

Heterogeneous Photophysics and Photochemistry of Luminescent Ruthenium Complexes: Implications in Sensor Design

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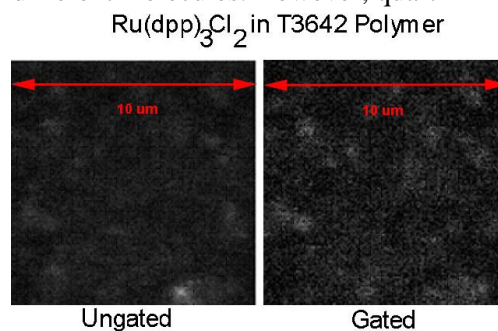
Proposal Title: Heterogeneity effects in luminescence based sensors

Research Achievements

Luminescence-based sensors are tools in bio and environmental analysis including nanosensors that can be incorporated into cells and patterned microsensors used in DNA identification. Many sensors use immobilized luminophores. Polymers are a common support because of easy fabrication and versatile properties. Unfortunately, sensor properties frequently fail to make a clean transition from solution to the solid state. The basic chemistry and photophysics can change (e.g., pKs, binding constants, lifetimes) and well-behaved solution responses can become highly non-ideal with no good models for these changes. These problems complicate the rational design of new sensor systems, and the prevalent view is that much of the non-ideality arises from heterogeneity of the domains in which the sensor molecules partition.¹ As sensors become smaller heterogeneity issues become a more important factor in predicting performance and obtaining reproducible calibrations. For example, using fluorescence microscopy we have established that **microheterogeneity** is the source of nonlinear Stern-Volmer responses in some quenching based oxygen sensors, and that the non-ideal behavior at the microscope's optical resolution is consistent with nanoheterogeneity (i.e., heterogeneity that is below optical resolution).² The ultimate, and probably most common, form of nanoheterogeneity in polymer supports is where every molecule including nearest neighbors can be in spectroscopically distinguishable environments. Fundamental questions concern the existence, the structure, and origins of underlying nanoheterogeneity as well as its role in the design of well-behaved sensors.

Our work is devoted towards the development of biological and environmental sensors based on luminescent transition metal (e.g., Ru(II), Os(II), Re(I) and Ir(III)) complexes. In particular, we pioneered their use in oxygen and pH sensing and are applying them to metal ion sensing.³ These sensors can be used in a wide variety of analyses where another response can be converted into one directly measurable by our complexes. Applications include diabetic glucose microsensors where glucose oxidase catalyzing glucose oxidation with consumption of oxygen is monitored with an oxygen sensor. Non-ideality is still one of the largest problems in developing new systems and refining existing ones. While we successfully developed microheterogeneity analyses,² we had considered direct observation of nanoheterogeneity in these systems out of reach. However, Hochstrasser recently demonstrated that our sensor molecules could be studied at the single molecule level on quartz and the responses of each molecule could be measured independently.⁴ A heterogeneous response was obtained for the different molecules. However, quartz surfaces are not polymers and their results do not address the issues in real sensors. Here we examine heterogeneous photochemistry and photophysics of sensor at submonolayer surface levels as well as preliminary results on single molecule studies on glass surfaces and in polymers.

The figure shows single ruthenium $\text{Ru}(\text{dpp})_3^{2+}$ molecules (dpp= 2,2'-4,7-diphenyl-phenanthroline) in a **polymer matrix** (T3642 =poly(timethylsilyl-methylmethacrylate)) oxygen sensors. This is a major accomplishment due to the larger polymer backgrounds. The figure compares ungated and time gated confocal images obtained using pulsed excitation and time-gated



photon detection. This approach allows substantial background reduction due to the long lifetimes of the Ru(II) complexes. These preliminary measurements establish unambiguously that we can detect single Ru(dpp)₃²⁺ molecules against the polymer background.

Because SMD with Ru complexes is very difficult due to their long lifetimes (microsecond), we have been developing the confocal lifetime system to examine nanoheterogeneity photophysics and photochemistry using submonolayers rather than SMD. Using the related Ru(bpy)₃Cl₂ (bpy=2,2'-bipyridine) and Ru(dpp)₃²⁺ we find that the lifetimes are multi-exponential and the Stern-Volmer quenching plots for oxygen quenching are nonlinear indicating that different molecules have different sensing properties.

Further, the photochemistry is heterogeneous with the extent of photolysis as shown by the non-exponential emission intensity as a function of photolysis time and nonlinear Stern Volmer plots (see figures). The photochemistry is dependent on the complex and the presence of oxygen. Oxygen can either stabilize or destabilize different complexes, presumably due to the relative stability of the complex to attack by reactive singlet oxygen formed on quenching and the degree of quenching of the excited state before it can directly decompose.

Longer lived, more sensitive molecules can photobleach more readily than shorter lived molecules. Thus, on partial photodecomposition, the oxygen sensing properties of Ru(bpy)₃Cl₂ change. It has been widely assumed that lifetime-based sensors are largely immune to photodecomposition; this work establishes that this is not always true. This result has implications for low level sensors in cellular imaging and biomedical analysis where photodecomposition is likely to be a more serious problem.

Finally we found one sample of Ru(dpp)₃²⁺ on quartz that aggregated into domains, possibly nanocrystals. These domains showed oxygen quenching behavior similar to that of single molecules. This is totally unexpected as macro and microcrystals show no oxygen quenching.² The figure shows these particles, which are quenched like single molecules but are clearly some sort of Ru(dpp)₃²⁺ aggregate as evidenced by their high emission rates and the absence of single step photobleaching.

This submonolayer work demonstrates that we can study nanoheterogeneity in polymer-supported sensors without SMD. Implications to sensor design will be discussed.

Future Work

We will improve the S/N in SMD to get quantitative quenching data (e.g., better filters and polymer clean up). We will examine individual single sensor molecule responses and measure the responses of ensembles of individual molecules. This will allow direct proof of sensor nanoheterogeneity and provide the underlying fundamental distribution. These data can help develop reliable behavior modeling and design of improved systems. We will explore the originals of the quenchable nanofeatures.

References

- ¹ B. A. DeGraff, J. N. Demas, *Reviews in Fluorescence 2005*, Volume 2, Eds. C. Geddes and J. R. Lakowicz, Springer Science, 125-151.
- ² R. D. Bowman, K. A. Kneas, J. N. Demas, A. Periasamy, *Microsc. Microanal.*, **2003**, 211, 112-120.
- ³ J. R. Bacon and J. N. Demas, *Anal. Chem.* **1987**, 59, 2780-85.
- ⁴ Erwen Mei, Sergei Vinogradov, Robin M. Hochstrasser, *J. Am. Chem. Soc.* **2003**, 125, 13198-13204

