Covalent and non-covalent binding of metal complexes to RNA

Elena Alberti, Marianthi Zampakou and Daniela Donghi[✉]

University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich,
Switzerland

[✉] daniela.donghi@chem.uzh.ch

Abstract

Targeting nucleic acids with metal complexes is an exciting and widely explored field of research. Following the discovery of the anticancer drug cisplatin, a number of metal complexes have been designed, synthesised, and tested for their DNA binding properties. On the contrary, the interaction of metal complexes with RNA has been much less investigated. RNA is an essential biomolecule, involved in a variety of crucial cellular functions, which offers a much wider structural diversity than DNA. As such, RNA represents an attractive target for the design and the development of structure-selective therapeutic and diagnostic agents. A few recent publications describe the ability of various metal complexes to interact with RNA, and the binding of cisplatin and derivatives to RNA is being currently investigated. This short review offers an overview of some recent advances on both covalent and non-covalent interactions of metal complexes with RNA and addresses the potential of targeting RNA non-duplex structures.

Keywords: metal complexes; RNA; covalent binding; non-covalent binding

1. Introduction

Nucleic acids are attractive targets for metal complexes [1-9]. The latter have a great diversity of structures and functions, due to nature, oxidation state and coordination geometry of the metal centre as well as to the variety of organic ligands bound to the metal centre. They are highly modular systems. On the one hand, the ligands can be functionalized to control cellular uptake, accumulation and specific biomolecule targeting. On the other hand, the metal centre can exhibit spectroscopic, photophysical and electrochemical properties, which may be also tuned by ligand modifications. All of these properties can be exploited for both diagnostic and therapeutic applications [10-13]. Binding of metal complexes to nucleic acids can be achieved in two different ways, either covalently, by direct coordination of the metal centre (*e.g.* platinum drugs [14, 15]), or non-covalently, by supramolecular interaction of the organic ligands (*e.g.* the numerous metal complexes used in bio-imaging [10-12]). In the first case, the metal centre

directly binds to, for example, the nitrogen atoms of the nucleobases, as observed for platinum drugs [14, 15]. **On the contrary**, in the second case, the interaction is guided by the organic ligands, and can be of different kinds, including hydrophobic groove binding, electrostatic interaction, **hydrogen-bonding**, intercalation, insertion, or a combination of **all of** them [1-9]. Numerous applications for metal complexes interacting with nucleic acids have been proposed, spanning from their use as therapeutic drugs [13], as fluorescent imaging probes [12] and as footprinting agents [16], **and** also include applications in nanotechnology [17]. Most of the studies on metal complexes binding to nucleic acids were performed using DNA, since they were fuelled by the pioneering discovery of the covalent binding of the anticancer drug cisplatin to DNA [1-9]. Metal complexes can bind to nucleic acids by targeting specific sequences, but they can also be designed to target specific structural elements, as it was recently reviewed by Keene and co-workers [3]. In fact, even if DNA occurs mainly as duplex structure, recent research efforts have been dedicated to the design of metal compounds able to target DNA non-canonical structures. These compounds include, for instance, bulky ruthenium(II) complexes able to bind bulged DNAs [18], iron(II) supramolecular helicate binding to DNA **three-way junctions** [19], and a series of metal containing polycyclic planar aromatic compounds, like metal-salphen [20] and platinum(II) terpyridine [21] **complexes**, that bind to G-quadruplex DNAs [22]. It is clear that **all of these** non-canonical structures represent a valid target for structure- and shape-selective binding agents [3].

Even if less explored as a target for metal complexes, RNA is even richer in non-canonical structural elements than DNA. RNA is an exciting molecule, for which several functions are continuously revealed [23-25]. Besides the early discovered messenger, ribosomal and transfer RNAs, that allow the flow of genetic information from DNA to proteins, a great variety of non-coding RNAs **have** been identified in the last decades [25]. **Examples are represented by** the short microRNAs (miRNA) [26, 27] and the riboswitches [28], involved in gene regulation, as well as the ribozymes, catalytically active RNAs able to perform catalysis without the aid of a

protein [25, 29]. The great variety of non-coding RNAs is paralleled by the variety of their functions, and still much work has to be done to comprehensively understand the way these fascinating molecules work [25]. RNA function is invariably associated to its structure [30]. The latter is so rich in non-canonical elements that their targeting represents a challenging but powerful way to interfere with RNA function. Along this line, a few small molecules have been developed that are able to target RNAs [31-34]. For example, different classes of antibiotics, including, *e.g.*, aminoglycosides and tetracyclines, are known to exert their effect by targeting bacterial RNA [31]. Although successful, these compounds show only moderate affinity and selectivity for RNA [32]. Thus, a constant effort is being made to identify the main features ruling the interaction between small molecules and RNA, in order to design compounds displaying selective binding affinity for a specific RNA target. It seems that high-throughput screening followed by synthetic optimization may lead to the development of molecules with high RNA affinity and specificity [31-35]. Some successful examples include the design of cyclic peptides able to target HIV-1 (human immunodeficiency virus) RNA [36] and of modularly assembled compounds targeting expanded nucleotide repeats that cause neuromuscular diseases [37]. Also, small molecules able to modulate miRNA functions were identified [33, 38]. Surprisingly, the RNA binding abilities of small metal complexes, which can bind to RNA both covalently and non-covalently, have been much less explored [3, 39, 40]. However, a few recently published papers highlight the renewed interest of the scientific community for this topic [41-47].

The aim of this review is to summarize recent studies on both covalent and non-covalent interaction of metal complexes with RNA. We will at first present a brief overview on RNA structure, including its intrinsic need for metal ions (section 2). This will be followed by the discussion of some recently published examples of direct metal binding to RNA, focusing on platinum binding (section 3), and of supramolecular non-covalent RNA binding of complexes with different metal centres (section 4).

2. RNA structure and its intrinsic need for metal ions

RNA offers a much wider structural diversity than DNA. It is transcribed as a single strand and it folds in a complex **three-dimensional** structure to minimize its overall energy. In addition to **double-helical** regions, it forms several non-duplex secondary structural elements, including bulges, hairpins and junctions (Figure 1a). The presence of the **2'-OH group influences the sugar pucker in favour of a C3'-endo conformation**, resulting in RNA adopting an A-form conformation in double helical regions (Figure 1b). As a consequence, unlike DNA, which is mostly found in B-form (Figure 1c), the major groove is deep and narrow, while the minor groove is wide and shallow. RNA grooves are thus differently accessible to ligand binding compared to DNA, and the nucleobases that are not involved in regular **base-pairing** can induce double helical widening and local perturbation which may offer unique binding sites for small molecules [3, 31, 32, 40].

Figure 1. (a) Schematic representation of RNA secondary structural elements; (b) A-RNA double helix; (c) B-DNA double helix. Panels (b) and (c) have been prepared with MOLMOL [48] using PDB ID 1RNA [49] and 1BNA [50] respectively.

The structural complexity of RNA is critical for its functioning [30]. The various secondary structural elements provide a scaffold of subunits that direct the complex tertiary RNA architecture, in which various regions far away in primary sequence are brought together via long-range interactions. These include, *e.g.*, pseudoknots, kissing loops, coaxial stacking, tetraloop/receptor interactions, sequence specific contacts, and define the correct and active RNA

tertiary fold [30]. Such complex structures cannot be achieved in the absence of metal ions. Metal ions have structural and functional roles in RNA, being critical for both folding and catalysis [51]. On the one hand, they serve as general charge screeners of the negatively charged phosphate backbone. On the other hand, metal ions could bind to specific binding pockets generated upon formation of the complex **three-dimensional** RNA structure, and may eventually participate in catalysis [52-54]. K(I) is the most abundant intracellular monovalent metal ion, and is often associated to RNA for charge neutralization, while Mg(II) is considered a true RNA cofactor, and may be involved in catalysis in the so-called **two-metal-ion** mechanism [55]. There are different ways to investigate the interactions between metal ions and RNA [56, 57]. Biochemical and spectroscopic techniques used to study such interactions include, for instance, **metal ion** induced cleavage experiments, sulphur rescue and nucleotide analogue interference mapping experiments, X-ray crystallography, EPR and NMR spectroscopy, and have been recently reviewed [56, 57]. Transition metal ions (*e.g.* Fe(II)-EDTA) and lanthanides (*e.g.* Tb(III)) are currently used in RNA footprinting experiments to map secondary and tertiary structure as well as to identify metal ion binding sites [58, 59]. Also, different transition metal ions, like Cd(II) and Mn(II), as well as cobalt(III)hexammine are used as Mg(II) mimics in NMR studies [60-65]. These aspects of metal ion-RNA interactions go beyond the scope of this review and will not be further discussed.

3. Platinum binding to RNA

The therapeutic success of the anticancer drugs cisplatin and its analogues, oxaliplatin and carboplatin (Figure 2a), that are in clinical use worldwide [15], stimulated intense research dedicated to the design and the development of metal-based compounds as anticancer agents [13, 15]. Since cellular DNA is regarded as the main target of cisplatin and its derivatives [14, 15], great research effort has been directed towards the rational design of metal complexes able to bind DNA [1-9]. The most common binding mode of cisplatin to DNA is the direct coordination of the platinum centre to N7 of adjacent guanines, resulting in a strong structural distortion

which eventually leads to cell apoptosis [14, 15, 66, 67] (Figure 2b). Even if cellular DNA is considered the main therapeutical active target for cisplatin, the latter could also interact with other biomolecules, like RNA [39] and proteins [67, 68]. Studying the interaction of cisplatin with biomolecules other than DNA is important to obtain a clearer picture of its complex biological activity [39, 67, 68]. Moreover, it is worth noting that intense research is being currently dedicated to the identification of new cellular targets for metallodrugs in general [69]. Due to the chemical similarity between DNA and RNA, cisplatin and its derivatives are expected to easily bind also to RNA. Since RNA is already available in the cytosol, it may act as an effective scavenger for cisplatin [70]. Therefore interactions between cellular RNAs and platinum drugs may influence drug toxicity and resistance, and their investigation may provide a more complete understanding of drug activity [39].

Figure 2. (a) Chemical structure of the anticancer drugs cisplatin, carboplatin and oxaliplatin. (b) NMR solution structure of a DNA dodecamer containing a 1,2-intrastrand crosslink between cisplatin and two adjacent guanines. **Panel (b) has been** prepared with MOLMOL [48] using PDB ID 1A84 [66].

Early studies showed that RNA-dependent processes like splicing [71] and translation [72, 73] are inhibited in cell extracts upon administration of cisplatin. A recent study also demonstrated that cisplatin, oxaliplatin and carboplatin have different effects on mRNA translation *in vitro* [74]. Moreover, RNA-cisplatin adducts have been shown to inhibit the function of some RNA processing enzymes *in vitro* [75]. Interestingly, inductively coupled plasma mass spectrometry (ICP-MS) was recently used to show that platinum accumulates in total 4- to 20-fold more in

total cellular RNA than in genomic DNA upon cisplatin treatment of *Saccharomyces cerevisiae* (*S. cerevisiae*) [76].

Given the wide variety of RNA secondary and tertiary architectures, and the importance of RNA structure to fulfil its functions, studies aimed at elucidating RNA-platinum interactions are challenging but of great interest. Platinum binding can indeed strongly distort RNA structure, thus interfering with its function. Moreover, the RNA structure itself may direct and influence metal binding. A number of biochemical investigations have been performed to study platinum-RNA interactions, and to possibly understand the effects of platinum binding on RNA biology, as recently reviewed by DeRose and co-workers [39]. In the sections below the most recent advances in the elucidation of platinum-RNA interactions are summarized, mainly focusing on examples subsequent to the above-mentioned review [39]. In particular, the following aspects are covered: (i) RNA platination kinetics [45, 70, 77-79] (sections 3.1 and 3.2); (ii) some examples of platinum binding to structured RNAs [80], focusing on latest insights into platinum binding to ribosomal RNA [44, 76, 81] (section 3.3); (iii) the effect of platination on RNA interference [82-84] (section 3.4); (iv) the use of Pt-RNA adducts to study RNA structure and dynamics [85] (section 3.5) and (v) recent methods to detect RNA-platinum adducts in cells [47, 86, 87] (section 3.6).

3.1 Kinetics of RNA-platinum binding: the example of tRNA

Upon cell membrane penetration, either by passive diffusion or mediated by copper transporter proteins or organic cation transporters [15, 67], cisplatin is hydrolysed to its active form, *cis*-[Pt(NH₃)₂Cl(H₂O)]⁺ (mono-aquated cisplatin), due to the reduced intracellular concentration of chloride ions (4-20 mM) [14, 15]. This step is of crucial importance, since hydrolysis of the first chloride was shown to represent the rate-limiting step for initial binding [88]. Accordingly, hydrolysis rate constants should be considered when predicting toxicity of new platinum drugs [89]. Once the positively charged mono-aquated species is formed, its DNA interaction is facilitated by electrostatic association to the negatively charged phosphate backbone. This

preassociation precedes covalent interaction [90, 91], and was shown to influence the binding kinetics also in model systems using short single-stranded DNAs [92]. This finding suggests that short RNAs may also represent good targets for cisplatin [77, 78], and prompted further studies to better elucidate the basis of platinum-RNA interaction. Interestingly, *in vitro* studies performed using mono-aquated platinum complexes have shown that RNA platination kinetically competes or is even preferred over DNA platination [80, 93]. Elmroth and co-workers investigated the interaction of cisplatin with the full length tRNA^{Ala} and a short hairpin model (Mh^{Ala}) of its GC rich acceptor stem region (Figures 3a and 3b). Partial hydrolysis and enzymatic cleavage assays were used to identify the platination sites. Interestingly, the rate of initial product formation was found similar to that of cisplatin hydrolysis in the two cases, and a kinetic preference for adduct formation was observed at the wobble pair region in both constructs (Figures 3a and 3b), despite their differences in size and flexibility [77].

Figure 3. Secondary structure of tRNA^{Ala} (a) and its model systems: the acceptor stem Mh^{Ala} (b) and the anticodon loop acMh^{Ala} (c) [77, 78]. The GC-rich acceptor stem is indicated in grey; the GU wobble base-pair is indicated with a box; the UGC/GGC modification in the anticodon loop is indicated with contours.

The study was then further extended, and it was shown that the initial adduct formation in Mh^{Ala} can be inhibited by substituting the GU wobble in the acceptor stem region with a regular GC base-pair (Figure 3b). This confirms kinetic preference for platination at the wobble pair region. Additionally, the platination of a short hairpin model of the anticodon region (acMh^{Ala}, Figure

3c) was investigated, and a different platination pattern was observed when the natural UGC in the terminal loop was substituted with a GGC sequence (Figures 3a and 3c). Moreover, GG sequences seem to be more readily platinated when they are in **single-stranded** regions than when they are involved in **base-pairing** like in the stem region. **All of these** findings suggest that small sequence variations, solvent accessibility and overall RNA structure may strongly influence platinum binding [78].

*3.2 Kinetics of RNA-platinum binding studied via UV-Vis: **how different parameters can influence platination***

Platination of RNA and DNA duplexes strongly influences their melting profiles. In fact, the destabilization caused by platinum binding induces a loss of **base-pair** stacking and **hydrogen-bond** interaction which leads to a consequent decrease of the melting temperature. Elmroth and co-workers measured via UV-Vis the destabilization induced by oxaliplatin and cisplatin in the two duplexes RNA-1 and RNA-2 (Figure 4), and the corresponding DNA duplexes. They showed that both the two platinum complexes strongly destabilize the duplexes, **resulting in melting temperature reduction**, with a much larger destabilization observed for the GXG containing duplexes than for the GG containing ones, especially for the RNA species [94]. Following this study, they established a quick methodology **to investigate the platination kinetics of different RNA duplexes**, that relies on monitoring **platinum-induced** duplex destabilization via UV-Vis spectroscopy **in the presence of the mono-aquated form of cisplatin**. Platination rate constants are obtained by following the changes of RNA absorbance in the presence of **mono-aquated** cisplatin as a function of time [79]. They first applied this method to study the platination kinetics of three RNA duplexes: two **15-nucleotide-long** model RNAs, containing a GG and a GUG internal binding site, and a mature miRNA (RNA-1, RNA-2 and miR-146a in Figure 4).

Figure 4. RNA duplexes used by Elmroth and co-workers in the platinumation studies commented in section 3.2 [45, 70, 79, 94]. The main platinumation sites for duplexes RNA-1 to RNA-7 are indicated with a box.

The three duplexes showed similar reactivity at low salt concentration (29 mM Na(I)), while at higher salt concentration (129 mM Na(I)) RNA-1 proved to be **the most reactive among the three**. These findings suggest that **mono-aquated** cisplatin is able to discriminate in biological relevant salt concentration between different RNA targets [79]. The same approach was used to evaluate how platinumation kinetics **are** affected by the length of the duplex and the nature of bases surrounding the central GG platinumation site [70]. Five different duplexes were used in this study: RNA-1, [79] **and RNA-3 to RNA 6 which differ from RNA-1 either in length or in the nature of bases around the central GG (boxes in Figure 4)**. Differences in reactivity were observed. **In particular RNA-6, which contains four mismatched uracils, was the most reactive**. These results suggest that local structure variations have a strong influence on duplex reactivity [70]. A similar study was performed on four duplexes (RNA-1, RNA-3, RNA-5 and RNA-7 in Figure 4) to evaluate the effect of cation nature **on the interaction between mono-aquated cisplatin and RNA** (ca. 0.1 M K(I) *vs* Na(I) were used to mimic the intracellular and the extracellular environment, respectively). Moreover, kinetic data were collected in the presence of ethanol (to mimic *in vivo* conditions), to evaluate the effect of hydrophobicity on duplex reactivity. Interestingly, all tested duplexes show a small increase in reactivity in the presence of K(I), compared to Na(I), while ethanol seems to decrease the reactivity. These results suggest that the reaction between **the mono-aquated form of** cisplatin and short RNA duplexes is favoured in the intracellular rather

than in the extracellular environment, but also show that the increased hydrophobicity of the environment reduces the reactivity [45].

All of these studies underline the possibility of rapid and effective platination of short RNA duplexes in the cytosol, including biologically important miRNAs involved in gene regulation. Moreover, they confirm that platination may be strongly affected by different parameters, including local RNA structure, cation nature and concentration, and hydrophobicity of the environment [45, 70, 79].

3.3 Platinum binding to structured RNAs: the case of spliceosomal and ribosomal RNA

A few studies were performed in order to understand the ability of cisplatin to target specific RNA structures, other than duplexes. These include, for example, early X-ray investigations of platinated yeast tRNA^{Phe} [95, 96] and a spectroscopic study on cisplatin and a platinum(II)-estradiol adduct with yeast tRNA [97]. DeRose and co-workers recently explored the platination profile of a 41-nucleotide-long RNA construct containing a large asymmetric internal loop (Figure 5a), as model system for the catalytic core of the U2:U6 spliceosomal RNA [80]. The spliceosome is a complex molecular machine responsible for splicing and maturation of mRNA in eukaryotes [98], and earlier studies performed in cell extracts showed that spliceosome activity is inhibited upon addition of platinum(II) compounds [71]. Interaction of their model system with mono-aquated cisplatin resulted in the platination of two opposite guanines in the internal loop, leading to the formation of a novel platinum intrastrand crosslink (Figure 5a), as confirmed by partial alkaline hydrolysis mapping. This rapid crosslink formation in an internal loop of a structurally complex RNA may in principle affect RNA dynamics and function [80]. However, the effect of RNA-platinum binding on cell functions is still largely unknown [39, 75]. Investigation of ribosomal RNA platination was also the subject of a number of studies. For instance, DeRose and co-workers found significantly larger amounts of platinum in rRNA compared to mRNA in *S. cerevisiae* upon cisplatin administration *in cellulo* [76]. Mapping of platination sites in rRNA using reverse transcription primer extension revealed the presence of

preferential binding sites in regions that are conserved between yeast and humans [76]. Moreover, a more recent study showed that cisplatin accumulates in the sarcin-rich loop and at the peptidyl transferase centre of rRNA, thus suggesting potential ribotoxic role for platinum [87].

Chow and co-workers investigated the platination of rRNA from *E. coli* in a previous study [99], with the aim of using cisplatin as a molecular probe to identify solvent exposed, *i.e.* easily accessible, purine residues in bacterial ribosome and to map potential small molecule binding sites. Reverse transcriptase stop assays were used to identify the platination sites in 16S rRNA in its free form and in the context of 30S subunit and 70S ribosome, both *in vitro* and *in vivo*. The region corresponding to helix 24 was chosen for this study, because it is located close to the ribosome decoding site and X-ray structures suggest there a high solvent accessibility [99] (Figure 5b). Two consecutive guanosines were identified as a preferential binding site both in free 16S rRNA and when it was complexed with the ribosomal proteins (contour nucleotides in Figure 5b), indicating similar accessibility at this site. Interestingly, DeRose and co-workers showed that platination at this site does not take place in rRNA from *S. cerevisiae*, where C783-G799 is reversed into G783-C799 (boxes in Figure 5b and 5c), thus suggesting sequence specificity of the binding [76].

Figure 5. (a) Secondary structure of the RNA construct used as model for the catalytic core of the U2:U6 spliceosomal RNA (BBD RNA) by DeRose and co-workers [80]. Only platinum crosslinking in the

internal loop is indicated. (b) Secondary structure of helix 24 of the *E. coli* 16S rRNA used by Rijal and Chow in their studies [99]. The two consecutive guanines targeted by cisplatin in free and complexed 16S rRNA (see section 3.3) are indicated as contour nucleotides. (c) Secondary structure of helix 18 of *S. cerevisiae* small ribosomal subunit investigated by DeRose and co-workers [76]. The boxes in (b) and (c) indicates the **base-pair** that is inverted in *E. coli* and *S. cerevisiae* [76] (see section 3.3).

Chow and co-workers further investigated rRNA platination by MALDI mass spectrometry and chemical and enzymatic mapping. They identified platination sites and **platinum-induced** distortion on two highly conserved hairpins of *E. coli* rRNA, namely the pseudouridine-modified helix 69 from 23S rRNA, and the 790 loop of helix 24 from 16S rRNA. The results show preferential binding of **mono-aquated** cisplatin with GG motifs, and chemical probing suggests structural changes in the loop upon platination [81]. They also used helix 24 of 16S rRNA to show that modifications in size and charge distribution of cisplatin derivatives can strongly influence **the** RNA platination profile [44]. They studied the reactivity of the **mono-aquated** form of four aminoacid-linked platinum complexes (Figure 6) with 16S rRNA using primer extension analysis, and compared their platination profile with the one of **mono-aquated** cisplatin [99].

Figure 6. Different platinum(II) complexes used by Chow and co-workers in reaction with *E. coli* 16S rRNA [44].

Consecutive GGs that were the preferential binding sites for cisplatin are less prone to platination with the other investigated platinum complexes. For example, Oplatin and Kplatin, **which only differ in one CH₂ group, show overall similar reactivity, with** preferential binding to A, AG or GA in loops or bulges. Rplatin, **which contains an arginine group, shows different reactivity, with preferential coordination to a bulge nucleotide. This resembles the interaction of the arginine-rich Tat protein with the three-nucleotide bulge region of TAR RNA (see section 4.3). Finally,** Dplatin shows no significant reactivity, likely due to electrostatic repulsion.

Further, HPLC investigation confirmed the preference of Oplatin for adenosine coordination over guanosine, suggesting the use of these compounds to assess adenosine accessibility in structured RNAs [44]. This study showed that the increased bulkiness of the *cis* ligands and the different charge distribution may significantly influence RNA platination profiles.

The above-commented findings may seem in contradiction, at least partially, with a recent study by Theile and Kos, in which rRNA platination patterns induced by equitoxic concentrations of cisplatin, oxaliplatin and carboplatin in mammalian cells were investigated [100]. It was shown that equitoxic concentrations of the three platinum complexes lead to same rRNA platination patterns and under pharmacokinetically relevant concentrations none of the drugs inhibit translation efficiency. Even if a deeper sequencing of the platinated rRNA might have given more details on platination profiles, this study suggests that rRNA platination is unlikely to contribute to the clinical toxicity of these three drugs [100].

In general, the studies commented in this section give evidences towards the ability of different platinum complexes to interact with structured RNAs, but at the same time underline the complexity of platination of complex RNAs and call for further and more detailed investigations.

3.4 *Effect of RNA platination on siRNA silencing*

Short-interference RNAs (siRNAs), as well as miRNAs, are short non-coding RNAs (around 20-30 nucleotides in length) able to silence gene expression [26]. MiRNAs are derived from RNA hairpins and are of endogenous nature; they target mRNA by only partial complementarity, and inhibit mRNA translation. On the contrary, siRNAs are derived from long double stranded RNAs and are often of exogenous nature; they target mRNA by full complementarity and lead to mRNA degradation [101, 102]. In both cases, the silencing mechanism relies on the incorporation of one of the two strands, the antisense strand, into a ribonucleoprotein complex, the RNA-induced silencing complex (RISC). This is followed by recognition of the target mRNA which leads to either translation repression or mRNA degradation [84, 101, 103].

MiRNAs have strong potential as biomarkers, since their dysregulation has been associated with the occurrence of several pathologies, including cancer [27, 102, 104], whereas synthetic analogues of siRNAs are currently being developed as biological tools to suppress protein production [82-84, 102, 105].

The Elmroth group investigated the effect of siRNA platination in silencing ability. They first showed that sense strand platinated siRNAs with both cisplatin and oxaliplatin still maintain their silencing abilities, likely due to the fact that the RISC machinery is still able to correctly identify the platinated siRNA via the unplatinated antisense strand [82, 83]. In a more recent study they used cellular luciferase expression assays to evaluate the silencing ability of pre-platinated siRNA antisense strands in positions outside the seed regions, with both cisplatin and oxaliplatin. Interestingly, they observed that even if platinated siRNAs are still active in inducing silencing, there is an overall decrease of silencing activity, with oxaliplatin having a more pronounced effect than cisplatin. These results suggest that platinum drugs may interfere with siRNA processing, with subtle differences between the different drugs used. Moreover, the authors speculate that the differences between cisplatin and oxaliplatin could be even more pronounced for endogenous miRNA silencing, and that the different activity and toxicity of the two drugs might be somehow partly related to their ability to interfere with miRNA activity [84].

3.5 Using platinum(II) crosslinking to study RNA structure and dynamics

Site-specific platinum(II) crosslinking was recently proposed by DeRose and co-workers as a promising tool for exploring RNA structure and dynamics [85]. As a proof of concept, they introduced a phosphorothioate substitution at the scissile bond in the catalytic core of the Hammerhead ribozyme (HHRz). The sulphur atom was used to anchor a cisplatin core. The second exchangeable ligand of the platinum core was then substituted by a nitrogen atom of a close-by RNA nucleotide. This gave rise to a stable metal-mediated crosslink that could be mapped after isolation. This method can be successfully used to obtain information on nucleotides that are close in space to the scissile backbone upon tertiary structure formation [85].

3.6 Identifying platinum-RNA adducts in cells

DeRose and co-workers have recently developed a convenient and potent method to identify platinum cellular targets [47, 86, 87]. They showed that platinum complexes containing an azide unit (Figure 7a) can be used for post-labelling with an alkyne containing fluorophore, using azide-alkyne dipolar cycloaddition (click reaction; reaction scheme in figure 7b).

Figure 7. (a) Platinum(II) complexes used by DeRose and co-workers for post-treatment labelling by means of click chemistry (right), including their non-azido analogues (left) [47, 86]. (b) Scheme of click chemistry reaction involving a biomolecule-bound picazoplatin and a fluorophore [86].

The use of post-treatment labelling to localize platinum drugs is convenient to investigate the biological distribution of native drugs. In fact, it avoids the issues associated with the use of platinum compounds tagged with big fluorescent molecules which can influence their cellular distribution [39, 106]. Application of this method to RNA-platinum interaction was first reported in a study in which picazoplatin, an azide functionalized derivative of picoplatin (Figure 7a), was successfully used to label an RNA construct model of the ribosomal sarcin-ricin loop via acetonitrile-assisted click reaction. The method proved also successful for the labelling of rRNA extracted from *S. cerevisiae* after treatment with picazoplatin [86]. Subsequently, the authors used click chemistry to label platinum bound to rRNA and tRNA, after extraction from picazoplatin treated *S. cerevisiae* with Alexa Fluor 488 DIBO alkyne, which can be visualized using in-gel fluorescence. Their study showed for the first time *in vivo* Pt(II) accumulation in tRNA. Even if the functional consequences of tRNA platination are not known yet, a possible

influence of platination on tRNA regulatory functions can be hypothesised [87]. More recently, they also showed the use of another platinum complex, namely *cis*-[Pt(2-azido-1,3-propanediamine)Cl₂] (Figure 7a), for the detection of long-lived Pt-RNA adducts in rRNA [47]. All in all this non-invasive post-labelling strategy proves to be a potent tool to follow the fate of platinum drugs *in vivo*, and to investigate platinum-RNA adducts, with the aim of understanding their biological functions.

4. Non-covalent binding of metal complexes to RNA

In section 3 we described the interaction of platinum complexes with RNA, which proceeds via direct RNA-metal coordination. On the contrary, non-covalent interactions between metal complexes and RNA may be achieved via a variety of modes, including intercalation, hydrogen-bonding, hydrophobic and electrostatic interactions, as observed in the case of DNA [1-9]. RNA non-duplex structures provide an attractive scaffold for supramolecular interaction. However, even if the potential of small organic molecules to bind to RNA is well acknowledged [31, 32], non-covalent interactions of metal complexes with RNA have been less investigated [3, 40]. Some examples of metal complexes interacting with RNA have been commented in recent comprehensive reviews on metal complex binding to nucleic acid non-duplex regions, which however mainly focused on DNA binding [3, 7]. In the sections below some recent advances in non-covalent binding of metal complexes to RNA are described and discussed. In particular, the following aspects are covered: (i) the use of metal complexes as RNA structural probes [40, 107, 108] (section 4.1); (ii) the structure of a metal complex interacting with an RNA three-way junction [41] (section 4.2); (iii) the development of metal complexes targeting viral RNA [109-112] (section 4.3); (iv) the combination of metal complexes with small RNA-binding organic molecules (section 4.4) [113-117]; (v) the use of RNA-targeting metal complexes in cell imaging [42, 43, 118-121] (section 4.5); (vi) comparison between DNA and RNA binding properties of some metal complexes [122-125] (section 4.6); (vii) the RNA binding ability of some metal

surfactants and metal polymers [126-129] (section 4.7) and (viii) the binding of metal complexes to RNA triplexes [46, 130-133] (section 4.8).

4.1 Metal complexes as shape selective probes for RNA structure

Several metal complexes have been developed as probe for RNA tertiary structure [40]. For example, early studies showed that $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ (phen = 1,10-phenanthroline, phi = 9,10-phenanthrenequinone diimine) (Figure 8a) does not bind to RNA double-helical regions, nor to unstructured **single-stranded** RNA regions, but it is able to bind in regions that are involved in tertiary interactions, where the major groove is wider and thus allows for stacking interaction [40, 134]. This complex was successfully used to identify tertiary interaction sites in tRNA [135] and regions in *Xenopus* oocyte **5S** rRNA which are critical for protein binding [134].

Figure 8. (a) $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ (phen = 1,10-phenanthroline, phi = 9,10-phenanthrenequinone diamine) and (b) $[\text{Rh}(\text{DIP})_3]^{3+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline), used as shape selective agents to investigate RNA tertiary structure [40].

A similar Rh(III) complex, $[\text{Rh}(\text{DIP})_3]^{3+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline) (Figure 8b), was also used to specifically target GU mismatches and exposed guanines in small and large RNA molecules [107, 136]. The information on RNA structures **is** obtained owing to the ability of these rhodium complexes to promote photoactivated strand scissions at their binding sites, which can be monitored with gel electrophoresis. Other complexes containing different metal centres, including lanthanides [16, 137], can induce RNA strand scissions via different chemical reactions, like, *e.g.*, hydrolysis and sugar or guanine oxidation [138-141]. This ability of metal

complexes to cause backbone cleavage can be used both to investigate secondary and tertiary structural features in RNA [16, 40] and to induce RNA degradation [137].

Other properties of metal complexes can be used to investigate RNA structure. For instance, Mascareñas, Vázquez and co-workers recently reported on the use of a short peptide functionalized with a luminescent DOTA[Tb³⁺] (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) complex and a tryptophan antenna as selective probe for a short RNA hairpin (Figure 9). In this case, the presence of the hairpin structure was identified following the changes in emission properties of the used metal complex.

Figure 9. Schematic representation of a short peptide functionalized with a tryptophan antenna and a DOTA[Tb³⁺] complex developed by Mascareñas, Vázquez and co-workers. Upon binding to RNA, the distance between the antenna and the DOTA[Tb³⁺] complex becomes smaller thus maximizing energy transfer [108].

They investigated the interaction between an arginine-rich motif derived from the transcriptional antitermination protein N of phage P22, functionalized with a DOTA[Tb³⁺] complex and a tryptophan sensitizer, and its biological target *boxB*, a short RNA hairpin [108]. Binding of the peptide to the folded hairpin resulted in close proximity of the tryptophan unit and the luminescent DOTA[Tb³⁺] complex, thus maximizing the lanthanide sensitization process leading to higher emission. Owing to the exceptional luminescent properties of the lanthanide ions, and the high sensitivity and selectivity of the described probe, the authors suggest that this approach could be used for the development of additional probes able to detect other structured RNAs, to possibly study similar biological processes [108].

These examples confirm the potential of metal complexes as reporters of RNA structures.

4.2 Crystal structure of a metal complex bound to a three-way junction RNA

Hannon, Coll and co-workers recently described the binding of a tetra-cationic dinuclear Fe(II) supramolecular metal complex ($[\text{Fe}_2\text{L}_3]^{4+}$, cylinder) to the central cavity of a three-way junction (3WJ) DNA [19]. This cylinder is a nano-sized metal complex, of about 2 nm length and 1 nm diameter, which is synthesised upon reaction of FeCl_2 and bis-pyridylimine ligands (Figure 10a). The structure of the adduct between the 3WJ DNA and the iron(II) cylinder represents an almost perfect shape-fit, in which the original structure of the two components remain relatively unperturbed [2, 19]. Since RNA is much richer in junctions than DNA [3, 31, 32], it was of interest to explore the ability of these cylinders to bind a three-way junction RNA. Freisinger, Hannon, Spingler, Sigel and co-workers recently reported the crystal structure of a dinuclear Fe(II) cylinder with a palindromic hexanucleotide RNA sequence (Figure 10b) [41]. The structure contains exclusively the *M* enantiomer of the cylinder, and shows one cylinder sitting in the heart of a three-way junction RNA, to optimize π -stacking interactions between the phenylene rings and the RNA bases (Figure 10b). Additional cylinder molecules are located at the termini of the three arms of the junctions. This structure represents the first crystallographic example of a designed metal complex bound to RNA [41]. The authors also explored the ability of the cylinder to induce RNA three-way junction formation in solution. They used RNA sequences that potentially form similar 3WJ as previously observed for DNA. Native gel electrophoresis and circular dichroism (CD) experiments confirmed that the cylinder strongly stabilizes RNA three-way junctions also in solution. Finally, competition assays between analogues DNA and RNA 3WJs showed that the two nucleic acids compete with one other for cylinder binding [41].

This example demonstrates the potential of metal complexes in supramolecular recognition of nucleic acid junctions.

Figure 10. (a) Iron cylinder developed by Hannon and co-workers. Only the *M* enantiomer is represented [142]. (b) Crystal structure of the adduct between an RNA three-way junction and the iron cylinder (top view). Only the cylinder sitting in the heart of the junction is depicted (see section 4.2). The figures have been prepared with MOLMOL [48] using PDB ID 4JIY [41].

4.3. Targeting viral RNA with metal complexes

The design of small molecules able to target viral RNA represents a good strategy for the development of antiviral agents [31, 32]. Along this line, several metal complexes were investigated for their ability to interact with viral RNA. One important binding site in HIV-1 RNA is a **three-nucleotide** bulge in the TAR (*trans* activation response) element. The latter is a short **stem-loop** RNA located at the 5'-end of the HIV-1 transcript which serves as a binding site for the Tat protein (Figure 11a) [3, 31, 143].

Figure 11. Secondary structure of the Tat protein binding site of HIV-1 TAR RNA (a) [143, 144] and of the **minimized construct RRE JW derived from RRE RNA** used in binding studies by Tor and co-workers (b) [112, 113].

The formation of the TAR-Tat complex is crucial for viral transcription. Consequently, inhibiting the formation of such complex by small molecule binding to TAR RNA has potential

as antiviral strategy [31, 144]. Neenhold and Rana first used $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ (Figure 8a) to show that the presence of the **three-nucleotide** bulge is necessary to widen the major groove at the Tat binding site, so facilitating the TAR-Tat complex formation [143]. $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ was also found able to target the Tat binding site in bovine immunodeficiency virus TAR RNA [145].

More recently, Kimura and co-workers described the ability of zinc(II)-macrocyclic tetraamine complexes to inhibit the formation of HIV-1 TAR-Tat complex, by strongly binding the U rich bulge in HIV-1 TAR RNA [109]. Keene and co-workers also showed that the dinuclear ruthenium complex $[\{\text{Ru}(\text{Me}_2\text{bpy})_2\}_2(\mu\text{-bpm})]^{4+}$ (Me_2bpy = 4,4'-dimethyl-2,2'-bipyridine; bpm = 2,2'-bipyrimidine) (Figure 12) is a potential probe for RNA bulges [146], and is also able to bind selectively the three-base bulge of a modified TAR sequence of the HIV-1 virus [110].

Figure 12. $\Delta\Delta\text{-}[\{\text{Ru}(\text{Me}_2\text{bpy})_2\}_2(\mu\text{-bpm})]^{4+}$ (Me_2bpy = 4,4'-dimethyl-2,2'-bipyridine; bpm = 2,2'-bipyrimidine) developed by Keene and co-workers, which bind to RNA bulges [146] and was also shown able to bind the three-base bulge region of a modified TAR RNA construct [110].

Another important region which can be targeted for therapeutical purposes is represented by the Rev Response Element (RRE) RNA. The interaction of the latter with the Rev protein is essential for viral maturation [31]. Tor and co-workers showed that $\Delta\text{-}$ and $\Lambda\text{-}$ $[\text{Ru}(\text{bpy})_2(\text{eilatin})]^{2+}$ (bpy = 2,2'-bipyridine; **eilatin is an heptacyclic aromatic marine alkaloid, able to act as metal chelator** [147]) complexes can inhibit HIV-1 Rev-RRE interaction *in vitro*, likely due to their interaction with RRE bulged regions (Figure 11b) [111, 112]. A detailed analysis of the nucleic acid binding properties of free eilatin and these two Ru-eilatin complexes showed that they preferentially bind via intercalation and with strong preference for **RNA single-**

stranded regions. Interestingly, free eilatin shows selectivity for electron-poor polymeric purines while Ru-eilatin complexes exhibit a preference for the polypyrimidine r(U) [112]. This behaviour shows how binding preferences of an intercalating ligand can change upon **incorporation into octahedral metal complexes**. Moreover, different RNA binding preferences for the two enantiomers were observed, with a slight preference of the RRE construct for Λ -[Ru(bpy)₂(eilatin)]²⁺ vs Δ -[Ru(bpy)₂(eilatin)]²⁺ [111].

These examples illustrate how the ability of metal complexes to bind thermodynamically destabilized regions like bulges can be used to target biologically relevant RNA molecules.

4.4 Combining metal centre and small organic RNA binders for selective RNA targeting

The conjugation of metal centres to ligands which display, for example, intercalating or groove binding properties, or sequence specificity [1, 6], has been widely exploited to develop metal compounds with enhanced DNA targeting abilities. Similarly, conjugating a metal centre to an RNA binder may offer a valid strategy for the design of RNA selective binding molecules, as suggested by the examples commented below [113-115]. Aminoglycoside antibiotics possess selectivity for RNA binding over DNA binding, and their conjugation to a metal centre may indeed lead to potent RNA binding agents. In this regard, Tor and co-workers explored the possibility of functionalizing aminoglycosides and guanidinoglycosides with a platinum unit to induce selective platination, and subsequent damage, of the RRE RNA motif. They prepared platinum conjugates of neomycin B and guanidinoneomycin B (Figure 13) and reacted them with a minimized Rev-binding RRE construct (RRE JW) (Figure 11b). Interestingly, even if different binding sites were found in the two cases, the two platinum adducts were able to bind RRE RNA also in the presence of an excess of calf thymus DNA (CT-DNA), generating RNA selective covalent crosslinks. This suggests that the non-covalent interaction abilities of such conjugates can be used to direct covalent binding on therapeutically significant RNA targets [113].

Figure 13. Platinum(II) adducts of neomycin B and **guanidinoneomycin B** developed by Tor and co-workers [113].

Marchan and co-workers prepared conjugates between a cytotoxic organometallic ruthenium(II) complex and neomycin B and guanidinoneomycin B, and observed different cytotoxicity and cellular accumulation of the two conjugates in cancerous and healthy cells. The authors speculate that RNA could be involved as a target for these conjugates, and suggest that the conjugation of cytotoxic Ru(II) complexes to guanidinoglycosides could lead to more selective and less toxic anticancer metallodrugs [114]. Later on they conjugated a platinum(IV) photoactivatable complex to guanidinoneomycin. Interestingly, they observed enhanced cellular accumulation in two cancer cell lines for the Pt(IV)-guanidinoneomycin conjugate. Higher phototoxicity was observed in the case of melanoma cancer cells, thus suggesting some selectivity over the human prostate carcinoma cells. However, the observed phototoxicity was not increased with respect to the parent compounds, despite the higher intracellular accumulation. The authors suggest that the presence of guanidinoneomycin, far of being a passive carrier, could favour RNA targeting, thus modifying the photocytotoxic activity of the Pt(IV) parent pro-drug [115].

A similar strategy was used by Bradford and Cowan in the development of a catalytic metallodrug. They incorporated a copper centre in a short peptide able to selectively recognize the **stem-loop** IIb of the IRES (internal ribosomal entry site) RNA of the hepatitis C virus. Interestingly, this complex induces specific degradation in the target RNA, and shows good antiviral activity, that was not observed for the targeting peptide alone [116]. Also, Rusak and

co-workers showed that a complex between a flavonoid quercetin and lanthanum(III) is able to bind a plant viral satellite dsRNA with higher affinity than the ligand and the metal ion alone. They suggest that such increased binding is due to a combination of ligand driven intercalation and electrostatic attraction of the positively charged lanthanum(III) centre with the negatively charged RNA backbone [117].

This strategy can also be used for the development of potential RNA-imaging probes, as proved by, for example, the conjugation of a phenanthridine moiety with a ruthenium(II) bipyridine complex (see section 4.5.3) [121].

These examples show the potential of combining the properties of metal centres with those of organic ligands for the development of agents able to bind biologically significant RNA targets.

4.5 Metal complexes in bio-imaging

The use of luminescent metal complexes for cell imaging applications is well acknowledged [9-12]. Since their emission often originates from a triplet metal-to-ligand charge transfer state, it may be strongly influenced by solvent polarity, by hydrophobicity, mobility and viscosity of the environment, and as such metal complexes can report on local modifications and are potential probes for cell imaging. Metal complexes possess favourable photo-physical properties. These include large Stokes shifts and long lifetimes, that facilitate distinction of their emission from cell auto-fluorescence, and reasonable quantum yield in aqueous solutions. Moreover, they have a red shifted absorption profile, which allows for the use of lower energy excitation with better tissue penetration and less damage, and relatively good photo-stability. The kinetic inertness of the metal centre often ensures only slight toxicity, thus allowing their use in imaging [10]. Although most of the newly synthesised metal complexes are investigated for their ability to bind DNAs [1-9], their RNA binding cannot be excluded, as underlined by the fact that often they accumulate in RNA-rich regions in cells [118, 119].

4.5.1 Dinuclear polypyridylruthenium(II) complexes preferentially target non-duplex nucleic acid structures and accumulate in RNA-rich regions

Keene and co-workers recently reported on cellular localization of dinuclear polypyridylruthenium(II) complexes [118, 119] (Figure 14) previously developed as potential antimicrobial agents [148]. In particular, the duplex Rubb_{16} (Rubb_n with $n = 16$, Figure 14) was found to accumulate in rRNA in bacteria *in vivo* [118]. Confocal microscopy was used to show that this class of complexes penetrate the nucleus and accumulate in RNA-rich nucleoli rather than in chromosomal DNA in eukaryotic cells, without enantiomeric preference [119]. The ability of these and other bulky dinuclear ruthenium complexes (Figures 12 and 14) to preferentially bind non-canonical DNA regions, like bulges and hairpins, was previously shown. For instance, the ability of the dinuclear ruthenium(II) complexes $\Delta\Delta/\Lambda\Lambda\text{-}[\{\text{Ru}(\text{phen})_2\}_2(\mu\text{-bb}_n)]^{4+}$ (Rubb_n , phen = 1,10-phenanthroline; $\text{bb}_n = \text{bis}[4(4'\text{-methyl-2,2'-bipyridyl})]\text{-1},n\text{-alkane}$, $n = 2, 5, 7, 10, 12, 14, 16$; Figure 14) to bind non-duplex nucleic acid structures *in vitro* was assessed by a combination of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) displacement assays and ^1H NMR spectroscopy studies [18]. Moreover, the complex $[\{\text{Ru}(\text{Me}_2\text{bpy})_2\}_2(\mu\text{-bpm})]^{4+}$ ($\text{Me}_2\text{bpy} = 4,4'\text{-dimethyl-2,2'-bipyridine}$; bpm = 2,2'-bipyrimidine, Figure 12) was found to preferentially bind to DNA and RNA bulges [146, 149]. Interestingly, an approximately 50-fold increase of the RNA binding affinity was observed in the presence of a single adenine bulge site for both enantiomers [146].

Figure 14. The structure of the dimeric complexes $[\{\text{Ru}(\text{phen})_2\}_2(\mu\text{-bb}_n)]^{4+}$ (Rubb_n ; phen= 1,10-phenanthroline; $\text{bb}_n = \text{bis}[4(4'\text{-methyl-2,2'-bipyridyl})]\text{-1},n\text{-alkane}$, $n = 2, 5, 7, 10, 12, 14, 16$), developed by Keene and co-workers to bind destabilized duplex sequences [18].

The high amount of non-canonical structural elements in RNA and an higher linear negative charge density of A-RNA compared to B-DNA may account for the cellular distribution and RNA binding observed for these recently developed dinuclear ruthenium complexes [118, 119].

4.5.2 RNA binding properties of ruthenium(II) complexes containing the intercalating moiety dppz (Ru-dppz complexes)

The compounds $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine, Figure 15a) behave as molecular light switches, and show remarkable photo-switching upon interaction with DNA [1, 9, 150, 151]. They are luminescent in organic solvents but their emission is quenched by water. Conversely, when the complexes intercalate into DNA, the dppz ligand is shielded from water and the emission properties are restored [1, 9, 150, 151]. Early studies showed that little or no luminescence is observed upon interaction with A-RNA [150].

Figure 15. (a) Molecular light switches $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ (left) and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (right) (bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine) [150, 151]; (b) Ru-dppz complexes in which the dppz unit has been functionalized with long alkyl chains [152].

However, Barton and co-workers have recently explored the use of this complex as potential probe for RNA mismatches [120]. They investigated the interaction of *rac*- $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ with short RNA and DNA hairpins containing single mismatches. They observed that while no emission of *rac*- $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ is observed in the presence of matched RNA, a sensitively enhanced emission is observed in the presence of a CA mismatch. On the contrary, no significant discrimination is observed for matched and mismatched DNA. This behaviour suggests that, although the overall emission intensity is higher for DNA than for RNA, this complex is a better

probe for RNA than for DNA mismatches. Based on comparison with ethidium bromide binding the authors suggest that *rac*-[Ru(bpy)₂(dppz)]²⁺ bind to mismatched RNA via minor groove metalloinsertion, a binding mode previously observed for octahedral metal complexes to thermodynamically destabilized mismatches in DNA [120].

In a recent paper reporting on the effect of ionic strength, temperature and DNA sequence and conformation on the emission abilities of Δ -[Ru(phen)₂dppz]²⁺ (Figure 15a), Tuite and co-workers comment an overall weak emission enhancement in the presence of RNA poly(A)·poly(U), likely due to groove binding of the metal complex to the A-form RNA duplex [153].

Svensson, Lincoln and co-workers functionalized three Ru-dppz complexes with alkyl ether chains of different lengths (Figure 15b) [152]. It is known that ligand modifications influence cellular uptake and accumulation, and also nucleic acid binding properties of this class of compounds [6, 9, 152, 154, 155]. Interestingly, the length of the alkyl ether chain influences the nucleic acid binding properties of the three metal complexes. The complex of intermediate lipophilicity accumulates in RNA-rich nucleoli, and *in vitro* studies confirmed that it has higher binding affinity for rRNA vs CT-DNA [152]. The authors suggest that the increased lipophilicity of the complex may account for this binding preference.

These examples show that, besides its intercalating ability to B-DNA, the dppz moiety can interact with RNA in thermodynamically destabilized regions, and its functionalization can direct RNA binding.

4.5.3 Recently developed potential RNA-binding probes

A few recent publications show the potential of metal complexes to image RNA in cells. For example, Turro and co-workers developed a potential RNA imaging probe by combining a ruthenium(II) bipyridine complex with a phenanthridine moiety (RuEth, Figure 16a). Fluorescence steady state and time-resolved emission studies demonstrated that lifetime and luminescence of RuEth complex are strongly influenced by yeast tRNA. Moreover, confocal

microscopy images on mammalian cells show a greater accumulation of this complex in RNA-rich regions, such as nucleoli and cytoplasm [121].

Sun, Peng and co-workers reported in a recent paper on the potential use of a hetero dinuclear metal complex for rRNA imaging in living cells [43]. Their dual luminescence probe contains a Ru(II) and an Ir(III) centre, $[(bpy)_3Ru(CH_2)_{10}Ir(F_2ppy)_2]^{3+}$ (bpy = 2,2'-bipyridine, ppy = 2-phenylpyridine; RuIr, Figure 16b), and the emission intensity of the latter is strongly enhanced upon RNA interaction. RNA binding experiments confirmed that RuIr has higher affinity for RNA compared to other investigated biomolecules, including DNA and HSA (human serum albumin), and imaging studies confirmed that this complex readily stains cytoplasm and nucleoli [43].

Figure 16. (a) Adduct between a phenanthridine moiety and a ruthenium complex (RuEth) proposed by Turro and co-workers as RNA imaging probe, owing to its changes in emission intensity and lifetime upon RNA binding [121] (b) Dinuclear RuIr complex recently developed by Sun, Peng and co-workers for rRNA imaging in living cells [43] (c) Platinum(II) complex recently developed by Che and co-workers, that showed different emission properties upon binding to dsRNA vs dsDNA [42].

Finally, Che and co-workers recently showed that the complex $[Pt(C^{\wedge}N^{\wedge}N)(C\equiv NtBu)]^{+}$ ($C^{\wedge}N^{\wedge}N$ = 6-phenyl-2,2'-bipyridine Figure 16c), besides displaying potent anticancer activity, exhibits different changes in emission properties upon binding to double-stranded DNA (dsDNA) vs double-stranded RNA (dsRNA) [42]. The complex has a weak emission at 525 nm in aerated CH_3CN /saline phosphate buffer solution; this emission is highly increased and red shifted to 610 nm in the presence of dsDNA. On the other hand, addition of dsRNA leads to an increase in emission intensity but no shift in the emission maximum. This behaviour is likely due to

structural differences between A-RNA and B-DNA, and can be exploited to differentiate between dsRNA and dsDNA [42].

These very recent studies highlight the potential of metal complexes to discriminate between RNA and DNA, and suggest their use as RNA imaging probes.

4.6 A variety of metal complexes showed different DNA vs RNA binding properties

There are a few studies in which RNA binding abilities of metal complexes are investigated spectroscopically, and compared with DNA binding properties. In an early study, UV-Vis, CD and fluorescence spectroscopy were successfully used to investigate the interaction of $[\text{Ru}(\text{phen})_2\text{MHPIP}]^{2+}$ (phen = 1,10-phenanthroline, MHPIP = 2-(3-methoxy-4-hydroxyphenyl)imidazo[4,5-f][1,10]phenanthroline) with yeast tRNA. The binding of this complex was found to be enantioselective and likely to proceed via intercalation [156].

Figure 17. The complex $[\text{Ru}(\text{phen})_2\text{PMIP}]^{2+}$ (phen = 1,10-phenanthroline, PMIP = 2-(4-methylphenyl)imidazo[4,5-f][1,10]phenanthroline), which shows stronger binding to yeast tRNA than to CT-DNA [122].

Similarly, UV-Vis, CD and fluorescence spectroscopy, isothermal titration calorimetry (ITC), viscosity measurements and equilibrium dialysis experiments were used to study the RNA binding affinity of $[\text{Ru}(\text{phen})_2\text{PMIP}]^{2+}$ (phen = 1,10-phenanthroline, PMIP = 2-(4-methylphenyl)imidazo[4,5-f][1,10]phenanthroline) (Figure 17) to yeast tRNA. Comparison with DNA binding using CT-DNA was also performed. The data suggest that the binding proceeds via intercalation in both cases, and that RNA binding is stronger than DNA binding [122].

A comparative study on RNA and DNA binding abilities of the heterobimetallic $[(\text{phen})_2\text{Ru}(\text{bpibH}_2)\text{Co}(\text{phen})_2]^{5+}$ (phen = 1,10-phenanthroline, bpibH₂ = 2,2'-(1,4-phenylene)-bis-

imidazo[4,5-f][1,10]phenanthroline) complex was also recently published [123]. The complex was shown to bind both CT-DNA and tRNA, but a stronger interaction was observed in the latter case, again suggesting that the structure on the nucleic acids affect the binding of metal complexes.

Also, the binding affinities to double helical DNA and RNA of a series of polypyridine Pt(II) complexes, $[\text{Pt}(\text{bpy})(n\text{-Rpy})_2]^{2+}$ and $[\text{Pt}(\text{Me}_2\text{bpy})(2\text{-Rpy})_2]^{2+}$ (bpy = 2,2'-bipyridine; Me_2bpy = 4,4'-dimethyl-2,2'-bipyridine; $n = 2\text{-}4$; $\text{R} = \text{H}$ or CH_3), were compared using UV-Vis and CD spectroscopy, and viscosity measurements. All the complexes, except $[\text{Pt}(\text{bpy})(2\text{-Mepy})_2]^{2+}$ (bpy = 2,2'-bipyridine; 2-Mepy = 2-methylpyridine), bind to both CT-DNA and RNA poly(A)·poly(U), used as model nucleic acids, in a non-covalent way, likely through intercalation *via* the bipyridyl ligand. The binding constants for DNA resulted generally higher than for RNA, and the nature of the metal complex seems to affect more RNA than DNA binding. $[\text{Pt}(\text{bpy})(2\text{-Mepy})_2]^{2+}$ binds DNA but does not interact significantly with RNA [124].

In a recent study the ability of a platinum-modified perylene complex to bind G-quadruplex structures was explored using a combination of techniques, including UV/Vis and CD spectroscopy, ITC, and electrospray mass spectrometry (ESI-MS). The compound under investigation was found to selectively recognize the antiparallel form of the telomeric G-quadruplex formed by the sequence d(TTAGGG)₄. Lower affinity was found for the RNA G-quadruplex formed by the sequence r(UUAGGG)₄, which has a parallel form and dimerises in solution [125].

The studies commented above show the importance of a detailed investigation of DNA and RNA binding properties of metal complexes, and call for further studies to rationalize the origin of the differences observed in RNA and DNA binding.

4.7 Binding of metal surfactants to RNA

RNA binding properties of some metal surfactants were recently reported. For example, Arunachalam and co-workers recently investigated the interaction of the two cobalt(III) surfactants $cis-[Co(ip)_2(DA)_2]^{3+}$ and $cis-[Co(dpq)_2(DA)_2]^{3+}$ (ip = imidazo[4,5-*f*][1,10]phenanthroline, dpq = dipyrido[3,2-d:2',3'-*f*]quinoxaline, DA = dodecylamine) with yeast tRNA [126]. They suggest that the two metal surfactants mainly bind to RNA via intercalation, and that both the aromaticity of the diamine and the long aliphatic chains favour hydrophobic interactions with tRNA. They also observed a stronger interaction with tRNA than with CT-DNA. Similarly, stronger RNA binding compared to DNA was found for the complex $cis-[Co(trien)(4CNP)(DA)]^{3+}$ (trien = triethylenetetramine, 4CNP = 4-cyanopyridine, DA = dodecylamine) [127]. There are also a few recent reports on the interaction of Cu(II) containing polymers and surfactants with RNA [128, 129]. For example, the water soluble copper(II) complex $[Cu(phen)(sal-ala)(DA)]$ (phen = 1,10-phenanthroline, sal-ala = salicylalanine, DA = dodecylamine) was shown able to bind similarly DNA and RNA *via* intercalation driven by hydrophobicity of the ligands [128]. Also, DNA and RNA binding properties of various polyethylenimine copper(II) complexes of general formula $[Cu(phen)(L-val)(BPEI)]Cl \cdot H_2O$ (phen = 1,10-phenanthroline, L-val = L-valine, BPEI = branched polyethyleneimine) were studied. The polymers interact similarly with CT-DNA and yeast tRNA, mostly through surface binding [129].

These recent publications underline the importance of comparative studies of DNA and RNA binding properties for different classes of compounds.

4.8 Metal complexes binding to RNA triple helical structures

Triple helical structures have been found to play important roles in, *e.g.*, DNA metabolism and gene function, transcriptional regulation and RNA processing [157, 158]. Moreover, they find application as tools in molecular biology and in gene therapy [157, 159, 160]. In triple helical structures, a third strand is bound to the double helix via Hoogsteen base-pairing, and great effort has been dedicated to develop small molecules able to recognize, bind and stabilize triplexes

[159]. Tan and co-workers have recently reported on ruthenium(II) complexes able to stabilize triplex RNAs. They used various biophysical techniques to prove the stabilizing effect of the complex $[\text{Ru}(\text{phen})_2(\text{mdpz})]^{2+}$ (**phen** = 1,10-phenanthroline, **mdpz** = 7,7'-methylenedioxyphenyl-dipyrido-[3,2-*a*:2',3'-*c*]-phenazine) on the Hoogsteen based pair strand in the RNA triplex poly(U)·poly(A)*poly(U) [130]. Steady-state luminescence experiments showed that the emission of this metal complex is much better enhanced upon addition of triplex than of duplex RNAs, thus suggesting its use as molecular light switch for poly(U)·poly(A)*poly(U) triplexes [130]. The effect of the ancillary ligand on triplex stabilization was also explored, comparing the behaviour of $[\text{Ru}(4,7\text{-dmp})_2(\text{mip})]^{2+}$ vs $[\text{Ru}(\text{phen})_2(\text{mip})]^{2+}$ (4,7-dmp = 4,7-dimethyl-1,10-phenanthroline, **phen** = 1,10-phenanthroline, **mip** = 2-(1,3-benzodioxol-5-yl)imidazo[4,5-*f*][1,10]phenanthroline [131] and $[\text{Ru}(\text{bpy})_2(\text{pnip})]^{2+}$ vs $[\text{Ru}(\text{phen})_2(\text{pnip})]^{2+}$ (**bpy** = 2,2'-bipyridine, **phen** = 1,10-phenanthroline, **pnip** = 2-(*N*-(*p*-phenyl)-1,8-naphthalimide)imidazo[4,5-*f*][1,10]phenanthroline) (Figure 18a) [46]. The complexes have the same intercalative ligand but different ancillary ligand. A stronger stabilization of the triplex structure was observed for the complexes with phenanthroline as ancillary ligands, thus indicating that the ancillary ligand has an effect in the stabilization [46, 131].

Figure 18. Examples of metal complexes developed by Tan and co-workers, used as RNA triplex stabilizers [46, 133].

Similarly, the ability of the two complexes $[\text{Ru}(\text{bpy})(\text{btip})]^{2+}$ and $[\text{Ru}(\text{phen})(\text{btip})]^{2+}$ (**bpy** = 2,2'-bipyridine; **phen** = 1,10-phenanthroline, **btip** = 2-(benzo[*b*]thien-2-yl)imidazo[4,5-

f][1,10]phenanthroline) to stabilize RNA triplexes was studied by a combination of experimental and density functional theory (DFT) studies. The results suggest that the slightly different RNA affinity of the two complexes may derive from the planarity of the intercalative moiety and the LUMO level of the complexes [132].

The effect of changing the intercalative ligand was also studied on the stabilization of the third strand [133]. Various biophysical techniques were used to compare the interaction of the two complexes $[\text{Ru}(\text{bpy})_2(\text{mip})]^{2+}$ and $[\text{Ru}(\text{bpy})_2(\text{bdip})]^{2+}$ (bpy = 2,2'-bipyridine, mip = 2-(1,3-benzodioxol-5-yl)imidazo[4,5-*f*][1,10]phenanthroline; bdip = 2-(1,3-benzodioxol-4-yl)imidazo[4,5-*f*][1,10]phenanthroline) (Figure 18b) with poly(U)·poly(A)*poly(U) triplex. On the contrary to what commented above, these two complexes contain the same ancillary ligand but different intercalative ligands. Interestingly, a stronger RNA binding for $[\text{Ru}(\text{bpy})_2(\text{mip})]^{2+}$ was observed, thus suggesting that also the intercalative ligand plays a role in RNA triplex stabilization [133]. On the other hand, metal complexes can also destabilize the triplex, as it occurs for a platinum derivative of proflavine, which shows a higher destabilizing effect of the triplex structure compared to pure proflavine [161].

These results show that little modifications of the organic ligands surrounding the metal centre can strongly influence the interaction with the RNA target, and once again underline the complexity of the RNA-metal complex system.

5. Conclusions

In this review we have described and discussed some of the latest results on the interaction between metal complexes and RNA. On one side, the rich structural diversity of RNA, the manifold roles of RNA in cell functioning and the fact that RNA structure is intrinsically linked to its function, offer an exciting challenge to develop structure-selective RNA binders [31, 32, 34]. On the other side, metal complexes, which due to their chemical properties can be employed in therapy and diagnosis [10-13], may be designed to target specific RNA structural elements. Surprisingly, they have been extensively investigated for their ability to bind DNA [1-9], but

their RNA binding properties still remain rather unexplored. The examples here summarized highlight the strong potential in targeting RNA with metal complexes, but also show the complexity of the system, that unavoidably makes this research field extremely challenging.

Metal complexes can interact with RNA both covalently and non-covalently. Several examples of covalent binding of platinum complexes to RNA have been herein commented [44, 45, 47, 70, 76-87]. Even if the existence of platinum-RNA adducts is undeniable, further biochemical and structural studies are needed to better understand the nature of platinum-RNA adducts as well as their impact on RNA structure and on RNA biological functions. Any information in this regard would be particularly valuable to obtain a more comprehensive picture of the biological activity of cisplatin and its analogues, and may provide information for the design of new platinum therapeutics [39, 44, 81, 84, 87]. Moreover, the combination of platinum or other metal complexes to small RNA binders may provide a potential strategy for the development of new metallodrugs [113-117]. Besides covalent interaction, metal complexes can bind non-covalently to numerous non-canonical RNA structural elements [3]. Such metal complexes can have various applications, as described in the examples commented above, including for instance rhodium(III) complexes used as RNA shape-selective tools [40, 107, 108, 134-136] and several metal complexes with different metal centres which may be used in bio-imaging [42, 43, 118-121]. Studies performed with different analytic techniques confirmed non-covalent interactions of these metal complexes to RNA structures. In some cases, distinct RNA vs DNA binding properties were identified, thus offering a good opportunity for developing selective RNA targeting agents. However, further studies are needed to rationalize the bases of metal complex-RNA recognition, and identify preferential RNA targets.

Overall, data reported in this review suggest that coordination chemistry is likely to give an important contribution to design structure-specific RNA targeting agents, aiming at the development of novel therapeutic and diagnostic tools.

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Metal complexes for diagnostic and therapeutic applications

Graphical abstract synopsis

Covalent and non-covalent binding of metal complexes to RNA

Elena Alberti, Marianthi Zampakou and Daniela Donghi✉

University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland

✉ daniela.donghi@chem.uzh.ch

RNA binding of metal complexes is much less explored than DNA binding. However, RNA is an emerging biomedical target, due to its involvement in crucial cellular functions and its very high structural diversity. This review summarizes some recent advances on both covalent and non-covalent binding of metal complexes to RNA.