## **Supporting Information**

## pH-responsive dual fluorescent core-shell microspheres fabricated *via* a one-step emulsion polymerization

Bin Bao, Fengyu Li,\* Heng Li, Linfeng Chen, Changqing Ye, Jinming Zhou, Jingxia Wang, Yanlin Song\* and Lei Jiang



**Fig. S1** Fluorescence image of aqueous and organic phases with two fluorescent dyes. The bottom layer is FRA in ammonium bicarbonate aqueous solution (water phase) and the upper layer is styrene monomer containing PER (oil phase). In the emulsion polymerization process water in oil system would form and the hydrophobic PER would be embedded into the PS core and hydrophilic FRA would be anchored onto the PMMA-PAA shell of the as-prepared microspheres.



**Fig. S2** (a), (c) and (e) SEM images and (b), (d) TEM images of the microspheres prepared with acceptor-donor ratios as (a) and (b) 4.64, (c) and (d) 10.21, (e) 13.78, respectively. The monodispersity of the microspheres became worse with the increase of FRA which would influence the nucleation of the microspheres during the emulsion polymerization.



**Fig. S3** Normalized absorption and emission spectra of PER and FRA. The absorption and emission spectra were measured using singular fluorescent microspheres PER@PSMA and FRA@PSMA. FRA@PSMA is in buffer solution at pH 9.0. At 365 nm, PER have a moderate absorption but the absorption of FRA is small, indicating PER can be excited at 365 nm whereas FRA can't be excited at this wavelength. There is a considerable overlap between the absorption spectrum of FRA and emission spectrum of PER, which is one of the prerequisites for FRET.



**Fig. S4** Fluorescence spectra of FRA@PSMA singular fluorescent microspheres under the excitation of (a) 470 nm and (b) 365 nm at different pH levels. The microspheres can be excited at 470 nm in alkaline environment but can hardly be excited at 365 nm regardless of the pH level of the solution.



**Fig. S5** Fluorescence spectra and images of the microsphere films at different pH values. The microsphere latexes were spin-coated into a thin film. The film was dipped into aqueous solutions with different pH values and subsequently observed by fluorescence spectrophotometer and fluorescent microscopy.



Fig. S6 Confocal images of fluorescent microspheres embedded in polyacrylamide hydrogel films. The hydrogel films are at pH (a), (b) 6.0 and (c), (d) 9.0. (a) and (c) are blue channel images collected at wavelength of 400-500 nm. (b) and (d) are green channel images collected at wavelength of 500-600 nm. The solid content of fluorescent microspheres in the dry hydrogel is  $2 \times 10^{-2}$  wt%. The scale bars are 20  $\mu$ m.