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# 3D Imaging of Membrane Networks in a Bacterial Cell Using Electron Tomography

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Electron tomography allows determination of the three-dimensional architecture of organelles and macromolecular assemblies that are too heterogeneous to be amenable to either crystallographic methods or single particle averaging methods [1]. In current implementations of electron tomography, a complete series of projection images spanning angular ranges as much as ± 70° is obtained by tilting the specimen relative to the electron beam in a transmission electron microscope. The images are then used to computationally reconstruct the 3D structure of the object being imaged. There is great excitement in this field with the emerging realization that the gradual advances in microscope automation, Field Emission Gun (FEG) electron sources and increases in computing power may make it possible to obtain an unprecedented level of resolution in biological three-dimensional microscopy [2]. Here we show an application of electron tomography to study the architecture of *E.coli* cells engineered to overproduce the bacterial chemotaxis receptor Tsr [3].

Tomograms constructed from fixed, cryo-sectioned cells reveal that the overproduction of the full-length chemotaxis receptor Tsr leads to the formation of a remarkable internal membrane network composed of stacks and rounded structures that are reminiscent of the sites of vesicle budding in eukaryotic cells. X-ray crystallographic studies have already established atomic structures for the periplasmic [4] and cytoplasmic [5] domains of the receptor, providing a foundation to interpret the electron microscopic results. Electron microscopic studies of membranes isolated from lysed cells indicate the presence of similar membranous morphologies as observed in the tomograms, and small crystalline arrays, thus provide constraints on the possible modes of Tsr packing in the membrane. By placing individual Tsr molecules into the density map derived from tomography, we have constructed a three-dimensional molecular model for the membrane protein network, and show how it is stabilized by specific interactions between Tsr molecules in apposing membranes.

References

[1] O. Medalia et al., Science 298 (2002) 1209-1213.
[2] J. Bohm et al., Curr Biol 11 (2001) 1168-75.
[3] G. Li & R.M. Weis, Cell 100 (2000) 357-65.
[4] M.V. Milburn et al., Science 254 (1991) 1342-1347.
[5] K.K. Kim et al., Nature 400 (1999) 787-92.

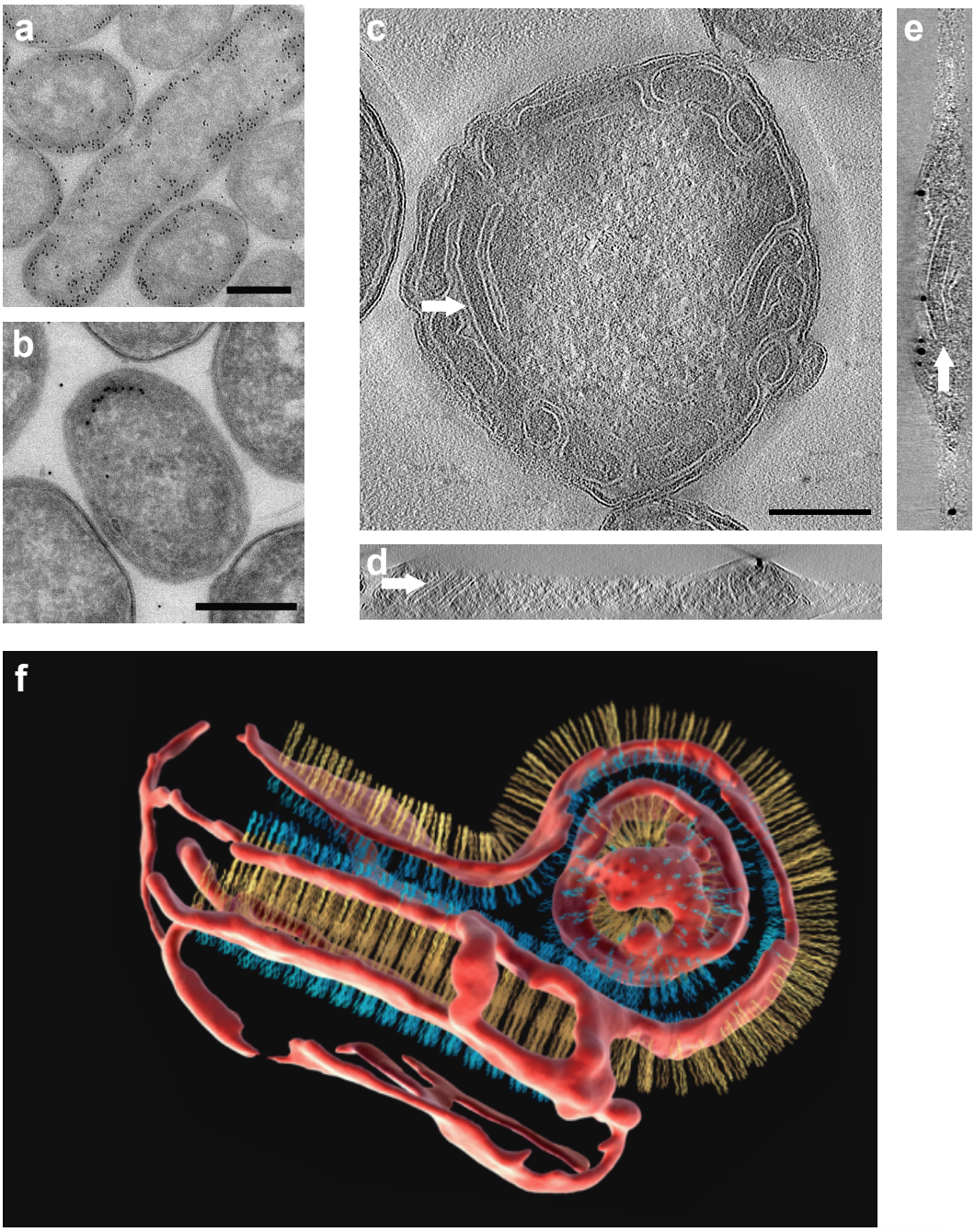


**Figure 1.** Projection images from thin sections of fixed specimens of either the *E. coli* Tsr overexpressor (a) or wild-type *E.coli* (b). The dark spots are from gold-conjugated Protein-A used to immunolabel Tsr. The scale bars in both panels are 0.5 μm. (c) – (e) Slices in three mutually orthogonal directions from the electron tomogram. The white arrows pointing to the zippered layers in the three slices correspond to the same region in the tomogram. The scale bars are 0.2 μm wide in all three panels. (f) Segmented representation of a small region from a cellular tomogram with individual Tsr receptor molecules placed in the membrane. The entire membrane network seems to be stabilized by the interactions between the cytoplasmic domains (shown in yellow) and between the periplasmic domains (shown in blue).

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