Electronic Supplementary Material (ESI) for RSC Advances

BSA Binding to Silica Capped Gold Nanostructures: Effect of Surface Cap and Conjugation Design on Nanostructure – BSA Interface

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Additional Experimental Procedures

The coating of silica and its functionalization with amine group was carried out by four different methods.

Method I: To 1.5 mL of GNP, a few drops of ammonia were added to adjust the pH to 10.0. Four variations of silica coating were evaluated. For this, to GNP adjusted to pH 10.0, 500 µL of ethanol and 52, 104, 312 and 416 µL of TEOS was added. The mixture was subjected to a vortex for 2 h, following which it was left undisturbed for 24 h. The solution thus obtained after ageing was centrifuged at 3,500 rpm, washed thrice with ethanol and finally resuspended in ethanol for amine functionalization. To each of the four suspensions, 10 µL of APTES was added and mixed by gentle shaking for 2 h. The solution was then heated at 50 ^{0}C for one hour. This was again centrifuged and washed with water thrice and finally resuspended in water.

Method 2: To 1 mL of GNP solution, a few drops of ammonia were added and the solution made up to 2 mL with ethanol. Four variations of silica coating were evaluated. For this, to each of the made up GNP solution, 10, 20, 30 and 40 µL of 1M TEOS was added and the resulted mixture subjected to vortex for 3 h, following which it was centrifuged thrice at 3,500 rpm for 30 min and finally resuspended in ethanol. Amine functionalization procedure was similar to that of Method 1.

Method 3: To 1.5 mL of GNP solution, 5 mL of isopropanol and 100 µL of ammonia were added under vigorous stirring. The contents were subjected to vortex for 15mins and 200, 400, 800 and 1600 µL of 20 mM TEOS was added in four equal instalments over a period of 18 h. The reaction was allowed to continue for a further 18 h, following which it was centrifuged thrice at 3,500 rpm for 30 min and finally resuspended in ethanol. Amine functionalization procedure was similar to that of Method 1.

Method 4: To 10 mL of GNP, 20 mL of ethanol followed by 200 µL of ammonia was added. After thorough mixing, 500 µL of 20 mM TEOS was added and subjected to vortex for 3 h. The solution was then centrifuged and suspended in ethanol, similar to other methods. To the

suspension, 20 µL of APTES was added for amine functionalisation. Rest of the procedure was similar to other methods.

Determination of Binding Constant

$$\frac{F_o - F}{F - F\alpha} = \left(\frac{[M]}{K_{diss}} \right)$$

Where F_o, F_α and F are the tryptophan residue fluorescence intensities of BSA in pure water, saturated with functionalized GNP and F the fluorescence intensity of an intermediate concentration between F_o and F_α. Apart from the fluorescence intensities F_o, F_α and F, in the above equation, [M] denotes the GNPs concentration, 'n' the number of binding sites/particle and K_{diss} the reciprocal value of the binding constant K_b. The binding constant and 'n' was determined through linear fitting of the logarithmic plot employing Origin 8.5. The concentration of $Au@Si@NH_2$, $Au@Si@NH_2^{edc}$, $Au@Si@NH_2^{glu}$, $Au@Si@NH_2^{ele}$ in solution was estimated as a factor of Au concentration, by employing the Beer-Lambert's law, assuming an extinction coefficient of 3×10^8 M^{-1} cm^{-1}.[1]

Information on Peak Positions in Raman Spectra of BSA

The Raman spectra of BSA is characterized by the presence of bands in the wavelength region of 1580 – 1720 cm^{-1} and 1400 – 1580 cm^{-1} corresponding to Amide I (characteristic of α helical content, C O stretching vibration), Amide II (N–H bending coupled with C–N stretching) and Amide III (C–N stretching mixed with N–H bending vibration) regions.[2] The major signals observed in the Raman spectra of BSA, i.e at 605.4 cm^{-1}, 1566.23 cm^{-1}, 1212.16 cm^{-1} and 713.45 cm^{-1} are due to the aromatic amino acids like Phe (Phenylalanine), at ~620, 1005, and 1033 cm^{-1}, Trp (Tryptophan) at 1011 and 1560 cm^{-1}, Tyr (Tryrosine) at 645, 825, 855, 1210, and 1620 cm^{-1} and the bands at at 655, 672, and 720 cm^{-1} are due to cysteine.

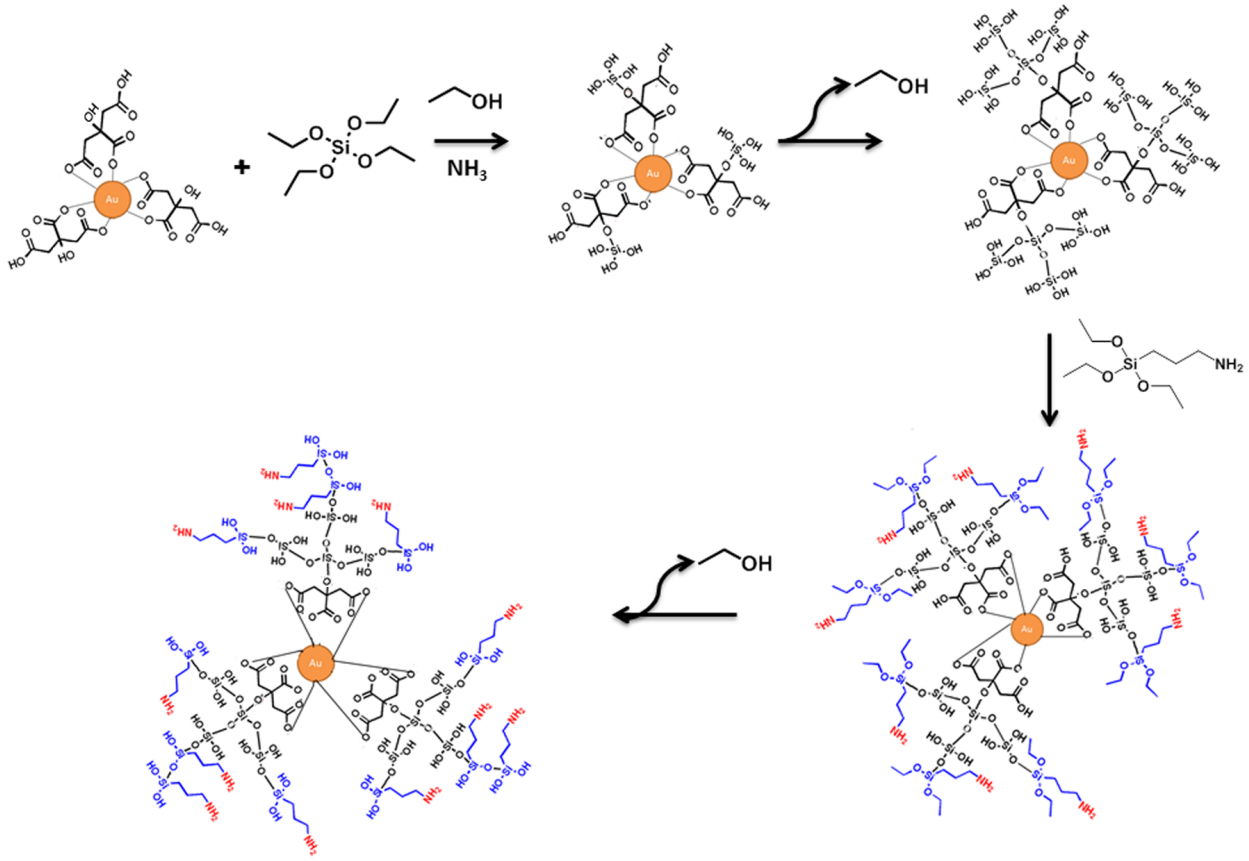
Determination of unbound BSA by Bradford Assay

The percentage of free BSA associated with the $Au@Si@NH_2$ – BSA nanostructures obtained through different conjugation strategies (ele, edc and glu) was estimated by Bradford Assay[3-5]. For this study, the final amount of BSA was maintained at 200 µg/mL. For a given concentration

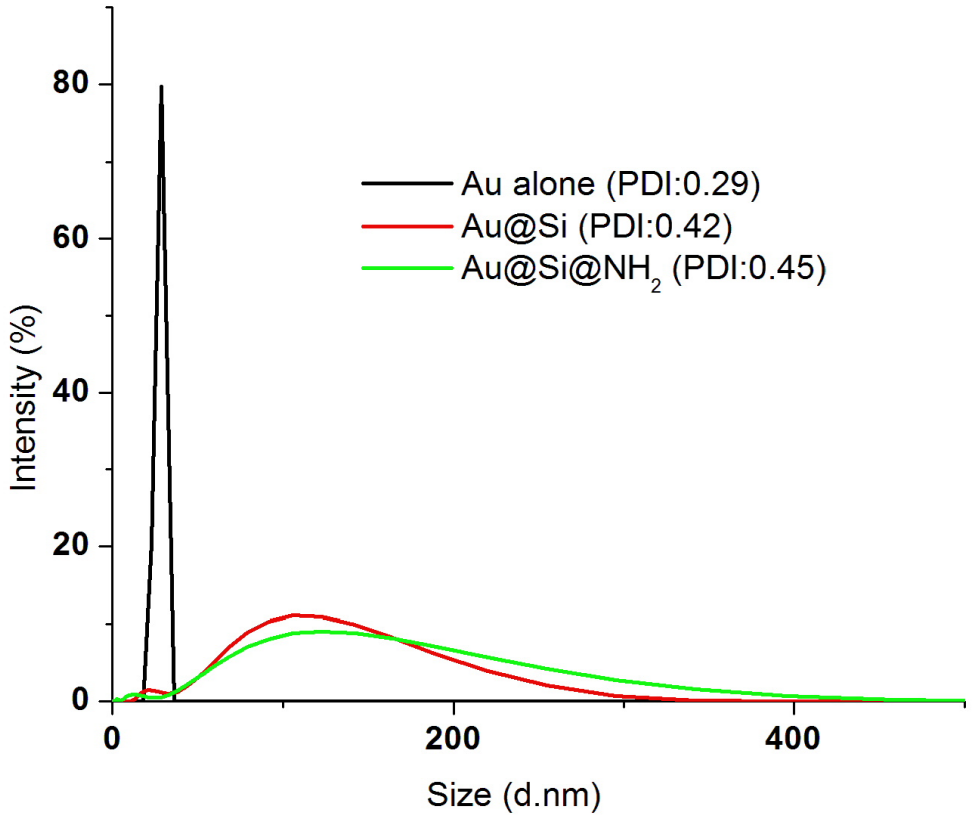
of $Au@Si@NH_2$, the percentage of free BSA was found (experiment in triplicate) to be 26.89% ± 3.07, 10.11% ± 4.60 and 17.78 % ± 1.54 respectively. The percentage of free BSA associated with the protein – nanostructure network follows the order $Au@Si@NH_2^{ele}$ - BSA > $Au@Si@NH_2^{glu}$ - BSA > $Au@Si@NH_2^{edc}$ - BSA.

Supplementary Figures

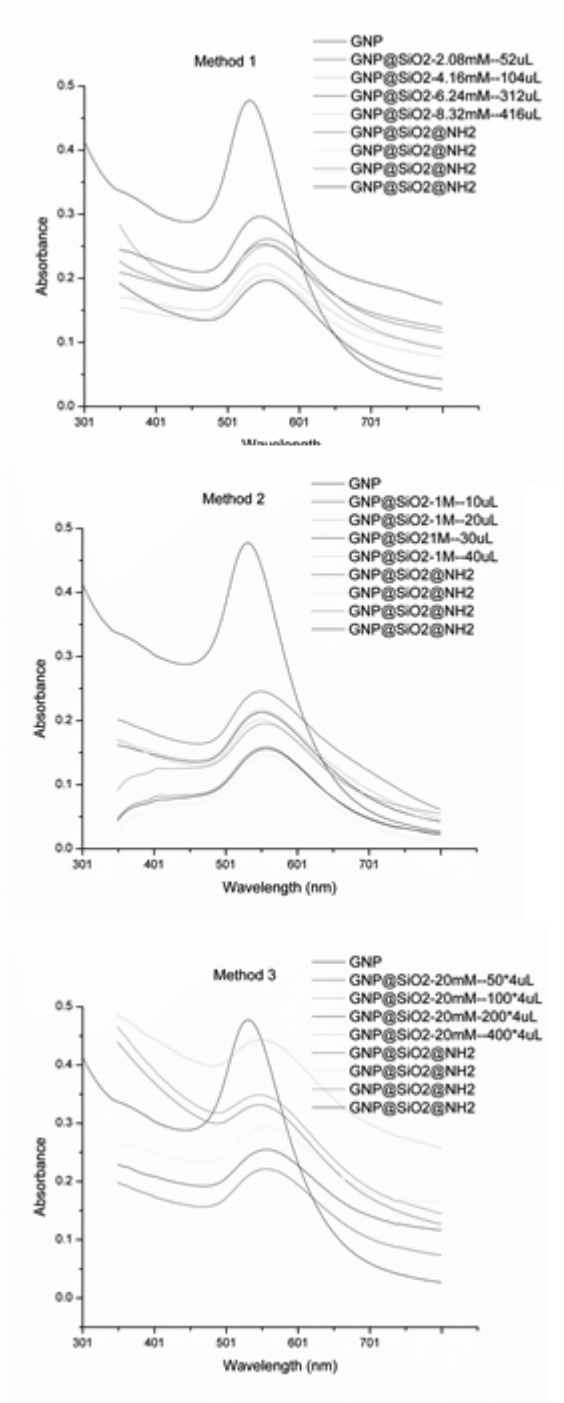
Scheme 1. Scheme chemical representation of silica coating to citrate capped gold nanoparticles by TEOS and amine functionalisation of silica coated nanostructures by APTES (References[6-8]) .



Supplementary Figure 1. Particle size distribution (Intensity) plot for Au nanoparticles, Au@Si and Au@Si@NH_2.



Supplementary Figure 2. UV–vis spectra of gold nanoparticles (Au) surface capped nanostructures (Au@Si) amine coated silica (Au@Si@NH_2) using three methods



Supplementary Table 1. EDAX analysis of gold nanoparticles and silica coated gold nanostructures

Element	Atomic Wt (%)	
	Gold Nanoparticles	Silica Coated Gold Nanostructures
Carbon	21.7	0.0
Oxygen	08.60	37.30
Gold	12.20	50.10
Sodium	57.40	0
Silica	0.0	12.70

Supplementary Table 2. Hydrodynamic diameter of various conjugated systems as determined by dynamic light scattering

Conjugate System	Hydrodynamic diameter (nm)[1]
Gold nanoparticles alone	27.7±2.1
Au@Si	82.3±11.2
Au@Si@NH_2	97.7±4.7
Au@Si@NH_2^{edc}	116.0±2.6
Au@Si@NH_2^{ele}	113.3±1.5
Au@Si@NH_2^{glu}	116.7±2.08

[1] Average of three measurements

Supplementary Table 3. Binding constant values and the number of binding sites and regression coefficient values for all the methods followed

Method of Conjugation	K_b (M^{-1})	n	R value
1			
1	1.41×10^{12}	0.702	0.97
2	1.01×10^{12}	0.595	0.97
3	0.87×10^{12}	0.916	0.93
2			
1	5.9×10^{12}	0.907	0.93
2	0.5×10^{12}	0.951	0.92
3	0.6×10^{12}	1.49	0.88
4	1.0×10^{12}	0.924	0.62
3			
1	0.24×10^{12}	3.08	0.98
2	0.29×10^{12}	1.38	0.88
3	0.17×10^{12}	1.20	0.96
4	0.30×10^{12}	1.93	0.90

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