Electronic supplementary information

On-demand one-step synthesis of small-sized fluorescent-magnetic-bifunctional microparticles on a droplet-splitting chip

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Contents

S1 Synthesis of acryloyl rhodamine B

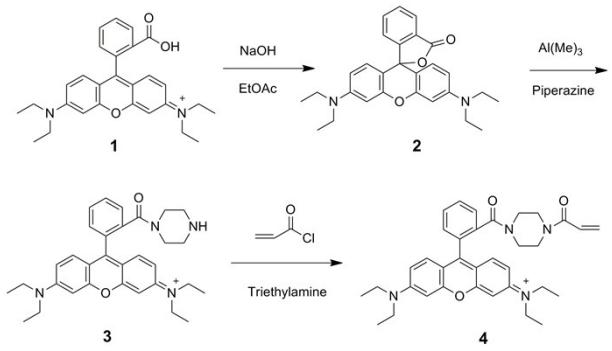
As scheme S1, addition of rhodamine B base which was prepared from 1 to $Al(Me)_3$ and piperazine resulted in rhodamine B piperazine amide(3).[1] Finally, acryloyl rhodamine B was obtained by reaction between crude 3 and acryloyl chloride .

Rhodamine B base (2): Rhodamine B (1, 1.65 g, 3.44 mmol, Sigma-Aldrich) was dissolved and partitioned between 1 M NaOH and EtOAc (Sinopharm Chemical Reagent Co., Ltd, China). After isolation of organic layer, aqueous layer was extracted by EtOAc. The combined organic layers were washed by NaOH and brine, then dried with Na_2SO_4, filtered, and concentrated to yield 1.37 g of purple foam (90%). The product was used to next step reaction without purification.

Rhodamine B piperazine amide (3): A 2.0 M solution of $Al(Me)_3$ in hexane was added into a solution of piperazine (0.78 g, 9.1 mmol) in 35 mL of CH_2Cl_2 at room temperature. After 1 h stirring, a white precipitate was observed. A solution of rhodamine B base (2, 1.0 g, 2.26 mmol) in 20 mL of anhydrous CH_2Cl_2 was dropwise added to above mixture solution. Gas evolution was observed during the addition period. After 24 h reflux, a 0.1 M solution of HCl was slowly added at the ice bath until the gas evolution ceased. The mixture solution was filtered and the retained precipitate was washed repeatedly by CH_2Cl_2 and CH_2Cl_2/MeOH. The combined filtrate was concentrated, then dissolved in CH_2Cl_2, filtered to remove insoluble salts, and concentrated again. The resulting solid was dissolved and partitioned in dilute aqueous $NaHCO_3$ and EtOAc. After isolation of organic layer, aqueous layer was extracted with EtOAc to remove residual starting materials. The retained aqueous layer was saturated with NaCl, acidified with 1 M HCl, and extracted with 2:1 ^{i}PrOH/CH_2Cl_2 until the slight pink color was observed in the aqueous layer. The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to get purple solid. The solid was dissolved in a minimum amount of MeOH, precipitated in a large volume of Et_2O and repeated three times. A 0.52 g of dark purple product was collected by filtration (45%). The product was used to next step reaction without purification.

Acryloyl Rhodamine B (4). Triethylamine (0.17 mL, 1.2 mmol) and acryloyl chloride (0.1 mL, 1.2 mmol, Aladdin Reagent Co., Ltd, China) were added to a solution of rhodamine B piperazine amide (3, 0.52 g, 1.02 mmol) in CH_2Cl_2 (20 mL). After stirring at room temperature for 12 h, the reaction was terminated with H_2O. The reaction solution was partitioned when a solution of 1 M HCl was added. The organic layer was washed with a solution of 1 M Na_2CO_3 until the aqueous layer was colorless. After washing with H_2O, the aqueous layer was removed and the organic layer was dried with anhydrous Na_2SO_4, concentrated to get 0.52 g of solid.

The product was purified by column chromatography and confirmed by ^{1}H NMR (Fig.S1). The NMR result suggested acryloyl-RhB was successfully synthesized.



Scheme S1 Synthesis of acryloyl-RhB

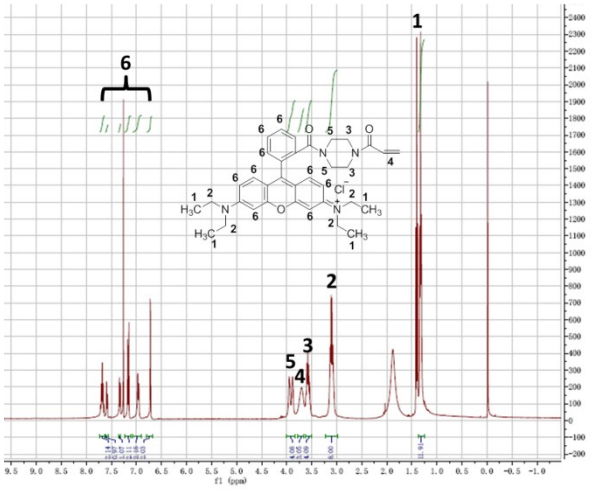


Fig. S1 NMR analysis of acryloyl-RhB.

S2 Synthesis and Characterization of PEI-Fe_3O_4 Nanoparticles

The hydrophilic Fe_3O_4 nanoparticles were synthesized following the coprecipitation method. Briefly, 2.71 g of $FeCl_3 \cdot 6H_2O$ (Sinopharm Chemical Reagent Co., Ltd, China) and 1.08 g of $FeCl_2 \cdot 4H_2O$ was dissolved in 100 mL ultra-pure water. The mixture was stirred under nitrogen atmosphere for 30 min at room temperature. Then concentrated ammonia solution was dropwise added into the above solution until the pH reached 10.0 under vigorous stirring. After 400 μL of poly(ethylene imine) (*M*n 1.8 k, 0.1 g mL^{-1}, Sigma-Aldrich) was added, the mixture solution was slowly heated to 60°C and maintained at 60°C for 1 h under nitrogen atmosphere. Afterward, the mixture was processed by water bath crystallization at 80°C for 30 min. After cooling down to room temperature, the black PEI-Fe_3O_4 product was magnetically separated and washed several times by water and ethanol, respectively. Finally, the PEI-Fe_3O_4 product was centrifuged (6000 rpm, 5 min) to remove larger particles, and the supernatant suspension was collected and stored at 4°C. TEM (Tecnai G^2 20 TWIN, FEI Company, USA) and dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern Instruments, Inc., UK) were used to characterize the PEI-Fe_3O_4 nanoparticles. TEM image (Fig. S2a) showed that the size of PEI-Fe_3O_4 nanoparticles was 14.2±1.3 nm. The hydrodynamic size of the PEI-Fe_3O_4 nanoparticles was about 45.3 nm with the polydispersity index (PDI) of 0.176, and zeta potential was 32.8 mV which were determined by the DLS (Fig. S2c-d). The magnetization curve showed PEI-Fe_3O_4 nanoparticles were of superparamagnetism (Fig. S2b). The results indicated that the positive charge of PEI-Fe_3O_4 nanoparticles had good stability and superparamagnetism.

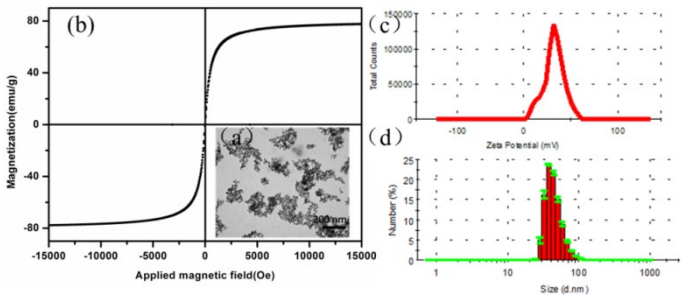
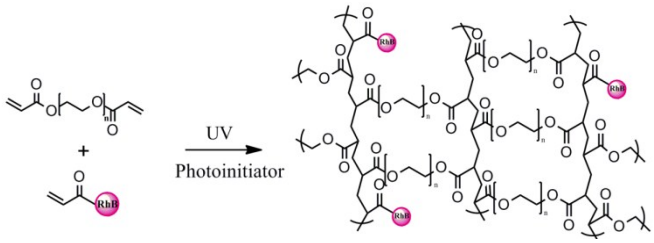


Fig. S2 The characterization of superparamagnetic PEI-Fe_3O_4 nanoparticles (a) TEM image (14.2±1.3 nm) (b) Magnetization curve (c) Zeta potential (32.8 mV) and (d) Hydrodynamic particle size (45.3±0.6 nm) distributions.

S3 Photopolymerization principle



Scheme S2 Schematic illustrating the formation of PEG@ RhB hydrogel via photopolymerization.

S4 Characterization of multifunctional microparticles

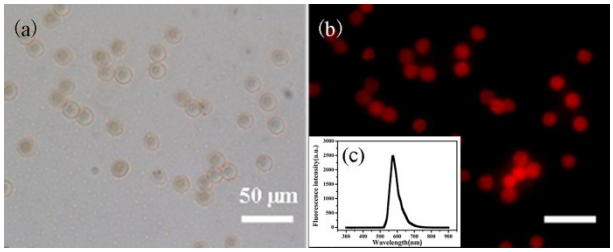


Fig. S3 Images of PEG@Fe_3O_4@RhB microparticles. (a) Bright field image of PEG@Fe_3O_4@RhB microparticles. (b) Fluorescence image of PEG@Fe_3O_4@RhB microparticles. (C) Fluorescence spectrum of single PEG@Fe_3O_4@RhB microparticle collected by portable fiber optic spectrometer.

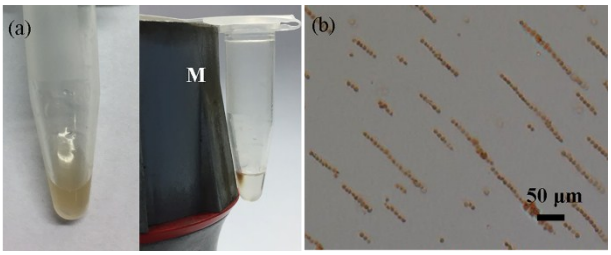


Fig. S4 (a) Photographs of PEG@Fe_3O_4@RhB microparticles in centrifuge tube captured by a magnetic scaffold at 30 s. (b) Microscopy image of self-assembly of PEG@Fe_3O_4@RhB microparticles in response to homogeneous magnetic fields.

S5 Size control of microparticles

In our work, the size of microparticles prepared using droplet templates could be fabricated by adjusting the volumetric flow rate of the oil and water phases from syringe pump. The size of PEG@Fe_3O_4@RhB microparticles obtained was 19.2±0.9, 14.5±0.6, 13.1±0.3, 10.8±0.2, 9.0±0.2, 8.0±0.1, 6.6±0.3, 6.0±0.2, 5.4±0.1, 4.6±0.3, 2.8±0.2 μm, respectively.

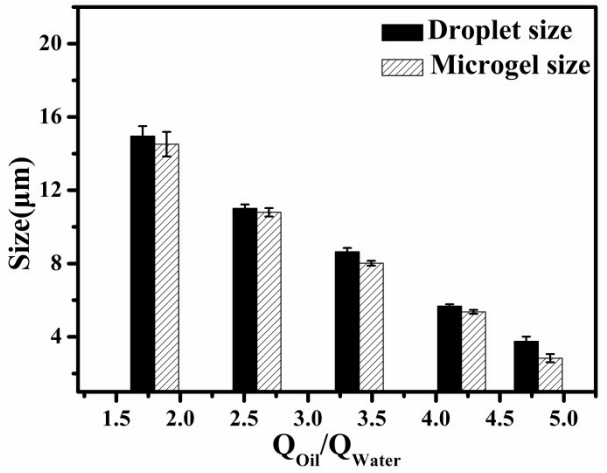


Fig. S5 Influence of flow rate on the size of droplets and microparticles before and after photopolymerization.

S6 Protein detection

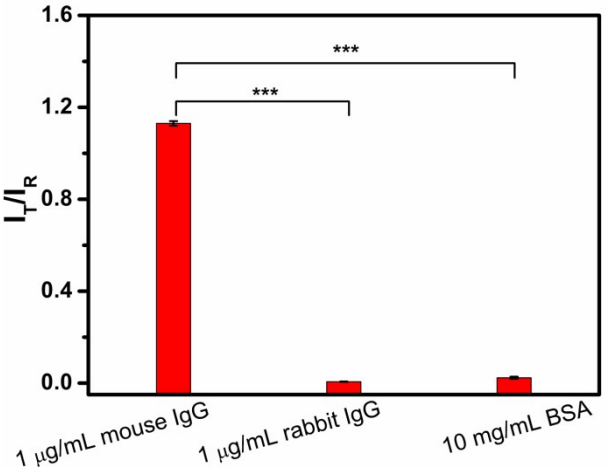


Fig. S6 Ratiometric fluorescence analysis of special test. P<0.001.

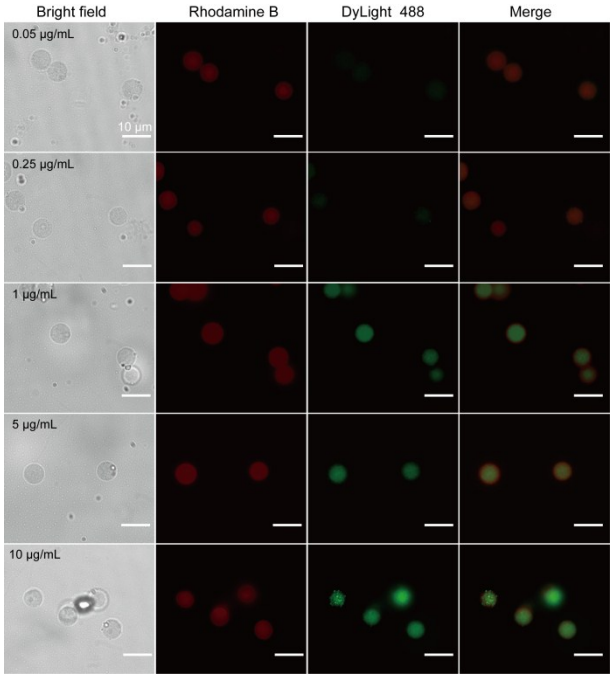


Fig. S7 Microscopy images of different concentrations of mouse IgG captured by PEG@Fe_3O_4@RhB microparticles.

Reference

1 T. Nguyen and M. B. Francis, *Org. Lett.*, 2003, **5**, 3245-3248.