Lipid bilayers decorated with photosensitive ruthenium complexes

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1

General Introduction

Abstract

In this chapter an overview is given concerning photosensitive polypyridyl ruthenium complexes. The photosubstitution reactions of these complexes and their applications as light-controlled molecular machines and light-activatable anticancer compounds are presented. Lipid bilayers are introduced as a link between these two research fields. Lipid bilayers can be used on the one hand as surfaces where the molecular motion of ruthenium complexes can occur, and on the other hand as molecular carriers for drug delivery of anticancer ruthenium compounds.

1.1. Photosensitive polypyridyl ruthenium(II) complexes

1.1.1. Photoreactivity and photophysical properties

Ruthenium(II) complexes with polypyridyl ligands have been extensively studied because they show a variety of interesting properties in the excited-state, such as photosubstitution, photoluminescence, photo-redox chemistry, and photoisomerization processes. The unique photophysical and photochemical properties of these complexes allow them to be used in numerous medicinal and technological applications.[1-3]

Ru^{II} is a d^6 octahedral system; the polypyridine ligands usually have σ donor orbitals localized on the nitrogen atoms, and π donor and π* acceptor orbitals delocalized on the aromatic rings. Transition of an electron from a t_{2g} metal-based orbital to a π^*_L ligand orbital typically results in a metal-to-ligand charge transfer (MLCT) excited states, whereas promotion of an electron from the t_{2g} to the e_g orbitals gives rise to a metal-centered (MC) excited state (Figure 1.1). The geometry of the metal center in a ^{3}MC excited state is strongly modified with respect to the ground state geometry notably along the metal-ligand bonds. When the lowest excited state has ^{3}MC character, it usually undergoes either fast, radiationless deactivation to the ground state, or ligand dissociation reactions (Figure 1.2a). Thus, the excited state lifetime is very short at room temperature and no radiative decay (luminescence) to the ground state is observed. On the other hand, since the ground state (GS) and MLCT states do not involve a change in e_g orbital occupation, their corresponding potential wells are usually not significantly modified along the Ru–L coordinates. Consequently, when the lowest excited state is ^{3}MLCT it does not undergo fast radiationless decay to the ground state and luminescence is usually observed (Figure 1.2b). In such a case, the lifetime of the ^{3}MLCT excited state is typically temperature dependent, as it can be promoted to the ^{3}MC state thermally, which leads to photosubstitution reaction or rapid non-radiative decays to the ground state.[3-4] Overall, the photochemical behavior of ruthenium(II) complexes, *i.e.*, either their excellent luminescence properties or their ability for photochemical ligand exchange, is strongly influenced by the relative energy levels of the ^{3}MC and ^{3}MLCT excited states.

Many strategies have been considered to modify the energy difference between the ^{3}MLCT and ^{3}MC states of the complex and get the desired behavior under light irradiation. One strategy is the adjustment of the electronic properties of the polypyridyl ligands, which affects the energy of the ^{3}MLCT state and also the ligand

field splitting energy.[4] The second strategy is to vary the steric properties of the ligand to increase or reduce the energy difference between the ^{3}MC and ^{3}MLCT excited states. Thus, the relative energy levels of the various excited states, and thereby the nature of the lowest excited state, can be controlled by tuning the properties of the polypyridyl ligands in ruthenium(II) complexes.[5-6]

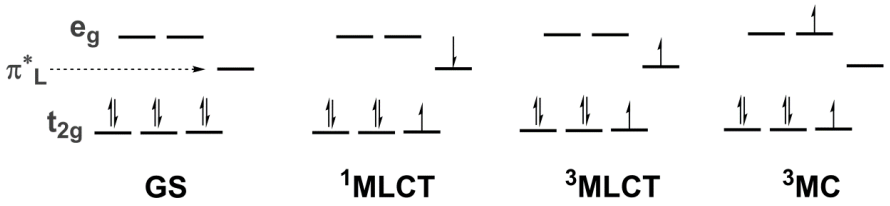


Figure 1.1. Schematic orbital diagram for the electronic ground state (GS) and the excited states for $[Ru(bpy)_3]^{2+}$ complex. Adapted from reference [4].

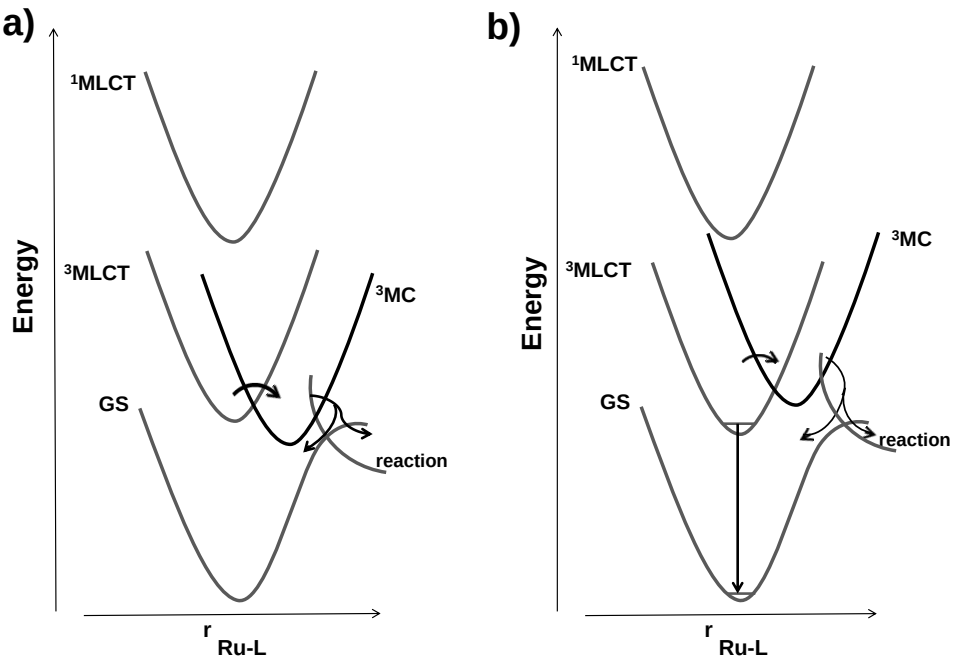


Figure 1.2. Potential well diagrams showing the relative energies of the ^{3}MC and ^{3}MLCT for Ru(II) polypyridyl complexes. (a) The ^{3}MC is the lowest excited state, and (b) the ^{3}MLCT is the lowest excited state. Ru-L is a coordination bond, where L is a nitrogen- or sulfur-donor ligand. Adapted from reference [3].

$[Ru(bpy)_3]^{2+}$ is one of the most investigated polypyridyl ruthenium(II) complexes (bpy = 2,2'-bipyridine).[3, 7] This complex has D_3 symmetry and its lowest excited state is of ^{3}MLCT character with a long lifetime at room temperature (~1 μs). It thus undergoes relatively slow radiationless transitions and rather intense emission.[3] By replacing one bpy ligand with a constrained bipyridyl ligand like 1,2-di(pyridin-3-yl)ethane, the ligand field splitting energy decreases due to the modification of the N-Ru-N bite angle. Distortion of the complex and lower ligand field splitting energy reduces the energy of ^{3}MC state. A decrease in the energy gap between the ^{3}MLCT and ^{3}MC is observed and the ^{3}MC becomes thermally accessible from the ^{3}MLCT state, which facilitates non-radiative decay back to the ground state (GS). As a result at room temperature the emission intensity of the ruthenium complex with 1,2-di(pyridin-3-yl)ethane is much lower (almost no emission in acetonitrile) than the emission of $[Ru(bpy)_3]^{2+}$.[8]

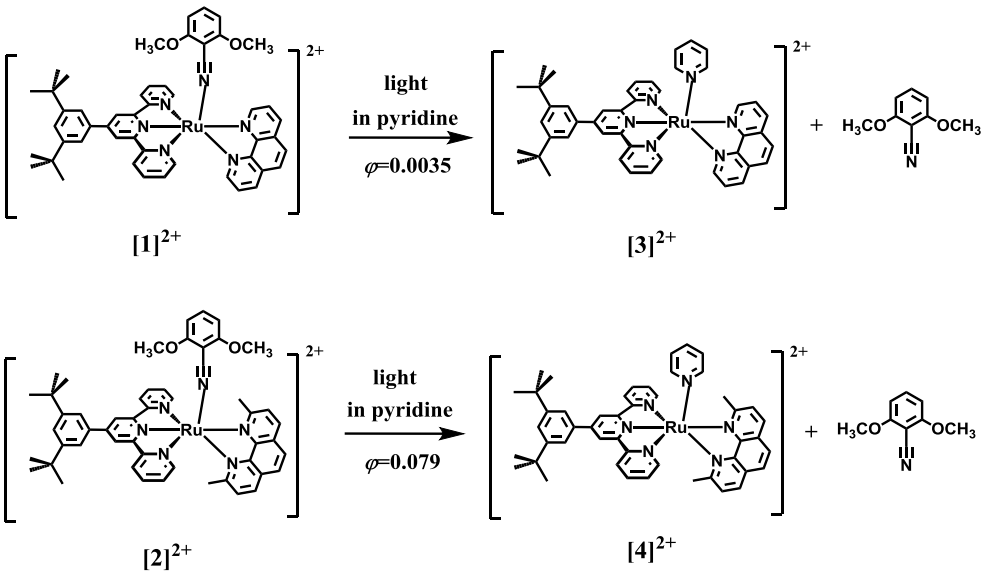
Similarly, using rigid tridentate ligands such as 2,2′;6′,2″-terpyridine (terpy) induces an even greater distortion from the ideal octahedral geometry compared to the Ru(II) complexes containing only bidentate ligands, since the N–Ru–N *trans* angles are significantly smaller than 180° with coordinated terpyridine ligands.[9] As a result, the complex $[Ru(terpy)_2]^{2+}$ for example is only luminescent at 77 K, whereas at room temperature the ^{3}MLCT excited state is quenched.[10-11] In the extreme case, Ru[(6,6"-dptpy)]$^{2+}$ (dpterpy = 6,6"-dipheny1-2,2';6',2''-terpyridine) does not show any luminescence even at 77 K. A possible explanation is the presence of inter-ligand steric repulsions, which may further weaken the ligand field splitting, and as a consequence lower the energy of the ^{3}MC state below that of the ^{3}MLCT state, to fully quench emission.[10] Overall, more distortion in the coordination octahedron results in lower luminescence intensity for Ru(II) complexes.

1.1.2. Photosubstitution reactions

Photochemically labile ruthenium(II) complexes are capable of selectively photosubstituting a given ligand upon visible light irradiation.[12-15] Decreasing the energy of the ^{3}MC state, for example by introducing distortion in the coordination octahedron, not only renders non-radiative processes more efficient, but also allows for the thermal population of the ^{3}MC state from the ^{3}MLCT state. Such thermal population of ^{3}MC states may lead to photocleavage of one ligand L of the

coordination sphere, followed more or less simultaneously by the coordination of an incoming ligand L', typically a coordinating solvent molecule.[16]

Ruthenium complexes of the $[Ru(terpy)(N\text{-}N)(L)]^{2+}$ family, where N–N is a bidentate diimine ligand like 1,10-phenanthroline (phen) or 2,2'-bipyridine (bpy), and L is a neutral monodentate ligand, typically have enough distortion in their coordination sphere to selectively photosubstitute the monodentate ligand L.[17] In a study by Collin *et al.*, the photosubstitution of 2,6- -dimethoxybenzonitrile (MeOBN) by pyridine in a pyridine solution of $[Ru(terpy^*)(N\text{-}N)(L)]^{2+}$ was investigated, where terpy* is 4'-(3,5-ditertiobutylphenyl)- 2,2';6',2''-terpyridine and N-N is phen or 2,9-dimethyl-1,10-phenanthroline (dmp). The study showed that using a sterically hindered dmp ligand, instead of the non-hindered ligand phen, resulted in an increase of the photosubstitution quantum yield by a factor 20 (Scheme 1.1). More steric interactions between dmp and MeOBN led to more efficient photoexpulsion of MeOBN from the octahedral coordination sphere of the metal.[13]

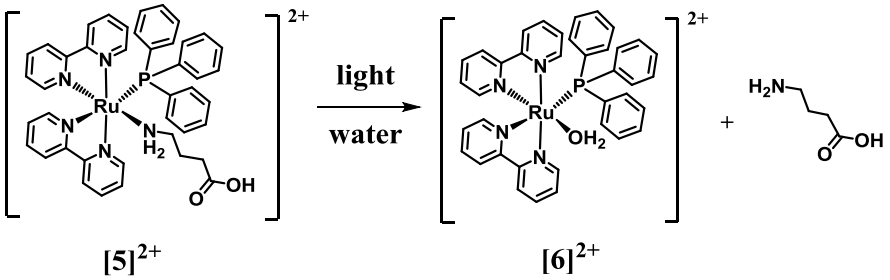


Scheme 1.1. Increasing the photosubstitution quantum yield by distorting the coordination sphere of the ruthenium complex. φ represents the photosubstitution quantum yield. Adapted from reference [13].

The electronic properties of the ligands can also affect the rate and efficiency of photosubstitution processes. In a recent study by Turro *et al.*,[18] the role of the ^{3}MLCT

state in photosubstitution reactions was investigated by changing the electronic properties of the leaving ligands. In this study, the photosubstitution of ligand L in $[Ru(bpy)_2(L)]^{2+}$, where L is a bidentate sulfur-donor ligand like 3,6-dithiaoctane or a bidentate nitrogen-donor ligand like 1,2-diaminoethane, was investigated. Higher photosubstitution quantum yields were reported in the former case. Based on DFT calculation, it was shown that the elongation of the Ru-S bond in the ^{3}MLCT triplet state is larger than that of a Ru-N bond, which means that the Ru-S bonds are weaker in the ^{3}MLCT excited state than Ru-N bonds, and will lead more efficiently to photosubstitution.

In the ruthenium(II) complexes of the $[Ru(bpy)(X)(Y)]^{2+}$ family, the monodentate ligands X and Y can be efficiently photosubstituted by solvent molecules. Modifying the properties of these monodentate ligands helps promoting the photodissociation of one of them, while allowing the other one to be photochemically stable. Typically, weaker σ donor ligands like phosphites, thioethers, or triazoles, were reported to be photoreleased faster than stronger σ donors such as pyridines, amines, or phosphines. Etchenique and co-workers have investigated the properties of these complexes to apply them as phototriggered caged molecules.[19] In complex $[\mathbf{5}]^{2+}$ (Scheme 1.2), PPh_3 is a weaker σ-donor and stronger π-acceptor than the amino group of γ-aminobutyric acid. Thus, upon irradiation with visible light the amine ligand is substituted by a water molecule to give $[\mathbf{6}]^{2+}$, but the phosphine ligand in $[\mathbf{6}]^{2+}$ remains coordinated even upon further irradiation.[20]



Scheme 1.2. Amine *vs.* phosphine reactivity in the photosubstitution of a monodentate ligand in complex $[\mathbf{5}]^{2+}$ upon visible light irradiation[20].

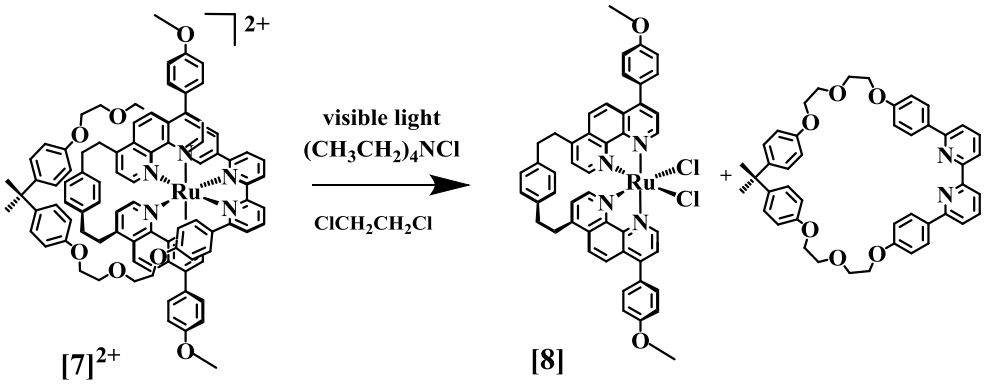
1.1.3. Ruthenium-based molecular machines

A molecular machine can be defined as an assembly of different molecular components, *i.e.*, a supramolecular structure, designed to perform a specific mechanical function in response to an appropriate external stimulus such as light, electricity, or chemical energy.[21-22] The extension of the concept of machine from the macroscale to the molecular level is believed to be valuable for the development of nano-sized devices. Furthermore, it helps understanding the complex behavior of biological molecular machines such as ATPases or myosin, by mimicking their functions.[23-25] With such a concept in mind, the controlled unidirectional motion of single molecules is an ultimate goal that has been challenged mostly by organic chemists. For example, unidirectional motion in a mechanically interlocked assembly (molecular rotor)[26] and 'walking' of a two-legged molecular unit on a four-foothold molecular track (linear molecular machine),[27] have been reported by Leigh and co-workers.

Light irradiation, in particular, is a powerful tool to induce molecular motion. Several molecular machines have been reported that are powered by photonic stimuli.[28-31] Transition metal-containing catenanes and rotaxanes for example have been considered for building such systems, and among them multicomponent ruthenium(II) complexes, in which one part of the molecule can be set in motion photochemically with respect to the other part.[32-35] These systems take advantage of the dissociative, metal-centered ^{3}MC state described in Section 1.1.1 to perform the motion in one direction by photosubstitution of one ligand. The reverse motion usually occurs thermally, to reset the molecule into the initial, photosensitive state. In such systems, sterically hindered chelating ligands are necessary to distort the octahedral geometry of the ruthenium(II) complexes and allow thermal population of the ^{3}MC sate from the photochemically generated ^{3}MLCT state.[17, 36] Complexes of the $[Ru(diimine)_2(N\text{-}N)]^{2+}$ family with hindered N-N ligands have been reported by the group of Sauvage and co-workers.[37-41] Two examples from this family are discussed below.

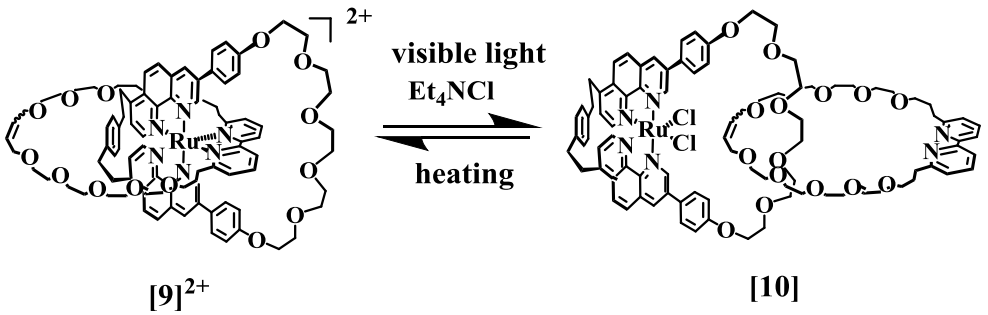
A rotaxane-based ruthenium complex forms by threading a N-N-containing macrocycle onto a $Ru(diimine)_2$-containing helical axis. Rigidity of the macrocycle is important for obtaining only the *endo*-coordinated isomer, where the helical axis passes through the macrocycle. As shown in Scheme 1.3, a $Ru(phen)_2$-based complex ($[\mathbf{8}]^{2+}$) can act as an axis, and a 6,6'-diphenyl-2,2'-bipyridine-based (dpbpy) macrocycle is threaded through the ruthenium axis to form the pseudo-rotaxane ruthenium complex $[\mathbf{7}]^{2+}$.

Under visible light irradiation, de-coordination of the dpbpy-containing ring was observed, leading to the separate fragments.[40]



Scheme 1.3. Photoinduced dissociation of the macrocycle from a pseudo-rotaxane $[Ru(diimine)_3]^{2+}$ complex. Adapted from reference [40].

The second example consists of the catenane-based ruthenium complex $[\mathbf{9}]^{2+}$ containing two interlocked rings. A macrocycle is usually used as a templating element in order to incorporate the $[Ru(diimine)_3]^{2+}$ core in the catenane (see Scheme 1.4).

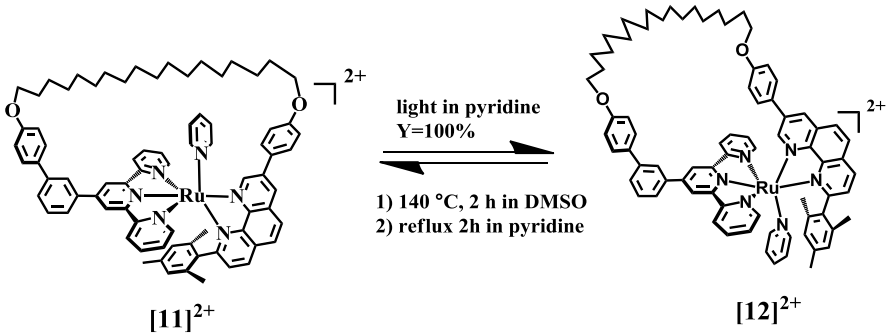


Scheme 1.4. The catenane-based ruthenium complex $[\mathbf{9}]^{2+}$ undergoes a thermally reversible and complete rearrangement upon visible light irradiation [17].

The $[Ru(diimine)_2]$ -containing fragment is a 63-membered ring incorporating two phen units, whereas the N-N-bidentate fragment is a 42-membered ring containing a 6,6'-disubstituted bipyridine ligand. Light irradiation leads to the dissociation of the bpy ligand from the ruthenium center, to form complex [**10**]. The starting complex

[**9**]$^{2+}$ was recovered by heating the system. The size of the macrocyclic ring has a strong influence on the photoreactivity of the ruthenium complex: a catenane with a smaller ring than in complex [**9**]$^{2+}$ was reported to be less photoreactive.[36] Recently, a biisoquinoline-based 39-membered macrocycle was shown to improve the shuttling kinetics in this kind of mechanically interlocked coordination compounds.[42]

Another strategy reported by the same group[43] is to build a macrocycle using the Ru(terpy)(phen) core instead of Ru(phen)$_2$. In the sixth coordination position a monodentate ligand that can be photosubstituted should be included inside the macrocyclic cavity (Scheme 1.5).[43] In complex [**11**]$^{2+}$ a Ru(terpy)(phen) macrocyclic core was formed by connecting the terpy unit to the phen unit by a $(CH_2)_{18}$ linker and the monodentate pyridine ligand is included inside the ring. White light irradiation of this isomer induces the formation of a "photochemical" isomer [**12**]$^{2+}$ where the phen moiety has rotated by an angle of 90° compared to the terpy chelate. Such rotation leads to a major rearrangement of the alkyl linker chain. The reverse rotation of the phen chelate was achieved by heating the photochemical isomer in dimethylsulfoxide to recover, after ligand exchange, the initial "thermal" isomer [**11**]$^{2+}$. This is an example of quantitative, light-induced isomerization of a ruthenium polypyridyl complex.[43]



Scheme 1.5. Re-organization of a flexible $(CH_2)_{18}$ chain by the photoinduced rotation of the phen chelate in [**11**]$^{2+}$. The reverse motion is obtained by heating the complex in DMSO, followed by ligand exchange in pyridine [43].

In all of these examples the light-controlled motion of ruthenium-based molecules was performed in homogeneous solutions. Linear motion in homogeneous solution can be achieved for rotaxane-based transition-metal complexes when the ring moves from a

given position on the rotaxane axle to another position and vice versa.[35] These type of linear motors have been developed in order to mimic natural linear molecular machines such as myosin or kinesin, which move along the linear track of actin filament or microtubules, respectively, using ATP as a fuel (Figure 1.3a).[44] Ideal mimicking of the linear motion of natural molecular machines would be obtained by the development of molecules walking on a surface or on an artificial molecular track.

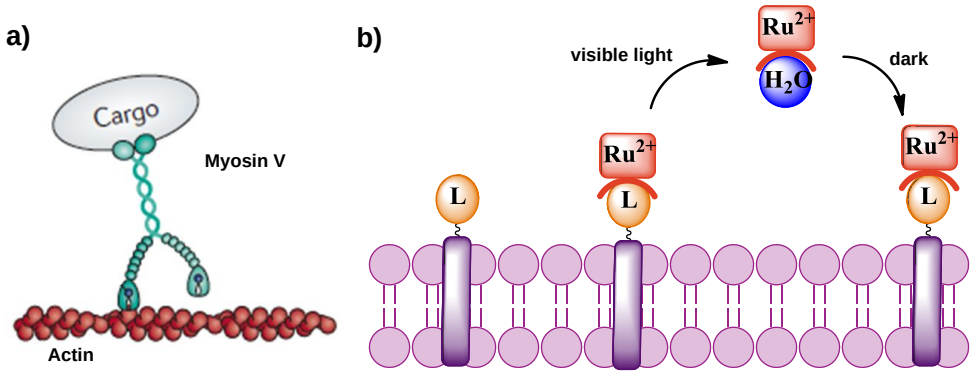


Figure 1.3. a) Myosin V works as a dimer that transports intracellular cargos along actin filaments. Adapted from reference [44]. b) Schematic cartoon proposed for the molecular motion of a photosensitive ruthenium complex at the surface of a lipid bilayer. The ruthenium carrier is detached from the lipid bilayer surface upon visible light irradiation (forming an aqua ruthenium complex), while it binds to the membrane embedded ligand L in the dark.

In the research reported in this thesis, such an artificial road was envisioned as being self-assembled at the surface of lipid bilayer membranes. Model membranes do not have the complexity of natural membranes, and their size, geometry, and composition can be optimized.[45] In such a vision, photosensitive ruthenium polypyridyl complexes would be used as molecular carriers to move a load unidirectionally at the surface of an artificial membrane. As shown in Figure 1.3b, the surface of a lipid bilayer can be functionalized with monodentate ligands L that may coordinate to ruthenium complexes. The idea was to use visible light to substitute ligand L by an aqua ligand, thus detaching the ruthenium carrier from the surface of the lipid bilayer. The aqua ruthenium species would diffuse freely near the surface and bind back to the membrane-embedded ligand L under thermal conditions, *i.e.*, in the absence of light.[46] By making the artificial road dissymmetric, the light-controlled motion of the ruthenium carrier from ligand to ligand would occur preferentially in one direction. In

the design of such a supramolecular system, understanding the reactivity of photosensitive ruthenium complexes must be deepened, and the dynamic interaction of the ruthenium complex with a model membrane should be fully understood. Thus, in Section 1.2 the dynamic interaction of metal cations and lipid bilayers will be discussed.

1.2. Lipid bilayers

1.2.1. Liposomes as model for cellular membranes

The self-assembly of lipid molecules in aqueous solution usually results in the formation of amphiphilic bilayers. In such an assembly the hydrophilic polar heads orient towards the aqueous phase while the hydrophobic part of the lipids form the inner hydrophobic core of the bilayer. Closed, spherical bilayers form structures called vesicles. Artificially synthesized vesicles are usually named liposomes.[47] Liposomes are dynamic systems with flexible surfaces; they have a great variety of topologies and shapes and can be unilamellar, multilamellar or oligovesicular (Figure 1.4).[48]

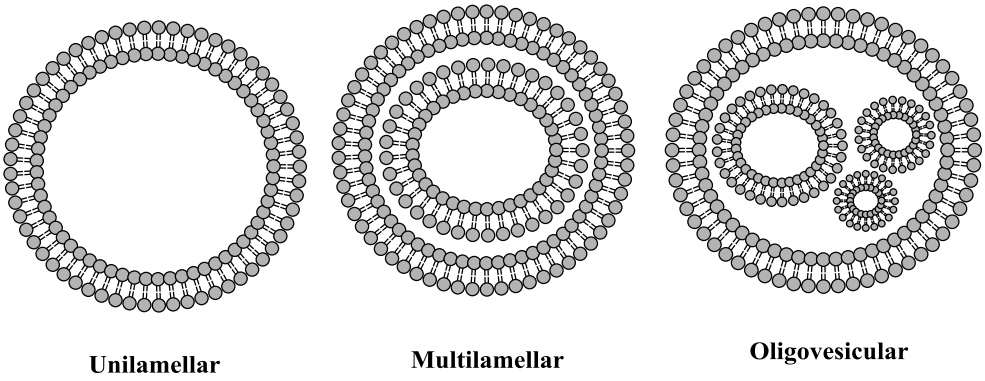


Figure 1.4. Schematic presentation of liposome structures of bilayer membranes.

Cell membranes play a crucial role in biological systems and many fundamental molecular processes are controlled by them. Membranes also act as a boundary between the extracellular and intracellular environments of a cell, and represent an essential functional unit for the transportation of materials, energy, and information. Liposomes formed of phospholipids or synthetic lipids have been widely used to mimic the functions and shape of biological membranes,[49-50] and also to develop biomimetic

systems such as nano-scale carrier systems,[51] reaction containers,[52] switchable assemblies,[53] sensors,[54-56] or supramolecular catalysts.[57]

Chemical recognition events on cellular membranes are the initial steps toward cellular signaling, and mimicking these functions is an important goal in the development of nano-scale molecular systems.[58] Usually, synthetic receptors are incorporated into liposomes that can interact with guest molecules or metal ions, which mostly leads to vesicular aggregation or fusion.[59] Interactions between liposomes can be controlled using electrostatic interactions,[60] hydrogen bonding,[61] but also metal ion coordination.[58-59] Metal ion coordination reactions in liposomal systems are more specifically discussed in the next section.

1.2.2. Dynamic systems involving liposomes and metals

In nature, important biological functions depend on metal ions interactions with cellular membranes. For example, it is known for a long time that calcium ions can bind to biological cell membranes containing phospholipids to induce liposome aggregation, and ultimately liposomal fusion.[62] Artificial membranes (liposomes) can be equipped with membrane-embedded ligands to control interaction with metal ions or complexes, in particular those involving transition metals.[63] Metal ion coordination to several membrane-embedded ligands can occur either on the same vesicle (intravesicular binding) or between two different vesicles (intervesicular binding). Only intervesicular binding induces aggregation, adhesion, or fusion of vesicles.[64]

The interaction between metal ions and lipid vesicles depends on several factors such as the charge of the lipid bilayer, the nature of the metal ions, or the nature and number of coordination sites of the membrane-embedded ligand (monodentate, bidentate, *etc.*). In addition, the ligand conformation and orientation in the lipid bilayer, and the strength of the metal-ligand coordination, can have an effect on the metal-bilayer interaction. It is noteworthy to briefly discuss these factors as an introduction to Chapters 2, 4, and 5 of this thesis.

Negatively charged phospholipids are known to aggregate or fuse in presence of metal cations such as Ca^{2+}, Mg^{2+}, or lanthanoid ions.[65] The nature of these interactions is believed to be mostly electrostatic and involves coordination of the phosphate head groups of the lipids to the metal ion. However, better selectivity and stronger metal-lipid interactions can be obtained with membrane-embedded ligands. For example,

intervesicular interaction has been reported for vesicles functionalized with terpyridine ligands (terpy), which aggregated in the presence of Fe^{2+} ions. The aggregation process proved to be reversible, as the addition of the strongly chelating ligand Na_2H_2edta (disodium salt of ethylendiaminetetraacetic acid) recovered the initial situation of non-aggregated vesicles (Figure1.5).[66] Lehn and co-workers[58] have reported similar aggregation phenomena for vesicles equipped with bipyridine (bpy) ligands in presence of Ni^{2+} or Co^{2+} cations. The coordination reaction first induced vesicle aggregation, which was followed by vesicle fusion.

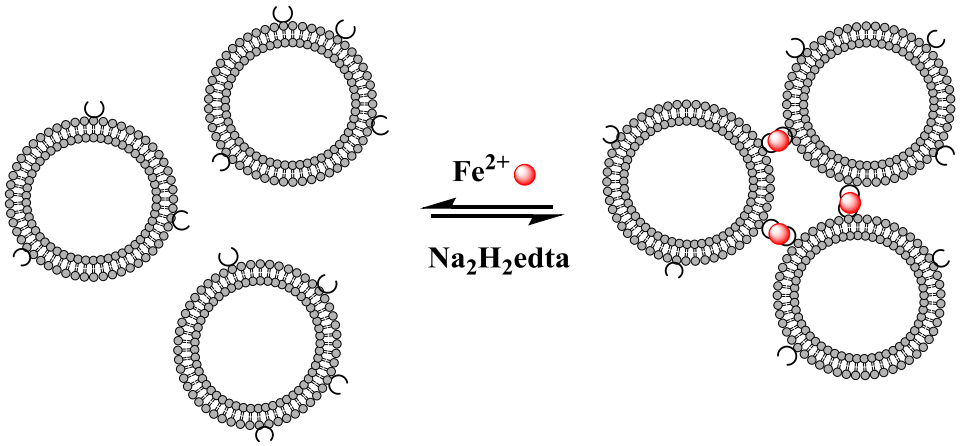


Figure 1.5. Aggregation of terpyridine-modified liposomes upon addition of iron(II) cations. Adapted from reference [66].

Besides the nature and number of coordination sites of the embedded ligands, the strength of the coordination bond plays a role in driving metal-lipid interactions. In other words, different metal ions may interact differently with one given ligand receptor incorporated in liposomes. In a study reported in 2007,[67] liposomes composed of amphiphilic cyclodextrins containing adamantyl-functionalized ethylenediamine ligands (L) were prepared. When Cu^{2+} was added to the liposome sample, intravesicular interactions resulted in the formation of $[CuL_2]^{2+}$ complexes at the membrane, and no sign of aggregation was observed (Figure 1.6a). In contrast, after addition of Ni^{2+} a mixture was formed comprising L, NiL and $[NiL_2]^{2+}$, and intervesicular interactions resulted in vesicle aggregation (Figure 1.6b). In fact, the stronger metal-ligand coordination bond in $[CuL_2]^{2+}$ resulted in exclusively

intravesicular interaction, while the weaker metal-ligand coordination bond in $[NiL_2]^{2+}$ resulted in predominantly intervesicular interaction.

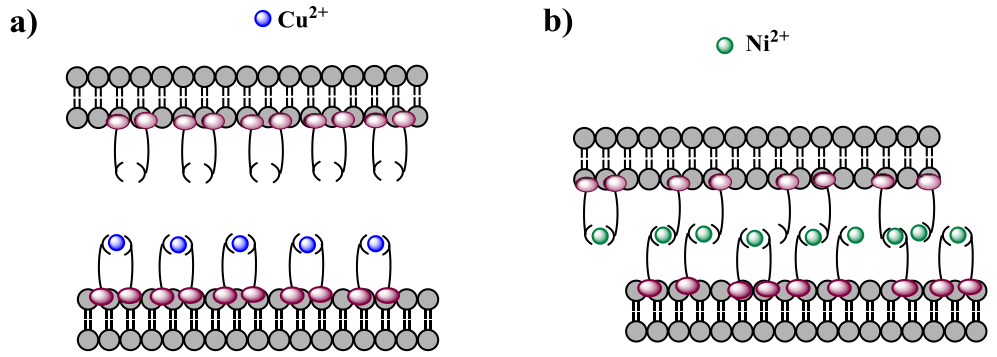


Figure 1.6. Orthogonal multivalent interactions within one bilayer and between two different bilayers of amphiphilic cyclodextrin-based liposomes. (a) Vesicle surface saturated with $[CuL_2]^{2+}$ (intravesicular interaction). (b) Two vesicles interacting *via* multiple coordination sites on Ni^{2+} and L (intervesicular interaction). Adapted from reference [67].

Conformational changes of ligands inserted in a membrane, in response to metal coordination and/or external stimuli, can be used to control the reactivity of liposomes towards metal ions. For example, light irradiation can induce photoisomerization of membrane-embedded ligands, which might influence ligand coordination to metal ions. Kikuchi and co-workers reported supramolecular systems that mimic information processing in biological signal transduction systems.[49, 68] Molecular communication occurs between a molecular emitter and a molecular receiver (see Figure 1.7). A molecular switch based on an azobenzene-containing peptide lipid was embedded in a lipid bilayer. This molecular switch exhibited photoresponsive recognition behavior towards Zn^{2+}, which allowed for controlling the binding of a small liposome to a giant liposomal receiver. Upon UV light irradiation, the azobenzene ligand embedded in the small and giant liposomes significantly changed their configuration through photoisomerization of the N=N double bond, from the *trans* form to the *cis* form. As the metal-binding affinity of the *cis* isomer is much higher than that of the *trans* isomer; after addition of Zn^{2+} the metal ion was stabilized by forming a complex with two ligands in the *cis* conformation only. Thus, the small liposome equipped with *cis* ligands bound to a receiver liposome that had the same molecular conformation. In contrast, visible light irradiation converted the *cis* isomer to the *trans* isomer, which has a lower metal-binding affinity. Thus, light-induced *cis-trans* isomerization of the

ligand modified the adhesion of the small liposomes to the receiver liposomes, *i.e.*, the metal-ligand interaction at the lipid membrane was modulated using light as external stimulus.[68]

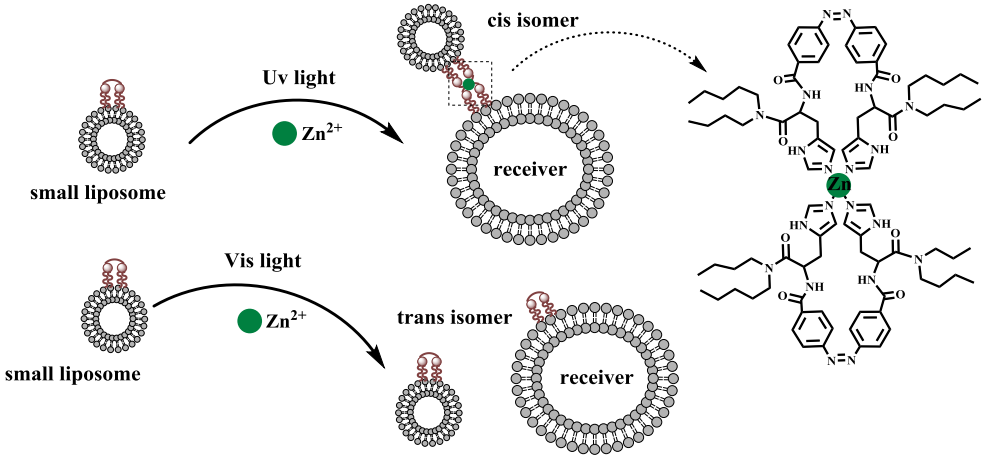


Figure 1.7. Photonic control of the binding of a molecular capsule (small liposome) to a molecular receiver (large liposome) by using a molecular switch. Adapted from reference [68].

Metal coordination can also influence ligand conformation, which can be used to regulate the association and dissociation of adhering liposomes. In a study by Ravoo *et al.*[64] a p-tert-butylbenzyl dimer with a flexible N,N'-bis(3-aminopropyl)ethylenediamine spacer was used as a non-covalent linker between cyclodextrin-functionalized liposomes (Figure 1.8). This linker induces adhesion of the liposomes by the formation of hydrophobic cyclodextrin/^{t}Bu-phenyl inclusion complexes in absence of metal ions. In the presence of Cu^{2+}, the tetraamine linker molecule formed a stable coordination complex and switched its conformation from linear to bent, which led to the dissociation of the intervesicular complexes and to the dispersion of the vesicle clusters. This process was reversible, as in presence of a strong chelating ligand such as Na_2H_2edta the Cu^{2+} ions were removed from the system and liposomal adhesion was re-established (Figure 1.8). Overall, ligand shape changes, lipid bilayers, and metal coordination influence each other, and such interactions would need to be understood and controlled when building a molecular machine at a bilayer surface based on metal coordination.

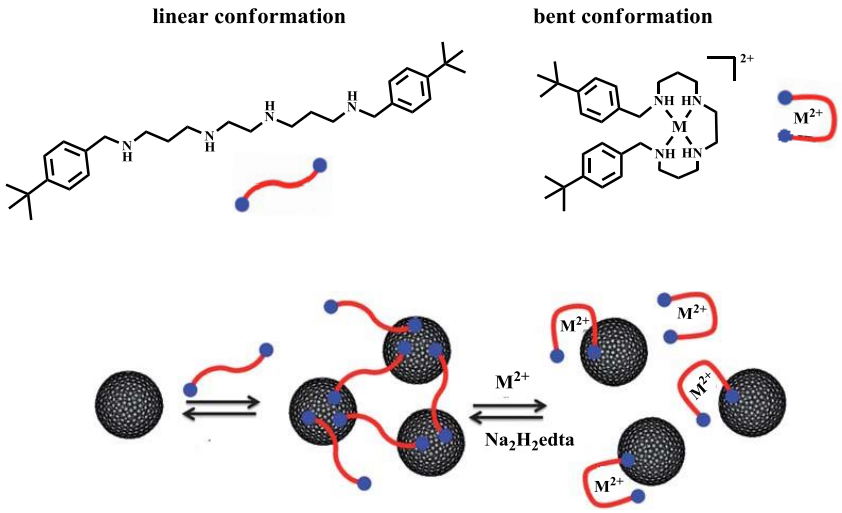


Figure 1.8. Coordination of Cu^{2+} to a tetraamine ligand and a schematic representation of the metal ion responsive supramolecular system, in which vesicle adhesion or dispersion is controlled by the reversible conformational change of the spacer induced by metal ion coordination. Adapted from reference [64].

Metal binding to ligands embedded in neutral membranes can induce ligand dispersion and prevent ligand aggregation in the lipid bilayer membranes due to electrostatic repulsion between the cationic metals at the membrane surface. Arnold and co-workers in 1995 reported a liposomal sensor system that was able to detect Cu^{2+} ions based on this principle.[69] The system relies on the excimer–monomer equilibrium of a pyrene dye. Neutral liposomes were functionalized with a lipid conjugate containing a pyrene moiety that was inserted into the lipophilic part of the membrane, and that was attached to a ligand facing the aqueous phase (Figure 1.9). The lipid conjugates with neutral head groups formed clusters in the liposomal bilayer in absence of Cu^{2+}, which showed the typical pyrene excimers emission. After addition of Cu^{2+} ions and subsequent metal-ligand coordination the positively charged coordination complexes at the membrane repelled each other, which induced the dispersion of the membrane-embedded ligands and disrupted the pyrene eximers. The pyrene monomer and its excimer show very distinguishable emission spectra, which was used to detect coordination of the copper ions.

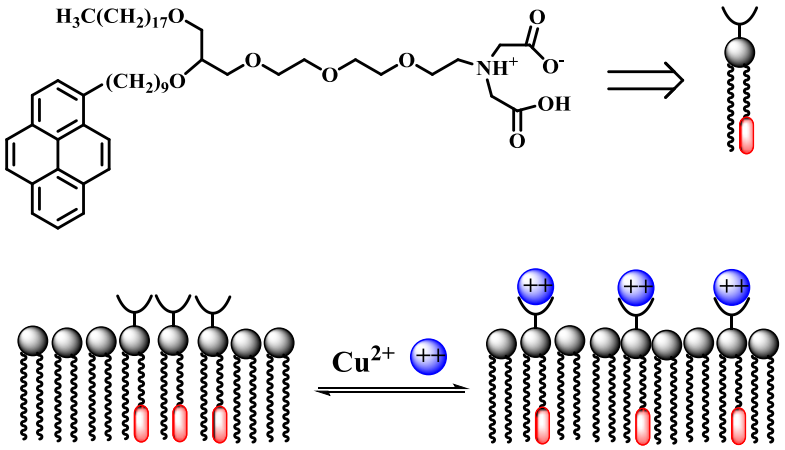


Figure 1.9. Metal ion sensor based on the switching of the monomer–excimer equilibrium of a pyrene moiety in a neutral liposome. The equilibrium is modified by the electrostatic repulsion between positive charges upon binding of Cu^{2+} at the membrane surface. Adapted from reference [63].

Coordination of metal cations to membrane-embedded ligands can also modify the membrane permeability for metal cations. For example a 2004 study[65] showed that coordination of Eu^{3+} ions to membrane-embedded diketonate ligands promotes the transportation of the Eu^{3+} ions across the lipid bilayer surface. It was an artificial functional system mimicking the selective transport of metal ions by ionophores in biology.

The last factor to take into account in the design of a metal-based molecular transporter at the surface of a lipid bilayer is the site of metal-ligand coordination, which may be either the bilayer-water interface of the lipid membrane, or its lipophilic region. The latter type of coordination has been used to create liposomal ion sensors that mimic ion transportation through biological membranes *via* ion channels.[70] Webb and co-workers[71] have reported such kind of ion channels that can be gated "open" or "closed" by the addition or removal of palladium(II) ions. In the example shown in Figure 1.10 a pyridyl-cholate moiety was incorporated in unilamellar liposomes composed of neutral phospholipids. These liposomes also encapsulated a pH-sensitive dye (Figure 1-10a). Addition of $PdCl_2$ led to the linkage of two pyridyl-cholate moieties *via* coordination of the pyridine subunits to Pd^{2+}. The palladium(II) bis(pyridyl) motives created a channel through the membrane, which facilitated alkali metal ion transport. After addition of NaOH the transportation of the Na^+ ion resulted in an increase in pH, which was detected by a fluorescence increase of the encapsulated

dye. Subsequent addition of a palladium(II)-chelating agent (hexathia-18-crown-6 (18S6)) disconnected the channels, which stopped the flow of sodium ions and the evolution of fluorescence.

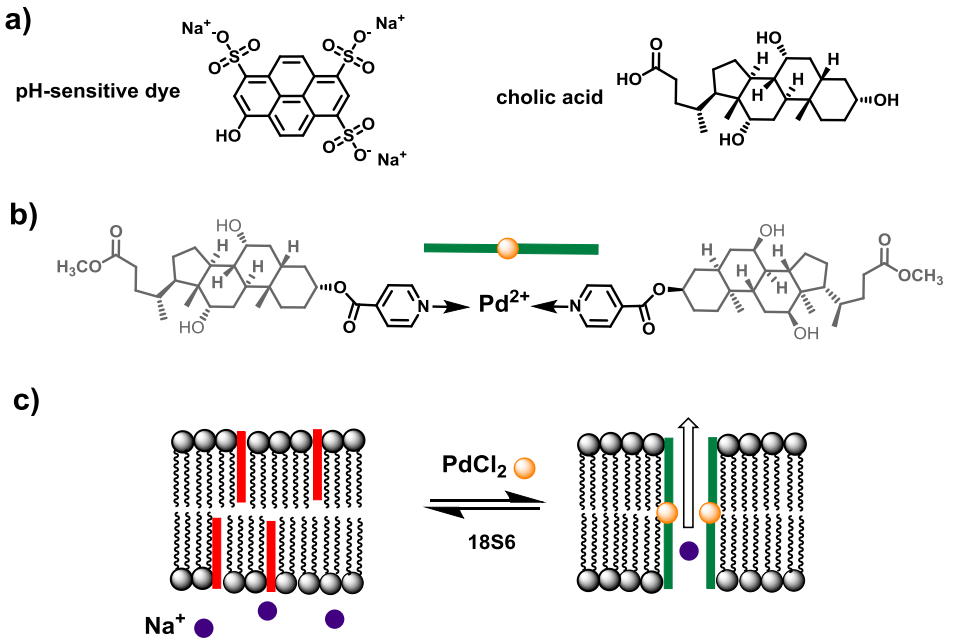


Figure 1.10. a) Chemical structure of a pH-sensitive encapsulated dye and cholic acid, b) pyridyl-cholate conjugate and coordination to a Pd^{2+} ion , c) a schematic representation showing the gating of an artificial ion channels; either opened by the addition of $PdCl_2$, or closed by the addition of the hexathia-18-crown-6 ligand (18S6, bottom). Adapted from reference [71].

Overall, the examples detailed above illustrate the many options available when designing dynamic systems involving liposomes and metals. The dynamics of systems involving ligands, metal, and lipid bilayers, depend on a variety of factors that should be controlled in order to control molecular motion of the metal center at the membrane surface. In particular, intervesicular interactions like aggregation or fusion, ligand conformational changes, coordination in the lipophilic region of the membrane, or deep insertion of the ruthenium complex into the lipid bilayers, may reduce or impair the motion of ruthenium compounds at the membrane. In addition, neutral ligands may aggregate in the membrane and be dispersed upon coordination of the positively charged ruthenium complex, which would add another level in the complexity of the motion of the complexes. Finally, the ruthenium-ligand coordination bond should be

light-sensitive and stable in the dark if one wants to control the motion using light. For this PhD project, neutral monodentate thioether-cholesterol conjugates with flexible polyethyleneglycol linker were chosen, as there are flexible enough not to have one preferred conformation or configuration, do not significantly interact with protons in water, and may disperse homogeneously in the two dimensions of the membrane.

Next to their potential as metal sensors or as surfaces where molecular motion could occur, liposomes are mostly known for their application in drug delivery, as they can notably improve drug targeting towards cancer cells. In the next section the advantages of liposomal drug carrier systems in medicinal chemistry are introduced, before discussing the potential of ruthenium complexes as anticancer drugs.

1.3. Ruthenium-decorated liposomes as light-activatable prodrugs

1.3.1. Liposomes as drug carriers in cancer therapy

The major goal in drug delivery is to effectively deliver molecular drugs to their biological target in order to avoid toxic side effects for the patient. Three basic requirements for a successful drug delivery system in anticancer research are: (I) prolonged blood circulation of the drug, (II) sufficient accumulation of the drug in the tumor, and (III) controlled drug release and uptake by tumor cells.[72] Nano-sized drug delivery systems like micelles, liposomes, and nanoparticles, can be modified to incorporate targeting moieties that allow for specific delivery of the drug to cancer cells expressing specific receptors at their surface. Gregoriadis *et al.* [73] in 1974 proposed the first liposomal-based drug carrier in cancer chemotherapy, and since then the interest in liposomal drug carriers has increased significantly.[72] One of the most acknowledged advantage of liposomes is their ability to deliver both hydrophobic and hydrophilic drugs, as well as mixtures of these. Water-soluble drugs can be encapsulated in the internal aqueous compartment of the liposome, whereas lipophilic drugs can be included within the hydrophobic part of the phospholipid bilayer.[74] Moreover, liposomes tend to accumulate at cancer tumor sites rather than at normal tissues. The structure of the microvasculature in tumors has large openings (up to 500 nm), which allows liposomes diffusion inside the tumors.[75] Beside their size, the surface charge of liposomes and their lipid composition play critical roles in their circulation lifetimes in the blood.[76] It has been proven that “stealth” liposomes, *i.e.*, liposomes coated with synthetic polyethyleneglycol polymers (PEG), have

significantly increased half-life in the blood compared to liposomes of the same composition but deprived of PEG chains. Such long circulation half-life times allow efficient delivery of this kind of liposomes to cancer cells via the so-called "Enhanced Permeability and Retention" (EPR) effect.[77-78]

There are two main strategies for efficient targeting of liposomes to tumors and drug-release: (I) site-specific delivery, which can be achieved by coating the liposomes with ligands or antibodies that target overexpressed receptors in the tumor tissue; (II) site-specific triggering by external stimuli like pH,[79-80] temperature,[81] or light,[82-83] to release the encapsulated drug.[72] Using light as a triggering signal, for example, is possible with photosensitive liposomes made of lipids that can either isomerize, fragment, or polymerize upon light irradiation.[84]

Light-triggered drug activation is a basic concept used primarily in a treatment modality called "photodynamic therapy" (PDT). In PDT a photosensitizer is applied to the diseased tissue. This photosensitizer absorbs photons and transfers its energy to the triplet ground state of the dioxygen molecule, to form the excited state of O_2 called singlet oxygen ($^1O_2$). The high oxidizing properties of $^1O_2$ can then induce cell death by fast reactions with proteins, lipids, or nucleic acids.[85-87] Most photosensitizers applied in clinical treatments are rather hydrophobic and tend to form aggregates in aqueous media, which reduces their photosensitizing efficacy as only monomeric species are usually photoactive. Liposomes have been used in PDT since they can significantly decrease photosensitizer aggregation. A variety of photosensitizer drugs, such as tetramethyl hematoporphyrin (TMHP), fullerene (C60/C70), and zinc phthalocyanine (ZnPc), have been used in combination with liposomes.[87-89] In a recent study by Lissi *et al.*[90] the photophysical and photochemical properties of ZnPc photosensitizers in THF was compared with those of ZnPc incorporated in phosphatidylcholine liposomes. The results showed that dye incorporation into liposomes decreases ZnPc aggregation and provide a better photodynamic activity on HeLa cancer cell line (cervical cancer cells).

Despite the variety of liposomal drug delivery systems reported in the scientific literature, there are only few examples of liposomes used for encapsulating metal-based drugs. Hence, some of the few systems described so far will be briefly discussed here. Anticancer platinum compounds, in particular cisplatin (*cis*-diamminedichloridoplatinum), are one of the few metal-based anticancer agents that

have been considered for liposomal drug delivery. The antitumor property of cisplatin is largely due to its binding to nuclear DNA. However, cisplatin tends to bind to blood plasma proteins as well, particularly those with thiol groups such as human serum albumin and other proteins with high cysteine content. Such binding mostly leads to deactivation of cisplatin, and it induces side effects during cisplatin chemotherapy. [91-92] Liposomal drug delivery is believed to be able to solve or at least reduce these problems. In the literature, mostly poorly water-soluble platinum compounds such as cisplatin have been incorporated into the hydrophilic core of liposomes (Figure 1.11a).[93-94] However, in a recent study by Kaluderovic *et al.*[95] a water-insoluble platinum drug was incorporated into the lipophilic part of lecithin liposomes (Figure 1.11b) and the cytotoxicity of this formulation was tested on several tumor cell lines as well as normal cells. The results showed that a liposome-incorporated cisplatin drug had higher cytotoxicity and selectivity for some cancer cell lines such as human thyroid carcinoma cells SW1736, compared to non-encapsulated complex [**14**] or cisplatin [**13**].

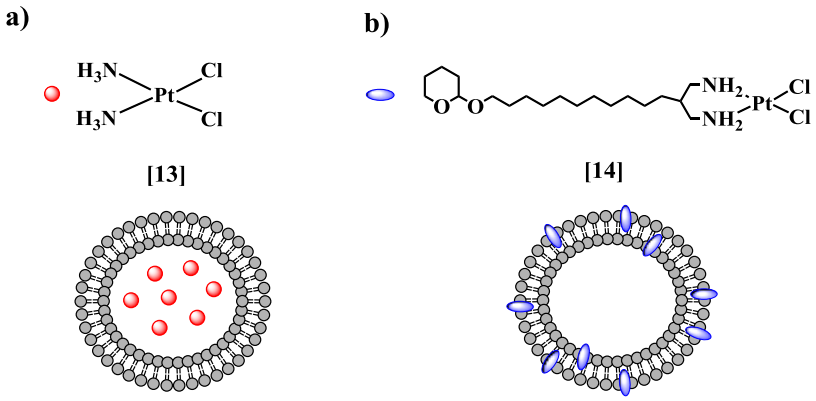


Figure 1.11. a) Cisplatin loaded in the hydrophilic core of a liposome. b) Lipophilic cisplatin analog loaded in the lipid bilayer of a liposome. Adapted from reference [95].

Most drugs are toxic in high dosage, which restricts their clinical application in cancer therapy. In order to overcome the high dosage toxicity, the drug activity needs to be controlled, for example by encapsulation in liposomes. In 2006 Halloran *et al.* [96] developed a liposomal system for encapsulating arsenic-based drugs. Arsenic trioxide (As_2O_3) is a promising agent for the treatment of blood and bone marrow cancers. However, clinical application of this drug to other cancers has been limited due to its

toxicity at higher doses. This problem was solved by encapsulation of high doses of As_2O_3 in phospholipid liposomes that were able to release the drug in a controlled fashion, *i.e.*, upon pH variation. While the therapeutic agent remained in the liposome at physiological pH (7.4), it was released at lower pH (4.0), typical of the endocytic compartments involved in the cellular uptake of liposomes.

The cellular uptake pathway can also be changed by encapsulation of metallodrugs in liposomes, which sometimes leads to better cellular uptake of a liposome formulation compared to the non-encapsulated drug. For example, gallium nitrilotriacetate is a therapeutic agent that has been proven to be effective for the treatment of several cancer types. Ga^{3+} ions are mostly taken up by cancer cells via a transferrin (TF) receptor pathway, and it competes with iron cellular uptake. The transferrin-independent uptake mechanism is also possible, but this accounts for only 10% of the total Ga^{3+} uptake. In a study from 1993[97] it was reported that encapsulation of gallium nitrilotriacetate in negatively charged liposomes provided a transferrin-independent route for the delivery of Ga^{3+} ions to cancer cells.

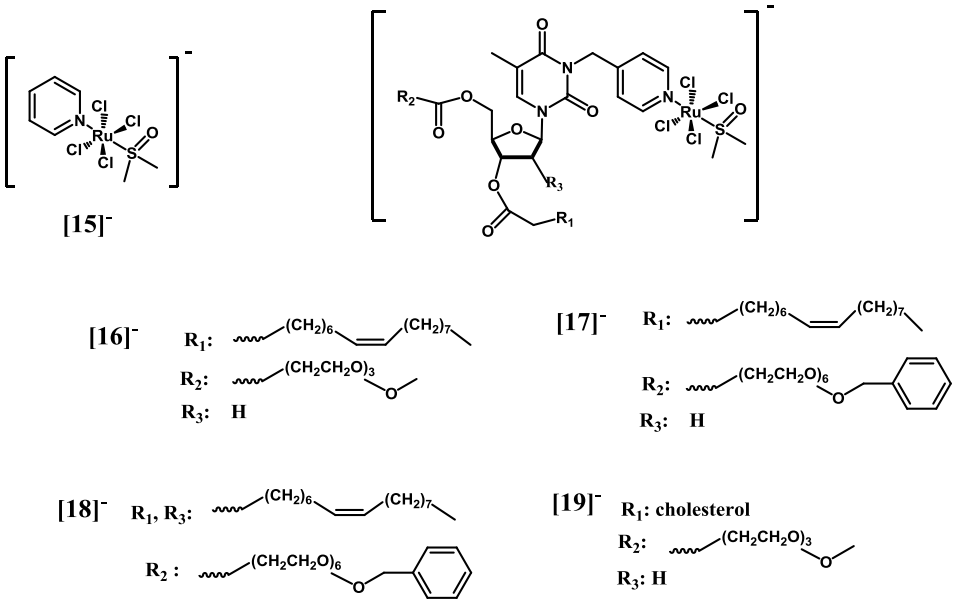


Figure 1.12. Structural formula of complex AziRu [**15**]$^-$ and AziRu functional nucleolipids [**16**]$^-$, [**17**]$^-$, [**18**]$^-$, and [**19**]$^-$. Adapted from references [98-99].

Until recently no study has been reported for the liposomal drug delivery of ruthenium-based anticancer compounds. In 2012 Paduana and co-workers[98-99] reported the first systems of this kind. Ruthenium(III) complexes functionalized with different amphiphilic nucleosides (Figure 1.12) were incorporated in the lipophilic phase of neutral liposomes. The ruthenium complex [**15**]$^{-}$ (named AziRu) was chemically linked to the nucleolipid (a hybrid molecule containing a nucleic acid unit and amphiphilic moieties) via an Ru-N coordination bond. The anticancer activity of these ruthenium-functionalized liposomes was investigated on several cancer cell lines and compared with free AziRu. [98-99] The results showed higher *in vitro* anti-proliferative activities for the ruthenium-containing liposomes than for free AziRu. It was reported that the liposomal formulation facilitated the internalization of the ruthenium complex and postponed its hydrolysis in physiological conditions. This work showed for the first time the capacity of ruthenium-decorated liposomes to be used in drug delivery.

1.3.2. Ruthenium complexes as anticancer drugs

1.3.2.1. Cytotoxicity of ruthenium complexes and mechanism of action

Since the discovery of cisplatin, many transition metal complexes have been synthesized and tested for their anticancer activity. In recent years, ruthenium-based molecules have attracted much attention as promising antitumor agents. Ruthenium complexes have three properties that make them potentially suitable for medicinal use: I) slow ligand-exchange kinetics similar to those of Pt(II) complexes, II) multiple accessible oxidation states allowing prodrug activation strategies, and III) the ability to mimic iron binding to certain biologic molecules such as albumin and transferrin.[100] Since rapidly dividing cells, such as cancer cells, have a greater demand for iron compared to normal cells, transferrin receptors are over-expressed in tumors, which may allow for more effective delivery of ruthenium-based drugs to cancer cells.[101-102] Moreover, Ru(II) complexes have octahedral coordination spheres, in contrast to the square-planar geometry of Pt(II) compounds, which may allow for obtaining different toxicity profiles for ruthenium compounds and addressing cisplatin-resistant cancer cells.[103-104]

Among the many ruthenium complexes that have been investigated only two compounds, namely NAMI-A[105] and KP1019,[106] have entered human clinical trials

(Figure 1.13). Despite their structural and chemical similarities, these two Ru(III) complexes show different antitumor behavior. In pre-clinical studies, NAMI-A has shown inhibitory effects against the formation of metastases in a variety of animal tumor models, although it appeared to lack direct cytotoxicity towards human tumors.[107] In contrast, KP1019 has proven to be cytotoxic against a wide range of primary human tumors by inducing apoptosis.[106]

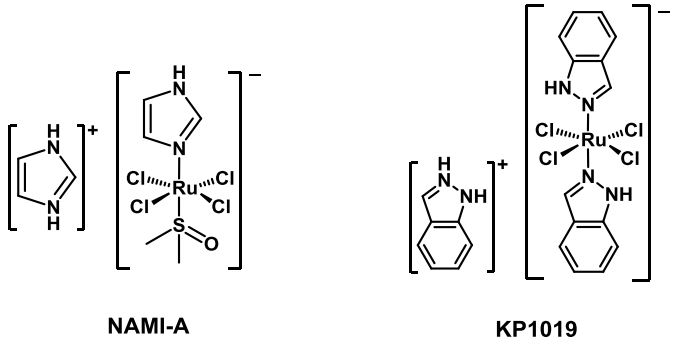


Figure 1.13. Chemical structures of anticancer ruthenium complexes NAMI-A and KP1019.

Most ruthenium complexes investigated for medicinal purposes, including NAMI-A and KP1019, undergo ligand exchange in biological media. Usually the metal complex is first hydrolyzed to give an aqua complex, which is often believed to interact with DNA through the formation of coordination bonds between the metal center and nitrogen ligands or DNA phosphate groups on the DNA bases,[108] leading to metal-DNA adduct formation and cell death (Scheme 1.6). This mechanism is quite often called "irreversible binding" because it involves the formation of a coordination bond.[109] Binding of the ruthenium(II) center to DNA has been hypothesized for a wide range of ruthenium-based analogues of cisplatin, such as for example $[RuCl_2(DMSO)_4]$,[107] $[Ru(bpy)_2Cl_2]$, $[Ru(terpy)Cl_3]$,[110-111] or complexes of the type $[Ru(terpy)(N\text{-}N)(L)]^{2+}$, where N-N is a bidentate diimine ligand like bpy or phen.[112] However, in the case of substitutionally inert polypyridyl Ru(II) complexes of $[Ru(diimine)_3]^{2+}$ family , cytotoxic effects were also obtained *via* van der Waals interactions with DNA.[113-115] All interactions with DNA not involving coordination to the metal center are usually called "reversible" binding, and are divided into four categories: I) electrostatic interaction, II) intercalation, III) groove binding (molecules

occupy the minor or major groove of DNA), and IV) binding to non-canonical DNA such as mismatch, G-quadruplex, or triplex DNA structures, which involves a combination of electrostatic and van der Waals interactions.[109]

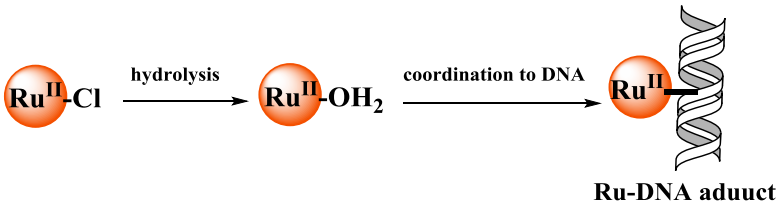


Figure 1.14. Hydrolysis and coordination of a Ru(II) complex to the nucleophilic DNA binding sites.

In recent years innovative studies have shown that other mechanisms such as topoisomerase enzymes inhibition,[116] or mitochondria-mediated apoptosis,[117-118] may be responsible for the cytotoxicity of metallodrugs, in particular for saturated complexes unable to coordinate to DNA. In a study by Gazzer *et al.*, the cytotoxicity mechanism of the coordinatively saturated Ru(II) complex $[Ru(dppz)_2(CppH)]^{2+}$ (CppH =2-(2′-pyridyl)pyrimidine-4-carboxylic acid; dppz = dipyrido[3,2-a:2′,3′-c]phenazine) was investigated in detail.[119] It was proposed that this compound exerted its toxicity through a mitochondria-related pathway rather than *via* binding to nuclear DNA. Although the complex was shown to bind to calf thymus DNA by intercalation, this interaction is not involved in the toxicity mechanism *in vitro*.

1.3.2.2. Photoactivated chemotherapy

Photoactivated chemotherapy (PACT) consists in the light-controlled activation of a drug at the tumor site, which results in greater specificity for the action of a drug. The concept of an inactive precursor, or "prodrug", is important in this field.[120] The challenge is to develop compounds that are thermally stable, but can be triggered by low energy light irradiation to generate toxic species with anticancer properties similar to that of other chemotherapeutics.[121] The activity of light-produced cytotoxic agents ideally depends on their ability to interact with biopolymers or bio-aggregates such as cell membranes, proteins, or DNA. Damage to DNA can occur by photoinduced electron transfer between the excited state of the photoactivated molecule and DNA.[122] Another method is photodynamic therapy (PDT).[85-86] Since in PDT the toxicity is oxygen-dependent and tumor cells are generally hypoxic, new approaches

based on photoinduced ligand substitution in transition metal complexes are interesting alternatives, where a coordinatively saturated metal complex would either bind to nucleic acids or proteins after photochemically losing a biologically inactive ligand (Figure 1.15-I), and/or releasing photochemically a biologically active organic ligand (Figure 1.15-II).[108, 123]

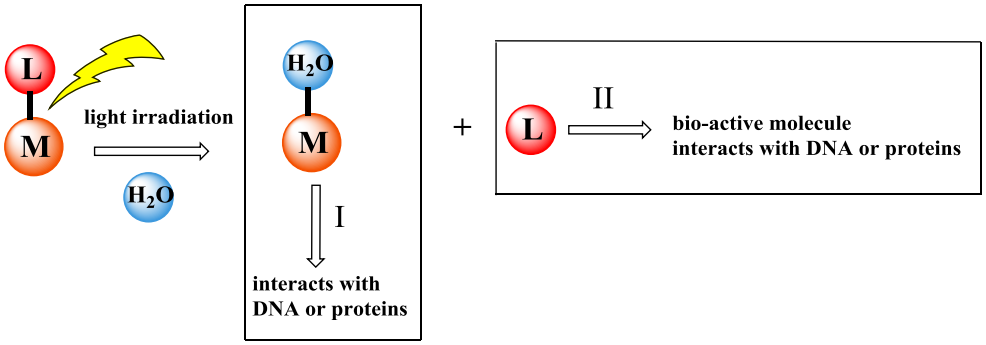


Figure 1.15. Photochemotherapy using a photosensitive metal-based prodrug and two possible cytotoxicity mechanisms involving photosubstitution. M: metal complex, L: photosubstituted ligand, M-H_2O: hydrolyzed metal complex.

Ruthenium complexes are particularly attractive for photoactivated chemotherapy (PACT), as their photophysical properties can be tuned, they strongly absorb in the visible region (400-600 nm), and are kinetically inert.[123] As mentioned in Section 1.1.2. complexes with distorted octahedral geometry are prone to ligand dissociation under visible light irradiation. Thus, steric and electronic properties of the ligands can be tuned to obtain Ru(II) complexes suitable for PACT.[124] For example, in a recent publication by Glazer and co-workers[121] the light-induced cytotoxicity of three $[Ru(bpy)_2(N\text{-}N)]^{2+}$ complexes, where N-N is a sterically hindered bidentate diimine ligand, was investigated and compared with that of cisplatin. A high cytotoxicity was reported for the more strained Ru(II) compounds $[\mathbf{21}]^{2+}$ and $[\mathbf{22}]^{2+}$ (Figure 1.16), compared to the less strained complex $[\mathbf{20}]^{2+}$ and cisplatin. As both hindered complexes were inert in the dark and only became cytotoxic by visible light irradiation, the phototoxicity is believed to result from the photosubstitution of the hindered N-N ligand, followed by covalent binding of ruthenium to DNA.

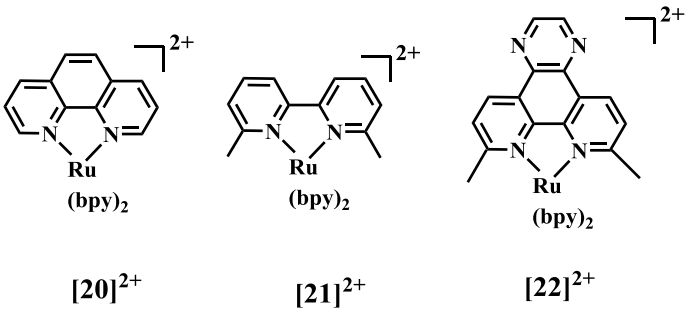


Figure 1.16. Structures $[Ru(bpy)_2(N\text{-}N)]^{2+}$ complexes with reported anticancer activity. [121]

The photoinduced cytotoxicity of polypyridyl Ru(II) complexes also depends on the electronic properties of the spectator ligands. Nair and co-workers[125] have recently investigated the cytotoxicity of a series of Ru(II) complexes of the type $[Ru(Rterpy)(N\text{-}N)Cl]^+$ (Figure 1.17). The Ru-Cl bond can be cleaved by light and Cl^- be photosubstituted by the nucleobase of a DNA fragment. It was shown that the electronic properties of the substituent X on the Xterpy ligand influence the ground state properties of its ruthenium complex, and thus the photolability of the Ru-Cl bond. As benzimidazole is more electron withdrawing than imidazole, compounds $[\mathbf{23}]^+$ and $[\mathbf{24}]^+$ with an imidazole substituent on the Xterpy ligand were found to be more phototoxic towards cancer cells under irradiation at 440 nm than $[\mathbf{25}]^+$ and $[\mathbf{26}]^+$.

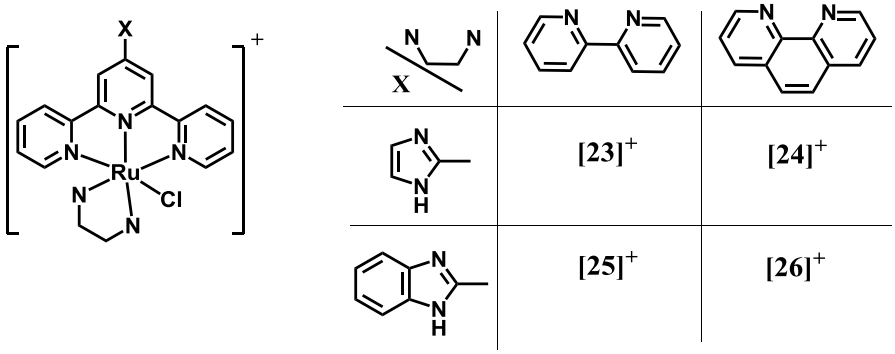
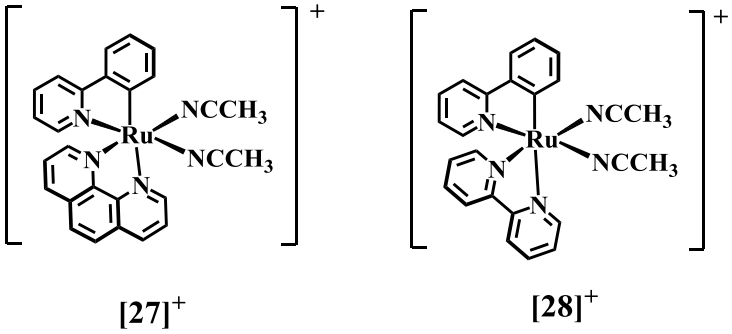


Figure 1.17. $[Ru\ (Rterpy)(N\text{-}N)Cl]^+$ complexes with different light-induced cytotoxic properties. Adapted from reference [125].

Effective light absorption by the photoactive drug inside human tissues is another significant challenge in PACT. The penetration depth of light in human tissue is highly

wavelength dependent, and significant penetration only takes place in the range of 600–850 nm, which is referred to as the "photodynamic window".[126-127] Many efforts have been dedicated to achieve photochemical activation of ruthenium complexes with low-energy photons. Changing the electronic properties of the polypyridyl ligands can extend the light activation of the ruthenium complexes towards longer wavelengths, as discussed in a recent review by Turro *et al.* [128] It was shown that in ruthenium complexes $[Ru(N\text{-}N)_2(L)_2]^+$ (L=NH_3, pyridine, or CH_3CN, N-N=bpy or phen), if one of the N-N ligands is replaced by a cyclometallating ligand such as $phpy^-$ (see Figure 1.18) the negative charge of the carbon-based ligand induces an increase in the energy of the HOMO orbital of the complex, and thus reduces the energy needed to promote an electron to the π* orbital of the diimine ligand. As a result the MLCT absorption band is red-shifted to 690 nm. Compound $[\mathbf{27}]^+$ (Figure 1.18) showed very good phototoxicity on advanced ovarian epithelial cancer cells upon irradiation at 690 nm.[128] The cytotoxicity of this compound upon low-energy light irradiation enhanced the potential of this compound as a phototherapeutic agent.[129]



[27]$^+$ **[28]$^+$**

Figure 1.18. Chemical formulae of $[Ru(phen)(phpy)(CH_3CN)_2]^+$ ($[\mathbf{27}]^+$) and $[Ru(bpy)(phpy)(CH_3CN)_2]^+$ ($[\mathbf{28}]^+$).

In the development of light-activated ruthenium-based cytotoxic compounds, efficient targeting is also a great challenge. Mesoporous silica nanoparticles (MSNPs) have recently been reported by Sauvage and coworkers to be efficient nano-carriers for ruthenium dipyridophenazine (dppz) complexes.[130] As shown in Figure 1.19. the ruthenium complexes were grafted on the surface of the nanoparticles *via* nitrile ligand **29**. The resulting supramolecular assembly showed fast cellular uptake, and while the ruthenium-modified nanoparticle was unreactive in the dark, upon visible light

irradiation the Ru-nitrile coordination bond was cleaved to release the ruthenium complex from the surface of the nanoparticles. The resulting cytotoxic aqua complex [**30**]$^{2+}$ was able to form mono-adducts with DNA and induce cytotoxicity. As discussed in session 1.3.1. liposomes also have great potential to be used as metallodrug carriers that improve drug targeting to tumors. Liposomes functionalized with photosensitive ruthenium complexes have been proposed by our group as a support for the molecular motion of ruthenium-based molecular machines.[46] However, they have not been used until now for the delivery of phototoxic ruthenium complexes to cancer cells, and no toxicity or phototoxicity data have been reported yet. Ideally, ruthenium-functionalized liposomes might be taken up by cancer cell, where light irradiation would release the ruthenium aqua complex (Figure 1.20). In Chapter 5 of this thesis the initial efforts in this direction are described.

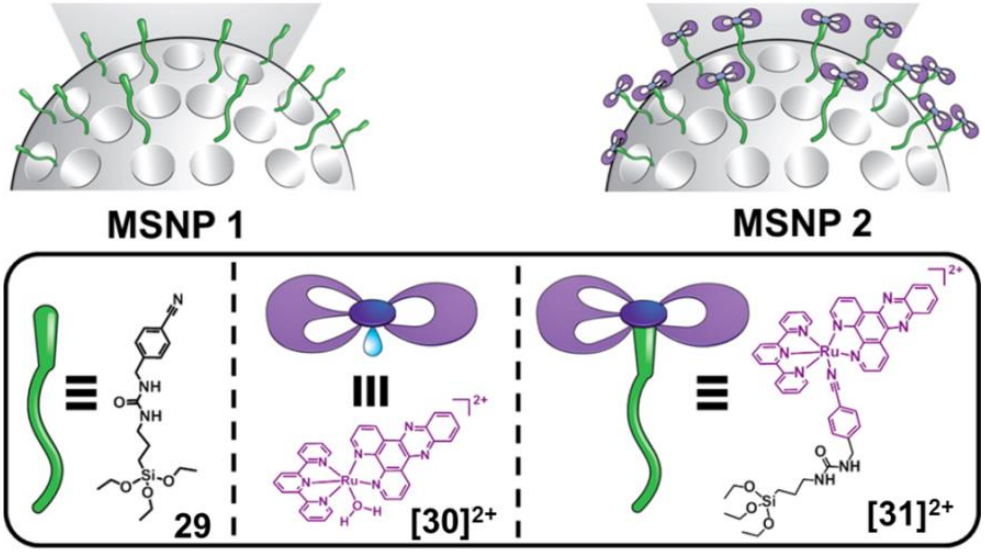


Figure 1.19. Structural formula of the nitrile ligand **29**, ruthenium-aqua complex [**30**]$^{2+}$, and ruthenium−dppz complex [**31**]$^{2+}$. Ligand **29** is grafted onto the surface of nanoparticles (MSNP 1), followed by coordination of [**30**]$^{2+}$ in the dark to form ruthenium-functionalized nanoparticle (MSNP 2). Image taken from reference [130].

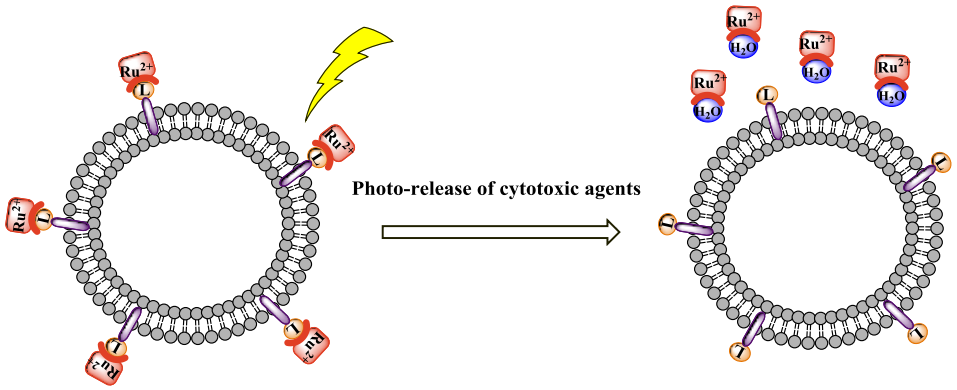


Figure 1.20. Liposomes decorated with photosensitive ruthenium-based anticancer prodrugs. Cleavage of the Ru-L coordination bonds upon light irradiation leads to release of the potentially cytotoxic ruthenium-aqua complexes.

1.4. Aim and scope of this thesis

Polypyridyl ruthenium(II) complexes of the $[Ru(terpy)(N\text{-}N)(L)]^{2+}$ family, where N-N is a diimine ligand and L is a monodentate ligand, have been known for a long time. However, there are very few studies on liposomes functionalized with these complexes, and on the interaction of ruthenium complexes with lipid bilayers. The research described in this thesis focuses on the photoreactivity and coordination chemistry of $[Ru(terpy)(N\text{-}N)(L)]^{2+}$ complexes both in homogenous aqueous solutions and at the surface of lipid bilayers. Their potential application either for the building of light-controlled molecular machines (chapters 2, 3, and 4), or as light-activatable anticancer prodrugs (chapters 5 and 6), is described.

In Chapter 2 the coordination chemistry of $[Ru(terpy)(dcbpy)(SRR')]^{2+}$ complexes (dcbpy=6,6'-dichloro-2,2'-bipyridine and SRR'=thioether ligand), is reported in homogeneous aqueous media. The Ru-S coordination bond was found to form spontaneously in the dark and to be efficiently broken by light irradiation. The potential of this system in supramolecular chemistry is presented by describing the repeatable formation and breakage of the Ru-S bond at the surface of anionic lipid bilayers.

In Chapter 3 an attempt to optimize the dynamics of the light-sensitive interconversion between $[Ru(terpy)(N\text{-}N)(SRR')]^{2+}$ (RuSRR') and $[Ru(terpy)(N\text{-}N)(H_2O)]^{2+}$ ($RuOH_2$) species in homogeneous aqueous media is reported. The effect of the steric hindrance

of the spectator diimine N-N ligand on the kinetics and thermodynamic of the Ru-S bond formation and hydrolysis is discussed, both in the dark and under light irradiation.

In Chapter 4 the mechanism of the coordination of ruthenium polyryridyl complexes to sulfur ligands embedded in lipid bilayers is described. The kinetics of the coordination reaction at the membrane interface was found to be highly dependent on the charge of the lipid bilayer. This study highlights the differences between coordination chemistry at membranes and coordination chemistry in homogeneous conditions.

In Chapter 5 the application of ruthenium-decorated liposomes in photochemotherapy is described. The photoreactivity of a series of photosensitive ruthenium complexes incorporated in liposomes with different surface charge (neutral or negative) is reported. The dark stability of the liposomes, their cellular uptake, and their cytotoxicity in the dark and under visible light irradiation are discussed.

In Chapter 6 the functionalization of a $[Ru(terpy)(N\text{-}N)(SRR')]^{2+}$ complex with a fluorescent rhodamine dye is reported. The dye-functionalized ruthenium complex was initially considered for monitoring the molecular motion of ruthenium complexes at the surface of a lipid bilayer. However, the emission of the dye appeared to be quenched by the nearby ruthenium complex, leading to the sensitization of ligand photosubstitution reactions with low-energy photons. This study demonstrates that efficient cleavage of the Ru-S bond can be obtained with yellow photons that, in theory, do not have enough energy. Our results provide thorough understanding of the effect of irradiation wavelength on ruthenium-based photosubstitution reactions.

Parts of this thesis have been published,[131-132] have been submitted,[133-134] or are in preparation for publication. [135]

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