Interaction of azide ion with hemin and cytochrome *c* immobilized on Au and Ag nanoparticles

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Supporting information (1)

Calculation of the number of molecules per nanoparticle

Theory

20mL of nanoparticle solution was mixed with 5mL of 0.03mM (0.02 mg/mL) hemin chloride in 20 % aqueous ethanol.

Molar concentration hemin chloride (C_t) = (0.03 X 5/25) mM in 25 mL (0.025 L)

The number molecules (N_t) = $6 \times 10^{-6} \times 6.023 \times 10^{23} \times 0.025$

$= 9.03 \times 10^{16}$

concentration of adsorbed hemin (C_a) = Total concentration taken for the reaction (C_t) - concentration of undsorbed hemin (C_u)

Fraction of hemin molecule get adsorbed on the surface of the nanoparticle

$= (C_a / C_t)$

Number of nanoparticles adsorbed on the surface of nanoparticles

$$(N_a) = N_t \times (C_a / C_t) \qquad \text{———} \quad (1)$$

Concentration (C) is directly proportional to the absorbance of sorret band at 400 nm (A) in the UV– vis spectrum

ie $C_t \alpha A_t$ (The absorbance of 0.03 mM hemin chloride in 20 % aqueous ethanol)

similarly, $C_a \alpha A_a$ (The absorbance of adsorbed hemin chloride)

Thus, $(C_a / C_t) = (A_a / A_t)$ ——— (2)

From (1) and (2) $(N_a) = N_t \times (A_a / A_t)$ ——— (3)

$A_a = A_t - A_u$ ——— (4)

From (3) and (4) $(N_a) = N_t \times ((A_t - A_u) / A_t)$ ——— (5)

Case(1) Au particles

The concentration of $HAuCl_4$ used for the synthesis is 2.5×10^{-4} M

Assuming complete reduction, the weight of Au present in the 20 mL (0.02 L) of Au colloid is
$W_{Au} = 2.5 \times 10^{-4} \times 197 \times 0.02 = 9.85 \times 10^{-4}$ g
Au density, ρ =19.3 gcm^{-3}
Au atomic weight = 197 Da
Volume of 15 nm sized gold nanoparticle, $V_{np} = (4\pi r^3 / 3)$, where r = 7.5 nm = 7.5×10^{-7}cm
Thus $V_{np} = (4\pi(7.5 \times 10^{-7} cm)^3 / 3) = 1.77 \times 10^{-18}\ cm^3$
Weight of a nanoparticle, $W_{np} = V_{np} \times \rho = 1.77 \times 10^{-18}\ cm^3 \times 19.3\ gcm^{-3}$
$= 34.16 \times 10^{-18}$ g
Number of nanoparticles = $(W_{Au} / W_{np}) = (9.85 \times 10^{-4}\ g / 34.16 \times 10^{-18}\ g)$
$= 2.88 \times 10^{13}$

A_t = 1.427
(a) In the case of Hem binding with Au (15nm) particle, A_u =1.318
Thus from equation (5) $N_a = 9.03 \times 10^{16} \times [(1.427 – 1.318) / 1.427]$
$= 6.875 \times 10^{15}$
Number molecules per nanoparticle = $(6.875 \times 10^{15} / 2.88 \times 10^{13})$.
= 238.72 ~ 240
Surface area of nanoparticle = $4\pi r^2 = 4 \times 3.14 \times (7.5\ nm)^2 = 706.5\ nm^2$
The area occupied by a single Hem molecule on the surface of nanoparticle
$= (706.5\ nm^2 / 240) = 2.90\ nm^2$

Case(2) Ag (II) particles

The concentration of $AgNO_3$ used for the synthesis is 7.5×10^{-4} M
Assuming complete reduction the weight of Ag present in the 20 mL (0.02 L) of Ag colloid is
$W_{Ag} = 7.5 \times 10^{-4} \times 107.87 \times 0.02 = 16.17 \times 10^{-4}$ g
Ag density, ρ =10.5 gcm^{-3}
Ag atomic weight = 107.87 Da
Volume of 60nm sized silver nanoparticle, $V_{np} = (4\pi r^3 / 3)$, where r = 30 nm = 30×10^{-7}cm
Thus $V_{np} = (4\pi(30 \times 10^{-7} cm)^3 / 3) = 113.04 \times 10^{-18}\ cm^3$
Weight of a nanoparticle, $W_{np} = V_{np} \times \rho = 113.04 \times 10^{-18}\ cm^3 \times 10.5\ gcm^{-3}$
$= 1.187 \times 10^{-15}$ g
Number of nanoparticles = $(W_{Ag} / W_{np}) = (16.17 \times 10^{-4}\ g / 1.187 \times 10^{-15}\ g)$
$= 1.36 \times 10^{12}$

Case(3) Ag (I) particles

The concentration of $AgNO_3$ used for the synthesis is 7.5×10^{-4} M
Assuming complete reduction the weight of Ag present in the 20 mL (0.02 L) of Ag colloid is
$W_{Ag} = 7.5 \times 10^{-4} \times 107.87 \times 0.02 = 16.17 \times 10^{-4}$ g
Ag density, ρ =10.5 gcm^{-3}
Ag atomic weight = 107.87 Da
Volume of 4 nm sized silver nanoparticle, Vnp= $(4\pi r^3 / 3)$, where r = 2nm = 2×10^{-7}cm
Thus $V_{np} = (4\pi(2 \times 10^{-7} cm)^3 / 3) = 0.10 \times 10^{-18}\ cm^3$
Weight of a nanoparticle, $W_{np} = V_{np} \times \rho = 0.10 \times 10^{-18}\ cm^3 \times 10.5\ gcm^{-3}$
$= 1.05 \times 10^{-18}$ g
Number of nanoparticles = $(W_{Ag} / W_{np}) = (16.17 \times 10^{-4}\ g / 1.05 \times 10^{-18}\ g)$

$= 1.54 \times 10^{15}$

$A_t = 1.427$

(a) In the case of Hem binding with Ag (I) particle, $A_u = 0.896$

Thus from equation (5) $N_a = 9.03 \times 10^{16} \times [(1.427 - 0.896)/1.427]$

$= 34.48 \times 10^{15}$

Number molecules per nanoparticle = $(34.48 \times 10^{15} / 1.54 \times 10^{15})$

$= 22.747 \sim 23$

Surface area of nanoparticle = $4\pi r^2 = 4 \times 3.14 \times (2\text{ nm})^2 = 50.24\text{ nm}^2$

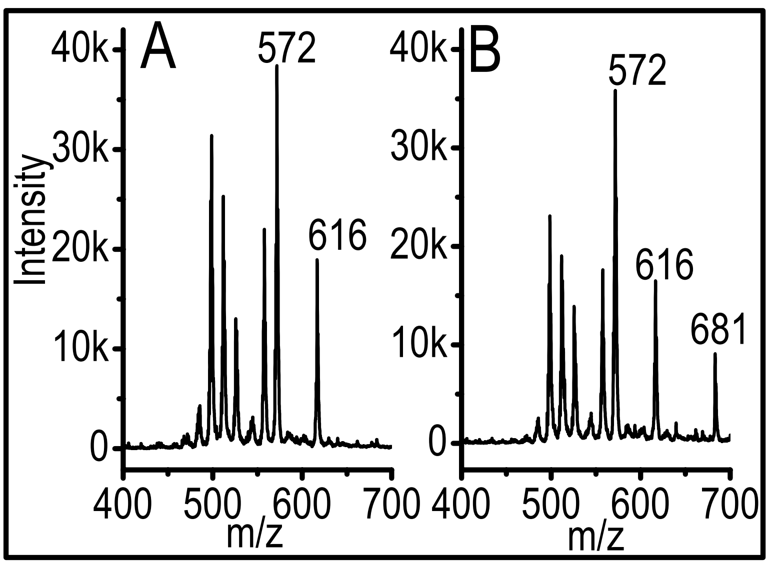
The area occupied by a single Hem molecule on the surface of nanoparticle

$= (50.24\text{ nm}^2 / 23) = 2.18\text{ nm}^2$

The same methodology was applied in the case of Ag (II) particle for calculating the number molecules /nanoparticle.

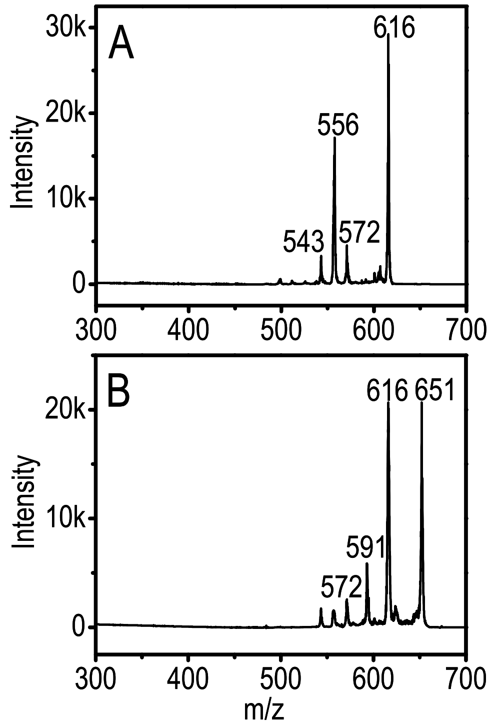
(2)The number of Cyt *c* molecule / nanoparticle was calculated using the above method. The molecular weight of Cyt *c* (12574 Da) was obtained from MALDI-TOF MS analysis. The absorbance of sorret band at 408 nm is taken as a measure for determining the amount of Cyt *c* in water.

Supporting information (2)



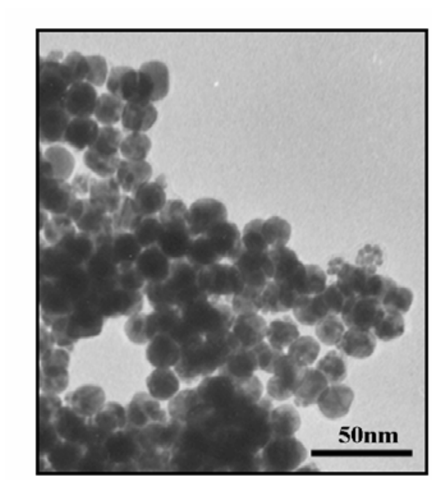
Figures (A) and (B) represent LDI – TOF mass spectra recorded in the positive ion mode for Ag(I)@Hem and Ag(I)@Hem–N_3, respectively.

Supporting information (3)



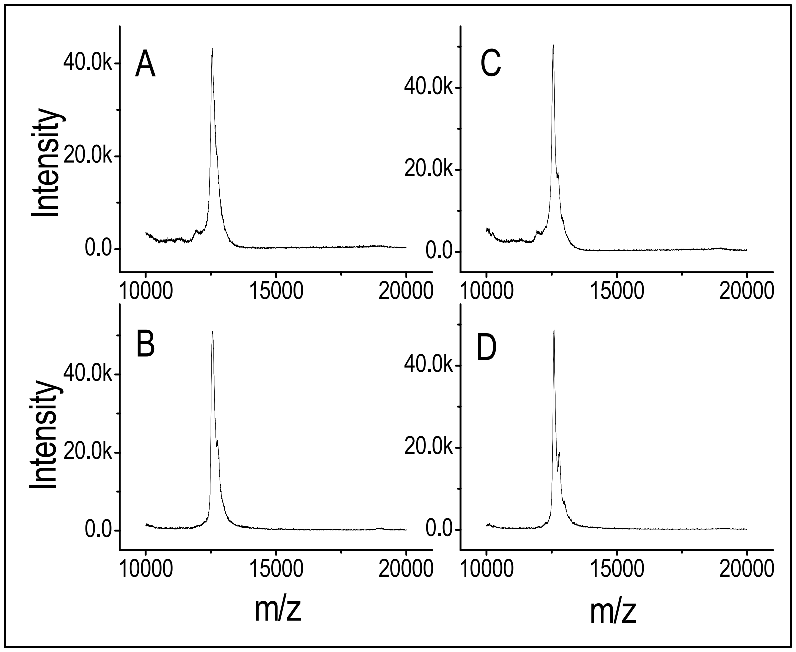
PSD mode analysis of LDI-TOF mass spectrum for the peaks at m/z 616 (Hem)(A) and m/z 651 (Hem-Cl) (B).

Supporting information (4)



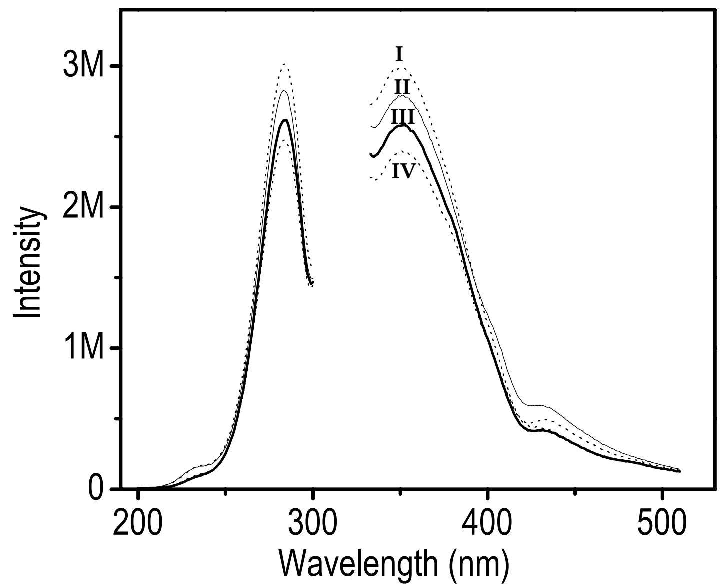
Transmission electron micrograph of Cyt *c* capped gold nanoparticles Au@Cyt *c*. This shows a group of uniform sized (15 nm) nanoparticles.

Supporting information (5)



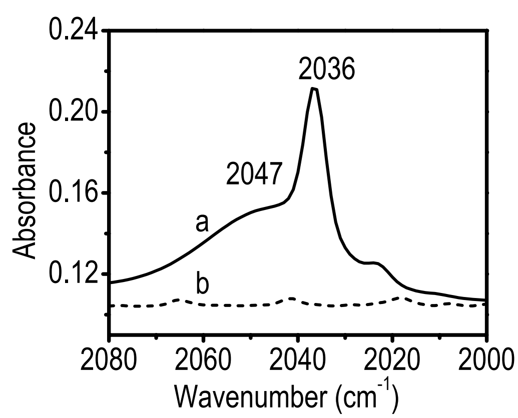
MALDI –TOF mass spectra of the sample measure using 337 nm N_2 laser using sinapinic acid matrix. Figures (A) and (B) represent MALDI – TOF mass spectra recorded in the negative and positive ion modes for Cyt *c*. Figures (C) and (D) represent MALDI – TOF mass spectra recorded in the negative and positive ion modes for Au(I)@Cyt *c*.

Supporting information (6)



Excitation and emission spectra recorded in aqueous medium. The traces I, II, III and IV represent the samples free Cyt. *c*, Ag (II)@Cyt. *c*, Ag (II)@Cyt. *c*-N_3 and Au@Cyt. *c*, respectively. The excitation wavelength was set at 283 nm and the slit width was 8 nm. All the samples contain the same number of Cyt. *c* molecules. All the samples show emission at 352 nm. There was no significant enhancement observed in the case of samples containing Ag (II) particles.

Supporting information (7)



FTIR spectra of Ag(I)@Cyt *c*-N_3 (a) and Ag(I)@Cyt *c* (b) recorded in KBr matrix.