Supporting information

Dual stimuli-responsive upconversion nanoparticle–poly-*N*-isopropylacrylamide/DNA core–shell microgels

Qi Gao[a], Xiaowen Wang[a], Shanjin Hu[a], Ping-Ping He[a], Siyu Gou[a], Shuo Liu[a], Xiaoxue Du[a,b,*] and Weiwei Guo[a,*]

[a] *Research Center for Analytical Sciences, Tianjin Key Laboratory of Biosensing and Molecular Recognition, College of Chemistry, Nankai University, Tianjin 300071, P. R. China; Smart Sensing Interdisciplinary Science Center, School of Materials Science and Engineering, Nankai University, Tianjin 300350, P. R. China. E-mail: weiweiguo@nankai.edu.cn; Tel: +86-15822298109*

[b] *Handan Key Laboratory of Novel Nanobiomaterials, College of Materials Science and Engineering, Hebei University of Engineering, Handan, 056000, P. R. China. E-mail: duxiaoxue@hebeu.edu.cn*

Experimental section

Chemicals and materials

Yttrium chloride (YCl_3), Ytterbium chloride ($YbCl_3$), Thulium chloride ($TmCl_3$), *N*-isopropylacrylamide (NIPAM), *N,N'*-methylenebisacrylamide (BIS), ammonium persulfate (APS), *N, N, N′, N′*-tetramethylethylenediamine (TEMED), 1-Octadecene (ODE, 90%), oleic acid (OA, 90%), 3-(trimethoxysilyl)propyl methacrylate (MPS) and tetraethoxysilane (TEOS) were purchased from Sigma-Aldrich (Shanghai). 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), Tris(hydroxymethyl)methyl aminomethane (Tris), sodium chloride (NaCl), sodium hydroxide (NaOH), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$), boric acid and ammonium fluoride (NH_4F) were purchased from Aladdin. Anhydrous ethanol (AR), acetone (AR), hydrochloric acid (AR), cyclohexane (GR) and methanol (GR) were purchased from Tianjin Bohua Chemical Reagent. Igepal CO-520 was purchased Maclin. All DNA were purchased from Shanghai Sangon Biotechnology. All other reagents were at least analytical grade. All of the solutions were prepared with ultrapure water purified by a Mill-Q system (Millpore, Bedford, MA, USA).

Instruments and characterizations

Transmission electron microscopy (TEM) images were recorded with a Tecnai G2 F20 field-emission transmission electron microscopy (FEI, U.S.A.). SEM images were taken by a MERLIN Compact scanning electron microscope (ZEISS, Germany) with an accelerating voltage of 5 kV. DLS analyses and Zeta potential were carried out by a Zetasizer Nano ZS0303081003 Nanoparticle size analyzer (Malvern, U.K.). Fluorescence intensity spectra was recorded by a F-4600 fluorescence spectrophotometer (HITACHI, Japan). UV-Vis absorbance spectra were recorded with a UV-3900 spectrophotometer (HITACHI, Japan). IR absorption spectra was recorded with a Nicolet iS50 Fourier transform infrared spectrometer (Thermo Fisher Scientific, America). Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was measured by iCAP 7400 ICP-OES analyzer (Thermo Fisher Scientific, America).

Preparation of core $NaYF_4$: Yb, Tm nanoparticles

In a typical synthesis of monodisperse $NaYF_4$: Yb, Tm, YCl_3 (135.7 mg), $YbCl_3$ (83.8 mg), $TmCl_3$ (1.3 mg) were added to a 100 mL three-necked flask that containing oleic acid (6 mL) and 1-octadecene (15 mL). The mixture was

∗ Corresponding author.
E-mail address: duxiaoxue@hebeu.edu.cn (X. Du), weiweiguo@nankai.edu.cn (W. Guo).

purged with N_2 for 15 min, then heated to 150 ºC with constant stirring for 1 h. Then the solution was cooled down to 45ºC under N_2 atmosphere. 10 mL of methanol solution containing NH_4F (148 mg) and NaOH (100 mg) was added slowly to the reaction solution in 10 min. Then the mixture was stirred at 45 ºC for 30 min. After that, the solution was heated to 110ºC for 30 min to remove methanol. Then the solution was further heated to 300 ºC and the temperature was maintained for 80 min. Finally, the reaction solution was cooled down to room temperature with constant stirring. The product was precipitated by the addition of acetone and collected by centrifugation at 10000 revolutions per minute for 5 min. The product was redispersed with cyclohexane, precipitated by acetone, and then collected by centrifugation. After washing three times, the final product was redispersed in 10 mL cyclohexane for further use.

Preparation of core-shell $NaYF_4$: Yb, Tm@$NaYF_4$ (UCNP) nanoparticles

The preparation of $NaYF_4$:Yb,Tm@$NaYF_4$ nanoparticles was carried out according to a literature procedure with minor modifications. In brief, YCl_3 (156 mg) was added to a 100 mL three-necked flask containing oleic acid (6 mL) and 1-octadecene (15 mL). The mixture was cooled down to 45 ºC under N_2 atmosphere. 10 mL of the above-synthesized core nanoparticles were added to the reaction, heated to 80 ºC to remove cyclohexane, and cooled down to 45ºC. 8 mL of methanol solution containing NH_4F (118 mg) and NaOH (80 mg) was added slowly to the reaction solution in 10 min. Then the solution was further heated to 300ºC and the temperature was maintained for 80 min. Finally, the reaction solution was cooled down to room temperature with constant stirring, washed with the same procedure as the core nanoparticles.

Silica modification of UCNPs

The surface modification of UCNPs was synthesized by adopting an inverse microemulsion method. 30 mg of UCNPs and 1g of Igepal CO-520 were dispersed in 10 mL cyclohexane and stirred for 30 min. Then added 0.15 mL of NH_4OH and stirred for 30 min. Afterward, 0.02 mL of TEOS was added to the reaction solution and stirred for 24 h. 10 mL of ethanol was added to the solution to precipitate the products (denoted as UCNP-S) by centrifugation and redispersed in 10 mL of ethanol. Subsequently, 0.13 mL of MPS was added to the reaction solution and stirred for 24 h. Finally, the products (denoted as UCNP-SM) were collected by centrifugation, washed several times with ethanol and water, and then redispersed in 1 mL of water.

Synthesis of UCNP–pNIPAM/DNAzyme (UCPD) microgels

All UCPD microgels were synthesized by modified precipitation polymerization. 47.5 mM NIPAM, 2.5 mM BIS were mixed with 0.01 mM 5'-acrydite DNAzyme and 0.15 mL UCNP-SM in 1mL aqueous solution. The solution was purged with N_2 gas and heated to 45 ºC with vigorously stirring for 1 h. Then 1 mM initiator (APS and TEMED) was injected into the reaction. The reaction mixture was polymerized under N_2 gas protection at 45ºC with constant stirring for 5 h. The resulting turbid mixture was allowed to cool down at room temperature and then purified with a Microcon (Millipore) spin filter unit (MWCO:100KD) to remove unreacted monomers and initiator. For microgels with different monomer concentrations of 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, and 70 mM, fixed the molar proportion of BIS at 5% relative to NIPAM, UCNP-SM concentration at 0.5 mg/mL and DNA concentration at 0.01 mM. The other procedures were the same as described above. For microgels with different crosslinker contents of 2.5%, 5%, 7.5%, 10%, 12.5% and 15%, fixed the total monomer concentrations of NIPAm and BIS at 50 mM, UCNP-SM concentration at 0.5 mg/mL and DNA concentration at 0.01 mM. The other procedures were the same as described above.

Synthesis of UCNP–pNIPAM (UCP) microgels

The UCP microgels was synthesized with the same procedures as the UCPD microgels without the addition of DNAzyme.

Synthesis of UCPD-L microgels

UCPD-L microgels was synthesized by mixed UCPD microgels and Lock strand in buffer solution (20 mM HEPES, 500 mM NaCl, 6 mM $MgCl_2$, pH 7.4) overnight at room temperature. Then the reaction solution was purified with a Microcon (Millipore) spin filter unit (MWCO:100 KD) to remove excess Lock strand.

Synthesis of pNIPAM/DNAzyme (PD) microgels and pNIPAM/DNAzyme-Lock (PD-L) microgels

The same procedures were conducted to obtain the PD microgels and PD-L microgels without UCNPs, except for the addition of UCNP-SM.

Synthesis of UCNP-DNAzyme

As-synthesized UCNP was stirred in 0.1 M hydrochloric acid solution to remove the original oleate ligands and obtained ligand-free UCNP. Then the ligand-free UCNP was added to a solution containing C10-DNAzyme. The mixture was sonicated for 30 min to promote the adsorption of DNA on nanoparticles. Subsequently, 10×Tris-BA buffer (890 mM tris-base, 890 mM boric acid, 1 M NaCl, pH~8) was added to the mixture every 20 min to obtain a final concentration of 1×Tris-BA buffer (89 mM tris-base, 89 mM boric acid, 0.1 M NaCl, pH~8). The solution was stirred for 12 h. Then the solution was centrifuged at 14800 rpm and washed with ultrapure water to remove the free C10-DNAzyme. The obtained UCNP-DNAzyme solution was redispersed in ultrapure water and the concentration of DNAzyme was measured with UV spectrophotometer.

Catalytic activity evaluation of the UCPD-L microgels under dual stimuli-regulation

Reaction buffer solution (20 mM HEPES, 500 mM NaCl, 6 mM $MgCl_2$, pH 7.4) containing 1 μM substrate and 40 μL UCPD-L microgels were added into a PCR cube. Then the reaction system was incubated at four modes: 25 ºC without NIR light irradiation; 35 ºC without NIR light irradiation; 25 ºC with NIR light irradiation; 35 ºC with NIR light irradiation. After irradiation, the reaction systems are incubated at 25 ºC for 90 min. Then the fluorescence intensity at 608 nm of the reaction system was measured.

Table S1. Oligonucleotide sequences used in this work.

Name	Sequence（5’-3’）
DNAzyme	Acrydite-TTTTTCTCATTCAGCGATCCGGAACGGCACCCATGTTCTGTGA
Lock-12	CACAGA-pc-ACATGG
Lock-14(Lock)	CACAGAA-pc-CATGGGT
Lock-16	CACAGAAC-pc-ATGGGTGC
Substrate	ROX-TCACAGATrAGGAATGAG-BHQ2
C_{10}-DNAzyme	CCCCCCCCCCCTCATTCAGCGATCCGGAACGGCACCCATGTTCTGTGA

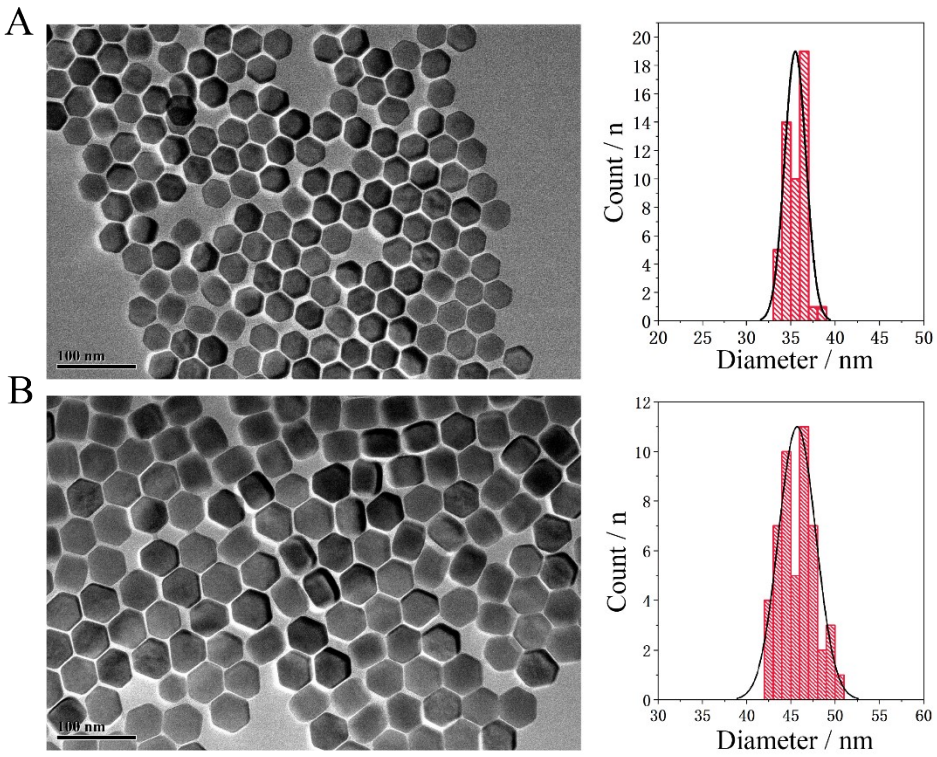

Figure S1. TEM image and the corresponding size distribution of (A) $NaYF_4$:Yb,Tm. (B) $NaYF_4$:Yb,Tm@$NaYF_4$.

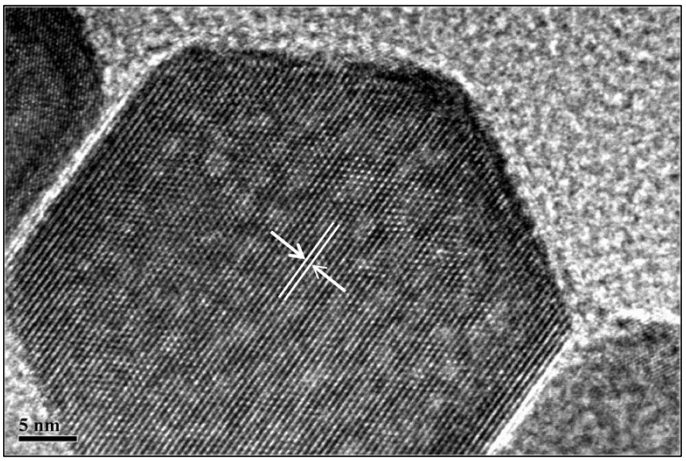

Figure S2. HRTEM image of $NaYF_4$:Yb,Tm@$NaYF_4$.

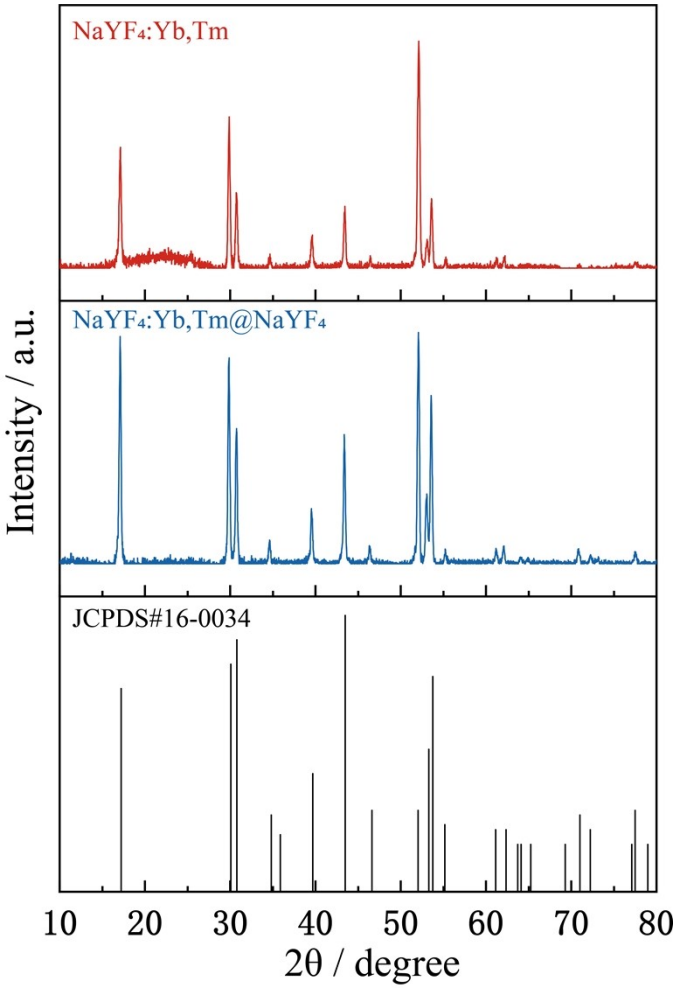

Figure S3. XRD patterns of $NaYF_4$:Yb,Tm (red), $NaYF_4$:Yb,Tm@$NaYF_4$ (blue) and standard hexagonal $NaYF_4$ structure of JCPDS#16-0034 (black).

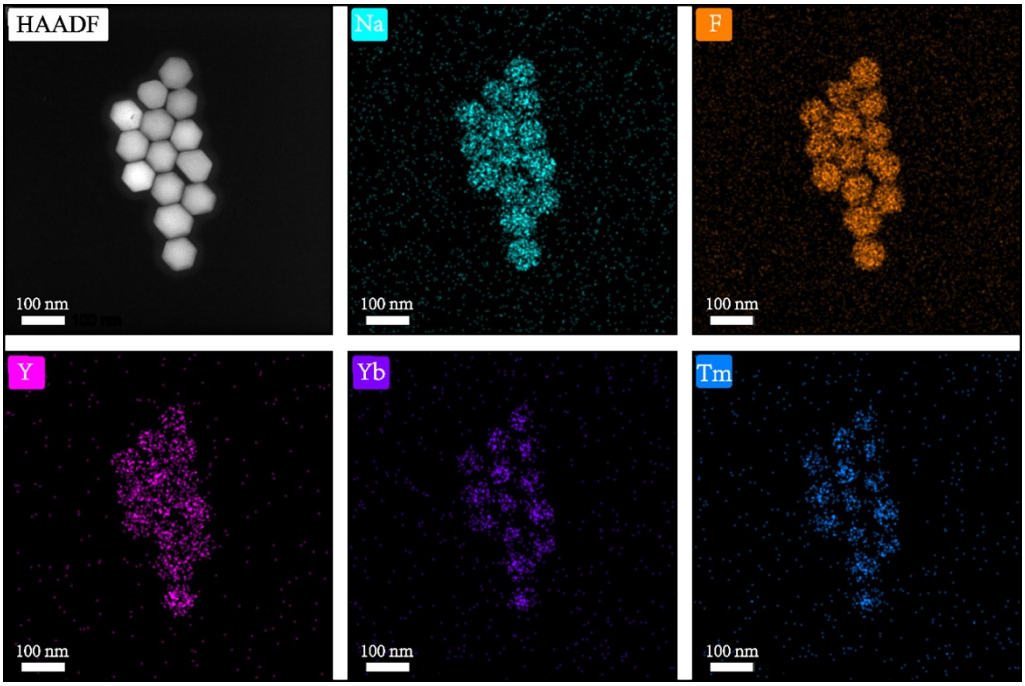

Figure S4. HADDF-STEM images and corresponding EDS elemental mapping analysis of $NaYF_4$:Yb,Tm@$NaYF_4$, illustrating the spatial distribution of Na, F, Y, Yb and Tm elements in $NaYF_4$:Yb,Tm@$NaYF_4$.

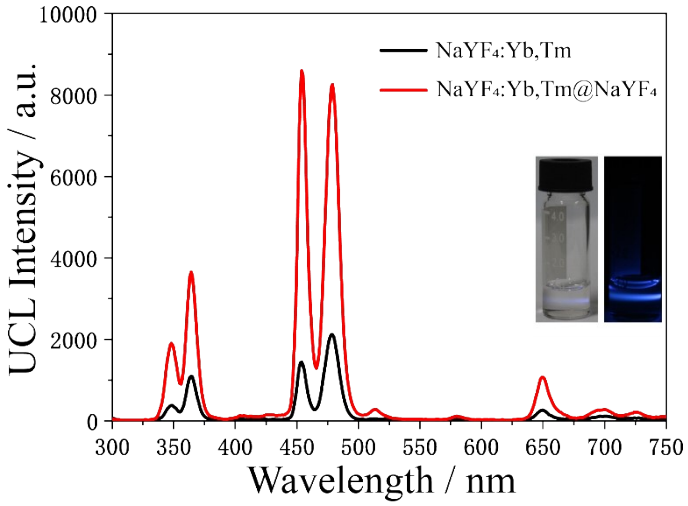

Figure S5. The UCL emission spectra of $NaYF_4$:Yb,Tm and $NaYF_4$:Yb,Tm@ $NaYF_4$ under 980 nm laser excitation. Insert: the photographs show the UCL emission of $NaYF_4$:Yb,Tm@ $NaYF_4$ under 980 nm laser excitation.

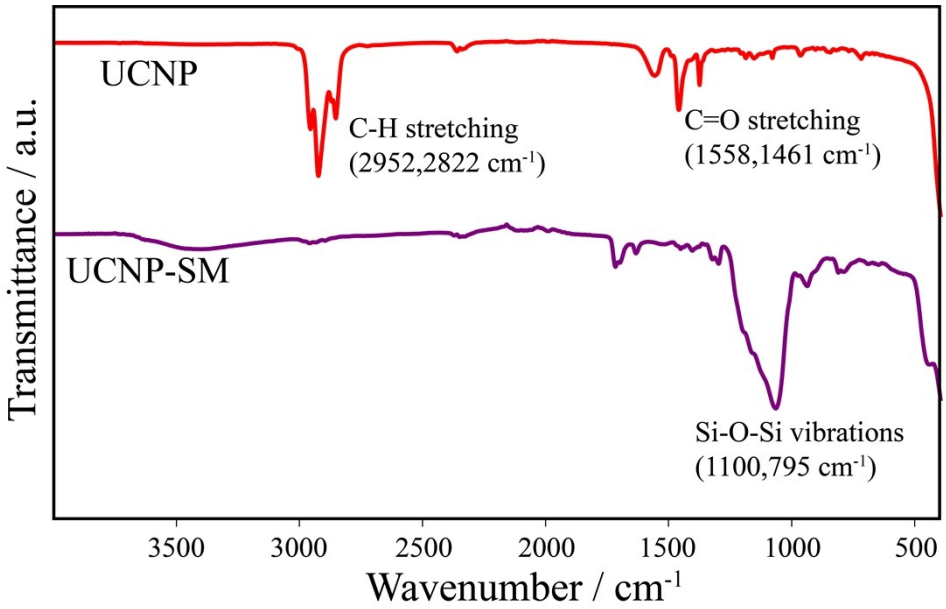

Figure S6. FT-IR spectra of UCNP and UCNP-SM.

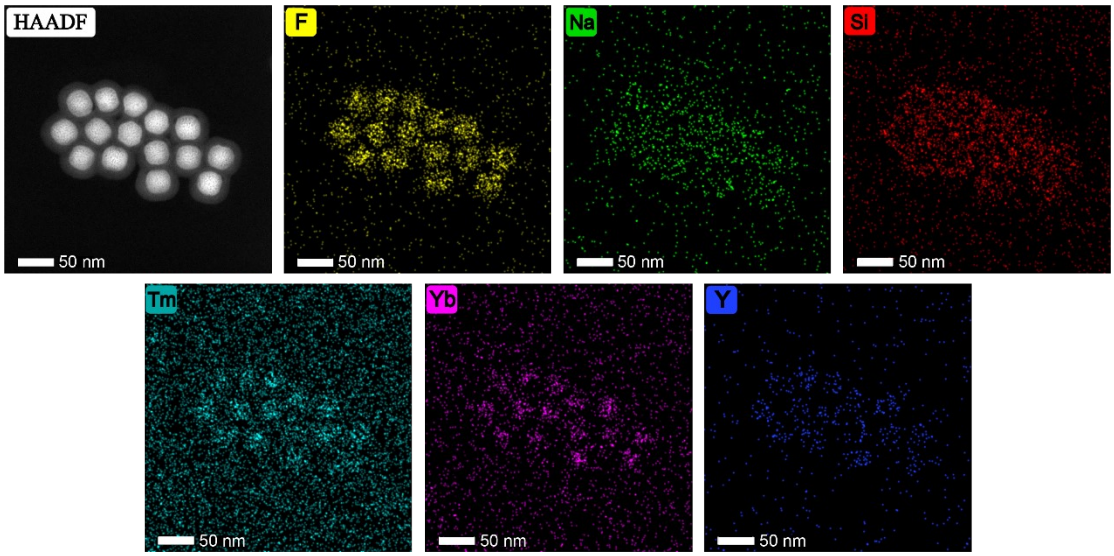

Figure S7. HADDF-STEM images and corresponding EDS elemental mapping analysis of UCNP-SM, illustrating the spatial distribution of F, Na, Si, Tm, Yb and Y elements in UCNP-SM.

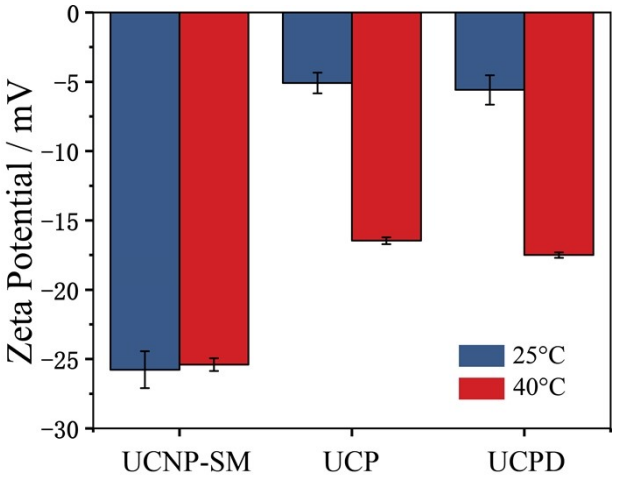

Figure S8. Zeta potentials of UCNP-SM, UCP microgel and UCPD microgel at 25 °C and 40 °C, respectively.

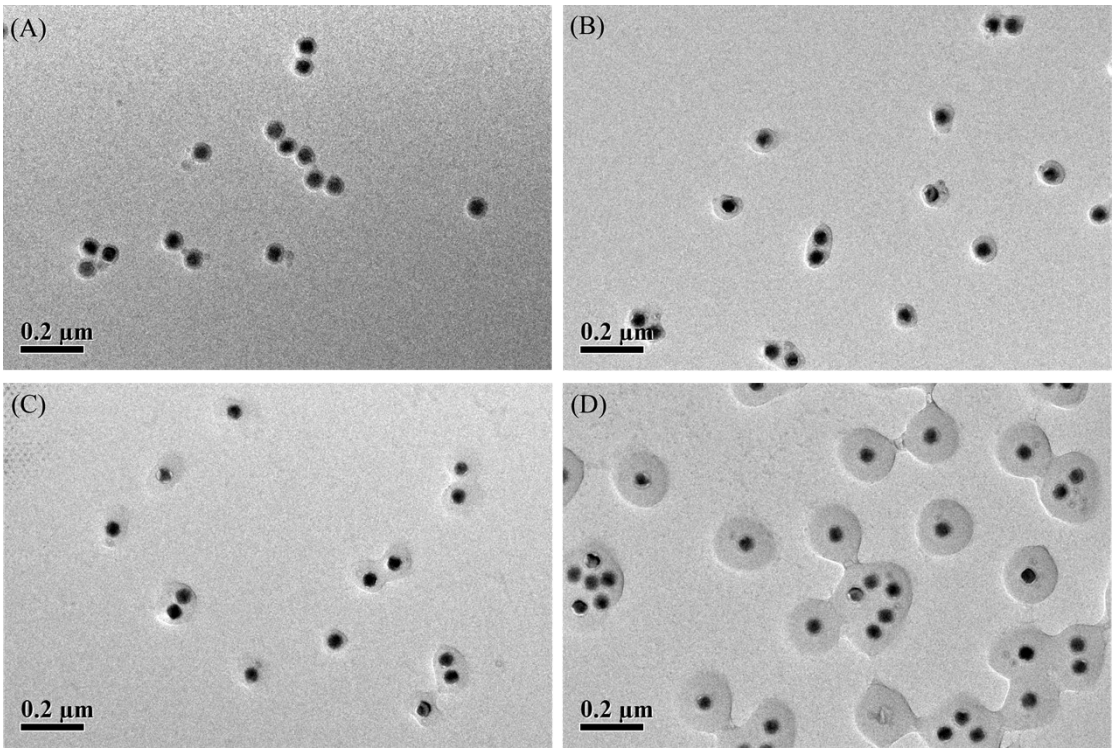

Figure S9. TEM images of UCPD microgels synthesized at monomer concentration of (A) 20 mM, (B) 40 mM, (C) 60 mM and (D) 70 mM.

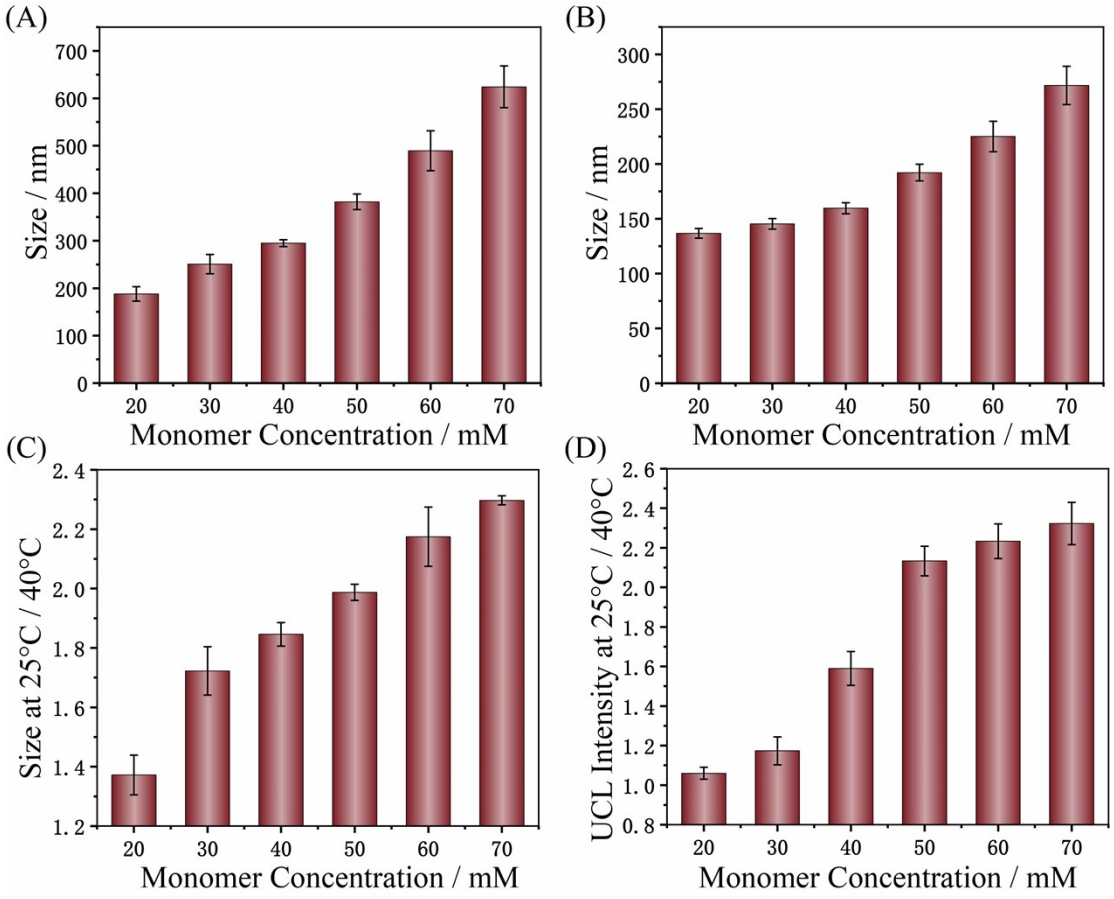

Figure S10. The hydrodynamic diameters of UCPD microgels synthesized with different monomer concentration at 25 °C (A) and 40 °C (B), respectively. (C) The swelling ratio of UCPD microgels synthesized with different monomer concentrations from 20 to 70 mM. (D) UCL intensity ratio between 25 °C and 40 °C of the UCPD microgels with different monomer concentrations from 20 to 70 mM.

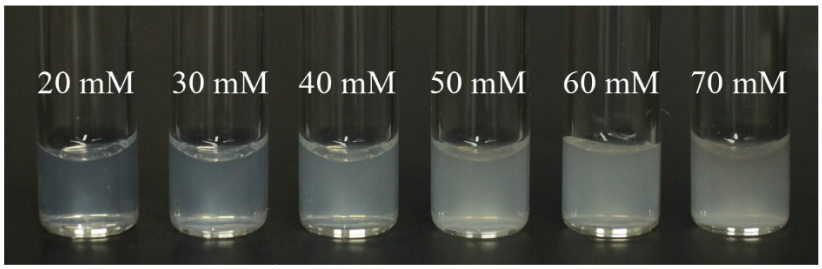

Figure S11. Photographs of the UCPD microgels synthesized with different monomer concentrations from 20 to 70 mM.

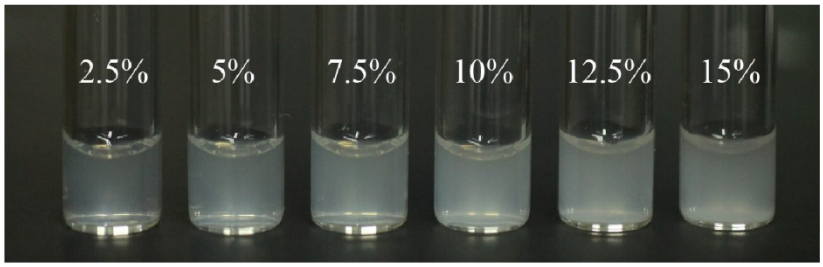

Figure S12. Photographs of the UCNP microgels synthesized with different crosslinker contents from 2.5% to 15%.

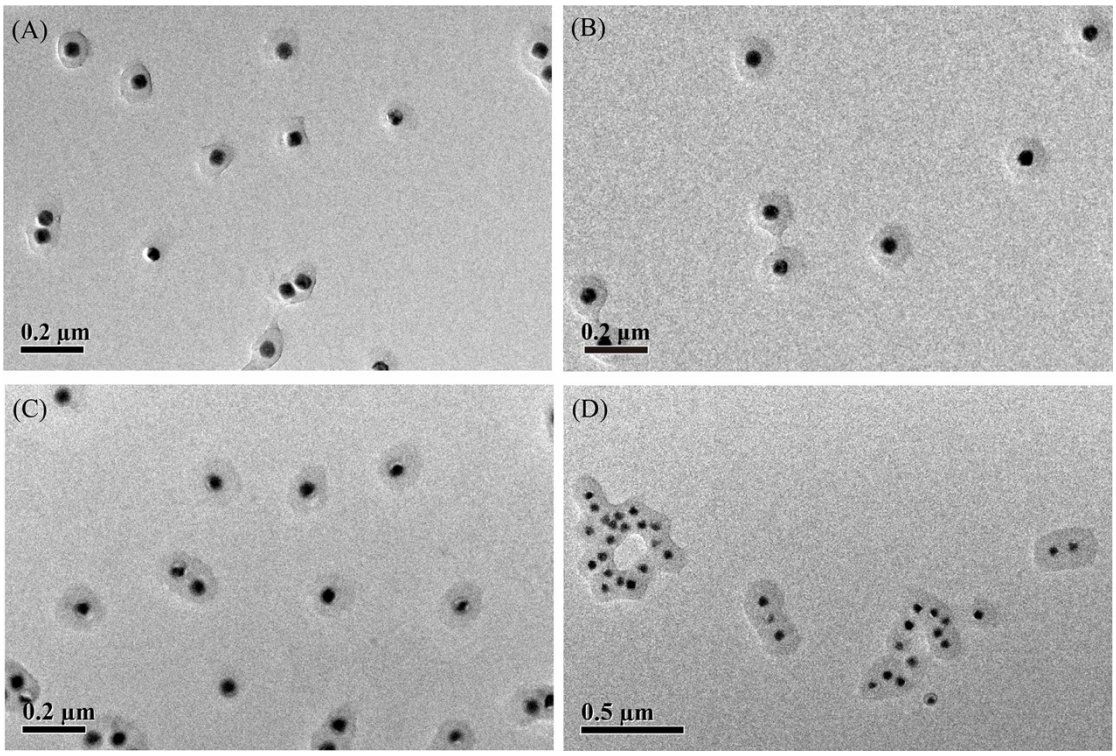

Figure S13. TEM images of UCPD microgels synthesized with different crosslinker content of (A) 2.5%, (B) 7.5%, (C) 10% and (D) 15%.

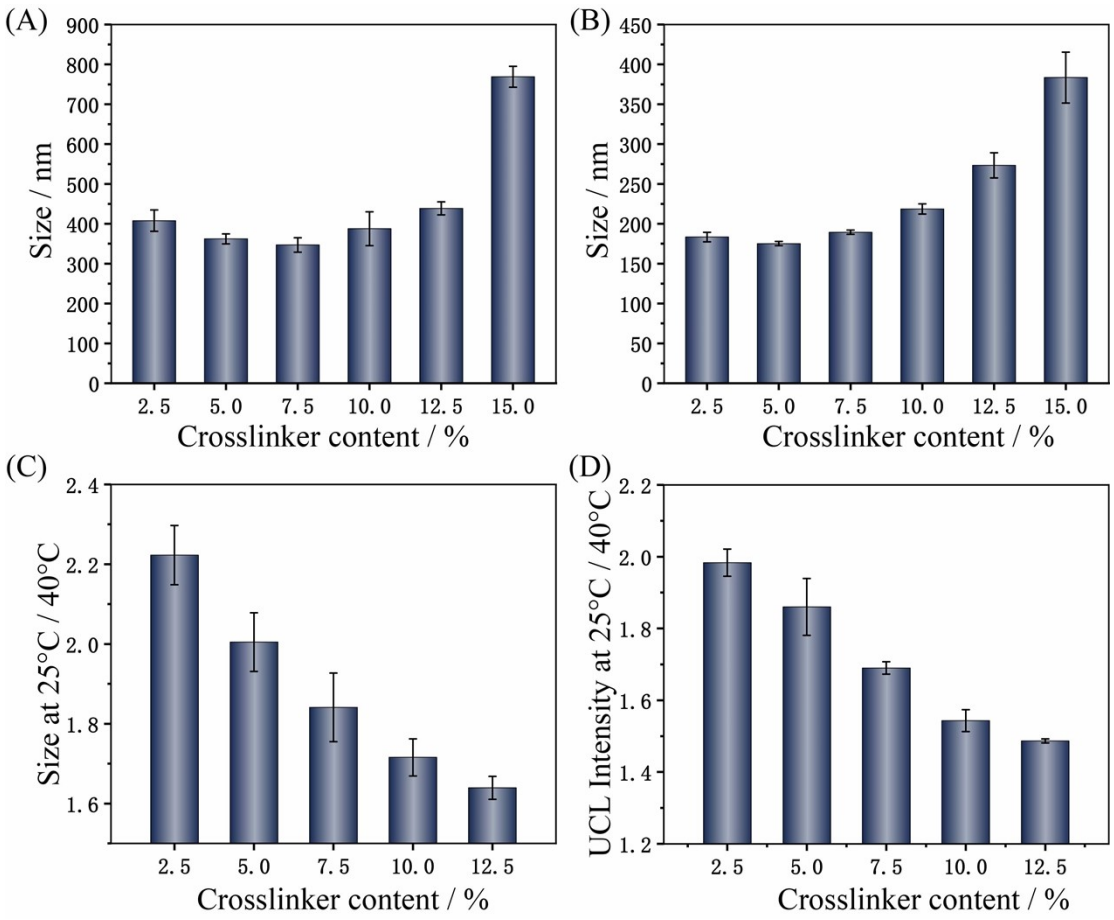

Figure S14. The hydrodynamic diameters of UCPD microgels synthesized with different crosslinker content at 25 °C (A) and at 40 °C (B), respectively. (C) The swelling ratio of UCPD microgels synthesized with different crosslinker contents from 2.5% to 12.5%. (D) UCL intensity ratio between 25 °C and 40 °C of the UCPD microgels with different crosslinker contents from 2.5% to 12.5%.

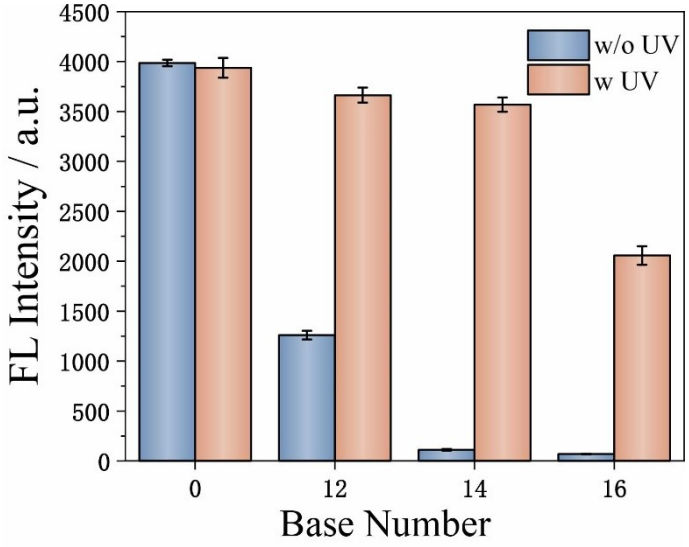

Figure S15. FL intensity of the reaction system catalyzed by DNAzyme-L with different Lock strand lengths.

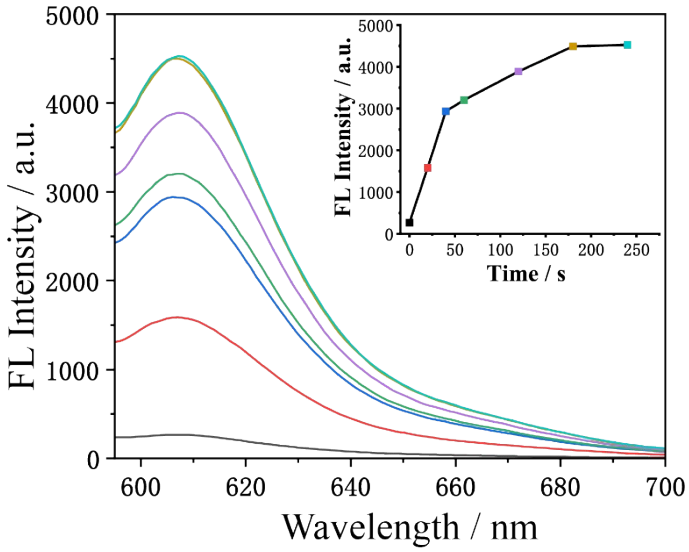

Figure S16. Fluorescence spectra of DNAzyme-L catalytic system upon different UV light irradiation times (with a UV irradiation intensity of 2 mW/cm^2).