INSTITUT DES SCIENCES ANALYTIQUES

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 (SilP), a responder and membrane sensor performing two-component transcription regulation (SilRS) and three periplasmic silver-binding proteins SilE, SilF and SilG.

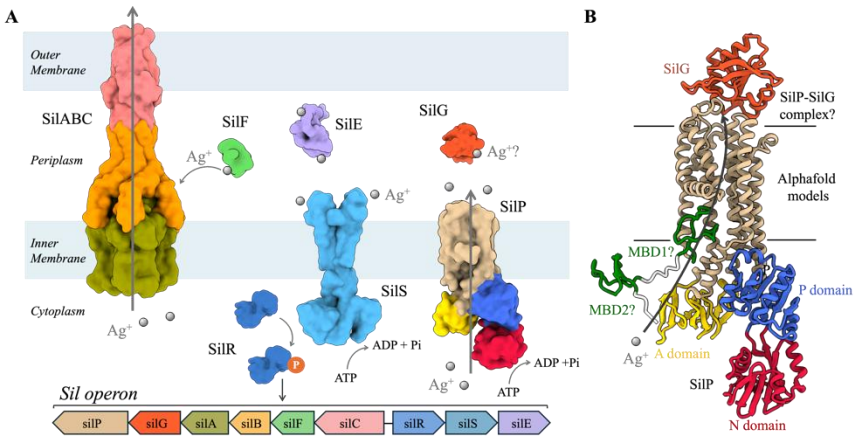

Figure 1: Composition of the sil system

Our group is interested in understanding the complete mechanism of silver ions eviction through the efflux pump system *sil*. Until now, we tried to decipher how the interplay between SilB, SilF and SilE proteins contribute to the silver efflux pump mechanism[1-5]. The next challenge will be the understanding of the role of SilP and particularly its metal binding domains and its interplay with SilG. Indeed, the specific role and exact number of MBDs is still enigmatic in terms of metal uptake or regulatory function. An AlphaFold model of SilP informs us about the nature of the long flexible N-ter (158 AA). It highlights the presence of two presumed MBDs (MBD1, ^{28}His-Asp^{77}; MDB2, ^{92}Val-Ser^{148}, Fig. 1B), each containing three cysteine residues forming together a possible silver ion interaction site. The candidate will produce and purify ^{15}N and ^{15}N/^{13}C labeled MBDs and will use NMR spectroscopy to resolve the structure of the two predicted MBDs of SilP and to investigate the interaction between Ag^+ and the two MBDs. The protein SilG possesses the conserved CxCC motif involved in metal interaction. An Alphafold model of the SilG/SilP complex reveals a confident interaction site involving this CxCC motif and a conserved Met residue of SilP in a periplasmic flexible 15 residues loop. This putative exit pathway suggests a silver-dependent transfer from cytoplasm to periplasm. Finally, our project proposes to elucidate the silver transport mechanism from the cytoplasm to the periplasm, by studying the structural and dynamical features of SilP, SilG and their interplay.

The project will be hosted by the analytical science institute located in Lyon/Villeurbanne (France). This new institute comprise around 150 researchers and is among the largest analytical science center in Europe. 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.

References

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