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

Supplementary information

Probing protein adsorption on nanoparticle surface by second harmonic light scattering

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Synthesis of Au-NPs

The synthesis of 15-60 nm Au-NPs has been performed following a seed mediated growth mechanism. At first 2.5 mL of $HAuCl_4.3H_2O$ solution (0.2% w/v) was diluted to 50 mL of water taken in a 250 mL double necked round bottom flask. The solution was then heated to boiling followed by a quick addition of 2 mL of sodium-citrate solution (1% w/v, containing 0.05% w/v citric acid) under vigorous stirring. The color of the solution slowly turned from yellow to red. The solution was boiled for 10 min and then allowed to cool down to room temperature resulting in 15 nm Au-NPs.

For the synthesis of 30 nm Au-NPs, 15 nm Au-NPS were used as seeds. A volume of 6 mL of the 15 nm Au-NP solution was diluted to a volume 40 mL in a 250 mL round bottom flask. To this 0.8 mL of 0.2% w/v $HAuCl_4.3H_2O$ solution diluted with water to a total volume of 20 mL (henceforth called solution A) and 0.2 mL 1% w/v ascorbic acid and 0.1 mL 1% w/v TSC solutions diluted to a total volume of 20 mL (henceforth called solution B) were added separately at room temperature over an hour under vigorous stirring. After the addition of solutions A and B to the 15 nm seed solution was complete, the mixture was heated to boiling. The solution was boiled for about 30 min and then allowed to cool down to room temperature. By this method we have prepared 30 nm Au-NPs.

For the synthesis of 45 nm Au-NPs 6 mL of the 15 nm Au-NPs solution was diluted to a volume 40 mL in a 250 mL round bottom flask. To this a 20 mL aliquot of the precursor solution A containing $HAuCl_4.3H_2O$, and 20 mL of the solution B containing trisodium citrate as capping and ascorbic acid as reducing agent, were added separately at room temperature over an hour under vigorous stirring. The precursor solution A was prepared by dilution of 4 mL of 0.2% w/v $HAuCl_4.3H_2O$ solution to 20 mL. The precursor solution B was prepared by dilution of 1 mL 1% w/v ascorbic acid and 0.5 mL 1% w/v TSC solutions to 20 mL. After the addition of solution A and B was complete, the solution in the round bottom flask was heated to boiling. The solution was boiled for about 30 min and then allowed to cool down to room temperature. By this method we have prepared 45 nm Au-NPs.

The procedure for preparation of 60 nm size Au-NPs was carried out via second growth step where the 45 nm Au-NPs of the first step growth is used as seed solution. At first 9 mL of the 45 nm Au-NPs solution was diluted to a volume 40 mL in a 250 mL round bottom flask. To this a 20 mL aliquot of the precursor solution A containing $HAuCl_4.3H_2O$, and 20 mL of the solution B containing trisodium citrate as capping and ascorbic acid as reducing agent, were added separately at room temperature over an hour under vigorous stirring. The precursor solution A was prepared by dilution of 1.2 mL of 0.2% w/v $HAuCl_4.3H_2O$ solution to 20 mL. The precursor solution B was prepared by dilution of 0.3 mL 1% w/v ascorbic acid and 0.15 mL 1% w/v TSC solutions to 20 mL. After the addition of solution A and B was complete, the solution in the round bottom flask was heated to boiling. The solution was boiled for about 4 hr and then allowed to cool down to room temperature. By this method we have prepared 60 nm Au-NPs.

Desorption of proteins from Au-NP surface studied via SHLS from Au-NPs

Three sets of solutions of 1.5 mL volume containing 1 nM 15 nm Au-NPs or 0.13 nM 45 nm Au-NPs in the absence or presence of ADH or insulin were prepared. One set of solutions were diluted to 10 times before measurement. Remaining two sets of solutions were centrifuged for 30 min at A) 15000 rpm in the case where 15 nm Au-NPs were present and at B)

6000 rpm in the case where 45 nm Au-NPs were present. The centrifuged was discarded in both sets. In one set the supernatant was diluted to 10 times prior to SHLS measurement. In the last set the supernatant was diluted 10 times in presence of A) 1.5 mL 15 nm Au-NPs of concentration 1 nM in case where the starting solution contained 15 nm particles and B) 1.5 mL 45 nm Au-NPs of concentration 0.13 nM in case where the starting solution contained 45 nm particles. Then the SHLS signal was recorded. The SHLS signal was monitored at 532 nm. The input energy was maintained below 5 mJ/pulse. In the final 15 mL volume of 15 nm 0.1 nM Au-NPs the concentration for A) ADH was 0.11 µM, B) insulin was 1.55 µM. In the final 15 mL volume of 45 nm 0.013 nM Au-NPs the concentration for A) ADH was 0.18 µM, B) insulin was 2.41 µM.

Figures

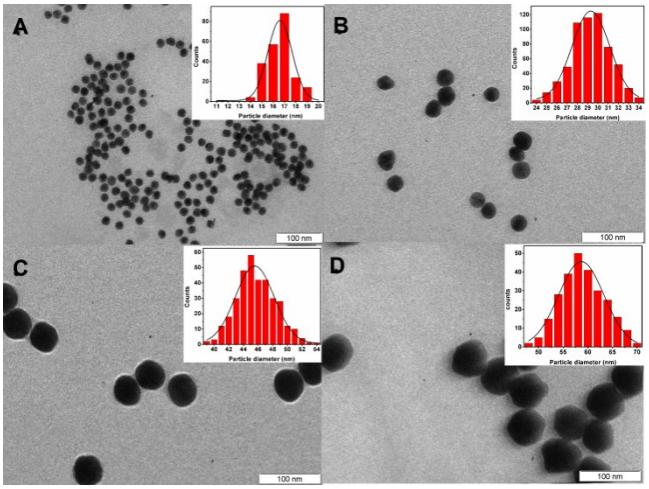

Fig. S1. TEM images of (A) 15 nm (16.6 ± 1.3 nm), (B) 30 nm (29.3 ± 2.3 nm), (C) 45 nm (45.5 ± 3.1 nm), and (D) 60 nm (58.6 ± 5.1 nm) gold nanoparticles (insets are showing the histogram and the size distribution).

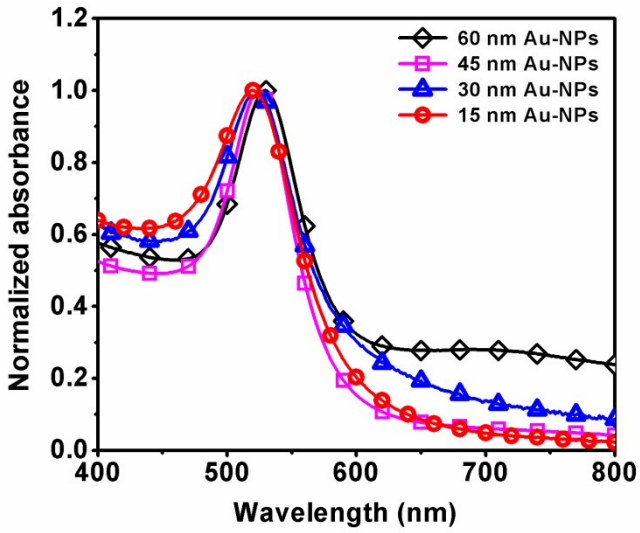

Fig. S2. UV-Vis spectra of gold sols of various particle sizes.

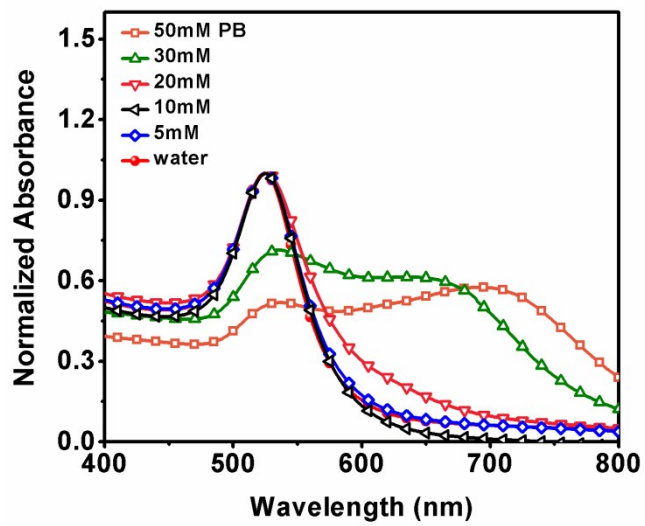

Fig. S3. Normalized absorption spectra of 45 nm gold nanoparticles in phosphate buffer at pH = 7.0. With phosphate buffer concentration above 10 mM, the red shift and broadening of the spectrum indicate the onset of aggregation of nanoparticles.

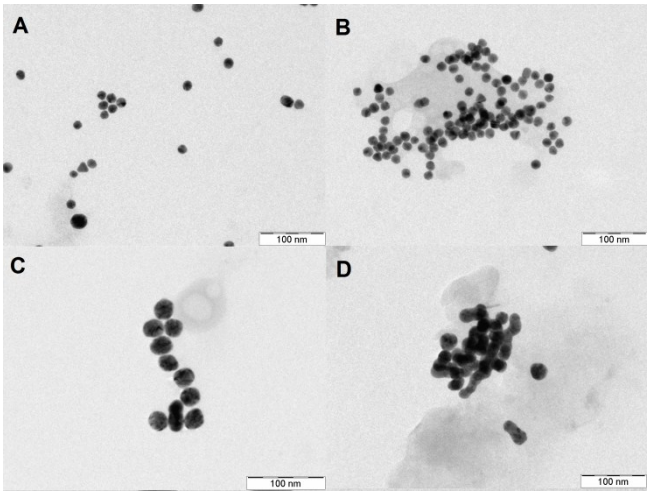

Fig. S4. TEM images of gold nanoparticles in solution with varied concentrations of the buffer: (A) 15 nm Au-NP in 10 mM phosphate buffer, (B) 15 nm Au-NP in 40 mM phosphate buffer, (C) 30 nm Au-NP in 10 mM phosphate buffer, (D) 30 nm Au-NP in 30 mM phosphate buffer.

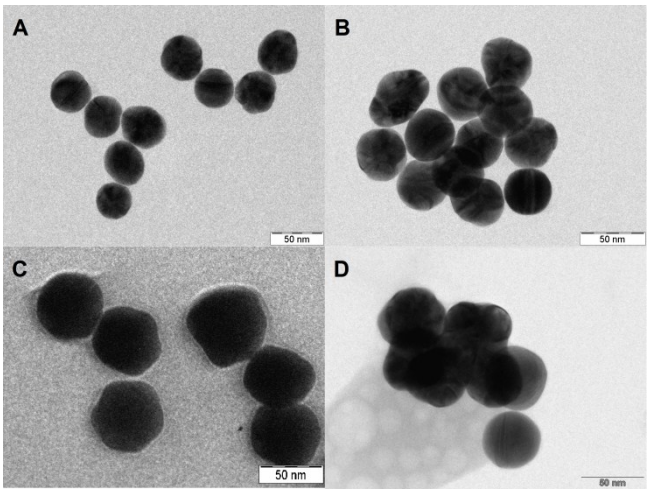

Fig. S5. TEM images of gold nanoparticles in solution with varied concentrations of the buffer: (A) 45 nm Au-NP in 10 mM phosphate buffer, (B) 45 nm Au-NP in 30 mM phosphate buffer, (C) 60 nm Au-NP in 10 mM phosphate buffer, (D) 60 nm Au-NP in 50 mM phosphate buffer.

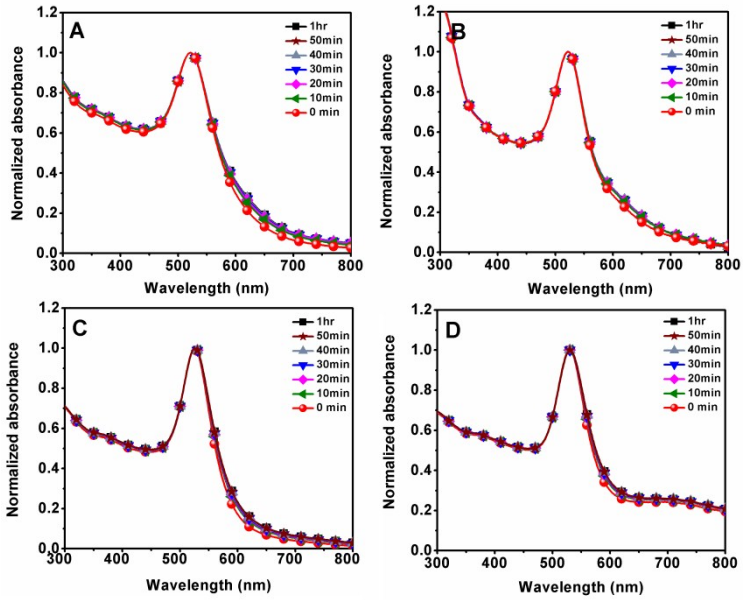

Fig. S6. Normalized absorption spectra of gold nanoparticles of (A) 1 nM 15 nm, (B) 0.067 nM 30 nm, (C) 0.05 nM 45 nm, and (D) 0.01 nM 60 nm, in 10 mM phosphate buffer at pH = 7.0. Spectra were recorded as a function of time at different intervals. The SPR peak position and broadening remain unchanged with time indicating homogeneous dispersion of Au-NPs in solution.

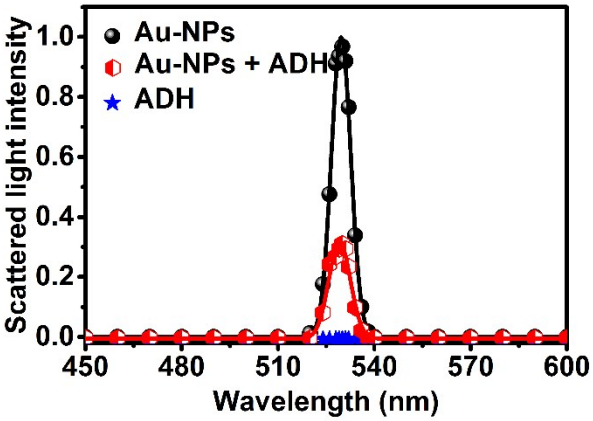

Fig S7.A typical monochromator scan of normalized second harmonic scattered light intensity from 0.34 µM ADH and 45 nm Au-NPs (0.013 nM) in 10 mM pH 7 buffer in the absence and presence of 0.34 µM ADH.The wavelength scan shows that no single or two photon fluorescence is interfering with the detected SH signal.

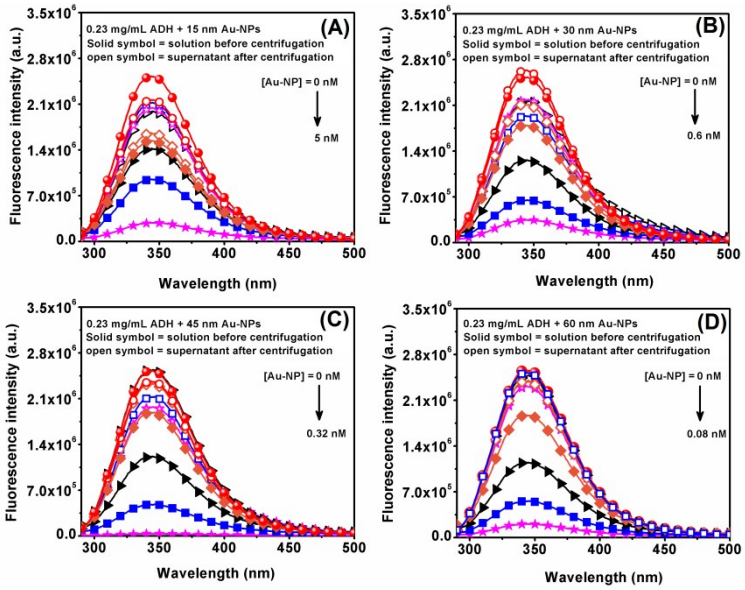

Fig S8. Fluorescence of ADH alone and in the presence of Au-NPs of size A) 15 nm, B) 30 nm, C) 45 nm and D) 60 nm before and after centrifugation of the solutions. 1 mL solutions of 0.23 mg/mL ADH in 10 mM pH 7 buffer were prepared in the absence and presence of different concentrations of a) 15 nm, b) 30 nm, c) 45 nm and d) 60 nm Au-NPs. Afterwards 500 µL of the solution were centrifuged for 30 min at a speed of i) 15000 rpm for solution containing 15 nm Au-NPs, ii) 9000 rpm for solution containing 30 nm Au-NPs, iii) 6000 rpm for solution containing 45 nm Au-NPs and iv) ca 5000 rpm for solution containing 60 nm Au-NPs. Fluorescence spectra of the supernatants were recorded and compared with those of the solutions before centrifugation.The fluorescence intensity at the maxima, 336 nm decreases in the presence of nanoparticles due to the adsorption of ADH on the surface of Au-NPs and the position of the fluorescence maxima don't change. The extent of quenching increases with increasing concentration of Au-NPs due to the increased adsorption. After centrifugation of nanoparticle-ADH conjugate solution, the fluorescence signal was recovered completely in the supernatant with the position of the fluorrescence maxima unchanged. These indicate complete desorption of ADH from Au-NP surface during the centrifugation process.

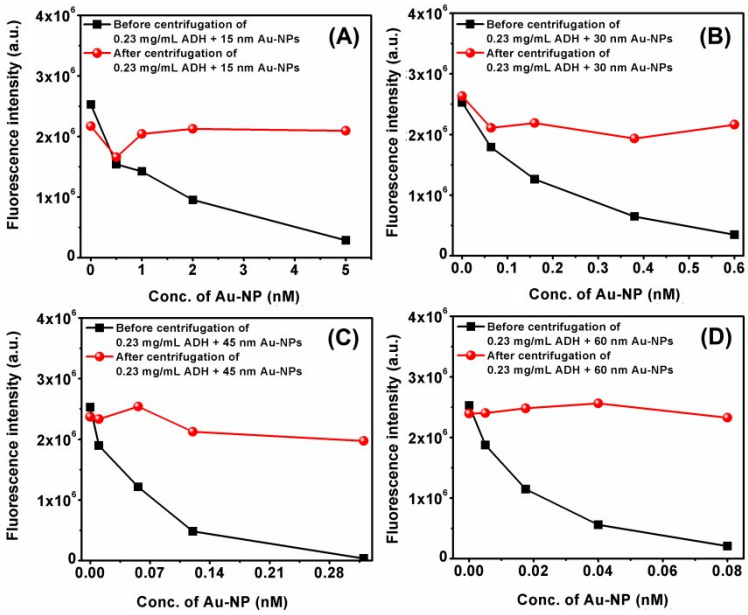

Fig S9. Quantitative analysis of tryptophan fluorescence of 0.23 mg/mL ADH alone and in the presence of 15 (A), 30 (B), 45 (C) and 60 nm (D) Au-NPs in 10 mM pH 7 buffer at 25 ^{o}C before and after centrifugation. The fluorescence signal at the maxima, 336 nm are plotted as a function of concentration of Au-NPs. The solid line connects the experimental data points.

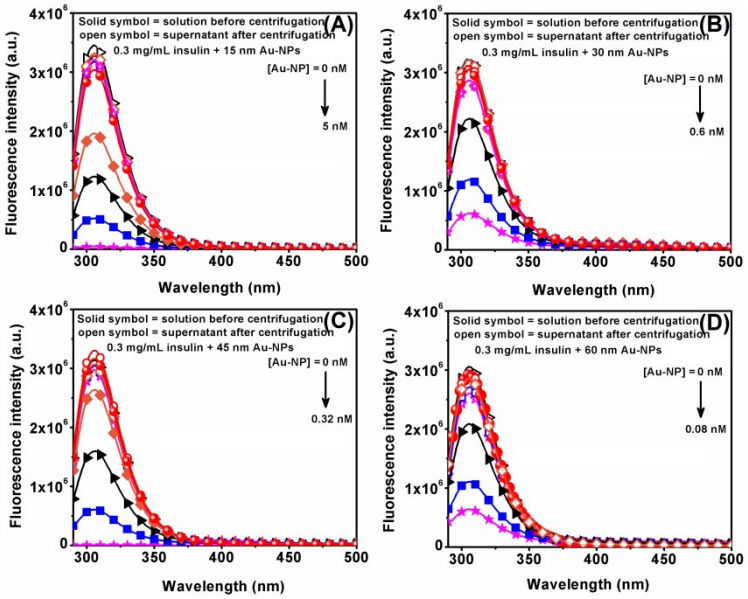

Fig S10. Fluorescence of insulin, alone and in the presence of Au-NPs of size A) 15 nm, B) 30 nm, C) 45 nm and D) 60 nm before and after centrifugation. 1 mL solution of 0.3 mg/mL insulin in 10 mM pH 7 buffer were prepared in the absence and presence of different concentrations of a) 15 nm, b) 30 nm, c) 45 nm and d) 60 nm Au-NPs. Afterwards 500 µL of the solution were centrifuged for 30 min at a speed of i) 15000 rpm for 15 nm Au-NPs, ii) 9000 rpm for 30 nm Au-NPs, iii) 6000 rpm for 45 nm Au-NPs and iv) ca 5000 rpm for 60 nm Au-NPs. Fluorescence spectra of the supernatant were recorded and compared with that of solutions before centrifugation.

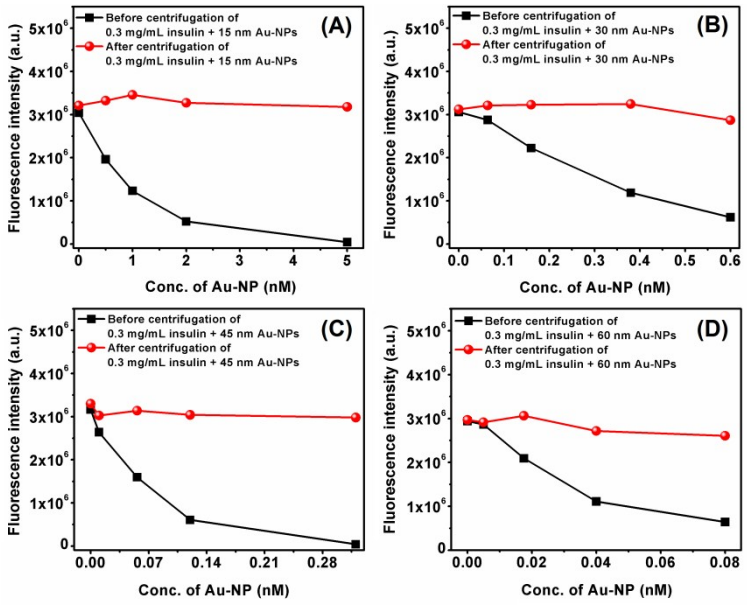

Fig. S11. Quantitative analysis of tyrosine fluorescence of 0.3 mg/mL insulin alone and in the presence of Au-NPs of size A) 15 nm, B) 30 nm, C) 45 nm, D) 60 nm in 10 mM buffer at 25 ^{o}C before and after centrifugation. The fluorescence intensity at the peak maximum at 305 nm are plotted as a function of concentration of Au-NPs. The solid line connects the experimental data points.

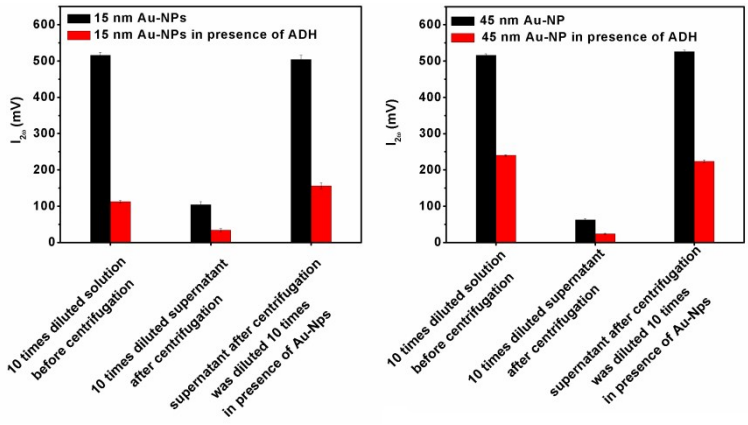

Fig S12. SHLS intensity at 532 nm from Au-NPs in the absence and presence of ADH and before or after centrifugation. A) 1.5 mL 1 nM 15 nm Au-NPs, in the absence and presence of 1.1 µM ADH was prepared in 10 mM pH 7 buffer and the SH intensity was recorded i) after 10 times dilution of the solution ii) after centrifugation of the solution at 15000 rpm for 30 min and then dilution of the supernatant to 10 times, iii) after centrifugation of the solution at 15000 rpm for 30 min and then dilution of the supernatant to 10 times in the presence of 1.5 mL 1nM 15 nm Au-NPs. B) 1.5 mL 0.13 nM 45 nm Au-NPs, in the absence and presence of 1.8 µM ADH was prepared in 10 mM pH 7 buffer and the SH intensity was recorded i) after 10 times dilution of the solution ii) after centrifugation of the solution at 6000 rpm for 30 min and then dilution of the supernatant to 10 times, iii) after centrifugation of the solution at 6000 rpm for 30 min and then dilution of the supernatant to 10 times in the presence of 1.5 mL 0.13 nM 45 nm Au-NPs. The concentration of ADH was chosen following the results in Table 1 in such a way that the adsorbed ADH can fully cover the surface of all the nanoparticles in the solution. The SHLS signal of the supernatant obtained after centrifugation and treated with Au-NPs was found to be almost similar in magnitude with the signal obtained from Au-NP-ADH conjugate solution, before centrifugation.

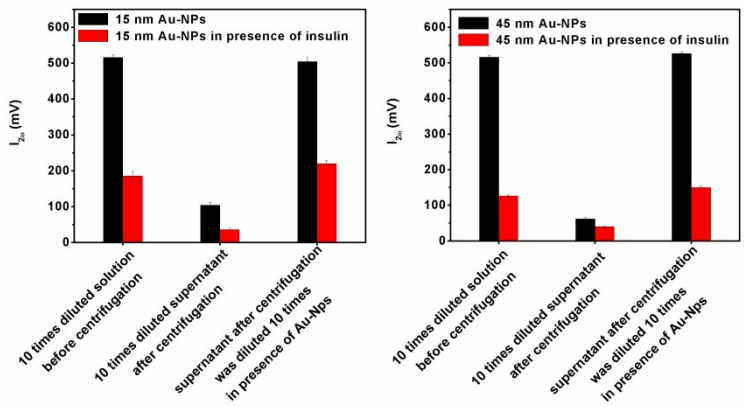

Fig. S13. SH intensity at 532 nm from Au-NPs in the absence and presence of insulin and before or after centrifugation. A) 1.5 mL 1 nM 15 nm Au-NPs, in the absence and presence of 15.5 µM insulin was prepared in 10 mM pH 7 buffer and the SH intensity was recorded i) after 10 times dilution of the solution ii) after centrifugation of the solution at 15000 rpm for 30 min and then dilution of the supernatant to 10 times, iii) after centrifugation of the solution at 15000 rpm for 30 min and then dilution of the supernatant to 10 times in the presence of 1.5 mL 1nM 15 nm Au-NPs. B) 1.5 mL 0.13 nM 45 nm Au-NPs, in the absence and presence of 24.1 µM insulin was prepared in 10 mM pH 7 buffer and the SH intensity was recorded i) after 10 times dilution of the solution ii) after centrifugation of the solution at 6000 rpm for 30 min and then dilution of the supernatant to 10 times, iii) after centrifugation of the solution at 6000 rpm for 30 min and then dilution of the supernatant to 10 times in the presence of 1.5 mL 0.13 nM 45 nm Au-NPs. The concentration of insulin was chosen following the results in Table 2 in such a way that the adsorbed insulin can fully cover the surface of all the nanoparticles in the solution.