BIOLOGICAL LABELLING
REAJECTS AND PROBES
DERIVED FROM LUMINESCENT
RHENIUM(I) POLYPYRIDINE
COMPLEXES

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Biological Labelling Reagents and Probes
Derived from Luminescent Rhenium(I)
Polypyridine Complexes
從具發光性錸多吡啶絡合物衍生出之生物標記試劑及探測器

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Abstract

A series of luminescent rhenium(I) polypyridine complexes containing an isothiocyanate moiety, [Re(N–N)(CO)₃(py-3-NCS)](CF₃SO₃), has been synthesised and characterised. The X-ray crystal structure of [Re(bpy)(CO)₃(py-3-NCS)](CF₃SO₃) has been investigated. Upon photoexcitation, these complexes display intense and long-lived green to orange-red photoluminescence. The emission wavelengths vary from 502 to 650 nm and the emission lifetimes range from 0.05 to 9.15 µs. The luminescence is assigned to a triplet metal-to-ligand charge-transfer (³MLCT) (dπ(Re) → π*(diimine)) excited state. The incorporation of an isothiocyanate moiety into the pyridine ring enables these complexes to target the amine groups of modified oligonucleotides and protein molecules. An amine-modified oligonucleotide and human serum albumin have been labelled with one of the complexes, [Re(phen)(CO)₃(py-3-NCS)](CF₃SO₃), to form bioconjugates. These bioconjugates exhibit strong and long-lived luminescence in aqueous buffers under ambient conditions. In addition, a heterogeneous competitive assay for biotin has been developed using avidin labelled with one of the complexes and biotinylated microspheres.

A new class of luminescent rhenium(I) polypyridine maleimide complexes [Re(N–N)(CO)₃(py-3-mal)](CF₃SO₃) has been synthesised, characterised and their photophysical and electrochemical properties studied. The crystal structure of [Re(2,9-Me₂-phen)(CO)₃(py-3-mal)](CF₃SO₃) has been established. These complexes exhibit intense and long-lived emission in fluid solutions at 298 K upon photoexcitation. The emission wavelengths vary from 514 to 654 nm and the emission lifetimes fall in the microsecond
time scale. On the basis of the photophysical data, the emission is assigned to an $^3\text{MLCT} \ (d\pi(\text{Re}) \rightarrow \pi^*(\text{diimine})$ excited state. The maleimide group allows these complexes to be used as sulfhydryl-specific luminescent labels for biomolecules such as thiolated oligonucleotides, the reduced form of glutathione, bovine serum albumin and human serum albumin. The photoluminescence properties of biological species labelled with these complexes have been investigated.

A family of biotin-containing luminescent rhenium(I) polypyridine complexes, [Re(N–N)(CO)$_3$(py-L-biotin)](PF$_6$)$_2$, with various diimine ligands and spacers of different lengths and hydrophobicity between the monopyridine ring and the biotin group, has been prepared. The photophysical and electrochemical properties of these complexes have been studied. Excitation of the complexes in fluid solutions at 298 K results in intense and long-lived luminescence. The photophysical studies indicate that the emissive states of the complexes possess predominantly $^3\text{MLCT}$ ($d\pi(\text{Re}) \rightarrow \pi^*(\text{diimine})$) character. The interactions of these rhenium(I)–biotin complexes with avidin have been investigated by 4’-hydroxyazobenzene-2-carboxylic acid (HABA) assays, luminescence titrations and competitive association and dissociation assays using native biotin. Results from HABA assays show that all the complexes bind to avidin with a stoichiometry of 4:1. In sharp contrast to common biotin–fluorophore conjugates, all the rhenium(I)–biotin complexes display emission enhancement and lifetime elongation upon binding to avidin. A homogeneous competitive assay for biotin has been designed. Furthermore, a homogeneous assay for avidin based on the resonance energy-transfer principle using a quencher-conjugated polypeptide has been developed.
A series of luminescent rhenium(I)–indole conjugates that can function as probes for indole-binding proteins has been designed. These luminescent rhenium(I) polypyridine indole complexes [Re(N=NN(CO)₃(py-3-CO-NH-en-indole)](CF₃SO₃) exhibit moderately intense and long-lived $^3\text{MLCT (d}_\pi\text{(Re)} \rightarrow \pi^*(\text{diimine})$ emission in fluid solutions at 298 K and in low-temperature glass upon irradiation. The X-ray crystal structure of one of the complexes, [Re(3,4,7,8-Me₄-phen)(CO)₃(py-3-CO-NH-en-indole)](CF₃SO₃), has been studied. The photophysical and electrochemical properties of these indole-containing complexes have been compared to those of their indole-free counterparts [Re(N=NN(CO)₃(py-3-CO-NH-Et)](CF₃SO₃). The binding of the complexes to indole-binding proteins including bovine serum albumin and lysozyme has been studied by spectroscopic titrations. The inhibition of an indole-binding protein, tryptophanase, by the complexes has been investigated and compared to that of unmodified indole.
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