Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis

Thomas Simon Shimizu*†, Nicolas Le Novère*†, Matthew Daniel Levin*, Andrew J. Beavil‡, Brian J. Sutton‡ and Dennis Bray*§

*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
‡The Randall Centre, New Hunt's House, King's College London, Guy's Campus, London Bridge, London SE1 1UL, UK
†These authors contributed equally to this work
§e-mail: d.bray@zoo.cam.ac.uk

Coliform bacteria detect chemical attractants by means of a membrane-associated cluster of receptors and signalling molecules. We have used recently determined molecular structures, in conjunction with plastic models generated by three-dimensional printer technology, to predict how the proteins of the complex are arranged in relation to the plasma membrane. The proposed structure is a regular two-dimensional lattice in which the cytoplasmic ends of chemotactic-receptor dimers are inserted into a hexagonal array of CheA and CheW molecules. This structure creates separate compartments for adaptation and downstream signalling, and indicates a possible basis for the spread of activity within the cluster.

An important challenge of contemporary biology is to deduce the spatial arrangement of protein molecules in a living cell. Genome-sequencing projects, in conjunction with high-throughput X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, have provided us with a large and rapidly growing database of high-resolution protein structures. But fitting these molecules together into the large protein complexes found throughout the cell — such as multimeric enzymes involved in metabolism or genetic processes, cytoskeletal structures responsible for cell shape and motility, and assemblies of signalling proteins that mediate the responses of the cell to environmental stimuli — presents considerable difficulties. X-ray-diffraction analysis of a multiprotein structure usually requires that the molecular aggregates are small, stable and well-defined, whereas electron microscopy has insufficient resolution to allow the packing at an atomic level to be deduced.

A third option, at least in principle, is to use the information contained in the molecular structures themselves to identify binding relationships and hence deduce spatial location. Automated methods of protein–protein docking exist but are of limited value for large complexes, being frequently overwhelmed by enormous combinatorial possibilities[1]. An alternative approach is to investigate interactions using physical models[2,3] and here we present an approach in which accurate solid models generated by rapid prototyping were used to investigate surface complementarity and protein–protein interactions in a large signalling complex. The manipulation of solid models in real space, guided by clues from experimental data, offers a powerful and intuitive means of exploring the structure and functional implications of protein–protein interactions. We re-created selected configurations, identified by means of hand-held models, with computer modelling packages in order to conduct a more detailed analysis.

In this study, we examined the cluster of chemotactic receptors associated with the plasma membrane of *Escherichia coli*[4]. In particular, we focused on the association of the serine receptor Tsr with the autophosphorylating kinase CheA and the small transducing protein CheW[5,6]. This signalling complex detects concentration changes of serine and other substances in the environment of the cell and transmits this information into the cell in the form of a phosphorylation signal that regulates flagellar rotation[7]. The receptors are thought to exist in thermal equilibrium between two conformational states — one enhances the activity of CheA, whereas the other inhibits it. The output of the complex, which is usually measured in terms of phosphorylation levels of CheA or of its phosphoacceptor CheY, is related to this equilibrium[6]. From the standpoint of signal processing, the complex produces an amplified output that, because of associated adaptational machinery, is proportional to the rate of change of receptor occupancy. The molecular structures of most of its components are now known, but their detailed spatial arrangement and stoichiometry, which are crucial for the signalling properties of the complex, remain to be determined.

Results

Orientation of CheA. The starting point for our model building was the structure of the core region of *Thermotoga maritima* CheA, which has recently been determined at 0.26-nm resolution[8]. This core region, which crystallizes as a dimer, comprises two chains of ~380 amino acids each, containing a dimerization domain, a catalytic domain and a regulatory domain. An interesting feature of this structure is that the two CheA monomers show slightly different conformations, which are interpreted as corresponding to the active and inactive forms[8]. We made a solid model of this structure as two monomers, which we subsequently joined at their dimerization domains (Fig. 1). We examined this model for possible clues to the interaction of CheA with other components of the receptor complex.

CheA uses the γ-phosphate of ATP to phosphorylate itself at a histidine residue, and then transfers this active phosphoryl group to a molecule of the flagellar regulator CheY. The site of ATP-binding has been identified as a cleft on the surface of the core molecule, on the basis of sequence and structural similarity to a type II topoisomerase, gyrase B[8]. The CheY-binding site is actually located in a separate domain (P2), which is linked to the core through a linker of 25 amino acids[9]. This linker is thought to function as a flexible tether[10] and therefore does not have a defined position, but its most probable location is close to the tether site on the core molecule (Ser293). Strikingly, the area around Ser293 lies on the same side of the CheA molecule as the ATP-binding site, so that all four reactive

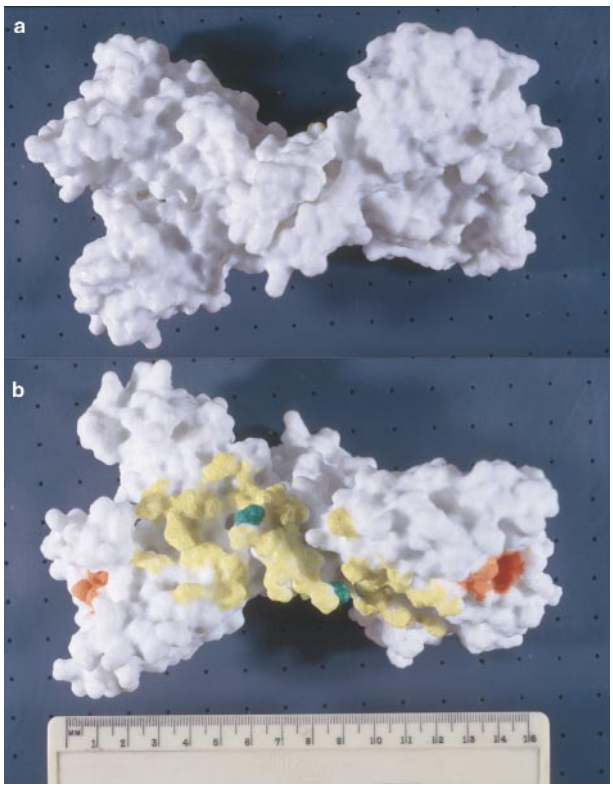

Figure 1 **Core region of CheA. a**, Photograph of a solid model of CheA made to a scale of 1:15,000,000, according to Protein Data Bank file 1B3Q. **b**, The same model, rotated by 180° about a horizontal axis in the plane of the page. The positions of the ATP-binding cleft (orange) and the Ser293 to which the CheY-binding P2 domain is attached (green) are indicated. The region within which diffusion of P2 is limited, assuming a linker length of 2 nm, is also highlighted (yellow). Note that all four reactive sites on the dimer lie on the same surface of the molecule, which we therefore assume on the basis of kinetic arguments to face away from the plasma membrane and towards the cytoplasm.

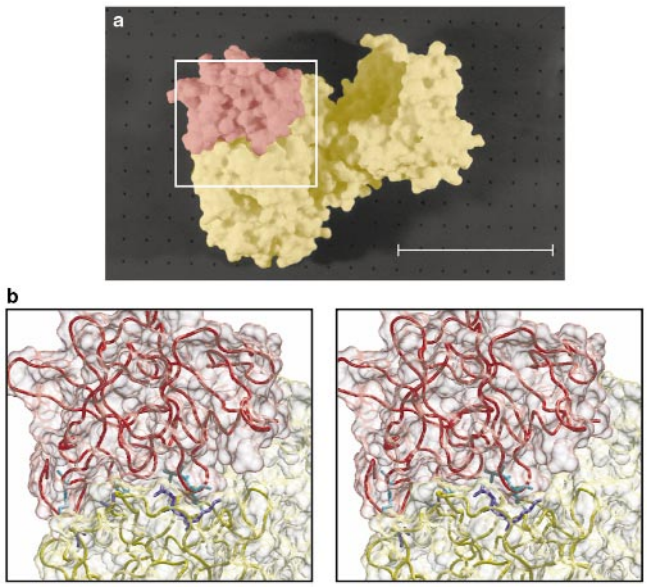

Figure 2 **Proposed binding of CheA to CheW. a**, Photograph of solid models of CheA (highlighted in yellow) and CheW (pink) in the bound state. Scale bar represents 10 cm. **b**, Stereo view of the boxed region around the binding interface in **a**, generated using the reconstructed computer model. The backbone of CheW (red) and the regulatory domain of CheA (yellow) are shown as tubes beneath their respective transparent surfaces. Side chains of residues that may form hydrogen bonds at the binding interface are highlighted in green (CheW; residues Lys9, Thr86, Lys87 and Thr112) and blue (CheA; residues Ser568, Asp573, Lys604, Glu605 and Asp627).

sites on the dimeric molecule face in the same direction (Fig. 1b). We reasoned that this reactive surface of CheA would be likely to face into the cell and away from the plasma membrane, as a rapid flux of CheY molecules occurs between CheA and the flagellar motors, and the more rapidly this can occur, the faster will be the response time of the bacterium.

Positioning of CheW. The next step was to decide on the probable location of the small coupling protein CheW, which is thought to bind both to the regulatory domain of the CheA core[11,12] and to the cytoplasmic tail of the chemotactic receptors[13]. We used the atomic coordinates of *T. maritima* CheW (provided by F. W. Dahlquist) to construct a solid model with which we investigated possible docking sites on the CheA core.

In the absence of a structure for CheW, it has been proposed that a hydrophobic face at the distal end of the CheA regulatory domain (containing residues Val582, Arg586, Gly587 and Pro591) may be the functional interface for CheW binding[8]. The basis of this proposal is that this region forms contacts between CheA regulatory domains in the crystal lattice, and that given the similarity of sequence between CheW and the CheA regulatory domain, this symmetrical interface could have evolved in an ancestral homodimer. However, a search for regions of surface complementarity using plastic models of CheA and CheW led us to a second site between the membrane-facing surface of the CheA regulatory domain and a groove on the CheW surface, containing residues Lys9, Phe11, Asp28 and Lys87 (Fig. 2). In this new binding, a larger amount of the surface area of CheW is buried (~8 nm^2) than at the other site (~3 nm^2), as calculated using the Lee–Richards algorithm[14], and CheW is positioned in a favourable orientation for receptor interaction (see below).

Positioning of Tsr. We then considered the interaction between the CheA–CheW complex and the cytoplasmic tails of the chemotactic receptor. The structure of most of the cytoplasmic portion of the *Escherichia coli* serine receptor Tsr has recently been determined[15], confirming that it is a long α-helical coiled coil. In the crystal structure, the tails of three Tsr dimers come together into a tripartite structure, and we assumed that the same trimeric structure is present in the cluster of receptors in the cell. From the atomic coordinates of the Tsr dimer (provided by S. H. Kim), we reconstructed the trimeric assembly using the program MOLMOL[16]. We then made a solid model of the cytoplasmic tip of this structure (residues 348–427) — the region that is implicated in CheW binding[13,17]. This region is also known as the highly conserved domain because of the striking sequence similarity among known homologues of methyl-accepting chemotaxis proteins (MCPs), even outside the bacterial kingdom[18].

Previous studies involving phenotypic suppression of missense mutations in CheW and Tsr[13], as well as cysteine-scanning experiments in Tar[17] have identified several residues that are likely to be close to the binding interface of CheW and Tsr. We used this information, in conjunction with the solid models, to investigate possible functional contacts between the two proteins. One particular assembly was found in which CheW docked firmly into a groove between two Tsr dimers, near the extremity of the helical hairpin (Fig. 3). This configuration maintains the dimerization domain of the CheA dimer parallel to the axis of the Tsr bundle, which is consistent both with the symmetrical role of CheA and with the further extension of the network. Furthermore, in this arrangement, a protruding

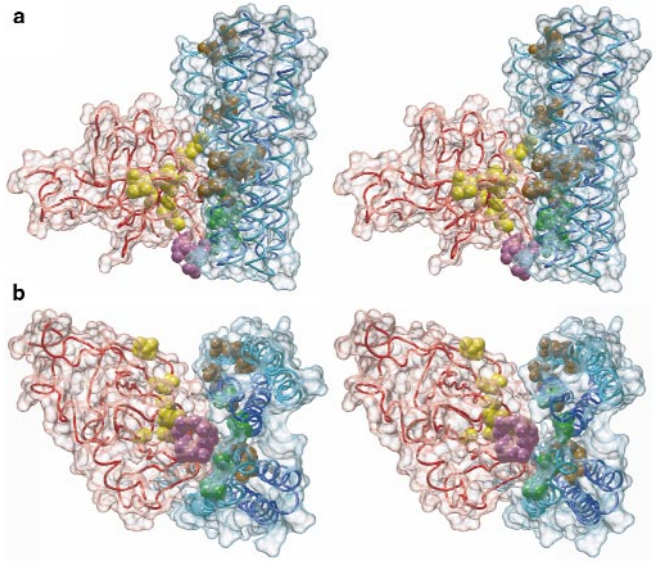

Figure 3 **Proposed binding of CheW to Tsr. a**, Stereo 'side view', in which the line of sight is parallel to the plasma membrane. **b**, Stereo 'bottom view', looking towards the membrane from the cytoplasm. CheW is shown in red; Tsr dimers are shown in blue and cyan. Residues identified by phenotypic-suppression studies[13] are shown in yellow (CheW; residues Met32, Lys79, Ile81, Gly92, Val98 and Val101), purple (CheW; residues Arg56, Gly57 and Arg58) and brown (Tsr; residues Ser357, Gln374, Thr375, Ala400, Glu402 and Ala413). Residues identified by mutational studies as essential for activity[17] are shown in green (Tsr; residues Ala383, Ala387, Ala397 and Ala400). Ala400 was identified by both procedures. Note the purple projection (the 'light switch' (see text)) that is inserted between two adjacent Tsr dimers.

loop in the CheW molecule containing residues Arg56, Gly57 and Arg58 (all of which have been identified in phenotypic-suppression experiments) is tightly locked between the helical hairpins of two Tsr dimers. This configuration indicates a sterically activated 'light switch' for the transmission of the signal from Tsr to CheA. Movement of the Tsr signalling domains could move the CheW loop, and this change could be transmitted through the protein to the interface with CheA. The species difference between the crystal structures we have used (Tsr from *E. coli*; CheW and CheA from *T. maritima*) is unlikely to affect this interaction, as six putative *T. maritima* MCPs (identified by sequence similarity) maintain 70% sequence identity with *E. coli* Tsr in the region of interaction (residues 370–416). Furthermore, all but one of the ten *E. coli* residues implicated by the phenotypic-suppression[13] and cysteine-scanning[17] studies are conserved in these sequences.

Hexagonal network composed of trigonal units. We arranged three CheA–CheW complexes around a Tsr bundle, according to the configuration described above. The result is a triangular 'vault' formed from CheA and CheW, with Tsr as the 'keystone' (Fig. 4). An important criterion for us in selecting this arrangement was that it could be extended laterally into a two-dimensional lattice lining the plasma membrane. The structural unit of the lattice is identical to the vault structure, but with three CheA monomers rather than dimers (Fig. 4). These trigonal units can be joined at the CheA dimerization domains (according to the crystal structure of the dimer) to produce a large-scale hexagonal pattern, representing the clusters of receptors and associated proteins observed in *E. coli*[4] (Fig. 5a). At the centre of each hexagon, there is a small pore of ~10 nm in diameter, surrounded by CheA molecules. Molecules of CheA and CheW together form a layer that is parallel to the plasma membrane but is separated from it by a distance of ~26 nm — the length of the cytoplasmic domain of Tsr (Fig. 4a).

Estimates of the number of chemotactic receptors in a single *E. coli* cell range from ~1,500 to ~4,500 (refs 19–22). If these were all incorporated into a network such as that described in Fig. 4, they would create a patch of 0.2–0.6 μm in diameter, which is in the previously reported range[4]. More realistically, we would expect the extent of network formation to vary temporally, and perhaps also to be influenced by the recent signalling history of the cell[23].

The stoichiometry of the receptor complex is not well established, and has been assigned values ranging from a canonical ternary complex 1T:1W:1A (where T, W and A refer to monomers of receptor, CheW and CheA, respectively) to ~7T:2W:1A[19,24,25]. In our proposed model, the proportion of receptors in an extended (infinite) network would be 2T:1W:1A. However, it is easy to imagine smaller aggregates based on this pattern having a different composition. For example, a small unit composed of two trimers of receptor dimers on either

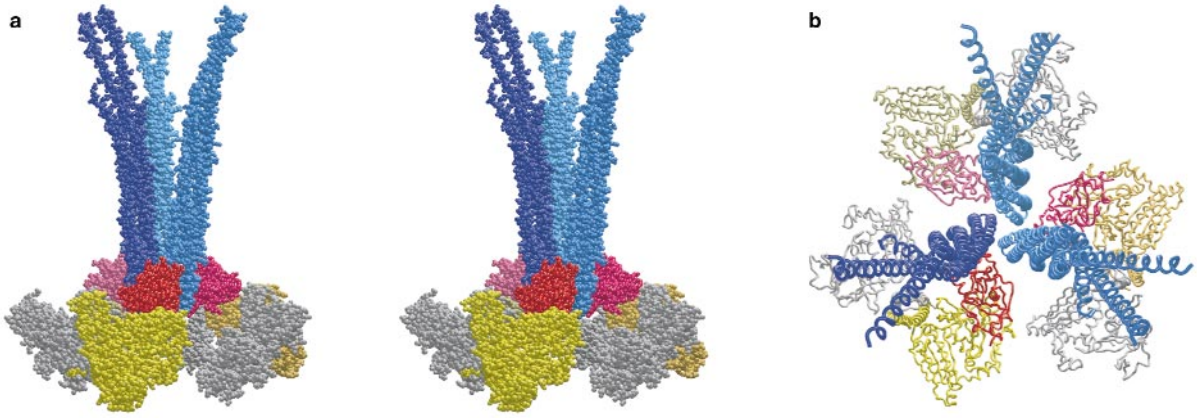

Figure 4 **Structural unit of the lattice.** The repeating trigonal unit of the receptor lattice contains three receptor dimers, three CheW monomers and three CheA monomers. Here we show this trigonal unit with three CheA dimers, rather than monomers, to illustrate the fact that the trigonal unit is not stable on its own. **a**, Stereo 'side view' of the space-filling representation, in which the line of sight is parallel to the plasma membrane. **b**, 'Plan view' of the tube representation, looking towards the cytoplasm from the membrane. In both **a** and **b**, components of the trigonal unit are represented in colour (different shades of blue for receptors, red for CheW and yellow for CheA), whereas the CheA monomers that would be components of adjacent units are shown in grey. Figs 2–4 were created using VMD and Raster3D[42].

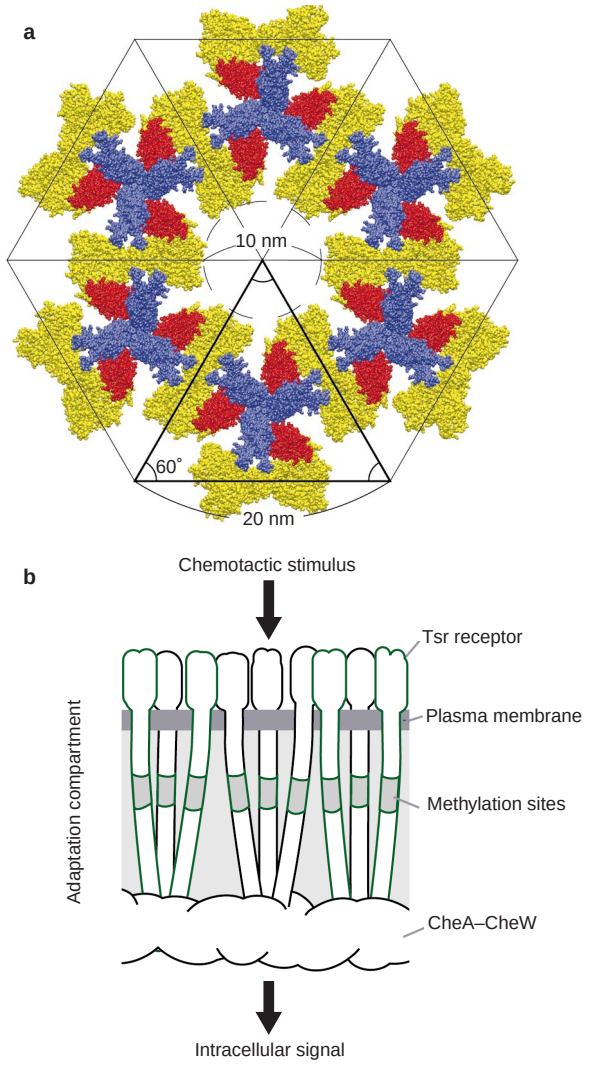

Figure 5 **Hexagonal network. a**, 'Plan view', as seen from the plasma membrane looking into the cell. Receptors are shown in blue, CheW in red and CheA in yellow. Note the 'pores' that form where the vertices of the trigonal units meet in the network. The molecular model was constructed in MOLMOL and the image was generated using VMD. **b**, Schematic side view of the network, showing the proposed 'adaptation compartment' (grey).

side of a CheA dimer, to which they are linked by CheW molecules, would have the composition 6T:1W:1A and could correspond to the receptor-containing complexes prepared by *in vitro* aggregation[25].

Discussion

The proposed structure has several interesting features from the standpoint of chemotaxis, including the new idea of an adaptation compartment. The densely packed layer of CheA and CheW molecules creates, in effect, a compartment on the inner face of the plasma membrane that encloses the long α-helical cytoplasmic domains of the receptors (Fig. 5b). This portion of the receptor carries the four sites at which methyl groups are added or removed during adaptation[26,27]. It seems logical to suppose that this compartment would also contain the two enzymes that catalyse the modification reactions — the methyltransferase CheR (which uses S-adenosylmethionine as a methyl donor) and the methylesterase CheB. These enzymes would function more efficiently if they were sequestered in a compartment with their substrates, especially in the case of CheR, which is present in low copy number in the cell[28]. Both CheR[29] and CheB[30] have been shown to dock to a conserved five-amino-acid sequence (the NWETF motif) at the carboxy terminus of the receptor, close to the membrane. The compartment proposed here would serve to limit the diffusion of the two enzymes away from their docking sites, thus enhancing their sequestration near to their substrates.

The case of CheB is interesting, as this is also a substrate for CheA phosphorylation and competes with CheY for binding to the P2 domain of CheA[31]. If CheB is indeed located in the adaptation compartment as we propose, then this would restrict its access to the catalytic face of CheA. However, the rate of phosphorylation of CheB is some seven times slower than that of CheY (R. C. Stewart, personal communication) and could probably be achieved by the passage of CheB molecules through the small pores in the lattice shown in Fig. 4 to the reactive face of the CheA molecules.

The strategy of making an 'adaptation sandwich' also makes sense from the standpoint of kinetics. A swimming bacterium is continually subjected to thermal buffeting and must make very rapid shifts in flagellar rotation in order to achieve a directed motion. Experimentally, responses to sudden changes in chemoattractant occur in <0.2 s (refs 32, 33). A sandwich that has the ligand-binding domains on one side and catalytic sites on the other — with the slower-acting machinery of adaptation in between — would provide an efficient segregation of function. Indeed, it could be argued that the advantages of an adaptation compartment might have driven the evolution of the remarkably long α-helical cytoplasmic tails, which are a distinctive feature of this class of receptors.

Lastly, the proposed structure provides a possible route by which conformational changes could spread from one receptor to the next. Conformational spread has been proposed as a mechanism to increase the sensitivity and range of the chemotactic response[34,35], and recent evidence supports the idea that interactions between receptors are mediated by formation of a complex with CheA and CheW[23]. The symmetry of the trigonal unit 6T:3W:3A (Fig. 4) would allow it to form a quaternary structure that is able to switch between conformational states in a concerted manner. The close contact between CheA monomers would then allow this 'signal' to pass to the three surrounding units, resulting in a spread of activity across the receptor network. Note, however, that a crucial link in the spread of conformational change would be its progression from one half of a CheA dimer to the other. If this view is correct, then CheA will be seen to have two functions — first as a generator of phosphoryl groups, and second as an essential link in the spread of conformational signals through the network.

Our model should be applicable not only to Tsr, but to all MCP transducers, including the aerotaxis receptor, because of the high degree of sequence similarity in the region that interacts with CheW. The pairwise identity between residues 370–416 of *E. coli* Tsr and all other *E. coli* MCPs ranges between 85.1% and 95.7%, and all residues identified by phenotypic suppression[13] and cysteine scanning[17] are conserved. Moreover, as the length of the cytoplasmic domain is also highly conserved among these transmembrane transducers[18], a laterally extended receptor complex could contain more than one type of MCP. Such a hybrid lattice would have at least two consequences for signal transduction. Firstly, the lattice could act as an integrator of different signals, in which each signal affects the response of the system to others through the coupling of trigonal units. Second, this interaction of signals, as well as the proximity of transducers in the lattice, could also affect the adaptation process. *E. coli* cells expressing only the low-abundance transducers (Trg and Tap) cannot adapt to changing stimulus concentrations[36–38], although normal adaptation is observed when the high-abundance transducers (Tsr and Tar) are co-expressed. By

providing a structural basis for the crosstalk[27] between the different transducer species, the lattice could help to explain signalling and adaptation by the low-abundance transducers. □

Methods

Generation of solid models.

To create the solid models of molecules, triangulated solvent accessible surface files were generated (in the STL file format) from atomic coordinates in PDB format using the program VMD[39]. Coordinates of CheA and the Tsr cytoplasmic domain may be found in Protein Data Bank files 1B3Q and 1QU7, respectively. The STL file format is common to almost all manufacturers of rapid-prototyping equipment, and companies that offer an engineering bureau service are easy to find. Models were manufactured using a Stratasys Genisys 3D printer. This printer uses fused-deposition modelling (FDM) technology and operates as if it were a 3D pen-plotter, extruding a thin bead of thermosetting plastic (Polyester-P1500) to define a shape layer by layer. In all cases, models were made to a scale of 15 mm per nm, and a resolution of 0.3 mm (0.02 nm), thereby allowing the contours of atoms to be compared with the same structures displayed on the computer screen using programs such as RasMol[40], MOLMOL, Swiss-PdbViewer[41] and VMD.

Searching for docking interfaces using solid models.

The solid models were used to explore the geometric complementarity between the components of the complex. Plastic models were manoeuvred by hand to rapidly search through translational and rotational orientations. In searching for CheA–CheW binding, we assumed that CheW makes contact with the regulatory domain of CheA and that the core region of CheA is orientated with the reactive sites facing the cytoplasm (see Results). For CheW–Tsr binding, the residues that have been identified as likely sites of interaction by phenotypic-suppression[13] and cysteine-scanning[17] experiments were used as clues to determine the interface.

Reconstruction of complexes in molecular graphics programs.

The structures of bound complexes identified using the solid models were reconstructed *in silico* using molecular graphics packages. Swiss-PdbViewer was used to reconstruct the CheA–CheW complex (Fig. 2) on the basis of the binding selected using the solid models. The distances between three pairs of residues on the solid models were first measured and converted to nm using the scale at which the model was built. The computer models of the proteins were then manoeuvred so as to match these distances. Rotamer combinations of side chains that were within 0.3 nm of the interface were explored in a heuristic fashion, using the rotamer library of Swiss-PdbViewer to minimize energy at the interface. The complex between CheW and the Tsr bundle (Fig. 3) was constructed in a similar manner using MOLMOL. The vault-shaped trigonal structure (Fig. 4) was constructed in MOLMOL by rotations (120° and 240°) of the CheA–CheW complex around the Tsr bundle.

RECEIVED 16 JUNE 2000; REVISED 10 JULY 2000; ACCEPTED 20 JULY 2000;
PUBLISHED 4 OCTOBER 2000.

1. Dixon, J. S. Evaluation of the CASP2 docking section. *Proteins* **1 (suppl.),** 198–204 (1997).
2. Skawinsky, W. J. *et al.* The application of stereolithography to the fabrication of accurate molecular models. *J. Mol. Graphics* **13,** 126–135 (1995).
3. Bailey, M. J., Schulten, K. & Johnson, J. E. The use of solid physical models for the study of macromolecular assembly. *Curr. Opin. Struct. Biol.* **8,** 202–208 (1998).
4. Maddock, J. R. & Shapiro, L. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. *Science* **259,** 1717–1723 (1993).
5. Eisenbach, M. Control of bacterial chemotaxis. *Mol. Microbiol.* **20,** 903–910 (1996).
6. Falke, J. J., Bass, R. B., Butler, S. L., Chervitz, S. A. & Danielson, M. A. The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Annu. Rev. Cell Dev. Biol.* **13,** 457–512 (1997).
7. Armitage, J. P. Bacterial tactic responses. *Adv. Micro. Phys.* **41,** 229–289 (1999).
8. Bilwes, A. M., Alex, L. A., Crane, B. R. & Simon, M. I. Structure of CheA, a signal-transducing histidine kinase. *Cell* **96,** 131–141 (1999).
9. McEvoy, M. M., Hausrath, A. C., Randolph, G. B., Remington, S. J. & Dahlquist, F. W. Two binding modes reveal flexibility in kinase/response regulator interactions in the bacterial chemotaxis pathway. *Proc. Natl Acad. Sci. USA* **95,** 7333–7338 (1998).
10. Morrison, T. B. & Parkinson, J. S. Liberation of an interaction domain from the phosphotransfer region of CheA, a signaling kinase of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **91,** 5485–5489 (1994).
11. Bourret, R. B., Davagnino, J. & Simon, M. I. The carboxy-terminal portion of the CheA kinase mediates regulation of autophosphorylation by transducer and CheW. *J. Bacteriol.* **175,** 2097–2101 (1993).
12. Morrison, T. B. & Parkinson, J. S. A fragment liberated from the *Escherichia coli* CheA kinase that blocks stimulatory, but not inhibitory, chemoreceptor signaling. *J. Bacteriol.* **179,** 5543–5550 (1997).
13. Liu, J. & Parkinson, J. S. Genetic evidence for interaction between CheW and Tsr proteins during chemoreceptor signaling by *Escherichia coli*. *J. Bacteriol.* **173,** 4941–4951 (1991).
14. Lee, B. & Richards, F. M. The interpretation of protein structures: estimation of static accessibility. *J. Mol. Biol.* **55,** 379–400 (1971).
15. Kim, K. K., Yokota, H. & Kim, S.-H. Four-helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor. *Nature* **400,** 787–792 (1999).
16. Koradi, R., Billeter, M. & Wüthrich, K. MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graphics* **14,** 51–55 (1996).
17. Bass, R. B., Coleman, M. D. & Falke, J. J. Signaling domain of the aspartate receptor is a helical hairpin with a localized kinase docking surface: cysteine and disulfide scanning studies. *Biochemistry* **38,** 9317–9327 (1999).
18 Le Moual, H. & Koshland, D. E. Jr Molecular evolution of the C-terminal cytoplasmic domain of a superfamily of bacterial receptors involved in taxis. *J. Mol. Biol.* **261,** 568–585 (1996).
19. Gegner, J. A., Graham, D. R., Roth, A. F. & Dahlquist, F. W. Assembly of an MCP receptor, CheW, and kinase CheA complex in the bacterial chemotaxis signal transduction pathway. *Cell* **70,** 975–982 (1992).
20. Scharf, B. E., Fahrner, K. A. & Berg, H. C. CheZ has no effect on flagellar motors activated by $CheY^{13DK106YW}$. *J. Bacteriol.* **180,** 5123–5128 (1998).
21. DeFranco, A. L. & Koshland, D. E. Jr Molecular cloning of chemotaxis genes and overproduction of gene products in the bacterial sensing system. *J. Bacteriol.* **147,** 390–400 (1981).
22. Hazelbauer, G. L. & Harayama, S. Sensory transduction in bacterial chemotaxis. *Int. Rev. Cytol.* **81,** 33–70 (1983).
23. Li, G. & Weis, R. M. Covalent modification regulates ligand binding to receptor complexes in the chemosensory system of *Escherichia coli*. *Cell* **100,** 357–365 (2000).
24. Bray, D. & Bourret, R. B. Computer analysis of the binding reactions leading to a transmembrane receptor-linked multiprotein complex involved in bacterial chemotaxis. *Mol. Biol. Cell* **6,** 1367–1380 (1995).
25. Liu, Y., Levit, M., Lurz, R., Surette, M. G. & Stock, J. B. Receptor-mediated protein kinase activation and the mechanism of transmembrane signaling in bacterial chemotaxis. *EMBO J.* **16,** 7231–7240 (1997).
26. Borkovich, K. A., Alex, L. A. & Simon, M. I. Attenuation of sensory receptor signaling by covalent modification. *Proc. Natl Acad. Sci. USA* **89,** 6756–6760 (1992).
27. Hazelbauer, G. L., Park, C. & Nowlin, D. M. Adaptational "crosstalk" and the crucial role of methylation in chemotactic migration by *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **86,** 1448–1452 (1989).
28. Subbaramaiah, K. & Simms, S. A. Photolabeling of CheR methyltransferase with S-adenosyl-L-methionine (AdoMet). Studies on the AdoMet binding site. *J. Biol. Chem.* **267,** 8636–8642 (1992).
29. Wu, J., Li, J., Li, G., Long, D. G. & Weis, R. M. The receptor binding site for the methyltransferase of bacterial chemotaxis is distinct from the sites of methylation. *Biochemistry* **35,** 4984–4993 (1996).
30. Barnakov, A. N., Barnakova, L. A. & Hazelbauer, G. L. Efficient adaptational demethylation of chemoreceptors requires the same enzyme-docking site as efficient methylation. *Proc. Natl Acad. Sci. USA* **96,** 10667–10672 (1999).
31. Li, J., Swanson, R. V., Simon, M. I. & Weis, R. M. The response regulators CheB and CheY exhibit competitive binding to the kinase CheA. *Biochemistry* **34,** 14626–14636 (1995).
32. Segall, J. E., Manson, M. D. & Berg, H. C. Signal processing times in bacterial chemotaxis. *Nature* **296,** 855–857 (1982).
33. Khan, S., Amoyaw, K., Spudich, J. L., Reid, G. P. & Trentham, D. R. Bacterial chemoreceptor signaling probed by flash photorelease of a caged serine. *Biophys. J.* **62,** 67–68 (1992).
34. Bray, D., Levin, M. D. & Morton-Firth, C. J. Receptor clustering as a cellular mechanism to control sensitivity. *Nature* **393,** 85–88 (1998).
35. Duke, T. A. J. & Bray, D. Heightened sensitivity of a lattice of membrane receptors. *Proc. Natl Acad. Sci. USA* **96,** 10104–10108 (1999).
36. Hazelbauer, G. L. & Engström, P. Parallel pathways for transduction of chemotactic signals in *Escherichia coli*. *Nature* **283,** 98–100 (1980).
37. Feng, X., Baumgartner, J. W. & Hazelbauer, G. L. High- and low-abundance chemoreceptors in *Escherichia coli*: differential activities associated with closely related cytoplasmic domains. *J. Bacteriol.* **179,** 6714–6720 (1997).
38. Weerasuriya, S., Schneider, B. M. & Manson, M. D. Chimeric chemoreceptors in *Escherichia coli*: signaling properties of Tar–Tap and Tap–Tar Hybrids. *J. Bacteriol.* **180,** 914–920 (1998).
39. Humphrey, W., Dalke, A. & Schulten, K. VMD — visual molecular dynamics. *J. Mol. Graphics* **14,** 33–38 (1996).
40. Sayle, R. & Milner-White, E. J. RasMol: biomolecular graphics for all. *Trends Biochem. Sci.* **20,** 374 (1995).
41. Guex, N. & Peitsch, M.C. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* **18,** 2714–2723 (1997).
42. Merritt, E. A. & Bacon, D. J. Raster3D: photorealistic molecular graphics. *Methods Enzymol.* **277,** 505–524 (1997).

ACKNOWLEDGEMENTS
We thank F. W. Dahlquist, A. M. Bilwes and S. H. Kim for atomic coordinates of chemotaxis proteins, and F. W. Dahlquist for criticism of the manuscript. T.S.S. was supported by a Glaxo International Scholarship and an ORS Award from CVCP. N.L.N. was supported by an EMBO long-term fellowship. This work was supported by a grant from the UK Medical Research Council (to D.B.).
Correspondence and requests for materials should be addressed to D.B.