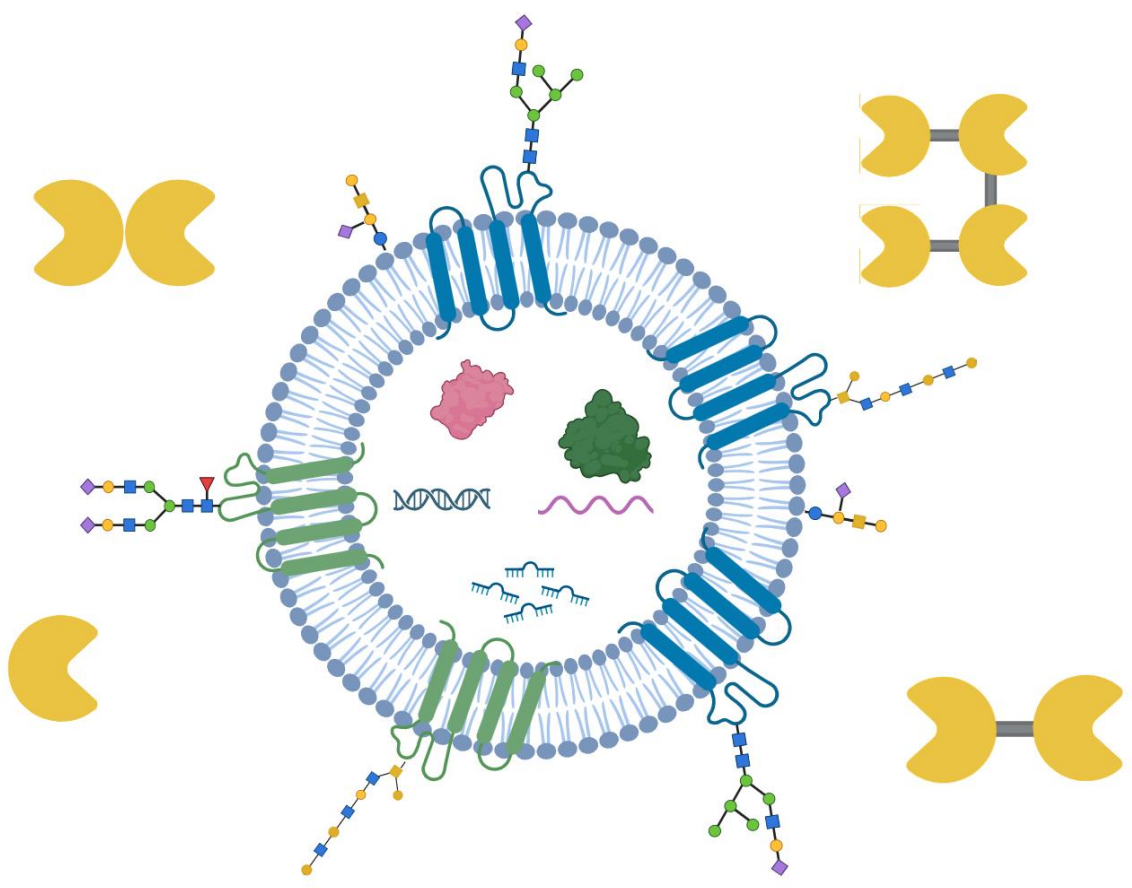


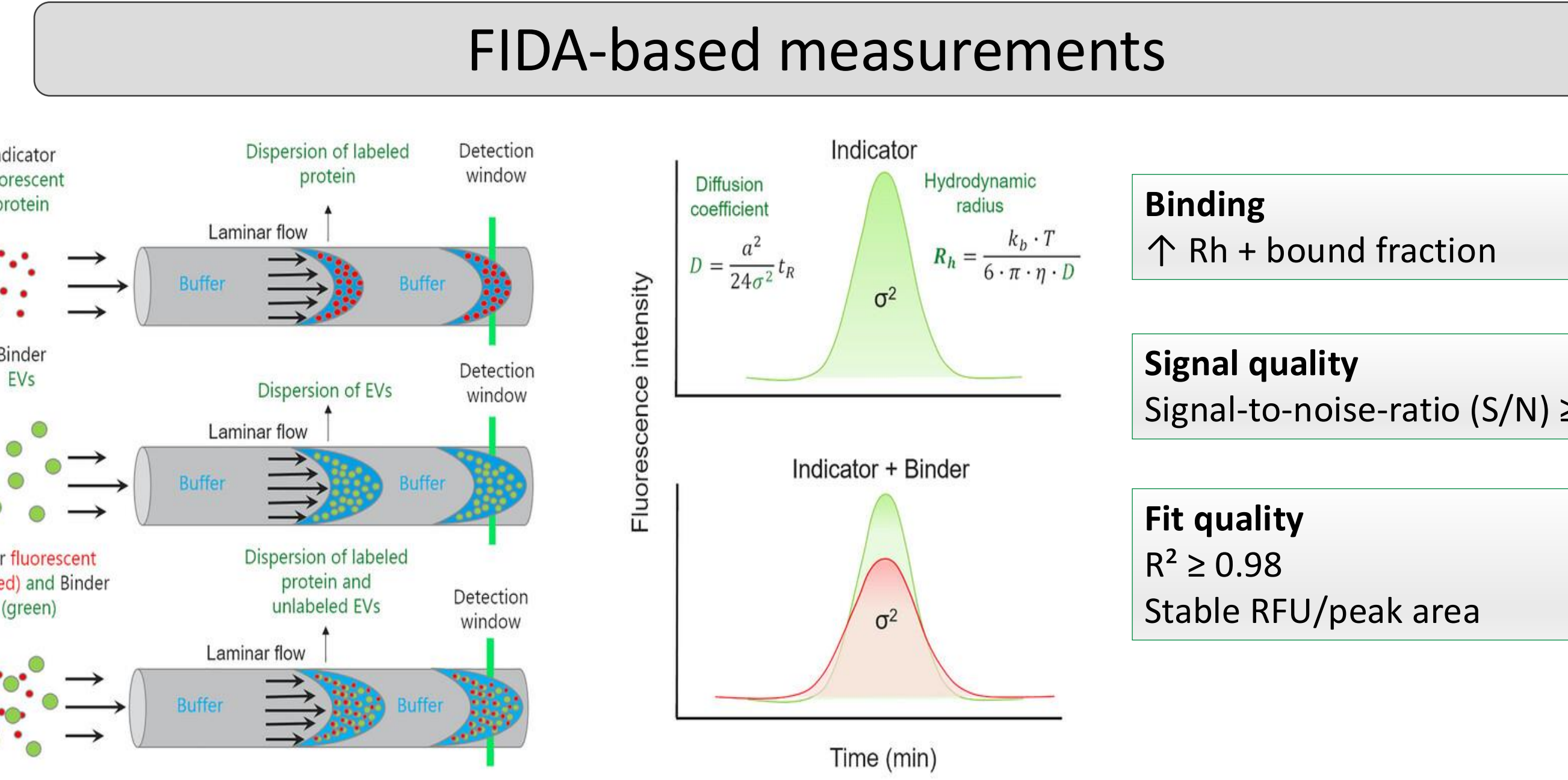
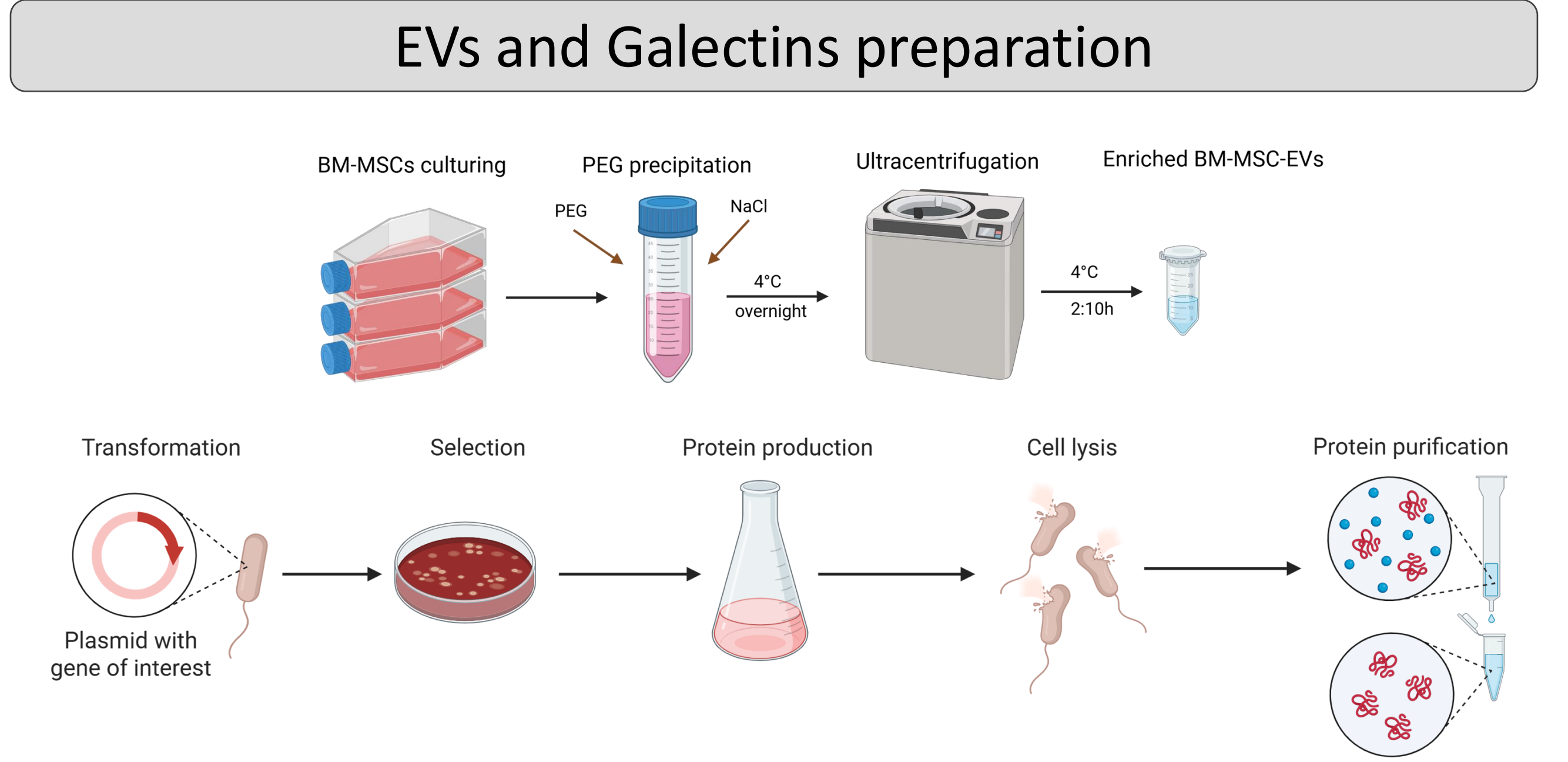
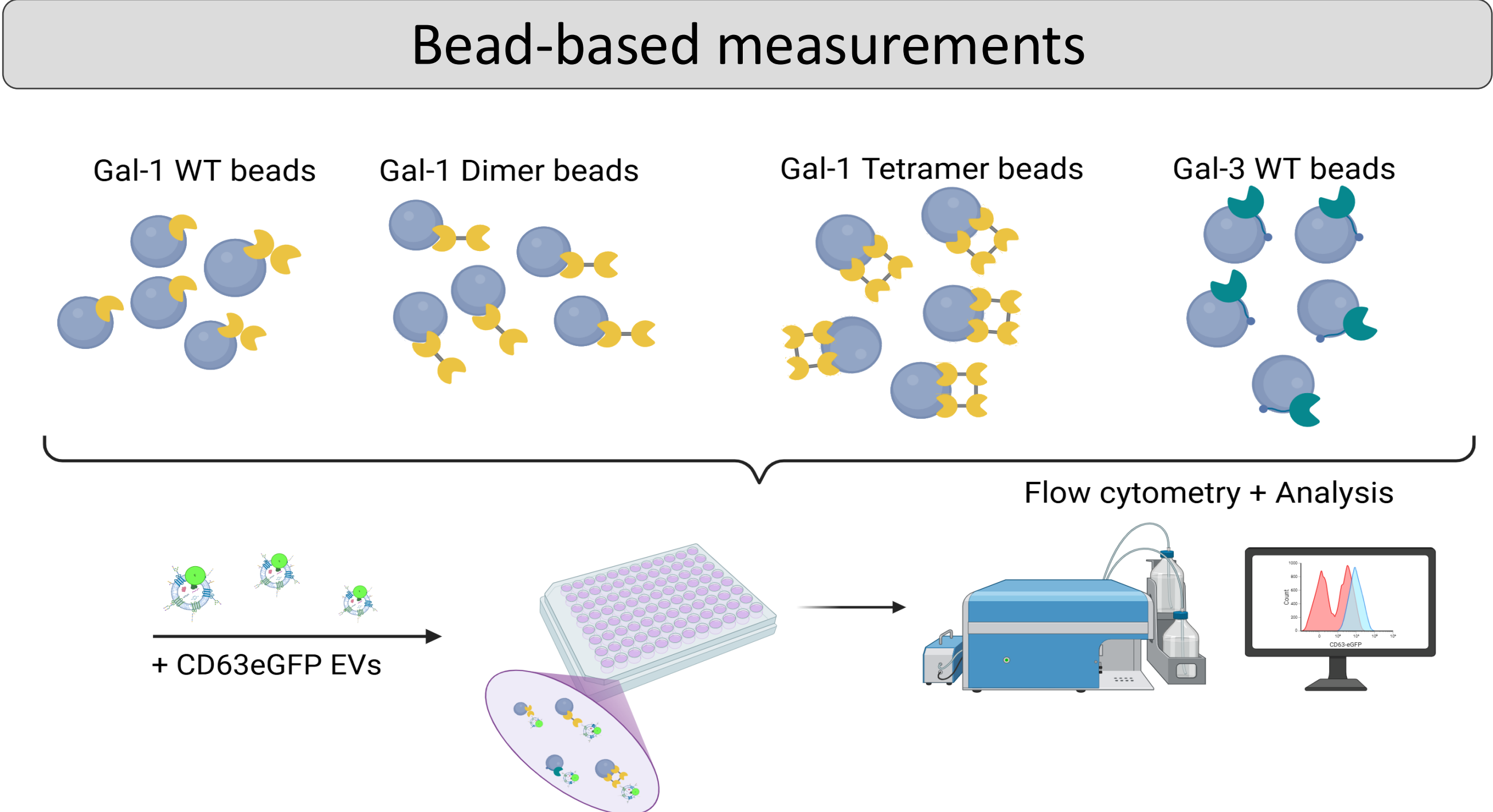
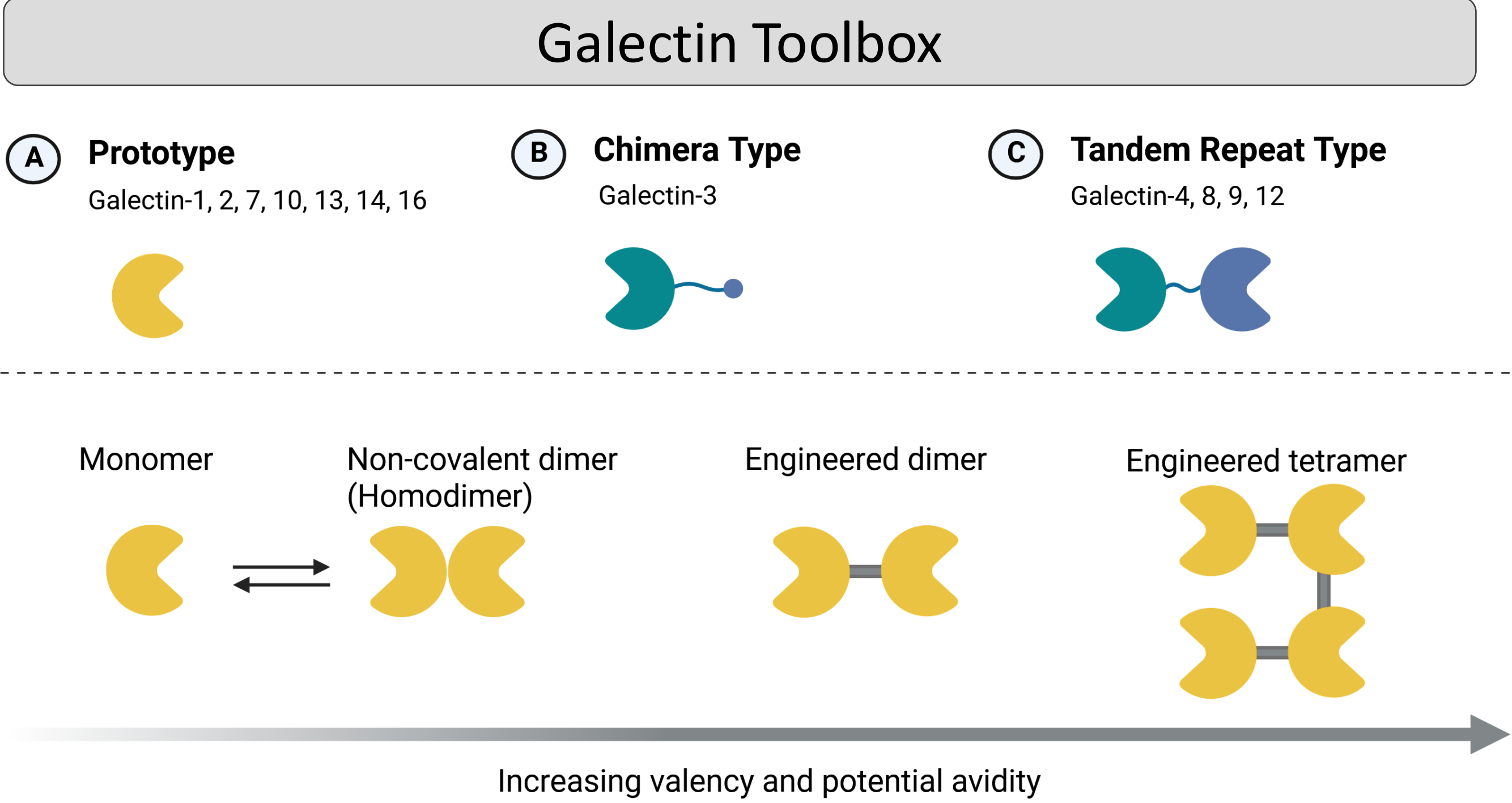
Background

Galectin-1 (Gal-1) is a β -galactoside-binding lectin that recognizes glycans on cell surfaces. Because extracellular vesicles (EVs) display a distinct glycan density and organization, efficient Gal-1 association with EVs may require increased lectin valency and avidity. We hypothesized that multivalent Gal-1 constructs promote stable binding to MSC-derived EVs, thereby acting as molecular bridges or anchors that could enhance cellular interactions and potentially influence EV uptake, clearance, or aggregation through cross-bridging of vesicle surfaces.

AIM: To determine whether engineered dimeric and tetrameric Gal-1 constructs enhance glycan-dependent association with MSC-derived EVs compared with wild-type Gal-1.

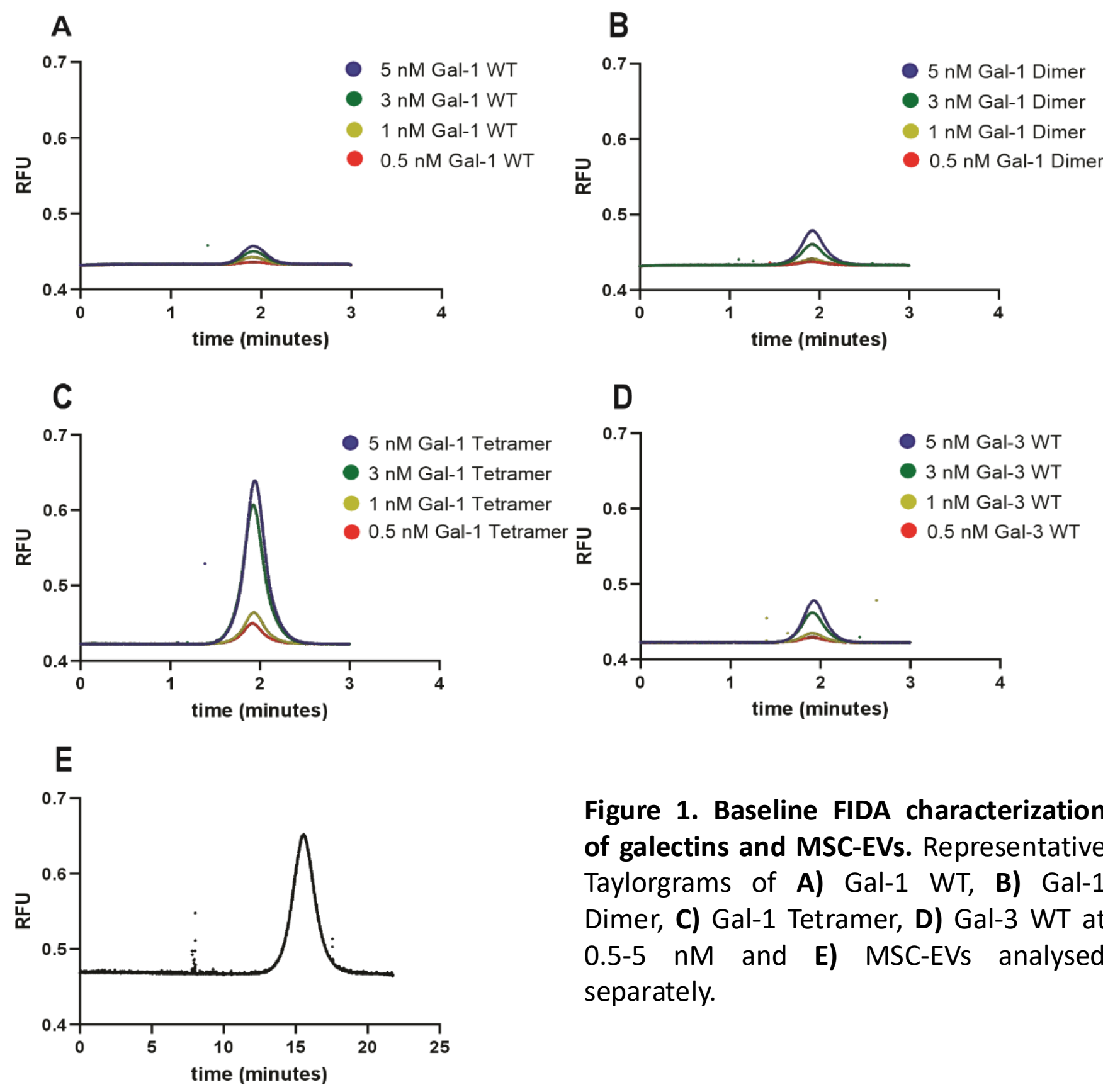


Methods



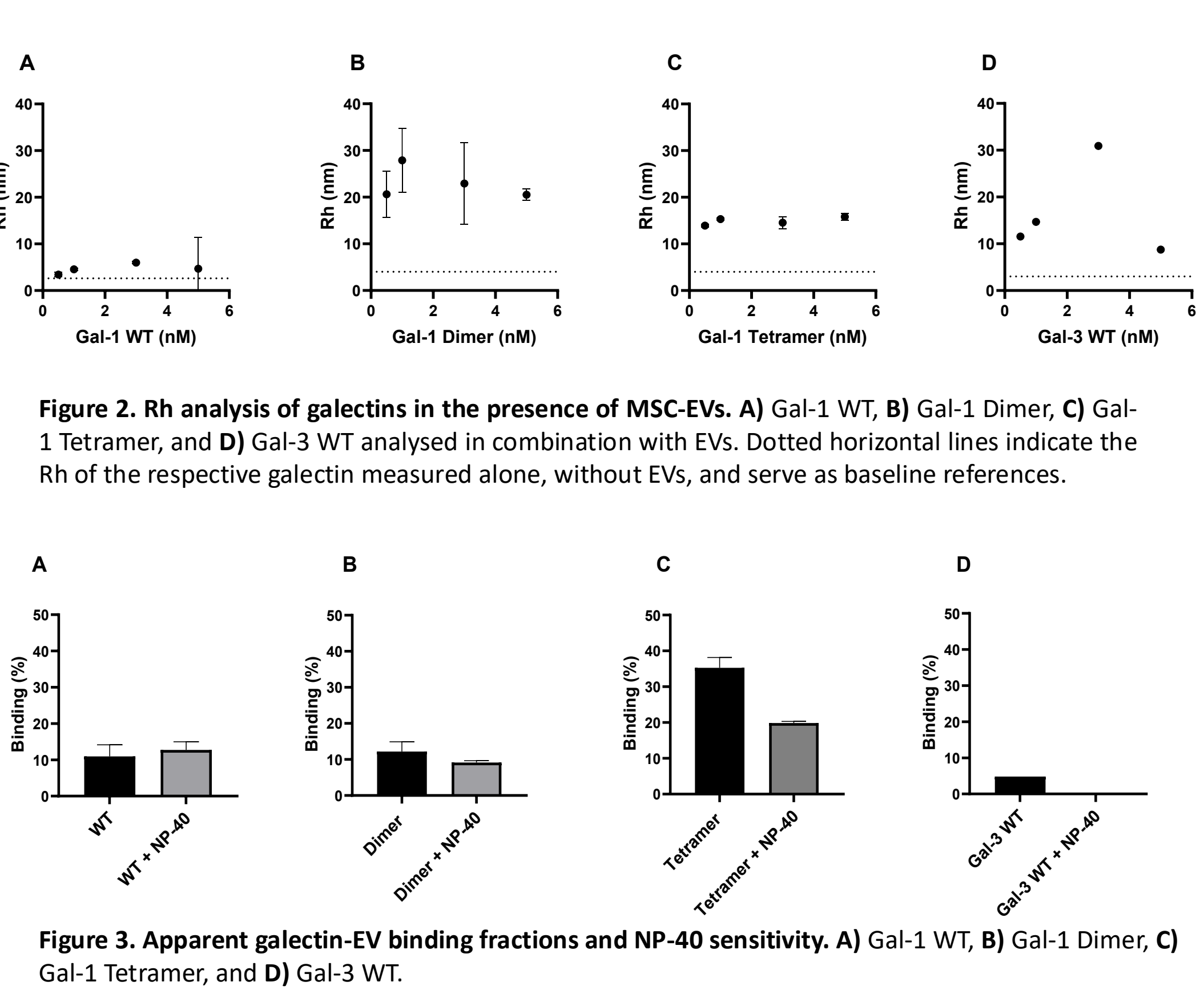
Results

Characterisation by FIDA



Galectins and MSC-EVs met the predefined FIDA quality criteria and were suitable for subsequent interaction analysis.

FIDA-based binding



Increasing Gal-1 valency enhanced EV association, with the tetramer showing the strongest interaction.

Bead-based binding

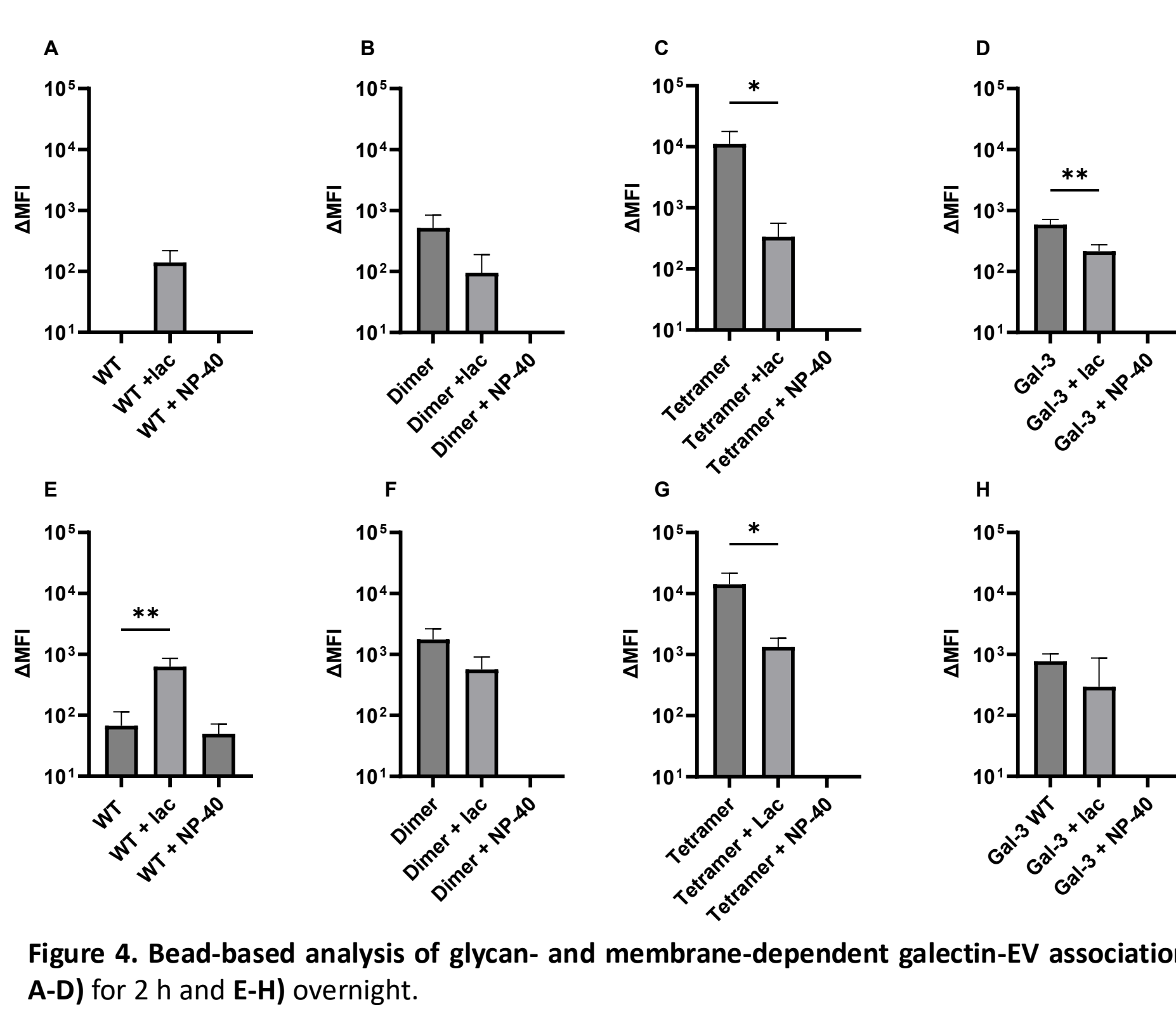


Figure 4. Bead-based analysis of glycan- and membrane-dependent galectin-EV association. A-D) for 2 h and E-H) overnight.

Multivalent Gal-1 showed stronger, carbohydrate-dependent binding to intact EVs than WT.

Conclusion

Gal-1 WT showed limited MSC-EV association, whereas increasing Gal-1 valency enhanced binding. The Gal-1 Tetramer showed the highest apparent EV-associated fraction by FIDA, reaching approximately 35%. This was supported by the bead assay, in which the tetramer produced a higher EV-associated fluorescence signal than the dimer and WT. Lactose inhibition and NP-40 disruption further support glycan-dependent binding to intact EVs, identifying valency and avidity as key determinants of Gal-1-EV association.

Engineering Gal-1 valency may provide a strategy to modulate EV-cell interactions by stabilizing vesicle docking and promoting cellular uptake, thereby offering a potential route toward targeted EV delivery.

References

Kakavoulia, M.-A., P. Spies, T. J. Kutzner, et al. 2026. "In-Solution Characterization of Extracellular Vesicles: A New Approach to Evaluating Antibody Binding and Surface Interactions." *Journal of Extracellular Vesicles* 15, no. 4: e70269.

