New Insights Into Bacterial Chemoreceptor Array From Electron Cryotomography

Wen Yang[1], C Keith Cassidy[2], Simon Ringgaard[3], Sandy Parkinson[4], Ariane Briegel[1]

[1.] Institute of Biology, Leiden University, Leiden, The Netherlands
[2.] Department of Physics, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[3.] Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
[4.] Biology Department, University of Utah, Salt Lake City, UT, USA

The ability to quickly sense and interpret the environmental signals is crucial for microbes to survive. Most motile bacteria have evolved delicate protein networks to detect the gradient changes of surrounding conditions and switch the direction of flagellar rotation in response [1]. Within this chemotaxis pathway, the signal transduction and regulation is carried out by the large, highly ordered supramolecular complexes termed chemoreceptor arrays. The Chemoreceptor arrays have been most intensively studied in *Escherichia.coli.* In this organism, receptors, the histadine kinase CheA and coupling protein CheW form extended arrays with strict stoichiometry [2]. These clusters form highly ordered, hexagonally packed arrays with a strict 12-nm spacing at cell pole, which is proposed to be the structural basis for signal amplification, cooperativity and sensitivity adaptation [3].

Although bacterial chemotaxis is the best understood cellular signalling system to date, many questions on how the chemoreceptor array responds to the environmental signals and switch between active and inactive states through conformational dynamics is still unclear. We carried out electron cryotomography (ECT) studies combined with subtomogram averaging in *E.coli* cells with well-defined activity states (Fig. 1A). This allowed us to study the conformational changes in intact arrays in different signalling states at unprecedented resolution.

Unlike *E.coli,* which contains only one chemotaxis system, *Vibrio cholerae* possess three chemotaxis distinct clusters. Structurally, 2 of these clusters have been investigated using ECT. The proteins from the chemotaxis Cluster I in *V.cholerae* form cytoplasmic arrays in which a unique receptor, DosM, directly connect the two layers of chemorecepor array together (Fig 1B)[4]. Cluster II forms a typical membrane bound array at the cell pole. So far, this cluster is the only one shown to be involved in chemotatic behaviour [5]. Our recent results confirm that although the cluster II chemoreceptor array exhibited the same general hexagonal packing of the receptors with a 12 nm spacing, the protein composition of this array was different compared to the array in *E.coli*. In addition to CheA and CheW, ParP directly integrate into the baseplate of chomoreceptor array [6]. Not only does ParP promote cluster II array formation and stabilize the array lattice, it also plays an important role in localizing the array at the cell pole. Here we show, for the first time, that the arrays are indeed structurally distinct from the well-understood *E. coli*, which suggests different adaptations of the system to each cell's preferred environment.

The new insights into bacterial chemoreceptor arrays we gained haven been largely derived from the ECT data, which provide an outstanding example of how ECT can be used not only for revealing the architecture of macromolecular apparatuses *in situ* but also for investigating the molecular mechanism of those apparatuses at a higher resolution. Our research also highlights the necessity of extending the

chemotaxis studies in different microorganism, as the variance of spatial organization and structural architecture of different chemotaxis arrays are beyond the simplicity of the chemotaxis model in *E.coli*. To explore how microorganism use auxiliary chemotaxis system to allow their high adaptability to the diverse environment will lead to a better understanding of the chemotactic signalling process.

References:

[1] Turner, L., W.S. Ryu, and H.C. Berg, J Bacteriol, 2000. **182**(10): p. 2793-801.
[2] Parkinson, J.S., G.L. Hazelbauer, and J.J. Falke, Trends Microbiol, 2015. **23**(5): p. 257-66.
[3] Briegel, A., et al., Proc Natl Acad Sci U S A, 2012. **109**(10): p. 3766-71.
[4] Briegel, A., et al., Proc Natl Acad Sci U S A, 2016. **113**(37): p. 10412-7.
[5] Gosink, K.K., et al., J Bacteriol, 2002. **184**(6): p. 1767-71.
[6] Alvarado, A., et al., Elife, 2017. **6**: p. e31058.
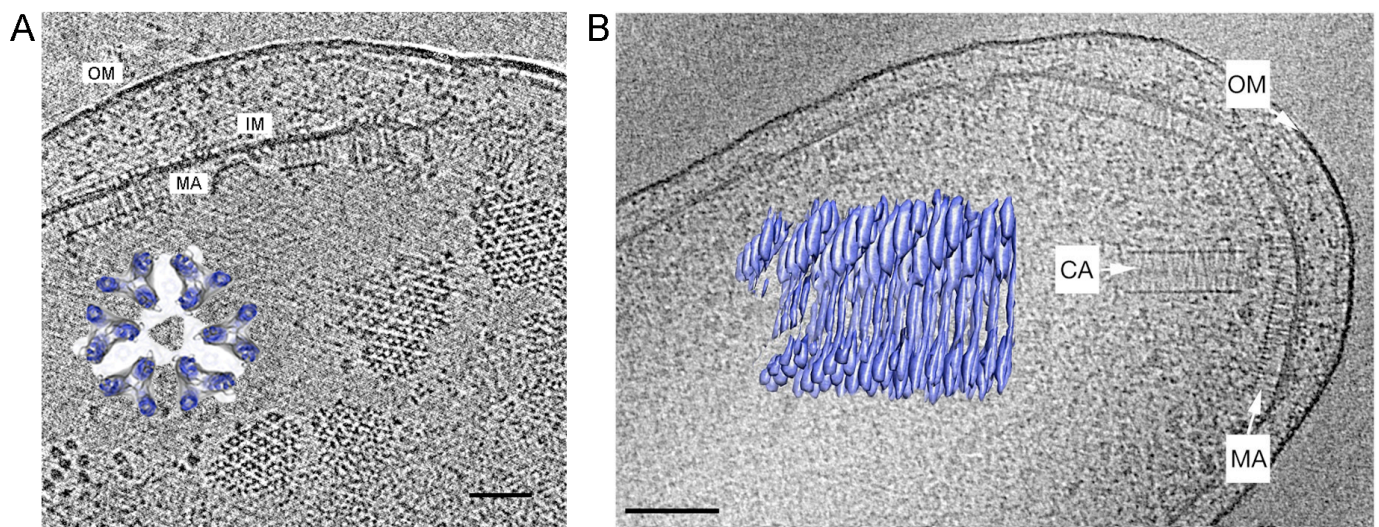
Figure 1. Chemoreceptor arrays visualized in bacterial cells by electron tomography. (A) Membrane-bound array in *E.coli* shown in both side view and top view, in top view the receptor trimmers of dimers from hexagonally packed lattice. Insert shows the receptor crystal structure fitted in to density map of triple signaling core unit. Scale bar is 50 nm. (B) Both membrane-bound array and cytoplasmic array exist in *V.colerae;* insert shows the subtomogram averaging result of the cytoplasmic array. Scale bar is 100 nm. OM, outer membrane; IM, inner membrane; MA, membrane-bound array; CA, cytoplasmic array.