FIDA: A novel technique for determination of Protein binding on Extracellular Vesicles

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Introduction

The binding of e.g., proteins, proteoglycans, nucleic acids, chemokines or, cytokines to the surface of EVs influences their activity and targeting direction. EV preparations from different sources can contain diverse vesicles, making it challenging to detect and analyze the binding of associated molecules to the EVs. Antibody-based methods like flow cytometry, high-resolution microscopy, and solid-phase assays are commonly used to measure and characterize the molecules bound to EVs. While these methods have improved EV research, they have limitations, especially when analyzing molecules with lower affinities. In body fluids, several proteins, including galectins, can interact with EVs and affect their functionality. Galectins, unlike antibodies, have lower affinity to their ligands and often require high concentrations for detection in vitro. Here, we introduce the **F**low **I**nduced **D**ispersion **A**nalysis (FIDA) to analyze both the binding of human soluble lectins (galectins) and antibodies to EVs derived from mesenchymal stem cells and blood serum.

Method

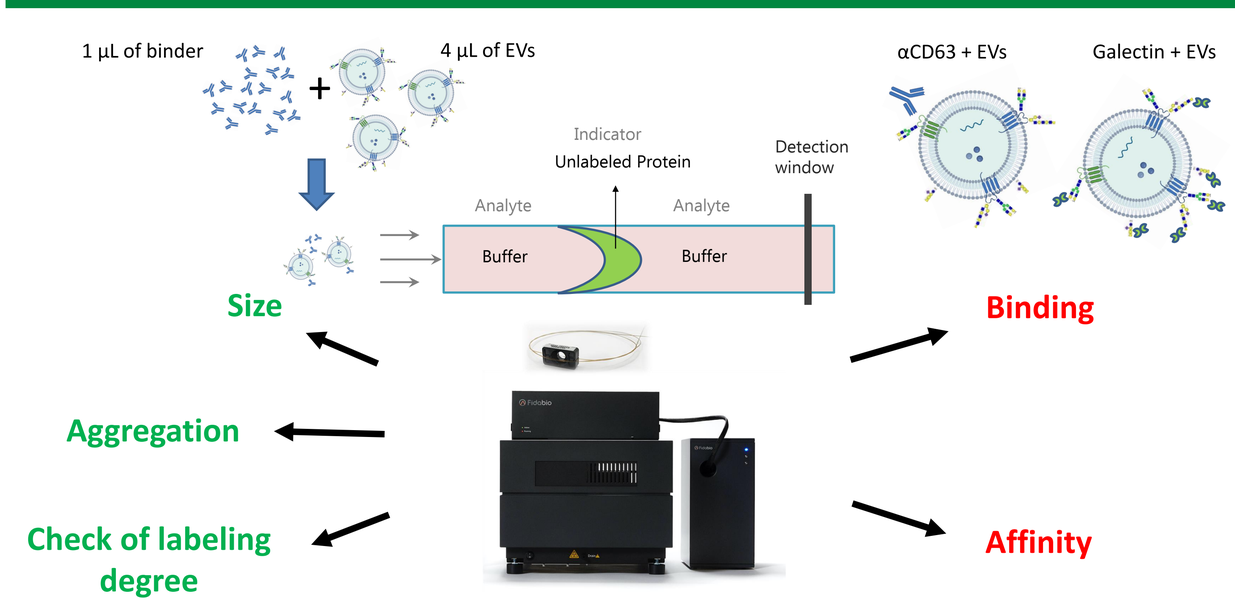

Figure 1. Principle of the **F**low **I**nduced **D**ispersion **A**nalysis (FIDA). Combines sample quality check (green) with protein-molecule binding analysis (red).

Quality control

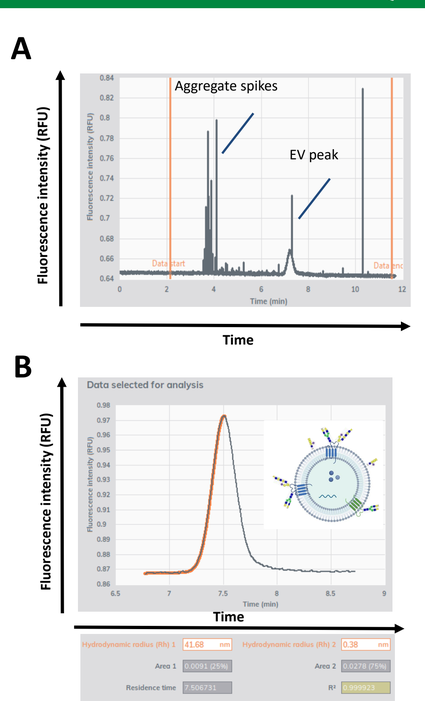

Figure 2. Analysis of Size and aggregation content in the EV preparations. A) EV preparation with several aggregates. B) Representative EV preparation.

Table 1: Size in nm of analyzed MSC EV samples.

	Diameter	
	nm	nm
BM1	65.28	83.36
BM2	61.34	40.66
WJ	69.78	44.24

Results

Analysis of the protein labeling degrees

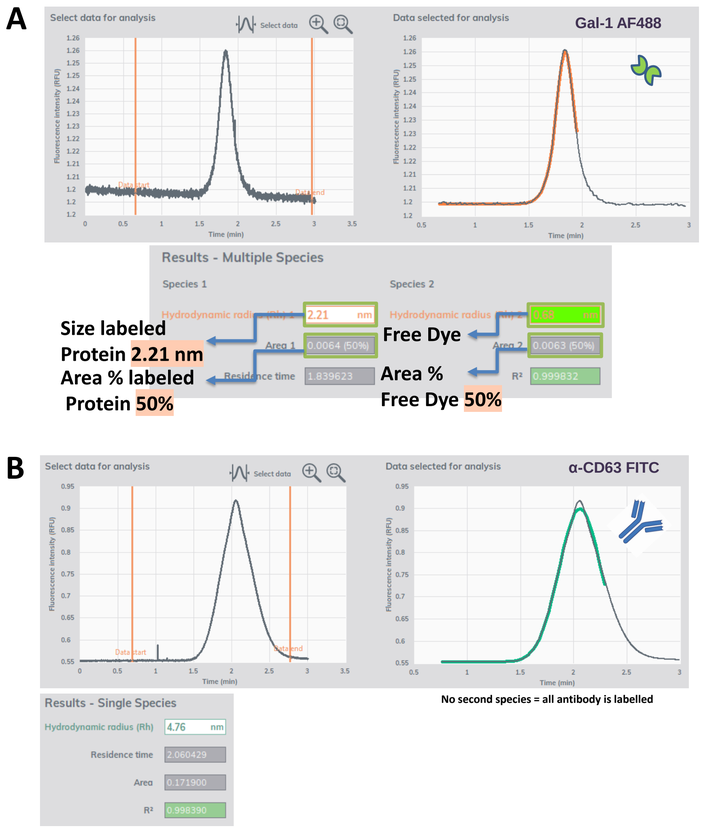

Figure 3. A) Representative Graph for hydrodynamic radius (Rh) and labeling degree of galectins. Galectins showed a labeling degree from 12% - 50% (Table 2). B) Hydrodynamic radius (Rh) and labeling degree of antibodies. Used antibodies had a labeling degree of 100% (Table 2).

Table 2: Size and labeling degree of galectins and antibodies.

	Binder		Free fluorophore	
	Rh (nm)	Area %	Rh (nm)	Area %
Gal-1	2.21	50	0.68	50
Gal-3	2.53	12	0.71	88
Gal-3CRD	2.05	45	0.75	55
α-CD63	4.76	100		

Binding of fluorescent labeled proteins to EVs

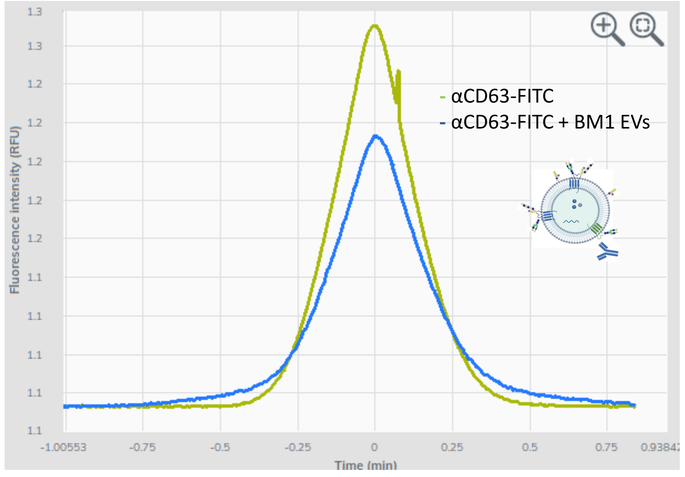

Figure 4. Representative curve of binding of fluorescently labeled α-CD63 to EVs derived from different MSCs

Table 3: Change of the hydrodynamic radius of binders on Serum EVs.

Standard EVs Serum	EVs		Binder		Free fluorophore	
1 x 10^{10} particles	Rh (nm)	Area %	Rh (nm)	Area %	Rh (nm)	Area %
α-CD63 (2.5 nM)	60.33	26	5.49	69	0.61	5
Gal-1 (5 nM)	54.39	62	5.42	33	0.92	5
Gal-3 (20 nM)	47.69	46	4.76	30	0.71	24
Gal-3 CRD (20 nM)	29.05	44	3.03	48	0.77	7

Table 4: Change of the hydrodynamic radius of binders on MSC EVs.

α-CD63 (5nM)	EVs		Binder		Free fluorophore	
MSC EVs	Rh (nm)	Area %	Rh (nm)	Area %	Rh (nm)	Area %
BM1	32.64	12	5.28	83	0.49	4
BM2	30.67	16	5.17	79	0.48	5
WJ	34.89	33	5.43	61	0.52	6
α-CD63			4.57	100		
Gal-3 (40 nM)	EVs		Binder		Free fluorophore	
MSC EVs	Rh (nm)	Area %	Rh (nm)	Area %	Rh (nm)	Area %
BM1			3.25	10	0.74	90
BM2			3.74	9	0.69	91
WJ			5.06	13	0.72	87
Gal-3			2.53	12	0.71	88

EC_{50} calculation of EV binding

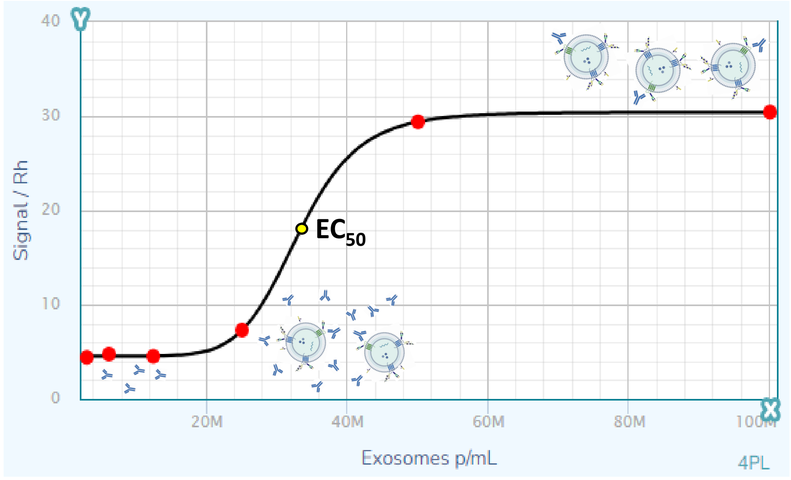

Figure 5. Concentration dependent binding of serum EVs to α-CD63 (2.5 nM). EC_{50} for the EVs: 3.3 x $10^9 \pm$ 5.15 x 10^7 particels/mL (p/mL). Signal/Rh show the change of the hydrodynamic radius in nm.

Perspectives

The FIDA technology provides fast and reproducible results for the sizing and labeling of proteins and EVs (Fig. 3, Table 2) with minimal sample volume requirements. Detection of antibody and galectin binding to EVs was quantifed (Table 3 and 4) and EC_{50} analysis of EV-antibody binding was performed (Fig. 5) .

Outlook for optimizing the measurements:

- improve labeling of galectins to a min. of 60-100% to reduce the presence of unbound dye and unlabeled protein
- standardize EV concentration to 2.5 x 10^9 p/µL

Perpectives:

- determine Kd values of EV galectin binding
- identify subpopulations of EVs

Conclusion

Flow induced dispersion analysis is an excellent technology to combine quality checks of your samples with binding analysis between two molecules. The detection of binding between small proteins like galectins and large particles such as EVs and the measurement under more physiological conditions and concentrations than with other methods make the FIDA very attractive for EV analysis. Our results demonstrate the successful detection and quantification of EV-protein interactions for the tested proteins and antibodies. In summary, FIDA represents a powerful tool for studying EV-protein interactions, shedding light on the complex mechanisms underlying intercellular communication mediated by EVs. Overall, this study demonstrates the significance of FIDA technology in advancing our knowledge of EV biology and its potential medical implications.

Acknowledgement

We thank Prof. Bernd Giebel, Dr. Tanja Kutzner, Prof. George Koliakos and Prof. Kokkona Kouzi-Koliakou for providing the immortalized MSC cell lines.

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