# Dissipation of Self-assemblies by Fusion of Complementary Gels: An Elegant Strategy for Programmed Enzymatic Reaction

Deep Mandal, Pritam Choudhury, Deblina Sarkar and Prasanta Kumar Das*

*Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata-700032, India. E-mail: bcpkd@iacs.res.in*

## Electronic Supplementary Information (ESI)

**Materials.**

Silica gel of 100-200 mesh, dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), 4-carboxyphenylboronic acid, triethylamine ($Et_3N$), solvents and all other reagents were procured from SRL, India. $\delta$-D(+)-glucono-1,5-lactone was procured from Alfa Aesar-Johnson Matthey company. Milli-Q water was used throughout the study. Thin layer chromatography was performed on Merck precoated silica gel 60-$F_{254}$ plates. $CDCl_3$ and other deuteriated solvents for NMR and FTIR, cholesteryl chloroformate, hexan-1,6-diamine, dodecane-1,12-diamine, 8-anilino-1-naphthalenesulfonic acid (ANS), *Chromobacterium viscosum* lipase (E.C.3.1.1.3 type XII) was purchased from Millipore, India. Chloramphenicol sodium succinate (CPS) and chloramphenicol (CP) were procured from Sigma Aldrich. Substrate for *CV* lipase *p*-Nitrophenyl-*n*-octanoate (substrate) was synthesized by following the reported protocol.[1]

**Synthesis of Amphiphile-1 and 2**.

Cholesterol-based amphiphiles (**1** and **2**) were synthesized by following methods (Scheme S1, ESI). In case of amphiphile-**1**, one end of hexan-1,6-diamine was coupled with cholesteryl chloroformate in dry dichloromethane (DCM) using equivalent amount of dry triethylamine. For amphiphile-**2**, the spacer used was dodecane-1,12-diamine and one end was coupled with same cholesteryl chloroformate. The cholesteryl chloroformate was added drop wise to the DCM

solution of diamine under cold conditions (0–5 $^{o}$C) over a period of 4–5 h. After complete addition, the solution was stirred for further 8 h and then worked up with water and brine to remove excess base and diamine. The DCM part was vacuum evaporated and the product obtained was purified by column chromatography (100-200 mesh silica gel) and methanol (7% v/v) -chloroform was used as the eluent. For synthesis of **1**, NHS linked 4-carboxyphenylboronic acid was prepared by using 4-carboxyphenylboronic acid, DCC (1.5 equivalent) and NHS (1.1 equivalent) in dry DMF (8 mL) and stirred overnight under $N_2$ atmosphere. To this activated 4-carboxyphenylboronic acid mixture, mono-cholesterol coupled dodecane-1,12-diamine and dry pyridine were added. The reaction mixture was stirred overnight and the DMF was distilled out under vacuum. The residue mixture was then column purified using 100-200 mesh silica gel and methanol (3% v/v)-chloroform as the eluent to obtain pure **1**. On the other hand mono cholesteryl coupled hexan-1,6-diamine was then refluxed with $\delta$-D(+)-glucono-1,5-lactone in dry methanol for 16 h. The product obtained was column purified using methanol (10% v/v) -chloroform as the eluent. The product obtained in two cases were characterized by HRMS and $^{1}$H-NMR spectra**.**

**$^{1}$H-NMR of amphiphile-1 (500 MHz, methanol-d4, 25 °C).**

δ /ppm = 0.82-1.66 (m, 39H, cholesteryl), 1.22-1.46 (m, 20H, -(C$\underline{H}_2$-C$\underline{H}_2$)$_5$-), 1.78-1.82 and 1.88-1.96 (m, 4H, allylic cholesteryl protons), 1.87-2.05 (broad, 2H, -B(O$\underline{H}$)$_2$), 2.60-2.65 (m, 2H, Chol-CONH-C$\underline{H}_2$-CH$_2$-), 3.15-3.18 (m, 2H, PBA-CONH-C$\underline{H}_2$-CH$_2$-), 4.30-4.32 (m, 1H, -C$\underline{H}$-O-(CO)- of cholesteryl proton), 5.32-5.34 (t, 1H, vinylic proton of cholesteryl group); 7.01-7.10 (m, 4H, phenyl ring of PBA); (Elemental analysis calculated (%) for $C_{47}H_{77}N_2O_5$: C, 74.19; H, 10.20; N, 3.68; found: C, 74.18; H, 10.22; N, 3.66. MS (ESI): m/z calculated for $C_{47}H_{77}N_2O_5$: 760.59; found: 783.68 [$M^+ + Na^+$]

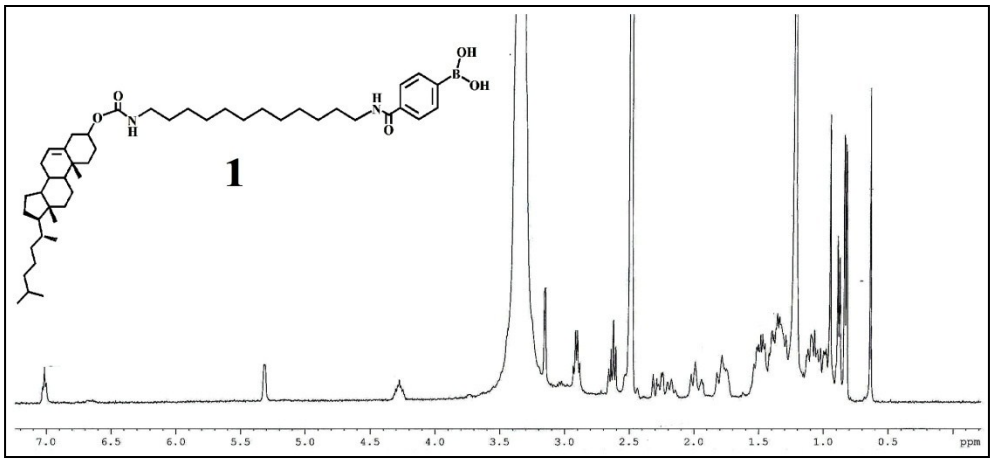



**Fig. S1** [1]H-NMR spectra of amphiphile-**1** in DMSO-d6.

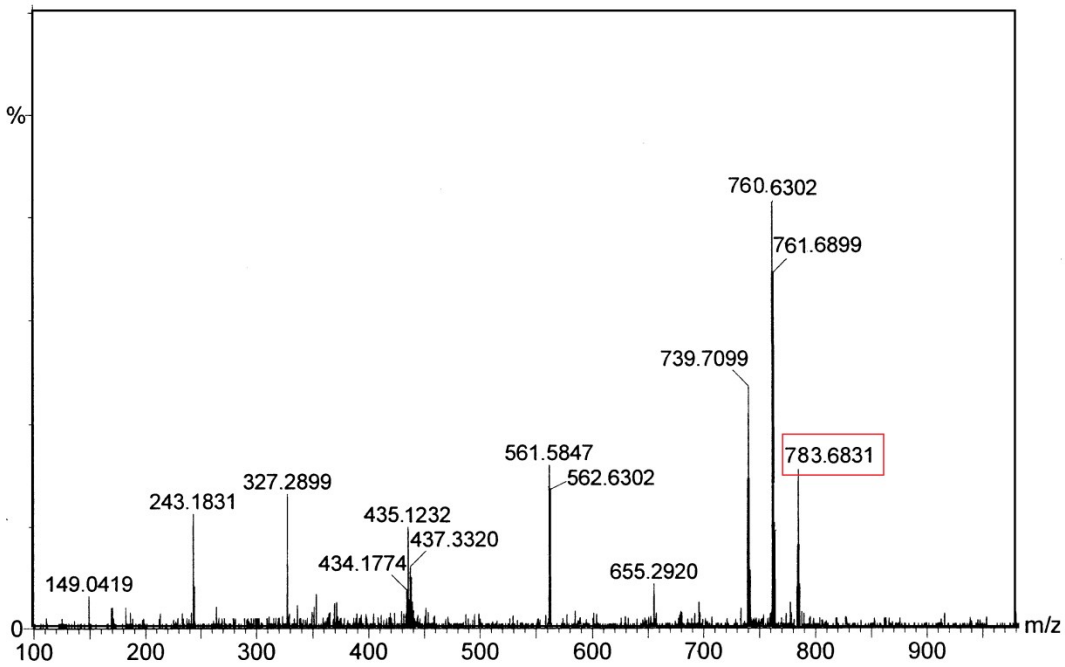



**Fig. S2** HRMS spectra of amphiphile-**1**.

**[1]H-NMR of amphiphile-2 (500 MHz, methanol-d4, 25 °C).**

δ /ppm = 0.81-1.69 (m, 39H, cholesteryl), 1.28-1.45(m, 4H, -C$\underline{H}_2$-C$\underline{H}_2$-), 1.41-1.60 (m, 4H, -NH-$CH_2$-C$\underline{H}_2$-), 1.80-1.96 and 2.26-2.34 (m, 4H, allylic cholesteryl protons), 2.03-2.10 (m, 5H, glucose-O$\underline{H}$), 3.02-3.12 (m, 2H, Chol-CONH-C$\underline{H}_2$-), 3.22-3.29 (m, 2H,-C$\underline{H}_2$-CONH-glucose),

3.52-3.65 (m, 3H, C-3, C-4 and C-5 protons of glucose), 3.69-3.74 (m, 2H, C-6 proton of glucose). 4.08-4.11 (m, 1H, -CH-O-(CO)- of cholesteryl proton), 4.32-4.40 (d, 1H, C-2 proton of glucose), 5.38-5.41 (t, 1H, vinylic proton of cholesteryl group); (Elemental analysis calculated (%) for $C_{40}H_{70}N_2O_8$: C, 67.95; H, 9.98; N, 3.96; found: C, 67.91; H, 9.96; N, 3.91. MS (ESI): m/z calculated for $C_{40}H_{70}N_2O_8$: 706.51; found: 729.50 [$M^+ + Na^+$]

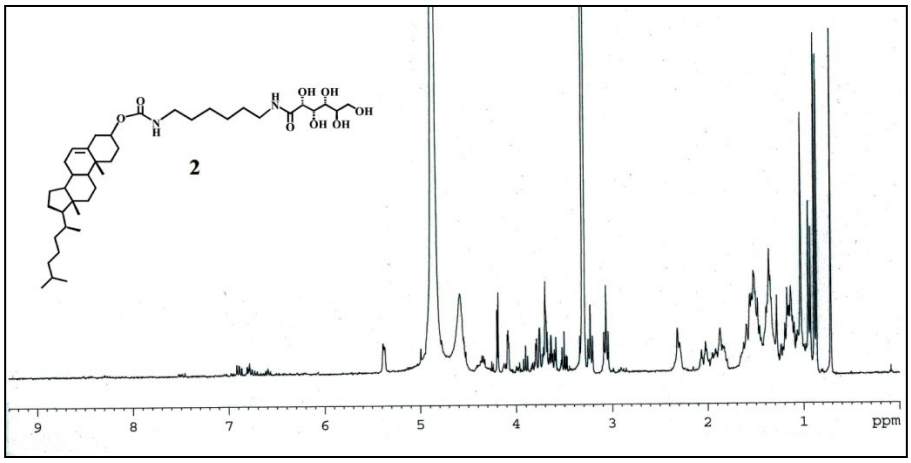



**Fig. S3** $^1$H-NMR spectra of amphiphile-**2** in methanol-d4.

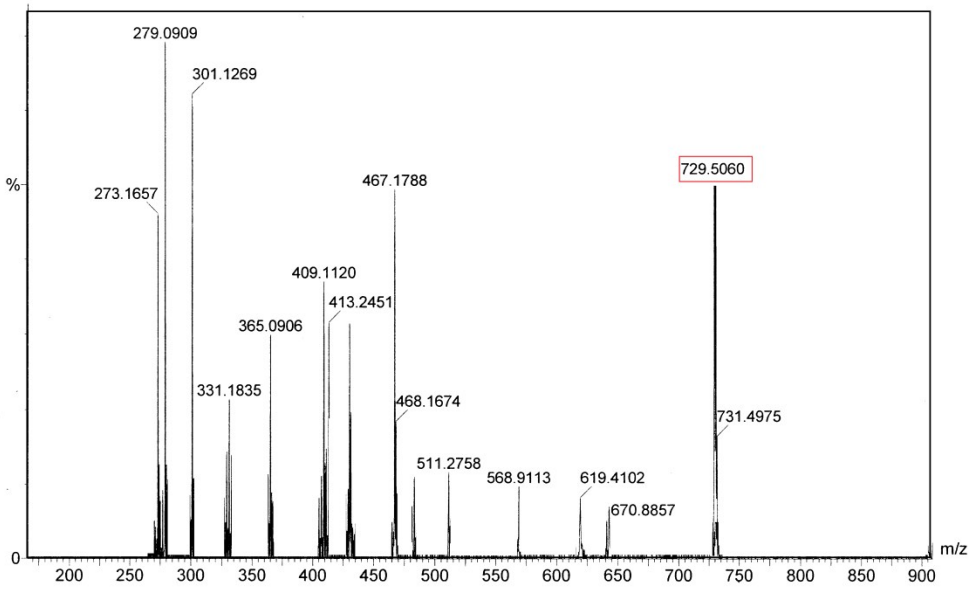



**Fig. S4** HRMS spectra of amphiphile-**2**.

**Preparation of gel**.

Requisite amount of amphiphile was taken in a screw capped vial having an internal diameter (i.d.) of 10 mm and slowly heated to dissolve in 1 mL of desired solvent. The solution was then allowed to cool slowly (undisturbed) to room temperature. The gelation was checked by "stable to inversion" of the aggregated material in the glass vial.

**Gel dissolution upon mixing of gels**.

The prepared gels **1** and **2** (at its corresponding MGCs in DMF-water (2:1v/v)) were placed one over another, incubated for hours and gradual change in the physical state was macroscopically noticed. In case of gel degradation study with dye entrapped gels, Oil Red O dye was entrapped in gel-**1** and Comassie Brilliant blue R 250 was entrapped in gel-**2**. Gel-**1** was placed over gel-**2** and degradation was observed with time.

**FTIR Study.**

FTIR measurements were performed with amphiphile-**1** and **2** in non-self-assembled state using KBr pellets and in the self-assembled state (gelators taken in DMF-$D_2O$, 2:1 v/v) using 1 mm $CaF_2$ cell. All the experiments were carried out in a Perkin–Elmer Spectrum 100 FTIR spectrometer.

**Fluorescence spectroscopy.**

The emission spectra of free ANS in DMF and ANS-doped gel-**1** and **2** were recorded with a Varian Cary Eclipse luminescence spectrometer. The probe molecules were added to the solutions of amphiphile **1** and **2** at various concentrations at room temperature. ANS stock solution was prepared in MeOH (stock solution) and the required amount of ANS solution was added to the gelators so that the final concentration of ANS solution was $1\times10^{-6}$ M. The ANS solution was excited at 360 nm ($\lambda_{ex}$). The excitation and emission slit widths were 5 nm.

**Field-emission scanning electron microscopy (FESEM).**

FESEM images were obtained on a JEOL-6700F microscope. 6 μL solutions of **1** and **2** taken in DMF-water mixture (1.1 mg/mL and 0.82 mg/mL, respectively) was placed separately on a piece of cover slip and dried overnight. It was then kept few hours under vacuum before imaging. The morphologies of dissolute gels upon fusion of the complementary gels were also observed under FE-SEM. The gels (prepared at their MGCs) were mixed and incubated for hours. With regular time interval the resulting gel mixture was collected from the system, diluted ten times with DMF-water (2:1v/v), dried over cover slip and FESEM images were captured.

**Temperature dependent $^1$H-NMR Measurement.**

Temperature dependent $^1$H-NMR spectra of **1** and **2** was recorded in AVANCE 300 MHz (Bruker) spectrometer and temperature was varied from 25 to 85 °C (in benzene-d6).

**Rheology and complex viscosity (η*) measurement.**

The rheological experiments were conducted in cone and plate geometry (diameter = 40 mm) on the rheometer plate by using an Anton Paar, MCR 302. Gel-**1**, **2** and their mixture (gels were prepared at its corresponding MGCs in DMF-water (2:1 v/v) and incubated for hours) were placed on the rheometer plate so that there was no air gap with the cone. A frequency sweep experiment was done as a function of angular frequency (0.1–500 rad $s^{-1}$) at a fixed strain of 0.01% at 25 °C and the storage modulus ($G'$) and the loss modulus ($G''$) were plotted against the angular frequency ($\omega$). To measure complex viscosity, a frequency sweep experiment was done as a function of angular frequency (0.001-10 $rads^{-1}$) at a fixed strain of 1% at 25 °C and the complex viscosity was plotted against the angular frequency (ω).

**X-ray Diffraction (XRD).**

XRD spectra of the dried gel-**1**, **2** and gel mixture were obtained on a Bruker D8 Advance diffractometer and the source used was CuKα radiation (λ = 0.15406 nm) with a voltage 40 kV and current 30 mA.

**UV-vis Study.**

To understand the molecular arrangement present in the complementary gels before and after mixing, UV-vis spectroscopic study was carried out using 8-anilino-1-naphthalenesulfonic acid (ANS) as the probe. The UV-vis spectra of ANS doped **1**, **2** and its mixture were recorded on a PerkinElmer Lambda 25 spectrophotometer at varying incubation period. We have taken the UV-vis spectra of ANS in presence of **1** and **2** (1.01 mg/mL), [ANS] =$1\times10^{-5}$ M) in pure DMF (non-self-assembled condition) and DMF-water (self-aggregating solvent).

**Monitoring *CV* lipase catalyzed hydrolysis within complementary gel mixture.**

The lipase catalyzed hydrolysis of *p*-nitrophenyl-*n*-octanoate was examined in Perkin Elmer Lambda 25 spectrophotometer. The aqueous lipase solution (20 μg/mL) was included in gel-**1** (500 μL) without disturbing the internal stability of the gel. Similarly, *p*-nitrophenyl-*n*-octanoate (30 mM) was incorporated within gel-**2** (500 μL). The enzyme/substrate included hybrid gels were placed one over another. The liberation of yellow coloured *p*-nitrophenol was evinced through naked eye upon gradual dissolution of the mixed complementary gels. For spectroscopic analysis of the enzymatic reaction, *CV* lipase was included within the self-assembled gelator-**1** solution (2.02 mg/mL, five time lower than the MGC) and corresponding substrate was included within self-assembled gelator-**2** solution (1.64 mg/mL, five time lower than the MGC). To avoid turbidity related error we have taken the gelator concentration below their MGCs. Now these solutions were mixed within the cuvette and the absorbance of liberated *p*-nitrophenol owing to the enzymatic reaction was measured at 316 nm immediately with regular time interval. The

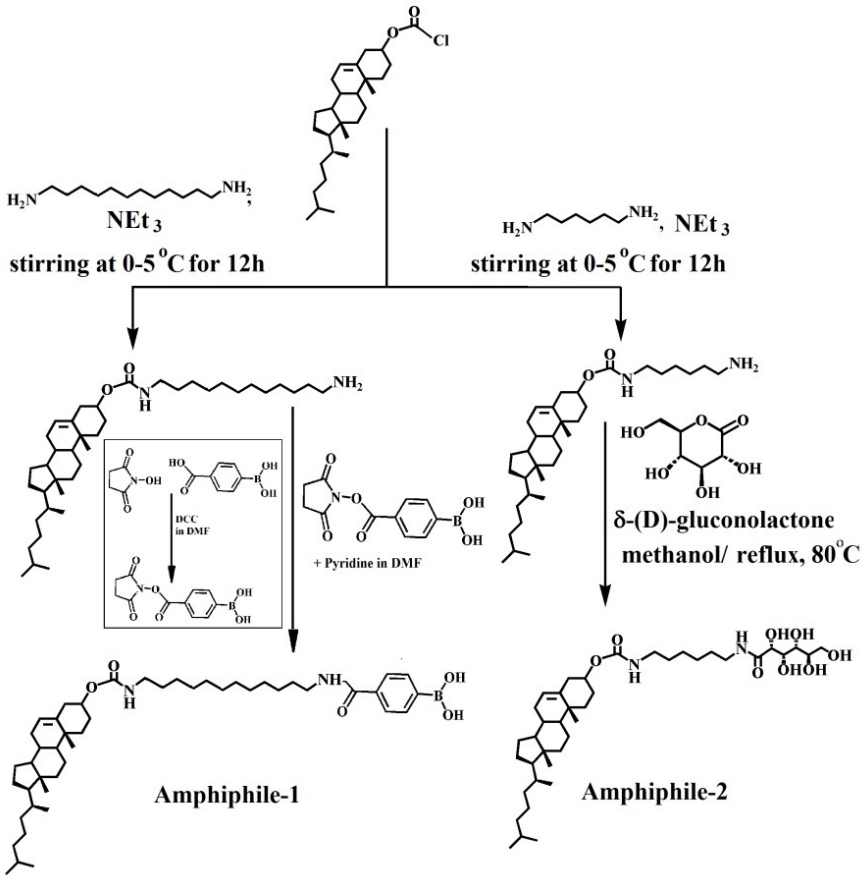
overall concentrations of lipase and *p*-nitrophenyl-*n*-octanoate within the cuvette (after mixing of the two solution) were maintained as 1.02 μg/mL and 3 mM, respectively.

**Monitoring *CV* lipase catalyzed activation of chloramphenicolsuccinate sodium salt (CPS) within complementary gel mixture.**

The activation of pro-drug chloramphenicolsuccinate sodium salt (CPS) was monitored through lipase catalysed hydrolysis of CPS to chloramphenicol (CP). *CV* lipase was included within the self-assembled gelator-**1** solution (2.02 mg/mL) and corresponding CPS was included within self-assembled gelator-**2** solution (1.64 mg/mL). These solutions were mixed within the cuvette and the absorbance of produced CP owing to the enzymatic reaction was measured at 278 nm immediately with regular time interval. The overall concentrations of lipase and CPS within the cuvette (after mixing of the two solutions) were maintained as 1.02 μg/mL and 6 mM respectively. The $\lambda_{max}$ value of CP produced during the enzymatic reaction (within complementary gel mixture) was compared with the native CP (as purchased) which confirmed the formation of CP due to enzymatic reaction within dissolute complementary gel mixture.

**References.**

1. A. Shome, S. Roy, P. K. Das, *Langmuir*, 2007, **23**, 4130.




**Scheme. S1** Synthetic procedure of amphiphile-**1** and **2**.

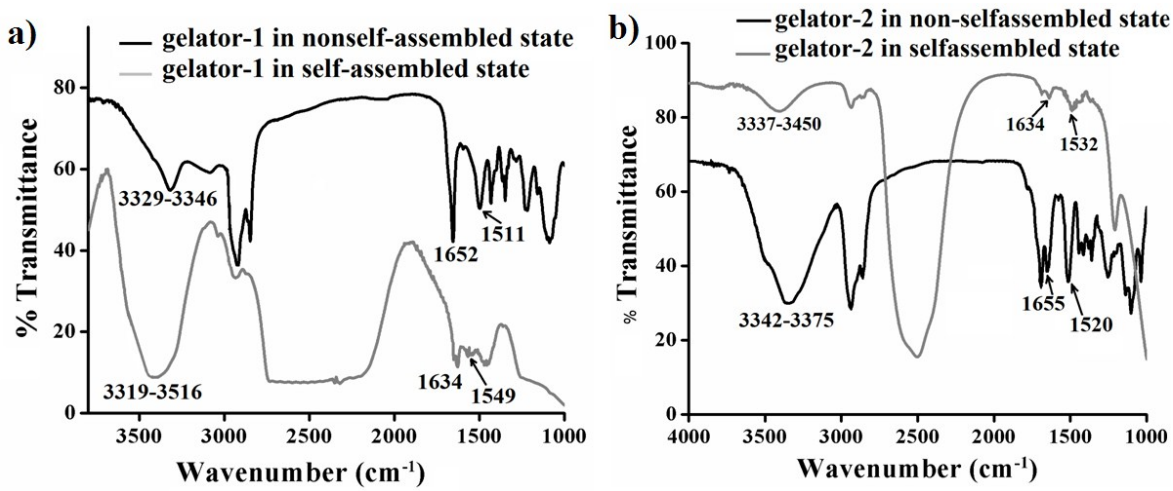



**Fig. S5** FTIR spectra of (a) amphiphile-**1** and (b) **2** in its non-self-assembled state (solid samples fused in KBr pellet) and self-assembled state (gelators taken in DMF-$D_2O$ solvent mixture 2:1 v/v).

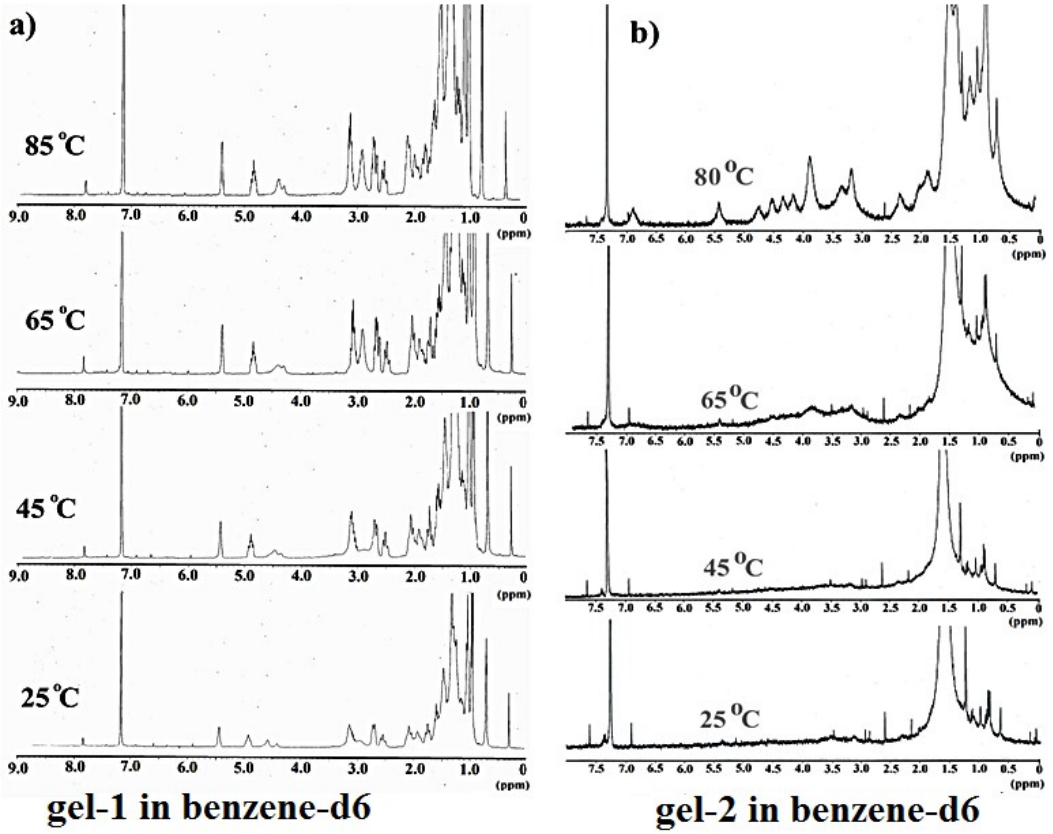



**Fig. S6** Temperature dependent $^{1}$H-NMR of amphiphile **1** and **2**.

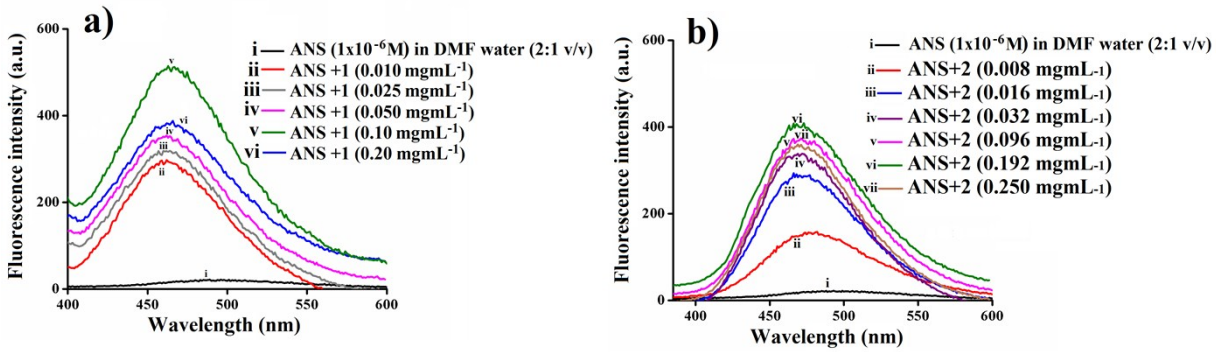



**Fig. S7** Fluorescence spectra of ANS within (a) **1** and (b) **2** with varying concentrations of gelators doped with ANS dye ([ANS] = $1\times10^{-6}$ M).

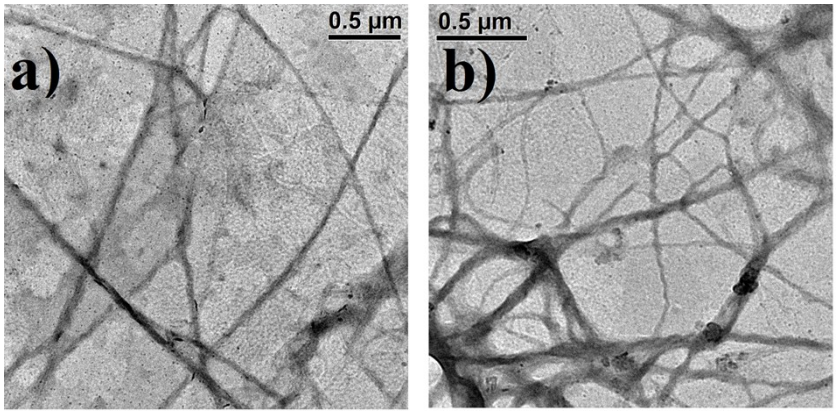



**Fig. S8** TEM images of the native (a) gel-**1** and (b) gel-**2** in DMF-water (2:1 v/v).

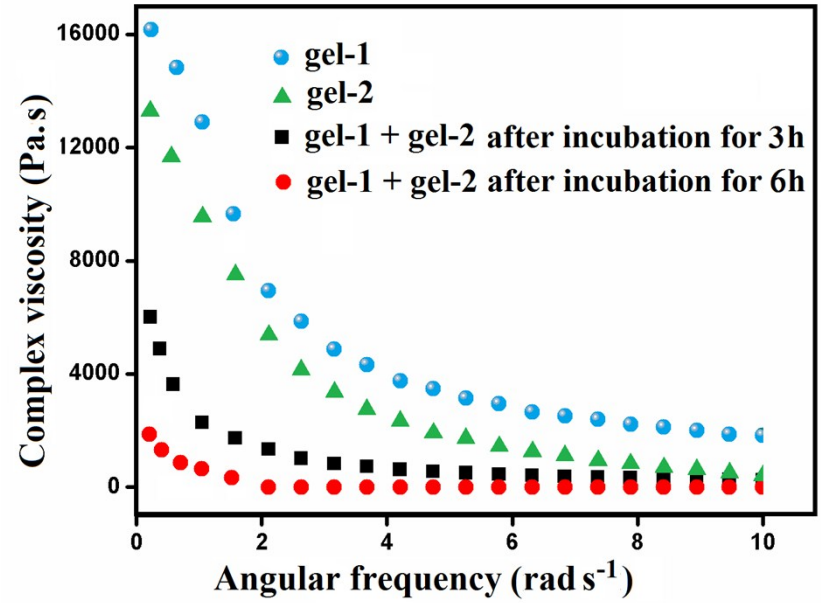



**Fig. S9** Complex viscosity (η*) vs. angular frequency plot of the gel-**1**, **2** and its mixed state at different incubation period.

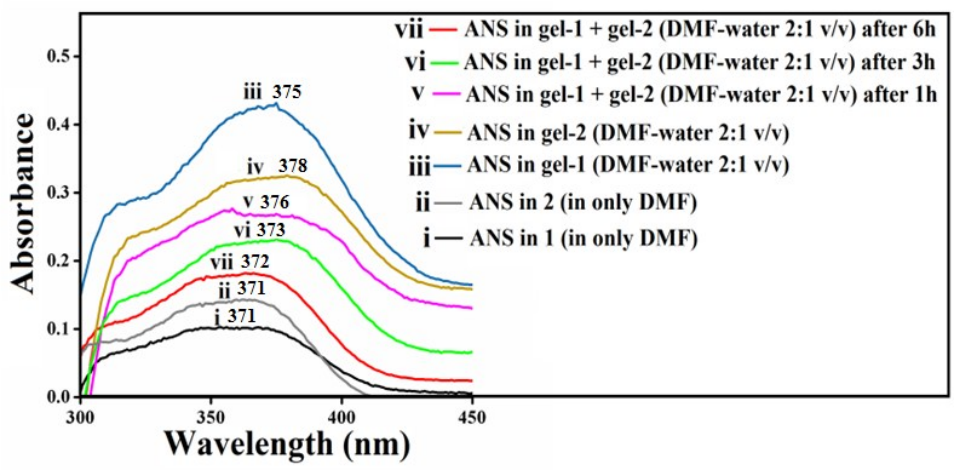



**Fig. S10** UV spectra of ANS ($1\times10^{-5}$ M) doped in different solutions of gelator-**1**, **2** and its mixed state.

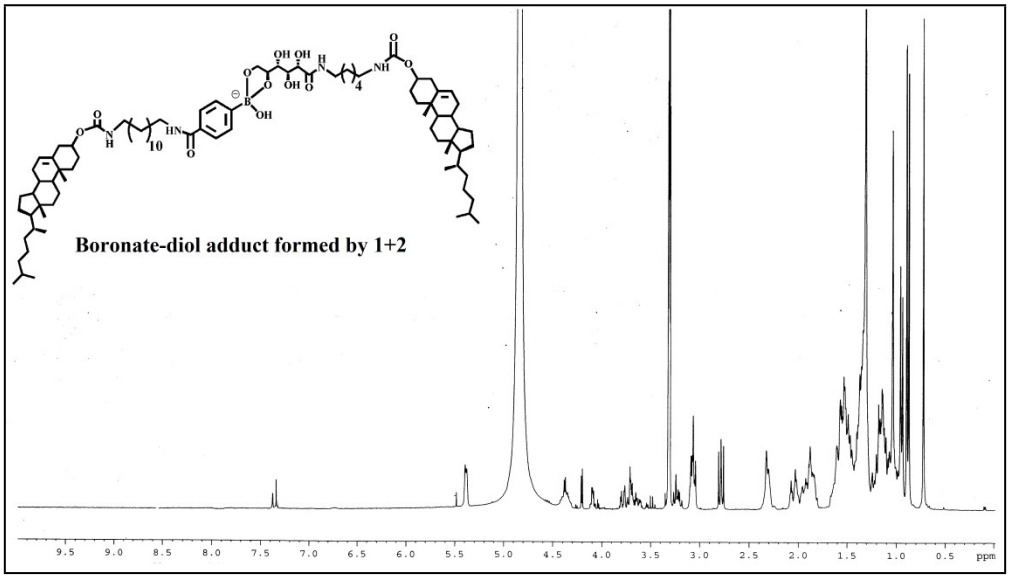



**Fig. S11** $^{1}$H-NMR spectra of boronate diol adduct of (**1**+**2**) in methanol-d4.

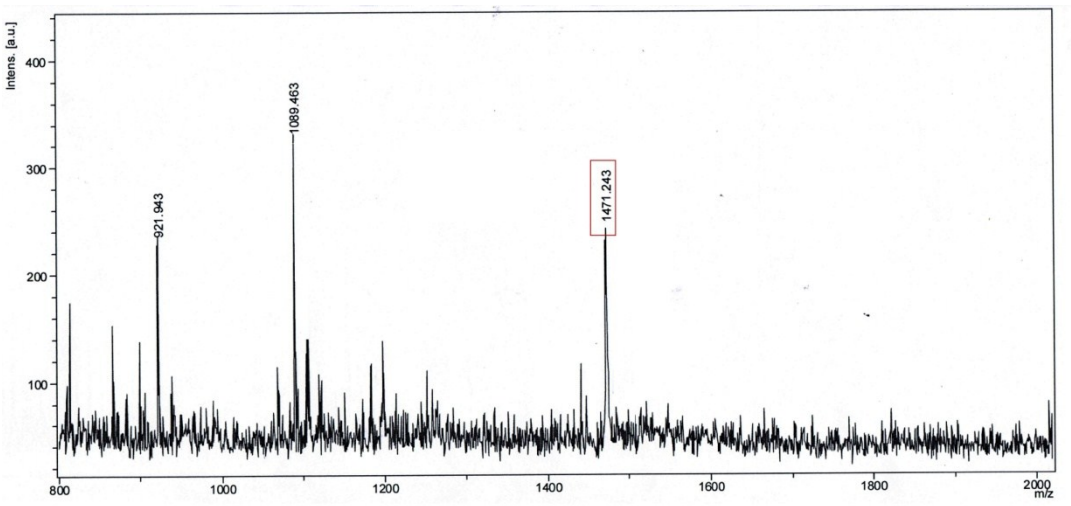



**Fig. S12** MALDI of boronate-diol adduct of (**1**+**2**) in methanol-d4. Calculated Mass of **1**+**2** = 1448.09, Found Mass: 1471.68 [$M^+ + Na^+$].

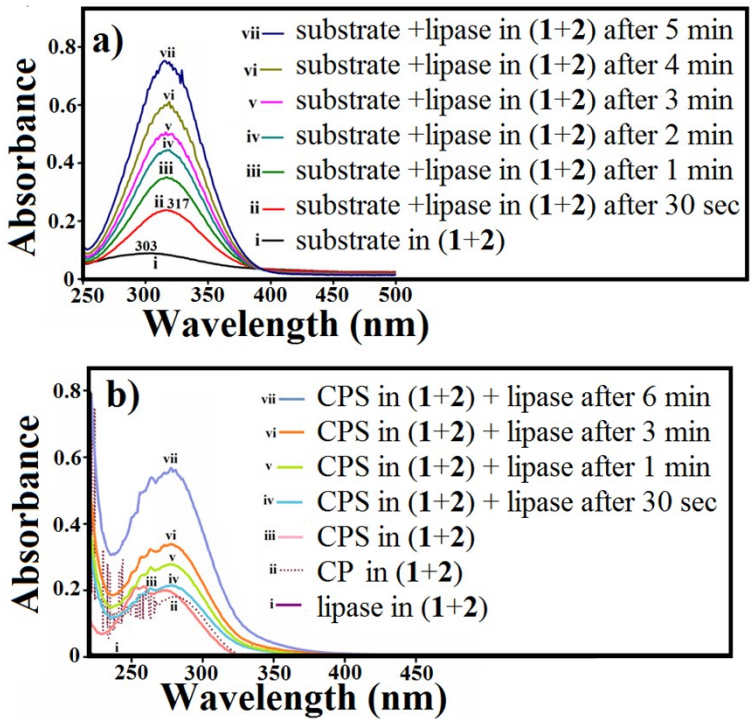



**Fig. S13** a) UV-vis spectra of liberated *p*-nitrophenol upon lipase catalyzed hydrolysis of the substrate (*p*-nitrophenyl-*n*-octanoate) within dissolute gels; b) UV-vis spectra of liberated chloramphenicol (CP) upon enzymatic hydrolysis of the pro-drug chloramphenicol succinate Na-salt (CPS) within dissolute gels.