Supplementary Information

Multi-stimuli Responsive Self-healing Metallo-Hydrogels: Tuning of the Gel Recovery Property

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Experimental Section

Materials

Palmitic acid, Myristic acid, Lauric acid, L-Tyrosine and L-Valine were purchased from Aldrich. HOBt (1-hydroxybenzotriazole) and DCC (*N, N′*-dicyclohexylcarbodiimide) were purchased from SRL, India.

Methods

Amphiphiles were synthesized by conventional solution phase methods using racemization free fragment condensation strategy. The C-terminus was protected as a methyl ester. Couplings were mediated by DCC/HOBt. All compounds were purified by column chromatography using silica gel (100-200 mesh size) as stationary phase and chloroform and ethyl acetate as eluent.

Instrumentation

NMR experiments: All 500 MHz NMR studies were carried out on a Bruker DPX 500 MHz spectrometer at 300 K using cryo probe in $CDCl_3$ and DMSO-d_6 maintaining the concentration 4–10 mM.

Mass spectrometry: Mass spectra were recorded on a Qtof Micro YA263 high-resolution mass spectrometer.

Transmission electron microscopy (TEM): The morphology of gels was investigated by TEM. A drop of dilute solution of the gel was placed on carbon-coated copper grid (300 mesh) and dried by slow evaporation. Each grid was then allowed to dry in a vacuum for two days. TEM images were recorded on a JEM 2010 electron microscope at an accelerating voltage of 200 KV.

FT-IR Spectroscopy: FT-IR spectroscopy was performed using Nicolate 380 FT-IR spectrophotometer (Thermo Scientific). All reported FT-IR spectra were taken using the spectroscopic cell with CaF_2 window. During the recording of IR spectra using the CaF_2 cell, 100 scans were performed.

XRD study: XRD study of the xerogel was carried out by placing the sample on a glass plate. Experiments were carried out by using an X-ray diffractometer (Bruker AXS, Model No. D8 Advance). The instrument was operated at a 40 kV voltages and 40 mA current using Ni-filtered CuK_α radiation and the instrument was calibrated with a standard Al_2O_3 (corundum) sample before use. For scan 1°–5°, the scintillation counts detector was used with scan speed 2s and step size 0.02°. In another scan 5°–60°, the LynxEye super speed detector was used with scan speed 0.3s and step size 0.02°.

Rheology: The rheology experiment was performed by using an Anton Paar Modular Compact Rheometer MCR 302 at 25 °C.

Determination of T_{gel}: Gel melting temperature determination was performed by heating gels in a thermostat controlled water bath at a heating rate of 2 °C/ 5 minute until the gel was melted. The calculated error range in T_{gel} determination was found to be ±1 °C.

Amphiphile synthesis

(1) $CH_3(CH_2)_{10}CONHCH(CH_2PhOH)COOMe$

Lauric acid (2.00 g, 10 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Tyr-OMe was isolated from the corresponding methyl ester hydrochloride (4.6 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentrate to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (2.06 g, 10 mmol) and HOBt (1.53 g, 10 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (1×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 3.2 g (8.4 mmol, 84%).

^{1}H NMR (500 MHz, $CDCl_3$, TMS, 25 °C) 6.93–6.91 (aromatic, meta to OH, 2Hs, J = 8.0), 6.74–6.72 (aromatic, ortho to OH, 2Hs, J = 8.0), 6.06–6.04 (NH, 1H, d, J = 8.0), 4.89–4.85 ($C^{\alpha}H$, 1H, q), 3.72 (OCH_3, 3H, s), 3.08–2.95 ($C^{\beta}H$, 2H, m), 2.19–2.16 ($^{\alpha}CH_2$, 2H, t, J = 7.5), 1.58–1.55 ($^{\beta}CH_2$, 2H, m), 1.30-1.23 ($8CH_2$, 16H, m), 0.88–0.85 (CH_3, 3H, t, J = 7); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 172.61, 172.54, 155.81, 130.32, 126.97, 115.72, 53.33, 52.50, 37.37, 36.67, 33.52, 32.01, 29.72, 29.58, 29.44, 29.40, 29.29, 25.69, 24.83, 22.79, 14.21. HRMS: (m/z) 378.2009 $(M+H)^+$, 400.1781 $(M+Na)^+$, 416.1573 $(M+K)^+$.

(2) $CH_3(CH_2)_{10}CONHCH(CH_2PhOH)COOH$ (P_1)

$CH_3(CH_2)_{10}CONHCH(CH_2PhOH)COOMe$ (3.2 g, 8.4 mmol) was dissolved in MeOH (20 mL) and then 2M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulfate, and evaporated in vacuum to yield as a white solid sample.

Yield: 2.8 g (7.7 mmol, 91%).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 12.51 (COOH, 1H, s), 9.17 (Tyr-OH, 1H, s), 8.01–8.00 (NH, 1H, d, *J* = 8.0) 7.00–6.98 (aromatic, meta to OH, 2Hs, *J* = 8.5), 6.64–6.63 (aromatic, ortho to OH, 2Hs, *J* = 8.0), 4.33–4.30 ($C^{\alpha}H$, 1H, m), 2.93–2.69 ($C^{\beta}H$, 2H, m), 2.05–2.02 ($^{\alpha}CH_2$, 2H, t, *J* = 7.5), 1.40–1.36 ($^{\beta}CH_2$, 2H, m), 1.27-1.13 ($8CH_2$, 16H, m), 0.87–0.84 (CH_3, 3H, t, *J* = 6.75). ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 173.90, 172.71, 156.44, 130.50, 128.32, 115.47, 54.20, 36.63, 35.66, 31.88, 29.62, 29.59, 29.50, 29.38, 29.30, 29.09, 25.77, 22.67, 14.52. HRMS: (m/z) 364.4085 $(M+H)^+$, 386.3947 $(M+ Na)^+$.

(3) $CH_3(CH_2)_{12}CONHCH(CH_2PhOH)COOMe$

Myristic acid (2.28 g, 10 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Tyr-OMe was isolated from the corresponding methyl ester hydrochloride (4.6 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentrate to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (2.06 g, 10 mmol) and HOBt (1.53 g, 10 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (1×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 3.6 g (8.8 mmol, 88%).

^{1}H NMR (500 MHz, $CDCl_3$, TMS, 25 °C) δ 7.51 (Tyr-OH, 1H, s), 6.93–6.90 (aromatic, meta to OH, 2Hs, *J* = 8.3), 6.75–6.72 (aromatic, ortho to OH, 2Hs, *J* = 8.4), 6.08–6.06 (NH, 1H, d, *J* = 8.0), 4.87–4.85 ($C^{\alpha}H$, 1H, q), 3.72 (OCH_3, 3H, s), 3.05–2.98 ($C^{\beta}H$, 2H, m), 2.20–2.15 ($^{\alpha}CH_2$, 2H, t, *J* = 7.6), 1.59–1.55 ($^{\beta}CH_2$, 2H, m), 1.24 ($10CH_2$, 20H, s), 0.89–0.85 (CH_3, 3H, t, *J* = 6.57); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 173.64, 172.53, 155.90, 134.49, 130.29, 126.81, 115.72, 53.32, 52.51, 52.48, 37.33, 36.66, 33.95, 32.02, 29.79, 29.75, 29.57, 29.46, 29.39, 29.28, 25.68, 24.97, 22.78, 14.22. HRMS: (m/z) 406.2565 $(M+H)^+$, 428.2345 $(M+Na)^+$, 444.2050 $(M+K)^+$.

(4) $CH_3(CH_2)_{12}CONHCH(CH_2PhOH)COOH$ (P_2)

$CH_3(CH_2)_{12}CONHCH(CH_2PhOH)COOMe$ (3.6 g, 8.8 mmol) was dissolved in MeOH (20 mL) and then 2M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with

diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulfate, and evaporated in vacuum to yield as a white solid sample.

Yield: 3.2 g (8.1 mmol, 92%).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 12.53 (COOH, 1H, s), 9.15 (Tyr-OH, 1H, s), 8.00–7.98 (NH, 1H, d, J = 8.0) 6.99–6.97 (aromatic, meta to OH, 2Hs, J = 8.3), 6.63–6.61 (aromatic, ortho to OH, 2Hs, J = 8.3), 4.34–4.28 ($C^{\alpha}H$, 1H, m), 2.92–2.67 ($C^{\beta}H$, 2H, m), 2.04–2.00 ($^{\alpha}CH_2$, 2H, t, J = 7.26), 1.40–1.36 ($^{\beta}CH_2$, 2H, m), 1.28-1.13 ($10CH_2$, 20H, m), 0.86–0.83 (CH_3, 3H, t, J = 6.4). ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 173.35, 172.15, 155.88, 129.94, 127.72, 114.89, 53.64, 36.05, 35.08, 31.34, 29.09, 28.97, 28.85, 28.76, 28.54, 25.22, 22.13, 13.95. HRMS: (m/z) 414.3504 $(M+Na)^+$, 430.3329 $(M+K)^+$.

(5) $CH_3(CH_2)_{14}CONHCH(CH_2PhOH)COOMe$

Palmitic acid (2.56 g, 10 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Tyr-OMe was isolated from the corresponding methyl ester hydrochloride (4.6 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentrate to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (2.06 g, 10 mmol) and HOBt (1.53 g, 10 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (1×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 3.1 g (7.1 mmol, 71%).

^{1}H NMR (500 MHz, $CDCl_3$, TMS, 25 °C) δ 6.92–6.91 (aromatic, meta to OH, 2Hs, J = 8.0), 6.74–6.72 (aromatic, ortho to OH, 2Hs, J = 8.0), 6.09–6.08 (NH, 1H, d, J = 8.0), 4.89–4.85 ($C^{\alpha}H$, 1H, q), 3.72 (OCH_3, 3H, s), 3.08–2.95 ($C^{\beta}H$, 2H, m), 2.19–2.16 ($^{\alpha}CH_2$, 2H, t, J = 7.5), 1.58–1.55 ($^{\beta}CH_2$, 2H, m), 1.30-1.23 ($12CH_2$, 24H, m), 0.88–0.86 (CH_3, 3H, t, J = 6.75); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 173.70, 172.53, 155.91, 130.29, 126.82, 115.72, 53.35, 52.47, 37.32, 36.63, 32.01, 29.79, 29.76, 29.57, 29.45, 29.38, 29.27, 25.68, 22.77, 14.19. HRMS: (m/z) 434.1394 $(M+H)^+$, 456.1119 $(M+Na)^+$, 472.0970 $(M+K)^+$.

(6) $CH_3(CH_2)_{14}CONHCH(CH_2PhOH)COOH$ (P_3)

$CH_3(CH_2)_{12}CONHCH(CH_2PhOH)COOMe$ (3.1 g, 7.1 mmol) was dissolved in MeOH (20 mL) and then 2M NaOH (10 mL) was added. The reaction mixture was stirred and the

progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulfate, and evaporated in vacuum to yield as a white solid sample.

Yield: 2.5 g (5.9 mmol, 83%).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 12.53 (COOH, 1H, s), 9.15 (Tyr-OH, 1H, s), 7.99–7.98 (NH, 1H, d, J = 8.0), 6.98–6.97 (aromatic, meta to OH, 2Hs, J = 8.0), 6.62–6.61 (aromatic, ortho to OH, 2Hs, J = 8.5), 4.33–4.28 ($C^{\alpha}H$, 1H, m), 2.91–2.67 ($C^{\beta}H$, 2H, m), 2.03–2.00 ($^{\alpha}CH_2$, 2H, t, J = 7.25), 1.40–1.34 ($^{\beta}CH_2$, 2H, m), 1.25-1.12 ($12CH_2$, 24H, m), 0.85–0.82 (CH_3, 3H, t, J = 6.75). ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ 173.91, 172.70, 156.43, 130.50, 128.31, 115.45, 54.19, 36.61, 35.65, 31.31.86, 29.63, 29.50, 29.38, 29.28, 29.08, 25.76, 22.66, 14.51. HRMS: (m/z) 442.3143 $(M+Na)^+$.

(7) $CH_3(CH_2)_{12}CONHCH(CHMe_2)COOMe$

Myristic acid (2.28 g, 10 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Val-OMe was isolated from the corresponding methyl ester hydrochloride (3.35 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentrate to 10 mL. Then it was added to the reaction mixture, followed immediately by DCC (2.06 g, 10 mmol) and HOBt (1.53 g, 10 mmol). The reaction mixture was stirred for three days. The reaction mixture was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 1M HCl (3×50 mL), brine (2×50 mL), 1M sodium carbonate (1×50 mL), and brine (2×50 mL) and then dried over anhydrous sodium sulfate and evaporated in vacuum to yield peptide as a white solid. Purification was done by silica gel column (100–200 mesh) using chloroform and ethyl acetate as eluent.

Yield: 2.9 g (8.5 mmol, 85%).

^{1}H NMR (300 MHz, $CDCl_3$, TMS, 25 °C) δ 6.05–6.02 (NH, 1H, d, J = 8.6), 4.57–4.53 ($C^{\alpha}H$, 1H, m), 3.70 (OCH_3, 3H, s), 2.22–2.17 ($^{\alpha}CH_2$, 2H, t, J = 7.5), 2.12-2.10 ($C^{\beta}H$, 1H, m), 1.62–1.58 ($^{\beta}CH_2$, 2H, m), 1.25-1.21 ($10CH_2$, 20H, m), 0.91–0.82 ($3CH_3$, 9H, m); ^{13}C NMR (75 MHz, $CDCl_3$, TMS, 25 °C) δ 173.17, 172.85, 56.88, 52.14, 52.10, 36.74, 31.98, 31.35, 29.70, 29.67, 29.55, 29.41, 29.32, 25.78, 22.74, 18.99, 17.90, 14.16. HRMS: (m/z) 342.2222 $(M+H)^+$, 364.1911 $(M+Na)^+$.

(8) $CH_3(CH_2)_{12}CONHCH(CHMe_2)COOH$ (P_4)

$CH_3(CH_2)_{12}CONHCH(CH(CH_3)_2)COOMe$ (2.9 g, 8.5 mmol) was dissolved in MeOH (20 mL) and then 2M NaOH (10 mL) was added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were dried over anhydrous sodium sulfate, and evaporated in vacuum to yield as a white solid sample.

Yield: 2.5 g (7.6 mmol, 89.4%).

^{1}H NMR (300 MHz, DMSO-d_6, 25 °C) δ 12.46 (COOH, 1H, s), 7.88–7.85 (NH, 1H, d, J = 8.5), 4.16–4.11 ($C^{\alpha}H$, 1H, m), 2.49–2.06 ($^{\alpha}CH_2$, 2H, m), 1.48–1.46 ($^{\beta}CH_2$, 2H, m), 1.22 ($10CH_2$, 20H, m), 0.87–0.82 ($3CH_3$, 9H, m); ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ 173.18, 172.45, 56.96, 34.93, 31.29, 29.72, 29.00, 28.76, 28.70, 28.58, 25.35, 22.08, 19.12, 18.00, 13.90. HRMS: (m/z) 328.0565 $(M+H)^+$, 350.0257 $(M+Na)^+$.

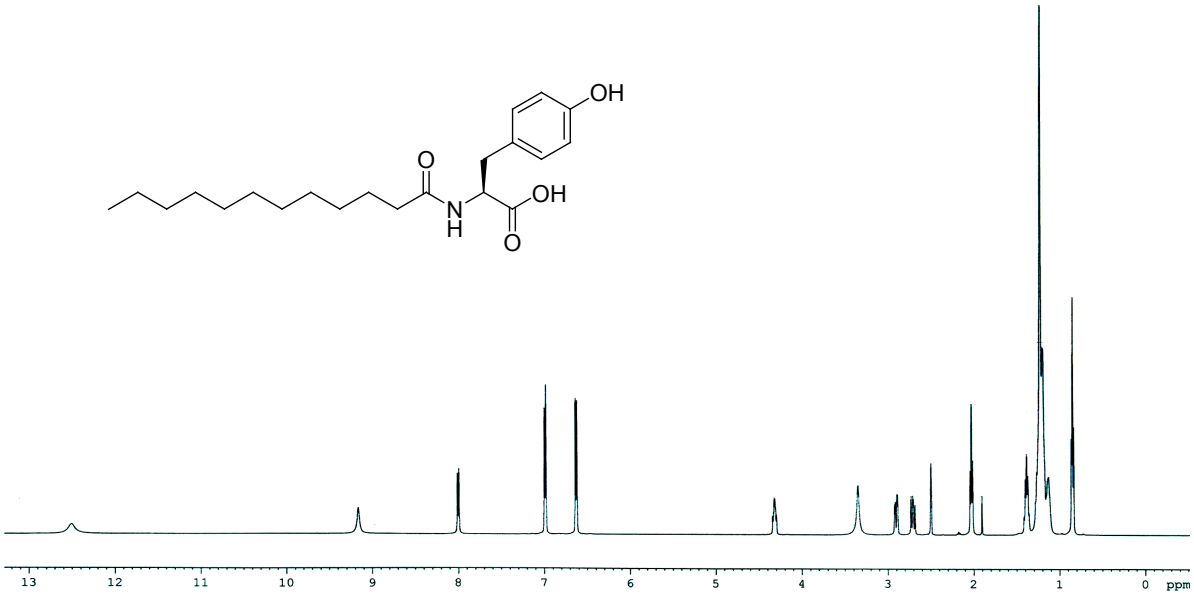

Figure S1: ^{1}H NMR (500 MHz) Spectra of **P_1** in DMSO-d_6.

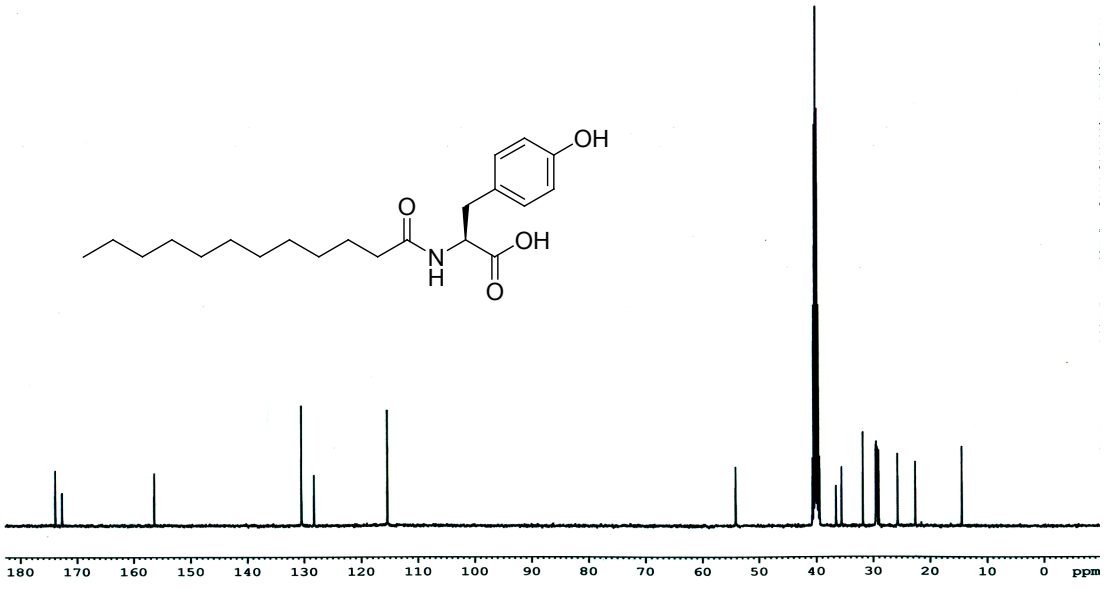

Figure S2: ^{13}C NMR (500 MHz) Spectra of **P_1** in DMSO-d_6.

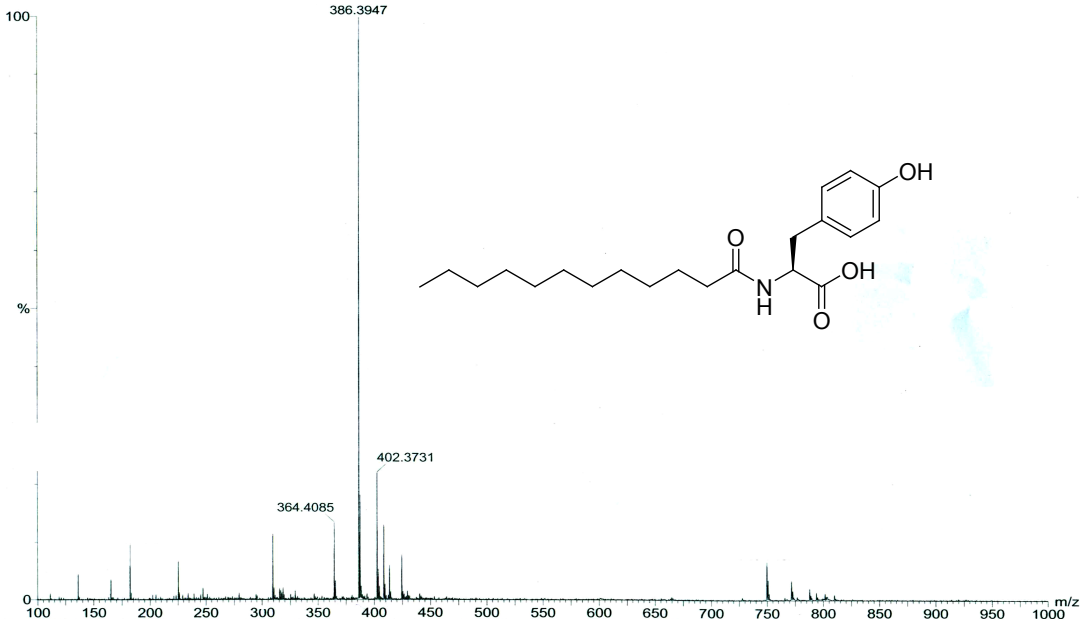

Figure S3: HRMS Spectra of $\mathbf{P_1}$.

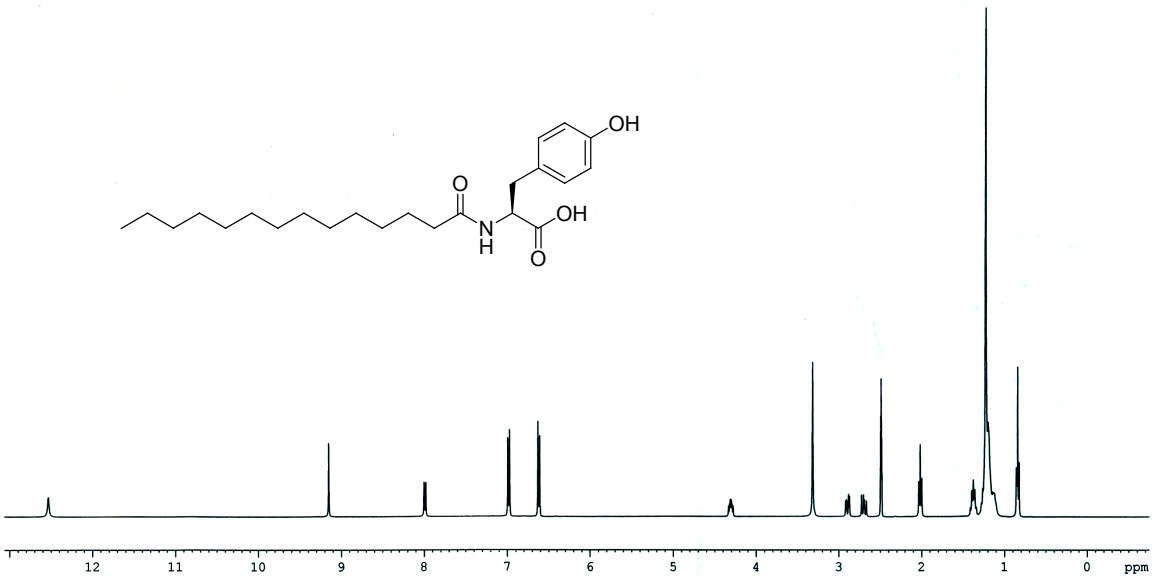

Figure S4: ^{1}H NMR (500 MHz) Spectra of $\mathbf{P_2}$ in DMSO-d_6.

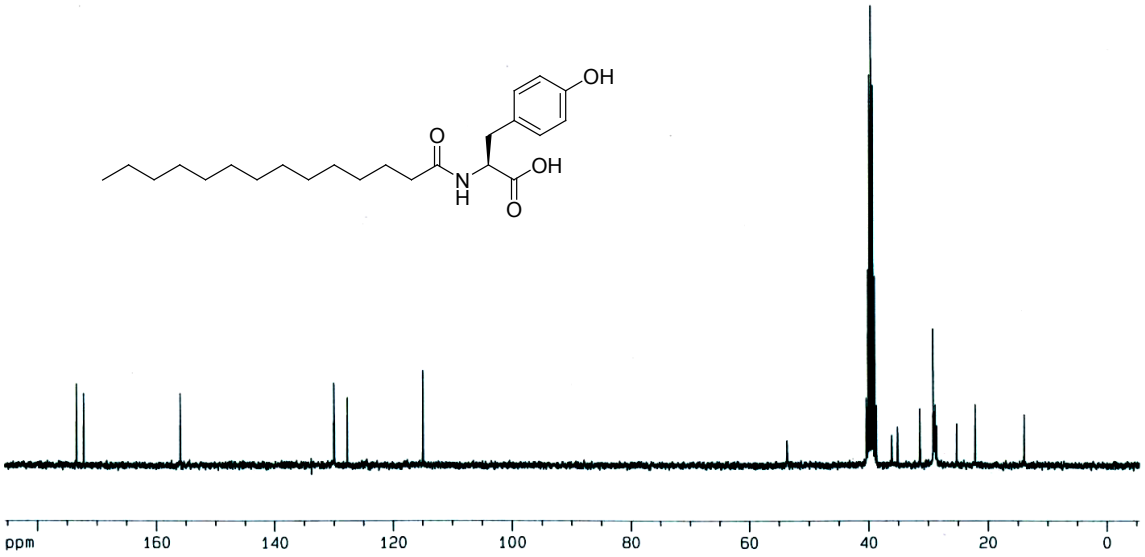

Figure S5: ^{13}C NMR (500 MHz) Spectra of $\mathbf{P_2}$ in DMSO-d_6.

Figure S6: HRMS Spectra of $\mathbf{P_2}$.

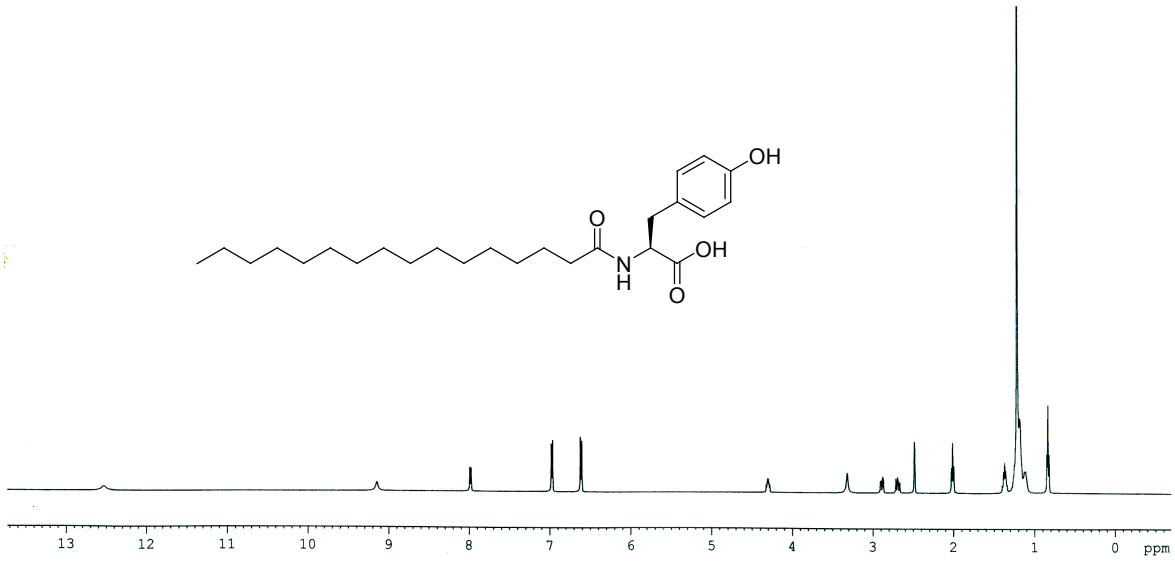

Figure S7: ^{1}H NMR (500 MHz) Spectra of **P_3** in DMSO-d_6.

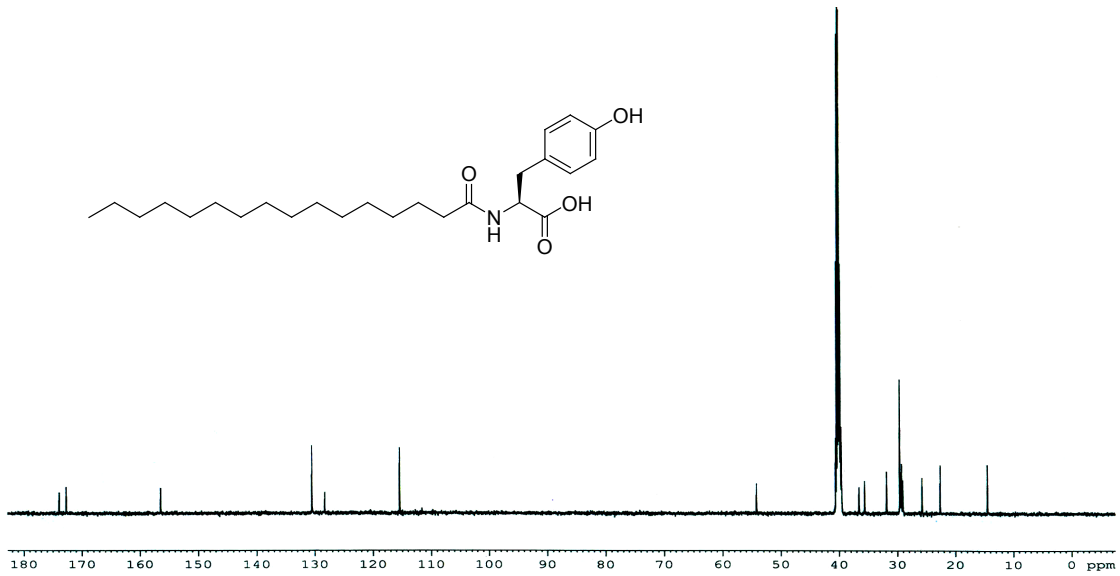

Figure S8: ^{13}C NMR (500 MHz) Spectra of **P_3** in DMSO-d_6.

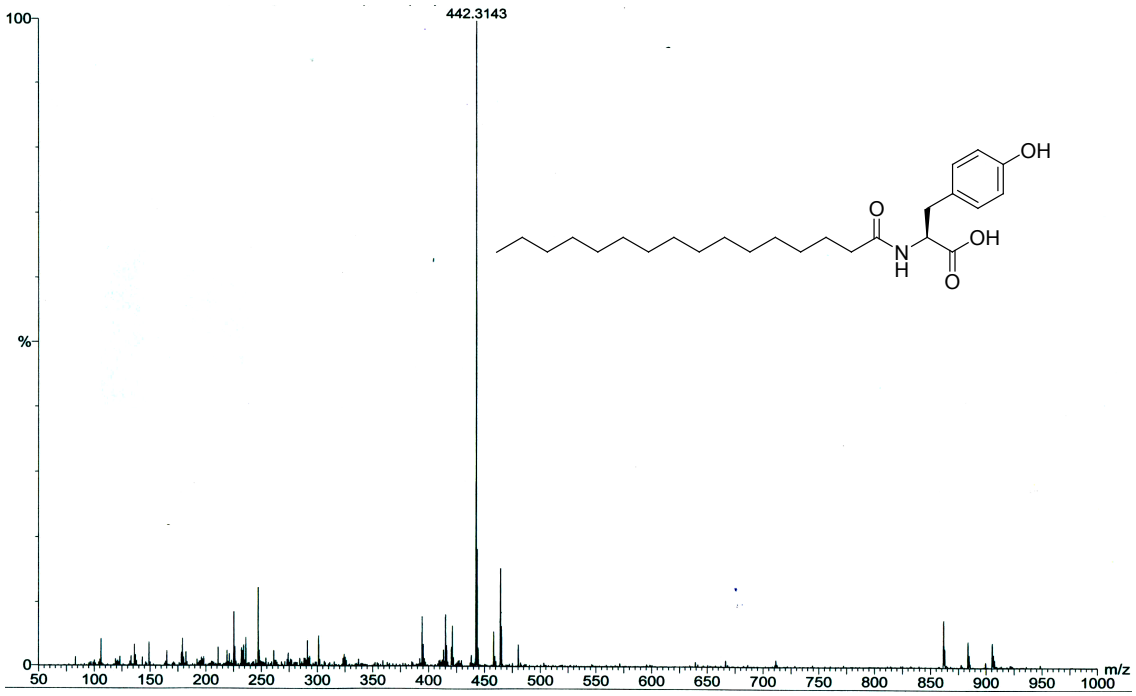

Figure S9: HRMS Spectra of $\mathbf{P_3}$.

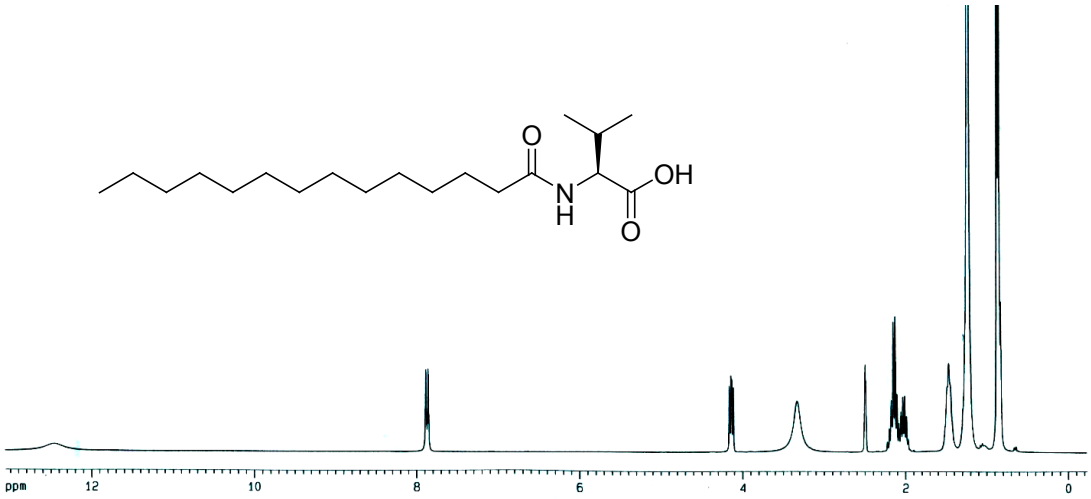

Figure S10: ^{1}H NMR (500 MHz) Spectra of $\mathbf{P_4}$ in DMSO-d_6.

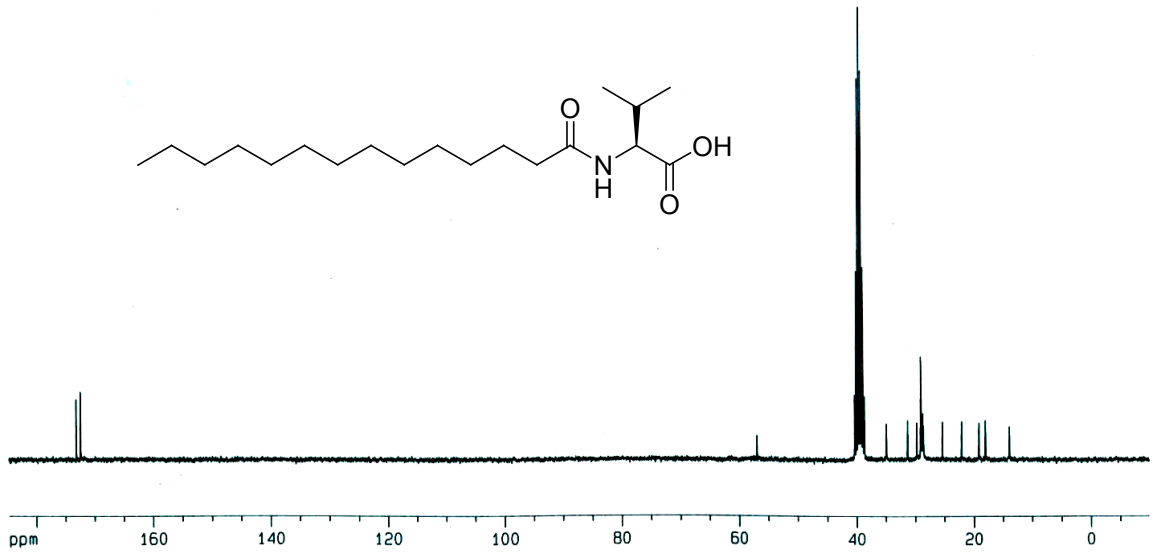

Figure S11: ^{13}C NMR (500 MHz) Spectra of **P$_4$** in DMSO-d$_6$.

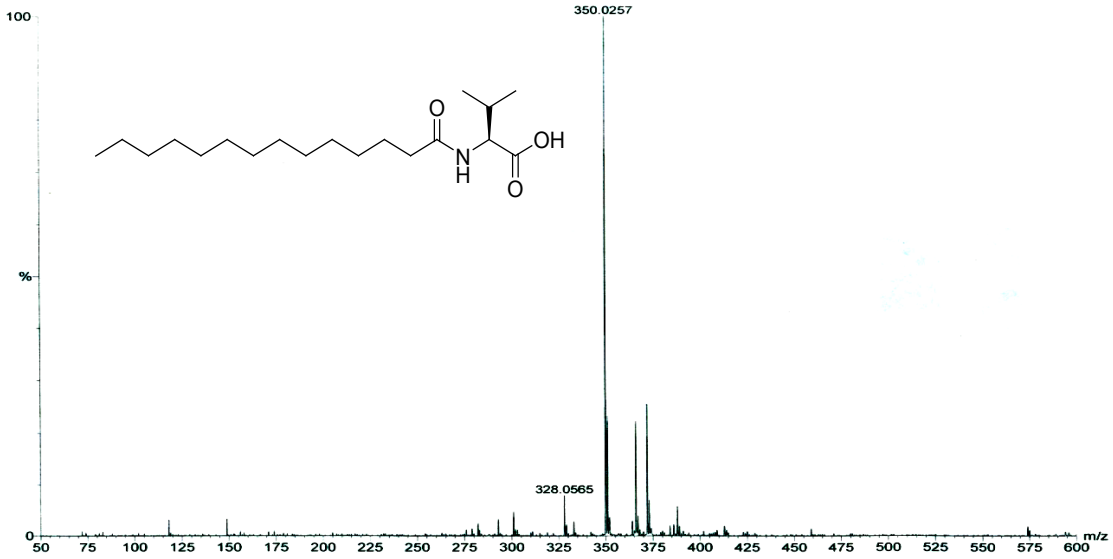

Figure S12: HRMS Spectra of **P$_4$**.

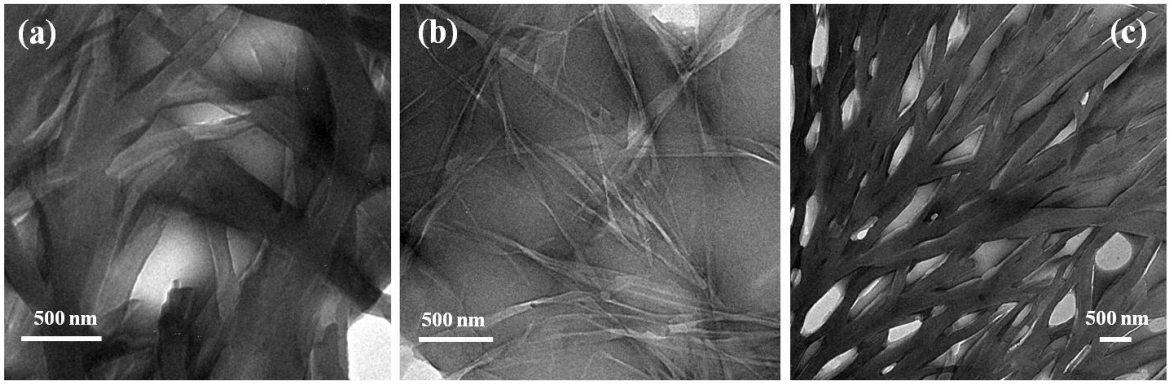

Figure S13: TEM images of the metallo-hydrogels obtained from (a) $\mathbf{P_1}$, (b) $\mathbf{P_2}$ and (c) $\mathbf{P_3}$ in phosphate buffer solution of pH 7.46 show thick fibriller nature.

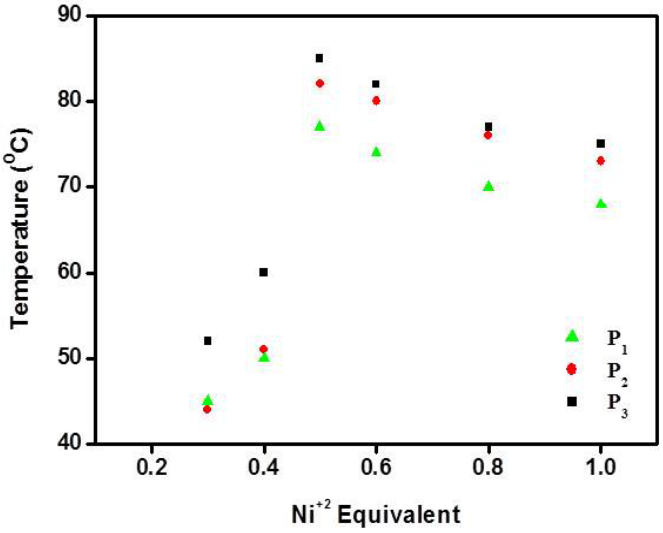

Figure S14: Plot of T_{gel} with different Ni^{2+} ion concentrations at constant amphiphile concentration (10 mM) at pH 7.46.

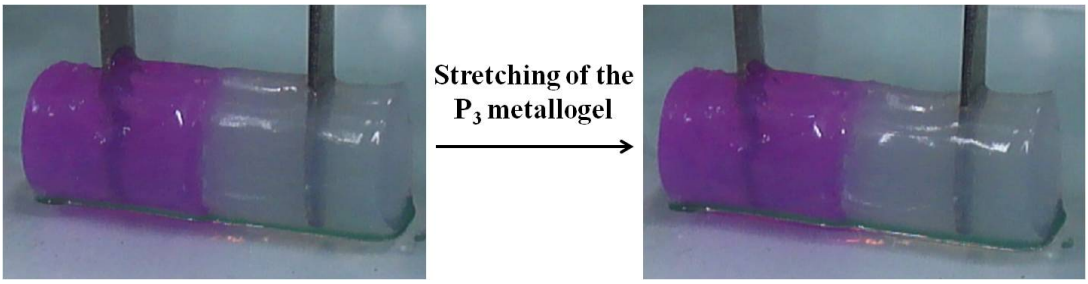

Figure S15: Dragging of two metallo-gel blocks from two different ends after self-healing, indicates the elasticity of the metallo-gel (coloration has been done by using rhodamine-B).

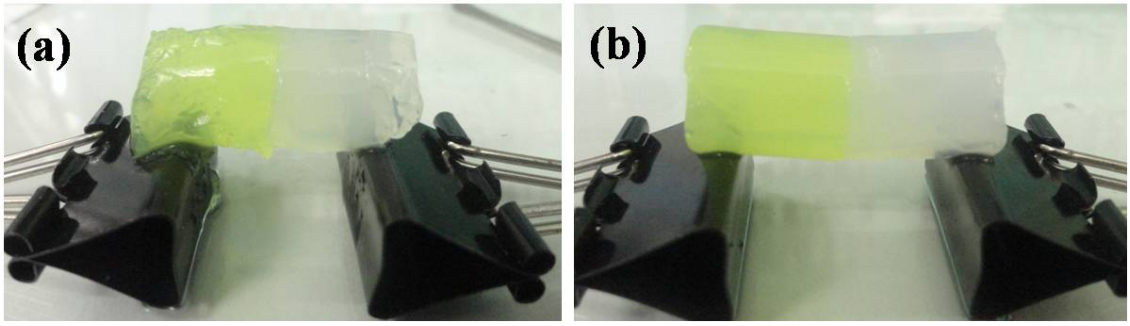

Figure S16: Illustration of self-healing behavior shown by the metallo-hydrogelator $\mathbf{P_1}$ (a) and $\mathbf{P_2}$ (b). Coloration has been done by using thioflavin T.

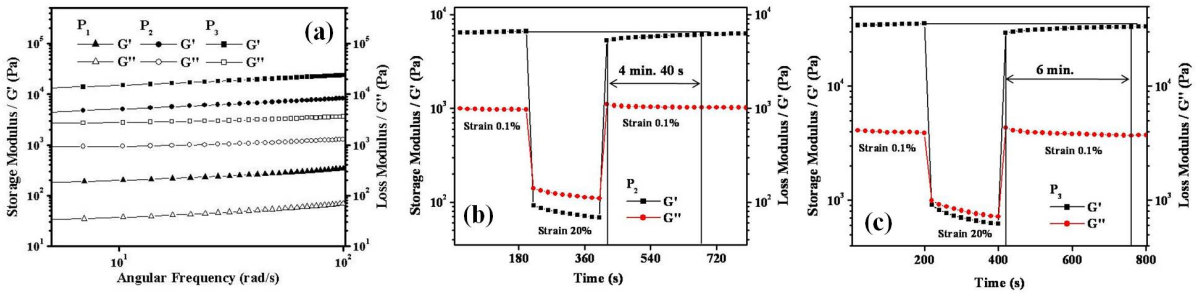

Figure S17: (a) The frequency sweep experimental data showing no crossover point throughout the experimental region for all three gels. (b) and (c) are the step strain experiment data obtained from $\mathbf{P_2}$ and $\mathbf{P_3}$ metallo-hydrogels respectively. Concentration was maintained at 10 mM in 1: 0.5 gelator and Ni^{2+} ratio respectively.

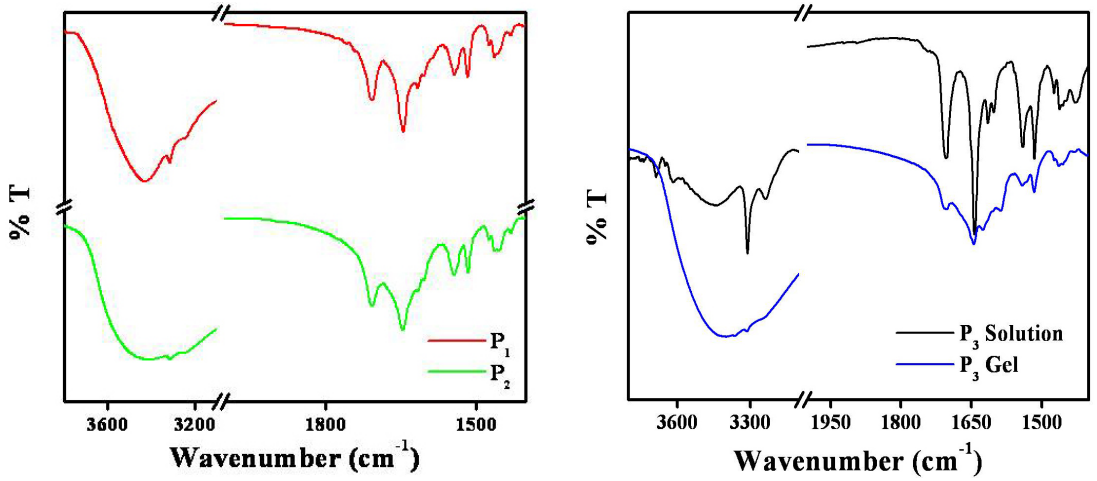

Figure S18: The FT-IR spectrum of the hydrogels obtained from **P_1**, **P_2** and **P_3** respectively.

Table S1 FT-IR frequency obtained from aggregate of **P_3** and gel state of **P_1**, **P_2** and **P_3** respectively.

Compounds	**FT-IR frequencies (cm^{-1})**
P_1 (Gel)	3425, 3314, 1706, 1643, 1543
P_2 (Gel)	3415, 3314, 1707, 1645, 1543
P_3 (Gel)	3400, 3312, 1705, 1644, 1540
Aggregated P_3 (Nongel)	3440, 3312, 1703, 1644, 1540

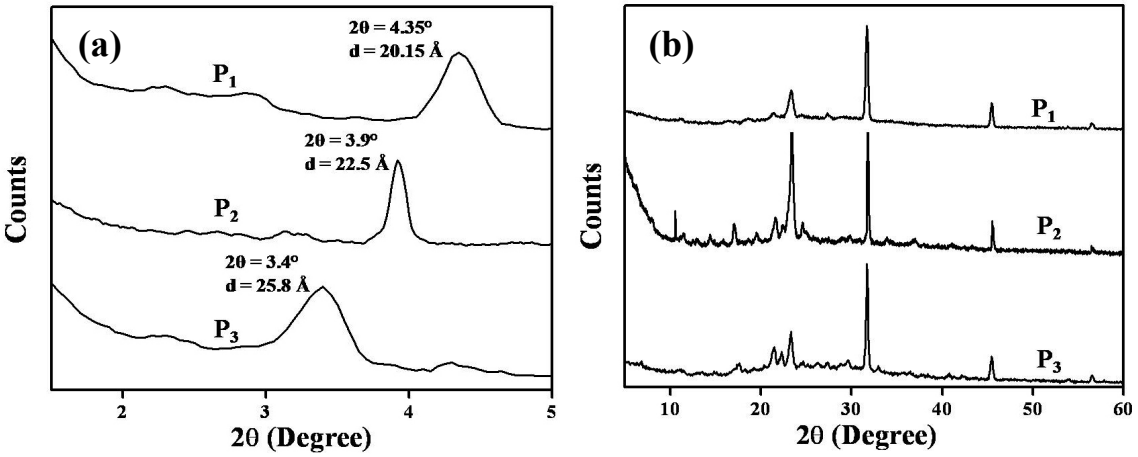

Figure S19: (a) Small angle XRD and (b) wide angle XRD pattern in xerogel state of the metallo-hydrogels obtained from $\mathbf{P_1}$, $\mathbf{P_2}$ and $\mathbf{P_3}$ in phosphate buffer solution of pH 7.46.

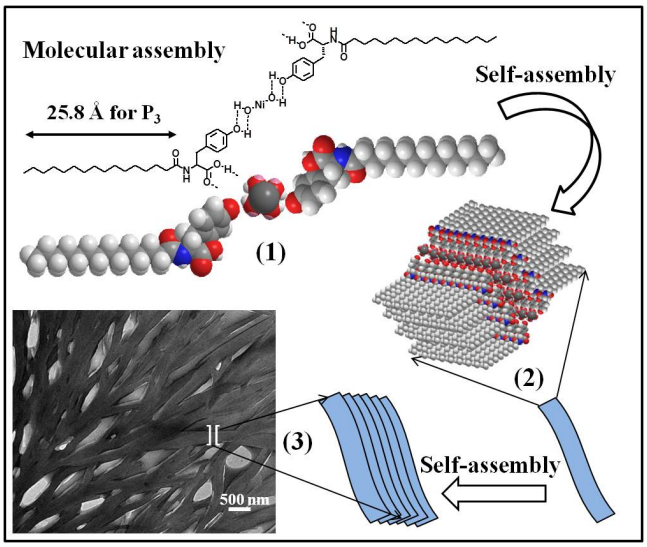

Figure S20. A probable self-assembly of gelator molecules with nickel hydroxide that upon further assembly forms tape-like structure as illustrated in the figure.

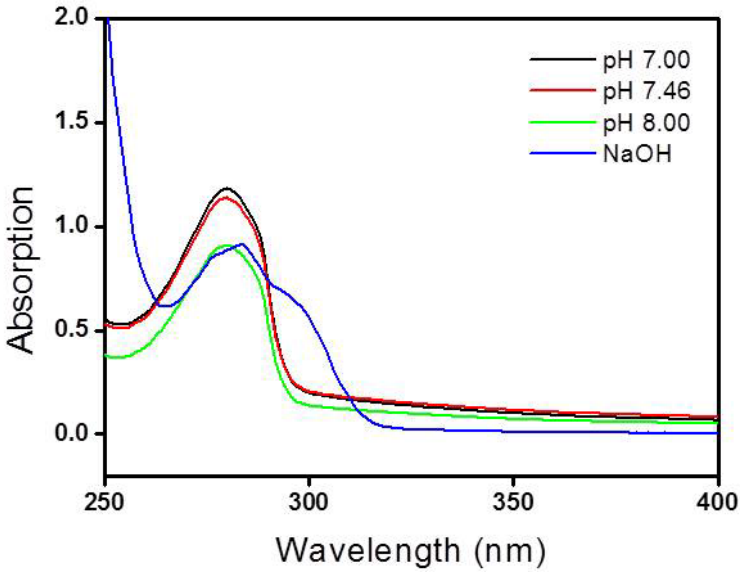

Figure S21. The UV-Vis spectra obtained from amphiphile $\mathbf{P_3}$ at different pH ranging from 7.00-8.00. The peak at 280 nm in the pH range 7.00-8.00 indicates that the phenolic OH group of tyrosine is not deprotonated. However, in presence of NaOH, a significant change is occured . This indicates that the phenolic -OH group of tyrosine deprotonates easily at the basic pH (pH greater than 8.0). Concentration was maintained at 0.5 mM of gelator $\mathbf{P_3}$ throughout the entire experiment.