IMAGING AND VISUALIZATION

All that glitters but does not blink

Silver nanoclusters are bright, stable and well suited for single-molecule applications.

The use of fluorescence microscopy to monitor the dynamic behavior of cellular macromolecules requires fluorophores that are bright, stable and not toxic to the cell. Probe properties are even more relevant in the case of single-molecule studies. Presently available fluorophores each have their own spectrum of strengths and weaknesses, and there is no class of emitters that can be considered ideal for all biological applications.

Small clusters of silver atoms have been known for decades to have fluorescence properties, and these clusters are the subject of a recent report from the laboratory of Robert Dickson at the Georgia Institute of Technology, Atlanta. "We showed some years ago that we could make silver nanoclusters on silver oxide surfaces," Dickson says, "and they were extremely bright. We were very motivated to get them into solution so they could be used as probes for microscopy, and we've been working on that ever since." In their most recent report on this effort, Dickson and colleagues prepared complexes of silver atoms with single-stranded DNA. Silver salts precipitate out of solution in aqueous conditions and thus silver clusters need to be stabilized. DNA works for this purpose, Dickson points out, because its cytosines bind the silver atoms very tightly, rendering them chemically stable in biological buffers. Other scaffolds, such as peptides, have also been used for this purpose, but the DNA-scaffolded clusters have properties that make them potentially very useful for single-molecule applications.

Organic dye fluorophores are small and usually nontoxic, but they have the disadvantage of rapid bleaching and are not bright enough for some applications. Also, most dyes blink—that is, show intermittent dark states—with characteristic time scales, thus making them less than ideal for single-molecule measurements. Semiconductor quantum dots are nanoparticles with bright, very stable and size-tunable emission, but this comes at the expense of large size (10–20 nm), problems with cellular toxicity, polyvalence in terms of attachment sites for biological macromolecules and extremely strong blinking behavior at all time scales. "Silver nanoclusters are sort of an intermediate," says Dickson. "They're small, they're bright, and they have complementary properties in many ways to the existing probes out there."

The researchers showed that DNA-stabilized silver nanoclusters containing two or three silver atoms emit in the near-infrared, that they do not blink on biologically relevant time scales (0.1 to >1,000 ms), that they are an order of magnitude more photostable than the fluorophore Cy5, and that, in organic films, they yield an order of magnitude more photons than Cy5. The photophysical properties in aqueous solution are similar, although not identical, to those in the immobilized state. Moreover, the clusters are equivalent to Cy5 in terms of size and chemical stability in aqueous buffers.

Silver nanoclusters have been visualized in bulk in cells and can be conjugated to proteins. The size of the cluster or the nature of the stabilizing scaffold can be manipulated to affect the fluorescence properties. "In the case of gold, as the cluster gets bigger, the emission wavelength gets longer, and that makes sense," explains Dickson. "But for silver it doesn't seem to be so simple. The scaffold seems to have a much stronger effect." In the future, a better understanding of this size-scaling as well as of the other factors that influence chemical and photophysical stability of these intriguing entities could help in the design of a range of small, size-tuned, stable fluorophores with no blinking and no toxicity to cells.

"Silver nanoclusters are a new class of emitters with really good chemical and photophysical properties," says Dickson. "It's going to take a while before they can be routinely used in cells, but we hope that we can improve them enough so that they will solve some issues with current probes."

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RESEARCH PAPERS

Vosch, T. *et al*. Strongly emissive individual DNA-encapsulated Ag nanoclusters as single-molecule fluorophores. *Proc. Natl. Acad. Sci. USA*; published online 22 May, 2007.

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