Luminiscent Metal Complexes for Diagnostic Applications
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Summary

The aim of this Thesis was to design and investigate luminescent metal complexes for diagnostics applications. As outlined in Chapter 1 (Section 1.1), there is a strong interest in the development of luminescent probes for highly sensitive assays, such as immunoassays and DNA-probe assays. Population of the luminescent excited state can be achieved by a chemical reaction between two species of sufficient energy (e.g. electrochemiluminescence (ECL)) or by photoexcitation. High emission quantum yield of the probe is a fundamental requirement for highly sensitive analysis. Furthermore, emission decay-times of a few microseconds are also required in order to avoid background signal by means of time-gated detection, in particular for the assays where the probe is excited upon irradiation. A promising class of compounds for luminescent probe technologies are transition metal complexes with a lowest triplet metal-to-ligand charge-transfer (3MLCT) excited state, such as ruthenium and osmium polypyridyl complexes. 3MLCT states can indeed be long-lived, exhibit relatively high emission quantum yields and are in general stable with respect to cleavage of metal-ligand bonds. Furthermore, due to their low energy they can be populated by a strongly exergonic reaction, such as electron transfer between two redox species (e.g. in ECL reactions).

In diagnostics, the ECL-assays are usually performed in aqueous solution, employing [Ru(bpy)_3]^{2+} (bpy = 2,2'-bipyridine) as label for the biological molecules and tripropylamine (TPrA) as sacrificial reductant. In an attempt to improve the sensitivity (signal-to-noise ratio) of the ECL-assays, we decided to study the behaviour of a series of modified ruthenium complexes with 2,2'-bipyridine ligands bearing substituents in 4,4'- and 5,5'-positions (Chapter 3). The spectroscopic and ECL properties of the increasingly hydrophobic complexes [Ru(4,4'-di-CH_3-bpy)_3]^{2+}, [Ru(4-CH_3-4'-CH_2C(CO_2Et)_3-bpy)_3]^{2+} and [Ru(4,4'-di-CH_2C(CO_2Et)_3-bpy)_3]^{2+} and their 5,5'-substituted derivatives [Ru(5,5'-di-CH_3-bpy)_3]^{2+} and [Ru(5,5'-di-CH_2C(CO_2Et)_3-bpy)_3]^{2+} are discussed in Chapter 3. An enhancement of the efficiency for the ECL reaction was expected due to hydrophobic interactions with non-ionic surfactant present in the assay buffer (see Chapter 1). Even though for [Ru(5,5'-di-CH_3-bpy)_3]^{2+} the ECL intensity is enhanced by 10% as compared to the reference [Ru(bpy)_3]^{2+}, no direct proof for the influence of the hydrophobicity of the complexes on the ECL reaction could be found. Substitution of the bipyridine ligands with the bulky -CH_2C(CO_2Et)_3 groups at the 4,4'-positions resulted in steric hindrance of the metal complex toward the reaction with TPrA. However, substitution at the 5,5'-positions causes much less steric hindrance and the complex then reacts more readily with TPrA.

A more successful approach to enhance the sensitivity of the ECL-assays regards larger number of ECL active centres contained within the label. Dendrimers are a convenient tool for the design of large structures with several metal centres. Particularly promising are...
multinuclear systems with the metal units at the periphery of the dendrimer, as their redox reactions are not hindered. In Chapter 4 the syntheses, spectroscopic data and ECL behaviour of two di- and trinuclear homometallic complexes are described. The compounds contain ruthenium tris(2,2'-bipyridine) units covalently linked by the amino acid lysine (Lys) or the corresponding dipeptide (LysLys). The electrochemical and spectroscopic results prove that the bridging ligands (Lys and LysLys) do not affect the electronic properties of the metal centres, their spectroscopic properties being similar to those of [4-carboxypropyl-4'-methyl-2,2'-bipyridine]bis(2,2'-bipyridine)ruthenium(II) complex, [Ru-Ref]$_2^{2+}$, isostructural with the ruthenium moieties in the two oligonuclear complexes. The ECL studies showed that 30% enhancement of the ECL intensity can be achieved for the dinuclear and trinuclear complexes, as compared to [Ru-Ref]$_2^{2+}$. A greater improvement is prevented by their slow diffusion. Heterogeneous ECL studies, performed on larger dendritic complexes, containing up to eight ruthenium units, showed that limitations due to slow diffusion can be easily overcome by means of the nanoparticle technology, leading to ECL intensities proportional to the number of ruthenium moieties in the complex (Chapter 4). However, care must be taken when considering large multimetallic complexes, as hydrophobic interactions with nanoparticles and biological moieties in the assay buffer may dramatically increase the background signal.

Dendrimers can also be important means to prolong the excited state lifetime of a luminescent metal core by protecting it from molecular oxygen and other quenchers. In Chapter 5 the syntheses and photophysical investigations of three ruthenium complexes with dendritic ligands are presented. As metal core [Ru(4,7-(SO$_3$-Ph)$_2$-phen)$_3$]$^{4+}$ (4,7-(SO$_3$-Ph)$_2$-phen = 4,7-(diphenylsulfonate)-1,10-phenanthroline) was used, known to emit with lifetime of 3.8 $\mu$s in deaerated water at room temperature. The dendritic complexes exhibit lifetimes of ca. 7 $\mu$s at room temperature in deaerated acetonitrile. In agreement with a shielding effect by the dendritic branches on the central metal core, the oxygen quenching rate constants decrease upon increasing the length of the branches.

A different approach for the elongation of the excited state lifetime is the design of metal complexes with a large aromatic (appended or chelating) ligand possessing long-lived triplet excited state of suitable energy. In particular, a good matching between the energies of $^3$MLCT (triplet metal-to-ligand charge transfer) state of the metal centre, that is often the lowest excited state in ruthenium and osmium polypyridyl complexes, and the $^3$IL (triplet intraligand) state of the large aromatic ligand, could lead to an equilibrium between these states, resulting in longer emission decay time. In Chapter 6 the redox and photophysical properties of two homonuclear metal complexes [(bpy)$_3$Ru(bidppz)Ru(bpy)$_3$]$^{4+}$ and [(bpy)$_2$Os(bidppz)Os(bpy)$_2$]$^{4+}$ (bpy = 2,2'-bipyridine, bidppz = 1,1'-dipyrido[3,2-a:2',3'-c]phenazin-1,1'-ylidipyrido[3,2-a:2',3'-c]phenazine) are described. The bridging bidppz ligand has long-lived $^3$IL state at low energy. Strong solvent dependence of the spectroscopic properties of the two complexes was observed. In particular, [(bpy)$_3$Ru(bidppz)Ru(bpy)$_3$]$^{4+}$
shows long excited state lifetime (9.7 \mu s) in deaerated dichloromethane that becomes much shorter (360 ns) in deaerated butyronitrile. (Sub)picosecond transient absorption spectra in dichloromethane showed that thermal population of the long-lived triplet intraligand (3IL) state of the bridging ligand from the slightly higher-lying 3MLCT state takes place and is responsible for the observed long emission decay time. In butyronitrile the energy of the 3MLCT state is lowered due to the polarity of the medium and the 3IL state is not thermally accessible. For [(bpy)_2Os(bidppz)Os(bpy)_2]^{4+} the 3MLCT state lies low in energy even in dichloromethane and no interaction of this state with the 3IL state of the bridging ligand occurs.

Due to the large interest on photoinduced processes for vectorial and long range charge and energy transfer within covalently linked units, we decided to study the (spectro)electrochemical and the photophysical behaviour of the heteronuclear [(bpy)_2Ru(bidppz)Os(bpy)_2]^{4+} (Chapter 7). Steady-state emission and transient absorption measurements are in agreement with photoinduced energy transfer occurring from the excited ruthenium moiety to the osmium unit. Although the electrochemical results and the steady-state absorption spectra show only weak electronic interaction between the ruthenium and osmium metal centres, a large energy transfer rate constant \( k_{\text{en}} > 10^{11} \text{ s}^{-1} \) was found. Such a fast process is most probably due to a Dexter-type energy transfer mechanism.