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Constructing Initial Models From Subnanometer Density Maps With Pathwalker and Additional Protein Backbone Constraints

Ian Rees[1], Mariah R. Baker[1], Steven J. Ludtke[1], Wah Chiu[1], Matthew L. Baker[1]
(1) Verna and Marrs McLean Department of Biochemistry and Microbiology, Baylor College of Medicine and National Center for Macromolecular Imaging (2) Structural Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030

Macromolecular assemblies are critical for nearly every biological process, and accurate structural information is important for understanding functional mechanisms and discovering drug targets. X-ray crystallography has traditionally been the main tool obtaining high-resolution structural information, but many important biological complexes either do not crystallize or require conditions significantly different from in vivo. Cryo-electron microscopy (cryo-EM) permits imaging and 3D reconstruction of very large complexes and without disruptive solvent environments, and a significant number of structures have reached 3.5-6Å. This resolution often permits identification of secondary structural elements, however, direct tracing of backbones with traditional methods remains difficult at this resolution. To address this need, we have recently released Pathwalker, a semi-automated protocol for modeling subnanometer cryo-EM data without the need for a structural template or sequence-structure correspondence.

Pathwalker is an iterative process that involves placing pseudoatoms (representing C-alpha positions) in the density map, connecting these pseudoatoms into a backbone model using an adapted form of the Traveling Salesman Problem (TSP), then evaluating and refining the resulting model. The Pathwalker protocol was validated using a number of real and simulated cryo-EM density maps, however, few restrictions based on domain knowledge of allowed protein backbones geometries were incorporated. We are currently incorporating additional backbone geometry constraints into Pathwalker to eliminate “impossible” conformations and decrease the amount of manual intervention needed. Additionally, we are making the refinement step more integrated into the process, and results will include a score that flags strained bond angles.