

## 7 SUMMARY

*Pseudomonas aeruginosa* is an antibiotic resistant opportunistic bacterial pathogen that remains one of the leading causes of death in immunocompromised patients. *P. aeruginosa* synthesizes an arsenal of virulence factors, one of which is the bluish phenazine derivative pyocyanin. The Latin name “*aeruginosa*”, meaning “copper rust”, was given to this species because of its characteristic blue-green color, mostly resulting from the combined excretion of pyocyanin and the yellow metabolite pyoverdine. The physiological function of pyocyanin seems to be complex: it is a respiratory pigment, an antibiotic against other microbials, a signal for *P. aeruginosa* and a prominent virulence factor in pathogenesis as well.

In this work, two proteins with unknown molecular function, PqsE and PA0803 were studied *in vitro* and *in vivo*, using a broad spectrum of techniques. Crystal structures of both proteins alone and in complex with ligands/substrates were determined at high resolution. Together with other methods, potential molecular functions of both proteins were investigated. The structural and functional insights gained here will guide further studies in search for the natural ligands/substrates.

### **Molecular Function of PqsE**

The first project studies one of the molecular mechanisms through which *P. aeruginosa* controls the biosynthesis of pyocyanin as well as some other virulence factors, via a specific signaling molecule termed *Pseudomonas* quinolone signal (PQS). Specifically, the gene product of *pqsE*, a member of the PQS biosynthetic *pqsABCDE* operon, was characterized. Previous studies showed that *pqsE* is not required for the biosynthesis of PQS, but deletion of its gene leads to loss of virulence production, including pyocyanin. This suggested that *pqsE* is a potential target for therapeutic intervention against *P. aeruginosa* infections. It is therefore compelling to understand the gene product at molecular level.

Since its discovery in 1999, PqsE has drawn extensive attention in bacterial quorum sensing research. Many reports in the literature demonstrated the importance of PqsE as a critical link between quorum sensing and virulence, yet despite recent progress providing significant insight into the PQS system, the molecular function of PqsE remains a mystery. In this study, recombinant PqsE was cloned, expressed, purified and characterized.

Surprisingly, PqsE does not interact with PQS at all and is therefore not a PQS signal response protein. Since previous findings, mostly obtained by genetic experiments, did not provide a comprehensive starting point, the crystal structure of PqsE was first determined to 1.6 Å resolution, expecting the three dimensional structure to point out directions of possible molecular mechanisms.

PqsE is a binuclear metallohydrolase with an Fe(III)M(II) mixed-valent activity center. A copurified ligand was assigned as benzoate and may indicate that PqsE exerts its regulatory effect by converting a chorismate-derived molecule. Further, PqsE was found to slowly hydrolyze phosphodiesterases including single- and double-stranded DNA as well as mRNA and also the thioester S-(4-nitrobenzoyl)mercaptoethane. Higher activity was observed after incubation with  $\text{Co}^{2+}$  and, to lesser extent,  $\text{Mn}^{2+}$ , suggesting that the Fe(II)Fe(III) center of recombinant PqsE may be an artifact of heterologous expression. A crystal complex of the E182A mutant with bis-pNPP was obtained and suggests a catalytic mechanism for hydrolysis. Transcriptome analysis of PqsE deletion mutant and a complement strain was carried out. Data suggested that the PqsE natural substrate may be related to anthranilate or catechol.

### **Pyocyanin-Binding Activity of a Hypothetical Protein PA0803**

The 6 mega base-pair *Pseudomonas aeruginosa* genome contains about 5500 ORFs, many of which have never been studied and are annotated as hypothetical proteins. However, lack of apparent connection to known pathways does not mean that they play less important roles for the bacteria.

In the second project reported here, a gene with locus ID PA0803 was investigated. PA0803 has low sequence similarity to a known phenazine resistance protein EhpR from *P. agglomerans* but is not located near either of the two phenazine-biosynthesis gene clusters of *P. aeruginosa*. To clarify whether it can bind phenazines or not, recombinant PA0803 was cloned, expressed, purified and characterized. Crystal structure of PA0803 was determined to 1.6 Å resolution. ITC experiments and the determination of PA0803:PYO complex structure confirmed that PA0803 can bind pyocyanin with low micromolar affinity. This protein, among a few other gene products, may serve as escort proteins of aromatic small molecules.