**Supporting Information**

**for**

# Fluorescence quenching by lipid encased nanoparticles shows that Amyloid-β has a preferred orientation in the membrane

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**Contents:**

## 1. Synthesis and characterization of peptides and lipid coated nanoparticles

**1.1 Synthesis of $A\beta_{40}$ and fluorescein labeling:** All the $A\beta_{40}$ peptides used were synthesized on Rink Amide MBHA resin using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry in an automated peptide synthesizer (PS3, Protein technologies, USA). The peptides were subsequently purified and characterized in the laboratory following a well-established protocol described previously[1]. The sequence of the $A\beta_{40}$ peptide used in the N-terminal fluorescein-labeled $A\beta_{40}$ sample (Fl-$A\beta_{40}$) was DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV, and the fluorescein (succinimidyl ester) was covalently linked to the free amine at the N-terminal. For the C-terminal fluorescein-labeled $A\beta_{40}$ sample ($A\beta_{40}$-Fl), the amino acid sequence of the peptide used was DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVK, i.e., with an extra lysine at the C-terminal, and the fluorescein was linked to the side chain amine of the C terminal lysine.

**1.2 Synthesis of silver nanoparticle (Ag NP) suspension:** The synthesis and characterization of silver nano particles and preparation of lipid encased nano particles has been described earlier[1]. Briefly, 1 mM of $AgNO_3$ solution (50 mL) was heated in a 250 mL round-bottom flask until it started boiling. 1 ml of 39 mM of tri-sodium citrate solution (1 mL from a freshly made stock solution of 0.114 g $C_6H_5O_7Na_3.2H_2O$ in 10 mL of water) was added to this boiling solution in one shot. During the process, the solution was stirred vigorously, and the color of the solution changed from colorless to pale yellow and ultimately turbid yellow with a little greenish tinge. After about 30 min of reflux, the solution was cooled down to room temperature to obtain the colloidal nanoparticle suspension. $AgNO_3$ was purchased from Merck (Schuchardt, Germany) and tri-sodium citrate dihydrate was purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

**1.3 Characterization of the Ag NP colloid solution using the UV-Visible spectroscopy:** UV-Visible absorption spectroscopy is quite sensitive to the presence of silver nanoparticles (350 nm to 450 nm) because they exhibit an intense absorption peak due to the surface Plasmon excitation. With the increasing size of particles, the Plasmon absorption shifts toward the red-side of the spectrum. The absorption spectrum of freshly prepared Ag nanocolloids is shown in Figure 1. The spectra exhibit a Plasmon absorption band at ~ 407 nm which is the characteristic of silver nanoparticles of ~ 30 – 40 nm size. UV-visible absorption spectrum was recorded on an Analytikjena, SPECORD 205 UV-Vis spectrophotometer.

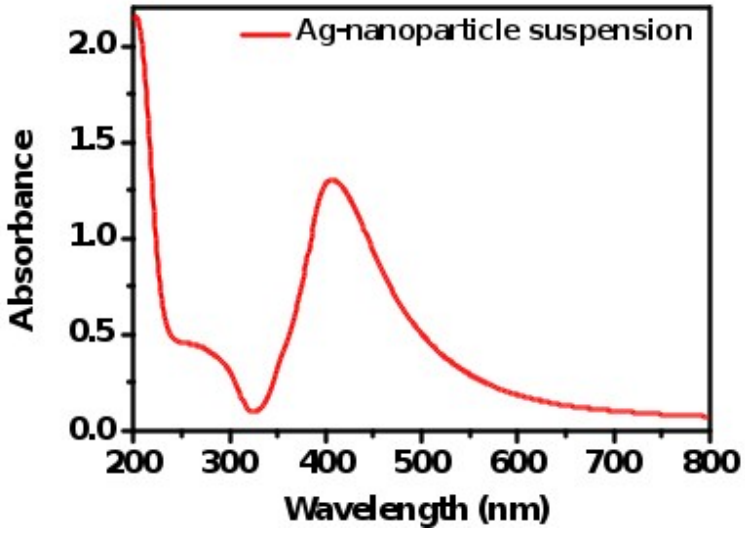


**Figure S1.** The UV-Visible absorption spectrum of Ag NPs (after 6x dilution of the stock)

**1.4 Preparation of Lipid-coated silver nanoparticles:** A thin film of lipid was prepared in small glass containers by mixing equimolar amount of POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), POPG (1-palmitoyl-2-oleoyl-sn-glycero-3 phosphoglycerol) and cholesterol (PPC111; 7.5 mg in total) in chloroform, followed by drying under nitrogen flow until the solvent had visibly dried. Residual portions of the chloroform were removed by vacuum suction for an overnight. The container was then filled with nitrogen gas, capped and stored at -20°C until use. The lipid films were again thawed prior to the encapsulation of particles by lipids. Silver nanoparticle suspensions were synthesized in the lab as described earlier. To coat these Ag NPs with lipid bilayers, a previously established method was followed, namely first 8 mL of the silver nanoparticle (Ag NP) solution was centrifuged @4000g for 10 min to precipitate down the nanoparticles and the supernatant was discarded. Then, the precipitate was re-suspended in 1 mL of distilled water and vortexed in the presence of PPC111 (7.5 mg) lipid film for about 5-6 min. The above mixture was then sonicated for about 20 min in a water bath sonicator. This process of sonication is expected to coat a lipid bilayer on top of Ag NP, as shown earlier by Bhowmik *et. al*[1]. A reasonable fraction of the lipids used ends up as lipid coats on the nano particles. We therefor estimate the peptide to lipid ratio to be < 1:10000. These lipid-coated Ag NPs were stable against a high salt concentration. Coated AgNP were separated by mild centrifugation (2000g, 12 min) followed by resuspension in salted phosphate buffer of pH ~7.4. The oligomeric solution of C-terminal and N-terminal fluoroscein-labelled $A\beta_{40}$ was then separately mixed with

lipid bilayer coated AgNP solution. Aβ oligomers were allowed to interact with the lipid bilayer coat for 30 mins.

**1.5 TEM Measurements:** Ten microliters of the sample solutions (either AgNP or Lipid-coated AgNP) were added on top of the carbon-coated 100 mesh copper grids (Electron Microscopy Sciences, Hatfield, PA). After 5 min of incubation, excess solvents were soaked out with clean tissue paper and 5 μL of 0.1% of uranyl acetate was added for sample staining (negative staining) and incubation for another 1-2 min, followed by similar blotting with a filter paper. The grids were then dried under an infrared lamp for 6 hours and examined under a transmission electron microscope (LIBRA 120, EFTEM, Carl Zeiss, Germany).

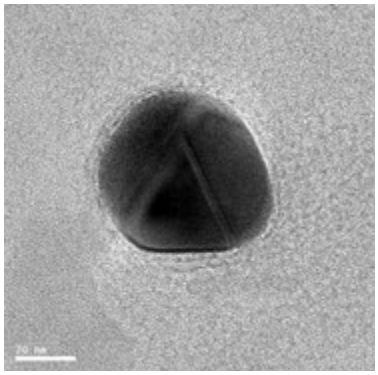

**Figure S2.** The TEM image of a lipid-coated silver nanoparticle, Scale bar: 20 nm.

**2. Instrumental set up for fluorescence life-time measurements:** The basic design of our time correlated single photon counting (TCSPC) instrument consists of a confocal fluorescence setup, with a stationary pulsed laser beam. Briefly, a picosecond pulsed diode laser (480 nm, PDL 800-D, Picoquant) was used as an excitation source, of which repetition rate was 80 MHz, but could be reduced by a factor of 2, 4, 8, 16 or 32, as required. As the excitation beam is small in size and divergent, a lens was used to make the beam size larger and collimated. An objective lens (NA = 1.2, 60x Olympus America Inc, Center Valley, PA, USA) was used to focus the collimated beam into the sample. The fluorescence was collected back with the aid of the same objective lens, separated from the excitation light by using a suitable dichoric mirror, and focused into a fiber coupled single photon avalanche photodiode with high time resolution (SPAD, Micro Photon Devices, Italy). The instrument response function was recorded using a sample consisting of a mixture of chalk powder and bleaching powder. The timing of the

excitation pulses (the 'sync' signal) was generated by a fast photodiode (PDL800, PicoQuant, Germany). Both the sync signal and the MPD signal were relayed to a signal analyzer (SPC730). The data were analyzed using the Fluofit software (PicoQuant, Germany).

**3. Lifetime measurement and data fitting:** We have fitted all the data with the Fluofit software (PicoQuant, Germany) taking into account the measured IRF. However, for C-terminal fluorescein labeled $A\beta_{40}$ with lipid coated nanoparticles, the major lifetime came out to be ~60 – 80 ps. As the width of the IRF is ~160ps, this value of the lifetime is 2-3 times shorter than the IRF. The exact value of a lifetime may be difficult to determine if it is so much shorter than the IRF. To verify the fitting of the data, which was done with the commercial software Fluofit, we have also fitted the dataset with an additional fitting method, namely a C-language based laboratory developed program (developed by N. Periasamy[2], ) (Fig. S3).

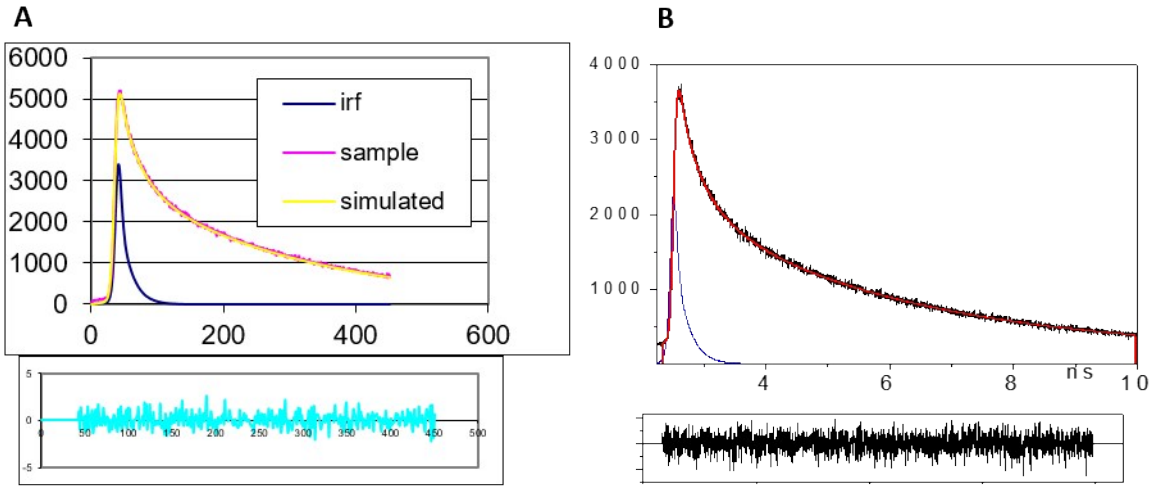


**Figure S3:** (A) TCSPC decay curve of C-terminal fluorescein labeled $A\beta_{40}$ in the presence of lipid coated nanoparticle and its fitting with Prof. Periasamy's program[2]. (B) TCSPC decay curve of C-terminal fluorescein labeled $A\beta_{40}$ in the presence of lipid coated nanoparticle and its fitting with Fluofit program.

The lifetime values are listed in the table below. Mean lifetimes are amplitude weighted averages.

1

2 **Table 1:** Comparison of different fitting models for TCSPC data

| System | Fluofit | Laboratory developed program[2] |
|---|---|---|
| N-terminal fluorescein labeled $A\beta_{40}$ | $A_1$: 0.85<br>$T_1$: 3.75 ns | $A_1$ : 0.87<br>$T_1$: 3.43 ns |
| | $A_2$: 0.15<br>$T_2$: 0.35 ns | $A_2$ : 0.12<br>$T_2$ : 0.28 |
| | Mean : 3.24 ns | Mean : 3.02 ns |
| Average of the Mean* | 3.25 ± 0.08 ns | |
| C-terminal fluorescein labeled $A\beta_{40}$ | $A_1$: 0.88<br>$T_1$: 3.86 ns | $A_1$ : 0.89<br>$T_1$ : 3.52 ns |
| | $A_2$: 0.12<br>$T_2$: 0.36 ns | $A_2$ : 0.11<br>$T_2$ : 0.27ns |
| | Mean : 3.43 ns | Mean : 3.16 ns |
| Average of the Mean* | 3.41 ± 0.16 ns | |
| N-terminal fluorescein labeled $A\beta_{40}$ with vesicle | $A_1$: 0.75<br>$T_1$: 3.63 ns | $A_1$ : 0.81<br>$T_1$ : 3.61 ns |
| | $A_2$: 0.25<br>$T_2$: 0.275 ns | $A_2$ : 0.19<br>$T_2$ : 0.28 ns |
| | Mean : 2.79 ns | Mean : 2.97 ns |
| Average of the Mean* | 2.62 ± 0.24 ns | |
| C-terminal fluorescein labeled $A\beta_{40}$ with vesicle | $A_1$: 0.79<br>$T_1$: 3.83 ns | $A_1$ : 0.62<br>$T_1$ : 3.45 ns |
| | $A_2$: 0.21<br>$T_2$: 0.26 ns | $A_2$ : 0.38<br>$T_2$ : 0.12 ns |
| | Mean : 3.1 ns | Mean : 2.19 ns |
| Average of the Mean* | 2.55 ± 0.4 ns | |
| N-terminal fluorescein labeled $A\beta_{40}$ Lipid coated nanoparticle | $A_1$: 0.69<br>$T_1$: 3.55 ns | $A_1$ : 0.70<br>$T_1$ : 3.58 ns |
| | $A_2$: 0.31<br>$T_2$: 0.281 ns | $A_2$ : 0.30<br>$T_2$ : 0.26ns |
| | Mean : 2.52 ns | Mean : 2.6 ns |
| Average of the Mean* | 2.58 ± 0.06 ns | |
| C-terminal fluorescein labeled $A\beta_{40}$ Lipid coated Nanoparticle | $A_1$: 0.85<br>$T_1$: 0.06 ns | $A_1$ : 0.15<br>$T_1$ : 3.26 ns |
| | $A_2$: 0.05<br>$T_2$: 0.48 ns | $A_2$ : 0.06<br>$T_2$ : 0.45ns |
| | $A_3$: 0.1<br>$T_3$: 3.5 ns | $A_3$ : 0.79<br>$T_3$ : 0.06ns |
| | Mean: 0.41 ns | Mean : 0.55 ns |

| | | |
|---|---|---|
| Average of the Mean* | 0.38 ± 0.02 ns | |
| N-terminal fluorescein labeled $A\beta_{40}$ In Vesicle + external KI | $A_1$: 0.3<br>$T_1$: 3.6 ns | $A_1$ : 0.33<br>$T_1$ : 3.19 ns |
| | $A_2$: 0.66<br>$T_2$: 0.08 ns | $A_2$ : 0.67<br>$T_2$ : 0.1 ns |
| | $A_3$: 0.05<br>$T_3$: 0.64 ns | |
| | Mean: 1.01 ns | Mean : 1.113 ns |
| Average of the Mean* | 1.1 ± 0.08 | |
| C-terminal fluorescein labeled $A\beta_{40}$ In Vesicle + external KI | $A_1$: 0.63<br>$T_1$: 3.67 ns | $A_1$ : 0.61<br>$T_1$ : 3.84 ns |
| | $A_2$: 0.37<br>$T_2$: 0.15 ns | $A_2$ : 0.39<br>$T_2$ : 0.15 |
| | Mean : 2.38 ns | Mean : 2.397 ns |
| Average of the Mean* | 2.14 ± 0.22 ns | |

The exact value of lifetimes or the number of lifetime components differ somewhat between the two fitting algorithms, but the variation between the two programs is not very large. Most importantly, the interpretation of the data does not change, as the trend is similar in both cases.

**4. Surface enhanced Raman spectroscopy (SERS) studies with Fl-$A\beta_{40}$ and $A\beta_{40}$-Fl:** We recorded the SERS spectra of Fl-$A\beta_{40}$ and $A\beta_{40}$-Fl using home-built line source Raman spectrometer. The 100 nM of peptide was incubated with the lipid encased nanoparticles for 30 min, and then it was subjected to SERS measurements.

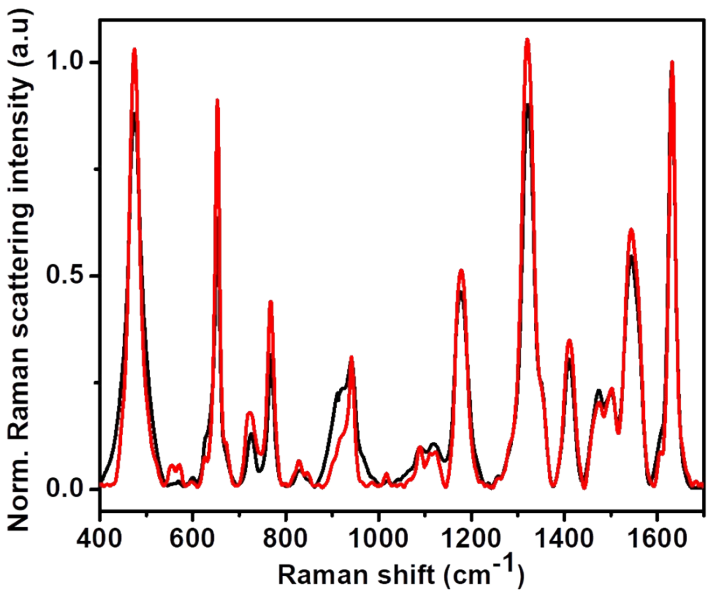

**Figure-S4:** SERS studies of 100 nM Fl-A$\beta_{40}$ (black) and 100 nM A$\beta_{40}$-Fl (red) with lipid membrane encased Ag nanoparticles. All the major peaks are identified to be from fluorescein.

**References:**


1. D. Bhowmik, K. R. Mote, C. M. MacLaughlin, N. Biswas, B. Chandra, J. K. Basu, G. C. Walker, P. K. Madhu and S. Maiti, *ACS Nano*, 2015, **9**, 9070-9077.
2. R. Swaminathan, G. Krishnamoorthy and N. Periasamy, *Biophys J*, 1994, **67**, 2013-2023.