

Quantum dots shed light on processes in living cells

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New reagents and techniques in cell biology make it possible to visualize cell-signaling mechanisms in real time.

Water-soluble, biofunctionalized, semiconductor quantum dots (QDs) are fluorescent nanoparticles that have the advantage of much greater photostability compared with conventional fluorescent dyes. As a consequence, single QDs can easily be detected in living cells, and their localization can be monitored over minutes to days.¹⁻⁴ We have shown that QDs coupled to growth-factor ligands behave similarly to unmodified growth factors, and serve as highly fluorescent probes of the erbB family of tyrosine kinase growth factor receptors.¹

Single epidermal growth factor (EGF) peptides attached to QDs were used to activate epidermal growth factor receptors (EGFR, erbB1) by binding to the receptors. This caused the to form pairs (dimers) that, in turn, induced the uptake of the resulting QD-EGFR complex into internal vesicles, or endosomes.¹ Continuous confocal laser scanning microscopy and flow cytometry measurements of QDs, combined with fusing visible fluorescent proteins to the receptors, allowed us to visualize individual steps in the signaling cascades initiated by these receptors in living cells (see Figure 1).

This approach has led to the exciting new discovery of a novel retrograde transport mechanism of the activated receptors on filopodia.¹⁻³ The cytoskeleton of mammalian cells comprises a dynamic network of polymerized actin and tubulin, and their associated proteins, that mediates protein trafficking, cell motility, and endocytosis. The filopodia are fine sensory processes that extend from the cell body. They have a core of F-actin bundles that undergo growth and exchange by the addition of actin monomers to one end, at the tips of the filopodia, and depolymerization from the other, a process referred to as treadmilling.⁵ The process also involves active transport of the actin filaments by myosin molecular motors, culminating in a net flow of F-actin directed toward the interior of the cell. Association of other macromolecules with an actin subunit within the filament of a

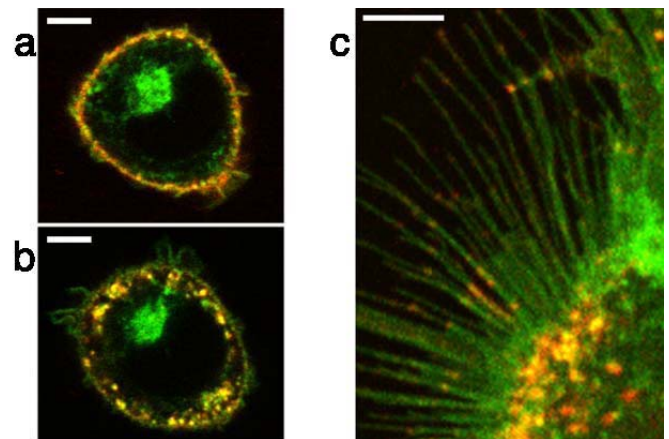


Figure 1. A QD-EGF binds and activates cells expressing a green fluorescent protein (GFP)-fused EGFR. (a) Red QD-EGF and green GFP-EGFR colocalize on the cell membrane within minutes and (b) are rapidly internalized together into endosomes in the cytoplasm. (c) Individual receptors on long sensory filaments called filopodia can be visualized by QD-EGF binding. Images are single confocal sections through living cells.

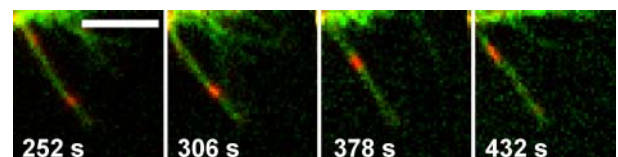


Figure 2. Snapshots in time showing retrograde transport of QD-EGF-receptor-activated complexes on a filopodium.^{1,2}

filopodium causes that macromolecule to move toward the cell body in a process known as retrograde transport.

Using inhibitors of EGFR kinase as well as cytochalasin (a drug that depolymerizes actin), we showed that retrograde transport of the QD-EGFR complex occurs only after EGF-loaded receptors dimerize and couple to the actin treadmill.

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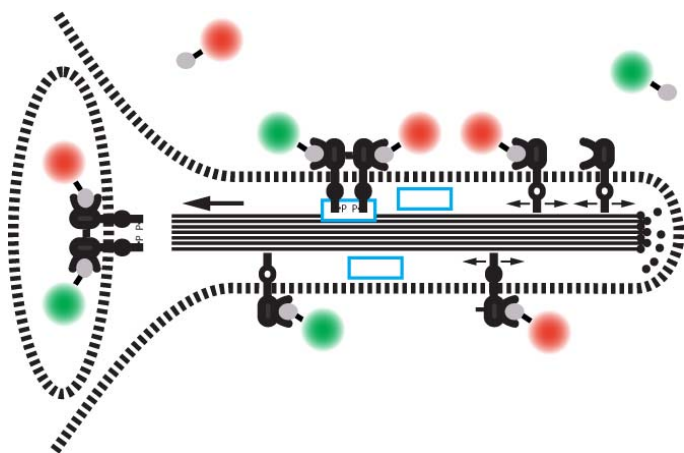


Figure 3. The scheme depicts the mechanism of filopodial retrograde transport. QD-EGF binds to EGFR monomers diffusing in the membrane. Dimerization of two liganded receptors activates the receptor tyrosine kinase, leading to autophosphorylation of the EGFRs and their attachment to the actin filament bundle in the filopodium through an adaptor protein complex denoted by the blue box. The QD-EGFR complex stops diffusing and is transported by actin treadmilling toward the cell body, into which it endocytoses through a clathrin-coated pit.²

Moreover, the receptors ‘surf’ on the external surface of the filopodia and are only endocytosed at their base after retrograde transport (Figure 2).² These results suggest that the receptors situated on the filopodia play an important role as a sensory system (natural ‘biosensors’) for the organism. Such a system would assist in regulating signal transduction pathways through transport of receptors only after a given threshold of ligand binding is attained (see the model shown in Figure 3).

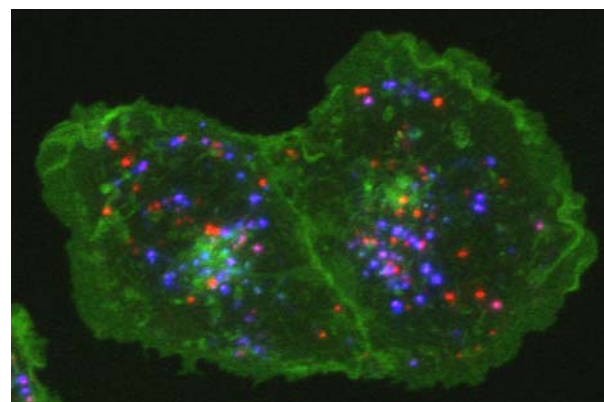


Figure 4. In pulse-chase experiments using two different colors of QD-EGF, binding and endocytosis of orange QD-EGF by EGFR was followed 22min later by blue QD-EGF. Few endosomes contain both colors of QDs (purple dots) when the pulses are separated by more than 15min.³

Following uptake into the cell via clathrin-coated pits at the base of the filopodia, endocytic vesicles containing EGF-QD surrounded by GFP-EGFR undergo Brownian movement, a directed linear motion mediated by microtubule-associated motor proteins, vesicular fusion, and finally sorting of the QDs to the lysosomes.¹ By varying the time between additions of different colored QD-EGF complexes in pulse-chase experiments (Figure 4), we were able to measure the lifetime of the fusing endosomes.³

Absorption of a photon by the QD semiconductor core anywhere in a continuum rising toward the ultraviolet generates an

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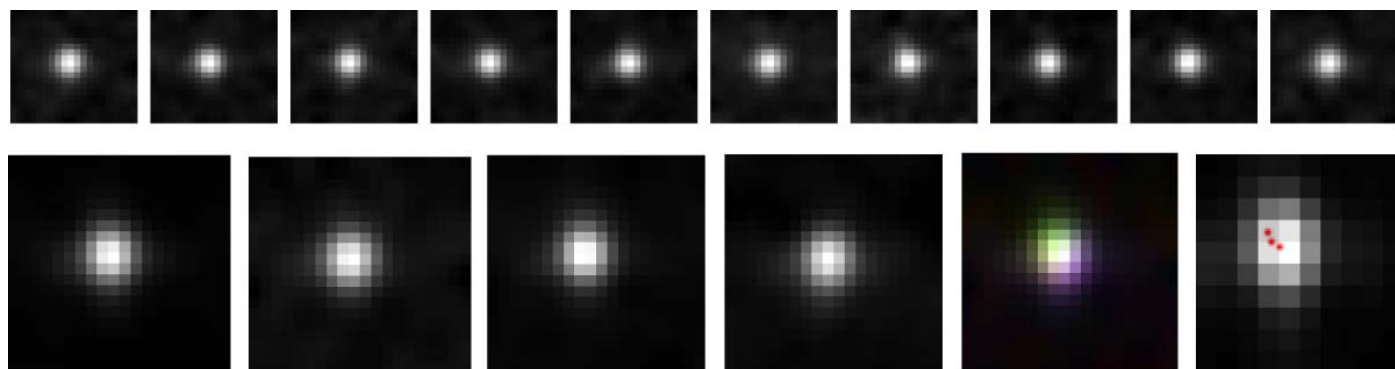


Figure 5. Independent component analysis (ICA) decomposition of blinking QD data sets showing the resolution of three QDs in one locus. The upper panel shows 10 frames from a time series. The lower panel shows left to right: time average of the series; ICA-returned component 1; ICA component 2; ICA component 3; color-coded overlay of components 1, 2, and 3; position overlay of 1, 2, and 3.⁶



electron-hole pair, which on recombination results in emission of a less-energetic photon. The emission wavelength depends on the size of the core (smaller produces lower wavelength). Although the blinking behavior of QDs adds complexity to some live cell- imaging applications,³ it can be used to achieve super-resolution in the light microscope⁶ (Figure 5). A more serious problem is that posed by QDs emitting >600nm: greater size owing to outer shells for passivation and for biocompatibility limit the ability of the QDs to form tight cell junctions.³ Noble metal nanodots constitute an attractive alternative to QDs in that they are much smaller and nontoxic.^{7,8} We have developed a new electrochemical method for synthesizing gold nanodots that allows us to reproducibly control cluster size.³

The in-vivo measurements using QDs described above provide new insights into processes and interactions that previously could only be studied on fixed cells or by biochemical fractionation.⁹ The combination of different QDs and even smaller fluorescent noble metal nanodots coupled to cellular constituents promises single-molecule resolution of the locus, aggregation state, and dynamics of those constituents in live cells.

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