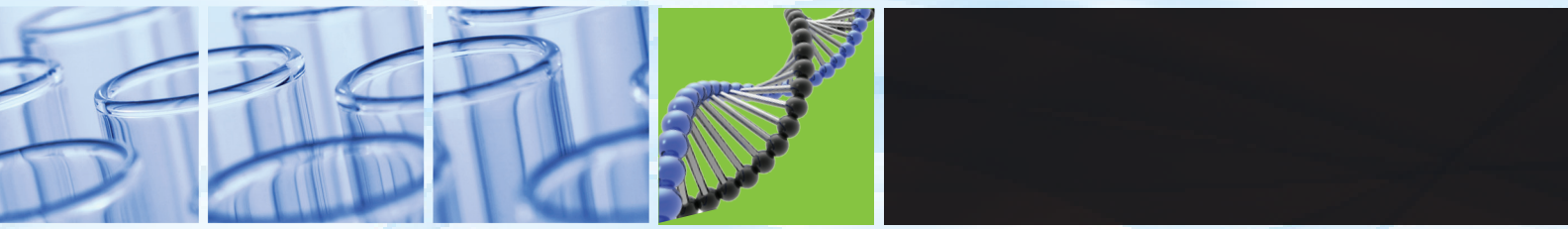
HORIBA Scientific

Fluorescence of Molecular Beacons

FL-02

ELEMENTAL ANALYSIS
FLUORESCENCE
GRATINGS & OEM SPECTROMETERS
OPTICAL COMPONENTS
FORENSICS
PARTICLE CHARACTERIZATION
RAMAN
SPECTROSCOPIC ELLIPSOMETRY
SPR IMAGING



Introduction

Biological processes can be traced through study of gene-expression via a "molecular beacon" (single-stranded DNA, ssDNA), a hairpin-shaped oligonucleotide with a fluorophore (donor) and a quencher (acceptor). The hairpin's stem has two ends of complementary DNA (cDNA) that pair up. When hybridized, the fluorophore and quencher are close, producing little or no fluorescence. Molecular beacons are used to study enzyme interactions, cDNA sequencing, and biosensing.[1]

Molecular beacons exhibit two forms of quenching (energy-transfer): direct and FRET (Förster resonance energy-transfer). Donor-quencher contact causes direct energy-transfer, dissipating heat energy. Over longer distances (2–10 nm, 20–100 Å), spectral overlap between the donor's emission and the quencher's absorption causes FRET.[2] When the ssDNA loop encounters cDNA, the hairpin spontaneously opens and the ssDNA hybridizes to this cDNA—separating fluorophore and quencher—increasing fluorescence (Fig. 1). The amount of hybridization is related to fluorescence intensity. Heat also an open ssDNA. When heated, the ssDNA's arms separate, moving the donor and acceptor ends apart, causing fluorescence.

Hybridization experiments

A two-fluorophore molecular beacon was used: the donor end was coumarin (λ_{exc}= 348 nm; λ_{em} = 447 nm); the acceptor end was 6-carboxy-fluorescein (6-FAM, λ_{em} = 518 nm). A Fluorolog® spectrofluorometer recorded emission scans of ssDNA (100 p*M*) alone. To a solution of 100 n*M* ssDNA, 500 n*M* cDNA was added, and the spectrum recorded. Lastly, deoxyribonuclease I hydrolyzed the molecular beacon, and a spectrum was taken (Fig. 2).

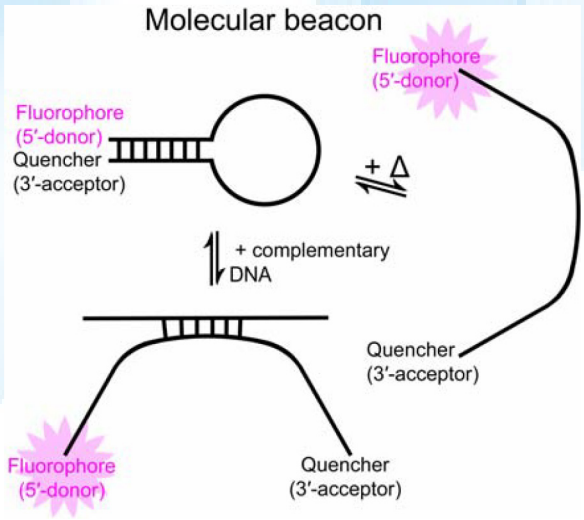


Fig. 1. Two processes that open a molecular beacon, enhancing fluorescence: (left) hybridization with cDNA; (right) heat input.

The donor (Fig. 2), near the quencher when the molecular beacon is intact, fluoresces weakly. When the ssDNA hybridizes to cDNA, the donor gives a larger signal, indicating a greater distance from the quencher. When the molecular beacon is hydrolyzed, the donor and quencher are so far apart in solution that FRET ceases, and the donor fluoresces strongly.

Another ssDNA hybridization experiment used rhodamine 6G (λ_{exc} = 527 nm; λ_{em} = 560 nm) as the fluorophore, and non-fluorescent 4-(dimethylaminoazo) benzene-4-carboxylic acid as the quencher. An emission spectrum (Fig. 3; integration time = 0.5 s, λ_{exc} = 525 nm, slits = 3 nm bandpass) shows that when ssDNA pairs with cDNA,

[1] X. Liu, *et al., Anal. Biochem.* 2000, **283**, 56– 63; X. Fang, *et al., Anal. Chem.* 2000, **72**(14), 3280–3285.
[2] X. Fang, *et al., Anal. Chem.* 2000, **72**(23), 747A–753A.

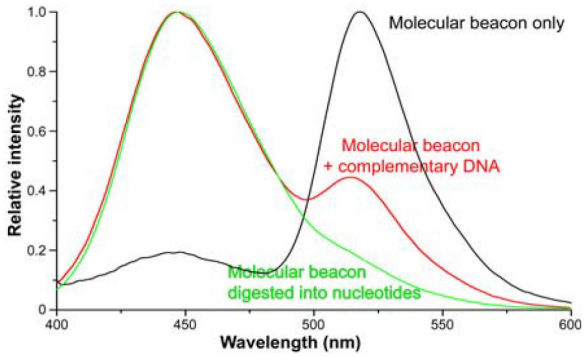


Fig. 2. Emission spectra (λ_{exc} = 350 nm) of: (black) 100 pM ssDNA only; (red) 100 nM ssDNA hybridized with 500 n*M* cDNA; and (green) ssDNA hydrolyzed by deoxyribonuclease I. Spectra are normalized to the fluorophore λ_{em} (447 nm). The 447-nm peak's height increases relative to the 518 nm quencher peak as fluorophore and quencher separate. Data from Dr. Weihong Tan, University of Florida.

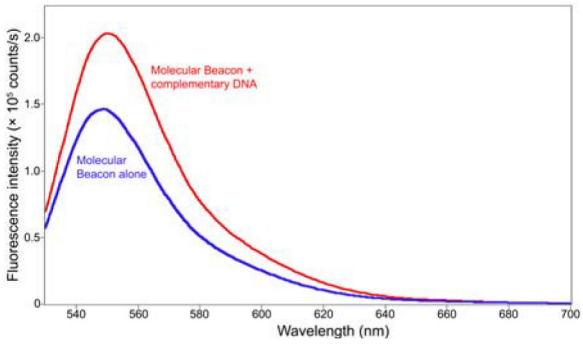


Fig. 3. Comparison of emission spectra between (blue) ssDNA only; (red) ssDNA hybridized with cDNA. The peak's relative height increases as fluorophor separates from the quencher.

the rhodamine 6G's fluorescence increases. Hybridization opens the ssDNA, separating fluorophore from quencher, allowing the fluorophore to fluoresce more intensely.

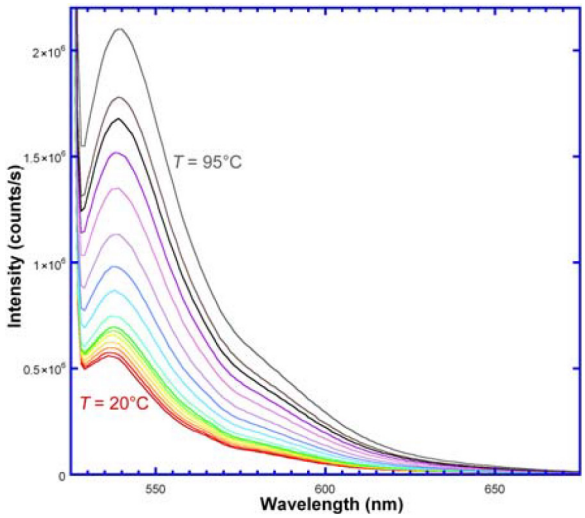


Fig. 4. Emission spectra of ssDNA with TET (fluorophore) and QSY (acceptor); λ_{exc} = 521 nm. With rising temperature, fluorescence intensity rises, meaning greater distance between donor and quencher.

Annealing experiment

A fluorescent dye (tetrachloro-6-carboxyfluorescein, TET; λ_{em} = 447 nm) was attached to a 5'-end of ssDNA, and a quencher (QSY) was bound to the 3'-end. With a FluoroMax®-4 spectrofluorometer, the ssDNA was excited at 521 nm. Emission spectra (Fig. 4) were recorded from 525–675 nm between 20–95°C. As the temperature rises—forcing the hairpin's arms apart—the TET and QSY separate, increasing fluorescence.

Conclusions

Fluorescence measurements with HORIBA Scientific spectrofluorometers are a sensitive tool for probing biochemical interactions such as molecular beacons and DNA.

JOBIN YVON Technology

HORIBA Scientific

info.sci@horiba.com www.horiba.com/fluorescence

USA: +1 732 494 8660
UK: +44 (0)20 8204 8142
China: +86 (0)21 6289 6060
France: +33 (0)1 69 74 72 00
Italy: +39 2 5760 3050
Brazil: + 55 11 2923 5400
Germany: +49 (0) 6251 8475-0
Japan: +81 (75) 313-81231
Other: +1 732 494 8660