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Coordination Chemistry Reviews 249 (2005) 1434–1450
COORDINATION CHEMISTRY REVIEWS
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Biological labelling reagents and probes derived from luminescent transition metal polypyridine complexes

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Received 31 July 2004; accepted 17 October 2004
Available online 8 December 2004

Contents

Abstract

Many transition metal polypyridine complexes display intense and long-lived metal-to-ligand charge-transfer emission with a large Stokes' shift. This property renders them promising candidates as luminescent labelling reagents and probes for biological molecules. In view of this, we have designed various biological labels that are derived from luminescent rhenium(I) and iridium(III) polypyridine complexes. These complexes contain various functional groups that can react with the amine and sulfhydryl groups of biomolecules such as oligonucleotides, peptides and protein molecules to form luminescent bioconjugates. In other studies, we have incorporated biotin into luminescent rhenium(I) polypyridine complexes to form new probes for the protein avidin. These new luminescent conjugates and biological probes have been utilised in the development of various bioassays.

Keywords: Labelling reagents; Polypyridine complexes; Bioassays; Luminescence; Rhenium(I); Iridium(III)

Abbreviations: bpy-CHO, 4-carboxy-4′-methyl-2,2′-bipyridine; bpy-IAA, 4-iodoacetamido-2,2′-bipyridine; bpy-ITC, 4-isothiocyanato-2,2′-bipyridine; bpy-NH$_2$, 4-amino-2,2′-bipyridine; BSA, bovine serum albumin; dppn, benzo[*i*]dipyrido[3,2–*a*:2′,3′–c]phenazine; dppz, dipyrido[3,2–*a*:2′,3′–c]phenazine; HABA, 4′-hydroxyazobenzene-2-carboxylic acid; Hmppz, 3-methyl-1-phenylpyrazole; Hpba, 4-(2-pyridyl)benzaldehyde; Hpq, 2-phenylquinoline; HSA, human serum albumin; IL, intra-ligand; MLCT, metal-to-ligand charge-transfer; phen-IAA, 5-iodoacetamido-1,10-phenanthroline; phen-ITC, 5-isothiocyanato-1,10-phenanthroline; phen-NH$_2$, 5-amino-1,10-phenanthroline; py-3-mal, 3-maleimidopyridine; py-3-NCS, 3-isothiocyanatopyridine; py-3-NH$_2$, 3-aminopyridine; py-CH$_2$─NH-biotin, *N*-((4-pyridyl)methyl)biotinamide

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doi:10.1016/j.ccr.2004.10.004

1. Introduction

Traditionally, radioactive isotopes have been commonly used for labelling of biomolecules for DNA sequencing, hybridisation studies and immunological applications owing to their high detection sensitivity. However, due to the relatively long experimental time, short shelf-lives of expensive reagents and increasing concern about the potential hazards of radioactive materials, alternative reagents derived from organic fluorophores and luminescent lanthanide chelates have been developed [1]. A number of fluorescent organic compounds have been designed and labelling of oligonucleotides, amino acids, peptides, proteins, antibodies and biological tissues with these fluorophores has been well documented [2]. However, the use of many organic dyes has general limitations including their high photobleaching rates, strong pH dependence, short fluorescence lifetimes and small Stokes' shifts. Thus, various lanthanide chelates have been employed as biological labelling reagents owing to their intense and extraordinarily long-lived luminescence [1]. However, the design of new lanthanide chelates is a challenge because the chelate must (i) protect the luminescent lanthanide centre from quenching by water molecules and (ii) act as an energy sensitiser to enable energy transfer to the lanthanide centre [1].

By virtue of their variable oxidation states, flexible coordinating geometry, and rich photophysical and electrochemical properties, many transition metal complexes have been covalently linked to biomolecules for various purposes [3–9]. The studies include photo-induced electron transfer in metalloproteins [3]; recognition, photocleavage and cross-linking of nucleic acids [4,5]; automated synthesis of metal-containing oligonucleotides [6]; investigations of protein hydrodynamics using anisotropy probes [7]; development of artificial nucleases [8]; and folding kinetics and thermodynamics of proteins [9].

We are interested in the possibility of using luminescent transition metal complexes, such as rhenium(I) and iridium(III) polypyridines, as biological labels and probes because many of these complexes show intense and long-lived photoluminescence in the visible region, and the emission energy of these complexes can be controlled using various diimine and/or cyclometallating ligands. Attachment of reactive functional groups or biologically important molecules to these luminescent complexes is anticipated to generate a new class of labelling reagents and probes for biomolecules.

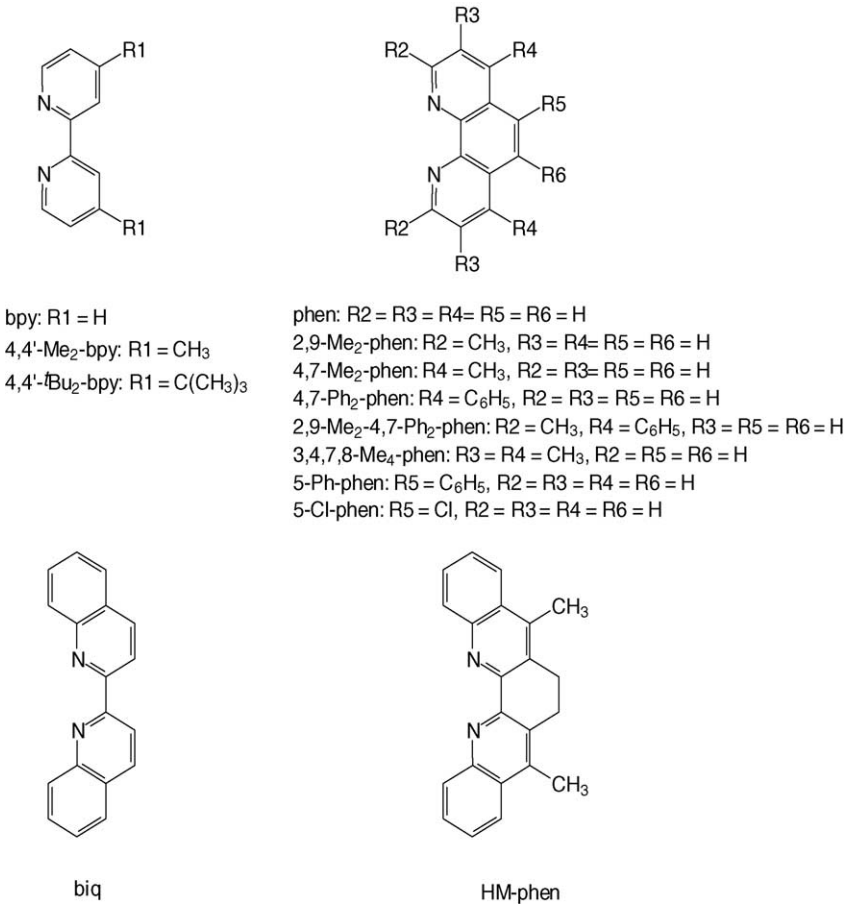

Fig. 1. Structures of the diimine ligands (N─N) of the rhenium(I) polypyridine complexes $[Re(N{-}N)(CO)_3(py\text{-}3\text{-}NCS)](CF_3SO_3)$ and $[Re(N{-}N)(CO)_3(py\text{-}3\text{-}mal)](CF_3SO_3)$.

This review article summarises our recent work in these areas.

2. Rhenium(I) polypyridine isothiocyanate and maleimide complexes

2.1. [Re(N–N)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) and [Re(N–N)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$)

The isothiocyanate and maleimide moieties can react readily with primary amines and sulfhydryls to form stable thiourea and thioether groups, respectively [10]. Many organic fluorophores have been equipped with these moieties to form specific biological labels. We are interested in exploiting the rich ^{3}MLCT emission properties of rhenium(I) polypyridine complexes in the development of amine- and sulfhydryl-specific luminescent biological labels. Thus, a series of rhenium(I) polypyridine isothiocyanate [Re(N–N)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) [11] and maleimide [Re(N–N)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) [12] complexes have been synthesised and characterised. The isothiocyanate complexes were obtained from the reaction of [Re(N–N)(CO)$_3$(py-3-NH$_2$)](CF$_3$SO$_3$) with CSCl$_2$ whilst the maleimide complexes were obtained from [Re(N–N)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) with py-3-mal. The structures of the diimine ligands (N–N) used are shown in Fig. 1. The X-ray crystal structures of [Re(bpy)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) and [Re(2,9-Me$_2$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) have been studied, and the perspective views of the cations of these two complexes are shown in Figs. 2 and 3, respectively. All the complexes showed typical ^{1}IL ($\pi \rightarrow \pi^*$) (N–N and py-3-NCS or py-3-mal) absorption bands in a high-energy region (ca. 250–350 nm) and ^{1}MLCT ($d\pi(Re) \rightarrow \pi^*$(N–N)) absorption features in a lower energy region. Upon irradiation, all the complexes displayed intense and long-lived emission under ambient conditions. The photophysical data of selected complexes are shown in Table 1. It can be seen that complexes with electron-donating substituents on the diimine ligands emitted at higher energy than those with electron-withdrawing substituents or a more conjugated diimine ligand. On the basis of this emission energy trend, and the observed positive solvatochromism, it is evident that the emission of the complexes originates from an excited state of ^{3}MLCT ($d\pi(Re) \rightarrow \pi^*$(N–N)) character [13–23].

2.2. Bioconjugation studies

The bioconjugation properties of these luminescent complexes have been studied. First, the universal M13 reverse sequencing primer (16-mer) modified with an aminohexyl group at the 5′-end, **M13** (Fig. 4), has been labelled with [Re(phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$). The labelled primer

Table 1
Photophysical data (emission wavelength and quantum yields) of selected [Re(N⁀N)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$) and [Re(N⁀N)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$) at 298 K

Complex	Medium	λ_{em} (nm) (τ_0 (μs))	Φ_{em}
[Re(phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)	CH_2Cl_2	530 (2.51)	0.54
	CH_3CN	546 (1.35)	
	Solid	534 (0.32)	
[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)	CH_2Cl_2	492 sh, 510 (3.74)	0.17
	CH_3CN	514 (2.32)	
	Solid	480, 504 (max), 544 sh (0.32)	
[Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)	CH_2Cl_2	544 (6.54)	0.59
	CH_3CN	558 (3.74)	
	Solid	568 (2.02)	
[Re(biq)(CO)$_3$(py-3-NCS)](CF$_3$SO$_3$)	CH_2Cl_2	642 (0.10)	0.0024
	CH_3CN	650 (0.05)	
	Solid	613, 644 sh (0.32)	
[Re(phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$)	CH_2Cl_2	530 (2.30)	0.51
	Acetone	546 (1.84)	
	CH_3CN	546 (1.95 (5%), 0.19 (95%))	
[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$)	CH_2Cl_2	514 (5.21)	0.13
	Acetone	518 (2.45)	
	CH_3CN	518 (9.60 (70%), 1.64 (30%))	
[Re(4,7-Ph$_2$-phen)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$)	CH_2Cl_2	542 (7.42)	0.53
	Acetone	558 (2.48)	
	CH_3CN	558 (5.81 (15%), 1.06 (85%))	
[Re(biq)(CO)$_3$(py-3-mal)](CF$_3$SO$_3$)	CH_2Cl_2	632 (0.10)	0.0020
	Acetone	654 (0.05)	
	CH_3CN	650 (0.06)	

Reproduced from ref. [11,12], with permission of The Royal Society of Chemistry and The American Chemical Society.

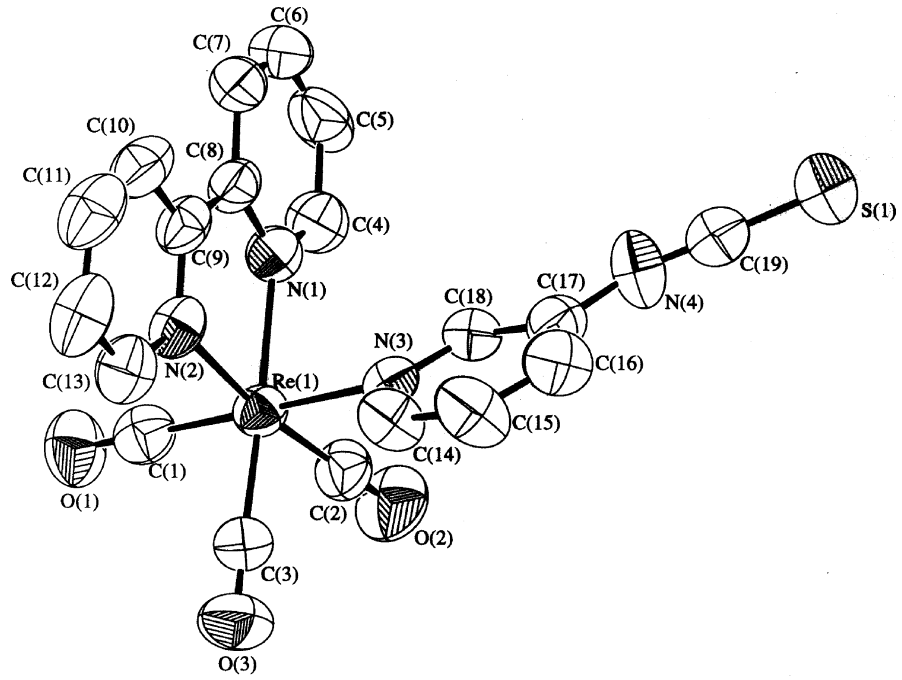

Fig. 2. Perspective view of the complex cation of $[Re(bpy)(CO)_3(py\text{-}3\text{-}NCS)](CF_3SO_3)$. Reproduced from ref. [11], with permission of The Royal Society of Chemistry.

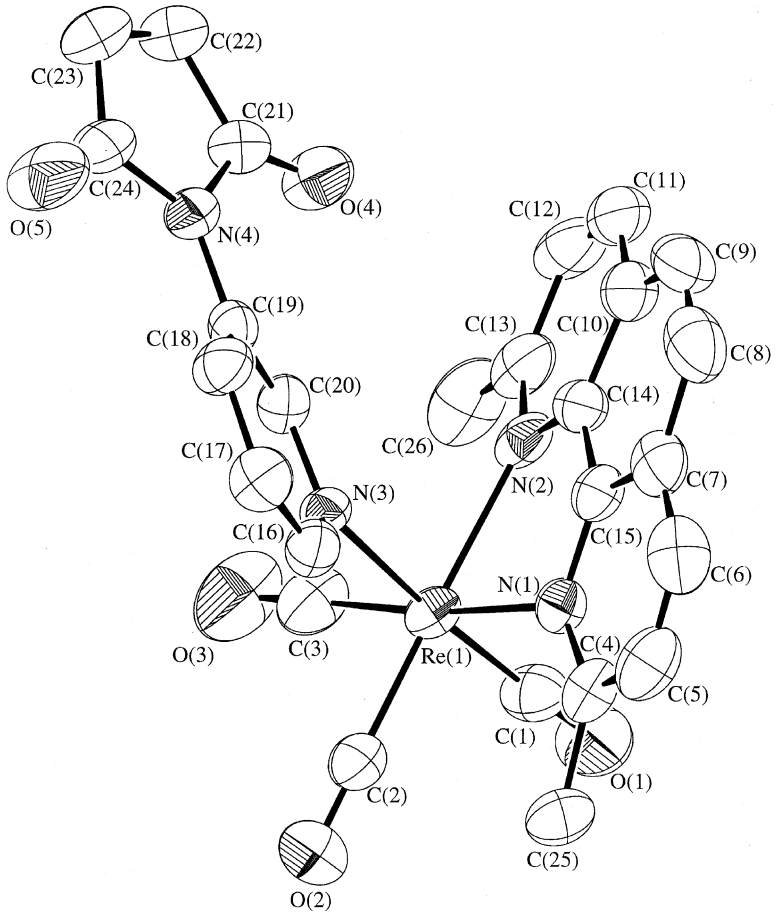

Fig. 3. Perspective view of the complex cation of $[Re(2,9\text{-}Me_2\text{-}phen)(CO)_3(py\text{-}3\text{-}mal)](CF_3SO_3)$. Reproduced from ref. [12], with permission of The American Chemical Society.

M13:

5'-H_2N-$(CH_2)_6$-AACAGCTATGACCATG-3'

M13-20:

5'-TAATCATGGTCATAGCTGTT-3'

M13-50:

5'-GAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTT-3'

M13-80:

5'-AATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTT-3'

Fig. 4. DNA sequences of **M13**, **M13–20**, **M13–50** and **M13–80**. The underlined sequence is complementary to that of M13. Reproduced from ref. [11], with permission of The Royal Society of Chemistry.

Re-M13 has been isolated, purified and shown to exhibit yellow luminescence (λ_{em} = 548 nm, τ_0 = 0.52 μs) in degassed 50 mM Tris–Cl buffer pH 7.4 at 298 K upon irradiation. The melting temperature (40 °C) of the duplex formed between this primer and its complementary DNA strand showed a decrease of only 2 °C compared to the unmodified double-stranded DNA molecule, suggesting that minimal changes occurred in the tertiary structure of the duplex after conjugation. The labelled primer has been used as a luminescent probe to target unmodified oligonucleotides. In this experiment, three different single-stranded DNA molecules (**M13–20** (20 mer), **M13–50** (50 mer) and **M13–80** (80 mer)) (Fig. 4), all of which contain a region that is complementary to **M13**, have been hybridised with **Re-M13**. The polyacrylamide gel (Fig. 5) showed three yellow luminescent bands, indicating the formation of three heteroduplexes (**Re-M13**)·(**M13–20**), (**Re-M13**)·(**M13–50**), and (**Re-M13**)·(**M13–80**). These results reveal that the hybridisation abilities of **M13** have been retained upon conjugation of the luminescent label.

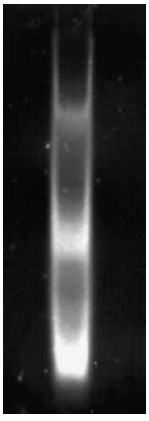

Fig. 5. Polyacrylamide gel electrophoresis result of a hybridisation mixture of **M13**, **M13–20**, **M13–50** and **M13–80**. Reproduced from ref. [11], with permission of The Royal Society of Chemistry.

Since the isothiocyanate moiety can react with the amine group of lysine [10], we have labelled the protein HSA with [Re(phen)$(CO)_3$(py-3-NCS)](CF_3SO_3) to further investigate the bioconjugation properties of the rhenium(I) isothiocyanate complexes. The polyacrylamide gel electrophoresis result showed an intense yellow luminescent band corresponding to a molecular weight of 66 kDa. A similar luminescent band was not observed in a control experiment in which an isothiocyanate-free rhenium(I) complex was used, indicating that the band was associated with the rhenium-labelled protein. The labelled conjugate displayed intense and long-lived yellow ^{3}MLCT ($d\pi(Re) \rightarrow \pi^*$(phen)) emission ($\lambda_{em}$ = 534 nm, bi-exponential decay: τ_1 = 0.79 μs, τ_2 = 0.13 μs) upon irradiation.

Since the maleimide moiety can undergo an addition reaction with the sulfhydryl group to form a stable thioether, we have used the rhenium(I) polypyridine maleimide complexes to label a thiolated oligonucleotide, a cysteine-containing tripeptide glutathione, and the serum albumins HSA and BSA. All the labelled biomolecules showed intense and long-lived ^{3}MLCT emission upon photoexcitation. The observation of long emission lifetimes, in the microsecond timescale, of these bioconjugates renders the rhenium(I) maleimide labels and their isothiocyanate counterparts promising candidates for time-resolved bioassays.

3. Iridium(III) polypyridine aldehyde, isothiocyanate and iodoacetamide complexes

3.1. *[Ir(pba)$_2$(N–N)](PF$_6$)*

Recently, there has been much interest in the utilisation of iridium(III) complexes as dopants in the fabrication

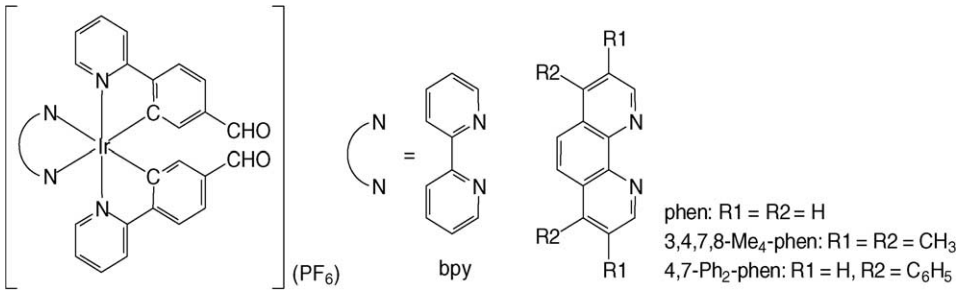

Fig. 6. Structures of $[Ir(pba)_2(N{-}N)](PF_6)$. Reproduced from ref. [52], with permission of Wiley-VCH.

of organic light-emitting diodes [24–26]. In fact, the photophysical properties of both Werner-type and cyclometallated iridium(III) complexes have been receiving much attention [27–55]. To investigate the potential of these complexes as biological labelling reagents, we have attached amine- and sulfhydryl-specific functional groups to various luminescent iridium(III) complexes. The first series of complexes $[Ir(pba)_2(N{-}N)](PF_6)$ (Fig. 6) involved different diimine ligands (N–N) and the aldehyde-containing cyclometallating ligand pba^- as it can react with the primary amine of biomolecules to form an imine that can be readily reduced to a stable secondary amine [52]. All the iridium(III)-pba complexes have been synthesised from the reaction of $[Ir_2(pba)_4Cl_2]$ with (N–N), followed by metathesis with KPF_6 and purification by recrystallisation. X-ray crystallographic studies of the complex $[Ir(pba)_2(bpy)](PF_6)$ revealed a separation of ca. 9 Å between the two aldehyde oxygen atoms (Fig. 7), suggesting that the aldehydes are sufficiently far apart to react with amine groups of two biomolecules, and that the complexes can function as luminescent biological cross-linkers. Unexpectedly, all the four complexes displayed very similar luminescence properties at 298 and 77 K, respectively. The photophysical data are tabulated in Table 2. The emission spectra of the bpy complex in CH_3CN at 298 K and in alcohol glass at 77 K are shown in Fig. 8. In fluid solutions at room temperature, the complexes showed vibron-

Table 2
Photophysical data (emission wavelength, lifetimes and quantum yields) of $[Ir(pba)_2(N{-}N)](PF_6)$

Complex	Medium (T (K))	λ_{em} (nm)	τ_0 (μs)	Φ_{em}
$[Ir(pba)_2(bpy)](PF_6)$	CH_2Cl_2 (298)	529, 563 sh	5.60	0.25
	CH_3CN (298)	534, 565 sh	4.71	0.27
	Glass[a] (77)	520 (max), 562, 612 sh	7.19	
$[Ir(pba)_2(phen)](PF_6)$	CH_2Cl_2 (298)	529, 564 sh	5.73	0.26
	CH_3CN (298)	534, 567 sh	5.42	0.21
	Glass[a] (77)	520 (max), 563, 612 sh	6.92	
$[Ir(pba)_2(3,4,7,8\text{-}Me_4\text{-}phen)](PF_6)$	CH_2Cl_2 (298)	532, 565 sh	5.35	0.27
	CH_3CN (298)	536, 568 sh	4.32	0.24
	Glass[a] (77)	520 (max), 563, 607 sh	6.62	
$[Ir(pba)_2(4,7\text{-}Ph_2\text{-}phen)](PF_6)$	CH_2Cl_2 (298)	530, 565 sh	5.32	0.25
	CH_3CN (298)	534, 566 sh	5.41	0.24
	Glass[a] (77)	520 (max), 566, 612 sh	6.90	

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[a] EtOH/MeOH (4:1, v/v).

Table 3
Photophysical data (emission wavelength, lifetimes and quantum yields) of $[Ir(pba)_2(N{-}N)](PF_6)$-$(Ala)_2$ in water and $[Ir(pba)_2(N{-}N)](PF_6)$-(Av) in 50 mM Tris–Cl pH 7.4 at 298 K

Conjugate	λ_{em} (nm)	τ_0 (μs)	Φ_{em}
$[Ir(pba)_2(bpy)](PF_6)$-$(Ala)_2$	565	0.17	0.059
$[Ir(pba)_2(phen)](PF_6)$-$(Ala)_2$	558	0.40	0.10
$[Ir(pba)_2(3,4,7,8\text{-}Me_4\text{-}phen)](PF_6)$-$(Ala)_2$	480 (max), 510, 550 sh	2.75	0.11
$[Ir(pba)_2(4,7\text{-}Ph_2\text{-}phen)](PF_6)$-$(Ala)_2$	570	0.42	0.15
$[Ir(pba)_2(bpy)](PF_6)$-(Av)	560	0.31	0.060
$[Ir(pba)_2(phen)](PF_6)$-(Av)	554	0.73	0.14
$[Ir(pba)_2(3,4,7,8\text{-}Me_4\text{-}phen)](PF_6)$-(Av)	486 (max), 514, 556 sh	3.56	0.15
$[Ir(pba)_2(4,7\text{-}Ph_2\text{-}phen)](PF_6)$-(Av)	570	0.65	0.16

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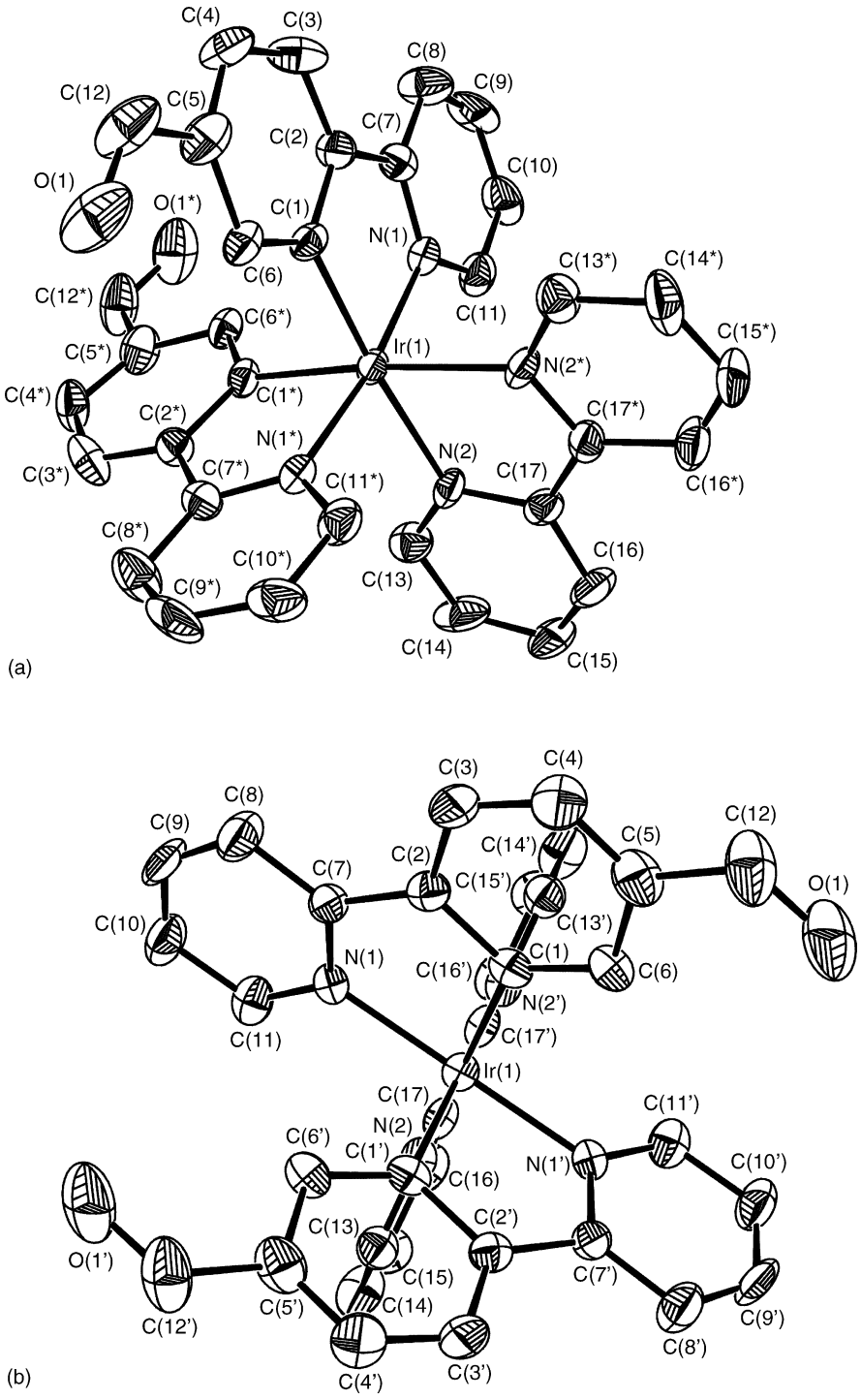

Fig. 7. Perspective views of the complex cation of $[Ir(pba)_2(bpy)](PF_6)$. Reproduced from ref. [52], with permission of Wiley-VCH.

ically structured spectra with very long emissive lifetimes. When the samples were cooled to 77 K, the emission spectra did not show a significant blue shift as commonly exhibited by cyclometallated iridium(III) diimine MLCT emitters. These observations strongly suggest that the emissive state of the complexes is ^{3}IL ($\pi \rightarrow \pi^*$) (pba^-) in nature. We have made use of the reactivity of the aldehyde groups of the complexes towards primary amines, and cross-linked L-alanine and the glycoprotein avidin, respectively. The cross-linked products have been isolated and characterised. Interestingly, the four cross-linked alanine conjugates showed various emission colours depending on the identity of the diimine ligands (Table 3). Specifically, the 3,4,7,8-Me_4-phen conjugate emitted with a green colour whilst the 4,7-Ph_2-phen analogue shows orange–yellow emission (Fig. 9). It is apparent that the conversion of the aldehyde moiety to

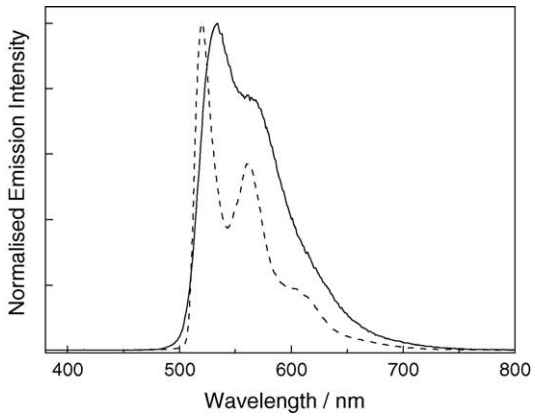

Fig. 8. Emission spectra of $[Ir(pba)_2(bpy)](PF_6)$ in degassed CH_3CN at 298 K (—) and EtOH/MeOH (4:1, v/v) at 77 K (- - -). Reproduced from ref. [52], with permission of Wiley-VCH.

a secondary amine has changed the emissive-state character from ^{3}IL ($\pi \rightarrow \pi^*$) (pba^-) to ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*(N–N)$). However, given the structured emission spectrum and the very long lifetime of the 3,4,7,8-Me_4-phen alanine conjugate, the emissive state of this labelled alanine should possess a high parentage of ^{3}IL ($\pi \rightarrow \pi^*$) (3,4,7,8-Me_4-phen) character [19]. The cross-linked avidin conjugates also showed long-lived and intense luminescence. Although the emission energy was similar to that of their alanine counterparts, higher emission quantum yields and longer emission lifetimes were observed (Table 3). These observations are attributed to the more hydrophobic environment associated with the protein molecule.

In view of the interesting photoluminescence properties of the avidin conjugates, a simple heterogeneous competitive assay for biotin using one of the conjugates and biotinylated microspheres has been developed. The assay was based on the competition between the immobilised biotin molecules and the free biotin analyte on binding to the luminescent avidin conjugate (Fig. 10). After the recognition reaction, the microspheres were removed by centrifugation and the emission intensity of the supernatant containing the mobile luminescent conjugate was measured. In our experiments, the emission of the supernatant was measured over a concentration range of biotin analyte from 1×10^{-3} to 1×10^{-9} M. Under the optimised conditions, biotin with a concentration from $1 \times 10^{-4.5}$ to 1×10^{-6} M could be measured by this assay (Fig. 11).

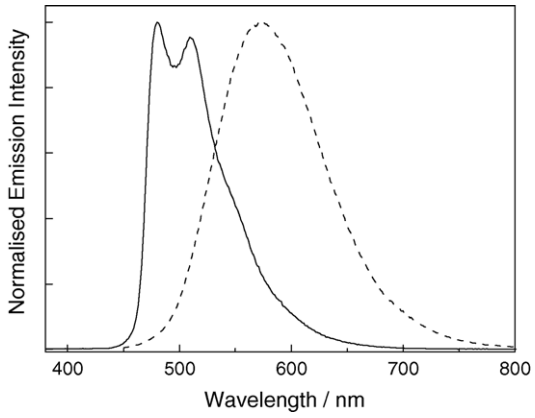

Fig. 9. Emission spectra of bioconjugates $[Ir(pba)_2(3,4,7,8\text{-}Me_4\text{-}phen)](PF_6)$-$(Ala)_2$ (—) and $[Ir(pba)_2(4,7\text{-}Ph_2\text{-}phen)](PF_6)$-$(Ala)_2$ (- - -) in degassed water at 298 K. Reproduced from ref. [52], with permission of Wiley-VCH.

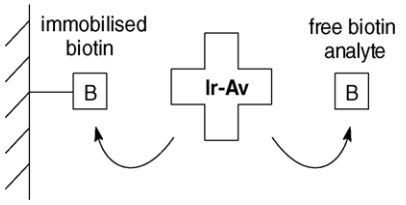

Fig. 10. Heterogeneous competitive assay for biotin using $[Ir(pba)_2(phen)](PF_6)$-(Av) (**Ir-Av**) and biotinylated microspheres. Reproduced from ref. [52], with permission of Wiley-VCH.

3.2. *[Ir(N–C)$_2$(N–N–R)](PF$_6$) (R = CHO, NCS and NHCOCH$_2$I)*

In addition to modifying the cyclometallating ligand, we have also incorporated aldehyde, isothiocyanate and iodoacetamide groups (R) into the diimine ligands (N–N) of the complexes $[Ir(N–C)_2(N–N–R)](PF_6)$ ($N–C^-$ = cyclometallating ligands, R = CHO, NCS and $NHCOCH_2I$) [50,53,54]; the structures of these complexes are shown in Fig. 12. The aldehyde complexes were obtained from the reaction of $[Ir_2(N–C)_4Cl_2]$ and bpy-CHO followed by anion exchange, whilst the isothiocyanate and iodoacetamide complexes were synthesised from the reactions of the precursor amine complexes $[Ir(N–C)_2(N–N–NH_2)](PF_6)$ ($N–N–NH_2$ = 4-amino-2,2′-bipyridine and 5-amino-1,10-phenanthroline) with $CSCl_2$ and iodoacetic anhydride

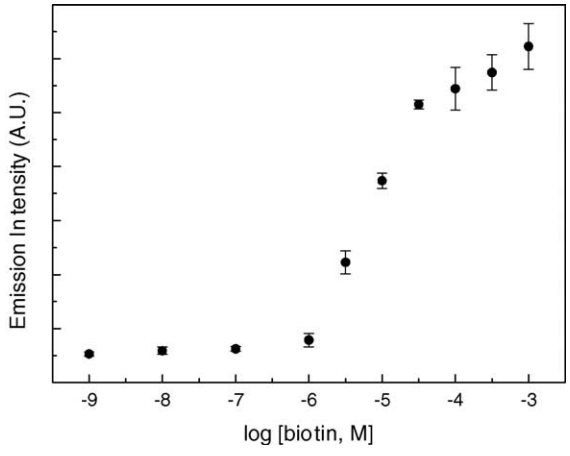

Fig. 11. Results of a heterogeneous competitive assay for biotin using biotinylated microspheres and the conjugate $[Ir(pba)_2(phen)](PF_6)$-(Av). The emission intensities of the supernatant are average of triplicate experiments ±1 standard deviation. Reproduced from ref. [52], with permission of Wiley-VCH.

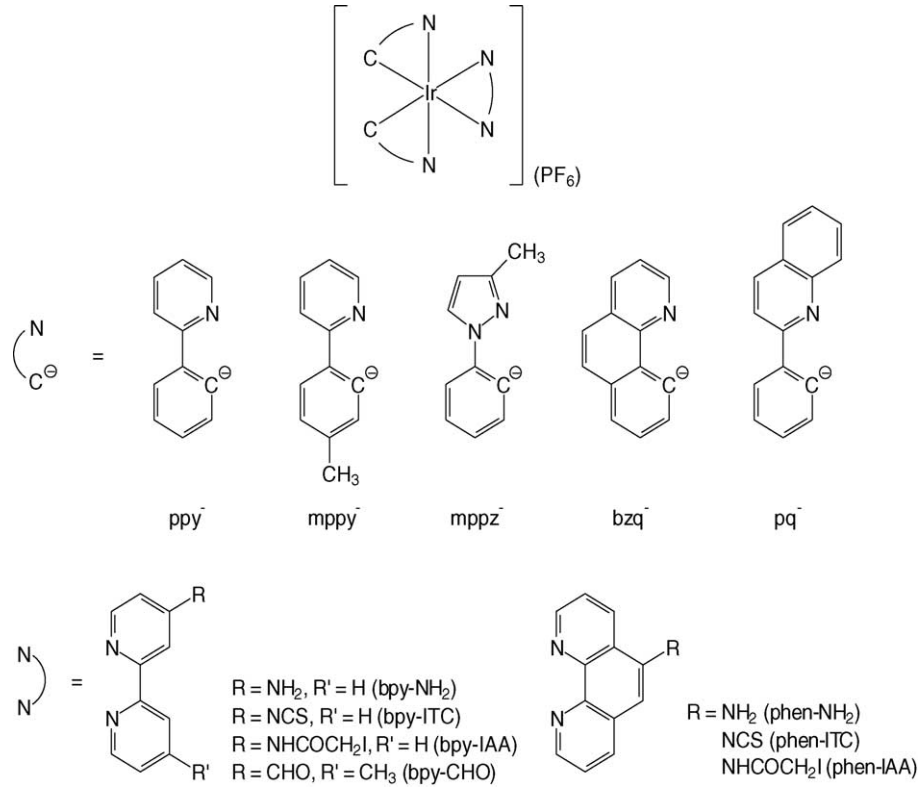

Fig. 12. Structures of $[Ir(N{-}C)_2(N{-}N{-}R)](PF_6)$. Reproduced from ref. [53], with permission of The American Chemical Society.

(or iodoacetyl chloride), respectively. Some of the complexes have been structurally characterised. The perspective views of the complex cations $[Ir(ppy)_2(bpy\text{-}CHO)]^+$ and $[Ir(mppz)_2(bpy\text{-}NH_2)]^+$ are shown in Figs. 13 and 14.

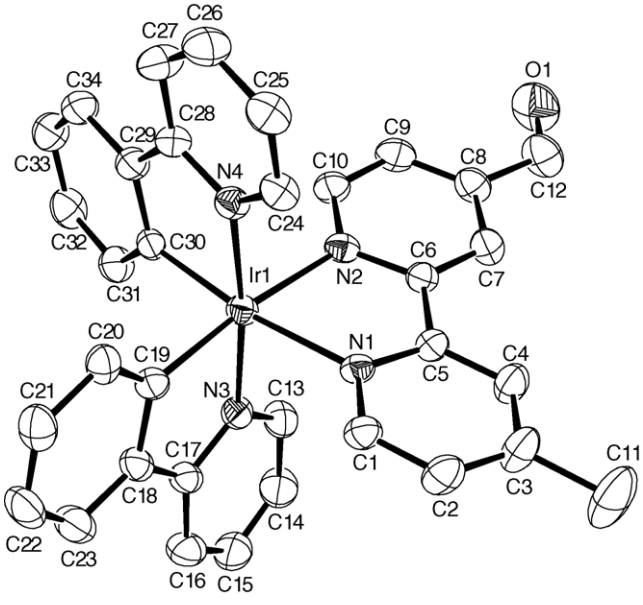

Fig. 13. Perspective view of the complex cation of $[Ir(ppy)_2(bpy\text{-}CHO)](PF_6)$. Reproduced from ref. [54], with permission of Elsevier Science.

Upon irradiation, all the complexes displayed intense and long-lived orange–red to greenish-yellow luminescence under ambient conditions and in alcohol glass at 77 K. The photophysical data of selected complexes are listed in Table 4. With reference to previous spectroscopic studies on luminescent iridium(III) systems, the emission of $[Ir(N{-}C)_2(N{-}N{-}R)](PF_6)$ was generally assigned to a triplet MLCT ($d\pi(Ir) \rightarrow \pi^*(N{-}N{-}R)$) excited state [27–29,31,35,38,41,46,50,53–55]. The assignment is supported by the findings that the amine-containing complexes emitted at the highest energy, followed by their aldehyde and iodoacetamide counterparts, whilst the isothiocyanate complexes emitted at the lowest energy. These observations are in line with the lowering π^* levels of the diimine ligands. To illustrate this trend, the emission spectra of $[Ir(mppz)_2(bpy\text{-}NH_2)](PF_6)$, $[Ir(mppz)_2(bpy\text{-}IAA)](PF_6)$ and $[Ir(mppz)_2(bpy\text{-}ITC)](PF_6)$ in CH_2Cl_2 at 298 K are shown in Fig. 15.

It is noteworthy that two groups of this family of complexes showed special emissive properties. First, the phen-NH_2 complexes $[Ir(N{-}C)_2(phen\text{-}NH_2)](PF_6)$ exhibited extraordinarily long emission lifetimes (from ca. 3.3 and 25.0 μs) in fluid solutions at 298 K. Also, the emission maxima of these complexes showed exceptionally small blue-shifts upon cooling to 77 K. It is likely that the emission of these phen-NH_2 complexes at room temperature originates from an excited state of substantial ^3IL ($\pi \rightarrow \pi^*$) (phen-NH_2) character [37,38,43–45,50,53]. The pq^- com-

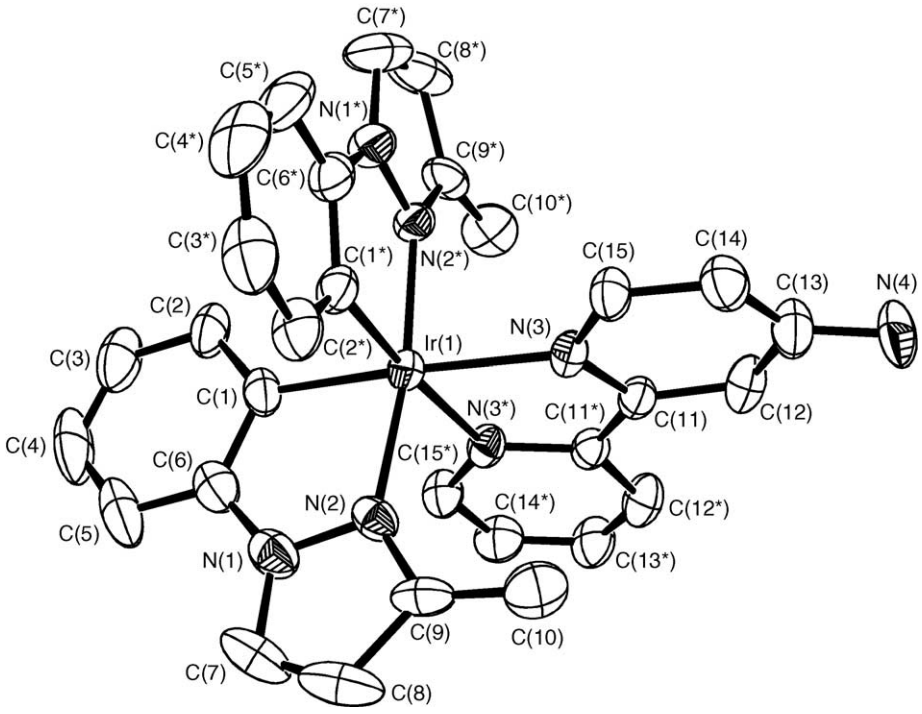

Fig. 14. Perspective view of the complex cation of $[Ir(mppz)_2(bpy\text{-}NH_2)](PF_6)$. Reproduced from ref. [53], with permission of The American Chemical Society.

plexes $[Ir(pq)_2(N{-}N{-}R)](PF_6)$ all showed very similar structural features in their emission spectra, irrespective of the identity of the diimine ligands. The emission spectra of $[Ir(pq)_2(bpy\text{-}NH_2)](PF_6)$ in CH_2Cl_2 at 298 K and in low-temperature alcohol glass are shown in Fig. 16. The emission lifetimes of these pq^- complexes were apparently longer than those of complexes with other cyclometallating ligands (except $[Ir(pq)_2(phen\text{-}NH_2)](PF_6)$). The emissive state of these complexes is likely to possess much ^3IL ($\pi \rightarrow \pi^*$) (pq^-) character and perhaps some ^3MLCT ($d\pi(Ir) \rightarrow \pi^*(pq^-)$) character as well [24,53–55]. The emission spectra of all these $[Ir(N{-}C)_2(N{-}N{-}R)](PF_6)$ complexes in alcohol glass at 77 K displayed structural features. In general, the emission is assigned to an ^3MLCT ($d\pi(Ir) \rightarrow \pi^*(N{-}N{-}R)$) excited state, with the probable mixing of some ^3MLCT ($d\pi(Ir) \rightarrow \pi^*(N{-}C^-)$) character. The two groups of complexes $[Ir(N{-}C)_2(phen\text{-}NH_2)](PF_6)$ and $[Ir(pq)_2(N{-}N{-}R)](PF_6)$ mentioned above also showed photophysical properties that were remarkably different from the other complexes, and ^3IL emissive states associated with the phen-NH_2 and pq^- ligands have been identified.

3.3. Bioconjugation studies

We have labelled amine- and sulfhydryl-containing biological molecules with a selection of the luminescent irid-

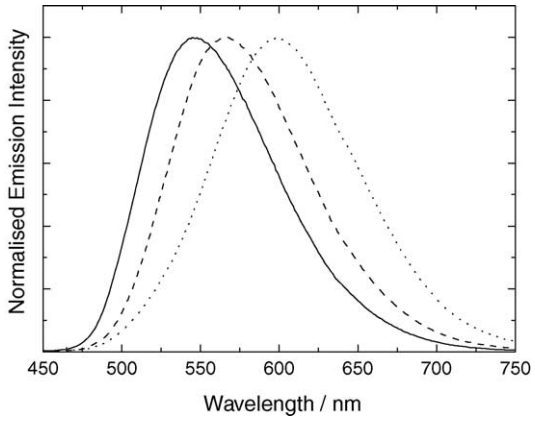

Fig. 15. Emission spectra of $[Ir(mppz)_2(bpy\text{-}NH_2)](PF_6)$ (—), $[Ir(mppz)_2(bpy\text{-}IAA)](PF_6)$ (- - -) and $[Ir(mppz)_2(bpy\text{-}ITC)](PF_6)$ (· · ·) in degassed CH_2Cl_2 at 298 K. Reproduced from ref. [53], with permission of The American Chemical Society.

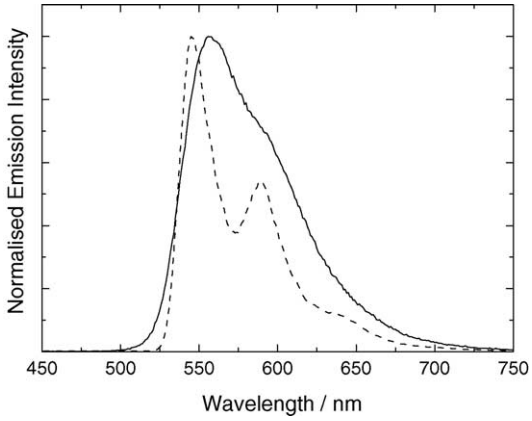

Fig. 16. Emission spectra of $[Ir(pq)_2(bpy\text{-}NH_2)](PF_6)$ in degassed CH_2Cl_2 at 298 K (—) and EtOH/MeOH (4:1, v/v) at 77 K (- - -). Reproduced from ref. [53], with permission of The American Chemical Society.

Table 4
Photophysical data (emission wavelength and quantum yields) of selected $[Ir(N\text{—}C)_2(N\text{—}N\text{—}R)](PF_6)$

Complex	Medium (T (K))	λ_{em} (nm)	τ_0 (μs)	Φ_{em}
$[Ir(mppz)_2(bpy\text{-}NH_2)](PF_6)$	CH_2Cl_2 (298)	546	1.05	0.285
	CH_3CN (298)	555	0.72	0.236
	Glass[a] (77)	486 (max), 518, 562 sh	9.50	
$[Ir(mppz)_2(bpy\text{-}CHO)](PF_6)$	CH_2Cl_2 (298)	567	0.58	0.013
	CH_3CN (298)	579	0.27	0.0071
	Glass[a] (77)	506, 543 sh	4.39	
$[Ir(mppz)_2(bpy\text{-}IAA)](PF_6)$	CH_2Cl_2 (298)	567	0.29	0.059
	CH_3CN (298)	575	0.21	0.024
	Glass[a] (77)	480 sh, 511, 537 sh, 609 sh	4.70	
$[Ir(mppz)_2(bpy\text{-}ITC)](PF_6)$	CH_2Cl_2 (298)	599	0.30	0.079
	CH_3CN (298)	595	0.26	0.033
	Glass[a] (77)	503 sh, 534	4.16	
$[Ir(pq)_2(bpy\text{-}NH_2)](PF_6)$	CH_2Cl_2 (298)	556, 602 sh	2.60	0.361
	CH_3CN (298)	562, 602 sh	2.58	0.270
	Glass[a] (77)	546 (max), 589, 639 sh	4.90	
$[Ir(pq)_2(bpy\text{-}CHO)](PF_6)$	CH_2Cl_2 (298)	559, 599 sh	2.16	0.098
	CH_3CN (298)	564, 599 sh	2.08	0.15
	Glass[a] (77)	543 (max), 583, 635 sh	4.87	
$[Ir(pq)_2(bpy\text{-}IAA)](PF_6)$	CH_2Cl_2 (298)	555, 596 sh	0.93	0.237
	CH_3CN (298)	559, 599 sh	1.02	0.147
	Glass[a] (77)	541 (max), 585, 640 sh	4.84	
$[Ir(pq)_2(bpy\text{-}ITC)](PF_6)$	CH_2Cl_2 (298)	556, 602 sh	2.02	0.240
	CH_3CN (298)	563, 604 sh	2.09	0.179
	Glass[a] (77)	542 (max), 581, 637 sh	4.82	
$[Ir(mppz)_2(phen\text{-}NH_2)](PF_6)$	CH_2Cl_2 (298)	554	10.36	0.253
	CH_3CN (298)	564	10.34	0.016
	Glass[a] (77)	560 (max), 606, 658 sh	308.83	
$[Ir(mppz)_2(phen\text{-}IAA)](PF_6)$	CH_2Cl_2 (298)	576	0.66	0.153
	CH_3CN (298)	586	0.39	0.078
	Glass[a] (77)	501 (max), 514, 535, 580 sh	15.57 (28%), 4.19 (72%)	
$[Ir(mppz)_2(phen\text{-}ITC)](PF_6)$	CH_2Cl_2 (298)	587	0.63	0.153
	CH_3CN (298)	602	0.28	0.132
	Glass[a] (77)	512 (max), 552, 598	44.82 (19%), 4.28 (81%)	
$[Ir(pq)_2(phen\text{-}NH_2)](PF_6)$	CH_2Cl_2 (298)	555, 599 sh	4.07	0.359
	CH_3CN (298)	559, 599 sh	5.99	0.169
	Glass[a] (77)	540 sh, 570, 615 sh, 681 sh	389.15	
$[Ir(pq)_2(phen\text{-}IAA)](PF_6)$	CH_2Cl_2 (298)	555, 599 sh	1.90	0.271
	CH_3CN (298)	558, 600 sh	2.22	0.281
	Glass[a] (77)	539 (max), 583, 630 sh	4.95	
$[Ir(pq)_2(phen\text{-}ITC)](PF_6)$	CH_2Cl_2 (298)	560, 599 sh	1.50	0.248
	CH_3CN (298)	559, 599 sh	1.13	0.172
	Glass[a] (77)	540 (max), 582, 626 sh	4.96	

Reproduced from ref. [53,54], with permission of The American Chemical Society and Elsevier Science.
[a] EtOH/MeOH (4:1, v/v).

ium(III) aldehyde, isothiocyanate and iodoacetamide complexes. The electronic absorption spectra of the protein conjugates showed an intense absorption band at ca. 280 nm, attributed to both the protein molecule and the complex absorption, and other absorption shoulders in the visible region mainly due to the iridium(III) complex. Upon photoexcitation, all the bioconjugates displayed intense and long-lived emission in aqueous buffer at 298 K. The emission origins of most of the conjugates have been identified as ^{3}MLCT ($d\pi(Ir) \rightarrow \pi^*$(diimine)) in nature. One of the iridium(III)–avidin conjugates $[Ir(pq)_2(phen\text{-}ITC)](PF)_6$-Av has been employed to recognise biotinylated DNA molecules by making use of resonance-energy transfer (RET) quenching [56]. Specifically, a degassed buffer solution of the conjugate showed emission quenching in the presence of a double-stranded DNA molecule in which the 3′-end of one strand had

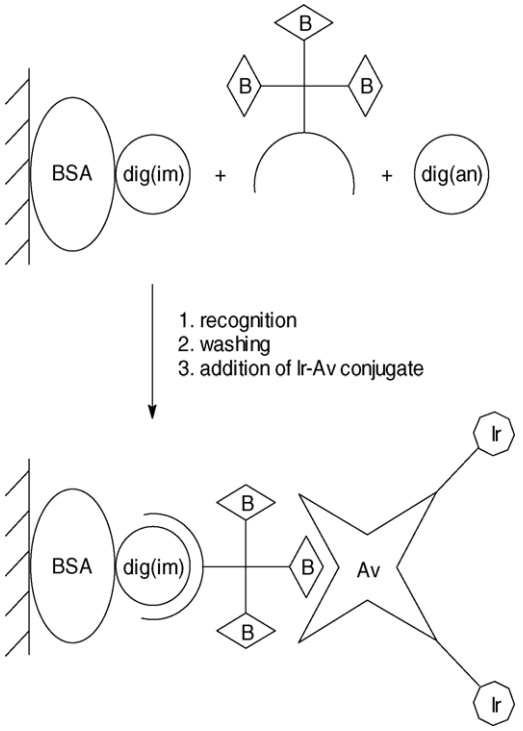

Fig. 17. Heterogeneous competitive assay for digoxin (BSA, immobilised bovine serum albumin as carrier protein; dig(im), immobilised digoxin; B, biotin; dig(an), digoxin analyte; Av, avidin; Ir, luminescent iridium labels). Reproduced from ref. [53], with permission of The American Chemical Society.

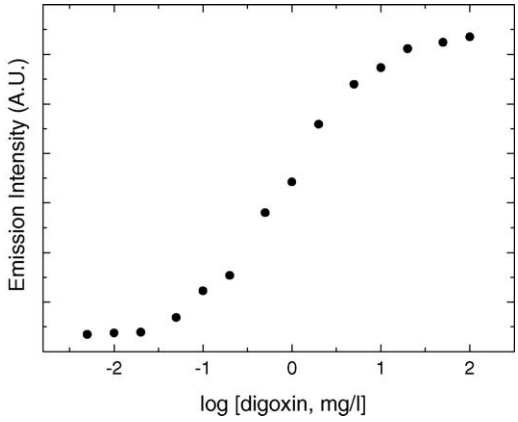

Fig. 18. Standard curve for the optimised heterogeneous competitive assays for digoxin using digoxin-modified microspheres, biotinylated anti-digoxin and the conjugate $[Ir(pq)_2(phen\text{-}ITC)](PF_6)$-Av. Reproduced from ref. [53], with permission of The American Chemical Society.

been biotinylated and the 5′-end of the other strand had been modified with the non-fluorescent energy-acceptor dye QSY-7 carboxylic acid hydroxysuccinimidyl ester. The dye QSY-7 was chosen because its absorption spectrum (λ_{max} = 560 nm) overlaps significantly with the emission spectrum of the iridium(III)–avidin conjugate (λ_{em} = 560, 603 (sh) nm) in aqueous buffer. It appears that distance-dependent RET plays an important role in this emission quenching since the lifetime decreased from ca. 3.16 to 2.06 μs whereas a similar decrease in the emission lifetime was not observed when a similar heteroduplex containing a non-biotinylated oligonucleotide was used (τ = 3.10 μs). We believe that these interesting results could form the basis of new time-resolved DNA hybridisation assays.

A new bioassay for the cardiac drug digoxin has been developed using the same luminescent iridium(III)–avidin conjugate. The assay is based on the competition between digoxin immobilised on microspheres and free digoxin analyte on binding to the biotinylated anti-digoxin (Fig. 17). After the competition and washing steps, the biotinylated anti-digoxin captured on the solid phase was recognised by the luminescent avidin conjugate. After incubation, the microspheres were removed and the emission intensity of the supernatant was measured. A higher concentration of the digoxin analyte resulted in a lower number of immobilised biotinylated antibodies, a higher number of luminescent avidin molecules in the supernatant, and thus, more intense emission. Under the optimised conditions, the concentration range of digoxin that could be measured was between ca. 0.05 and 20 mg/l (6.4×10^{-8} and 2.6×10^{-5} M) (Fig. 18). We expect that the detection limit of the assay can be improved using other approaches; for example, the iridium–avidin conjugates can be incorporated into other larger biomolecules to form luminescent macromolecular species, which is then used as the detection agent.

4. Rhenium(I) polypyridine biotin complexes

Avidin is a tetrameric glycoprotein (MW = 66 kDa) that consists of four identical subunits. This protein can bind up to four molecules of biotin (Vitamin H) non-cooperatively with exceptionally high affinity (K_d = ca. 10^{-15} M). Owing to the strong binding interaction, the avidin–biotin system has been widely utilised in bioanalytical applications [57,58]. Biomolecules can be modified with a wide range of biotinylating reagents and can then be recognised by avidin molecules that have been labelled with reporters such as fluorescent compounds or enzymes [59]. Since avidin has four biotin-binding sites, in theory, biotinylated biomolecules can be detected and quantified by fluorophore–biotin conjugates when avidin is used as a bridge. However, this approach is not feasible because most conventional fluorescent biotin molecules suffer from efficient self-quenching upon binding to avidin, unless long spacers are present between the fluorophore and biotin [60–64].

We anticipate that this problem can be solved using luminescent transition metal complexes as the reporter in view of their characteristic photophysical properties, in particular, the large Stokes' shifts. Although there have been reports on conjugation of the biotin moiety to different d-block metal complexes [65–68], the possibility of employing biotin–transition metal complex conjugates

Table 5
Photophysical data of [Re(N—N)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$) at 298 K

Complex	Medium	λ_{em} (nm[a])	τ_0 (μs[a])	Φ_{em}[a]	I (τ (μs))[b,c]	I (τ (μs))[b,d]	I (τ (μs))[b,e]
[Re(phen)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$)	CH$_3$CN	552	1.37	0.079	1.00 (0.56)	1.42 (0.90)	0.98 (0.55)
	CH$_2$Cl$_2$	536	2.69	0.25			
[Re(3,4,7,8-Me$_4$-phen)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$)	CH$_3$CN	518	7.32	0.072	1.00 (1.23)	2.25 (2.96)	1.04 (1.25)
	CH$_2$Cl$_2$	510	7.81	0.16			
[Re(2,9-Me$_2$-4,7-Ph$_2$-phen)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$)	CH$_3$CN	550	6.78	0.079	1.00 (1.84)	2.98 (2.70)	0.96 (1.90)
	CH$_2$Cl$_2$	540	7.22	0.22			

Reproduced from ref. [69], with permission of The American Chemical Society.
[a] In degassed solvents.
[b] Relative emission intensities in aerated 50 mM potassium phosphate buffer pH 7.4.
[c] [Re] = 15.2 μM, [avidin] = 0 μM, [unmodified biotin] = 0 μM.
[d] [Re] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 0 μM.
[e] [Re] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 380.0 μM.

as luminescent probes has not been investigated. Thus, we designed a new class of luminescent rhenium(I)–biotin complexes. The complexes [Re(N–N)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$) were synthesised from the reaction of [Re(N–N)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) with py-CH$_2$—NH-biotin, followed by metathesis with KPF$_6$ and purification by column chromatography [69].

These rhenium(I)–biotin complexes exhibited intense and long-lived ^{3}MLCT luminescence upon photoexcitation (Table 5). The binding of the complexes to avidin has been studied using the standard HABA assay [70]. The results revealed that all three complexes bound to avidin with the same stoichiometry as unmodified biotin (Re:avidin = 4:1). Interestingly, luminescence titrations using the rhenium(I)–biotin complexes as titrants showed that the complexes displayed enhanced emission intensity in the presence of avidin. At [Re]:[avidin] = 4:1, the emission intensity was ca. 1.4–3.0 times that of the complexes in the absence of avidin (Table 5). The emission lifetimes also showed an increase of ca. 1.5- to 2.4-fold. Emission titration curves for the 2,9-Me$_2$-4,7-Ph$_2$-phen complex are shown in Fig. 19. The increase in emission intensity and lifetimes is ascribed to the binding of the complex to the biotin-binding sites of avidin because no increase was observed when excess unmodified biotin was present (Table 5). These observations are in contrast to most fluorophore–biotin conjugates, which suffer from severe emission quenching upon binding to avidin due to RET, unless exceptionally long spacers such as poly(ethylene gly-

Table 6
Photophysical data of rhenium(I)–biotin complexes containing extended planar diimine ligands

Complex	Medium (T (K))	λ_{em} (nm)	τ_0 (μs)	Φ_{em}	I (τ (μs))[a,b]	I (τ (μs))[a,c]	I (τ (μs))[a,d]
[Re(dppz)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$)	CH$_2$Cl$_2$ (298)	558, 601 sh	4.90	0.0049	1.00 (0.39)	39.87 (0.91)	0.85 (0.39)
	CH$_3$CN (298)	556, 599 sh	7.12	0.0016			
	Glass[e] (77)	547 (max), 562 sh, 592, 614 sh, 645 sh	12887				
[Re(dppz)(CO)$_3$(py-CH$_2$—NHCOC$_5$H$_{10}$NH-biotin)](PF$_6$)	CH$_2$Cl$_2$ (298)	556, 607 sh	4.50	0.0049	1.00 (0.28)	1.92 (0.50)	0.89 (0.26)
	CH$_3$CN (298)	554, 597 sh	8.01	0.0031			
	Glass[e] (77)	544 (max), 559 sh, 590, 612 sh, 638 sh	12405				
[Re(dppn)(CO)$_3$(py-CH$_2$—NH-biotin)](PF$_6$)	CH$_2$Cl$_2$ (298)	587	17.88	0.20	1.00 (0.26)	3.65 (0.40)	1.28 (0.27)
	CH$_3$CN (298)	595	31.47	0.13			
	Glass[e] (77)	517, 556 (max), 603 sh	5.77				
[Re(dppn)(CO)$_3$(py-CH$_2$—NHCOC$_5$H$_{10}$NH-biotin)](PF$_6$)	CH$_2$Cl$_2$ (298)	588	22.15	0.23	1.00 (0.22)	2.39 (0.29)	1.09 (0.20)
	CH$_3$CN (298)	595	33.09	0.095			
	Glass[e] (77)	524, 558 (max), 607 sh	6.72				

Reproduced from ref. [73], with permission of The American Chemical Society.
[a] Relative emission intensities in degassed 50 mM potassium phosphate buffer at pH 7.2.
[b] [Re] = 15.2 μM, [avidin] = 0 μM, [unmodified biotin] = 0 μM.
[c] [Re] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 0 μM.
[d] [Re] = 15.2 μM, [avidin] = 3.8 μM, [unmodified biotin] = 380.0 μM.
[e] EtOH/MeOH (4:1, v/v).

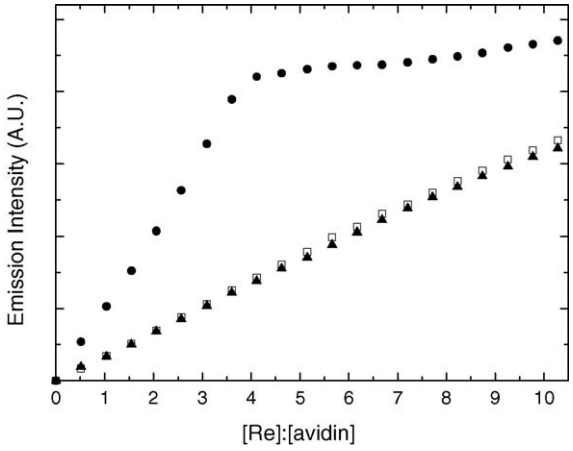

Fig. 19. Luminescence titration curves for the titrations of (i) 3.8 μM avidin (●), (ii) 3.8 μM avidin and 380.0 μM unmodified biotin (▲), and (iii) a blank phosphate buffer solution (□) with [Re(2,9-Me_2-4,7-Ph_2-phen)$(CO)_3$(py-CH_2-NH-biotin)](PF_6). Reproduced from ref. [69], with permission of The American Chemical Society.

col) are present between the fluorophore and the biotin units [60–64]. The spacers provide sufficient separation between the fluorophores bound to the same avidin molecule to eliminate the quenching effects. The quenching is in line with the observations that biomolecules labelled with organic fluorophores do not become more highly fluorescent with higher degrees of labelling [71]. The absence of emission quenching in the present case is due to the insignificant overlap between the absorption and emission spectra of the rhenium(I)–biotin complexes, which disfavours RET quenching. Since both the emission intensity and lifetimes of the excited complexes are sensitive to the hydrophobicity of the environment, indicated by the fact that the luminescence quantum yields and lifetimes of all three complexes increased from CH_3CN to CH_2Cl_2 (Table 5), it is conceivable that the enhancement results from the hydrophobicity associated with the binding pockets of avidin. Another possible reason is the increased rigidity of the surroundings of the complexes upon the binding event because such an increase in rigidity can lead to lower non-radiative decay efficiency. It is worth mentioning that the organic compound 2-anilinonaphthalene-6-sulfonic acid also exhibits avidin-induced fluorescence enhancement and it has been used as an apolar probe to investigate the environment of the biotin-binding sites of this protein [72]. However, the binding of this molecule does not rely on native biotin–avidin interactions and is thus apparently much weaker (K_d = ca. 10^{-4} M) [72] than the current rhenium(I)–biotin complexes (K_d = ca. 10^{-9}–10^{-11} M) [69] and other organic fluorophore–biotin conjugates (K_d = ca. 10^{-9} M) [61].

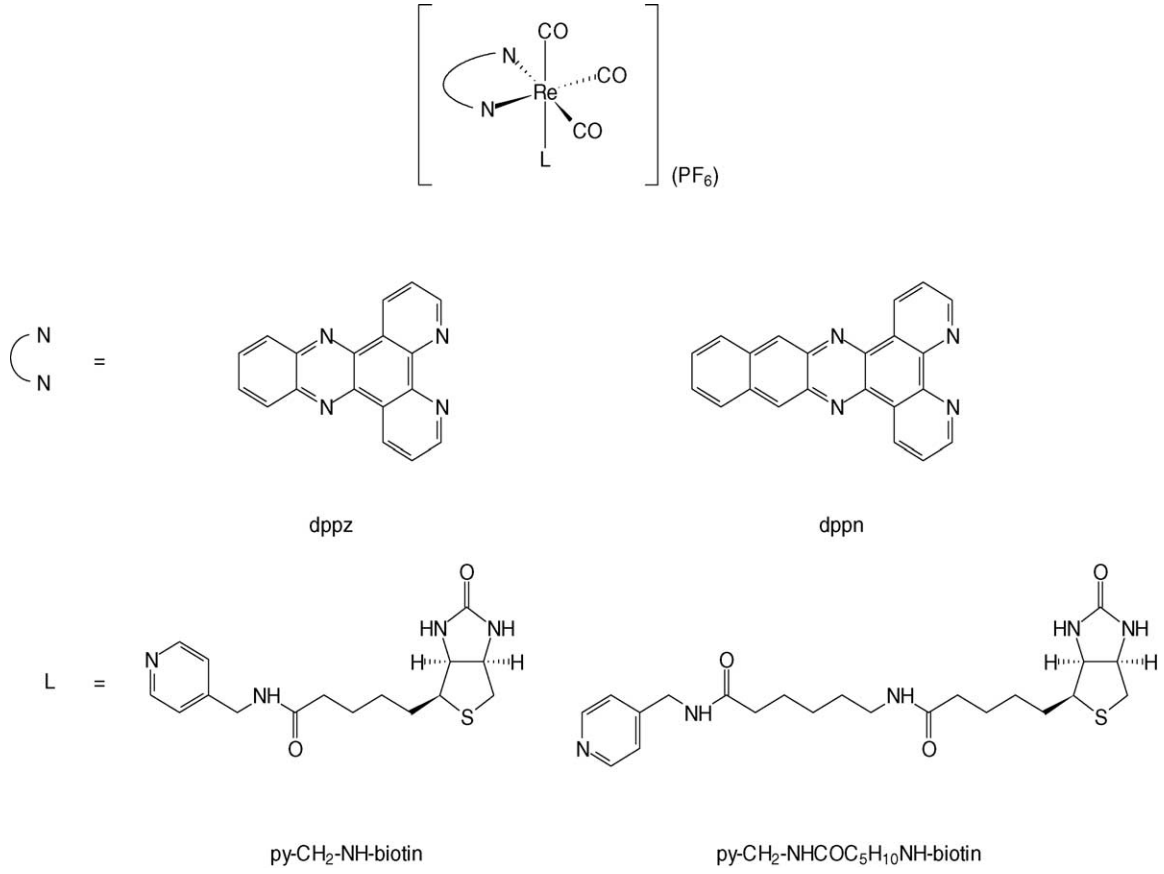

Fig. 20. Structures of rhenium(I)–biotin complexes containing extended planar diimine ligands. Reproduced from ref. [73], with permission of The American Chemical Society.

We have utilised the complexes as probes for biotin and biotinylated species using quencher-labelled avidin. The 2,9-Me_2-4,7-Ph_2-phen complex in a degassed buffer solution exhibited a decrease of emission lifetime from 6.02 to 4.90 μs in the presence of QSY-7-modified avidin. The quenching is due to distance-dependent RET because a similar decrease was not observed when excess unmodified biotin had been present initially (τ = 5.94 μs). These results could lead to the design of new time-resolved assays for biotinylated species and avidin.

Another aim of our project is to develop bi-functional luminescent probes for both DNA and protein molecules. To achieve this, we have recently expanded this family of rhenium(I)–biotin complexes by making use of extended planar diimine ligands dppz and dppn, and the incorporation of a spacer-arm into the biotin-containing pyridine ligands (Fig. 20) [73]. We expected that the extended planar diimine ligands would allow the complexes to bind to double-stranded DNA molecules [4,74], whilst the biotin moieties would enable the complexes to bind to avidin. Excitation of the complexes in solutions at room temperature and in low-temperature alcohol glass gave rise to intense and long-lived greenish-yellow to orange luminescence. The photophysical data are summarised in Table 6. Upon addition of double-stranded calf thymus DNA, the low-energy absorption bands of all the complexes exhibited pronounced hypochromism and a small bathochromic shift, and the emission of the complexes was substantially enhanced. The absorption and emission spectral traces of [Re(dppz)$(CO)_3$(py-CH_2–NH-biotin)](PF_6) are shown in Fig. 21 to illustrate these changes. These observations are ascribed to the binding of the complexes to the double helical DNA molecules by intercalation [4,74–83]. Similar to other rhenium(I)–biotin complexes described above, the HABA assays showed that all the complexes bind to avidin with a stoichiometry of 4:1. The emission intensity and lifetimes of the complexes also exhibited an increase in the presence of avidin (Table 6). The emission intensity enhancement factors varied from ca. 1.9 to 40. The emission spectral changes of [Re(dppz)$(CO)_3$(py-CH_2–NH-biotin)](PF_6) upon addition of avidin are shown in Fig. 22. The results of the titrations indicate that the complexes bind to both double-stranded DNA with an intrinsic binding constant of ca. $10^4\ M^{-1}$, and avidin with dissociation constant in the order of ca. 10^{-9} M. To the best of our knowledge, these complexes act as the first luminescent sensory systems that are responsive to both DNA molecules and avidin.

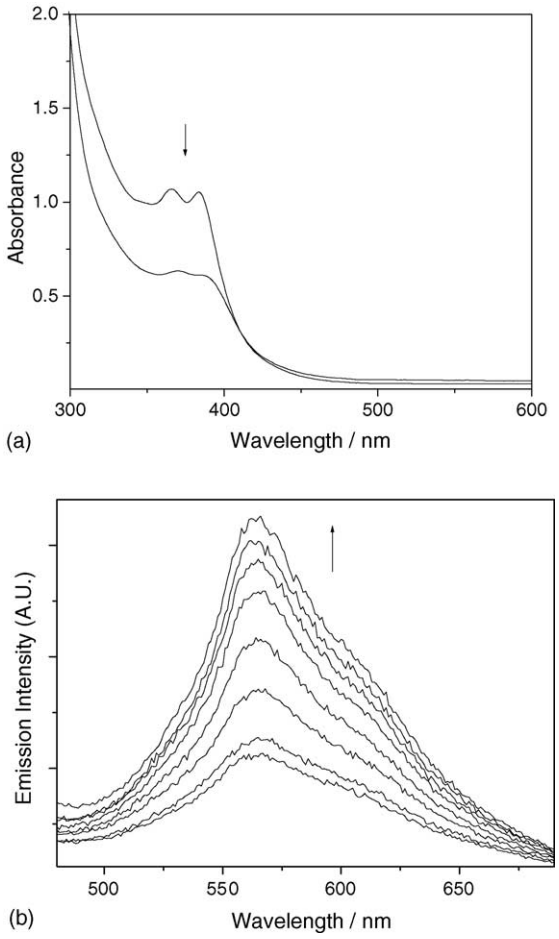

Fig. 21. (a) Absorption and (b) emission spectral traces of [Re(dppz)$(CO)_3$(py-CH_2–NH-biotin)](PF_6) in Tris–Cl buffer/methanol upon addition of double-stranded calf thymus DNA. Reproduced from ref. [73], with permission of The American Chemical Society.

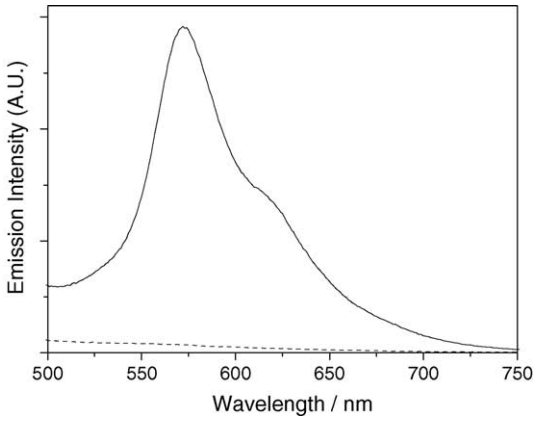

Fig. 22. Emission spectra of [Re(dppz)$(CO)_3$(py-CH_2–NH-biotin)](PF_6) in the absence (- - -) and presence (—) of avidin in degassed potassium phosphate buffer. Reproduced from ref. [73], with permission of The American Chemical Society.

5. Concluding remarks

This review article describes our recent work on the design of luminescent transition metal complexes as biological labels and probes. Although the most commonly studied ruthenium(II) polypyridine complexes emit with a broad emission band in the orange–red region, recent studies have clearly shown that the emission of structurally related complexes

such as those of iridium(III) can occur from the blue to the red regions, spanning the entire visible spectrum [84]. These properties enable the possibility of multi-colour detection and thus multiplexed bioassays. Although common d-block metal complexes cannot compete with many organic fluorophores in terms of emission quantum yields, the interesting electrochemiluminescence properties of transition metal polypyridine complexes have been documented [85–88]. Without the requirement of light excitation, the detection sensitivity of these complexes can be significantly enhanced concomitant with a lower limit of detection. In fact, biological reagents based on electrochemiluminescent transition metal complexes have been reported [89–91] and related systems would receive further attention in the near future.

Concerning the luminescent biotin system, we believe that the avidin-induced emission enhancement is not limited to rhenium(I) polypyridine complexes, but is common to other transition metal complexes that show minimal overlap between the absorption and emission spectra, and those that display environment-sensitive emission. We have recently demonstrated that related iridium(III)– [55] and ruthenium(II)–biotin complexes [92] also exhibit similar avidin-binding properties. From the results, it is apparent that more hydrophobic complexes can give rise to stronger binding, but too high hydrophobicity will substantially lower the solubility of complexes in aqueous solution. Additionally, a longer spacer-arm between the luminophore can enhance the binding affinity, but at the same time it renders the complex more exposed to the aqueous environment after the binding and thus lowers the enhancement factors of emission intensity (I/I_o) and lifetimes (τ/τ_o). These remarks will be taken into consideration in the future design of related biotin-containing luminescent complexes.

Overall, the emission properties of transition metal complexes can be described as a combination of those of organic fluorophores and lanthanide chelates. Instead of replacing these two classes of compounds, luminescent transition metal complexes offer a new and supplementary choice of biological labels and probes, and possess unique properties that can be exploited for specific applications.

Acknowledgements

We thank the Hong Kong Research Grants Council (Project Numbers CityU 1116/00P, CityU 1113/02P and CityU 101603) and the City University of Hong Kong (Project Numbers 7100212, 7100272 and 7001456) for financial support. W.-K.H., C.-K.C. and K.H.-K.T. acknowledge the receipt of a Postgraduate Studentship and a Research Tuition Scholarship, both administered by the City University of Hong Kong. We are grateful to Professor Vivian W.-W. Yam of The University of Hong Kong for access to the equipment for photophysical measurements.

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