Etude par RMN de la structure et la fonction d'un régulateur moléculaire impliqué dans la résistance bactérienne à l'argent

Structural and functional investigation of a molecular regulator involved in bacterial silver resistance using NMR

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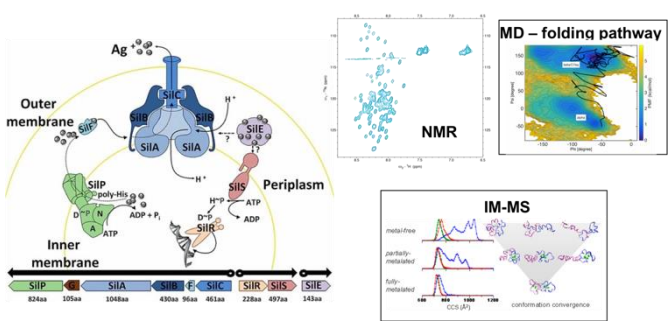

Like werewolves and vampires, bacteria have a weakness: silver. The antimicrobial properties of this precious metal have extensively been used for thousands of years. Despite this long-standing history and its demonstrated activity against Gram-negative bacteria, the complete bactericidal mode of action of silver remains unclear. Nevertheless, silver misuse can damage the cells and a note of caution is mandatory about its potential toxicity. To counteract the toxic effect of silver, Gram-negative bacteria have developed different resistance mechanisms, including the efficient efflux of the metal out of the cell. The first silver-resistant plasmid pMG101 was isolated from *Salmonella* strain after the death of patients in the burn ward at the Massachusetts General Hospital. The silver-resistant gene cluster is composed of nine genes: a chemiosmotic efflux pump (SilCBA), an ATPase efflux pump (SilRS) and two periplasmic silver-binding proteins SilE and SilF. SilE is an interesting target to understand the silver resistance because this protein is only synthesized during bacterial growth in the presence of silver ions and its structure, as well as its role are unknown.

Until now, only macroscopic techniques as far UV-CD have been used to characterize structural arrangement of Ag-SilE complex, whose stoichiometry has been determined by mass spectrometry. In the literature, the stoichiometry varies between 5 to 38 silver ions per protein, depending on the experimental conditions. SilE has been described as “molecular sponge” but without clear experimental characterization of the associated mechanisms. In particular, high resolution structural data on the Ag-SilE complex is lacking to gain insight into the coordination mechanisms at the molecular level. To date, only Ag-SilE derived peptide structures have been resolved and binding affinities have been extracted by our group and our Swiss partners. This allowed to emphasize that histidine and methionine residues are involved in the helical folding of the peptides upon silver binding. Beyond structure, pending questions concern the role of SilE in the efflux pump, and the way silver ions are effectively transferred out of the cell. In this context, Mirolo et *al.* proposed a mode of action of SilE for the silver ions transport: at acidic pH, SilE will release silver ions and SilB (part of the tripartite SilCBA pump), through an increase of silver in the cell, will undergo a conformational change allowing silver ions to enter into the pump. Moreover, Urbina et *al.* recently studied the periplasmic protein, SilB, which provides a continuous channel for the extrusion of silver out of the cell. The role and function of SilB

in the pump has been investigated using different constructs of SilB with a multi-technique approach. However, no structural data concerning the SilB/SilE interaction has been described in the literature. Building on those grounds, our project proposes to use NMR to tackle the above questions.

The project will be hosted by the analytical science institute located in Lyon/Villeurbanne (France). This new institute comprise around 200 researchers and is among the largest analytical science center in Europe. Cutting edge instruments are available like High field NMR spectrometers (From 600 to 1000MHz). The thesis project will be developed inside the Biosys group and will mainly make use of NMR and will benefit from the expertise of the group members. A part of the project will be dedicated to the production of isotope labeled proteins. The successful candidate should have completed (or in stage of completion) M.Sc. degree either in biochemistry, structural biology, biology, physical chemistry or related fields. Willingness to learn NMR will be strongly appreciated.[1-2]

References

1. Chabert, V., et al., *Chem Commun (Camb)* **2017,** *53* (45), 6105-6108.https://doi.org/10.1039/c7cc02630g
2. Chabert, V., et al., *Chem Commun (Camb)* **2018,** *54* (74), 10419-10422.https://doi.org/10.1039/c8cc03784a