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Supporting information for

Stimuli-Responsive Single-Chain Polymeric Nanoparticles Towards Development of Efficient Drug Delivery Systems

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Experimental Section

Materials

Uracil-diamidopyridine-functionalized poly[oligo(ethylene glycol) methyl methacrylate (POEGMA-U-DPy) was prepared by a combination of atom transfer radical polymerization (ATRP) and an alkyne-azide click reaction as shown in Scheme S1. Synthetic procedures for POEGMA-U-DPy have been described in detail in our previous report.[4g] All chemicals including catalysts, reagents and organic solvents used for the chemical synthesis of the POEGMA-U-DPy

were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification unless otherwise noted. The human embryonic kidney (HEK) 293 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Characterizations

Ultraviolet-visible (UV–Vis) spectroscopy. UV–Vis spectra were obtained using a Hewlett Packard Model 8453 diode array spectrophotometer. (Waldbronn, Germany). *Dynamic light scattering (DLS).* The particle size distribution and average particle size of the aqueous samples were measured using a Nano Brook 90 Plus PALS (Brookhaven Instruments Corp., Holtsville, NY, USA). Calibration of the DLS instrument was conducted with standard latex microspheres ranging in diameter from 60 nm to 500 nm. *Transmission electron microscopy (TEM).* TEM was carried out on a Tecnai 12 electron microscope (FEI, Phillips, Eindhoven, the Netherlands) operated at 20-120 k. For TEM analysis, all aqueous samples were prepared by placing drops of solutions onto a carbon-coated copper grid and drying under ambient conditions overnight, then the samples were stained with ruthenium tetroxide vapor to enhance electron contrast.

Preparation of fluorouracil (FU)-loaded POEGMA-U-DPy SCNPs

FU (2 mg) was dissolved in water (6 ml) and slowly dropped into aqueous POEGMA-U-DPy solution (1 mg/ml) at 15 °C while stirring. The mixture was stirred for 6 h at 15 °C, subsequently transferred into a dialysis membrane (molecular weight cut-off = 6000-8000 Da) and then dialyzed for 24 h against 2 L of PBS pH 7.0 at 4 °C to remove free FU. The FU-loading content of POEGMA-U-DPy was determined by UV–Vis spectroscopy at 268 nm, then lyophilized to

obtain the total weight of FU-loaded SCNPs. The FU-loading content was calculated directly using the following formula:

$$\text{FU-loading content (wt\%)} = W_{FU}/ W_{total} \times 100\%$$

where W_{FU} is the weight of FU loaded into SCNPs and W_{total}, the weight of the FU-loaded SCNPs after dialysis and lyophilization.

UV-Vis assessment of lower critical solution temperature (LCST)

The thermo-responsive properties of POEGMA and POEGMA-U-DPy in aqueous solution or PBS buffer (1 mg/ml) were monitored by examining transmittance in the UV–Vis spectra at 500 nm over the temperature range of 40-80 °C at a heating rate of 0.5 °C/min. The LCST values of the sample solutions were determined as the temperature at which transmittance decreased to 50% of the original value.

Kinetic stability studies of FU-loaded POEGMA-U-DPy SCNPs

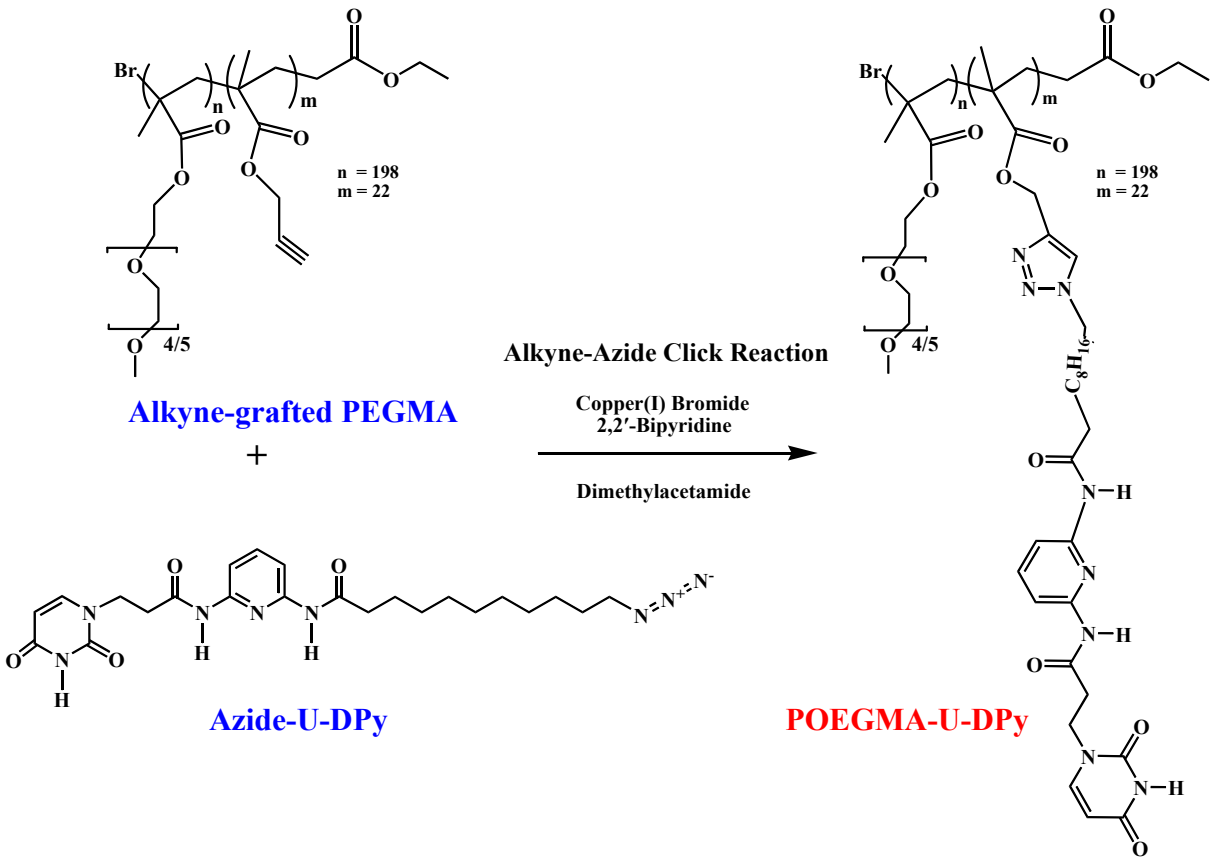
The long-term kinetic stability of FU-loaded SCNPs upon dilution in PBS buffer in the presence of the strong micellar destabilizing agent sodium dodecyl sulfate (SDS) was evaluated by DLS. These kinetic experiments were performed as previously described.[3b,d]

Cell culture

HEK 293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Mediatech, Fairfax, VA, USA) and maintained at 37 °C in a 5% CO_2 humidified atmosphere.

In vitro FU release assay

FU release studies using a dialysis technique were performed in PBS buffer at different temperatures and pH values. The amount of FU released was monitored by UV–Vis spectroscopy measurements at a wavelength of 268 nm. The resulting absorbance values were determined by comparison to a calibration curve generated from free UF dissolved in the same buffer. The release rate and percentage of FU at different environmental conditions were calculated and plotted versus time after release, as shown in Fig. 4a.



Scheme S1 Synthetic procedures for POEGMA-U-DPy.[4g]

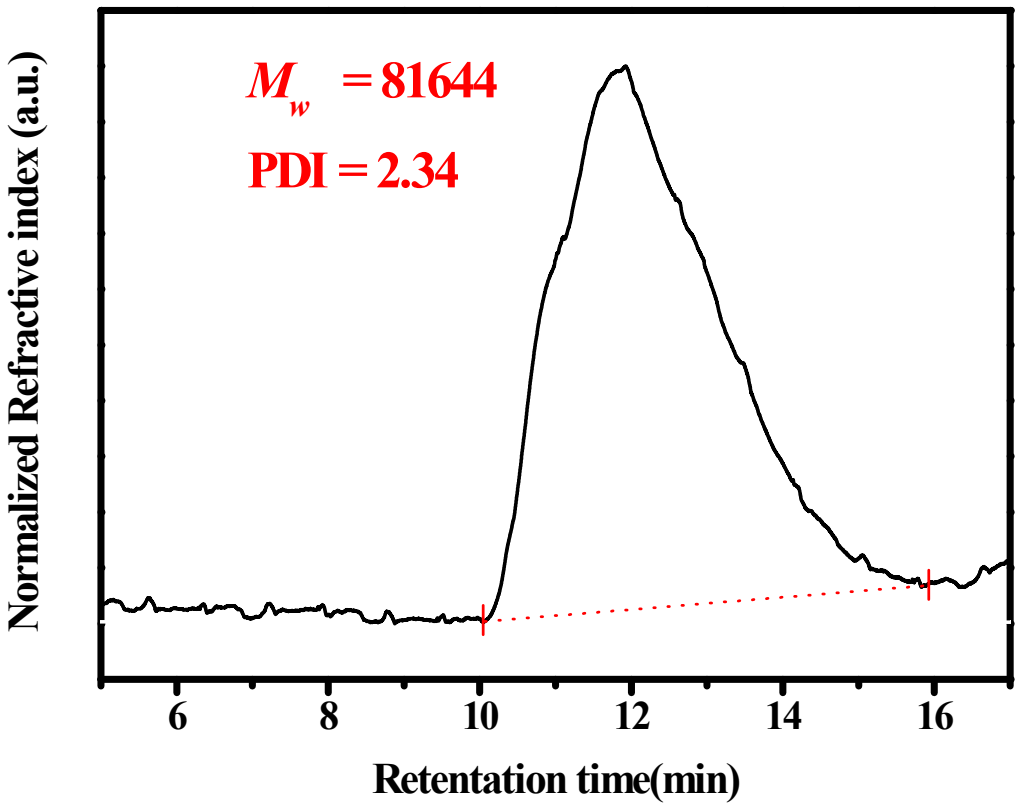


Fig. S1 SEC trace of POEGMA-U-DPy in tetrahydrofuran at 40 °C.

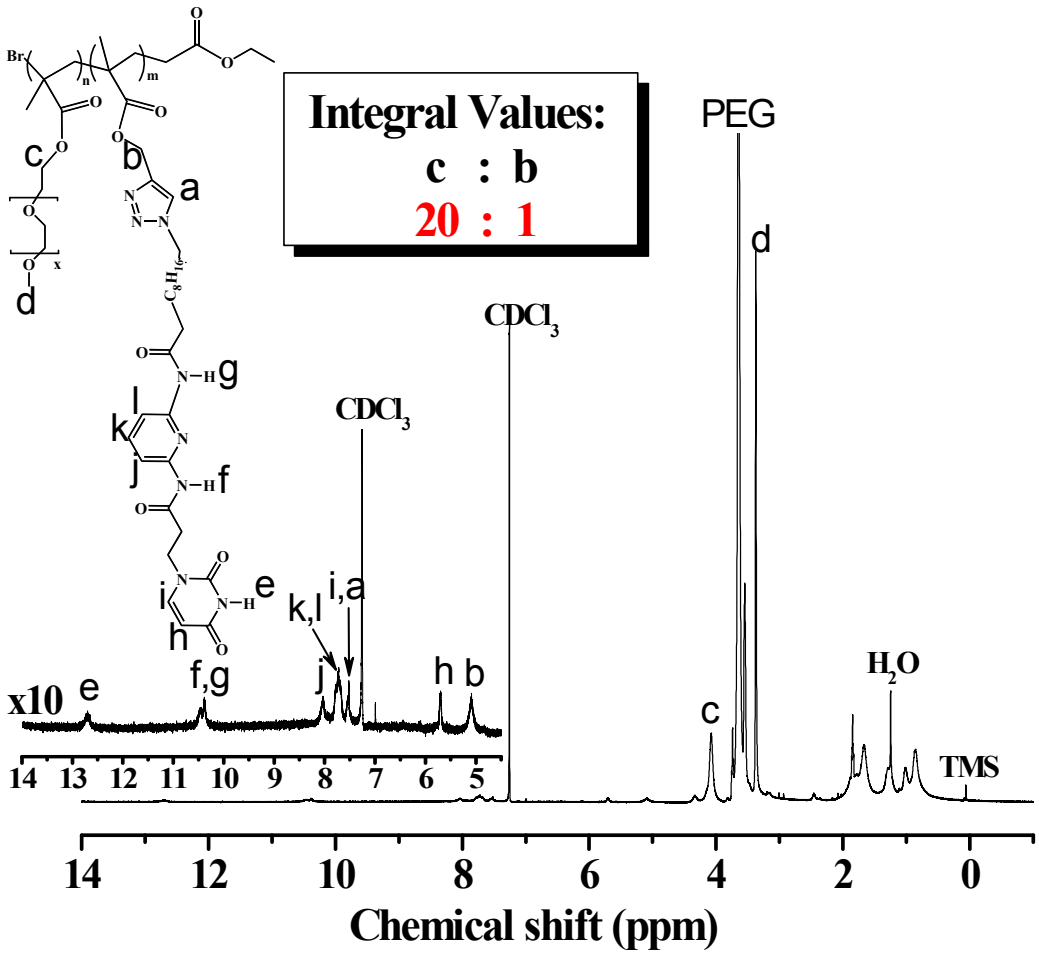


Fig. S2 ^{1}H-NMR spectrum of POEGMA-U-DPy in deuterated chloroform ($CDCl_3$)

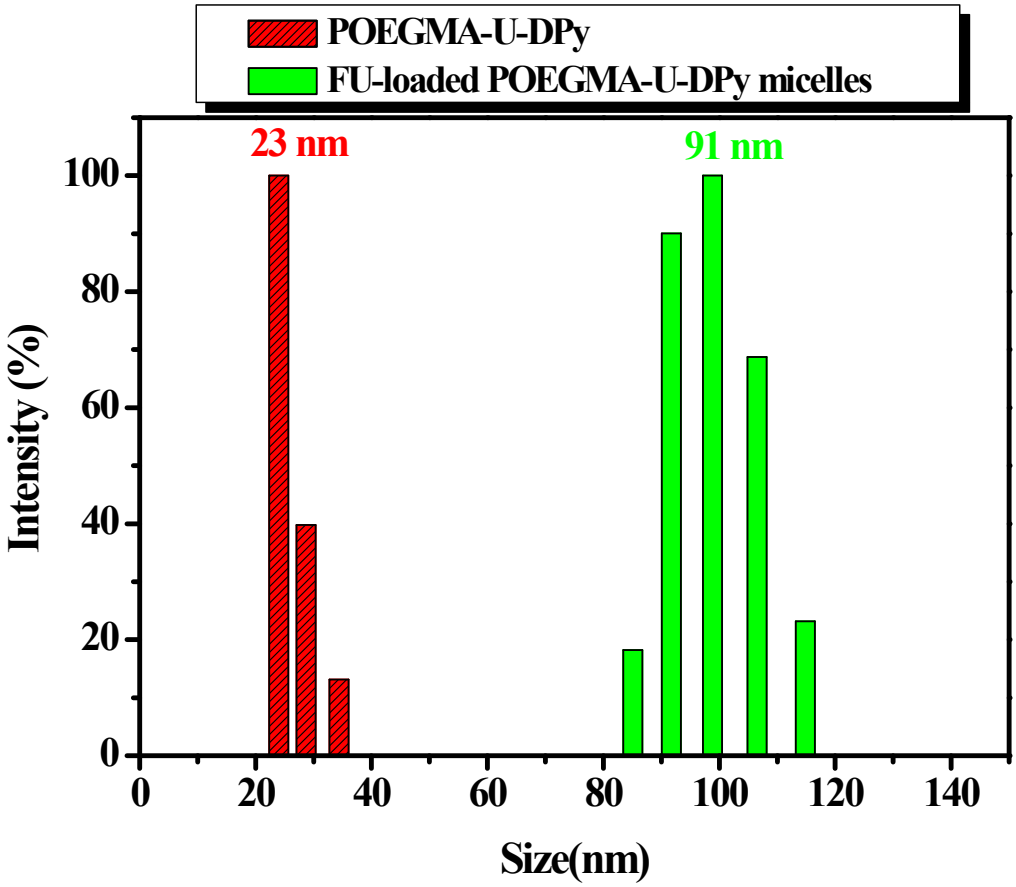


Fig. S3 DLS analyses of blank and FU-loaded POEGMA-U-DPy SCNPs in PBS buffer.