Electronic Supplementary Information

Thermodynamics of Adsorption of Alcohol Dehydrogenase on Gold Nanoparticle Surface: A Model Based Analysis Versus Direct Measurement

Akriti Mishra[1], Kamini Mishra[1], Dipayan Bose[2], Abhijit Chakrabarti[2] and Puspendu K. Das[1*]

[1*]Department of Inorganic and Physical Chemistry Indian Institute of Science, Bangalore 560012 India

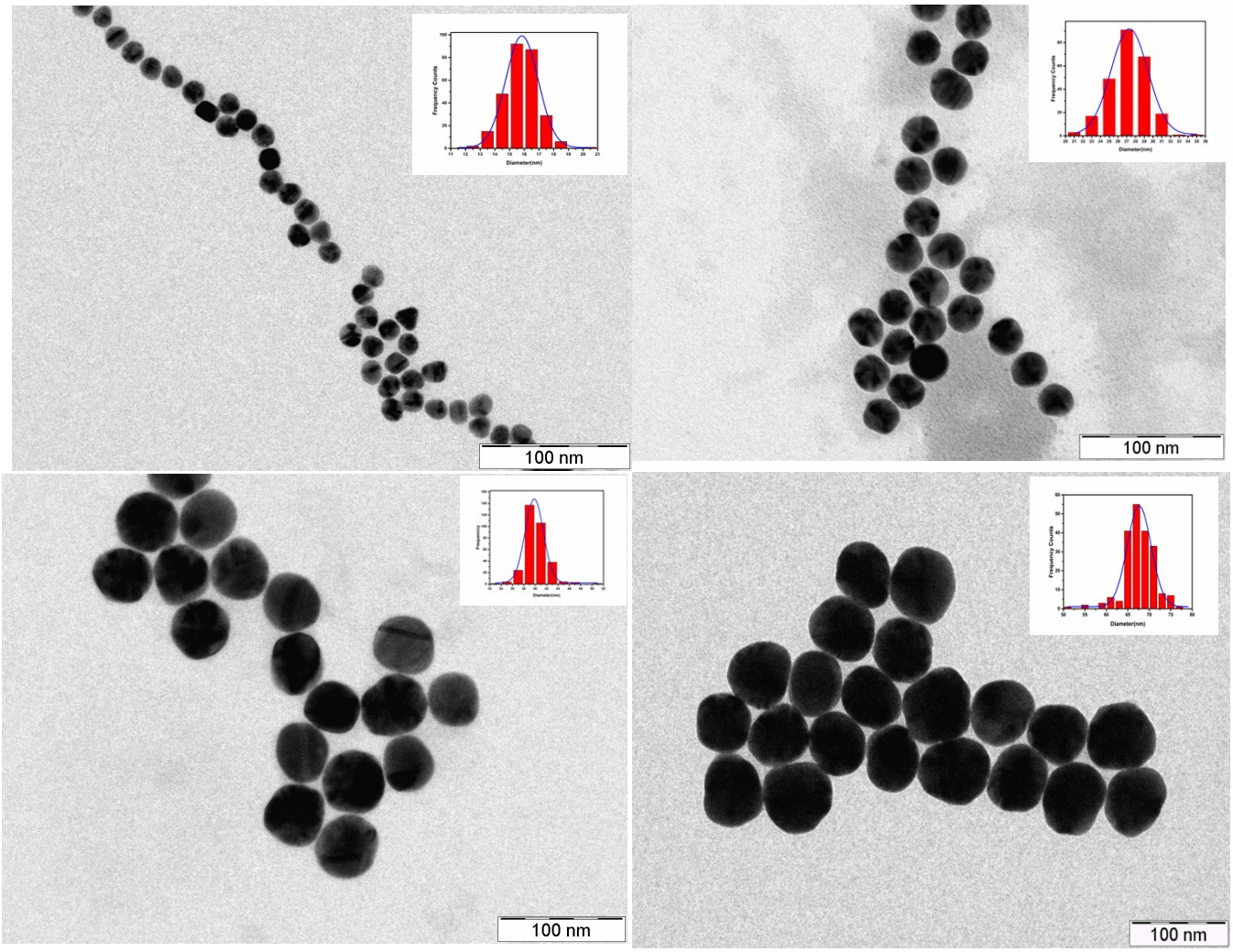
[2]Crystallography & Molecular Biology Div., Saha Institute of Nuclear Physics, HBNI, 1/AF Bidhannagar, Kolkata 700 064, India.

Email of the corresponding author: pkdas@iisc.ac.in

S1. Synthesis of Gold Nanoparticles

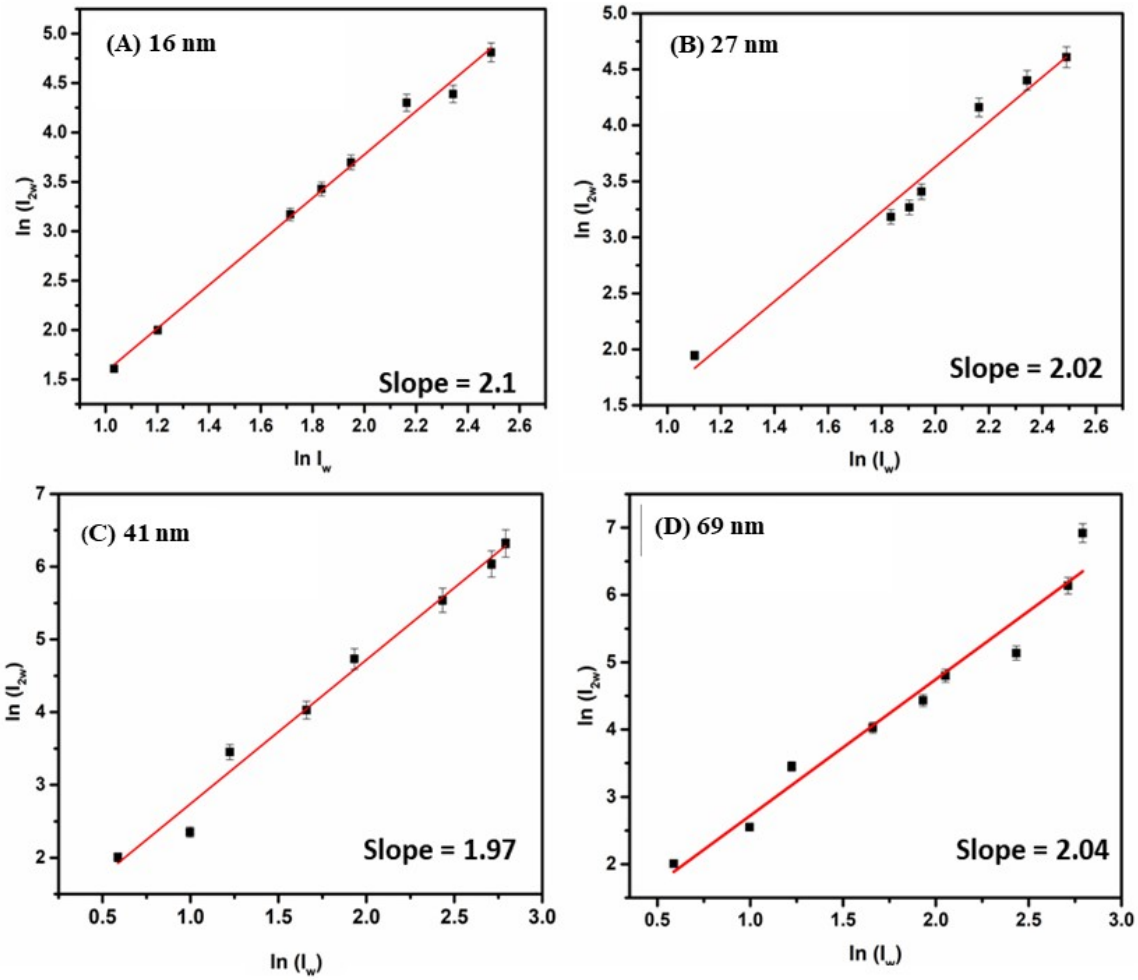
To start, 5 mL of $HAuCl_4.3H_2O$ solution (0.2% w/v) was taken in 100 mL of water in a double necked round bottom flask and heated till boiling followed by addition of 4 mL of sodium-citrate solution (1% w/v, containing 0.05% w/v citric acid) with vigorous stirring. The solution mixture was boiled for about 10-15 min until the color changed to deep red and it was then left for cooling overnight. This deep red solution was marked as "SEED" which has 16 nm GNPs dispersed in aqueous medium.[1] From the SEED solution 6 mL was taken and diluted to 20 mL with water in a round bottom flask. To this 10 mL solution A containing $HAuCl_4.3H_2O$ and 10 mL solution B containing trisodium citrate (1% w/v) and ascorbic acid (1%w/v) were added slowly at 60uL/min under continuous stirring. The relative volume ratio of $HAuCl_4.3H_2O$, ascorbic acid and tri-sodium citrate solutions were maintained at 8: 2: 1. We have synthesized two sizes, 27 nm and 41 nm GNP via SEED mediated synthesis. Synthesizing GNPs with larger diameter than 41 nm via the SEED mediated synthesis resulted in octahedral and non-spherical shapes. Therefore, we followed a different protocol suggested by Xia et al. for the synthesis of 69 nm GNP with some modifications. In a 100 mL round bottom flask, 0.178 mL $HAuCl_4.3H_2O$ (0.2%w/v), 0.015 mL $AgNO_3$ (0.1%w/v) and 0.107 mL trisodium citrate (1%w/v) were taken and heated to boil. After 40-45 s 0.714 mL of Tris buffer (0.10 M) was added to the boiling mixture followed by addition of 17.8 mL boiling water. The resulting solution was allowed to boil for another 40-60 minutes. When the colour changed from light yellow to violet to dark pink after 30 minutes, the heating was stopped. Then, the reaction mixture was allowed to cool overnight. The GNPs produced by this method was 69 nm diameter. The synthesized GNPs of all sizes were found to be stable for more than a month. The monodispersed spherical particles were purified by centrifugation and redispersed in fresh mQ water.

S2. TEM images and Histogram of GNPs



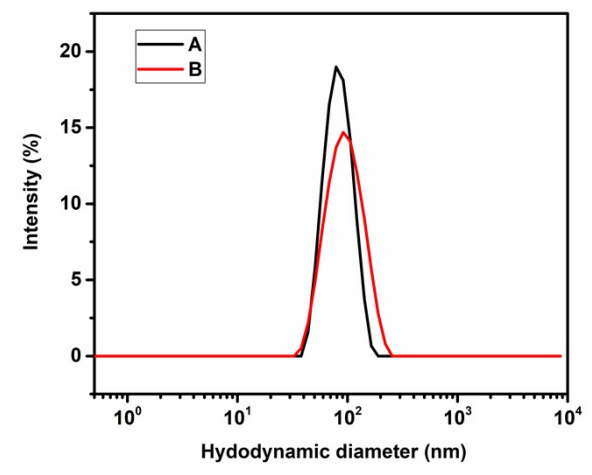
TEM Images of 16 nm (15.84 ± 1.10 nm), 27 nm (27.3 ± 2.10 nm), 41 nm (41 ± 2.5 nm), 69 nm (69.2 ± 2.7 nm). Inset shows Histograms generated by considering more than 300 particles.

S3. Incident intensity dependence of the SH signal



Quadratic dependence of the second-harmonic signal on the incident laser intensity for GNPs in 5 mM phosphate buffer at pH 7 for (A) 16 nm, (B) 27 nm, (C) 41 nm, and (D) 69 nm at 1064 nm.

S4. DLS signal for 69 nm GNP before and after ADH addition



S4. DLS size distribution by intensity for (A) 41 nm GNP and (B) 41 nm GNP after addition of ADH at saturation. The corresponding z-average hydrodynamic diameter of GNPs obtained from the cumulant fit is reported.

TS1. DLS data for adsorption of ADH on GNPs of different size

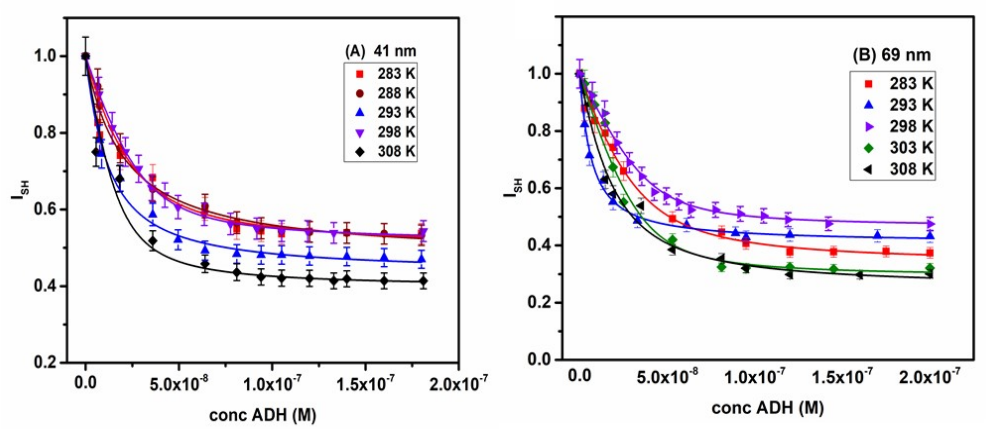
GNP size	Concentration of GNP (nM)	Change in Hydrodynamic diameter (nm)	Number of ADH/GNP from volume calculation
16	8	5 ± 1	5
27	0.81	5.9 ± 0.8	16
41	0.14	7.7 ± 0.8	47
69	0.002	9.5 ± 0.6	158

TS2. Number of ADH molecules (n_{sat}) adsorbed on GNP surface measured by three different methods.

Size of GNP (nm)	SHLS	Fluorescence	DLS

16	180 ± 20	147 ± 11	5 ± 2
27	933 ± 66	387 ± 31	50 ± 7
41	2307 ± 384	1820 ± 145	390 ± 23
69	10750 ± 750	12000 ± 918	31816 ± 1411

S5. SHLS decay curves at different temperatures



Smooth lines are MLM fits of SHLS decay as a function of ADH concentration at different temperatures for (A) 41 nm and (B) 69 nm GNPs.

TS3. The binding parameters N_{max} and n_{sat} values obtained from the MLM fits for different temperatures.

	41 nm		69 nm	
Temperature (K)	N_{max} (10^{-8} M)	n_{sat}	N_{max} (10^{-8} M)	n_{sat}
283	1 ± 0.2	769 ± 153	3 ± 0.4	7000 ± 1000
288	1.85 ± 0.2	1423 ± 153	-	-
293	2 ± 0.4	1538 ± 307	3.15 ± 0.3	7875 ± 750
298	3.1 ± 0.5	2384 ± 384	4.3 ± 0.3	10750 ± 750
303	-	-	4.63 ± 0.5	11575 ±1250
308	2.8 ± 0.5	2153 ± 384	4 ± 0.8	10000 ± 2000

References

1 C. Ziegler and A. Eychmüller, *Journal of Physical Chemistry C*, **2011**, 115, 4502–4506.