

Crossing the Blood–Brain Barrier with Aco-Dyes™: Fluorogenic Visualisation of Extracellular Vesicle Nanocarriers in the Brain

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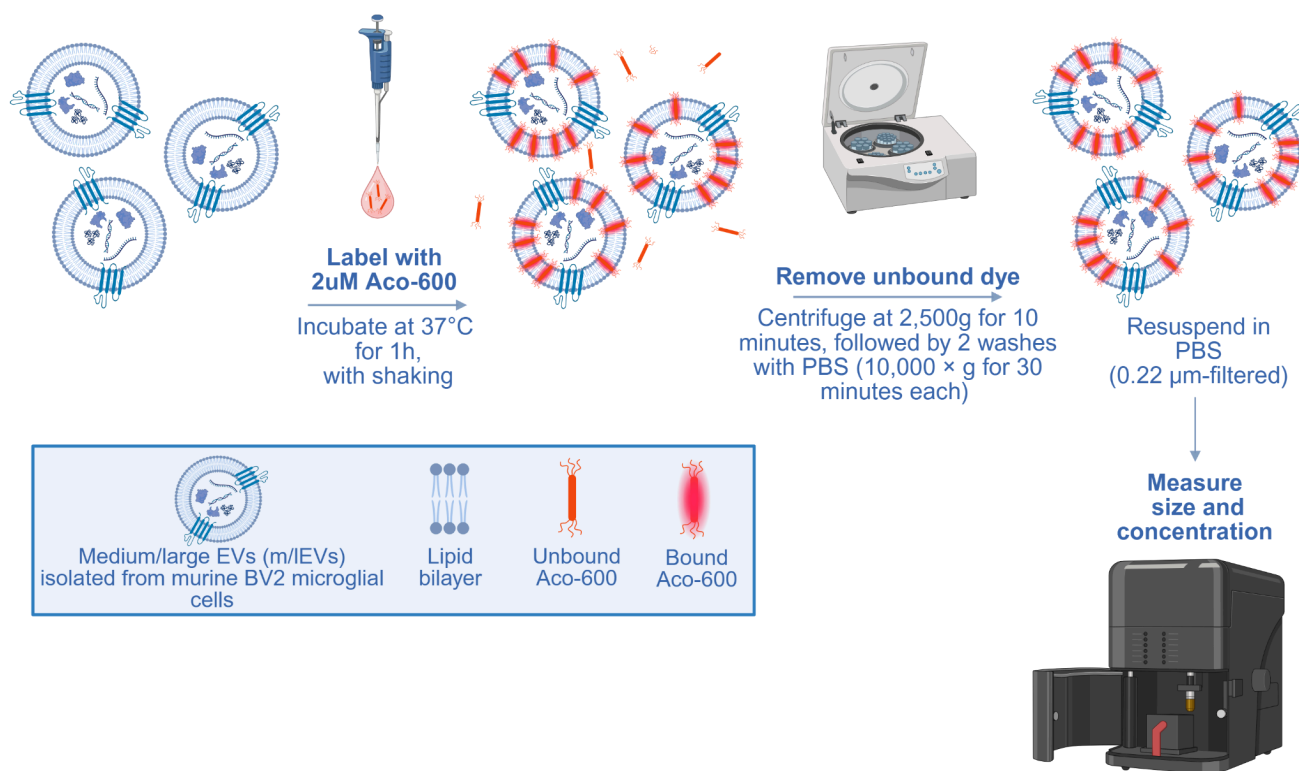
Introduction

The use of extracellular vesicles (EVs) as nanocarriers is rapidly expanding due to their biocompatibility, ability to transport bioactive cargo, and potential to cross the blood–brain barrier [1]. However, reliable in vivo EV tracking remains challenging, as conventional lipophilic dyes can aggregate, form micelles, and generate high background fluorescence, making EV-associated signals difficult to distinguish from free dye artifacts [2].

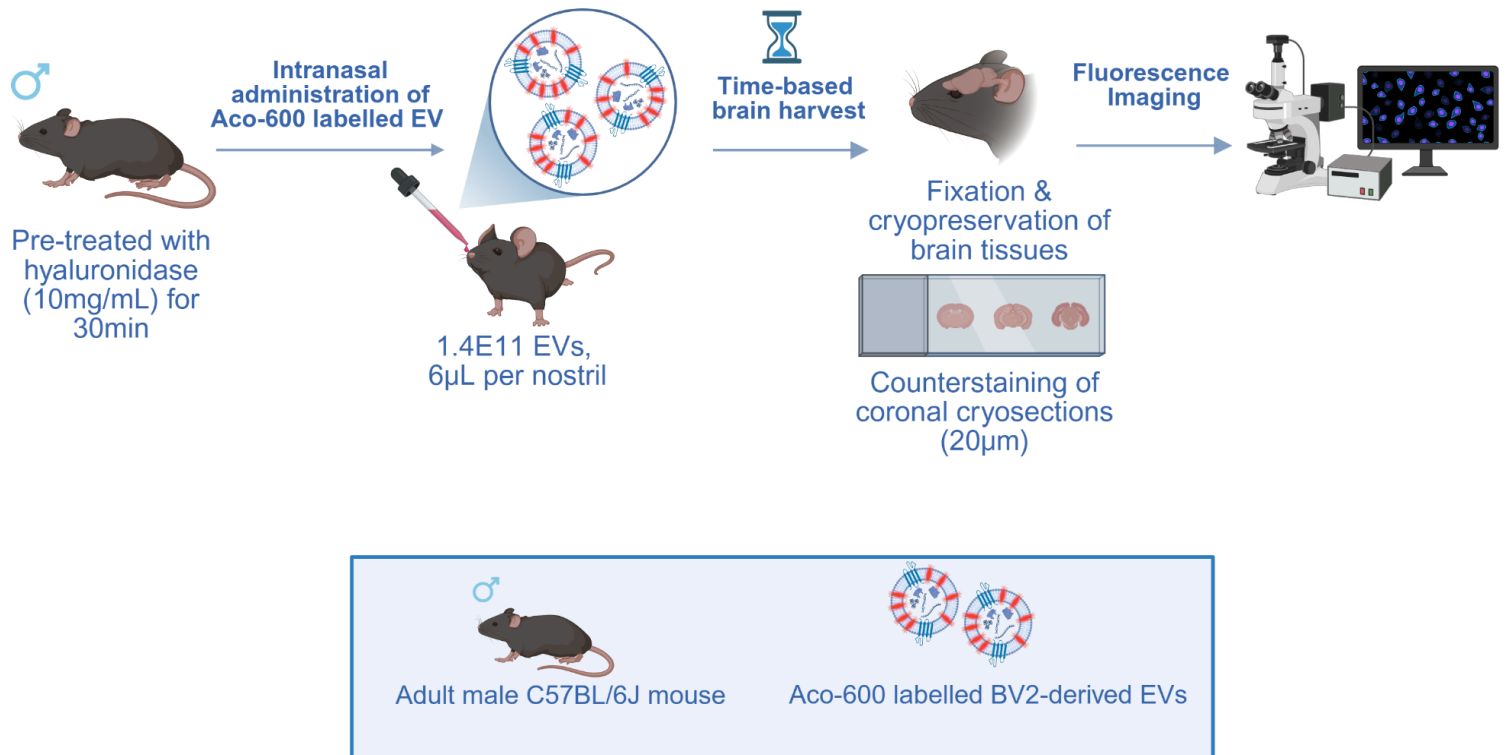
Here, we highlight the work of Rinaldi & Catalano [3], who used Aco-600™ from the range of Aco-Dyes™ (Acoerela, Singapore), which are water-soluble, fluorogenic membrane dyes designed for stable, low-background EV labelling to track intranasally administered EVs in the mouse brain over 24 hours. EV-associated fluorescence peaked at approximately 60 minutes, demonstrating the utility of fluorogenic membrane labelling for sensitive, time-resolved imaging of EV biodistribution in the central nervous system.

Materials and Methods

EV Labelling and Characterisation



In Vivo EV Administration



Created in <https://BioRender.com>

Results

m/IEVs exhibited a mean diameter of 183 ± 2 nm, as determined by NTA, confirming successful vesicle isolation (Fig. 1A). Following fluorogenic membrane labelling with Aco-600, $45.6 \pm 1.9\%$ of EVs exhibited fluorescence, resulting from the dye insertion into the lipid bilayer of the EV membrane. Importantly, Aco-600 labelling did not significantly alter EV particle concentration or size distribution (Fig. 1B–C), indicating that membrane labelling preserved vesicle integrity and physicochemical properties.

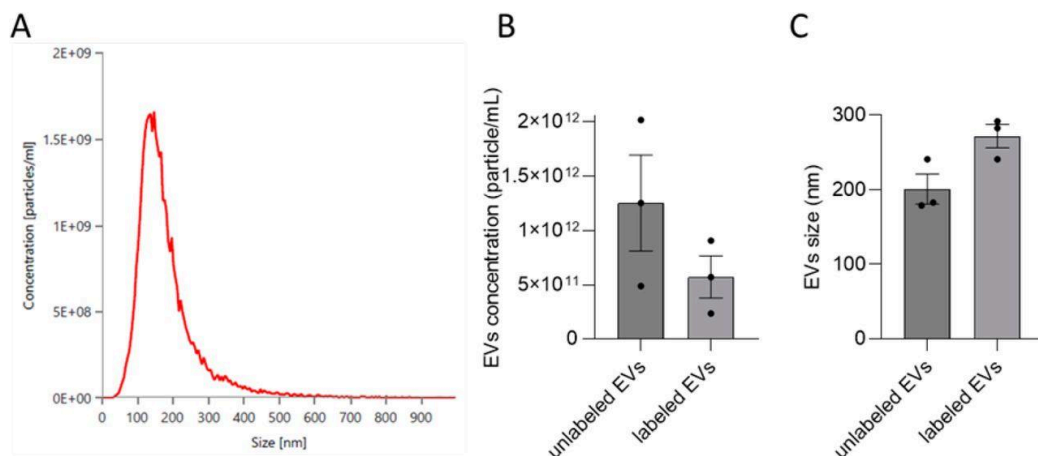
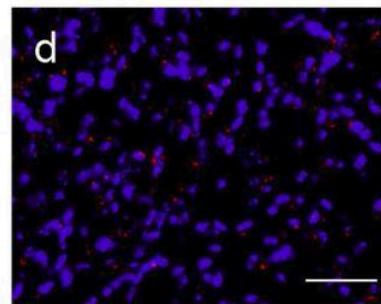
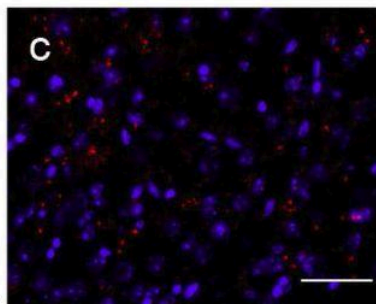
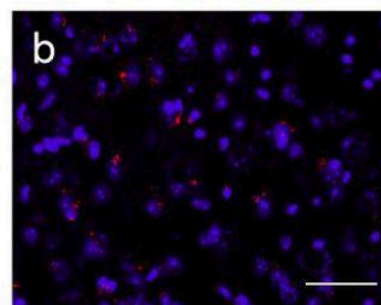
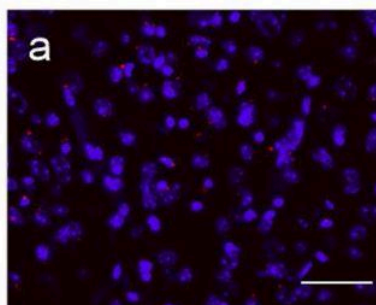
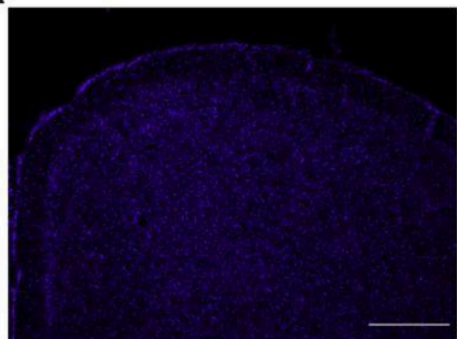


Figure 1. (A) Size distribution profile of m/IEVs isolated from the murine microglia-like cell line BV2, as determined by NTA. (B) Quantification of Aco-600 negative (unlabelled) and positive (labelled) EVs. (C) Size distribution of Aco-600 negative (unlabelled) and positive (labelled) EVs.

A



B

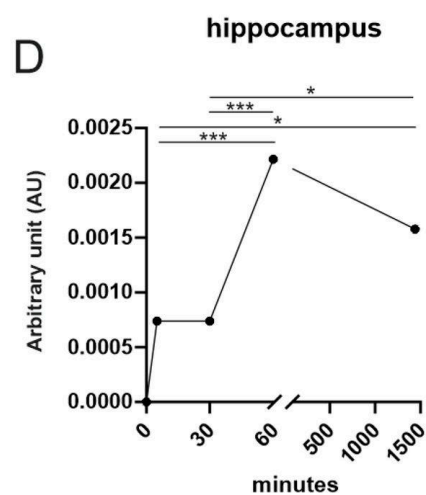
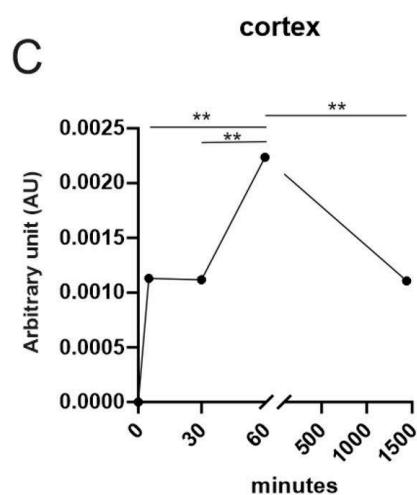
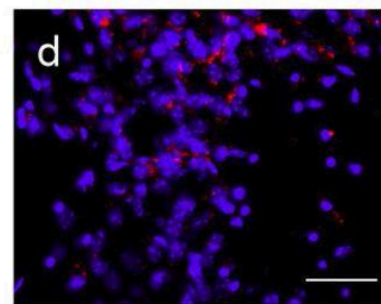
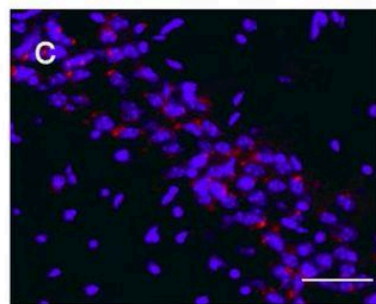
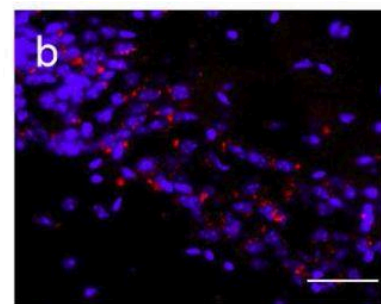
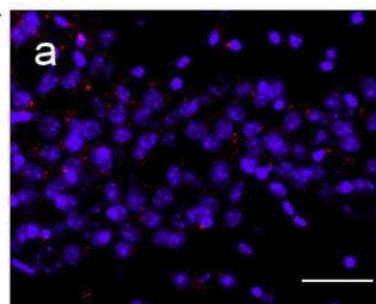
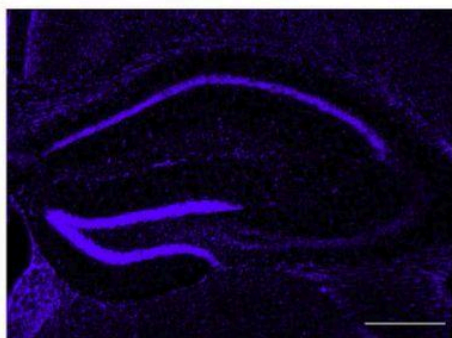


Figure 2.

(A–B) Representative fluorescence images of coronal cortical (A) and hippocampal (B) brain sections from mice intranasally administered with Aco-600 labelled BV2-derived EVs (red); nuclei were stained with Hoechst (blue). Scale bars: 400 μ m (left panels) and 40 μ m (right panels: higher magnification at 5 (a), 30 (b), 60 (c) and 1440 minutes (d)). (C–D) Quantification of Aco-600 fluorescent signal normalised to the number of nuclei in the cortex (C) and in the hippocampus (D), expressed as arbitrary unit (AU). Data are the mean $\pm$ SEM, n = 3 animals per time point. Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparisons test *p=0,0487, **p $\leq$ 0,0071, ***p=0,0002.

Following intranasal administration, Aco-600-labelled EVs were detected in both the cerebral cortex and hippocampus as early as 5 minutes post-administration (Fig. 2A–B). Quantitative analysis revealed a comparable spatiotemporal biodistribution profile across both brain regions, with fluorescence remaining relatively stable between 5 and 30 minutes, peaking at 60 minutes, and declining thereafter (Fig. 2C–D). While fluorescence decreased more rapidly in the cortex, the hippocampus retained higher signal intensity at 24 hours, suggesting prolonged EV retention. These findings identify 60 minutes as the optimal time point for detecting EV accumulation in the brain following intranasal delivery.

Conclusion

The fluorogenic properties of Aco-600 enable sensitive and specific tracking of EV biodistribution in the brain with minimal background interference, without affecting EV integrity.

Guidelines for Using Aco-Dyes

- Sonicate the reconstituted Aco-Dye for 15 minutes before each use.
- Each Aco-Dye has its own unique brightness and staining efficiency. Titrate and optimise the labelling concentration for the chosen Aco-Dye, EV type and study condition.
- EVs perform best when used fresh after isolation or preparation. If diluting the stock, make a fresh dilution on the day of the experiment as particle counts tend to drop over time.
- Filter all buffers through a 0.2 μ m filter before use.

References

1. Du S, Guan Y, Xie A, Yan Z, Gao S, Li W, Rao L, Chen X, Chen T. Extracellular vesicles: a rising star for therapeutics and drug delivery. *J Nanobiotechnology*. 2023 Jul 20;21(1):231. doi: 10.1186/s12951-023-01973-5. PMID: 37475025; PMCID: PMC10360328.
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3. Rinaldi, A., & Catalano, M. (2026, June 22). Fluorogenic labelling for tracking extracellular vesicle nanocarriers in the brain. *bioRxiv*. 2026.06.17.732894; doi: <https://doi.org/10.64898/2026.06.17.732894>.

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