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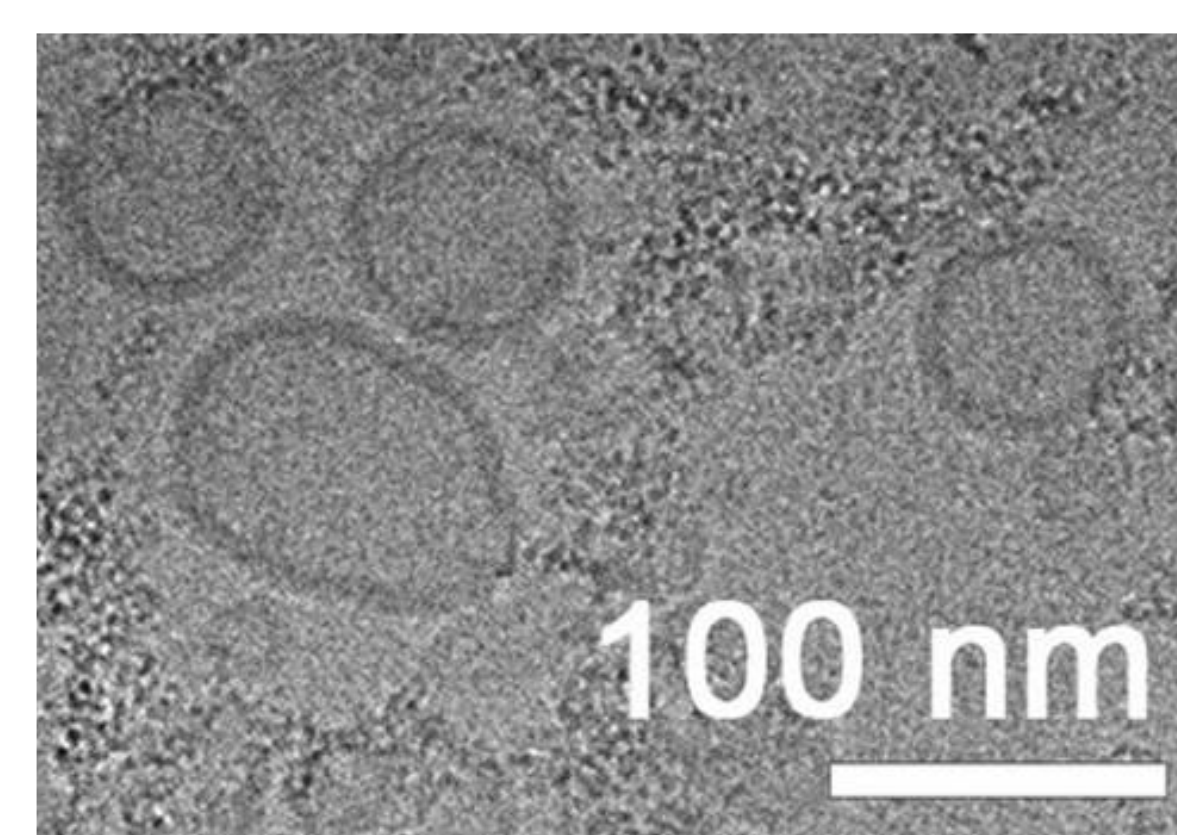
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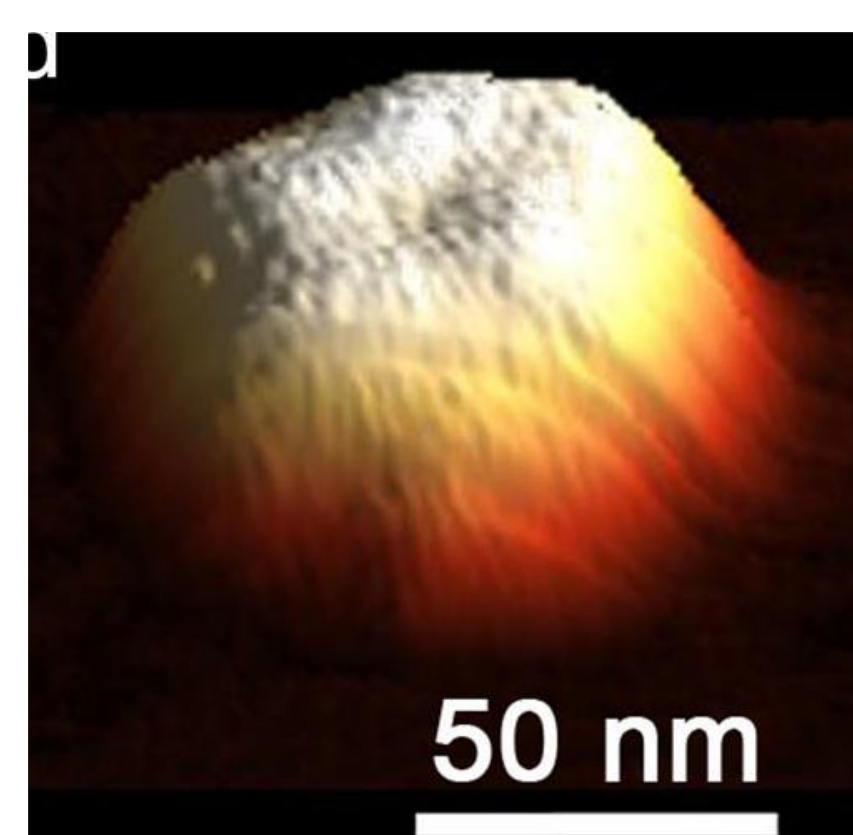


We recently identified nanoalgosomes (NA), that are small EVs secreted by microalgae, a sustainable natural source [Picciotto *et al. Biomaterials Science* 2021; Adamo *et al. JEV* 2021]. The study was led mainly on microalgae strain *Tetraselmis chuii*, but the multitechnique approach is exploitable on EVs from other strains and provides the means for a comprehensive structural characterization of EVs, which is expected to be crucial in the design of engineered vesicles to be employed in different fields of life science.

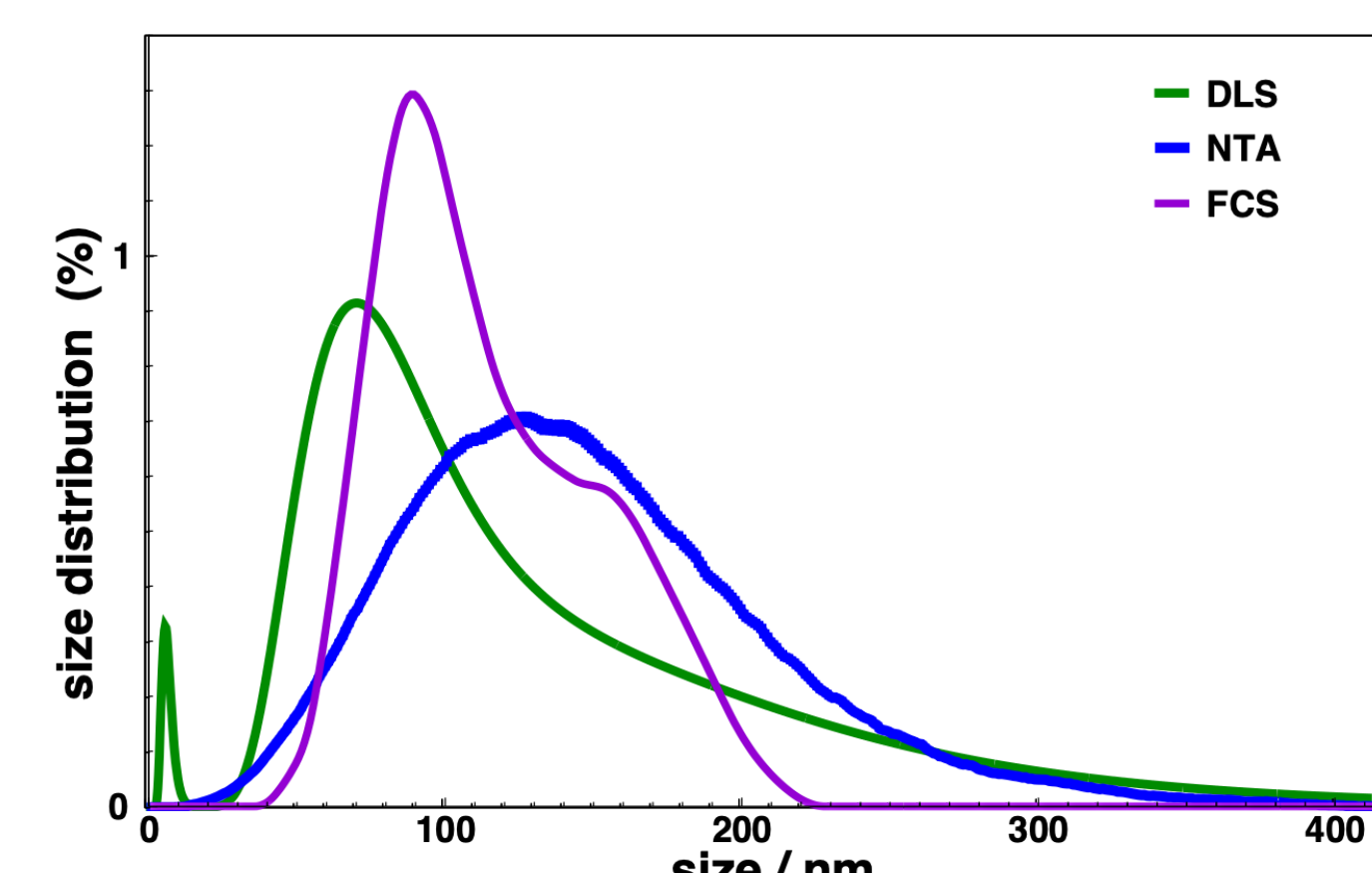
## Biophysical and biochemical characterisation



Cryo-TEM



AFM



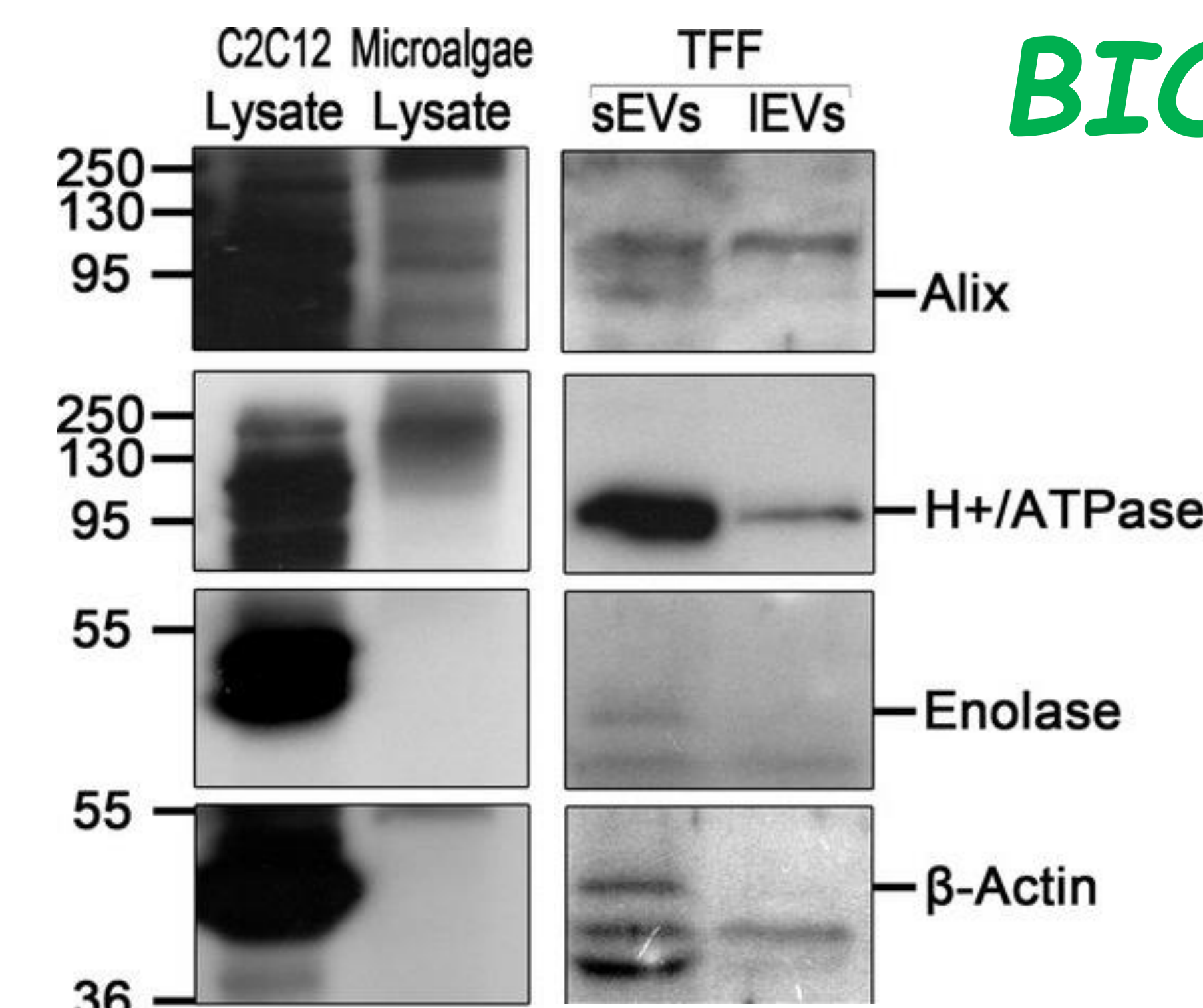
Ensemble techniques

Minimal information for studies on Evs (MISEV)  
[Théry *et al. JEV* 2018]



Standard Operating Procedures (SOPs)

## BIOMARKERS



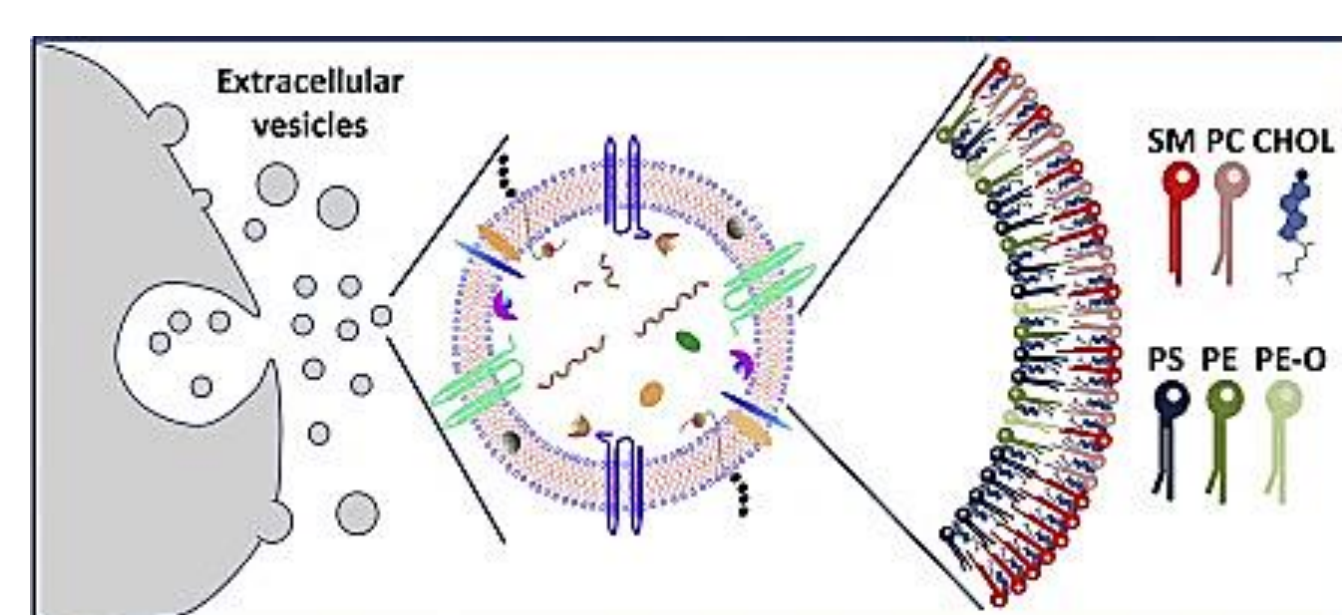
Immunoblot Analysis

H+/ATPase is specific for EVs from microalgae

[Adamo *et al. JEV* 2021]

## SIZE AND MORPHOLOGY

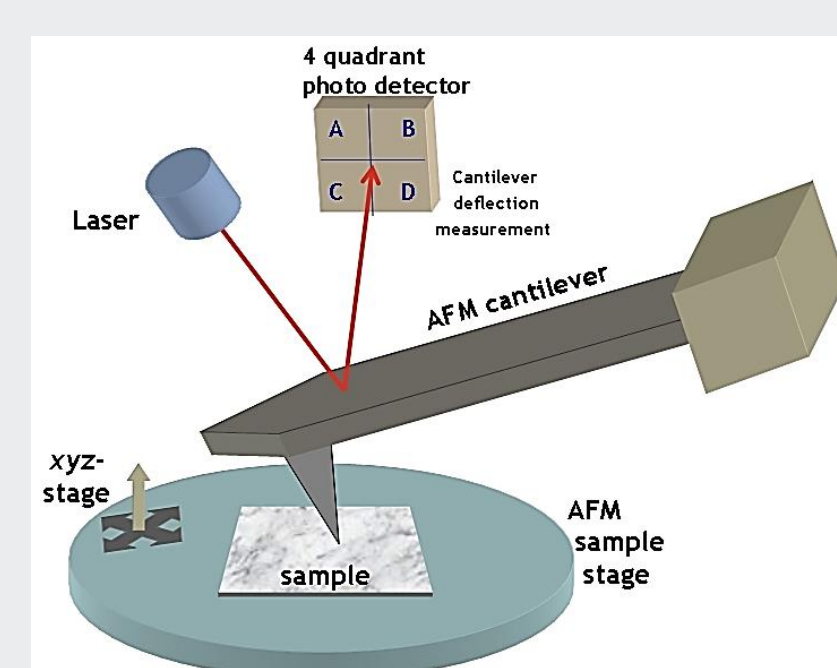
## Membrane topology



Different chemical species on the membrane

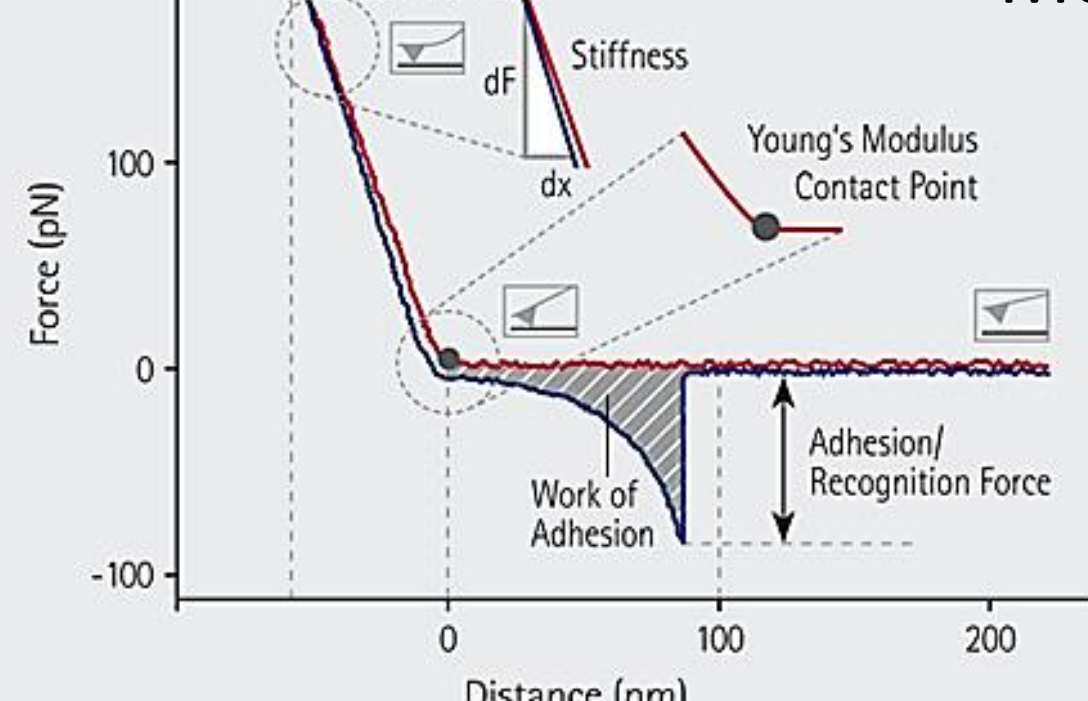
Different functionalisation strategies

### Atomic Force Microscopy (AFM)



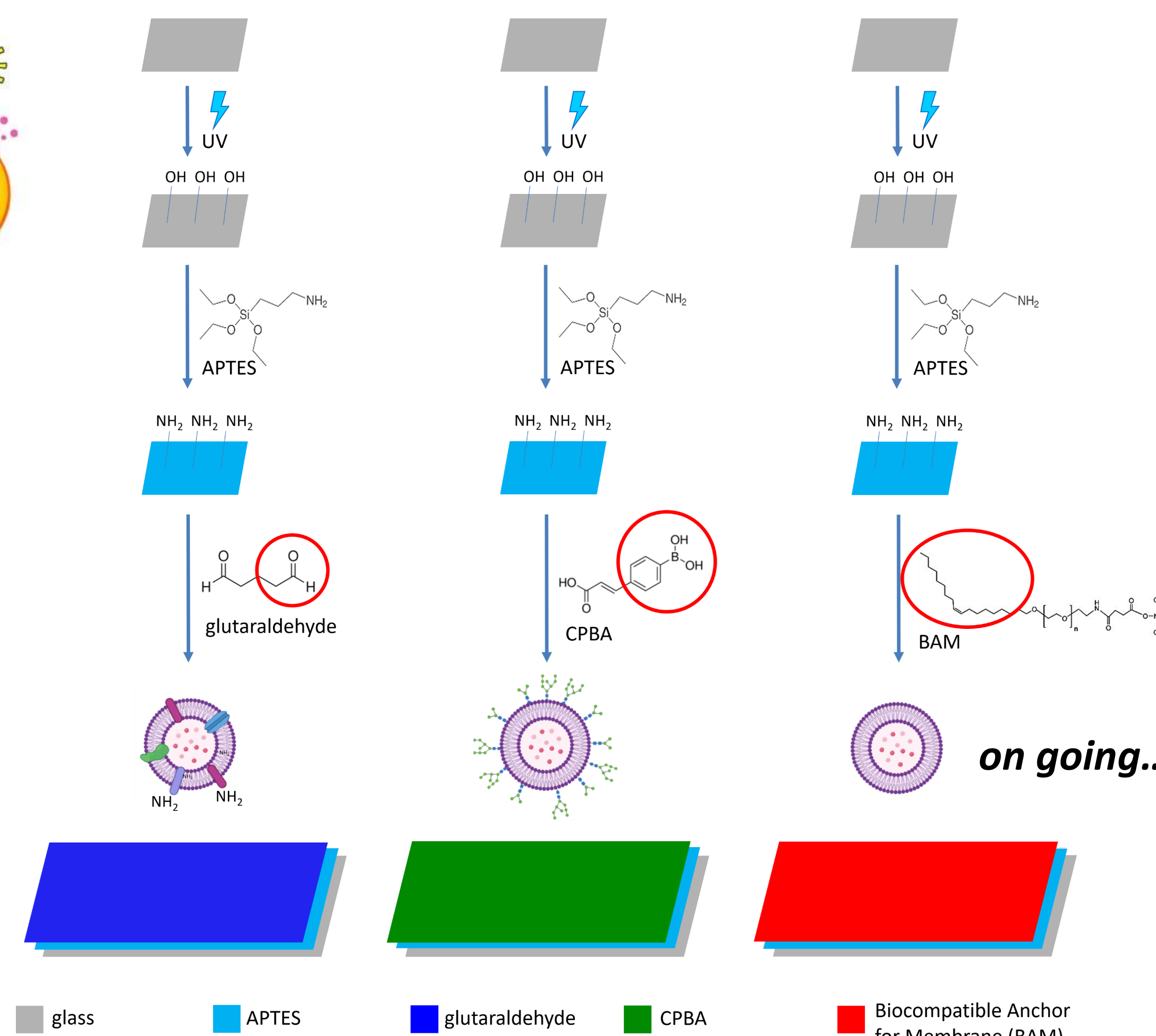
### Quantitative Imaging (JPK)

- No drag force
- Mechanical features



Force curve

## Functionalisation strategies

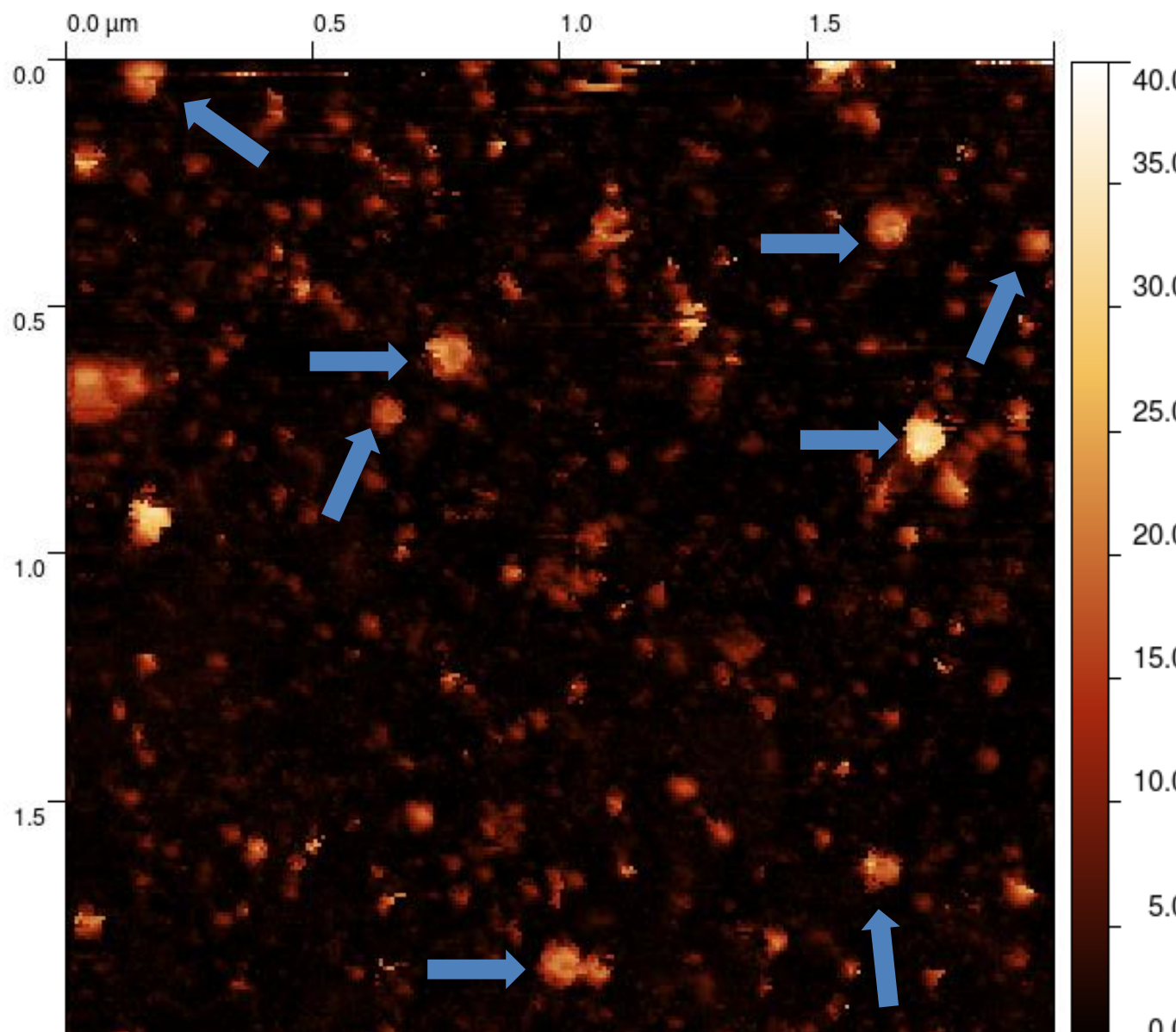


subpopulations?

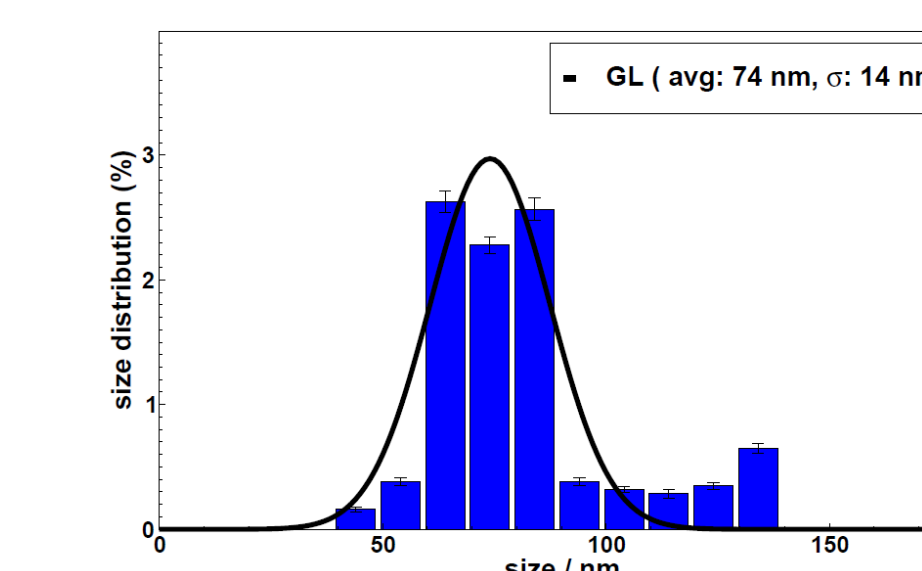


## BIOMECHANICS

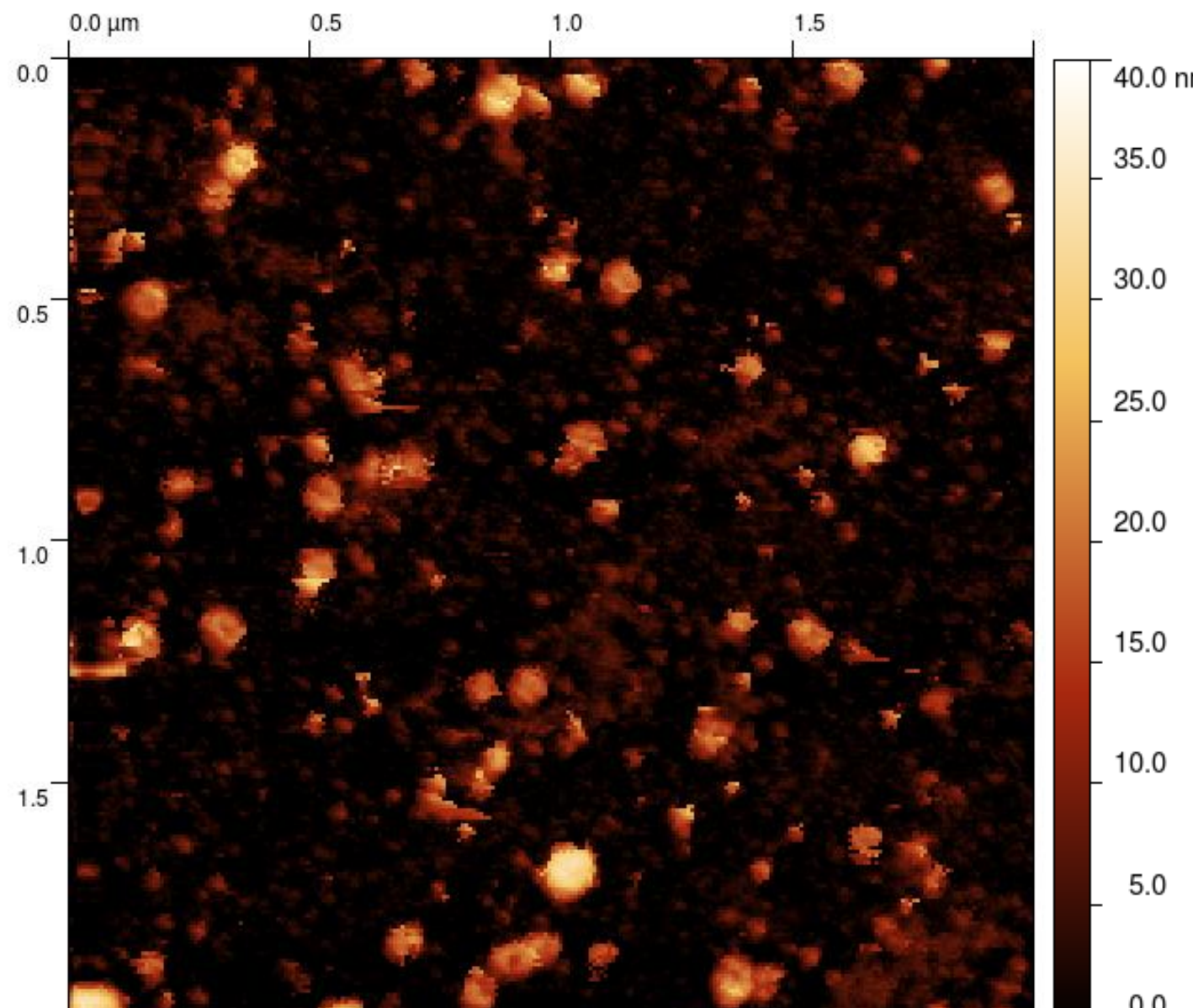
### NA on APTES/glutaraldehyde



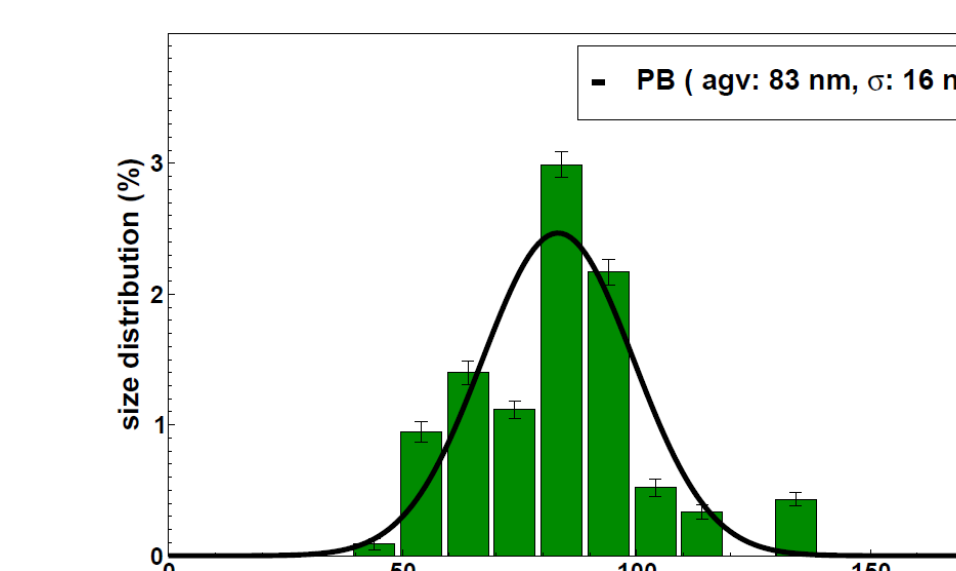
$h = 22 \pm 6$  nm ;  $\phi = 74 \pm 14$  nm ;  
 $6.7 \pm 2.5$  vesicles/frame QI in PBS



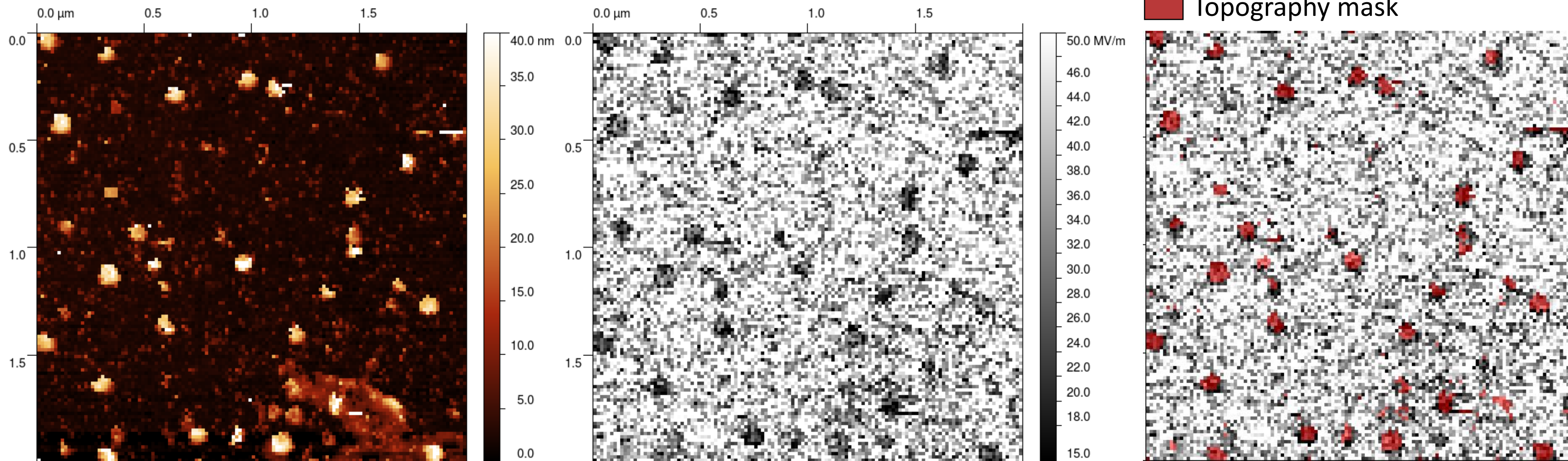
### NA on APTES/CPBA



$h = 22 \pm 5$  nm ;  $\phi = 83 \pm 16$  nm ;  
 $17 \pm 4$  vesicles/frame QI in PBS



### NA on APTES/glutaraldehyde



Topography

Stiffness

Merge

## CONCLUSIONS

- Functionalisation to bind glycans allows to fix more vesicles: higher sensitivity in detection
- Mechanical study makes EVs detection more precise and could be useful to identify EVs subpopulations with different membrane properties

## Acknowledgements

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