

# Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA

Rong-guang Zhang<sup>+</sup>, Terina Pappas<sup>†</sup>, Jennifer L. Brace<sup>+</sup>, Paula C. Miller<sup>‡</sup>, Tim Oulmassov<sup>‡</sup>, John M. Molyneaux<sup>‡</sup>, John C. Anderson<sup>‡</sup>, James K. Bashkin<sup>‡</sup>, Stephen C. Winans<sup>‡</sup> & Andrzej Joachimiak<sup>\*</sup>

<sup>\*</sup> Bioscience Division/Structural Biology Center, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, Illinois 60439, USA  
<sup>†</sup> Department of Microbiology, Cornell University, Ithaca, New York 14853, USA  
<sup>‡</sup> Monsanto Company, 800 N. Lindbergh Boulevard Street, Louis, Missouri 63167, USA  
<sup>§</sup> Present address: Pharmacia Corporation, St. Louis, Missouri, USA

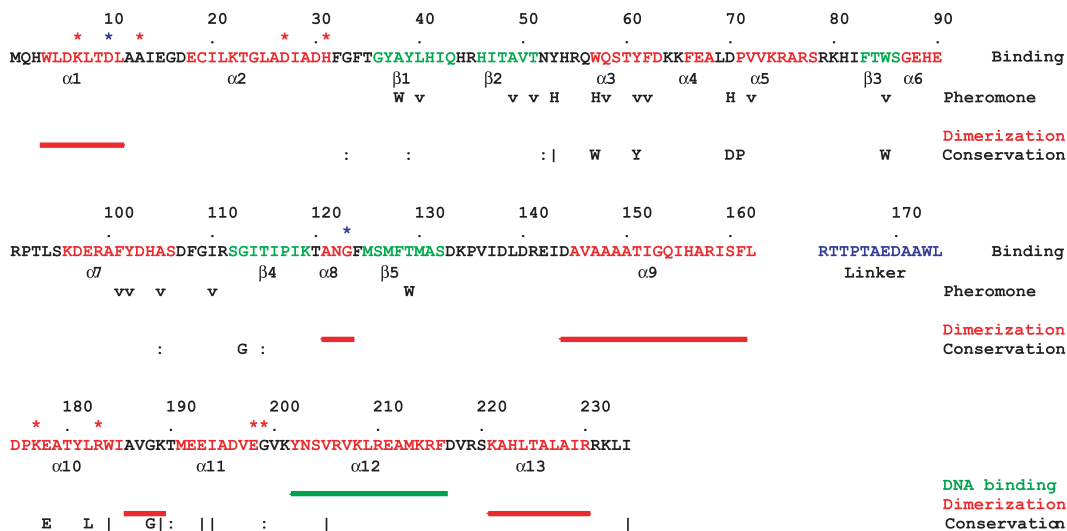
Many proteobacteria are able to monitor their population densities through the release of pheromones known as *N*-acylhomoserine lactones. At high population densities, these pheromones elicit diverse responses that include bioluminescence, biofilm formation, production of antimicrobials, DNA exchange, pathogenesis and symbiosis<sup>1</sup>. Many of these regulatory systems require a pheromone-dependent transcription factor similar to the LuxR protein of *Vibrio fischeri*. Here we present the structure of a LuxR-type protein. TraR of *Agrobacterium tumefaciens* was solved at 1.66 Å as a complex with the pheromone *N*-3-oxooctanoyl-L-homoserine lactone (OOHL) and its TraR DNA-binding site. The amino-terminal domain of TraR is an  $\alpha/\beta/\alpha$  sandwich that binds OOHL, whereas the carboxy-terminal domain contains a helix–turn–helix DNA-binding motif. The TraR dimer displays a two-fold symmetry axis in each domain; however, these two axes of symmetry are at an approximately 90° angle, resulting in a pronounced overall asymmetry of the complex. The pheromone lies fully embedded within the protein with virtually no

solvent contact, and makes numerous hydrophobic contacts with the protein as well as four hydrogen bonds: three direct and one water-mediated.

Many bacteria communicate by releasing specific chemical signals (pheromones) whose concentrations increase at high population densities. Bacteria are thought to use these signals to induce expression of particular target genes only at high population sizes, a phenomenon referred to as quorum sensing<sup>1</sup>. A large family of regulatory systems has been described, each resembling the LuxI and LuxR proteins of *V. fischeri*. LuxI-type proteins synthesize *N*-acylhomoserine lactone pheromones (AHLs, also referred to as autoinducers), which diffuse from the bacteria that produce them either passively or by means of active efflux, and accumulate at high population densities. AHLs are thought to bind to and activate LuxR-type receptor proteins, which function as cytoplasmic transcription factors, either as transcriptional activators or repressors<sup>2</sup>.

LuxR is composed of two domains. An *Escherichia coli* strain that overexpresses the N-terminal fragment of LuxR sequesters its cognate AHL, indicating that this domain is sufficient for pheromone binding<sup>3</sup>. Overexpression of this fragment also has a dominant negative effect on the activity of the full-length protein, suggesting that this domain can mediate protein oligomerization<sup>4</sup>. The C-terminal domain of LuxR-type proteins has a predicted helix–turn–helix (HTH) motif and is thought to make sequence-specific contacts with DNA. The C-terminal domain, when overexpressed *in vivo*, is sufficient to activate transcription<sup>5</sup>, and has been shown *in vitro* to facilitate RNA polymerase binding to a target promoter<sup>6</sup>. Studies of LuxR-type proteins have been hindered by a lack of structural data.

TraR of *Agrobacterium tumefaciens* is a close homologue of LuxR and displays similar properties. The protein is coded for by the tumour-inducing (Ti) plasmid of this plant-pathogenic bacterium, where it activates *tra* genes, required for interbacterial plasmid transfer<sup>7</sup>. The *traR* gene is induced by nutrients called opines that are released from plant tumours, ensuring that quorum sensing by *A. tumefaciens* occurs only in the tumour environment. Both LuxR



**Figure 1** Functional and structural roles of amino acid residues in TraR. Residue colour scheme: red, helical; green, form  $\beta$ -strands; blue, indicate an inter-domain linker; black, random coils. Residues participating in pheromone binding are indicated using the letters v (van der Waals interactions), H (direct protein–pheromone hydrogen bonding), and W (water-mediated hydrogen bonding). Residues that participate in protein dimerization are marked with a red bar, whereas the DNA binding helix is indicated with a green bar.

Residues that are identical in at least 95% of LuxR-type proteins are indicated using a letter, whereas residues that are at least 80% or 60% identical in LuxR-type proteins are marked with a vertical line or a colon, respectively (see ref. 1 for a recent compilation). Residues required for positive control are marked with a blue asterisk; those involved in N- and C-terminal domain interactions are marked with a red asterisk.

and TraR require their respective pheromones for DNA binding *in vivo*<sup>8,9</sup>. The TraR N-terminal domain acts as a transdominant mutant and inactivates the full-length protein *in vivo* and *in vitro* by forming heterodimers<sup>9,10</sup>. We previously purified TraR as a complex with the pheromone OOHL bound at a one-to-one stoichiometry<sup>11</sup>. TraR is a dimer in solution and binds target promoters *in vitro* as a dimer<sup>12,13</sup>. TraR recognizes specifically 18-base pair (bp) palindromic DNA sequences called 'tra boxes', one of which is centred 42 bp upstream of the divergent *traA* and *traC* promoters<sup>11</sup>. A similar *tra* box is found upstream of the *traI* promoter, which is regulated by TraR<sup>7</sup>.

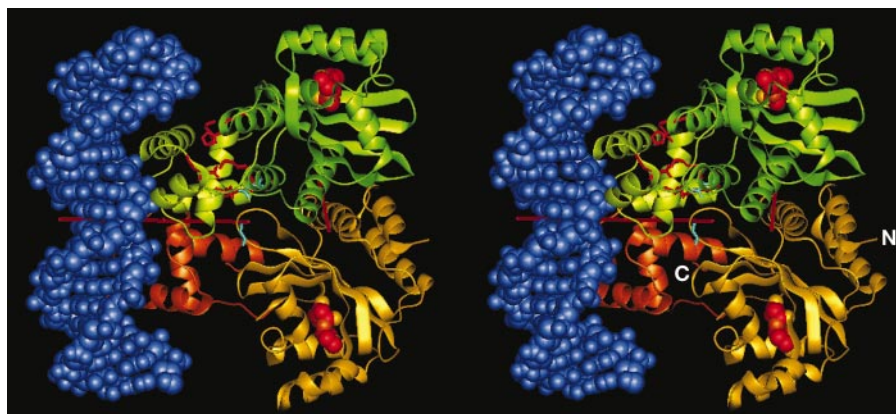
We crystallized TraR in the presence of OOHL and a self-complementary oligonucleotide containing the canonical *tra* box sequence. The crystal asymmetric unit contains two structurally similar TraR dimers, each binding two molecules of OOHL and one duplex DNA fragment. The N-terminal domain of each monomer (residues 1–162) binds one molecule of OOHL, whereas the C-terminal domain (residues 175–234) binds to half of a *tra* box. A twelve-residue amino acid linker joins these domains (Fig. 1, residues indicated in blue). The two N-terminal domains and OOHL molecules of the TraR dimer show a two-fold rotational symmetry, and similarly, the two C-terminal domains and bound *tra* box DNA also show a two-fold rotational symmetry. However, the two axes of rotation are at an approximately 90° angle to each other (red lines in Fig. 2), creating a pronounced asymmetry for the entire complex (see below).

The N-terminal domain consists of an  $\alpha/\beta/\alpha$  sandwich. The central  $\beta$ -sheet has five anti-parallel strands and is curved, with the pheromone bound to its concave surface. The pheromone is fully embedded within the protein and has no significant contact with solvent (Figs 2 and 3a). Hydrophobic and aromatic residues constitute most of the OOHL binding pocket. The fatty acyl chain runs parallel to the  $\beta$ -sheet surface and is accommodated by several hydrophobic side chains (Leu 40, Thr 51, Tyr 53, Tyr 61, Phe 62, Val 72, Trp 85, Ile 110). Several of these residues are highly conserved among LuxR family members (Tyr 53, Tyr 61, Val 72, Trp 85, Ile 110) and make extensive van der Waals contacts with the pheromone. In addition, the highly conserved residue Gly 113 positions the side chain of Trp 85. Hydrogen bonds contribute to pheromone binding, including one between Trp 57 and the ring keto group, another between Asp 70 and the imino group, and another between Tyr 53 and the 1-keto group (Fig. 3b). A fourth hydrogen bond is made between the 3-keto group of OOHL and a water molecule that is

stabilized by hydrogen bonding to Thr 129 and Ala 38 (Fig. 3b). All of these residues are highly conserved<sup>1</sup>.

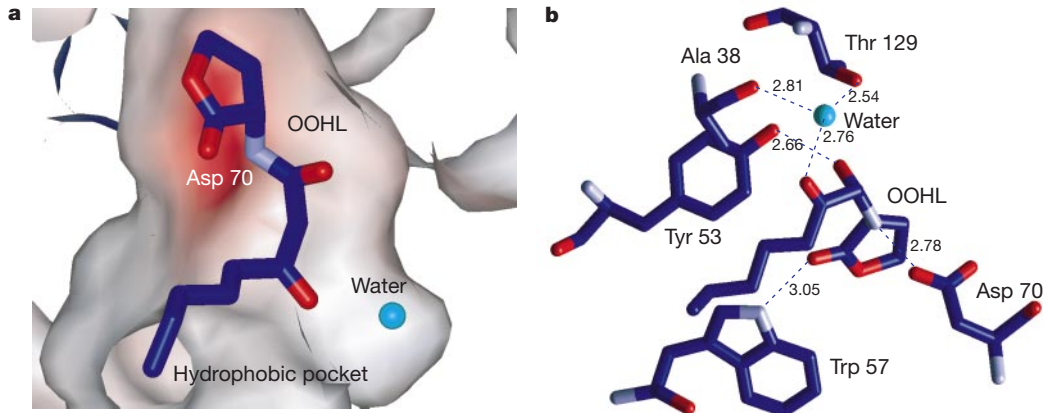
The complete encapsulation of the pheromone by TraR is similar to the binding of the insect pheromone bombykol to its cognate receptor protein<sup>14</sup>. This encapsulation helps to explain why OOHL is bound virtually irreversibly to TraR<sup>11,13</sup>. We have previously shown that TraR synthesized in *E. coli* in the absence of OOHL is proteolysed rapidly by the Clp and Lon proteases, and that its half-life both in *E. coli* and in *A. tumefaciens* is extended 20-fold by the addition of OOHL<sup>11,12</sup>. These experiments indicate that TraR may require OOHL as a scaffold to help it acquire a protease-resistant tertiary structure. However, this is probably not true for all regulatory system members, as some LuxR-type proteins form multimers even in the absence of pheromone<sup>15</sup> and EsaR protein is thought to bind DNA only in the absence of pheromone<sup>16</sup>. Furthermore, these DNA-bound apoproteins remain receptive to pheromone, indicating that their pheromone binding sites must be accessible.

The DNA-binding domain of TraR is a four-helix bundle containing a HTH DNA-binding motif<sup>7</sup>. The DNA in the complex has a canonical B conformation and is slightly bent, following the surface of the protein (Fig. 2). We confirmed this DNA bend by gel mobility assays using permuted DNA fragments containing *tra* box DNA (data not shown). TraR recognizes the *tra* box DNA primarily through a recognition helix (residues 202–216) that lies perpendicular to the DNA axis and penetrates the surface of the major groove (Fig. 4). The orientation of the recognition  $\alpha$ -helix is stabilized by a salt bridge formed between the conserved residues Arg 215 and Glu 178. TraR makes sequence-specific contacts with six bases in each half-site of the *tra* box. Sequence-specific hydrogen bonds are made primarily by Arg 206 and Arg 210. Arg 206 contacts G<sub>-5</sub> and C<sub>-4</sub> of one DNA strand and G<sub>4</sub> of the complementary strand. Arg 206 also forms a hydrogen bond with Asn 203, which in turn contacts phosphate<sub>-7</sub>, while Arg 210 contacts G<sub>-7</sub>, T<sub>-6</sub> and G<sub>-5</sub> of one strand and C<sub>5</sub> of the complementary strand. Additional van der Waals contacts are made between Val 207 and G<sub>-7</sub>, Val 207 and T<sub>-8</sub>, and Glu 211 and T<sub>-8</sub>. Tyr 202 makes a water-mediated hydrogen bond with T<sub>3</sub> and hydrogen bonds with Arg 206. Residues Met 191 (main chain imino group), Asn 203, Ser 204, Lys 208 and Lys 221 contact the DNA backbone. The completely conserved residue Gly 188 is a part of the DNA-binding domain dimerization interface. It permits the close approach of the protein to the minor groove, although no contact is made with DNA bases.



**Figure 2** Stereo view of the structure of the TraR–OOHL–DNA complex. Domains in the two monomers are shown in different colours (light/dark orange and light/dark green), whereas the DNA is coloured blue and the OOHL is coloured red. Note that the two-fold dyad axis of the DNA and DNA-binding domains lies in the plane of the page (horizontal red line), whereas that relating to the pheromone-binding domains is swiveled by

approximately 90° (short red line). Side chains of residues in the upper monomer (light/dark green) that mediate interaction between DNA-binding and pheromone-binding domains are shown in red and residues that affect transcription activation are shown in light blue. The N terminus and C terminus of the lower subunit are labelled.



**Figure 3** The pheromone-binding site. **a**, Surface around the pheromone, which is coloured by the pK (red for acidic and blue for basic residues) of the residues of the pheromone-binding cavity. **b**, Four hydrogen bonds between the pheromone and TraR.

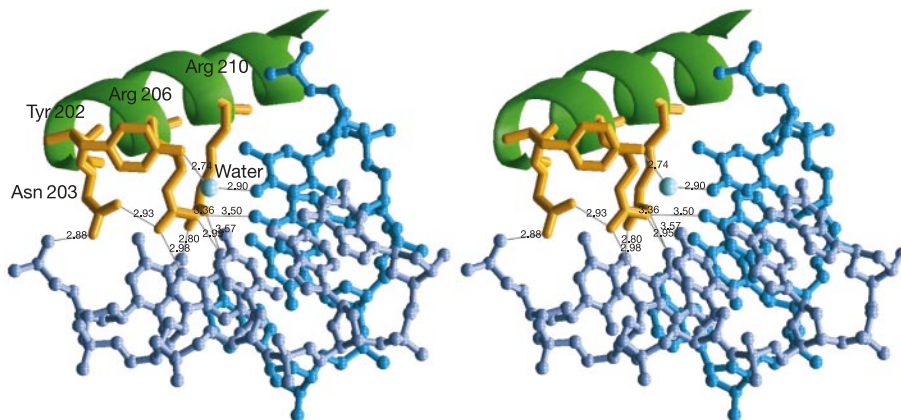
The hydrogen bond between the 3-keto group and protein is water-mediated. The distance between interacting atoms is shown in Å.

The N-terminal and C-terminal domains both contribute to protein dimerization. The two N-terminal domains dimerize chiefly through  $\alpha$ -helix 9 of each protomer (the longest  $\alpha$ -helix in the protein), forming a coiled-coil, whereas  $\alpha$ -helix 1 of each subunit contacts  $\alpha$ -helix 8 of the opposite subunit. There are a number of direct contacts between domains, including a salt bridge between Asp 6 and Lys 119, a cation- $\pi$  interaction between Arg 158 and Phe 161, hydrogen bonds between Gln 154 and the carbonyl of Lys 119 and between Asp 10 and the amide of Asn 122, as well as water-mediated and van der Waals contacts. This structural model is supported by genetic and biochemical studies showing that these domains are sufficient to promote protein dimerization<sup>10,13</sup>. The two C-terminal domains dimerize through  $\alpha$ -helix 13 of each subunit, which form a second coiled-coil. Additional contacts are made by salt bridges between the C-terminal carboxylate of each subunit and Arg 230 of the opposite subunit. The DNA-binding domain shows a pronounced structural similarity to that of the *E. coli* NarL protein<sup>18</sup>; however, unlike the DNA binding domains of TraR, those of NarL do not appear to form a dimer in the crystal.

In contrast, few contacts are made between the N-terminal and C-terminal domains of the protein. Most of such interactions occur within one monomer in the asymmetric dimer: two salt bridges (Lys 7 and Glu 198, Asp 27 and Lys 177) and two hydrogen bonds (His 31 to the carbonyl of Gly 199, Arg 183 to the carbonyl of Ala 13) (Fig. 2b). In this monomer, the linker region (residues 163–174) between

the N- and C-terminal domains is disordered. In contrast, the second monomer shows almost no contacts between the N- and C-terminal domains.

The TraR protein of the nopaline-type Ti plasmid pTiC58 (over 90% identical to TraR of the octopine-type Ti plasmid) has been subjected to extensive mutagenesis. Deletion of the N-terminal 2–4 residues abolishes transcriptional activity and has dominant negative effects *in vivo*<sup>9</sup>. Our structure shows that residue Trp 4 is buried within the domain and may contribute to structural integrity. Deletion of two amino acids from the C terminus abolishes *tra*-box binding *in vivo* and causes a strong dominant negativity against wild-type TraR<sup>9</sup>. Both side chains are buried within the domain, forming part of the hydrophobic core, and may contribute to the integrity of this domain. Similarly, the double mutations M213I-R215H and M191I-R206H also block DNA binding *in vivo*<sup>9</sup>. Arg 215 forms a salt bridge with Glu 178, whereas, as described above, Arg 206 makes sequence-specific DNA contacts. The C-terminal domain of TraR also binds with high affinity to the antiactivator protein TraM<sup>19</sup>. Residues Pro 176, Leu 182 and Ala 195 were reported to be important for this interaction. Structural analysis of this region shows that these residues are scattered throughout the domain and are unlikely to contact TraM simultaneously<sup>19</sup>. Residues Asp 10 and Gly 123 are essential for transcription activation but not for specific DNA binding<sup>9</sup>, suggesting that these residues could make specific contact with RNA polymerase (RNAP). Both



**Figure 4** Specific protein–nucleic acid contacts between the recognition helix and *tra* box DNA. Side chains of Tyr 202, Asn 203, Arg 206 and Arg 210 are shown. A water

molecule (yellow) forms a hydrogen bond between the DNA and Tyr 202. Distances are shown in Å.

residues are exposed to solvent, and Asp 10 of each subunit is closely juxtaposed to Gly 123 of the opposite subunit. Owing to the asymmetry of the complex, one of the Asp 10–Gly 123 pairs lies close to the DNA-binding domain (and could contact RNAP), whereas the other Asp 10–Gly 123 pair lies far away from the DNA-binding domain. Mutational studies of LuxR suggest that residues in the DNA-binding domain are involved in interactions with RNAP<sup>20</sup>.

Unexpectedly, TraR is strongly asymmetric. In the asymmetric TraR dimer the DNA-binding C-terminal domains and pheromone-binding N-terminal domains behave as distinct units, and the asymmetry of the complex is instigated by a small number of contacts between pheromone- and DNA-binding domains. These interactions are sufficient to maintain a specific orientation in the crystal, as the crystal lattice contacts are predominantly between the N-terminal domains and DNA. The asymmetry of the TraR dimer is reminiscent of that of NarL, whose N-terminal receiver domain contacts one side of the C-terminal DNA-binding domain<sup>18</sup>. The asymmetry of NarL was presented as confirmatory evidence that the N-terminal domain inhibits the function of the C-terminal domain. In contrast, as the TraR crystals described here contain OOHL and *tra* box DNA, TraR must be in an active conformation. TraR could show the same asymmetry *in vivo*, and it also seems likely that the observed asymmetry reflects the conformational freedom between domains allowing TraR to function on bi-directional promoters *in vivo*.

We describe here the structure of a functional complex of quorum-sensing transcriptional regulator TraR from *A. tumefaciens*, a member of LuxR family. Our structural studies support earlier work that TraR requires OOHL as a scaffold for folding and dimerization<sup>11,12</sup>. The pheromone-binding domains appear to provide an extensive dimerization interface, while the DNA-binding domains contribute additional interactions. As a result the pheromone indirectly affects gene activation by increasing stability of TraR and the formation of functional dimers that are predisposed to decode specific TraR-binding sites and activate transcription. The TraR–OOHL–*tra* DNA ternary complexes described here should serve as a prototype for the large family of AHL-induced transcription activators. □

## Methods

### TraR production and purification

TraR was purified from *A. tumefaciens* strain B21(DE3) harbouring pJZ358 (ref. 11) cultured in LB broth supplemented with 5  $\mu$ M OOHL. TraR production was induced using 200  $\mu$ M isopropyl- $\beta$ -D-thiogalactopyranoside. Cells were suspended in a buffer containing 10 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 7.0), 150 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol 5% glycerol, and 100 nM OOHL, and lysed using a French Press. The resulting cleared lysate was purified by successive heparin Sepharose, SP-Sepharose, and MonoS chromatography columns (Pharmacia). TraR was eluted from each using linear gradients from 150 mM to 1 M NaCl. Selenomethionyl (SeMet) TraR was purified as described above from strain B834(DE3) harbouring pJZ358. This strain is a derivative of BL21(DE3), which is auxotrophic for methionine. The strain was cultured in AT minimal medium<sup>21</sup> supplemented with 10 mg l<sup>-1</sup> thiamine, 40 mg l<sup>-1</sup> L-selenomethionine (Sigma), and 5  $\mu$ M OOHL.

### Crystallization, structure determination and refinement

The DNA constructs for co-crystallization were designed using rules described previously<sup>22</sup>. Crystals were produced by vapour diffusion in the presence of the self-complementary oligonucleotide GATGTGCAGATCTGCACATC (underlined residues represent the canonical 18-nucleotide TraR-binding site; nucleotides run from -10 through to 10 (left to right, as in text)). These crystals belong to space group P2<sub>1</sub> and diffract X-rays to 1.66 Å resolution using synchrotron radiation. A three-wavelength multiple anomalous dispersion (MAD) data set was collected to 1.9 Å resolution at the Structural Biology Center 19ID beamline using a protocol described earlier<sup>23,24</sup>. Data were processed and scaled with the HKL2000 software package<sup>25</sup>. A heavy atom search using CNS<sup>26</sup> yielded 8 of the 12 Se sites predicted from the sequence, and these sites were used in phasing; the figure of merit was 0.468. The electron density was calculated to 1.9 Å resolution. After density modification, the DNA region, protein region, pheromone, and ordered solvent were fully interpretable. Protein model building was carried out using a semi-automated approach. The initial model (90%) was built with wARP<sup>27</sup>. Missing sections and poorly fitted regions of the protein were built or corrected manually using QUANTA<sup>28</sup>. The DNA structure and the pheromone model were also built manually using

QUANTA. After most of the model was built, unassigned electron density was found in the interior of the N-terminal domain and was assigned to bound pheromone. The structure was refined with CNS with a crystallographic R-factor of 23.0% and R<sub>free</sub> value of 25.2%.

Received 23 January; accepted 18 April 2002; doi:10.1038/nature00833.

- Whitehead, N. A., Barnard, A. M. L., Slater, H., Simpson, N. J. L. & Salmond, G. P. C. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* **25**, 365–404 (2001).
- Fuqua, C., Parsek, M. R. & Greenberg, E. P. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* **35**, 439–468 (2001).
- Hanzelka, B. L. & Greenberg, E. P. Evidence that the N-terminal region of the *Vibrio fischeri* LuxR protein constitutes an autoinducer-binding domain. *J. Bacteriol.* **177**, 815–817 (1995).
- Choi, S. H. & Greenberg, E. P. Genetic evidence for multimerization of LuxR, the transcriptional regulator of *Vibrio fischeri* luminescence. *Mol. Mar. Biol. Biotechnol.* **1**, 408–413 (1992).
- Choi, S. H. & Greenberg, E. P. The C-terminal region of the *Vibrio fischeri* LuxR protein contains an inducer-independent lux gene-activating domain. *Proc. Natl Acad. Sci. USA* **88**, 1115–1119 (1991).
- Stevens, A. M., Dolan, K. M. & Greenberg, E. P. Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the lux promoter region. *Proc. Natl Acad. Sci. USA* **91**, 12619–12623 (1994).
- Fuqua, W. C. & Winans, S. C. A LuxR–LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumour metabolite. *J. Bacteriol.* **176**, 2796–2806 (1994).
- Egland, K. A. & Greenberg, E. P. Conversion of the *Vibrio fischeri* transcriptional activator, LuxR, to a repressor. *J. Bacteriol.* **182**, 805–811 (2000).
- Luo, Z. Q. & Farrand, S. K. Signal-dependent DNA binding and functional domains of the quorum-sensing activator TraR as identified by repressor activity. *Proc. Natl Acad. Sci. USA* **96**, 9009–9014 (1999).
- Chai, Y., Zhu, J. & Winans, S. C. A defective TraR-like protein of *Agrobacterium tumefaciens* forms heterodimers with TraR *in vitro*, thereby blocking TraR-mediated quorum sensing. *Mol. Microbiol.* **40**, 414–421 (2001).
- Zhu, J. & Winans, S. C. Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters *in vitro* and decreases traR turnover rates in whole cells. *Proc. Natl Acad. Sci. USA* **96**, 4832–4837 (1999).
- Luo, Z. Q. & Winans, S. C. The quorum-sensing regulator TraR of *Agrobacterium tumefaciens* requires autoinducer for protein folding, dimerization, and protease resistance. *Proc. Natl Acad. Sci. USA* **98**, 1507–1512 (2001).
- Qin, Y. *et al.* Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J.* **19**, 5212–5221 (2000).
- Sandler, B. H., Nikonova, L., Leal, W. S. & Clardy, J. Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. *Chem. Biol.* **7**, 143–151 (2000).
- Welch, M. *et al.* N-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J.* **19**, 631–641 (2000).
- von Bodman, S. B., Majerczak, D. R. & Coplin, D. L. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *Proc. Natl Acad. Sci. USA* **95**, 7687–7692 (1998).
- Nelson, H. C. Structure and function of DNA-binding proteins. *Curr. Opin. Genet. Dev.* **5**, 180–189 (1995).
- Baikalov, I. *et al.* NarL dimerization? Suggestive evidence from a new crystal form. *Biochemistry*. **37**, 3665–3676 (1998).
- Luo, Z.-Q., Qin, Y. & Farrand, S. K. The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interacting with the C-terminal region of the quorum-sensing activator. *J. Biol. Chem.* **275**, 7713–7722 (2000).
- Egland, K. A. & Greenberg, E. P. Quorum sensing in *Vibrio fischeri*: analysis of the LuxR DNA binding region by alanine-scanning mutagenesis. *J. Bacteriol.* **183**, 382–386 (2001).
- Tempe, J., Petit, A., Holsters, M., Van Montagu, M. & Schell, J. Thermosensitive step associated with transfer of the Ti plasmid during conjugation: possible relation to transformation in crown gall. *Proc. Natl Acad. Sci. USA* **74**, 2848–2849 (1977).
- Joachimski, A. & Sigler, P. B. Crystallization of protein–DNA complexes. *Methods Enzymol.* **208**, 82–99 (1991).
- Walsh, M. A., Dementieva, I., Evans, G., Sanishvili, R. & Joachimski, A. Taking MAD to the extreme: ultrafast protein structure determination. *Acta Crystallogr. D* **55**, 1168–1173 (1999).
- Walsh, M. A., Evans, G., Sanishvili, R., Dementieva, I. & Joachimski, A. MAD data collection—current trends. *Acta Crystallogr. D* **55**, 1726–1732 (1999).
- Otwinowski, Z. & Minor, W. Processing of x-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307–326 (1997).
- Brünger, A. *et al.* Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr. D* **54**, 905–921 (1998).
- Perrakis, A., Morris, R. & Lamzin, V. S. Automated protein model building combined with iterative structure refinement. *Nature Struct. Biol.* **6**, 458–463 (1999).
- QUANTA, Molecular Simulations Inc, San Diego. (2000).

## Acknowledgements

This work was supported by Monsanto Company, the US Department of Energy, Office of Biological and Environmental Research, and a National Research Service Award to S.C.W.

## Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to A.J. (e-mail: andrzej@anl.gov). The atomic coordinates have been deposited with Research Collaboratory for Structural Bioinformatics (RCSB) into the Protein Data Bank (PDB) under code 1L3L for the PDB and 015611 for RCSB.