Alternatives to FRET: Design of Fluorescence-quenched Oligonucleotide Probes with Extremely High Signal / Noise Ratios

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Introduction

Fluorescence Quenched Oligonucleotide probes are used for:
- Gene quantitation
- Allelic determination
- Expression analysis
- SNP analysis

Probe types:
- linear probes
- molecular beacons

Traditional probes use quenchers (e.g. Dabcyl) that work via the FRET mechanism.

This poster introduces Black Hole Quenchers (BHQs), which have been developed to give extremely high signal/noise ratios. Selected reporter dye- BHQ pairs form a *ground state complex*. This alternate quenching mechanism, together with FRET quenching, leads to very low BHQ probe background fluorescence.

FRET Quenching

1st step: The dye molecule absorbs light generating the dye excited state.

$$\text{Dye} \xrightarrow[\text{absorption}]{\text{light}} \text{Dye}^*$$

2nd step: In the absence of quenching - fluorescence!

$$\text{Dye}^* \xrightarrow[\text{emission}]{\text{light}} \text{Dye}$$

2nd step when FRET occurs: The dye excited state transfers energy to the quencher ground state generating quencher excited state.

$$\text{Dye}^* + \text{Q} \xrightarrow[\text{transfer}]{\text{energy}} \text{Dye} + \text{Q}^*$$

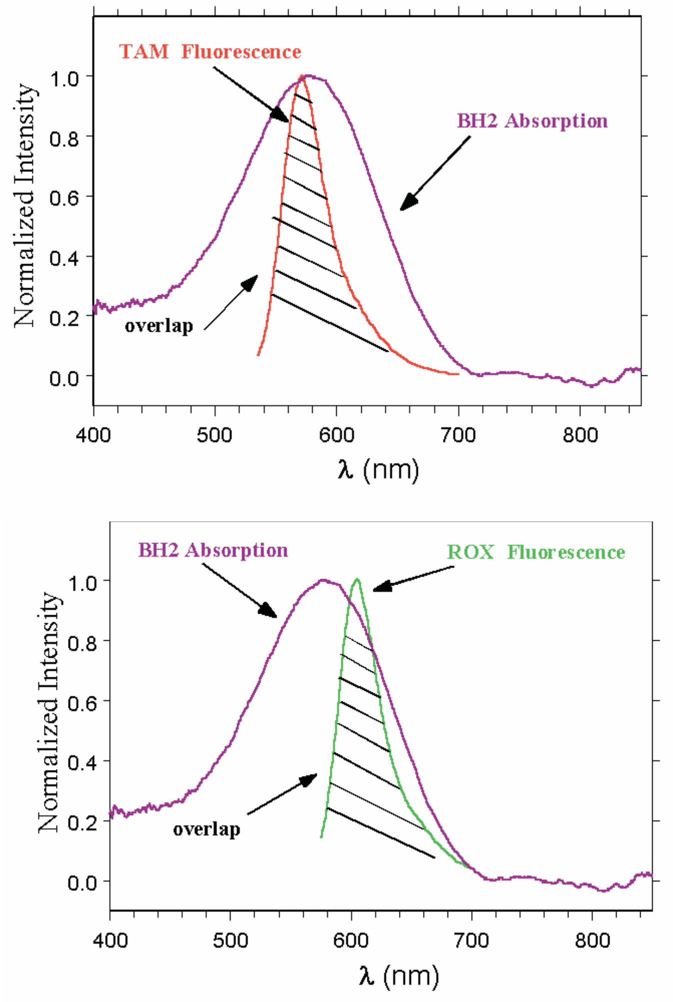
3rd step: The quencher returns to the ground state.

$$\text{Q}^* \rightarrow \text{Q}$$

The efficiency of FRET energy transfer is given by:

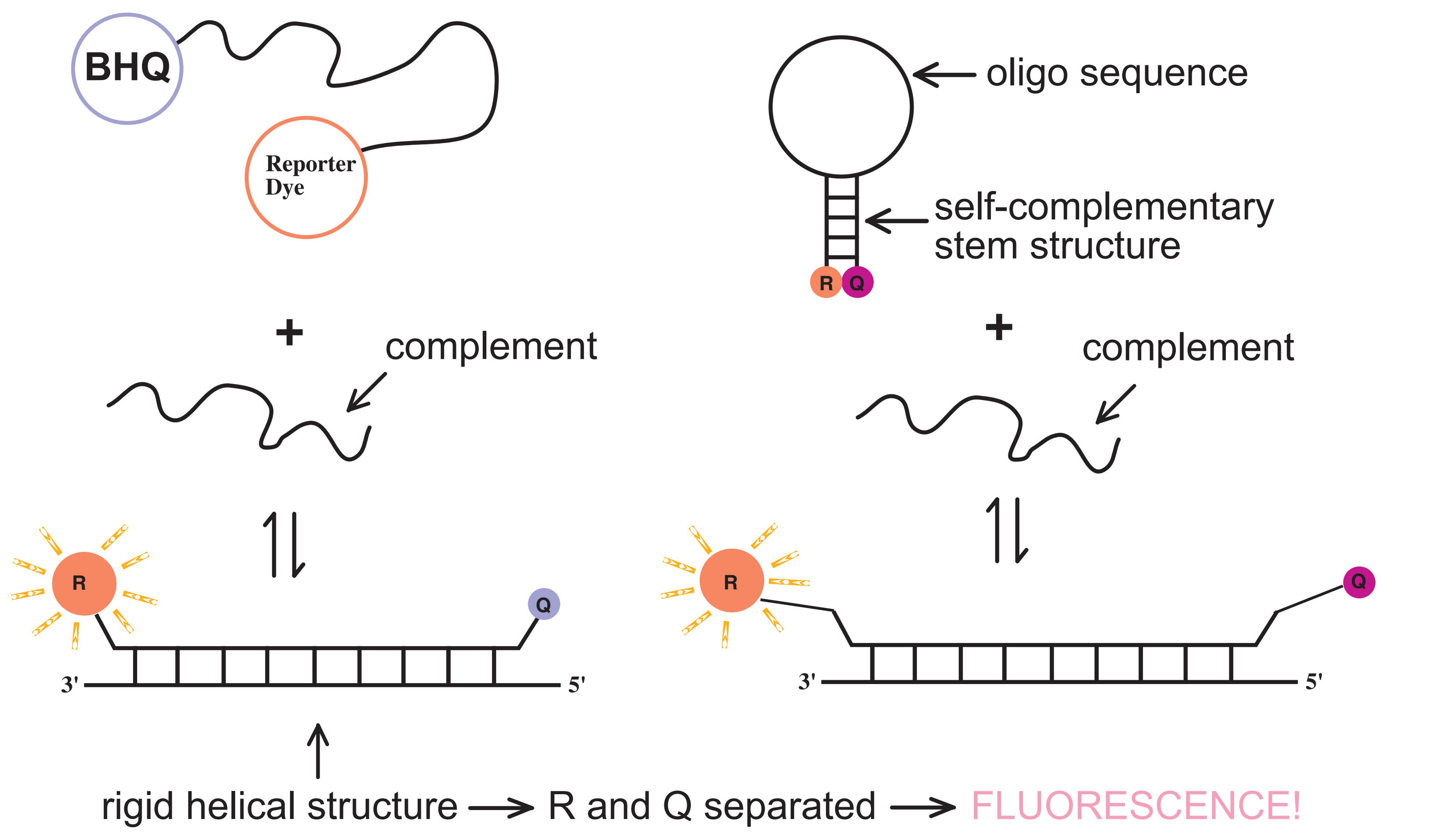
$E = R_o^6/(R_o^6 + r^6)$ where R_o = Förster distance, r = donor-quencher distance

$R_o^6 \propto Q_D . J$ where Q_D = donor fluorescence efficiency, J = overlap integral



The reporter dyes TAMRA and ROX both have large overlap integrals with BH2 quencher ($J \approx 1$)

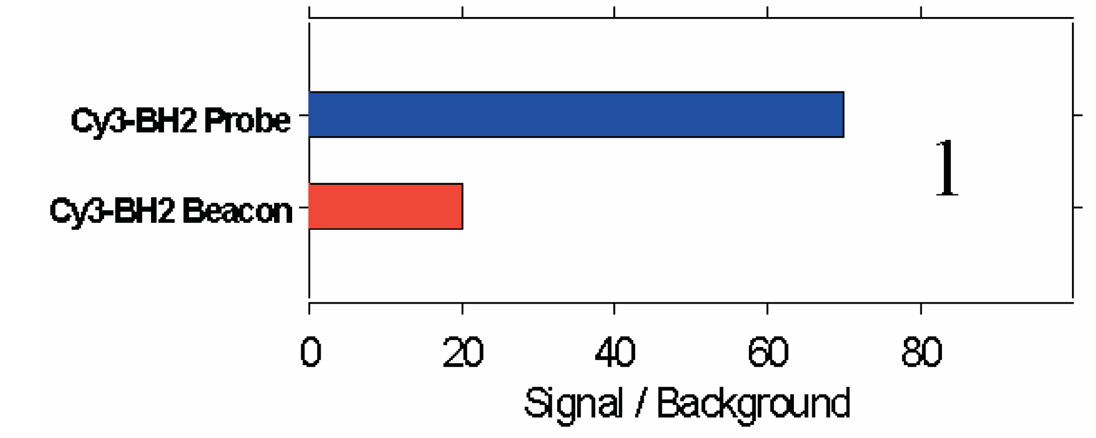
BHQ Probes vs. Beacons

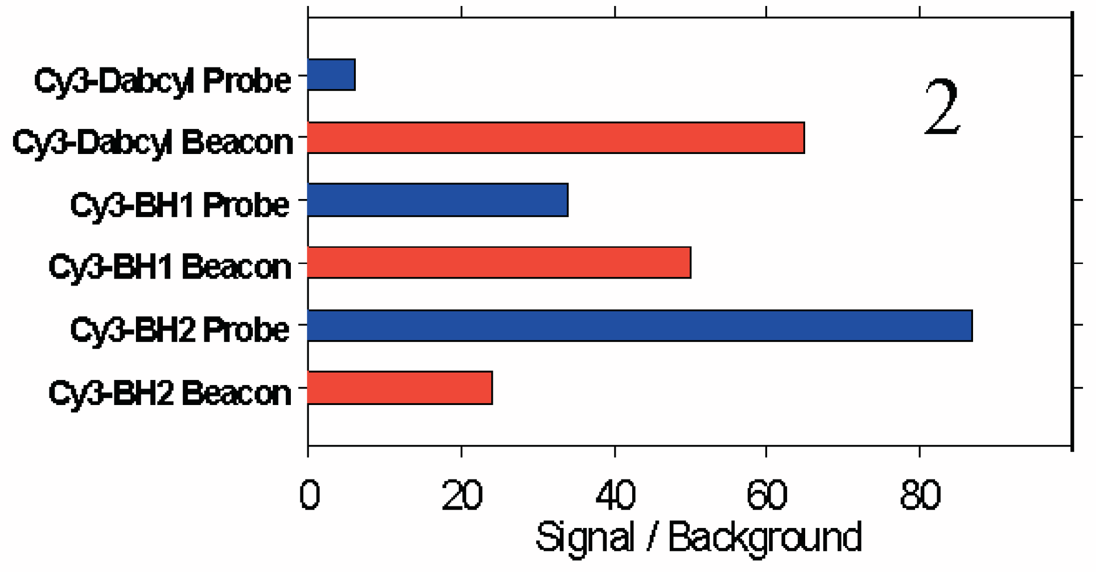


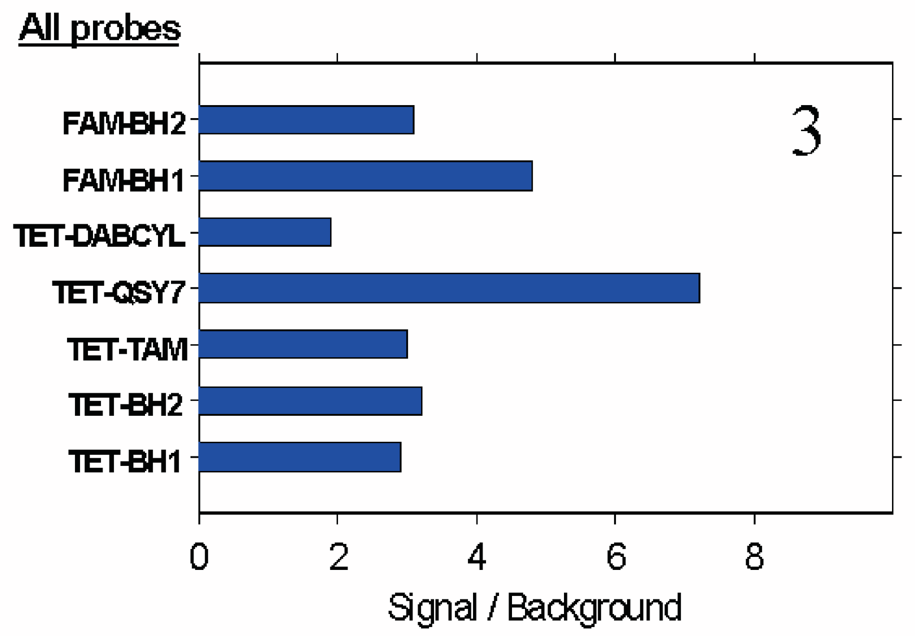
The beacon stem structure holds the dye and quencher close together, in order to maximize FRET quenching.

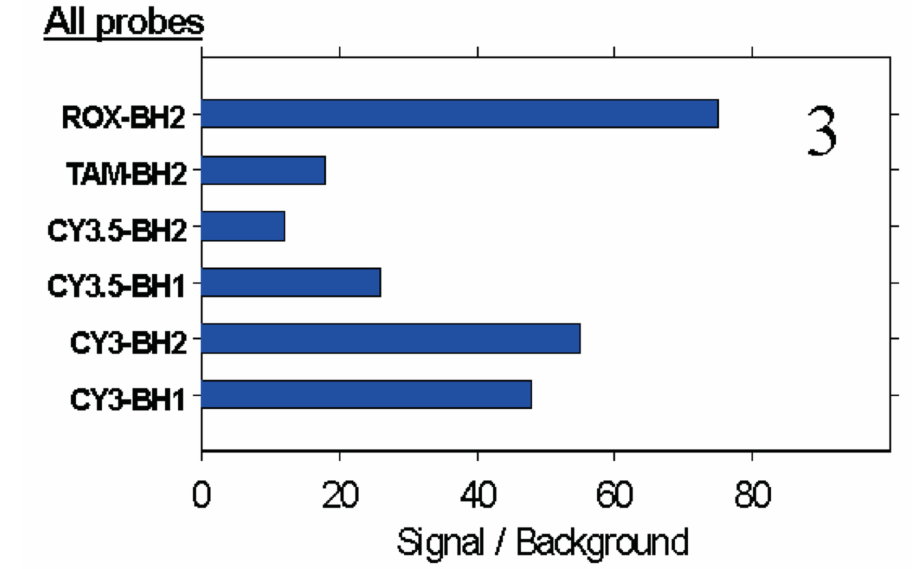
BHQ Probe / Beacon Endpoint Analysis

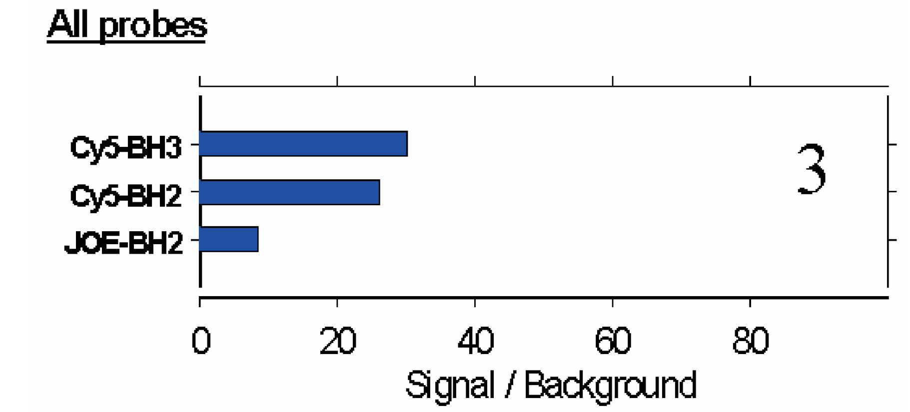
Sequence 1: 5'-GGAACCGACTACTTTGGGTGTCCGTG-3'
Sequence 2: 5'-GCATTCTTCACACCATGTTCAG-3'
Sequence 3: 5'-ATGCCCTCCCCCATGCCATCCTGCG-3'
All samples 5'Dye - 3'Quencher labeled.
Fluorescence Intensity measurements made before and after complement (with 3 extra T's on each end) added.
Beacon stem: 5'-CGCGAC - .... - GTCGCG-3'





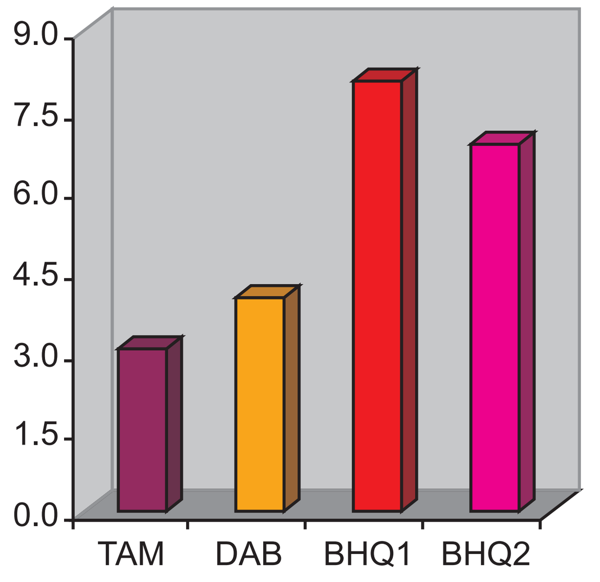






The above three probes were treated with exact complement

For these FAM probes (with TAMRA, Dabcyl, BH1 and BH2), exact complement was added.



Observation: Some BHQ-dye probes have higher signal/background ratios than the corresponding beacons (e.g. Cy3-BH2).

Conclusion: Since probes are less costly to manufacture, it's important to understand why some BHQ probes are so effective.

Observation: Some probe signal/background ratios cannot be accounted for by changes in J or Q_D or oligo sequence.

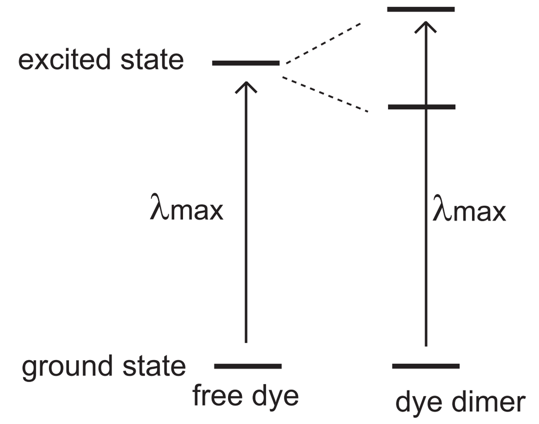
Conclusion: There is some other quenching mechanism besides FRET!

Observation: The dye absorption spectrum changes for BHQ - dye probes with especially low background fluorescence.

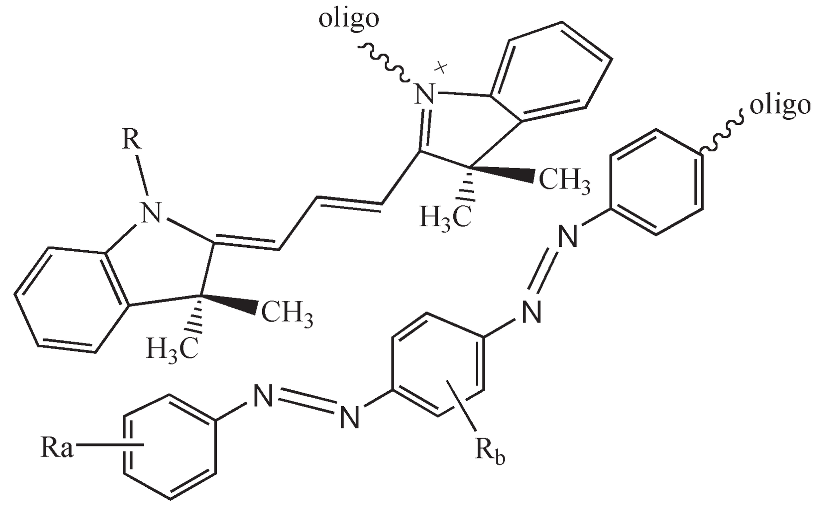
Conclusion: Ground state quenching occurs for certain BHQ - dye combinations. Furthermore, ground state complex formation can reduce beacon fluorescent signal because it is more difficult for complement to open the beacon.

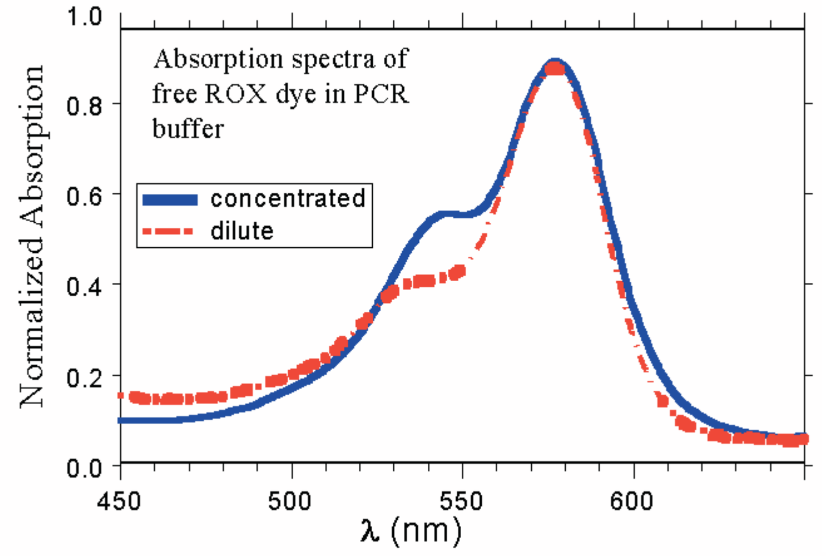
Quenching by G. S. Complex Formation

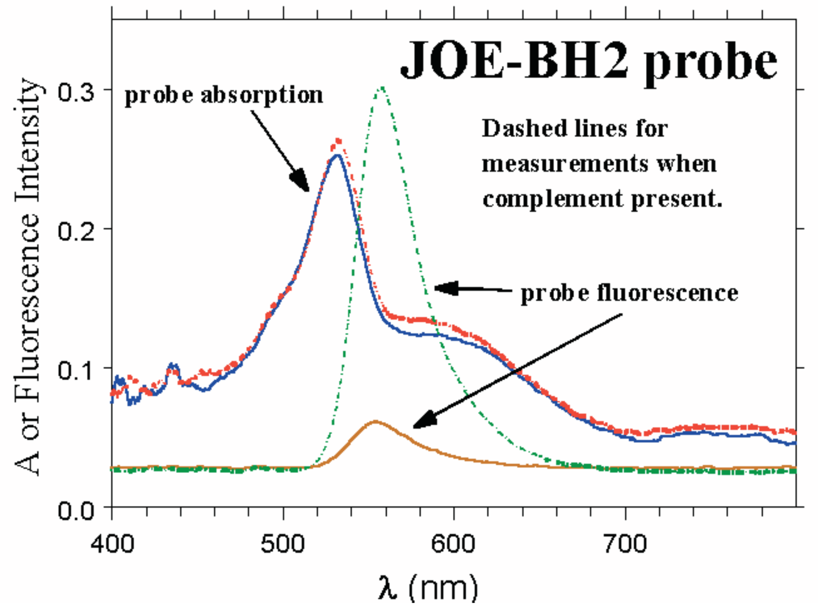
Background: Many dyes, including cyanines and fluorescein derivatives, form dimers and higher aggregates in aqueous solutions. At high dye concentrations, hydrophobic and electrostatic effects bring the dye molecules together.

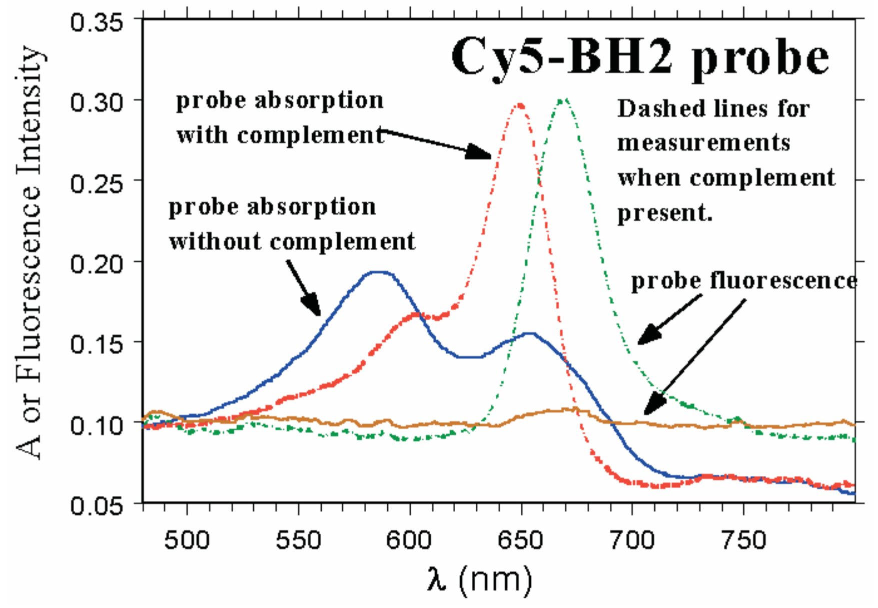


Optical effects: When two dye molecules form a complex, the absorption and emission spectra change. The complex has its own unique properties. Many dyes form nonfluorescent complexes.









Conclusions

Dyes that are known to self-aggregate, forming nonfluorescent ground state complexes, can also form nonfluorescent complexes with BHQ dyes. This is evident from distinct changes in the dye absorption spectra. Quenching via ground state complex formation gives linear BHQ probes extremely low background fluorescence.

References

Molecular beacons: Tyagi et al., *Nature* Biotech., **1996**, *14*, 303.
TagMan assay: Holland et al., *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 7276.
BHQs: patent filed 2/2000.
Cyanine dimers: W. West and S. Pearce, *J. Phys. Chem.*, **1965**, *69*, 1894.
Fluorescein dimer: I. L. Arbeloa, *J. Chem. Soc., Faraday Trans. 2*, **1981**, *77*, 1725.

For more information on BHQs or other Biosearch products,please visit booth 117 or our website **www.biosearchtech.com**.