Supporting Information

Ashley et al. 10.1073/pnas.1215498110

SI Text

Multiarm PEGs were purchased from NOF America Corp. or JenKem Technology. Dibenzocyclooctyne (DBCO) and bicyclononyne (BCN) reagents were from Click Chemistry Tools and SynAffix, respectively. DBCO–*N*-hydroxysuccinimide (NHS) ester was purified before use by silica gel chromatography using 1% isopropanol in 1:1 EtOAc/hexane. Solutions of DBCO were quantitated spectrophotometrically using $\varepsilon_{308} = 13{,}800\ M^{-1}{\cdot}cm^{-1}$, and azide concentrations were determined from the decrease in absorbance at 308 nm upon reaction of with ∼2.5-fold excess (500 μM) of DBCO acid using $\Delta\varepsilon_{308} = 11{,}800\ M^{-1}{\cdot}cm^{-1}$ and a 2-mm path-length cuvette. Dialyses were performed using SpectraPor 2 membranes (12- to 14-kDa cutoff). Azido linker–*N*-hydroxysuccinimido carbonates (HSC), azido-linker–carbamoyl-aminoacetamido fluorescein and trinitrobenzene sulfonate (TNBS) assays to quantify amines have been described (1). Two-component analyses of solubilized drug and erosion probe were determined as described in *Two-Component Analysis* using Eq. **S12**.

BODIPY Azide. A 100-mM solution of 11-azido-3,6,9-trioxaundecan-1-amine in acetonitrile (ACN) (13 μL, 1.3 μmol) was added to a 12.8-mM solution of BODIPY TMR-X SE (Invitrogen) in DMSO (100 μL, 1.28 μmol). After 30 min at ambient temperature, the mixture was diluted to 2 mL with 0.1 M KP_i, pH 7.4, and loaded on a 500-mg C18 BondElut extraction column (Varian). The column was washed successively with 5-mL portions of water and 20% ACN/water, then eluted with 50% ACN/water and concentrated to dryness. The residue was dissolved in 1.0 mL of ACN, and the concentration (1.0 mM) was determined using $\varepsilon_{546\ nm} = 60{,}000\ M^{-1}{\cdot}cm^{-1}$.

Dinitrophenyl-Azide. A solution of 1-fluoro-2,4-dinitrobenzene (94.5 mg, 0.51 mmol, 1.2 eq) in THF (2 mL) was added to 11-azido-3,6,9-trioxaundecan-1-amine (92.3 mg, 0.42 mmol, 1 eq) followed by Et_3N (0.18 mL, 131 mg, 1.3 mmol, 3 eq). The resulting mixture was kept at room temperature for 100 min, then concentrated under reduced pressure to 1 mL and dissolved in EtOAc (10 mL). The solution was washed with 0.1 N HCl (10 mL), water (10 mL), then brine (5 mL), and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography using 2:1 hexanes:EtOAc, then 1:1 hexanes:EtOAc as the mobile phase. The fractions containing product were concentrated to dryness under reduced pressure to give dinitrophenyl (DNP)-azide (123 mg, 0.32 mmol, 76% yield) as a yellow oil. HPLC (reverse-phase C18, 20–100% acetonitrile with 0.1% TFA linear gradient elution, retention volume (RV) = 8.1 mL).

Preparation of End Group-Modified Multivalent PEGs. ***PEG_{20kDa}(linker-azide)$_4$.*** In a typical example, a solution of 25 μmol of the azido linker–HSC [modulator (Mod) = $MeSO_2-$] in 1 mL of ACN was added to 5 μmol (100 mg) of 20-kDa four-arm PEG-amine·HCl in 1 mL of water and 40 μL of 1.0 M $NaHCO_3$ (40 μmol). After 1 h at ambient temperature, TNBS assay indicated <2% of the amino groups remained, and the solution was dialyzed against 1 L of 50% methanol followed by 1 L of methanol. After evaporation, the residue (109 mg) was dissolved in 2.12 mL of sterile-filtered 10-mM NaOAc, pH 5.0, and stored frozen at −20 °C. The azide concentration determined by reaction with DBCO acid was 9.5 mM.

PEG_{20kDa}(DBCO)$_4$. A 60-mM solution of DBCO-NHS in acetonitrile (0.5 mL, 30 μmol, 1.5 eq) was added to a solution of 20-kDa four-arm PEG-amine·HCl (100 mg, 5 μmol), and diisopropylethylamine (0.010 mL, 57 μmol) in acetonitrile (1 mL). After stirring 2 h at ambient temperature, TNBS assay indicated ≤2% of free-amine groups remaining, and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in 50% aqueous methanol (4 mL) and dialyzed against 50% aqueous methanol followed by methanol. After evaporation, the residue (100 mg) was dissolved in water to give a 50 mg/mL stock (10 mM DBCO by spectrophotometric assay), which was stored frozen at −20 °C.

PEG_{40kDa}(DBCO)$_8$. One milliliter of 40-mM solution (40 μmol) of DBCO-NHS in THF was added to a solution of 168 mg (4.2 μmol) of 40-kDa eight-arm PEG-amine·HCl (tripentaerythritol core; JenKem Technology) and 12.9 μL diisopropylethylamine (74 μmol) in 0.6 mL of ACN, and the mixture was kept at ambient temperature overnight. TNBS assay of a sample (4 μL) showed <0.4% free amine remaining (lower limit of detection = 0.30 μmol amine). The reaction mixture was dialyzed against 2 L of 50% methanol followed by 1 L of methanol. After evaporation, the residue (149 mg) was dissolved in 1.49 mL water and stored frozen at −20 °C. The DBCO concentration determined spectrophotometrically was 16 mM.

PEG_{40kDa}(BCN)$_8$. A solution of 200 mg of 40-kDa eight-arm PEG-amine·HCl (JenKem Technology; 40 μmol NH_2), 20 mg of BCN p-nitrophenyl carbonate (63 μmol), and 20 μL of *N*,*N*-diisopropylethylamine (115 μmol) in 2 mL of DMF was stirred 16 h at ambient temperature. After quenching with 0.5 mL of 100 mM taurine in 0.1 M KP_i, pH 7.5, for 1 h, the mixture was dialyzed sequentially against water, 1:1 methanol/water, and methanol. After evaporation, the residue was dissolved in 2 mL of THF and precipitated with 10 mL of methyl *tert*-butyl ether. The product, containing <2% free amine by TNBS assay, was collected and dried (190 mg).

Hydrogel Drug Release and Degradation. ***Gel casting, drug release, and gel dissolution.*** To form gels, components were mixed by vortexing, quickly pipetted into 64-μL (9 × 1 mm) circular rubber-perfusion chambers (Grace Bio-Labs) mounted on a silanized glass microscope slide, and allowed to cure overnight. Gel discs were suspended in 2–6 mL of an appropriate buffer [PBS or Hepes (pH 7.4 or pH 7.8), BICINE (pH 8.1, 8.4, or 8.7), Na borate (pH 9.0 or 9.3)] in 4- or 20-mL screw-cap air-tight vials and maintained at 37 °C in a water bath. A 1-mL aliquot was periodically removed and analyzed for protein (280 nm), fluorescein (493 nm), DNP (360 nm), or BODIPY (546 nm) directly or as described in Eq. **S12** before returning the sample to the vessel.

Protein diffusion in encapsulating gels. Stock solutions of ∼90 OD_{280}/mL myoglobin (17.7 kDa), carbonic anhydrase (29.0 kDa), and BSA (66.4 kDa) were prepared in 0.1 M KP_i, pH 7.4. PEG hydrogels (4% wt/vol PEG) were prepared by adding 100 mg/mL $PEG_{20kDa}(L\text{-}N_3)_4$ (50 μL) to a mixture of 100 mg/mL $PEG_{20kDa}(DBCO)_4$ (50 μL), protein stock (50 μL), and 10× PBS (100 μL). Cast gels were suspended in 2 mL of 0.1 M KP_i, pH 7.4, at 37 °C, and OD_{280} in the solution was periodically measured. The $t_{1/2}$ values for release into solution were ∼20 min for myoglobin, 24 min for carbonic anhydrase, and 150 min for BSA.

Gel degradation with β-eliminative cross-links. A 50-mg/mL solution of $PEG_{20kDa}(DBCO)_4$ (250 μL, 2.50 μmol DBCO end-groups) in water was mixed with 25 μL of a 10-mM solution of the azide linker-aminoacetylfluorescein (AAF) (0.25 μmol azide) erosion probe in DMF and kept 30 min at ambient temperature. The 50-μL aliquots (0.42 μmol DBCO) were mixed with 28 μL of 10 mM NaOAc, pH 5.0, followed by 45 μL of 50 mg/mL $PEG_{20kDa}(L2\text{-}Mod\text{-}N_3)_4$ (0.42 μmol azide). Cast gels were suspended in 2 mL

of 0.1 M KP_i, pH 7.4, at 37 °C, and the OD_{493} of the supernatant was periodically measured to monitor fluorescein solubilization.

Hydroxide-catalyzed hydrogel degradation. Hydrogels were made as above and suspended in 6 mL of 0.1 M Hepes (pH 7.8), 0.1 M BICINE (pH 8.1, pH 8.4, pH 8.7), or 0.1 M Borate (pH 9.0) at 37 °C, and the OD_{493} of the supernatant was periodically measured to monitor fluorescein solubilization (Fig. S1).

SEC-HPLC analysis of degrading gel. A gel disk prepared using $PEG_{20kDa}(DBCO)_4$ labeled with ~0.1 mol fluorescein as an erosion probe and $PEG_{20kDa}(L2\text{-}PhSO_2\text{-}N_3)_4$ was suspended in 5 mL of 0.1 M sodium borate, pH 9.3, and kept at 37 °C. Aliquots (50 μL) were periodically injected on a SEC column (Phenomenex Bio-Sep SEC-S2000 7.8 × 300 mm), eluted with 50:50:0.1 ACN:water/CF_3CO_2H, and monitored by fluorescence detection (440 nm excitation/521 nm emission wavelength). At early stages, the released gel fragments were primarily 20-kDa monomers, but the proportion of 40-kDa dimers and larger fragments increased dramatically at t_{RGEL}.

Drug release from β-eliminative linkers. A solution (99.6 μL) containing 50 μL of 100 mg/mL $PEG_{40kDa}(DBCO)_8$ (1.0 μmol DBCO end-groups) in water was mixed with 6.2 μL of 12.5 mM of azide-Mod-AAF (0.078 μmol) in 1:1 DMF:acetonitrile with various modulators, 15 μL of 1.0 mM BODIPY-azide (0.015 μmol) in ACN as an erosion probe, 20 μL of 20 mM O-(2-azidoethyl) heptaethylene glycol (0.40 μmol) in water, and 8.4 μL water. After 10 min at ambient temperature, the solution containing 0.5 μmol uncommitted DBCO groups was mixed with 50 μL of a 50-mg/mL solution of $PEG_{20kDa}(L2\text{-}Mod\text{-}N_3)_4$ (0.5 μmol azide groups) in 10 mM NaOAc, pH 5.0. Duplicate cast gels were suspended in 2 mL of 0.1 M Hepes, pH 7.4, at 37 °C, and OD_{493} for fluorescein and OD_{546} for BODIPY in the solution were periodically measured. The t_{RGEL}, as determined by complete solubilization of the BODIPY erosion probe, was 630 ± 39 (SD) h (n = 8). Solubilization of fluorescein followed the first-order rate law $[F]_t/F_{tot} = \exp(-k_{obsd}t)$ and gave apparent k_{obsd} ± SE for best fits of the total released fluorescein of 0.021 ± 0.00014 h^{-1} for Mod = $ClPhSO_2-$, 0.011 ± 0.00031 h^{-1} for Mod = $PhSO_2-$, 0.0053 ± 0.00022 h^{-1} for Mod = $MeOPhSO_2-$, and 0.0033 ± 0.00012 h^{-1} for Mod = $MeSO_2-$. The rate data were converted to plots for the fluorescein released directly from the gel (Fig. S2) using Eq. **S6** (below).

Hydroxide-catalyzed drug release. A solution (333 μL) containing 208 μL of 80 mg/mL $PEG_{40kDa}(DBCO)_8$ (3.33 μmol DBCO end-groups) in water was mixed with 20 μL of 12.5 mM of azide-$ClPhSO_2$-AAF (0.25 μmol) in 1:1 DMF:acetonitrile, 25 μL of 10 mM DNP-azide (0.25 μmol) in DMSO, 58.3 μL of 20 mM *O*-(2-azidoethyl)heptaethylene glycol (1.17 μmol) in water, and 21.7 μL water. After 15 min at ambient temperature, the solution containing 1.66 μmol uncommitted DBCO groups was mixed with 167 μL of a 50 mg/mL solution of $PEG_{20kDa}(L2\text{-}MeSO_2\text{-}N_3)_4$ (1.7 μmol azide groups) in 10 mM NaOAc, pH 5.0. Cast gels were suspended in 4 mL of 0.1 M Hepes (pH 7.8), 0.1 M BICINE (pH 8.1, pH 8.4, pH 8.7), or 0.1 M Borate (pH 9.0) at 37 °C, and OD_{493} for AAF and OD_{360} for DNP in the solution was periodically measured. Fig. S3 shows the best fit of data to a first-order rate expression.

Degradation of gels with varying cross-linking density. A mixture of 100 mg/mL $PEG_{40kDa}(BCN)_8$ [20 mM BCN end-groups] in water was combined with appropriate amounts of 50 mg/mL $PEG_{20kDa}(L2\text{-}ClPhSO_2\text{-}N_3)_4$ (10 mM azide) to prepare 4% wt/vol PEG hydrogels having cross-link densities (Xld) of 4.0, 4.4, 4.8, 5.1, or 5.3 average cross-links per node (Table S1). Non–cross-linked end-groups (non-Xl end-groups) were labeled with an erosion probe by including 0.0015 μL of 10 mM 5-(6-azidohexyloxy)-carbonylamino) acetamido-fluorescein in DMF and the remainder capped with stoichiometric amounts of 50 mM *O*-azidoethyl-heptaethylene glycol in water before addition of $PEG_{20kDa}(L2\text{-}ClPhSO_2\text{-}N_3)_4$. Cast gels were placed in 1 mL of 0.1 M borate (pH 9.2), 37 °C, and the supernatant monitored at 493 nm.

Gels dissolved at pH 9.2 with t_{RGEL}, as indicated as Table S1, with t_{RGEL} at pH 7.4 calculated as ${t_{RGEL}}^{7.4} = {t_{RGEL}}^{9.2} \times 10^{(9.2-7.4)}$; the calculated t_{RGEL} of the 4 Xld gel at pH 7.4 (39 h) is in reasonable accord with the experimental value determined at pH 7.4 for the 4 × 4 gel of the same Xld in Fig. 3*C* (31 h). As expected (2), t_{RGEL} increased with increasing cross-link density. Xld is defined as the average number of cross-links per node.

Derivation and Use of Equations. ***Modeling of drug release and gel erosion.*** Drug release and gel degradation occurs as follows, with the final products being the free drug and gel monomers:

$$\text{Gel} - (\text{Drug})_n \rightarrow \text{Drug} + \text{EP - gel fragment - Drug} \rightarrow \text{Drug} + \text{EP - monomers}$$

The drug or drug surrogate released into solution may emanate directly from L1 cleavage from the gel, or from solubilized fragments that arise from gel erosion via cleavages of L2. To distinguish the drug released from the intact gel vs. solubilized gel fragments, it is necessary to determine the distribution of drug-bearing nodes between the intact gel and solution at time t. In the present study, we used a modification of a reported approach to monitor and model gel degradation (2). The appearance of an erosion probe (EP) permanently attached to nodes of the gel allows calculation of the fraction of nodes in solution as $EP(t)/EP_\infty$; the concentration of drug originally present on these solubilized nodes, $D_s(t)$, is thus given by Eq. **S1:**

$$D_s(t) = D_\infty \times EP(t)/EP_\infty \;\; \text{or} (D_\infty/EP_\infty) \times EP(t). \qquad \textbf{[S1]}$$

The drug released from the intact gel at time t, $D_g(t)$, is the difference between the total drug released, $D(t)$ and the drug either contained in or released from solubilized gel fragments $D_s(t)$, as in Eq. **S2**.

$$D_g(t) = D(t) - D_s(t) = D(t) - (D_\infty/EP_\infty) \times EP(t) \qquad \textbf{[S2]}$$

Calculation of the first-order rate of drug release from intact gel nodes is not straightforward from measuring $D(t)$ because of the changing quantity of gel from erosion, but can be calculated based on the fraction of drug remaining on intact gel. Based on released erosion probe $EP(t)$, the fraction of gel remaining is $1 - EP(t)/EP_\infty$. The amount of drug originally carried by this amount of gel is thus given by $D_\infty \times (1 - EP(t)/EP_\infty)$. Because the drug remaining on the intact gel is $D_\infty - D(t)$, the fraction of drug remaining on intact gel, $D_{f,gel}(t)$ is given as Eqs. **S3** and **S4**.

$$D_{f,gel}(t) = [D_\infty - D(t)]/[D_\infty \times (1 - EP(t)/EP_\infty)] \qquad \textbf{[S3]}$$

$$= [1 - D(t)/D_\infty]/[1 - EP(t)/EP_\infty] \qquad \textbf{[S4]}$$

For a first-order release of drug from the gel, $D_{f,gel}(t)$ will show an exponential decay having a rate constant k_{L1} that describes the rate of drug release from the intact gel (Eq. **S5**). Merging Eqs. **S4** and **S5** provides **S6**, which can be used to experimentally estimate the rate of drug release directly from the intact gel.

$$D_{f,gel}(t) = e^{-k_{L1}t} \qquad \textbf{[S5]}$$

$$D_{f,gel}(t) = [1 - D(t)/D_\infty]/[1 - EP(t)/EP_\infty] = e^{-k_{L1}t} \qquad \textbf{[S6]}$$

The amount of drug directly released from the intact gel over time depends on the rate of release, k_{L1}, together with the erosion rate of the gel. If the solubilization of the erosion probe can be approximated by a first-order process between times t = 0 and

t_1 with rate k_{sol}, the quantity of drug released from the gel during that time can be approximated as Eq. **S7**.

$$D_g(t_1) = D_\infty \times (k_{L1}/k_{sol}) \times \left[1 - e^{-k_{sol}t_1}\right] \quad \textbf{[S7]}$$

If the drug remaining on the intact gel is negligible at time t_1, then the total fraction of drug directly released from the gel is given in Eq. **S8**:

$$D_g(t_1)/D_\infty = k_{L1}/k_{sol} = t_{1/2,sol}/t_{1/2,L1}. \quad \textbf{[S8]}$$

Two-component analysis. The total absorbance, A_{tot}, λ, of a mixture of two components satisfying Beer's Law at each of two wavelengths, λ_1 and λ_2, is determined by the concentrations c_1 and c_2 and extinction coefficients ε_1 and ε_2. The concentrations c_1 and c_2 can be determined by solving the simultaneous linear equations for

$$A_{tot,\lambda} = c_1 \times \varepsilon_{1,\lambda} + c_2 \times \varepsilon_{2,\lambda}, \text{ where } \lambda \text{ is either } \lambda_1 \text{ or } \lambda_2, \quad \textbf{[S9]}$$

which gives Eq. **S10**:

$$c_1 = \left(\varepsilon_{1,\lambda 2} \times A_{tot,\lambda 1} - \varepsilon_{2,\lambda 1} \times A_{tot,\lambda 2}\right) / \left(\varepsilon_{1,\lambda 1} \times \varepsilon_{2,\lambda 2} - \varepsilon_{1,\lambda 2} \times \varepsilon_{2,\lambda 1}\right). \quad \textbf{[S10]}$$

To determine the contribution to absorbance of component 1 in the mixture at λ1, defined as $A_{1,\lambda 1,mix} = \varepsilon_{1,\lambda 1} \times c_1$, Eq. **S10** can be simplified. We use absorbance ratios $A_{1,\lambda 2}/A_{1,\lambda 1} = \varepsilon_{1,\lambda 2}/\varepsilon_{1,\lambda 1}$ and $A_{2,\lambda 1}/A_{2,\lambda 2} = \varepsilon_{2,\lambda 1}/\varepsilon_{2,\lambda 2}$, where A_1 and A_2 are the absorbance values of pure components 1 and 2, respectively, at the indicated wavelength λ1 or λ2. Substitutions convert Eq. **S10** to **S11**.

$$A_{1,\lambda 1,mix} = \left(A_{tot,\lambda 1} - \left(A_{2,\lambda 1}/A_{2,\lambda 2}\right) \times \left(A_{tot,\lambda 2}\right)\right) / \left(1 - \left(A_{1,\lambda 2}/A_{1,\lambda 1}\right) \times \left(A_{2,\lambda 1}/A_{2,\lambda 2}\right)\right) \quad \textbf{[S11]}$$

When component 1 has negligible absorbance at λ_2, this becomes

$$A_{1,\lambda 1,mix} = A_{tot,\lambda 1} - \left(A_{2,\lambda 1}/A_{2,\lambda 2}\right) \times A_{tot,\lambda 2}. \quad \textbf{[S12]}$$

Relevant $A_{2,\lambda 1}/A_{2,\lambda 2}$ ratios for BODIPY were $A_{492nm}/A_{546nm} = 0.482$, and $A_{280\ nm}/A_{546nm} = 0.473$.

Exenatide–PEG Hydrogel. ***Preparation of exenatide–hydrogel.*** Exenatide linked at the α-terminus to an azide linker having a $MeSO_2$–modulator was synthesized by solid-phase peptide synthesis at AnaSpec as previously described (1). Azide-linker($MeSO_2$)–exenatide (1.2 mg, 270 nmol) in 30 μL of 1.0 M phosphate, pH 7.8, and eight-arm $PEG_{40kDa}(BCN)_8$ (5 mg; 50 μL, 1,000 nmol BCN end-groups) in 50 μL of water was kept for 1 h at ambient temperature, then 20 μL of a 1-mM BODIPY-azide (20 nmol) in ACN and four-arm PEG_{20kDa}(linker(CN)-azide)$_4$ (3.55 mg; 710-nmol end-groups) in 71 μL water was added. The gels were allowed to cure overnight, and then stored in 1 mL of PBS, pH 7.4, at 4 °C. Note that this gel was designed to have an average of 4.7 cross-links per node.

Exenatide release from and degradation of PEG hydrogel. A gel disk was placed in 1.0 mL of 0.1 M borate buffer, pH 8.8, and kept at 37 °C. Solubilization of exenatide by release and/or gel erosion was monitored at 280 nm and gel erosion at 546 nm by periodic sampling of the supernatant. Direct release from the gel was calculated as solubilization adjusted for gel erosion as described in *Modeling of Drug Release and Gel Erosion*. Exenatide solubilization was a first-order process with $t_{1/2}$ = 20.7 h at pH 8.8, which, because the reaction is first-order in hydroxide ion, corresponds to a $t_{1/2}$ of 520 h (21.7 d) at pH 7.4; a $t_{1/2}$ of 23.6 h at pH 8.8, corresponding to 593 h (24.7 d) at pH 7.4 was calculated for the drug directly released from the gel, which accounted for ∼88% of the total exenatide. Reverse gelation was observed at 172 h at pH 8.8, corresponding to t_{RGEL} 180 d at pH 7.4.

1. Santi DV, Schneider EL, Reid R, Robinson L, Ashley GW (2012) Predictable and tunable half-life extension of therapeutic agents by controlled chemical release from macromolecular conjugates. *Proc Natl Acad Sci USA* 109(16):6211–6216.

2. DuBose JW, Cutshall C, Metters AT (2005) Controlled release of tethered molecules via engineered hydrogel degradation: Model development and validation. *J Biomed Mater Res A* 74(1):104–116.

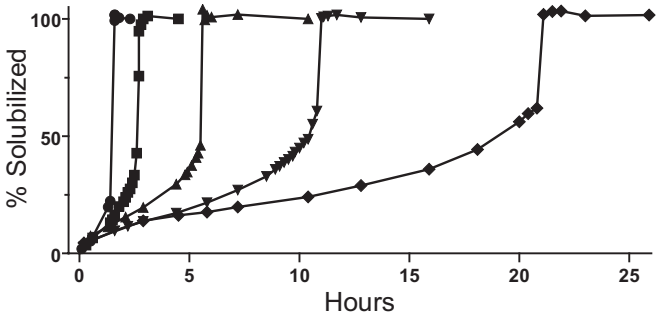

Fig. S1. Plots of degradation for 4 × 4 gels with Mod = $ClPhSO_2$– at various pH values and 37 °C. t_{RGEL} were 20.9 h at pH 7.8 (–◆–), 10.9 h at pH 8.1 (–▼–), 5.59 h at pH 8.4 (–▲–), 2.75 h at pH 8.7 (–■–), and 1.49 h at pH 9.0 (–●–). Assuming t_{RGEL} reflects a pseudo–first-order process at a given pH (i.e., linker cleavage), the reaction was first-order in hydroxide ion with k_{OH} = 5.06 ± 0.30 (SD) × 10^4 $M^{-1}{\cdot}h^{-1}$.

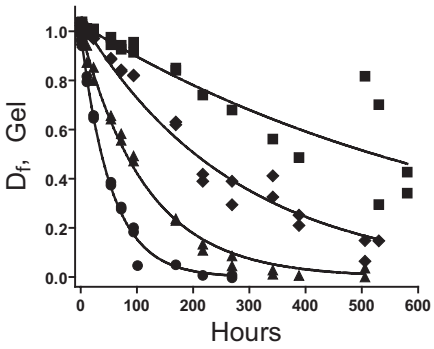

Fig. S2. First-order plot of estimated AAF remaining on intact gel from experimental data and Eq. **S6**. The k_{obsd} ± SE for the best fits are 0.019 ± 0.00063 h^{-1} for Mod = $ClPhSO_2$– (–●–), 0.0087 ± 0.00030 h^{-1} for Mod = $PhSO_2$– (–▲–), 0.0036 ± 0.00016 for Mod = $MeOPhSO_2$– (–◆–), and 0.0014 ± 0.00015 h^{-1} for Mod = $MeSO_2$– (–■–).

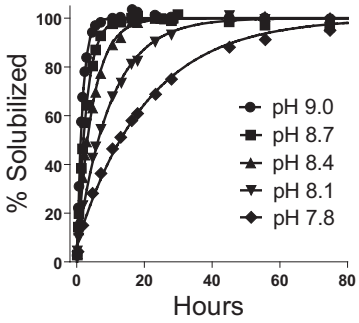

Fig. S3. First-order plots of AAF release from a 4 × 8 gel with Mod = $ClPhSO_2$– at various pH values and 37 °C. The k_{obsd} values were 0.050 h^{-1} at pH 7.8 (–◆–), 0.10 h^{-1} at pH 8.1 (–▼–), 0.22 h^{-1} at pH 8.4 (–▲–), 0.36 h^{-1} at pH 8.7 (–■–), and 0.63 h^{-1} at pH 9.0 (–●–). Reactions were first-order in hydroxide ion with k_{OH} = 7.6×10^4 ± 0.90 (SD) × 10^4 $M^{-1}{\cdot}h^{-1}$.

Table S1. t_{RGEL} of gels with varying cross-linking densities

M_a, mM*	M_b, mM†	Total EG, mM‡	Non-Xl EG, mM§	Xld¶	$t_{RGEL}^{pH9.2}$	$t_{RGEL}^{pH7.4}$
0.67	0.67	8.04	2.7	4.0	0.62	39
0.62	0.77	8.04	1.9	4.4	0.77	49
0.57	0.86	8.00	1.1	4.8	0.83	52
0.53	0.93	7.96	0.52	5.1	0.88	56
0.51	0.99	8.04	0.12	5.3	0.97	61

EG, end group; Xl, cross-linking; Xld, cross-linking density.
*Concentration of monomer a (eight-arm PEG-CO).
†Concentration of monomer b (four-arm azide PEG).
‡Total end-group concentration determined as [(M_a × $arms_a$) + (M_b × $arms_b$)].
§Non–cross-linked end-group concentration determined as [(M_a × $arms_a$) – (M_b × $arms_b$].
¶Average cross-links per node (total EG – non-Xl EG)/(M_a + M_b).