Electronic Supplementary Information (ESI)

Biomolecularly stimuli-responsive tetra-poly(ethylene glycol) that undergoes sol–gel transition in response to a target biomolecule

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^{1}H-NMR measurements

Succinimidyl Tetra-PEG, amine-PEG_2-biotin, and biotinylated Tetra-PEG were dissolved in $CDCl_3$, and then ^{1}H NMR measurements were carried out with a JEOL JNM-AL 400 spectrometer using tetramethylsilane as the internal standard. Their ^{1}H-NMR spectra were assigned as follows:

(a) Succinimidyl Tetra-PEG

^{1}H-NMR (400MHz, $CDCl_3$) δ (ppm from TMS): 4.2(t, 8H, $CH_2C\underline{H_2}OC{=}O$), 3.796-3.291 (m, 4165H, $C\underline{H_2}C\underline{H_2}$-O-), 2.814 (br, 12H, $C\underline{H_2}(C{=}O)NO$), 2.696-2.454(q, t, 16H, $O(C{=}O)C\underline{H_2}$), 2.069 (m, 8H, $CH_2C\underline{H_2}CH_2$).

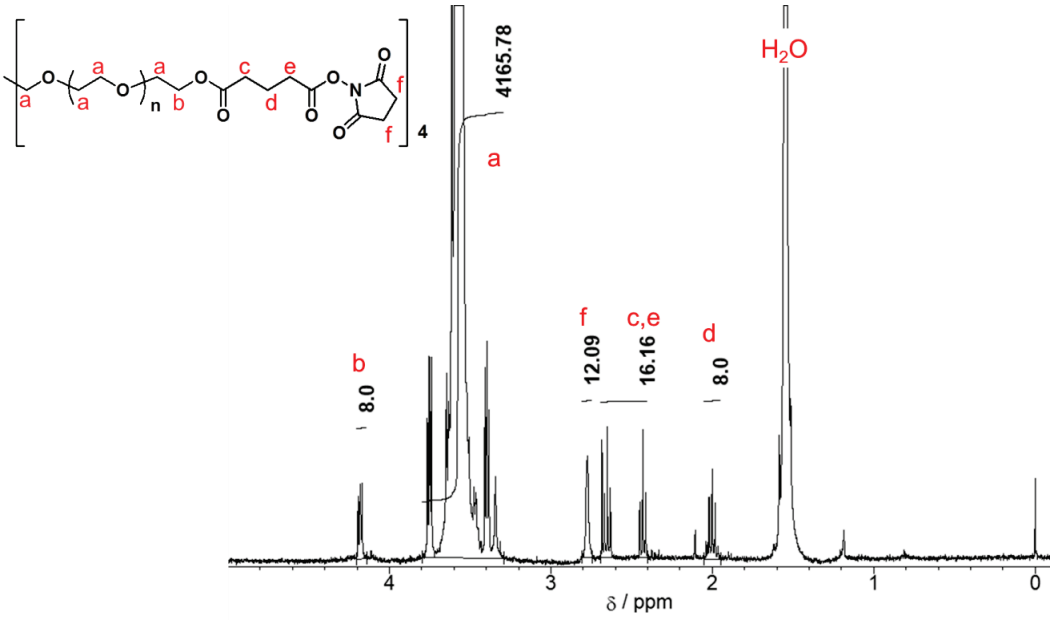

Figure S1. ^{1}H NMR spectrum of succinimidyl Tetra-PEG.

(b) Amine-PEG_2-biotin

^{1}H-NMR (400MHz, $CDCl_3$) δ(ppm from TMS): 4.56 (q, 1H, C=ONH-C$\underline{H}$CH of biotin), 4.37 (q, 1H, C=ONH-C$\underline{H}$CH of biotin),3.66 (m, 8H, $C\underline{H_2}C\underline{H_2}O$), 3.48 (m, 2H, $CH_2C\underline{H_2}NHC{=}O$), 3.198 (m, 1H, $CH_2C\underline{H}$-S), 2.97 (m, 2H -$CH_2C\underline{H_2}NH_2$), 2.77-2.43 (d, 1.67H, -S$C\underline{H_2}$CH), 1.83-1.36 (m, 6H, C=O$C\underline{H_2}C\underline{H_2}C\underline{H_2}$CH).

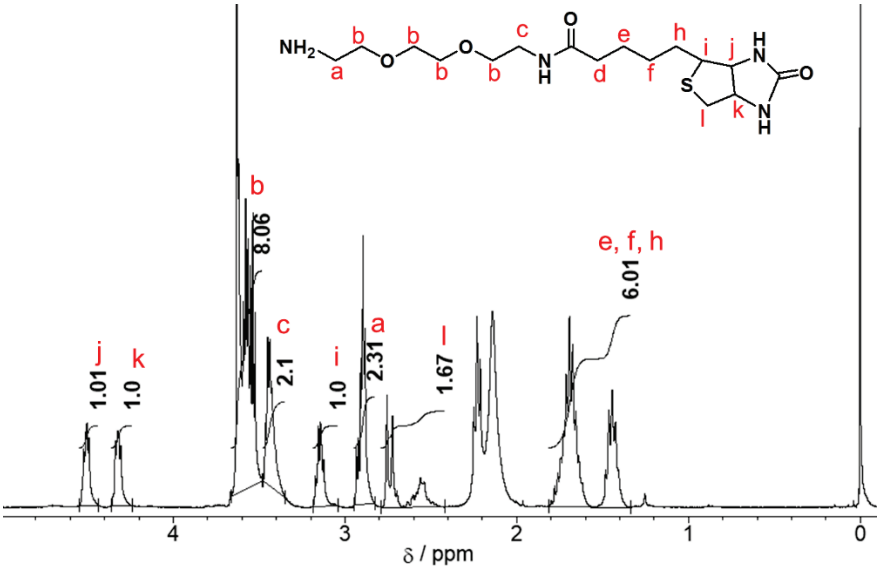

Figure S2. ^{1}H NMR spectrum of amine-PEG$_2$-biotin.

(c) Biotinylated Tetra-PEG.

^{1}H-NMR (400MHz, $CDCl_3$) δ(ppm from TMS): 4.53 (q, 3.7H, C=ONH-C<u>H</u>CH of biotin), 4.35 (q, 3.6H, C=ONH-C<u>H</u>CH of biotin), 4.26 (t, 8H, $CH_2C\underline{H}_2OC{=}O$), 3.94-3.35 (m, 4752H, $C\underline{H}_2C\underline{H}_2$-O-, $C{=}ONHC\underline{H}_2C\underline{H}_2OC\underline{H}_2$, -$C\underline{H}_2$NHC=O-), 3.20 (m, 3.6H, $CH_2C\underline{H}$-S), 2.97 (q, 4H, NH-CH_2-), 2.79 (d, 3.7H, -S$C\underline{H}_2$CH), 2.45 (m, 24H, $OC{=}OC\underline{H}_2$), 1.35 (m, 18H, $C{=}OC\underline{H}_2C\underline{H}_2C\underline{H}_2CH$).

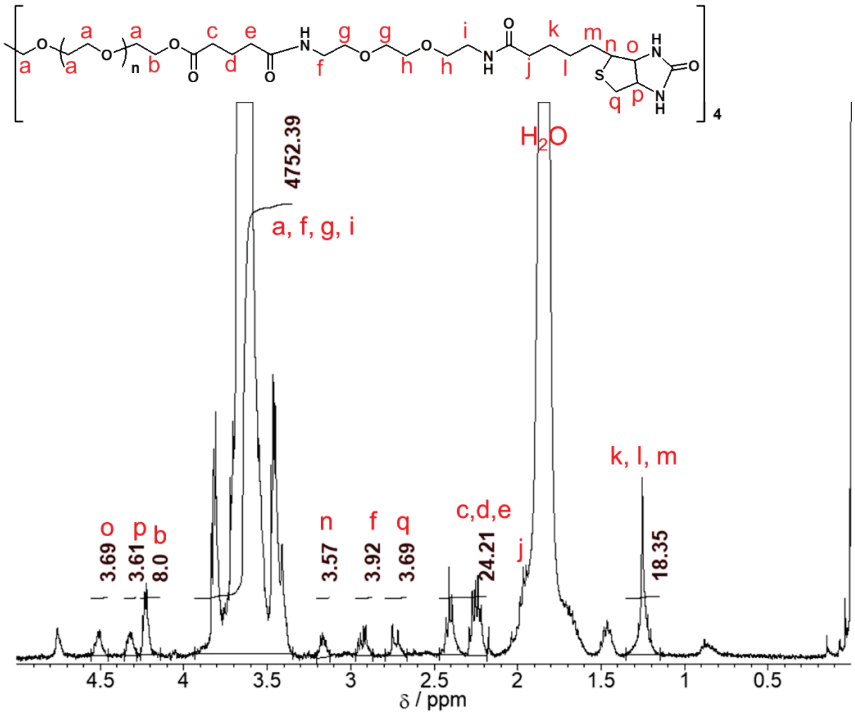

Figure S3. ^{1}H NMR spectrum of biotinylated Tetra-PEG.

Gel permeation chromatography (GPC)

The molecular weights of succinimidyl Tetra-PEG and biotinylated Tetra-PEG were determined using GPC (TOSOH, 8020). Their polymers were dissolved in a phosphate buffer solution with a polymer concentration of 1mg/mL (pH7.4, 20mM). Operating temperature was 30 °C. Columns were TSK-GEL G5000PW$_{XL}$ No H3322 and No G3362. The GPC eluent was a phosphate buffer solution (pH7.4, 20mM) at a flow rate of 0.5 mL min^{-1}. Calibration was conducted using a series of linear PEG standards (Mw=982–87,900). The GPC measurements revealed that succinimidyl Tetra-PEG and biotinylated Tetra-PEG had Mw=42,448 and 48,627, respectively, which almost agreed with the molecular weight described by NOF.

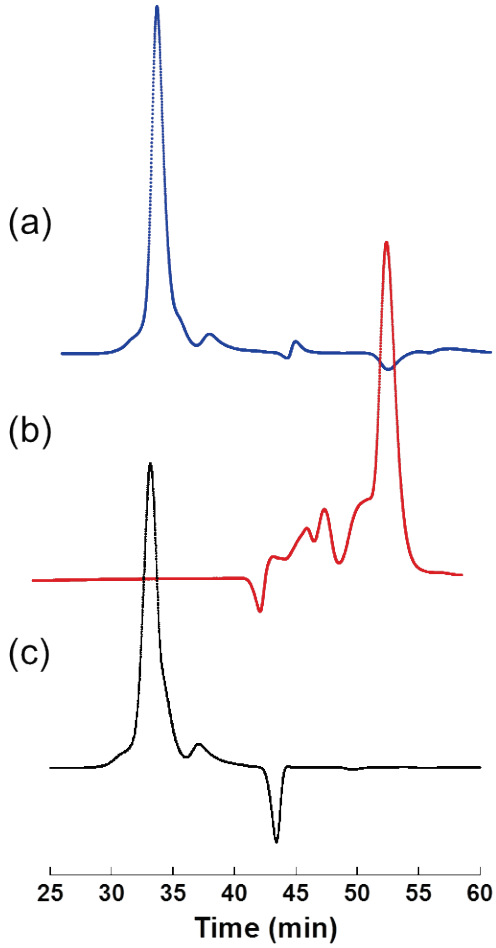

Figure S4. GPC traces of (a) succinimidyl Tetra-PEG, (b) amine-PEG$_2$-biotin, and (c) biotinylated Tetra-PEG. GPC condition

Fourier transform infrared (FT-IR) spectroscopy.

The chemical structures of succinimidyl Tetra-PEG, amine-PEG_2-biotin and biotinylated Tetra-PEG were examined by the KBr method with a Fourier transform infrared spectrophotometer (FT-IR, Perkin Elmer, Waltham, MA, USA). All the spectra represent an average of 32 scans taken in the wavenumber range of 4000-600 cm^{-1}. The FT-IR spectra supported the successful synthesis of biotinylated Tetra-PEG, which was confirmed by ^1H NMR spectra.

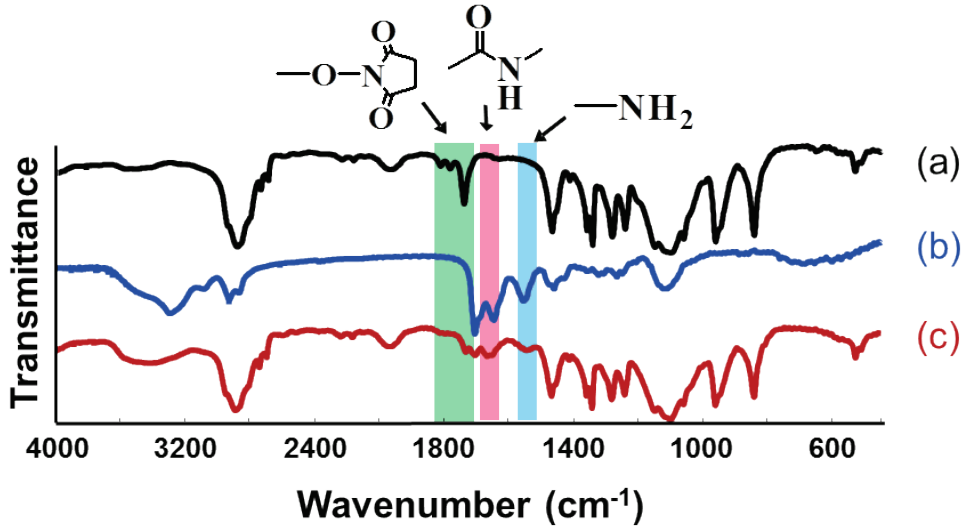

Figure S5. FT-IR spectra of (a) succinimidyl Tetra-PEG, (b) amine-PEG_2-biotin, and (c) biotinylated Tetra-PEG.