Electronic Supplementary Information

Phase Transfer of Fatty Acids into Ultrasmall Nanospheres for Colorimetric Detection of Lipase and Albumin

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Reagents

Pluronic F-127 (F127), bis(2-ethylhexyl) sebacate (DOS), sodium tetrakis[3,5-bis(trifluoromethyl)-phenyl]borate (NaBARF)，pocine pancreas lipase (PPL，Type II), human immunoglobulin G(IgG), human serum albumin (fatty acid free, HSA), glyceryl trioleate, oleic acid, acetonitrile, and chromoionophore I (CHI) were obtained from Sigma-Aldrich. Glycerol trilinoleate was from Macklin Inc. Bovine serum albumin (BSA) was obtained from Bovostar. Tetrahydrofuran (THF) and glyceryl trilinolenate were purchased from J&K Scientific Ltd. (China). Phosphate and octanoic acid were from Aladdin (China). Deionized water was purified by Milli-Q Integral 5. The PPL (3 mg) was added directly in 5 mL of 10 mM phosphate buffer (pH = 7.5). The mixture was stirred (800 rpm) for 2 hours at room temperature and diluted to the desired concentrations with the phosphate buffer. HSA stock solution was prepared at 5 mg/mL in water and stored at 4 °C before use. Serum samples were provided by Qian Xi Nan People's Hospital and stored at -20 °C.

Preparation of the nanospheres containing CHI

A cocktail was prepared by adding 2.0 mg of F127, 4.0 mg of DOS, and 0.7 mg of CHI into 1.0 mL THF. Then 50 μL of the cocktail was carefully pipetted into 2.0 mL of H_2O on a vortex spinning at 1000 rpm, forming a visually homogeneous suspension without aggregates. The mixture was blown with compressed air to remove THF leading to a concentrated stock solution of the nanospheres.

Lipase activity assay

To prepare the suspended substrate, 250 μL of 50 mg/mL triglyceride (in THF) was injected into 2.0 mL of H_2O on a vertex spinning at 1000 rpm, and blown with compressed air to remove THF. Different concentrations of lipase stock solutions (200 μL) were mixed with 200 μL of PB solution (10 mM, pH 7.5) and 200 μL of 6.25 mg/mL of triglyceride in a cuvette. After shaking at room temperature, the color of samples was captured with a digital camera (Canon 5D EOS Mark IV). To obtain a calibration curve, calculated amounts of oleic acid was additionally added and mixed with the PB solution and the nanospheres containing CHI with a volume ratio of 1:1:1. The final

concentrations of oleic acid were adjusted to be 0, 5, 10, 15, 20, 30, 40, 50, 70, 90, 110, 130, 150 and 170 μg/mL.

Colorimetric detection of HSA

A cocktail was prepared by adding 2.0 mg of F127, 4.0 mg of DOS, 1.5 mg of oleic acid, and 0.7 mg of CHI into 1.0 mL THF to form a homogeneous solution. Then the nanospheres were prepared by the abovementioned solvent displacement method. The nanospheres (100 μL) were then mixed with 100 μL of PB and 100 μL of HSA solution (or diluted serum, 100x). After 5 minutes of shaking, the color of the mixture was captured and the hue values were extracted for quantitative analysis.

Serum deproteinization

The 10-fold diluted human serum samples (200 μL) was mixed with acetonitrile (200 μL), shaken for 30 s, and centrifuged for 5 min at 8000 r/min. After removing the precipitates, acetonitrile was evaporated with compressed air. The steps were repeated four times, and finally, the total volume was fixed at 200 μL.

Instrumentation and measurements

The hydrodynamic size distribution and zeta potential (ζ) of the nanospheres were performed on a Zetasizer Nano ZSE (Malvern Instruments). Absorption spectra were measured by a UV-Vis absorption spectrometer (Evolution 220, ThermoFisher Scientific). The transmission electron microscope images were recorded with a 100 kV HT-7700 TEM (Hitachi). For TEM sample preparation, a drop of the nanosphere suspension was deposited onto carbon-coated copper grids and dried at room temperature. The photos or videos of the samples were recorded by a digital camera (Canon 5D Mark IV). The hue values were extracted by the software ImageJ (Fiji).

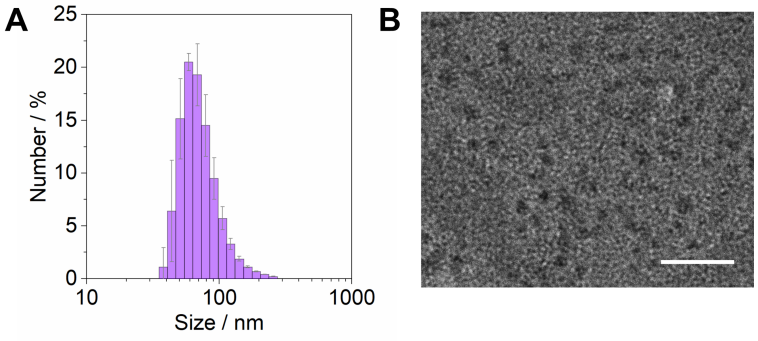

Figure S1. Size characterizations of the nanospheres for fatty acids detection from (A) dynamic light scattering and (B) TEM (scale bar, 100 nm).

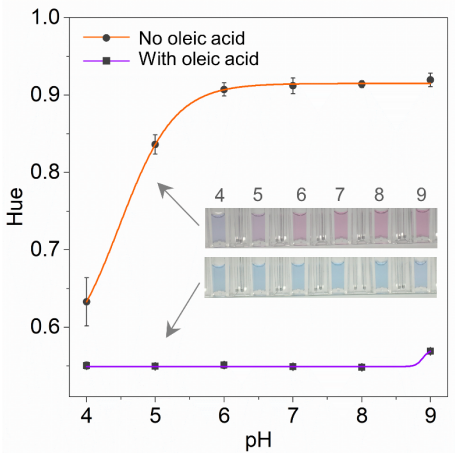

Figure S2. The pH responses of the nanospheres with or without the oleic acid (170 μg/mL) in 3.3 mM PB solutions.

Response Model

The relationship between the total concentration of fatty acids, denoted as R_T, and the degree of protonation of the chromoionophore CHI, denoted as 1-α, is modeled here based on the phase transfer equilibra.

1-α, as expressed in Eqn. S1, is the percentage of the protonated CHI in the nanospheres, where Ind_T and *HInd* represent the total concentration of CHI and the concentration of the protonated CHI, respectively.

$$1 - \alpha = \frac{HInd}{Ind_T} \qquad (S1)$$

The acidity constant K_a of CHI is expressed in Eqn. S2, where *H* and *Ind* represent the concentration of hydrogen ions and the deprotonated CHI, respectively.

$$K_a = \frac{HInd}{Ind\ H} \qquad (S2)$$

The transfer of fatty acids should abide by the partition equilibrium in and outside the nanospheres. However, the concentration of the fatty acids inside the nanospheres, denoted as R_{in}, will approach a maximum value due to many factors including the solubility, surface tension, etc. This partition could be approximated with Eqn. S3, where R_{max} represents the maximum concentration of the fatty acids in the nanospheres and the constant *k* controls the readiness of the transfer.

$$R_{in} = R_{max}(1 - Exp[-k\ R_T]) \quad (S3)$$

The electroneutrality in the interior of the nanospheres is expressed in Eqn. S4, where *J* represents the concentration of the competing cation in the nanospheres, such as Na^+.

$$R_{in} = J + HInd + H \qquad (S4)$$

Note that the competing cation J enters the nanospheres through the ion-exchange with H^+ (Eqn. S5), which is dictated by an exchange constant *K*. Here, *aJ* and *aH* represent the activity of the hydrogen ions and the competing cations in the aqueous phase.

$$K = \frac{H\ aJ}{aH\ J} \qquad (S5)$$

From Eqn. S1 to S5, we could establish the relation between α and R_T, which is expressed in Eqn.

S6.

$$R_T = \frac{1}{k} ln \left[\frac{aH\ \alpha\ K\ K_a R_{max}}{(\alpha - 1)\left(aJ + aH\ K(1 + \alpha K_a Ind_T)\right) + aH\ \alpha\ K\ K_a R_{max}} \right] \qquad (S6)$$

From Eqn. S6, the response of the nanospheres to different concentrations total fatty acid R_T is shown in Figure S3, where an increase of *k* indicates that the fatty acid is more readily transferred to the nanosphere, i.e., more hydrophobic.

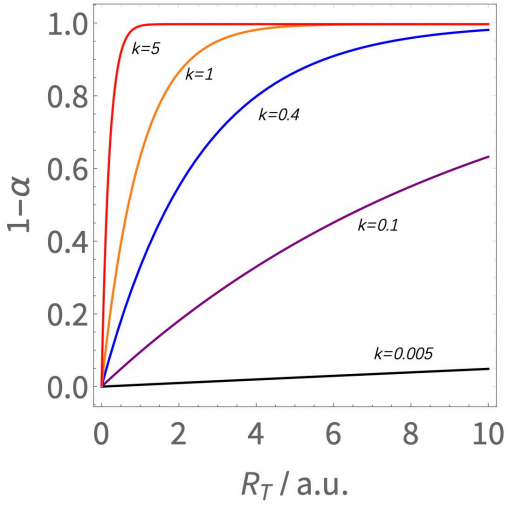

Figure S3. Modeled signal change as a function of the total concentration of the fatty acids (R_T) in a suspension of the nanospheres containing CHI according to Eqn. S6.

Parameters for the simulation: aH = 10^{-7} M, Ind_T = R_{max} = 1 a.u. K_a = 10^9, K = 10, aJ = 0.01 M, and k as indicated.

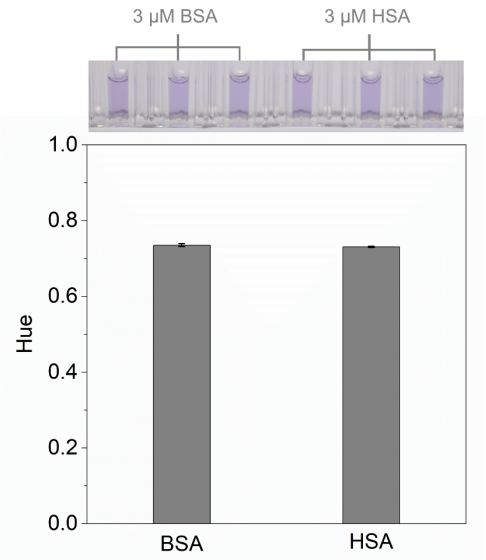

Figure S4. Comparison of the response of nanospheres to the same added amount of BSA and HSA. The images indicate three replicates for BSA and HSA, respectively, and the hue values are presented for comparison.

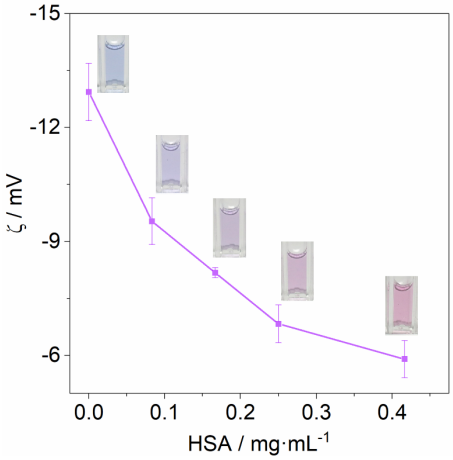

Figure S5. Zeta-potential (ζ) measurements for the nanospheres containing oleic acid and CHI at different HSA concentrations.

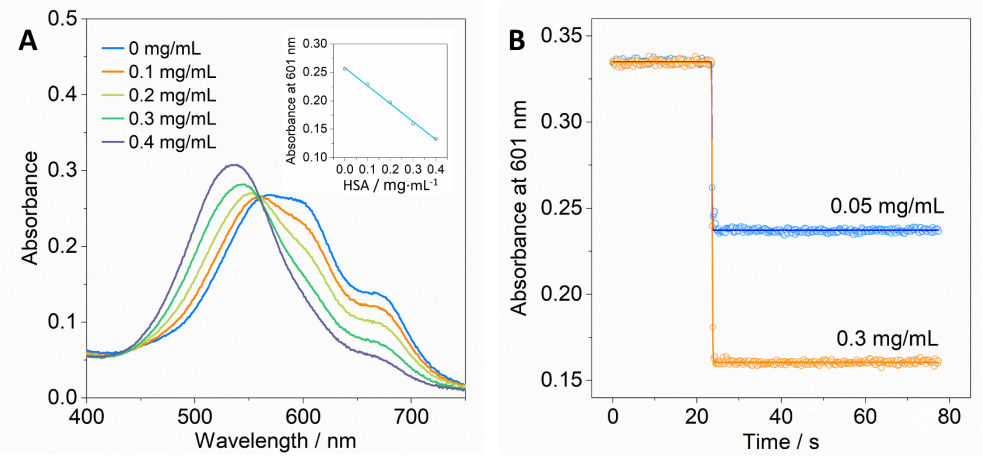

Figure S6. (A) Absorption spectra of the nanospheres containing oleic acid and CHI at different HSA concentrations. (B) Kinetic monitoring of the absorbance at 601 nm upon mixing 1.8 mL of the nanospheres with 0.6 mL of HSA solutions (0.05 mg/mL and 0.3 mg/mL, respectively).

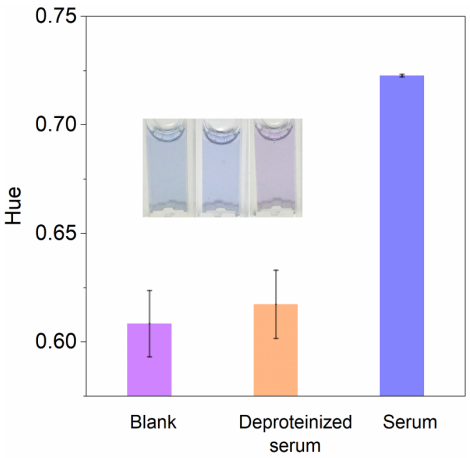

Figure S7. The color of the nanospheres containing oleic acid and CHI in the presence and absence of serums.

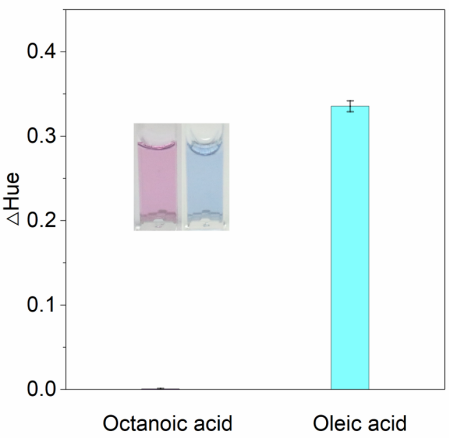

Figure S8. The color change (Δhue) of the nanospheres to octanoic acid and oleic acid. The inset is a picture of the nanosphere suspensions.

Table S1. The determination of HSA in serum samples with the nanospheres containing oleic acid and CHI

Sample	HSA / mg·mL^{-1}
1	52±1
2	66±2
3	57±1