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A General Fluorescence Resonance Energy Transfer (FRET) Method for Observation and Quantification of Organometallic Complexes under Reaction Conditions

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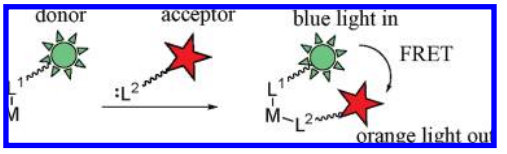
Summary: A two-fluorophore FRET system provided a more general approach than previously described fluorescence techniques to observing and quantifying organometallic complexes under reaction conditions. Over the concentration range of 3×10^{-7} *to* 5×10^{-6} *M, this method provided quantification with faster time resolution and greater sensitivity than is possible with NMR spectroscopy.*

Small quantities of transition-metal complexes play key roles in catalytic reactions,[1] and therefore new methods for the sensitive detection of transition-metal complexes can lend insight into catalytic and other metal-mediated processes. For example, Sohn and Ihee recently reported that the metal-based quenching of a ligand's fluorescence could be used to monitor formation of an intermediate in the Grubbs enyne metathesis reaction at low concentration.[2] The increased sensitivity of a fluorescence measurement over an NMR spectroscopy measurement[3] was critical for their detection. We envisioned a fluorescence resonance energy transfer (FRET)[4,5] method more general than previously reported fluorescence detection methods. Specifically, a two-fluorophore system would be employed: a donor fluorophore attached to a ligand through an alkyl spacer and an acceptor fluorophore similarly attached to a second ligand. When both ligands were bound to the metal center, their proximity would result in a strong FRET signal (Scheme 1), permitting quantification of that specific complex at low concentrations. In contrast to the extensive literature pertaining to the detection of specific metal ions that relies on fluorescence quenching and enhancement by the metal atom,[2,6−12] this new method would use *spectator* fluorophores which would not rely on changing photophysical properties from interaction with the metal, thereby creating a more general method for studying reactions of diverse substrates and metal centers. The current dearth of fundamental studies and applications of two-fluorophore FRET in transition-metal systems stands in sharp contrast to the abundant studies and applications in biochemical systems.[13−15] Two-fluorophore FRET's employment in transition-metal complexes will open up new areas of study and application; however, the fundamental properties and feasibility of the method had not been demonstrated previously. Herein, we report fundamental studies of this method, with the goal of establishing its useful concentration range, determining its sensitivity in comparison to NMR spectroscopy, quantifying its FRET efficiency, and discovering the role of the metal (if any) in moderating the FRET process. These fundamental studies provide the groundwork for the general employment of two-fluorophore FRET to study transition-metal complexes at low concentrations.

Scheme 1. Tagging of One Ligand with a Donor Fluorophore and a Second Ligand with an Acceptor Fluorophore To Permit Quantification of a Metal Complex with Both Ligands Bound, through FRET



In order to explore the useful concentration range of this two-ligand FRET method, palladium complex **4** was synthesized with a donor and an acceptor fluorophore. Complexes analogous to **4** are intermediates in palladium-catalyzed nucleophilic allylic substitution reactions,[16] and therefore **4** was chosen as a representative complex for this fundamental study. We examined the tetramethyl BODIPY (dipyrromethene boron difluoride) core as a green FRET donor (λ_{ex} = 498 nm, λ_{em} = 504 nm) and the tetrahydroindole BODIPY core as an orange FRET acceptor (λ_{ex} = 541 nm, λ_{em} = 545 nm) (eq 1).[17] Displacement of

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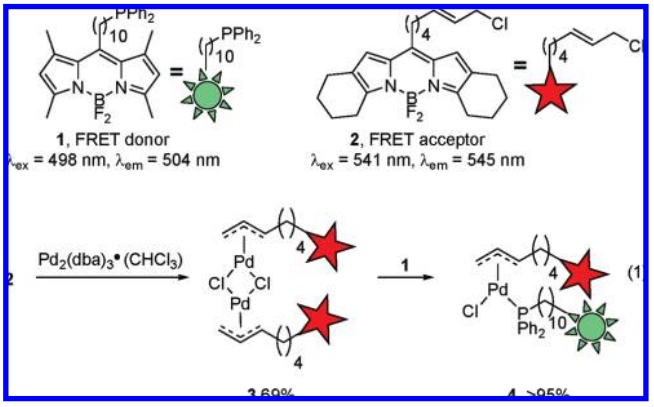
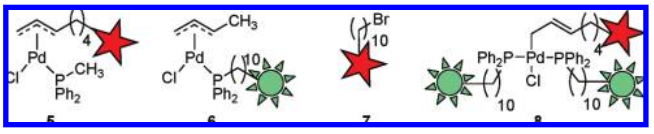
chloride from complex **3** with the tagged phosphine **1** produced complex **4** in quantitative yield (eq 1).



We next investigated FRET as a tool to detect **4**, which we anticipated would occur only when both fluorophores were attached through ligands to the palladium center. No significant changes in the photophysical properties of the BODIPY cores of **1** or **2**[18] were observed upon separate coordination to the metal through a ligand and a spacer in the monotagged complexes **6** and **5**, respectively, confirming their behavior as photophysical spectators.[19] Excitation of a 2.5×10^{-6} M solution of **4** at 460 nm resulted in a decrease of the emission signal at 504 nm (green) and a concomitant increase in the emission signal at 545 nm (orange), in comparison to the emission intensities of **1** and **2**. This decrease at 504 nm corresponded to a FRET efficiency of 92% (Figure 1A).



A control experiment examined the excitation of a mixed solution of **1** and bromide **7**, both at 2.5×10^{-6} M. Bromide **7** was examined as a nonreactive surrogate to allyl chloride **2** and had indistinguishable photophysical properties. No decrease in the signal at 504 nm occurred upon mixing of **1** and **7**. Thus, the decrease in the signal at 504 nm from a solution of **4** arose from intramolecular FRET within complex **4**, since intermolecular FRET was not significant at this concentration.

Complexes analogous to the (η^1-allyl)chlorobis(phosphine)palladium species **8**, which was formed upon exposure of complex **3** to 4 equiv of phosphine **1**, have been proposed as the reactive electrophiles in palladium-catalyzed nucleophilic allylic substitution reactions.[20] In order to build a predictive model for the photophysical properties of metal complexes tagged with two donors and one acceptor,[4] we investigated the fundamental FRET properties of complex **8**. In contrast to Watanabe's report that labeling DNA with two BODIPY donors and one acceptor enhanced the signal

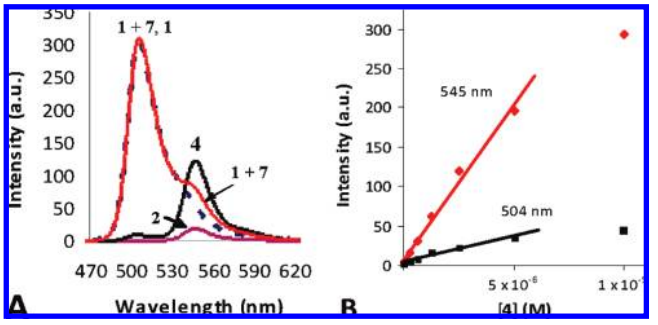


Figure 1. (A) Comparison of fluorescence intensities of separate solutions of **1**, **1** + **7**, **2**, and **4**, at 2.5×10^{-6} M, showing FRET in **4**. (B) Red and black lines denoting the linear regions for detection of **4** at 545 and 504 nm, respectively.

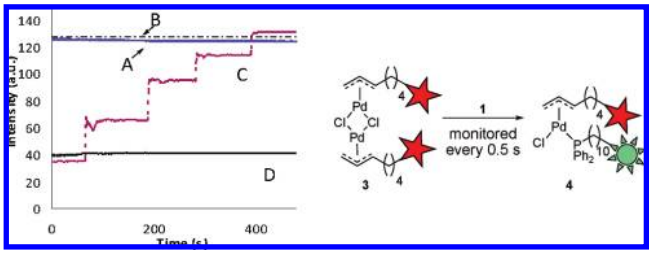


Figure 2. (A) Addition of **1** to **3** in one portion. (B) Calculated intensity corresponding to full conversion to **4**. (C) Addition of **1** to **3** in four portions. (D) Control, with **1** added to **7** in four portions.

from FRET,[21] the emission intensity of **8** at 545 nm was not significantly changed relative to **4**, establishing different criteria for FRET in palladium complexes than in labeled DNA (119 au for **8**; 119 au for **4**).

The emission intensity of a solution of **4** at 504 and 545 nm was examined over 4 orders of magnitude: 1×10^{-4} to 1×10^{-8} M. The solution's emission intensity was proportional to the concentration of **4** over the range 3×10^{-7} to 5×10^{-6} M (Figure 1B). For example, at 3.1×10^{-7} M, the fluorescence intensity at 545 nm was 16.2 ± 1.2 au and at 1.3×10^{-5} M the intensity was 62.8 ± 5.5 au. Standard deviations from triplicate runs were consistently below 10% of the value of the measurement. At concentrations greater than 1×10^{-5} M, intermolecular FRET and self-quenching became competitive with the desired intramolecular FRET, as determined by detection of FRET in a mixed solution of free ligands **1** and **7** at 1.0×10^{-5} M and self-quenching[22] in a separate solution of **1** at 1.0×10^{-5} M.[19] At concentrations less than 5×10^{-7} M, quantification of the small differences in emission signal approached the limits of the spectrometer.

With the fundamental concept established, the FRET method was applied to the quantification of complex **4** during a chemical reaction. Addition of equal volumes of a 5.0×10^{-6} M solution of phosphine **1** to a 2.5×10^{-6} M solution of complex **3** was monitored using 460 nm excitation and detection of fluorescence intensity at 545 nm (Figure 2, line A). Data were acquired every 0.5 s. In the first experiment, solutions of **1** and **3** were mixed in one portion. The first data point, acquired 10 s after the mixing of **1** and **3**, showed a high fluorescence intensity that did not change over

(18) Mixture of regioisomers (see the Supporting Information for details).

(19) See the Supporting Information for data, comparisons, and calulations.

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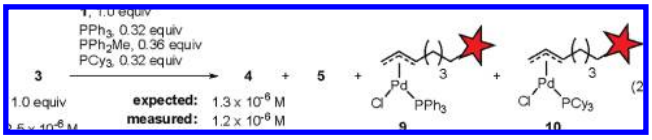
time, implying an extremely rapid reaction between **1** and **3** to form **4**, even at these low concentrations. The measured fluorescence intensity of the solution, 125 au, corresponded to 2.5×10^{-6} M **4** and was within 2% of the expected fluorescence value for full conversion (127 au, Figure 2, line B). Half-second resolution of the initial stages of the reaction was obtained by adding four aliquots of a 5.0×10^{-6} M solution of **1** (2.0 equiv) to a 2.5×10^{-6} M solution of **3** (1.0 equiv) inside the fluorimeter. Addition of each sequential aliquot resulted in a rapid increase in FRET, consistent with a reaction rate to fully form **4** of less than 2 s (Figure 2, line C). Since mixing was slow inside the fluorimeter, the signal required about 10 s to fully stabilize after each addition, during which time the solution homogenized. Intensity measurements at less than 10 s were within 15% of the homogenized value. A control experiment examined the fluorescence intensity of a 5.0×10^{-6} M solution of **7** when four aliquots of a 5.0×10^{-6} M solution of **1** were added, in the absence of palladium, and established that no increase in fluorescence intensity was observed (Figure 2, line D).

Formation of **4** could be observed and quantified in a reaction that produced a mixture of products (eq 2). Addition of a mixture of phosphines to **3** was anticipated to produce a mixture of complexes **4**, **5**, **9**, and **10**. During the reaction, dimer **3** produces 2 equiv of (η^3-allyl)palladium; thus, a total of 2 equiv of phosphines was added. After mixing, excitation of the solution at 460 nm resulted in a fluorescence intensity of 13 au at 504 nm and 65 au at 545 nm, corresponding to a measured concentration of **4** of 1.2×10^{-6} M (1.3×10^{-6} M expected, on the basis of complete and rapid consumption of phosphine **1** as identified in Figure 2).[19] This result established that low concentrations of side products did not interfere with this method's quantitative application: for example, by excited-state quenching.[23−25]



To directly compare the sensitivity of the FRET technique with that of ^{31}P and ^{1}H NMR spectroscopy, a mixture of phosphines was added to dimer **11** at 2.1×10^{-3} M to produce analogous nonfluorophore-tagged complexes **12**−**14** at concentrations high enough for NMR spectroscopic detection (eq 3). After confirmation of the reaction by ^{31}P NMR spectroscopy (Figure 3A), the mixture of products was then diluted to the identical concentrations of the previous FRET experiment. At these low concentrations, the less sensitive technique of ^{31}P NMR spectroscopy could not detect any of the resonances from the complexes, even with long

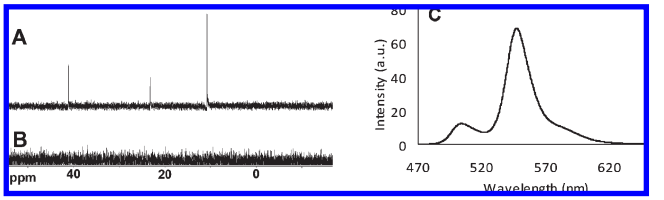
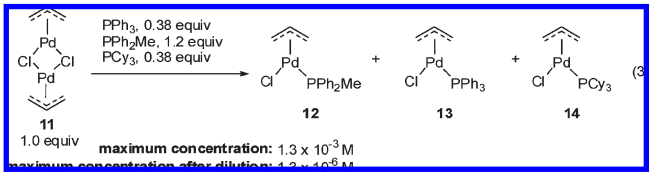


Figure 3. (A) Concentrated ^{31}P NMR spectrum at 1.3×10^{-3} M, 312 scans, 8 min acquisition (eq 3). (B) After dilution, ^{31}P NMR spectrum at 1.3×10^{-6} M, 1086 scans, 27 min acquisition; no signal observed (eq 3). (C) Fluorescence spectrum at 1.3×10^{-6} M (eq 2).

acquisition times (1086 scans, 27 min, ^{31}P; Figure 3B). Similarly, ^{1}H NMR spectroscopy was not able to identify or quantify the complexes, even with long acquisition times (512 scans ^{1}H, see the Supporting Information). In contrast, our FRET method can detect and quantify **4** in 1 s (Figure 3C; full spectrum clearly observable at 1.2×10^{-6} M). This result confirmed that the FRET technique permitted rapid quantification of complex **4** at concentrations where the lower sensitivity of NMR spectroscopy was not adequate.



In conclusion, FRET with spectator fluorophores provided a more general method for studying transition-metal complexes than the metal−ligand quenching method previously reported. The high signal from FRET permitted quantification of complexes with the rapid time resolution of 1 s at 1.3×10^{-6} M, a concentration at which the lower sensitivity of NMR spectroscopy was not sufficient. No evidence for photophysical involvement of the metal was observed. More broadly, these fundamental studies provide the groundwork and feasibility demonstration for future applications, such as studying transition-metal intermediates using ensemble FRET methods, or single-molecule FRET studies of transition-metal reactivity,[17] in analogy to single-molecule biophysical FRET studies.[13,26]

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Supporting Information Available: Text and figures giving experimental procedures, compound characterization data, and sample fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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