crystallized so that every subunit is in an identical environment. Electron microscopy has been one of the main techniques used to study such polymers, but resolution has been limited by disorder and heterogeneity, and in many cases three-dimensional reconstructions have not even been possible due to very weak scattering. An interative single-particle type approach to 3D reconstruction has proven to be remarkably successful in studying actin filaments, protein-DNA filaments, bacterial pili, filamentous bacteriophage, etc. A number of examples will be shown, and some general conclusions drawn. One is that subunits in these filaments frequently have the ability to switch among multiple different states. The notion that there is a single 'structure' for a polymer may thus be misleading. Another conclusion is that small changes in sequence can lead to large changes in the quaternary structure of these polymers, and this may be an important mechanism in the evolutionary divergence of organisms.

Keywords: helical polymers, three-dimensional reconstruction, electron cryo-microscopy

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Structural and functional significance of the N-terminus of Cx26 gap junction channels

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Investigations of gap junction channels have used varying experimental approaches to elucidate the gating mechanism of these intercellular conduits. Here, we report two structures of mutant connexin26 channels by electron cryo-crystallography. Full length connexin26 (Cx26M34A) and an N-terminal deletion mutant (Cx26M34Adel2-7) were overexpressed using Sf9 insect cells. Purified proteins were reconstituted into the lipid bilayers that formed two dimensional crystals. Three dimensional maps of the two Cx26 isotypes were obtained at 10 Å resolution and revealed that the crystals from both constructs were comprised of three lipid bilayers with the channels forming a $P22_12_1$ lattice. The structure of Cx26M34A clearly showed a prominent density we refer to as a "plug", which resides in each hemichannel pore and contacts the innermost helices of surrounding subunits at the bottom of the vestibule. These channels showed no dye transfer and dramatically reduced conductance, although the voltage gating characteristics of the residual conductance was normal. In the 3D structure of Cx26M34Adel2-7, the density in the pore was significantly reduced along with a partially reduced density of the cytoplasmic domain which bridges the adjacent four helix bundles. These results demonstrate that the N-terminus of Cx26 contributes to the structure and/or organization of pore plug and cytoplasmic domains. However, while the N-terminal deletion mutant was expressed on the cell surface, it failed to form functional channels, suggesting that the positioning of the plug inside the channel may also play a role in stabilization of open gap junction channels in situ. While the pore plug may contribute to the closure of the Cx26M34A mutant, this may represent one of multiple gating configurations.

Keywords: membrane protein channels, electron microscopy, two-dimensional protein crystals

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Merging data from Cryo-EM and X-ray crystallography to reveal biomolecular function

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We have developed a novel method to flexibly fit atomic structures into EM maps using molecular dynamics simulations. The simulations incorporate the EM data as an external potential defined on a grid that is added to the molecular dynamics force field, allowing all internal features present in the EM map to be used in the fitting process, while the model remains fully flexible and stereochemically correct; harmonic restraints are also applied to enforce secondary structure and to prevent overfitting. Validation for the method using available crystal structures of protein and RNA in different conformations, as well as measures to assess and monitor the fitting process, will be introduced. I will present example applications, including the the ribosome and poliovirus. Emphasis will be placed on E. coli ribosome structures obtained in various functional states imaged by cryo-EM, including a structure derived from a 6.7-Å resolution EM map that reveals the interaction between the ribosome and a bound ternary complex at unprecedented detail.

Keywords: flexible fitting, electron microscopy, ribosome structure and function

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The search for good crystals: How far have we come?

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Crystallization is acknowledged to be a rate-limiting step in structure determination of biological macromolecules. This lecture will give an overview of the past decade's developments to examine how far the field has come and how far it has yet to go in solving the crystallization problem. Two significant developments have been the miniaturization and automation of the crystallization trials. Although automation has become affordable even for small laboratories, the cost of screening has nevertheless increased because more conditions are tested. The pros and cons of this will be discussed. Automation also generates an enormous number of crystallization experiments. A vast pool of data is available, but has this led to more rational strategies for crystallization? Bioinformatics can be used to analyze crystallization feasibility. Ideally, one would like to be able to predict if a protein can crystallize simply by looking at the amino acid sequence or some easily measurable physico-chemical property. How successful are the current prediction programs? Biophysical diagnostic tools will also be reviewed. How well does the information from methods like mass spectrometry, dynamic light scattering, Thermofluor, microcalorimetry, etc., correlate with the crystallization results? Finally, microfluidics, diagnostic tools to detect the onset