Supporting Information

A Thermo-responsive Supramolecular Hydrogel that Senses Cholera Toxin via Color-Changing Response

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This Supplementary Information file replaces that originally published on 02 April 2020. The original version contained some poor resolution NMR images which have been improved in this updated version. This does not affect the results or conclusions in the article.

Physical measurements and instrumentation

Material and Methods. All chemicals, solvents and silica gel for TLC were obtained from well-known commercial sources and were used without further purification, as appropriate. Cholera Toxin was obtained from Sigma Aldrich. Solvents were distilled and dried by standard procedure before use. Melting points was measured in open capillaries and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Bruker-400 Advance NMR spectrometer. Chemical shifts were reported in ppm downfield from the internal standard tetramethyl silane (TMS). Mass spectrometry of individual compounds was performed using a Micro Mass ESI-TOF MS instrument. Elemental analysis was recorded in Thermo Finnigan EA FLASH 1112 SERIES.

Gelation Studies. A detailed procedure of gelation has been discussed in the main manuscript. The formation of the gel was confirmed using the tube inversion method. If a gel was formed, it was evaluated quantitatively by determining the critical gelator concentration (CGC) which is the minimum amount of gelator required to immobilize 1 mL of a particular solvent or solvent mixtures. A glass tube with a capacity of 10 x 75 mm has been used for the CGC measurement.

FT-IR Spectroscopy. Prepared solution and gels were drop casted on the CaF_2 cell and dried under vacuum and FT-IR spectra were recorded in Perkin Elmer Spectrum BX FT-IR system. For the solid sample we have taken the powder sample and then FT-IR was recorded.

UV/Vis and Fluorescence Spectroscopy. The UV-Vis Spectroscopy and Fluorescence Spectroscopy of described solutions/suspensions were recorded on a Shimadzu model 2100 spectrophotometer and Cary-Eclipse spectrofluorimeter respectively, both equipped with a temperature controller bath.

Circular Dichroism Spectroscopy (CD). All the CD spectra of described solutions were recorded on a JASCO instrument, Model J-815-150S. Experiments were performed by purging dry N_2 gas continuously. Data were collected in a quartz cuvette of 1 mm path length between 200 to 450 nm.

Atomic Force Microscopy (AFM). A dilute solution of the sample was deposited on freshly cleaved mica surface and then carefully air-dried. Each of the samples was analyzed using a JPK 00901 AFM instrument and Nano-Wizard software. Tapping mode off 10 nm tip radius, silicon tip, 292 KHz resonant frequency, 0.7-1 Hz scan speed, 256 x 256 and 512 x 512- pixels.

Transmission Electron Microscopy (TEM). A dilute solution of the aggregate at specified concentration were drop coated on carbon-coated copper grids and it was then air-dried carefully. Each sample was stained with 0.1% uranyl acetate solution and the TEM images were taken at an accelerating voltage of 200 kV using a TECNAI F30 instrument.

Scanning Electron Microscopy (SEM). The gels or the specified solution were carefully drop casted onto the brass stubs and were allowed to air-dry overnight. The samples were then coated with gold vapor and analyzed on a Quanta 200 SEM operated at 10-15 kV.

Fluorescence Microscopy. A diluted solution of **PyLac** was drop casted on a pre-cleaned glass slides and it was left overnight for drying in a dust free environment and finally evacuated. The fluorescence microscopic images were taken on an Olympus IX-71 microscope with the excitation of 300-400 nm UV light.

Dynamic Light Scattering (DLS). DLS measurements were performed at room temperature using a Malvern Zetasizer Nano ZS particle sizer (Malvern Instruments Inc., Westborough, MA). Samples were prepared and examined under a dust-free conditions. Average hydrodynamic diameters (D_h) reported were obtained from the Gaussian analysis of the intensity-weighted particle size distributions.

X-ray Diffraction. The gel sample of the specified concentration was prepared and was carefully taken on glass slide and dried under vacuum for the corresponding XRD measurement. These samples were examined using Bruker D8 Advance instrument (θ, 2θ geometry with Scintillation Detectors). The X- ray beam generated with a rotating Cu anode at the wavelength of KR beam at 1.5418 was directed towards the film directed toward the film edge and scanning was done up to a 2θ value of 30°. Data were then analyzed and interpreted using the Bragg equation.

Energy Minimization. Energy minimization of the compound was performed using B3LYP/6-31G* level of computations.

Rheological studies. Rheology experiments of the gels were carried out using Antor Paar MCR 52 with a cone and plate geometry (CP 25-2) having an adjustable peltier temperature controlling system. The distance between the cone and plates was kept fixed at 0.105 mm for all the measurement. The gels were taken on the plate of the rheometer. An oscillatory strain amplitude sweep experiment was performed at a constant oscillation frequency of 1 Hz for the applied strain range 0.01-100 % at 20 °C. The rheometer has inbuilt software that converts the

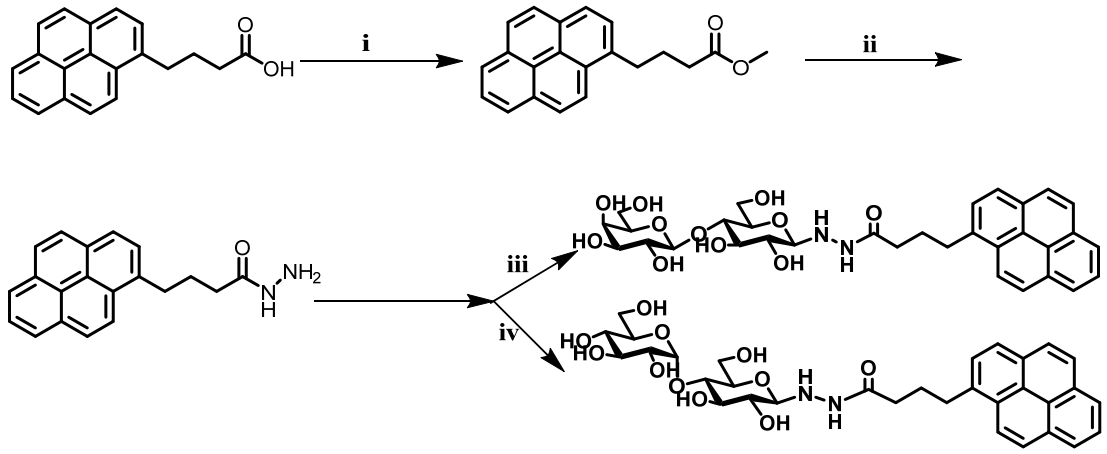
torque measurements into either G' (the storage modulus) and G" (the loss modulus) which is represented either as strain or shear stress. Oscillatory frequency sweep experiments were performed in the linear viscoelastic region (strain 0.01%) to ensure that calculated parameters correspond to an intact network structure.

Fluorescence-Decay Experiments. Fluorescence lifetimes were measured by using a time-correlated single-photon-counting fluorimeter (Horiba Jobin Yvon). The system was excited with nano LED light (Horiba–Jobin Yvon) with a pulse duration of 1.2 ns. (slit width: 2/2, λ_{em}=345 nm). The average fluorescence lifetimes (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (α_i) by using Equation (1), where $a_{1\text{-}3}$ are the relative amplitudes and τ_{1-3} are the lifetime values.

$$\tau_{av} = (\alpha_1\tau_1^2 + \alpha_2\tau_2^2 + \alpha_3\tau_3^2)/(\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3)$$

Preparation of Test Strip: The paper strip (10.5 cm x 2 cm) was dipped in the solution containing **PyLac** (0.8 mM) for 40 sec followed by cooling at room temperature. The remaining solvent was then completely removed under reduced pressure and the dried strip was cut into pieces and used further for the detection of CT.

Scheme 1[a]



[a]**Reagents, Conditions and Yields.** (i) MeOH, c. H_2SO_4 (cat.), 65 °C, 6 h, 98 %; (ii) $NH_2NH_2.H_2O$, MeOH, 65 °C, 12 h, 97 %; (iii) D-Lactose. H_2O, $CHCl_3$/ MeOH (1:3 v/v), 65 °C, 36 h, 65 %; (iv) D-Maltose.H_2O, $CHCl_3$/ MeOH (1:3 v/v), 65 °C, 36 h, 69 %.

Synthetic procedure and characterization: The synthesis of **2** and **3** were carried out following the procedures reported in the literature.[Sa-c]

Synthesis of PyLac. 4-(pyren-1-yl) butanehydrazide (1 g, 3.47 mmol) and D-lactose monohydrate (1.6 g, 4.20 mmol) were taken in a round bottom flask. The mixture was dissolved by adding 35 mL of MeOH/$CHCl_3$ (3:1 v/v) to it and refluxed at 65 °C for 24 h. The resultant mixture was cooled, and the solvent was evaporated in vacuum or under reduced pressure to afford an off- white residue. The residue so obtained was purified by repetitively washing with chloroform and water. The resultant residue was vacuum dried to get a white solid product with 65 % isolated yield. M.P. (170 °C) FT-IR (KBr cm^{-1}) 3414, 3298, 3064, 2871, 1654, 1527, 1459, 1302.26, 1120, 1081; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.98-2.00 (m, 2H), 2.17-2.26 (m, 2H), 3.01-3.06 (m, 2H), 3.26-3.29 (m, 5H), 3.34 (m, 1H), 3.36 (m, 2H), 3.49 (m, 2H), 3.60 (m, 3H), 4.15-4.17 (m, 2H), 4.60-4.71 (m, 2H), 4.77-4.87 (m, 2H), 5.20 (m, 1H), 5.30 (m, 1H), 5.62 (m, 1H) 7.90-7.93 (m, 1H), 8.01-8.03 (m, 3H), 8.17-8.26 (m, 4H), 8.32-8.34 (m, 1H), 9.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.87, 32.57, 33.30, 60.76, 68.53, 70.90, 71.23, 73.62, 75.17, 75.91, 76.44, 79.03, 79.69, 81.43, 90.74, 104.25, 123.87, 124.55, 124.63, 125.21, 125.34, 126.54, 126.92, 127.69, 127.86, 127.96, 128.54, 129.72, 130.83, 131.28, 136.81, 172.27. HRMS: *m/z* calcd. for $C_{32}H_{38}N_2O_{11}$ ($M + Na^+$) 649.2373, found: 649.2371. Anal.: calcd. For $C_{32}H_{38}N_2O_{11}$: C 61.33, H 6.11, N 4.47; found: C 61.14, H 6.38, N 4.25.

Synthesis of PyMal. 4-(pyren-1-yl) butanehydrazide (1 g, 3.47 mmol) and D-maltose mono hydrate (1.6 g, 4.20 mmol) were taken in the round bottom flask. The mixture was dissolved by adding 35 mL of MeOH/$CHCl_3$ (3:1 v/v) to it and then stirred under refluxing conditions for 24 h. The resultant mixture was cooled, and the solvent was evaporated in vacuum to furnish a yellowish white residue. The residue so obtained was purified by washing with chloroform followed by water. The resultant residue was vacuum dried to get a yellowish white solid product with 69 % isolated yield. M.P. (168 °C) FT-IR (KBr cm^{-1}) 3415, 3297, 3066, 2870, 1650, 1528, 1457, 1301,1122, 1083; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.00-2.06 (m, 2H), 2.20-2.28 (m, 2H), 2.99-3.08 (m, 2H), 3.19-3.31 (m, 4H), 3.41-3.48 (m, 5H), 3.54-3.83 (m, 4H), 4.26-4.29 (m, 1H), 4.52-4.55 (m, 1H), 4.99-5.01 (m, 3H), 5.20-5.21 (m, 1H), 5.44-5.46 (m, 1H), 5.54-5.55 (m, 1H), 5.76 (m, 1H), 7.94-7.96 (m, 1H), 8.04-8.14 (m, 3H), 8.22-8.29 (m, 4H), 8.37-8.39 (m, 1H), 9.48 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.41, 33.11, 33.87, 61.79, 70.85, 71.52, 73.43, 74.25, 74.47, 77.24, 77.32, 79.75, 80.82, 81.10, 91.70, 101.96, 124.42, 125.10, 125.18, 125.77, 125.90, 127.10, 127.48, 128.26, 128.41, 128.51, 129.10, 130.28, 131.38, 131.84, 137.34, 172.98. HRMS: *m/z* calcd. for $C_{32}H_{38}N_2O_{11}$ ($M + Na^+$) 627.2554, found: 627.2554. Anal.: calcd. For $C_{32}H_{38}N_2O_{11}$: C 61.33, H 6.11, N 4.47; found: C 61.48, H 6.53, N 4.10.

References:

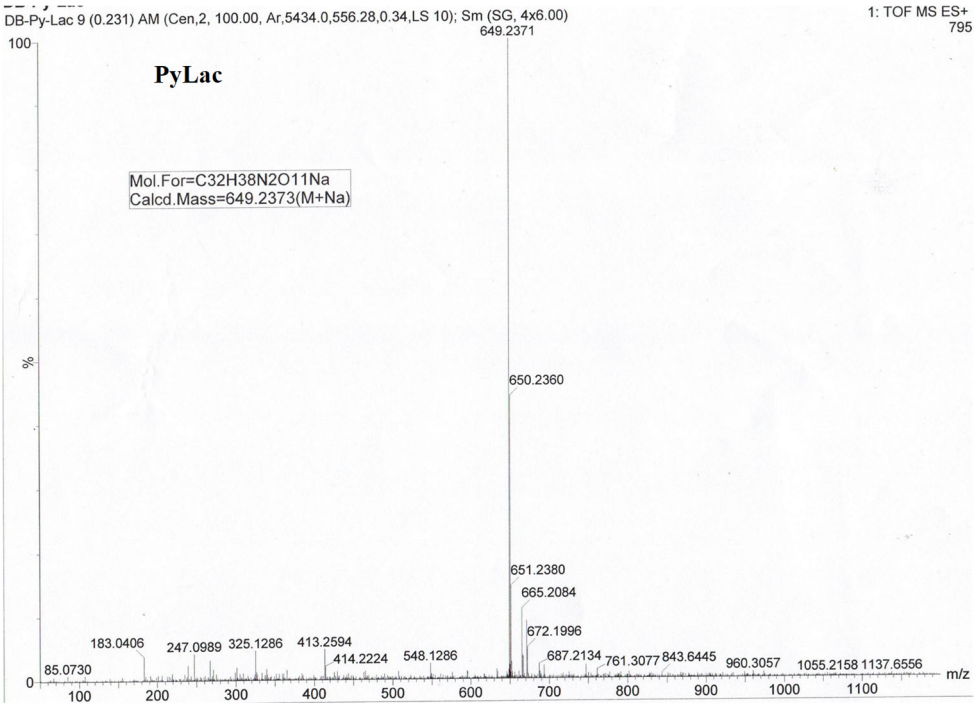
Sa. M. R. Molla and S. Ghosh, *Chem. Eur. J.*, 2012, **18**, 9860-9869.

Sb. S. Akama, T. Makia and M. Yamanaka, *Chem. Commun.*, 2018, **54**, 8814-8817.

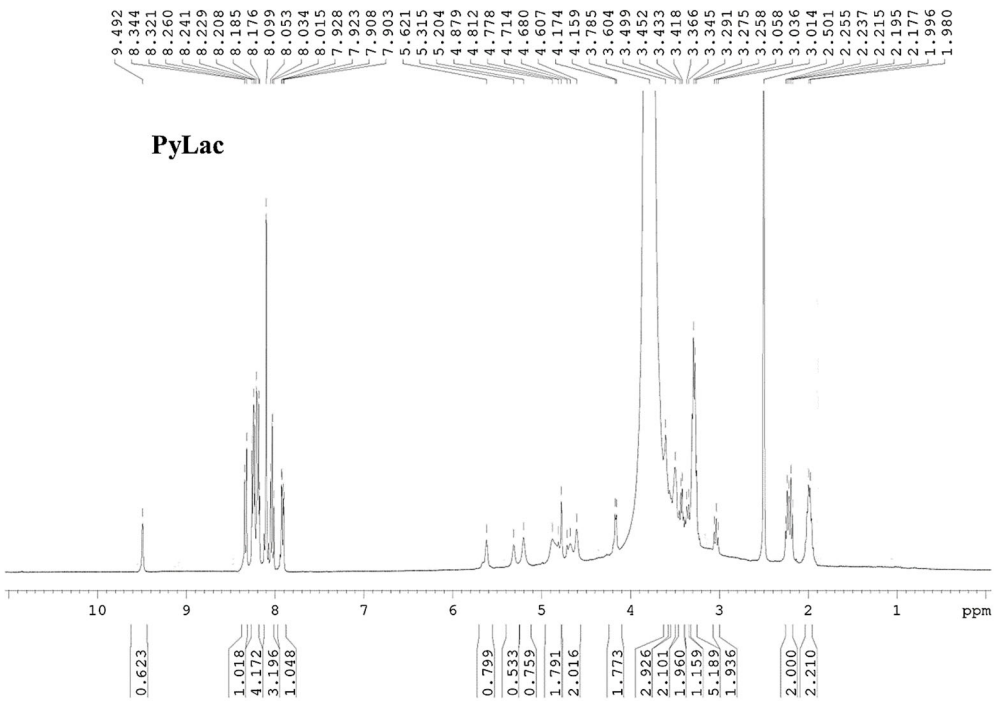
Sc. P. Rajamalli, P. S. Sheeta and E. Prasad, *Chem. Commun.*, 2013, **49**, 6758-6760.

Characterization of PyLac.

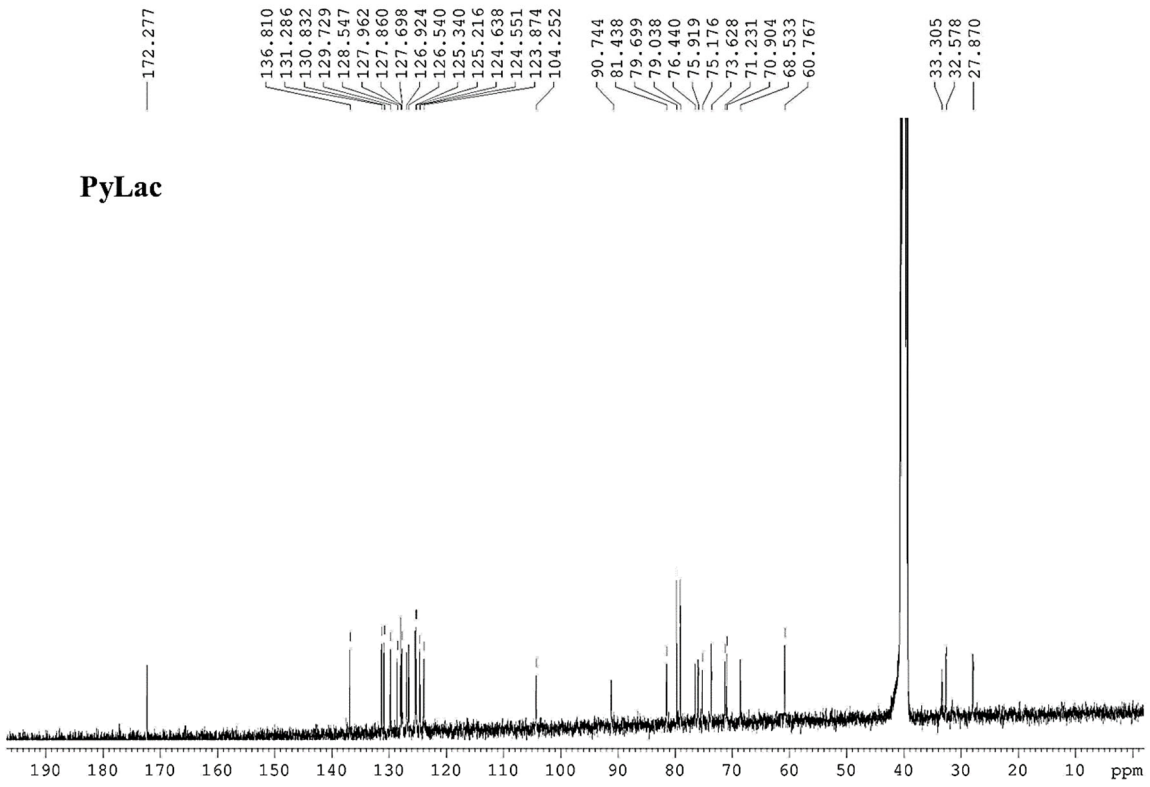
Mass spectrum



^{1}H NMR

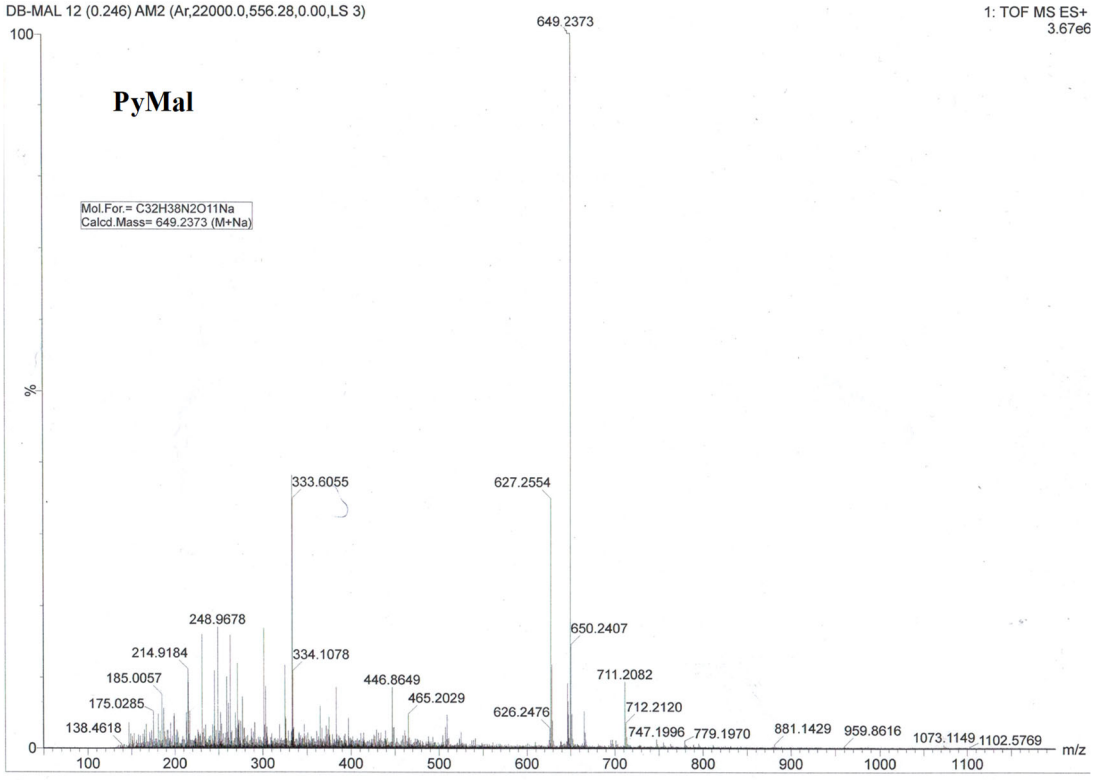


^{13}C NMR

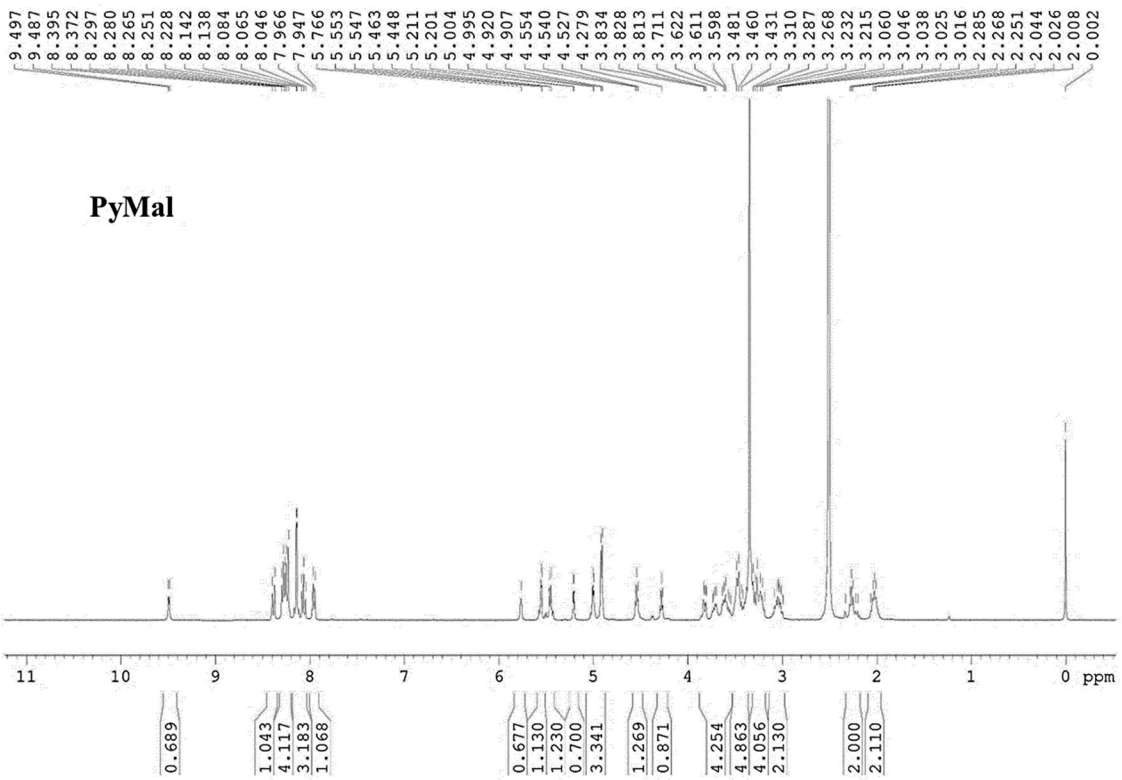


Characterization of PyMal

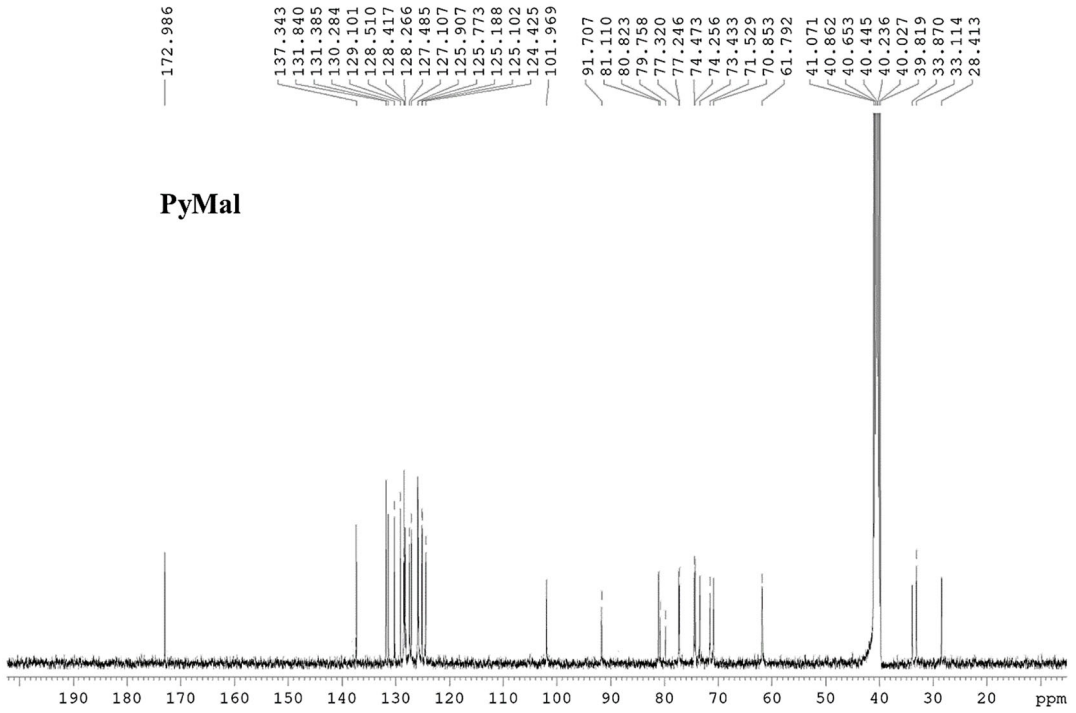
Mass spectrum



^{1}H NMR



^{13}C NMR



System Used	Medium	Detection limit	Application	Ref
Lactose-stabilized gold nanoparticles	Tris buffer solution (10 mM, pH 7.6)	54 nM	CT in electrolyte solutions that mimic the watery stool of cholera patients	*Anal. Chem.*, 2007, **79**, 1356-1361.
Antibody-conjugated gold nanoparticle	Water	10 nM	Cholera toxin detection in local lake water	*Anal. Chim. Acta*, 2015, **892**, 167-174
Tetraphenylethylene (TPE)-based glycoconjugates	50 mm PBS solution, pH 7.3	-	-	*Chem. Asian J.*, 2011, **6**, 2376-2381
Lactose based hydrogel*	Pure Water	0.4 μM	Detection in water stool sample, different water sample, Paper strips-based detection, Gel-to-sol transition	Present work

* This is the first report for the detection of CT in the hydrogel system.

Table S1. Comparison table of detection limits by different material including present work.

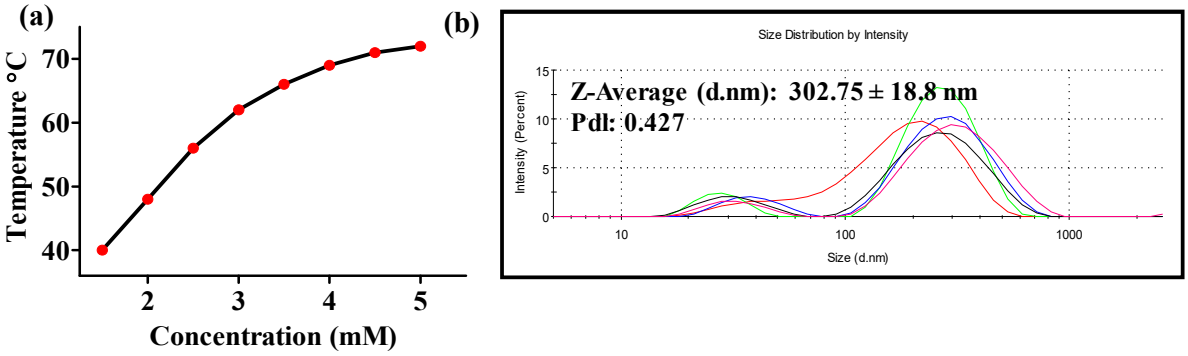


Figure S1. (a) Concentration dependent gel melting temperature (T_{gel}) of **PyLac**. (b) Measurements of an average hydrodynamic diameter (D_h) of the solution of **PyLac** (0.09 mM) in water.

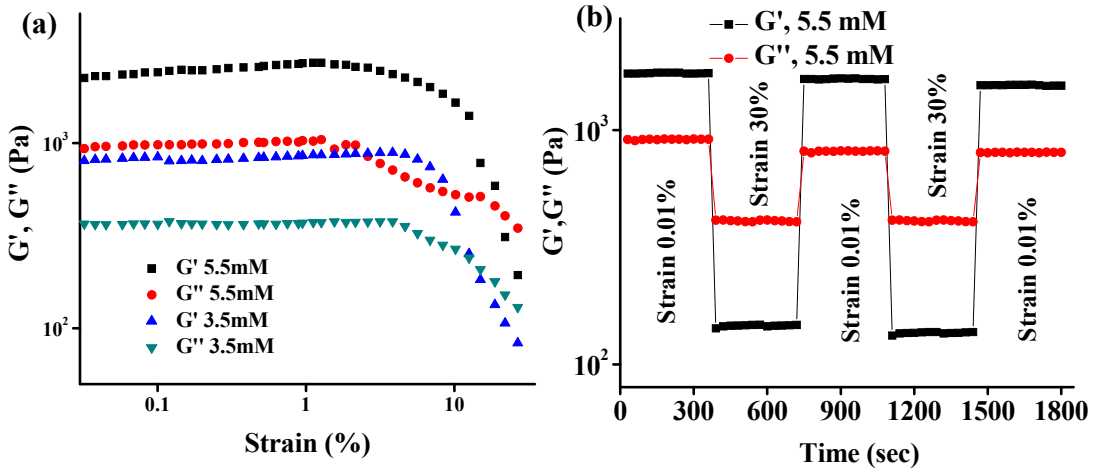


Figure S2. (a) Concentration dependent oscillatory amplitude sweep rheology data of **PyLac** (3.5 and 5.5 mM). (b) Hysteresis Loop test rheology data of **PyLac** (5.5 mM).

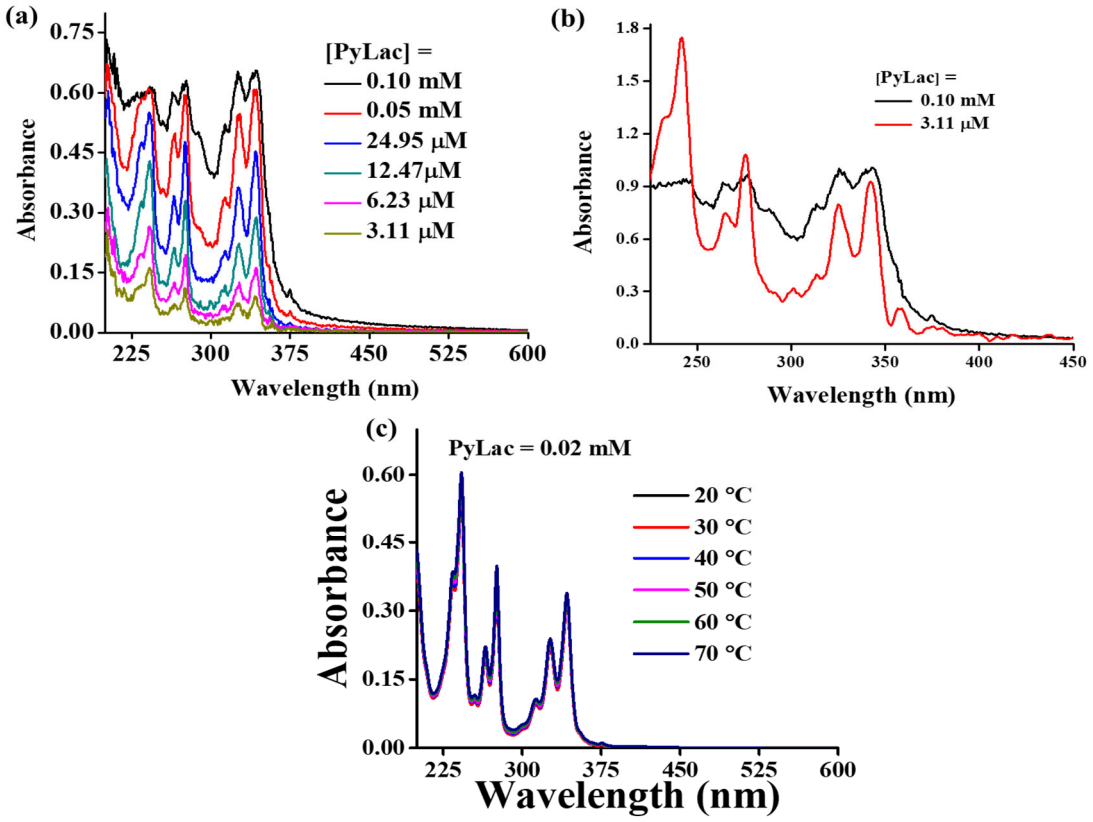


Figure S3. (a) Variable concentration absorption spectra of **PyLac**. (b) Absorbance plot of **PyLac** at low (3.11 µM) vs. high (0.10 mM) concentration in water. (c) Temperature dependent absorption spectra of **PyLac** (0. 02 mM)

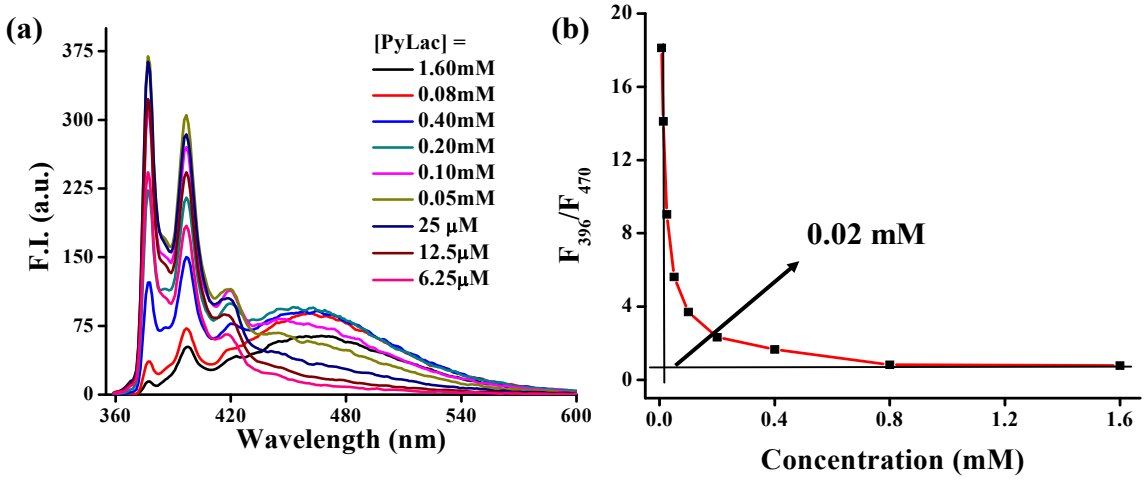


Figure S4. (a) Variable concentration emission spectra (λ_{ex} = 345 nm) of **PyLac** (6.25 µM – 1.60 mM) in water. (b) Plot of relative intensities of monomer/excimer (F_{396}/F_{470}) band versus concentration of **PyLac** (6.25 µM – 1.60 mM).

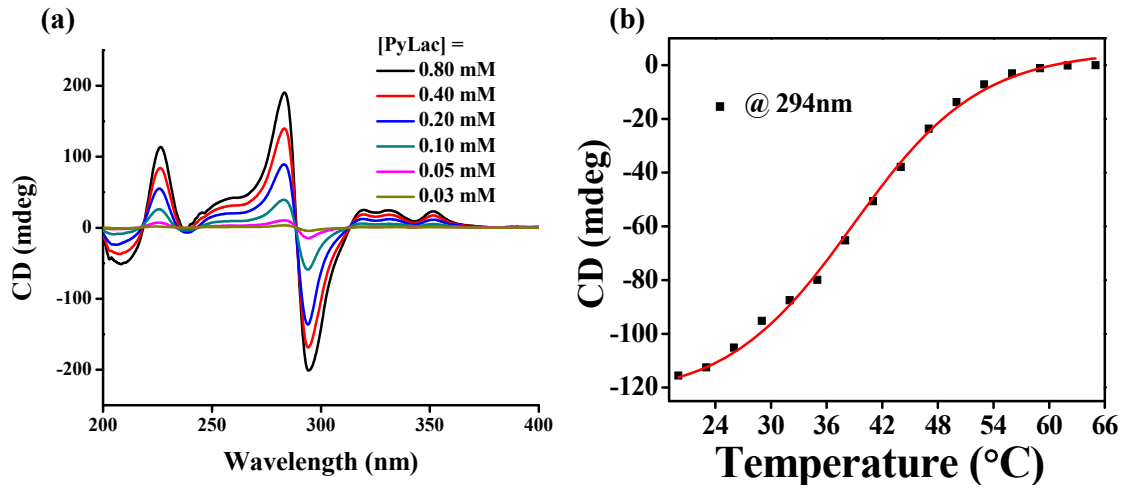


Figure S5. (a) Variable concentration CD spectra of **PyLac** (0.03 mM- 0.80 mM) in water. (b) Plot of CD signal vs. temperature of **PyLac** (0.20 mM) in water.

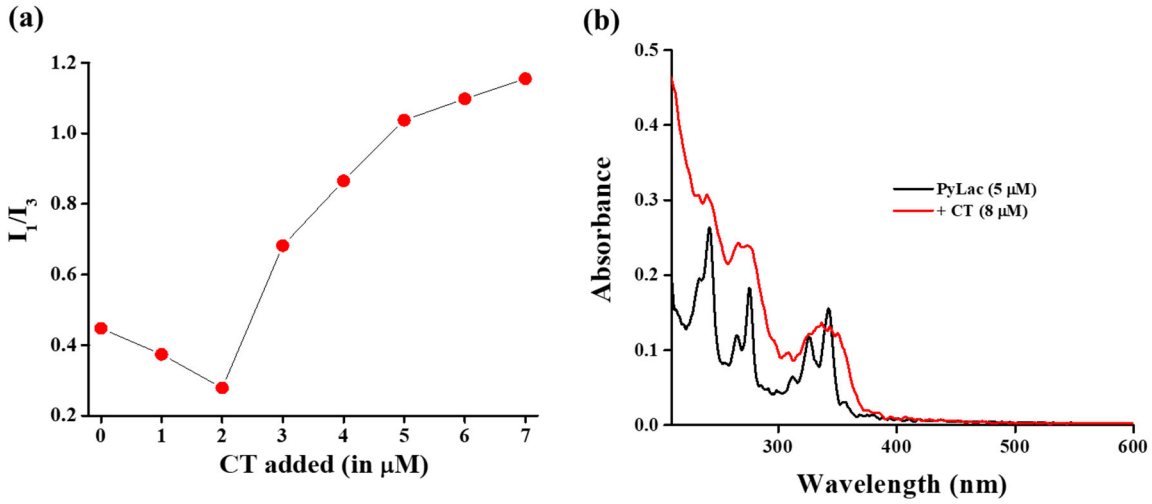


Figure S6. (a) Changes in I_1/I_3 ratio of **PyLac** (5 μM, λ_{ex} = 345 nm) on addition of increasing CT (0-7 μM) concentration. (b) Absorbance spectra of **PyLac** (5 μM) in the presence of CT (8 μM) in water.

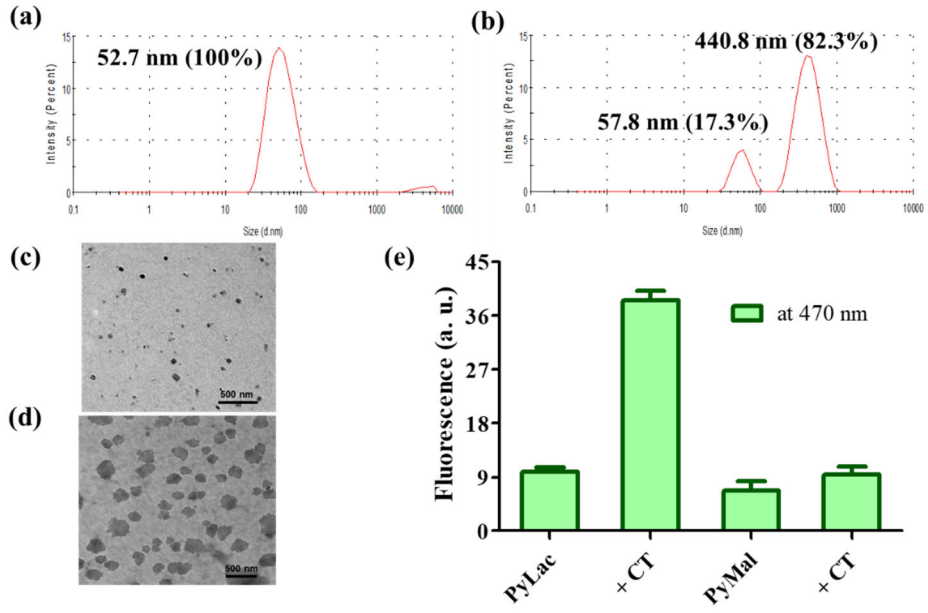


Figure S7. Hydrodynamic diameter of (a) **PyLac** (5 μM) alone, (b) **PyLac** (5 μM) in the presence of CT (8 μM) in water. TEM images of (c) **PyLac** (5 μM), (d) **PyLac** (5 μM) in the presence of CT (8 μM). (e) Interaction of **PyLac** (5 μM, λ_{ex} = 345 nm) and **PyMal** (5μM, λ_{ex} = 345 nm) with CT (8 μM) in water (number of independent experiments: 2).

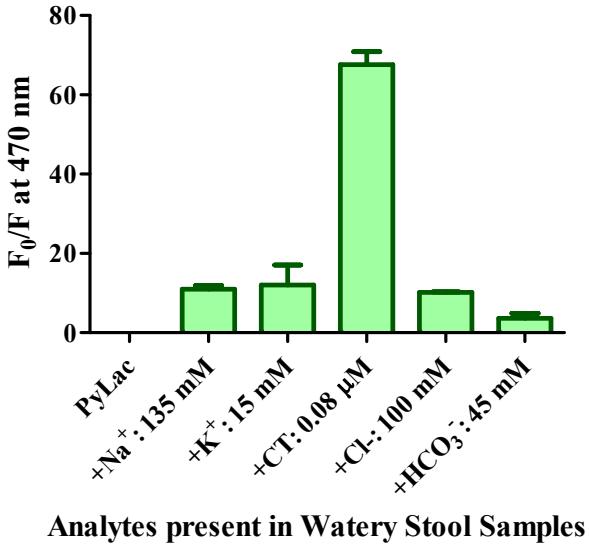


Figure S8. Interaction of **PyLac** (5 μM, λ_{ex} = 345 nm) with different analyte present in stool samples (number of independent experiments: 2).

(a)

	CT (µM) Added	F_0/F	CT (µM) Calcd.	Difference (%)
Tap water	1	1.22	0.91	9.0
	2	1.52	1.87	6.5
	3	2.17	3.28	9.3
	4	2.83	4.91	2.3
Pool water	1	1.27	1.04	4.0
	2	1.42	1.73	1.4
	3	2.11	3.13	4.3
	4	3.15	4.21	5.2
Sea water	1	1.13	0.93	7.0
	2	1.32	1.77	1.2
	3	1.81	3.19	6.3
	4	2.40	3.85	3.8

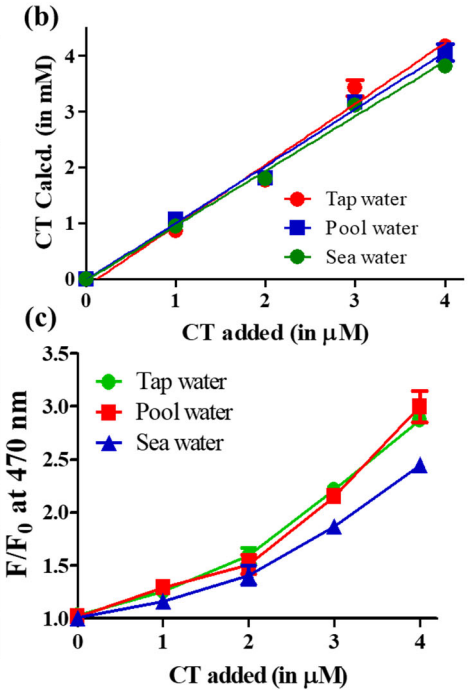
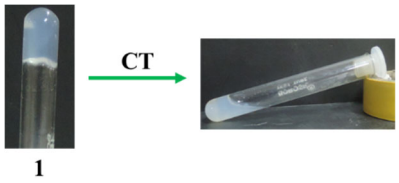


Figure S9. (a) Quantitative estimation of CT using **PyLac** (5 µM) in different water sample (number of independent experiments: 2). (b) Recovery plot of **PyLac** (5 µM, λ_{ex} = 345 nm) upon interaction of with CT in different water samples (number of independent experiments: 2). (c) Change in the emission intensity of **PyLac** (5 µM, λex = 345 nm) upon interaction of with CT in different water samples (number of independent experiments: 2).

Structure	Medium of Gelation	Fluorescence Response with	Effect on CT on gelation	Ref.
	Water	Blue to Black	Gel-to-sol	Present work
	No Gelation	No change was observed	NA	Present work
	EtOH/H_2O (98% v/v water)	NA	Gel-to-sol	*Chem. Mater.*, 1999, **11**, 3504-3511
	EtOH/H_2O (98% v/v water)	NA	No change in gelation	*Chem. Mater.*, 1999, **11**, 3504-3511



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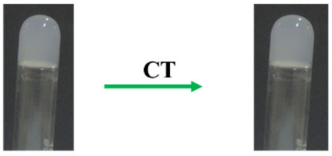

2

Table S2. Table shows the comparison of different hydrogel on interaction with CT.