Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supporting Information

Thermodynamics of Adsorption of Lysozyme on Gold Nanoparticles from Second Harmonic Light Scattering

Kamini Mishra and Puspendu K. Das*

Dept. of Inorganic and Physical Chemistry

Indian Institute of Science, Bangalore 560012

India

Email: pkdas@iisc.ac.in

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

S1. Synthesis of Gold Nanoparticles : In the seed mediated method, 5 mL of $HAuCl_4.3H_2O$ solution (0.2% w/v) in 100 mL of water was taken and heated till boiling in a double necked round bottom flask followed by quick addition of 4 mL of sodium-citrate solution (1% w/v, containing 0.05% w/v citric acid) under vigorous stirring. The solution was allowed to boil until deep red colour appeared (10-15 min) and was cooled down overnight. This prepared solution was marked as "SEED". For growth of SEED, addition of salt and reducing agent onto the seed solution at room temperature was done. Fixed volumes of SEED (6 mL) was diluted to 20 mL and kept for stirring in a round bottom flask. Now 10 mL solution A containing $HAuCl_4.3H_2O$ and 10 mL B containing trisodium citrate (1% w/v) and ascorbic acid (1%w/v) were added slowly. The relative volume ratios of $HAuCl_4.3H_2O$, ascorbic acid and tri-sodium citrate solutions were strictly maintained at 8: 2: 1 followed by which 28 nm and 41 nm monodispersed spherical gold nanoparticles were prepared. Seeding higher sizes resulted in poor quality puckered octahedral and mixed shaped morphologies.

For 69 nm synthesis, protocol by Haibing Xia was followed with minor modifications. A reaction mixture of 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) was prepared at room temp. Tris buffer (0.10 M) was added to the reaction mixture at an interval of 40-45 s followed by addition of boiling water. The solution was further allowed to boil for 40-60 minutes. When the colour changed gradually from light yellow to dark pink, the reaction was over and the reaction mixture was allowed to cool overnight. All glasswares were thoroughly cleaned with aqua regia and fresh mQ water was used in the entire synthesis. All particles were found to be stable for more than a month.The monodispersed spherical particles of varying sizes were purified by centrifugation and redispersed in fresh mQ water.

S2. TEM Images and Histogram of Gold Nanoparticles

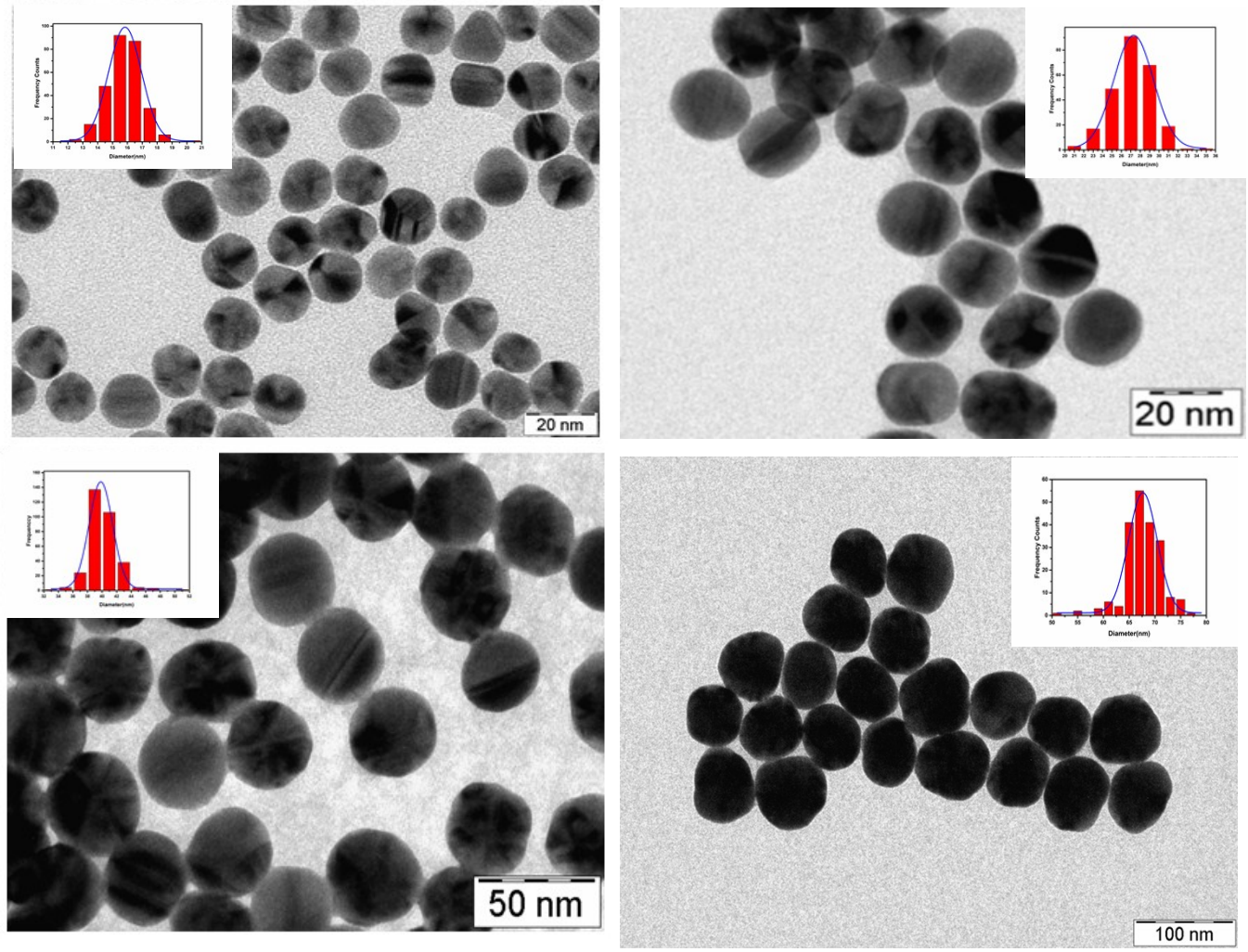


Figure S1. TEM Images of 16 nm(15.84±1.10 nm),28 nm (27.3±2.10 nm), 41 nm(39.80±2.5 nm), 69 nm(69.2±2.7 nm). Inset shows Histogram on accounting more than 300 particles.

S3. Monochromator scan of SHLS

Wavelength scan from 510 nm to 550 nm was done by keeping a monochromator (automated, Czerny-Turner, Horiba Jobin Yvon TRIAX 550, resolution = 0.024 nm) in between the 532±4 nm interference filter and the PMT (see SH signal scan shown below).

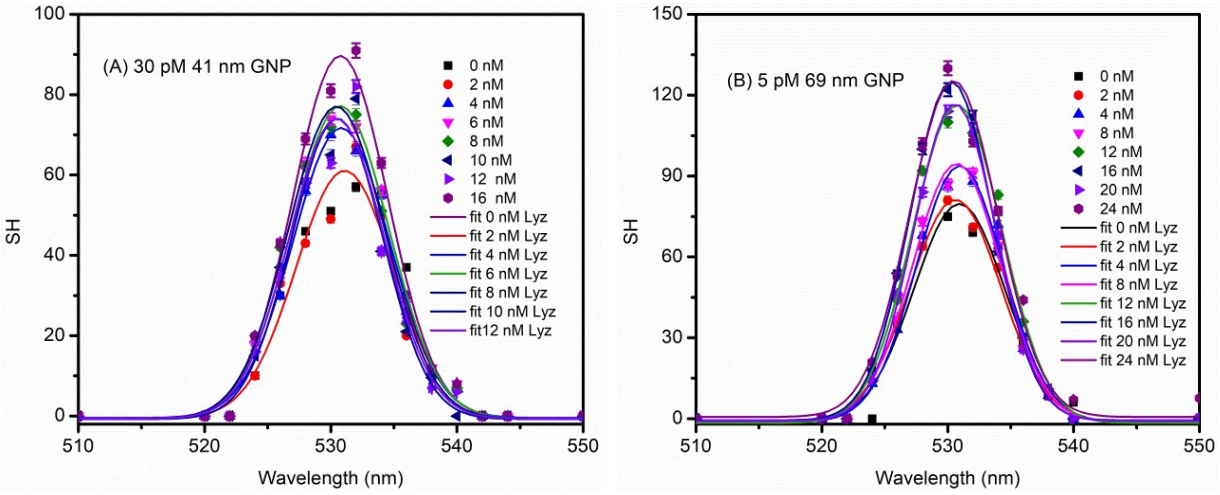


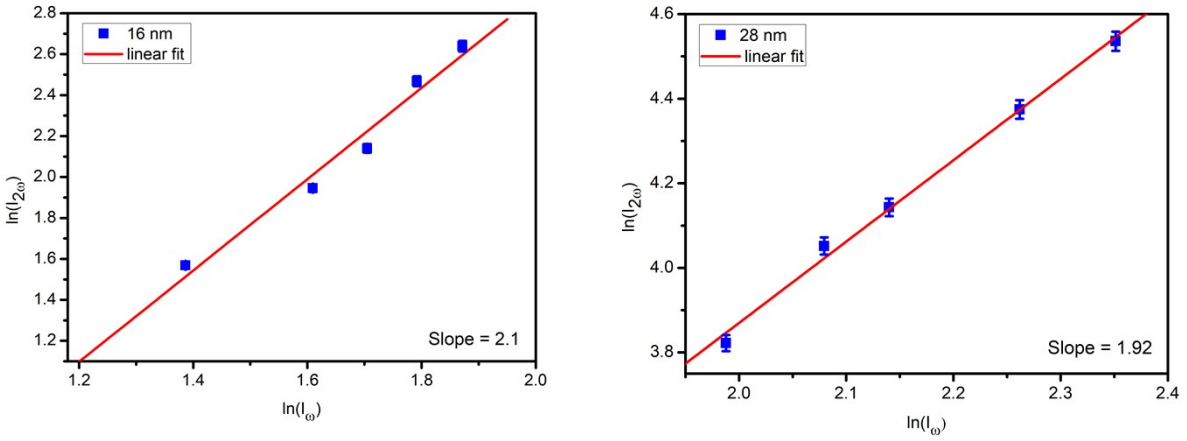
Figure S2 Wavelength scan of (A) 41 and (B) 69 nm GNPs at different concentration of Lyz to a fixed concentration of GNPs solution in phosphate buffer.

S4. Quadratic Power Dependence of the SH Signal

Quadratic power dependence check was done with GNPs. The output SH signal was fitted to

$$\ln I_{2\omega} = \ln A + n \ln I_{\omega} \qquad (1)$$

Where $I_{2\omega}$ is the SH output power, I_{ω} is the input power, A is a proportionality constant and n is the order of process. Experimental data points were fitted to eq. 1 which gives a straight line with the value of n indicated inside Figure S3.



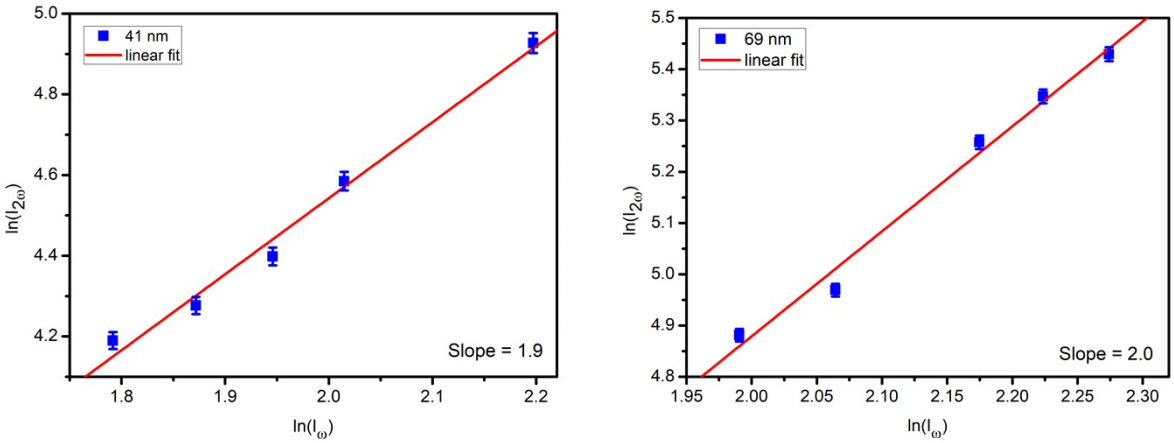
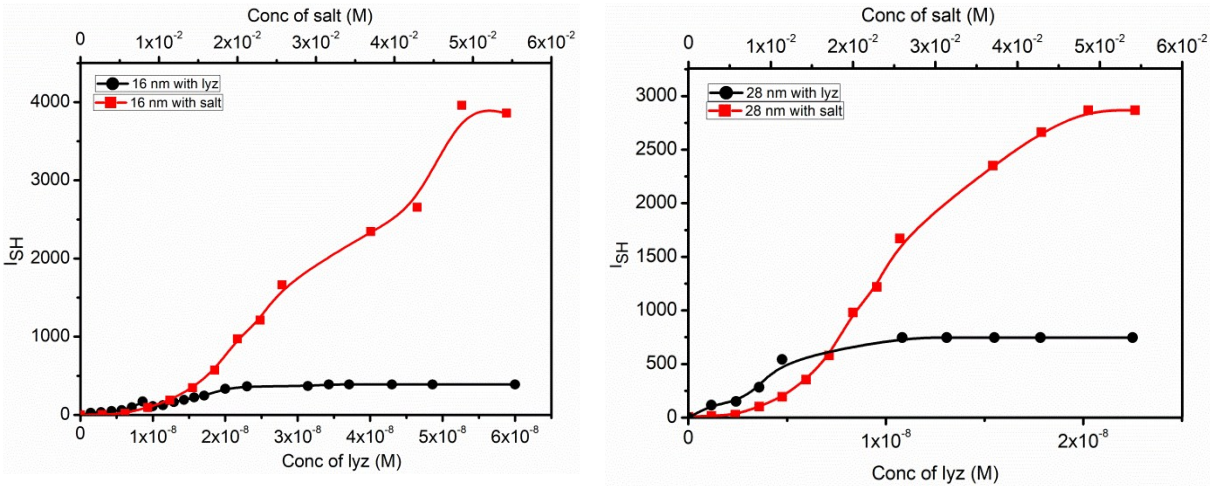


Figure S3. *Quadratic dependence of the second-harmonic signal on the incident laser intensity for GNPs in 10 mM phosphate buffer at pH 7 for 16 nm, 28 nm, 41 nm and 69 nm, respectively, at 1064 nm.*

S5. Comparison of Salt Induced *vs* Lyz Mediated GNP Aggregation

Same concentration of GNPs was taken for all sizes as was taken in case of Lyz mediated aggregation. Small aliquots of NaCl were added to the GNP solutions in a stepwise manner.The SH signal increased quickly after the first few additions which on further addition reached saturation and finally precipitate out. The comparison of salt mediated aggregation and Lyz mediated aggregation is shown in figure S3. To show the difference between the Lyz and salt mediated aggregations, unnormalized background subtracted SH signal is plotted with respect to concentration.



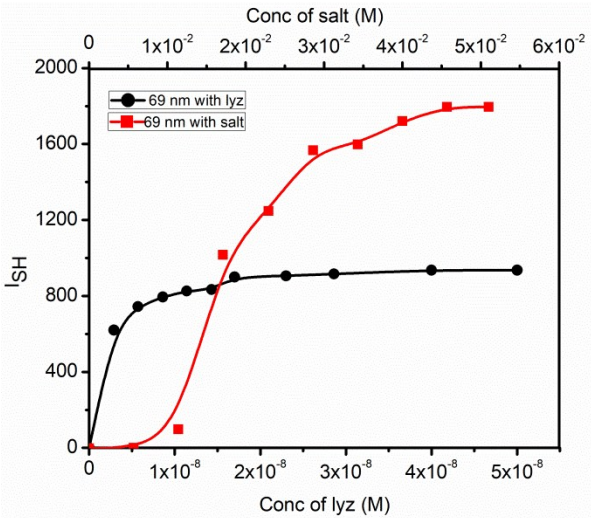


Figure S4. Comparison of increase in the SH intensity of 16, 28, 69 nm GNPs after addition of Lyz and NaCl to a fixed concentration of GNP solution.

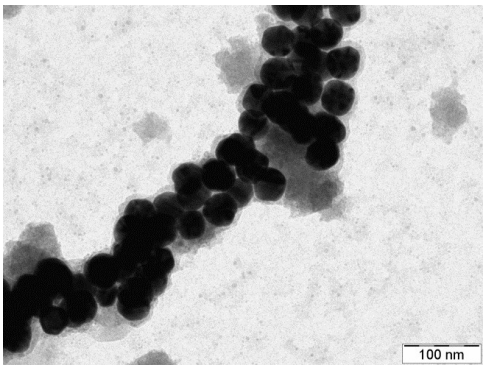


Figure S5. TEM image of salt mediated aggregation of 41 nm GNP

S6. Variable Temperature SH Data

To see clearly, the change in the SH signal wrt to temperature, the concentration of 69 nm was increased upto 12 pM and SH measurements were done in the same way as earlier. There is an increase in the binding constant with decrease in temperature and also the N_{max}(also n_{sat}) increases at low temperatures. Although there is no trend in the N_{max}values but the binding constant follows a trend. The values taken for K_b and N_{max} are those where the model fits well with the experimental data. Comparison of SH data at different temperatures for 69 and 41 nm GNP is shown in figure S5.

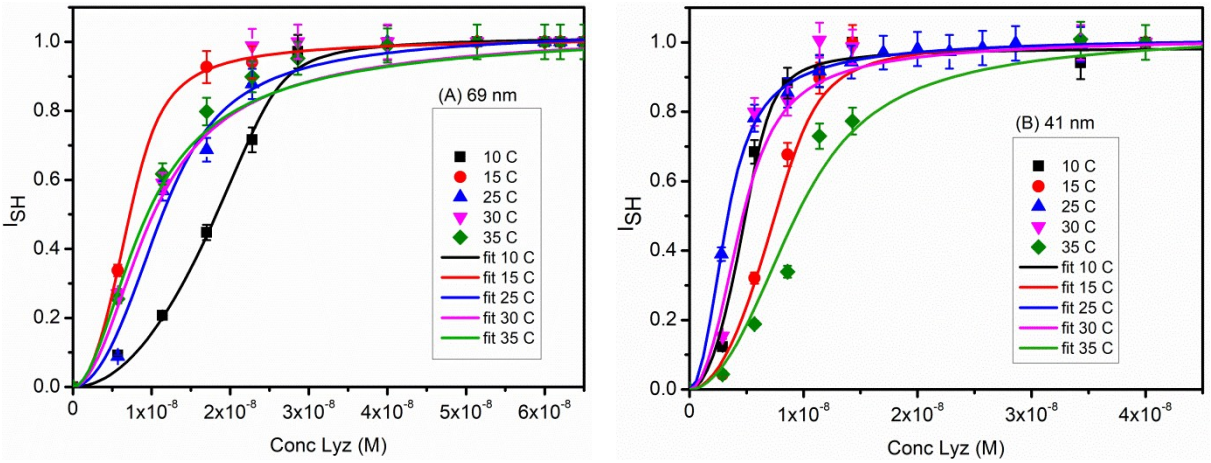


Figure S6. Normalized SH intensity w.r.t. concentration of Lyz at different temperatures (A) 12 pM 69 nm GNP solution and (B)30 pM 41 nm GNP solution in phosphate buffer at pH 7