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CheA and CheW, peptides exhibit protection from exchange at long times (16 hours) that is greater in the kinase-on state. HDX-MS of complexes prepared using different means of shifting the signaling state will reveal which changes correlate with kinase activity and will quantify stabilization of receptor subdomains and binding interfaces that contribute to receptor control of kinase activity. Thus HDX-MS provides an important tool in a hybrid approach for understanding structure and mechanism in membrane-bound, multi-protein complexes.
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203-Plat
Disulfide Trapping and Spectroscopic Studies of Bacterial Chemosensory Core Signaling Complexes: Probing Molecular Mechanisms of Complex Assembly and Receptor-Regulated On-Off Switching
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The core unit of the bacterial chemosensory array is multi-protein complex comprised of 6 transmembrane chemoreceptor homodimers, 1 CheA His kinase homodimer, and 2 CheW adaptor protein monomers. We are reconstituting core units on isolated bacterial membranes, yielding individual core units and oligomers of core units ranging in size up to small hexagonal arrays. This approach generates functional, membrane-bound core complexes and allows incorporation of modified kinase and adaptor proteins possessing pairs of engineered Cys residues for disulfide trapping, or spectroscopic probes for fluorescence or EPR studies. The resulting core complexes display native receptor-stimulated kinase activities that are fully regulated by attractant binding to the receptor, or covalent modification of the receptor adaptation sites. The findings shed new light on the kinetics and order of core complex assembly, and provide insights into the molecular mechanisms underlying receptor-mediated kinase on-off switching.

204-Plat
Flagellar Motor Architecture
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The cytoplasmic or C-ring of the bacterial flagellar motor plays central roles in transmitting torque from peptido-glycan anchored membrane protein complexes MotA and MotB and in controlling the sense of rotation of the motor. The C-ring is made up of three proteins, FliN, FliM and FliG with differing copy numbers. The C-ring is attached to the MS-ring via interactions between FliF and the N-terminal domain of FliG. We have used a combination of NMR, x-ray diffraction and mutant analysis to investigate the structures of the domains of FliG and of the N-terminal domain of FliG with FliF. These results will be discussed in the context of various models that have been proposed to account for the assembled structure of the C-ring and the mechanism of torque transmission and the control of the sense of rotation of the flagellar motor.

205-Plat
Structure and Dynamics of the Receptor:Kinase Complex that Mediates Bacterial Chemotaxis
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Bacterial chemotaxis, the ability of bacteria to adapt their motion to external stimuli, has long stood as a model system for understanding transmembrane signaling, intracellular information transfer, and motility. The sensory apparatus underlying chemotaxis displays remarkably sensitivity, robustness, and dynamic range. These properties stem from a highly cooperative excitation response and an integral feedback mechanism for adaptation to changing surroundings. Although the molecular components of the chemotaxis system are well characterized, we still do not fully understand the biophysical mechanisms responsible for function. This is because the sensory apparatus comprises an extensive multi-component transmembrane assembly of chemoreceptors, histidine kinases (CheA) and coupling proteins (CheW), whose architecture is just emerging. We will discuss efforts to understand the detailed structure of the chemoreceptor:CheA:CheW complex and how chemoreceptors transmit signals across the membrane to regulate CheA activity. To address these issues, studies have been undertaken on isolated components, reconstituted complexes, and native receptor arrays. Soluble, chemoreceptor maquettes that mimic receptor oligomeric states have been particularly useful for studying kinase activation. Evidence will be presented to support the notion that changes to both molecular structure and dynamics are involved in signal transduction by the receptor:kinase assemblies.

206-Plat
HAMP: The CPU Domain of Bacterial Chemoreceptors
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The transmembrane chemoreceptors that mediate chemotactic behaviors in E. coli contain a HAMP domain at the cytoplasmic face of the membrane that governs their input-output signaling transactions. The four-helix HAMP bundle receives stimulus signals from the periplasmic chemoeffector-binding domain via a five-residue control cable connection to a transmembrane helix (TM2). HAMP in turn, through its structural interactions with an adjoining four-helix methylation (MH) bundle, modulates the activity of CheA, a cytoplasmic histidine autokinase bound at the membrane-distal tip of the receptor molecule.
To investigate the mechanism of HAMP signaling in Tsr, the E. coli serine chemoreceptor, my lab has characterized the serine sensitivities and response cooperativities of a large collection of mutant receptors that have amino acid replacements in the TM2 - control cable - HAMP - MH bundle region, using an in vivo FRET-based assay of CheA kinase activity.
Signaling by wild-type Tsr follows a two-state model of shifts between kinase-activating and kinase-deactivating outputs. Both states correspond to ensembles of mutationally distinct HAMP conformations. A variety of HAMP structural lesions, including ablation of the entire domain, shift receptor output toward the kinase-on state, indicating that the signaling role of HAMP is not to activate CheA, but rather to down-regulate kinase activity in response to chemoattractant ligands. Stimulus signals from TM2 and the control cable probably trigger output responses by modulating the packing stability of HAMP: A loosely packed HAMP bundle allows kinase activity; a tightly packed HAMP bundle deactivates CheA. These signaling shifts occur through an opposing structural interplay of packing stability in the HAMP and MH bundles. Loosely packed methylation helices produce kinase-off output and serve as substrates for subsequent receptor modifications that enhance MH packing during the sensory adaptation phase of an attractant response.

207-Plat
Signal Integration by Bacterial Chemosensory Complexes
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Chemotaxis receptors in bacteria are organized in large clusters that play an essential role in signal processing. Allosteric interactions within these clusters allow activities of individual receptors to be coupled. The resulting cooperative signaling is well investigated and can be mathematically described using Monod-Wyman-Changeux (MWC) or Ising models, but its importance in the overall signal processing by the chemotaxis pathway is not fully understood. One established function of the cooperative signaling is to amplify chemotactic stimuli, whereby ligand binding to one receptor molecule can stabilize inactive state of multiple neighboring receptors. Using FRET-based reporter of the pathway activity, we have recently investigated another function of the allosteric interactions between receptors, namely in integration and coordination of responses to multiple stimuli sensed by different receptors. We showed that such signal integration could be explained by a simple summation of free energy changes that are elicited by individual ligands. Moreover, receptor clusters also integrate metabolism-related signals that are transmitted through the cytoplasmic domains of the receptors. Finally, receptor clustering also allows cells to align adaptation kinetics for different attractants, ensuring a robust and optimal time scale of the short-term memory that is required for efficient navigation in chemoeffector gradients.

208-Plat
Architecture and Assembly of Chemoreceptor Arrays as seen by Electron Cryotomography
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Most motile bacteria as well as many archaea sense and respond to their environment through arrays of chemoreceptors. While X-ray crystallography and NMR spectroscopy and other high-resolution structural methods have produced atomic models of components and sub-complexes of these arrays, we have used electron cryotomography to visualize their basic architecture in their native state within intact cells and reconstituted in vitro systems to ~2 nm resolution, revealing principles of array structure and assembly. First, receptors cluster in a