ApoE3 Lipid Particles: Assembly, Dynamics, and Biological Roles

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KEY FINDING

ApoE3-POPC (1:90)/ApoE3-POPC-Cholesterol (1:90:5) nanoparticles significantly enhance the efflux of fluorescent-tagged saturated fatty acids and cholesterol

INTRODUCTION

- Apolipoproteins are a diverse class of lipid-binding proteins that regulate lipid transport, metabolism, and cellular signaling.
- Major families, including ApoA, ApoB, ApoC, ApoD, ApoE, ApoH, ApoJ (clusterin), ApoL, and ApoM, serve as structural components of lipoproteins and modulators of lipid-associated pathways.
- Apolipoprotein E (ApoE) is critically involved in the pathogenesis of Alzheimer's disease and exists in three major isoforms (ApoE2, ApoE3, and ApoE4) which differentially influence the disease risk and progression.
- ApoE3 exhibits relatively stable lipid interactions and supports more effective amyloid clearance compared to pathogenic isoforms, thereby reducing neurotoxicity.
- Since ApoE3 drives receptor-mediated interactions, ApoE3:POPC:cholesterol nanoparticles provide a physiologically relevant and tunable platform to study receptor-mediated lipid efflux and cellular lipid trafficking.

ApoE3: EXPRESSION, PURIFICATION & CHARACTERIZATION LIPID NANOPARTICLE ASSEMBLY

ApoE3 Expression

- Transient transfection in Expi293 cells using Expi-fectamine reagents.
- Expressed for 3 days and culture media purified using affinity purification.
- ApoE3 (37.7 kDa) is an intrinsically self-aggregating protein with a strong tendency to precipitate.

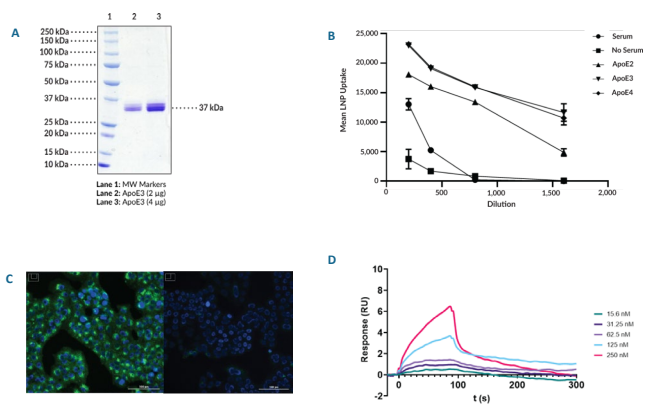


FIGURE 1. ApoE3 characterization. A. Purity gel of recombinant ApoE3. B. LNP uptake. Fluorescent lipid nanoparticles with SM-102 (LNP-102; Item No. 33474) were diluted in media with 10% serum, no serum, or different ApoE proteins (Item Nos. 37225, 37226, or 37227) at 1 µg/ml. A549 lung epithelial cells were incubated with diluted SM-102 samples, washed, and stained with Hoeschst 33342 dye for nuclei. The cells were imaged on a Cytation V imaging plate reader and analyzed for mean per-cell BODIPY fluorescence, as a measure of LNP uptake. **C. ApoE3 activity in cell-based assay.** ApoE3 (left) increases the uptake of LipidLaunch™ SM-102 LNP (GFP) (Item No. 39320) compared with no serum (right). **D. ApoE3 binds LDLR.** ApoE3 binds human LDLR with nanomolar affinity. LDLR (Fc-tagged) was immobilized on CM5 *via* anti-human IgG Fc antibodies. SPR analysis was used to determine ApoE3 binding affinity on a Biacore™ 8K using multi-cycle kinetics with five concentrations of ApoE3.

ApoE3: LIPID NANOPARTICLE ASSEMBLY

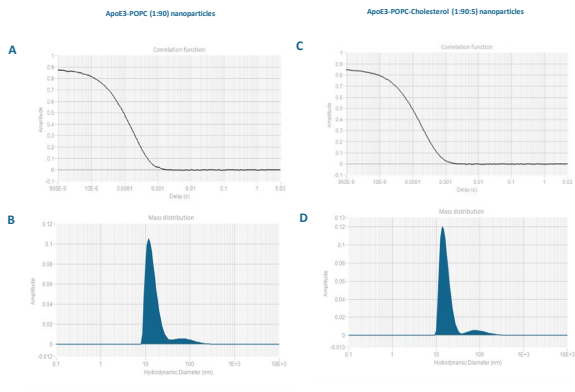
Day 1: Lipid/Cholesterol preparation

Day 2: ApoE3-POPC/ApoE3-POPC-Cholesterol assembly

Day 3-5: ApoE3-POPC/ApoE3-POPC-Cholesterol assembly & concentration

Day 6: ApoE3-POPC/ApoE3-POPC-Cholesterol characterization

ApoE3 NANOPARTICLE: CHARACTERIZATION



	Polydispersity Index (PDI)	Peak of Interest Mode Diameter (nm)	Peak of Interest Est. MW (kDa)	Calculated Concentration (mg/ml)
ApoE3-POPC-NP	0.29	17.81	495.5	0.65
ApoE3-POPC-Cholesterol-NP	0.34	17.17	481	0.66

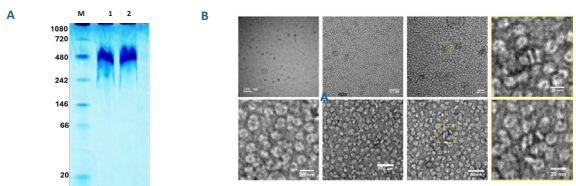


FIGURE 2. Top panel (A-D): Dynamic light scattering (DLS) analysis of ApoE3-lipid particles. Panels A-B & C-D: ApoE3-POPC- and ApoE3-POPC-Cholesterol-nanoparticles. The top panel (A & C) shows the intensity autocorrelation function. The lower panel (B & D) presents the corresponding mass-weighted size distribution. **Middle panel. Table showing DLS parameters.** Values shown represent averaged (n=2-3) measurements. **Bottom panel Left (A): Analysis of purified lipid-nanoparticle (Lipid-NP):** A. Native PAGE of purified ApoE3 lipid nanoparticle (Lipid-NP). Lane M - NativeMark™ Unstained Protein Standard, Lane 2 – ApoE3-POPC-NP, Lane 3 – ApoE3-POPC-Cholesterol-NP. **B. TEM image (Reference - Ioannou Lab).** Representative TEM images of discoidal human ApoE3-POPC-cholesterol nanoparticles at different magnifications. Particles were 100x diluted in 20 mM HEPES (~6.6 µg/ml). 5 µl of diluted sample was stained with 1% uranyl acetate in a glow-discharged carbon film supported copper square grid, 400 mesh. Images were taken with a 200kv JEOL 2100 TEM in the Cross Cancer Institute.

ApoE3: LIPID RELEASE ASSAY

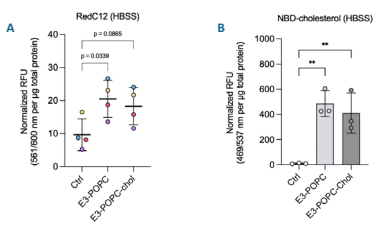


FIGURE 3: The measurements of fluorescent intensity of lipids released into the media in the presence of lipidated ApoE nanoparticles. Primary rat hippocampal neurons were preloaded with fluorescent lipid analogs overnight; saturated fatty acids RedC12 (left) or NBD-cholesterol (right). The cells were washed vigorously, stabilized in fresh media, then incubated in HBSS for 4 hours. The conditioned media were collected and spun at 16,000 g to remove dead cells and debris. The relative fluorescence of the collected media in black 96-well plates was measured using the Synergy Mx Multi-Mode Microplate Reader, normalized to total protein contents; λex/em = 561/600 nm (RedC12) and 469/537 nm (NBD-cholesterol). n = 3-4 independent experiments; mean ± SD. One-way ANOVA with Tukey's post-test; *p < 0.05, **p < 0.01.

CONCLUSIONS

- ApoE3-POPC and ApoE3-POPC-Cholesterol nanoparticles enhance the efflux of fluorescently tagged saturated fatty acids and cholesterol

REFERENCES

Ralhan, I., Do, A.D., Bae, J.-Y., Feringa F.M., *et. al.* (2025). Protective ApoE variants support neuronal function by effluxing oxidized phospholipids . *Neuron*. **114(4)**:661-678

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