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Belling the Cat—Tagging Live Cells with Quantum Dots

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Featured article: Jaiswal JK, Mattoussi H, Mauro JM, Simon SM. Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat Biotechnol 2003;21:47–51.[3]

Fluorescence imaging allows high-resolution, noninvasive monitoring of live cells and organisms and their responses to the environment. Organic fluorophores have been the staple for most of these developments; however, some of the limitations of organic fluorophores have generated a need for other fluorescent probes for live and multicolor imaging. Quantum dots (QDs)[4] are inorganic fluorophores that offer many improvements over the organic fluorophores. These nanoparticles, first synthesized in 1980s, were not tested for biological applications until the late 1990s [reviewed in *(1)*], and the limitations of QDs, such as poor long-term stability in aqueous solution, toxicity associated with their composition, and safe delivery of QDs into live cells, prevented the full realization of the live cell–imaging potential of QDs. In 1990, a few coatings were developed to stabilize QDs in water and conjugate them with biomolecules, such as avidin and antibodies, for targeted labeling. One such coating developed by our collaborators at the Naval Research Laboratory in Washington, DC, used the negatively charged molecule dihydroxylipoic acid (DHLA). They also developed positively charged recombinant molecules that allowed the electrostatic linking of antibodies and streptavidin to DHLA-coated QDs *(2)*. Through the use of a biotin tag and a protein-specific antibody, these investigators achieved specific labeling with QDs in vitro *(2)*.

To evaluate the stability of DHLA-coated QDs in culture media and the specificity of labeling live cells with these QDs, we used P-glycoprotein (Pgp)—a cell surface multidrug transporter protein that provides chemoresistance to tumor cells. We obtained a population of cells that showed mixed expression of Pgp fused to the green fluorescent protein (Pgp-GFP). Owing to the GFP tag, all cells expressing the Pgp transporter were marked by GFP fluorescence. For targeting QDs to bind Pgp, we used avidin–biotin binding and electrostatic binding to generate QDs conjugated to an antibody that recognizes the extracellular part of Pgp. In the mixed population of live cells expressing Pgp-GFP, QD–antibody conjugates bound only the Pgp-GFP–expressing cells. This result demonstrated that QD conjugates prepared in this way have the specificity required to label molecules in live cells. These QDs were also stable in cell culture, and cells efficiently endocytosed DHLA-capped QDs. We were able to enhance the endocytosis by using cationic lipids that increased the association of QDs with cell surfaces. This result thus allowed endocytosis to become a viable approach for the intracellular delivery of QDs into live cells.

A key test of the utility of QDs for live imaging was to determine if labeling with QDs or extended imaging after QD labeling affected a cell's health. First, we monitored the proliferation of human cells that had been labeled with QDs via endocytosis. As we monitored the cells for more than a week, we found the survival and growth of labeled and unlabeled cells to be indistinguishable. Next, we monitored whether QD affected cell movement, differentiation, and development. The cellular development of the slime mold *Dictyostelium* requires chemotaxis and differentiation of its cells. We found that QD-labeled *Dictyostelium* cells chemotaxed and differentiated in the same manner as unlabeled cells. To test the effect of QD labeling on the behavior of mammalian cells in vivo, we evaluated the ability of metastatic mouse tumor cells that had been endocytotically labeled with QDs to survive circulation in the blood, extravasate out of the blood stream, migrate, grow, and form tumors in mice *(3)*. In this intense competitive environment with strong selection for survival, the performance of QD-labeled and unlabeled cells in the live mice were indistinguishable.

While we were assessing the utility of DHLA-capped QDs, physicists across the campus who were working with phospholipid micelle–encapsulated QDs contacted us about testing the utility and biosafety of these QDs. These QDs were not functionalized, which precluded specific labeling; however, on the basis of our observations and discussions, these researchers took the approach of microinjecting QD-containing

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[3] This article has been cited more than 1100 times since publication.
[4] Nonstandard abbreviations: QD, quantum dot; DHLA, dihydroxylipoic acid; Pgp, P-glycoprotein; GFP, green fluorescent protein.

micelles into cells. They observed that QD micelle–labeled and unlabeled cells in embryos from frog eggs participated similarly in embryo development *(4)*. Two other studies, conducted in parallel in laboratories across the continent (in California) and using different QD coatings, independently observed that QDs could be used for the specific labeling of live and fixed cells *(5, 6)*. Together, these studies and over 1000 subsequent studies that have used QDs to label cells are testament to the fact that the transition of QDs from material science laboratories to clinical and biological research laboratories has occurred.

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