Fluorescence of Molecular Beacons

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 can 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-carboxyfluorescein (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).

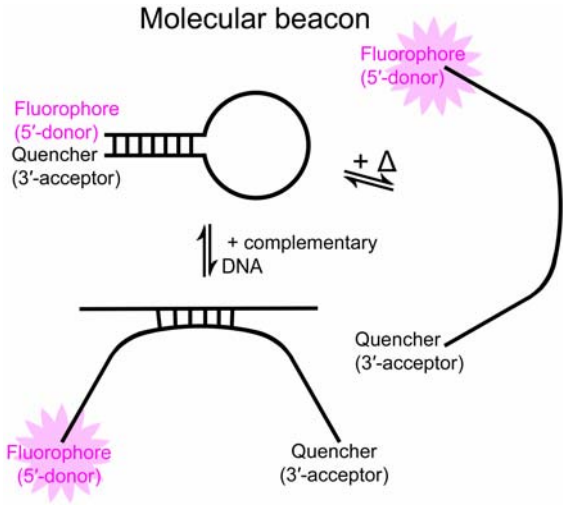

Fig. 1. *Two processes that open a molecular beacon, enhancing fluorescence: (left) hybridization with cDNA; (right) heat input.*

Fig. 2 shows that the donor, 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-(dimethylamino-

[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.

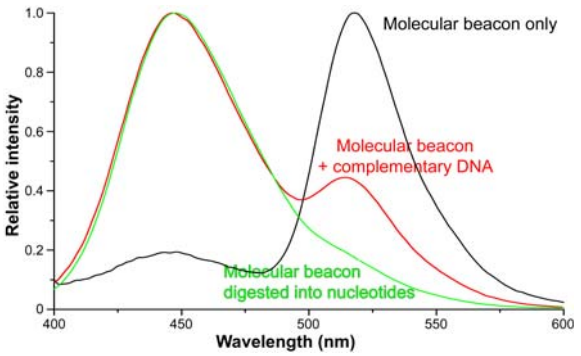

Fig. 2. *Emission spectra (λ_{exc} = 350 nm) of: (black) 100 p*M *ssDNA only; (red) 100 n*M *ssDNA hybridized with 500 n*M *cDNA; and (green) ssDNA hydrolyzed by deoxyribonuclease I. Spectra are normalized to the fluorophore λ_{em} (447 nm). The 447-nm peak's height increases relative to the 518 nm quencher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

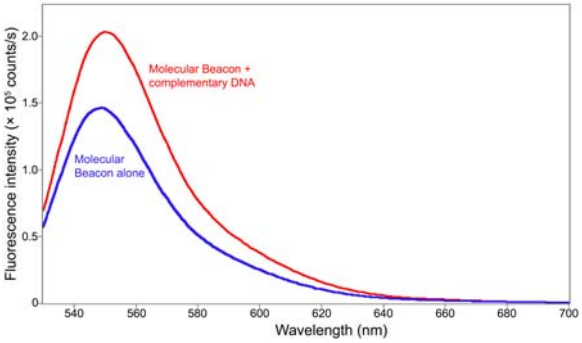

Fig. 3. *Comparison of emission spectra between (blue) ssDNA only; (red) ssDNA hybridized with cDNA. The peak's relative height increases as fluorophor separates from the quencher.*

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

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, and emission spectra were recorded from 525–675 nm between 20–95°C. As the temperature increases—forcing the hairpin's arms apart—the TET and QSY separate, increasing fluorescence (Fig. 4).

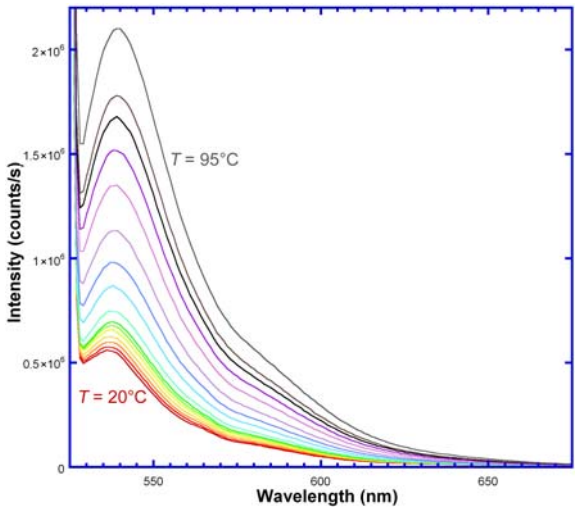

Fig. 4. *Emission spectra of ssDNA with TET (fluorophore) and QSY (acceptor); λ_{exc} = 521 nm. With rising temperature, fluorescence intensity rises, meaning greater distance between donor and quencher.*

Conclusions

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

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