Selective Detection of Membrane-Bound Amyloid-β Oligomers Using SERS "Hot-Spots": Toward Early Diagnostics of Alzheimer's Disease

Supporting Information

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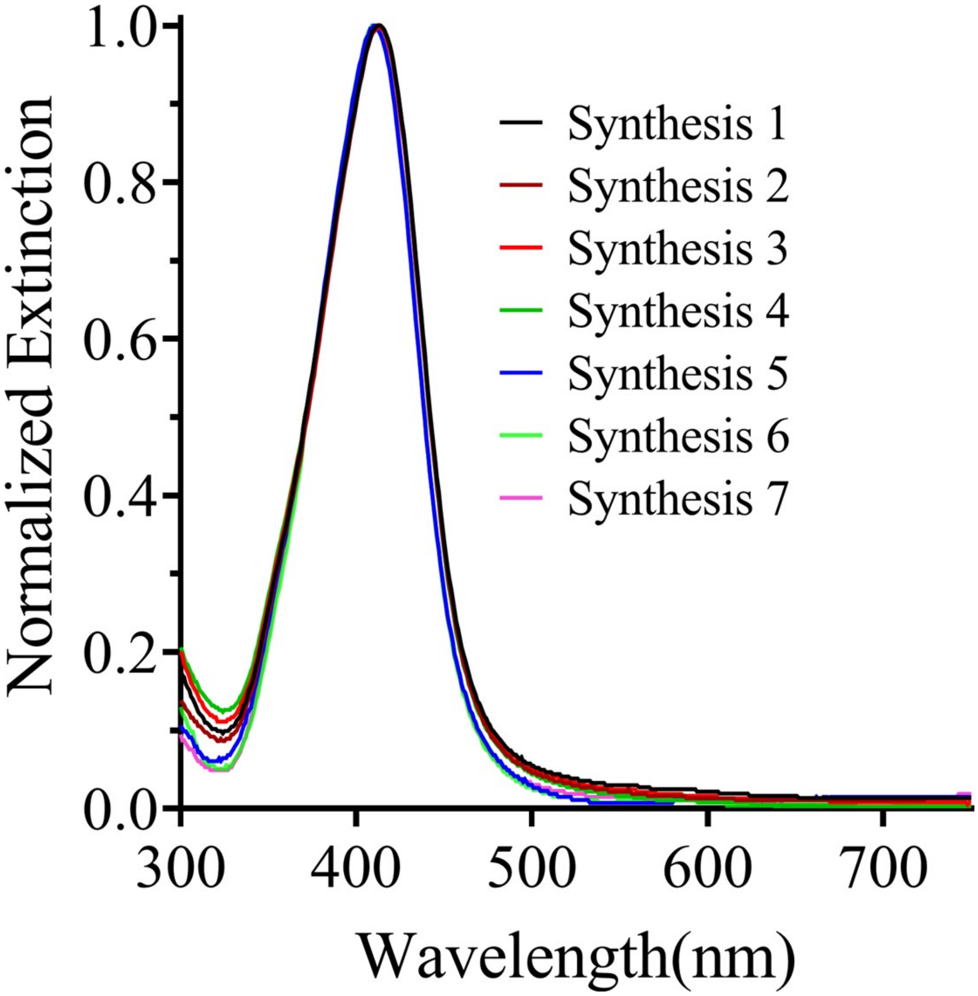

Figure S1. Representative absorption spectra of separately synthesized batches of monodisperse silver nanoparticles, highlighting their consistent optical properties**.**

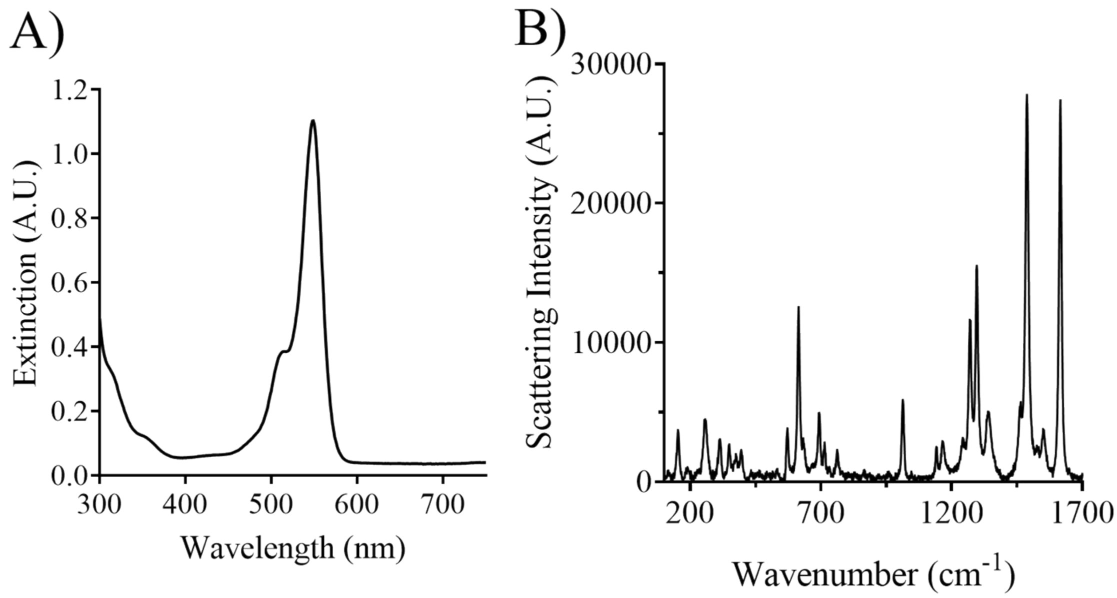

Figure S2 Optical characteristics of Rose Bengal (RB). (A) Absorption spectrum of RB dye obtained via UV-Vis spectroscopy. **(B)** Raman spectrum of RB dye collected under 633 nm laser excitation, showing characteristic vibrational modes.

Loading of Rose Bengal dye on AgNP-construct

In the absorbance spectra of the RB-nanoconstructs, a prominent peak was observed at 550 nm across different activation times. Using Beer-Lambert's Law $A = \varepsilon Cl$, where A is absorbance (a unitless measure of light absorbed), ε is the molar absorptivity or extinction coefficient, l is the path length or thickness of the absorbing medium in centimeters, and C is the molar dye concentration; the concentrations of RB were calculated. The resulting concentrations were 314 nM, 68 nM, and 41 nM for constructs activated for 5, 10, and 15 minutes, respectively.

$$C\ (5\text{minutes}\ activation) = \frac{A}{\varepsilon l} = \frac{0.030798}{98{,}000} = 314\ nM$$

$$C\ (10\text{minutes}\ activation) = \frac{A}{\varepsilon l} = \frac{0.006657}{98{,}000} = 68\ nM$$

$$C\ (15\ minutes\ activation) = \frac{A}{\varepsilon l} = \frac{0.004031}{98{,}000} = 41\ nM$$

Using the same Beer-Lambert equation, the concentration of the RB-nanoconstructs was also calculated to be 0.12 nM = 120 pM.

$$Number\ of\ RB\ molecules\ per\ AgNP = \frac{Concentration\ of\ RB\ dye\ on\ the\ construct}{Concentration\ of\ RB - nanoconstruct},$$

$$RB\ molecules\ per\ AgNP\ (5\ minute\ activation) = \frac{314\ nM}{0.12\ nM} = 2617$$

$$RB\ molecules\ per\ AgNP\ (10\ minute\ activation) = \frac{68\ nM}{0.12\ nM} = 567$$

$$RB\ molecules\ per\ AgNP\ (15\ minute\ activation) = \frac{41\ nM}{0.12\ nM} = 342$$

Table S1: Calculation of loading of Rose Bengal dye molecules on RB-nanoconstruct.

Activation time	Concentration of RB per 120 pM AgNP	Number of RB per nanoparticle
5 minutes	314 nM	2617
10 minutes	68 nM	567
15 minutes	41 nM	342

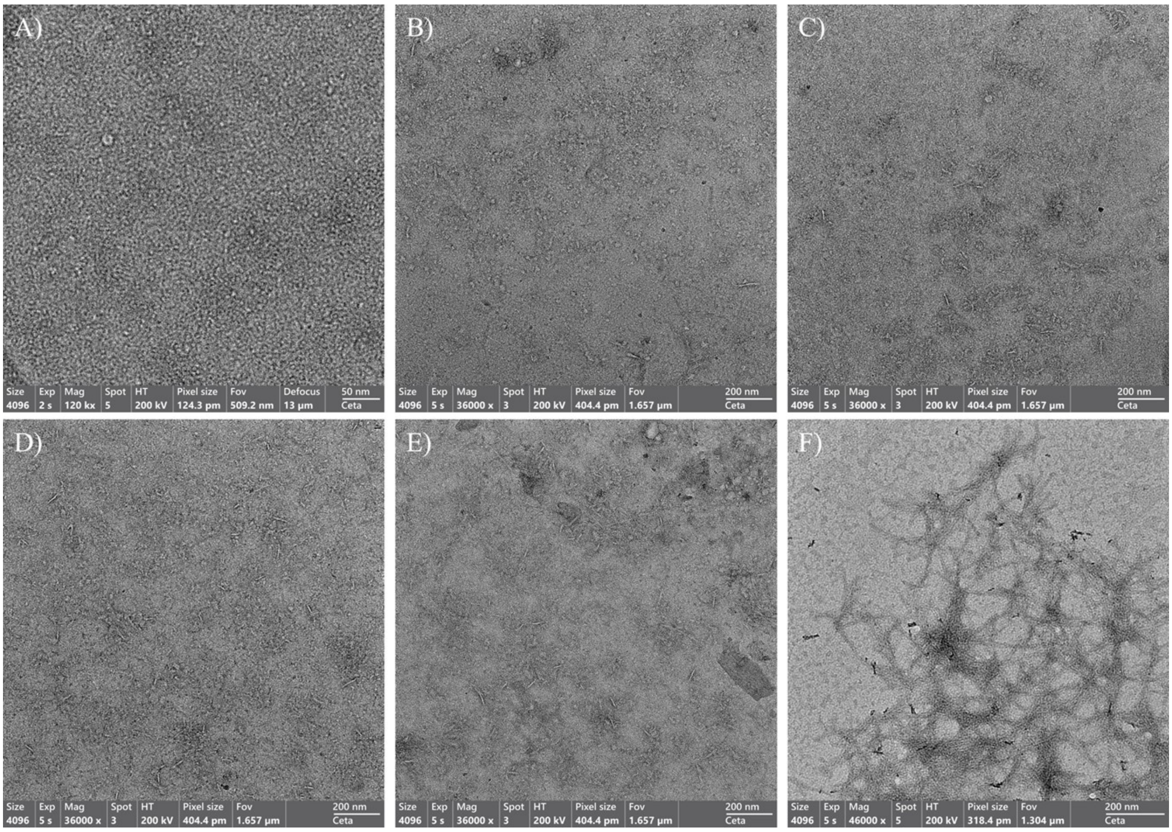

Figure S3. TEM images showing the morphological evolution of oligomers, protofibrils, and fibrils during Aβ_{42} aggregation. (A) Early-stage aggregation showing predominantly oligomers. **(B)** Predominantly oligomers with a few small protofibrils. **(C)** A mixture of oligomers and protofibrils in approximately equal proportions. **(D and E)** Primarily small protofibrils observed after ~6 hours of aggregation. **(F)** Large protofibrils formed after ~19 hours of aggregation.

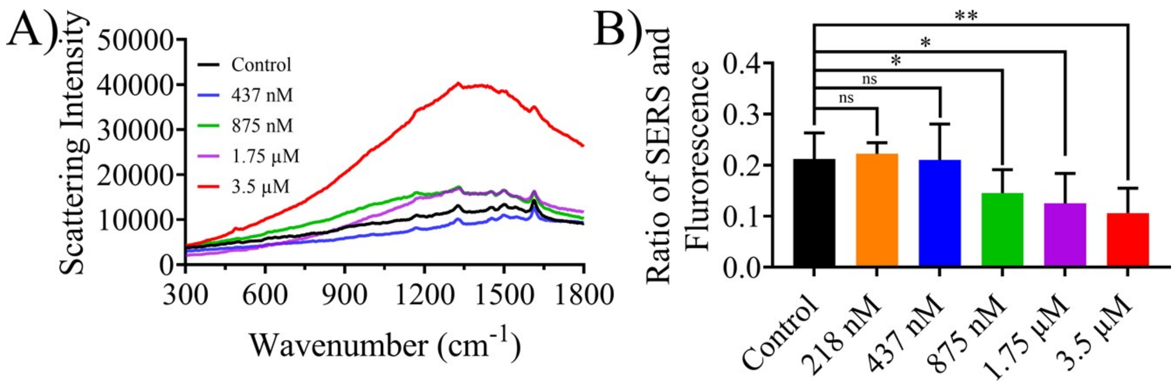

Figure S4. Probing Small Oligomers of Aβ_{42} Using RB-Nanoconstructs. (A) Representative spectra illustrating the variation in SERS intensity at ~1614 cm^{-1} with different concentrations of Aβ_{42} oligomers. As the concentration of Aβ_{42} decreased from 3.5 µM to 437 nM, the SERS signal at ~1614 cm^{-1} increased, while the fluorescence background decreased, indicating reduced interaction between oligomers and the RB-nanoconstructs. **(B)** Bar graph quantifying the ratio of SERS intensity to fluorescence at ~1614 cm^{-1} for varying oligomer concentrations, measured after 1 hour of peptide aggregation. A clear increase in the SERS-to-fluorescence ratio is observed as the concentration of soluble oligomers decreases, consistent with reduced RB displacement from the nanoparticle surface.

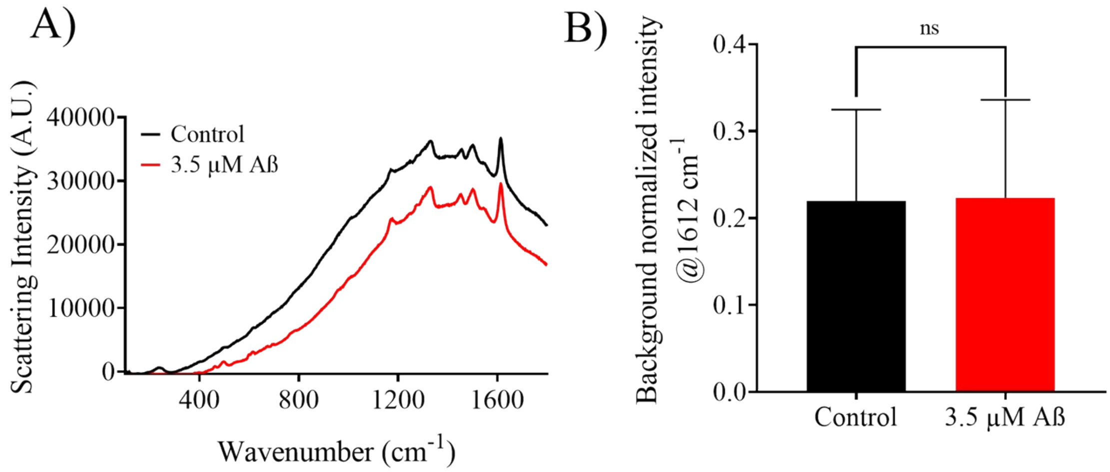

Figure S5. Probing fibrillar aggregates of Aβ$_{42}$ Using RB-Nanoconstructs. (A) Comparison of SERS spectra recorded for the control (no Aβ$_{42}$) and a sample containing 3.5 µM Aβ$_{42}$ after 4 days of aggregation, focusing on the signal near 1614 cm^{-1}. No significant difference in SERS intensity is observed between the control and the fibril-containing sample, indicating that mature fibrils do not alter the SERS response of the RB-nanoconstructs. **(B)** Bar graph showing background-normalized SERS intensity near 1614 cm^{-1} for both control and 3.5 µM Aβ$_{42}$. The identical intensity values confirm that RB-nanoconstructs do not respond to fibrillar Aβ$_{42}$, consistent with their selectivity for early oligomers.

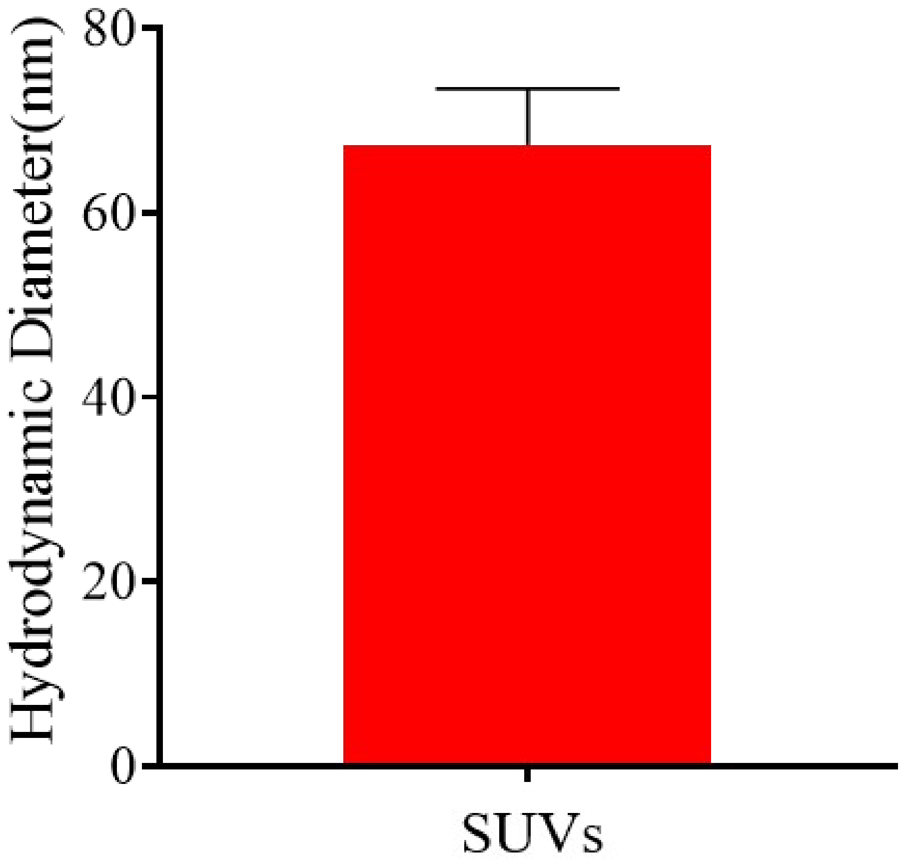

Figure S6. Hydrodynamic diameter of SUVs (67 ± 6 nm**) measured using Dynamic Light Scattering.**

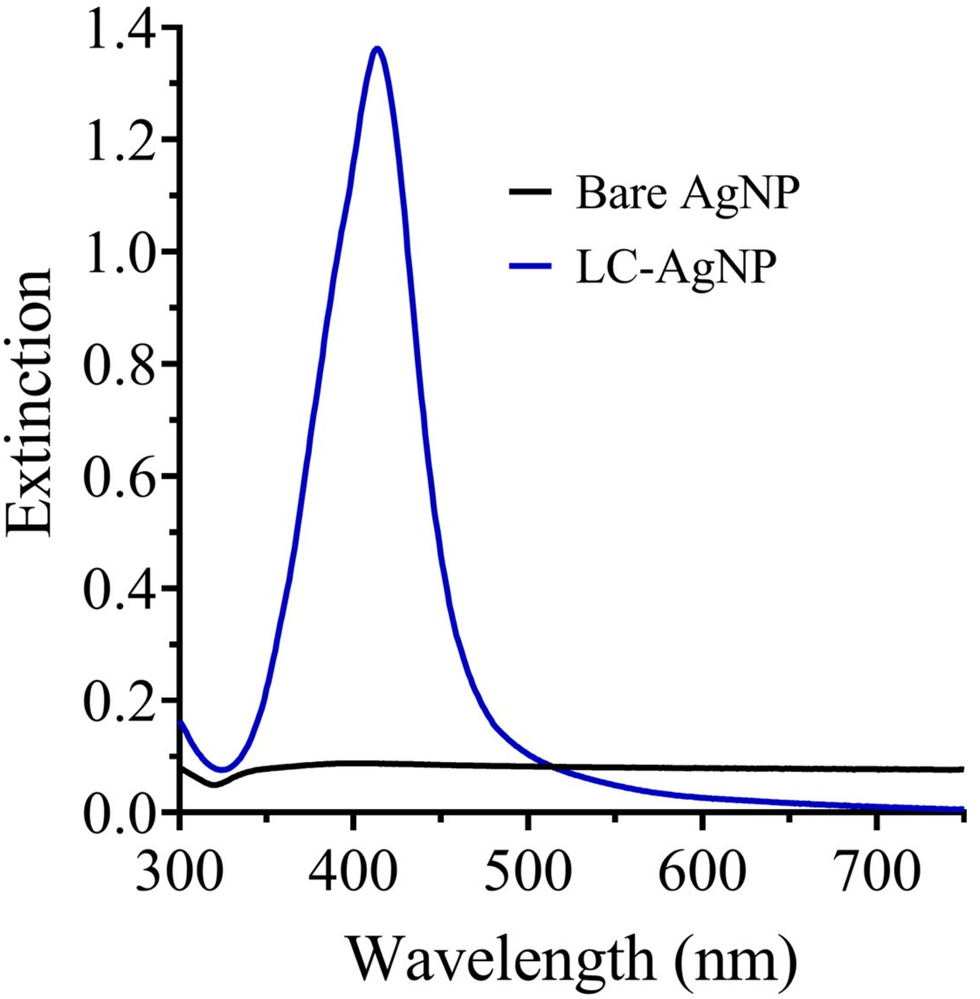

Figure S7. Salt stability test comparing the absorption spectra of bare AgNPs (black) and lipid-coated AgNPs (blue) following the addition of 1X phosphate buffer (final concentration).

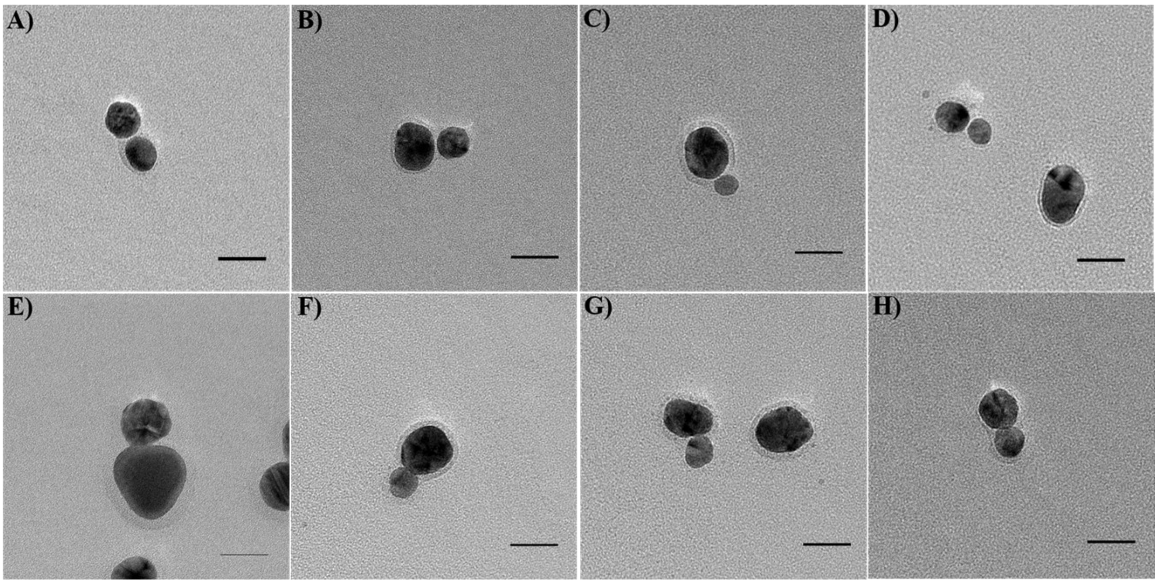

Figure S8. TEM images showing the formation of SERS "hot spots" during interactions between Aβ-loaded lipid-coated AgNPs (LC-AgNPs) and Rose Bengal-functionalized silver nanoparticles (RB-AgNPs). Two distinct interaction patterns were observed: separated configurations with a visible interparticle gap (panels A–D) and overlapping configurations (panels E–H). The transparent layer surrounding the AgNPs in the LC-AgNPs (but absent in RB-AgNPs) indicates the presence of a lipid bilayer. Scale bar: 25 nm.

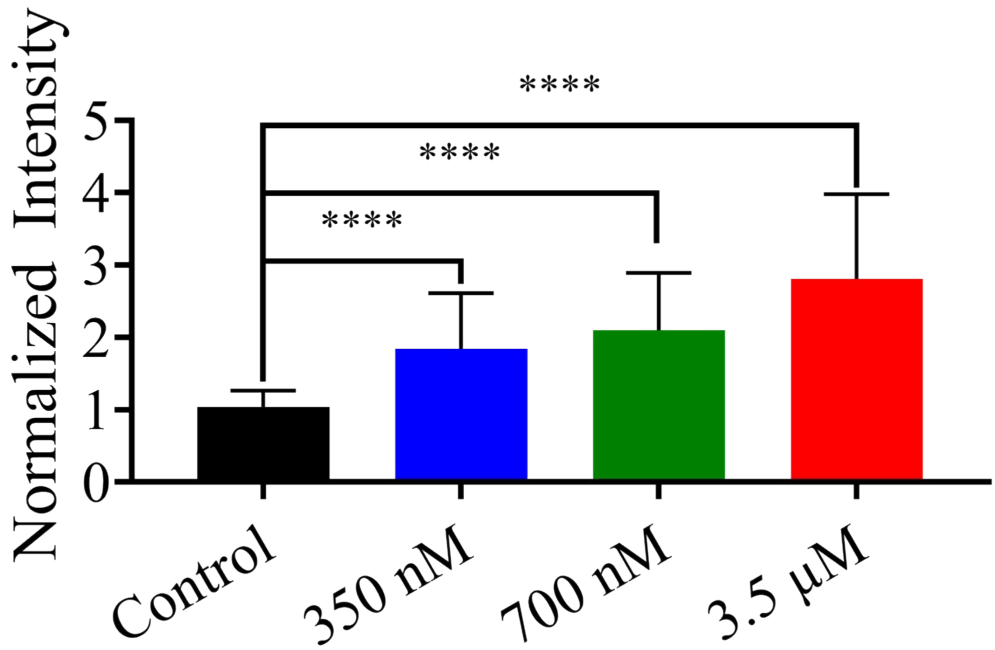

Figure S9. Increase in the RB-SERS with increasing loading of Aβ_{42} oligomers on LC-AgNP. Bar graph quantifying the normalized SERS intensity of RB for lower concentrations (3.5 μM, 700 nM, 350 nM) of Aβ_{42} oligomer at ~1614 cm^{-1}.

Calculation for the number of $A\beta_{42}$ being detected:

The prolate-shaped diffraction-limited spot in our experimental setup has a radius along the z-axis:

$$R_z = \frac{2n\lambda}{N_A^2} = \frac{2 \times 1.33 \times 532}{0.5^2} = 5660 \text{ nm}$$

The radius along the lateral (short) axes is:

$$R_{xy} = \frac{0.61 \times \lambda}{N_A} = \frac{0.61 \times 532\, nm}{0.5} = 649\, nm$$

The volume of this diffraction-limited focal region is:

$$V = \frac{4}{3}\pi \times a \times b \times c = \frac{4}{3}\pi \times 649 \times 649 \times 5660\, nm^3 = 9986066046\, nm^3$$

$Now,\ 1\, nm^3 = 10^{-24}\, liters = 10^{-9} fL$

and,

$$V = 9.986\, fL$$

Estimating the Number of Particles in the Focal Volume

For a concentration of **0.3 nM**, the number of particles within this $9.986\, fL$ Volume is calculated as follows:

Concentration $C = 0.3\, nM = 0.3 \times 10^{-9}\, mol/L$

Volume $V = 9.986\, fL = 9.986 \times 10^{-15}\, L$

Avogadro's number $N_A = 6.022 \times 10^{23}\, particles/mol$

$Moles = C \times V = (0.3 \times 10^{-9}) \times (9.986 \times 10^{-15}) = 2.996 \times 10^{-24}\, mol$

$Number\ of\ particles = moles \times N_A = 2.996 \times 10^{-24} \times 6.022 \times 10^{23} = 18.04 \times 10^{-1} = 1.8$

Therefore, during a 10-second data acquisition, on average, 1.8 LC-AgNP particles are present in the focal volume. This is equivalent to acquiring data from 1 particle over 18 seconds, assuming linear scaling.

Diffusion Characteristics of LC-AgNPs

The diffusion constant D is given by the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta r}$$

Where k_B is the Boltzmann constant $= 1.380649 \times 10^{-23} J/K$, T is the absolute temperature (in Kelvin), η is the dynamic viscosity of the fluid (in Pa·s), r is the radius of the particle (in meters).

$$D = \frac{1.380649 \times 10^{-23} JK^{-1} \times 297.15K}{6 \times \pi \times 0.0009107\, Pa.S \times 23 \times 10^{-9} m} = 1.04 \times 10^{-11} m^2 s^{-1}$$

Diffusion Time Through Focal Volume

Using the relation $< r^2 >= 4 \times D \times \tau_D$, and the lateral radius $r = 649\, nm$:

$$\tau_D = \frac{< r^2 >}{4D} = \frac{(649 \times 10^{-9})^2 m^2}{4 \times 1.04 \times 10^{-11} m^2 s^{-1}} = \frac{1.012 \times 10^{-13}}{10^{-11}} s = 10.13\, msec$$

Assuming each LC-AgNP leaves the focal volume after this diffusion time and does not return within the 18-second data acquisition period, the maximum number of distinct LC-AgNPs detected in this time is:

$$\frac{18\, sec}{10.13\, msec} = 1777 \text{ particles.}$$

Signal Scaling with $A\beta_{42}$ Concentration

Therefore, at a sample concentration of 3.5 μM $A\beta_{42}$, the data could include signals from up to 1777 $A\beta_{42}$-loaded LC-AgNPs. Therefore, at the detection limit of 218 nM $A\beta_{42}$, the number of detected $A\beta_{42}$-loaded LC-AgNPs is: $(1777 \times 218\, nM \div 3500\, nM) = 110$.

Thus, at our detection limit, an average of 110 $A\beta_{42}$-loaded LC-AgNPs contributes to the measured signal during each 10-second.

Table S2: Comparison of the our SERS-based detection platform with other reported methods for detecting lipophilic-$A\beta_{42}$ oligomers.

Sl. No.	Reference	Method of Detection	Target Species	Detection Limit	Specificity towards lipophilic/ vesicle associated oligomers
1	High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nakamura et al., 2018,* Nature, 554, 249-254.	Immunoprecipitation-mass spectrometry	$A\beta_{42}$, $A\beta_{40}$, $APP_{669–711}$ (ratios)	Lower limit based on standard curves (~40 –160 pM range, depending on peptide).	**No:** Targets primarily soluble peptides; no focus explicitly on oligomeric or membrane-bound forms.
2	New ELISAs with high specificity for soluble oligomers of amyloid β-protein detect natural Aβ oligomers in human brain but not CSF. *Yang et al.*, Alzheimer's & Dementia, 2013, 9, 99–112.	Sandwich ELISA	Soluble Aβ oligomers	~39 pg/ml (using synthetic Aβ dimer standards)	**No:** Explicitly targets soluble oligomers in brain extracts; no mention of membrane-associated forms.
3	Subtyping of circulating exosome-bound amyloid β reflects brain plaque deposition. *Lim et al.,* Nature Communication, 2019, 10, 1144.	Amplified plasmonic exosome platform (with SPR)	Exosome-carrying prefibrillar $A\beta_{42}$ aggregates ($A\beta_{42}$+ and CD63+ exosomes).	~200 exosomes (10^5-fold improvement over Western blot; 10^3-fold over standard ELISA).	**Yes:** Targets Aβ oligomers bound to exosomes (smallest of the extra-cellular vesicles); distinct from free soluble oligomers.
4	Bifunctional Fluorescent/ Raman Nanoprobe for the Early Detection of Amyloid. *Xia et al.,* Nature Scientific Report, 2019, 9, 8497.	Surface-enhanced Raman scattering and fluorescence.	$A\beta_{42}$ aggregates/ peptides.	Not explicitly stated; linear detection range 1–2 μM (saturation >2 μM).	**No**: No explicit distinction or preference for lipophilic forms.
5	Our method	Surface-enhanced Raman scattering	Lipophilic, membrane-bound small oligomers	~110 $A\beta_{42}$-loaded-Lipid-bilayer coated AgNPs.	**Yes:** Specifically designed to detect lipophilic membrane associated Aβ

			of $A\beta_{42}$		oligomers.