Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Interaction of monomeric and self-assembled aromatic amino acids with model membranes: Self-reproduction phenomena

Table of contents

1. Materials and Methods:

1.1 Materials: All the phospholipids, namely, DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), DMPG (1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)), DOTAP (1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)) and NBD-PE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt)) were purchased from Avanti Polar Lipids. The other chemicals, namely, N,N-dimethyl-6-propionyl-2-naphthylamine (PRODAN); 4-(2-hydroxyetyl)-1-piperazineethanesulfonic acid (HEPES); Thioflavin T (ThT) and the amino acids L-Phenylalanine (Phe), L-Tryptophan (Trp) and L-Histidine (His) were purchased from Sigma-Aldrich. The chemical structure of all the chemicals is shown in **Scheme S1**. All the solvents used in the experiments were of the spectroscopic grade obtained from Merck and used without further purification. Milli-Q water was used to prepare all of the solutions. The resistivity of the mili-Q water was 18.2 MΩ·cm at 25 °C in our set up. The required amount of ethanolic solution of PRODAN was taken in a volumetric flask and dried under vacuum to create a thin film of PRODAN. Then, an appropriate amount of buffer was added to it and sonicated for 2 hours. The final concentration of PRODAN was fixed at 1 μM.

1.2 Preparation of lipid vesicles: Lipid vesicles (DMPC, DMPC/DMPG and DMPC/DOTAP) were prepared by the thin-film hydration method. The ratio of zwitterionic and charged lipid was fixed at 8:2 for preparing the DMPC/DMPG and DMPC/DOTAP lipid vesicles. The required amount of lipids was dissolved in a mixture of chloroform and ethanol, and then the solvents were removed completely in a rotary evaporator under gentle conditions (P = 180 mBar, T = 30 °C). The flask containing a dry lipid film was placed under a high vacuum overnight to remove any residual solvent. The film was hydrated with a preheated HEPES buffer (I= 10 mM) solution, vortexed and stirred for 1 hour above the phase transition of the respective

lipid. Lipid dispersion was sonicated for clarity using ultrasonic cell disruptor. For the samples that included preparation of PRODAN-loaded lipid vesicles, we used freshly prepared PRODAN solution (1 μM) to hydrate the lipid film. To unravel any kind of formation of amyloid structures in solution phase we perform the Thioflavin T (ThT) binding assay. A stock solution of 40 μM ThT in PBS was added to the lipid-amino acids mixture to a final concentration of 20 μM ThT while lipid and amino acid concentration were fixed. For confocal microscopy, 1 mol% NBD-PE was mixed with the required amount of lipid vesicles, and then the thin film was hydrated with HEPES buffer.

1.3 Preparation of Lipid vesicles-amino acid mixture: For all steady-state and time-resolved experiments, the lipid concentration was fixed at 1 mM, and amino acids concentration varied from 0 to 10 mM. For confocal microscopy imaging, 1 mM lipid vesicles were incubated with 10 mM of amino acid. The excess vesicles and unbound amino acids were removed via centrifugation (10000 r/min, 1 min) and exchanged with mili-Q water 3 times. The final concentration of amino acid was around 3.5 and 6.1 mM for DMPC and DMPC/DMPG, respectively. Finally, the lipid-amino acid mixture was re-dispersed in water and vortex for 30 sec, to overcome any vesicle aggregation during the centrifugation process.

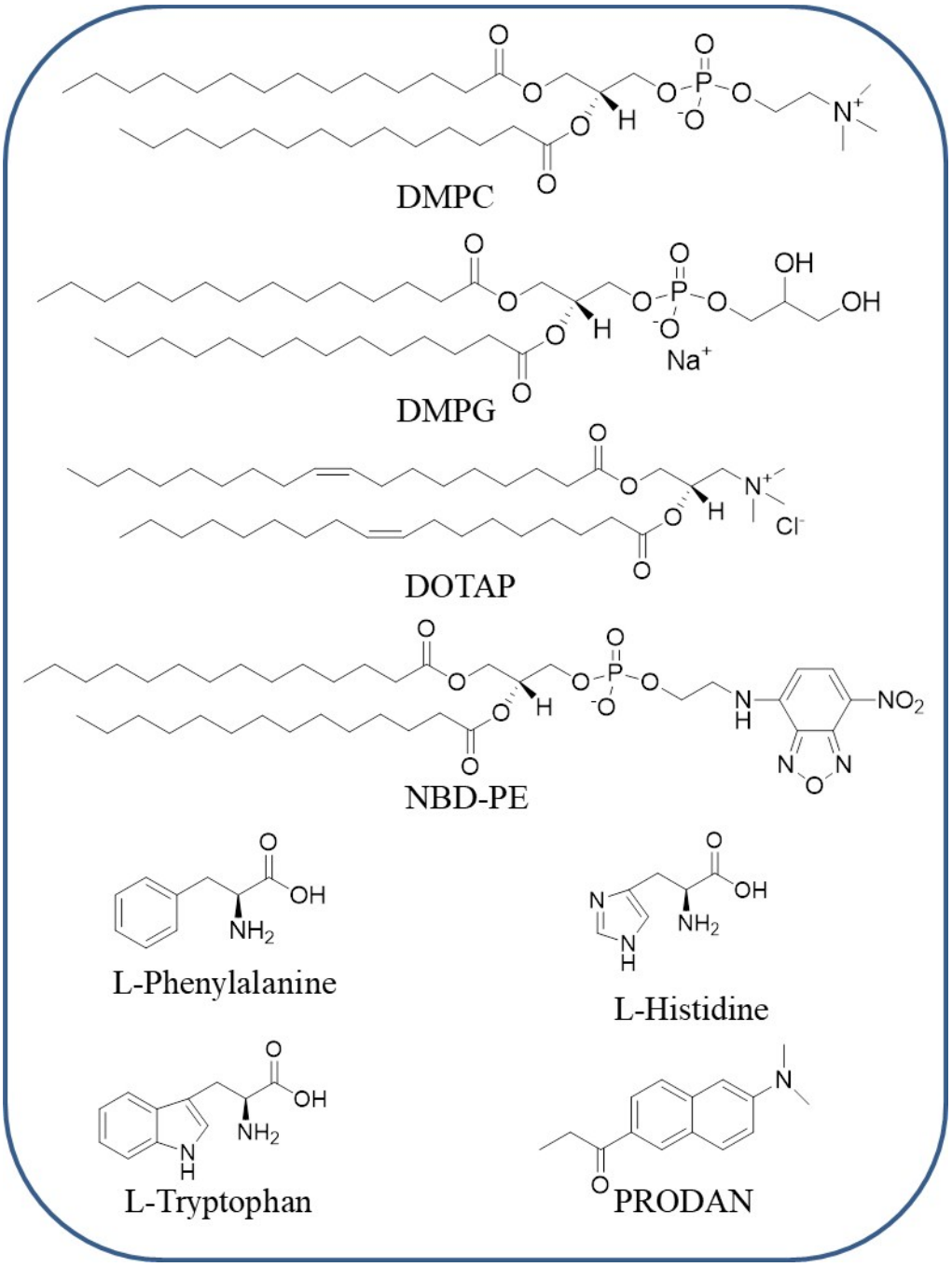
2. Instrumentation:

2.1 Steady-State Fluorescence Measurements: Steady-state fluorescence spectra were recorded using a Fluoromax-4p spectrofluorometer from Horiba JobinYvon (model: FM-100). The samples were excited at 375 nm. The fluorescence spectra were corrected for the spectral sensitivity of the instrument. The excitation and emission slits were 2/2 for almost all the PRODAN emission measurements. We maintained temperature (T) at 25°C throughout all the titration experiments. Before conducting the experiment, nitrogen gas was passed through the solution to remove any dissolved gas. For ThT binding assay, ThT emission spectra (excitation at 430 nm) were collected at 485 nm for each of the lipid-amino acid mixtures.

PRODAN exhibits an emission maximum at 540 nm in the aqueous medium. In the presence of lipid vesicles, another band emerges at 440 nm wavelength. The appearance of the new band at 440 nm is assigned to be the local excited (LE) state of PRODAN and it is a characteristic band of the gel phase of the lipid bilayer. Using this special spectral feature of PRODAN, general polarization plots are a very important tool to investigate the phase transition between liquid crystalline phase and gel phase of the lipid bilayer. the General polarization value provides a quantitative measurement of what proportion of the PRODAN molecules are surrounded mainly by the liquid-crystalline or mainly by gel-phase phospholipids. We estimated the General polarization (GP) by flowing equation.

$$GP = \frac{A_{440} - A_{490}}{A_{440} + A_{490}} \quad (1)$$

Where $A440$ and $A490$ are the emitted area under 440 nm and 490 nm, respectively.



Scheme S1: Chemical structures of DMPC, DMPG, DOTAP, NBD-PE, Phenylalanine, Tryptophan, Histidine and PRODAN.

2.2 Time-Resolved Fluorescence Measurements: For lifetime measurements, we used a picosecond TCSPC (time-correlated single

photon counting) machine from Horiba (Fluorocube-01-NL). The samples were excited at 375 nm using a picosecond diode laser (model: Pico Brite-375L), and the decays were collected at 440 nm. We used a filter on the emission side to eliminate the scattered light. The signals were collected at magic angle (54.75°) polarization using a photomultiplier tube (TBX-07C) as a detector with instrument response function ~140 ps. The data analysis was performed using IBH DAS Version 6 decay analysis software. Throughout all the titration experiments we maintained temperature (T) at 25 °C. The decays were fitted with a non-exponential function.

$$D(t) = \sum_{i=1}^{n} a_i exp(\frac{-t}{\tau_i}) \quad (2)$$

Here D(t) denotes normalized fluorescence decay and a_i is the normalized amplitude of decay components τ_i, respectively. The quality of the fit was judged by reduced chi-square (χ2) values and corresponding residual distribution. The acceptable fit has a χ^2 near unity.

The same setup was used for anisotropy measurements. For the anisotropy decays, we used a motorized polarizer in the emission side. The emission intensities at parallel and perpendicular polarizations were collected alternatively until a certain peak difference between parallel and perpendicular decay was achieved. The same software was also used to analyze the anisotropy data. The time-resolved anisotropy decay was described with the following equation:

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad (3)$$

Where *r(t)* is the rotational relaxation correlations function, $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the parallel and perpendicular components of the fluorescence and G is the correlation factor.

2.3 Confocal microscopy: For the confocal imaging of samples, we used a confocal microscope from OLYMPUS, model no. IX-83. A Multiline Ar laser (gas laser) with an excitation wavelength of 488 nm was used. The observation mode was LSM (laser scanning microscopy), the scan mode was XY, and the scan direction was one way. The samples were immobilized on a clean cover slide by spin coating at 750 rpm for 3 minutes and it was fixed by a glass slide in a sandwich manner by using transparent nail polish before imaging. To perform the time-lapse confocal imaging, an aliquot of the samples was drop cast on the imaging slide where a fixed particular area was chosen and monitored over a period of time during the drying of lipid-amino acid system. It is important to mention that, we study the amyloid-like fibril formation upon drying of the solution (presumably yielding a supersaturated solution).[1] To avoid photobleaching, we kept the illumination intensity very low. All the images and videos were processed through ImageJ software, with linear adjustments of brightness and contrast. All images studies were performed at room temperature.

Formation of amyloid-like fibril structures of these amino acids was also studied by confocal microscopy where amino acids (10 mM) are dyed with 0.2 mM ThT. The sample was prepared by adding 10 µL of 40 mM ThT dye to a 2 mL volume of amino acids, with excitation and emission wavelengths of 455 and 485 nm, respectively.

Atomic Force microscopy: Atomic force microscopy (AFM) images were taken by drop-casting the sample on mica substrates via tapping mode at a scan frequency of 0.65−1.0 Hz and were recorded using SmartScan software (model park NX10). It is important to mention that we are unable to perform the AFM microscopy at the same condition as for confocal microscopy. Formation higher aggregates of lipids or amino acid make the mica surface very rough and we find imaging at that condition is very difficult. So, to overcome this limitation we decide to use a very much lower concentration of amino acids. For AFM microscopy we add only 1 mM Phe to the zwitterionic or negatively charged lipid bilayer, incubated

for 48 hours and then an aliquot of the sample drop cast on a mica substrate followed by spin coating at 750 rpm for 3 minutes.

3. Supporting Table:

Table S1: Time-resolved lifetime data of PRODAN in DMPC:DMPG (8:2) system in presence of different amino acids collected at 440 nm at different time intervals, where [lipid] = 1 mM and [amino acid] = 10 mM.

DMPC/DMPG in presence of 10 mM amino acid	**χ2**	**τ_1 (ns)**	**τ_2 (ns)**	**a_1**	**a_2**	**< τ > (ns)**
DMPC/DMPG	1.10	1.50	4.23	0.48	0.52	2.92
Trp T 1 H	1.19	1.69	5.18	0.45	0.55	3.25
Trp T 24 H	1.12	1.86	6.49	0.42	0.58	4.55
Trp T 48 H	1.05	1.89	6.98	0.32	0.68	5.33
Phe T 1 H	1.16	1.76	5.08	0.53	0.47	3.33
Phe T 24 H	1.09	2.00	6.21	0.49	0.51	4.16
Phe T 48 H	1.08	2.28	6.85	0.38	0.62	5.12
His T 1 H	1.09	1.69	4.83	0.51	0.49	3.21
His T 24 H	1.19	1.77	5.49	0.50	0.50	3.61
His T 48 H	1.04	2.11	6.40	0.42	0.58	4.59

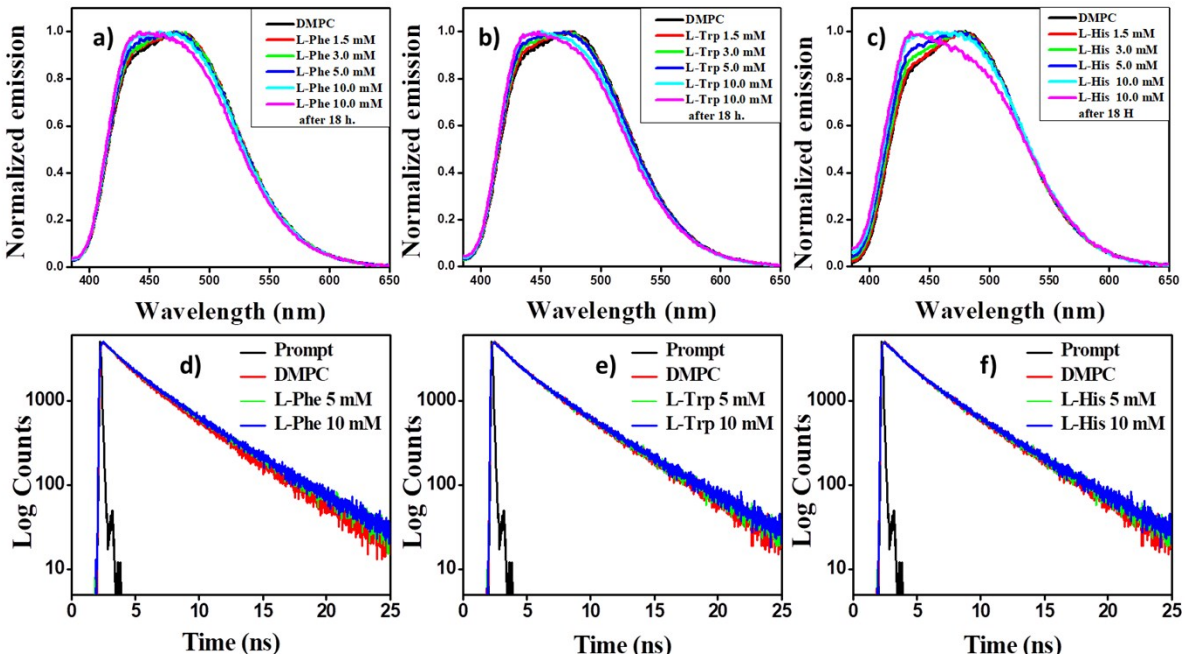


Fig. S1: Normalized emission spectra (a-c) and time-resolved lifetime decay curves (d-f) of PRODAN in DMPC vesicles at different concentration of amino acids (0-10 mM) for L-Phe (a, d), L-Trp (b, e), and L-His (c, f) respectively.

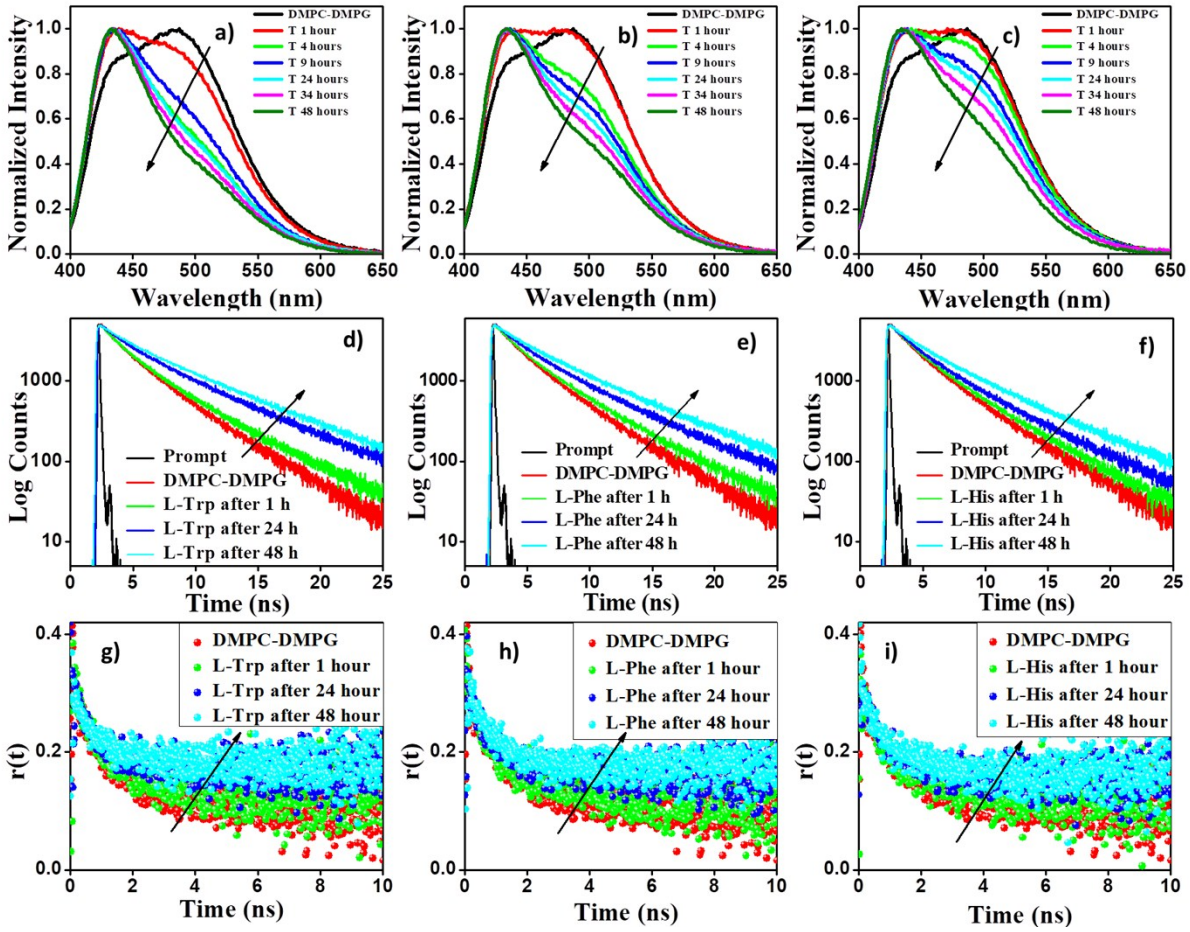


Fig. S2: Time-dependent normalized emission spectra (a-c), time-resolved lifetime decay (d-f) and anisotropy decay (g-i) of PRODAN in DMPC:DMPG (8:2) vesicles in presence of 10 mM amino acids for L-Trp (a, d, g), L-Phe (b, e, h), and L-His (c, f, i) respectively.

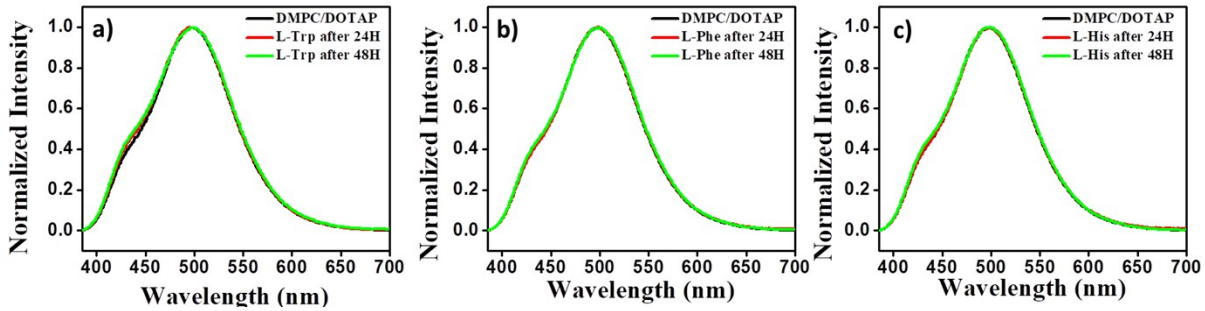


Fig. S3: Normalized emission spectra of PRODAN in DMPC:DOTAP (8:2) vesicles at different concentration of amino acids (0-10 mM) for L-Trp (a), L-Phe (b), and L-His (c) respectively.

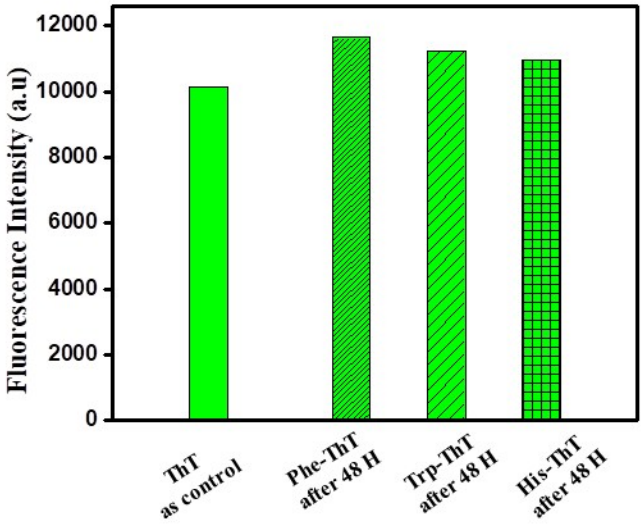


Fig. S4: Steady-state fluorescence emission spectra of Thioflavin T (ThT) in the presence of different amino acids after 48 hours in a fixed lipid concentration. ThT emission intensity was collected at 485 nm while excited at 430 nm. The negligible increase in fluorescence intensity of ThT indicates that simply mixing the amino acids in the aqueous medium does not influence the formation of amyloid structure.

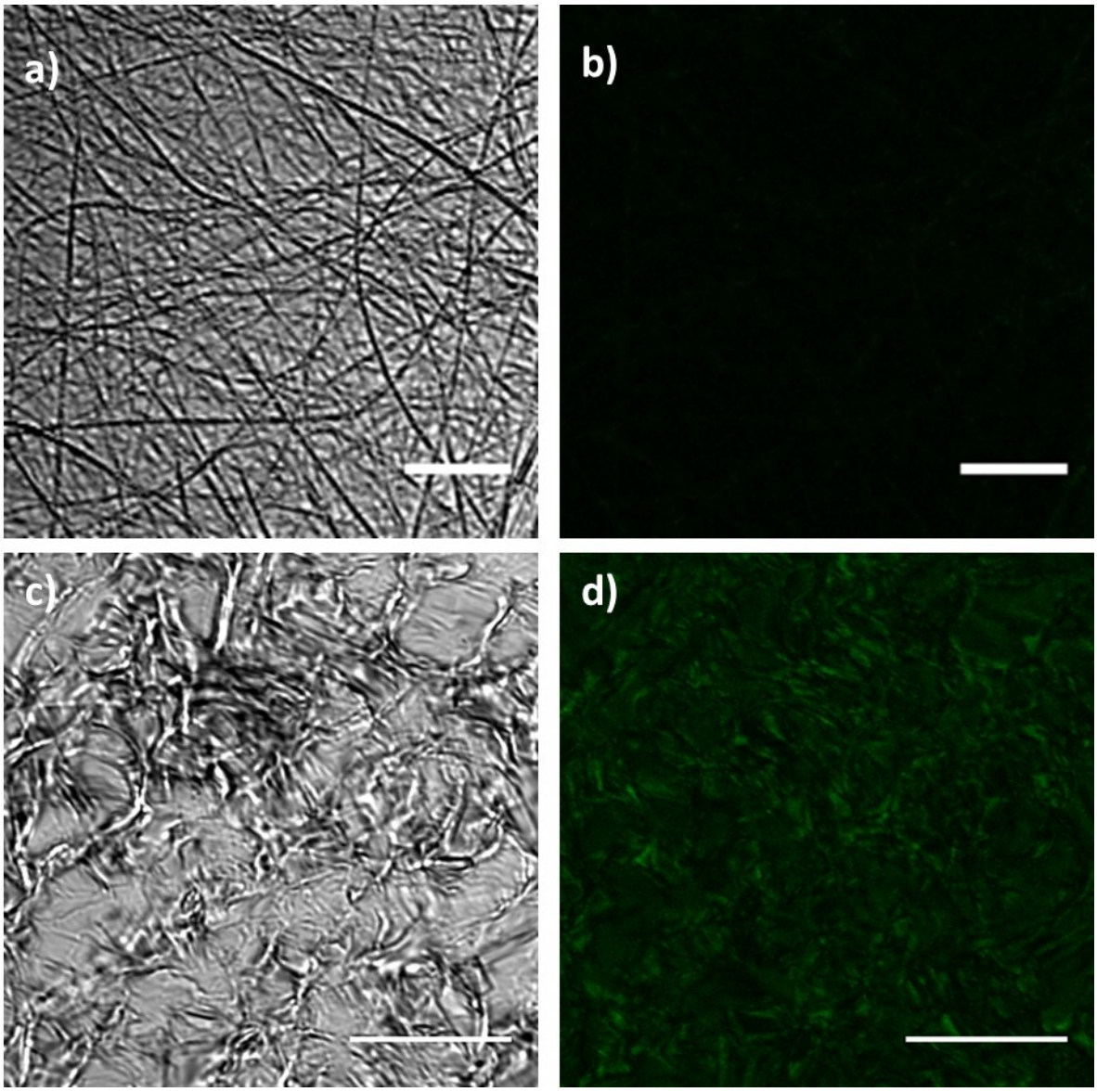


Fig. S5: Bright field and confocal images of amyloid aggregates of Phe (a, b) and Trp (c, d) upon excitation at 488 nm laser. (Scale bar 10 µm)

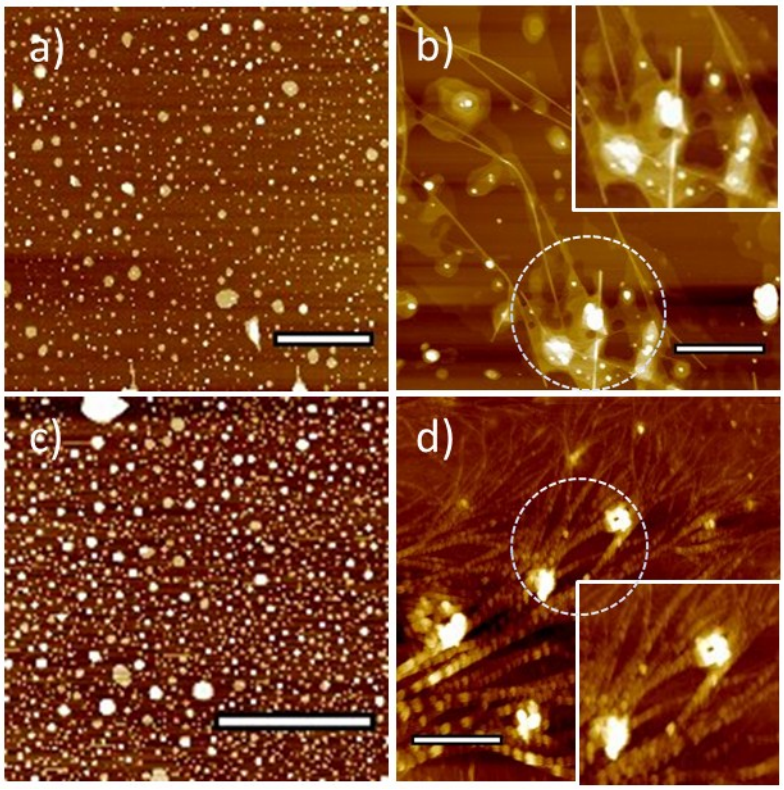


Fig. S6: Atomic force microscopy (AFM) images of zwitterionic lipid vesicles (DMPC) in absence and presence of Phe (a-b) and negatively charged lipid (DMPC/DMPG) vesicles in absence and presence of Phe. (Scale bar 2 µm)

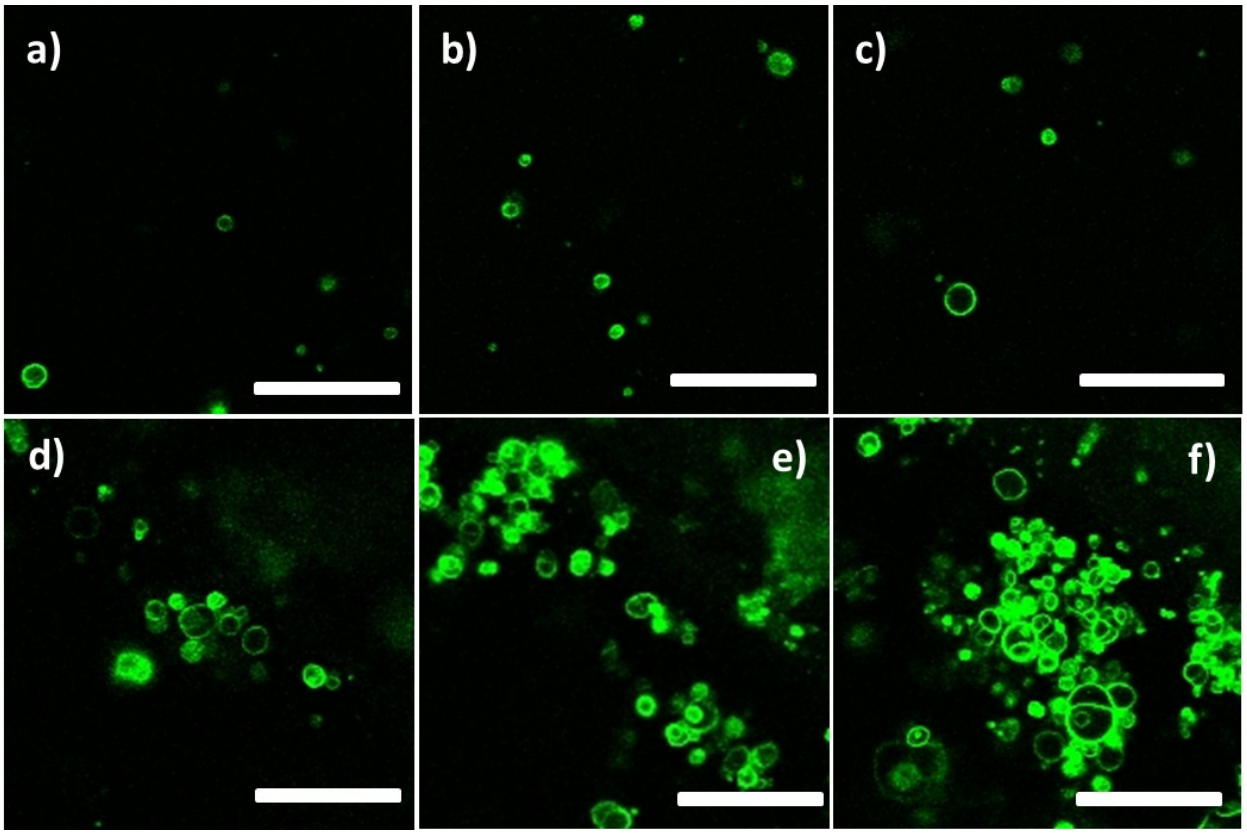


Fig. S7: Confocal microscopy images of DMPC lipid vesicles in presence of Phe with 1 mol% NBD-PE. Non-aggregated DMPC vesicles in presence of Phe at the initial stage of time-lapse imaging (a-c) and aggregated DMPC vesicles during the formation of amyloid aggregates (d-f). (Scale bar 5 µm)

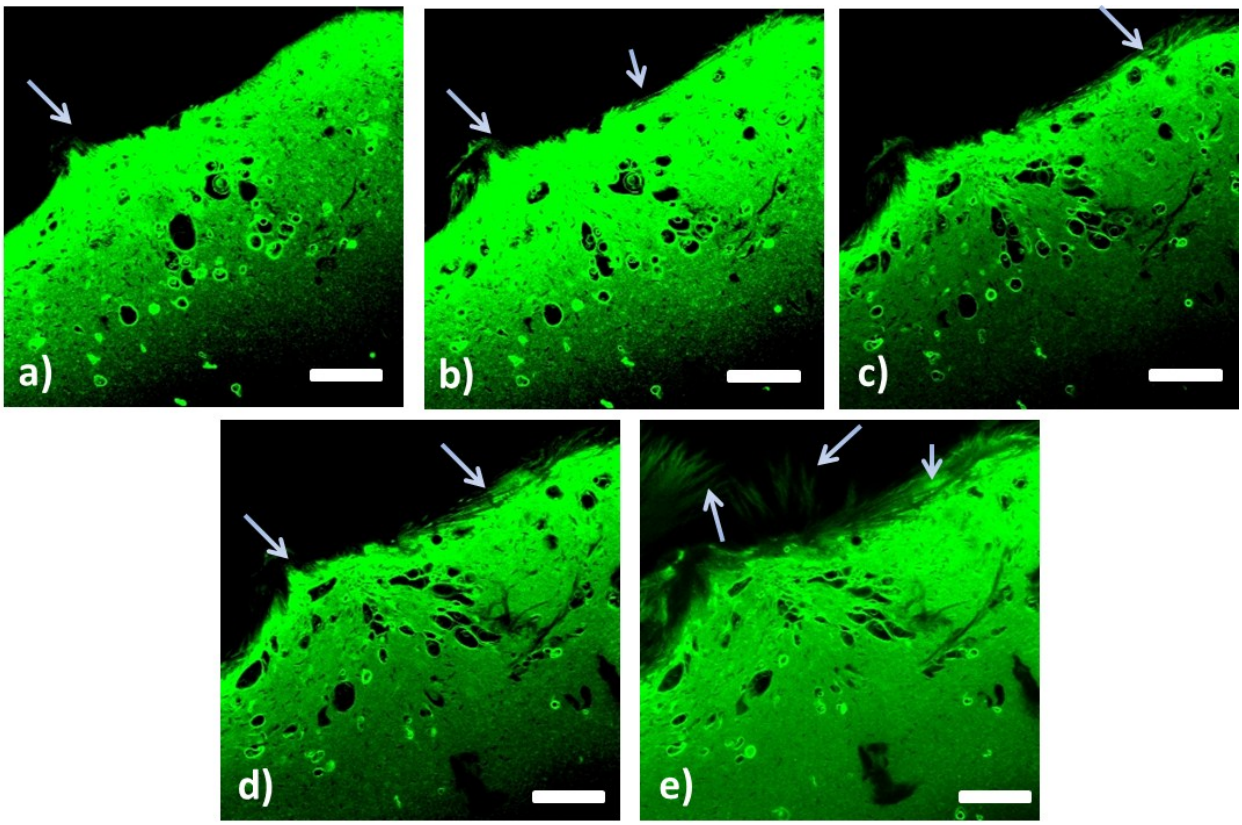


Fig. S8: Confocal microscopy images of DMPC:DMPG (8:2) lipid vesicles after the formation of the amyloid aggregate of Phe with 1 mol% NBD-PE. Blue arrow indicates the formation of fibril structures. Deformed lipid vesicles (white circle) and fluorescence from the fibril structure (blue arrows) are also evident from these figures. (Scale bar 5 µm)

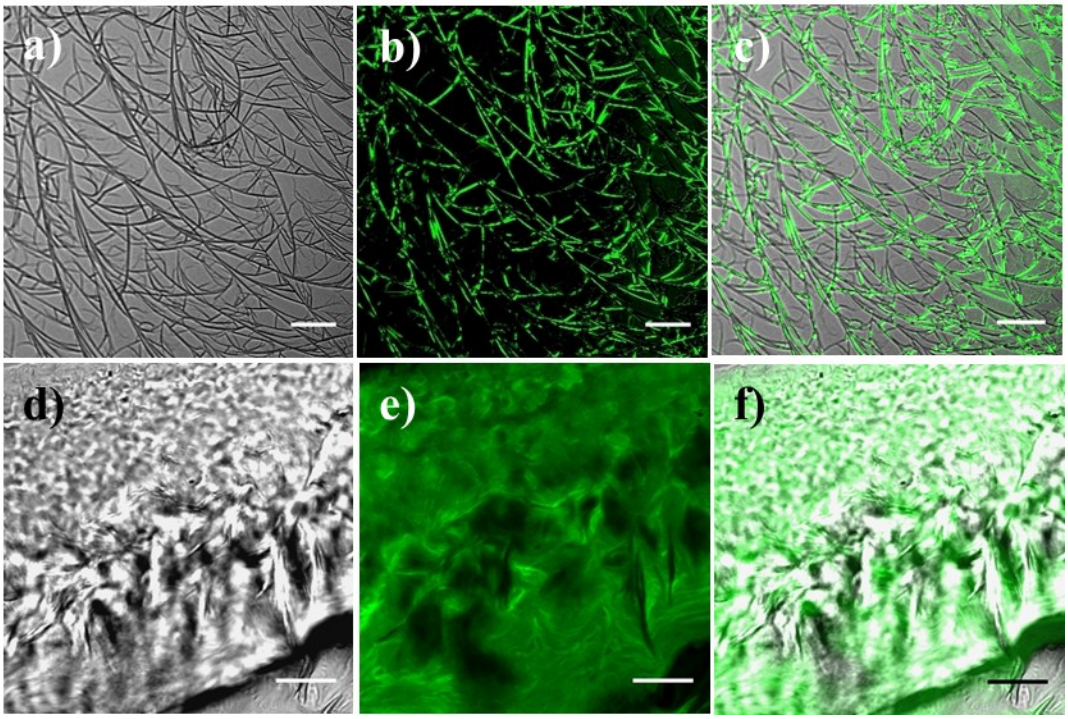


Fig. S9: Bright field, confocal, and merge images of 10 mM Phe (a-c) and Trp (d-e) stained with ThT. (Scale bar 5 µm)

Referrences:

1. E. C. Griffith, R. J. Perkins, D.-M. Telesford, E. M. Adams, L. Cwiklik, H. C. Allen, M. Roeselová, and V. Vaida, *J. Phys. Chem. B*, 2015, **119**, 9038.
2.