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New and Notable

High-Speed Atomic Force Microscopy Tracks Toxin Action

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In this issue of *Biophysical Journal*, the article “Real-Time Visualization of Assembling of a Sphingomyelin-Specific Toxin on Planar Lipid Membranes” by Yilmaz et al. (1) describes a high-speed atomic force microscopy (HS-AFM) analysis of the assembly and the conformational changes of pore-forming toxin lysenin (2) on a supported lipid bilayer. Pore-forming toxins are ubiquitous in bacteria, fungi, and animals, inducing cell death through membrane permeation. They have been structurally (3) and functionally intensively studied (4). However, essential information concerning conformational changes, assembly dynamics, and membrane insertion of the toxin action has remained unknown, probably due to the lack of a technique that can analyze molecules with both high spatial and temporal resolution.

Using HS-AFM, pioneered by the authors (5), they study the addition of lysenin toxins onto supported lipid bilayers and report the subsequent steps of toxin action: Monomer diffusion is extremely fast, and HS-AFM imaging could not localize or track single subunits (which would quickly result in oligomer and cluster formations). These clusters are initially heterogeneously arranged and display a variety of short-distance

orientations. The authors are able to capture pore sub-elements like half-rings within these initial clusters, probably the unique observation of assembly intermediates of the toxin self-assembly process. Subsequently, lysenin self-assembly adjusts interactions, a process that is rather slow and takes place from seconds to up to minutes, leading to the formation of hexagonal close-packed assemblies, reminiscent of hexagonal two-dimensional crystals. The authors state

“Initially, both individual and small domains of lysenin clusters formed randomly at different locations on the membrane. While some of these domains grew continuously, most of them reorganized by subsecond dissociation/reassociation of the clusters.”

This indicates that the toxin assembly remains in a dynamic equilibrium of association/dissociation at the cluster edges over time, and that stable toxin assemblies gather in the hexagonally dense-packed regions.

Most impressive is the dynamic observation of membrane interaction:

“Thus, the observation of the bright (taller) oligomers just before the dissociation and after association supports the possibility of conformational changes in lysenin... Considering the size of lysenin, which is about 10 nm in length, a change in its height up to 3 nm seems to be resulting from the reversible partial insertion of clusters into the membrane by vertical collapse. Therefore, the height change might be an indication of the transition from prepore to pore.”

where single toxin rings undergo a conformational change that brings protein structure toward the bilayer plane, reported by the AFM by a height decrease of 2.4 nm. Doubtlessly, the

authors have captured the dynamic transition of the toxin ring assembly into the membrane-inserted prepore. Perhaps the solid support used in this work might have hampered complete pore formation, and a support with nanoholes in which certain membrane regions are nonsupported (6) could serve as a sample stage that allows acquisition of further insights into toxin action.

At this stage, HS-AFM image acquisition was between 1 and 10 frames per second. This feat is outstanding compared to the most commonly used AFM setups that monitor biological assemblies at frame rates of minutes. However, even at 100-ms frame acquisition time, some aspects, like the behavior of the toxin monomers, are still elusive. Future technical developments in the field of HS-AFM will certainly push this limit (7), and give the researchers access to even faster biological processes.

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