DOUBLE FUNCTIONALIZATION OF MICROBEADS

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Introduction

The functionalization of microbeads can be used as cell or virus mimetics for *in vitro* studies of cell/virus surface interactions.

Our group studies the biophysical interactions between blood cells and the blood vessel wall.

Therefore, the microbeads will be functionalized with the proteins CD44 and PSGL-1 that bind to the blood vessel wall.

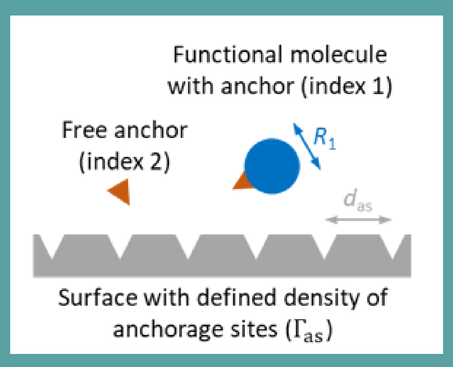
Our goal: To control the surface density of the two proteins separately and then perform a double functionalization

Competitive anchoring

Principle:

A flat surface composed of specific binding sites in a perfect and laminar flow.

Two different populations with the anchor tag to attach, are in competition to bind onto the surface :



The binding of the proteins is controlled by the diffusion leading to the following equation:

$$\frac{c_2}{c_1} = \left(\frac{R_2}{R_1}\right)^{\frac{2}{3}} \left(\frac{\Gamma_{as}}{\Gamma_{1,sat}} - 1\right)$$

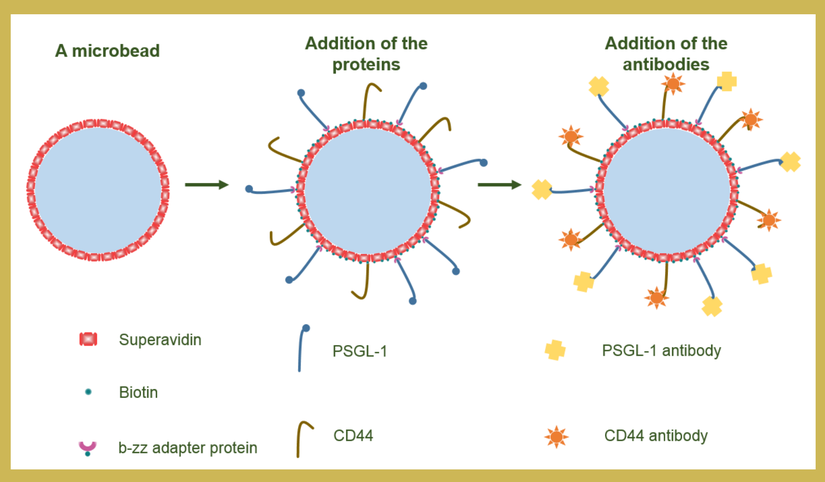
C_i: concentration of the protein
R_i: hydrodynamic radius
$\Gamma_{1,sat}$: molar surface density of the protein at saturation
Γ_{as}: total molar surface density ($\Gamma_1 + \Gamma_2$)

With this equation, we can control the surface density of the protein on the beads by adjusting the relative concentrations.

Why this method?

- Specific and strong interactions as biotin/Superavidin or zz/PSGL-1.
- Less dependent of the number of beads in the solution than only putting the protein of interest.
- A theory that relates the mix in solution to the relative concentrations on the surface:
  →quantitative
  →doable for more than one ligand (our objective).

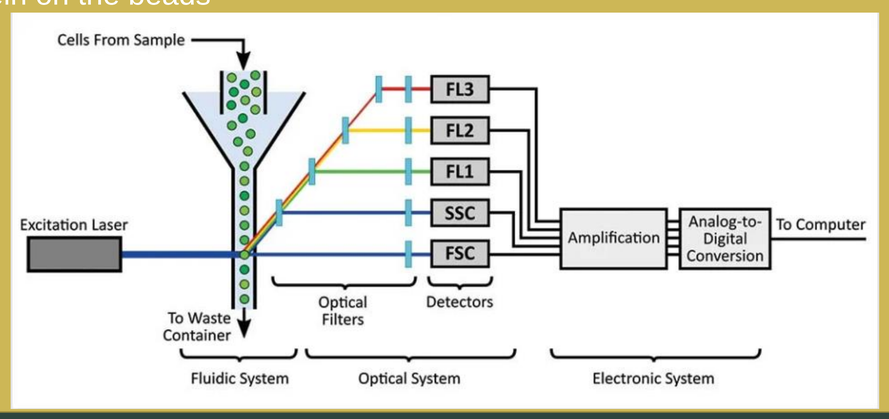
Functionalization process



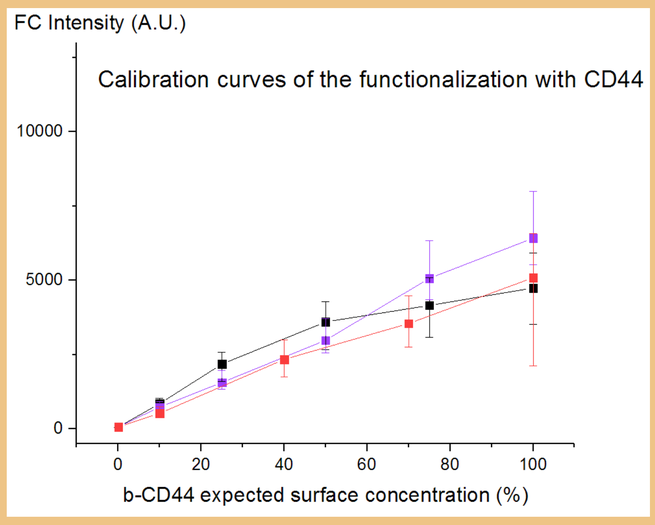
Flow cytometry

Fluorescent antibodies targeting our protein of interest are used in order to measure quantitatively its surface density on the beads with a flow cytometer.

→ The intensity we measure is proportional to the surface density of the protein on the beads



Functionalization with CD44



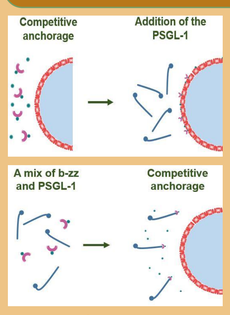
- Same shape between the curves
- Values between experiments are quite same

→ Reasonably well reproducible

Functionalization with PSGL-1

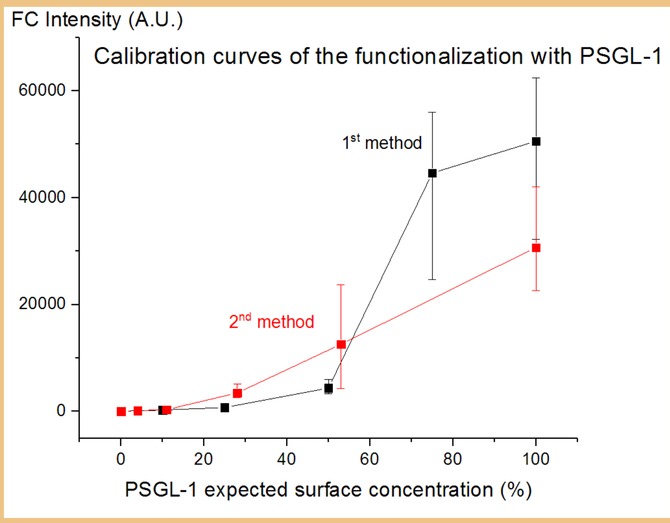
1st METHOD

2nd METHOD

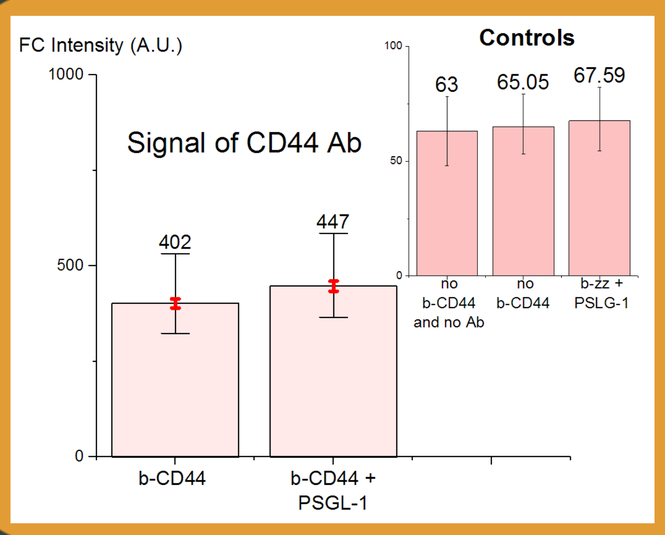


- At low concentration, reasonably same values
→ Reproducible

→ 2nd method kept: more effective and precise despite less signal at high concentration

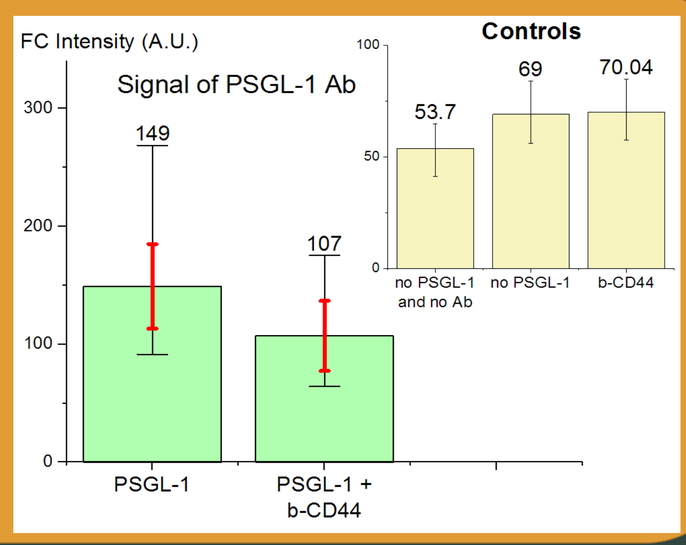


Double functionalization



- Functionalization at 10%
- Negligible non-specific interactions between the protein and the opposite antibody
- Sensibly same values between single and double functionalization

→ The control of two different proteins on the beads is a success



Conclusion

- Single functionalization of each protein permits good and reproducible values of calibration
- Double functionalization is well controlled

Publication

Kirichuk, O., Srimasorn, S., Zhang, X., Roberts, A. R., Coche-Guerente, L., Kwok, J. C., ... & Richter, R. P. (2023). Competitive specific anchorage of molecules onto surfaces: quantitative control of grafting densities and contamination by free anchors. *bioRxiv*, 2023-06.