Electronic Supplementary Material (ESI) for Soft Matter.


# Supporting Information for

# Peptide-induced RAFT polymerization via an amyloid-$\beta_{17-20}$-based chain transfer agent

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## Experimental Section

### Materials

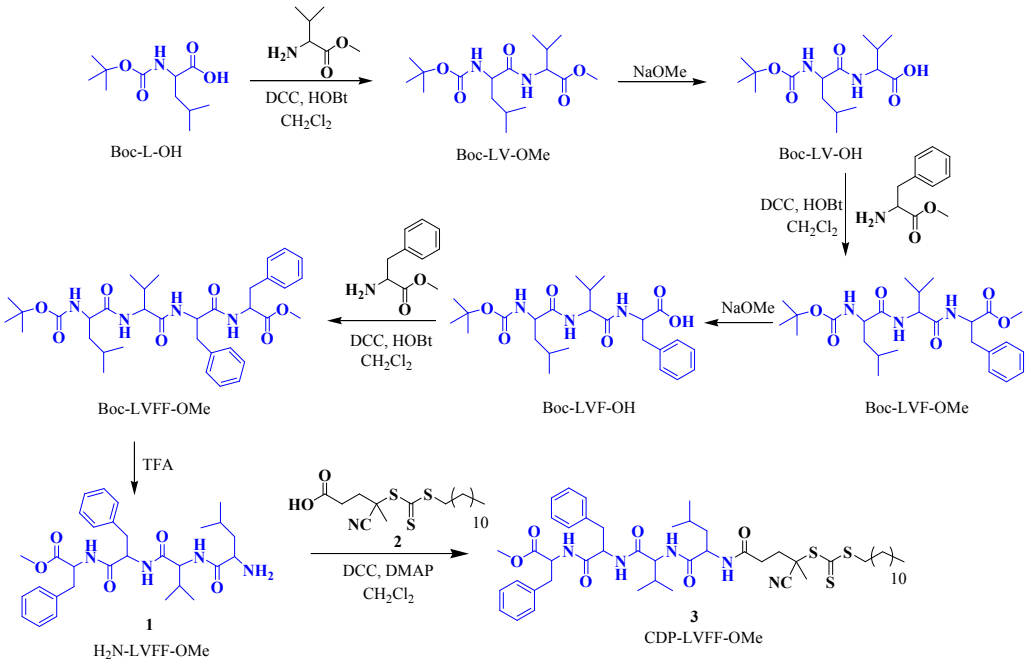
Boc-L-Leucine monohydrate (Boc-L-OH.$H_2O$, 99%) was purchased from TCI chemicals, andL-Phenylalanine methyl ester hydrochloride (HCl.$H_2$N-F-OMe, 98%) and L-Valine methyl ester hydrochloride (HCl.$H_2$N-V-OMe, 98.5%), were received from Carbolution chemicals.1-Di(ethylene glycol) methyl ether methacrylate (DEGMA, 95%), poly(ethylene glycol) methyl ether methacrylate (PEGMA, average $M_n$ = 300), and di(ethylene glycol) dimethacrylate (DEGDMA, 95%) was received from Sigma-Aldrich and passed through a basic alumina column prior to polymerization. Hydroxybenzotriazole hydrate (HOBt, 97%), trifluoroacetic acid (TFA, 99%), triethylamine ($Et_3N$, 99%), 4-(dimethylamino)pyridine(DMAP, 99%), *N*,*N*-Diisopropylethylamine (DIPEA, 99%),and anhydrous *N*,*N*-dimethylformamide (DMF, 99.9%) were purchased from Sigma-Aldrich and used as received. *N*,*N*-Dicyclohexylcarbodiimide (DCC, 99%) were received from Alfa Aesar. The initiator azobisisobutyronitrile (AIBN, 98%) was recrystallized twice from methanol. Reaction solvents were dried and distilled according to the standard procedures.$H_2$N-LVFF-OMe was adopted from our recent work published elsewhere.[1a]The synthesis of 4-cyano-4-((dodecylsulfanylthiocarbonyl)sulfanyl)pentanoic acid (CDP) was done as per reported procedure elsewhere.[1b]

## Instrumentation and Measurements

Gel permeation chromatography (GPC) based studies were carried out using a Viscotek GPCmax VE 2001 (Viscotek$^{TM}$) having a set of column $H_{HR}$-H-Guard-17369 and $GMH_{HR}$-N-18055 and in DMF solution with 10 mM $LiNTf_2$with the flow rate of 1.0 mL $min^{-1}$ (at 60 °C).External calibration was done by using polystyrene standards, and datas were analysed by using OmniSEC software (V 4.5.6). Nuclear magnetic resonance (NMR) studies for

solution state $^1$H NMR spectroscopy were doneby using a Varian Gemini 2000 (400 MHz) or on a Varian Unity Inova 500 (500 MHz) NMR spectrometer in $CDCl_3$ or DMSO-$d_6$ as solvent at 27 °C. Dataswere analysed by applyingMestRec-C software (version 4.9.9.6). Electrospray ionization time-of-flight mass spectroscopy (ESI-TOF MS) based measurements wereperformedona Focus Micro TOF of Bruker Daltonicsunder positive mode by applying 4.5 kV accelerator voltagewith a transfer line of 190 °C at the spectral rate of 1 Hz. The obtained spectra were processed by using Brucker Daltonic ESI compass 1.3 for microTOF (Data Analysis 4.0). Rheology studies were carried out on an Anton Paar (Physica) MCR 101-DSO using a parallel plate-plate geometry (plate diameter 8 mm) at 25 °C. Fourier transformation infrared spectroscopy (FTIR) measurements were performed on VERTEX 70 IR spectrometer (Bruker) by using a KBr pellet or single reflex-diamond attenuated total reflectance unit (ATR-FTIR) for solid-state investigations. Transmission electron microscopy (TEM) based studies were undertakenby using an EM 900 transmission electron microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany)for taking micrographs of self-assembled structure of polymer in solution.

**Scheme S1.** Full synthetic scheme forthe LVFF peptide-based macro-CTA**3**.




**Synthesis of Dipeptide Boc-LV-OMe.** Initially, $H_2N$-V-OMe (4.2 g, 32.02 mmol) was isolated from their corresponding methyl ester hydrochloride by its neutralization followed by extraction with ethyl acetate and subsequent drying. Then to its stirring solution in 250 mL dry dichloromethane ($CH_2Cl_2$) in an ice-water bath condition7.4 g of Boc-L-OH (32.02 mmol) was added followed by DCC (6.6 g, 32.02 mmol) and HOBt (4.9 g, 32.02 mmol)additions. The reaction mixture was further allowed to stir for 24 h at room temperature and then it was filtered by suction filteraton to remove the formed insoluble *N*,*N*′-dicyclohexylurea (DCU) byproduct. The filterate was evaporated and dissolved in 150 mL of ethyl acetate (EtOAc) and then organic layer was washed three times with 100 mL of 1N HCl, saturated $NaHCO_3$ and brine solution,followed by drying over anhydrous $Na_2SO_4$and evaporation in vacuum.Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture hexane:EtOAc (9:1), resulting final product dipeptide Boc-LV-OMe as a white solid with a yield of 85%, and was further characterized by $^1$H NMR(Figure S1).

**Synthesis of Dipeptide Boc-LV-OH.**To a stirring solution of dipeptide Boc-LV-OMe (7.0 g, 20.32 mmol) in60 mL of methanol (MeOH),45 mL aqueous solution of 2M NaOH was added and stirred at room temperature. Thin layer chromatography (TLC) was used to moniter the progress of saponification reaction. Then methanol was removed from the reaction mixture by applying vacuum via rotary evaporator followed by addition of 100 mL water. 1M HCl was added dropwise to adjust the pH of the obtained aqueous layer to 2 and was further extracted with ethyl acetate followed by drying over anhydrous $Na_2SO_4$ and evaporation under vacuum. Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture $CH_2Cl_2$:EtOAc (9:1), resulting final product dipeptideBoc-LV-OH with a yield of 83%, and was further characterized by $^1$H NMR (Figure S2).

**Synthesis of Tripeptide Boc-LVF-OMe.** Initially,$H_2$N-F-OMe (4.6 g, 22.64 mmol) was isolated from their corresponding methyl ester hydrochloride by its neutralization followed by extraction with ethyl acetate and subsequent drying. Then to its stirring solution in 200 mL dry $CH_2Cl_2$ in an ice-water bath condition6.8 g of Boc-LV-OH (20.58 mmol) was added followed by DCC (4.24 g, 20.58 mmol) and HOBt (3.2 g, 20.58 mmol)additions. The reaction mixture was further allowed to stir for 24 h at room temperature and then it was filtered by suction filteraton to remove the formed insoluble DCU byproduct. The filterate was evaporated and dissolved in 160 mL EtOAc and then organic layer was washed three times with 100 mL of 1N HCl, saturated $NaHCO_3$ and brine solution,followed by drying over anhydrous $Na_2SO_4$and evaporation in vacuum.Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture hexane:EtOAc (8:2), resulting final product tripeptide Boc-LVF-OMe as a white solid with a yield of 81%, and was further characterized by $^1$H NMR(Figure S3).

**Synthesis of Tripeptide Boc-LVF-OH.** Toa stirred solution of tripeptide Boc-LVF-OMe (4.0 g, 8.14 mmol) in35 mL of MeOH,25 mL aqueous solution of 2M NaOH was added and stirred at room temperature. TLC was used to moniter the progress of saponification reaction. Then methanol was removed from the reaction mixture by applying vacuum via rotary evaporator followed by addition of 100 mL water. 1M HCl was added dropwise to adjust the pH of the obtained aqueous layer to 2 and was further extracted with ethyl acetate followed by drying over anhydrous $Na_2SO_4$ and evaporation under vacuum. Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture $CH_2Cl_2$:EtOAc (8:2), resulting final product tripeptideBoc-LVF-OH with a yield of 80%, and was further characterized by $^1$H NMR (Figure S4).

**Synthesis of Boc-ProtectedTetrapeptide Boc-LVFF-OMe.** Initially,$H_2$N-F-OMe (3.9 g, 21.65 mmol) was isolated from their corresponding methyl ester hydrochloride by its neutralization followed by extraction with ethyl acetate and subsequent drying. Then to its stirring solution in 200 mL dry $CH_2Cl_2$ in an ice-water bath condition9.4 g of Boc-LVF-OH (19.68 mmol) was added followed by DCC (4.5 g, 21.65 mmol) and HOBt (3.3 g, 21.65 mmol)additions. The reaction mixture was further allowed to stir for 24 h at room temperature and then it was filtered by suction filteraton to remove the formed insoluble DCU byproduct. The filterate was evaporated and dissolved in 170 mL of EtOAc and then organic layer was washed three times with 100 mL of 1N HCl, saturated $NaHCO_3$ and brine solution,followed by drying over anhydrous $Na_2SO_4$and evaporation in vacuum.Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture hexane:EtOAc (7:3), resulting final product tetrapeptide Boc-LVFF-OMe as a white solid with a yield of 78%, and was further characterized by $^1$H NMR(Figure S5).

**Synthesis of Amino-Group Modified Tetrapeptide $H_2$N-LVFF-OMe (1).** To a stirred solution of 3.7 g Boc-protected tetrapeptide Boc-LVFF-OMe(5.79 mmol) in 50 mL

$CH_2Cl_2$in ice-water bath condition,4.4 mL TFA (57.46 mmol) was added drop-wise and the reaction mixture was allowed to stir for 2 hat room temperature. Then the reaction mixture was driedfollowed by the addition of 150 mL EtOAc. The organic solution was thoroughly washed several times with $NaHCO_3$ solution followed by drying over anhydrous $Na_2SO_4$and in vacuum by rotary evaporator.Silica gel column chromatography was further used to purify the crude product by applying the eluent solvent mixture $CH_2Cl_2$:EtOAc (7:3), resulting final product tetrapeptide $H_2N$-LVFF-OMe (**1**) with a yield of 75%, and was further characterized by $^1H$ NMR (Figure S6).

**Synthesis of LVFF Peptide-Based RAFT Agent 3.**The as-synthesized peptide $H_2N$-LVFF-OMe, **1** (50 mg, 0.09 mmol) and CDP, **2**(41 mg, 0.10 mmol) was dissolved in 30 mL of dry $CH_2Cl_2$and the solution was purged with dry $N_2$for 20 min. To this stirring solution DCC (23 mg, 0.11 mmol), HOBt (17 mg,0.11 mmol) and DIPEA (19 μL, 0.11 mmol) was added under ice-water bath condition and was allowed to react at room termperature for 18 h in $N_2$ atmosphere. Then the formed reaction mixture was filtered by suction filteration to remove insoluble DCU and the organic layer was sequencially washed for one time with 100 mL of 1N HCl, saturated $NaHCO_3$ and brine solution,and dried over anhydrous $Na_2SO_4$. The removal of organic solvent was done by rotary evaporation and the crude product was purified by silica gel column chromatography using $CH_2Cl_2$:MeOH as mobile phase (4:1, v/v), to obtain a yellowish solid product CDP-LVFF-OMe(**3**)with an yield of 77 %, and was fully characteried by $^1H$ NMR, ESI-TOF MS and FTIR spectroscopy (Figure 1 and Figure S8).

**Synthesis of Polymers via RAFT Polymerization.**as a typical example, solution polymerization of DEGMA monomer was carried out by using LVFF-based chain-transfer agent (CTA) **3**. DEGMA (100 mg, 0.531 mmol), macro-CTA **3** (9.82mg, 10.626μmol),

AIBN (0.175 mg, 1.063μmol; 0.1 mL solution of 1.75 mg AIBN in 1 mL DMF),trioxane (1.91 mg, 21.252μmol) as an internal reference for measurement of monomer conversion via $^1$H NMR, anddry DMF (0.3 mL) were taken in a 20 mL septa sealed vial equipped with a magnetic stir bar. Then the reaction mixture was thoroughly deoxygenated by applying three consecutive freeze-pump-thaw cycles and placed in an oil-bath thermostated at 70 ºCwith stirring under $N_2$ atmosphere.Around 0.1 mL reaction mixture was periodically takenfrom the reaction mixture by a $N_2$ purged syringe to determine the number average molecular weight ($M_{n,GPC}$) by GPC measurement, and the monomer conversion by $^1$H NMR spectroscopy by comparing the integration of the monomer vinyl protons with the trioxane protons at $\delta$ 5.12 ppm. Then the reaction was quenched by plunging the vial into liquid nitrogen and exposing the solution to air,followed by subsequent precipitationfor thrice into excess of hexane for purification. Then, the obtained peptide-polymer conjugate PDEGMA-LVFF-OMe (**4**)was dried under vacuum and characterized by $^1$H NMR and GPC (Figure 2 and 3B).A similar procedure was followedfor the RAFT polymerization for synthesis of LVFF-based macro-CTA **3** derived peptide-polymer conjugate PMMA-LVFF-OMe (**5**), and CDP (**2**) derivedunfunctionalized homopolymers PDEGMA (**7**) and PMMA (**8**) (see Table S1, Scheme 1 and S2).

The number average molar masses by $^1$H NMR ($M_{n,NMR}$)of the purified polymers were evaluated from the relative integration of the characteristic $\omega$-chain end terminalprotons (corresponds to CDP segment)-C$H_2$C$H_2$C(CN)(C$H_3$)- (4H, $\delta$=2.6-2.3 ppm)with respect to the repeating unit protons-C(O)OC$H_2$C$H_2$- (2nH, $\delta$=4.4-3.9 ppm, with n = degree of polymerization) for polymer **7** and conjugate **4**, and -C(O)OC$H_3$ (3nH, $\delta$=3.8-3.5 ppm) for polymer **8** and conjugate **5** (Table S1).Furthermore, $^1$H NMR based number average molar mass calculations were also made for conjugate **4** and **5** in a similar way, however by using

peptidic segment ($M_{n,NMR(Peptide)}$) methylene protons from benzyl groups (Ph-C$H_2$-)$_2$(4H, $\delta$ = 3.06-2.97 ppm) instead of CDP segment protons.

**Synthesis of Cross-Linked Gels via RAFT Polymerization.**As a typical example, RAFT polymerization of PEGMA monomer in the presence of divinyl cross-linker DEGDMA was carried out by using LVFF-based CTA **3** to afford the chemically cross-linked gel. PEGMA (0.4 g, 1.333 mmol), DEGDMA (13 mg, 53.33 μmol), macro-CTA **3** (12.3 mg, 13.33 μmol), AIBN (0.657 mg, 4 μmol), and 0.1 mL of dry DMF were placed in a 20 mL septa sealed vial equipped with a magnetic stir bar. Then the reaction mixture was thoroughly deoxygenated by applying three consecutive freeze-pump-thaw cycles and placed in an oil-bath thermostated at 70 ºC with stirring under $N_2$ atmosphere for 18 h.Then the reaction was quenchedby plunging the vial into liquid nitrogen and exposing to air, and the purification of the obtained semi-solid crude gel was done by placing it in a 250 mL beaker and washing/dialysing againstDI water (24 h) and acetone (12 h) with consecutive cycles(6×150 mL) to remove unreacted monomers, DMF and other impurities. Finally, the achieved peptide-functional gel PPEGMA$_{gel}$-LVFF-OMe (**6**) was dried in air for 6 h followed by under vacuum for 2 days (Scheme 1). A similar procedure was applied for the synthesis of RAFT-made gel PPEGMA$_{gel}$(**9**) by using CTA **2**(Scheme S3).

Gelation time was noted (90 and 140 min for **6** and **9**, respectively) when a highly viscous gel formed during reaction and magnetic bar stirring was stopped. Monomer conversion was calculated (90 and 87% for **6** and **9**, respectively) via gravimetric method by comparing the weight of purified dry gel relative to the monomer feed.The amount of swelling (*w*/*w*) occurred, swelling degree at equilibrium ($SD_e$) forthe hydrogels were investigated (480% and 360% for **6** and **9**, respectively)by soaking a measured amount of purified dried gel in DI water at room temperature for 24 h to reach the maximum swelling and then by calculating according to the following equation:

$$SD_e\ (\%) = \frac{W_{eq} - W_0}{W_0} \times 100\%$$

Where $W_0$ and $W_{eq}$ are the weight of the dry gel prior to swelling initially and at equilibrium swelling, respectively.

**Micellar Aggregates Formation by Conjugate PDEGMA-LVFF-OMe.** 5 mg of amphiphilic polymer-peptide conjugate PDEGMA-LVFF-OMe (**4**) were dissolved in 2 mL acetone, and the solution was transferred to a dialysis bag (Spectra/por dialysis membrane, molecular weight cutoff 1 kDa) and was extensively dialyzed against cold DI water for 72 h with replacing water after every 2-6 h. The prepared micellar aggregation solution was further diluted ($c$ = 0.2 mg mL$^{-1}$), and then a small aliquot was placed on a Cu grid coated with a carbon film, stained with uranyl acetate solution (2%) and air dried for undertaking TEM studies. The above dialysis and sample preparations were carried out by maintaining temperature at 20 °C.

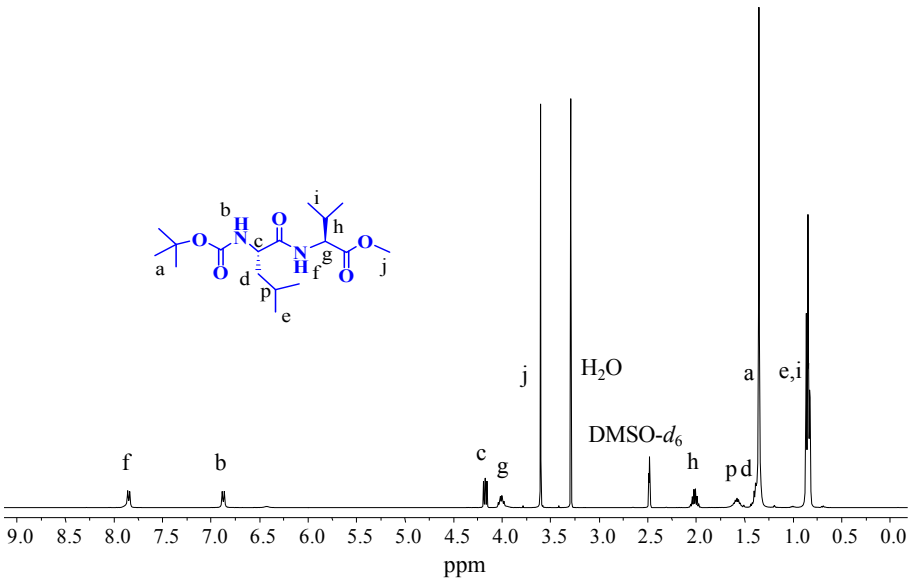



**Fig. S1.** $^1$H NMR spectrum of dipeptide Boc-LV-OMe.

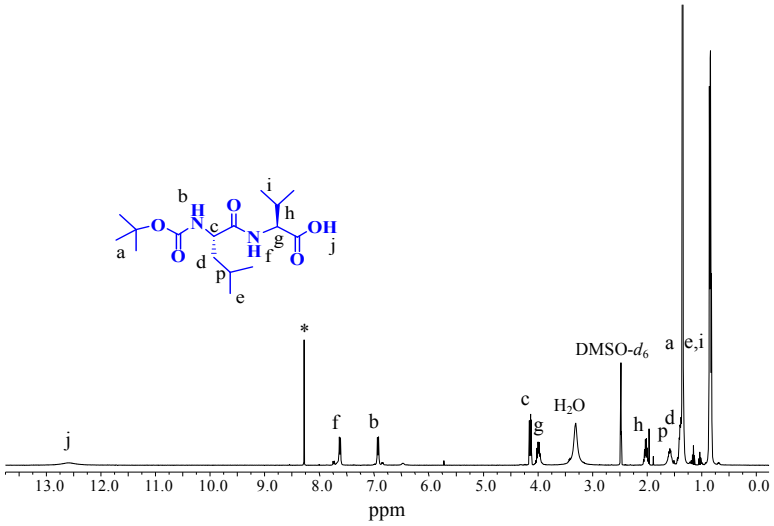



**Fig. S2.**$^1$H NMR spectrum of dipeptide Boc-LV-OH(* denote the solvent resonance of chloroform).

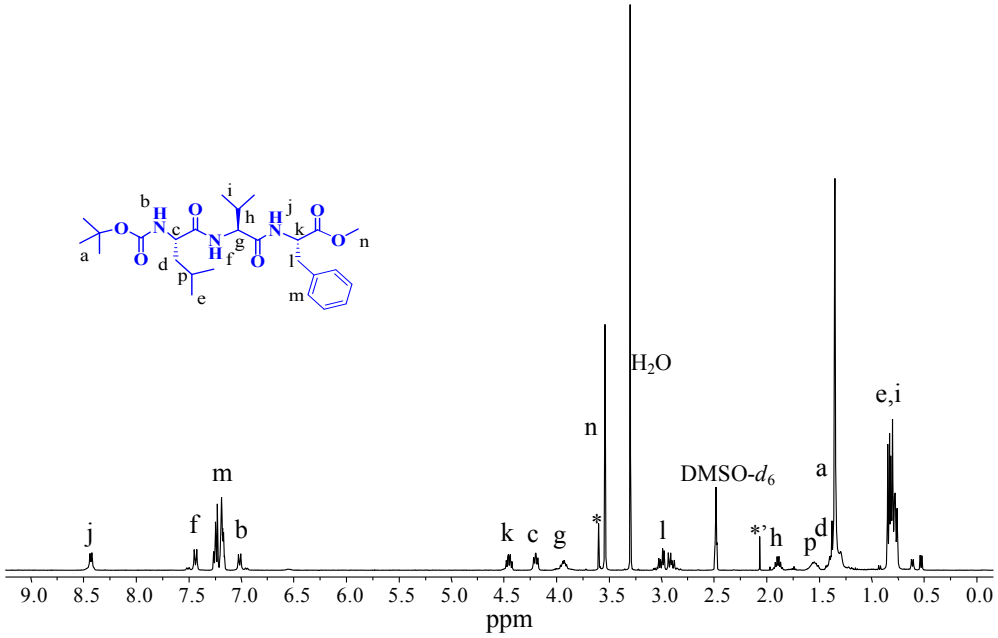



**Fig. S3.**$^1$H NMR spectrum of tripeptide Boc-LVF-OMe(* and *' denote the solvent resonance of 1,4-dioxane and acetonitrile, respectively).

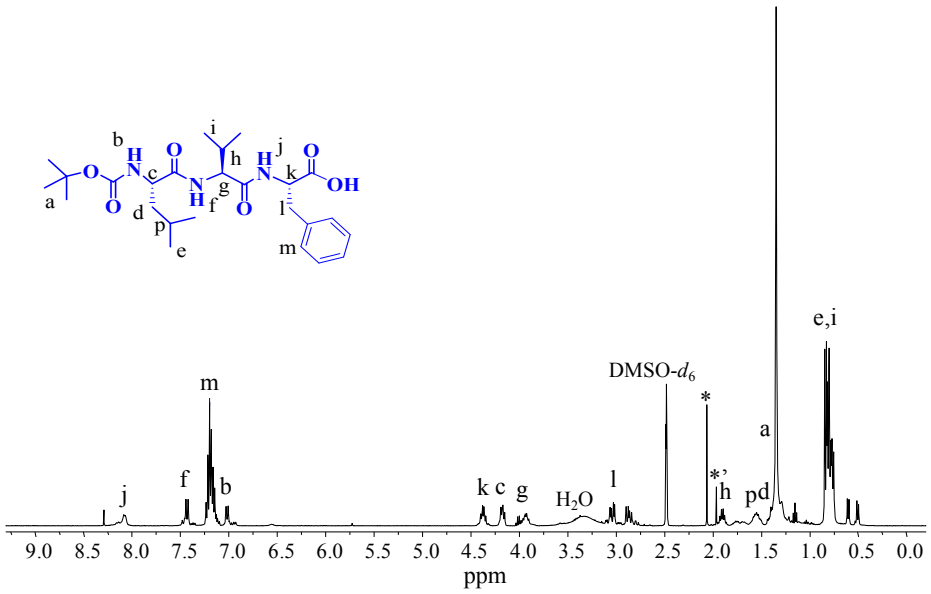



**Fig. S4.**[1]H NMR spectrum of tripeptide Boc-LVF-OH(* and *' denote the solvent resonance of acetonitrile and ethyl acetate, respectively).

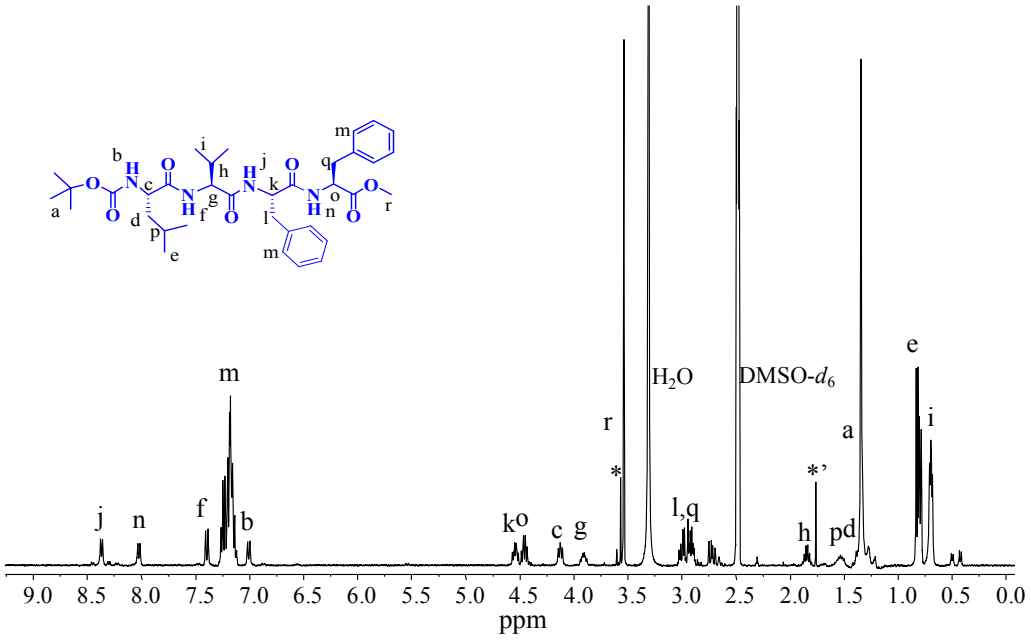



**Fig. S5.**[1]H NMR spectrum of tetrapeptide Boc-LVFF-OMe (* and *' denote the solvent resonance of THF).

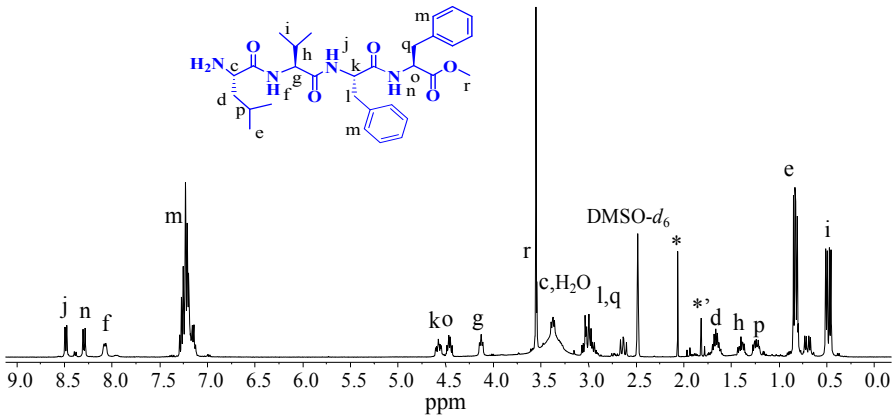



**Fig. S6.** $^1$H NMR spectrum of tetrapeptide $H_2N$-LVFF-OMe (**1**) (* and *' denote the solvent resonance of acetonitrile and THF, respectively).

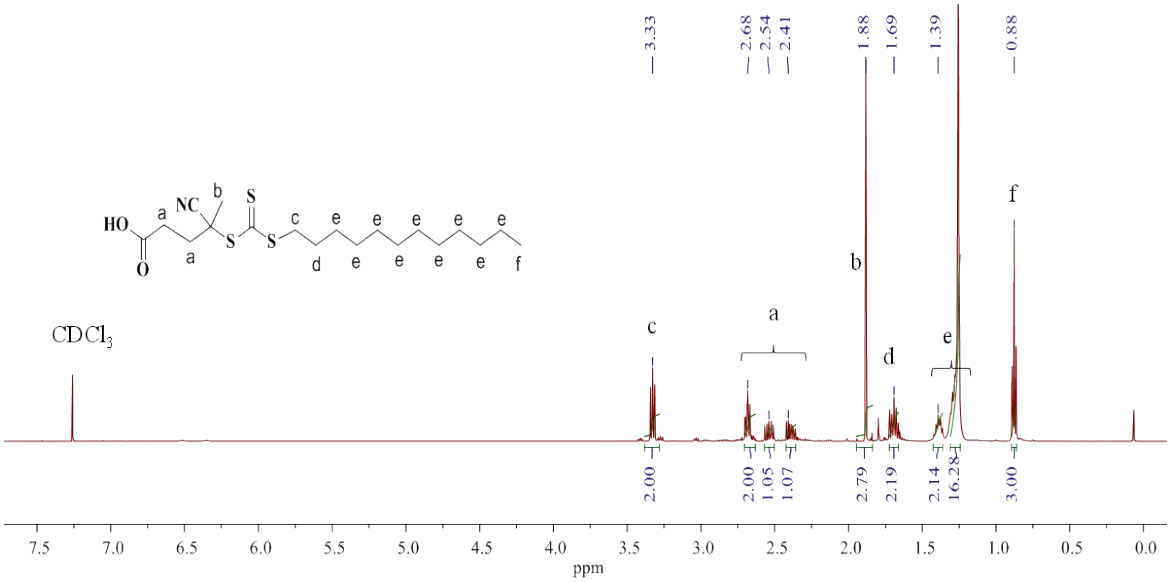



**Fig.S7.** $^1$H NMR spectrum of CDP-based RAFT agent (**2**).

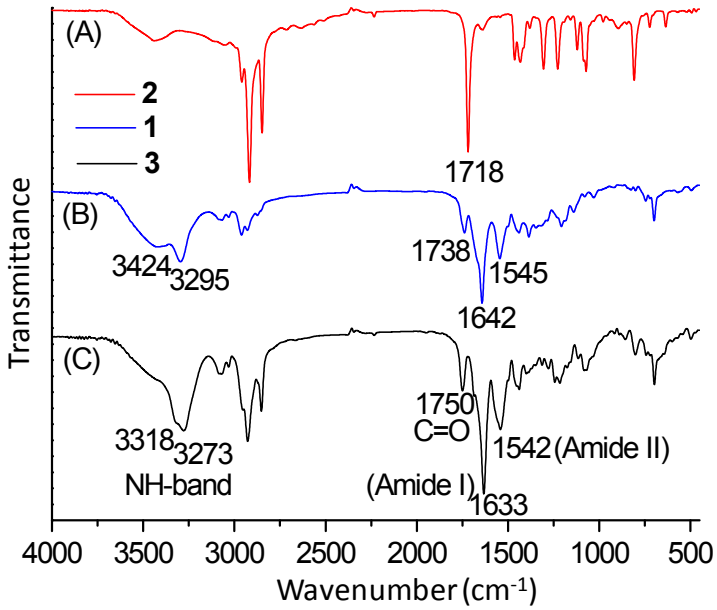



**Fig.S8.**Solid-state FTIRspectra of CTA **2** (A), peptide **1** (B), and LVFF-based macro-CTA **3** (C).

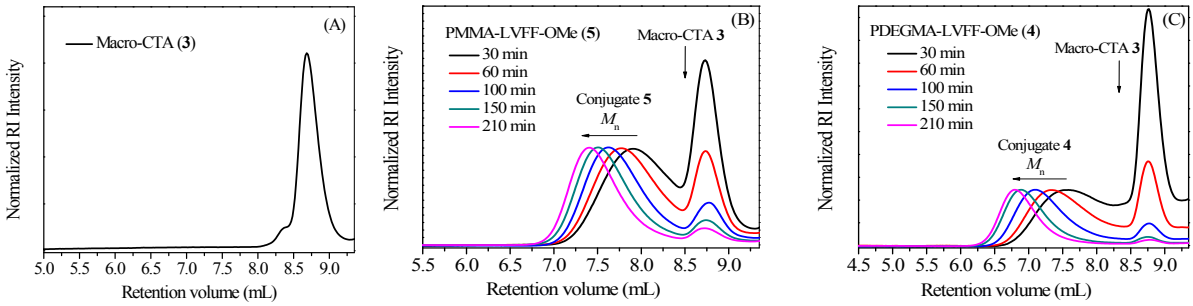



**Fig. S9.** GPC RI traces for (A) peptide-based RAFT agent **3** ($M_{n,GPC}$ = 1100 g mol$^{-1}$, $Đ$ = 1.1), (B and C) RAFT polymerization based synthesis of conjugate **5** and **4**, respectively (represents as extended spectra of Fig. 2C and D, respectively).

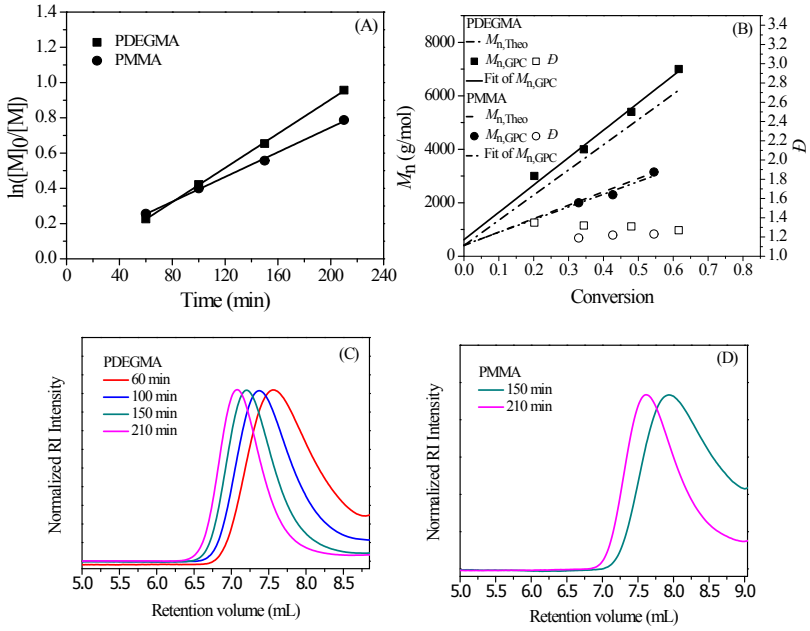
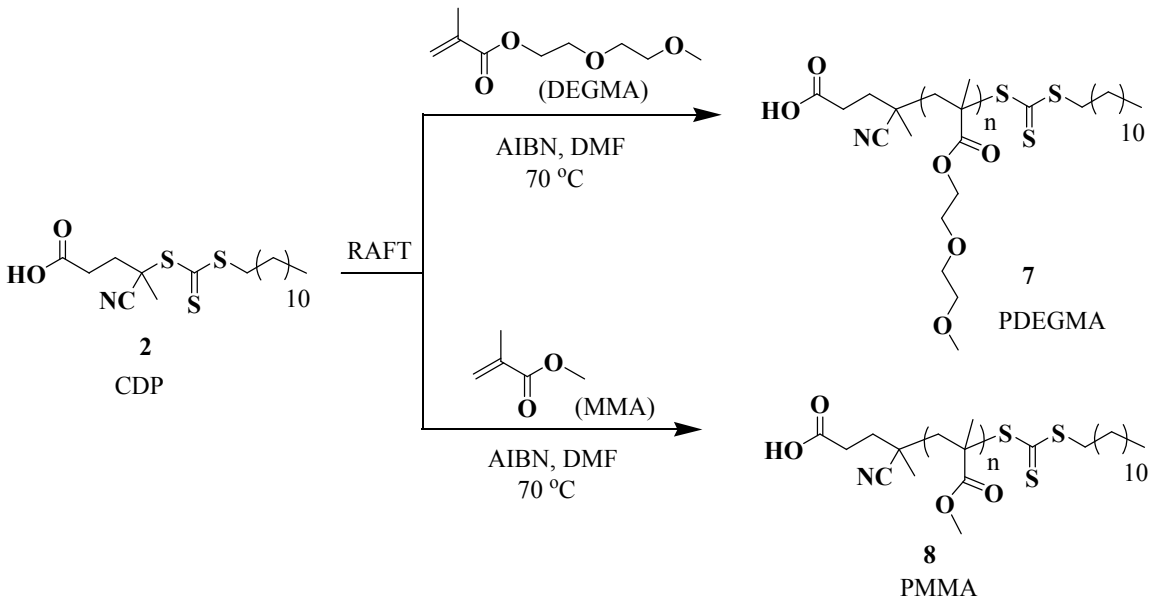



**Fig. S10.** Pseudo-first-order kinetics plot(A), and corresponding $M_n$ versus monomer conversion plot (B) for CDP **2** induced RAFT polymerizations for preparing homopolymer PDEGMA (**7**) and PMMA (**8**). (C and D) Evolution of the GPC RI traces as a function of retention volume for the synthesis of polymer **7** and **8**, respectively. (In D, a representable RI trace for time 100 min could not be achieved due to the formation of very low $M_n$ value polymer)



**Scheme S2.** Synthesis of CDP mediated RAFT polymerization based homopolymer PDEGMA (**7**) and PMMA (**8**).

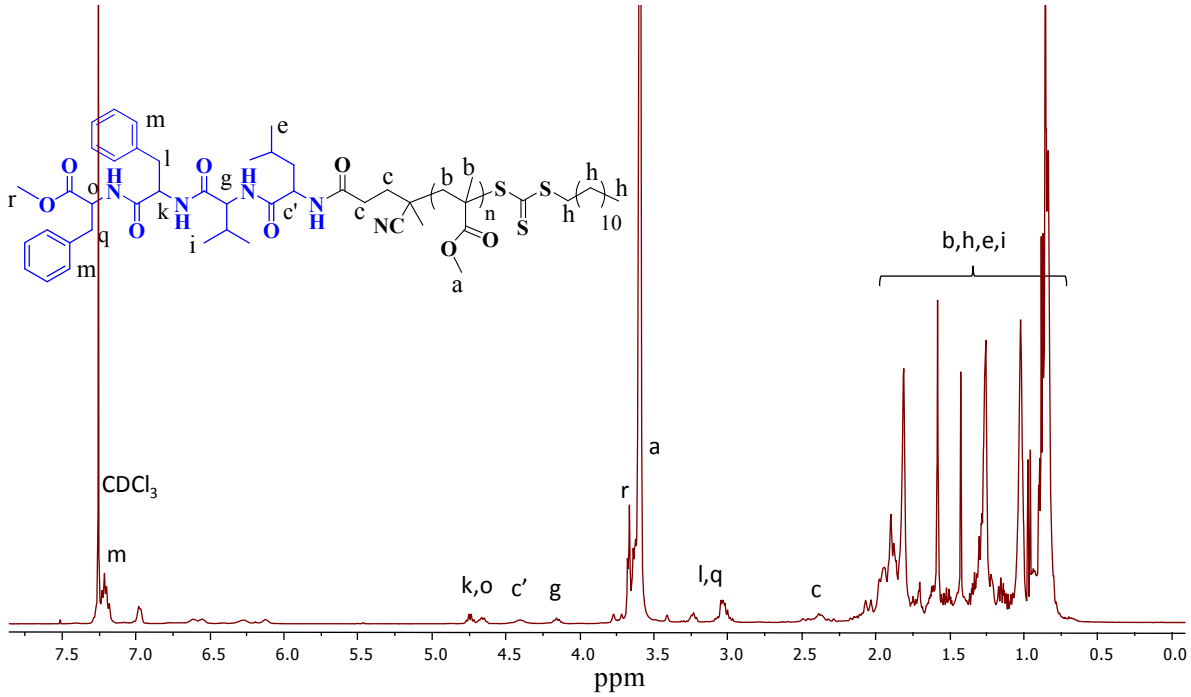



**Fig. S11.** $^{1}$H NMR spectrumof macro-CTA **3** derived polymeric conjugate PMMA-LVFF-OMe (**5**).

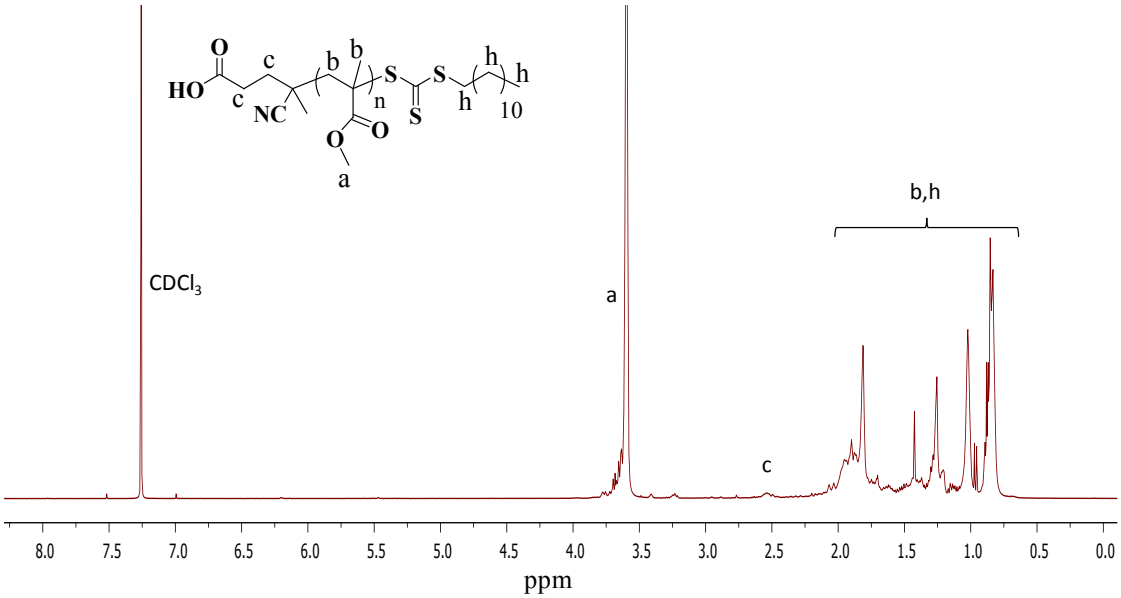



**Fig. S12.** $^{1}$H NMR spectrum of homopolymer PMMA (**8**).

**Table S1.** Results from the RAFT polymerization at 70 °C in DMFfor 210 min for the synthesis of homopolymers and peptide-polymer conjugates.

| CTA | Polymer/con-jugate | [M]/[CTA]/[I] | Conv.[a] (%) | $M_{n,GPC}$[b] (g mol$^{-1}$) | $Đ$[b] | $M_{n,theo}$[c] (g mol$^{-1}$) | $M_{n,NMR}$[d] (g mol$^{-1}$) | $M_{n,NMR(Peptide)}$[e] (g mol$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **3** | PDEGMA-LVFF-OMe (**4**) | 50/1/0.1 | 68.4 | 10000 | 1.3 | 7360 | 7050 | 6670 |
| **3** | PMMA-LVFF-OMe (**5**) | 50/1/0.1 | 68.8 | 4700 | 1.2 | 4372 | 3930 | 3600 |
| **2** | PDEGMA (**7**) | 50/1/0.1 | 61.6 | 7000 | 1.2 | 6208 | 5650 | NA |
| **2** | PMMA (**8**) | 50/1/0.1 | 54.5 | 3150 | 1.2 | 3130 | 3240 | NA |

[a]Calculated by $^1$H NMR spectroscopy in $CDCl_3$.[b]Measured by GPC using PS standard in DMF. [c]The theoretical molecular weight ($M_{n,theo}$) = ($[M]_0/[CTA]_0$×MW of M) × conversion + (MW of CTA). [d]Calculated via$^1$H NMRbased on CDP segment protons as discussed above in RAFT based polymer synthesis section.[e]Calculated via$^1$H NMRbased on peptide segment protons as discussed above in RAFT based polymer synthesis section.(M = monomer, CTA = chain transfer agent, MW = molecular weight, I = AIBN as Initiator, NA = not applicable)

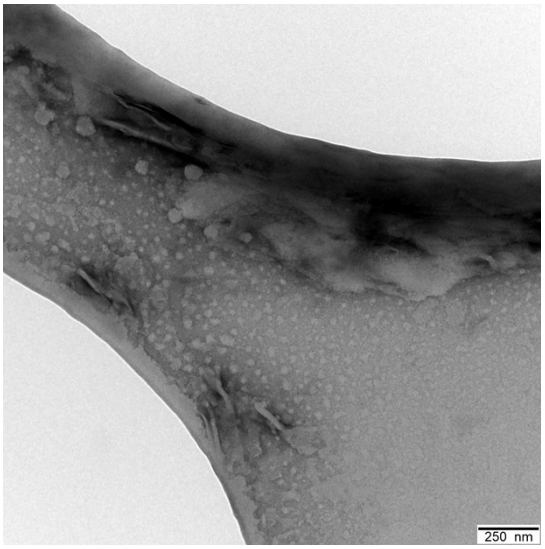
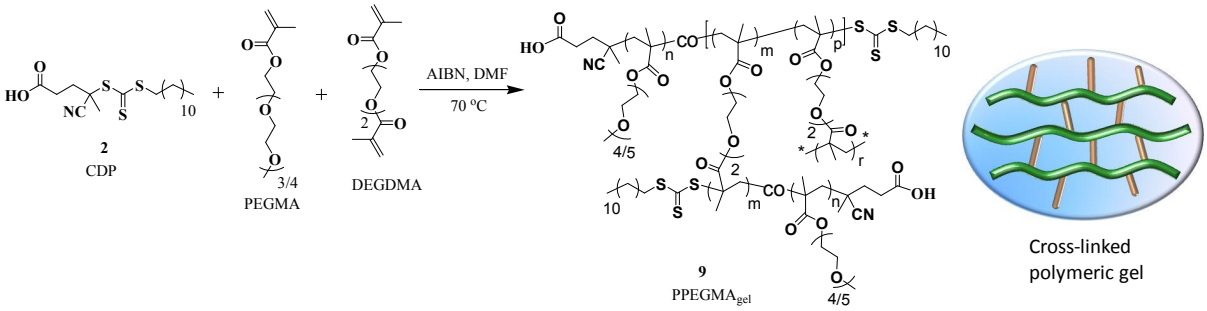



**Fig. S13.** TEM image for conjugate **4** in aqueous solution.




**Scheme S3.** Synthesis of CDP mediated RAFT polymerization based cross-linked polymeric hydrogel PPEGMA$_{gel}$(**9**).

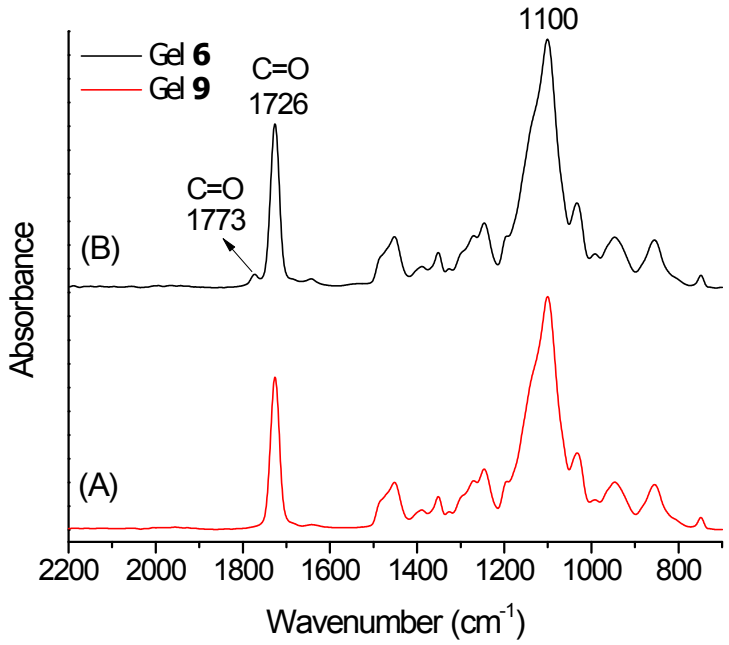



**Fig. S14.** ATR-FTIR spectra of dry gel **9** (A) and **6** (B).

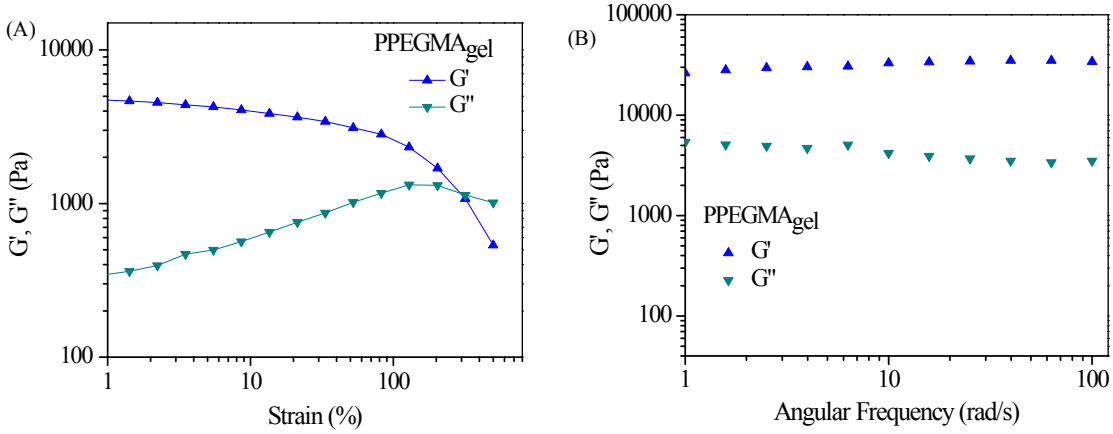



**Fig. S15.**Storage modulus (G') and loss modulus (G") versus (A) strain sweep, and (B) angular frequency (strain 2%) for the CDP mediated RAFT polymerization based cross-linked hydrogel **9**.

## References

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