Supplementary Information

External stimuli responsive syneresis of amino acid-based bioactive hydrogels: a sustainable platform for environmental remediation

Abhinandan Bera[a], Ritu Raj Patel[b], S. Daisy Precilla[c], Meenakshi Singh[b], B. Agiesh Kumar[c], Sudipta Bhowmik[c,d], Mayank Varshney[e] and Subhasish Roy*[a]

[a.] *A. Bera, Dr. S. Roy, Department of Chemistry, Birla Institute of Technology and Science-Pilani, K K Birla Goa Campus, NH 17B, Zuarinagar, Sancoale, Goa 403726, India, E-mail: subhasishr@goa.bits-pilani.ac.in*

[b.] *R. R. Patel, Dr. M. Singh, Department of Medicinal Chemistry, Institute of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, U.P, India.*

[c.] *S. D. Precilla, Dr. B. A Kumar and Dr. S. Bhowmik, Mahatma Gandhi Medical Advanced Research Institute (MGMARI), Central Inter-Disciplinary Research Facility (CIDRF), Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, 607402, India.*

[d.] *Dr. S. Bhowmik, Department of Biophysics, Molecular Biology and Bioinformatics, University of Calcutta, 92, A.P.C. Road, Kolkata, 700009, India.*

[e.] *M. Varshney, Senior Application Specialist, Characterization Division, Anton Paar India Pvt. Ltd., Phase V, Udyog Vihar Industrial Area, Gurgaon-122016, Haryana, India.*

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Instrumentations, sample preparation, and method of analysis:

Preparation of metal ions solutions. For the syneresis study, 1 mg mL^{-1} stock solutions were prepared by adding 20 mg of metal salts [$Hg(OAc)_2$ and $NiCl_2.6H_2O$] in 20 mL of Milli Q water. Other concentrations including 0.1 mg mL^{-1}, 0.01 mg mL^{-1}, 0.01 mg mL^{-1} and 0.001 mg mL^{-1} were prepared diluting these stock solutions.

FT-IR study. FT-IR spectra were recorded in IR Affinity-1 (Shimadzu, Japan) instrument for **Fmoc-^{1}Nap-A**, **Fmoc-^{2}Nap-A** solid compound, and all the xerogels of gels produced in the experiments.

Field emission scanning microscopic (FE-SEM) study. 50 µL of **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogels were taken from hydrogels obtained from **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogels at 0.08% (w/v) in phosphate buffer of pH 7.4. 500 µL of Milli Q water in different vials were added for dilution. 2 µL from each vial were drop casted on clean glass coverslips and dried at room temperature for 48 hours. The cover glasses were coated with gold before FE-SEM imaging. FE-SEM measurement of the shrink hydrogels were done by taking 50 µL from each hydrogel and diluting with millii Q water by adding 500 µL of millii Q water. 2 µL were taken on clean glass coverslips and FE-SEM was carried out following the protocols as described above. The released water due to the syneresis were directly drop casted for FE-SEM imaging on clean glass coverslips. We have used 20 kV accelerating voltage to acquire FE-SEM images by using FEI Quanta FEG 250 scanning electron microscope.

X-ray powder diffraction Study. Xerogels obtained from **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogels and the xerogels obtained from the hydrogels formed after syneresis were used for the XRPD analysis. The experiment was done using (Bruker D8 Advance) with Cu-Kα (λ=0.154 nm).

UV study. UV experiment of the gel-to-sol systems were recorded by using JASCO V-550 instrument.

Fluorescence study. Fluorescence spectroscopic measurements were recorded by using Jasco Spectrofluorometer (FP-8500).

Rheological Study. Rheological studies were done at 0.1% (w/v) concentrations of both **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogels. All the measurements were done by using Anton Paar, MCR 702e Space. The minimum torque limit of the instrument is 0.5 nNm under oscillation mode and 1 nNm in rotation mode and the maximum torque limit is 230 mNm.

pH measurement. Preparation of phosphate buffer solution was done using THERMO SCIENTIFICTM EUTECHTM pH meter.

ICP-MS sample preparation and analysis. 700 µL of Hg^{II} salt [$Hg(OAc)_2$] of concentration 1 mg mL^{-1} was added in a 2 mL of **Fmoc-^{1}Nap-A** gel of concentration 0.08 % (w/v). After the syneresis (after 3 days) of the **Fmoc-^{1}Nap-A** hydrogel in the presence of Hg^{II} ions, 50 µL of the water part was taken and diluted to 5 mL by adding Milli Q water. Now, in that 5 mL solution, 5 mL of nitric acid (67-69 %, pico-pure) was added and then digested in a microwave for 30 minutes at 220 ^{0}C. After digestion that 10 mL solution was further diluted to 20 mL by adding 9.8 mL Milli Q water and 0.2 mL internal reference solution of concentration 1000 ppb (Bi 209). Now this solution was used for analysis. A calibration curve was drawn through 1 ppb, 2 ppb, 5 ppb, 10 ppb, 20 ppb, and 50 ppb of standard Hg^{II} solutions. ICP-MS analysis was performed through **ICPMS-2040 LF (SHIMADZU)**.

Absorbance Calculation Method for TT dye and Hg^{II} metal ions

The **TT** dye and Hg^{II} metal ions absorbance experiment, after the addition of **TT** dye and Hg^{II} ions solution on the top of the gels, the gel shrinks and water expels from the matrix. Here, volume of the water and gel matrix changes. Now after completion of the syneresis process, the water left outside of the shrink gel matrix is taken for analysis and termed as the final concentration (C_F). A theoretical initial concentration is considered based on **TT** dye or Hg^{II} metal ions present only in that water part outside of the shrink gel matrix, termed as Initial concentration (C_I). This way we have removed the volume change effect.

Culture preparation

In this study, four bacterial strains were utilized *i.e.*, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 13883). Before experimentation, the stored bacterial strains were revived by streaking onto nutrient agar plates and incubating at 37 °C for 24 hours. Cultures were maintained on agar slants at 4 °C, with sub-culturing performed every two weeks.

Agar disk diffusion test

The agar disk diffusion assay was conducted to evaluate the antibacterial activity of hydrogels performed according to the NCCLS guidelines, 1993.[S1] The antibacterial efficacy of the hydrogels, **Fmoc-^{1}Nap-A** [1 % (w/v), 2.2 mM] and **Fmoc-^{2}Nap-A** [1 % (w/v), 2.2 mM] were determined against four bacterial strains, *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCC 13883). For this assay, Mueller-Hinton agar (MHA) plates were prepared and inoculated with a standardized bacterial suspension, adjusted to approximately 1×10^6 CFU/mL. Using a sterile cotton swab, the bacterial suspension was evenly spread to make bacterial lawn over the surface of the agar plates to ensure uniform bacterial coverage. After that, sterile paper disks (6 mm in diameter) were then placed onto the surface of the inoculated agar and impregnated with 10 µL of hydrogel samples and controls.[S2] The plates were incubated at 37 °C for 24 hours. After

incubation, the presence of clear zones of inhibition surrounding the disks was measured in millimeters. Standard antibiotics specific to each bacterial strain were used as positive controls, while phosphate-buffered saline (PBS) (50 mM phosphate buffer, pH 7.4) was used as the negative control. The experiment was performed in triplicate.

Hemolytic assay

The hemolytic activity of the hydrogels *i.e.,* **Fmoc-^{1}Nap-A** [1 % (w/v), 2.2 mM] and **Fmoc-^{2}Nap-A** [1 % (w/v), 2.2 mM] was assessed using a blood agar plate assay.[S3] Freshly prepared blood agar plates containing 5% (v/v) defibrinated human blood was used. The hydrogel was aseptically applied onto sterile filter paper disks (6 mm in diameter) and placed on the surface of the blood agar plates using sterile forceps.[S4] The plates were then incubated at 37 °C for 24 hours. After incubation, the plates were examined for hemolysis around the hydrogel-impregnated disks. Hemolysis can be classified as alpha (partial hemolysis, indicated by a greenish discoloration around the disk), beta (complete hemolysis, indicated by a clear zone around the disk), or gamma (no hemolysis, no change in the appearance of the agar around the disk). A known hemolytic agent, Tween 80, was used as the positive control, and PBS (phosphate-buffered saline) served as the negative control.

Antioxidant Assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the free radical scavenging activity of antioxidants. DPPH is a stable free radical with a characteristic absorption peak at 517 nm, which can be reduced upon receiving an electron or hydrogen atom from an antioxidant.[S5] In this assay, 10 µL of the synthesized compound (5 mg/mL) is added incrementally to 190 µL of DPPH solution, resulting in a final concentration of 50 µg/mL. After incubating the mixture at 37°C for about 30 minutes, the absorbance is measured at 517 nm using a microplate reader.[S6] A reduction in absorbance reflects the compound's radical scavenging activity, allowing the antioxidant efficiency to be quantified. The percentage of free radical scavenging activity is calculated using a specific formula. The DPPH assay is performed in duplicate, with methanol as the blank and ascorbic acid as the positive control.

% Free radicle scavenging activity = Abs control-Abs test compound/ Abs control *100

Cell Lines & Culture Conditions. PANC-1 (Human pancreatic cancer cell line) (Passage No: 29) was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in DMEM with 10% FBS and 1% penicillin/streptomycin antibiotic solution and sustained at 37ºC under humidified 5% CO_2 - 95% O_2. At their logarithmic growth phase, the cells were harvested using 0.25% Trypsin and 0.53 mM EDTA, counted by trypan-blue dye exclusion assay, and seeded in 24-well or 96-well plates according to the experiments being performed. Stock solution of the compounds was diluted to the required working concentrations (µl) using distilled H_2O (1:100 dilution) as per the assays performed.

Cytotoxicity Analysis- MTT assay. The cytotoxic effect of the compounds was assessed on PANC-1 cancer cells using MTT assay. Briefly, PANC-1 cells were cultured in a 96-well plate at a density of 5×10^3 cells/well. After 24 hours of seeding, the cells were treated with the varying concentrations of the compounds (**Fmoc-^{1}Nap-A & Fmoc-^{2}Nap-A**: 20, 40, 60, 80, and 100 µl) respectively. After 24 hours of treatment, 20 µl of MTT (5 mg/ml in PBS) reagent was added to each well and the cells were incubated in dark at 37ºC for 4 hours. To dissolve the purple formazan crystals formed, 120 µl of DMSO was added to each well. The absorbance at 570 nm was recorded using ELISA-Multiplate reader (Thermo Scientific, South America) and the absorbance values were obtained. To perform relative quantification, absorbance obtained from each group was normalized to that of the control group. The percentage viability was calculated using the formula:
% Viability = [(Optical density {OD} of treated cell − OD of blank)/ (OD of control − OD of blank) × 100].

IC_{50} calculation. IC_{50} denotes the concentration at which 50% percentage of the tumor cells have been inhibited by the compounds. Based on the trend line in the scattered plots obtained from the MTT assay, the IC_{50} value of the compounds was elucidated employing the mathematical formula:

$$y = mx + c$$

where, 'm' denotes the slope, 'x, y' are the coordinates of the X-axis and Y-axis, and 'c' denotes the intercept.

AO/EB staining. To examine the effect of the compounds on the induction of apoptosis in pancreatic cancer cells, Acridine Orange/Ethidium Bromide (AO/EB) staining was performed. Briefly, PANC-1 cells were cultured in a 24-well plate (25×10^3 cells/well) and treated with the IC_{50} values of the compounds obtained from the MTT assay. Following 24 hours of drug treatment, the cells were stained with 100 µl of freshly prepared AO/EB solution (AO- 100 µg/ml and EB- 100 µg/ml in PBS). Photographs of the stained cells were acquired immediately using a fluorescence microscope (Axiovert Carl Zeiss, Germany) at 20x magnification and the fluorescence intensities were quantified using Fiji software. A minimum of 200 cells were counted in each group. The cells were categorized as live/healthy, and apoptotic cells, based on the fluorescence emitted and cellular morphology.

Statistical Analysis. All the experiments were done in triplicates. Experimental data were analyzed using GraphPad Prism v.8.0.1 (GraphPad Software, San Diego, CA). Data are represented as Mean ± Standard Deviation (SD). Statistical significance between the control and various experimental groups was compared using Two-Way Analysis of Variance (ANOVA). P value <0.05 was considered to be statistically significant.

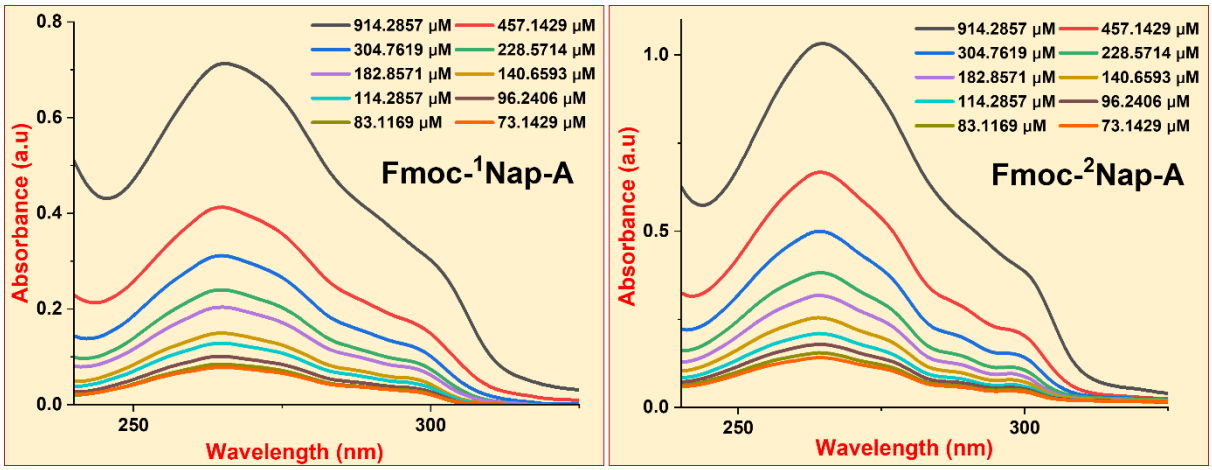

Fig. S1: UV-Vis spectroscopic profiles of **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** gel to sol transition.

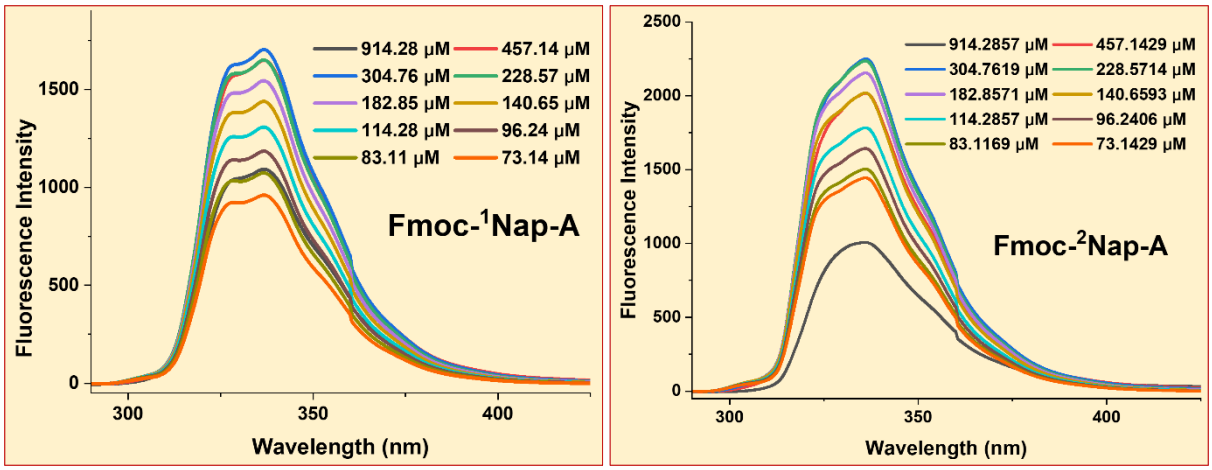

Fig. S2 Fluorescence spectroscopic profiles of **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** gel to sol transition.

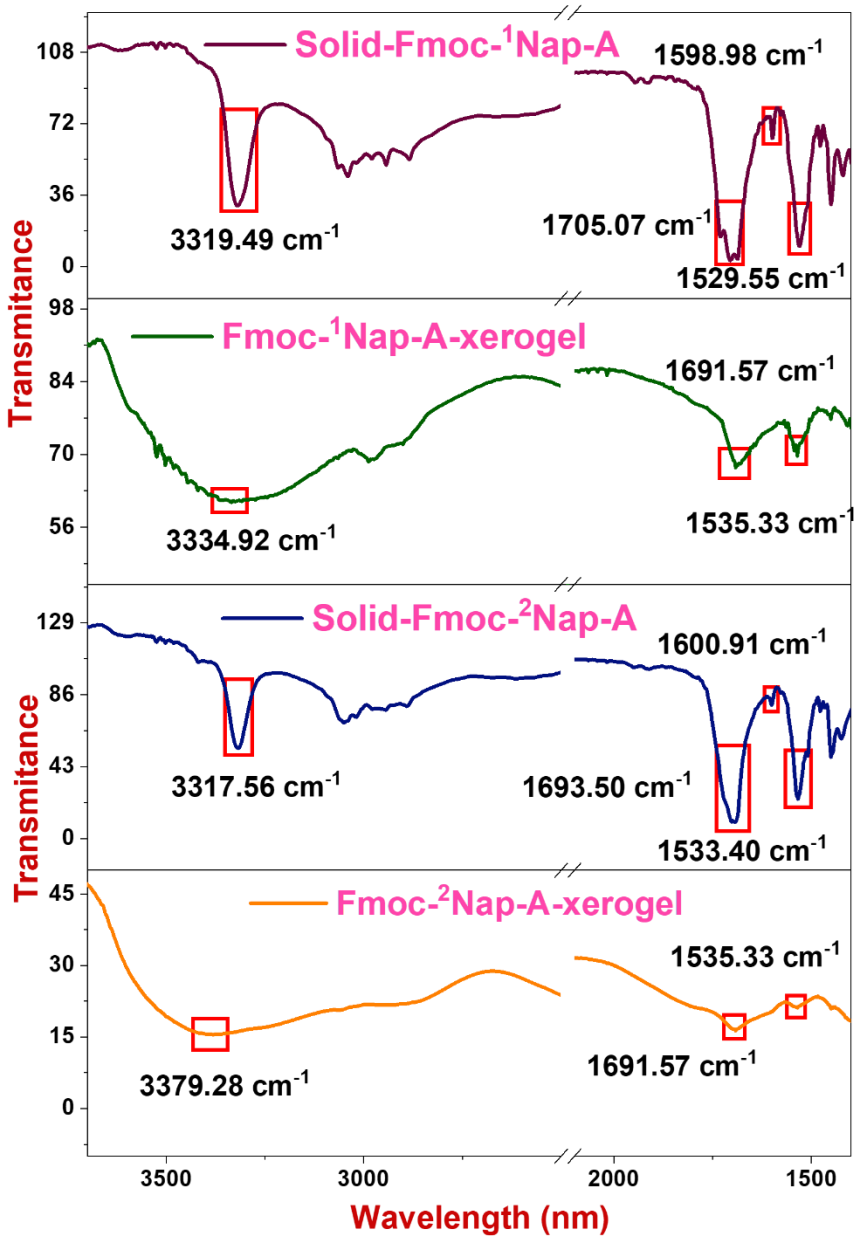

Fig. S3 FT-IR spectroscopic profiles of solid **Fmoc-^{1}Nap-A**, **Fmoc-^{1}Nap-A-xerogel**, solid **Fmoc-^{2}Nap-A** and **Fmoc-^{2}Nap-A** xerogel.

Table S1 FT-IR stretching frequency values of solid **Fmoc-^{1}Nap-A**, **Fmoc-^{1}Nap-A-xerogel**, solid **Fmoc-^{2}Nap-A** and **Fmoc-^{2}Nap-A-xerogel**.

Hydrogelator	N-H stretching frequency of amide group / cm^{-1}	Carbonyl stretching frequency of amide bond / cm^{-1}	N-H bending frequency of amide group / cm^{-1}	C=C double bond stretching frequency of aromatic ring / cm^{-1}
Solid-**Fmoc-^{1}Nap-A**	**3319.49**	**1705.07**	**1529.55**	**1598.98**
Fmoc-^{1}Nap-A-xerogel	**3334.92**	**1691.57**	**1535.33**	**-**
Solid-**Fmoc-^{2}Nap-A**	**3317.56**	**1693.50**	**1533.40**	**1600.91**
Fmoc-^{2}Nap-A-xerogel	**3379.28**	**1691.57**	**1535.33**	**-**

Table S2 X-ray diffraction peak positions of **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** xerogels.

Fmoc-^{1}Nap-A-xerogel			Fmoc-^{2}Nap-A-xerogel		
2θ (^{0})	Distance (Å)	Abbreviation	**2θ (^{0})**	**Distance (Å)**	**Abbreviation**
	17.03	D		**17.97**	**D**
9.47	9.33	D/2	**8.77**	**10.07**	**D/2**
15.75	5.62	D/3	**14.67**	**6.03**	**D/3**
19.22	4.61	D/4	**19.00**	**4.66**	**D/4**
26.14	3.40	D/5	**26.10**	**3.41**	**D/5**

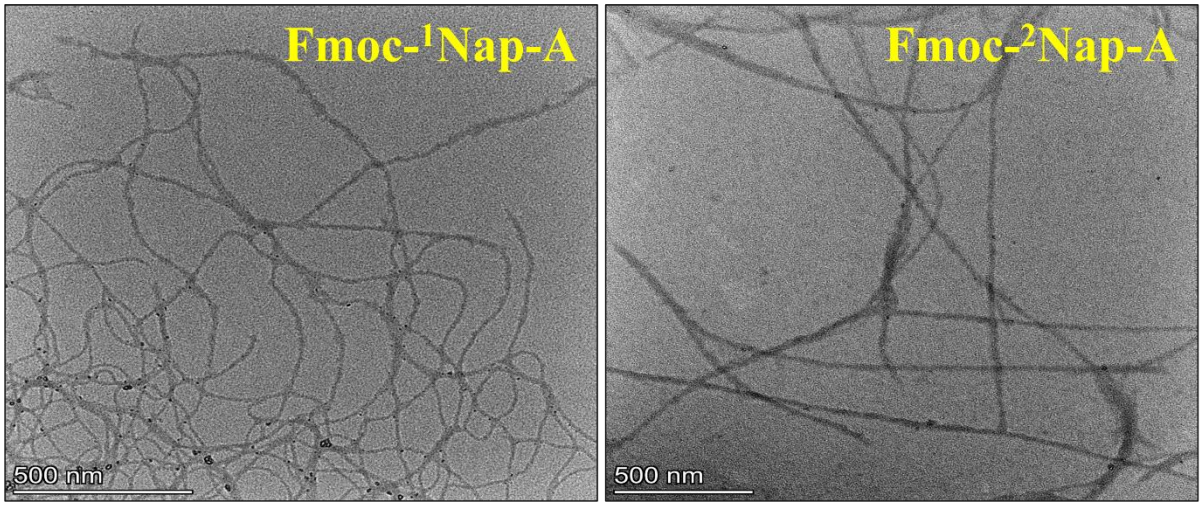

Fig. S4 HR-TEM images of the **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogels in their dried gel forms.

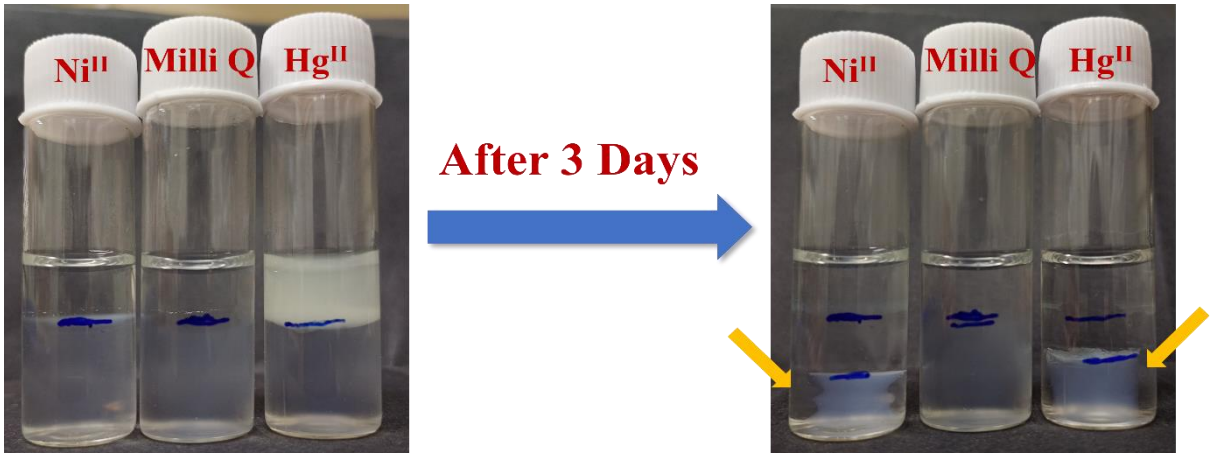

Fig. S5 A control syneresis study of **Fmoc-^{1}Nap-A** hydrogel in the presence of Ni^{II} ions, milli Q water and Hg^{II} ions solutions.

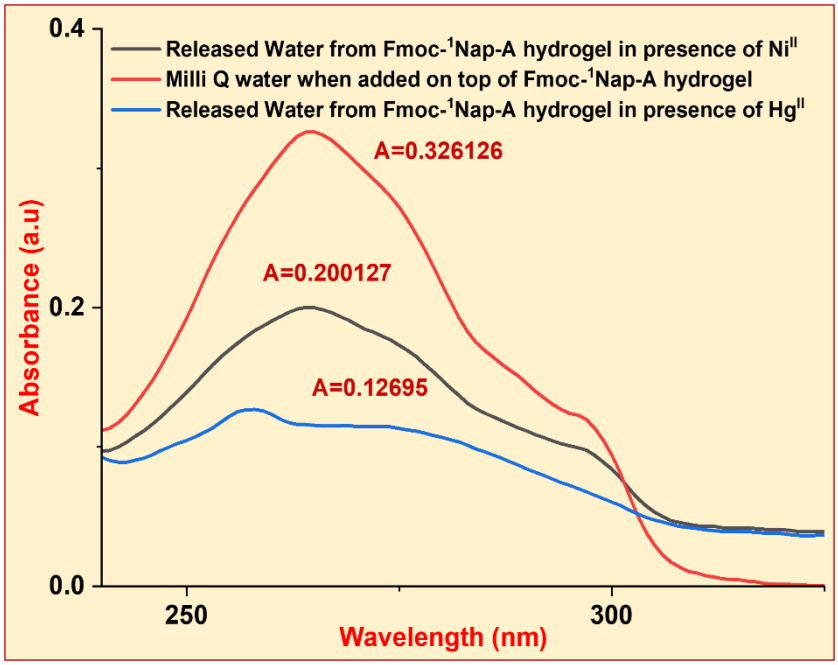

Fig. S6 Absorbance of **Fmoc-¹Nap-A** hydrogelator due to dissolution in the water part above **Fmoc-¹Nap-A** hydrogels after three days.

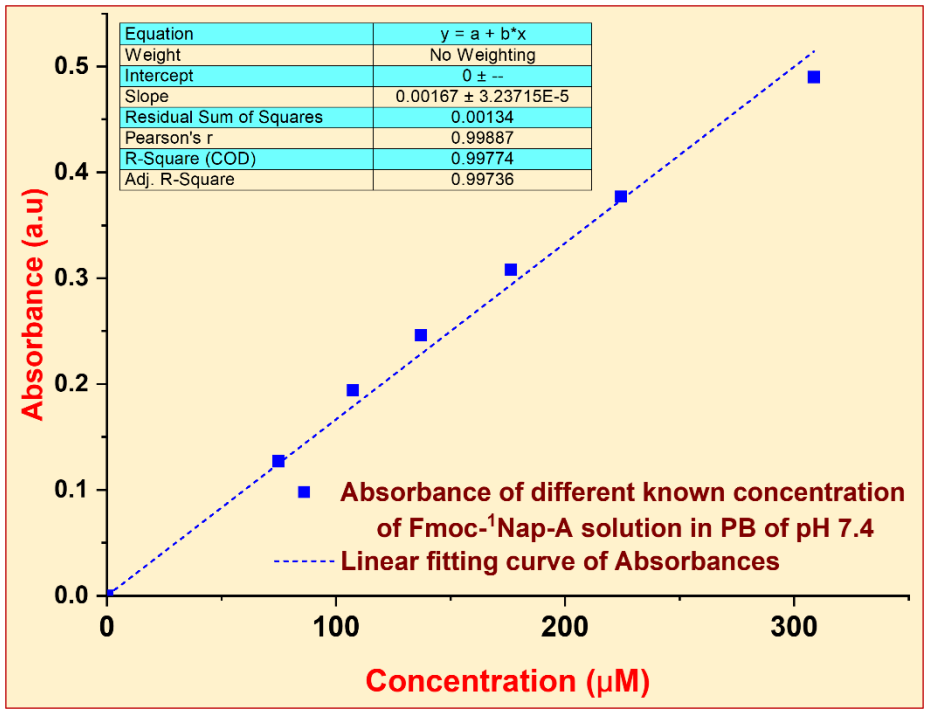

Fig. S7 Standard absorbance calibration curve of different concentrations of **Fmoc-¹Nap-A** hydrogel solutions.

Table S3 Amount of gelator molecules that can dissolute in the process of syneresis or aging in the presence of Hg^{II}, Ni^{II} ions, and Milli Q water.

Salt solutions (1 mg mL^{-1} concentration) and Milli Q water (1 mL)	Absorbance of water part after 3 days	Concentration of released water / µM	Excess volume of water after syneresis / mL	Dissolution of hydrogelators from gel to water (µg)	Amount of hydrogelator molecules present in 2 mL of gel before addition of salt solution or MQ water (µg)
Ni^{II}	0.200127	119.83	2.4	125.82	1600
Milli Q	0.326126	195.28	1.08	92.26	1600
Hg^{II}	0.12695	76.01	2.21	73.49	1600

Table S4 Hg^{II} removal percentage by **Fmoc-^{1}Nap-A** hydrogel and the amount of Hg^{II} ions can be removed by the shrink hydrogel.

The concentration of the water sample measured in ICP-MS / ppb	Concentration correction (C_F)/ppb (X 400)	Theoretical initial concentration (C_I) /ppb	Removal Percentage (C_F) / $R\,\% = \frac{c_I - c_F}{c_I} \times 100$
275	110000	208621.27	47.2

Total Amount of Hg in the system (2.7 mL) (µg unit)	The total amount of Hg in the water part (2.112 mL) after syneresis (µg unit)	The total amount of Hg present in the Gel Part (0.588 mL) (µg unit)	Removal of Hg metal ions / 1 mL of shrink gel (µg unit)
440.61	232.32	208.29	354.23

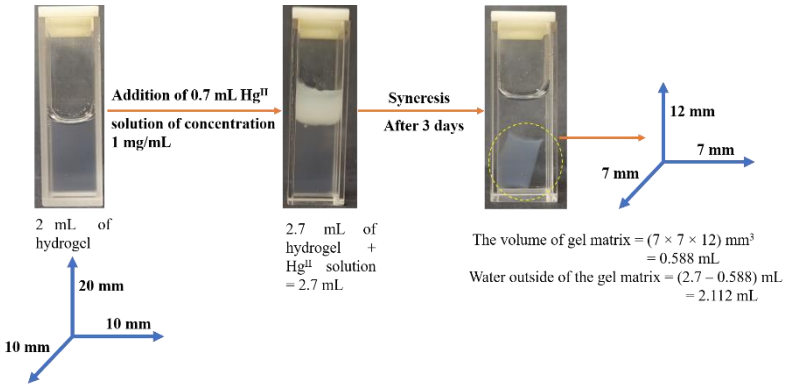

Fig. S8 Estimation of volume of the shrink gel and water outside of the gel matrix.

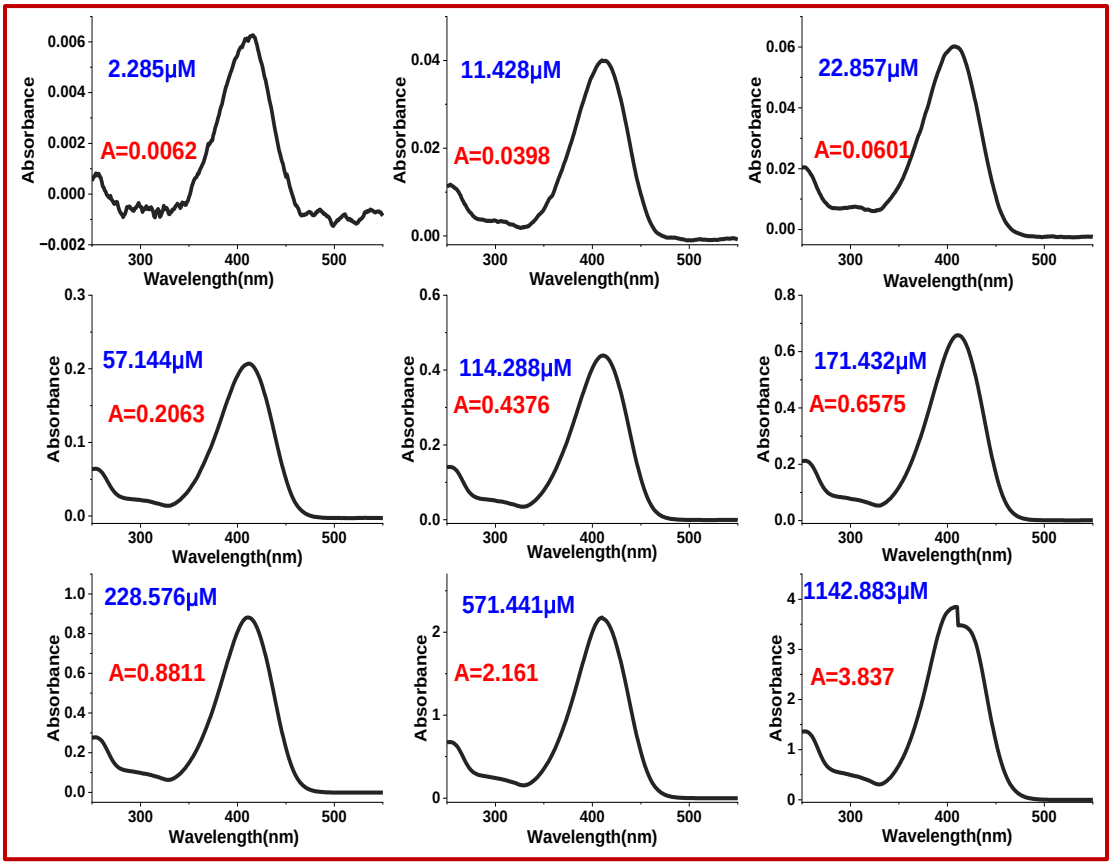

Fig. S9 Absorbance of different standard **TT** solutions.

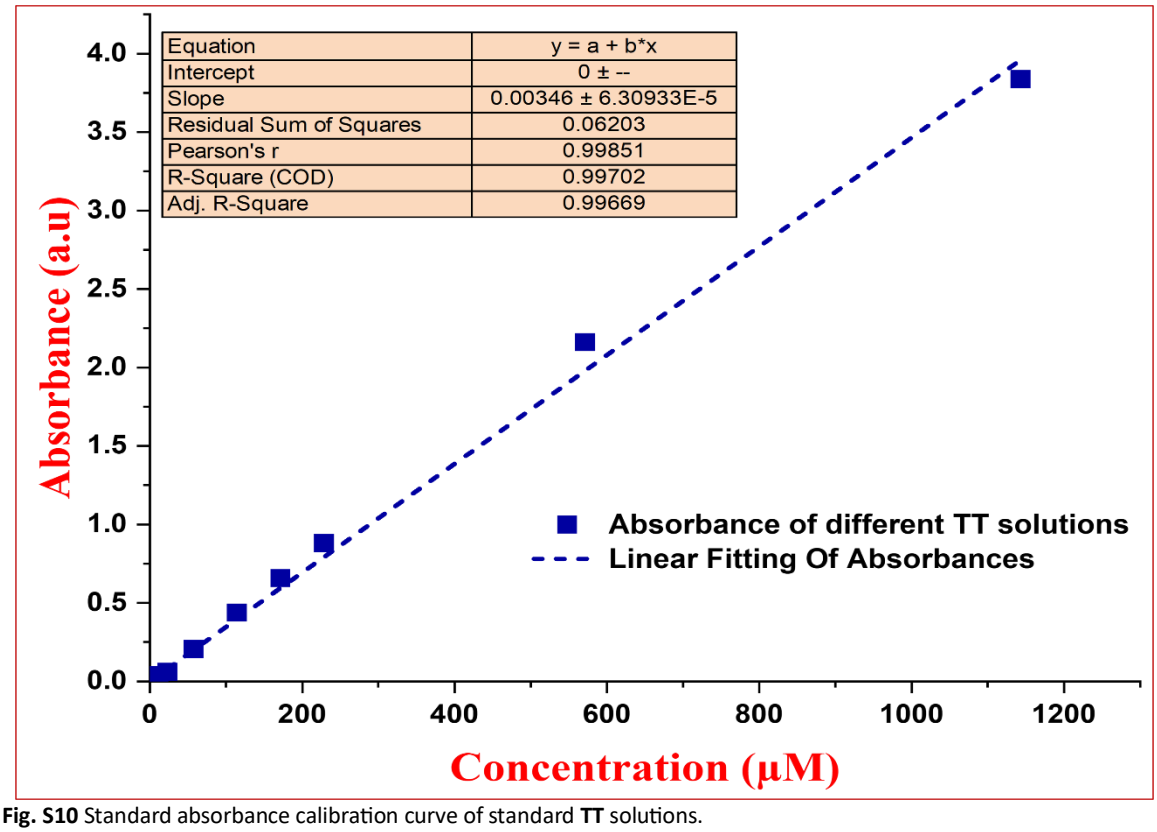

Fig. S10 Standard absorbance calibration curve of standard **TT** solutions.

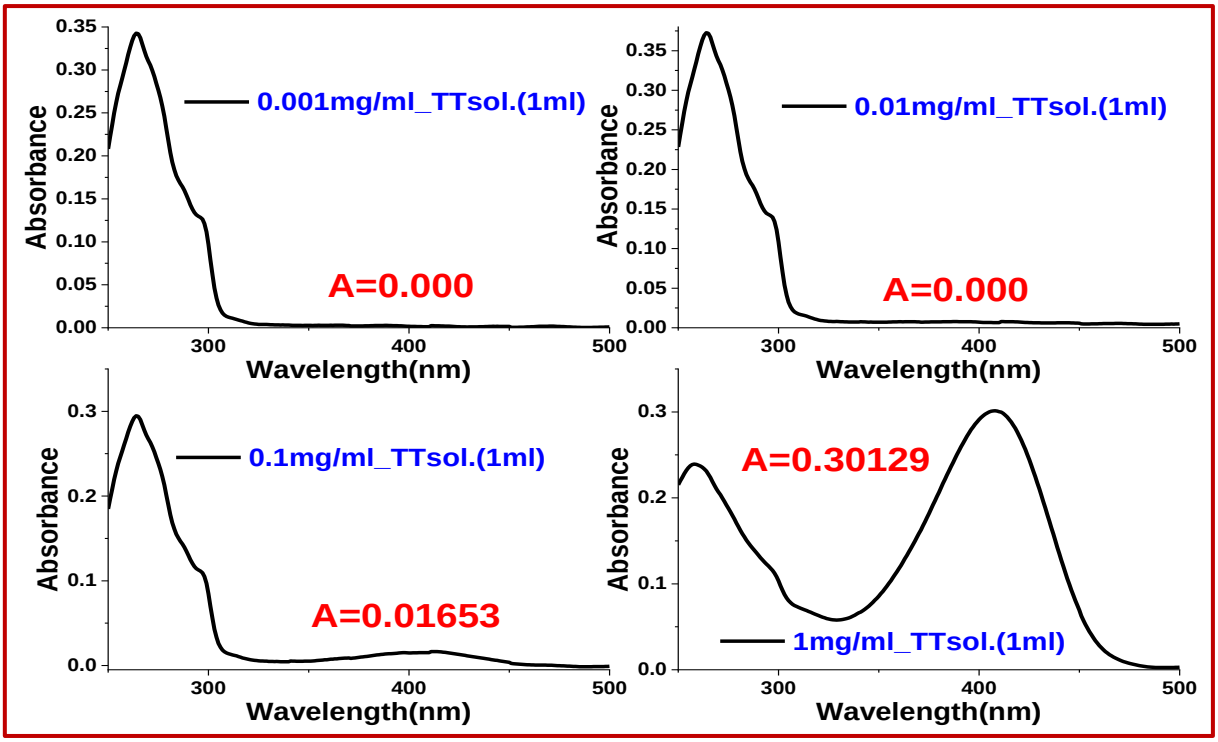

Fig. S11 Absorbance of excess water obtained from concentration dependent syneresis experiment in the presence of **TT** dye for **Fmoc-^{1}Nap-A** hydrogel.

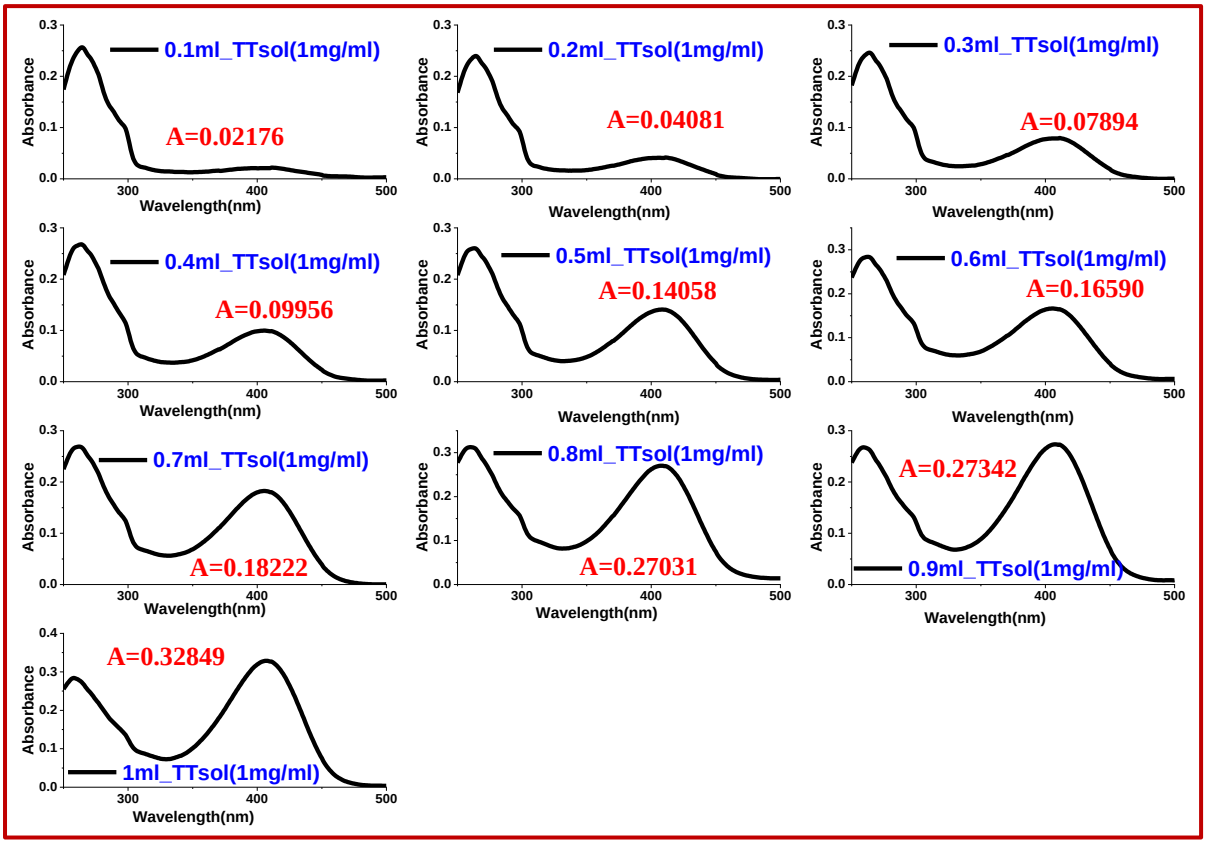

Fig. S12 Absorbance of excess water obtained from volume dependent syneresis experiment in presence of **TT** dye for **Fmoc-^{1}Nap-A** hydrogel.

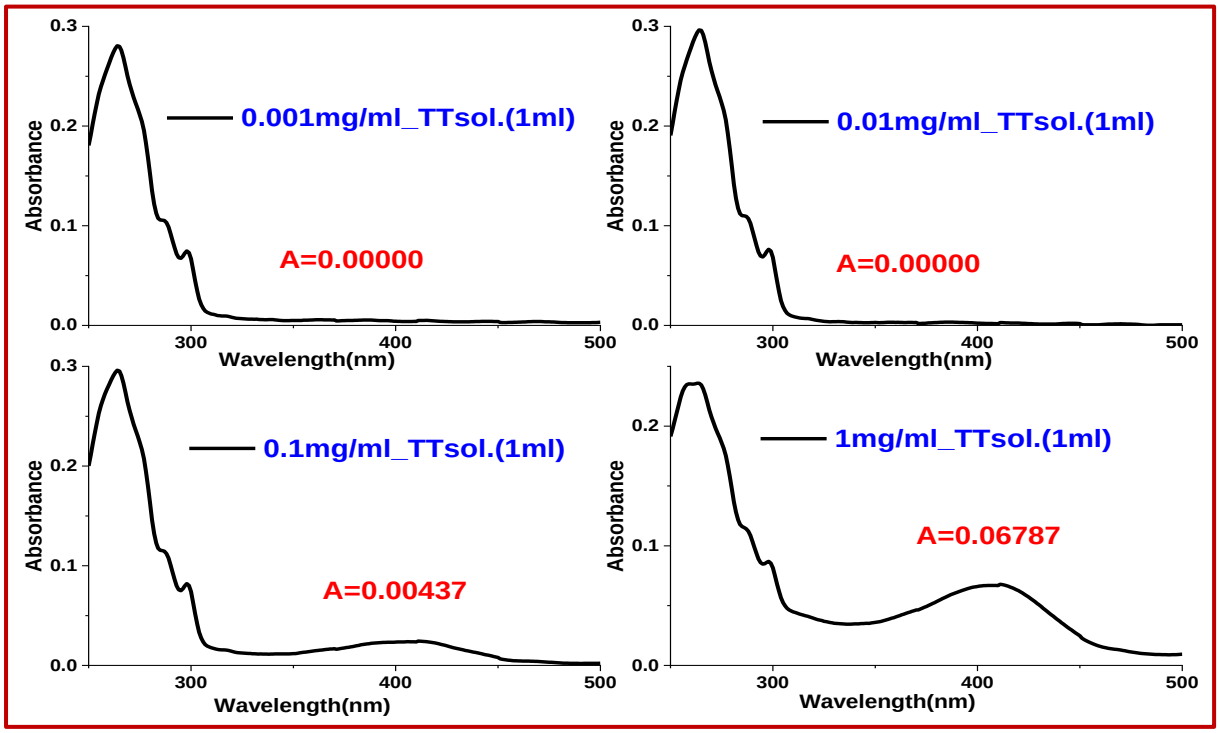

Fig. S13 Absorbance of excess water obtained from concentration dependent syneresis experiment in presence of **TT** dye for **Fmoc-^{2}Nap-A** hydrogel.

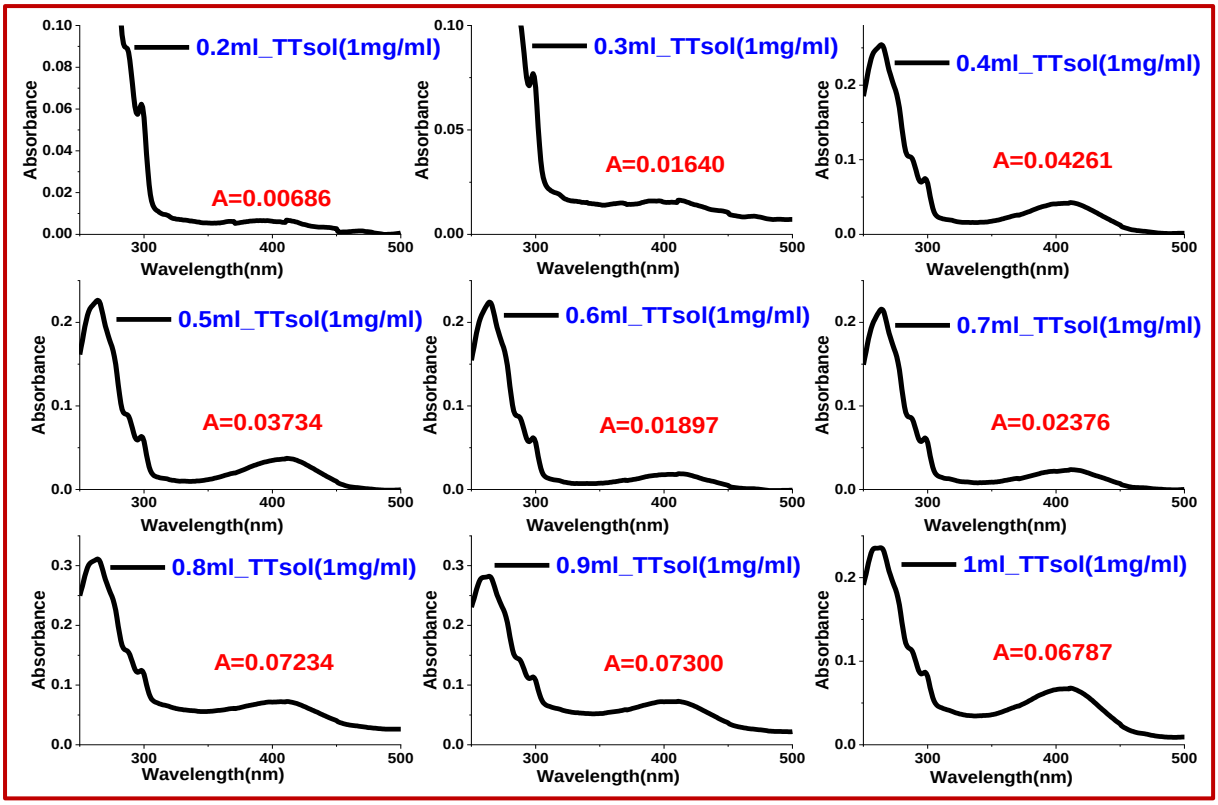

Fig. S14 Absorbance of excess water obtained from volume dependent syneresis experiment in presence of **TT** dye for **Fmoc-^{2}Nap-A** hydrogel.

Table S5 TT dye removal percentage calculation from the released water by **Fmoc-^{1}Nap-A** hydrogel in concentration dependent syneresis experiment.

Conc. of TT solution (mg/mL)	Absorbance after syneresis	Concentration of released water after syneresis (C_F)/ μM	Excess volume of water after syneresis /mL	Theoretical initial Concentration (C_I) / μM	Removal percentage $R\,\% = \frac{c_I - c_F}{c_I} \times 100$
0.001	**0.0000**	**0.0000**	**1**	**2.2858**	**100**
0.01	**0.0000**	**0.0000**	**1**	**22.8577**	**100**
0.1	**0.01653**	**4.77745**	**2.17**	**105.3281**	**95.46**
1	**0.30129**	**87.07803**	**2.890**	**790.9209**	**87.99**

Table S6 TT dye removal percentage calculation from the released water by **Fmoc-^{1}Nap-A** hydrogel in volume dependent syneresis experiment.

Vol. of TT solution in mL from 1 mg mL^{-1}	Absorbance after syneresis	Concentration of excess water after syneresis (C_F)/ μM	Excess volume of H_2O after syneresis / mL	Theoretical initial Concentration (C_I) / μM	Removal Percentage $R\% = \frac{c_I - c_F}{c_I} \times 100$
0.1	**0.02176**	**6.2890**	**1.520**	**150.3577**	**95.82**
0.2	**0.04081**	**11.7947**	**1.870**	**244.4627**	**95.17**
0.3	**0.07894**	**22.8150**	**2.000**	**342.8650**	**93.26**
0.4	**0.09956**	**28.7745**	**2.180**	**419.3924**	**93.14**
0.5	**0.14058**	**40.6300**	**2.340**	**488.3997**	**91.68**
0.6	**0.16590**	**47.9479**	**2.430**	**564.3786**	**91.50**
0.7	**0.18222**	**52.6647**	**2.430**	**658.4379**	**92.00**
0.8	**0.27031**	**78.1242**	**2.650**	**690.0272**	**88.68**
0.9	**0.27342**	**79.0231**	**2.765**	**743.9941**	**89.38**
1	**0.32849**	**94.9393**	**2.890**	**790.9209**	**87.99**

Table S7 TT dye removal percentage calculation from the released water by **Fmoc-^{2}Nap-A** hydrogel in concentration dependent syneresis experiment.

Vol. of TT solution in mL from 1 mg mL^{-1}	Absorbance after syneresis	Concentration of excess water after syneresis (C_F)/ μM	Excess volume of H_2O after syneresis / mL	Theoretical initial Concentration (C_I) / μM	Removal Percentage $R\% = \frac{c_I - c_F}{c_I} \times 100$
0.001	**0.0000**	**0.0000**	**1**	**2.2858**	**100**
0.01	**0.0000**	**0.0000**	**1**	**22.8577**	**100**
0.1	**0.00437**	**1.26300**	**1.6**	**142.8604**	**99.11**
1	**0.06787**	**19.61560**	**2.57**	**889.3918**	**97.79**

Table S8 TT dye removal percentage calculation from the released water by **Fmoc-^{2}Nap-A** hydrogel in volume dependent syneresis experiment.

Vol. of TT solution in mL from 1 mg mL^{-1}	Absorbance after syneresis	Concentration of excess water after syneresis (C_F)/ µM	Excess volume of H_2O after syneresis / mL	Theoretical initial Concentration (C_I) / µM	Removal Percentage $R\% = \frac{C_I - C_F}{C_I} \times 100$
0.2	**0.00686**	**1.9826**	**0.60**	**761.9146**	**99.74**
0.3	**0.01640**	**4.7398**	**1.60**	**428.5812**	**98.89**
0.4	**0.04261**	**12.3150**	**1.30**	**703.3075**	**98.25**
0.5	**0.03734**	**10.7919**	**1.56**	**732.6110**	**98.53**
0.6	**0.01897**	**5.4826**	**1.96**	**699.7189**	**99.22**
0.7	**0.02376**	**6.8670**	**2.40**	**666.6167**	**98.97**
0.8	**0.07234**	**20.9075**	**2.12**	**862.5340**	**97.58**
0.9	**0.07300**	**21.0982**	**2.14**	**91.302**	**97.80**
1	**0.06787**	**19.6156**	**2.57**	**889.3918**	**97.79**

Table S9 Total amount of TT dye that can be removed by 2 mL of **Fmoc-^{1}Nap-A** and **Fmoc-^{2}Nap-A** hydrogel of concentration 0.085 % w/v (1600 µg gelator molecules).

	Volume of Gel (0.08% w/v)	Total amount of gelator present in that 2 mL gel (µg)	Volume of TT solution (1 mg/mL concentration)	Concentration of TT in Water after Syneresis (µM)	Total Volume of Water after Syneresis (mL)	Total amount of TT dye present in that water (µg)	Total amount of TT dye was added in the system (µg)	Amount of TT dye removed by the 2 mL of gel (µg)
Fmoc-^{1}Nap-A	**2 mL**	**1600**	**1 mL**	**94.9393**	**2.890**	**87.4898**	**1000**	**912.5102**
Fmoc-^{2}Nap-A	**2 mL**	**1600**	**1 mL**	**19.6156**	**2.57**	**18.0764**	**1000**	**981.9236**

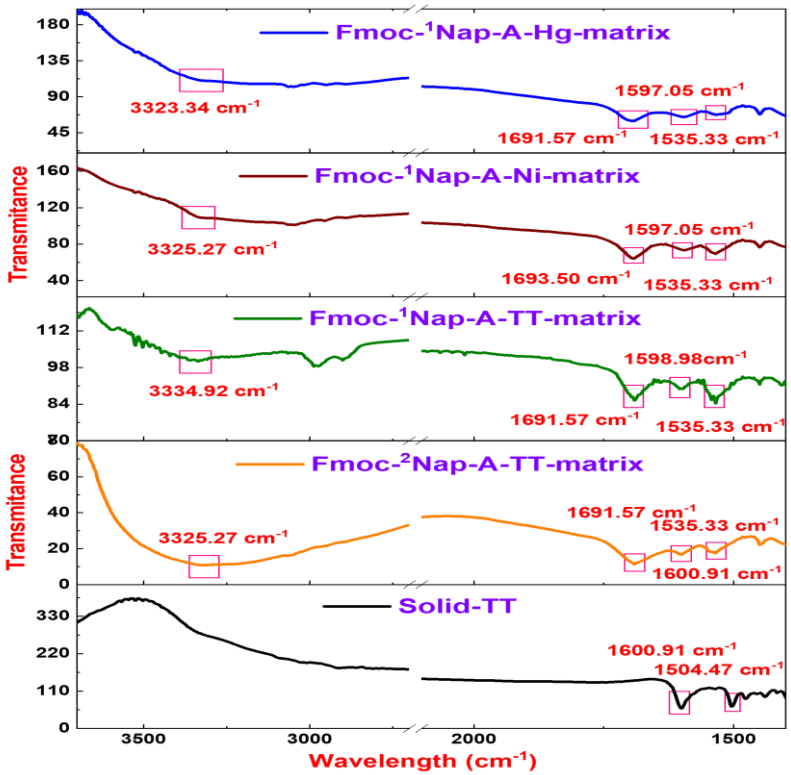

Fig. S15 FT-IR profile of solid **TT** dye, **Fmoc-¹Nap-A-Hg** matrix, **Fmoc-¹Nap-A-Ni** matrix, **Fmoc-¹Nap-A-TT** matrix, and **Fmoc-²Nap-A-TT** matrix.

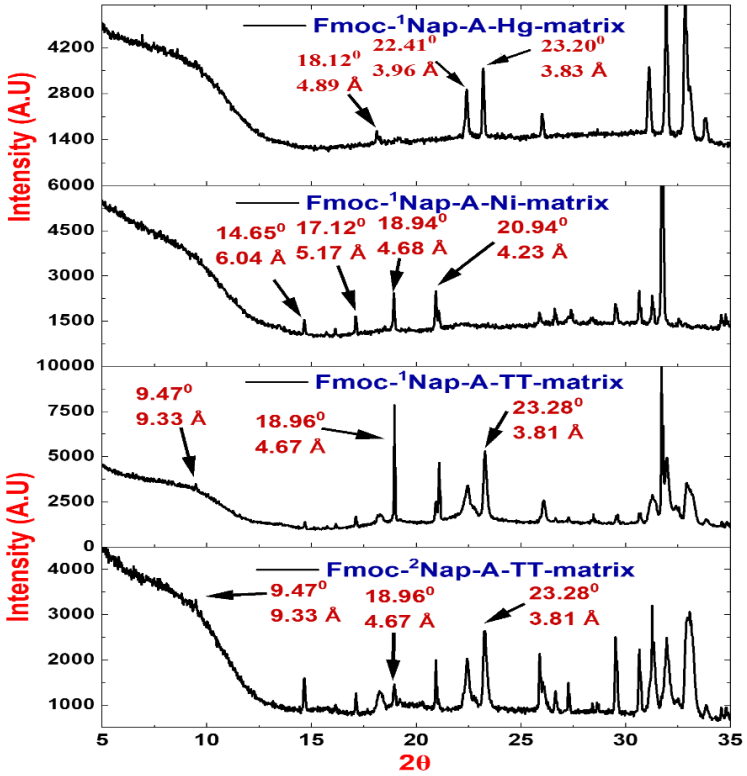

Fig. S16 XRD profile of **Fmoc-¹Nap-A-Ni** matrix, **Fmoc-¹Nap-A-Hg** matrix, **Fmoc-¹Nap-A-TT** matrix, and **Fmoc-²Nap-A-TT** matrix.

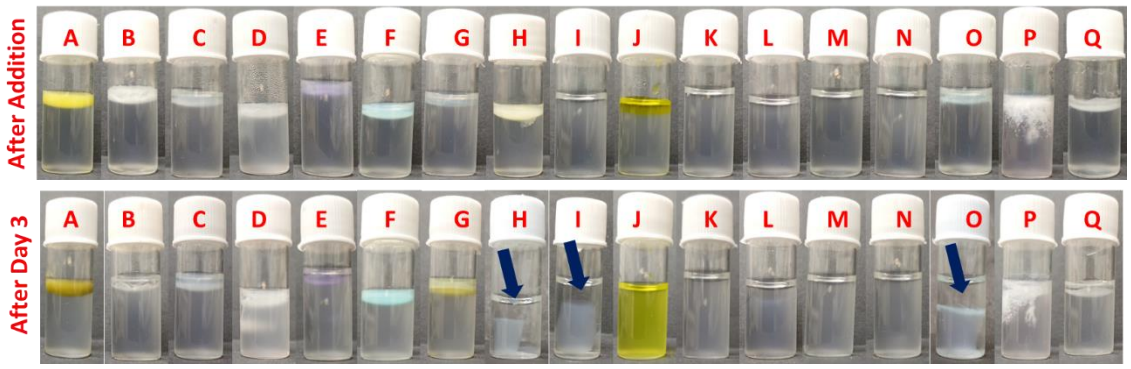

Fig. S17 Syneresis experiment for **Fmoc-^{1}Nap-A** hydrogel in the presence of different metal salts, $AgNO_3$ (A), $BaCl_2$ (B), $CaCl_2$ (C), $Cd(OAc)_2$ (D), $Co(NO_3)_2$ (E), $CuSO_4$ (F), $FeSO_4$ (G), $Hg(OAc)_2$ (H), $HgCl_2$ (I),K_2CrO_4 (J), KBr(K), $MgSO_4$ (L), NaCl(M), NaOAc(N), $NiCl_2$ (O), $Pb(OAc)_2$ (P), $ZnSO_4$ (Q).

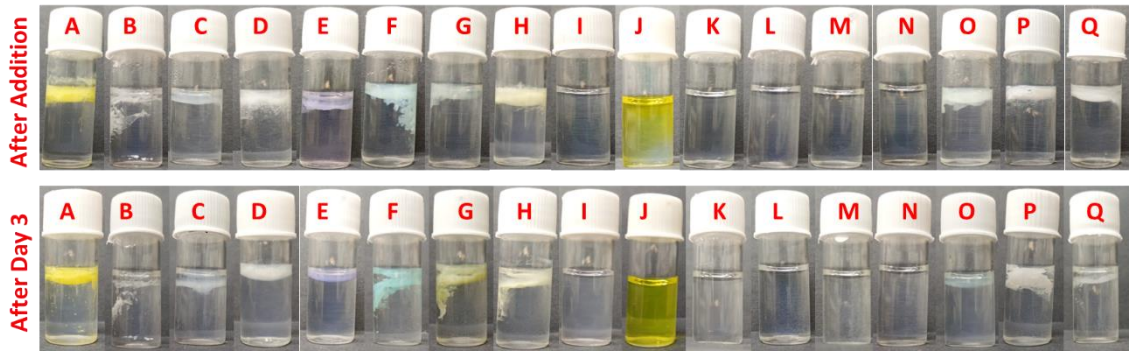

Fig. S18 Syneresis experiment for **Fmoc-^{2}Nap-A** hydrogel in the presence of different metal salts, $AgNO_3$ (A), $BaCl_2$ (B), $CaCl_2$ (C), $Cd(OAc)_2$ (D), $Co(NO_3)_2$ (E), $CuSO_4$ (F), $FeSO_4$ (G), $Hg(OAc)_2$ (H), $HgCl_2$ (I),K_2CrO_4 (J), KBr(K), $MgSO_4$ (L), NaCl(M), NaOAc(N), $NiCl_2$ (O), $Pb(OAc)_2$ (P), $ZnSO_4$ (Q).

Table S10 Measured zone of inhibition of prepared hydrogels *i.e.*, **Fmoc-^{1}Nap-A** [1 % (w/v), 2.2 mM] and **Fmoc-^{2}Nap-A** [1 % (w/v), 2.2 mM] and standard drugs *i.e.,* Linezolid (30 µg/mL), Ampicillin (25 µg/mL), Levofloxacin (5 µg/mL) and Ciprofloxacin (1 µg/mL) against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, respectively.

Bacteria	**Zone of inhibition (mm)**				
	Positive control		**Test Samples**		**Negative control (PBS)**
			Fmoc-^{1}Nap-A	**Fmoc-^{2}Nap-A**	
Staphylococcus aureus	Linezolid (30 µg/mL)	20 ±1	18 ± 2	10 ± 1	-
Enterococcus faecalis	Ampicillin (25 µg/mL)	20 ±2	16 ± 2	11 ± 1	-
Pseudomonas aeruginosa	Levofloxacin (5 µg/mL)	27 ±1	24 ± 1	21 ± 2	-
Klebsiella pneumoniae	Ciprofloxacin (1 µg/mL)	21 ±1	11 ± 2	08 ± 1	-

References:

S1. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard. NCCLS document M2-A5. Wayne, Pa: National Committee for Clinical Laboratory Standards; **1993**.

S2. E. D. Beer and MB Sherwood, *J Bacteriol*, 1945, **50**(4), 459-467.

S3. G. E. Rowe and R. A. Welch, *Methods Enzymol.*, 1994, **235**, 657–667.

S4. Vidyasagar, R. R. Patel, S. K. Singh, D. Dehari, G. Nath, and M. Singh, *Heliyon.*, 2024, **10**(10), e31116.

S5. M. Javaid, I.-U. Haq, H. Nadeem, H. Fatima, A.-U. Khan, and N. Irshad, *Front. Pharmacol.*, 2023, **14**, 1084181.

S6. K. Thongchai, P. Chuysinuan, T. Thanyacharoen, S. Techasakul, and S. Ummartyotin, *J. Mater. Res. Technol.*, 2020, **9**(3), 6512–6520.