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Studies of interactions between aptamers/antibodies and bacteria by flow cytometry

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Introduction

This work focuses on the **interaction between aptamers or antibodies and bacteria**. It is part of an ambitious project aiming to produce a **nanovector** containing **antimicrobial peptides (AMPs)** and decorated with aptamers. AMPs will be enclosed within **mesoporous silica nanoparticles** by utilizing the complementary sequence of the aptamer to "lock" the pores. Subsequently, the aptamers will specifically **recognize the target bacteria**, leading to a stronger affinity between the aptamer and the bacteria compared to its complementary sequence. This will facilitate the **release of AMPs in large quantities** to prevent the development of **bacterial resistance**. The first objective of this project is to **characterize an aptamer sequence able to bind, specifically a bacterial strain**.

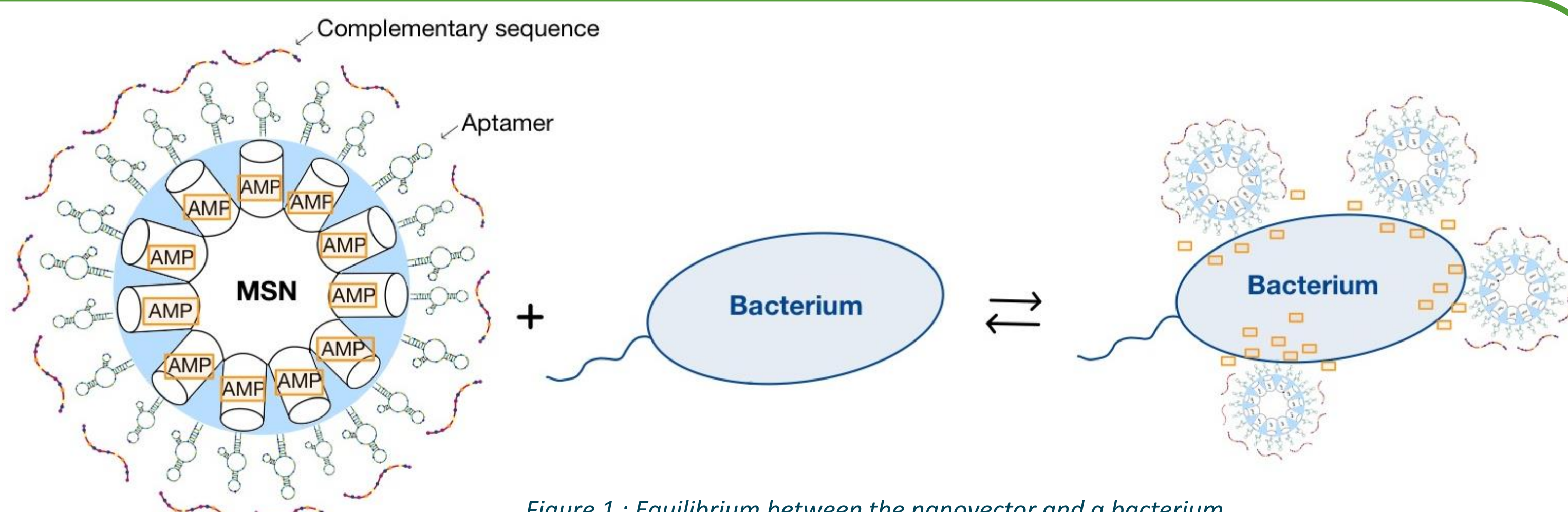


Figure 1 : Equilibrium between the nanovector and a bacterium



Aptamers & Antibodies

Aptamers are **simple DNA or RNA sequences** selected by SELEX procedures. They can specifically recognize a target with a **high affinity and specificity**. Compared to antibodies, aptamers have many advantages. The Table 1 below outlines several benefits associated with aptamers and antibodies¹:

Aptamers	Antibodies
Small size enables an effective entry into biological compartments	Large size prevents renal filtration
Low variability between batches	Not susceptible to nuclease degradation
Non-immunogenic	Technology widely distributed

Table 1 : Advantages of aptamers and antibodies.

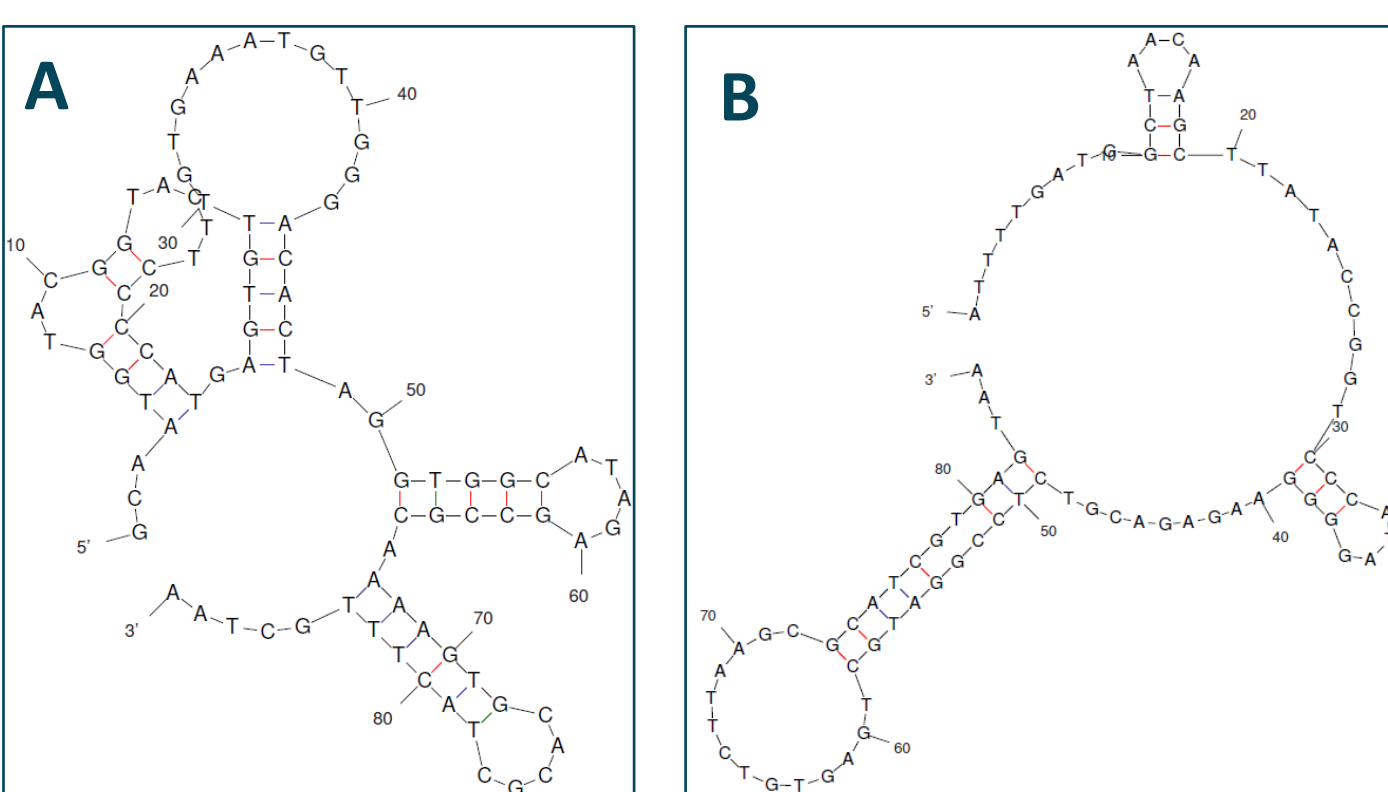
Several aptamer sequences previously isolated by research groups were selected to assess their ability to bind to bacteria, more specifically to *E. coli* strain.²⁻⁴ They were modified by attaching a fluorophore in order to track them using flow cytometry.

Aptamers	Targeted bacteria : <i>E. coli</i>
EcoJin ²	<i>E. coli</i> ATCC 8739
Eco4R ³	<i>E. coli</i> ATCC 8739
Apta1 & Apta5 ⁴	<i>E. coli</i> O157:H7 (ATCC 03820)

Table 2 : Selected aptamers and their targeted bacteria.

The laboratory (DPM) has a strain library with five *E. coli* strains:

- ATCC 700728 (O157:H7)
- ATCC 25922 (O6)
- ATCC 35218
- ATCC 8739
- ATCC 10536



Scrambles (randomized sequences) were designed to assess the specificity of the sequences to *E. coli* strains.

Figure 2 : Secondary structure of EcoJin (A) and EcoJin scramble (B).

Negative controls for specificity: *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Listeria innocua*, *Staphylococcus xylosum*...

Positive control: « Anti-*E. coli* » antibody

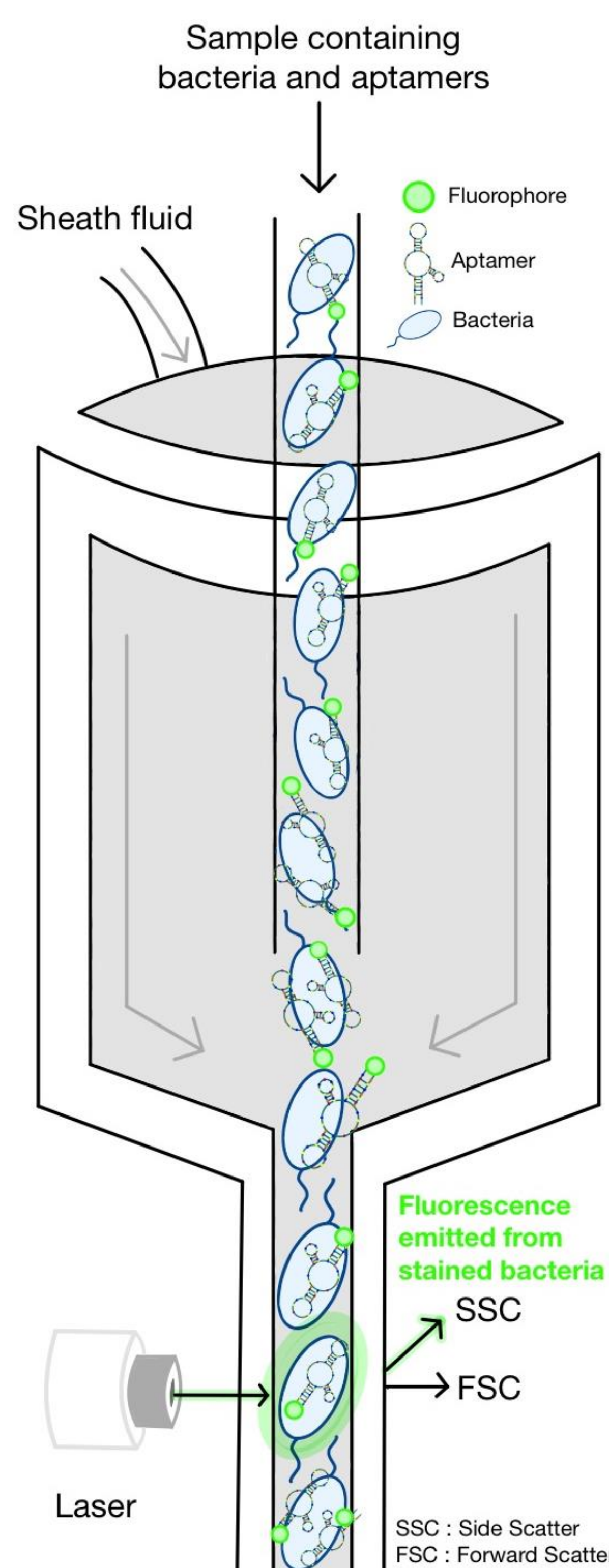


Figure 3 : Use of cytometry to detect the labeling of bacteria using aptamers.

Incubation process

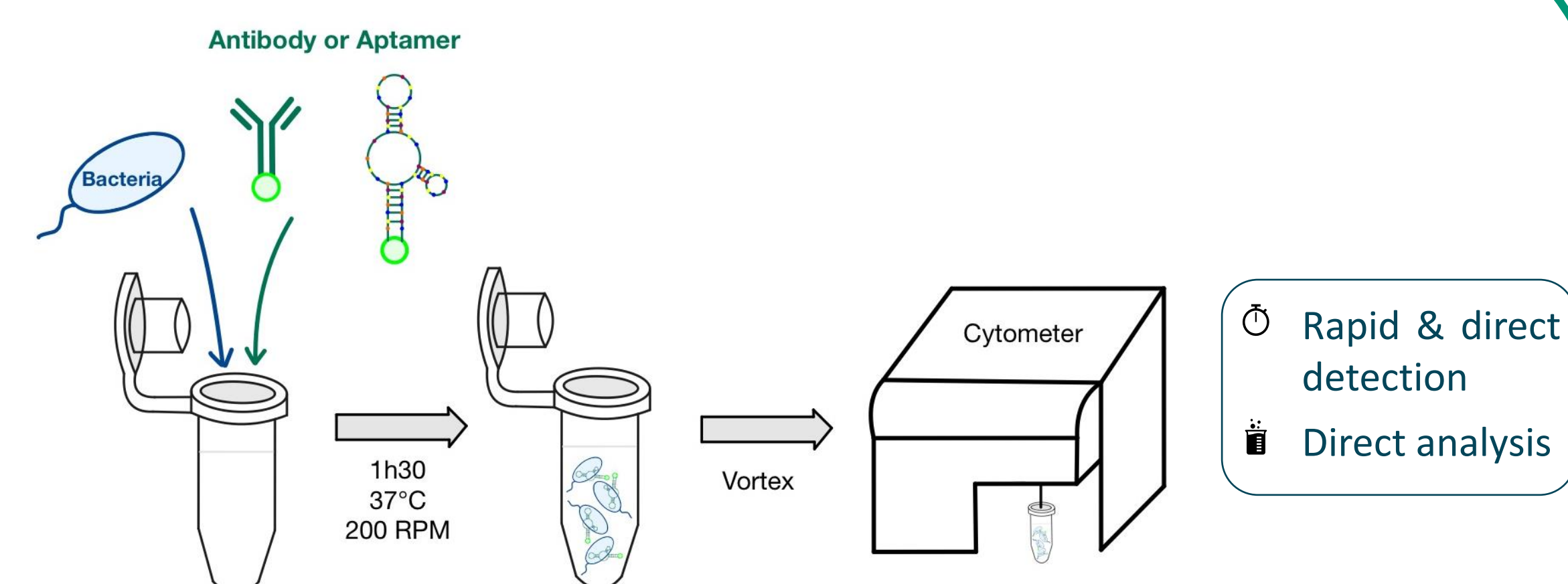


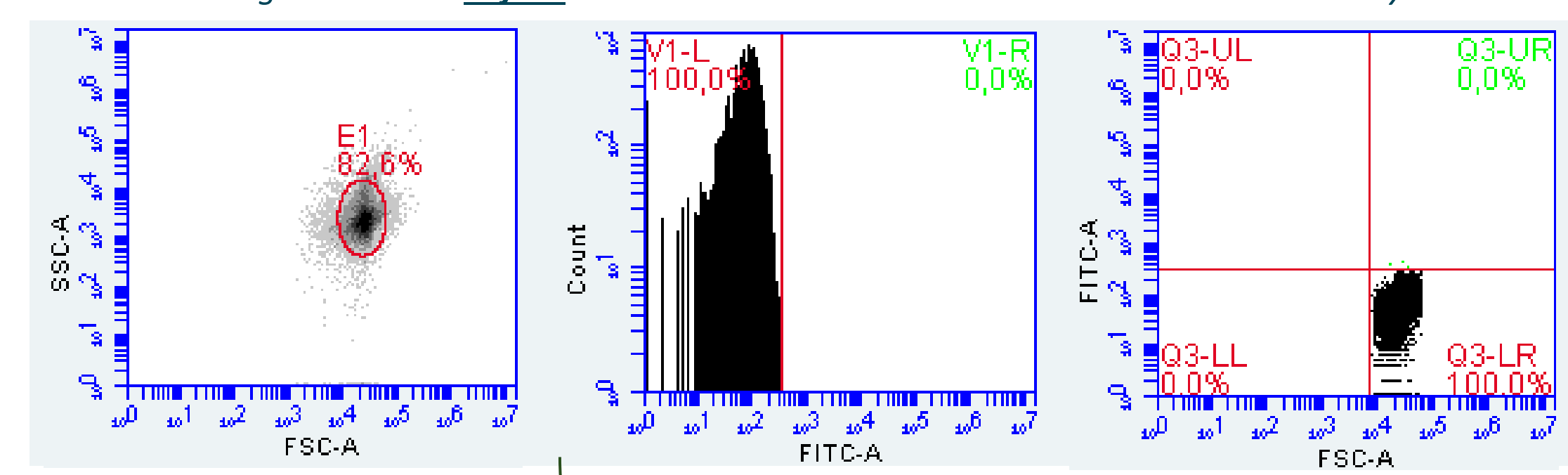
Figure 4 : Method used to observe an interaction between aptamer/antibody and bacteria.

Experimental conditions :

- Fluorophore-tagged aptamers or antibody (0 to 400 nM)
- Bacterial solution containing 10⁶ cfu/mL
- Binding buffer according to literature conditions (e.g., EcoJin : PBS, pH=7.4)

Data analysis

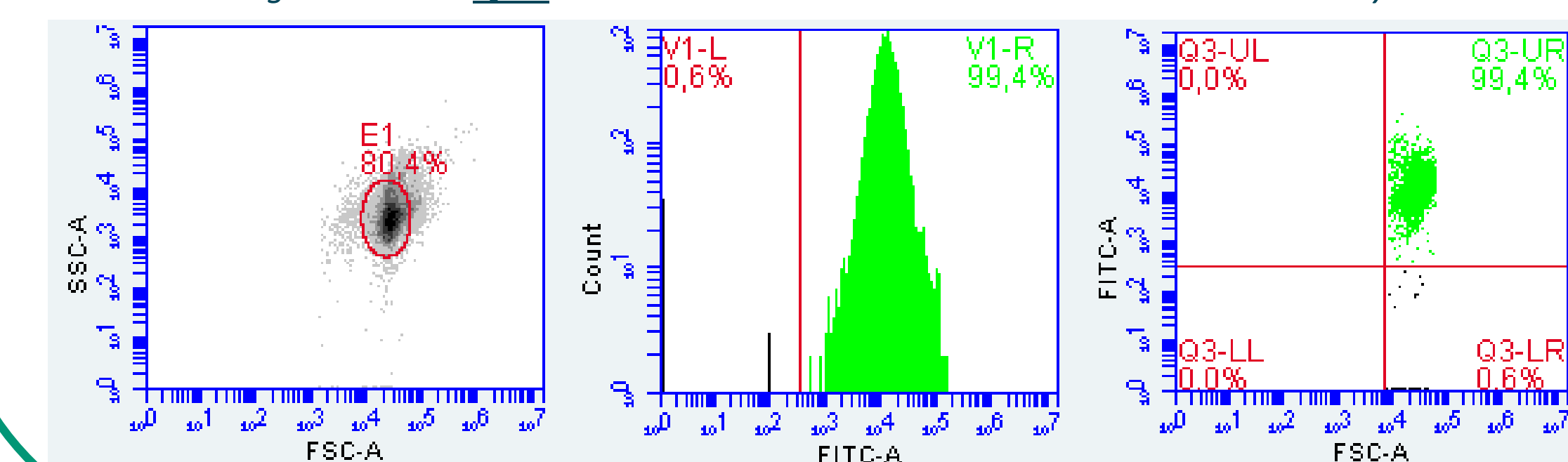
Figure 5 : Plots **before** incubation between *E. coli* ATCC 8739 and the antibody.



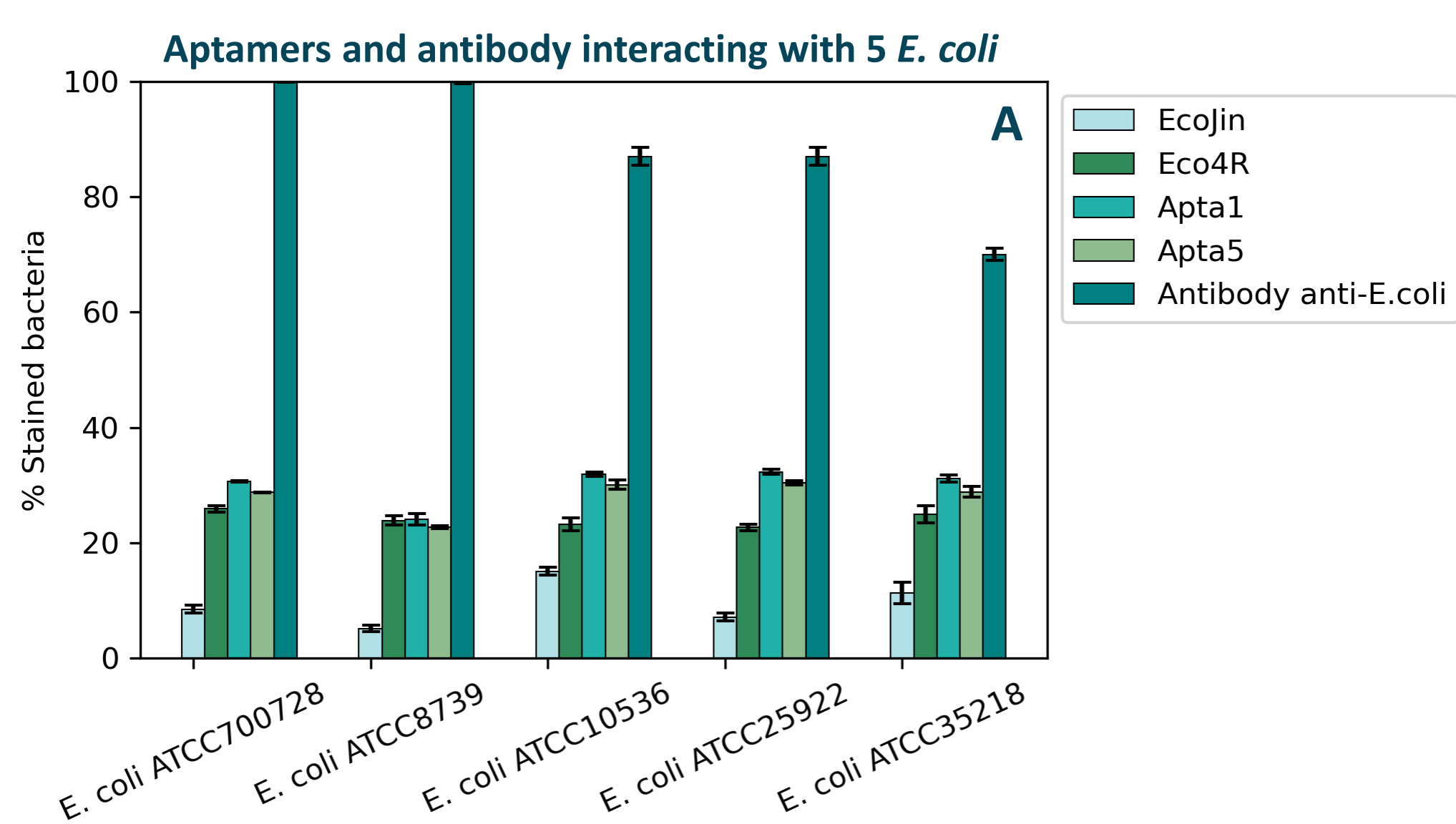
Selection of the population density corresponding to the target bacteria.

Gated plots using the population density to observe the fluorescent gain.

Figure 6 : Plots **after** incubation between *E. coli* ATCC 8739 and the antibody.

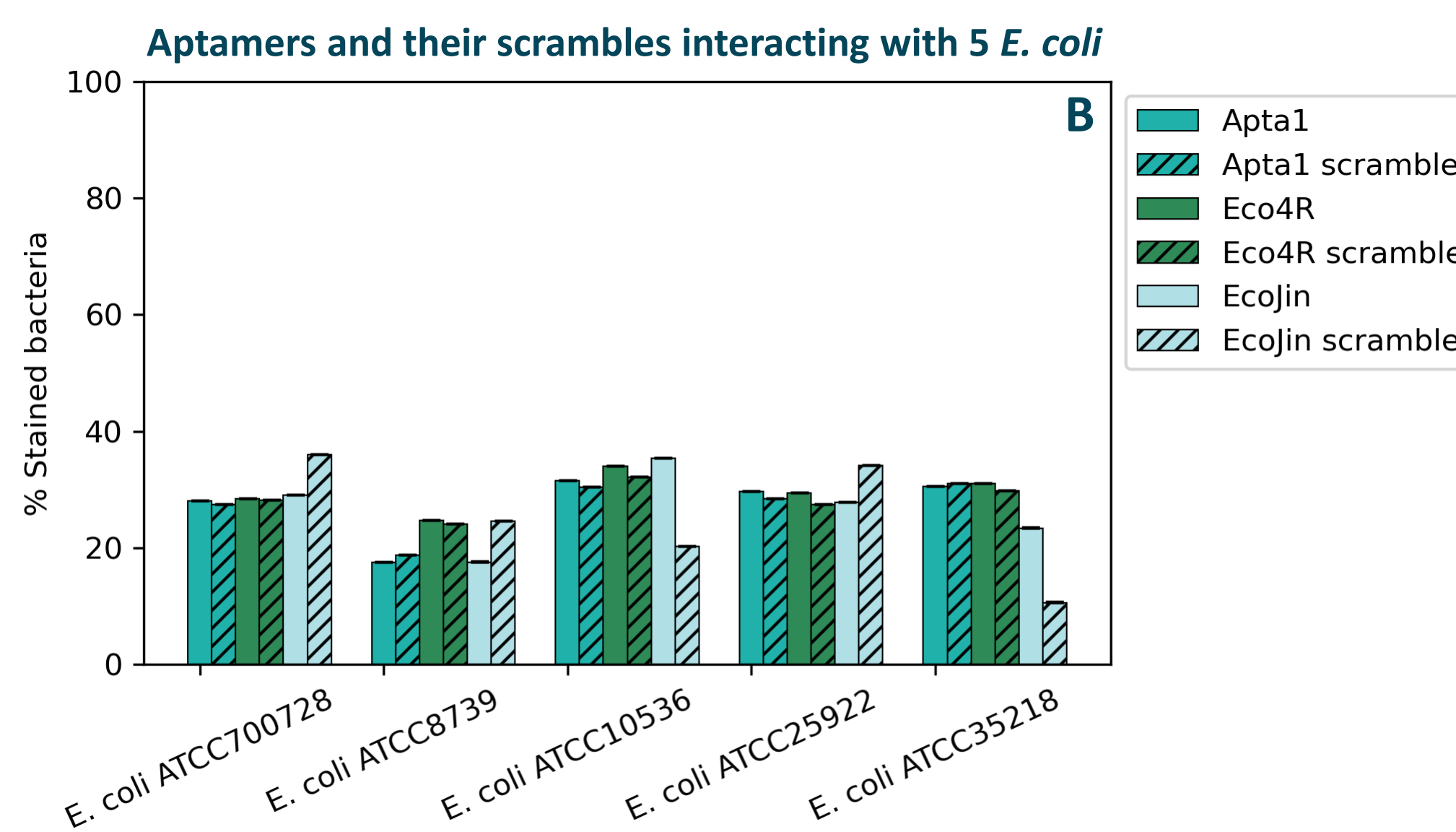


Results

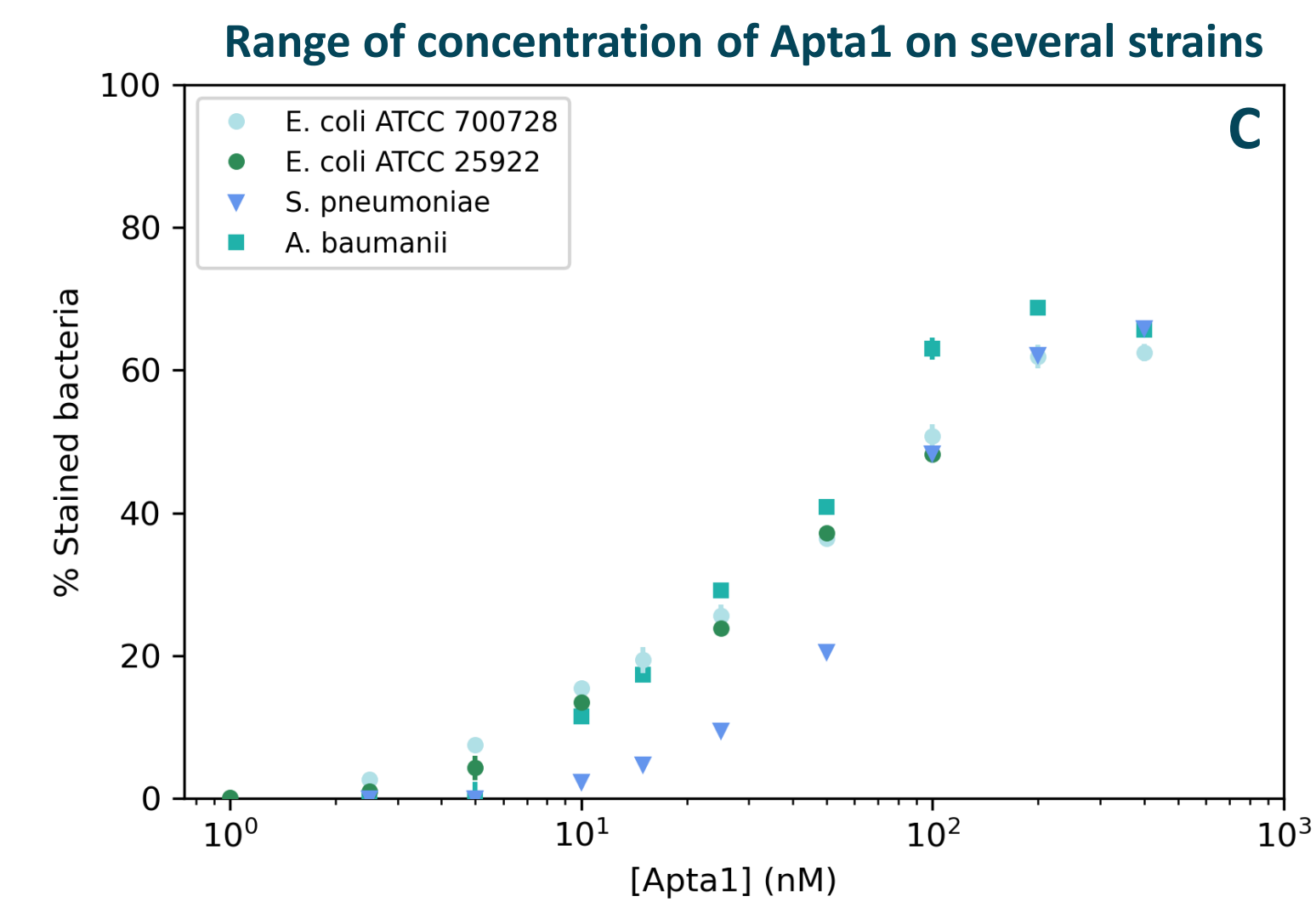


Experimental conditions : (A & B) [Aptamers] = 100 nM, [Bacteria] = 10⁶ cfu/mL, (C) [Apta1] = 0 to 400 nM, [Bacteria] = 10⁴ cfu/mL. Binding buffers : (A) EcoJin = PBS, pH = 7.4 ; Eco4R = Tris/KCl/MgCl₂ (10/500/1 mM) pH = 7.5 to 7.6 ; Apta1 & Apta5 = Tris/KCl/NaCl/MgCl₂ (50/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4 ; (B) Tris/KCl/NaCl/MgCl₂ (50/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4 for all aptamers ; (C) Tris/KCl/NaCl/MgCl₂ (50/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4.

- Percentage of labeled bacteria around **35%** for « Apta1 »
- Antibody « anti-*E. coli* » looks like a nice positive control



- Percentage of labeled bacteria seems to be the **same between aptamers and their scrambles**



- Variable interactions depending on the strain
- Weaker interaction with *S. pneumoniae* compared to the others

Conclusion

Flow cytometry appears to be an effective analytical technique for studying the **interactions between aptamers/antibodies and bacteria**. Every aptamer demonstrates **interaction with *E. coli* bacteria**, and the employed antibody seems to be an **effective positive control**. When testing the specificity of aptamers against its scrambled sequence using the conditions described in the literature, **similar percentages of labeled bacteria were observed**. Further experimentation involved the use of different bacterial strains and increasing concentrations of Apta1. Under these modified conditions, a higher binding rate of **63%** was achieved, compared to the previous conditions where it was **35%**. However, it remains challenging to establish a distinct differentiation window between *E. coli* and the other strains that were tested. Future experiments will be conducted to assess the **specificity of alternative aptamers (EcoJin, Eco4R & Apta5) across different strains, not limited to *E. coli***.

References

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