Sociedad Argentina de Microbiología General

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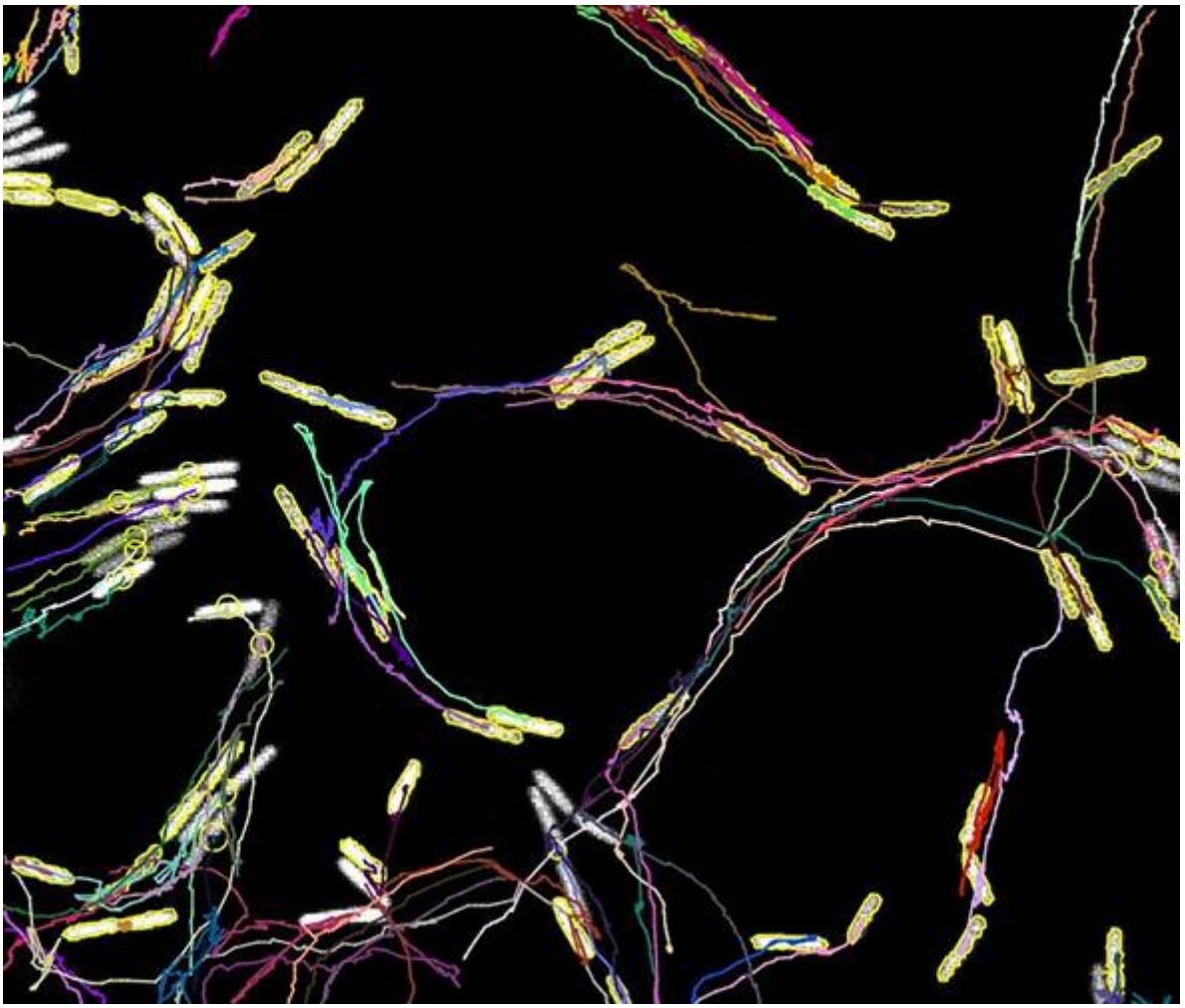

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

A POWERFUL TOOLKIT TO EXPLORE HOW VraS FROM Staphylococcus aureus DETECTS ANTIBIOTICS AND UNCOVER THE CRITICAL ROLE OF THE REGULATORY PROTEIN VraT

Peralta, Melani D.[1] - Rodriguez Aieta, Constanza[1] - Suarez, Irina P.[1] - Llarrull, Leticia I.[1,2]

1) Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR) - Rosario - Santa Fe - Argentina.
2) Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario - Rosario - Santa Fe - Argentina.
Contacto: suarez@ibr-conicet.gov.ar

Staphylococcus aureus is a global clinical threat, primarily due to its multidrug-resistant nature. Amidst the arsenal of mechanisms employed by this bacterium, the VraTSR three-component system has garnered attention for its role in conferring resistance to cell-wall active antibiotics, particularly vancomycin. Comprising three vital proteins —VraS, a membrane histidine kinase; VraR, a cytoplasmic response regulator; and VraT, an uncharacterized membrane protein— the VraTSR system regulates the cell wall stress stimulon. Despite its prominence, the molecular signal initiating VraTSR activation has remained unknown. To define the mechanism of this putative tripartite regulatory system, our research delved into the interactions between VraS, VraT, and antibiotics. In this project we employ biophysical tools to study full-length membrane proteins *in vitro*. We overexpress the proteins in E. coli, purify in detergents micelles and determine their interaction with different antibiotics by means of saturation transfer difference (STD) NMR spectroscopy. On the other hand, we study the topology of the VraS/VraT complex by introducing lanthanide binding tags (LBTs) which allow luminescence resonance energy transfer (LRET) with fluorescently labelled antibiotics. By determining the change in the luminescence lifetime upon complex formation, we deduce the possible topology for the accessory protein VraT with respect to the histidine kinase VraS. We optimized the expression and purification of the full-length histidine kinase VraS in DDM micelles. Size exclusion chromatography confirmed that the protein was in the expected dimeric form. In the STD experiments showed direct interaction between vancomycin and ampicillin with the kinase in micelles. We observed no interaction with antibiotics which do not activate the system in vivo. On the other hand, we cloned and expressed two versions of VraS and VraT harboring LBTs in strategic positions that allow LRET experiments. The LBT-VraS protein was purified in DDM micelles and its affinity for terbium (III) was determined. We then titrated the sample with the fluorescent antibiotic Bocillin FL and determined the distance between the LBT and the antibiotic. We have also co-expressed VraS and VraT and the combinations of LBT constructs with wild type partner, to test co-purification and luminescence on membrane extracts. Our results allow us to

conclude that VraS is a direct ?-lactam and glycopeptide sensor. The STD NMR experiments open a new opportunity to screen for VraS ligands that could prevent antibiotic binding. In addition, the LBT constructs will provide us with the necessary toolkit for determination of conformational changes upon activation of the VraS/VraT complex by LRET and electron paramagnetic resonance spectroscopy.

Palabras clave: Staphylococcus aureus – VraTSR – glycopeptides – ?-lactams - NMR