Supporting Information

A novel AIE-active dye for fluorescent nanoparticles by one-pot combination of Hantzsch reaction and RAFT polymerization: synthesis, molecular structure and application in cell imaging

Yali Chen,[a,b] Zengfang Huang,*[a,b] Xiaobo Liu,[b] Liucheng Mao,[c] Jinying Yuan,[c] Xiaoyong Zhang,[d] Lei Tao,[c] and Yen Wei,[c]

[a]School of Materials & Food Engineering，Zhongshan Institute, University of Electronic Science & Technology of China，Zhongshan, 528402, P. R. China. hzf105@163.com

[b]School of Materials and Energy, University of Electronic Science & Technology of China，Chengdu, 610054. P. R. China.

[c]Department of Chemistry, the Tsinghua Center for Frontier Polymer Research, Tsinghua University, Beijing 100084, P. R. China.

[d] Department of Chemistry, Nanchang University, Nanchang 330047, P. R. China.

Experimental Section

The experiment exploring the kinetics process of Hantzsch reaction and RAFT polymerization was shown as described below: TPDA (0.113 g, 0.200 mmol), PEGMA (0.475 g, 1.00 mmol), AEMA (42.8 mg, 0.200 mmol), ammonium acetate (23.2 mg, 0.300 mmol), glycine (2.20 mg, 0.0300 mmol), 5,5-dimethyl-1,3-cyclohexadione (28.0 mg, 0.200mmol), AIBN (2.00 mg, 0.0122 mmol), CTA (3.20 mg, 0.012 mmol), trimethylbenzene (0.200 g) and a mixture of acetonitrile (2.00 mL) and tetrahydrofuran (2.00 mL) were added to a Schlenk tube which was deoxygenated five times in a vacuum-nitrogen cycle after being frozen by liquid nitrogen. The Schlenk tube was continuously reacted for 36 h in an oil bath at 70°C with magnetic stirring. During the polymerization, samples were taken at different reaction times (0, 0.5, 1, 2, 4, 7, 14, 24, 36 h) for ^{1}H NMR analysis.

Kinetics of RAFT polymerization

The conversion of TPDA and PEGMA monomers were monitored by intensity change of the peak at 6.14 ppm assigned to PEGMA and AEMA together with the

peak at 10.08 ppm assigned to TPDA with the peak at 6.80 ppm of trimethylbenzene as an internal standard. From the ^{1}H NMR spectra of **Fig. S1 (A)**, it was observed that the relative intensity of the peaks at 6.14 ppm and 10.08 ppm gradually reduced, indicating that both TPDA, PEGMA and AEMA were successfully incorporated into the polymer as expected. The monomers reached a very high conversion rate after 36 hours of polymerization. The kinetic study presented a linear pseudo-first-order kinetic curve versus time as shown in **Fig. S1 (B)**. During the first 7 hours of the polymerization, the reaction rate of Hantzsch reaction was higher than that of RAFT polymerization, resulting in a gradual increase in the molar fraction of TPDA in the polymer. As the polymerization progressed, the conversion of TPDA and PEGMA remained almost the same, and the molar fraction of TPDA in the polymers gradually decreased, because the concentration of TPDA in the reaction mixture was less and less, as compared with that of PEGMA.

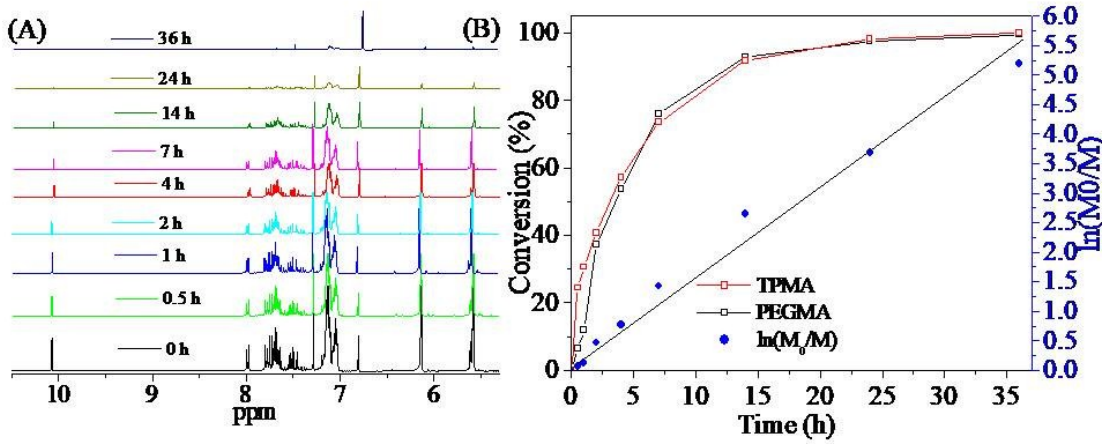

Fig. S1. Kinetics study of RAFT polymerization and Hantzsch reaction: (A) ^{1}H NMR spectra ($CDCl_3$) of reaction mixture at various reaction time; (B) conversion and the kinetic curve of TPDA and PEGMA vs reaction time.

Fluorescence emission properties of TPDA

The fluorescence intensity of TPDA is significantly enhanced with distinct red shift of emission wavelength compared with the TPB dye. As shown in **Fig. S2**, the emission wavelength of TPDA increases to 509 nm with a marked increase in fluorescence intensity, while the emission wavelength of TPB dye was 470 nm under the same condition, which indicates that TPDA is more advantageous for

biological imaging.

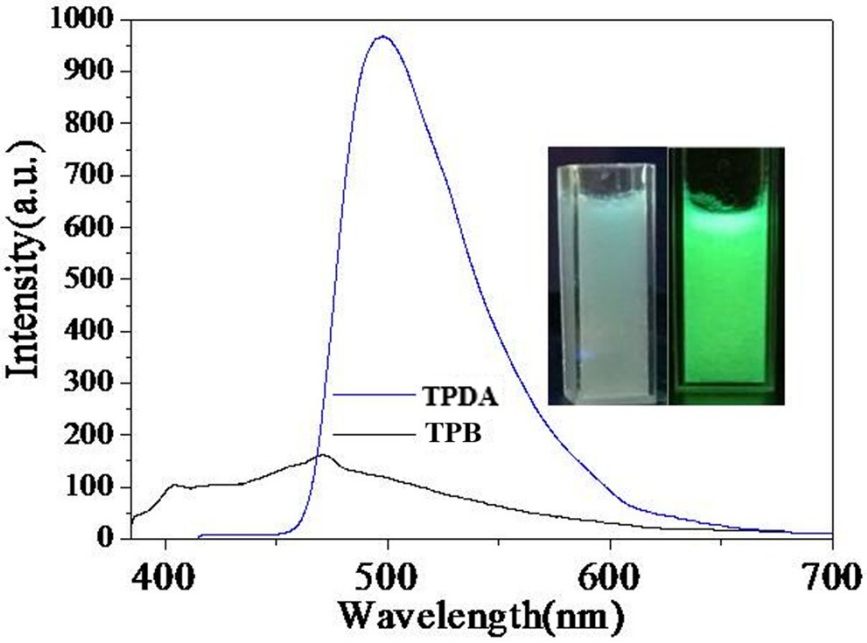

Fig. S2. Fluorescence emission difference of TPDA and TPB at same temperature and concentration.

Biocompatibility evaluations of PEG-TPD

To explore the possibility of biomedical applications of PEG-TPD2 FPNs, their biocompatibility was evaluated. As shown in **Fig. S3**, it can be clearly seen from the optical microscope images that all treated cells remained normal morphology. Even though the concentrations of PEG-TPD2 FPNs in the cell culture increased from a lower concentration of 20 μg mL^{-1} to a higher 80 μg mL^{-1}, there was no significant change in cell morphology.

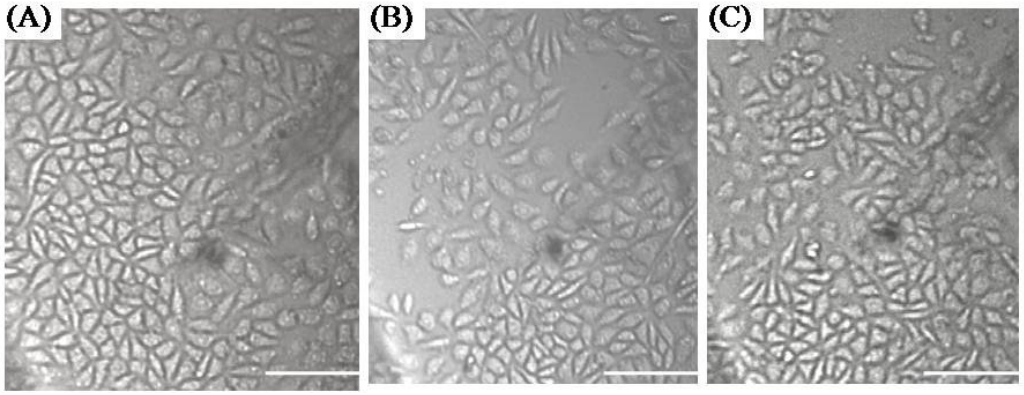

Fig. S3. Biocompatibility evaluations of PEG-TPD2 FPNs. (A-C) Optical microscopy images of L929 cells culturedwith different concentrations of PEG-TPD2 FPNs for 24

h: (A) control cells, (B) L929 cells cultured with 20 μg mL $^{-1}$ PEG-TPD2 FPNs, (C) L929 cells cultured with 80 μg mL $^{-1}$ PEG-TPD2 FPNs. (Scale bar =100 μm)