Electronic Supplementary Material (ESI) for Soft Matter.

Electronic supplementary information

Phenylalanine dimer assembly structure as basic building block of amyloid like photoluminescent nanofibril network

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Material and Methods

Materials

Phenylalanine (L-Phe-OH) was purchased from Sigma-Aldrich and was used as such. Milli-Q water (resistivity ~18.2 MΩ) was used for all experiments.

UV-Visible spectroscopy

Optical spectroscopy was done using Agilent Cary Bundle UV-Visible spectroscope at different concentration of phenylalanine aqueous solutions.

Photoluminescence spectroscopy

Photoluminescence emission spectra of the phenylalanine aqueous solutions at different concentrations was recorded using Agilent made Cary Bundle fluorescence spectrophotometer. The PL spectra were recorded for different excitation wavelengths using same slit parameters.

FE-SEM analysis

The FE-SEM micrographs were recorded investigate the morphology of the self-assembled structure. In a general procedure, 10 µl of freshly prepared phenylalanine solution at concentration 1 mg/mL was air dried on

the surface of Ag film pasted on FE-SEM step. Pt coating was applied on the samples to make it conductive and followed by FE-SEM analysis on a Hitachi, SU8010 electron microscope, operating at 10–15 kV.

SAXS analysis of phenylalanine self-assembly in both solution and deposited phase

The small angle x-ray scattering data was recorded using SAXSpace Anton Paar with generator model no. ID3003 and Eiger R1M vertical detector. The line collimation analysis was done to obtain SAXS data for both solution as well as solid phase phenylalanine self-assembly. The phenylalanine aqueous solution was placed in 1mm quartz capillary and self-assembled fibrils was placed between scotch tape for the SAXS analysis. The scattering background for capillary tube, deionized water for solution phase and scotch tape in case of deposited phase was subtracted prior to the SAXS analysis of phenylalanine assembly. The beam stop length of 0 and 0.1 mm was used for solid and liquid samples, respectively. The scattering data points were selected from 50 to 900 q values for the GIFT analysis. The lower and higher marginal q values were ignored for obtaining the noise free PDDFs. The Lagrange multiplier for the stability of PDDFs was selected at 0.00.

Density function theory (DFT) analysis

The density function theory optimization of the Phe-DA was performed using Gaussian software with model 3 and 3-21G as basis set. The solvent free calculations were carried out to obtain optimized Phe-DA structure.

Confocal Laser scanning microscopy

The CLSM images were captured by depositing 10 µl of air dried hydrogel fibrils on microscopic glass slide and images were captured using Zeiss, LSM 510 confocal laser scanning microscope.

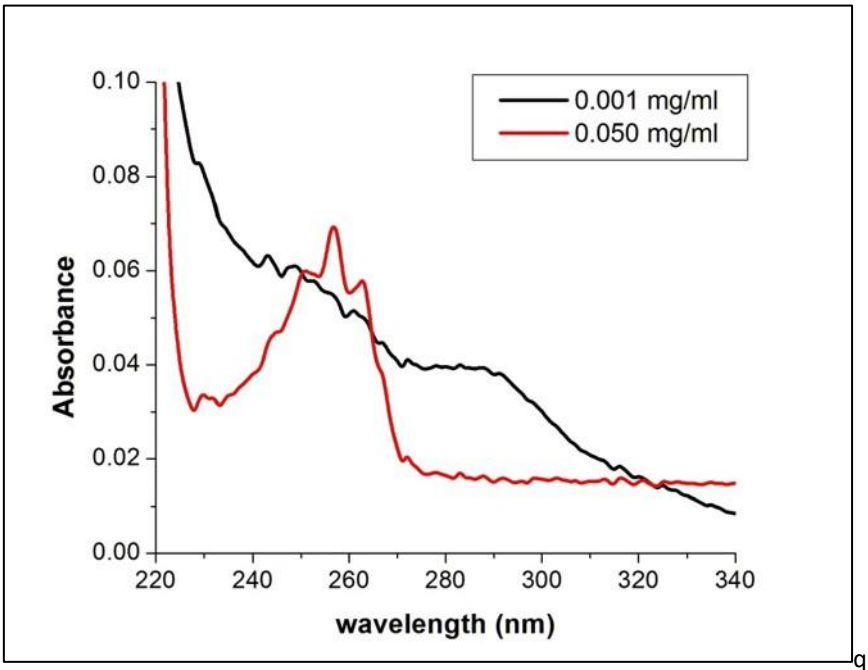

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Figure S1: - UV Vis spectra of phenylalanine confined self-assembly (red) at 0.05 mg/mL and non-confined phenylalanine (black) at 0.001 mg/mL in aqueous medium.

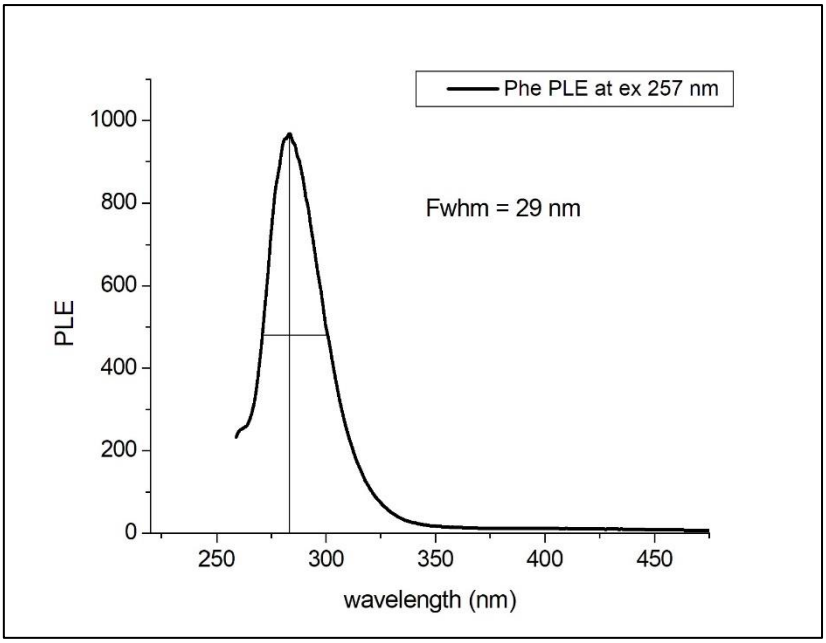


Figure S2: - Full width half maxima (FwHm) of PL spectrum of phenylalanine at excitation wavelength of 257 nm.

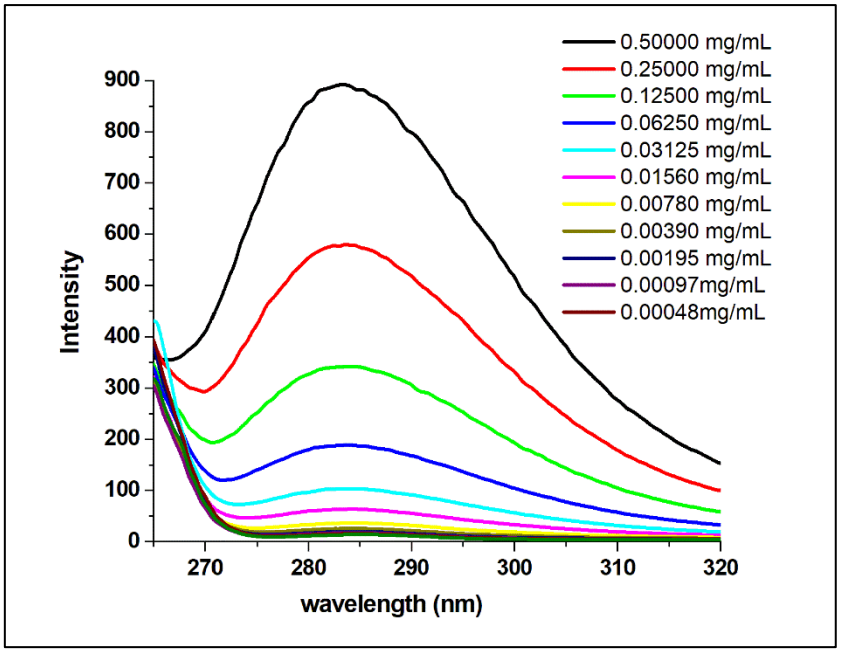


Figure S3: - PL spectra of Phe-DA at different concentration when excited at 257 nm.

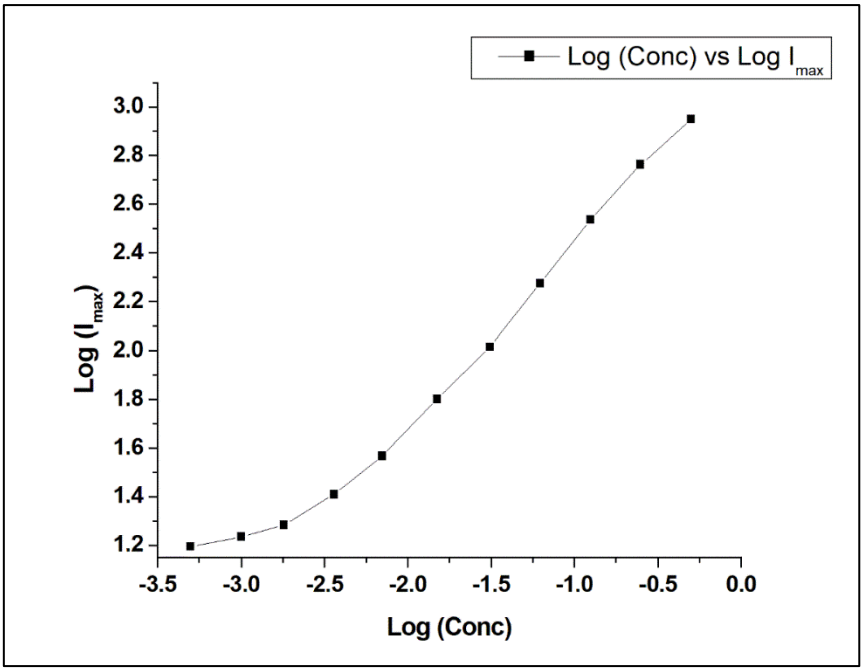


Figure S4: - Plot between Log (phenylalanine concentration) in mg/mL and maximum intensity (I_{max}) of phenylalanine PL emission at varying concentration using excitation wavelength of 257 nm.

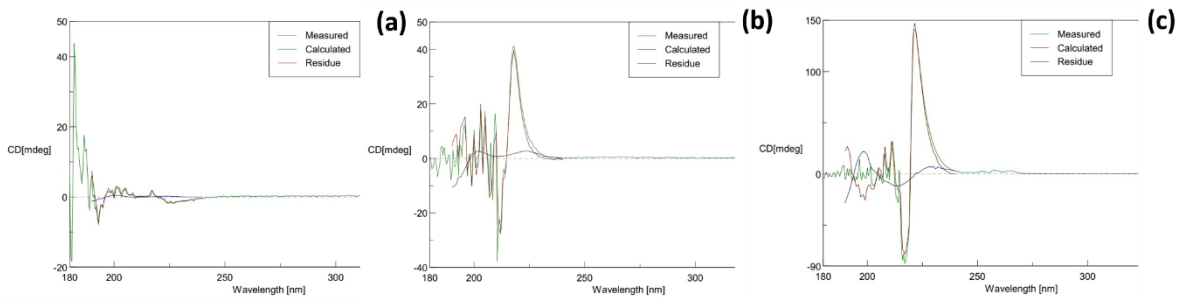


Figure S5: - Circular dichorism spectra for phenylalanine solutions at concentrations 0.0025 mg/mL (a); 1 mg/mL (b) and 10 mg/mL (c).

Equation S6

$$R = \pi r_B^0 \sqrt{\frac{\frac{m_0}{M}}{\frac{\mu}{m_0 \varepsilon_\infty^2} - \frac{E_{ex}^{QD}}{R_y}}}$$

where r_B^0 = 0.52 Å is Bohr radius of hydrogen atom, R_y = 13.56 eV is the Rydberg constant, M = m_e + m_h is translational mass of exciton and hole (m_e and m_h are effective mass of electron and hole, respectively). The effective mass of an electron (m_e) is almost equal to that of a hole (m_h) which is quite approximate to 0.5 m_0 (where m_0 is free electron mass).[14] Thus, M = m_0 which provides reduced mass (μ) equal to 0.25 m_0 (where μ = $m_e m_h/m_e + m_h$) (Calculation S6 in Supporting Information). The parameter ε_∞ = n^2 is the dielectric of medium where n is the refractive index of the material (for aqueous phenylalanine n = 1.35).[23]

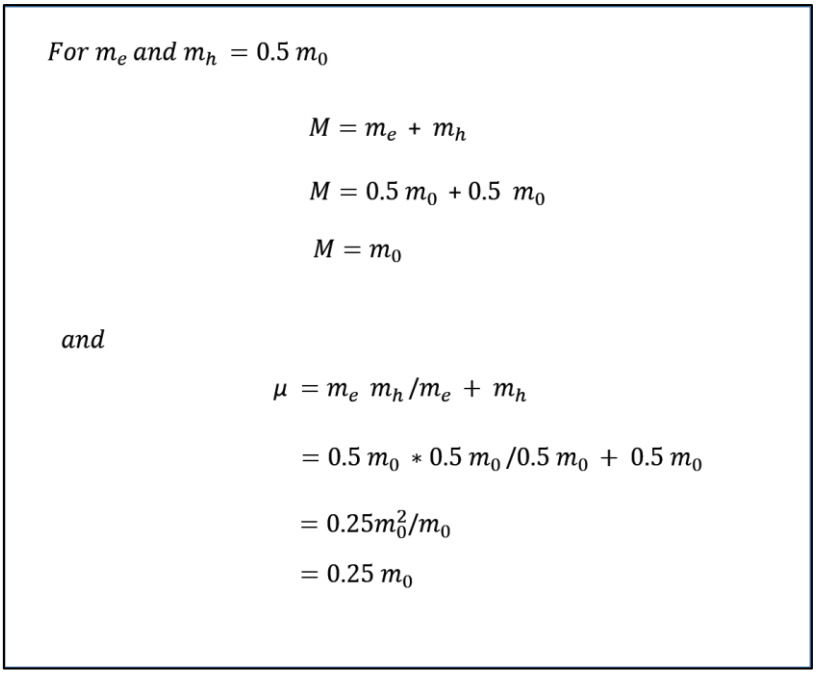


Figure S7: - Scheme depicting methodology used for calculating translational mass and reduced mass.

For zero dimensional Quantum well model

$$R = \pi r_B^0 \sqrt{\frac{\frac{m_0}{M}}{\frac{\mu}{m_0\varepsilon_\infty^2} - \frac{E_{ex}^{QD}}{Ry}}}$$

$$R = 3.14 * 0.052 \sqrt{\frac{1}{\frac{0.25m_0}{m_0\varepsilon_\infty^2} - \frac{E_{ex}^{QD}}{13.56}}} \quad \text{nm}$$

$For\ \varepsilon_\infty = n^2, and\ n = 1.35$

$\varepsilon_\infty^2 = 3.32$

$$R = 3.14 * 0.052 \sqrt{\frac{1}{\frac{0.25}{3.32} - \frac{E_{ex}^{QD}}{13.56}}} \quad \text{nm}$$

$For E_{ex}^{QD} = 0.567\ eV$

$$R = 3.14 * 0.052 \sqrt{\frac{1}{\frac{0.25}{3.32} - \frac{0.567}{13.56}}} \quad \text{nm}$$

$$R = 0.892\ nm$$

Figure S8: - Scheme depicting methodology used for calculating radius of Phe-DA using zero dimensional quantum well model.

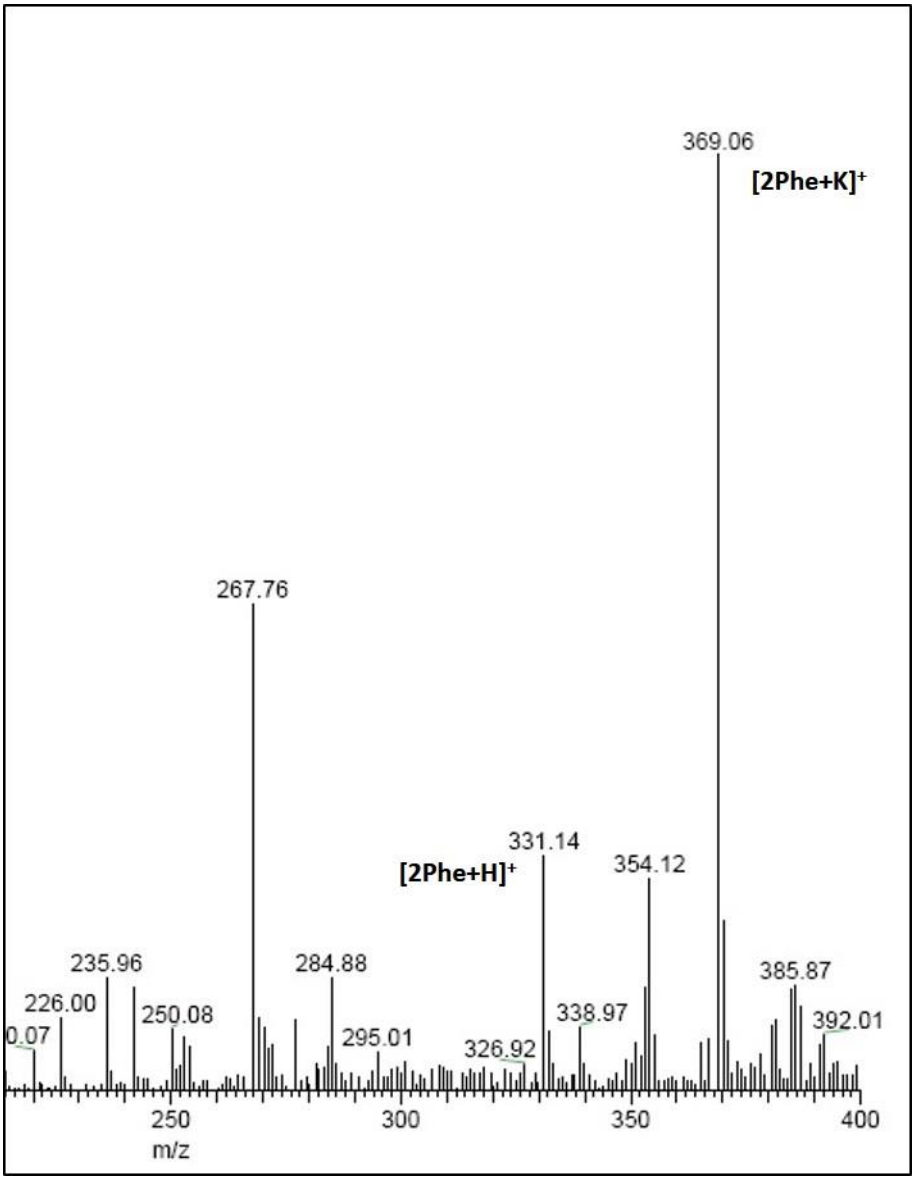


Figure S9: - ITMS-ESI mass spectrum depicting self-assembled dimer peaks of Phe-DA at m/z = 331.14 and 369.06.

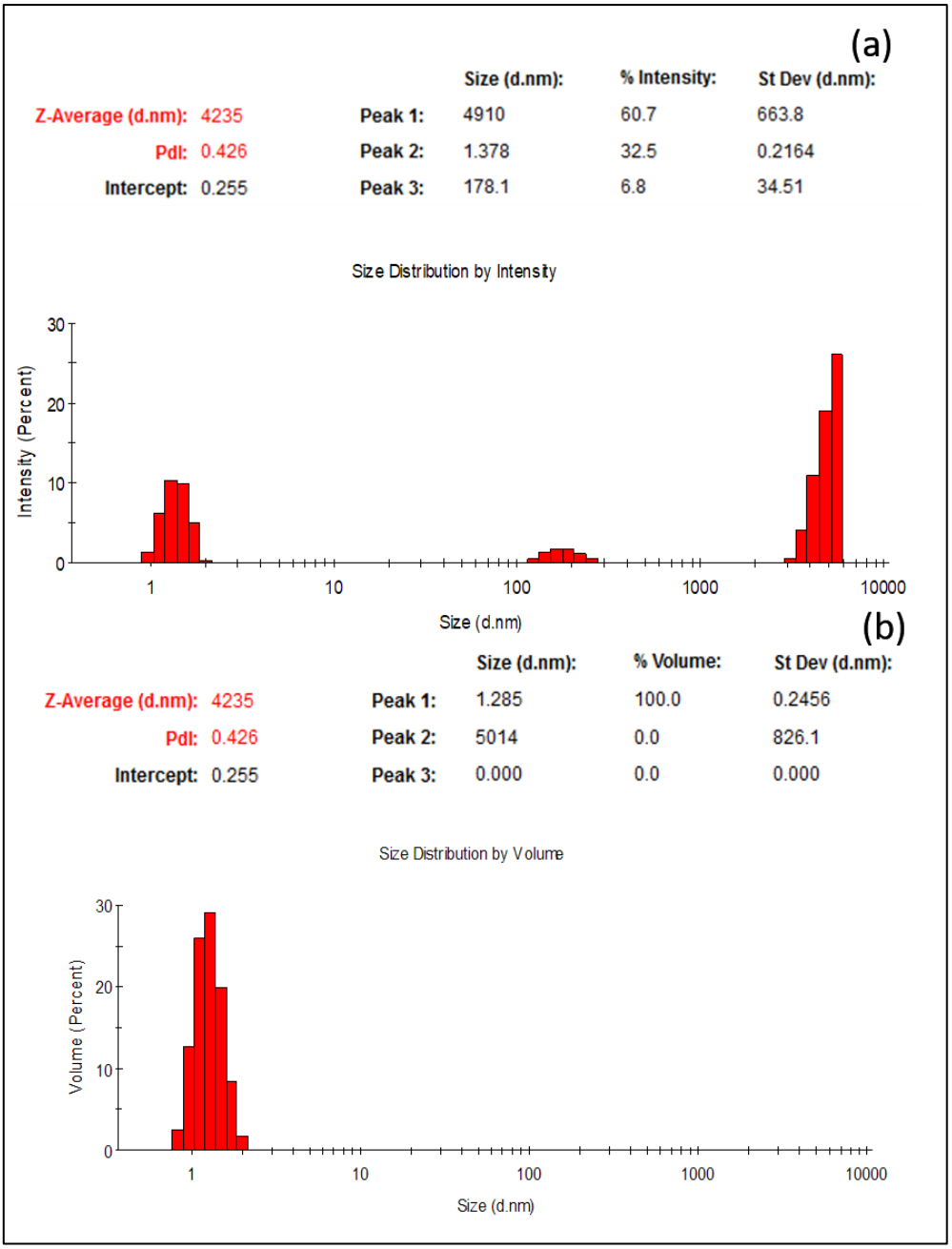


Figure S10: - DLS data depicting size distribution by intensity percentage (a); and volume percentage (b); for Phe-DA in aqueous medium at 1 mg/mL concentration.

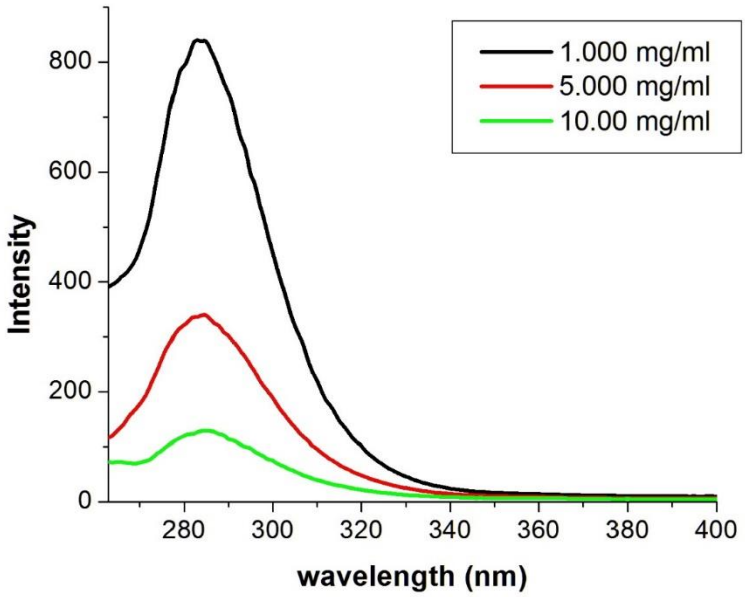


Figure S11: - PL spectra of phenylalanine aqueous solutions at excitation wavelength of 257 nm.

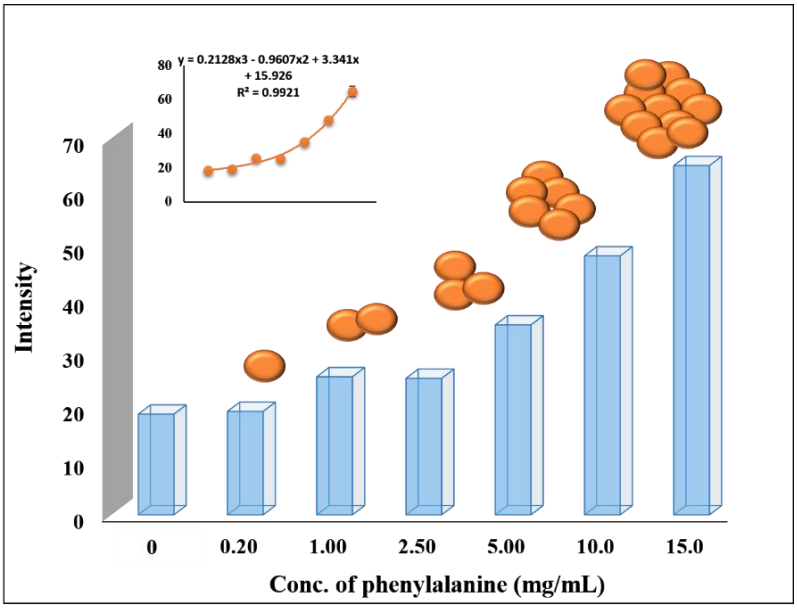


Figure S12: - Thioflavin T fluorescence assay depicting concentration dependent increase in the emission intensity for phenylalanine aqueous solutions.

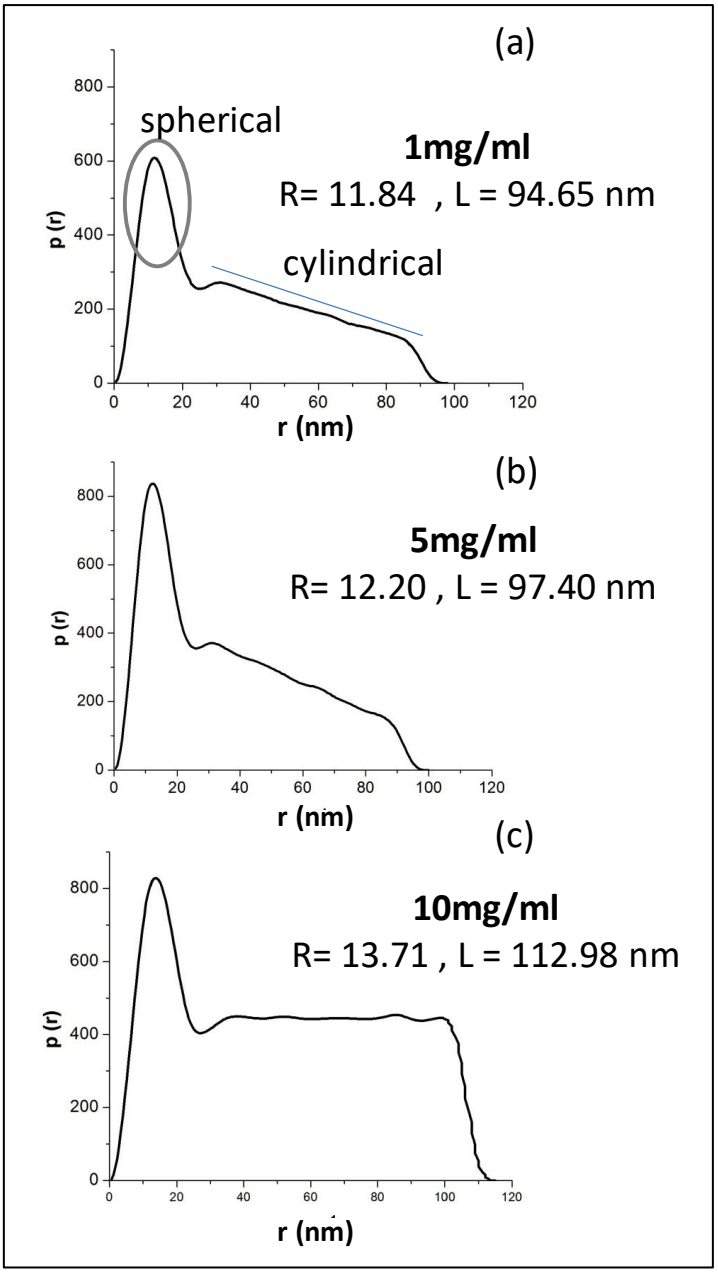


Figure S13: - Pair distribution distance function (PDDF) obtained by SAXS analysis for 1 mg/mL (a); 5 mg/mL (b); 10 mg/mL (c) phenylalanine solution. (R: radius; L: length of proposed nano cylinders).

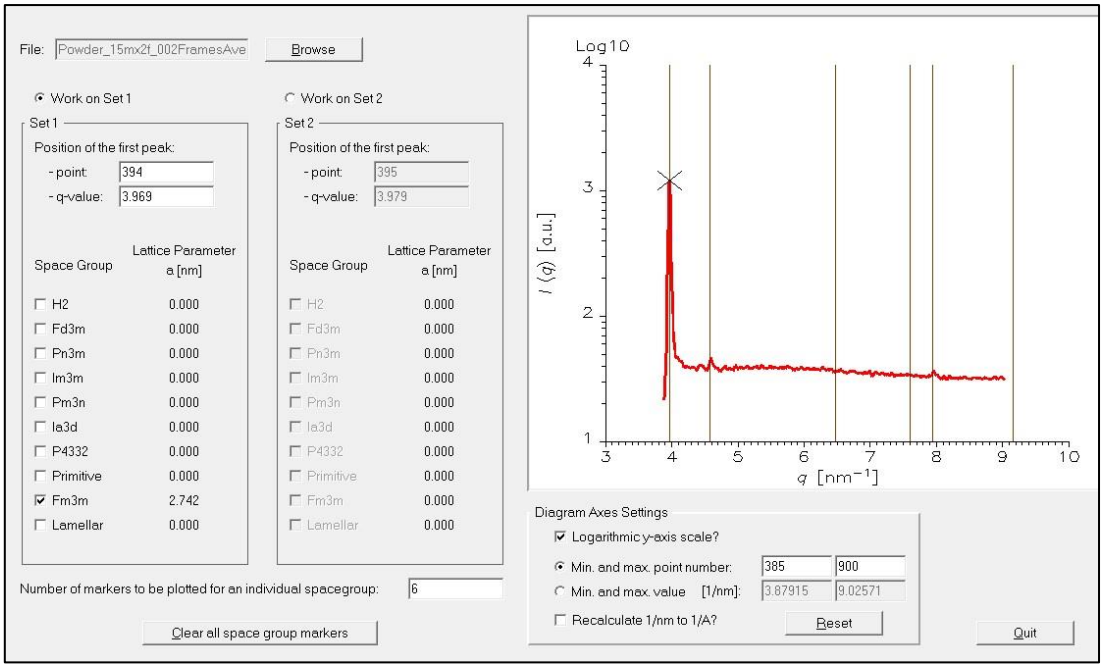


Figure S14: - Space group indexing for self-assembled phenylalanine fibrils using SAXS analysis correlating Fm3m as space group for Phe-DA units.

For FCC lattice,

If unit cell parameter $= 2.742\ nm$
and radius of lattice point (r)

$$4r = \sqrt{(2.742)^2 + (2.742)^2}$$

$$4r = \sqrt{15.036}$$

$$4r = 3.877$$

$$r = 0.969\ nm$$

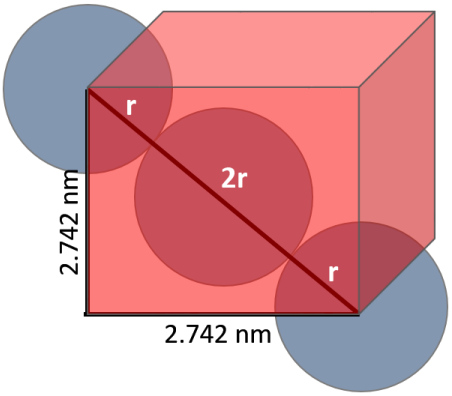
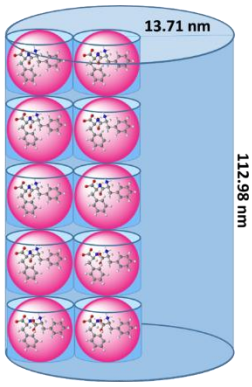


Figure S15: - Scheme depicting methodology to calculate the radius of lattice point (Phe-DA) using space group indexing data obtained by SAXS analysis of phenylalanine fibrils.

$At \frac{10\ mg}{mL} phenylalanine, nanocylinder\ with\ radius = 13.71\ and\ length = 112.98\ nm$



$$Volume = \pi r^2 h$$

$$Volume = 3.14 * (13.71)^2 * 112.98$$

$$Volume = 66{,}681.61\ nm^3$$

$For\ Phe - DA\ (as\ sphere)\ with\ radius = 0.892\ nm$

$$Volume = \frac{4}{3}\pi r^3$$

$$Volume = \frac{4}{3} * 3.14 * (0.892)^3$$

$$Volume(Vs) = 2.97\ nm^3$$

$Assuming\ that\ each\ Nanocylinder\ made\ up\ of\ tiny\ cyminders\ each\ encapsulating\ one\ Phe - DA\ sphere\ completely$

$Thus, For\ Volume\ of\ tiny\ cylinder\ (Vc)\ and\ volume\ of\ sphere\ (Vs)$

$$\frac{V_s}{V_c} = \frac{2}{3}$$

$$V_c = \frac{2.97 * 3}{2} = 4.455\ nm^3$$

$Thus, total\ number\ of\ tiny\ cylinders = Phe - DA\ structures\ (N) in\ one\ nanocylinder\ (10\ mg/mL):-$

$$N = \frac{66{,}681.61}{4.455}$$

$$N = 14{,}967.81 = 14{,}968\ Phe - DA\ structures$$

$At \frac{5\ mg}{mL} phenylalanine, nanocylinder\ with\ radius = 12.20\ and\ length = 97.40\ nm$

$$Volume = 45{,}520.6\ nm^3$$

$$Volume\ of\ Phe - DA\ (Vs) = 2.97\ nm^3$$

$$Volume\ of\ tiny\ cylinder\ V_c = \frac{2.97 * 3}{2} = 4.455\ nm^3$$

$Thus, total\ number\ of\ tiny\ cylinders = Phe - DA\ structures\ (N) in\ one\ nanocylinder\ (5\ mg/mL):-$

$$N = \frac{45520.6}{4.455}$$

$$N = 10218\ Phe - DA\ structures$$

$At \frac{1\ mg}{mL} phenylalanine, nanocylinder\ with\ radius = 11.84\ and\ length = 94.65\ nm$

$$Volume = 41{,}663.3\ nm^3$$

$$Volume(Vs) = 2.97\ nm^3$$

$$Volume\ of\ tiny\ cylinder\ V_c = \frac{2.97 * 3}{2} = 4.455\ nm^3$$

$Thus, total\ number\ of\ tiny\ cylinders = Phe - DA\ structures\ (N)\ in\ one\ nanocylinder\ (1\ mg/mL):-$

$$N = \frac{41663.3}{4.455}$$

$$N = 9352\ Phe - DA\ structures$$

Figure S16: - Scheme depicting methodology adopted for quantification of Phe-DA units present in one proposed self-assembled nano cylinder (for 10, 5 and 1 mg/mL concentration).

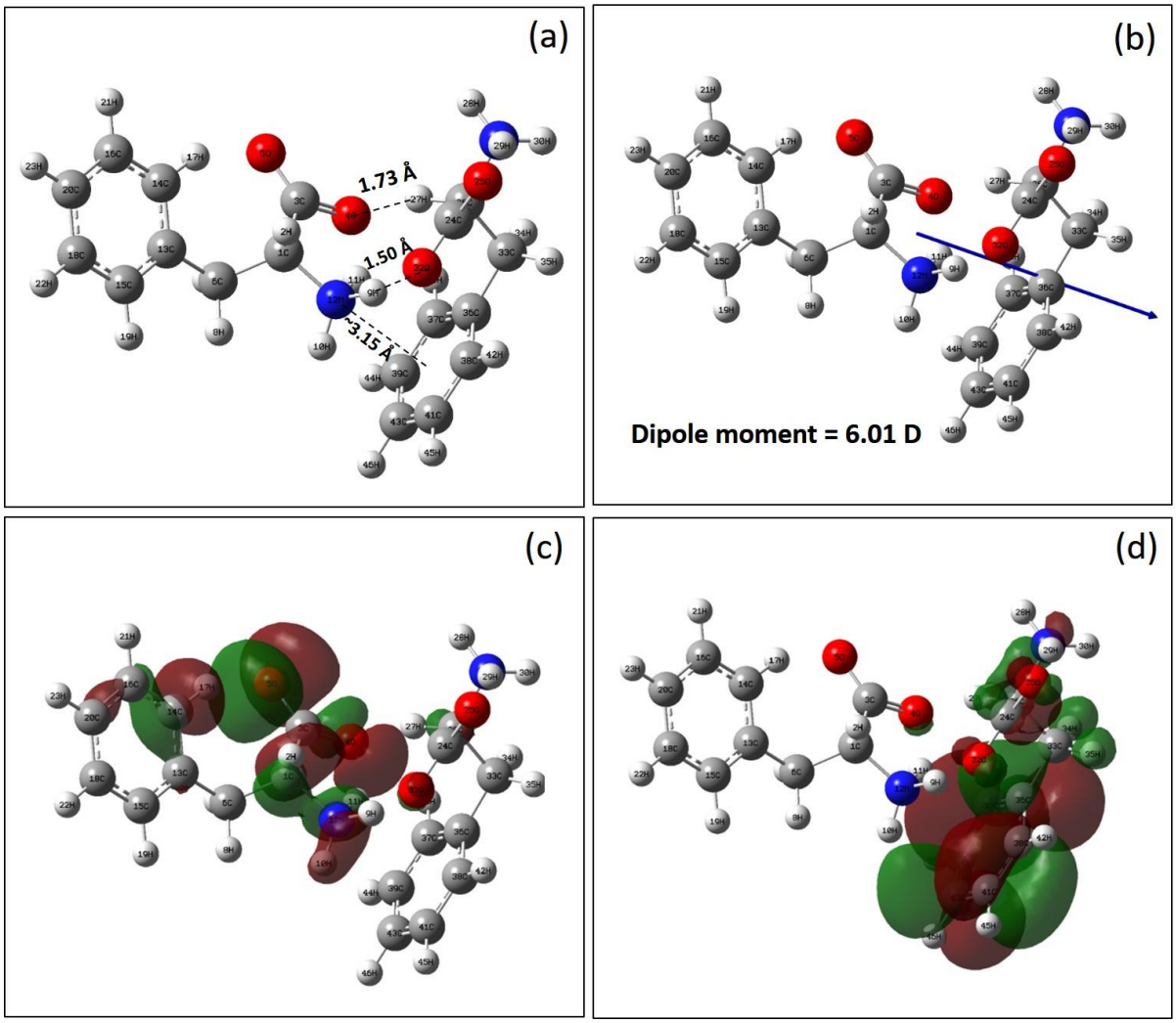


Figure S17: - DFT optimized Phe-DA structure (a); dipole moment (b); quantum confined HOMO orbital and LUMO energy level (d).

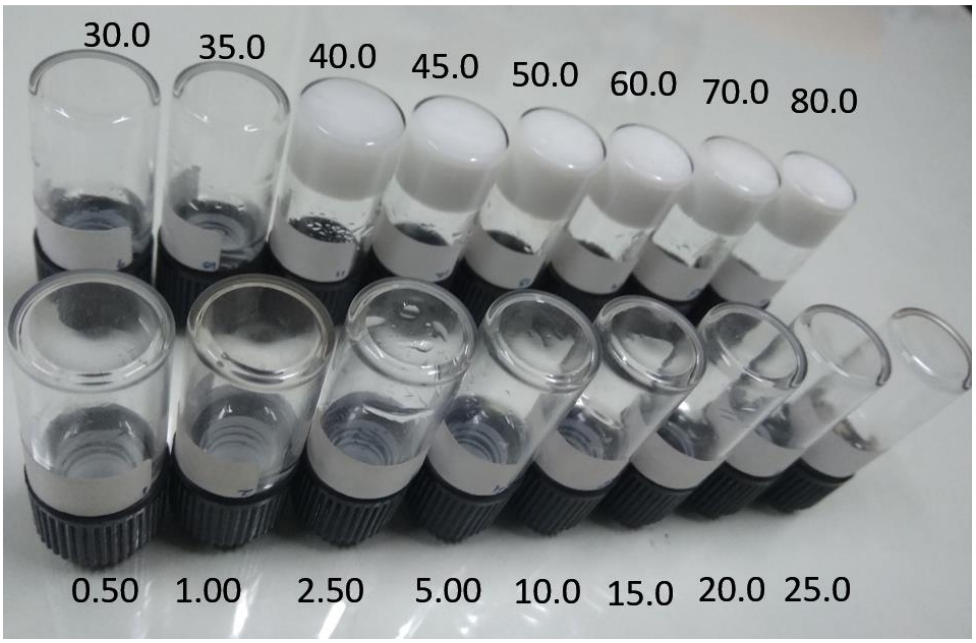


Figure S178: - Inverted Vial Hydrogel formation for phenylalanine at different concentrations from 0.5 mg/mL to 80 mg/mL after incubation for 2 hours at room temperature.

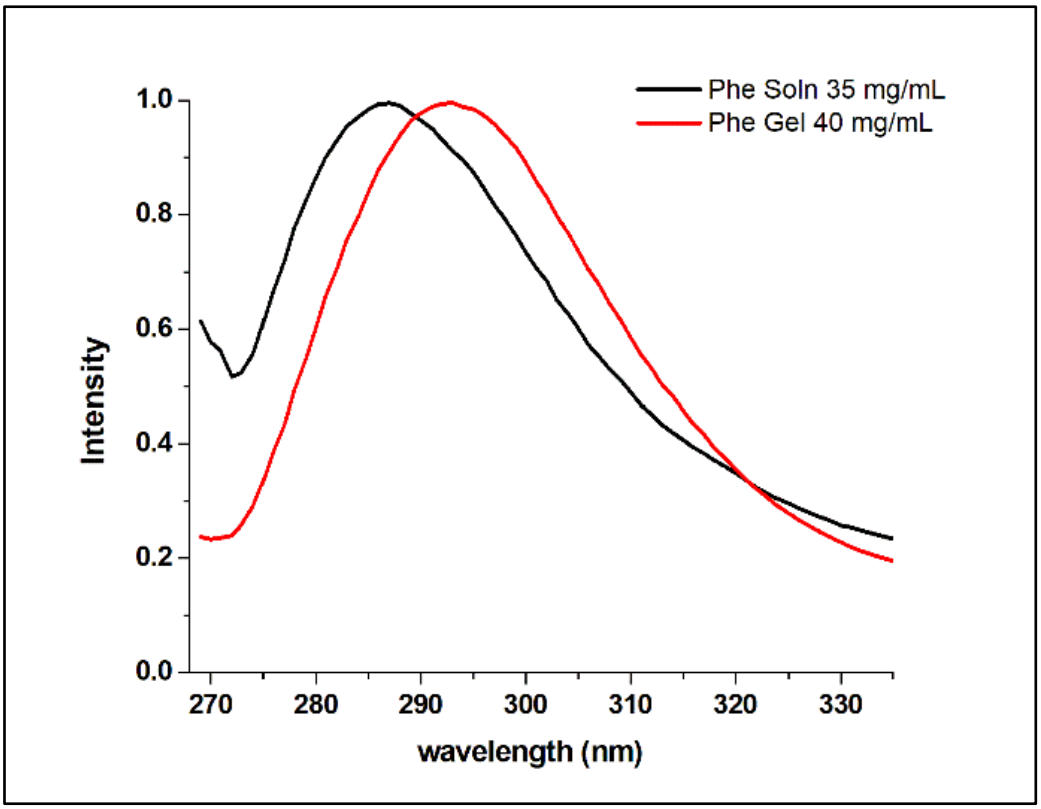


Figure S19: - PL spectra (at excitation 257 nm) depicting red shift in emission from phenylalanine aqueous solution at 35 mg/mL and hydrogel at 40 mg/mL after 2 hour incubation of freshly prepared solutions.

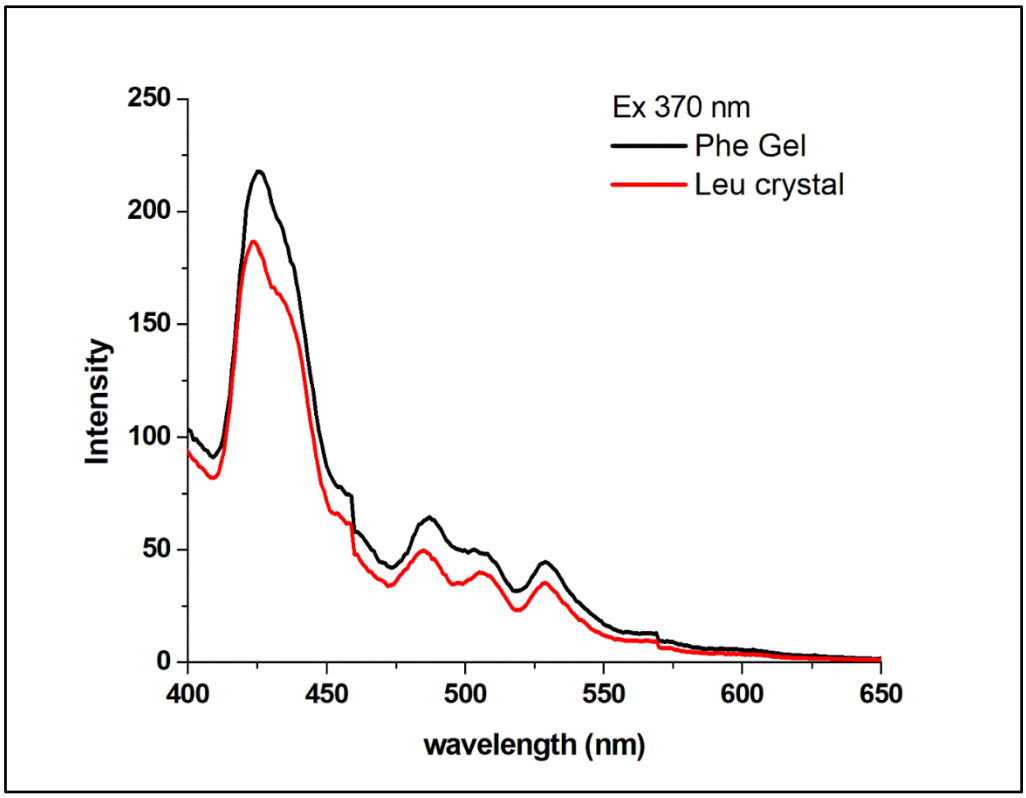


Figure S20: - PL of phenylalanine hydrogel and leucine crystallite at 370 nm excitation wavelength.