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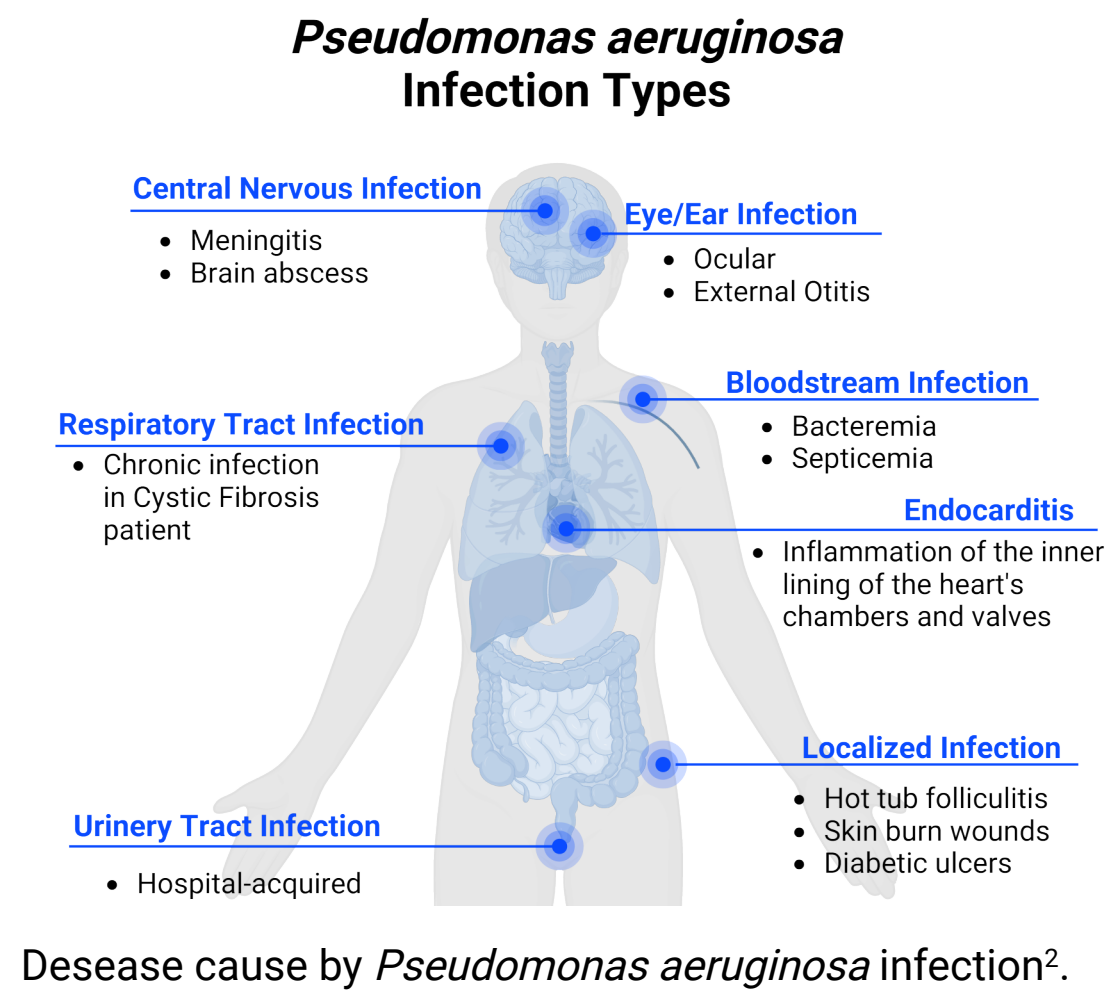
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Resistance to the Last-Resort Antibiotic Colistin in *Pseudomonas aeruginosa*

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INTRODUCTION

Pseudomonas aeruginosa is among 'the 3 most critical antibiotic-resistant priority pathogens list' of the World Health Organisation (WHO)¹. Besides being resistant, it causes several chronic infections including biofilm formation in cystic fibrosis patients.



Colistin (polymyxin E) is an antibiotic used as a last-resort treatment for multidrug-resistant (MDR) Gram-negative infections including pneumonia and *P. aeruginosa*. As a last resort antibiotic, it is in the WHO list of 'the critically important antimicrobials for human medicine'¹.

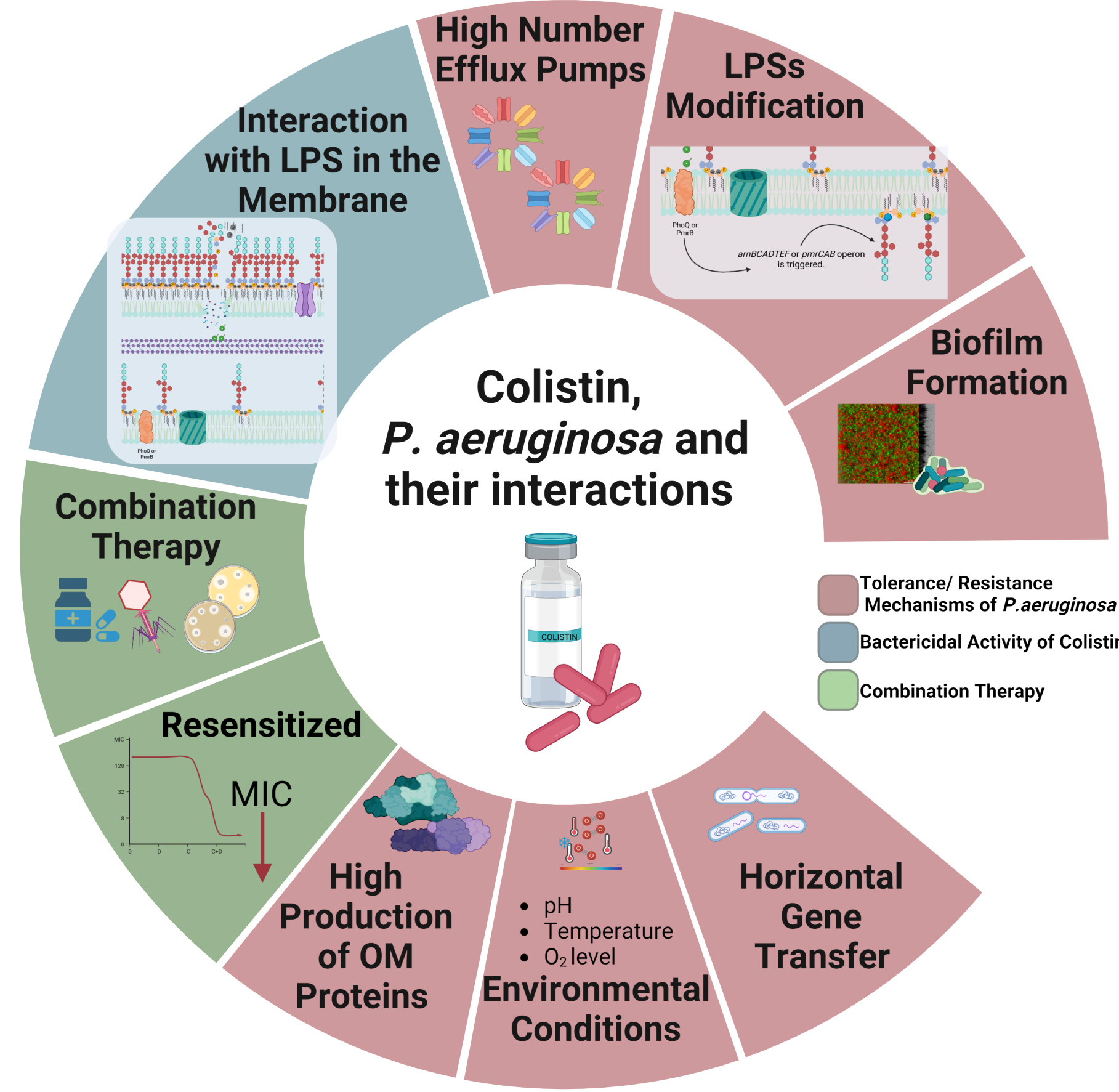
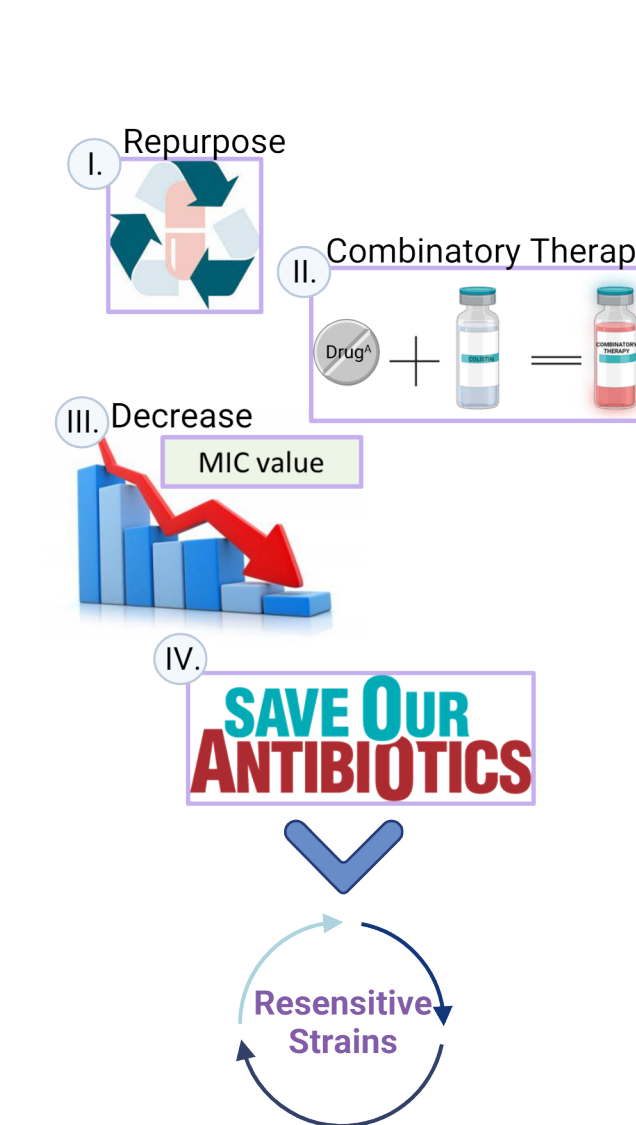


Illustration of Colistin mode of action, resistant/tolerance mechanisms of *P. aeruginosa*³ and new combination therapies.

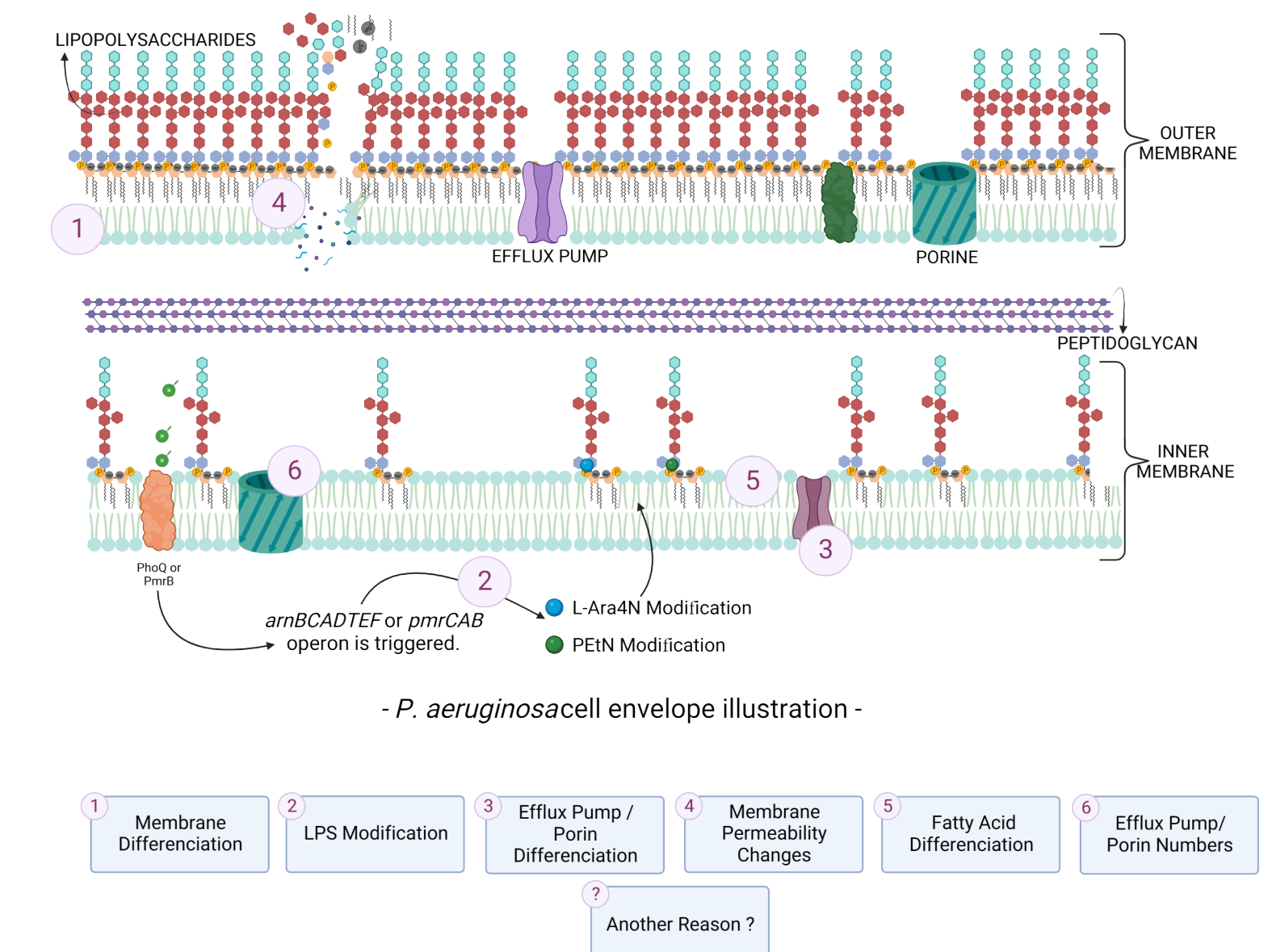
OBJECTIVE

Recent studies show an increase in the number of *P. aeruginosa* isolates less sensitive to colistin in chronic infections⁴. **Drug^A** is a new candidate to **restore** colistin sensitivity in *P. aeruginosa*. It is the mainstone of the project: it has no antimicrobial activity on its own in *P. aeruginosa* but has synergistic antibacterial activity with colistin. Preliminary results show that it **restores** sensibility to colistin in resistant strains. The main aim of the project is hence to decipher the molecular processes involved in the colistin-resensitivation mechanisms to better control it.

Why is the Colistin-Drug^A synergy important?



Hypothesis



METHODS

I. Developing a toolbox for *P. aeruginosa* fluorescent imaging

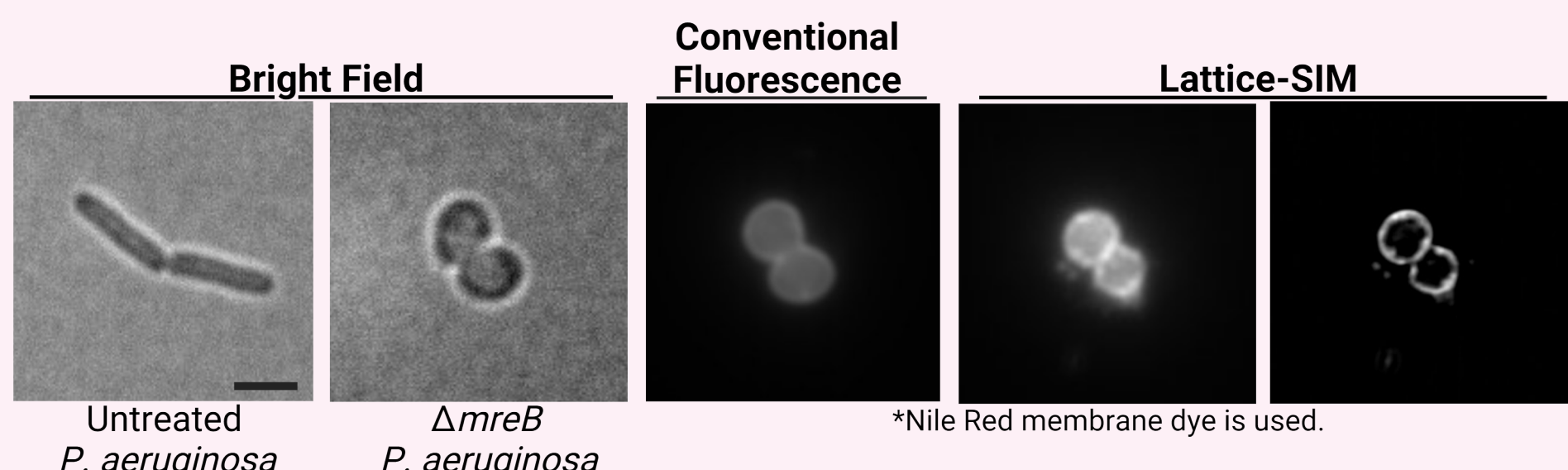
a. Fluorescent Colistin

- To monitor cellular uptake
- Determine diffusion rate in the presence and the absence of drug^A

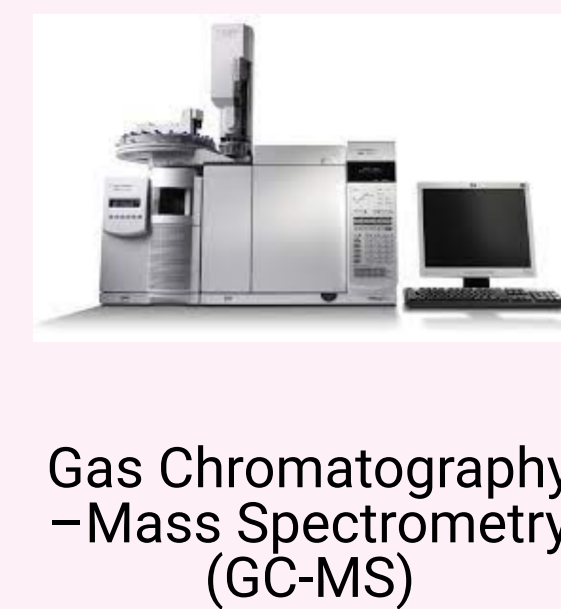
- FADDI-285
- FADDI-286 (Yun B et al., 2017)⁵

c. Engineering *P. aeruginosa* strains

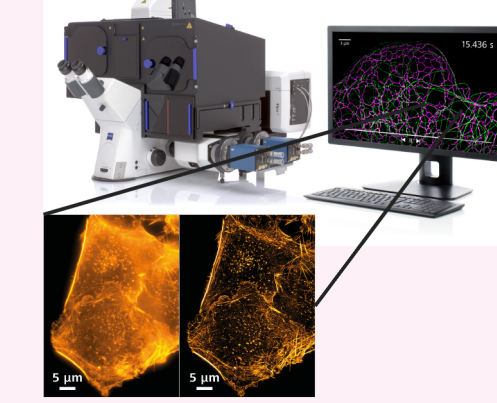
- Fluorescent protein addition
- Gene deletion



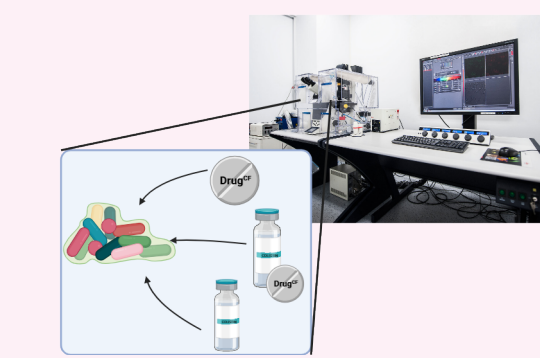
II. Mode of action in planktonic cells



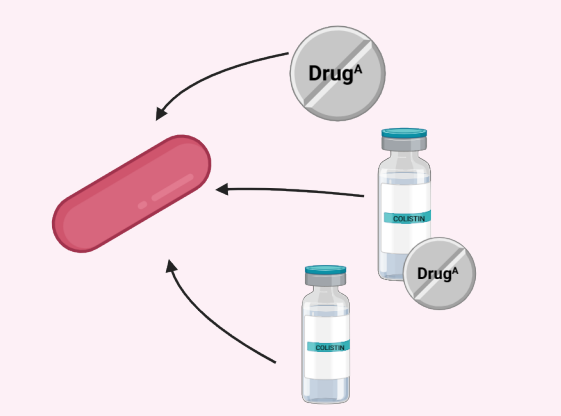
Gas Chromatography-Mass Spectrometry (GC-MS)



Lattice-Structure Illumination Microscopy (SIM)



SP8 Confocal Microscopy - Flow Cell

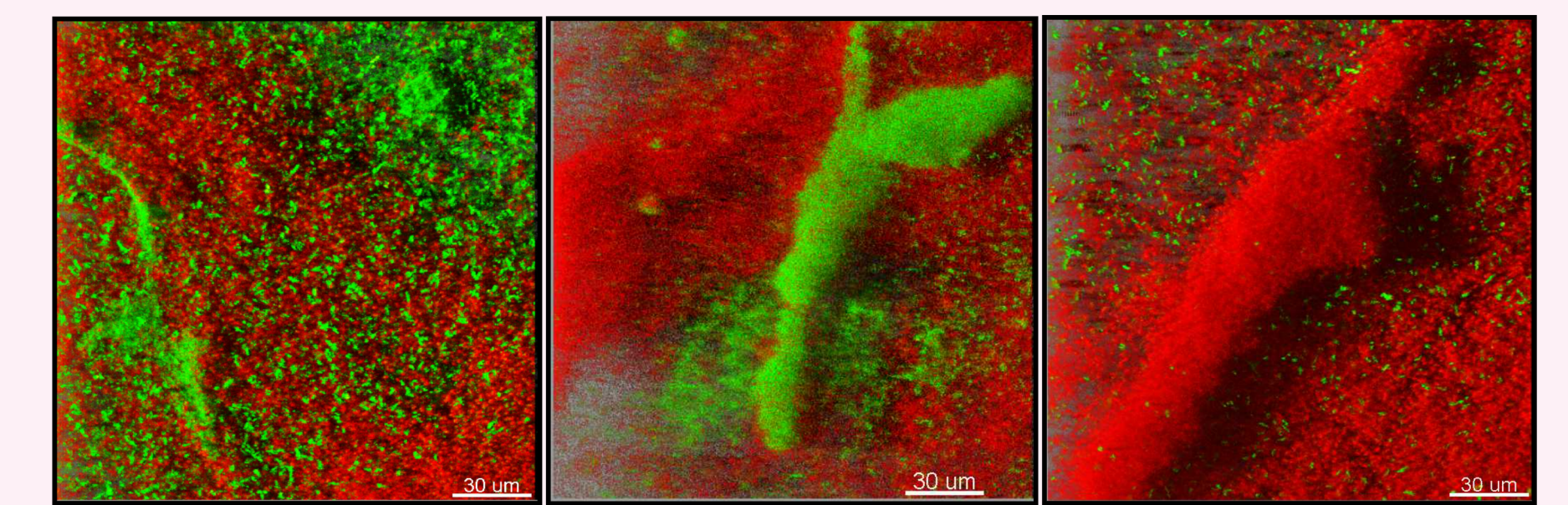


Conditions that will be observed in single cell.

- Effect on the growth rate
- Membranes permeability
- Metabolic activity
- ROS production
- Cell death differences

III. Mode of action in biofilms

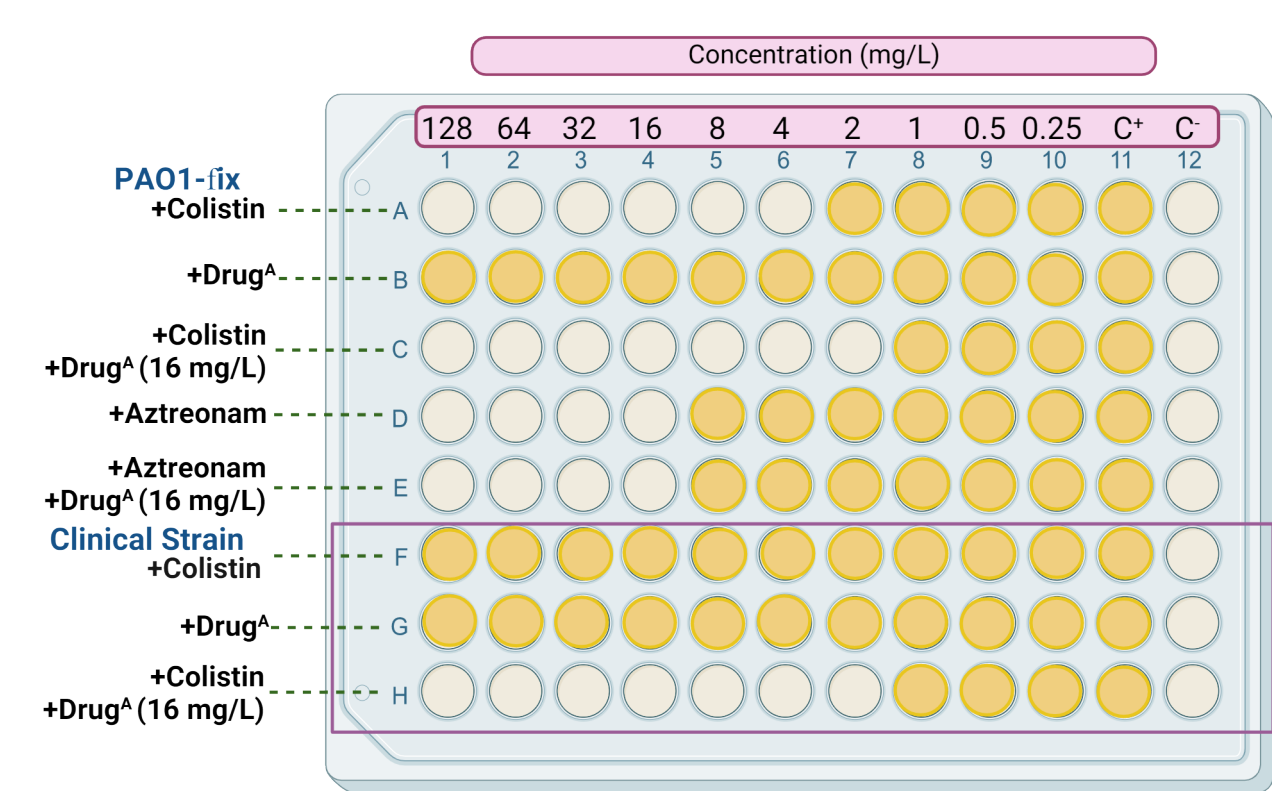
- Effect on biofilm formation
- Biofilm structural dynamics
- Local expression of resistance/tolerance genes with fluorescent reporters



20 hours biofilm PAO1-fix Live/Dead Staining. Left to right, untreated control, 2 hours incubated with 8 mg/L colistin and 3 hours incubated with 2 mg/L colistin + 16 mg/L Drug^A PAO1-fix biofilm z-stack images. Green refers to all population (Syto9), red refers to dead subpopulation (Propidium iodide).

FIRST RESULTS

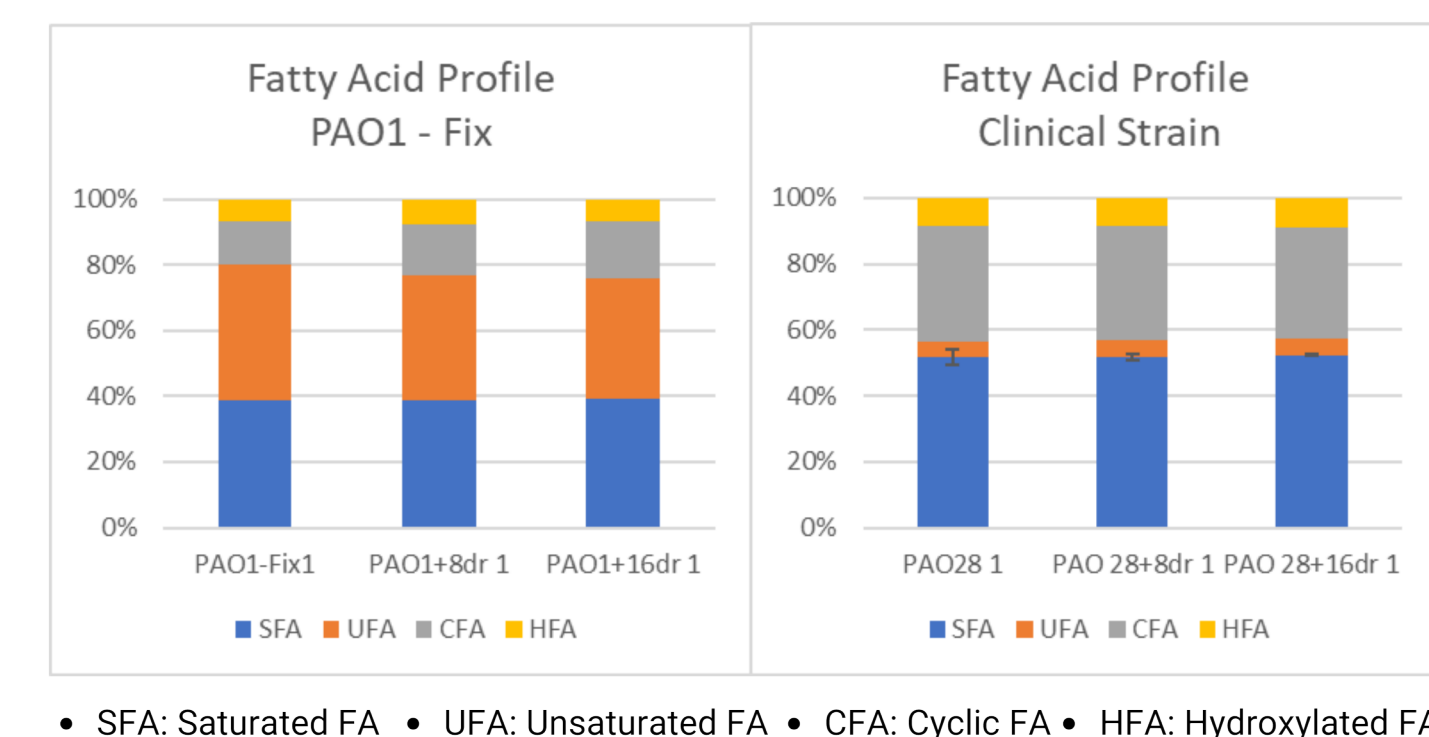
I. Minimum Inhibitory Concentration (MIC) Determination for 69 clinical and control strains. For *P. aeruginosa* the breakpoints of colistin is set at Sensitive (S) ≤4, Resistant (R) >4 mg/L. As a control, aztreonam is used and its breakpoint is 16 mg/L.



- Growth
 - No Growth
 - C⁺ — Positive Control
 - C⁻ — Negative Control
- Why do we use 16 mg/L Drug^A?
It is the minimum value that we reach the highest sensitivity.

> Drug^A decreasing MIC value of colistin for both sensitive and resistance *P. aeruginosa* strains while it has no effects on aztreonam MIC value.

II. Membrane Fatty Acid Profiles of PAO1-fix and clinical *P. aeruginosa* strain with/out and different concentration of Drug^A.



> A Closer look to Hydroxylated Fatty Acid Profiles <

Peak Name	PAO1-fix	PAO1-fix + 8 mg/L DrA	PAO1-fix + 16 mg/L DrA
Me 3-OH C10	1.65	1.78	1.77
Me 2-OH C12	2.38	2.43	2.33
Me 3-OH C12	2.61	3.48	2.73
Me 2-OH C14	0.00	0.00	0.00
Me 3-OH C14	0.00	0.00	0.00
HFA	6.64	7.70	6.83

Peak Name	Clinical Strain	CS + 8 mg/L DrA	CS + 16 mg/L DrA
Me 3-OH C10	0.00	0.00	0.00
Me 2-OH C12	0.00	0.00	0.00
Me 3-OH C12	0.00	0.00	0.00
Me 2-OH C14	0.93	1.09	1.17
Me 3-OH C14	7.35	7.61	7.58
HFA	8.28	8.71	8.75

> There is no significant differences when Drug^A is added.

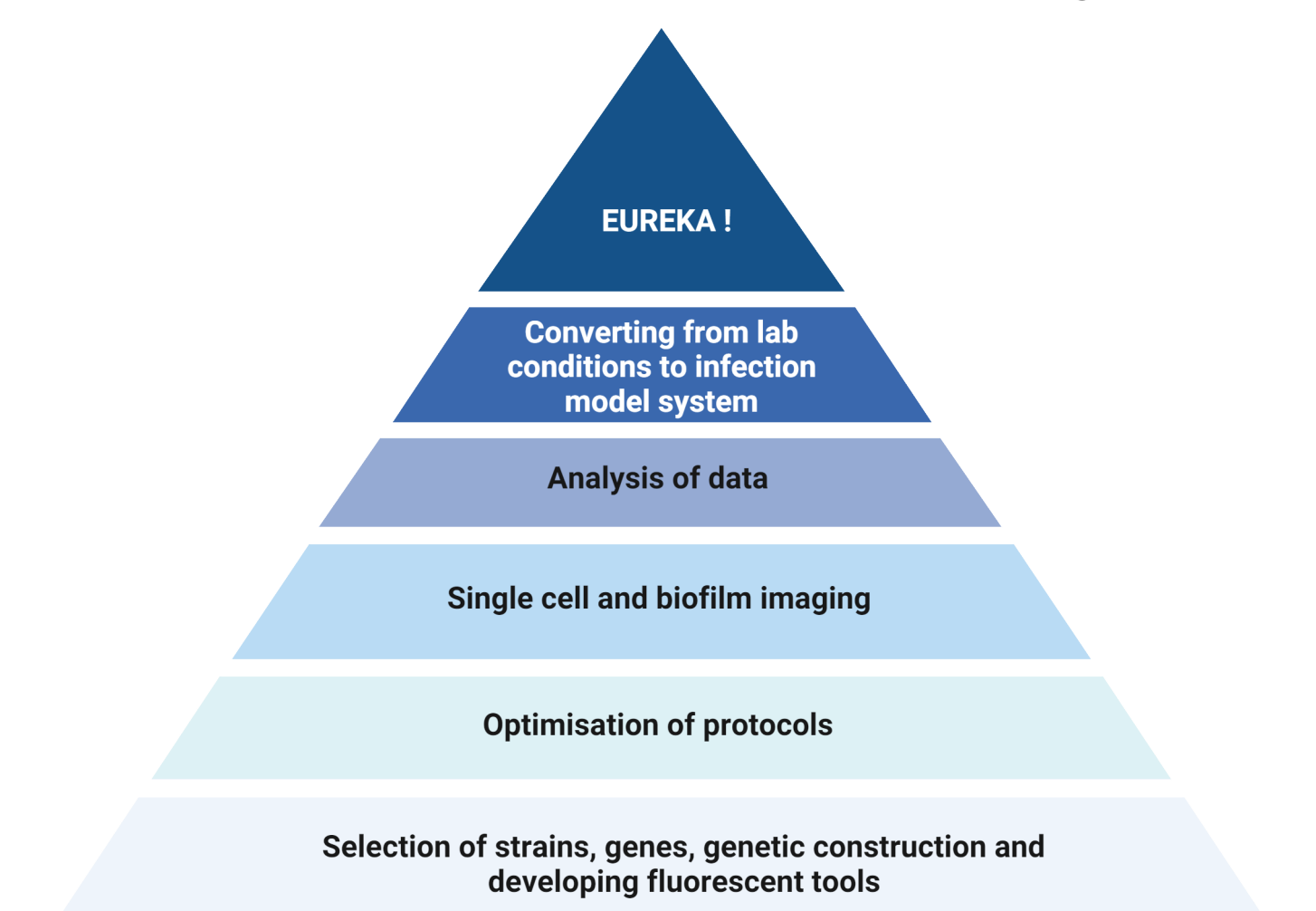
> More clinical strains must be checked to see if hydroxylated fatty acids are important for colistin resistance/tolerance.

CONCLUSION/ PERSPECTIVES

Even though the bactericidal mechanism of colistin is not fully known yet, several hypotheses could be involved to explain the synergistic interaction of colistin and drug^A. The first results suggest that the leading cause of this synergistic interaction is not related to fatty acids modification.

The first steps were taken for the construction of the strains required for the test of bacterial membrane structure and permeability. To check these hypotheses; Lattice-SIM, 4D-LSC Microscopy and SEM, cytometry techniques will be used, fluorescent tools will be developed and analyses of these data will be done.

The Pathway to Reach the Mechanism of Resensitivity



ACKNOWLEDGEMENTS

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