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Next-generation quantum dots

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Abstract

Quantum dots that are small and non-blinking offer new opportunities for dynamic single-molecule imaging in live cells.

Quantum dots are brightly fluorescent nano-crystals that have found use across a broad spectrum of biological imaging applications. When observed individually under a fluorescence microscope, these particles show a rapid on-and-off ‘blinking’ of their emission, an attribute that is often detrimental, especially for single-molecule imaging, as the molecules being monitored exhibit frequent loss of signal. In a recent paper in *Nature*, Wang *et al.*¹ reported that quantum dots with an alloyed composition gradient from the core to the surface do not blink but rather remain continuously ‘on’. This finding is both surprising and profound, and represents considerable progress toward the next generation of fluorescent intracellular probes.

Fluorescent dyes and proteins have been invaluable for visualizing the dynamics and interactions of biomolecules within living cells. Unfortunately, light emission by these tags rapidly decays during observation, and the weak intensity of the emitted light cannot be readily detected from single molecules. Single quantum dots, however, are immensely bright and easily observed using standard fluorescence microscopes, and they emit light hundreds to thousands of times longer than do fluorescent dyes and proteins. These two key characteristics have spurred intense interest in the use of these particles for live-cell imaging, but the utility of these particles has been limited, in part, because current commercially available quantum dots rapidly blink, are hydrodynamically large, are multivalent when functionalized with biological molecules and often aggregate inside cells.

Scientists have attempted to control the blinking behavior of quantum dots for over a decade, operating under the assumption that these nanocrystals turn off when they lose electrons, that is, when they become ionized. Before the work of Wang *et al.*¹, other researchers observed that blinking can be attenuated if a quantum dot core is coated with a thick crystalline shell that electronically insulates the core to prevent ionization^{2,3}. Although it is not possible to completely eliminate blinking using a thick shell, the optimized core-shell particles are in the ‘on’ state for >97% of the time and are never ‘off’ for longer than tens of milliseconds. Wang *et al.*¹ were able to completely eliminate blinking by preparing core-shell particles in which there is a smooth composition gradient from the core to the shell, a structure that never blinks because it can emit light when it is ionized (Fig. 1). A crucial difference between the thick-shell and gradient structures is the overall size of the resulting nanocrystals. Growth of a thick shell structure invariably leads to large quantum dots (generally >13 nm), whereas the particles with a gradient structure can be produced at a more compact size of 5–7 nm.

The hydrodynamic size of a nanoparticle probe has a large impact on its behavior in cellular environments. This is especially true for intracellular applications, as the cellular cytoplasm is a crowded maze of macromolecular structures that act as a sieve to limit diffusion of large

molecules. Experiments with polysaccharides suggest that the cytoplasmic diffusion in a cell of a molecule larger than ~15 nm in diameter is a small fraction of its mobility in aqueous solution, and a particle as large as 50 nm is effectively immobile⁴. Current quantum dots are generally 15–35 nm in diameter, with an elongated shape, and fall well within the range of strongly limited mobility⁵. Interestingly, on the other end of the spectrum, particles that are exceptionally small (<3 nm) may be actively transported into the nuclei of some cell types⁶. For cytoplasmic imaging, this leaves a small window for ‘optimal’ probe sizes. Until recently, probes in the 4–15 nm range have not been available.

Although it may seem that the new gradient structure introduced by Wang *et al.*¹ is ideal for generating long-awaited non-blinking quantum dots, the earlier thick-shell structure may actually be quite useful for certain applications. This is because the shell thickness can be readily adjusted to finely tune the blinking dynamics, which can then, in turn, be used as an unambiguous optical signature of a single molecule. In certain circumstances, such optimized blinking should not impair the tracking of molecules in motion if the blinking dynamics are engineered such that the off-time is much shorter than the average residence time of a quantum dot in the focal volume, but longer than the data acquisition rate. Of course this will only be beneficial for nanoparticles with limited diffusion, such as particles bound to membrane receptors or large particles in the cellular cytoplasm; small quantum dots (<15 nm) will diffuse too fast for blinking to be of practical use.

The large size of current quantum dots is due to both the shell structure and the thick organic bilayer coatings used for stabilization in water. These coatings are second-generation nanocrystal coatings; quantum dots were originally stabilized in water using small ligand surfactants, but these thin layers deteriorate quickly. To reduce the size of quantum dots, researchers have recently returned to this first class of thin coating and substantially improved its stability by increasing the ligand affinity through multivalent interactions. A monolayer of multi-dentate ligands, such as di-thiols conjugated to ethylene glycol oligomers^{7,8} or low-molecular-weight multidentate polymers⁹, has tremendously reduced the overall sizes to yield probes similar in size to globular proteins (5–10 nm). These coatings are expected to form the basis for the next generation of fluorescent particles that can diffuse similarly to biological macromolecules.

Because current quantum dots are much larger than most soluble biological macromolecules, when they are conjugated to bioaffinity molecules such as antibodies, the nanoparticle can dominate the behavior of the conjugate by hindering diffusion, reducing bioaffinity and increasing the propensity for nonspecific interactions. In addition, bioaffinity ligands are usually attached to quantum dots through chemical schemes that are inherently stochastic, such that the number and geometric orientation of conjugated molecules vary widely across the nanoparticle population. This means that within a population of quantum dot-antibody conjugates, some nanoparticles will have a large number of active antibodies that can cross-link multiple target molecules, which can damage the biological system under study. Researchers have recently found ways to isolate conjugates with precise valency (for example, monovalent streptavidin), resulting in particles with reduced steric hindrance and decreased cross-linking of target proteins¹⁰.

The next challenge will be to assimilate the individual characteristics of continuous (nonblinking) light emission, compact size and defined valency into a single particle with tunable emission. A secondary challenge is minimization of quantum-dot cytotoxicity. Quantum dots are composed of potentially toxic metal atoms and may also elicit unexpected cytotoxicity owing to colloidal effects and photon-induced free-radical formation¹¹. Nevertheless, quantum dots have been used for several years to track and monitor membrane

receptors, and the translation of this success to intracellular targets has already been demonstrated⁵.

Quantum dots offer a powerful new tool for illuminating the complex labyrinth of signal transduction pathways and uncovering the intricacies of biomolecular interactions within cells. Remarkably, quantum dot-based intracellular probes have advanced concurrently with super-resolution optical imaging techniques¹²; a combination of the two techniques promises to reveal the mysteries of cellular biology in unprecedented detail. Indeed, optical nanoscopy should benefit considerably from new quantum dots whose optical properties are uniquely tailored for imaging beyond the diffraction limit¹³.

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Figure 1.

Comparison of current and next-generation quantum dots. **(a)** Current quantum dots are elongated core-shell nanocrystals coated with a thick organic bilayer and are limited by signal intermittency (blinking), a bulky size and undefined valency (depicted here with tetrameric streptavidin conjugates). **(b)** Through nanocrystal engineering, Wang *et al.*¹ produced smaller quantum dots that do not blink owing to a gradient alloy structure. In addition, these quantum dots can be coated with a compact monolayer of ligands and conjugated with a defined biomolecular valency (illustrated here with monovalent streptavidin).