



Study of interactions between aptamers/antibodies and bacteria

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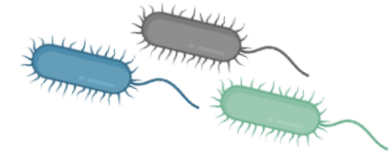
25 June 2024



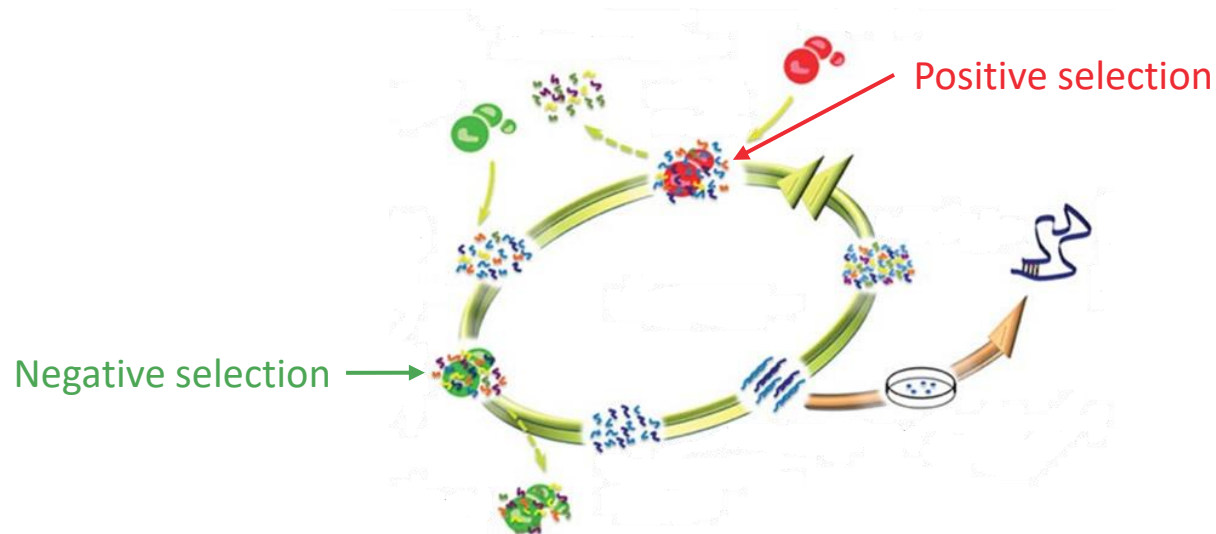
Aptamers



- Aptamers can be directed against a variety of molecular or cellular targets with high specificity and selectivity
- Number of **aptamers** have been developed to target various **bacteria strains** (*E. coli*, *S. aureus*, *M. Tuberculosis*...)



From Web of Science : 2000-2010 : 69 / 2010-2020 : 585 / 2020-2024 : 611



Determination of K_d using binding saturation curve



S : Substrate
L : Ligand
SL : Complex

Simple model assuming that all ligands bind the interaction sites independantly with the same K_d

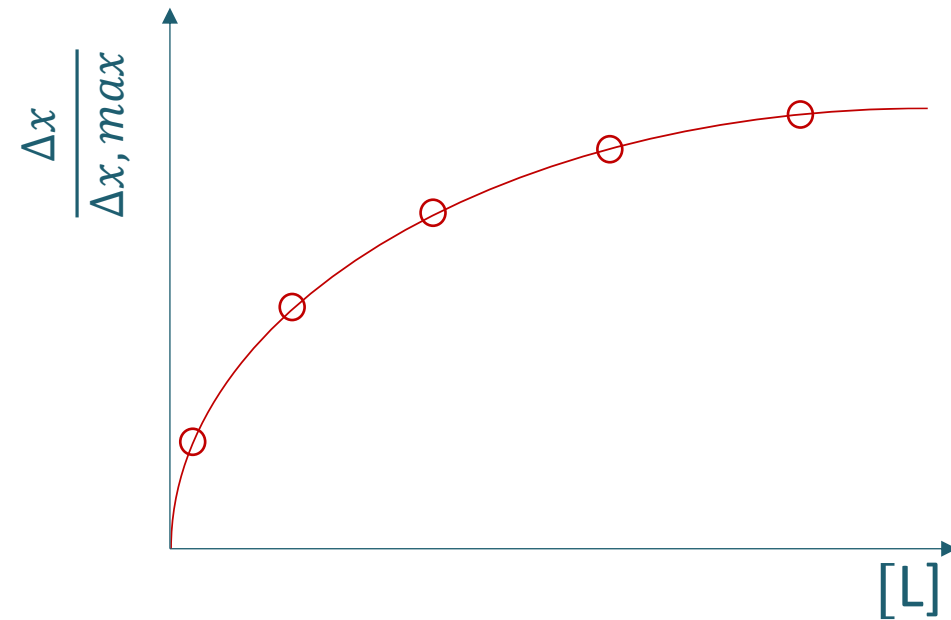
$$K_d = \frac{[S][L]}{[SL]} \Rightarrow \frac{n_{SL}}{n_{S,0}} = \frac{[L]}{K_d + [L]} = \frac{\Delta x}{\Delta x, \max}$$

Δx = signal change $\propto n_{SL}$

$\Delta x, \max$ = maximum change of the signal $\propto n_{S,0}$

$n_{S,0}$ = number of binding site

n_{SL} = number of bound ligand



Signal: Fluorescence, anisotropy, absorbance, radioactivity⁽²⁾.....

Determination of interaction parameters (k, n) using the adsorption isotherm



S : Substrate
L : Ligand
SL : Complex

Multisite model of independant energies ^(3,4)

$$\bar{n} = \frac{[L]_T - [L]_f}{[S]_T} = \sum_i n_i \frac{k_i [L]}{1 + k_i [L]}$$

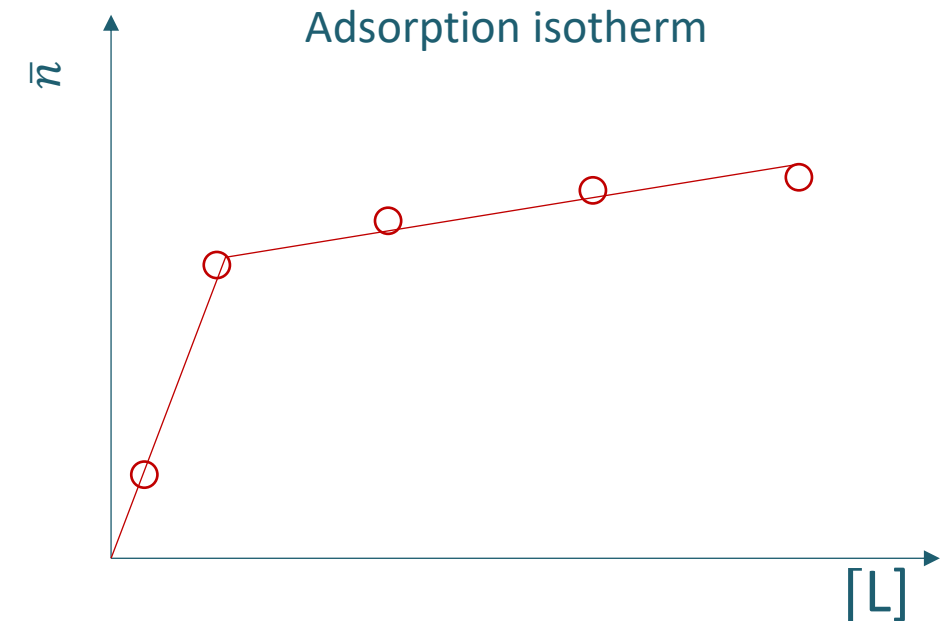
n_i = number of independant sites having the same binding constant k_i

\bar{n} = average number of bound ligand/substrat

$[L]_T$ = total ligand concentration

$[S]_T$ = total substrate concentration

$[L]_f$ = total free ligand concentration



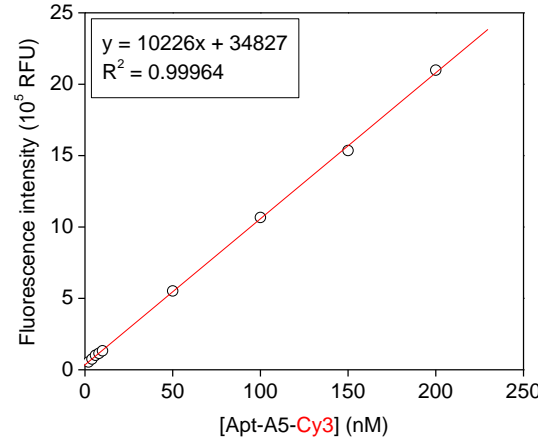
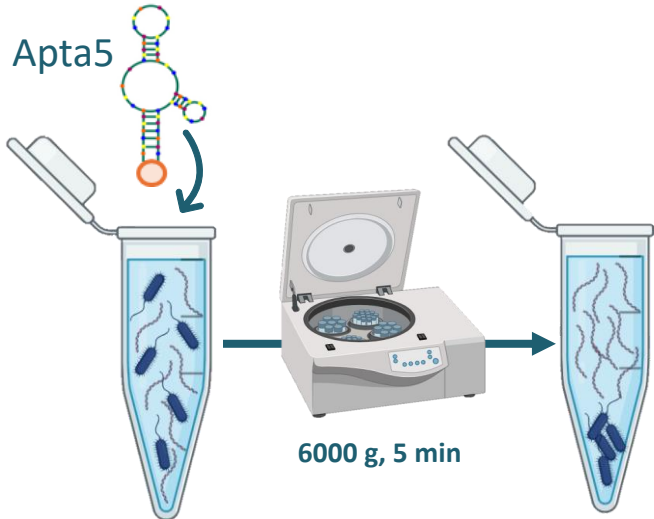
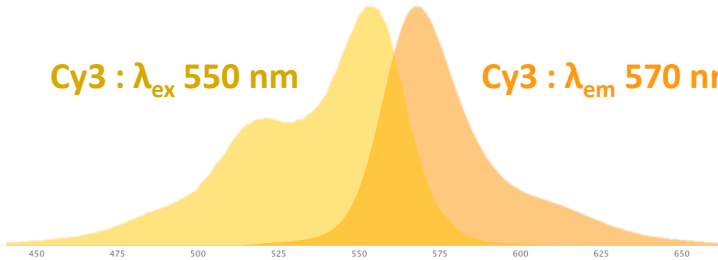
$[L]_f \longrightarrow$ Centrifugation, filtration, HPLC, CE

Quantification of the free ligand $[L]_f$ by fluorescence spectroscopy

Apta5: anti-*E. coli*⁴ : 5' - **Cy3** - TGA-GCC-CAA-GCC-CTG-GTA-TGC-GGA-TAA-CGA-GGT-ATT-CAC-GAC-TGG-TCG-TCA-GGT-ATG-GTT-GGC-AGG-TCT-ACT-TTG-GGA-TC 3'

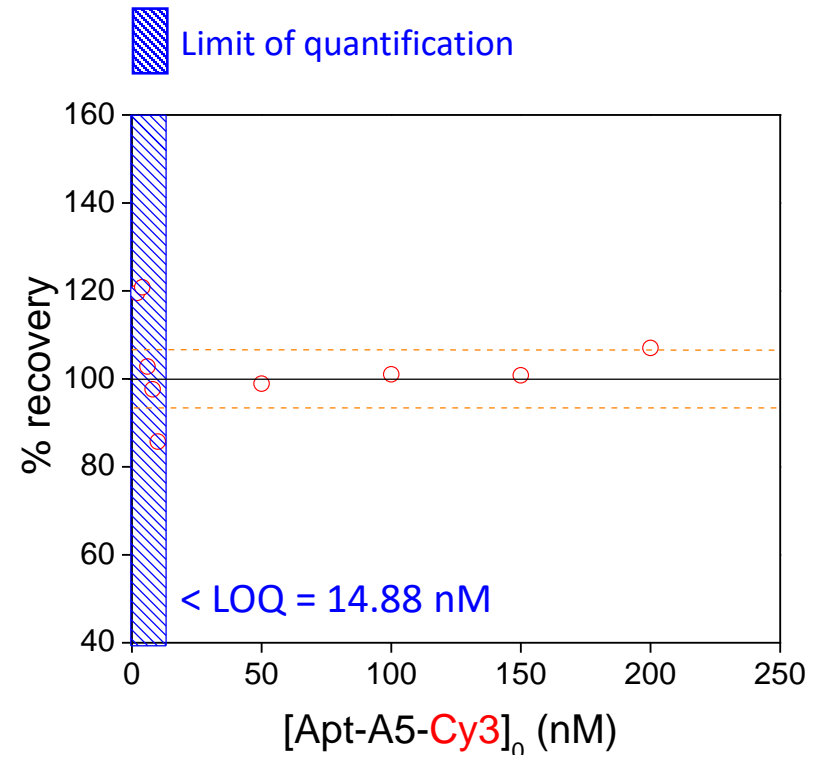
Cy3 : λ_{ex} 550 nm

Cy3 : λ_{em} 570 nm



The calibration curve was used
to quantify [Apta5] in the supernatant

$$\% \text{ recovered} = 100 \times \frac{[\text{Apta5}]_{\text{recovered}}}{[\text{Apta5}]_0}$$

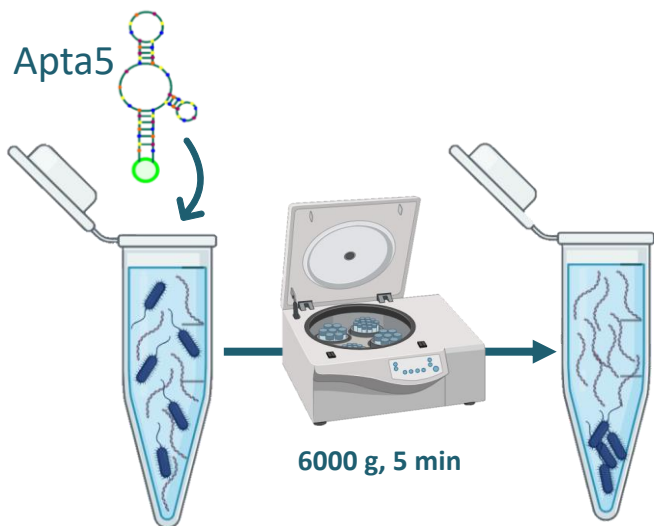
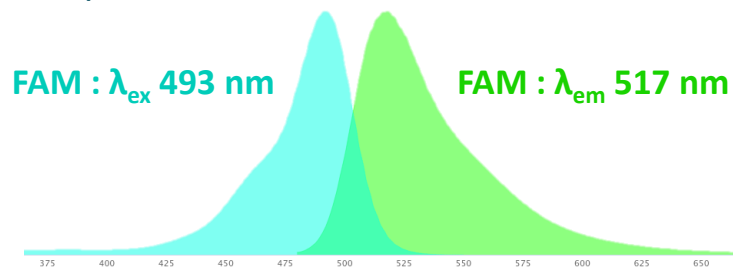


Buffer : Tris, MgCl₂, NaCl, KCl, HCl (50/1/100/5/45 mM), pH = 7,5

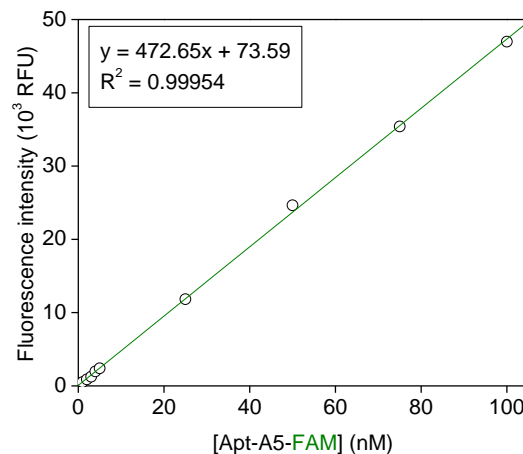
❖ Beyond the limit of quantification of the method, the quantities of aptamer initially introduced into the medium were entirely recovered in the supernatant

Quantification of the free ligand $[L]_f$ by fluorescence spectroscopy

Apta5: anti-*E. coli*⁴ : 5' FAM - TGA-GCC-CAA-GCC-CTG-GTA-TGC-GGA-TAA-CGA-GGT-ATT-CAC-GAC-TGG-TCG-TCA-GGT-ATG-GTT-GGC-AGG-TCT-ACT-TTG-GGA-TC 3'

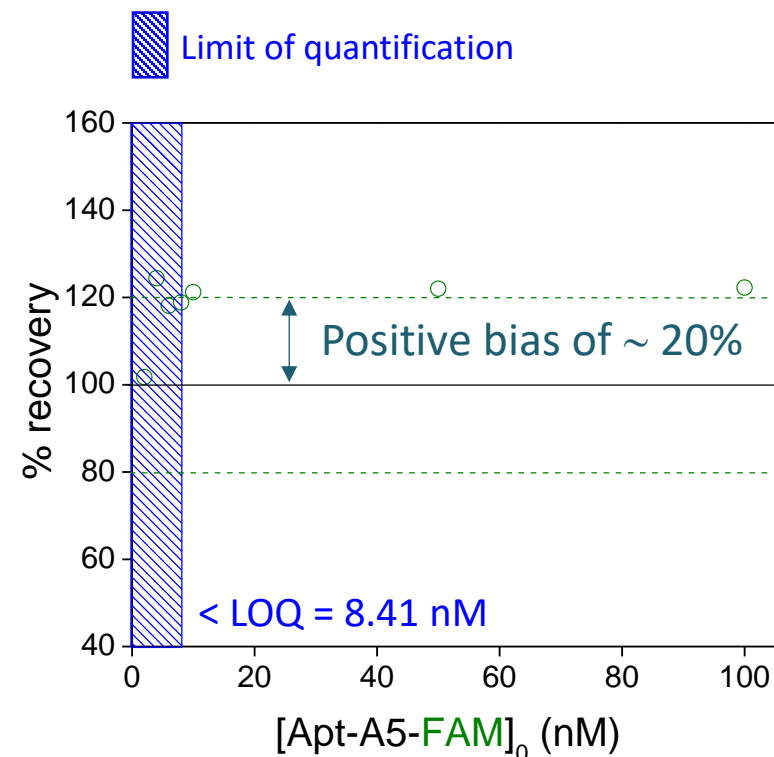


Buffer : Tris, $MgCl_2$, NaCl, KCl, HCl (50/1/100/5/45 mM), pH = 7,5



The calibration curve was used
to quantify [Apta5] in the supernatant

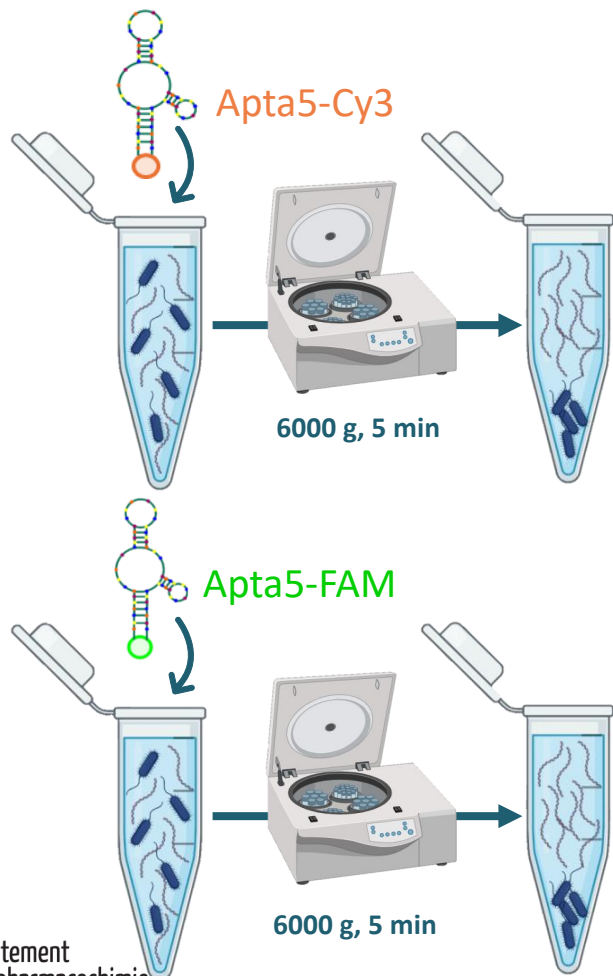
$$\% \text{ recovered} = 100 \times \frac{[\text{Apta5}]_{\text{recovered}}}{[\text{Apta5}]_0}$$



❖ Using a green excitation wavelength, a positive bias of ~ 20% is systematically observed

Quantification of the free ligand $[L]_f$ by fluorescence spectroscopy

- ❖ The free ligand concentration was found to be not significantly different from its initial concentration



1/ The pellet is not equilibrium

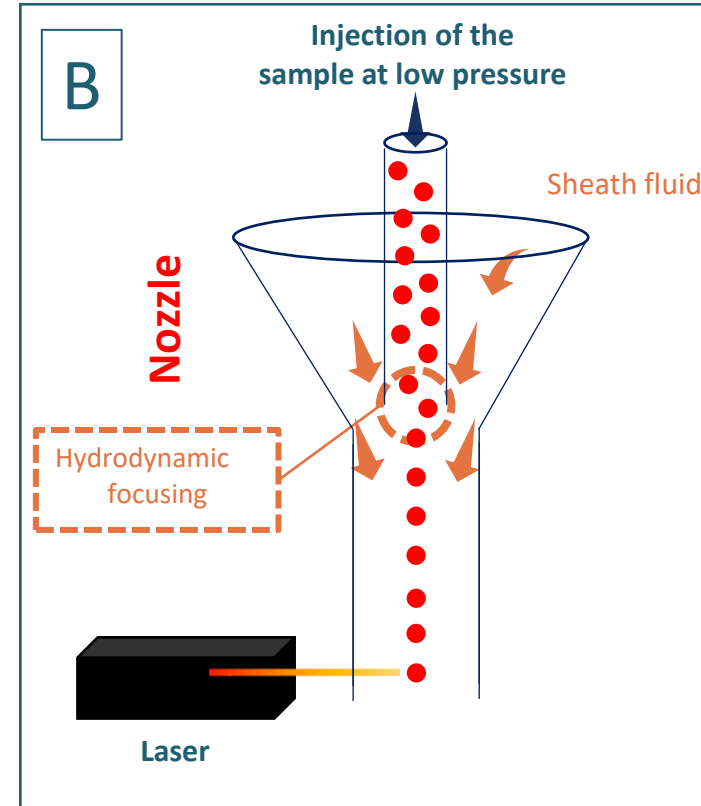
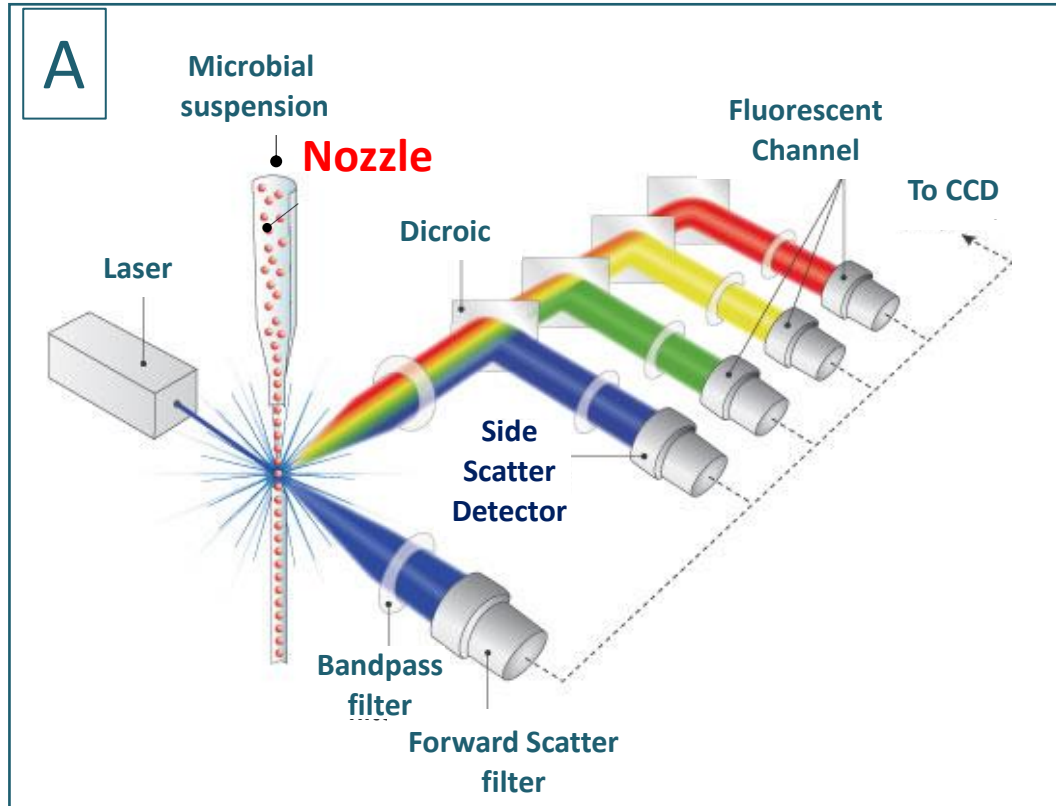
- Low affinity interaction ($K_d \sim 1 \text{ mM}$)
- High dissociation rate constant ($k > 1 \text{ s}^{-1}$)

2/ Interaction is very high, but the number of binding sites is low

- At 10^8 cell/mL , if 10,000 aptamers bind to each bacterium, the total amount of the adsorbed ligand will be as low as 1.7 nM.

3/ Release of the intracellular contents of some bacteria

Study of interactions, without disturbing equilibria, using flow cytometry

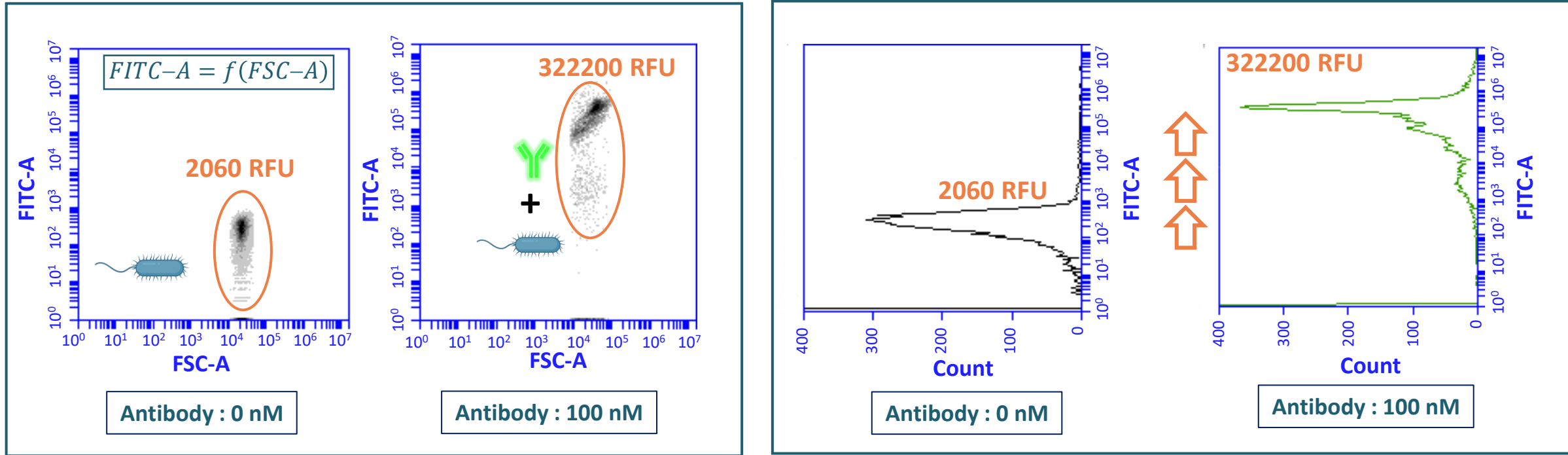


$$\Delta P_{\text{sample}} > \Delta P_{\text{sheath fluid}}$$

- ❖ The aptamer/bacteria complex remains in equilibrium because the ligand (free aptamer) is also present in the system during the the bacteria mobilisation

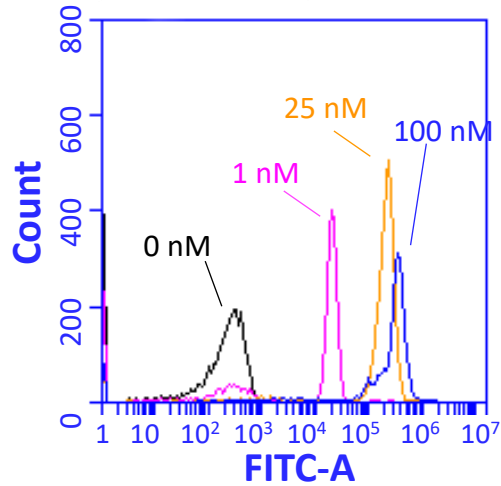
Study of interactions, without disturbing equilibria, using flow cytometry

❖ Validation of the methodology using a FITC-labelled anti-*E. coli* polyclonal antibody



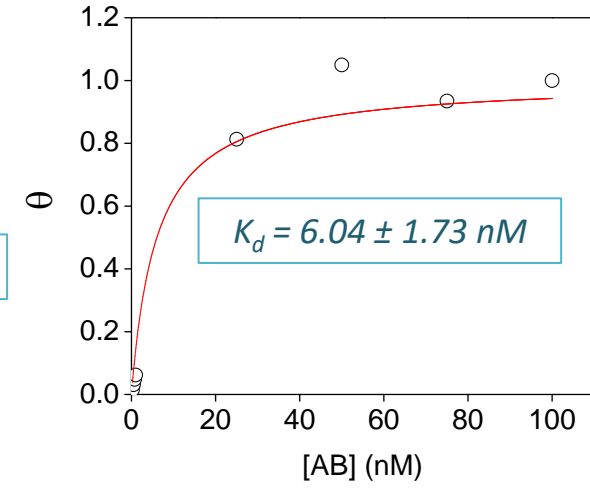
Experimental conditions: *E. coli* ATCC 700728 at 10⁴ CFU/mL diluted in PBS (Phosphate buffered saline, pH 7.4). *E. coli* serotype O/K Polyclonal Antibody was provided by Invitrogen.

Study of interactions between (AB)/bacteria using flow cytometry



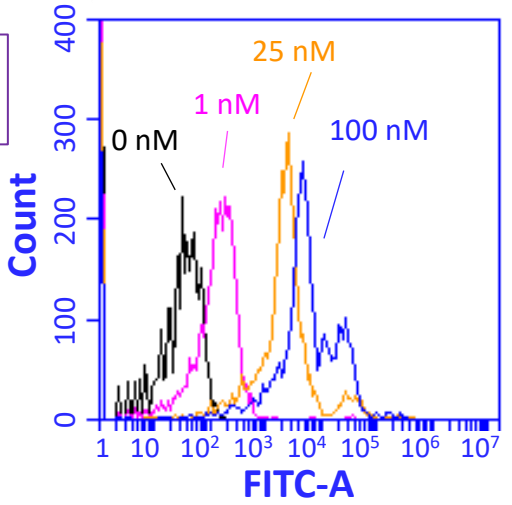
$$\theta = \frac{F - F_0}{F_{\max} - F_0} = \frac{[AB]}{K_d + [AB]}$$

E. coli ATCC 700728 at 10⁴ CFU/mL in PBS



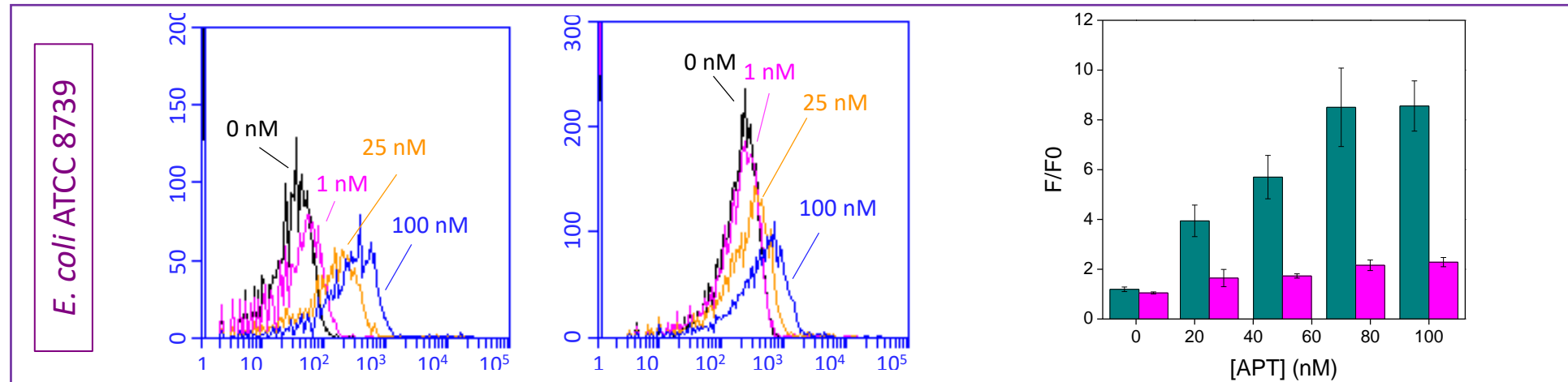
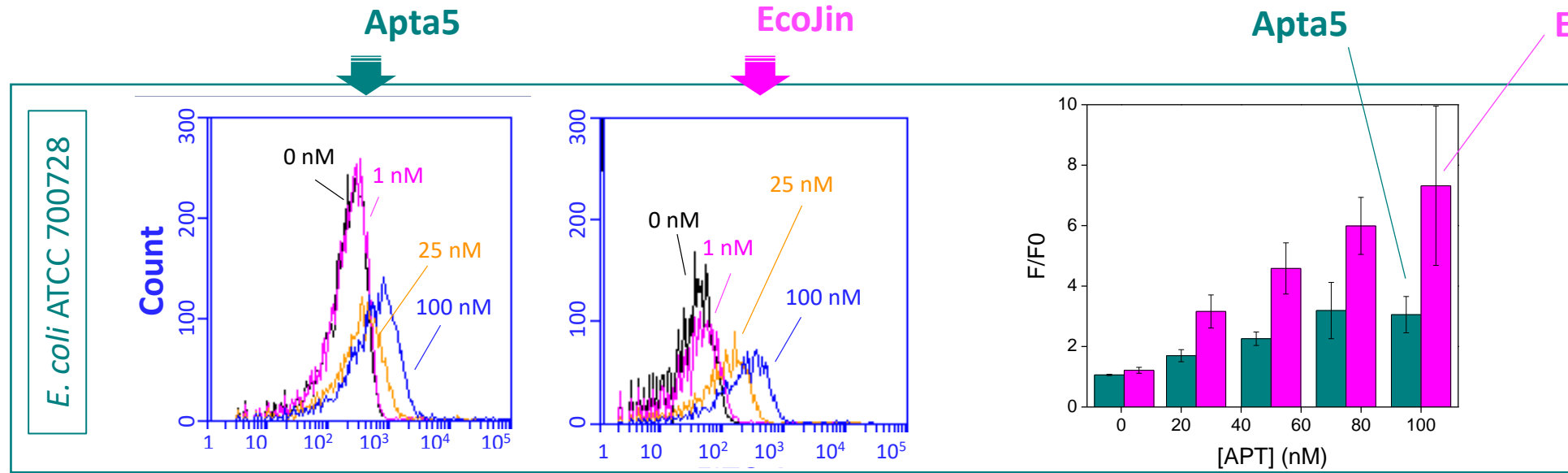
The antibody also interacts with the other *E. coli* strains (ATCC 8739), but to a lesser extent

E. coli ATCC 8739 at 10⁴ CFU/mL in PBS



- ❖ A shift in fluorescence is observed in the presence of antibodies
- ❖ Additional experiments
 - Calculate K_d
 - Study the specificity of the AB against different bacteria strains

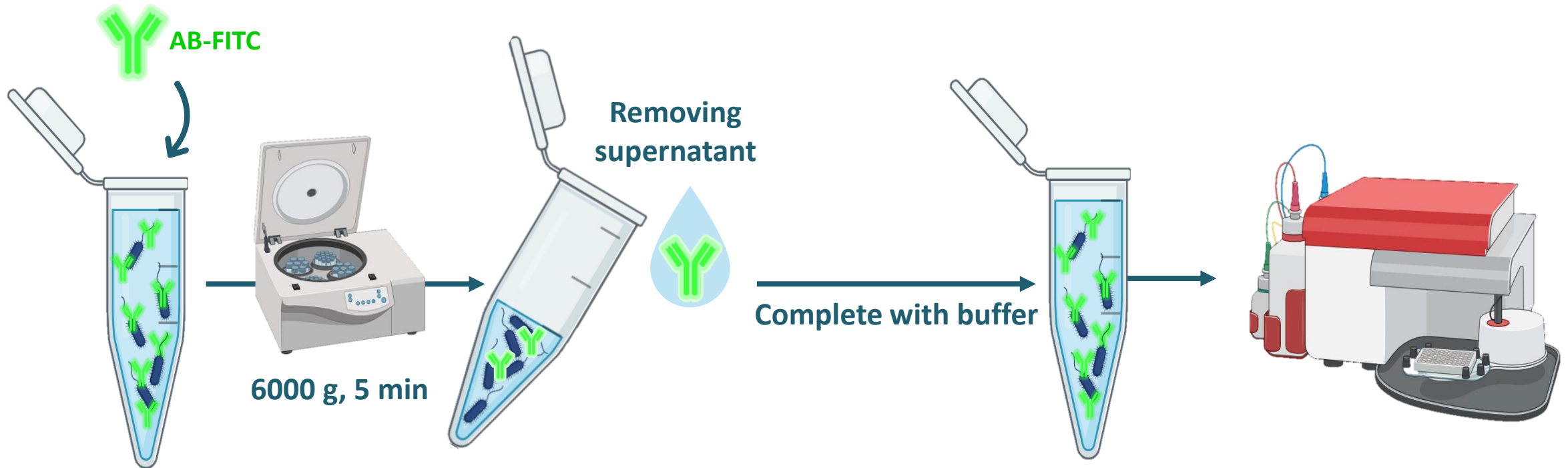
Study of interactions between aptamers/bacteria using flow cytometry



❖ Ratio F/F_0 provides no information on affinities

- Study of interactions, under non equilibrium conditions, by flow cytometry

- ❖ Purification process to remove free ligand – non equilibrium conditions

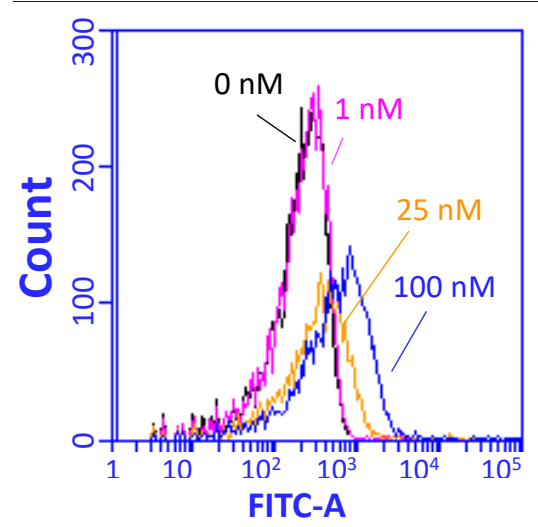


Study of interactions, under non equilibrium conditions, by flow cytometry

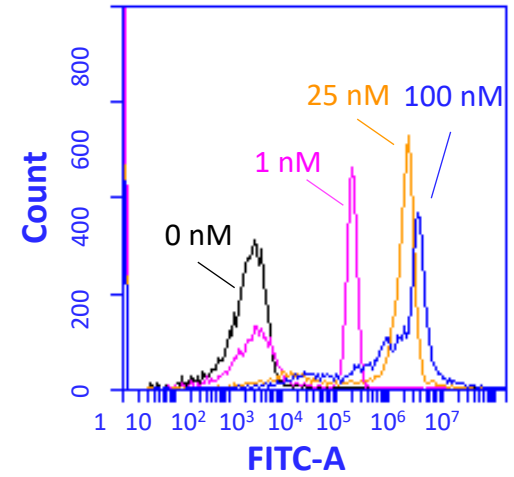
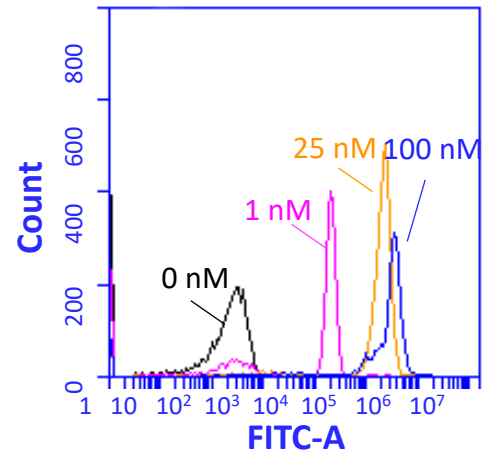
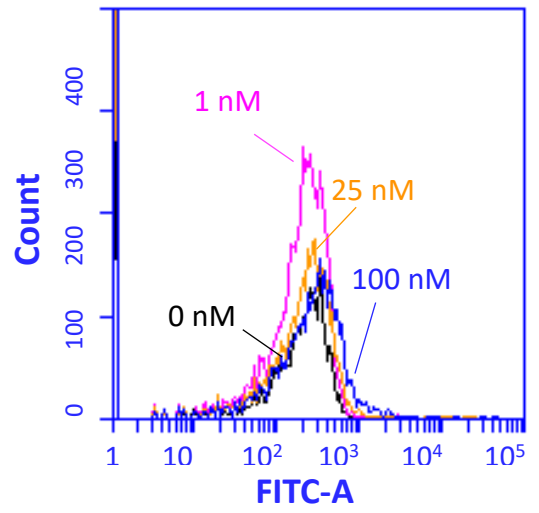
Apta5 + *E. coli* ATCC 700728

AB + *E. coli* ATCC 700728

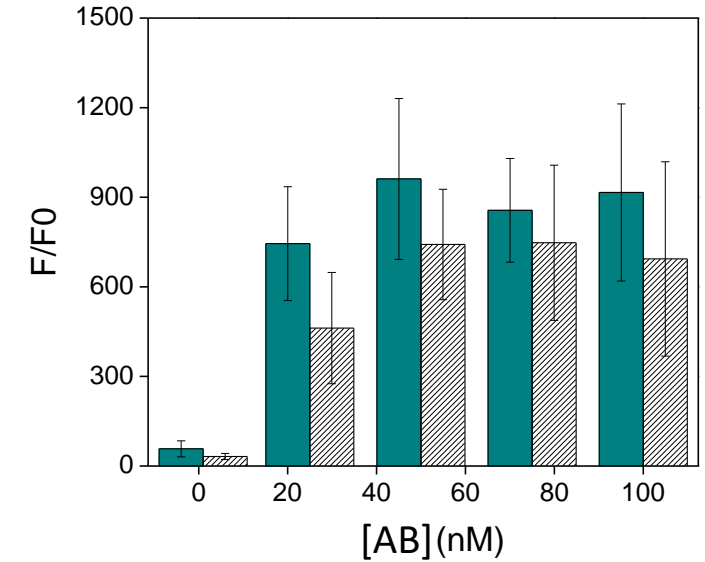
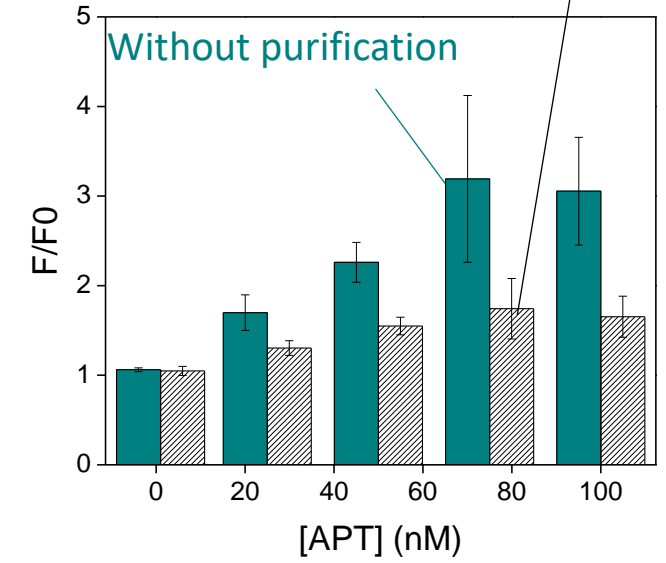
Under equilibrium



Under non equilibrium conditions



After purification



Conclusion & perspectives

Fluorescence spectroscopy cannot quantify free ligand concentration

Study of interactions between biomolecules and bacteria using flow cytometry is more appropriate

- The maximum number of binding sites on bacteria is not very high to differentiate the free ligand concentration from the initial concentration.
- Release of the intracellular contents can interfere with the results when a green fluorescent wavelength is used.

- Aptamers & antibody interact with the two *E. coli* strains tested
- Ratio $IF/IF_0 \longrightarrow$ no information on affinities
Only on average number of bound ligand/substrat
- No disruption of complexes is observed by separating the AB from *E. coli* ATCC 700728 by centrifugation
 \longrightarrow strong interaction
- In the concentration range studied, the number of AB at the bacteria surface is at least 50 times higher than that of aptamer sequence studied

Additional experiments are required to calculate K_d and to study the specificity

Acknowledgments



Aurélie Hennebique



Véronique BLANC-MARQUIS
& all NOVA team

