Secrets of plague revealed

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In work that is pushing the "diffraction barrier" associated with microscopic imaging of living cells, researchers at Sandia National Laboratories in Albuquerque, NM demonstrated the power of a new super-resolution microscopy technique called Stochastic Optical Reconstruction Microscopy (STORM), which can simultaneously image multiple molecules in living immune cells.

As described today at the 55th Annual Biophysical Society Annual Meeting in Baltimore, MD, Jesse Aaron and Jerilyn Timlin used this new technique to reveal the changes in the concentration of certain proteins in the membranes of human immune cells that encounter toxins from E.coli and showed that the same changes did not occur in immune cells that encountered toxins from Y.pestis, the bacteria that causes plague.

This work is significant because it addresses how our bodies are often able to naturally fight off some bacteria, such as E.coli, while the bacteria that cause plague are able to circumvent our immune systems. Moreover, this is the first time such differences have been detected because molecular changes like these are too small to be seen by conventional imaging methods, which can only reveal the microscopic world down to what is known as the diffraction limit -- essentially the smallest features of the microscopic world that can be resolved using visible light.

"[This] is a way to image biological samples at resolutions that, historically, were thought to be unachievable," says Aaron, who is a postdoctoral fellow at Sandia.

In particular, they were able to image the organization of a key human receptor protein called TLR4, which adorns the outside of immune cells in the body as they prowl for foreign invaders. These receptors recognize lipopolysaccharide (LPS), a toxic chemical that marks the presence of certain types of bacterial invaders, and the TLR4 proteins are key mediators of our bodies' early, "innate" immune responses to these sorts of bacterial infections.

"A cell membrane is a complex, heterogeneous system, so oftentimes you have many proteins that are interacting with each other simultaneously and the scale of those interactions is way below the diffraction limit," says Jerilyn Timlin, a principal scientist at Sandia National Laboratories. "Until the super-resolution methods were discovered, there really was not a way to visualize those interactions."

Now, by employing a novel, simultaneous dual-color imaging system based on the STORM technique and by using an objective-based TIRF microscope and filter-based image splitter, Timlin and Aaron have imaged how TLR4 receptors is organized after it encounters toxic bacterial LPS.

Resolving these molecular interactions at or below 40 nanometers (about 10 times finer than the highest resolution images that can be obtained with light microscopes), they showed that TLR4 receptors cluster together when they detect the toxin. Moreover they compared this clustering behavior for different types of toxins from different bacteria, including Y.pestis, the bacteria that causes plague.

Say Timlin and Aaron, this difference is evident when you look at the higher resolution, as they did in their study.

When TLR4 receptors encounters the toxins produced by E.coli, for instance, they increase in number and clusters on the cell membrane --

changes that are only detectable below the diffraction limit and are not evident using conventional imaging methods.

More information: The presentation, "SUPER-RESOLUTION MICROSCOPY REVEALS PROTEIN SPATIAL REORGANIZATION IN EARLY INNATE IMMUNE RESPONSES" by Jesse S. Aaron et al is at 11:45 a.m. on Tuesday March 8, 2011 in Ballroom IV of the Baltimore Convention Center. ABSTRACT: tinyurl.com/67ptfqs

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