Electrochemiluminescence (ECL)

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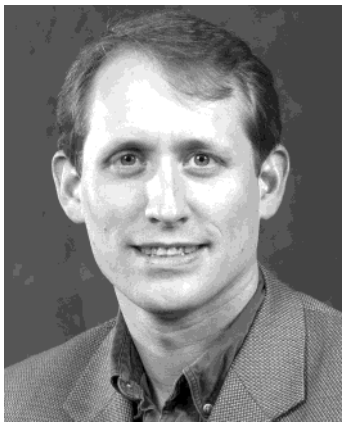

Mark Richter received his bachelor's degree in chemistry from Gustavus Adolphus College (St. Peter, MN) in 1989 and his Ph.D. degree in inorganic chemistry from Washington State University (Pullman, WA) in 1993. He then accepted a postdoctoral fellowship at the University of Texas at Austin with Prof. Allen J. Bard, where he began working with ECL. Following a brief stint in industry as a Senior Scientist with Boehringher-Mannheim Corp., he bolted back to academia and accepted a position at Southwest Missouri State University, where is he currently an Associate Professor of Chemistry.

1. Introduction

Electrochemiluminescence (also called electrogenerated chemiluminescence and abbreviated ECL) involves the generation of species at electrode surfaces that then undergo electron-transfer reactions to form excited states that emit light. For example, application of a voltage to an electrode in the presence of an ECL luminophore such as $Ru(bpy)_3^{2+}$ (where bpy = 2,2′-bipyridine) results in light emission and allows detection of the emitter at very low concentrations ($\leq 10^{-11}$ M).[1] By employing ECL-active species as labels on biological molecules, ECL has found application in immunoassays and DNA analyses.[2] Commercial systems have been developed that use ECL to detect many clinically important analytes (e.g., α-fetoprotein, digoxin, thyrotopin, protein and steroidal hormones, and various antibodies) with high sensitivity and selectivity.[2]

Since the first detailed studies in the mid-1960s, over 1000 papers, patents, and book chapters have been published on ECL, ranging from the very fundamental to the very applied. Also, several excellent reviews covering various aspects of ECL have appeared.[1−17] Therefore, this paper will cover the principles, light-emitting systems, and applications of ECL with a particular focus on the past decade.

2. Principles of ECL

ECL is a means of converting electrical energy into radiative energy. It involves the production of reactive intermediates from stable precursors at the surface of an electrode. These intermediates then react under a variety of conditions to form excited states that emit light.

For example, ECL from $Ru(bpy)_3^{2+}$ (Figure 1) was first reported in 1972[18] in acetonitrile (MeCN) using tetrabutylammonium tetrafluoroborate ($TBABF_4$) as the electrolyte. ECL was generated by alternate pulsing of an electrode potential to form oxidized $Ru(bpy)_3^{3+}$ and reduced $Ru(bpy)_3^{+}$ (Figure 1):

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \quad E°_{Ru}{}^{2+/3+} = +1.2 \text{ V vs SCE} \quad (1)$$

$$Ru(bpy)_3^{2+} + e^- \rightarrow Ru(bpy)_3^{+} \quad E°_{Ru}{}^{2+/1+} = -1.4 \text{ V vs SCE} \quad (2)$$

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

$$Ru(bpy)_3^{\bullet 2+} \rightarrow Ru(bpy)_3^{2+} + h\nu \ (\sim 2.1 \text{ eV}, 610 \text{ nm}) \quad (4)$$

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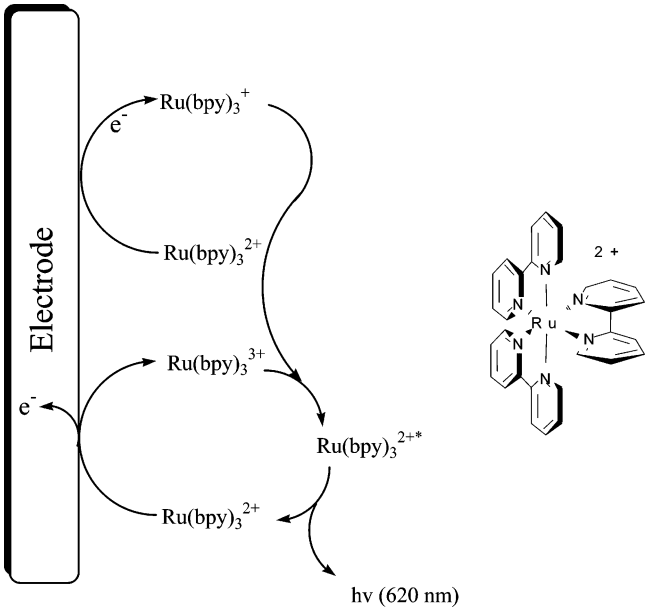

Figure 1. Structure of $Ru(bpy)_3^{2+}$ and proposed mechanism for $Ru(bpy_3^{3+}/Ru(bpy)_3^{+}$ ECL system.

$Ru(bpy)_3^{\bullet 2+}$ represents the excited molecule that emits light, and $h\nu$ is a photon of light. The excited state formed in this ECL reaction is similar to that formed during photoexcitation (i.e., photoluminescence or PL). In PL, an electron is excited from metal-based dπ orbitals to ligand-based π^* orbitals [a metal-to-ligand charge-transfer (MLCT) transition].[19,20] The excited electron then undergoes intersystem crossing to the lowest triplet states of $Ru(bpy)_3^{\bullet 2+}$ from where emission occurs.[19,21] The MLCT excited state may be formed in ECL if an electron is transferred to the π^* orbital of one of the bipyridine ligands. $Ru(bpy)_3^{\bullet 2+}$ can then decay to the ground state, producing the same luminescence as obtained from photoluminescence spectroscopy.[18]

It is also important to distinguish ECL from chemiluminescence (CL). Both involve the production of light by species that undergo highly energetic electron-transfer reactions. However, luminescence in CL is initiated and controlled by the mixing of reagents and careful manipulation of fluid flow. In ECL, luminescence is initiated and controlled by switching an electrode voltage.

2.1. General Reaction Mechanisms

2.1.1. Annihilation ECL

The first detailed studies on ECL involved electron-transfer reactions between an oxidized and a reduced species, both of which were generated at an electrode by alternate pulsing of the electrode potential.[22−25] This approach is typically called "annihilation", and a general mechanism is outlined below:

$$A - e^- \rightarrow A^{\bullet +} \text{ (oxidation at electrode)} \quad (5)$$

$$A + e^- \rightarrow A^{\bullet -} \text{ (reduction at electrode)} \quad (6)$$

$$A^{\bullet -} + A^{\bullet +} \rightarrow A^{\bullet} + A \text{ (excited-state formation)} \quad (7)$$

$$A^{\bullet} \rightarrow A + h\nu \text{ (light emission)} \quad (8)$$

For example, the potential of the working electrode is quickly changed between two different values to generate the oxidized, $A^{\bullet +}$, and reduced, $A^{\bullet -}$, species (eqs 5 and 6, respectively) that will react near the electrode surface to form the emissive state, $A^{\bullet}$ (eq 7).

A classic example involves rubrene (RUB, Figure 2).[3,4,26,27] ECL is generated when a double-potential step is applied to an electrode (such as platinum, gold, or glassy carbon), producing the radical cation ($RUB^{\bullet +}$) upon anodic oxidation and the radical anion ($RUB^{\bullet -}$) upon cathodic reduction. The resulting electrogenerated products can then react and undergo annihilation (i.e., reaction 7) to produce an excited state ($RUB^{\bullet}$) that is then able to emit light.

$$RUB - e^- \rightarrow RUB^{\bullet +}$$
$$E^\circ_{RUB/RUB^{\bullet +}} = +0.95 \text{ vs SCE} \quad (9)$$

$$RUB + e^- \rightarrow RUB^{\bullet -}$$
$$E^\circ_{RUB/RUB^{\bullet -}} = -1.37 \text{ vs SCE} \quad (10)$$

$$RUB^{\bullet +} + RUB^{\bullet -} \rightarrow RUB + RUB^{\bullet} \quad (11)$$

$$RUB^{\bullet} \rightarrow RUB + h\nu \quad (12)$$

For rubrene the emission maximum (λ_{max}) occurs at ~540 nm and the ECL spectrum (i.e., a plot of ECL emission versus wavelength) is identical to its PL (Figure 2). This indicates that the ultimate product of charge transfer, and hence luminescence, is the lowest singlet RUB species, $^1RUB^{\bullet}$. There is considerable evidence that the reactions outlined in eqs 10 and 11 are oversimplifications and that several mechanistic steps intervene between the electron-transfer and photon emission steps. The identification of these mechanisms has been a focus of past work in the field and is thoroughly reviewed elsewhere.[3,4]

A cyclic voltammogram of rubrene is also shown in Figure 2. Cyclic voltammetry (CV) is a convenient method for determining the potentials at which the desired reactants can be generated.[5] Also, CV is an excellent probe of the stability of the oxidized and reduced forms of the complex (i.e., the radical anions and cations) and their ability to undergo electron-transfer reactions. Stable, "reversible" electrochemistry (as well as high photoluminescence lifetimes and/or efficiencies) is often used to determine whether a compound shows promise as an ECL luminophore. In Figure 2, starting at 0.0 V, the potential is scanned linearly in a positive direction until rubrene starts to be oxidized near +1.0 V and anodic current is generated. Reversal of the scan at ~+1.5V causes re-reduction of the cation (at ~+0.8V). Comparison of the anodic and cathodic currents (i_{pa} and i_{pc}) should result in $i_{pa}/i_{pc} \cong 1$ for a well-behaved, electrochemically reversible system. As the potential is scanned in a negative direction, rubrene is reduced to its radical anion near −1.3 V. Subsequent scan reversal will cause oxidation of the radical anion species. Again, $i_{pa}/i_{pc} \cong 1$.

In a typical annihilation experiment, a platinum electrode is immersed in a quiescent solution, and

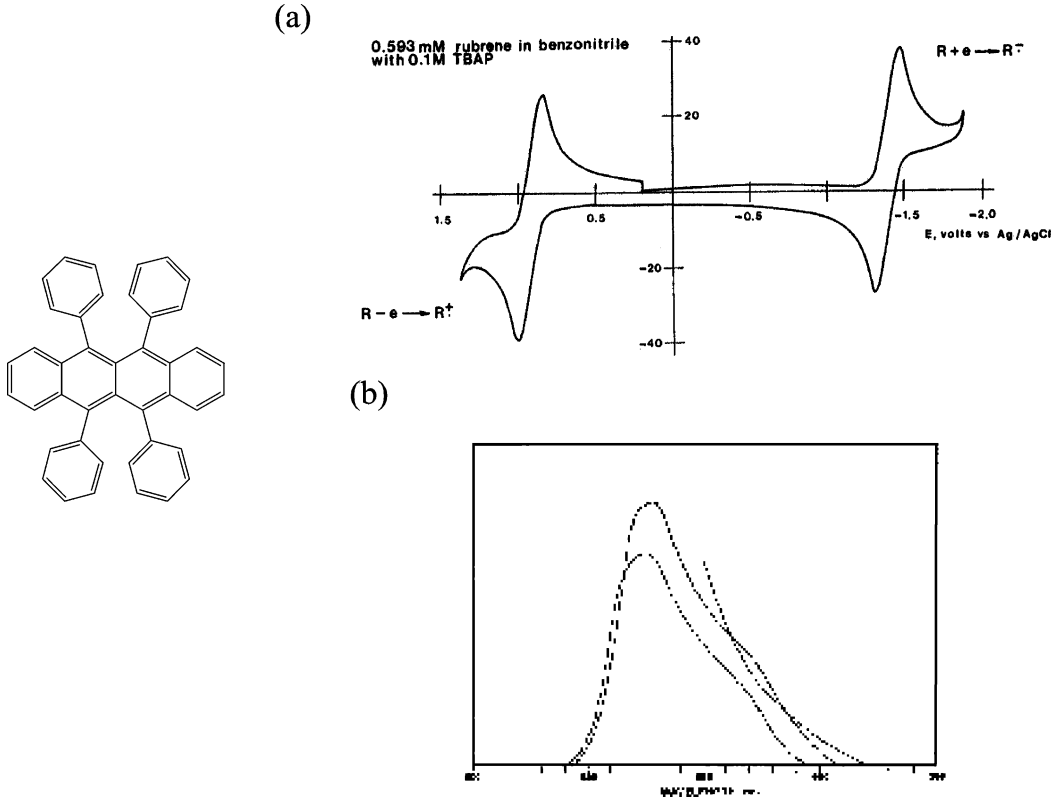

Figure 2. (Left) Structure of rubrene (RUB). (a) Cyclic voltammetric curve of 0.593 mM rubrene in benzonitrile with 0.1 M tetra-*n*-butylammonium perchlorate (Reprinted with permission from ref 3. Copyright 1977 Marcel Dekker, Inc.) (b) Corrected fluorescence (—) and ECL (— - —) spectra for rubrene in benzonitrile (Reprinted with permission from ref 27. Copyright 1972 American Chemical Society.)

the electrode potential is held at a value where no electrochemical activity occurs (such as 0.0 V). The potential is then stepped anodically or cathodically to a value where the first reagent is produced (e.g., $RUB^{\bullet+}$ in eq 9). The electrode is held at this value for a certain period of time (t_f, which lasts from microseconds to seconds); the potential is then switched to a value at which the opposing reagent is generated for t_f (eq 10) and then returned to the initial value. This sequence can then be repeated as many times as desired. During the second step both reagents exist in the diffusion layer near the electrode and can react, resulting (it is hoped) in light emission (eqs 11 and 12).

Rubrene, and several other polyaromatic hydrocarbons including diphenylanthracene, were among the first complexes studied using ECL[22−25] because they were known to undergo chemically and electrochemically reversible one-electron oxidation and reduction at easily attainable potentials (e.g., ≤2V vs SHE, where SHE = standard hydrogen electrode), display photoluminescence efficiencies (ϕ_{em} = number of photons emitted per photons absorbed by the compound) of near unity, and undergo electron-transfer reactions.

RUB is an example of an "energy sufficient" system. An energy sufficient system is one in which $\Delta H°$ of the electron-transfer reaction in eq 7 is larger than the energy required to produce the excited singlet state from the ground state (eq 8). In RUB, one of the products of the reaction is therefore produced with excess energy that can be emitted as light (e.g., $^1RUB^{\bullet}$). When the luminescence is emitted by a species in an excited singlet state, this process is known as the singlet or S-route. Because the free energy ($\Delta G°$) available for exciting the product is the energy available from the redox reaction producing the ground-state products (i.e., eqs 5 and 6), $\Delta H°$ can be calculated from the reversible standard potentials of the redox couples[3,4] with the equation

$$\Delta H° = \Delta G° + T\Delta S° = (E°_{A+/A} - E°_{A/A-}) - 0.1 \text{ eV} \quad (13)$$

where $E°_{A+/A}$ and $E°_{A/A-}$ represent the cyclic voltammetric peak potentials for the oxidative and reductive redox couples, respectively. As discussed elsewhere,[3,4] the precise value for $T\Delta S°$ is unknown, but it is estimated to be ∼0.1 ± 0.1 eV. In RUB (eqs 9 and 10), for example, annihilation results in an enthalpy for the electron-transfer reaction of 2.32 eV. When we compare this to the energy of the emitted light in RUB obtained spectroscopically (λ_{max} = ∼540 nm or 2.30 eV), we see that the emitting state is accessible and $^1RUB^{\bullet}$ may be populated directly in the reaction.

Another example of an energy sufficient system is the inorganic species $Ru(bpy)_3^{2+}$ (Figure 1; eqs 1−4).[18] The reaction of eq 4 has ∼2.6 V of free energy to place into an excited product. The charge-transfer triplet lies at 2.1 eV, so it is readily accessible to the homogeneous redox process.[28,29] No other excited states of appreciable lifetime can be populated, reducing the opportunities for alternate mechanistic pathways.

$Ru(bpy)_3^{2+}$ has an ECL efficiency (ϕ_{ecl}, photons generated per redox event) of 0.0500.[28,29] Under

certain conditions, the reaction of eq 3 produces the emitting charge-transfer triplet with an efficiency approaching unity[30,31] and is comparable with photoluminescence data,[19,32] showing that ~5% of the excited states produce luminescence. This demonstrates that photoluminescence and electrochemical data can be used to predict compounds that may show promise as ECL light emitters.

The ECL quantum efficiency, ϕ_{ecl}, is defined as the ratio of the number of photons emitted to the number of annihilations between the oxidized and reduced species. In the case of $Ru(bpy)_3^{2+}$, this would involve annihilations between the 3+ and 1+ forms of the ruthenium complex (eq 3). Values of ϕ_{ecl} are obtained using the following expression[27,28,30,31,33]

$$\phi_{ecl} = \frac{\int_t^0 I\,dt}{\int_{t'}^0 i_{c,a}\,dt} = \frac{\int_{t'}^0 I\,dt}{Q_{c,a}} \quad (14)$$

where I (einsteins/s) represents the total ECL intensity integrated over a finite period of time t' and $i_{c,a}$ represents integration of the cathodic or anodic current, i_c or i_a, respectively, over the same time period that results in the total cathodic or anodic charge (e.g., $Q_{c,a}$ for the latter). Experimental methods for obtaining ϕ_{ecl} have been described in detail elsewhere,[5,27,28,30] and the interested reader is encouraged to consult the literature.

Often a relative ECL efficiency is reported, using a compound such as $Ru(bpy)_3^{2+}$ as a reference system. For example, the ECL quantum efficiencies of $Ru(dpbpy)_3^{2+}$ and $Ru(dp\text{-}phen)_3^{2+}$ (dp-bpy = 4,4′-diphenyl-2,2′-bipyridine; dp-phen = 4,7-diphenyl-1,10-phenanthroline) were calculated relative to that of $Ru(bpy)_3^{2+}$ (ϕ_{ecl} = 0.0500 or 5%) using the equation[34]

$$\phi_{ecl} = \frac{\int_t^0 I\,dt}{\int_t^0 i\,dt\,(N_A/F)} \quad (15)$$

where I is the intensity in photons per second, i is the current in amperes (coulombs per second), F is Faraday's constant, and N_A is Avogadro's constant. If the same electrode is used for each experiment, luminophore and electrolyte concentrations are approximately the same, and the number of electrons transferred is equivalent between the target molecules, then the charge passed for each complex should be about the same and direct comparisons among the systems possible using a relationship such as

$$\phi_{ecl} = \phi^\circ_{ecl}(IQ^\circ/I^\circ Q) \quad (16)$$

where ϕ°_{ecl} is the ECL efficiency of the standard, I and I° are the integrated ECL intensities of the target luminophore and standard systems, and Q and Q° are the charges passed (in coulombs) for the luminophore and standard, respectively.

It is also possible to use two different precursors to generate ECL.

$$A + e^- \rightarrow A^{\bullet -} \quad (17)$$

$$D - e^- \rightarrow D^{\bullet +} \quad (18)$$

$$A^{\bullet -} + D^{\bullet +} \rightarrow A^\bullet + D\ (\text{or } A + D^\bullet) \quad (19)$$

$$A^\bullet\ (\text{or } D^\bullet) \rightarrow A\ (\text{or } D) + h\nu \quad (20)$$

ECL is achieved via "cross-reactions" between the radical cation of one species ($A^{\bullet -}$) and the radical anion of a different species ($D^{\bullet +}$). A classic example is the electron-transfer reaction and subsequent chemiluminescence between the anion radical of 9,10-diphenylanthracene (DPA) and the cation radical of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine [Wurster's Blue (TMPD)] in dimethylformamide.

$$DPA + e^- \rightarrow DPA^{\bullet -}\ E^\circ_{DPA-/DPA} = -1.89\ \text{V vs SCE} \quad (21)$$

$$TMPD - e^- \rightarrow TMPD^{\bullet +}\ E^\circ_{TMPD/TMPD+} = +0.24\ \text{V vs SCE} \quad (22)$$

$$DPA^{\bullet -} + TMPD^{\bullet +} \rightarrow {}^1DPA^\bullet + TMPD \quad (23)$$

$${}^1DPA^\bullet \rightarrow DPA + h\nu \quad (24)$$

where $^1DPA^\bullet$ is the excited singlet state the excess energy of which is emitted as light. In theory, light may be emitted from either $^1DPA^\bullet$ or $TMPD^\bullet$ (e.g., either $A^\bullet$ or $D^\bullet$ in eq 19). However, the emitted light generated via ECL is identical to DPA photoluminescence, indicating that $^1DPA^\bullet$ is the ultimate product of charge transfer.[3] The fact that the DPA/TMPD system undergoes ECL at all is surprising because the enthalpy for the electron-transfer reaction is 2.03 eV, much less than that required to reach the emitting singlet excited state for DPA of 3.00 eV. Therefore, the ultimate emitter is not directly populated. Such systems are called "energy deficient", and a more complicated scheme than that depicted in eq 23 is required to generate the emitter. The proposed mechanism involves triplet intermediates, the so-called triplet or T-route.

$$A^{\bullet -} + D^{\bullet +} \rightarrow {}^3A^\bullet + D \quad (25)$$

$${}^3A^\bullet + {}^3A^\bullet \rightarrow {}^1A^\bullet + A\ (\text{triplet}-\text{triplet annihilation}) \quad (26)$$

Reaction 26 is generally called "triplet−triplet annihilation",[3,4,35] where the energy from two electron-transfer reactions is pooled to provide sufficient energy to form the singlet excited state.[36,37] For the DPA/TMPD system:[35]

$$TMPD^{\bullet +} + DPA^{\bullet -} \rightarrow TMPD + {}^3DPA^\bullet \quad (27)$$

$${}^3DPA^\bullet + {}^3DPA^\bullet \rightarrow DPA + {}^1DPA^\bullet \quad (28)$$

Many ECL reactions with different precursors follow this route, and several examples are given in the

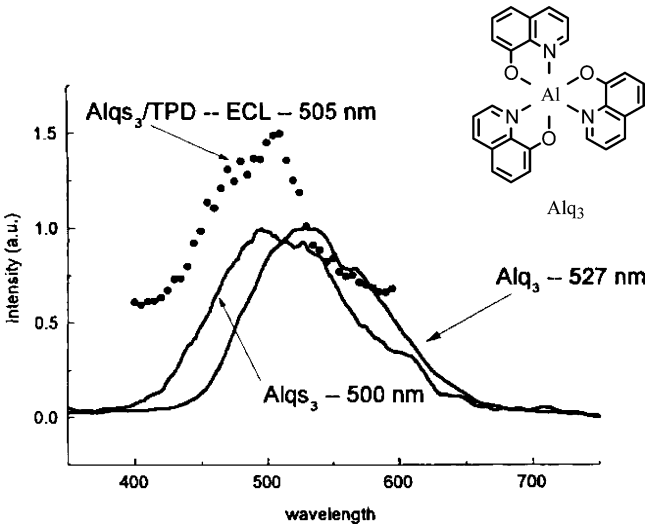

Figure 3. ECL response for a typical double-potential step experiment involving $Al(qs)_3^{\bullet-}/TPD^{\bullet+}$ (···; qs = sulfonamide derivative of q) and the electroemissive response from OLEDs (—) involving either Alq_3 or $Al(qs)_3$. (Reprinted with permission from ref 38. Copyright 2000 American Chemical Society.)

literature.[3,6] The T-route may also operate in energy sufficient systems.

A recent example is the annihilation cross-reaction between tris(8-hydroxyquinoline)aluminum (Alq_3) and a triarylamine such as 4,4′-bis(*m*-tolylphenylamino)-biphenyl (TPD) (Figure 3).[38] The motivation behind these studies was to probe the mechanism of light emission and to develop a method to rapidly screen candidates for potential use in organic light-emitting diode (OLED) assemblies.

$$Alq_3 + e^- \rightarrow Alq_3^{\bullet-} \quad (29)$$

$$TPD \rightarrow TPD^{\bullet+} + e^- \quad (30)$$

$$TPD^{\bullet+} + Alq_3 \rightarrow Alq_3^{\bullet+} + TPD \quad (31)$$

$$Alq_3^{\bullet+} + Alq_3^{\bullet-} \rightarrow {}^1Alq_3^{\bullet} + Alq_3 \quad (32)$$

$$Alq_3^{\bullet-} + TPD^{\bullet+} \rightarrow {}^1Alq_3^{\bullet} + TPD \quad (33)$$

$$^1Alq_3^{\bullet} \rightarrow Alq_3 + h\nu \quad (34)$$

Voltammetric and ECL studies clearly show that the excited state formed in ECL is the same as that formed using solid-state OLED devices (i.e., electroluminescence) (Figure 3) and that the reaction presented in eq 33 is primarily responsible for excited-state production.

Experiments focused on annihilation ECL of radical ions are carried out in fairly conventional electrochemical apparatus.[3] However, cells, electrodes, and experimental procedures must be modified to allow electrogeneration of two reactants, rather than one, while taking into account constraints imposed by optical measurement equipment and the exclusion of stray light (i.e., "light-tight" experiments). In addition, one must pay scrupulous attention to the purity of the solvent/supporting electrolyte system, especially with organic systems (such as polyaromatic hydrocarbons). Water and oxygen are particularly harmful to these experiments because they can quench ECL. Thus, cells and electrodes are constructed to allow transfer of solvent and degassing on high-vacuum lines or in inert-atmosphere ("glove") boxes. Experimental details for annihilation systems, including electrode configurations and sample cells, have been thoroughly reviewed.[3,6]

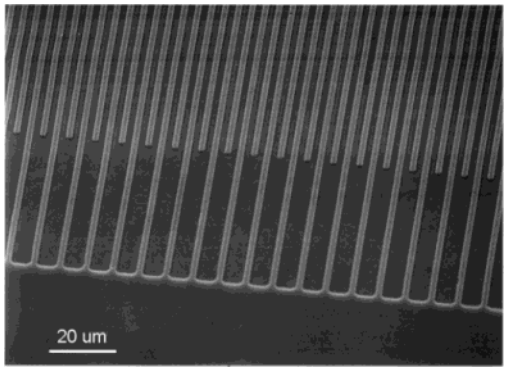

Figure 4. Scanning electron micrograph of a portion of a carbon interdigitated microelectrode array (C-IDA), showing individual electrodes. Each carbon electrode is 2 μm wide, 0.4 μm high, and 1 mm long. (Reprinted with permission from ref 42. Copyright 1998 American Chemical Society.)

The ECL systems discussed above generate oxidized and reduced species at a single electrode. It is also possible to obtain emission at two different electrodes that are close enough to allow the electrogenerated reactants to interdiffuse and undergo annihilation (e.g., eq 7).[39,40] For example, a rotating ring disk electrode (RRDE) was used to generate one reactant, such as $A^{\bullet-}$, at the central disk while $A^{\bullet+}$ was generated at the ring. These are then swept together by diffusion and convection, resulting in a ring of light on the inner edge of the ring electrode.[3,39] Other experiments have employed dual-working electrode systems[40−42] with interdigitated electrodes,[42] thin-layer geometry, or flowing streams to move the reactants together and individually accessible microarrays[43] with potential for use in remote ECL analyses.

For example, annihilation ECL of $Ru(bpy)_3^{2+}$ was reported in aqueous solution containing no electrolyte using a microfabricated interdigitated carbon dual-electrode system (Figure 4).[42] Each electrode is biased to form the reduced, $Ru(bpy)_3^{+}$, or oxidized, $Ru(bpy)_3^{3+}$, species. The electrodes are in close enough proximity (2 μm width and spacing) that the simultaneously produced reactants can diffuse together and undergo annihilation (eq 3).

The generation of ECL at micro- and ultra-microelectrodes is well established.[44−46] ECL with microelectrodes has also been coupled to scanning probe techniques such as scanning electrochemical microscopy (SECM) to image surfaces.[47,48] High-frequency ECL has also been used to image the surfaces of

microelectrodes.[49] More recently, ECL has been used as a light source for near-field scanning optical microscopy using ultramicroelectrodes with effective diameters from 1 μm to <100 nm.[50] This technique was used to image an interdigitated array with resolution comparable to that observed via near-field scanning optical microscopy (NSOM), suggesting this technique may be used for near-field optics in solution. Annihilation ECL with microelectrodes has also been used to study sol−gel composites containing $Ru(bpy)_3^{2+}$ [51] and diode-like chemiluminescence in frozen concentration gradients of the polymer poly-$[Ru(vbpy)_3](PF_6)_2$.[52]

2.1.2. Coreactant ECL

It is also possible to generate ECL in a single-potential step using a coreactant. A coreactant is a species that, upon oxidation or reduction, produces an intermediate that can react with an ECL luminophore to produce excited states. Usually, this occurs upon bond cleavage of the coreactant to form strong oxidants or reducants. For example, oxalate ion ($C_2O_4^{2-}$) was the first coreactant discovered[53] and is believed to produce the strong reductant $CO_2^{\bullet-}$ upon oxidation in aqueous solution:

$$C_2O_4^{2-} - e^- \rightarrow [C_2O_4^{\bullet-}] \rightarrow CO_2^{\bullet-} + CO_2 \quad (35)$$

The oxidizing potential can also oxidize an ECL luminophore [such as D, where D is, for example, $Ru(bpy)_3^{2+}$].

$$D - e^- \rightarrow D^{\bullet+} \quad (36)$$

$D^{\bullet+}$ and $CO_2^{\bullet-}$ may then react to produce an excited state capable of emitting light.

$$CO_2^{\bullet-} + D^{\bullet+} \rightarrow D^{\bullet} + CO_2 \quad (37)$$

$$D^{\bullet} \rightarrow D + h\nu \quad (38)$$

Oxalate is often referred to as an "oxidative" or "oxidative−reductive" coreactant due to its ability to form a strong reducing agent upon electrochemical oxidation. In coreactant ECL the electrode typically only oxidizes *or* reduces the reagents in a *single* potential step, whereas in annihilation schemes a double-potential step (e.g., oxidation followed by reduction) is required to generate the highly energetic precursors. For example, in the oxalate system the electrode oxidizes both the oxalate and the ECL reactant D; the reducant, $CO_2^{\bullet-}$ is then generated upon bond cleavage of oxalate via eq 35. This strategy is used in analytical and biotechnology applications, with the reactant D being $Ru(bpy)_3^{2+}$.[1,2]

Another example of an "oxidative−reductive" system is the commercially important $Ru(bpy)_3^{2+}$/TPrA system [TPrA = tri-*n*-propylamine; $(CH_3CH_2CH_2)_3N$].[1,38]

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \quad (39)$$

$$TPrA - e^- \rightarrow [TPrA^{\bullet}]^+ \rightarrow TPrA^{\bullet} + H^+ \quad (40)$$

$$Ru(bpy)_3^{3+} + TPrA^{\bullet} \rightarrow Ru(bpy)_3^{\bullet 2+} + \text{products} \quad (41)$$

$$Ru(bpy)_3^{\bullet 2+} \rightarrow Ru(bpy)_3^{2+} + h\nu \quad (42)$$

ECL is produced upon concomitant oxidation of $Ru(bpy)_3^{2+}$ and TPrA (Figure 5). Electrochemical studies of various aliphatic amines indicate a possible reaction pathway for the oxidation of TPrA.[54] Upon oxidation, the short-lived TPrA radical cation ($TPrA^{\bullet+}$) is believed to lose a proton from an α-carbon to form the strongly reducing intermediate $TPrA^{\bullet}$ (Figure 6). This radical can then reduce $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{\bullet 2+}$. Recent work utilizing cyclic voltammetric simulations and SECM, ECL, and SECM−ECL ex-

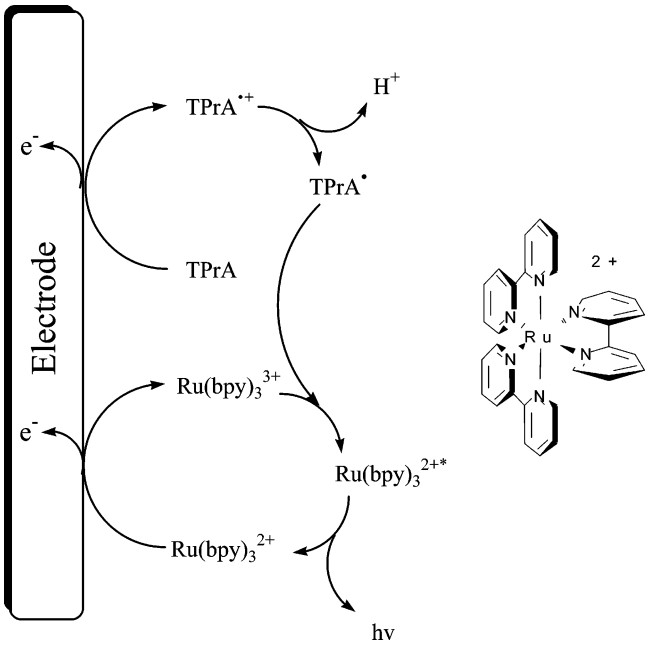

Figure 5. Proposed mechanism for $Ru(bpy)_3^{2+}$/TPrA ECL system.

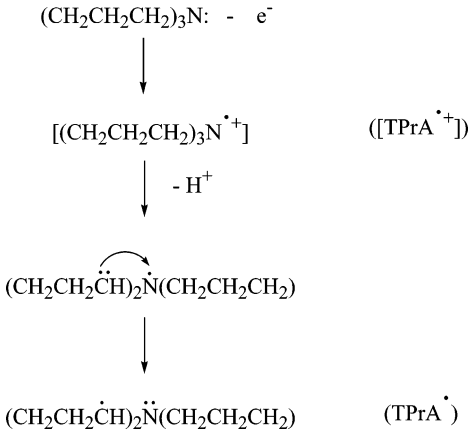

Figure 6. Proposed tri-*n*-propylamine oxidation/reaction sequence with abbreviations in parentheses.

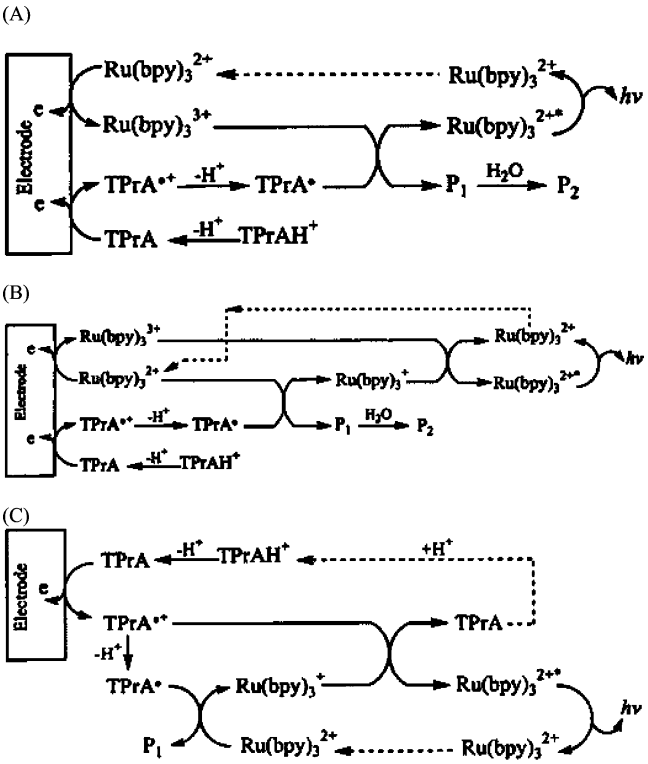

Figure 7. Proposed mechanisms of $Ru(bpy)_3^{2+}$/TPrA ECL excited state formation and light emission. (A) Electrogenerated $Ru(bpy)_3^{3+}$ reacts with TPrA as well as by direct reaction of TPrA at the electrode; (B) $Ru(bpy)_3^{2+}$ reacts with $TPrA^{\bullet}$ to form $Ru(bpy)_3^{+}$, which can then interact with $Ru(bpy)_3^{3+}$ to form ECL via annihilation; (C) generation of ECL within the potential range before the oxidation of $Ru(bpy)_3^{2+}$ at the electrode, involving formation of excited state on reaction of $TPrA^{\bullet+}$ with $Ru(bpy)_3^{+}$ [formed by reaction of $Ru(bpy)_3^{2+}$ with $TPrA^{\bullet}$]. (Reprinted with permission from ref 55. Copyright 2002 American Chemical Society.)

periments on the $Ru(bpy)_3^{2+}$/TPrA system indicates that $[TPrA^{\bullet}]^{+}$ may also play a role in the generation of ECL.[55] The half-life of $[TPrA^{\bullet}]^{+}$ was estimated at 0.2 ms, and direct evidence for the existence of the intermediate was found via flow-cell electron spin resonance experiments.

Other reaction mechanisms for the production of the excited state have also been proposed, and several are outlined in Figure 7.[55] For example, reduction of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{1+}$ by $TPrA^{\bullet}$ followed by annihilation:

$$Ru(bpy)_3^{2+} + TPrA^{\bullet} \rightarrow Ru(bpy)_3^{+} \quad (43)$$

$$Ru(bpy)_3^{1+} + Ru(bpy)_3^{3+} \rightarrow Ru(bpy)_3^{2+} + Ru(bpy)_3^{\bullet 2+} \quad (44)$$

Although the details of the coreactant ECL mechanism (eqs 39−42) to generate light emission are still under study,[55−57] the origin of the light emission from $Ru(bpy)_3^{2+}$ has been well documented.[1,4,29,34] Because the photoluminescent and ECL spectra are nearly identical, the emission process in ECL involves the MLCT state of $Ru(bpy)_3^{2+}$. This state may be formed if the reducing agent (e.g., $TPrA^{\bullet}$ or $TPrA^{\bullet+}$) transfers an electron to the π^* orbital of one of the bipyridine ligands. $Ru(bpy)_3^{\bullet 2+}$ can then decay to the ground state, producing the same luminescence as obtained from photoluminescence spectroscopy.

Numerous other amine-based coreactants have been studied, including primary, secondary, and tertiary systems,[1] and attempts made to understand the electron-donating and -withdrawing properties that might lead to optimal coreactant efficiency.[58] To date, TPrA still provides the optimum ECL in the $Ru(bpy)_3^{2+}$ system.[1,34]

"Reductive−oxidative" coreactants are also used to generate ECL. For example, in the case of peroxydisulfate ($S_2O_8^{2-}$), reduction produces the strong oxidant $SO_4^{\bullet-}$, which then undergoes an electron-transfer reaction with an ECL luminophore such as $Ru(bpy)_3^{2+}$ to generate light:[59,60]

$$Ru(bpy)_3^{2+} + e^- \rightarrow Ru(bpy)_3^{+} \quad (45)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{\bullet-} + SO_4^{2-} \quad (46)$$

$$Ru(bpy)_3^{+} + SO_4^{\bullet-} \rightarrow Ru(bpy)_3^{\bullet 2+} + SO_4^{2-} \quad (47)$$

As well as being of practical interest, ECL reactions of this type also demonstrate the intermediacy of species such as $TPrA^{\bullet}$ and $CO_2^{\bullet-}$.

It is hard to overstate the importance of coreactants to the growth and development of ECL. Although analytical uses for ECL are possible with annihilation systems (such as display devices), most annihilation systems require the use of rigorously purified and deoxygenated nonaqueous solvents, because the available potential range in water is too narrow to generate the required energetic precursors. The potential window for the electrochemical oxidation and reduction of water is simply too small to conveniently generate both species (i.e., the radical anion and cation) needed for annihilation ECL. For example, $Ru(bpy)_3^{2+}$ is oxidized at a Pt electrode to form $Ru(bpy)_3^{3+}$ at ~1.2 V vs SCE. $Ru(bpy)_3^{2+}$ is reduced at a Pt electrode in aqueous solution to form $Ru(bpy)_3^{+}$ at ~ −1.4 V, a potential not easily attainable at Pt electrodes in aqueous solution without the evolution of large amounts of hydrogen gas. The products of water oxidation and/or reduction interfering with the annihilation reaction (eqs 3 and 7) such that little to no light is observed. Coreactants, on the other hand, are generated using potential sweep or step in only one direction, allowing the generation of ECL in aqueous solution.

As discussed above, the ECL quantum efficiency for annihilation systems is generally described as the number of photons emitted per redox event. Unfortunately, the complexity of coreactant systems, the irreversible nature of coreactant electrochemistry, and the high concentrations of coreactants in solution compared to luminophore (typically 10−100-fold excess of coreactant compared to luminophore) make the direct measurement of ϕ_{ecl} nearly impossible. Therefore, several methods have been used to estimate ECL efficiencies in coreactant systems.

For example, in the $Ru(bpy)_3^{2+}/S_2O_8^{2-}$ system the coulombic ECL efficiency (taken as the photons generated per electron injected) was estimated.[59] This

was done by comparing the integrated intensity of the $Ru(bpy)_3^{2+}/S_2O_8^{2-}$ reductive−oxidative system (eqs 45−47) to that of the $Ru(bpy)_3^{3+}/Ru(bpy)_3^{+}$ (eqs 1−4) annihilation system and the total coulombs passed during the respective experiments. It was found that the ECL intensity of the $Ru(bpy)_3^{2+}/S_2O_8^{2-}$ system was 6−7 times larger than that observed in the $Ru(bpy)_3^{2+}$ annihilation system. However, the charge obtained in the coreactant system was also 12−14 times higher, leading to an overall coulombic efficiency for $Ru(bpy)_3^{2+}/S_2O_8^{2-}$ of about half that of the annihilation system.

The recent trend in the coreactant literature is to report relative ECL efficiencies using a standard such as $Ru(bpy)_3^{2+}$ and a relationship such as eq 16, where $\phi°_{ecl}$ is the ECL efficiency of the standard, I and $I°$ are the integrated ECL intensities of the target luminophore/coreactant and standard/coreactant systems, and Q and $Q°$ are the charges passed (in coulombs) for the forward sweeps of the luminophore/coreactant and standard/coreactant, respectively.[34,61] If the same experimental parameters (e.g., electrodes, solvent, electrolyte, coreactant) and concentrations are used for each compound and the number of electrons involved in the redox chemistry is the same among the target complex/coreactant and standard/coreactant systems, then Q and $Q°$ are about the same.

However, one must be careful when comparing coreactant efficiencies between systems, as different values for $\phi°_{ecl}$ have been used. For example, $\phi°_{ecl}$ of $Ru(bpy)_3^{2+}$ obtained via annihilation is taken as 0.05 in MeCN at 25 °C,[29−31] and this value has been used for the value of $\phi°_{ecl}$ in many $Ru(bpy)_3^{2+}$/coreactant studies. The recent trend, however, is to assign the $Ru(bpy)_3^{2+}$/coreactant (or other suitable standard/coreactant) a value of 1.0 or 100% because the absolute value of $\phi°_{ecl}$ is unknown.[62]

Electrochemical apparatus for coreactant ECL are, in many instances, identical to those used in annihilation ECL.[6] However, the constraints of working with nonaqueous systems (e.g., vacuum lines) are alleviated. The earliest experiments were carried out in electrochemical batch cells designed to fit into optical spectrophotometer chambers.[53] As the development of coreactant ECL for use in diagnostics and for flow injection and liquid chromatographic applications increased, many ECL flow cell configurations were developed.[2,6] Electrode configurations, cells incorporating them, and experimental details for both annihilation and coreactant systems have been thoroughly reviewed.[3,5,6]

2.1.3. Cathodic Luminescence

Light emission has also been observed at oxide-covered metal (such as aluminum and tantalum) electrodes under various conditions.[63−66] For example, emission from Dy(III), Sm(III), and Tb(III) has been observed at oxide-covered aluminum electrodes during the reduction of hydrogen peroxide, persulfate, or oxylate in aqueous solution.[64] It was proposed that this type of high-voltage cathodic luminescence results from the injection of hot electrons into the aqueous electrolyte solution with the possible formation of hydrated electrons.[63,64] Subsequent studies in nonaqueous solution have provided experimental evidence for the production of hot electrons in an acetonitrile solution from a Ta_2O_5-covered Ta electrode.[65,66] Although this method is often called "electrogenerated chemiluminescence" in the literature and has found use in analytical applications, its mechanism is distinctly different than those described above (e.g., the direct formation of the excited states at the electrode surface rather than electron-transfer chemiluminescent reactions of electrochemically generated reactants). Therefore, it will not be discussed in further detail, and the interested reader is encouraged to consult the literature.

3. ECL Luminophores

3.1. Inorganic Systems

Inorganic complexes, and in particular tris(bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$, have played a pivotal role in transforming ECL from a "laboratory curiosity" to a useful analytical technique. This is not surprising, considering many metal complexes and clusters display the electrochemical and spectroscopic qualities required of ECL luminophores. The ECL of complexes and/or clusters containing Ag, Al, Au, Cd, Cr, Cu, Eu, Hg, Ir, Mo, W, Os, Pd, Pt, Re, Ru, Si, Tb, and Tl have been reported. A comprehensive listing of ECL-active inorganic compounds is presented in Table 1.

Due to the number of ECL-active inorganic compounds, and a recent review of inorganic ECL,[15,67] only a few examples will be discussed here.

Ironically, the first inorganic complex to show ECL has also proved to be the most valuable in both fundamental studies and commercial applications, namely, $Ru(bpy)_3^{2+}$. This is for a number of reasons, including its strong luminescence and solubility in a variety of aqueous and nonaqueous solvents at room temperature and its ability to undergo reversible one-electron-transfer reactions at easily attainable potentials. Following the first report of $Ru(bpy)_3^{2+}$ ECL[18] many other studies followed. For example, two groups working independently discovered that the ECL efficiency of $Ru(bpy)_3^{3+}$ is 0.0500 and that the annihilation reaction produces an emitting charge-transfer state with an efficiency approaching 100%.[28,29] This is comparable to PL data and shows that ∼5% of the excited states produce luminescence. Other studies have followed, and much of this work has been reviewed.[3,4]

The first report of ECL with a coreactant in aqueous solution also involved $Ru(bpy)_3^{2+}$.[53] The coreactant in question was the oxalate ion ($C_2O_4^{2-}$). Subsequently, other species were shown to act as coreactants with $Ru(bpy)_3^{2+}$, among them peroxydisulfate[59] and tri-*n*-propylamine[1] (TPrA). TPrA was an especially important discovery for practical applications because it allowed efficient ECL not only in aqueous media but also at physiological pH (∼7.4). By attaching suitable groups to the bipyridine moieties (Figure 8), $Ru(bpy)_3^{2+}$ can be linked to biologically interesting molecules, such as antibodies, where it serves as a label for analysis in a manner analo-

Table 1. Spectroscopic and ECL Properties of Inorganic Complexes[a]

complex/reactant	solvent	λ_{em} (nm)	λ_{ecl} (nm)	ϕ_{em}	ϕ_{ecl}	ref
	Aluminum					
Alq_3/TPD (cross-R)	50:50 v/v MeCN/toluene (0.1 M $TBAPF_6$)	527	510		0.09	38[a,b]
Alq_3/$TPDF_2$ (cross-R)	50:50 v/v MeCN/toluene (0.1 M $TBAPF_6$)	527	510		0.18	38
$Al(qs)_3$/TPD (cross-R)	50:50 v/v MeCN/toluene (0.1 M $TBAPF_6$)	505	510		0.03	38[b]
$Al(qs)_3$/TPDF (cross-R)	50:50 v/v MeCN/toluene (0.1 M $TBAPF_6$)	505	510		0.10	38[b]
$Al(HQS)_3$/TPrA	H_2O (0.2 M KH_2PO_4)	499	499	0.06	0.002	122[c]
	Cadmium					
Cd(II)-phen/TPrA	H_2O (0.15 M NaH_2PO_4)					371
	Chromium					
$Cr(bpy)_3^{2+}$	MeCN (0.1 M TBAP)	730	730			372
$Cr(CN)_6^{3-}$	MeCN (0.1 M TBAP)	800	800		$\sim 3 \times 10^{-4}$	372
$Cr(bpy)_3^{3+}/S_2O_8^{2-}$	H_2O (0.1 M NaCl)	727	727	0.02	0.25	373[c]
$Cr(4,4'\text{-}Me_2bpy)_3^{3+}/S_2O_8^{2-}$	H_2O (0.1 M NaCl)	727	727	0.06	0.45	373[c]
$Cr(phen)_3^{3+}/S_2O_8^{2-}$	H_2O (0.1 M NaCl)	727	727	0.10	0.08	373[c]
$Cr(5\text{-}Cl\text{-}phen)_3^{3+}/S_2O_8^{2-}$	H_2O (0.1 M NaCl)	727	727	0.08	0.07	373[c]
	Copper					
$[Cu(pyridine)I]_4$	CH_2Cl_2 (0.1 M $TBABF_4$)	698	698	0.05		110
$Cu(dmp)_2(PF_6)_2$/TPrA	MeCN (0.1 M $TBAPF_6$)	519,642		0.04	0.004	118[c]
$Cu(dmp)_2(PF_6)_2$/TPrA	MeCN/H_2O (50:50 v/v; 0.1 M KH_2PO_4)	519,642		0.04	0.002	118[c]
$Cu(dmp)_2(PF_6)_2$/TPrA	H_2O (0.1 M KH_2PO_4)	519,642		0.04	0.001	118[c]
Cu(II)−3,4(DAT)	H_2O (0.2 M KH_2PO_4)					369
	Europium					
Eu(dibenzoylmethide)$_3$·piperidine	MeCN (0.1 M $TBAClO_4$)	612				374[d]
Eu(dinaphthoylmethide)$_3$·piperidine	MeCN (0.1 M $TBAClO_4$)	610				374[d]
$Eu(TTFA)_3$(phen)	MeCN (0.05 M $TBABF_4$)	610				110[d]
$(pipH^+)Eu(DBM)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	611	611	0.099	0.052	124
$(TBA)Eu(DBM)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	611	611	0.20	0.06	124
$(TBA)Eu(TTA)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612	612	1.01	0.006	124
$(TBA)Eu(HFAC)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	611	611	0.095	0.01	124
$(TBA)Eu(BA)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612	612	0.13	0.02	124
$(TBA)Eu(BTA)_4/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	611	611	1.2	0.01	124
$Eu[2.2.2](NO_3)_3/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612		0.0004		124
$Eu[2.2.1](NO_3)_3/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612				124
$Eu[2.2.1](DBM)(NO_3)_2/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612	612	0.0002	2×10^{-4}	124
$Eu[2.2.1](BA)(NO_3)_2/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612	612	0.0013	5×10^{-5}	124
$Eu[2.2.1](TTA)(NO_3)_2/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	614		0.0011		124
$Eu[2.2.1](BTA)(NO_3)_2/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	612		0.046		124
$Eu[2.2.1](HFAC)(NO_3)_2/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	613		0.0014		124
	Gold					
Au(I)−(2,4-DAT)	H_2O (0.2 M KH_2PO_4)					369
	Iridium					
$Ir(ppy)_3$	MeCN (0.1 M $TBABF_4$)	495				110
$Ir(ppy)_3$	benzonitrile	530	530			112
$Ir(ppy)_3$	MeCN (0.1 M $TBAPF_6$)	510	510		0.14	113[b]
$Ir(ppy)_3$/TPrA	MeCN (0.1 M $TBAPF_6$)	517	517	0.14	0.33	114
$Ir(ppy)_3$/TPrA	MeCN/H_2O (50:50 v/v; 0.1 M KH_2PO_4)	507, 532	517	0.10	0.0044	114
$Ir(ppy)_3$/TPrA	H_2O (0.1 M KH_2PO_4)	507, 532	517	0.08	0.00092	114
$Ir(ppy)_3$/2-cyanofluorene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.67	134
$Ir(ppy)_3$/4-cyano-4′-methylbiphenyl	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.59	134
$Ir(ppy)_3$/4-cyanobiphenyl	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.62	134
$Ir(ppy)_3$/2-cyanonaphthalene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.29	134
$Ir(ppy)_3$/1-cyanonaphthalene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.36	134
$Ir(ppy)_3$/4-acetylbiphenyl	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		517		0.26	134
$Ir(ppy)_3$/2-acetylnaphthalene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.37	134
$Ir(ppy)_3$/9-cyanophenanthrene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.33	134
$Ir(ppy)_3$/1-acetylnaphthalene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.30	134
$Ir(ppy)_3$/benzophenone	scetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.25	134
$Ir(ppy)_3$/1,2-dicyanobenzene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.20	134
$Ir(ppy)_3$/4,4′-dicyanobiphenyl	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		0.23	134
$Ir(ppy)_3$/4-cyanobenzoic acid methyl ester	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		3.7×10^{-2}	134
$Ir(ppy)_3$/4-acetylbenzoic acid methyl ester	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		6.0×10^{-4}	134
$Ir(ppy)_3$/1,4-dicyanobenzene	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		2.2×10^{-3}	134
$Ir(ppy)_3$/4-acetylbenzonitrile	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		4.0×10^{-5}	134
$Ir(ppy)_3$/4-acetylacetophenone	acetonitrile/dioxane (1:1 v/v); (0.1 M $TBAPF_6$)		~517		1.7×10^{-5}	134
$[Ir(COD)(\mu\text{-pz})]_2$	THF (0.3 M $TBABF_4$)	682	694			375
$[Ir(COD)(\mu\text{-mpz})]_2$	THF (0.3 M $TBABF_4$)					375
$[Ir(COD)(\mu\text{-dmpz})]_2$	THF (0.3 M $TBABF_4$)	714	719			375
$[Ir(COD)(\mu\text{-pz})]_2/C_2O_4^{2-}$	THF (0.3 M $TBABF_4$)	682	694			375
$[Ir(COD)(\mu\text{-dmpz})]_2/C_2O_4^{2-}$	THF (0.3 M $TBABF_4$)	714	719			375

Table 1 (Continued)

complex/reactant	solvent	λ_{em} (nm)	λ_{ecl} (nm)	ϕ_{em}	ϕ_{ecl}	ref
	Mercury					
Hg-DALM/TPrA	H_2O (0.15 M NaH_2PO_4)					376, 377
	Molybdenum					
$Mo_6Cl_{14}^{2-}$	MeCN (0.1 M TBAP)	700	700	0.19		378
$Mo_6Cl_{14}^{-}/D^{-}$ (cross-R)	CH_2Cl_2 (0.1 M TBAP)	~700	~700			379, 380, 381, 382[e]
$Mo_6Cl_{14}^{3-}/A^{+}$ (cross-R)	CH_2Cl_2 (0.1 M TBAP)	~700	~700			379, 380, 381, 382[f]
$Mo_2Cl_4(PMe_3)_4$	THF (0.1 M $TBABF_4$)	680	680	0.13	0.002	383
$Mo_2Cl_4(PMe_3)_4/S_2O_8^{2-}$	THF (0.1 M $TBABF_4$)	680	680	0.13		383
$Mo_2Cl_4(PMe_3)_4/C_2O_4^{-}$	THF (0.1 M $TBABF_4$)	680		0.13		383
	Osmium					
$Os(bpy)_3^{2+}$	MeCN	724	720	0.00462		102
$Os(bpy)_2(diphos)^{2+}$	MeCN	612	610	0.0550	~0.7	102
$Os(bpy)_2(dppene)^{2+}$	MeCN	605	606	0.0699		102
$Os(bpy)_2(DMSO)_2^{2+}$	MeCN	575				102
$Os(bpy)_2dpae^{2+}$	MeCN	632	633			102
$Os(bpz)_3^{2+}$	MeCN	700	700			104
$Os(phen)_3^{2+}$	DMF	690	691	0.0159		102
$Os(phen)_3^{2+}/S_2O_8^{2-}$	MeCN		740	0.32	0.40	60
$Os(phen)_2(diphos)^{2+}$	MeCN	601	600	0.138		102
$Os(phen)_2(dppene)^{2+}$	MeCN	597	599	0.239		102
$Os(phen)_2dpae^{2+}$	MeCN	622		0.121		102
$Os(bpy)_2(dppene)^{2+}$/ TPrA	CH_3CN/H_2O (50:50 v/v)	368	585	0.088	0.95	103
$Os(bpy)_2(dppene)^{2+}$/TPrA	H_2O (0.2 M KH_2PO_4)	368	589	0.157	2.0	103[c]
[Os(4,4′-distyryl-2,2′-bipyridine)$_2$(bis-1,2-phenylphoninoethane)]$^{2+}$ polymer	CH_3CN ($TBAClO_4$)	635	635			107
	Palladium					
Pd(TPP)	CH_2Cl_2 (0.1 M $TBAPF_6$)	700	700			384
	Platinum					
Pt(TPP)	CH_2Cl_2 (0.1 M $TBAPF_6$)	654	656			384
$Pt_2(P_2H_2O_5)_4^{4-}$	MeCN (0.1 M $TBABF_4$)	517	517			385
$Pt_2(P_2H_2O_5)_4^{4-}/Bu_4N^{+}$	MeCN (0.1 M $TBABF_4$)	512	512			386
$Pt(thpy)_2$	DMF (0.1 M $TBAPF_6$)	580	580	1.2	0.005	387
$Pt(thpy)_2/S_2O_8^{2-}$	DMF (0.1 M $TBAPF_6$)	580				387
$Pt(q)_2$	MeCN (0.005 M $TBABF_4$)	650	>530	0.01		110
$Pt_2(dba)_3$	CH_2Cl_2 (0.1 M $TBAPF_6$)	782	~780	0.95		388[g]
$Pt_3(tbaa)_3$	CH_2Cl_2 (0.1 M $TBAPF_6$)	782	~780	1.05		388[g]
$Pt_2(dba)_3$	CH_2Cl_2 (0.1 M $TBAPF_6$)	726		0.29		388[g]
$Pt_3(tbaa)_3$	CH_2Cl_2 (0.1 M $TBAPF_6$)	724		0.28		388[g]
Pt(OEP)	CH_2Cl_2 (0.1 M $TBAPF_6$)	619	619		0.02	113
	Rhenium					
$Re(CO)_3Cl(phen)$	MeCN (0.1 M $TBAClO_4$)	598	598			389
$Re(CO)_3Cl(4,7\text{-diphenylphen})$	MeCN (0.1 M $TBAClO_4$)	610	610			389
$Re(CO)_3Cl(phen)$/THPO (cross-R)	MeCN (0.1 M $TBABF_4$)	610	610			110, 390
$Re(CO)_3Cl(phen)$	MeCN (0.1 M $TBAPF_6$)	601	601	0.0026	6.4×10^{-4}	61
$Re(CO)_3Cl(2,9\text{-}Me_2phen)$	MeCN (0.1 M $TBAPF_6$)	595	595	0.012	5.1×10^{-4}	61
$Re(CO)_3Cl(5,6\text{-}Me_2phen)$	MeCN (0.1 M $TBAPF_6$)	599	599	0.022	4.2×10^{-4}	61
$Re(CO)_3Cl(4,7\text{-}Me_2phen)$	MeCN (0.1 M $TBAPF_6$)	588	588	0.044	0.0011	61
$Re(CO)_3Cl(3,4,7,8\text{-}Me_4phen)$	MeCN (0.1 M $TBAPF_6$)	599	599	0.0044	3.1×10^{-5}	61
$Re(CO)_3Cl(bpy)$	MeCN (0.1 M $TBAPF_6$)	609	609	0.023	2.9×10^{-4}	61
$Re(CO)_3Cl(4,4'\text{-}Me_2bpy)$	MeCN (0.1 M $TBAPF_6$)	599	599	0.017	6.9×10^{-4}	61
$Re(CO)_3Cl(phen)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	601	601	0.0026	0.0011	61[c]
$Re(CO)_3Cl(2,9\text{-}Me_2phen)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	595	595	0.012	0.0012	61[c]
$Re(CO)_3Cl(5,6\text{-}Me_2phen)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	599	599	0.022	0.0054	61[c]
$Re(CO)_3Cl(4,7\text{-}Me_2phen)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	588	588	0.044	0.030	61[c]
$Re(CO)_3Cl(3,4,7,8\text{-}Me_4phen)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	599	599	0.0044	0.0049	61[c]
$Re(CO)_3Cl(bpy)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	609	609	0.023	0.0031	61[c]
$Re(CO)_3Cl(4,4'\text{-}Me_2bpy)/S_2O_8^{2-}$	MeCN (0.1 M $TBAPF_6$)	599	599	0.017	0.0043	61[c]
$Re(CO)_3Cl(phen)$/TPrA	MeCN (0.1 M $TBAPF_6$)	601	601	0.0026	0.087	61[c]
$Re(CO)_3Cl(2,9\text{-}Me_2phen)$/TPrA	MeCN (0.1 M $TBAPF_6$)	595	595	0.012	0.12	61[c]
$Re(CO)_3Cl(5,6\text{-}Me_2phen)$/TPrA	MeCN (0.1 M $TBAPF_6$)	599	599	0.022	0.12	61[c]
$Re(CO)_3Cl(4,7\text{-}Me_2phen)$/TPrA	MeCN (0.1 M $TBAPF_6$)	588	588	0.044	0.22	61[c]
$Re(CO)_3Cl(3,4,7,8\text{-}Me_4phen)$/TPrA	MeCN (0.1 M $TBAPF_6$)	599	599	0.0044	0.0027	61[c]
$Re(CO)_3Cl(bpy)$/TPrA	MeCN (0.1 M $TBAPF_6$)	609	609	0.023	0.52	61[c]
$Re(CO)_3Cl(4,4'\text{-}Me_2bpy)$/TPrA	MeCN (0.1 M $TBAPF_6$)	599	599	0.017	0.40	61[c]
	Ruthenium					
$Ru(bpy)_3^{2+}$	MeCN	608	608	0.062	0.05	18, 29−30
$Ru(bpy)_3^{2+}/C_2O_4^{2-}$	H_2O (0.1 M H_2SO_4)	610	610		0.02	53
$Ru(bpy)_3^{2+}/C_2O_4^{2-}$	H_2O (0.1 M NaCl + 0.1 M KH_2PO_4)		591	0.062	0.0011	97
$Ru(bpy)_3^{2+}/S_2O_8^{2-}$	$MeCN/H_2O$ (50:50 v/v)	625	625		0.025	53, 59

Table 1. (Continued)

complex/reactant	solvent	λ_{em} (nm)	λ_{ecl} (nm)	ϕ_{em}	ϕ_{ecl}	ref
		Ruthenium (Continued)				
$Ru(bpy)_3^{2+}$/TPrA	H_2O (0.2 M KH_2PO_4)	610	610		1.0	1[h]
$Ru(dmbp)_3^{2+}/C_2O_4^{2-}$	H_2O (0.1 M NaCl + 0.1 M KH_2PO_4)		594	0.045	5×10^{-3}	97
$Ru(phen)_3^{2+}$	MeCN	590	590			391
$Ru(phen)_3^{2+}/C_2O_4^{2-}$	H_2O (0.1 M NaCl + 0.1 M KH_2PO_4)		585	0.065	0.0015	97
$Ru(terpy)_3^{2+}$	MeCN		660			391
$Ru(TPTZ)_3^{2+}$	MeCN					391
$Ru(bpz)_3^{2+}$	MeCN	585	585	~0.03	~0.04	90, 91
$Ru(bpz)_3^{2+}/S_2O_8^{2-}$	H_2O (0.1 M Na_2SO_4)	585	590			92
$Ru(dp\text{-}bpy)_3^{2+}$	MeCN	635	635	0.26	0.14	108
$Ru(dp\text{-}phen)_3^{2+}$	MeCN	615	615	0.31	0.24	108
$Ru(dp\text{-}bpy)_3^{2+}$/TPrA	H_2O (0.2 M phosphate buffer)					108
$Ru(dp\text{-}phen)_3^{2+}$/TPrA	H_2O (0.2 M phosphate buffer)					108
$Ru(dmphen)_3^{2+}/C_2O_4^{2-}$	H_2O (0.1 M NaCl + 0.1 M KH_2PO_4)		591			97
$(bpy)_2Ru(bphb)^{2+}$	MeCN	624	624	0.08	0.0066	62
$(bpy)_2Ru(bphb)^{2+}$/TPrA	MeCN (0.1 M $TBAPF_6$)	624	624	0.08	1.5	62
	MeCN/H_2O (50:50 v/v; 0.1 M $TBAPF_6$)				1.6	62
	H_2O (0.2 M KH_2PO_4)				0.058	62
$(bpy)_2Ru(bphb)^{2+}/S_2O_8^{2-}$	MeCN	624	624	0.08	0.4	62[c]
	MeCN/H_2O (50:50 v/v)				0.7	62[c]
$[(bpy)_2Ru]_2(bphb)^{4+}$	MeCN	624	624	0.11	0.16	62
$[(bpy)_2Ru]_2(bphb)^{4+}$/ TPrA	MeCN	624	624	0.11	2.6	62[c]
	MeCN/H_2O (50:50 v/v)				2.8	62[c]
	H_2O (0.2 M KH_2PO_4)				2.0	62[c]
$[(bpy)_2Ru]_2(bphb)^{4+}/S_2O_8^{2-}$	MeCN	624	624	0.11	0.6	62[c]
	MeCN/H_2O (50:50 v/v)				0.8	62[c]
$(bpy)_2Ru(AZA\text{-}bpy)^{2+}$/TPrA	MeCN/H_2O (0.1 M KH_2PO_4)	603	603	0.062	0.84	96
$(bpy)_2Ru(AZA\text{-}bpy)^{2+}$/TPrA	H_2O (0.2 M KH_2PO_4)	613	613	0.062	0.51	96
$(bpy)_2Ru(CE\text{-}bpy)^{2+}$/TPrA	H_2O (0.1 M Tris)		650		1.0	95[i]
$(bpy)_2Ru(CE\text{-}bpy)^{2+}$/TPrA	MeCN (0.1 M $TBAClO_4$)		655		~0.5	95[j]
$(bpy)_2Ru(bpy\text{-}C_{19})^{2+}/C_2O_4^{2-}$	H_2O (0.5 M Na_2SO_4)	600	680			392
$(bpy)_2Ru(bpy\text{-}O{-}C_8)^{2+}$	MeCN (0.1 M $TBAPF_6$)	618	618	0.75	1.0	98[k]
Den-8-Ru	MeCN (0.1 M $TBAPF_6$)	618	618	0.75	~5	98[l]
$Ru(v\text{-}bpy)_3^{2+}$	MeCN (0.1 M $TBAClO_4$)	~630	~650			393
$(bpy)_2Ru(DC\text{-}bpy)^{2+}$	H_2O (0.1 M KH_2PO_4)	629	629	0.030	0.73	101
	MeCN/H_2O (50:50 v/v)	624	624	0.025	0.60	101
$(bpy)_2Ru(DM\text{-}bpy)^{2+}$	H_2O (0.1 M KH_2PO_4)	605	605	0.020	0.84	101
	MeCN/H_2O (50:50 v/v)	606	606	0.024	0.95	101
		Silicon				
[l]$SiPc(OR)_2$	CH_2Cl_2 (0.1 M $TBAPF_6$)	~684	725			394
[l]$SiNc(OR)_2$	CH_2Cl_2 (0.1 M $TBAPF_6$)	~792	828			394
[l]$RO(SiPcO)_2R$	CH_2Cl_2 (0.1 M $TBAPF_6$)					394
Si−NC	DMF + MeCN (0.1 M $THAClO_4$)	420	640			127
CdSe NC	CH_2Cl_2 (0.1 M $TBAClO_4$)	545	~745			128
		Silver				
Ag^+-DALM	H_2O (0.15 M KH_2PO_4)					377
Ag(I)-HDEA	H_2O (KOH−KCl, pH 12.8)					395
		Terbium				
$Tb(TTFA)_3(phen)$	MeCN (0.05 M $TBABF_4$)	565				110[d]
$Tb(TTFA)_4^-$	MeCN (0.05 M $TBABF_4$)	565				110[d]
		Thallium				
Tl-tunichrome/TPrA	H_2O (0.15 M KH_2PO_4)					396
		Tungsten				
$W_6X_8Y_6^{2-}$ (X, Y = Cl, Br, I; X = Cl, Y−Br; X−I, Y = Br)	MeCN (0.1 M TBAP)					382

[a] Ligand abbreviations are listed at the end of the article in section 7. Coreactants are listed after the backslash (e.g., $Ru(bpy)_3^{2+}$/TPrA, where TPrA is the coreactant). If no coreactant is listed, then ECL was generated via annihilation. In certain instances annihilation ECL was generated using cross-reactions between two different species (e.g., eqs 17−20). In those cases the cross-reactant is listed after the backslash and the reaction identified with (cross-R). The spectroscopic and ECL data listed were either reported directly in the cited paper or extrapolated from data. In those instances where no ECL spectra and/or efficiencies were reported, only the compound, conditions, and reference are listed. Photoluminescence efficiencies (ϕ_{em}) and ECL efficiencies (ϕ_{em}) are relative to $Ru(bpy)_3^{2+}$ at 0.062 and 0.05, respectively, unless otherwise noted. Reference notes: [a]Volts vs Fc/Fc^+ (0.631 V vs NHE); [b]relative to diphenylanthracene (ϕ_{ecl} = 6.3%); [c]relative ECL efficiency with respect to $Ru(bpy)_3^{2+}$/Coreactant (ϕ_{ecl} = 1); [d]weak ECL such that no spectrum was obtained; [e]ECL generated via cross-reaction between $Mo_6Cl_{14}^-$ and electroactive donor species (D^-; nitroaromatic radical anions); [f]ECL generated via cross-reaction between $Mo_6Cl_{14}^{3-}$ and electroactive acceptor species (A^+; aromatic amine radical cations); [g]relative to $Cr(bpy)_3(ClO_4)_2$; [h]assigned a value of 1.0 for comparison purposes; [i]set to 1.0 for comparison to CE-bpy in MeCN, relative ϕ_{ecl} vs $Ru(bpy)_3^{2+}$/TPrA not reported; [j]relative to CE-bpy in H_2O (ϕ_{ecl} = 1); [k]set to 1.0 for comparison with Den-8-Ru; [l]relative to $(bpy)_2Ru(bpy\text{-}O{-}C_8)^{2+}$ at 1.0; [l][SiPc(OR)$_2$] = bis(tri-*n*-hexylsiloxy)(2,3-phthalocyaninato-)silicon; $RO(SiPcO)_2R$ = dimer of [$SiPc(OR)_2$]; [$SiNc(OR)_2$] = bis(tri-*n*-hexylsiloxy)(2,3-naphthalocyaninato)silicon.

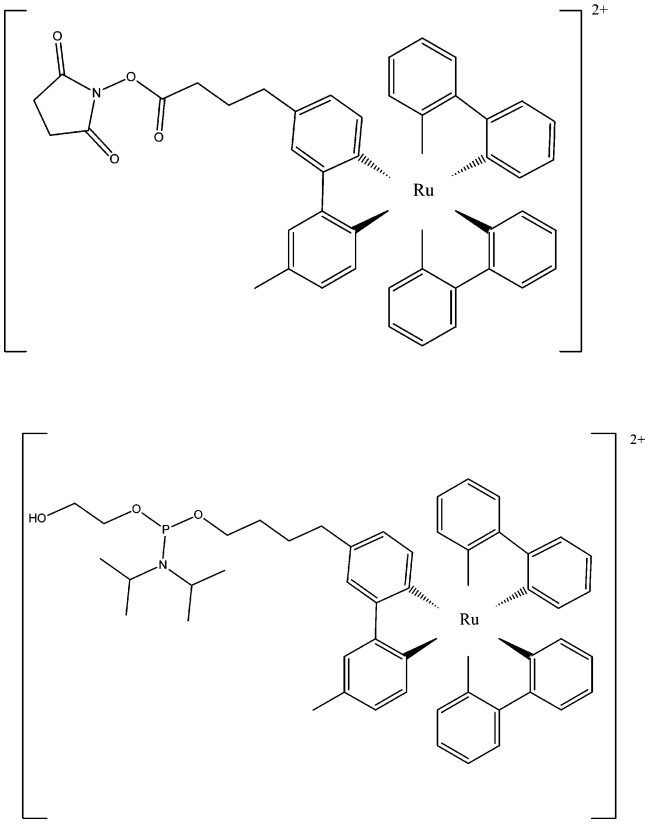

Figure 8. (Top) $Ru(bpy)_3^{2+}$−NHS ester for ECL labeling of biological molecules and (bottom) $Ru(bpy)_3^{2+}$−phosphoramadite linker for ECL labeling of DNA and RNA.

gous to that of radioactive or fluorescent labels.[2] This combination has resulted in a wide range of analytical applications for ECL in clinical diagnostic assays in which $Ru(bpy)_3^{2+}$/TPrA plays a key role (see section 4). Added to this is the study of $Ru(bpy)_3^{2+}$ for use in liquid chromatography detection,[68] the measurement of biologically and pharmacologically important compounds (such as alkylamines, antibiotics, antihistamines, and opiates),[2,69] and environmental applications,[70,71] to name a few.

Another area of active research using $Ru(bpy)_3^{2+}$ has been the "surfactant effect" on ECL. Solubilization of $Ru(bpy)_3^{2+}$ in aqueous nonionic surfactant solutions leads to significant, and potentially useful, changes in the electrochemiluminescence properties.[34,72] For example, up to 8-fold increases in ECL efficiency were observed in surfactant media upon oxidation of $Ru(bpy)_3^{2+}$ and TPrA.[72] The mechanism of the surfactant effect appears to involve adsorption of surfactant on the electrode surface. In the case of Triton X-100 [polyoxyethylene(10) isooctylphenyl ether], adsorption of surfactant on Pt and Au electrodes renders the surface more hydrophobic, facilitating coreactant oxidation and leading to increased ECL intensities in the $Ru(bpy)_3^{2+}$/TPrA system.[73] A study of the effects of nonionic chain lengths on $Ru(bpy)_3^{2+}$/TPrA ECL[74] and enhanced ECL of $Ir(ppy)_3$/TPrA[75] and $Os(phen)_2(dppene)^{2+}$/TPrA[76] (see below) in the presence of Triton X-100 confirm these results. Although the effects of micelles and discrete complexation of the surfactants with ECL luminophore and TPrA cannot be ruled out, these studies indicate that increases in ECL intensity are probably due to changes in electrode hydrophobicity upon formation of a surfactant adsorption layer and less likely due to micelle interactions.[73,74] The precise mechanism of the surfactant effect is still under study, but the dramatic increases in ECL intensity, coupled with work on more efficient ECL labels and coreactants, may have profound impacts on the sensitivity of ECL for a variety of applications.

Ultrasonic irradiation on $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ aqueous coreactant and $Ru(bpy)_3^{2+}$ nonaqueous (acetonitrile) annihilation ECL results in highly stable and reproducible ECL signals, increases in $\phi_{ecl} \geq 100\%$, and less electrode fouling.[77,78] The dramatic increases appear to be due to agitation of the system, leading to greater mass transport across the electrode double layer and less dependence on diffusion to get material to the electrode surface. Also, it is speculated that the degassing effects of sonication reduce the aggregation of gas bubbles at the electrode surface and prevent the formation of passivating films. The mechanisms of ECL under sonification are the same as under conventional conditions, but the high reproducibility of the signal has allowed the measurement of $Ru(bpy)_3^{2+}$ ECL quenching via oxygen to be measured with greater precision than previously possible.[77]

Numerous studies of electrode surface effects (e.g., surface oxidation, adsorption, electrode hydrophobicity) on electrochemistry have been reported, and this was recently extended to $Ru(bpy)_3^{2+}$/TPrA ECL.[57] Hydrophobicity was controlled by the modification of gold and platinum electrodes with thiol monolayers that contained different terminal groups. Significant increases in TPrA oxidation rate and ECL intensity were observed at alkanethiol-modified electrodes, whereas the influence of the thiol layer with a hydrophobic terminal group on both TPrA oxidation and ECL was much less.

A different approach for DNA detection has also been reported.[79,80] Single-stranded DNA was immobilized directly on aluminum(III) alkanebiphosphate modified electrodes with $Ru(bpy)_3^{2+}$/TPrA detection. In another report, $Ru(bpy)_3^{2+}$-derivatized antibodies or antigens interacted with biomolecules that were immobilized on screen-printed gold electrodes.[81] The biomolecules were incorporated into self-assembled monolayers of thiol or F_c-specific binding protein G.

$Ru(bpy)_3^{2+}$ labels coupled with TPrA have also been used for the detection of DNA derived from *Bacillus anthracis*.[82] In these experiments Au(111) electrodes were coated with a self-assembled thiol monolayer of 3-mercaptopropanoic acid (3-MPA). Then, a synthetic 23-mer single-stranded DNA (ssDNA) was covalently attached to the monolayer via an amino group on the 5′-terminal end followed by hybridization with a target ssDNA tagged with the $Ru(bpy)_3^{2+}$ ECL labels. The general principles of ECL detection using this methodology are outlined in Figure 9. ssDNA (Figure 9a) is immobilized on the surface of the electrode (or substrate), and then the complementary target strand of ssDNA tagged with the ECL label hybridizes with the immobilized ssDNA strand (Figure 9a). The electrode assembly is placed in a

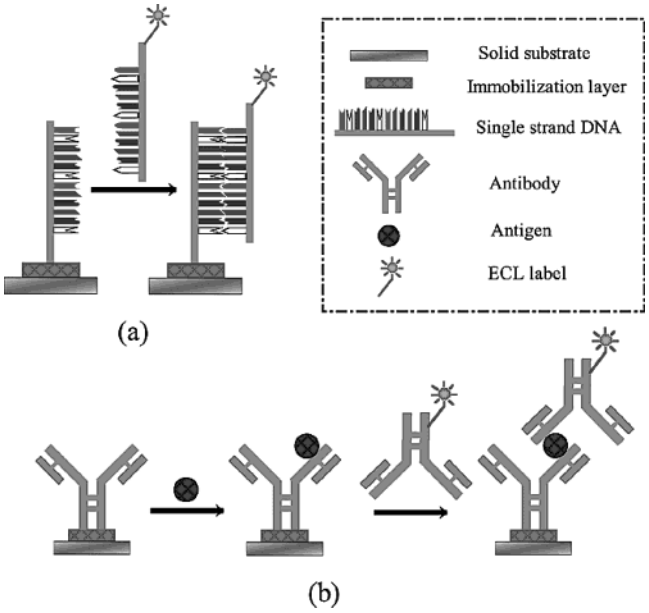

Figure 9. Schematic diagram of solid state ECL detection of (a) DNA hybridization and (b) sandwich type immunoassay. (Reprinted with permission from ref 82. Copyright 2003 American Chemical Society.)

solution or flow cell containing a coreactant solution, followed by application of a potential pulse or sweep and the measurement of ECL.

Direct ECL involving DNA has been demonstrated using guanine residues as the coreactant.[83] In these studies, ultrathin films (~10 nm) of the catalytic polymer $[Ru(bpy)_2PVP_{10}](ClO_4)_2$ [PVP = poly(vinylpyridine)] were assembled layer by layer with DNA. A square wave voltammetric waveform oxidized the Ru^{2+} sites to Ru^{3+}, and ECL was measured. Interestingly, significant ECL generation occurred only when guanine bases were present in the oligonucleotide films. Proposed mechanisms for light emission involve the interaction of guanine radicals with Ru^{3+} to generate the $Ru^{\bullet 2+}$ excited state and the reduction of Ru^{2+} to Ru^{+} by the guanine radicals, followed by annihilation of Ru^{3+} and Ru^{+}.[83] Guanine radicals can be formed upon one-electron oxidation, either via electrode processes or by reaction with Ru^{3+}.[84]

The determination of C-reactive protein (CRP) concentrations in human plasma and serum using $Ru(bpy)_3^{2+}$ as the ECL label and TPrA as the coreactant has also been demonstrated.[82] CRP is an "acute phase protein" found in human serum and may play a role in predicting the onset of coronary events (e.g., angina).[85] In this study, biotinylated anti-CRP species were immobilized onto a Au(111) substrate that had been precoated with a layer of avidin covalently linked to a thiol monolayer composed of 3-MPA and 16-mercaptohexadenoic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and *N*-hydroxysuccinimide. CRP and anti-CRP tagged with $Ru(bpy)_3^{2+}$ labels were then conjugated to the surface layer, followed by immersion of the modified electrode in a TPrA solution for ECL generation. A schematic diagram of this assay is shown in Figure 9b.

A recent study reported the ECL of $Ru(bpy)_3^{2+}$ as a function of solution viscosity in *N*,*N*-dimethylformamide and glycerin mixtures.[86] Annihilation of the +1 and +3 forms (eqs 1−4) was used to generate ECL. Interestingly, the ECL intensity decreases with increasing solution viscosity and tracks diffusion and electron transfer until it reaches an upper limit. However, when the ECL intensity is normalized for diffusion weak enhancement with increasing solution viscosity is observed. Such studies, while providing valuable information on the basic ECL mechanism, might also provide insight into matrix effects.

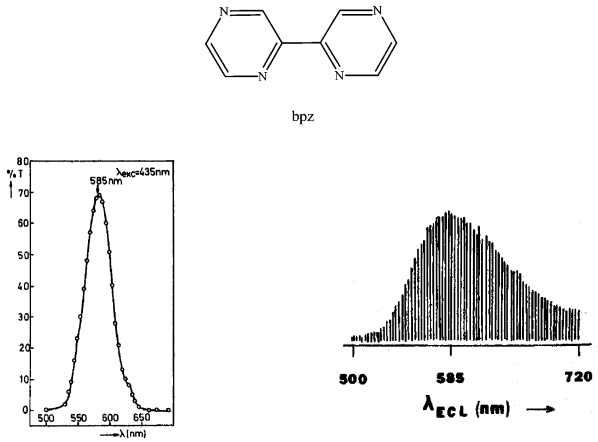

Figure 10. Structure of 2,2′-bipyrazine (bpz); luminescence spectrum of a 10^{-5} M solution of $Ru(bpz)_3^{2+}$ in MeCN at room temperature (left) and ECL spectrum of a 1 mM $Ru(bpz)(PF_6)_2$/MeCN/0.1 M $TBAPF_6$ solution (pulsing limits of −0.8 to +1.85 vs Ag-wire reference electrode at +0.5 Hz). (Reprinted with permission from ref 90. Copyright 1983 American Chemical Society.)

ECL from gel-entrapped $Ru(bpy)_3^{2+}$,[87] in sol−gel derived glasses[88] and in Nafion-silica composite films[89] using coreactants, has also been observed with the potential for using both coreacant and annihilation ECL in display device technology.

Obviously the number of studies on, and commercial applications of, $Ru(bpy)_3^{2+}$ ECL has eclipsed those of other inorganic complexes and clusters, including other ruthenium chelates. However, a number of papers exist on both mono- and multimetallic ruthenium systems. One ruthenium chelate that has attracted attention is $Ru(bpz)_3^{2+}$ (bpz = 2,2′-bipyrazine) and is shown in Figure 10). Of particular interest in $Ru(bpz)_3^{2+}$ is that the oxidation and reduction potentials occur at ~0.5 V more positive than $Ru(bpy)_3^{2+}$, and this might facilitate electrochemical, photochemical, and ECL studies in aqueous solutions. The first report on $Ru(bpz)_3^{2+}$ noted ECL characteristic of $Ru(bpz)_3^{\bullet 2+}$ in acetonitrile upon formation of the +3 and +1 states (Figure 10).[90] It was also reported that the shift in the +3 state compared to $Ru(bpy)_3^{2+}$ resulted in greater stabilization of the +2 versus +3 species. The temperature dependence of ϕ_{ecl} in MeCN has also been reported,[91] with the aim of further understanding the mechanism of $Ru(bpz)_3^{2+}$ annihilation ECL. A bright orange luminescence characteristic of $Ru(bpz)_3^{\bullet 2+}$ ECL was observed in aqueous solution using $S_2O_8^{2-}$ as a reductive−oxidative coreactant.[92] $Ru(bpz)_3^{2+}/S_2O_8^{2-}$ methodology has also been used to determine persulfate with nanomolar (nM) detection limits.[93]

Ru(II) diimine complexes having phosphonic acid substituents have been shown to adsorb (via the

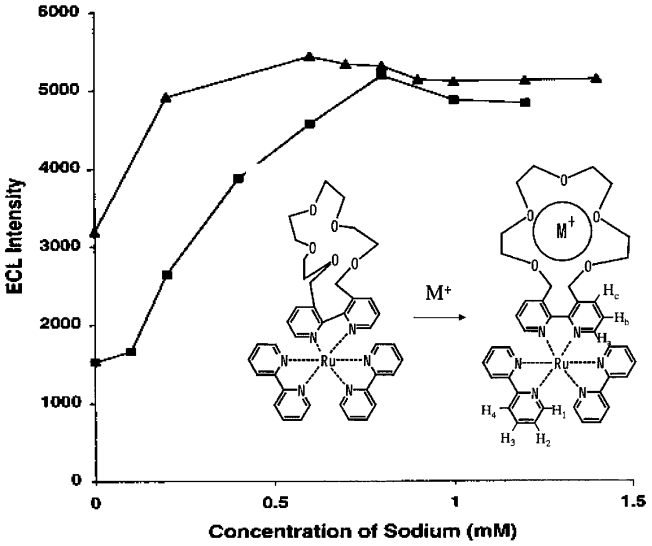

Figure 11. ECL signal intensity as a function of sodium concentration for solutions containing 0.3 $Ru(bpy)_2(CE\text{-}bpy)^{2+}$ and 30 mM TPrA in 0.1 M $TBAClO_4$, MeCN (■) and in 0.1 M, pH 7.0, Tris buffer (▲). (Reprinted with permission from ref 95. Copyright 2002 American Chemical Society.)

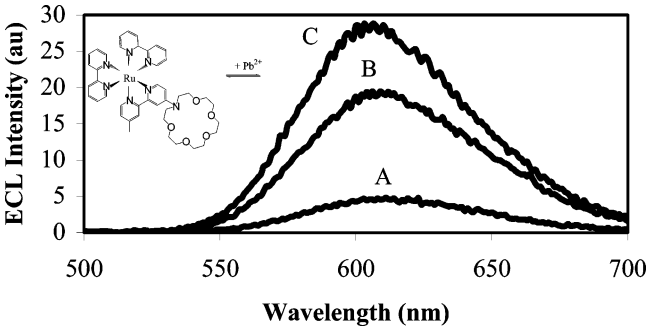

Figure 12. Perturbation of ECL emissioni spectrum of $(bpy)_2Ru(AZA\text{-}bpy)(PF_6)_2$ (0.1 mM) in 50:50 (v/v) CH_3CN/H_2O upon addition of Pb^{2+}: (A) 0 mM Pb^{2+}; (B) 0.5 mM Pb^{2+} (5-fold excess); (C) 1 mM Pb^{2+} (10-fold excess). (Reprinted with permission from ref 96a. Copyright 2002 American Chemical Society.)

phosphonic acid substituents) to TiO_2-modified indium−tin oxide (ITO) electrodes and undergo coreactant ECL with $C_2O_4^{2-}$.[94] Oxalate ion was used as the coreactant in these systems. It was shown that optically transparent electrodes containing TiO_2 can serve as solid supports for the generation of ECL from adsorbed ruthenium chelates (and potentially other ECL luminophores) and that the stability of the modified surfaces for sustained ECL depends on the number of substituents per chromophore.

With the goal of sensing clinically and environmentally important metal ions not directly involved in redox reactions, the ECL of monometallic ruthenium complexes has also been extended to systems containing a crown ether moiety covalently bonded to a bipyridyl ligand. For example, the ECL of $Ru(bpy)_2(CE\text{-}bpy)^{2+}$ [CE-bpy is a bipyridine ligand in which a crown ether (15-crown-5) is bound to the bpy ligand in the 3- and 3′-positions][95] and $(bpy)_2Ru(AZA\text{-}bpy)^{2+}$ [bpy = 2,2′-bipyridine; AZA-bpy = 4-(*N*-aza-18-crown-6-methyl −2,2′-bipyridine)][96] has been reported. $Ru(bpy)_2(CE\text{-}bpy)^{2+}$ is sensitive to sodium ions

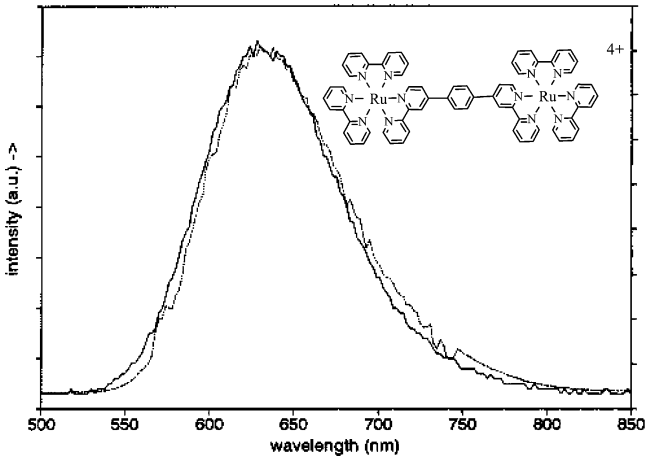

Figure 13. ECL emission spectra of 1 mM $[(bpy)_2Ru]_2(bphb)(PF_6)_4$ generated via annihilation (- - -) and in the presence of TPrA (—). The annihilation spectrum (- - -) is offset 5 nm to the red for clarity. (Reprinted with permission from ref 62. Copyright 1998 American Chemical Society.)

in aqueous buffered solution (Figure 11), whereas $(bpy)_2Ru(AZA\text{-}bpy)^{2+}$ has been shown to be sensitive to Pb^{2+}, Hg^{2+}, Cu^{2+}, Ag^+, and K^+ in 50:50 (v/v) CH_3CN/H_2O (0.1 M KH_2PO_4 as electrolyte) and aqueous (0.1 M KH_2PO_4) solution (Figure 12).

Structure−activity relationships may prove to be useful in both designing new ECL systems and improving the performance of existing systems. Therefore, in an effort to understand the structure−activity relationships for ECL in coreactant systems, a series of ruthenium bipyridine complexes were used to probe the homogeneous oxidation of oxalate and the effect of electrochemical steps on the ECL emission intensity.[97] ECL was generated by oxidation of $Ru(bpy)_3^{2+}$, $Ru(phen)_3^{2+}$, $Ru(bpy)_2(dmbp)^{2+}$, $Ru(dmphen)_3^{2+}$, or $Ru(dmbp)_3^{2+}$ (dmbp = 4,4′-dimethyl-2,2′-bipyridine; dmphen = 4,7-dimethyl-1,10-phenanthroline) and oxalate in aqueous solution. The luminescent emission was related to the driving force for the electron-transfer reactions and the different pathways for coreactant (i.e., $CO_2^{\bullet-}$) reaction.

ECL has also been extended to multimetallic ruthenium systems. Solution and solid-phase coreactant ECL is quite sensitive, with detection limits as low as 10^{-18} M possible.[2] However, there are systems requiring an even greater sensitivity, such as in environmental (where preconcentration of samples is often necessary) and clinical/molecular diagnostics applications, when the detection of as few as 10 molecules would eliminate the need for sample amplification (e.g., via the Polymerase Chain Reaction). With the goal of increasing ECL sensitivity and lowering detection limits, the ECL of the bimetallic ruthenium system $[(bpy)_2Ru]_2(bphb)^{4+}$ [bphb = 1,4-bis(4′-methyl-2,2′-bipyridin-4-yl)benzene] was studied (Figure 13).[62] bphb is capable of binding two independent metal centers through a "bridging ligand" framework. This bimetallic species produced 2−3-fold more intense emission than $Ru(bpy)_3^{2+}$ in aqueous and nonaqueous solutions using annihilation and coreactant methods (Table 1). It was apparent from this study that there must be small electronic cou-

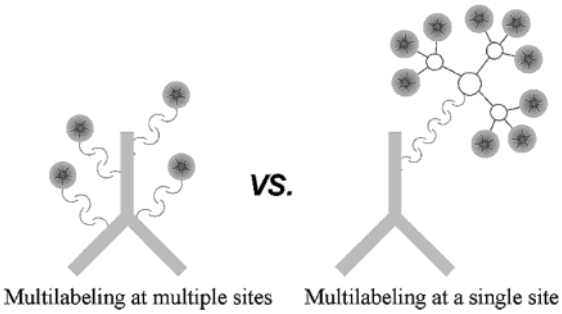

Figure 14. Multilabeling of a biomolecule at a single site with dendritic label bearing multiple signal-generating units. (Reprinted with permission from ref 98. Copyright 2003 American Chemical Society.)

pling between metal centers via the bridging ligand for enhanced ECL to be possible.

This work was extended to dendrimeric systems containing eight $Ru(bpy)_3^{2+}$ units at the periphery,[98] $(bpy)_2Ru(bpy\text{-}O\text{-}8)^{2+}$ in Table 1, of a carbosilane dendrimer platform. The ECL of the $Ru(bpy)_3^{2+}$ dendrimer is 5 times that of a reference monometallic species (Den-8 in Table 1). As with the bimetallic study, spectroscopic and electrochemical studies show that the $Ru(bpy)_3^{2+}$ units do not interact in either the ground or excited state and that PL and ECL emission can be amplified with multimetallic species.

With the goal of multilabeling biomolecules at a single site, a dendritic molecule containing three [$Ru(bpy)_3^{2+}$] units linked by a succinimidyl group was synthesized.[99] In many applications multiple labels are used to increase the signal of the target analyte and the binding of these labels occurs at numerous sites (Figure 14). However, such multilabeling can result in the loss of biological activity of the molecules and the precipitation of target analytes (such as proteins). From photophysical, electrochemical, and ECL studies it was clear that there was little electronic coupling among the [$Ru(bpy)_3^{2+}$] units in the dendrimer and that enhanced ECL and PL were observed compared to a single $Ru(bpy)_3^{2+}$ molecule.[99] Labeling of the protein bovine serum albumin (BSA) by the dendrimer at one NH_2 position was demonstrated with no subsequent loss of biological activity or precipitation of the BSA−dendrimer complex. These use of multilabels at a single site shows much promise and may find use in affinity-based bioanalytical assays in which lower detection limits and increased signal-to-noise ratios (S/N) are required.

ECL in multimetallic assemblies has also been extended to homometallic complexes containing two and three [$Ru(bpy)_3^{2+}$] units linked by the amino acid lysine (Lys) and the dipeptide LysLys, $[Ru2\text{-}Lys]^{4+}$ and $[Ru3\text{-}LysLys]^{6+}$, respectively.[100] As in the multimetallic systems discussed above, little electronic communication between the ruthenium−bipyridyl units was observed in the electrochemical and photophysical data. ECL was measured in phosphate buffer solutions containing nonionic surfactant and excess TPrA, and an increase of 30% was achieved for equimolar solutions of $[Ru2\text{-}Lys]^{4+}$ and $[Ru3\text{-}LysLys]^{6+}$ with respect to the mononuclear reference compound[4-carboxypropyl-4′-methyl-2,2′-bipyridine]-bis(2,2′-bipyridine)ruthenium(II)]. The lower than expected enhancement of the bi- and trimetallic systems was attributed to slow diffusion of $[Ru2\text{-}Lys]^{4+}$ and $[Ru3\text{-}LysLys]^{6+}$ to the working electrode surface. Interestingly, the presence of surfactant does not affect the spectroscopic properties of these two complexes but does significantly increase the ECL signal. This phenomemon has been observed in other monometallic systems[72−76,101] and was discussed above for $Ru(bpy)_3^{2+}$.

ECL experiments have also been performed on dendritic complexes containing two, four, and eight ruthenium units in both homogeneous and heterogeneous assay formats.[100] Using nanoparticle technology (i.e., attaching the dendrimeric units to a paramagnetic bead that can then be magnetically attracted to the electrode), the ECL in these systems has been shown to increase linearly with the number of active ruthenium centers, thus overcoming the problems associated with the slow diffusion of multimetallic assemblies to the electrode surface. However, intense background signals due to nonspecific binding were observed in the dendrimer containing eight ruthenium units, lowering the final ECL intensity and sensitivity.

Osmium polypyridine complexes have also been studied.[60,76,102−104] The development of osmium-based sensors would be advantageous because osmium systems are more photostable than their ruthenium analogues and usually oxidize at less anodic potentials than analogous ruthenium systems. This latter property could prove to be useful in designing DNA-labeling agents as it has been well documented that oligonucleotide sequences undergo irreversible oxidative damage at potentials ≥ 1 V.[105] However, extension of $Ru(bpy)_3^{2+}$ ECL to osmium systems has been somewhat limited due to the larger spin−orbit coupling in osmium systems that typically results in shorter excited-state lifetimes and weaker emission efficiencies.[106]

The first report of ECL in an osmium complex was $Os(phen)_3^{2+}$ using $S_2O_8^{2-}$ reduction to generate the excited state.[60] ECL intensities were obtained in DMF that were ~40% of a $Ru(bpy)_3^{2+}$ standard. The mechanism of the reaction was believed to be electron transfer between electrogenerated $Os(phen)_3^{+}$ and $SO_4^{\bullet -}$. Much higher ECL intensities were observed via annihilation in a series of osmium complexes containing bpy and phen ligands.[102,107] The complexes were of the general form $Os(bpy)_2L^{2+}$ or $Os(phen)_2L^{2+}$ with L = 1,2-bis(dihenylphosphino)ethane (diphos) 1,2-bis(diphenylphosphino)methane (dppm), CH_3CN, dimethyl sulfoxide (DMSO), 1,2-*cis*-bis(2-diphenylphosphinoethylene) (dppene), and 1,2-bis(diphenylarsinoethane) (dpae). Unlike $Os(bpy)_3^{2+}$ and $Os(phen)_3^{2+}$, many of these complexes show very intense photoluminescence with efficiencies 2−3 orders of magnitude higher than that of $Os(bpy)_3^{2+}$.[102] ECL spectra confirmed the identity of the ECL emission as being MLCT in nature. A subsequent study on several of these complexes probed the mechanism of ECL and found that there was a good correlation between the observed ECL intensity and the photoluminescence quantum yield.[107] This indicates that the direct formation of the ECL excited state may occur upon

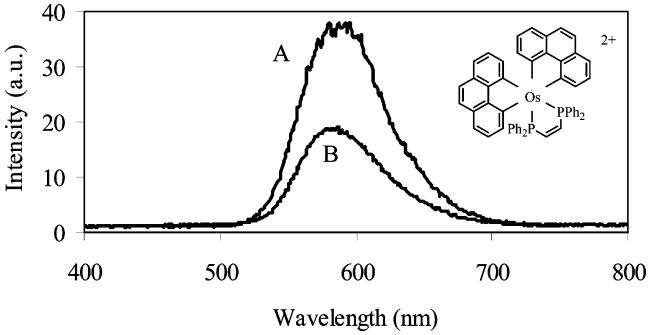

Figure 15. Photoluminescence (A) and ECL (B) spectra of 0.01 mM $Os(phen)_2(dppene)^{2+}$ in aqueous solution (0.1 M KH_2PO_4, 0.05 M TPrA). (Reprinted with permission from ref 103. Copyright 2002 American Chemical Society.)

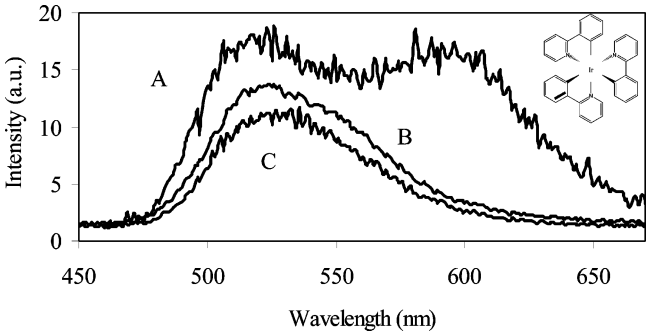

Figure 16. Structure of $Ir(ppy)_3$ (ppy = 2-phenylpyridine). ECL spectra of (A) a 10 μM $Ir(ppy)_3$ *and* 10 μM $Ru(bpy)_3^{2+}$ solution in CH_3CN containing 0.05 M TPrA (0.1 M Bu_4NPF_6 as electrolyte), (B) 10 μM $Ir(ppy)_3$ (0.05M TPrA) in CH_3CN (0.1 M Bu_4NPF_6), and (C) 10 μM $Ir(ppy)_3$ (0.05 M TPrA) in Ch_3CN/PH_2O [50:50 (v/v), 0.1 M KH_2PO_4]. (Reprinted with permission from ref 114. Copyright 2002 American Chemical Society.)

annihilation without intervening deactivation pathways.

Experiments were designed to study the microenvironmental effects of micelles on the electrochemical and ECL behavior of $Os(bpy)_3^{2+}$.[108] The one-electron oxidation of $Os(bpy)_3^{2+/3+}$ near +0.6 V versus SCE allowed the studies to be carried out in aqueous solution using oxalate to generate $Os(bpy)_3^{\bullet 2+}$. Anionic [sodium dodecyl sulfate (SDS)], cationic [cetyltrimethylammonium bromide (CTAB)], and neutral (Triton X-100) species were chosen as representative surfactants. Measurements of changes in oxidation peak current and ECL intensities at different electrolyte concentrations indicated a strong interaction of $Os(bpy)_3^{2+}$ with SDS. Above the critical micelle concentration (cmc), strong hydrophobic interactions between the osmium chelate and SDS micelles suppress both peak current and ECL intensity.

$Os(phen)_2(dppene)^{2+}$ (Figure 15) exhibits electrochemiluminescence in aqueous and mixed aqueous/nonaqueous solutions using TPrA as coreactant.[103] In fact, the ECL emission quantum efficiency is 2-fold greater than that of $Ru(bpy)_3^{2+}$ in aqueous buffered solution. The ECL spectra were identical to photoluminescence spectra (Figure 15), indicating formation of the same MLCT excited states in both ECL and PL. The ECL is also linear over several orders of magnitude in aqueous and mixed solution with theoretical detection limits (blank plus 3 times the standard deviation of the noise) of 16.9 nM in H_2O and 0.29 nM in $MeCN/H_2O$ (50:50 v/v). These observations may prove to be useful in diagnostic or environmental applications requiring greater sensitivity and detection limits than $Ru(bpy)_3^{2+}$ can provide.

Ortho-metalated complexes of Ir(III) display strong visible absorptions and ground- and excited-state redox potentials that, like their Ru(II) counterparts, make them of interest in fundamental and applied studies (such as solar energy conversion, molecular sensing, and ECL).[109] Weak ECL was reported for $Ir(ppy)_3$ (ppy = 2-phenylpyridine) in acetonitrile via annihilation between the reduced, $Ir(ppy)_3^-$, and oxidized, $Ir(ppy)_3^+$, species.[110] However, no spectra were reported, making assignments of the ECL emission difficult. Although the ECL emission was weak, the corresponding electroluminescent emission (EL) is quite intense (≤7%), such that $Ir(ppy)_3$ has been incorporated into numerous EL devices.[111] This has prompted renewed interest in the solution ECL of ortho-metalated iridium systems. In one study ECL was observed for $Ir(ppy)_3$ in benzonitrile via annihilation.[112] In this work, a thin-layer cell composed of glass/indium−tin oxide(ITO)/emitting solution/ITO/glass was used, and an ECL emission spectrum characteristic of $Ir(ppy)_3^{\bullet}$ was observed. In another, the role of the triplet excited state on the annihilation ECL of $Ir(ppy)_3$ was explored.[113] The light generated in this manner was stable, and the emission spectrum was the same as that reported for OLED devices based on this compound, indicating formation of the same excited state using both methodologies.

In a separate study the ECL of $Ir(ppy)_3$ was reported in acetonitrile (MeCN), mixed $MeCN/H_2O$ (50:50 v/v), and, for the first time, aqueous (0.1 M KH_2PO_4) solutions using TPrA as an oxidative−reductive coreactant.[114] The ECL intensity peaks at potentials of ∼+0.8V. At these potentials, oxidation of both TPrA ($E° \sim +0.83-0.95$ V vs Ag/AgCl)[55] and $Ir(ppy)_3$ ($E° \sim +0.7$ V) has occurred. ECL efficiencies of 0.00092 in aqueous, 0.0044 in mixed, and 0.33 in MeCN solutions were obtained using $Ru(bpy)_3^{2+}$ as a relative standard ($\phi_{ecl} = 1$). ECL emission spectra are identical to photoluminescence spectra (Figure 16), indicating the same MLCT excited state is formed in both experiments. Although the ECL emission quantum efficiency was weaker than that of $Ru(bpy)_3^{2+}$ under similar conditions, the green ECL emission maximum of $Ir(ppy)_3$ and the red/orange emission of $Ru(bpy)_3^{2+}$ are far enough removed that it is possible to distinguish both signals in a single ECL experiment (Figure 16).[114] This ability may prove to be useful in applications when an ECL internal standard or multianalyte determination is desired. The ECL was also linear over several orders of magnitude in mixed and acetonitrile solution with theoretical detection limits (blank plus 3 times the standard deviation of the noise) of 1.23 nM in CH_3CN and 0.23 μM in CH_3CN/H_2O (50:50 v/v). As with the osmium systems discussed above, the lower potentials required to excite $Ir(ppy)_3$ (i.e., ≤1 V) compared to $Ru(bpy)_3^{2+}$ (i.e., ∼1.2−1.6 V) may prove

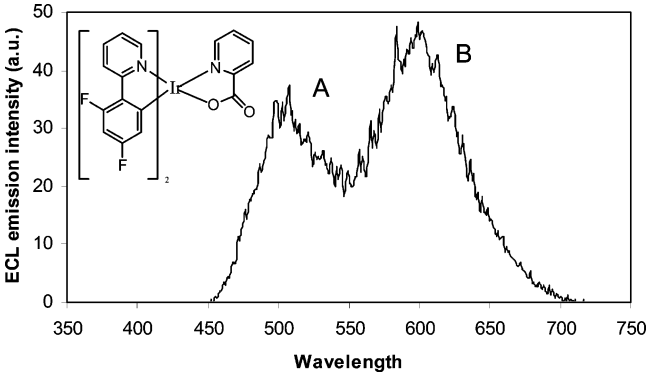

Figure 17. ECL emission spectra of (A) 100 μM F(Ir)pic (structure shown) and (B) 100 μM $Ru(bpy)_3^{2+}$ in the same MeCN solution with 0.05 M TPrA. (Reprinted with permission from ref 117. Copyright 2004 American Chemical Society.)

to be useful in DNA diagnostic applications.[105,115]

The ECL of $Ir(ppy)_3$ has also been generated in acetonitrile/dioxane (1:1 v/v) solutions containing 0.1 M $TBAPF_6$ as the electrolyte using annihilation and coreactant methodologies.[116] The coreactants in question were radical anions of aromatic nitriles and ketones (Table 1). Using $Ru(bpy)_3^{2+}$ as a relative standard, ϕ_{ecl} values of up to 0.67 $\pm$ 0.10 were obtained in this solvent system, which is comparable to the reported photoluminescence efficiency of 0.75.

Work on $Ir(ppy)_3$ has been extended to F(Ir)pic [bis(3,5-difluoro-2-(2-pyridyl)phenyl-(2-carboxypyridyl)-iridium III] (Figure 17) and $(btp)_2Ir(acac)$ [bis[2,(2′-benzothienyl)pyridinato-*N*,*C*3′](acetylacetonate)-Ir(III)] in acetonitrile (MeCN), mixed MeCN/H_2O (50:50, v/v), and aqueous solutions using TPrA.[117] ECL was also studied in the presence of the nonionic surfactant Triton X-100 [poly(ethylene glycol) *tert*-octylphenyl ether]. F(Ir)pic is a blue emitter ($\lambda_{ECL} \sim$ 470 nm) and $(btp)_2Ir(acac)$ emits in the red ($\lambda_{ECL} \sim$ 600 nm). The ECL spectrum of each compound is identical to its photoluminescence spectrum, indicating the same metal-to-ligand (MLCT) excited states. Much like $Ir(ppy)_3$ (Figure 16), the ECL emission spectrum of F(Ir)pic can be distinguished from that of $Ru(bpy)_3^{2+}$ when both are present in the same solution (Figure 17), raising the possibility of using these compounds for the detection of multiple analytes in the same solution. In fact, the ECL spectra of F(Ir)pic and $Ru(bpy)_3^{2+}$ show better resolution than those of $Ir(ppy)_3$ and $Ru(bpy)_3^{2+}$. ECL intensity increased in the presence of surfactant up to 6-fold for F(Ir)pic and up to 20-fold for $(btp)_2Ir(acac)$. Oxidative current also increased for both compounds, supporting the theory of surfactant adsorption at the electrode surface, leading to greater concentrations of TPrA and Ir species near the electrode surface and higher ECL intensities.

ECL has also been extended to copper bis(phenanthroline) systems.[118,119] For example, ECL has been observed from aqueous solutions of $Cu(dmp)_2^+$ generated in situ from copper ions and the ligand 9,10-dimethylphenanthroline (dmp).[119] ECL is generated by reducing Cu^{2+} ions (the common oxidation state found in nature) to Cu^+ with hydroxylamine hydrochloride and then complexing with dmp, followed by oxidation in the presence of tri-*n*-propylamine. The ECL intensity peaks at potential corresponding to oxidation of both TPrA and $Cu(dmp)_2^+$. Intensity versus potential curves were identical whether the $Cu(dmp)_2^+$ was formed in situ[119] or the complex was prepared and isolated separately.[118] This indicates that the emission is from a $Cu(dmp)_2^+$ metal-to-ligand charge-transfer excited state. A calibration curve was linear over the 0.1−5 mg/L (ppm) range with a theoretical limit of detection of 6 μg/L (ppb) and a practical limit of detection of 0.1 ppm. This is well below the U.S. EPA federal standard of 1.3 ppm for copper in drinking water. This work suggests that Cu/dmp ECL may find use as a detection method in analytical applications such as liquid chromatography, flow injection analysis, or the determination of copper in aqueous samples. The latter application is of interest because copper is one of a small group of metallic elements that are essential to human health. These elements, along with amino and fatty acids and vitamins, are required for normal biochemical processes such as respiration, biosynthesis and metabolism.[120] However, excess copper may also potentially cause adverse health effects (e.g., stomach and intenstinal distress and liver and kidney damage) when present at levels >1.3 mg/L (parts per million, ppm) Therefore, water utilities and companies that provide water are required to analyze for the amount of copper in water supplies.[121]

In a similar manner, tris(8-hydroxyquinoline-5-sulfonic acid)aluminum(III) [$Al(HQS)_3$] has been generated in situ in aqueous, buffered solutions and its ECL measured.[122] Of particular interest in this study was the determination of Al^{3+}. This is due to its role as a neurotoxic agent and the potential accumulation of this ion in humans.[123] For example, Al(III) has been shown to play a role in bone and neurological disorders, and its role in Alzheimer's disease is a matter of some debate within the medical and research community.[123] Therefore, the ability to detect Al(III) in situ from clinical and environmental samples using ECL would be advantageous. The ECL of $Al(HQS)_3$ was measured using TPrA in aqueous buffered solution. Conditions for ECL emission were optimized with the ECL correlating to aluminum concentration over several orders of magnitude. The ECL of several metal ions other than aluminum with HQS and the effects on $Al(HQS)_3$ ECL were also examined. Only two, cadmium(II) and zinc(II), showed any significant effect, and both led to an ~50% enhancement of the ECL emission.

Transition metal species such as $Ru(bpy)_3^{2+}$, $Ir(ppy)_3$, and $Os(phen)_2(dppene)^{2+}$ typically have broad emission spectra stretching ~200 nm. This can be a disadvantage in applications where an ECL internal standard or multianalyte determinations are desired. The ECL of a series of europium chelates, cryptates, and mixed-ligand chelate/cryptate complexes was studied using persulfate as a "reductive−oxidative" coreactant[124] because many trivalent lanthanides display high photoluminescence efficiencies, large shifts between absorption and emission maxima (~300 nm), and narrow emission spectra.[125,126] ECL appears to occur via a "ligand-sensitization" route,

where electron transfer between the coreactant and chelate occurs in the organic ligands, with subsequent transfer to the f-orbitals of the metal centers.[124] Unfortunately, very low ECL efficiencies were observed in nonaqueous solvents.

ECL is also possible in sterically stabilized silicon nanocrystals (NCs). Electron transfer reactions between positively and negatively charged NCs (i.e., annihilation) or between charged NCs and coreactants results in ECL.[127] Light emission in Si NCs peaked at 640 nm, significantly red-shifted from the PL maximum of 420 nm. Interestingly, higher intensity light emission was observed from the NC solutions when coreactants (the oxidative−reductive coreactant oxalate and the reductive−oxidative coreactant persulfate) were added to solution. A mechanism was proposed that involved electron/hole annihilation through electron transfer between NCs or NCs with redox-active coreactants. These preliminary studies demonstrate that the Si NCs not only have the ability to store charge in solution but can emit light under the proper conditions. The potential use of Si NCs in sensor technologies was proposed.

Work on Si NCs has been extended to TOPO-capped CdSe nanocrystals (TOPO = trioctylphosphineoxide) dissolved in methylene chloride containing 0.1 M tetra-*n*-butylammonium perchlorate ($TBAClO_4$).[128] Light emission was observed via annihilation, and the ECL spectrum was substantially red-shifted (~200 nm) compared to the PL spectrum, suggesting that surface states play an important role in the emission process. These surface effects are currently being explored via surface passivation and the fabrication of core/shell NC structures.[128]

3.2. Organic Systems

The first detailed studies on ECL involved organic systems.[22−25] The excited states of polyaromatic hydrocarbons (such as anthracene and rubrene) were generated in aprotic media via annihilation. Many studies on polyaromatic hydrocarbons (PAHs) followed,[27,129−137] and this work was extended to annihilation cross-reactions[138−153] and coreactants.[154,155] Various other organic systems also produce ECL including polymers,[156,157] the amino acid tryptophan,[158] and acridinium esters,[159] to name a few. A listing of the ECL-active organic compounds studied to date is presented in Table 2. Much of the pre-1997 work in this area has been the subject of reviews,[3,4,6,17] and a fairly comprehensive compilation of pre-1993 compounds has been published.[6] Therefore, this discussion will center on the more recent literature.

One limitation of PAHs for practical applications/studies is their poor water solubility and the need for rigorously purified and deoxygenated nonaqueous solvents. To address these problems, the ECL behaviors of 9,10-diphenylanthracene-2-sulfonate (DPAS) and 1- and 2-thianthrenecarboxylic acid (1- and 2-THCOOH) in aqueous solution using TPrA as coreactant were studied.[155] The reduction of DPAS using $S_2O_8^{2-}$ as coreactant also produces ECL. In the case of DPAS, ECL spectra were characteristic of PL spectra, indicating formation of the same excited state in both experiments. Also, the emission maximum occurs at ~420 nm, considerably blue shifted when compared to $Ru(bpy)_3^{2+}$. In contrast, ECL spectra for 1- and 2-THCOOH were red shifted compared to PL spectra. It was proposed that systems such as these for aqueous ECL might prove to be useful in the design of new labels for ECL analysis of biomolecules.[155]

Evidence has also been obtained for laser action driven by ECL.[160] In this experiment, a pair of sputter-deposited platinum film electrodes were positioned facing each other with a distance between them of 2−7 μm (Figure 18). One electrode functioned as the mirror while the other was a half-mirror with a transmittance of ~0.8%. A solution of DPA in DMF was continuously introduced into the assembly, and the DPA molecules were oxidized and reduced at each electrode by applying a potential between the electrodes. The electrodes were in close enough proximity that $DPA^{\bullet+}$ and $DPA^{\bullet-}$ could diffuse together and undergo annihilation, with the subsequent emission of blue light (λ_{ecl} = 420 nm). The output intensity of the ECL showed a clear threshold value as the drive current increased, and spectral narrowing was also observed as the intensity increased, indicating that lasing action occurred.[160] An ECL laser might provide several advantages over traditional, optically pumped, lasers including power, tunability, and range of available wavelengths. Also, an ECL laser would not require an additional laser source to optically pump the dye into the required excited state.

Non-PAHs have also been studied; for example, perylene diimide radical anions and cations were generated by pulsing the applied potential between the parent compound's first oxidation and reductive waves.[161] The excited states formed in this manner are believed to occur via triplet−triplet annihilation (T-route), because the energy difference between the oxidation and reduction is not enough to populate the emitting state directly (eqs 25 and 26). The ECL of both indole and tryptophan using hydrogen peroxide to generate the excited state has also been reported.[162] The limit of detection (LOD) for both species was 1.0×10^{-7} M with a linearity observed in the 1.0×10^{-7}−8.4×10^{-5} M range for tryptophan and in the 1.0×10^{-7}−8.0×10^{-5} M range for indole.

ECL has also been extended to the detection of thiamin (vitamin B_1) using two different methodologies.[158,163] In the first, rhodamine B was used as a sensitizer to enhance the weak intrinsic ECL of thiamin in alkaline solution. The addition of rhodamine B resulted in a LOD of 0.08 μg/mL.[158] The mechanism involves energy transfer between the excited oxidation product of thiamin and rhodamine B. In the second, thiamin was used as an oxidative−reductive coreactant to generate ECL from ruthenium complexes.[163]

Due to their high PL efficiencies several laser dyes were studied in the 1970s in aprotic media using annihilation[164,165] and annihilation cross-reaction ECL.[165] More recently, the ECL of a heptamethine cyanine dye was reported in MeCN with TPrA as coreactant.[166] Emission was observed in the near-infrared (NIR) region of the electromagnetic spectrum (λ_{em} and λ_{ecl} = 805 nm; Figure 19). This work was

Table 2. Organic Electrochemiluminescent Compounds

compound[a]	emitting species	ref
Annihilation (For Example, Equations 5−8)		
acenaphthalene	acenaphthalene	135
acridinium esters	acridinium esters	159
aryl derivatives of *N,N*-dimethylanaline	complexes	397
aryl derivatives of isobenzofurans and indoles	complexes	398
binaphthyl	binaphthyl	134
bis(2,4,6-trichlorophenyl)peroxyoxalate	complex	399
boron-containing dipyrromethene−BF_2 (PM-BF_2) laser dyes	dye	165
BPQ-PTZ	BPQ-PTZ	186
chrysene	chrysene	134
coronene	coronene	134
cyclic hydrazides in a micellar medium	cyclic hyrazides	137
decaphenylnaphthalene (DecPA)	decPA	137
9,10-diphenylanthracene (DPA)	DPA	24, 27, 129−130, 160, 165
1,8-diphenyl-1,3,5,7-octatetracene	1,8-diphenyl-1,3,5,7-octatetracene	134
donor-substituted polyaromatic hydrocarbons	PAHs	136
DPBF	DPBF	180−181
DPBF-2	DPBF-2	181
DPBF-3	DPBF-3	180−181
DPBF-4	DPBF-4	182
fluoroanthene	fluoroanthene	134
laser dyes (e.g., coumarin − 2)	dye	164
luminol	luminol	398−402
luciferace	luciferace	400
lucigenin	lucigenin	169−170, 176
1-methyl-2,5-diphenylindene	complex	401
octaphenylnaphthalene (OPN)	OPN	137
PDI	PDI	402
peralenetetracaroxylic diimide	complex	402
perylenedicarboxylic imide (PI)	PI	402
perrylene dimer salt (Pe_2ClO_4)	Pe_2ClO_4	403, 404
perylenetetracarboxylic diimide (PDI)	PDI	402
phenanthrene	phenanthrene	132
9-phenylanthracene	9-phenylanthracene	134
phenothiazene	phenothiazene	134
PI	PI	402
poly(9,9-dioctylfluorene) polymer	polymer	178
poly(vinyl-9,10-diphenylanthracene) polymer	Polymer	156
pyrene	pyrene	133
pyrine/phenothiazine-substituted peptides	peptide	179
QDI	QDI	402
quaterrylenecarboxylic diimide (QDI)	QDI	402
rubrene (RUB)	RUB	26−27, 165
TDI	TDI	402
terrylenetetracarboxylic diimide (TDI)	TDI	402
thiamin (vitamin B1)	thiamin	152
TPP	TPP	3
trans-stilbene derivative	complex	192
tricarbocyanine dye IR125	IR 125	166
tricarbocyanine dye IR144	IR 144	166−167
Annihilation Cross-Reactions (Equations 17−20)		
DPA−1,4-dihydropyridines	DPA	133
DPA−TMPD	DPA	139
DPA−halogen ions	DPA	140
DPA−9,10-dichloro-9,10-dihydro-9,10-DPA	DPA	141
RUB−TMPD	RUB	142
RUB-amines, H_2O, or DMF	RUB	143
tetracene−TMPD	tetracene	144
aromatic hydrocarbons−tetraphenylporphins	hydrocarbons	145
aromatic hydrocarbons−tetrathiafulvalene	hydrocarbons	146
fluoranthrene−10-methylphenothiazine	fluoranthrene	147
thianthrene−2,4-diphenyl-1,3,4-oxadiazole	thianthrene	148
TPP−TMPD	TPP	3
anthracene−TMPD	anthracene	3
p-benzoquinone−rubrene	rubrene	3
10-MP−fluoranthene	fluoranthene	147,161, 184
10-MP−radical anions	10-MP	186
TTF−anthracene	anthracene	146
thianthrene−PPD	thianthrene	148
perylene (PE)−various aromatic compounds	aromatic compounds	149
PE−pyrene (PY)	(PE−PY) exciplex	150−151
thiamin−rhodamine B	rhodamine B	152

Table 2. (Continued)

compound[a]	emitting species	ref
Annihilation Cross-Reactions (Equations 17−20) (Continued)		
rhodamine 6G−tubrene		164
rhodamine 6G−DPA	both compounds	164
TCPO/$C_2O_4^{2-}$/DPA	DPA	153
lucigenin/riboflavin	lucigenin	171
Coreactant (For Example, Equations 36−38)		
polyaromatic hydrocarbons/persulfate	PAHs	154
MEH−PPV polymer/TPrA	MEH−PPV	157
indole/trytophan	indole	162
indole/H_2O_2	indole	162
MFPA/ H_2O_2	MFPA	172
lucigenin-hemin/H_2O_2	hemin	173
heptamethine cyanine dye/TPrA	dye	166
DPAS/TPrA	DPAS	155
DPAS/$S_2O_8^{2-}$	DPAS	155
1-THCOOH/TPrA	2-THCOOH	155
2-THCOOH/TPrA	2-THCOOH	155
BADE/H_2O_2	BADE	174
SPBA/H_2O_2	SPBA	175
ABEI/H_2O_2	ABEI	188
luminol/H_2O_2	3-aminophthalate	189−196, 198−203, 209−214
luminol derivatives/H_2O_2	luminol decomposition products	205−208

[a] Compound abbreviations are listed at the end of the article in section 7.

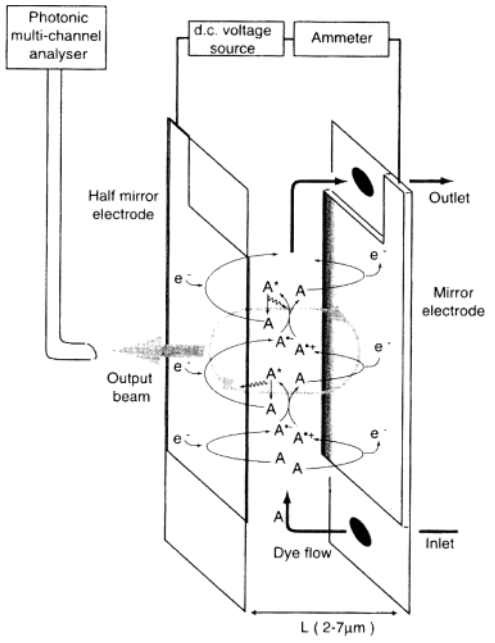

Figure 18. Diagram of ECL laser. A, A•, A•−, and A•+ indicate the ground, excited, anion radical, and cation radical states of 9,10-diphenylanthracene. (Reprinted with permission from *Nature* (http://www.nature.com), ref 160. Copyright 1998 Nature Publishing Group.)

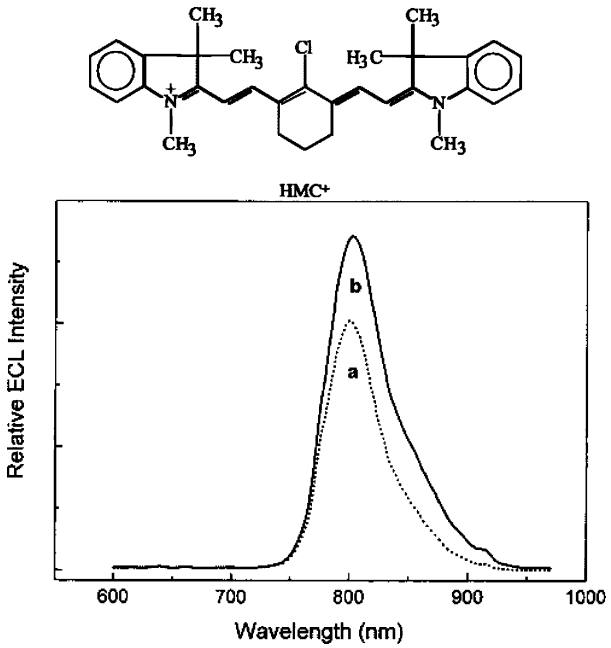

Figure 19. Structure of HMC^+ (top) and uncorrected (a) and corrected (b) ECL spectra of 2.0×10^{-4} M HMC^+ with 0.06 M TPrA in MeCN using TPrA as coreactant. (Reprinted with permission from ref 166. Copyright 1997 American Chemical Society.)

extended to two tricarbocanine NIR dyes (IR125 and IR144).[167] Investigations were carried out in organic, aqueous, and micellar solutions. IR144 showed sufficient ECL intensity in aqueous solution, but the formation of dimers and higher order aggregates decreased the ECL efficiency. The addition of a surfactant, however, shifted the equilibrium back to monomers. This work may prove to be important in the development of new ECL labels to complement visible emitters such as $Ru(bpy)_3^{2+}$ and $Ir(ppy)_3$ with the possibility of multianalyte assays using more than one label. The photochemistry, electrochemistry, and ECL of five highly fluorescent boron-based laser dyes have been investigated.[168] Moderately intense ECL was also observed via annihilation from three of the dyes that could be traced to electron-donating substituents in key positions on the dipyrromethene rings. Dyes with a lower degree of substitution showed irreversible cyclic voltammetric waves, presumably due to a decrease in the stability of the cation and anion forms of the compounds. This instability gave rise to lower ECL efficiencies in three of the dyes and lack of ECL in two others.

Acridinium esters have also been well studied with lucigenin (*N*,*N*′-dimethyl-9,9-bisacridinium) being the classic example.[169] Lucigenin is ECL active in the presence of hydrogen peroxide[170] and has been used

to detect riboflavin,[171] human chorionic gonadotropin,[172] and hemin.[173] In fact, hemin retained its catalytic ECL activity when conjugated to a protein, and the use as a label for bioassays was proposed. The ECL reaction of methyl-9-(*p*-formylphenyl)acridinium carboxylate fluorosulfonate (MFPA) and its applications as a label in bioassays has been demonstrated.[172] MFPA could be detected with a LOD of 2.1×10^{-10} M in the presence of H_2O_2. Lucigenin itself contains no functional groups that allow conjugation to biological molecules of interest. However, two lucigenin derivatives containing conjugation groups, 9,9′-bisacridinium-*N*,*N*′-diaceticacid ethyl ester (BADE) and *N*,*N*′-di-(3-sulfopropyl)-9,9′-bisacridinium (SPBA), have been synthesized,[174,175] and SPBA was immobilized on aminocellulose and in HYPAN hydrogels via a sulfopropyl moiety.[176] Lucigenin ECL has also been extended to surfaces.[177] Enhanced ECL of lucigenin has been observed by the modification of electrodes with self-assembled monolayers (SAMs) and in solutions containing Triton X-100 surfactant molecules. The enhancement of ECL at SAM- and surfactant-modified electrodes has been traced to concentration effects of the positively charged lucigenin adsorbed at or near the surface of the electrode.[177]

Organic polymers have also attracted attention.[156,157] For example, the thin film electrochemistry and electrochemiluminescence of 4-methoxy-(2-ethylhexoxy)-2,5-polyphenylenevinylene (MEH-PPV) were obtained using annihilation and coreactant methods.[157] Orange luminescence characteristic of MEH-PPV ($\lambda_{max} = 622$ nm) was observed by pulsing between +0.40 and −2.35V versus Fc/Fc^+ as well as by oxidizing MEH-PPV in the presence of TPrA and reducing MEH-PPV in the presence of persulfate, $S_2O_8^{2-}$. Excimer emission from poly(9,9-dioctylfluorene) was reported in benzeneacetonitrile solutions.[178] ECL was generated via annihilation, and the spectrum was characterized by emission bands centered at 438 nm, assigned to intrapolymer emission, and 610 nm, assigned to excimer emission. The ratio of excimer to intrapolymer emission depends on polymer concentration.[178]

Monomer, exciplex, and excimer emission has also been observed in donor−acceptor functionalized luminescent hairpin peptides.[179] The ECL of pyrene/phenothiazine-substituted optically active peptide systems was compared with PL spectra. Interestingly, two of the peptides showed intramolecular chromophore-based through-space interactions resulting in excimer bands, whereas another displayed bands characteristic of exciplex formation. Due to the relationship between structural features and signal response, it was proposed that these peptides might prove to be useful for probing molecular recognition events.

Within the past few years, several papers have appeared on the synthesis and light-emitting properties of 7,12-diphenylbenzo[*k*]fluoranthene (DPBF)[180,181] and structurally related complexes.[182] For example, upon oxidation DPBF undergoes intermolecular dehydrogenative coupling to form bis[4,4′-(7,12-diphenyl)benzol[*k*]fluoranthene] (DPBF-2).[181] Further

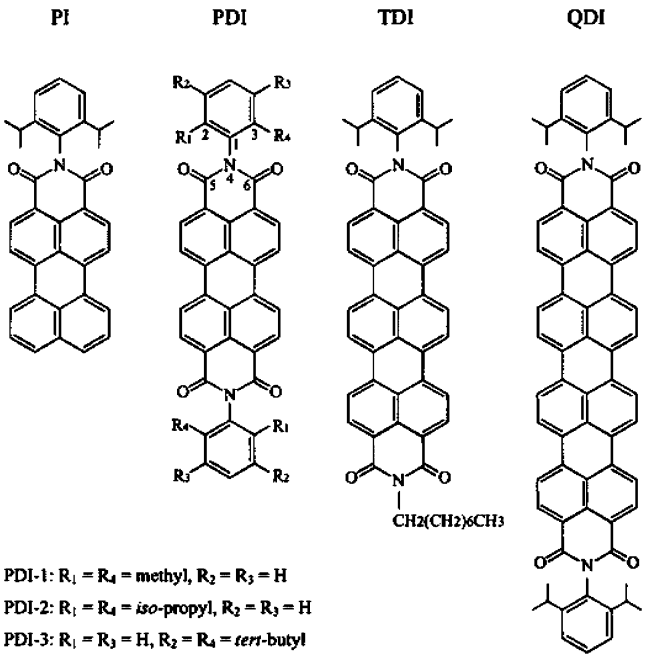

Figure 20. Structures of PI, PDI, TDI, and QDI. (Reprinted with permission from ref 183. Copyright 1999 American Chemical Society.)

oxidation of DPBF-2 results in dibenzo{[*f*,*f*′]-4,4′,7,7′-tetraphenyl}diindeno[1,2,3-*cd*:1′,2′,3′-*lm*]perylene (DPBF-3). All three compounds show ECL via annihilation (e.g., formation of the dication and dianion of DPBF-2). Research in this area has been motivated by the desire to develop multiple wavelength labels to facilitate simultaneous ECL detection in bioanalytical assays and the design of new, highly efficient electroluminescent compounds for use in organic light emitting devices (OLEDs).

The electrochemistry and ECL of diimide complexes have also generated interest.[183] This is due to their long wavelengths for emission, which may be important in the design of ECL labels that emit in the red and near-IR regions. Perylenedicarboxylic imide (PI), perylenetetracarboxylic diimide (PDI), terrylenetetracarboxylic diimide (TDI), and quaterrylenecarboxylic diimide (QDI) (Figure 20) undergo two reversible one-electron reductions and one reversible one-electron oxidation reaction. PDI and TDI in particular generated very stable ECL spectra by sequential production of the radical cation and radical anion at an electrode that were identical to PL spectra. It was concluded that ECL emission occurred via the T-route (eqs 25 and 26).

The ECL of 10-methylphenothiazine (MP) was first reported in the early 1970s,[147,184,185] and a more recent report has also appeared.[186] MP continues to generate interest because it can form an emissive state that is not identical to the PL singlet excited state. It was necessary to generate the excited state via annihilation cross-reaction methods (i.e., a second compound is added to react with the stable radical cation; eqs 17−20) because the radical anion of MP could not be generated prior to the reduction of the nonaqueous solvents. Recently, however, electron-accepting phenylquinoxoline groups were covalently attached to the 3- and 7-positions of 10-methylphenothiazine to form

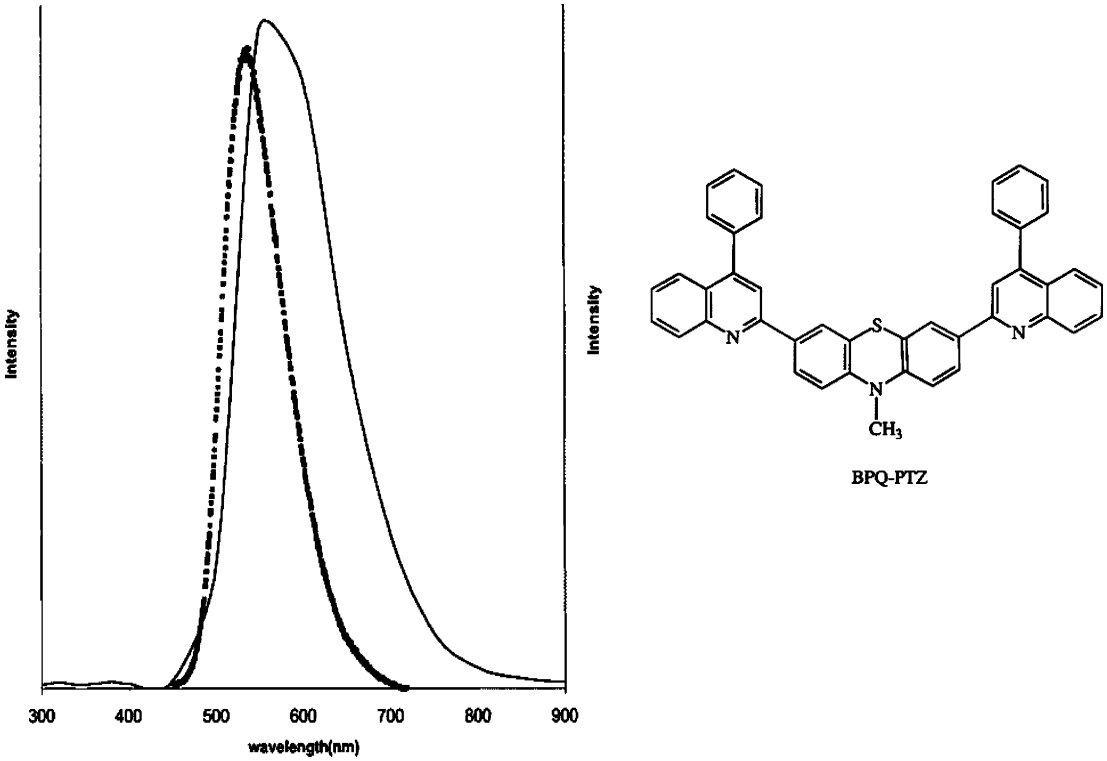

Figure 21. Fluorescence (squares) and ECL spectra of BPQ-PTZ in benzene/acetonitrile. ECL generated via annihilation. (Reprinted with permission from ref 187. Copyright 2001 American Chemical Society.)

3,7-[bis[4-phenyl-2-quinolyl]]-10-methylphenothiazine (BPQ-PTZ), and the ECL was observed via direct annihilation of the reduced and oxidized forms (Figure 21).[187] The ECL spectrum indicates good agreement with the PL spectrum, indicating the excited state that is formed in ECL is the same as that formed via photoluminescence methods.

N-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) produces ECL when oxidized at ~+1.0 V versus Ag/AgCl in alkaline aqueous solution. The addition of hydrogen peroxide improved the ECL efficiency with a detection limit of 2.2×10^{-12} M.[188] ABEI was also used as a probe to identify target ssDNA immobilized on an electrode.[188] In DNA hybridization assays, a probe DNA sequence is labeled with an ECL luminophore. This probe is specific for a complementary or target sequence (Figure 9a) and will form only a double-stranded DNA (dsDNA) sequence with the target. If the target sequence is immobilized on a working electrode, application of a potential should result in ECL only when the dsDNA probe-target sequence has undergone hybridization. Therefore, ABEI was attached to a known oligonucleotide sequence that was complementary to the target ssDNA. Upon formation of a double-stranded DNA target-probe sequence ECL could be measured. A three-base mismatch sequence and noncomplementary sequence produced no ECL response, indicating ABEI's potential usefulness in DNA hybridization analyses.

Luminol (2,3-aminophthalhydrazide) is another classic organic species that continues to generate interest. Light emission from luminol at electrodes was first reported in 1929 upon application of +2.8 V in aqueous alkaline solution.[189] Several reports followed,[190−196] and a proposed mechanism is shown in Figure 22. The ECL reaction of luminol with hydrogen peroxide in alkaline medium is similar to the chemiluminescence (CL) that is generated upon chemical oxidation.[197] Luminol deprotonates in basic solution to form an anion that can undergo electrochemical oxidation (Figure 22). This intermediate species undergoes further electro-oxidation in the presence of hydrogen peroxide to produce 3-aminophthalate in an excited state. 3-Aminophthalate then produces the characteristic "luminol" emission at 425 nm. Different mechanistic pathways have been proposed[198,199] depending on the applied potential. However, all result in the irreversible oxidation of luminol to nonrenewable species. This, the high nonspecific background ECL—possibly due to the formation of oxygen at the anode in aqueous solution, followed by chemiluminescent reactions involving oxygen—and the extremely basic conditions (pH >11) that are needed to generate sufficient light emission have resulted in fewer practical applications for the luminol/H_2O_2 system compared to $Ru(bpy)_3^{2+}$ (see section 4). In fact, the number of publications on luminol ECL has tapered off in recent years compared to $Ru(bpy)_3^{2+}$. However, the reaction of luminol with hydrogen peroxide does allow the detection of a variety of analytes. For example, hydrogen peroxide can be detected with picomolar sensitivity[200−203] and luminol itself can be detected at sub-nanomolar concentrations.[200,204] Luminol derivatives capable of covalently bonding to biomolecules and generating ECL have also been studied,[205−208] and their use in combination with separation techniques such as liquid chromatography for biosensing has been reported. An interesting area of luminol ECL is the study of electrogenerated catalysts that promote both the conventional CL reaction and the luminol/H_2O_2 ECL reaction. This allows the detection of analytes

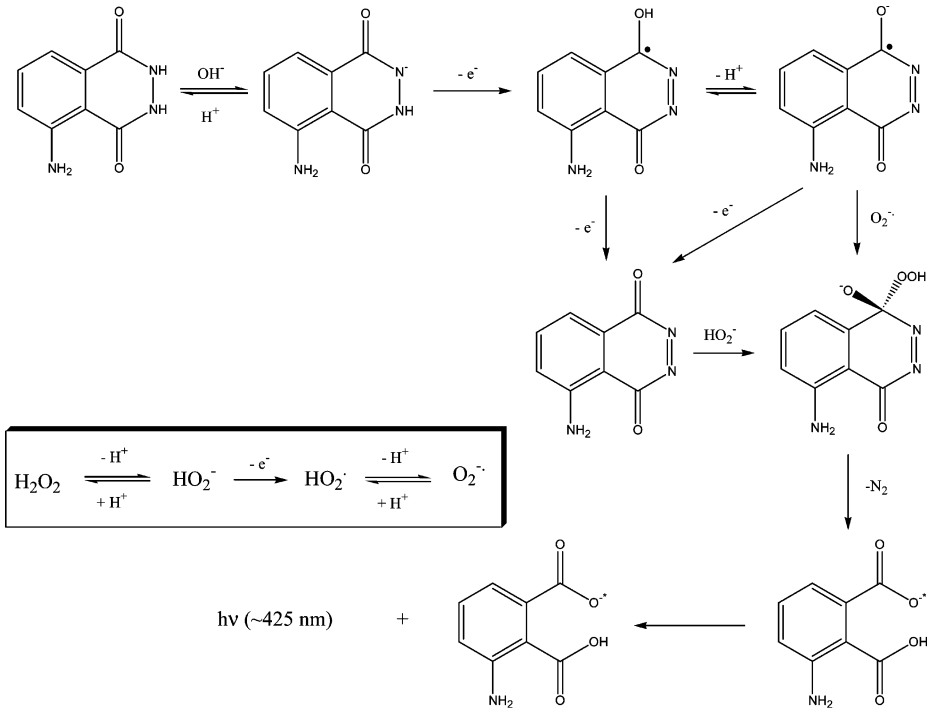

Figure 22. Proposed ECL mechanism for luminol in alkaline solution.

directly[209,210] or by the enhancement of luminol ECL for the detection of analytes.[211−214] A recent example is the enhancing effect of hydrazine on luminol ECL based on hydrazine's in situ electrochemical modification at a preanodized Pt electrode.[213] A method for the determination of phloroglucinol was also reported with a detection limit of 1.2×10^{-4} g/L.[214] The proposed mechanism involves the sensitization of the weak luminol anodic ECL signal in basic (0.1 M $NaHCO_3$) medium. Hydrogen peroxide is an integral part of most luminol studies. Its role in the ECL mechanism appears to involve the peroxide anion, HOO^-, and the electrochemically formed superoxide radical, $O_2^{\bullet -}$ (Figure 22). Because many enzymes produce hydrogen peroxide during their substrate-specific enzymatic activity, sensitive and selective detection in the presence of luminol may be possible.[203] However, to fully exploit this chemistry a compromise has to be found between the basic conditions required for luminol/H_2O_2 ECL and the destruction of the enzyme in alkaline solution.

4. Applications

Coreactant ECL is being used in a wide range of analytical applications including clinical diagnostics, environmental assays such as food and water testing, and biowarfare agent detection.[2,6,215] The overwhelming majority of commercially available tests use a Ru(bpy)$_3^{2+}$ derivative (such as those shown in Figure 8) as the ECL luminophore or label and TPrA as the coreactant (eqs 40 and 41 and Figures 5−7). One advantage of using the Ru(bpy)$_3^{2+}$/TPrA reaction sequence is that upon formation of the luminescent excited state, Ru(bpy)$_3^{\bullet 2+}$, emission of a photon regenerates Ru(bpy)$_3^{2+}$ in its ground state near the electrode surface. Therefore, a single Ru(bpy)$_3^{2+}$ molecule may participate in multiple ECL reaction cycles to produce multiple photons, thereby increasing sensitivity and lowering detection limits.

Figure 23. Commercially available electrochemiluminescence and analyzer—M-series by BioVeris Corp. (Image courtesy of BioVeris Corp.)

Typically, the label is covalently linked to one of the binding partners in the assay and provides the means for detecting the coupling of the binding partner to the analyte. Several classes of binding partners are used including antibody/antigen, enzyme/inhibitor, carbohydrate/lectin, and nucleic acid/complementary nucleic acid.[216] Several commercial instruments are available for ECL assays (Figure 23)[215,217] and are currently based on the use of magnetic bead separation technology. The use of magnetic beads for immunomagnetic separations are well established, and excellent reviews exist.[218−221] In the context of ECL, magnetic beads allow for the separation of the analyte and ECL label onto a solid support (i.e., the bead) followed by collection of the labeled beads on an electrode surface. Most magnetic beads used in ECL systems are paramagnetic (i.e., magnetic only in the presence of an external magnetic field) and consist of a core of magnetite (Fe_3O_4) surrounded by a polystyrene shell. These micrometer-sized particles may be purchased commercially (e.g., BioVeris Corp., Dynal Corp.) with a variety of surface

Table 3. Assays That Relate Coreactants to Analyte Concentration

analyte	coreactant	detection method	ref
carbon dioxide	NADH	$Ru(bpy)_3^{2+}$/NADH	232
cholesterol	$C_2O_4^{2-}$	$Ru(bpy)_3^{2+}/C_2O_4^{2-}$	232
ethanol	NADH	$Ru(bpy)_3^{2+}$/NADH	232, 233
glucose	NADH	$Ru(bpy)_3^{2+}$/NADH	232, 233
lactate	NADH	$Ru(bpy)_3^{2+}$/NADH	233
β-lactamase	hydrolyzed β-lactamase	$Ru(bpy)_3^{2+}$/hydrolyzed β-lactamase	233

immobilization chemistries including preconjugated streptavidin, amines, hydrazides, and long-chain alkyl linkers, to name a few.

4.1. Clinical Applications

The most common and, arguably, the most important commercial application to date for ECL is its use in diagnostic assays. Currently, >50 assays, including those for thyroid diseases, tumor and cardiac markers, fertility therapies, and analytes relevant to infection diseases, are commercially available (Tables 3−5).[2,215]

Because ECL emission intensity is usually proportional to the concentration of the emitter[222] or coreactant,[1] ECL can be used to analyze for both. For example, the concentration of an analyte can be determined by measuring the emission of a label, usually $Ru(bpy)_3^{2+}$, in the presence of a high and constant concentration of coreactant. Alternately, some assays rely on the detection of coreactants in the presence of constant $Ru(bpy)_3^{2+}$ concentrations.

4.1.1. Assays That Relate Coreactants to Analyte Concentration

An example of the detection of coreactants using ECL was the determination of oxalate and peroxydisulfate in aqueous solution at sub-picomolar levels[223] using $Ru(bpy)_3^{2+}$. In fact, the ability of $Ru(bpy)_3^{2+}$ to generate emission in the presence of oxalate has led to the selective determination of this coreactant in synthetic urine samples.[224] $Ru(bpz)_3^{2+}$ (where bpz = bipyrazine) has also been used for the determination of peroxydisulfate with nanomolar (nM) detection limits.[93]

Another class of compounds that act as coreactants are amines.[1,2,6,225] ECL assays for amines find many applications because amine groups are prevalent in numerous biologically and pharmacologically important compounds including alkylamines, antibiotics, antihistamines, opiates, nicotinamide, and the reduced form of NADH (i.e., adenine dinucleotide).[6,69,215] In general, these compounds contain no chromophore and therefore cannot undergo luminescence unless an ECL-active compound is present. As a general rule, the ECL signal from alkylamine coreactants follows the order tertiary > secondary > primary.[1] Therefore, when primary and secondary amines are analyzed, it is often necessary to derivatize them prior to analysis. For example, primary amines have been detected using $Ru(bpy)_3^{2+}$ coreactant ECL after prior derivatization with divinyl sulfone ($CH_2{=}CHSO_2{-}CH{=}CH_2$). The primary amines undergo a cycloaddition reaction resulting in the formation of acyclic tertiary amines[226] that then act as efficient coreactants. Other examples of cyclic amines that undergo ECL include nictone, atropine, and sparteine.[227] It is also possible to quantitatively measure amino acids, peptides, and proteins such as proline and valine. In fact, detection limits of 20 pM for proline[228] and 30 pM for valine[229] using flow injection techniques have been achieved. Although the ability of numerous amines to act as coreactants makes ECL a versatile technique for their detection, it also makes selectivity for the presence of a specific amine problematic. However, chlorpromazine (a commonly prescribed dopamine inhibitor) was used as an oxidative−reductive coreactant with $Ru(bpy)_3^{2+}$ as the ECL luminophore. High selectivity was obtained by preconcentration of the chlorpromazine at a lauric acid-modified carbon paste electrode with a detection limit of 3.1×10^{-9} M.[230]

ECL has also been used to monitor enzymatic reactions. In such systems, the reaction is often coupled to the generation or consumption of an ECL coreactant. An example is the coenzyme nicotinamide adenine dinucleotide (NADH). NADH contains an amine moiety that can act as a coreactant for $Ru(bpy)_3^{2+}$. However, NAD^+, the oxidized form of NADH, is not a coreactant.[231] Because numerous NADH-dependent enzymes are known, this allows for the detection of a variety of analytes including glucose, carbon dioxide, ethanol, and lactate (Table 3).[232,233] In the glucose example, NADH is formed by glucose dehyrogenase in the presence of glucose (Figure 24), thus allowing a diagnostic test for diabetes mellitus.

β-Lactamase activity can also be detected via ECL.[234] Pencillin and its derivatives do not act as coreactants with $Ru(bpy)_3^{2+}$ to produce ECL. However, β-lactamase-catalyzed hydrolysis of pencillin forms a molecule with a secondary amine that can act as a coreactant. The efficiency of the ECL process has been increased by covalent attachment of a β-lactamase substrate to a $Ru(bpy)_3^{2+}$ derivative.[235] The ECL behaviors of aminopeptidase and esterase cleavage products have also been reported by covalently attaching such species as ligands to bis(bipyridine)ruthenium (II). $Ru(bpy)_2^{2+}$ has little to no intrinsic ECL, but attachment of a third ligand leads to enhanced ECL.[235]

ECL with coreactants has also been used in detector cells for high-performance liquid chromatography (HPLC). These generally involve the ECL of $Ru(bpy)_3^{2+}$ for the detection of species that act as coreactants, such as amino acids, amines, and NADH,[236] and this area has recently been reviewed.[237] One technique that has acheived picomole detection limits uses postcolumn ECL detection. A solution of $Ru(bpy)_3^{2+}$ is steadily injected into the solution stream containing separated species coming from the HPLC column. The mixed stream flows into

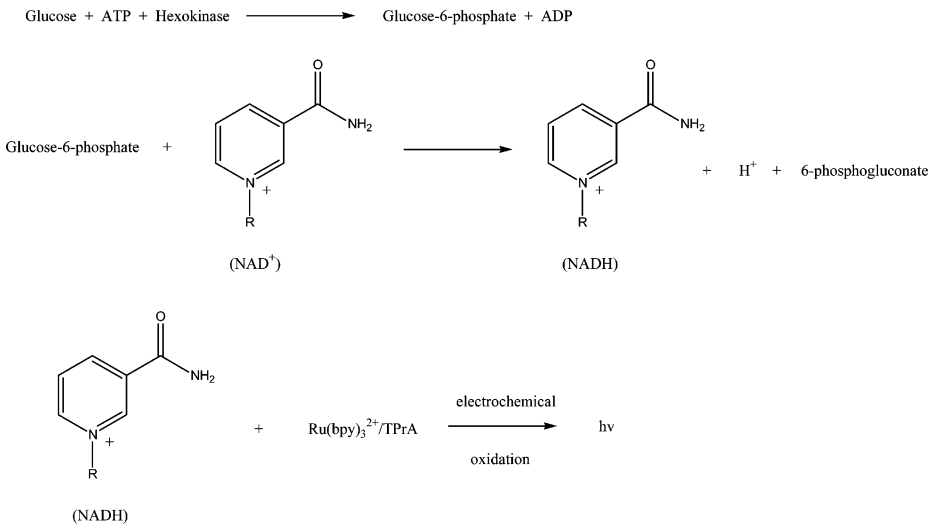

Figure 24. Proposed mechanism for the ECL reaction of NADH with $Ru(bpy)_3^{2+}$/TPrA for the measurement of glucose.

an electrochemical flow cell where the ECL reaction occurs and emission can be measured.[238] $Ru(bpy)_3^{2+}$ can also be immobilized in a thin film of polymer (e.g., Nafion) deposited on the working electrode.[239,240] This eliminates the need for a constant stream of $Ru(bpy)_3^{2+}$. In this technique, ECL results when a species that can act as a coreactant is in the solution coming from the HPLC column and reacts with the immobilized $Ru(bpy)_3^{2+}$ in the detector cell.[68]

In the past decade, ECL has also been coupled with capillary electrophoresis (CE).[241−246] Biochemical applications of ECL have rapidly expanded in the past 10 years, so coupling ECL with CE should provide rapid, efficient, and versatile methods of separating and detecting biochemicals using extremely small sample volumes. For example, an ITO electrode-based $Ru(bpy)_3^{2+}$ ECL detector for microchip CE and flow injection analysis was demonstrated.[246,247] Proline was detected with a limit of detection of 1.2 μM and a linear range from 5 to 600 μM. In another study, CE−ECL was used to study the chemical oxidation of the biologically and clinically significant molecule *p*-hydroxyphenylpyruvic acid (pHPP) by dissolved oxygen in aqueous solution.[248] Interestingly, not only was pHPP observed to be readily oxidized by oxygen in alkaline solution, but one of the byproducts of this oxidation was oxalate ion, which resulted in strongly enhanced $Ru(bpy)_3^{2+}$ ECL.

Many examples exist in which the analyte is incorporated into the ECL sequence as the coreactant and generates ECL from a label such as $Ru(bpy)_3^{2+}$ or luminol (a complete listing is in Table 4). For example, certain amino acids, aliphatic amines, cyclic amines, aromatic amines, pharmaceuticals, and proteins contain secondary or tertiary amines and produce measurable ECL with $Ru(bpy)_3^{2+}$.[2] Also, thiamin (vitamin B1) can be determined using the ECL reaction of ruthenium complexes with thiamin as the coreactant.[249]

4.1.2. Assays That Relate Emitters to Analyte Concentration

In the methods described in section 4.1.1, ECL was measured in the presence of high, predetermined concentrations of ECL emitters. However, the vast majority of clinical tests use a format in which the label concentration is measured. At the present time, all commercial assays use a $Ru(bpy)_3^{2+}$ derivative (Figure 8).[2,215] The $Ru(bpy)_3^{2+}$ derivative is covalently attached to one of the species involved in an affinity-binding reaction, and ECL is detected in the presence of excess coreactant. The coreactant, typically TPrA, is present in high concentrations, so the amount of luminescence depends on the concentration of the ECL emitter present in the assay. Because the emitters are bound to the analyte of interest, the amount of luminescence can be correlated with the concentration of the analyte.

A list of ECL-based immunoassays that relate ECL luminophore emission to analyte concentration is in Table 5. Tumor markers,[250−262] fertility and hormone tests,[252,263−265] thyroid function,[252,266−270] cardiac,[252,271−274] heptatitis,[275−277] bone markers,[278−280] Alzheimer's disease,[281] anemia,[282] diabetes,[283] and tests for infectious diseases[252,276,277,284−289] have been areas of active research. For example, tumor markers such as α-fetoprotein (AFP),[250,251] carcinoembryonic antigen (CEA),[251,252] prostate-specific antigen (PSA),[251,253−255] messenger ribonucleic acid (mRNA) coding for various cancer markers,[256−259] cytokeratin 19,[260] and des-γ-carboxyprothrombin[261,262] are rapidly growing areas of diagnostic analyses. Tests for these analytes are used for screening and diagnosing cancers and to monitor the effectiveness of drug treatments and surgery.

An expanding area of ECL is in molecular diagnostics or molecular probe assays. Molecular diagnostics include tests and methods to identify a disease or the predisposition for a disease by analyzing the DNA or RNA of an organism. Reported assays have used either an *N*-hydroxysuccinimide (NHS) ester linked $Ru(bpy)_3^{2+}$ conjugate ["TAG NHS Ester" available from IGEN International Inc. or a $Ru(bpy)_3^{2+}$ phosphoramidite conjugate (Figure 8)] to label probes that bind analytes. As in immunoassays, assays for a variety of analytes have been reported in the areas of infectious diseases,[290−305] tumor markers,[306−309] metabolism,[310] venous thromboembo-

Table 4. Assays in Which Analyte Is the Coreactant

analyte/coreactant	ECL luminophore	ref
AFP	$Ru(bpy)_3^{2+}$	313
alcohols	$Ru(bpy)_3^{2+}$	405, 406, 407
alicyclic tertiary amines	$Ru(bpy)_3^{2+}$	227
amines (primary), derivatized with DVS	$Ru(bpy)_3^{2+}$	226
amino acids	$Ru(bpy)_3^{2+}$	228
amitrypyline	$Ru(bpy)_3^{2+}$	408
amino acids (dansylated)	$Ru(bpy)_3^{2+}$	68
antihistamines	$Ru(bpy)_3^{2+}$	68
arginine	$Ru(bpy)_3^{2+}$	68
ascorbic acid	$Ru(bpy)_3^{2+}$	68
atropine	$Ru(bpy)_3^{2+}$	68
bupivicaine	$Ru(bpy)_3^{2+}$	68
carcinoembryonic antigen	$Ru(bpy)_3^{2+}$	313
chlorpromazine	$Ru(bpy)_3^{2+}$	408
citrate	$Ru(bpy)_3^{2+}$	409
clindamycin-2-phosphate	$Ru(bpy)_3^{2+}$	68
codeine	$Ru(bpy)_3^{2+}$	68
cystic fibrosis ΔF-508 deletion mutation	$Ru(bpy)_3^{2+}$	410
dextromethrophan	$Ru(bpy)_3^{2+}$	68
digoxin	$Ru(bpy)_3^{2+}$	313
digoxin	Luminol	411
doxepin	$Ru(bpy)_3^{2+}$	408
EDTA	$Ru(bpy)_3^{2+}$	412
emeline dithiocarbamate copper(II)	$Ru(bpy)_3^{2+}$	17
erythromycin	$Ru(bpy)_3^{2+}$	69
glycophosphate and related systems	$Ru(bpy)_3^{2+}$	413
fatty amine ethoxylate surfactants	$Ru(bpy)_3^{2+}$	353
heroin	$Ru(bpy)_3^{2+}$	68
HIV 1 gag gene	$Ru(bpy)_3^{2+}$	410
hydrazine and hydrazine derivatives	$Ru(bpy)_3^{2+}$	17
lactate	$Ru(bpy)_3^{2+}$	414
leucine	$Ru(bpy)_3^{2+}$	68
lignocaine	$Ru(bpy)_3^{2+}$	415
malonate	$Ru(bpy)_3^{2+}$	416
morpholine fungicides (dodemorph and tridemorph)	$Ru(bpy)_3^{2+}$	417
nortriptyline	$Ru(bpy)_3^{2+}$	408
oxalate ($C_2O_4^{2-}$)	$Ru(bpy)_3^{2+}$	53, 68
oxaprenolol	$Ru(bpy)_3^{2+}$	68
persulfate	$Ru(bpy)_3^{2+}$	59
persulfate	$Ru(bpz)_3^{2+}$	92−93
procaine	$Ru(bpy)_3^{2+}$	415
procyclidine	$Ru(bpy)_3^{2+}$	68
promazine	$Ru(bpy)_3^{2+}$	408
pyruvate	$Ru(bpy)_3^{2+}$	68, 418
serine	$Ru(bpy)_3^{2+}$	68
tartrate	$Ru(bpy)_3^{2+}$	419
thiazide compounds	$Ru(bpy)_3^{2+}$	68
thyrotrophin	$Ru(bpy)_3^{2+}$	313
trialkylamines	$Ru(bpy)_3^{2+}$	420
tri-*n*-propylamine (TPrA)	$Ru(bpy)_3^{2+}$	1
L-tryptophan	$Ru(bpy)_3^{2+}$	421
valine	$Ru(bpy)_3^{2+}$	68, 422

lism,[311] and cystic fibrosis[312] (Table 4). For example, the ECL assay for HIV 1 gag gene has been reported with detection limits of <10−30 gene copies.[290,313] Coupling ECL with Polymerase Chain Reaction (PCR) amplification has lowered the detection limit of HIV 1 gag DNA to <5 copies.[305] Other assays and applications incorporating both PCR and ECL for nucleic acid based analyses have been reported,[291,312,314−316] including the quantitation of varicella-zoster DNA in whole blood, plasma, and serum[317] and the detection of viable oocysts of *Cryptosporidium parvum*.[318] This will undoubtedly continue to be an area of intense research activity.

Table 5. Assays That Relate Emitter to Analyte Concentration

analyte	detection method	ref
AFP	$Ru(bpy)_3^{2+}$/TPrA	250, 251
anti-Borna disease antibodies	$Ru(bpy)_3^{2+}$/TPrA	285−287
β-amyloid peptide	$Ru(bpy)_3^{2+}$/TPrA	334
apo 8-100 gene mutation	$Ru(bpy)_3^{2+}$/TPrA	310
cancer antigen 15-3	$Ru(bpy)_3^{2+}$/TPrA	251, 352
cancer antigen 19-9	$Ru(bpy)_3^{2+}$/TPrA	251
cancer antigen 72-4	$Ru(bpy)_3^{2+}$/TPrA	350
cancer antigen 125	$Ru(bpy)_3^{2+}$/TPrA	353, 354
carcinoembryonic antigen	$Ru(bpy)_3^{2+}$/TPrA	251, 252
β-crosslaps	$Ru(bpy)_3^{2+}$/TPrA	278, 279
C-telopeptides	$Ru(bpy)_3^{2+}$/TPrA	280
CKMB, creatine base	$Ru(bpy)_3^{2+}$/TPrA	252, 271
anti-CMV antibodies	$Ru(bpy)_3^{2+}$/TPrA	284
CMV DNA	$Ru(bpy)_3^{2+}$/TPrA	292
CMV mRNA	$Ru(bpy)_3^{2+}$/TPrA	215
cytokeratin 19	$Ru(bpy)_3^{2+}$/TPrA	260
coxsackievirus B3 RNA	$Ru(bpy)_3^{2+}$/TPrA	423
cytomegalovirus	$Ru(bpy)_3^{2+}$/TPrA	288
des-γ-carboxy	$Ru(bpy)_3^{2+}$/TPrA	261, 262
dengue virus RNA	$Ru(bpy)_3^{2+}$/TPrA	295
enterovirus	$Ru(bpy)_3^{2+}$/TPrA	297
Epstein−Barr virus DNA	$Ru(bpy)_3^{2+}$/TPrA	293
estradiol	$Ru(bpy)_3^{2+}$/TPrA	263
ferritin	$Ru(bpy)_3^{2+}$/TPrA	251
FSH, follitropin	$Ru(bpy)_3^{2+}$/TPrA	263
foot-and-mouth disease	$Ru(bpy)_3^{2+}$/TPrA	424
glucose	$Ru(bpy)_3^{2+}$/TPrA	425
HCG	$Ru(bpy)_3^{2+}$/TPrA	252, 265
creatine kinease muscle brain	$Ru(bpy)_3^{2+}$/TPrA	252, 265
HBsAg, hepatitis B virus surface antigen	$Ru(bpy)_3^{2+}$/TPrA	275−277, 289
HAsAg, hepatitis A virus surface antigen	$Ru(bpy)_3^{2+}$/TPrA	215
HIV 1 p7 antigen	$Ru(bpy)_3^{2+}$/TPrA	334
HIV 1 RNA	$Ru(bpy)_3^{2+}$/TPrA	291, 301
HIV DNA	$Ru(bpy)_3^{2+}$/TPrA	290, 300−302
IgE, immunoglobulin E	$Ru(bpy)_3^{2+}$/TPrA	426
influenza virus RNA	$Ru(bpy)_3^{2+}$/TPrA	298−299
insulin	$Ru(bpy)_3^{2+}$/TPrA	283, 427
IL (interleukin)-18 binding protein	$Ru(bpy)_3^{2+}$/TPrA	428
IL (interleukin)-6	$Ru(bpy)_3^{2+}$/TPrA	251
IL (interleukin)-8	$Ru(bpy)_3^{2+}$/TPrA	251
IL (interleukin)-10	$Ru(bpy)_3^{2+}$/TPrA	428, 429
Legionella antigen	$Ru(bpy)_3^{2+}$/ triethylamine	346
LH, lutropin	$Ru(bpy)_3^{2+}$/TPrA	263
osteocalcin	$Ru(bpy)_3^{2+}$/TPrA	280
mRNA	$Ru(bpy)_3^{2+}$/TPrA	306−308
pancreatic phospholipase A2	terbium chelate	319
parathyroid hormone	$Ru(bpy)_3^{2+}$/TPrA	430
prolactin	$Ru(bpy)_3^{2+}$/TPrA	263
prostrate-specific antigen	$Ru(bpy)_3^{2+}$/TPrA	252, 255
prothrombin	$Ru(bpy)_3^{2+}$/TPrA	261, 262
prothrombin gene mutation	$Ru(bpy)_3^{2+}$/TPrA	311
serum interferon-α	$Ru(bpy)_3^{2+}$/TPrA	428
St. Louis encephalitis	$Ru(bpy)_3^{2+}$/TPrA	296
T4, thyroxine	$Ru(bpy)_3^{2+}$/TPrA	252, 266, 268, 270
T3, triiodothyronine	$Ru(bpy)_3^{2+}$/TPrA	252, 266, 268
thyroid-stimulating hormone	$Ru(bpy)_3^{2+}$/TPrA	252, 266, 268
thyroid-stimulating hormone	terbium chelate	269
testosterone	$Ru(bpy)_3^{2+}$/TPrA	263, 264
troponin	$Ru(bpy)_3^{2+}$/TPrA	252, 271−274
tumor necrosis factor-α	$Ru(bpy)_3^{2+}$/TPrA	431
Varicella-Zoster virus DNA	$Ru(bpy)_3^{2+}$/TPrA	295
West Nile virus RNA	$Ru(bpy)_3^{2+}$/TPrA	296
ΔF508 deletion	$Ru(bpy)_3^{2+}$/TPrA	312

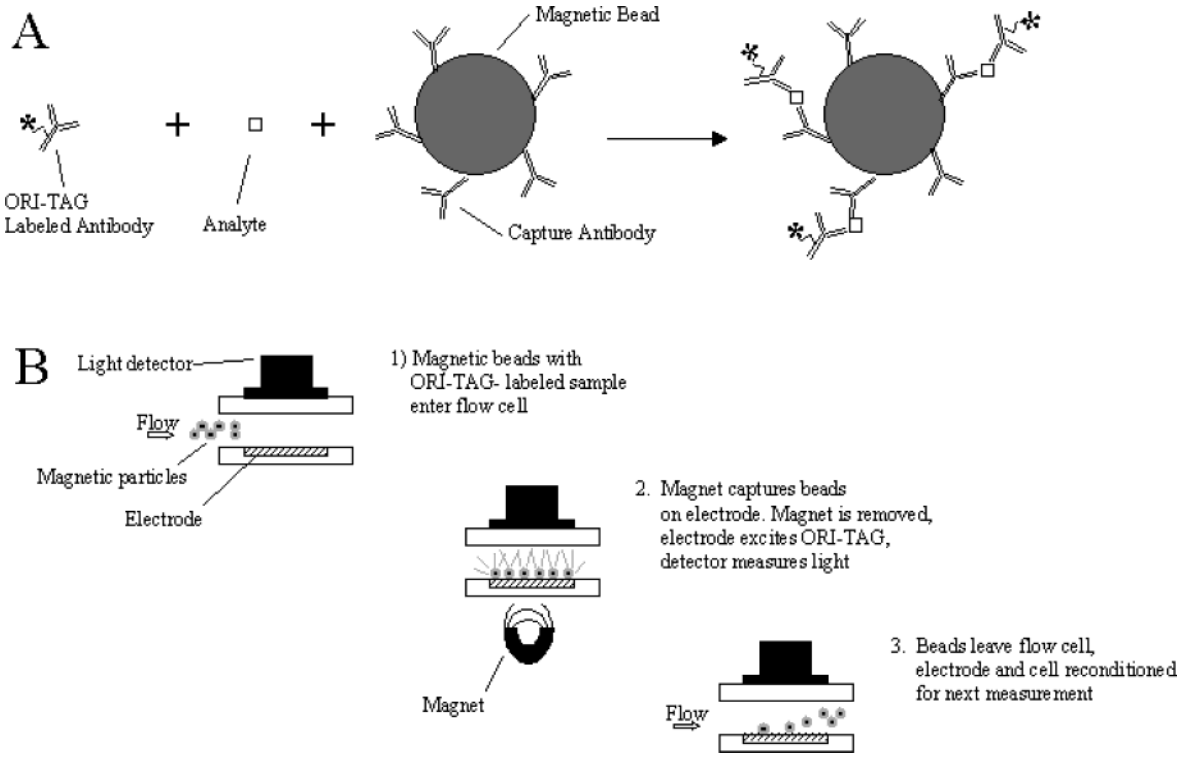

Figure 25. (A) Representation of ECL "sandwich" (antibody−antigen/analyte−antibody) assay. ORI-TAG refers to ECL label. (B) Magnetic bead/flow cell ECL process (courtesy of BioVeris Corp.).

ECL methods to detect other clinically relevant analytes not grouped under immunoassays and molecular assays including heavy metals[96] (toxicological) and electrolytes[95,96] have also appeared (CE-bpy and AZA -bpy in Table 1 and Figures 11 and 12). For example, ECL has been used to measure electrolytes such as sodium and potassium in the parts per million range by coupling a $Ru(bpy)_3^{2+}$ luminophore to crown ether moieties capable of binding the electrolytes of interest. These types of complexes might also be useful in water safety applications.

Although $Ru(bpy)_3^{2+}$ is the most widely used ECL luminophore, assays have also been developed for pancreatic phospholipase[319] and thyroid-stimulating hormone[266] that use a terbium chelate. Organic ECL complexes have been proposed for a range of antioxidant assays that quench anthracene ECL upon electrolysis of sodium citrate, methanol, and dissolved oxygen.[320] Indole and tryptophan have also been shown to generate ECL upon electrolysis in the presence of hydrogen peroxide, with detection limits of 0.1 μM for both indole and tryptophan,[162] and sensors have been reported for a range of alcohols and saccharides because hydroxyl compounds have been shown to generate ECL directly.[321]

ECL is a valuable tool in life science research laboratories to study basic physiology mechanisms and to detect, discover the causes for, and find potential cures for diseases.[322−335] For example, sandwich immunoassays have been used to detect numerous cytokines in serum,[324] cell culture supernatant,[322,323] and blood[325] for potential use in diagnostic testing. Cytokines are regulatory proteins produced by white blood cells and regulate embryogenesis, hameotosis, tissue repair, inflammation, and immune response. The principles of a typical sandwich assay for an antigen are outlined in Figure 25A. Magnetic beads modified by attaching an antibody for a particular antigen of interest (e.g., α-fetoprotein, AFP), the sample of interest, and $Ru(bpy)_3^{2+}$-labeled antibodies are mixed. If the antigen of interest is present, it acts as a bridge to form the "sandwich" structure, and the antibody labeled with ECL luminophore becomes attached to the magnetic bead. If no antigen is present, the labeled antibody does not attach to the bead. These labeled and unlabeled beads are then flushed into an ECL flow cell, where they are captured on the working electrode by positioning a magnet beneath the working electrode (Figure 25B). The beads are washed to remove any unattached $Ru(bpy)_3^{2+}$-labeled antibodies as well as other reaction components, and a solution of the appropriate composition containing a coreactant (usually TPrA) is pumped into the cell. The concentration of the coreactant is kept constant and high (e.g., ≥50 mM) to maximize the sensitivity of the detection and to prevent fluctuations in concentration of the coreactant from changing the ECL. The electrode is then swept to positive potentials to initiate ECL, and the intensity of the emitted light is measured with a photomultiplier tube. In these measurements, the number of photons generated by the ECL labels is directly proportional to the concentration of analyte. The magnetic beads are then washed from the cell, which is cleaned and made ready for the next sample. Typically, the electrode is cleaned by first flushing the flow cell with a dilute (∼0.1 M) solution of sodium hydroxide and then applying a potential to the working electrode sufficiently cathodic and anodic to destroy any material that has built up on the electrode.[2,34] Sulfuric acid has also been used to clean electrodes in ECL experiments. Typically, the work-

ing and auxiliary electrodes are placed on concentrated sulfuric acid and the potential swept to positive (~+2.0 V) and negative (~-2.0 V) potentials using cyclic voltammetry. This effectively cleans the electrode by producing reactive intermediates in the oxidation cycle (e.g., hydroxyl radicals) that oxidize organics.[336] The extreme potentials also desorb species from the electrode surface. The oxidation and reduction of the Pt surface in H_2SO_4 also breaks it up to produce a more active surface.

DNA has also been detected in a microfabricated cell.[337−339] The cell was composed of glass and silicon, with thin-film Pt working electrodes. A silicon PIN photodiode was used as the light collector. Using streptavidin-coated magnetic beads to capture the biotinylated primers with attached $Ru(bpy)_3^{2+}$ labels, significantly greater detection limits could be observed for the microfabricated system compared to commercial flow cell systems (e.g., Origen Analyzer).

Fundamental studies aimed at the development of portable sensors and probes for use in point-of-care clinical analyses (such as hospital examining rooms)[2] and environmental applications[221] have also been reported. For example, a gold-coated fiber optic probe for the measurement of $Ru(bpy)_3^{2+}$ in the presence of peroxydisulfate has been developed.[340] Also, a miniaturized fiber optic sensor has been developed and applied to the determination of oxalate using $Ru(bpy)_3^{2+}$ in urine samples with a limit of detection of 3×10^{-5} M.[341] A thin-layer flow cell using a planar optical waveguide coated with an ITO layer was fabricated. The ITO layer was modified by covalently attaching glucose oxidase and ECL measured from luminol at the end of the waveguide.[342] The generation of $Ru(bpy)_3^{2+}$ ECL on interdigitated gold microelectrodes mounted above a photodiode has been observed, with a limit of detection for $Ru(bpy)_3^{2+}$ of 0.5 μM.[42] An ECL cell with gold and optically transparent indium tin oxide coated glass electrodes incorporating a photodiode has also been fabricated[343] for use in the quantification of DNA labeled with $Ru(bpy)_3^{2+}$. Reported limits of detection were 1 nM, with a cell volume of 85 μL. Cell volumes as low as 100 nL have also been reported[344] with sub-picomolar $Ru(bpy)_3^{2+}$ detection limits using a cell composed of a sandwich of poly(methyl methacrylate) layers containing two platinum thin-film electrodes, connected to a conventional flowing systems using photomultiplier tube detection. In fact, a commercial ECL system approximately $^1/_{20}$ the size of the original Origen instrument has been developed (the TRICORDER detection system, BioVeris Corp.). This system is self-contained and has an accuracy and sensitivity equal to those of the Origen analyzer. A miniaturized ECL cell based on the ECL of $Ru(bpy)_3^{2+}$ has also been developed for the determination of peptides having proline at the amino terminal,[345] and a membrane strip immunosensor utilizing a $Ru(bpy)_3^{2+}$ label used for the determination of *Legionella antigen* has been reported.[346]

4.2. Food and Water Safety and Military/Defense Applications

ECL coupled with magnetic bead separation has also been used to develop assays for a variety of biotoxoids that are important for environmental, food, and water industries and military applications. For example, assays for bovine leutenizing hormone show greater sensitivity with ECL than standard radioimmunoassays.[347] Also, several authors have reported extremely sensitive ECL assays for bacterial and viral species[348−350] as well as cancer antigens[351−354] in a variety of matrices using both immunoassays and nucleic acid amplification techniques. Species such as anthrax (*Bacillus anthracis*),[82,355−360] *Escherichia coli* O157,[215,357,358,361−364] and *Salmonella typhimurium*,[215,361,365] *Cryptosporidium parvum* oocysts,[318,366,367] campylobacter,[215] listeria monocytogenes,[215] *Staphylococcus aureus* enterotoxins,[215,82,368] Botulinus A toxin,[82,356] cholera toxin,[82,356] and ricin toxin[82,356] have been reported with limits of detection and assay sensitivities equal to or greater than those of conventional assays using flow cytometry, enzyme-linked immunosorbent assays (ELISA), and radioallergosorbent test (RAST). *E. coli* 0157 tests, for example, have been reported for drinking[363] and creek[362] water, feces,[328] various food and environmental water matrices,[215,357−359] and ground beef, chicken, fish, juices, and milk.[361,364]

Another potential military application for ECL is in the detection of explosives or explosives' degradation products. For example, diaminotoluene isomers form weakly electrochemiluminescent compounds in the presence of Au^+ and Cu^{2+} ions.[369] Because amino-aromatic compounds such as diaminotoluene are often associated with the degradation of explosives such as TNT, this approach may find use in military applications.

Interestingly, magnetic particle ECL has been used in combination with systematic evolution of ligands by exponential enrichment (SELEX).[370] In lieu of protein-based antibodies for immunoassays, highly specific receptor ligands (known as aptamers) were formed from oligonucleotides. The aptamers were formed with a library of randomly synthesized oligonucleotides coupled with amplification of promising binding receptors using PCR. An assay for anthrax spores using this technology and an Origen analyzer resulted in a dynamic range of $10-6 \times 10^6$ spores.

5. Concluding Remarks

Since the first detailed studies in the mid-1960s ECL has moved from being a laboratory curiosity to a useful, and commercially viable, analytical technique. It offers many advantages for the detection of a wide range of analytes. It is clear from the amount of activity in the past decade alone that ECL continues to be an area of active research. It provides a powerful tool for understanding fundamental questions in chemistry, biology, and physics. For example, studies to find new ECL light-emitting molecules and coreactants, to elucidate the nature of excited state formation and light emission as well as coreactant mechanisms, and to improve the sensitivity and selectivity of ECL will undoubtedly continue. The commercial interest in using ECL reactions in clinical and biomedical diagnostics has catalyzed interest in expanding ECL to detect numerous chemical and biochemical analytes including those important for

food and water safety, environmental monitoring, and military/defense applications.

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7. Abbreviations

[2.2.2] = 4,7,13,16,21,24-hexaoxa-1,10-diazacyclo[8,8,8]-hexacosane
[2.2.1] = 4,7,13,16,21-hexaoxa-1,10-diazacyclo[8,8,5]tricosane
ABEI = *N*-(4-aminobutyl)-*N*-etylisoluminol
AFP = α-fetoprotein
anti-HAV = antibody heptatitis A virus
anti-HBc = antibody hepatitis B core antigen
anti-HBe = antibody hepatitis B "e" antigen
anti-HBs = antibody hepatitis B surface antigen
AZA-bpy = 4-(*N*-aza-18-crown-6-methyl-2,2′-bipyridine
BA = benzoyl acetate
BADE = 9,9′-bisacridinium-*N*,*N*′-diacetic acid ethyl ester
bphb = 1,4-bis(4′-methyl-2,2′-bipyridin-4-yl)benzene
BPQ-PTZ = 3,7-[bis(4-phenyl-2-quinolyl)]-10-methylphenothiazine
bpy = 2,2′-bipyridine
bpz = 2,2′-bipyrazine
BSA = bovine serum albumin
BTA = benzoyltrifluoroacetate
Bu_4N^+ = tetra-*n*-butylammonium ion
$C_2O_4^{2-}$ = oxalate ion
CE-bpy = bipyridine ligand where a crown ether (15-crown-5) is bound to the bpy ligand in the 3- and 3′-positions
5-Cl-phen = 5-chloro-1,10-phenanthroline
cmc = critical micelle concentration
COD = 1,5-cyclooctadiene
crypt = a cryptand ligand, e.g., 4,7,13,16,21-pentaoxa-1,-10-diazabicyclo[8,8,5]tricosane
CTAB = cetyltrimethylammonium bromide
CYFRA = cytokeratin fragment
DALM = diazoluminomelanin with a luminol pendant group
2,4-DAT = 2,4-diaminotoluene
3,4-DAT = 3,4-diaminotoluene
dba = dibenzylideneacetone
DBM = dibenzoylmethide
DC-bpy = 4,4′-dicarboxy-2,2′-bipyridine
diphos = 1,2-bis(dihenylphosphino)ethane
dmbp, 4,4′-Me_2bpy, and DM-bpy = 4,4′-dimethyl-2,2′-bipyridine
2,9-Me_2phen = 2,9-dimethyl-1,10-phenanthroline
4,7-Me_2phen = 4,7-dimethyl-1,10-phenanthroline
5,6-Me_2phen = 5,6- dimethyl-1,10-phenanthroline
3,4,7,8-Me_4phen = 3,4,7,8-tetramethyl-1,10-phenanthroline
DMF = dimethylformamide
dmp = 2,9-dimethyl-1,10-phenanthroline
DMSO = dimethyl sulfoxide
dmphen = 4,7-dimethyl-1,10-phenanthroline
Dmpz = 3,5-dimethylpyrazole
DNA = deoxyribonucleic acid
dpae = bis(diphenylarsinoethane)
DPAS = 9,10-diphenylanthracene-2-sulfonate
DPBF = 7,12-diphenylbenzo[*k*]fluoranthene
DPBF-2 = 4,4′-(7,12-diphenyl)benzol[*k*]fluoranthene
DPBF- 3 = dibenzo{[*f*,*f* ′]-4,4′,7,7′-tetraphenyl}diindeno-[1,2,3-*cd*:1′,2′,3′-*lm*]perylene
DPBF-4 = 9,10-dimethyl sulfone-7,12-diphenylbenzo[*k*]-fluoranthene
dp-bpy = 4,4′-biphenyl-2,2′-bipyridyl
dp-phen = 4,7-diphenyl-1,10-phenanthroline
dppene = 1,2-*cis*-bis(2-diphenylphosphinoethylene)
dppm = 1,2-bis(diphenylphosphino)methane
dsDNA = double-stranded DNA
ELISA = enzyme-linked immunoadsorbent assay
FDA = U.S. Food and Drug Administration
HBeAg = hepatitis B "e" antigen
HDEA = 6-(2-hydroxy-4-diethylaminophenylazo)-2,3-diydro-1,4-phthalazine-1,4-dione
HFAC = hexafluoroacetylacetate
HQS = 8-hydroxyquinoline-5sulfonic acid
IA = immunoassay
IgM = immunoglobulin M
IM = immunomagnetic
ITO = indium-doped tin oxide electrode
Lys = lysine
LysLys = lysine dipeptide
4′-Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine
MLCT = metal-to-ligand charge transfer
MFPA = methyl-9-(*p*-formylphenyl)acridinium carboxylate fluorosulfonate
mpz = 3-methylpyrazole
mRNA = messenger RNA
NCs = nanocrystals
NSOM = near-field optical scanning microscopy
NSE = neuron-specific enolase
OEP = octaethyl porphyrin
OR = OSi(*n*-C_6H_{13})$_3$
PCR = Polymerase Chain Reaction
PI = perylenedicarboxylic imide
PDI = perylenetetracarboxylic diimide
phen = 1,10-phenanthroline (often referred to as o-phen in the literature)
pHPP = *p*-hydroxyphenylpyruvic acid
$pipH^+$ = piperidine cation
ppy = 2-phenylpyridine
$P_2O_5H_2$ = pyrophosphito
ProBNP = brain natriuretic peptide
Pz = pyrazole
q = 8-hydroxyquinoline
QDI = quaterrylenecarboxylic diimide
RNA = ribonucleic acid
SAM = self-assembled monolayer
SDS = sodium dodecyl sulfate
SECM = scanning electrochemical microscopy
SELEX = systematic evolution of ligands by exponential enrichment
[SiPc(OR)$_2$] = bis(tri-*n*-hexylsiloxy)(2,3-phthalocyanato)-silicon
$S_2O_8^{2-}$ = persulfate or peroxydisulfate
SPBA = *N*,*N*′-di-(3-sulfopropyl)-9,9′-bisacridinium
ssDNA = single-stranded DNA
tbaa = tribenzylideneacetylacetone.
TCPO = bis(2,4,6-trichlorophenyl)oxalate
terpy = 2,2′,2″-terpyridine
TDI = terrylenetetracarboxylic diimide
1- and 2-THCOOH = 1- and 2-thianthrenecarboxylic acid
$THAClO_4$ = tetra-*n*-hexylammonium perchlorate
THF = tetrahydrofuran
$TPAPF_6$ = tetra-*n*-butylammonium hexaflurophosphate
TPD = 4,4′-bis(*m*-tolylphenylamino)biphenyl

TPOP = trioctylphosphineoxide
TPP = α,β,γ,δ-tetraphenylporphyrin complexes
TPrA = tri-*n*-propylamine
TPTZ = 2,4,6-tripyridyl-2-triazine
Triton X-100 = poly(ethylene glycol) *tert*-octylphenyl ether
TTA = thenoyltrifluoroacetate
TTFA = 4,4,4-trifluoro-1-(2-thienyl)-1,5-butanediono/thenoyltrifluoroacetonato
v-bpy = 4-vinyl-4′-methyl-2,2′-bipyridine

8. References

(1) Leland, J. K.; Powell, M. J. *J. Electroanal. Chem.* **1991**, *318*, 91.
(2) Bard, A. J.; Debad, J. D.; Leland, J. K.; Sigal, G. B.; Wilbur, J. L.; Wohlstadter, J. N. *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; Wiley: Chichester, U.K., 2000; p 9842.
(3) Faulkner, L. R.; Bard, A. J. *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1977; Vol. 10, pp 1−95.
(4) Faulkner, L. R.; Glass, R. S. In *Chemical and Biological Generation of Excited States*; Waldemar, A., Giuseppe, C., Eds.; Academic Press: New York, 1982; Chapter 6.
(5) (a) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980. (b) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods Fundamentals and Applications*, 2nd ed.; Wiley: New York, 2001.
(6) Knight, A. W.; Greenway, G. M. *Analyst* **1994**, *119*, 879.
(7) Gerardi, R. D.; Barnett, N. W.; Lewis, A. W. *Anal. Chim. Acta* **1999**, *378*, 1.
(8) Kukoba, A. V.; Bykh, A. I.; Svir, I. B. *Anal. Bioanal. Chem.* **2000**, *368*, 439.
(9) Mitschke, U.; Bauerle, P. *J. Mater. Chem.* **2000**, *10*, 1471.
(10) Aboul-Enein, H.; Stefan, R. I.; van Staden, J. F.; Zhang, X. R.; Garcia-Campana, A. M.; Baeyens, W. R. G. *Crit. Rev. Anal. Chem.* **2000**, *30*, 271.
(11) Knight, A. W. *Electrogenerated Chemiluminescence in Chemiluminescence in Analytical Chemistry*; Grarcia-Campana, A. M., Baeyens, W. R. G., Eds.; Dekker: New York, 2001; pp 211−247.
(12) Andersson, A.-M.; Schmehl, R. H. Sensors based on electrogenerated chemiluminescence. In *Molecular and Supramolecular Photochemistry 7: Optical Sensors and Switches*; Ramamurthy, V., Schanze, K. S., Eds.; 2001, pp 153−187.
(13) Fahnrich, K. A.; Pravda, M.; Guilbault, G. G. *Talanta* **2001**, *54*, 531.
(14) Richter, M. M. Electrochemiluminescence. In *Optical Biosensors: Present and Future*; Ligler, F. S., Rowe-Taitt, C. A., Eds.; Elsevier: New York, 2002; pp 173−205.
(15) *Electrogenerated Chemiluminescence*; Bard, A. J., Ed; Dekker: New York, in press.
(16) Kulmala, S.; Suomi, J. *Anal. Chim. Acta* **2003**, *500*, 21.
(17) Knight, A. W. *Trends Anal. Chem.* **1999**, *18*, 47.
(18) Tokel, N.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 2862.
(19) (a) Demas, J. N.; Crosby, G. A. *J. Mol. Spectrosc.* **1968**, *26*, 72. (b) Demas, J. N.; Crosby, G. A. *J. Am. Chem. Soc.* **1971**, *93*, 2841.
(20) Roundhill, D. M. *Photochemistry and Photophysics of Coordination Complexes*; Plenum: New York, 1994; Chapter 5.
(21) Van Houten, J.; Watts, R. J. *J. Am. Chem. Soc.* **1975**, *98*, 4853.
(22) Hercules, D. M. *Science* **1964**, *143*, 308.
(23) Chandross, E. A.; Visco, R. E. *J. Am. Chem. Soc.* **1964**, *86*, 5350.
(24) Santhanam, K. S. V.; Bard, A. J. *J. Am. Chem. Soc.* **1965**, *87*, 139.
(25) Bader, J. M.; Kuwana, T. *J. Electroanal. Chem.* **1965**, *10*, 104.
(26) Maricle, D. L.; Maurer, A. *J. Am. Chem. Soc.* **1967**, *89*, 188.
(27) Bezman, R.; Faulkner, L. R. *J. Am. Chem. Soc.* **1972**, *94*, 6324.
(28) Luttmer, J. D.; Bard, A. J. *J. Phys. Chem.* **1981**, *85*, 1155.
(29) Glass, R. S.; Faulkner, L. R. *J. Phys. Chem.* **1981**, *85*, 1160.
(30) Wallace, W. L.; Bard, A. J. *J. Phys. Chem.* **1979**, *83*, 1350.
(31) Itoh, I.; Honda, K. *Chem. Lett.* **1979**, 99.
(32) Meyer, T. J. *Acc. Chem. Res.* **1978**, *11*, 94.
(33) Laser, D.; Bard, A. J. *J. Electrochem. Soc.* **1975**, *122*, 632.
(34) McCord, P. M.; Bard, A. J. *J. Electroanal. Chem.* **1991**, *318*, 91.
(35) Ritchie, E. L.; Pastore, P.; Wightman, R. M. *J. Am. Chem. Soc.* **1997**, *119*, 11920.
(36) Parker, C. A. *Photoluminescence of Solutions*; Elsevier: Amesterdam, the Netherlands, 1968.
(37) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: New York, 1970.
(38) Gross, E. M.; Anderson, J. D.; Slaterbeck, A. F.; Thayumanavan, S.; Barlow, S.; Zhang, Y.; Marder, S. R.; Hall, H. K.; Flore Nabor, M.; Wang, J.-F.; Mash, E. A.; Armstrong, N. R.; Wightman, R. M. *J. Am. Chem. Soc.* **2000**, *122*, 4972.
(39) Maloy, J. T.; Prater, K. B.; Bard, A. J. *J. Am. Chem. Soc.* **1971**, *93*, 5959.
(40) Brilmyer, G. H.; Bard, A. J. *J. Electrochem. Soc.* **1980**, *127*, 104.
(41) Bartelt, J. E.; Drew, S. M.; Wightman, R. M. *J. Electrochem. Soc.* **1992**, *139*, 70.
(42) Fioccabrino, G. C.; Koudelka-Hep, M.; Hsueh, Y.-T.; Collins, S. D.; Smith, R. L. *Anal. Chem.* **1998**, *70*, 4157.
(43) Chovin, A.; Garrigue, P.; Vinatier, P.; Sojic, N. *Anal. Chem.* **2004**, *76*, 357.
(44) Collinson, M. M.; Wightman, R. M. *Anal. Chem.* **1993**, *65*, 2576.
(45) Collinson, M. M.; Wightman, R. M. *Science* **1995**, *682*, 1883.
(46) Maness, K. M.; Wightman, R. M. *J. Electroanal. Chem.* **1995**, *396*, 85.
(47) Fan, F.-R. F.; Cliffel, D.; Bard, A. J. *Anal. Chem.* **1998**, *70*, 2941.
(48) Maus, R. G.; McDonald, E. M.; Wightman, R. M. *Anal. Chem.* **1999**, *71*, 4944.
(49) Wightman, R. M.; Curtis, C. L.; Flowers, P. A.; Maus, R. G.; McDonald, E. M. *J. Phys. Chem. B* **1991**, *102*, 9991.
(50) Zu, Y.; Ding, Z.; Zhou, J.; Lee, Y.; Bard, A. J. *Anal. Chem.* **2001**, *73*, 2153.
(51) Sykora, M.; Meyer, T. J. *Chem. Mater.* **1999**, *11*, 1186.
(52) Maness, K. M.; Terrill, R. H.; Meyer, T. J.; Murray, R. W.; Wightman, R. M. *J. Am. Chem. Soc.* **1996**, *118*, 10609.
(53) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 512.
(54) Smith, P. J.; Mann, C. K. *J. Org. Chem.* **1969**, *34*, 1821.
(55) Miao, W.; Choi, J.-P.; Bard, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 14478.
(56) Kanoufi, F.; Zu, Y.; Bard, A. J. *J. Phys. Chem. B* **2001**, *105*, 210.
(57) Zu, Y.; Bard, A. J. *Anal. Chem.* **2000**, *72*, 3223.
(58) Knight, A. W.; Greenway, G. M. *Analyst* **1996**, *121*, 101R.
(59) White, H. S.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *104*, 6891.
(60) Bolletta, F.; Rossi, A.; Balzani, V. *Inorg. Chim. Acta* **1981**, *53*, L23.
(61) Richter, M. M.; Debad, J. D.; Striplin, D. R.; Crosby, G. A.; Bard, A. J. *Anal. Chem.* **1996**, *68*, 4370.
(62) Richter, M. M.; Bard, A. J.; Kim, W.; Schmehl, R. S. *Anal. Chem.* **1998**, *70*, 310.
(63) Kulmala, S.; Ala-Kleme, T.; Vare, L.; Helin, M.; Lehtinen, T. *Anal. Chim. Acta* **1999**, *398*, 41.
(64) (a) Kankare, J.; Falden, K.; Kulmala, S.; Haapakka, K. *Anal. Chim. Acta* **1992**, *256*, 17. (b) Kankare, J.; Haapakka, K.; Kulmala, S.; Nanto, V.; Eskola, J.; Takalo, H. *Anal. Chim. Acta* **1992**, *266*, 205.
(65) Sung, Y.-E.; Gaillard, F.; Bard, A. J. *J. Phys. Chem. B* **1998**, *102*, 9797.
(66) Gaillard, F.; Sung, Y.-E.; Bard, A. J. *J. Phys. Chem. B* **1999**, *103*, 667.
(67) Richter, M. M. Metal chelates. In *Electrogenerated Chemiluminescence*; Bard, A. J., Ed.; Dekker: New York, 2004 (in press); Chapter 6.
(68) See, for example: Downey, T. M.; Nieman, T. A. *Anal. Chem.* **1992**, *64*, 261.
(69) See, for example: (a) Danielson, N. D.; He, L.; Noffsinger, J. B.; Trelli, L. *J. Pharm. Biomed. Anal.* **1989**, *7*, 1281−1285. (b) Knight, A. W. *Trends Anal. Chem.* **1999**, *18*, 47.
(70) Dixon, S. B.; Sanford, J.; Swift, B. W. *Principles and Practices for Petroleum Contaminated Soils*; Calabrese, E. J., Kosteki, P. T., Eds.; Lewis Publishers: Boca Raton, FL, 1993; p 85.
(71) (a) Alexander, C.; Richter, M. M. *Anal. Chim. Acta* **1999**, *402*, 105. (b) McCall, J.; Alexander, C.; Richter, M. M. *Anal. Chem.* **1999**, *71*, 2523. (c) McCall, J.; Richter, M. M. *Analyst* **2000**, *125*, 545.
(72) Workman, S.; Richter, M. M. *Anal. Chem.* **2000**, *72*, 5556.
(73) Zu, Y.; Bard, A. J. *Anal. Chem.* **2001**, *73*, 3960.
(74) Factor, B.; Muegge, B.; Workman, S.; Bolton, E.; Bos, J.; Richter, M. M. *Anal. Chem.* **2001**, *73*, 4621.
(75) Cole, C.; Muegge, B. D.; Richter, M. M. *Anal. Chem.* **2003**, *75*, 601
(76) Walworth, J.; Brewer, K. J.; Richter, M. M. *Anal. Chim. Acta* **2004**, *503*, 241.
(77) Malins, C.; Vandeloise, R.; Walton, D.; VanderDonckt, E. *J. Phys. Chem. A* **1997**, *101*, 5063.
(78) Walton, D. J.; Phull, S. S.; Bates, D. M.; Lorimer, J. P.; Mason, T. J. *Ultrasonics* **1992**, *30*, 186.
(79) Xu, X. H.; Bard, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2627.
(80) Xu, X. H.; Yang, H. C.; Mallouk, T. E.; Bard, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 8386.
(81) Fahnrich, K.; O'Sullivan, C. K.; Guilbault, G. G. *SAC99*; Dublin, Ireland, July 1999; p PC20.
(82) Miao, W.; Bard, A. J. *Anal. Chem.* **2003**, *75*, 5825.
(83) Dennany, L.; Forster, R. J.; Rusling, J. F. *J. Am. Chem. Soc.* **2003**, *125*, 5213.
(84) Geise, B. *Acc. Chem. Res.* **2000**, *33*, 631.
(85) Benzaquen, L. R.; Yu, H.; Rifai, N. *Crit. Rev. Clin. Lab. Sci.* **2000**, *39*, 459.
(86) Scott, A. M.; Pyati, R. *J. Phys. Chem. B* **2001**, *105*, 9011.
(87) Collinson, M. M.; Taussig, J. S.; Martin, S. A. *Chem. Mater.* **1999**, *11*, 2594.
(88) Collinson, M. M.; Novak, B.; Martin, S. A.; Taussig, J. S. *Anal. Chem.* **2000**, *72*, 2914.
(89) Khramov, A. N.; Collinson, M. M. *Anal. Chem.* **2000**, *72*, 2943.
(90) Gonzales-Velasco, J.; Rubinstein, I.; Crutchley, R. J.; Lever, A. B. P.; Bard, A. J. *Inorg. Chem.* **1983**, *22*, 822.
(91) Gonzalez-Velasco, J. *J. Phys. Chem.* **1988**, *92*, 2202.

(92) Yamazaki-Nishida, S.; Harima, Y.; Yamashita, K. *J. Electroanal. Chem.* **1990**, *283*, 455.
(93) Yamashita, K.; Yamazaki-Nishida, S.; Harima, Y.; Segawa, A. *Anal. Chem.* **1991**, *63*, 872.
(94) Anderson, A.-M.; Isovitsch, R.; Miranda, D.; Wadhwa, S.; Schmehl, R. H. *J. Chem. Soc., Chem. Commun.* **2000**, 505.
(95) Lai, R. Y.; Chiba, M.; Kitamura, N.; Bard, A. J. *Anal. Chem.* **2002**, *74*, 551.
(96) (a) Muegge, B. D.; Richter, M. M. *Anal. Chem.* **2002**, *74*, 547−550. (b) Bruce, D.; Richter, M. M. *Analyst* **2002**, *127*, 1492.
(97) Kanoufi, F.; Bard, A. J. *J. Phys. Chem. B* **1999**, *103*, 10469.
(98) Zhou, M.; Roovers, J. *Macromolecules* **2001**, *34*, 244.
(99) Zhou, M.; Roovers, J.; Robertson, G. P.; Grover, C. P. *Anal. Chem.* **2003**, *75*, 6708.
(100) Staffilani, M.; Hoss, E.; Giesen, U.; Schneider, E.; Harti, F.; Josel, H.-P.; De Cola, L. *Inorg. Chem.* **2003**, *42*, 7789.
(101) Bruce, D.; McCall, J.; Richter, M. M. *Analyst* **2002** *127*, 125.
(102) Abruna, H. D. *J. Electroanal. Chem.* **1984**, *175*, 321.
(103) Bruce, D.; Richter, M. M.; Brewer, K. J. *Anal. Chem.* **2002**, *74*, 3157.
(104) Lee, C.-W.; Ouyang, J.; Bard, A. J. *J. Electroanal. Chem.* **1988**, *244*, 319.
(105) Heller, A. *Faraday Discuss.* **2000**, *116*, 1.
(106) (a) Creutz, C.; Chou, M.; Netzel, T. L.; Okumara, M.; Sutin, N. *J. Am. Chem. Soc.* **1980**, *102*, 1309. (b) Kober, E. M.; Meyer, T. J. *Inorg. Chem.* **1982**, *21*, 3967.
(107) Abruna, H. D. *J. Electrochem. Soc.: Electrochem. Sci. Technol.* **1985**, *132*, 842.
(108) Ouyang, J.; Bard, A. J. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 17.
(109) King, K. A.; Spellane, P. J.; Watts, R. J. *J. Am. Chem. Soc.* **1985**, *107*, 1431.
(110) Vogler, A.; Kunkely, H. Electrochemiluminescence of organometallics and other transition metal complexes. In *High Energy Processes in Organometallic Chemistry*; Suslick, K. S., Ed.; ACS Symposium Series 333; American Chemical Society: Washington, DC, 1987; p 155.
(111) (a) Baldo, M. A.; Lamansky, S.; Burrows, P. E.; Thompson, M. E.; Forrest, S. R. *Appl. Phys. Lett.* **1999**, *75*, 4. (b) Grushin, V. V.; Herron, N.; LeCloux, D. D.; Marshall, W. J.; Petrov, V. A.; Wang, Y. *Chem. Commun.* **2001**, 1494.
(112) Nishimura, K.; Hamada, Y.; Tsujioka, T.; Shibata, K.; Fuyuki, T. *Jpn. J. Appl. Phys.* **2001**, *40*, L945.
(113) Gross, E. M.; Armstrong, N. R.; Wightman, R. M. *J. Electrochem. Soc.* **2002**, *149*, E137.
(114) Bruce, D.; Richter, M. M. *Anal. Chem.* **2002**, *74*, 1340.
(115) Palecek, E. In *Topics in Bioelectrochemistry and Bioenergetics*; Milazzo, G., Ed.; Wiley: London, U.K., 1983; Vol. 5, p 65.
(116) Kapturkiewicz, A.; Angulo, G. *Dalton Trans.* **2003**, 3907).
(117) Muegge, B. D.; Richter, M. M. *Anal. Chem.* **2004**, *76*, 73.
(118) McCall, J.; Bruce, D.; Workman, S.; Cole, C.; Richter, M. M. *Anal. Chem.* **2001**, *73*, 4617.
(119) High, B.; Bruce, D.; Richter, M. M. *Anal. Chim. Acta* **2001**, *449*, 17.
(120) Environmental Health Criteria, No. 200; World Health Organization: Geneva, Switzerland, 1998.
(121) U.S. Environmental Protection Agency Consumer Fact Sheet on Copper, 2001; www.epa.gov/safewater/dwh/c-ioc/copper.html.
(122) Muegge, B. D.; Brooks, S.; Richter, M. M. *Anal. Chem.* **2003**, *75*, 1102.
(123) Martin, R. B. *Acc. Chem. Res.* **1994**, *27*, 204.
(124) Richter, M. M.; Bard, A. J. *Anal. Chem.* **1996**, *68*, 2641.
(125) Crosby, G. A.; Whan, R. E.; Allire, R. M. *J. Chem. Phys.* **1961**, *34*, 743.
(126) Sinha, A. P. B. *Spectrosc. Inorg. Chem.* **1971**, *2*, 255.
(127) Ding, Z.; Quinn, B. M.; Haram, S. K.; Pell, L. E.; Korgel, B. A.; Bard, A. J. *Science* **2002**, *296*, 1293.
(128) Myung, N.; Ding, Z.; Bard, A. J. *Nano Lett.* **2002**, *11*, 1315.
(129) Kim, J.; Faulkner, L. R. *J. Electroanal. Chem. Interfacial Electrochem.* **1988**, *242*, 107.
(130) Kim, J.; Faulkner, L. R. *J. Electroanal. Chem. Interfacial Electrochem.* **1988**, *242*, 123.
(131) Bezman, R.; Faulkner, L. R. *J. Am. Chem. Soc.* **1972**, *94*, 6317.
(132) Zweig, A.; Maricle, D. L.; Brinen, J. S.; Maurer, A. *J. Am. Chem. Soc.* **1967**, *89*, 473.
(133) Lotnik, S. V.; Kazakov, V. P. Deposited Doc. 1982 No. VINITI 5069-82 USSR; *Chem. Abstr.* **1983**, *99*, 221604w.
(134) Fleet, B.; Kirkbright, G. F.; Pickford, C. J. *Talanta* **1968**, *15*, 566.
(135) Schaper, H. *J. Electroanal. Chem. Interfacial Electrochem.* **1981**, *129*, 335.
(136) Zweig, A.; Maurer, A. H.; Roberts, B. G. *J. Org. Chem.* **1967**, *32*, 1322.
(137) Debad, J. D.; Lee, S. K.; Qiao, X. X.; Pascal, R. A.; Bard, A. J. *Acta Chem. Scand.* **1998**, *52*, 45.
(138) Ludvik, J.; Volke, J.; Pragst, F. *J. Electroanal. Chem. Interfacial Electrochem.* **1986**, *215*, 179.
(139) Faulkner, L. R.; Bard, A. J. *J. Am. Chem. Soc.* **1969**, *91*, 209.
(140) Kihara, T.; Sukigara, M.; Honda, K. *Electrochim. Acta* **1973**, *18*, 639.
(141) Siegel, T. M.; Mark, H. B., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 6281.
(142) Kim, J.; Faulkner, L. R. *J. Am. Chem. Soc.* **1988**, *110*, 112
(143) Hercules, D. M.; Lansbury, R. C.; Roe, D. K. *J. Am. Chem. Soc.* **1966**, *88*, 4578.
(144) Tachikawa, H.; Bard, A. J. *Chem. Phys. Lett.* **1973**, *19*, 287.
(145) Tokel, N.; Keszthelyi, C. P.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 4872.
(146) Wallace, W. L.; Bard, A. J. *J. Electrochem. Soc.* **1978**, *125*, 1430.
(147) Bezman, R.; Faulkner, L. R. *J. Am. Chem. Soc.* **1972**, *94*, 6331.
(148) Keszthelyi, C. P.; Tachikawa, H.; Bard, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 1522.
(149) Oyama, M.; Mitani, M.; Ozazaki, S. *Electrochem. Commun.* **2000**, *2*, 363.
(150) Oyama, M.; Okazaki, S. *Anal. Chem.* **1998**, *70*, 5079.
(151) Oyama, M.; Okazaki, S. *J. Electrochem. Soc.* **1997**, *144*, L326.
(152) Zhang, C.; Zhou, G.; Zhang, Z.; Aizawa, M. *Anal. Chim. Acta* **1999**, *394*, 165.
(153) Brina, R.; Bard, A. J. *J. Electroanal. Chem.* **1987**, *238*, 277.
(154) Becker, W. G.; Seung, H. S.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1984**, *167*, 127.
(155) Richards, T. C.; Bard, A. J. *Anal. Chem.* **1995**, *67*, 3140.
(156) Fan, F.-R. F.; Mau, A.; Bard, A. J. *Chem. Phys. Lett.* **1985**, *116*, 400.
(157) Richter, M. M.; Fan, F. F.; Klavetter, F.; Heeger, A. J.; Bard, A. J. *Chem. Phys. Lett.* **1994**, *226*, 115.
(158) Zhang, C.; Zhou, G.; Zhang, Z.; Aizawa, M. *Anal. Chim. Acta* **1999**, *394*, 165.
(159) Littig, J. S.; Nieman, T. A. *Anal. Chem.* **1992**, *64*, 1140.
(160) Horiuchi, T.; Niwa, O.; Hatakenaka, N. *Nature* **1998**, *394*, 659.
(161) Lee, K. S.; Zu, Y.; Herrmann, A.; Geerts, Y.; Mullen, K.; Bard, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 3513.
(162) Chen, G. N.; Lin, R. E.; Zhao, Z. F.; Duan, J. P.; Zhang, L. *Anal. Chim. Acta* **1997**, *341*, 251.
(163) Chen, X.; Chen, W.; Wang, X. R. *Acta Chim. Sinica* **2000**, *58*, 563.
(164) Park, S.-M.; Bard, A. J. *J. Electroanal. Chem.* **1977**, *77*, 137.
(165) Keszthelyi, C. P.; Bard, A. J. *J. Electrochem. Soc.: Electrochem. Sci. Technol.* **1973**, *120*, 241.
(166) Lee, S. K.; Richter, M. M.; Strekowski, L.; Bard, A. J. *Anal. Chem.* **1997**, *69*, 4126.
(167) Lee, S. K.; Bard, A. J. *Anal. Lett.* **1998**, *31*, 2209.
(168) Lai, R. Y.; Bard, A. J. *J. Phys. Chem. B* **2003**, *107*, 5036.
(169) Legg, K. D.; Hercules, D. M. *J. Am. Chem. Soc.* **1969**, *91*, 1902.
(170) Haapakka, K. E.; Kankare, J. J. *Anal. Chim. Acta* **1981**, *130*, 415.
(171) Zhang, C.; Qi, H. *Anal. Sci.* **2002**, *18*, 819.
(172) Lin, J. M.; Yamada, M. *Microchem. J.* **1998**, *58*, 105.
(173) Chen, G. N.; Zhang, L.; Lin, R. E.; Yang, Z. C.; Duan, J. P.; Chen, H. Q.; Hibbert, D. B. *Talanta* **2000**, *50*, 1275.
(174) Werner, T.; Fahnrich, K.; Huber, C.; Wolfbeis, O. S. *Photochem. Photobiol.* **1999**, *70*, 585.
(175) Huber, C.; Fahnrich, K.; Huber, C.; Wolfbeis, O. S. *J. Photochem. Photobiol. A* **1999**, *128*, 111.
(176) Fahnrich, K. A. Academic Dissertation, Regensurg, Germany, 1998.
(177) Okajima, T.; Oshaka, T. *J. Electroanal. Chem.* **2002**, *534*, 181.
(178) Prieto, I.; Teetsov, J.; Fox, M. A.; Vanden Bout, D. A.; Bard, A. J. *J. Phys. Chem. A* **2001**, *105*, 520.
(179) Strauss, J.; Daub, J. *Adv. Mater.* **2002**, *14*, 1652.
(180) Debad, J. D.; Morris, J. C.; Lynch, V.; Magnus, P.; Bard, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 2374.
(181) Debad, J. D.; Morris, J. C.; Magnus, P.; Bard, A. J. *J. Org. Chem.* **1997**, *62*, 530.
(182) Fabrizio, E. F.; Payne, A.; Westlund, N. E.; Bard, A. J.; Magnus, P. P. *J. Phys. Chem. A* **2002**, *106*, 1961.
(183) Lee, S. K.; Zu, Y.; Herrmann, A.; Geerts, Y.; Mullen, K.; Bard, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 3513.
(184) Freed, D. J.; Faulkner, L. R. *J. Am. Chem. Soc.* **1971**, *93*, 2097.
(185) Freed, D. J.; Faulkner, L. R. *J. Am. Chem. Soc.* **1971**, *93*, 3565.
(186) Slaterbeck, A. F.; Meehan, T. D.; Gross, E. M.; Wightman, R. M. *J. Phys. Chem. B* **2002**, *106*, 6088.
(187) Lai, R. Y.; Fabrizio, E. F.; Lu, L.; Jenekhe, S. A.; Bard, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 9112.
(188) Yang, J.; Liu, C.; Qian, K.; He, P.; Fang, Y. *Analyst* **2002**, *127*, 1267.
(189) Harvey, N. *J. Phys. Chem.* **1929**, *33*, 1456.
(190) Bernanose, A.; Bremer, Th.; Goldfinger, P. *Bull. Soc. Chim. Belg.* **1947**, *56*, 269.
(191) Vojir, V. *Collect. Czech. Chem. Commun.* **1954**, *19*, 872.
(192) Kuwana, T.; Epstein, B.; Seo, E. T. *J. Phys. Chem.* **1963**, *67*, 2243.
(193) Vitt, J. E.; Johnson, D. C.; Engstrom, R. C. *J. Electrochem. Soc.* **1998**, *138*, 1637.
(194) Haapakka, K. E. *Anal. Chim. Acta* **1982**, *139*, 229.
(195) Haapakka, K. E.; Kankare, J. J. *Anal. Chim. Acta* **1980**, *118*, 333.
(196) van Dyke, D. A.; Cheng, H. Y. *Anal. Chem.* **1989**, *61*, 633.
(197) (a) Bowie, A. R.; Sanders, M. G.; Worsfold, P. J. *J. Biol. Chemilum.* **1996**, *11*, 61. (b) Zhu, R. H.; Kok, W. T. *J. Pharm.*

Biomed. Anal. **1998**, *17*, 985. (c) Dodeigne, C.; Thunus, L.; Lejeune, R. *Talanta* **2000**, *51*, 415.
(198) Sakura, S. *Anal. Chim. Acta* **1992**, *262*, 49.
(199) (a) Lin, X. Q.; Sun, G.; Cui, H. *Chinese J. Anal. Chem.* **1999**, *27*, 497. (b) Sun, Y. G.; Cui, H.; Lin, X. Q. *Acta Chim. Sinica* **2000**, *58*, 567.
(200) Jirka, G. P.; Martin, A. F.; Nieman, T. A. *Anal. Chim. Acta* **1993**, *284*, 345.
(201) Leca, B.; Blum, L. J. *Analyst* **2000**, *125*, 789.
(202) Sakura, S.; Imai, H. *Anal. Sci.* **1988**, *4*, 9.
(203) Maruqette, C. A.; Blum, L. J. *Anal. Chim. Acta* **1999**, *381*, 1.
(204) Kearney, N. J.; Hall, C. E.; Jewsbury, R. A.; Timmis, S. G. *Anal. Commun.* **1996**, *33*, 269.
(205) Spurlin, S.; Cooper, M. M. *Anal. Lett.* **1986**, *19*, 2277.
(206) Nakashima, K.; Suetsuga, K.; Yoshida, M.; Imai, K.; Akiyama, S. *Anal. Sci.* **1991**, *7*, 815.
(207) (a) Ishida, J.; Sonezaki, S.; Yamaguchi, M. *J. Chromatogr.* **1992**, *598*, 203. (b) Ishida, J.; Sonezaki, S.; Yamaguchi, M. Yoshitake, T. *Analyst* **1992**, *117*, 1719.
(208) Steijger, O. M.; Ligeman, H.; Brinkman, U. A. T.; Holthuis, J. J. M.; Smilde, A. K.; Doornbos, D. A. *J. Chromatrogr.* **1993**, *615*, 97.
(209) Wilson, R.; Schiffrin, D. J. *J. Electroanal. Chem.* **1998**, *448*, 125.
(210) Taylor, C. E., IV; Creager, S. E. *J. Electroanal. Chem.* **2000**, *474*, 82.
(211) Ouyang, C. S.; Wang, C. M. *J. Electrochem. Soc.* **1998**, *145*, 2654.
(212) Ouyang, C. S.; Wang, C. M. *J. Electroanal. Chem.* **1999**, *474*, 82.
(213) Zheng, X.; Zhang, Z.; Guo, Z.; Wang, Qi. *Analyst* **2002**, *127*, 1375.
(214) Guo, Z.; Zheng, X.; Zhang, Z. *Fenxi Huaxue* **2002**, *30*, 461.
(215) Debad, J. D.; Glezer, E. M.; Leland, J. K.; Sigal, G. B.; Wohlstadter, J. Chemical and biological applications of ECL. In *Electrogenerated Chemiluminescence*; Bard, A. J., Ed.; Dekker: New York, 2004 (in preparation); Chapter 8.
(216) Wild, D., Ed. *The Immunoassay Handbook*; Macmillan Press: Basingstoke, U.K., 1994.
(217) Origen Analyzer, M-8 and M-1 Instruments, and Tricorder by BioVeris Corp. (www.bioveris.com); Nuclisens Reader and System by bioMerieux (http://www.biomerieux-usa.com/clinical/nucleicacid/reader.htm); PicoLumi by Eisai Co. (http://www.eisai.com); Elecsys 1010, Elesys 2010, and E170 module coupled with Modular Analytics clinical analyzer by Roche Diagnostics (http://www.roche-diagnostics.com/products_services/elecsys.html); Sector HTS and Sector PR Instruments by Meso Scale Diagnostics, LLC.
(218) Uhlen, M., Hornes, E., Olsvik, O., Eds. *Advances in Biomagnetic Separation*; Eaton Publishing: Westborough, MA, 1994.
(219) Olsvik, O.; Popovic, T.; Skjerve, E.; Cudjoe, K. S.; Hornes, E.; Ugelstad, J.; Uhlen, M. *Clin. Microbiol. Rev.* **1994**, *7*, 43.
(220) Safarikova, S. M.; Forsythe, S. J. *J. Appl. Bacteriol.* **1995**, *78*, 575.
(221) Bruno, J. G. *Recent Research Developments in Microbiology*; Pandalai, S. G., Ed.; Research Signpost: Trivandrum, India, 1998; Vol. 1, p 25.
(222) Cruser, S. A.; Bard, A. J. *Anal. Lett.* **1967**, *1*, 11.
(223) Ege, D.; Becker, W. G.; Bard, A. J. *Anal. Chem.* **1984**, *56*, 2413.
(224) Rubinstein, I.; Martin, C. R.; Bard, A. J. *Anal. Chem.* **1983**, *55*, 1580.
(225) Noffsinger, J. B.; Danielson, N. D. *Anal. Chem.* **1987**, *59*, 865.
(226) Uchikura, K.; Kirisawa, M.; Sugii, A. *Anal. Sci.* **1993**, *9*, 121.
(227) Uchikura, K.; Kirisawa, M. *Anal. Sci.* **1991**, *7*, 803.
(228) He, L.; Cox, K. A.; Danielson, N. D. *Anal. Lett.* **1990**, *23*, 195.
(229) Brune, S. N.; Bobbitt, D. R. *Talanta* **1991**, *38*, 803.
(230) Xu. G.; Dong, S. *Anal. Chem.* **2000**, *72*, 5308.
(231) Downey, T.-M.; Nieman, T. A. *Anal. Chem.* **1992**, *64*, 261.
(232) Jameison, F.; Sanchez, R. I.; Dory, L.; Leland, J. K.; Yost, D.; Martin, M. T. *Anal. Chem.* **1996**, *68*, 1298.
(233) Martin, A. F.; Nieman, T. A. *Biosens. Bioelec.* **1997**, *12*, 479.
(234) Liang, P.; Dong, L.; Martin, M. T. *J. Am. Chem. Soc.* **1996**, *118*, 9198.
(235) Dong, L.; Martin, M. T. *Anal. Biochem.* **1996**, *236*, 344.
(236) Jackson, W. A.; Bobbitt, D. R. *Microchem. J.* **1994**, *49*, 99.
(237) Lee, W. Y. *Mikrochim. Acta* **1997**, *127*, 19.
(238) Holeman, J. A.; Danielson, N. D. *J. Chromatogr. Sci.* **1995**, *33*, 297.
(239) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1980**, *102*, 6641.
(240) Martin, C. R.; Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 4817.
(241) Gilman, S. D.; Silverman, C. E.; Ewing, A. G. *J. Microcolumn. Sep.* **1994**, *6*, 97.
(242) Dickson, J. A.; Ferris, M. M.; Milofsky, R. E. *J. High Resolut. Chromatogr.* **1997**, *20*, 643.
(243) Forbes, G. A.; Nieman, T. A.; Sweedler, J. V. *Anal. Chim. Acta* **1997**, *347*, 289.
(244) Bobbit, D. R.; Jackson, W. A. U.S. Patent 5,614,073, 1997.
(245) Tsukagoshi, K.; Miyamoto, K.; Saito, E.; Nakajima, T.; Hara, K.; Fujinaga, K. *Anal. Sci.* **1997**, *13*, 639.
(246) Liu, J.; Yan, J.; Yang, X.; Wang, E. *Anal. Chem.* **2003**, *75*, 5435.
(247) Liu, J.; Yan, J.; Yang, X.; Wang, E. *Anal. Chem.* **2003**, *75*, 3637.
(248) Chen, G.; Chi, Y.; Wu, X.; Duan, J.; Li, N. *Anal. Chem.* **2003**, *75*, 6602.
(249) Chen, X.; Chen, W.; Wang, X. R. *Acta Chim. Sinica* **2000**, *58*, 563.
(250) Namba, Y.; Usami, M.; Suzuki, O. *Anal. Sci.* **1999**, *15*, 1087.
(251) Yilmaz, N.; Erbagci, A. B.; Aynacioglu, A. S. *Acta Biochim. Pol.* **2001**, *48*, 775.
(252) Stockmann, W.; Bablok, W.; Luppa, P. *Wien. Klin. Wochenschr.* **1998**, *110*, 10.
(253) Xu, X.-H. N.; Jeffers, R. B.; Gao, J.; Logan, B. *Analyst* **2001**, *126*, 1285.
(254) Butch, A. W.; Crary, D.; Yee, M. *Clin. Biochem.* **2002**, *35*, 143.
(255) Haese, A.; Dworschack, R. T.; Piccoli, S. P.; Sokoll, L. J.; Partin, A. W.; Chan, D. W. *Clin. Chem.* **2002**, *48*, 944.
(256) Miyashiro, I.; Kuo, C.; Huynh, K.; Iida, A.; Morton, D.; Bilchik, A.; Giuliano, A.; Hoon, D. S. B. *Clin. Chem.* **2001**, *47*, 505.
(257) O'Connell, C. D.; Juhasz, A.; Kuo, C.; Reeder, D. J.; Hoon, D. S. *Clin Chem.* **1999**, *44*, 1161.
(258) Taback, B.; Chan, A. D.; Kuo, C. T.; Bostick, P. J.; Wang, H. J.; Giuliano, A. E.; Hoon, D. S. B. *Cancer Res.* **2001**, *61*, 8845.
(259) Hoon, D. S. B.; Kuo, C. T.; Wen, S.; Wang, H.; Metelitsa, L.; Reynolds, C. P.; Seeger, R. C. *Am. J. Pathol.* **2001**, *159*, 493.
(260) Sanchez-Carbayo, M.; Espasa, A.; Chinchilla, V.; Herrero, E.; Megias, J.; Mira, A.; Soria, F. *Clin. Chem.* **1999**, *45*, 1944.
(261) Shimizu, A.; Shiraki, K.; Ito, T.; Sugimoto, K.; Sakai, T.; Ohmori, S.; Murata, K.; Takase, K.; Tameda, Y.; Nakano, T. *Int. J. Mol. Med.* **2002**, *9*, 245.
(262) Sassa, T.; Kumada, T.; Nakano, S.; Uematsu, T. *Eur. J. Gastroenterol. Hepatol.* **1999**, *11*, 1387.
(263) Gassler, N.; Peuschel, T.; Pankau, R. *Clin. Lab.* **2000**, *46*, 553.
(264) Sanchez-Carbayo, M.; Mauri, M.; Alfayate, R.; Miralles, C.; Soria, F. *Clin. Chem.* **1998**, *44*, 1744.
(265) Ehrhardt, V.; Assman, G.; Baetz, O.; Bieglmayer, C.; Mueller, C.; Neumeier, D.; Roth, H. J.; Veys, A.; Yvert, J. P. *Wien. Klin. Wochenschr.* **1998**, *110*, 61.
(266) Sanchez-Carbayo, M.; Mauri, M.; Alfayate, R.; Miralles, C.; Soria, F. *Clin. Biochem.* **1999**, *32*, 395.
(267) Sanchez-Carbayo, M.; Mauri, M.; Alfayate, R.; Miralles, C.; Soria, F. *Clin. Biochem.* **1999**, *32*, 395.
(268) Luppa, P. B.; Reutemann, S.; Huber, U.; Hoermann, R.; Poertl, S.; Kraiss, S.; Von Vuelow, S.; Neumeier, D. *Clin. Chem. Lab. Med.* **1998**, *36*, 789.
(269) Kulmala, S.; Hakansson, M.; Spehar, A.-M.; Nyman, A.; Kankare, J.; Loikas, K.; Ala-Kleme, T.; Eskola, J. *Anal. Chim. Acta* **2002**, *458*, 271.
(270) Sapin, R.; Schlienger, J.-L.; Gasser, F.; Noel, E.; Lioure, B.; Grunenberger, F.; Goichot, B.; Grucker, D. *Clin. Chem.* **2000**, *46*, 418.
(271) Klein, G.; Kampmann, M.; Baum, H.; Rauscher, T.; Vukovic, T.; Hallermayer, K.; Rehner, J.; Muller-Bardorff, M.; Katus, H. A. *Wien. Klin. Wochenschr.* **1998**, *110* (Suppl. 3), 40.
(272) Hetland, O.; Dickstein, K. *Clin. Chem.* **1998**, *44*, 1348.
(273) Ishii, J.; Ishikawa, T.; Yukitake, J.; Nagamura, Y.; Ito, M.; Wang, J. H.; Kato, Y.; Hiramitsu, S.; Inoue, S.; Kondo, T.; Morimoto, S.; Nomura, M.; Watanabe, Y.; Hishida, H. *Clin. Chim. Acta* **1998**, *270*, 183.
(274) Collinson, P. O.; Jorgensen, B.; Sylven, C.; Haass, M.; Chwallek, F.; Katus, H. S.; Muller-Bardorff, M.; Derhaschnig, U.; Hirschl, M. M.; Zerback, R. *Clin. Chim. Acta* **2002** *307*, 197.
(275) Kobayashi, Y.; Hayakawa, M.; Fukumara, Y. *Igaku to Yakugaku* **1999**, *42*, 749.
(276) Kashiwagi, S.; Hayashi, J.; Asai, T.; Nishimura, J.; Arai, N.; Kanashima, M.; Asai, Y. *Igaku to Yakugaku* **1998**, *40*, 119.
(277) Takahashi, M.; Hoshino, H.; Ohuchi, Y.; Ryan, S.; Shimoda, K.; Yasuda, K.; Tanaka, J.; Yoshizawa, K.; Hino, K.; Iino, S. *Igaku to Yakugaku* **1998**, *40*, 483.
(278) Seck, T.; Diel, I.; Bismar, H.; Ziegler, R.; Pfeilschifter, J. *Bone* **2002**, *30*, 217.
(279) Okabe, R.; Nakatsuka, K.; Inaba, M.; Miki, T.; Naka, H.; Masaki, H.; Moriguchi, A.; Nishizawa, Y. *Clin. Chem.* **2001**, *47*, 1410.
(280) Scheunert, K.; Albrecht, S.; Konnegen, V.; Wunderlich, G.; Distler, W. *Chemiluminescence at the Turn of the Millennium*; Schweda-Werbedruck: Dresden, Germany, 2001; p 347.
(281) Khorkova, O. E.; Pate, K.; Heroux; J. Sahasrabudhe, S. *J. Neurosci. Methods* **1998**, *82*, 159.
(282) Yilmaz, N.; Erbagci, A. B.; Aynacioglu, A. S. *Acta Biochim. Pol.* **2001**, *48*, 775.
(283) Sapin, R.; Le Galudeck, V.; Gasser, F.; Pinget, M.; Grucker, D. *Clin. Chem.* **2001**, *47*, 602.
(284) Ohlin, M.; Silvestri, M.; Sundqvist, V.-A.; Borrebaeck, C. A. K. *Clin. Diag. Lab. Immunol.* **1997**, *4*, 107.
(285) Yamaguchi, K.; Sawada, T.; Yamane, S.; Haga, S.; Ikeda, K.; Igata-Yi, R.; Yoshiki, K.; Matsuoka, M.; Okabe, H.; Horii, Y.; Nawa, Y.; Waltrip, R. W.; Carbone, K. M. *Ann. Clin. Biochem.* **2001**, *38*, 348.
(286) Horii, Y.; Garcia, J. N. P.; Novianna, D.; Kono, F.; Sawada, T.; Naraki, T.; Yamaguchi, K. *J. Vet. Med. Sci.* **2001**, *63*, 921.
(287) Fukuda, K.; Takahashi, K.; Iwata, Y.; Mori, N.; Gonda, K.; Ogawa, T.; Osone, K.; Sato, M.; Ogata, S.-I.; Horimoto, T.;

Sawada, T.; Tashiro, M.; Yamaguchi, K.; Niwa, S.-I.; Shigeta, S. *J. Clin. Microbiol.* **2001**, *39*, 419.
(288) Ohlin, M. *Clin. Diag. Lab.* **1997**, *4*, 107.
(289) Weber, B.; Bayer, A.; Kirch, P.; Schluter, V.; Schlieper, D.; Melchior, W. *J. Clin. Microbiol.* **1999**, *37*, 2639.
(290) Kenten, J. H.; Gudibande, S.; Link, J.; Willey, J. J. Curfman, B.; Major, E. O.; Massey, R. J. *Clin. Chem.* **1992**, *38*, 873.
(291) Van Gemen, B.; Van Beuningen, R.; Nabbe, A.; Van Strijp, V.; Jurriaans, S.; Lens, P.; Schoones, R.; Kievits, T. *J. Virol. Methods* **1994**, *49*, 157.
(292) Boom, R.; Sol, C.; Weel, J.; Gerrits, Y.; de Boer, M.; van Dillen, P. W. *J. Clin. Microbiol.* **1999**, *37*, 1489.
(293) Stevens, S. J. C.; Vervoort, M. B. H. J.; van den Brule, A. J. C.; Meenhorst, P. L.; Meijer, C. J. L. M.; Middeldorp, J. M. *J. Clin. Microbiol.* **1999**, *37*, 2852.
(294) de Jong, M. D.; Weel, J. F. L.; Schuurman, T.; Wertheim-van Dillen, P. M. E.; Boom, R. *J. Clin. Microbiol.* **2000**, *38*, 2568.
(295) Wu, S.-J. L.; Lee, E. M.; Putvatana, R.; Shurtliff, R. N.; Porter, K. R.; Suharyono, W.; Watts, D. M.; King, C.-C.; Murphy, G. S.; Hayes, C. G.; Romano, J. W. *J. Clin. Microbiol.* **2001**, *39*, 2794.
(296) Lanciotti, R. S.; Kerst, A. J. *J. Clin. Microbiol.* **2001**, *39*, 4506.
(297) Fox, D.; Han, S.; Samuelson, A.; Zhang, Y.; Neale, M. L.; Westmoreland, D. *J. Clin. Virol.* **2002**, *24*, 117.
(298) Collins, R. A.; Ko, L.-S.; So, K.-L.; Ellis, T.; Lau, L.-T.; Yu, A. C. H. *J. Virol. Methods* **2002**, *103*, 213.
(299) Collins, R. A.; Ko, L. S.; Fung, K. Y.; Chang, K. Y.; Xing, J.; Lau, L. T.; Yu, A. C. *Biochem. Biophys. Res. Commun.* **2003**, *300*, 507.
(300) Yu, H.; Bruno, J. G.; Cheng, T.-C.; Calomiris, J. J.; Goode, M. T.; Gatto-Menking, D. L. *J. Biolum. Chemilum.* **1995**, *10*, 239.
(301) Schutzbank, T. E.; Smith, J. *J. Clin. Microbiol.* **1995**, *33*, 2036.
(302) Oprandy, J. J.; Amemiya, K.; Kenten, J. H.; Green, R. G.; Major, E. O.; Massey, R. Technical Advances in AIDS Research in the Human Nervous System. *Proceedings of an NIH Symposium on Technical Advances in AIDS Research in the Human Nervous System, Washington, DC, Oct 4*−*5*, 1993; U.S. GPO: Washington, DC, 1995; p 281.
(303) Reetoo, K. N.; Osman, S. A.; Illavia, S. J.; Banatvala, J. E.; Muir, P. *J. Virol. Methods* **1999**, *82*, 145.
(304) Collins, R. A.; Ko, L. S.; Fung, K. Y.; Lau, L. T.; Xing, *J. Biochem. Biophys. Res. Commun.* **2002**, *297*, 267.
(305) Schutzbank, T. E.; Smith, J. *J. Clin. Microbiol.* **1995**, *33*, 2036.
(306) Miyashiro, I.; Kuo, C.; Huynh, K.; Iida, A.; Morton, D.; Bilchik, A.; Giuliano, A.; Hoon, D. S. B. *Clin. Chem.* **2001**, *47*, 505.
(307) O'Connell, C. D.; Juhasz, A.; Kuo, C.; Reeder, D. J.; Hoon, D. S. *Clin. Chem.* **1999**, *44*, 1161.
(308) Taback, B.; Chan, A. D.; Kuo, C. T.; Bostick, P. J. Wang, H. J.; Giuliano, A. E.; Hoon, D. S. B. *Cancer Res.* **2001**, *61*, 8845.
(309) Hoon, D. S. B.; Kuo, C. T.; Wen, S.; Wang, H.; Metelitsa, L.; Reynolds, C. P.; Seeger, R. C. *Am. J. Pathol.* **2001**, *159*, 493.
(310) Klingler, K. R.; Zech, D.; Wielckens, K. *Clin. Lab.* **2000**, *46*, 41.
(311) Gellings, A.; Holzem, G.; Wielckens, K.; Klingler, K. R. *Laboratoriumsmedizin* **2001**, *25*, 26.
(312) Stern, H. J.; Carlos, R. D.; Schutzbank, T. E. *Clin. Biochem.* **1995**, *28*, 470.
(313) Blackburn, G. F.; Shah, H. P.; Kenten, J. H.; Leland, J.; Kamin, R. A.; Link, J.; Pterman, J.; Powell, M. J.; Shah, A.; Talley, D. B.; Tyagi, S. K.; Wilkins, E.; Wu, T.-G.; Massey, R. J. *Clin. Chem.* **1991**, *37*, 1626.
(314) Heroux, J. A.; Szczepanik, A. M. *PCR Methods Appl.* **1995**, *4*, 327.
(315) Wilkinson, E. T.; Cheifetz, S.; De Grandis, S. A. *PCR Methods Applications* **1995**, *4*, 363.
(316) Gudibande, S.; Kenten, J. H.; Link, J.; Friedman, K.; Massey, R. J. *J. Mol. Cell. Probes* **1992**, *6*, 495.
(317) De Jong, M. D.; Weel, J. F. L.; Schuurman, T.; Wertheim-van Dillen, P. M. E.; Boom, R. *J. Clin. Microbiol.* **2000**, *38*, 2568.
(318) Baeummer, A. J.; Humiston, M. C.; Montagna, R. A.; Durst, R. A. *Anal. Chem.* **2001**, *73*, 1176.
(319) Kankare, J.; Haapakka, K.; Kulmala, S.; Nanto, V.; Eskola, J.; Takalo, H. *Anal. Chim. Acta* **1992**, *266*, 205.
(320) Chmura, J.; Slawinski, J. *J. Biolum. Chemilumin.* **1994**, *9*, 1.
(321) Egashira, N.; Nabeyama, Y.; Kurauchi, Y.; Ohga, K. *Anal. Sci.* **1996**, *12*, 793.
(322) Blohm, S.; Kadey, S.; McKeon, K.; Perkins, S.; Sugasawara, R. *Biomed. Prod.* **1996**, April.
(323) Shapiro, L.; Heidenreich, K. A.; Meintzer, M. K.; Dinarello, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7422.
(324) Obenauer-Kutner, L. J.; Jacobs, S. J.; Kolz, K.; Tobias, L. M.; Bordens, R. W. *J. Immunol. Methods* **1997**, *206*, 25.
(325) Puren, A. J.; Razeghi, P.; Fantuzzi, G.; Dinarello, C. A. *J. Infect. Dis.* **1998**, *178*, 1830.
(326) Motmans, K.; Raus, J.; Vandevyver, C. *J. Immunol. Methods* **1996**, *190*, 107.
(327) Gopalakrishnan, S. M.; Warrior, U.; Burns, D.; Groebe, D. R *J. Biomol. Screen.* **2000**, *5*, 369.
(328) Weinreb, P. H.; Yang, W. J.; Violette, S. M.; Couture, M.; Kimball, K.; Pepinsky, R. B.; Lobb, R. R.; Josiah, S. *Anal. Biochem.* **2002**, *306*, 305.
(329) Hughes, S. R.; Khorkova, O.; Goyal, S.; Knaeblein, J.; Heroux, J.; Riedel, N. G.; Sahasrabudhe, S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3275.
(330) Horiuchi, H.; Lippé, R.; McBride, H. M.; Rubino, M.; Woodman, P.; Stenmark, H.; Rybin, V.; Wilm, M.; Ashman, K.; Mann, M.; Zerial, M. *Cell* **1997**, *90*, 1149.
(331) Mathew, A.; Mathur, S. K.; Jolly, C.; Fox, S. G.; Kim, S.; Morimoto, R. I. *Mol. Cell. Biochem.* **2001**, *21*, 7163.
(332) Zhang, L.; Schwartz, G.; O'Donnell, M.; Harrison, R. K. *Anal. Biochem.* **2001**, *293*, 31.
(333) Zhang, L.; Song, L.; Terracina, G.; Liu, Y.; Pramanik, B.; Parker, E. *Biochemistry* **2001**, *40*, 5049.
(334) De Baer, M. P.; van der Horn, K. H. M.; Goudsmit, J.; de Ronde, A.; de Wolf, F. *J. Clin. Microbiol.* **1999**, *37*, 63.
(335) Khorkova, O. E.; Pate, K.; Heroux, J.; Sahasrabudhe, S. *J. Neurosci. Methods* **1998**, *82*, 159.
(336) Woods, R. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1979; Vol. 9, p. 1.
(337) Hsueh, Y. T.; Smith, R. L.; Northrup, M. A. *Transducers '95*−*Eurosensors IX*; Stockholm, Sweden, June 1995; p 768.
(338) Hsueh, Y. T.; Smith, R. L.; Northrup, M. A. *Sens. Actuators, B: Chem.* **1996**, *33*, 110.
(339) Hsueh, Y. T.; Collins, S. D.; Smith, R. L. *Sens. Actuators, B: Chem.* **1998**, *49*, 1.
(340) Kuhn, L. S.; Weer, A.; Weber, S. G. *Anal. Chem.* **1990**, *62*, 1631.
(341) Egashira, N.; Kumasako, H.; Ohga, K. *Anal. Chem.* **1990**, *6*, 903.
(342) Kremeskotter, J.; Wilson, R.; Schiffrin, D. J.; Luff, B. J.; Wilkinson, J. S. *Measurement Sci. Technol.* **1995**, *6*, 1325.
(343) Hsueh, Y. T.; Smith, R. L.; Northrup, M. A. *Sens. Actuators, B: Chem.* **1996**, *33*, 110.
(344) Arora, A.; DeMello, A. J.; Manz, A. *Anal. Commun.* **1997**, *34*, 393.
(345) Egashira, N.; Piao, J.; Hifumi, E.; Uda, T. *Bunseki Kagaku* **2000**, *49*, 1029.
(346) Yoon, C. H.; Cho, J.-H.; Oh, H.-I.; Kim, M.-J.; Lee, C.-W.; Choi, J.-W.; Paek, S.-H. *Biosens. Bioelectron.* **2003**, *19*, 289.
(347) Deaver, D. R. *Nature* **1995**, *377*, 758.
(348) Henchal, E. A.; Teska, J. D.; Ludwig, G. V.; Shoemaker, D. R.; Ezzel, J. W. *Clin. Lab. Med.* **2001**, *21*, 661.
(349) Higgins, J. A.; Ibrahim, M. S.; Knauert, F. K.; Ludwig, G. B.; Kijek, T. M.; Ezzell, J. W.; Courtney, B. C.; Henchal, E. A. *Ann. N. Y. Acad. Sci.* **1999**, *894*, 130.
(350) Bruno, J. G.; Kiel, J. L. *BioTechniques* **2002**, *32*, 178.
(351) Filella, X.; Friese, S.; Roth, H. J.; Nussbaum, S.; Whenl, B *Anticancer Res.* **2000**, *20*, 5229.
(352) Stiebler, P.; Molina, R.; Chan, D. W.; Fritsche, H. A.; Beyrau, R.; Bonfrer, J. M. G.; Filella, X.; Gornet, T. G.; Hoff, T.; Inger, W.; Van Kamp, G. J.; Nagel, D.; Peisker, K.; Sokoll, L. J.; Troalen, F.; Untch, M.; Domke, I. *Clin. Chem.* **2001**, *47*, 2162.
(353) Hubl, W.; Chan, D. W.; Van Ingen, H. E.; Miyachi, H.; Molina, R.; Filella, X.; Pitzel, L.; Ruibal, A.; Rymer, J. C.; Bagnard, G.; Domke, I. *Anticancer Res.* **1999**, *19*, 2727−2733.
(354) Ingen, H. E.; Chan, D. W.; Hubl, W.; Miyachi, H.; Molina, R.; Pitzel, L.; Ruibal, A.; Rymer, J. C.; Domke, I. *Clin. Chem.* **1998**, *44*, 2530−2536.
(355) Gatto-Menking, D. L.; Yu, H.; Bruno, J. G.; Goode, M. T.; Miller, M.; Zulich, A. W. *Biosens. Bioelectron.* **1995**, *10*, 501.
(356) Yu, H.; Raymonda, J. W.; McMahon, T. M.; Campagnari, A. A. *Biosens. Bioelectron.* **2000**, *14*, 829.
(357) Yu, H. *Proceedings of SPIE*−*The International Society for Optical Engineering 2982 (Optical Diagnostics of Biological Fluids and Advanced Techniques in Analytical Cytology)*; Bellingham, WA, 1997; p 168.
(358) Bruno, J. G.; Yu, H. *Appl. Environ. Microbiol.* **1996**, *62*, 3474.
(359) Yu, H.; Bruno, J. G. *BioMedical Prod.* **1995**, *20*, 20.
(360) Bruno, J. G.; Kiel, J. L. *Biosens. Bioelectron.* **1999**, *14*, 457.
(361) Yu, H.; Bruno, J. G. *Appl. Environ. Microbiol.* **1996**, *62*, 587.
(362) Shelton, D. R.; Karns, J. S. *Appl. Environ. Microbiol.* **2001**, *67*, 2908.
(363) Min, J.; Baeumner, A. J. *Anal. Biochem.* **2002**, *303*, 186.
(364) Crawford, C. G.; Wijey, C.; Fratamico, P.; Tu, S. I.; Brewster, J. *J. Rapid Methods Automation Microbiol.* **2000**, *8*, 249.
(365) Yu, H. *J. Immunol. Methods* **1996**, *192*, 63.
(366) Lee, Y. M.; Johnson, P. W.; Call, J. L.; Arrowood, M. J.; Furness, B. W.; Pichette, S. C.; Grady, K. K.; Resh, P.; Michell, L.; Bergmire-Sweat, D.; MacKenzie, W. R.; Tsang, V. C. W. *Am. J. Trop. Med. Hyg.* **2001**, *65*, 1.
(367) Call, J. L.; Arrowood, M. J.; Xie, L.-T.; Hancock, K.; Tsang, V. C. W. *J. Parasitol.* **2001**, *87*, 203.
(368) Kijeck, T. M.; Rossi, C. A.; Moss, D.; Parker, R. W.; Henchal, E. A. *J. Immunol. Methods* **2000**, *236*, 9.
(369) Bruno, J. G.; Cornette, J. C. *Microchem. J.* **1997**, *56*, 305.
(370) Bruno, J. G.; Kiel, J. L. *Biosens. Bioelectron.* **1999**, *14*, 457.
(371) (a) Taverna, P. J.; Mayfield, H.; Andrews, A. R. J. *Anal. Chim. Acta* **1998**, *373*, 111. (b) Whitchurch, C.; Andrews, A. R. J. *Anal. Chim. Acta* **2002**, *454*, 45.
(372) Kane-Maguire, N. A. P.; Guckert, J. A.; O'Neill, P. J. *Inorg. Chem.* **1987**, *26*, 2340.

(373) Bolletta, B.; Ciano, T.; Balzani, V.; Serpone, N. *Inorg. Chim. Acta* **1982**, *62*, 207.
(374) Hemingway, R. E.; Park, S.-M.; Bard, A. J. *J. Am. Chem. Soc.* **1975**, *95*, 200.
(375) Rodman, G. S.; Bard, A. J. *Inorg. Chem.* **1990**, *29*, 4699.
(376) Bruno, J. G.; Collard, S. B.; Andrews, A. R. J. *J. Biolumin. Chemilumin.* **1997**, *12*, 155.
(377) Bruno, J. G.; Parker, J. E.; Holwitt, E.; Alls, J. L.; Kiel, J. L. *J. Biolumin. Chemilumin.* **1998**, *13*, 117.
(378) Nocera, D. G.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 824.
(379) Mussell, R. D.; Nocera, D. G. *Polyhedron* **1986**, *5*, 47.
(380) Mussell, R. D.; Nocera, D. G. *J. Am. Chem. Soc.* **1988**, *110*, 2764.
(381) Mussell, R. D.; Nocera, D. G. *J. Phys. Chem.* **1991**, *95*, 6919.
(382) Mussell, R. D.; Nocera, D. G. *Inorg. Chem.* **1990**, *29*, 3711.
(383) Ouyang, J.; Zietlow, T. C.; Hopkins, M. D.; Fan, F.-R. F.; Gray, H. B.; Bard, A. J. *J. Phys. Chem.* **1986**, *90*, 3841.
(384) Tokel-Takvoryan, N. E.; Bard, A. J. *Chem. Phys. Lett.* **1974**, *25*, 235.
(385) Vogler, A.; Kunkely, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 316.
(386) Kim, J.; Fan, F. F.; Bard, A. J.; Che, C.-M.; Gray, H. B. *Chem. Phys. Lett.* **1985**, *121*, 543.
(387) Bonafede, S.; Ciano, M.; Bolletta, F.; Valzani, V.; Casshot, L.; von Zelewsky, A. *J. Phys. Chem.* **1986**, *90*, 3836.
(388) Kane-Maguire, N. A. P.; Wright, L. L.; Gucker, J. A.; Tweet, W. S. *Inorg. Chem.* **1988**, *27*, 2905.
(389) Luong, J. C.; Nadjo, L.; Wrighton, M. S. *J. Am. Chem. Soc.* **1978**, *100*, 5790.
(390) Vogler, A.; Kunkely, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 469.
(391) Tokel-Takvoryan, N. E.; Hemingway, R. E.; Bard, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 6582.
(392) Zhang, X.; Bard, A. J. *J. Phys. Chem.* **1988**, *92*, 5566.
(393) Abruna, H.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 2641.
(394) Wheeler, B. L.; Nagasubramanian, G.; Bard, A. J.; Schechtman, L. A.; Dininny, D. R.; Kenney, M. E. *J. Am. Chem. Soc.* **1984**, *106*, 7404.
(395) Jungru, A.; Jinming, L. *Chem. Res. Chinese Univ.* **1991**, *7*, 32.
(396) Bruno, J. G.; Collard, S. B.; Andrews, A. R. J. *J. Biolumin. Chemilumin.* **1997**, *12*, 155.
(397) Kapturkiewicz, A. *J. Electroanal. Chem. Interfacial Electrochem.* **1991**, *302*, 131.
(398) Zweig, A.; Metzler, G.; Maurer, A.; Roberts, B. G. *J. Am. Chem. Soc.* **1967**, *89*, 4091.
(399) Brian, R.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1987**, *238*, 277.
(400) Munakata, H. Jpn. Patent JP 86-259286 A2 (861117), 1986.
(401) Wilson, J. R.; Park, S. M.; Daub, G. H. *J. Electrochem. Soc.* **1981**, *128*, 2085.
(402) Lee, S. K.; Zu, Y.; Herrmann, A.; Geerts, Y.; Mullen, K.; Bard, A. J. *J. Am. Chem. Soc.* **1999**, *121*, 3513.
(403) Oyama, M.; Mitani, M.; Washida, M.; Masuda, T.; Okazaki, S. *J. Electroanal. Chem.* **1999**, *473*, 166.
(404) Oyama, M.; Masuda, T.; Mitani, M.; Okazaki, S. *Anal. Chim. Acta* **1999**, *67*, 1211.
(405) Chen, X.; Jia, L.; Wang, X. R.; Hu, G. L.; Sato, M. *Anal. Sci.* **1997**, *13*, 71.
(406) Chen, X.; Sato, M.; Lin, Y. J. *Microchem. J.* **1998**, *58*, 13.
(407) Chen, X.; Jia, L.; Sato, M *Acta Chim. Sinica* **1998**, *56*, 238.
(408) Greenway, G. M.; Dolman, S. J. L. *Analyst* **1999**, *124*, 759.
(409) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(410) Kenten, J. H.; Gudibande, S. R.; Link, J.; Friedman, K.; Massey, R. J. *Mol. Cell. Probes* **1992**, *6*, 495.
(411) Qi, H.; Zhang, C. *Anal. Chim. Acta* **2004**, *501*, 31.
(412) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(413) Ridlen, J. S.; Klopf, G. J.; Nieman, T. A. *Anal. Chim. Acta* **1997**, *341*, 195.
(414) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(415) Knight, A. W.; Greenway, G. M. *Anal. Commun.* **1996**, *33*, 171.
(416) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(417) Gonzalez, J. M.; Greenway, G. M.; McCreedy, T.; Qijin, S. *Analyst* **2000**, *125*, 765.
(418) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(419) Knight, A. W.; Greenway, G. M. *Analyst* **1995**, *120*, 2543.
(420) Noffsinger, J. B.; Danielson, N. D. *J. Chromatogr.* **1987**, *387*, 520.
(421) Uchikura, K.; Kirisawa, M. *Anal. Sci.* **1991**, *7*, 971.
(422) Brune, S. N.; Bobbitt, D. R. *Talanta* **1991**, *38*, 419.
(423) Retoo, K. N.; Osman, S. A.; Illavia, S. J.; Banatvala, J. E.; Muir, P. *J. Virol. Methods* **1999**, *82*, 145.
(424) Collins, R. A.; Ko, L. S.; Fung, K. Y.; Lau, L. T.; Xing, J.; Yu, A. C. H. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 267.
(425) Wang, C.-Y.; Hsuan-Jung, H. *Anal. Chim. Acta* **2003**, *498*, 61.
(426) Kobrynski, L.; Tanimune, L.; Pawlowski, A.; Douglas, S. D.; Campbell, D. E. *Clin. Diag. Lab. Immunol.* **1996**, *3*, 42.
(427) Liebert, A.; Beier, L.; Schneider, E.; Kirch, P. *Chemiluminescence at the Turn of the Millennium*; Schweda Werbedruck: Dresden, Germany, 2001, 341.
(428) Novick, D.; Schwartsburd, B.; Pinkus, R.; Suissa, D.; Belzer, I.; Sthoeger, Z.; Keane, W. F.; Chvatchko, Y.; Kim, S. H.; Fantuzzi, G.; Dinarello, C. A.; Rubinstein, M. *Cytokine* **2001**, *14*, 334.
(429) Swanson, S. J.; Jacobs, S. J. Mytych, D.; Shah, C.; Indelicato, S. R.; Bordens, R. W. *Dev. Biol. Standardization* **1999**, *97*, 135.
(430) Hermsen, D.; Franzson, L.; Hoffman, J. P.; Isaksson, A.; Kaufman, J. M.; Leary, E.; Muller, C.; Nakatsuka, K.; Nishizawa, Y.; Reinauer, H.; Riesen, W.; Roth, H. J.; Steinmuller, T.; Troch, T.; Bergmann, P. *Clin. Lab.* **2002**, *48*, 131.
(431) Moreau, E.; Philippe, J.; Couvent, S.; Leroux-Roels, G. *Clin. Chem.* **1996**, *42*, 1450.

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