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Applications of Luminescent Transition Metal Complexes to Sensor Technology and Molecular Probes

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Transition metal complexes (TMCs) with long-lived excited states are revolutionizing much of modern photochemistry and photophysics. In particular, platinum-group metal complexes with α-diimine ligands have formed the basis for several entirely new areas. Here, we focus on systems exhibiting luminescence and the applications of these luminescence properties to practical devices—in particular, molecular probes and sensors.

Luminescent TMCs have been used as photosensitizers, in energy conversion and electron transfer (*1*), and as probes of heterogeneous binding dynamics (*2*) and macromolecular structures (*3, 4*). $Ru(II)L_3^{2+}$ complexes (L = α-diimine) have proven particularly versatile in these applications owing to their strong visible absorption, stability, efficient emissions, and long-lived excited states (*1, 5*). Further, their emitting-state energies and excited-state redox properties can be exquisitely sensitive to variations in the metal, coordinating ligands, and local environment (*1, 6–10*). Many of these sensitizers exhibit a variety of energetically accessible charge transfer (CT), ligand field (*d–d*), and intraligand excited states that can have quite different excited-state characteristics. Understanding these allows the rational design of new, more useful sensitizers and probes. Models of CT state behavior are based predominantly on the photophysics and photochemistry of $*Ru(bpy)_3^{2+}$ and related sensitizers. Figure 1 summarizes the ligand structures and abbreviations used here and shows a representative structure for one of our TMCs. In addition to the extremely popular Ru(II) complexes, Os(II) (*8, 11*), Ir(III) (*12*), Mo(0) and W(0) (*13*), and Re(I) (*14–16*) complexes are being increasingly studied.

TMCs can have many potential advantages as luminescence probes, including long excited-state lifetimes (τ's) and high luminescence quantum yields (*17*). Long τ's make them much easier to measure than the typical nanosecond organic probes and allow efficient time discrimination from the ubiquitous fluorescences of short-lived organics. Re(I) probes can have luminescence quantum yields in room-temperature fluid solutions of 0.4–0.7 with τ's >10–100 μs (*18*). Such extraordinarily high yields and long τ's make these molecules extremely attractive as molecular probes for sensors.

States of Metal Complexes and Design Considerations

Crucial to understanding the luminescence and applications of TMCs is knowledge of the different types of excited states and their characteristics. There are three different types of excited states of relevance here: (i) metal center or *d–d* excited states that involve excitation of *d* electrons in unfilled metal orbitals; (ii) ligand localized excited states involving promotion of electrons from filled to unfilled ligand orbitals; (iii) charge transfer transitions involving transfer of electron density between the metal and ligand. If an electron is transferred from the metal to ligand, it is a metal-to-ligand charge transfer transition (MLCT), and if an electron is transferred from the ligand to the metal, it is a ligand-to-metal charge transfer transition (LMCT). Details of excited-state types and their characteristics are given elsewhere (*19, 20*).

To first order, control of the TMC luminescence properties hinges on control of the nature of the lowest excited state and the energies and natures of the upper excited states (*1, 6*). The primary goal of sensor probe design is creating molecules that have high luminescence efficiencies and long excited state τ's, are easily pumped, have specific environmental sensitivity and binding properties, and are chemically and photochemically robust (*17*).

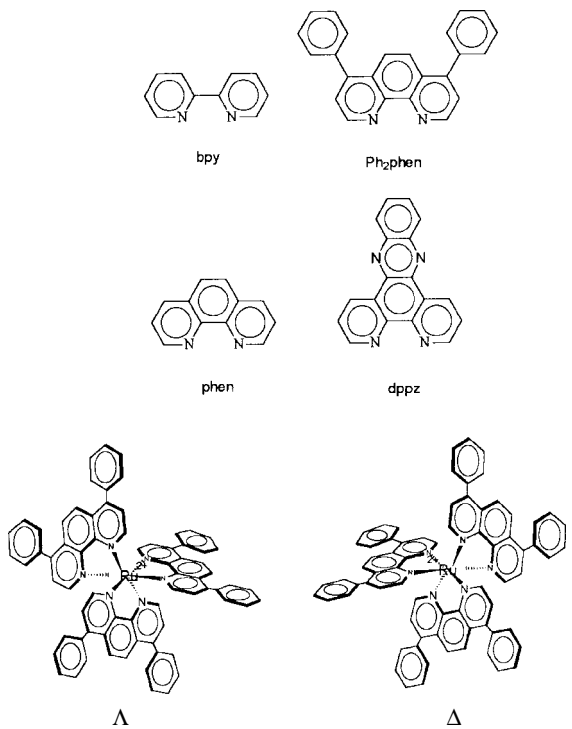

Figure 1. Structures of ligands and their abbreviations used in this paper, and a typical structure for a Ru(II) sensor/probe.

Sensors

Sensors are molecules that respond to some property or analyte. We focus on luminescence-based sensors employing TMCs. Widely used luminescence detection methods are emission intensity, spectrum, excited state τ's, and emission polarization. Basic sensing strategies for detecting and quantitating analytes include:

1. Specific excited state deactivation with a change in emission intensity or τ. Example: O_2.
2. Reaction of the ground or excited state species with the analyte to yield a species with different properties. Example: pH.
3. Variations on the previous cases where a nonemissive portion of the luminescence species can react with the analyte and then alter the luminescence properties. Example: pH.
4. Incorporation of a nonluminescent, analyte-sensitive molecule in a matrix that alters a transmitter species' luminescence. Reaction of the nonemissive species with the analyte alters the rate of deactivation of the luminescence species. Example: pH.

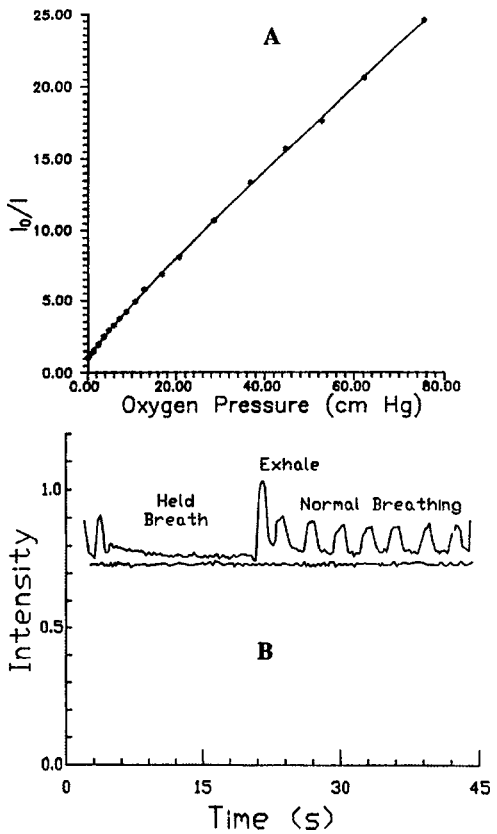

Figure 2. (A) Intensity Stern–Volmer quenching plot for Ru(4,7-Ph_2phen)$_3^{2+}$ in silicone rubber (*22*). The solid line is the best fit for a two-site model with different quenching constants. (B) Luminescence intensity of Ru(4,7-Ph_2phen)$_3^{2+}$ in silicone rubber while it is being breathed over (*21*). Copyright 1991 and 1987 American Chemical Society.

Oxygen Sensors

Oxygen deactivation or quenching of excited states has been recognized since the early days of luminescence. It was generally considered a nuisance that necessitated its removal to permit luminescence to be observed. However, since detection and quantitation of O_2 is exceptionally important in industry and in the biomedical field, the quenching can serve as an analytical method. Quenchometric O_2 sensors are based on the bimolecular quenching of excited states. The basic processes, shown here for O_2, are

$$D + h\nu \longrightarrow D^* \quad \text{excitation} \tag{1a}$$

$$D^* \xrightarrow{k_1} D + h\nu \text{ or } \Delta \quad \text{luminescence or deactivation} \tag{1b}$$

$$D^* + O_2 \xrightarrow{k_2} D + {}^*1O_2 \quad \text{quenching} \tag{1c}$$

For O_2, the primary quenching path is energy transfer to form singlet O_2. Any bimolecular processes with the analyte that deactivate the excited state will reduce emission intensity and τ.

For diffusional quenching (eq 1c), the τ's and luminescence intensities are related to the quencher concentration [Q] by the lifetime and intensity Stern–Volmer equations.

$$\tau_0/\tau = 1 + K_{SV}[Q] \tag{2a}$$

$$I_0/I = 1 + K_{SV}[Q] \tag{2b}$$

$$K_{SV} = k_2\tau_0 \tag{2c}$$

where τ's and I's are luminescence τ's and intensities, respectively. The subscript "0" denotes the measurement in the absence of quencher. K_{SV} is the Stern–Volmer quenching constant, and k_2 is the bimolecular quenching constant. Ideally, plots of τ_0/τ or I_0/I vs. [Q] will be linear, with identical slopes of K_{SV}.

Since the excited states of many O_2 quenched species are also highly sensitive to environment and will respond to metal ions, oxidants, reductants, surfactants, DNAs, proteins, etc., practical systems must separate the sensor from all interferents while still allowing O_2 access. The simplest common method is to put the sensor into a gas-permeable, solvent-impermeable membrane. Silicone elastomers based on polydimethylsiloxane are especially useful because they can have excellent O_2 diffusion and quenching and good biocompatibility.

Because of their long τ's, large shifts between excitation and emission, and efficient luminescences, TMCs have become important O_2 sensors, particularly $[Ru(Ph_2phen)_3]^{2+}$ (*21, 22*). Figure 2 shows a Stern–Volmer plot for $[Ru(Ph_2phen)_3]^{2+}$ immobilized in GE RTV-118, a silicone bathtub caulking compound. It has excellent sensitivity over the physiologically interesting range of 0–1 atm of O_2. The emission intensity decreases about 8-fold on going from no O_2 to an atmosphere of air and 25-fold at an atmosphere of pure O_2. These dramatic changes are easily quantitated. $[Ru(Ph_2phen)_3]^{2+}$ is the sensor in a commercial critical-care system.

The nonlinear Stern–Volmer quenching plot is characteristic of solid state (as opposed to solution) supports, where the predicted linear behavior is observed (eq 2). Nonlinearity is a consequence of polymer heterogeneity. This heterogeneity causes sensor molecules to exist in multiple sites with different quenching constants and results in downward curved Stern–Volmer plots. The system shown has relatively little heterogeneity.

Figure 2B shows the sensor film being breathed over. At one point, the subject held his breath and then resumed breathing. Note the large burst in emission intensity (lower

O_2 concentration) after the held breath due to the greater exchange time in his lungs. The irregularities of the next few breaths are due to the elevated CO_2 levels, but equilibrium is reached quickly. We include in this issue a dramatic teaching demonstration of luminescence O_2 quenching (*23*). Another application of O_2 sensors is in wind tunnels as pressure sensors (*24*).

pH Sensors

Changes in absorption spectra for species having pH-dependent ground state chemistry are used for both quantitative pH determinations and as titration indicators. Absorption spectra are rather insensitive and difficult to use in remote fiber optics and small pH-based sensors. However, luminescent molecules that show pH-dependent changes in luminescence spectra can be used as sensitive pH indicators. The basic reactions are shown in Figure 3A.

HA and A^- are the acid and its conjugate base. HA* and A^-* are the excited acid and its conjugate base. K_d and K_d* are the acid dissociation constants for the ground and excited state processes, respectively, and are frequently different. Acid–base properties in the excited state can be completely different from those of the ground state molecule because the excited state has substantially more energy (45 kcal/mole for a visible emission) and generally a completely different electron distribution. Both factors can cause p*K** to differ from p*K*, sometimes by an extraordinarily large amount (5–10 p*K* units).

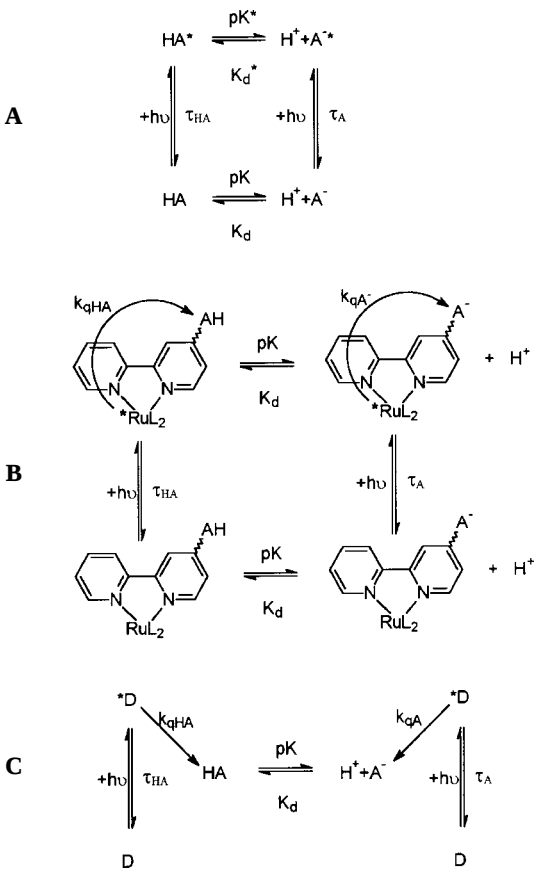

Figure 3. Some strategies used to measure pH by luminescence. A: Basic reactions. B: Reactions involving a pH-sensitive fragment. C: Indirect quenching.

$RuL_2(CN)_2$ was the first diimine complex of a platinum-group metal to display an excited state acid–base reaction (*25*). The two cyanides can be protonated at the N end, which causes a dramatic blue shift in the MLCT excited states. The p*K*s are on the order of 0 to 1. However, on excitation the complex becomes a super acid with a highly negative p*K*. Other pH-sensitive groups include amines and phenols. For example, $RuL_2(4,7\text{-}(HO)_2\text{-phen})^{2+}$ (L = α-diimine). $Ru(3,4,7,8\text{-}Me_4phen)_2(4,7\text{-}(HO)_2\text{-phen})^{2+}$ exhibits a break in the ground state absorption titration at 5 and a break at 2 in the emission titration (*26*).

One can also build a composite molecule having a luminescent piece covalently attached to a fragment that is pH sensitive and can quench the excited portion differently depending on protonation (Fig. 3B). The common version of this sensor uses electron transfer quenching.

Examples of such systems involve α-diimine ligands with CH_2 coupled with pendant phenol groups, where phenolate is a much more efficient electron-transfer quencher than phenol (*27*). The systems' usable pH range is 6–10. Alternatively, $-CH_2NR_2$ groups are used, as amines can be good reductive excited-state quenchers of RuL complexes (*28*).Variations on this mechanism include situations in which the emission varies with the charge on the pendant groups or forms intramolecular exciplexes (*exci*ted state com*plexes*) with the pendant group.

A final strategy for pH sensing is indirect quenching. This depends on very efficient dipole–dipole or Förster resonance energy transfer occurring between an excited- and a ground-state molecule, where the emission of the donor overlaps well with a strong absorption band of the acceptor (up to 30–90 Å) (*29*, *30*). See Figure 3C. The sensor, a two-component system, contains a pH-sensitive dye HA. HA and A^- have different spectra with significantly different overlaps with the donor's (D) emission. As pH varies, the fraction of dye in the different forms varies and the rate of *D quenching varies. One can monitor the emission intensity or the τ.

Carbon Dioxide Sensors

Most CO_2 sensors are actually pH sensors (*31*). A carbonate–bicarbonate buffer is separated from the analyte fluid by a CO_2-permeable membrane. The buffer pH changes in response to the CO_2 concentration in the analyte. A fluorescent dye that is pH sensitive in the correct range tracks the buffer pH and allows the CO_2 concentration to be inferred.

An alternative approach is based on energy-transfer quenching of a TMC by a pH-sensitive dye in polymer systems. Colorimetric CO_2 sensors based on pH-sensitive organic dyes (*32*) exist. The addition of a luminescent TMC that undergoes differential quenching to the two dye forms would provide a CO_2 sensor.

Specific Metal Ion Sensors

To have sensitive, specific metal-ion optical sensors is a major goal, especially for those interested in biomedical applications. Ions of interest include sodium, potassium, calcium, and magnesium. This technology is invaluable in clinical analyses and in such areas as in-vivo imaging of cellular components. Metal-specific chemistry that can then be translated into a luminescence signal change is needed (*33*).

Temperature Sensors

The significant and predictable temperature dependencies of the TMC emissions even at room temperature suggests their use as temperature probes. Their multi-microsecond τ's and ease of excitation with blue LEDs should

make them ideal remote temperature-sensors. This effect is frequently due to the quenching of the emitting state via thermal access to an upper *d–d* level. To aid in the design of such sensors, we have carried out a theoretical analysis on the molecular parameters of a sensor molecule for optimizing response for different temperatures (*34*).

In a special case, Crosby suggested Ru diimine complexes as cryogenic sensors (*35*). The temperature coefficients of these complexes are very large at liquid nitrogen temperatures and huge at liquid He temperatures.

Immunoassay

In clinical laboratories, the most common analytic methods are based on immunoassay (*36*). Immunoassays employ an antibody (Ab) specific for the desired analyte, which is an antigen (Ag) for the antibody. The antigen is labeled, Ag–D. If Ag–D is added to Ab, it will bind to form a tagged complex Ab---AgD, where --- stands for a noncovalent bond, which can be quite strong.

$$\text{Ab} + \text{AgD} \rightleftharpoons \text{Ab---AgD} \qquad (3)$$

If the analyte is added to the solution, it will competitively bind with the antibody and displace the labeled antigen.

$$\text{Ab---AgD} + \text{Ag} \rightleftharpoons \text{Ab---Ag} + \text{AgD} \qquad (4)$$

By determining the amount of AgD bound to Ab as a function of the analyte (Ag) concentration, the Ag analysis is available. Antibody–antigen reactions can be made exquisitely selective, and labeled antibodies are readily produced for a wide variety of antigens.

For environmental and simplicity reasons, the original radioisotope approach is rapidly being replaced by luminescence methods. Method development depends on measuring the ratio of bound to unbound AgD. If binding directly alters the luminescence properties, a method is available; but interactions between the probe and the binding site frequently adversely affect binding.

There are two better approaches. The first is luminescence polarized immunoassay (*37*), in which the fluorescence polarization of AgD in the solution is monitored. Emission polarization of molecules depends on their rotational correlation times compared to their emission τ's. Since large molecules tumble more slowly than small ones, they will exhibit more polarized emissions than smaller molecules with the same emission τ. In the above equilibria (eqs 3 and 4), the polarization of the AgD emission will decrease as it is displaced from Ab---AgD by adding increasing amounts of the competitive binder Ag.

The second approach is based on luminescence energy transfer (*38*). Here the antibody is labeled with a quencher (Q) for D. The critical equilibrium is then

$$\text{Q-Ab---AgD} + \text{Ag} \rightleftharpoons \text{Q-Ab---Ag} + \text{AgD} \qquad (5)$$

In the antibody-bound AgD, the emission of D is partially or completely quenched by Q on Ab. The average τ and intensity of D will depend on the amount bound to Ab. This τ or intensity signal can then be translated into the amount of Ag in solution. The simplest, most reliable form of quenching is Förster energy transfer. Because this requires no probe-quencher contact, contact interactions that can interfere with specificity are avoided.

Ru(II) complexes have been used in both luminescence depolarization (*37*) and energy transfer (*38*) immunoassay. The long τ's of TMCs increase the molecular weight limit of detectable analytes from less than several thousand for a typical 4 ns τ probe to $>10^6$ for a 400-ns Ru(II) probe. Long τ's greatly simplify measurements.

Molecular Probes

Chirality and Conformational Probes

A fertile area is elucidating the nature and dynamics of small molecules binding to DNA. In particular, the design of site/conformation-specific probes (*39*), selective cleaving agents for mapping and fingerprinting (*40*), and the potential in drug design (*41*) drive this work. Traditionally, work has focused on small intercalative binders with extended π system heterocycles having high DNA affinity (e.g., ethidium bromide) (*42, 43*). See the literature for details (*44–47*).

Rigidity Probes

An important area of interest is the rigidity of materials. An example is the curing of polymeric systems, where luminescent Re(I) complexes have proved a powerful tool for real-time monitoring of polymer curing (*48*). The sharpness and energy of many emission spectra are highly dependent on solvent rigidity. The basis of the effect is the difference in energy of thermally equilibrated and unrelaxed excited states along with the relaxation times relative to the excited state τ (*29*). Although such behavior was well known in many systems, the first use of the term "rigidochromic" was for Re(I) diimine complexes (*15, 16*).

Water Exposure

Determining the degree of water exposure of a probe molecule is a powerful tool in studying binding sites in biopolymers, polymers, bilayers, and micelles. We have developed a method for measuring the degree of water exposure in binding of TMCs in such systems. It depends on the fact that OH vibrations of water are more effective at quenching the metal complex than are lower-frequency OD vibrations of heavy water. By comparing the τ's of the complex in H_2O and in D_2O in the presence and absence of the binder, one can estimate the fraction of the surface of the molecule exposed to water (*49, 50*).

Dynamics

Determining the local viscosity and range of motions available to molecules bound to proteins, DNAs, other biopolymers, and vesicles and bilayers is an exceptionally important mechanistic and fundamental tool (*29*). Dynamic luminescence polarization measurements provide this data. Using pulsed or phased methods, one excites the probe with polarized light and, in effect, monitors the time dependence of the polarized emission. The method hinges on the excited state τ being comparable to rotational correlation times. If rotation is too slow, the emission shows no depolarization. If rotation is too fast, the emission will be depolarized during the entire decay and will yield no information. The short lifetimes of organic fluorophores (ca. 1–20 ns) permit studying motions of small biomolecules and localized motions in larger molecules. However, they are too short for large molecules (e.g., large proteins, viruses, and DNAs). The long τ's of TMCs are ideal dynamic polarization probes for larger systems. For example, Ru(II) complexes were used to study horse serum albumin proteins (*38*) and DNA (*51*).

Problem Areas

Several problems are associated with the use of luminescent TMCs in sensor and probe technology. These include photostability, ease of optical excitation, attachment chemistry, mixed sensor responses, understanding support–substrate interactions, and the difficult problem of mechanistically interpreting complex systems.

Photostability

Sensor photostability is critical to long-term sensor reliability. In luminescence measurements, high fluxes are generally required to provide good signal-to-noise ratio (S/N) and, since small amounts of sensor material are used in fiber optic systems, relatively few molecules must handle the high light exposures. Thus, exceptional photochemical stability is required to avoid decomposition on extended use. Several strategies can minimize this problem. First, to minimize light exposure, the light source can be on only during the measurement. Second, τ measurements can use significantly less light than intensity measurements. Although decomposition will reduce emission intensity, it will not affect the measured τ.

However, the highest goal for sensors is a more stable molecule. Dürr (*52*) has claimed greater stability for pendant Ru(II) complexes with attached long polyethyleneoxides, but no quantitative data were provided. It is possible that the long tails entangle the ligands and prevent rapid loss of ligand on photodissociation; a longer residence time would then permit self-healing by ligand reattachment. Os(II) sensor molecules promise higher photochemical stability than their Ru(II) analogues, owing to reduced accessibility of ligand-dissociating *d–d* states (*17*).

Excitability

A major shortcoming of Ru(II) and Re(I) sensors is that they generally must be excited in the blue and UV regions, respectively. These have traditionally been difficult regions for low-cost excitation sources—especially those employed in fiber-optic probes, where small source size and relatively high intensity are important.

While a number of promising excitation sources, such as blue and violet LEDs and frequency-doubled laser diodes, are appearing, the most promising immediate approach is still chemical. For example, Os(II) complexes analogous to the Ru(II) sensors can be excited efficiently by low-cost, red, near-IR laser diodes (*53, 54*) The downside is that, although they are still practical, they generally have shorter τ's and lower luminescence yields than analogous Ru(II) complexes. New ligand systems will probably prove valuable in enhancing performance by better tuning of excited-state energies and quenching properties.

Attachment

The problem of selective attachment to substrates is a critical issue. One must design the necessary chemistry into the metal complex to allow attachment to the target. Since the ligands are generally organic, all the tools currently used to attach organic probes should be equally applicable to TMCs (*55*).

Covalent attachment as well as intercalation, ionic attraction, hydrophobic interactions, and guest–host interactions provide means of attaching probes to substrates. While such attachment is generally weaker than covalent systems, it does not preclude very successful utilization, as shown by widely used organic probes using these binding modes. The problem is creating metal complexes that bind tightly enough to the substrate, and this is a synthetic issue.

Mixed Responses

The feature that makes TMCs so valuable is also a major weakness. The long emission τ's make them susceptible to interferences by other species. This can yield mixed responses with multiple analytes. For example, oxygen quenching in a pH sensor could be a major problem. It is not obvious how to circumvent this problem.

One possible solution is to use Re(I) and Ir(III) complexes that are substantially less sensitive to O_2 quenching than Ru(II) and Os(II) complexes of comparable τ's. Alternatively, although this has not yet been demonstrated, it may be possible to put an O_2-impervious barrier around the metal complex. TMCs with suitably engineered O_2-impervious foliage on the ligands might achieve this shielding if an asymmetric ligand combination is used and the excitation resides in the shielded ligand. This has been demonstrated with Re(I) complexes, where, if the α-diimine portion of the complex is covered by cyclodextrin (CD), O_2 shielding is very efficient; but if the cyclodextrin binds to an unexcited portion of the molecule, quenching is only slightly reduced (*56*). Suitable shields might be covalently bound CDs, alkanes, and polyethylene oxides.

Substrate–Support Interactions

A major difficulty of sensor design has been the lack of a clear understanding of the support–probe interactions that play a critical role in performance. The support system can show complex variations in microstructure, and the precise distribution of probe molecules within this microheterogeneous system can affect behavior. We have examined a number of systematically varied polymer systems with different quenchometric O_2 sensors (*57, 58*). Although complex, in many cases a domain structure is a useful tool for predicting behavior (*58, 59*). Specifically, polar domains were found necessary to permit solubilization of polar probes. However, this approach can lead to systems that are clearly more complicated (*58*).

Other problems involve fundamental rate processes. These include the determination of true rate constants based on the currently unknown internal O_2 solubility and the effect of polymer structure on diffusion, which is a critical factor in response time.

The Uniqueness Problem

It is well known that it is extremely difficult to accurately fit experimental decay curves to sums of exponentials, especially for relatively close τ's (differing by less than factor of 2) (*60–62*). The problem is severe and can make it impossible to reliably build binding–quenching models.

Drawing mechanistic conclusions from uncritical modeling is another area fraught with pitfalls. For example, there are two common 3-parameter models for fitting intensity-quenching data in polymers. In one, the sensor is treated as existing in two discrete sites each with a different Stern–Volmer quenching constant (eq 6) (*63*). In the other, all molecules are assumed to be equivalent, but the solubility of O_2 in the polymer is inferred to be nonlinear and to obey the common nonlinear solubility equation for gases in glassy polymers (eq 7) (*64*).

$$\frac{I_o}{I} = \frac{1}{\dfrac{f_{01}}{1 + K^p_{SV1} p_{O_2}} + \dfrac{f_{02}}{1 + K^p_{SV2} p_{O_2}}} = \frac{1}{\dfrac{f_{01}}{1 + K^p_{SV1} p_{O_2}} + \dfrac{1 - f_{01}}{1 + K^p_{SV2} p_{O_2}}} \quad (6)$$

$$\frac{I_o}{I} = 1 + A p_{O_2} + \frac{B p_{O_2}}{1 + b p_{O_2}} \quad (7)$$

where the I is intensity, f is the fractional contribution to the unquenched emission, the K_{sv}'s are Stern–Volmer quenching constants expressed in pressure units, p is pressure, A and B are composite parameters including gas solubility parameters and rate constants, and b is the parameter in the nonlinear gas solubility equation (*64*).

Despite the fact that the equations look completely different and have radically different physical models, they are mathematically equivalent (*63*). That is, either equation fits data exactly the same! Although we favor (based on other evidence) the two-site model in elastomer supports, the models cannot be distinguished on the basis of intensity quenching data alone.

These results demonstrate that one should use extreme caution in accepting "proof" of models in microheterogeneous systems. This ambiguity can be reduced by using different types of measurements that probe the system in different ways.

Acknowledgments

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