

## BIOCHEMISTRY

## Crowds of Syntaxins

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In the 1970s, Frye and Edidin (1) demonstrated two-dimensional diffusion of fluorescently labeled surface antigens in the plasma membranes of fused mouse and human cells. This observation led to the idea that membranes are a mosaic composed of a fluid lipid bilayer with embedded membrane proteins, which diffuse freely in the plane of the bilayer (2–4). This concept received early support from measurements of the diffusion of rhodopsin molecules in the rod outer segment photoreceptors of the eye (5). But questions soon arose about restriction of protein mobility as a result of protein-protein interactions or attachment of membrane proteins to other cell components (6). The questions have not abated, as evidenced by the debates about partitioning of selected proteins into lipid rafts (7, 8) and restriction of membrane protein mobility by submicrometer compartments in the cell membrane (9, 10).

On page 1072 of this issue, Sieber, Lang, and co-workers (11) refreshingly turn our attention back to the problem of simple diffusion and clustering of proteins in fluid membranes. They show that the clustering of syntaxin 1 in the plasma membrane of neuroendocrine PC12 cells (12) can be described by a simple physical model. The work is particularly notable because of the importance of syntaxin 1 in the docking and fusion of secretory vesicles to allow external release of their contents (exocytosis).

Syntaxins are members of the SNARE (13) family of proteins that mediate membrane fusion events associated with intracellular membrane trafficking (14). All SNARE proteins contain highly conserved heptad-repeat amino acid sequences that readily form coiled-coil structures that link secretory vesicles to target membranes during fusion. The active link structure, required for exocytosis, is a four-helix coiled-coil structure known as the SNARE core complex. This complex is formed from SNARE proteins on the secretory vesicles (vesicle-associated membrane protein or VAMP) and a soluble SNARE protein (synaptosome-associated protein or SNAP). Syntaxins and VAMPs are anchored

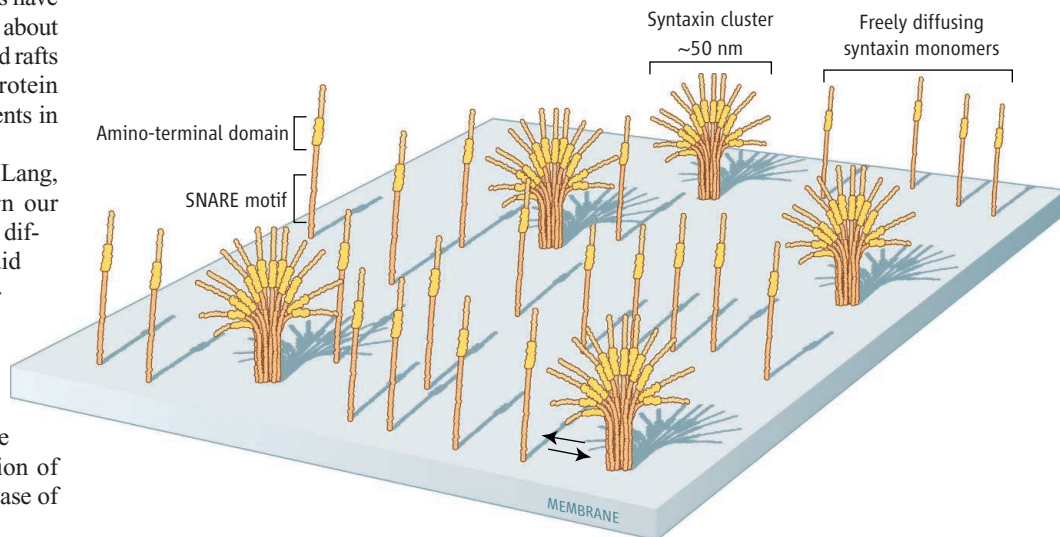
to their membranes by single carboxyl-terminal transmembrane helices. The two proteins are distinguished from one another by the subdomain structures of their amino-terminal domains, but in both cases the SNARE heptad-repeat sequences are immediately adjacent to the transmembrane helix anchors.

Lang *et al.* (15) have shown that effective exocytosis of PC12 secretory vesicles requires syntaxin 1 to form clusters. Harkening back to an earlier time when changes in lipid bilayer properties—especially lipid composition—were believed to be primarily responsible for the aggregation of membrane proteins (16), these clusters disperse when the normally high

Diffusion of the membrane-fusion protein syntaxin in cell membranes can be explained by a simple model of attraction and repulsion.

and computer simulations. The results show that freely diffusing syntaxins exchange dynamically with syntaxin clusters containing ~75 densely crowded syntaxins, with a cluster diameter of 50 to 60 nm (see the figure).

The technically demanding procedure is simple in concept. First, measure the size and number of clusters per  $\mu\text{m}^2$ . This is where stimulated emission depletion fluorescence microscopy (18) is invaluable, because it allows features with diameters below the diffraction limit to be resolved. Second, determine the total number of clusters from measurements of the average cell surface area using confocal microscopy. Third, establish



**How syntaxin clusters form.** Syntaxins freely diffuse in the plasma membrane to form clusters of ~75 molecules (11). Clustering appears to obey a simple physical principle: mutual attraction due to SNARE domains balanced by repulsion due to crowding. The clusters likely play an important role in vesicle secretion processes.

concentration of cholesterol in the cell membrane is reduced. Cluster dispersal through membrane cholesterol depletion strongly reduced the vesicle secretion rate. But even though cholesterol affects clustering, it is not directly responsible for it. Lang and co-workers recently showed that the syntaxin 1 SNARE motif drives clustering through self-association (17).

This observation set the stage for the experiments presented by Lang and co-workers in this issue. In a technical tour de force, they have quantitatively characterized cluster formation with a combination of stimulated emission depletion fluorescence microscopy, fluorescence recovery after photobleaching,

the total number of syntaxin 1 copies per cell with quantitative immunoblotting. Combined, these measurements yield the cluster diameter and syntaxin:cluster ratio.

Measurements of fluorescence recovery after photobleaching of living PC12 cells showed that the mobility of native syntaxin 1 was highly restricted. In contrast, mutant syntaxins that lacked most of the amino-terminal cytoplasmic domain were highly mobile. Restricted mobility was recovered when only the SNARE domain was added back to the transmembrane domain. Further observations of small numbers of clusters suggested that fluorescence recovery occurred via exchange of syntaxin molecules between the clusters,

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implying a dynamic equilibrium between monomers and clusters.

The coup de grace was delivered by Brownian dynamics simulations with a simple interaction potential between individual syntaxins. The photobleaching observations were reproduced perfectly by a simple model of weak homophilic attraction between monomers balanced by a repulsive potential due to steric repulsion. One can readily imagine the arms of the resulting anemone-like structures (see the figure) snaring passing vesicles in preparation for exocytosis.

The image of syntaxin clusters that emerges from this study is far more complex than textbook images, which generally show only two copies of the SNARE core complex bringing the vesicle and target membranes

together for fusion. One must wonder how the components of the core complex can locate one another in the protein tangle presented by the syntaxin cluster. How many SNARE core complexes are necessary for docking and fusion? Do the interactions proceed in the coordinated, structurally attractive manner of textbook cartoons? The impressions gained from the report by Lang and co-workers will be influential in designing experiments to answer these questions.

#### References and Notes

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## GEOCHEMISTRY

# The Oldest Fossil or Just Another Rock?

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The history of the past 542 million years of life on Earth can be read from shells, bones, teeth, and leaf casts preserved in the geological record. For the preceding 4 billion years, more subtle clues, such as remnants of microbial cells, biomolecules, and the impressions left by soft-bodied organisms, may be preserved in sedimentary rocks. But in sediments deposited in the first billion years of Earth history, these traces have been destroyed by metamorphism (recrystallization without melting, often accompanied by reaction and chemical exchange). Here, the only widely recognized evidence for life comes from measurements of carbon isotopes in kerogen and graphite (1). Because metabolic carbon fixation discriminates against  $^{13}\text{C}$ , the  $^{13}\text{C}/^{12}\text{C}$  ratio in biogenic carbon is ~3% lower than in inorganic carbon. This signature can be preserved through metamorphic processes that destroy microfossils and biomolecules.

In 1996, Mojzsis *et al.* reported the oldest indications of life on Earth to date (2) in a rock collected from a patch of intensely folded and metamorphosed quartz-rich rocks on Akilia, a tiny, barren island off the southwest coast of Greenland. The authors suggested that these

quartz-rich rocks are banded iron formations (a type of iron-rich marine sediment) deposited more than 3860 million years ago, and that at least one of them contains  $^{13}\text{C}$ -poor graphite derived from organic matter. Rocks nearly this old from elsewhere also contain  $^{13}\text{C}$ -depleted carbon (1).

The graphitic, quartz-rich rocks on Akilia have been widely discussed and intensely scrutinized. Much of this scrutiny has been critical and has eroded confidence in the original finding (3–11). But this year, the authors of the original study have punched back in a pair of papers (12, 13) that address the critics' most serious charges.

The ages of old, metamorphosed sediments can be constrained through isotope dating of igneous rocks that cut through, or contain inclusions of, those sediments. The originally reported age of the Akilia quartz-rich rocks [(2) and references therein] was based on the isotopic age of the mineral zircon in such a cross-cutting granitoid (an igneous rock rich in quartz and feldspar). But these zircons could be minerals from an unknown older rock that were entrained in the igneous rock while it was still liquid (3). It has also been suggested that the intense deformation undergone by nearly all Akilia island rocks prevents confident identification of places where granitoids cross-cut

Recent evidence strengthens the case for signs of ancient life in rocks from Akilia, Greenland, but important questions remain.

older volcanic or sedimentary rocks (4).

Manning *et al.* (12) refute the first criticism by showing that the trace-element contents of the suspect zircons (redated at 3820 to 3840 million years ago) are consistent with them having crystallized from their host rocks; thus, at least some granitoids on Akilia very likely are as old as originally claimed. The authors also strive to address the second critique, but against long odds: No cross-cutting relations between granitoids and the quartz-rich rocks have been observed. In some places, granitoids to cross-cut volcanic strata that are part of the same original set of strata as the quartz-rich rocks, providing a minimum age for the whole stratigraphic section. However, even these cross-cutting relations are partially obscured or otherwise ambiguous. Barring discovery of further cross-cutting relations, it is difficult to foresee how this part of the debate can be more definitively resolved.

Even if the Akilia rocks are as old as originally suggested, it is not obvious that any of them are sediments that could contain primary deposits of organic matter. Akilia island is largely composed of metamorphosed volcanic rocks and the granitoids that appear to intrude them; the thin layer of quartz-rich rocks is an anomaly (12). Moreover, the one sample of quartz-rich rock in which  $^{13}\text{C}$ -poor

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