BHQs Efficiently Quench a Wide Variety of Fluorophores Including IR Dyes, Lanthanides and Ru Complexes

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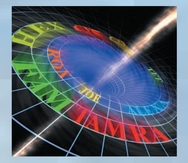


Overview

We report on:

(1) The mechanisms by which Black Hole Quenchers, BHQs, stop fluorescence

(2) Hybridization assays with a series of 5'Reporter-oligo-3'BHQ probes.

All Reporter-BHQ pairs investigated make profluorescent probes with high signal/background ratios.

BHQs and Quenching Mechanisms

FRET Quenching

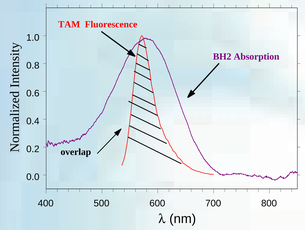
The dye excited state transfers energy to the quencher ground state generating quencher excited state.

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

Note: this occurs without emission and absorption of light.

If a dark quencher is used, the quencher returns to the ground state without the emission of light.

$Q^* \rightarrow Q$



Förster Resonance Energy Transfer

The efficiency of FRET is given by:

$E = R_0^6/(R_0^6 + r^6)$

where R_0 = Förster distance

r = donor-quencher distance

$R_0^6 \propto Q_D \cdot J$

where Q_D = donor fluorescence efficiency

J = overlap integral

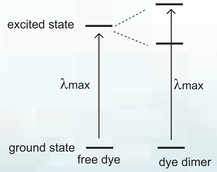
The spectral overlap between the emission of the reporter dye TAMRA and the absorption BH2 quencher is large ($J \approx 1$).

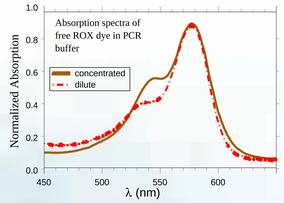
Quenching via Intramolecular Dimers

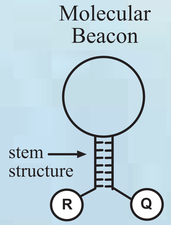
Background: Many dyes, including cyanines and fluorescein derivatives, form dimers and higher aggregates in aqueous solutions. At high dye concentrations, hydrophobic and electrostatic effects bring the dye molecules together.

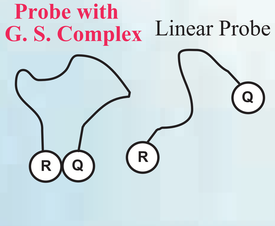
An alternative quenching mechanism: Fluorophore-quencher pairs on the ends of an oligo sequence can form intramolecular dimers, also known as ground state complexes. (Ref 1)

Optical effects: When two dye molecules form a dimer, their absorption and emission spectra change. The dimer has its own unique properties. So far, we have found that dye-BHQ heterodimers are nonfluorescent.







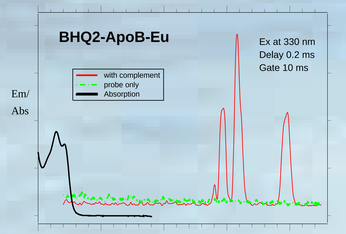


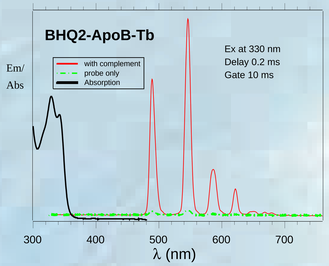
BHQs Quench Lanthanide Luminescence

Background on Lanthanides

- atomic emission involving transitions between f orbitals.
- luminescence is long-lived (ms), allowing gated detection which uses time as a filter to eliminate background signal
- Tb^{+3}, Eu^{+3}, Sm^{+3}, Dy^{+3} are all luminescent lanthanides
- lanthanides are poor absorbers and need to be sensitized

These probes were prepared using the lanthanide reporter DTPA(Ln)-carbostyril. The synthesis in Ref. 2 was modified.



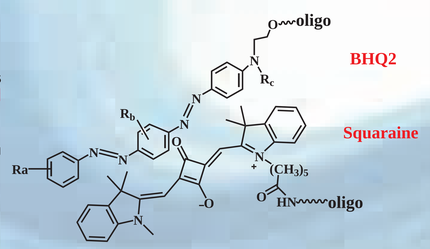


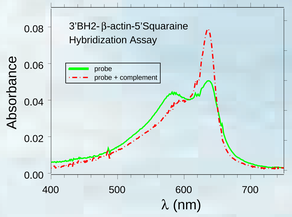
BHQs Quench Squaraine Fluorescence

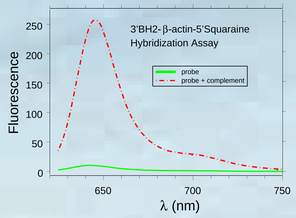
Squaraine-COOH was prepared via a modification of Ref. 3 and coupled to an amine-labeled oligo.

The following suggest that there is significant quenching via ground state complex formation:

- high signal/background despite minimal spectral overlap between BHQ-2 abs and squaraine emission
- changes in probe absorption spectrum after addition of complement.





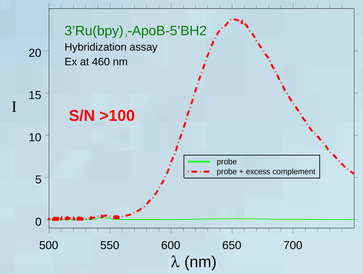


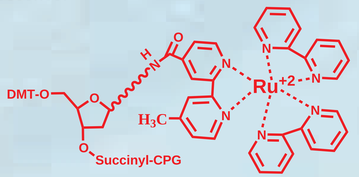
BHQs Quench $Ru(bpy)_3$ Luminescence

Background

- When $Ru(bpy)_3$ absorbs light at 460 nm, there is a metal to ligand charge transfer.
- Emission occurs around 640 nm.
- $Ru(bpy)_3$ complexes are known to have long excited state lifetimes (ca. 1 μs).

⇒Therefore, optical gating (using time as a filter) should be possible to reduce background fluorescence.





The $Ru(bpy)_3$ was added as a 3' label via a CPG (Ref 4)

Experimental

Sequences used:

Apo B: 5'-CGA-GAA-TCA-CCC-TGC-CAG-ACT-TCC-G_n-3'.

β-actin: 5'-d-ATG-CCC-TCC-CCC-ATG-CCA-TCC-TGC-G-3'.

Hybridization assays were done with complement that has 3 extra T bases on each end.

References

1) Johansson, M. K.; Fidder, H.; Dick, D.; Cook, R. *J. Am. Chem. Soc.* **2002**, in press.
2) Li, M.; Selvin, P. R. *Bioconjugate Chem.* **1997**, *8*, 127.
3) Terpetschnig, E.; Szmacinski, H.; Ozinskas, A.; Lakowicz, J. R. *Analytical Biochemistry*, **1994**, *217*, 197.
4) Johansson, M.K.; Lyttle, M.; Cook, R. **2002**, patent application in progress.

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