# Development of $Ru(bpy)_3$ CPG for Solid-phase Synthesis of 3'-labeled Oligonucleotides

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## Objectives

The goal of this study is to develop a CPG solid support for DNA synthesis that is labeled with a ruthenium tris 2,2'-bipyridyl complex. We have prepared this CPG, made 3'-labeled oligonucleotides and performed some functional assays.

## Introduction

Fluorescent dyes traditionally used as oligonucleotide labels (fluoresceins, rhodamines, cyanines) share some advantages(+) and disadvantages(-):

- \+ absorb light efficiently ($\varepsilon$ > 50,000)
- \+ emit light efficiently ($\phi_F$ > 0.1)
- +/- different degrees of photostability
- \- small Stokes shift (absorption and emission curves overlap)
- \- self-quenching
- \- must excite at long wavelengths to get near-IR emission

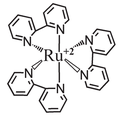
**While $Ru(bpy)_3$ labels have the following characteristics:**

- +/- moderate $\varepsilon$ (*ca.* 15,000) and $\phi_F$ (*ca.* 0.1)
- \+ photostable
- \+ large Stokes shift (excite at 460 nm and get emission at 610 nm)
- \+ not self-quenching
  → can use multiple labels
- \+ long fluorescence lifetimes (*ca.* 1 μs)
- \+ useful redox properties with applications including:
  electrochemistry, electrochemical detection, chemiluminescence

## Properties of Ru Complexes

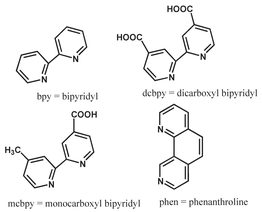
### Structural


Ruthenium is a transition metal. Ru is below iron in the periodic table. $Ru^{+2}$ binds to six ligands to form an octahedral complex. These complexes are typically stable to organic synthesis conditions.




one enantiomer of $Ru(bpy)_3$

Common Bidentate Ligands:

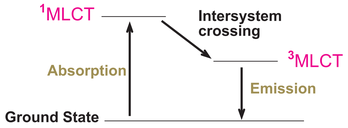



### Electronic

With **organic dyes** (FAM, Cy5, etc), the same excited state is involved in the absorption and emission of light:


The emission of light from **ruthenium complexes** is not simple fluorescence (i.e. more than singlet states are involved). When $Ru(bpy)_3^{+2}$ absorbs light at 450 nm, **Metal to Ligand Charge Transfer (MLCT)** occurs:

$Ru^{+2}(bpy)_3 \rightarrow Ru^{+3}(bpy)_2(bpy^{\bullet -})$

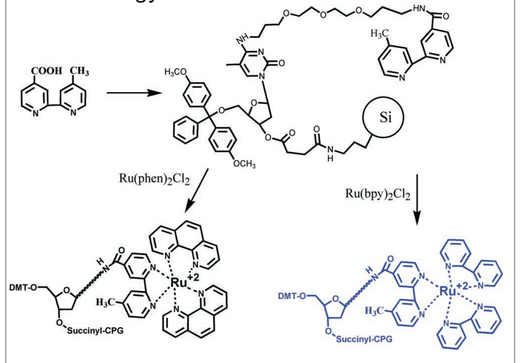



## Synthesis of CPG with a Ru Complex

### Background

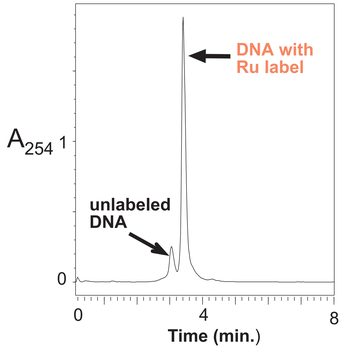
There have been several reports of labeling oligos with transition metals, most often as a 5'-modification. Grinstaff and coworkers at Duke University have prepared a $Ru(bpy)_3$ phosphoramidite. To our best knowledge, **a CPG has not previously been prepared.**

### Our Strategy:

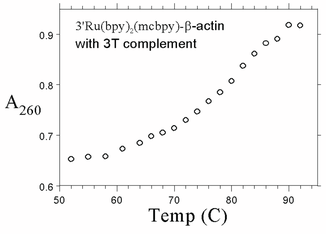


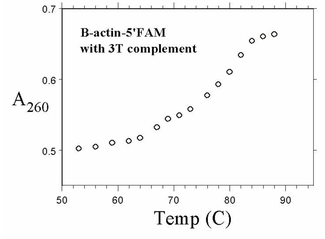

## Preparation and Evaluation of 3'-$Ru(bpy)_3$ labeled Oligos

- The Ru complexes are stable to standard DNA synthesis conditions, including ammonia deprotection.
- Obtained high yields of labeled oligos
- Preparative reversed-phase (RP) HPLC purification gave >98% pure samples (according to both analytical anion-exchange and RP HPLC).
- Oligos 3'-labeled with Ru complexes are photostable. They are also stable and luminesce at high temperatures.

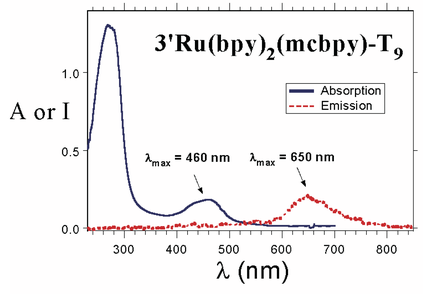



A reversed-phase HPLC chromatogram of crude 3'$Ru(bpy)_2$(mcbpy)-β–actin (a 26-mer). The $Ru(bpy)_3$ CPG underwent standard DNA synthesis conditions and deprotection of 2 hours at 60 °C in concentrated ammonia.

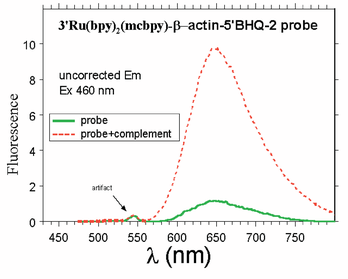



The melting temperatures for duplexes with the β-actin sequence (a 26-mer) are the same whether it is 3'-labeled with a ruthenium complex or 5'-labeled with FAM.




The absorption and emission curves of the ruthenium complex are very well separated.




An endpoint assay of a dual-labeled probe, 5'-labeled with a Black Hole Quencher™. The emission of the ruthenium complex depends on its distance from the Black Hole Quencher.

## Conclusions

We have successfully prepared a $Ru(bpy)_3$-labeled CPG. This CPG has been used to make a variety of 3'-$Ru(bpy)_3$ labeled oligos in high yield. These labeled oligos are stable and have behaved well in complementation assays. It has also been shown, using a 5'quencher-3'reporter dual-labeled probe, that $Ru(bpy)_3$ efficiently transfers energy to a dark quencher, a Black Hole Quencher™. The quenching of $Ru(bpy)_3$ by BHQ-2 probably occurs via the FRET mechanism.

## References

Lakowicz, J. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1999; Chapter 20.

Hu, X.; Smith, G. D.; Sykora, M.; Lee, S. J.; Grinstaff, M. W. *Inorg. Chem.* **2000**, *39*, 2500, and reference contained therein.