Supplemental Material for
Anomalous diffusion in associative networks of high sticker-density polymers

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A. Supplementary Figures cited in the Main Text

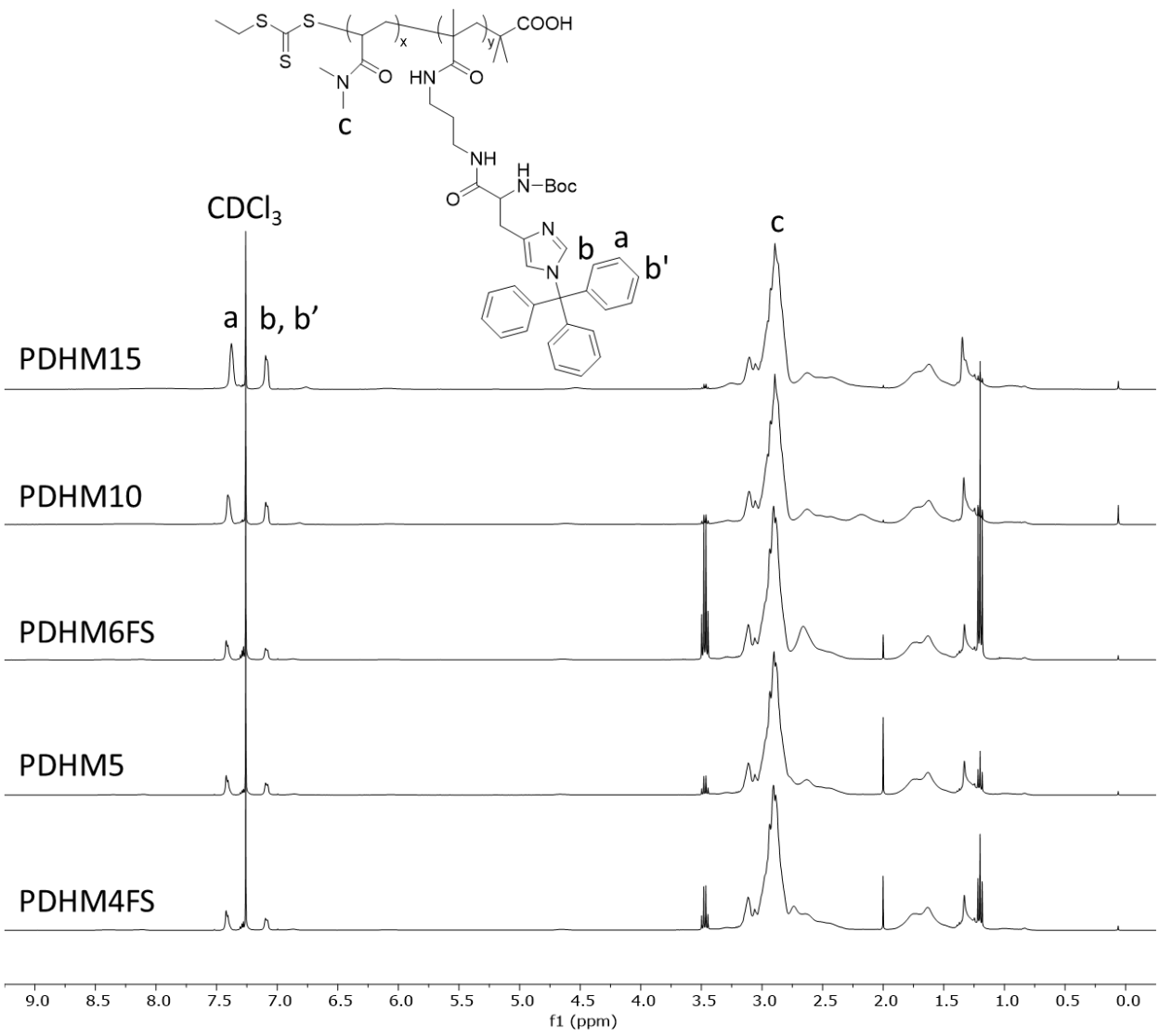


Figure S1. ^{1}H NMR characterization of PDMA polymers with protected histidine side groups P1 ($CDCl_3$, 400 MHz). Peaks assigned in the spectrum are used for calculating the monomer ratio in the polymers. For peak labelled c, the integration is from δ = 2.75 – 3.20 ppm.

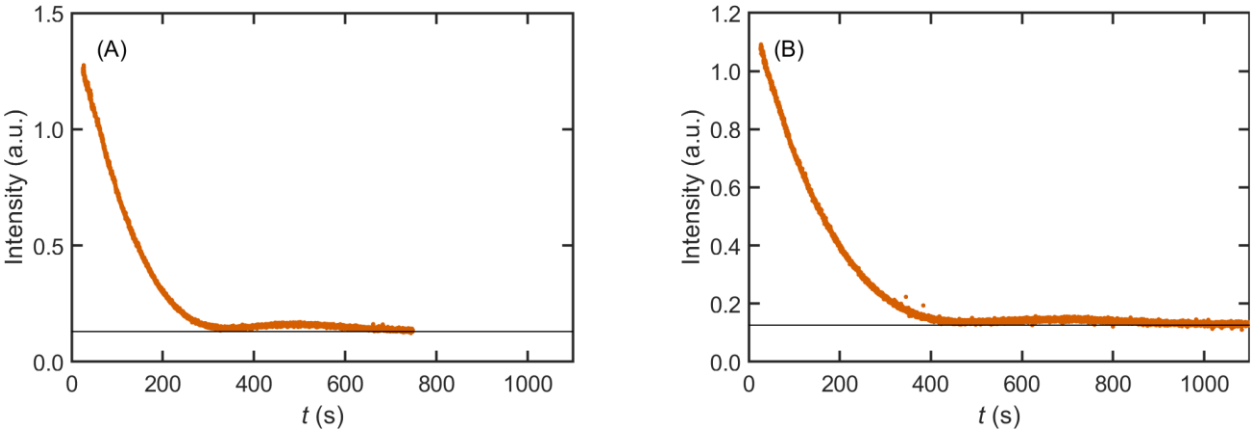


Figure S2. Sample data showing additional relaxation processes seen as a decay-growth-decay profiles for samples that were not equilibrated. Plot in panel A and B were collected from the same sample, but at different positions. Data acquired for PDHM10 30% (w/v) at $\theta = 10°$ ($d^2 = 61.3\ \mu m^2$) and temperature of 35 °C.

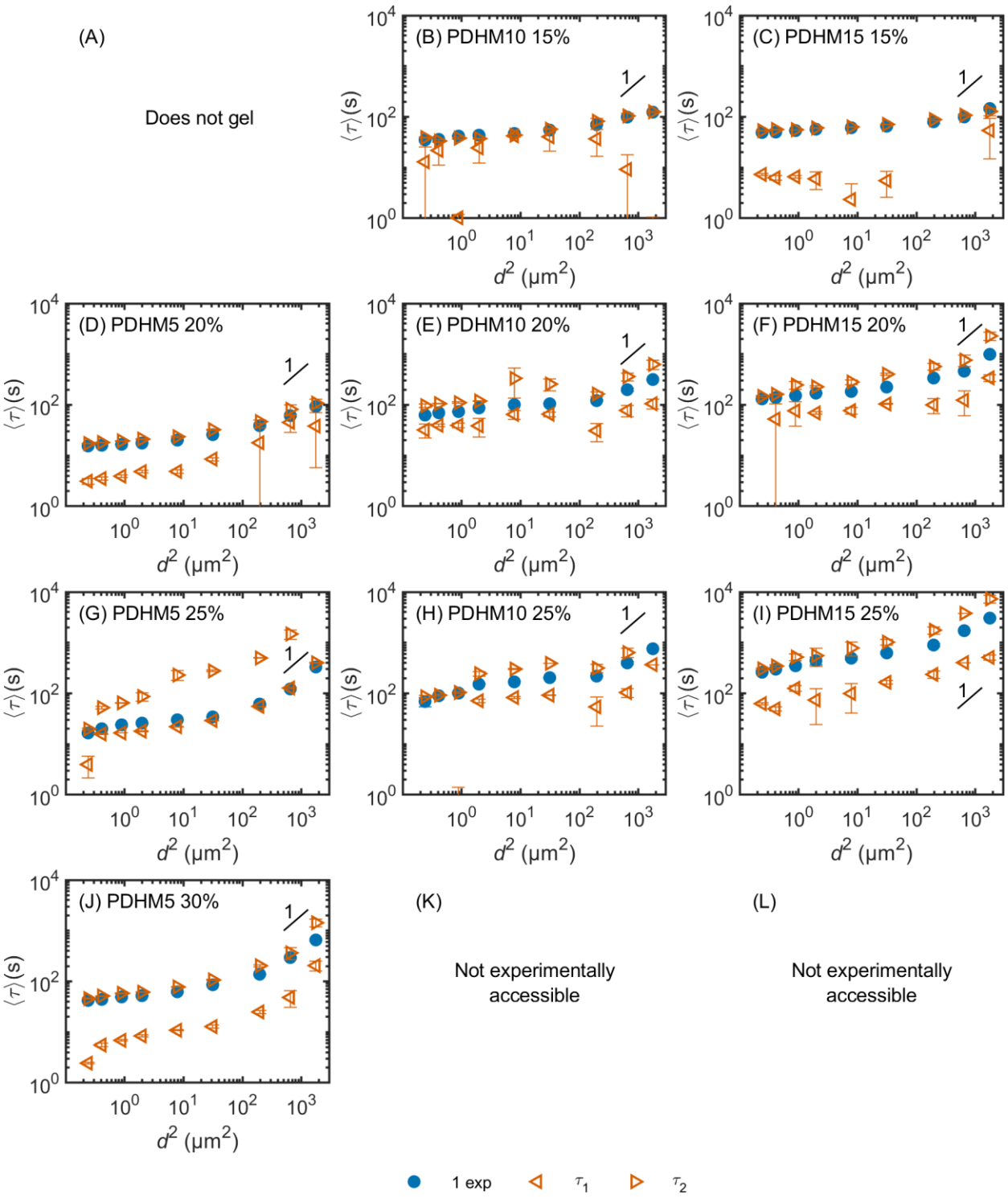


Figure S3. The plot of $\langle\tau_1\rangle$ and $\langle\tau_2\rangle$ from the fits to two exponential function, compared to $\langle\tau\rangle$ from the fit to a single exponential function for PDHM5 (D, G, J), PDHM10 (B, E, H) and PDHM15 (C, F, I) for concentrations ranging from 15% to 30% (w/v) as noted in each plot.

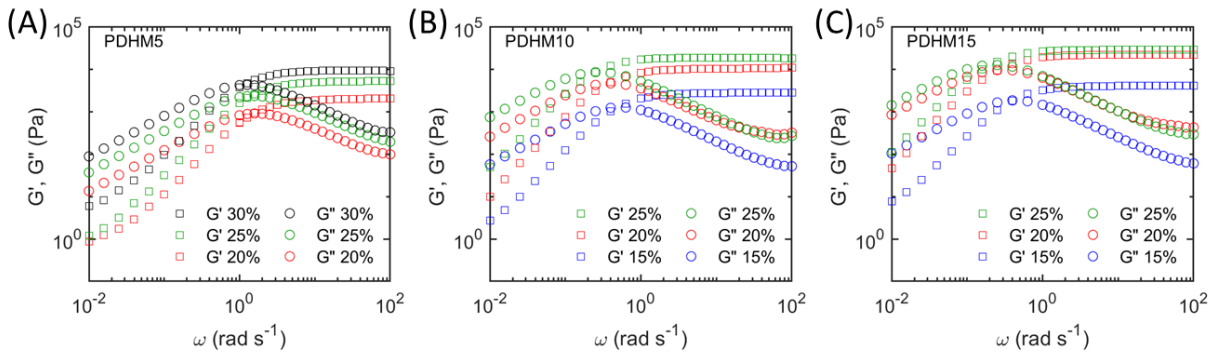


Figure S4. Frequency sweeps for (A) PDHM5, (B) PDHM10 and (C) PDHM15 at 15, 20, 25 and 30% (w/v). All experiments were conducted at 35 °C.

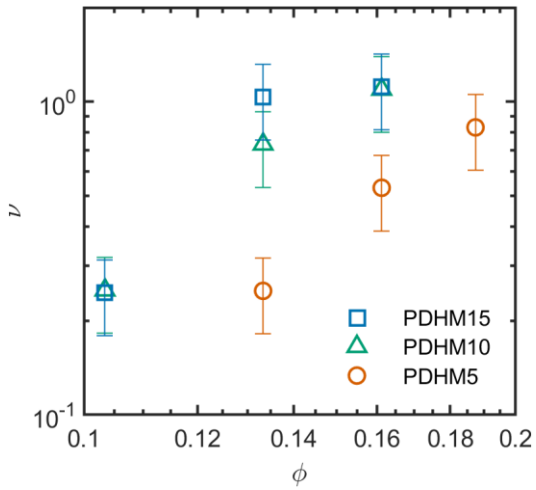


Figure S5. Estimated number fraction of elastically active strands as a function of concentration (v/v), based on the affine network model. Error bars are from uncertainty in the determination of the monomer size, a = 1.3 ± 0.1 nm.[1-2]

B. Calculation of the Debye length and distance between stickers

The Debye length, κ^{-1} is given by

$$\kappa^{-1} = \sqrt{\frac{\varepsilon\varepsilon_0 RT}{e^2 \sum_i z_i^2 c_i}} \quad (1)$$

where ε is the permittivity of water, ε_0 is the permittivity of free space, e is the electronic charge and $I = 0.5 \sum_i z_i^2 c_i$ is the ionic strength from all the ions in solution.

$$\kappa^{-1} = \sqrt{\frac{(8.85 \times 10^{-12}) \times 80 \times 8.31 \times 308}{(1.6 \times 10^{-19})^2 \times 2 \times 0.056}} = 1.3\ nm \quad (2)$$

The highest concentration of histidine-Ni^{2+} complexes used in this work was 0.05 M. The average spacing between the complexes in solution can be estimated as

$$Average\ spacing = \sqrt[3]{\frac{3(1 \times 10^{24}/(0.05 \times 6 \times 10^{23}))}{4\pi}} = 2\ nm \quad (3)$$

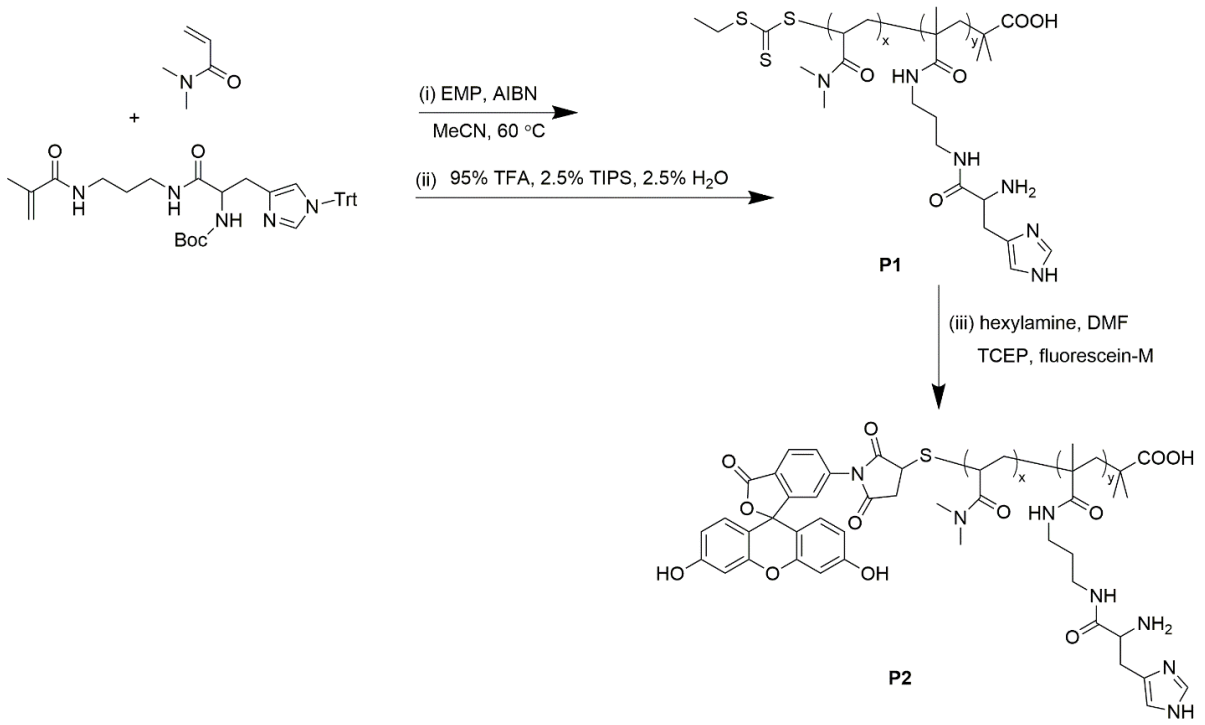
The smallest estimated spacing between complexes is larger than the Debye length.

C. Polymer synthesis and characterization

Materials. Boc-His(Trt)-OH was purchased from Chem-Impex International Inc. (WoodDale, IL). N-(3-Aminopropyl)methacrylamide hydrochloride was purchased from Polysciences Inc.(Warrington, PA). Fluorescein-5-maleimide was purchased from ThermoFisher Scientific. N-Boc-Nim-trityl-N-3-methacrylamidopropyl-L-Histidinamide (HisMA) [3] and 2-(ethylthio-carbonothioylthio)-2-methylpropionic acid (EMP) [4] were synthesized following published procedures. *N,N*-Dimethyl-acrylamide (DMA) was purified through a basic alumina column to remove inhibitor before polymerization. All other chemical reagents were purchased from Sigma-Aldrich or VWR and used as received.

Characterization. NMR spectra were recorded on a Varian 400 MHz spectrometer. The residual undeuterated solvent peaks were used as references (7.27 ppm for $CDCl_3$ and 4.79 ppm for D_2O). Gel permeation chromatography (GPC) measurements were performed on an Agilent 1260 LC system with two ResiPore columns (300 × 7.5 mm, Agilent Technologies, Santa Clara, CA) in series at a flow rate of 1 mL/min at 70 °C, where DMF with 0.02 M LiBr was used as the mobile phase. The molecular weights were determined using a Wyatt miniDAWN TREOS multiangle light scattering detector and a Wyatt Optilab T-rEX differential refractive index detector. Liquid chromatography−mass spectrometry (LC-MS) analysis was performed using an Agilent 1260 Infinity LC system coupled with a 6130 quadrupole mass spectrometer. A mixture of 0.1% formic acid in water and MeCN was used as the mobile phase.

Scheme 1. Synthesis of Linear PDMA Polymers with Pendant Histidine Side Groups



Synthesis of PDMA Polymers with Pendant Histidine Side Groups (P1). Copolymers of DMA and HisMA were synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization (Scheme 1). The total monomer concentration in polymerization was 2.0 M, and the ratio of EMP/azobisisobutyronitrile (AIBN) was 1:0.2. For polymers with fixed molecular weight, the ratio of monomers/EMP was 215:1. The amount of HisMA added varied between 2 – 7.5 mol% of the total monomer concentration to achieve the targeted sticker densities. All polymerizations were performed in MeCN at 60 °C for approximately 8 h. Once the desired conversion of around 80% was achieved, as determined by DMF GPC, the reaction flask was exposed to air. Polymers were purified by precipitation into diethyl ether and dried under vacuum. The mole fraction of HisMA in the polymer P1 was determined by ^{1}H NMR (Figure S1). The molecular weight of polymer P1 was characterized by DMF GPC prior to the deprotection step (Figure S6, Table S1).

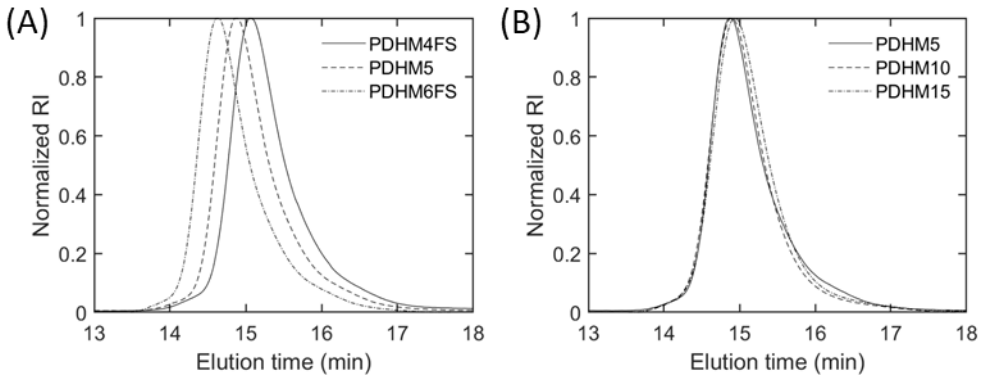


Figure S6. DMF GPC characterization of all polymers prior to deprotection. All samples were prepared at 10 mg/mL in DMF with 0.02 M LiBr. All polymers have dispersity below 1.05.

Table S1. The weight average molar mass and dispersity of each polymer determined by DMF GPC as polymerized, the calculated molar mass after deprotection and the radius of gyration for each polymer.

Polymer	M_w (kg/mol)	Đ	M_w after deprotection (kg/mol)	R_g (nm)[a]	R_g (nm)[b]
PDHM4FS	21.1	1.03	19.6	9 ± 1	9
PDHM5	26.6	1.03	24.7	11 ± 1	10
PDHM6FS	36.4	1.03	34.0	12 ± 1	12
PDHM10	30.7	1.04	27.3	10 ± 1	11
PDHM15	35.8	1.03	30.4	11 ± 1	11

[a] From tandem gel permeation chromatography (GPC) (Waters)-multiangle laser light scattering (MALLS) (Wyatt Technologies). [b] Estimated from scaling arguments, based on ref. [5].

To remove the Boc and Trt protecting groups, the resulting polymers (1000 mg) were dissolved in DCM (16.7 mL). Water (416.7 µL), triisopropylsilane (TIPS, 416.7 µL), and trifluoracetic acid (TFA, 16.7 mL) were sequentially added to the solution. The mixture was stirred at room temperature for 2h. The volatiles were then removed under vacuum, and the residue was dissolved in MeOH. The polymers were recovered by precipitation into diethyl ether twice. The polymers were then dissolved in 140 mL water, transferred to a centrifugal filter (3 kDa MWCO), and spun at 4000 g for 1 hr. A further 140 mL was added, and the filtration was repeated four times. The polymers were then filtered through a 0.45 µm hydrophilic PVDF syringe filter and lyophilized. Complete removal of the Boc and Trt groups was evidenced by ^{1}H NMR (Figure S7). See supporting information for mass and volumes of reactants used and the yield for each polymer (Table S2 and Table S3).

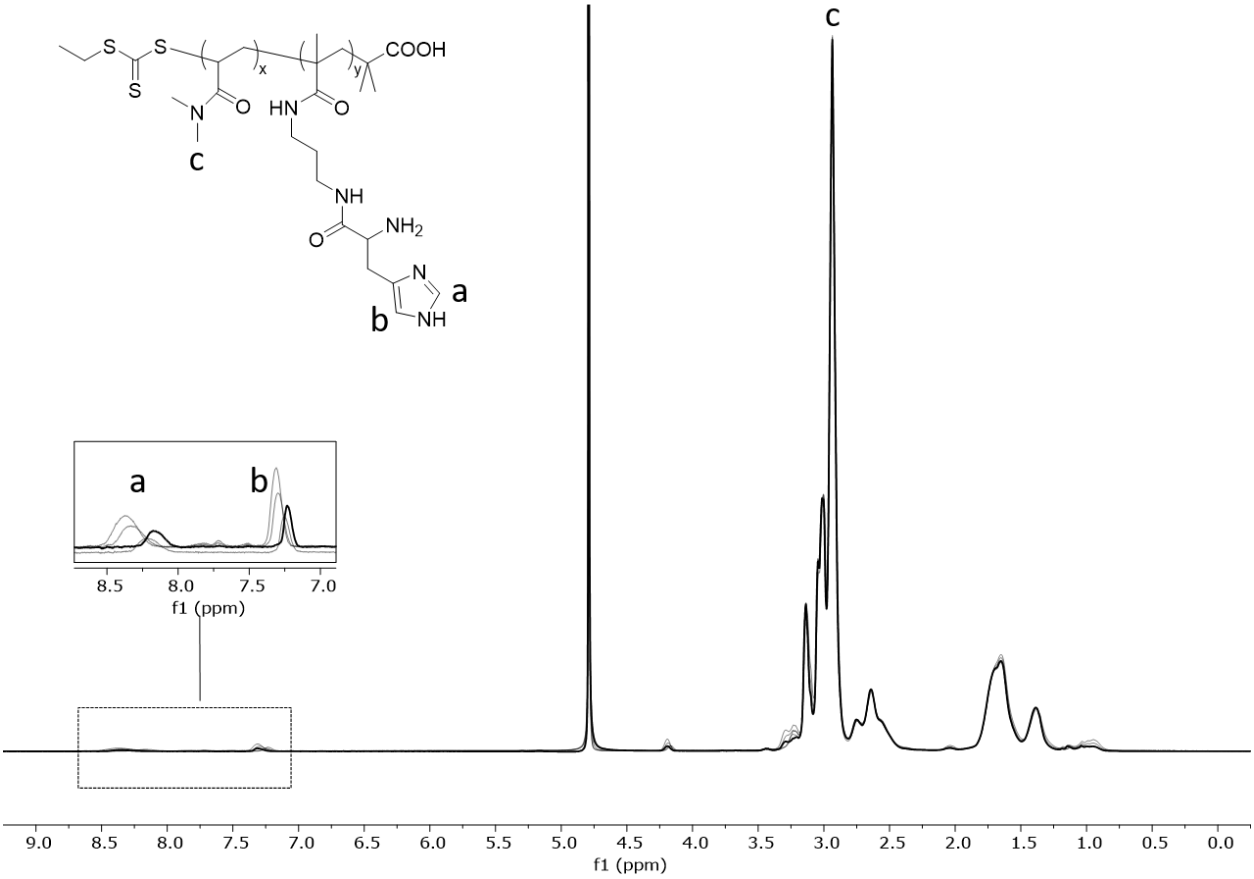


Figure S7. ^{1}H NMR characterization of PDMA polymers with histidine side groups, after deprotection (D_2O, 400 MHz). The positions of peaks a and b shift slightly due to hydrogen bonding with residual TFA in the polymer.

Table S2. The mass and volume of reactants used for the synthesis of PDMA polymers with pendant histidine side groups.

Polymer	Volume of DMA (mL)	Mass of HistMA (mg)	Mass of EMP (mg)	Mass of AIBN (mg)	Volume of MeCN (mL)	Yield (g)
PDHM4FS	2.27	334	27.13	4.0	11.3	2.20
PDHM5	2.27	334	22.61	3.3	11.3	2.21
PDHM6FS	2.27	334	16.96	2.5	11.3	2.22
PDHM10	1.00	236	10.54	1.54	5.05	1.03
PDHM15	0.98	349	10.54	1.54	5.05	1.16

Table S3. The mass and volume of reactants used for the deprotection of PDMA polymers with pendant histidine side groups.

Polymer	Mass of polymers (mg)	Volume of DCM (mL)	Volume of water (μL)	Volume of TIPS (μL)	Volume of TFA (mL)	Yield (mg)
PDHM4FS	1000	16.7	416.7	416.7	16.7	842.2
PDHM5	1000	16.7	416.7	416.7	16.7	858.3
PDHM6FS	1000	16.7	416.7	416.7	16.7	809.2
PDHM10	900	16.7	416.7	416.7	16.7	726.5
PDHM15	900	18.3	458.4	458.4	18.3	724.5

Synthesis of Fluorescein-Labeled PDMA Polymers (P2). Note that the mass and volume of reactants used is calculated based on each polymer's molecular weight after deprotection (Table S4). For PDHM5, the deprotected polymers (100 mg, 4 μmol) were first dissolved in DMF, and then hexylamine (10.6 μL, 80 μmol) was added. The reaction was stirred overnight under a

nitrogen atmosphere to ensure complete aminolysis and to minimize undesirable cysteine oxidation. Then tris(2- carboxyethyl)phosphine hydrochloride (TCEP·HCl, 11.5 mg, 40 μmol) and maleimide-functionalized fluorescein (17.4 mg in 400 μL of DMSO, 40 μmol) were added to the reaction mixture. After the reaction was stirred overnight in the dark, the solution was diluted with 5% DMSO in water. The mixture was transferred to a centrifugal filter (3 kDa MWCO), spun at 4000g for 1 h at 4 ℃, and more solution of 5% DMSO in water was added. This process was repeated several times until the spin-through fraction was colorless. A final spin with water was then used to remove the DMSO. Polymers were filtered through a 0.45 μm hydrophilic PVDF syringe filter and lyophilized. See supporting information for mass and volumes of reactants used and the yield for each polymer.

Table S4. The mass and volume of reactants used for the synthesis of fluorescein labelled PDMA polymers with pendant histidine side groups.

Polymer	Mass of polymers (mg)	Volume of DMF (mL)	Volume of hexylamine (μL)	Mass of TCEP-HCl (μL)	Mass of fluorescein-M (mL)	Yield (mg)
PDHM4FS	25	1.3	3.3	3.6	5.3 (in 123 μL DMSO)	10.2
PDHM5	100	5.0	10.6	11.5	17.4 (in 400 μL DMSO)	45.0
PDHM6FS	25	1.3	1.9	2.1	3.1 (in 72 μL DMSO)	15.3
PDHM10	50	2.5	4.5	4.9	7.4 (in 170 μL DMSO)	32.9
PDHM15	50	2.5	3.8	4.1	6.3 (in 145 μL DMSO)	22.4

Rheology. Frequency sweep experiments were performed on an Anton Paar 301 Physica rheometer with a stainless-steel cone and plate geometry (25 mm in diameter, 1° angle). Inertial calibration and motor adjustment were performed before each measurement. Gel samples were centrifuged at 21,100g for 10 min at 4 °C to remove bubbles before loading onto the rheometer. Mineral oil was added to the sample edge to minimize dehydration. Experiments were performed at four temperatures, 5, 15, 25, and 35 °C, where the temperature was controlled by a Peltier plate. Experiments were performed at 1% strain, which was within the linear viscoelastic (LVE) region as determined by strain sweep experiments (Figure S8).

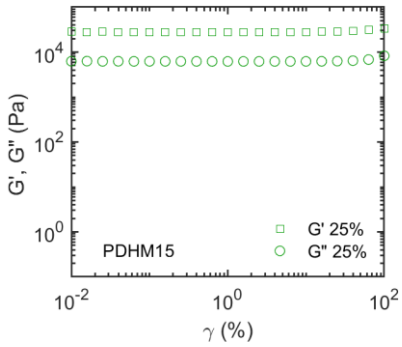


Figure S8. Typical data for amplitude sweeps. Data obtained for PDHM15 at 25% (w/v). Experiment performed with $\omega = 10 rad/s$ and at 35 °C.

D. Note on the use of TFA for deprotection and pH of the gels

While TFA is a common choice for deprotection chemistry, it is difficult to completely remove TFA from the product following the reaction. For the gels used in this study, the residual TFA alters the pH of the gels and the properties of histidine-nickel coordinate bonds have been shown to strongly depend on pH.[6] To avoid issues with reproducibility of the gel properties, the polymers are dissolved in Milli-Q water and spun down using centrifugal filters with a MWCO of 3 kDa. This process is repeated four times and is successful at removing most of the residual TFA, as evidenced by the shift in the position of peaks a and b in Figure S7. Prior to the filtration steps, the peak positions correspond to histidine-TFA salt at approximately 7.4 ppm and 8.75 ppm.[3]

Following the filtration step, the volume of 1 M NaOH in 100 mM bis-tris required to adjust the pH of the solution to seven was determined by titration with dilute solution. The polymers were dissolved in a solution of 0.1 M KCl, with a concentration of approximately 15 mg in 1 mL. The pH was recorded with a pH probe while aliquots of 0.1 M KOH were titrated into the solution (Figure S9). The volume of 0.1 M KOH needed to arrive at pH 7 is used to calculate the volume of 1 M NaOH stock solution added for each polymer.

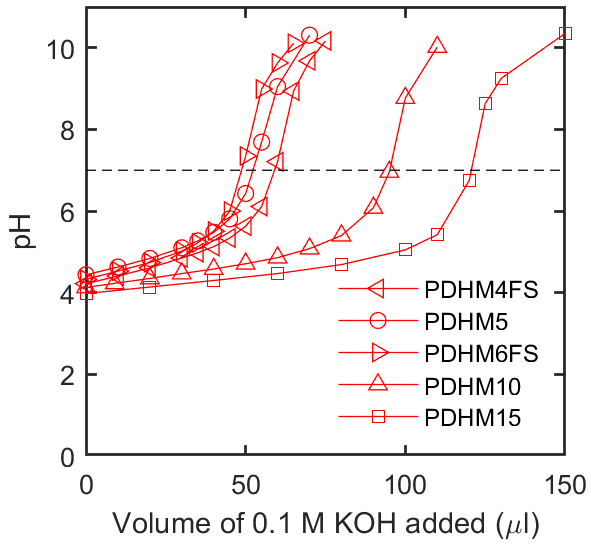


Figure S9. After deprotection and removal of residual TFA by filtration with water, ~15 mg of the polymers [PDHM4FS (15.0 mg), PDHM5 (15.1 mg), PDHM6FS (15.5 mg), PDHM10 (15.1 mg) and PDHM15 (15.0 mg)] was dissolved in 1 mL of 0.1 M KCl. To each solution, the appropriate amount of Ni^{2+} was added such that the ratio of His:Ni^{2+} was 2:1. The pH values were then recorded as aliquots of 0.1 M KOH were added.

E. Error estimation for fits using one or sum of two exponential functions

The effect of using the values of $\langle\tau\rangle$ instead of $\langle\tau_2\rangle$ for the PDHM10 gels can be seen in the plots of $\langle\tau\rangle$ vs d^2 in Figure S10 and the two-state model parameters in Figure S11. Figure S10 (B) shows that the scatter in the plot for the PDHM10 gels, most apparent for gels at 20 and 25% (w/v), leads to two-state model fits that are skewed by the scatter. Additionally, comparison of two-state model fit parameters (Figure S11) for the PDHM10 gels to the PDHM5 and PDHM15 gels further indicate that these results are skewed by the error associated with the fits of the decay curves to the sum of two exponential fits for the PDHM10 gels, and these deviations are not related to the sticker density itself.

From Figure S3, the largest difference between $\langle\tau\rangle$ and $\langle\tau_2\rangle$ is seen with PDHM15 gels at the highest concentration of 25% (w/v). Thus, an error calculation for this data set represents the upper bound of the error associated with the approximation when using the single exponential fit. From the two-state model fit to the $\langle\tau_2\rangle$ plot of Figure S10 (C), the model parameters were extracted and compared to the parameters obtained for $\langle\tau\rangle$ (Figure S11). The difference between the parameters were approximately, $D_{\mathrm{M,eff},\langle\tau\rangle} \sim 2.5 \times D_{\mathrm{M,eff},\langle\tau_2\rangle}$, $k_{,off,\langle\tau\rangle} \sim 1.5 \times k_{off,\langle\tau_2\rangle}$ and $\gamma K_{eq,\langle\tau\rangle} \sim 0.39 \times \gamma K_{eq,\langle\tau_2\rangle}$. Compared to the order of magnitude difference to the values of these parameters for the PDHM5 polymers, a factor of two is not a significant error.

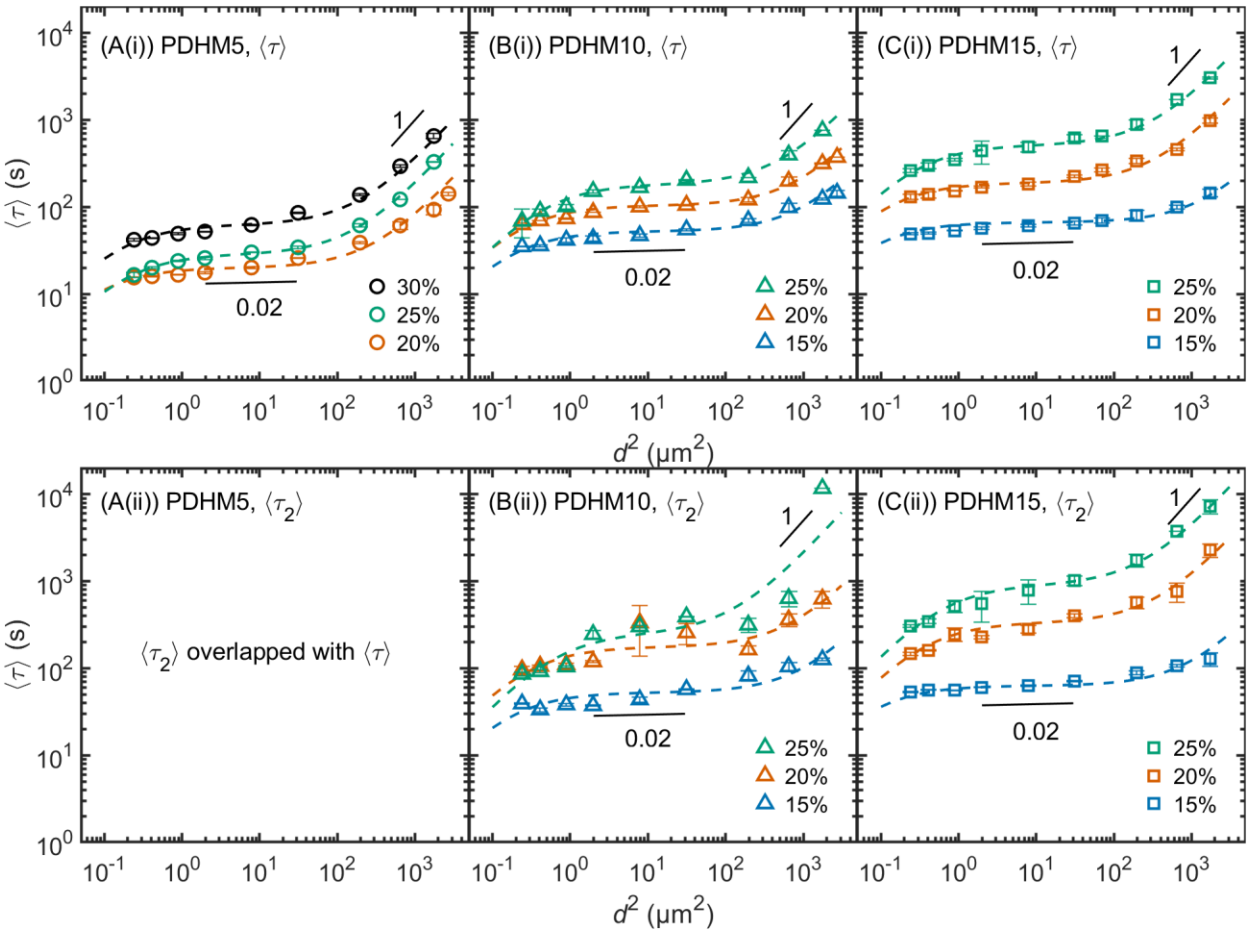


Figure S10. Plot of $\langle\tau\rangle$ vs d^2 for (A) PDHM5, (B) PDHM10 and (C) PDHM15 at 15%, 20%, 25% and 30% (w/v), measured at 35 °C. The dashed lines are fits to the two-state model. Error bars represent standard error of measurements performed in triplicate. Top row (A(i), B(i), C(i)) shows $\langle\tau\rangle$ from single exponential fits for all the gels, while bottom row (B(ii), C(ii)) show $\langle\tau_2\rangle$ from the sum of two exponential fits for PDHM10 and PDHM15.

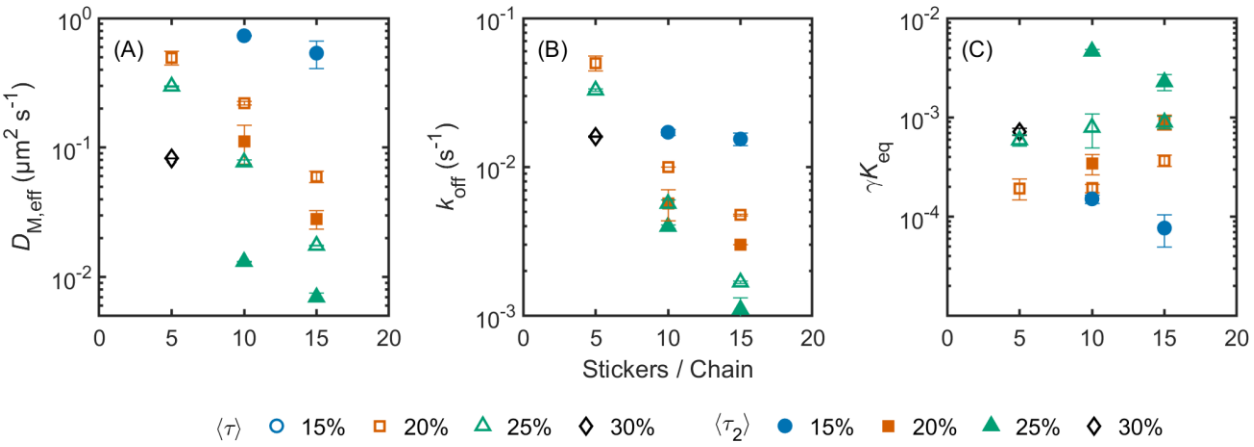


Figure S11. Effect of sticker density on (A) the effective diffusivity in the large-length-scale Fickian regime, $D_{M,eff}$ (B) the molecular dissociation rate k_{off}, and (C) γK_{eq}. The parameters were obtained by fitting the analytical solution of the two-state model to the experimentally derived relation $\langle\tau\rangle$ vs d^2 for gels at various concentrations. Error bars represent 95% confidence intervals from fits to the two-state model. Unfilled symbols show two-state model fit parameters to $\langle\tau\rangle$ from single exponential fits for all the gels, while filled symbols show two-state model fit parameters to $\langle\tau_2\rangle$ from the sum of two exponential fits for PDHM10 and PDHM15.

F. Scaling of diffusivity from sticky Rouse model

The scaling for the chain diffusivity in the sticky Rouse model can be derived from the relation proposed by de Gennes where $D \approx R^2/\tau$, with R as the radius of gyration and τ is the sticky Rouse time. Assuming R has no dependence on the sticker density, then $D \sim \tau^{-1}$ and τ has a dependence on sticker density that varies with the concentration relative to the sticker overlap concentration ϕ_s, as defined in the sticky Rouse model. For $\phi < \phi_s$, $\tau \sim S^{-0.675}$ so that $D \sim S^{0.675}$ and for $\phi > \phi_s$, $\tau \sim S^3$ such that $D \sim S^{-3}$. Thus, the diffusivity is predicted to increase with sticker density for lower concentrations, before it decreases with sticker density for gels formed at above the sticker overlap concentration.

G. Additional NMR Spectra

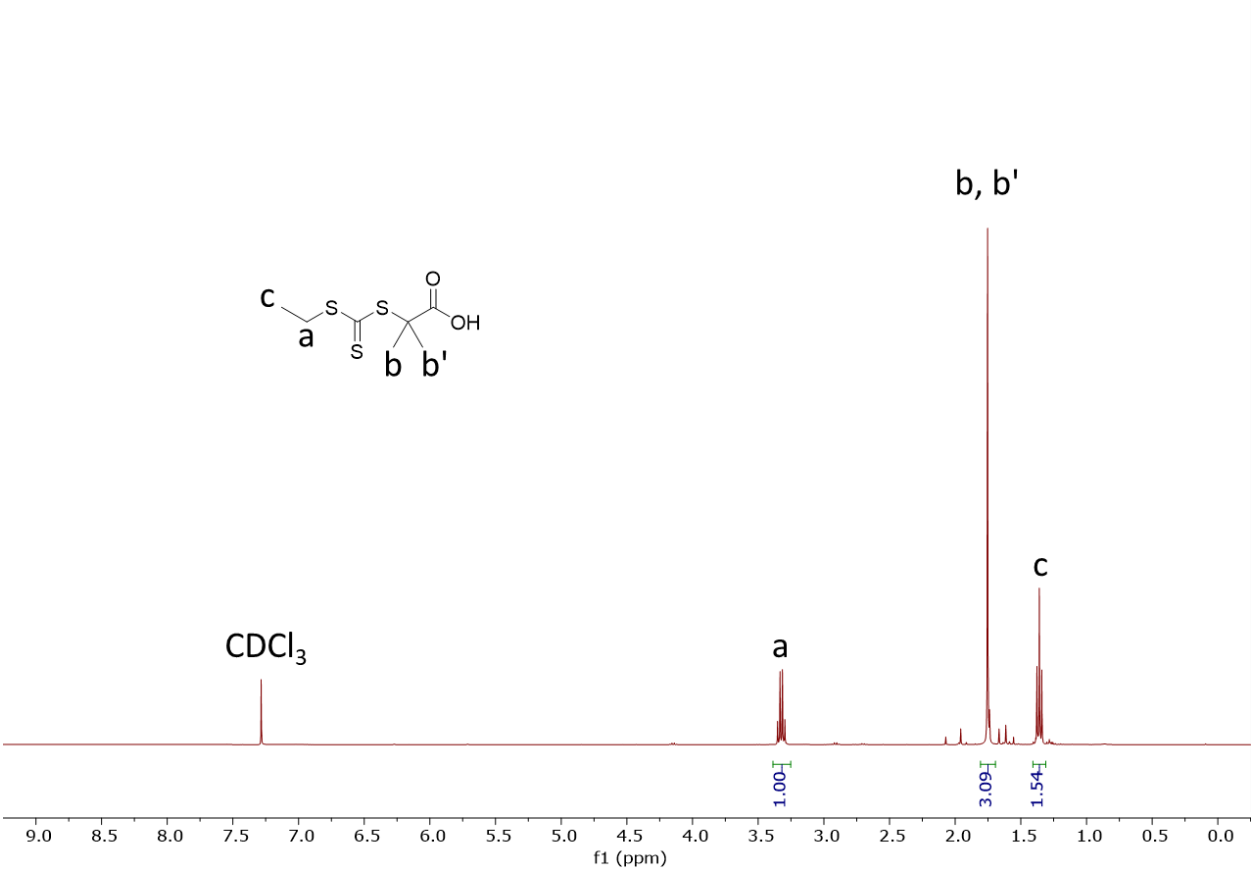


Figure S12. ^{1}H NMR characterization of 2-(((ethylthio)carbonothioyl)thio)-2-methylpropanoic acid ($CDCl_3$, 400 MHz).

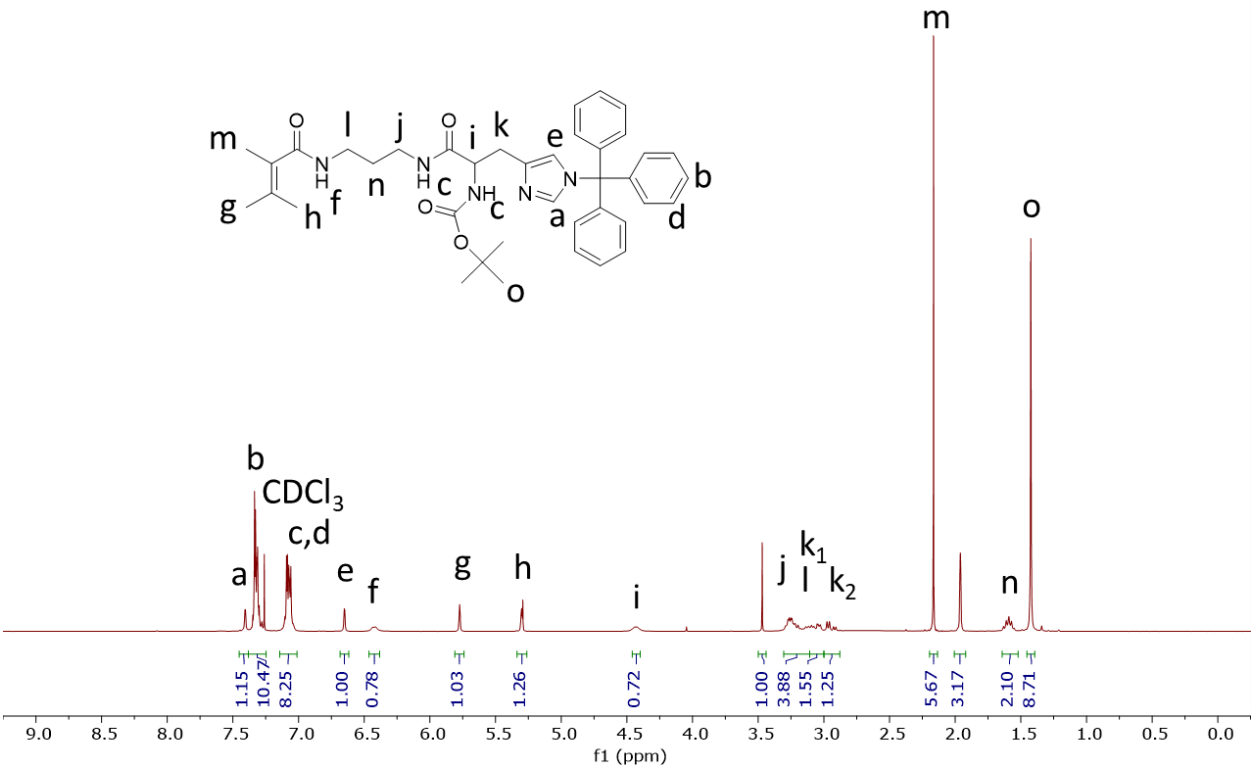


Figure S13. ^{1}H NMR characterization of Boc-Nim-trityl-N-3-methacrylamidopropyl-L-Histidinamide ($CDCl_3$, 400 MHz).

H. References

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