Supporting Information

Peptide-Functionalized Click Hydrogels with Independently Tunable Mechanics and Chemical Functionality for 3D Cell Culture

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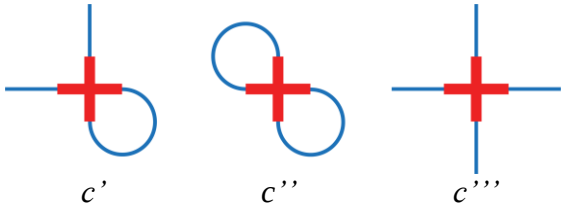
Table S1. Amount of cyclic non-idealities in step-growth hydrogel.

For a step-growth network, we define the fraction of pairs of the azide functional groups that are part of primary cycles as c.[3-5] From this, the probability of having azide monomers with 1, 2, or 0 primary cycles is given as c', c'', and c''', respectively:



$$c' = 2c(1-c) \tag{2}$$

$$c'' = c^2 \tag{3}$$

$$c''' = (1-c)^2 \tag{4}$$

In a perfectly ideal network containing no primary cycles, $c = c' = c'' = 0$ and $c''' = 1$. For these networks, the ideal crosslinking density during network formation ($\rho_{x,0}$) is equal to twice the concentration of PEG tetraazide multiplied by r, the ratio of azide to alkyne functionalities such that $0 \leq r \leq 1$, since each arm of the PEG azide is attached to half an infinite chain. The formation of primary cycles decreases the network crosslinking density and the experimentally measured compressive moduli from values predicted using $c''' = 1$. By comparing the ratio of the initial bulk moduli (K_0) and the predicted bulk moduli (K_{theo}), we can estimate the degree of cyclization present in the network. K_0 can be calculated from the swelling ratio during network formation (q_{NF}), the initial swelling ratio (q_0), and the measured swollen bulk moduli ($K_{measured}$).

$$\rho_x = \frac{2(\text{moles of PEG tetraazide})r}{\text{total polymerization volume}} \tag{5}$$

$$\frac{K_0}{K_{theo}} = (1-c)^2 = c''' \tag{6}$$

$$K_0 = K_{measured}(q_0)^{1/3}(q_{NF})^{2/3} \tag{7}$$

The theoretical bulk modulus (K_{theo}) is readily calculated from the $\rho_{x,0}$ and the Poisson ratio (v_p, assumed = 0.42), [3,4] where R is the universal gas constant and T is the absolute temperature.

$$E_{theo} = 3RT\rho_{x,0} \quad (9)$$

$$K_{theo} = \frac{E_{theo}}{3(1-2v_p)} \quad (10)$$

In addition, we must calculate the obtained bulk moduli ($K_{measured}$) from the measured shear elastic moduli ($G_{measured}$).

$$K_{measured} = \frac{2G_{measured}(1+v_p)}{3(1-2v_p)} \quad (11)$$

With these equations, as well as known values for $\rho_{x,0}$, $G_{measured}$, and swelling ratios (q_0 and q_{NF}), we can calculate *c, c', c'', and c'''* for our different formulations. For mean values of $G_{measured}$ and the swelling ratios, the values for *c* and *c'''* are calculated as:

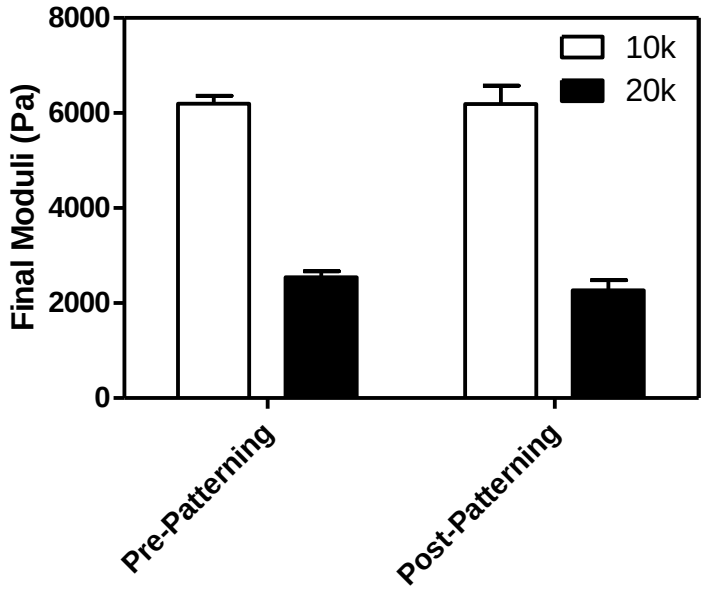
Azide: Alkyne	**10k**		**15k**		**20k**	
	c	*c'''*	*c*	*c'''*	*c*	*c'''*
1.5:1	0.075	0.86	0.106	0.80	0.122	0.77
1.25:1	0.066	0.87	0.024	0.95	0.093	0.82
1:1	0.035	0.93	0.015	0.97	0.057	0.89
1:1.25	0.023	0.95	0.005	0.99	0.134	0.75
1:1.5	0.038	0.92	0.071	0.86	0.133	0.75

We find that the networks are highly ideal (>95%) for the 1:1 stoichiometry case. As expected, ideality decreases as the system is formed further off-stoichiometry.

[3] A. E. Rydholm, S. K. Reddy, K. S. Anseth, C. N. Bowman, *Polymer* **2007**, *48*, 4589.

[4] B. D. Johnson, D. J. Beebe, W. Crone, *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* **2004**, *24*, 575.

Figure S1. Equilibrium shear moduli and swelling ratios post patterning (2.2 mM I2959 and a 6 J cm^{-2} dosage of 365 nm light).



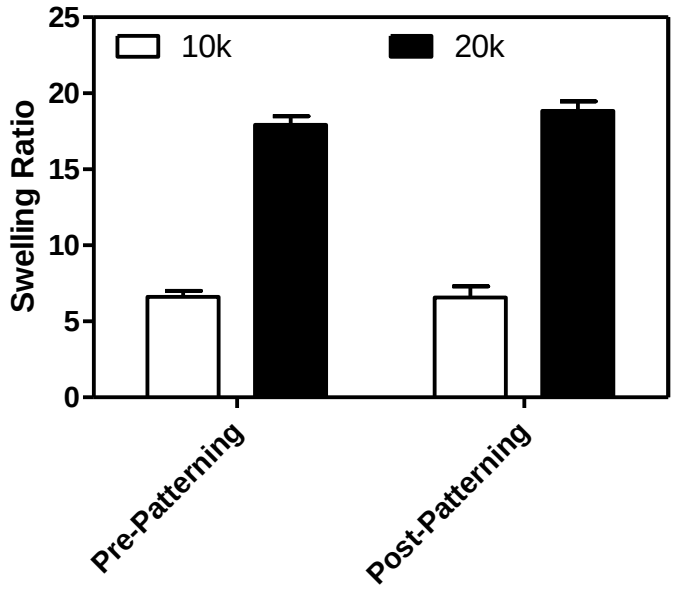


Figure S2. Total amount of photoinitiator consumed over photofunctionalization process.

The volume-averaged initiator concentration versus irradiation time[1] is given as:

$$\frac{[\mathrm{I}]}{[\mathrm{I}]_0} = \frac{1}{\varepsilon[\mathrm{I}]_0 L}\ln\left[1-\left(1-e^{\varepsilon[\mathrm{I}]_0 L}\right)e^{-\varepsilon\Phi I_0 t}\right] \qquad (1)$$

where:

[I] = photoinitiator concentration as a function of time

$[I]_0$ = initial photoinitiator concentration (chosen here as 2.2 mM)

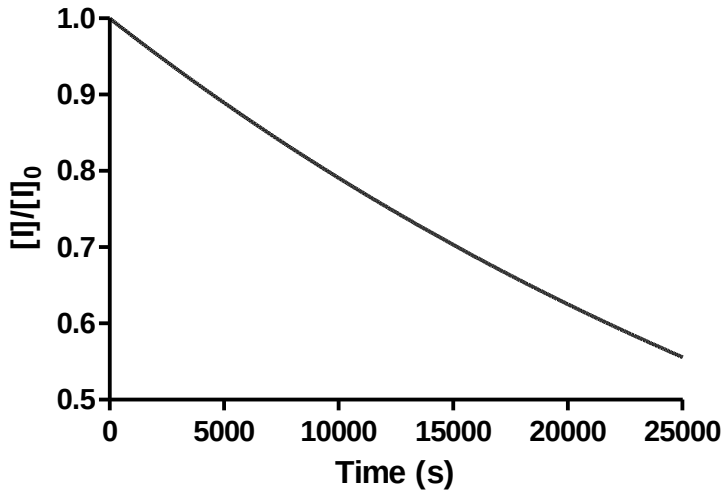
I_0 = irradiance at the base of the sample = 3.05 x 10^{-8} moles photons s^{-1} cm^{-2} for 365 nm light at 10 mW cm^{-2}

ε = the wavelength dependent absorption coefficient = 2.302 x extinction coefficient (6.7 L mol^{-1} cm^{-1} for I2959)

L = sample thickness = 1 mm

Φ = quantum yield of the photoinitiator consumption = 0.05 for I2959 at 365 nm[2]

Plotting Eq. 1 as a function of time gives the representative curve:



Even for our longest reaction time (600 s), <2% of the total photoinitiator is consumed.

[1] S. Asmussen, G. Arenas, W. D. Cook, C. Vallo, *European Polymer Journal* **2009**, *45*, 515.

[2] N. S. Allen, M. C. Marin, M. Edge, D. W. Davies, J. Garrett, F. Jones, S. Navaratnam, B. J. Parsons, *Journal of Photochemistry and Photobiology a-Chemistry* **1999**, *126*, 135.

Scheme S1. Hydrogels are exposed to collimated UV light (365 nm at 10 mW cm^{-2}) while a moving photomask covers the sample.[55] This rate of coverage is easily controlled and enables different gradients of light to be imposed on the hydrogel. This ultimately results in well-defined gradients of patterning concentrations across relatively large distances.

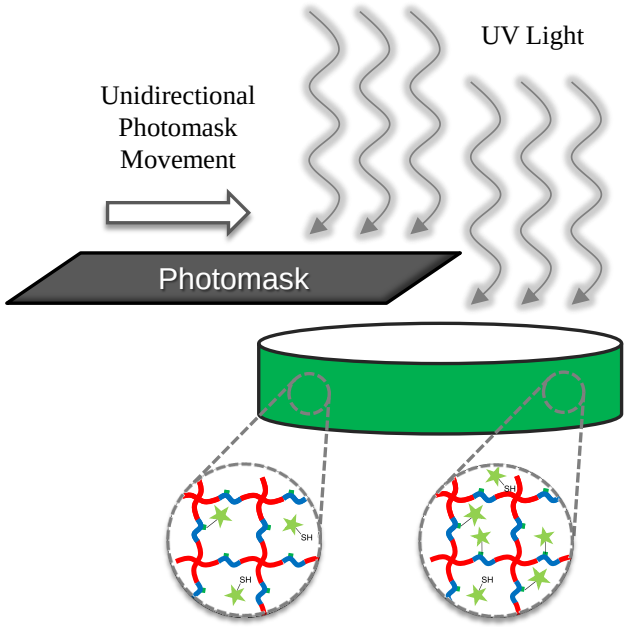


Figure S3. ^{1}H-NMR spectrum of PEG tetraazide (here, M_n ~ 10,000 g/mol).

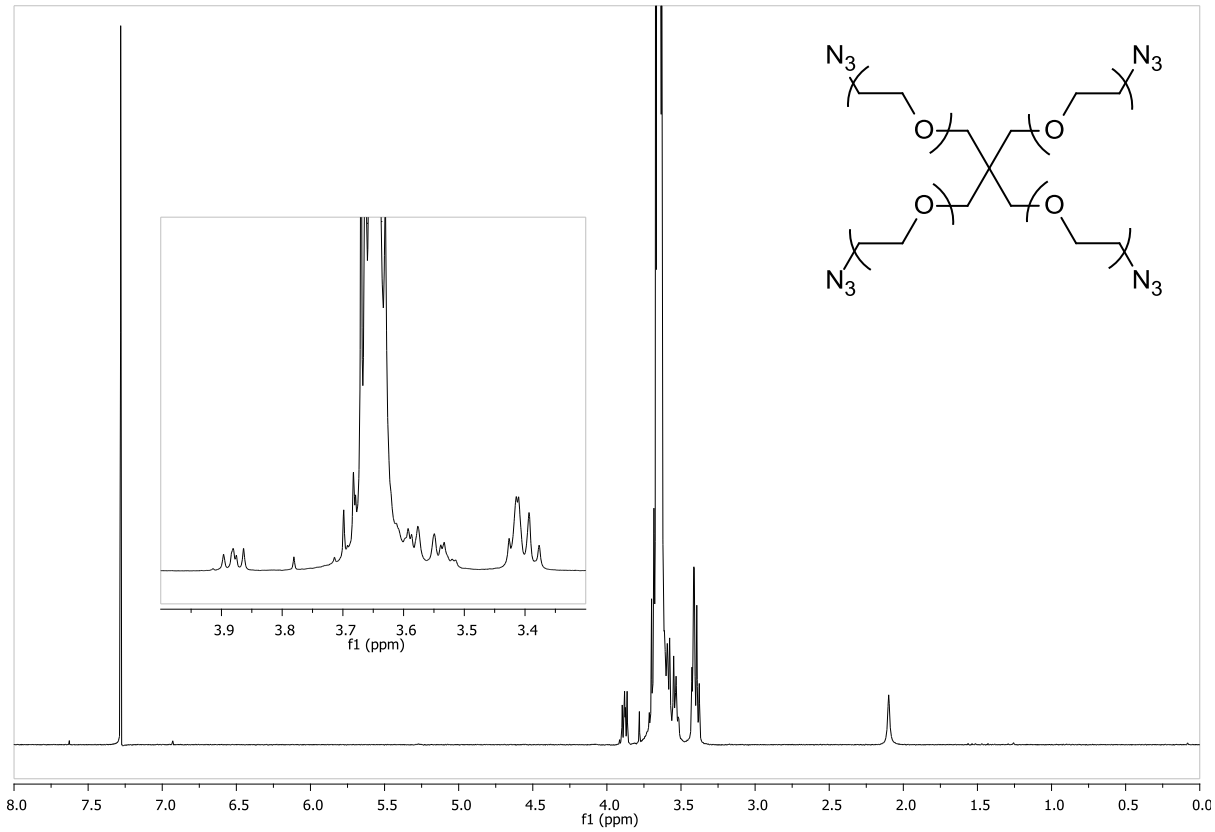


Figure S4. ^{1}H-NMR spectrum of DIFO3.

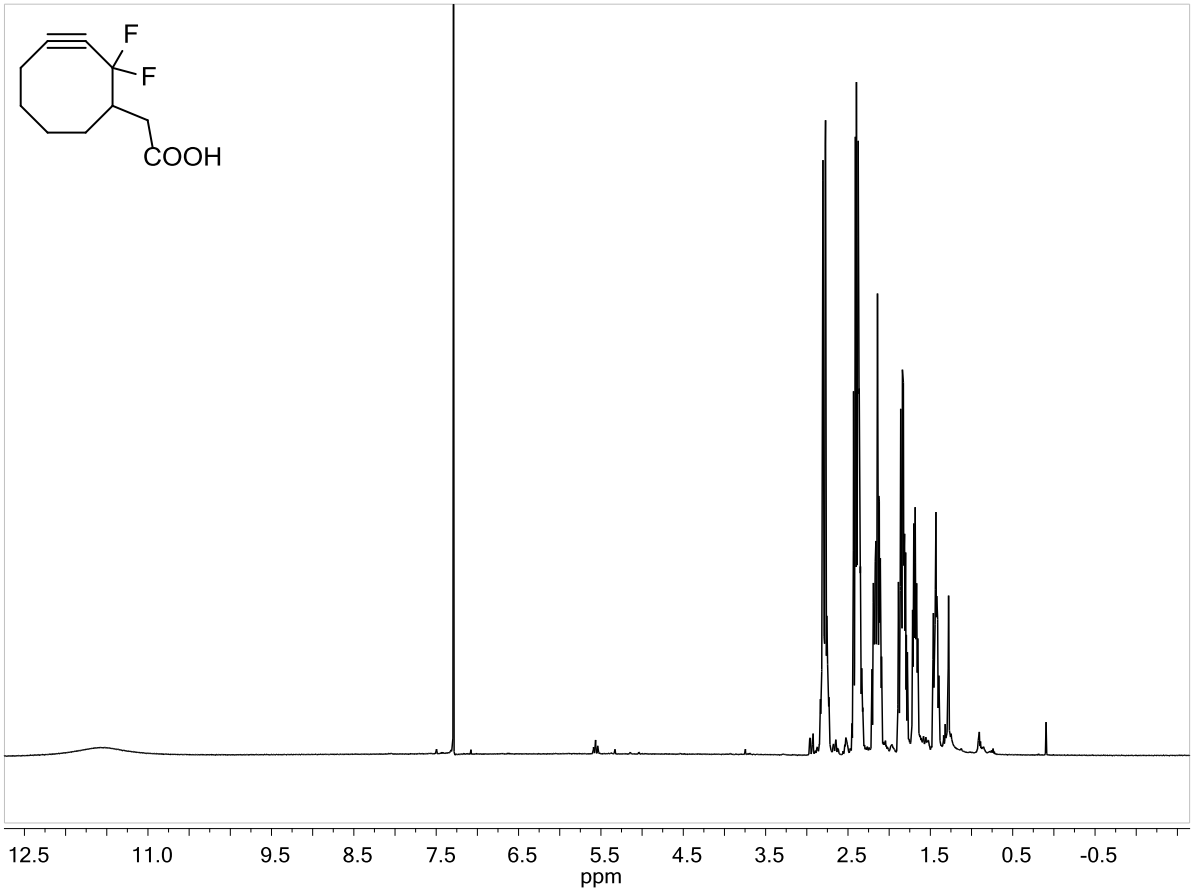


Figure S5. ^{13}C-NMR spectrum of DIFO3.

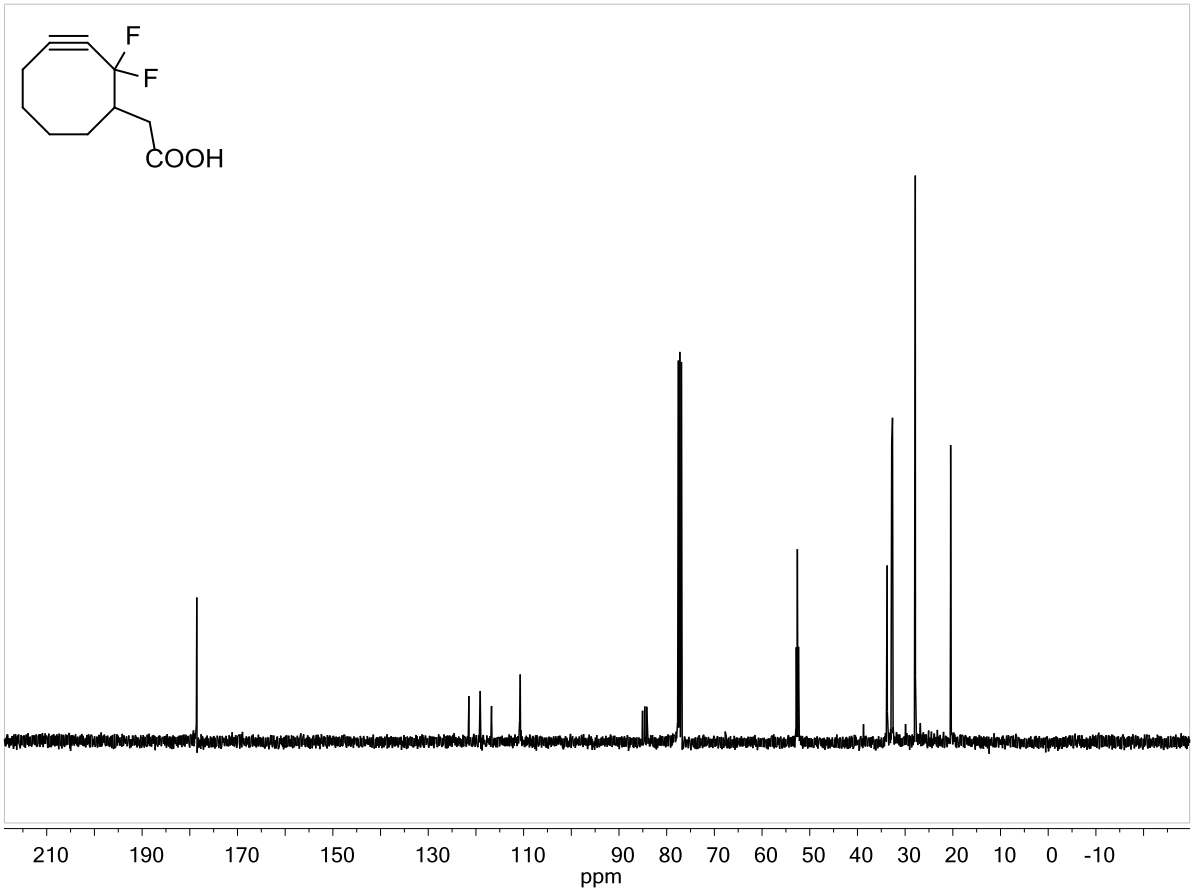


Figure S6. ^{19}F-NMR spectrum of DIFO3.

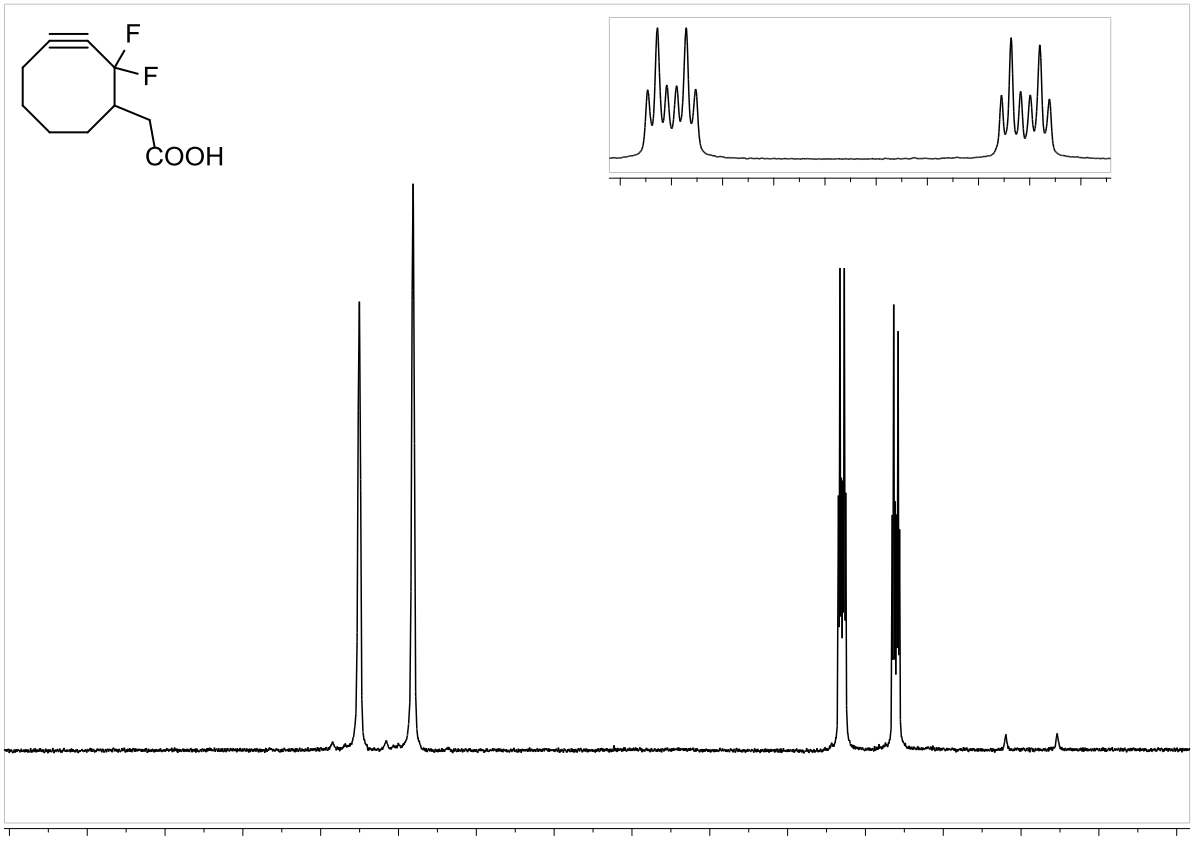


Figure S7. MALDI-TOF spectrum of regular peptide (+4 charge)

Ac-K(DIFO3)RRGGK(alloc)GGPQGILGQRRK(DIFO3)-NH_2

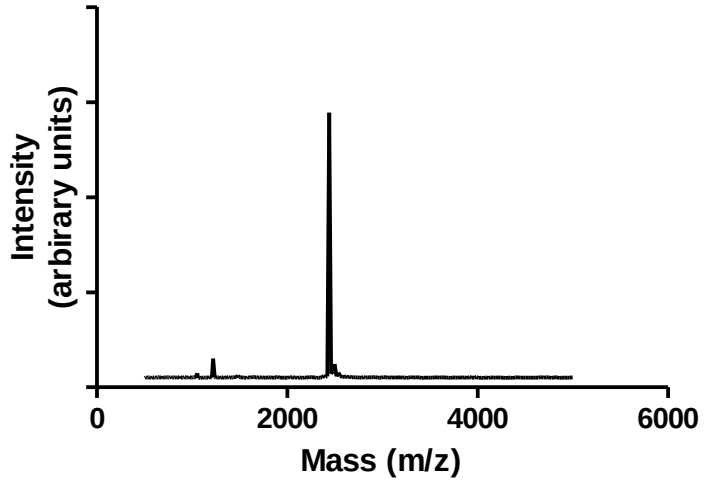


Figure S8. MALDI-TOF spectrum of scrambled peptide (+4 charge):

Ac-K(DIFO3)QGK(alloc)RIPGRRLGGRGQGK(DIFO3)-NH_2

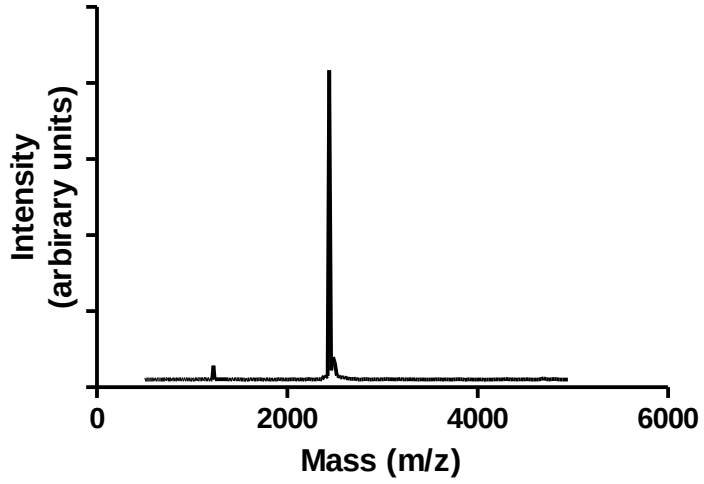


Figure S9. MALDI-TOF spectrum of neutral peptide (no net charge):

Ac-K(DIFO3)REGGK(alloc)GGPQGILGQERK(DIFO3)-NH_2

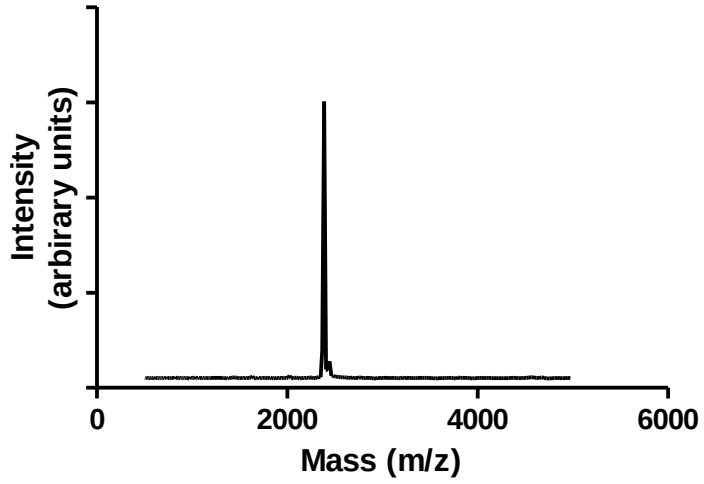


Figure S10. MALDI-TOF spectrum of negatively charged peptide (-4 charge):

Ac-K(DIFO3)EEGGK(alloc)GGPQGILGQEEK(DIFO3)-NH_2

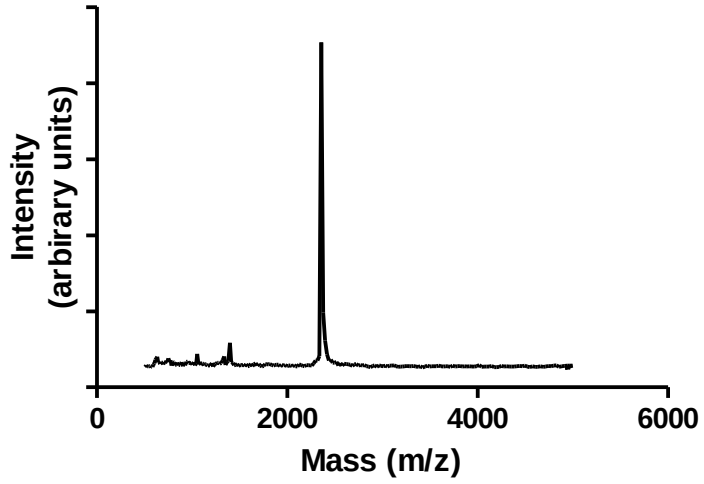


Figure S11. Calibration curve of fluorescence versus concentration of patterning agent swollen into network.