Electronic Supplementary Material (ESI) for Sensors & Diagnostics.


Supporting information

# Colorimetric assay based on iron (III) ion triggering the aggregation of poly(tannic acid) coated Au nanocomposite for carbonic anhydrase II detection

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## 1. Materials

Polyethylene Glycol (PEG, $M_n$ = 1000), propargylamine, hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4 \cdot 4H_2O$) and tannic acid (TA) were purchased from Innochem (Beijing, China). 4-Sulfamylbenzoic acid (SABA), 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC·HCl), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), *N, N'*- dicyclohexylcarbodiimide (DCC), phenylmagnesium bromide (PhMgBr), hexadecyltrimethylammonium chloride (CTAC), triethylamine (TEA), tetraethoxysilane (TEOS), Triethanolamine (TEOA) and (3-aminopropyl) triethoxysilane (APTES) were purchased from J&K (Beijing, China). *p*-Toluenesulfonyl chloride (TsCl) and carbon disulfide ($CS_2$) were purchased from Fuchen (Tianjin) Chemical Reagent (Tianjin, China). 1-Hydroxybenzotriazole hydrate (HOBt), 5-sulfosalicylic acid dihydrate and 4-dimethylaminopyridine (DMAP) were purchased from Beijing OUHE technology (Beijing, China). L-Ascorbic acid sodium salt (VcNa) were purchased from Alfa-Aesar. Carbonic Anhydrase II (CA II), human serum albumin (HSA), β-2-microglobulin (β-MG), streptavidin (SA) and transthyretin (TTR) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Deionized water was used throughout the experiment. The used solvents and reagents in the experiment process were subjected to water and oxygen removal treatment according to the requirements of the reaction conditions. Anhydrous $CaCl_2$ particles were added to dichloromethane (DCM) to remove water. After filtration, the filtrate was distilled at atmospheric pressure to obtain anhydrous DCM. Acetonitrile and anhydrous calcium hydride were refluxed under normal pressure for 8 hours, and then distilled to obtain anhydrous acetonitrile. Anhydrous magnesium sulfate was added to *N, N*-dimethylformamide (DMF) and stir to remove water. Anhydrous DMF was distilled from the filtrate at atmospheric pressure. Ethyl ether, dioxane and tetrahydrofuran (THF) were refluxed with Na to remove water. PEG was added to toluene and azeotropically removed water.

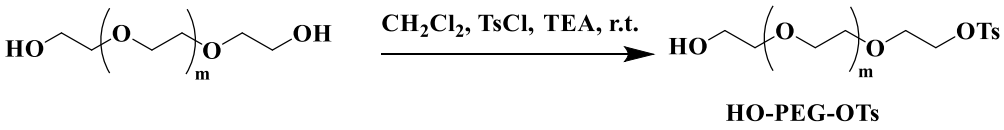
## 2. Methods

The chemical structures of the mid-products and polymers were characterized by the German 500 MHz Bruker NMR spectrometer. The morphology of nanoparticles was characterized by the Hitachi HT-7700 TEM. The British Malvern Panalytical dynamic light scattering particle size analyzer was used to characterize the particle size and zeta potential of the nanoparticles. The mesoporous structure of MSNs was characterized by the American Mike ASAP2460 MP nitrogen adsorption-desorption instrument. The Hitachi U-3010 UV-Vis spectrophotometer was used to detect the UV signal.

**3. Preparation of SABA-labeled mercaptopolyethylene glycol 1000 (SABA-PEG-HS).**

The preparation of the final product was carried out via multiple-step synthesis. The detailed procedures were described as following.

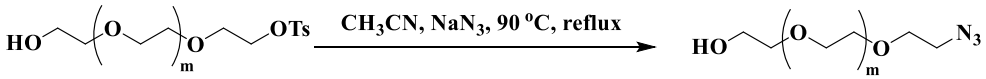
*3.1 Preparation of p-toluenesulfonate polyethylene glycol (HO-PEG-OTs)*




**HO-PEG-OTs**

In an anhydrous and oxygen-free environment, anhydrous PEG (1.0 g, 1 mmol) was dissolved in 30 mL anhydrous DCM. Anhydrous TEA (404.76 mg, 4 mmol) was added to the reaction system, and TsCl (1.049 g, 1.1 mmol) in THF solution was added dropwise to the reaction system. After stirring at 25 °C for 24 h, the reaction system was washed with HCl, $Na_2CO_3$ and NaCl successively. Finally, the product is obtained by precipitation. $^1$H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.86-7.33 (d, 2H, $CH_3C_6H_4$), 7.44-7.35 (d, 2H, $CH_3C_6H_4$), 4.71-4.60 (m, 2H, $CF_3SO_3CH_2$), 4.18-4.11 (m, 2H, $SO_3CH_2$), 4.00-3.53 (s, nH, $CH_2$), 2.55-2.41 (s, 3H, $CH_3$).

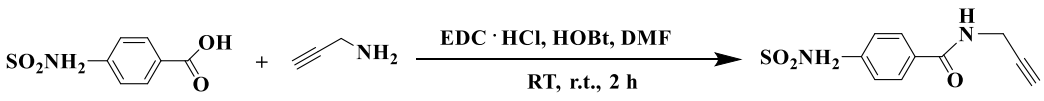
*3.2 Preparation of azide polyethylene glycol (HO-PEG-$N_3$)*




**HO-PEG-$N_3$**

HO-PEG-OTs (500 mg, 0.4 mmol) and $NaN_3$ (104 mg, 1.6 mmol) were added to 50 mL anhydrous acetonitrile, and then refluxed at 90 °C for 36 h. Acetonitrile was removed in vacuum and the crude product was dissolved in DCM. The reaction solution was washed 3 times with saturated NaCl solution. Anhydrous $MgSO_4$ was added to the organic phase and stir to remove water. The organic phase was filtered, and concentrated. The concentrated solution was precipitated by a mixed solution of anhydrous ether and n-hexane, and obtain HO-PEG-$N_3$. $^1$H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.67-4.64 (m, 2H, $CF_3SO_3CH_2$), 3.99-3.62 (m, nH, $CH_2$), 3.49-3.39 (m, 2H, $N_3CH_2$).

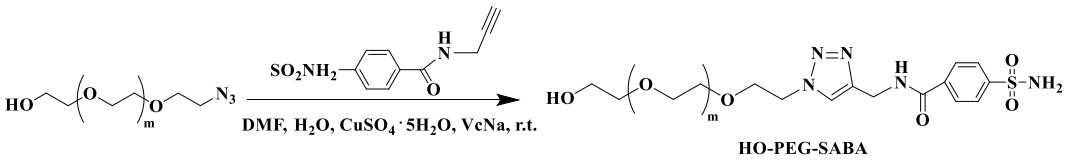
*3.3 Preparation of Alkynylated benzenesulfonamide (PA-SABA)*




**PA-SABA**

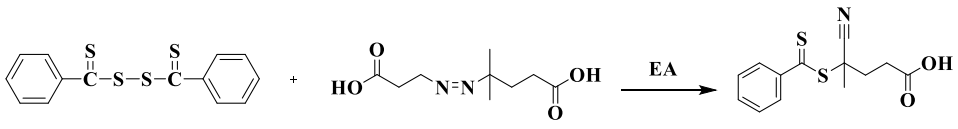
In anhydrous and oxygen-free environment, SABA (1.0 g, 4.9 mmol), HOBt (1.1 g, 7.45 mmol), EDC·HCl (1.42 g, 7.5 mmol) were stirred to dissolve in anhydrous DMF (10 mL). Propargylamine (0.41 g, 7.5 mmol) was added to the reaction device, stirred and reacted at room temperature for 2 h. The reaction solution was extracted 3 times with ethyl acetate. The ethyl acetate solution was washed with saturated NaCl solution 3 times. Anhydrous $MgSO_4$ was added to the ethyl acetate solution and stirred to remove $H_2O$. The ethyl acetate solution was filtered to remove the anhydrous $MgSO_4$ powder, and concentrated by rotary evaporation. The concentrated solution was precipitated with anhydrous ether and obtain PA-SABA. $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$ ppm): 9.18 (t, $J$ = 5.36 Hz, 1H, NH), 7.99 (d, $J$ = 8.26 Hz, 2H, *ArH*), 7.92 (d, $J$ = 8.30 Hz, 2H, *ArH*), 7.49 (s, 2H, N$H_2$), 4.08 (dd, $J$ = 5.27, 2.18 Hz, 2H, C$H_2$), 3.11 (d, $J$ = 2.14 Hz, 1H, C≡C*H*).

*3.4 Preparation of SABA-labeled polyethylene glycol (SABA-PEG-HO)*




In anhydrous and oxygen-free environment, $N_3$-PEG-OH (0.35 g, 0.35 mmol) was added in 40 mL DMF, followed by the addition of VcNa (13.8 mg, 0.07 mmol) and $CuSO_4{\cdot}5H_2O$ (8.75 mg, 0.035 mmol). The DMF solution of PA-SABA was added dropwise to the reaction system. After stirring at room temperature for 24 h, disodium edetate was added to remove $Cu^{2+}$. The product was extracted with DCM, and then washed with NaCl solution. Anhydrous $MgSO_4$ was added to the organic phase to remove water. The organic phase was filtered to remove $MgSO_4$ powder, and concentrated by rotary evaporation. The concentrated solution was precipitated by a mixed solution of anhydrous ether and n-hexane, and obtain HO-PEG-SABA. $^1$H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.10-7.94 (m, 4H, ArH), 7.94-7.82 (t, 1H, NH), 7.82-7.66 (s, 1H, CH), 6.30-6.16 (s, 2H), 4.79-4.67 (d, 2H), 4.61-4.43 (t, 2H), 3.96-3.27 (m, nH).

*3.5 Preparation of 4-cyanopentanoic acid dithiobenzoate (CPADB)*

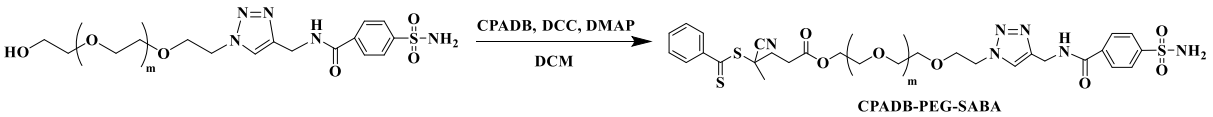



PADB were prepared according to the previously reported method.[1] In anhydrous and

oxygen-free environment, ether solution of PhMgBr (5.4 g, 30 mmol) and anhydrous THF was added to the device in ice bath. THF solution of $CS_2$ (2.5 g, 33 mmol) was added dropwise, and the reaction was stirred at room temperature for 10 h. Then ice bath the reaction device and dilute HCl was added until the solution is separated. The reaction solution was extracted with cold ether for 3 times, the ether solutions were combined, the NaOH solution was added to the ether solution. The aqueous phase was collected by standing for liquid separation. Sufficient potassium ferricyanide solution was added dropwise to the water phase, filtered, recrystallized with hot absolute ethanol, and a purple solid product of di(thiobenzoyl) disulfide was afford.

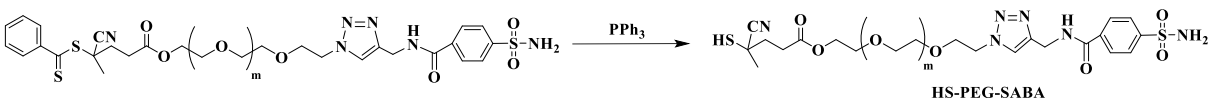
Dithiobenzoyl (2 g, 13.3 mmol), 4,4'-azobis (4-cyanovaleric acid) (2.5 g, 9 mmol) and ethyl acetate were added to the reflux device at 80 °C reaction for 10 h. The crude product was isolated by column chromatography using the mixture of ethyl acetate and hexane (v/v = 2/3) as eluent, which is followed by recrystallization in hot toluene to afford a pink solid. $^1$H NMR (400 MHz, δ ppm): 7.95-7.83 (s, 2H, ArH), 7.63-7.40 (s, 1H, ArH), 7.48-7.27 (s, 2H, ArH), 2.78-2.60 (s, 2H, $CH_2$), 2.75 -2.28 (s, 2H, $CH_2$), 2.05-1.95 (s, 3H, $CH_3$).

*3.6 Preparation of SABA-labeled 4-cyanodithiobenzate capped polyethylene glycol (CPADB-PEG-SABA)*




SABA-PEG-OH (1.0 g, 0.78 mmol)，DCC (635.5 mg, 3.12 mmol)，DMAP (45.8 mg, 0.39 mmol) and CPADB (637 mg, 2.34 mmol) were added to 50 mL anhydrous DCM. The reaction was stirred at room temperature for 36 h, and the product was obtained by precipitation with ether to remove impurities. $^1$H NMR (400 MHz, δ ppm): 8.18-7.77 (d, 8H, ArH, NH,CH), 7.61-7.50 (s, 1H, ArH), 7.44-7.34 (s, 1H, ArH), 6.37-6.22 (s, 2H, $NH_2$), 4.79-4.67 (s, 2H, $CH_2$), 4.57-4.49 (s, 2H, $CH_2$), 4.30-4.20 (s, 2H, $CH_2$), 3.93-3.42 (s, nH, $CH_2O$), 2.81-2.05 (m, 4H, $CH_2CH_2$), 1.99-1.82 (d, 3H, $CH_3$).

*3.7 Preparation of SABA-labeled mercaptopolyethylene glycol (SABA-PEG-HS)*



SABA-PEG-CPADB (0.2 g, 0.13 mmol) was added to 20 mL anhydrous THF, and n-propylamine (1.5 mg, 0.026 mmol) and triphenylphosphine (3.4 mg, 0.013 mmol) were slowly added to the reaction. After stirring for 20 min at room temperature, the product was precipitated with ether. $^1$H NMR (400 MHz, δ ppm): 8.16-7.68 (d, 6H, ArH, NH,CH), 6.38-6.26 (s, 2H, $NH_2$), 4.79-4.67 (s, 2H, $CH_2$), 4.57-4.49 (s, 2H, $CH_2$), 4.30-4.20 (s, 2H, $CH_2$), 3.93-3.42 (s, nH, $CH_2O$), 2.81-2.05 (m, 4H, $CH_2CH_2$), 1.99-1.82 (d, 3H, $CH_3$).

**4. Preparation of AuNPs@PTA**

All glass instruments used in the preparation of AuNPs were soaked in aqua regia ($HCl/HNO_3$ = 3/1) for 30 min, and the residual aqua regia was rinsed with ultrapure water.

50 mL $HAuCl_4$ solution was added to a clean reflux device and heated to 110 °C, and TA solution with a molar ratio of 2/1 to $HAuCl_4$ was added to the reaction system. The solution rapidly changed to red. pH of AuNPs solution was adjusted to 7-8, and stirred for 12 h to obtain AuNPs@PTA.

**5. Preparation of SABA-labeled PEGylated AuNP@PTA**

6 mg SABA-PEG-HS was added to $3.56\times10^{-4}$ mol/L 10 mL AuNP@PTA and incubated with shaking for 12 h. AuNP@PTA were washed 3 times with ultrapure water to get SABA-labeled PEGylated AuNP@PTA.

**6. Preparation of aminated mesoporous silica nanoparticles ($NH_2$-modified MSNs)**

MSNs were prepared by the reported method.[2] CTAC (25 wt%, 24 mL) and 0.18 g TEOA were added to 36 mL ultrapure water, stirred at 65 °C for 1 h to make the solution evenly mixed. Then, TEOS in cyclohexane solution (5 v/v%, 20 mL) was slowly added in the reaction system, and stirred slowly at 60 °C for 17 h. Calcination at 550 °C to remove the template CTAC to obtain MSNs.

100 mg MSNs and 1.5 mL APTES were added to 50 mL anhydrous toluene, and refluxed at 80 °C for 20 h, then washed with absolute ethanol, and dried to obtain $NH_2$-modified MSNs.

**7. Preparation of SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs**

4 mg $NH_2$- modified MSNs were added to 5 mL 0.6 mol/L $FeCl_3$ solution and incubated for 12 h, and then washed 3 times to remove excess $Fe^{3+}$. 3 mg $Fe^{3+}$-loading MSNs was added to 5 mL

ultrapure water and its pH was quickly adjusted to 6. SABA-labeled PEGylated AuNP@PTA solution ($4.55\times10^{-3}$ mol/L, 5mL) with identical pH was added and mixed uniformly for 8 h. The SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system had a volume of 1 mL for detection.

**8. Efficiency evaluation of $Fe^{3+}$ loaded in MSNs.**

5-Sulfosalicylic acid dihydrate as chromogenic agent was selected to complex with the $Fe^{3+}$ in the supernatant before and after encapsulation. $Fe^{3+}$ aqueous solutions at various concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 M were added into $NH_2$-modified MSNs (4.0 mg), sonicated for 30 min, and incubated at room temperature for 8 h. After centrifugation (3000 rpm, 10 min), the supernatant was added into 5-sulfosalicylic acid dihydrate, and the solution pH was adjusted by $NH_3\cdot H_2O$ to 8.0 for the UV measurement. As control, the blank experiments were performed using the corresponding $Fe^{3+}$ concentrations reacting with 5-sulfosalicylic acid dihydrate without MSNs. Comparing the two-group results, the difference in UV absorbance at 420 nm ($\Delta Abs_{420}$) was measured for encapsulation efficiency of $Fe^{3+}$ in the MSNs.

**9. Detection of CA II**

Different concentrations of CA II solution (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 nmol/L) were added to the SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system, and shaked at 37 °C for 3.5 h. After the reaction, the solution was centrifuged (3000 r/min, 10 min) to obtain the supernatant. ([$Fe^{3+}$-loading@ MSN] = 0.2 mg/mL, [SABA-labeled PEGylated AuNP@PTA] = 2.93 mM)

PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs and SABA-labeled PEGylated AuNP@PTA@ MSNs detection systems were performed as control.

**9. Specificity investigation of the detection system**

1 mL CA II, human serum albumin (HSA), transthyretin (TTR), β-microglobulin (β-MG) and streptavidin (SA) with a concentration of 300 nM were added to SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system, respectively, and incubated with shaking at room temperature for 3.5 h, centrifuged (3000 r/min, 10 min). The supernatant was taken for testing. 1 mL ultrapure water was added to the SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system as control.

The concentration ratios of CA II and β-MG to 1/0.25, 1/0.5, 1/0.75, 1/1, 1/1.25 mixed protein solution (CA II concentration was 300 nM) was added to the SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system ([$Fe^{3+}$-loading MSN] = 0.2 mg/mL, [SABA-labeled PEGylated AuNP@PTA] = 2.93 mM). Comparatively, 75, 150, 225, 300, 375 nM β-MG solution was added to the SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system. Incubation was maintained at room temperature for 3.5 hours, centrifuged (3000 r/min, 10 min), and took the supernatant for testing. The SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs detection system was added with 1.0 mL ultrapure water as control.

Identically, the detection experiments were performed at varying concentration ratios of CA II and HSA.

**References**.


1. Y. Misukami, M. S. Donovan, A. B. Lowe, et al. *Macromolecules*, **2001**, *34(7):* 2248-2256.
2. D. Shen, J. Yang, X. Li, et al. *Nano Lett.*, **2014**, *14(2)*: 923-932.

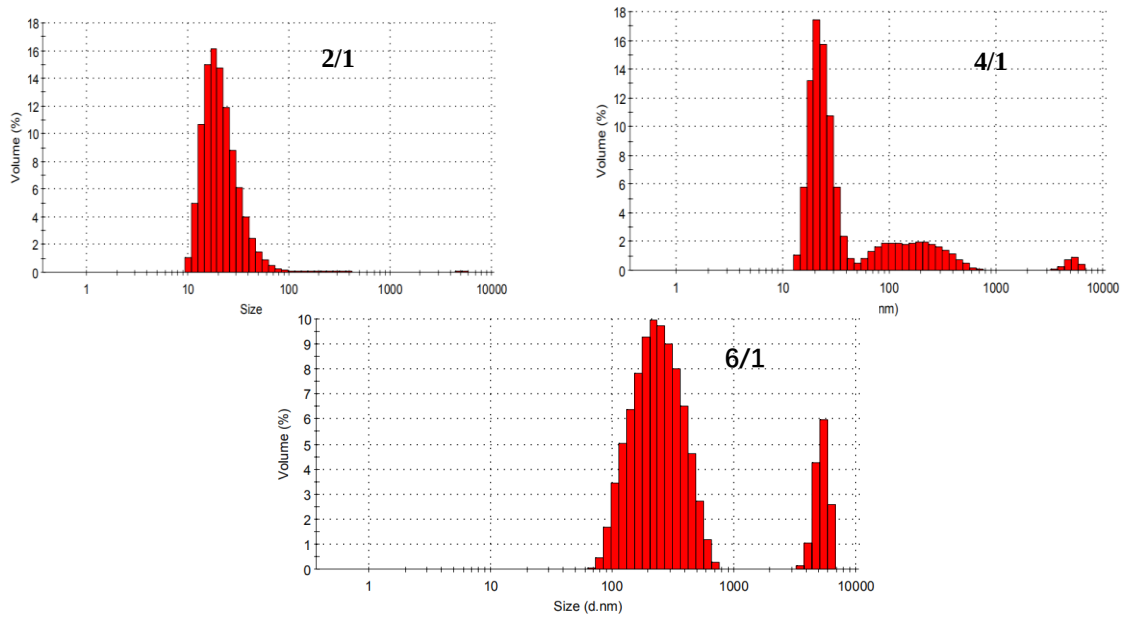



**Figure S1**. DLS at varying the molar ratios of TA and $HAuCl_4$.

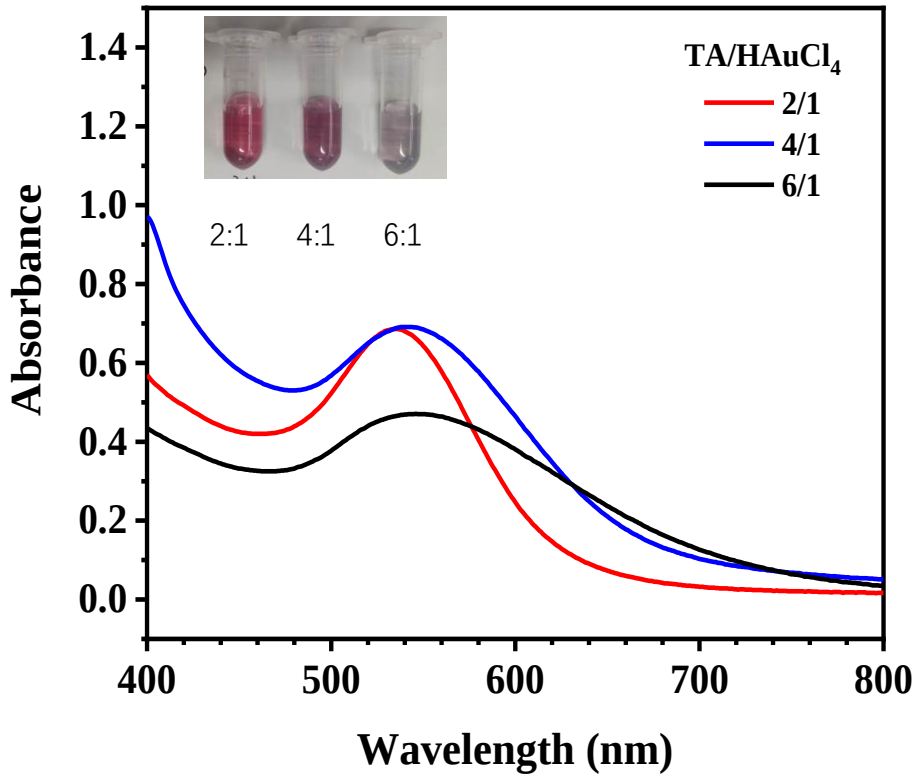



**Figure S2**. UV spectra at varying the molar ratios of TA and $HAuCl_4$.

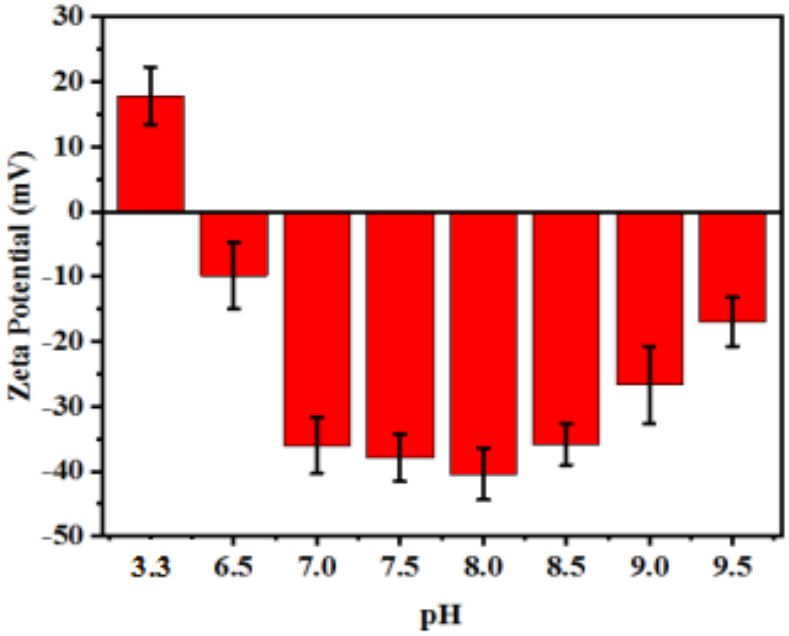



**Figure S3**. Zeta potential of AuNP@PTA dependence of environmental pHs.

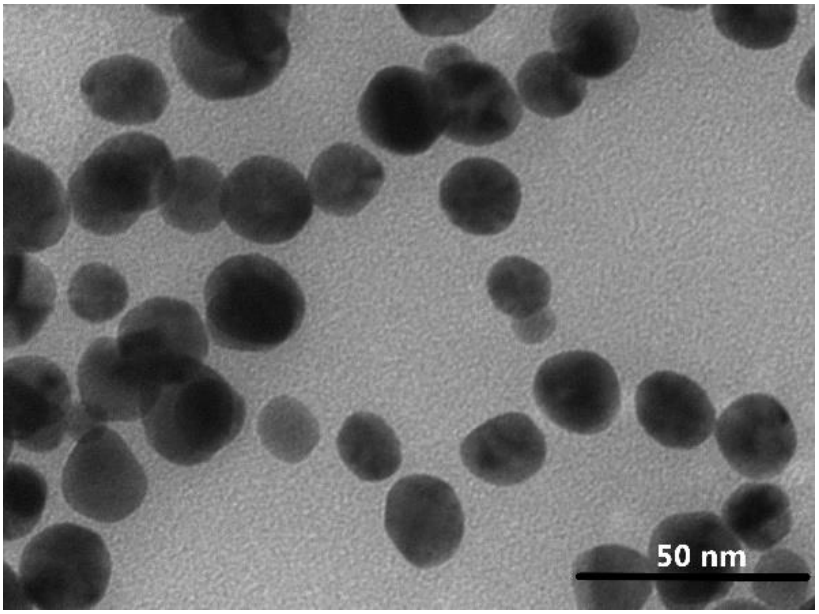



**Figure S4**. TEM images prepared at the molar ratio of TA and $HAuCl_4$ to 2/1.

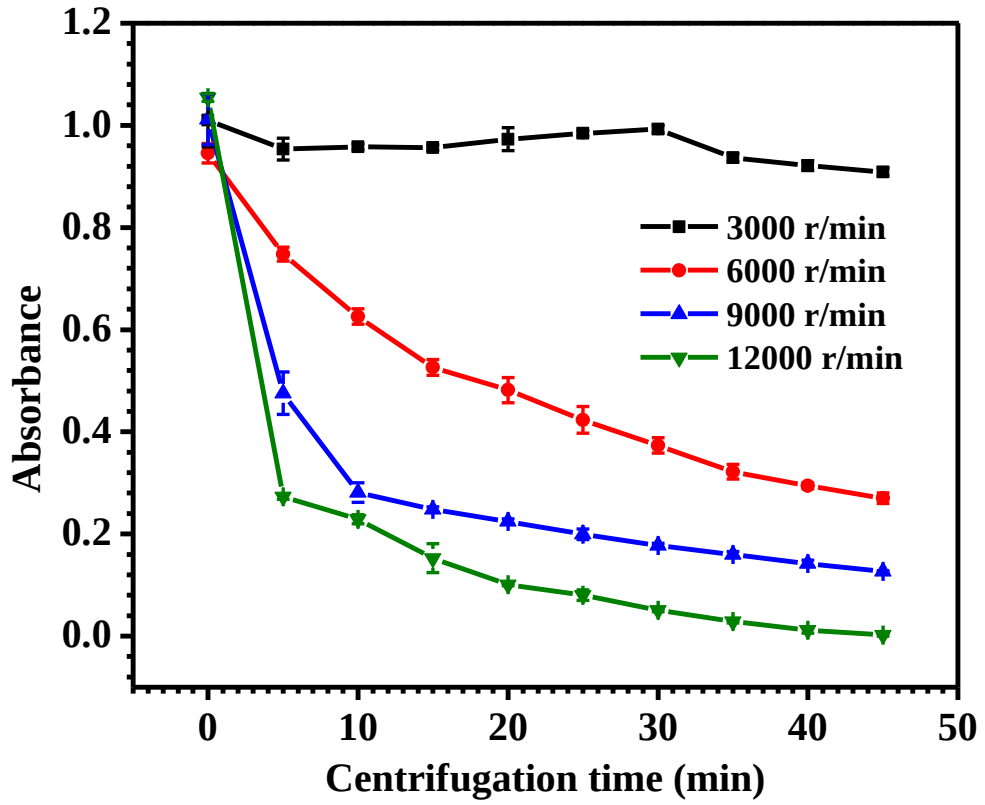



**Figure S5**. The effect of centrifugation speed and time on the UV absorbance of AuNP@PTA in the solution.

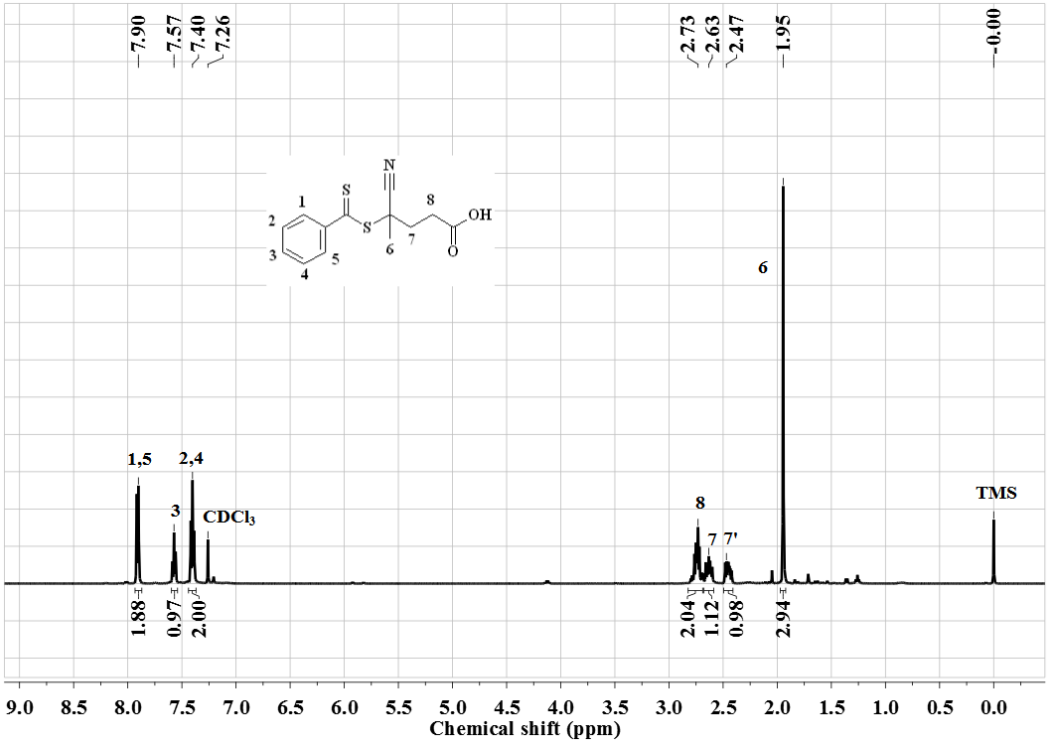



**Figure S6**. $^{1}$H NMR spectrum of CPADB.

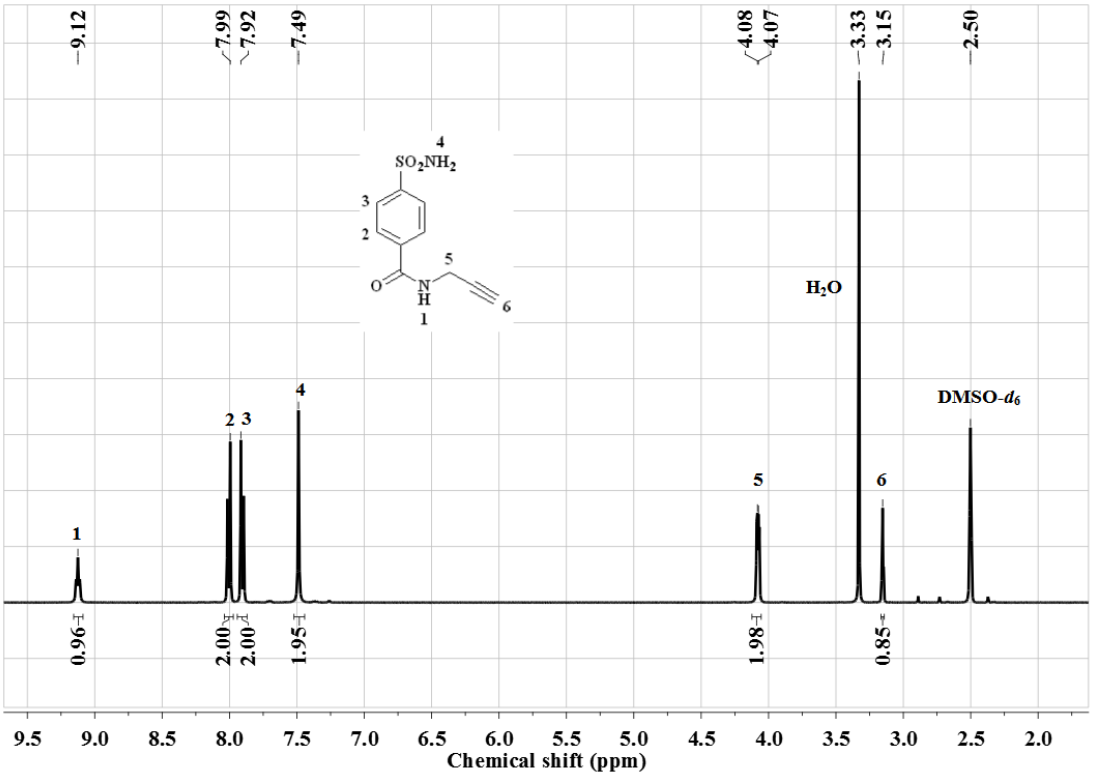



**Figure S7**. $^{1}$H NMR spectrum of PA-SABA.

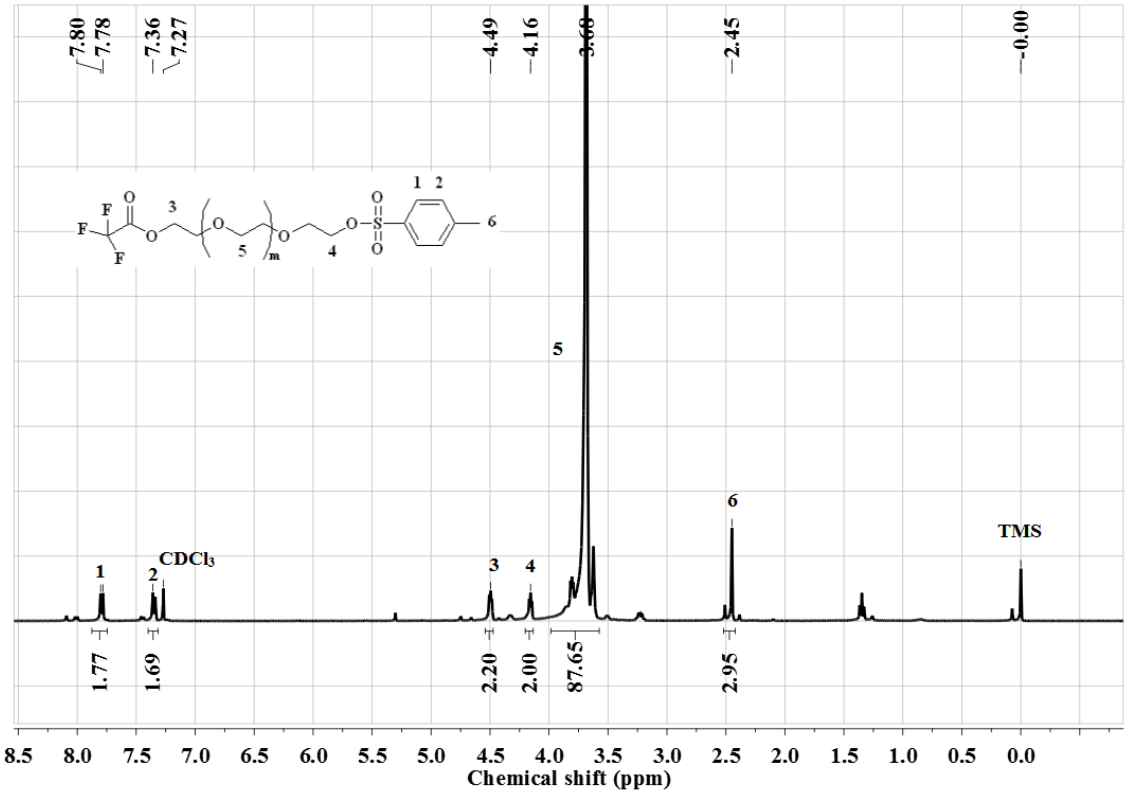



**Figure S8**. $^{1}$H NMR spectrum of HO-PEG-OTs.

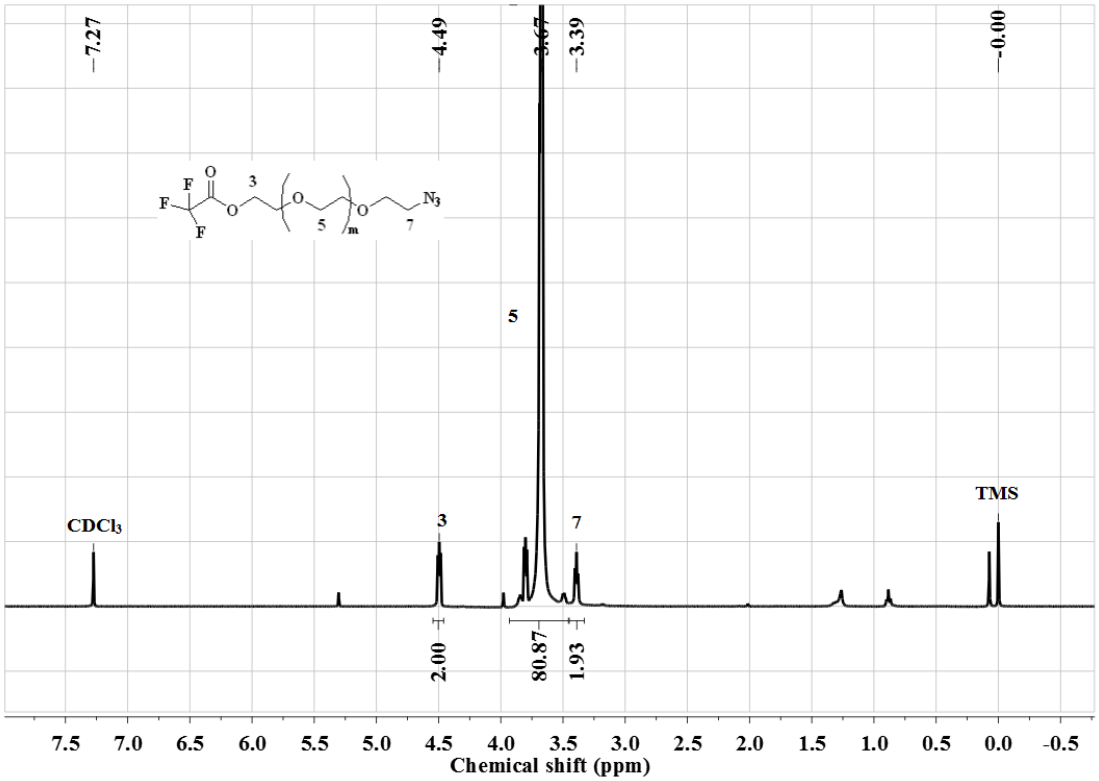



**Figure S9**. $^{1}$H NMR spectrum of HO-PEG-$N_3$.

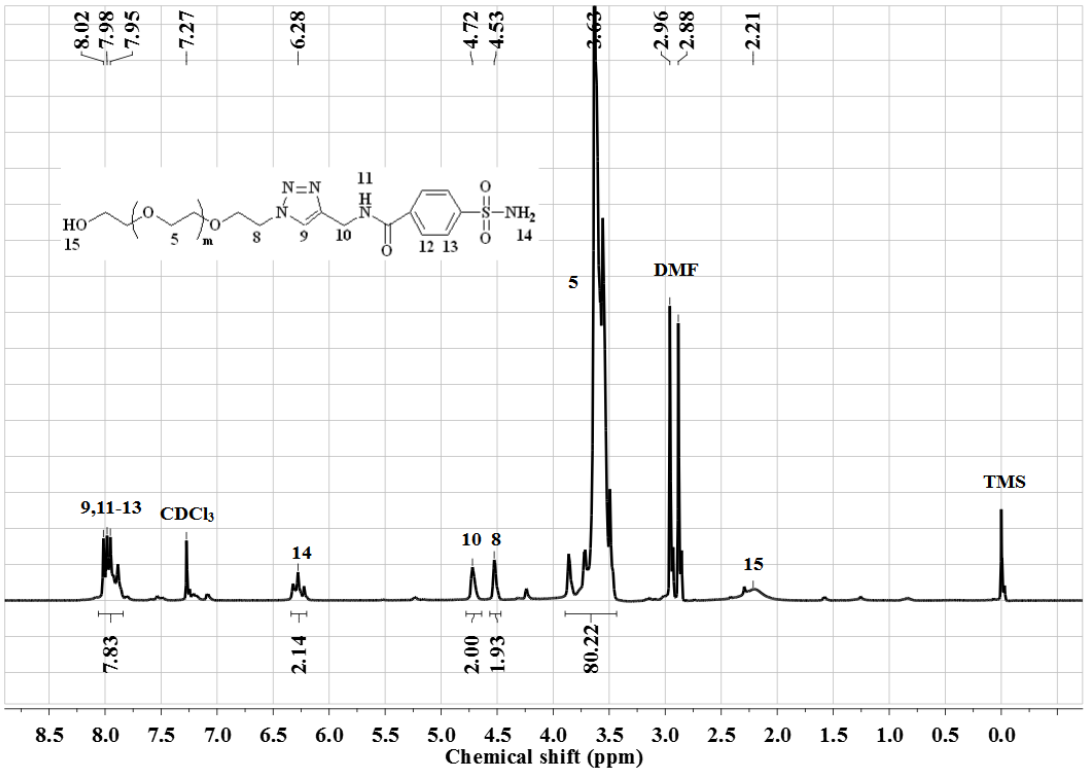



**Figure S10**. $^{1}$H NMR spectrum of HO-PEG-SABA.

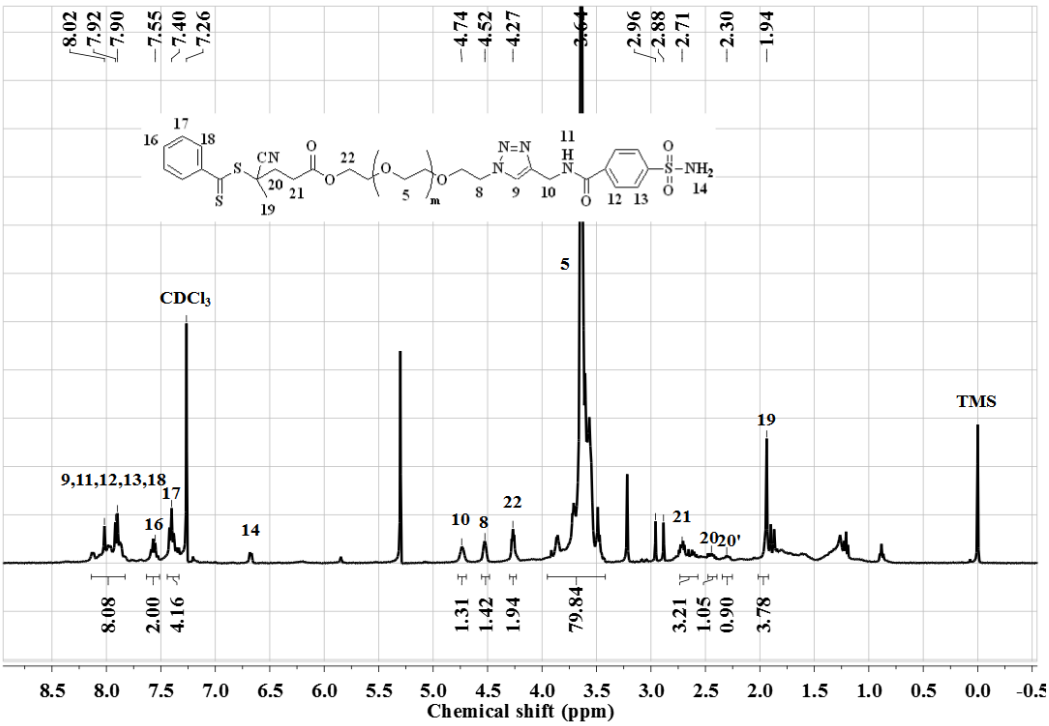



**Figure S11**. $^{1}$H NMR spectrum of CPADB-PEG-SABA.

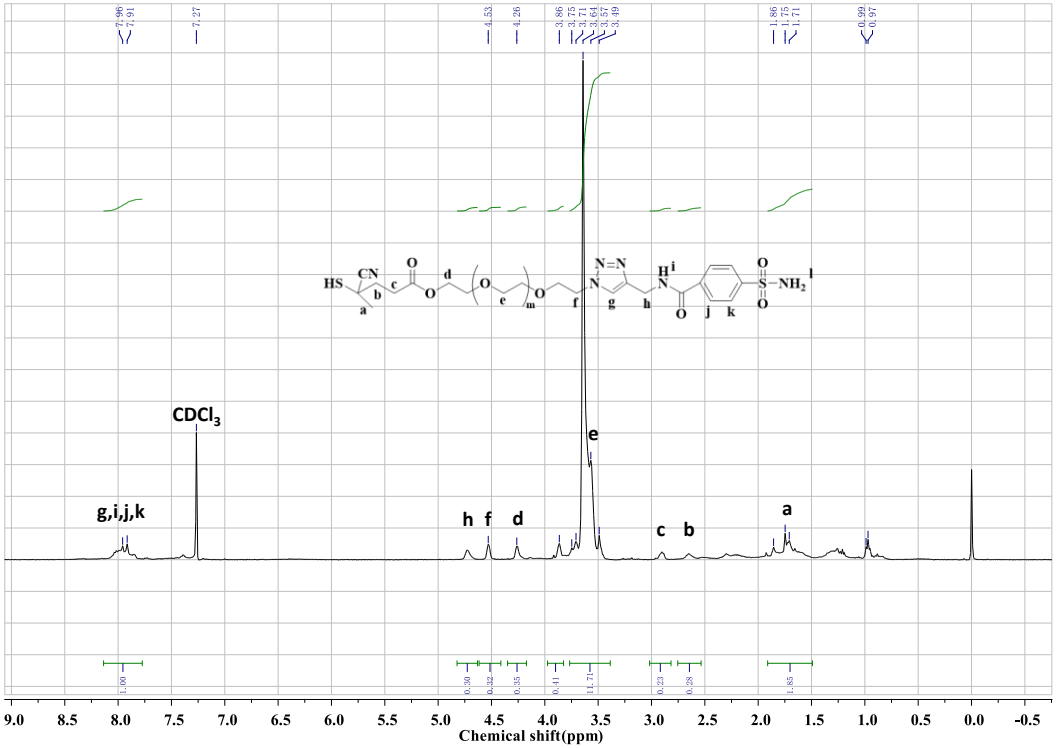



**Figure S12**. $^{1}$H NMR spectrum of SABA-PEG-SH.

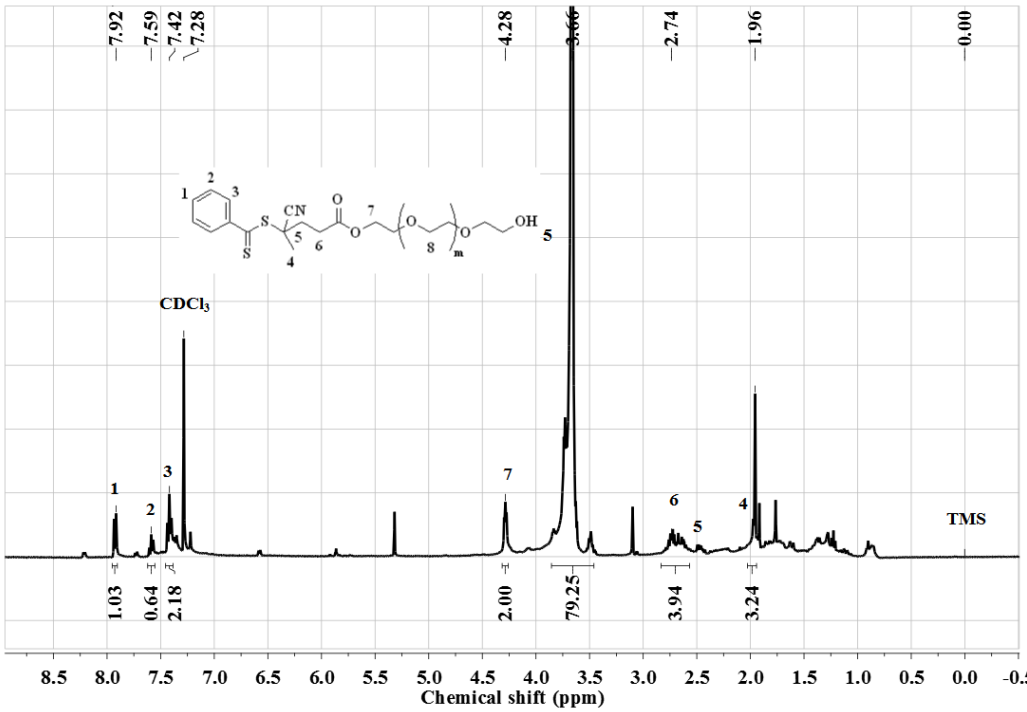



**Figure S13**. $^{1}$H NMR spectrum of HO-PEG-CPADB.

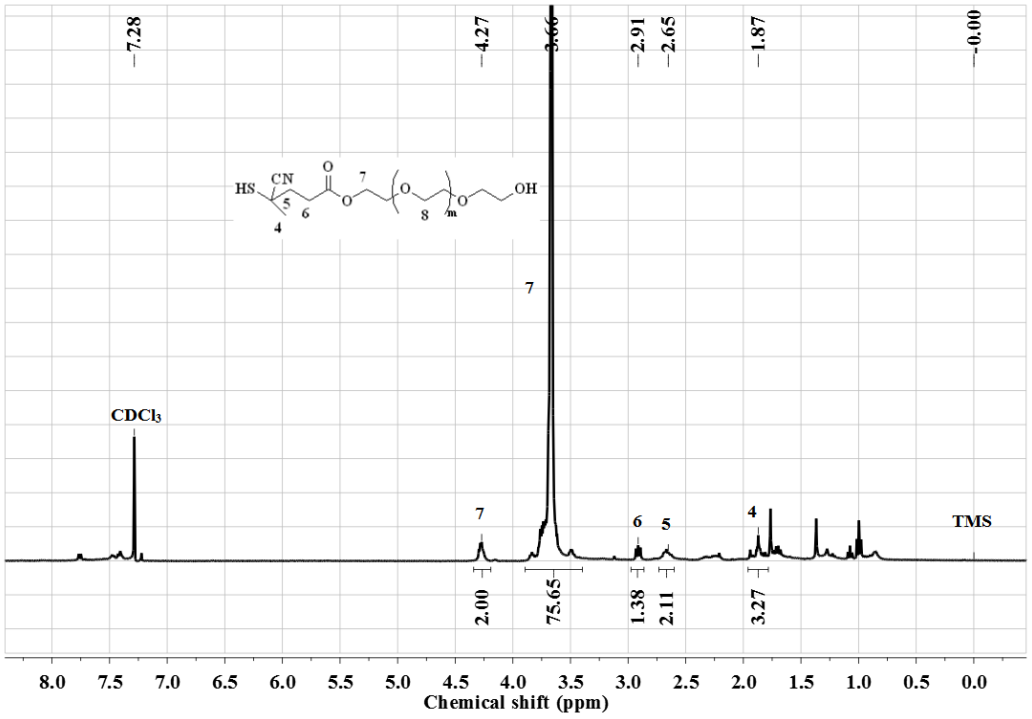



**Figure S14**. $^{1}$H NMR spectrum of HO-PEG-SH.

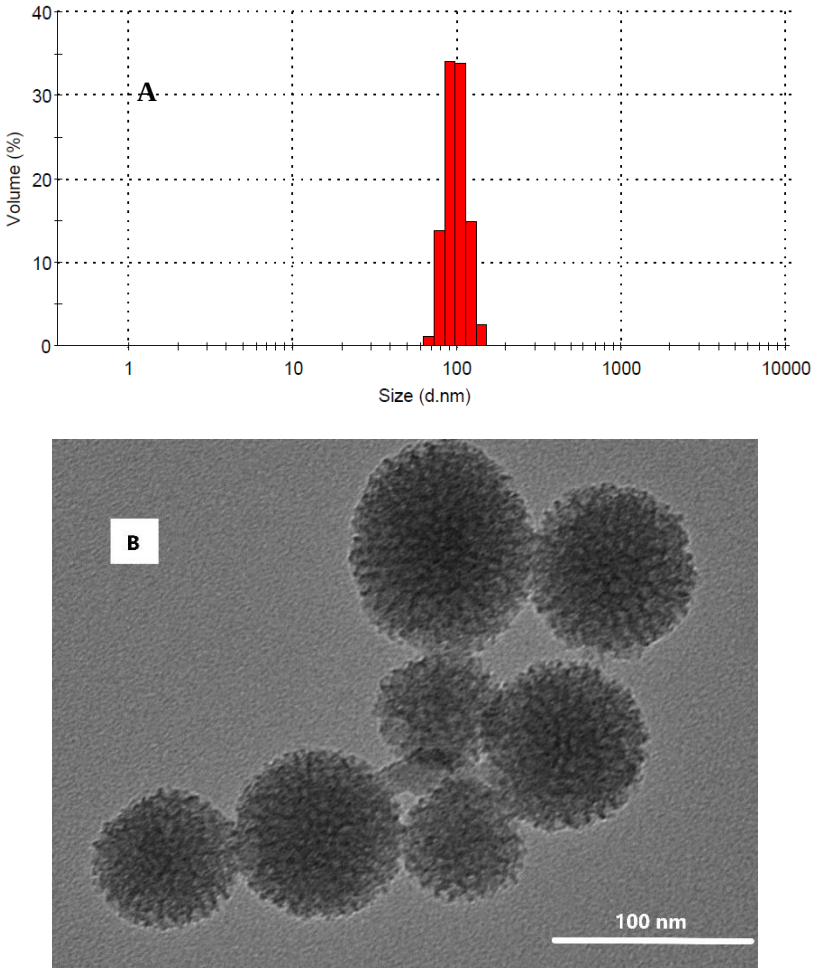



**Figure S15**. (A) DLS and (B) TEM image of MSNs.

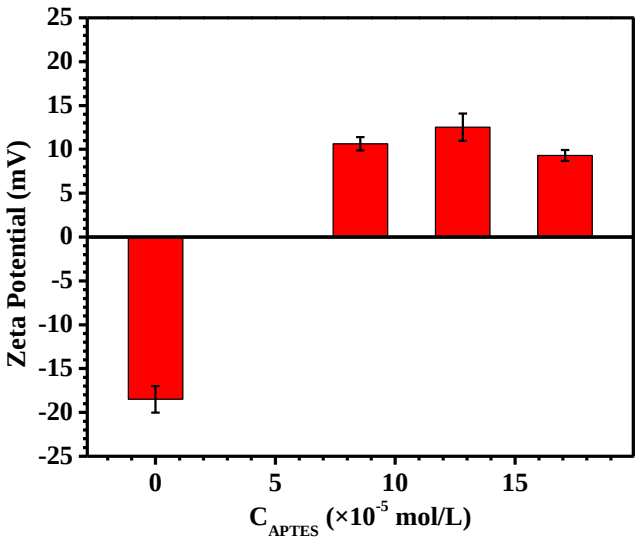



**Figure S16**. Zeta potential of $NH_2$-modified MSNs dependence of the given APTES concentrations.

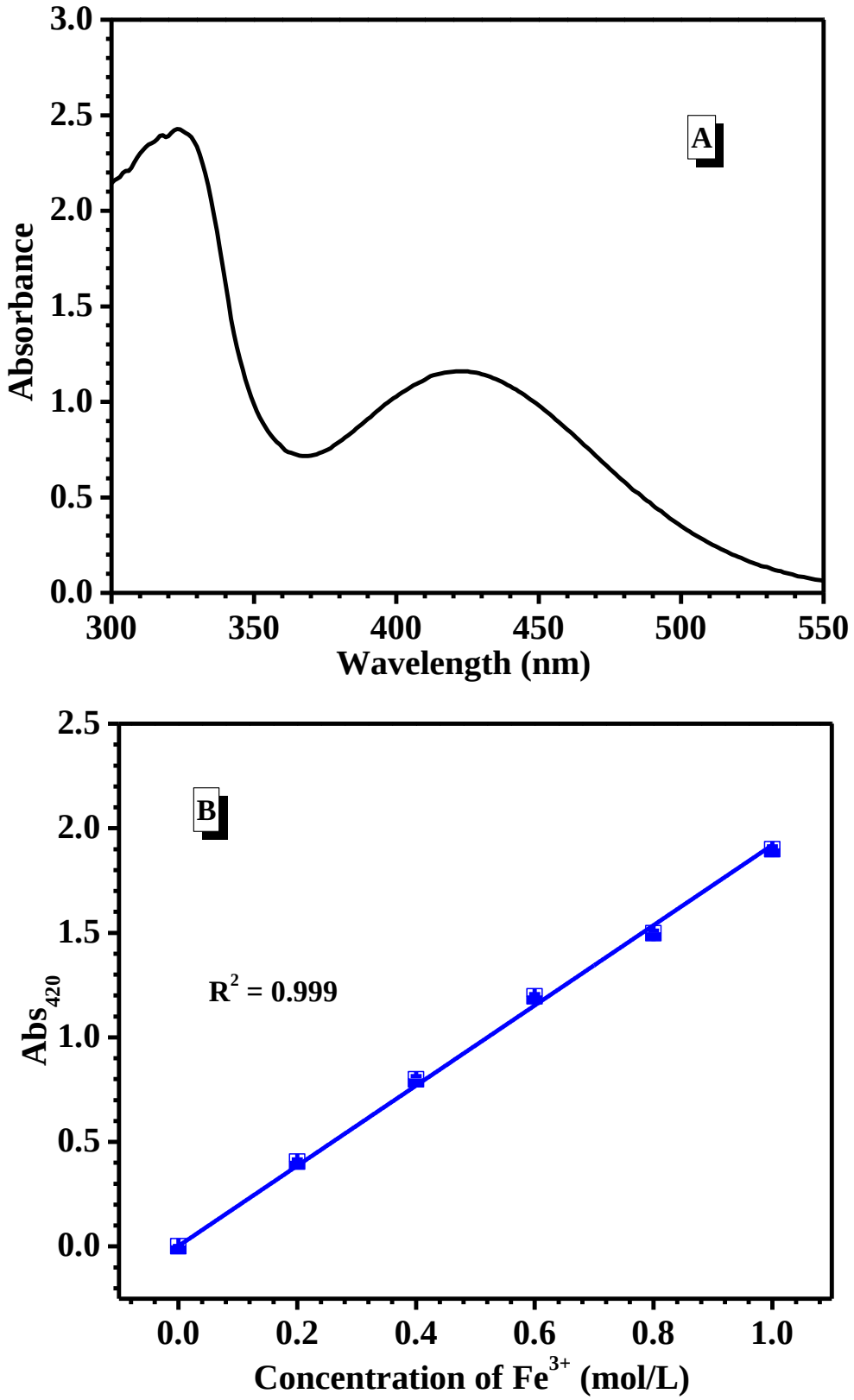



**Figure S17**. (A) UV-vis spectrum of $Fe^{3+}$ complexing with 5-sulfosalicylic acid dihydrate. (B) the standard curve of $Fe^{3+}$ absorbance with varying its concentrations.

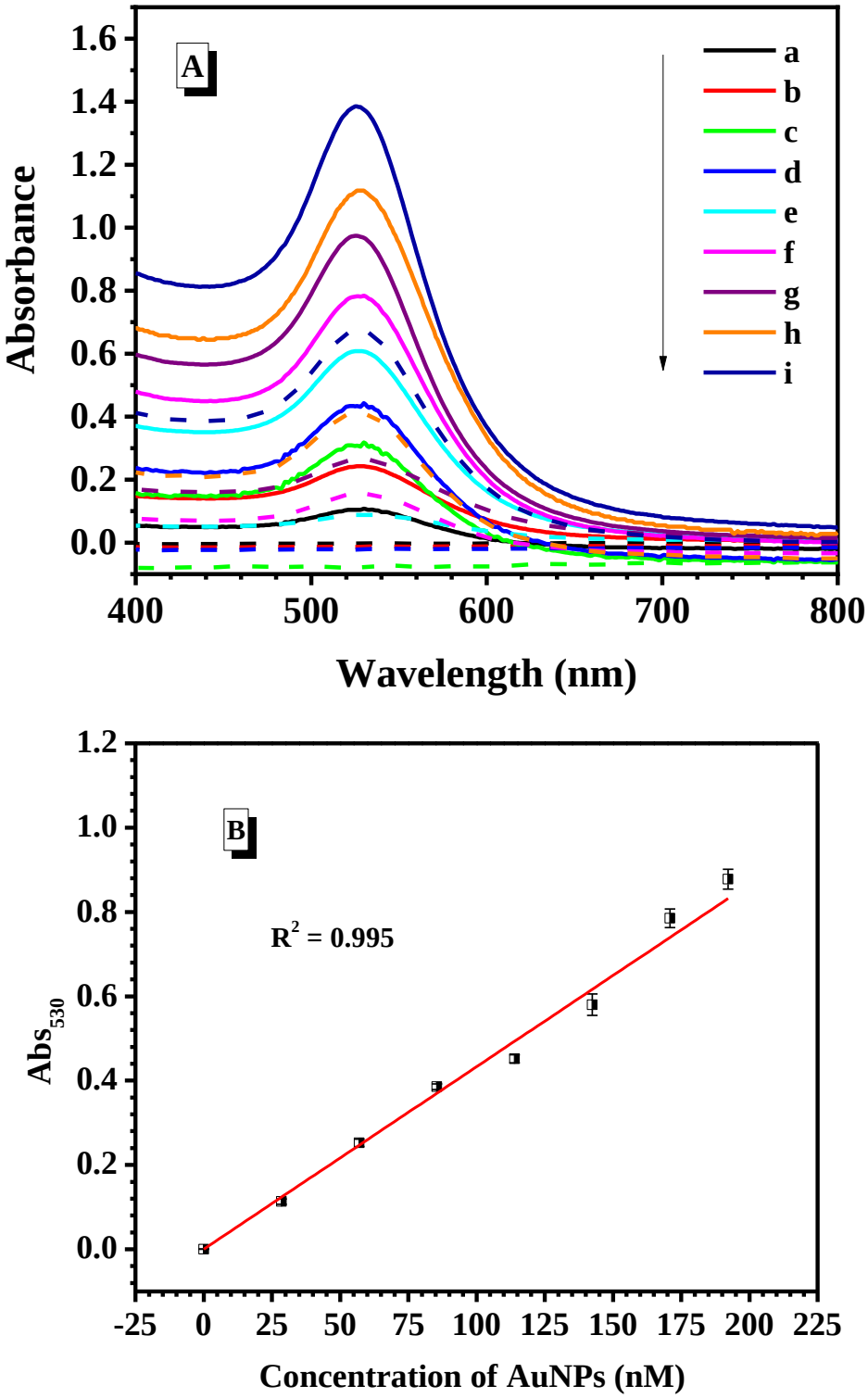



**Figure S18.** (A) UV spectra of SABA-labeled PEGylated AuNP@PTA incubated with $NH_2$-modified MSNs before (solid line) after (dotted line). The concentrations of SABA-labeled PEGylated AuNP@PTA: (a) 0.57 mM; (b) 1.14 mM; (c) 1.70 mM; (d) 2,28 mM; (e) 2.85 mM; (f) 3.42 mM; (g) 3.98 mM; (h) 4.55 mM; (i) 5.12 mM. ([$NH_2$-modified MSNs] = 0.3 mg/mL, 10 mL). (B) Standard curve of AuNP absorbance dependence of its concentrations.

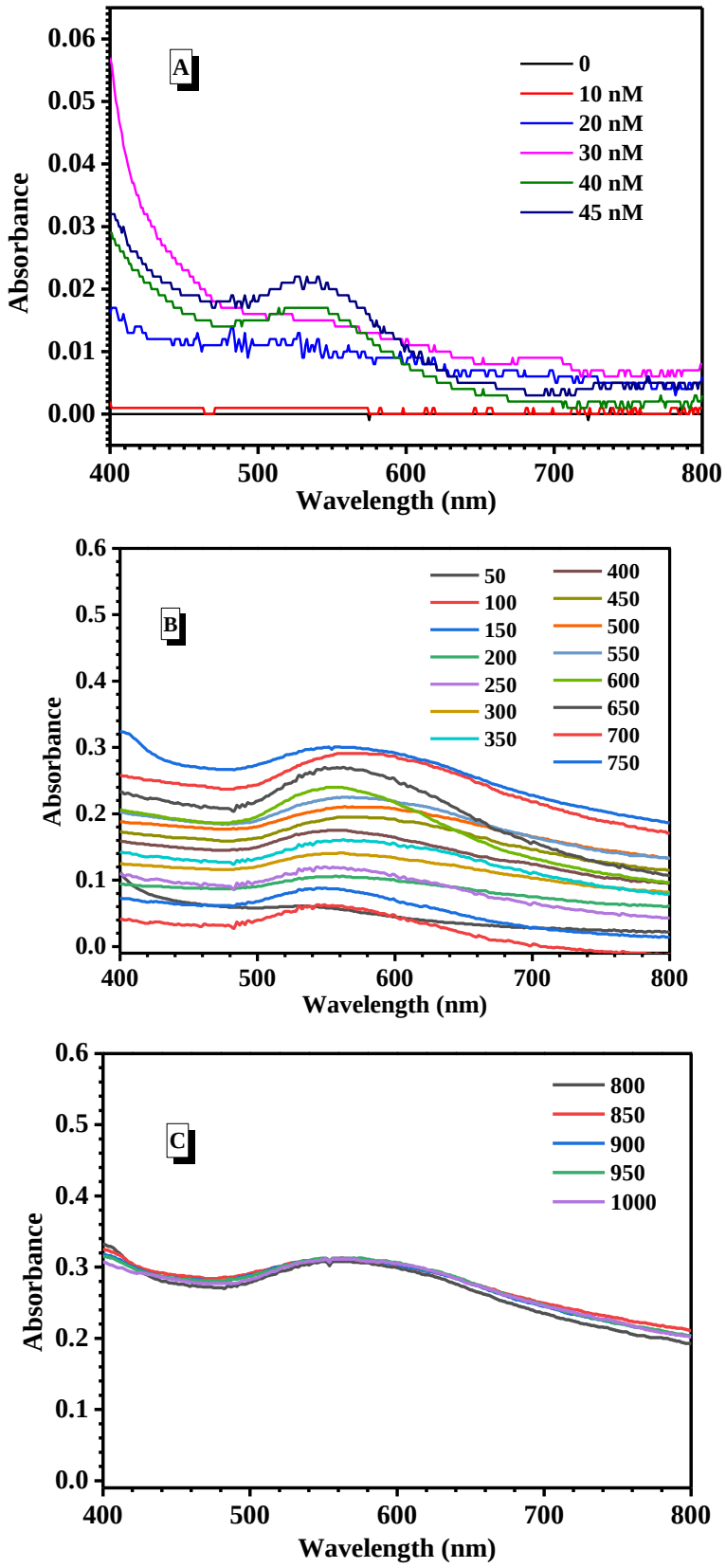



**Figure S19**. UV spectra of SABA-labeled PEGylated AuNP@PTA@$Fe^{3+}$-loading MSN-$NH_2$ varying CA II concentrations. (A) below 50 nM of CA II; (B) between 50 and 750 nM; (c) above 750 nM.

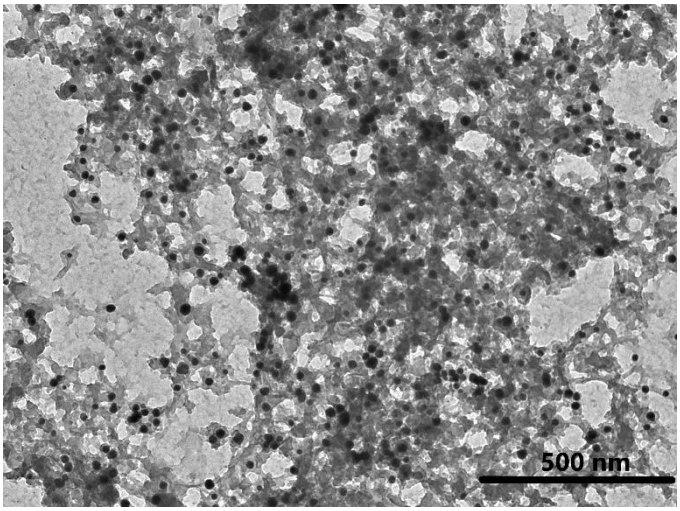



**Figure S20**. TEM images of the supernatant after detecting CA II.

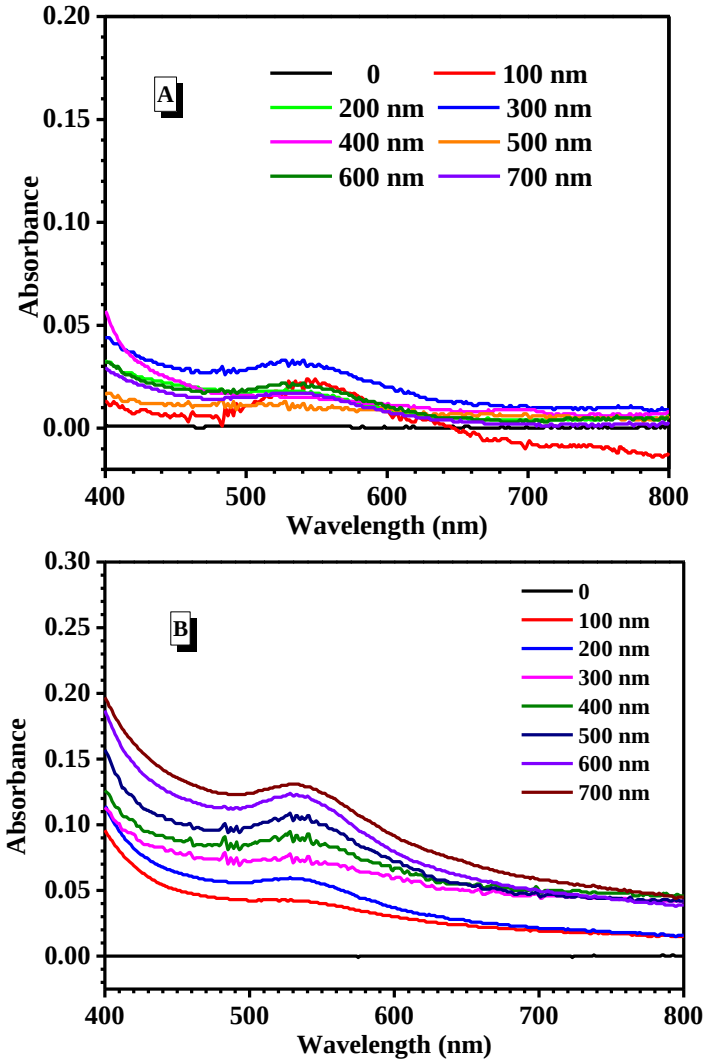



**Figure S21**. (A) UV spectra of PEGylated AuNP@PTA@$Fe^{3+}$-loading MSNs without SABA labeling varying CA II concentrations. (B) UV spectra of SABA-labeled PEGylated AuNP@PTA@MSN without loading $Fe^{3+}$ varying CA II concentrations.

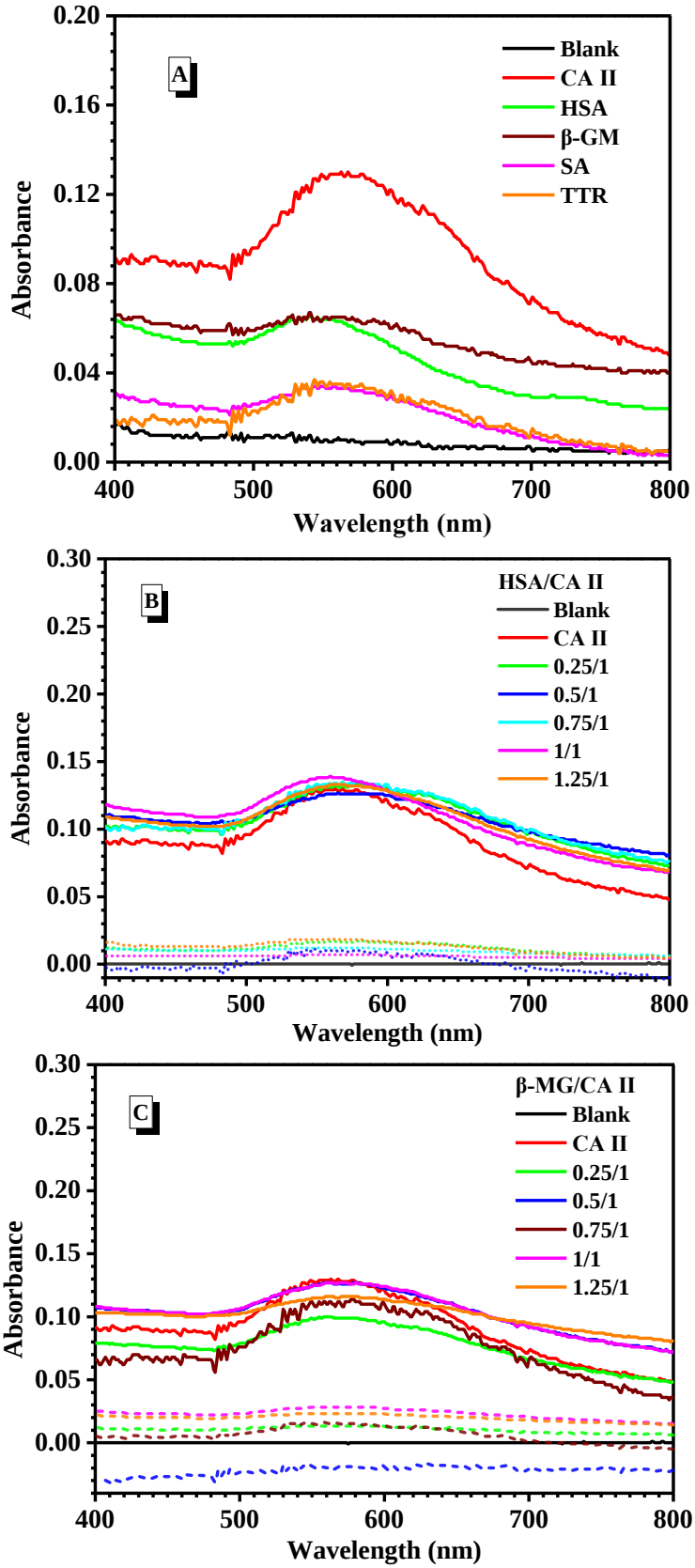



**Figure S22**. UV spectra of (A) SABA-labeled PEGylated AuNP@PTA@ $Fe^{3+}$-loading MSN in the presence of various protein. (B) the different molar ratios of CA II to HSA. (C) the different molar ratios of CA II to β-GM. ([CA II] = 300 nM