

## QUANTUM DOTS

## DNA detectives

The use of quantum dots as DNA nanosensors promises to significantly enhance the sensitivity of fluorescence-based DNA detection for medical diagnosis and biomolecular investigations.

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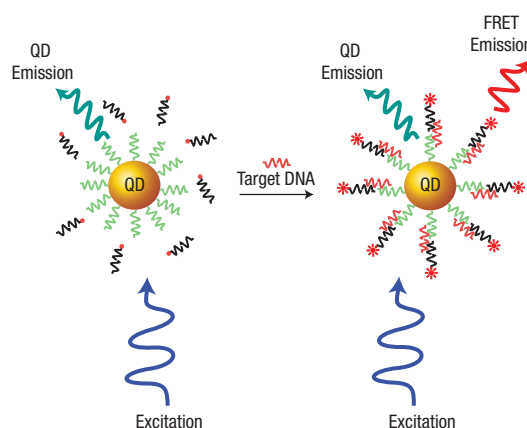
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**D**etection of minute concentrations of nucleic acid sequences is important for medical diagnosis and to understand biomolecular mechanisms. On page 826 of this issue, Zhang *et al.*<sup>1</sup> unveil an original scheme based on single-particle detection in solution that has two advantages compared with the classical DNA-chip technique: it is label-free, and it can directly detect nucleic acid sequences.

One of the great challenges of our time is to be able to quantify the amount of given RNA sequences present in a cell. The determination of RNA sequences is important, as protein production is orchestrated by the cell genome through the synthesis of RNA sequences. The challenge is hard indeed because an RNA sequence can be present in very little quantity in the cell — sometimes as small as a few tens of copies. Most common detection methods are based on the transformation of RNA sequences into DNA sequences, followed by an amplification step obtained by a polymerase chain reaction that produces multiple copies of the original DNA. The amplified product is then labelled with a fluorophore (or a radioactive marker) and analysed on DNA chips. In this setup the DNA sequences are sorted on a solid substrate according to their affinity with other DNA sequences, and detected through quantitative fluorescence measurements. Because of the noise induced by the amplification step and the necessary labelling of the probes, DNA-chip techniques are not well adapted for the quantitative detection of low-abundance sequences. This severely limits the applications of DNA-chip techniques.

In the setup of Zhang *et al.*, the sensing of the target sequence occurs when it is sandwiched between a reporter strand of DNA (labelled with a fluorophore) and another strand linked to a nanometre-sized semiconductor quantum dot (QD; Fig. 1). The idea introduced by Zhang *et al.* is to



**Figure 1** Nanosensors based on quantum dots. The target DNA is selectively linked to quantum dots and detected by FRET.

detect the target DNA through the fluorescence emission of the QD simultaneously at two different wavelengths. One wavelength corresponds to the QD emission, the other to the emission of the fluorophore, serving as a reporter probe. In the absence of the DNA target sequence, the nanoparticle and the reporter probe are unlinked and only the QD fluorescence is detectable. However, on addition of target DNA, the reporter probes are linked to the QD surface, and emission occurs at both colours because of a resonant energy transfer from the QD to the organic fluorophores attached to the QD.

Fluorescent resonant energy transfer (FRET) is a technique that has made recent qualitative and quantitative improvements<sup>2</sup> in terms of sensitivity. One of its main characteristics is that this phenomenon is very sensitive to the distance between the two interacting fluorophores. So far, energy transfer between a QD and an organic fluorophore has been observed with QDs bearing a surface chemistry specifically designed to minimize the distance between the nanoparticle and the organic fluorophore<sup>3</sup>. Interestingly here, the energy transfer is observed using commercially available QDs. This is quite unexpected because the relatively large size of these commercial QDs (including the polymer coating) has been measured to be around 30 nm

in diameter<sup>4</sup>, which makes the energy transfer very inefficient. The trick here is to use the QD as a DNA concentrator by linking several DNA sequences to it. According to Zhang *et al.*, one QD can be linked to up to 54 DNA sequences. This makes the energy transfer much more efficient to detect.

Label-free DNA detection schemes in a separation-free format have already been developed using nanomechanical cantilever arrays<sup>5</sup> or array-based electrical detection<sup>6</sup>. Although these methods have advantages compared with the classical DNA-chip approach, they use probes immobilized on a solid-state substrate and cannot be implemented in solution. Detection in solution has several advantages: mixing and therefore reaction kinetics are faster, and non-specific adsorption due to large surface areas is greatly reduced. Furthermore, the probe concentration can be finely tuned so that low target concentrations can be detected.

In particular, about 10 years ago Tyagi *et al.* introduced the molecular beacons<sup>7</sup>, a label-based and separation-free detection scheme that uses probes that fluoresce upon DNA binding. This detection technique has shown its advantages in terms of specificity<sup>8</sup>, but its sensitivity is limited by the probe signal/background ratio. The advantages of the work presented by Zhang *et al.* is that their single-molecule detection scheme gives a lower detection threshold than the molecular beacon strategy.

The price Zhang *et al.* have to pay though is that as their detection is based on single QD particle fluorescence detection, several QD events are needed (a few thousands) and some form of statistical analysis is required. Detections will therefore be longer

than for ensemble measurements. Also, to get low detection thresholds, the concentrations of the probe and of the reporter DNA have to be carefully chosen to take advantage of the concentrator effect. It should also be noted that one important aspect of DNA detection is the need to detect several sequences in parallel. Although it would be possible to detect a few (three to four) sequences simultaneously using QDs emitting at different wavelengths, this is far from the few thousands of sequences that can be detected with classical DNA-chip methods.

Despite its limitations, the method proposed here lays down an additional stone on the road of macromolecule detection using single particles. The advantages in terms of sensibility are clear, but further experiments will be needed to go beyond the proof of concept. It should be possible to use different QD species acting as an optical 'bar code' to take advantage of the sensitivity increase and to have access to multiplexed detection. In any case, these studies show that the use of QDs and their specific optical properties are a promising basis for new and enhanced fluorescence-based detection techniques.

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