



Supporting Information

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Gelation Kinetics and Mechanical Properties of Thiol-Tetrazole Methylsulfone Hydrogels Designed for Cell Encapsulation

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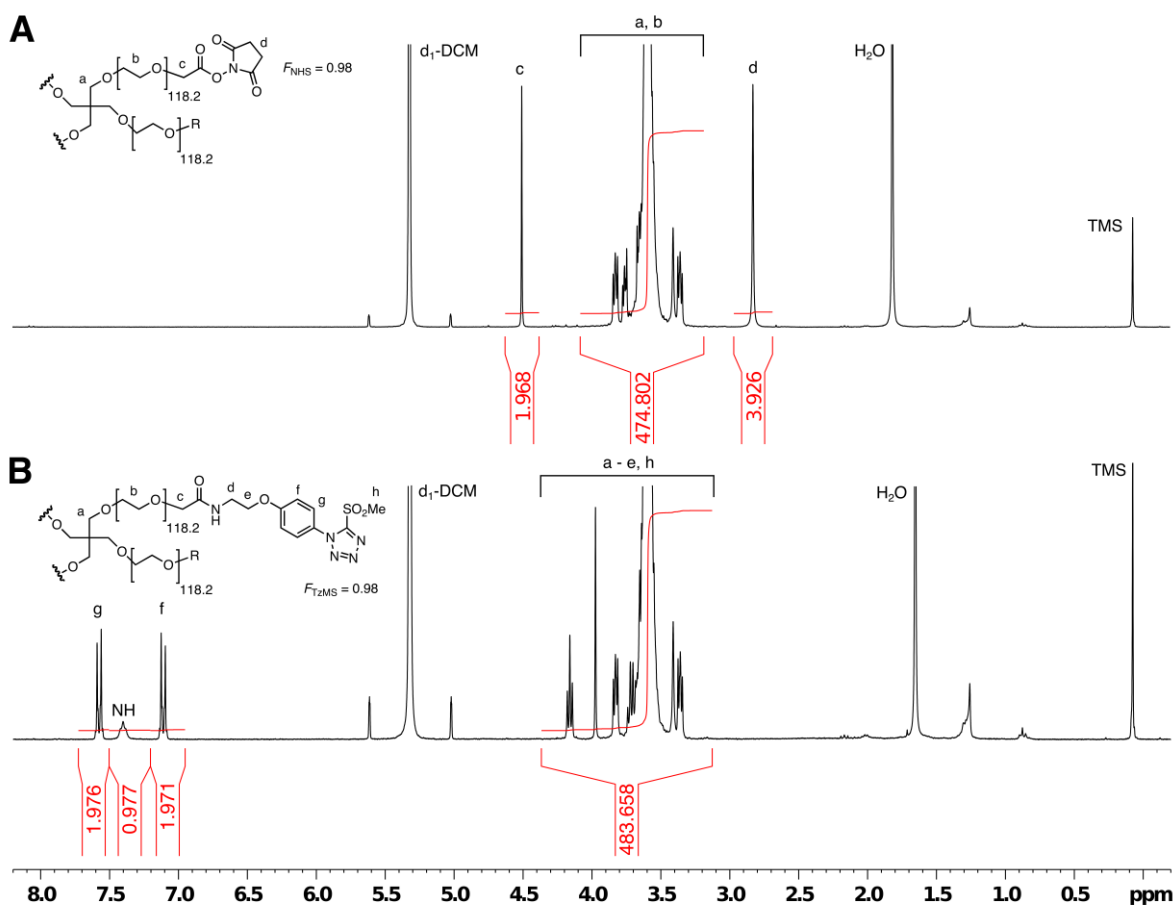


Figure S1. ^1H NMR of A) PEG-4NHS and B) PEG-4TzMS. For A), the $F_{\text{NHS}} = 0.984$ from the supplier was taken as the reference value for the integral of proton signal ‘c’ (4.5 ppm), i.e. $I_c = 2 \times F_{\text{NHS}} = 2 \times 0.984 = 1.968$. The integral of proton signals ‘a’ and ‘b’ from the PEG repeat units and 4-arm core was then used to determine the number of monomer repeat units n , i.e. $n = I_b / 4 = (I_{a,b} - I_a) / 4 = (474.802 - 2.00) / 4 = 118.2$. The molar mass of the polymer ($M_n = 21.6$ kDa) was then determined from the molecular structure, assuming that the non-functional end groups were carboxylic acid groups. For B), the integral for proton signals ‘a – e, h’ was set to the value of I_{a+b} plus expected values from protons ‘c’, ‘d’, ‘e’, and ‘h’, assuming that all the NHS groups had been converted to TzMS groups. Signals from protons ‘c’, ‘d’, ‘e’, and ‘h’ are negligible compared with I_{a+b} and this assumption is therefore reasonable even if conversion was not quantitative. The F_{TzMS} was then calculated from the signals of the aromatic protons ‘f’ and ‘g’ (7.1 and 7.6 ppm), i.e. $F_{\text{TzMS}} = (I_f + I_g) / 4 = (1.971 + 1.976) / 4 = 0.986$, which we took to be 0.984 given it cannot exceed F_{NHS} . The molar mass was then calculated in the same manner as for PEG-4NHS, giving $M_n = 22.2$ kDa.

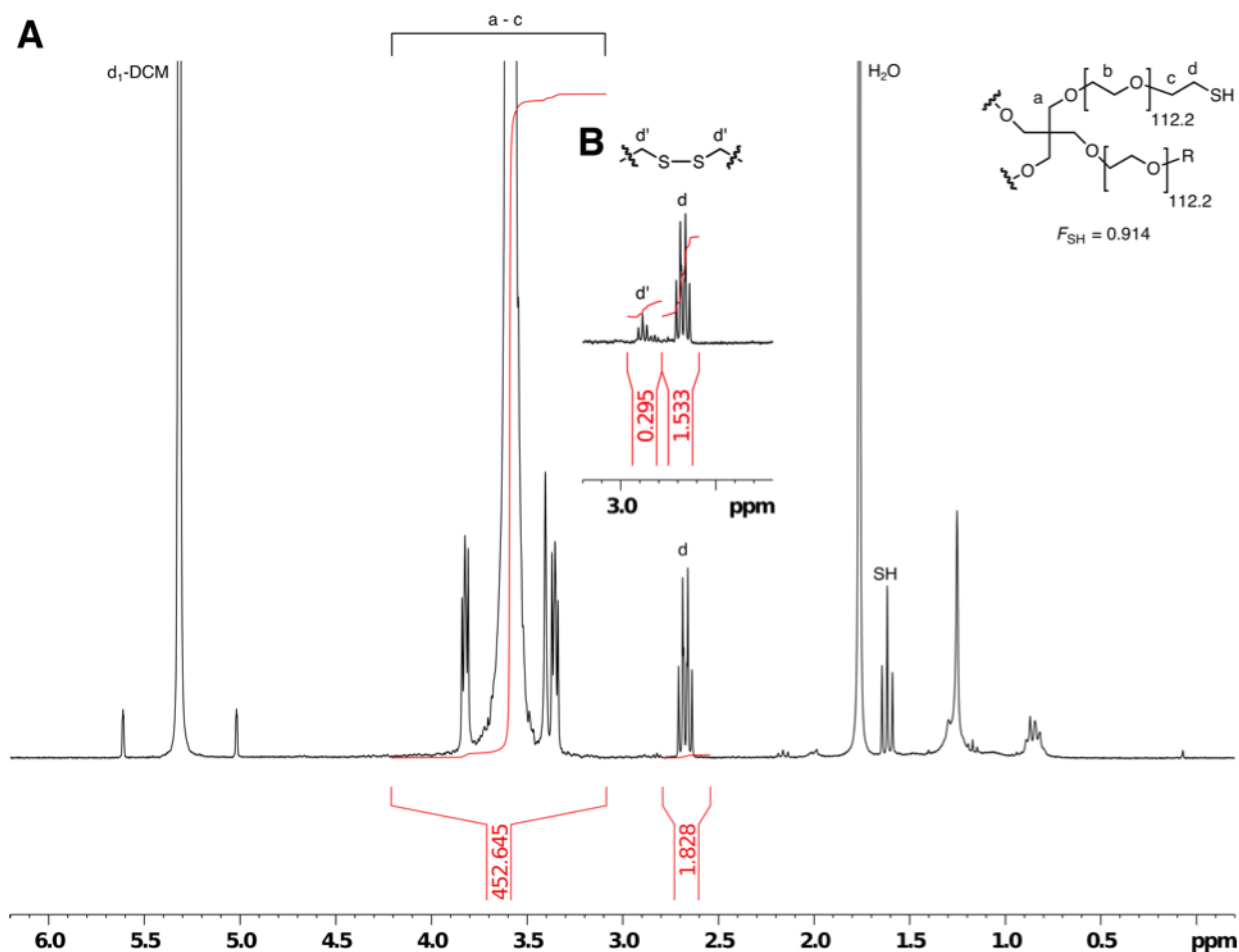


Figure S2. A) ¹H NMR of commercial PEG-4SH. The integral of the signal from the methylene protons ‘d’ adjacent to the SH terminus were set based on the reported degree of functionality, i.e. $I_d = 2 \times F_{SH} = 2 \times 0.914 = 1.828$. The integral of the signals from the star core and arms ‘a-c’ (3.1 – 4.2 ppm) was then used to determine the number of repeat units per arm, i.e. $n = I_b / 4 = (I_{a,b,c} - I_a - I_c) / 4 = (452.645 - 2.000 - 1.828) / 4 = 112.2$. The molar mass of the polymer ($M_n = 20.1$ kDa) was then determined from the molecular structure, assuming that the non-functional end groups were hydroxyl groups. The same protocol was followed for the linear PEG-dithiols. B) ¹H NMR spectrum of PEG-4SH in which ca. 20 % of the thiols had been converted to disulfides after long-term storage (under Ar, -20 °C, > 12 months).

Table S1. Degree of functionality and molar mass of PEG polymers used in this study.

Species	Degree of functionality from supplier (%)	Molar mass by ¹ H NMR spectroscopy (Da)
PEG-4NHS 20 kDa	98.4	21 579
PEG-4TzMS 20 kDa ^{a)}	98.4 / 96.4	22 243 / 22 219
PEG-4SH 20 kDa	91.4	20 127
PEG-dithiol 10 kDa	95.0	12 072
PEG-dithiol 1 kDa	99.0	996

^{a)} Two different batches of PEG-4TzMS were used in this work.

Table S2. Purity of peptides used in this study as determined by HPLC. ^{a)}

Species	Purity (%)
c[RGDfC]	97.3
VPM	98.8

^{a)} Peaks from the 220 nm channel were integrated for the quantification. Analysis conditions are described in the Experimental section.

Calculation of prepolymer concentrations for rheology experiments

In all rheology experiments, we imposed equal moles (n) of methylsulfone (MS) and thiol (SH) functional groups:

$$n(\text{MS}) = n(\text{SH})$$

This can be re-expressed as:

$$n(\text{P} - \text{MS}) \cdot a(\text{MS}) \cdot F(\text{MS}) = n(\text{P} - \text{SH}) \cdot a(\text{SH}) \cdot F(\text{SH})$$

where $n(\text{P-MS})$ is the number of moles of PEG-4TzMS, $n(\text{P-SH})$ is the number of moles of thiol-containing crosslinker, a is the number of chain ends per molecule, and F is the degree of functionality, i.e. the proportion of chain ends carrying the desired function.

This equation can be re-expressed as:

$$\frac{m(\text{P} - \text{MS}) \cdot p(\text{P} - \text{MS})}{M_n(\text{P} - \text{MS})} \cdot a(\text{MS}) \cdot F(\text{MS}) = \frac{m(\text{P} - \text{SH}) \cdot p(\text{P} - \text{SH})}{M_n(\text{P} - \text{SH})} \cdot a(\text{SH}) \cdot F(\text{SH})$$

where m is the mass, p is the purity (on a mass basis), and M_n is the number-average molar mass of each polymer component P-MS and P-SH.

Rearranging gives:

$$m(\text{P} - \text{MS}) = \frac{M_n(\text{P} - \text{MS}) \cdot p(\text{P} - \text{SH}) \cdot a(\text{SH}) \cdot F(\text{SH})}{M_n(\text{P} - \text{SH}) \cdot p(\text{P} - \text{MS}) \cdot a(\text{MS}) \cdot F(\text{MS})} \cdot m(\text{P} - \text{SH}) \quad (1)$$

In all rheology experiments, we also imposed a total polymer mass fraction (x) of 0.05 in the final rheology solutions, i.e.

$$x(\text{P} - \text{MS}) + x(\text{P} - \text{SH}) = 0.05$$

That is:

$$\frac{m(\text{P} - \text{MS})}{m_{\text{solution}}} + \frac{m(\text{P} - \text{SH})}{m_{\text{solution}}} = 0.05$$

Rearranging gives:

$$m(\text{P} - \text{SH}) = 0.05 \cdot m_{\text{solution}} - m(\text{P} - \text{MS}) \quad (2)$$

Substituting (2) into (1) gives:

$$m(\text{P} - \text{MS}) = \frac{M_n(\text{P} - \text{MS}) \cdot p(\text{P} - \text{SH}) \cdot a(\text{SH}) \cdot F(\text{SH})}{M_n(\text{P} - \text{SH}) \cdot p(\text{P} - \text{MS}) \cdot a(\text{MS}) \cdot F(\text{MS})} \cdot [0.05 \cdot m_{\text{solution}} - m(\text{P} - \text{MS})]$$

Rearranging gives an expression for the mass of P-MS required in a given mass of rheology solution:

$$m(\text{P} - \text{MS}) = 0.05 \cdot m_{\text{solution}} \cdot \frac{\left[\frac{M_n(\text{P} - \text{MS}) \cdot p(\text{P} - \text{SH}) \cdot a(\text{SH}) \cdot F(\text{SH})}{M_n(\text{P} - \text{SH}) \cdot p(\text{P} - \text{MS}) \cdot a(\text{MS}) \cdot F(\text{MS})} \right]}{\left[1 + \frac{M_n(\text{P} - \text{MS}) \cdot p(\text{P} - \text{SH}) \cdot a(\text{SH}) \cdot F(\text{SH})}{M_n(\text{P} - \text{SH}) \cdot p(\text{P} - \text{MS}) \cdot a(\text{MS}) \cdot F(\text{MS})} \right]} \quad (3)$$

The mass of P-SH can then be found from (2).

In order to facilitate mixing of the two reactive components, we also targeted equal volume aliquots of each precursor solution, which defined the concentrations of stock solutions of P-MS and P-SH.

Table S2. Buffer 2 pH values prior to dissolving PEG-4TzMS and the crosslinkers in order to attain the target initial pH values for rheology experiments.

Compound	Target initial pH	Initial buffer pH without compound	pH after dissolving compound
PEG-4TzMS	8.0	7.80	7.95
	7.5	7.20	7.59
	7.0	6.59	7.09
PEG-4SH	8.0	7.90	8.02
	7.5	7.27	7.54
	7.0	6.59	7.10
PEG-dithiol 10 kDa	8.0	7.98	8.03
	7.5	7.36	7.58
PEG-dithiol 1 kDa	8.0	8.04	8.06
	7.5	7.35	7.53
VPM	8.0	9.97	7.93
	7.5	9.47	7.50

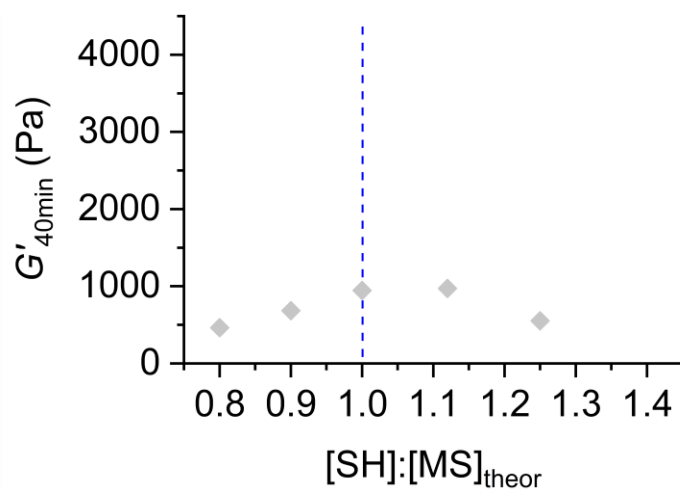


Figure S3. Effect of reactive group stoichiometry on $G'_{40\text{min}}$ for PEG-4TzMS crosslinked with 10 kDa PEG-dithiol at pH 8.0 (identical to **R3** at $[\text{SH}]:[\text{MS}] = 1$), $n = 1$. The vertical dotted line shows theoretical 1:1 stoichiometry.

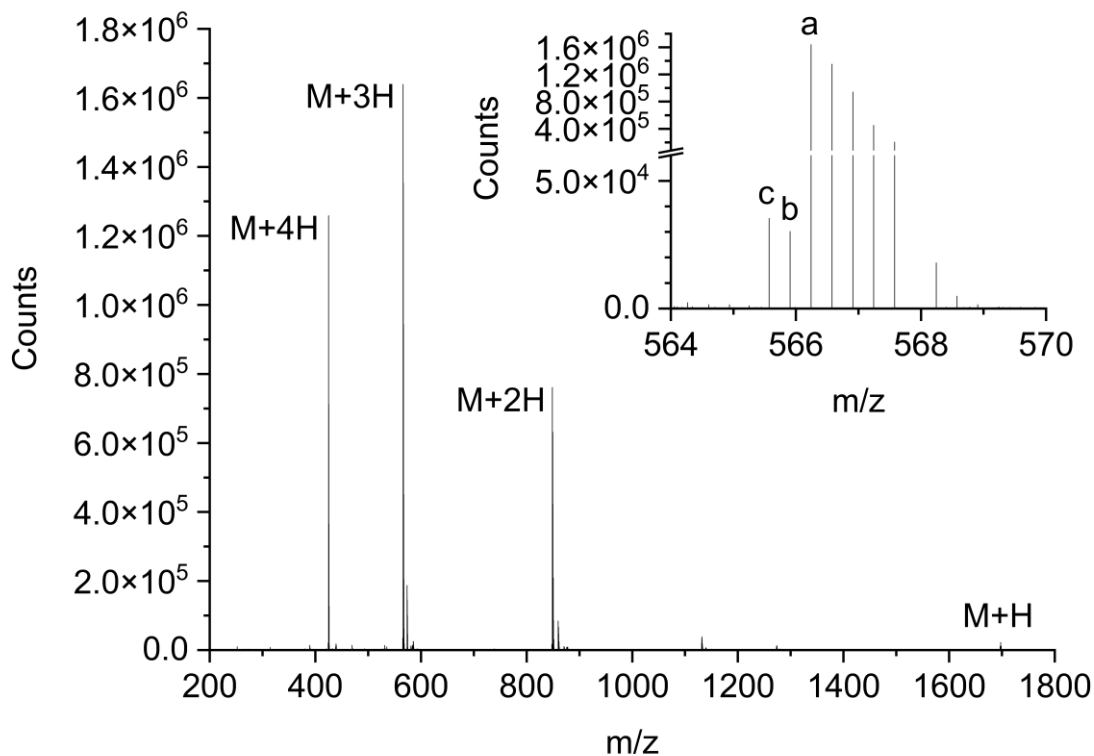


Figure S4. ESI-QTOF-MS spectrum of 61.5 min VPM solution, taken at 3.126 min retention time in the liquid chromatogram. The inset shows the region corresponding to a) VPM, b) Linear VPM dimer, and c) Cyclic VPM.

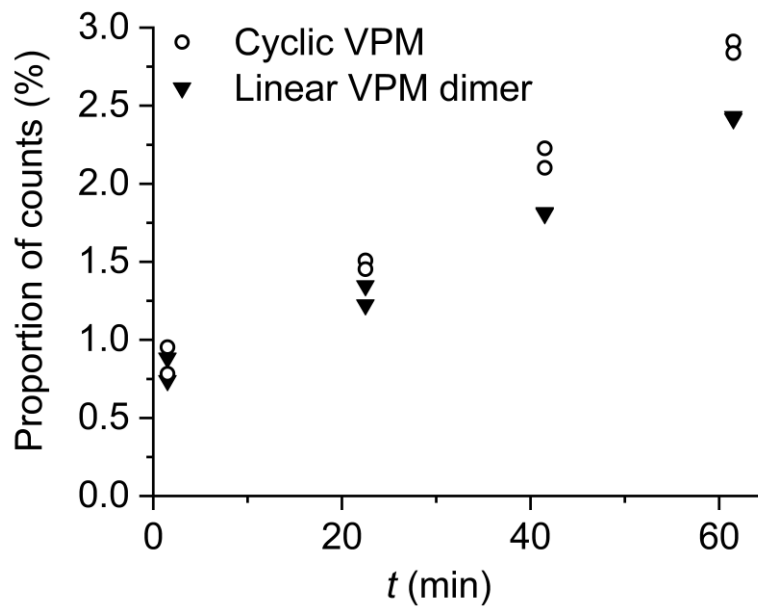


Figure S5. Detector counts for linear ($m/z = 565.905$) and cyclic ($m/z = 565.571$) disulfide, divided by the sum of counts for VPM ($m/z = 566.243$) and the disulfide species. The VPM solution was prepared identically to in the **R5** rheology experiment at pH 8.0.