BHs 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

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

**BHs Quench Lanthanide Luminescence**

Background on Lanthanides
- Atomic emission involving transitions between orbitals.
- Lanthanide luminescence is long-lived (ms).
- Forster Resonance Energy Transfer (FRET) which uses time as a filter to eliminate background signal.
- Tb(III), Eu(III), Sm(III), Dy(III) are all luminescent lanthanides
- Lanthanides are poor absorbers and need to be sensitized

These probes were prepared using the lanthanide reporter: DTPA(In/Carboxyl). The synthesis in Ref. 2 was modified.

**BHs Quench Squaraine Fluorescence**

Squaraine:COOH was prepared via a modification of Ref. 3 and coupled to an amino-terminated oligo.

The following suggest that there is significant quenching via ground state complex formation:
- High signal/background despite minimal overlap between BHQ-abs and squaraine emission
- Changes in probe absorption spectrum with addition of complement.

**BHs Quench Ru(bpy)_3 Luminescence**

Background
- When Ru(bpy)_3 absorbs light at 450 nm, there is a metal to ligand charge transfer (MLCT).
- Emission occurs around 500-550 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.

**Experimental**

**Sequences used:**
- APC-F: CGAA-TCA-CCC-TGC-CA-G-AC-TCC-G-3
- APC-R: 5’-ATG-CCC-TCC-ATG-CCA-TTC-GG-3

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

**References**

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