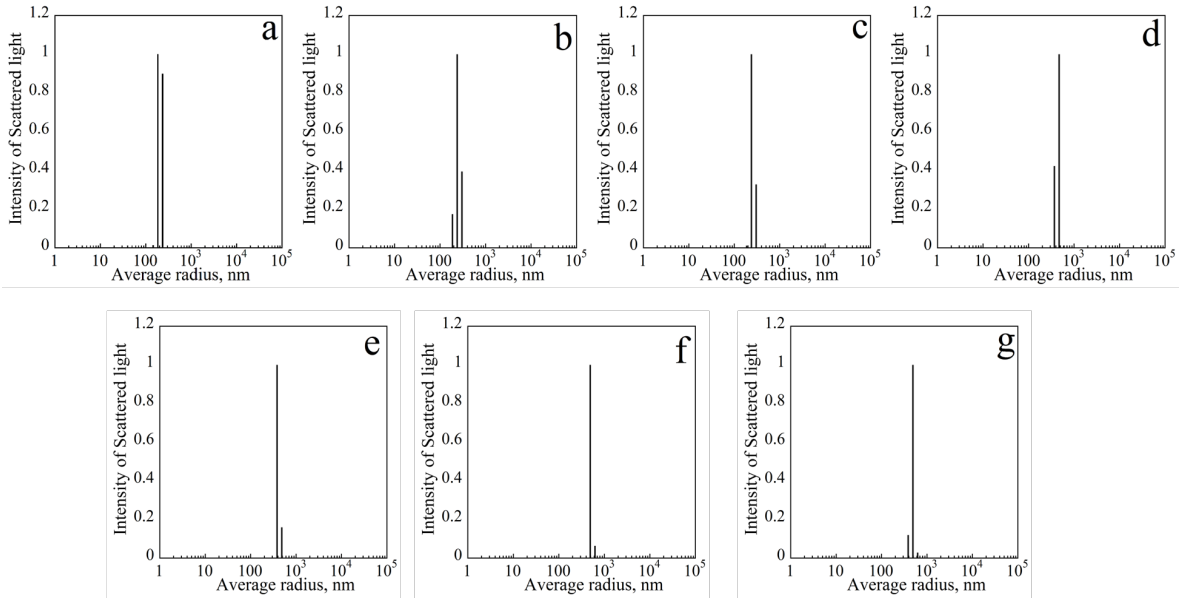

Figure S6: DLS-revised Hydrodynamic radii size distribution for different particles and particle-conjugates. (a) Silica nanoparticles (SiNPs); (b) SiNPs and human serum albumin (HSA) conjugates; (c) SiNPs and glucose oxidase (GOx) conjugates; (d) Artificial antibodies of HSA (AA_{HSA}); (e) Artificial antibodies of GOx (AA_{GOx}); (f) AA_{HSA} and HSA conjugates; (g) AA_{GOx} and GOx conjugates.

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