Liposome-crosslinked hybrid hydrogels for glutathione-triggered delivery of multiple cargo molecules

Supporting Information

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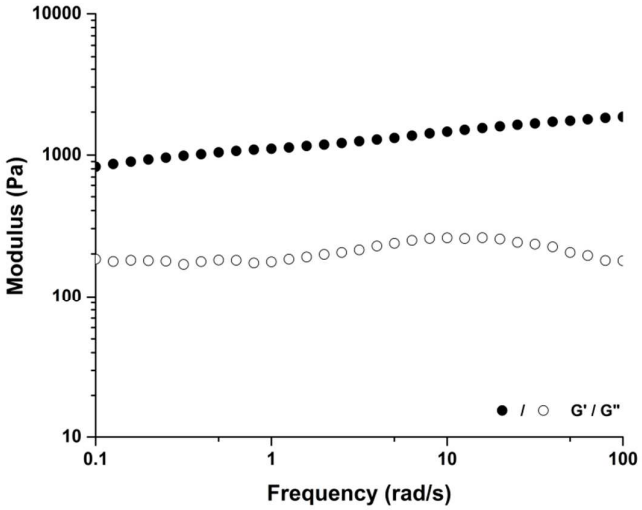

Figure S1. Representative oscillatory frequency sweep of the arylthiol-based, liposome-crosslinked hydrogels (1:2 Mal:SH ratio) in pH=7.0 bis-tris buffer at 37°C. Modest difference between G' and G" was observed at low frequency range and frequency dependence of both moduli was shown due to the hydrophobic interactions between the aromatic moieties of PEG arylthiol and lipid bilayers.

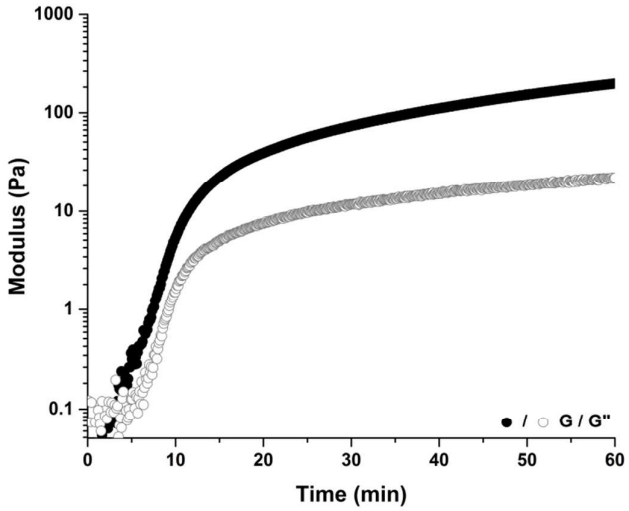

Figure S2. Representative oscillatory time sweep of the liposome-crosslinked hydrogels (1:2 Mal:SH ratio) in pH=7.0 bis-tris buffer at 37°C.

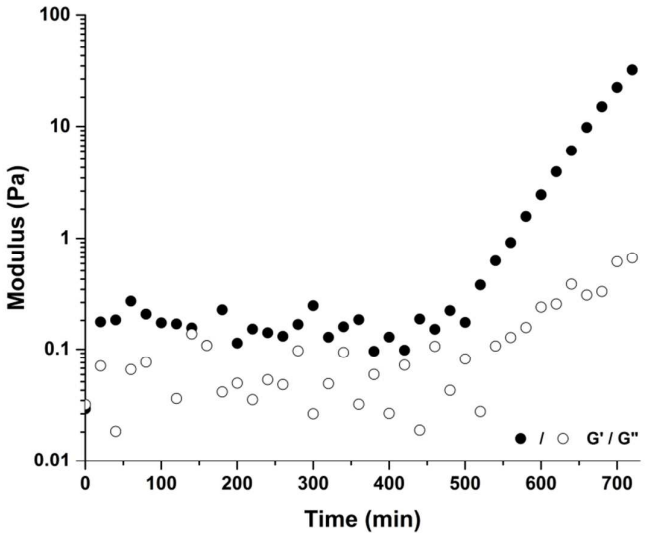

Figure S3. Oscillatory time sweep of 4-arm alkyl PEG-SH polymers (6wt%, polymer only) as a disulfide control, in pH=7.0 bis-tris buffer at 37°C.

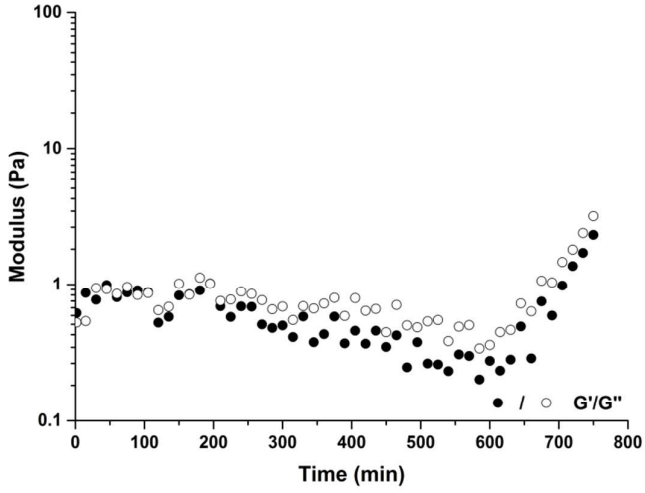

Figure S4. Oscillatory time sweep of the mixture of 10mM L-α-phosphatidylcholine (Egg PC) liposomes and 4-arm alkyl PEG-SH polymers (6wt%) as a non-maleimide liposome control, in pH=7.0 bis-tris buffer at 37°C.

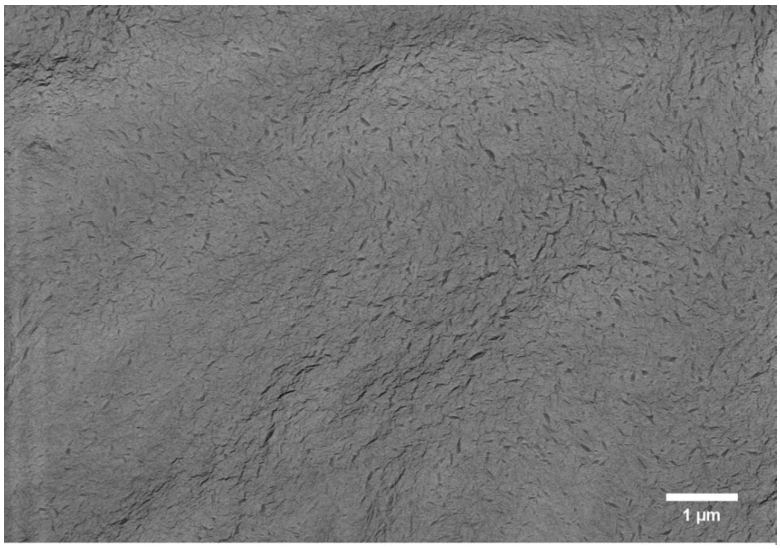

Figure S5. Scanning electron microscopy (SEM) images of 6wt% PEG hydrogel control lacking liposomes.

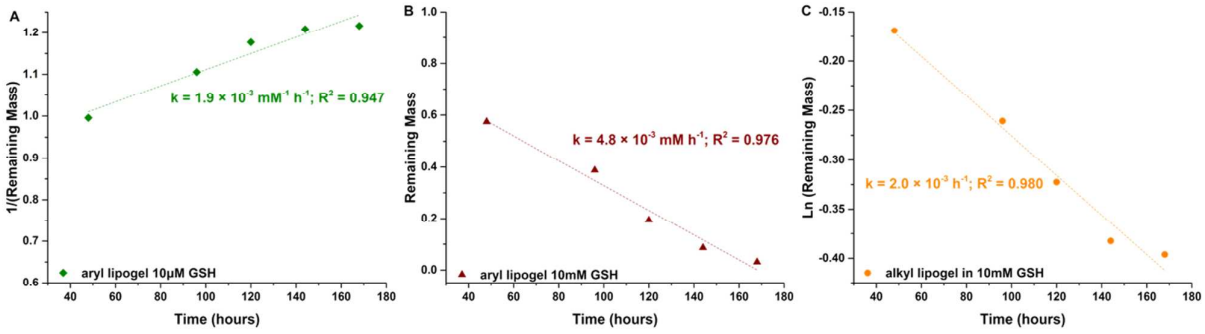

Figure S6. Fitting of the degradation profiles from liposome-crosslinked hybrid hydrogels synthesized from arylthiol-PEG and alkylthiol-PEG respectively. A) Degradation kinetics of the aryl lipogel in 10µM GSH; fitting is performed based on the rate law for second order kinetics ($-\frac{d[M]}{dt} = k[M][GSH] \approx k[M]^2$) considering the comparable concentration of GSH and crosslinks. B) Degradation kinetics of the aryl lipogel in 10mM GSH; fitting is performed based on zero-order kinetics ($-\frac{d[M]}{dt} = k$) given the almost linear release observed. C) Degradation kinetics of the alkyl lipogel in 10mM GSH; fitting is performed based on first-order kinetics ($-\frac{d[M]}{dt} = k[M]$) because of the excess amount of GSH, which renders its concentration unchanged over the course of the experiment.

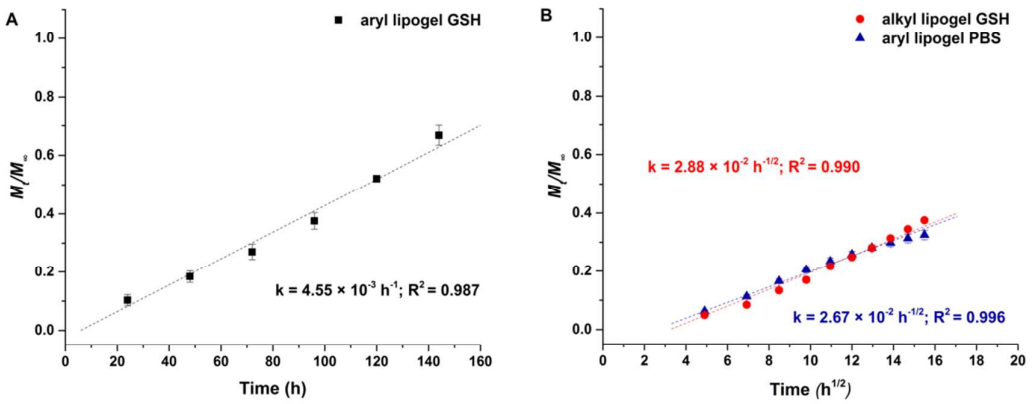

Figure S7. Fitting of the DOX release profiles from the liposome-crosslinked hybrid hydrogels (A) with network degradation and (B) lacking network degradation. A) Fitting of DOX release data is based on the the Ritger–Peppas equation (n=1). Data plotted as M_t / M_∞ versus t. B) Fitting of DOX release data is based on the early-time approximation of Fickian diffusion. Data plotted as M_t / M_∞ versus $t^{1/2}$.

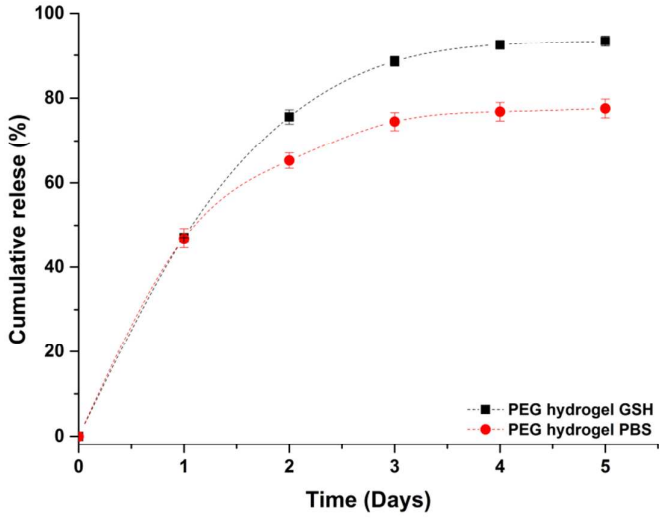

Figure S8. In vitro DOX release profiles from 6wt% PEG hydrogel control prepared by reacting 4-arm aryl PEG-SH and 4-arm PEG-maleimide. Release experiments were carried out in 10mM GSH in PBS and PBS solutions alone respectively at 37°C. Mean and S.D. are shown (n = 2).

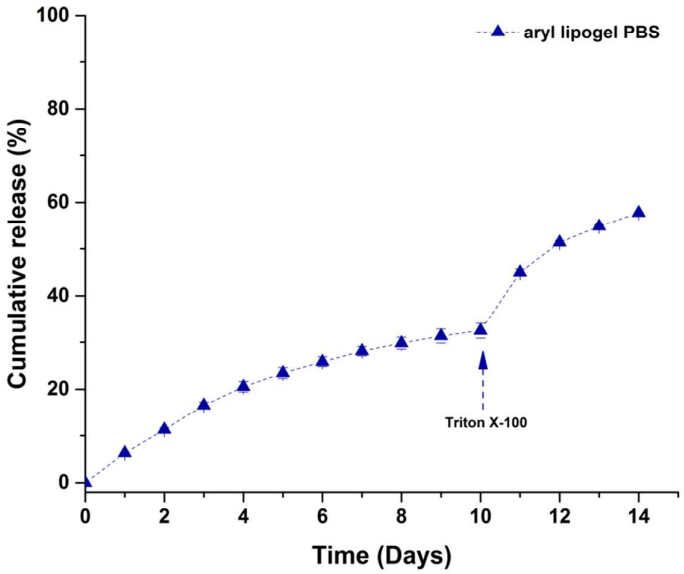

Figure S9. In vitro release of DOX from the liposome-crosslinked hydrogels at 37°C in PBS. 10% Triton™ X-100 was applied to the aryl lipogels incubated in PBS at Day 10, resulting in the liberation of more liposome-encapsulated DOX. Mean and S.D. are shown (n = 3).

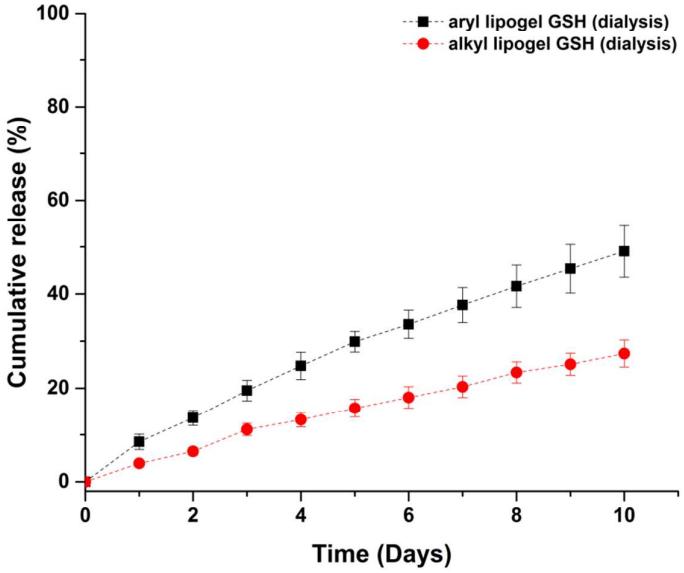

Figure S10. In vitro release of freely diffusing DOX from the liposome-crosslinked hydrogels in 10mM GSH solutions, as monitored after dialysis in a cup-like mini dialysis device (MWCO 3.5K) at 37°C. Mean and S.D. are shown (n = 3).

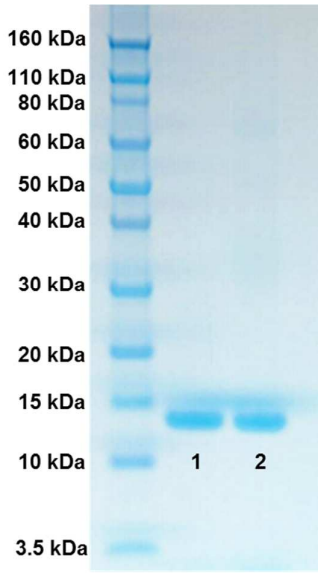

Figure S11. SDS-PAGE electrophoresis of cytochrome c (Lane 1) and a mixture of cytochrome c and 4-arm PEG arylthiol (Lane 2). The cytochrome c was incubated with PEG arylthiol polymers (1mg cytochrome c and 3mg of PEG polymer, the same amount as was employed in hydrogel formation, were incubated for 1h at 37°C), and the mixture electrophoresed almost identically to native cytochrome c, demonstrating the side reactions between the protein and polymers were insignificant within the timescale of crosslinking.

Fitting of the release profiles of DOX and cytochrome *c*

The release constant *k* of DOX from the aryl lipogel in 10mM GSH was calculated based on the linear fitting of the release data based on the Ritger–Peppas equation:

$$\frac{M_t}{M_\infty} = kt^n$$, where *n*=1 for zero-order release. **(1)**

The release constant *k* of DOX from the lipogels with first order release (lacking network degradation) was calculated based on the early-time approximation of Fickian diffusion (Higuchi model, $0 \leq \frac{M_t}{M_\infty} \leq 0.6$):

$$\frac{M_t}{M_\infty} = 4\,(\frac{Dt}{\pi h^2})^{1/2}, k = 4\,(\frac{D}{\pi h^2})^{1/2};$$ **(2)**

The release constant *k* of cytochrome *c* was calculated based on the late-time approximation of Fickian diffusion ($0.4 \leq \frac{M_t}{M_\infty} \leq 1$):

$$\frac{M_t}{M_\infty} = 1 - \left(\frac{8}{\pi^2}\right)\exp[(-\pi^2 Dt)/h^2], k = (-\pi^2 D)/h^2;$$ **(3)**

M_t is the amount of drug released at time t, M_∞ is the total mass of drug loaded into the hydrogel, D is the diffusion coefficient of the drug within the polymer matrix, π is 3.14, and h is the thickness of the hydrogel.