Supporting information:

A Spontaneous In Situ Thiol-Ene Crosslinking Hydrogel with Thermo-Responsive Mechanical Properties

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^{1}HNMR spectroscopy

P(NIPAAM-CO-HEAACR)

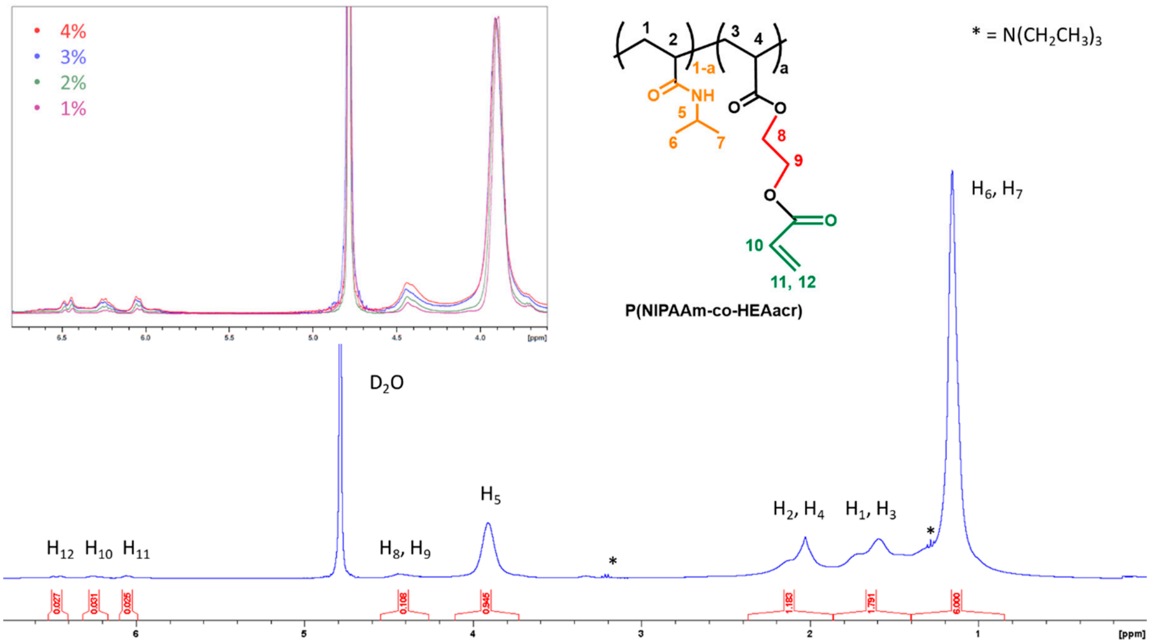

Fig. S1: Spectra of HEAacr: inset represents different incorporation ratio. Normalized to peak 3.91 ppm.

DIFFERENT RATIOS HEAACR

$\delta(ppm)$	*Proton*	*# protons*	*Intensity HEAacr1*	*Intensity HEAacr2*	*Intensity HEAacr3*	*Intensity HEAacr4*
6.46	H_{12}	1	0.007	0.019	0.027	0.030
6.24	H_{10}	1	0.006	0.019	0.031	0.039
6.05	H_{11}	1	0.007	0.017	0.025	0.030
4.79	D_2O	/	/	/	/	/

4.40	H_8, H_9	4	0.035	0.072	0.108	0.154
3.91	H_5	1	1.000	1.000	1.000	1.000
2.12	H_2, H_4	2	1.108	1.137	1.183	1.397
1.63	H_1, H_3	4	1.864	1.814	1.791	1.800
1.13	H_6, H_7	6	5.922	5.82	5.682	5.688

Table S1: Overview intensities measured in NMR spectra P(NiPAAm-co-HEAacr)

$$\frac{HEA}{NIPAAm} = \frac{I(\mathrm{H_8}, \mathrm{H_9})}{4} \quad e.g.(HEAacr3) = \frac{0.108}{4} = 2.7\ \%$$

$$\frac{HEAacr}{NIPAAm} = \frac{I(\mathrm{H_{10}}, \mathrm{H_{11}}, \mathrm{H_{12}})}{4}$$

$$e.g.(HEAacr3) = \frac{0.025 + 0.031 + 0.027}{3} = 2.7\ \%$$

Name	*Feed*	*Measured HEA (NMR)*	*Measured HEAacr (NMR)*
HEAacr 1%	1 %	0.88 %	0.67 %
HEAacr 2%	2 %	1.8 %	1.8 %
HEAacr 3%	3 %	2.7 %	2.7 %
HEAacr 4%	4 %	3.8 %	3.3 %

Table S2: Obtained incorporation ratios from NMR spectra for P(NiPAAm-co-HEAacr)

P(NiPAAm-co-cys)

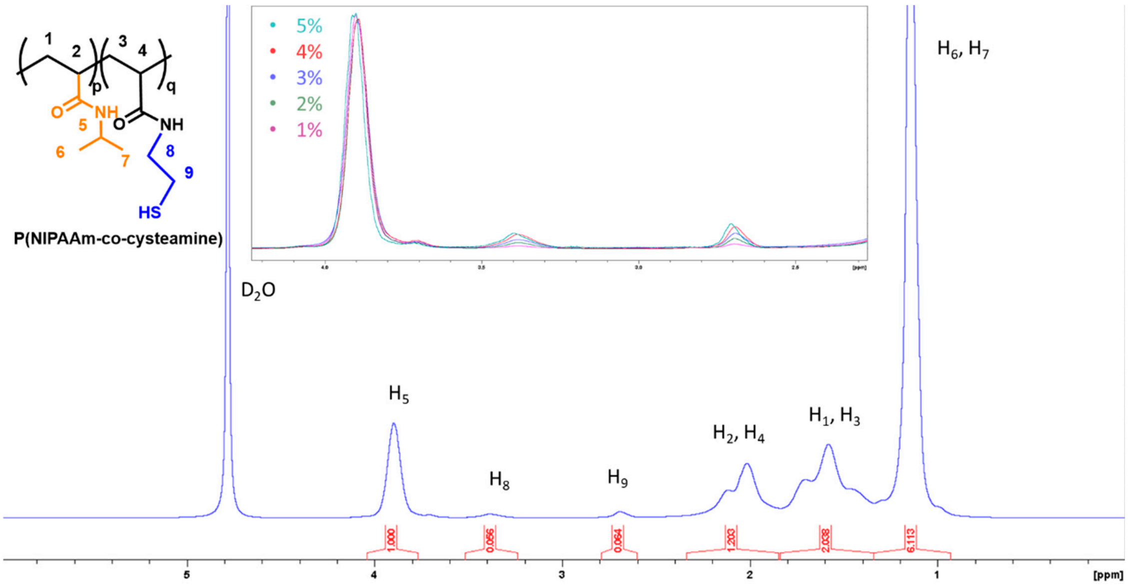

Fig.S2: Spectra of Cys3; inset represents different incorporation ratio. Normalized to peak 3.91 ppm.

$\delta(ppm)$	*Proton*	*# protons*	*Intensity Cys 1*	*Intensity Cys 2*	*Intensity Cys 3*	*Intensity Cys 4*	*Intensity Cys 5*
4.79	D_2O		/	/	/	/	/
3.90	H_5	1	1.000	1.000	1.000	1.000	1.000
3.39	H_8	2	0.017	0.035	0.056	0.076	0.095
2.69	H_9	2	0.021	0.039	0.064	0.078	0.091
2.10	H_2, H_4	2	1.131	1.193	1.203	1.235	1.126
1.60	H_1, H_3	4	1.909	1.875	2.038	1.880	1.977
1.14	H_6, H_7	6	6.024	6.091	5.988	6.192	6.000

Table S3: Overview intensities measured in NMR spectra P(NiPAAm-co-Cys)

$$\frac{Cys}{NIPAAm} = \frac{I(\mathrm{H_8}, \mathrm{H_9})}{4} \quad e.g.(Cys3) = \frac{0.056 + 0.064}{4} = 3.0\ \%$$

Name	*Feed*	*Measured (NMR)*
Cys 1%	1 %	0.95 %
Cys 2%	2 %	1.85 %
Cys 3%	3 %	3.00 %
Cys 4%	4 %	3.85 %
Cys 5%	5%	4.65 %

Table S4: Obtained incorporation ratios from NMR spectra for P(NiPAAm-co-Cys)

ELLMAN'S METHOD

Name	*Concentration solution (mmol/L)*	*Absorbance (A)*	*Percentage free thiols*	*Conversion*
CYS1	7.35	0.774	0.74%	74%
CYS2	2.77	0.690	1.76%	88%
CYS3	7.33	2.74	2.64%	88%
CYS4	3.41	1.88	3.89%	97%
CYS5	7.31	4.36	4.22%	84%

Table S5: : Obtained incorporation ratios from Ellman's method for P(NiPAAm-co-Cys)

$$molar\ absorption\ coefficient\ (\varepsilon) = 14\ 150\ M^{-1}cm^{-1}$$

$$Percentage\ free\ thiols\ = \frac{\frac{Absorbance\ (A)}{molar\ absorption\ coefficient\ (\varepsilon)}}{concentration\ solution}$$

$$Conversion = \frac{Percentage\ free\ thiols}{feed\ ratio}$$

FTIR SPECTROSCOPY

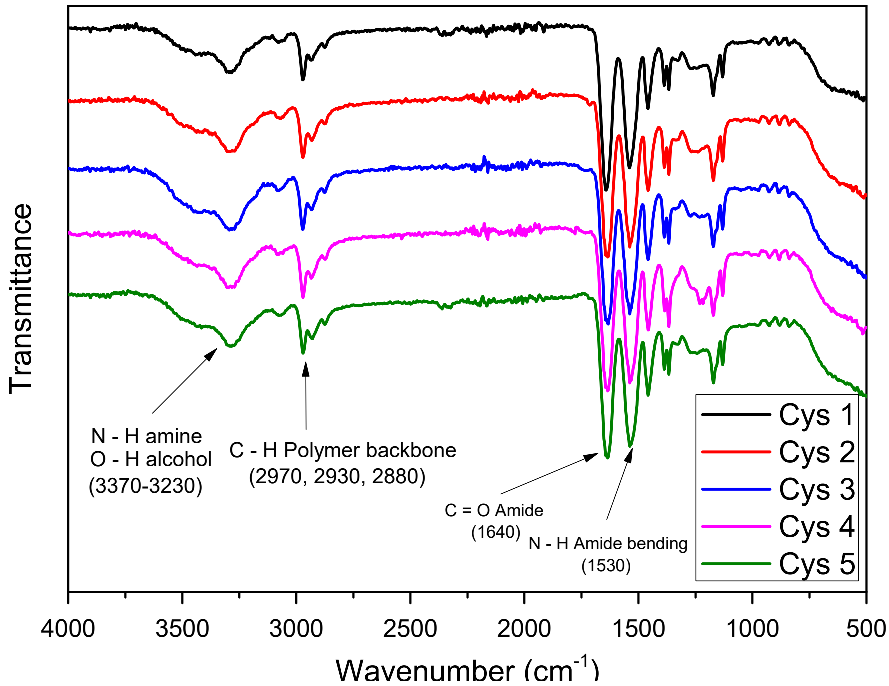

Fig.S3: FITR of Cys with different incorporation ratio and annotated peaks.

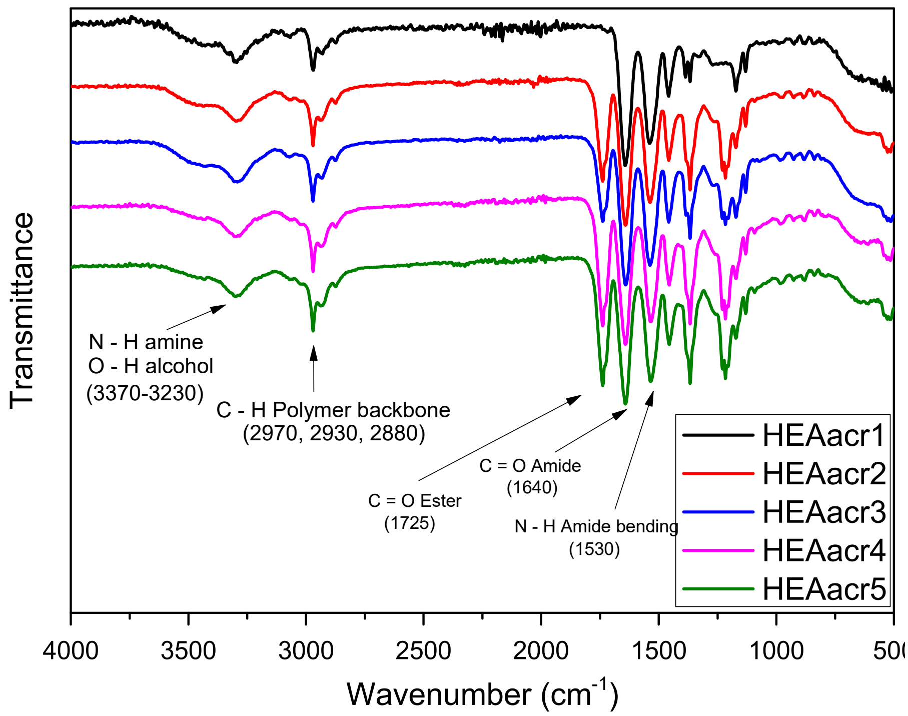

Fig.S4: FITR of HEAacr with different incorporation ratio and annotated peaks.

	Cold 4 °C				Hot 45 °C			
Name	Storage modulus G' (kPa)	Loss modulus G'' (kPa)	Complex modulus G* (kPa)	Loss factor tan(δ)	Storage modulus G' (kPa)	Loss modulus G'' (kPa)	Complex modulus G* (kPa)	Loss factor tan(δ)
Hydr 1%	0.33 ± 0.08	0.056 ± 0.002	0.34 ± 0.08	0.17 ± 0.04	10.1 ± 1.6	14.7 ± 2.8	18.8 ± 2.9	1.45 ± 0.23
Hydr 2%	6.55 ± 0.59	0.442 ± 0.163	6.56 ± 0.59	0.068 ± 0.03	*29.7 ± 5.5*	34.7 ± 6.6	45.7 ± 8.6	1.17 ± 0.01
Hydr 3%	11.0 ± 2.7	0.269 ± 0.129	11.0 ± 2.7	0.025 ± 0.01	*44.3 ± 11.9*	31.5 ± 9.9	54.4 ± 15.3	0.71 ± 0.07
Hydr 4%	3.08 ± 0.49	0.155 ± 0.054	3.08 ± 0.49	0.05 ± 0.01	*14.4 ± 1.4*	12.3 ± 1.3	18.9 ± 1.9	0.86 ± 0.03

Table S6: Numerical values of Figure 4, mechanical properties of hydrogels consisting of polymers with a different incorporation ratio determined by rheology

Frequency and strain sweep Hydr2%

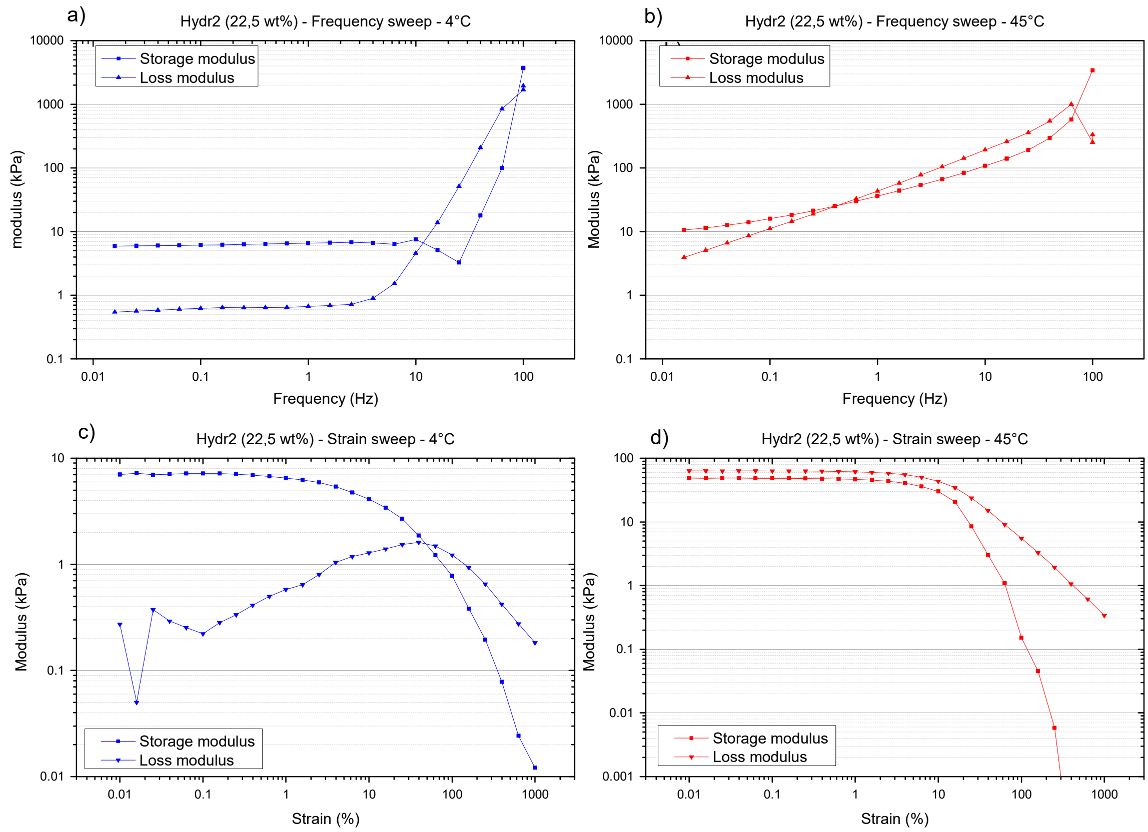

Fig.S5: Frequency and strain sweep for Hydr2% at 4°C and 45°C; Frequency and strain sweep for Hydr2% at 4°C (blue) and 45°C (red), a) frequency sweep at 4°C, b) frequency sweep at 45 °C, c) strain sweep at 4 °C, d) strain sweep at 45 °C

	Cold 4 °C		Hot 45 °C	
Name	**Young's modulus (kPa)**	**Relaxation modulus (kPa)**	**Young's modulus (kPa)**	**Relaxation modulus (kPa)**
Hydr 2%	**9.86 ± 1.56**	**5.98 ± 1.41**	***94.5*** **± 30.6**	**6.12 ± 1.14**
Hydr 3%	**21.7 ± 4.3**	**12.6 ± 3.4**	***181.1 ± 31.7***	**55.4 ± 26.1**

Table S7: Numerical values of Figure 9, mechanical properties of hydrogels consisting of polymers with a different incorporation ratio determined by rheology;

Compression – relaxation: data

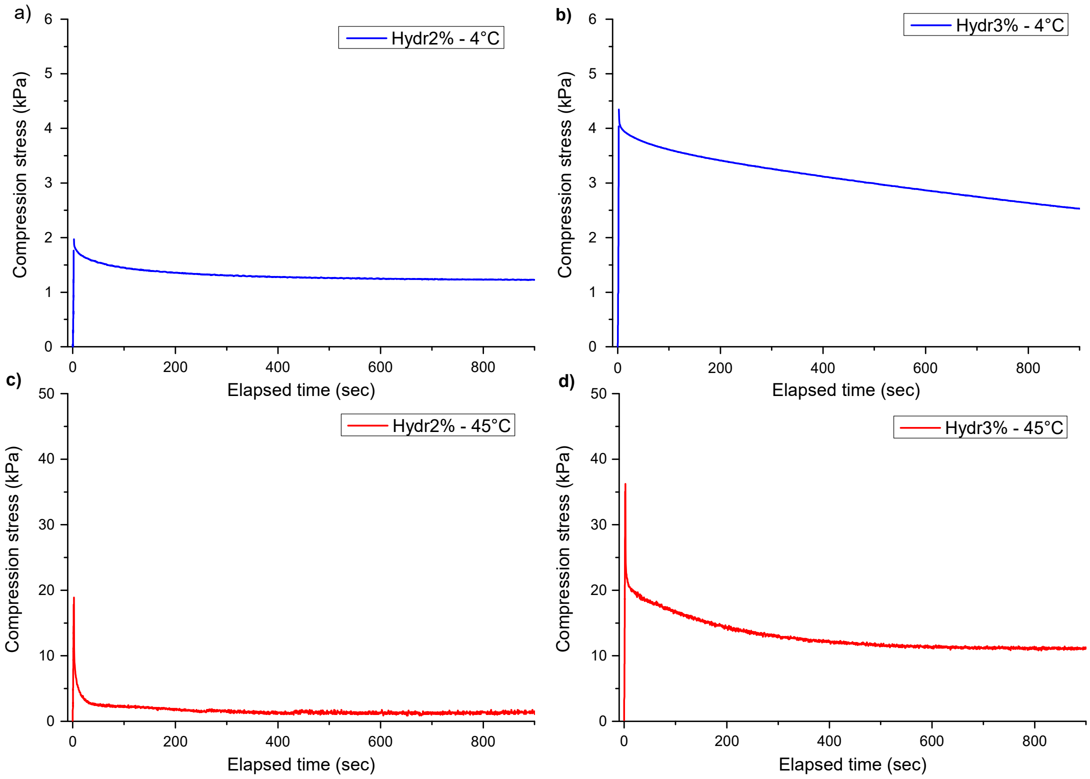

Fig.S6: Compression-relaxation test of hydr2% and hydr3% at 4 °C and 45 °C; a) hydr2% at 4 °C, b) hydr3% at 4 °C, c) hydr2% at 45 °C, d) hydr3% at 45 °C