

Fluorescent, Phosphorescent, Magnetic Resonance Contrast and Radioactive Tracer Labelling of Extracellular Vesicles

Kartika Wardhani^{a,b}, Aviva Levina^a, Georges E. R. Grau^{c-f}, Peter A. Lay^{a-e,g*}

Figure 1. Extracellular Vesicle Biogenesis

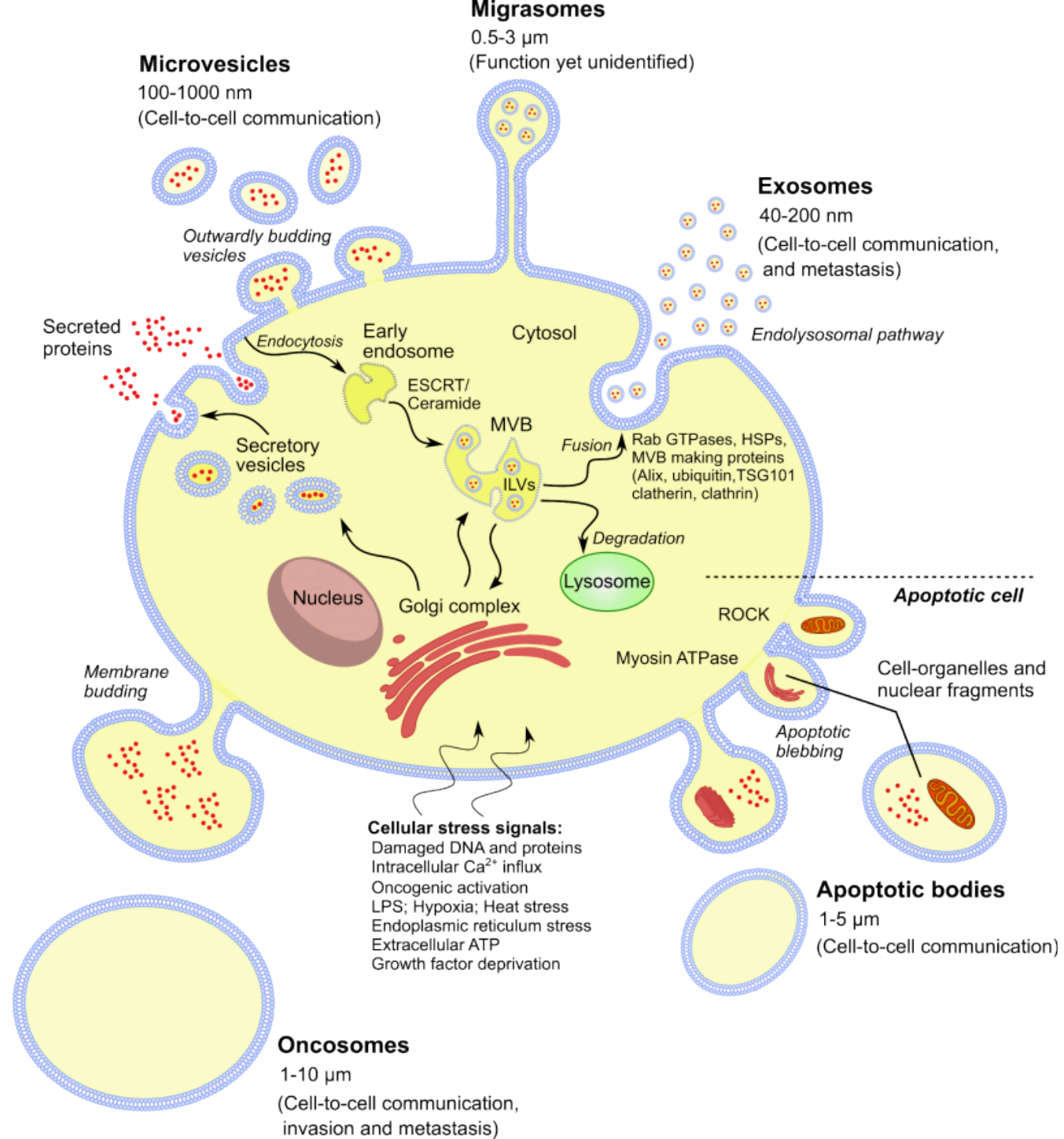
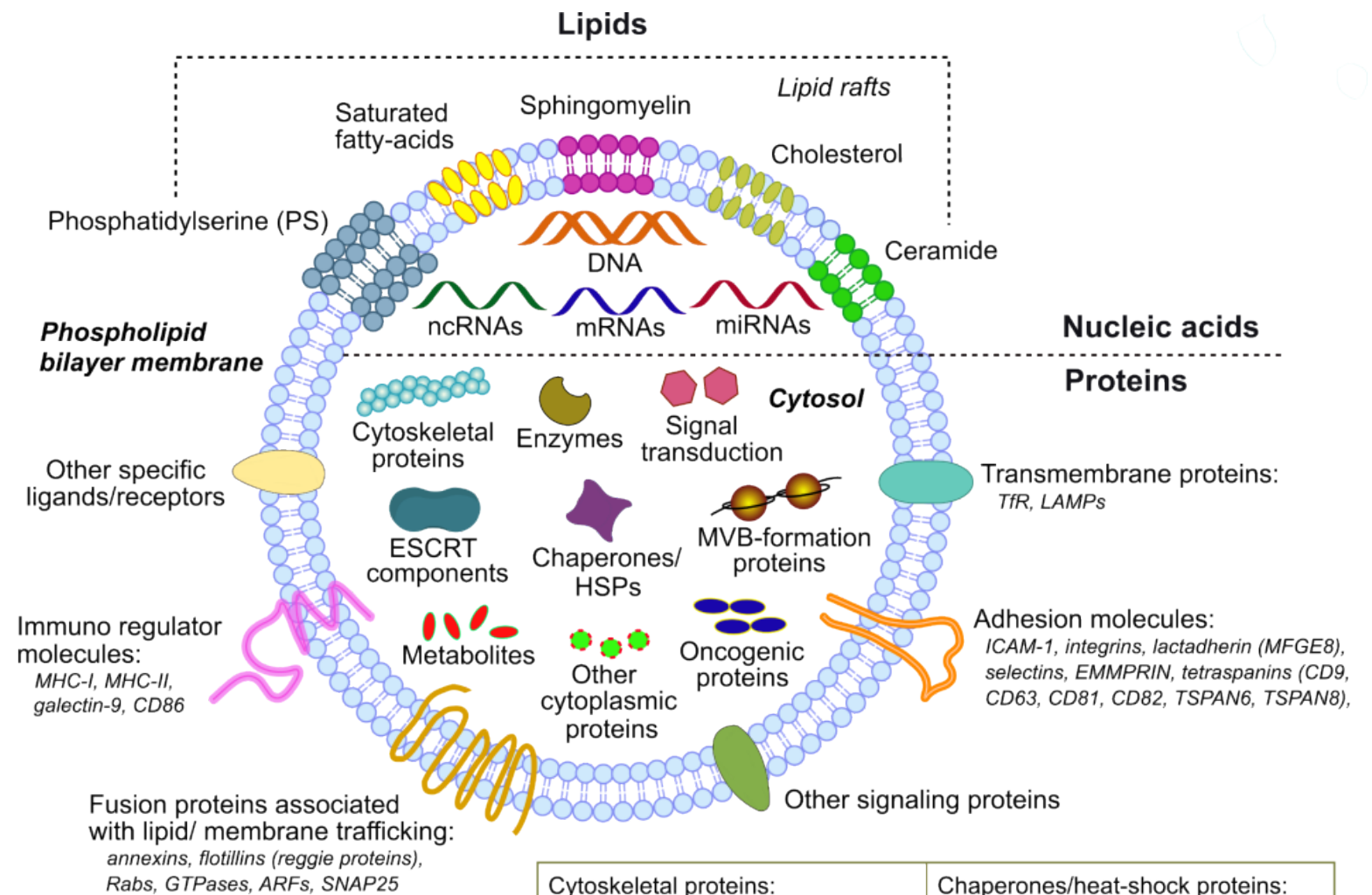


Figure 2. EV Molecular Composition



<p>Cytoskeletal proteins: actin, myosin, tubulins, moesin, keratins, fibronectin, vimentin, cofilin, DIAPH3, profilin, talin, gelsolin, ezrin</p> <p>Enzymes: enolase, ROCKs, GAPDH, ATPase, catalase, PGK1, pyruvate kinase, peroxidases, AChE, elongation factors</p> <p>Signal transduction: syntenin, EGFR, G-proteins, PI3K, ARF-1, ARF-6, protein kinase B (Akt), CDC42, β-catenin, 14-3-3, MUC1</p> <p>ESCRT components: VPS4, VPS28, ESCRT-0, -I, -II, and -III</p>	<p>Chaperones/heat-shock proteins: HSP60, HSP70, HSC70, HSP84, HSP90 (HSP90AA1)</p> <p>MVB-formation proteins: TSG101, alix, clathrin, ubiquitin</p> <p>Metabolites: carboxylic acids, peptides, nucleotides, sugars, amino acids, cyclic alcohols, nucleosides, biogenic amines, carnitines</p> <p>Oncogenic proteins: K-Ras, EGFRvIII, EpCAM, survivin, MET oncogene (HGFR), FAK</p> <p>Other cytoplasmic proteins: histones, proteasome, ribosomal proteins</p>
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Figure 3. EV-Mediated Intercellular Communication

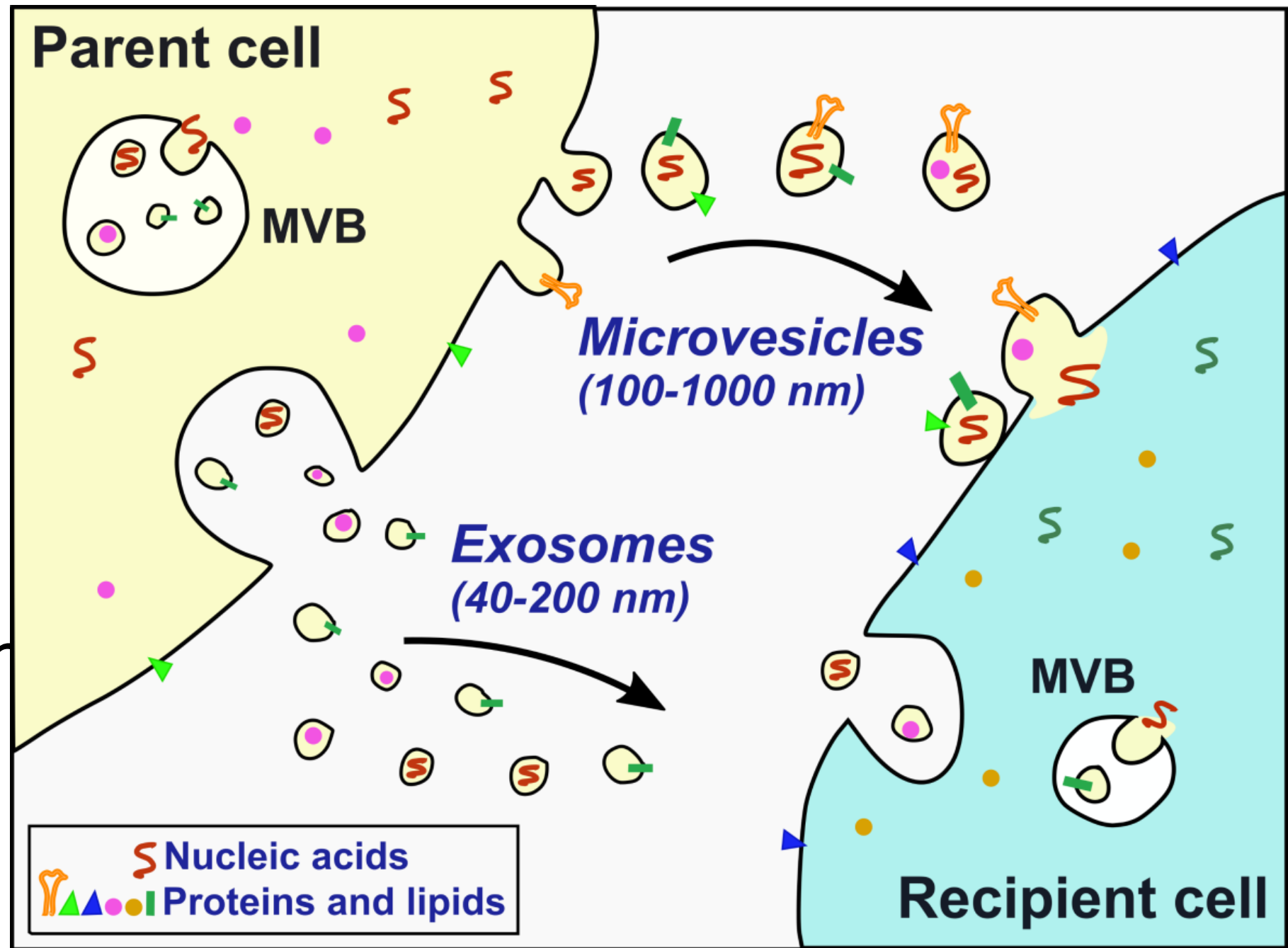
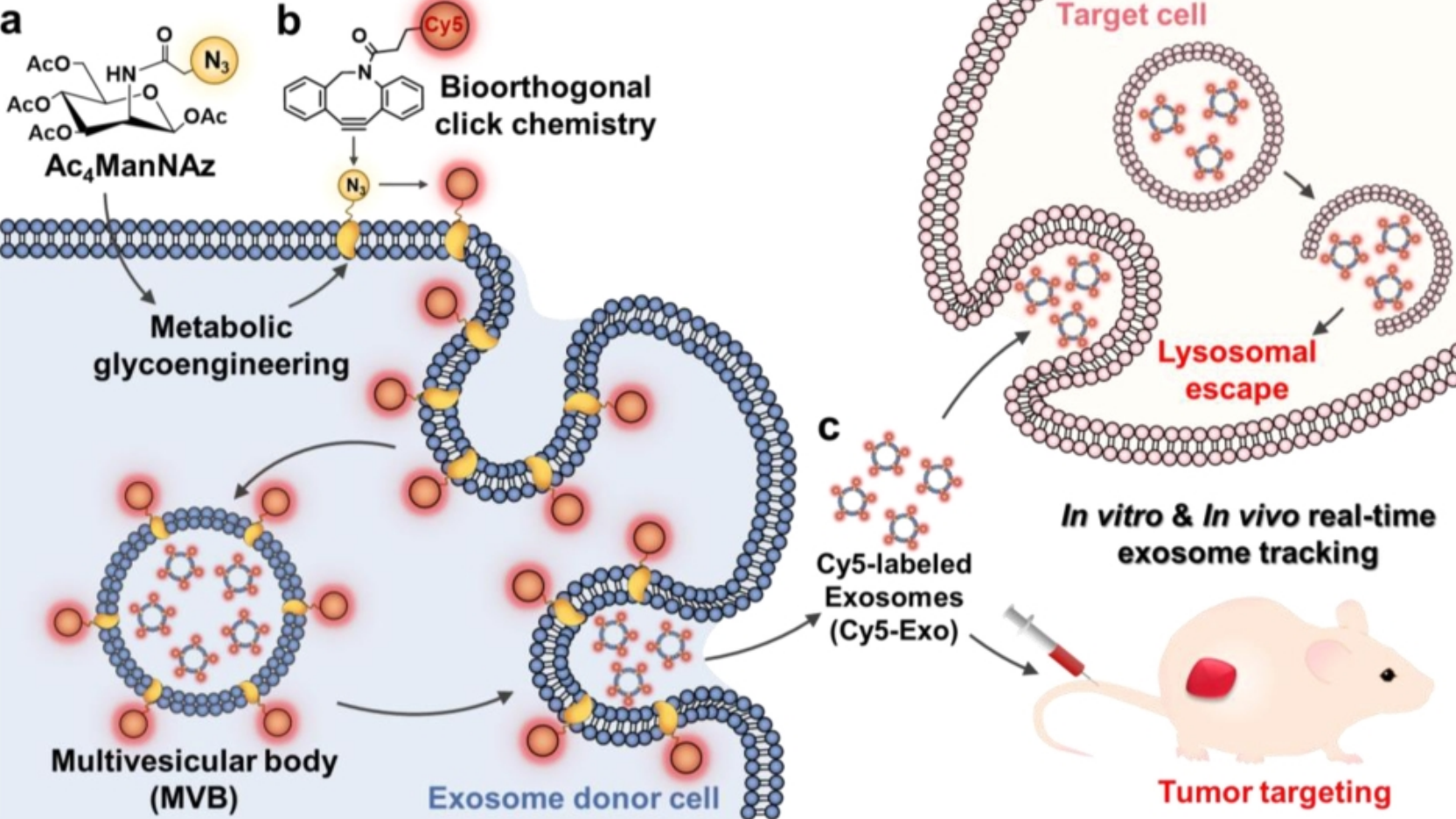
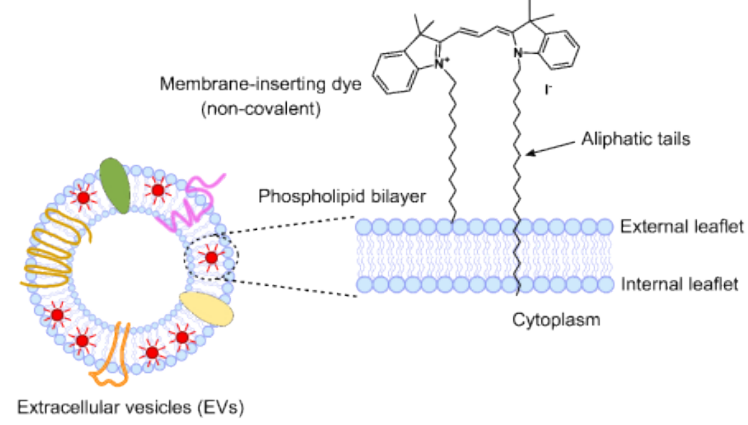


Figure 4. Indirect Labelling of EVs





EV Membrane Labelling (Staining) by Lipophilic Dyes

Lipophilic fluorescent dyes

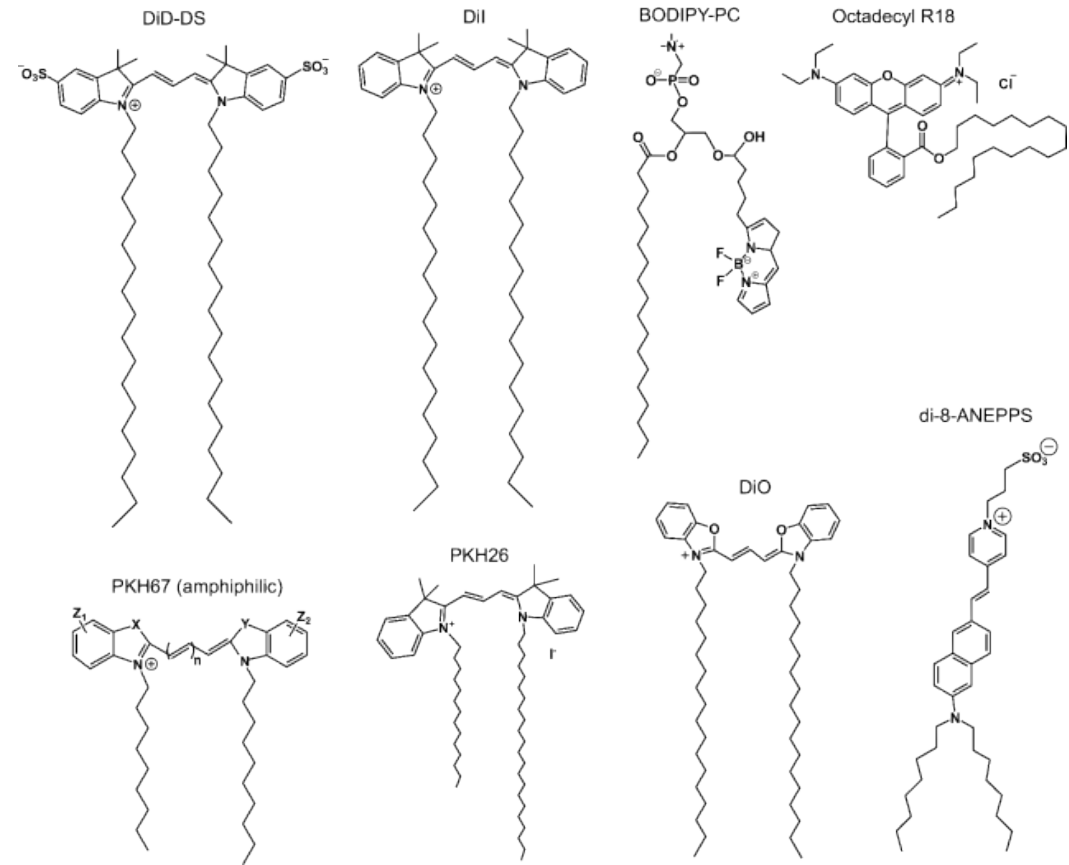

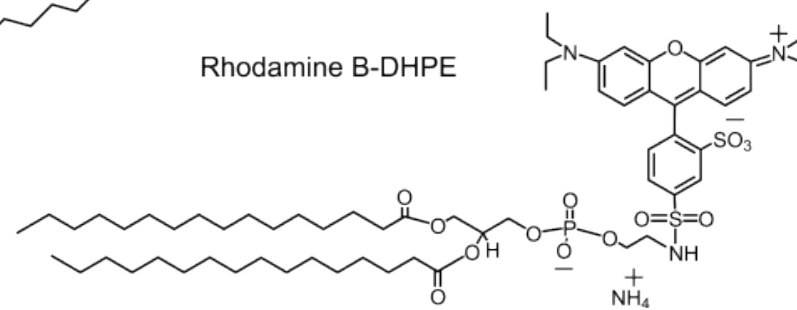
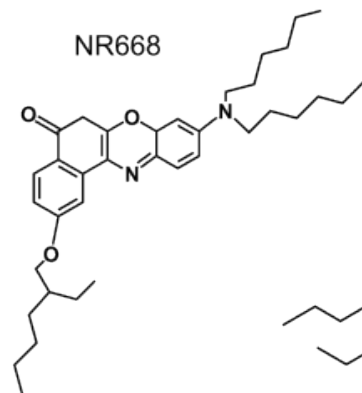
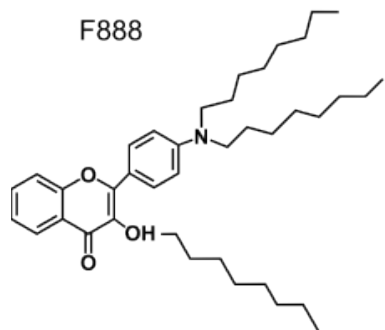
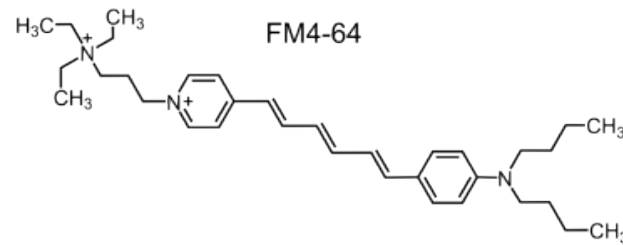
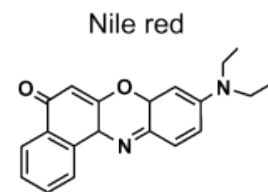
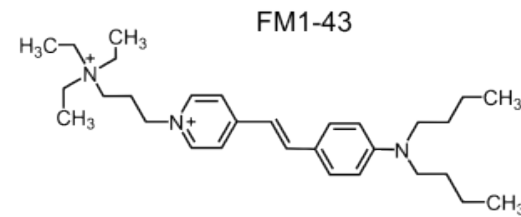
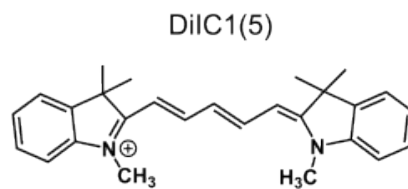
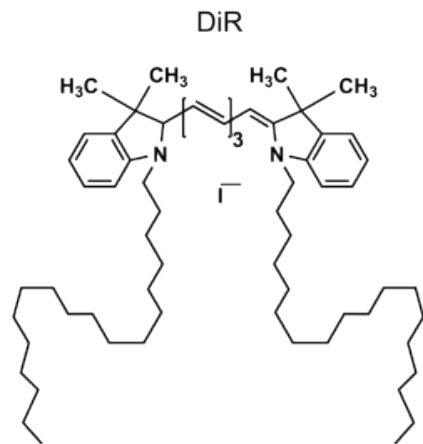
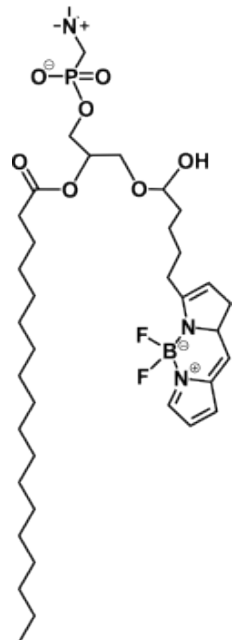


Figure 5. EV Membrane Labelling by Lipophilic Dyes

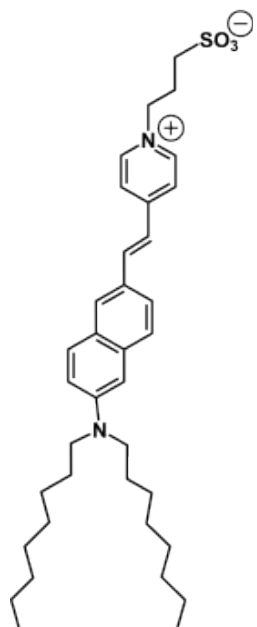
 Lipophilic fluorescent dyes



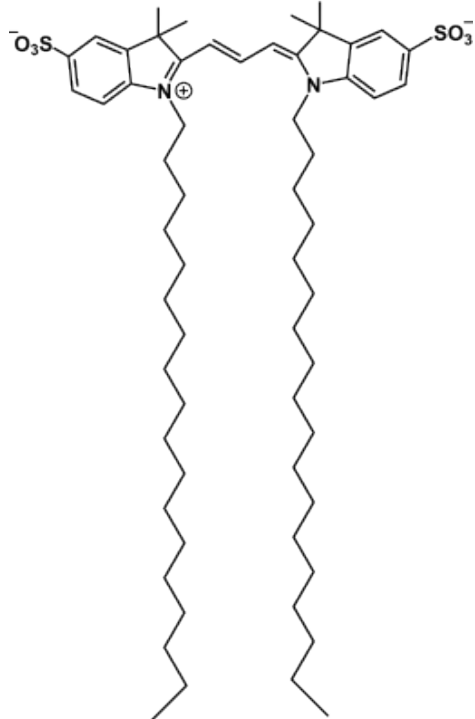
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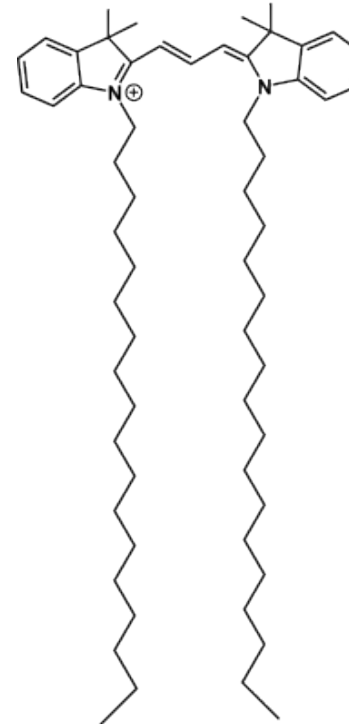
di-8-ANEPPS



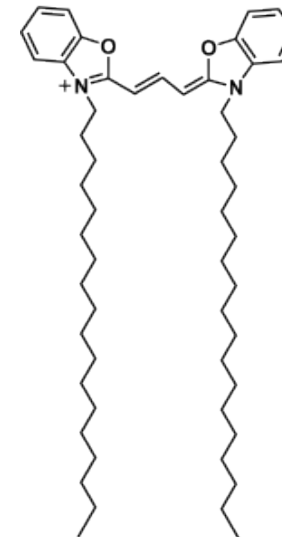
DiD-DS



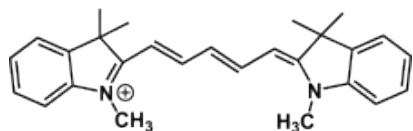
Dil



DiO



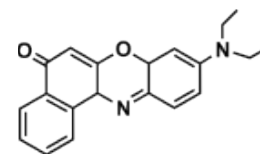
DiIC1(5)



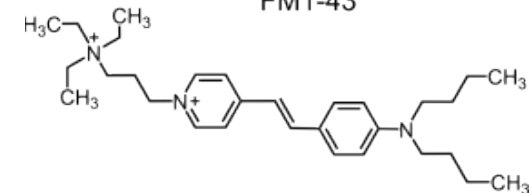
FM4-64

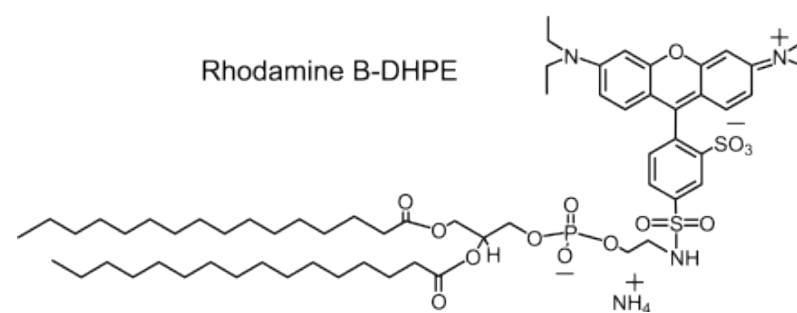
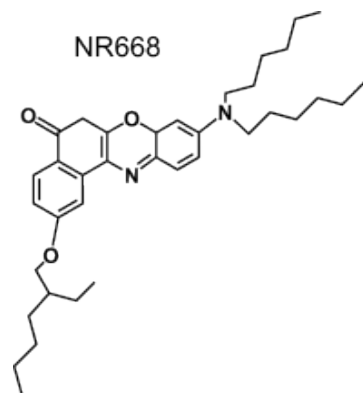
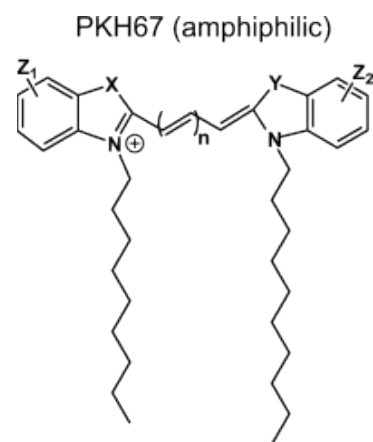
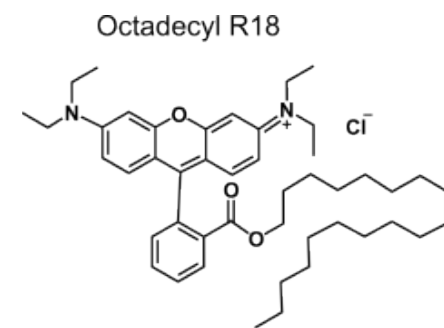
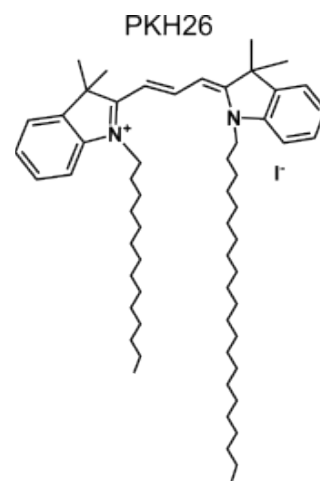
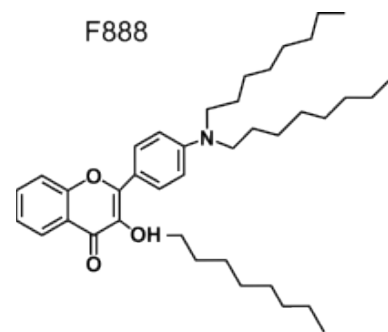
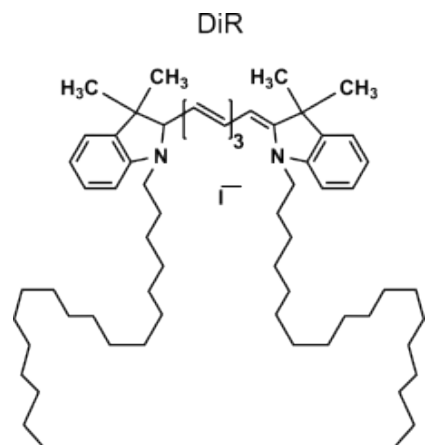


Nile red



FM1-43








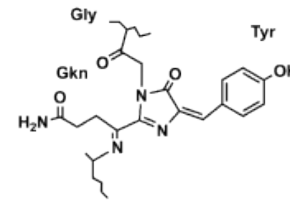
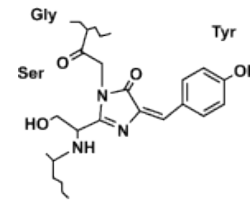
Fluorescent proteins conjugated with reactive chemical moieties to covalently label proteins of interest present on the EV surface

Extracellular vesicles (EVs)

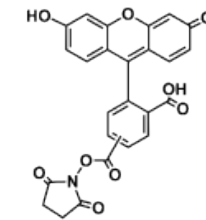
EV Surface Protein Labelling

 Fluorescent proteins

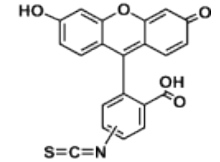
Green and red fluorescent protein (GFP and RFP)



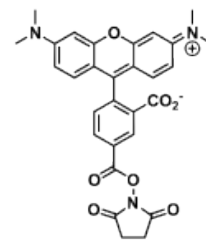
NHS-Fluorescein



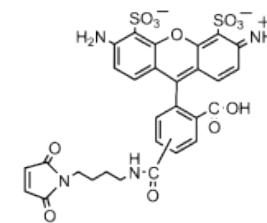
FITC



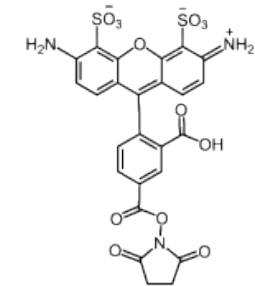
TAMRA NHS ester, 5-isomer (amine-reactive TAMRA-NHS)



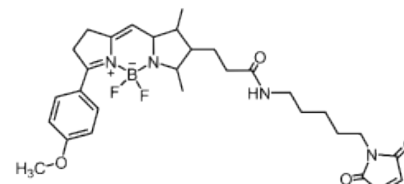
Alexa488-maleimide



Alexa 488 succinimidyl ester



BODIPY TMR C5-maleimide



Phycocerythrin

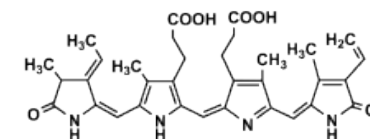
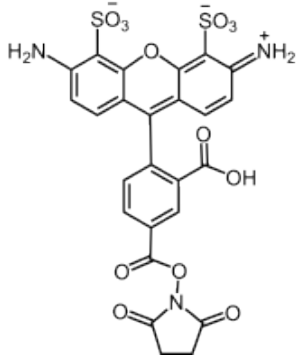
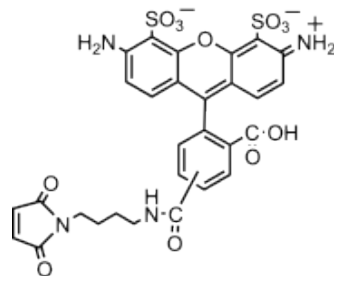


Figure 6. EV Surface Protein Labelling

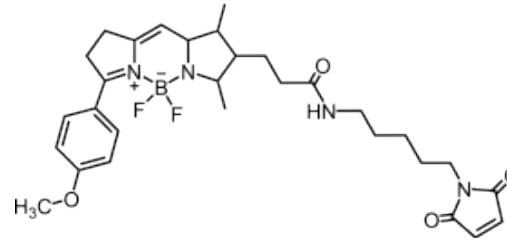
Alexa 488 succinimidyl ester



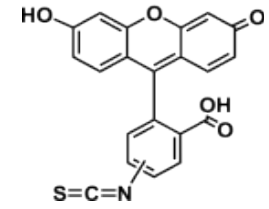
Alexa488-maleimide



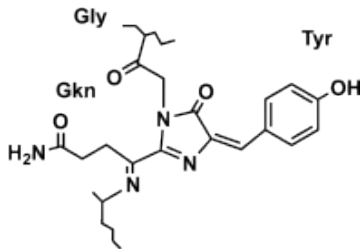
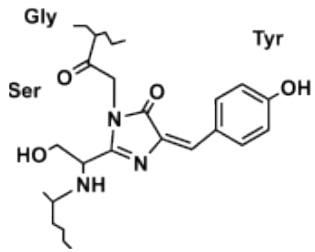
BODIPY TMR C5-maleimide



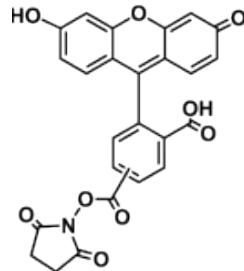
FITC



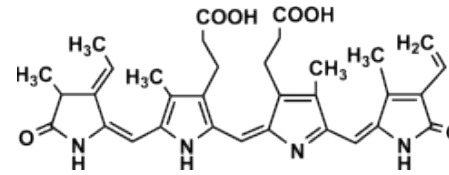
Green and red fluorescent protein (GFP and RFP)



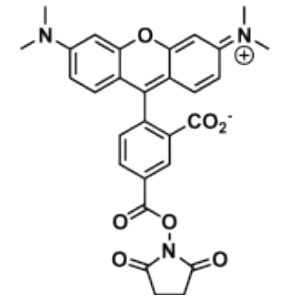
NHS-Fluorescein

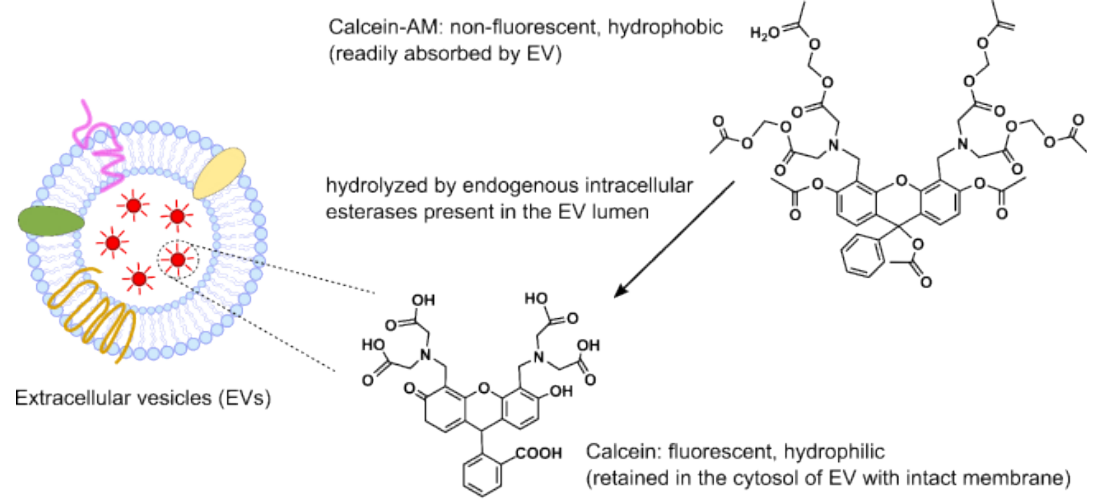


Phycocerythrin



TAMRA NHS ester, 5-isomer (amine-reactive TAMRA-NHS)





EV Luminal labelling

☀ Membrane-permeant fluorescent dyes

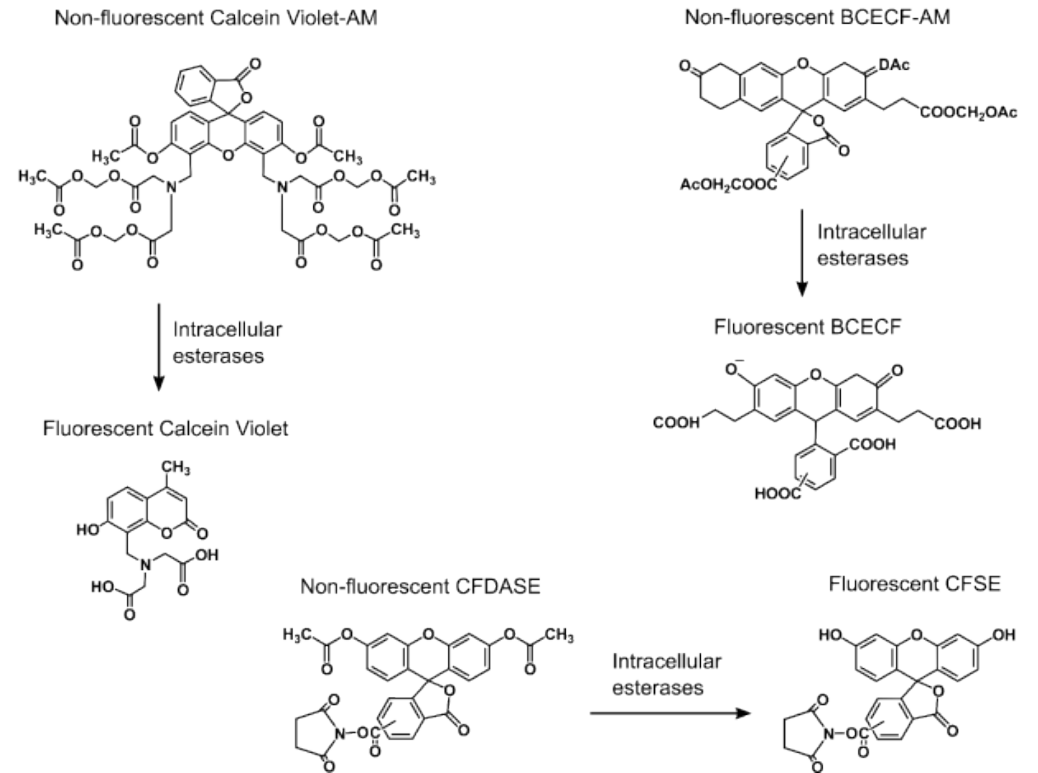
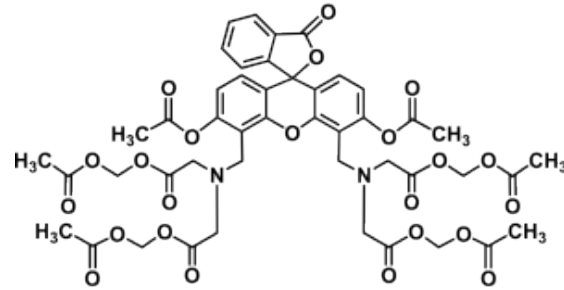


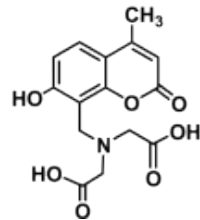
Figure 7. Luminal Labelling by Membrane-Permeant Dyes

Non-fluorescent Calcein Violet-AM



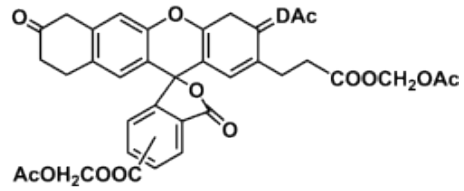
Intracellular
esterases

Fluorescent Calcein Violet



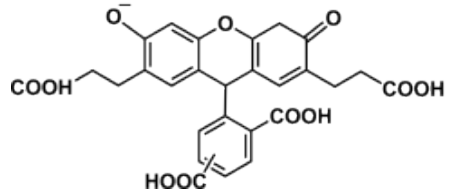
Non-fluorescei

Non-fluorescent BCECF-AM

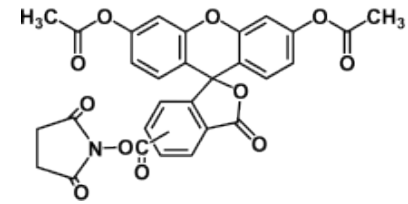


Intracellular
esterases

Fluorescent BCECF

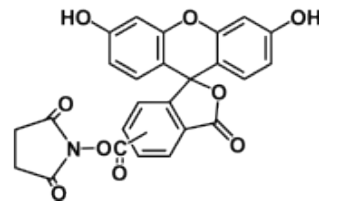


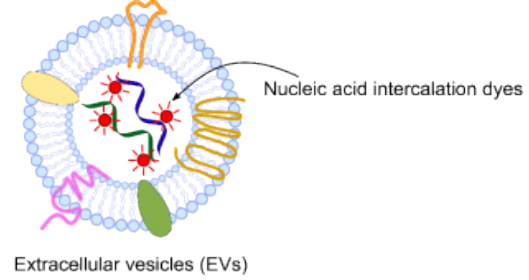
Non-fluorescent CFDASE



Intracellular
esterases

Fluorescent CFSE





Fluorescent labelling of nucleic acid molecules within EV

☀ Nucleic acid fluorescent dyes

Figure 8. Labelling of Nucleic Acids within Extracellular Vesicles

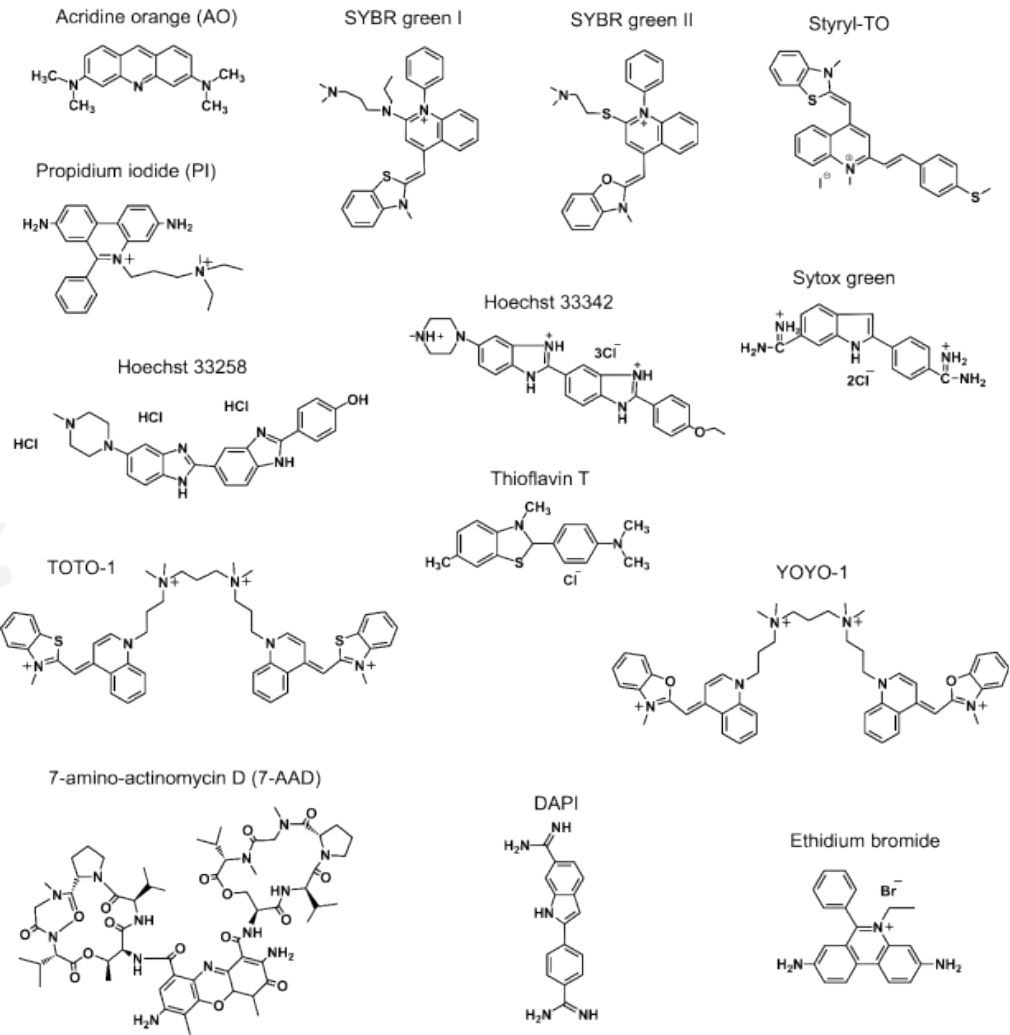


Figure 9. Wardhani et al. Study

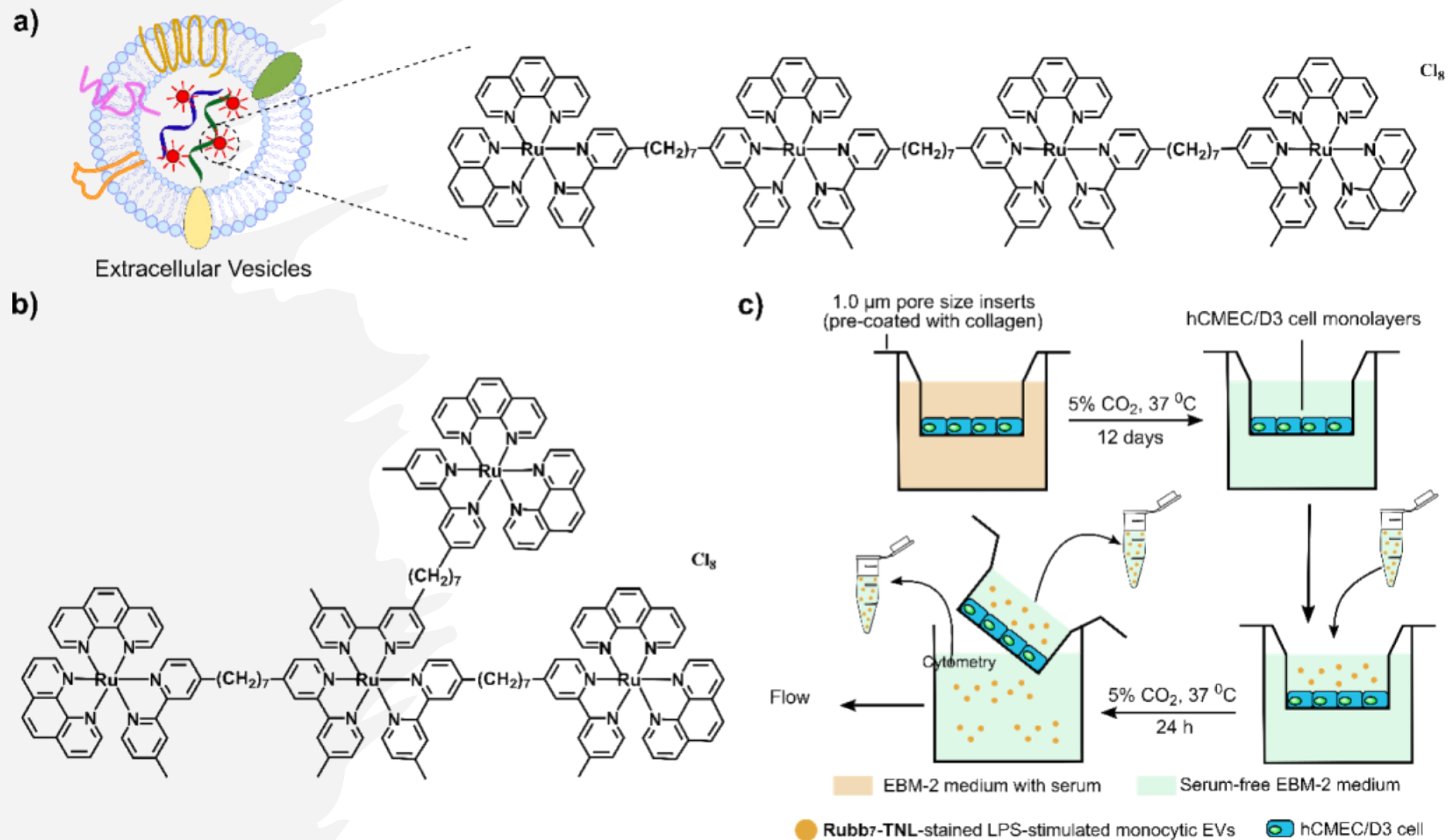
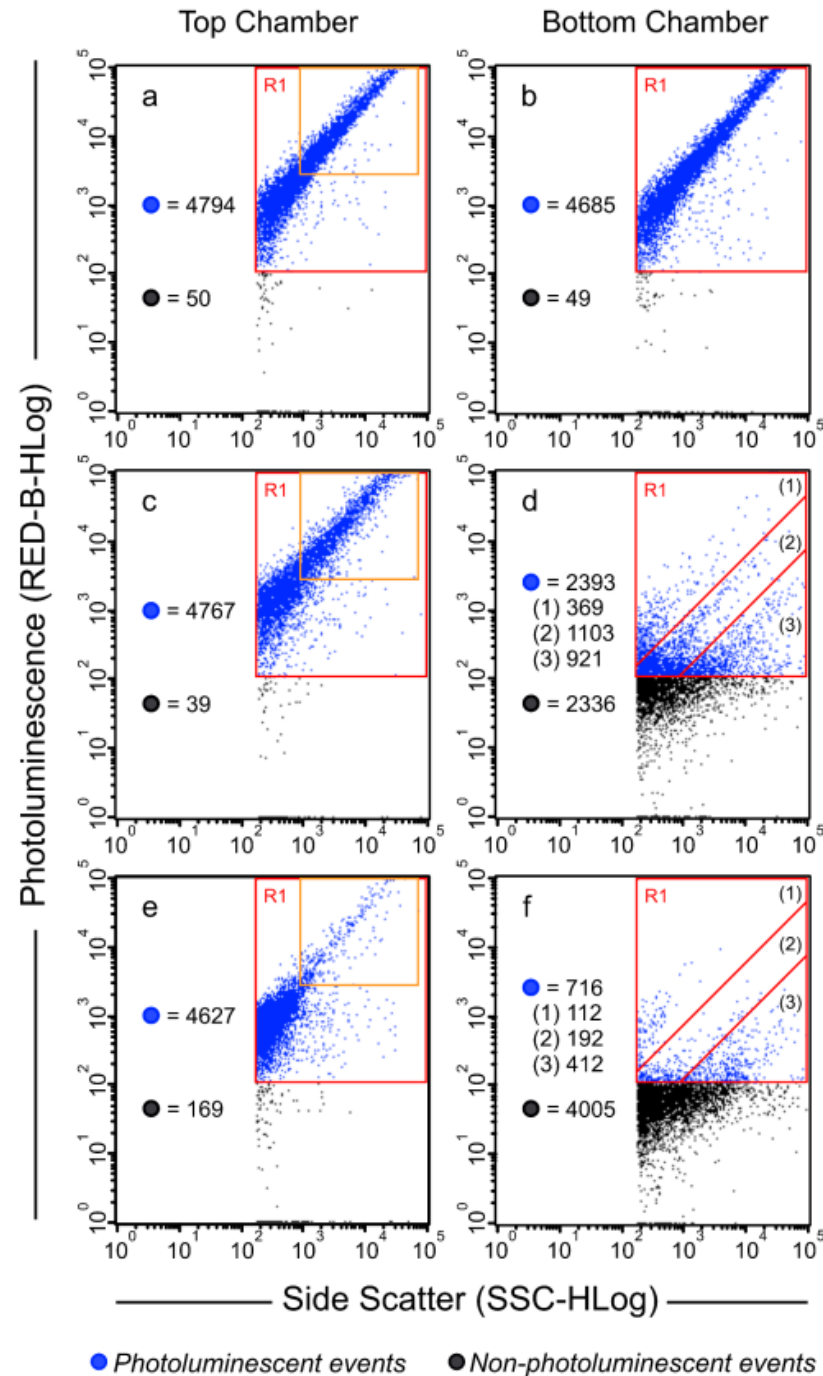


Figure 10. EV- Blood Brain Barrier Study



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