Utilizing Quantum Dots to Label DNA

ABSTRACT

By using a modified version of polymerase chain reaction (PCR), researchers from China's Wuhan University were able to show that one-to-one labeling of DNA with quantum dots could be achieved. Using a Wavelength Electronics temperature controller, thermal testing of quantum dots was done to ensure the stability of the quantum dots during the drastic temperature fluctuations of PCR. This individually labeled DNA has the ability to only bind to complementary binding sites. Thus, by labeling DNA with quantum dots, the ability to image or look at very specific regions is enabled. Moving forward, this hyper-sensitive labeling scheme can to be applied in biology, medicine, and nanomaterial fabrication.

WHY?

Quantum dots, due to their small size, and fluorescent properties, are increasingly being utilized in fundamental scientific research, medical diagnosis, and nanomaterial development.

Quantum dots provide advantages over previously used fluorescent labels. They are brighter than organic fluorophores, which are widely used in imaging. Additionally, quantum dots exhibit less photobleaching. These two qualities are giving researchers hope that quantum dots can be a solution to present problems stemming from labels that aren't bright enough, or that undergo irreversible photobleaching quickly.

The fluorescent properties of quantum dots are of particular use if the quantum dots can be bound to a region of interest. In this case study, the researchers were able to bind a single quantum dot to a single long strand of DNA.

The choice to bind quantum dots to DNA was made due to the properties of DNA. Using DNA will allow for better cell imaging, and sequence-specific DNA detection. Both of these properties are of particular importance in biology and medicine.

Binding quantum dots to DNA will also be beneficial for the development and improvement of nanomaterials. The natural ability of DNA to bind to many different types of materials makes it a promising candidate for developing functional nanomaterials.

HOW?

In order to bind the quantum dots to the DNA, the researchers chose to use polymerase chain reaction (PCR) as the basis for their experiment. PCR allows for exponential reproduction of DNA. This yields a better chance of a quantum dot binding to the DNA due to the greatly increased number of binding sites available.

PCR has a specific cycle of heating and cooling (breaking down and putting back together strands of DNA). This heating and cooling has a temperature differential of approximately 80°C. This means that the quantum dots that were chosen needed to be able to withstand large changes in temperature without losing their fluorescent properties, be water soluble, and able to bind with the DNA through specifically designed primers.

The basic process followed was comprised of three steps:

1. Quantum dots were made water soluble and coated with octylamine-modified polyacrylic acid (OPA).
2. Specially designed primers were then conjugated to the OPA-coated quantum dots.
3. PCR was carried out, attaching DNA to the quantum dots.

The OPA coating allowed the succesful binding of primers to the quantum dots. After PCR was carried out, a thorough investigation to confirm the one-to-one binding between the QDs and the DNA was carried out.

RESULTS

To confirm that a single QD was bound to a single strand of DNA, the researchers utilized two methods: gel electrophoresis analysis and atomic force microscopy (AFM). Fluorescence spectroscopy was then used to ensure that the binding of the QDs to the DNA didn't shift the fluorescence spectrum of the QD.

The first check of the one-to-one binding was via gel electrophoresis analysis, as shown in **Figure 1**. Through comparison with a well-known DNA sample, gel electrophoresis analysis allows the estimation of the size or number of base pairs (bp) of a given sample. By applying a voltage across the gel, the samples will move through the gel. The smallest samples will move the farthest through the gel, while the larger samples will have more difficulty moving through the gel, and thus, won't travel as far. By comparing normal PCR products, OPA-coated QDs, QDs with primers, and the hypothesized one-to-one QDs bound to DNA, it was found that the DNA bound QDs had the largest size, as expected.

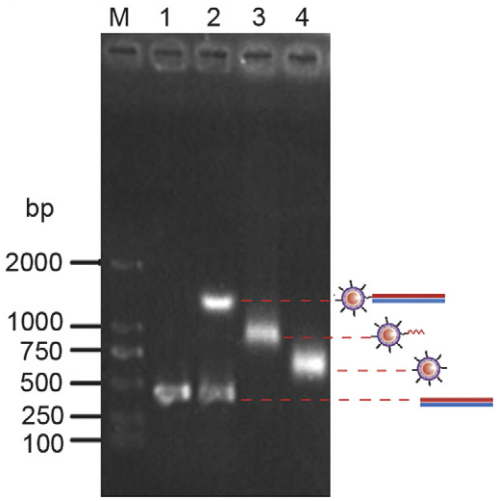

Figure 1. Gel electrophoresis measurements showed successful binding of QDs to DNA. Lane M is a DNA marker. Lane 1 is normal PCR products. Lane 2 is QD-based PCR products. Lane 3 contains QDs with primers attached. Lane 4 contains OPA-coated QDs.

The next check was to use AFM to image the actual bound samples. Utilizing this method allowed the researchers to actually see the bound QDs, with the single long strand of DNA attached. A large scale image was acquired with multiple succesful bound QDs to DNA, as well as a zoomed-in image showing a single QD attached to the DNA strand. Via AFM, the researchers were able to estimate both the height and width of the DNA strand. This allowed estimation of the number of DNA base pairs attached to the QD. **Figure 2** shows the AFM results.

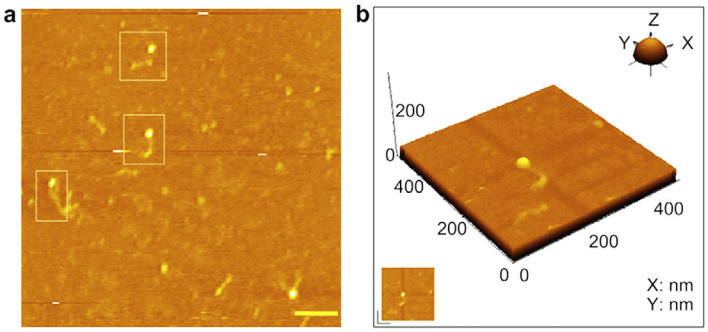

Figure 2. AFM confirmed that QDs were successfully bound to single-long DNA strands. (a) is a 1500 nm x 1500 nm image showing multiple successful binding sites (enclosed by rectangles). Scale bar is 200 nm. (b) shows a 3D representation of a single binding site, zoomed in to 500 nm x 500 nm.

Finally, the fluorescence spectra was measured for various steps of the process. Spectra were measured for the QDs after OPA coating, after the primers were attached, and after PCR was performed (see **Figure 3**). Ideally, the spectrum shouldn't shift, allowing the same excitation and emission wavelengths to be used to study the bound QDs. The measured spectrum indicated that the binding of QDs to DNA shouldn't show a measurable shift in the fluorescence spectrum.

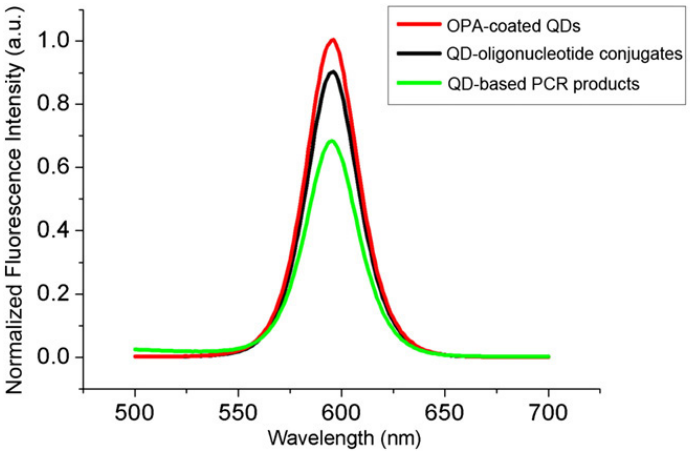

Figure 3. Spectra were measured after the first three steps of the process. None showed a measureable shift in the resulting fluorescence, indicating suitability for probes.

The researchers then showed that the labeled DNA probes would bind to complementary sites on a single copy gene, but would not bind to noncomplementary regions. This shows that this process of labeling DNA with quantum dots can be customized to fit other materials, and that this process is applicable in many different areas of science.

ENSURING STABILITY – WAVELENGTH ELECTRONICS LFI3751

The ability of the quantum dots to withstand the extreme temperature changes that are inherent to PCR was key to the success of the research. Once the researchers were sure that the primers were bound to the quantum dots, the next step was to ensure that the dots could handle the temperature cycling of PCR.

In order to simulate the temperatures of PCR, the researchers used a Wavelength Electronics LFI3751 temperature controller to put the quantum dots through repeated thermal cycling. Over the course of three hours, seven full cycles (15°C to 95°C and back) were made as shown in **Figure 4**. During this test, the LFI3751 was able to accurately and repeatably control the sample temperature between the temperature limits. This ensured a quality test that simulated PCR temperature changes as accurately as possible.

During the temperature testing, the QDs showed remarkable stability, and recovery from the high temperature limit. Note that the high temperature limit is where the minimum fluorescence signal occurs, while the maximum signal is measured at the lower temperature limit. Due to incomplete coating of OPA on a small portion of the QDs, the first temperature cycle did not achieve full fluorescence recovery. The QDs which were not fully coated became irreversably quenched during this first temperature increase.

Doing this simulation allowed the researchers to know that the quantum dots would be able to withstand the temperature changes of PCR. By using the LFI3751 to simulate PCR, the researchers were able to save time and materials, while ensuring that the quantum dots that they chose to move forward with would not be compromised by the extreme temperature changes of PCR.

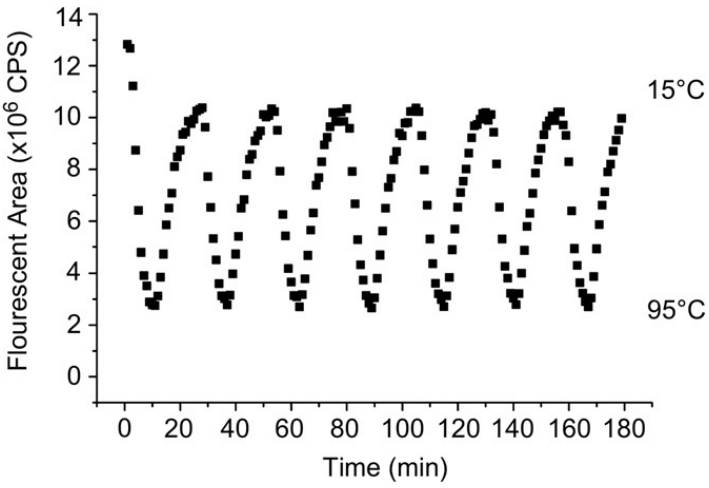

Figure 4. Using a Wavelength Electronics LFI3751, the researchers put the prospective QDs through temperatures that simulate the extreme conditions of PCR. The maximum signals occur at 15°C, while the minimum signals are at 95°C.

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PRODUCTS USED
LFI3751

KEYWORDS
quantum dots, temperature control, thermal cycling, polymerase chain reaction, PCR, DNA, fluorescence, spectroscopy, atomic force microscopy, AFM, photostability, nanomaterials, nanobiotechnology, life science research

USEFUL LINKS

- LFI3751 Product Page

REFERENCES

1. Shibin He, Bi-Hai Huang, Junjun Tan, Qing-Ying Luo, Yi Lin, Jun Li, Yong Hu, Lu Zhang, Shihan Yan, Qi Zhang, Dai-Wen Pang, Lijia Li, "One-to-one quantum dot-labeled single long DNA probes," Biomaterials **32**(23), 5471-5477 (2011). https://doi.org/10.1016/j.biomaterials.2011.04.013

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