

Multiplexed Nucleic Acid Hybridization Assays Using Single Tb-to-Quantum Dot Pair Distance-Tuning

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Multiplexed photoluminescence (PL) detection plays an important role in chemical and biological sensing. Different to most frequently developed approaches, which use spectral separation (color multiplexing) of different fluorophores [1-3], we demonstrate the applicability of a single Förster resonance energy transfer (FRET) pair (Tb donor and quantum dot (QD) acceptor) for multiplexed biosensing using multiple time-gated (TG) PL intensity detection windows. We accomplished tunable Tb-QD distances by two strategies: 1) DNA-polyhistidine self-assembly and streptavidin-biotin-DNA interaction on the QD surfaces (Figure 1A); 2) Specific location of the Tb donor by using different lengths of DNA. Both systems used acceptor-sensitization and donor-quenching for quantifying the biomolecular recognition and modification of the donor-acceptor distance for tuning the PL decays. We also show that TG detection of a single Tb-to-QD pair can be used to selectively quantify low nanomolar concentrations of multiple microRNAs in a single sample. TG intensity detection provided extremely low background noise and a quick and simple one-step assay format. Single TG-FRET pair multiplexing can be combined with spectral (color) and spatial (microarrays) resolution, paving the way for biosensing with unprecedented high order multiplexing capabilities.

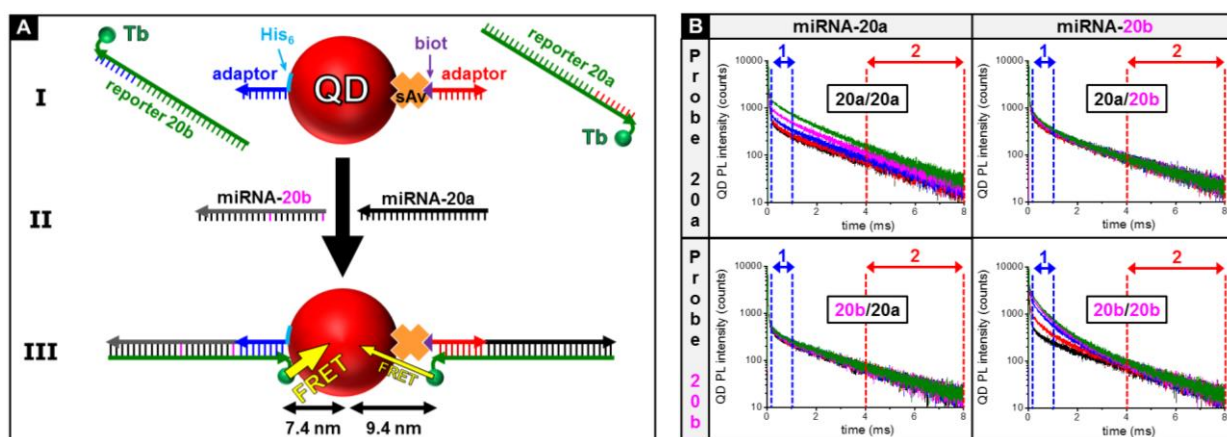


Fig. 1 Tb-to-QD TG-FRET sensor for the specific quantification of two miRNAs. **A:** Adaptor DNAs (conjugated to the QD via hexahistidine, His₆, or streptavidin-biotin, sAv-biot) and Tb-conjugated reporter DNAs (I) were mixed with miRNA-20a and 20b (II). This mixture resulted in the formation of stable double-strands and Tb-to-QD FRET, in which the Tb-QD distance for the miRNA-20a-specific double-strand was 9.4 nm and the one for the miRNA-20b-specific double-strand was 7.4 nm (III). **B:** QD PL decay curves of increasing concentrations (0 to 25 nM from black to green) of miRNA-20a (left) or miRNA-20b (right) at constant concentrations (0.5 nM DNA-QD and 20 nM Tb-DNA) of the miRNA-20a (top) or 20b (bottom) probes. PL intensities only increased for perfectly matching probe/target pairs (20a/20a and 20b/20b), whereas the PL intensities remained at background levels for unmatched combinations (20a/20b and 20b/20a). The distinct decays could be used to specifically quantify the different miRNAs from a single sample by simple detection of PL intensities in two distinct time-windows (1, 2) after the excitation pulse and a mathematical correlation of these two windows.

- 1) N. Hildebrandt, K. D. Wegner, and W. R. Algar. Luminescent Terbium Complexes: Superior Förster Resonance Energy Transfer Donors for Flexible and Sensitive Multiplexed Biosensing. *Coordination Chemistry Reviews*, **2014**, 273–274, 125–138.
- 2) X. Qiu and N. Hildebrandt. Rapid and Multiplexed MicroRNA Diagnostic Assay Using Quantum Dot-Based Förster Resonance Energy Transfer. *ACS Nano*, **2015**, 9 (8), 8449–8457.
- 3) Z. Jin, D. Geißler, X. Qiu, K. D. Wegner, and N. Hildebrandt. Rapid, Amplification-Free, and Sensitive Diagnostic Assay for Single-Step Multiplexed Fluorescence Detection of MicroRNA. *Angewandte Chemie International Edition*, **2015**, 54, 10024–10029.