Supporting Information for

Synthetic Polymeric Variant of *S*-Adenosyl Methionine Synthetase

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EXPERIMENTAL SECTION

Materials. Boc-L-methionine (Boc-L-Met-OH, 99%, Sisco Research Laboratories Pvt. Ltd., India), trifluoroacetic acid (TFA, 99.5%, Sisco), 4-dimethylaminopyridine (DMAP, 99%, Sigma), anhydrous *N*, *N*′-dimethylformamide (DMF, 99.9%, Sigma), dicyclohexylcarbodiimide (DCC, 99%, Sigma) and 2-hydroxyethyl methacrylate (HEMA, 97%, Sigma) were used without any further purification. Methyl methacrylate (MMA, Aldrich, 99%) and PEGMA (molecular weight 300 g/mol, Aldrich, 99%) were purified through a basic alumina column prior to polymerization. The 2, 2′-azobisisobutyronitrile (AIBN, Sigma, 98%) radical initiator was purified by recrystallization from methanol. The NMR solvents such as $CDCl_3$ (99.8% D), D_2O (99.9% D) and dimethylsulfoxide-d_6 (DMSO-d_6, 99.8% D) were obtained from Cambridge Isotope Laboratories, Inc., USA. The synthesis of 4-cyanopentanoic acid dithiobenzoate (CTP) was conducted according to an earlier reported procedure.[1] The solvents such as hexanes (mixture of isomers), acetone, ethyl acetate, methanol (MeOH), dichloromethane (DCM) and tetrahydrofuran (THF) were purified by standard procedures.[2]

Instrumentation. Gel permeation chromatography (GPC) measurements were carried out in DMF at 35 ^{o}C using a flow rate of 1.0 mL/min (Viscotek GPC pump; columns: two Visco Gel I-Series G4000). Detection consisted of a Viscotek refractive index (RI) detector operating at 660 nm, and a Viscotek model 270 series platform consisting of a laser light scattering detector (detection angles of 7^{o} and 90^{o}) and a four-capillary viscometer. Narrow

molecular weight polystyrene standards were used to calibrate the system. NMR spectra were acquired in a Bruker Avance^{III} 500 MHz spectrometer. Positive mode electrospray ionization mass spectrometry (ESI-MS) was recorded on a Q-Tof Micro YA263 high resolution mass spectrometer from Waters Corporation. Dynamic light scattering (DLS) and zeta potential (ξ) values were determined using a dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instrument Ltd., UK) instrument equipped with a He–Ne laser beam at 658 nm. Polymer solutions were filtered through a 0.45 μm syringe filter prior to analysis and each experiment was repeated at least three times to obtain the average value. Field emission scanning electron microscopy (FE-SEM) samples were prepared as follows: an aliquot of sample solution was drop casted on a silicon wafer, dried, coated with gold:palladium (20:80). Finally, images were recorded using a Carl Zeiss-Sigma instrument. Photo-catalytic radical formation reaction was carried out in a laboratory-scale photo reactor (Luzchem Research Inc., Quebec, Canada) at light intensity of 365 nm. FT-IR spectra were recorded in a Perkin-Elmer Spectrum 100 FT-IR spectrometer. UV-Visible spectroscopic analysis was carried out using a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was carried out on a Bruker ultrafleXtreme^{TM} instrument equipped with a smart beam-II laser in the reflector mode and 22 kV acceleration voltages. 2,5-Dihydroxybenzoic acid (DHB, Bruker) was used as matrix.

Synthesis of monomer. We synthesized side-chain Boc protected methionine monomer (METMA) as described elsewhere.[3] Typically, to a 250 mL double-necked round-bottom flask on an ice-water bath, 12.41 g (60.16 mmol) DCC dissolved in 50.0 mL of ethyl acetate was added and the solution was purged for 10 min with dry N_2. Next, 7.82 g (64.8 mmol) HEMA was added in one shot under stirring, followed by drop-wise addition of Boc-L-Met-OH (15.00 g, 60.16mmol) in 70 mL ethyl acetate over 15 min. Then, DMAP (0.80 g, 6.4 mmol) dissolved in 5.0 mL ethyl acetate was added drop-wise *via* a syringe. This reaction mixture was stirred in the ice-water bath for 30 min and then at room temperature for 24 h. After removing insoluble *N*,*N*′-dicyclohexylurea by filtration, the organic layer was thoroughly washed with 2 × 60 mL 1.0 N HCl solution, 2 × 60 mL saturated $NaHCO_3$ solution, and finally with 2 × 60 mL brine solution. Then, the organic layer was dried over anhydrous Na_2SO_4, concentrated by a rotary evaporator and purified by column chromatography using 6% ethyl acetate in hexanes to a get a pale yellow viscous liquid, yield = 80%. ^{1}H NMR (Fig. S1, $CDCl_3$, δ, ppm): 6.12 and 5.59 (C=CH_2, 2H, s), 5.12 (N*H*COO, 1H, s), 4.46−4.34 (OCH_2CH_2O, and CH_2C*H*(NH)CO, 5H, m), 2.5 (CH_3SC$H_2$$CH_2$-, 1H, m),

2.07 (C=CCH_3, 3H, s), 2.03(CH_3S, 3H, s) 1.93 [($CH_3SCH_2CH_2$-, 2H, m], 1.44 [$COO(CH_3)_3CH_2$, 9H, s]. ESI-MS (Fig. S2): observed *m*/*z* for [M + Na^+] = 384.

RAFT Polymerization of METMA. A typical polymerization procedure is described as follows: METMA (1.0 g, 2.6 mmol), CTP (30.94 mg, 0.10 mmol), AIBN (3.63 mg, 22.0 μmol, from THF stock solution) and 4.0 g THF were added to a 20 mL septa sealed glass vial equipped with a magnetic bar and purged with dry N_2 gas for 15 min. The reaction vial was placed in a preheated reaction block at 60 ^oC and after predetermined time the polymerization reaction was quenched by cooling the vial in ice-water bath. The polymer (PMETMA) was purified by reprecipitation in hexanes at least five times (from acetone solutions) and dried under high vacuum at 35 ^oC for 8 h.

Synthesis of block copolymers. A typical block copolymerization procedure is described as follows: PEGMA (0.5 g, 1.6 mmol), PMETMA macro-CTA ($M_{n,GPC}$ = 5100 g/mol, PDI = 1.17, 153 mg, 0.03 mmol), AIBN (1.09 mg, 6.0 μmol, from stock solution), and THF (0.87 g) were added in a 20 mL polymerization vial equipped with a magnetic bar and purged with dry N_2 gas for 15 min. The reaction vial was put in a preheated reaction block at 60 ^oC for 7 h. The resulting block copolymer, PMETMA-*b*-PPEGMA, was purified as mentioned above for the homopolymer.

Deprotection of Bocprotecting groups. 1.0 mL TFA was added to the solution containing 0.1 g of polymer in 1.0 mL DCM in a 20 mL glass vial. The solution was stirred for 2 h at room temperature, precipitated four times in hexanes (from acetone solutions), and finally dried under vacuum at 35 ^oC for 8 h.

Reaction of polymers with ATP. Deprotected polymer DPMETMA (0.22mmol, 80 mg) was dissolved in freshly prepared phosphate buffer solution pH 7.0 in a 20 mL glass vial. ATP (0.22mmol, 110 mg) was added to the vial and the reaction mixture was stirred at room temperature for 48 h. Then, the solution was dialysed against de-ionized (DI) water by using a dialysis bag (Spectra/por^R dialysis membrane, molecular weight cut-off (MWCO): 2 kDa). During the dialysis, water was replaced after every 2-8 h for 6 times. Finally, water was removed *via* freeze drying, Operon, Model: FDU 8606, Korea).

Similarly, both protected (0.2 mmol) and deprotected (0.2 mmol) block copolymers were reacted with 0.2 mmol of ATP in 3.0 mL freshly prepared phosphate buffer solution pH 7.0 in a 20 mL glass vial. After 48 h reaction time at room temperature, the reaction mixture was dialyzed and lyophilized.

Methyl transfer reaction. Typically, the polymer-ATP complex (in case of DPMETMA, 50 mg, 0.13 mmol), cytosine (30 mg, 0.26 mmol) and H_2O_2 (19.6 mM) were taken in acidic medium (3.0 mL, pH = 3.0) in a 20 mL septa sealed vial equipped with a small magnetic stir bar. The reaction vial was purged with dry nitrogen for 12 min. After 3 h reaction under the UV photo reactor, a portion of the solution was analyzed by MALDI-TOF measurement.

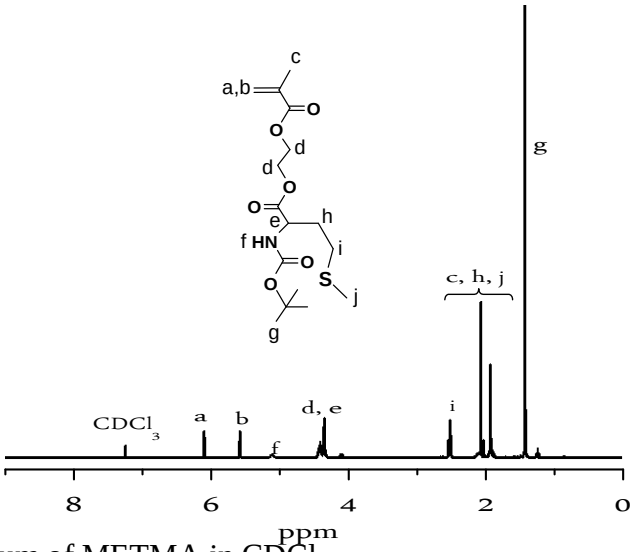


Fig. S1[1]H NMR spectrum of METMA in $CDCl_3$.

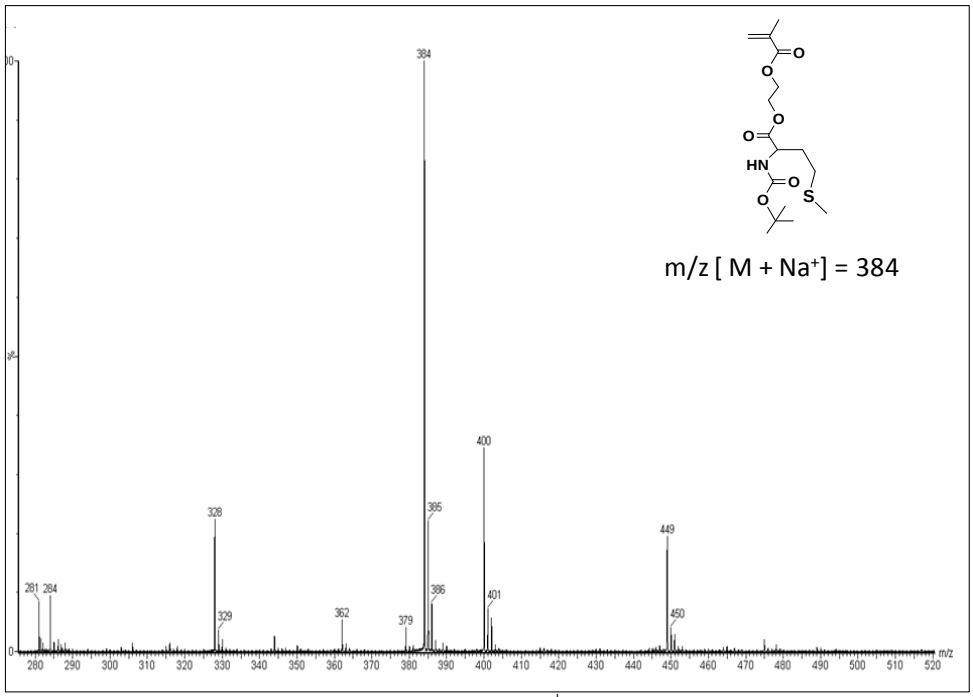


Fig. S2 ESI-MS spectrum of METMA, [M + Na^+ = 384 *m*/*z*, calculated for M + Na^+ = 384*m*/*z*].

Table S1 Results from the synthesis of PMETMA homopolymer and corresponding block copolymers in THF at 60 °C.

Expt. No.	[M]/[CTA]/[AIBN]	Time (h)	Conv.[d] (%)	$M_{n,\ GPC}$[e] (g/mol)	$Đ$[e]	$M_{n,\ NMR}$ (g/mol)	$M_{n,theo}$[g] (g/mol)
1	15:1:0.2[a]	14	84	5100	1.13	5500[f]	4800
2	25:1:0.2[a]	14	60	6100	1.17	5700[f]	5700
3	40:1:0.2[a]	15	75	10200	1.25	11700[f]	11000
4	50:1:0.2[a]	15	76	12700	1.29	13400[f]	14000
5	50:1:0.2[b]	7	77	9700	1.24	10400[g]	8950
6	50:1:0.2[c]	7	78	15300	1.33	15900[g]	16800
7	100:1:0.2[c]	6	45	20500	1.18	17,700[g]	18600
8	100:1:0.2[c]	7	55	23600	1.26	20400[g]	21600

[a][Monomer (M)]/[CTA] = [METMA]/[CTP]. [b][M]/[CTA] = [MMA]/[PMETMA-macroCTA, $M_{n,GPC}$ = 5100 g/mol]. [c][M]/[CTA] = [PEGMA]/[PMETMA-macroCTA, $M_{n,GPC}$ = 5100 g/mol]. [d]Determined by gravimetric analysis. [e]Measured by GPC. [f]Obtained from ^{1}H NMR study as mentioned in the main article. [g]$M_{n,NMR}$ values were determined by using the following formula: molecular weight (MW) of PMETMA macro-CTA + degree of polymerization of MMA (or PEGMA) × MW of MMA (or PEGMA). [h]$M_{n,theo}$ = (([M]/[CTA] × molecular weight (MW) of M × conversion) + (MW of CTA)).

Table S2 Solubility of PMETMA and DPMETMA in different solvents.[a]

Solvent	**PMETMA**	**DPMETMA**
Water	-	+
Hexanes	-	-
Pet ether	-	-
Diethyl ether	-	-
Ethyl acetate	+	-
Benzene	+	-
Toluene	+	-
THF	+	-
DMF	+	+
DMSO	+	+
Methanol	+	+
Ethanol	+	+
Dichloromethane	+	-
Acetone	+	+
Acetonitrile	+	-
Chloroform	+	-
Dioxane	+	-

[a]The symbols (+) and (-) indicate soluble and insoluble, respectively.

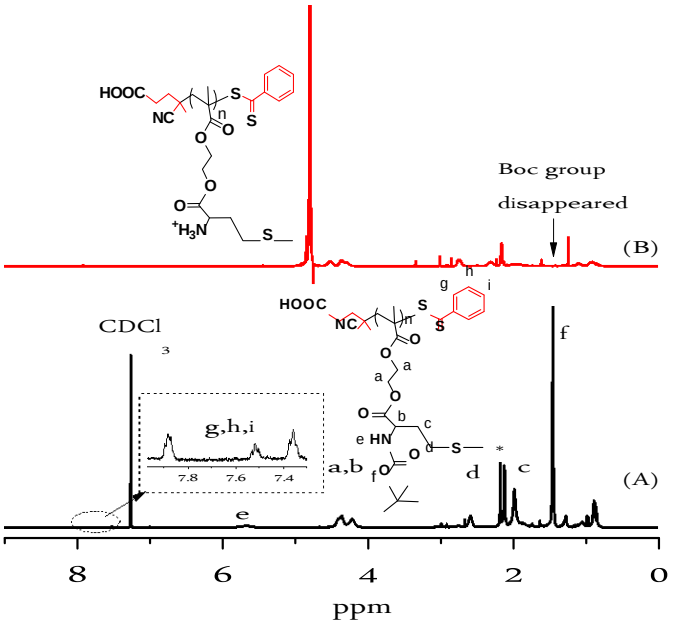
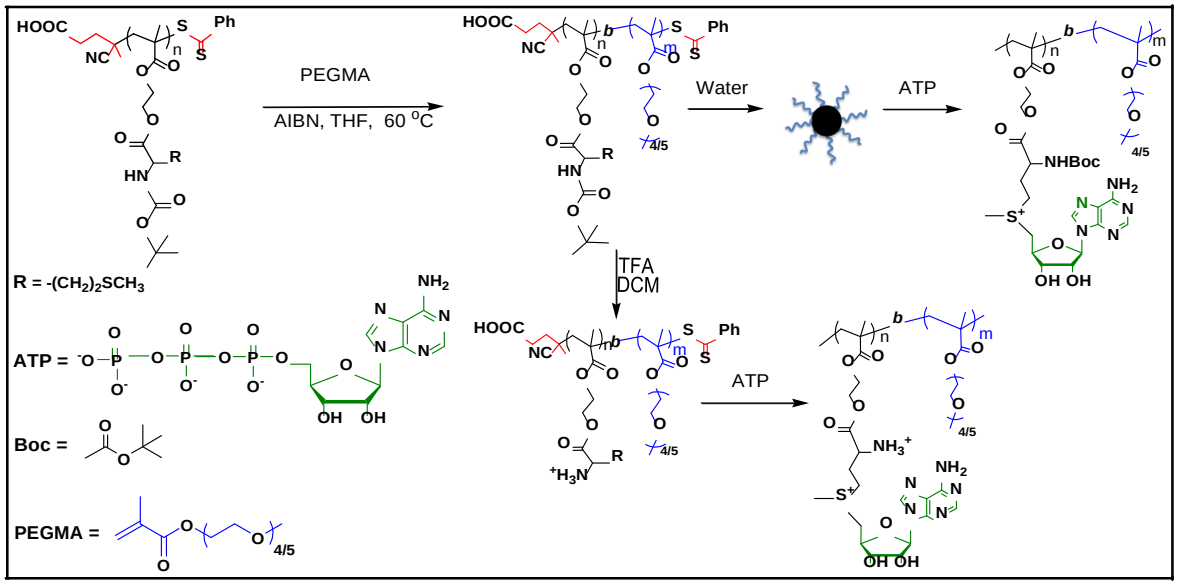


Fig. S3 ^{1}H NMR spectra of (A) PMETMA in $CDCl_3$ and (B) DPMETMA in D_2O.



Scheme S1 Synthesis of PMETMA-*b*-PPEGMA block copolymers, reaction of side-chain methionine moiety with ATP in the Boc protected and deprotected block copolymers in aqueous medium at room temperature.

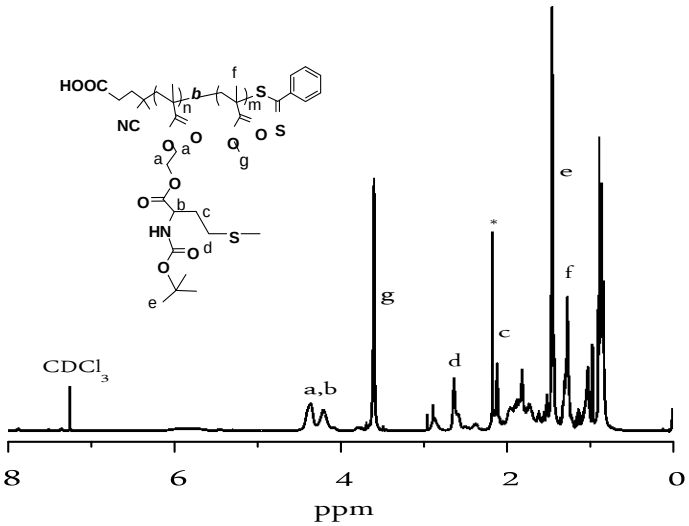


Fig. S4 ^{1}H NMR spectrum of PMETMA-*b*-PMMA block copolymer.

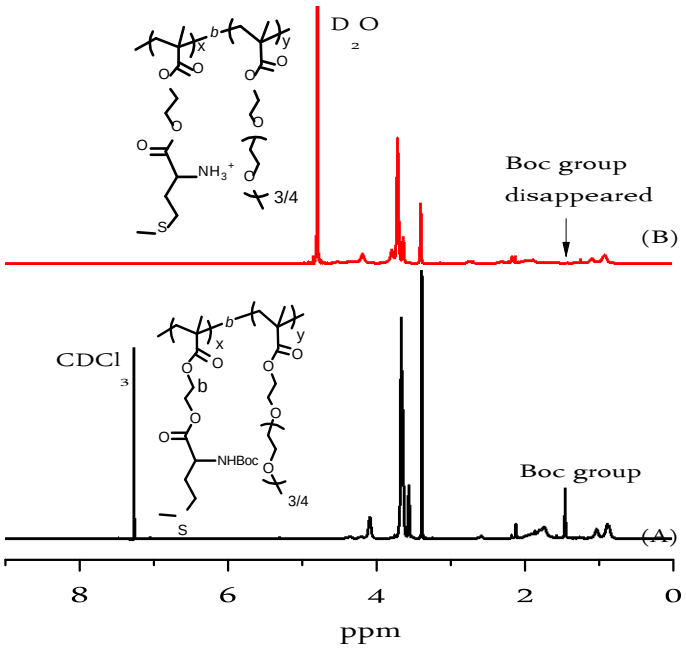


Fig. S5 ^{1}H NMR spectra of (A) PMETMA-*b*-PPEGMA in $CDCl_3$ and (B) DPMETMA-*b*-PPEGMA in D_2O.

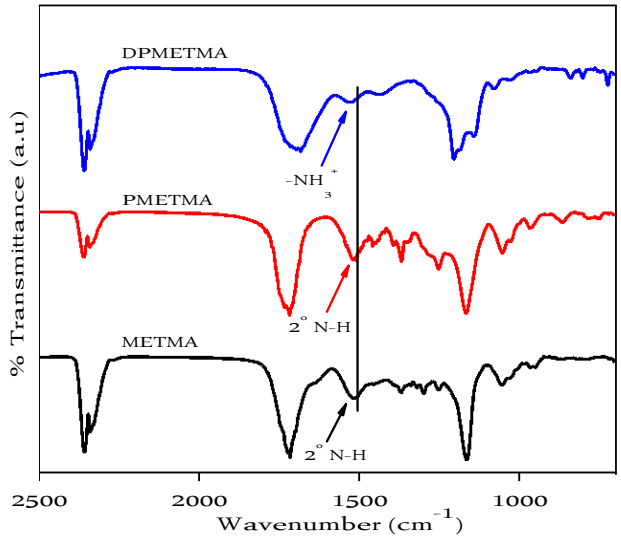


Fig. S6 FT-IR spectra of METMA, PMETMA, and DPMETMA.

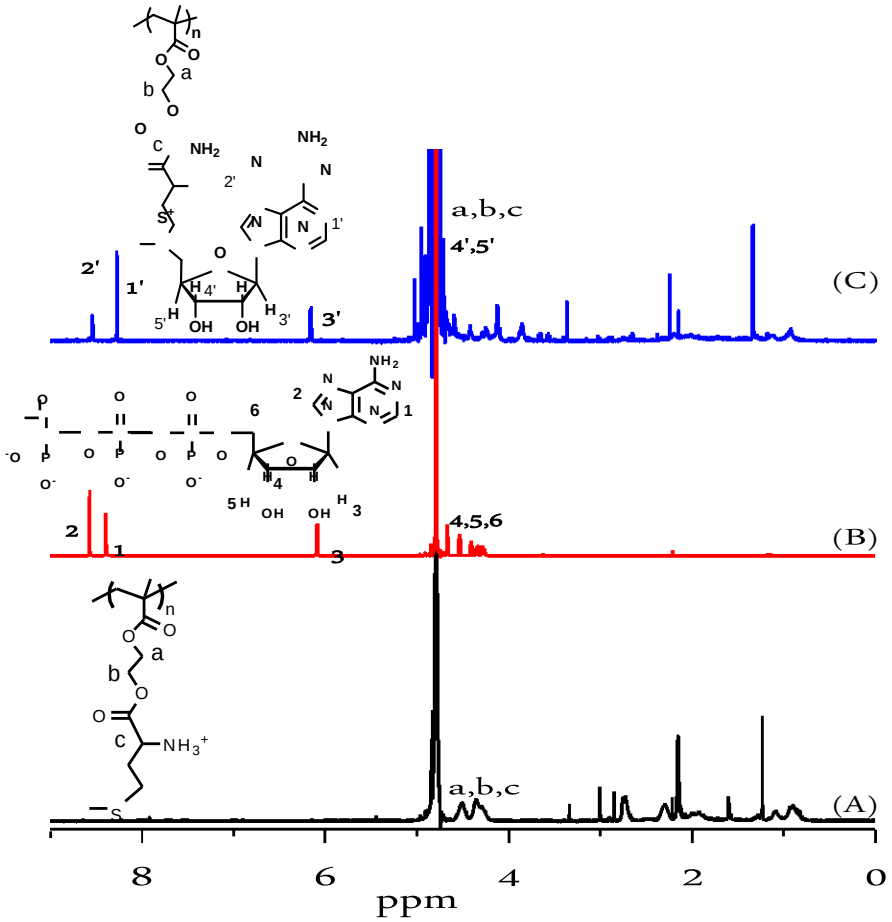


Fig. S7 ^{1}H NMR spectra of (A) DPMETMA, (B) ATP, and (C) DPMETMA-ATP complex in D_2O.

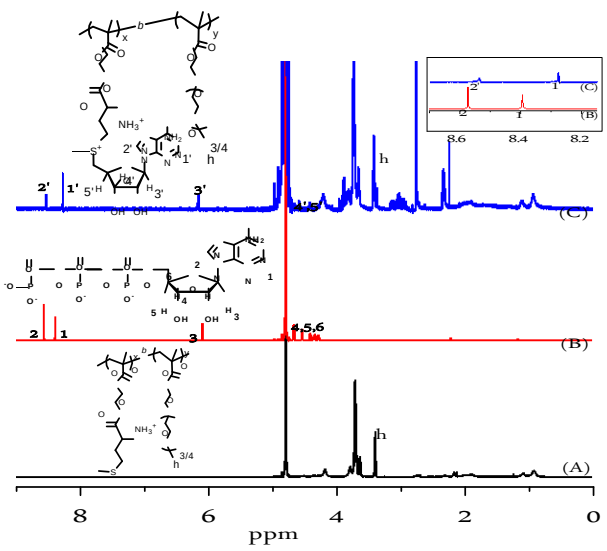


Fig. S8 ^{1}H NMR spectra of (A) DPMETMA-*b*-PPEGMA, (B) ATP, and (C) DPMETMA-*b*-PEGMA-ATP complex in D_2O.

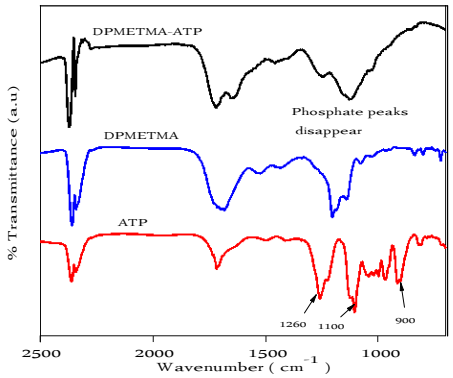


Fig. S9 FT-IR spectra of ATP, DPMETMA, and DPMETMA-ATP complex.

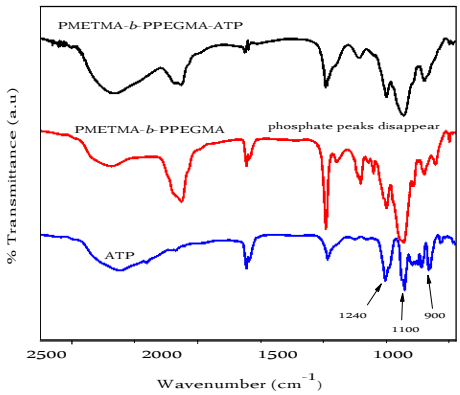


Fig. S10 FT-IR spectra of ATP, $PMETMA_{14}$-*b*-$PPEGMA_{42}$, and $PMETMA_{14}$-*b*-$PPEGMA_{42}$-ATP.

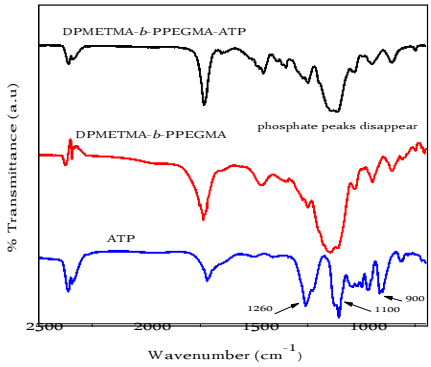


Fig. S11 FT-IR spectra of ATP, DPMETMA$_{14}$-*b*-PPEGMA$_{42}$, and DPMETMA$_{14}$-*b*-PPEGMA$_{42}$-ATP.

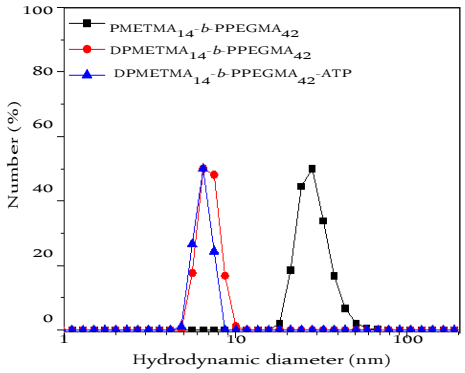


Fig. S12 Size distributions detected by DLS for (A) PMETMA$_{14}$-*b*-PPEGMA$_{42}$, (B) DPMETMA$_{14}$-*b*-PPEGMA$_{42}$ and (C) DPMETMA$_{14}$-*b*-PPEGMA$_{42}$-ATP complex in aqueous medium at 25 °C (solution concentration: 1.0 mg/mL).

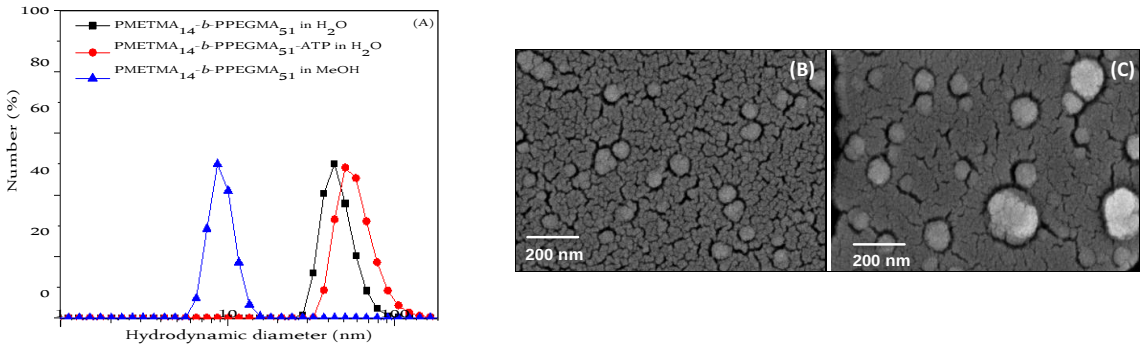


Fig. S13 (A) Size distributions detected by DLS for PMETMA$_{14}$-*b*-PPEGMA$_{51}$ in aqueous medium and methanol, and PMETMA$_{14}$-*b*-PPEGMA$_{51}$-ATP in aqueous medium at 25 °C. FE-SEM images of (B) PMETMA$_{14}$-*b*-PPEGMA$_{51}$ and (C) PMETMA$_{14}$-*b*-PPEGMA$_{51}$-ATP. During the drying of the thin film, some cracks appeared.

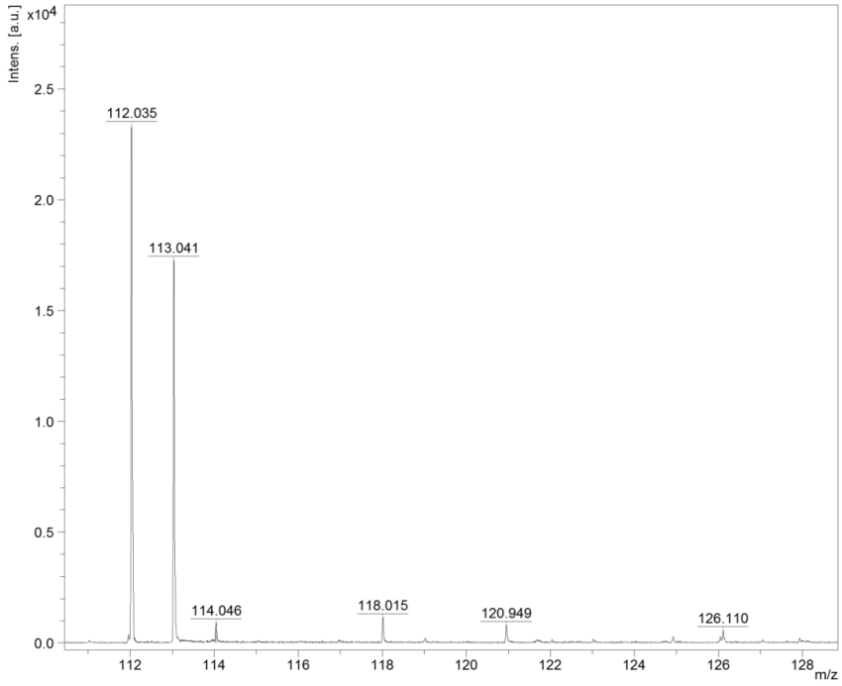


Fig. S14 MALDI-TOF spectrum of the reaction mixture after the methyl transfer reaction to cytosine in the presence of H_2O_2 and $PMETMA_{14}$-*b*-$PPEGMA_{51}$-ATP complex.

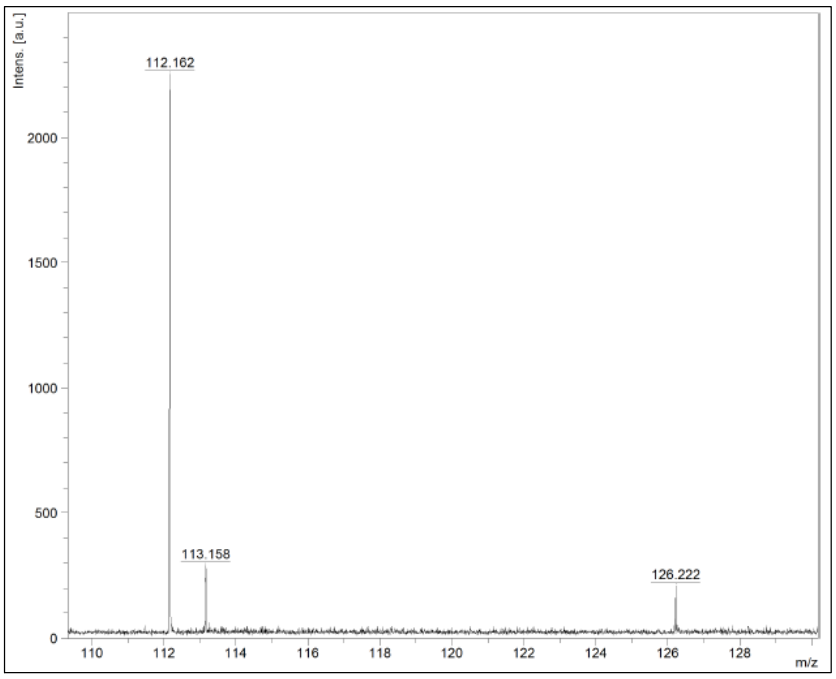


Fig. S15 MALDI-TOF spectrum of the reaction mixture after the methyl transfer reaction to cytosine in the presence of H_2O_2 and DPMETMA-ATP complex.

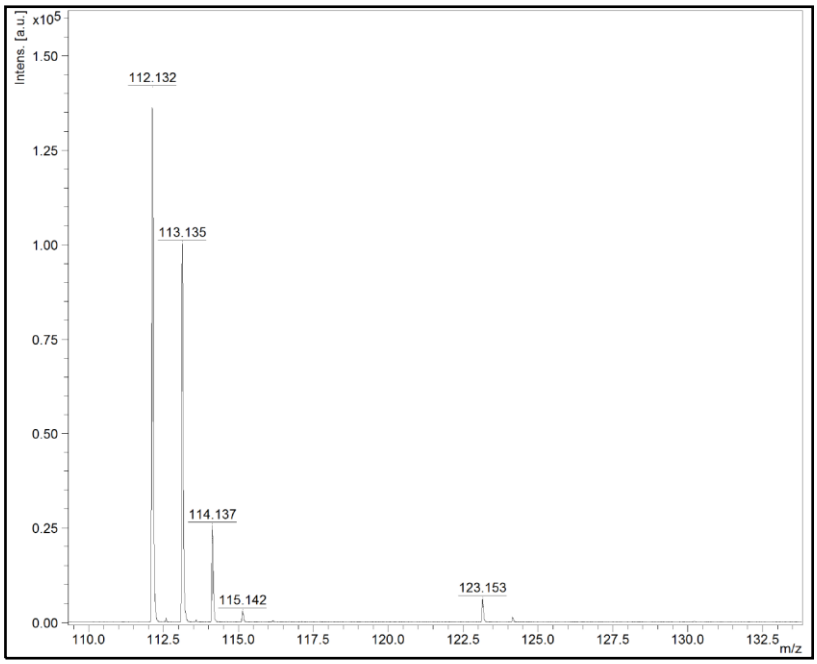


Fig. S16 MALDI-TOF spectrum of the reaction mixture after the photo reaction between cytosine and H_2O_2.

References

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