Gold nanorod in reverse micelles: a fitting fusion to catapult lipase activity

Subhabrata Maiti, Moumita Ghosh and Prasanta Kumar Das*

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India. E-mail: bcpkd@iacs.res.in

Electronic Supplementary Information (ESI)

Materials.

Chromobacterium viscosum lipase (E.C.3.1.1.3 Type XII), $HAuCl_4$ (30 wt. %) in dilute hydrochloric acid solution, FITC and uranyl acetate were purchased from Sigma-Aldrich Chemical Company, USA and were used as received. Analytical grade CTAB was purchased from Spectrochem (India) and it was crystallized three times from methanol/diethyl ether. Recrystallized CTAB was without minima in its surface tension plot. HPLC-grade isooctane, *n*-hexanol, ascorbic acid, $NaBH_4$, sodium salt of fluorescein and all other reagents were purchased from SRL (India) and were of highest analytical grade. Trisodium citrate, silver nitrate was procured from Merck (India). The UV-visible absorption spectra were recorded on a Varian Cary-50 spectrophotometer. Bruker Avance DPX-300 spectrophotometer was used to perform ^1H NMR spectra. Substrate, *p*-nitrophenyl-*n*-octanoate was synthesized following previously reported protocols.[1]

Preparation of gold nanorod (GNR). GNR-solution having different aspect ratio was synthesized by following protocols described elsewhere (Reference 5 in the main manuscript). Briefly, 5 mL of aqueous solution of cetyltrimethylammonium bromide (CTAB) was prepared with $HAuCl_4$. The final concentrations of CTAB and $HAuCl_4$ were 0.1 M and 1 mM, respectively. Next, ascorbic acid and $AgNO_3$ were added in this solution so that reaction

concentration of ascorbic acid was 2 mM and that of $AgNO_3$ was 0.10, 0.15 and 0.20 mM, respectively for GNR-1, GNR-2 and GNR-3. The solution color changed from orange to colorless upon the addition of ascorbic acid. Finally, 50μL of 1mM freshly prepared aqueous $NaBH_4$ solution was added. The solution color started to appear pink-violet within few min indicating the formation of gold nanorods.

This GNR-solution was ultracentrifugated at 40000 rpm for 30 min to remove excess CTAB by discarding the supernatant. The GNR-residue was then dispersed with phosphate buffer (pH=6, 20mM), which was used to prepare microemulsions, to check enzyme activity and for all other studies.

Preparation of reverse micelles and GNR-doped reverse micelles.

The requisite quantity of CTAB was dispersed in isooctane in a 2 mL volumetric flask, to which the calculated amount of *n*-hexanol was added to attain the corresponding z ([*n*-hexanol]/[surfactant]) value and shaken vigorously. Finally, aqueous buffer (phosphate) solution was added (to reach the corresponding W_0), and the whole suspension was vortexed to obtain a clear homogeneous solution of CTAB (50 mM)/isooctane/*n*-hexanol/water reverse micelle. GNR-doped reverse micelle was prepared by following the same procedure where abovementioned GNR-solution was used to attain desired concentration and rest of the volume was made up with buffer as when needed to achieve corresponding W_0.

Activity of lipase in reverse micelle.

The second-order rate constant (k_2) in lipase-catalyzed hydrolysis of *p*-nitrophenyl-*n*-octanoate in w/o microemulsions was determined spectrophotometrically at the isosbestic points (Table S1) as described previously.[1,2] In a typical experiment, 4.5 μL of the aqueous enzyme stock solution (0.34 mg/mL) followed by 10 μL of the substrate stock solution (0.45 M) in isooctane were added to the previously prepared 50 mM CTAB or GNR doped CTAB reverse micelle solution (1.5 mL) at pH=6 (pH refers to the pH of the aqueous buffer

solutions used for preparing the w/o microemulsions; pH within the water pool of w/o microemulsions did not vary significantly, <1 unit),[1] in a cuvette to make the particular W_0 and reactant concentrations. It was then shaken by hand until solution becomes clear. The initial linear rate of increase in absorbance of liberated *p*-nitrophenol was then recorded at their respective isosbestic points (λ_{iso}). The overall concentrations of lipase and *p*-nitrophenyl-*n*-octanoate were 1.02×10^{-6} g/mL and 3×10^{-3} M, respectively. Although the lipase was essentially confined to the dispersed water droplets (at the oil/water interface), for simplicity, the concentration of reactants were referred to the overall concentration to avoid the complexity of the volume fraction of water droplet in the w/o microemulsions and the partitioning coefficient of the substrate.[1,2] Furthermore we measured the second-order rate constant (k_2) instead of first-order Michaelis–Menten catalytic constant (k_{cat}), since the initial rate of lipase catalyzed hydrolysis of *p*-nitrophenyl-*n*-octanoate was observed to be first order with respect to the substrate concentration (Figure S2).[1]

Circular Dichroism (CD) Spectra.

The CD spectra of Lipase in CTAB and GNR-doped CTAB reverse micelles with varying W_0 were recorded in Jasco J-815 using 2 mm path length cell at wavelength 220-300 nm with a scan speed of 50 nm/min (CD spectra could not be measured below 220 nm due to off scale signal). All the spectra were corrected by subtracting a blank spectrum (without enzyme) and accumulated 6 times. Results were expressed in terms of mean residue ellipticity ($degcm^2dmol^{-1}$). The final concentration of the lipase was 25 μg/mL.

TEM Studies.

4 μL of the solution containing the GNRs (either in water or reverse micelles) were placed on 300-mesh Cu coated TEM grid and dried under vacuum for 4 h before taking TEM images. For the negatively stained images of lipase in CTAB reverse micelles in absence and presence of GNR, aqueous solution (4 μL) of freshly prepared uranyl acetate (2 %, w/v) was

used. Final concentration of protein and Au was 2.5 μg/mL and 20 μM within reverse micelle. TEM measurements were performed on JEOL JEM 2010 microscope.

Fluorescence studies

The luminescence spectra of FITC were taken by exciting at wavelength of 495 nm in water, isooctane and the CTAB reverse micelles at different z and W_0 both in absence and presence of GNR solution. FITC in DMSO (5 μL) was diluted to 1 mL solution (water, isooctane or the microemulsion of CTAB) before taking the luminescence spectra. Final concentration of FITC was 5 μg/mL. The excitation and emission slits were kept at 5 nm. Fluorescence spectra were recorded in Varian Cary Eclipse luminescence spectrometer.

Table S1 : W_0 range of CTAB-reverse micelle in absence and presence of GNR-3.

z-value	W_0 in **absence** of GNR	W_0 in **presence** of GNR
4.8	40-56	40-56
6.4	28-48	32-64
8.0	18-48	20-80
11.2	8-44	12-92
16.0	8-36	8-120

Table S2: Isosbestic point (λ_{iso}) and molar extinction co-efficient (ε) of *p*-nitrophenol/*p*-nitrophenolate couple in different GNR-doped reverse micelles.

System	[Au] (μM)	λ_{iso} (nm)	ε values ($M^{-1}cm^{-1}$)
GNR-doped CTAB reverse micelle	0	340	4370
	8-10	342	4350
	16-20	341	4040
	32-36	341	3550
	40	342	3300
	80	341	3100

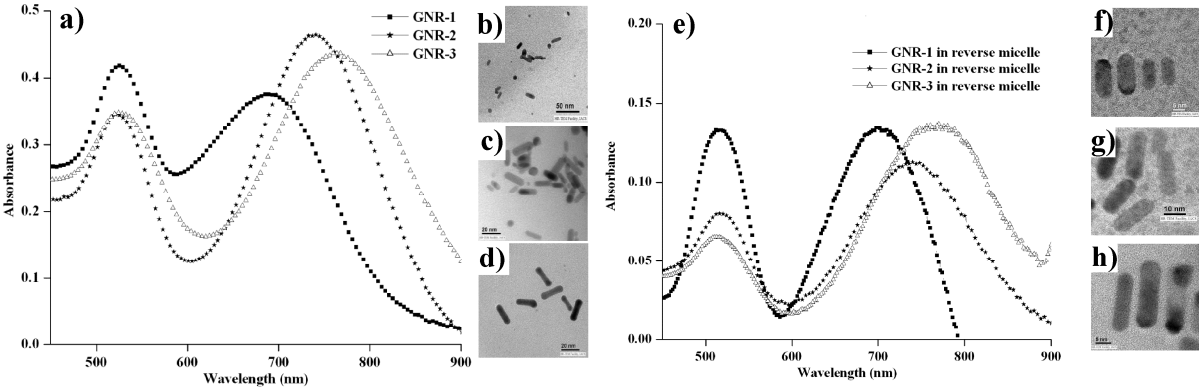

Fig. S1 a) UV-vis spectra of the synthesized GNR. (b-d) TEM images of GNR-1, GNR-2 and GNR-3, respectively. e) UV-Vis spectra of GNR-1, GNR-2 and GNR-3 in CTAB-reverse micelle (z= 8.0, W_0=40, [Au]=40 μM). (f-h)TEM images of GNR-1, GNR-2 and d) GNR-3, respectively in reverse micelle at same condition.

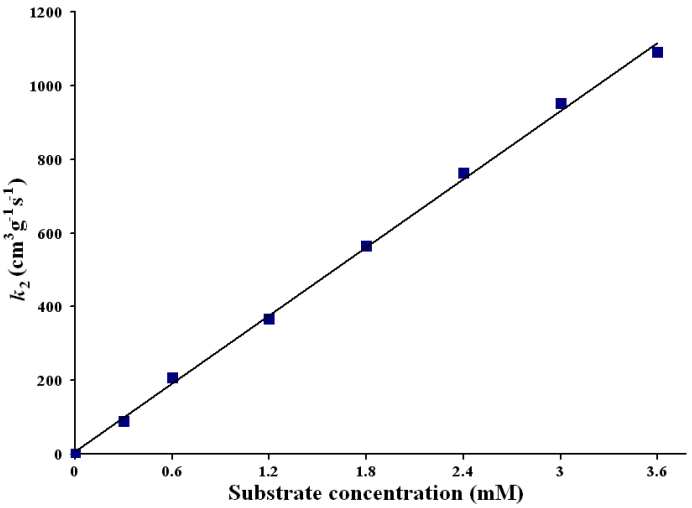

Fig. S2 Plot of second order rate constant (k_2) of lipase hydrolysis of *p*-nitrophenyl-*n*-octanoate in GNR-doped CTAB-reverse micelle with varying substrate concentration at z = 6.4 and W_0 = 44. [CTAB] = 50mM

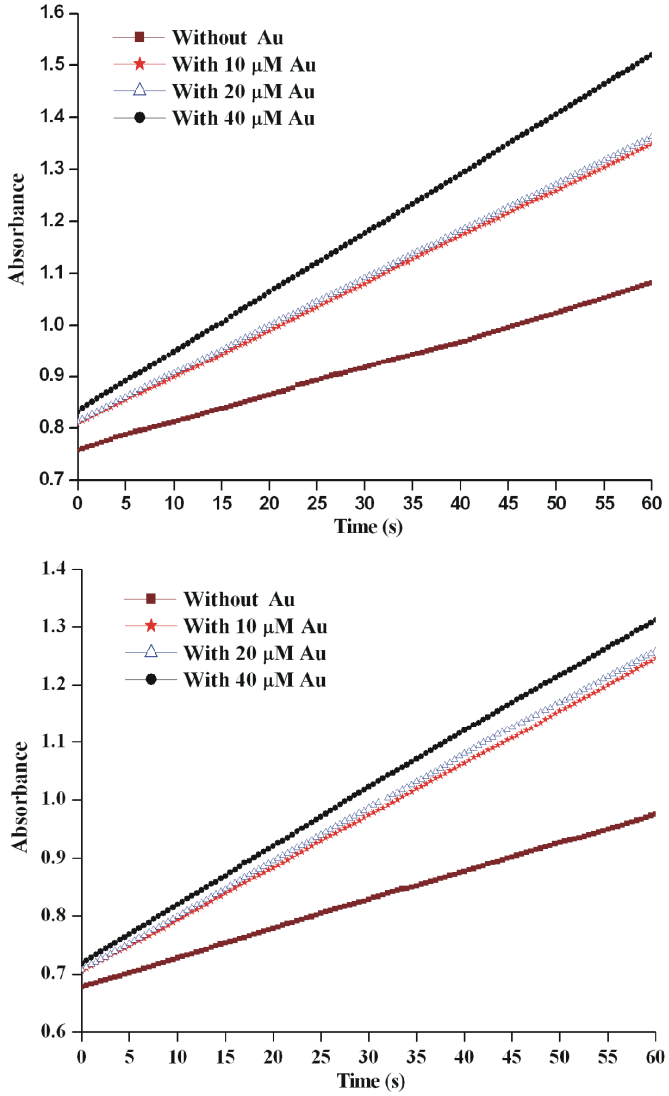

Fig. S3 Absorbance v time plot during lipase catalyzed hydrolysis of *p*-nitrophenyl octanoate in absence and presence of GNR-3 doped in CTAB reverse micelle at a) z=4.8, b) z=6.4. Absorbance values were measured at their respective isosbestic points given in Table S2.

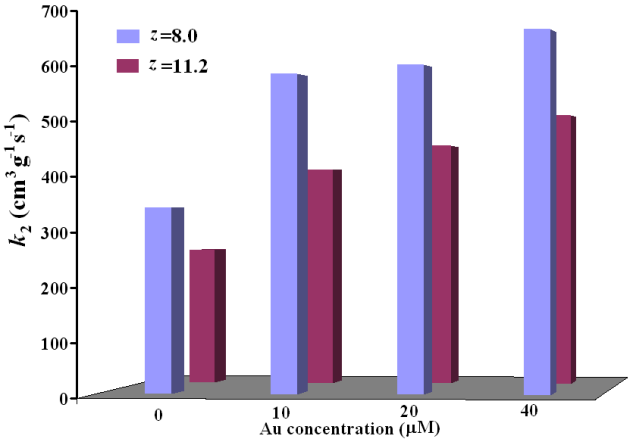

Fig. S4 Variation of the second-order rate constant k_2 for the lipase-catalyzed hydrolysis of *p*-nitrophenyl-*n*-octanoate in GNR-3 doped CTAB reverse micelles with varying Au concentrations at different z and 25 °C. [CTAB]=50 mM, [enzyme]=1.02 μgmL^{-1}, [substrate]= 3 mM.

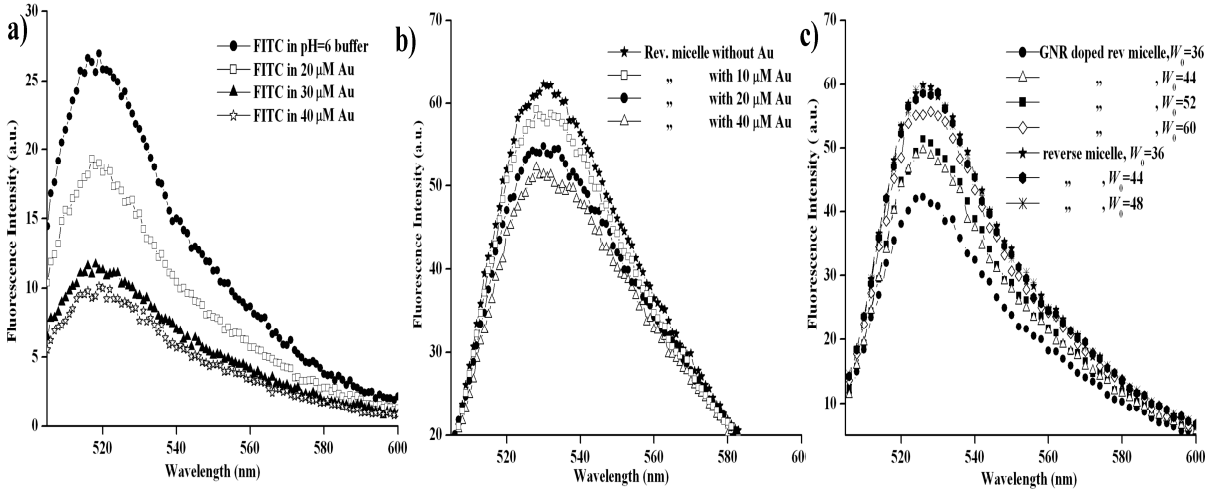

Fig. S5 Fluorescence spectra of FITC a) in aqueous pH=6 phosphate buffer in absence and presence of varying concentration of GNR; b) in 50mM CTAB-reverse micelle at z=6.4, W_0=44 in absence and presence of different GNR concentration; c) in CTAB-reverse micelle in absence and presence of GNR at z=6.4 with increasing W_0 and constant [Au]=40 μM. The excitation and emission slit width were 5 nm.

References:

1. D. Das, P. K. Das, *Langmuir*, **2003**, *19*, 9114; D. Das, S. Roy, R. N. Mitra, A. Dasgupta, P. K. Das, *Chem. Eur. J.*, **2005**, *11*, 4881.

2. A. Shome, S. Roy, P. K. Das, *Langmuir*, **2007**, *23*, 4130; P. D. I. Fletcher, B. H. Robinson, R. B. Freedman, C. Oldfield, *J. Chem. Soc. Faraday Trans.*, **1985**, *81*, 2667.