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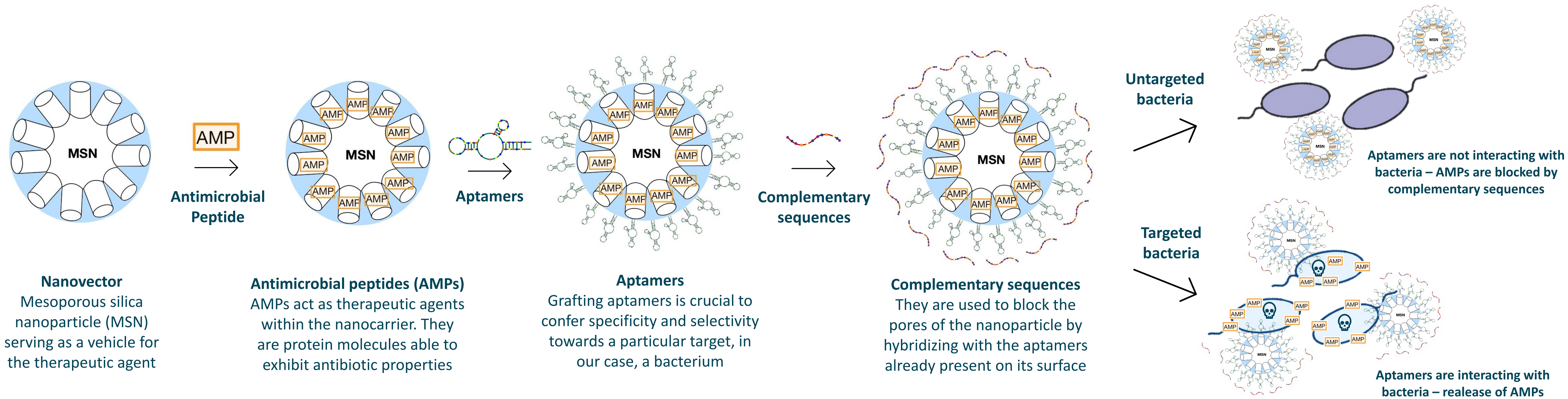
Studies of interactions between aptamers/antibodies and bacteria by flow cytometry

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Develop antimicrobial peptides delivery systems with a high affinity and specificity to target bacterium



Selected aptamers from literature

Fluorophore-tagged aptamers	Targeted bacteria
	<i>E. coli</i> ATCC 8739
	<i>E. coli</i> O157:H7 (ATCC 700728)
	<i>E. coli</i> O157:H7 (ATCC 700728)

Table 1 : Selected aptamers and their targeted bacteria.

Setting up a flow cytometry method to study fluorescent aptamer interaction with bacteria

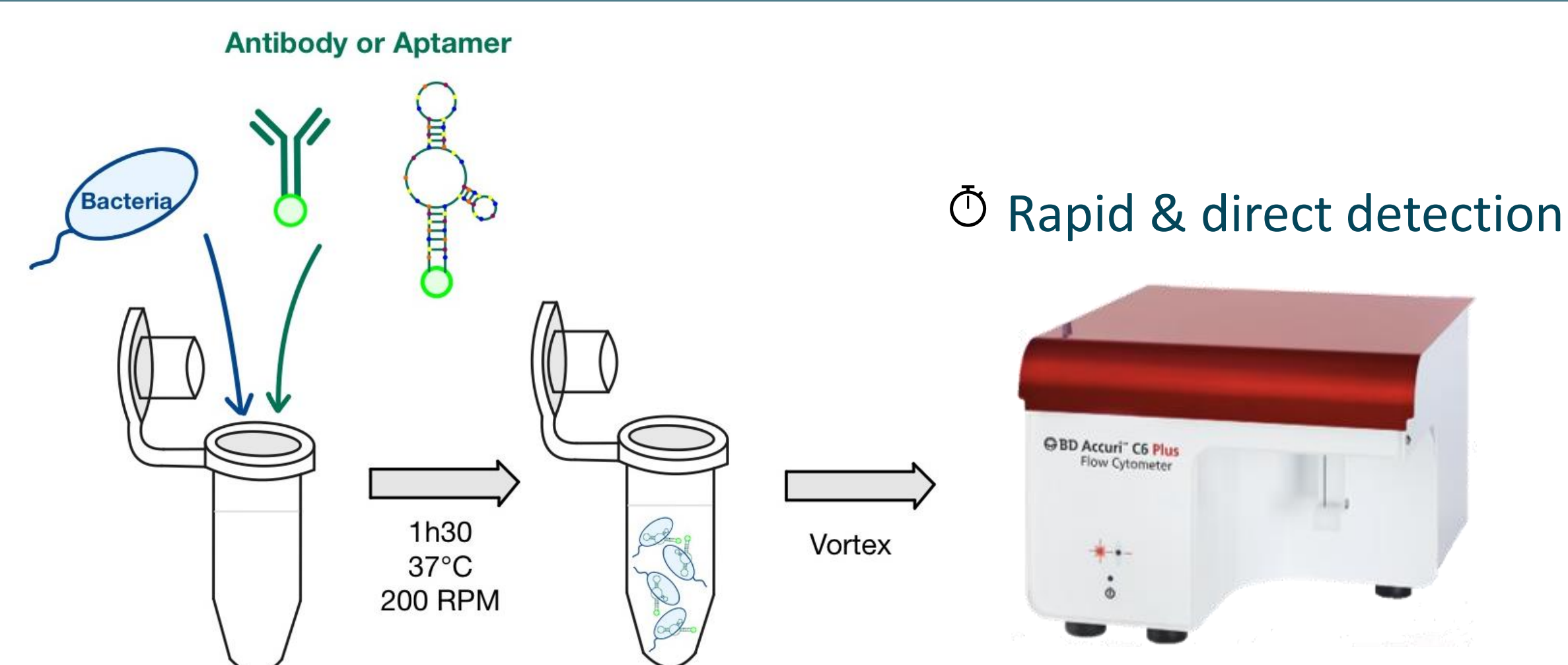


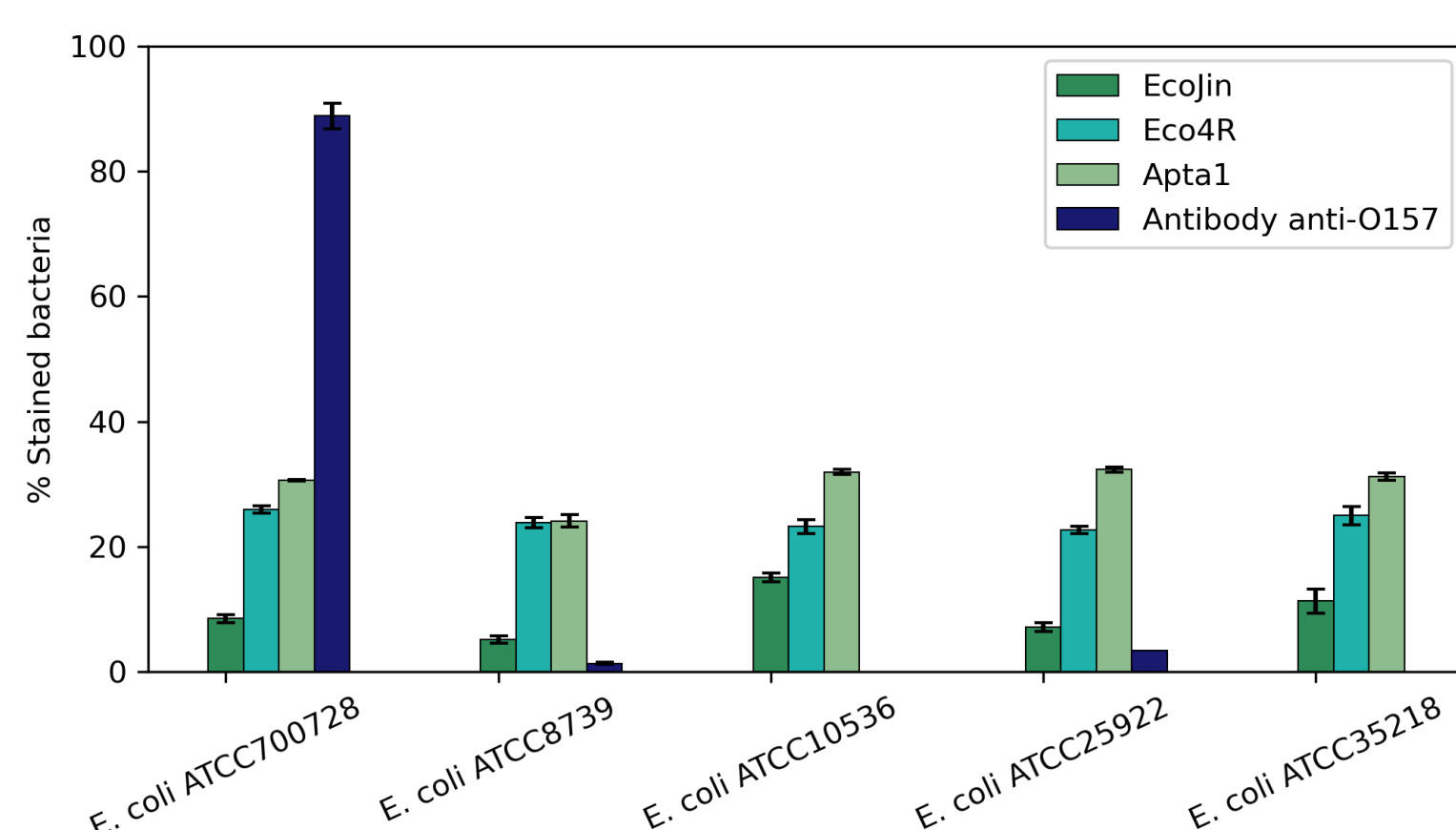
Figure 1 : Method used to observe an interaction between aptamer/antibody and bacteria.

Experimental conditions :

- Fluorophore-tagged aptamers or antibody
- Bacterial solution
- Binding buffer according to literature conditions

Results

Comparative study between aptamers and antibody with 5 *E. coli*

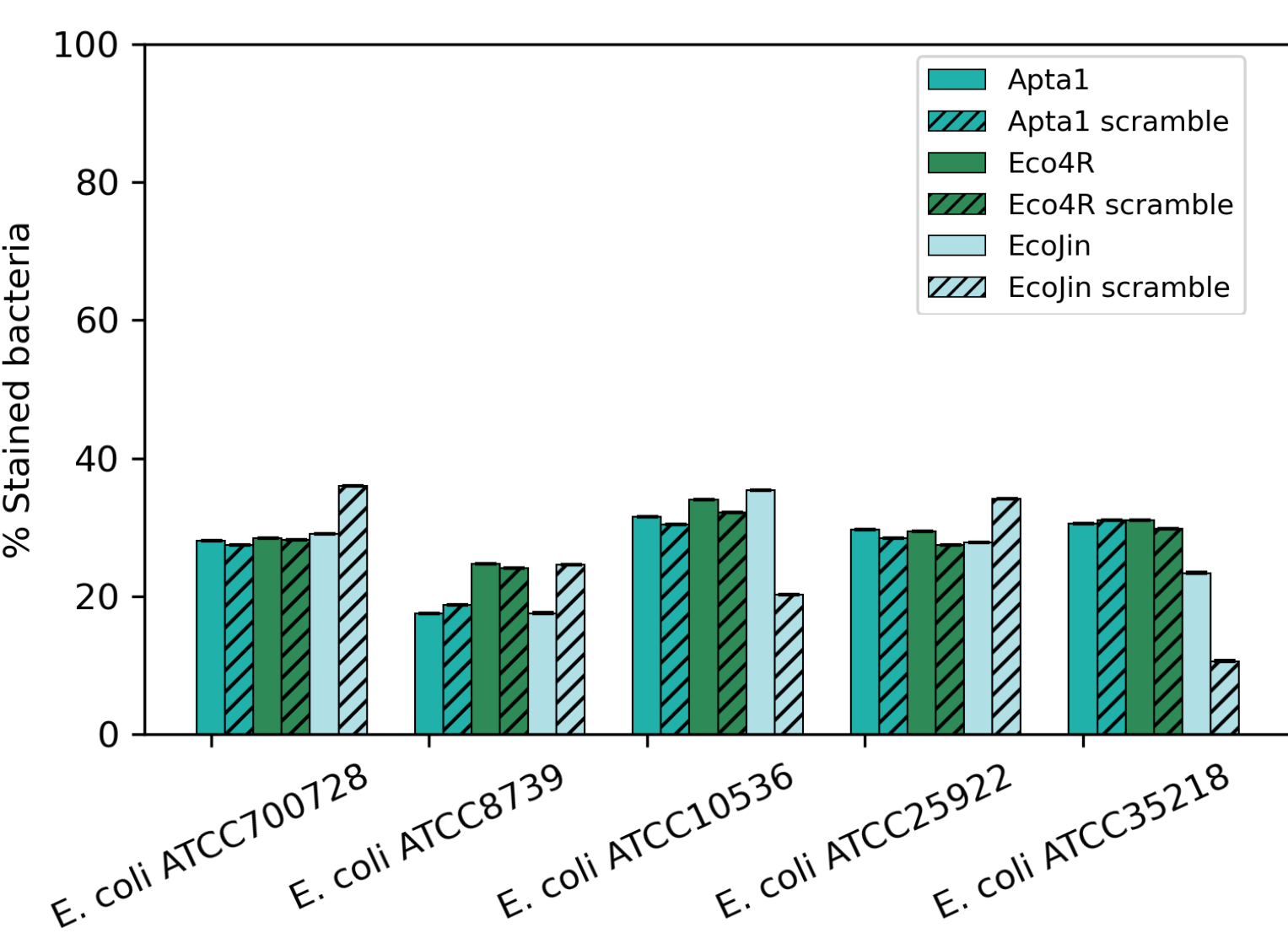


- Percentage of labeled bacteria around 35% for « Apta1 »
- Antibody « anti-O157 » looks like a nice positive control

Experimental conditions:

- [Aptamers] = 100 nM, [Antibody] = 500 pM
- [Bacteria] = 10⁶ cfu/mL (Aptamers) ; 10⁴ cfu/mL (Antibody)
- Binding buffers:
 - EcoJin = PBS 1X, pH = 7.4
 - Eco4R = Tris/HCl/NaCl/MgCl₂.6H₂O (10/9/500/1 mM) pH = 7.5 to 7.6
 - Apta1 = Tris/HCl/KCl/NaCl/MgCl₂.6H₂O (50/42.5/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4

Study of specificity between aptamers and their scrambles with 5 *E. coli*

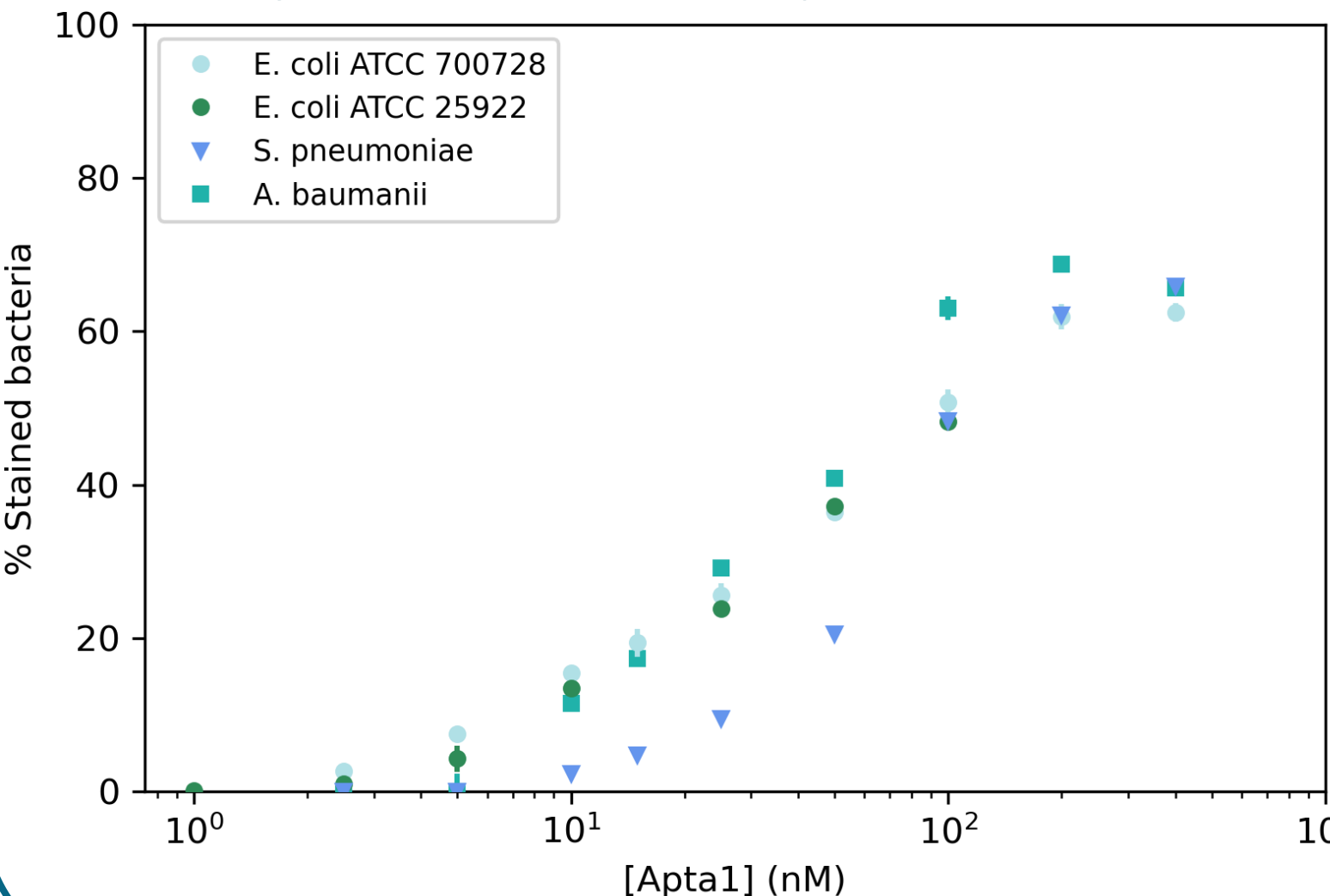


- Scrambles are randomized sequences of selected aptamers
- Percentage of labeled bacteria seems to be the same between aptamers and their scrambles

Experimental conditions:

- [Aptamers] = 100 nM
- [Bacteria] = 10⁶ cfu/mL
- Binding buffer: Tris/HCl/KCl/NaCl/MgCl₂.6H₂O (50/42.5/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4

Study of concentration with Apta1 on several strains

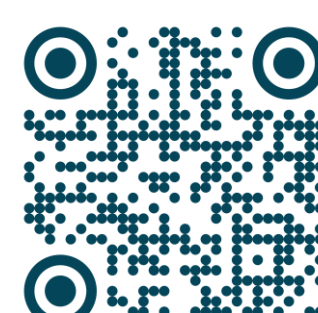


- Variable interactions depending on the strain
- Weaker interaction with *S. pneumoniae* compared to the others

Experimental conditions:

- [Apta1] = 0 to 400 nM
- [Bacteria] = 10⁴ cfu/mL
- Binding buffer: Tris/HCl/KCl/NaCl/MgCl₂.6H₂O (50/42.5/5/100/1 mM), BSA 1 mg/mL, 500 nM yeast tRNA, pH = 7.4

More results on Pauline CHEVALIER's poster

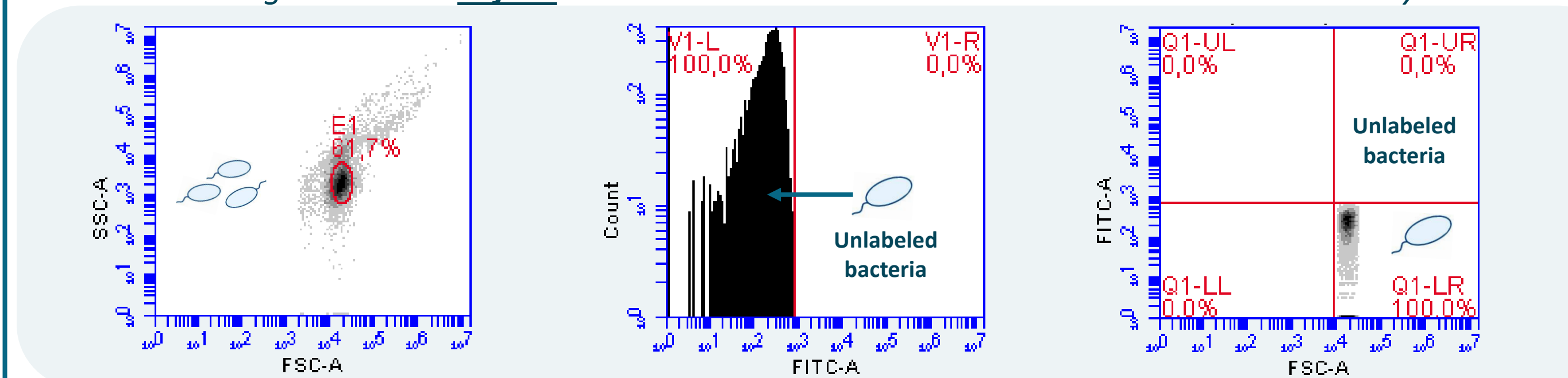


Data analysis



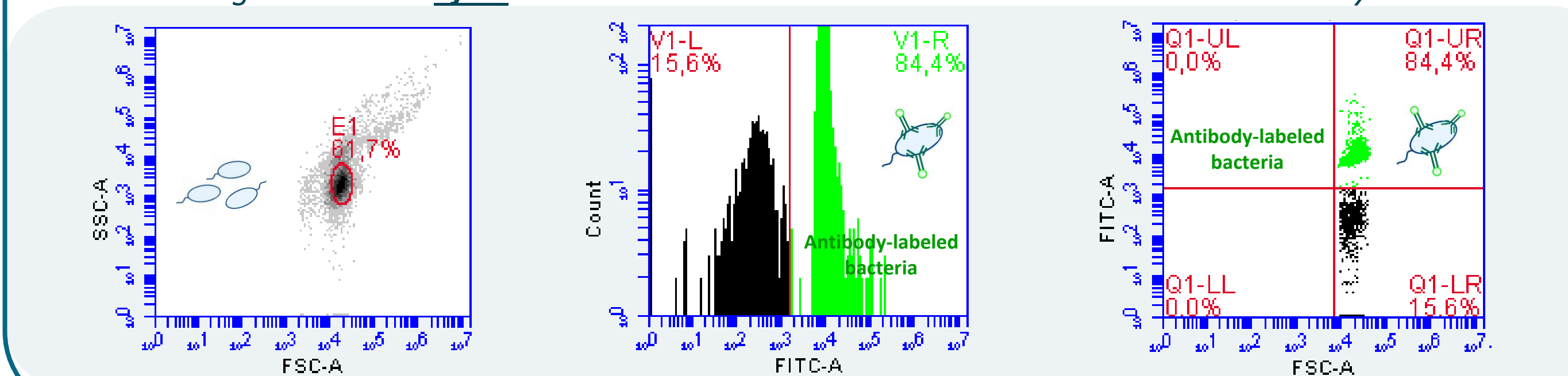
Figure 2 : Detection of aptamer-bacteria interaction by flow cytometry. Focusing on the laser beam of the device which allows the fluorescence detection.

Figure 3 : Plots before incubation between *E. coli* ATCC 700728 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 4 : Plots after incubation between *E. coli* ATCC 700728 and the antibody.



Conclusion

- Flow cytometry : effective analytical technique for studying the interactions between aptamers/antibodies and bacteria
- Every aptamer demonstrates interaction with *E. coli* bacteria
- It remains challenging to establish a distinct differentiation window between *E. coli* and the other strains that were tested
- Future experiments : study specificity of alternative aptamers across different strains.