Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Materials

Metabolites (tryptophan and tyrosine purity ≥98%; adenine purity ≥99%), thiazolyl blue tetrazolium bromide 98%, Tris(hydroxymethyl)aminomethane (TRIZMA base buffer, C4H11NO3) and sodium dithionite were purchased from Sigma-Aldrich (Rehovot, Israel). Dulbecco's modified Eagle's medium (DMEM), high glucose, was purchased from Biological Industries (BI) (Kibbutz Beit Haemek, Israel). Dimethylformamide (DMF) was purchased from Bio-lab (Jerusalem, Israel). Sodium n-dodecyl sulfate (SDS) 99% was purchased from Tzamal D-Chem (Petah Tikva, Israel). 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), L-α-phosphatidylserine (Brain, Porcine) (PS) and fluorescent dyes N-(7- nitrobenz-2-oxa-1,3-diazol-4-yl)1,2-dihexadecanoyl-sn-glycero-3 phospho-ethanolamine, triethylammonium salt (NBD-PE) were purchased from Avanti (Alabaster, AL). The diacetylenic monomer 10,12- tricosadiynoic acid (TRCDA) was purchased from Alfa Aesar (Karlsruhe, Germany). The powder was washed in chloroform and purified through a nylon 0.45 μm filter (Whatman) before use. 1-(4-Trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) was obtained from Molecular Probes, Inc. (Eugene, Oregon).

Methods

Preparation of metabolites assemblies' and alanine solutions

Tryptophan (4 mg/ml), tyrosine (4 mg/ml) adenine (2 mg/ml) and alanine (4 mg/ml) were dissolved at 90 °C in PBS or cell medium, to obtain monomeric solutions of the metabolites. Next, the solutions were left to cool down gradually overnight.

Transmission electron microscopy

Subsequently to the preparation of metabolites assemblies' and alanine solutions as mentioned above, 10 μl samples were placed on 400-mesh copper grids. After 1 min, excess fluids were removed. Samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV.

Cytotoxicity analysis

Human embryo kidney (HEK)-293 cells (2×10^5 cells/ml) were cultured in 96-well tissue microplates (100 μl per well) and allowed to adhere overnight at 37 °C. Half of each plate was cultured with cells, with the other half later serving as a control for the solutions alone. The treatment solutions were prepared as described above. As a control, medium with no metabolites

was treated in the same manner. On the next day, the cells were washed and treated with the metabolites or control solutions (100 µl per well), followed by overnight incubation at 37°C. Cell viability was evaluated using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 10 µl of 5 mg/ml MTT dissolved in PBS were added to each well. After a 4 h incubation at 37°C, 100 µl extraction buffer [20% SDS dissolved in a mixture of 50% DMF and 50% DDW (pH 4.7)] were added to each well, and the plates were incubated again at 37°C for 30 min. Finally, color intensity was measured using an ELISA reader at 570 nm. The data are presented as mean ± SEM.

Preparation of vesicles for model membrane system interaction analysis

Vesicles containing the diacetylene monomer 10,12-tricosadiynoic acid (TRCDA) and the lipid components (DMPC/TRCDA at 2:3 molar ratio, or PS/DMPC/TRCDA at 1:1:3 molar ratio) were dissolved in chloroform/ ethanol (1:1) and dried together in vacuo to a constant weight, followed by addition of deionized water to a final concentration of 1 mM, and probe sonicated at 40 W at 70 °C for 3 min. The vesicle solution was subsequently cooled at room temperature and kept at 4 °C overnight. The solution was then irradiated at 254 nm for 30 s, resulting in intense blue color appearance due to polymerization of the diacetylene units. Vesicles containing only phospholipids (DMPC, DMPS/DMPC at 1:1 molar ratio) were sonicated at 26 W for 10 min at room temperature and then kept at 4 °C overnight.

Fluorescence spectroscopy

Samples were prepared for fluorescence measurements by adding 30 µl of each tested solution (PBS, metabolite assemblies and alanine solutions) to 30 µl of the of lipid/PDA vesicles, followed by addition of 30 µl 50 mM Tris-base buffer (pH 8.0). The samples were incubated for 24h at 28 °C. Fluorescence measurements (excitation, 485 nm; emission, 555 nm) were carried out on a Fluscan Ascent microplate reader in a 96-well plate (grainer), using LP filters with normal slits. The background fluorescence of the vesicles alone was negligible. The fluorescent chromatic response (FCR) was calculated according to the following formula: %FCR = [EmI/ Emred]×100%, where EmI is the value obtained for the vesicle treated with the test compounds, and Emred is the value obtained for the vesicles treated with the positive control (1 M NaOH). The %FCR presented is after reduction of the PBS blank signal. The displayed results are representative of three independent experiments.

Fluorescence anisotropy

The fluorescent probe TMA-DPH was incorporated into the small unilamellar vesicles (SUVs) (DMPC or PS/DMPC (1:1)) by adding the dye dissolved in THF (1 mM) to the vesicles to a final concentration of 1.25 μM. The samples (with either PBS, metabolite assemblies or alanine) were incubated for 24h at 28 °C. TMA-DPH fluorescence anisotropy was measured at 430 nm (excitation 360 nm) before and after the addition of the tested solution (metabolite assemblies and alanine) using a FL920 spectrofluorimeter (Edinburgh Co., Edinburgh, UK). Anisotropy values were automatically calculated by the spectrofluorimeter software using default parameters. Results are presented as means ± SEM. The displayed results are representative of three independent experiments.

Fluorescence quenching measurements

The fluorescent probe NBD-PE was dissolved in chloroform, added to phospholipids at 1 mol %, and the sample was dried in vacuum before sonication. The samples (with either PBS, metabolite assemblies or alanine) were incubated for 24h at 28 °C. The quenching reaction was initiated by adding sodium dithionite (Na2S4O2; Aldrich) in 50 mM Tris base buffer, pH 11.0, to a final concentration of 10 mM. The fluorescence emission was recorded for 5 min at 27 °C using 469 nm excitation and 535 nm emission on an Edinburgh Co. (Edinburgh, Scotland, U.K.) FL920 spectrofluorimeter. The fluorescence decay was calculated as a percentage of the initial fluorescence, as measured before the addition of dithionite.

Molecular dynamics simulations

Simulations

Bulk crystal structures for tryptophan, tyrosine, and adenine[1–3] were chosen. Membranes were constructed using the CHARMM GUI software.[4] SOPC lipid molecules were chosen for the PC membrane, and 90% SOPC and 10% SOPS lipid molecules were chosen for the PC/PS combination membrane. Initially, three different orientations (facet A,B,C) were chosen for each crystal structure. For tryptophan and tyrosine, the head groups were in direct contact with the membrane in facet A and perpendicular to the membrane in facets B and C. For both of these crystals, another orientation was chosen, such that the zwitterions were in direct contact with the membrane. In the case of the adenine crystal, facet A was determined such that the plane of the molecules in the crystal was parallel to that of the membrane. In facets B and C, the planes of the molecule were oblique to that of the membrane.

Simulations were performed using the NAMD2[5] package, using the CHARM force field[6–12]. The systems were simulated in [NaCl] = 0.15 M aqueous solution, in order to imitate physiological conditions. The Langevin dynamics with a damping coefficient of 1 ps^{-1} and a time step of 2 fs was used to describe the systems in a NPγT ensemble at a pressure of 1 atm, surface tension of 40 dyne/cm, and a temperature of 310 K. During minimization, pre-equilibration, and equilibration, Particle Mesh Ewald[13] was used with a grid spacing of 1.0. The non-bonded interactions used the SHAKE switching algorithm with the switch on/off distance at 10/12 Å. Non-bonded pairs lists were 13.5 Å, with the list updated every 20 steps; 1-4 non-bonded interactions were not scaled. There were 50,000 steps of minimization. Production runs for each system lasted 30 ns.

Interaction energies

Average interaction energies between each crystal and membrane were calculated from the simulated systems. The CHARMM force field[6–12] and NAMDenergy plugin in VMD[14] were used to determine the strength of total interaction energy for each combination of metabolite crystal and membrane calculated as a sum of the electrostatic and van der Waals (vdW) interaction energies. Electrostatic interaction energies were calculated for a dielectric constant of 1 and dispersion interactions were approximated by the 12-6 Lennard Jones potential. Long range electrostatic interactions were modeled by the Particle Mesh Ewald method[13].

In order to determine the crystal-membrane interactions, interaction energies per contact area were calculated. Throughout the simulations, a significant number of metabolites was found to dissociate from the crystal. Many of these molecules were later absorbed in the membrane. These free molecules had to be omitted from the calculations. Contact area between metabolite crystal and membrane was determined by calculating solvent accessible surface area (SASA) with command in VMD. Contact Area is given by equation S1, where SASA is the solvent accessible surface area of the membrane, crystal, or the union of both species, as indicated. Interaction energy between metabolite crystal and membrane was normalized to the contact area in each snapshot.

$$Contact\ Area = \frac{SASA_{membrane} + SASA_{crystal} - SASA_{membrane\ +\ crystal}}{2} \quad \text{(S1)}$$

Supporting Figures

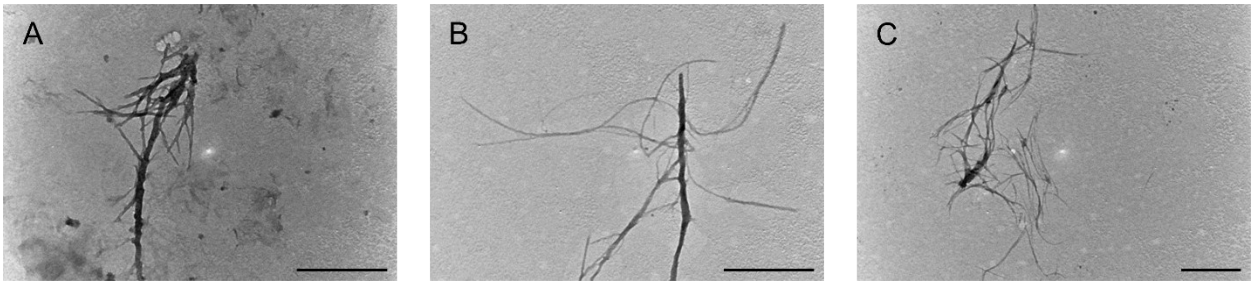

Figure S1. TEM images of metabolite amyloid assemblies. (**A**) Tryptophan (4 mg/ml). (**B**) Tyrosine (4 mg/ml). (**C**) Adenine (2 mg/ml).

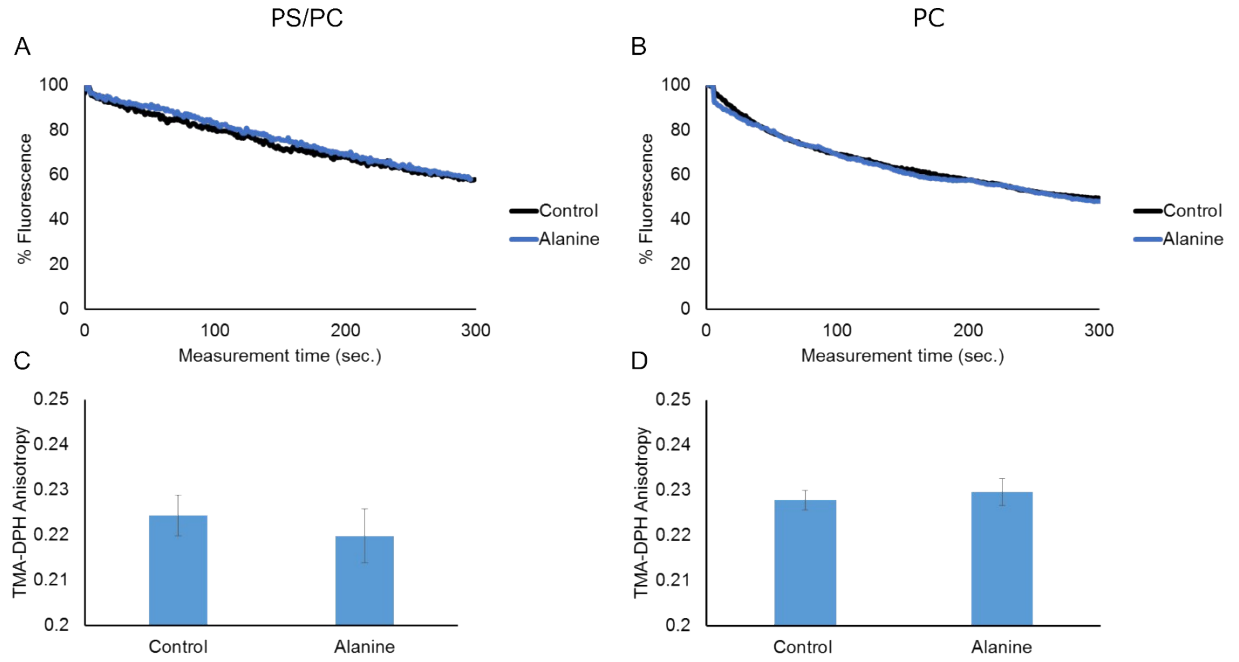

Figure S2. Alanine interaction with membrane model systems as compared to untreated control. Alanine (4 mg/ml) and PBS, served as an untreated control, were examined using two phospholipid compositions of membrane model systems, PC and a combination of PC with PS. (**A-B**) Quenching of NBD fluorescence as measured after 24 h incubation with examined solutions, following an addition of dithionite quencher. Fluorescence values represent the percentage of initial emission reading. (**C-D**) Fluorescence anisotropy of TMA-DPH, incubated for 24 h with examined solutions (**A**) PS/PC (**B**) PC (**C**) PS/PC (**D**) PC.

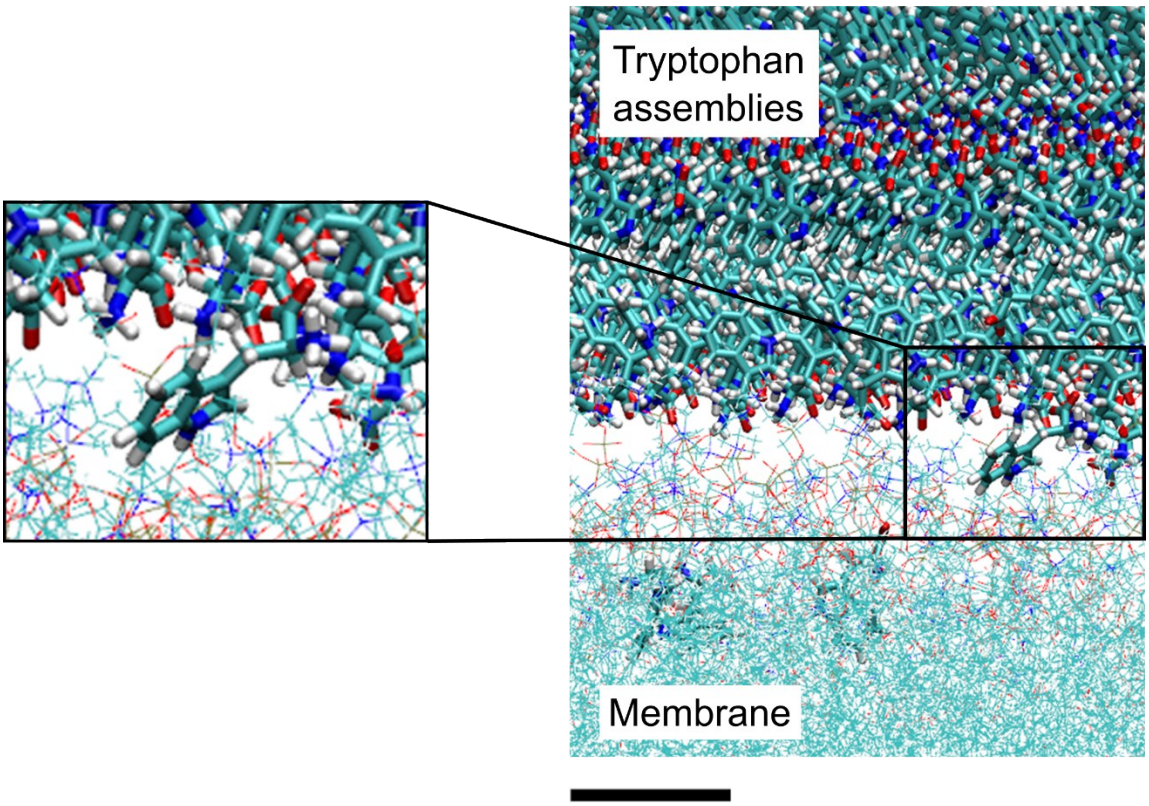

Fig. S3 Molecular dynamic simulations of tryptophan assemblies with membrane systems. Enlarged figure taken from Fig. 3A. The most stable orientations of the tryptophan assemblies (zwitterion layer) are presented.

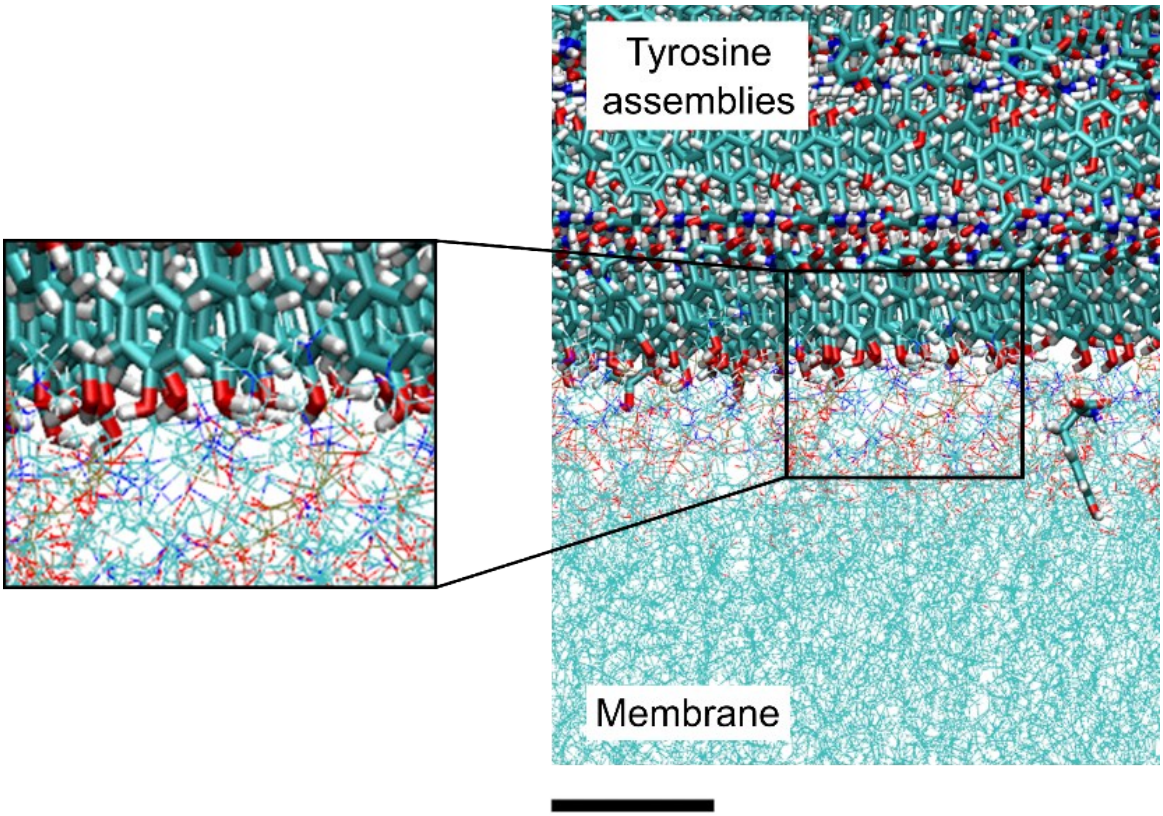

Fig. S4 Molecular dynamic simulations of tyrosine assemblies with membrane systems. Enlarged figure taken from Fig. 3B. The most stable orientations of the tyrosine assemblies (facet A) are presented.

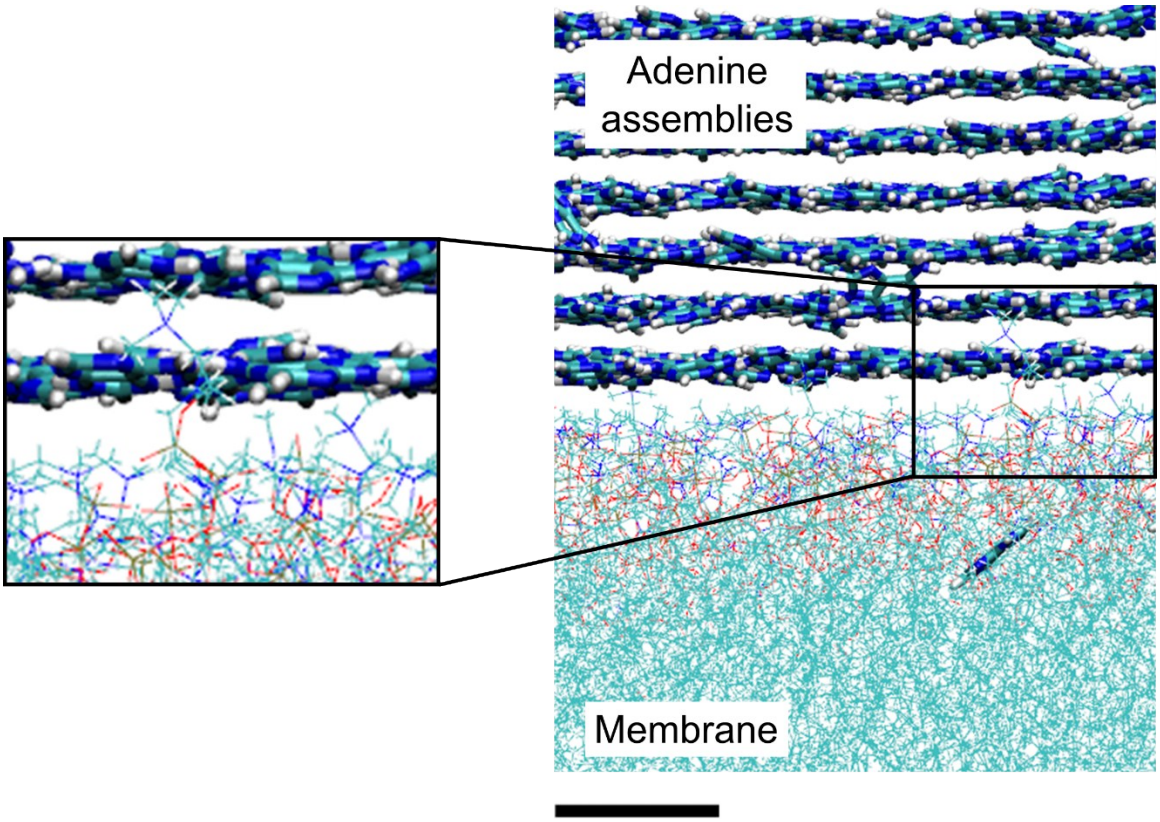

Fig. S5 Molecular dynamic simulations of adenine assemblies with membrane systems. Enlarged figure taken from Fig. 3C. The most stable orientations of the adenine assemblies (facet A) are presented.

References

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