Biochemical and biophysical characterizations of Outer Membrane Vesicles through the NanoBioAnalytical Platform

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The Outer-Membrane Vesicles (OMVs) are spherical buds (20-250 nm) that display multiple offensive and defensive functions (Fig 1; [1]). Such versatility makes them an excellent tool for therapy, bioengineering (vaccines, protein production, drug delivery; [2]) and development of new antimicrobial peptides. However, many uncertainties still exist at the level of biogenesis and function mechanisms of these vesicles, hence there is a strong interest for better understanding of their composition, structure and diversity.

Recombinant OMVs were purified from *E. coli* laboratory strain BL21 (DE3) overexpressing or not the virulence gene haemolysin F, protein which boost OMVs synthesis [3] (OE-HlyF and Δ- HlyF respectively). In order to compare OMV structure and composition in OE HlyF versus Δ- HlyF, they were biochemically and then biophysically characterized in solution and on biochip using the NanoBioAnalytical platform (NBA) [4]. The latter resides on the combination of two label free techniques: the Surface Plasmon Resonance (SPR) and the Atomic Force Microscopy (AFM), which allow a fine characterization of nanoscale biological objects captured on the surface of a biochemically functionalized biochip; it is coupled to mass spectrometry (MS) for a proteomics analysis (Proteomics Platform CLIPP, Dijon).

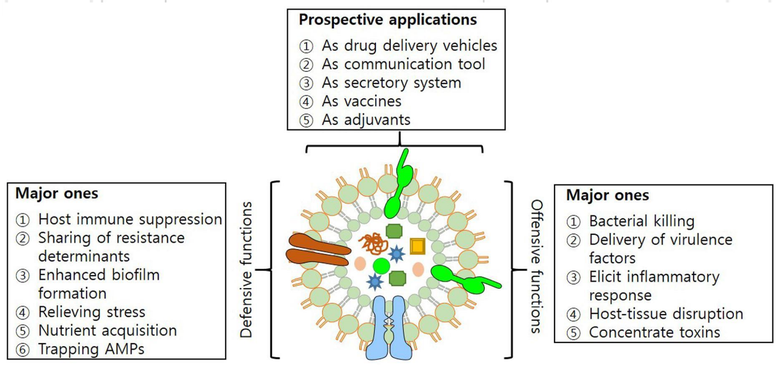
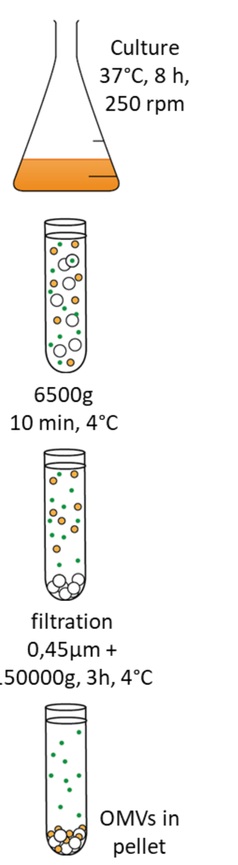


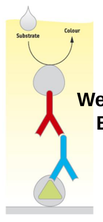
Fig 1: Offensive and defensive functions and prospective applications of OMVs [5]

Methods [3,4]

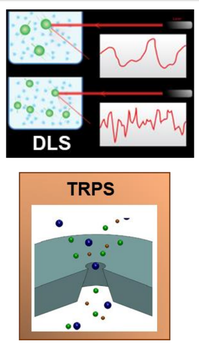
PREPARATION



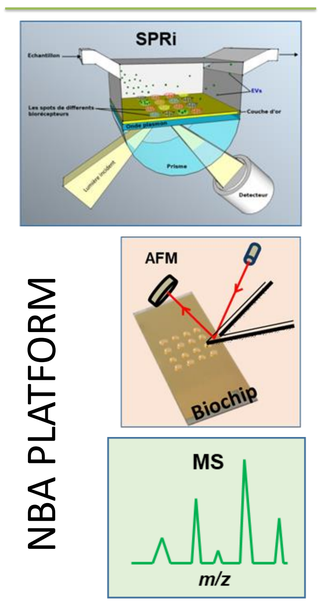
BIOCHEMISTRY



METROLOGY



NBA PLATFORM



Biochemical characterizations

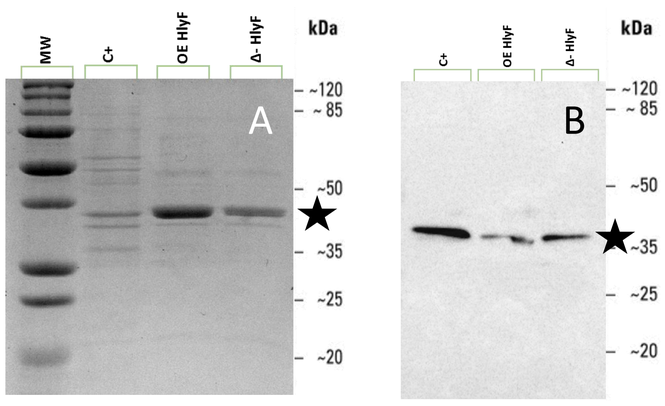


Fig 2 : SDS-Page (A) and Western Blot (B) analysis with an anti-OmpA antibody (d1/10000[e]). 20 µg of total membrane proteins were loaded on a 12% acrylamide gel. C+ = positive control (total bacterial membrane protein)

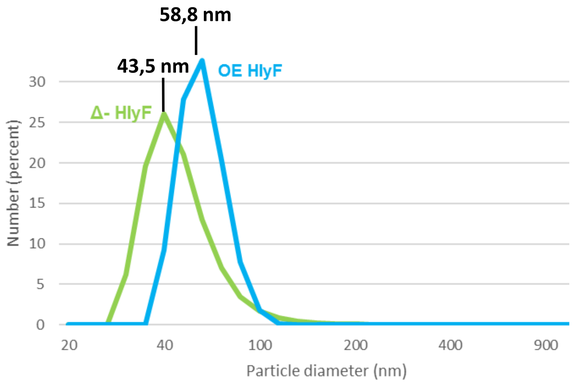
Strain [5]	Characteristics
OE HlyF	Overexpression HlyF
Δ- HlyF	HlyF deletion mutant

→ Revelation of OmpA (★) present in both OE HlyF and Δ- HlyF

→ Looking for other biomarkers for specific capture of OMVs

Metrology in solution

Dynamic Light Scattering



TRPS

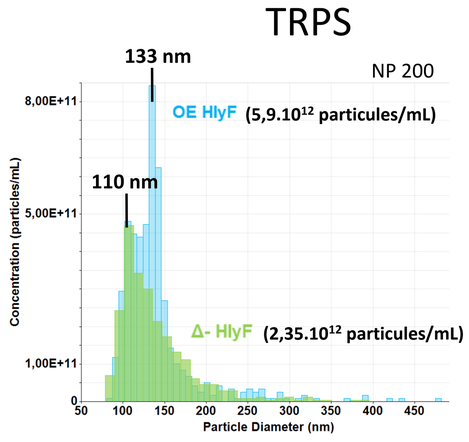


Fig 3 : Size distribution profiles of OMV diameters of OMVs from both types by DLS (200 particles measured) and TRPS (n=500 particles measured)

→ OE-HlyF OMVs are larger than Δ-HlyF and OMVs concentration is compatible with biophysical approaches on biochip

→ DLS data are in accordance with TEM and AFM results [5] but small OMVs can not be evaluated by TRPS due to the cut-off of the technique (80 nm)

MS analysis (nanoLC-MS/MS) in solution

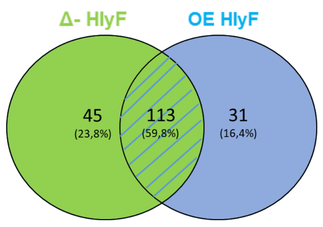


Fig 4 : Venn diagram of proteomic analysis of OMVs from both types of samples (500 ng of proteins)

→ Most of proteins are common to both types of OMVs

→ OmpA is among the 104 common proteins → potential candidate for SPRi experiments

→ Absence of HlyF due to databases used for analysis

On-chip characterization via NBA platform

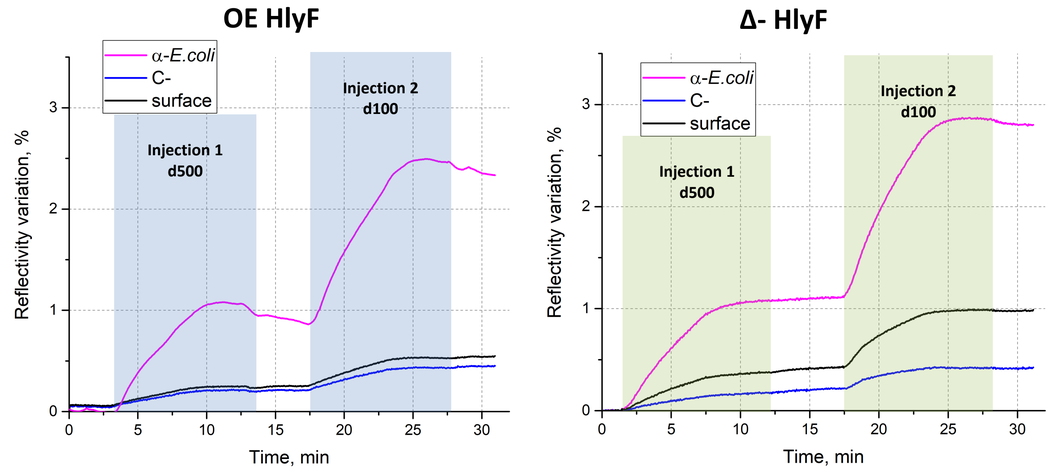


Fig 5: SPRi sensorgrams of specific capture by antibody α-*E. coli* of OE HlyF (left) and Δ- HlyF (right) on a multiplex gold biochip. C- : negative control (irrelevant IgG). N=2

→ OMVs are specifically captured on a biochip via specific antibodies

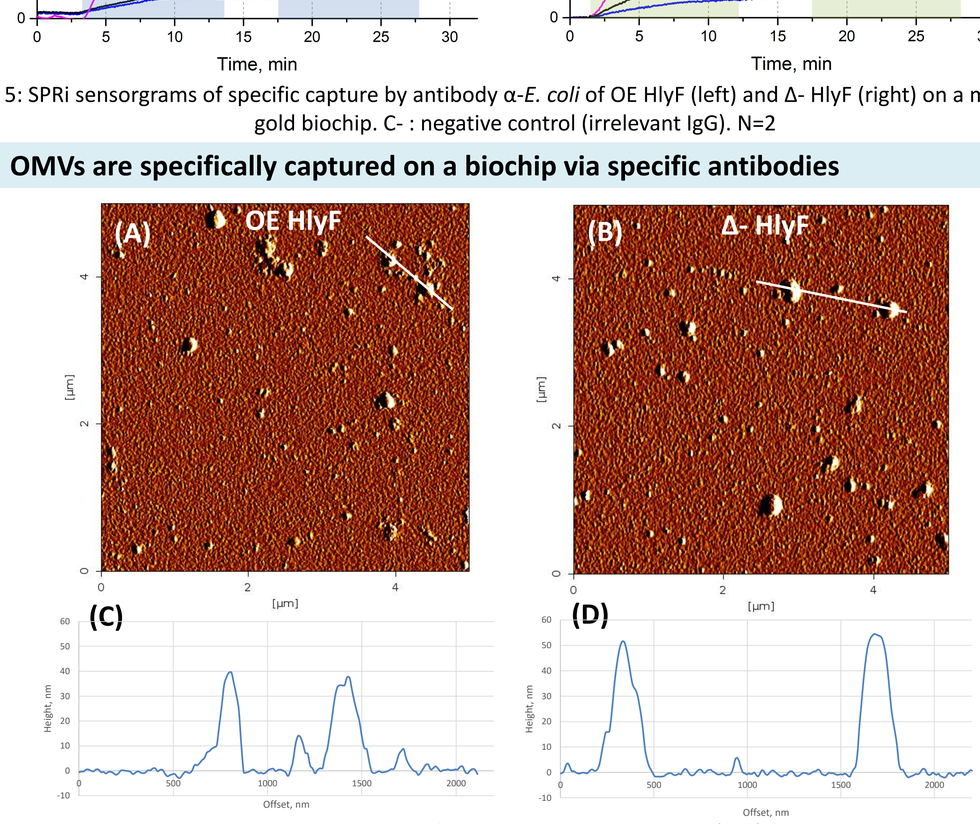


Fig 6: AFM imaging in air in contact mode of OMVs captured on α-*E.coli*. (A-B): Error signal images of OE HlyF (A) and Δ-HlyF (B); (C-D): profile sections of selected zones. The particles are fixed with 0.5% glutaraldehyde.

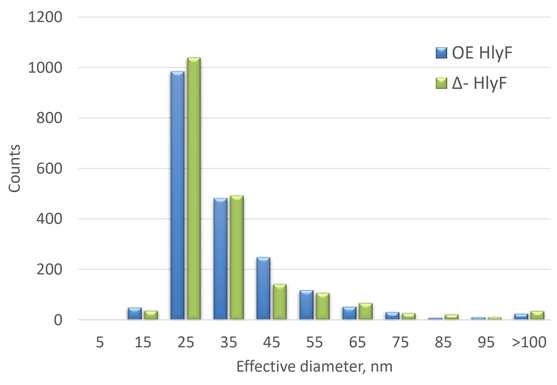


Fig 7: Particles size distribution histogram calculated from AFM imaging (2000 particles measured)

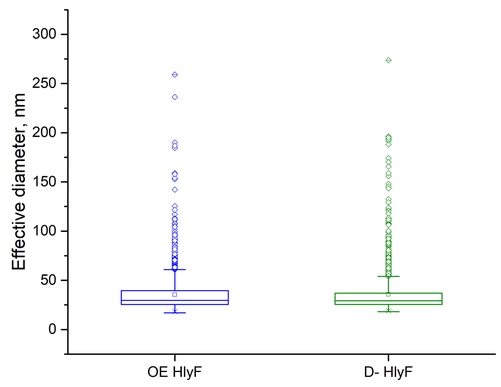


Fig 8: Box and whisker plot of OMVs effective diameter defined from AFM imaging.

→ Similar size distribution for OMVs from OE-HlyF and Δ-HlyF ; 90% of OMVs between 25 and 70 nm / 1% larger than 100 nm

Conclusion

- ✓ Adequation of size determination of OMVs in solution and through biochip specific capture
- ✓ Data from these studies are in accordance with previous results obtained by TEM [3]
- ✓ Complementary analysis is required for completed characterization of OMVs (lipidomics,…)
- ✓ NBA platform suitable for other bacterial EVs characterization

References

[1] Schwechheimer C, Kuehn MJ (2015) Nat Rev Microbiol. 13:605-19.
[2] Acevedo R et al. (2014) Front Immunol. 5:121.
[3] Murase et al. (2016) J Infect Dis. 213:856-65
[4] Obeid S et al. (2016) Biosens Bioelectron. pii: S0956-5663(16)30856-9.
[5] Jan (2017) Frontiers in Microbiology 8:1053.

Perspectives

- Biochemical and biophysical characterizations of other recombinant OMVs to study functions of protein(s) implicated in OMVs biogenesis
- Analysis of samples from patients suffering from bacterial infection and sepsis
- Determination of function/pathogenicity of OMVs