ESRF	**Experiment title:** Structure determination of the Quorum-Sensing Regulator TraR bound to its natural DNA promoter	**Experiment number**: **LS1933**
Beamline: ID14-2	**Date of experiment**: from 12-07-2001 to 13-07-2001 (3 shifts)	**Date of report**: 20-07-01
Shifts to BAG: 6	**Local contact(s)**: Stephanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

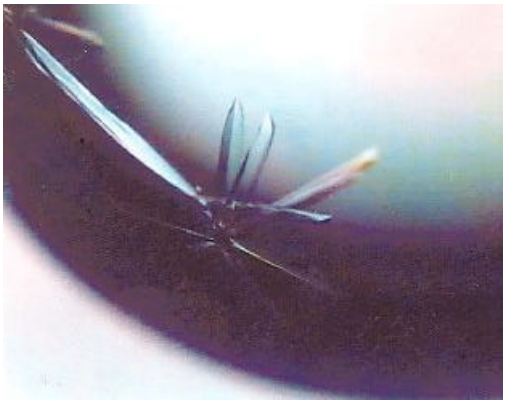
(*)Alessandro Vannini, (*) Cinzia Volpari, Stefania Di Marco
Dept. of Biochemistry
IRBM P. Angeletti
Pomezia, Rome
Italy

Many bacteria are able to regulate the expression of specific gene system in response to the size of their population by a strategy called “quorum sensing”. Such regulation provides that, in response to the amount of bacterial population, a diffusible molecule (acting as a “signal”) can bind and activate a transcription factor, which binds a recognition site in the promoter of the target genes, enhancing their expression. TraR is an *Agrobacterium tumefaciens* transcriptional regulator involved in this paradigm and belongs to the family of LuxR-type proteins.

For activity TraR requires the pheromone N-3-oxooctanoyl-L-homoserine lactone (AAI). TraR-pheromone complexes bind to a single DNA site and activates two promoters that flank this site.

TraR is a 27 KDa protein, which in the presence of the cofactor dimerizes as assayed by light scattering and gel filtration. The recombinant protein is highly soluble and monodisperse and fully active with a Kd for DNA binding (18 bp) of 4 nM. The protein is mainly alpha-helical, as assayed by CD spectroscopy and there are no evidence of post-translational modification, as assayed by mass spectrometry. It binds the cofactor with a 1:1 stoichiometry (1 AAI per protein monomer), as determined by a multiple reaction monitoring mass spectrometry experiment.

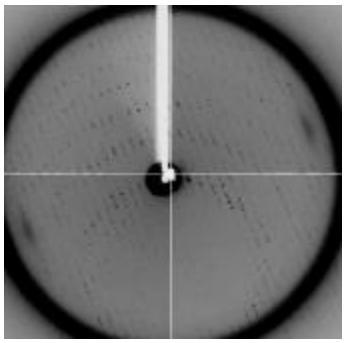
We have obtained diffraction quality crystals of the ternary complex Trar/AAI/DNA (dimensions of the crystals are about 0.7 x 0.1 x 0.1 mm.) with the native protein (see fig below).



Previously, we have screened native crystals at our home x-ray source and only for few of them we got diffraction (6 Å max resolution), in addition the pattern was very anisotropic. Also some crystals were brought at ESRF and tested at ID14-H1 (see previous report LS1933) and again some crystals did not diffract at all, but some of them diffracted to 4.0-4.5 Ang (very anisotropic diffraction) and we were able to collect some images for indexing and spacegroup determination. In the meantime we worked on crystals optimisation and Se-Met production. We got crystals of the Se-Met protein and we did a fluorescence scan at ID29 (2 hour test) of these new crystals. The Se is present and is all reduced.

During the experiment of this report (LS1933, the first aimed to this project), we have screened a lot of crystals (about 60, native and SeMet derivative) grown in several different conditions. All these crystals diffract at about 3 Å with low anisotropy, but often they get cracked in cryosolutions. For this reason, we have screened also several conditions for cryocooling. We collected one data-set of the native complex at 3.9 Å resolution. Crystal have been indexed as tetragonal with unit cell dimension of a =b =76.161 Å, c =208.415 Å, a =ß =? =90° (see table 1).

Furthermore, we have collected a data-set of the SeMet crystal at 3.4 Å resolution (see diffraction pattern below). Unfortunately, radiation damage has occurred early in this crystal. Crystal has been indexed as the native one (tetragonal, same unit cell dimensions) and the processing is ongoing.



At the moment, we are trying to improve conditions for cryoprotection of these crystals in order to extend the resolution limit and to decrease radiation damage.

Furthermore, we have obtained diffracting crystals of the ternary complex also in presence of Br-substituted bases in the DNA sequence. We intend to solve the structure by MAD phasing at the Se-edge and/or Br-edge.

Solving the structure of the complex TraR/AAI/DNA will allow us to investigate about the structural determinants who drives the "switch" from inactive to active form of TraR (i.e. dimerization), and the residues involved in the site-specific recognition on DNA. In addition, this structure will be the first representative of the LuxR-type family and using the TraR structure as a model we could extend the structural work to truncated forms of TraR, as well as to other proteins of this family, e.g. LuxR. This project can be further extended to mutagenesis studies.

Understanding more about the quorum sensing pathway, is important not only for improving some biotechnological methods, but has also medical implications as the quorum-sensing system is present in many pathogenic organism, e.g. *Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus*.

Table 1

Crystallographic Data Collection Statistics of native TraR crystal

Unit cell parameters (Å)	a = b=76.161 c = 208.415 $\alpha = \beta = \gamma = 90$
Space group	P4(3)2(1)2 or P4(1)2(1)2
Resolution range (Å)	20 – 3.9
No. reflections measured	42,159
No. unique reflections	6,448
completeness (%)	97.5 (98.3)
R_{merge}(%)	17.0 (66.1)
$\langle I \rangle / \langle sI \rangle$	9.8 (3.0)

Postal address: User Office, ESRF, B.P. 220, F-38043 GRENOBLE Cedex, France
Street address: 6 rue Jules Horowitz, F-38043 GRENOBLE Cedex
Telephone: +33 (0)4 7688 2552; Fax: +33 (0)4 7688 2020; e-mail: useroff@esrf.fr