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LXII REUNIÓN ANUAL DE LA SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

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BUENOS AIRES, VOL 77 Supl. I - 2017

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LXII ANNUAL MEETING OF ARGENTINE SOCIETY OF CLINICAL INVESTIGATION (SAIC)

LIII ANNUAL MEETING OF ARGENTINE SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (SAIB)

LXV ANNUAL MEETING OF ARGENTINE SOCIETY OF IMMUNOLOGY (SAI)

MEETING OF ARGENTINE SOCIETY OF ANDROLOGY (SAA)

XLVI ANNUAL MEETING OF ARGENTINE SOCIETY OF BIOPHYSICS (SAB)

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November 13 -17, 2017
Palais Rouge– Buenos Aires

(1277) **DESK THERMOSENSING RELIES ON A COILED COIL SWITCH**

Emilio Saita (1), Luciano Abriata (2), Diego de Mendoza (1), Daniela Albanesi (1)
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Propagation of a signal through transmembrane segments of a sensor protein is key at the initial stage of many complex signaling processes. The thermosensor histidine kinase DesK from *Bacillus subtilis* senses changes in membrane fluidity initiating an adaptive response. Structural changes in DesK have been implicated in transmembrane signaling, but direct evidence is still lacking. Based on structure-guided mutagenesis, we have proposed a mechanism of DesK-mediated signal sensing and transduction. Specifically, stabilization/destabilization of a 2-helix coiled coil, which connects the transmembrane sensory domain of DesK to its cytosolic catalytic region, is crucial to control its signaling state. Computational modeling and simulations reveal couplings between protein, water and membrane mechanics favoring such conformational changes. We propose that membrane thickening is the main driving force for signal sensing, and that it acts by inducing helix stretching and rotation prompting an asymmetric kinase-competent state. At present, we are dedicating our efforts to crystallize full-length DesK for X-ray structure determination, expecting to shed light on DesK's transmembrane sensing mechanics.

(1327) **ARCHITECTURE OF MECR1: A MEMBRANE-EMBEDDED METALLOPROTEASE DOMAIN POISED TO RECEIVE INFORMATION FROM THE EXTRACELLULAR SENSOR DOMAIN**

Bruno Salvador Belluzo (1), Luciano Abriata (2), Estefania Giannini (1), Matteo Dal Peraro (2), Leticia Irene Llarrull (1)
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Abstract: **The signal transducer protein MecR1 from methicillin resistant *Staphylococcus aureus* regulates the expression of PBP2a, a protein that is not inhibited by clinical concentrations of most β-lactam antibiotics**. In this study we aimed at identify a structural model that present a better comprehension of the signal transduction mediated by MecR1, which is an attractive target for drug development.

By integrating homology modeling, residue co-evolution analysis, dynamic simulations and docking, we generated a model for the full length MecR1 protein. This model was used to design a series of constructions to further evaluate the topology in-vitro. Fluorescence spectroscopy and Proteinase K susceptibility assays in MecR1. eGFP truncated fusions and TEV peptidase susceptibility assays in MecR1.E205A.TEV insertions were carried out. As a whole, these data allowed us to corroborate the orientation of most transmembrane helixes, it showed that the peptide S63-T102 is extracellular (and not a TM helix) and that Loop 4 in the metalloprotease domain N194 to D213 has low flexibility, which could be due to compaction or membrane interaction. To further evaluate if the effector domain has membrane localization, the cytoplasmic domain (cytMecR1), was expressed in *E. coli*, and we found it was membrane embedded. These results support the existence of a reentering alpha-helix in the metalloprotease domain that tightly anchors this domain to the membrane, in accordance with our computational model.

In conclusion, we presented a model for full length MecR1 in which the metalloprotease domain is embedded in the membrane, defining a hydrophilic chamber. A reentering loop connecting the metal ligands tightly anchors the metalloprotease domain to the membrane (even in the absence of the rest of the TM helixes) and reaches to the outer leaflet of the membrane where it is posed to interact with the sensor domain loops that show altered mobility upon antibiotic binding.

Keywords: Staphylococcus aureus, b-lactam antibiotics, resistance, MecR1, signal transduction.

(1285) **CHARACTERIZATION OF THE MECHANISM OF ACTIVATION OF THE *vraSRT* SYSTEM OF *Staphylococcus aureus* USING ANTIBIOTIC-DERIVATED PHOTOPROBES**

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Staphylococcus aureus is the leading cause of both nosocomial and community-acquired infections. In *S. aureus* the *vraSRT* three-component system acts as a sentinel that can rapidly sense cell wall peptidoglycan damage and coordinate a response that leads to resistance to β-lactam and glycopeptide antibiotics. VraS and VraR encode a histidin-kinase and a response regulator, respectively. However, the rol of VraT is yet unknown. We still do not understand how VraS is activated in response to cell wall-active antibiotics.

The interaction between VraS (the possible sensor), VraT and different photoprobes derived from ampicillin was studied. The photoaffinity probes were used for covalent labelling of VraS and VraT in *E. coli* spheroplasts, and the interaction was evidenced by an electrophoretic mobility shift in the case of VraS, although no interaction was seen with VraT. We used a *S. aureus* reporter strain to confirm that the ampicillin-derived photoprobes effectively activate the *vraSRT* system. The VraS-photoprobe complexes were purified and analyzed by MALDI-TOF/TOF. In this study we also addressed the topology of VraT in membranes. Using a Proteinase K susceptibility assay we determined that the C-terminal domain of VraT has extra-cytoplasmatic location.

Keywords: Staphylococcus aureus; *vraSRT*; photoaffinity ampicillin-derived probes.

(1381) **FUNCTIONAL EXPRESSION OF HUMAN PARK9-ATP13A2 P5-ATPase IN *Saccharomyces cerevisiae*.**

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IQUIFIB FFyB (UBA-CONICET)

The P-type ion pumps are membrane transporters energized by ATP-hydrolysis. They were classified into five subfamilies termed P1-P5; the substrate specificity of P5 subfamily is still unknown. Five genes named *ATP13A1-ATP13A5* that belong to the P5-ATPases are present in humans, while two named Spf1p and Ypk9p were found in the yeast *S. cerevisiae*. By DNA sequence alignment it was shown that P5-ATPases were classified in groups P5A and P5B; the mouse gene Atp13a1 and the yeast gene coding Spfp1 (Yel031w) are members of the first group, while the mouse genes Atp13a2-Atp13a5 and the yeast gene coding Ypk9p (Yor291w) are clustered into the second one. Mutations of the ATP13A2 gene, also known as PARK9, are associated with a form of Parkinson's Disease (Kufor-Rakeb syndrome), a form of Neuronal Ceroid Lipofuscinosis (CNL12) and hereditary spastic paraplegia (SPG78). ATP13A2 is localized in lysosomes and late endosomes (LEs). Dysfunction of this protein diminishes the lysosomal degradation, the autophagic flux and the exosome externalization. In order to advance the biochemical characterization of ATP13A2, *S.c* cells were transformed with the pMP625 vector coding for GFP tagged-ATP13A2 protein and the clone exhibiting the highest GFP-fluorescence intensity selected. Yeasts expressing GFP-ATP13A2 showed a reduced growing rate and an autophagic phenotype characterized by vacuolar multivesicularization. The recombinant fusion protein was purified from microsomal membranes by pseudo-affinity chromatography. Analysis of the eluate by SDS-PAGE and western blot indicated that ATP13A2 was successfully expressed and had the expected molecular size. Preliminary experiments measuring the release of free phosphate from ATP by the Baginsky's assay showed that the purified GFP-ATP13A2 is a functional ATPase.