Supporting Information

Fluorescent protein-reactive polymers via one-pot combination of the Ugi reaction and RAFT polymerization

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Experimental Section

1. Materials

Triethylamine (Aladdin, 99.7%), cyclohexyl isocyanide (J&K, 98%), dansyl chloride (J&K, 98%), 3-((methylamino)methyl)aniline (J&K, 98%), 2-(4-hydroxyphenylazo) benzoic acid (HABA, J&K, 98%), avidin (Sigma Aldrich, 98%), bovine serum albumin (BSA, Sangon Biotech, 97%), NuPAGE® Novex® 4-12% Bis-Tris Protein Gels, 1 mm, 10 well, NuPAGE® MOPS SDS Running Buffer (20 ×), NuPAGE® LDS Sample Buffer (4 ×), NuPAGE® Sample Reducing Agent (10 ×) were used as purchased. 2,2'-Azobis(2-methylpropionitrile) (AIBN, J&K, 99.9%) was recrystallized from acetone twice prior to use.

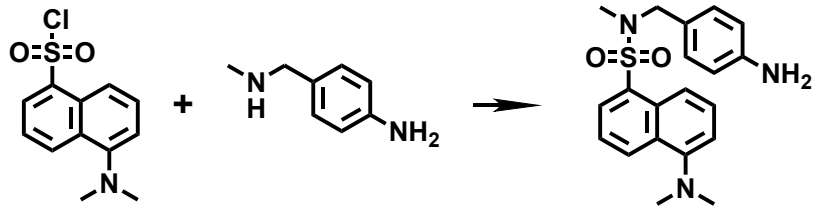
6-(4-Formylphenoxy)hexyl 4-cyano-4-(((ethylthio)carbonothioyl)thio)pentanoate[1], 3-(pyridin-2-yldisulfanyl)propanoic acid[2], N-(2-hydroxypropyl) methacrylamide (HPMA)[3] were synthesized as previous literatures.

2. Instrumental Analysis

Gel permeation chromatography (GPC) analyses of polymers were performed using N,N-dimethyl formamide (DMF) containing 50 mM LiBr as the eluent. The GPC system was a Shimadzu LC-20AD pump system consisting of an auto injector, a MZ-Gel SDplus 10.0 μm guard column (50 × 8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size column (50 – 10^6 Å, linear), a Shimadzu RID-10A refractive index detector and a Shimadzu SPD-10A UV detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}. The *dn/dc* and $M_{n,\ LLS}$ of the model polymers were collected through a Wyatt DAWN HELEOS-II detector (658 nm, 100 mW). ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-ECA400 (400M Hz) spectrometer for all samples. The ESI-MS data were collected using a Micro TOF-QII Bruker. The FT-IR spectra were made in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). UV-Visible absorption spectra were recorded on UV/Vis/NIR Perkin-Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz cuvettes of 1 cm path length. The fluorescence measurements were obtained on a Perkin-Elmer LS-55 spectrometer equipped with quartz cuvettes of 1 cm path length. The HPLC analyses were performed using Reverse phase high performance liquid chromatography (RP-HPLC) which was two Shimadzu LC-6AD pump systems consisting of an auto injector, an Agilent Zorbax 300SB-C18 column, a Shimadzu SPD-M20A diode array detector. The mobile phases were phase A (99.9% H_2O, 0.1% TFA) and phase B (99.9% acetonitrile, 0.1% TFA) respectively. The gradient of the mobile phase is 30% - 70% phase B (30 min) (BSA) and 20% - 50% phase B (30 min) (avidin). Matrix-assisted laser desorption ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded on an AXIMA-PerformanceMA in a linear mode.

3. Methods

3.1. Synthesis of N-(4-aminobenzyl)-5-(dimethylamino)-N-methylnaphthalene-1-sulfonamide:



Under nitrogen, dansyl chloride (5.05 g, 18.72 mmol) was placed in a dry 250 mL round bottom flask equipped with a stir bar. Dry dichloromethane (50 mL) was added via syringe, the system was cooled to 0°C. Then, 4-((methylamino)methyl)aniline (2.80 g, 20.59 mmol), triethylamine (2.27 g, 22.46 mmol), and 75 mL of dry dichloromethane were dissolved, and the solution was added dropwise with stirring over 30 min. The solution was stirred for an additional 30 min at 0 °C, then kept at 25°C for 14 hours. After removing the precipitation by filtration and the solvent by rotavapor, the product was isolated using flash chromatography with ethyl acetate/petroleum ether (1/4) to get a pale yellow solid (5.67 g, 82%).

^{1}H NMR (400 MHz, DMSO-d_6) : δ 8.51 (d, J = 8.5 Hz, 1H, C<u>H</u>CCN$(CH_3)_2$), 8.29 (d, J = 8.6 Hz, 1H, C<u>H</u>CHCHCN$(CH_3)_2$), 8.11 (d, J = 7.3 Hz, 1H, C<u>H</u>CHCHCCN$(CH_3)_2$), 7.64 (dt, J = 11.5, 8.1 Hz, 2H, C<u>H</u>CHCCN$(CH_3)_2$), 7.28 (d, J = 7.6 Hz, 1H, C<u>H</u>CN$(CH_3)_2$), 6.89 (d, J = 8.0 Hz, 2H, Ph), 6.48 (d, J = 8.0 Hz, 2H, Ph), 5.09 (s, 2H, N<u>H</u>$_2$), 4.15 (s, 2H, SNC<u>H</u>$_2$), 2.84 (s, 6H, N(C<u>H</u>$_3)_2$), 2.61 (s, 3H, SNC<u>H</u>$_3$).

^{13}C NMR (100 MHz, DMSO-d_6) : δ 151.42, 148.36, 134.55, 129.83, 129.55, 129.39, 129.27, 128.80, 128.10, 123.73, 122.28, 119.11, 115.26, 113.72, 52.53, 45.70, 45.08, 33.31.

FT-IR cm^{-1}: 3460, 3376, 2941, 1571, 1517, 1454, 1312, 913, 789, 740.

[M+Na^{+}]：392.1403 (theoretical value: 392.1402).

3.2. HABA test:

HABA titration: The number of active sites on avidin surface was tested by titration using 2-(4-Hydroxyphenylazo) benzoic acid (HABA) which binds to avidin at the same position as biotin with much weaker affinity (Kd = 10^{-6} M).

Briefly, HABA solution (1 mL, H_2O, 0.0354 mg/mL) and avidin solution (1 mL, H_2O, 0.1 mg/mL) were freshly prepared. Then the HABA solution (5 µL, molar ratio to the avidin: 0.5) was added in the avidin solution, and the mixture was scanned from 400 nm to 600 nm by a UV spectrometer. The procedure was repeated until the absorbance did not increase (Figure S5).

Competitive binding experiment: HABA was dissolved (0.1 mL, H_2O, 1 mg mL-1, UVmax = 350 nm), and mixed with avidin solution (1 mL, H_2O, 1 mg mL-1). The colourless avidin solution immediately changed to red, and the solution was used for the UV analyses. After adding the Biotin-Flu-poly(HPMA) solution (0.2 mL, H_2O, 10 mg/mL) into the avidin-HABA complex solution, the red color (avidin-HABA) faded instantly, and the solution was used for the UV analyses.

Supporting data:

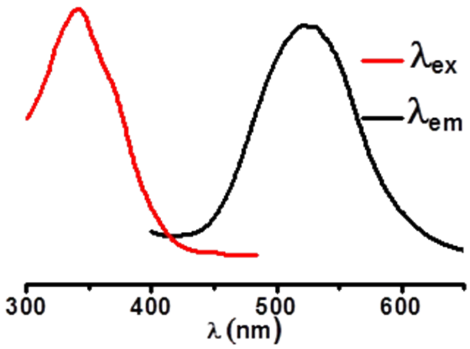


Figure S1. Excitation wavelength (340 nm) and emission wavelength (520 nm) of the PDS-Flu-poly(HPMA).

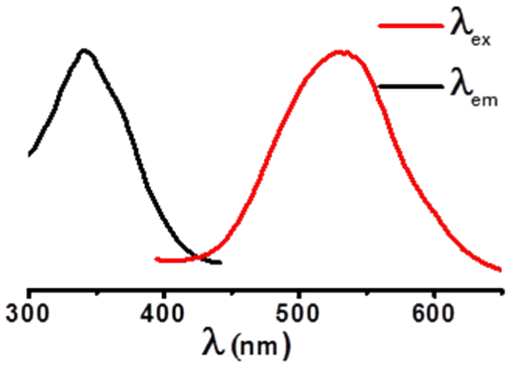


Figure S2. Excitation wavelength (350 nm) and emission wavelength (530 nm) of the Biotin-Flu-poly(HPMA).

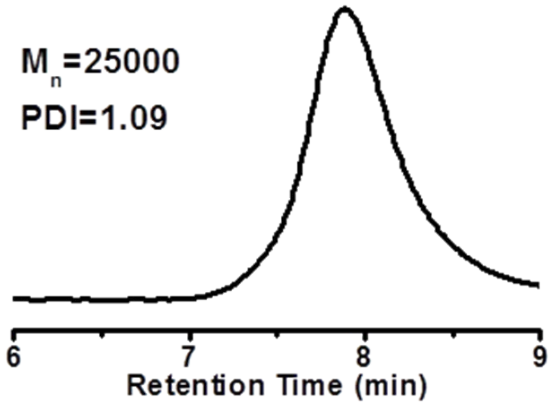


Figure S3. The GPC curve of the PDS-Flu-poly(HPMA).

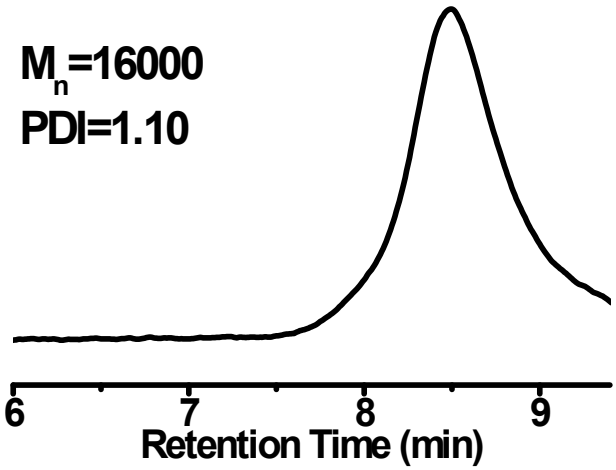


Figure S4. The GPC curve of the Biotin-Flu-poly(HPMA).

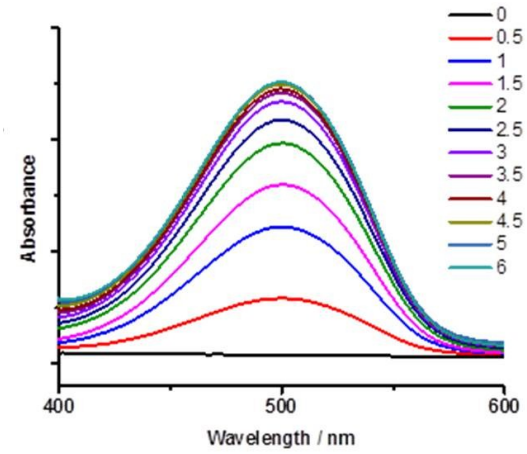


Figure S5. The reaction sites of the avidin tested by HABA titration.

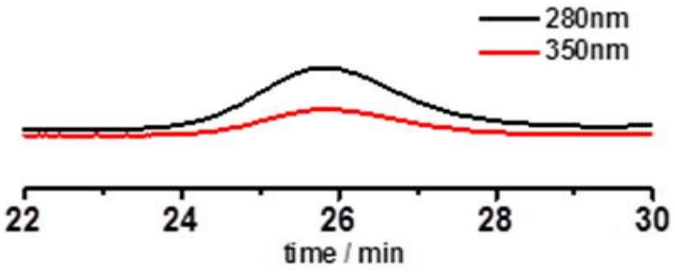


Figure S6. The Re-HPLC curve of the Biotin-Flu-poly(HPMA).

References:

1. X. Ren, Y. Zhao, B. Yang, X. Wang, Y. Wei and L. Tao, *RSC Adv.*, 2015, **5**, 54133-54137.
2. Y. Hattori, Y. Nagaoka, M. Kubo, H. Yamasaku, Y. Ishii, H. Okita, H. Nakano, S. Uesato and Y. Maitani, *Chem. Pharm. Bull.*, 2011, **59**, 1386-1392.
3. M. F. Ebbesen, D. H. Schaffert, M. L. Crowley, D. Oupický and K. A. Howard, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 5091-5099.