Apolipoprotein E: Purification, Characterization, and Lipid Nanoparticle Uptake Enhancement

Alfredo J. Guerra, Kendall M. Muzzarelli, J. Paige Gronevelt, Julie M. Rumble, and Zahra Assar
Cayman Chemical Company, Ann Arbor, MI

KEY FINDING

Cayman's recombinant ApoEs aid in the uptake of lipid nanoparticles.

INTRODUCTION

Apolipoprotein E (ApoE) is a crucial player in lipid metabolism. However, ApoE has roles beyond lipoprotein metabolism. Of the three major isoforms (ApoE2, ApoE3, and ApoE4), the ApoE4 isoform has been extensively implicated in an elevated risk of neurodegenerative, cardiovascular, and infectious diseases.[1-3] Beyond its implications in disease, ApoE has been implicated in the intricate process of lipid nanoparticle (LNP) uptake. Recent studies have revealed the essential role of ApoE in facilitating the cellular internalization of LNPs, providing a potential avenue for targeted drug delivery and therapeutic interventions.[4] In this study we have expressed and purified all three ApoE isoforms and characterized their binding properties to cognate ligands by surface plasmon resonance (SPR). Furthermore, we show that Cayman-produced LNP uptake is enhanced by addition of exogenous ApoE in a cell-based assay with lung epithelial cells.

PROTEIN EXPRESSION AND PURIFICATION

C-terminally His-tagged human ApoE2, ApoE3, and ApoE4 (Item Nos. 37225, 37226, and 37227, respectively) were expressed in Expi293 cells and harvested 3 days post-transfection. Proteins were purified by immobilized metal chelate affinity chromatography (IMAC) to a final purity of 73-97% as determined by densitometry.

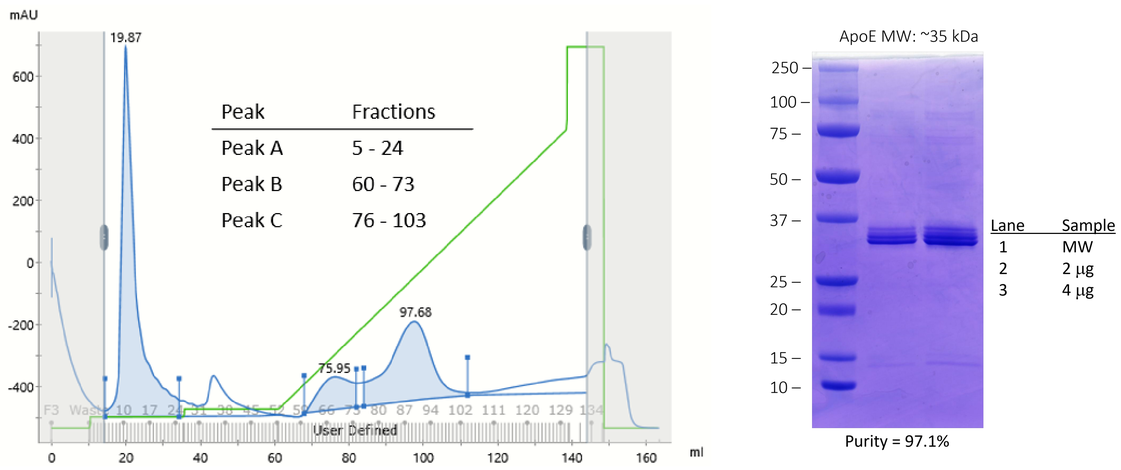

FIGURE 1 – ApoE3 purification by IMAC and final purity gel.

APOE TREM2/LDLR BINDING DETERMINED BY SPR

- The ApoE4 allele of the *APOE* gene stands out as the most potent established genetic risk factor for late-onset Alzheimer's disease.
- Triggering receptor expressed on myeloid cells 2 (TREM2) represents another significant risk factor influencing the progression of Alzheimer's disease.[5]
- Recent findings suggest TREM2's potential role as a receptor for ApoE, suggesting that interactions between ApoE and TREM2 may influence the pathogenesis of Alzheimer's disease.[5]
- We immobilized Fc-tagged TREM2 to a protein G chip through direct capture method and tested the binding of all ApoE isoforms by SPR.
- We also tested all the binding of each isoform to Fc-tagged LDLR.

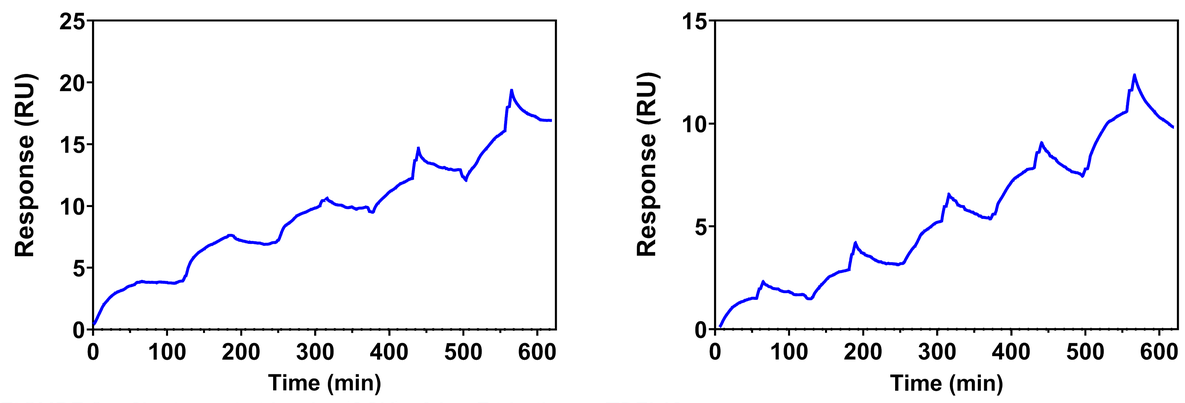

FIGURE 2 – Single-cycle kinetics (SCK) of ApoE binding to TREM2.
ApoE3 **(left)** and ApoE4 **(right)** binding to human TREM2 captured on a protein G chip.

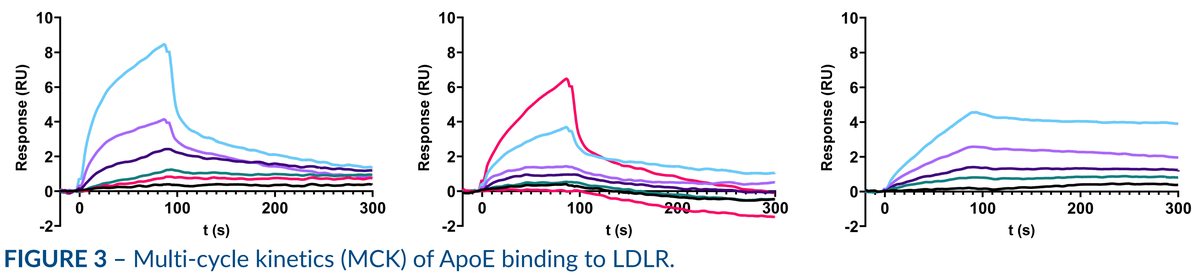

FIGURE 3 – Multi-cycle kinetics (MCK) of ApoE binding to LDLR.
ApoE2 **(left)**, ApoE3 **(middle)**, and ApoE4 **(right)** binding to human LDLR (Fc-tag) captured on an α-Fc previously immobilized on CM5 chip.

Table 1 – ApoE SPR binding summary.

Protein	TREM2 K_D (nM)	LDLR K_D (nM)
ApoE2	n.d.	75 ± 6
ApoE3	36 ± 7	114 ± 59
ApoE4	197 ± 17	51 ± 38

LNP UPTAKE IS ENHANCED WITH APOE

- Fluorescent LNPs were prepared using LipidLaunch™ LNP-102 Uptake Kit (Green Fluorescence) (Item No. 38218) using a Nunchuck microfluidics device (SM-102:DSPC:cholesterol:BODIPY-cholesterol: DMG-PEG of 50:10:36:2.5:1.5, respectively, TFR of 15 ml/min, 3:1 aqueous:lipid).
- A549 cells were plated at 2,500 cells per well and allowed to attach overnight.
- LNPs were diluted in media with no serum or ApoE proteins at 1 µg/ml without additional serum.
- Diluted LNPs were added to cells and incubated at 37°C for 48 h.
- Media were removed and cells were stained with Hoechst dye and imaged on a BioTek Cytation 5 imaging plate reader.

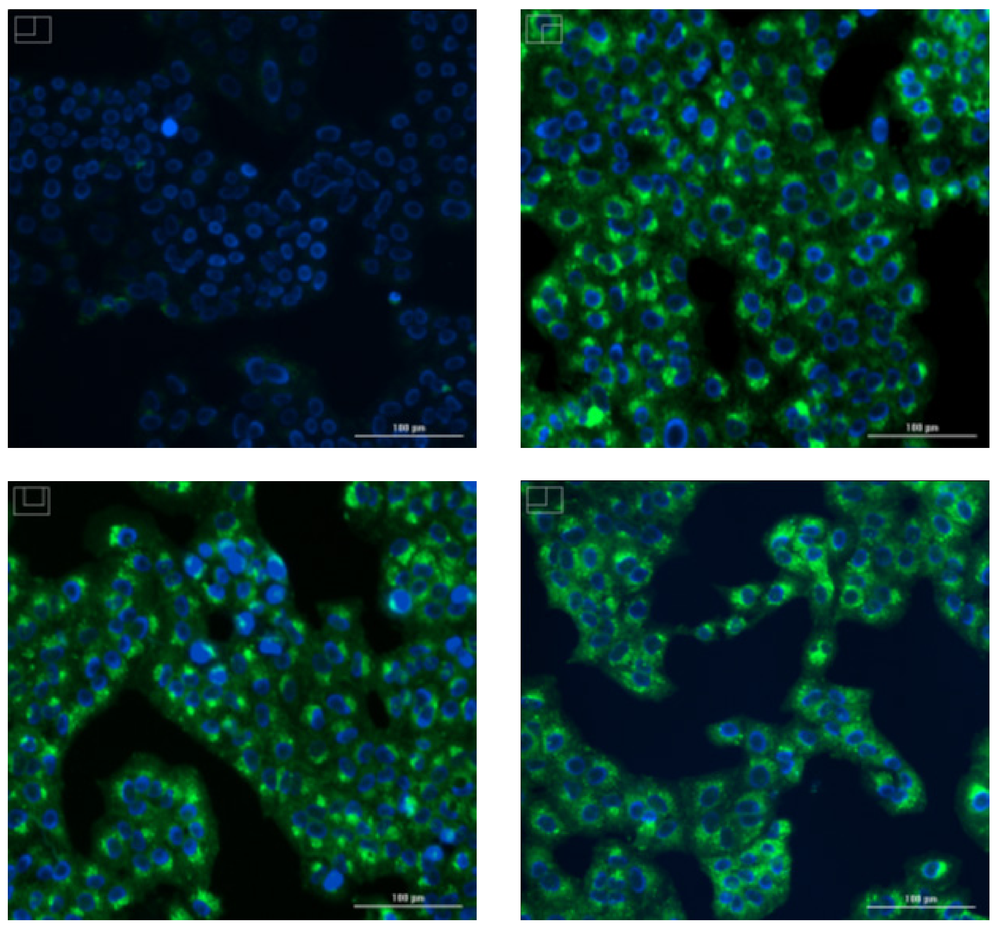

FIGURE 4 – LNP-102 uptake by A549 lung epithelial cells is enhanced with ApoE.
Top left: no serum, **Top right**: ApoE2 without serum, **Bottom left**: ApoE3 without serum, **Bottom right**: ApoE4 without serum.

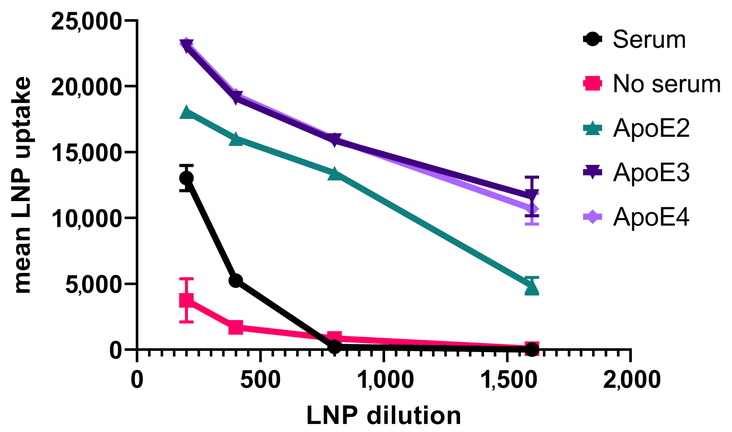

FIGURE 5 – Summary of LNP-102 uptake by A549 lung epithelial cells.

CONCLUSION

- We have expressed and purified all three ApoE isoforms to a high degree of purity.
- ApoE3 and ApoE4 bind to TREM2 with affinities comparable to those previously reported in the literature.
- Addition of exogenous, recombinant ApoE aids in LNP uptake in lung epithelial cells.

References

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