



## **Bioinformatic prediction and experimental validation of signals recognised by sensor domains in bacterial receptors: the case of amino acid specific dCache\_1AA domain**

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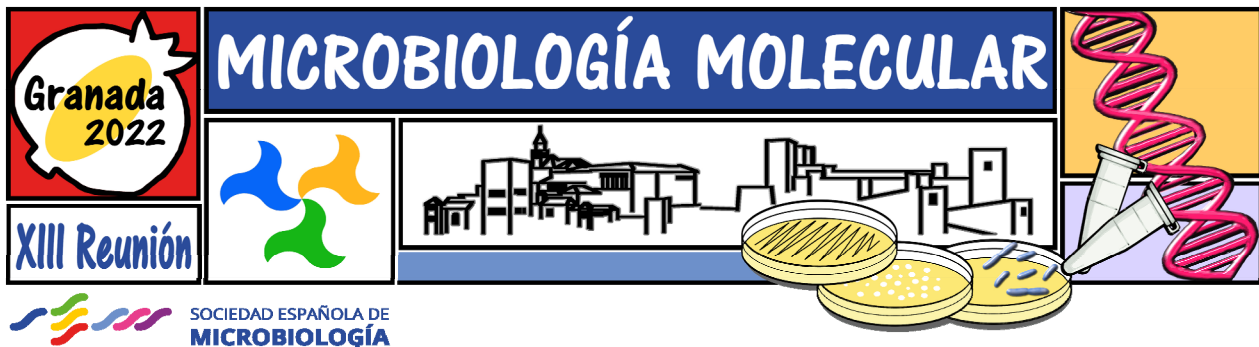
Bacteria have evolved a large number of signal transduction receptors that are essential for an optimal adaptation to changing environmental conditions. These receptors are typically stimulated by the binding of signals to dedicated ligand binding domains (LBDs). However, the nature of the signals that are recognized by most of sensor domains is unknown and information on receptors' function generally derives from the phenotypic characterization of bacterial mutants. The difficulty to identify the cognate signals is also given by the high degree of sequence divergence between characterized homologous LBDs. Previously, we solved the 3D structures of the LBDs of PctA, PctB and PctC - three amino acid specific chemoreceptors of *Pseudomonas aeruginosa* containing a dCache-type sensor domain - which allowed us to identify the key residues for amino acid binding (1). Here, we report the first study to successfully predict the signals that are recognized by sensor domains. We discovered a subclass of dCache-type LBDs, named dCache\_1AA, that contains a motif that recognizes the amino and carboxyl groups of amino acids ligands. Bioinformatics analyses showed that dCache\_1AA domains are present in thousands of bacterial and archeal receptors, including important clinical and agricultural pathogens. dCache\_1AA domains were identified in all major classes of sensor proteins, including chemoreceptors, histidine kinases, diguanylate cyclases and phosphodiesterases, serine/threonine kinases and phosphatases. To verify our bioinformatics predictions, we purified the individual dCache\_1AA LBDs of a selection of different receptors types from diverse microbial taxonomic groups and conducted biochemical assays based on differential scanning fluorimetry and isothermal titration calorimetry. We found that all the target proteins analyzed bound amino acids. The dCache\_1AA domain was also identified in eukaryotic receptors, indicating that amino acids are central signal molecules throughout the Tree of Life. Our study highlights the power of structural biology and bioinformatics to predict the signals recognized by sensor domains based on amino acid composition of the ligand binding sites. Analogous approaches may be employed to identify the signals of other sensor domain families, which permits to tackle a central bottleneck in understanding signal transduction mechanisms.

### **References**

1. Gavira, J.A., Gumerov, V.M., Rico-Jiménez, M., Petukh, M., Upadhyay, A.A., Ortega, A., Matilla, M.A., Zhulin, I.B., Krell, T. (2020) How bacterial chemoreceptors evolve novel ligand specificities. *mBio*, 11: e03066-19.
2. Gumerov, V.M., Andrianova, E.P., Matilla, M.A., Page, K.M., Monteagudo-Cascales, E., Dolphin, A.C., Krell, T., Zhulin, I.B. (2022) Amino acid sensor conserved from bacteria to human. *Proceedings of the National Academy of Sciences of the United States of America*, 119: e2110415119.

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# XIII REUNIÓN DEL GRUPO DE MICROBIOLOGÍA MOLECULAR DE LA SEM

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