

Non-conventional fluorescent biogenic and synthetic polymers without aromatic rings

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Experimental Methods

General information

Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Ile-OH, Rink Amide-AM resin were purchased from GL Biochem (Shanghai) Ltd. *N,N*-Diisopropylethylamine (99%; DIPEA), *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), hydroxybenzotriazole (HOBT), Abeta, L-alanine, L-isoleucine, L-valine, NIPAM, NtBA, mesitylene were purchased from Meryer (Shanghai) Chemical Technology Co., Ltd. Papain was purchased from Merck. Trifluoroacetic acid (TFA), triisopropylsilane (TIPS), BSA, GSH, PPRO, PALA, PLYS, azobisisobutyronitrile (AIBN), potassium bromide (FT-IR grade; KBr) were purchased from Sigma-Aldrich. H₃PO₄ (85%) and n-hexane were purchased from VWR chemicals. Tetrahydrofuran (THF), diethyl ether, *N,N*-dimethylamide (DMF) were purchased from RCI labscan Ltd. THF was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use. Diethyl ether was purified by passing through an aluminum oxide column. NIPAM was recrystallized from hexane/toluene. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States).

Synthesis of Oligopeptides (OALA, OVAL and OILE)

The synthetic procedure was followed by a typical solid-phase peptide synthesis protocol using Rink amide-AM resin¹. Deprotection of Fmoc group was carried out in 20% piperidine in DMF for 15 mins × 2. The coupling of amino acids was carried out for 2.5 h with molar ratio of Resin : Fmoc-protected amino acid : HBTU : HOBT : DIPEA = 1:4:4:4:8. Double coupling of amino acid was applied after 3rd position. For the last

deprotection of Fmoc group, 30 mins of 20% piperidine in DMF \times 2 and 20% piperidine in THF \times 2 was carried out to ensure the complete deprotection of Fmoc group. The resin was then washed with DMF \times 3, ethanol \times 3, THF \times 3 and DCM \times 3, and finally air dried.

A cleavage cocktail of TFA/water/TIPS (96:2:2) was freshly prepared and added to the dried resin for cleavage of oligopeptides. After 1 h, the cleavage cocktail was filtered and the filtrate was added dropwise to cold ether (\sim 45 mL) to precipitate the oligopeptides. The precipitate was collected by centrifuge at 7000 rpm, and further resuspended in \sim 45 mL cold ether, washed twice and dried in vacuum. The crude oligopeptide was then dissolved in minimal amount of TFA and precipitate in \sim 45 mL solvents. After that, the transparent precipitate was dried in vacuum. The process was repeated with solvents including THF \times 3 and then water \times 3. The precipitate must be vacuum-dried before dissolution in minimal amount of TFA, as otherwise an extra amount of TFA would be needed and it might result in dissolution of peptide in THF/water. The precipitate was finally dried in vacuum and stored at -20 °C before use.

Synthesis of PALA-SS

To a 100 mL round bottom flask was added L-alanine (1.0 g; 11 mmol), HBTU (4.6 g; 12 mmol), HOBT (1.6 g; 12 mmol), DIPEA (4.2 mL; 24 mmol) in 70 mL DMF. The reaction was stirred at room temperature for 20 h until all the suspended L-alanine consumed and the solution turned transparent. The solution was centrifuged at 7000 rpm and a transparent gel was collected. The transparent gel was further dispersed in 50 mL DMF, sonicated for 15 mins and collected by centrifuge for 3 times. The extra DMF was

removed by resuspending the gel in 50 mL diethyl ether and centrifuging at 7000 rpm for 10 mins. The precipitate was collected and dried in vacuum. The crude polypeptide was then dissolved in minimal amount of TFA and precipitate in ~45 mL solvents. After that, the precipitate was dried in vacuum. The process was repeated with solvents including THF \times 3 and then water \times 3. The precipitate must be vacuum-dried before dissolution in minimal amount of TFA, as otherwise an extra amount of TFA would be needed that it might result in dissolution of polypeptide in THF/water. The precipitate was finally dried in vacuum and stored at -20 °C before use.

Synthesis of PALA-HT

PALA-HT was synthesized by acid-catalyzed polycondensation as reported². Briefly, to a 100 mL round bottom flask was added L-alanine (1.67 g; 0.0188 mol) and H_3PO_4 (0.06 mL; 0.94 mmol) in 60 mL mesitylene. The suspension was frozen by liquid nitrogen and degassed 3 times with dry nitrogen. Then the reaction was refluxed under nitrogen for 5 h. Water formed in the reaction was removed using a Dean-Stark trap with a reflux condenser. Afterwards, the reaction was cooled to room temperature. The solvent was removed by filtration and the precipitate was washed with 200 mL methanol and 200 mL water. The precipitate was then dissolved in minimal amount of TFA and precipitated from 100 mL THF three times. The precipitate was further washed with water \times 3 by sonication for 10 mins. The precipitate was collected and dried at 40 °C under vacuum.

Synthesis of PNIPAM and PNtBA

PNIPAM and PNtBA were synthesized as reported^{3,4}. Briefly, to a 100 mL two-neck round bottom flask was added monomer (15.7 mmol; 1.78 g NIPAM or 2.00 g NtBA) and AIBN (0.125 mmol; 20.5 mg). The flask was evacuated under vacuum and flushed with dry nitrogen for three times. Then 60 mL distilled THF was injected and the reaction was stirred at 70 °C for 4 h. Afterwards, the reaction was stopped and cooled to room temperature. The THF solution was concentrated to ~10 mL under reduced pressure and added dropwise to 150 mL bad solvents (n-hexane for PNIPAM and water for PNtBA). The precipitates were collected by filtration. The precipitates were further dissolved in minimal amount of THF and precipitated from their corresponding bad solvents for 4 times. The precipitates were collected by filtration and dried under vacuum. The weight-averaged molecular weights for PNIPAM and PNtBA are 6320 and 8965 with PDI of 1.52 and 1.70, respectively.

Characterization

FTIR spectra were recorded on a PerkinElmer 16 PC FTIR spectrophotometer. Confocal microscopy images were performed on a Zeiss Laser Scanning Confocal Microscope, LSM7 DUO. The images were taken using a 405 nm laser (4.0% power) with a 429-485 nm filter. Solid state UV-vis absorbance and CD spectra were obtained on Chirascan equipped with solid state station under nitrogen. The fluorescence quantum yields were measured using a calibrated integrating sphere.^{5,6} A transparent KBr film (1 cm in diameter) was freshly prepared by pressing ~60 mg 1 wt% of homogeneously-mixed

peptides in KBr at 10 ton for 1 min. ^1H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using TFA-*d*, CDCl_3 , $\text{DMSO-}d_6$ as solvent and TFA ($\delta = 11.5$), tetramethylsilane (TMS; $\delta = 0$), TMS ($\delta = 2.5$) as internal reference, respectively. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 Mass Spectrometer System operated in a MALDI-TOF mode. Weight-average molecular weights and polydispersities (PDI) of the polymers were estimated on a Waters gel permeation chromatography (GPC) system using THF as eluent. Details about the experimental setup can be found in our previous publication⁷. Steady-state PL spectra and fluorescence lifetimes of peptides were determined with a Hamamatsu C11367-11 Quantaurs-Tau time-resolved spectrometer. Solution-state PL spectra of biomimetic polymers were recorded on a Perkin-Elmer spectrofluorometer LS 55.

Supplementary Figures and Tables

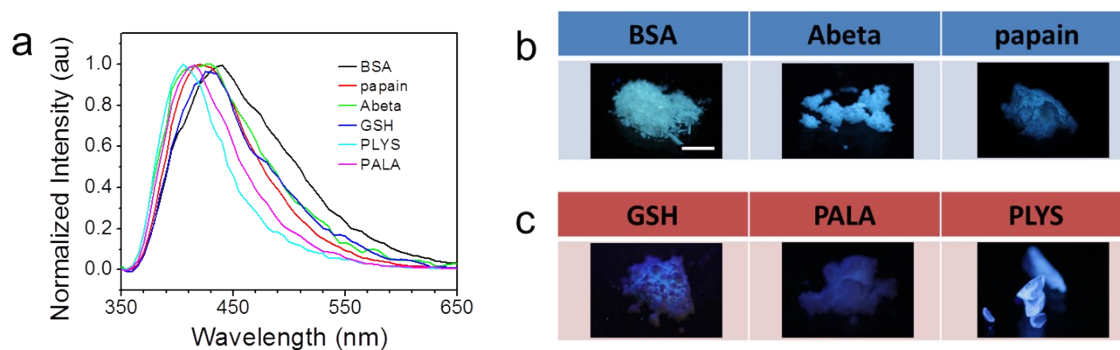


Figure S1. Luminescence in nature. (a) Normalized PL emission of natural or synthetic peptide and protein excited at 325 nm. (b) Physical appearance of natural protein containing aromatic amino acid without extended conjugation under a 365 nm UV lamp. (c) Physical appearance of synthetic peptides without aromatic amino acid under a 365 nm UV lamp. The scale bar is 5 mm.

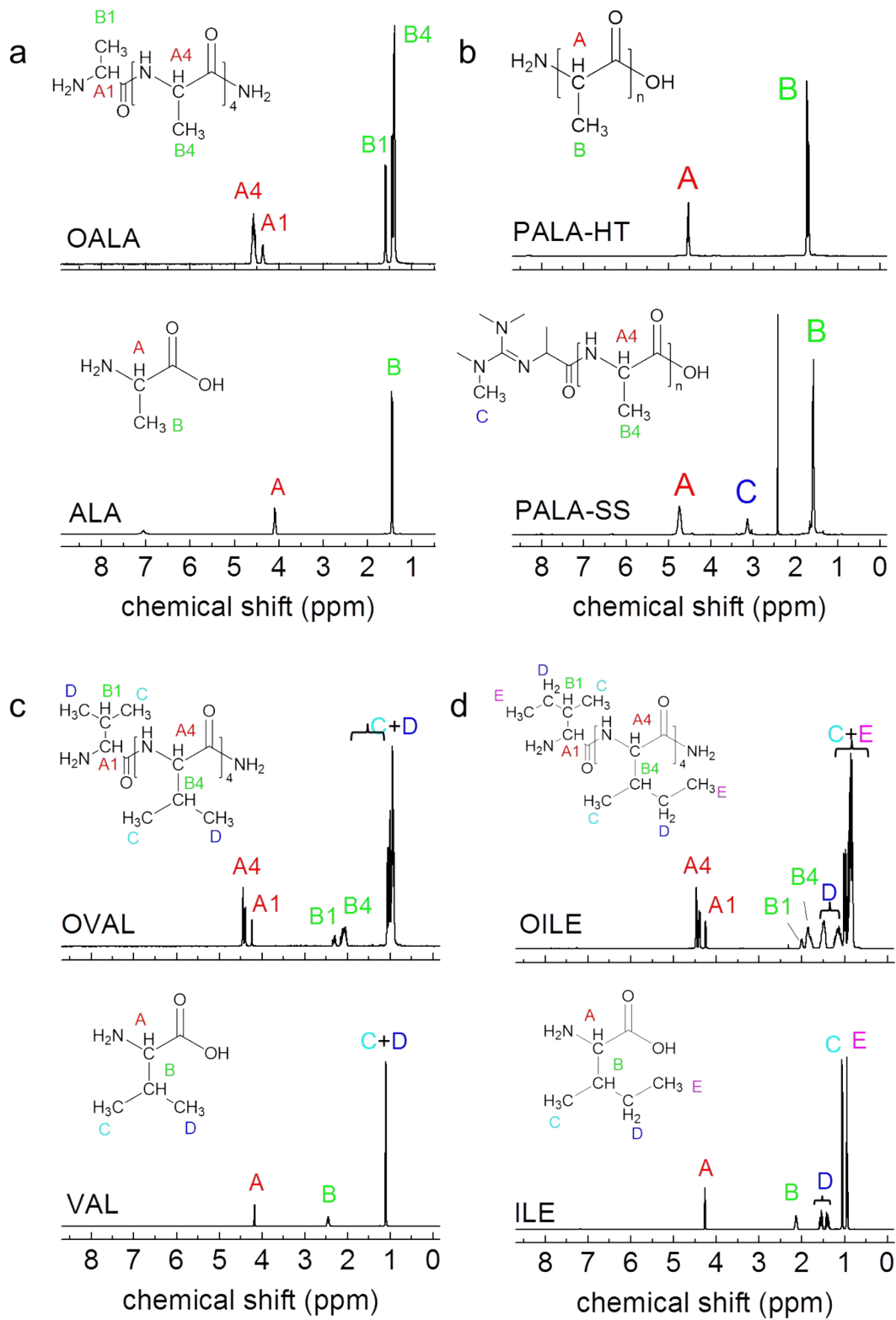
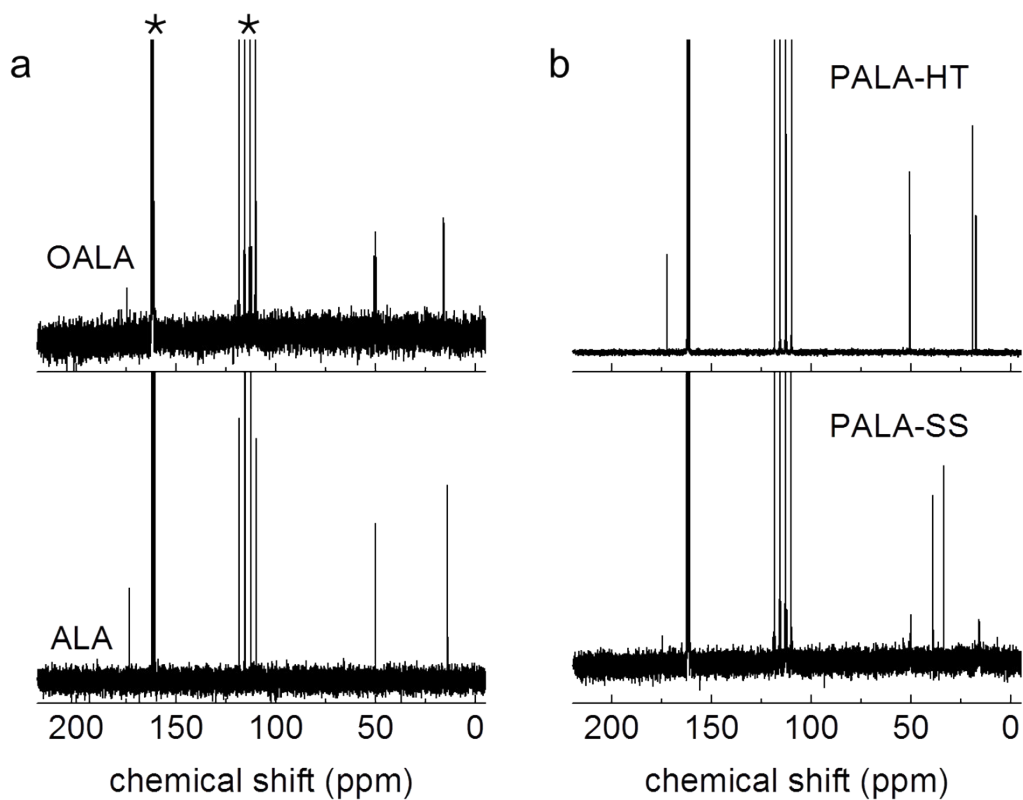


Figure S2. H-NMR spectra of (a) ALA and OALA (b) PALA-SS and PALA-HT. It is noticeable that a peak corresponding to guanidine structure ($-\text{N}=\text{C}(\text{N}(\text{CH}_3)_2)_2$) at the N-terminus is observed at δ 3.1 for PALA-SS. (c) VAL and OVAL; (d) ILE and OILE. The internal standard is TFA with $\delta = 11.5$.



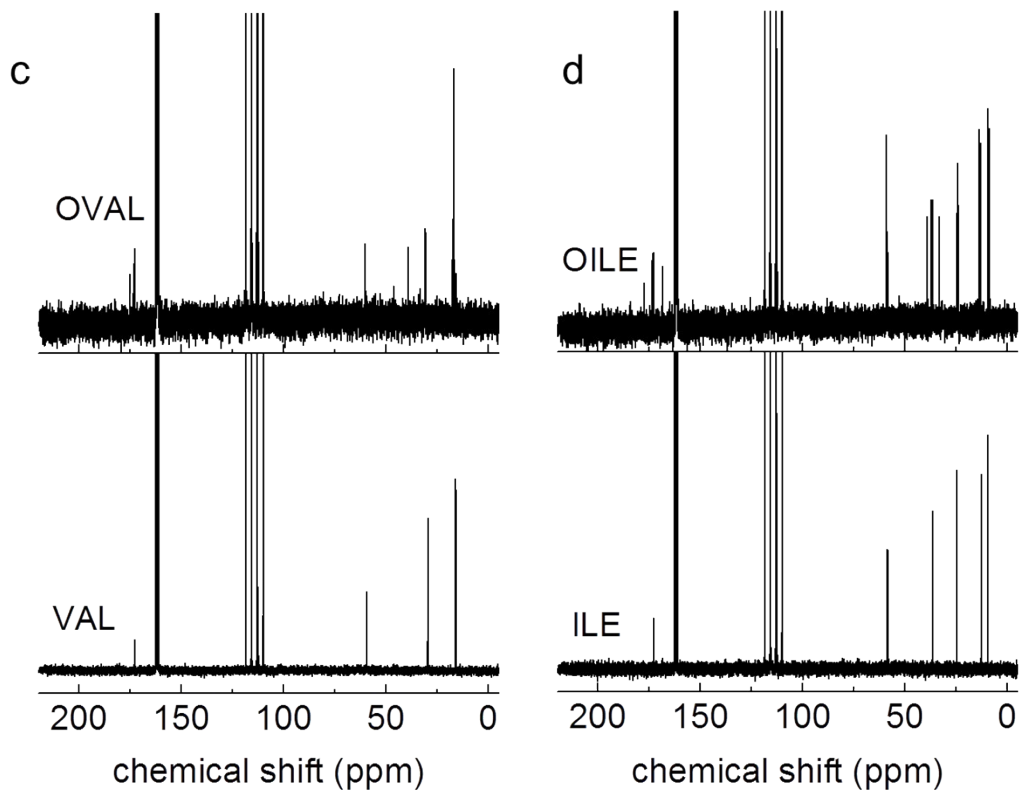


Figure S3. ^{13}C -NMR spectra of (a) ALA and OALA; (b) PALA-SS and PALA-HT; (c) VAL and OVAL; (d) ILE and OILE. The internal standard is TFA marked with asterisk.

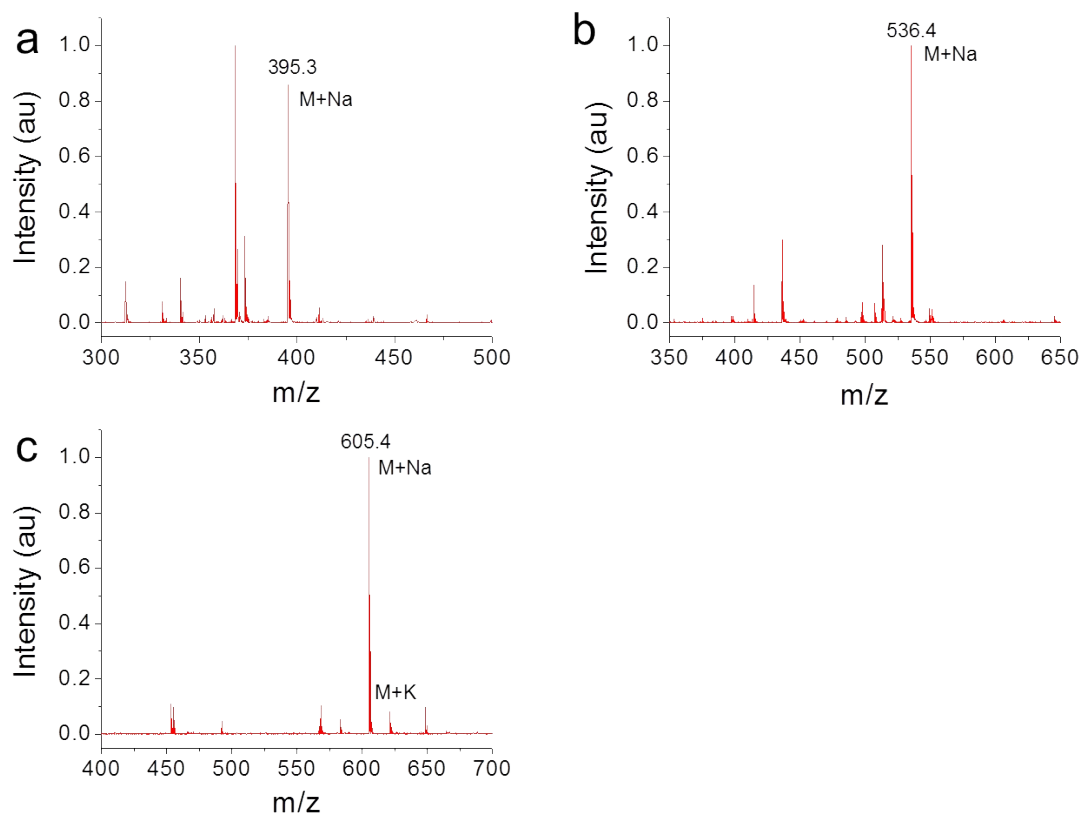


Figure S4. MALDI-TOF of (a) OALA (b) OVAL and (c) OILE. The matrixes are DHB for OALA and OVAL, and CHCA for OILE.

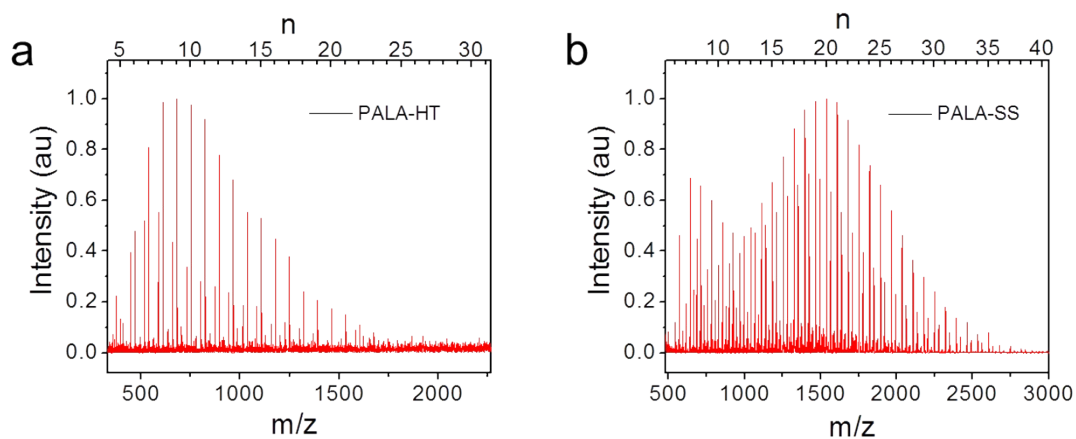


Figure S5. Degree of polymerization and molecular weight distribution of (a) PALA-HT and (b) PALA-SS determined by MALDI-TOF with DHB as matrix. The degree of polymerization for PALA-HT is approximately calculated as $(M-18)/71$, as the end groups are $-\text{NH}_2$ and $-\text{COOH}$. The degree of polymerization of PALA-SS is

approximately calculated as (M-117)/71, as the N-terminus is ended with guanidine structure ($-N=C(N(CH_3)_2)_2$).

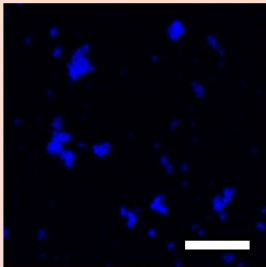
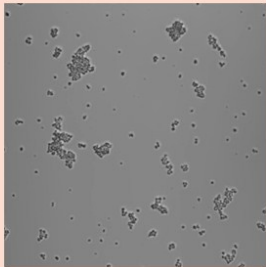
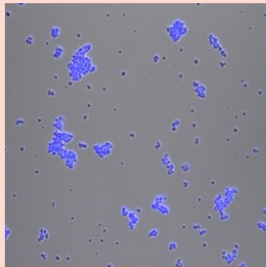
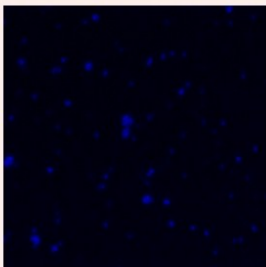
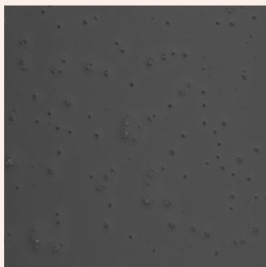
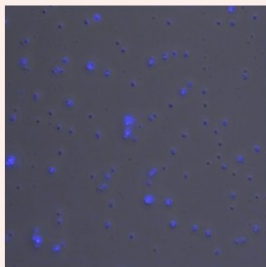
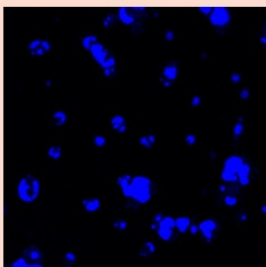
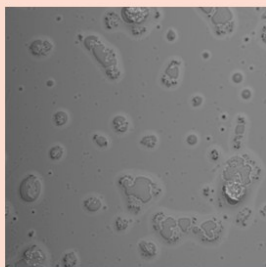
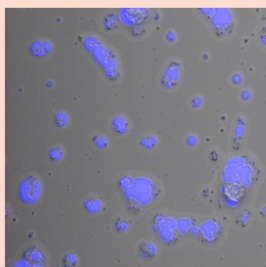
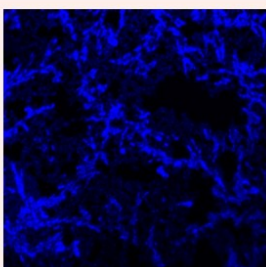
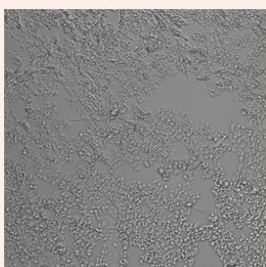
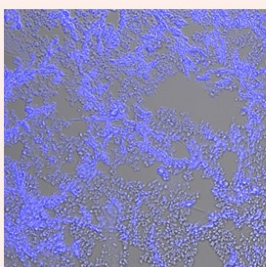
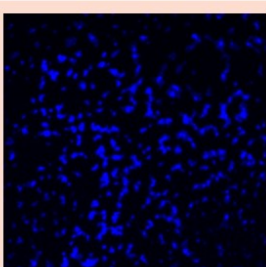
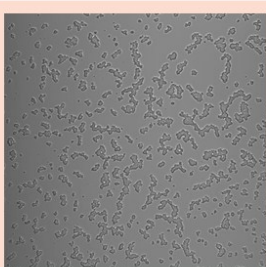
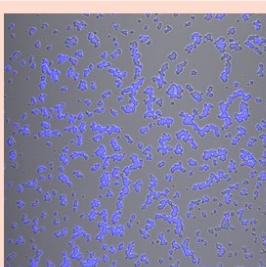
Sample	Fluorescence Image	Bright Field Image	Merged Image
OALA			
OVAL			
OILE			
PALA-HT			
PALA-SS			

Figure S6. Confocal microscopy images of oligopeptide and polypeptide. The samples are prepared by casting their TFA solutions on a glass slide and drying overnight in air. The scale bar is 20 μm . The fluorescence images were taken using a 405 nm laser (4.0% power) with a 429-485 nm filter.

Table S1. Summary of fluorescence lifetimes of synthetic oligopeptides and polypeptides.

Sample	τ (ns)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)
OALA	7.20	3.81	8.41	17.28
OVAL	8.56	4.37	9.64	40.82
OILE	8.58	4.31	9.52	34.33
PALA-HT	4.92	2.50	6.89	23.80
PALA-SS	4.34	1.73	5.70	20.05

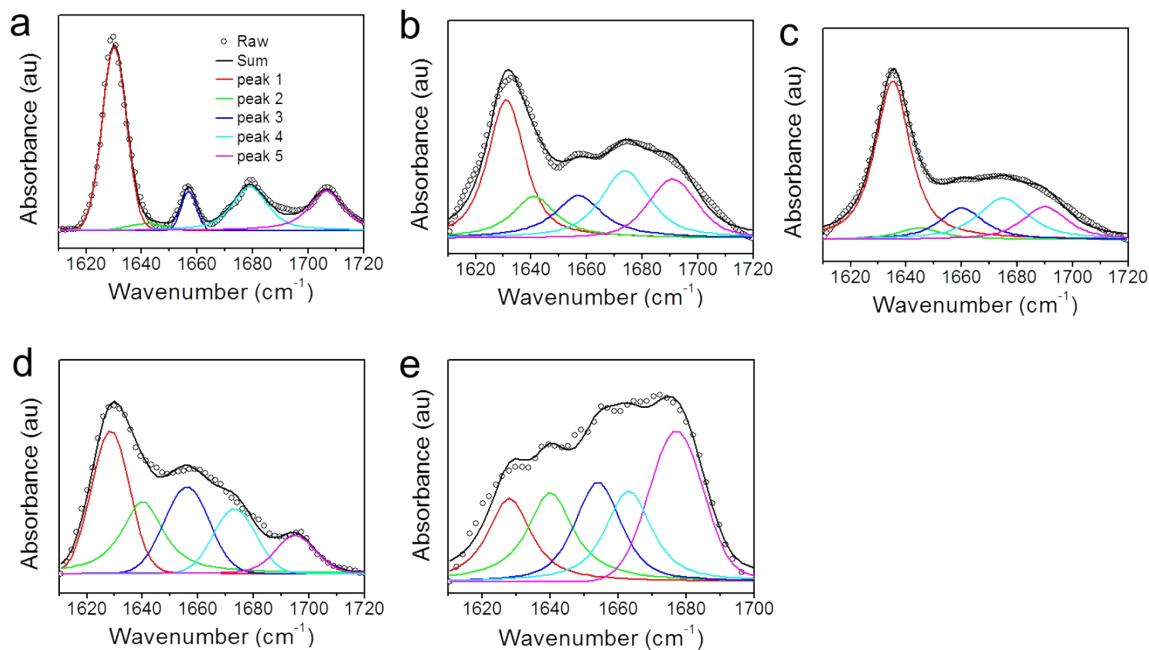


Figure S7. Deconvolution of the amide I FTIR band of (a) OALA (b) OVAL (c) OILE (d) PALA-SS (e) PALA-HT.

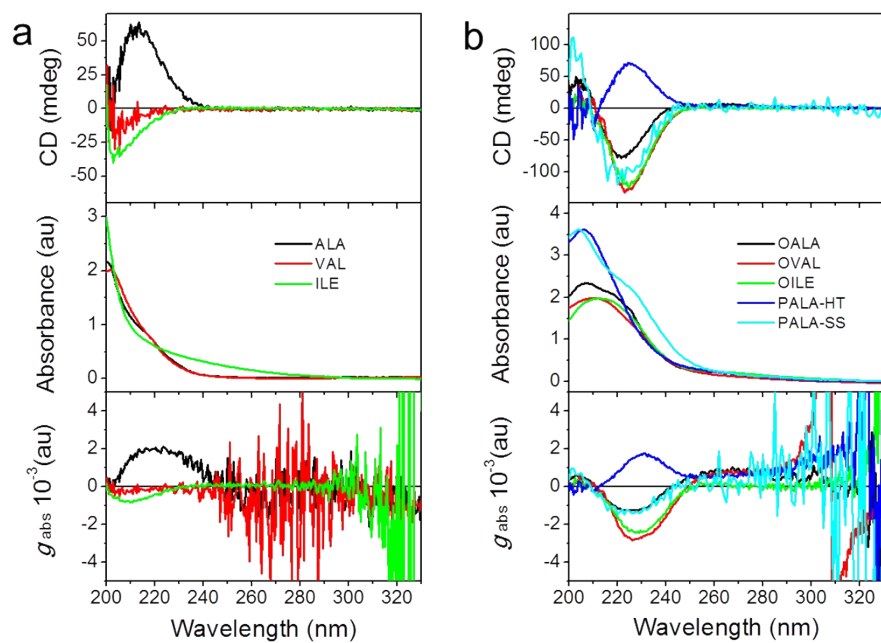


Figure S8. The CD, UV-vis absorbance, and g_{abs} spectra of (a) amino acid and (b) synthetic peptides in KBr film with a concentration of 1 wt%.

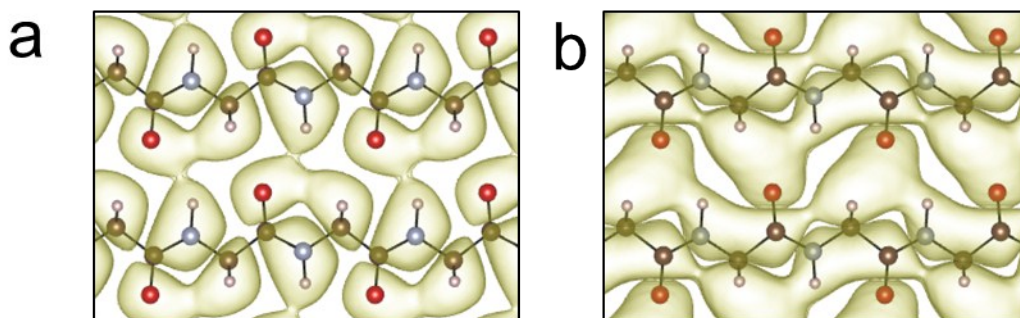


Figure S9. Charge density distribution of **a**, the valence band maximum and **b**, conduction band minimum states from the DFT calculation. Red: O; Blue: N; Grey: C; White: H.

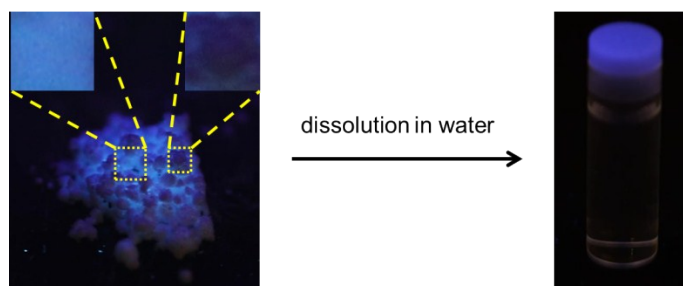


Figure S10. Luminescence of GSH in response to humidity. The luminescence of outer part of GSH is dimmer as compared to the inner part. Dissolution of GSH in water leads to complete loss of luminescence.

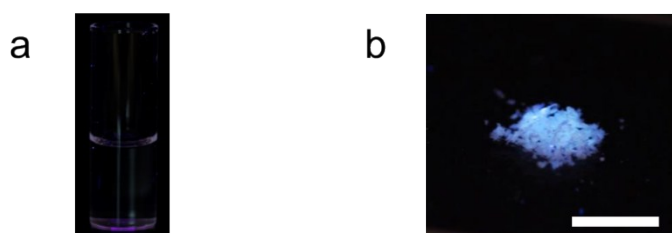


Figure S11. Fluorescence of PPRO in solution state and in condensed powder form. (a) a TFA solution of PPRO under 365 nm UV lamp. (b) condensed PPRO powder under 365 nm UV lamp. The powder is prepared by first dissolution of PPRO in minimal amount of TFA and then precipitation from its TFA solution into ether. The precipitate was further sonicated and washed with ether 3 times. Scale bar: 1 cm.

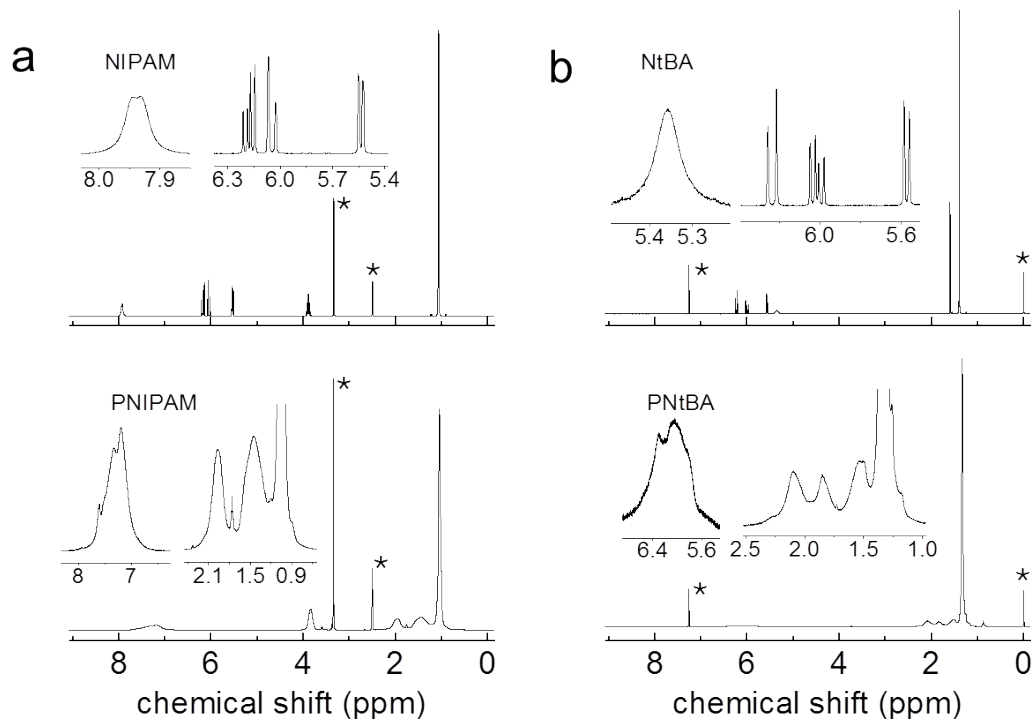


Figure S12. ^1H NMR spectra of (a) NIPAM and PNIPAM in $\text{DMSO-}d_6$ with TMS ($\delta = 2.5$) as internal standard, (b) NtBA and PNtBA in CDCl_3 with TMS ($\delta = 2.5$) as internal standard. The solvent peaks are marked with asterisks.

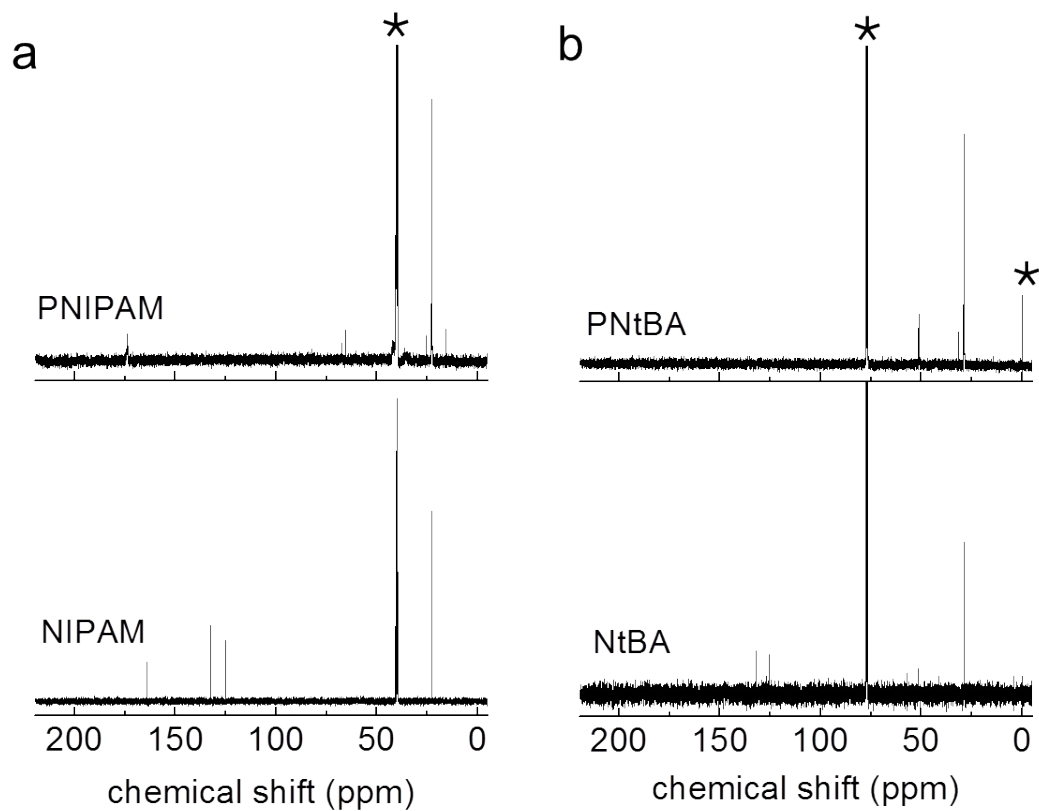


Figure S13. ^{13}C -NMR spectra of (a) NIPAM and PNIPAM in $\text{DMSO-}d_6$ with TMS as internal standard, (b) NtBA and PNtBA in CDCl_3 with TMS as internal standard. The solvent peaks are marked with asterisks.

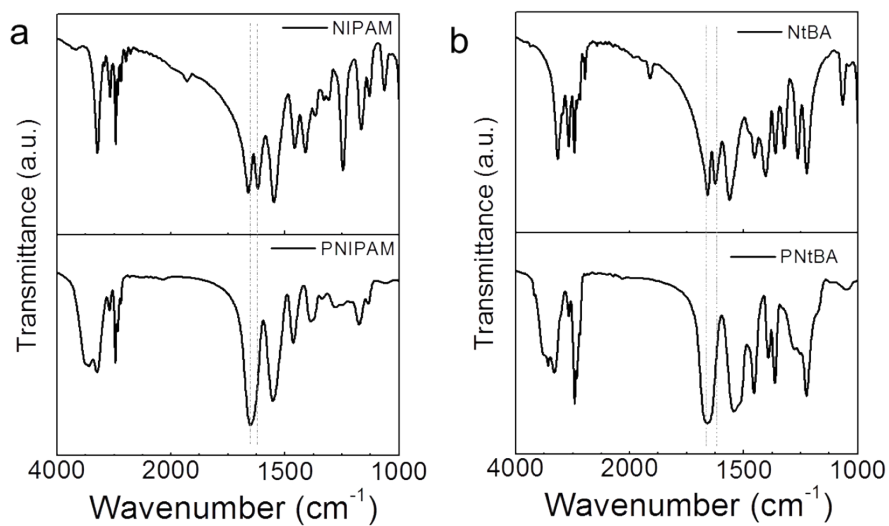


Figure S14. FTIR spectra of (a) NIPAM and PNIPAM; (b) NtBA and PNtBA.

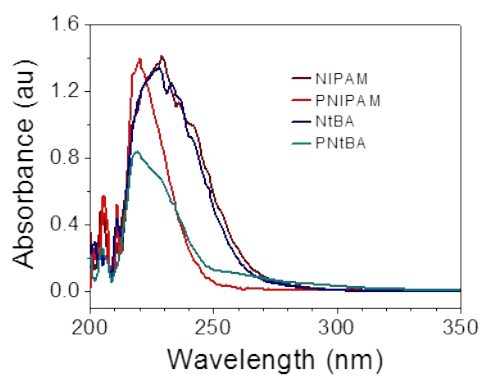


Figure S15. UV-vis spectra of monomers and polymers in THF at a concentration $\sim 10^{-4}$ M for monomer and $\sim 5 \times 10^{-3}$ M for polymer.

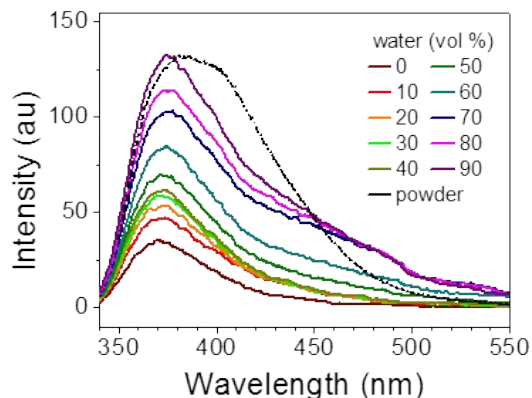


Figure S16. PL emission of PNtBA in THF:water mixture with a concentration of 10^{-4} M and in powder forms.

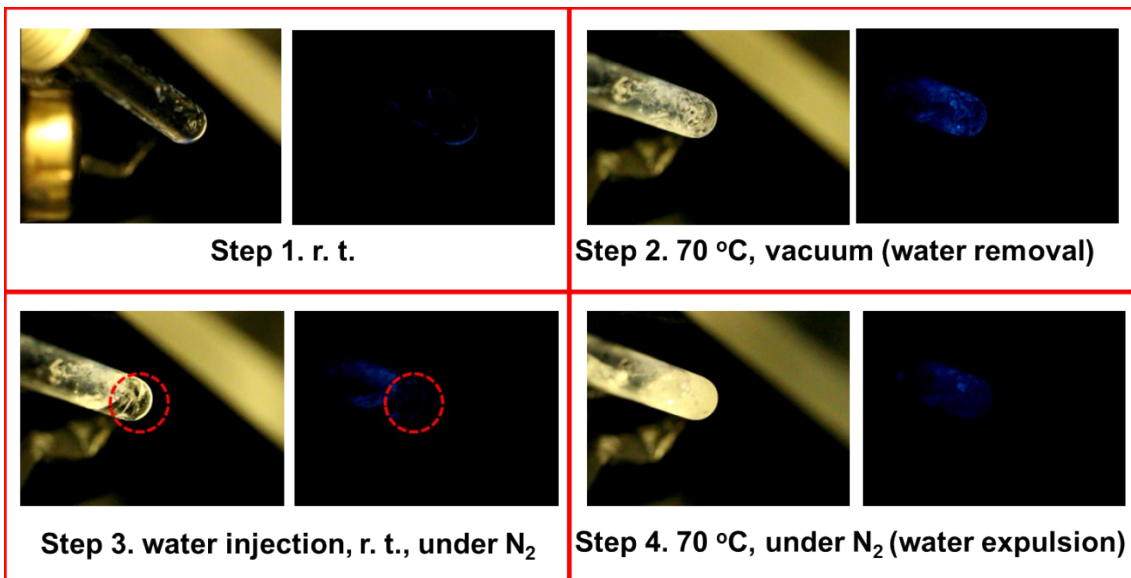


Figure S17. Reversible on/off fluorescence of PNIPAM hydrogel on dehydration/hydration. Images taken at different time frame in supplementary movie 2. In each step, the left image is taken under day light and the right one is under 365 nm UV lamp. **Step 1**, a PNIPAM hydrogel is prepared by first dissolving PNIPAM in water and then removing partially the water at r.t. under vacuum. **Step 2**, a complete removal of water is by heating the hydrogel at 70 °C under vacuum. **Step 3**, the dehydrated PNIPAM is partially dissolved by injection of water. The red circle indicates the solvated part that is non-emissive under UV light. **Step 4**, heating the tube at 70 °C leads to expulsion of water from PNIPAM matrix and the fluorescence is turned on.

Reference

1. W. C. Chan and P. D. White, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, Oxford, UK, 2000.
2. M. Tomida, T. Nakato, S. Matsunami and T. Kakuchi, *Polymer*, 1997, **38**, 4733-4736.
3. B. Asikoglu, S. Kilic and B. M. Baysal, *Journal of Polymer Science Part a-Polymer Chemistry*, 1985, **23**, 1017-1029.
4. Y. J. Zhang, S. Furyk, D. E. Bergbreiter and P. S. Cremer, *J. Am. Chem. Soc.*, 2005, **127**, 14505-14510.
5. Y. L. Zhang, J. Li, B. Z. Tang and K. S. Wong, *J. Phys. Chem. C*, 2014, **118**, 26981-26986.
6. J. C. deMello, H. F. Wittmann and R. H. Friend, *Adv. Mater.*, 1997, **9**, 230-232.
7. C. Y. K. Chan, N. W. Tseng, J. W. Y. Lam, J. Z. Liu, R. T. K. Kwok and B. Z. Tang, *Macromolecules*, 2013, **46**, 3246-3256.