Identification of molecular determinants required for the interaction between the nucleoid associated proteins H-NS and Hha

C. Fernández de Alba[1], T.N. Cordeiro[1], J. García[1], M. Pons[1,2]

[1] Laboratory of Biomolecular NMR, Institut de Recerca Biomèdica-Parc Científic de Barcelona, 08028 Barcelona, Spain.
[2] Departament de Química Orgànica, Universitat de Barcelona, 08028, Spain

Nucleoid associated proteins Hha and H-NS participate in the regulation of transcription of many genes involved in bacterial pathogenicity. For example, Hha interacts with H-NS enhancing its ability to repress under non-permissive conditions the expression of the *E. coli* hemolysin toxin [1].

H-NS is organized in two independent structural domains linked by a flexible region: an N-terminal domain, involved in homomeric and heteromeric protein - protein interactions, and a DNA-binding, C-terminal domain [2].

The Hha binding region was previously identified around helices H1 and H2 of the H-NS N-terminal domain, and Arg12 of H-NS was found to be essential for the interaction [3].

In this study, we report the use of mutagenesis of Hha negative charged residues combined with NMR and fluorescence anisotropy to identify critical residues for H-NS binding. Substitution of Asp48 for Asn results in a complete loss of Hha/H-NS interaction while the Glu25Gln mutation causes a drastic decrease in the binding affinity.

The results described here provide new insights into the contribution of individual amino acids in the binding event that are currently being used to derive a structural model of the H-NS/Hha heterocomplex by computational docking.

[1] Madrid, C., Balsalobre, C., García, J., Juárez, A. *Mol. Microbiol.* **2007**, *63*, 7-14
[2] Dorman, C. J. *Nat. Rev. Microbiol.* **2004**, *2*, 391-400
[3] García, J., Madrid, C., Juárez, A., Pons, M. *J. Mol. Biol.* **2006**, *359*, 679-689