IMAGING AND VISUALIZATION

Scattering gold

Gold nanoparticles are used to monitor caspase activity at the single-molecule level in living cells.

If a biologist could be a fly on the membrane, peering into the interior of the cell, watching single molecules as they move, interact and execute their functions, a new view of the cell would probably emerge.

But it is not easy to observe the activity of single molecules inside living cells. Fluorophores, the labels typically used for visualization, are prone to bleaching and blinking, and thus detecting them in the complex environment of the cell is challenging, in particular over long time periods.

Paul Alivisatos at Lawrence Berkeley National Laboratory and colleagues, now use a different probe to monitor enzyme activity in the live cell, one that is based on light scattering rather than fluorescence emission. Previous work has shown that the effective light scattering by gold nanoparticles can be used to examine single-molecule enzyme activity *in vitro*. To visualize the scattering signal in the live cell milieu, the researchers designed a crown-shaped cluster of gold nanoparticles, with a single central particle surrounded by five satellites, as mutual proximity of the particles increases the intensity of the scattered light.

The particles were linked by peptides containing the DEVD sequence, which is cleaved by the apoptotic protease caspase-3. Cleavage of the linker, and consequent disassembly of the nanoparticle, results in reduced scattering and also in a blue-shift of the scattered light.

The researchers delivered the probes to cultured cells (and most probably to the cytoplasm) by coating them with a cell-penetrating peptide. The particles could be visualized without a loss of signal over several hours under constant illumination, which is not possible with standard fluorophores. Upon induction of apoptosis, a drop in signal intensity was detectable much earlier than with standard population assays.

As the crown particles have a diameter of about 100 nanometers, they do not diffuse freely within the cell, facilitating the imaging of single particles over time. The addition of apoptotic inducers resulted in sharp, step-wise drops in signal intensity, which are thought to correspond to single cleavage events.

Although the researchers applied light scattering nanoparticles here to detect protease activity, it is conceivable that alternative design strategies could enable different types of studies, such as of protein interactions or of conformational changes, at single-molecule resolution, within the living cell.

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RESEARCH PAPERS

Jun, Y. *et al*. Continuous imaging of plasmon rulers in live cells reveals early-stage caspase-3 activation at the single-molecule level. *Proc. Natl. Acad. Sci. USA* **106**, 17735–17740 (2009).