Introduction

The detection of nucleic acids has numerous applications ranging from pathogen identification, genetic analysis, and forensic chemistry. The most widely used method for nucleic acid detection is based on hybridization of two complementary strands of nucleic acid. Hybridization methods can be broken down into either multi-step or single-step methods. Polymerase chain reaction-based methods, blotting, microarrays and fluorescence in-situ hybridization-based methods fall into the multi-step category. Molecular beacons, quenched auto-ligation probes, and various energy transfer methods fall into the single-step category. Currently, multi-step methods are more time-consuming, while many single-step methods are limited due to the possibility of non-specific signal yielding high background. Therefore, the development of more rapid, highly sensitive, solution-phase nucleic acid detection methods is needed.

In this report, nucleic acid is detected in solution phase using bioluminescence resonance energy transfer between the luminescent enzyme Renilla luciferase (Rluc), and quantum dots (QD). To perform the assay, Rluc was conjugated to an oligonucleotide with the same sequence as the target, while QDs were conjugated to oligonucleotides with a sequence complementary to the target. In this assay, once target nucleic acid is mixed simultaneously with Rluc-probe and QD-probe, the target and Rluc-probe compete to hybridize with the QD-probe. Therefore, upon addition of coelenterazine (ctz), the substrate that binds to Rluc giving off light at 485 nm, the more target there is present in the sample, the lower the emission signal from the QD-probe due to the Rluc and QD not being within close proximity.

Experimental

- phRI-CMV plasmid was mutated to include a 6X His tag for purification
- Rluc was expressed and purified via Ni²⁺ affinity chromatography
- Rluc was conjugated to a thiol-modified oligonucleotide (T1) with the same sequence as the target
- QD was conjugated to an amine-modified oligonucleotide (CT1) with a sequence complementary to the target
- Hybridization time and temperature were optimized to be 37°C for 30 min
- 4 pmoles of Rluc-T1 and QD-CT1 were hybridized in borate buffer, ctz added, and a luminescence scan recorded from 450-750 nm
- Varying amounts of target were combined with a fixed amount of Rluc-T1 and QD-CT1 (4 pmoles), ctz added, and the BRET ratio recorded
- 4 pmoles of Rluc-T1 and QD-CT1 were hybridized in ER2566 cellular extract, ctz added, and a luminescence scan recorded from 450-750 nm

Results

- BRET Luminescence Scan in Buffer Matrix

Conclusions

- Rluc and QD conjugation does not alter their luminescence properties. Rluc emits at 485 nm and QD emits at 710 nm both before and after conjugation
- In the absence of target, Rluc-T1 and QD-CT1 hybridize in a buffer matrix and upon addition of ctz, emission peaks at 485 nm and 710 nm, corresponding to Rluc and QD, respectively are seen
- In the presence of target, Rluc-T1 and QD-CT1 hybridize with QD-CT1, resulting in a decrease in 710 nm emission due to the fact that QD and Rluc are not within close proximity in order for BRET to occur
- As amount of target is increased, 485 nm emission increases, coupled with a decrease in 710 nm emission, thus increasing the BRET ratio (I₂₅₀/I₇₁₀)
- In an E. coli cellular matrix, 4 pmoles of Rluc-T1 and QD-CT1 hybridize, and upon addition of ctz, reveal a luminescence scan comparable to the scan in the buffer matrix, thus showing the assay's compatibility in more complex matrices
- In the future, multiple nucleic acids will be detected simultaneously using QDs with spectrally distinct emission wavelengths

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