Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Self-Assembled Lipoprotein Based Gold Nanoparticles for Detection and Photothermal Disaggregation of β-Amyloid Aggregates

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1. Supplementary Figures
2. Materials and Methods
3. References

1. Supplementary Figures

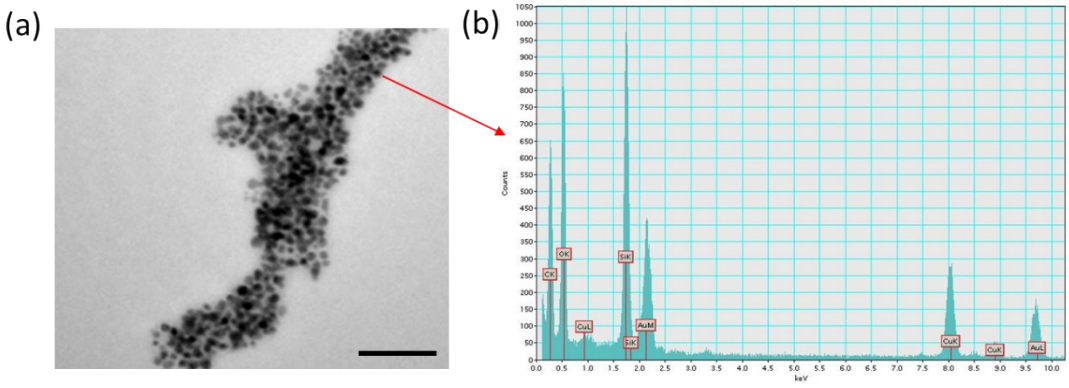


Fig. S1 (a) Electron microscopy (scale bar: 200 nm) and (b) EDAX spectra of ApoE3-AuNP-rHDL.

	Size (d, nm)	Zeta potential (mV)
ApoE3-rHDL	65.20 ± 11.71	- 11.4 ± 4.2
ApoE3-AuNP-rHDL	41.03 ± 7.24	- 17.3 ± 5.0
Curcumine-ApoE3-rHDL	68.33 ± 17.32	-11.7 ± 4.7
Curcumine-ApoE3-AuNP-rHDL	40.07 ± 8.31	-16.9 ± 4.5

Fig. S2 Size and zeta potential of the pristine and curcumin loaded ApoE3-rHDL and ApoE3-AuNP-rHDL particles.

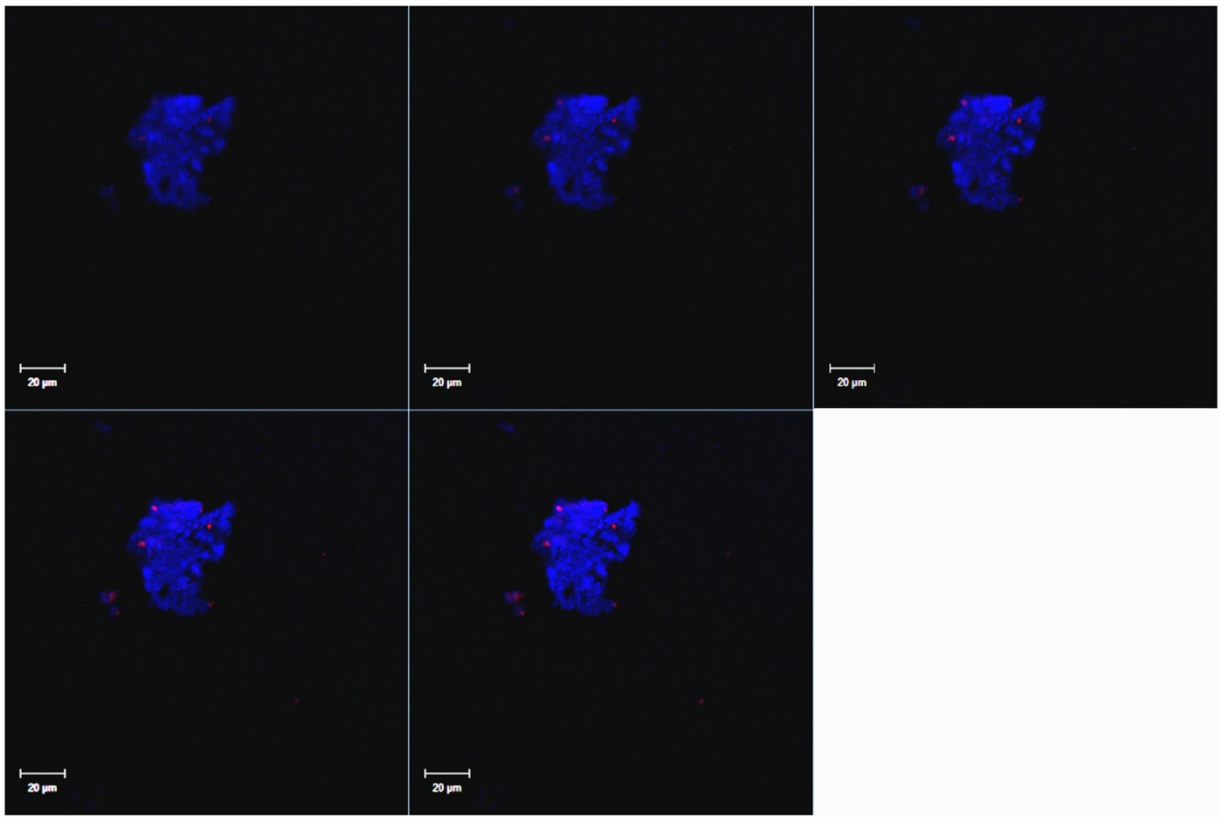


Fig. S3 Serial confocal sections of amyloid aggregates labeled with ThT after incubation with ApoE3-rHDL prepared with 10% RhB labeled DMPC. The representative confocal images were taken every 1 µm section from top to bottom.

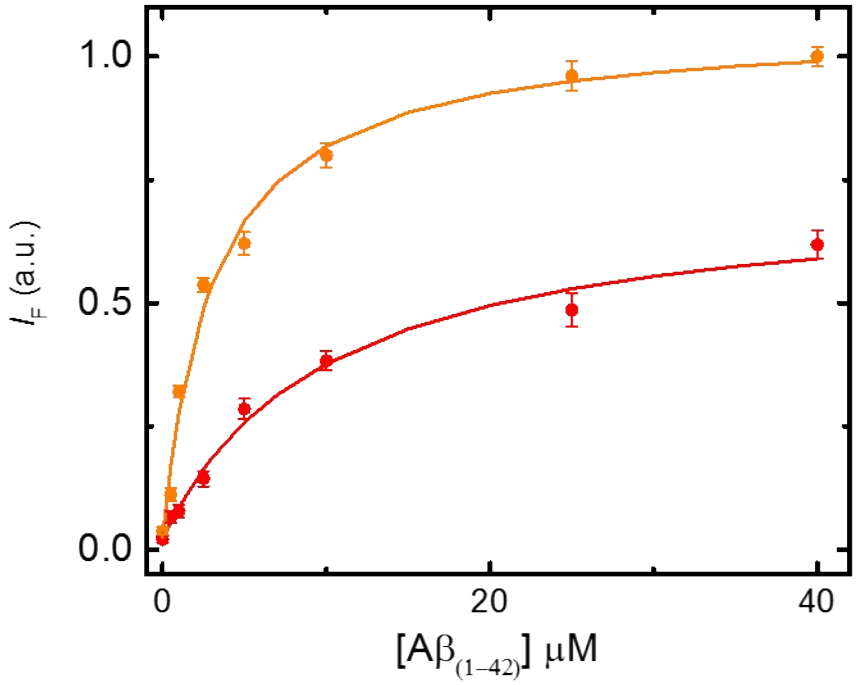


Fig. S4 Normalized average values, and standard deviation, of the fluorescence intensity of 2.5 μM curcumin loaded in ApoE3-AuNP-rHDL as a function of Aβ concentration for Aβ monomers (red) and Aβ aggregates, incubated for 80 h (orange). The recovered apparent binding constant was K_b^{app} = (9.9±0.7) × 10^4 M^{-1} for Aβ monomers and K_b^{app} = (3.2±0.2) × 10^5 M^{-1} for Aβ aggregates.

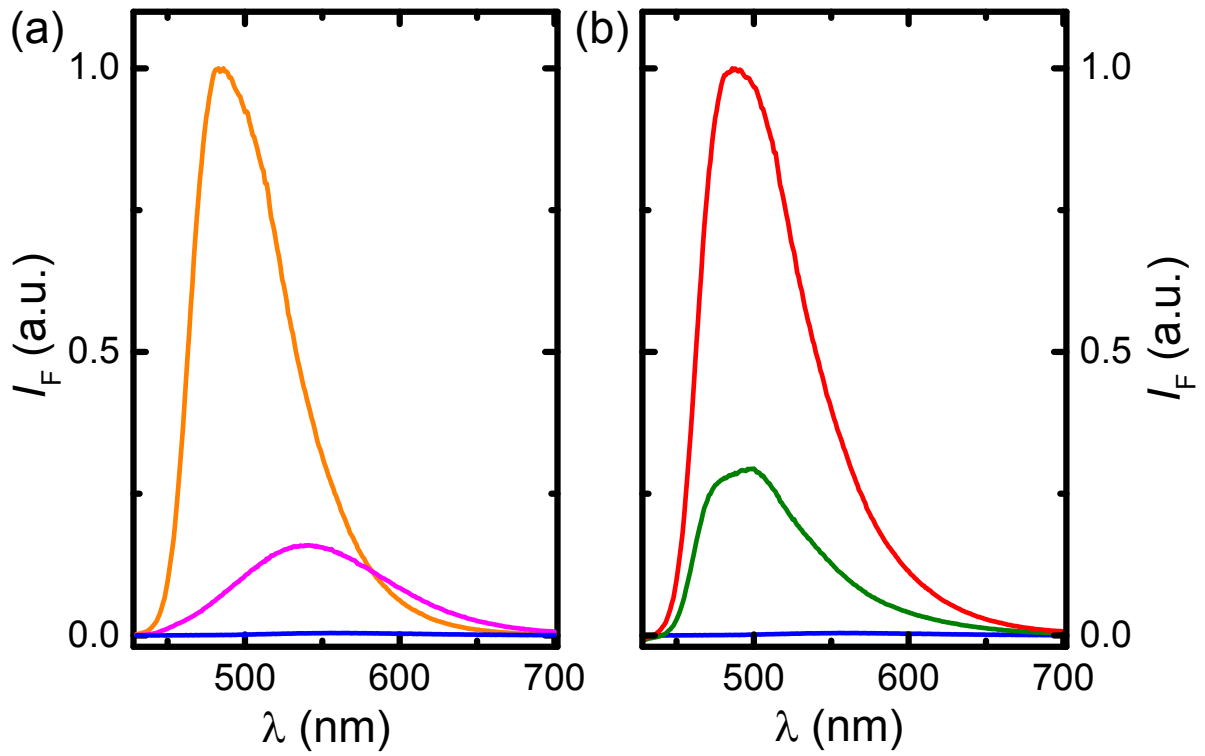
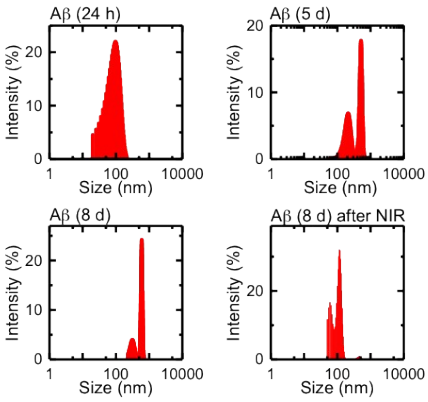


Fig. S5 Fluorescence emission spectra of curcumin in solvents with different polarity such as (a) pure water (blue), methanol (pink) and chloroform (orange) and (b) in buffer (blue), after incubation with ApoE3-rHDL (green) and with Aβ aggregates (red). The fluorescence spectra were recorded between 430 and 700 nm following excitation at 420 nm.



	Size (d, nm)	
Aβ (24 h) (N = 7)	97.0 ± 43.4	
Aβ (5 days) (N = 4)	486.5 ± 51. 6	182.4 ± 49.3
Aβ (8 days) (N = 3)	570.7 ± 37.0	283.1 ± 52.7
Aβ (8 days) after NIR (N = 4)	86.9 ± 18.6	30.1 ± 12.2

Fig S6. Size of amyloid beta peptide incubated at aggregative conditions up to 8 days and after incubation with ApoE3-AuNP-rHDL and irradiation with NIR for 15 minutes. In all cases more than three independent experiments were measured.

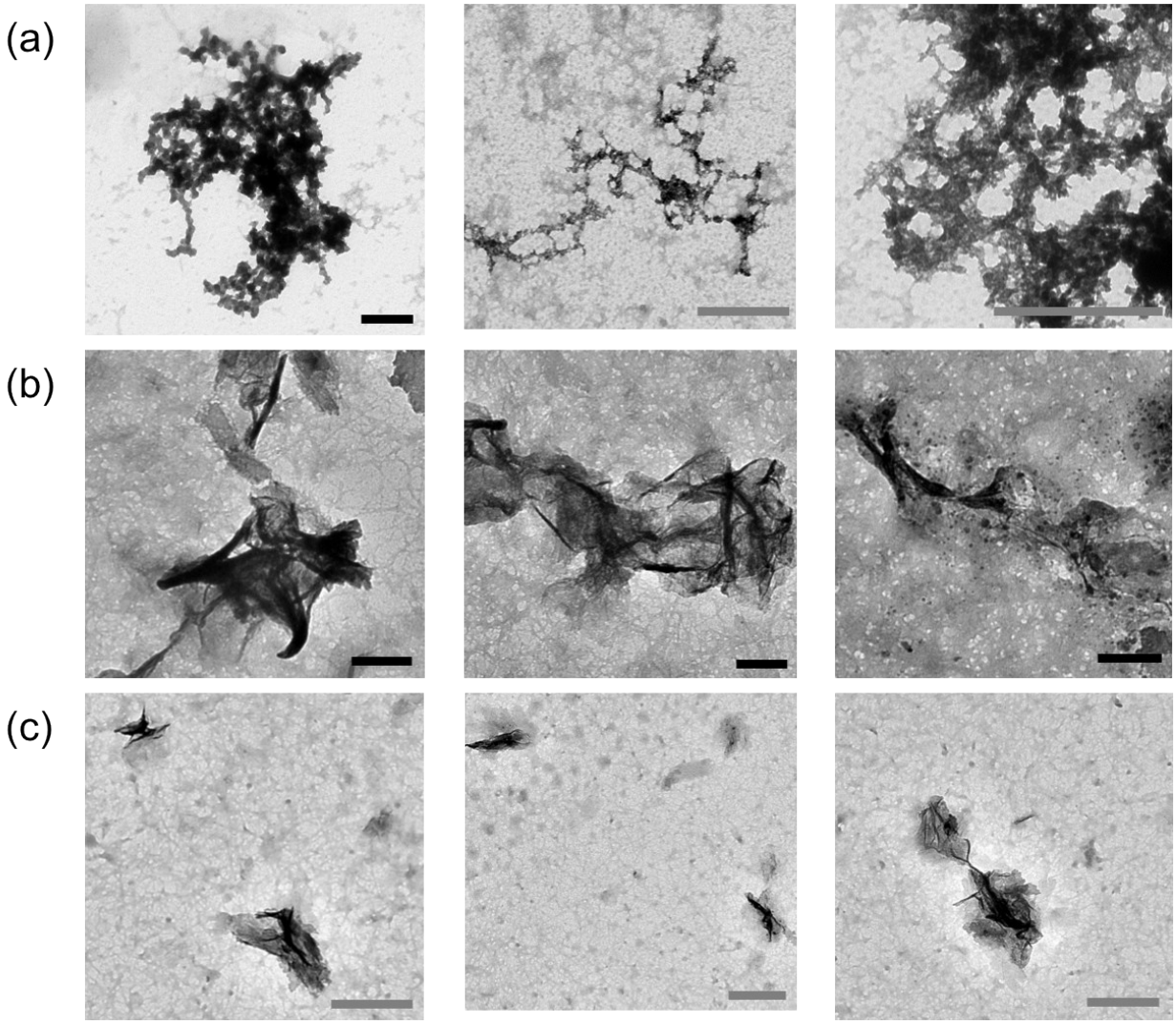


Fig S7. TEM images of Aβ(1-42) incubated under aggregative conditions for (a) 24 hours, (b) 8 days and (c) after 15 minutes irradiation with NIR light followed by 3 hours equilibration. Scale bars: 200 nm (black) and 500 nm (grey).

2. Materials and Methods

Curcumin was obtained from Sigma-Aldrich (Taufkirchen, Germany) and used with no further purification. Dimyristoyl phosphatidylcholine (DMPC) was obtained from Avanti Polar Lipids (AL, US). Apolipoprotein E3 (ApoE3) was obtained from BioVision (CA, US) as a lyophilized sample, it was hydrated according to manufacturer's suggestions and aliquots of 5 mg/mL were stored at – 20 °C until usage. Amyloid beta peptide, Aβ(1-42) was obtained from American Peptide Company (CA, USA), dissolved in hexafluoroisopropanol (HFIP) at 1mg/mL and stored in aliquots at – 20 °C until usage. All other reagents and solvents were of high purity and no further purification was needed.

Preparation of reconstituted HDL with ApoE3. Aqueous suspensions of DMPC were prepared by evaporating a lipid solution in azeotropic mixture composed of chloroform:methanol 9:1 (v:v), by blowing dry nitrogen over the heated (blowing hot air over the external surface of the vessel) solution and then leaving the residue in vacuum for at least 8 h at room temperature. The solvent free residue, as a thin film in the vessel wall, was hydrated with HEPES buffer (10 mM, pH 7.4) with 0.15 M NaCl, 1 mM EDTA and 0.02 % (m/v) NaN_3 (hereafter termed buffer). The hydrated lipid was subjected to several cycles of vortex/incubation for at least 1 h, to produce a suspension of multilamellar vesicles, that was then extruded in a water-jacketed extruder (Lipex Biomembranes, Vancouver, British Columbia, Canada) using a minimum of ten passes, through two stacked polycarbonate filters (Nucleopore) with a pore diameter of 0.1 μm^1. The hydration and extrusion steps were performed at 30 °C and resulted in 100 nm diameter unilamellar lipid vesicles or liposomes. The same procedure was followed for liposomes containing curcumin, except that an appropriate volume of a stock solution of curcumin 5 mM in azeotropic mixture was added to the lipids and thoroughly mixed prior to vesicle preparation. Following the preparation of liposomes, ApoE3 0.01 mM was added and the solution subjected to bath sonication for 10 minutes

followed by incubation at 37 °C for 36 h to allow the self-assembly of apolipoprotein and phospholipids into reconstituted lipoproteins (ApoE3-rHDL). The final solution was sterilized through a 0.22 μm filter to remove particles larger than 220 nm.

AuNP synthesis and modification. Gold nanoparticles were prepared following the experimental procedure described by Baiker and co-workers (1993)[2]. The resulting orange-brown hydrosols of gold were then characterized by HR-TEM and EDAX (Figure S1).

AuNP surface was then substituted with acyl chains containing 8 carbons. For that, 100 μL of octane thiol were dissolved in cyclohexane to a final concentration of 1.67 % (v/v) and added to the solid gold nanoparticles dispersed in water. Two phase mixture (water:cyclohexane) was bath sonicated at 45 °C for one hour after which it was stirred for an extra 3 hours and left resting overnight to allow for complete phase separation. Before reaction the organic layer was colorless and the aqueous phase containing the THPC stabilized AuNPs was red and translucid but after the reaction, the opposite was detected, the organic solution became red and the aqueous phase transparent and colorless indicating the successful transfer of gold nanoparticles to cyclohexane as a result of coating with octanethiol. The resulting organic phase containing AuNP-C_8 was quantified for gold using ICP-OES with a final concentration of 0.75 μg/mL, in cyclohexane. The diameter of modified gold nanoparticles was determined to be 25.83 ± 4.145 nm using dynamic light scattering.

Preparation of AuNP templated reconstituted HDL with ApoE3. To assemble the AuNP core lipoprotein-like particles (ApoE3-AuNP-rHDL), C_8 modified AuNP prepared previously were dried under nitrogen and incubated with 0.5 mg ApoE3 in buffer, overnight, at 37 °C to produce an ApoE3-AuNP solution. In parallel, DMPC in azeotropic mixture was dried to film and after vacuum, for at least 8 h, this film was hydrated with the ApoE3-AuNP aqueous solution and incubated for 24 h, at 37 °C.

The same procedure was followed for ApoE3-AuNP-rHDL containing curcumin, except that an appropriate volume of a stock solution of curcumin 5 mM in chloroform:methanol 9:1 (v/v) was added to DMPC and

thoroughly mixed prior to film preparation or incubated a posteriori with the preformed reconstituted lipoproteins, adding a concentrated aliquot of curcumin in MeOH for a final volume of MeOH never above 0.5 % (v/v).

Preparation of Aβ(1-42) Monomers and Oligomers. Aβ(1-42) monomer solution was prepared by evaporating HFIP (boiling point = 58.2 °C) and allowing the traces of solvent to be removed under vacuum for at least 2 h, the peptide was then resuspended in dimethyl sulfoxide (DMSO) at a final concentration of 5 mM and bath sonicated for 10 minutes[3].

Oligomerization was initiated by diluting the monomeric peptide in DMSO to a final concentration of 100 µM in buffer and incubation the solution for 24 h, a 4°C[4]. The presence of oligomers using this protocol has been previously confirmed and carefully characterized by other authors[5]. For the purpose of the experiments done in this work, time zero is considered when the oligomer solution is taken to room temperature, after the 24 h oligomerization period.

Analytical procedures. For quantifying curcumin in the lipoprotein samples, a 4mg/mL stock solution was prepared in DMSO. A standard curve, generated by serial dilution of the stock solution was used to determine curcumin concentrations in unknown samples following the transfer of an aliquot to DMSO. Sample absorbance was measured at 430 nm on a Cary 5000 UV-Vis-NIR spectrophotometer (Varian, NC). ApoE3 concentration was assessed by its absorbance at 280 nm considering a molar extinction coefficient of 9377 $M^{-1}cm^{-1}$ in aqueous solution. The final phospholipid concentrations in the reconstituted lipoproteins were determined using a modified version of the Bartlett phosphate assay[6]. All fluorescence measurements were performed on a Cary eclipse fluorescence spectrophotometer equipped with a thermostated cell holder (Varian, Cary, NC).

Characterization of reconstituted lipoproteins. The average size/hydrodynamic diameter and zeta potential of the reconstituted particles obtained, both discoidal (ApoE3-rHDL) and spheroidal with the AuNP core (ApoE3-AuNP-rHDL) were measured at 25 °C by photon correlation spectroscopy on a Zetasizer

Nano-ZS (Malvern Instruments, UK) utilizing a 4.0 mW He-Ne laser operating at 633 nm and a detector angle of 90°. Seven independent samples were characterized for ApoE3-rHDL and nine for ApoE3-AuNP-rHDL.

The morphology of reconstituted HDL was observed using transmission electron microscopy after negative staining with 1% sodium phosphotungstate solution, as described by Gursky and co-workers[7].

NIR-Triggered Photothermal Effect of ApoE3-AuNP-rHDL. A NIR laser was obtained from Changchun New Industries Optoelectronics Technology Co., Ltd. (cnilaser). After 8 days incubation, Aβ(1-42) 100 μM aggregates were mixed with ApoE3-AuNP-rHDL containing curcumin, at a final curcumin concentration of 15 μM and allowed to equilibrate for 2 h. Sample was subjected to irradiation with continuous NIR laser (0.5 W cm−2 , 808 nm) for periods of 5 minutes until a maximum of 15 minutes. Controls were made with ApoE3-rHDL. The real-time temperature was recorded by a Neoptix Reflex fiber optic signal conditioner (Neoptix Inc.).

3. References

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