Controlled self-assembly of modified aromatic amino acids

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Abstract

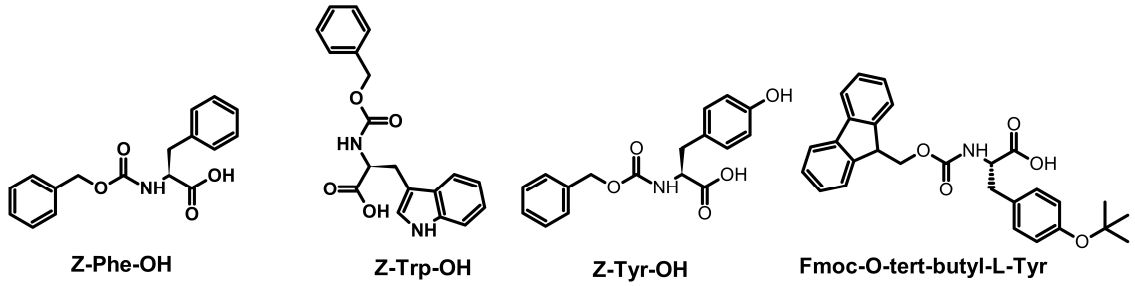
We report the self-assembly of carbobenzoxyphenylalanine (**Z-Phe-OH**), carbobenzoxytryptophan (**Z-Trp-OH)**, carbobenzoxytyrosine (**Z-Tyr-OH**) and *N*-(9-Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-tyrosine **(Fmoc-Tyr(tbu)-OH)** to well-defined morphologies such as fibers, spherical and flower-like self-assembled structure. The self-assembled structure formed by modify single amino acids were characterized through various microscopic and spectroscopic techniques. The self-assembled structure formation was studied extensively under varying concentrations and temperature and the mechanism of self-assembled structure formation was extensively studied through solution state ^{1}H NMR. The self-assembly studies on modified amino acid provide an important avenue for the simple fabrication and design of novel materials with immense applications. Hence, **Z-Phe-OH, Z-Trp-OH, Z-Tyr-OH,** and **Fmoc-Tyr(tbu)-OH** self-assembly studies may be a pertinent extension in this direction.

Keywords: Self-assembly; modified amino acid; varying concentration; varying temperature; spheres, fibers.

Introduction

Gazit and coworkers for the very first time reported the formation of well-ordered tubular structures by dipeptide diphenylalanine (FF) and used it for the fabrication of silver nanowires.[1] Following, this research the group has studied extensively the self-assembling

behaviour of short aromatic peptides and suggested the crucial role of aromatic stackings in their self-assembly.[2] The group further reported amyloid-like self-assembled structure formed by Boc-Phe–Phe-COOH, Z-Phe–Phe-COOH, and Fmoc-Phe–Phe-COOH.[3] Panda et al. reported self-assembly of N-(tertButoxycarbonyl)-S-trityl-L-cysteine to nanobowls type of structure which can be used for the anticancer drug delivery.[4] In the past few decades, there has been a considerable amount of research which is pursued in single amino acids and modified single amino acids.[5-12] In this context, Gazit et al reported amyloid-like structure formed by phenylalanine,[5] tyrosine[6], and tryptophan.[7] Sarkar et al. reported the fibrillar structure of glycine.[13] Wangoo and co-workers demonstrated the self-assembly of aromatic single amino acids,[14-16] and studied them extensively via various biophysical assays such as FTIR, NMR, TGA, and XRD.[8, 9, 13, 14]



Scheme 1: Chemical structure of **Z-Phe-OH, Z-Trp-OH, Z-Tyr-OH,** and **Fmoc-Tyr(tbu)-OH.**

Since our group has been working on the molecular self-assembly and assessing the self-assembly of the single amino acids,[8, 9, 17, 18] modified single amino acids,[19] peptides,[20-26] nanoparticle/nanomaterial,[27-29] and heterocyclic[30-35] compounds. Our group reported for the very first time the amyloid-like self-assembled structure formed by the non-aromatic single amino acid cysteine and methionine[8]. Recently our group has also reported the amyloid-like structure formed by proline and hydroxyproline.[9] Hence, from their study we were motivated to assess the self-assembling propensity of **Z-Phe-OH, Z-Trp-OH, Z-Tyr-OH,** and **Fmoc-Tyr(tbu)-OH.** Our study revealed that **Z-Phe-OH** gives a fiber-like self-assembled structure

at room temperature and at higher temperature near 70 ^{0}C, while **Z-Trp-OH** gives the bunch of flowers like self-assembled structure at room temperature which on heating at 70 ^{0}C becomes more distinct. However, **Z-Tyr-OH** do not form any self-assembled structure at lower and higher concentrations at room temperature and on heating as well, **Fmoc-Tyr(tbu)-OH,** on the other hand forms a sphere-like self-assembled structure at room temperature while on heating at 70 ^{o}C there is a morphological transition from sphere to fibers.

Result and Discussion

Our group has been interested to study the self-assembly of single amino acids and modified single amino acids. Hence, we studied the self-assembling properties of **Z-Phe-OH, Z-Tyr-OH, Z-Trp-OH, and Fmoc-Tyr(tbu)-OH** under various concentration and temperature. A 20 mM stock solution of **Z-Phe-OH, Z-Tyr-OH, and Z-Trp-OH** was prepared in 50 % aqueous methanol, while the stock solution of **Fmoc-Tyr(tbu)-OH** prepared in only methanol due to its lower solubility. The self-assembly studies were done by diluting the stock solution in deionized water at various concentrations. The self-assembled morphology of **Z-Phe-OH, Z-Tyr-OH, Z-Trp-OH and F-moc-Tyr(tbu)-OH** were studied from the 1 to 10 mM concentration range.

Z-Phe-OH (3mM) self-assemble to dense fibre like structures under all concentrations and temeperature. We cannot observe any morphological change under varying concentration and temperature (Figure 1). The fibres have non crystalline nature as can be assessed by phase contrast microscopy (Figure 2).. The fibres also bind ThT dye (Figure 3).

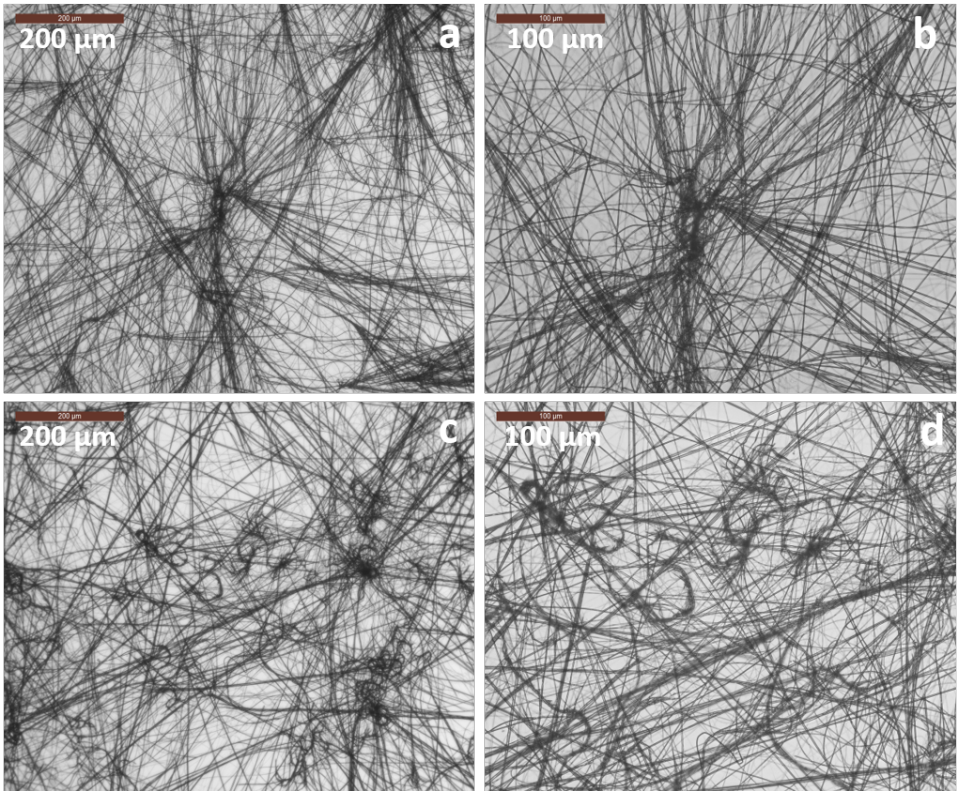


Figure 1: Optical microscopy images of self-assembled structure of **Z-Phe-OH** at 3 mM concentration (a) at room temperature under 10X, scale bar 200 µm; (b) at room temperature under 20X, scale bar 100 µm; (c) at 70 ^{O}C under 40 X, scale bar 50 µm; (d) at 70 ^{O}C under 63X, scale bar 20 µm.

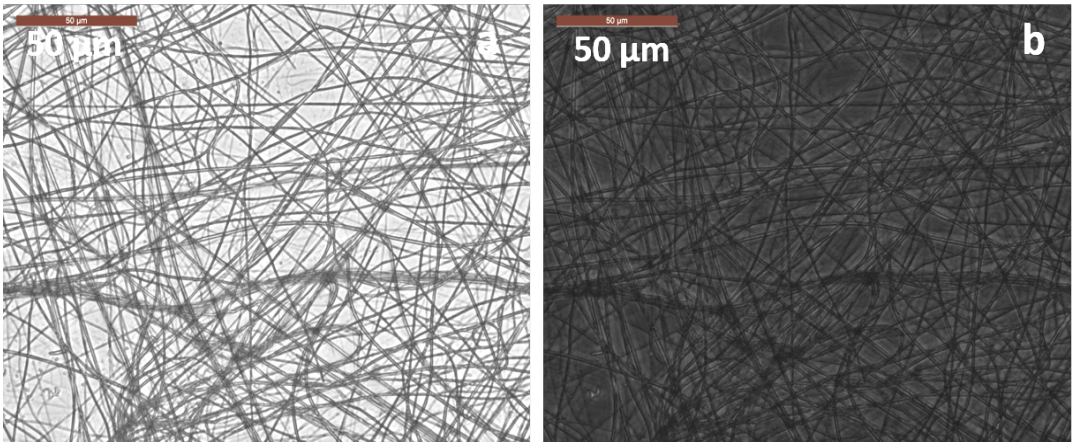


Figure 2: Microscopy image of **Z-Phe-OH** at 3 mM (a) under bright field; (b) under phase contrast.

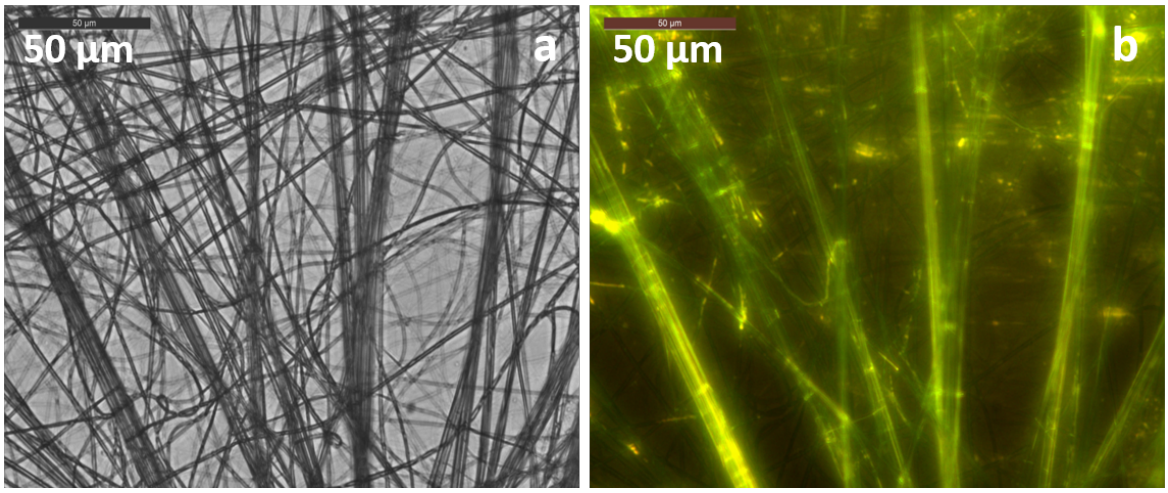


Figure 3: ThT binding with **Z-Phe-OH** aggregates (a) under bright field; (b) in the green filter.

The self-assembled structure of the other modified aromatic amino acid **Z-Trp-OH** was also studied and it revealed flower-like morphologies. At room temperature, **Z-Trp-OH** formed the bunch of flowers while at the higher temperature these bunch of flowers may be converted into distinct flower and more clarity could be observed in the self-assembled structure. To compare the morphologies formed by the self-assembly of **Z-Phe-OH** and **Z-Trp-OH**, we were motivated to assess the self-assembly of **Z-Tyr-OH** too. However, in **Z-Tyr-OH** we could not observe any assembly under varying concentration or temperature. This may be because of free –OH group in **Z-Tyr-OH** which is absent from both **Z-Phe-OH** and **Z-Trp-OH**, The extra –OH group might destabilize the pi-pi stacking interaction which are operating in case of **Z-Tyr-OH** which is the main force responsible for inducing self-assembly in aromatic amino acids. Hence, we also studied the self-assembly of **F-moc-Tyr(tbu)-OH** to assess if it could form self-assembly.

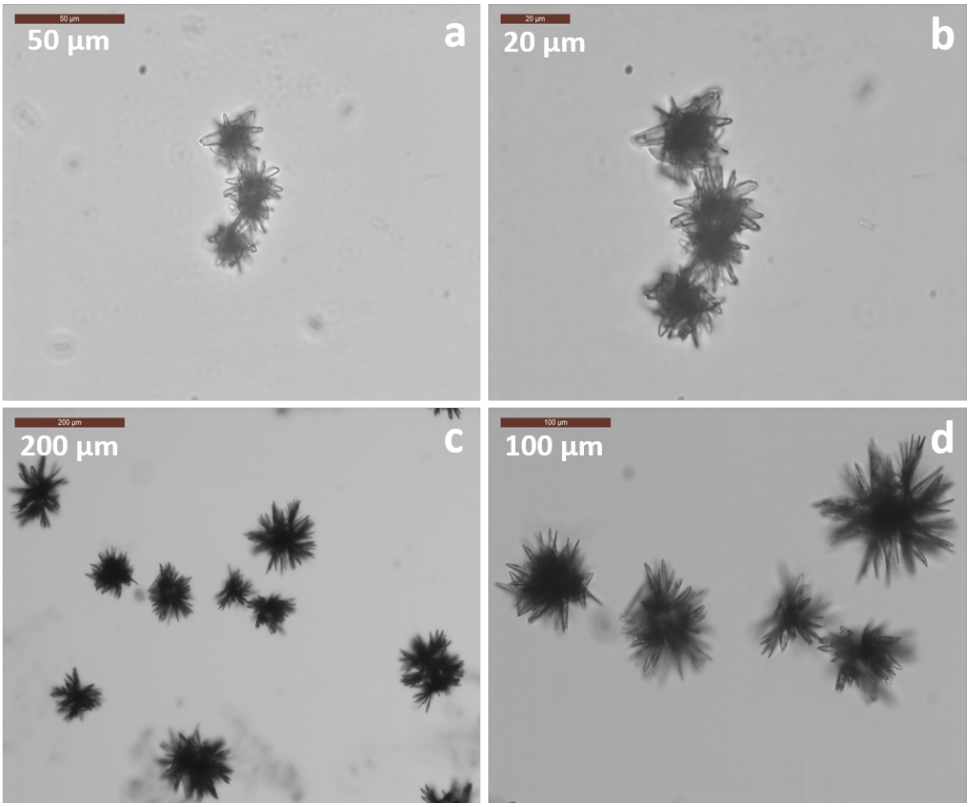


Figure 5: Optical microscopy images of self-assembled structure of **Z-Trp-OH** (3mM) at room temperature in water different magnification (a) under 40X, scale bar 50 µm; (b) under 63X, scale bar 20 µm; Heating at 70 ^{0}C(c) under 10 X, scale bar 200 µm; (d) under 20X, scale bar 100 µm.

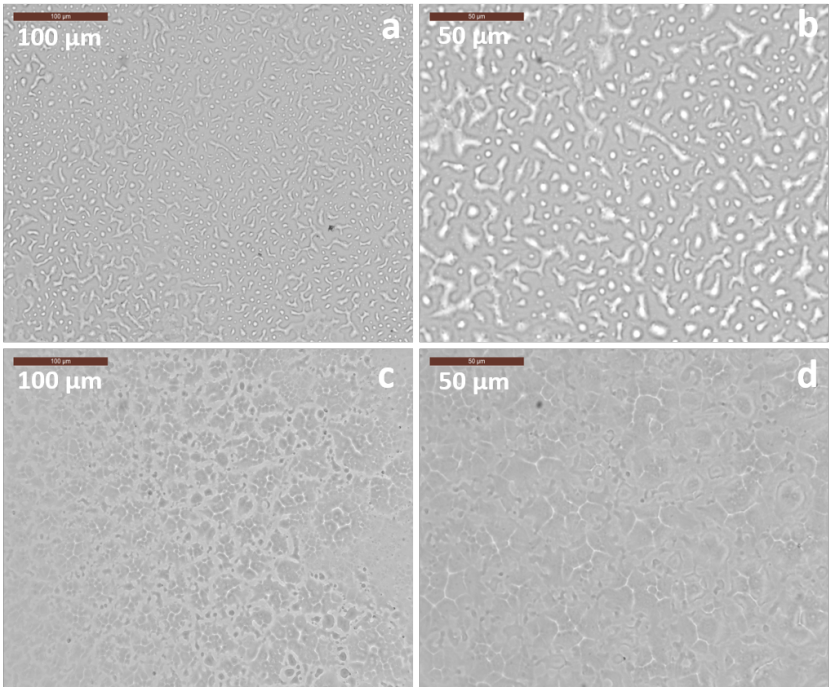


Figure 6: Optical microscopy images of self-assembled structure of **Z-Tyr-OH** (3mM) at room temperature in water different magnification (a) under 40X, scale bar 50 µm; (b) under 63X, scale bar 20 µm; Heating at 70 ^{0}C(c) under 10 X, scale bar 200 µm; (d) under 20X, scale bar 100 µm.

Interestingly, **F-moc-Tyr(tbu)-OH** (5mM) forms sphere-like assemblies at room temperature which may be converted to fibers-like structure on heating at 70 ^{0}C. As the concentration was increased the thickness and the density of the nano/microtubes kept on increasing without leading to any morphological transition in **Z-Phe-OH.** Moreover, **Fmoc-Tyr(tbu)-OH** we could not see any fibril formation at the concentration below 5 mM. In the case of **Fmoc-Tyr(tbu)-OH,** it formed a destructive aggregate at lower concentration instated of fibrils structure. We can observe which may be combined and formed the self-assembled fibrils at 5 mM concentration.

Further, the phase-contrast study also supports the fibrils would be amorphous in nature because crystalline material would be easily seen under phase-contrast microscopy. Interestingly, **Z-Phe-OH** assemblies could also bind with Thioflavin T (ThT) and fibers show green fluorescence indicating efficient binding.

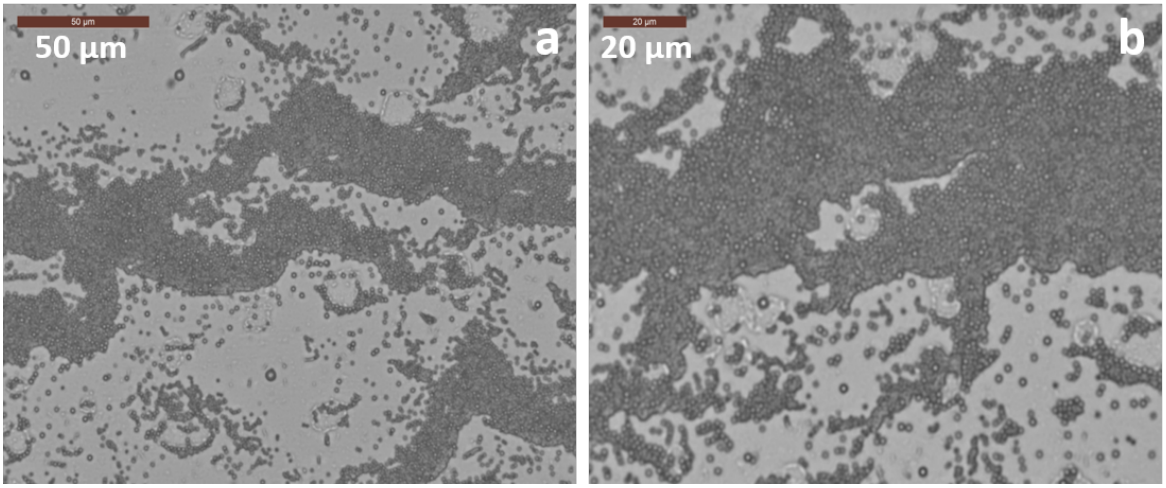


Figure 7: Optical microscopy images of the self-assembled structure of **Fmoc-Tyr(tbu)-OH** at 5 mM concentration under different magnification at room temperature (a) under 40X, scale bar 50 µm; (b) under 63X, scale bar 20 µm.

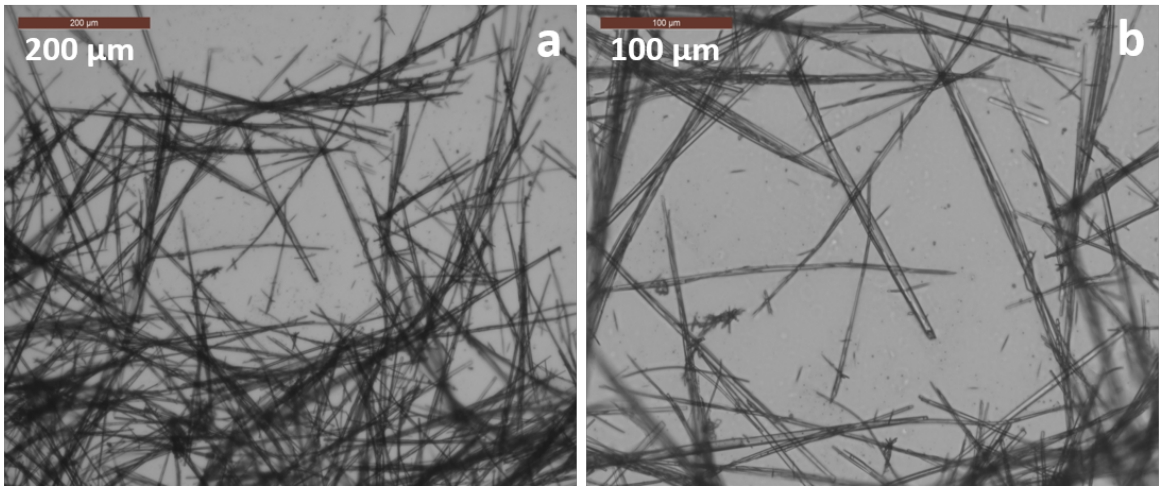


Figure 8: Optical microscopy images of self-assembled structure of **Fmoc-Tyr(tbu)-OH** at 5 mM concentration under different magnification at 70 ^{0}C (a) under 10X, scale bar 200 µm; (b) under 20X, scale bar 100 µm.

Table 1: Describe the recap of the self-assemble structure formed by **Z-Phe-OH, Z-Tyr-OH, Z-** and **Fmoc-Tyr(tbu)-OH** in different conditions.

Name of sample	Concentration	Morphology	Temperature
Z-Phe-OH	Lower concentration	Fibrils	RT

Z-Phe-Oh	Higher concentration	dense fibrils	RT
Z-Phe-OH	Lower concentration	Fibrils	70 ^{0}C
Z-Phe-Oh	Higher concentration	dense fibrils	70 ^{0}C
Z-Tyr-OH	Low concentration	Assembly not observed	RT
Z-Tyr-OH	Higher concentration	Assembly not observed	RT
Z-Tyr-OH	Lower concentration	Assembly not observed	70 ^{0}C
Z-Tyr-OH	Higher concentration	Assembly not observed	70 ^{0}C
Z-Trp-OH	Low concentration	Flower	RT
Z-Trp-OH	Higher concentration	Flower	RT
Z-Trp-OH	Lower concentration	Bunch of flowers	70 ^{0}C
Z-Trp-OH	Higher concentration	Bunch of flowers	70 ^{0}C
Fmoc-Tyr(tbu)-OH	Lower concentration	Assembly not observed	RT
Fmoc-Tyr(tbu)-OH	Higher concentration	Spheres	RT
Fmoc-Tyr(tbu)-OH	Lower concentration	Assembly not observed	70 ^{0}C
Fmoc-Tyr(tbu)-OH	Higher concentration	Fibrils	70 ^{0}C

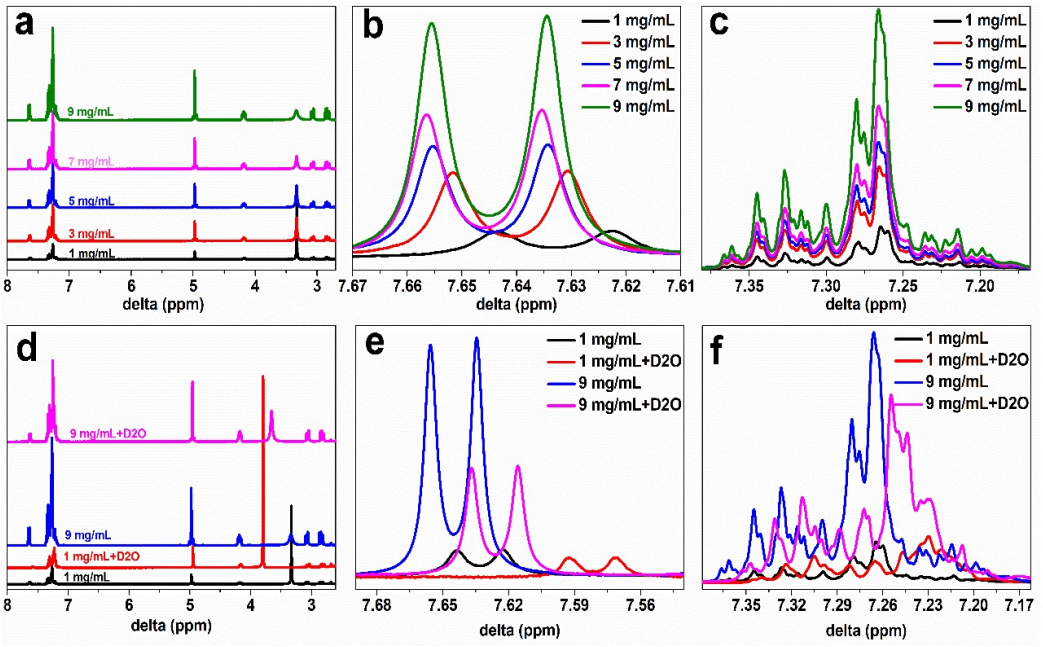


Figure 9: ^{1}H NMR spectra of **Z-Phe-OH** at concentration 1, 3, 5, 7, and 9 mg/mL in DMSO-d_6 (a) Full spectra; expansion in (b) 7.67-7.61 ppm; (c) 7.35-7.20 ppm; on addition of drop of D_2O in DMSO-d_6 sample (d) full spectra; expansion (e) 7.68-7.56 ppm; (f) 7.35-7.14 ppm.

To understand the mechanism of the self-assembly and structure formation we performed the concentration dependent[36, 37] liquid-state ^{1}H NMR..

The concentration-dependent ^{1}H NMR study was performed at concentrations 1, 3, 5, 7, and 9 mg/mL in DMSO-d_6 at ambient temperature. The results revealed that as we increased the concentration, peak broadening was observed between 7.19-7.36 ppm in the aromatic region, and no significant shifting. The -NH proton of carbamate group on the other hand shows significant deshielding as we increased the concentration of **Z-Phe-OH** from 1 to 9 mg/mL in DSMO-d_6. The carbamate -NH proton shows the doublet at 7.63 ppm in 1 mg/mL concentration whereas at 9 mg/mL this doublet shifted to 7.65 ppm which may occur due to the presence of hydrogen bonding. To understand more about the self-assembled structure formation, we have added the drop of D_2O in the DMSO-d_6 sample and recorded the NMR

spectra. The study demonstrated that the aromatic peak of **Z-Phe-OH** becomes shielded and shows the upfield shifting on the addition of D_2O. The aromatic peak in 1 mg/mL **Z-Phe-OH** shows the peak at 7.17-7.37 ppm which shifted on the addition of D_2O to 7.18-7.35 ppm which clearly shows that there might be the presence of pi-pi stacking, also the main peak shifting from 7.26 to 7.23 ppm which indicated the presence of pi-pi stacking in the aggregated state. There are various literature which suggest presence of pi-pi stacking causes upfield shifting of the peak as more aggregates are formed due to increased concentration.[35, 36]The details of peak shifting on increasing the concentration of **Z-Phe-OH** in DMSO-$d_6$and on the addition of a drop of D_2O described in table 1.

Table 2: NMR peak value of **Z-Phe-OH** in ppm in DMSO-d_6 and on the addition of a drop of D_2O.

Concentration of sample in mg/mL	**Characteristic peak**	**Peak value (ppm) In DSMO-d_6**	**Peak value (ppm) after addition of a drop of D_2O in DMSO-d6 sample**
1	Aromatic	7.26(m)	7.23(m)
	-NH	7.63(d)	7.58 (d)
3	Aromatic	7.28 (m)	7.26 (m)
	-NH	7.64(d)	7.57 (d)
5	Aromatic	7.28(m)	7.26 (m)
	-NH	7.64(d)	7.60 (d)
7	Aromatic	7.28(m)	7.25(m)
	-NH	7.65(d)	7.61(d)
9	Aromatic	7.28(m)	7.25(m)
	-NH	7.65(d)	7.63(d)

Conclusions

In conclusion, we report the self-assembly of **Z-Phe-OH, Z-Tyr-OH , Z-Trp-OH,** and **Fmoc-Tyr(tbu)-OH** to the formed sphere and fibers like self-assembled structures at different concentrations and temperatures. The self-assembled structure formation has been characterized by optical microscopy, ^{1}H-NMR spectroscopy. Our future endeavors will be focused to assess the self-assembly of **Z-Phe-OH** and **Fmoc-Tyr (tbu)-OH** in more detail by other spectroscopic and microscopic techniques.

Materials and Methods

General: All the modified amino acids were purchased from Sigma and used without further purification. The purity of all the amino acids procured was a minimum of 99%. Deionized water was used for preparing all the solutions.

Optical microscopy (OM):

A 20 mM stock solution of modified amino acids was prepared in 50 % aqueous methanol solution while **Fmoc-Tyr(tbu)-OH** was prepared in only methanol due to its less solubility in aqueous methanol and given a sonication of 5 minutes to improve the solubility. Further, dilution was done using deionised water to prepare various concentrations of modified amino acids. These solutions turned into turbid solutions after some sometime which further indicated presence of assemblies in solution. The structures formed by self-assembly were observed under an optical microscope (OM) by drop-casting 20 µL solution on a clean glass slide. Furthermore, the same solutions were heated at 70°C and then drop-casting of 20 µL solution was done on a glass slide. The optical microscopy (OM) images were taken under Leica DM2500 upright fluorescence microscope with different magnifications and under different modes. The sample solutions were dried at room temperature before imaging.

Concentration-dependent ^{1}H NMR spectroscopy: The concentration-dependent ^{1}H NMR study was performed for **Z-Phe-OH** at 1, 3, 5, 7 and 9 mg/mL concentration in DMSO-d_6 in subsequently drop of D_2O addition by using 400 MHz NMR instrument at 25 $^{\circ}$C.

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Conflicts of interest

There is no conflict of interest to declare.

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