ESRF

ESRF	**Experiment title:** Transcriptional regulator TraR bound to its autoinducer and to target DNA	**Experiment number**: LS1933 and LS2183
Beamline: ID14-EH2 ID29 ID14-EH3 ID14-EH1	**Date of experiment**: from: 12/07/2001 to: 12/07/2001 from: 16/09/2001 to: 16/09/2001 from: 1/12/2001 to: 1/12/2001 from: 28/02/2002 to: 28/02/2002	**Date of report**: 9 September 2002
Shifts: 3 (EH2) 1,5 (ID29) 2 (EH3) 3 (EH1)	**Local contact(s)**: Dr. Stéphanie MONACO Dr. Andrew THOMPSON Dr Joanne MCCARTHY Dr. Elspeth GORDON	*Received at ESRF:*

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Report:

The LuxR family of transcriptional regulators requires an acyl-homoserine lactone signal molecule to control the regulation of gene expression in response to fluctuations in cell-population density by a mechanism called quorum sensing. In *Agrobacterium tumefaciens*, quorum sensing controls conjugal transfer of the tumour-inducing plasmid,

responsible for plant crown gall disease. The core components of this system are the transcriptional regulator TraR and its inducing ligand N-(3-oxo-octanoyl)-L-homoserine lactone. This complex binds DNA and activates gene expression. We have determined the crystal structure of TraR in complex with its autoinducer and target DNA (PDB code 1h0m). The crystals belong to space group P21 21 21, with unit-cell parameters a = 66.99, b = 94.67, c = 209.66 A, with two [TraR/AAI]2/TraBox complexes in the asymmetric unit. A three-wavelength MAD data set for the seleno-l-methionine-substituted form has been collected to a resolution of 3 Å at beamline ID29.

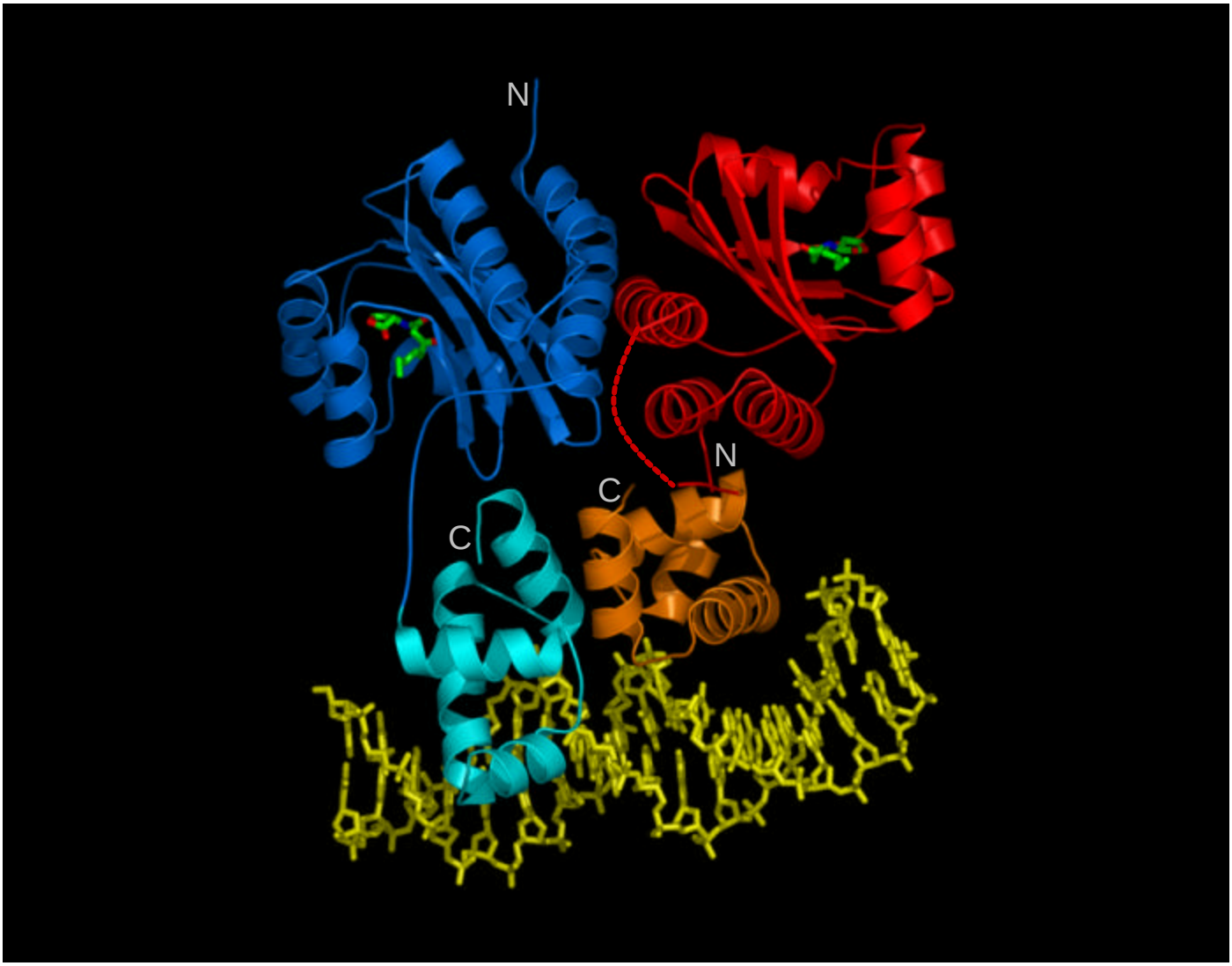
The structure of this ternary complex, $[TraR/AAI]_2$/TraBox, reveals one completely enclosed AAI molecule in each N-terminal ligand-binding domain. The AAI is not directly involved in dimerization. The C-terminal DNA-binding domain binds the DNA duplex with the typical helix-turn-helix motif. In addition, the structure reveals an asymmetric dimer with a different arrangement of the N- and C-terminal domains relative to each other, resulting in one monomer being more elongated than the other. The N-terminal ligand-binding domain has structural similarities with the ubiquitous GAF/PAS domains, which show considerable evolutionary mobility and are involved in the generation of many lineage-specific domain architectures in prokaryotic and eukaryotic cells. The TraR structure suggests that a gene fusion between a GAF/PAS domain and a DNA-binding domain resulted in a transcriptional regulator with a central function in quorum sensing.

The key finding is that, surprisingly, the signal molecule, N-(3-oxo-octanoyl)-L-homoserine lactone (AAI), is completely embedded into a narrow cavity of about 200 $Å^3$, formed by a cluster of hydrophobic and aromatic residues, without any possible solvent contact. In addition, the presence of a completely buried aspartate residue (Asp 70) in this cavity, which stabilizes the binding of the pheromone *via* a hydrogen bond, suggests the key role of the autoinducer in the correct folding of the nascent TraR protein and agrees with results from previous biochemical studies. We propose that the accumulation of the pheromone in the environment and its subsequent diffusion into bacterial cells, leads to the stabilization of the nascent TraR, which is otherwise rapidly degraded by proteases. The correct folding of TraR, mediated by the autoinducer, results in the exposure of the hydrophobic face of the long helix α6 (residues 145-162), thus resulting in dimerization of the ligand-binding domains. Dimerization at the DNA-binding domain does not seem correlated with the dimerization of the ligand-binding domains, induced by the pheromone. However, the dimeric interface of the DNA-binding domains is an indispensable requirement for the binding of TraR to its DNA target sequence, *tra* box, which contains a perfect dyad symmetry. In this scenario, the role of the autoinducer seems to be that of guiding the folding and subsequent formation of stable TraR dimers, which become protease-resistant and therefore bind *tra* box operators *via* their dimeric DNA-binding domains.

In summary, the $[TraR/AAI]_2$/TraBox structure unravels the molecular determinants by which LuxR-type proteins recognize autoinducers and afterwards bind to specific DNA sequences. As documented by the increasing number of publications in the field of bacterial quorum sensing, the exceptional variety of physiological functions controlled by quorum sensing in prokaryotes has just begun to emerge. The structure of this ternary complex could greatly enhance the ability to design new inhibitory compounds to fight pathogenic bacteria of many different species. Furthermore,

the structure reveals the modular organization of the LuxR-family of transcriptional activators, making it a promising candidate for genetic manipulations, which would change a prokaryotic transcription factor into a ligand-dependent eukaryotic transcriptional activator.

Structure of the ternary complex TraR / AAI / *tra*-box



Publications:

- Crystallization and preliminary X-ray diffraction studies of the transcriptional regulator TraR bound to its cofactor and to a specific DNA sequence.
  Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., De Francesco, R., Neddermann, P. and Di Marco, S*. (2002). Acta Cryst. D58, 1362-1364.
- The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA.
  Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., Cortese, R., De Francesco, R., Neddermann, P. and Di Marco, S*. (2002). EMBO J., 21, 1-9.