State specific localization of AcrAB-TolC in E. Coli using subtomogram averaging

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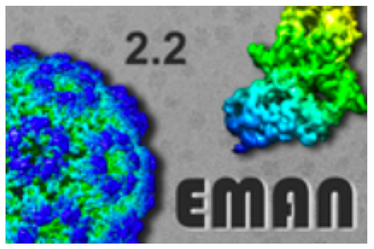
Abstract

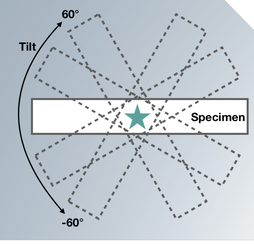
Multidrug efflux pumps (MEP) expel a wide variety of toxic substances across the membrane of bacterial cells. In Gram-negative bacteria, MEP form tripartite complexes spanning the cellular envelope; however, the *in situ* structure and assembly mechanism of many such pumps remain unknown. Using cryo-electron tomography (cryoET) and subtomogram averaging, we have solved the first *in situ* structure of the AcrAB-TolC tripartite complex and the AcrAB bipartite complex at better than 2nm resolution. Here we discuss the computational workflow in EMAN2 used to obtain these two structural states and how these structures can be mapped back to the cell, facilitating state-specific localization *in situ*. In addition to demonstrating the current state of the art in cellular subtomogram averaging, our findings also uncover the assembly mechanism of this tripartite MEP in living bacterial cells, ultimately providing a basis for the design of MEP inhibitors.

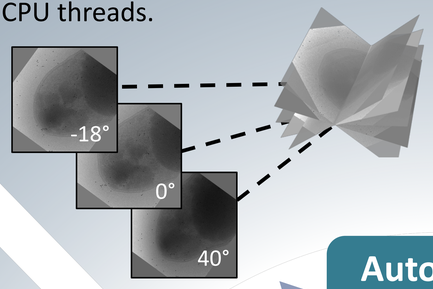


1

CryoET is an imaging technique in which tilted images of a vitrified specimen are recorded in an electron microscope and reconstructed in 3D.

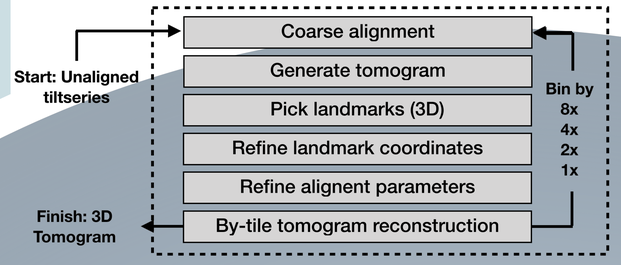
A **tilt series** is a collection of micrographs recorded over a range of angles – usually from -60° to 60° in increments of ~2°.

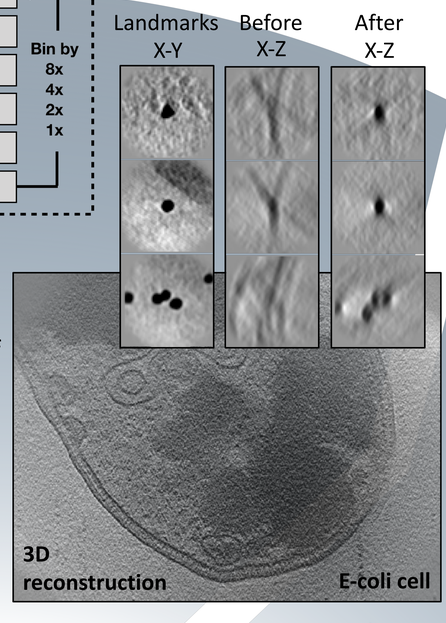




2

EMAN2 facilitates automated 3D tomogram reconstruction via the series of algorithmic steps described in the workflow above. Depending on the quality of the tilt series data, typical alignment errors range from 0 to ~4 pixels depending on the quality of the data, and the time required to align full sampling 8k x 8k data is ~10 minutes on 12 CPU threads.





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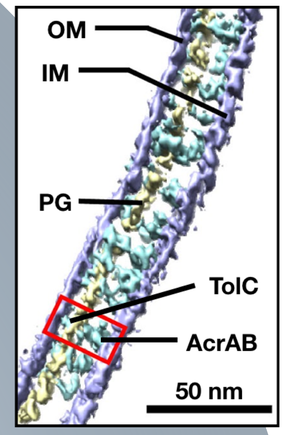
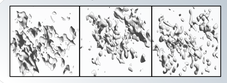
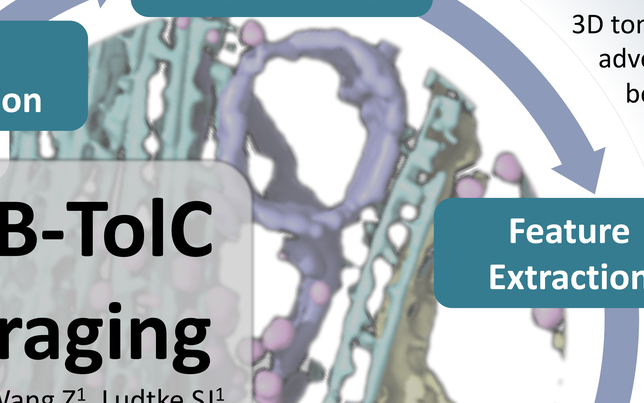
Subtomogram boxing & cellular annotation

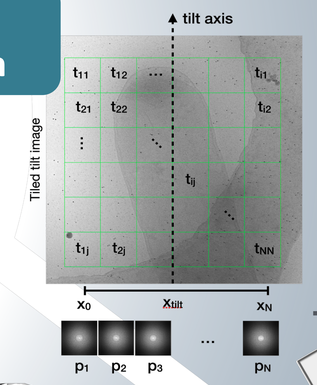




3D tomograms can be automatically segmented using an adversarial-CNN approach. Individual particles can also be extracted for further processing. Next we correct for microscope distortions on an individual particle basis using each particle's location and geometric info from our automated 3D reconstruction procedure.

Data Acquisition → **Automated 3D Reconstruction** → **Feature Extraction**

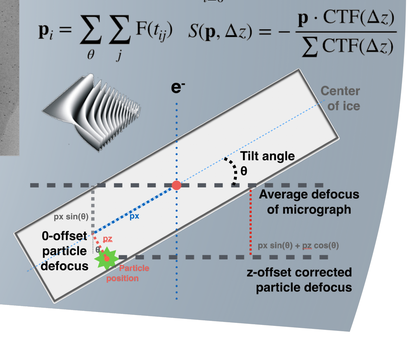




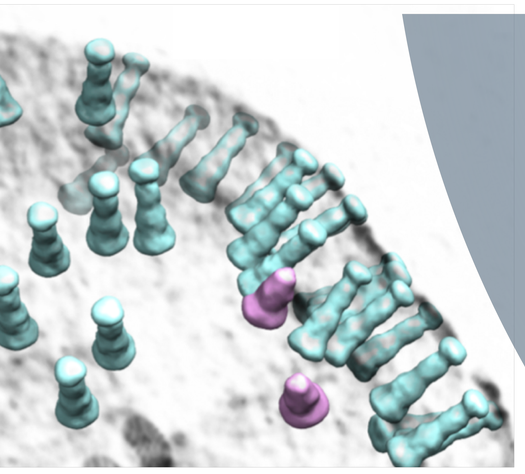
Contrast transfer function (CTF) correction

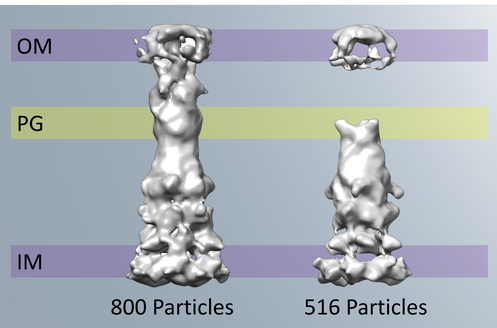
$$\Delta z_{avg} = \underset{\Delta z}{\operatorname{argmin}} \sum_{i=0}^{N} S(\mathbf{p}_i, \Delta z + \Delta x_i \sin(\theta))$$

$$\mathbf{p}_i = \sum_{\theta} \sum_{j} \mathrm{F}(t_{ij}) \quad S(\mathbf{p}, \Delta z) = -\frac{\mathbf{p} \cdot \mathrm{CTF}(\Delta z)}{\sum \mathrm{CTF}(\Delta z)}$$

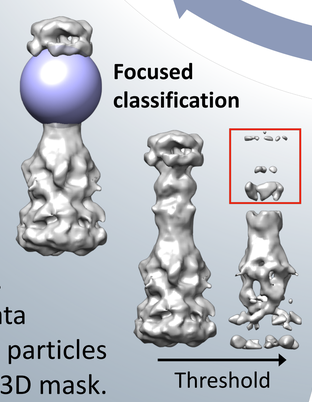


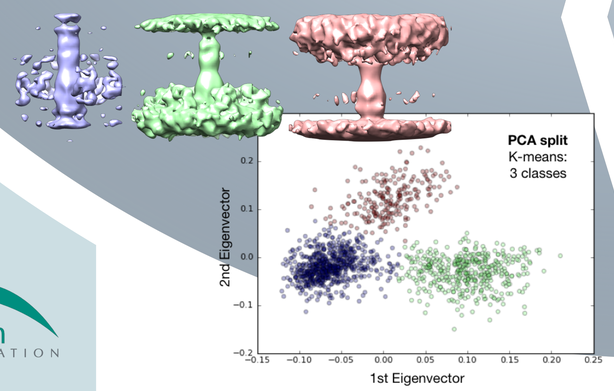
Heterogeneity Analysis ← **Subtomogram Averaging**





To analyze structural variability among particles, we classify particles in a PCA subspace and reconstruct each cluster. We can further subdivide particle data using focused techniques to label particles according to the contents of a 3D mask.





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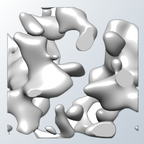
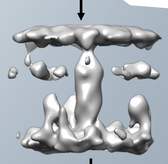
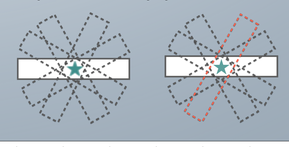
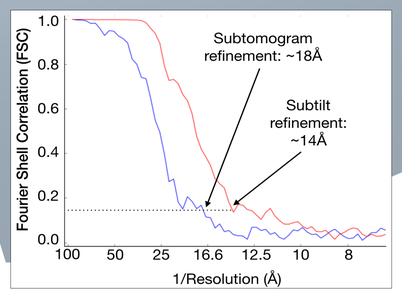
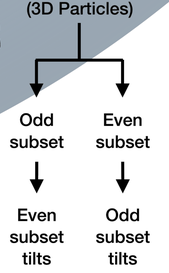
We utilize a stochastic gradient descent approach to generate de novo, reference-free initial models, which are used to seed multiple iterations of 3D refinement. "Subtilt" (per-particle per-tilt) decomposition methods are then used to refine individual particles to obtain higher resolutions than previously possible.











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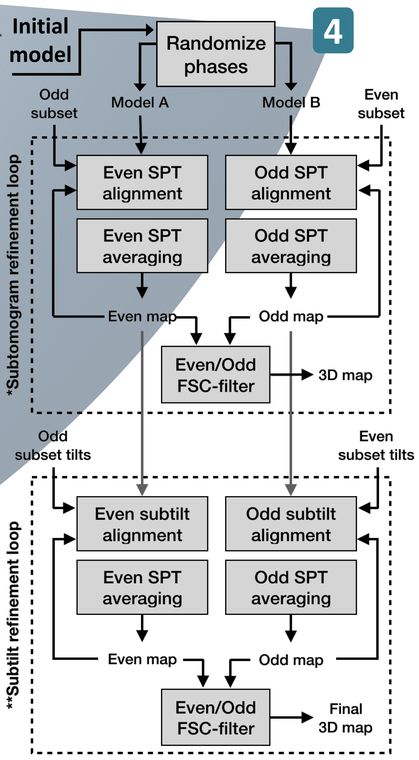

Acknowledgements

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