BioFeedback

Response to the Letter to the Editor by Igor L. Medintz, Lorenzo Berti, and Richard A. Mathies

I am gratified by the positive response to my recent review article (1), especially when it comes from the scientific group that made very significant contributions to the field of fluorescence resonance energy transfer probes. The papers mentioned in the letter are important and were not cited in the review because they were published after the final version of the review had already been submitted. This fast appearance of the new and interesting papers is just another sign of the rapid progress in the field of energy transfer probes.

REFERENCE

1. **Didenko, V.V.** 2001. DNA probes using fluorescence resonance energy transfer (FRET): designs and applications. BioTechniques *31*:1106-1121.

Vladimir V. Didenko
Baylor College of Medicine
Houston, TX, USA
vdidenko@bcm.tmc.edu

Early Shape-Shifting FRET Probe

It was brought to my attention that in my recent review of DNA probes using fluorescence resonance energy transfer (FRET) (2) I did not mention an early UniFluor probe (1). I will briefly discuss it here to correct this omission and give a better historical perspective of the homogenous FRET assays for hybridization detection.

Homogenous assays are performed in a closed-tube format and do not require additional washing steps to get rid of the unbound probe. The first homogenous assays employing FRET for hybridization detection appeared in the 1980s (3,5). However the FRET probes used in them consisted of two labeled oligonucleotide species. Positioning of both donor and acceptor fluorophores on the same DNA strand is more advantageous and ensures efficient energy transfer within the probe.

The UniFluor probe was the first single-molecule oligonucleotide probe designed to detect specific nucleic acid sequences with the help of FRET. The probe changes conformation when in contact with specific target sequences. This induces separation of fluorophore tags with cessation of FRET and results in the fluorescence increase. This mechanism is in general similar to that employed in molecular beacons, introduced by Tyagi and Kramer in 1996 (6). In this regard, the UniFluor can be viewed as their early precursor. However, the long dumbbell-shaped probe formed by two imperfect hairpins (1) is topologically different from molecular beacons. In addition, the "specificity sequence", which should hybridize to the target, is placed in the stem of the dumbbell (molecular beacons carry a single-stranded hybridization sequence in the loop). To maintain the stem, the probe also contains a complementary sequence, which is almost identical to the target it is supposed to detect. This design lessens the sensitivity of the probe and is disadvantageous in a multiplex simultaneous detection of various targets, which is one of the most attractive features of molecular beacons.

Shortly after the paper (1) was published, it was found that a linear oligonucleotide labeled with donor and acceptor fluorophores at its 5′ and 3′ ends makes a much simpler FRET probe (4). This resulted in the development of the more versatile and efficient TaqMan probes. Together with molecular beacons, these practical and convenient systems completely overshadowed the early UniFluor probe, which can still be viewed as a first attempt to employ the probe conformation change to signal-specific sequence detection.

REFERENCES

1. **Bagwell, C.B., M.E. Munson, R.L. Christensen, and E.J. Lovett.** 1994. A new homogeneous assay system for specific nucleic acid sequences: poly-dA and poly-A detection. Nucleic Acids Res. *22*:2424-2425.
2. **Didenko, V.V.** 2001. DNA probes using fluorescence resonance energy transfer (FRET): designs and applications. BioTechniques *31*:1106-1121.
3. **Heller, M.J. and L.E. Morrison.** 1985. Chemiluminescent and fluorescent probes for DNA hybridization, p. 245-256. *In* D.T. Kingsbury and S. Falkow (Eds.), Rapid Detection and Identification of Infectious Agents. Academic Press, New York.
4. **Livak, K.J., S.J.A. Flood, J. Marmaro, W. Giusti, and K. Deetz.** 1995. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl. *4*:1-6.
5. **Morrison, L.E., T.C. Halder, and L.M. Stols.** 1989. Solution-phase detection of polynucleotides using interacting fluorescent labels and competitive hybridization. Anal. Biochem. *183*:231-244.
6. **Tyagi, S. and F.R. Kramer.** 1996. Molecular beacons—probes that fluoresce upon hybridization. Nat. Biotechnol. *14*:303-308.

I thank Dr. Mark Munson for bringing the UniFluor publication to my attention. This work was supported by the Baylor College of Medicine-Karolinska Institutet Research Exchange Program sponsored by the DeBakey Medical Foundation and by grant no. 004949-054 from the Texas Higher Education Coordinating Board.

Vladimir V. Didenko
Baylor College of Medicine
Houston, TX, USA
vdidenko@bcm.tmc.edu

Corrigendum

The caption describing the cover of the January 2002 issue of *BioTechniques* was incorrect. We regret this error. The correct caption follows.

Laser confocal microscope images of fluorescent and DIC channels of hnRNPA1-dsRed1 and eGFP-NLS-β-gal in a bikaryon with the green and DIC channels (right panels) and the red and DIC channels (left panels) merged. Pre-bleach (top), 0′ post-bleach (middle), and 30′ post-bleach (bottom) panels are indicated. Fluorescence recovery after photobleaching (FRAP) can be seen for hnRNPA1-dsRed1 by comparing the right (acceptor) nucleus in the left center and bottom panels. No recovery is seen for the eGFP-NLS-β-gal protein (right center and bottom panels). See article by Howell and Truant, p. 80.