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, there is still 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 biocomponent functionalized biochip; it is coupled to mass spectrometry (MS) for a proteomics analysis (Proteomics Platform CLIPP, Dijon).

**Methods [3,4]**

**Biochemical characterizations**

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

**Dynamic Light Scattering**

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)

**TRPS**

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

**MS analysis (nanoLC-MS/MS) in solution**

Absence of HlyF Δ HlyF

Presence of HlyF OE HlyF

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

**Conclusions**

- 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

**On-chip characterization via NBA platform**

OMVs are specifically captured on a biochip via specific antibodies

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

- **Fig 6:** AFM imaging in air in contact mode of OMVs captured on a E. coli - (A) and Δ HlyF - (B) (C-01) pressure zones of selected zones. The particles are fixed with 0.5% glutaraldehyde.

- **Fig 7:** Particles size distribution histogram calculated from AFM imaging (1000 particles measured)

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

- **Fig 2:** Size distribution profile of OMV diameters of OMVs from both types by DLS (200 particles measured) and TRPS (200 particles measured)

**References**


**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