Nitric Oxide sensing by the DNR transcription factor from *Pseudomonas aeruginosa*: a novel heme-dependent mechanism

**Serena Rinaldo, Nicoletta Castiglione, Giorgio Giardina, Manuela Caruso and Francesca Cutruzzolà**

Department of Biochemical Sciences, University of Rome La Sapienza, 00185 Rome, Italy.

*Pseudomonas aeruginosa* is a well-known pathogen able to grow under oxygen-limited conditions using the anaerobic metabolism of denitrification (nitrate reduction into dinitrogen via nitric oxide). Denitrification is activated by a cascade of redox-sensitive transcription factors, including the DNR protein. This regulator belongs to the CRP-FNR superfamily and is active under low oxygen tension in the presence of N-oxides; the NO-dependence of its activity suggested that it may act as a NO sensor *in vivo*.

To gain further insight into the mechanism of NO-sensing by DNR, we have developed an *E. coli*-based reporter system to investigate different aspects of the DNR activity. In such background, DNR transactivates the *Pseudomonas aeruginosa norCB* promoter only in the presence of NO; moreover, the NO-dependent DNR activity specifically requires heme biosynthesis. These data support previous *in vitro* studies which demonstrated that DNR binds heme. The *E. coli*-based reporter system was also used to demonstrate that DNR responds specifically to NO, being able to discriminate between NO and CO, two different diatomic heme iron ligands. *In vitro* spectroscopic studies indicate that two histidines coordinate the heme iron; characterization of DNR His mutants *in vitro* and *in vivo* allows the proposal of a novel heme-dependent mechanism for DNR activation.