

Kinetic characterization of nanobody interactions with extracellular vesicles using the novel Octet® R8e

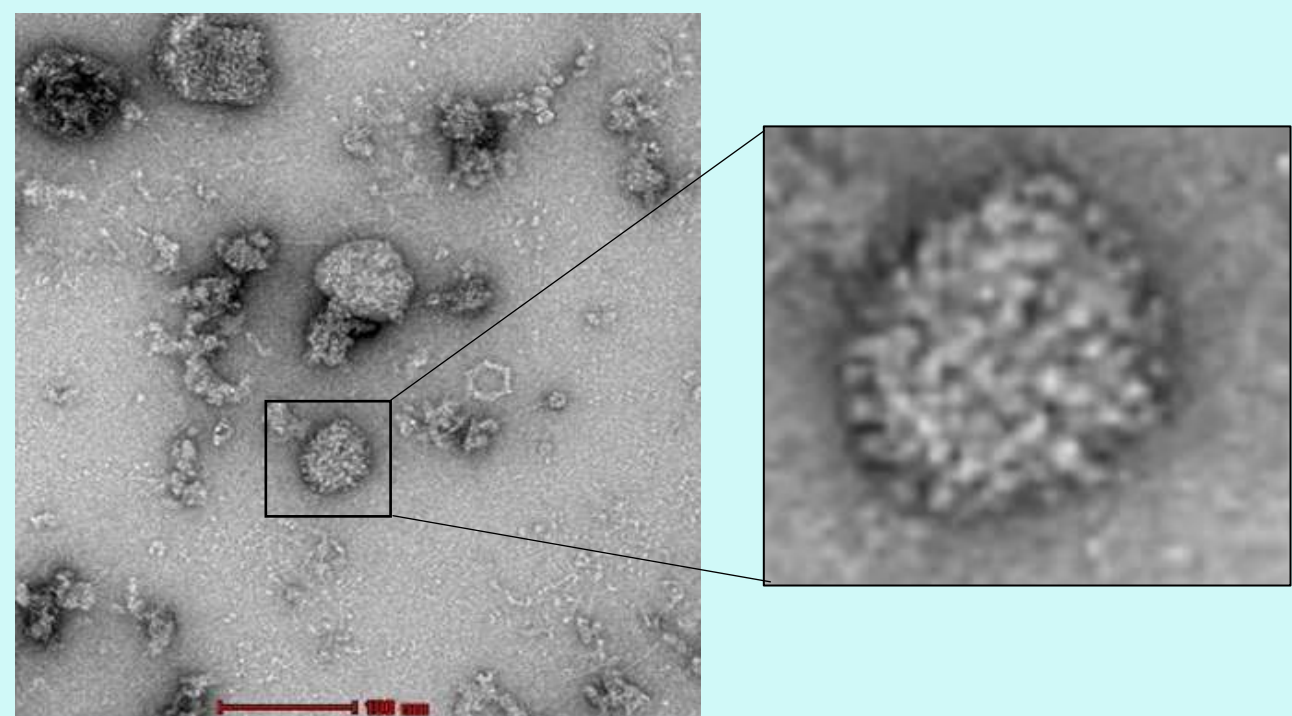
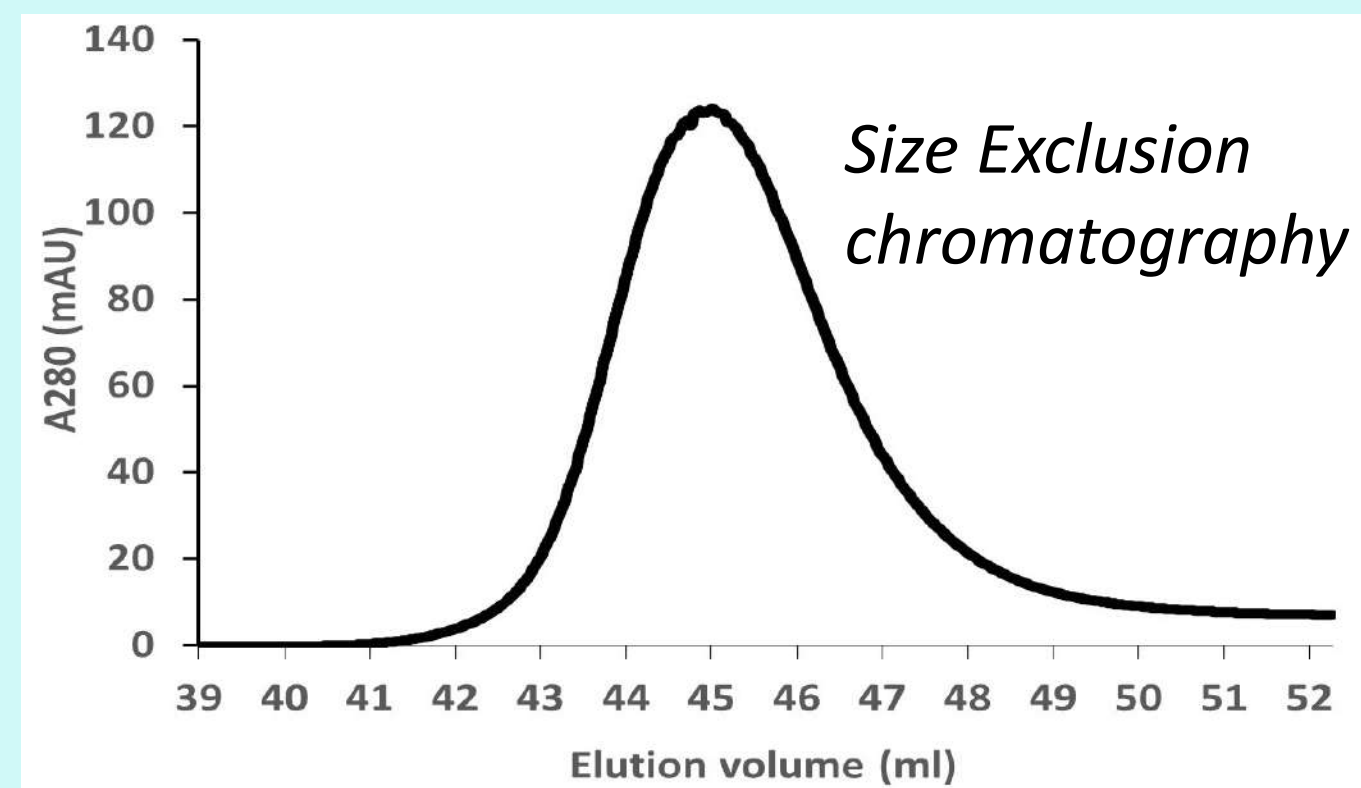
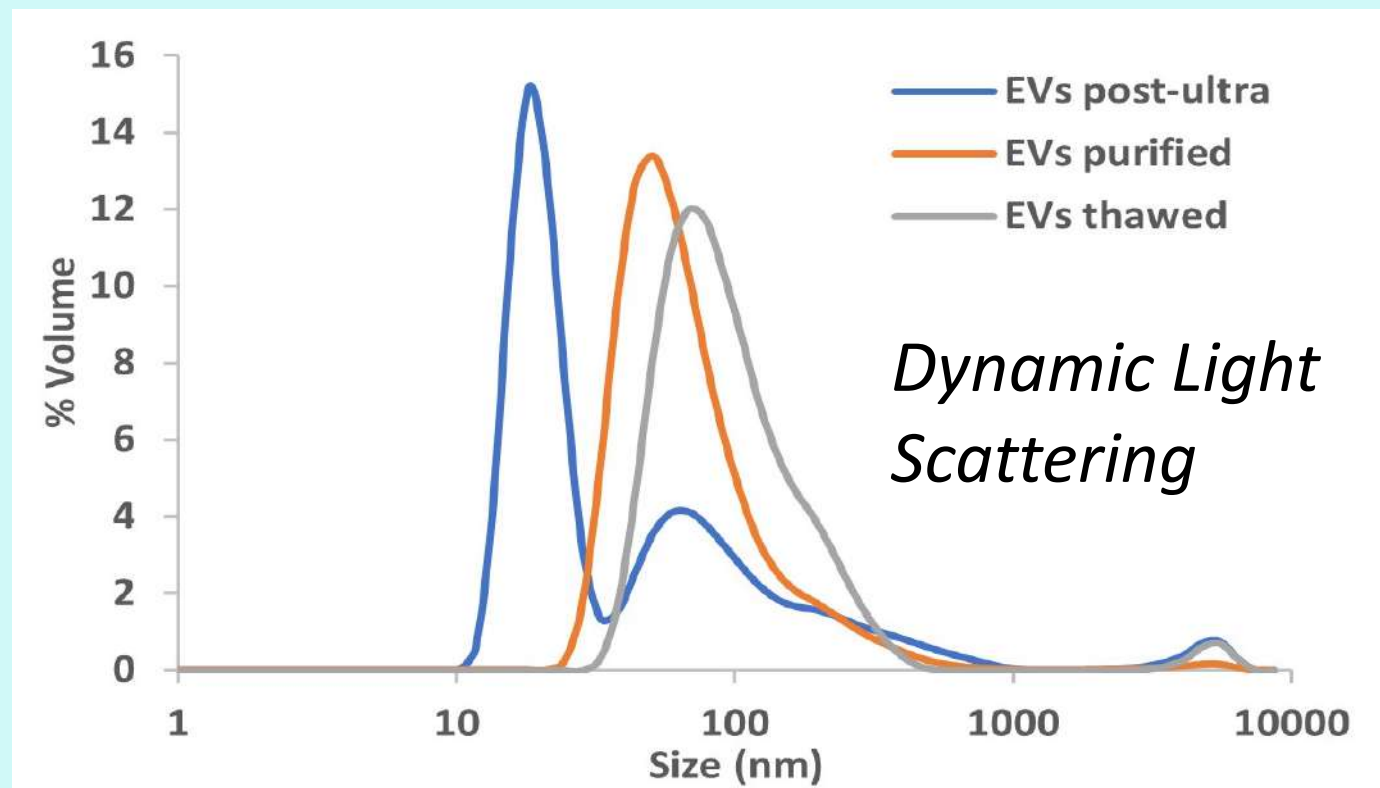
Josiane KASSIS¹, Christophe QUETARD², Laura FRADALE¹, Clara BOUYX³ and Alain ROUSSEL^{1,3}

¹ CNRS, LISM, Marseille, France. ² SARTORIUS. ³ NVDIAG, Marseille, France

Handling **membrane proteins** (MPs) in their native environment is crucial for ensuring proper folding and stability. **Extracellular vesicles** (EVs) offer a superior alternative to detergents and artificial lipid environments. Expressing recombinant MPs on the EVs surface allows for their display in a native context, making them **ideal for llama immunization to generate Nanobodies (Nbs) targeting MPs**. However, **the large difference in size** between the vesicles, measuring around 100 nanometers and weighing several million Daltons, and a small 15 kDa nanobody posed a problem for characterization using Bio Layer Interferometry. Data obtained very recently with the new **Octet® R8e** has made it possible to **overcome this challenge**.

1- EVs preparation

- EVs expressing recombinant MPs on their surface are
 - produced by transient transfection of HEK293 cells
 - isolated by differential centrifugation & purified by SEC
 - characterized by DLS, western blot & electron microscopy

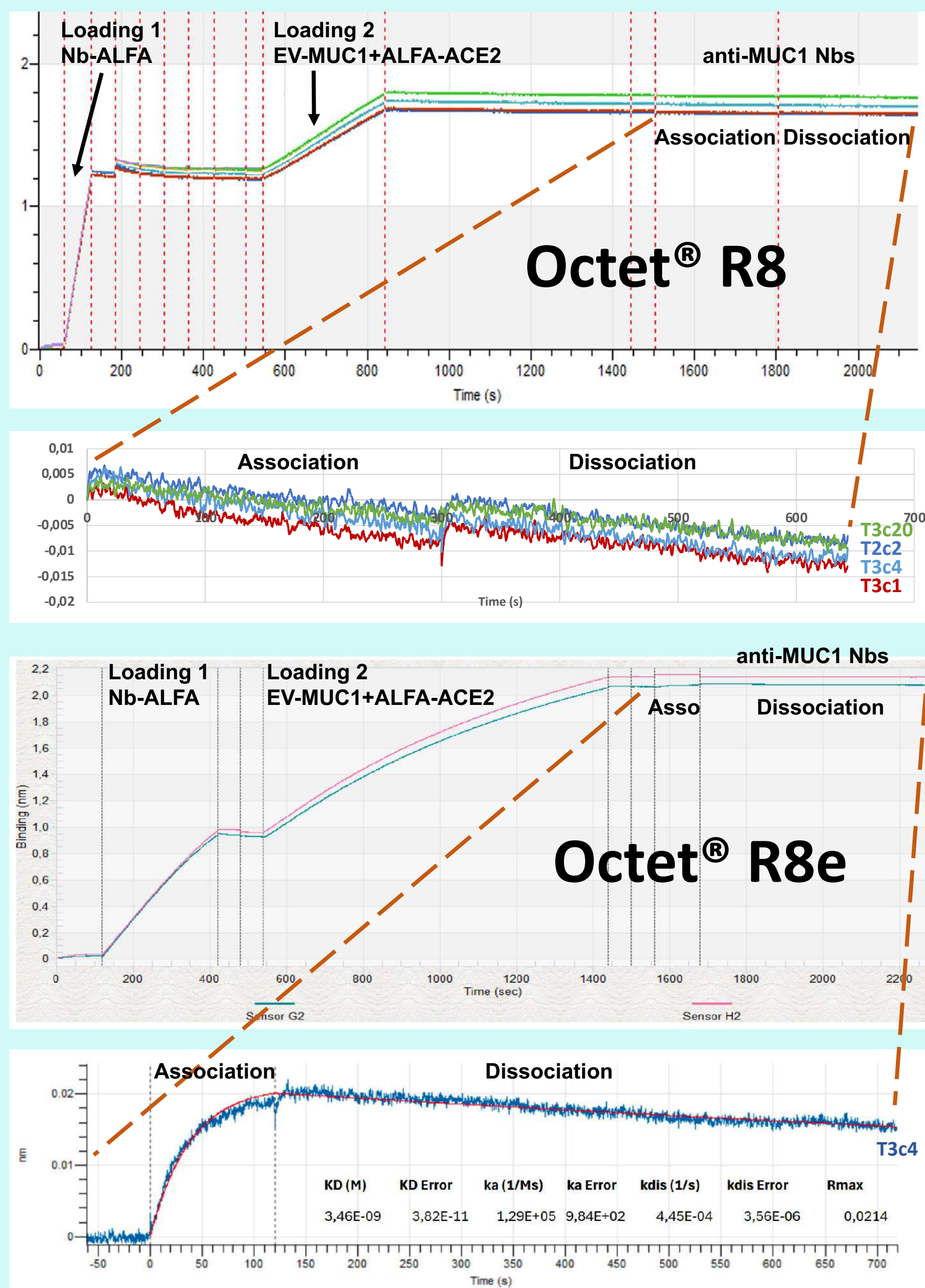


Negative staining-EM



Llamas are immunized with EVs in order to generate Nbs targeting the MP of interest

3- MUC1 project



MUC1 is a member of the human mucin family. MUC1 EVs were produced and used to generate three anti-MUC1 Nbs. Tests to characterize the Nbs on the EVs were conducted on an Octet® R8. Briefly, Nb-ALFA was loaded onto Strep biosensors (loading 1). Next, the EVs carrying MUC1 and the ALFA-ACE2 anchor protein were loaded (loading 2). Finally, association and dissociation steps were performed with the three anti-MUC1 Nbs (T2c2, T3c1 and T3c4). Unfortunately, no signal was detected.

The experiment was repeated on an Octet® R8e. After loading Nb-ALFA and MUC1+ALFA-ACE2 EVs, the association and dissociation steps were performed with anti-MUC1 Nb T3c4. In this case, a very clear signal was obtained, allowing the kinetic parameters of the interaction to be calculated.

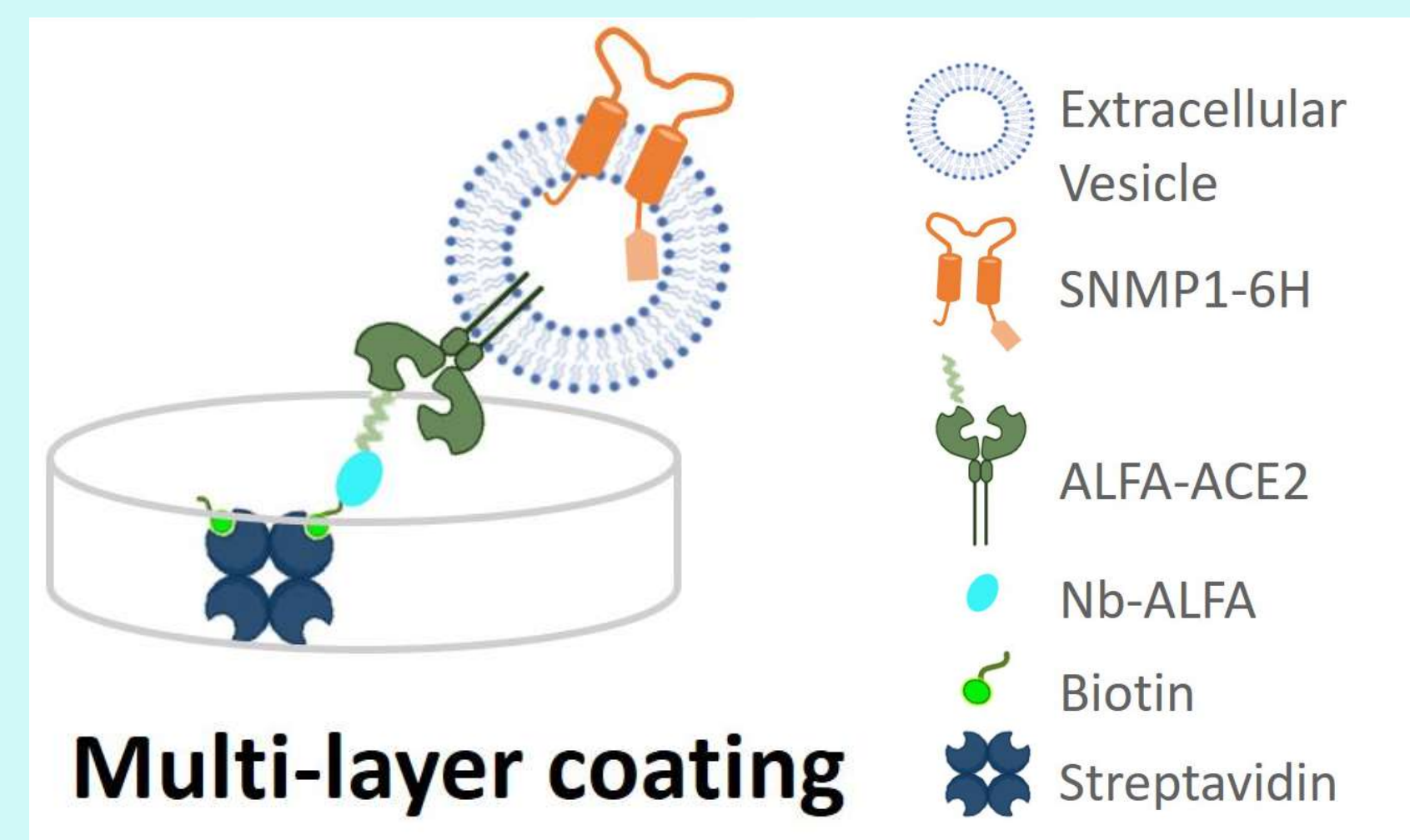
Conclusion

Working with **membrane proteins** (MPs) in their native environment is crucial for ensuring proper folding and stability. **Extracellular vesicles** (EVs) offer a superior alternative to detergents and artificial lipid environments. Expressing recombinant MPs on the EVs surface allows for their display in a native context, making them **ideal for antibody generation**.

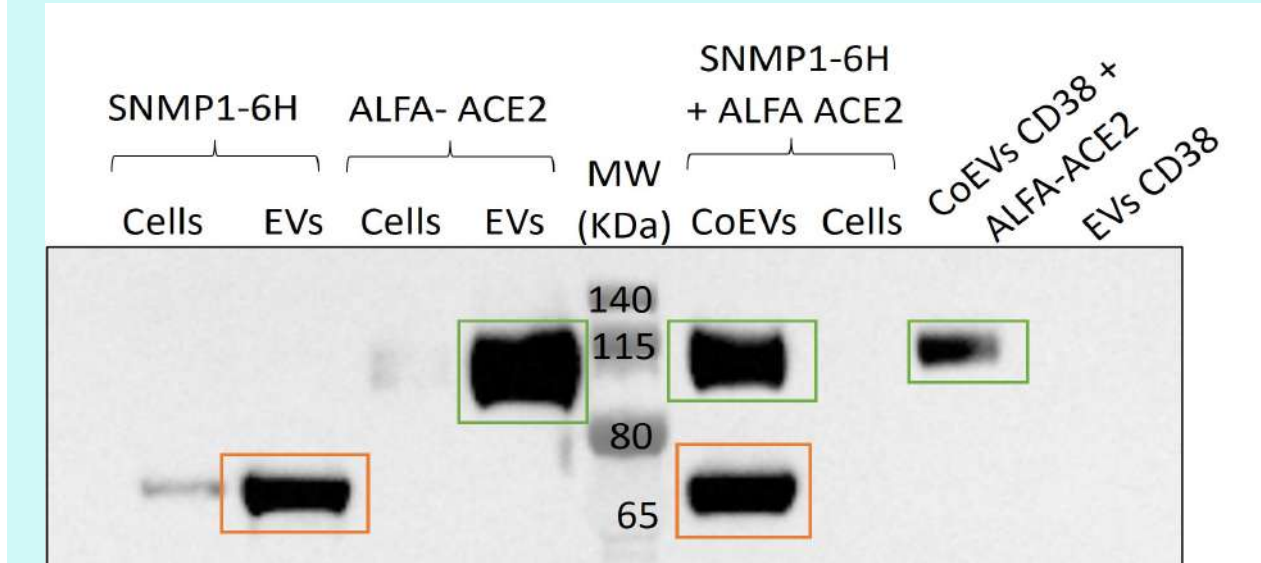
In this study, we showed that EVs could also be used to perform kinetic characterizations on MPs in their native environment, i.e. in a natural membrane. The **increased sensitivity** provided by the **Octet® R8e** compared to the Octet® R8 was decisive in obtaining data that was not previously accessible by BLI, as shown by the example of the MUC1 project.

To our knowledge, very few antibodies have been characterized under native conditions. The use of EVs and Octet R8e paves the way for a new type of study in this field.

2- Multi-layer approach



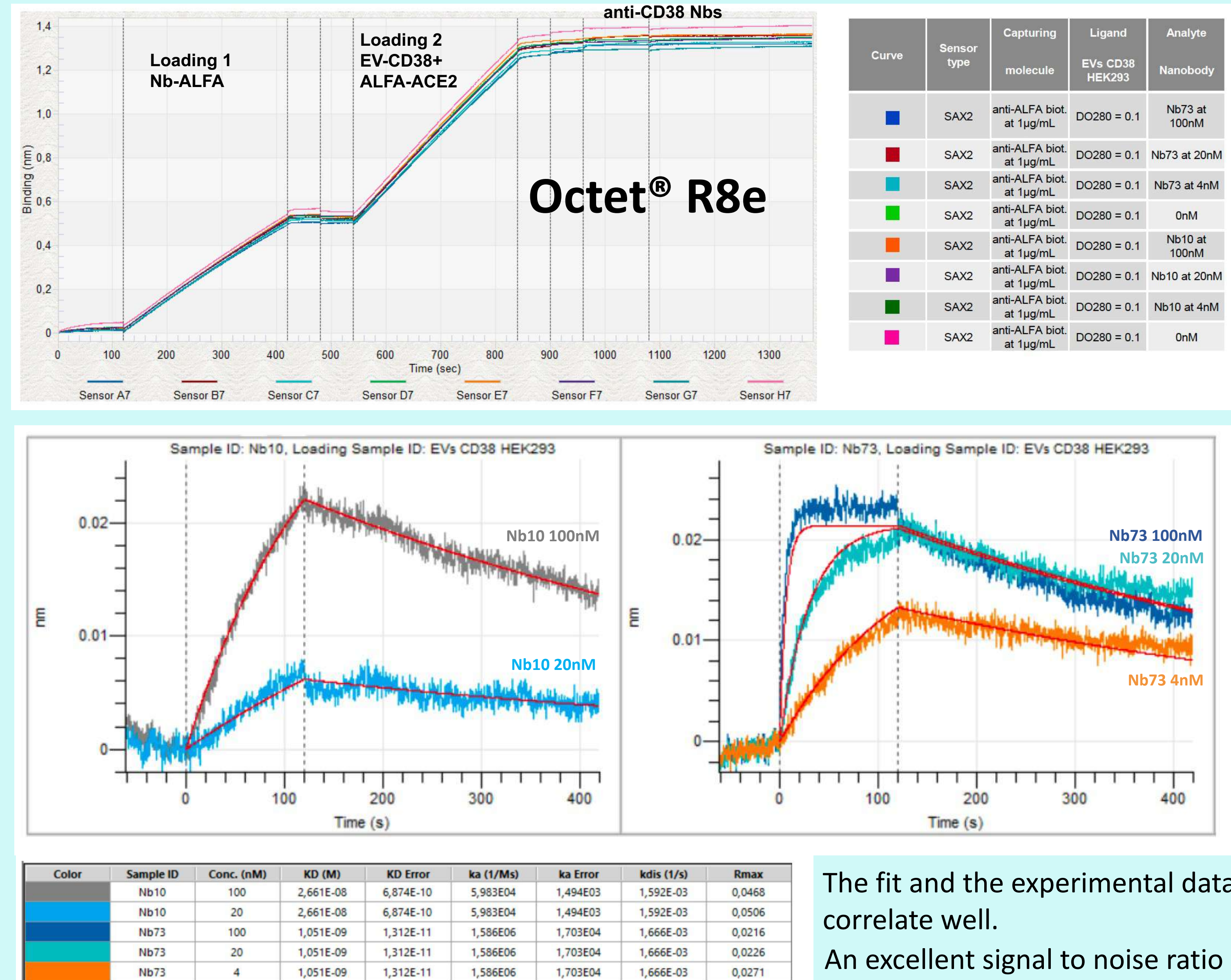
The co-expression of the protein of interest (in this case, the SNMP1 protein) with ALFA-ACE2 is verified by Western blot.



The binding of EVs to surfaces is necessary throughout the Nanobody generation process, whether for amplification by panning, selection by ELISA or characterization by BLI. We chose to add an anchoring protein (ACE2) carrying an ALFA tag by co-transfection and we use the ALFA Nanobody to perform a multi-layer approach.

4- CD38 project

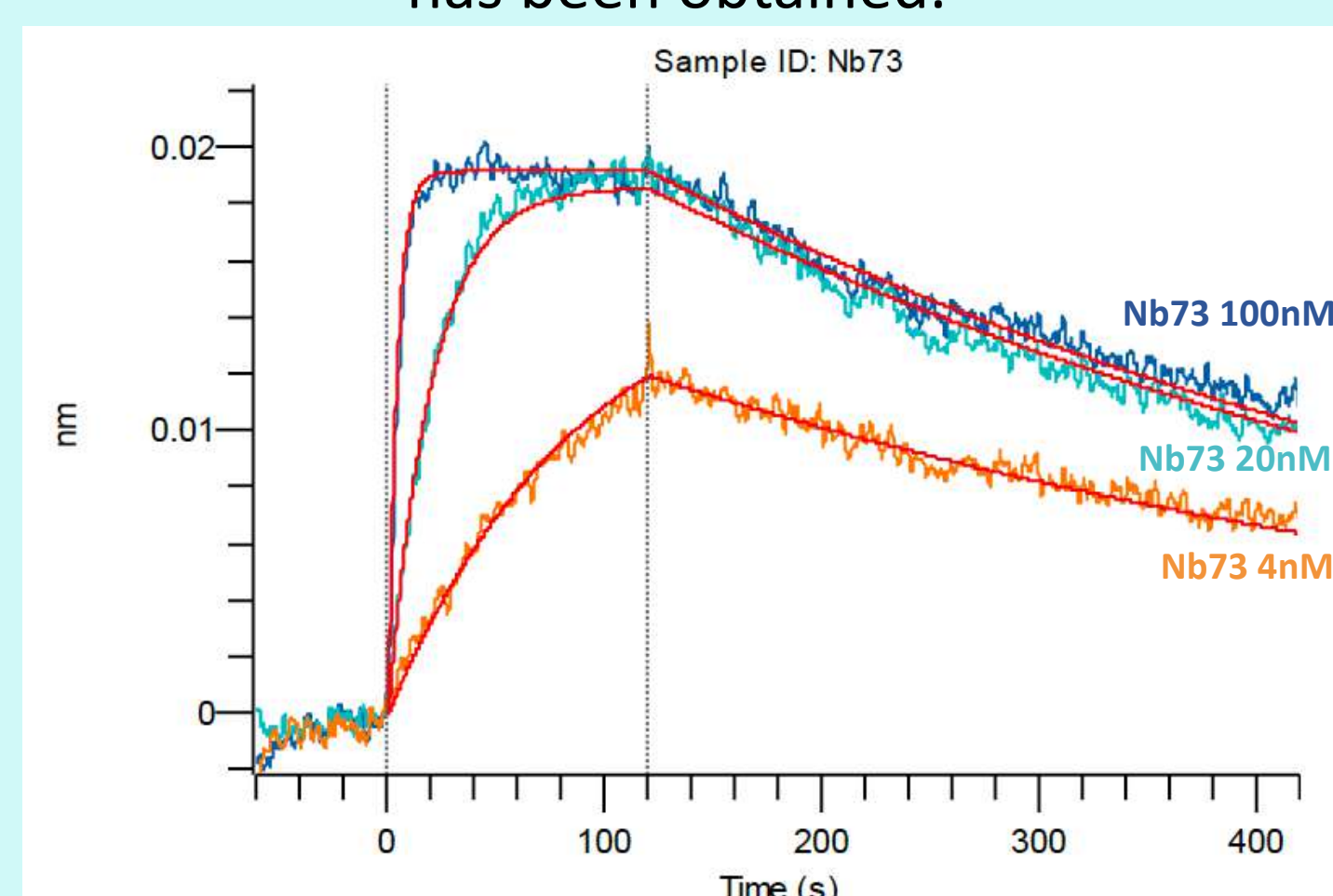
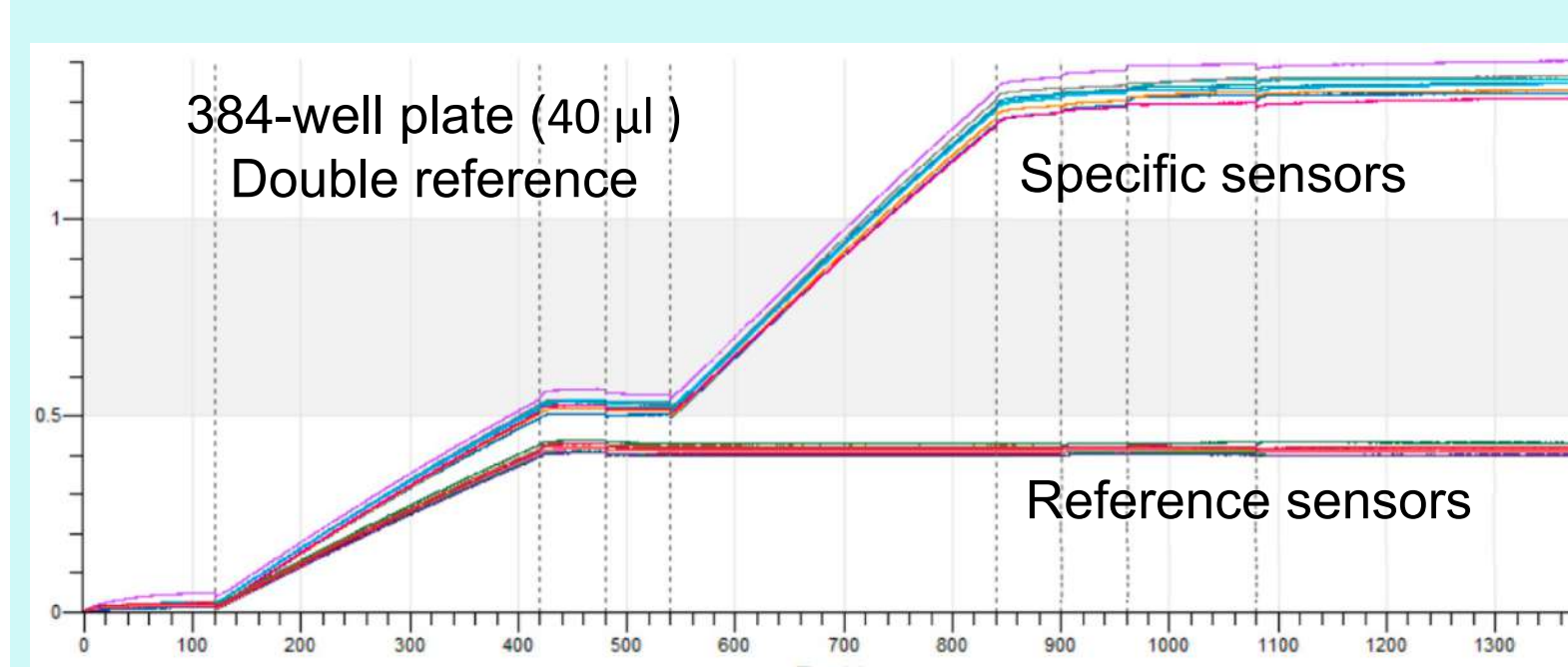
CD38 is a glycoprotein most frequently found on the surface of B cells. CD38 is used as a prognostic marker in leukemia. CD38 EVs were produced and used to generate several anti-CD38 Nbs. Among them, Nb10 and Nb73 exhibit different behaviours in flow cytometry. We have tried many times to characterize their interaction with CD38 EVs, but without success so far. For the Octet® R8e tests, we proceeded as for MUC1, with a first loading of Nb-ALFA and a second loading of CD38+ALFA-ACE2 EVs. We then used three concentrations of Nbs: 100 nM, 20 nM and 4 nM for the association and dissociation steps.



The fit and the experimental data correlate well.

An excellent signal to noise ratio with a high level of confidence has been obtained.

We also tested Octet® R8e in a 384-well plate with a volume of 40 µl using a double-reference protocol, i.e. with a set of specific sensors and a set of reference sensors.



References

- V. Delauzun *et al.*, « Extracellular vesicles as a platform to study cell-surface membrane proteins », *Methods*, 2020
- Desmyter *et al.*, « Camelid nanobodies: killing two birds with one stone », *Current Opinion in Structural Biology*, 2015
- A. Dogliani *et al.*, « Generation of Nano-antibodies targeting the SNMP1 membrane receptor of *Spodoptera littoralis* using Extracellular Vesicles », *Journal of Extracellular Biology*, In press