Supporting Information

Modulating Phe-Phe dipeptide aggregation landscape by covalent attachment of an Azobenzene photoswitch

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1. Synthesis of dipeptide (L)-AzoPhe-Phe-OH:

The protocol for the synthesis of Boc-L-AzoPhenylalanine was followed from a previous work.[1] Briefly, L-N-tert- Butoxycarbonyl-p-aminophenylalanine (1 g, 3.6 mmol) was dissolved in glacial acetic acid (200 mL) at room temperature. Nitrosobenzene (578 mg, 5.4 mmol) was added to this solution. The color of the reaction changes from green to orange to deep red within the first 30 mins. The reaction is allowed to stir for 8-12 hours. The volume of acetic acid is reduced by evaporation on a high vacuum rotary evaporator. The reaction mixture was then quenched with satd. $NaHCO_3$ solution (300 mL) until no effervescence is observed. The compound is extracted with ethyl acetate (3x 200 mL). The organic layers were then combined, dried (anhydrous $MgSO_4$) and concentrated on a rotary evaporator. The crude material (bright orange) was then purified by silica gel column chromatography (CH_2Cl_2-MeOH. 90:10, Yield~77%) as used as such for the synthesis of the dipeptide described next.

Boc-L-AzoPhenylalanine (347mg, 1mM) was dissolved in 100ml of dry tetrahydrofuran (THF) and cooled to -20°C (in a salt ice mix). To it was added N-methyl morpholine (NMM) (145 µl) and Isobutyl chloroformate (IBCF) (154 µl) one after other while stirring the reaction. The reaction appears turbid and bright orange. The reaction is allowed to continue for 30 mins while maintaining the temperature at -20°C. To the mixed anhydride formed, a mix of H-Phe-OH (181mg, 1.1mM) and NaOH (44mg, 1.1mM) was added and the reaction is removed from ice. The reaction is allowed to stir for 8-12 hrs until completion. The residual THF was evaporated on a rotary evaporator and the compound was precipitated with sat. citric acid (50ml). The N-terminus protected dipeptide was then extracted with ethyl acetate (3x 100 ml). The organic layers were then combined, dried (anhydrous $MgSO_4$) and concentrated on a rotary evaporator. The compound was lyophilized once to obtain the powder.

The N-terminal deprotection was carried out by dissolving the compound (obtained above) in 50ml of dichloromethane (DCM) and 50ml of trifluoroacetic acid (TFA) and stirred over ice for 30mins. The solvent and acid was then removed under vacuum and the N–terminal free dipeptide was precipitated with 100ml petroleum ether (Boiling Point: 45-60 °C). The precipitate was washed thrice in petroleum ether and dried in vacuum. Finally, the peptide was solubilized in a minimum volume of acetic acid (~10 ml), diluted in deionized water (~150 ml) (a turbid suspension is observed). The mix is frozen in ice and lyophilized. Purity of the molecule was ascertained using reverse phase HPLC (see Fig S4) with a gradient of Acetonitrile: Water (5%:95%) to Acetonitrile: Water (95%:5%). The compound typically elutes between 30-45% ACN with two peaks (corresponding to the cis-/trans-isomers). ^{1}H-NMR (Fig S2), ^{13}C-NMR (Fig S3) and ESI MS (Fig S5) were performed to characterize the dipeptide. The molecule was found to be 98% pure based on the HPLC analysis shown in Figure S4.

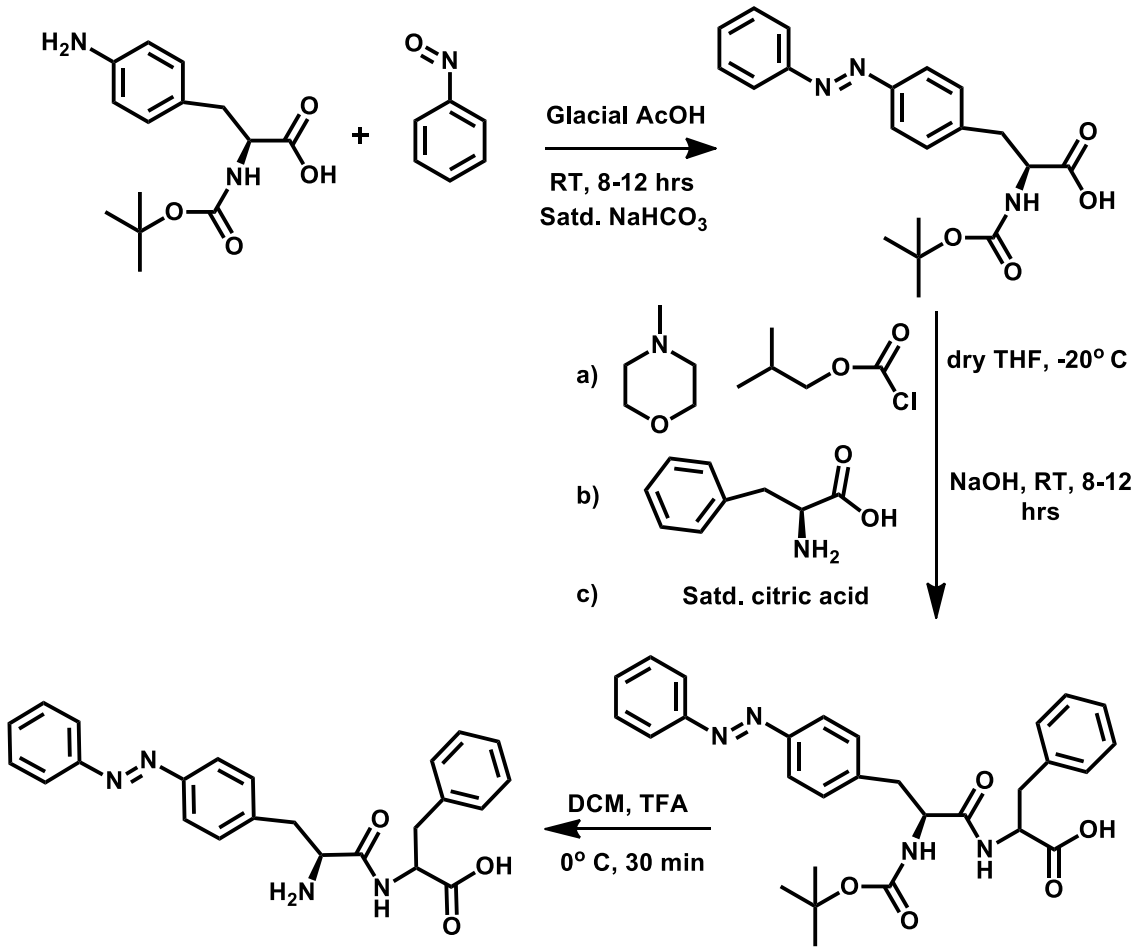

Figure S1. The figure shows the synthesis scheme of the peptide AzoPhe-Phe-OH.

2. Experimental details:

Sample Preparation. For preparing monomer solution of AzoPhe-Phe-OH, 0.75 mg of the peptide was added to 1 ml of methanol solvent. For the aggregation of the peptide, initially 0.25mg of the sample was made completely dissolved in 50 µl of methanol and later water was added in 1:12 v/v ratio to get a turbid solution (1 mM). The turbid solution was kept for 3 hour after sample preparation to facilitate aggregation. The *cis* isomer of the peptide monomer was obtained after irradiation of the peptide solution in a cuvette (4 cm *1 cm) for 15 min using a broadband UV lamp source (350 nm- 390 nm) with peak at 365 nm (1.656 mW/cm^2). The self-assembly of *cis* isomer in methanol solvent is done by addition of water in 1:12 v/v ratio in the presence of UV irradiation. The solution was kept for 10 hours to

monitor the aggregation of the peptide. The aggregated peptide in *trans* isomeric form, i.e., fibril was illuminated with UV source to monitor the morphology switching due to trans-to-cis photo-isomerization of the azo functional group within aggregate. The *cis-trans* isomerization was done by illumination of sample in monomeric and aggregated form using light emitting diode centered at 457 nm (203mW/cm^2). Steady-state absorption measurements were done for sample prepared in above mentioned protocol. Surface Enhanced Raman Spectroscopy measurements were done using negatively charged silver nanoparticles that were prepared by Lee and Meisel method (details given below).[2] Nanoparticles were concentrated to 1/10 volume fraction by centrifugation for 10 min at 25^{0}C with 7200 rpm. The aggregated peptide solution was mixed with the concentrated silver nanoparticle solution in 1:5 v/v ratios and a 10 μL of mixture was dropped on the surface of glass slide to measure Raman spectra.

Steady State Absorption. The steady-state absorption measurements of peptide were carried out in JASCO V-670 spectrophotometer using a cuvette with path length of 1 mm.

High-Performance Liquid Chromatography (HPLC). The peptide H-Azo-Phe-Phe was methanol: water solvent (1:12 v/v ratio) was injected into a Prominence series ultra-high performance liquid chromatography (model UFLC; Shimadzu, Columbia, MD) system equipped with photodiode array detector (PDA) in order to obtain the fraction of *trans* and *cis* isomer present in the sample kept at room temperature. HPLC purification was done using a Reverse phase column C18 (Varian, 250 mm x10 mm, 10 μm) maintained at 4°C. A binary solvent system was established using mobile phase A with acetonitrile and the mobile phase B with water. Isocratic flow of Acetonitrile and water (50: 50) with a flow rate of 0.4 ml/min was run for 20 min time. The *trans* and *cis* isomer were resolved and identified using λ_{max} previously known for the sample through steady-state absorption measurements. Then the sample was illuminated with UV light for different timescales, and HPLC purification was done to obtain fraction between two isomers. HPLC purification was also done at these time durations for *cis*-to-*trans* isomerization process using LED at 457 nm.

Dynamic Light Scattering (DLS). The hydrodynamic radius of *trans* isomer in aggregated form in a solvent system of methanol and water in 1:12 ratio was performed using a DAWN 8+, eight angle light scattering instrument (Wyatt Technology, Santa Barbara, CA) at room temperature. DLS measurements were also done to monitor the aggregation of *cis* isomer of the peptide in methanol: water solvent system.

Surface Enhanced Raman Spectroscopy (SERS). Silver nanoparticles were used as SERS substrates for detecting vibrational spectra from the dipeptides. The nanoparticles were prepared by the Lee and Meisel method.[2] To 100 ml of Milli-Q water, 18 mg of silver nitrate ($AgNO_3$) was added to yield a resistance 18.2 MΩcm at 25^{o}C. The solution was boiled under reflux conditions to maintain constant water level. About 2 mL of reducing agent 1% sodium citrate was added to the boiling solution and maintained for 1 hour of constant boiling and stirring the solution. The reduced silver nitrate to form plasmonic nanoparticles in solution was brought back to the room temperature. Silver nanoparticles were characterized by UV-

Vis absorption spectroscopy, Zeta potential measurements using dynamic light scattering (DLS) and transmission electron microscopy (TEM) methods. The surface plasmon resonance absorption appeared at 415 nm and the size of nanoparticles is approximately 75 nm with zeta potential -31 meV..The SERS measurements were performed on the dipeptide prepared in methanol: water (v/v 1:12) mixed with the silver nanoparticle colloidal solution (v/v 1:5) followed by the excitation at 532 nm of 0.15 mW power near the sample.

We recorded Surface Enhanced Raman Spectra of the peptide assembly using a confocal Raman microscope (alpha300R, WITech, Germany). The frequency doubled DPSS Nd:YAG laser at 532 nm was used to excite a drop of peptide solution mixed with silver nanoparticle, on a glass slide. The scattered light in the focal plane was collected through a 100 μm core multimode fiber as pinhole. The spectra ware collected using lens based ultrahigh throughput spectrometer (UTHS300, 1800 grooves/ mm grating) coupled to a back illuminated CCD- camera (1024 × 128 pixels, 2 cm^{-1} per pixel). The spectra were collected for 20 accumulations with 1s integration time. Laser power of 0.15 mW was focused to the sample using 20X Zeiss microscopic objective. The data was processed with the help of WITec project software and plotted using IGOR 5 software.

NMR. ^{1}H NMR and ^{13}C NMR spectra were collected at 25 °C on either Avance Bruker 500 MHz or Varian 600 MHz spectrometers at the National NMR facility, Tata Institute of Fundamental Research, Mumbai, India. The solvent used was CD_3OD (Cambridge Isotope Laboratories, Cambridge, MA). All chemical shifts are reported in the standard notation of parts per million (ppm) using either the proton peaks of residual solvents or tetramethylsilane as internal reference.

ESI-MS. Mass spectral analyses were carried out on a liquid chromatography mass spectrometer (LCMS-2020, Shimadzu Corp.) with an ESI probe (positive and negative ion modes).

Transmission Electron Microscope (TEM). A 20 μL drop of the self-assemblies prepared as mentioned by the above process was placed on a 300 mesh copper grid coated with Formvar and carbon. After allowing the drop to sit on the grid for 10 minutes within a humidified box (~Relative humidity 95 %) at 25 ºC, excess sample was drained from the side of the grid using a Whatman filter paper. The sample was stained using 1 % aqueous solution of Uranyl formate for 30 seconds. Excess stain was drained off the grid using Whatman filter paper. The grid was imaged on a 120 kV Tecnai Twin (FEI, Netherlands) with a Tungsten filament operating at 120 kV. The scale bar (image inset) was previously calibrated to correspond to the magnification at which the sample is imaged. The image was slightly enhanced with Differential Contrast Enhancement (DCE) filter in the software.

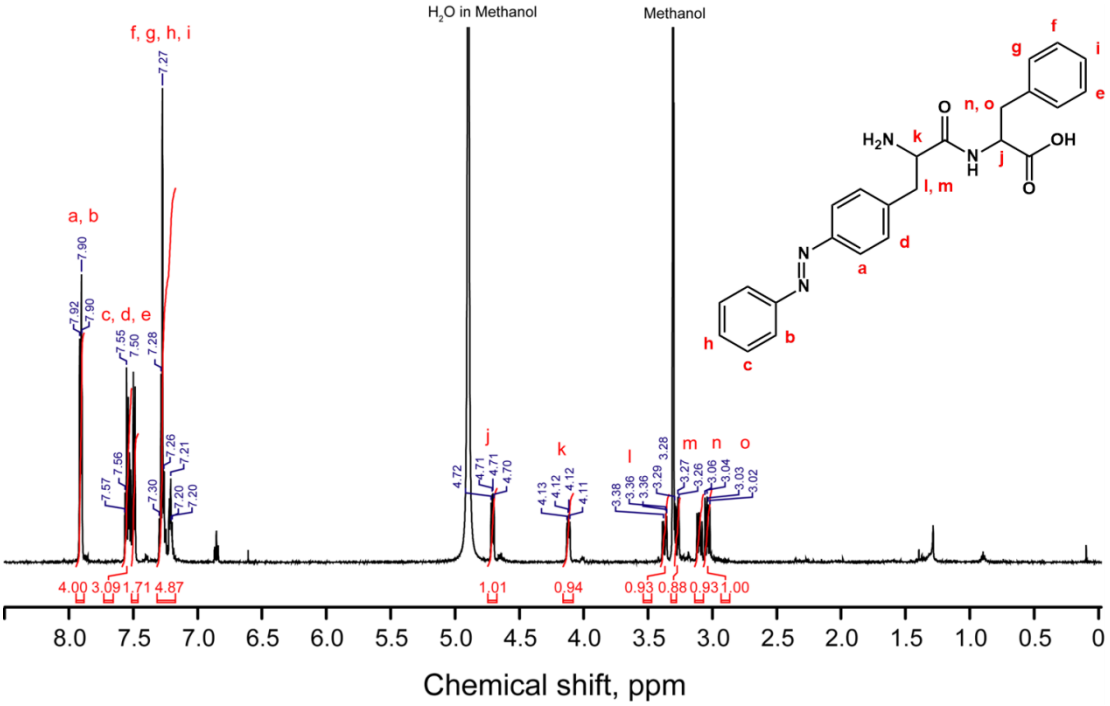

Figure S2. Figure shows the ^{1}H NMR of Azo-Phe-Phe-OH in methanol (8 mM). The peak at 7.90 is from aromatic protons *a, b*. The multiplet at 7.55 is from *c, d* and *e*. The peak at ~7.26 is from *f, g, h* and *i*. The aliphatic protons at position *j* and *k* are tertiary protons giving rise to two doublet of doublet (dd) while *l, m* and *n, o* are diastereotopic –CH_2 protons showing doublet of doublet (dd) for each.

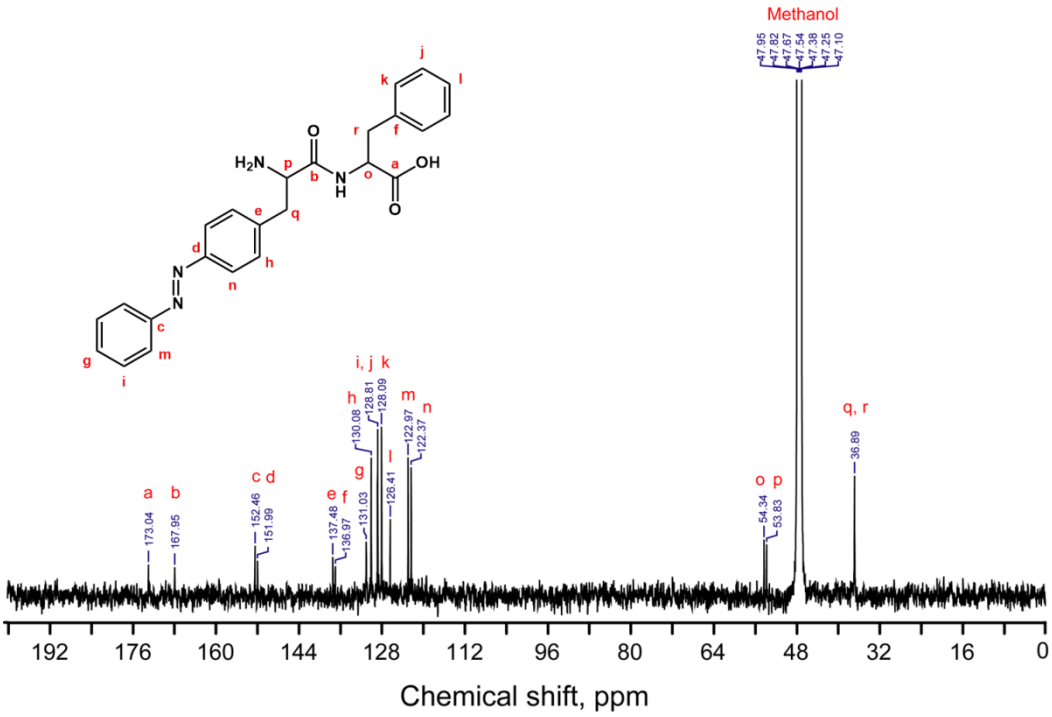

Figure S3. ^{13}C NMR of AzoPhe-Phe-OH in methanol (8 mM); ^{13}C peaks are at 173, 168, 152.5, 152, 137.5, 137, 131, 130, 128.8, 128, 126.4, 123, 122.4, 54.3, 53.8 and 37 ppm, all the marked peaks are assigned for the molecule; solvent methanol peak is at 47.54 ppm.

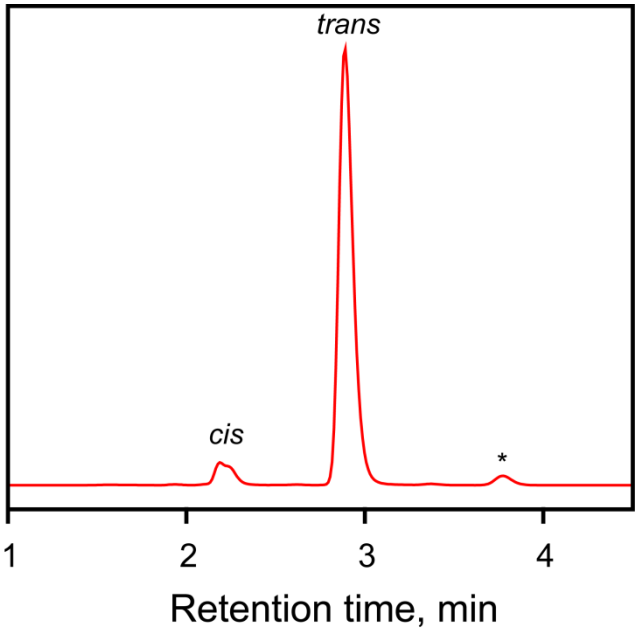

Figure S4. HPLC trace of *trans*-AzoPhe-Phe-OH (purity 98%) in ACN-water showing major *trans* and minor *cis* population; an impurity of *trans*-azobenzene is present in 2% content, marked as *.

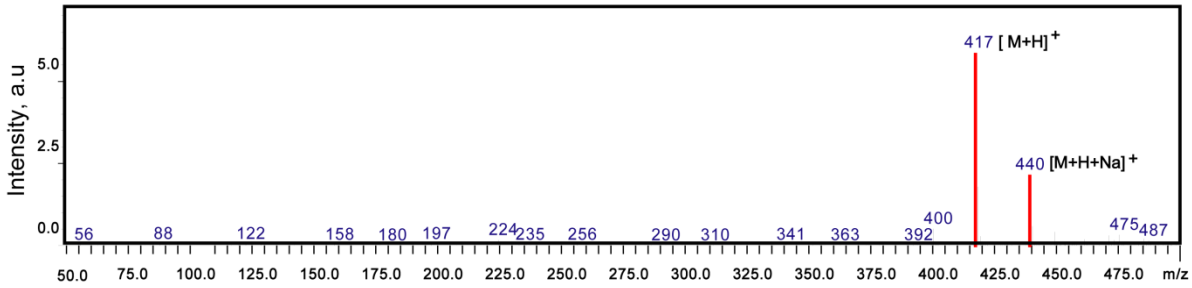

Figure S5. ESI-MS spectra of AzoPhe-Phe-OH (MW 416) in methanol.

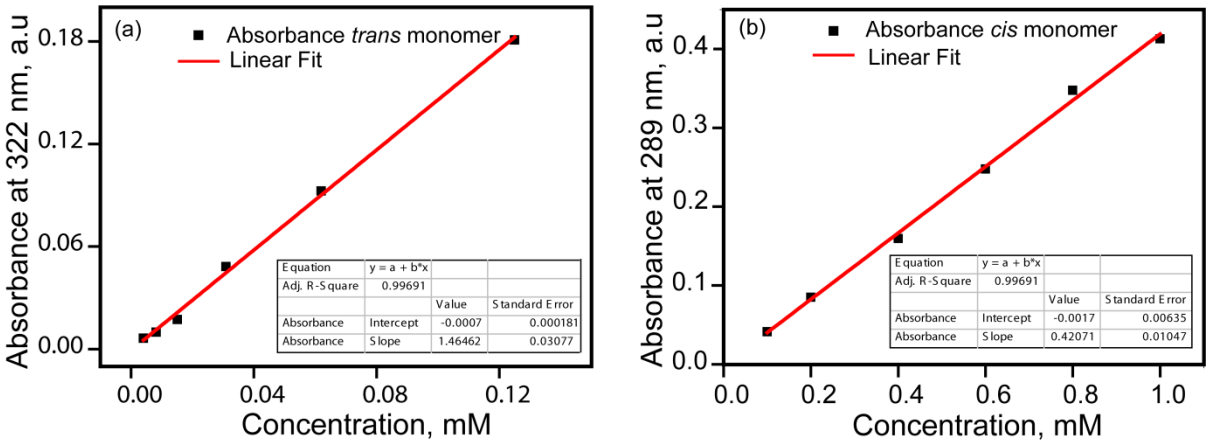

Figure S6. (a) The plot of absorbance for *trans* monomer at 322 nm (π - π* transition) versus the concentration of peptide in MeOH solvent and linear fit of the plot (red trace). **(b)** The plot of absorbance for monomer of *cis* isomer at 289 nm (π - π* transition) versus the concentration of peptide in MeOH solvent and linear fit of the plot (red trace).[3]

Absorbance, A = ϵ. C. d, where 'C' is concentration, 'd' is length of cuvette.

Molar extinction coefficient, ϵ = (Δ A/ ΔC) * d

The length of the cuvette used for measurement is 1 mm.

Molar extinction coefficient for *trans* isomer in monomeric form, ϵ =14646 ± 307 $M^{-1}cm^{-1}$

Molar extinction coefficient for *cis* isomer in monomeric form, ϵ = 4207 ± 104 $M^{-1}cm^{-1}$

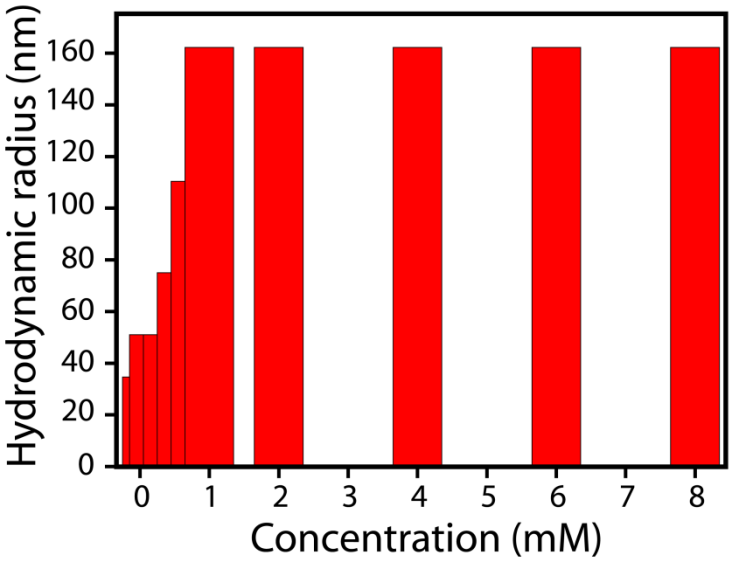

Figure S7. The histogram shows the size of the aggregated peptide at different concentration in methanol: water solvent system.

The *trans-to-cis* photo-isomerization of azo functional group within the aggregate shows a red shift in π - π* transition of azo functional group to 298 nm (ϵ =1720 ± 180 $M^{-1}cm^{-1}$) as compared to monomer of *cis* isomer, and n - π* transition has a maxima at 430 nm (Figure S8a)

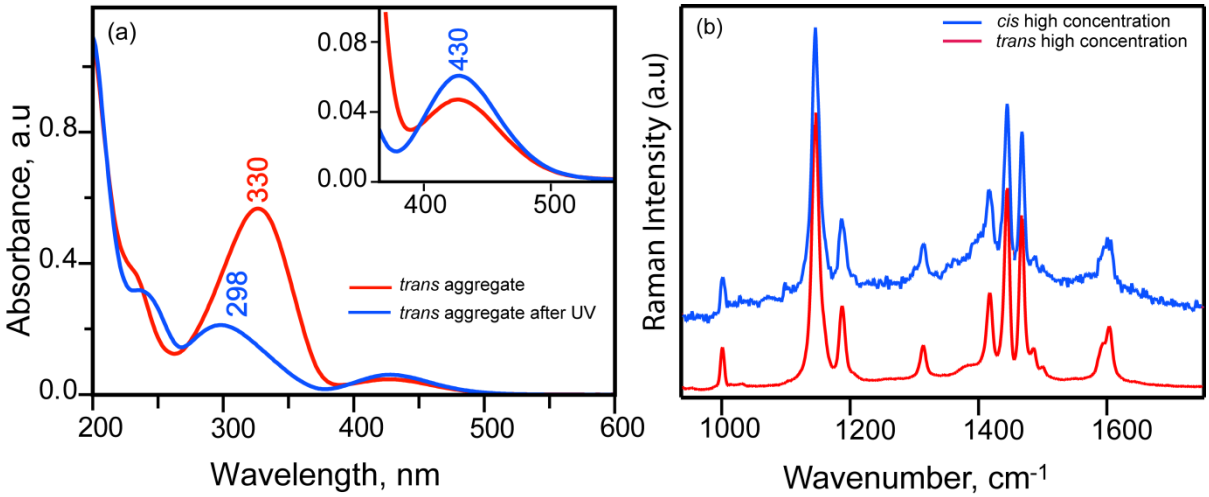

Figure S8. (a) Main: Red trace shows the steady state absorption spectra of *trans* isomer in aggregated form (1 mM). Inset: zoomed in region showing n-π* transition of Azo functional group. Blue trace corresponds to absorption spectra after the *trans*-to-*cis* photoisomerization of the azo functional group within the aggregated peptide using UV illumination (1 mM). **(b)** Red trace corresponds to SERS spectrum for *trans* aggregate and blue stands for the *cis* aggregate, formed by UV illumination on *trans* aggregate.

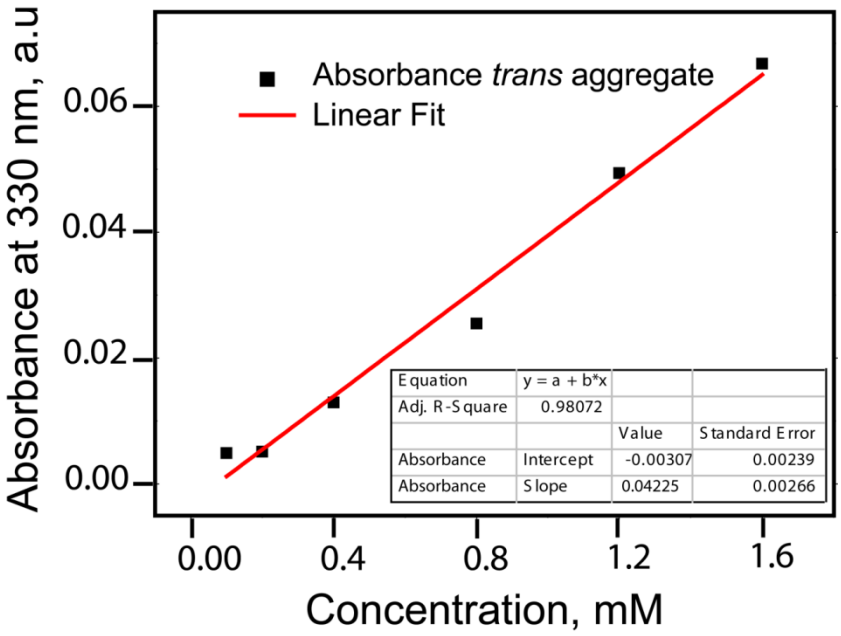

Figure S9. The plot of absorbance for *trans* aggregate at 330 nm (π - π* transition) versus the concentration of peptide in MeOH: H_2O solvent (1:12 ratio) and linear fit of the plot (red trace). The length of the cuvette used for measurement is 1mm.

Absorbance, A = ϵ. C. d, where 'C' is concentration, 'd' is length of cuvette.

Molar extinction coefficient, $\epsilon = (\Delta A / \Delta C) * d$

The length of the cuvette used for measurement is 1mm.

Molar extinction coefficient for *trans* isomer in aggregate form, $\epsilon = 422 \pm 26\ M^{-1}cm^{-1}$

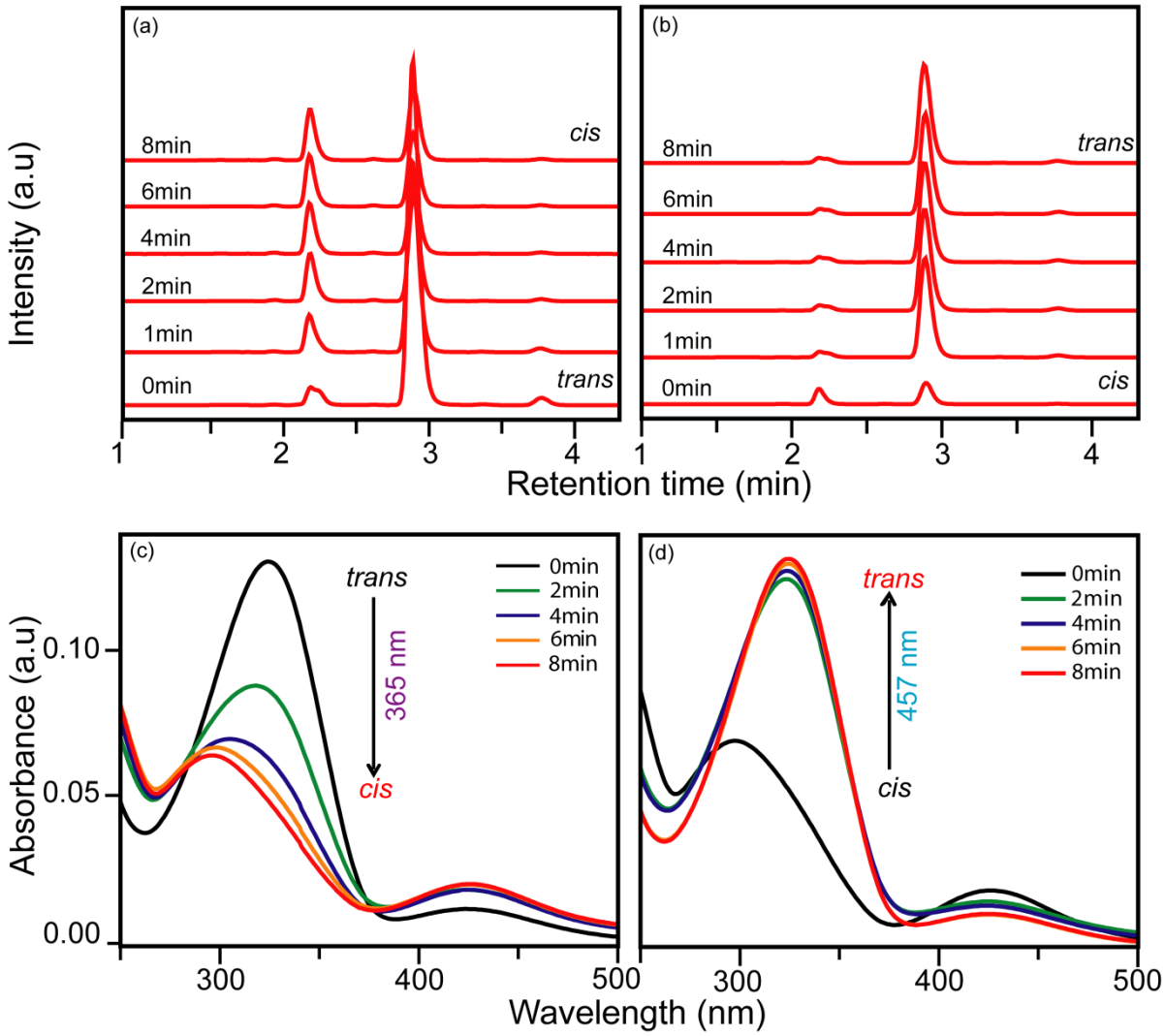

Figure S10. (a) HPLC traces of *trans*-to-*cis* isomerization at different durations of light illumination using UV source (365 nm, 1.656 mW/cm^2). **(b)** HPLC traces of *cis*-to-*trans* isomerization at different durations of light illumination by LED source (457 nm, 203 mW/cm^2). **(c)** The transient changes in the absorption spectra during *trans*-to-*cis* photo-isomerization. **(d)** The transient changes in the absorption spectra during *cis*-to-*trans* photo-isomerization.

Quantum yield of isomerisation for *trans*-to-*cis* isomerisation using 365 nm UV lamp (8 min) is given by

$$\Phi = \frac{\text{Number of moles of } cis \text{ isomer formed(N)}}{\text{Number of moles of photons absorbed by molecule /unit time/unit area}}$$

$$N = \frac{\text{Change in area under } cis \text{ isomer peak} * \text{volume injected}}{\text{molar extinction coefficient of } \pi - \pi^* \text{ transistion} * \text{optical pathlength}}$$

Number of moles of photons absorbed by molecule/ unit time/ unit area = $0.0984898 * 10^{-4}$

$$\Phi = \frac{1995.992 * 10 * 10^{-6}\text{L}}{4207\ \text{mol}^{-1}\text{cm}^{-1} * 1\ \text{cm} * 0.0984898 * 10^{-4}}$$

$$\boldsymbol{\Phi = 0.492}$$

Quantum yield of isomerisation for *cis*-to-*trans* isomerisation using 457 nm LED source (2 min) is given by

$$\Phi = \frac{\text{Number of moles of } trans \text{ isomer formed(N)}}{\text{Number of moles of photons absorbed by molecule /unit time/unit area}}$$

$$N = \frac{\text{Change in area under } trans \text{ isomer peak} * \text{volume injected}}{\text{molar extinction coefficient of } \pi - \pi^* \text{ transistion} * \text{optical pathlength}}$$

Number of moles of photons absorbed by molecule/ unit time/ unit area = $0.4748 * 10^{-4}$

$$\Phi = \frac{262243 * 10 * 10^{-6}\text{L}}{14646\ \text{mol}^{-1}\text{cm}^{-1} * 1\ \text{cm} * 0.4748 * 10^{-4}}$$

$$\boldsymbol{\Phi = 0.3765}$$

We tried to modulate the morphology of the fibril by performing *trans*-to-*cis* photo-isomerization of the pendant azo group within the fibrillar aggregate. To track the morphology switching we have performed SERS measurements at different timescales of UV irradiation. Figure S11, S12 show the SERS spectra at 0 min, 5 min, 15 min, 30 min, and 60 min of UV irradiation. The new features at 1645 cm^{-1}, 1512 cm^{-1}, and 1362 cm^{-1}significant to structural transition are observed only after 15 min of UV irradiation. In the low frequency spectral region new feature corresponding to *cis* azobenzene is observed at 772 cm^{-1}. A significant enhancement is observed for peak at 611 cm^{-1}, one of the characteristic of *cis* azo moiety.

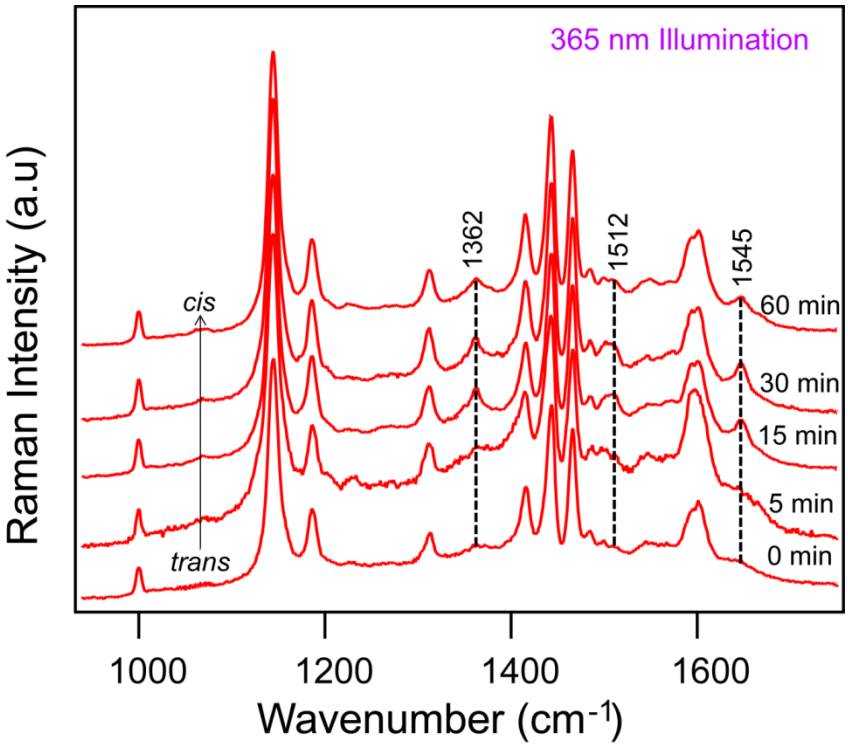

Figure S11. SERS traces of *trans*-to-*cis* isomerisation of aggregated AzoPhe-Phe-OH (4 mM) at different durations of light illumination using UV source at 365 nm.

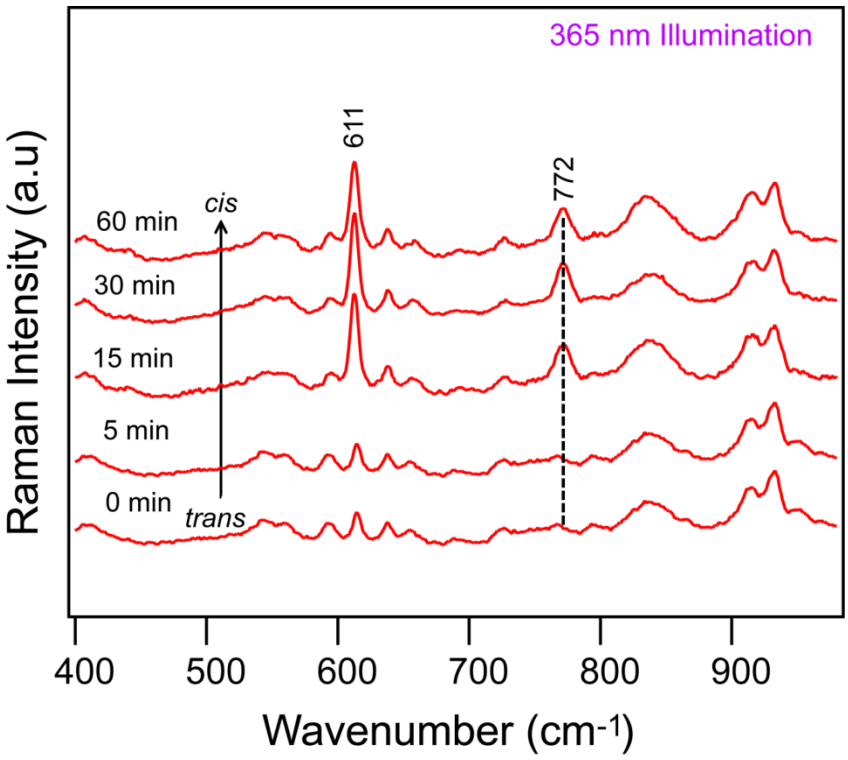

Figure S12. SERS traces of *trans*-to-*cis* isomerisation of aggregated AzoPhe-Phe-OH (4 mM) at different durations of light illumination using UV source at 365 nm.

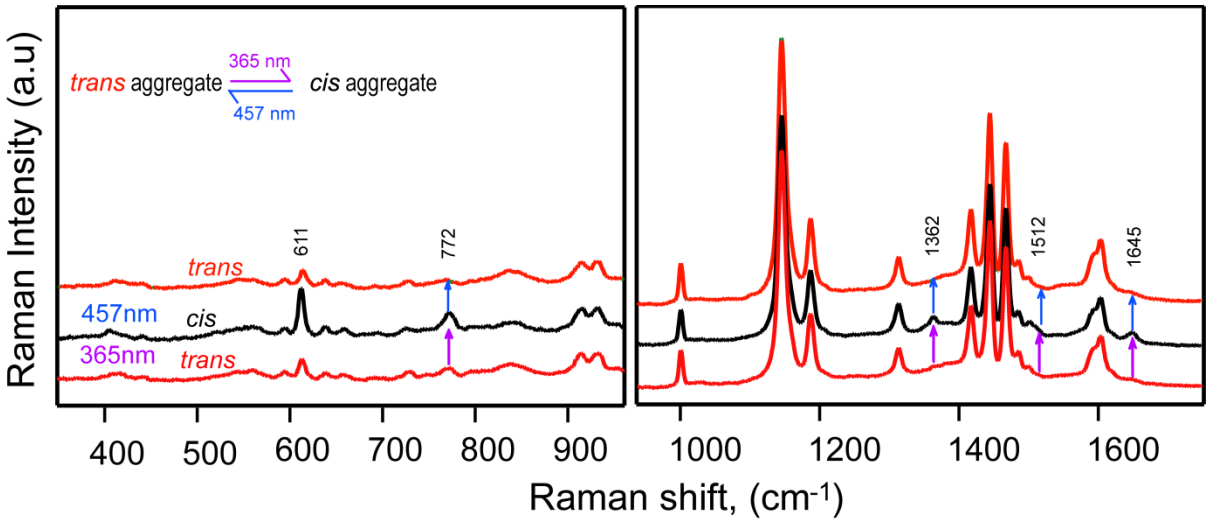

Figure S13. SERS spectra showing the reversibility of the morphology switch. The SERS spectra of aggregated peptide in *trans* isomeric form with fibrillar morphology (bottom red trace), SERS spectra corresponding to the aggregated peptide after *trans*-to-*cis* isomerization of azo functional group (black trace), SERS spectra of the aggregated peptide after *cis*-to-*trans* isomerization of the azo functional group using 457 nm LED illumination (top red trace) and it shows spectra similar to that of initial fibril morphology.

We have also performed centrifugation of the aggregated peptide in *trans* isomeric form (fibril) with 13000 rpm for 30 min to separate out the aggregated fraction of the peptide and the soluble fraction (preferably monomer) from the same peptide solution. SERS measurements were also done for both the aggregated (precipitate) and the clear solution before and after UV irradiation. SERS shows changes in the peptide secondary structure upon UV irradiation only for the aggregated fraction and it shows vibrational features corresponding to fibril-to-vesicle morphology switching (1362 cm^{-1}, 1512 cm^{-1}, 1645 cm^{-1}). The vibrational feature does not change for the soluble portion of the peptide upon isomerization. This hints towards the formation of vesicle from fibrillar starting states not from aggregation of *cis* monomer.

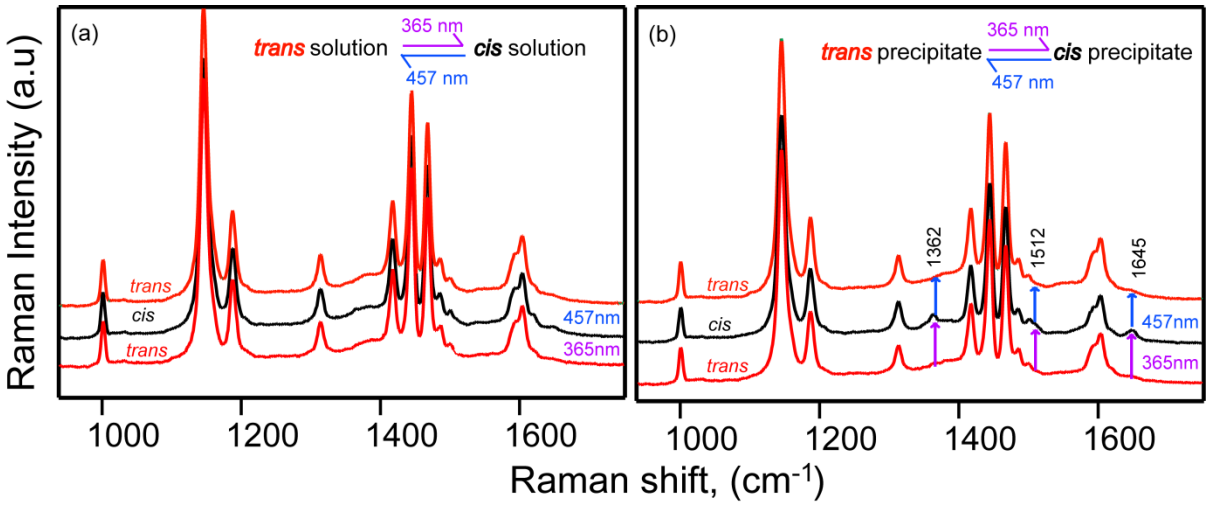

Figure S14. Figure shows the SERS spectra of the reversible *trans*-to-*cis* photo-isomerization of the monomer and aggregated fraction of peptide in solution. Figure **(a)**: SERS spectra of photo-isomerization of soluble fraction (preferably monomer) of the peptide solution after centrifugation. Figure **(b):** SERS spectra of photo-isomerization of precipitated fraction of the peptide solution (aggregate). The SERS spectra of peptide in *trans* isomeric form (bottom red trace); SERS spectra of peptide after *trans*-to-*cis* isomerization of azo functional group (black trace); SERS spectra of the peptide after *cis*-to-*trans* isomerization of the azo functional group using 457 nm LED illumination (top red trace).

Table 1

Raman Frequency (cm^{-1}) of *trans* monomer	**Raman Frequency (cm^{-1}) of *trans* aggregate**	**Mode Assignments**
1002	1002	Symmetric ring stretch (29,30,31,32)
1020	-	δ(CH) (29,30,34,35,36)
-	1060	τ NH_2(30)
1143	1143	ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1183	1183	ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1308	1308	δ (CCH)+ δ(NCC)(31,32)
1390	-	ν(COO)+ δ(C_α-H)(29,34,35,36)
1412	1412	COO symmetric stretch (30,34)
1443	1443	ν(NN)+ δ(CNN)+δ(NCC)+δ(CCH) (31,32)
1465	1465	ν(NN)+ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1484	1484	ν(CC)+ν(NN)+δ(CCH)(31,32)
-	1540	ν(C-N),δ(N-H)(Amide II)(37), β sheet
1592	1592	Ring vibrations(Phe)(29,31,32,36)
1601	1601	ν(C-N),ν(C-O) (Amide I), Ring vibrations (Phe)(29,33,37)
-	1634 to 1685	ν(C-N),ν(C-O)(Amide I), antiparallel β sheet (37,)

Table 2

Raman Frequency (cm^{-1}) of *trans* aggregate	**Raman Frequency (cm^{-1}) of *cis* aggregate**	**Mode Assignments**
611	611	δ (CCC)+ τ(CNNC)(31,32)
-	772	τ(CCCH)+ τ(CCCC)+ τ(CNNC)(31,32)
1002	1002	Symmetric ring stretch(29,30,31,32)
1060	1060	τ NH_2(30)
1143	1143	ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1183	1183	ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1308	1308	δ (CCH)+δ (NCC) (31,32)
-	1362	δ (C_α-H)+ν(C-N)+ τ(NH)(33,37)
1412	1412	COO symmetric stretch (30,34)
1443	1443	ν(NN)+ δ(CNN)+δ(NCC)+δ(CCH) (31,32)
1465	1465	ν(NN)+ν(CN)+δ(CNN)+δ(NCC)+δ(CCH)(31,32)
1484	1484	ν(CC)+ν(NN)+δ(CCH)(31,32)
-	1512	ν(CC)+ δ(CCC)+(δ(CCH)(31,32)
-	1535	ν(C-N),δ(N-H)(Amide II)(29,37)
1540	1540	ν(C-N),δ(N-H)(Amide II) (29,33,37)
1592	1592	Ring vibrations(29,31,32,36)
1601	1601	ν(C-N),ν(C-O) Amide I, Ring vibrations

		(Phe)(29,31,32,36)
-	1645	ν(C-N),ν(C-O)(Amide I) (33,36,37) α helix or random coil

References:

1. M. Bose, D. Groff, J. Xie, E. Brustad and P. G. Schultz, *J. Am. Chem. Soc.*, 2006, **128**, 388-389.
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