DOI: 10.1002/ejic.201100469

Recent Exploitation of Luminescent Rhenium(I) Tricarbonyl Polypyridine Complexes as Biomolecular and Cellular Probes

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Keywords: Labels / Luminescence / N ligands / Probes / Rhenium

The interesting emission properties of rhenium(I) tricarbonyl polypyridine complexes have been exploited in the development of various sensors and probes for analytes. Luminescent probes targeting biomolecules have also been developed. Additionally, there has been a fast-growing interest in the cellular uptake properties of these complexes with a focus on their potential as cellular imaging reagents. In this Microreview, we describe the fundamental emission characteristics of luminescent rhenium(I) tricarbonyl polypyridine complexes and explain why they hold promise for use as luminescent sensors. Additionally, we summarize the recent design of these complexes as biomolecular and cellular probes, with an emphasis on studies of their structure–property relationships, bioconjugation, biomolecular binding, cellular uptake, cytotoxicity, and bioimaging applications.

1. Introduction

Common rhenium(I) tricarbonyl polypyridine complexes exhibit rich photophysical and photochemical properties.[1–14] In particular, many complexes display intense and long-lived emission in the visible region upon photoexcitation. This emission property has been exploited in the development of various sensors and probes for analytes such as oxygen,[15–17] protons,[18,19] and ions.[20–25] Additionally, luminescent probes targeting biomolecules such as nucleosides,[26] nucleic acids,[27–35] amino acids,[36] peptides,[37,38] and proteins[32,39–52] have been developed. In the past few years, there has also been a fast-growing interest in the cellular uptake properties of luminescent transition metal

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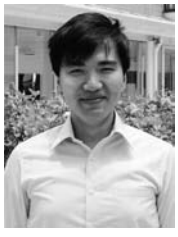
Kenneth Lo obtained his PhD degree from The University of Hong Kong in 1997. From 1997 to 1999, he worked as a Croucher Foundation Postdoctoral Research Fellow in the Inorganic Chemistry Laboratory, University of Oxford. He joined the Department of Biology and Chemistry of the City University of Hong Kong as Assistant Professor in 1999 and became Associate Professor (Scale B) in 2003, Associate Professor (Scale A) in 2007, and Professor in 2011. He received The APA Prize for Young Scientists from the Asian and Oceanian Photochemistry Association in 2005 and The Distinguished Lectureship Award from the Chemical Society of Japan in 2011. The main theme of his research work is the utilization of luminescent transition metal complexes as biomolecular and cellular probes.

Kenneth Zhang was born in Jiangsu Province, P. R. China, in 1982. He graduated with a first class honors BSc degree from the City University of Hong Kong in 2005. He obtained his MPhil and PhD degrees under the supervision of Prof. Kenneth Lo in 2007 and 2010, respectively. His research projects were on luminescent cyclometalated iridium(III) estradiol and biotin complexes as biological probes. He is currently working as a Senior Research Assistant in Prof. Lo's research group.



Steve Li was born in Hong Kong, P. R. China, in 1984. He obtained his BSc degree from the City University of Hong Kong in 2009 and is currently an MPhil student under the supervision of Prof. Kenneth Lo. His project focuses on the design of luminescent iridium(III) PEG and PEI complexes as biocompatible labeling reagents and gene delivery agents.

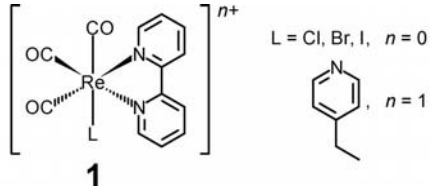
complexes with a focus on their potential as cellular imaging reagents.[25b,28,29,37,38,51–62] Luminescent rhenium(I) tricarbonyl polypyridine complexes are among the most extensively studied systems.[25b,28,29,37,38,51–57] In this Microreview, we describe the fundamental emission characteristics of luminescent rhenium(I) tricarbonyl polypyridine complexes and explain why they hold promise for use as luminescent sensors. Additionally, we summarize the recent design of these complexes as biomolecular and cellular probes, with an emphasis on studies of their structure–property relationships, bioconjugation, biomolecular binding, cellular uptake, cytotoxicity, and bioimaging applications.

2. Emission Characteristics of Rhenium(I) Tricarbonyl Polypyridine Complexes

The spectroscopic and photophysical properties of rhenium(I) tricarbonyl polypyridine complexes have attracted considerable interest since their emission behavior was reported in the mid-1970s.[7] Complexes of the general formula $[Re(N^\wedge N)(CO)_3(L)]$ (N^N = diimine ligands, L = monodentate ligands) show intense spin-allowed intraligand (^{1}IL) absorption bands in the UV region and spin-allowed broad metal-to-ligand charge-transfer (^{1}MLCT) bands in the visible region with lower intensity. Similar to many other transition metal polypyridine complexes, the ^{1}MLCT bands of these complexes display solvatochromism and occur at higher energy with increasing solvent polarity. Upon irradiation, these complexes show intense and long-lived emission in the visible region. In most cases, the emission has been assigned to a ^{3}MLCT [$d\pi(Re)\rightarrow\pi^*(N^\wedge N)$] excited state. The emission characteristics of these complexes are very sensitive to the polarity of the solvents; for example, the emission occurs in lower energy with a shorter lifetime in more polar solvents. The exponential dependence of the nonradiative decay rate constants (k_{nr}) on the emission energy is described by the energy gap law; for example, the k_{nr} values of the complexes $[Re(N^\wedge N)(CO)_3(L)]^+$ are dependent on the emission energy while the radiative decay rate constants (k_r) remain relatively constant.[8] Thus, it is possible to tune the emission energy and lifetime of these complexes by modifying the σ-donating and π-accepting properties of the ligands N^N and L. Also, the emission of luminescent rhenium(I) tricarbonyl polypyridine complexes is very sensitive to the rigidity of their local environments.[2,4,7] In a rigid matrix, stabilization of the dipole moment of the excited complex by reorientation of the surrounding solvent molecules becomes impossible. Thus, upon increasing the rigidity of the environment, the emission of these complexes is shifted to higher energy with significant enhancement in intensity. This interesting phenomenon has been applied to the design of luminescent sensors for rigidity; for example, the ^{3}MLCT emitter [$Re(Ph_2$-phen)$(CO)_3Cl$] has been used to monitor the acrylate polymerization process.[9]

The excited-state nature of rhenium(I) tricarbonyl polypyridine complexes is not strictly limited to ^{3}MLCT; for example, triplet halide-to-ligand charge-transfer ^{3}XLCT and ligand-to-ligand charge transfer ^{3}LLCT excited states have been reported, and the charge-transfer character renders the properties of these emissive states similar to those of ^{3}MLCT.[3] Also, mixing of triplet intraligand (^{3}IL) ($\pi\rightarrow\pi^*$) (N^N or L) character in the ^{3}MLCT emissive state is not uncommon for luminescent rhenium(I) tricarbonyl polypyridine complexes.[10–12] In fact, the presence of both ^{3}MLCT and ^{3}IL states can be detected by many experimental approaches. Evaluation of the radiative decay rate constant ($k_r = \Phi_{em}/\tau_{em}$) is a simply and widely applicable method, as the k_r values for typical ^{3}MLCT emitters are approximately $8 \pm 4 \times 10^4$ s^{-1}. In other words, experimental values of k_r which are substantially smaller than 10^4 s^{-1} suggest the involvement of ^{3}IL character in the emissive states.[10] Thus, the existence of ^{3}IL character in the emission is generally reflected by low emission quantum yields and/or extraordinarily long emission lifetimes, and in many cases the observation of structured emission spectra in fluid solutions at room temperature. For example, the temperature-dependent emission properties of the complex $[Re(Me_4\text{-phen})(CO)_3(py)]^+$ are very different to those of its structural analogues, which has been attributed to a ^{3}IL state that is slightly higher in energy than the ^{3}MLCT emissive state.[11] Also, ^{3}IL excited states are common in rhenium(I) complexes coordinated with diimine ligands with an extended delocalized π system; examples include dipyridophenazine (dppz) and its derivatives.[12,30–32]

The properties of the ^{1}MLCT excited state of rhenium(I) tricarbonyl polypyridine complexes have been studied in the picosecond and femtosecond timescales.[13] Analysis of data obtained from time-resolved emission spectra of the complexes $[Re(bpy)(CO)_3(L)]^{n+}$ (L = Cl, Br, I, n = 0; L = py-et, n = 1) (**1**) reveals three spectral components, namely the decay from the initially excited singlet state ^{1}MLCT, a higher triplet state ^{3}IL, and the lowest triplet state ^{3}MLCT.[13a,13b]



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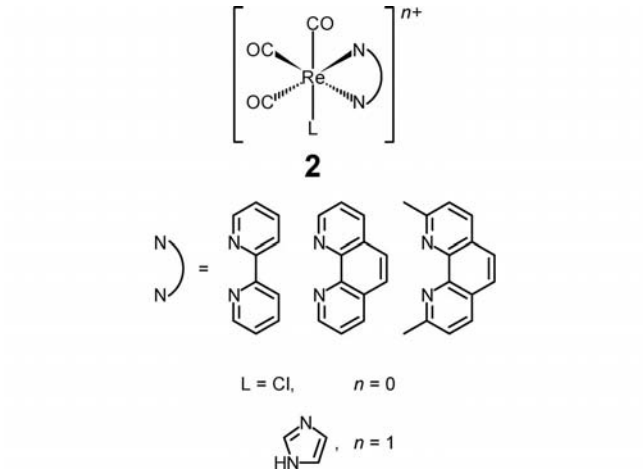
The lifetimes of the first two components (τ_1 = 85–150 fs, τ_2 = 0.34–1.2 ps) correspond to the times for the intersystem crossing from singlet to triplet states and the internal conversion from ^{3}IL to ^{3}MLCT states, respectively. The intersystem crossing rates are solvent-independent, but the internal conversion is slower in DMF than in CH_3CN, as a result of different polarities of the two triplet states and selective stabilization of ^{3}MLCT in the course of solvent dielectric relaxation. The effects of diimine ligands on the ultrafast excited state dynamics of the complexes $[Re(N^\wedge N)(CO)_3(L)]^{n+}$ (N^N = bpy, phen, Me_2-phen; L = Cl, n = 0; L = imidazole, n = 1) (**2**) have been investigated.[13c]



The femtosecond intersystem crossing is essentially independent on the diimine ligands, and is faster for Cl complexes (ca. 100 fs) than the imidazole complexes (ca. 150 fs) (Figure 1). Internal conversion simultaneously results in two triplet states that reach equilibrium within 1 to 3 ps. Analysis of the time-resolved infrared spectra of the complexes [Re(N^N)(CO)$_3$(Cl)] reveals decreasing MLCT character (bpy > phen > Me$_2$-phen) in their lowest triplet states.

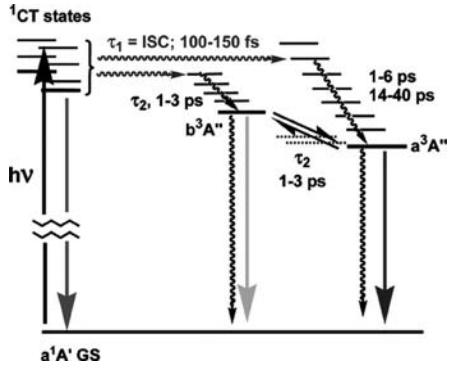
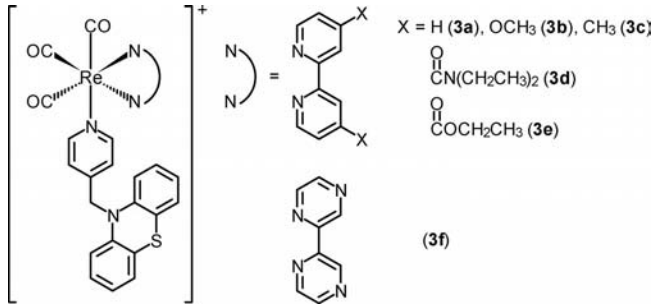
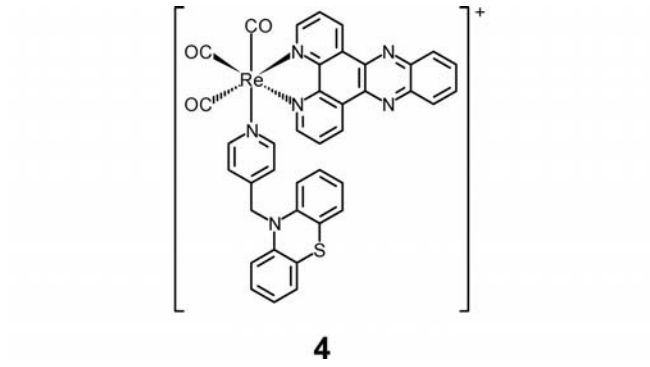


Figure 1. Excited-state relaxation mechanism of [Re(N^N)(CO)$_3$(L)]$^{n+}$ complexes.

In addition to the emission behavior, the photoredox properties of luminescent rhenium(I) tricarbonyl polypyridine complexes have been extensively investigated.[14] The photophysical properties of [Re(bpy)(CO)$_3$(py-PTZ)]$^+$ (**3a**) containing a reductive quencher PTZ (phenothiazine) are essentially the same as those of its PTZ-free analogue [Re(bpy)(CO)$_3$(py-et)]$^+$ at 77 K in a 4:1 (v/v) EtOH/MeOH glass, but are profoundly different in fluid solutions.[14a] The PTZ complex displays a reduced emission quantum yield and shorter lifetime due to reductive quenching. The transient absorption spectrum of this complex shows an intense absorption feature at approximately 500 nm, which has been ascribed to the charge-separated state [Re(bpy$^-$)(CO)$_3$(py-PTZ$^+$)]$^+$. Similar intramolecular electron transfer from the py-PTZ ligand to the rhenium center has also been observed in the complexes [Re(X$_2$-bpy)(CO)$_3$(py-PTZ)]$^+$ [X = OCH$_3$ (**3b**), CH$_3$ (**3c**), CONEt$_2$ (**3d**), COOEt (**3e**)] and [Re(bpz)(CO)$_3$(py-PTZ)]$^+$ (**3f**).[14a] The excited complexes return to their ground states by intramolecular back-electron-transfer processes that have rate constants decreasing logarithmically with $-\Delta G°$. Interestingly, the excited state of the complex [Re(dppz)(CO)$_3$(py-PTZ)]$^+$ (**4**) undergoes both electron and energy transfer processes, resulting in the formation of the charge-separated state [Re(dppz$^-$)(CO)$_3$(py-PTZ$^+$)]$^+$ and the dppz-localized ^{3}IL state, respectively (Figure 2).[14b] The competition ratio for the electron and energy transfer processes is approximately 2.4:1.





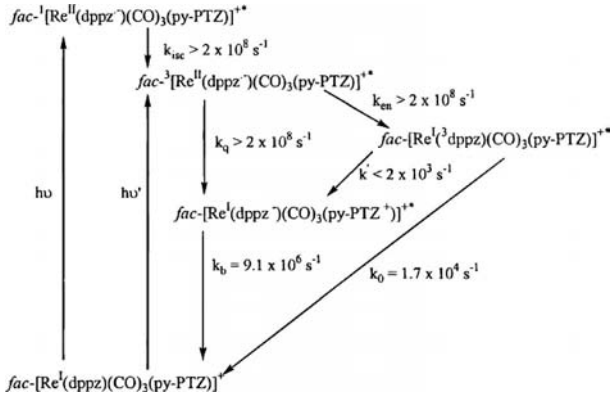


Figure 2. Photophysical processes of [Re(dppz)(CO)$_3$(py-PTZ)]$^+$ in CH_2Cl_2 at 298 K.

Rhenium(I) tricarbonyl polypyridine complexes have very high potential to function as luminescent sensors for several important reasons. (1) The use of various diimine and spectator ligands not only allows control of the structures and physical properties of the complexes, but also their emission energy, which is useful in the design of multicolor luminescent probes. (2) Owing to the phosphorescence nature of the emission, these complexes usually exhibit large Stokes shifts, which can minimize self-quenching

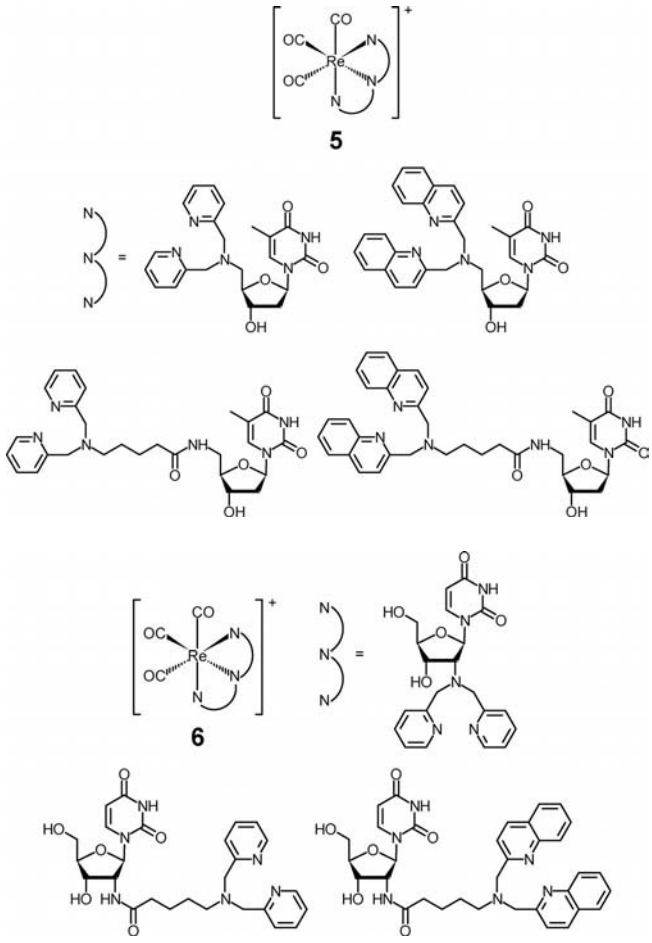
that is commonly encountered in multiple labeling of biomolecules with fluorescent organic dyes. (3) The long emission lifetimes of rhenium(I) tricarbonyl polypyridine complexes could be applied in time-resolved detection techniques such as fluorescence lifetime imaging microscopy, which offers enhanced detection sensitivity. (4) Compared to common organic dyes that suffer from significant photobleaching, luminescent rhenium(I) tricarbonyl polypyridine complexes are generally much more photostable. (5) Since the coordination chemistry of the group 7 congeners rhenium and technetium is similar, the same set of polypyridine ligands (in particular tridentate chelators) can be coordinated to the $[Re(CO)_3]^+$ and $[^{99m}Tc(CO)_3]^+$ cores to yield luminescent probes and radiopharmaceuticals (and radioimaging reagents), respectively.

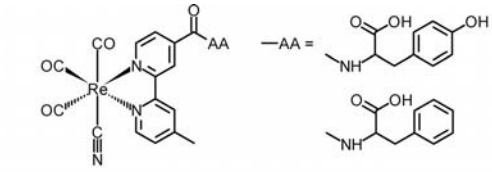
In the following sections, the use of luminescent rhenium(I) tricarbonyl polypyridine complexes in various biological studies is described. It is noteworthy that most of the photophysical data are measured under nonphysiological conditions, for example in degassed organic solvents, which may be very different from the in cellulo environments. However, these data can give an outlined picture of the emission properties of the complexes.

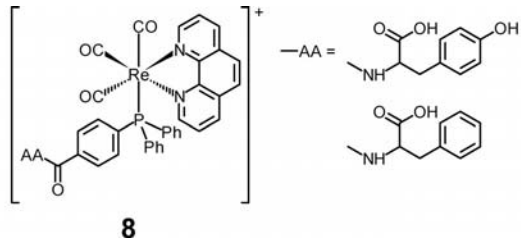
3. Covalent Modification of Nucleosides, Amino Acids, Peptides, Oligonucleotides, and Proteins

The incorporation of reactive functional groups into luminescent rhenium(I) tricarbonyl polypyridine complexes affords labeling reagents for various biomolecules; for example, the C2′ of uridine and C5′ of thymidine have been modified with a primary amine and reacted with 2-pyridinecarboxyaldehyde and 2-quinolinecarboxaldehyde to form chelating nucleosides. These nucleosides have been coordinated to a rhenium(I) tricarbonyl unit to yield the metallonucleosides $[Re(N\hat{}N\hat{}N\text{-thymidine})(CO)_3]^+$ (**5**) and $[Re(N\hat{}N\hat{}N\text{-uridine})(CO)_3]^+$ (**6**), respectively.[26] These modified nucleosides absorb strongly in the UV and visible region. Upon irradiation, they emit at 554–565 nm with a very long lifetime (14.4–18.6 μs), and in some cases an additional higher energy band is observed at approximately 418–429 nm. The low- and high-energy emission features have been assigned to ^{3}MLCT and ^{3}IL excited states, respectively.

In addition to nucleosides, amino acids have been appended to these complexes; for example, phenylalanine (Phe) and tyrosine (Tyr) have been attached to the bipyridine and phosphane ligands of the complexes [Re(bpy-Phe/Tyr)$(CO)_3$(CN)] (**7**) and [Re(phen)$(CO)_3$(phosphane-Phe/Tyr)]$^+$ (**8**), respectively.[36] Excitation of the Phe complexes in aqueous solution at room temperature results in emission at 524 and 633 nm (τ_o = 3900 and 59.4 ns, respectively). Both Phe complexes are powerful photooxidants, as reflected by their high excited-state redox potentials [$E°(Re^{+*/0})$ = +1.59 and +1.78 V vs. NHE, respectively]. Emission quenching of the bpy-Tyr complex due to photooxidation of the tyrosine phenol occurs only upon its deprotonation at pH 12, and a quenching rate constant (k_q) of 8.2×10^8 s^{-1} has been determined. On the contrary, the phosphane-Tyr complex displays emission quenching in aqueous solution of a much wider pH range (from acidic to alkaline), and the formation of tyrosine radicals is observed at pH 7 with a k_q of 2.0×10^8 s^{-1}.

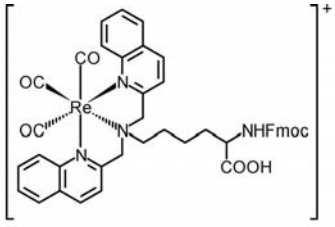






In the following two examples, a rhenium(I) tricarbonyl polypyridine unit has been incorporated into peptides. The rhenium(I) tricarbonyl polypyridine complex $[Re(N\hat{}N\hat{}N\text{-K})(CO)_3]^+$ (**9**) has been designed by using a lysine-derived bis(quinoline) amine as a tridentate ligand.[37] This complex

shows intense absorption in the UV and blue-visible regions with a maximum at 301 nm (ε_{max} = 13,200 M^{-1} cm^{-1}). Photoexcitation results in dual emission bands at 452 and 580 nm with a lifetime of 4.31–9.76 μs. Since this complex is an amino acid analogue, it can be incorporated within peptide-based targeting agents by using a conventional automated synthesizer. As an example, it has been linked to the peptide fMLF, which is a targeting sequence for the formyl peptide receptor (FPR). Images of human leukocytes coincubated with the rhenium–fMLF conjugate and a known FPR targeting fluorophore reveal colocalization. Additionally, the 99m-technetium labeled conjugate has been synthesized, and this modified peptide has been shown to be highly stable in aqueous solution.



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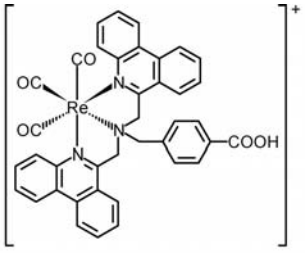
The rhenium(I) tricarbonyl bis(phenanthridinylmethyl)-amine complex [Re(bpm-$CH_2C_6H_4COOH$)$(CO)_3$]$^+$ (**10**) with a tridentate ligand has been synthesized and characterized.[38]



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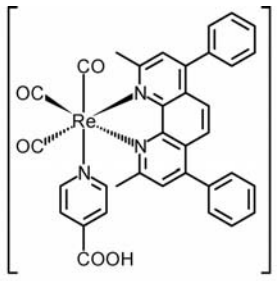
The complex absorbs strongly in the UV region between 310 and 380 nm. It displays greenish-yellow ^{3}MLCT emission at approximately 570 nm in CH_3CN and at a longer wavelength in water. By means of solid-phase peptide synthesis, the complex is conjugated to neurotensin(8–13), which is a shortened version of the native neuropeptide neurotensin. The ^{3}MLCT emission of this conjugate occurs at 560 nm in CH_3CN. The interesting emission properties of both this peptide conjugate and the methyl ester derivative of the complex allow them to serve as cellular imaging reagents.

In general, amine- and thiol-specific functional groups can be attached to luminescent rhenium(I) tricarbonyl polypyridine complexes to yield labels for oligonucleotides and proteins; for example, the isonicotinic acid complex [Re(Me_2Ph_2-phen)$(CO)_3$(py-COOH)]$^+$ (**11**) (λ_{em} = 559 nm, τ_o = 0.99 μs in aerated 0.1 M PBS) has been activated with *N*-hydroxysuccinimide and used to label the proteins HSA and IgG.[39]



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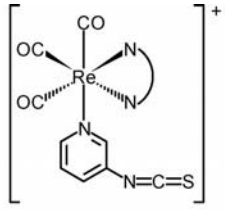
Also, the isothiocyanate complexes [Re(N^N)$(CO)_3$(py-NCS)]$^+$ (**12**) (λ_{em} = 502–650 nm, τ_o = 0.05–9.15 μs in deaerated solutions) have been conjugated to amine-modified oligonucleotides and the protein BSA.[40]



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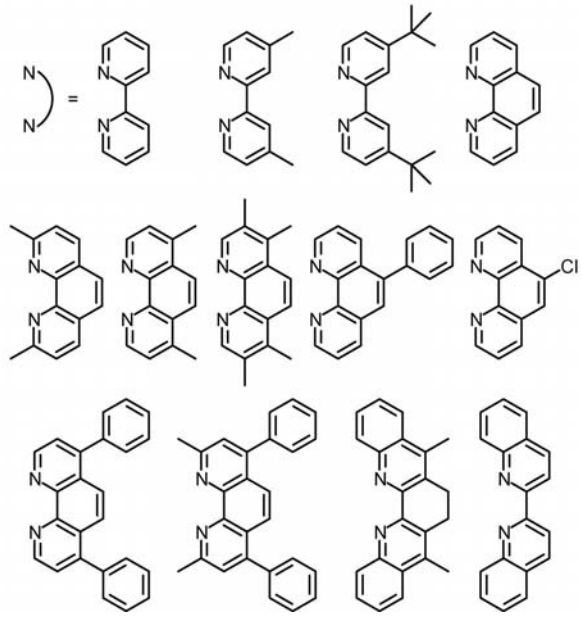
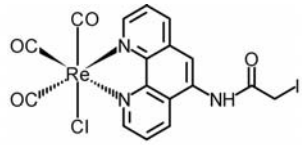


The iodoacetamide complex [Re(phen-IAA)$(CO)_3$Cl] (**13**) (λ_{em} = 600 nm, τ_o = 436 ns in deaerated CH_2Cl_2)[41] and maleimide complexes [Re(N^N)$(CO)_3$(py-mal)]$^+$ (**14**) (λ_{em} = 514–654 nm, τ_o = 0.05–9.60 μs in deaerated solutions)[42] have also been reacted with the sulfhydryl group of cysteine residues of HSA through substitution and addition reactions, respectively.

Upon irradiation, all these rhenium-labeled oligonucleotides and proteins display intense and long-lived ^{3}MLCT [dπ(Re)→π*(N^N)] emission in aqueous solution. Most importantly, the biological properties and activity of the biomolecules have been retained after the bioconjugation; for example, the rhenium–HSA bioconjugate is recognized



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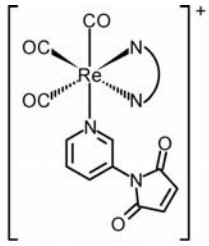

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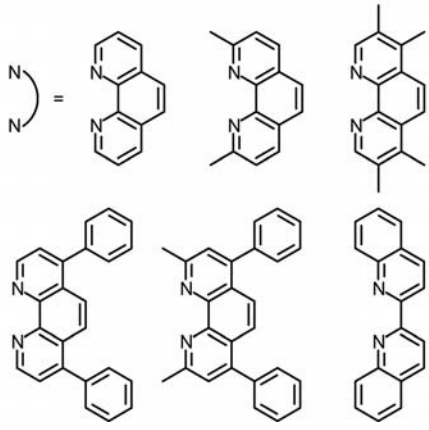
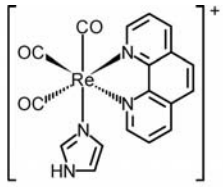


by anti-HSA,[39b] the rhenium–oligonucleotides undergo hybridization with their complementary strands, and the resultant duplexes only show a small decrease in their melting temperature, which is indicative of minimum structure perturbation.[40]

In addition to their rich luminescence properties, the ^{3}MLCT excited states of rhenium(I) tricarbonyl polypyridine complexes are usually very oxidizing, which can be used to study electron transfer in biological systems.[43a–43c] The complex [Re(phen)(CO)$_3$(imidazole)]$^+$ (**15**), synthesized from the aqua complex [Re(phen)(CO)$_3$(H$_2$O)]$^+$, emits at 590 nm with a lifetime of 120 ns in aqueous solution upon irradiation.[43a]



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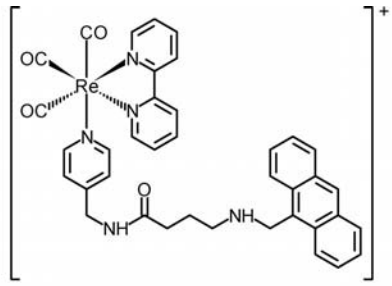
This complex is a powerful photooxidant [$E°$(Re$^{+*/0}$) = +1.2 V vs. SCE]. The histidine-83 residue of azurin (Az) from *Pseudomonas aeruginosa* has been reacted with the aqua complex to yield the bioconjugate [Re(phen)(CO)$_3$-(His83-Az)]$^+$. Upon photoexcitation, the [Re(phen)(CO)$_3$-(His83)]$^{+*}$ moiety of the modified azurin causes direct intramolecular oxidation of the copper center in the protein with an electron-transfer rate constant of approximately 3×10^6 s^{-1} ($-\Delta G° = 0.95$ eV). The faster electron transfer compared to that in the ruthenium(II)-modified azurin has been accounted for by a stronger electron coupling in the rhenium–copper system.[43a] In other studies, the carbonyl ligands of rhenium(I) polypyridine complexes have also been used to probe the structural dynamics of related azurin mutants.[43d,43e]

4. DNA Metallointercalators

Most of the noncovalent interactions between luminescent rhenium(I) tricarbonyl polypyridine complexes and double-stranded (ds) DNA molecules reported to date are intercalative in nature.[27–34] These complexes are either appended with a planar aromatic unit via a spacer[27–29] or coordinated with an extended planar diimine ligand.[30–34] An intercalating anthracene has been covalently linked to a rhenium(I) tricarbonyl 2,2′-bipyridine chromophore to yield the complex [Re(bpy)(CO)$_3$(py-spacer-anthracene)]$^+$ (**16**).[27]



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In aqueous solution, the spacer adopts such a conformation that the hydrophobic anthracene group is in close proximity to the rhenium(I) tricarbonyl bipyridine core. For this reason, the ^{3}MLCT emission of the complex is strongly quenched by the triplet state of anthracene by the Dexter exchange mechanism (intramolecular energy transfer). Interestingly, after ds DNA is added to the sample, the quenched ^{3}MLCT emission at 580 nm is restored (Figure 3). This finding has been attributed to the intercalation of the anthracene unit into the base pairs of ds DNA and hence an increased separation distance between the organic pendant and the rhenium(I) core. A binding constant of $4.6 \pm 0.5 \times 10^5$ M^{-1} ($n = 2 \pm 0.5$) has been determined.

The rhenium(I) complexes [Re(N^N^N-acridine-*N*)-(CO)$_3$]$^{2+}$ (**17a**),[28a] [Re(N^N^N-acridine-*C*)(CO)$_3$]$^+$ (**17b**),[28a] [Re(N^N^N-pyrene)(CO)$_3$]$^+$ (**17c**),[28b] and [Re(N^O–COOH)(CO)$_3$(NC-acridine)]$^+$ (**18**),[29] containing a planar acridine or pyrene unit, have been designed as probes for ds DNA. Complex **17a** binds to DNA by intercalation, as confirmed by the bathochromic shift of its absorption band from 496 to 506 nm upon addition of the biopolymer. The emission intensity of this complex at approximately 525 nm is enhanced, and a positive band at 516 nm and negative bands at 475 and 789 nm appear in

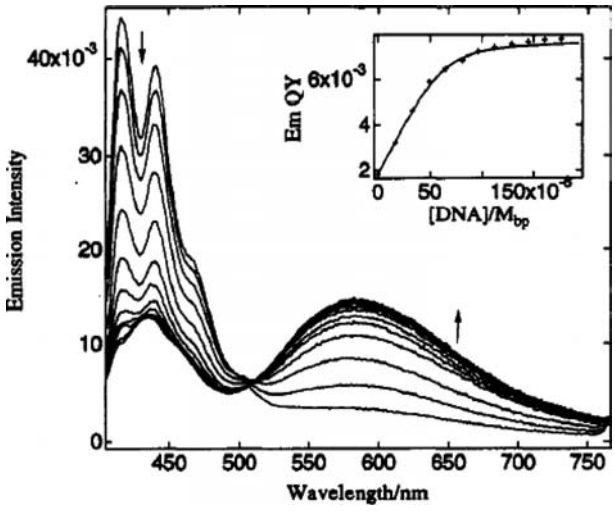
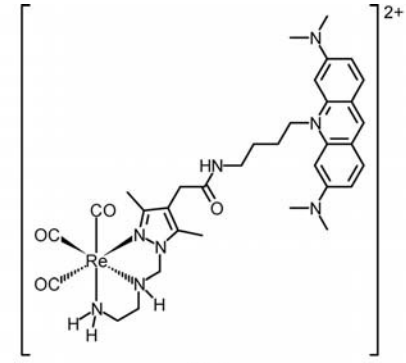
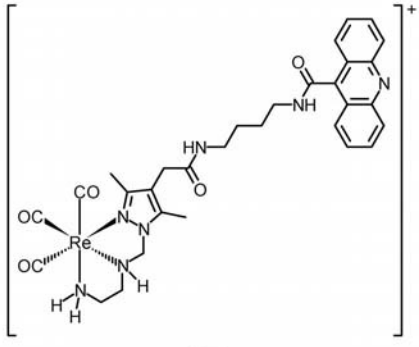


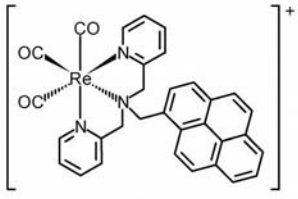
Figure 3. Corrected emission spectra of $[Re(bpy)(CO)_3(py\text{-}spacer\text{-}anthracene)]^+$ as a function of [DNA]/[Re]. The inset shows a plot of $\Phi_{em,\,MLCT}$ vs. [DNA] for the emission titration experiment.



17a

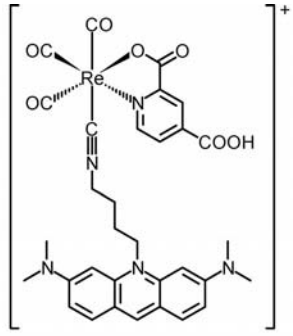


17b



17c

the induced circular dichroism spectra upon addition of ds DNA. All these changes are not observed for complex **17b**, which indicates its poor affinity to DNA. Complex **17c** displays emission bands characteristic of those of pyrene with an emission lifetime shorter than 1 ns. The absorption spectrum of this complex undergoes a drastic change upon addition of fish sperm DNA, as a result of the intercalation of the pyrene pendant into the DNA ($K_b = 1.5 \pm 0.2 \times 10^5\ M^{-1}$, $n = 3.2 \pm 0.2$). The emission band of complex **18** at approximately 525 nm exhibits intensity enhancement and a small redshift upon addition of calf thymus DNA, indicative of intercalative binding. A K_b value of $1.13 \times 10^5\ M^{-1}$ has been determined. This intercalative binding mode has been confirmed by linear dichroism measurements.



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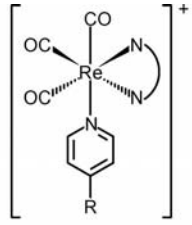
The extended planar diimine ligands of the complexes $[Re(N\text{^}N)(CO)_3(py\text{-}R)]^+$ (N^N = dppz, dppn; R = H, Me) (**19**) enable them to intercalate into ds DNA molecules.[30,31a,31b]



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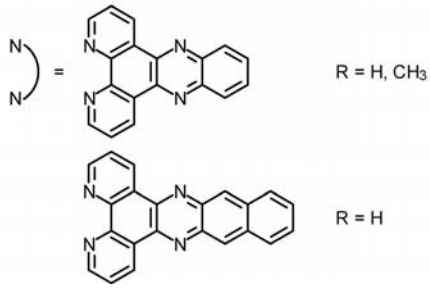
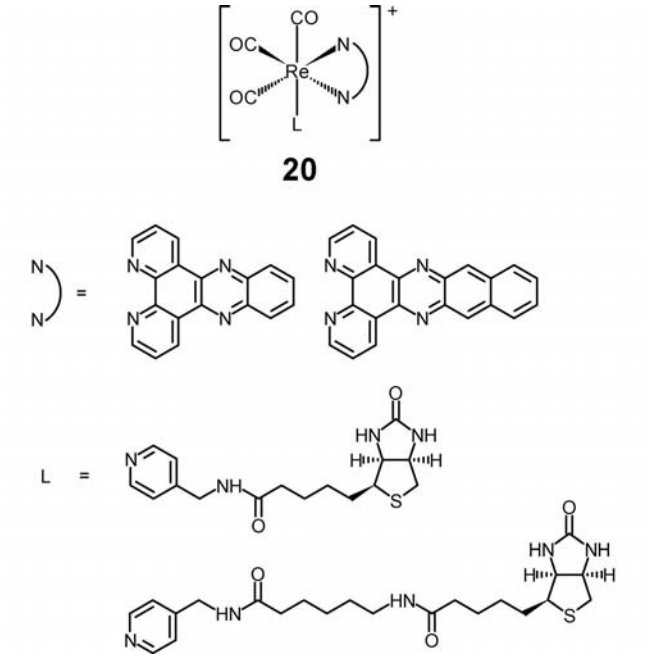


Emission spectroscopy and transient absorption studies indicate that the emission of the dppz [λ_{em} = 556, 598 (sh) nm] and dppn (λ_{em} = 588 nm) complexes in CH_2Cl_2 originates from a state of ^3IL ($\pi \rightarrow \pi^*$) (N^N) character. Intercalation of these complexes into ds calf thymus DNA and synthetic oligonucleotides results in emission enhancement and lifetime extension.[30,31a] Additionally, incubation of the

complexes with plasmid pBR322 upon visible light irradiation leads to photocleavage of the biomolecule.[31b] A range of quenching and inhibition experiments indicate that the excited dppz complex is the active species that causes the cleavage of DNA. However, in the case of the dppn complex, superoxide and hydroxy radicals have been identified to be the reactive species involved in the photocleavage of the biomolecule.

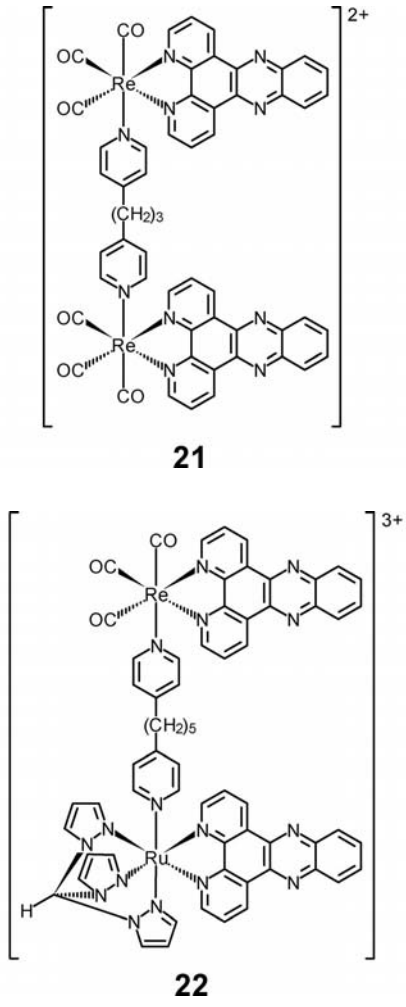
The rhenium(I)-dppz and -dppn units have been functionalized with a biotin unit to yield the complexes $[Re(N^{\wedge}N)(CO)_3(py\text{-}spacer\text{-}biotin)]^+$ (N^N = dppz, dppn) (**20**).[32]



These complexes show emission at 554–607 nm with lifetimes of 4.50–33.09 μs (Φ_{em} = 0.0016–0.23) in degassed CH_2Cl_2 and CH_3CN solutions. However, in aqueous solutions, they are weakly emissive or nonemissive. Both complexes bind to ds DNA by intercalation, as evidenced by pronounced hypochromism and a small bathochromic shift in their UV/Vis absorption spectra. Addition of DNA also leads to emission enhancement.

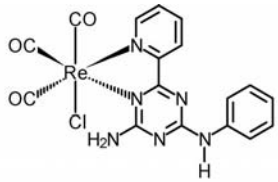
The rhenium(I)-dppz complex has been further modified with another metal-ligand unit; for example, the DNA-binding properties of the homo- and heterodinuclear dppz complexes $[Re(dppz)(CO)_3(py(CH_2)_3py)Re(dppz)(CO)_3]^{2+}$ (**21**)[33] and $[Ru(tpm)(dppz)(py(CH_2)_5py)Re(dppz)(CO)_3]^{3+}$ (**22**)[34] have been studied by absorption titrations.

The titration curve for the nonemissive bis-rhenium(I) complex shows that the hypochromicity appears to approach saturation at [DNA]/[rhenium] ratios of about 10:1 ($K_b = 7 \times 10^5$ M^{-1}, n = 4.5).[33] However, it does not reach saturation even at high [DNA]/[rhenium] ratios. Apparent saturation in the first binding event for this dimetallic complex occurs when the percentage of hypochromicity is half that observed for the monomeric systems. This indicates that the two rhenium(I)-dppz units cannot intercalate into the same duplex by intrastrand interactions because of the insufficiently long propane tether. However, the second rhenium center may be free to interact with other DNA duplexes, which is reflected by the second binding event observed in the titration experiments. The heterodimetallic rhenium(I)–ruthenium(II) complex shows ^{1}IL absorption at 277 and 315 nm, and ^{1}MLCT absorption at wavelengths longer than 320 nm.[34] Excitation of the complex results in ^{3}MLCT [dπ(Ru)→π*(dppz)] emission at 680 nm (τ_o = 77 ns) in CH_3CN. The absence of ^{3}MLCT [dπ(Re)→π*(dppz)] emission has been attributed to energy transfer from the rhenium(I) to ruthenium(II) units. Similar to other dppz complexes, this heterometallic complex displays DNA light-switch properties and emits at 650 nm (τ_o = 117 and 36 ns) upon intercalating into DNA. Additionally, it causes photocleavage of pBR322 plasmid DNA as shown by gel electrophoresis.



In addition to intercalation, other modes of binding have been discovered; for example, the binding of the nonemissive complex $[Re(appt)(CO)_3Cl]$ (**23**) to DNA has been investigated.[35] Addition of ds calf thymus DNA to this complex leads to an increase in the DNA melting temperature, modest hypochromism of the absorption bands at 376 and 450 nm, and insignificant shifts in the absorption maxima. Addition of the complex to a calf thymus DNA solution does not increase its viscosity. These findings, together with

the results of the competition dialysis and modeling study, reveal a binding preference for AT sequences and a minor-groove binding mode.



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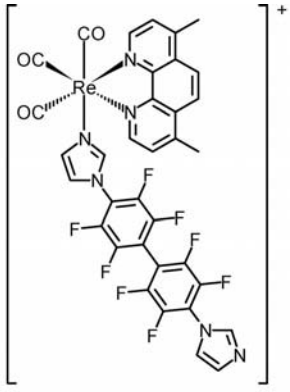
5. Noncovalent Interactions with Protein Molecules

In general, noncovalent binding between a transition metal complex and a protein receptor is based on hydrophobic interactions; for example, mammalian inducible nitric oxide synthase (iNOS) catalyzes the production of L-citrulline and nitric oxide from L-arginine and oxygen.[63] The rhenium(I) tricarbonyl polypyridine complexes $[Re(Me_2\text{-phen})(CO)_3(phenyl_2F_8\text{-imidazole})]^+$ (**24**)[44] and $[Re(Me_2\text{-phen})(CO)_3(im\text{-}C8\text{-}argNO_2)]^+$ (**25**)[45] have been designed as probes for this protein.



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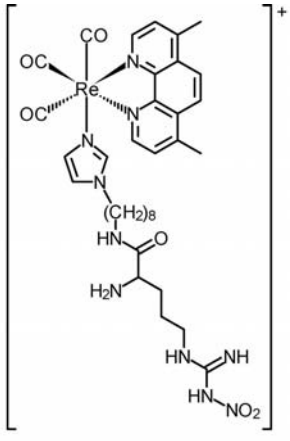

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Addition of these complexes to $iNOS_{oxy}$ causes partial displacement of the water ligand in the active site, resulting in the change from the six-coordinate resting-state iron(III) to the high-spin five-coordinate iron(III). The binding results in a decrease of the emission intensity of the rhenium(I) complexes at 560–570 nm (Figure 4). Emission spectral data and Scatchard analysis give K_d values of 0.1 and 2.0 μM for complexes **24** and **25**, respectively. The decrease in emission intensity has been ascribed to reductive quenching by a nearby tryptophan residue (Trp-490 or Trp-84), generating a rhenium(0) species and a Trp radical cation. Direct coupling-limited electron tunneling from rhenium(0) to iron(III) (over a distance of ca. 20 Å) proceeds on the microsecond time scale, producing iron(II)-$iNOS_{oxy}$, which is stable for hours before reoxidation occurs.

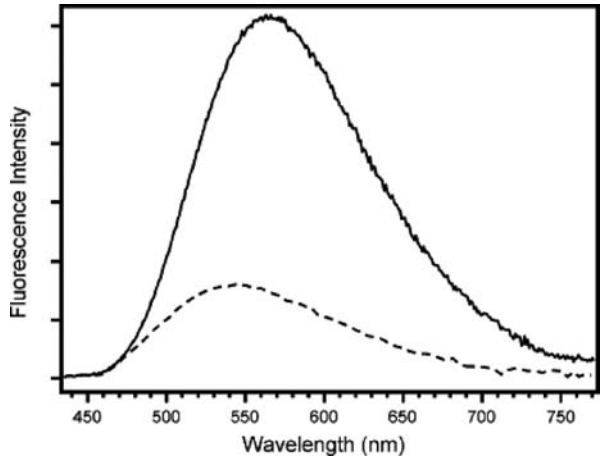
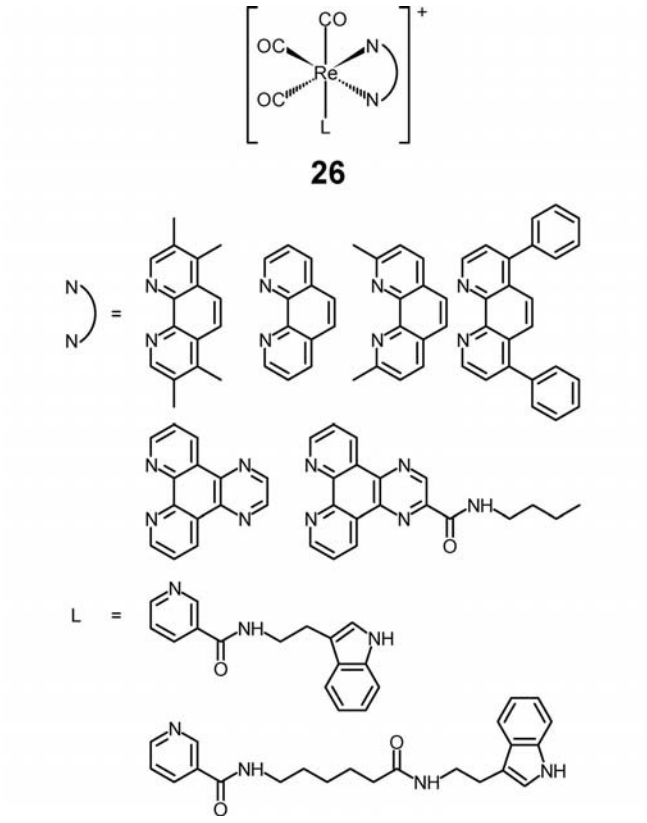
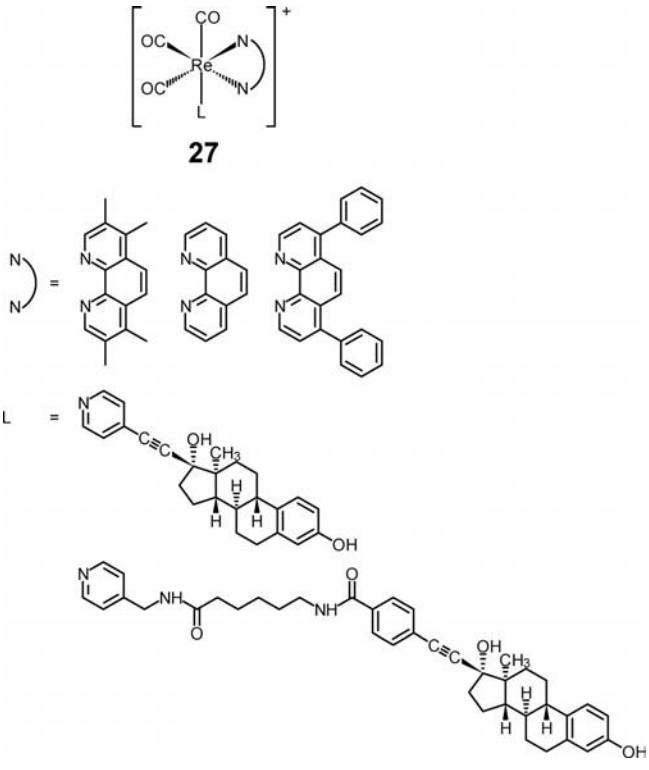


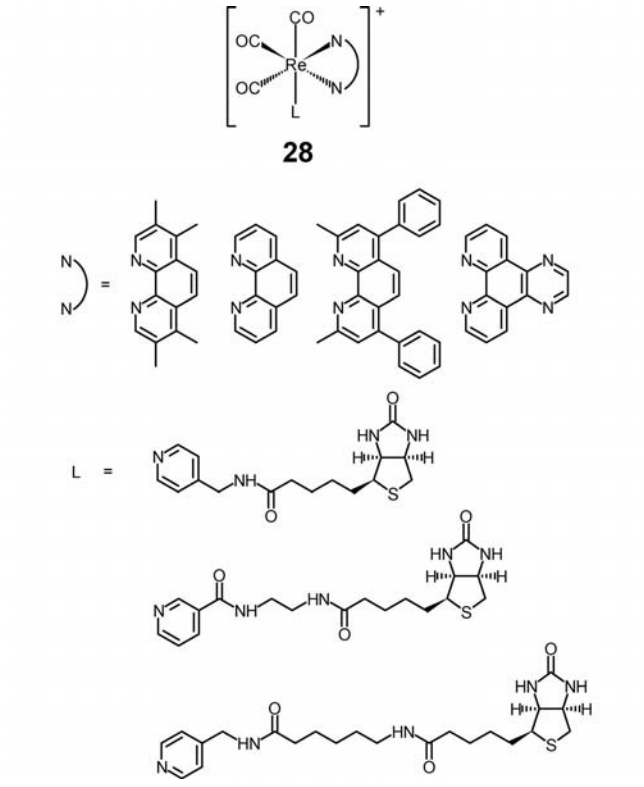
Figure 4. Steady-state luminescence traces for $[Re(Me_2\text{-phen})(CO)_3(im\text{-}C8\text{-}argNO_2)]^+$ in buffer (—) and 1:1 $[Re(Me_2\text{-phen})(CO)_3(im\text{-}C8\text{-}argNO_2)]^+$-$iNOS_{oxy}$ (---).

Due to the strong dependence of the emission properties of rhenium(I) tricarbonyl polypyridine complexes on their local environment, different biological substrates have been attached to these complexes to develop luminescent probes for protein receptors.[46–52] Binding of the complexes to the proteins generally leads to an increase in the hydrophobicity and rigidity of the surroundings of the complexes, which is reflected by a change in the emission properties. Biological recognition units, including indole, estradiol, and biotin, have been attached to the pyridine ligands of the complexes $[Re(N^\wedge N)(CO)_3(py\text{-}spacer\text{-}R)]^+$ [R = indole (**26**),[46] estradiol (**27**),[47] biotin (**28**)[48]] through spacer arms of different lengths.

Upon excitation, the complexes emit at 483–572 nm in degassed solutions with a lifetime of 0.13–15.48 μs. As the emission of the complexes usually originates from ^{3}MLCT and/or ^{3}IL excited states related to the diimine ligands, these biological substrates appended to the pyridine ligands do not significantly change the emission properties of the complexes. However, one exception is the rhenium(I) indole complexes, which exhibit concentration-dependent emission lifetimes and much lower emission quantum yields compared to their indole-free counterparts.[46] These findings suggest a self-quenching process in which the emission of the rhenium–diimine moiety is quenched by the indole unit via intermolecular electron transfer. The binding of these rhenium–substrate complexes to their corresponding recep-







elongation, which have been ascribed to a less polar and more rigid local environment. Generally speaking, complexes with a longer spacer arm exhibit stronger binding affinities but smaller emission enhancement.[46–48] Similar to unmodified indole, the rhenium(I) indole complexes inhibit the tryptophanase-catalyzed conversion of L-serine to pyruvate in a noncompetitive fashion.[46]

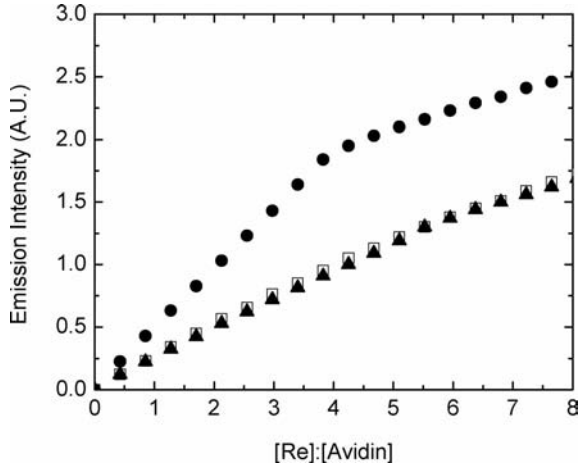


Figure 5. Emission titration curves for the titrations of (i) 3.8 μM avidin (●), (ii) 3.8 μM avidin and 380.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with $[Re(Me_2Ph_2\text{-}phen)(CO)_3(py\text{-}4\text{-}CH_2NH\text{-}C6\text{-}NH\text{-}biotin)]^+$.

tors has been studied by emission titrations. The emission titration curves for one of the biotin complexes are shown in Figure 5 as an example. Upon binding to BSA,[46] estrogen receptor α (ERα),[47] and avidin,[48] respectively, these complexes display emission enhancement and lifetime

The bifunctional luminescent rhenium(I) tricarbonyl polypyridine complexes $[Re(N^\wedge N)(CO)_3(py\text{-}spacer\text{-}biotin)]^+$ (N^N = dppz, dppn) (**20**) with an extended planar diimine ligand and a pyridine-biotin ligand have been designed as probes for ds DNA and avidin.[32] As described

above, these rhenium(I) complexes bind to ds DNA by intercalation. The biotin units of these complexes bind to avidin, resulting in an increase in the emission intensity of the complexes with an enhancement factor as large as 40 (Figure 6). These changes have been attributed to the increase of hydrophobicity and rigidity of the local environment of the complexes.

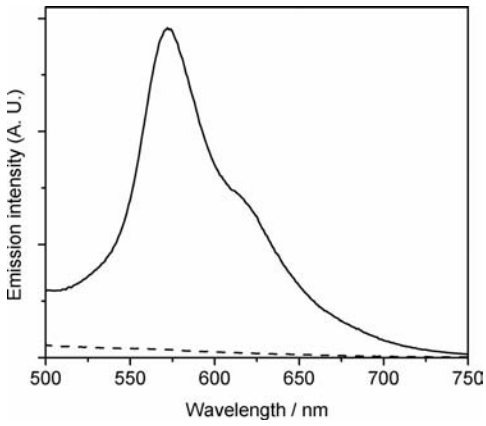
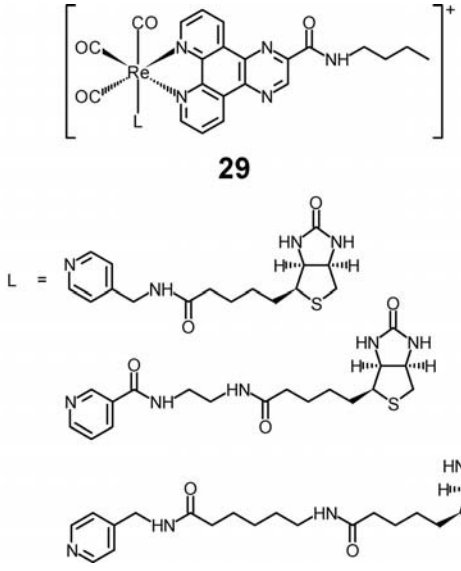


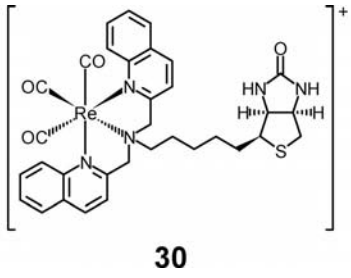
Figure 6. Emission spectra of $[Re(dppz)(CO)_3(py\text{-}CH_2NH\text{-}biotin)]^+$ (15.2 μM) in the absence (---) and presence (—) of avidin (3.8 μM) in degassed 50 mM potassium phosphate buffer at pH 7.2 at 298 K.

If the emission of a biotin complex is selectively suppressed in the free form compared to the protein-bound form, the avidin-induced emission enhancement factor will be significantly increased. One strategy is to identify systems that show very weak emission in aqueous solution but intense luminescence in more hydrophobic media, such as the substrate-binding sites of proteins. Thus, rhenium(I) dpqa biotin complexes $[Re(CO)_3(dpqa)(py\text{-spacer-biotin})]^+$ (**29**) have been synthesized and characterized.[49]

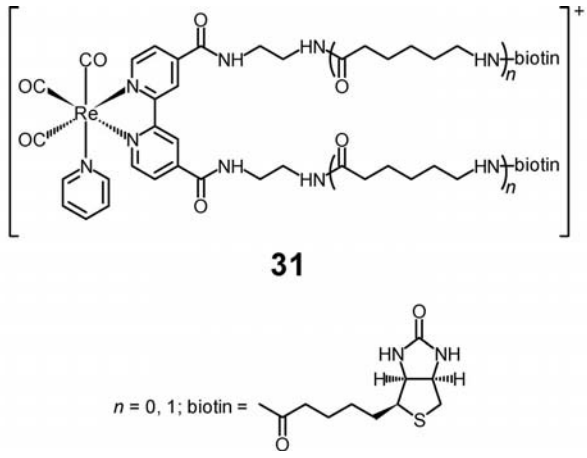


They emit at 550–577 nm in degassed fluid solutions (τ_o = 0.23–1.03 μs). Interestingly, the complexes in aqueous solutions are very weakly emissive ($\Phi_{em} \approx 10^{-3}$). Emission enhancement is observed after the complexes bind to avidin, and the enhancement factors (I/I_o) of 3.05–8.05 are much larger than those of other rhenium–biotin complexes, for example, the dpq analogues (I/I_o = 1.15–1.75).

The rhenium(I) complex $[Re(N^\wedge N^\wedge N\text{-biotin})(CO)_3]^+$ (**30**) coordinated with a tridentate biotin ligand has been used for fluorescence imaging.[50] Upon photoexcitation, this complex exhibits dual emission (λ_{em} = 420 and 580 nm, τ_o = 11.4 μs), and the lower energy band has been assigned to a ^{3}MLCT emissive state. The binding of this complex to avidin has been confirmed by the observation of the luminescence from avidin-modified microbeads that have been incubated with a solution of this complex. The binding affinity is so strong that the bound complex is not dissociated from the beads upon incubation of more than 1000-fold unmodified biotin at 37 °C for 30 min. The 99m-technetium analogue has also been prepared by reacting the tridentate biotin ligand with $[^{99m}Tc(CO)_3(H_2O)_3]^+$.



Since avidin has four biotin-binding sites, multibiotin compounds may crosslink this protein and assist in the construction of polymeric bioarchitecture.[64] The diimine ligands of the two rhenium(I) tricarbonyl polypyridine complexes $[Re(N^\wedge N\text{-spacer-biotin}_2)(CO)_3(py)]^+$ (**31**) have been modified with two biotin units.[51]



The emission of these bis(biotin) complexes at 596–616 nm (τ_o = 0.040–0.15 μs, Φ_{em} = 0.0039–0.016) is much lower in energy compared to that of the mono(biotin) complexes,[48] since the π* orbitals of the diimine ligands are stabilized by the two electron-withdrawing amide groups. Similar to their mono(biotin) counterparts, these bis(biotin) complexes exhibit emission enhancement and lifetime extension in the presence of avidin. The potential use of the complexes as signal amplifiers for heterogeneous recogni-

tion assays has been demonstrated by using avidin-coated microspheres (Figure 7).

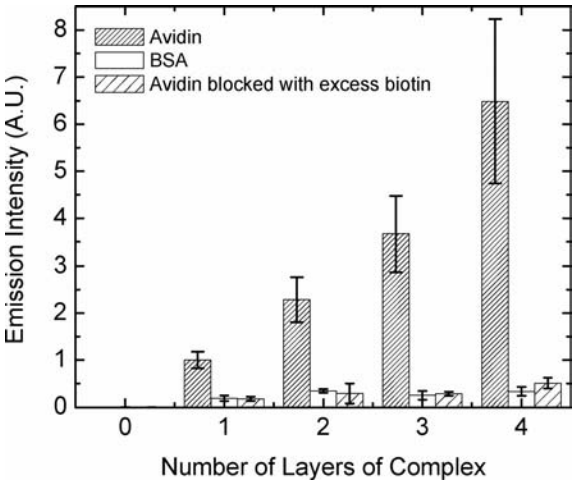
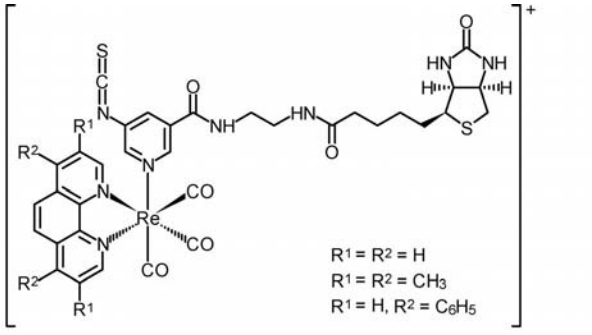


Figure 7. Averaged emission intensities of avidin-modified microspheres (N = 6) upon incubation of zero to four layers of [Re(bpy-C2-C6-biotin$_2$)(CO)$_3$(py)]$^+$. The results of the control experiments (BSA was used instead of avidin or when the avidin was presaturated with biotin from the outset) are included.

Although biotinylation reagents have been developed, those with luminescence properties have not been fully explored. These reagents not only endow the biotinylated biomolecules with emission properties, but also the extent of biotinylation can be directly determined by more sensitive spectrofluorometric methods.[65] Additionally, they can be employed to biotinylate small molecular substrates and allow (1) the isolation of the specific biological receptors by affinity chromatography, and (2) monitoring of the biological uptake of the biotinylated compounds by luminescence spectroscopy and microscopy. The amine-reactive isothiocyanate group has been introduced to rhenium(I) tricarbonyl polypyridine biotin complexes to yield the first luminescent biotinylation reagents [Re(N^N)(CO)$_3$(py-biotin-NCS)]$^+$ (**32**).[52]



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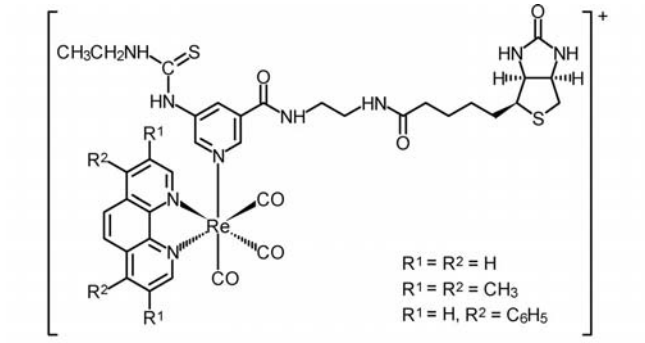
These luminescent biotinylation reagents have been reacted with a model substrate, ethylamine, which resulted in the formation of the thiourea complexes [Re(N^N)(CO)$_3$-(py-biotin-Et)]$^+$ (**33**). These complexes emit at 482–560 nm (τ_o = 0.18–14.67 μs, Φ_{em} = 0.010–0.56) upon irradiation. The isothiocyanate complexes have been used to biotinylate BSA, and the resultant rhenium–BSA bioconjugates display intense and long-lived orange-yellow to greenish yellow ^{3}MLCT emission in aqueous buffer. The biotin/BSA ratios of the bioconjugates have been determined by emission spectroscopy. The avidin-binding properties of the thiourea complexes have also been investigated by HABA assays and emission titrations.



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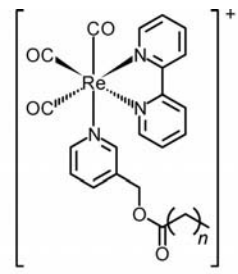
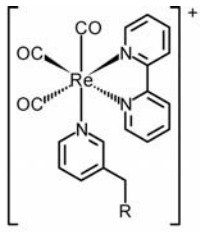
6. Cellular Uptake and Bioimaging

In the past few years, there has been a fast-growing interest in the cellular uptake properties of luminescent transition metal complexes with a focus on their potential as cellular imaging reagents.[25b,28,29,37,38,51–62] Luminescent rhenium(I) tricarbonyl polypyridine complexes are among the most extensively studied systems.[25b,28,29,37,38,51–57] Investigations have been focused on the effects of charges and lipophilicity of these complexes on their cellular uptake, localization, and cytotoxic activity. Confocal microscopy images reveal that most of the cationic complexes enter mammalian cells by passive diffusion.[54] In some cases, the complexes bind to the glycocalyx and cannot enter the lipid bilayers. Specifically, the cationic lipophilic complexes [Re(bpy)(CO)$_3$(py-CH$_2$OCO(CH$_2$)$_n$CH$_3$)]$^+$ (n = 6, 12, 16) (**34**) (λ_{em} ≈ 550 nm) localize in hydrophobic membranes of the organelles or other cytoplasmic structures,[54a,54b] whereas the electrophilic complexes [Re(bpy)(CO)$_3$(py-R)]$^+$ (R = CH$_2$OH, CH$_2$Cl) (**35**) (λ_{em} ≈ 551–556 nm) distribute in the mitochondria.[54c,54d] The anionic complexes [Re((PhSO$_3$)$_2$-phen)(CO)$_3$(py-R)]$^-$ (R = H, CH$_2$OH, CH$_2$O-COC$_{13}$H$_{27}$) (**36**) (λ_{em} ≈ 560 nm) accumulate on the outer face of the plasma membrane or show no uptake at all even for the very lipophilic complexes.[54b,54d]



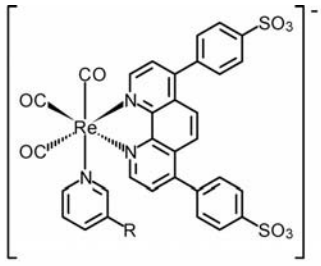
34

n = 6, 12, 16



35

R = OH, Cl



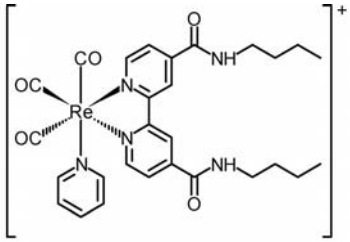
36

R = H, CH_2OH, $CH_2OC(=O)(CH_2)_{12}CH_3$

The methyl ester of the rhenium(I) complex [Re(bpm-$CH_2C_6H_4COOH$)$(CO)_3]^+$ (**10**) mentioned above and the neurotensin conjugate of the carboxyl complex are readily internalized by various cell lines including HeLa, HT-29, IMIM-PC2, and PT-45, as evidenced by fluorescence microscopy.[38] Whereas the lipophilic free complex enters the cells by passive diffusion, the bioconjugate shows more pronounced differences in efficiency across different cell lines, which has been ascribed to a receptor-mediated uptake mechanism.

The cytotoxicity (IC_{50} values, after exposure for 48 h; the same below) of the DNA-binding complex [Re(appt)$(CO)_3$-Cl] (**23**) towards different human cancer cell lines, including KB-3-1, KB-V-1, HepG2, and HeLa, and noncancerous normal lung fibroblasts, CCD-19Lu, has been evaluated.[35] The complex exhibits moderate cytotoxic activity towards KB-3-1, HepG2, and HeLa cells (IC_{50} = 30.9–50.3 μM), but is less cytotoxic towards the multi-drug-resistant cancer KB-V-1 cells and the noncancerous CCD-19Lu cells (IC_{50} = 195 and 112 μM, respectively).

The cytotoxicity and cellular uptake of the rhenium(I)–bis(biotin) complexes [Re(N^N-spacer-biotin$_2$)$(CO)_3$(py)]$^+$ (**31**),[51] mono(biotin) complexes [Re(N^N)$(CO)_3$(py-biotin-Et)]$^+$ (**33**),[52] and biotin-free complex [Re(N^N-$(C4)_2$)-$(CO)_3$(py)]$^+$ (**37**)[51] have been studied by using HeLa cells as a model.



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The cytotoxicity depends on the number of biotin units in the complexes; for example, the bis(biotin) complexes **31** are basically noncytotoxic (IC_{50} > 250 μM), the mono(biotin) complexes **33** have cytotoxicity (IC_{50} = 17.5–28.5 μM) similar to that of cisplatin (IC_{50} = 25.6 ± 2.3 μM), and the biotin-free complex **37** exhibits the highest cytotoxic activity (IC_{50} = 7.7 ± 1.0 μM). The lower cytotoxicity of the biotin-containing complexes is due to the polar biotin moieties and hence the lower lipophilicity of these complexes. On the basis of the fluorescence and laser-scanning confocal microscopy images, it can be inferred that all these complexes retain their emission properties within the cells. It appears that the bis- and mono(biotin) complexes bind to lipophilic organelles such as the Golgi apparatus and endoplasmic reticulum (Figure 8).

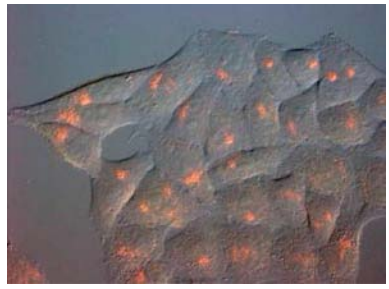
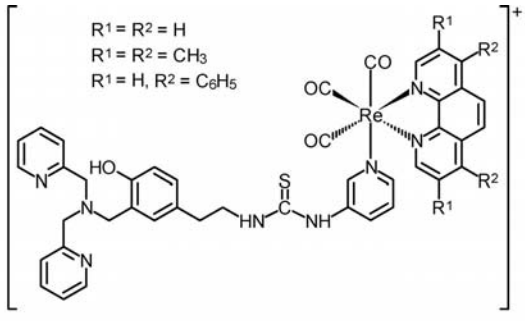


Figure 8. Fluorescence and brightfield overlaid microscopy image of HeLa cells incubated with [Re(Ph_2-phen)$(CO)_3$(py-biotin-Et)]$^+$ (10 μM) at 37 °C for 24 h.

The zinc ion participates in many important biological controls such as gene expression, neurotransmission, and bioinorganic catalysis.[66] Three rhenium(I) tricarbonyl polypyridine complexes [Re(N^N)$(CO)_3$(py-DPAT)]$^+$ (**38**) containing a tyramine-derived 2,2′-dipicolylamine (DPAT) unit have been designed as intracellular zinc ion sensors.[25b]



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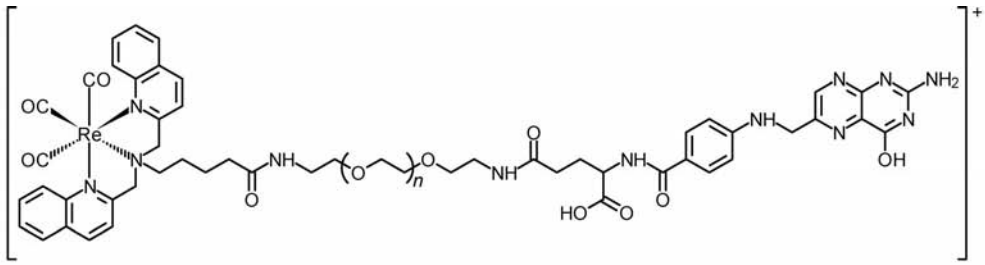
Excitation of these complexes leads to emission at 489–554 nm with a lifetime of 0.54–3.15 μs in degassed fluid solutions. The appended DPAT unit effectively quenches the emission through electron transfer, which results in very low quantum yields for these complexes (Φ_{em} = 0.0085–0.028). Upon addition of Zn^{2+} or Cd^{2+} ions, the DPAT complexes show emission enhancement and lifetime extension due to suppression of the self-quenching process.



39

Treatment of HeLa cells with the complexes leads to almost full cytoplasmic staining but negligible localization in the nuclei. After treatment with Zn^{2+} and Cd^{2+} ions, the emission intensity of an average HeLa cell increases by approximately 2.2- and 1.8-fold (Figure 9). These results indicate that the complexes can function as intracellular luminescent ion probes.

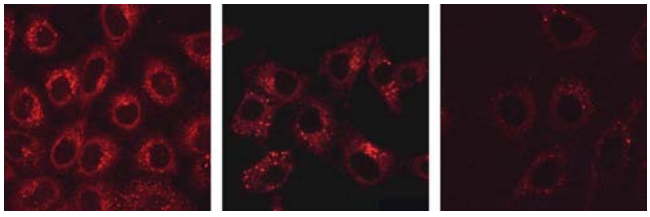
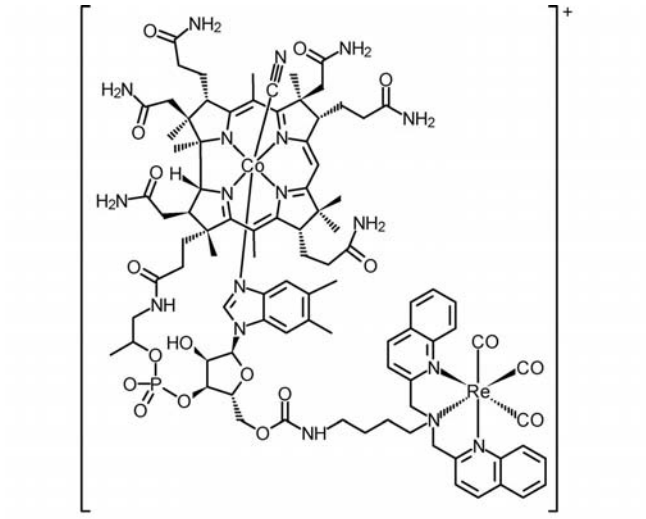


Figure 9. Laser-scanning confocal microscopy images of HeLa cells incubated with $[Re(Ph_2\text{-phen})(CO)_3(py\text{-}DPAT)]^+$ (5 μM) at 37 °C for 1 h followed by incubation with zinc(II) chloride/2-mercaptopyridine *N*-oxide (MPO) (25 μM) (left), cadmium(II) chloride/MPO (25 μM) (middle) and MPO (25 μM) only (right) for 5 min.

Biologically active substrates have been attached to rhenium(I) tricarbonyl polypyridine complexes for enhanced cellular uptake and specific targeting of cellular receptors; for example, complexes containing folate (vitamin B_9) and cubilin (vitamin B_{12}) moieties have been synthesized to target folate and cubilin receptors, respectively.[55] The cellular uptake of the folate complex $[Re(N^N^N\text{-}B_9)(CO)_3]^+$ (**39**) has been investigated by using folate receptor (FR) overexpressing A2780/AD ovarian cancer cells.[55a] CHO cells are used as a negative control as they do not express FR. Uptake of the complex by A2780/AD cells has been studied by confocal microscopy. No internalization of the complex was observed when excess folate was added to the incubation medium or when CHO cells were used. As revealed by WSK-8 colorimetric assays, the cytotoxicity of this folate complex towards the multi-drug-resistant A2780/AD cells is much higher than that of cisplatin. On the contrary, the cytotoxicity of the complex towards non-FR-expressing CHO cells is 17 times lower than that of cisplatin. Since no internalization of the complex in CHO cells is observed, the cytotoxic activity has been ascribed to nonspecific cell surface interactions. The rhenium–vitamin B_{12} complex $[Re(N^N^N\text{-}B_{12})(CO)_3]^+$ (**40**) binds to intrinsic factor (IF), a 44 kDa glycosylated protein that specifically binds vitamin B_{12}, through the B_{12}–IF interaction as indicated by absorption titrations.[55b]



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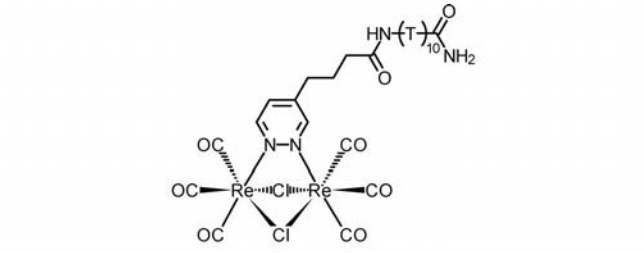
Confocal microscopy shows that the rhenium–B_{12}–IF conjugate is internalized by BeWo cells and accumulates in the nuclear and cytosolic region. The uptake and accumulation are completely inhibited by the addition of excess vitamin B_{12} to the incubation medium, indicative of the endocytotic mechanism of cubilin, a 460 kDa protein that binds B_{12}–IF. The involvement of this receptor has been supported by the observation that siRNA specific for cubilin mRNA totally inhibits the uptake of the rhenium–B_{12}–IF conjugate.

Although many transition metal complexes show cellular uptake properties, the observation of nuclear uptake is scarce. Nevertheless, the uptake and localization of transition metal complexes in the nucleus have been achieved by introducing a nucleic acid binding group to the complex molecules. As mentioned above, the emission intensity of the complexes $[Re(N^N^N\text{-acridine-}N)(CO)_3]^{2+}$ (**17a**)[28a] and $[Re(N^O\text{-}COOH)(CO)_3(NC\text{-acridine})]^+$ (**18**)[29] at approximately 525 nm is enhanced upon intercalation of the acridine moiety into ds DNA. Efficient uptake of both complexes by B16-F1 cells has been observed by using fluorescence microscopy. Interestingly, colocalization experi-

ments with DAPI staining confirm that both complexes accumulate in the cell nuclei. Introduction of the gastrin-releasing peptide (GRP) receptor-specific peptide, Bombesin, to complex **18** yields a trifunctional rhenium–acridine–Bombesin complex, which is only taken up by PC-3 cells that express the GRP receptor, indicative of a receptor-mediated active uptake. Interestingly, the rhenium–acridine–Bombesin complex localizes in the cytoplasm instead of the nuclei. The 99m-technetium–acridine–Bombesin conjugate has also been synthesized and its stability in the presence of histidine and cysteine has been evaluated.

Peptide nuclei acid (PNA), which in general exhibits stronger and more selective binding affinity for complementary nucleic acid strands than natural nucleic acids,[67] has been utilized in the design of luminescent nuclear stains. The dinuclear rhenium(I) complex $[(CO)_3Re(pyridazine\text{-}PNA)(Cl)_2Re(CO)_3]$ (**41**) bearing a PNA unit has been synthesized by a solid-phase synthetic methodology.[56]



41

Upon excitation, this complex shows emission at 610 nm (τ_o = 316 ns, Φ_{em} = 0.017) in deaerated aqueous acetonitrile. HEK-239 cells treated with the rhenium complex reveal whole cell staining, and the emission inside the nucleus appears to be blueshifted with respect to that in the cytoplasm, probably due to the reduced mobility and the more hydrophobic character of the nuclear environment compared to that of the cytoplasm (Figure 10).

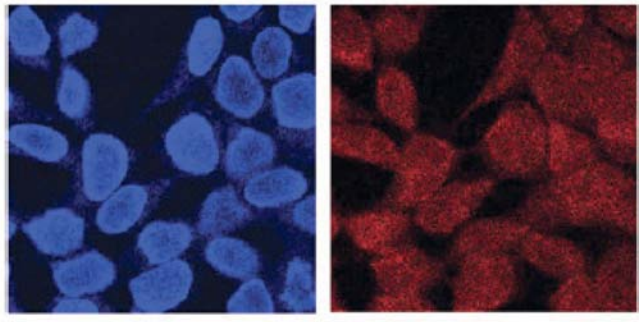
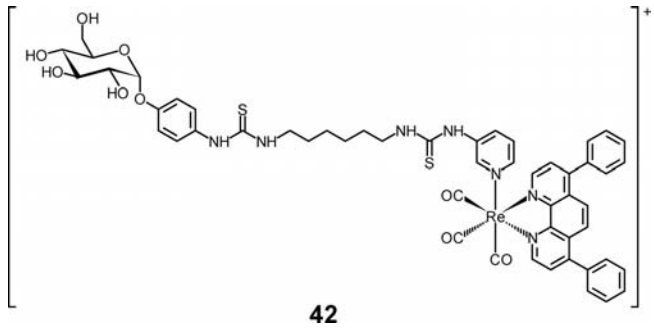


Figure 10. Images of HEK-293 cells stained with the Re–PNA conjugate recorded about 10 min after the addition of the complex, through a 485/30 (left) and a 600/40 (right) band pass filter. Note the enhanced visibility of the nucleus in the blue-filtered image on the left, which indicates efficient nuclear uptake.

One of the most characteristic phenotypes of rapidly growing cancer cells is their propensity to catabolize glucose at high rates, possibly due to the overexpression of glucose transporters (GLUTs).[68] Thus, the in vitro and in vivo monitoring of glucose utilization in cancer cells has attracted much attention. Novel rhenium(I) glucose complexes such as $[Re(Ph_2\text{-}phen)(CO)_3(py\text{-}3\text{-}glucose)]^+$ (**42**) have been synthesized and characterized.[57] Upon irradiation, complex **42** shows greenish-yellow ^{3}MLCT



42

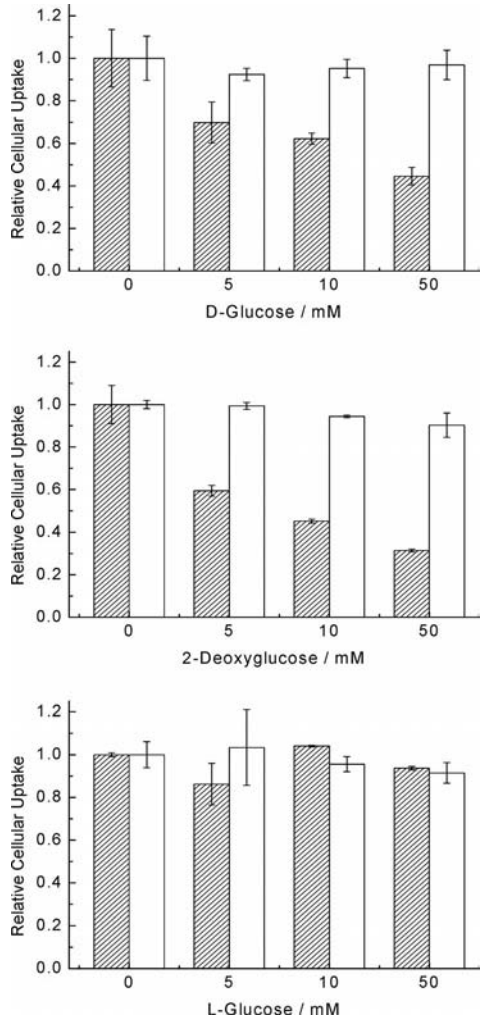


Figure 11. Relative cellular uptake of rhenium associated with an average HeLa cell upon incubation with complex **42** (shaded) and its glucose-free counterpart (empty) (100 μM) at 37 °C for 5 min in a glucose-free medium containing various concentrations of D-glucose (top), L-glucose (middle), and 2-deoxyglucose (bottom) (n = 3).

[dπ(Re)→π*(N^N)] emission at 543–552 nm (τ_o = 0.63–1.84 μs, Φ_{em} = 0.0023–0.034). The uptake of the complex by HeLa cells is inhibited by D-glucose and deoxyglucose, but not by L-glucose (Figure 11). These results, together with findings from other cell-line dependence and inhibition studies, indicate that the uptake of the glucose complex is via a GLUT-mediated pathway. Costaining experiments show that the complex localizes in the mitochondria.

7. Conclusion

In this Microreview, we describe the basic photophysical properties of luminescent rhenium(I) tricarbonyl polypyridine complexes that allow them to function as luminescent probes. We include and discuss recent reports on the use of these complexes as labels and probes for biomolecules. Importantly, the rhenium(I)-labeled biomolecules display intense and long-lived emission originating from the metal complexes, and the activity and binding selectivity of the biomolecules are retained in almost all cases. In the development of noncovalent probes, luminescent rhenium(I) tricarbonyl polypyridine complexes have the advantage of environment-sensitive emission properties, which can report binding of the complexes to various biomolecules. Additionally, cellular studies show that most of these complexes are internalized by mammalian cells effectively, and the uptake can be readily examined by ICP-MS and confocal microscopy. Since the cellular uptake of these complexes is closely related to their charge and lipophilicity, the possible use of a wide range of diimine and monodentate ligands means that the structural and physical properties can be controlled, which will affect the cellular uptake efficiency and possibly specificity. Also, the use of various ligands can vary the emission properties of these complexes. This is an advantage for these complexes, as it enables them to function as cellular probes and imaging reagents. Furthermore, the incorporation of various recognition groups and biologically relevant substrates into these complexes is also anticipated to result in the development of new cellular probes for ions, molecules, and intracellular structures and processes. One of the important challenges is the design of new reagents with lower cytotoxicity and high organelle specificity. With their high photooxidizing properties, these complexes are also anticipated to serve as new photodynamic therapeutics.

Acknowledgments

We thank the Hong Kong Research Grants Councils (CityU 102410) and the City University of Hong Kong (Project No. 7002679) for financial support. K. Y. Z. and S. P.-Y. L. both acknowledge the receipt of a Postgraduate Studentship, Research Tuition Scholarship, and Outstanding Academic Performance Award, all administered by the City University of Hong Kong.

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Received: May 4, 2011
Published Online: July 21, 2011

