Supporting Information for:

Hydrogels Cross-Linked by Catalyst-Free Native Chemical Ligation

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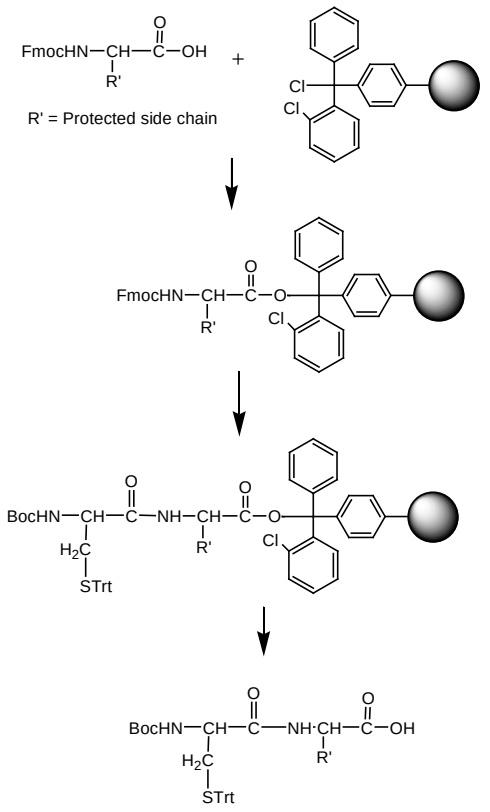

Scheme S1. Solid-phase synthesis of protected cysteine dipeptides.

Table S1. Effect of polymer concentration, stoichiometry, buffer and temperature on gel formation* of **1** and **2** by native chemical ligation.

Test	C[1]	Buffer system	R[2]	T (ºC)	Gelation time
1	2	GIBCO PBS	1:1	23	3 days
2	5	0.1 M PBS, pH 7.6	1:1	23	2 hrs
3	5	0.1 M PBS, pH 7.6	1:2	23	2 hrs
4	5	0.1 M Phosphate, pH 7.6	1:1	23	2 hrs
5	5	0.1 M Phosphate, pH 7.6	1:2	23	2 hrs
6	20	0.1 M PBS, pH 7.6	1:1	37	25 min
7	20	0.1 M PBS, pH 7.6	1:1	23	35 min
8	20	50 mM Tris buffer, pH 8.2	1:1	23	> 60 min
9	20	50 mM Tris buffer, pH 8.2	1:1	37	> 60 min
10	20	0.1 M $NaHCO_3$, pH 8.3	1:1	23	15 min
11	20	0.1 M $NaHCO_3$, pH 8.3	1:1	37	6 min
12	20	0.1 M $NaHCO_3$, pH 8.3	0:1[3]	23	>3 hrs

* gel formation measured by visual inspection

[1] Total polymer concentration (%, w/v).

[2] Molecular ratio of **1** to **2** (TFA salt of **2** was used throughout).

[3] Experiment employed compound **2** only

Table S2. Gel formation for equimolar mixtures of **1** with different *N*-terminal cysteine-polymers*

Test	N-terminal cysteine-polymer	GT[1]	GT[2]
1	Cys-PEG4A, **2**	5'30"	3'47"
2	Cys-Gly-PEG4A, **3a**	5'40"	3'42"
3	Cys-Glu-PEG4A, **3b**	4'40"	3'30"
4	Cys-Asp-PEG4A, **3c**	7'20"	6'34"
5	Cys-Trp-PEG4A, **3d**	8'05"	Gel[3]
6	Cys-Trp-PEG4A, **3d**		9'43"[4]
7	Cys-Trp-PEG4A, **3d**		11'41"[5]
8	Cys-Arg-PEG4A, **3e**	n/d	3'38"

*20% **1** in H_2O and 20% *N*-terminal cysteine-polymer (**2**, **3a**-**e**) in 0.2 M $NaHCO_3$ pH 8.3 at 37ºC. Gel formation measured by visual inspection

[1]Gelation time with TFA salt of *N*-terminal cysteine-polymer.

[2]Gelation time with salt-free *N*-terminal cysteine-polymer.

[3] Salt-free *N*-terminal cysteine-polymer gelled immediately after addition of H_2O and vortex.

[4] Salt-free *N*-terminal cysteine-polymer was dissolved in 20 mM TCEP aqueous solution and **1** in 0.2 M $NaHCO_3$.

[5] Salt-free *N*-terminal cysteine-polymer was dissolved in 200 mM 2-mercaptoethanol aqueous solution and **1** in 0.2 M $NaHCO_3$.

n/d: not determined.

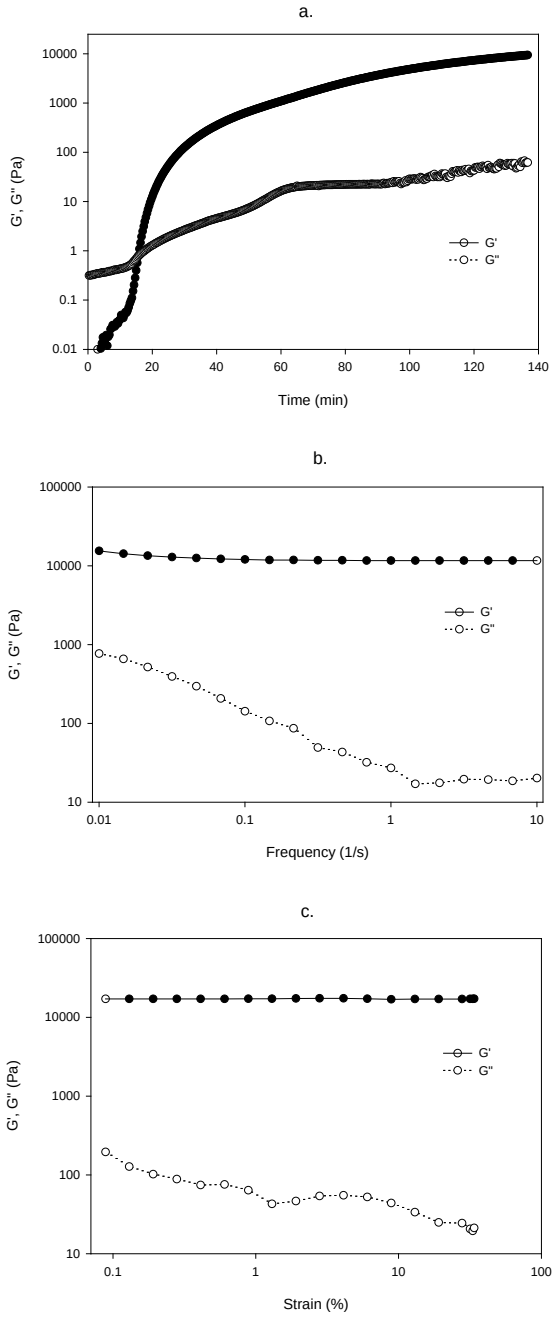

Figure S1. Oscillatory rheology of hydrogel containing 20% macromonomer **1** and 20% macromonomer **3c** (1:1) in 100 mM NH_4HCO_3, pH 8.3. a. storage modulus versus time during crosslinking; b. frequency sweep at 1% strain after 180 minutes crosslinking at 20°C; c. strain sweep at 1 Hz frequency after frequency sweep experiment. G' = storage modulus; G'' = loss modulus (rheology method 1).

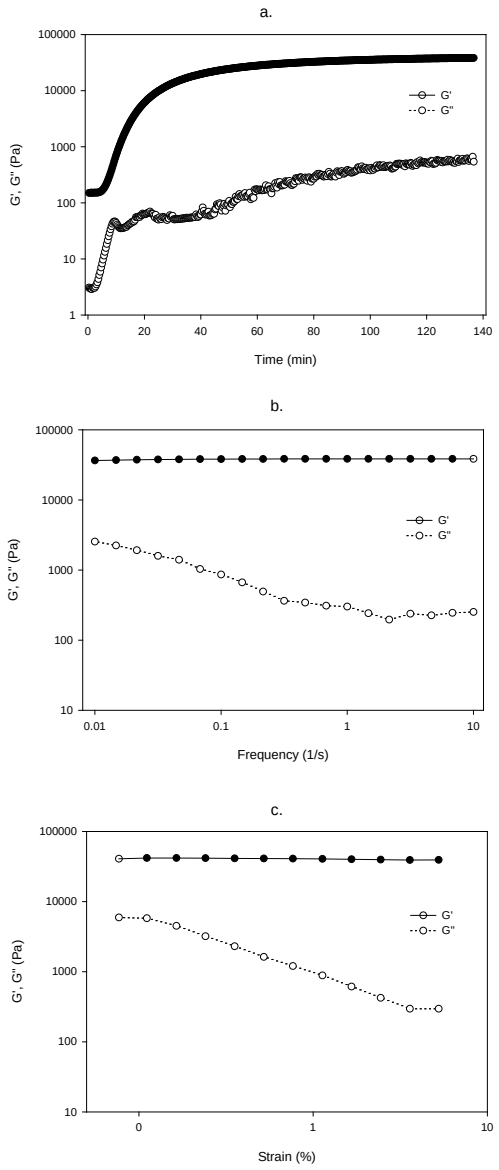

Figure S2. Oscillatory rheology of hydrogel containing 20% macromonomer **1** and 20% macromonomer **3b** (1:1) in 100 mM NH_4HCO_3, pH 8.3. a. storage modulus versus time during crosslinking; b. frequency sweep at 1% strain after 180 minutes crosslinking at 20°C; c. strain sweep at 1 Hz frequency after frequency sweep experiment. G' = storage modulus; G" = loss modulus (rheology method 1).

Figure S3. Picture of the hydrogel containing 20% macromonomer **1** and 20% macromonomer **3b** (1:1) in 100 mM NH_4HCO_3, pH 8.3 after oscillatory rheology (rheology method 1).

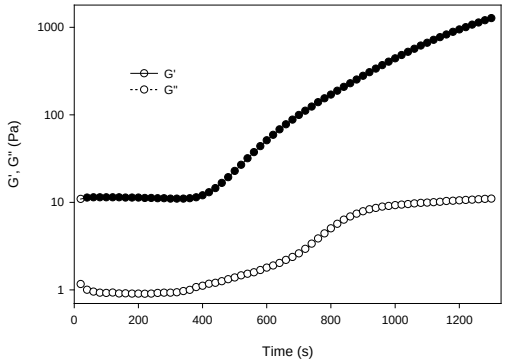

Figure S4. Oscillatory rheology of hydrogel containing 10% macromonomer **1** and 10% macromonomer **3b** (1:1) in 100 mM NH_4HCO_3, pH 8.3. Storage modulus versus time during crosslinking; G' = storage modulus; G" = loss modulus (rheology method 1).

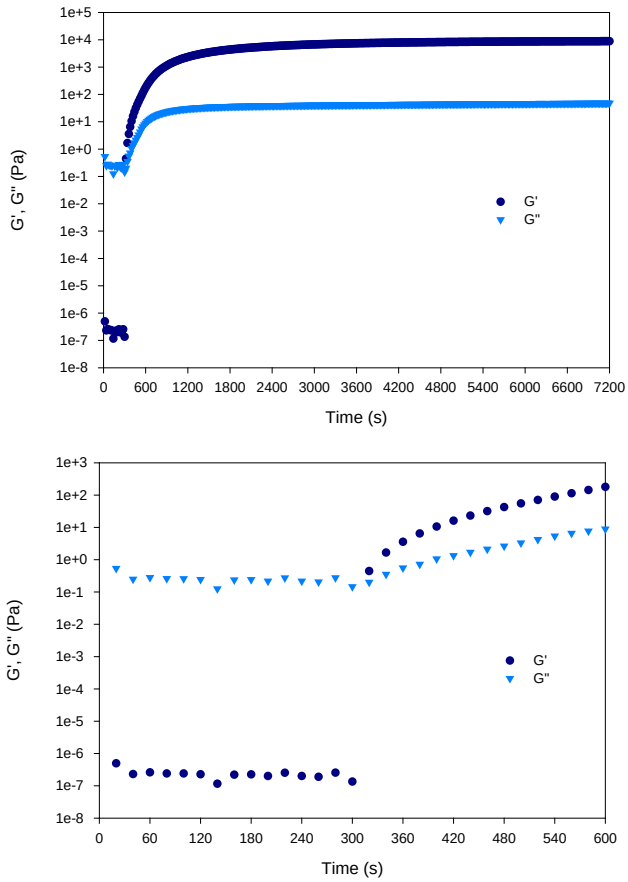

Figure S5. Oscillatory rheology of hydrogel containing 10% macromonomer **1** and 10% macromonomer **3a** (1:1) in 100 mM NH_4HCO_3, pH 8.3. Storage (G') and loss (G") modulus versus time during crosslinking are shown, with detail of the first 600 seconds shown at bottom. (Rheology method 2).

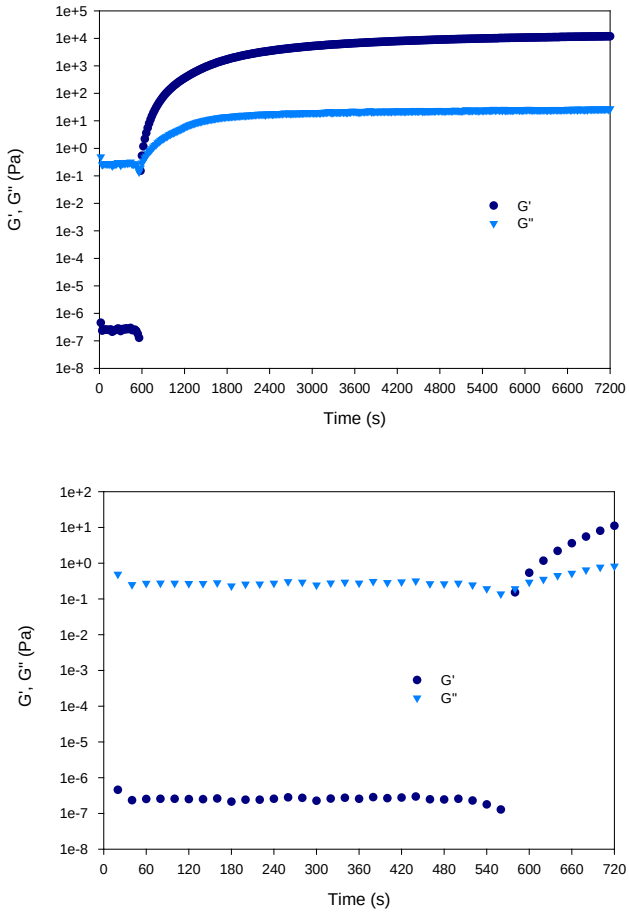

Figure S6. Oscillatory rheology of hydrogel containing 10% macromonomer **1** and 10% macromonomer **3c** (1:1) in 100 mM NH_4HCO_3, pH 8.3. Storage (G') and loss (G") modulus versus time during crosslinking are shown, with detail of the first 720 seconds shown at bottom. (Rheology method 2).

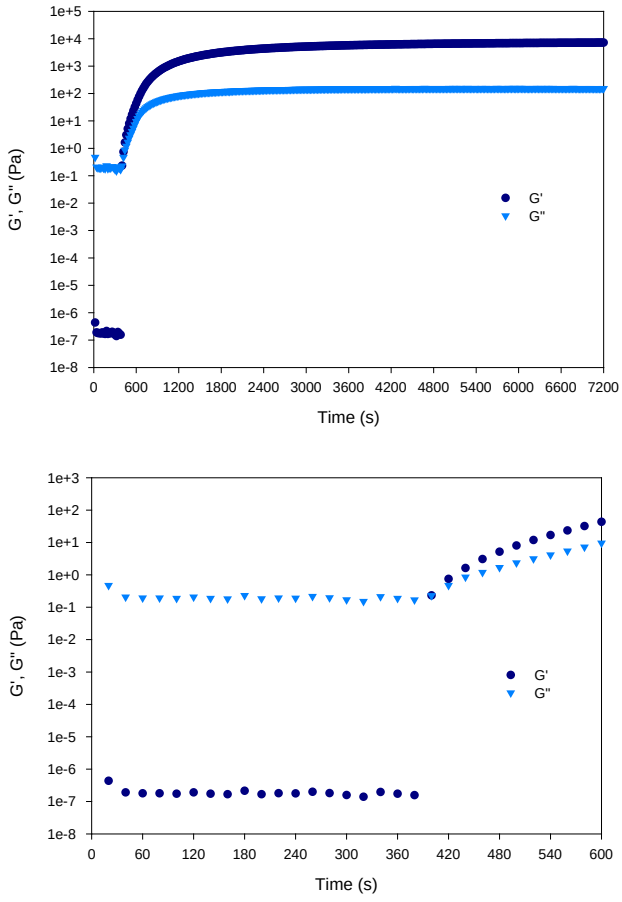

Figure S7. Oscillatory rheology of hydrogel containing 10% macromonomer **1** and 10% macromonomer **3d** (1:1) in 100 mM NH_4HCO_3, pH 8.3. Storage (G') and loss (G") modulus versus time during crosslinking are shown, with detail of the first 600 seconds shown at bottom. (Rheology method 2).

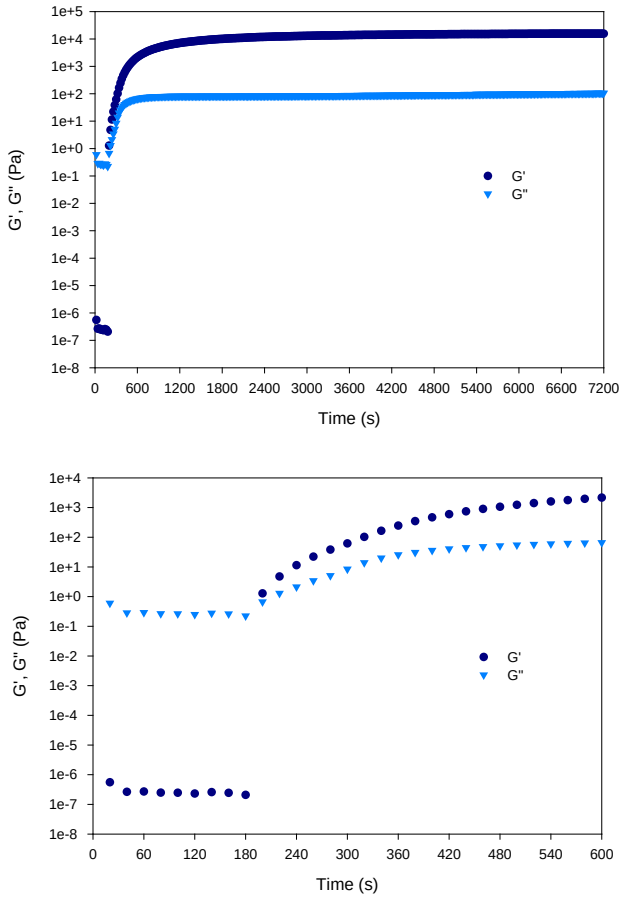

Figure S8. Oscillatory rheology of hydrogel containing 10% macromonomer **1** and 10% macromonomer **3e** (1:1) in 100 mM NH_4HCO_3, pH 8.3. Storage (G') and loss (G'') modulus versus time during crosslinking are shown, with detail of the first 600 seconds shown at bottom. (Rheology method 2).