A Cocktail of Vitamins for Aqueous RAFT Polymerization in an Open-to-Air Microtiter Plate

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Experimental Section

Materials

N,N-dimethylacrylamide (DMA, 99%), *N,N*-diethylacrylamide (DEA, 99%), 4- acryloylmorpholine (NAM), 2-hydroxylethyl acrylate (HEA), oligo(ethylene glycol) methyl ether acrylate (OEGA), and oligo(ethylene glycol) methyl ether methacrylate (OEGMA) were all acquired from Sigma-Aldrich and deinhibited by passing through a column of basic alumina (Ajax Chemicals, AR) prior to usage. 2-(n-butyltrithiocarbonate)propionic acid (BTPA) and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB) were synthesized according to literature procedures.[1-2] *N,N*-dimethylformamide (DMF, 99.8%, Ajax Chemicals), dimethyl sulfoxide (DMSO, Ajax Chemicals) and acetonitrile (MeCN) were used as received. Riboflavin 5'-monophosphate (FMN, 95%),ascorbic acid (AscA, 99%) and terephthalic acid (98%), were purchased from Sigma-Aldrich and used as received. Corning® 96-well polypropylene microtiter plates (well volume ~ 300 µL) were acquired from Sigma-Aldrich. Vitamin B_2 (*Nature's Own*™ Vitamin B2) and Vitamin C (*Nature's Own*™ High Strength Vitamin C) were used as received.

Instrumentation

Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectra were performed using a Bruker 300, 400 or 600 MHz spectrometer and all chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS), referenced to the chemical shifts of residual solvent resonances. All ^{1}H NMR samples were prepared in d_6-DMSO or D_2O.

Size Exclusion Chromatography (SEC)

The molecular weight and molecular weight distribution of the synthesized polymers were determined by Size Exclusion Chromatography (SEC). The eluent was DMAc (containing 0.03% w/v LiBr and 0.05% w/v 2,6-dibutyl-4-methylphenol (BHT)) at 50 °C (flow rate of 1 mL/min) with a Shimadzu modular system comprising an SIL-20A auto-injector, a Polymer Laboratories 5.0 μm bead-size guard column (50×7.5 mm^2) followed by three linear PL (Styragel) columns (10^5, 10^4 and 10^3) and an RID-10A differential refractive-index (RI) detector. The calibration was based on commercial poly(methyl methacrylate) (PMMA) standards with molecular weights of 200 to 10^6 g mol^{-1}.

Fourier Transform Near-Infrared (FTNIR) spectroscopy

Online FTNIR spectroscopy was used to determine monomer conversions following the decrease in integration of the vinylic C-H stretching overtone of the monomer at ~6100 cm^{-1}. A Brucker Vertex 70 Fourier transform spectrometer equipped with a CaF_2 beam splitter, and room temperature DLaTGS detector was used. Polymerizations were carried out in a glass cuvette (10 mm × 2 mm). At different time intervals, the cuvette was manually placed in the sample holder with each spectrum acquired with 16 scans at a wavenumber resolution of 4 cm^{-1}. Spectra were analyzed with OPUS software (Version 7.5).

UV-Vis spectroscopy

UV-Vis absorption spectra were recorded using a CARY 300 Spectrophotometer (Agilent) from 200 to 800 nm at a scan rate of 600 nm/min. Spectra were acquired in 0.9 mL quartz cuvettes with a 2 mm pathlength.

Fluorescence spectroscopy

Fluorescence spectroscopy was performed using a Shimadzu RF-5301 PC Spectrofluorophotometer. Emission spectra were acquired using an excitation wavelength of 312 nm with an emission range of 320 - 800 nm. All spectra were acquired with excitation and emission slit widths of 5 nm.

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS analysis was performed on a Bruker ultrafleXtreme mass spectrometer in positive ion, linear mode using acetonitrile as the solvent. For each sample, 1 μL of polymer solution (1 mg/mL in acetonitrile) was mixed with 1 μL α-cyano-4- hydroxycinnamic acid solution (CHCA, 10 mg/ml, in acetonitrile) before spotting on the MALDI target plates.

Photopolymerization

Unless otherwise stated, all aqueous RAFT photopolymerizations were performed in either: **(A)** a rubber septum sealed FTNIR glass cuvette (10 × 2 mm) at a reaction volume of 0.6 mL or **(B)** a 96-well polypropylene microtitre plate at a reaction volume of 300 μL. Reaction mixtures were irradiated using a Thorlabs M470L3 LED (λ_{max} = 470 nm, 6.4 mW/cm^2) equipped with a collimation adapter and powered by a DC4100 driver. The light intensity was measured using a Newport 843-R power meter.

Methods

General procedure for FMN initiated RAFT polymerization in a cuvette

Polymerization kinetics for the aqueous RAFT polymerization of DMA in a cuvette ([DMA]:[BTPA]:[FMN] = 200:1:0.1 and [M] = 50 v/v%) was conducted as follows: BTPA (3.47 mg, 14.56 μmol), DMA (0.3 mL, 2.91 mmol), FMN (0.7 mg, 69.6 μL of a 10 mg/mL stock solution) and water (230.37 μL) were added to a glass vial. The reaction mixture was transferred to a 0.9 mL quartz cuvette (10 × 2 mm) and sealed with a rubber septum. For deoxygenated experiments, oxygen was then removed by sparging with nitrogen for 20 min at 0 ^{o}C. The polymerization was initiated by irradiating the cuvette with blue LED light (λ_{max} = 470 nm,

6.4 mW/cm^2) at room temperature. To determine monomer conversion, the cuvette was manually transferred to a FTNIR sample holder every 30 mins. After irradiation for 240 mins, the cuvette was exposed to air and kept in the dark. A small aliquot was removed for determination of the number average molecular weight ($M_{n,exp}$) and polymer dispersity (M_w/M_n) by SEC (DMAc as eluent).

General procedure for studying the polymerization kinetics of FMN initiated RAFT polymerization in a 96-well microtiter plate

A typical experiment conducting FMN initiated aqueous RAFT polymerization ([DMA]:[BTPA]:[FMN] = 200:1:0.05) in a 96-well plate was set up as follows: a reaction stock solution containing BTPA (4.63 mg, 19.41 μmol), DMA (0.4 mL, 3.88 mmol), FMN (0.46 mg, 46.41 μL of a 10 mg/mL stock solution) and water (307.16 μL) was added to a glass vial. 200 μL aliquots were then transferred to four wells of a polypropylene 96-well plate. The plate was covered with a transparent polystyrene lid and irradiated with blue LED light (λ_{max} = 470 nm, 6.4 mW/cm^2) at room temperature. Every 60 mins, the reaction mixture from a single well was removed and analysed to determine the monomer conversion, $M_{n,exp}$ and M_w/M_n by NMR and SEC (DMAc as eluent).

General procedure for studying the polymerization kinetics of two-component FMN/AscA initiated RAFT polymerization in a 96-well microtiter plate

A typical experiment conducting FMN/AscA initiated aqueous RAFT polymerization ([DMA]:[BTPA]:[FMN]:[AscA] = 200:1:0.05:0.5) in a 96-well plate was set up as follows: a reaction stock solution containing BTPA (10.41 mg, 43.67 μmol), DMA (0.9 mL, 8.73 mmol), FMN (1.04 mg, 104.44 μL of a 10 mg/mL stock solution), AscA (3.85 mg, 384.56 μL of a 10 mg/mL stock solution) and water (410.99 μL) was added to a glass vial. 200 μL aliquots were then transferred to seven wells of a polypropylene 96-well plate. The plate was covered with a transparent polystyrene lid and irradiated with blue LED light (λ_{max} = 470 nm, 6.4 mW/cm^2) at room temperature. Periodically, the reaction mixture from a single well was removed and analysed to determine the monomer conversion, $M_{n,exp}$ and M_w/M_n by NMR and SEC (DMAc as eluent).

Detection of hydroxyl radical by terephthalic acid trapping

A solution of FMN (2.9 mg, 290.12 µL of a 10 mg/mL stock solution), terephthalic acid (0.4 mg, 400 µL of a 1 mg/mL stock solution in DMSO) and water (4.3 mL) was prepared in a glass vial. The solution was subsequently irradiated by blue LED light (λ_{max} = 470 nm, 6.4 mW/cm^2). At given time points, 2 mL of the reaction solution was transferred to a fluorescence cuvette and fluorescence emission spectra were acquired from 320 - 800 nm at an excitation wavelength of 312 nm.

Detection of hydrogen peroxide by NMR spectroscopy

A reaction stock solution consisting of FMN (0.23 mg, 23.21 µL of a 10 mg/mL stock solution), AscA (0.85 mg, 42.73 µL of a 20 mg/mL stock solution) and Milli-Q water (334.06 µL) was prepared in a glass vial. A 200 µL aliquot was transferred to a single well of a polypropylene 96-well plate which was subsequently covered with a transparent polystyrene lid and irradiated with blue LED light (λ_{max} = 470 nm, 6.4 mW/cm^2) at room temperature. After 24 h, 100 µL of the reaction mixture was transferred to 500 µL of DMSO-d_6 for analysis with a Bruker Avance III, 600 MHz Cryo NMR. The presence of hydrogen peroxide (H_2O_2) was detected by cooling the sample down to -15 ^oC and comparing with a standard hydrogen peroxide solution (H_2O_2, 0.003%) from a commercial source.

General procedure for studying the chain extension of a PDMA macro-RAFT in a 96-well microtiter plate

A typical chain extension experiment in a 96-well plate was set up as follows: a PDMA macro-RAFT with DP = 100 was synthesized using FMN/AscA initiated aqueous RAFT polymerization ([DMA]:[BTPA]:[FMN]:[AscA] = 100:1:0.05:0.5) under blue LED irradiation for 12 hours. The crude PDMA was transferred and diluted with 200 µL DI water, then, 100 µL of this macro-RAFT stock solution was added to a 96 well plate and mixed with additional DMA (100 µL, [DMA]:[macro-RAFT] = 400:1). The chain extension was performed under blue LED irradiation for 12 hours to reach high conversion. DMF was added as an internal NMR standard to calculate monomer conversion.

MALDI-TOF MS analysis

A matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used for end group confirmation of polymer samples synthesized via FMN initiated RAFT polymerization in the presence and absence of AscA. For each sample, 1 mg of polymer was dissolved in acetonitrile (1 mg/mL). Then, 1 μL of polymer was mixed with 1 μL α-cyano-4- hydroxycinnamic acid solution (CHCA, 10 mg/ml, in acetonitrile) before spotting on the MALDI target plates. MALDI-TOF MS reveals evenly distributed mass peaks (separated by 99.13 m/z) and highly symmetric mass distribution. Magnified into the detailed structure of each mass peak set, we were able to distinguish the main peaks which corresponds to PDMA with BTPA chain-end plus Na^+ and secondary peaks to PDMA with BTPA chain-end plus K^+ in excellent agreement with theoretical values within instrumental error (< 1 m/z), showing no evidence for end-group loss.

Table S1. Experimental and characterization data for FMN mediated RAFT polymerization of DMA conducted in a glass cuvette. Polymerizations were conducted at a monomer concentration of 50 v/v% for 4 hours under blue LED light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2).

[DMA]/[BTPA]/ [FMN]	**Deoxygenated**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
200:1:0.1	No	4	15	3 100	5 500	1.12
200:1:0.1	Yes	4	3	900	n/r	n/r
200:1:0.1	No*	4	43	8 800	12 400	1.15

**Note: every 30 min, 10 mL of ambient air was injected into the reaction solution via syringe*

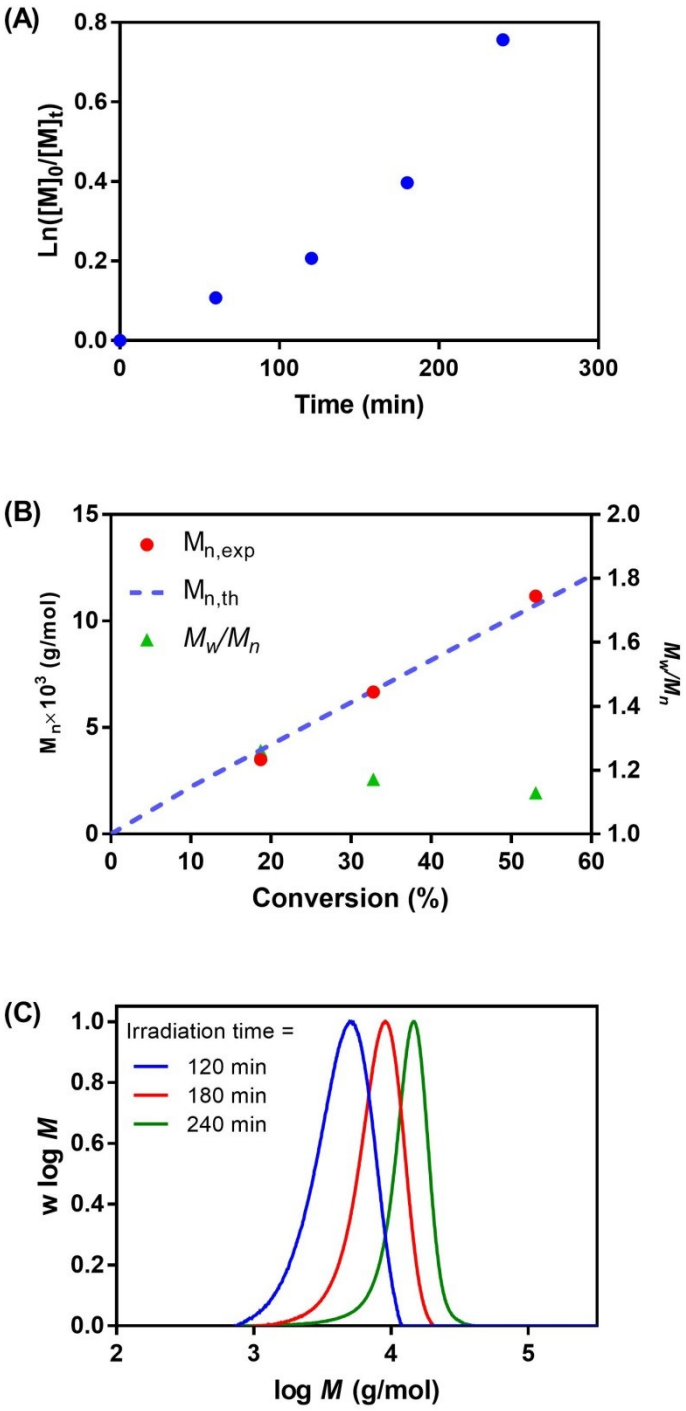

Figure S1. Polymerization kinetics of FMN mediated aqueous RAFT polymerization of DMA conducted in an open-to-air 96-well plate. Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a monomer concentration of 50 v/v% using a ratio of [DMA]:[BTPA]:[FMN] = 200:1:0.05. Evolution of **(A)** $\ln([M]_0/[M]_t)$ with irradiation time and **(B)** molecular weight and dispersity values versus monomer conversion. **(C)** SEC derived molecular weight distributions of PDMA acquired at different irradiation times.

Table S2. Experimental and characterization data for FMN mediated aqueous RAFT polymerization of DMA conducted in a 96-well plate under various conditions. Polymerizations were conducted at a monomer concentration of 50 v/v% for 4 hours under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2).

Monomer	**[DMA]/[BTPA]/ [FMN]/[AscA]**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
DMA	200:1:0.1:0	4	44	9 000	10 000	1.18
DMA	200:1:0:0	4	5	1 200	n/r	n/r
DMA	200:0:0.1:0	4	64	n/r	22 800	2.23
DMA	200:1:0.1:1	4	63	12 600	13 500	1.14
DMA	200:1:0:0	4	5	1 200	n/r	n/r
DMA	200:1:0:0.5	4	5	1 200	n/r	n/r
DMA	200:1:0.05:0	4	52	10 500	11 300	1.15
DMA	200:1:0.05:0.5	4	80	16 100	17 300	1.12

Table S3. Experimental and characterization data for FMN mediated aqueous RAFT polymerization of DMA conducted in a 96-well plate at varying FMN concentrations. Polymerizations were conducted at a monomer concentration of 50 v/v% for 4 hours under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2).

Monomer	**[DMA]/[BTPA]/ [FMN]**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
DMA	200:1:0	4	5	1 200	n/r	n/r
DMA	200:1:0.01	4	46	9 400	10 200	1.14
DMA	200:1:0.02	4	48	9 800	10 900	1.13
DMA	200:1:0.05	4	52	10 500	11 300	1.15
DMA	200:1:0.1	4	45	9 100	10 700	1.19
DMA	200:1:0.2	4	35	7 100	11 400	1.23
DMA	200:1:0.5	4	18	3 800	8 100	1.28

Table S4. Experimental and characterization data for two-component FMN/AscA mediated aqueous RAFT polymerization of DMA conducted in a 96-well plate at varying AscA concentrations. Polymerizations were conducted at a monomer concentration of 50 v/v% for 4 hours under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2).

Monomer	**[DMA]/[BTPA]/ [FMN]/[AscA]**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
DMA	200:1:0.05:0.005	4	43	8 800	9 000	1.15
DMA	200:1:0.05:0.01	4	43	8 800	8 700	1.16
DMA	200:1:0.05:0.05	4	57	11 500	11 300	1.13
DMA	200:1:0.05:0.1	4	66	13 200	13 400	1.12
DMA	200:1:0.05:0.5	4	77	15 500	14 300	1.10
DMA	200:1:0.05:1	4	57	11 400	9 400	1.11
DMA	200:1:0.05:2	4	45	9 200	7 000	1.12
DMA	200:1:0.05:5	4	38	7 800	4 600	1.17
DMA	200:1:0.05:10	4	25	5 100	3 100	1.24

Table S5. Experimental and characterization data for the two-component FMN/AscA mediated aqueous RAFT polymerization of DMA in a 96-well plate at varying FMN concentrations. Polymerizations were conducted at a monomer concentration of 50 v/v% for 4 hours under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2).

Monomer	**[DMA]/[BTPA]/ [FMN]/[AscA]**	**Irradiation time (h)**	**α (%)**	$M_{n,th}$ **(g/mol)**	$M_{n,exp}$ **(g/mol)**	M_w/M_n
DMA	200:1:0:0.5	4	5	1 200	n/r	n/r
DMA	200:1:0.01:0.5	4	42	8 500	8 100	1.13
DMA	200:1:0.02:0.5	4	61	12 300	12 200	1.11
DMA	200:1:0.05:0.5	4	80	16 100	17 300	1.12
DMA	200:1:0.1:0.5	4	82	16 500	17 500	1.13
DMA	200:1:0.2:0.5	4	78	15 600	18 200	1.17
DMA	200:1:0.5:0.5	4	35	7 100	9 800	1.22

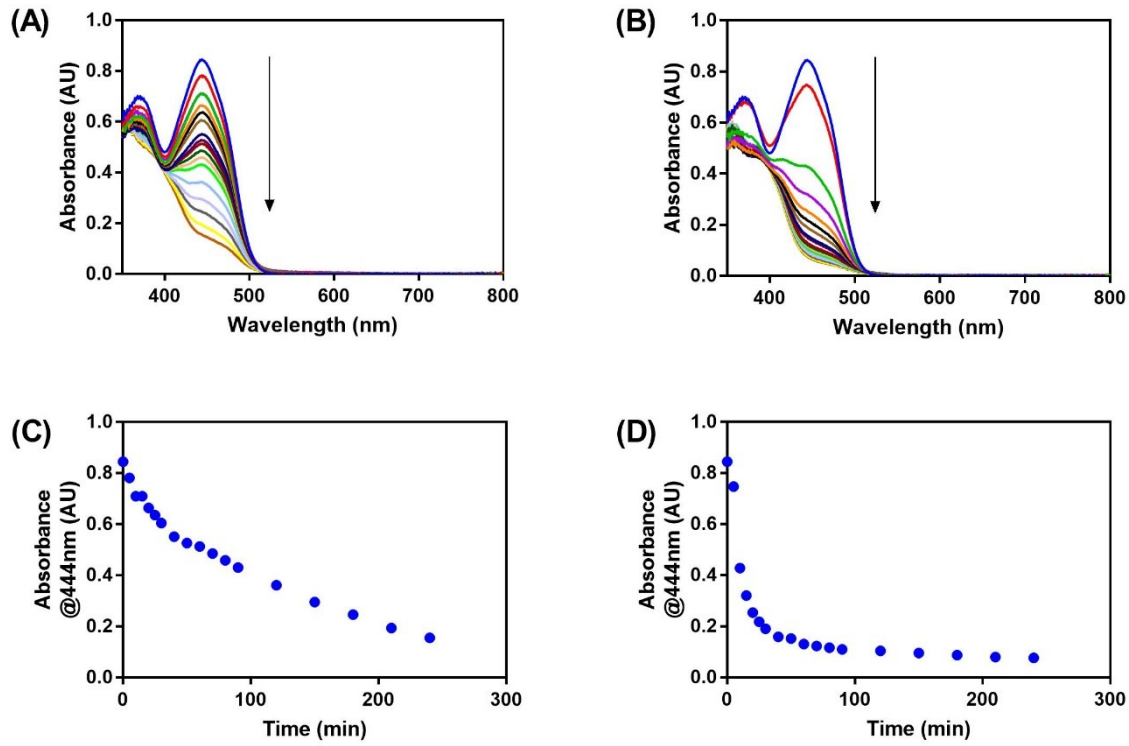

Figure S2. UV-Vis kinetic study of a non-deoxygenated solution of FMN under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) in the **(A)** presence and **(B)** absence of AscA. Change in FMN absorbance at 444 nm with increasing irradiation time in the **(C)** presence and **(D)** absence of AscA. Photoreduction of FMN was carried out in a 0.9 mL quartz cuvette with a 2 mm pathlength under a non-deoxygenated condition using a [FMN] = 1.21 mM and [AscA] = 12.13 mM.

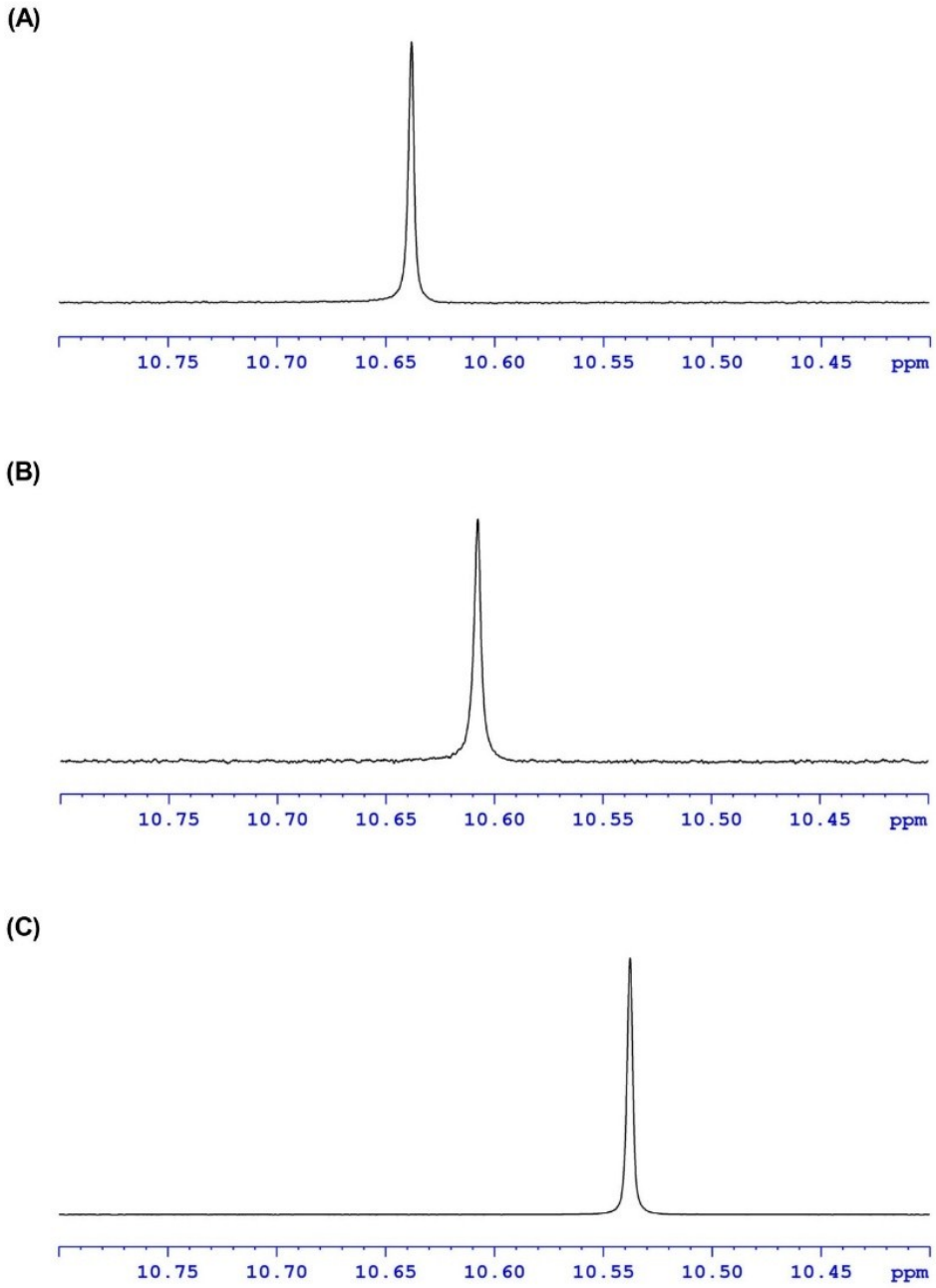

Figure S3. ^{1}H NMR spectrum of **(A)** commercial hydrogen peroxide solution (final concentration = 0.0005%), **(B)** a mixture of FMN and AscA after 24 h of blue light irradiation and **(C)** after spiking the resulting photoreduced solution with a drop of diluted 0.003% hydrogen peroxide. All spectra were acquired in d_6-DMSO at a temperature of -15 ^{o}C. For clarity, only the spectral range from 10.4 to 10.8 ppm is shown.

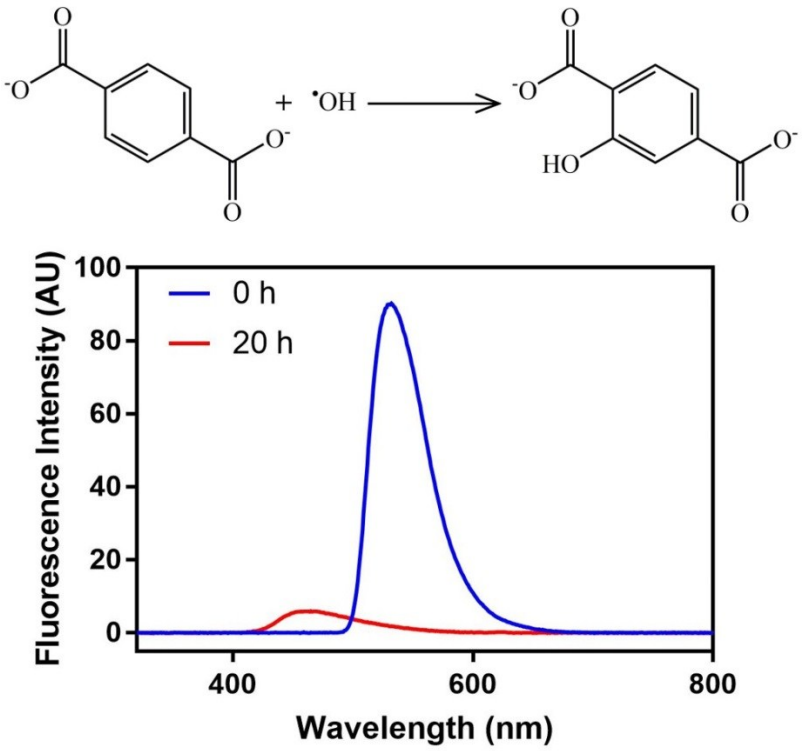

Figure S4. (Top) Chemical scheme showing the trapping reaction of hydroxyl radicals with terephthalic acid to generate 2-hydroxyterephthalate. (Bottom) Fluorescence emission of an aqueous solution of FMN in the presence of terephthalic acid after irradiation for 0 and 20 h under blue light (λ_{max} = 470 nm, 6.4 mW/cm^2). The appearance of a fluorescence emission band at 430 nm indicates the formation of 2-hydroxyterephthalate via hydroxyl radical trapping by terephthalic acid. Emission spectra were acquired with an excitation wavelength of 312 nm.

Note: The strong fluorescence in the initial reaction mixture (0 h) is due to the fluorescence emission of FMN at 530 nm. After photoreduction of FMN (20 h), this peak disappears and a peak associated with the formation of 2-hydroxyterephthalate is observed at 430 nm.

Table S6. Experimental and characterization data of PDMA of varying target DPs synthesized using two-component FMN/AscA mediated RAFT polymerization of DMA in a 96-well plate with [BTPA]:[FMN]:[AscA] = 1:0.05:0.5. The concentrations of FMN and AscA were variable relative to the RAFT concentration. Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a monomer concentration of 50 v/v%.

Monomer	**[DMA]:[BTPA]**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
DMA	1000	4	70	70 000	48 800	1.71
DMA	500	4	65	32 400	32 300	1.45
DMA	400	4	79	31 400	28 100	1.21
DMA	200	4	75	15 100	16 800	1.11
DMA	100	4	35	3 700	3 300	1.17
DMA	50	4	5	500	n/r	n/r

Table S7. Experimental and characterization data of PDMA of varying target DPs synthesized using two-component FMN/AscA mediated RAFT Polymerization of DMA in a 96-well plate with [FMN] = 1.21 and [AscA] = 12.13 mM. Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a monomer concentration of 50 v/v%.

Monomer	**[DMA]:[BTPA]**	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
DMA	1000	4	77	76 500	45 100	1.41
DMA	500	4	80	39 800	31 100	1.27
DMA	400	4	83	33 300	27 000	1.20
DMA	200	4	82	16 400	16 900	1.11
DMA	100	4	44	4 500	4 800	1.13
DMA	50	4	9	700	n/r	n/r

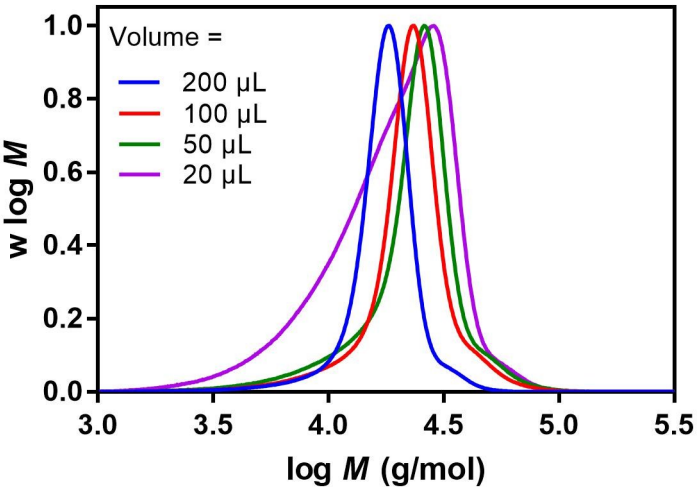

Figure S5. SEC derived molecular weight distributions of PDMA synthesized at variable reaction volumes using two-component FMN/AscA mediated aqueous RAFT polymerization of DMA in an open-to-air 96-well plate. Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a monomer concentration of 50 v/v% using a [DMA]:[BTPA]:[FMN]:[AscA] = 200:1:0.05:0.5. Further characterisation data can be found in **Table 1 (Main Text).**

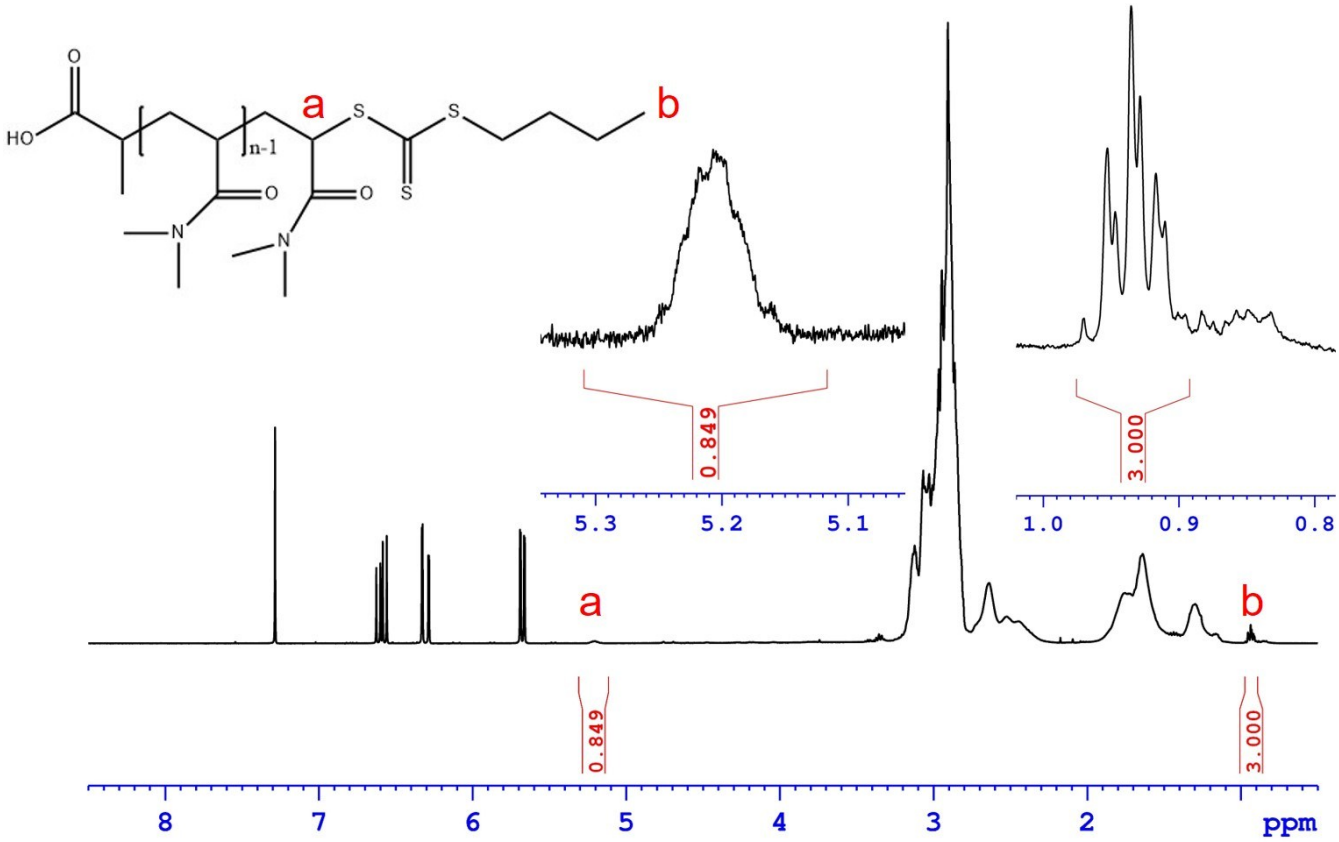

Figure S6. ^{1}H NMR spectrum (400 MHz, D_2O) of crude PDMA synthesized via two-component FMN/AscA mediated aqueous RAFT polymerization. RAFT end group fidelity was calculated as follows: end-group fidelity (%) = 100 × ($I^{5.1\ ppm-5.3\ ppm}/1$)/($I^{0.9\ ppm-1.0\ ppm}/3$)). Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) using a ratio [DMA]:[BTPA]:[FMN]:[AscA] = 100:1:0.02:0.5 in a 96-well plate.

Table S8. Experimental and characterization data of homopolymers synthesized with different FMN concentrations and their subsequent chain extension into diblock copolymers. Polymerizations were conducted under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) using [DMA] = 4.85 M, [BTPA] = 48.52 mM, and [AscA] = 24.26 mM.

Polymer	Targeted DP	[FMN] (mM)	α (%)	$M_{n,\,th}$ (g/mol)	$M_{n,\,exp}$ (g/mol)	M_w/M_n	End-Group Fidelity
PDMA	100	0.97	82	8 400	10 300	1.13	85%
PDMA-*b*-PDMA	400	0	68	35 500	35 700	1.32	n/r
PDMA	100	2.43	90	9 200	12 600	1.13	74%
PDMA-*b*-PDMA	400	0	82	41 700	43 200	1.43	n/r
PDMA	100	4.85	96	9 800	14 100	1.16	69%
PDMA-*b*-PDMA	400	0	94	47 000	51 400	1.66	n/r
PDMA	100	9.70	82	8 300	14 200	1.26	48%
PDMA-*b*-PDMA	400	0	97	46 800	51 600	1.87	n/r

*Note: *End group fidelities were calculated based on NMR spectrum (See **Figure S6**); n/r not reported.*

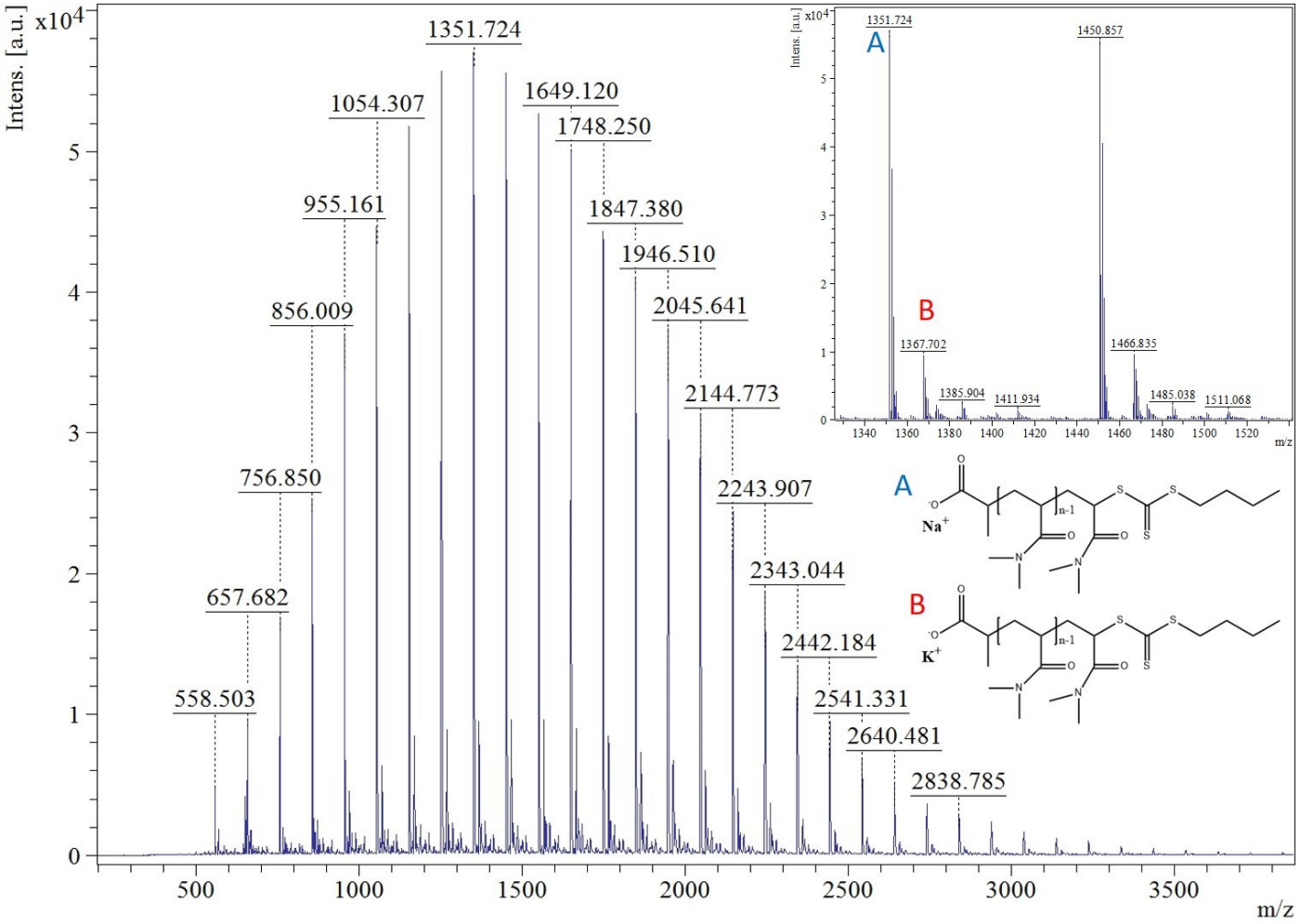

Figure S7. MALDI-TOF MS of a PDMA sample synthesized via FMN initiated polymerization in an open-to-air 96-well plate. Polymerizations were conducted under blue LED light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a fixed monomer concentration of 50 v/v%. Polymerizations were stopped at approximately 10% monomer conversion (degree of polymerization ~ 20) to obtain a suitable molecular weight range for MALDI-TOF MS analysis. Magnifying into the molecular weight range of 1320-1540 m/z (inset), we observe that the major peaks correspond to the theoretical predictions of PDMA with BTPA chain-end group plus Na^+ (A, n = 11, $M_{n,theo}$ = 1351.811 ($M_{n,Maldi}$ = 1351.724), n = 12, $M_{n,theo}$ = 1450.941 ($M_{n,Maldi}$ = 1450.857)), within experimental error (< 1 g/mol). The minor peaks correspond to PDMA with BTPA chain-end group plus K^+ (B, n = 11, $M_{n,theo}$ = 1367.811 ($M_{n,Maldi}$ = 1367.702), n = 12, $M_{n,theo}$ = 1466.941 ($M_{n,Maldi}$ = 1466.941)), within experimental error (< 1 g/mol).

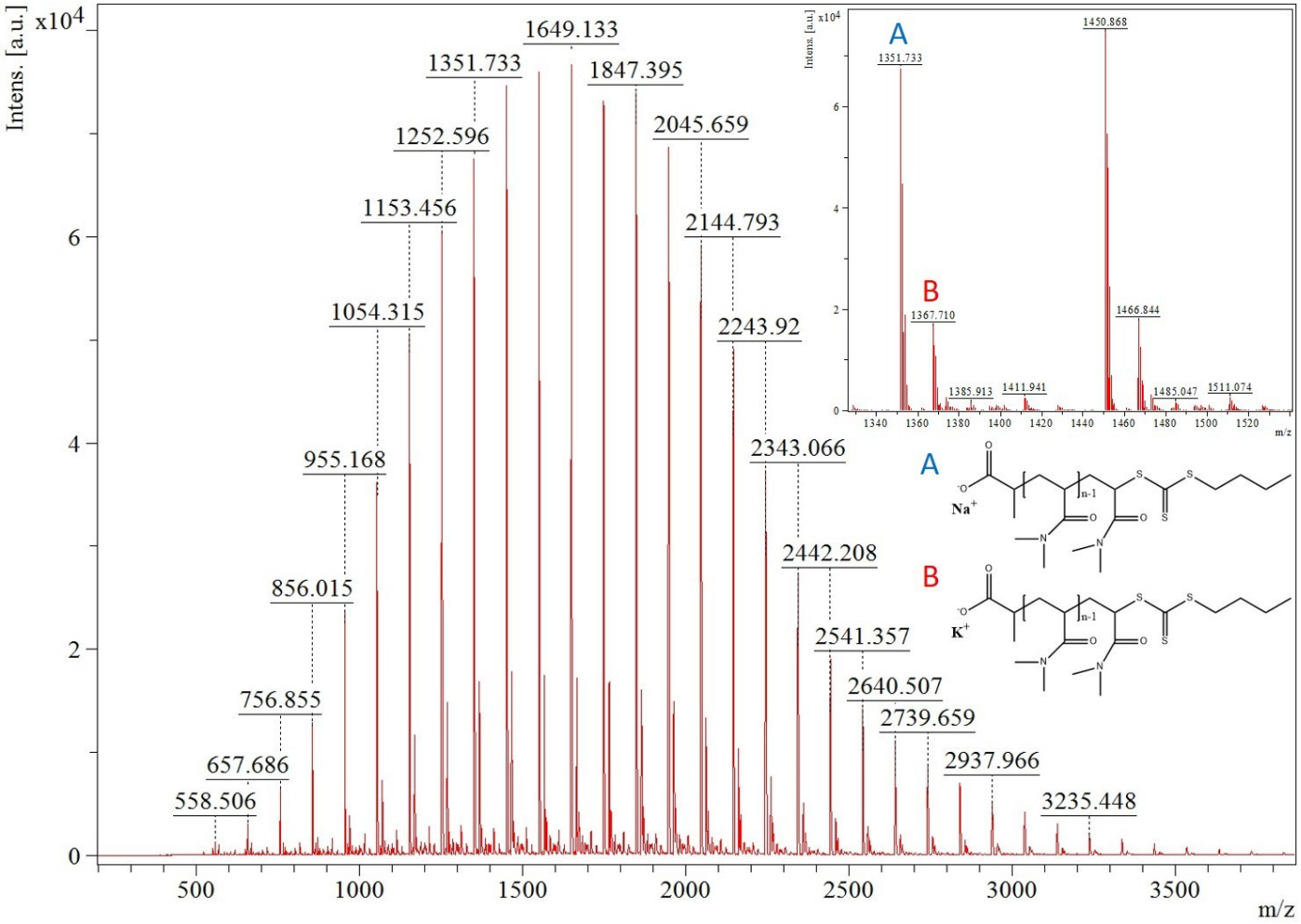

Figure S8. MALDI-TOF MS of a PDMA sample synthesized via FMN/AscA initiated polymerization in an open-to-air 96-well plate. Polymerizations were conducted under blue LED light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a fixed monomer concentration of 50 v/v%. Polymerizations were stopped at approximately 10% monomer conversion (degree of polymerization ~ 20) to obtain a suitable molecular weight range for MALDI-TOF MS analysis. Magnifying the molecular weight range of 1320-1540 m/z (inset), we observed that the major peaks correspond to the theoretical predictions of PDMA with BTPA chain-end group plus Na^+ (A, n = 11, $M_{n,theo}$ = 1351.811 ($M_{n,Maldi}$ = 1351.733), n = 12, $M_{n,theo}$ = 1450.941 ($M_{n,Maldi}$ = 1450.868)), within experimental error (< 1 g/mol). The minor peaks correspond to PDMA with BTPA chain-end group plus K^+ (B, n = 11, $M_{n,theo}$ = 1367.811 ($M_{n,Maldi}$ = 1367.710), n = 12, $M_{n,theo}$ = 1466.941 ($M_{n,Maldi}$ = 1466.844)), within experimental error (< 1 g/mol).

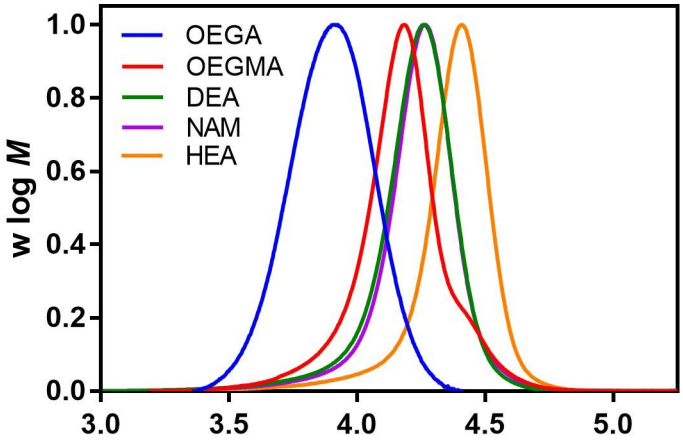

Figure S9. SEC derived molecular weight distributions of a range of monomer families synthesized using two-component FMN/AscA mediated aqueous RAFT polymerization in an open-to-air 96-well plate. Polymerizations were conducted under blue LED light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2) at a fixed monomer concentration of 50 v/v%. Further characterisation data can be found in **Table 2 (Main Text)**.

Table S9. Experimental and characterization data for vitamin B_2/vitamin C** mediated aqueous RAFT polymerization of DMA conducted in a 96-well plate under various conditions.

[DMA]/[BTPA]/ [Vitamin B_2]/[Vitamin C]	**Irradiation time (h)**	**α (%)**	$M_{n,\,th}$ **(g/mol)**	$M_{n,\,exp}$ **(g/mol)**	M_w/M_n
200:1:0:0	10	5	1 200	n/r	n/r
200:1:0.05:0.5	4	11	2 500	2 100	1.24
200:1:0.05:0.5	6	22	4 600	37 00	1.26
200:1:0.05:0.5	8	54	11 000	10 800	1.17
200:1:0.05:0.5	10	85	17 200	19 100	1.20

*Note: *Polymerizations were conducted at a monomer concentration of 50 v/v% under blue light irradiation (λ_{max} = 470 nm, 6.4 mW/cm^2). **Vitamins B_2 and C were sourced from Nature's Own™ Vitamin B_2 and Nature's Own™ High Strength Vitamin C tablets, respectively.*

Supporting References

1. Ferguson, C. J.; Hughes, R. J.; Nguyen, D.; Pham, B. T.; Gilbert, R. G.; Serelis, A. K.; Such, C. H.; Hawkett, B. S. J. M., Ab initio emulsion polymerization by RAFT-controlled self-assembly. **2005,** *38* (6), 2191-2204.

2. Mitsukami, Y.; Donovan, M. S.; Lowe, A. B.; McCormick, C. L. J. M., Water-soluble polymers. 81. Direct synthesis of hydrophilic styrenic-based homopolymers and block copolymers in aqueous solution via RAFT. **2001,** *34* (7), 2248-2256.