

Department of Chemistry

## Bacterial gene regulation

BioProt /Bioin- Bacterial Mechanisms of Environmental Adaptation Group

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## Objectives

- Identification and isolation of bacterial genes involved in Mo responsive two component signal transduction systems.
- Heterologous expression, purification and characterization of the target protein, a Mo responsive regulatory protein (*MorR*)
- Protein-DNA binding studies. Our aim is to elucidate how *MorR* interacts with DNA, and if post-translational modification, such as phosphorylation, alters the protein conformation and binding.

## Methodology

- Molecular cloning for gene isolation: genetic engineering (PCR, restriction enzymes, and ligation of the target gene with pET vector);
- Protein expression: competent cells are used for recombinant plasmid isolation and protein expression.
- Protein purification: different chromatographic steps are used to purify the target protein
- Protein- DNA interaction: EMSA, footprinting, and fluorescence spectroscopy.
- Post translational modification: small phosphodonors have been used and *MorR* conformational changes have been monitored by gel filtration chromatography and fluorescence spectroscopy

## Expected Results

- Cloning, expression and purification of the target protein.
- Understand the *MorR*/DNA binding mechanism.
- Understand the *MorR* regulation mechanism.
- Investigate the existence of any conformational and functional alteration by post translational modification (phosphorylation)
- Study the interaction of Mo with *MorR*

