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## Architecture and Signal Transduction Mechanism of the Bacterial Chemosensory Array: Progress, Controversies, and Challenges

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### Abstract

Recent research has deepened our understanding of the ancient, conserved chemosensory array that detects small molecule attractants and repellents, and directs the chemotaxis of bacterial and archaeal cells towards an optimal chemical environment. Here we review advances towards a molecular description of the ultrastable lattice architecture and ultrasensitive signal transduction mechanism of the chemosensory array, as well as controversies and challenges requiring further research. Ultimately, a full molecular understanding of array structure and on-off switching will foster (i) the design of novel therapies that block pathogenic wound seeking and infection, (ii) the development of highly specific, sensitive, stable biosensors, and (iii) the elucidation of general functional principles shared by receptor patches in all branches of life.

### Keywords

two-component signaling pathway; chemotaxis; chemosensory receptor; methyl-accepting chemotaxis protein; histidine kinase; Tsr; Tar; CheA; CheW; ultrastable

### The Bacterial Chemosensory Pathway

The bacterial chemosensory pathway is an ancient, ubiquitous signaling system that controls cell migration up or down chemical attractant or repellent gradients, respectively, towards an optimal living environment (reviewed in (1-4)). The resulting chemotactic behavior is essential for cell survival under nutrient stress, and to pathogenic processes including wound-seeking (5-7). The pathway components are localized within a two-dimensional, hexagonal signaling lattice termed the chemosensory array, which is remarkable for its highly sensitive detection of one or a few ligand molecules, its positive cooperativity involving dozens of coupled receptors, its multi-week kinetic stability, and its millisecond signaling speed (3,8-13). In short, the chemosensory array functions as an

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ultrasensitive, ultrastable biological integrated circuit or sensory chip. The array is conserved throughout Bacteria and Archaea (14-16), and it belongs to the ubiquitous two-component family of prokaryotic signaling pathways. However, its unique specializations for array structure and function set it apart from other family members, and yield similarities to clustered receptor patches now recognized as important elements in eukaryotic signaling (17-20). The present review focuses on the best-characterized bacterial chemosensory arrays of *Escherichia coli*, *Salmonella typhimurium*, and *Thermatoga maritima*.

## Chemosensory Array Components, Assembly, Architecture, and Ultrastability

### Components

All components of the bacterial chemotaxis pathway localize to the chemosensory array, which typically contains five protein functional classes: (i) transmembrane chemoreceptors for attractants and repellents, (ii) a pair of His and Asp protein kinases that form a phosphotransfer-relay (CheA and CheY, respectively), (iii) an adaptor protein that stabilizes array architecture (CheW), (iv) adaptation enzymes that covalently modify the chemoreceptors (CheR, CheB), and (v) a phosphatase that speeds hydrolysis of phosphoproteins as required for rapid responses to changing stimuli (CheZ) (1-4). High-resolution structures are available for all functional classes, and for several multi-protein complexes (1,4,21-25).

### Core Unit

The first three of the above components constitute the structural and functional core of the array (1,4,26-28). Specifically, the core unit contains two receptor oligomers, the homodimeric His-kinase CheA, and two copies of the adaptor protein CheW (Figures 1A-C). As the receptor oligomer is a trimer-of-homodimers, the resulting receptor:kinase:adaptor stoichiometry is 2:1:2 (oligomers), or 12:2:2 (polypeptide chains). In the current model of core unit architecture, the kinase and adaptor proteins bridge the cytoplasmic tips of the two receptor oligomers (Figs 1B,C) (1,21-28). Core units have been directly detected by cryo-EM imaging of reconstituted complexes on bacterial membranes (1). Furthermore, core units reconstituted in a water-soluble nanodisc system are stable for hours and possess near-native, receptor-regulated kinase activity (29).

### Array Assembly

Figure 1A shows a hypothesized array assembly mechanism in which core units join together to form partial hexagons, then full hexagons, and ultimately the full array, which may contain hundreds of core units and hexagons. The proposed early intermediates have been detected by cryo-EM studies of in vitro reconstitutions that assemble the three core proteins on bacterial membranes (1).

### Array Architecture

The structural framework of the array is provided by the cell membrane, by the long, three-pronged transmembrane receptor oligomers, and by the kinase-adaptor base plate located in

a plane near the receptor cytoplasmic tips (Figs. 1B,C) (1,4,21-28). Diffusion of the receptors in the membrane is prevented by their tight coupling to the base plate via specific contacts with both the kinase and adaptor proteins. The base plate is an interconnected network of six-membered, kinase-adaptor rings, each of which possesses three kinase regulatory domains (P5) alternating with three adaptor proteins (Fig. 1A, right). Since the kinase regulatory domain is structurally homologous to the adaptor protein (both are dual-SH3-like-fold domains (30,31)), the six-membered ring possesses pseudo-6-fold symmetry, and each member binds to one of the surrounding six receptor oligomers.

The thickness of the *E. coli* array is ~350 Å from receptor tip-to-tip (Fig. 1B) (27). The cytoplasmic region resembles a proto-organelle wherein an aqueous, pseudo-compartment of height ~220 Å lies between the membrane and kinase-adaptor base plate (15); this height can vary between species possessing receptor cytoplasmic domains of different lengths (15,32). The edges of the array and the centers of the base plate hexagons remain open to the cytoplasm, but their narrowness can limit the rate of macromolecular diffusion between bulk cytoplasm and the internal compartment (26,27). Within this compartment the adaptation enzymes diffuse between receptors and modify their covalent adaptation sites. The resulting negative-feedback adaptation system enables the array to zero out a constant background concentration of attractant while responding to a superimposed attractant gradient (4).

Some species, such as *Rhodobacter sphaeroides* (33), possess soluble, double-layered cytoplasmic arrays in which two hexagonal lattices formed by soluble receptors, kinases and adaptor proteins are sandwiched back-to-back. Similar double-layered, soluble arrays can be formed by reconstituting the isolated cytoplasmic domains of transmembrane receptors with the kinase and adaptor proteins (1). In these soluble arrays the two-layered architecture appears to provide the structural integrity normally provided by the membrane in cell surface arrays.

### Protein-Protein Contacts at the Array Vertices

Cryo-EM has provided a wealth of information about global array architecture (1,26,27). At higher resolution, crystallographic and NMR studies of complexes between core protein fragments have provided valuable insights into key array protein-protein contacts, while disulfide mapping has identified essential contacts in functional, full-length, membrane-bound core complexes (21-25). Four types of repeating contacts anchor the array at its vertices. Some or all of these contacts must also transmit signals between proteins in the same or different core units (see below). (i) The kinase-receptor contact is formed by the tight coupling between the kinase regulatory domain and the N-terminal helix of the receptor protein-interaction region (Figs. 1E, and 2B below). Structural studies of core protein fragments originally provided two alternative models of this contact (23,26). Disulfide mapping studies carried out in functional, full-length, membrane-bound, reconstituted core complexes revealed the correct model, illustrating the synergistic information provided by high resolution studies of soluble fragment complexes and disulfide mapping of intact, membrane-bound core complexes (21,23). (ii) The adaptor-receptor contact is believed to be structurally homologous to the kinase-receptor contact, based on structural studies of fragment complexes and the homologous folds of the kinase regulatory domain and the

adaptor protein (Fig. 1F) (23,26). (iii) The structure of kinase-adaptor interface 1 has been fully defined by crystallographic and disulfide mapping studies (22,23). This core unit interface also provides half of the contacts within the kinase-adaptor ring (Fig. 1D). (iv) Kinase-adaptor interface 2 provides the other kinase-adaptor ring contacts (crystallographic model in Fig. 1D) and bridges adjacent core units in higher order assemblies (23,26,27).

### Ultrastability

Arrays formed in cells then isolated in bacterial membranes, as well as core complexes reconstituted on isolated bacterial membranes, are kinetically ultrastable and continue to exhibit receptor-regulated kinase activity for long periods up to weeks at room temperature (10,11). This remarkable long term stability is believed to arise from the extensive network of protein contacts provided by core unit and lattice organization (10,11). Eventually, the kinase activity decays as the bound kinase molecules are clipped by a protease endogenous to the membranes, leading to loss of the N-terminal substrate domain and kinase function (10). Reconstituted core complexes exhibit both quasi- and ultra-stable components with lifetimes of ~1.5 days and ~3 weeks, respectively. Incorporation of a tryptophan bump at the kinase-receptor interface or at kinase-adaptor interface 1 preferentially destabilizes the ultrastable component, such that the entire population becomes quasi-stable. In contrast, a disulfide bond covalently coupling the latter interface shifts the quasi-stable component into the ultra-stable state, such that the entire population becomes ultrastable with a longer exponential lifetime spanning at least 6 weeks (34). Overall, the evidence indicates that ultrastability protects the kinase from proteolysis, and requires well-formed contacts within the core unit.

### Complementary Systems for Studying Core Units and Arrays

Rapid progress in the field has been facilitated by a diverse set of core unit and array preparations that each offer unique advantages as well as limitations. Native, functional hexagonal arrays formed in cells can be analyzed *in vivo* by cryo-EM or specialized chemical methods (1,26,27,40,46,68,69), or can be isolated in cell membranes for *ex vivo* studies (11). Membrane-bound core complexes can be reconstituted by combining isolated, receptor-containing cell membranes with purified CheA kinase and CheW adaptor protein (11,21,22,34). The resulting, functional complexes allow incorporation of chemically and chromophore-modified components, enabling unfettered disulfide chemistry and spectroscopic analysis of core unit structure, assembly and signaling mechanism. The reconstituted core complexes range in size from core units and small oligomers, including individual hexagons formed by three core units, up to small hexagonal arrays (1). Present also are nonfunctional receptor aggregates termed zippers, but stoichiometric analysis indicates that at least 2/3 of the receptors are in functional core complexes; moreover, the zippers are invisible in the standard kinase activity-based assays (11,21,22,34). Individual core units can be reconstituted in the nanodisc bilayer system that provides the simplest functional complex, eliminates interactions between core units, and also enables incorporation of modified components (4,29). Most recently, studies of soluble, membrane-bound, and crystallized complexes of core component fragments have utilized EPR, NMR, mass spec and crystallographic methods to probe core complex structure

(1,23-26,33,39,52,61). Although these fragment systems lack attractant regulation, they provide the highest resolution structural information.

### Structural Controversies and Challenges

The robust combination of cryo-EM imaging of native arrays, crystallographic and NMR studies of soluble fragment complexes, and disulfide mapping studies of intact, functional, reconstituted core complexes on membranes has revealed many key elements of array architecture. Not yet tested, however, is the hypothesized array assembly mechanism. In addition, the proposed adaptor-receptor and kinase-adaptor 2 contacts have not yet been tested by disulfide mapping in intact core complexes. Perhaps the main outstanding architectural question is whether the adaptor protein fills the empty hexagons in the array with a symmetric 6-membered ring homologous to the pseudo-symmetric kinase-adaptor ring (Fig. 1A, right). Turning to ultrastability, the importance of contacts within core units is now well established, but the contributions of contacts between core units has not been tested. Moreover, the available evidence suggests that in live cells the array-bound kinase and adaptor components exchange with bulk cytoplasmic copies more rapidly than the bulk exchange observed for complexes on isolated cell membranes (10,11,35). This faster exchange is hypothesized to arise from an unidentified cytoplasmic array disassembly machinery, perhaps Hsp70 or Hsp90 (36,37), that is lost when membranes are isolated. In cells, such machinery would ensure that the ultrastable array does not exhibit unregulated growth when protein synthesis is operating.

## Signal Transduction within Core Units, and Cooperative Signaling Between Core Units

### Minimal Effects of Signaling on Global Array Structure

In the absence of attractant, apo-receptors stimulate the His-kinase activity of native arrays and of core complexes built from the three core components, while attractant binding to the chemoreceptors inhibits the kinase (4). Cryo-EM comparison of the on- and off-states of native arrays has shown that the receptor and base plate lattices are maintained and do not undergo major, detectable rearrangements during on-off switching (38), consistent with previous evidence that receptor on-off switching involves subtle structural and/or dynamical changes (39-43). Detectable changes are observed near the cytoplasmic surface of the base-plate, likely arising from receptor-regulated changes in the positions or dynamics of CheA kinase domains outside the base-plate (40). The lattice structure likely constrains the magnitudes of conformational changes that can occur during on-off switching.

### Signaling within the Core Unit

Receptor-regulated, kinase on-off switching is an inherent feature of the core unit in isolated nanodiscs, in reconstituted core complexes, and in native arrays. In all three systems, in the absence of attractant the activated kinases exhibit similarly large specific activities, whereas the addition of attractant inhibits these specific kinase activities about  $10^2$ -fold in each system (11,29). On-off switching in all three systems can be regulated by covalent receptor adaptation as well as attractant binding (4). In short, the signaling properties of the core unit

and higher order assemblies, including arrays, are similar – except in attractant titrations wherein the arrayed assemblies exhibit significantly higher positive cooperativity (2,9,44,45).

**Receptor on-off switching mechanism**—For simplicity, the mechanism of on-off switching is discussed herein for a single chemoreceptor homodimer, noting its native context is the receptor trimer-of-dimers within a core unit. Broadly, the receptor can be divided into (i) a transmembrane signaling module containing the periplasmic ligand binding domain and the transmembrane helices, and (ii) a cytoplasmic signaling module containing the HAMP, adaptation, Gly hinge, and protein interaction regions (Figure 2A) (4).

Multiple, independent lines of evidence support a conserved, piston-type signaling mechanism in the transmembrane signaling module of chemoreceptors (4,46). In the apo state of the receptor, the attractant binding site is, on average, closed, and the transmembrane signaling helix (TM2) is in its “up” position. During a thermal fluctuation that opens the pocket, attractant binds and traps the transmembrane signaling helix in its “down” position translocated  $\sim 1.6 \text{ \AA}$  towards the cytoplasm (Fig. 2A). The signal can be asymmetric due to negative cooperativity between the two attractant binding sites of the homodimer, resulting in attractant binding and displacement of the TM2 helix in one subunit only. Alternatively, the signal can be symmetric and displace TM2 in both subunits. Other mechanisms, including scissors and rotational, are disfavored by extensive data (4,46). In contrast to the array-forming chemoreceptors, the other major class of two-component pathway receptors – sensor His-kinase receptors – are structurally distinct from chemoreceptors, generally do not form arrays, and are proposed to undergo a large-amplitude scissors rearrangement during transmembrane signaling (47). Thus, CheA-linked chemoreceptors and CheA-independent sensor His-kinase receptors appear to possess different signaling mechanisms evolved for their different contexts within an array, or in the absence of an array, respectively.

In the long, cytoplasmic signaling module an alternating static-dynamic mechanism (also termed yin-yang) has been proposed for signal transmission through adjacent structural regions (42,43,48-52). The model postulates that when one region switches from its more tightly packed, more static state to its less tightly packed, more dynamic state, the adjacent region makes the opposite transition from dynamic to static, or vice-versa. Up to three cytoplasmic regions – the HAMP domain, the adaptation region, and the protein interaction region – are hypothesized to be coupled in this way, such that the kinase-activating and -inhibiting states possess dynamic-static-dynamic and static-dynamic-static configurations in these three regions, respectively. Notably, the three regions are separated by flexible regions important for signal transmission: (i) the junction between HAMP and the adaptation region is susceptible to proteolysis (53) and contains a Gly hotspot where large sidechains lock the receptor in the kinase-activating on-state (54), and (ii) the junction between the adaptation and protein interaction regions contains a multi-Gly hinge where substitutions can trap either the on- or off-state (55).

The alternating static-dynamic model has been tested independently by multiple methods in intact, membrane-bound, core complexes and arrays. Mutational, functional and structural

studies of HAMP support the model, as do local charge engineering, disulfide trapping and engineered socket studies of the adaptation region, and an engineered socket study of the protein interaction region (42,43,49-52). In addition, the latter socket study (50) indicates that the crystal structure of the protein interaction region (56) represents the kinase off-state stabilized by multiple socket (knob-in-holes) interactions between pairs of helices both within and between the two identical subunits. The loss of even one socket interaction, which would presumably increase local helix dynamics, typically prevents attractant-triggered kinase inhibition as predicted by the alternating static-dynamic model (50).

A recent MD simulation of the protein interaction region has revealed a Phe ring flip at the buried homodimer interface, involving the most highly conserved residue in chemoreceptors (F396 Tsr, F394 Tar) (57). The ring flip is correlated with distance changes between adjacent helices, and with the receptor signaling state. The conserved Phe is located near the contact surface thought to transmit information from receptor to kinase, and this same position is the location of the only engineered, intersubunit disulfide bond in the region that locks the kinase-on state (50). These findings suggest that the conserved Phe may play an important role in receptor signal transmission, and could be directly involved in static-dynamic or conformational on-off switching.

Many chemoreceptors detect small molecule attractants (such as Ser, Asp for the two commonly studied amino acid receptors) with small binding energies, while sending a signal over 300 Å to the kinase (4,46). Both the transmembrane piston and cytoplasmic alternating static-dynamic signals are small amplitude, low energy transitions well-suited for such signal transmission (22,50,58,59). In the piston model the incompressibility of an  $\alpha$ -helix (which is much easier to bend than to compress), together with the isoenergetic nature of the 1.5 Å piston displacement, ensures that the small displacement is carried the length of the signaling helix with little damping. Such a small helix sliding movement costs little energy since specific side chain contacts with adjacent helices are maintained by side chain flexibility (60). Similarly, the alternating static-dynamic model proposes a nearly isoenergetic transition between adjacent receptor regions, since each state possesses a set of tight and loose helix-helix interactions that are simply swapped during on-off switching.

The signal exiting the receptor protein interaction region can, in principle, be transmitted to other receptor dimers, and to a bound kinase or adaptor protein (4,46). The apo receptor homodimer possesses two-fold rotational symmetry about its long axis, but when incorporated into the trimer-of-dimers this symmetry is lost. One face binds the two other receptors in the same oligomer, leaving the opposite face open to bind a kinase or adaptor protein. Each of these faces is a potential site of signal transmission from a receptor dimer to other core proteins.

**Receptor-Kinase Signal Transfer**—In principle, receptor signals could be transmitted to the kinase by either a direct receptor-to-kinase path, or an indirect receptor-to-adaptor-to-kinase path, or both (61). Disulfide mapping studies of the receptor-kinase interface in functional core complexes have revealed an attractant triggered displacement of the tight complex formed between the receptor protein interaction region and the kinase regulatory

domain (Fig. 2C) (34). It follows that the direct receptor-to-kinase path is utilized, but the contribution of the indirect path remains unknown.

**Kinase on-off switching mechanism**—Each subunit of the homodimeric kinase possesses five structural and functional domains: the substrate domain containing the His autophosphorylation site (P1), the response regulator binding domain (P2), the dimerization domain (P3), the ATP-binding catalytic domain (P4), and the kinase regulatory domain (P5) (4,30,62,63). When the kinase is active, it catalyzes a trans-autophosphorylation reaction in which the catalytic domain transfers the  $\gamma$ -phosphate of ATP to the target His on the substrate domain of the other subunit. Subsequently this same phosphate is transferred to a response regulator protein, either the motor regulator CheY or the adaptation enzyme CheB.

A recent cryo-EM study compared native arrays in kinase-on and -off states trapped by signal locking receptor mutations, revealing detectable protein density, termed a “keel”, projecting below the cytoplasmic surface of the base plate (40). The keel is presumably comprised by large, mobile CheA kinase domains including the P1 substrate and P4 catalytic domains. Notably, the on-state keel appears significantly smaller than the off-state keel, indicating the on-state keel domains are more difficult to detect, most likely because they are more mobile, or exhibit static, heterogeneous positions (Fig. 2C).

Currently, two classes of models (which are not mutually exclusive) exist for the mechanism of kinase on-off switching. In Class I models, the signal is allosteric and travels through protein domains to the kinase active site, as proposed by a recent NMR study (Fig. 2D,E) (39). In Class II models, the signal regulates the mobility or location of the P1 substrate domain, as for example in a model in which P1 is bound to the active site of the P4 catalytic domain in the on-state, but is bound to a separate inhibitory site on P4 in the off-state (Fig. 2E) (64). Both Class I and Class II models would explain changes in apparent keel density (Fig. 2C) by proposing that the keel domains are more mobile or heterogeneous in the kinase activating on-state than in the kinase inhibiting off-state (see legend Fig. 2E). Moreover, in both Class I and II models, it appears likely that tethered diffusion of the P1 substrate domain via the exposed, extended conformations of the P1-P2 and P2-P3 linkers is limited to transient events (Fig. 2E) given the striking inaccessibility of these linkers to proteases in ultrastability (10,34).

### Cooperative Signaling between Core Units

The ultrasensitivity of the chemosensory array arises in part from extensive positive cooperativity between receptors, which yields an extremely steep dependence of kinase activity on attractant concentration (Hill coefficients up to  $\sim 15$ ) (9,44,45,65). Circuit modeling studies suggest this cooperativity is generated by signal spread through the array, enabling attractant binding at a single receptor homodimer to downregulate 20-30 receptors and their associated kinases (2,3,8). On a molecular level, the receptor trimers-of-dimers are not believed to contact each other directly in the array, thus positive cooperativity is hypothesized to spread through the array via the network of interconnected kinase-adaptor rings. Presumably such signals must be transmitted through kinase-adaptor interface 2, which bridges different core units, but little is currently known about this type of signaling.

## Mechanistic Controversies and Challenges

Significant progress has been made in elucidating the mechanism of receptor transmembrane signaling, and the piston model is widely accepted (4,46). Strong evidence supporting the alternating static-dynamic hypothesis of signal transduction in the receptor cytoplasmic domain has been generated (42,43,50-52), but additional direct structural and dynamical studies in functional core complexes are needed to fully test this hypothesis. One laboratory continues to champion an alternative, rotational model (66). Analysis of receptor switching mechanisms face technical challenges: studies of receptors in intact core complexes are less amenable to high resolution analysis but ensure retention of native membrane interactions, packing constraints and switching mechanism, while studies of receptor fragments enable high resolution analysis but may lack native constraints and mechanism.

Direct signal transmission from receptor to kinase has been detected (21), but it is not yet clear whether or not an indirect transmission route from receptor to adaptor to kinase also exists, and if so whether both routes are essential for receptor regulation of kinase activity. Further research is also needed to elucidate the mechanism of kinase on-off switching. Even less well understood is the mechanism of signal spread between core units underlying positive cooperativity and ultrasensitivity.

As summarized herein, recent findings in the field have emphasized the importance of both conformational changes and dynamics changes in receptor and kinase on-off switching. In reality, switching mechanisms are likely to generally involve changes in both average conformation and dynamics. A major challenge will be to distinguish whether one of these two components is more important to native regulation, or whether both are essential. In short, the pursuit of a molecular understanding of signal transduction will continue to provide controversies and challenges for years to come.

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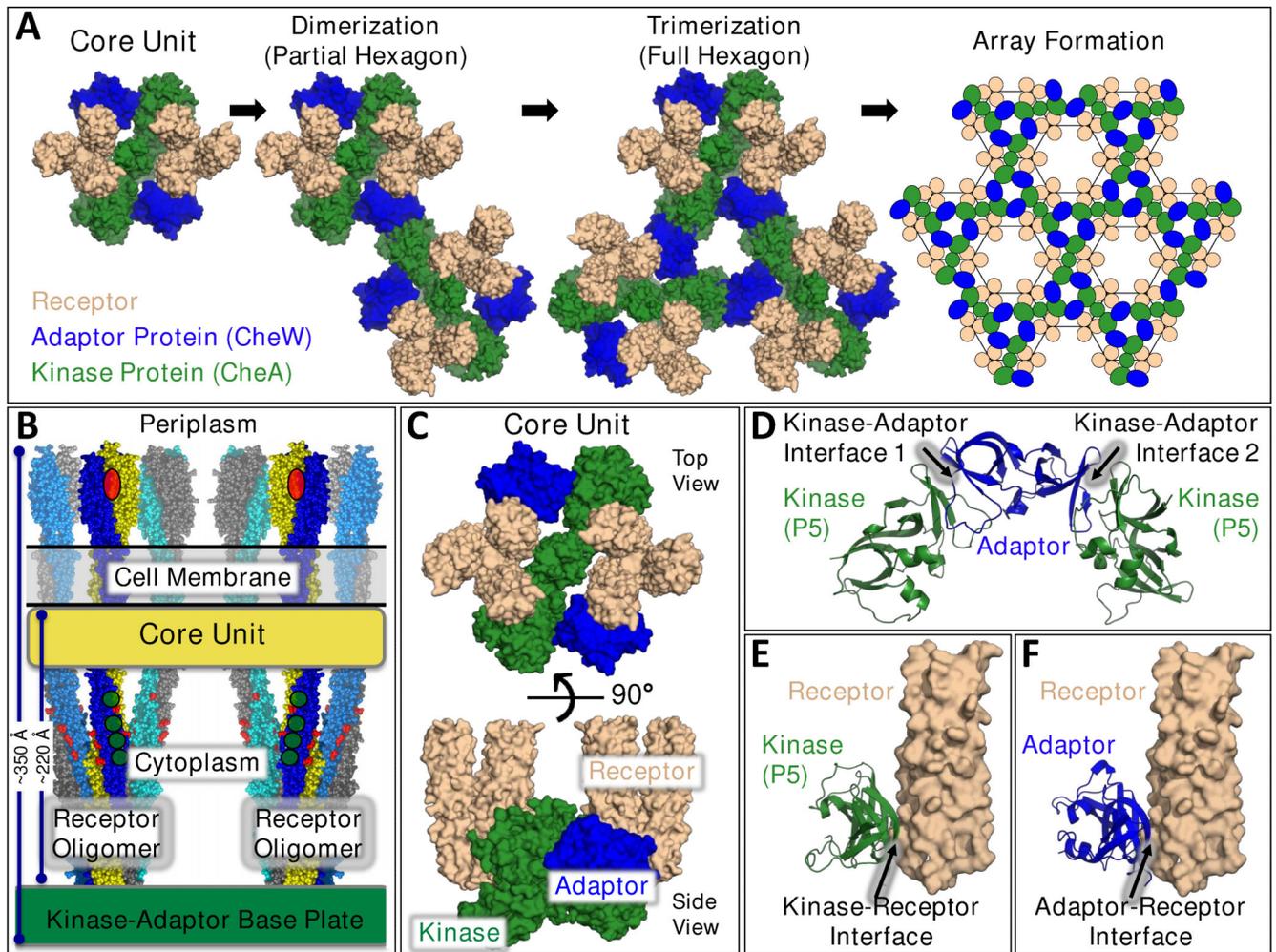
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**HIGHLIGHTS**

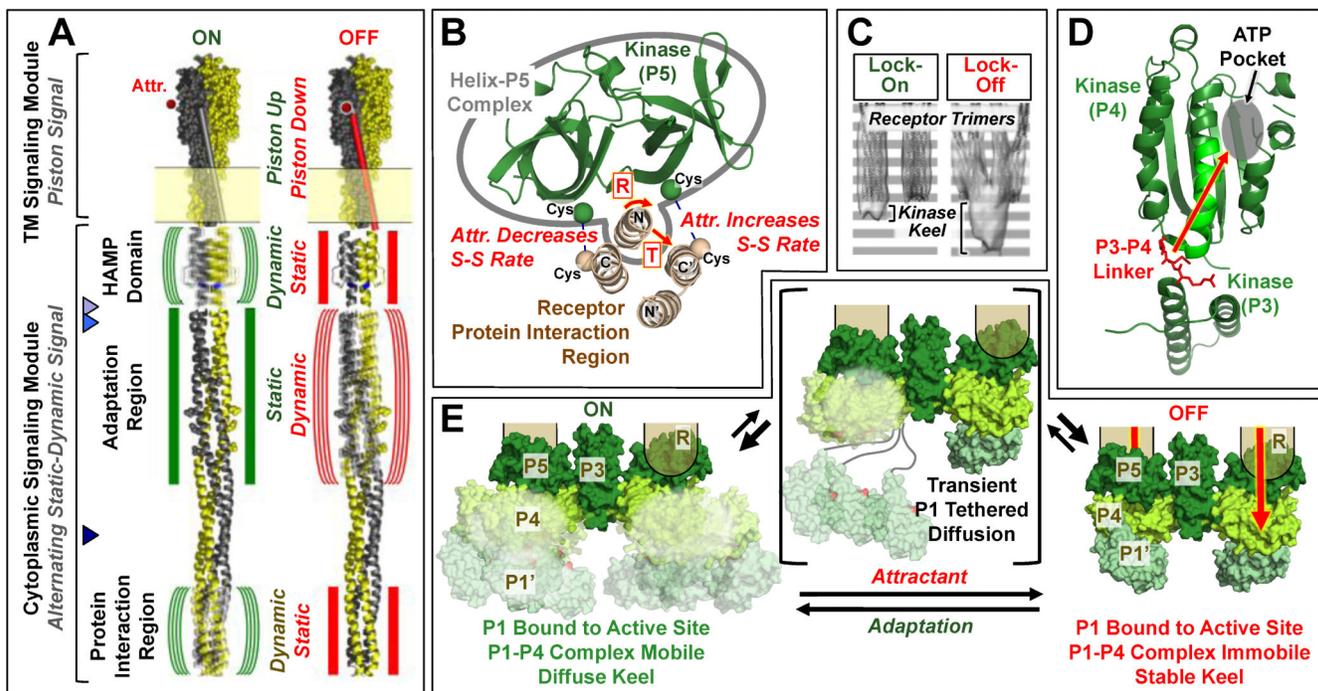
- A conserved, ultrasensitive, ultrastable chemosensory array guides bacterial motility
- Three core proteins form the array framework: receptor, His-kinase, and adaptor
- Complementary approaches are developing a molecular model of array architecture
- Recent progress has furthered the mechanistic understanding of receptor signaling
- Early studies are investigating the mechanism of kinase on-off switching



**Figure 1. Chemosensory Array Components, Assembly, Architecture, and Stabilizing Contacts**

**A**) A core unit (far left) is comprised of the three core components: two receptor oligomers (each a trimer-of-homodimers, tan), a homodimeric His-kinase (CheA, green), and two copies of a monomeric adaptor protein (CheW, blue) (28-31,56,63). Core units are hypothesized to associate, forming dimers and then trimers during assembly of individual hexagons (1). Continued assembly forms a hexagonal array (far right) with receptor oligomers located at the vertices (21-23,26,27). In this array, the kinase and adaptor proteins are arranged in a system of interconnecting rings that stabilize individual hexagons and, more globally, the full lattice. In each hexameric ring, the structurally homologous kinase regulatory domain (P5) and the adaptor protein alternate, yielding pseudo-6-fold symmetry with contacts to each of the six surrounding receptor oligomers. Signals can be transmitted within individual core units, or between core units via the kinase-adaptor ring system. **B**) Schematic side view of the core unit, showing the two receptor trimers-of-dimers, the periplasmic attractant binding sites (red ovals), the cell membrane, the cytoplasmic adaptation sites (green circles), and the kinase-adaptor base plate region (4,23,26,27,46). **C**) Top view (from periplasm, upper) and side view (lower) of the molecular core unit model, focusing on the receptor protein interaction region and the kinase and adaptor proteins that

stably bind to this region (21-23,26,27). **D)** Current models for the two types of interfaces, 1 and 2, between the kinase regulatory domain (P5) and the adaptor protein in the kinase-adaptor ring system (22,23,26,27). **E), F)** Current models for the kinase-receptor and adaptor-receptor interfaces, respectively (21,23,26,27).



**Figure 2. Models of signal transduction in the membrane-bound core unit**

**A)** Schematic chemoreceptor on- and off-states (green and red symbols, respectively), shown for simplicity in a single chemoreceptor homodimer by omitting the other two dimers of the receptor oligomer. The transmembrane and cytoplasmic signaling modules signal via an established piston mechanism (4,46) and a hypothesized alternating static-dynamic mechanism, respectively (42,43,48-52). The transmembrane signal begins in the periplasm where attractant binds to the apo-receptor and displaces the transmembrane signaling helix (TM2) towards the cytoplasm, thereby driving the piston-up, kinase-activating state into the piston-down, kinase-inhibiting state. The cytoplasmic HAMP domain converts this piston signal into a static-dynamic signal, such that the piston-up, kinase-on state possesses greater HAMP dynamics, while the piston-down, kinase-off state possesses less HAMP dynamics. The model further proposes that in the adaptation region the static-dynamic states are reversed in polarity relative to HAMP, and another polarity reversal is postulated in the protein interaction region. Triangles point to two proposed hinge regions: the major proteolysis site (53) and the nearby hotspot Gly (54) (light and medium blue, respectively), and the multi-Gly hinge (55) (dark blue). **B)** Shown is the tight complex formed between the kinase regulatory domain (P5) and the N-terminal helix of the receptor protein interaction region (see also Fig. 1E), as well as the attractant-triggered displacement of this complex (either a rotation or translation) detected by disulfide mapping in functional core complexes (21,23). This displacement is believed to transmit, at least in part, the on-off switching signal from the receptor to the kinase. **C)** Cryo-EM average density maps for signaling arrays containing a lock-on (I241E) or lock-off (A413T) mutant receptor, respectively (40). Within the average core unit, the two receptor trimers of dimers are easily observed, and an additional “keel” of protein density below the base plate region is less evident in locked-on arrays than in locked-off arrays. **D)** Shown is a hypothesized Class I (see text) allosteric on-

off signal, based on NMR studies of CheA kinase, transmitted from the linker between the dimerization (P3) and catalytic (P4) domains, through the catalytic domain to the active site (39). **E**) Three-state picture of kinase on-off switching compatible with Class I and II models (see text). In Class I, the substrate domain (P1') of one subunit is proposed to be bound much of the time to the active site on the catalytic domain of the other subunit (P4) awaiting trans-autophosphorylation, yielding a stable P1'-P4 complex regulated by an allosteric on-off signal traveling through the kinase to the P4 active site (28,67). In Class II, the substrate domain P1' is bound most of the time either to the active site or to a separate inhibitory site on P4 in the on- and off-states, respectively, such that on-off signals regulate the ratio of binding to the two sites (64). In both Class I and II models, the keel domains including the P1'-P4 complex are proposed to be more mobile in the on-state than in the off-state, explaining the low keel density of the on-state in cryo-EM (see (C)) (40). Loss of mobility in the off-state could involve additional constraints on inter-domain linkers, or steric hindrance due to changes in the relative positions of kinase, adaptor and receptor regions. In both models, tethered diffusion of the substrate domain via the long P1-P2 and/or P2-P3 linkers could occur as shown, but would likely be transient to explain the known proteolytic protection of the linkers (10,11,34).