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STRUCTURE AND FUNCTION OF BACTERIAL CHEMOSENSORY ARRAYS

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EXECUTIVE SUMMARY

Chemotaxis—the ability of a cell or organism to direct its movement in response to environmental chemicals—is a ubiquitous biological behavior. In chemotactic bacteria, extended transmembrane protein clusters called chemosensory arrays process complex sensory signals to affect motile behavior and thereby aid in survival. Remarkably, the molecular architecture of the chemosensory array is universally conserved across species, providing a novel target for broadly applicable antimicrobial therapies. Despite significant effort, however, the array's large size and dynamic nature have thwarted high-resolution structural characterization by conventional techniques, preventing a detailed description of array function. Utilizing large-scale molecular simulations on Blue Waters, we have constructed the first atomically resolved model of a complete chemosensory array, enabling the study of critical aspects of sensory signal transduction as never before.

RESEARCH CHALLENGE

Chemotaxis plays a critical role in colonization and infection by many human and plant pathogens [1]. With antimicrobial-resistant infections projected to kill more people worldwide per year than cancer by 2050 [2], a comprehensive understanding of the chemotaxis response could have a significant impact on societal wellbeing.

The streamlined chemotaxis machinery of the bacterium *Escherichia coli* is the best understood biological signal transduction system, and serves as a powerful tool for investigating cellular behavior and motility [3]. Essential to their chemotaxis response, *E. coli* cells possess a highly ordered sensory apparatus known as the chemosensory array (Fig. 1), which consists of hundreds of basic core-signaling units comprised of three components: transmembrane chemoreceptors, the histidine kinase CheA, and the adaptor protein CheW [4,5]. Despite important progress in the characterization of chemosensory arrays using a battery of genetic, biochemical, and structural techniques, the lack of a high-resolution description of the array's intact, multicomponent structure has prevented a detailed understanding of the molecular mechanisms underlying array function. Hence, the immediate goal of this project is to provide a high-fidelity, atomistic model of the complete chemosensory array. The resulting model will enable computer simulations of the molecular mechanisms underlying cooperative sensory signal transduction, and thereby aid in the development of novel antibiotics to control multidrug-resistant strains of bacteria.

METHODS & CODES

To characterize the structural and dynamical properties of the chemosensory array, we carried out extensive all-atom molecular dynamics (MD) simulations using NAMD [6], a highly scalable MD code optimized to make peak use of Blue Waters' petascale features. The all-atom model depicted in Fig. 1 was constructed using a variety of so-called hybrid modeling techniques [7]. We analyzed simulation trajectories using several dimensionality-reducing techniques, especially k-medoids clustering [8].

RESULTS & IMPACT

To understand the underlying molecular mechanism of chemosensory array activation, regulation, and cooperativity, it is essential to understand the precise interactions among chemoreceptors, CheA, and CheW in the context of their native array organization and as they undergo dynamical changes in response to chemical signals. Recently, our collaborator Peijun Zhang developed a novel *in vitro* reconstituted-monolayer system, producing array specimens ideal for high-resolution characterization by cryo-electron microscopy (cryoEM). This analysis resulted in a density map of the cytoplasmic portion of the *E. coli* core-signaling unit (CSU) at 9 Å resolution. Using Molecular Dynamics Flexible Fitting (MDFF) simulations [9], we integrated homology models based on existing crystallographic structures with our cryoEM data to produce an atomic model of the intact *E. coli* cytoplasmic CSU. Using Generalized Simulated Annealing [10] to further explore the conformational spaces of especially flexible array components, we were able to identify a number of novel CheA conformations contained within the averaged cryoEM data and, thereby, to significantly advance the mechanistic description of kinase activation. These findings have produced a number of residue-based predictions that have subsequently been validated via functional assays in live cells by our collaborator Sandy Parkinson (manuscript forthcoming).

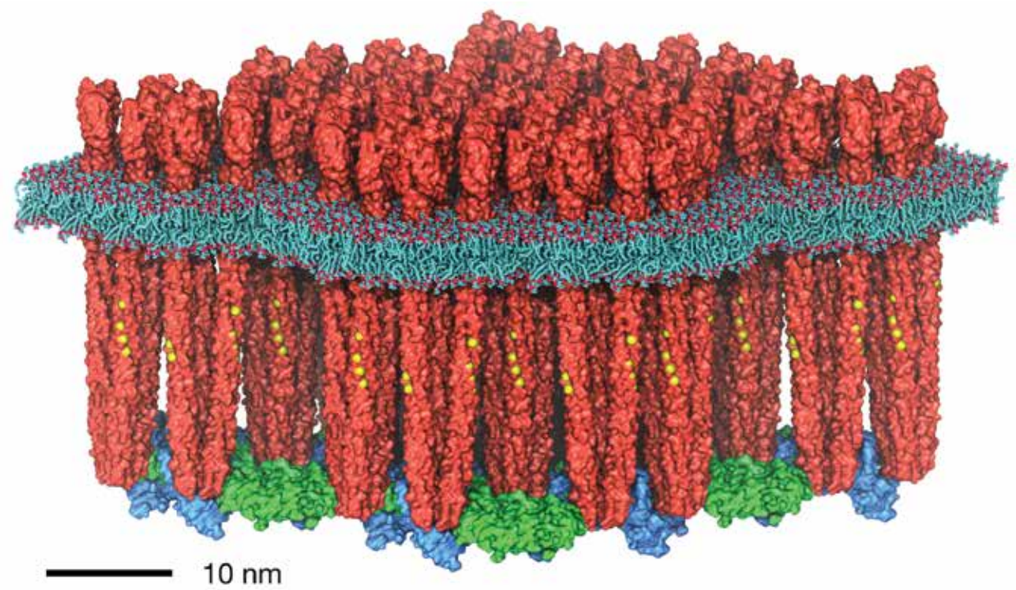

Figure 1: All-atom model of a complete bacterial chemosensory array (20 million atoms). Environmental ligands are sensed by binding to chemoreceptors (red), which then transmit signals across the cell's inner membrane to cooperatively regulate the activity of the CheA kinase (blue) with the help of the adaptor protein CheW (green).

In addition, based on extensive mutagenesis data from the Parkinson Lab, as well as crystallographic and disulfide crosslinking data, we have developed a model of the complete *E. coli* serine chemoreceptor, including the previously uncharacterized transmembrane bundle and HAMP domains. Using this model, along with *in vivo* cryoEM data from our collaborator Ariane Briegel, which provided a view of the membrane-proximal regions of the chemoreceptors in multiple signaling states, we were able to extend our cytoplasmic model to include intact chemoreceptors and membranes as well as to elucidate critical signaling-related interactions among chemoreceptors. Taken together, the boundary-pushing simulations described above represent the first major effort to bring the immense power of modern supercomputing to bear on the problems of bacterial chemotaxis. These simulations have already helped to fill in the missing atomistic detail necessary for elucidating several long-standing problems in the field and provide in general a much-needed molecular platform on which to generate wholly new hypotheses.

WHY BLUE WATERS

To properly investigate the long-range and highly cooperative mechanisms of sensory signal transduction within the chemosensory array, it is necessary to simulate large, multicomponent molecular systems comprised of millions of atoms on the order of microseconds. Such simulations require sustained access to thousands of tightly coupled processors and are able to benefit tremendously from GPU acceleration. Thus, the unique petascale capabilities of the Blue Waters machine have provided a premier computing infrastructure without which this work could not have been completed.

PUBLICATIONS & DATA SETS

Cassidy, C.K., et al., CryoEM and computer simulations reveal a novel kinase conformational switch in bacterial chemotaxis signaling. *Elife*, 4:e08419 (2015).