Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Self-assembly of naturally occurring stigmasterol in liquids yielding fibrillar network and gel

Braja Gopal Bag* and Abir Chandan Barai

Department of Chemistry and Chemical Technology, Vidyasagar University,

Midnapore, 721102, West Bengal, India

Email: brajagb@gmail.com

Table of contant **Pages**

1. Energy minimized structure of stigmasterol

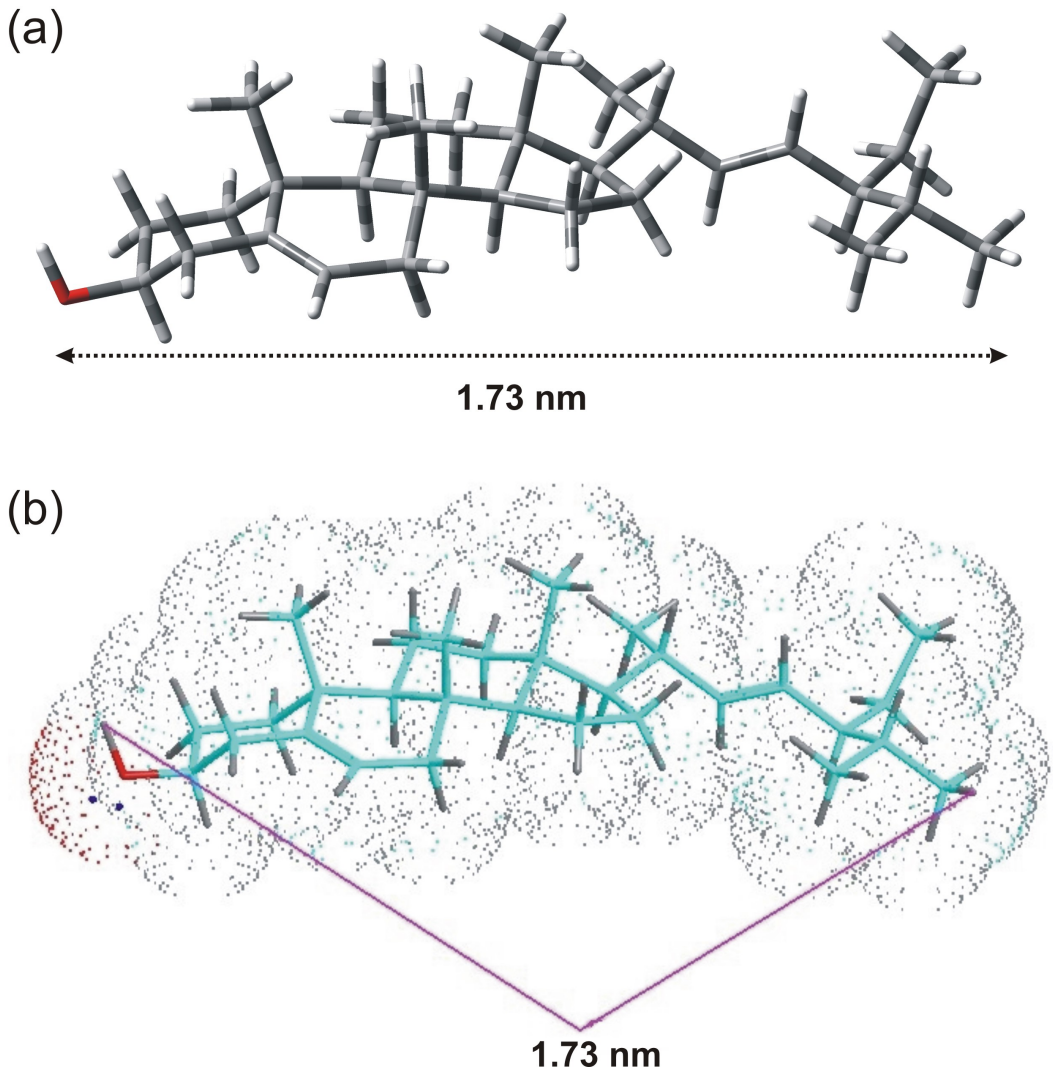


Figure S1: Energy minimized structure of stigmasterol **1** obtained by (a) DFT calculation using Gaussian 09 software, the molecular length is 1.73 nm and (b) MMX force field as implemented in PC MODEL version 9.2 (Serena Software), the molecular length is 1.73 nm.

2. Calculation the thermodynamic parameter

The thermoreversible melting of a gel can be expressed as:

$$\text{Gel} \leftrightarrow \text{liquid}$$

The equilibrium constant can be expressed as:

$$K = [\text{Gelator}]/[\text{Gel}]$$

Assuming unit activity of the gel, the equilibrium constant can be expressed as:

$$K = [\text{Gelator}]$$

The Gibbs free energy change during gel melting can be expressed as:

$\Delta G^0 = -RT \ln K = \Delta H^0 - T\Delta S^0$,

Hence, $\ln K = -\Delta H^0/R.\ (1/T) + \Delta S^0/R$

Thermodynamic parameters (ΔH^0, ΔS^0) and free energy (ΔG^0) at 298 ^{0}K for different Stigmasterol gels

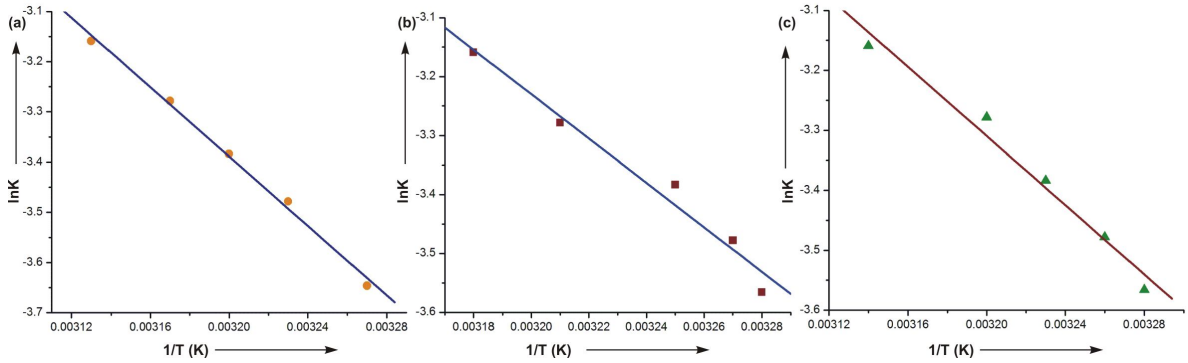


Fig. S2 : ln K vs 1/T (K) plot of stigmasterol in (a) DMSO; (b) n-heptane and (c) n-hexane

3. Scanning electron micrographs

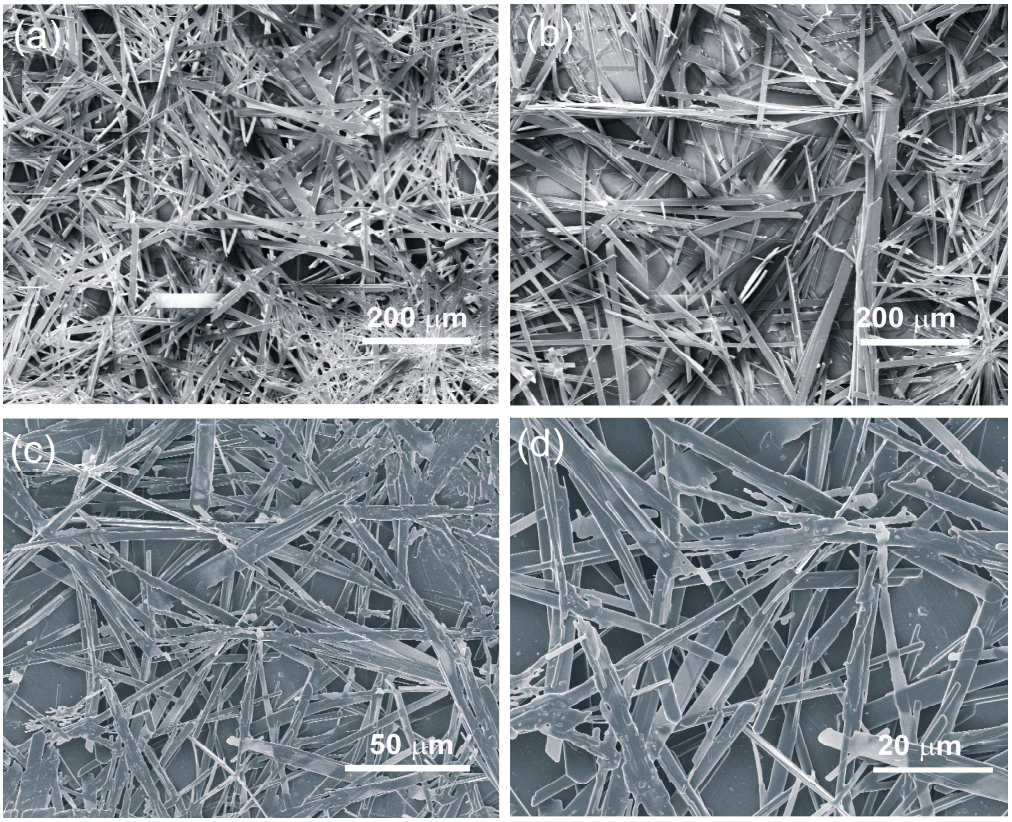


Figure S3: Scanning electron micrographs of the dried self-assemblies of stigmasterol prepared from dilute solution in (a–b) nitrobenzene (1.07 % w/v), (c-d) in n-hexane (1.10% w/v).

4. FTIR spectrum of stigmasterol

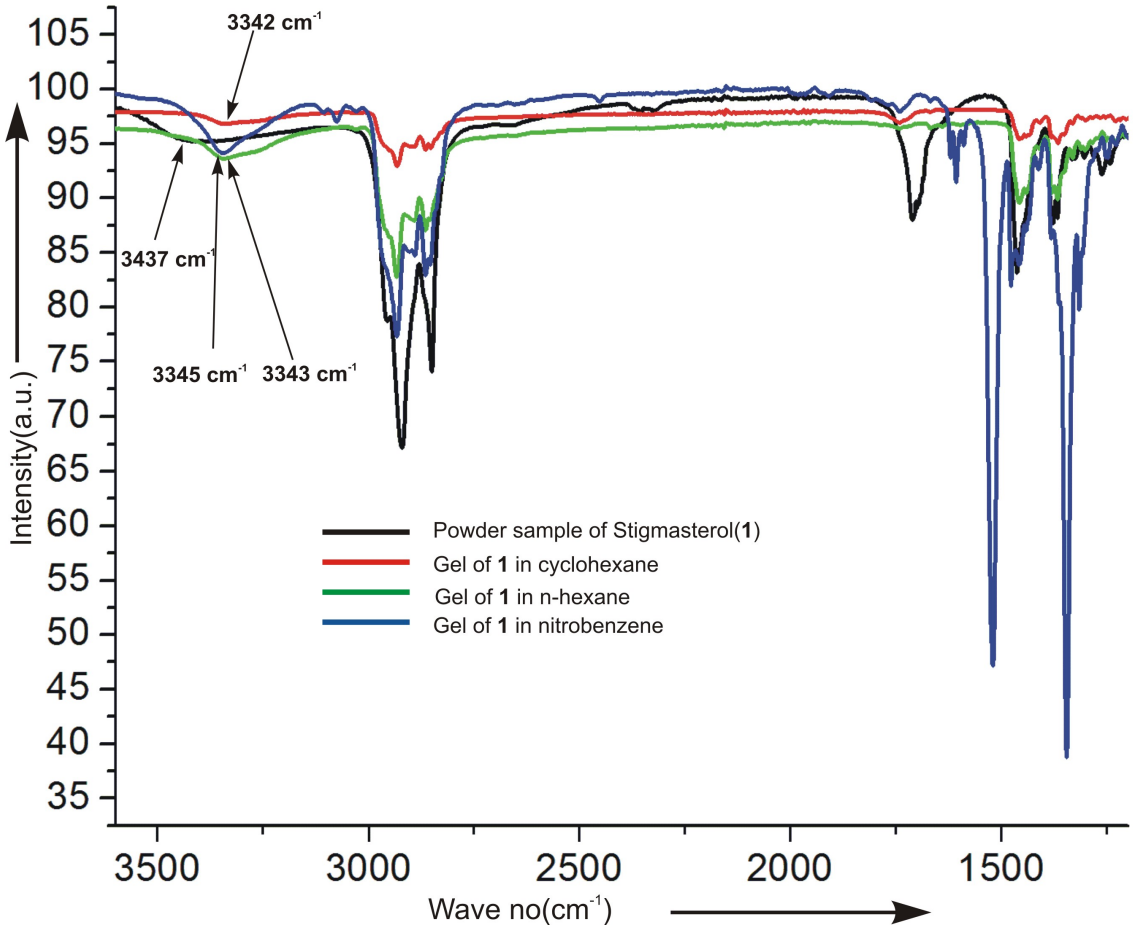


Figure S4: FTIR spectra of stigmasterol (powder) and its gels in cyclohexane, n-hexane and nitrobemzene

5. X-ray powder diffraction peaks generated from the X-ray crystal structure of stigmasterol using Mercury 4.2.0 program

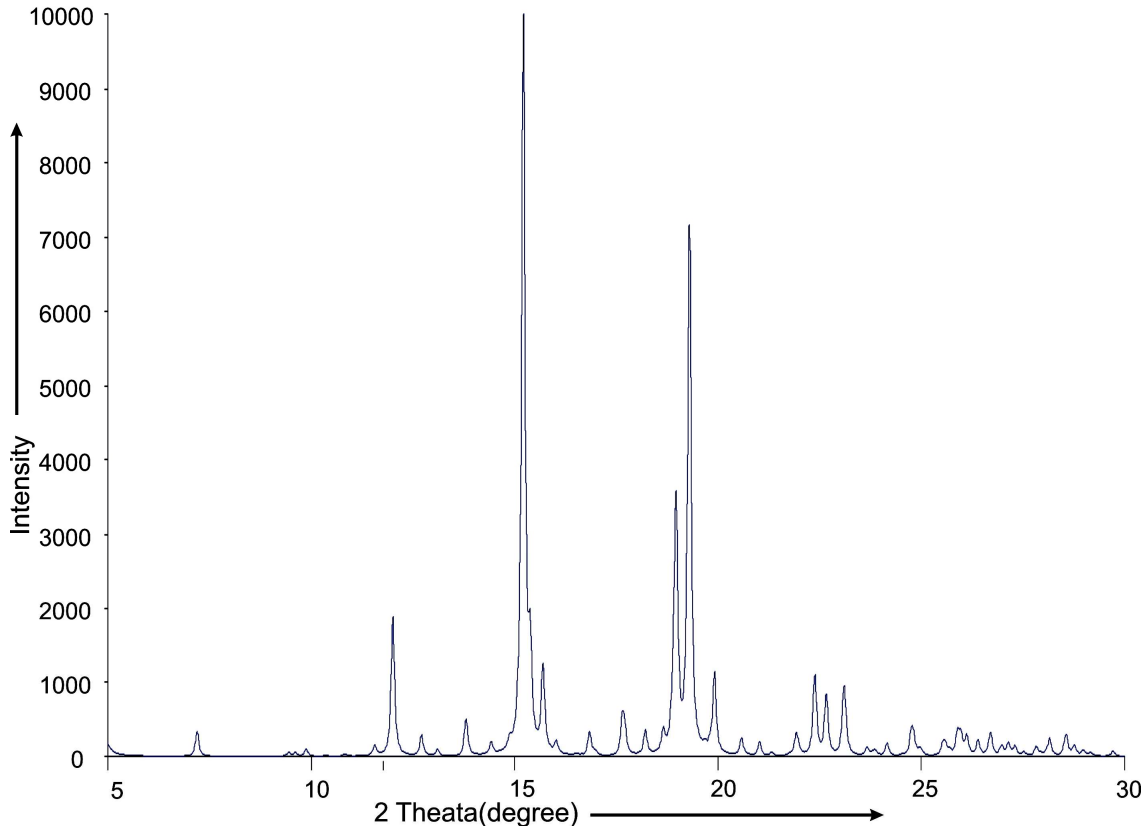


Fig. S5: The computer generated X-ray powder diffraction peaks obtained from the X-ray crystal structure. (Mercury 4.2.0 (Build 257471); http:// www.ccdc.cam.ac.uk/ mercury)

6. Various modes of assembly of stigmasterol observed in its crystal structure

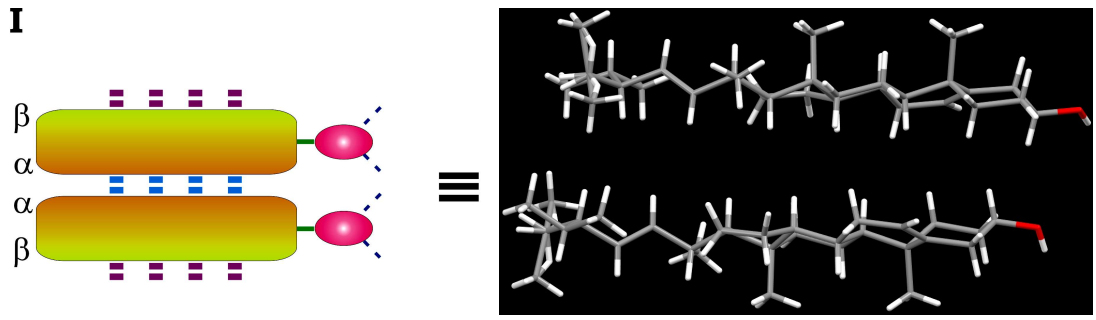


Fig. S6: Schematic representation of two interacting stigmasterol molecules within van der Waals contact having steroid α-face facing each other ($0.5H_2O$ present as solvent of crystallization is not shown for clarity)

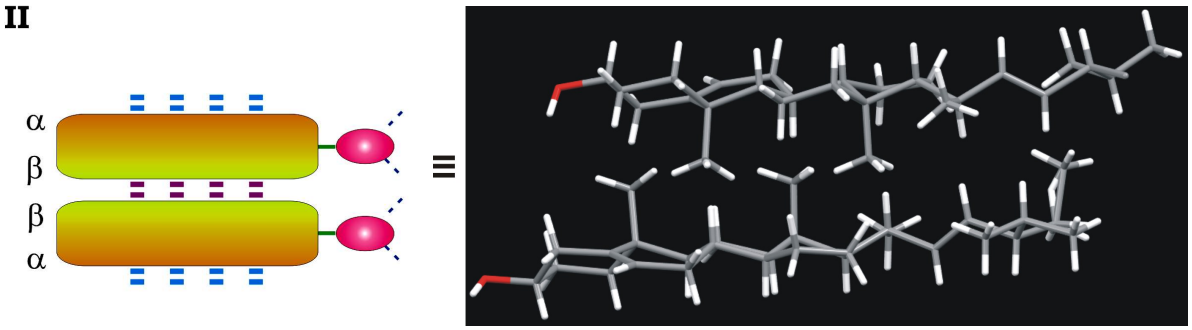
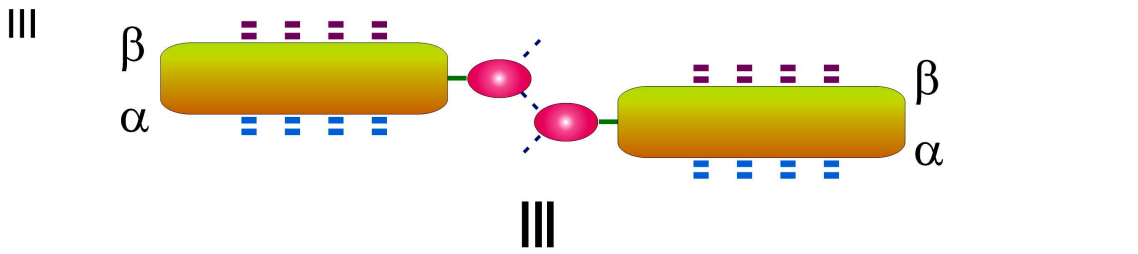


Fig. S7: Schematic representation of two interacting stigmasterol molecules within van der Waals contact having steroid β-face facing each other ($0.5H_2O$ present as solvent of crystallization is not shown for clarity)



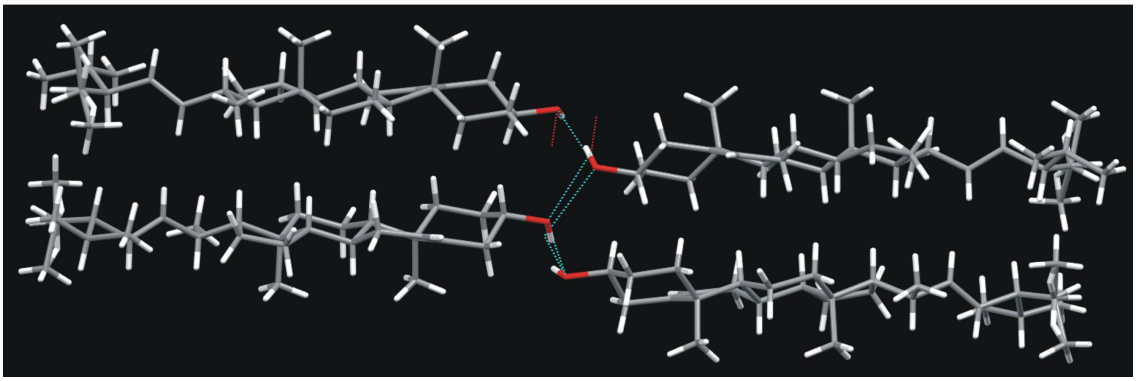


Fig. S8: Schematic representation of two interacting stigmasterol molecules within H-bonding ($0.5H_2O$ present as solvent of crystallization is not shown for clarity)

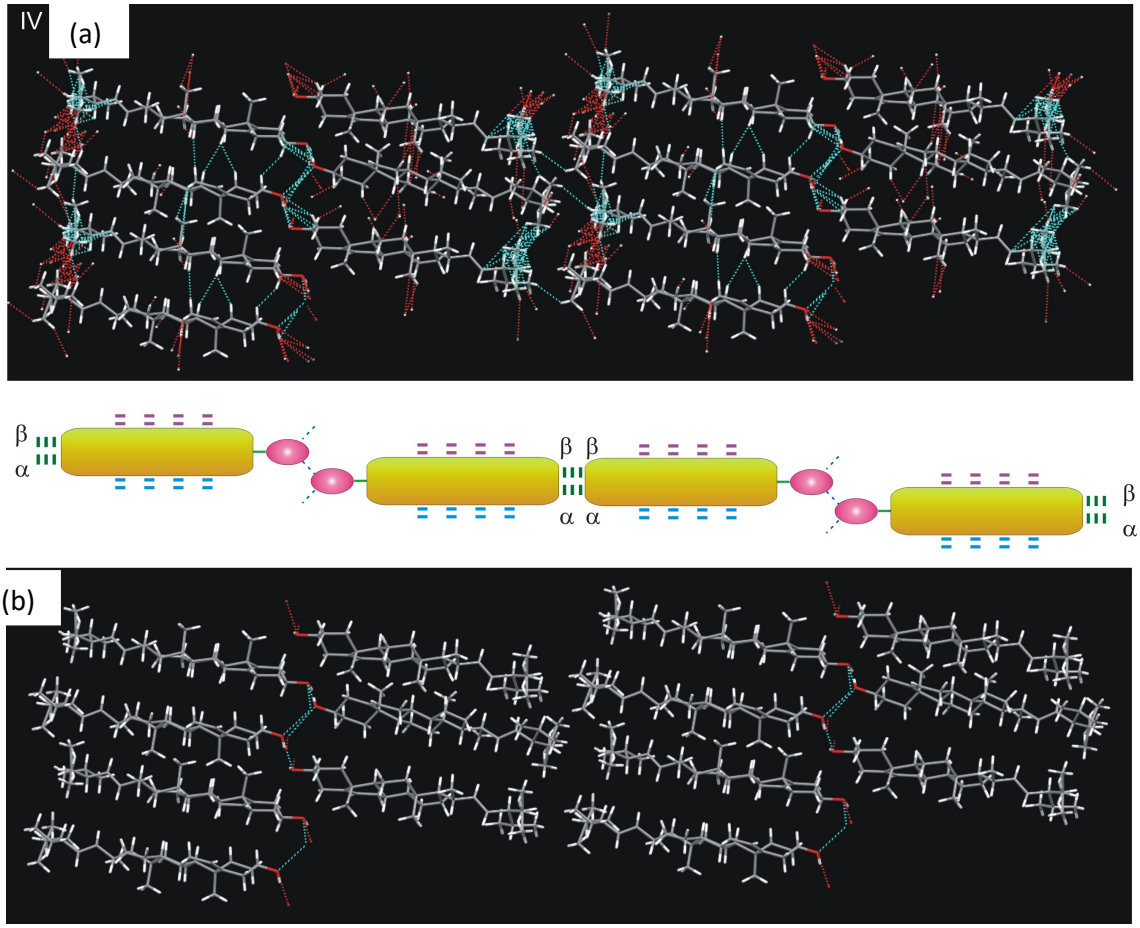


Fig. S9 Schematic representation of interacting stigmasterol molecules forming 1D, 2D and 3D architecture (a) within van der Waals contact (b) with OH participating in H-bonding ($0.5H_2O$ present as solvent of crystallization is not shown for clarity)

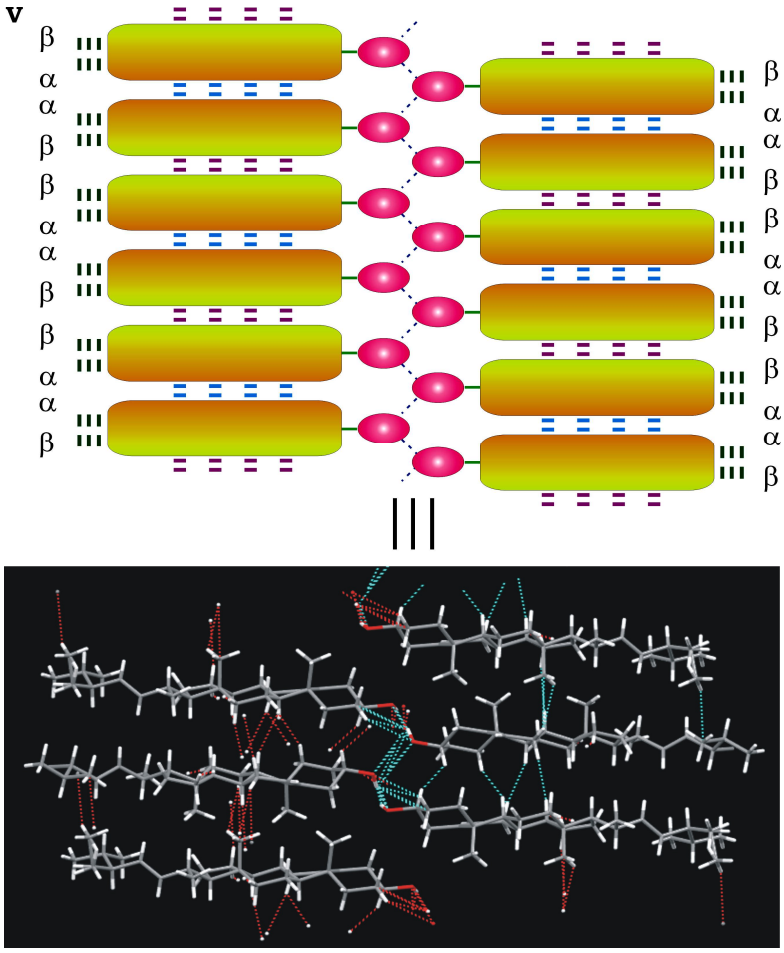


Fig. S10: Schematic representation of interacting stigmasterol molecules forming 1D, 2D and 3D architecture within van der Waals contact and OH participating in H-bonding ($0.5H_2O$ present as solvent of crystallization is not shown for clarity)

7 Experimental

7.1 Materials.

All solvents used for purification purposes were commercial grade and were distilled before use. The liquids used for gelation studies were laboratory-grade reagents and dried using standard literature methods and distilled before use.

7.2 Method of Sample Preparation and Characterization.

For self-assembly studies, 1−5 mg of compound 1 contained in a vial (1 cm id) was heated with a liquid with continuous magnetic stirring over a hot plate until a clear solution was obtained. The solution was then allowed to cool at room temperature (24−25 °C). When the material did not flow as observed by turning the vial upside down we called it a gel. The self-assemblies have been characterized by various spectroscopic and microscopic techniques. Scanning electron microscopy samples were prepared by placing a dilute solution of the sample on a aluminium foil and then allowing it to dry initially in air for 24 h and then under reduced pressure for 12 h and then sputter coated with Au before use for 120 s and studied using a Zeiss field-emission scanning electron microscope (FESEM). For optical microscopy, an aliquot of sample was taken on a glass plate and covered with a coverslip and observed both under normal and polarized light using a Nikon LV100 POL microscope. TEM images of the self-assemblies were recorded on dilute solution samples placed on 300 mesh carbon coated copper grids and dried at ambient temperatures in the air for 24 h and then under reduced pressure for 24 h and studied using JEOL transmission electron microscopy. For wide-angle X-ray scattering (WAXS) experiment, a thin layer of self-assemblies was placed on a glass plate and the volatiles were removed initially in air and then under reduced pressure and the diffractions were recorded in a Bruker X-ray diffractometer at 25 °C using Co−Kα filament (λ = 1.789 Å). For the measurement of diffraction pattern of a powder sample, it was taken directly in the diffractometer cell and measured. For FTIR spectra of the neat powder and self-assemblies were analyzed by using a PerkinElmer Spectrum.

7.3 Calculation of diffusion coefficients for the release of drug/fluorophore from loaded gel

The diffusion coefficient of the fluorophore /drug molecule was determined on the basis of a non-steady state diffusion model equation[1]

$$(M_t/M_\alpha) = 4\,(Dt/\pi\lambda^2)^{1/2}$$

where M_t is the total amount of drug released during the measurement, M_α is the total amount of drug that was kept within the gel matrix, λ represents the gel thickness, t is the time of measurement, and D is the diffusion constant of the dye/drug molecule. The diffusion coefficients for the release of dye molecule from Rho-B and CF loaded gel of stigmasterol calculated from Fig. 11, were 2.88 X 10^{-10} m^2 s^{-1} and 4.25 X10^{-10} m^2 s^{-1} respectively.

Similarly, the diffusion coefficient value for release of the anti cancer drug doxorubicin was 4.04 X 10^{-10} m^2s^{-1}.

[1] A. Baral, S. Roy, A. Dehsorkhi, I. W. Hamley, S. Mohapatra, S. Ghosh, A. Banerjee, *Langmuir* 2014, 30, 929–936.