Supporting Information

Substrate-Induced Phase Transition within Liquid Condensates Reverses Catalytic Activity of Nanoparticles

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Experimental

Materials and Methodology:

All the chemicals unless mentioned are procured from Sigma-Aldrich. Bovine serum albumin (BSA) and sodium sulphate are purchased from Hi-Media. Pyrogallol is procured from TCI chemicals. Amine reactive second generation sensors were procured from the ForteBio. Double Distilled water has been used to carry out all the experiments.

1.Synthesis of BSA gold nanocluster (AuNC@BSA): We synthesized AuNC@BSA using the protocol described by Xie et al. with slight modification[1]. Briefly, 12 mg/ml BSA was incubated with 5 mM $HAuCl_4$ for 30 min under vigorous stirring at 37˚C. After half an hour, 0.1 N NaOH was added to the solution of BSA and $HAuCl_4$. The resulting solution was incubated and continuously stirred for 12 hrs under the same condition and then dialysed against water for 24 hrs using 10 kDa cut-off membrane (SnakeSkin Dialysis Tubing, Thermo scientific) to attain the neutral pH of the solution.

2. Characterisation and phase separation of AuNC@BSA: We recorded the absorption spectrum of the AuNC@BSA using Cary UV-Vis compact Peltier spectrophotometer (Agilent, USA). The fluorescence property of the AuNC@BSA was checked on UV-illuminator under 312 nm light and further, the fluorescence spectrum was recorded with fluorimeter (Edinburg spectrometer FS5, U.K.) at the excitation wavelength of 450 nm. For the agarose gel electrophoresis, we prepared 1.5% of agarose gel and 20 µL samples were loaded after mixing them with loading dye containing 2mg/ml Coomassie brilliant blue G250 and 40% glycerol.

For fluorescence microscopic studies, BSA was tagged with amine-reactive NHS ester-Texas red dye by mixing dye and protein in 1:10 molar ratio. The sample was incubated overnight at 4°C overnight followed by the removal of unreactive dye by excess dialysis using 10 kDa cutoff membrane (SnakeSkin Dialysis Tubing, Thermo scientific). The resulting Texas red-labeled BSA was then used to synthesize AuNC@BSA using the above-mentioned protocol. To induce phase separation, 5% polyethylene glycol (MW 6000, PEG 6000)[2], was used in combination with 500 mM of Na_2SO_4, following the previous report[3]. For the microscopic study, 1mg/ml mixed AuNC@BSA (1% TR labeled AuNC@BSA with unlabelled AuNC@BSA) was incubated with 5% PEG 6000 and 500 mM Na_2SO_4 for 10 minutes at room temperature and then drop casted on the glass slide. The formation of AuNC@BSA condensates was visualised using fluorescence microscope (Model: Leica DMi8).

3. Biolayer interferometry: Interaction between AuNC@BSA, BSA and pyrogallol was checked using Fortebio Octet K2 (Molecular devices USA). Amine reactive second-generation (AR2G) biosensors were activated using a solution containing 2:1 molar ratio of EDC and NHS. 1mg/ml either of AuNC@BSA or BSA was then loaded on the activated sensor. The

association and dissociation kinetics were recorded by immersing the sensor in increasing concentrations of pyrogallol and water respectively. Next, to calculate the K_D, global fitting of the association and dissociation was performed using Data analysis HT 9.0.0.33 software provided with the instrument.

4. Assay of peroxidase activity in dispersed and phase separated condition: Peroxidase activity of AuNC@BSA was checked from oxidation of pyrogallol to purpurogallin in presence of high excess H_2O_2 in dispersed and phase separated condition. In dispersed condition, reaction mixtures contain 1 mg/ml AuNC@BSA, different concentration of pyrogallol (100 µM -3000 µM) and 0.1 M H_2O_2. Oxidation of the substrate was tracked at absorption maxima of purpurogallin i.e., 420 nm using TECAN INFINITE MPLEX reader at kinetic mode continuously for 30 minutes. The same procedure was followed for determination of peroxidase activity in phase separated condition in presence of 5% PEG 6000 and 500 mM Na_2SO_4. Rate of the reaction was calculated from the rate of change of absorbance (420 nm) per unit time ($\Delta A_{420}/\Delta t$). For dispersed condition, data was fitted with Michaelis-Menten kinetics equation using Origin pro 2020b. To check whether the oxidation of pyrogallol to purpurogallin impart any effect on the condensate, we imaged AuNC@BSA (labelled with Texas red) condensate in presence of different concentrations of substrate and 0.1 M H_2O_2. Next, we measured size of the condensate at each substrate concentration using ImageJ.

For the determination of partition coefficient, we phase separated AuNC@BSA with different concentration of substrate (pyrogallol) and then we centrifuged at 8000 rpm for 10 minutes at RT using Allegra 64R centrifuge (Beckman coulter). On centrifugation, the condensates get sedimented at the bottom of the micro centrifuge tube. We then recorded UV-Vis spectra of the sedimented condensate and the supernatant separately. We determined the partition coefficient of pyrogallol by analysing the absorbance ratio at 267 nm (λ_{max} of pyrogallol) between the condensed and diluted phases.

5. Substrate-catalyst interaction in condensate: We checked the interaction between pyrogallol and AuNC@BSA within the condensate by recording fluorescence spectra using spectrofluorometer (Edinburg spectrofluorometer FS5, UK). Samples were prepared by mixing 1 mg/ml AuNC@BSA, 5% PEG 6000, 500 mM Na_2SO_4 and different concentrations of pyrogallol (100 µM, 500 µM, 1000 µM, 1500 µM, 2000 µM, 2500 µM and 3000 µM). The samples were incubated at RT for 10 minutes then, fluorescence spectra were recorded by exciting the sample at 388 nm using band width of 2 nm.

6. Molecular docking and visualisation analysis: We docked pyrogallol with BSA (PDB ID: 3V03) using AutoDock Vina[4,5]. The resulting docked complex was then visualised and analysed using UCSF Chimera[6]. The obtained results were further validated using LigPlot Plus[7].

7. Change in phase behaviour of AuNC@BSA condensate in the presence of substrate: We first monitored the rate of formation of droplets with and without substrate from the rate of change in turbidity@700 nm per unit time ($\Delta A_{700}/\Delta t$). Next, the change in LCST (Lower Critical Solution Temperature) was checked using the thermal scan mode of Cary UV-Vis compact Peltier spectrophotometer (Agilent, USA). Condensate of AuNC@BSA was taken in a cuvette of 1 cm pathlength and the change in turbidity was measured at 700 nm with the change in temperature from 30°C-70°C. LCST was calculated from the plot of dA_{700}/dT vs T plot (where dA_{700}/dT was a change in turbidity with temperature and T was temperature). The change in LCST value ($\Delta LCST = LCST_{no\ substrate} - LCST_{substrate}$) with increasing concentrations of the substrate was also determined.

Supplementary tables and figures

Substrate Concentration (μM)	Reaction Rate in dispersed condition (min^{-1})	Reaction Rate in condensate condition (min^{-1})
100	$(25\pm4)x10^{-4}$	$(110\pm5)\ x10^{-4}$
500	$(8\pm2)\ x10^{-3}$	$(15\pm2)\ x10^{-3}$
1000	$(12\pm2)\ x10^{-3}$	$(23\pm1)\ x10^{-3}$
1500	$(14\pm3)\ x10^{-3}$	$(18\pm1)\ x10^{-3}$
2000	$(18\pm2)\ x10^{-3}$	$(9\pm3)\ x10^{-3}$
2500	$(18\pm0.5)\ x10^{-3}$	$(6\pm1)\ x10^{-3}$
3000	$(18\pm0.7)\ x10^{-3}$	$(6\pm0.2)\ x10^{-3}$

Table S1: Rate of oxidation of pyrogallol at different substrate concentrations in dispersed and phase separated condition.

Substrate Concentration (μM)	Average droplet size (μm)
Control	32.38404
100	32.95448
1000	32.14076
1500	32.50465
2000	33.96073

Table S2: Average droplet size determined with increasing substrate concentrations.

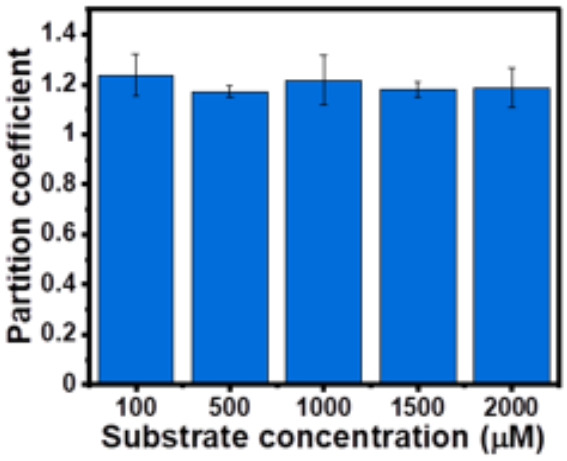

Figure S1: Partition coefficients of substrate with increasing substrate concentration showing no change in substrate uptake within the condensate.

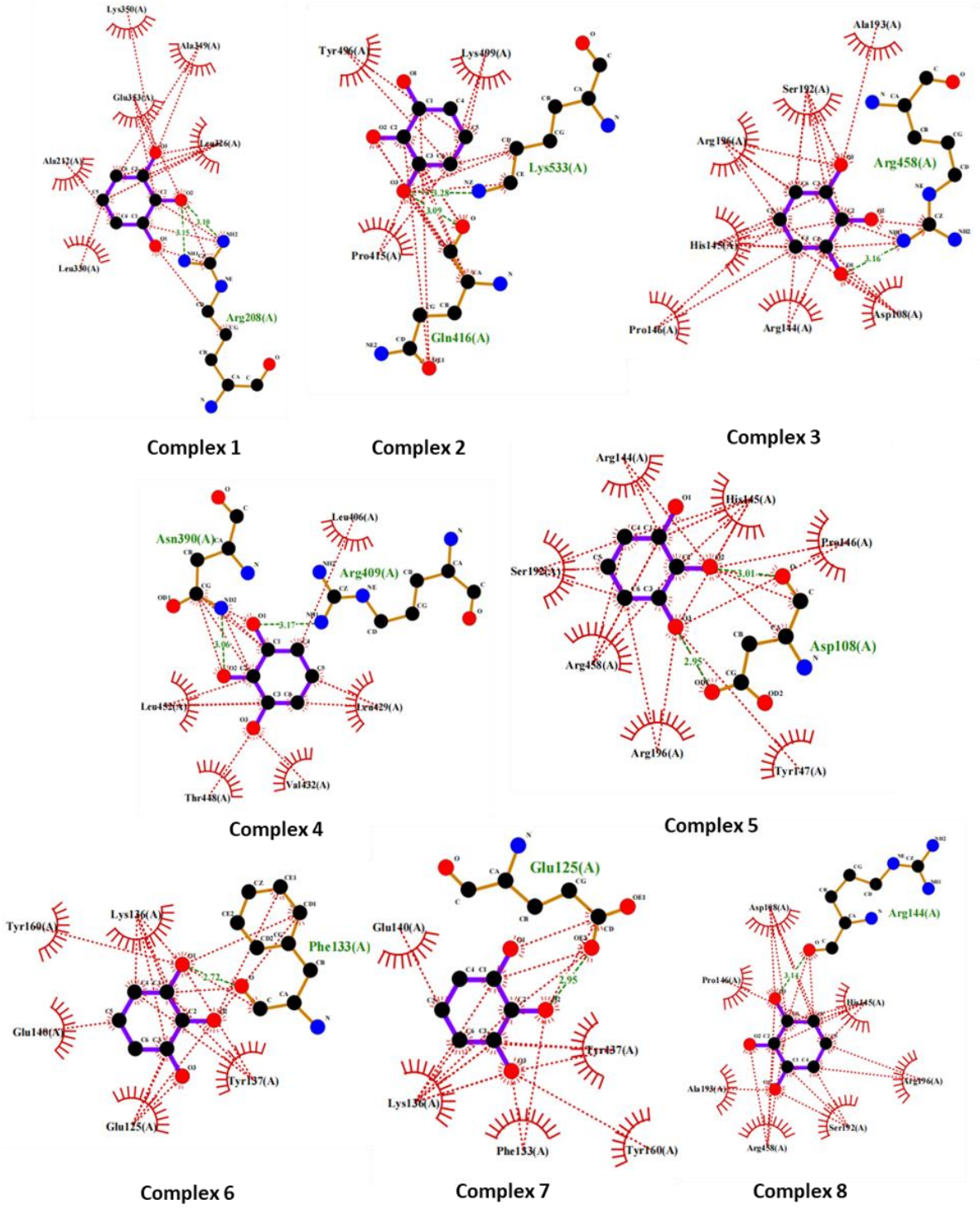

Figure S2: 2D diagrams of docked complexes of pyrogallol-BSA showing hydrogen bonding (green dotted lines) between the hydroxyl group of pyrogallol and specific amino acids of BSA. Red dotted lines are indicating hydrophobic interaction between pyrogallol and BSA. Notably, the polar hydroxyl groups of the substrate are being shielded by hydrogen bonding interactions, potentially altering the environment to be more hydrophobic.

References

1 J. Xie, Y. Zheng and J. Y. Ying, *J. Am. Chem. Soc.*, 2009, 131, 888–889.

2 S. Arora and S. R. K. Ainavarapu, *Biophys. J.*, 2024, **123**, 193a–194a.
3 S. Bedi, G. Kumar, S. M. Rose, S. Rakshit and S. Sinha, *Chem. Commun.*, 2022, **58**, 8634–8637.
4 J. Eberhardt, D. Santos-Martins, A. F. Tillack and S. Forli, *J. Chem. Inf. Model.*, 2021, **61**, 3891–3898.
5 O. Trott and A. J. Olson, *J. Comput. Chem*, 2010, **31**, 455–461.
6 E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem*, 2004, **25**, 1605–1612.
7 R. A. Laskowski and M. B. Swindells, *J. Chem. Inf. Model.*, 2011, **51**, 2778–2786.