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Supplementary Information

Facile Preparation of Polydiacetylene-Based Uniform Porous Fluorescent Microspheres for Potential Immunoassay Application

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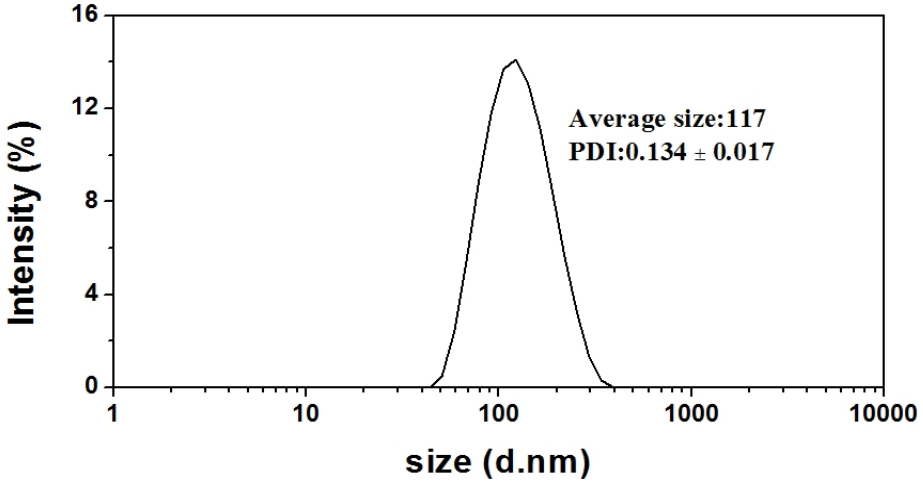

Fig. S1. Particle size by relative light intensity of DA vesicles with an initial monomer concentration of 1 mM.

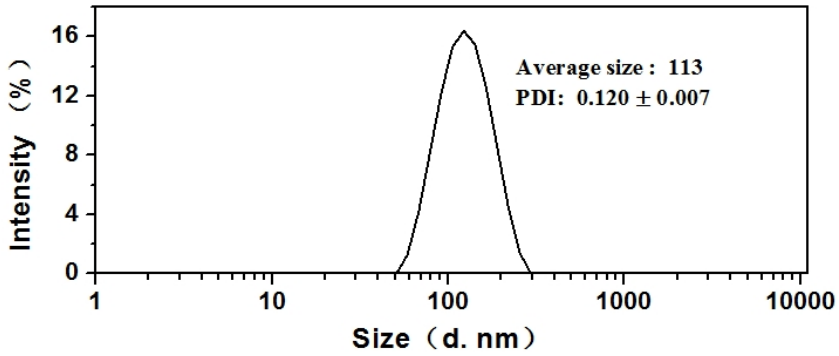

Fig. S2.Particle size by relative light intensity of blue PDA vesicles obtained by polymerizing DA monomers with an initial monomer concentration of 1 mM.

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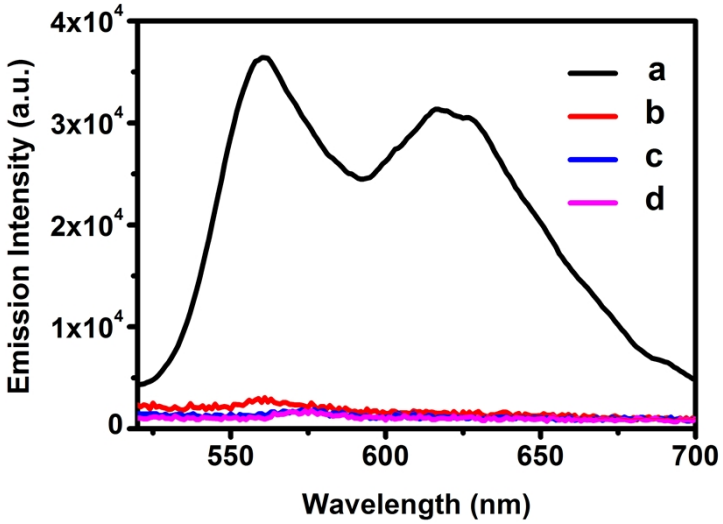

Fig. S3. Emission intensity of 3 mL of initial red PDA vesicles as obtained (a)，the filtrate after reacting with 15 mg of AMPGMA microspheres (b), and the filtrates of the resulting AMPGMA-PDA being washed by water for the first time (c) and for the second time (d). Note that these fluorescent microspheres were washed with 6 mL of deionized water each time in an oscillator with the rotating speed of 1000 r/min for 10 min. 2 mL of the eluent was added into a cuvette to measure its fluorescence.

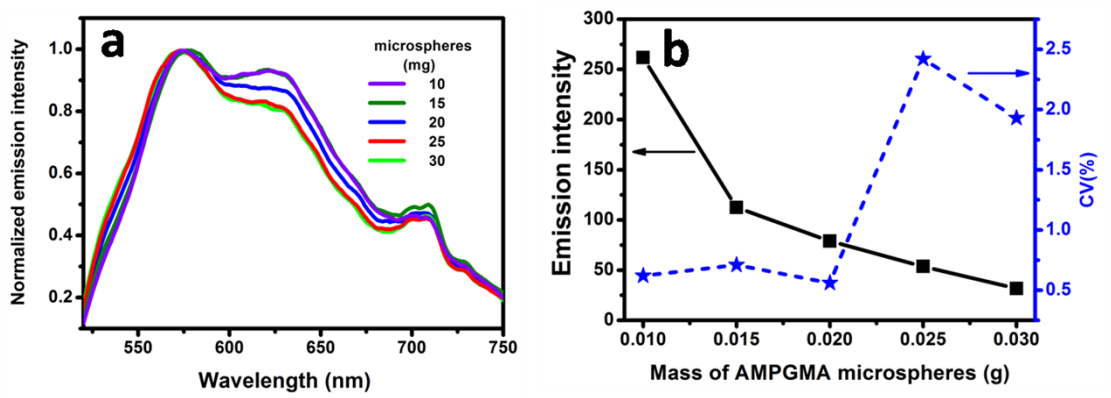

Fig. S4. (a) Normalized emission spectra from a steady fluorescence measurement on a fluorometer excited at 500 nm and (b)emission intensity and intensity CV obtained from a flow cytometer at channel 625 nm (excited at 488 nm) of red APGMA-PDA microspheres with different mass of APGMA microspheres and 3 mL of PDA vesicles.

Note：As shown in Fig. S4a, all the products from different feeding ratios display similar profile in the emission spectra, having a peak around 575 nm and a shoulder around 625 nm, with slight variation in intensity ratios between the peak and the shoulder. The more PDA loaded onto the microspheres, the more closer the emission is to that of polydiacetylene in solid film.[1] Fig. S4b shows emission intensities and the corresponding coefficient of variance values (CV) from flow cytometry measurements. The intensity decrease with increasing the amount of substrate spheres, which is very reasonable since less PDA was loaded on each microsphere.

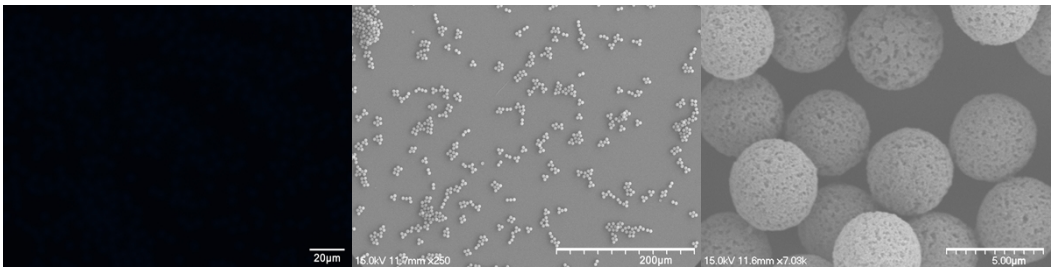

Fig. S5. Fluorescence microscope image (left, nonfluorescent) and SEM images with different scales (middle and right) and of the initial amino-modified PGMA microspheres.

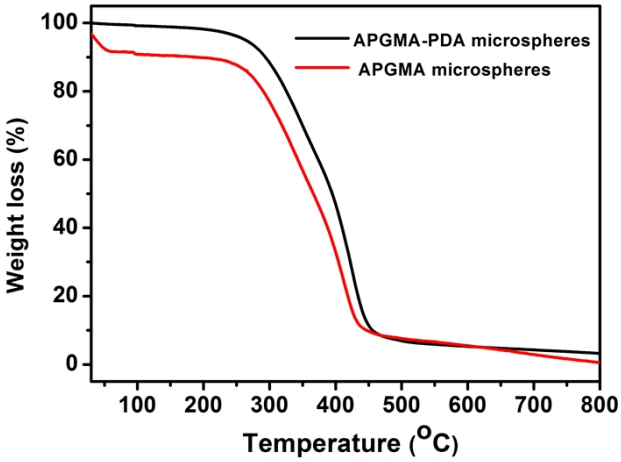

Fig. S6. TGA curves of APGMA microspheres and APGMA-PDA microspheres.

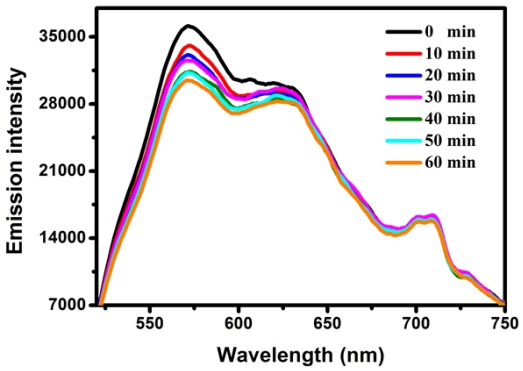

Fig. S7 Emission intensity of resulting red APGMA-PDA microspheres after laser irradiation for different time.

Table S1 The emission intensity of resulting red microspheres[a] and after being washed with deionized water for different times.

	Original microspheres	Microspheres after first washing	Microspheres after Second washing
Intensity of microspheres[b]	108.6	109.4	107.9

[a]obtained by reacting 3 mL of red PDA vesicles and 15 mg of APGMA microspheres
[b] emission intensities obtained from a flow cytometer at channel 575 nm when excited at 488 nm.

Data for Route 2

The experimental data for route 2 are very similar to those obtained from route 1 and are supplied as follows.

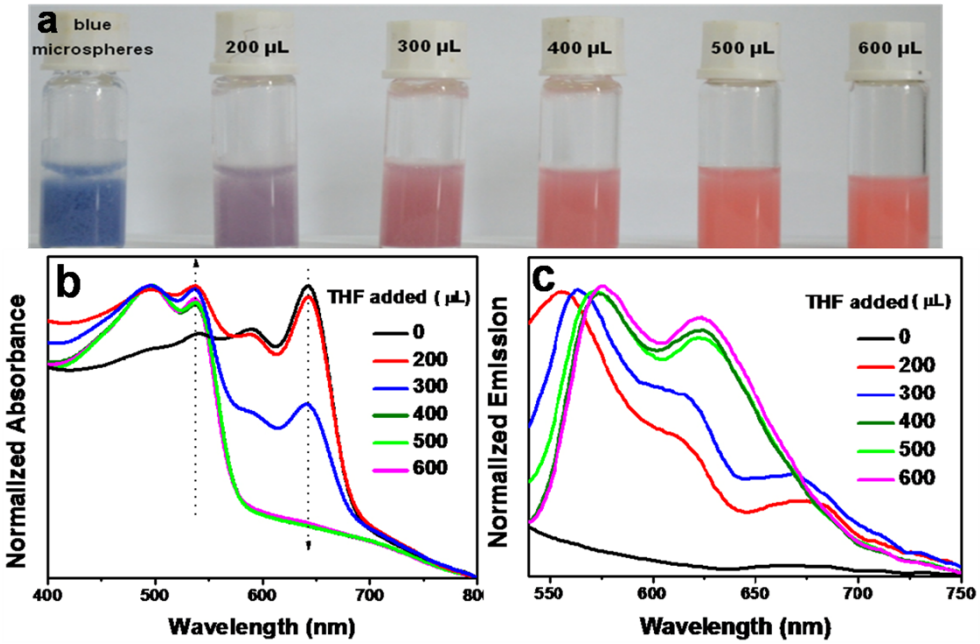

Fig. S8 Photographs (a), absorption (b) and the emission (c) spectra (excited at 500 nm) of blue PDA vesicles and vesicles treated with 200, 300, 400, 500 and 600 μL of THF, respectively.

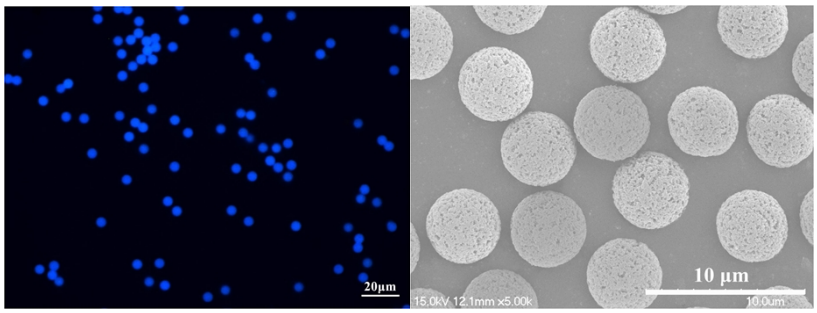

Fig. S9 Fluorescence microscope image (left) and SEM image (right) of red APGMA-PDA microspheres obtained by reacting different mass of APGMA microspheres with 3 mL of red PDA vesicles.

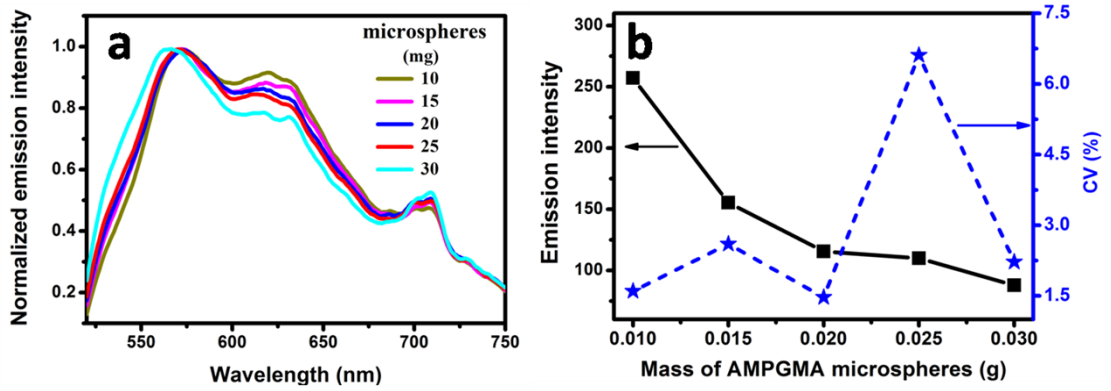

Fig. S10 Normalized emission spectrum (a), emission intensity and CVs (b) of red APGMA-PDA microspheres obtained by reacting different mass of APGMA microspheres with 3 mL of red PDA vesicles.

Reference

1. J. Olmsted III and M. Strand, *J. Phys. Chem.*, 1983, **87**, 4790-4792.