

Electronic Supplementary Information

Amyloid Beta Peptide inside a Reconstituted Cell-like Liposomal System: Aggregation, FRET, Fluorescence Oscillations and Solvation Dynamics

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1. Experimental Section

1.1 Methods:

Preparation of physiological relevant buffers: Liposome contains two types solution. The solution inside the liposome is Internal Buffer (IB) which was prepared by mixing Phosphate buffer saline (PBS 1X: 140 mM of NaCl, 2.7 mM of KCl, 8.1 mM Na₂HPO₄·2H₂O and 1.8 mM of KH₂PO₄ at pH 7.2), 2 M sucrose solution, dextran solution (400 mg/ mL) in Milli Q water. The solution outside the liposome is External Buffer (EB) was prepared by mixing PBS (1X), 20% glucose in water and BSA solution (100 mg/mL) in PBS. To prepare a liposome containing Aβ solution, we have prepared a solution by mixing HiLyte Fluor 488 labeled Aβ with internal buffer. Osmolarity of both the buffers and solution of IB containing Aβ were measured by Osmometer. For maintaining the shape of the liposome, osmolarity of EB was kept approximately 5-10 mOsm below than that of the IB. Isoosmotic condition results change in the shape of the liposome.¹

Preparation of Lipid-oil mixture: Chloroform solution of 2.5 mg of Egg PC, 0.5 mg of DOPS, 0.5 mg of POPG, 1% of biotin-DHPE and TRITC-DHPE was taken in a 10 mL round bottomed

flask. A thin film of lipid mixture was prepared by nitrogen flush and then the flask was kept in high vacuum pump for 40 min. Then, the vacuum was released under nitrogen atmosphere. 5 mL of mineral oil was added into the round bottomed flask containing lipid film. It was sonicated for 30 min in cold water followed by incubation in hot air oven at 50 °C for 3 h. Next, the flask was cooled down at room temperature and stored at 4 °C. For immobilization of liposome on biotin surface, biotin-DHPE lipid was used and for visualization of the membrane of liposome TRITC-DHPE lipid is used.

Preparation of biotin functionalized Surface: Glass coverslips (50 X 50 mm) were cleaned by sonicating them with 3 M NaOH for 30 min. Next, washed them with plenty of water and further treated with piranha (2:3 mixture of hydrogen peroxide and sulphuric acid) and sonicate them for 45 min under fume hood. Piranha solution was discarded and glass slides were thoroughly cleaned with water and dried under stream of nitrogen gas. Surface fictionalization was done by following stepwise methods. GOPTS was treated at 75 °C for 90 min for the silanization of glass surfaces. Next, silanised glass surfaces were treated with diamino-polyethylene glycol and heated at 75 °C for overnight. The polyethylene glycol functionalized surfaces were washed with plenty of water for complete removal of excess and unreacted diamino-polyethylene glycol from surfaces. Finally, polyethylene glycol surface was treated with Biotin-NHS for 1 h at 75 °C. Biotin functionalized surfaces were washed with DMF and plenty of water followed by drying under stream of air.¹

Construction of the flow chamber and Immobilization of liposomes: A flow chamber of 50 µL was constructed onto a microscopic glass slide using double sticky tape (Tesa, Hamburg, Germany) and biotin functionalized glass surface. Flow chamber was washed with EB before loading liposomes. Then, neutravidin solution (300 nM) in EB was loaded into the flow chamber and incubated for 10 min for the attachment of liposome to the biotin glass surface through biotin-avidin interaction. After 10 min flow chamber was washed with EB, liposomes with only 500 nM A β and 500 nM A β with 500 nM NV (A β peptide aggregation inhibitor) were loaded into the flow chamber and it was sealed and observed under inverted fluorescence microscope.

Preparation of Beta-amyloid peptide stocks: 1.0 mg of A β 42 peptide was dissolved in 100 µL of 1,1,1,3,3,3-Hexafluoro-2-propanol and 10 µL of aliquots were prepared and stored at -20 °C.

Similarly 0.1 mg of A β 42 labeled with HiLyte Fluor™ 488 peptide was dissolved in 100 μ L of 1,1,1,3,3,3-Hexafluoro-2-propanol and 10 μ L of aliquots were prepared and stored at -20 °C.

A β peptide aggregation study inside of the liposome: In an eppendorf tube 0.01 mg of A β 42 peptide and 0.001 mg of A β 42 with HiLyte Fluor™ 488 labeled peptides were taken as 1,1,1,3,3,3-Hexafluoro-2-propanol solution. The solution was dried by nitrogen flush. A mixture of 200 μ L of IB and 0.7 μ L of 1% NH₄OH (supplied along with the peptides from Anaspec as a peptide reconstitution solvent) solution was prepared and adjusting its osmolarity with sucrose (2 M) solution. 28 μ L of this solution was added to the tube containing the A β 42 peptide mixture. Peptide mixture was dissolved by vortex and sonication. Now the solution contained the mixture of A β 42 (~80 μ M) and HiLyte Fluor™ 488 labeled A β 42 (~8 μ M) peptides in a 10:1 ratio. 5 μ L of this solution was added into the 600 μ L of lipid-oil mixture and an emulsion were prepared. Liposomes were prepared with this emulsion containing A β 42 peptide inside the liposomes.

2. Lifetime of Single and Aggregated A β in Bulk and inside the Liposome

HiLyte Flour 488 display wavelength dependent fluorescence decays. Fluorescence decays of A β peptides inside the liposome are shown in Figure S4. Corresponding decay parameters are listed in Table S1. From Table S1, it is readily seen that lifetime of A β peptides in monomer form display two components- 1 ns (0.45) and 3.3 ns (0.55). Aggregated forms of A β peptides in bulk exhibit identical lifetime components as that of monomer. It may be mentioned that steady state emission spectra shows even in the aggregated form, HiLyte Flour 488 remains completely exposed to the bulk. Thus similar lifetime of A β peptides in monomer and aggregated form is in good agreement with the emission spectra.

In the bilayer region of liposome, HiLyte display shorter lifetime component of 0.75 ns (0.3) along with a long component of 3.2 ns (0.7). Shorter lifetime component of HiLyte arises due to FRET between HiLyte and TRITC. Occurrence of FRET is further confirmed by a distinct rise component of 170 ps in the fluorescence decay of TRITC. Rise component of acceptor are often used to evaluate rate constant of FRET.²⁻³ Following same strategy, we have determined the rate constant of FRET between HiLyte and TRITC in the bilayer region and found to be 0.006 ps⁻¹.

Table S1. Emission maxima and fluorescence lifetimes of HiLyte Flour 488 labeled A β in bulk and also inside the liposome.

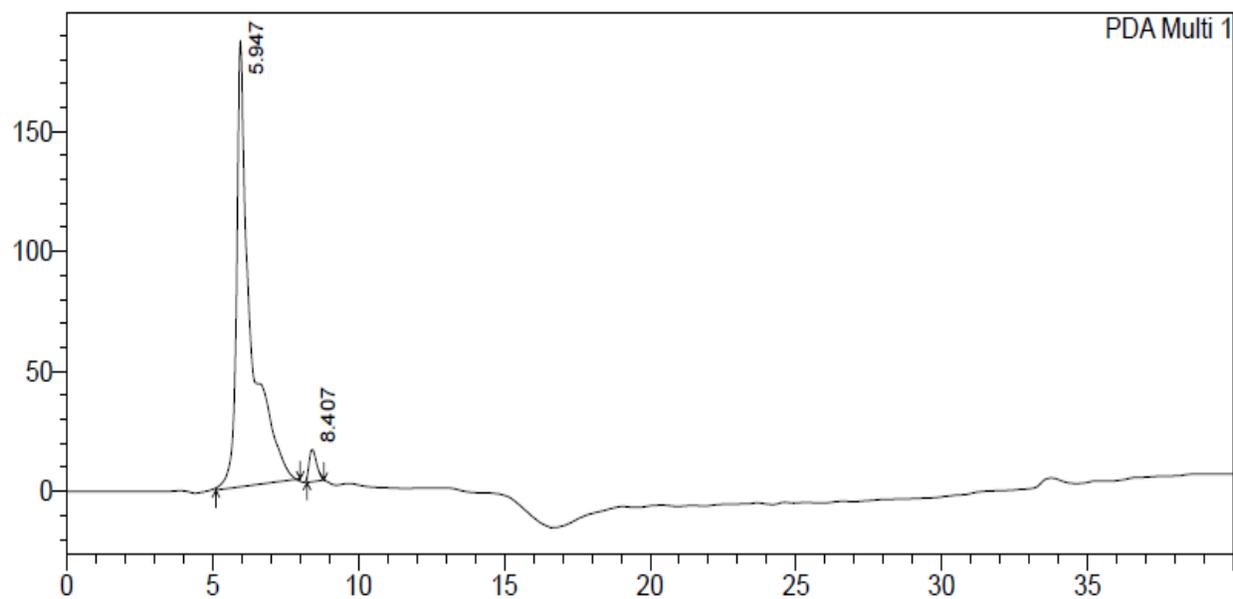
| System | | Emission Maxima (nm) | Lifetime | |
|-------------------|----------------------------|----------------------|-------------------------------|-------------------------------|
| | | | $\tau_1^{[a]}$ (a_1) (ns) | $\tau_2^{[b]}$ (a_2) (ns) |
| A β (37 °C) | Bulk | 538 (HiLyte) | 1.00 (0.45) | 3.3 (0.55) |
| | With NV | 538 (HiLyte) | 0.95 (0.30) | 3.2 (0.70) |
| | Bilayer region of Liposome | 537 (HiLyte) | 0.76 (0.30) | 3.2 (0.70) |
| | | 580 (TRITC) | 0.17 (-0.90) | 2.9 (1.90) |

^[a] ± 0.1 ns, ^[b] ± 0.2 ns

Reference:

1. A. Biswas, A. Saha, B. Jana, P. Kurkute, G. Mondal, and S. Ghosh, *ChemBioChem*, 2013, **14**, 689.
2. D. K. Das, A. K. Das, T. Mondal, A. K. Mandal, and K. Bhattacharyya, *J. Phys. Chem B*, 2010, **114**, 13159.
3. A. Adhikari, D. K. Das, D. K. Sasmal, and K. Bhattacharyya, *J. Phys. Chem A*, 2009, **113**, 3737.

Supplementary Figure



PDA Ch1 210nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|-------|-----------|---------|--------|---------|----------|
| 1 | 5.947 | 6284279 | 185900 | 96.333 | 93.233 |
| 2 | 8.407 | 239217 | 13492 | 3.667 | 6.767 |
| Total | | 6523496 | 199392 | 100.000 | 100.000 |

Figure S1: HPLC chromatogram of NV peptide showing 96 % of purity

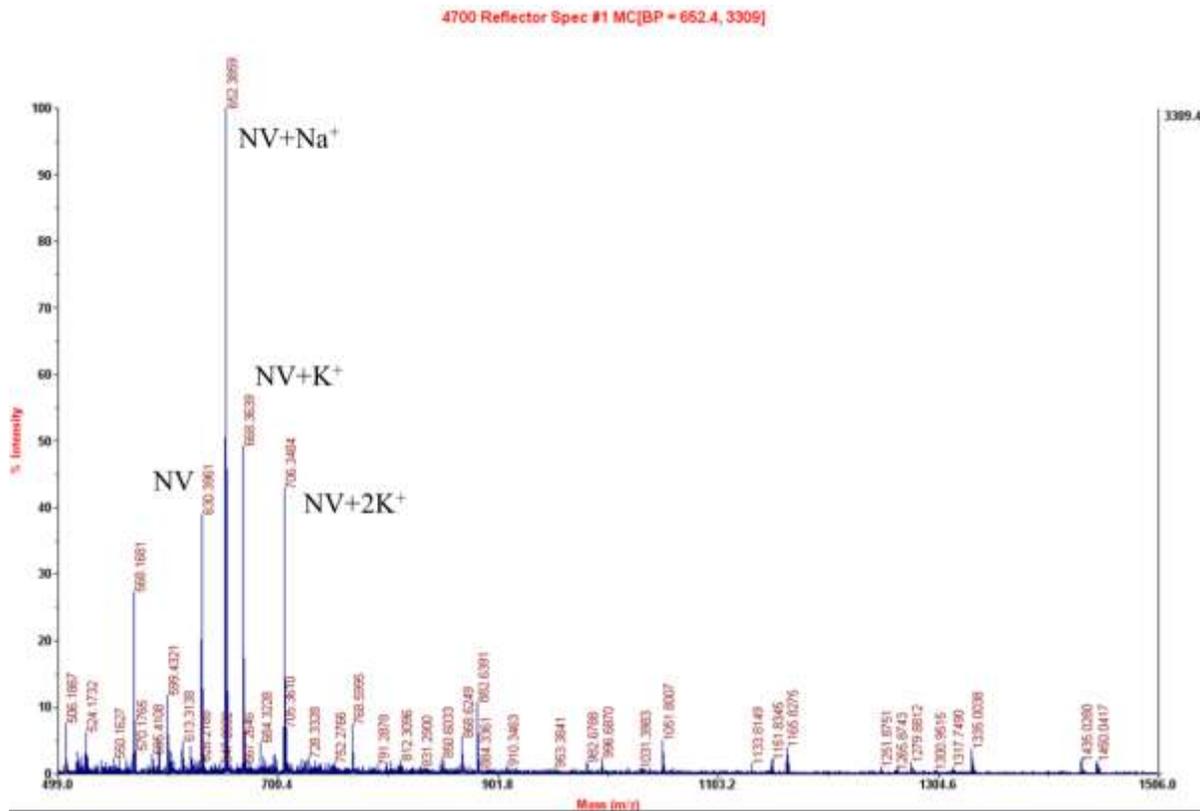


Figure S2. MALDI-TOF Mass spectrum of NV peptide (Molecular Mass-630 Da)

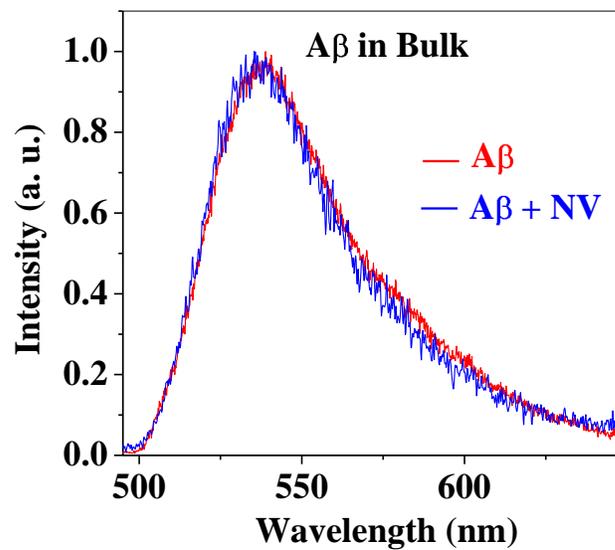


Figure S3. Emission spectra of A β in bulk solution.

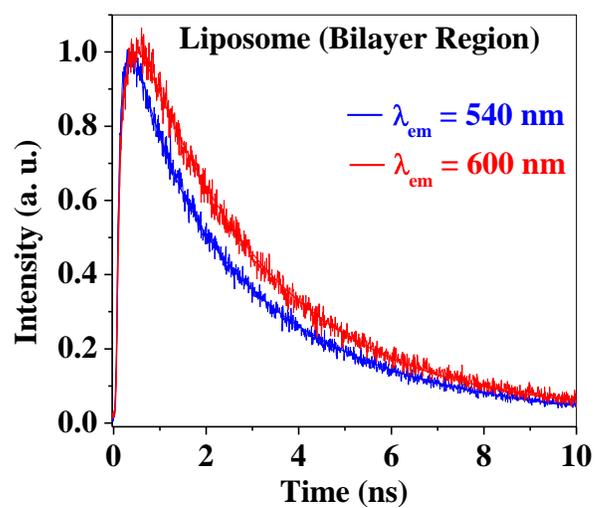


Figure S4. Fluorescence decay inside the liposome ($\lambda_{ex} = 470 \text{ nm}$).