Toward the Single-Molecule Investigation of Organometallic Reaction Mechanisms: Single-Molecule Imaging of Fluorophore-Tagged Palladium(II) Complexes

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Summary: The single-molecule fluorescence microscopy imaging of individual palladium(II) complexes is reported and the requisite high-quantum-yield BODIPY fluorophore tags are synthesized and shown to act as spectators when bound to metal complexes. These combined experimental results lay the fundamental groundwork for studying organometallic reaction chemistry at the single-molecule level using fluorophore tags.

Over the past 10 years, single-molecule fluorescence microscopy (SMFM) has provided substantial insight into biochemical processes.[1–6] In contrast, the potential of SMFM to provide insight into catalytic processes outside of biological systems remains virtually untapped. The development of a general SMFM method for investigating chemical reactions has tremendous potential, especially in the area of transition-metal catalysis, where ensemble averaging complicates the determination of active catalysts and mechanisms. A general SMFM method to study reaction mechanisms at catalytic metal centers would be particularly powerful, because it would aid in the rational design of new catalysts. The overarching goal of developing this method is to create live "movies" of chemical reactions, in which the changing spectroscopic signal indicates the reaction mechanism and the composition of intermediates. Hofkens' recent report of imaging the hydrolysis reaction of diacylfluorescein at the surface of a lithium/aluminum hydroxide crystal provides important precedent for extending this technique to nonbiological chemical systems.[7] Hofkens' imaging technique, while powerful, relies on the chemical modification of a fluorophore to turn "on" and "off" its fluorescent signal, which limits the method's applicability in terms of fluorophores and reactions.

We now describe a *general* dipyrromethene boron difluoride (BODIPY) tagging based method for observing single transition-metal complexes that does not require the fluorophore to undergo a chemical reaction for imaging. Instead, a fluorophore-tagged ligand or substrate will come on or off the metal, producing an "on/off" fluctuation in fluorescence signal that characterizes what is bound to the catalytic center at each point in the reaction. To develop this assay for organometallic SMFM, we first needed to establish a general synthesis of the fluorophore-tagged metal complexes and confirm that they could be imaged on the single-molecule level. The SMFM imaging of fluorophore-tagged transition metals is challenging for three reasons. (1) Coordination to a metal quenches the fluorescence of dyes under many conditions;[8–11] therefore, we needed to establish conditions that avoid this quenching for the specific metals and complexes that would be the most interesting to study (e.g., important catalysts). (2) It is a challenge to visualize individual complexes due to the fluorescent impurities present in even the highest grades of commercial organic solvents used for sample preparation. (3) The requisite fluorophore-tagged ligands have not been previously reported. We now report the successful SMFM imaging of palladium(II) complexes that gain their fluorescence from fluorophore-tagged ligands. To our knowledge, this represents the first SMFM imaging of fluorophore-tagged transition-metal complexes outside of biological systems.[12–14]

The BODIPY class of fluorophores[15] is appealing due to its members' solubility in organic solvents and lack of accessible lone pairs, which makes them unlikely to coordinate to metals. Additionally, their sharp fluorescence emission spectra and high quantum yields make them suitable for single-molecule studies.[16] Therefore, we sought a divergent synthesis of BODIPY-fluorophore-tagged ligands for transition metals. We opted to start with the known BODIPY bromide **1**,[15] available in one step from commercially available precursors (eq 1). A 10-methylene spacer was selected to sterically and electronically distance the fluorophore from the desired reactive center.[17]

Conversion of bromide **1** to amine **2**, thiourea **3**, and alcohol **4** proceeded smoothly (eqs 2 and 3). The fluorophore-tagged

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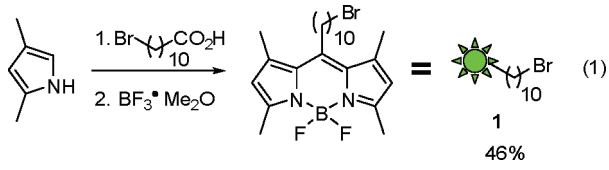
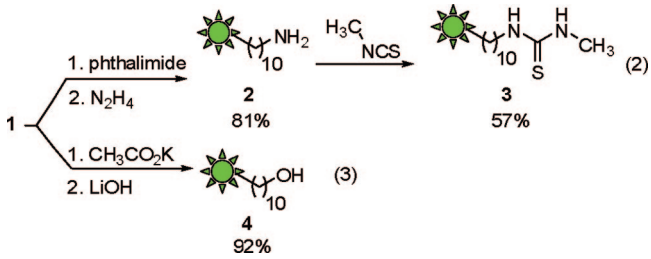
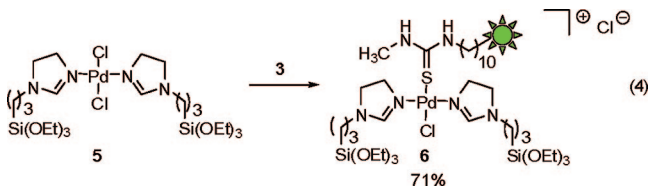


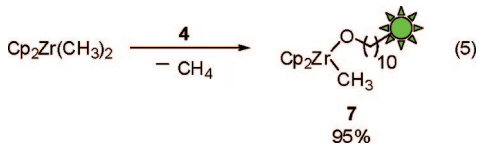
ligands **2**−**4** exhibit the desired high solubility in organic solvents and high quantum yields (Φ > 0.88). Conveniently, each is excited by the 488 nm line of an argon ion laser (λ_{ex} 492−496 nm, λ_{em} 500−504 nm).



We next explored if the BODIPY thiourea **3** retained its high quantum yield when coordinated in palladium complex **6** (eq 4). Thioureas are known to be highly palladaphilic[18,19] and have recently proven to be synthetically useful ligands for the palladium catalysis of the carbonylation of alkynes[20] and Heck and Suzuki cross-coupling reactions.[21] The fluorescence properties of **6** are essentially unchanged relative to those of unbound thiourea **3** (**3**, λ_{ex} 492 nm, λ_{em} 500 nm, Φ = 0.93; **6**, λ_{ex} 492 nm, λ_{em} 501 nm, Φ = 0.91). This result shows that the palladium in complex **6** does not significantly quench or alter the spectrum of the fluorophore.[22] The lack of quenching permits use of BODIPY-tagged reagents to image palladium complexes on the single-molecule level.



Gratifyingly, this lack of significant quenching or spectral shift appears to be quite general with respect to the nature of the metal complex, as attachment of BODIPY alcohol **4** to zirconium(IV) also does not significantly change the fluorescence properties of **4** (**4**, λ_{exc} 493 nm, λ_{em} 499 nm, Φ = 0.93; **7**, λ_{exc} 491 nm, λ_{em} 500 nm, Φ > 0.95) (eq 5).



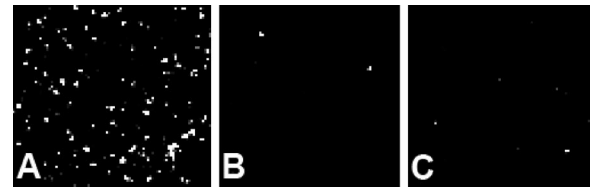
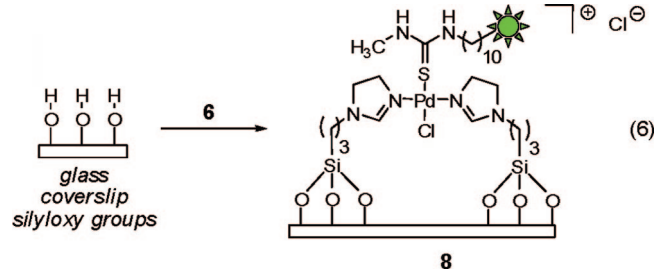


Figure 1. (A) Individual molecules of BODIPY-tagged palladium complex **8**. (B) Blank slide, showing <1% as much fluorescence signal. (C) Control, exposure to bis(imidazole)palladium dichloride **5** instead of **6**.

Given the prolific role that palladium complexes play in modern catalysis,[23,24] we turned our attention to imaging palladium complex **6** on the single-molecule level. We anticipated that the exposure of borosilicate glass microscope coverslips to a solution of complex **6** would produce immobilized silyloxy-tethered complex **8**, on the basis of extensive literature precedent for attaching metal complexes and functional groups to silica and glass slides (eq 6).[25−27] After soaking in a 10^{-6} M solution of **6**, the coverslips were rinsed, oven cured, and analyzed.



Glass coverslips were imaged using an inverted optical microscope with 488 nm excitation and a EM-CCD camera with a 500 ms exposure/frame. Optimal imaging conditions for single molecules occurred after 5 min of photobleaching, which decreased the overlap between molecules in the field of view. Figure 1 shows the images produced when the glass coverslip is treated with **6** (Figure 1A) compared to a blank slide (Figure 1B). Each single molecule is one bright spot. There are significantly more single molecules in A than in B, establishing that tethered complex **8** can be imaged above background levels.

We considered the possibility that the single-molecule fluorescence signal in A could arise from impurities recruited to the surface in the presence of palladium, creating a false positive. We therefore compared the signal in A to the signal from glass coverslips treated with the bis(imidazole)palladium dichloride **5** as a control. In this case, only a low level of single-molecule background impurities are observed (Figure 1C), confirming that the signal in A arises from the intentionally added fluorophore.

Over nine experiments, in a 2844 μm^2 area there were 569 ± 367 single molecules in A, compared to 3 ± 2 single-molecule impurities in the same area of the blank (B) and 4 ± 2 single-molecule impurities in the same area of control (C). Therefore, in comparison to control C, a given molecule in A has a 99 ± 1% probability of being complex **8** rather than an impurity.

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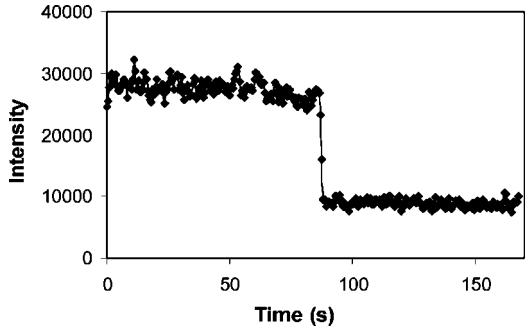
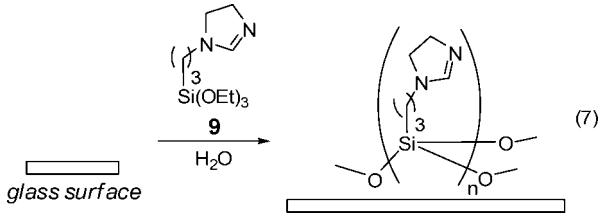


Figure 2. Representative graph of fluorescence intensity vs time, showing the photobleaching event of one molecule of **8**.

When a coverslip is treated with a solution of thiourea **3**, with no metal present, a large number of single molecules are observed, similar to that observed in A, presumably due to hydrogen bonding between the strong hydrogen bond donor and acceptor thiourea group and the surface hydroxyl groups on the coverslip. We thus examined the possibility that the single-molecule signals in Figure 1A result from free thiourea that has been displaced from the metal complexes; therefore, the presence of complex **8** on the surface of the coverslip in A was confirmed by X-ray photoelectron spectroscopy (XPS), which showed that palladium, chlorine, and sulfur were present in a ratio consistent with the complex, rather than free thiourea **3**. The loading of **8** on the coverslip was estimated by XPS as 1 molecule/20 Å^2 (i.e., one monolayer).

Time-lapse imaging showed complex **8** undergoing the quantized blinking and photobleaching events that characterized each as an individual molecule rather than a cluster of several molecules.[27] A representative example is shown in Figure 2.

Polarization-modulation Fourier transform IR reflection−absorption spectroscopy (PM-FTIRRAS) was used to examine the binding mode of ligand **9** to the glass surface.[28,29] Two options were considered as potential binding modes to the coverslip: physisorption or covalent attachment.[25−27] Covalent attachment could occur by condensation between the triethoxysilane groups and the surface hydroxyls with release of ethanol (eq 6) or by cross-polymerization of neighboring silyloxy groups in the presence of water, also with release of ethanol (eq 7).[30]



To reduce the Si−O stretching background from glass, gold slides coated with a monolayer of 11-mercaptoundecanol (MUD) were used as model systems for the surface hydroxyl groups on glass slides.[28] A comparison of cured and uncured slides (120 °C, 1 h) showed that the curing process resulted in a significant shift in the Si−O stretching frequency from 1057 cm^{-1} to 1145 cm^{-1} (Figure 3), corresponding to the disappearance of Si−O−C bonds and the appearance of Si−O−Si

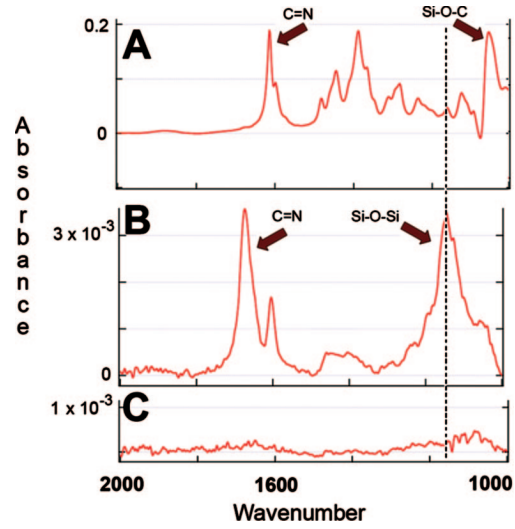


Figure 3. PM-FTIRRA spectra: (A) spin-coated, multilayer sample of **9** on gold slide without curing; (B) Sample of 2−4 layers of **9** on Au−MUD slide after curing for 1 h at 120 °C, followed by rinse, showing significant Si−O frequency shift; (C) sample of **9** on Au−MUD slide without curing, followed by rinse, showing lack of attachment.

bonds.[31] The formation of Si−O−Si bonds is consistent with a chemical reaction leading to attachment of ligand **9** onto the surface with loss of ethanol. Additionally, in the absence of a curing step, **9** could be rinsed from the MUD slides, whereas after curing, the same rinsing conditions did not remove **9**, consistent with a chemical reaction that leads to attachment rather than physisorption. A control reaction employing 1-mercaptooctadecane rather than MUD on the gold slide's surface showed that the surface hydroxyl groups were not required for a condensation reaction, suggesting operation of a cross-polymerization reaction between neighboring siloxy groups in the presence of water (eq 7). The magnitude of the absorbance spectrum after curing is consistent with a thin film (∼2−4 layers) (Figure 3B). Finally, since PM-FTIRRAS employs polarized light, information regarding the orientation of the molecules on the surface could be obtained. The presence of observable C═N resonances establishes that the heterocycle does not lay flat on the surface, in contrast to one option for physisorption. Thus, attachment of complex **8** to the glass coverslips is likely due to condensation with the surface or cross-polymerization between neighboring silyloxy groups or a mixture of both, where an extended network of Si−O bonds is created that involves some Si−O attachment points to the surface of the slide, rather than physisorption. Although the exact nature of the covalent binding mode is as of yet undetermined, complex **8** remains immobilized for at least 2 h, making this immobilization technique sufficient for the time-lapse imaging of chemical reactions.

In summary, we report the first SMFM imaging of fluorophore-tagged transition-metal complexes outside of biological systems. The development of high-quantum-yield, organic-solvent-soluble, spectator-fluorophore-tagged ligands permitted the establishment of this method. PM-FTIRRA spectroscopic analysis of model systems is consistent with the immobilization of complex **8** through covalent bond formation rather than physisorption. These results provide the fundamental groundwork for the development of a general method for studying organometallic chemistry at the single-molecule level.

We are currently exploring the scope of the fluorophore-tagging method on a range of transition-metal complexes, with

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an emphasis on developing methods for the live imaging of individual bond-forming events.

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

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