Commentary

Quantum dots for robust and simple assays using single particles in nanodevices

Peixuan Guo, PhD,a,* Chiming Wei, MD, PhDb

aPurdue University, West Lafayette, Indiana
bJohn Hopkins University School of Medicine, Baltimore, Maryland

Fluorescence microscopy, which enables the direct observation and detection of biologic materials, has become a powerful tool in biologic research. Concise evaluation, accurate quantification, and precise localization with ultimate sensitivity in the analysis of biomolecules can be achieved when single molecule microscopy is used. Classically, studies of biomolecules at the single molecule level often involve large teams of specialized biophysicists, sophisticated and finely tuned equipment, and long periods of time to record and analyze relatively few events. In the meantime, these studies also suffer from the limitations of certain organic dyes, such as photobleaching, instability, and low quantum efficiency. It is very desirable now to have fast, robust assays with single molecule sensitivity using photo-stable components readily available for routine diagnostics in clinical or scientific applications. For the sake of speed and simplicity, such assays would ideally be separation free and capable of multiplexing.

The combination of nanotechnology with biology and medicine is expected to lead to major advances in molecular diagnostics, molecular biology, and therapeutics [1,2]. Nanoparticles have been recently used for the detection of specific nucleic acid sequences [3-5]. The study described by Yeh et al [6] in the current issue of the Journal represents a step toward single particle bioassays in a simple, robust, and rapid format; combining advances in several scientific fields into this one assay is a novel and promising approach. The authors took advantage of the unique optical properties of core shell semiconductor nanoparticles [quantum dots (QD)] (Figure 1, A and B), which are pioneering achievements in nanotechnology [1]. Quantum dots are nanocrystals; the term emphasizes the quantum confinement effect and generally refers to the subclass of fluorescent nanocrystals that are small enough to exist in the quantum confinement regimen. Such nanoparticles are stable, bright, and do not photobleach like organic dyes. These nanoparticles display different colors corresponding to their sizes but with a single excitation wavelength, which makes the setup for multicolor detection simple. In their study, the authors also used microfluidic channels and up-to-date optical techniques for rapid, robust biomolecule detection, recording, and analysis [6]. The combination of the advanced optical technique with separation-free assay and automatic recording greatly improved the sensitivity of nucleic acid detection. The result is an assay that shows the potential to be made available as a robust routine test in molecular diagnostics yet with single molecule sensitivity. We are therefore witnessing continued robust signs signifying that interdisciplinary approaches including nanotechnology will have a profound impact on how we diagnose and treat medical conditions.

It is also important to note that as the ex vivo or in vitro applications of QDs continue to mature, the potential in vivo applications also represent a tremendous opportunity (Figure 1, C and D). Biocompatible or biodegradable nanoparticles or QDs in cells or tissue of interest in vivo can have a wide variety of applications in very early detection and identification of disease, and they represent one of the next frontiers of applications of nanotechnology in medicine. Biocompatible QDs have been applied for labeling cells and tissues for long-term cell trafficking, multicolor cell imaging, and fluorescence energy transfer–based sensing [2,4,7] (Figure 2). Because of the requirement of sample flow and single fluorescent complex sorting with the technique described, the approach by Yeh et al [6] cannot be applied directly to intracellular analysis or imaging by using the intact cells. However, this does not exclude potential future applications in intercellular analysis. For example, this technique might be applicable to the analysis of cell lysates.
The report by Yeh et al [6] describes the application of QD probes for the fluorescent detection of DNA sequences. It can further our knowledge concerning the impact of DNA mutation, damage and repair information, and mechanisms in the development of cancer or other genetic diseases. With further improvement and additional design, it might be possible to use this method as a standard technique for protein, RNA, and DNA quantification because new QD developments will draw on such unique biophysical properties to strengthen the quantitative data obtained and reduce the amount of processing analysis required to compensate for photobleaching. One concern in the biologic application of QDs is the nonspecific binding to biologic materials. This is in part due to the hydrophobicity of the QD itself. Current approaches, including coating the particle with biologic or chemical materials containing both hydrophobic and hydrophilic domains or moieties, will continue to improve the solubility of the complex and solve the problem of nonspecific binding [9].

**Acknowledgments**

We thank Drs Dieter Moll, Hui Zhang, Rashid Bashir, and John Turek for insightful comments on this article. We appreciate Drs Paul Alivisatos and Hedi Mattoussi for providing figures and feedback. This work in P. G.’s laboratory was supported by National Institutes of Health Nanosciences and Nanotechnology in Biology and Medicine grant No. R01-EB003730.

**References**

Fig 2. Function and properties of the 560QD-MBP nanosensor. A, Diagram showing the 560QD-MBP nanosensor. Each 560-nm emitting quantum dot (QD) is surrounded by an average of 10 MBP moieties; only one MBP is shown here. QD-MBP-β-cyclodextrin-QSY-9 formation results in the quenching of QD emission. The addition of maltose displaces -cyclodextrin-QSY-9 and results in an increase in direct quantum dot emission. B, Graph illustrating 560QD-10MBP maltose sensing. 560QD-10MBP/QD conjugate titration (with a quantum yield ~39%) is shown with preassembly of 1 μmol/L β-CD-QSY9 at increasing concentrations of maltose. C, Titration data transformation with fractional saturation shown on the left axis. The right axis represents PL at 560 nm. The 50% saturation point was used to solve for the maltose apparent dissociation constant (K_app). (Courtesy of Hedi Mattoussi. Figures adapted from reference 4 with permission.)