Signaling Recognition Events with Fluorescent Sensors and Switches

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Contents

I. Introduction

The crucial recognition events of chemistry, biology, and materials science occur in a much smaller world than the one we are accustomed to. Information about these events can be conveniently transmitted to us via light signals emitted by purpose-built molecular devices.[1−8] Besides this sensory role,[9−30] such molecular devices also have potential for information processing since their emission can be switched between two distinguishable states by environmental stimuli. In this review, we highlight the uniqueness and utility of such systems. It is remarkable that these devices serve several categories of beneficiary with very different needs and methods. Analytical chemists and environmental scientists usually require populations of sensor molecules which need to be bound to surfaces such as optical fibers in some instances.[31,32] Analytical biochemists, clinical and medical scientists as well as cell biologists prefer to work with populations of freely mobile sensor molecules under a microscope,[33,34] although fiber optic work is also possible now.[35,36] On the other hand, information technologists would wish to deal with optical switches at the single molecule level with the help of photon scanning tunneling microscopy[37] or near-field scanning optical microscopy.[38,38a] The reality of individual molecules and their orbitals is no longer in doubt.[39]

II. Using Fluoro/Lumophores for Signaling: Why and How

The advantages of molecular fluorescence or luminescence for sensing and switching can be summarized;[7,8] high sensitivity of detection down to the single molecule,[38a,40−44] "on−off" switchability, feasibility of human−molecule communication, subnanometer spatial resolution with submicron visualization[34−38] and submillisecond temporal resolution. Furthermore, many of the structural features which control (reduce) fluorescence efficiency have been delineated;[45−51] double-bond torsion, low energy nπ* levels, "heavy" atoms, weak bonds, and opportunities for photoinduced electron transfer (PET) or electronic energy transfer (EET). The importance of extensive delocalization in compensating for these negative effects has also been appreciated. Therefore, considerable opportunities exist for modulating these structural features via chemical or physical means at the molecular level.

Our emphasis will be on the transduction of discrete and stoichiometric recognition events into fluorescence signals. However, nonstoichiometric interactions such as solvation have much to teach us in this field. In fact, it will become clear that the information gleaned from the fluorescence effects due to nonstoichiomeric interactions is not only important in its own right. Such data can be adapted, sometimes rather directly, by designers of molecular and atomic recognition systems.

The plan of the review is as follows. First, we consider emissive photophysical effects arising from monofluoro/lumophore components. Additional components if present will only serve in auxiliary capacities. The supramolecular aspects will mainly arise from the necessary interaction of the environment or guest with the fluoro/lumophore. Most of the principal types of excited states encountered by organic and inorganic chemists will be represented in sections III−VII. From then on, the photophysical phenomena themselves take on a supramolecular character, i.e. two or more components are essential for the phenomenon. Their perturbation by the environment/guest will form the second layer of supramolecularity. One of these phenomena, photoinduced electron transfer (PET) can occur in monofluoro/lumo-

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Standing (left to right): Terry Rice, A. P. de Silva, Allen Huxley, and Nimal Gunaratne. Seated (left to right): Jude Rademacher, Thorri Gunnlaugsson, and Colin McCoy.

A. P. de Silva was born and raised on the pearl/teardrop of the Indian Ocean. His learning, teaching, and research experiences have been a tale of two cities and their universities. The University of Colombo, Sri Lanka (1971–1976 and 1980–1986), and the Queen's University of Belfast, Northern Ireland (1976–1980 and 1986 to the present), have played host to him alternately. He appreciates and enjoys (in alphabetical order): basketball, conversations with friends, education, percussion, photophysics/chemistry, providence, rock, serendipity, and supramolecular chemistry.

Nimal Gunaratne was born in Sri Lanka and received his B.Sc. degree from the University of Colombo. He obtained his Ph.D. from the Queen's University of Belfast with Professor R. Grigg. After a brief stay at the University of Colombo, Sri Lanka, he returned to Belfast to carry out research into sensing of molecular recognition with A. P. de Silva. His research interests include supramolecular chemistry, molecular recognition, and molecular devices.

Thorfinnur Gunnlaugsson was born in 1967 and brought up in Iceland. He undertook his preuniversity qualification at a local grammar and high school in the town of Hafnarfjordur before obtaining a B.Sc.(Hons) in chemistry from the University of Iceland, where he also worked on research with Dr. Christopher H. Evans on Intramolecular photoreactions of 3-nitroanisole-complexed cyclodextrins. He joined the Queen's University of Belfast in early 1993 to commence his Ph.D. studies with A. P. de Silva, on luminescent sensors and reagents for ions and neutral molecules. In October 1996 he joined the group of Professor David Parker at the University of Durham, England as a research assistant working on luminescent lanthanide complexes for physiological ion detection. His main chemical interests include molecular recognition and the mimicking of the photosynthetic reaction center. Outside work he enjoys cooking, entertaining, hiking, and playing golf.

Allen Huxley was born in Larne, Northern Ireland, and received his B.Sc. degree from the Queen's University of Belfast. He is currently in the second year of his Ph.D. with A. P. de Silva. His interests in life include most sports (participating and watching), the botanical world, ornithology, and baking.

Colin McCoy was born in Belfast, Northern Ireland, where, after a five-year holiday, he was educated for 19 years. His education included a B.Sc. in chemistry (1991), followed by a Ph.D. (1994) in molecular signaling via fluorescence with A. P. de Silva, who had triggered his interest in the field as an undergraduate at the Queen's University of Belfast. In 1995 he moved to Paris to work on photochromism at the Collège de France with Professor Jean-Marie Lehn as a Royal Society European Scientific Exchange Fellow and in 1996 returned to the Queen's University of Belfast to his current post of Instructor in organic chemistry.

phore systems (section VIII). Others require multifluorophore assemblies for their operation. Monomer/excimer equilibria and electronic energy transfer (EET) are taken up in sections IX and X, respectively.

Jude T. Rademacher was born in Lansing, MI. He received his B.A. degree at Michigan State University in 1989 and his Ph.D. in 1994 with Professor Anthony W. Czarnik at The Ohio State University in the synthesis of polyaza-aromatics and their charge-transfer behavior in aqueous solutions with cyclodextrins. After two years of postdoctoral work with A. P. de Silva at the Queen's University of Belfast involved in the synthesis of fluorescent sensors, and a year with Professor Daniel Nocera at Michigan State University preparing fluorescent cyclodextrin derivatives, he is currently Assistant Professor at the College of Wooster, Wooster, Ohio. His research interests are in fluorescent sensors and molecular recognition.

Terry Rice was born in 1971 in High Wycombe, England, but has been a resident of Newry, Northern Ireland, since 1979. He received his B.Sc. degree from the Queen's University of Belfast and will obtain his Ph.D. in early 1997, with A. P. de Silva, mainly working on PET sensors based on lanthanide luminescence. His outside interests include sporting activities of a diverse nature, fishing, and bowling.

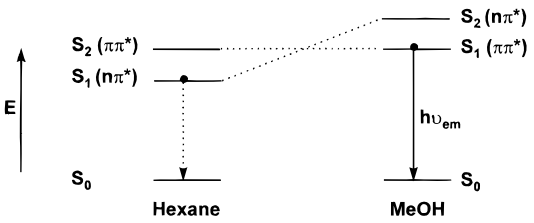
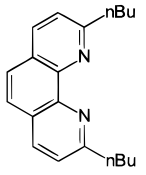


Figure 1. Hydrogen bond-induced $n\pi^*-\pi\pi^*$ state inversion.

III. $n\pi^*$ Excited States

Molecules with lowest excited singlet states of the $n\pi^*$ type are typically nonemissive in fluid solution,[48−51] with the exception of small carbonyl compounds such as acetone and biacetyl.[52,53,53a] Therefore such systems can be persuaded to switch "on" their fluorescence if the $n\pi^*$ state is perturbed (usually in a destabilizing manner) such that the lowest energy singlet excited state is of the $\pi\pi^*$ type. Figure 1 illustrates this situation in terms of hydrogen bonding—a major determinant of recognition in host−guest[54] and solvent−solute systems.[55] The hydrogen bond-donating solvent methanol increases the energy of the $n\pi^*$ excited state of pyrene-1-carboxaldehyde to such an extent that the fluorescence quantum yield increases over a 100-fold compared to the apolar (and aprotic) solvent hexane.[56,57] 9-Acetylanthracene possesses a $\pi\pi^*$ lowest excited singlet state but remains poorly emissive (except at rather low temperatures) because of intersystem crossing to an energetically close triplet state.[58] $n\pi^*-\pi\pi^*$ state inversions are easier to arrange if stronger forces can be brought into play. Azaaromatics such as acridine,[59] 1,10-phenanthroline derivative **1**,[60] and quinolines **2**[60,61] and **3**[62] demonstrate this when they engage in Lewis base−acid interactions with BF_3, Li^+, and Na^+ besides direct protonation. The 2,2′-bipyridyl-based macrocycle **4**[63] acts similarly except that the fluorescence switching "on" effect is large (1000-fold increase) and selective for Li^+. The presence of hydrogen atoms on the bridgehead carbons in related systems had led to tautomerization and colorimetric sensors over a decade ago.[64] We close this section by noting that polyacrylates carrying acylbenzo-18-crown-6 ether fluorophores display emission assigned to $n\pi^*$ excited states which are quenched upon K^+ complexation.[65] This case



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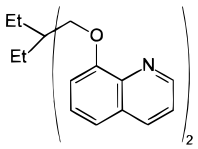

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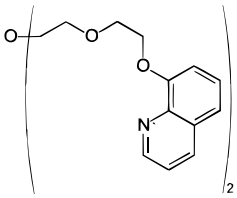

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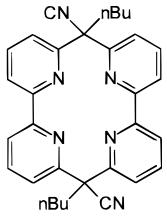

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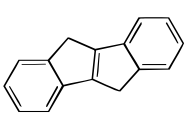
deserves further study because of the unusual emission behavior. K^+ binding is augmented by the polyanionic environment around the crown ether receptor.

IV. $\pi\pi^*$ Excited States

Non-alkenic hydrocarbon $\pi\pi^*$ excited states are largely insensitive to solvents. Nevertheless, the fluorescence of discoidal π-electron systems, e.g. pyrene, show a particularly subtle form of solvent control. The 0−0 vibrational band (I) intensity is strongly dependent on solvent dipolarity,[55] whereas e.g. 0−2 band (III) is not.[66] Apolar solvents cause minimal perturbation to the symmetric, nearly vibrationless excited state. Such symmetric states are poor emitters; hence, the 0−0 band is of low intensity in apolar media. Polar solvents perturb and desymmetrize the vibrationless excited state and the 0−0 band intensity rises accordingly. Therefore, the I/III band intensity ratio serves to sense the net effect of dipoles in the neighborhood.[66−69] Attempts have been made to apply this to recognition studies.[70] A different, but not unrelated, effect is found in inorganic systems. The intensity of the lowest energy electronic emission band ($^5D_0-{}^7F_6$) of Eu(III) is also sensitive to the symmetry of its ligand environment, whereas the other emission bands are not.[71] Conjugated alkenes such as 1,6-diphenyl-1,3,5-hexatriene display useful fluorescence only in semirigid membrane environments when double-bond torsion is obstructed in the excited state.[48,72−74] In other words, they serve as sensors for microviscosity.

Such obstruction to double-bond torsion may also occur upon tight encapsulation as seen to some extent in β-cyclodextrin complexed *trans*-stilbene.[75] Of course, torsion is best prevented by structural rigidification via covalent bridges.[73,76,77] For instance, **5** is a maximally fluorescent analogue of *trans*-stilbene and sets the standard for sensor/switch designers who wish to follow this avenue but with noncovalent regimes. An elegant example which employs covalent but reversible interactions is **6**.[78] Compound **6** switches "on" its fluorescence upon rigidification via macrocyclization with disaccharide guests. The situation was less clear-cut in pathfinding cases such as **7**−**9** when rigidification and conformational effects were invoked to explain the small fluorescence changes



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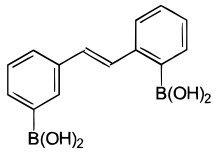

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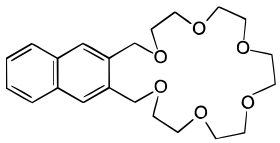
observed upon ion binding near $\pi\pi^*$ fluorophores.[79,80] The clearest effect in these experiments was due to "heavy" atom-induced spin−orbit coupling which can even happen in a remote fashion.[81−86]



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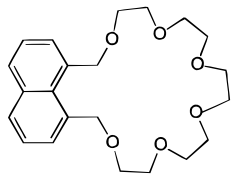

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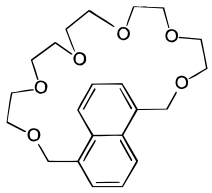

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V. Metal-Centered (MC) Excited States

The huge influence of emissive MC excited states on photophysics[3−5,71,87−90] and assay procedures[91−94] is largely due to two species Eu(III) and Tb(III). This influence is set to grow as the special features of these excited states are exploited for signaling purposes. We offer the following status report as a spur in this direction. Signaling with MC states in conjunction with other mechanisms is deferred to a later section.

Since the excitation of lanthanide(III) ions is locked away in inner f orbitals, environmental influences are few. This is critical for their application as luminescent labels since a label must remain constant in all its parameters whatever environment it finds itself in.[91] However, hydration of these trications couples the electronic excitation into the vibrational manifold of O−H bonds resulting in luminescence loss.[95] The problem is compounded by the rather high frequency of O−H vibrations and the rather low energy of the lowest MC state [especially in the case of Eu(III) $^5D_0$] since the Born−Oppenheimer hole[96−98] is easily accessed. This energy sink can be plugged by replacing the hydration shell with a set of hard ligating atoms devoid of O−H connections. The metal ion−ligand recognition is thus signaled with a enhanced luminescence. However this interaction is not rapidly reversible in many cases to be useful as sensors and switches. Most of these were originally designed for use as labels and reagents only. Nevertheless, such replacement of the hydration shell is totally successful in only a few instances; since some water molecules hang onto the trications rather stubbornly.[88,99] The Born−Oppenheimer hole is even harder to eliminate than the hydration shell since N−H and even C−H vibrational manifolds can lead to significant luminescence quenching. These can be mini-

mized by perdeuteration to reduce the size of the vibrational quantum.[100,101]

Additional problems remain. Since f−f electronic transitions are intrinsically difficult, lanthanide(III) ions are poor absorbers. Laser excitation is one way of overcoming this difficulty.[95] This has opened up the active sites of metalloproteins to investigation provided that the metal ion could be replaced with Ln(III). Quite separately, there is an advantage within the disadvantage of disallowed f−f transitions. In fact, this advantage is perhaps the one driving the popularity of pure and applied lanthanide photophysics. Once a lanthanide MC excited state has been populated one way or another, the difficulty of the f−f downward transition forces a longer excited state lifetime. Quite commonly, this extends to the millisecond time scale. Since fluorescence and light scattering operate over much faster time scales, these common optical interferences arising from chemical and biological matrices can be easily time-resolved out from the Ln(III) signal.[91−94,99] This results in exquisitely low limits of detection.

A simple solution to the difficulty of exciting f−f transitions is the use of a photon antenna.[71,87,88] In fact, the beginnings of lanthanide luminescence research employed this strategy half a century ago.[102] Many of the classical analytical reagents for lanthanide(III) ions also operate this way.[15] Photon antennas are also finding use in all-organic photochemistry.[103] Basically, the principle is that an intrinsically strong photon absorber with a relatively high excited-state energy will funnel the photonic energy to populate a MC excited-state held nearby. Such electronic energy transfer (EET) processes can be very efficient.

A particularly venerable example of this phenomenon has been used to signal the presence of DNA. The signaling agent is the simplest possible: aquo terbium(III). The polyanionic nature of the DNA holds the Tb(III) close and strips part of its first hydration shell. Its aromatic nucleobase components provide the photon antenna. So it is not surprising that DNA could be assayed by its switching "on" effect on Tb(III). Eu(III) fails in this regard, [104] presumably owing to quenching by PET (photoinduced electron transfer; see section VIII) from nucleobases to Eu(III). This general solution has enjoyed considerable popularity and various antenna−ligand systems have seen service with or without intervening spacers. These include acetylacetonates,[15,102,105] acyclic[106−120] and macrocyclic polyaminocarboxylates,[88,99] crown ethers,[121−123] cryptands,[124−126] calixarene amides, acids,[127a] and ethers,[125,126] polypyridine carboxylates,[115−119,128−130] polypyridine arrays,[124,125,131,132] polypyridine *N*-oxide sets,[125,126] phosphonates,[133] phosphinates,[132,134] aneN$_4$ amides,[135,476] and combinations thereof. As far as we are aware, the antenna has been an organic π-electron system without exception so far. We see no reason why suitable metal-containing systems with MC, MLCT, or LMCT excited states cannot fill this role.

While many of the above cases produce excellent luminescent labels, few of them show sensory/switching behavior. The ternary systems,[118,128,136,137] e.g., diaza-15-crown-5 ether *N*,*N*-diacetate, Eu(III), and

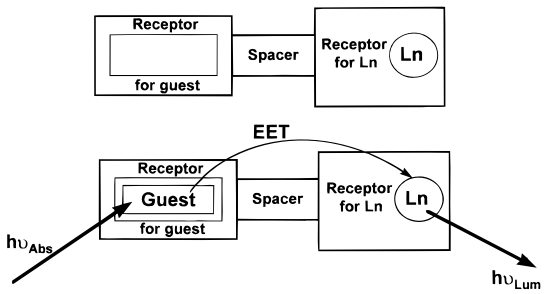
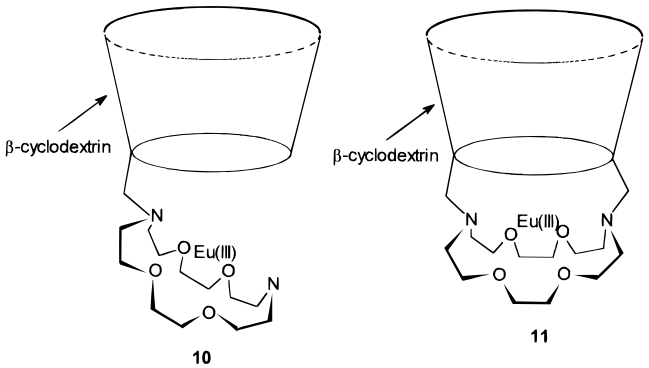


Figure 2. Luminescent sensors based on MC excited states for light-absorbing aromatics.

1,10-phenanthroline-2,9-dicarboxylate, are particularly interesting but usually show complicated and sluggish kinetics. Nevertheless, the principle of signaling the aromatic component by means of the emission from the assembly is clear. pH-induced disassembly of the ternary complexes causes emission loss[128] but the use of multicomponent sensors are fraught with reversibility problems especially when employed in microheterogeneous environments.

The basic message of the previous paragraph is that a photon antenna is essential for the widespread exploitation of lanthanide(III) luminescence. This point is underlined by the fact that lanthanide(III) ion concentrations as high as 0.01 M are necessary for luminescence experiments with aminopolycarboxylate ligands devoid of antennas.[138] Sensors/switches with lanthanide complexes can be designed on this basis for photon absorbing aromatic compounds. Figure 2 shows how the lanthanide(III) complex lacks a photon antenna until an aromatic molecule takes up residence in the receptor for the guest. Thus the arrival of the aromatic molecule is signaled by strong luminescence. The heterobireceptor system needs to possess orthogonal guest selectivities at its terminii for proper operation. It is important that the guest does not trigger new deactivation channels for the MC excited state it has just helped to create. Photoinduced electron transfer (PET) is constantly lurking in the shadows.[99,113] Low-lying ligand to metal charge transfer (LMCT) excited states also take their toll.[87] Furthermore, energetic proximity of guest lowest excited states can lead to temperature-dependent emission at best[87,131,132] and complete quenching at worst.[99,134] Sensors **10**,[139] **11**,[140] and **12**[141] show the evolution of this approach at Nocera's laboratory.[141,142] Although the heterobireceptor system in **10** has been known before along with several other β-cyclodextrins capped with crown ethers,[143] no optical investigations were conducted. The antenna action in **10** is limited by the relatively large distance between the complexed benzene guest and the Eu(III) center. Compound **11** solves this problem but the hydrophobic environment needed for efficient guest inclusion is spoiled by the presence of the charge-uncompensated trication in the cyclodextrin cap. The three carboxylate side arms in the cap of **12** cure this difficulty provided that Tb(III) is used as the lanthanide center apparently in order to avoid charge transfer problems from the aminocarboxylates to Eu(III). Nocera has also developed physical sensing schemes with these and other delayed luminescent systems for velocimetry in turbulent fluids.[141,142] Gouterman's work is also interesting in this respect.[144,145]



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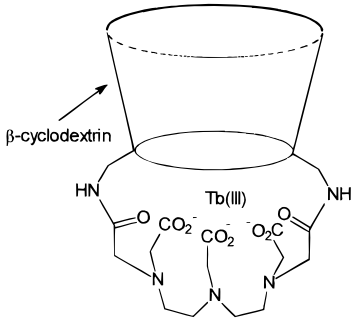

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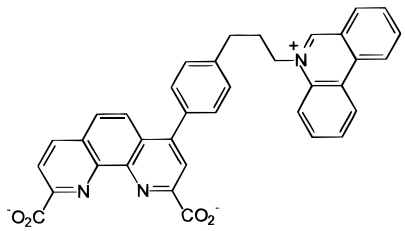
The threads in the previous two paragraphs come together nicely in Sammes' homogeneous assay for a target base sequence in single-stranded DNA.[129,130,146,147] The probe DNA strand carries an aliphatic polyaminocarboxylate to hold Eu(III) which remains poorly emissive until the photon antenna (1,10-phenanthroline-2,9-dicarboxylate in this case) arrives and remains close enough to sensitize luminescence. The effective concentration of the antenna near the Eu(III) center can be enhanced by attaching it via a short spacer to an intercalator (e.g., phenanthridinium) as soon as the double strand becomes available upon hybridization of the probe strand with the target DNA strand. Figure 3 represents this approach with **13** and relatives. Even point mutations in base sequences have been recognized in this way.[129]



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Even though several lanthanide(III) ions exhibit luminescence, Tb(III) and Eu(III) appear to have divided the signaling field between them. Other valence states are hardly represented either. The only stirrings of life that we have seen are represented by Eu(II)[148] and Tl(I).[149] Even though Eu(II) is unstable to oxidation in air, it is interesting that partial isolation from methanol solvation with 15-crown-5 ether leads to switching "on" of luminescence. The opposite is true of Tl(I) when it is presented with dibenzo-30-crown-10 ether and several other ionophores in that strong quenching is seen. Addition of alkali cations results in competition for the ionophore and luminescence recovery. A band shift rather than quenching is found if the saturated ionophore dicyclohexyl-18-crown-6 ether is used. Although the shortness of the communication wavelengths limits the popularity of Tl(I), this work over 20 years ago by Haynes et al. is remarkable for its foresight concerning several principles in the luminescent sensor/switch field including time-resolved detection. Time will tell if any of the other luminescent heavy metal ions can add some diversity to the role of MC excited states in this field.

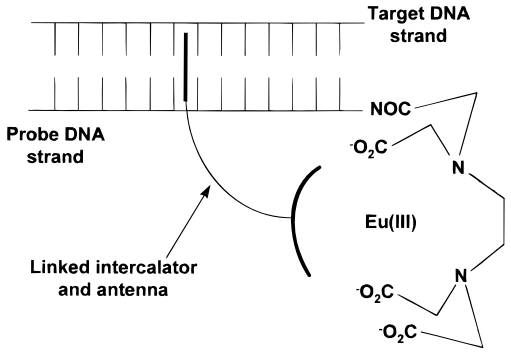


Figure 3. The principle behind Sammes' homogeneous assay for a DNA strand.

VI. Charge-Transfer (CT) Excited States and Relatives

A. All-Organic Internal Charge Transfer (ICT) Excited States

Non-hydrocarbon (i.e., heteroatom containing) π-electron systems[150] have very different dipole moments in their ground and lowest energy singlet excited states due to internal charge transfer (ICT). We note that within the context of this review it is preferable to refer to ICT as internal rather than intramolecular charge transfer. The word "intramolecular" is poorly focused when supermolecules with multiple components are being addressed. On the other hand, the word "internal" can be restricted to a given fluorophore π-electron system. A further qualifier is that some of the systems featured in this section may turn out, upon further study, to involve twisting in the excited state. Currently clear instances of the latter are deferred to section VI.C. Most common examples of heteroatom-containing π-systems have enlarged dipoles in their excited states,[151−153] although the opposite is true for betaines such as the popular solvatochromic, but non-fluorescent, dye $E_T(30)$.[154] The influence of the solvent−solute dipole interaction on the various states of a sensor/switch is shown in Figures 4 and 5. For maximum clarity, we consider an extreme case where the ground state (S_0) has a much smaller dipole moment compared to that of the lowest energy singlet ICT excited state (S_1). The orientation of the solvent dipoles neighboring the thermally equilibriated S_0 state would therefore be distributed in an unbiased manner. Upon photon absorption, the Franck−Condon S_1 state is populated with the solvent dipole orientational distribution being conserved. Hence

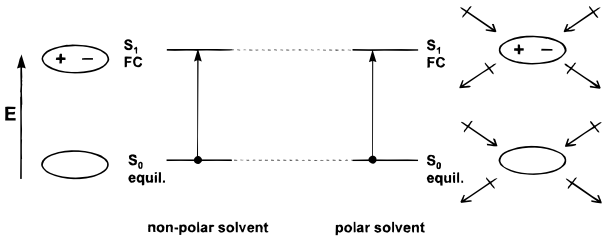


Figure 4. Solvent effect on absorption spectral wavelength for a fluorophore with an ICT excited state. FC represents a Franck−Condon excited state. "Equil." represents a thermally equilibriated electronic ground state. The dipole of the ground state is assumed to be small. S_0 and S_1 represent the electronic ground and lowest energy excited states, respectively.

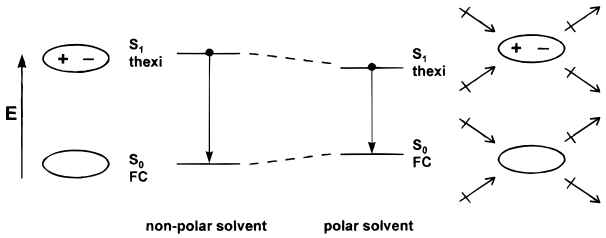
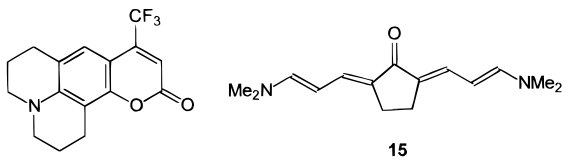


Figure 5. Solvent effect on emission spectral wavelength for a fluorophore with an ICT excited state. "Thexi" represents a thermally equilibriated electronic excited state. The other terms are defined under Figure 4.

the solvent dipolar effects on the charge separation within the S_1 state largely cancel out within the time scale of the absorption experiment. In contrast, the longer time scale of the emission experiment means that the solvent dipoles can relax around the S_1 state in response to the charge separation. The field due to the oriented solvent dipoles is now non-zero and is responsible for perturbation of the thermally equilibriated S_1 and Franck−Condon S_0 states. The message we wish to take is that solvent or external effects can show up in emission even when they are hardly visible in absorption. Although extreme, this situation can be found in 1,3-diaryl-2-pyrazolines.[155] Red-shifted fluorescence induced by polar solvents can be observed to evolve over a few picoseconds following excitation due to the rotational relaxation of solvent molecules around the fluorophore mentioned above.[156,157] Hydrogen bonding can also contribute to the situation.[158] Many of the better performers in this category are π-electron systems with push−pull substituent pairs, e.g., **14**,[159] **15**,[160] and **16**.[161] Aminophthalimides,[152,153] bimanes,[162] diarylalkenes,[163] and furocoumarins[164] are further sets of



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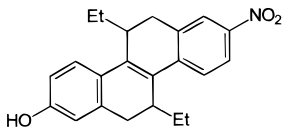

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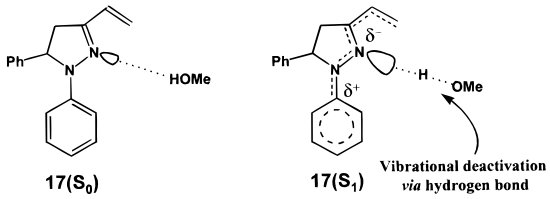


Figure 6. Hydrogen-bond donation to **17** from protic solvents.

examples. Even pyrene-1-carboxaldehyde fits this category (even though it is monosubstituted) provided that protic solvents are used to avoid low-lying nπ* excited states.[56] Several of these cases give rise to linear solvation energy relationships of emission maximum wavenumber versus Kamlet−Taft solvent parameters.[161,163,164] Other solvent properties can also serve as independent variables.[165−167]

Some fluorescent ICT sensors can report on two quasi-independent microenvironmental parameters by means of two fluorescence spectral parameters. Such two-dimensional sensing capability becomes available if an environmentally induced quenching channel is opened up in addition to the red-shift discussed above.[168] As Figure 6 shows, **17** develops substantial electron density on N(2) which gives a strengthened hydrogen bond with protic solvents. This opens a fluorescence deactivation pathway. Related, but different, fluorescence quenching channels arising from intramolecular hydrogen bonds are common in commercial ultraviolet stabilizers.[169] The fluorescence quantum yield of **17** fits a linear solvation energy relationship which is dominated by solvent hydrogen bond acidity (α). On the other hand, the emission maximum wavenunber fits a relation which involves dipolarity (π*) and α equitably. Thus the effective π* and α of a single microenvironment can be separately estimated by measuring the emission maximum wavelength and the quantum yield of **17**.[168] Methyl 9-anthroate shows some similarity in that the fluorescence lifetime is controlled by solvent hydrogen bonding, whereas the emission redshift is controlled by overall polarity of the solvent.[170] A related inorganic example[171] is deferred to section VI.B.

In a valuable development, Walt et al. immobilize the environmentally sensitive ICT fluorophore Nile Red in different polymers at different points on a fiber optic tip so that each element of the array responds differently to a pulse of a solvent vapor.[172] These spectral and temporal responses can be used to train a neural network so that it will recognize the vapor. This study, which emulates the olfactory system in some ways, follows related work with electrically conducting polymer arrays.[173]

We have just seen how the electric field of the solvent shell (along with hydrogen bonding) controls the emission maximum wavelength of a sensor/switch molecule with an ICT excited state. Ions with their uncompensated charge can exert stronger electric effects. However, ions are likely to be present in far smaller concentrations than the solvent molecules. The effective molarity of ions needs to be increased if their monopole nature is to be properly exploited. This can be done rather efficiently by trapping the ion in a receptor built into the fluorophore with the

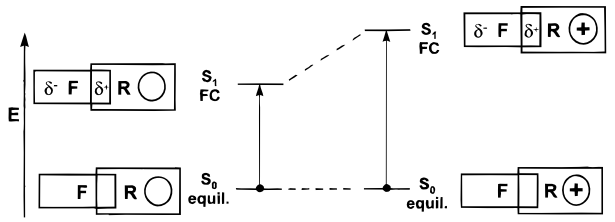


Figure 7. Cation effect on absorption or excitation spectral wavelength for a fluorophore with an ICT excited state. The terms are defined under Figures 4 and 5.

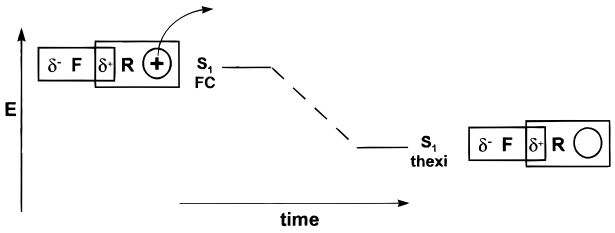
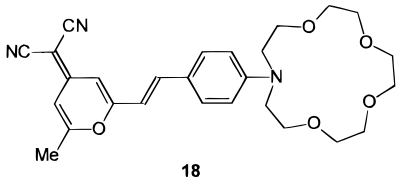


Figure 8. Dampening of the cation effect on emission spectral wavelength for a fluorophore with an ICT excited state. The terms are defined under Figures 4 and 5.

ICT excited state. In fact the fluorophore and the receptor share some critical atoms.

Most of the examples carry a cation receptor at the electron-rich terminal of the fluorophore. This leads to the situation illustrated in Figure 7 where a partial positive charge is photogenerated adjacent to the cation i.e. a cation-induced blue-shift is seen in the absorption or fluorescence excitation spectra. Fluorescence emission spectra are less affected due to cation ejection during the excited state (Figure 8). It is interesting that the cation which was initially attracted to the receptor is no longer welcome due to the new photogenerated repulsion. These principles were first crystallized to rationalize the increased acidity of phenols in the excited state[174,175] and later exploited in the pH jump method for photogenerating proton (or hydroxide) pulses.[175a,176,177] However the generality of these principles and their application to metal ion-sensing phenomena was not realized until Valeur[178−180] and Lapouyade and Rettig[181−184a] had conducted extensive steady state and kinetic studies on **18** and **19**, respectively. Alfimov's systems



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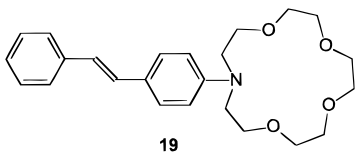

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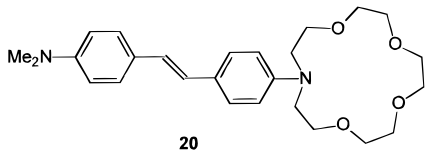
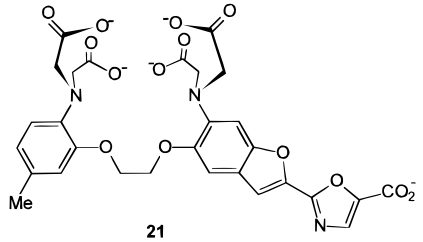
also display photocontrolled cation binding, although most of them are weakly fluorescent (see **37**[233] for an example). These are further distinguished by their reliance on *E*−*Z* photoisomerization to modulate the ligating ability of a side chain. Newer examples related to **18** and **19** which carry the cation acceptor at the electron-poor terminal of the fluorophore such as **20** produce Ca^{2+}-induced red-shifts in the emission spectra.[185]



20

The integrated fluorophore−receptor system is one of several approaches connecting fluorophores and receptors for sensing and switching applications (Figure 9).[8] Integrated fluorophore−receptor systems are in wide use. For instance, many fluorescent pH indicators based on aniline and phenolate derivatives are of this type.[175,187] Lipoidal versions of these have helped to illuminate membrane-bounded proton concentrations.[188,189] Polymer-bound versions which are well retained in cells have permitted sharp imaging of intracellular pH.[190] However the strongest reason for the popularity of this class is due to the success of low molecular weight sensors in physiological monitoring of H^+, Ca^{2+}, Na^+, and Mg^{2+} largely due to Tsien and his collaborators.[191−196] The fact that these sensors can be internally calibrated against several common microenvironmental variables (other than ion concentration) by dual excitation wavelength monitoring and ratioing of the two intensities is a contributor to their success.[197] Compound **21** for Ca^{2+},[198] **22** for Na^+,[199,200] **23** for Mg^{2+},[201−203] and fluorescein for H^+ [204] function on this basis. Their



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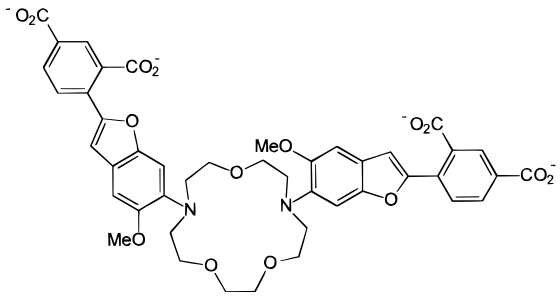

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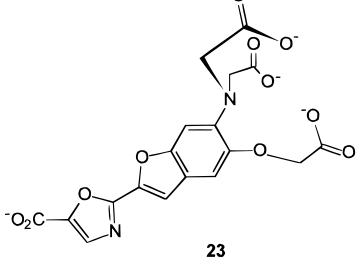

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sensing efficiency is augmented by the metal ion-

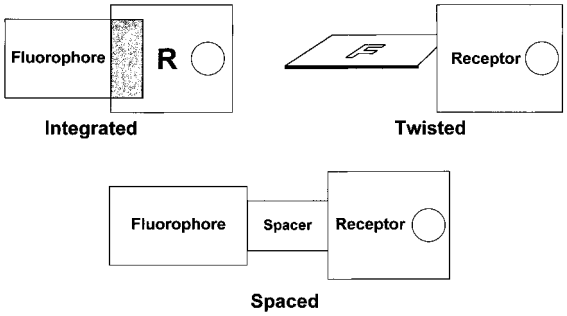
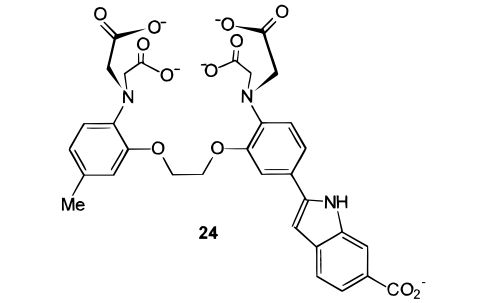


Figure 9. Three different ways of connecting fluorophores (F) and receptors (R).

induced decoupling of a key nitrogen from the rest of the fluorophore π-electron system. Their appeal to practicing biologists is enhanced by a painless method for smuggling them into living cells in the fully esterified form.[204−206] A minority of cases also show cation-induced shifts in emission as in the case of **20** although in the opposite direction. These are **24** for Ca^{2+},[198] **25** for Na^{+},[207] and **26** for H^{+}.[208] Compound **27**[209] is particularly interesting since its rhodamine-type fluorophore is transformed into a fluorescein-type emitter upon Ca^{2+}-induced deconjugation mentioned above. Thus intensity ratioing between two familiar emissions becomes possible. The applicability of several of these ICT-based systems is strengthened by time-resolved experiments.[210−214] A related study is available for 2,2′-bipyridyl-3,3′-diol when it binds Zn(II).[215] For instance, the weakened cation binding in the excited state (which leads to cation disengagement)[178−184] can be quantitated in this way.[211−213]

Prior to this benevolent invasion of biology by molecular fluorescent sensors/switches, there were examples quietly serving analytical chemistry. 8-Hydroxyquinoline,[216] 2,2′-dihydroxyazobenzene,[217] and 1,8-dihydroxyanthraquinone[218] are classical Mg^{2+} reagents of this type. The growing influence of macrocyclic systems broke into the field of fluorescent sensors with two studies on dibenzo-18-crown-6 ether.[62,219] More highly functionalized macrocyclic sensors **28**[220,221] and **29**[222] were also in the vanguard.



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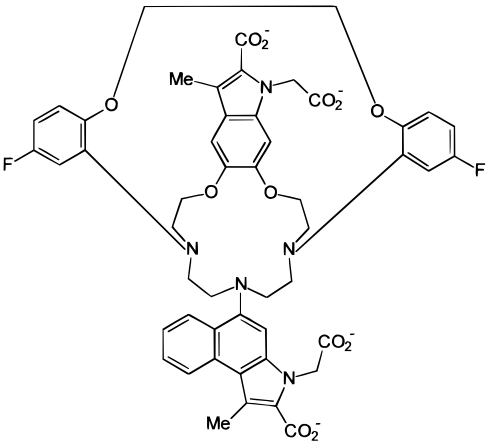

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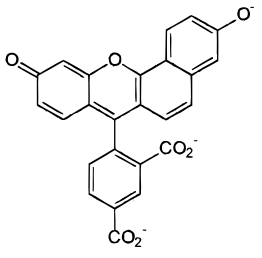

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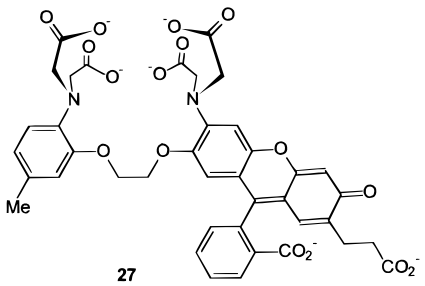

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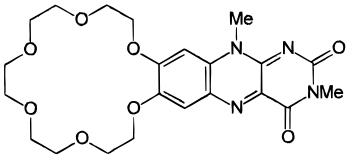

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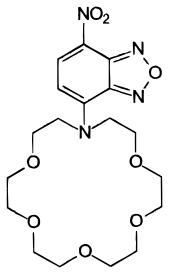
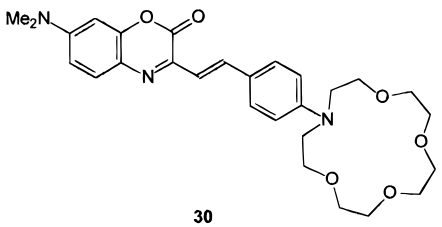


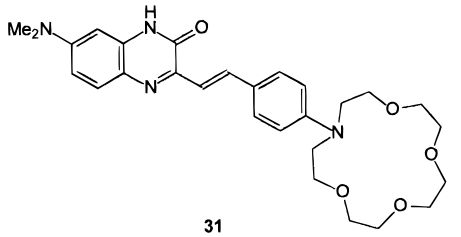
29

Naturally, these pioneering cases only showed small responses to alkali cations, but a valuable principle was thereby established. Compound **30**[223−225] has something in common with **29**, i.e., both are structural elaborations of previously known solvent polarity sensors. The possible involvement of TICT (twisted internal charge transfer) excited states in **30** has been examined.[225] Compound **18**, which is of laser dye parentage, has been similarly scrutinized.[226] Whatever their mechanistic classification, there is no



30

doubt about their cation-responsive fluorescence. Two close relatives of **30**, and a more distant one,[226a] also show similar cation responses. The use of benzodiazinone in **31**[227] and **32**[228] instead of benzoxazinone moieties permits the attachment of extra functionalities on the new nitrogen atom.



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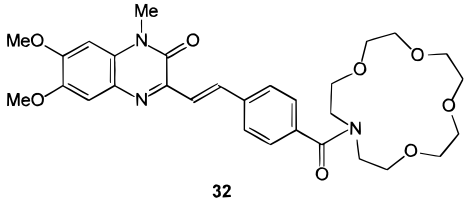
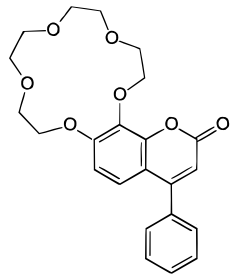


32

Macrocyclic crown receptors continue to feature heavily in the growth of integrated fluorophore–receptor systems with ICT excited states. Coumarin fluorophores form more of the common ground in **33**–**36**.[229–232,122] Compound **35**[230] distinguishes itself by



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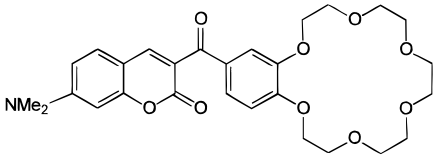

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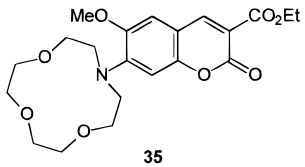

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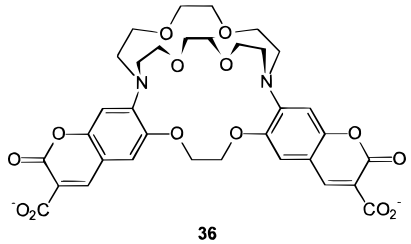
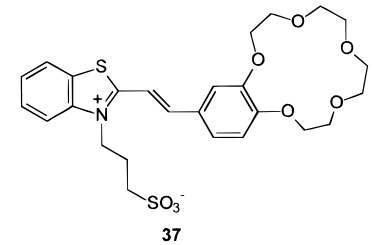


36

targeting Li^+, whereas **36** does the same by tackling extracellular K^+.[232] Lariat ether **37**[233] has several interesting features such as cation-controlled absorption spectra and photocontrollable cation binding. However the photoisomerization process responsible for the latter also causes reduced fluorescence quantum yields. Compound **38**[234,235] is structurally related to popular thermotropic liquid crystal media. This



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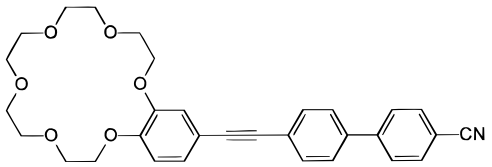

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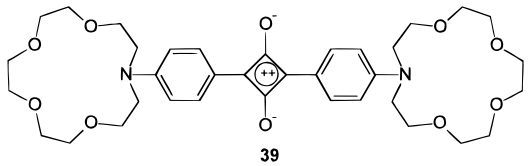
parentage may be responsible for some of its spectral characteristics even in homogeneous solution. K^+ causes a substantial blue-shifted fluorescence. Long communication wavelengths are achieved by **39**[236] and **40**,[237] but small ion-induced spectral effects remain a problem.



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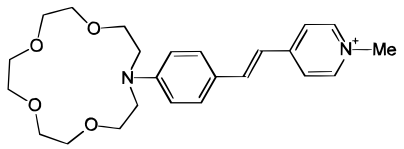

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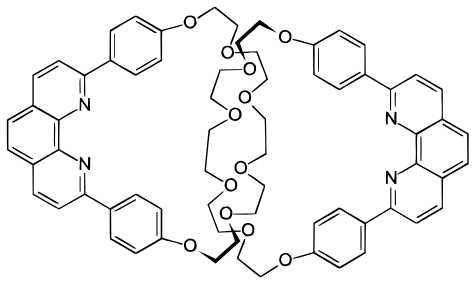
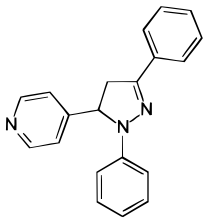
Catenane **41**[238] contains poly(oxyethylene) straps but the effective receptors are the phenanthroline moieties. The latter are also fluorophores with ICT excited states. The mechanical interlocking brings



41

about a special enhancement of the basicity of **41** leading to proton catenates. The various protonation states of **41** have very different fluorescence wavelengths, quantum yields, and lifetimes. Furthermore, the luminescence spectrum of **41** can be tuned from 380 to 780 nm with various cationic guests. Such sensors with tunable spectra are rare. Voyer's

bis[porphyrinzinc(II)] peptide[239] gives smoothly tunable absorption spectra in response to α,ω-diaminoalkanes of various lengths, whereas our substitutionally tunable proton sensor family **42**[240] has broad emission spectra which can be useful from 450 to 600 nm. It is particularly interesting that the proton-induced fluorescence and absorption spectral variations of bis(phenanthroline) **43**[241] can be understood in terms of a dimer entwined about two protons.



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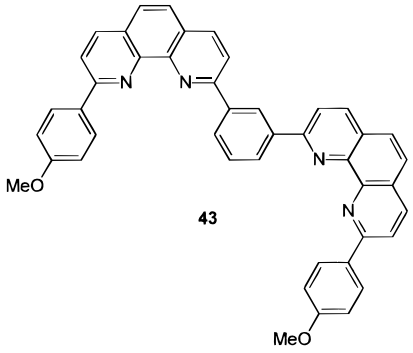

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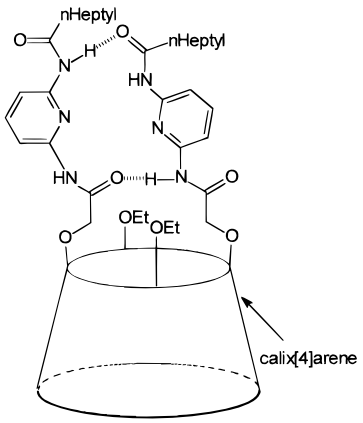
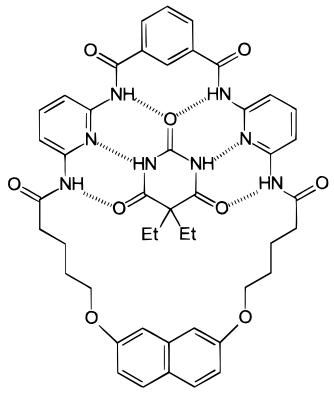
An indirect but clever approach to the fluorescent sensing of Na^+ with **44**[242] employs the macrocyclic calix[4]arene tetraester to bind the cation. During



44

this binding, Na^+ breaks the internal hydrogen-bonding array and allows the diaminopyridine moieties to reorientate toward an external flavin. Hydrogen bonding deactivates the ICT excited state of the flavin. Such deactivation is known for flavins,[243] pyridylindoles,[244,245] and other fluorophores with ICT excited states.[168] In fact, the binding isotherms involving several hydrogen-bonded arrays[246] such as **45**[247] and **46**[248] have been quantitated by means of the fluorescence changes that accompany the processes. In an interesting departure, self-assembling monolayers of alkanethiols on gold surfaces have been elaborated into relatives of **45**.[249] Small but



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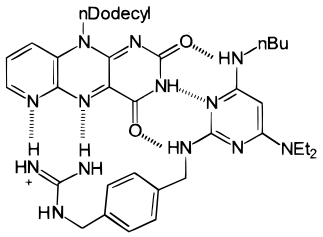
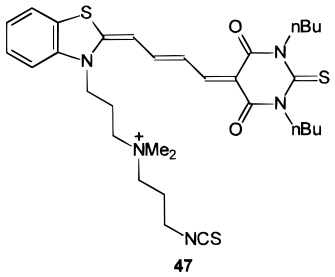


46

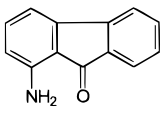
significant emission wavelength shifts were induced by the barbiturate guest. While most of these studies have been conducted in noncompetitive media like chloroform, the observation of hydrogen-bonded arrays within surfactant micelles in aqueous solution[250−252] bodes well for the future.

The Ca^{2+}-binding protein calmodulin has been derivatized with the long wavelength dye **47**.[253,254]



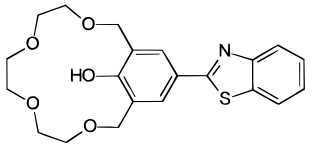
47

Ca^{2+}-induced conformational changes move the ICT fluorophore to a more hydrophobic region. The Ca^{2+} response is measured from the shifts in the excitation spectrum which also allows ratiometry. A similar philosophy and format can be identified in a zinc finger domain peptide outfitted with a fluorophore of the TICT type (see section VI.C).[366] We also note a fluorescein-appended β-cyclodextrin[254a] which may show related effects when its guest responsive fluorescence is examined. The fluorescence response of nonmacrocyclic but internally hydrogen bonded **48**[255] to high concentrations of Li^+ in acetonitrile has some similarities to the Mg^{2+}-binding 1,8-dihydroxyanthraquinone mentioned previously.[218]



48

We end the discussion of cation-responsive ICT excited states by featuring some examples which involve cation-induced proton ejection. The basicity of the medium is poised such that the phenol unit remains intact until the alkali cation arrives at the receptor. Substantial fluorescence enhancements can be observed in this way especially by employing excitation wavelengths which are transparent to the phenol form of the fluorophore. The emissive ICT state corresponds to the tight ion pair of the alkali cation−phenolate. Compounds **49**[256−258] and **50**[259] are the simplest examples. The fluorophore in **49** features again in a calix[4]arene ether receptor.[260] Compound **51**[261] has its crown ether receptor spaced from the fluorophore even though the latter can act as a lariat especially upon deprotonation. A conceptually related system contains a [5-(dimethylamino)-naphthalene-1-sulfonamido]ethyl lariat which deprotonates upon Zn(II) entry into a 12aneN$_4$ macrocycle.[261a] This results in fluorescence enhancement and a blue-shift.



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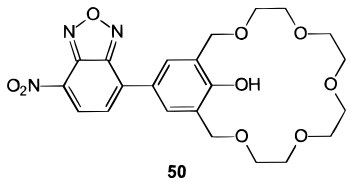

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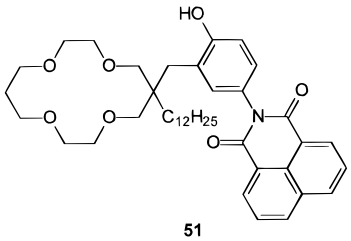
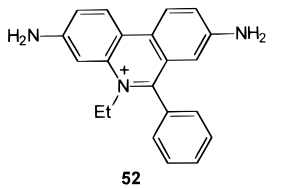


51

Fluorophores with ICT excited states are successful in responding dramatically to polyanionic DNA, although apparently via several mechanisms. For instance, the excited state of **52**[262−265] suffers enough electron drainage from the primary amine units to allow deprotonation when exposed to water. This fluorescence deactivation channel is closed off upon intercalation of **52** in double-stranded DNA, resulting in a large fluorescence enhancement along with a blue-shift in emission. Dimers of **52** linked via the quaternary nitrogen center are even stronger DNA binders.[266] A similar excited-state effect may also control the fluorescence switching "on" of **53** where the displacement of water molecules from the minor groove binding site is crucial.[267] On the other hand,



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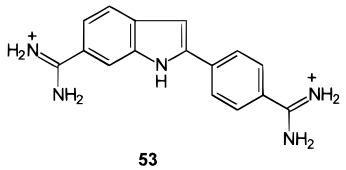
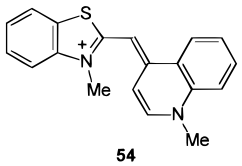


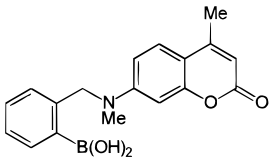
53

the large DNA-induced switching "on" in **54**[268] is more likely to arise from obstruction of double bond torsion (of the type discussed in section IV). The reversibility of binding is sacrificed in dimers of **54** with quaternized spermine linkers but extremely high detection sensitivity is gained.[269] It is particularly interesting that heterodimeric relatives of **52** permitted the estimation of the nucleobase composition of DNA via a fluorescence excitation ratio method nearly two decades ago[270] (cf., cation sensing **21**−**23** and relatives).



54

The near-absence of ICT fluorophores responsive to small anionic or neutral molecules is an embarrassing fact. Some headway is being made by Shinkai with **55**[271] which shows a small spectral shift upon binding fructose. It may be symptomatic that similar structural motifs give rise to much larger fluorescence responses when coupled to different photochemical mechanisms (see section VII). The cyanine dye **56** is more promising.[272] Its ICT excited state is poorly fluorescent until monosaccharide binding obstructs its torsion. The switching "on" of fluorescence by visible excitation and the detection of a previously intractable analyte are two very positive developments. Macrocyclic rigidification of a related cyanine such as **57**[273] can reduce nonradiative rates a 1000-fold in spite of this being dimeric (see section IX).



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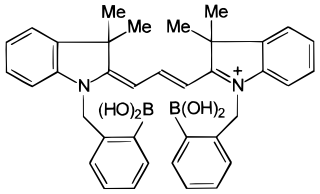

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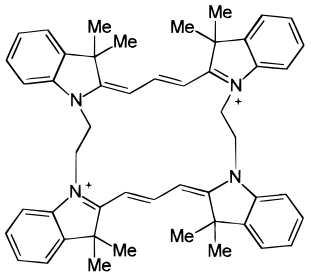
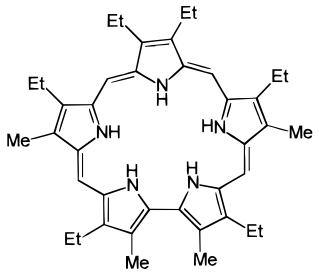


57

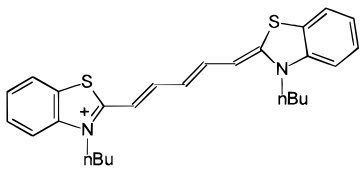
The F^--selective fluorescence enhancement of diprotonated **58**[274] also fits best here even though porphyrins (expanded or not) do not usually display the spectral properties we expect of ICT fluorophores.



58

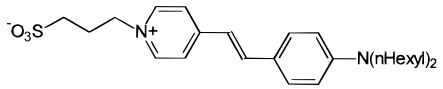
Nevertheless there is no escaping the fact that such systems with their multiple heteroatoms have ample opportunity for electron density redistribution. Coupling of the inner N−H protons to the solvent leads to fluorescence quenching. The situation discussed previously for **17** is not unrelated. F^- nesting in the central cavity cuts off this coupling to the solvent, causing the fluorescence enhancement.

It is clear even from the preceding pages that much of the success of fluorescent sensors has arisen from ion recognition phenomena. Quite separately, ion movements control nerve impulses and, through them, our thought and motion. Therefore it is natural that fluorescent signaling of membrane potential has evolved into a vital research area. However it must be emphasized that these signaling systems recognize the net electric field of these ions rather than their chemical nature. Interestingly, almost all the fluorescent systems developed for this purpose possess ICT excited states. Although some have formally neutral π-electron systems, many of them carry a positive charge to help anchoring in negative membranes. The older, and slower, fluorophores, e.g., **59**,[275] show rather large reductions in emission quantum yield due to accumulation and subsequent concentration quenching in hyperpolarized cell membranes. However we note that the ICT



59

nature of the excited state is not expected to be critical to this mechanism. On the other hand, the ICT phenomenon lies at the heart of the younger, and faster, fluorophores which can act on the microsecond timeframe e.g. **60**.[276,277] The pyridinium nitrogen



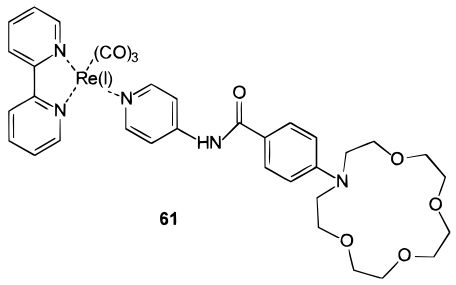
60

center can be expected to lie close to the membrane headgroups with the alkyl sulfonate segment stretched toward water. The di-*n*-hexylamino moiety will burrow among the hydrocarbon chains in the membrane interior. It is important that the long axis of the fluorophore is oriented perpendicular to the membrane. As we have seen previously, population of the ICT excited state results in considerable displacement of electron density. In cases like **60**, this results in a shift of positive charge from being near the membrane surface to the interior where it is shielded from the electrical effects. Thus membrane potential will influence the wavelength position of the emission band.[278] These electrochromic fluorophores represent another strong application of fluorescent sensors and switches in the biological sciences.

B. Metal to Ligand Charge Transfer (MLCT) Excited States

There is a wealth of metal complex-based lumophores with MLCT excited states[279−284] which can be harnessed for the design of sensors and switches. The best known MLCT lumophore is tris(2,2′-bipyridyl)-ruthenium(II) where photon absorption results in the generation of a lowest energy excited state which corresponds to a Ru(III) center and a radical anion of one bipyridyl moiety. In this section, we restrict ourselves to those cases whose switchability is a direct result of the charge transfer nature of the excited state. Quenching of excited states by protic solvents is not limited to the organics featured in section VI.A. Tris(2,2′-bipyridyl)ruthenium(II) shows this effect as unveiled by solvent deuteration studies.[285] Tetrakis(3-phenylpyridyl)dioxorhenium(V) can be used to gauge water penetration in membranes by means of related effects.[171] The MLCT excited state of (2,2′-bipyridyl)tetracyanoruthenium(II)[166,167] is also subject to solvent effects involving hydrogen bonding similar to those discussed for e.g. **17**.[168]

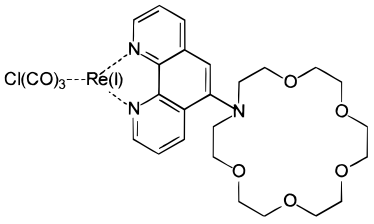
We begin our discussion of ion recognition phenomena with a set that contains a Re(I) polypyridyl lumophore and a crown ether-type receptor for metal ion guests: **61**−**63**.[286−288] Ca^{2+} causes a 8-fold enhancement in the luminescence of **61**[286] which is half that achieved by protonation of the *N*-aryl aza-15-crown-5 ether receptor. The rationalization of the



61

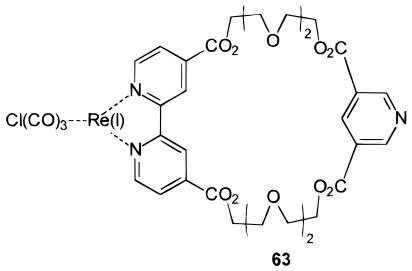
low luminescence quantum yield and lifetime is the evolution of the Re(I) to bipyridine MLCT state into

a nonemissive arylazacrown to bipyridine LLCT (ligand to ligand charge transfer) state. However, photoinduced electron transfer (PET) across the amide linker (section VIII.A) could serve as the luminescence quenching route. In either case, cation binding into the crown ether is expected to disfavor such deactivation. Compound **62**[287] shows significant



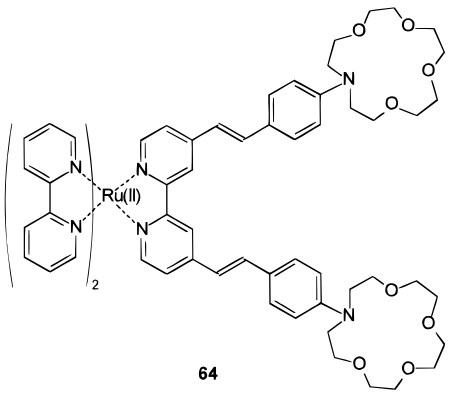
62

luminescence enhancement with Pb^{2+} in spite of the "heavy atom" nature of the latter, perhaps because the sensor is already significantly spin−orbit coupled due to the Re(I) center. Unlike **61**, the direct link between the azacrown ether and the phenanthroline ligand forces considerable overlap between the electron systems of the lumophore and the receptor. So **62** is a direct metal-based analogue of the cases discussed in section VI.A. The functionalized phenanthroline ligand in **62** is itself a fluorescent sensor of the TICT type for Zn^{2+} and H^+(see section VI.C). K^+ causes a 3-fold drop in luminescence lifetime of **63**[288]



63

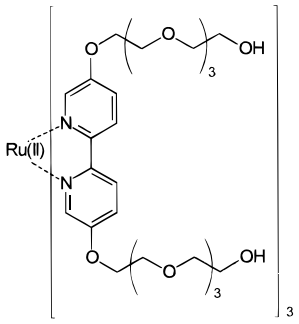
upon nestling in the central cavity. The reasons for this effect are currently unknown. More detailed examination of guest-induced spectral shifts and lifetime effects in this family would be welcome from a sensor perspective. Relatives of **63** present interesting possibilities for guest mediation of transmolecular movements of electrons and/or energy, a challenge which is being taken up elsewhere.[289−293] Compound **64**[294] also belongs here since it contains an *N*-arylaza-15-crown-5 ether receptor and a tris-(2,2′-bipyridyl)ruthenium(II) lumophore integrated into one another. Na^+-binding causes blue-shifts in



64

emission spectra even though the alkali cation would be expected to electrostatically stabilize the MLCT excited state if sufficient charge is transferred into the receptor-functionalized bipyridine moiety.

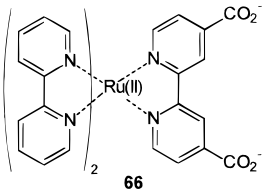
While **65** is devoid of any crown ether receptors, it does possess an array of poly(oxyethylene) chains which can organize around a guest cation, e.g., Ca^{2+}.[295] The MLCT excited state of tris(6,6′-alkoxy-



65

3,3′-bipyridazine)ruthenium(II) is thus perturbed to result in small increases of the luminescence quantum yield and lifetime. It is conceivable that the presence of the guest dication close to the ligand π-electron system can preferentially stabilize the MLCT state relative to the nonemissive MC excited state.[295a]

Nonmacrocyclic receptors such as carboxylate and 2,2′:6′,2″-terpyridyl can also be infused into poly-(pyridyl)ruthenium(II) lumophores, e.g., **66**[296,297] and **67**.[298] Protonation of these receptors induces signifi-



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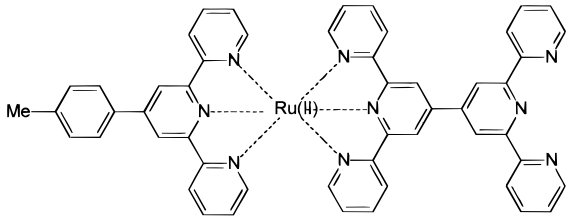


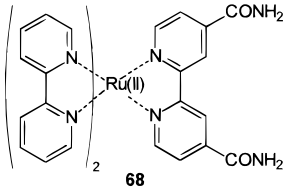
67

cant changes in the luminescence phenomena. These changes are particularly large in **67** where protonation serves to increase the electron-withdrawing nature of the "substituent" on the terpyridyl ligand.[298] Such substituents on ruthenium(II) terpyridyl complexes are known to revive luminescence,[300] most probably by preferentially stabilizing the MLCT excited state compared to the nonemissive metal-centered excited state. We take this opportunity to point out that the same outcome arises from the use of ligands with extended π-electron systems.[299] On the other hand, **66** shows some quenching of luminescence and a concomitant lifetime reduction upon protonation in spite of the electron-withdrawing nature of the carboxylic acid group.[297] The proton-induced red-shift is evidence of the latter effect,

however. Such quenching is even more prominent in an isomeric complex with a nonsymmetrically substituted bipyridine dicarboxylate ligand.[301] Evidently this behavior can be assigned to the coupling of the carboxylic acid group (and to some extent, the carboxylate) to the solvent via hydrogen bonds. The observation of negative activation energies is intriguing, however.[301,302]

If protons are considered as the simplest of guests to interact with metallolumophores, octaprotonated 32aneN$_8$ must be one of the most complex among nonpolymers. This "guest" wraps around the 2,2′-bipyridyl, tetracyanoruthenium(II) lumophore with high affinity and produces blue-shifted emission of much higher quantum yield.[303] Since the "guest" occupies most of the first solvation shell of the lumophore, the observed behavior can be understood as a specially augmented solvent effect.[166,167] Specifically, the "guest" ammonium centers hydrogen bond to the cyano ligands causing a destabilization of the ruthenium(II) center in the MLCT excited state.

A discussion of sensory lumophores with MLCT excited states is best rounded off with some attention to anionic guests which have so far been the poor cousins of supramolecular chemistry.[304] During his extensive studies, Beer[289,305−307] has unearthed some significant luminescent responses to Cl^- and $H_2PO_4^-$ with systems such as **68** and **69**. The approach here



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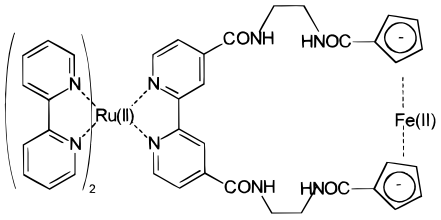
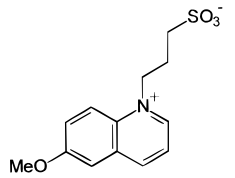


69

is to get effective anion binding with the combined forces of electrostatic attraction and hydrogen bonding acidity. Anionic perturbation of the adjacent MLCT excited state does the rest, although the mechanisms remain to be elucidated. Compound **69** is particularly interesting in several aspects. First, the macrocyclic receptor permits effective selection of Cl^- against the larger $H_2PO_4^-$. Second, the ferrocene moiety distal to the tris(2,2′-bipyridyl)ruthenium(II) can encourage photoinduced electron transfer (PET) or electronic energy transfer (EET) which the Cl^- guest can mediate, cf., **63**.[288] Third, the Cl^--induced switching "on" of luminescence is remarkable, though quantum yields remain low and the spectral shape is anomalous. This is in marked contrast to previous all-organic systems such as **70** which involve PET-type fluorescence quenching with Cl^- (see section VIII.B).

Polyanionic double-stranded DNA can be targeted with cationic metal complexes such as **71**[308−310] and



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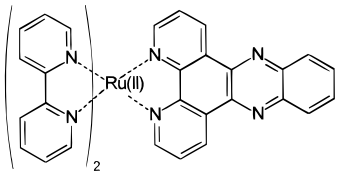
72[311] containing extended planar π-electron systems for intercalation between the base pairs. The popu-



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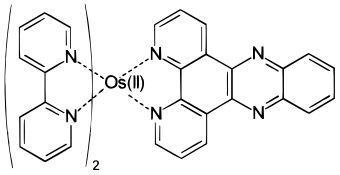
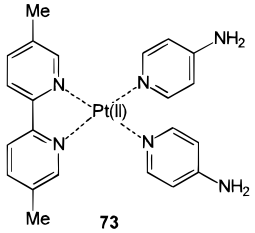


72

lation of the MLCT excited state deposits significant electron density on the metal-free nitrogen centers of the dipyridophenazine ligand, i.e., the basicity is significantly augmented upon excitation. Subsequent protonation of the excited state therefore opens up a luminescence-quenching channel provided that the crucial nitrogen centers are exposed to water. Hence, DNA intercalation serves to protect the MLCT states of **71** and **72** from water and switch "on" their luminescence. The Os(II) derivative **72** extends the luminescence into the low nanosecond time scale and the far red region of the spectrum.[311] Protection from the detrimental effects of aqueous media can also be achieved by hiding the sensitive MLCT excited state of **71** within anionic detergent micelles.[312] Conversely, **71** and **72** serve as sensitive detectors for these important anionic assemblies.

When complexes with less functionalized bipyridines, e.g., tris(2,2′-bipyridyl)ruthenium(II) are employed to probe DNA and other polyanionic species, less dramatic luminescence enhancements and lifetime extensions are seen.[310] This residual effect can be understood as follows. The ligand which serves as the acceptor in the MLCT excited state is likely to point away from the anionic surface toward water (as well as the counter cation atmosphere, in the context of the Debye−Hückel/Gouy−Chapman models). This stabilizing influence is behind the small red-shifts observed and also increases the barrier to reach the nonemissive MC excited state.[313] Racemic tris(2,2′-bipyridyl)ruthenium(II) also binds to the pockets of polyclonal antibodies raised against structurally related complexes.[313a] Significant increases in luminescence intensity and lifetime are found upon binding. The fact that the bound species displays essentially single exponential luminescence decay leads to the important deduction that the antibody population is responding homogeneously, at least with respect to the photophysics experiment.

Compound **72** is not the only instance where the monopoly held by ruthenium(II) complexes in this area has been broken. The Pt(II) complex **73**[314] and the Au(III) complexes **74**[315] and **75**[315] (with or without cyclometalation respectively) display all three qualitative possibilities in turn: "off−on", "on−off", and no change. Evidently, there is life beyond ruthenium(II) in this area but more exploration will be required to understand it. Interestingly, previous all-organic luminescent switches for double-stranded DNA, e.g., **52**, share some of the mechanistic platform occupied by ruthenium(II) complexes like **71**. Compound **73**[314] may involve a similar effect.



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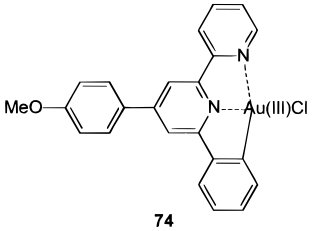

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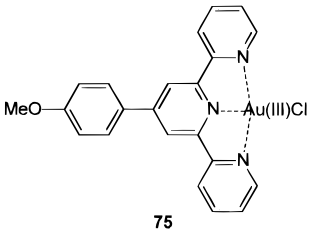

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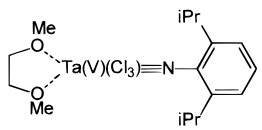
We conclude this section with two additional points. First, we are unaware of any sensory/switchable luminescent systems based on LMCT (ligand to metal charge transfer) excited states. Perhaps this is because the portfolio of emissive LMCT excited states is still small, at least for those operating in fluid solution at room temperature. It is to be hoped that **76**,[316] **77**,[317] and earlier relatives[318] represent the tip of an iceberg waiting to be exposed. The commoner examples of LMCT excited states such as **78** are nonluminescent.[87] Second, several MLCT excited states such as tris(2,2′-bipyridyl)ruthenium(II) are subject to quenching by O_2, at least partly because of their relatively long intrinsic lifetimes.[319,320] This has led to practical sensors for this lifegiving gas.[321−323] While no recognition processes are involved, the delocalization of the excitation over a ligand must aid collisional deactivation by an external agent.



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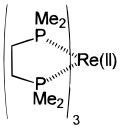

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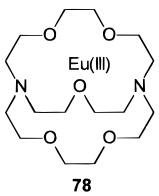
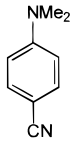


78

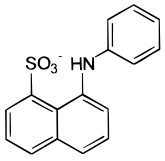
C. Twisted Internal Charge Transfer (TICT) Excited States

Sections VI.A and VI.B catalogued some of the uses to which photogenerated charge separation (or shift or even destruction) can be put in a fluorescence/luminescence context. Obviously, any such phenomena can be enhanced yet further if the charge separation in the excited state can be maximized. Grabowski[324,325] realized that decoupling of the donor and acceptor components of a push−pull π-electron system in the excited state by twisting through 90° can achieve full charge separation. A further advantage of full charge separation is that its thermodynamic feasibility can be assessed independently with electrochemical data.[324−329] Compound **79**[324−329] is perhaps the most celebrated of these systems and gives emission from its TICT excited state along with a shorter wavelength emission from a ICT-type excited state. The latter has the more delocalized excitation even though it was formerly referred to as a LE (locally excited) state. Compound **79** has a planar skeleton and hence a well conjugated π-system. The ICT-type excited state is similarly planar, whereas the TICT excited state has the dimethylamino unit orthogonalized from the rest of the π-system. The result is a dimethylamino radical cation and an adjacent benzonitrile radical anion.



79

Although emissive TICT excited states were instrumental in convincing sceptics of their existence,[324,325] poorly emissive counterparts[330,331] have been in the vanguard regarding applications. The destabilization of red-shifted but poorly emissive TICT excited states would favor the strongly emissive, less polar, ICT-type delocalized excited state. Lowering the solvent polarity is one way of causing such a destabilization. For instance, **80**[331,332] and relatives[333] have been serving the biochemical community since 1954 as sensors for environmental "polarity". Compound **80** shows dramatic switching



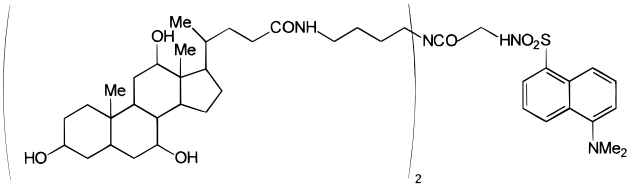
80

"on" of fluorescence upon being moved from an aqueous to a hydrophobic environment. A blue-shift also accompanies this switching action. The temptation of such a powerful switching phenomenon has proved to be too much for a host of workers in the molecular recognition area.

For instance, calix[*n*]arene amino acids include anionic **80** provided that the pH value is not basic enough to produce an anionic host.[70] Host concentrations are such that aggregation complications are

absent. The same cannot be said about a study with octakis[poly(oxyethylene)]calix[8]arene.[334] On the other hand, aggregation was the object of study when poly(vinylbenzo-18-crown-6 ether) transformed from a neutral micelle to cationic version upon treatment with alkali cations. Compound **80**'s binding increases under the latter conditions causing switching "on" of fluorescence.[335] Comparison of the last two investigations is educational because the same type of double reciprocal plot of fluorescence intensity−host concentration data is used to draw different conclusions concerning aggregation[335] or stoichiometric complexation.[334] Not only can **80** detect hydrophobic microenvironments in water, it can also indirectly detect anything that arranges or blocks such regions. The former situation is illustrated by Schneider's[336,337] and de Shayes'[338] work on metal ion-induced cyclophane formation from nonmacrocyclic precursors. Compound **80** takes up residence in the rather hydrophobic cavity of the cyclophane and signals its new status by switching "on" its fluorescence. The latter situation can be seen during the binding of ribose by β-cyclodextrin.[339] Since **80** is displaced from the interior of the cyclodextrin to the aqueous medium during this process, the fluorescence is switched "off".

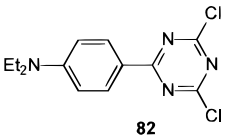
The 5-(dimethylamino)naphthalene-1-sulfonamide group,[340] which is a close relation of **80**, can be built into complex supramolecular structures which undergo large conformational changes upon environmental command. This fluorophore is the key reporter of a solvent-switchable molecular umbrella **81** devised by Regen.[341] Facially amphiphilic cholic acid



81

units surround the fluorophore in largely aqueous conditions but move away to expose the reporter in less polar media. Thanks to the clear fluorescence switching evidence, other applications of this interesting "open−close" function can now be addressed. While there is no doubt about the elegance and potential of Regen's supermolecule, it is important to note that a similar protection of a fluorescent reporter system in water-rich media by a copolymer was outlined by Tazuke as far back as 1982.[342] 5-(Dimethylamino)naphthyl-1-sulfonyl derivatives[165] are also useful as fluorescent reporters when attached to cross-linked poly(dimethylsiloxane) materials for swelling agents such as ethanol.

Although aminonaphthalene sulfonic acid derivatives like **80** appear to have stolen the show, there are others waiting in the wings. Cowley's **82**[157,343] shows strong switching "off" of fluorescence in polar media irrespective of their proticity. Corresponding red-shifts are also found. Substitutional tuning of these phenomena is an added advantage. The presence of the pyridine nitrogen center in **83**[344] endows it with a special sensitivity to quenching by protic solvents, although polar aprotic media also exert a moderate influence. It is worth comparing **83** with



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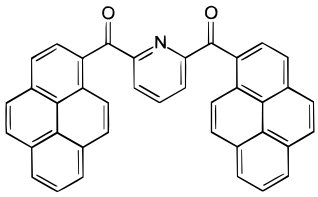
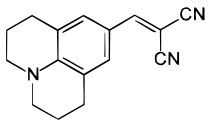


83

the older and ICT-based **17** which is selectively quenched by hydrogen bonding rather than dipolarity.[168]

The twisting motion essential for the birth of TICT states can be retarded by obstructions in their neighborhood. While this can include intramolecular bridging[345] as seen in corresponding alkene $\pi\pi^*$ excited states such as **5**,[76] the more popular focus has been on microviscosity or (especially in the case of polymers) free volume.[346,347] Examples like **84**[345] with poorly emissive TICT states show sharp switching "on" of fluorescence corresponding to the untwisted excited state as the environmental restrictions increase during polymerizations proceeding to high conversion. Others signal local viscosity by means of spectral blue-shifts.[348] Some cases will also permit ratio measurements with the delocalized ICT and TICT bands for added reliability.[347] The traditions of this field go back at least 40 years with examples like **85**[349] and are being kept alive by current examples such as **86**[350,351] and **87**.[352] Although the emphasis in all these sensors and switches is on rotation about formal single bonds, we must not forget that they also possess alkene units. As discussed in section IV, alkenes can also use rotation about double bonds as a deexcitation mode if the lowest excited state is of the $\pi\pi^*$ type. In passing, we note the structural similarity between **86** and the membrane potential sensor **60**.[276] This reminds us that the relatively rigid membrane environment is essential for **60** to fluoresce in the first place. The voltage-dependent wavelength shifts require this underpinning.



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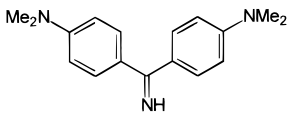

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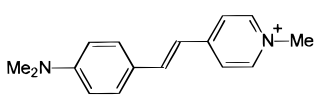

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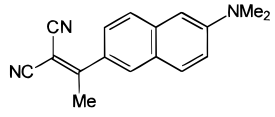
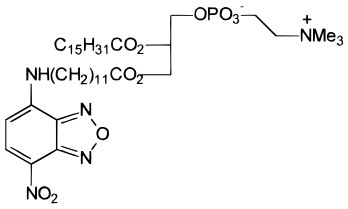


87

Just as fluorescent molecular viscometry is growing (see also section IX), fluorescent molecular thermometry is also gaining in prominence. Even though chemical recognition features are not usually involved, we use this opportunity to give a snapshot of the field since it represents the fluorescent signaling of a chemically crucial parameter. Compound **79** in aqueous β-cyclodextrin solution displays a temperature-invariant TICT emission, whereas the fluores-

cence from the ICT-type delocalized ICT state decreases significantly at higher temperatures.[353] Although the system is complicated by the different locations available to **79** in each of its electronic states, it permits ratioing of two emissions to give self-calibrated sensing. TICT state involvement is also suspected to control the thermal fluorescent response of **88** and **89** embedded within membranes of live cells.[354] The TICT excited state of **88** is



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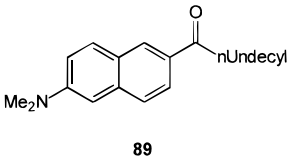
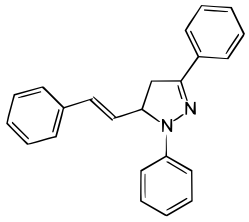


89

nonemissive but is expected to lie energetically above the ICT-type delocalized state. So increasing temperature biases the Boltzmann distribution of excited states toward the TICT state which results in fluorescence loss. The energetic positioning of a nonemissive excited state just above an emissive one is an approach to fluorescent molecular thermometers whose validity transcends TICT states. The nonemissive state within thermal reach can be a ligand centered triplet in some Tb(III) complexes.[87,131,132] It can be a dissociative MC (metal-centered) excited state in tris(2,2′-bipyridyl)ruthenium(II).[313] It can also be a repulsive $\pi\sigma^*$ or $\sigma\sigma^*$ state in fluorescent systems like **90** which show largely reversible fission−recombination sequences.[355] Compound **90** can be substitutionally tuned to adjust the critical energy gap and, in turn, the working temperature range. The opposite of this principle is found in the delayed fluorescence of e.g. **91**.[356] The emissive singlet state lies above the nonemissive triplet. Thermally driven back EET (electronic energy transfer) replenishes the singlet excited state and enhances the fluorescence at higher temperatures.



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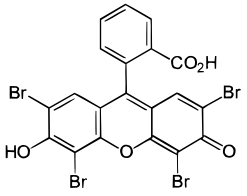
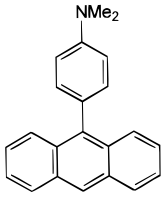


91

Our final concern in this section is with ion and molecule recognition observed via TICT excited states. As noted at the outset, the maximized charge separation in these states should be sensitively perturbed by external agents. The host−guest pairs **19**−Ca^{2+},[181,182,347] **92**−H^+,[357] **93**−H^+,[358] **94**−H^+,[359] **95**−Ca^{2+},[360,361] **96**−H^+,[362−364] and **96**−Ag^+ [363,364] attest to the success of this approach. The last pair is the first example known to us where fluorescence is switched "on" by a redox-active metal ion, and we have commented on its success.[365] With the exception of **94**, all the other cases involve cation reception near the electron-donor site of the TICT excited state and



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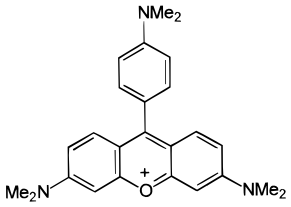

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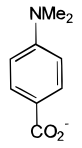

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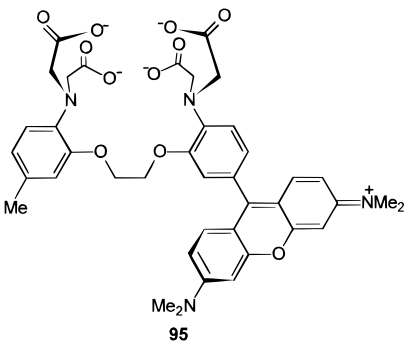

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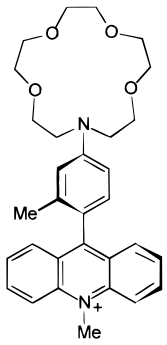
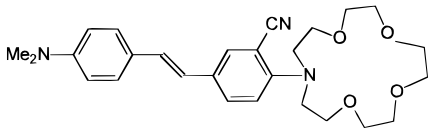


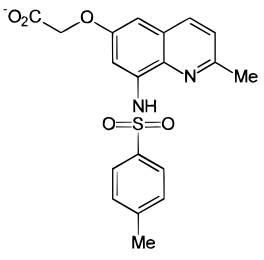
96

display cation-induced switching "on" of the ICT-type emission. Cation reception near the electron acceptor site of the TICT state is arranged in **97**[185] and leads to emission red-shifts upon Ca^{2+} addition. Some examples due to Valeur (e.g. **115**) which may be related to **97** will be considered in section VIII.A. The crowned phenanthroline ligand in **62**[287] which switches "off" its fluorescence upon protonation is apparently another example. Evidently, the TICT state is nonemissive in this case.



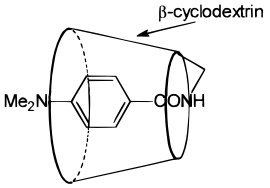
97

A more indirect, but clever, example of ion recognition with TICT excited states is due to Walkup and Imperiali.[366] The peptide finger domains organized by zinc[367] can be exploited for signaling by derivatization with the 5-(dimethylamino)naphthalene-1-sulfonamide fluorophore. The tertiary structure of the peptide undergoes large alterations upon binding nanomolar levels of Zn(II) which forces the fluorophore from a hydrated to a hydrophobic environment. The TICT fluorophore shows considerable enhancement of emission along with a significant blue-shift useful for ratiometry. The Zn(II) response of this supramolecular system compares very well with that of the more classically designed sensor **98**[368,369] of the ICT type which has much in common with 8-hydroxyquinoline and others discussed earlier.[216−218]



98

Molecule recognition by molecular fluorescent sensors and switches by whatever mechanism remains a major challenge. Generic solutions are few and one of the most successful is due to Ueno and his co-workers. Various cyclodextrins are employed as receptors for organic guests in aqueous media. The cyclodextrins are appended with various fluorophores and signaling is achieved via several mechanisms. Some of these systems can be found in section IX. A subset involving TICT excited states is considered here and can be represented by **99**.[370,371] The self-



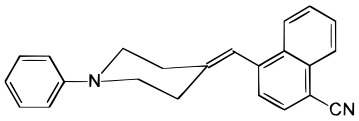
99

complexation of the fluorophore within the rather apolar β-cyclodextrin cavity produces substantial emission from the TICT excited state.[370] The use of 1-adamantanol as a guest displaces the fluorophore out into the aqueous environment causing a major reduction in the TICT emission. The emission from the ICT-type delocalized state is affected much less by this operation. Thus emission-ratioable sensing of 1-adamantanol and related molecules becomes possible. The change in residency is well-supported by circular dichroism and NMR spectroscopies. The smaller α-cyclodextrin counterpart of **99** cannot accommodate such self-complexation.[371] So its fluorescence is opposite in direction and smaller in magnitude upon addition of 1-pentanol as a guest. These experiments were guided by data gathered from intermolecular interactions between cyclodextrins and TICT fluorophores.[372−374] Newer data are also available.[353] The 5-(dimethylamino)naphthalene-1-sulfonamide fluorophore also makes an appearance in another version of **99**.[375] Elaboration of **99** with a biotin unit gives a very interesting triad whose guest sensitivity is inverted in the presence of avidin.[376] A huge increase of guest binding is also seen. Enhancements of alcohol binding are also found when the monensin residue is connected to a relative of **99** carrying a 5-(dimethylamino)naphthalene-1-sulfonamide fluorophore with further augmentation upon addition of Na^+.[376a] Hydrophobic capping appears to be responsible for these effects.

D. Through-Bond Charge-Transfer Excited States

Although intermolecular charge-transfer interactions have a long history,[377] intramolecular counterparts between separated components is of much more recent vintage. Such through-bond interactions have been brought to light by the efforts of Verhoeven,[378] Paddon-Row,[379] and Closs and Miller.[380] Essentially complete charge transfer can be achieved in this way with the attendant advantages for fluorescent signaling mentioned in section VI.C. This becomes a reality in cases which display radiative charge recombination. These through-bond interactions depend on rigid all-trans σ-bond frameworks which link the donor and acceptor components. Due to the active participation of the linker, these systems are discussed here rather than in section VIII. Section VIII will deal with cases where the linker plays the role of a spacer—a much more passive behavior.

Even systems as small as **100**[381] produce CT excited states which emit in the near UV−visible region: evidence of considerable electron delocalization. The response of the emission wavelength to solvent polarity is also considerable, corresponding to an excited-state dipole of 13 D. The fluorescence quantum yields are rather low, even when these systems are optimally excited by irradiating into their CT absorption bands. Nevertheless, these cases set an important precedent. Rather high quantum yields approaching unity can be achieved while preserving the other special features if the basic structure of **100** is elaborated with aromatic units into **101**[382] or **102**.[383] Some fluorescence loss is seen



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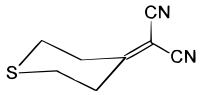

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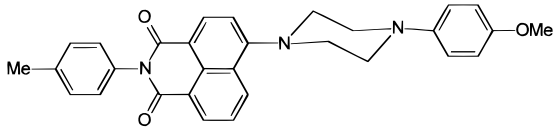


102

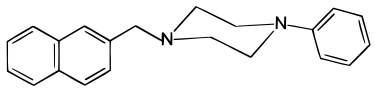
at extremes of polarity where aprotic solvents are concerned. Protic solvents cause complete, but subdiffusional, quenching. Hydrogen bonding to the radical anion site in the CT excited state is a likely culprit. Comparisons with **17**[168] and **83**[344] can be drawn from a sensing perspective. The solvent polarity range producing the large fluorescence can be rationally tuned by paying attention to the redox potentials of the donor and acceptor components of **101**. This is one of the bonuses of working with complete charge transfer, i.e. electron transfer (see sections VI.C and VIII). Electrochemical rationalizations can also be made for solvent-induced excited state interactions seen in some cases. The lowest excited state of **103** is usually CT but not in aliphatic hydrocarbon solvents where the locally excited (LE) state of the 2-vinylnaphthalene unit wins out. The excellent sensitivity of **101**[382] to local "polarity" can be applied to illuminate biochemical problems by attaching a thiol reactive maleimide unit to the 4-position of the aniline moiety.[384] However, ingress of water will kill off the emission.[382] Hence, only hydrophobic microenvironments can be targeted at the moment.

The apparent rigidity of **101** belies the fact that the excited state must undergo some conformational adjustment following complete charge transfer, e.g., flattening of the initially pyramidal nitrogen in the piperidine ring and of the vinyl naphthalene unit. Hence a degree of fluorescence response to viscosity is expected.[385,386] The small conformational changes mean that **101** can detect changes in the high viscosity regime.[386] Compound **84**[345] and related

TICT systems[346,347] have several similarities with **101** in their behavior.

An example of ionic perturbation of a CT excited state is also provided by **103**.[382] The state inversion mentioned above can be easily arranged by protonation of the aniline component. The broad CT emission is then replaced by the blue-shifted and vibrationally structured emission from the LE state. A particularly important case of proton-controlled switching of fluorescence is found in **104**.[387] Careful acidification neutralizes the central aliphatic amine unit from its participation in the three-component CT interaction. A long-range aniline to naphthalene CT process survives however. Experimentally this is indicated by the extremely red-shifted emission being replaced by a shorter wavelength, but still weak and broadened fluorescence. Such ion-directed switching of electron transfer route is unprecedented.

103



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VII. All-Organic Triplet Excited States

The last type of excited state which we meet in luminescent signaling research shifts the focus from orbital occupancy or charge separation to spin multiplicity. All of the all-organic excited states considered so far were singlets. Metallic systems can introduce states of higher multiplicity though spin begins to lose its meaning as spin−orbit coupling takes hold lower down the periodic table. Nevertheless, a triplet designation can be attached to the MLCT excited state of tris(2,2′-bipyridyl)ruthenium(II) for instance.[388] Our emphasis here is on all-organic systems however.

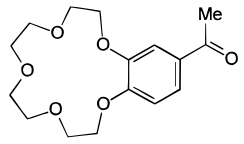
Organic triplet states can be generated by direct excitation with "heavy" atom auxiliaries to aid the required intersystem crossing (ISC).[28,48] Direct excitation in the presence of O_2 is another approach,[28] although the requirement of high gas pressures and the fear of photoxidation are drawbacks. Some molecules such as aromatic carbonyl compounds need no external help since ISC is rapid anyway. These can assist the birth of other organic triplets by intermolecular electronic energy transfer (EET).[28,48]

Phosphorescence is the most visual photophysical signal from a triplet excited state.[389−391] However its observation is not straightforward due to the fragility of triplet excited states with regard to collisional quenching with other triplets. Embedding it in a glass is a solution which is most successful at low temperatures,[389] making it less useful for general sensing and switching purposes. Adsorption of the phosphor on certain surfaces is also fruitful.[391] The problem is more intransigent in fluid solution and schemes employing statistical or steric protection of the triplet excited state become necessary. The former scheme requires microenvironmental compartmentation as provided by aqueous micellar solutions.[14,16,392−394] Since the organic phosphor and the quencher (most commonly residual O_2) will take up residence in the micelle according to a Poisson distribution,[14,16,395] a significant fraction of organic triplet excited states will not have a quencher within the same micelle when they are photogenerated. Double occupancy of a given micelle by organic triplet states is also disfavored. This clever solution is only limited by the intermicelle diffusion rate of O_2 and by micelle dynamics. In practice this means that deaeration of samples becomes necessary.

The second solution of steric protection is also an elegant one. Insertion of a phosphor into a cyclodextrin cavity provides a transparent jacket for the triplet excited state. Material contact with the environment is minimized while permitting photon traffic in and out. Early successes involved halonaphthalene phosphors,[396−397] although even aromatic hydrocarbons were persuaded to emit phosphorescence by coincluding haloalkanes or relatives as "heavy" atom auxiliaries.[398−402] Some of the cases even operated in aerated solution with[402] or without[403] O_2 scavengers. There are limitations however. If phosphorescence is to be useful for sensing, the encapsulation of the triplet excited state away from the very environment to be sensed might seem paradoxical (see later and section VIII.C). Phosphors with $n\pi^*$ character can lead to irreversible hydrogen atom abstraction reactions with the encapsulant.[404−406]

Once a triplet excited state is persuaded to emit phosphorescence in room temperature fluid solution, two rewards present themselves. Both arise from the long millisecond lifetime caused by the spin change required during deexcitation (exceptionally short triplet lifetimes are usually caused by additional quenching processes). As in the case of lanthanide luminescence (section V), delayed observation will remove interference from fluorescence and scattering. The second reward, also potentially available to lanthanide systems, is that lifetime (rather than intensity)-based sensing is technically easy. There is a substantial literature on fluorescence/luminescence lifetime sensing of several guests[210−214,319−323] (see also section VIII.A) which can be adapted for the less demanding time scale. By its very nature, lifetime sensing is internally calibrated against environmental uncertainties that can bother intensity-based methods.

In spite of these rewards, phosphorescent sensors and switches represent a field in its infancy. After all, the observation of fluorescence emission in room temperature fluid solution had a head start of four centuries[407] over its delayed cousin. We begin this discussion of the small handful of examples with some cryogenic experiments. Sousa and Larson's pioneering investigations into macrocyclic systems **7**−**9** with cation-responsive emission included phosphorescence measurements on 77 K glasses.[79,80] The changes observed can be largely rationalized by "heavy" atom induced spin−orbit coupling. Shirai and Tanaka's study focused on the aryl ketone phosphor within **105**.[408] Under most conditions it



105

displays phosphorescence from a $\pi\pi^*$ triplet state as judged from the length of the lifetime. The possible exception is in the presence of Na^+ which fits well into the crown cavity when the emission component of $\pi\pi^*$ origin is significantly quenched. We can understand this since the aromatic ether oxygen electron pairs are held by Na^+ and, in effect, deconjugated from the rest of the π-electron system. The blue-shift of emission in this case is suggestive. Thus triplet-state inversion becomes feasible (see section III for singlet cases). Phosphorescence from the $n\pi^*$ triplet excited state occurs at much shorter time scales. Of course, "heavy" atom influences must play a part in the smaller enhancement of emission quantum yield of **105** with Cs^+.

As mentioned above, cyclodextrins can offer substantial steric protection for triplet excited states held within. However, in most cases, O_2 is capable of breaching this defense, i.e., the complex is nonemissive in aerated solution. Binding of an additional agent can cover these remaining deficiencies. Ternary complex formation with *tert*-butyl alcohol produces intense phosphorescence from 1-bromonaphthalene included in glucosyl β-cyclodextrin.[409] This example fom Nocera's laboratory is an excellent "off−on" switch induced by a neutral guest.[410] Hydrogen bonding of the guest functionalities to the rim of the macrocycle is considered to be responsible for cocomplexation. An interesting pH-dependent phosophorescence is seen when pivalic acid is substituted for *tert*-butyl alcohol because the ionized form of the acid is unable to offer complete steric protection from O_2. Less dramatic but older examples from Turro's research concern acetonitrile as the cocomplexant.[396] A somewhat related phosphorescent signaling system will be considered in section VIII.C.

VIII. Photoinduced Electron Transfer (PET) Systems

Due to its central role in photosynthesis, there is a rich mine of information on this subject.[411−416] Nevertheless, the exploitation of this information for the development of fluorescent sensors and switches has been confined to the last two decades with the few pioneering efforts being scattered across the first of these decades. We comprehensively reviewed this field in 1993.[365] We have updated this survey in a companion[417] to the present account. Several of the laboratories active in this field have summarized their own contributions along with related material. These include the groups of Czarnik,[418−421] Fabbrizzi,[422−424] Tsien,[33,192,425] Kuhn,[426] Desvergne and Bouas-Laurent,[427,428] Rettig and Lapouyade,[347] Valeur,[26,186,429] Shinkai,[430−433] and ourselves.[7,8,434−438] The reader interested in the wider picture is referred to the above material.

Fluorescent signaling via the PET strategy is distinguished by its intrinsically supramolecular

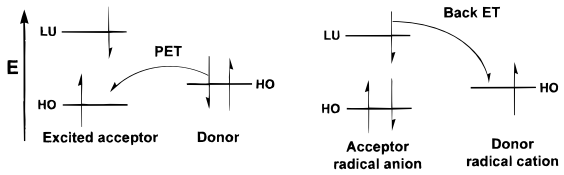
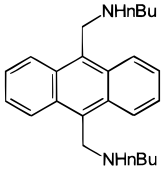


Figure 10. Frontier orbital energy diagrams illustrating thermodynamics of PET and back electron transfer.

nature since distinct components perform each one (or more) of the necessary functions. A fluorophore module is the site of both photonic transactions of excitation and emission. A receptor module is responsible for guest complexation and decomplexation. A spacer module holds the fluorophore and receptor close to, but separate from, each other. This also means that true molecular engineering applies, i.e., the optical, guest-binding, and redox properties of the components allow the quantitative prediction of the signaling parameters of the supramolecular system. Further, PET signaling systems have natural "all or none" switchability: guest-induced "off−on" and "on−off" fluorescence are both designable.

The pioneering work of Weller over a quarter of a century ago[439,440] is our starting point, since this provides the thermodynamic basis of PET. Figure 10 provides a summary in terms of frontier orbital energies. It also shows how PET systems employ thermal back-electron transfer as a self-repair mechanism following the potentially damaging PET process. Although allowance needs to be made for the extent of electron delocalization in the PET partners,[441] Weller's approach even allows estimates of kinetics in intermolecular situations.[440] However the PET kinetics in the intramolecular context, which applies to the signaling systems under discussion, are best described following Marcus.[442−444]

As indicated in sections VI.A−D, charge-separating processes, especially those separating a full electronic charge, are highly sensitive to environmental stimuli. Of course, PET is the ideal process fitting this description, even though charge shift (or translocation) can be seen in intrinsically charged systems. This environmental sensitivity shows up in fluorescence (quantum yield and lifetime but not wavelength except for exciplexes) since it competes with electron transfer to deactivate the photoproduced excited state.[445−448] For instance, the fluorescence quantum yield of **106**[449] shows a nearly step-function response to solvent polarity with switching "off" at high polarities. Solvent dipolarity (π^*) and hydrogen bond



106

basicity (β) are the controlling parameters. Not only is this linear solvation energy relationship understandable according to PET principles but substitutional tuning of the step-function to different solvent polarity ranges is also possible, cf. **101**.[382]

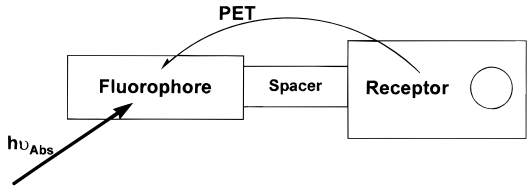


Figure 11. Spaced fluorophore−receptor system in the "off" state. The thermodynamic condition for PET is that the excited-state energy of the fluorophore must be sufficient to oxidize the receptor and reduce the fluorophore.

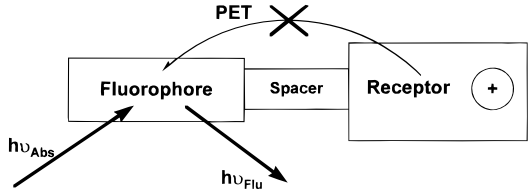


Figure 12. Spaced fluorophore−receptor system in the "on" state. PET is thermodynamically disfavored since the oxidation potential of the receptor is raised by cation entry.

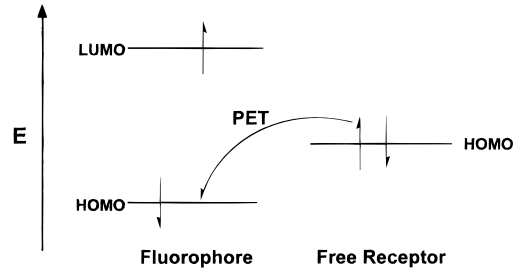


Figure 13. Frontier orbital energy diagram elaborating on Figure 11.

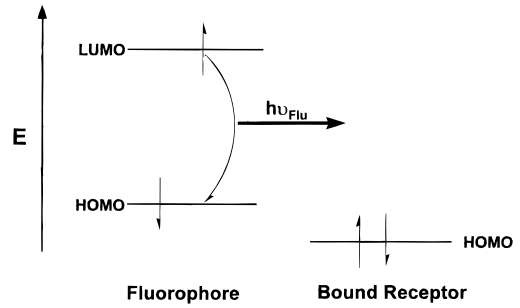
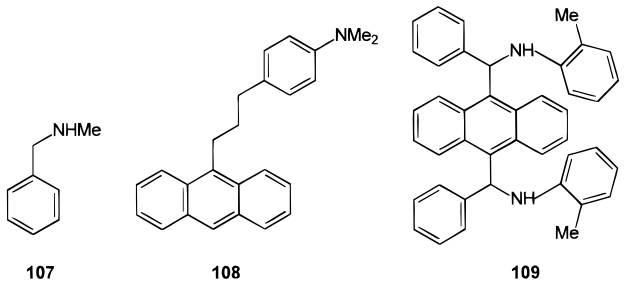


Figure 14. Frontier orbital energy diagram elaborating on Figure 12.

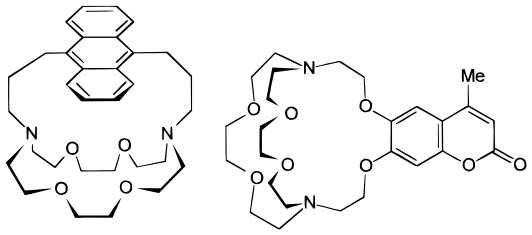
A. "Off–On" and "On–Off" Switches

Stoichiometric host−guest recognition is the more popular avenue for fluorescent PET signaling. This strategy is Schematized in Figures 11 and 12.[7,8,365] A somewhat more quantitative view is in terms of the frontier molecular orbital energies given in Figures 13 and 14.[8,365] The fact that cationic guests are chosen in these figures simply reflects their dominance in the literature thus far. One reason for this dominance is that receptor design for metal ions is relatively rational.[450] A substantial literature has built up concerning cation-induced alterations of frontier orbital energies as estimated from redox potentials.[294,451,452] The history of the field began with **107**[453] along with other phenylalkylamines[454] and **108**,[455] due to Morawetz,[456] Shizuka, and Selinger respectively. Davidson also reported the related case of *N*-butyl-*N*,*N*-bis(1-naphthylmethyl)amine in 1977.[457] All four reported the enhancement of fluorescence due to protonation: the first cases of guest-induced "off−on" switching. Nakaya's **109**[458] was a near miss since the low fluorescence was noted but not exploited. Of course the insights of Weller's investigations were not generally available in 1966. Compound **109** was ahead of its time.



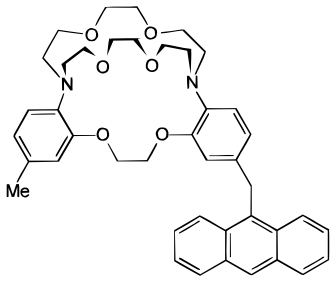
107 **108** **109**

The strength of the fluorescent PET signaling principle is that, given suitable redox activity, almost any receptor that is available for a given guest under a specific set of conditions can be converted into a sensor or switch. Indeed, this can be done in a range of absorption and emission colors. The converse can also be recognized in the examples that follow. A given fluorophore can be paired with a variety of receptors. The flexibility of the modular approach is the clear message. The favorable binding selectivities shown by cryptands toward alkali cations is exploited within **110**[459,460] and **111**[463] on the basis of anthracene and coumarin fluorophores, respectively.



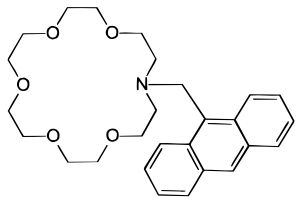
110 **111**

Both of these show substantial fluorescence enhancements upon guest entry. Compound **110** also binds Ag(I) because of cation−π interactions due to the anthracene lining the cryptand cavity, but no fluorescence is seen (see section VIII.C). A bifluorophoric version of **110** shows some similarities and also some interesting variations (see section IX).[461] Attachment of the diaza-18-crown-6 ether unit to the 1,4 positions of the anthracene reduces the intercomponent interactions compared to **110** resulting in smaller intensity effects due to alkali cations.[462] However, significant band shifts are seen, especially with protons. The orientation of the π-electron system in **111** is such that potential guests are presented with a classical [2.2.2] cryptand cavity. So it is not surprising that **111** can measure intracellular K^+ after careful pH adjustment.[463] On the other hand, perfluorination of the methyl group in the coumarin unit appears to lose this fluorescence "off−on" signaling capability toward alkali cations.[464] Further study seems necessary, especially because proton binding causes the expected fluoresence enhancement. N-Benzannelated cryptand **112** produces order of magnitude fluorescent enhancements with K^+.[465]



112

Azacrown ethers like **113**[466] are also unequivocal in delivering K^+-switched emission in spite of their structural simplicity. Compound **114** is an early example whose fluorescence responded to Ca^{2+} in a solvent extraction experiment.[467] Compound **115** is one of several examples from Valeur's laboratory which respond to a variety of metal ions from various regions of the periodic table.[468−470] Charge density



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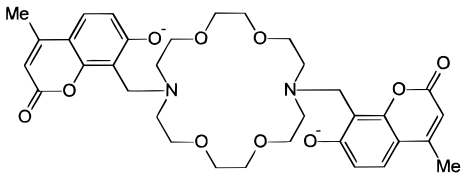

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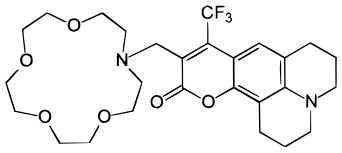
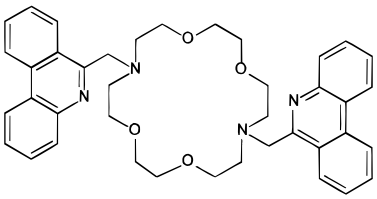


115

control of the metal ion-induced fluorescence enhancement is evident. Compound **115** has another point of interest since the ICT excited state of the fluorophore is stabilized by the metal ion when bound to the receptor. This leads to metal ion-induced red-shifts in the emission which are unusual among ICT-type fluorescent signaling systems in section VI.A and among the TICT counterparts in section VI.C. Zinic's **116** also delegates an auxiliary role in guest binding to the fluorophore as in **115** and offers X-ray crystallographic evidence as well.[471] Czarnik's **117**[472]



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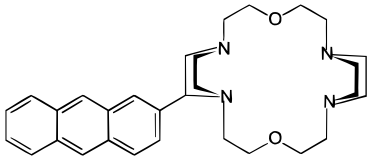
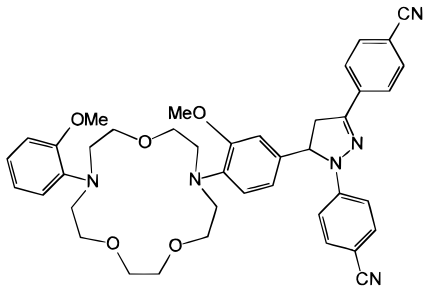
possesses a Pb(II)-selective receptor but no Pb(II)-induced fluorescence enhancement is found. Connection of the anthracene fluorophore through its 2-position seems to exert a negative regiochemical effect on PET (see section VIII.C). The aliphatic



117

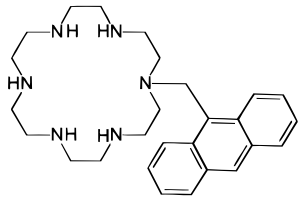
nitrogen centers in **113**−**117** naturally leads to large fluorescence enhancements with H^+. Compound **118**[473] controls this tendency by adapting the heart of Tsien's ICT-type fluorescent sensor **22**[199,200] for Na^+. Now the nitrogen centers in the diaza-15-



118

crown-5 ether unit are part of the aniline π-electron systems and therefore of reduced basicity. Nevertheless, its pseudo cryptand nature allows sufficiently strong Na^+ binding. Furthermore, the draping of this ligand around Na^+ causes decoupling of the nitrogen centers from the rest of the π-electron system of the receptor (cf. section VI.A). Thus the retardation of PET, and hence the Na^+-induced switching "on" of fluorescence, is as strong as ever.

The macrocycle theme continues with 18aneN_6 and 12aneN_4 systems **119**[474] and **120**,[475,476] respectively.



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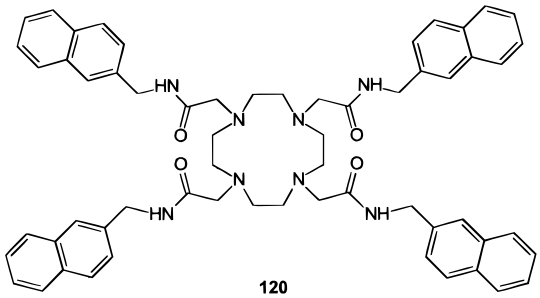
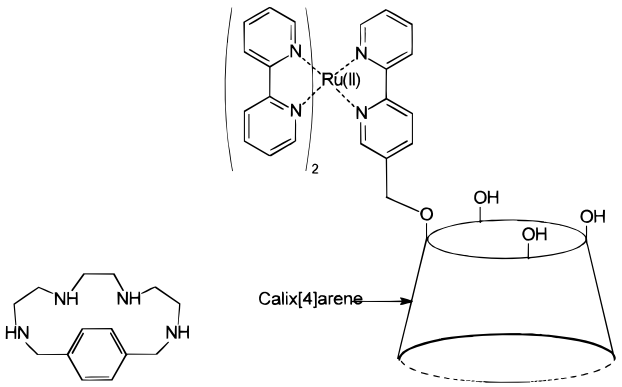


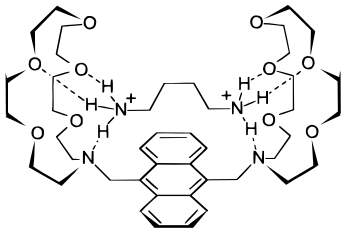
120

Both of them serve as fluorescent "off−on" switches with Zn(II) given adequate pH control. Zn(II) binding alters the relative spatial disposition of the four naphthalene fluorophores in **120** to produce different enhancement effects in the monomer and excimer bands. Therefore, self-calibration via ratiometry

becomes possible. Kubo and Sakurai[476a] find the same outcome for *N*,*N*-bis(1-pyrenylmethyl)diaza-18-crown-6 ether with a range of metal ions. The presence of multiple fluorophores exacts a price and large reductions of binding strength are seen relative to *N*-(1-pyrenylmethyl)aza-18-crown-6 ether, although this is partly caused by the change in the ligating atom set. Polyazacyclophane **121**[477] and calix[4]arene **122**[478] share the feature of proton-induced enhancement even though their receptor units are based on different atoms. Suitably positioned pairs of aza-15-crown-5 ether units can signal the presence of butane-1,4-diammonium ions via an intervening anthracene fluorophore due to the complex **123**.[479] Hydrogen-bonding blocks both nitrogen lone electron pairs and their possible PET activity.

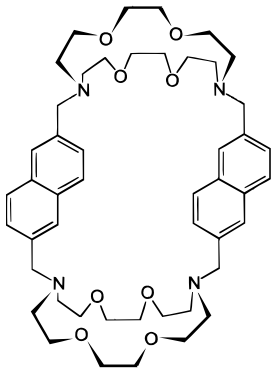


121 **122**



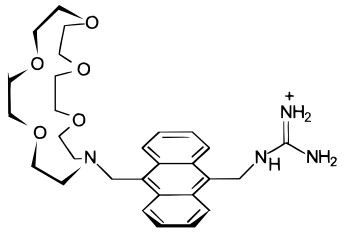
123

Compound **124** has two diaza-18-crown-6 ether units bridged by two 2,6-naphthylenedimethyl units.[480]



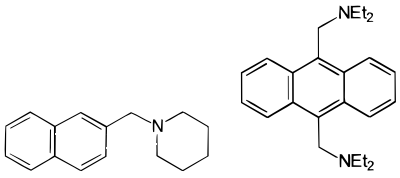
124

Binding of pentane-1,4-diammonium ions by **124** is supported by several lines of evidence, but fluorescence signaling is not one of them. Since protons are very successful in switching the emission "on", the failure of pentane-1,4-diammonium ions must be attributed to their inability to block all four nitrogen lone electron pairs sufficiently strongly. Compound **125** targets the neurotransmitter γ-aminobutyric acid zwitterion,[481] although improvements will be necessary if this target is to be sensed in its natural habitat.

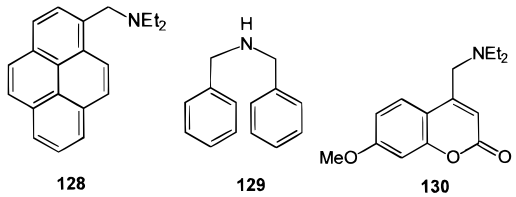


125

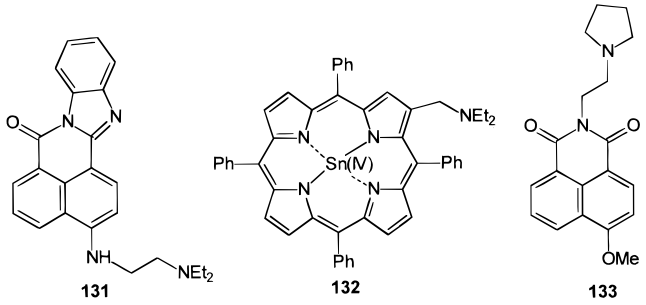
Nonmacrocyclic receptors play perhaps a larger part in serving as recognition components within fluorescent PET "off−on" signaling systems. The Brønsted basicity of aliphatic amines is exploited within the pH sensors/switches **126**−**133**[387,482−488] as in their predecessor **107**.[453,454] The signaling ability



126 **127**

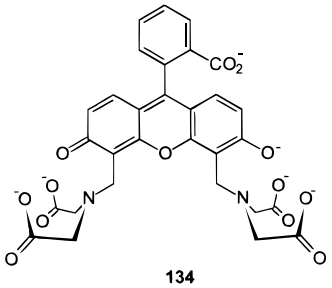


128 **129** **130**



131 **132** **133**

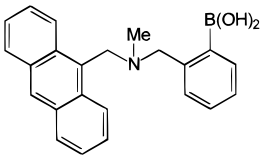
of **127**[483,484] and **133**[488] is also supported by time-resolved studies. Significant signaling action is not confined to methylene spacers.[454,486,489] Saeva's study with conformationally restricted systems is also relevant.[490] Compound **134**[491] even predates **107** in



134

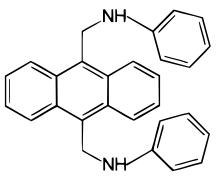
terms of its structural format, but its pH sensing

action is complicated by protonation of its ICT fluorophore with resulting fluorescence loss. The proton-induced fluorescence "off−on" switching behavior can be shifted to the alkaline region with cases like **135**[492] in the presence of sugars (see later) where the nitrogen electron pair is blocked by the boron center except at rather high pH values. Following



135

on from **108**,[455] aromatic amines feature in systems **136**[482] and **137**[493] responding to lower pH ranges. A



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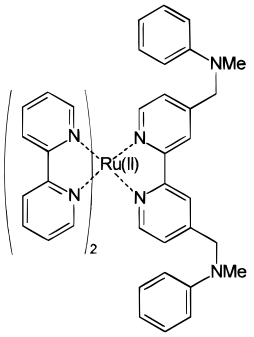
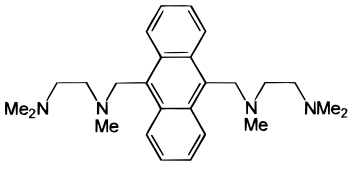


137

related case is distinguished by the presence of a β-turned dipeptide as part of the spacer module.[496] The difficulty of protonating polyamine receptors such as **138**[494] and **139**[495] also leads to a similar outcome.



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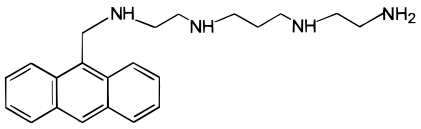
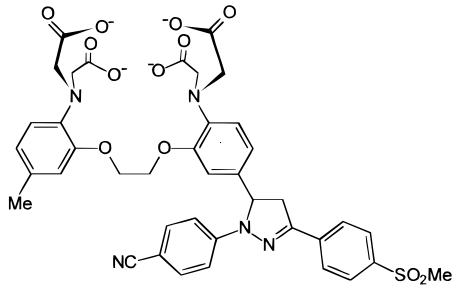


139

Compounds **129**, **138**, and **139** can also signal the presence of Zn(II) and bear comparison with **98**[368,369] and fluorescent zinc finger peptides[366] (also see section X). Morawetz's **129**[453,456,485] and derivatives are rather special because they helped to initiate several lines of research which are burgeoning today: pH and metal ion signaling (via PET principles), self-calibration via dual emission, and microviscosity signaling via monomer−excimer equilibria (section IX).

When elaborated with extra ligating sites, aromatic amine-based receptors are particularly suited for fluorescent systems sensing metal ions under physiological conditions. These systems are immune to normal fluctuations of pH. Such systems produce dramatic "off−on" responses which can allow sensitive of detection of small alterations in these metal ion concentrations. Ca^{2+} is targeted by **140**[497] and **141**,[426] both of which use Tsien's selective Ca^{2+} receptor[498] within **21**.[198] It is notable that the spacer



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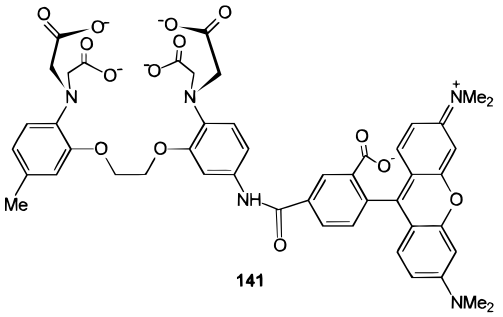
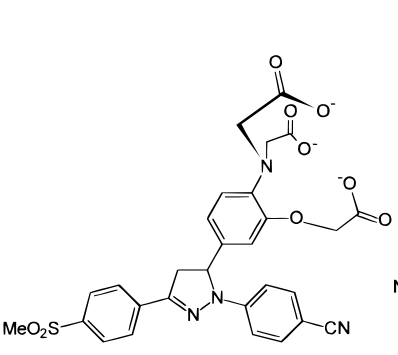


141

unit in **140** is clearly the 5-methine group (within the pyrazoline), whereas this function in **141** must be assigned to the amide with the orthogonally twisted aryl group (on the rhodamine) also assisting. Amides serve as connectors which encourage PET via superexchange in several photoactive supramolecular systems.[499,500] Bifluorophoric versions of **140** and **141** produce even larger fluorescence enhancements on encountering their guests.[426,501] London's selective Mg^{2+} receptor[502] within **23**[201] reappears in the Mg^{2+}-responsive signaling system **142**.[503] Almost complete freedom from proton-induced fluorescence switching can be achieved by using nitrogen-free receptors within PET systems. Compound **143** shows fluorescence switching "on" with Na^+ with excellent selectivity against protons.[504] This discrimination can be put to good use (see section VIII.C).



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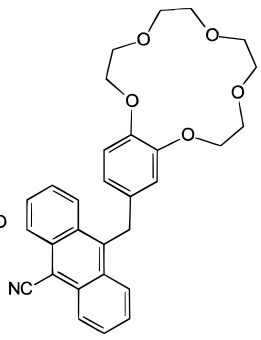
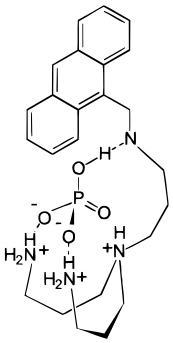


143

A clever adaptation of a partially protonated polyamine by Czarnik[505] to serve as a receptor whose PET channel is blocked upon arrival of HPO_4^{2-} guest can be seen in complex **144**. Two of these receptor units can be placed on the 1- and 8-positions of an

anthracene fluorophore as in **145**[506,507] in order to create a signaling PET system for pyrophosphate in the form of $H_2P_2O_5^{2-}$. Both of these cases require careful pH control for success. As a bonus, **145** will also assay the pyrophosphatase enzyme since the hydrolysis products will be released from the receptor pair with concomitant loss of fluorescence.



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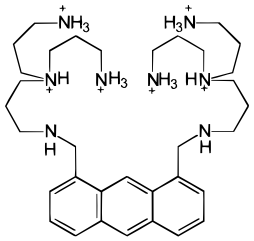
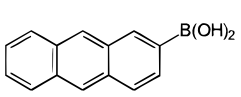


145

As seen above, judicious use of nonmacrocyclic receptors within fluorescent PET "off−on" signaling systems succeeds against cationic and anionic targets. An important class of neutral targets have also succumbed recently. Fluorescent PET sensing of sugars is a major success story in this general area.[430−433] Following on the heels of **146**[508] and **147**,[509] **135**[492] exploits the tried and tested PET signaling format in **127**[482] to produce strong fluorescence enhancements with fructose in neutral aqueous solution. Compound **148**[510] shows excellent selectiv-



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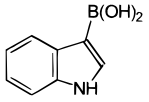
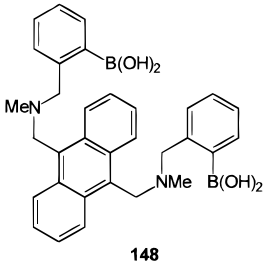


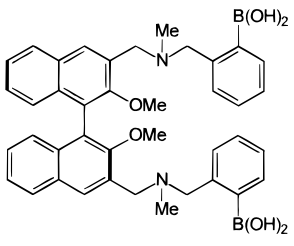
147

ity for glucose due to the geometric recognition capability of its bireceptor system reminiscent of **123**[479] and **145**.[506,507] A milestone in this area is **149**



148

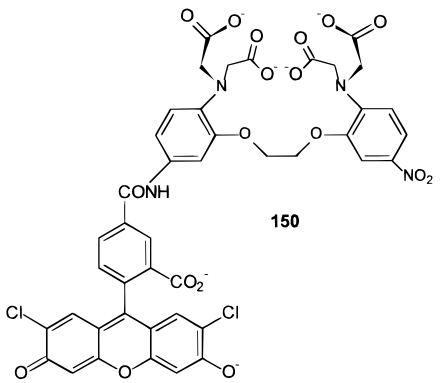
whose enantiomer pair demonstrates good discrimination between monosaccharide enantiomers via fluorescence.[511] Naphthylprolinol-derived calix[4]-



149

arenes can also provide a solution to the tough problem of chiroselective signaling via fluorescence, although different mechanisms are involved in this case.[512] The 1,1′-binaphthyl component within **149** serves in four crucial capacities: fluorophore, PET acceptor, rigid backbone for geometric selectivity, and chiral 'centre' for enantioselectivity. However, none of this would have been possible if the reception capability of boronic acids[513] and especially 2-aminomethylphenyl boronic acids[514] toward diols had gone unnoticed by Czarnik, Aoyama and Shinkai. Since it is only weakly bonded to boron, the amine lone electron pair is largely free to engage in PET activity with the fluorophore nearby in neutral solution. An attractive view is that sugar binding encourages the rehybridization (from sp^2 to sp^3) of the boron center forcing a strong boron−nitrogen bond. Thus the PET activity ceases and fluorescence is switched "on". There are bound to be further applications of these key ideas. Recent applications include (a) a wide spectrum (with regard to sugar structure) fluorescent sensor for high sugar concentrations common in the food and beverage industry,[515] and (b) competition betwen PET and excimer−monomer equilibria[516] (section IX) which may allow sugar assay with self-calibration as in **120**[475,476] and **129**.[453,456,485]

All the cases discussed so far in this section have exploited the blocking of PET in one way or another. Another avenue of enhancing the fluorescence emanating from a fluorophore−spacer−receptor PET system is to increase the effective length of the spacer. Guest binding to the central region of such systems can prevent close approach of the PET-active terminals. This scenario is observed in Ca^{2+}-responsive **150**[426] and Na^+-responsive **151**.[517] The acetyl-



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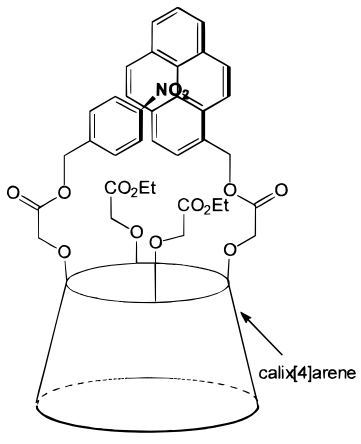
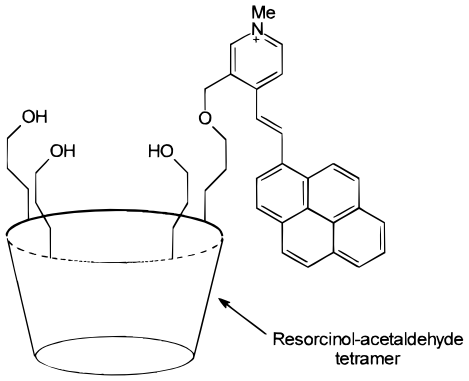


151

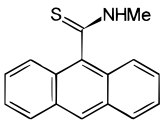
choline-responsive **152**[518] is an example of a fluorophore with an ICT excited state switching its environment from inside to outside of a resorcin[4]-arene receptor upon guest inclusion. The binding of acetylcholine is driven by cation−π interactions.[519] The fluorescence is enhanced 2-fold even though the corresponding intermolecular analogue (which is less useful as a sensor) gives a more visually dramatic response in alkaline solution. The larger response of the intermolecular case can be attributed to (a) rapid PET from the powerfully electron-donating tetradeprotonated resorcinarene to the complexed fluorophore and (b) the large spatial separation between the receptor and the free fluorophore once it is displaced by acetylcholine. Such a large separation is impossible in the tethered case of **152**. Similar participation of cavitand π-electron systems in PET processes with incarcerated fluorophores is known.[520]



152

Sophisticated sensors, such as engineered peptide sequences,[521] will also permit control of separation between PET active terminii by means of external stimuli such as temperature (see fluorescent molecular thermometry in section VI.C). Cooling below −8 °C causes a 3_{10}-helix to α-helix transition in a oligopeptide outfitted with a 1-methoxynaphthyl fluorophore and a piperidone electron acceptor.[522] The contribution of the shorter lifetime component to the fluorescence almost disappears as a result even though the opposite would have been expected on geometric arguments alone. Obviously, there are many mysteries about PET across peptides that need to be dispelled in further studies. Even without major conformational consequences, guest binding in the path of PET can influence superexchange interactions and hence, control luminescence quantum yields and lifetimes.[290,291,523−525]

We round off this discussion of fluorescence PET "off−on" switches with a few examples involving irreversible interactions. Compound **153**[526] produces large fluorescence enhancements with thiaphilic Hg(II) and Ag(I) even though the redox activity of these guests usually wrecks fluorescence. The origin of this



153

fluorescence enhancement and the irreversibility lies

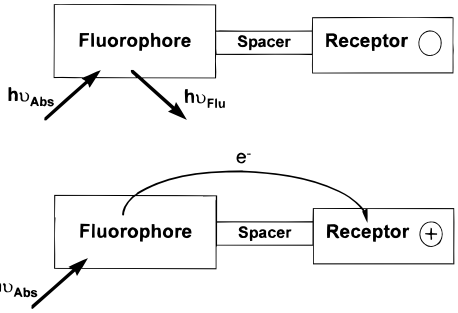
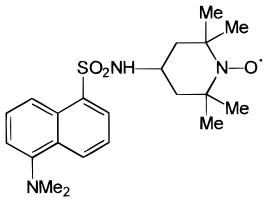


Figure 15. Fluorescent PET "on−off" switch.

in the guest promoted hydrolytic removal of the electron-rich thioamide moiety. PET or electron exchange quenches the fluorescence of nitroxyl-appended fluorophores in **154**[527] and **155**.[528] Trans-



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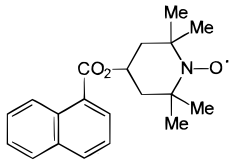
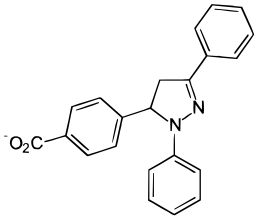


155

formation of the nitroxyl free radical moiety to a relatively inocuous closed shell oxime derivative can therefore cause large increases in fluorescence emission. The possible involvement of TICT excited states in **154** (see section VI.C) is immaterial to the present application. Therefore these two can act as free-radical detectors by radical−radical annihilation.[528] They can also serve as reduction indicators.[528]

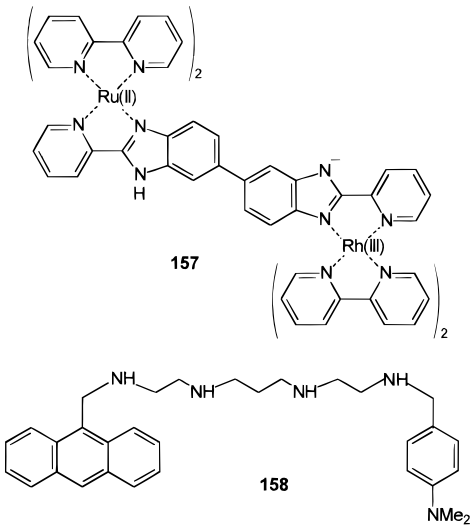
Fluorescent PET "on−off" switches are the opposite of the "off−on" switches discussed above both with regard to the nature of the phenomenon and their popularity. We have commented on the latter in the companion review.[417] In any event, the theoretical foundations of both types are equally strong. Figure 15 offers a schematic summary of fluorescent "on−off" switches. Compounds **42**[240] and **156**[529] appear to be the only simple representatives of this type so far (see section VIII.C). The action of both of these



156

families rely on the proton-induced conversion of receptor π systems which are hardly or weakly electron deficient into rather strong PET acceptors. Unlike the amine receptors discussed above, aryl carboxylates and pyridines permit the examination of the receptor's regiochemical preferences during PET processes. For instance, the coupling of tetraphenylporphyrin Zn(II) fluorophore to a dinitrobenzoyl electron acceptor via ortho, meta, or para disubstitution shows a minimum PET rate for the meta case which overrides simple geometric expectations.[530] Even subtler conformational causes can give

rise to large effects on the PET rate.[531,532] Interestingly, **42** and **156** maintain their switching efficiency whatever the regioisomer (see section VIII.C). A more elaborate example of proton-controlled "on−off" luminescence switching is available from Haga and his colleagues.[533] The benzimidazole moieties within **157**, especially the one on the Rh(III) center, are particularly prone to protonation−deprotonation equilibria. Therefore the redox potentials of the Rh(III) center are pH dependent[534] which, in turn, control the thermodynamics of PET from the ruthenium(II) center to Rh(III). Thus **157** itself is luminescent, whereas its protonated form is not.[533] Fabbrizzi's **158**[495] may also be a lead compound for the design of elaborate "on−off" fluorescent switches since it shows Zn(II)-induced folding so that the terminals are brought within range for rapid PET.



The same purpose can be served with the same stimulus [Zn(II)] if suitable synthetic amino acids are incorporated into peptides. The tried and tested PET pair of 9-cyanoanthracene fluorophore and dialkoxybenzene electron donor seen in **143**[504] has been recently employed by Torrado and Imperiali[534a] at widely spaced locations in an oligopeptide sequence. Two 2,2′-bipyridine amino acids are positioned in between. Micromolar levels of Zn(II) folds the oligopeptide reversibly so as to achieve tetrahedral coordination to the four 2,2′-bipyridine nitrogen centers. In so doing, the PET pair approach one another and cause fluorescence loss.

Even this curtailed survey serves to illustrate the wide scope of the fluorescent PET signaling principle in its most basic manifestation. We note that fluorophores with $\pi\pi^*$, ICT, TICT, and MLCT excited states were harnessed here (see section VIII.C for involvement of MC excited states). In section VI.C we saw how signaling systems based on TICT excited states could be interpreted according to PET principles at least as an approximation. Given the range of receptors and spacers accomodated, PET appears to be a signaling system for all seasons.

B. "On–Off" Switches using Redox-Active Guests

The extent of applicability of fluorescent PET systems can be expanded even further by involving the electron transfer abilities of the guests themselves. A schematic representation of this idea is available in Figure 16. The guest can trigger PET to or away from it or both depending upon its redox potentials relative to those of the fluorophore. In any event, "on−off" switching of fluorescence is the result. Evidently, the intrinsic redox activities of the guests used with the signaling systems in section VIII.A are insufficient to introduce guest-centered PET pathways.

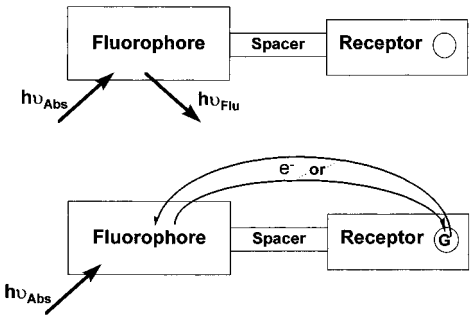
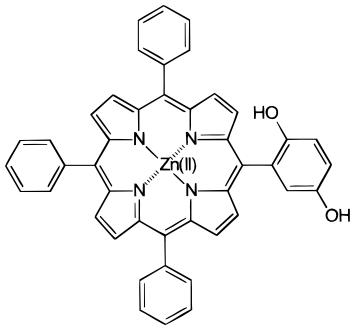


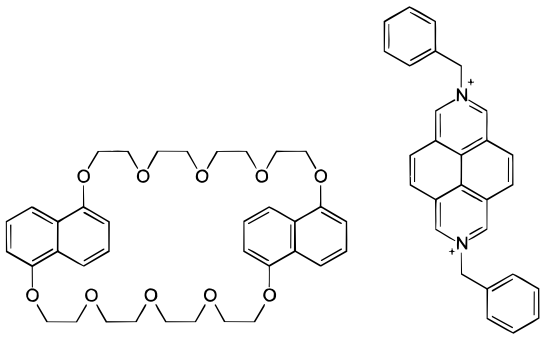
Figure 16. Fluorescent PET "on−off" switch responding to a redox-active guest.

It has been clear for some time that the metal ion−ligand coordination is only one of several bimolecular associations which can be exploited within the fluorescent PET signaling system framework. We set out a list of these in 1993.[365] To begin the present survey, we consider host−guest interactions driven by charge transfer (CT). Those readers with a classical chemistry education will remember the quinhydrone pH electrode, the precursor of which is the hydroquinone−benzoquinone CT complex with auxiliary hydrogen bonds. This CT interaction becomes accessible to fluorescence signaling by the attachment of (triphenylporphyrin)zinc(II) in an orthogonal manner to hydroquinone. The fluorescence intensity and lifetime of D'Sousa's **159**[535] fall by 1 order of magnitude upon encountering various aromatic quinones.



Cyclic voltammetry offers corroborative data. The reducibility of the quinone within the complex is less than that when free, but is sufficient to cause fluorescence "on−off" switching of **159**.

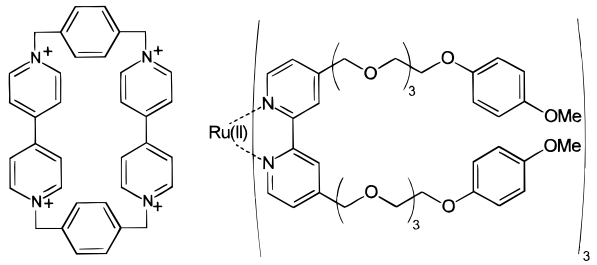
Macrocyclic **160** associates avidly with the electron-deficient π-electron system **161** with the loss of its fluorescence. The more red-shifted fluorescence of **161** is also lost in the process.[536] This system due to Stoddart and Balzani is distinguished by several other features, some of which are noted below. **160·161** is a pseudorotaxane and its mechanochemical capabilities can be tracked by the fluorescence



160 **161**

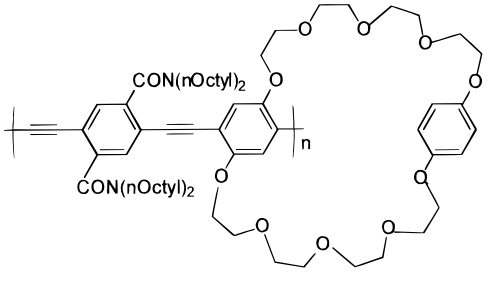
quenching phenomenon. "Unthreading" of the assembly can be affected by amines such as 1,4-diazabicyclo[2.2.2]octane since they compete successfully against **160** for the company of **161**. This is signaled by the reappearance of the fluorescence of **160.** Neutralization of the amine with trifluoroacetic acid reestablishes the status quo. All these fluorescence changes are mirrored, but oppositely, by a charge transfer absorption band characteristic of **160·161**.

Stoddart joins forces with Dürr and Willner to exploit the redox activity of guest **162** as it is threaded by any of the hydroquinone ether moieties in **163**.[537] The proximity of **162** to the tris(2,2′-



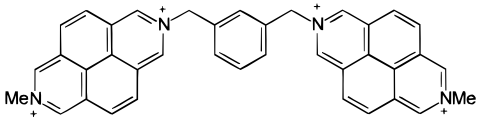
162 **163**

bipyridyl)ruthenium(II) lumophoric center triggers PET and luminescence "on−off" switching is observed along with shortened emission lifetimes. Again "unthreading" can be arranged by competition for one of the partners. β-Cyclodextrin binds to the hydroquinone ether moieties and displaces **162** with emission consequences similar to those in the previous paragraph. A closely related study with a similar conclusion involves the threading of **162** by a linear polyether with a hydroquinone ether station and a phthalocyanine fluorophore.[537a] The 4,4′-bipyridinium motif is recognizable within both **161** and **162**. The parent itself is involved as the principal redox-active guest when it is trapped by Swager's **164**.[538]



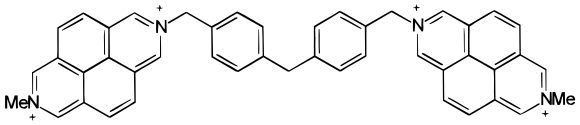
164

The fact that **164** is polymeric leads to its uniqueness. The quenching cross-section for the excitation is thereby increased and a very high efficiency for guest detection is the practical outcome. Harriman's **165**[539] is a dimeric relative of **161**. Not only was this



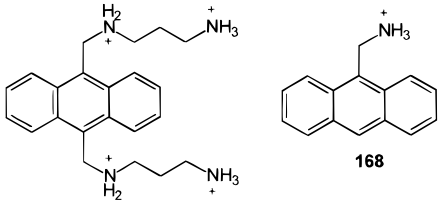
165

reported in 1992 but its response to nucleotides was characterized by fluorescence "on−off" switching and by picosecond absorption spectroscopy. The observation of the diazapyrenium radical cation by the latter method is proof of PET. Such proof is sadly lacking for most of the PET signaling systems collected here. Compound **165** also displays guest-induced folding which has some mechanochemical similarity with **160·161**. It must be noted that both these instances show the fluorophore also doubling as the receptor. Compound **166**, due to Lehn, is yet older and illustrates the same principle.[540,541]



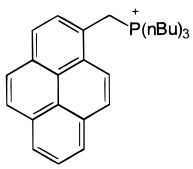
166

Even more elaborate binders for nucleotides and related anionic guests are available,[542] with correspondingly stronger affinities. Such π-stacking between nucleic acid bases and electron-deficient fluorophores also leads to the "on−off" switching of the anthrylmethylammonium systems **167** and **168**,



167 **168**

due to Czarnik[543] and Kumar[544] respectively, as they interact with DNA. Hydrogen bonding and electrostatic attractions also contribute to the binding energy in these cases. Compound **167** succeeds even with single-stranded DNA, whereas **168** is demonstrably bound into the double-helical variety in an intercalative fashion. Jones' **169**[631] also displays fluorescence quenching following intercalative binding with guanine-rich single-stranded polynucleotides. The unusually long lifetime of the singlet

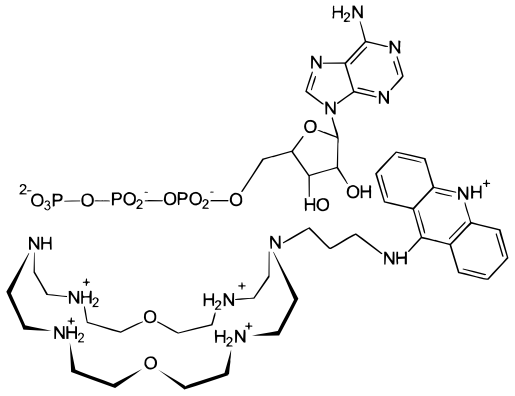


169

excited state in **169** must contribute to the quenching

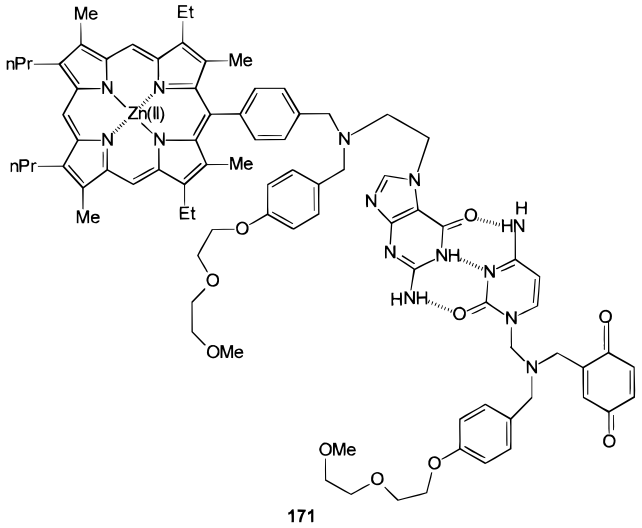
efficiency. Compound **167** shows a similar but smaller fluorescence quenching when bound to adenosine triphosphate,[545] the key player in bioenergetics. van Arman and Czarnik attribute this behavior to a PET process arising from a partial deprotonation of a benzylic ammonium center (section VIII.A) by the hydrogen-bonded triphosphate moiety. A π-stacking interaction between the fluorophore and the adenine unit is also geometrically feasible. Therefore we feel this case fits naturally at this point in our survey. Since **167** is a poorer binder of adenosine monophosphate with its lower charge density, an assay for the dephosphorylation enzyme apyrase becomes possible.[545]

All the examples discussed in the section so far concern guest-induced fluorescence quenching which can be rationalized in terms of stacking π-electron systems with a PET or CT-type contribution. It is refreshing to find cases where π stacking leads to fluorescence increases. Compound **170** is such a complex.[546] It is only the particularly oxidizable guanosine triphosphate which gives rise to a slight quenching.



170

We now collect examples where the fluorescent system and the redox active guest are brought together mainly by hydrogen bonding. Harriman and Sessler's collaboration has produced information on the assembly **171**[547] and its more rigid and compact cousin **172**.[548] Even though the driving force for PET is less exergonic, **172** produces stronger quenching (and binding). Nevertheless, the PET rate constants are not much different. Both cases are well-behaved during time-resolved emission studies with clear biexponential decays and constant lifetimes irrespective of component ratio. This shows that only two emitting species (complex **172** and its precursor porphyrin) are involved and that there is no diffusional quenching. Unlike in the case of **165**,[539] efforts to detect the radical ion intermediates were fruitless. PET via hydrogen-bonded arrays[549] is also addressed in Nocera's **173**.[550] While this structure may be structurally less biomimetic than **172**, it is no less informative. In fact, the observation of the porphyrin radical cation product of PET within **173** is a welcome addition. Furthermore, kinetic isotope effects suggest coupling of proton motion (within the hydrogen-bonded array) with that of the photodriven electron. The photoinduced coupled proton and electron transfer seen by Valeur in hydroxyquinolines is not



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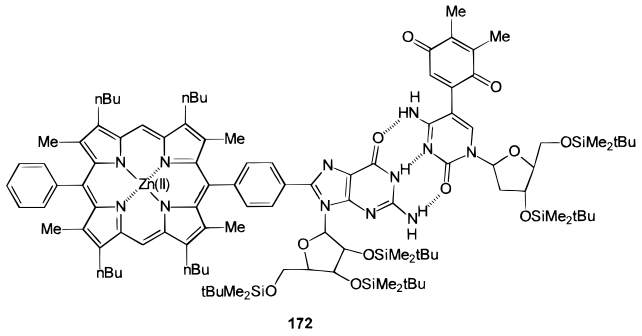
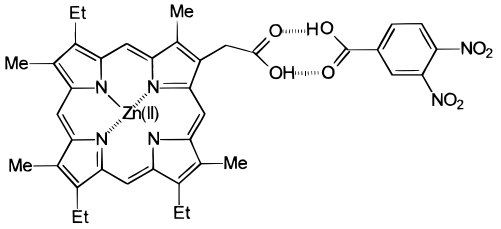


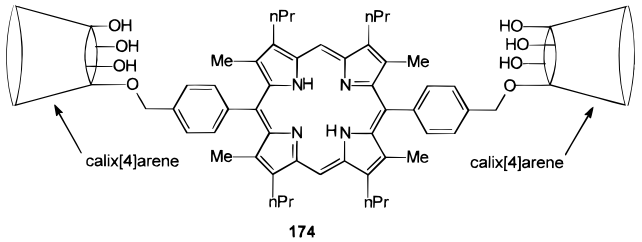
172

unrelated.[551] The asymmetric amidinium−carboxylate hydrogen-bonded interface is also an effective



173

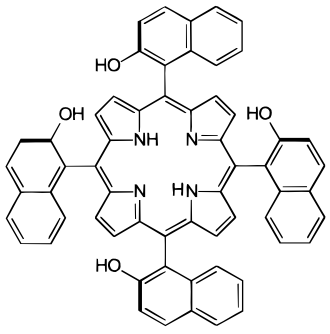
conduit for PET between photoactive units on either side of it, although it is noticeably less efficient than the symmetric carboxylic acid dimer versions.[552] All three cases **171**−**173** recover the porphyrin fluorescence upon disassembly by competition with hydrogen-bonding solvents. Compound **174**[553] is another effort by Sessler to arrange PET from the central porphyrin unit to a benzoquinone guest held by hydrogen bonds to one of the calix[4]arene phenol arrays. However, the weakness of the association leads to a large dynamic contribution to the guest-caused quenching of porphyrin emission.



174

PET via hydrogen-bonded arrays takes on a special significance where the latter is an entire sequence of double-stranded DNA. The recognition aspects remain at the fore bacause the two PET partners are covalently affixed at the 3′-position of each of the single but complementary strands before hybridization. Turro, Barton, and their colleagues find the emission of the lumophore is quenched by extraordinarily fast PET to the electron-accepting partner over 30 Å away provided that both the PET partners are rooted in the double helix by intercalation.[554,555] The lumophore is chosen to be a phenanthroline analogue of **71**,[308−310] the established ruthenium(II)-based intercalator, whereas the electron acceptor is a related Rh(III) complex. The covalent bonding of the partners to the strands is an insufficient condition for the DNA double helix to act as a molecular conduit for the transiting electron.

The pairing of porphyrin fluorophores with electron-accepting benzoquinones remains popular with modelers of photosynthetic reaction centers.[556] We have already met two relevant examples in **171**[547] and **172**.[548] Compound **175**[557] due to Hayashi and Ogoshi, explores the influence of cofaciality between the PET pair upon multipoint hydrogen bonding of tetramethoxy-*p*-benzoquinone by the convergent naphthol moieties. Extensive "on−off" switching is seen



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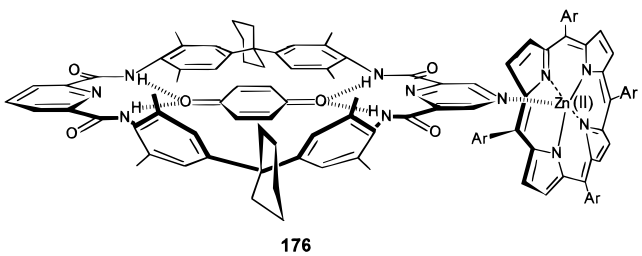
upon complexation.[557−559] Exchange of the 2-hydroxy-1-naphthyl ligating units for 7-hydroxy-1-naphthyl preserves much of this emission quenching but the complexation constant drops by 3 orders of magnitude due to entropic as well as enthalpic effects.[559]

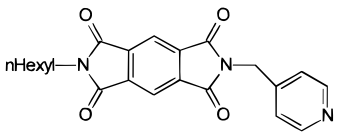
Hunter's **176**[560] provides a nice bridge between the hydrogen-bonded ensembles discussed above and our next target of metal ion−ligand PET systems. The



176

cyclophane holds the benzoquinone guest in its cavity with the aid of hydrogen bonds involving the amide moieties and also accommodates an exomacrocyclic guest, the porphyrinzinc(II) fluorophore, via coordination to one of its pyridine units. Both the PET partners are assembled on the cyclophane scaffold in an orthogonal fashion. Substantial quenching of fluorescence within the termolecular complex is the satisfying observation.

As implied at the start of the section, classical metal ion−ligand interactions have continuing applicability within the fluorescent PET signaling framework. In fact, signaling systems can be developed from the perspective of either partner. We commence with the minority of cases where the metal center is part of the assemblage containing the fluorophore and the PET partner is an all-organic guest ligand. The influence of PET rate on thermodynamic driving force[442] in the ever popular porphyrins with electron acceptors has been examined by Hunter, Sanders, et al.[561] with a major economy in synthetic effort. Instead of synthesizing each supermolecule, they assembled these from the much more accessible components such as **177** and **178** thanks to the capability of porphyrinzinc(II) to associate axially with a pyridine ligand. The coordinative



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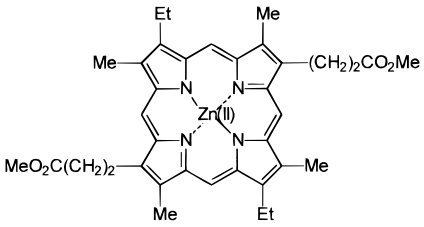
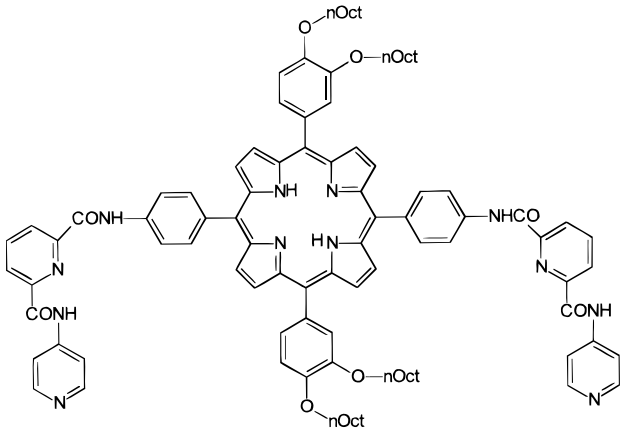


178

assembly step can use the full set of combinations of the families represented by **177** and **178.** This approach also permits variation of the spacer unit, from which valuable lessons can be learned. Furthermore, the collapse of the assembly by a competitive ligand such as piperidine recovers the fluorescence as seen in hydrogen-bonded systems **171**−**174** with competitive solvents. Perhaps the only potential problem is the relative weakness of the coordinate bond (binding constant $\sim 10^3$ M^{-1} in CH_2Cl_2). This loophole can be closed by taking advantage of cooperative coordination interactions between e.g. **179** and **180**.[562] Now the binding constant rises to 10^8 M^{-1}. PET occurs from the metal-free porphyrin unit in **179** to the naphthalene-1,8:4,5-tetracarboxydiimide moiety in **180** without complications of EET involving the porphyrinzinc(II) fluorophores.

Zn(II) features again, although with carboxylate ligand guests, when it is held by a trigonal-bipyramidal N_4 donor atom set with a deliberate vacancy. Fabbrizzi's **181** includes this idea with a fluorophore close by.[563] The synthetic accessibility of the fluorophore-appended ligand is notable, with a homolog being previously used by Czarnik for signaling HPO_4^{2-}.[505] Compound **181** shows "on−off" switching of fluorescence with 4-(*N*,*N*-dimethylamino)benzoate or 4-nitrobenzoate even though the PET direction would be opposite for these two guests. The use of 1-ferrocene carboxylate guest will introduce an ad-



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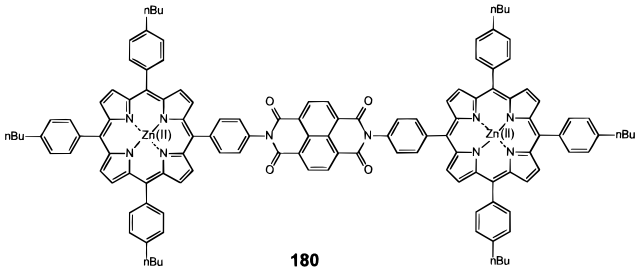
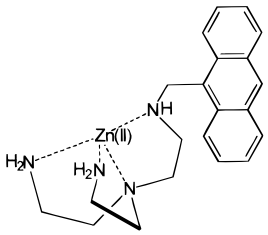


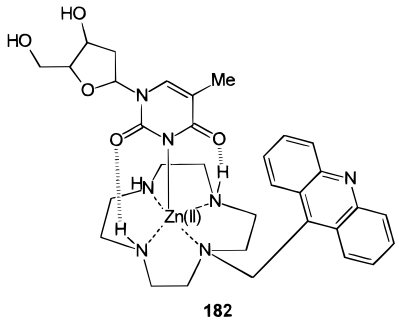
180

ditional emission quenching channel due to electronic energy transfer (EET) (section X).[564]



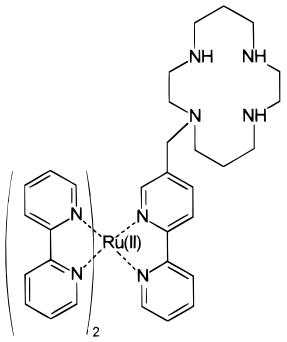
181

Fluorophore-appended cyclen−Zn(II) complexes undergo strong and selective axial ligation with N(3) deprotonated deoxythymine in neutral aqueous solution as shown by Kimura.[565] The demonstrable π stacking betwen the acridine fluorophore and the deprotonated nucleobase within **182** leads to augmented binding and also to small but significant quenching of fluorescence.



182

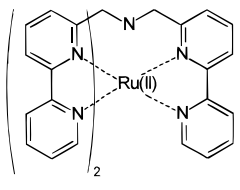
By far the larger number of PET signaling devices for redox active guests relying on metal ion−ligand association for the host−guest interaction targets metal ion guests. The signaling system itself is usually devoid of inorganic components even though there is no good reason why not, especially if care is taken to avoid labile metal complexes. We launch this segment of the survey with an exception of this type. Moore's **183**[566] contains the kinetically stable tris(2,2′-bipyridyl)ruthenium(II) lumophore. The cyclam moiety receives guests such as Cu(II) and Ni(II) with virtually complete switching "off" of the emission. Notably, the metal-free form of **183** is diprotonated under neutral pH conditions. However



183

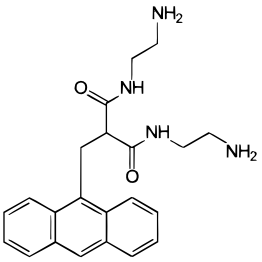
this metalation is too sluggish to allow the use of **183** as a real-time signaling device. Kimura reported a closely related tris(1,10-phenanthroline)ruthenium(II)−cyclam system and its emission quenching with Ni(II) two years earlier.[567] Both EET and PET probably contribute to the guest-induced quenching of luminescence.[564] The lumophore−receptor connectivity is critical in this instance since distortion of the coordination geometry around ruthenium(II) can bring nonemissive MC excited states into contention with the MLCT state responsible for the luminescence of **183** (section VI.B). Such problems are minimized by attachment of the receptor via the methylene spacer to the 5-position of the 2,2′-bipyridyl moiety. Connection via the 6-position leads to supressed luminescence even before guest arrival.[567−570] Even simple methylation at one of the 6-positions of tris(2,2′-bipyridyl)ruthenium(II) causes a decrease in luminescence lifetime by 2 orders of magnitude.[570] The lack of luminescence in **184** is also attributable to its deviation from an octahedral geometry caused by bonding constraints,[571] although the forced proximity of the aliphatic amine to ruthenium(II) can also contribute.

Fluorescence "on−off" switching of anthracene moieties in **119**,[474] **110**,[459,460,461] and **185**[572,573] is seen upon binding Hg(II), Ag(I), and Cu(II) or Ni(II), respectively. The multiprotonated form of **119** must



184

be employed for this "on−off" switching to be observed.[474] On the other hand, **185** functions by undergoing a metal ion-caused double deprotonation.[572] Ag(I) kills the porphyrinzinc(II)-based emission of the tetramacrocyclic **186**.[574] Porphyrinzinc(II) fluorescence also suffers at the hands of Eu(III) held



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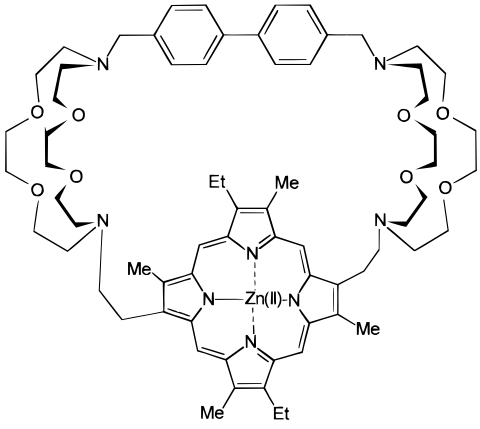
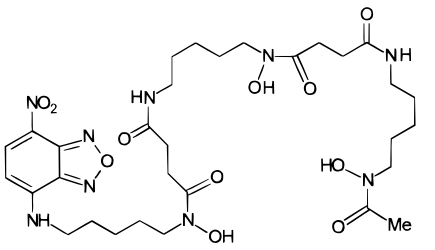


186

in a neighboring 15-crown-5 ether.[575] Along with PET, EET, and "heavy" atom induced spin−orbit coupling [576] are likely contributors to the "on−off" switching of fluorescence in these cases. Fabbrizzi's cryogenic investigations on cases related to **119** show that EET is the principal culprit behind the Cu(II)-induced emission quenching, whereas PET accounts for the behavior of **185**.[573] Transition metal ions also take their toll on the fluorescence of **115**[469,470] and **134**,[491] provided that the latter is maintained at a low enough pH value to suppress the PET process from the iminodiacetate to the fluorophore. As is necessary in **119** and also in **120**,[475] the metalation requires displacement of these bound protons.

While Fe(III) and many other d-block cations are powerful quenchers of classical fluorescent reagents,[15] examples of such activity in supramolecular sytems where PET is definable can be represented by **187**−**189**[577−582] and relatives.[579a] Of course the viability



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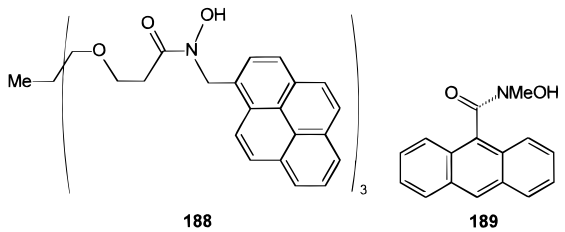
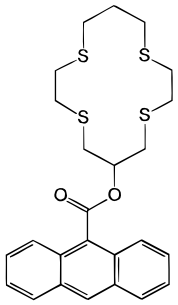


188 **189**

of EET in these cases must not be ignored.[564] Shanzer's **187**[577−579] and Fages' **188**[580,581] contain siderophore receptors for powerful and selective Fe(III) reception. However such power inevitably leads to poor reversibility. Nevertheless, these and similar signaling systems can be recycled by demetalation with acid,[583] although EDTA is also required in the case of **187**. The specificity of some siderophores is only marred by their attraction to other trications like Ga(III). Since Ga(III) is electrochemically more innocent than Fe(III), the binding of the former to **188** does not ruin the fluorescence. This permits the observation of the guest-induced biasing of the monomer−excimer equilibrium of the pyrene fluorophores[581] (see section IX). Czarnik's **189** has a similar, but monohydroxamate, receptor with a proportionately reduced selectivity.[582] In addition to Fe(III)-caused "on−off" switching in neutral solution, this system also switches "off" with Al(III) (to a lesser extent) and also in alkaline solution. The latter observations suggest that the orthogonalized 9-(*N*-methyl hydroxamate) substituent may serve as a PET donor, whether neutralized somewhat with Al(III) or when entirely free, to the anthracene segment.

Our final foray into PET signaling systems which target metal ion guests addresses an important outgrowth. Until now, the speciation of the metal ion guest has not been an issue. If two oxidation states are selected such that one serves as a redox-active guest, whereas the other is benign, interesting switching possibilities arise. Fabbrizzi and his team have successfully demonstrated such a redox controllable fluorescent switch. Compound **190**[584] contains the 14aneS$_4$ receptor which tolerates Cu(I) or Cu(II) guests. The d^{10} electron configuration in the latter



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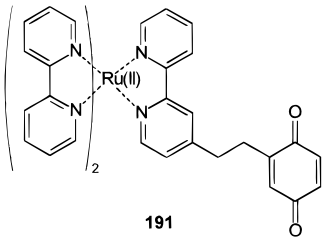
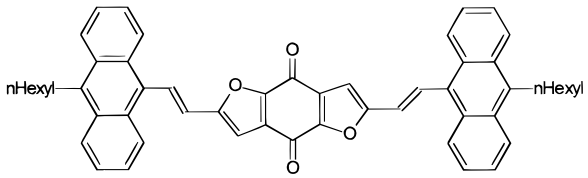
prevents any thermodynamically viable PET process involving the 9-anthroate fluorophore, i.e., **190**·Cu(I), is strongly emissive. Chemical or electrooxidation leads to **190**·Cu(II) where the d^9 center triggers a demonstrable PET process from anthroate to Cu(II) with essentially zero fluorescence. The stability of both the copper complexes permits reversible switching of the device. Similar concepts can be recognized within **191** due to Lehn[585] and a related structure due to Berthon,[586] the reports of which appeared nearly simultaneously in 1993, even though no host−guest recognition is involved. Compound **191** and metal-bound **190** assign opposite roles to the inorganic and organic components. An "inorganic" lumophore and a redox convertible all-organic PET partner can be discerned within **191**. The benzoquinone/hydroquinone redox couple leads to strong PET from the tris-



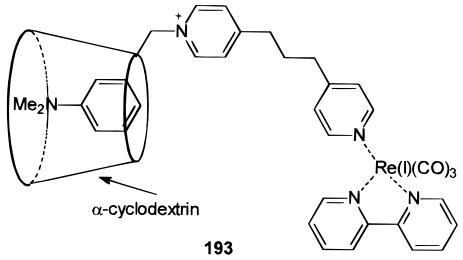
191

(2,2′-bipyridyl)ruthenium(II) lumophore to the all-organic partner in its higher oxidation state but not in the other state. The good reversibility of this couple in the presence of some water is, of course, underpinned by the classical quinhydrone electrode (mentioned previously in connection with **159**[535]). Compound **191** was the first supramolecular electrocontrolled luminescence switch. Simpler molecular versions are available within classical luminescent redox indicators such as tris(2,2′-bipyridyl)ruthenium(II) which need higher potentials for successful operation.[587] The third member of this trilogy is Daub's **192**[588] because, in contrast to **190** and **191**, metal centers are abandoned altogether with no sacrifice of concept or loss of performance. The emissive state of **192** is its hydroquinone dianion form obtained after two-electron reduction in aprotic medium.



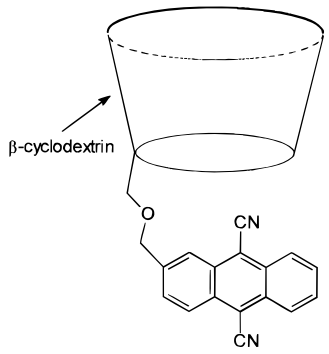
192

Hydrophobically driven association of hosts with redox active guests represent another category of fluorescent "on−off" switches. Most of these cases are united in their use of a cyclodextrin derivative as the receptor component in aqueous media. Nakamura's recent example **193**[589] is pictured as a representative of this appealing line of research.



193

Compound **193** also acts as a convenient bridge with the foregoing paragraphs since metal complexation and hydrophobic binding potential are coexistent within it. Its appeal stems from the fact that many PET active (or inactive) guests can be tested with a single photoexcitable host. The flexibility of noncovalent assemblies is the message. In their pioneering work, Weedon, Bolton, and their colleagues[590] employed porphyrin derivatives as fluorophores and quinones as guests in order to mimic the photosynthetic reaction center.[556] The early research of Nango et al. is also noteworthy, especially because it anchored the porphyrin fluorophore in a lipid membrane for enhanced biomimicry.[591] Kuroda, Ogoshi, and co-workers were able to magnify the guest-caused fluorescence "on−off" switching by employing a porphyrin which was rigidly and closely sandwiched by the primary faces of two cyclodextrin units.[592] Besides the steady-state fluorescence quenching, the analysis of biexponential fluorescence decays and the ESR detection of radical ion pair PET products in the host−guest complex contribute to the value of this work. Additionally, redox active "guests" with intrinsically poor binding capability were persuaded to participate in PET processes by the relatively simple covalent attachment to a tried and tested guest. The latter was chosen to be redox inactive. Compound **193** carries on this tradition but uses a polypyridyltricarbonylrhenium(I) lumophore and a *N*,*N*-dimethylaniline guest instead. Some dynamic quenching complicates the situation in this instance due to the relatively high guest concentrations required for the experiments. The relatively long spacer most contribute to the rather low PET rate observed. Interestingly, a closely related supermolecule was reported a year earlier but the stated aim of guest effects on photoproperties remain unrealized.[593] Compound **194**[594] is similar to **193**[589] as it shows both static and dynamic fluorescence quenching with electron-rich guest such as anthracene-2-sulfonate. The regioisomer of **194** where the fluorophore is attached to the wider rim via its secondary alcohol moieties shows weaker binding and quenching. A rigidly capped version of **194**[595] is the worst performer of the three (see **10** and **11** in section V). Calix[4]arenes can also replace cyclodextrins as receptors for quinone guests and cause quenching of porphyrin fluorescence.[596]



194

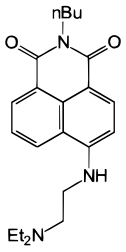
We bring this section to a close by turning the spotlight on two other forms of host−guest association which give rise to a PET-type fluorescence quenching: reversible covalent bonding and ion-pairing. We meet the former during the interaction of **146** with catechol and also the neurochemical DOPA.[597] As pointed out in section VIII.A, boronic acid−sugar association is an important avenue for "off−on" fluorescent PET sensing/switching. Czarnik's use of **146** in the "on−off" switching mode with catechols of low oxidation potential illustrates the flexibility of these designs. PET from the catechol−boronic acid ester to the anthracene unit is reasonable especially since the boron center is likely to be tetrahedral. Ion pairing may augment the dynamic quenching of the cationic fluorophore within **70**[598]

with Cl^-. Compound **70** represents a family of fluorescent sensors with an established track record in physiological Cl^- monitoring.[599, 600]

C. Switches with More Complex Behavior

The sheer number of examples discussed so far under section VIII underscores the powerful position of PET within the fluorescent signaling framework. However this is not all. The modular construction of fluorescent PET signaling systems allows substantial conceptual expansion. Some of the more complex devices encroach into research fields which are normally far removed from conventional chemistry. Since we concentrated on these complex systems in a companion review,[417] we will only offer a whistle-stop tour of this area in order to prevent overkill.

Even the simplest fluorescent PET sensor/switch can display complicated behavior due to subtle causes. For instance, ICT fluorophore modules naturally create local electric fields upon photoexcitation. Such fields will influence rates of electron flow and, in extreme cases, will bias their competitiveness vis-a-vis fluorescence. Galoppini and Fox find significant control of PET rate by the net electric field of an α-helical polypeptide.[601,602] Opposite regioisomers differ in their PET rates by up to a factor of 27. The PET thermodynamics also differ. This is why the push−pull 4-aminonaphthalimide-based **195** behaves as a normal "off−on" sensor toward protons, whereas its regioisomer **196** does not.[603] When built into



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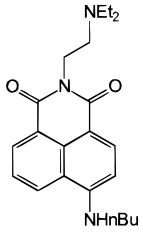
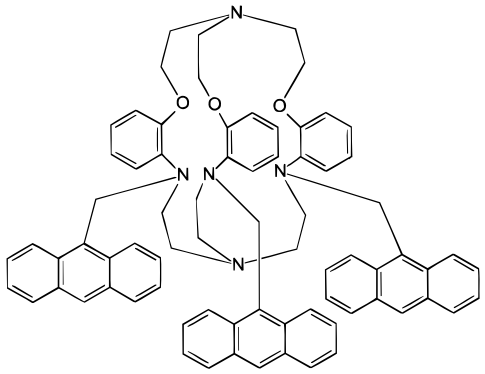


196

larger PET systems, such 4-aminonaphthalimides also produce Stark effects in a neighboring probe chromophore due to the field of the transient radical ion pair.[604] Wasielewski[383] has also used the same family of fluorophores within triads to photogenerate giant dipoles.

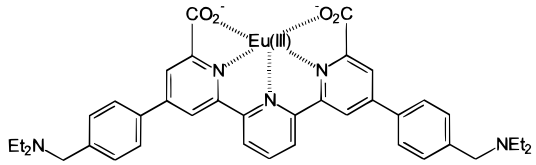
Some of the simple fluorescent PET switches also behave as OR logic gates when exposed to a selection of ionic inputs. Of course, molecule-based logic operations are a powerful approach to molecular information processing. Compounds **112**[465] and **197**[605,606] are examples which employ ions from different parts of the periodic table as guests. Any one or more of the ions produce "off−on" emission switching. Compound **197** is distinguished by the use of d-block ions such as Cu(II) to elicit an "off−on" switching response. As section VIII.B shows, Cu(II) usually causes the opposite effect. The rigid cryptand environment around Cu(II) appears to stifle its redox activity. Cation coordination to the aliphatic amine units would then be responsible for the "off−on" switching. Ideally, OR logic gates should produce the same output when they are switched "on". Since various ions have different charge densi-



197

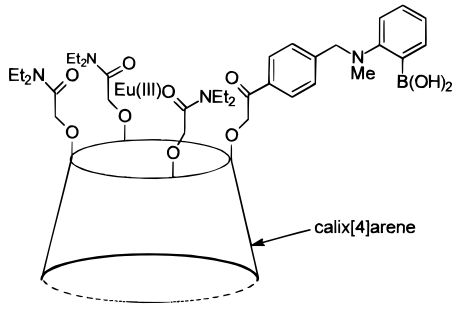
ties and coordination strengths, their abilities to enhance fluorescence will also differ. That is why **112** and **197** produce maximum fluorescence signals which are somewhat dependent on the nature of the ion. Compound **142**[503] surmounts this shortcoming by relying on a conformational change as the main mechanism for fluorescence switching (see section VIII.A). Then the charge density effects are relegated to a minor role.

Apparently simple luminescent PET "off−on" switches may also rely on additional EET processes to create emission in the first place. In such cases, the exact point at which PET diverts the excitation can be harder to locate. For example, emission from the MC excited state of the lanthanide complex **198**[607] is switched "on" with protons. However, PET could



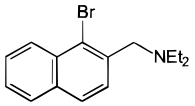
198

intervene to quench the ligand singlet state or the ligand triplet. Although its shorter lifetime would disfavor the involvement of the singlet, its higher energy would redress the balance. The dilemma would multiply in relatives of **198** containing the more reducible Eu(III) lumophore. Lanthanide-based PET signaling systems are also available from Shinkai's laboratory. Compound **199**[608] relies on the sugar−(aminomethyl)boronic acid interaction which has been so successful for the design of fluorescent systems discussed in section VIII.A. The aromatic nature of the calix[4]arene provides additional opportunities for EET other than from the ketone and the effective antenna appears to depend on the particular lanthanide ion that is receiving the energy.



199

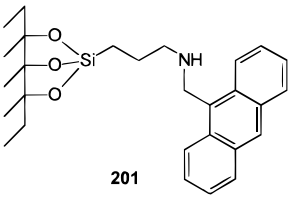
All-organic illustrations are also available. Compound **200**[609] is a phosphorescent PET system which requires steric protection (see section VII) for its operation. β-Cyclodextrin provides protection for the



200

triplet excited state mainly against triplet−triplet annihilation. PET between halonaphthalene triplet and aliphatic amines has been previously demonstrated by Davidson.[610] Protons tie up the nitrogen lone electrons to switch "on" the phosphorescence. Subtle but understandable regioselective self-assembly of **200** within the macrocyclic host is also necessary for its success. These mechanistic uncertainties in no way reduce the practical value of these systems for reliable sensing even in intrinsically fluorescent environments. Time-resolved observation accurately dissects out the sensor's delayed emission from the scatter and fluorescence which can plague optical sensing in biomatrices. From a conceptual viewpoint, it is important to note the increase in the number of functional components beyond the trio found in the simple sections of VIII.A and B.

Additional components are usually introduced because they usually endow the signaling system with new and desirable properties. The previous paragraph contained examples which provided protection and/or antenna action. Another important property absent within simple PET systems is a means of immobilization. As mentioned in section I, this is desired in industrially oriented chemical analysis. Compounds **201**[611] and **202**[612] are examples of simple PET signaling systems like **127**[482] and **113**[466] which have been connected to inorganic and organic polymers, respectively. The insolubility of the former



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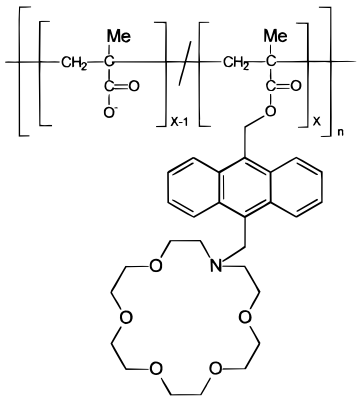
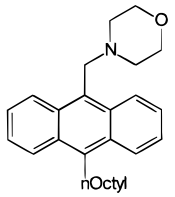


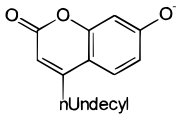
202

permits its reuse as a pH sensor. Cross-linked relatives of **202** would confer insolubility if desired. Compound **202**[612] is interesting as it stands because of the sensitivity amplification due to concentration of K^+ locally by the polyanion.[335,613] Covalent bonding is not always necessary to keep a sensor/switch anchored near a superstructure. Self-assembly can be a means to the same end. Molecular submarine **203**[614] relies on the hydrophobicity parameters[615] of its targeting modules neighboring the fluorophore and the receptor to access a range of locations on a continuum from a membrane surface to bulk water.



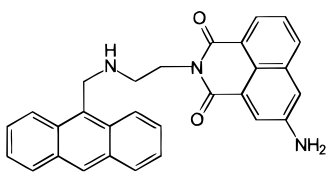
203

There are membrane-targeted versions of integrated fluorophore−receptor systems based on ICT excited states. For example, n-octadecylated versions of **21**[616] and **204**[188] are useful for measuring membrane-bounded Ca^{2+} and for dissecting electrical and dielectric effects of membranes on local pH. However, the spatial separation between the various components in PET systems allows fine positional control of the receptor while putting gross targeting groups on the fluorophore.



204

We have already met cases such as **129**,[453,485] *N*-butyl-*N*,*N*-bis(1-naphthylmethyl)amine,[457] **120**,[475] and *N*,*N*-bis(1-pyrenylmethyl)diaza-18-crown-6 ether[476a] where multiple but identical fluorophores provided two emission spectral bands with different guest responses. EET instead of monomer−excimer equilibria can be introduced into PET signaling systems if two different fluorophores are employed. Compounds **198**−**200** also combine PET and EET although their design and purposes are quite different. A simple PET system like **127**[482] can be elaborated with an aminonaphthalimide fluorophore via a second spacer to give **205**.[617] Anthracene and

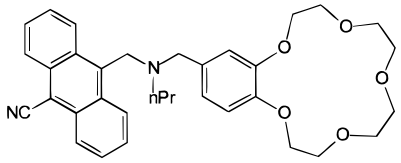


205

aminonaphthalimide fluorophores are complementary since they can be separately excited reasonably cleanly and because their spectral features are clearly distinguishable. Most importantly, anthracene will undergo PET from the aliphatic amine receptor, whereas aminonaphthalimide will do so only weakly according to both theory and experiment. Preferen-

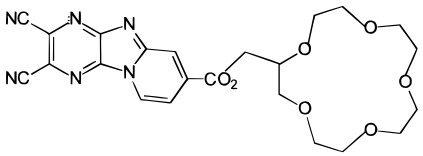
tial excitation of the anthracene component produces emissions due to it and also due to the aminonaphthalimide unit. The former is switched "on" with protons, whereas the latter shows a much smaller effect. Thus **205** has an internal reference channel against which the sensory signal can be calibrated as required in many applications. Cain and Murphy have previously described a polymeric signaling system for pH with an internal reference.[618]

The use of multiple, but identical, receptors within fluorescent PET signaling systems such as **123**,[479] **145**[506,507] and **148**[510] gives them improved guest selectivity via geometric recognition. Fluorescent PET systems can break into new research areas if this identity condition is relaxed. Compound **206**[619] is fitted with two different receptors targeting H^+ and Na^+. Switching "on" of fluorescence is only achieved



206

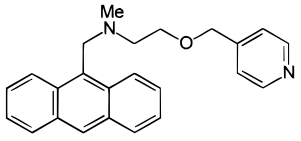
by the simultaneous provision of these two cations at sufficient concentrations near **206**, i.e. coincidence in time and near-coincidence in space (within a few angstroms) is required of the ionic inputs. This represents a wireless, self-selecting AND logic gate. As mentioned above under OR logic gates, these are vital starting points for molecular information processors. AND logic gates are particularly critical since they are needed for the achievement of arithmetic operations. A similar outcome is achieved by Iwata and Tanaka in **207**.[620] Although this also



207

employs PET, its design is different in that the arrival of the first ion (Ba^{2+}) in the only receptor prepares the ground for the arrival of the second (SCN^-). Such a sequential guest layering is also seen in Lehn's **170**[546] and Czarnik's **144**[505] where protons mediate the entry of phosphate derivatives in order to create the fluorescence response. Of course, the simpler one-input YES (pass) and NOT logic gates are already available in large numbers in the form of "off−on" and "on−off" switches respectively. Thus the family of photoionic logic gates already covers four basic operations. Other logic operations are likely to join this list as time passes.

The identity condition between multiple receptors can also be relaxed in a more subtle fashion. Both receptor modules within **208**[621] select protons but at different thresholds owing to basicity differences. Verhoeven and Fabbrizzi have also reported systems **104**[387] and **158**[495] with this feature. The distinctive feature of **208** is that the two receptors (aliphatic



208

amine and pyridine) encourage "off−on" and "on−off" switching respectively. Integration of both these switching functions within one molecule produces a sensor/switch with a "off−on−off" window response. Not only does **208** permit the immediate identification of microenvironments with a chosen guest concentration range but it also emulates the current−voltage characteristic of tunnel diodes in solid-state electronics.[622] S. A. de Silva and his colleagues have independently demonstrated the proton-induced "off−on−off" switching action in *N*-(9-anthrylmethyl)-*N*,*N*-bis(2′-pyridylmethyl)amine.[622a] This example is particularly attractive since the system is synthesized in one step and because it doubles as an "off−on" sensor for Zn(II).[622a] There are several examples of proton induced "off−on−off" switching in the literature where the switching at low pH is due to excited state protonation of the fluorophore itself.[10,491] Other systems show luminescence components with such behavior which must be resolved according to wavelength[623] or decay time.[298]

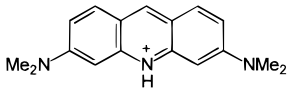
It is clear that most of these more complex switches have only dealt with the simplest guest of all: H^+. The previous parts of section VIII showed the range of guests which can be accommodated by the simple fluorescent PET signaling system. Combining these two streams of thought must surely cause a further flowering of this already fertile field.

IX. Monomer–Excimer Systems

Other than a few exceptions, all the discussion so far has centered on systems comprising of a single fluorophore. Now we pass on to phenomena which demand at least two fluorophores. When these are all identical, the excited monomer can associate with a ground-state partner to produce an intramolecular excimer state which is in equilibrium with its precursors.[28,624] It is driven by excitation and also by local concentration. Its emission spectral signature is distinct from that of the monomer due to a red-shift and a lack of vibrational features. The excimer evolves from the excited monomer if the interaction develops within the lifetime of the latter. It is therefore to be expected that excimers are more likely to be produced by relatively long-lived monomer excited states. Another expectation is that excimer formation will be controlled by the dynamics of chain cyclization[625] in a viscous medium. Diskoidal π-electron systems such as pyrene have extended excited singlet state lifetimes and are particularly suitable fluorophores in this arena.[624] α,ω-Bis(1-pyrenyl)-alkanes show excimer/monomer emission ratios which are viscosity controlled.[626] These and older cases like **129**[453,456,485,627] signal local viscosity by an easily measured, self-calibrated optical parameter ratio. Although the former systems show some complications in steady-state and time-resolved behavior,[628] the general utility of monomer/excimer systems for

this purpose is not in doubt. Even surface chemistry is a beneficiary due to the use of fluorophore end-capped polyethylene glycols as adsorbates.[629] We will meet related systems within a cation sensing context shortly and the advantage of self-calibration via dual wavelength monitoring will remain.

Spatial restriction of their microenvironments allows fine control of the monomer-excimer equilibrium in flexibly linked bifluorophoric systems or their unlinked counterparts. This idea is particularly appealing to designers of host−guest partners. Since excimer formation requires π-orbital contact between the pair of fluorophores, one can call upon various supramolecular forces to bring the pair close together or push them apart. We commence this survey with formally intermolecular examples. Polyanionic chains can host cationic dyes to produce pseudo intramolecular effects. The probability of excimer formation is enhanced under such one-dimensional regimes. Acridine orange (**209**) is the classic case of an emission color change upon encountering double-stranded DNA.[630] The (1-pyrenylmethyl)phosphonium cation **169**[631] shows a similar effect in the ultraviolet-blue region of the spectrum with single-strand polynucleotides but only under conditions of low ionic strength. The π-electron-free heparin is the target of Czarnik's **210**.[543] An optimum host−guest



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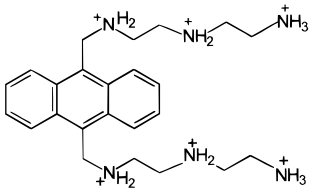
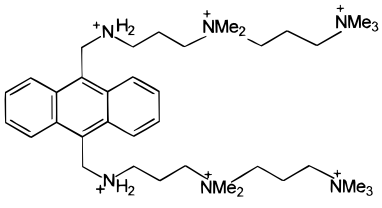


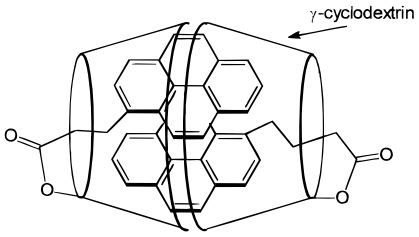
210

ratio applies in these examples since higher host concentrations dilute the effect. Host disassembly can also be monitored. Heparinase causes fluorescence recovery of **210** by hydrolyzing heparin into smaller oligosaccharides which lack the electrostatic field strength to host **210**. This approach should be rather general for assaying hydrolytic enzymes acting on polyanions.[543] The polyamino functionality of **210** precludes its use in situations which require the presence of d-block metal ions (section VIII.B). Compound **211** cures this defect,[632] since metal ion binding at the effectively monoamine sites are further restricted by steric and electrostatic effects. Compound **211** brings the sensitivity of fluorescence methods to the examination of a Ni(II)-activated nuclease.[632] We note in passing that concentration quenching, which can be considered as a photophysical extreme of excimer phenomena, is a useful tool for testing transport[633] or leakage[634,635] in membrane science.



211

When fluorophores are covalently attached to the 3′- and 5′-positions in two runs of polynucleotides, this combined sequence can hybridize with a complementary strand of DNA.[636] This causes the switching "on" of the excimer emission. Pyrene appended γ-cyclodextrin self-dimerizes to **212**[637] and displays an excimer band. This can be switched "off" above pH 13.5 due to repulsion between secondary alkoxide groups. The same effect can be arranged at neutral pH values by occupying the cyclodextrin cavity with borneol to give the complex **213**. Ueno has also



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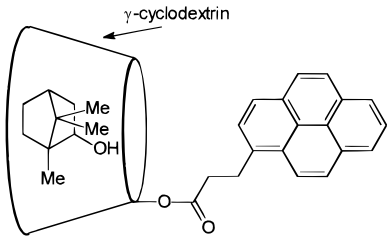
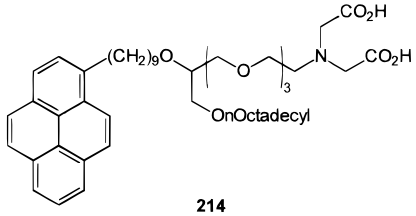


213

reported a naphthyloxy appended β-cyclodextrin derivative.[638] A similar outcome can be seen when 1-butanol displaces one of two pyrene fluorophores residing within γ-cyclodextrin.[639] No excimers are found with anthracene-2-sulfonate but the quenching of monomer emission and photodimerization[640] suggests a situation analogous to pyrene. The pyrene excimer within the chiral γ-cyclodextrin cavity displays clear evidence of its own chirality by emitting circularly polarized fluorescence, in complete contrast to the accompanying monomer emission.[641]

Lipid **214** is a case where Arnold and her colleagues use 1-pyrenyl and iminodiacetate units to impart photophysical and metal ion coordination properties respectively.[642] When dispersed in vesicles, **214** shows excimer emission due to domain-type



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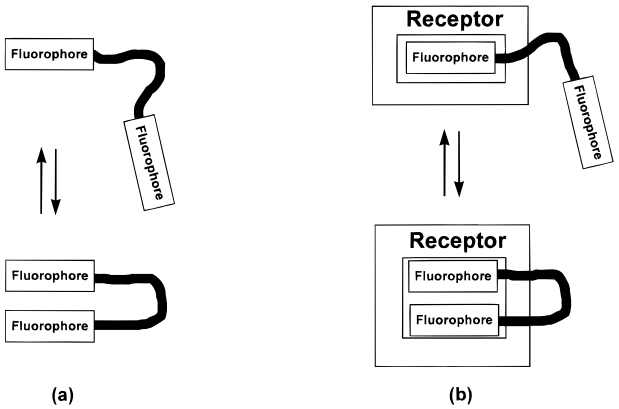
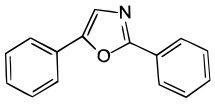


Figure 17. Biasing of monomer−ground state dimer equilibrium of a tethered bifluorophore by a receptor which stabilizes one or the other state.

aggregation perhaps via hydrogen-bonded carboxylic acid dimers. Cu(II) binding at nanomolar levels can induce the separation of **214** one from another to recover monomer emission at the expense of the excimer. The low detection limits can be ascribed at least partly to the local amplification of Cu(I) concentration at the anionic membrane surface.

A literal extension of some of the above ideas can be found in the work of Agbaria and Gill.[643,644] The quasi-linearity of the popular scintillant **215** is exploited to form repeating 2:1 (**215**:γ-cyclodextrin) assemblies. Compound **216** illustrates a possible structure. Such photoactive supramolecular polymers are very interesting not only because they exhibit excimer emission. Their reversible thermal behaviour and their relationship to molecular "necklaces" [645−647] are additional attractions.



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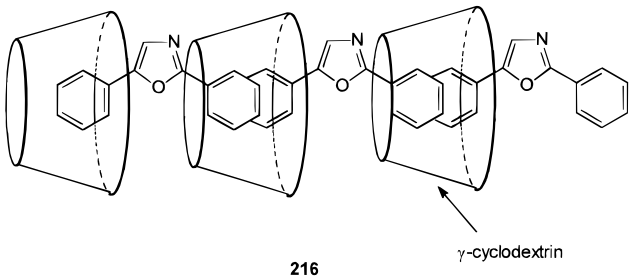


216

Our discussion of signaling systems based on intramolecular excimer formation can be schematically organized with Figures 17−20. Figure 17 illustrates how equilibria between monomers and ground-state dimers of flexibly tethered bifluorophoric systems can be biased by rigid receptors which stabilize either state. Hydrophobic fluorophores are likely to π stack in aqueous or other polar media to produce some degree of excimer emission. Emert,[648] Turro,[649] and their colleagues find predominantly monomer and excimer emission from 1,3-bis(1-pyrenyl)propane when it is hosted by the smaller β- and the larger γ-cyclodextrin respectively. The inclusion

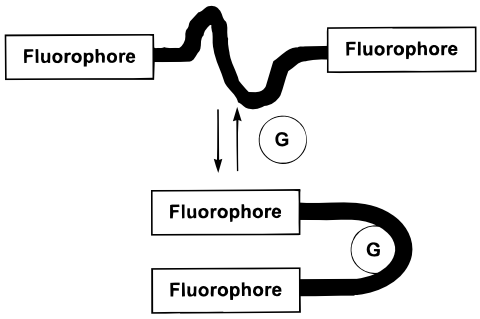
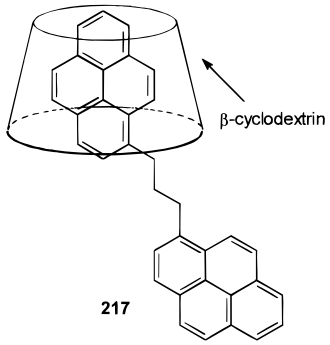


Figure 18. Guest-induced folding and stacking of a flexibly tethered bifluorophore.

complexes **217** and **218** are probably the key players. Diederich reports a cyclophane analogue of **217**.[650]



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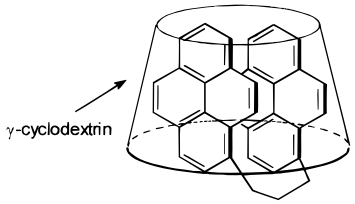

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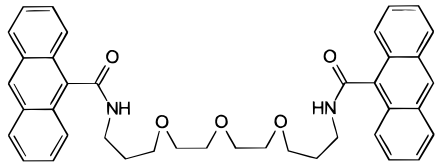
Guest-induced folding of flexible receptors which brings about π stacking of fluorophores is pictorially summarized in Figure 18. Nakamura's podand **219** exemplifies this approach with Ca^{2+}-induced enhancement of excimer emision.[651] Even though 9-mono-



219

substituted anthracenes are prone to photodimerization,[640,651a] the short tether in **219** prevents the usually necessary head-to-tail alignment especially in the guest-bound state. Van Arman and his colleagues find a Zn(II)-induced enhancement of the intramolecular excimer in the case of triethylenetetramine with 9-anthrylmethyl terminii.[651b] The fact that aqueous solutions (at pH 10.5) are used is a distinguishing feature. Besides the geometry changes illustrated in Figure 18, some degree of Zn(II)-induced PET suppression is also involved (section VIII.A). The latter point is responsible for the growth of the monomer emission accompanying the excimer. Oligo-[652] and polyethylene glycols[653] terminated with

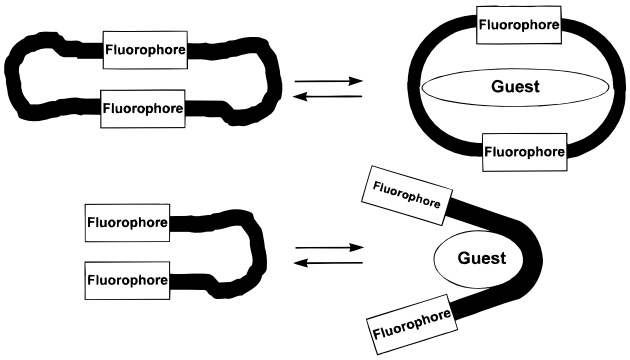
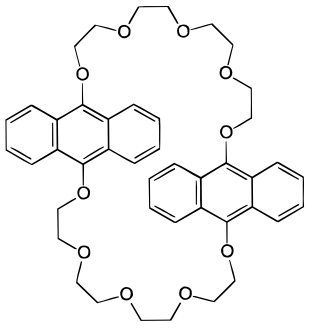


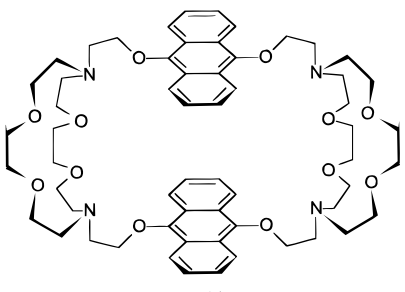
Figure 19. Guest-induced unstacking of a flexibly tethered bifluorophore.

naphthalene-derived fluorophores produce broadly similar results although guest ion-induced reduction of the monomer emission is not compensated by the growth of an excimer band.[652] A double receptor version of Figure 18 was described by Bouas-Laurent and Desvergne a decade ago.[654] Besides being an early illustration of guest-induced excimer formation, **220** is special for at least two reasons.[655,656] First,



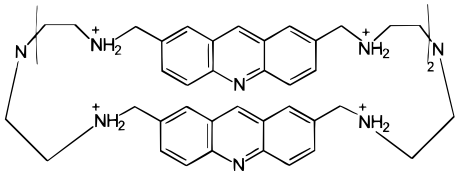
220

the binding constant for the entry of the second Na^+ is higher than that for the first in spite of statistical and electrostatic handicaps. This clear positive cooperativity means that the first Na^+ reorganizes the second pseudocrown ether for better reception of the second Na^+. The other reason is that higher binding constants are found in the excited state of **220**. Unlike in the cases with ICT excited states (section VI.A), electric field effects cannot be responsible. Rather, the relatively long-lived excimer state must also be preorganizing both pseudocrown ethers for Na^+ entry. These two important observations are best seen in the noncompetitive solvent acetonitrile, whereas they are clouded in methanol. A more elaborate version of **220** can be found in **221** when the latter interacts selectively with Rb^+ over other alkali cations.[657,658]



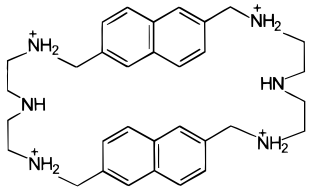
221

By suitable choice of guest−receptor combinations, the scenario opposite to that of Figure 18 can be arranged. Guest-induced unstacking of fluorophores is the design principle shown in Figure 19. The double receptor version (Figure 19a) has a clear example in Lehn and Bouas-Laurent's study of **221** and 1,6-hexanediammonium ion.[657,659] The interruption of the excimer interaction in **221** by the guest is total. The acridine-based cryptand **222**[660] is also from the Lehn stable. The excimer emission in this



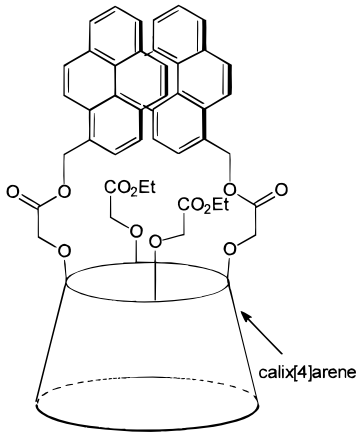
222

instance is rather weak. Nevertheless, the entry of terephthalate switches "on" the monomer emission by over an order of magnitude. The macromonocyclic relative **223**[661] has no discernible excimer band but shows strong PET-type quenching of the monomer fluorescence with guests like ATP (section VIII.B).



223

Bouas-Laurent and Desvergne have also reported bifluorophoric systems related to **110** and to **221** which showed weaker response to alkali cations.[461] Ag(I) understandably quenches the excimer emission (section VIII.B). Less extreme, but still very useful, guest-induced switching "off" of excimer emission (and corresponding switching "on" of monomer emission) can be achieved by realization of Figure 19b. Bis(1-pyrenylmethyl)calix[4]arene tetraester **224** shows strong excimer emission from the two adjacent fluorophores since dipole−dipole repulsion among the ester groups orients the carbonyl oxygen atoms outward. Na^+ binding reorients the latter atoms



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inward pivoting the fluorophores away from each other with a large loss of the excimer band.[662]

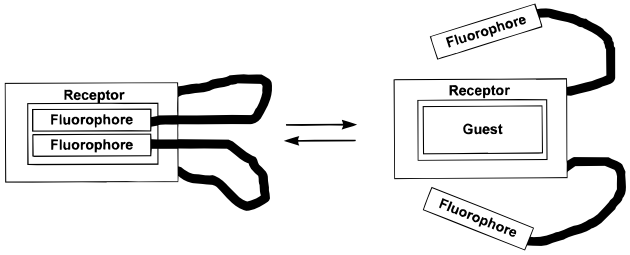
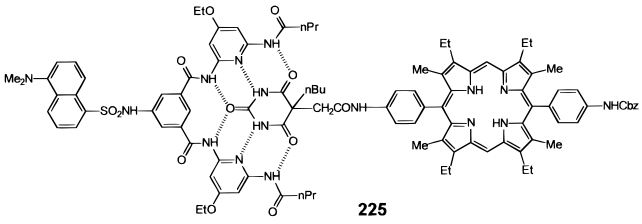


Figure 20. Self-complexing within a system with two fluorophores flexibly tethered to a rigid receptor.

Shinkai also finds a similar end-result when a closely related calix[4]arene diester binds to trifluoroacetic acid via hydrogen bonds to ester and other moieties.[663] Tetra(9-anthryl) versions of **224** show less drastic Na^+-induced spectral variations.[664−666] These cases owe part of their success to their cone conformation. Employment of some smaller appendages can permit rotations of calixarene phenyl units through the macrocycle to access other conformations of lower dipole moment. Solvent polarity will naturally control the position of equilibrium between these conformations and, consequently, the monomer/excimer emission ratio.[667] One of Shinkai's most recent efforts in this area provides an allosteric avenue to the biasing of an excimer−monomer equilibrium toward the latter. Very selective Na^+ binding at the lower rim to a calix[4]aryl crown ether flattens the two unbridged phenyl ether units carrying 1-pyrenyl moieties on the upper rim, thus increasing their separation and reducing their sandwiching.[668]

The excited-state interaction between two 1-pyrenyl fluorophores can also be attenuated by barbiturate binding to the central bis(2,6-diaminopyridine) receptor (cf. **45** and **225** in sections VI.A and X, respectively) of **226** in homogeneous[669] or two-phase[670] solution. This principle is general enough to encompass trifluorophoric systems based on C_3 symmetric calix[6]arenes[671] and partial cone conformations of homotrioxacalix[3]arenes[672] which target guanidinium and primary alkylammonium ions respectively. Bis(1-pyrenoxycarbonylmethyl)calix[4]-



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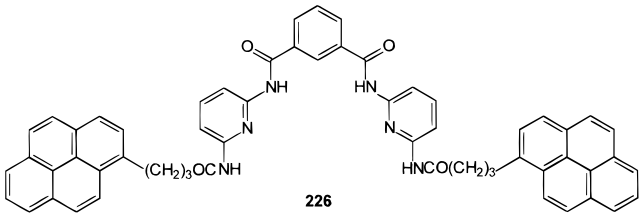

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arene derivatives like **227** can also be put to work as radiation dosimeters.[673] γ-Radiation will naturally wreck the molecule and the initial products of radiation damage are likely to be monofluorophoric. Hence the monomer/excimer ratio would increase.

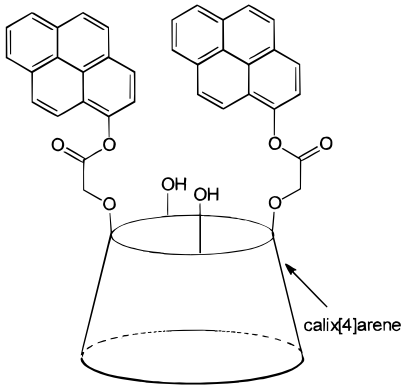

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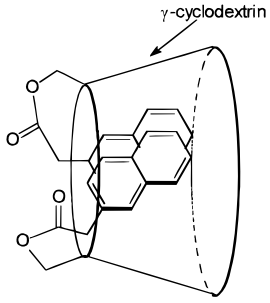
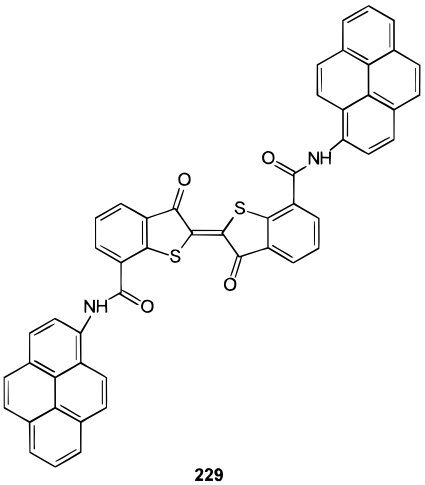
Figure 20 is a self-complexing version of Figure 19b. Another critical difference in the present instance is that the receptor is relatively rigid whereas Figure 19b required flexibility for conformational reorganization. Compound **228** illustrates some aspects of this approach.[674] In related cases such as



228

9-anthroate analogues of **228**,[677] the π-stacking of the two fluorophores causes some losses of intensity and vibrational fine structure in the absorption spectrum, and the self-dimerizing 1-pyrenyl systems, e.g., **212**, show the clearest effect of all.[637] Such absorption behavior is common during intercalation of dyes into DNA, for instance.[544,631] Absorptiometric clues to the association of π-systems are also found in bifluorophoric relatives of **115** which additionally display self-quenching of emission.[468−470] The guest (e.g. cyclododecanol)-induced switching of the monomer−excimer equilibrium is not strong in **228** because the fluorophores remain paired to form a cap over the guest. Compound **228** is only one of many fluorophore derivatized cyclodextrins to emerge from Ueno's laboratory.[675,676] The role of the linker can be critical for their detailed behavior.

Until now, excimer formation was aided or hindered by chemical entities, be they viscous solvents or guests. Physical influences such as light can be equally if not more effective. This results in reversibly photon-writeable molecular devices which can be read via fluorescence. Shimidzu combines the photoisomerizable thioindigo unit with two 1-pyrenyl fluorophores via amide linkages within the *E* isomer **229**.[678] Excimer emission arises only from the *Z* isomer where the fluorophores are adjacent in space (**230**). The *E*−*Z* equilibrium can be driven in either direction to a photostationary state with photons of appropriate wavelength. Zweig described the general ideas behind photofluorescence over two decades



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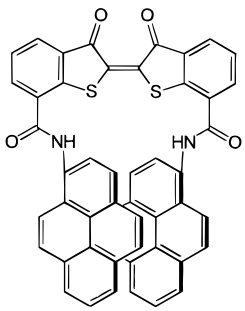
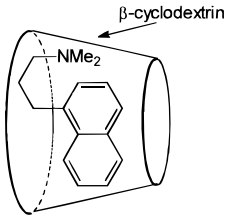


230

ago.[679] Nevertheless, the Vavilov−Kasha rule[680] must be contended with during the development of photon-write, photon-read molecular devices which are both reversible and efficient. The writing and reading steps require the population of different excited states within the same supermolecule. Usually EET will be rapid and only the lowest excited state of a given multiplicity will survive long enough to participate in photochemistry or fluorescence. While exceptions to the Vavilov−Kasha rule are many, they are either inefficient or arise from special circumstances.[680]

Exciplexes are closely related to excimers in that excited-state association[681] remains the focus except that the identity condition between the two π-electron systems is removed.[682] Only the π system with the lower excited state energy will be excited in order to avoid EET, since that will be our subject in section X. Since exciplexes involve unequal fluorophores it is natural that some charge separation will result. Thus solvent "polarity" will be a key controller of exciplex phenomena, in complete contrast to excimers. The wavelength of exciplex emission increases in more polar media whereas the emission quantum yield of monomer and exciplex fall off sharply in highly polar solvents.[439] Exciplex emission can be preserved in nominally aqueous media by embedding the emitter in a cyclodextrin or cyclophane of appropriate size[683−685] or within a relatively hydrophobic polymer microdomain.[342] Turro and Yang's example[684] exists in two possible situations **231** and **232** in basic and acidic aqueous solution respectively. In other words, monomer emission undergoes "off−on" switching with protons whereas the exciplex signature shows the opposite behaviour. To close, we note the interesting magnetic field effects on exciplex



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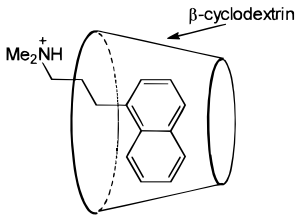


232

emission observed by Tanimoto et al.[686,687] The significant magnetic field-induced emission enhancement seen in phenanthrene−dimethylaniline supermolecules and polymers can be rationalized by considering the radical ion pair associated with the exciplex.

We met several forced ground-state associations between fluorophores during the excimer studies discussed above. As stated earlier, we will not generally consider intermolecular effects unless they were pseudointramolecular in nature (except for host−guest interactions, of course). Nevertheless, a useful example is provided for illustrative purposes. Porphyrin-diboronic acids aggregate in aqueous media with concurrent quenching of fluorescence. Reversible binding of hydrophilic saccharides to the boronic acid moieties causes deaggregation with recovery of fluorescence.[688]

Overall, we are left with the feeling that the basic ideas concerning signaling with excited-state association phenomena are yet to be exploited widely. Nevertheless, it is to be hoped that the pathfinding examples collected here will be a spur toward that wider exploitation.

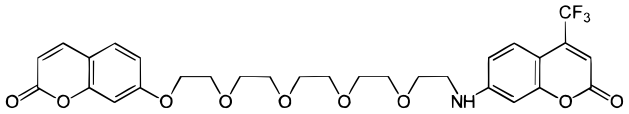
X. Electronic Energy Transfer (EET) Systems

Our final focus concerns bifluorophoric (or higher) systems where the photoactive units are nonidentical. Perhaps the earliest photochemical studies on supramolecular systems containing photoactive components linked by all-σ-bonded spacers belong in this category.[689−690a] The importance of EET within the photosynthetic reaction center[556] has prompted its mimicry within smaller supermolecules.[691,697] Since wires and fiber optics are essential in the information age, molecular energy carriers in one dimension are also sought after.[692−700] Many of these concern tris(2,2′-bipyridyl)ruthenium(II)/osmium(II) pairs or their bis(2,2′:6′,2″-terpyridyl) equivalents and porphyrinzinc(II)/metal-free porphyrin with or without a boron-dipyrromethene as the initial energy donor.[695,701] Polyalkynes[299,696] and linear porphyrinzinc(II) arrays[695] show very good transmission characteristics. Nonlinear arrays of porphyrinzinc(II)/metal-free porphyrin are also good candidates for EET, although some of these involve considerable PET as well.[562,702,702a] Mechanistic dilemmas are common because two or possibly three modes of EET are available to the larger supermolecules. Electron exchange, dipole−dipole coupling and emission−reabsorption are considered to be important at short-, medium-, and long-range respectively.[48,28] As usual, the borders are ill defined, and the fact that many supramolecular dimensions are in this borderland adds to the uncertainty. The situation is not helped

by the fact that the electron exchange model contains unquantifiable parameters.[690,703] The effects of orbital penetration were not realized until recently.[704] EET is of course essential to the efficient functioning of many signaling systems based on MC (section V) and triplet (section VII) excited states.

As the following pages will demonstrate, almost all the fluorescent EET-based signaling systems for analytes depend on conformational changes caused by the recognition process. In other words, the distance dependence of EET is being exploited. The other crucial controller of EET, the spectral overlap integral between donor emission and acceptor absorption, is hardly made use of. We begin with this minority. Walt employs an absorptiometric pH indicator as an EET acceptor whose efficiency is ionically adjustable.[705] The donor fluorophore and the acceptor are used as a double-labeled polymer system. When the EET pair is held at relatively small separation in a cross-linked gel, the EET efficiency is high and the behavior is as predicted.[705] The use of linear polymers leads to complications caused by predominantly electrostatic dye−dye interactions.[706]

Besides providing an important overview of this area,[690] Valeur is also responsible for a rare example of a small molecule which signals metal ions by modulation of EET.[707,708] The podand midsection of **233** curls up with Pb(II) to draw the terminal fluorophores closer together. The two coumarins have been adjusted with suitable substituents to behave as an EET donor−acceptor pair. Increase of the EET quantum efficiency is a key result.



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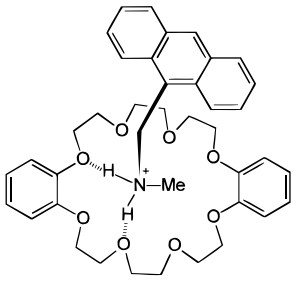
Fluorescent signaling of ions via EET can also benefit from highly selective natural receptors. Section VIII.B illustrated siderophore-based examples **187**[577−579] and **188**[580,581] for Fe(III) using PET. We met Walkup and Imperiali's Zn(II) signaling finger peptide[366] in section VI.C concerned with TICT excited states. An almost simultaneous report targets an almost identical system from the different angle of EET.[709] Godwin and Berg double label the peptide at the N-terminal and at a cysteine with rhodamine-type and fluorescein fluorophores, respectively. The popularity of this EET pair will be evident from later examples. Conformational rearrangements triggered by Zn(II) double the rhodamine-type emission intensity, whereas the fluorescein emission is virtually unaltered. This system is visibly excitable (cf., the case based on 5-(dimethylamino)naphthalene-1-sulfonamide)[366] and self-calibratable via emission ratioing besides the selective and strong (binding constant of 10^{12} M^{-1} at neutral pH) affinity for the analyte.

Hydrogen bonding, rather than metal ion coordination, is responsible for the assembly of **225**[710] and **234**.[711] Hamilton and co-workers exploit their 2,6-diaminopyridine receptor for barbiturate guests[54] in the design of **225**. As mentioned in section VIII.B, such assembly strategies are versatile because many



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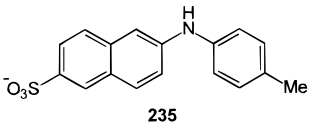
combinations can be generated once the smaller components have ben synthesized. While the binding is strong enough in chlorocarbon solvents to permit the use of components at micromolar levels, a significant amount of nonspecific quenching of the donor emission needs to be allowed for during the steady state and time resolved studies. A metal-free porphyrin fluorophore also serves as the energy acceptor in Sessler and Harriman's EET system[711a] which is closely related to their PET system **172**.[548] In fact, the metal-free porphyrin EET acceptor replaces the PET acceptor quinone unit within **172**. Balzani and his colleagues employ commercially available components to assemble **234**.[711] Self-assembly is successful only when the 9-anthrylmethylammonium cation is disengaged from its counteranion in the chlorocarbon solvent. The dialkoxybenzene units on the 24-crown-8 ether efficiently donate excitation energy to the 9-anthryl group within the ammonium guest.

Hydrophobic interactions involving cyclodextrin derivatives in water played a significant part in section VIII.B. In the present context, the cases under discussion contain the important addition of antenna action[712,713] (see section V). Both cases involve involve a β-cyclodextrin, appearing within a year of each other from either side of the Atlantic Ocean, and have their primary alcohol functions converted into seven naphthyloxy units with solubilizing groups. The ring of subunits comprising the antenna is reminiscent of natural antenna pigment assemblies.[714] The anionic solubilizing groups appear to suppress excimer formation within the antenna.[715] After matching the host antenna emission spectrum with the absorption spectrum of the energy acceptor, Gravett and Guillet[712] use the anionic TICT-based guest **235** (section VI.C), whereas Lehn, Valeur, and their colleagues choose the neutral laser dye derivative **236** related to **18**.[225,178] The latter shows par-



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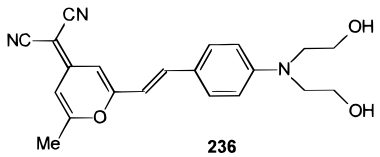

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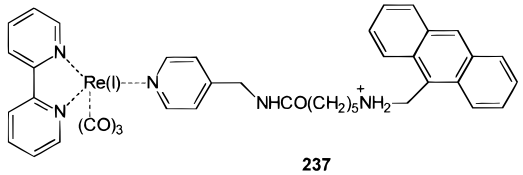
ticularly strong binding due to the extension of the host's inner hydrophobic surface by the antenna units. Compound **235** offers the compensation that any uncomplexed members of the population will be silent during fluorescence experiments. Both studies

display guest emission when the host antennas are optically pumped. The increase of **236** emission as more and more of it occupies the host molecules is mirrored by a quenching of the antenna emission.

We featured the quenching of (9-anthrylmethyl)-ammonium fluorescence upon intercalation into double stranded DNA[544] in section VIII.B. Excitation of the nucleobases also permit observation of this quenched fluorescence provided hetero adenine−thymine sequences are present.[716] Intercalation is found to be essential for EET and melting the double helix destroys the effect. It is interesting to note that PET along the double helix also requires intercalative burial of the donor and acceptor moieties among the nucleobase pairs.[554,555] Intercalation of a (9-anthrylmethyl)ammonium moiety into double-stranded DNA is also involved in Thornton and Schanze's experiments with **237**.[717] Compound **237** most probably



237

exists in a folded conformation in aqueous media. In the presence of DNA, the 9-anthryl unit enters the core of the double helix, whereas the Re(I) center is forced to stay outside. As in Kumar's work,[544,716] the 9-anthryl fluorescence is significantly quenched. The novelty arises from the concomitant "off−on" switching of the MLCT emission which is due to suppression of the electron exchange EET by the forcibly extended spacer. Transient absorption experiments with a 20 ns threshold shows only anthracene triplet states produced by EET. Even if PET processes were playing a part, radical ion intermediates would have disappeared earlier. Hybridization also interferes with EET fron acridine to fluorescein moieties attached to a single DNA strand presumably by a similar intercalative burial[718] of the donor within the double strand. The EET efficiency returns to normal if the double helix is melted.

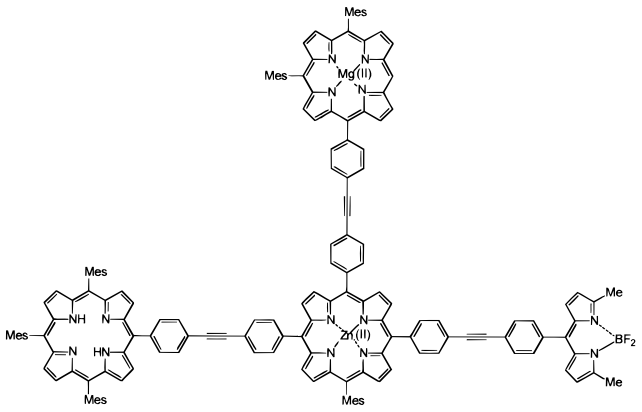
EET by the dipole−dipole coupling mechanism has been of particular relevance to biochemists studying relatively large structures. The "spectroscopic ruler" is suited to distance measurement over several tens of angstroms.[719,720] Self-calibration can be intrinsic to this type of signaling since a ratio between donor and acceptor emissions is being observed.

Relatively large biomolecules feature again in an elegant self-calibrating sensor for the important intracellular messenger cyclic adenosine monophosphate (cAMP). Tsien, Taylor, and colleagues derivatize the natural protein kinase for cAMP for this purpose.[721] Thus the issue of addressing physiological levels of the analyte is settled at a stroke. Since the protein kinase disassembles its regulatory and catalytic subunits upon binding cAMP, attaching an EET donor (fluorescein) to one subunit and the acceptor (rhodamine) to the other is a neat signaling strategy. The acceptor/donor emission ratio of the sensor is smaller when guest bound. This cAMP sensor can be used in combination with the Ca^{2+}-responsive **21**[198] to visualize the interplay between these two cellular second messengers.[721a]

Fluorescein and rhodamine-based fluorophores have been perennial favorites in biological assays. However, an exploitation of a strong interaction between the pair is rare. Herron and co-workers use this approach to develop a homogeneous immunoassay method.[722] These two fluorophores are attached to the N- and C- terminals of an oligopeptide which is recognized by an antibody raised against the important analyte hCG (human chorionic gonadotrophin). It is particularly notable that the double labeling of the relatively small oligopeptide is far easier to perform than on the large biomolecule. The flexibility of the oligopeptide linker means that the terminal dyes π stack together to destroy each other's fluorescence. Antibody binding to the central oligopeptide straightens it sufficiently to force the two fluorophores apart to a safe distance. Normal EET takes over and strong emission is seen from the rhodamine acceptor. The emission from the fluorescein donor remains weak whether the antibody is present or not i.e. ratioing of the two emission bands is feasible. Homogeneous DNA assays (section V) should also benefit from this general approach.

The spotlight remains on the oligopeptide linkers betwen EET pairs of photoactive components as we near the end of our journey along the recognition−fluorescence interface. In the present example developed by Krafft for protease assays,[723,724] the C-terminal carries a 2-aminonaphthalene-5-sulfonate fluorophore as EET donor and the N-terminal is elaborated into 4-[[4′-(dimethylamino)phenyl]azo]benzamide as the acceptor. This EET pair maintains considerable efficiency even across a decapeptide without special folding, i.e., the donor's fluorescence is substantially quenched. Application of Herron's approach with π-stacking fluorophore pairs[722] should allow the use of longer oligopeptides in selective protease assays if that becomes necessary. Hydrolysis of the peptide linker by the protease switches "on" the fluorescence since the EET acceptor has been disconnected. Rapid screening of protease inhibitors with such irreversible signaling systems could hold the key to therapies for disease such as AIDS and Alzheimer's.

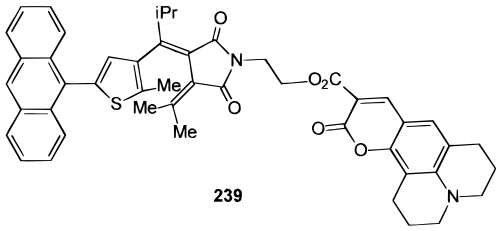
Lindsey's porphyrin arrays which carry energy efficiently over large (∼10 nm) distances can be fitted with redox switchable units to divert the productive EET into an energy sink upon command. Compound **238**[725] is one such example where the redox switchable unit sits on a transmissive component. The



238

porphyrinmagnesium(II) moiety is oxidizable at potentials which have negligible effect on the boron−dipyrromethene donor, porphyrinzinc(II) transmitter and metal-free porphyrin acceptor components. The nonfluorescent porphyrinmagnesium(II) radical cation has such a low-energy excited state that it takes over as the ultimate destination for the excitation energy in the array. The possibility of PET at this stage is yet to be evaluated. Of course, one electron reduction of this radical cation restores the ultimate energy acceptor status to the metal-free porphyrin which responds with strong fluorescence.

To close, we draw together some examples where EET is directed by physical, rather than chemical, stimuli. Reversibly photon-writeable, photon-readable molecular devices were encountered in section IX in the form of **229**.[678] The comments made there are equally valid here. Nevertheless, some landmark cases are available. Effenberger's **239**[726] is the earliest member of this set which combines photochromism[727] and fluorescence. EET from the 9-anthryl unit to a push−pull coumarin fluorophore is diverted only when the central fulgide is photoconverted to the colored form. The latter serves as the energy sink which prevents emission from the coumarin moiety. Unfortunately, long irradiation times are necessary to effect the transformation of the photochromic unit in the presence of EET from it to a lower energy chromophore nearby. A somewhat similar concept can be identified within Yamazaki and Ohta's research on interlayer EET across a photochromic indolinospiropyran in Langmuir−Blodgett multilayers.[728,729] The difficulties mentioned in section X are less relevant to this intermolecular situation and relatively efficient switching is seen. These ideas are extensible to performing parallel all-optical logic operations with molecular, although not molecular-scale, materials (cf. **206** in section VIII.C). Daub's **240**[730] is more compact than **239**[726] but the outcome is similar. The mode of operation is distinct since the only EET process occurs when the colored form of the photochromic unit in **241** acts as the energy sink for the anthracene donor. Thermal reversion of **241** to **240** is a negative aspect of this otherwise very interesting system. Compound **242**, due to Tsvigoulis and Lehn,[731] has no problems of thermal reversion from the colored form **243** since it employs an excellent photochromic core.[732] Essentially complete fluorescence switching "off" is found when the core is converted to the coloured form with short wavelength irradiation. Long wavelength irradiation into the absorption band of the coloured form retrieves the original core and fluorescence is switched back "on". Compound **244**[733] has also been proposed for photoswitchable EET experiments.



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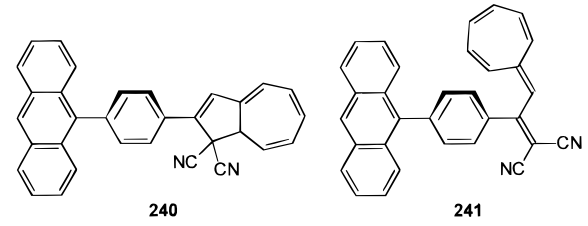
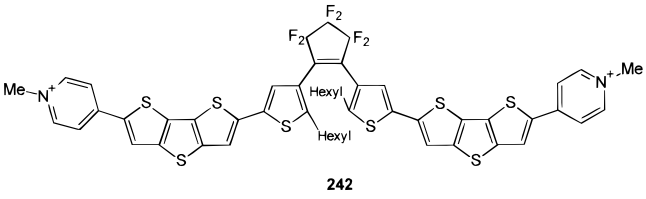


240 **241**



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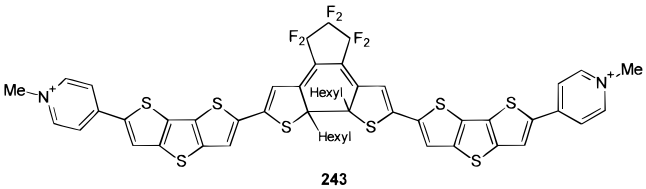

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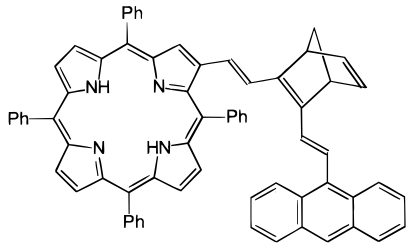

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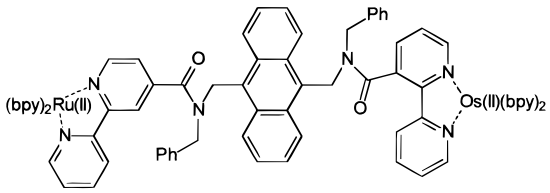
Compound **245**[734,735] is an inorganic version of **239**[726] with some interesting contrasts. Belser, De Cola, Balzani, and co-workers find that EET from the tris(2,2′-bipyridyl)ruthenium(II) moiety to its Os(II) counterpart is rapid across the anthracene unit.



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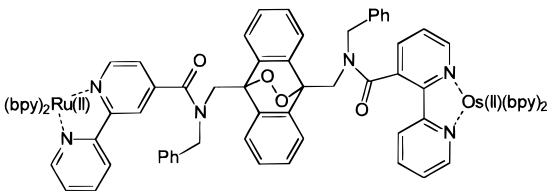
However, extended irrradiation in aerated solution with wavelengths to which the anthracene unit is transparent leads to its peroxidation. Olmsted reported a similar result for an anthracene-linked tris-(2,2′-bipyridyl)ruthenium(II) derivative.[736] Compound **246**, the oxygenated form of **245**, still displays intramolecular EET but at a rate 2 orders of magnitude slower. The aromaticity of the central ring of anthracene, lying as it does in the EET path, evidently controls energy traffic. The triplet excited



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state of anthracene is energetically bracketed by the emissive states of the two metal complexes. This favorable situation is lost upon destruction of the central benzene ring. Some parallels may be found in **205** where electron exchange EET is evidently mediated by an ionically neutralizable "stepping stone".[617] Reversible versions of **245** are eagerly awaited.

While the number of EET-based fluorescent signaling systems are still small (cf. PET systems), their ready applicability suggests there are many more to come.

XI. Conclusion

The foregoing pages have illustrated how chemists from diverse backgrounds have constructed fluorescent signaling systems for various purposes. Some of these have an academic flavor, i.e., mimicry of behavioral aspects of natural photosynthetic centers. Others are clearly application-oriented: switches with potential for information technology and sensors for monitoring species and properties in medicine, biology, environmental science, analytical chemistry, materials science, and even some branches of engineering. While the examples are numerous and their breadth is panoramic, it is comforting that only a relatively small number of design principles are involved. That is why a rather logical progression is possible through the different classes of active systems. Our hopes for this review are twofold. First, that it will be a handy reference for those already involved in this enterprise. Second, that it will be a point of entry for fresh minds into this enjoyable and rewarding field.

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XIII. References

(1) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89.
(2) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304.
(3) Lehn, J.-M. *Supramolecular Chemistry;* VCH: Weinheim, 1995.
(4) *Supramolecular Photochemistry*; Balzani, V., Ed.; Reidel: Dordrecht, 1987.
(5) Balzani, V.; Scandola, F. *Supramolecular Photochemistry*; Ellis-Horwood: Chichester, 1991.
(6) Balzani, V.; Credi, A.; Scandola, F. In *Transition Metals in Supramolecular Chemistry*; Fabbrizzi, L., Poggi, A., Eds.; Kluwer: Dordrecht, 1994.
(7) Bryan, A. J.; de Silva, A. P.; de Silva, S. A.; Rupasinghe, R. A. D. D.; Sandanayake, K. R. A. S. *Biosensors* **1989**, *4*, 169.
(8) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G.E. M.; Sandanayake, K. R. A. S. *Chem. Soc. Rev.* **1992**, *21*, 187.
(9) *Indicators*; Bishop, E., Ed.; Pergamon, Oxford, 1972.
(10) *Biochemical Fluorescence*: *Concepts*; Chen, R. F., Edelhoch, H., Eds.; Dekker: New York, 1976; Vols. 1 and 2.
(11) Fendler, E. J.; Fendler, J. H. *Catalysis in Micellar and Macromolecular Systems*; Academic: New York, 1975.
(12) *Fluorescent Probes*; Beddard, G. S., West, M. A., Eds.; Academic: London, 1981.
(13) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.
(14) Thomas, J. K. *Chemistry of Excitation at Interfaces*; ACS Monograph 191; American Chemical Society: Washington DC, 1983.
(15) Fernandez-Gutierrez, A.; Munoz de la Pena, A. In *Molecular Luminescence Spectroscopy. Methods and Applications*; Schulman, S. G., Ed.; Wiley, New York, 1985; Part 1, p 371.
(16) Kalyanasundaram, K. *Photochemistry in Microheterogeneous Systems*; Academic: New York, 1987.
(17) *Photochemistry in Organized and Constrained Media*; Ramamurthy, V., Ed.; VCH: New York, 1991.
(18) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Eugene, OR, 1996.
(19) *Fluorescent Chemosensors of Ion and Molecule Recognition*. Czarnik, A. W., Ed.; ACS Symp. Ser. 538; American Chemical Society: Washington DC, 1993.
(20) Valeur B. In *Molecular Luminescence Spectroscopy*; Schulman, S. G.; Ed.; Wiley, New York, 1993; Part 3, p 25.
(21) Probe Design and Chemical Sensing. *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R.; Ed.; Plenum, New York, 1994; Vol. 4.
(22) Early review on supramolecular aspects: Löhr, H.-G.; Vögtle, F. *Acc. Chem. Res.* **1985**, *18*, 65. See also: Herrmann, U.; Tumler, B.; Maass, G.; Mew, P. K. T.; Vögtle, F. *Biochemistry* **1984**, *29*, 4059.
(23) *Biophysical and Biochemical Aspects of Fluorescence Spectroscopy*; Dewey, T. G., Ed.; Plenum: New York, 1991.
(24) *Luminescence Techniques in Chemical and Biochemical Analysis*; Baeyens, W. R. G., de Keukelaire, D., Korkidis, K., Eds.; Dekker, New York, 1991.
(25) *Fluorescence Spectroscopy: New Methods and Applications*; Wolfbeis, O. S., Ed.; Springer, Berlin, 1993.
(26) Valeur, B.; Bardez, E. *Chem. Brit.* **1995**, *31*, 216.
(27) Biochemical Applications. *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Plenum, New York, 1992; Vol. 3.
(28) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: London, 1970.
(29) Pietraszkiewicz, M. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D. N., Ed.; Pergamon, Oxford, 1996; Vol. 10, p 225.
(30) Balzani, V.; Scandola, F. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D. N., Ed.; Pergamon, Oxford, 1996; Vol. 10, p 687.
(31) *Fibre Optic Chemical Sensors and Biosensors*; Wolfbeis, O. S., Ed.; CRC Press: Boca Raton, 1991; Vols. 1 and 2.
(32) *Biosensors with Fiberoptics*; Wise, D. L., Wingard, L. B., Eds.; Humana Press, Clifton, 1991.
(33) Tsien, R. Y., *Chem. Eng. News*, **1994**, July 18, 34.
(34) Dixon, A. J.; Benham, G. S. *Int. Lab.* **1988** (4), 38.
(35) Tan, W.; Shi, Z. Y.; Kopelman, R. *Anal. Chem.* **1992**, *64*, 2985.
(36) Tan, W.; Shi, Z. Y.; Smith, S.; Birnbaum, D.; Kopelman, R. *Science* **1992**, *258*, 778.
(37) Sharp, S. L.; Warmack, R. J.; Goudonnet, J. P.; Lee, I.; Ferrell, T. L. *Acc. Chem. Res.* **1993,** *26*, 377.
(38) Lewis, A.; Lieberman, K. *Anal. Chem.* **1991,** *63*, 625A. (a) Xie, X. S. *Acc. Chem. Res.* **1996**, *29*, 598.
(39) Avouris, P. *Acc. Chem. Res.* **1995**, *28*, 102.
(40) Goodwin, P. M.; Ambrose, W. P.; Keller, R. A. *Acc. Chem. Res.* **1996**, *29*, 607.
(41) Orrit, M.; Bernard, J. *Phys. Rev. Lett.* **1990**, *65*, 2716.
(42) Mets, U.; Rigler, R. *J. Fluoresc.* **1994**, *4*, 259.
(43) Moerner, W. E.; Basche, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 457. (a) Moerner, W. E. *Acc. Chem. Res.* **1996**, *29*, 563.
(44) Yeung, E. S. *Acc. Chem. Res.* **1994**, *27*, 209.
(45) Guilbault, G. G. *Practical Fluorescence*; 2nd ed.; Dekker: New York, 1990.
(46) *Modern Fluorescence Spectroscopy*; Wehry, E. L., Ed.; Plenum: New York, 1976−81; Vols. 1−4.
(47) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983.
(48) Turro, N. J. *Modern Molecular Photochemistry*; University Science Books: Mill Valley, CA, 1991.
(49) Kopecky, J. *Photochemistry. A Visual Approach*; VCH, New York, 1992.
(50) Wayne, R. P. *Principles and Applications of Photochemistry*; Oxford University Press: Oxford, 1988.
(51) Barltrop, J. A.; Coyle, J. D. *Excited States in Organic Chemistry*; Wiley: London, 1975.
(52) Sandros, K. *Acta Chem. Scand.* **1964**, *18*, 2355.
(53) Pina, F.; Parola, A. J.; Ferreira, E.; Maestri, M.; Armaroli, N.; Ballardini, R.; Balzani, V. *J. Phys. Chem.* **1995**, *99*, 12701. (a)

Parola, A. J.; Pina, F.; Ferreira, E.; Maestri, M.; Balzani, V. *J. Am. Chem. Soc.* **1996**, *118*, 11610.
(54) Hamilton, A. D. *Adv. Supramol. Chem.* **1990**, *1*, 1.
(55) Kamlet, M. J.; Abboud, J. L. M.; Abraham. M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877.
(56) Kalyanasundaram, K.; Thomas, J. K. *J. Phys. Chem.* **1977**, *81*, 2176.
(57) Lianos, P.; Lux, B.; Gerard, D. J. *Chim. Phys.* **1988**, *77*, 907.
(58) Matsumoto, T.; Sato, M.; Hirayama, S. *Chem. Phys. Lett.* **1972**, *13*, 13.
(59) Snyder, R.; Testa, A. C. *J. Lumin.* **1990**, *47*, 35.
(60) Hiratani, K.; Nomoto, M.; Sugihara, H.; Okada, T. *Chem. Lett.* **1990**, 43.
(61) Hiratani, K.; Nomoto, M.; Ohuchi, S.; Taguchi, K. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1349.
(62) Wolfbeis, O. S.; Offenbacher, H. *Monatsh. Chem.* **1984**, *115*, 647.
(63) Ogawa, S.; Tsuchiya, S. *Chem. Lett.* **1996**, 709.
(64) Ogawa, S.; Narushima, R.; Arai, Y. *J. Am. Chem. Soc.* **1984**, *106*, 5760.
(65) Shirai, M.; Ishimaru, S.; Tsunooka, M. *J. Fluoresc.* **1993**, *3*, 51.
(66) Winnik, M. A.; Dong, D. C. *Can. J. Chem.* **1984**, *62*, 2560.
(67) Street, K. W.; Acree, W. E. *Analyst* **1986**, *111*, 1197.
(68) Acree, W. E.; Tucker, S. A.; Wilkins, D. C. *J. Phys. Chem.* **1993**, *97*, 11199.
(69) Acree, W. E.; Wilkins, D. C.; Tucker, S. A.; Griffin, J. M.; Powell, J. R. *J. Phys. Chem.* **1994**, *98*, 2537.
(70) Nagasaki, T.; Tajirl, Y.; Shinkai, S. *Rec. Trav. Chim Pays Bas* **1993**, *112*, 407.
(71) Richardson, F. H. *Chem. Rev.* **1982**, *82*, 541.
(72) Bisby, R. H.; Cundall, R. B.; Davenport, L.; Johnson, I. D.; Thomas, E. W. In ref 12, p 97.
(73) Waldeck, D. H. *Chem. Rev.* **1991**, *91*, 415.
(74) Whitten, D. G. *Acc. Chem. Res.* **1993**, *26*, 502.
(75) Duveneck, L. G.; Sitzmann, E. V.; Eisenthal, K. B.; Turro, N. J. *J. Phys. Chem.* **1989**, *93*, 7166.
(76) Saltiel, J.; D'Agostino, J.; Megarity, E. D.; Metto, L.; Neuberger, K. R.; Wrighton, M.; Zafiriou, O. C. *Org. Photochem.* **1973**, *3*, 1.
(77) Saltiel, J.; Sun, Y.-P. In *Photochromism. Molecules and Systems*; Dürr, H.; Bouas-Laurent, H.; Eds.; Elsevier: Amsterdam, 1990; p 64.
(78) Sandanayake, K. R. A. S.; Nakashima, K.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1621.
(79) Sousa, L. R.; Larson, J. M. *J. Am. Chem. Soc.* **1977**, *99*, 307.
(80) Larson, J. M.; Sousa, L. R. *J. Am. Chem. Soc.* **1978**, *100*, 1943.
(81) Basu, G.; Kubasik, M.; Anglos, D.; Secor, B.; Kuki, A. *J. Am. Chem. Soc.* **1990**, *112*, 9410.
(82) Kavarnos, G.; Cole, T.; Scribe, F.; Dalton, J. C.; Turro, N. J. *J. Am. Chem. Soc.* **1971**, *93*, 1032.
(83) Turro, N. J.; Kavarnos, G.; Fung, V.; Lyons, A. L.; Cole, T. *J. Am. Chem. Soc.* **1972**, *94*, 1392.
(84) Davidson, R. S.; Bonneau, R.; Joussot-Dubien, J.; Trethewey, K. R. *Chem. Phys. Lett.* **1980**, *74*, 318.
(85) Harriman, A.; Rockett, B. W. *J. Chem. Soc., Perkin Trans. 2* **1974**, 217.
(86) Davidson, R. S.; Goodwin, J. W.; Kemp, G. *Tetrahedron Lett.* **1980**, 2911.
(87) Sabbatini, N.; Guardigli, M.; Lehn, J.-M. *Coord. Chem. Rev.* **1993**, *123*, 201.
(88) Balzani, V.; Sabbatini, N.; Scandola, F. *Chem. Rev.* **1986**, *86*, 319.
(89) Sabbatini, N.; Guardigli, M.; Manet, I.; Ungaro, R.; Casnati, A.; Ziessel, R.; Ulrich, G.; Asfari, Z.; Lehn, J.-M. *Pure Appl. Chem.* **1995**, *67*, 135.
(90) Bunzli, J.-C. G. In *Lanthanide Probes in Life, Chemical and Earth Sciences*; Bunzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; Chapter 7.
(91) Mayer, A.; Neuenhofer, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1044.
(92) Hemmila, I. *Clin. Chem.* **1985**, *31*, 359.
(93) Hemmila, I. *Applications of Fluorescence in Immunoassays*; Wiley: New York, 1991.
(94) Dickinson, E. F. G.; Pollak, A.; Diamandis, E. P. *J. Photochem. Photobiol. B: Biol.* **1995**, *27*, 3.
(95) Horrocks, W. DeW.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384.
(96) Dewar, M. J. S.; Dougherty, R. C. *The PMO Theory of Organic Chemistry*; Plenum: New York, 1975.
(97) Michl, J.; Bonacic-Koutecky, V. *Electronic Aspects of Organic Photochemistry*; Wiley: New York, 1990.
(98) Klessinger, M.; Michl, J. *Excited States and Photochemistry of Organic Molecules*; VCH: New York, 1995.
(99) Parker, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **1996**, 3613.
(100) Hemmila, I.; Mukkala, V.-M.; Takalo, H. *J. Fluoresc.* **1995**, *5*, 159.
(101) Dickins, R. S.; Parker, D.; de Sousa, A. S.; Williams, J. A. G. *Chem. Commun.* **1996**, 697.
(102) Weissman, S. *J. Chem. Phys.* **1950**, *18*, 1258.
(103) Morrison, H.; Agyin, K.; Tiang, A.; Xiao, C. *Pure Appl. Chem.* **1995**, *67*, 111.
(104) Stern, K. G.; Steinberg, M. A. *Biochem. Biophys. Acta* **1953**, *2*, 553.
(105) Frey, S. T.; Gong, M. L.; Horrocks, W. de W. *Inorg. Chem.* **1994**, *33*, 3229.
(106) Canfi, A.; Bailey, M. P.; Rocks, B. F. *Analyst* **1989**, *114*, 1405.
(107) Canfi, A.; Bailey, M. P.; Rocks, B. F. *Analyst* **1989**, *114*, 1407.
(108) Siepak, J. *Analyst* **1989**, *114*, 529.
(109) Saavedra, S. S.; Picozza, E. G. *Analyst* **1989**, *114*, 835.
(110) Selvin, P. R.; Rana, T. M.; Hearst, J. E. *J. Am. Chem. Soc.* **1994**, *116*, 6029.
(111) Li, M.; Selvin, P. R. *J. Am. Chem. Soc.* **1995**, *117*, 8132.
(112) Selvin, P. R.; Hearst, J. E. *Proc. Nat. Acad. Sci. USA* **1994**, *91*, 10024.
(113) Abu-Saleh, A.; Meares, C. F. *Photochem. Photobiol.* **1984**, *39*, 763.
(114) Remuinan, M. J.; Roman, H.; Alonso, M. T.; Rodriguez-Ubis, J. C. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1099.
(115) Mukkala, V.-M.; Kankare, J. J. *Helv. Chim. Acta* **1992**, *75*,1578.
(116) Mukkala, V.-M.; Sund, C.; Kwiatkowski, M.; Pasanen, P.; Hogberg, M.; Kankare, J.; Takalo, H. *Helv. Chim. Acta.* **1992**, *75*, 1621.
(117) Toner, J. L. U.S. Patent 1989, 4837169.
(118) Toner, J. L. In *Inclusion Phenomena and Molecular Recognition*; Atwood, J. L., Ed.; Plenum: New York, 1990; p 185.
(119) Saha, A. K.; Kross, K.; Kloszewski, E. D.; Upson, D. A.; Toner, J. L.; Snow, R. A.; Black, C. D. V.; Desai, V. D. *J. Am. Chem. Soc.* **1993**, *115*, 11032.
(120) Hoshino, H.; Utsumi, S.; Yotsuyanagi, T. *Talanta* **1994**, *41*, 93.
(121) Costa, S. M. de B.; Queimado, M. M.; Frausto da Silva, J. J. R. *J. Photochem.* **1980**, *12*, 31.
(122) Alonso, M. T.; Brunet, E.; Hernandez, C.; Rodriguez-Ubis, J. C. *Tetrahedron Lett.* **1993**, *34*, 7465.
(123) Sowinska, M.; Le Bris, M.-T.; Valeur, B. *J. Photochem. Photobiol. A Chem.* **1991**, *60*, 383.
(124) Alpha, B.; Lehn, J.-M.; Mathis, G. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 266.
(125) Paul-Roth, C. O.; Lehn, J.-M.; Guilhem, J.; Pascard, C. *Helv. Chim. Acta* **1995**, *78*, 1895.
(126) Pietraszkiewicz, M.; Karpiuk, J.; Rout, A. K. *Pure Appl. Chem.* **1993**, *65*, 563.
(127) Sato, N.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **1993**, 621. (a) Rudkevich, D. M.; Verboom, W.; Van der Tol, E.; van Staveren, C. J.; Kaspersen, F. M.; Verhoeven, J. W.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1995**, 131.
(128) Sammes, P. G.; Yahioglu, G.; Yearwood, G. D. *J. Chem. Soc., Chem. Commun.* **1992**, 1282.
(129) Coates, J.; Sammes, P. G.; West, R. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1107.
(130) Coates, J.; Sammes, P. G.; Yahioglu, G.; West, R. M.; Garman, A. J. *J. Chem. Soc., Chem. Commun.* **1994**, 2311.
(131) Sabbatini, N.; Guardigli, M.; Manet, I.; Bolletta, F.; Ziessel, R. *Inorg. Chem.* **1994**, *33*, 955.
(132) Sabbatini, N.; Guardigli, M.; Bolletta, F.; Manet, I.; Ziessel, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1501.
(133) Sato, N.; Goto, M.; Matsumoto, S.; Shinkai, S. *Tetrahedron Lett.* **1993**, *34*, 4847.
(134) Murru, M.; Parker, D.; Williams, J. A. G.; Beeby, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1116.
(135) Parker, D.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1581.
(136) Charles, R. G.; Riedel, E. P. *J. Inorg. Nucl. Chem.* **1966**, *28*, 527.
(137) Yu, J. A.; Lessard, R. B.; Nocera, D. G. *Chem. Phys. Lett.* **1991**, *187*, 263.
(138) Lis, S.; Konarski, J.; Hnatejko, Z.; Elbanowski, M. *J. Photochem. Photobiol. A Chem.* **1994**, *79*, 25.
(139) Pikramenou, Z.; Nocera, D. G. *Inorg. Chem.* **1992**, *31*, 532.
(140) Pikramenou, Z.; Johnson, K. M.; Nocera, D. G. *Tetrahedron Lett.* **1993**, *34*, 3581.
(141) Mortellaro, M. A.; Nocera, D. G. *Chemtech* **1996**, February, 17.
(142) Nocera, D. G. *New Sci.* **1996**, 13 January, 24.
(143) Fujii, I.; Kiyama, R.; Kanematsu, K. *Bioorg. Chem.* **1989**, *17*, 240.
(144) McLachlan, B. G.; Kavandi, J. R.; Callis, J. B.; Gouterman, M.; Green, E.; Khalil, G. E.; Burns, D. *Exp. Fluids* **1993**, *14*, 33.
(145) Gallery, J.; Gouterman, M.; Green, E.; Khalil, G. E. *Rev. Sci. Instrum.* **1994**, *65*, 712.
(146) Sammes, P. G.; Yahioglu, G. *Nat. Prod. Rep.* **1996**, *13*, 1.
(147) Mullins, S. T.; Sammes, P. G.; West, R. M.; Yahioglu, G. *J. Chem. Soc., Perkin Trans. 1* **1996**, 75.
(148) Adachi, G. Y.; Tomokiyo, K.; Sorita, K.; Shiokawa, J. *J. Chem. Soc., Chem. Commun.* **1980**, 914.
(149) Cornelius, G.; Gartner, W.; Haynes, D. H. *Biochemistry* **1974**, *13*, 3052.
(150) Krasovitskii, B. M.; Bolotin, B. M. *Organic Luminescent Materials*; VCH: Weinheim, 1989.
(151) Werner, T. C. In ref 46; Vol. 2, p 277.
(152) Langhals, H. *Z. Phys. Chem. Neue Folge* **1981**, *127*, 45.
(153) Langhals, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 724.
(154) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.

(155) Kutsyna, L. M.; Voevoda, L. V.; Tischenko, V. G.; Shepel, A. V. *Opt. Spectrosc.* **1969**, *26*, 91.
(156) Okamura, T.; Sumitani, M.; Yoshihara, K. *Chem. Phys. Lett.* **1983**, *94*, 339.
(157) Cowley, D. J. *J. Chem. Soc., Perkin Trans. 2* **1984**, 281.
(158) Tamaki, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3321.
(159) Jones, G.; Jackson, W. R.; Kanoktanaporn, S.; Bergmark, W. R. *Photochem. Photobiol.* **1985**, *42*, 477.
(160) Kessler, M. A.; Wolfbeis, O. S. *Spectrochim. Acta* **1991**, *47A*, 187.
(161) Anstead, G. M.; Carlson, G. E.; Kym, P. R.; Hwang, K. J.; Katznellenbogen, J. A. *Photochem. Photobiol.* **1993**, *58*, 785.
(162) Kosower, E. M.; Giniger, R.; Radkowsky, A.; Hebel, D.; Schusterman, A. *J. Phys. Chem.* **1986**, *90*, 5552.
(163) Shin, D. M.; Whitten, D. G. *J. Phys. Chem.* **1988**, *92*, 2945.
(164) Quanten, E.; Adrians, P.; De Schryver, F. C.; Roelandts, R.; Degreef, H. *Photochem. Photobiol.* **1986**, *43*, 485.
(165) Hoffman, D. A.; Anderson, J. E.; Frank, C. W. *J. Mater. Chem.* **1995**, *5*, 13.
(166) Bignozzi, C. A.; Chiobioli, C.; Indelli, M. T.; Rampi, M. A.; Varani, G.; Scandola, F. *J. Am. Chem. Soc.* **1986**, *108*, 7872.
(167) Indelli, M. T.; Bignozzi, C. A.; Marconi, A.; Scandola, F. *J. Am. Chem. Soc.* **1988**, *110*, 7381.
(168) de Costa, M. D. P.; de Silva, A. P.; Pathirana, S. T. *Can. J. Chem.* **1987**, *65*, 1416.
(169) Kloppfer, W. *Adv. Photochem.* **1977**, *10*, 311.
(170) Macanita, A. L.; Costa, F. P.; Costa, S. M. B.; Melo, E. C.; Santos, H. *J. Phys. Chem.* **1989**, *93*, 336.
(171) Thorp, H. H.; Kumar, C. V.; Turro, N. J.; Gray, H. B. *J. Am. Chem. Soc.* **1989**, *111*, 4364.
(172) Dickinson, T. A.; White, J.; Kauer, J. S.; Walt, D. R. *Nature* **1996**, *382*, 697.
(173) Hatfield, J. V.; Neaves, P.; Hicks, P. J.; Persaud, K.; Travers, P. *Sens. Actuators B Chem.* **1994**, *18*, 221.
(174) Van der Donckt, E. *Prog. React. Kinet.* **1970**, *5*, 273. More recent work on cyclodextrin complexes; Park, H. R.; Mayer, B.; Wolschann, P.; Kohler, G. *J. Phys. Chem.* **1994**, *98*, 6158.
(175) Ireland, J. F.; Wyatt, P. A. H. *Adv. Phys. Org. Chem.* **1976**, *12*, 131. (a) Clark, J. H.; Shapiro, S. L.; Winn, K. R. C. *J. Am. Chem. Soc.*, **1979**, *101*, 746.
(176) Gutman, M.; Huppert, D.; Pines, E. *J. Am. Chem. Soc.* **1981**, *103*, 3709.
(177) Pines, E.; Huppert, D.; Gutman, M.; Nachliel, M. *J. Phys. Chem.* **1986**, *90*, 6366.
(178) Martin, M. M.; Plaza, P.; Dai Hung, N.; Meyer, Y. H.; Bourson, J.; Valeur, B. *Chem. Phys. Lett.* **1993**, *202*, 425.
(179) Martin, M. M.; Plaza, P.; Meyer, Y. H.; Begin, L.; Bourson, J.; Valeur, B. *J. Fluoresc.* **1994**, *4*, 271.
(180) Martin, M. M.; Plaza, P.; Meyer, Y. H.; Badaoui, F.; Bourson, J.; Lefebvre, J. P.; Valeur, B. *J. Phys. Chem.* **1996**, *100*, 6879.
(181) Létard, J.-F.; Lapouyade, R.; Rettig, W. *Mol. Cryst. Liq. Cryst.* **1993**, *36*, 41.
(182) Létard, J.-F.; Lapouyade, R.; Rettig, W. *Pure Appl. Chem.* **1993**, *65*, 1705.
(183) Dumon, P.; Jonasauskas, G.; Dupuy, F.; Pee, P.; Rullière, C.; Létard, J.-F.; Lapouyade, R. *J. Phys. Chem.* **1994**, *98*, 10391.
(184) Mathevet, R.; Jonusauskas, G.; Rullière, C.; Létard. J-F.; Lapouyade, R. *J. Phys. Chem.* **1995**, *99*, 15709. (a) Jonusauskas, G.; Lapouyade, R.; Delmond, S.; Létard, J. F.; Rullière, C. *J. Chim. Phys. Phys. Chim. Biol.* **1996**, *93*, 1670.
(185) Delmond, S.; Létard, J. F.; Lapouyade, R.; Mathevet, R.; Jonusauskas, G.; Rullière, C. *New J. Chem.* **1996**, *20*, 861.
(186) Valeur, B. In ref 21; p 21.
(187) Kirkbright, G. F. In ref 9; p 685.
(188) Fernandez, M. S.; Fromherz, P. *J. Phys. Chem.* **1977,** *81*, 1755.
(189) Petrov, J. G.; Mîbius, D. *Langmuir* **1991**, *7*, 1495.
(190) Tanasugarn, L.; McNeil, P.; Reynolds, G. T.; Taylor, D. L. *J. Cell Biol.* **1984**, *98*, 717.
(191) Tsien, R. Y. *Annu. Rev. Biophys. Bioeng.* **1983**, *12*, 91.
(192) Tsien, R. Y. *Am. J. Physiol.* **1992**, *263*, C723.
(193) Tsien, R. Y. *Trends Neurosci.* **1988**, *11*, 419.
(194) Tsien, R. Y.; Poenie, M. *Trends Biochem. Sci.* **1986**, *11*, 450.
(195) Tsien, R. Y. *Annu. Rev. Neurosci.* **1989**, *12*, 227.
(196) Tsien, R. Y. In *Optical Methods in Cell Physiology*; de Weer, P.; Salzburg, B. M., Eds.; Wiley, New York, 1986; p 327.
(197) Kano, K.; Fendler, J. H. *Biophys. Biochim. Acta* **1978**, *509*, 289.
(198) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *206*, 3440.
(199) Minta, A.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 19449.
(200) Harootunian, A. T.; Kao, J. P. Y.; Eckert, B. K.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 19458.
(201) Raju, B.; Murphy, E.; Levy, L. A.; Hall, R. D.; London, R. E. *Am. J. Physiol.* **1989**, *256*, C540.
(202) London, R. E. *Annu. Rev. Physiol.* **1991**, *53*, 241.
(203) Illner, H.; McGuigan, J. C. S.; Luthi, D. *Pflugers Arch.* **1992**, *422*, 179.
(204) Thomas, J. A.; Kolbeck, P. C.; Langworthy, T. A. In *Intracellullar pH; Its Measurement, Regulation and Utilization in Cellular Functions*; Nuccitelli, R., Deamer, D. W., Eds.; Liss: New York; p 105.
(205) Thomas, J. A. In *Optical Methods in Cell Physiology*; de Weer, P., Salzberg, B. M., Eds.; Wiley: New York, 1986; p 311.
(206) Rotman, B.; Papermaster, B. W. *Proc. Nat. Acad. Sci. USA* **1966**, *55*, 134.
(207) Smith, G. A.; Hesketh, T. R.; Metcalfe, J. C. *Biochem. J.* **1988**, *250*, 227.
(208) Whitaker, J. E.; Haugland, R. P. Prendergast, F. G. *Anal. Biochem.* **1991**, *194*, 330.
(209) Smith, G. A.; Metcalfe, J. C.; Clarke, S. D. *J. Chem. Soc., Perkin Trans 2* **1993**, 1195.
(210) Szmacinski, H.; Lakowicz, J. R. *Anal. Chem.* **1993**, *65*, 1668.
(211) Van den Bergh, V.; Boens, N.; De Schryver, F. C.; Ameloot, M.; Steels, P.; Gallay, J.; Vincent, M.; Kowalczyk, A. *Biophys. J.* **1995**, *68*, 1110.
(212) Van den Bergh, V.; Boens, N.; De Schryver, F. C.; Gallay, J.; Vincent, M. *Photochem. Photobiol.* **1995**, *61*, 442.
(213) Meuwis, K.; Boens, N.; De Schryver, F. C.; Gallay, J.; Vincent, M. *Biophys, J.* **1995**, *68*, 2469.
(214) Kowalczyk, A.; Boens, N.; Van den Bergh, V.; De Schryver, F. C. *J. Phys. Chem.* **1994**, *98*, 8585.
(215) Rurack, K.; Resch, U.; Senoner, M.; Dahne, S. *J. Fluoresc.* **1993**, *3*, 141.
(216) Lieu, V. T.; Handy, C. A. *Anal. Lett.* **1974**, *7*, 267.
(217) Diehl, H.; Olsen, R.; Spielholtz, G. I.; Jensen, R. *Anal. Chem.* **1963**, *35*, 1144.
(218) Roman Ceba, M.; Fernandez-Guitierrez, A.; Mahadero, M. C. *Mikrochim. Acta (Wien)* **1983**, 85.
(219) Shizuka, H.; Takada, K.; Morita, T. *J. Phys. Chem.* **1980**, *84*, 994.
(220) Shinkai, S.; Ishikawa, Y.; Shinkai, H; Tsuno, T.; Manabe, O. *Tetrahedron Lett.* **1983**, *24*, 1539.
(221) Shinkai, S.; Ishikawa, Y.; Shinkai, H.; Tsuno, T.; Makishima, H.; Ueda, K.; Manabe, O. *J. Am Chem. Soc.* **1984**, *106*, 1801.
(222) Street, K. W.; Krause, S. A. *Anal. Lett.* **1986**, *19*, 735.
(223) Fery-Forgues, R.; Le Bris, M. T.; Guette, J.-P.; Valeur, B. *J. Phys. Chem.* **1988**, *92*, 6233; *J. Chem. Soc., Chem. Commun.* **1988**, 384.
(224) Fery-Forgues, R.; Bourson, J.; Dallery, L.; Valeur, B. *New J. Chem.* **1990**, *14*, 617.
(225) Fery-Forgues, S.; Le Bris, M. T.; Mialocq, J. C.; Pouget, J.; Rettig, W.; Valeur, B. *J. Phys. Chem.* **1992**, *96*, 701.
(226) Bourson, J.; Valeur, B. *J. Phys. Chem.* **1989**, *93*, 3871. (a) Mateeva, N.; Enchev, V.; Antonov, L.; Deligeorgiev, T.; Mitewa, M. *J. Inclusion Phenom.* **1994**, *20*, 323.
(227) Cazaux, L.; Faher, M.; Lopez, A.; Picard, C.; Tisnes, P. *J. Photochem. Photobiol. A: Chem.* **1994**, *77*, 217.
(228) Ahmad, A. R.; Mehta, L. K.; Parrick, J. *Tetrahedron* **1995**, *51*, 12899.
(229) Gocmen, A.; Bulut, M.; Erk, C. *Pure Appl. Chem.* **1993**, *65*, 447.
(230) Blackburn, C.; Bai, M.; Le Compte, K. A.; Langmuir, M. E. *Tetrahedron Lett.* **1994**, *35*, 7915.
(231) Crossley, R.; Goolamali, Z.; Gosper, J. J.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **1994**, 513.
(232) Crossley, R.; Goolamali, Z.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1615.
(233) Alfimov, M. V.; Gromov, S. P.; Lednev, I. K. *Chem. Phys. Lett.* **1991**, *185*, 455.
(234) He, G. X.; Wada, F.; Kikukawa, K.; Shinkai, S.; Matsuda, T. *J. Chem. Soc., Chem. Commun.* **1987**, 1294.
(235) He, G. X.; Wada, F.; Kikukawa, K.; Shinkai, S.; Matsuda, T. *J. Org. Chem.* **1990**, *55*, 548.
(236) Das, S.; Thomas, K. G.; Thomas, K. J.; Kamat, P. V.; George, M. V. *J. Phys. Chem.* **1994**, *98*, 9291.
(237) Thomas, K. J.; Thomas, K. G.; Manojkumar, T. K.; Das, S.; George, M. V. *Proc. Indian. Acad. Sci. (Chem. Sci.)* **1994**, *106*, 1375.
(238) Armaroli, N.; De Cola, L.; Balzani, V.; Sauvage, J.-P.; Dietrich-Buchecker, C. O.; Kern, J.-M.; Bailal, A. *J. Chem. Soc., Dalton Trans.* **1993**, 3241. See, also: Armaroli, N.; De Cola, L.; Balzani, V.; Sauvage, J.-P.; Dietrich-Buchecker, C. O.; Kern, J.-M. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 553. Armaroli, N.; Balzani, V.; De Cola, L.; Hemmert, C.; Sauvage, J.-P. *New J. Chem.* **1994**, *18*, 775.
(239) Voyer, N.; Maltais, F. *Adv. Mater.* **1993**, *5*, 568.
(240) de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 685.
(241) Dietrich-Buchecker, C. O.; Sauvage, J.-P.; Armaroli, N.; Ceroni, P.; Balzani, V. *New J. Chem.* **1996**, *20*, 801.
(242) Murakami, H.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1993**, 1533.
(243) Tamura, N.; Mitsui, K.; Nobeshima, T.; Yano, Y. *J. Chem. Soc., Perkin Trans. 2* **1994**, 2229.
(244) Herbich, J.; Waluk, J.; Thummel, R. P.; Hung, C. Y. *J. Photochem. Photobiol. A: Chem.* **1994**, *80*, 157.
(245) Thummel, R. P.; Hung, C. Y.; Hopfner, T.; Russel, J. *J. Chem. Soc., Chem. Commun.* **1994**, 857.
(246) Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010.
(247) Chang, S. K.; Van Engen, D.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640.

(248) Tamura, N.; Kajika, T.; Nabeshima, T.; Yano, Y. *J. Chem. Soc., Chem. Commun.* **1994**, 2583.
(249) Motesharei, K.; Myles, D. C. *J. Am. Chem. Soc.* **1994**, *116*, 7413.
(250) Nowick, J. S.; Chen, J. S. *J. Am. Chem. Soc.* **1992**, *114*, 1107.
(251) Nowick, J. S.; Chen, J. S.; Noronha, G. *J. Am. Chem. Soc.* **1993**, *115*, 7636.
(252) Nowick, J. S.; Cao, T.; Noronha, G. *J. Am. Chem. Soc.* **1994**, *116*, 3285.
(253) Hahn, K. M.; Waggoner, A. S.; Taylor, D. L. *J. Biol. Chem.* **1990**, *265*, 20335.
(254) Hahn, K. M.; DeBiasio, R.; Taylor, D. L. *Nature* **1992**, *359*, 736. (a) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Tetrahedron Lett.* **1993**, *34*, 4971.
(255) Thompson, P. A.; Broudy, A. E.; Simon, J. D. *J. Am. Chem. Soc.* **1993**, *115*, 1925.
(256) Nakashima, K.; Nakatsuji, S.; Akiyama, S.; Tanigawa, I.; Kaneda, T.; Misumi, S. *Talanta* **1984**, *31*, 749.
(257) Nakashima, K.; Nagaoka, Y.; Nakatsuji, S.; Kaneda, T.; Tanigawa. I.; Hirose, K.; Misumi, S.; Akiyama, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3219.
(258) Kaneda, T.; Shizaki, Y.; Misumi, S.; Kai, Y.; Hiro, G.; Kasai, N. *J. Am. Chem. Soc.* **1988**, *110*, 2970.
(259) Forst, H.; Pacey, R. E. *Talanta* **1989**, *36*, 355.
(260) Iwamoto, K.; Araki, K.; Fujimoto, H.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1885.
(261) Kimura, K.; Iketani, S.-I.; Shono, T. *Anal. Chim. Acta* **1987**, *293*, 85. (a) Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696.
(262) Le Pecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.
(263) Waring, M. J. *J. Mol. Biol.* **1965**, *13*, 269.
(264) Strothkamp, K. G.; Strothkamp, R. E. *J. Chem. Educ.* **1994**, *71*, 77.
(265) For related macrocyclic cases, see; Cudic, P.; Zinc, M.; Skaric, V.; Kiralj, R.; Kojic-Prodic, B.; Vigneron, J.-P.; Lehn, J.-M. *Croat. Chim. Acta* **1996**, *69*, 569.
(266) Markovits, J.; Roques, B.-P.; Le Pecq, J.-B. *Anal. Biochem.* **1979**, *94*, 259.
(267) Barcellona, M. L.; Cardiel, G.; Gratton, E. *Biochem. Biophys. Res. Commun.* **1990**, *170*, 270.
(268) Lee, L. G.; Chen, C. H.; Chiu, L. A. *Cytometry* **1986,** *7*, 508.
(269) Quesada, M. A.; Rye, H. S.; Gingrich, J. C.; Glazer, A. N.; Mathies, R. A. *Biotechniques* **1991**, *10*, 616.
(270) Gaugain, B.; Barbet, J.; Capelle, N.; Roques, B.-P.; Le Pecq, J.-B. *Biochemistry* **1978**, *17*, 5078.
(271) Sandanayake, K. R. A. S.; Imazu, S.; James, T. D.; Mikami, M.; Shinkai, S. *Chem. Lett.* **1995**, 139.
(272) Takeuchi, M.; Mizuno, T.; Shinmori, H.; Nakashima, M.; Shinkai, S. *Tetrahedron* **1996**, *52*, 1195.
(273) Tolmachov, A. I.; Zaitsev, A. K.; Kosta, N.; Schuster, G. B. *J. Photochem. Photobiol. A: Chem.* **1994**, *77*, 237.
(274) Shionoya, M.; Furuta, H.; Lynch, V.; Harriman, A.; Sessler, J. L. *J. Am. Chem. Soc.* **1992**, *14*, 5714.
(275) Waggoner, A. S. *Annu. Rev. Biophys. Bioeng.* **1979**, *8*, 47.
(276) Hassner, A.; Birnbaum, D.; Loew, L. M. *J. Org. Chem.* **1984**, *49*, 2546.
(277) Loew, L. M.; Bonneville, G. W.; Surow, J. *Biochemistry* **1978**, *17*, 4065.
(278) Loew, L. M., *Pure Appl. Chem.* **1996**, *68*, 1405.
(279) Juris, A.; Balzani, V.; Barigelletti, F.; Campagna, S.; Belser, P.; von Zelewsky, A. *Coord. Chem. Rev.* **1988**, *84*, 85.
(280) Horvath, O.; Stevenson, K. L. *Charge Transfer Photochemistry of Coordination Compounds*; VCH: New York, 1993.
(281) Balzani, V.; Juris, A.; Venturi, M.; Campagna, S.; Serroni, S. *Chem. Rev.* **1996**, *96*, 759.
(282) Kalyanasundaram, K. *Photochemistry of Polypyridine and Porphyrin Complexes*; Academic: London, 1992.
(283) Roundhill, D. M. *Photochemistry and Photophysics of Metal Complexes*; Plenum: New York, 1994.
(284) Demas, J. N.; DeGraff, B. A. In ref 21; p 71.
(285) Hauenstein, B. L.; Dressick, W. J.; Buell, S. L.; Demas, J. N.; De Graff, B. A. *J. Am. Chem. Soc.* **1983**, *105*, 4251.
(286) MacQueen, D. B.; Schanze, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6108.
(287) Shen, Y.; Sullivan, B. P. *Inorg. Chem.* **1995**, *34*, 6235.
(288) Yoon, D. I.; BergBrennan, C. A.; Lu, H.; Hupp, J. J. *Inorg. Chem.* **1992**, *31*, 3192.
(289) Beer, P. D. *J. Chem. Soc., Chem. Commun.* **1996**, 689.
(290) Iyoda, T.; Morimoto, M.; Kawasaki, N.; Shimidzu, T. *J. Chem. Soc., Chem. Commun.* **1991**, 1480.
(291) Martensson, J.; Sandros, K.; Wennerstrom, O. *J. Phys. Org. Chem.* **1994**, *7*, 534.
(292) Fees, J.; Kaim, W.; Moscherosch, M.; Matheis, W.; Klima, J.; Krejcik, M.; Zalis, S. *Inorg. Chem.* **1993**, *32*, 166.
(293) Bolger, J.; Gourdon, A.; Ishow, E.; Launay, J. P. *J. Chem. Soc., Chem. Commun.* **1995**, 1799.
(294) Beer, P. D.; Kocian, O.; Mortimer, R. J.; Ridgway, C. *J. Chem. Soc., Chem. Commun.* **1991**, 1460.
(295) Dürr, H.; Schwarz, R.; Willner, I.; Joselevich, E.; Eichen, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 1338. See, also: Bossmann, S.; Dürr, H. *New J. Chem.* **1992**, *16*, 769. (a) Durham, B.; Casper, J. V.; Nagle, J. K.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4803.
(296) Balzani, V.; Sabbatini, N.; Scandola, F. *Chem. Rev.* **1986**, *86*, 319.
(297) Lay, P. A.; Sasse, W. H. F. *Inorg. Chem.* **1984**, *23*, 4123.
(298) Barigelletti, F.; Flamigni, L.; Guardigli, M.; Sauvage, J.-P.; Collin, J.-P.; Sour, A, *Chem. Commun.* **1996**, 1329.
(299) Benniston, A. C.; Grosshenny, V.; Harriman, A.; Ziessel, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1884.
(300) Constable, E. C.; Cargill Thompson, A. M. W.; Armaroli, N.; Balzani, V.; Maestri, M. *Polyhedron* **1992**, *11*, 2707.
(301) Fernando, S. R. L.; Maharoof, U. S. M.; Deshayes, K. D.; Kinstle, T. H.; Ogawa, M. Y. *J. Am. Chem. Soc.* **1996**, *118*, 5783.
(302) Fernando, S. R. L.; Ogawa, M. Y. *Chem. Commun.* **1996**, 637.
(303) Rampi, M. A.; Indelli, M. T.; Scandola, F.; Pina, F.; Parola, A. J. *Inorg. Chem.* **1996**, *35*, 3355.
(304) Dietrich, B. *Pure Appl. Chem.* **1993**, *65*, 1457.
(305) Beer, P. D.; Dent, S. W.; Wear, T. J *J. Chem. Soc., Dalton Trans.* **1996**, 2341.
(306) Beer, P. D.; Graydon, A. R.; Sutton, L. R. *Polyhedron* **1996**, *15*, 2457.
(307) Szemes, F.; Hesek, D.; Chen, Z.; Dent, S. W.; Drew, M. G. B.; Goulden, A. J.; Graydon, A. R.; Grieve, R.; Mortimer, R. J.; Wear, T.; Weightman, J. S.; Beer, P. D. *Inorg. Chem.* **1996**, *35*, 5868.
(308) Homsi, A.; Chambron, J.-C.; Sauvage, J.-P. *J. Chem. Soc., Dalton Trans.* **1990**, 1841.
(309) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809.
(310) Turro, N. J.; Barton, J. K.; Tomalia, D. A. *Acc. Chem. Res.* **1991**, *24*, 332. Compare with: Kirsch-de Mesmaeker, A.; Orellana, G.; Barton, J. K.; Turro, N. J. *Photochem. Photobiol.* **1990**, *52*, 461.
(311) Holmlin, R. E.; Barton, J. K. *Inorg. Chem.* **1995**, *34*, 7.
(312) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett.* **1991**, *182*, 603.
(313) Dressick, W. J.; Cline, J.; Demas, J. N.; deGraff, B. A. *J. Am. Chem. Soc.* **1986**, *108*, 7567. (a) Shreder, K.; Harriman, A.; Iverson, B. L. *J. Am. Chem. Soc.* **1995**, *117*, 2673.
(314) Liu, H. Q.; Peng, S.; Che, C. M. *J. Chem. Soc., Chem. Commun.* **1995**, 509.
(315) Liu, H. Q.; Chung, T. C.; Peng, S. M.; Che, C. M. *J. Chem. Soc., Chem. Commun.* **1995**, 1787.
(316) Heinselmann, K. S.; Hopkins, M. D. *J. Am. Chem. Soc.* **1995**, *117*, 12340.
(317) Lee, Y. F.; Kirchoff, J. R. *J. Am. Chem. Soc.* **1994**, *116*, 3599.
(318) Bandy, J. A.; Cloke, F. G. N.; Cooper, G.; Day, J. P.; Girling, R. B.; Graham, R. G.; Green, J. C.; Grinter, R.; Perutz, R. N. *J. Am. Chem. Soc.* **1988**, *110*, 5039.
(319) Demas, J. N.; Diemente, D.; Harris, E. W. *J. Am. Chem. Soc.* **1973**, *95*, 6864.
(320) Bacon, J. R.; Demas, J. N. *Anal. Chem.* **1987**, *59*, 2780.
(321) Wolfbeis, O. S.; Leiner, M. J. P.; Posch, H. E. *Mikrochim. Acta* **1986**, *3*, 359.
(322) Hartmann, P.; Leiner, M. J. P.; Lippitsch, M. E. *Sens. Actuators B* **1995**, *29*, 251.
(323) Lippitsch, M. E.; Pusterhofer, J.; Leiner, M. J. P.; Wolfbeis, O. S. *Anal. Chim. Acta* **1988**, *205*, 1.
(324) Grabowski, Z. R.; Rotkiewicz, K.; Siemiarczuk, A.; Cowley, D. J.; Baumann, W. *Nouv. J. Chim.* **1979**, *3*, 443.
(325) Grabowski, Z. R.; Dobkowski, J. *Pure Appl. Chem.* **1983**, *55*, 245.
(326) Grabowski, Z. R. *Pure Appl. Chem.* **1992**, *64*, 1249.
(327) Rettig, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 971.
(328) Kosower, E. M.; Huppert, D. *Annu. Rev. Phys. Chem.* **1986**, *37*, 127. (a) Bhattacharyya, K.; Chowdhury, M. *Chem. Rev.* **1993**, *93*, 507.
(329) Rettig, W. *Top. Curr. Chem.* **1994**, *169*, 253.
(330) Compare with: Uphadyay, A.; Bhatt, T.; Tripathy, H. B.; Pant, D. D. *J. Photochem. Photobiol. A Chem.* **1995**, *89*, 201.
(331) Kosower, E. M. *Acc. Chem. Res.* **1982**, *15*, 266.
(332) Weber, G.; Laurence, D. J. R. *Biochem. J.* **1954**, *31*, 56.
(333) Cowley, D. J. *Nature* **1986**, *319*, 14.
(334) Shi, Y.; Wang, D.; Zhang, Z. *J. Photochem. Photobiol. A Chem.* **1995**, *91*, 211.
(335) Kimura, K.; Smid, J. *Makromol. Chem. Rapid Commun.* **1981**, *2*, 235.
(336) Schneider, H.-J.; Ruf, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1159.
(337) Baldes, R.; Schneider, H.-J.; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 321.
(338) Cole, K. L.; Farran, M. A.; DeShayes, K. *Tetrahedron Lett.* **1992**, *33*, 599.
(339) Aoyama, Y.; Nagai, Y.; Otsuki, J.; Kabayashi, K.; Toi, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 745.
(340) Meech, S. R.; O'Connor, D. V.; Phillips, D. *J. Chem. Soc., Faraday Trans.* **1983**, *79*, 1563.
(341) Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1573.
(342) Tazuke, S.; Iwaya, Y.; Hayashi, R. *Photochem. Photobiol.* **1982**, *35*, 621.

(343) Cowley, D. J.; Pasha, I. *J. Chem. Soc., Perkin Trans. 2* **1981**, 918.
(344) Kumar, C. V.; Tolosa, L. M. *J. Chem. Soc., Chem. Commun.* **1993**, 722; *J. Photochem. Photobiol. A: Chem.* **1994**, *78*, 63.
(345) Loutfy, R. O. *Macromolecules*, **1981**, *14*, 270.
(346) Rettig, W.; Fritz, R.; Springer, J. In *Photochemical Processes in Organized Molecular Systems*; Honda, K., Ed.; Elsevier: Amsterdam, 1991; p 61.
(347) Rettig, W.; Lapouyade. R. In ref 21; p 109.
(348) Leezenberg, P. B.; Fayer, M. D.; Frank, C. W. *Pure Appl. Chem.* **1996**, *68*, 1381.
(349) Oster, G.; Nishijima, Y. *J. Am. Chem. Soc.* **1956**, *78*, 1581.
(350) Wandelt, B.; Turkewitsch, P.; Stranix, B. R.; Darling, G. D. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 4199.
(351) Abdel-Mottaleb, M. S. A.; Sherief, A. M. K.; Ismail, L. F. M.; De Schryer, F. C.; van der Auweraer, M. A. *J. Chem. Soc., Faraday Trans. 2* **1989**, *85*, 1779.
(352) Jacobsen, A.; Petric, A.; Hogenkamp, D.; Sinur, A.; Barrio, J. R. *J. Am. Chem. Soc.* **1996**, *118*, 5572.
(353) El Baraka, M.; Garcia, R.; Quinones, E. *J. Photochem. Photobiol. A Chem.* **1994**, *79*, 181.
(354) Chapman, C. F.; Liu, Y.; Sonek, J. G.; Tromberg, B. J. *Photochem. Photobiol.* **1995**, *62*, 416.
(355) de Silva, A. P.; Gunaratne, H. Q. N.; Jayasekera, K. R.; O'Callaghan, S.; Sandanayake, K. R. A. S. *Chem. Lett.* **1995**, 123.
(356) Parker, C. A. *Adv. Photochem.* **1964**, *2*, 305.
(357) Shizuka, H.; Ogiwara, T.; Kimura, E. *J. Phys. Chem.* **1985**, *89*, 4302.
(358) Vogel, M.; Rettig, W.; Sens, R.; Drexhage, K. H. *Chem. Phys. Lett.* **1988**, *147*, 452.
(359) Jiang, J.-B. *J. Photochem. Photobiol. A Chem.* **1994**, *78*, 205.
(360) Tsien, R. Y.; Minta, A. Eur. Patent 1989, 0 314 480 A2.
(361) Minta, A.; Kao, J. P. Y.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 8171.
(362) Jonker, S. A.; Ariese, F.; Verhoeven, J. W. *Rec. Trav. Chem. Pays Bas* **1989**, *108*, 109.
(363) Jonker, S. A.; Verhoeven, J. W.; Reiss, C. A.; Goubitz, K.; Heijdenrijk, D. *Rec. Trav. Chem. Pays Bas* **1990**, *109*, 154.
(364) Jonker, S. A.; Van Dijk, S. A.; Goubitz, K.; Reiss, C. A.; Schuddeboom, W.; Verhoeven, J. W. *Mol. Cryst. Liq. Cryst.* **1990**, *183*, 273.
(365) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; McCoy, C. P.; Sandanayake, K. R. A. S. *Top. Curr. Chem.* **1993**, *168*, 223.
(366) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053.
(367) Vallee, B.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543.
(368) Zalewski, P. D.; Forbes, I. J.; Betts, W. H. *Biochem. J.* **1993**, *296*, 403.
(369) Coyle, P.; Zalewski, P. D.; Philcox, J. C.; Forbes, I. J.; Ward, A. D.; Lincoln, S. F.; Mahadevan, I.; Rofe, A. M. *Biochem. J.* **1994**, *303*, 781.
(370) Hamasaki, K.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, F.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1993**, *115*, 5035.
(371) Hamasaki, K.; Ueno, A.; Toda, F. *J. Chem. Soc., Chem. Commun.* **1993**, 331.
(372) Cox, G. S.; Hauptmann, P. J.; Turro, N. J. *Photochem. Photobiol.* **1984**, *39*, 597.
(373) Nag, A.; Bhattacharya, K. *Chem. Phys. Lett.* **1988**, *151*, 474.
(374) Nag, A.; Bhattacharya, K. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 53.
(375) Ueno, A.; Shingo, M., Suzuki, I.; Fukushima, M.; Ohkubo, M.; Osa, T.; Hamada, F.; Murai, K. *Chem. Lett.* **1990**, 605.
(376) Nakamura, M.; Ikeda, A.; Ise, N.; Ikeda, T.; Ikeda, H.; Toda, F.; Ueno, A. *J. Chem. Soc., Chem. Commun.* **1995**, 721. (a) Wang, J.; Nakamura, M.; Hamasaki, K.; Ikeda, T.; Ikeda, H.; Ueno, A. *Chem. Lett.* **1996**, 303.
(377) Bender, C. J. *Chem. Soc. Rev.* **1986**, *15*, 475.
(378) Verhoeven, J. W. *Pure Appl. Chem.* **1990**, *62*, 1585.
(379) Paddon-Row, M. N. *Acc. Chem. Res.* **1994**, *27*, 18.
(380) Closs, G. L.; Miller, J. R. *Science* **1988**, *240*, 440.
(381) Pasman, P.; Rob, F.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1982**, *104*, 5127.
(382) Hermant, R. M.; Bakker, N. A. C.; Scherer, T.; Krijnen, B.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 1214.
(383) Greenfield, S. R.; Svec, W. A.; Gosztola, D.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 6767.
(384) Verhey, H. J.; Hofstraat, J. W.; Bekker, C. H. W.; Verhoeven, J. W. *New J. Chem.* **1996**, *20*, 809.
(385) Jenneskens, L. W.; Van Ramesdonk, H. J.; Verhey, H. J.; VanHouwelingen, G. D.B.; Verhoeven, J. W. *Rec. Trav. Chem. Pays Bas* **1989**, *108*, 453.
(386) van Ramesdonk, H. J.; Vos, M.; Verhoeven, J. W.; Mohlmann. G. R.; Tissink, N.A.; Meesen, A. W. *Polymer* **1987**, *28*, 951.
(387) Mes, G. F.; Van Ramesdonk, H. J.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1984**, *106*, 1335.
(388) Crosby, G. A. *Acc. Chem. Res.* **1975**, *8*, 231.
(389) Kasha, M. *Radiat. Res. Suppl.* **1960**, *2*, 243.
(390) Vo-Dinh, T. *Room Temperature Phosphorimetry for Chemical Analysis*; Wiley: New York, 1984.
(391) Hurtubise, R. J. *Phosphorimetry. Theory Instrumentation and Applications*; VCH: Cambridge, 1990.
(392) Kalyanasundaram, K.; Grieser, F.; Thomas, J. K. *Chem. Phys. Lett.* **1977**, *51*, 501.
(393) Bolt, J. D.; Turro, N. J. *Photochem. Photobiol.* **1982**, *35*, 305.
(394) Cline Love, L. J.; Habarta, J. G.; Dorsey. J. G. *Anal. Chem.* **1984**, *56*, 1132A.
(395) Turro, N. J.; Gratzel, M.; Braun, A. M. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 675.
(396) Turro, N. J.; Bolt, J. D.; Kuroda, Y.; Tabushi, I. *Photochem. Photobiol.* **1982**, *35*, 69.
(397) Turro, N. J.; Okubo, T.; Chung, C. *J. Am. Chem. Soc.* **1982**, *104*, 1789.
(398) Scypinski, S.; Cline Love, L. J. *Anal. Chem.* **1984**, *56*, 322.
(399) Scypinski, S.; Cline Love, L. J. *Anal. Chem.* **1984**, *56*, 331.
(400) Femia, R. A.; Cline Love, L. J. *J. Colloid Interface Sci.* **1985**, *108*, 271.
(401) Hamai, S. *J. Am. Chem. Soc.* **1989**, *111*, 3954.
(402) Munoz de la Pena, A.; Duran Meras, I.; Salinas, F.; Warner, I. M.; Ndou, T.T. *Anal. Chim. Acta* **1991**, *255*, 351.
(403) Turro, N. J.; Cox, G. S.; Li, X. *Photochem. Photobiol.* **1983**, *37*, 149.
(404) Monti, S.; Flamigni, L.; Martelli, A.; Bartolus, P. *J. Phys. Chem.* **1988**, *92*, 4447.
(405) Bohne, C.; Barra, M.; Boch, R.; Abuin, A. B.; Scaiano, J. C. *J. Photochem. Photobiol. A Chem.* **1992**, *65*, 249.
(406) Bortolus, P.; Grabner, G.; Kohler, G.; Monti, S. *Coord. Chem. Rev.* **1993**, *125*, 261.
(407) Rancke-Madsen, E. In ref 9; p 1.
(408) Shirai, M.; Tanaka, M. *J. Chem. Soc., Chem. Commun.* **1988**, 381.
(409) Ponce, A.; Wong, P. A.; Way, J. J.; Nocera, D. G. *J. Phys. Chem.* **1993**, *97*, 11137.
(410) Hartmann, W. K.; Gray, M. H. B.; Ponce, A.; Nocera, D. G.; Wong, P. A. *Inorg. Chim. Acta* **1996**, *243*, 239.
(411) *Photoinduced electron transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Parts A−D.
(412) *Photoinduced electron transfer. Parts I-V*; Mattay, J., Ed.; *Top. Curr. Chem.* **1990**, *156*, *158*; **1991**, *159*; **1992**, *163*; **1993**, *168*.
(413) *Electron transfer. Part I;* Mattay, J., Ed.; *Top. Curr. Chem.* **1994**, *169*.
(414) Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.
(415) Kavarnos, G. J. *Fundamentals of photoinduced electron transfer*; VCH; Weinheim: New York, 1993.
(416) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.
(417) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. R. *Adv. Supramol. Chem.* **1997**, *4*, in press.
(418) Czarnik, A. W. In ref 19; p 104.
(419) Czarnik, A. W. *Adv. Supramol. Chem.* **1993**, *3*, 131.
(420) Czarnik, A. W. In ref 21; p 49.
(421) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302.
(422) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, *24*, 197.
(423) de Santis, G.; di Casa, M.; Fabbrizzi, L.; Licchelli, M.; Mangano, C.; Pallavicini, P.; Perotti, A.; Poggi, A.; Sacchi, D.; Taglietti, A. In *Transition Metals in Supramolecular Chemistry*; Fabbrizzi, L., Poggi, A., Eds.; Kluwer: Dordrecht, 1994.
(424) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Sacchi, D.; Taglietti, A. *Analyst* **1996**, *121*, 1763.
(425) Tsien, R. Y. In ref 19; p 130.
(426) Kuhn, M. A. In ref 19; p 147.
(427) Desvergne, J.-P.; Fages, F.; Bouas-Laurent, H.; Marsau, P. *Pure Appl. Chem.* **1992**, *64*, 1231.
(428) Bouas-Laurent, H.; Desvergne, J.-P.; Fages, F.; Marsau, P. In ref 19; p 59.
(429) Valeur, B.; Bourson, J.; Pouget, J. In ref 19; p 25.
(430) James, T. D.; Linnane, P.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1996**, 281.
(431) Sandanayake, K. R. A. S.; James, T. D.; Shinkai, S. *Pure Appl. Chem.* **1996**, *68*, 1207.
(432) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Supramol. Chem.* **1995**, *6*, 141.
(433) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.
(434) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; McCoy, C.P.; Maguire, G. E. M.; Sandanayake, K. R. A. S. In ref 19; p 45.
(435) de Silva, A. P.; McCoy, C. P. *Chem. Ind.* **1994**, 992.
(436) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; McCoy, C. P.; Maxwell. P. R. S.; Rademacher, J. T.; Rice, T. E. *Pure Appl. Chem.* **1996**, *68*, 1443.
(437) de Silva, A. P.; Gunnlaugsson, T.; Rice, T. E. *Analyst* **1996**, *121*, 1759.
(438) de Silva, A. P.; Gunnlaugsson, T.; McCoy, C. P. *J. Chem. Educ.* **1997**, *74*, 53.
(439) Weller, A. *Pure Appl. Chem.* **1968**, *16*, 115.
(440) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.
(441) Jacques, P.; Allonas, X. *J. Photochem. Photobiol. A Chem.* **1994**, *78*, 1.
(442) Marcus, R. A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1111.

(443) Sutin, N.; Creutz, C. *J. Chem. Educ.* **1983**, *60*, 809.
(444) Grampp, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 691.
(445) Brimage, D. R. G.; Davidson, R. S. *J. Chem. Soc., Chem. Commun.* **1971**, 1385.
(446) Ide, R.; Sakata, Y.; Misumi, S.; Okada, T.; Mataga, N. *J. Chem. Soc., Chem. Commun.* **1972**, 1009.
(447) Sugimoto, A.; Sumida, R.; Tamai, N.; Inoue, H.; Otsuji, Y. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3500.
(448) Wang, H.; Zhang, B. W.; Cao, Y. *J. Photochem. Photobiol. A: Chem.* **1995**, *92*, 29.
(449) Bissell, R. A.; de Silva, A. P.; Fernando, W. T. M. L.; Patuwathavithana, S. T.; Samarasinghe, T. K. S. D. *Tetrahedron Lett.* **1991**, *32*, 425.
(450) Hancock, R. D.; Martell, A. E. *Chem. Rev.* **1989**, *89*, 1875.
(451) Delgado, M.; Gustowski, D. A.; Yoo, H. K.; Gatto, V. J.; Gokel, G. W.; Echegoyen, L. *J. Am. Chem. Soc.* **1988**, *110*, 119.
(452) Plenio, H.; Diodone, R. *Inorg. Chem.* **1995**, *34*, 3964.
(453) Wang, Y. C.; Morawetz, H. *J. Am. Chem. Soc.* **1976**, *98*, 3611.
(454) Shizuka, H.; Nakamura, M.; Morita, T. *J. Phys. Chem.* **1979**, *83*, 2019.
(455) Selinger, B. K. *Aust. J. Chem.* **1977**, *30*, 2087.
(456) Morawetz, H. *J. Lumin.* **1989**, *43*, 59.
(457) Beddard, G. S.; Davidson, R. S.; Whelan, T. D. *Chem. Phys. Lett.* **1977**, *56*, 54.
(458) Nakaya, T.; Tomomoto, T.; Imoto, M. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1551.
(459) Konopelski, J. P.; Kotzyba-Hibert, F.; Lehn, J.-M.; Desvergne, J.-P.; Fages, F.; Castellan, A.; Bouas-Laurent, H. *J. Chem. Soc., Chem. Commun.* **1985**, 433.
(460) Fages, F.; Desvergne, J.-P.; Bouas-Laurent, H.; Marsau, P.; Lehn, J.-M.; Kotzyba-Hibert, F.; Albrecht-Gary, A. M.; Al Joubbeh, M. *J. Am. Chem. Soc.* **1989**, *111*, 8672.
(461) Desvergne, J.-P.; Lauret, J.; Bouas-Laurent, H.; Marsau, P.; Lahrahar, N.; Andrianatoandro, H.; Cotrait, M. *Recl. Trav. Chim. Pays Bas* **1995**, *114*, 504.
(462) Desvergne, J.-P.; Rau, J.; Cherkaoui, O.; Zniber, R.; Bouas-Laurent, H.; Lahrahar, N.; Meyer, U.; Marsau, P. *New J. Chem.* **1996**, *20*, 881.
(463) Golchini, K.; Mackovic-Basic, M.; Gharib, S. A.; Masilamani, D.; Lucas, M.E.; Kurtz, I. *Am. J. Physiol.* **1990**, *258*, F438.
(464) Kastenholz, F.; Grell, E.; Bats, J. W.; Quinkert, G.; Brand, K.; Lanig, H.; Schneider, F. W. *J. Fluoresc.* **1994**, *4*, 243.
(465) de Silva, A. P.; Gunaratne, H. Q. N.; Sandanayake, K. R. A. S. *Tetrahedron Lett.* **1990**, *31*, 5193.
(466) de Silva, A. P.; de Silva, S. A. *J. Chem. Soc., Chem. Commun.* **1986**, 1709.
(467) Nishida, H.; Katayama, Y.; Katsuki, H.; Nakamura, H.; Tagaki, M.; Ueno, K. *Chem. Lett.* **1982**, 1853.
(468) Bourson, J.; Borrel, M. N.; Valeur, B. *Anal. Chim. Acta* **1992**, *257*, 189.
(469) Bourson, J.; Pouget, J.; Valeur, B. *J. Phys. Chem.* **1993**, *97*, 4552.
(470) Bourson, J.; Badaoui, F.; Valeur, B. *J. Fluoresc.* **1994**, *4*, 275.
(471) Alihodzic, S.; Zinic, M.; Klaic, B.; Kiralj, R.; Kojic-Prodic, B.; Herceg, M.; Cimerman, Z. *Tetrahedron Lett.* **1993**, *34*, 8345.
(472) Chae, M. Y.; Cherian, X. M.; Czarnik, A. W. *J. Org. Chem.* **1993**, *58*, 5797.
(473) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. *Chem. Commun.* **1996**, 1967.
(474) Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590.
(475) Parker, D.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1305.
(476) Beeby, A.; Parker, D.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1565. (a) Kubo, K.; Sakurai, T. *Chem. Lett.* **1996**, 959.
(477) Bernardo, M. A.; Parola, A. J.; Pina, F.; Garcia-Espana, E.; Marcelino, V.; Luis, S. V.; Miravet, J. F. *J. Chem. Soc., Dalton Trans.* **1995**, 993.
(478) Grigg, R.; Holmes, J. M.; Jones, S. K.; Norbert, W. D. J. A. *J. Chem. Soc., Chem. Commun.* **1994**, 185.
(479) de Silva, A. P.; Sandanayake, K. R. A. S. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1173.
(480) Ballardini, R.; Balzani, V.; Credi, A.; Gandolfi, M. T.; Kotzyba-Hibert, F.; Lehn, J.-M.; Prodi, L. *J. Am. Chem. Soc.* **1994**, *116*, 5741.
(481) de Silva, A. P.; Gunaratne, H. Q. N.; McVeigh, C.; Maguire, G. E. M.; Maxwell, P. R. S.; O'Hanlon, E. *Chem. Commun.* **1996**, 2191.
(482) de Silva, A. P.; Rupasinghe, R. A. D. D. *J. Chem. Soc., Chem. Commun.***1985**, 1669.
(483) Draxler, S.; Lippitsch, M. E. *Sens. Actuators B. Chem.* **1995**, *29*, 199.
(484) Draxler, S.; Lippitsch, M. E. *Appl. Opt.* **1996**, *35*, 4117.
(485) Goldenberg, M.; Emert, J.; Morawetz, H. *J. Am. Chem. Soc.* **1978**, *100*, 7171.
(486) de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Patty, A. L.; Spence, G. L. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1611.
(487) Grigg, R.; Norbert, W. D. J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 1298.
(488) Pardo, A.; Poyato, J. M. L.; Martin, E.; Camacho, J. J.; Reyman, D. *J. Lumin.* **1990**, *46*, 381.
(489) Beeson, J. C.; Huston, M. A.; Pollard, D. A.; Venkatachalam, T. K.; Czarnik, A. W. *J. Fluoresc.* **1993**, *3*, 65.
(490) Saeva, F.; Luss, H.; Martic, P. *J. Chem. Soc., Chem. Commun.* **1989**, 1477.
(491) Wallach, D. F. H.; Steck, D. L. *Anal. Chem.* **1963**, *35*, 1035.
(492) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 477.
(493) Grigg, R.; Norbert, W. D. J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 1300.
(494) Huston, M. E.; Haider, K. W.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 4460.
(495) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Taglietti, A. *Inorg. Chem.* **1996,** *35*, 1733.
(496) Tamiaki, H.; Maruyama, K. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2431.
(497) de Silva, A. P.; Gunaratne, H. Q. N. *J. Chem. Soc., Chem. Commun.* **1990**, 186.
(498) Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396.
(499) Gust D.; Moore T. A. *Top. Curr. Chem.* **1991**, *159*, 103.
(500) Gust, G.; Moore, T. A.; Moore, A. L. *Acc. Chem. Res.* **1993**, *26*, 198.
(501) de Silva, A. P.; Gunaratne, H. Q. N.; Kane, A. T. M.; Maguire, G. E. M. *Chem. Lett.* **1995**, 125.
(502) Levy, L. A.; Murphy, E.; Raju, B.; London, R. E. *Biochemistry* **1988**, *27*, 4041.
(503) de Silva, A. P.; Gunaratne, H. Q. N.; Maguire, G. E. M. *J. Chem. Soc., Chem. Commun.* **1994**, 1213.
(504) de Silva, A. P.; Sandanayake, K. R. A. S. *J. Chem. Soc., Chem. Commun.***1989**, 1183
(505) Huston, M. E.; Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1989**, *111*, 8735.
(506) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397.
(507) Czarnik, A. W. *ACS Symp. Ser.* **1994**, *561*, 314.
(508) Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.
(509) Nagai, Y.; Kabayashi, K.; Toi, H.; Aoyama, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2965.
(510) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2207.
(511) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. *Nature* **1995**, *374*, 345.
(512) Grady, T.; Harris, S. J.; Smyth, M. R.; Diamond, D.; Hailey, P. *Anal. Chem.* **1996**, *68*, 3775.
(513) Lorand, J. P.; Edwards, J. D. *J. Org. Chem.* **1959**, *24*, 769.
(514) Wulff, G. *Pure Appl. Chem.* **1982**, *54*, 2093.
(515) Linnane, P.; James, T. D.; Imazu, S.; Shinkai, S. *Tetrahedron Lett.* **1995**, *36*, 8833.
(516) Sandanayake, K. R. A. S.; James, T. D.; Shinkai, S. *Chem. Lett.* **1995**, 503.
(517) Aoki, I.; Sakaki, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 730.
(518) Inouye, M.; Hashimoto, J.; Isagawa, K. *J. Am. Chem. Soc.* **1994**, *116*, 5517.
(519) Dougherty, D. A. *Science* **1996**, *271*, 163.
(520) Parola, A. J.; Pina, F.; Maestri, M.; Armaroli, N.; Balzani, V. *J. Phys. Chem.* **1994**, *18*, 659.
(521) Voyer, N.; Lamothe, J. *Tetrahedron* **1995**, *51*, 9241.
(522) Hungerford, G.; Martinez-Insua, M.; Birch, D. J. S.; Moore, B. D. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 326.
(523) Morimoto, M.; Fukui, K.; Kawasaki, N.; Iyoda, T.; Shimidzu, T. *Tetrahedron Lett.* **1993**, *34*, 95.
(524) Brun, A. M.; Atherton, S. J.; Harriman, A.; Heitz, V.; Sauvage, J.-P. *J. Am. Chem. Soc.* **1992**, *114*, 4632.
(525) Harriman, A.; Sauvage, J.-P. *Chem. Soc. Rev.* **1996**, *25*, 41.
(526) Chae, M. Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 9704.
(527) Bystryak, I. M.; Likhtenshtein, G. L.; Kotelnikov, A. I.; Hankovskii, H. O.; Hideg, K. *Zh. Fiz. Khim.* **1986**, *60*, 2796.
(528) Blough, N. V.; Simpson, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 1915.
(529) de Silva, A. P.; de Silva, S. A.; Dissanayake, A. S.; Sandanayake, K. R. A. S. *J. Chem. Soc., Chem. Commun.***1989**, 1054.
(530) Sirish, M.; Maiya, B. G. *J. Photochem. Photobiol. A: Chem.* **1994**, *77*, 189.
(531) Lewis, F. D.; Burch, E. L. *J. Am. Chem. Soc.* **1994**, *116*, 1159.
(532) Lewis, F. D.; Burch, E. L. *J. Phys. Chem.* **1996**, *100*, 4055.
(533) Nozaki, K.; Ohno, T.; Haga, M. *J. Phys. Chem.* **1992**, *96*, 10860.
(534) Haga, M.; Ano, T.; Ishizaki, T.; Kano, K.; Nozaki, K.; Ohno, T. *J. Chem. Soc., Dalton Trans.* **1994**, 263. (a) Torrado, A.; Imperiali, B. *J. Org. Chem.* **1996**, *61*, 8940.
(535) D'Sousa, F. *J. Am. Chem. Soc.* **1996**, *118*, 923.
(536) Ballardini, R.; Balzani, V.; Credi, A.; Gandolfi, M. T.; Langford, S. J.; Menzer, S.; Prodi, L.; Stoddart, J. F.; Venturi, M.; Williams, D. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 978.
(537) Seiler, M.; Dürr, H.; Willner, I.; Joselevich, E.; Doron, A.; Stoddart, J.F. *J. Am. Chem. Soc.* **1994**, *116*, 3399. (a) Kimura, M.; Misawa, Y.; Yamaguchi, Y.; Hanabusa, K.; Shirai, H. *Chem. Commun.* **1996**, 2785.
(538) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 7017.
(539) Coudret, C.; Harriman, A. *J. Chem. Soc., Chem. Commun.* **1992**, 1755.
(540) Blacker, A. J.; Jazwinski, J.; Lehn, J.-M. *Helv. Chim. Acta* **1987**, *70*, 1.

(541) Blacker, A. J.; Jazwinski, J.; Lehn, J.-M.; Wilhelm, F. X. *J. Chem. Soc., Chem. Commun.* **1986**, 1035.
(542) Claude, S.; Lehn, J.-M.; Schmidt, F.; Vigneron, J.-P. *J. Chem. Soc., Chem. Commun.* **1991**, 1182.
(543) Van Arman, S.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 5376.
(544) Kumar, C. V.; Asuncion, E. H. *J. Am. Chem. Soc.* **1993**, *115*, 8547.
(545) Van Arman, S. A.; Czarnik, A. W. *Supramol. Chem.* **1993**, *1*, 99.
(546) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896.
(547) Harriman, A.; Kubo, Y.; Sessler, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 388.
(548) Sessler, J. L.; Wang, B.; Harriman, A. *J. Am. Chem. Soc.* **1993**, *115*, 10418.
(549) de Rege, P. J. F.; Williams, S. A.; Therien, M. J. *Science* **1995**, *269*, 1409.
(550) Turro, C.; Chang, C. K.; LeRoi, G. E.; Cukier, R. I.; Nocera, D. G. *J. Am. Chem. Soc.* **1992**, *114*, 4013.
(551) Bardez, E.; Chatelain, A.; Larrey, B.; Valeur, B. *J. Phys. Chem.* **1994**, *98*, 2357.
(552) Roberts, J. A.; Kirby, J. P.; Nocera, D. G. *J. Am. Chem. Soc.* **1995**, *117*, 8051.
(553) Arimura, T.; Brown, C. T.; Springs, S. L.; Sessler, J. L. *Chem. Commun.* **1996**, 2293.
(554) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025.
(555) Turro, N. J. *Pure Appl. Chem.* **1995**, *67*, 199.
(556) *The Photosynthetic Reaction Center*; Diesenhofer, J., Norris, J. R., Eds.; Academic Press: San Diego, 1993; Vols. I and II.
(557) Hayashi, T.; Miyahara, T.; Aoyama, Y.; Kobayashi, M.; Ogoshi, H. *Pure Appl. Chem.* **1994**, *66*, 797.
(558) Ogoshi, H.; Kuroda, Y.; Mizutani,T.; Hayashi, T. *Pure Appl. Chem.* **1996**, *68*, 1411.
(559) Hayashi, T.; Miyahara, T.; Aoyama, Y.; Nonoguchi, M.; Ogoshi, H. *Chem. Lett.* **1994**, 1749.
(560) Hunter, C. A.; Shannon, R. J. *Chem. Commun.* **1996**, 1361.
(561) Hunter, C. A.; Sanders, J. K. M.; Beddard, G. S.; Evans, S. *J. Chem. Soc., Chem. Commun.* **1989**, 1765.
(562) Hunter, C. A.; Hyde, R. K. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1936.
(563) De Santis, G.; Fabbrizzi, L.; Licchelli, M.; Poggi, A.; Taglietti, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 202.
(564) Sauvage, J.-P.; Collin, J.-P.; Chambron, J.-C.; Guillerez, S.; Coudret, C.; Balzani, V.; Barigelletti, F.; De Cola, L.; Flamigni, L. *Chem. Rev.*, **1994**, *94*, 993.
(565) Shinoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848.
(566) Rawle, S. C.; Moore, P.; Alcock, N. W. *J. Chem. Soc., Chem. Commun.* **1994**, 684.
(567) Kimura, E.; Bu, X.; Shionoya, M.; Wada, S.; Maruyama, S. *Inorg. Chem.* **1992**, *31*, 4542.
(568) Fabian, R. H.; Klassen, D. M.; Sonntag, R. W. *Inorg. Chem.* **1980**, *19*, 1977.
(569) Kimura, E.; Wada, S.; Shionoya, M.; Takahashi, T.; Iitaka, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 397.
(570) Fujita, E.; Milder, S. J.; Brunschwig, B. S. *Inorg. Chem.* **1992**, *31*, 2079.
(571) Lehn, J.-M.; Ziessel, R. *J. Chem. Soc., Chem. Commun.* **1987**, 1292.
(572) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Sacchi, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1975.
(573) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem. Eur. J.* **1996**, *2*, 75.
(574) Gubelmann, M.; Lehn, J.-M.; Sessler, J. L.; Harriman, A. *J. Chem. Soc. Chem. Commun.* **1988**, 77.
(575) Krishnan, V. *J. Photochem. Photobiol. A: Chem.* **1994**, *84*, 233.
(576) McGlynn, S. P.; Azumi,T.; Kinoshita, M. *Molecular Spectroscopy of the Triplet State*; Prentice Hall: Englewood Cliffs, NJ, 1969.
(577) Lytton, S. D.; Cabantchik, Z. I.; Libman, J.; Shanzer, A. *Mol. Pharmacol.* **1991**, *40*, 584.
(578) Lytton, S. D.; Mester, B.; Libman, J.; Shanzer, A.; Cabantchik, Z. I. *Anal. Biochem.* **1992**, *205*, 326.
(579) Shanzer, A.; Libman, J. *Croat. Chim. Acta.* **1996**, *69*, 709. (a) Weizman, H.; Ardon, O.; Mester, B.; Libman, J.; Dwir, O.; Hadar, Y.; Chen, Y.; Shanzer, A. *J. Am. Chem. Soc.* **1996**, *118*, 12368.
(580) Bodenant, B.; Fages, F. *Tetrahedron Lett.* **1995**, *36*, 1451.
(581) Fages, F.; Bodenant, B.; Weil, T. *J. Org. Chem.* **1996**, *61*, 3956.
(582) Chae, M. Y.; Czarnik, A. W. *J. Fluoresc.* **1992**, *2*, 225.
(583) Barrero, J. M.; Camara, C.; Perez-Conde, M. C.; San Jose, C.; Fernandez, L. *Analyst* **1995**, *120*, 431.
(584) De Santis, G.; Fabrizzi, L.; Licchelli, M.; Mangano, C.; Sacchi, D. *Inorg. Chem.* **1995**, *34*, 3581.
(585) Goulle, V.; Harriman, A.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 1034.
(586) Berthon, R. A.; Colbran, S. B.; Moran, G. M. *Inorg. Chim. Acta* **1993**, *204*, 3.
(587) Kratochvil, B.; Zatko, D. A. *Anal. Chem.* **1964**, *36*, 527.
(588) Daub, J.; Beck, M.; Knorr, A.; Spreitzer, H. *Pure Appl. Chem.* **1996**, *68*, 1399.
(589) Nakamura, A.; Okutsu, S.; Oda, Y.; Ueno, A.; Toda, F. *Tetrahedron Lett.* **1994**, *35*, 7241.
(590) Gonzalez, M. C.; McIntosh, A. R.; Bolton, J. R.; Weedon, A. C. *J. Chem. Soc., Chem. Commun.* **1984**, 1138.
(591) Nango, M.; Higuchi, M.; Gondo, H.; Hara, M. *J. Chem. Soc., Chem. Commun.* **1989**, 1550.
(592) Kuroda, Y.; Ito, M.; Sera, T.; Ogoshi, H. *J. Am. Chem. Soc.* **1993**, *115*, 7003.
(593) Deschenaux, R.; Harding, M. M.; Ruch, T. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1251.
(594) Hubbard, B. K.; Beilstein, L. A.; Heath, C. E.; Abelt, C. J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1005.
(595) Acquavella, M. F.; Evans, M. E.; Farraher, S. W.; Nevoret, C. J.; Abelt, C. J. *J. Chem. Soc., Perkin Trans. 2* **1995**, 385.
(596) Milbradt, R.; Weiss, J. *Tetrahedron Lett.* **1995**, *36*, 2999.
(597) Yoon, J.; Czarnik, A. W. *Bioorg. Med. Chem.* **1993**, *1*, 267.
(598) Wolfbeis, O. S.; Urbano, E. *Fresenius Z. Anal. Chem.* **1983**, *314*, 577.
(599) Krapf, R.; Illsley, N. P.; Tseng, H. C.; Verkman, A. S. *Anal. Biochem.* **1988**, *169*, 142.
(600) Biwersi, J.; Verkman, A. S. *Biochemistry* **1991**, *30*, 7779.
(601) Galoppini, E.; Fox, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 2299.
(602) Whitesell, J. K.; Chang, H. K.; Fox, M. A.; Galoppini, E.; Watkins, D. M.; Fox, H.; Hong, B. *Pure Appl. Chem.* **1996**, *68*, 1469.
(603) de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J.-L.; McCoy, C. P.; Rice, T. E.; Soumillion, J.-P. *Angew. Chem., Int. Ed. Engl.* **1995**, ***34***, 1728.
(604) Debreczeny, M. P.; Svec, W. A.; Wasielewski, M. R. *New J. Chem.* **1996**, *20*, 815.
(605) Ghosh, P.; Bharadwaj, P. K.; Mandal, S.; Ghosh, S. *J. Am. Chem. Soc.* **1996**, *118*, 1553.
(606) Chand, D. K.; Ghosh, P.; Shukla, R.; Sengupta, S.; Das, G.; Bandyopadhyay, P.; Bharadwaj, P. K. *Proc. Indian Acad. Sci. Chem. Sci.* **1996**, *108*, 229.
(607) de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E. *Angew. Chem., Int. Ed. Engl.* 1996, **35**, 2116.
(608) Matsumoto, H.; Ori, A.; Inokuchi, F.; Shinkai, S. *Chem. Lett.* **1996**, 301.
(609) Bissell, R. A.; de Silva, A. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1148.
(610) Beecroft, R. A.; Davidson, R. S.; Goodwin, D.; Pratt, J. E.; Luo, X. J. *J. Chem. Soc., Faraday Trans 2* **1986**, *82*, 2393.
(611) Ayadim, M.; Habib-Jiwan, J.-L.; de Silva, A. P.; Soumillion, J.-P. *Tetrahedron Lett.* **1996**, *37*, 7039.
(612) Klok, H.-A.; Moller, M. *Makromol. Chem. Phys.* **1996**, *197*, 1395.
(613) Shirai, M.; Ishimaru, S.; Tsunooka, M. *J. Fluoresc.* **1993**, *3*, 51.
(614) Bissell, R. A.; Bryan, A. J.; de Silva, A. P.; McCoy, C. P. *J. Chem. Soc., Chem. Commun.***1994**, 405.
(615) Leo, A. *Chem. Rev.* **1993**, *93*, 1281.
(616) Etter, E. F.; Kuhn, M. A.; Fay, F. S. *Biophys. J.* **1993**, *64*, A364.
(617) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Lynch, P. L. M. *New J. Chem.* **1996**, *20*, 871.
(618) Cain, C. C.; Murphy, R. F. *J. Cell. Biol.* **1988**, *106*, 269.
(619) de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *Nature* **1993**, *364*, 42.
(620) Iwata, S.; Tanaka, K. *J. Chem. Soc., Chem. Commun.***1995**, 1491.
(621) de Silva, A. P.; Gunaratne, H. Q. N.; McCoy, C. P. *Chem. Commun.* **1996**, 2399.
(622) Ryder, J. D. *Engineering electronics*, 2nd ed.; McGraw-Hill: New York, 1967. (a) de Silva, S. A.; Zavaleta, A.; Baron, D. E.; Allam, O.; Isidor, E. V.; Kashimura, N.; Percarpio, J. M. *Tetrahedron Lett.* **1997**, *38*, 2237.
(623) Schulman, S. G.; Threatte, R.; Capomacchia, A.; Paul, W. *J. Pharm. Sci.* **1974**, *63*, 876.
(624) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587.
(625) Winnik, M. A. *Chem. Rev.* **1981**, *81*, 491.
(626) Zachariasse, K. A.; Vaz, W. L. C.; Sotomayor, C.; Kuhnle,W. *Biophys. Biochim. Acta* **1982**, *688*, 323.
(627) Wang, Y. C.; Morawetz, H. *J. Am. Chem. Soc.* **1976**, *98*, 3611.
(628) Snare, M. J.; Thistlethwaite, P. J.; Ghiggino, K. P. *J. Am. Chem. Soc.* **1983**, *105*, 3328.
(629) Chang, C.-H.; Webber, S. E. *J. Lumin.* **1989**, *44*, 113.
(630) Lee, H. C.; Forte, J. G.; Epel, D. In *Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions*; Nuccitelli, R., Deamer, D. W., Eds.; Liss: New York, 1982; p 136.
(631) Jones, G. R.; Oliviera, M. E.; Cundall, R. B. *Photochem. Photobiol.* **1990**, *52*, 451.
(632) Hong, S. Y.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 3330.
(633) Shanzer, A.; Samuel. D.; Korenstein, R. *J. Am. Chem. Soc.* **1983**, *105*, 3815.
(634) Okahata, Y. *Acc. Chem. Res.* **1986**, *19*, 3815.
(635) Fyles, T. M.; Zeng, B. *Chem. Commun.* **1996**, 2295.
(636) Ebata, H.; Masuko, M.; Ohtani, H.; Kashiwasake-Jibu, M. *Photochem. Photobiol.* **1995**, *62*, 836.
(637) Ueno, A.; Suzuki, I.; Osa, T. *J. Chem. Soc., Chem. Commun.* **1988**, 1373; *J. Am. Chem. Soc.* **1989**, *111*, 6391.
(638) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Tetrahedron* **1987**, *43*, 1571.

(639) Kano, K.; Takenoshita, I.; Ogawa, T. *Chem. Lett.* **1982**, 321.
(640) Tamaki, T. *Chem. Lett.* **1984**, 53.
(641) Kano, K.; Matsumoto, H.; Hashimoto, S.; Sisido, M.; Imanishi, Y. J. *Am. Chem. Soc.* **1985**, *107*, 6117.
(642) Sasaki, D. Y.; Shnak, D. R.; Pack, D. W.; Arnold, F. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 905.
(643) Agbaria, R. A.; Gill, D. *J. Phys. Chem.* **1988**, *92*, 1052.
(644) Agbaria, R. A.; Gill, D. *J. Photochem. Photobiol. A Chem.* **1994**, *78*, 161.
(645) Harada, A.; Li, J.; Kamachi, M. *Nature*, **1992**, *356*, 325.
(646) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803.
(647) Steinbrunn, M. B.; Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2139.
(648) Emert, J.; Kodali, D.; Catena, R, *J. Chem. Soc., Chem. Commun.* **1981**, 758.
(649) Turro, N. J.; Okubo, T.; Weed, G. C. *Photochem. Photobiol.* **1982**, *35*, 325.
(650) Diederich, F. *Cyclophanes*; Royal Society of Chemistry: Cambridge, 1991, p 113.
(651) Kakizawa, Y.; Akita, T.; Nakamura, H. *Chem. Lett.* **1993**, 1671. (a) Becker, H.-D. *Chem. Rev.* **1993**, *93*, 145. (b) Sclafani, J. A.; Maranto, M. T.; Sisk, T. M.; Van Arman, S. A. *Tetrahedron Lett.* **1996**, *37*, 2193.
(652) Kawakami, J.; Komai, Y.; Ito, S. *Chem. Lett.* **1996**, 617.
(653) Tung, C. H.; Wu, L. Z. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 1381.
(654) Bouas-Laurent, H.; Castellan, A.; Daney, M.; Desvergne, J.-P.; Guinand, G.; Marsau, P.; Riffaud, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 315.
(655) Marquis, D.; Desvergne, J.-P. *Chem. Phys. Lett.* **1994**, *230*, 131.
(656) Marquis, D.; Desvergne, J.-P.; Bouas-Laurent, H. *J. Org. Chem.* **1995**, *60*, 7984.
(657) Fages, F.; Desvergne, J.-P.; Kampke, K.; Bouas-Laurent, H,; Lehn, J.-M.; Konopelski, J.-P.; Marsau. P.; Barrans, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 655.
(658) Fages, F.; Desvergne, J.-P.; Bouas-Laurent, H.; Lehn, J.-M.; Barrans, Y.; Marsau, P.; Meyer, M.; Albrecht-Gary, A.-M. *J. Org. Chem.* **1994**, *59*, 5264.
(659) Fages, F.; Desvergne, J.-P.; Kampke, K.; Bouas-Laurent, H,; Lehn, J.-M.; Meyer, M.; Albrecht-Gary, A.-M. *J. Am. Chem. Soc.* **1993**, *115*, 3658.
(660) Teulade-Fichou, M.-P; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2169.
(661) Dhaenens, M.; Lehn, J.-M.; Vigneron, J.-P. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1379.
(662) Jin, T.; Ichikawa, K.; Koyama, T. *J. Chem. Soc., Chem. Commun.* **1992**, 499.
(663) Aoki, I.; Sakaki, T.; Tsutsui, S.; Shinkai, S. *Tetrahedron Lett.* **1992**, *33*, 89.
(664) Perez-Jimenez, C.; Harris, S. J.; Diamond, D. *J. Chem. Soc., Chem. Commun.* **1993**, 480.
(665) Perez-Jimenez, C.; Harris, S. J.; Diamond, D. *J. Mater. Chem.* **1994**, *4*, 145.
(666) Diamond, D.; McKervey, M. A. *Chem. Soc. Rev.* **1996**, *25*, 15.
(667) Aoki, I.; Kawabata, H.; Nakashima, K.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1991**, 1771.
(668) Matsumoto, H.; Shinkai, S. *Tetrahedron Lett.* **1996**, *37*, 77.
(669) Aoki, I.; Harada, T.; Sakaki, T.; Kawahara, Y.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 1341.
(670) Aoki, I.; Kawahara, Y.; Sakaki, T.; Harada, T.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 927.
(671) Takeshita, M.; Shinkai, S. *Chem. Lett.* **1994**, 41.
(672) Takeshita, M.; Shinkai, S. *Chem. Lett.* **1994**, 125.
(673) Broan, C. J. *Chem. Commun.* **1996**, 699.
(674) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 465.
(675) Ueno, A. *Adv. Mater.* **1993**, *5*, 132.
(676) Ueno, A. *ACS Symp. Ser.* **1993**, *538*, 74.
(677) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *J. Am. Chem. Soc.* **1988**, *110*, 4323.
(678) Saika, T.; Iyoda, T.; Honda, K.; Shimidzu, T. *J. Chem. Soc., Chem. Commun.* **1992**, 591.
(679) Zweig, A. *Pure Appl. Chem.* **1973**, *33*, 389.
(680) Turro, N. J.; Ramamurthy, V.; Cherry, W.; Farneth, W. *Chem. Rev.* **1978**, *78*, 125.
(681) De Schryver, F. C.; Boens, N.; Put, J. *Adv. Photochem.* **1977**, *10*, 359.
(682) Gordon, M.; Ware, W. R. *The Exciplex*; Academic: New York, 1975.
(683) Itoh, M.; Kujiwara, Y. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2261.
(684) Cox, G. S.; Turro, N. J.; Yang, N. C.; Chen, M. J. *J. Am. Chem. Soc.* **1984**, *106*, 422.
(685) Benson, D. R.; Fu, J. *Tetrahedron Lett.* **1996**, *37*, 4833.
(686) Tanimoto, Y.; Hasegawa, K.; Okada, N.; Itoh, M.; Iwai, K.; Sugioka, K.; Takemura, F.; Nakagaki, R.; Nagakura, S. *J. Phys. Chem.* **1989**, *93*, 3586.
(687) Cao, H.; Miyata, K.; Tamura, T.; Fujiwara, Y.; Tanimoto, Y.; Okazaki, M.; Iwai, K.; Yamamoto, M. *Chem. Phys. Lett.* **1995**, *246*, 171.
(688) Murakami, H.; Nagasaki, T.; Hamachi, I.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **1994**, 975.
(689) Filipescu, N. In *Molecular Luminescence*; Lim, E. C., Ed.; Benjamin: 1969; p 697.
(690) Valeur, B. In *Fluorescent Biomolecules: Methodologies and Applications*; Jameson, D. M., Reinhart, G. D., Eds.; Plenum: New York, 1989; p 269. (a) Speiser, S. *Chem. Rev.* **1996**, *96*, 1953.
(691) Chardon-Noblat, S.; Sauvage, J.-P.; Mathis, P. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 593.
(692) Vögtle, F.; Frank, M.; Nieger, M.; Belser, P.; von Zelewsky, A.; Balzani, V.; Barigelletti, F.; De Cola, L.; Flamigni, L. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1643.
(693) Barigelletti, F.; Flamigni, L.; Balzani, V.; Collin, J.-P.; Sauvage, J.-P.; Sour, A.; Constable, E. C.; Cargill Thompson, A. M. W. *J. Chem. Soc., Chem. Commun.* **1993**, 942.
(694) DeCola, L.; Balzani. V.; Barigelletti, F.; Flamigni, L.; Belser, P.; von Zelewsky, A.; Frank, M.; Vögtle, F. *Inorg. Chem.* **1993**, *32*, 5228.
(695) Wagner, R. W.; Lindsey, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 9759.
(696) Grosshenny, V.; Harriman, A.; Ziessel, R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1100.
(697) Harriman, A.; Sauvage, J.-P. *Chem. Soc. Rev.* **1996**, *25*, 41.
(698) Ward, M. D. *Chem. Soc. Rev.* **1995**, *24*, 121.
(699) Belser, P. *Chimia* **1994**, *48*, 347.
(700) Harriman, A.; Ziessel, R. *Chem. Commun.* **1996**, 1707.
(701) Wagner, R. W.; Lindsey, J. S. *Pure Appl. Chem.* **1996**, *68*, 1373.
(702) Anderson, S.; Anderson, H. L.; Bashall, A.; McPartlin. M.; Sanders, J. K. M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1096. (a) Prathapan, S.; Johnson, T. E.; Lindsey, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 7519.
(703) Scandola, F.; Balzani, V. *J. Chem. Educ.* **1983**, *60*, 814.
(704) Scholes, G. D.; Ghiggino, K. P. *J. Photochem. Photobiol. A. Chem.* **1994**, *80*, 355.
(705) Jordan, D. M.; Walt, D. R.; Milanovich, F. P. *Anal. Chem.* **1987**, *59*, 437.
(706) Yuan, P.; Walt, D. R. *J. Fluoresc.* **1992**, *2*, 231.
(707) Valeur, B.; Bourson, J.; Pouget, J.; Kaschke, M.; Ernsting, N. P. J. *Phys. Chem.* **1992**, *96*, 6545.
(708) Valeur, B.; Bourson, J.; Pouget, J. *J. Lumin.* **1992**, *52*, 345.
(709) Godwin, H. A.; Berg, J. M. *J. Am. Chem. Soc.* **1996**, *118*, 6514.
(710) Tecilla, P.; Dixon, R. P.; Slobodkin, G.; Alavi, D. S.; Waldeck, D. H.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 9408.
(711) Montalti, M.; Ballardini, R.; Prodi, L.; Balzani, V. *Chem. Commun.* **1996**, 2011. (a) Sessler, J. R.; Wang, B.; Harriman, A. *J. Am. Chem. Soc.* **1995**, *117*, 704.
(712) Gravett, D. M.; Guillet, J. E. *J. Am. Chem. Soc.* **1993**, *115*, 5970.
(713) Jullien, L.; Canceill, J.; Valeur, B.; Bardez, E.; Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2488.
(714) McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornethwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W., J.-M. *Nature* **1995**, *374*, 517.
(715) Berberan-Santos, M. N.; Canceill, J.; Brochon, J.-C.; Jullien, L.; Lehn, J.-M.; Pouget, J.; Tauc, P.; Valeur, B. *J. Am. Chem. Soc.* **1992**, *114*, 6427.
(716) Kumar, C. V.; Asuncion, E. H. *J. Chem. Soc., Chem. Commun.* **1992**, 470.
(717) Thornton, N. B.; Schanze, K. S. *New J. Chem.* **1996**, *20*, 791.
(718) Shinozuka, K.; Seto, Y.; Sawai, H. *J. Chem. Soc., Chem. Commun.* **1994**, 1377.
(719) Stryer, L. *Annu. Rev. Biochem.* **1978**, *47*, 819.
(720) Meares, C. F.; Wensel, T. G. *Acc. Chem. Res.* **1984**, *17*, 202.
(721) Adams, S. R.; Harootunian, A.; Buechler, Y. J.; Taylor, S. S.; Tsien, R. Y. *Nature* **1991**, *349*, 694. (a) DeBernardi, M. A.; Brooker, G. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4577.
(722) Wei, A.-P.; Blumenthal, D. K.; Herron, J. N. *Anal. Chem.* **1994**, *66*, 1500.
(723) Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. W. *Science* **1990**, *247*, 954.
(724) Kraft, G. A.; Wang, G. T. In ref 19; p 183.
(725) Wagner, R. W.; Lindsey, J. S.; Seth, T.; Palaniappan, V.; Bocian, D. F. *J. Am. Chem. Soc.* **1996**, *118*, 3996.
(726) Walz, J.; Ulrich, K.; Port, H.; Wolf, H. C.; Wonner, J.; Effenberger, F. *Chem. Phys. Lett.* **1993**, *213*, 321.
(727) *Photochromism. Molecules and Systems*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990.
(728) Minami, T.; Tamai, N.; Yamazaki, T.; Yamazaki, I. *J. Phys. Chem.* **1991**, *95*, 3988.
(729) Yamazaki, I.; Ohta, N. *Pure Appl. Chem.* **1995**, *67*, 209.
(730) Spreitzer, H.; Daub, J. *Liebigs. Ann.* **1995**, 1637.
(731) Tsvigoulis, G. M.; Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1119.
(732) Irie, M. *Pure Appl. Chem.* **1996**, *68*, 1367.
(733) Bonfantini, E. E.; Officer, D. L. *J. Chem. Soc., Chem. Commun.* **1994**, 1445.
(734) Belser, P.; Dux, R.; Baak, M.; De Cola, L.; Balzani, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 595.
(735) De Cola, L.; Balzani, V.; Belser, P.; Dux, R.; Baak, M. *Supramol. Chem.* **1995**, *5*, 297.
(736) Weinheimer, C.; Choi, Y.; Caldwell, T.; Gresham, P.; Olmsted, J. *J. Photochem. Photobiol. A: Chem.* **1994**, *78*, 119.

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