Supplementary Information:

Chemical Insights into Dodecylamine Spore Lethal Germination

Luo Mi, Andrew D.White, Qing Shao, P. Setlow, Yongqing Li and Shaoyi Jiang

Materials and Methods

Chemicals:

Dodecylamine (DDA), NaCl, $CaCl_2$, $NiCl_2$, $ZnSO_4$, $CuCl_2$, $FeCl_3 \bullet 6H_2O$, [2-(acryloyloxy)ethyl]trimethylammonium chloride (TMAEMA), 2-carboxyethyl acrylate (CAA), 3-[dimethyl-[2-(2-methylprop-2-enoyloxy) ethyl]azaniumyl] propane-1-sulfonate (SBMA), 2-mercaptoethanol, dodecyl trimethylammonium chloride (DTAC), 1H-pyrazole- 1- carboxamidine hydrochloride, 4,4'-azobis(4-cyanovaleric acid) (V-501), 4-cyano-4-[(dodecylsulfanylthiocarbonyl) sulfanyl]pentanoic acid (CTA), N,N'-methylenebis(acrylamide) (MBAA), 3,5-Dimethyladamantane-1-acetic acid (DMAA), 2-hydroxy-2-methylpropiophenone and 1,12-diaminododecane, methacrylic anhydride were purchased from Sigma-Aldrich Chemical Co. (MO, USA); 2-aminoethyl methacrylate hydrochloride (AMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), 2-hydroxyethyl methacrylate glycol methacrylate (HEMA), and urea were purchased from Acros Organics (PA, USA); acrylic acid was purchased from Alfa Aesar (MA, USA); sodium dodecyl sulfate was purchased from Fisher Scientific (PA, USA); N-[(3,5-dimethyl-1-adamantyl)methyl]guanidine hydrochloride (DMAG) was purchased from Chembridge Co. (CA, USA)

Nutrient Broth No1 was purchased from Fluka Analytical (MO, USA); Bacto Agar was purchased from BD (NJ, USA); *Bacillus megaterium* spore stock solution was purchased from Mesa Laboratories, Inc. (MT, USA).

Dodecyl guanidine (DDG) was synthesized based on a previously published method [1]. In a typical reaction, 2.93 g 1H-pyrazole- 1- carboxamidine hydrochloride, 3.71 g dodecyl amine (DDA), and 2.58 g N,N-diisopropylethlamine (DIEA) was added to 10ml dimethylformamide (DMF), and the reaction was vigorously stirred for 18 h. Dry ether was then added into the reaction to precipitate out the crude product. This crude product was then further purified with a silica gel column using methanol and dichloromethane mixed solvent as the eluent. The resulting compound was characterized by 1H NMR (300 MHz, MeOD): δ3.19 p.p.m. (t, 2H), δ1.56 p.p.m. (m, 2H), δ1.32 p.p.m. (m, 2H), δ1.13 p.p.m (m, 16H), δ0.86 p.p.m. (t, 3H), and 13C NMR (300 MHz, MeOD): δ 158.59, 42.56, 33.12, 30.54, 27.78, 23.80, 14.63.

12-methacrylamidododecan-1-aminium chloride (MDA) was synthesized based on a previously published method [2]. In a typical reaction, 5 g protonated 1, 12-diaminododecane was first mixed with 8.09 g unprotonated 1,12-diaminododecane in 100 ml H_2O. The suspension was vigorously stirred for 1 h. 100 ml methanol was then added to the reaction flask, and the reaction was cooled to 0^{o}C on an ice bath. In a separate flask, 5.47 ml methacrylic anhydride and trace amount of hydroquinone was mixed with 15 ml methanol. After adding the solution of the second flask to the original mixture, the whole reaction was kept at 0^{o}C for 2 h. Following this, the system was acidified to pH 1 and left for 24 h. At the end of the 24 h reaction, solvent was removed on a rotary evaporator; the crude product was washed extensively with diethylether and then dissolved in acetic acid. The insoluble portion was filtered out on a Buchner funnel, and organic phase was subsequently dried on a rotary evaporator, resulting a white powder. The product was characterized by 1H NMR (300 MHz, DMSO): δ5.79 p.p.m. (s, 1H), δ5.41 p.p.m. (s, 1H), δ3.33 p.p.m. (t, 2H), δ3.11 p.p.m. (t, 2H), δ1.98 p.p.m. (s, 3H), δ1.77 p.p.m. (m, 2H), δ 1.56 p.p.m. (m, 2H), δ 1.34 p.p.m. (m, 16H).

RAFT Polymerization of AMA Monomer

AMA monomer was polymerized via reversible addition-fragmentation chain-transfer polymerization (RAFT) based on a previously published procedure that was shown to have good control over the degree of polymerization (DP) and yield a narrow polydispersity [3]. In a typical reaction, 3.33 g AMA monomer was dissolved in 12ml H_2O/dioxane 3:1 mixed solvent. The amount of chain transfer agent (CTA) was added based on the desired DP. Initiator V501 amount was kept at 1/5 of the CTA in all RAFT reactions. The solution was then deoxygenated by bubbling with dry nitrogen gas for 1h. Following this, the reaction was heated to 70^{o}C and stirred vigorously. After 24 h, the reaction solution was characterized by 1H NMR to determine the polymerization conversion. The polymer was then purified by dialysis and finally dried via lyophilization. The degree of polymerization was calculated based on the initial monomer/CTA ratio and the conversion of the reaction.

Spore Lethal Germination Assay

In a typical assay of spore germination and lethality, bacterial spores were suspended in 100 mM NaCl or 150 mM PBS (Phosphate Buffer Saline) solutions containing the testing molecules for 30min under predetermined pH and temperature. After incubation, spore suspensions were, in the case of testing cationic surfactants, quenched with 1 mM SDS, diluted and spread onto Broth I agar plates (10g/L tryptone. 10g/L NaCl, 5g/L yeast extract and 1g/L glucose) for overnight culture at 37^{o}C. Broth I agar was verified in this study to completely germinate *B. megaterium* spores without the need for prior heat activation, thus ensuring that the difference in colony formation only comes from loss of viability in surfactant treatment and not from the incomplete germination during subsequent incubation. The viable spore number was determined by counting the colonies formed the following day as an indication for lethal germination potency. 100 mM NaCl was used in several experiments because PBS was found to cause precipitation with DDG; Tris buffer, another commonly used biological buffer, was not chosen because it has primary amine groups in the buffering agent, which could potentially interfere with the results.

For multivalent cation inhibition of DDA lethal germination, 0.1mM DDA in water was supplemented with various metal cations at various predetermined concentrations. *B. megaterium* spores were subsequently added to the mixtures and incubated at 40^{o}C for 30min before dilution and eventual plating as described above.

Molecular Dynamic Simulations

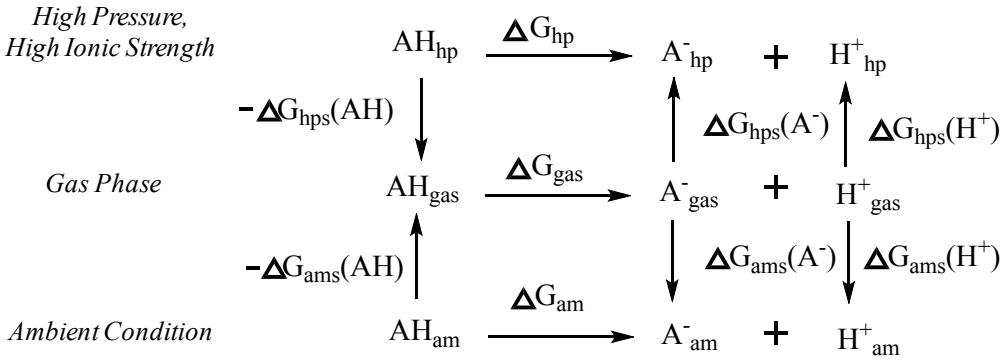
Molecular dynamics simulations were used to evaluate binding free energies in fixed volume (NVT) simulations with explicit water and complete orienatational freedom.

For the molecular dynamic simulation result reported in the main manuscript, a single cation (primary amine, quaternary amine, or guanidinium) and carboxylate anion were placed in 30 Å cubes with TIP4P-Ew water molecules and the equivalent of 100 mM NaCl. The large box size is important to allow binding and unbinding of the two ions. The interatomic forces for the simulations were calculated using the OPLS-AA force field which is parameterized to replicate properties of small organic molecules [4]. The systems were prepared with energy minimization, annealing, and 200 ps equilibration molecular dynamics in Parrinello-Rahman NPT [5]. Production simulations were conducted in NVT with replica-exchange. 40 replicas with temperatures from 300 K to 350 K for 20ns with exchanges attempted every 100 fs giving total simulation time of 800 ns. The stochastic Bussi-Donadio-Parrinello NVT thermostat (τ=0.5 ps) was used [6]. Particle-mesh Ewald summation was used for the long range coulombic force calculations [7] and a shifted, truncated Van der Waals potential was used. A cutoff of 8 Å was used for interatomic forces. All covalent hydrogen bonds were constrained using the LINCS algorithm [8]. The GROMACS simulation engine was used for the molecular dynamics simulations [9]. The potential mean force was calculated from the radial distribution function using $A(r) = -k_b T ln[g(r)]$.

The molecular dynamic simulation results presented in Figure 1S were acquired under a similar procedure but with the following modification: well-tempered metaynamics [10] was used to accelerate sampling and allow calculating of a potential of mean force (PMF) without replica-exchange as done in the main text. This creates a less-detailed PMF. The metadynamics parameters were a hill height of 0.1 kJ/mol, a bias factor of 10, and a sigma of 0.05 nm.

Quantum Chemical Calculations of Carboxyl and Primary Ammonium pKa

As the proposed salt-bridge formation occurs within the spore cortex section, a predominantly aqueous environment with high pressure and ionic strength [11,12], it would be relevant to look at the potential pKa change under such conditions. To this end, we used quantum chemical calculations and compare the protonation free energy change of the partaking head groups in response to environmental condition using a previously published protocol [13]:



In the above thermodynamic cycles, ΔG_{hps} stands for the solvation free energy of solute at high pressure and ionic strength, ΔG_{ams} stands for the solvation free energy of solute under ambient condition. As the acid disassociation constant pKa is given by:

$$pK_a = \Delta G^o / 2.303RT$$

where $\Delta G^o = \Delta G_{hp}\, or\, \Delta G_{am}$ for high pressure/ionic strength or ambient condition respectively. Thus, the change of acid dissociate constant:

$$\Delta pK_a = (\Delta G_{hp} - \Delta G_{am}) / 2.303RT$$

where

$$\Delta G_{hp} = -\Delta G_{hps}(AH) + \Delta G_{gas} + \Delta G_{hps}(A^-) + \Delta G_{hps}(H^+)$$

$$\Delta G_{am} = -\Delta G_{ams}(AH) + \Delta G_{gas} + \Delta G_{ams}(A^-) + \Delta G_{ams}(H^+)$$

The ΔG_{gas} terms cancel out, and the difference in solvation free energies of single protons is calculated to be negligibly small between the two conditions. The quantum chemical calculations for HA and A^- solvation free energies were performed using acetic acid as a model molecule.

In our calculation, 30 atm is taken as a reasonable estimation of osmotic pressure within spore cortex [14,15]. For practical reasons, we model this osmotic pressure to arise from 1:1 monovalent ion pairs, like Na^+ and Cl^-, and the ionic strength needed to generate a 30atm osmotic pressure is calculated to be close to 1.4 M, based on:

$$\pi = \left(-\frac{RT}{V_w}\right) * lna_w$$

where π is the osmotic pressure; R is the ideal gas constant; V_w is the molar volume of water and a_w is the water activity[16].

The quantum chemical calculation was performed in Gaussian 09® software package[17], using Density Functional Theory (DFT) B3LYP model and 6-31++G(d,p) basis set. SMD model (integral equation formalism polarizable continuum model with radii and non-electrostatic terms) was used to account for the solvent effect[18]. The pressure and ionic strength was set to be 1 atm and 0.15M for ambient condition and 30atm/1.4M to mimic the cortex environment. Under this setting, the calculated $(\Delta G_{hp} - \Delta G_{am})$ term is $-$ 4.176 kJ/mol, which corresponds to a shift of 0.73 in pKa at 25°C. A similar calculation can be

performed for primary ammonium protonation process using methyl amine as a model molecule, resulting a pKa change of 0.04. These two values are very close to or within the margin of error for quantum chemical calculations of ionic group pKa [13]. Hence, the high pressure and ionic strength alone are not expected to induce a major pKa shift in molecules.

This result acquired from quantum chemical calculation was also supported by a previous study directly measuring spore cortex carboxyl group pKa with time-resolved micropotentiometry [19]. The reported cortex carboxylate group apparent pKa of 4.7 is comparable to the isolated carboxyl group pKa in diluted aqueous solutions [19].

Figure 1S.

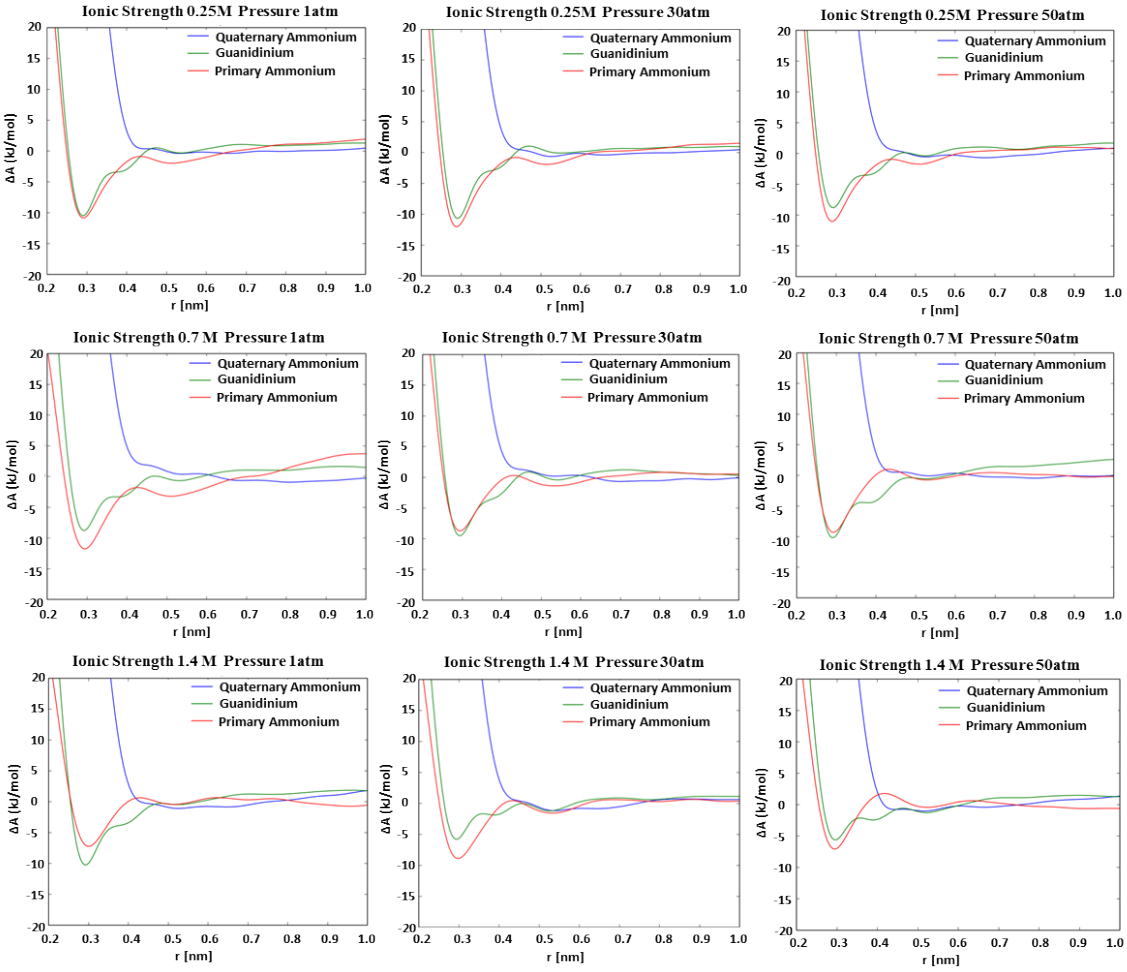
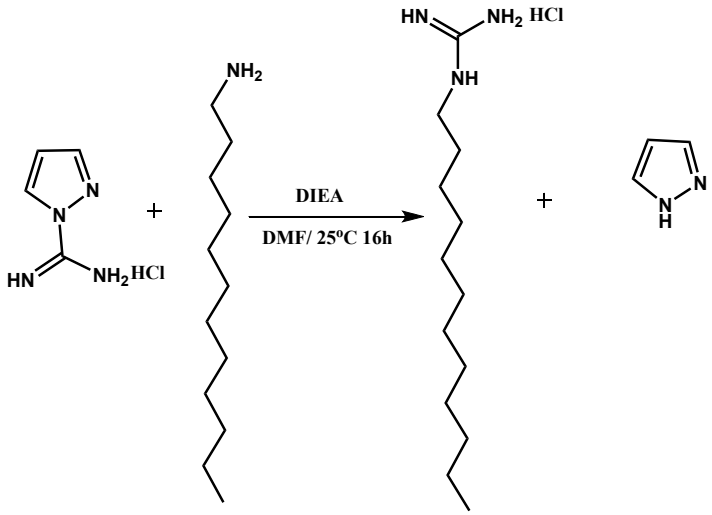


Figure 1S. The potential mean force free energy between carboxylate anion binding with primary ammonium, guanidinium or quaternary ammonium cations under various ionic strength and environmental pressure.

Figure 2S.

a.


HN
NH2 HCl
NH
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DIEA
DMF/ 25ºC 16h
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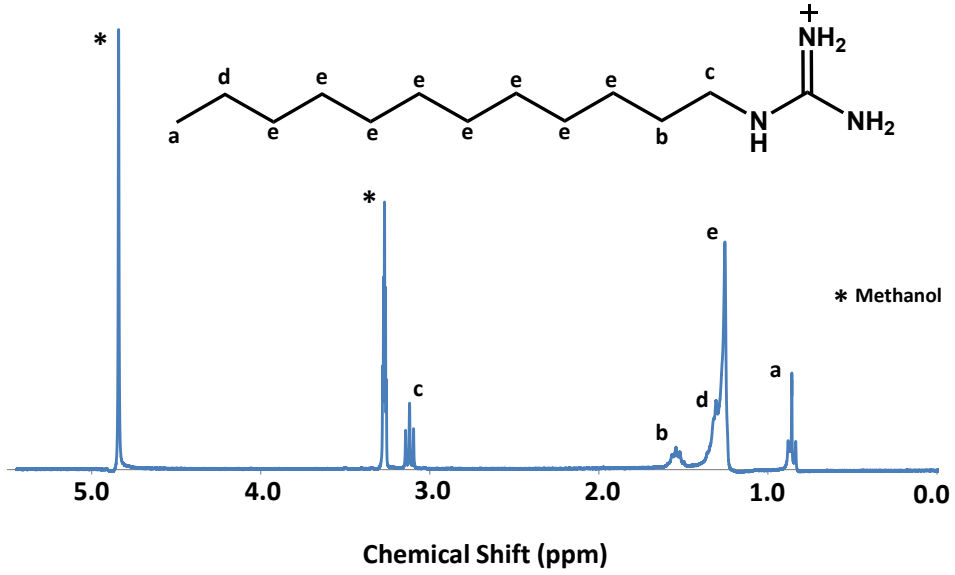
N
HN
NH2HCl
N
N
H

b.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d
e
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Chemical Shift (ppm)

c.

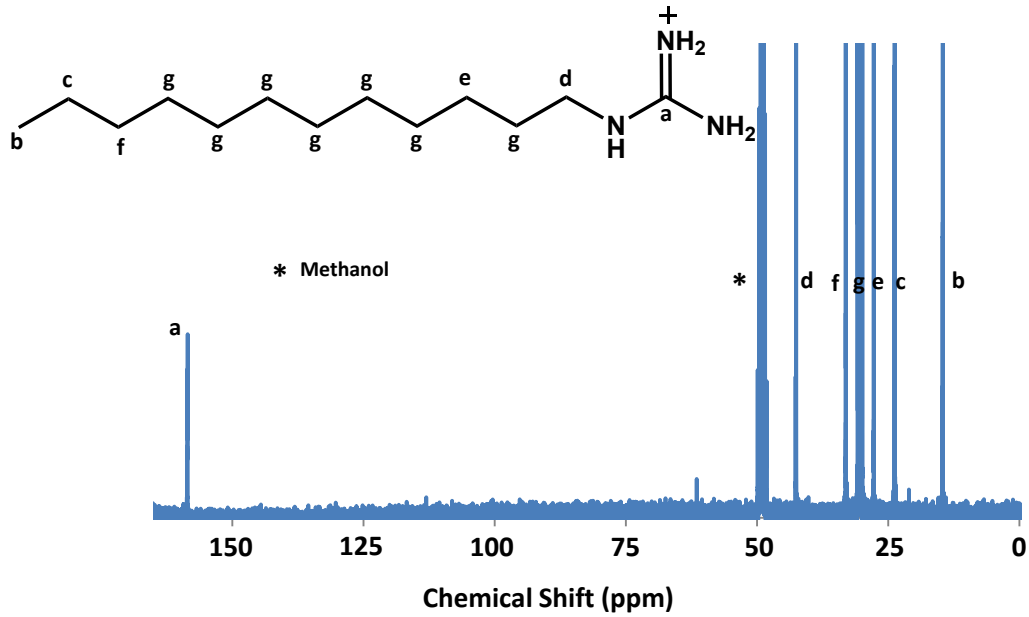
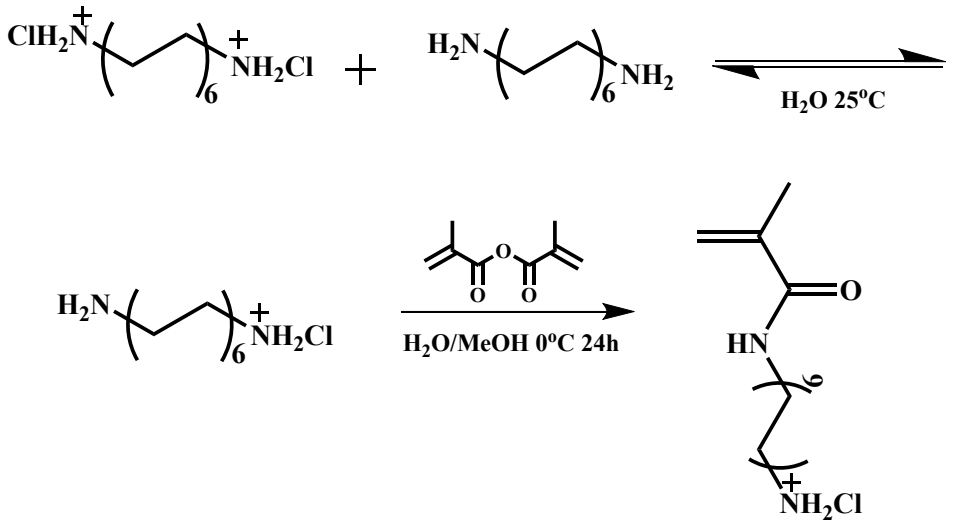


Figure 2S. a, The organic synthesis route for DDG. **b,** ^{1}H-NMR of DDG in MeOD. **c,** ^{13}C-NMR of MDA in MeOD.

Figure 3S.

a.



b.

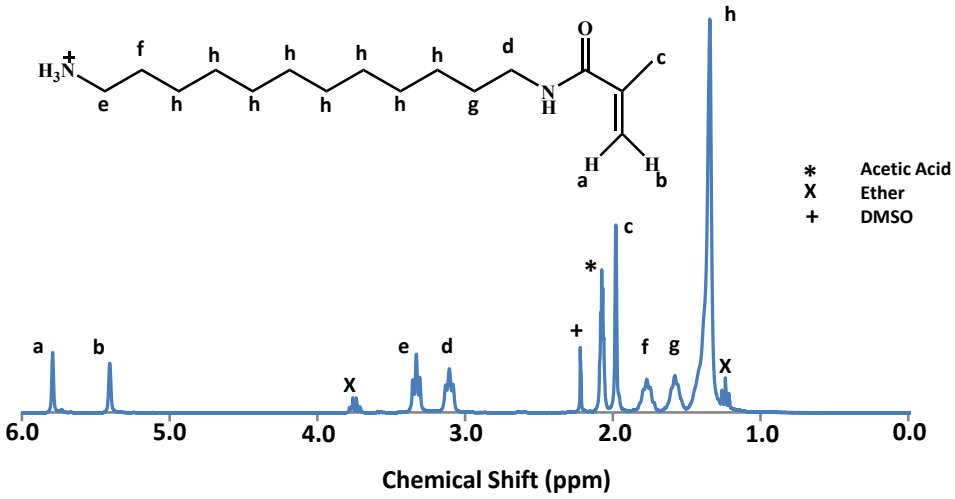
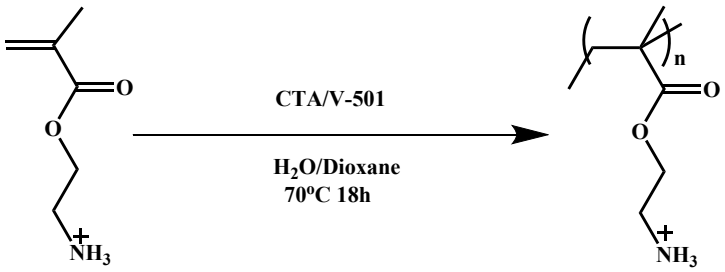
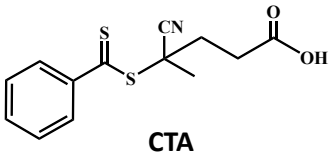


Figure 3S. a, The organic synthesis route for MDA. **b,** ^{1}H-NMR of MDA in DMSO.

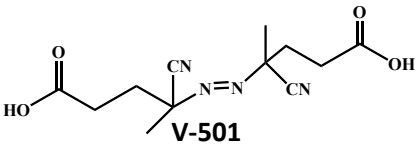
Figure 4S.



where



CTA



V-501

Figure 4S. The scheme of monomer AMA RAFT polymerization.

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