Polyamino-isoprenic Compound NV716 Selectively Disrupts the Outer Membrane of *Pseudomonas aeruginosa* and Revives Sensitivity to Cyclin Antibiotics

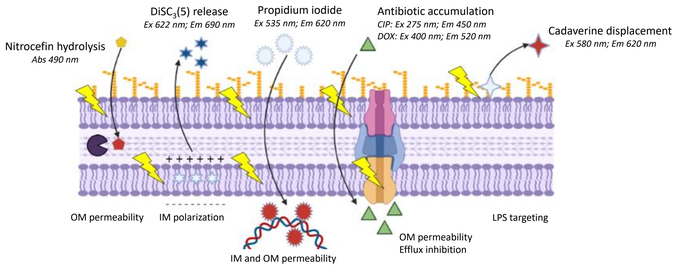
Margot Draveny[1,2], Marine Novelli[2], Hugo Chauvet[1], Fabienne Neulat-Ripoll[3], Jean-Michel Brunel[2], Jean-Michel Bolla[2], Frédéric Jamme[1] and Muriel Masi[2]
[1]DISCO Beamline, Synchrotron SOLEIL, L'Orme des Merisiers, Gif-sur-Yvette, France
[2]Membranes et Cibles Thérapeutiques, Aix-Marseille Université, UMR_MD1, Inserm U1261, Marseille, France
[3]Institut de Recherche Biomédicale des Armées, Département Microbiologie et Maladies Infectieuses, Unité Bactériologie, Brétigny-sur-Orge, France
margot.draveny@synchrotron-soleil.fr

Introduction

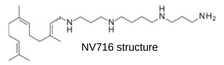
Increasing antibacterial resistance represents a major challenge in antibiotic discovery. Attractive targets in Gram-negative bacteria are (i) their unique asymmetric outer membrane, which acts as a permeability barrier and protects the cells from external stresses, such as antibiotics, and (ii) multidrug efflux pumps, which actively expel a wide range of antibiotic substrates. We previously identified novel polyamino-isoprenic compounds that act synergistically with doxycycline (DOX) against *Pseudomonas aeruginosa*. In this work, we describe the impact of compound NV716 on antibiotic accumulation and its mode of action on Gram-negative bacterial envelopes using *P. aeruginosa* and *E. coli* as model organisms.

Biochemical assays to characterize NV716

- **Methodology**



- **Results**



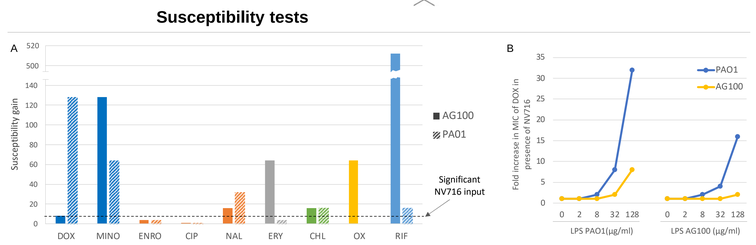


Figure 1 : (A) Susceptibility gain factors of several antibiotics on *P. aeruginosa* PAO1 and *E. coli* AG100 in presence of NV716. (B) MIC of DOX + NV716 in presence of exogenous LPS of PAO1 or AG100 on PAO1 and AG100.

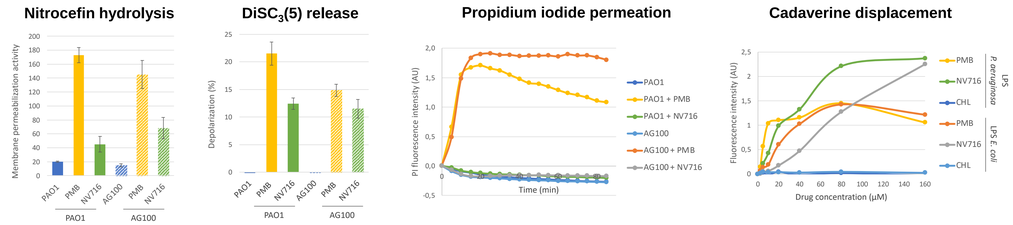
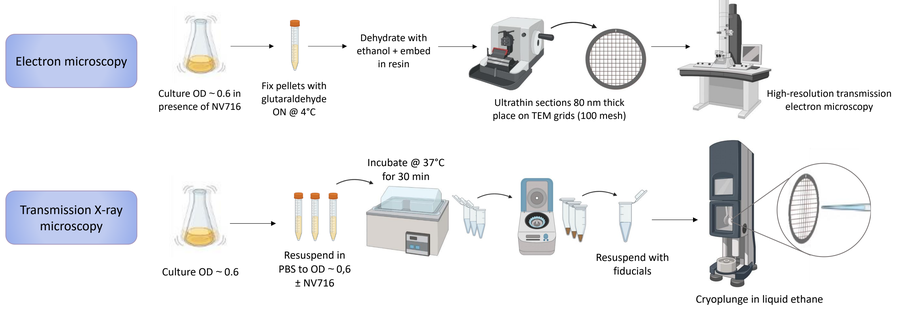


Figure 2 : Biochemical assays in *P. aeruginosa* PAO1 and *E. coli* AG100 to characterize NV716 *(PMB : Polymyxine B, disrupt both membranes ; CHL : negative control)*

- NV716 potentiates cyclines activity (DOX, MINO) but not fluoroquinolones (ENRO, CIP).
- NV716 seems to act on membrane permeability (RIF does not cross membranes but its activity increases widely in presence of NV716).
- NV716 acts on outer membrane probably by interacting with the LPS.

Effect of NV716 on bacterial envelopes

- **Methodology**



- **Results**

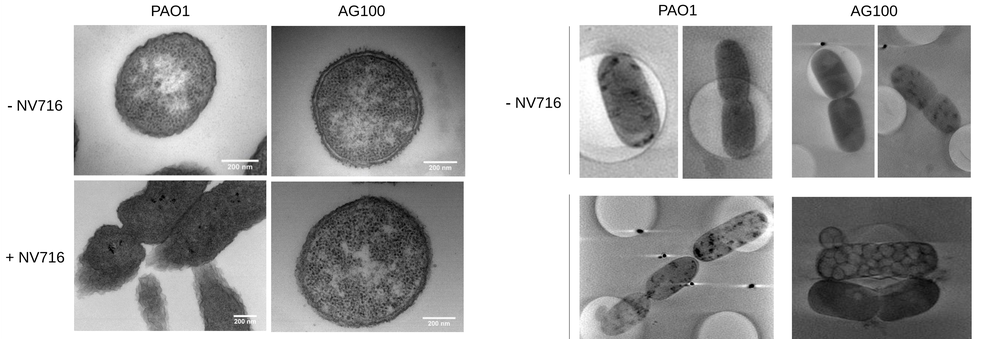


Figure 3 : Observation of *P. aeruginosa* PAO1 and *E. coli* AG100 in absence and in presence of NV716 treatment under electron microscope

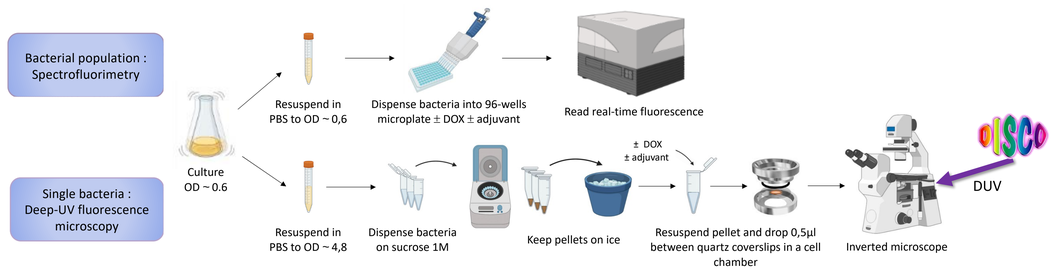
Figure 4 : Observation of *P. aeruginosa* PAO1 and *E. coli* AG100 in absence and in presence of NV716 treatment under soft X-ray microscope

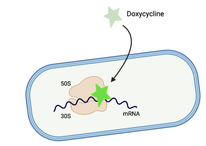
- NV716 induces membrane alteration in *P. aeruginosa* but not in *E. coli* in electron microscopy whereas in X-ray microscopy vesicles appears at the surface of *E. coli.*
- In X-ray microscopy, dark spots (lipid accumulation ?) are visible in both PAO1 and AG100 regardless the presence of NV716. Also in presence of NV716, internal vesicles can be observed.

Impact of NV716 on antibiotic accumulation

- **Methodology**

MCT and DISCO beamline at SOLEIL have developed innovatives approaches based on the intrinsc fluorescence properties of certain antibiotics to follow their accumulation in bacteria. These approaches can be used to test the efficiency of adjuvants molecules on antibiotic accumulation.





When doxycycline binds to its cytoplasmic target (the ribosomes) its fluorescence increases, allowing to record its accumulation over time in intact bacteria using spectrofluorimetry.

- **Results**

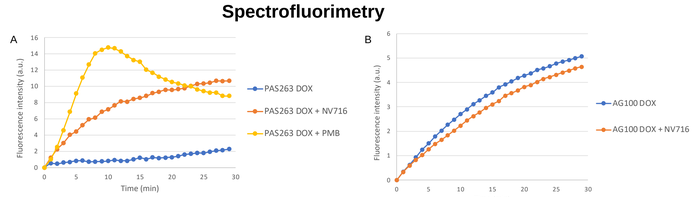


Figure 5 : Follow-up of doxycycline accumulation in *P. aeruginosa* PAS263 (A) and *E. coli* AG100 (B)

- NV716 significantly increases accumulation of doxycycline in *P. aeruginosa* but not in *E. coli.*

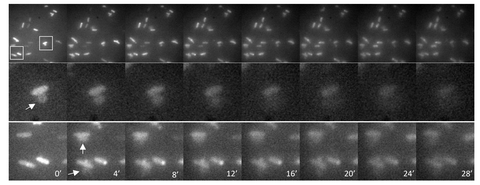


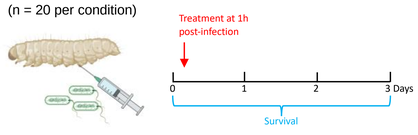
Figure 6 : Observation of doxycycline accumulation in *P. aeruginosa* in presence of NV716 under microscope over time.

- NV716 induces membrane alteration that lead to a leak of DOX fluorescence over time by dissipation in the environment.
- Spots of DOX fluorescence can be observed in some bacteria.

In vivo infection model

- **Methodology**

G. Mellonella infection model
(n = 20 per condition)



P. aeruginosa PAO1 (50 CFU)

- **Results**

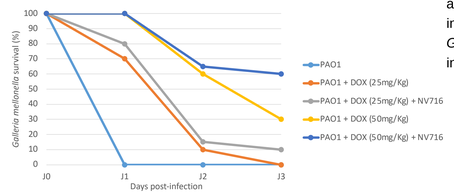


Figure 7 : Survival rates of *Galleria mellonella* larvae infected with *P. aeruginosa* PAO1

- NV716 potentiates the activity of doxycycline and increases the survival of *Galleria mellonella* larvae infected with PAO1.

Conclusion and Perspectives

- NV716 potentiates the activity of doxycycline and increases its accumulation in *P. aeruginosa.*
- NV716 acts on outer membrane permeability, primary barrier to antibiotic accumulation, probably by targeting the LPS.
- NV716 incerases the survival rate of *G. mellonella* infected with *P. aeruginosa.*
- Heterogeneity of the results in transmision X-ray microscopy → new proposal to ALBA in preparation.

Acknowledgments: We thank Martina Laudazzi (MCT, AMU) for carrying out the membrane depolarization assays, Eva Pereiro (Synchrotron ALBA) for her assistance and advices on the MISTRAL Beamline and Valérie Rouam (Synchrotron SOLEIL) for her continuous help.