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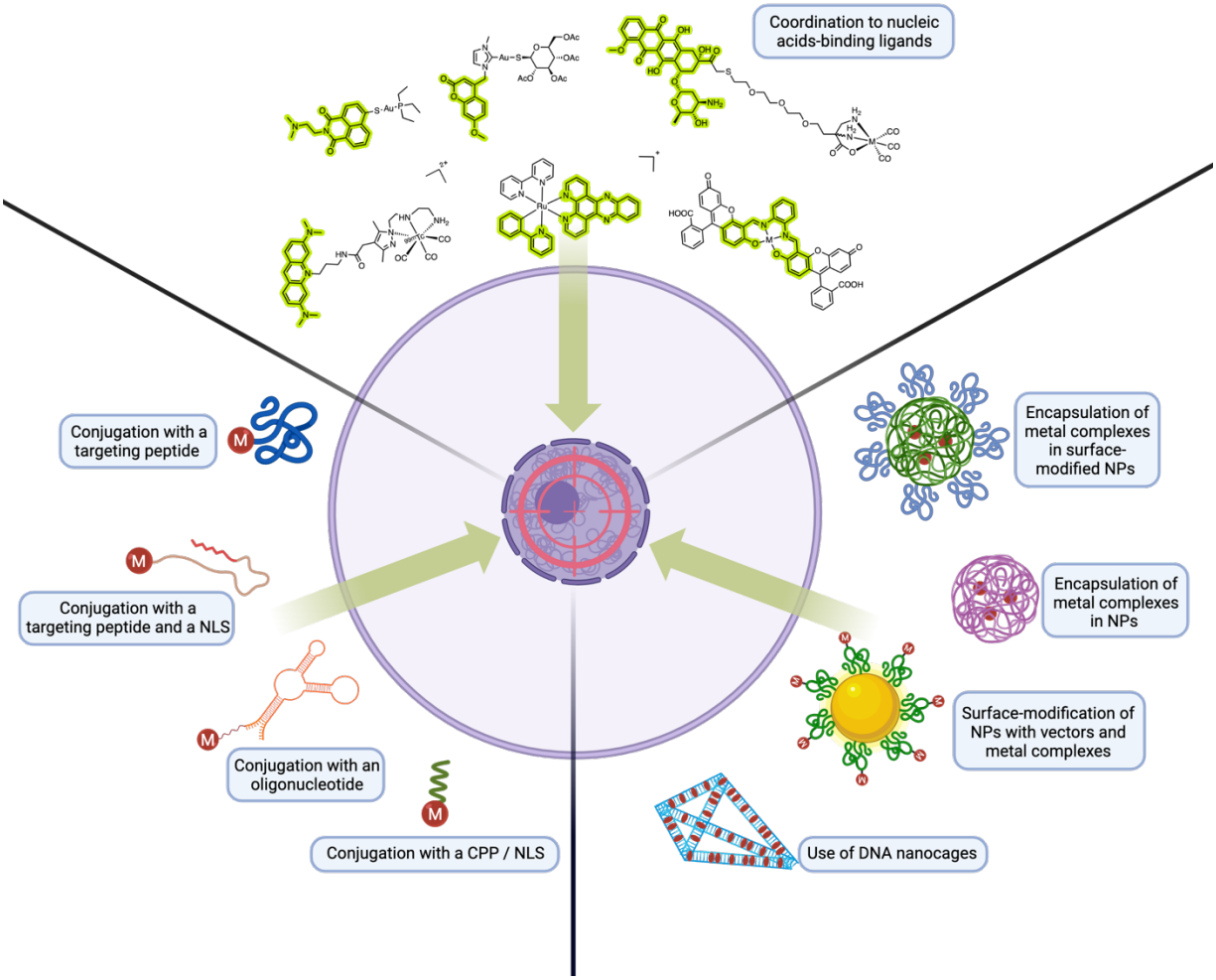
Abstract:

The nucleus is an essential organelle for the function of cells. It holds most of the genetic material and plays a crucial role in the regulation of cell growth and proliferation. Since many antitumoral therapies target nucleic acids to induce cell death, tumor-specific nuclear drug delivery could potentiate therapeutic effects and prevent potential off-target side effects on healthy tissue. Due to their great structural variety, good biocompatibility and unique physico-chemical properties, organometallic complexes and other metal-based compounds have sparked great interest as promising anticancer agents. In this review, strategies for specific nuclear delivery of metal complexes are summarized and discussed to highlight crucial parameters to consider for the design of new metal complexes as anticancer drug candidates. Moreover, the existing opportunities and challenges of tumor-specific, nucleus-targeting metal complexes are emphasized to outline some new perspectives and help in the design of new cancer treatments.



1. Introduction

The FDA approval of cisplatin in 1978 has sparked the interest in the use of metal complexes for potential biological and medical applications. Tremendous progress has been made since, giving rise to the discovery of numerous metal-based compounds with different purposes, ranging from molecular probes[1–4] to a variety of anti-microbial, anti-parasitic and anti-cancer drug candidates.[5–12]

Cisplatin represents one of the first lines of treatment by chemotherapy for early-stage disease and is routinely used against a variety of cancers such as ovarian, cervical, bladder or testis cancer. Cisplatin is known to interact with DNA following a series of spontaneous aquation reactions which involve the replacement of the *cis*-chloro ligands with water molecules. This newly formed, charged metal complex is then able to penetrate the nucleus and covalently bind with nucleophilic N7-sites of purine bases of duplex DNA to form mainly DNA-protein and DNA interstrand crosslinks, as well as some DNA intrastrand crosslinks. The subsequent DNA adducts are recognized by damage recognition proteins, triggering apoptosis through various molecular pathways. Treatment with cisplatin can be limited by the apparition of resistance mechanisms such as active efflux, thus increasing the concentration needed to kill cancer cells. However, the severe toxicity of cisplatin and other related compounds against the nervous system, digestive tract, and kidneys hinders the augmentation of doses. Since only about 1% of the intracellular cisplatin is reported to penetrate the nucleus and react with genomic DNA, this largely impairs therapeutic efficacy.[13] This problematic drove the research in the field of metal-based compounds towards an organelle-specific targeted delivery approach. A rising number of organelle-targeting compounds have thus been developed in recent years to increase the therapeutic effect of anti-cancer metal complexes, as well as to develop new cellular probes as an alternative to classical chemical imaging agents.[14,15]

In eukaryotic cells, organelles include the cytoplasmic membrane, lysosomes, mitochondria, Golgi apparatus, endoplasmic reticulum, and nucleus, located from the periphery to the core of the cells (**Figure 1**). These organelles are the house of vital biological mechanisms and each of these play a distinct role in maintaining the cell structure and homeostasis. Their dysfunction can lead to cell death, making them interesting targets for cancer therapeutics. Among the organelles found in cells, the nucleus is often considered as the most important. It is the host of most of the genetic material and is responsible for most of the essential biologic functions such

as gene expression, cell growth, differentiation, proliferation and apoptosis. Because of its fundamental role in cell function, minor damages to this organelle can be sufficient to trigger cell death. Therefore, it represents a highly valuable target and many efforts have been put towards the development of nucleus-targeted therapies. Various approaches can be used, ranging from ligand optimization to the use of bioconjugates or nanostructures. For clarification purposes, ligand will refer thereafter to the structure which binds non-covalently to the metal ion to form a metal complex.

In this review, the different approaches to target metal-based compounds to the nucleus of cells will be examined to identify key parameters influencing their subcellular localization. The opportunities and challenges of nuclear targeting of metal complexes will be brought up to emphasize their potential and inspire the development of new compounds. The aim of this review is to summarize and analyze strategies to bring metal complexes to the nucleus to aid in the design of new nucleus-targeting, metal-based drug candidates. Many metal complexes have been reported with potential nuclear uptake, although without specific nuclear accumulation. Such complexes thus fall out of the scope of this review, but an interested reader will be able to find data in other relevant works.[12,16–19]

2. The nucleus as a therapeutic target in cancer

The nucleus is an essential organelle in eukaryotic cells. It hosts most of the genetic material as well as various proteins involved in DNA replication and transcription.[20,21] Fundamental biological functions such as gene expression, cellular differentiation, cell growth and apoptosis take place within its vicinity. Because of its crucial role in cell growth and proliferation, it has been shown to be involved in mechanisms of carcinogenesis and therefore many cancer treatments have been developed to inhibit proliferation by targeting the nucleus.

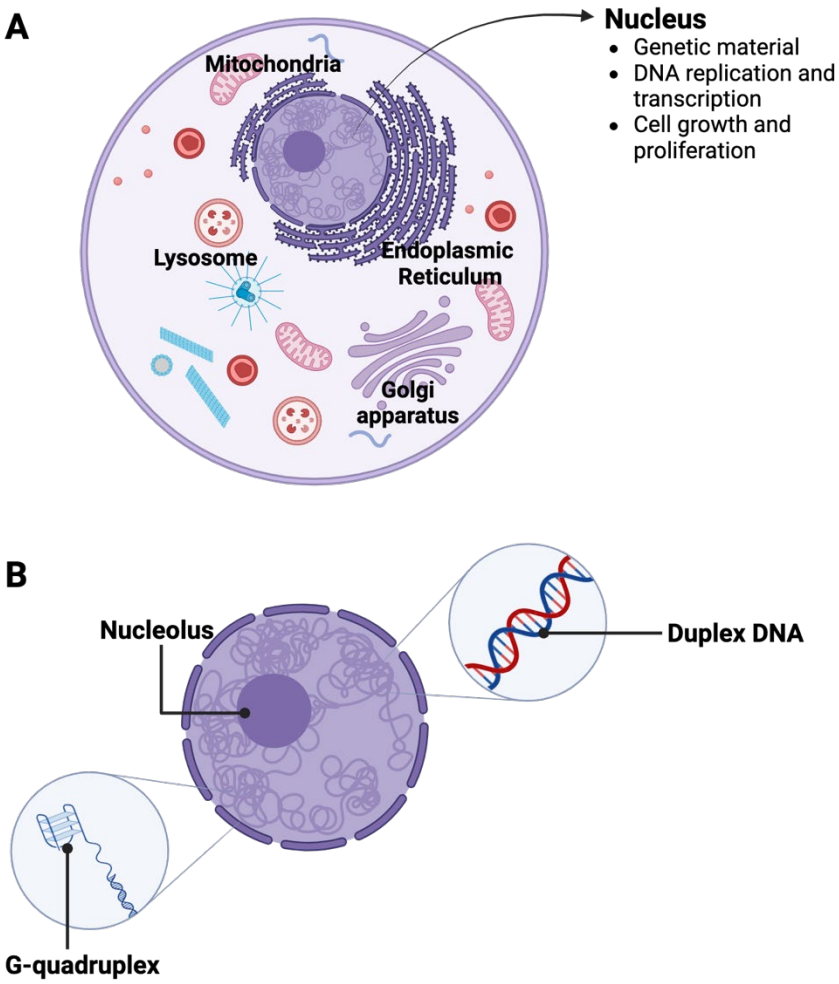


Figure 1. (A) Schematic illustration of organelles in an eukaryotic cell. **(B)** Schematic illustration of relevant biological targets in the nucleus: duplex DNA, G-quadruplexes, nucleolus.

Duplex DNA is the main target of the vast majority of antitumoral drugs currently used in the clinics (**Figure 1B**).[22–24] It is comprised of nucleotides – nitrogenous heterocyclic bases bound to a negatively charged furanose phosphate backbone, arranged in a double-stranded secondary structure. This double strand further takes a helix conformation that is stabilized by hydrogen bonding between opposite bases and π-π stacking interactions between planar aromatic rings of adjacent nucleotides.[25]

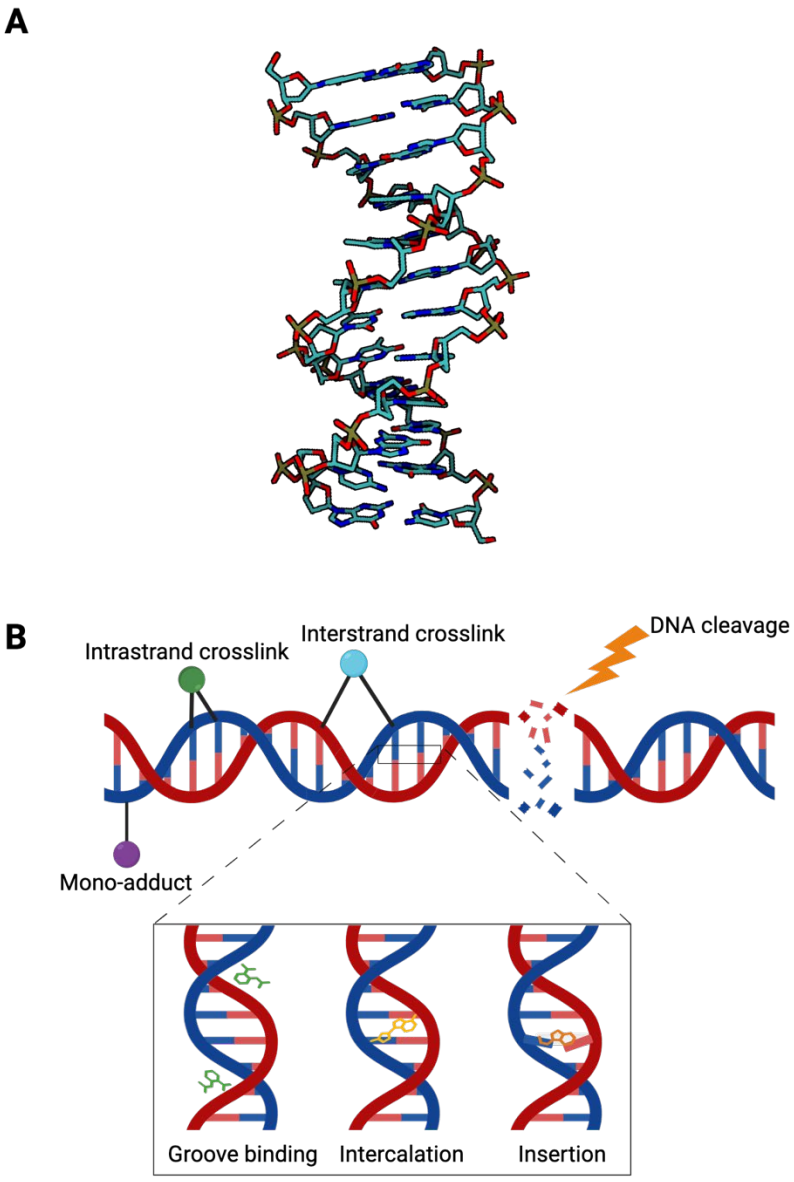


Figure 2. (A) Structure of B-DNA. The figure was generated using crystallographic data deposited in the PDB (PDB code: 1BDNA). **(B)** Modes of nucleic acid binding.

The conformational form of DNA most commonly encountered under physiological conditions is known as B-DNA, which is the form first discovered by Franklin and later on reported by Watson and Crick (**Figure 2A**).[26] B-DNA is a right-handed helix with a diameter of approximately 20 Å, 10.55 base pair *per* turn and a 3.4 Å step between base pairs. The separation of sugar-phosphate backbone chains due to base-pairing and stacking and the mutual repulsion of phosphate groups creates grooves that run through the length of duplex DNA, giving access to bases therein. In canonical B-DNA, there exist a wide (11.6 Å) major groove and a narrow (6 Å) minor groove, both of a similar depth (approx. 8 Å).[27] Other biologically

relevant conformations include A-DNA, which is a wider, more compact structure,[28] and Z-DNA, a left-handed double helix.[29] Although their role still needs to be determined, they have been identified to exist *in vivo*.[30,31]

Several modes of binding between nucleic acid and drugs can occur, such as covalent binding, groove binding, intercalation or insertion (**Figure 2B**).

Covalent binding to nucleic acids happens when an antitumoral agent forms a covalent bond with one or multiple atoms of a base (**Figure 2B**). Virtually all bases can be targeted, although it is most often guanine (*i.e.,* through the N7 atom). Small molecule drugs, such as chlorambucil or cyclophosphamide, known as alkylating agents fall within this category of interactions, as well as the well-known anticancer drug cisplatin and its derivatives.[32,33] Some metal complexes have also been found to interact with nucleic acids in such a way.[34] Usually, complexes initially interact with DNA *via* a reversible non-covalent binding (pre-association) to nucleic acids. This interaction is favored for cationic metal complexes due to the polyanionic nature of DNA. Subsequently, the metal complex can bind to a base to form DNA adducts. Metal complexes can form different types of DNA adducts depending on the bases that they interact covalently with. These adducts can be intrastrand or interstrand crosslinks, when binding occurs between bases on the same or opposite strand, respectively (**Figure 2B**). Mono-adducts can be formed when monofunctional binding to one base occurs (**Figure 2B**). Finally, although more rarely, protein-DNA crosslinks can occur when metal complexes are bound to a protein as well as a nucleotide. The formation of these adducts can trigger or disrupt various mechanisms, such as hindrance of DNA replication or the activation of several signal transduction pathways which ultimately lead to cell death.[35,36]

The three other types of interactions involve non-covalent binding to DNA. These interactions, which are predominantly observed for DNA-interacting metal complexes, are often reversible.[37]

Groove binding is a reversible intermolecular association based upon electrostatic and van der Waals interactions, coupled with potential hydrogen-bonding groups between molecules and bases (**Figure 2B**).[38] These interactions do not involve π-stacking between base pairs, which, in consequence, cause relatively minor changes to the structure of the double helix. Some minor groove binding small molecules are used as antineoplastic drugs due to their effect on stopping

the cell cycle in the G2/M phase.[39] As for metal complexes, they typically exhibit groove binding interaction as a pre-association rather than as a main mechanism of action, such as platinum complexes that associate closely to DNA grooves before forming adducts, or other ruthenium-, cobalt-, or zinc-based metal complexes that can cleave DNA.[40]

Intercalating agents are molecules that unwind DNA in order to π-stack between two base pairs (**Figure 2B**). Several well-known chemical anticancer drugs, such as camptothecin, doxorubicin and derivatives, are known to inhibit proliferation by consequence of their DNA intercalation.[24] Since the initial report by Lippard and co-workers of intercalation into DNA of inert platinum complexes containing aromatic ligands, there has been a tremendous interest in metal-based DNA intercalators, deemed metallo-intercalators.[41,42] These metallo-intercalators interact with DNA by means of one or several intercalating ligands coordinated to the metal center. The rigid three-dimensional structure of these metal complexes, stabilized by the coordination of ligands around the metal center, allows for good binding and recognition of DNA sequences.[43] Depending on the nature of the ligand, the complex can intercalate through the minor or the major groove. Generally, DNA intercalators disrupt enzyme activity by blocking DNA sites, thereby affecting DNA replication and transcription which results in the inhibition of cells growth and proliferation.[44]

Another type of DNA-binding metal complexes, deemed as metallo-insertors, were introduced by the Barton laboratory.[43] Metallo-insertors also contain planar polycyclic aromatic ligands that can be inserted into the π-stack; however, they differ from metallo-intercalators in that they displace the bases of a base-pair out of the π-stack (**Figure 2B**). These metallo-insertors have shown a stronger preference for DNA mismatch sequences and abasic and single bulge sites. These characteristics thus make them interesting as alternatives to other metal complexes for cells bearing a lot of DNA mismatch sequences, such as chemotherapy-resistant cancer cells deficient in mismatch mediated repair (MMR) proteins.[45]

All these types of DNA interactions are accessible with metallodrugs, sometimes with a simple change of ligands.[40] Dual binding modes can also be observed for some metal complexes. Such a feature highlights the great versatility that can be achieved using metal complexes, and the vast arrays of molecular responses that can be elicited depending on the type of interaction observed. An in-depth summary of the different binding modes of metal complexes to DNA can be found in a relevant review work by Andrezálová and Országhová.[46] Furthermore, the

impact of these drug-induced DNA destabilization and damage on molecular events within the cells have been studied and reported in several works that will offer the reader a more comprehensive view and understanding of the advantages and disadvantages of the use of different binding modes for antitumoral therapies.[44,47,16,48]

Another way to target duplex DNA is to induce a great number of single or double-strand cleavage, which triggers a chain of molecular events resulting in cell death.[22,49,50] In some cases, even a single double strand break is capable to induce cell death.[51] This approach is currently being developed for targeted therapies, with strategies such as using light to induce photocleavage in specific tissues – photodynamic therapy (PDT),[52] or the use of DNA damage-inducing radionuclides in targeted radiotherapies.[53] Depending on the mechanism responsible for cleavage, close association with DNA can be required or not.

Although duplex DNA is the most commonly targeted, other nuclear components have arisen as potential targets in antitumoral therapy.

Non-duplex DNA structures can also be targeted, notably G-quadruplexes (**Figure 1B, Figure 3**). They are characteristic structures consequent of the self-assembly of G-rich nucleic acid sequences into G-quartets, further stabilized by the presence of alkali-metal cations.[54] G-quadruplexes can form intramolecularly from a single nucleic acid sequence or intermolecularly by bringing together two or more strands. First identified by Blackburn and co-workers with the folding of G-rich repetitive sequences in the single stranded overhang of telomeres, which cap chromosomes' ends, the biological functions now associated with such structures make them appealing therapeutic targets.[55]

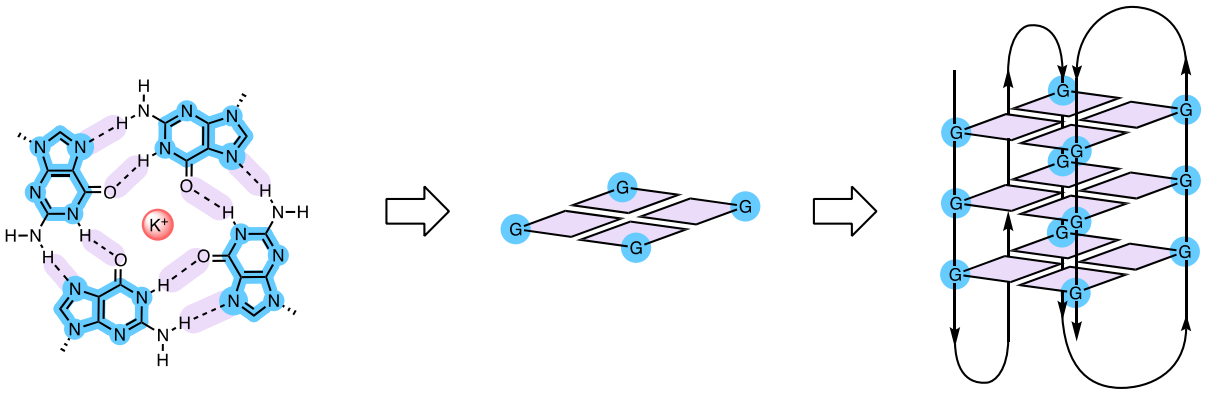


Figure 3. Schematic representation of a G-quartet and its stacking to form an intramolecular G-quadruplex structure.

It has been shown that telomerase, an enzyme responsible for telomere maintenance and reactivated in a large majority of cancers, is inhibited when the telomere extension is folded into a G-quadruplex.[56,57] Moreover, there is now mounting evidence that G-quadruplex formation in G-rich promoter regions of some oncogenes (such as *c-myc* and *c-kit*) can regulate and enhance their transcription.[58–61] Hence, the stabilization of such structures in either telomeres or promoter regions of oncogenes appears as an attractive strategy for antitumoral drug development.

The nucleolus is a subnuclear structure visible by microscopy as a distinct compartment within the nucleus (**Figure 1B**). Its canonical molecular function is the ribosome biogenesis, a well-orchestrated process in which all constituents of the ribosome are synthetized, modified and assembled, before being carried to the cytoplasm to build up the mature ribosomes.[62] Ribosome biogenesis plays a crucial role in the coordination of major cellular processes, as they are directly linked to protein biosynthesis. Moreover, recent advances have pointed out evidence that the nucleolus is also involved in non-canonical functions, independent from ribosome biogenesis. Genomic and proteomic studies have identified nucleolus as taking part in the regulation of major cellular processes including the maintenance, repair and stability of the genome,[63–65] the cell cycle,[66] cellular senescence,[67] response to stress,[68] telomere maintenance,[69] and the nuclear architecture.[63] Several human diseases have been associated with nucleolar dysfunction, and more particularly, alteration in both canonical and non-canonical functions of the nucleolus have been associated with several forms of cancer. [66,70]

Almost all cancer cell types display abnormalities in the morphology and number of nucleoli.[71] These abnormalities have been demonstrated to be a consequence of the over-activation of ribosome biogenesis.[72] This over-activation is directly linked to the need of cancer cells to synthetize tremendous amounts of proteins to sustain their altered growth. Targeting these structures could thus be a way to prevent cell proliferation and induce cell death.[73] Furthermore, the presence of supernumerary nucleoli in cancer cells compared to healthy tissue could provide a form of selectivity for these cell types.

The presence of these numerous molecular targets playing a relevant and crucial role into the determination and growth of cancer cells, in a single, well-identified organelle is highly appealing for the development of novel targeted therapies with increased specificity and efficiency. Over recent years, important efforts have been put towards the identification of strategies to bring relevant drugs to the nucleus of cancer cells to increase their therapeutic effect. Targeting the nucleus is indeed not an easy feat, as the nuclear envelope (NE), a double membrane bilayer, separates the nuclear contents from the cytoplasm. The transport of physiological components between the two compartments is mediated through nuclear pore complexes (NPCs) spread out throughout the NE. These NPCs act as selective, bidirectional transporters, which allow transit of a large range of appropriate cargoes whilst preventing the passage of non-specific macromolecules.[74]

This structural feature of the nucleus as well as the general functioning of eukaryotic cells imply to take into consideration many parameters when designing a drug destined to target this organelle. Firstly, compounds should be able to penetrate the cell. This entry can be mediated by various mechanisms; however, it should ultimately result in the presence of the compound within the cytoplasm. Thus, endosomal escape must be assessed for compounds internalized *via* endocytosis. Most importantly, once near the vicinity of the nucleus, compounds should be able to pass through NPCs. This can happen either through passive diffusion or facilitated transport. Ions, small molecules and small- to medium-sized macromolecules are able to penetrate the nucleus through passive diffusion, within a threshold that is thought to be between 30-60 kDa.[75] Facilitated transport inside of the nucleus is mediated by transport proteins of the karyopherin family, which bind to macromolecular cargos *via* specific sequences.[76] These sequences are short peptides deemed nuclear localization signals (NLS), and have been reported to induce nuclear addressing of various molecules.[77,78] Furthermore, evidence suggests that through facilitated transport, NPCs are able to translocate macromolecules with diameters of up to 39 nm.[79]

Reported strategies for nuclear targeting of various compounds and more specifically known drugs have been reviewed, mostly focusing on conjugation to NLS or encapsulation in nanoparticles.[3,80–83] In the field of metal-based compounds, pertinent reviews have listed works on metal complexes targeting specific organelles by means of different ligands.[14,15] This review proposes to bridge the gap between strategies used for small molecules and metal complexes to highlight parameters to consider when aiming for the nucleus of cells, and

hopefully provide some perspectives for the rational design of future metal-based drug candidates.

As such, an update and discussion on the influence of ligands on the localization of reported metal complexes as well as the use of bioconjugation and nanostructures to the field of metallodrugs will be discussed in the following parts.

3. Nucleus-targeting strategies for metal complexes

Before diving into more complex nucleus-targeting strategies such as bioconjugation or encapsulation, our attention will focus on smaller structures and the different parameters that can be tuned to impart metal complexes with an intrinsic affinity for the nuclear compartment.

3.1. Parameters influencing the subcellular localization of complexes

In contrast to the previous paradigm in the biological applications of metal-based compounds, where metallic centers were essentially used to improve the bioactivity of known pharmacophores, recent years have seen some metal complexes being placed at the center of the therapeutic strategy.[84] Thus, rather than using the metal center to modulate nuclear accumulation of reported drugs, new strategies focus on the modification of ligands to bring metal complexes to the desired organelle. The chemical structure of a ligand can influence the lipophilicity of the complexes and its interactions with nucleic acids. In addition, stereochemistry and formal charge can also play a part in the subcellular distribution of a complex. All these parameters are described below, and the extent of their influence illustrated by relevant works.

However, a note of caution must be added, as some of the complexes described below had their cellular localization assessed with fluorescence microscopy only. Phenomena such as the Aggregation Induced Emission (AIE) or the photoswitch effects could lead to an exaltation of the fluorescence in some conditions, for instance when complexes are bound to DNA. These effects would introduce a bias in the subcellular localization determination *via* fluorescence microscopy. It is unclear whether the nuclear accumulation is truly specific until further studies to evaluate the quantity of metal in each organelle are performed, such as inductively coupled

plasma mass spectrometry (ICP-MS). Nonetheless, these works have still been included to bring to light the role that ligands can play in the subcellular distribution of metal complexes.

3.1.1. The use of ligands known to bind with nucleic acids

One of the first approaches for the design of nucleus-targeting metal complexes is to use the interaction of a compound for a nuclear target, *i.e.* nucleic acids, to direct it to this specific organelle. To this end, metal-based compounds can be modified by changing their coordinating ligands.

The accumulation of a metal complex to the nucleus of cells by means of a DNA-intercalating ligand was first described by Dyson and co-workers.[85] They observed a seemingly preferential accumulation of complex **1** in the nucleus, likely on account of the anthracene moiety present on the ligand, known to interact with DNA by intercalation of its planar aromatic moiety into base pairs (**Figure 4**). Similar results were obtained by Santos and co-workers, who introduced anthracene motifs to ligands intended to coordinate rhenium(I) to yield complexes **2** and **3a-b** that also accumulated in cells nuclei (**Figure 4**).[86] The binding mode and binding constants of anthracene-based moieties have been reported to be influenced by substitutions, notably on positions 9 and 10, suggesting the possibility to further tune the affinity of these ligands for DNA.[87,88]

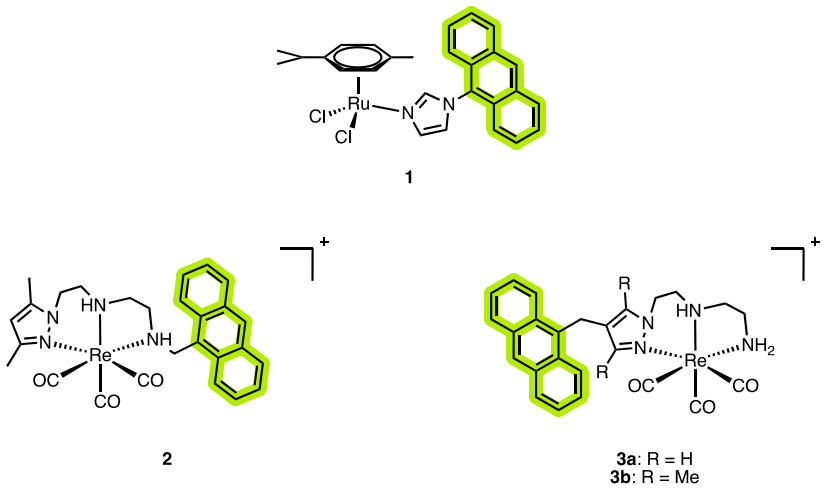


Figure 4. Nucleus-targeting ruthenium(II) and rhenium(I) complexes. The presence of a DNA-intercalating anthracenyl moiety (highlighted in green) on the ligand resulted in a preferential nuclear accumulation.

Shortly after, Ott et al. reported the gold(I) phosphine complex **4** derived from the approved drug auranofin bearing a naphthalimide ligand (**Figure 5**).[89] The latter ligand is derived from the antitumoral compounds mitonafide and amonafide, both known for their DNA-binding properties. Complex **4** displayed enhanced nuclear accumulation, in contrast with previous lead gold(I) compounds such as auranofin and related complexes, which mainly accumulated in mitochondria.[90,91] Following results confirmed their initial assumption that the DNA-binding properties of the naphthalimide ligand were responsible for the nuclear localization of these gold(I) phosphine complexes, whereas modifications of substituents on the phosphine only had influence on the cellular uptake.[92,93] The naphthalimide core has been reported as a strong binder of mononucleotides as well as duplex DNA structures and is thought to bind DNA partially through intercalation between base pairs. Other binding modes have also been reported over the years, and means to tune the affinity for DNA of various naphthalimide derivatives have been investigated, giving way to future opportunities for metal complexes with naphthalimide-based ligands.[94]

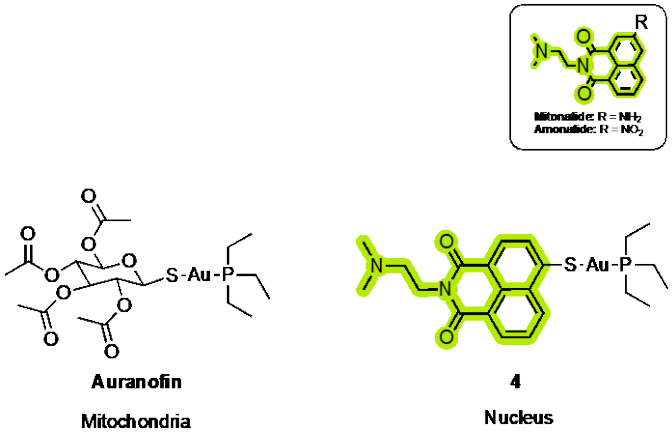


Figure 5. Structures of naphthalimide antitumor drugs, auranofin and derived nucleus-targeting gold(I) phosphine complex. The introduction of the naphthalimide core (highlighted in green) as a ligand resulted in enhanced nuclear accumulation compared to the parent compound auranofin which accumulates in the mitochondria.

Akin to the naphthalimide pharmacophore, chemical structures with extended planar aromatic cycles are very valuable as ligands, as they often display great potential for DNA intercalation.

Other nucleus-targeting silver(I) and gold(I) complexes were described by Casini, Pia Rigobello and co-workers, bearing ligands derived from known DNA-interacting agents such as anthracene for complexes **5a** and **5b**, or coumarin for complex **6 (Figure 6)**.[95,96] In contrast to other DNA-interacting moieties used as ligands described in the works reported in this review, coumarin has been reported to bind to DNA *via* minor groove binding, which suggests that various binding modes can be used to achieve nuclear localization and DNA interaction.[97]

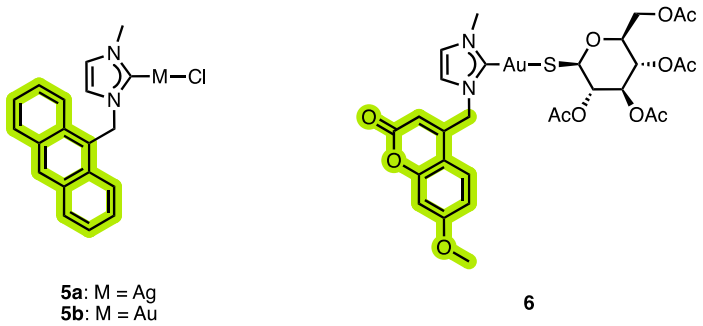


Figure 6. Nucleus-targeting silver(I) and gold(I) complexes coordinated to a DNA-binding ligand (highlighted in green).

Complexes coordinated with acridine, another pharmacophore known for its multimodal DNA-binding properties, notably through intercalation, were reported with gold(I) by Ruiz and co-workers, and ^{99m}Tc(I) by Paulo and co-workers (**Figure 7**).[98,99] In contrast to Cl-Au-P(Ph)$_3$ which showed no specific distribution in the nucleus, **7** coordinated with an acridine derivative instead of the chloro ligand accumulated specifically in the nucleus.[93] This highlighted the role of acridine in the nuclear localization of the complex. In a consistent manner, complex **8** coordinated with two acridine derivatives and complex **9** with an acridine moiety on its tridentate ligand also showed an enhanced accumulation in the nucleus of cells.

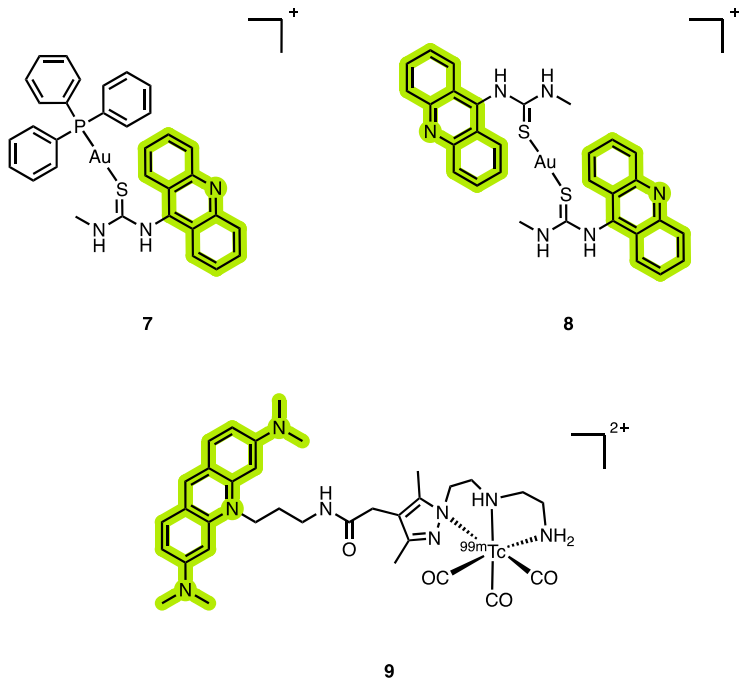


Figure 7. Nucleus-targeting gold(I) and ^{99m}Tc(I) complexes. The presence of acridine derivatives (highlighted in green) with DNA-intercalating properties on one or several ligands resulted in specific nuclear accumulation.

Rhenium(I) and ^{99m}Tc complexes were brought specifically to the nucleus by Alberto and co-workers using a ligand tethered *via* a linker to doxorubicin, an approved anticancer drug that intercalates into DNA and inhibits the activity of topoisomerase II (**10-12**; **Figure 8**).[100,101] Interestingly, the structure of the linker did not seem to impair the complexes' nuclear targeting abilities, although it did modulate cellular uptake.

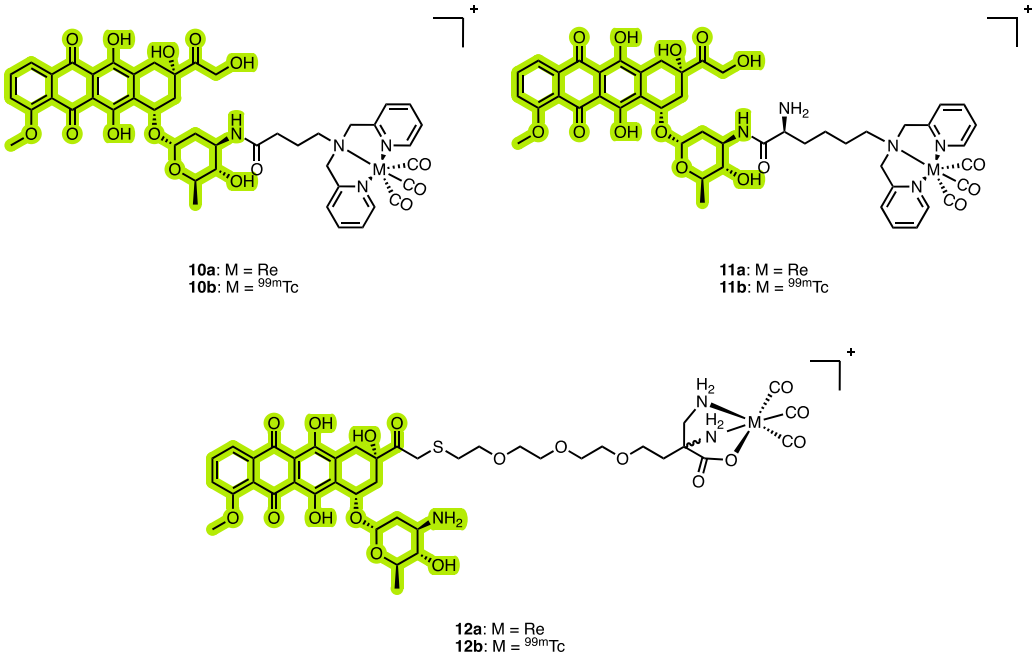


Figure 8. Nucleus-targeting rhenium(I) and ^{99m}Tc complexes. Nuclear localization was obtained through coordination to a ligand conjugated with DNA-intercalating antitumoral drug doxorubicin (highlighted in green).

Luminescent d^6 transition metal polypyridyl complexes have been extensively investigated due to their remarkable photophysical properties and DNA-binding abilities. Most complexes described in the literature function as "light-switch" probes, which accumulate in the whole cell but specifically light up upon interacting with DNA, making them advantageous for fluorescence microscopy. In most cases, the exact subcellular distribution of complexes was not investigated, and as such they fall out of the scope of this review. An interested reader will find further information on this kind of probes in relevant works by Coogan and Thomas.[19,102] Nonetheless, several of these complexes have been reported to accumulate specifically in the nucleus, and will be discussed thereafter.

$[Ru(bpy)_2(dppz)]^{2+}$ (**13**, bpy = bipyridine, dppz = dipyrido[3,2-*a*:2',3'-c]-phenazine; **Figure 9)** and its derivatives have attracted particular attention, due to their aforementioned DNA light-switch abilities. These molecules are known to intercalate between duplex DNA base pairs. However results by Wölfl and co-workers showed that $[Ru(bpy)_2(dppz)]^{2+}$ exhibits a poor cellular uptake, hindering their cytotoxic activity against cancer cells despite a high DNA binding affinity.[103] Subsequently, several groups, including ours, worked towards the synthesis of new derivatives with enhanced cellular uptake to increase their cytotoxic activity.

Among these, Chao and co-workers notably introduced a slight modification on the structure of $[Ru(bpy)_2(dppz)]^{2+}$ and incorporated a 2-phenylpyridine ligand to obtain a cyclometalated ruthenium(II) complex **14** that targets the nucleus of cancer cells (**Figure 9**).[104]

Modifications on the dppz ligand were also shown to modulate cellular uptake and distribution, as investigated by our group. We observed that the introduction of various substituents on the dppz ligand (**15a-f**; **Figure 9**) had an influence on the cellular uptake and subcellular localization of the complexes. Thus, complexes **15a** and **15b** showed a good uptake and preferential nuclear accumulation in cervical cancer cells.[105] Other derivatives displayed either poor stability in human plasma or poor cellular uptake. Interestingly, even slight modifications on the dppz ligand gave very different results between complexes **15b** and **15d**. Such results were also observed for the other ruthenium(II) dppz derivatives **16a** and **16b**, where the presence of hydroxyl groups seemed to hinder entry into cells.[106] The use of a dppz derivative of the same molecular size did not seem to be detrimental to the nuclear localization. Mei and co-workers reported a ruthenium(II) complex with a 6-chloro-5-hydroxylpyrido[3,2-*a*]phenazine ligand **17a** that accumulated specifically in the nucleus.[107] However, introduction of larger substituents on the dppz ligand (**16c-d; Figure 9**) by Lincoln and co-workers, resulted in a loss of affinity for DNA intercalation. This suggests caution when introducing substituents on known DNA-interacting moieties, as they can easily lose specificity for their nuclear target. Large, highly lipophilic substituents seemed to be more likely to result in a loss of nuclear targeting. This relationship between size, lipophilicity and subcellular localization will be further discussed in part 3.1.2.

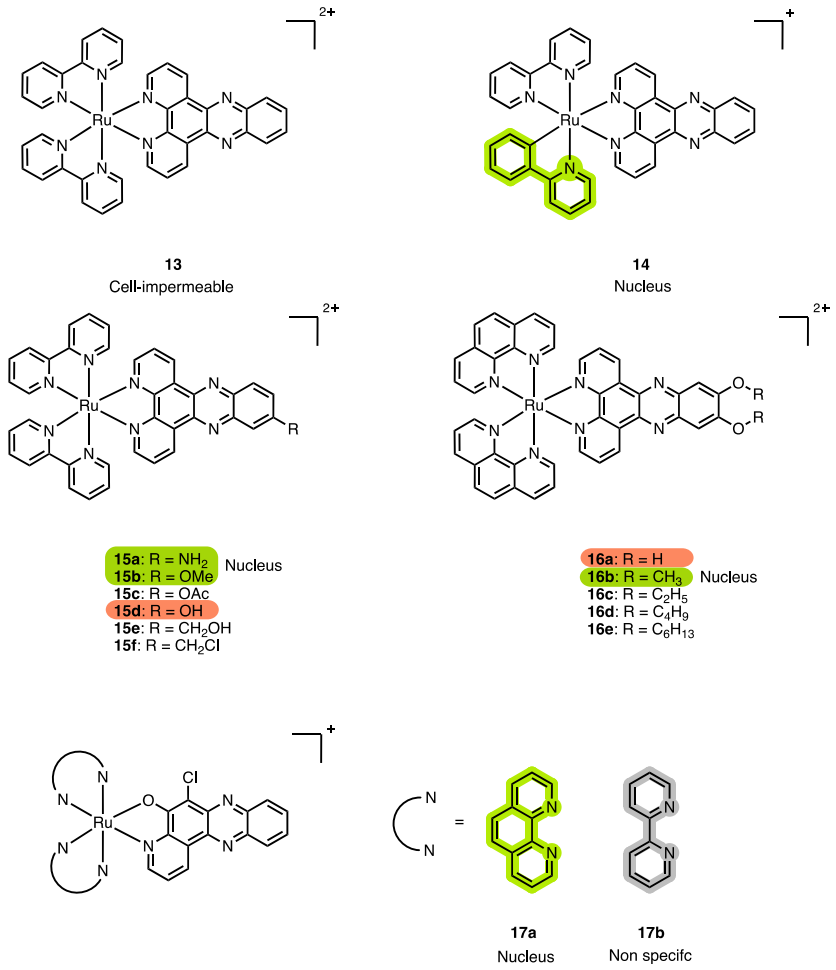


Figure 9. Changes of ligands on ruthenium(II) polypyridyl complexes coordinated with dppz derivatives can modulate their subcellular distribution. Ligands or substituents highlighted in green allowed for preferential nuclear accumulation whilst substituents highlighted in red hindered nuclear entry.

Tuning the selectivity of metal complexes for other DNA sequences, such as DNA mismatches for instance, can be done by changing ligands that are not involved in the intercalation. Mismatch specificity is thought to be attained by the introduction of bulkier ligands which would disfavor matching to well-matched binding sites due to steric clash with the DNA backbone.[108] This strategy was used by Vallis and co-workers to synthetize ruthenium(II) metallo-insertors which accumulated specifically in the nucleus of MMR-deficient cells (**18**; **Figure 10**).[109] Furthermore, this ability was used to bring Auger-emitter ^{111}In close to DNA, effectively increasing its radiotoxicity (**19**; **Figure 10**).

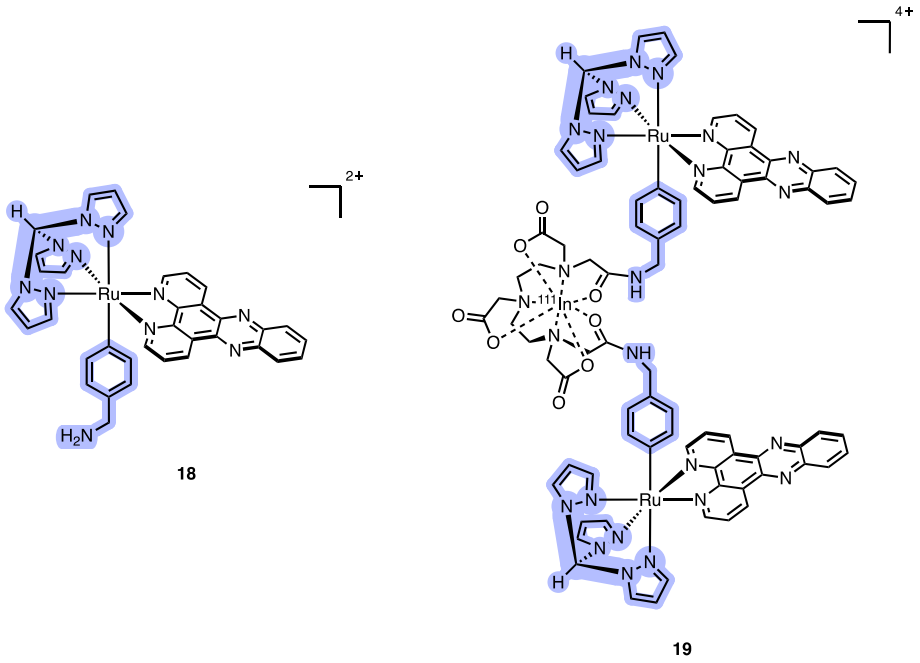


Figure 10. Introduction of bulkier non-intercalating ligands (highlighted in blue) on ruthenium(II) dppz complexes can confer selectivity for mismatch DNA sequences whilst maintaining nuclear accumulation.

Tetrapyrido[3,2-*a*:2‘,3‘-*c*:3‘‘,2‘‘-*h*:2‘‘‘,3‘‘‘-*j*]phenazine (tpphz) ligands are other derivatives known to interact with DNA thanks to their extended planar system, akin to dppz. They were used by Thomas and coworkers to synthetize a DNA-binding dinuclear ruthenium(II) complex **20a** that was able to specifically accumulate in the nucleus of live cells when coordinated to phenanthrolines ligands alongside tpphz.[110,111] The group then synthetized two other dinuclear tpphz complexes with iridium(III) and ruthenium(II) that were also found to localize in the nucleus (**21a-b**; **Figure 11**).[112] It is interesting to note that the introduction of an iridium(III) complex was enough to circumvent the poor cellular uptake observed with the use of bipyridine ligands in complex **20b** (**Figure 11**). This is thought to be due to the lower charge and higher lipophilicity conferred by the iridium(III) complex and its ligands.

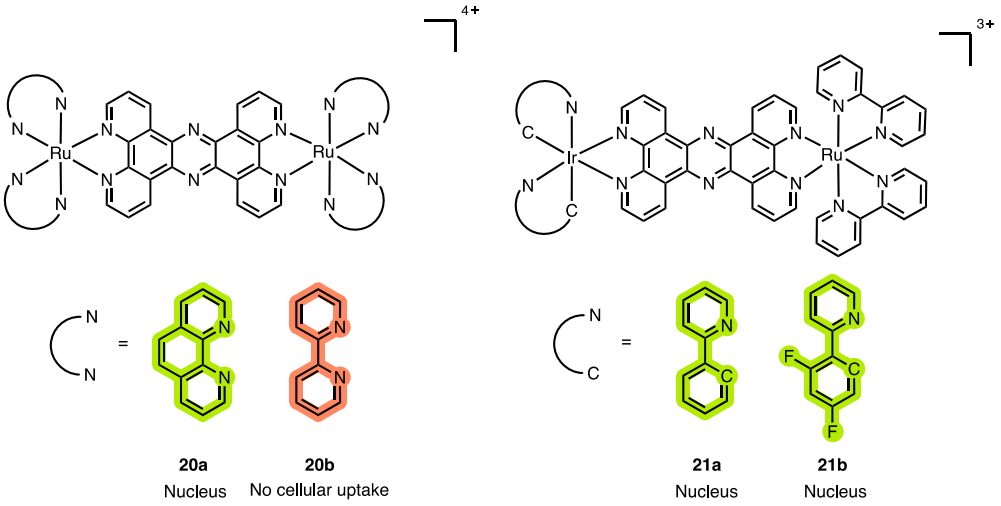


Figure 11. Dinuclear complexes or ruthenium(II) and iridium(III) coordinated to a tpphz ligand. Changes on the non-intercalating ligands (highlighted in green or red) and global charge can influence cellular uptake.

Other complexes were reported to localize in the nucleus of cells due to their interaction with nucleic acids. Our group identified two dioxo ruthenium(II) polypyridyl complexes that accumulated specifically in the nucleus (**22**, **23**; **Figure 12**).[113] Later, structure-activity relationship (SAR) studies showed that their cellular uptake could be improved by changing the dioxo ligand (**24**; **Figure 12**). However a loss of nuclear localization was observed.[114] Barton and co-workers reported a rhodium(III) metallo-insertor that targets DNA mismatches and displays selectivity towards MMR-deficient cells (**25a**; **Figure 12**).[115] As expected, the complex was found to accumulate in the nucleus due to its DNA-binding affinity, but interestingly, the introduction of a methyl substituent instead of an hydroxyl on two of the ligands redirected the resulting complex **25b** to the mitochondria (**Figure 12**).

Tian and co-workers synthetized a cyclometalated iridium(III)-based photosensitizer (PS) **26a** that first targets the cell nucleus, binds to DNA, and then is translocated to the mitochondria following two-photon irradiation (**Figure 12**).[116] Similarly, the replacement of the phenyl ester substituent with a methylbenzene changed the localization of the compound, with **26b** directly accumulating to mitochondria and remaining there even after irradiation. Very recently, a collaborative work between Visser's and our group focused on new rhenium(I) tricarbonyl complexes with an imidazo-phenanthroline derivative ligand. Complex **27b** was found to accumulate specifically in the nucleus, where it likely interacted with DNA (**Figure 12**). A change of the benzotiophene substituent to a methoxy indole resulted in the accumulation of

parent compound **27a** to the mitochondria. For some of these examples, this phenomenon seems to be linked, at least in part, to the lipophilicity of these complexes. This parameter will be brought up for further discussion in part 3.1.2.

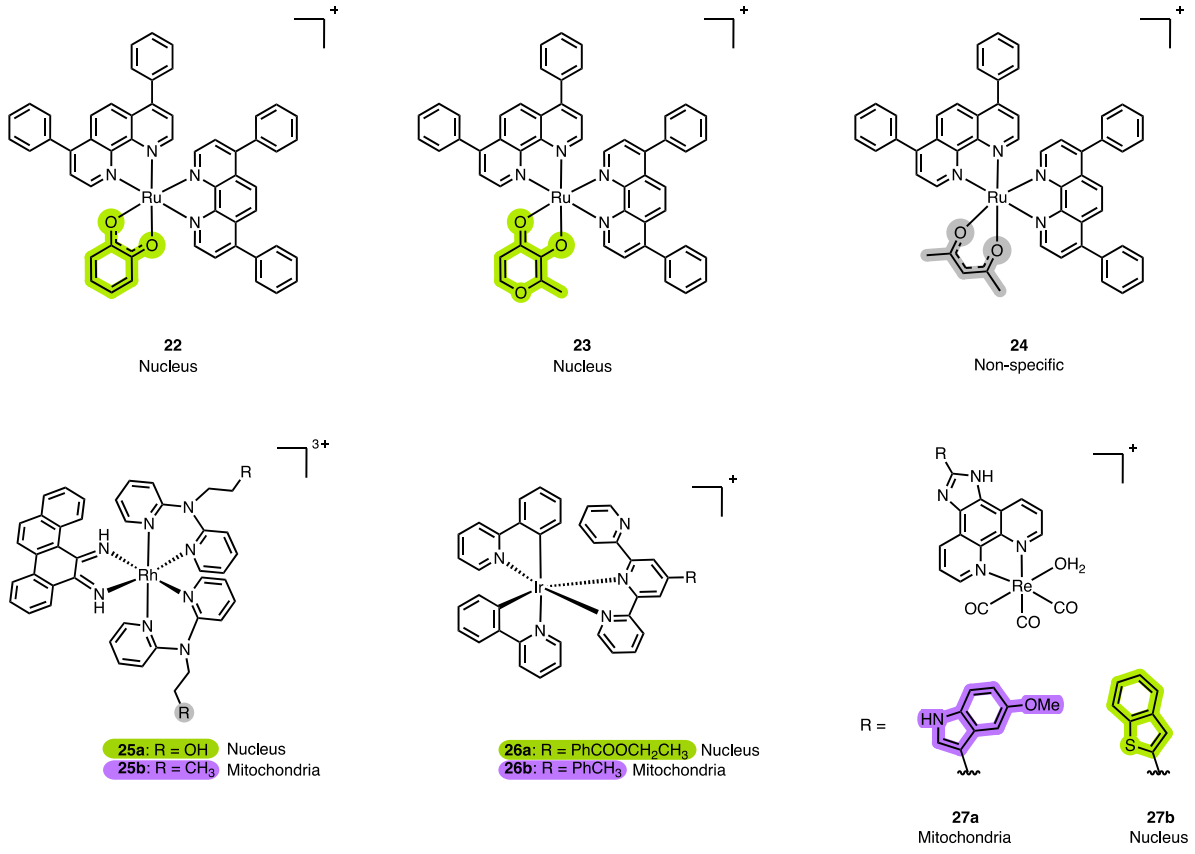


Figure 12. Varying the substituents on the ligands can result in a loss of nuclear targeting (highlighted in grey) or direct the corresponding complexes to the nucleus (highlighted in green) or the mitochondria (highlighted in purple).

Williams and co-workers synthetized and characterized several platinum(II) complexes for time-resolved emission imaging microscopy (TREM) that accumulated preferentially into the nucleus (**28a-b**, **Figure 13**), most likely due to interactions with DNA.[117] Changes in the terpyridine substituents did not seem to impair nuclear targeting, as the authors reported similar results for all other synthesized compounds. However, more significant changes such as the replacement of the terpyridine ligand on platinum(II) complex **29** by Kwok and co-workers resulted in a completely different distribution profile. The complex **29** accumulated rapidly in the cytoplasm but not in the nucleus (**Figure 13**). Further modifications with a triphenylphosphonium pendant (**30; Figure 13**) restored the affinity of the complex for the

nucleus, albeit with different molecular targets that were identified as nucleolar proteins. This was a rather unexpected result, given that the team introduced the triphenylphosphonium pendant on the basis of its capacity to deliver biomolecules to mitochondria.[118]

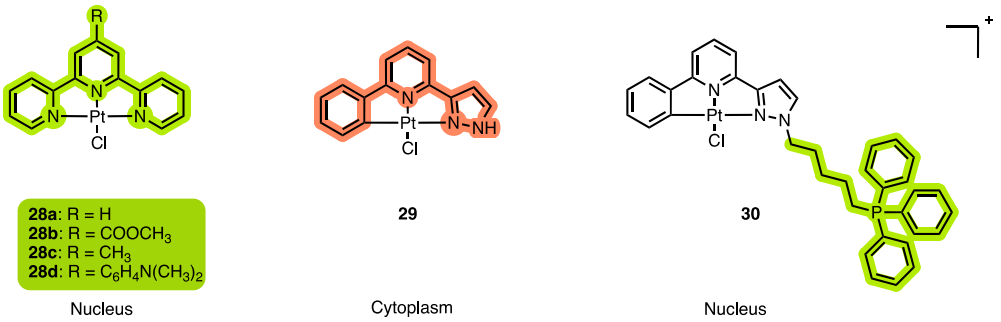


Figure 13. Varying the ligands on platinum(II) complexes can modulate their subcellular accumulation. Highlighted in green are ligands and substituents that result in nuclear accumulation, whereas ligands hindering nuclear accumulation are highlighted in red.

On other DNA-binding tripod platinum(II) complexes, a change of the N^N ligand afforded nuclear accumulation for **31a** whilst **31b** remained in the cytoplasm (**Figure 14**).[119] Interestingly, changes of isomers of the tripod ligand also produced different subcellular distribution patterns.[120] Isomers **32a** and **32b** accumulated mainly in the nucleus whereas **33a** and **33b** localized in the cytoplasm, suggesting that this change of structure prevented them from penetrating the nucleus (**Figure 14**).

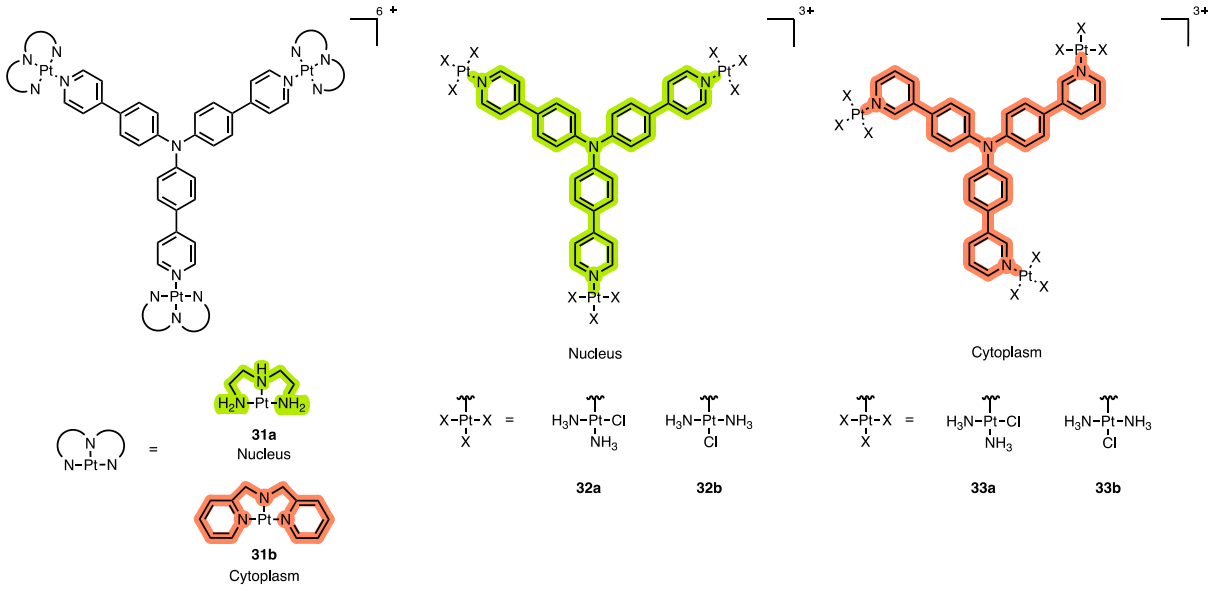
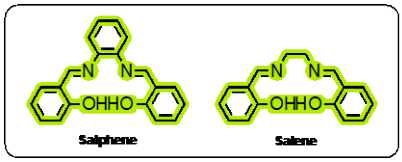
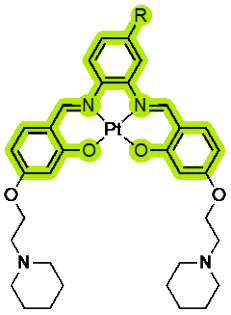


Figure 14. Variations of ligands or structures of tripod platinum(II) complexes can modulate their nuclear accumulation.

Salphen metal complexes have been reported and investigated as interesting scaffolds to target G-quadruplex DNA structures in several works describing their selectivity and binding properties *in vitro*.[121,122]. Vilar and co-workers synthetized the platinum(II) salphen complexes **34a** and **34b** as G-quadruplex binders and optical probes (**Figure 15**).[123] These complexes were found to preferentially localize in the nucleus and more precisely in the nucleolus. Bhattacharya and co-workers worked on other salphen metal complexes of nickel(II) and palladium(II) with fluorescein backbones.[124] The added fluorescein backbone did not seem to impair the complexes' ability to penetrate the nucleus, as both complexes **35a** and **35b** showed accumulation in this organelle and significant telomerase inhibition (**Figure 15**). However, the localization specificity lowered slightly, as they were found to localize in the mitochondria as well.

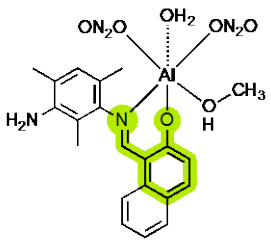
An aluminium(III) complex with a salen-like ligand **36** was found to stain the nucleus and nucleolus due to interactions with nucleic acids (**Figure 15**).[125] Binding to G-quadruplex structures has not been investigated, nonetheless, this salen-type structure for ligands seems to also favor direction of metal complexes to the nucleus and nucleolus.





34a: R = -O-(CH2)2-N(CH2)4
34b: R = SO_3H

35a: M = Ni
35b: M = Pd



36

Figure 15. Salphen and salen-like metal complexes targeting G-quadruplexes with a nuclear accumulation. Salphen and salen structures (highlighted in green) on the ligands appear to favor G-quadruplex targeting.

Zinc(II) terpyridine complexes were also reported to interact with G-quadruplexes, and two complexes were described by García and co-workers as preferentially accumulating in the nucleolus as a result (**37, 38**; **Figure 16**).[126] It is worth noting that **37** was found to have more affinity and selectivity towards human telomeric G-quadruplexes compared to **38**, despite bearing only one terpyridine ligand. Moreover, **37** was also shown to be more cytotoxic than **38**, suggesting that the addition of a second terpyridine ligand could result in a loss of affinity for G-quadruplex structures as well as a lower toxicity. It is unclear, however, whether this lower toxicity is the result of the loss of G-quadruplex selectivity.

Similar zinc(II) terpyridine complexes were synthetized by Tian and coworkers as potential imaging probes (**39a-b; Figure 16**).[127] Intriguingly, complex **39a** displayed an extremely low toxicity and very different accumulation pattern compared to complex **38**. In addition to the nucleus, membrane staining was observed. Moreover, complex **39b** only displayed membrane accumulation. G-quadruplex binding was not investigated for these complexes, however, the stark differences in cytotoxicity and staining patterns suggest a loss of affinity for these structures. This observation reinforces the statement that modifications on the backbone of a ligand coordinated to a nucleus accumulating complex can have a great influence on its subcellular distribution.

The mechanism by which complex **39a** accumulates in the nucleus and the membrane remains unclear, although it is thought to be recognized and transported to the nucleus through micro-tubular-like structures. Interestingly, this accumulation pattern was only observed in cancer cell lines, whereas **39a** was distributed throughout the whole cytoplasm in normal HELF cells (Human Embryo Liver Fibroblast). To the best of our knowledge, this is the sole example of a seemingly cancer cell-specific accumulation pattern for a metal complex. Albeit the reason for this specificity is unknown, a plausible hypothesis would involve the different characteristics displayed by different cell lines depending, amongst other things, on their type. Similar observations could thus occur with other metal complexes, which should encourage investigating subcellular distribution on several cell lines when designing nucleus-targeting agents.

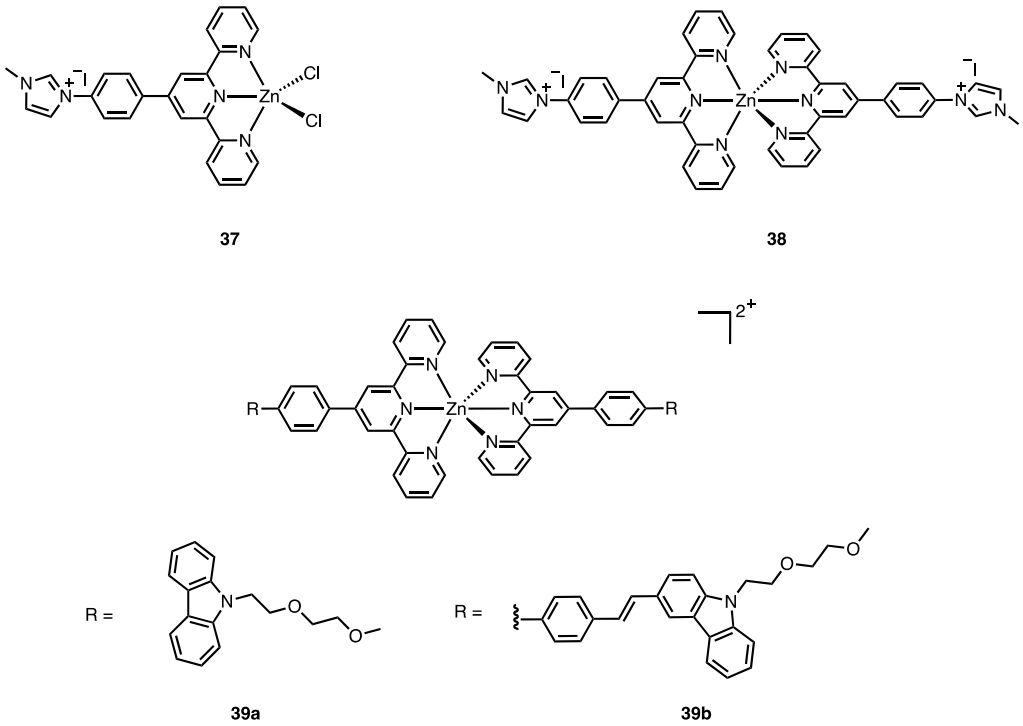


Figure 16. Zinc(II) terpyridine complexes targeting G-quadruplexes that accumulate in the nucleus.

Several groups reported ruthenium(II) polypyridyl complexes as G-quadruplex binders, with some of them accumulating in the nucleus and nucleolus of cells as a result (**40**, **41**; **Figure 17**).[128,129] Interestingly, chirality seemed to play an important part in the localization of such complexes; this will be discussed in section 3.1.3 below.

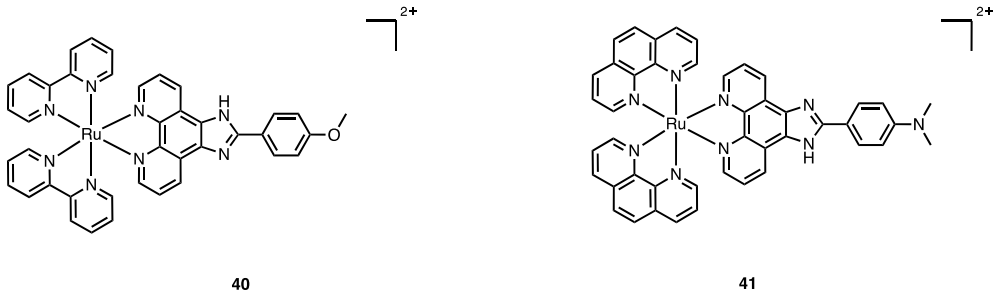


Figure 17. Ruthenium(II) polypyridyl complexes targeting G-quadruplexes that accumulate in the nucleus.

The study of several emissive lanthanide complexes by New, Parker and co-workers shed some light on the structural parameters that seemed to affect their distribution.[130,131] The authors highlighted the importance of the sensitizing chromophore and its mode of linkage in the subcellular localization of complexes above other parameters such as charge or lipophilicity. They found that complexes with a coordinated azaxanthone or azathiaxanthone sensitising moiety, such as complexes of europium reported by Cann, Parker and co-workers (**42**, **43**; **Figure 18**), were more likely to localize in organelles consistent with the nucleoli and ribosomes.[132,133] Similarly to examples above, this might be due to the capacity of these moities to interact with nucleic acids.

It is worth noting, however, that at a higher concentration, complexes that otherwise located in lysosomes could be directed to a nucleolar localization. This phenomenon is attributed to an enhanced nuclear membrane permeability induced by complexes themselves. Although the exact mechanism by which this enhancement occurs remains unknown, it could be a consequence of cells undergoing stress. This nucleolar accumulation subsequent to membrane permeabilization suggests that membrane permeability is one of the obstacles for the nuclear localization of lanthanide complexes. In accordance with this observation, Walton and co-workers reported that **44**, a terbium complex with azaxanthone ligands, is able to stain chromosomal DNA when cells undergo division in the M phase and the nuclear membrane integrity is somewhat compromised.[134]

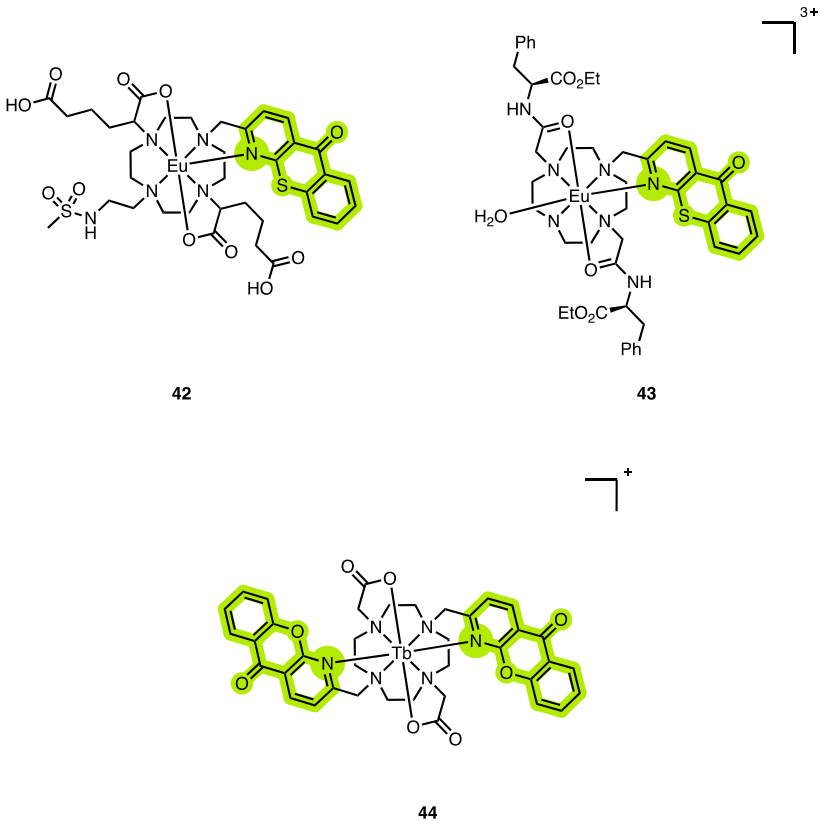
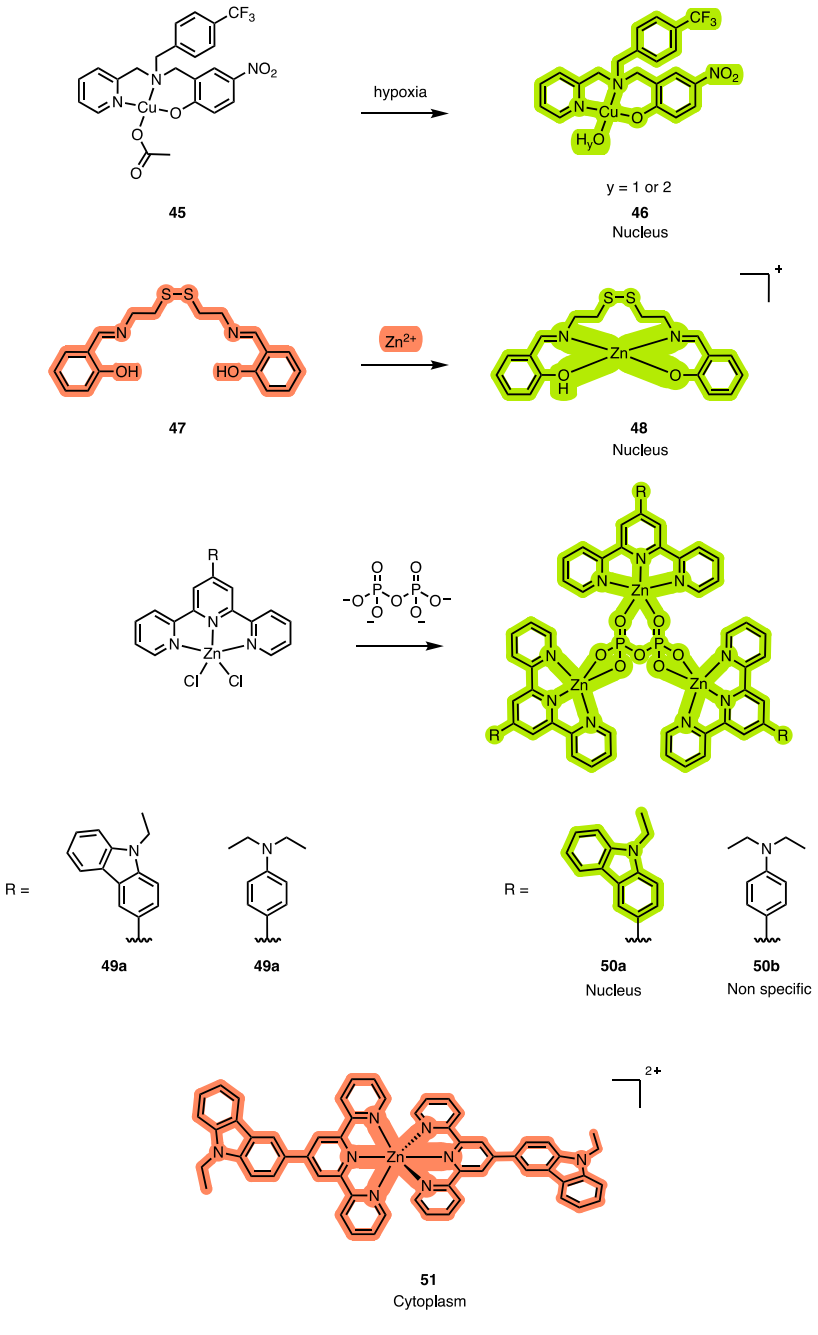


Figure 18. Lanthanide complexes that accumulate in the nucleus/nucleolus. Azaxanthone and azathiaxanthone moieties (highlighted in green) are important for specifc nucleolar accumulation.

Changes of ligands to redirect complexes to a nuclear target can also happen inside cells. It is the case for copper(II) pyridyl complexes synthetized by Walsby and co-workers, which work as hypoxia-selective, nucleus-targeting agents.[135] Exposition to a hypoxic environment triggers a ligand exchange from acetate to HO or H_2O and prompts an enhanced cellular uptake and nuclear accumulation of complex **46**, promoting selectivity for hypoxic cancer tissue (**Scheme 1**).

This strategy of in-cell synthesis was identified and used to yield pro-drug zinc(II) complexes by Mao and co-workers, as well as Chao and Ni. Mao and co-workers described the in-cell conversion of complex **48** upon coordination of Zn^{2+} ions to the nucleus impermeable ligand **47** (**Scheme 1**).[136] Akin to other salphen metal complexes (**34**, **35**; **Figure 15**), **48** localized in the nucleus by virtue of interaction with nucleic acids. Moreover, its in-cell conversion could be followed by confocal microscopy as it induces a characteristic green-to-blue fluorescence change.

While investigating for fluorescent probes to detect pyrophosphate (PPi) anions, Chao ad Ni discovered that the complexes formed upon coordination of the Zn complexes **49a** and **49b** with PPi were able to penetrate and light up the nucleus of cells (**Scheme 1**).[137] Change of substituent on the terpyridine ligand, from a carbazole to an aniline, resulted in a change of subcellular distribution of the corresponding complexes. Complex **50a** accumulated exclusively in the nucleus whereas **50b** stained the whole cell. In addition, coordination to PPi anions seemed essential for the nuclear redirection: bis-terpyridine zinc(II) complex **51** described by Tian and coworkers with the same substituent remained solely in the cytoplasm.



Scheme 1. Copper(II) and Zinc(II) complexes undergo ligand modifications inside live cells leading to their accumulation to the nucleus. Complexes highlighted in green accumulated specifically to the nucleus. In contrast, ligands, metal ions and complexes highlighted in red remained solely in the cytoplasm.

In light of examples listed above, it appears that the use of coordinating ligands that are known to interact with nucleic acids is a rather straightforward strategy to enhance the nuclear accumulation of metal complexes. Thus, pharmacophores from approved antitumoral drugs acting on nucleic acids seem particularly efficient at directing metal complexes to the nucleus of cells. They can be directly coordinated to the metal ion, or attached *via* a linker to a ligand, which allows for their use with various metal ions with different geometries. As a general observation, molecules with extended aromatic, planar structures often display DNA-intercalating properties. It is thus not surprising to find them as ligands on nucleus-targeting complexes, and the reader would be advised to consider using such structures to achieve nuclear accumulation.

Furthermore, ligands that do not interact with nucleic acids can have an influence on the subcellular localization of a complex. For polypyridyl complexes, the substitution of a bipyridine by a phenanthroline or a diphenyl-phenanthroline (DIP) appears to modulate cellular and nuclear uptake of complexes. Since there is no clear-cut factor in favor of one or the other, especially between bipyridine and phenanthroline, it is advised to test all combinations when possible. Moreover, Barton and co-workers reported that the introduction of bulkier ligands favored selectivity for DNA mismatches, due to steric hindrance in well-matched sites.[108] This highlights the role that non-intercalating ligands can play in favor or disfavor of nucleic acid binding by complexes and in consequence, their nuclear accumulation.

In addition to chemical structures known to interact with nucleic acids, molecular size and lipophilicity can play a significant part in the subcellular localization of metal complexes. These parameters are often tightly associated when designing and optimizing specific organelle-targeting, as we will see in the following part how they can heavily influence cellular uptake and distribution.

3.1.2. The importance of lipophilicity and its balance

The lipophilicity of molecules is an important parameter to consider, as it can determine their ability to cross biological barriers, including the cell membrane. Indeed, the interior of cells is separated from the exterior by a phospholipid bilayer that is mostly impermeable to hydrophilic and highly charged compounds. This represents the first obstacle for the use of metal complexes in live cells.

The increase in cellular uptake of metal complexes due to a greater lipophilicity is known and has notably been reported for ruthenium(II) complexes by Barton and Puckett,[138] or gold(I) phosphine complexes by Ott, Ruiz and co-workers.[92,93,98] Chao and co-workers used this strategy when designing the cyclometalated ruthenium(II) dppz complex **14** (**Figure 9**), which facilitated its cellular uptake and subsequent nuclear localization due to its DNA-binding affinity.[104] In a similar fashion, the introduction of more lipophilic phenanthroline ligands compared to bipyridines on dinuclear tpphz complexes by Thomas and co-workers (**20-21**; **Figure 11**) improved their cellular accumulation without impairing their DNA-binding activity.

Li and co-workers described a new strategy relying on the formation of stable ion-pairs. Pairing various negatively charged counter ions to $[Ru(bpy)_2(dppz)]^{2+}$ complexes successfully directed them to the nucleus of cells.[139,140] The ion-pairs (**52a-f**; **Figure 19**) displayed an increased lipophilicity and were subsequently able to be taken up by cells through passive diffusion, while retaining their DNA-intercalating properties and increased accumulation in the nucleus. Interestingly, nuclear uptake was negatively correlated with the lipophilicity of the ion-pairs, but positively correlated with their binding stability, suggesting an influence of the pairing for the nuclear as well as cellular entry.

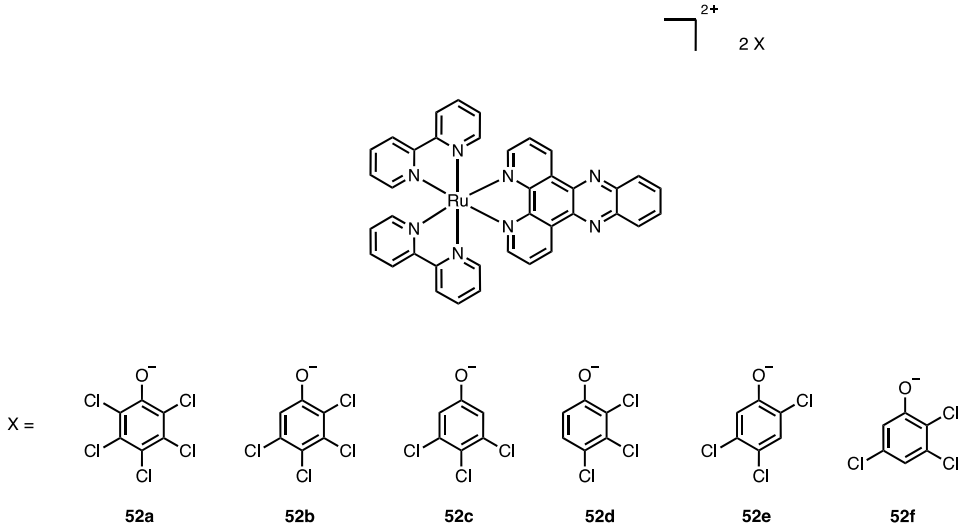


Figure 19. Using lipophilic ion-pairs allows for an increased cellular uptake and nucleus accumulation of $[Ru(bpy)_2(dppz)]^{2+}$.

However, changes in lipophilicity can also alter the localization of a complex inside the cell. To increase the lipophilicity of a complex, introduction of hydrophobic groups on ligands is often the preferred strategy. But changes on the ligand or the introduction of bulkier substituents can also lead to an exclusion from the nucleus, as seen on the cyclometalated iridium(III) derivatives described by Li and coworkers (**Figure 20**).[141,142] Complexes **53** and **54a** accumulated specifically in the nucleus of HeLa cells. However, an increase of the length of side carbon chain and branched chain at the 4-position of the phenyl on **54a** caused an accumulation of the corresponding derivatives in the cytoplasm rather than the nucleus (**53b-d**; **Figure 20**). In contrast to other iridium(III) and ruthenium(II) complexes mentioned previously, the authors hypothesized that rather than their affinity for a specific nuclear component, NPCs could selectively control the transport of complexes **53** and **54a** between the cytoplasm and nucleus. It is hypothesized that the nuclear localization of **54a** might be mediated through recognition and binding to molecular transporters, possibly karyopherins, involved in facilitated transport through NPCs. Substituent modifications on the ligands for complexes **54b-d** appeared significant enough to lose recognition by these molecules, prompting caution when introducing ligand changes.

Chen and co-workers observed that variations on the ligands of iron(II) polypyridyl complexes (**55a-e**; **Figure 20**) seemed to modulate nuclear accumulation.[143] Only the smallest, less hydrophobic complex **55a** reached the nucleus and appeared to accumulate preferentially there.

No precise mechanism was identified to explain such a behavior, however it could be hypothesized that the increased lipophilicity would play a part, or that similarly to iridium(III) complexes, recognition by molecular transporters was lost.

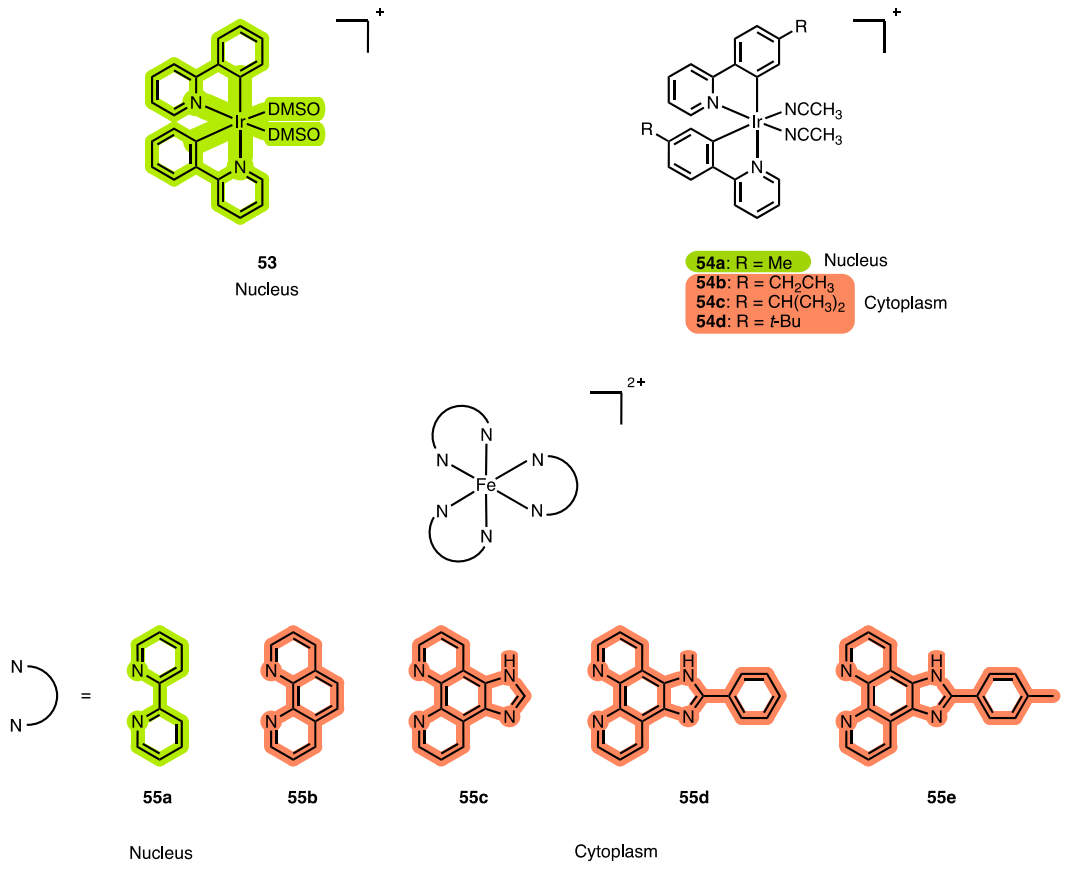


Figure 20. Variations of the lipophilicity and the substituents of cyclometallated iridium(III) complexes and polypyridyl iron(II) complexes appear to affect their subcellular localization.

Modulation of the lipophilicity can also change the localization of a complex by changing its affinity for cellular compartments. This was observed on $[Ru(phen)_2(dppz)]^{2+}$ (phen = phenanthroline) derivatives **16c-e** by Lincoln and co-workers (**Figure 9**).[144] The most hydrophilic complex **16c** accumulated in the nucleus, whereas the slightly more hydrophobic complex **16d** diffused in the nucleoli and cytoplasm. The most hydrophobic complex **16e** remained sequestered in membranes. Results suggested that complex **16c** specifically stained the nucleus by virtue of its strong DNA-binding activity. Complex **16d** was found in the nucleoli and cytoplasm, with a staining pattern like commercial RNA probes, most likely due to interactions with RNA. Complex **16e** remained in lipid-rich parts of the cell, *i.e.* membranes, because of its greater lipophilicity.

Besides membranes, another organelle where highly lipophilic complexes are likely to accumulate in are mitochondria. Given that both nucleus and mitochondria contain DNA, it is probable that the target is still DNA, and that this difference in localization resides in the capacity of complexes to penetrate either the nuclear or mitochondrial membrane – or both. The mitochondrial membrane is comprised of an inner and outer membranes, is highly lipophilic and displays a strong negative potential, which favors the accumulation of lipophilic, positively charged complexes that can accumulate *via* passive diffusion. In contrast, although the NE is also a phospholipid bilayer, it has a lower potential, and bi-directional transport in the nucleus happens exclusively through NPCs. These structural differences could account for the different abilities of complexes to accumulate in either organelle.

Mitochondrial DNA (mtDNA) is a circular, double stranded DNA that encodes genes of essential parts of this organelle.[145] MtDNA is more prone to DNA-damage compared to nuclear DNA, however it rarely results in rapid cell death and thus is discarded over other mitochondria-targeting strategies such as mitochondrial membrane permeabilization (MMP). MMP usually results in rapid cell death through apoptosis or necrosis of cells.[146] Thereby, the mitochondrial membrane represents the most interesting target for antitumoral agents, and mitochondrial accumulation of metal complexes is mostly desirable for complexes that are able to deal damage to the mitochondrial membrane rather than mtDNA.

A comparative study by Barton and co-workers of the rhodium(III) metallo-insertors **25a** and **25b** (**Figure 12**) reported the influence of changes in the structure of ligands on their permeability through mitochondrial membrane or NE and subsequent accumulation. Both complexes kept a high binding affinity for DNA, but the introduction of a methyl instead of an hydroxyl substituent on complex **25b** provided a facilitated uptake in the mitochondria, compared to **25a** which accumulated specifically in the nucleus instead.[115] A similar phenomenon happened with iridium(III)-based photosensitizers (PS) (**26a-b; Figure 12**) synthetized by Tian and colleagues, with the more lipophilic complex **26b** accumulating solely in mitochondria.[116]

SAR studies led on ruthenium(II) polypyridyl complexes (**22**-**24**; **Figure 12**) also showed that a slight change of dioxo ligand was enough to change the preferential localization of complex **24** to the mitochondria compared to complexes **22** and **23**.[113,114] It is unclear whether the

molecular target of all these compounds stayed the same, but an increase in lipophilicity is likely to be responsible for the higher cellular uptake observed and the mitochondrial localization.

Although it appears that more lipophilic, charged complexes will be taken up by mitochondria more easily, it is not a systematic phenomenon. It would thus be misleading to consider lipophilicity as the sole parameter for different cellular localizations.

In the case of rhenium(I) tricarbonyl complexes described by Visser's and our group (**27a-b**; **Figure 12**), despite having similar lipophilicity, complex **27a** accumulated preferentially in mitochondria whereas complex **27b** showed a strikingly higher cellular uptake and localized in the nucleus.[147]

Moreover, contrary to the belief that more lipophilic cationic complexes would be more likely to penetrate mitochondria, Mei and co-workers observed that the more lipophilc Ru(II) complex **17a** accumulated specifically in the nucleus, whereas the more hydrophilic complex **17b** accumulated in both nuclear and mitochondrial compartments (**Figure 9**).[107] In the same fashion, Liu and co-workers compared two half-sandwich iridium(III) and ruthenium(II) complexes bearing the same imino-pyridyl ligand. Whilst complex **56** (logP = 3.5 ± 0.6) was more lipophilic than **57** (logP = 1.7 ± 0.4), it still accumulated specifically in the nucleus, whereas **57** accumulated in the mitochondria (**Figure 21**).[148]

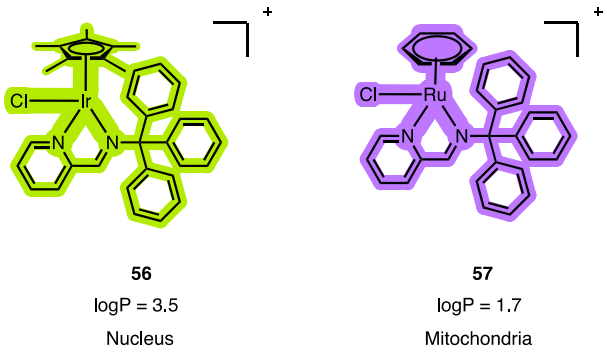


Figure 21. A higher lipophilicity is not the sole parameter for mitochondrial accumulation instead of nuclear.

In most works cited beforehand, synthesis and characterization of complexes were done on a mix of stereoisomers. However, as there is a clear influence of the geometry of the complex on

its interactions with DNA, it can be relevant to isolate stereoisomers to study their specific localization. Several works highlight how stereochemistry of complexes can play a part in their subcellular distribution.

3.1.3. Stereochemistry: a non-innocent parameter

As early as the 90's, the study of the influence of stereochemistry and in particular chirality of small inorganic complexes that bind to DNA brought to light the binding difference that could exist between two enantiomers of the same metal complex. In 1996, Mahadevan and Palaniandavar reported the chiral discrimination of $[Ru(phen)_3]^{2+}$ in the binding of calf thymus DNA (**Figure 22**).[149] They observed that the Δ enantiomer displayed a selectivy for B-DNA binding compared to the Λ enantiomer. This difference appears to be linked to the different binding mechanisms to DNA between the two enantiomers. Authors reported that the Δ enantiomer bound strongly to B-DNA by partial intercalation, whilst the Λ enantiomer bound weakly by a predominant electrostatic mode. Since then, similar phenomena have been observed for other ruthenium(II) complexes.

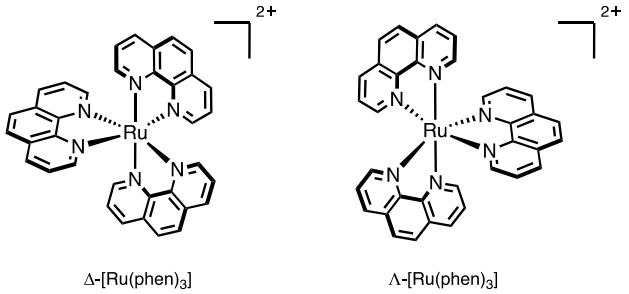


Figure 22. Structures of the Δ and Λ enantiomers of $[Ru(phen)_3]^{2+}$

In 2012, Lincoln and co-workers reported an instance of differential subcellular localization of pairs of enantiomers in fixed cells.[150] Interestingly, whilst the two structural isomers of $[\mu\text{-bipb}(phen)_4Ru_2]^{4+}$ (bipb = bis(imidazo [4,5-f]-1,10-phenanthrolin-2-yl)benzene, (**58**, **59; Figure 23**) displayed similar staining patterns, their enantiomers displayed substantial differences in localization. While all complexes were able to penetrate the nucleus, the ΔΔ enantiomers of both isomers showed preferential accumulation in the nucleoplasm compared to the ΛΛ enantiomers which remained mainly in the cytoplasm, although with a pronounced staining of the nucleoli. Surprisingly, this selectivity did not appear to be due to enantiomeric

differences in the strength of the interaction with DNA. No clear cause could be asserted; however it is possible than rather that strength of interaction, the nature of the interaction could explain the difference of subcellular localization. Nucleoli are structures which are mainly comprised of ribosomal RNA, that bear great differences in geometry to that of B-DNA. Thus, there might exist a selectivity for B-DNA over rRNA, or the contrary, between ΔΔ and ΛΛ enantiomers.

In live cells, however, only cytoplasmic staining could be observed for all complexes. Previous results pointed to an endocytic cellular uptake, suggesting that complexes were trapped in endosomes when incubated with live cells.

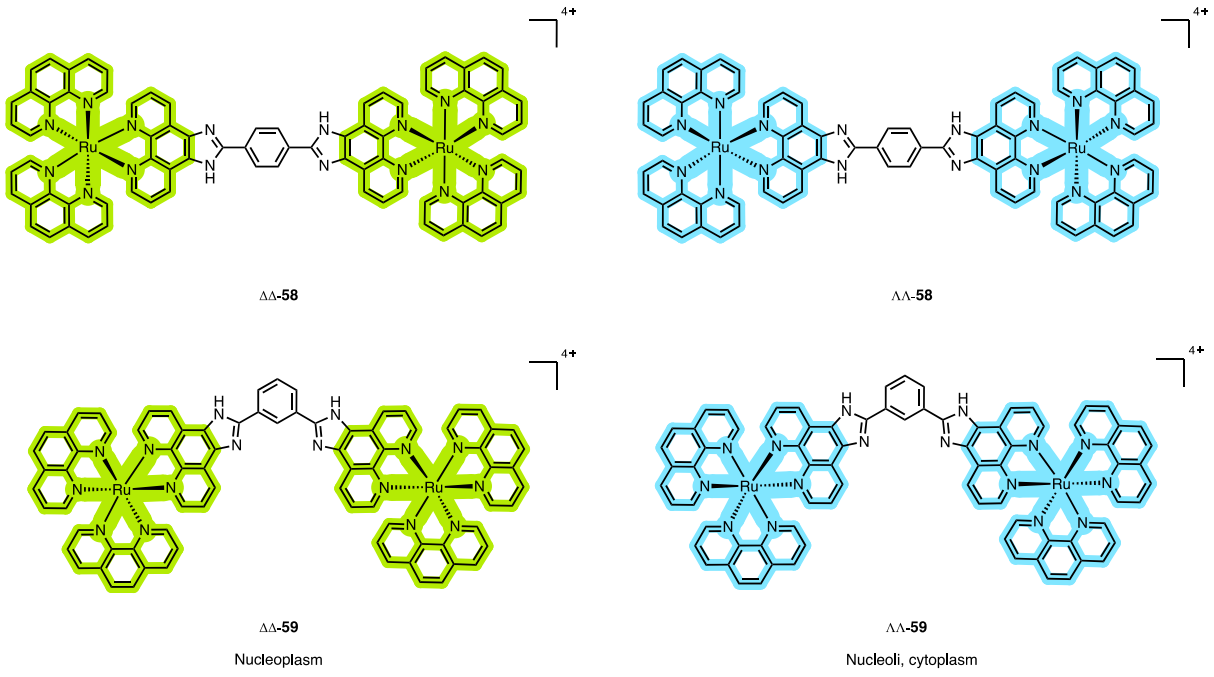


Figure 23. Changes on the stereochemistry of dinuclear ruthenium(II) complexes result in different subcellular localizations. ΔΔ enantiomers (highlighted in green) tend to accumulate in the whole nucleus and nucleoplasm whilst ΛΛ enantiomers (highlighted in blue) tend to accumulate in the cytoplasm and in nucleoli.

This work brought to light the influence of the stereochemistry of some complexes on their subcellular localization, which was subsequently touched on by several other groups. Liu and co-workers reported the synthesis of enantiomers of complex **41** (**Figure 24**). The Δ–**41** enantiomer accumulated to a lesser extend in the cells and remained almost exclusively in the

cytoplasm, whereas the Λ–**41** enantiomer accumulated preferentially in the nucleus.[129] This phenomenon is possibly due to the stronger binding to G-quadruplex DNA displayed by the Λ–**41** enantiomer compared to the Δ–**41** enantiomer.

Mei and co-workers described a similar trend for the enantiomeric pair Δ–**60** and Λ–**60** in MDA-MB-231 cells. The Λ–**60** enantiomer accumulated in the nucleus whereas Δ–**60** remained in the cytoplasm.[151] Recently, Zhu and co-workers reported this phenomenon with the ruthenium polypyridyl complex $[Ru(DIP)_2(dppz)]^{2+}$ (**61**; **Figure 24**). This time, only the Δ-**61** enantiomer was selectively directed to the nucleus.[152]

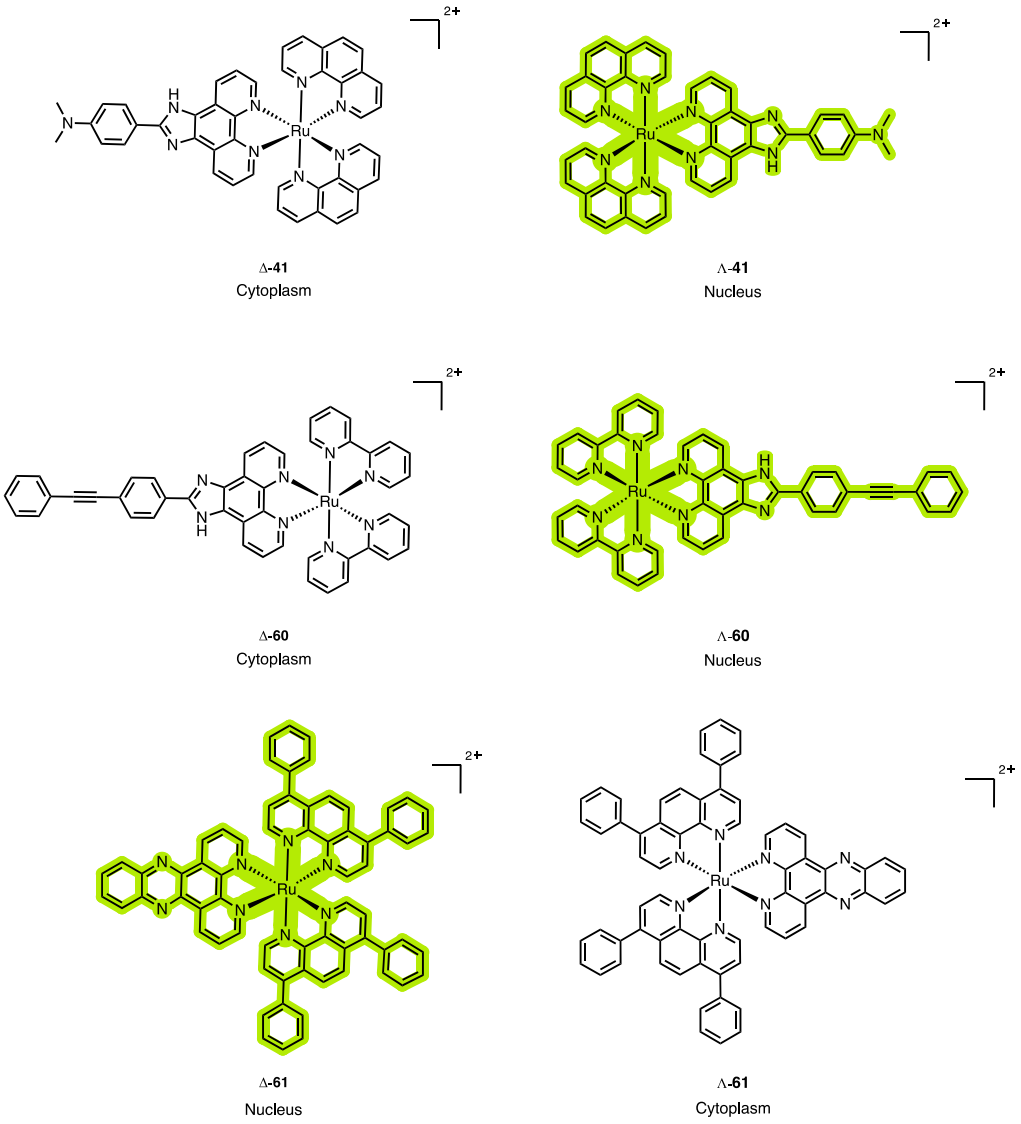


Figure 24. Changes in the stereochemistry of ruthenium(II) complexes has an influence on their nuclear accumulation. In each case, only one of the two enantiomers (highlighted in green) seems able to specifically accumulate in the nucleus.

Although no generalization can be made, it is likely in these examples that enantioselective binding affinities to nuclear components is partly responsible for the subcellular localization of the complexes. Stark differences in biological activity for two enantiomers of a pharmacological agent are not new; such things have been previously reported in small molecules with drugs like thalidomide and ketamine.[153,154] Therefore, it is not surprising to see similar behaviors when comparing enantiomers of a metal complex.

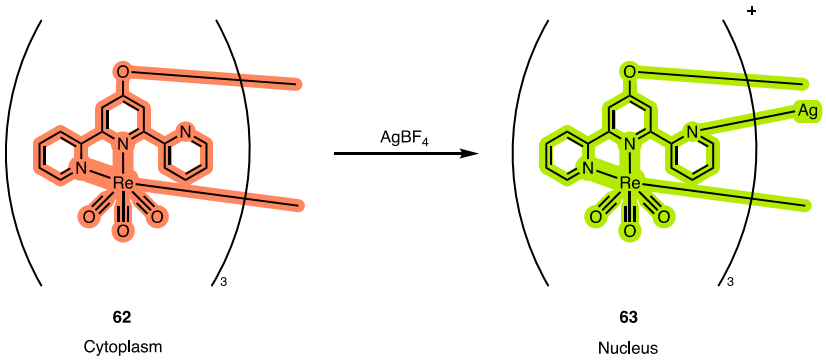
From these examples, it appears that for metal complexes that bind with B-DNA through intercalation, the Δ enantiomer displays a stronger, more selective binding. For other types of nucleic acids structures, such as rRNA or G-quadruplexes, it seems that the Λ enantiomer is preferred.

3.1.4. Charge of the complex

As mentioned before, the phospholipid bilayer that constitutes the plasma cell membrane acts as a barrier and prevents the entry of hydrophilic, highly charged compounds by passive diffusion. Still, some charged metal complexes are able to enter cells. Studies seem to indicate that cationic species in particular are taken up better by passive diffusion than anionic or neutral, due to the negative charge of the plasmic membrane.[102] This observation is particularly valuable since non-endocytic uptake pathways are to be favored for a nuclear targeting: this avoids the accumulation of complexes in endosomes, where they might stay trapped. These endosomes will eventually fuse with lysosomes, where these complexes will accumulate. Moreover, cationic complexes are also more likely to interact with negatively charged nucleic acids; as such, they make up most of the complexes reported so far in this review.

Based on these observations, Coogan and co-workers synthetized a trimeric, rhenium-based molecular vessel that becomes cell-permeable upon binding with metal cations.[155] Molecular vessel **62** bound to a silver cation yielded complex **63** which could penetrate cells and localize in the nucleoli (**Scheme 2**). It is hypothesized that this increased cellular uptake is due to charge-enhanced interaction with the glycocalyx periphery of the membrane, a polysaccharide-based

layer that acts as an interface between the extracellular matrix and the plasmic membrane. The cause of nucleolar localization remains unclear. The enhanced nuclear permeability, though, appears to be afforded by the binding of the silver cation.



Scheme 2. Coordination of rhenium complexes to a silver ion results in a nuclear localization.

Liu and co-workers synthetized iridium(III)-based PSs intended for dual-damage (**64, 65; Figure 25**).[156] These PSs were designed to accumulate first in mitochondria, and to inflict enough damage to this organelle upon light irradiation to be released into the cytosol, then redirected to the nucleus.

Interestingly, they observed that the mono-cationic complex **64** was not redirected to the nucleoli upon release from the mitochondrial compartment, unlike the bi-cationic complex **65** bearing a charged ligand, which could subsequently damage nucleic acids.

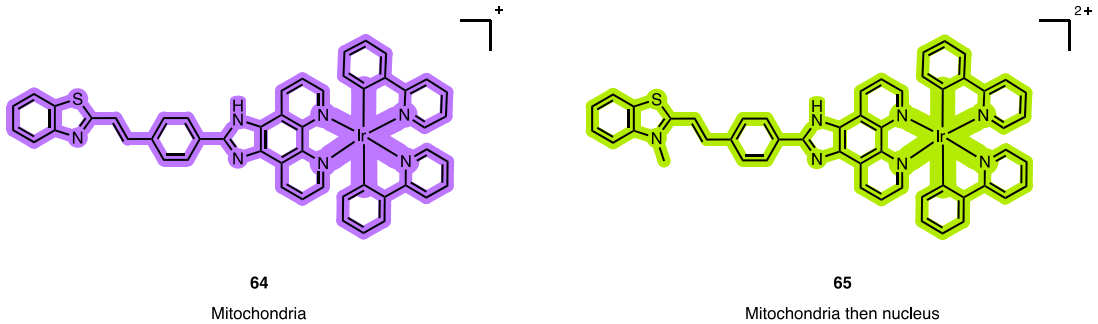


Figure 25. Variation of the charge of the complexes has an influence on the ability of iridium(III)-based PSs to be localized in the nucleus upon release from the mitochondrial compartment.

Although a global positive charge appears to increase DNA-binding affinity to metal complexes, notably through electrostatic interactions, it is not the determining factor for DNA selectivity, as many other cellular components such as the plasma membrane are also negatively charged. However, results suggest that the use of optimally positively charged metal complexes can help to overcome negatively charged barriers such as the plasmic membrane and NE.

Overall, it is apparent that the backbone of ligands and the lipophilicity of the complexes are the two parameters that play an essential role in determining subcellular localization. As expected, ligands that bear moieties known to interact with nucleic acids are more likely to afford nuclear accumulation, the main obstacle being the potential impermeability of complexes to cellular or nuclear membrane.

As a general trend, it appears that an increase in lipophilicity tends to result in an enhanced cellular uptake, and the modulation of this parameter is one the most widespread strategies. This method can be beneficial to let previously cell impermeable compounds arrive to their nuclear target but could also backfire. Indeed, it can lead to a loss of selectivity towards the nucleus. This can be directly linked to the increased lipophilicity, as more hydrophilic compounds seem to accumulate preferentially in the nucleus. This could also be due to larger sizes of complexes after the addition of lipophilic substituents, which can prevent nuclear entry. Finally, a loss of recognition by molecular transporters is possible as well, eliminating the means by which the complex can enter the nucleus.

A few examples have highlighted how other parameters could influence the localization of a complex, such as stereochemistry or global charge. When designing metal complexes intended for intercalation into B-DNA, one should favor Δ enantiomers, whilst Λ enantiomers could be useful to target other types of nucleic acids structures. As for charge, a global positive charge on the complex compared to neutral or negative appears to achieve better cellular and nuclear uptake. Although examples of the changes that these variations bring are scarce, this should encourage consideration of these parameters when trying to design or optimize nuclear-targeting complexes.

Moreover, it is expected that a rigorous quantification of the accumulation of metal complexes in the various organelles and a standardization of methods to perform this evaluation could help to further compare the effects of a change of ligands and unveil more parameters influencing

subcellular distribution. Therefore, quantification studies are strongly encouraged when assessing the characteristics of novel organelle-targeting metal complexes.

3.2. Nuclear targeting of metal complexes using biomolecules

A strategy to bring metal complexes to cancer cells is to use a macromolecular vector. Peptides and antibodies with a specific binding to relevant molecular structures are the most used, as many of them have been identified to interact with cancer biomarkers. These biomarkers mostly anchor in the external cell membrane or in the tumor microenvironment.[157–160] Several receptor-specific peptides and antibodies approved as antitumoral targeted therapies, such as octreotide and trastuzumab, are being used as vectors to improve the delivery of various drugs.[161–164]

In a similar way, biomolecules can be used to direct and facilitate the uptake of metal complexes in cancer cells, where they can subsequently be directed to the nucleus if they possess the right properties. Early instances of metal complex bioconjugates use a biomolecule for the sole purpose facilitating cellular uptake or achieving selectivity for cancer cells. Santos and co-workers (**66a-b; Figure 26**) synthetized rhenium(I) and ^{99m}Tc conjugates that accumulate specifically in the nucleus of GRPR (gastrin-releasing peptide receptor) positive cells, thanks to the presence of acridine orange on the ligand.[165] Similarly, Gano and colleagues synthetized an indium complex conjugated to an estrogen receptor (ER) ligand for breast cancer cells selectivity, which achieved nuclear accumulation thanks to acridine orange (**68; Figure 26**).[166]

In these examples, rather than the biomolecule, it is apparent that it is still the DNA-intercalating ligand that is responsible for the nuclear accumulation. This statement is strengthened by the fact that a change of moiety on the ligand results in a change of subcellular localization. This was observed by Paulo and co-workers on the complexes **67a** and **67b** (**Figure 26**) which accumulated in mitochondria instead.[167]

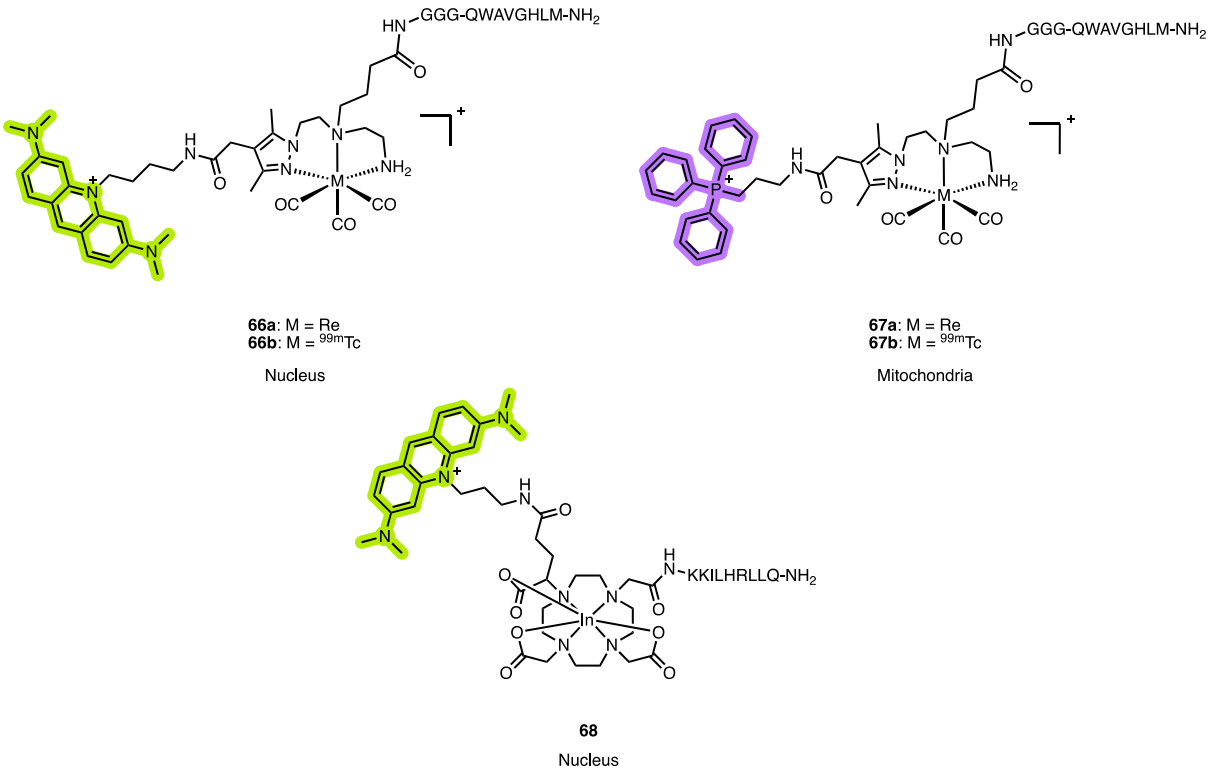


Figure 26. Biomolecules can provide selectivity for cancer cells but are not necessarily responsible for the nuclear accumulation. In these instances, ligands are responsible for the subcellular localization either in the nucleus (highlighted in green) or the mitochondria (highlighted in purple), as discussed in part 3.1.

The discovery of new biomolecules that are able to modulate cellular and nuclear uptake has however allowed for the design of new metal complex bioconjugates that, this time, could achieve nuclear accumulation thanks to the biomolecule. These structures were, in most cases, discovered in nature, and particularly in compounds whose mechanism of action relies on the access to the nucleus of cells. For instance, great inspiration has been taken from the structure of components of some viruses, which thrive and proliferate through the exploitation of the cellular machinery of eukaryotic cells.

Cell-penetrating peptides (CPP) are short sequences that possess the ability to interact with the plasmic membrane and be internalized through various pathways in most cell types. They are often found in the structures of venoms, as well as transduction domains of virus-associated proteins.[168] CPPs represent valuable tools for intracellular transport and have been used as vectors for the intracellular delivery of various molecules. Namely, poly arginine motifs and

sequences derived from the HIV Tat protein are the most well-known for their ability to facilitate the intracellular transport of different classes of molecules.[169]

CPPs can be internalized *via* different uptake pathways, both endocytic and non-endocytic, which subsequently lead to different subcellular localizations. Although mechanisms of cellular internalization of CPPs and CPP-cargo conjugates have yet to be fully understood and reported, it appears that they can be internalized through multiple pathways that depend on the structure, physico-chemical properties and concentration of the CPPs, as well as the characteristics of the cargo and the cell-type dependent composition of the plasma membrane.[170]

Octaarginine peptides have been shown to enhance the cellular and nuclear uptake of ruthenium(II),[171,172] iridium(III),[173] rhodium(III),[174] and osmium(II) (**70, 72; 73; 74; 75d respectively; Figure 27**) complexes.[175] In most cases, the conjugates accumulated more in the nucleus compared to complexes alone, although a good fraction remained in the cytoplasm. The addition of a fluorescein backbone to conjugate **69** by Puckett and Barton interestingly changed the subcellular localization of the resulting conjugate **70** from the cytoplasm to the nucleus.[171] Although not a systematic strategy, this highlights the possibility to attach another moiety to a conjugate to modulate its distribution. This prompts caution as well when using fluorescence to assess the localization of a complex, as it showed that the localization of a complex-fluorophore conjugate does not automatically correspond to that of the complex alone.

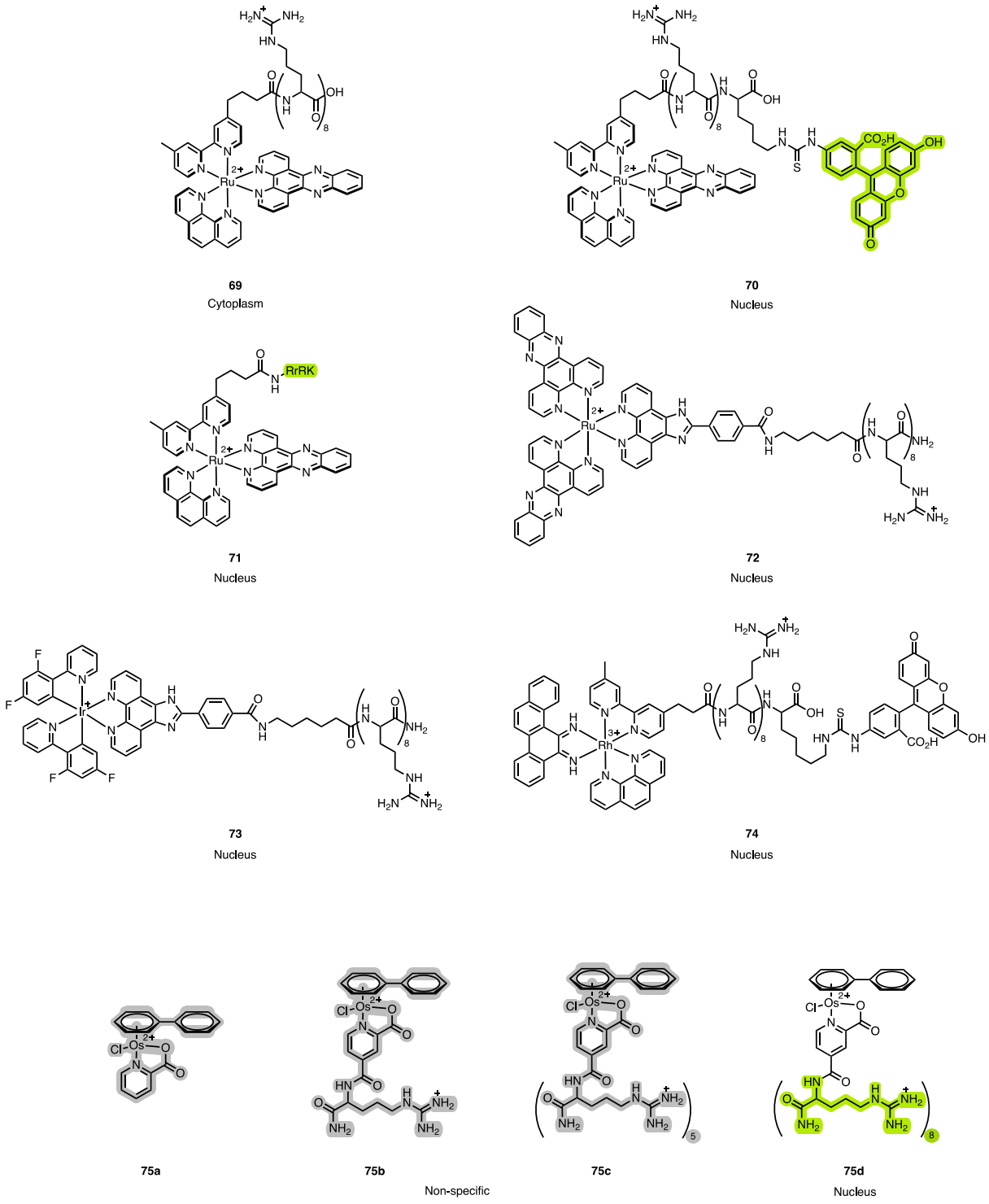


Figure 27. Peptide-conjugated metal complexes with preferential nuclear accumulation. In some cases, variations on the ligand or on the residues can enhance nuclear accumulation (highlighted in green) compared to other conjugates that remain with a non-specific subcellular localization (highlighted in grey).

It was also shown that the number of arginine residues can influence the cellular and nuclear uptake of conjugates. Sadler and co-workers observed with osmium(II) conjugates that the amount of osmium internalized in the cell and localizing in the nucleus increased with the arginine chain length (**75a < 75b < 75c < 75d; Figure 27**).[175] Puckett and Barton described a similar phenomenon when comparing the cellular and nuclear uptake of a ruthenium conjugate with a shorter peptide (RrRK) (**71; Figure 27**) to a ruthenium octaarginine conjugate (**69; Figure 27**).[176] The reduction of the overall charge of the complex linked to the reduction of the number of arginine residues in the peptide might play a role in this modulation. Interestingly, membrane potential has been shown to be an important factor in the cellular uptake of guanidium-rich peptides.[177] Reduction of the charge results in the partial loss of the membrane potential as a driving force for the entry of conjugates. Interestingly, when cells are incubated with a higher concentration of the conjugate **71** (**Figure 27**), the use of a shorter peptide appear to facilitate nuclear localization. It is thought to happen because it is less likely to interfere with the binding of the complex with DNA. Worthy of note, the same cellular uptake enhancing properties are not observed for oligolysines, that enter cells less effectively than oligoarginines despite bearing the same charge. In addition to the charge, the nature of the amino acids seems to play a big part in the ability of these peptides to vectorize a cargo molecule.

CPPs derived from the native hormone human calcitonin (hCT) have also been identified as potent delivery systems by Schatzschneider and co-workers. Conjugation to a cymantrene complex resulted in nuclear accumulation that could not be previously achieved by separate components (**76; Figure 28**).[178] Moreover, the resulting bioconjugate showed a significant cytotoxicity compared to both compounds separately. Human serum albumin (HSA) was shown to be useful as well as a carrier to facilitate cellular and nuclear entry of an iridium(III) complex. Sadler and co-workers observed that upon conjugation to HSA, complex **78** accumulated specifically in the nucleus compared to the unconjugated complex **77** that distributed in both cytoplasm and nucleus (**Figure 28**).[179] Intriguingly, rather than the whole conjugate, only the iridium complex part seemed to penetrate inside the nucleus; suggesting that HSA acts as a carrier up to the nuclear membrane but is released before entry. This is made possible by the reversible nature of the reaction between the thiol of the cysteine and the maleimide substituent of the ligand.

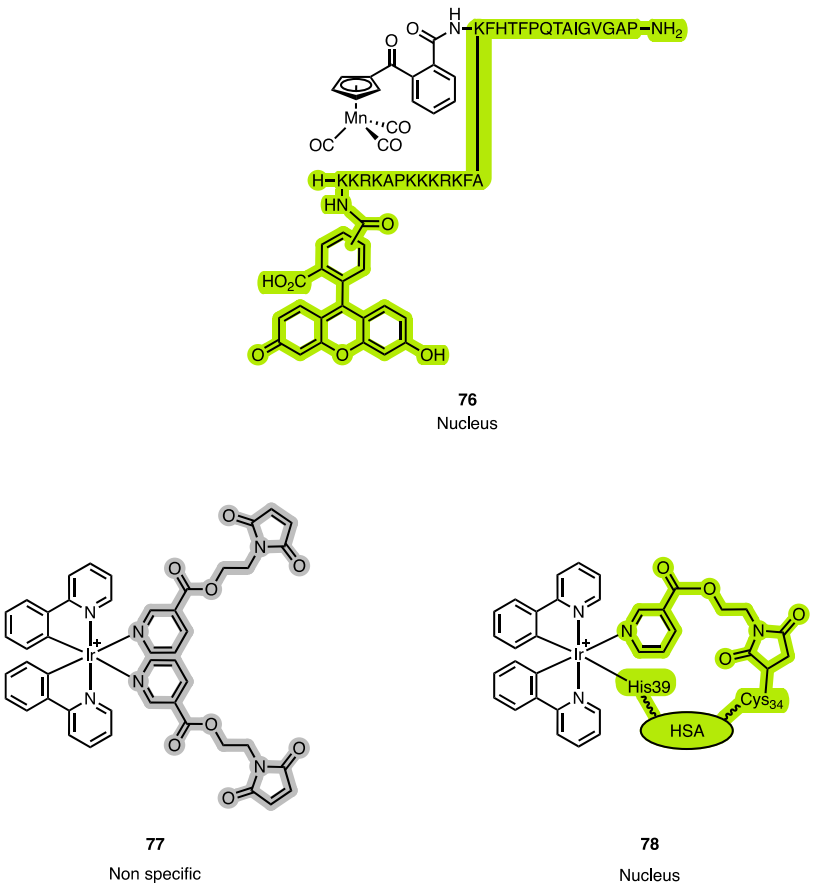


Figure 28. Conjugation to CPPs (highlighted in green) can help achieve nuclear accumulation.

Other short sequences were isolated from nuclear proteins and identified as essential for their importation into the nucleus. These sequences called nuclear localization signals (NLS) are present on proteins that need to be transported to the nucleus. The NLS are recognized by specific nuclear transporters to mediate nuclear entry through NPCs.[77] Several pathways have been identified involving different karyopherins, among them importin-α, importin-β and transportin-1.[180] Binding to these karyopherins represents an interesting strategy for intranuclear transport of drugs. Moreover, karyopherins have been shown to be overexpressed in multiple tumors and identified as interesting therapeutic targets. The design of new karyopherin substrates thus appears as a valuable strategy for the development of new antitumoral therapies.[181] The great diversity and complexity of signals recognized by karyopherins make the prediction of new substrates an arduous task. Nonetheless, efforts have been put towards the identification of rules for recognition of NLS by these specific proteins, to facilitate the design of new sequences.[180,182]

The binding abilities of NLS to karyopherins make them ideal candidates as vectors to bring various cargos inside the nucleus of cells. Moreover, these signals can be located at almost any part of a protein sequence, suggesting the possibility to add them at various positions of a bioconjugate.[183]

One of the first NLS identified is derived from the replicative helicase of the simian virus 40 (SV40), called the large T antigen, and which is responsible for virus replication. This NLS is comprised of seven amino acids (PKKKRKV) and has been reported to bring various cargos to the nucleus of cells.[184,185,81] It was subsequently used in various metal complex bioconjugates for that purpose. It was found to enhance nuclear uptake of Re(I) and ^{99m}Tc complexes by Alberto and co-workers (**79a-b; Figure 29**), a feature that could not be achieved by the sole presence of a DNA-intercalating pyrene as a ligand.[186] Metzler-Nolte and co-workers showed that conjugation of this NLS to cobalt or iron metallocenes effectively delivered the resulting bioconjugates to the nucleus of cells, regardless of charge (**80a-b; Figure 29**).[187,188] Moreover, the use of a NLS can be coupled with the presence of other relevant biomolecules. It was the case for cobalt-NLS conjugates bearing an additional peptide nucleic acid (PNA) sequence for DNA-targeting. Not only the cobalt-NLS conjugate **81** displayed an enhanced accumulation in the nucleus, but cobalt-PNA and furthermore cobalt-PNA-NLS also displayed a tremendous increase in nuclear accumulation (**82, 83; Figure 29**).[189] Similarly, Maecke and co-workers reported the synthesis of ^{111}In-labeled somatostatin-based bioconjugates bearing the SV40 large T antigen sequence, which also displayed a great increase in nuclear accumulation (**84; Figure 29**).[190]

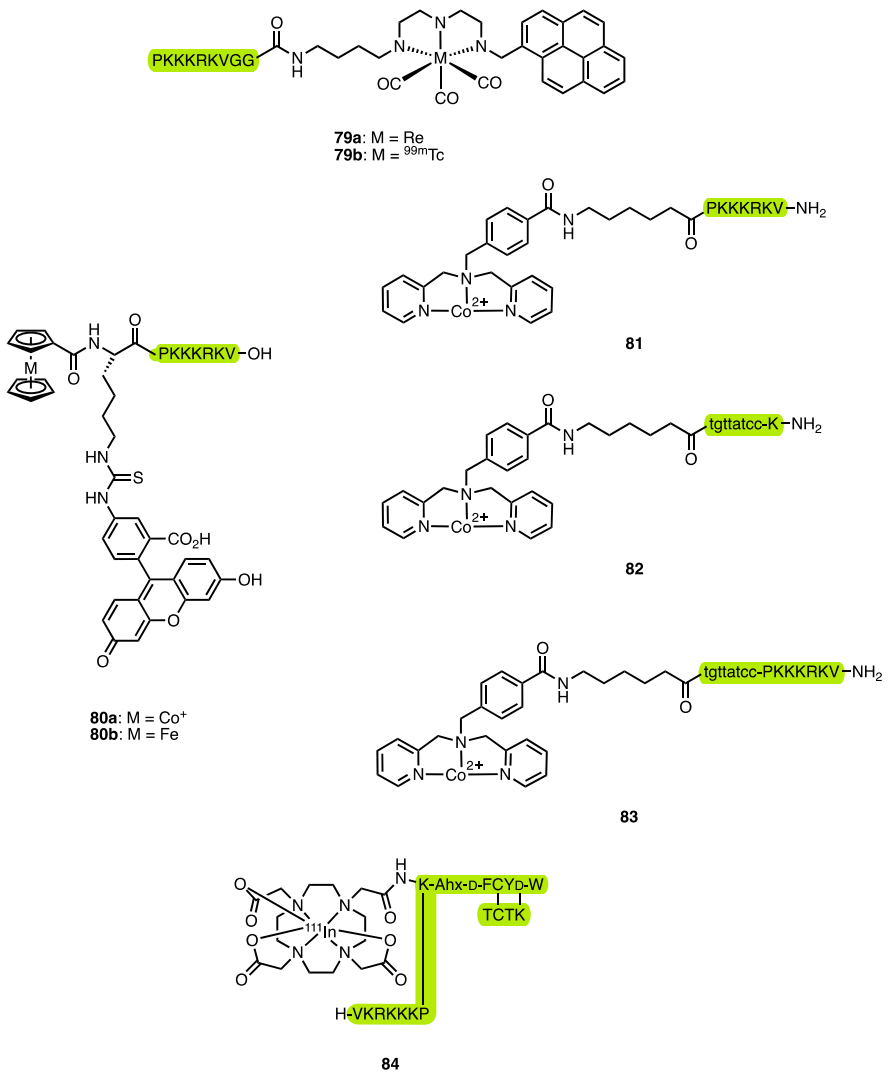
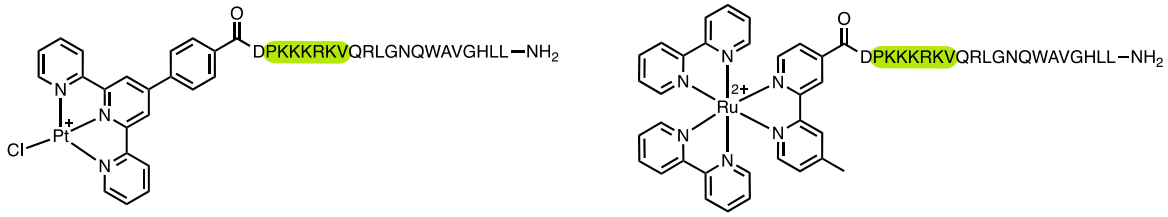


Figure 29. The introduction of NLS and PNA (highlighted in green) enhanced the nuclear accumulation of metal complexes.

Reilly and co-workers incorporated the seven amino acid sequence into a 13-residue peptide that retained nuclear-localizing properties and was shown to bring ^{111}In-antibody conjugates to the nucleus of cancer cells, effectively improving their radiotoxicity.[191–193] The use of that same peptide was also shown to be efficient to increase the nuclear accumulation of other ^{111}In- or ^{99m}Tc-labeled recombinant vascular endothelial growth factor (VEGF) bioconjugates, although this effect seemed to depend on the cell type. For cells over-expressing VEGF receptors, the translocation to the nucleus seemed to be prevalently obtained by the binding of VEGF to its receptor. Whereas for normal cells, the presence of NLS was responsible for the nuclear accumulation.[194] Similarly, Massaguer and co-workers incorporated the SV40 large T antigen NLS sequence to a bombesin derivative to improve the nuclear uptake of platinum

and ruthenium complexes (**85, 86; Figure 30**), to achieve nuclear targeting in addition to cancer cells targeting.[195] In that case, the presence of the NLS appeared to be the sole responsible for the enhanced nuclear accumulation. Heckl and Vogel also reported the nuclear accumulation of a gadolinium conjugate thanks to a combination of NLSs from the SV40 large T antigen and the protein ALL-1. This conjugate is, to the best of our knowledge, the first example of a nucleus-targeting gadolinium complex that could be used *in vivo* for imaging purposes.[196]



85 86

Figure 30. The introduction of NLS from the SV40 large T antigen (highlighted in green) enhanced the nuclear accumulation of metal complexes .

Other NLS derived from different proteins were used, notably derived from the HIV Tat peptide or the transcription factors NF-κB and c-Myc. Pedraza-López and co-workers synthetized a radiolabeled ^{99m}Tc-Tat-bombesin derivative (**87; Figure 31**) that effectively enhanced nuclear accumulation compared to the ^{99m}Tc-bombesin conjugate.[197] Keyes and co-workers reported the use of the NF-κB NLS sequence to bring various polypyridyl ruthenium(II) to the nucleus (**88-90; Figure 31**).[198,199] It is interesting to note that whilst the presence of a NLS did allow conjugates to breach the nuclear envelope, their intranuclear distribution pattern varied according to complexes' differences in ligands. Pope and co-workers showed that the addition of a c-Myc-derived NLS to a nuclear-impermeable iridium(III) could redirect it to the nucleus of cells (**91; Figure 31**).[200]

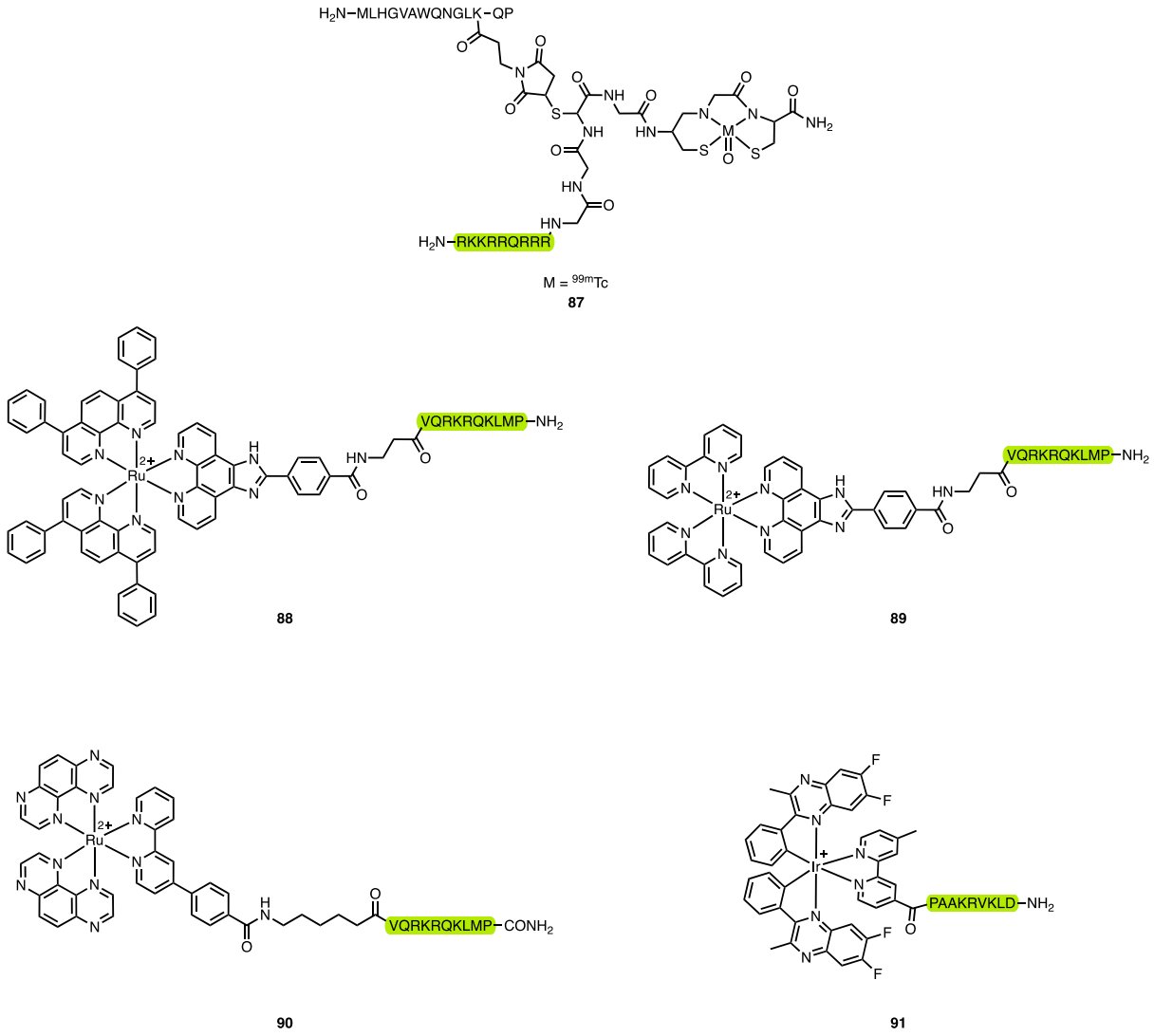


Figure 31. The introduction NLS derived from HIV Tat peptide, or transcription factors NF-κB or c-Myc (highlighted in green) enhanced the nuclear accumulation of metal complexes.

Previous works done in our group report the use of a short NLS for nuclear accumulation of a rhenium(I)-based PS (**92; Figure 32**);[201] as well as its use in metal-based pro-drugs, where conjugation of rhenium(I) or ruthenium(II) complexes to this NLS using an appropriate linker resulted in enhanced nuclear accumulation. Once in the nucleus, they could subsequently be released upon light irradiation to exert their cytotoxic action (**93, 94; Figure 32**).[202,203] This pro-drug approach represents a strategy to damage only cancer cells, without the use of a targeting vector.

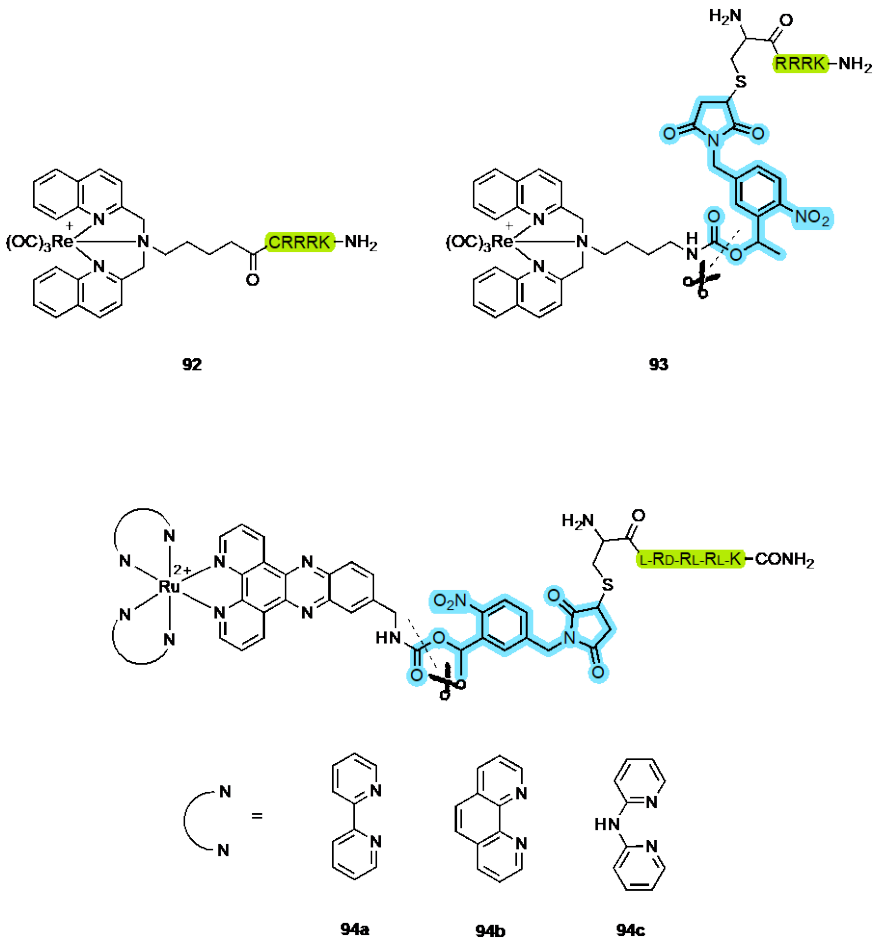


Figure 32. The introduction of NLS (highlighted in green) on rhenium(I) and ruthenium(II) complexes enhanced their nuclear accumulation. The addition of a linker (highlighted in cyan) allowed for the photo-triggered release of the complexes (the site of cleavage is indicated with a dashed line).

Nonetheless, the use of NLS must not be considered as a magic bullet to bring metal complexes to the nucleus. The sole presence of a NLS on a conjugate does not equate to systematic nucleus accumulation; the main obstacle residing in the necessity for the NLS to be in the cytoplasm to be recognized and brought to the nucleus.[204] A non-endocytic pathway of entry or endosomal escape thus becomes a requirement for the NLS-conjugate strategy to work. Conjugation to another biomolecule facilitating cellular entry and cytoplasmic localization is one efficient way to circumvent this issue, as was done in aforementioned works from Maecke and co-workers with somatostatin analogues, Reilly and co-workers with VEGF receptors substrates, and Massaguer with bombesin derivatives.[190,192–195]

Overall, bioconjugation represents a powerful and straightforward tool to facilitate the nuclear distribution of metal complexes in target cells. As highlighted in this part, they can serve multiple purposes: binding specifically to cancer cells, increase cellular uptake, bringing complexes inside the nucleus. In some cases, they could even be combined to achieve multiple of these effects. However, as they introduce important modifications on complexes, one must keep in mind that their biological effect might be impacted. Moreover, if the mode of action of a complex involves in-cell reactions, its conjugation to a biomolecule changing its subcellular localization or time of residence in the cell could yield very different results than the complex alone. For instance, in the case of carboplatin analogues conjugated with a NLS, Gibson and co-workers observed a decrease in the formation of DNA-adducts and a subsequent lower cytotoxicity, despite a higher quantity of complexes in the nucleus of cancer cells, suggesting that cytosolic activation was necessary for these complexes.[205] This highlights the many parameters that must be considered to refine the efficacy of metal-based drugs by modulating their cellular distribution.

3.3. Nuclear targeting of metal complexes using nanomedicine

In recent years, the application of nanotechnology for the diagnosis, treatment and of diseases, *i.e.*, nanomedicine, has brought significant advances in the medical field and further extended the diagnostic and therapeutic arsenal. Nanomedicine is a large field that includes various categories of nanoscale technologies such as biological mimetics, biomaterials or sensors, notably. In this work, our attention will be focused on nanocarriers such as nanoparticles (NPs), and their use for targeted drug delivery. Interested readers will be able to find more information on other technologies for cancer therapy in relevant literature reviews.[206–211]

NPs have emerged as attractive vectors for targeted drug delivery of antitumoral drugs thanks to their good solubility, bioavailability, lower renal excretion and supposedly selective accumulation in tissues subjected to the enhanced permeability and retention (EPR) effect, such as tumors.[212,213] Therapeutic agents can be thus encapsulated, covalently attached or adsorbed onto NPs to carry them to their target tissue and limit unspecific tissue uptake leading to side effects. Moreover, formulation with NPs also allows for nuclear delivery of encapsulated molecules. They are notably used as valuable non-viral carriers to deliver genetic material in gene therapy.[214] Currently, several NPs are used in clinics in cancer therapy, and many other nanomedicine formulations are currently undergoing pre-clinical and clinical trials.[215–217]

In this context, the development of tumor selective, nucleus-targeting nanocarriers for advanced therapeutics have become a hot topic in recent years. Thus, various NPs were reported as potent nucleus-targeting drug delivery systems, notably *via* surface modifications. These NPs could be synthetized using different materials and with different shapes. Lamprecht and co-workers reported for instance the synthesis of nucleus-targeting chitosan NPs modified with NLS (CPKKKRKV) for the nuclear delivery of encapsulated proteins.[218] Other works from Shi and co-workers and Qiao and co-workers reported successful tumor cell nuclear targeting with surface modified mesoporous silica NPs, conjugated with targeting ligands and NLS, respectively.[219,220] In both cases, these formulations resulted in the improved nuclear delivery of doxorubicin. Moreover, metal-based NPs, which will be discussed in more depth in a later part, as well as some polymeric NPs were also reported as potential formulations for the nuclear delivery of drugs. To the best of our knowledge, most of these systems have yet to be used for the encapsulation and nuclear delivery of metal complexes. Nonetheless, their potency with other drug payloads such as doxorubicin suggests that their use with therapeutic metal complexes could greatly improve nuclear accumulation. A detailed tutorial review by Shi and co-workers summarizes the most recent advances and discusses future perspectives in this field, which should be of great interest for readers working towards the design of tumor cell nucleus-targeting drug delivery nanosystems.[221]

Just like small-molecule drugs and nucleic acids, metal complexes can also be encapsulated in nanocarriers for selective delivery to the nucleus of cancer cells. A few works report the use of this strategy with polymeric NPs or DNA cages to encapsulate ruthenium, iridium or rhenium complexes. DNA cages are nanometer-scale polyhedral structures formed from the self-assembly of synthetic oligonucleotides. Tetrahedral DNA cages have been investigated by Tuberfield and co-workers for delivery of macromolecules into living cells and were found to accumulate in the cytoplasm.[222] Chen and co-workers used the excellent biocompatibility and robust structure of these tetrahedral DNA cages to load ruthenium(II) and iridium(III) polypyridyl complexes with antitumoral activity and bring them to the nucleus of cancer cells (**Figure 33**).[223,224] The DNA cages were conjugated with either biotin or with aptamers to achieve cancer cell selectivity and nuclear accumulation, respectively. Intercalation of the metal complexes into the DNA structure improved the drug loading and limited the undesirable release in the blood circulation. Interestingly, the loading of ruthenium(II) complexes on biotin-

conjugated DNA cages afforded a nuclear localization that was not present with the DNA cages alone.

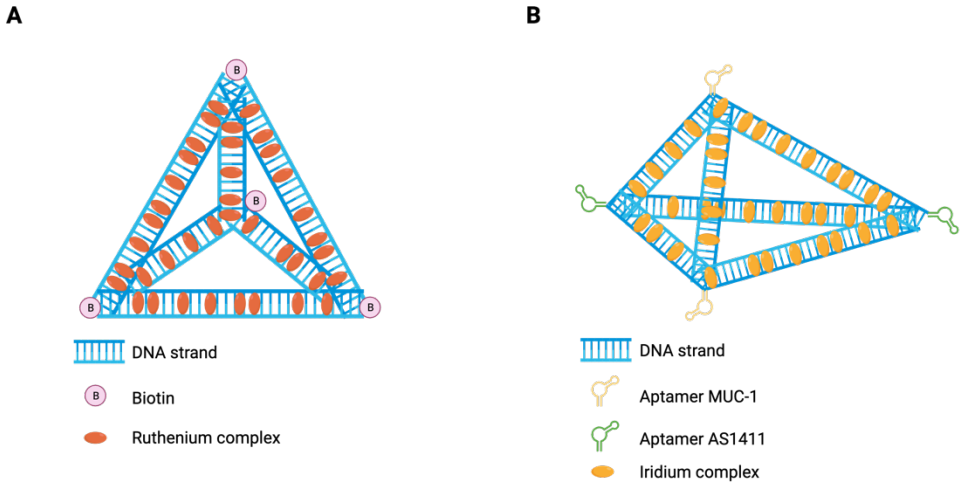


Figure 33. **(A)** Structure of a tetrahedral DNA cage conjugated with biotin and loaded with an antitumoral ruthenium(II) complex. Conjugation of the biotin served for cancer cells selectivity. Figure adapted from Huang et al., *Biomaterials*, **2016**,*103*,183-196 with permission.[223] Copyright 2016, Elsevier. **(B)** Structure of a tetrahedral DNA cage conjugated with aptamers MUC-1 and AS1411 and loaded with an antitumoral iridium(III) complex. Conjugation to aptamers MUC-1 and AS1411 served for cancer cells selectivity and nuclear targeting, respectively. Figure adapted from Tian et al. *Chem. Commun.*, **2018**,*54*, 9394-9397 with permission.[224] Copyright 2018, Royal Society of Chemistry.

Using a different kind of structure, Zeng and co-workers reported the synthesis of NPs prepared from amphiphilic co-polymers PDMAEMA-b-poly(PEGM)-b-PDMAEMA to encapsulate rhenium(I) complexes (**Figure 34**).[225] These co-polymers are made of blocks that are reported to be potent gene transfection vectors, hence their good cellular and nuclear membrane permeability.[226] Although these NPs were found throughout the whole cell, their structure offers the possibility to functionalize their surface. Such structures could therefore be envisioned for specific nuclear accumulation of NPs encapsulating metal complexes.

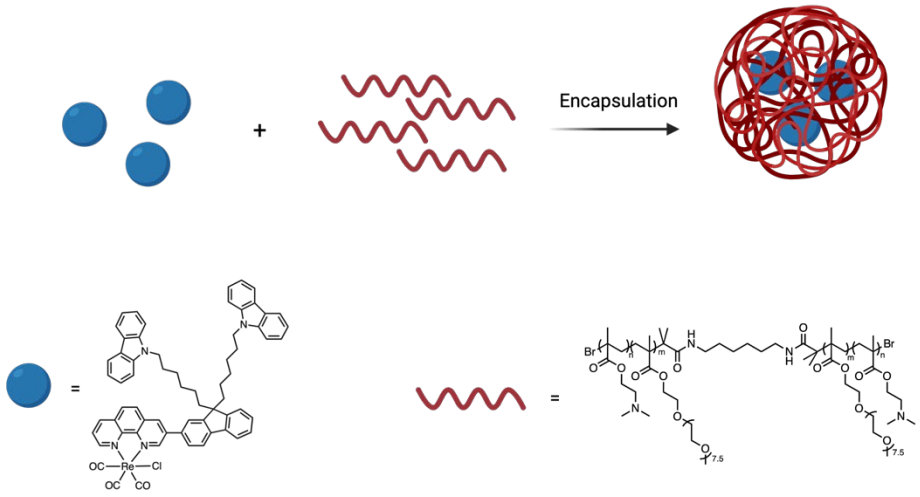


Figure 34. Structure of polymeric NPs encapsulating rhenium(I) complexes.

Vallis and co-workers used this surface-modification strategy with poly lactic-*co*-glycolic acid (PLGA) NPs encapsulating DNA-intercalating ruthenium(II) complexes (**Figure 35A**).[227] They introduced DTPA-hEGF (DTPA = diethylenetriaminepentaacetic acid, hEGF = human epidermal growth factor) to the surface of NPs for radiolabeling and to target EGF receptor (EGFR)-overexpressing cancer cells, respectively. The resulting ^{111}In-radiolabeled NPs were effectively able to target cancer cells overexpressing EGFR and co-deliver ^{111}In and ruthenium(II) complexes, which resulted in enhanced therapeutic effects in esophageal cancer cells *in vitro*. It is worth noting that only ruthenium complexes were found to accumulate specifically in the nucleus upon release from the NPs, likely due to their DNA-binding properties. In contrast, ^{111}In was mostly accumulating into the cytoplasm, along with the NPs upon release of the ruthenium(II) complex (**Figure 35B**). Nonetheless, the formulation displayed a cancer-cell selective, synergistic cytotoxic effect of ^{111}In and ruthenium(II) complexes.

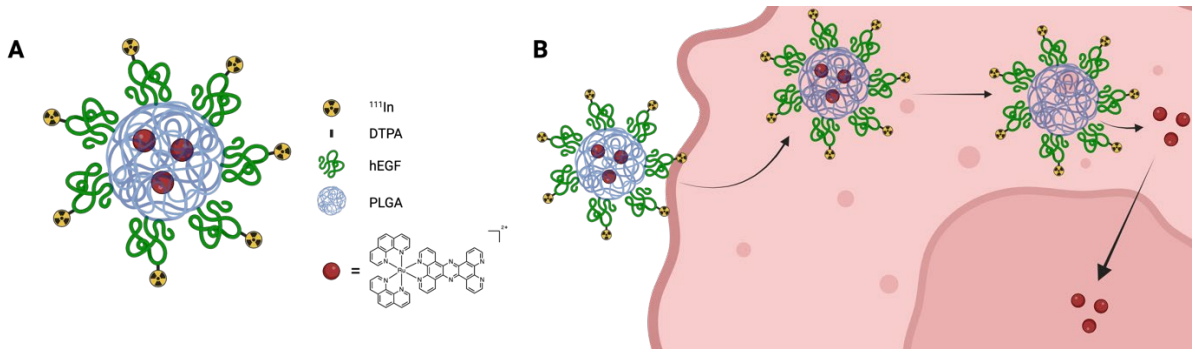


Figure 35. (A) Structure of radiolabeled PLGA NPs modified with DTPA-hEGF and encapsulating ruthenium(II) complexes**. (B)** Schematic illustration of the release and nuclear accumulation of ruthenium(II) complexes in the nucleus of cancer cells.

Similarly, Xiao and co-workers reported the synthesis of ethyl 2,6-diisocyanatohexanoate polymeric chains functionalized with oxaliplatin, a PS and a CPP R^8K (**Figure 36**).[228] Within an aqueous solution, these functionalized polymer chains assembled into spherical NPs, effectively encapsulating the oxaliplatin moieties. These NPs were found to accumulate in the nucleus of cancer cells, then upon irradiation, the platinum(IV) metal center of oxaliplatin was reduced to platinum(II) whilst the axially coordinated ligands were released (**Figure 36**). Cell death was triggered by the formation of DNA adducts, as well as the generation of ROS upon irradiation.

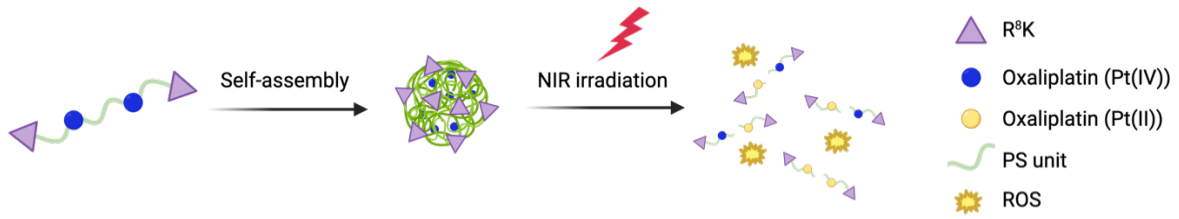


Figure 36. Structure of oxaliplatin(IV) conjugated polymers and mechanism of release upon NIR irradiation. The NIR irradiation triggers the PSs units, leading to ROS production and reduction from platinum(IV) to platinum(II). Figure adapted from Wei et al., *Angew. Chem. Int. Ed.* **2022**,*61*,e202201486.[228]

Chen and co-workers used the same strategy with PEG-benzoic imine-oligo-*L*-lysine copolymers functionalized with iridium(III) complexes that assemble in large NPs.[229] These large NPs are then conjugated to folate for cancer cells selectivity. Upon internalization by endocytosis and exposure to the acidic environment of endosomes or lysosomes, the PEG chains are released and polymers-iridium(III) complex conjugates assemble in smaller NPs. These smaller NPs, which display oligo-*L*-lysines at their surface for nuclear-targeting, can escape to the cytosol and accumulate into the nucleus where the iridium(III) complex is released.

In addition to aforementioned polymeric and DNA-based NPs, metal-based NPs are another type of formulation that is considered as attractive for targeted drug delivery. Moreover, they

are also investigated due to their interesting photothermal properties. Photothermal Therapy (PTT) is a non-invasive therapeutic strategy to treat cancers that relies on the conversion of light energy into heat upon NIR laser irradiation. This leads to an increase of temperature in the surrounding tissue that subsequently triggers cell death.[230] Metal-based nanomaterials, such as gold nanomaterials, quantum dots or metal oxide NPs, have been extensively investigated for this purpose, as some of them possess adequate properties such as good NIR absorbance, high photothermal efficiency and photostability. In contrast with other antitumoral therapies, PTT does not induce cell death by interacting with precise molecular targets, but rather relies on the higher heat sensitivity of cancer cells. The use of metal complexes and metal-based NPs for PTT alone thus falls out of the scope of nuclear targeting. An interested reader will found recent advances on nanomaterial-mediated PTT in a relevant review work from Han and Choi.[231] However, the photothermal properties of metal-based NPs remain interesting for the design of new multimodal treatments. Gold NPs for instance, have been recently investigated for PTT alongside drug delivery and other medical applications such as imaging, and radiosensitization.[232–236]

The use of gold NPs for cancer cells targeted delivery of antitumoral drugs, among other biomedical applications, is well known.[237] Among metal-based NPs, gold NPs represent a particularly attractive formulation due to their straightforward synthesis pathway, encapsulation properties and biocompatibility.[238] Thus, the research on gold NPs and other gold nanomaterials is very dynamic, in hopes to refine their use as new formulations.

Geometry and size of the gold nanomaterials seem to play an essential role in their intracellular fate. Stochaj and co-workers notably observed that gold nanoflowers, small and large gold nanospheres each possessed different distribution patterns depending on the cell type.[239] This observation is in accordance with works from Gaus and co-workers on other NPs made from block co-polymers, which suggests that shape of nanocarriers modulates the type of intracellular translocation they will go through.[240] For nanoparticles, it was found that smaller gold NPs (< 10 nm) displayed a higher accumulation in monolayer cells and a deeper penetration in tumor spheroids compared to larger gold NPs (> 10 nm).[241] This translated as well in tumor tissue, *in vivo*, with higher accumulations for smaller gold NPs after a single intravenous injection to tumor-bearing mice. Moreover, gold NPs smaller than 10 nm could penetrate in the nucleus of cancer cells *in vitro* whereas larger gold NPs remained solely in the cytoplasm where they formed aggregates. Encouragingly, ultrasmall gold NPs of a 2 nm size

were also found to be susceptible to cross the blood-brain barrier in 3D brain spheroid models, opening up the possibility to use them as carriers for tumors hardly accessible by conventional drugs.[242]

In addition to these size considerations, surface-modification of gold NPs has emerged as an efficient strategy to bring them specifically to the nucleus. Various gold NPs-peptide conjugates have been reported as nucleus-targeting agents using NLS or a combination of different peptides. The targeting strategy resembles what is described in part 3.2., with specific peptide sequences enhancing cellular or nuclear uptake upon recognition by relevant molecular actors. In most cases, the use of a NLS was the most efficient way to achieve nuclear accumulation.[243–247] So far, these strategies have mostly been used on gold NPs bearing no metal complexes. Nonetheless, they represent interesting approaches for the nuclear delivery of metal complexes that could be either encapsulated or conjugated on the gold NPs. To this end, more information on surface-modification of gold NPs can be found in other relevant works.[235,248–250]

To the best of our knowledge, encapsulation of metal complexes in gold NPs for targeted delivery has yet to be reported, although it holds great potential. However, conjugation of metal complexes on the surface of gold NPs has been reported. Wheate and co-workers synthetized platinum-tethered gold NPs that were able to enter the nuclei of cancer cells.[251] The gold NPs displayed a significantly higher cellular uptake compared to the parent, non-conjugated metal complex oxaliplatin, and were spotted in the nucleus. This resulted in an enhanced cytotoxic effect of the oxaliplatin-conjugated GNP in several cancer cell lines. Torres-García and co-workers synthetized gold NPs conjugated with bombesin and a NLS, that were subsequently radiolabeled with ^{99m}Tc or ^{177}Lu. The resulting NPs were internalized in the nucleus of GRPR overexpressing cancer cells and exhibited remarkable cytotoxic effects. This high cytotoxicity on cancer cells was achieved after laser irradiation on cells, through a combination of targeted radiotherapy, provided by radionuclides, and PTT, provided by the photothermal properties of gold NPs.[252]

Besides gold NPs, other metal-based NPs can accumulate specifically to the nucleus following surface-modification. For instance, Woloschak and co-workers reported the use of oligonucleotides to modulate the cellular distribution of TiO_2 NPs, with oligonucleotides matching either mitochondrial or nucleolar DNA being specifically retained in these respective organelles.[253] Surface modifications of metal-based NPs for biomedical applications and

targeted drug delivery are still an emerging field of research, nonetheless, some reviews have reported existing strategies and future perspectives for these formulations.[250,254,255]

Overall, the use of nanotechnologies for the targeted delivery of metal-based compounds appears as an emerging field that holds a lot of potential due to the great variety of existing strategies. Encapsulation of complexes in NPs could represent a reliable alternative to bioconjugates when conjugation to the biomolecule is susceptible to hinder the effect of a metal complex. In addition, the use of conjugated NPs represents a particularly promising strategy to provide selectivity whilst combining advantages of NPs. Research on metal-based drugs and nanotechnologies are both still quite recent, which explains the lower number of reported examples compared to the strategies described in parts 3.1. and 3.2. Nonetheless, it is a growing and dynamic field that can offer new onsets and broaden horizons for the nuclear targeting of metal-based drugs, particularly with the possibility of creating multifunctional and multitargeted systems.

4. Future perspectives of nucleus-targeting metal complexes

Resistance mechanisms and drug systemic toxicity are the most crucial challenges cancer therapies must overcome. In addition to the emergence of resistance mechanisms such as the overexpression of efflux proteins or increased DNA damage repair,[256] conventional chemotherapies often face insufficient specificity, leading to the apparition of tremendous side effects. Targeted therapies and personalized therapies have thus emerged as a dynamic field of research to provide patients with tailored, efficient, and safer treatments. Thanks to breakthrough discoveries in molecular biology on cancer biomarkers and the growing knowledge on the different tumor cells phenotypes, many targeted therapies have entered clinical trials, and some are currently used in the clinics.[257]

In parallel, the understanding of molecular mechanisms involved in the cytotoxic and antiproliferative properties of some compounds has uncovered another realm of possibilities to improve treatments' specificity. Knowledge of precise intracellular targets and effectors gives way to more accurate targeting and new mechanisms of action, particularly in the field of metal-based drugs that can be used in various ways owing to their remarkable physico-chemical properties. To translate into effective clinical improvements, new metal-based drugs must then comply to two-factors specificity: cancer cell and organelle. In this part, we will discuss the

perspectives of metal-based compounds mentioned in this work, in light of these two layers of specificity.

When developing unconjugated metal complexes, there are no clear parameters to predict whether they will specifically target cancer cells. Thus, one of the difficulties encountered when using unconjugated complexes such as those described in part 3.1. is accurate cancer cells targeting and sparing of healthy tissues. Some of them specifically target cancer cells by virtue of their mechanism of action rather than by specific accumulation: they distribute indiscriminately in cancer and healthy tissues, but their biological effect is exacerbated in the former. This is the case for metallo-insertors that show an enhanced cytotoxicity towards MMR-deficient cancer cells,[109,115] or G-quadruplex DNA stabilizers which can inhibit the activity of upregulated telomerases.[124] Thus, it is no surprise to find that such metallodrugs are currently being investigated as interesting candidates.[258] It is worth mentioning the interesting interplay that can exist between the subcellular localization of a complex and its cancer cells selectivity, illustrated by the work of Barton and co-workers on rhodium(III) metallo-insertors. In this study, the rhodium(III) metallo-insertors lost their specific activity towards MMR-deficient cells upon losing their nuclear accumulation.[115] Although it is the sole example of such a phenomenon, it seems essential to bear in mind this possibility when exploring the scope of a metal complex, as it can have a huge impact in the clinics.

Other similar strategies, going forward, could include the targeting of specific, critical regions of DNA. Small molecules that interact specifically with adenine-thymine base pairs (AT), such as bizelesin, have for instance been shown to target abnormal AT rich islands displayed by some tumorigenic cell phenotypes.[259] Furthermore, precise DNA sequences can now be targeted using triplex-forming nucleotides (TFOs), that bind through the major groove to form a somewhat stable triplex DNA structure.[260] Studies are currently ongoing to improve the stability of these TFOs in physiological conditions, as they hold great potential to open a pathway for the selective delivery of compounds that interact with DNA, notably metal complexes.[261]

Most of the other works focused their cytotoxicity studies on cancer cell lines only and give poor inconclusive indications on a potential selectivity. To circumvent this issue, strategies relying on the unique properties of some metal complexes have been developed, in which the metal complexes are specifically activated in tumor tissues using external stimuli (**Figure 37**).

The leading strategy is the use of PDT which relies on the photoactivation of a compound leading to reactive oxygen species (ROS) production, which can, induce damages to DNA. This way, specific cancer cell death can be achieved by shining light at an adequate wavelength at the tumor. Examples have shown that bringing PSs closer to duplex DNA could enhance their cytotoxic activity.[105,119,120,262] It is also worth noting that other organelles can be interesting targets when using PDT, notably mitochondria. Hence the existence of mitochondria-targeted complexes as well as dual-damage PSs that can localize in more than one organelle, all of which showed enhanced cytotoxic activity.[116,156] Other strategies include taking advantage of the properties of the tumor environment, notably the hypoxic conditions of cancer cells, to activate complexes *via* ligand-exchange for example.[135] The injection of a product directly within the tumor tissue to activate the drug could also be envisioned, although the use would be limited to solid, easily accessible tumors that have not metastasized.[136]

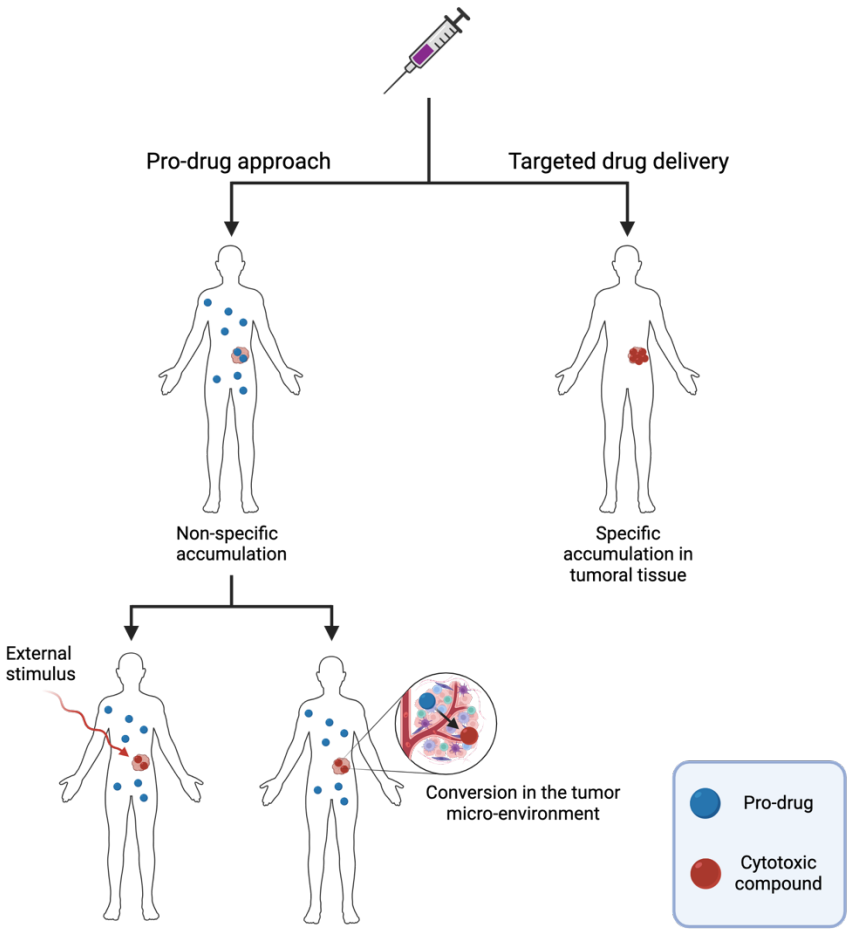


Figure 37. Schematic representation of possible approaches for cancer cell selectivity of nuclear-targeting metal complexes.

As more complex systems are developed such as those described in parts 3.2. and 3.3., it is possible to increase cancer cell targeting, in addition to nuclear targeting, by using relevant biomolecules and/or nanosystems (**Figure 37**). Conjugation to biomolecules known to target cancer cells such as antibodies or ligands of overexpressed receptors (*e.g.,* GRPR, EGFR) is a common strategy to achieve such a feature. Clinical benefits to this approach do not need to be proven anymore, as targeted therapy is emerging as a better alternative for the comfort and health of patients. Nonetheless, it is interesting to discuss added benefits that could arise from an additional nuclear targeting. Similar to what is reported in part 3.1. with metal complexes exerting their cytotoxic activity through DNA-binding, a direct positive correlation can be found between nuclear uptake and cytotoxicity in some cases. Although this effect must not prevail over cancer cell selectivity, its addition to tumor-selective complexes could yield particularly efficient treatments. Examples include osmium-polyarginine conjugates synthetized by Sadler and co-workers that displayed enhanced cytotoxicity as nuclear accumulation increased,[175] and other complexes inflicting direct or indirect damage to DNA such as PSs or Auger emitters.[179,186,191–195,197]

As mentioned in part 3.2., introduction of a great number of modifications for both tumor-cell selectivity and nuclear specificity to a conjugate is not always guaranteed with success. This was illustrated eloquently by Alberto and co-workers on ^{99m}Tc and rhenium-based conjugates with acridine orange and bombesin.[263] The presence of both moieties allowed for a specific uptake in targeted cells, but without any nuclear entry. Nuclear specificity was only achieved with complexes bearing only acridine orange on their ligand, however with loss of cancer cell selectivity. To circumvent this issue, the possibility of an intracellular cleavage leading to the release of the metal complex from bombesin was envisioned. The idea of cleavable linkers to release metal complexes opened a new set of possibilities, not only with bulky bioconjugates, but also pro-drugs that could be activated either by external triggers or in the tumor environment (**Figure 38**). This strategy was notably used in our group with the selective, photo-induced uncaging of rhenium and ruthenium complexes within the nucleus, which allowed for a decrease in toxicity in healthy cells compared to tumor cells.[202,203] Other triggers can be used such as the properties of the tumor micro-environment which is usually hypoxic and acidic, and many groups have started to investigate this subject.[264] Although reports of subsequent nuclear targeting are scarce, this outlines the potential of stimuli-responsive release of metal complexes in cancer therapeutics.

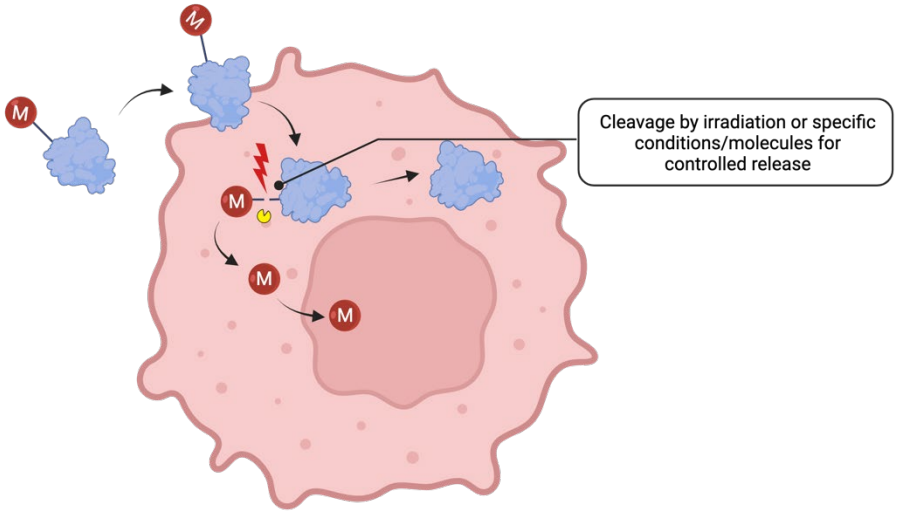


Figure 38. Schematic illustration of the controlled release of metal complexes following linker cleavage by irradiation or specific conditions / molecules in tumor cells / tumor microenvironment.

In light of all the works reported in this review, it appears that the introduction of chemical modifications on ligands of metal complexes can greatly increase their nuclear accumulation. Moreover, metal complexes are reported to be internalized in most cases through non-endocytic pathways, eliminating the issue of endosomal entrapment. It is however more difficult to provide tumor selectivity to these complexes in addition to nuclear affinity. Conjugation to vectors, on the other hand, allows for a better tumor selectivity, although sometimes to the detriment of nuclear accumulation. The introduction of biomolecules has been reported to influence subcellular distribution, notably due to bulky size and different modes of cellular uptake, which can prevent previously nuclear-targeted metal complexes to accumulate in the nucleus upon bioconjugation.

Considering the problematics depicted above, the benefits of nanomedicine can be highlighted as they allow for more intricate, multifunctional systems that can resolve some of the aforementioned issues. NPs are already used and investigated as an innovative formulation to improve drugs solubility, distribution, uptake, and selectivity. The use of this formulation for cancer cell selectivity partially relies on the observation that some tumor tissues display a peculiar vasculature system without supportive tissues, which leads to the formation of leaky vessels. This vasculature system is also characterized by a poor lymphatic system, which disables the drain of the intratumoral components, thus increasing their accumulation. These postulates form the basis of the rationale for the phenomenon called the EPR effect. Owing to

this EPR effect, NPs are supposedly more likely to accumulate greatly in tumor tissue and release their payload specifically in cancer cells rather than healthy ones. However, it appeared in recent years that the basis for the EPR effect is still incompletely understood, and that great variations of this effect exist depending on the tumor type and size.[265,266] As a result, it is advised not to solely rely on the EPR effect for increased cancer tissue selectivity but rather, find ways to improve this selectivity by using other properties of NPs. Perspectives for cancer tissue targeting beyond the EPR effect are discussed in this pertinent review work by Greish and co-workers.[267]

In addition to discussions about selectivity, safety of use of NPs in the human body is often brought up as a concern when envisioning their use in medicine, especially concerning metal-based NPs. Many means have been deployed to characterize their potential toxicity and ways to make safer NPs for clinical use.[268] Notably, shape, size and undesirable production of ROS in healthy tissues have been identified as parameters influencing the toxicity of gold NPs,[241] or magnetite NPs,[269] for instance. Such a toxicity is not inherently problematic, especially when researching antitumoral treatments, although it must be limited to tumoral tissue. This reinforces the idea that NPs would largely benefit from a cancer cell and nucleus targeting design for a use in the clinics.

Therefore, surface-modification of NPs represents a valuable strategy, as it allows for the addition of targeting moieties to refine cancer cell selectivity and nuclear accumulation. As observed in the works mentioned in part 3.3., introduction of targeting moieties on the surface of NPs of different nature yielded very interesting results in terms of treatment selectivity and efficacy. Moreover, the possibility to encapsulate metal complexes in addition to surface-modification allows for the design of multimodal therapeutic agents, such as what was done by Vallis and co-workers on ^{111}In-labeled polymeric NPs encapsulating ruthenium(II) complexes.[227]

In the same way as metal complexes and bioconjugates, NPs could also benefit from the pro-drug approach to improve tumor selectivity whilst carrying a nucleus-targeted metal complex. Triggered release of a drug payload in response to the tumor microenvironment represents an attractive feature for nanomedicine. Thus, this strategy has become a hot topic over recent years, and some interesting results have come forward, notably applied to the delivery of platinum(IV) pro-drugs.

Xiao, Jing and co-workers pioneered this field with the synthesis of polymer MPEG-b-PCL-b-PLL and cisplatin(IV) conjugates that assembled into nano-micelles. In the tumor micro-environment, cisplatin(II) could be released from these nano-micelles following acidity-triggered hydrolysis, then reduction by the presence of reducing agents.[270] A similar strategy was exploited by Xiao, Saltzman and co-workers with reductive-responsive polymers for the delivery of various platinum drugs. The group reported the synthesis of disulfide containing polymers poly-(CHTA-co-HD)-PEG (poly(1,2,4,5-cyclohexanetetracarboxylic dianhydride-co-hydroxyethyl disulfide)-polyethylene glycol) which could assemble in NPs with oxaliplatin(IV) or 56MESS.[271] The presence of reductive agents in the tumor cell triggered the release of these drugs via reduction of the disulfide bonds.

Moreover, these NPs, combined with the advanced convection-enhanced delivery (CED) technology, were reported to bypass the blood-brain barrier to deliver the platinum drugs to the target brain area. On patient-derived glioblastoma cells and patient derived cancer models, this strategy was shown to overcome Temozolomide-resistant glioblastoma.

Release or activation of metal complexes payloads could also be achieved using an external stimulus, notably light irradiation. This strategy was reported by Xiao and co-workers with triblock polymers with main chains containing platinum(IV) prodrugs and near-infrared light responsive units, discussed in part 3.3. (**Figure 36**).[209] The resulting NPs were shown to accumulate in the nucleus of cancer cells, and undergo photo-reduction which lead to the concomitant release and activation of platinum(IV) pro-drugs. Going further, the group adopted the same strategy with other polymers, pseudo-conjugated to the platinum pro-drug, which also showed light-triggered release of the metal complexes.[272] The development of a light-controlled nanoplatform for the nuclear delivery of molecules as well as nanoscale materials was also reported by Wu and coworkers using self-assembled NPs comprised of a polyamine-containing polyhedral oligomeric silsesquioxane unit, a hydrophilic polyethylene glycol chain, and the photosensitizer rose Bengal.[273] These NPs displayed light-induced lysosomal escape and nuclear membrane disruption upon irradiation, promoting the nuclear delivery of various payloads in tumor cells in a light-controlled manner. These works open up the pathway to controlled-release of therapeutic payloads as a mean to improve tumor selectivity and could perfectly be envisioned for the delivery of nucleus-targeting metal complexes.

Overall, the use of nanocarriers appears as an interesting way to introduce tumor selectivity to the delivery of metal complexes whilst avoiding structure changes on the ligands of the complex to retain an optimal nuclear accumulation.

In light of all the works discussed in this part, it seems that bringing cytotoxic metal complexes to the nucleus would result in a significant improvement in therapeutic efficacy, particularly for compounds interacting with DNA. This statement thus puts the emphasis on finding new ways to properly address relevant metal-based compounds to the nucleus, where they could exert their optimum activity.

In the field of nuclear medicine notably, the accumulation of alpha particles or Auger electron-emitting metal-based radionuclides within the vicinity of the nucleus and close to DNA is of particular interest. Indeed, alpha particles and Auger electrons display a high linear energy transfer which is deposited in extremely short ranges (< 100 μM and 100 nm respectively), making their use very attractive for targeted radiotherapies.[274,275] As nuclear medicine represents an increasingly relevant tool in the therapeutic arsenal against cancer, with FDA-approved drugs such as Lutathera® , Pluvicto® and Xofigo® being progressively incorporated into treatment plans,[276–279] the search for appropriate strategies to improve the efficacy of drug candidates becomes a crucial issue.

Perspectives for tumor-specific, nucleus targeting approaches for metal complexes first involve the inclusion of new molecular targets and new vectors. In this regard, the search for new macromolecular vectors with enhanced selectivity and specificity is very dynamic, with the inclusion of aptamers as innovative alternatives to peptides and antibodies for high affinity binding to relevant proteins.[280] This new class of vectors presents many unique advantages such as their small size, low production cost and facile chemical modification compared to antibodies, whilst conserving a similar binding affinity for their target. It is thus not surprising to find an increasing number of works using aptamers, notably in the field of nanomedicine, where various aptamer-coated NPs were found to target cancer cells, and in some cases accumulating into the nucleus.[223,224,281,282] The instability of aptamers in human plasma is however still an issue, and the increase of their stability remains the main improvement axis on which research is focused.[283,284]

Stimuli or tumor micro-environment responsive metal-based compounds are also appearing as attractive strategies for tumor-specific targeting, which could be tailored to target the nucleus as well. As mentioned before, this strategy is already in use with metal complexes conjugates, but also described in more complex systems using nanotechnologies for the delivery of small-molecule drugs. It is realistic to point out that, as we gain a deeper understanding of molecular mechanisms that could be taken advantage of in cancer cells, this could translate in another way to bring metal complexes to their nucleus.

The use of non-peptidic nuclear vectors could be envisioned, as they could be readily incorporated on the ligands of the metal complexes. Other than pharmacophores known to interact with nucleic acids, examples of non-peptidic, organelle-targeting vectors are scarce, with the most widely recognized being the triphenylphosphonium moiety that is able to drive most molecules to the mitochondria. Nonetheless, a recent work by Rotello and co-workers described the use of boronate tags to bring proteins specifically to the nucleus of cells.[285] The presence of these boronate tags enhanced greatly the accumulation of the protein, *via* facilitated transport through NPCs. Although only described for proteins so far, the use of this tag could perfectly be envisioned for metal complexes and incorporated to various ligands.

In recent years, gene therapy has emerged as another valuable tool for the treatment of various diseases, including cancer.[286,287] Gene therapy involves the modulation of the expression of a gene for therapeutic purposes, with the help of various technologies to access nuclear DNA. Various types of vectors for gene delivery have been identified and developed, starting from viral vectors which have progressively been cast aside in favor of non-viral vectors due to safety concerns.[288,289] Interestingly, some metal complexes have been identified amongst these non-viral gene delivery vectors, due to their DNA-condensing properties.[290–293] For the sake of clarity, they were not mentioned in previous parts, as no clear parameter for their nuclear localization could be singled out. Other materials have been identified as potent vehicles for nuclear delivery of genes or other macromolecules, such as polymers, lipids, peptides and nanomaterials.[294,295] Considering the extensive experience accumulated in the research of gene therapies, and particularly the efforts put towards the design and development of new transfection vectors, this field appears as very promising to find new ways to bring metal complexes to the nucleus of cancer cells.

Overall, different directions can be taken to improve nuclear delivery of metal complexes, depending on the starting strategies depicted in parts 3.1, 3.2, and 3.3. For unconjugated metal complexes, the design of ligands bearing moieties that target either nucleic acids or nuclear transporters (*e.g.*, karyopherins) seems the ideal way to achieve nuclear accumulation. Tumoral tissue selectivity however must not be overlooked, and the development of means to trigger cytotoxicity specifically in cancer cells (*e.g.*, PDT) is a great strategy.

For bioconjugated metal complexes, the search of new vectors that could display both cancer cell selectivity and nuclear accumulation is the most interesting strategy. For instance, the identification of the nucleolar protein nucleolin (NCL) as a cancer biomarker expressed on the plasma membrane of cells, has given way to the design and use of NCL-specific vectors for nuclear targeting of cancer cells.[296–298]

In the field of NPs, encapsulation of metal complexes and surface-modification of NPs are holding a great potential, as well as NPs with controlled release. The association of multiple modalities has already shown promising results in some of the works described, such as the combinational approach adopted by Vallis and co-workers as well as Torres-García and co-workers mentioned in part 3.3.[109,252] The increasing use of these strategies is thus to be expected and should yield very interesting results. Additionally, the application of gene therapy strategies to the nuclear delivery of metal complexes should also give way to efficient nuclear targeting.

5. Conclusion

Several problematics have been highlighted in this review as important to take in consideration when designing new metal-based compounds destined to be delivered to the nucleus of cancer cells. First, parameters influencing the subcellular localization for unconjugated complexes were identified, the most prominent being ligands backbones and the lipophilicity of the complexes. Ligands with a nuclear affinity, notably linked to interactions with nucleic acids, are expected to facilitate nuclear accumulation. Different non-covalent binding modes of metal complexes can be responsible for this affinity for DNA, notably groove-binding, metallo-intercalation, and metallo-insertion. As for lipophilicity, the trend points towards the fact that more hydrophilic compounds tend to locate in the nucleus whereas lipophilic counterparts tend to accumulate in the mitochondria although a more systematic study

would be required to confirm it. Changes in the conformation and stereochemistry of the metal complexes are also likely to influence binding affinity and/or the binding mode to nucleic acids, which can reverberate on the subcellular distribution of complexes. Other parameters can influence subcellular localization such as size and charge of the complex: bulkier complexes are expected to remain outside of the nucleus, and positively charged complexes are more likely to display better cellular and nuclear accumulation.

Some of these observations translate into the other types of metal-based compounds described in this review, particularly the influence of size and charge. Bioconjugation and the use of nanosystems can avoid some of the other issues by appending moieties that will lead complexes to specific cells and organelles. The use of peptide sequences for cancer cells targeting, facilitated cellular uptake or nuclear localization is well-represented in this work, as they represent robust, well-known strategies. Still, one must not discard the use of other vectors that can hold their own advantages over peptides, such as oligonucleotides. Overall, the combination of targeting moieties seems to yield encouraging results, be it for bioconjugates but also NPs.

A common problematic resides in the mode of cellular uptake, which is the main determinant of a compound's fate in the cell. To be relocated to the nucleus, compounds must be first released in the cytoplasm. In the case of an endocytic uptake pathway, compounds are first internalized in endosomes, from which they must then escape. Cellular uptake through non-endocytic pathways is therefore desirable for nucleus targeting compounds. It is noteworthy that even for metal complexes that are described to enter cells through non-endocytic pathways, conjugation to certain macromolecules can influence their mode of cellular uptake. CPPs have been described as more likely to be internalized by endocytosis, for instance, and are therefore not ideal.[169,299] This information can be exploited to pick the adequate kind of macromolecular vector for nuclear delivery. Alternatively, strategies towards endosomal escape are currently being investigated.[300–302]

Overall, it seems that a multimodal, combined approach holds the most chances of success in the tumor-specific, nuclear targeting delivery of metal-based compounds. Results tend particularly towards the use of bioconjugates and nanomedicine, which is not surprising as they exhibit great potential for applications in targeted therapies. Concerning nanomedicine, the use of more intricate systems seems to allow for more leverage to target the nucleus of cancer cells and escape resistance mechanisms. However, the accumulation of modifications can prevent

clinical translation of the nanodrugs. Indeed, the more complex a system becomes, the more issues it can face, notably in cost and production but also to assess pre-clinical efficacy and introduce it in clinical studies. Although more nanodrugs have been introduced to the market in recent years, there still remain regulatory challenges to their approval and clinical use, which are touched on by Vitorino and co-workers.[303] It seems thus preferable to focus on simpler, straightforward systems that will be more likely to be efficient in a clinical setting. As mentioned in part 4, exploitation of the knowledge accumulated in the field of gene therapy appears as one of the most promising strategies.

In this review, we have tried to emphasize existing as well as promising strategies used to bring metal complexes to the nucleus of cancer cells to strive towards safer, more efficient therapies. As resistance against antitumoral metal compounds is rising, particularly towards platinum-based drugs, the development of novel metallodrugs with various mechanisms of action steadily appears as an attractive therapeutic alternative. The great versatility in the biological activity of metal complexes is a valuable weapon but also a double-edged sword, with the development of novel, unpredictable resistance mechanisms by tumor cells under a pression of selection.[304] However, with the variety of targets that metallodrugs can act upon, it becomes less and less likely that tumors would develop cross-resistance to all of these compounds. Combination strategies with different metal drugs could thus be envisioned for synergistic effects. Because there are only a few resistance mechanisms specific to nucleus-targeting metal complexes, designing such compounds becomes even more valuable in the never-ending race against drug resistance. It is hoped that the clinical benefits that can advent from cancer specific, nucleus targeting of metal-based compounds will encourage research towards this objective.

Conflicts of interest:

The authors declare no conflicts of interest.

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References

[1] J.-C. G. Bünzli, *Chem. Lett.* **2009**, *38*, 104.
[2] N. Drude, L. Tienken, F. M. Mottaghy, *Methods* **2017**, *130*, 14.
[3] C. Hu, S. Xu, Z. Song, H. Li, H. Liu, *Chemosensors* **2023**, *11*, 125.
[4] K. K.-W. Lo, in *Photofunctional Transition Metal Complexes* (Ed.: V. W. W. Yam), Springer Berlin Heidelberg, Berlin, Heidelberg, **2006**, pp. 205–245.
[5] M. A. Jakupec, M. S. Galanski, V. B. Arion, C. G. Hartinger, B. K. Keppler, *Dalton Trans.* **2008**, 183.
[6] G. Süss-Fink, *Dalton Trans.* **2010**, *39*, 1673.
[7] W. Han Ang, P. J. Dyson, *Eur. J. Inorg. Chem.* **2006**, *2006*, 4003.
[8] D. Chen, V. Milacic, M. Frezza, Q. Dou, *CPD* **2009**, *15*, 777.
[9] P. C. A. Bruijnincx, P. J. Sadler, *Curr Opin Chem Biol* **2008**, *12*, 197.
[10] G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3.
[11] Y. K. Yan, M. Melchart, A. Habtemariam, P. J. Sadler, *Chem. Commun.* **2005**, 4764.
[12] A. G. Weidmann, A. C. Komor, J. K. Barton, *Comments on Inorganic Chemistry* **2014**, *34*, 114.
[13] R. P. Perez, *European Journal of Cancer* **1998**, *34*, 1535.
[14] K. Qiu, Y. Chen, T. W. Rees, L. Ji, H. Chao, *Coordination Chemistry Reviews* **2019**, *378*, 66.
[15] L. Xie, R. Guan, T. W. Rees, H. Chao, in *Advances in Inorganic Chemistry*, Elsevier, **2020**, pp. 287–337.
[16] A. C. Komor, J. K. Barton, *Chem. Commun.* **2013**, *49*, 3617.
[17] Q. Zhao, C. Huang, F. Li, *Chem. Soc. Rev.* **2011**, *40*, 2508.
[18] D.-L. Ma, H.-Z. He, K.-H. Leung, D. S.-H. Chan, C.-H. Leung, *Angew. Chem. Int. Ed.* **2013**, *52*, 7666.
[19] M. R. Gill, J. A. Thomas, *Chem. Soc. Rev.* **2012**, *41*, 3179.
[20] G. M. Cooper, *The Cell: A Molecular Approach*, ASM Press [U.A.], Washington, DC, **2000**.
[21] *Molecular Biology of the Cell*, Garland Science, Taylor And Francis Group, New York, **2015**.
[22] K. Cheung-Ong, G. Giaever, C. Nislow, *Chemistry & Biology* **2013**, *20*, 648.
[23] J. L. Delgado, C.-M. Hsieh, N.-L. Chan, H. Hiasa, *Biochemical Journal* **2018**, *475*, 373.
[24] S. Venugopal, V. Sharma, A. Mehra, I. Singh, G. Singh, *Chem Biol Drug Des* **2022**, *100*, 580.
[25] C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc., Perkin Trans. 2* **2001**, 651.
[26] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737.
[27] S. Neidle, *Nucleic Acid Structure and Recognition*, Oxford University Press, Oxford New York, **2002**.

[28] R. E. Franklin, R. G. Gosling, *Nature* **1953**, *172*, 156.
[29] A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. Van Boom, G. Van Der Marel, A. Rich, *Nature* **1979**, *282*, 680.
[30] J. Fohrer, M. Hennig, T. Carlomagno, *Journal of Molecular Biology* **2006**, *356*, 280.
[31] A. Jaworski, W. Zacharias, W.-T. Hsieh, J. A. Blaho, J. E. Larson, R. D. Wells, *Gene* **1988**, *74*, 215.
[32] D. Karati, K. R. Mahadik, P. Trivedi, D. Kumar, *ACAMC* **2022**, *22*, 1478.
[33] S. Ghosh, *Bioorganic Chemistry* **2019**, *88*, 102925.
[34] F. R. Keene, J. A. Smith, J. G. Collins, *Coordination Chemistry Reviews* **2009**, *253*, 2021.
[35] L. P. Bignold, *Anticancer Res* **2006**, *26*, 1327.
[36] Z. H. Siddik, *Oncogene* **2003**, *22*, 7265.
[37] C. Metcalfe, J. A. Thomas, *Chem. Soc. Rev.* **2003**, *32*, 215.
[38] Antonino Lauria, Paola Barraja, Gaetano Dattolo, Anna Maria Almerico, *CMC* **2007**, *14*, 2136.
[39] K. Mišković, M. Bujak, M. Baus Lončar, L. Glavaš-Obrovac, *Archives of Industrial Hygiene and Toxicology* **2013**, *64*, 593.
[40] B. J. Pages, D. L. Ang, E. P. Wright, J. R. Aldrich-Wright, *Dalton Trans.* **2015**, *44*, 3505.
[41] M. Howe-Grant, S. J. Lippard, *Biochemistry* **1979**, *18*, 5762.
[42] H.-K. Liu, P. J. Sadler, *Acc. Chem. Res.* **2011**, *44*, 349.
[43] B. M. Zeglis, V. C. Pierre, J. K. Barton, *Chem. Commun.* **2007**, 4565.
[44] G. Lenglet, M.-H. David-Cordonnier, *Journal of Nucleic Acids* **2010**, *2010*, 1.
[45] K. Pors, L. Patterson, *CTMC* **2005**, *5*, 1133.
[46] L. Andrezálová, Z. Országhová, *Journal of Inorganic Biochemistry* **2021**, *225*, 111624.
[47] A. Mukherjee, W. D. Sasikala, in *Advances in Protein Chemistry and Structural Biology*, Elsevier, **2013**, pp. 1–62.
[48] T. G. A. Reuvers, R. Kanaar, J. Nonnekens, *Cancers* **2020**, *12*, 2098.
[49] B. Kaina, *Biochemical Pharmacology* **2003**, *66*, 1547.
[50] W. P. Roos, B. Kaina, *Trends in Molecular Medicine* **2006**, *12*, 440.
[51] W. J. Cannan, D. S. Pederson, *Journal Cellular Physiology* **2016**, *231*, 3.
[52] T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *JNCI Journal of the National Cancer Institute* **1998**, *90*, 889.
[53] D. Schaue, W. H. McBride, *Nat Rev Clin Oncol* **2015**, *12*, 527.
[54] S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd, S. Neidle, *Nucleic Acids Research* **2006**, *34*, 5402.
[55] E. H. Blackburn, C. W. Greider, E. Henderson, M. S. Lee, J. Shampay, D. Shippen-Lentz, *Genome* **1989**, *31*, 553.
[56] K. Dhaene, E. Van Marck, R. Parwaresch, *Virchows Arch* **2000**, *437*, 1.
[57] A. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J.-F. Riou, J.-L. Mergny, *Biochimie* **2008**, *90*, 131.
[58] Y. Qin, L. H. Hurley, *Biochimie* **2008**, *90*, 1149.
[59] A. Rangan, O. Yu. Fedoroff, L. H. Hurley, *Journal of Biological Chemistry* **2001**, *276*, 4640.
[60] H. Fernando, A. P. Reszka, J. Huppert, S. Ladame, S. Rankin, A. R. Venkitaraman, S. Neidle, S. Balasubramanian, *Biochemistry* **2006**, *45*, 7854.
[61] A. Siddiqui-Jain, C. L. Grand, D. J. Bearss, L. H. Hurley, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11593.
[62] R. Nazar, *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)* **2004**, *56*, 457.
[63] M. Tsekrekou, K. Stratigi, G. Chatzinikolaou, *IJMS* **2017**, *18*, 1411.

[64] M. S. Lindström, D. Jurada, S. Bursac, I. Orsolic, J. Bartek, S. Volarevic, *Oncogene* **2018**, *37*, 2351.
[65] C. Schöfer, K. Weipoltshammer, *Histochem Cell Biol* **2018**, *150*, 209.
[66] R. Y. L. Tsai, T. Pederson, *FASEB j.* **2014**, *28*, 3290.
[67] S. Dillinger, T. Straub, A. Németh, *PLoS ONE* **2017**, *12*, e0178821.
[68] S. Boulon, B. J. Westman, S. Hutten, F.-M. Boisvert, A. I. Lamond, *Molecular Cell* **2010**, *40*, 216.
[69] O. V. Iarovaia, E. P. Minina, E. V. Sheval, D. Onichtchouk, S. Dokudovskaya, S. V. Razin, Y. S. Vassetzky, *Trends in Cell Biology* **2019**, *29*, 647.
[70] F.-M. Boisvert, S. van Koningsbruggen, J. Navascués, A. I. Lamond, *Nat Rev Mol Cell Biol* **2007**, *8*, 574.
[71] M. Derenzini, D. Trerè, A. Pession, L. Montanaro, V. Sirri, R. L. Ochs, *Am J Pathol* **1998**, *152*, 1291.
[72] L. Montanaro, D. Treré, M. Derenzini, *The American Journal of Pathology* **2008**, *173*, 301.
[73] P. Carotenuto, A. Pecoraro, G. Palma, G. Russo, A. Russo, *Cells* **2019**, *8*, 1090.
[74] S. R. Wente, M. P. Rout, *Cold Spring Harbor Perspectives in Biology* **2010**, *2*, a000562.
[75] B. L. Timney, B. Raveh, R. Mironska, J. M. Trivedi, S. J. Kim, D. Russel, S. R. Wente, A. Sali, M. P. Rout, *Journal of Cell Biology* **2016**, *215*, 57.
[76] D. Cowburn, M. Rout, *Biochemical Society Transactions* **2023**, *51*, 871.
[77] J. Lu, T. Wu, B. Zhang, S. Liu, W. Song, J. Qiao, H. Ruan, *Cell Commun Signal* **2021**, *19*, 60.
[78] E. Hébert, *Biology of the Cell* **2003**, *95*, 59.
[79] N. Panté, M. Kann, *MBoC* **2002**, *13*, 425.
[80] D. H. M. Dam, K. S. Culver, P. N. Sisco, T. W. Odom, *Therapeutic Delivery* **2012**, *3*, 1263.
[81] Y. Sun, L. Xian, H. Xing, J. Yu, Z. Yang, T. Yang, L. Yang, P. Ding, *Journal of Drug Targeting* **2016**, *24*, 927.
[82] M. F. Attia, N. Anton, J. Wallyn, Z. Omran, T. F. Vandamme, *Journal of Pharmacy and Pharmacology* **2019**, *71*, 1185.
[83] X. Qin, H. Zhang, X. Xing, P. Wang, J. Yan, D. Liu, Q. Gong, R. Zhang, H. Zhang, *Materials & Design* **2022**, *221*, 110999.
[84] B. Sharma, V. Kumar, *J. Med. Chem.* **2021**, *64*, 16865.
[85] C. A. Vock, W. H. Ang, C. Scolaro, A. D. Phillips, L. Lagopoulos, L. Juillerat-Jeanneret, G. Sava, R. Scopelliti, P. J. Dyson, *J. Med. Chem.* **2007**, *50*, 2166.
[86] R. F. Vitor, I. Correia, M. Videira, F. Marques, A. Paulo, J. Costa Pessoa, G. Viola, G. G. Martins, I. Santos, *ChemBioChem* **2008**, *9*, 131.
[87] H.-C. Becker, B. Nordén, *J. Am. Chem. Soc.* **1999**, *121*, 11947.
[88] M. R. Duff, V. K. Mudhivarthi, C. V. Kumar, *J. Phys. Chem. B* **2009**, *113*, 1710.
[89] I. Ott, X. Qian, Y. Xu, D. H. W. Vlecken, I. J. Marques, D. Kubutat, J. Will, W. S. Sheldrick, P. Jesse, A. Prokop, C. P. Bagowski, *J. Med. Chem.* **2009**, *52*, 763.
[90] P. J. Barnard, S. J. Berners-Price, *Coordination Chemistry Reviews* **2007**, *251*, 1889.
[91] S. J. Berners-Price, A. Filipovska, *Metallomics* **2011**, *3*, 863.
[92] C. P. Bagowski, Y. You, H. Scheffler, D. H. Vlecken, D. J. Schmitz, I. Ott, *Dalton Trans.* **2009**, 10799.
[93] H. Scheffler, Y. You, I. Ott, *Polyhedron* **2010**, *29*, 66.
[94] S. Banerjee, E. B. Veale, C. M. Phelan, S. A. Murphy, G. M. Tocci, L. J. Gillespie, D. O. Frimannsson, J. M. Kelly, T. Gunnlaugsson, *Chem. Soc. Rev.* **2013**, *42*, 1601.
[95] A. Citta, E. Schuh, F. Mohr, A. Folda, M. L. Massimino, A. Bindoli, A. Casini, M. P. Rigobello, *Metallomics* **2013**, *5*, 1006.

[96] B. Bertrand, A. de Almeida, E. P. M. van der Burgt, M. Picquet, A. Citta, A. Folda, M. P. Rigobello, P. Le Gendre, E. Bodio, A. Casini, *Eur. J. Inorg. Chem.* **2014**, *2014*, 4532.
[97] T. Sarwar, S. U. Rehman, M. A. Husain, H. M. Ishqi, M. Tabish, *International Journal of Biological Macromolecules* **2015**, *73*, 9.
[98] S. A. Pérez, C. de Haro, C. Vicente, A. Donaire, A. Zamora, J. Zajac, H. Kostrhunova, V. Brabec, D. Bautista, J. Ruiz, *ACS Chem. Biol.* **2017**, *12*, 1524.
[99] E. Pereira, L. Do Quental, E. Palma, M. C. Oliveira, F. Mendes, P. Raposinho, I. Correia, J. Lavrado, S. Di Maria, A. Belchior, P. Vaz, I. Santos, A. Paulo, *Sci Rep* **2017**, *7*, 42544.
[100] S. Imstepf, V. Pierroz, P. Raposinho, M. Bauwens, M. Felber, T. Fox, A. B. Shapiro, R. Freudenberg, C. Fernandes, S. Gama, G. Gasser, F. Motthagy, I. R. Santos, R. Alberto, *Bioconjugate Chem.* **2015**, *26*, 2397.
[101] S. Imstepf, V. Pierroz, P. Raposinho, M. Felber, T. Fox, C. Fernandes, G. Gasser, I. R. Santos, R. Alberto, *Dalton Trans.* **2016**, *45*, 13025.
[102] V. Fernández-Moreira, F. L. Thorp-Greenwood, M. P. Coogan, *Chem. Commun.* **2010**, *46*, 186.
[103] U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wölfl, *ChemMedChem* **2008**, *3*, 1104.
[104] H. Huang, P. Zhang, B. Yu, Y. Chen, J. Wang, L. Ji, H. Chao, *J. Med. Chem.* **2014**, *57*, 8971.
[105] C. Mari, V. Pierroz, R. Rubbiani, M. Patra, J. Hess, B. Spingler, L. Oehninger, J. Schur, I. Ott, L. Salassa, S. Ferrari, G. Gasser, *Chem. Eur. J.* **2014**, *20*, 14421.
[106] J. Hess, H. Huang, A. Kaiser, V. Pierroz, O. Blacque, H. Chao, G. Gasser, *Chem. Eur. J.* **2017**, *23*, 9888.
[107] Y. Ding, Q. Wu, K. Zheng, L. An, X. Hu, W. Mei, *RSC Adv.* **2015**, *5*, 63330.
[108] A. N. Boynton, L. Marcélis, J. K. Barton, *J. Am. Chem. Soc.* **2016**, *138*, 5020.
[109] M. R. Gill, M. G. Walker, S. Able, O. Tietz, A. Lakshminarayanan, R. Anderson, R. Chalk, A. H. El-Sagheer, T. Brown, J. A. Thomas, K. A. Vallis, *Chem. Sci.* **2020**, *11*, 8936.
[110] M. R. Gill, J. Garcia-Lara, S. J. Foster, C. Smythe, G. Battaglia, J. A. Thomas, *Nature Chem* **2009**, *1*, 662.
[111] E. Baggaley, M. R. Gill, N. H. Green, D. Turton, I. V. Sazanovich, S. W. Botchway, C. Smythe, J. W. Haycock, J. A. Weinstein, J. A. Thomas, *Angew. Chem. Int. Ed.* **2014**, *53*, 3367.
[112] A. Wragg, M. R. Gill, D. Turton, H. Adams, T. M. Roseveare, C. Smythe, X. Su, J. A. Thomas, *Chem. Eur. J.* **2014**, *20*, 14004.
[113] A. Notaro, M. Jakubaszek, S. Koch, R. Rubbiani, O. Dömötör, É. A. Enyedy, M. Dotou, F. Bedioui, M. Tharaud, B. Goud, S. Ferrari, E. Alessio, G. Gasser, *Chem. Eur. J.* **2020**, *26*, 4997.
[114] A. Gandioso, A. Vidal, P. Burckel, G. Gasser, E. Alessio, *ChemBioChem* **2022**, *23*, DOI 10.1002/cbic.202200398.
[115] A. G. Weidmann, A. C. Komor, J. K. Barton, *Phil. Trans. R. Soc. A.* **2013**, *371*, 20120117.
[116] X. Tian, Y. Zhu, M. Zhang, L. Luo, J. Wu, H. Zhou, L. Guan, G. Battaglia, Y. Tian, *Chem. Commun.* **2017**, *53*, 3303.
[117] S. W. Botchway, M. Charnley, J. W. Haycock, A. W. Parker, D. L. Rochester, J. A. Weinstein, J. A. G. Williams, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16071.
[118] R. A. J. Smith, C. M. Porteous, A. M. Gane, M. P. Murphy, *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5407.

[119] Y. Zhong, H. Zhang, W. Liu, X. Zheng, Y. Zhou, Q. Cao, Y. Shen, Y. Zhao, P. Z. Qin, L. Ji, Z. Mao, *Chem. Eur. J.* **2017**, *23*, 16442.
[120] Y.-F. Zhong, H. Zhang, G. Mu, W.-T. Liu, Q. Cao, C.-P. Tan, L.-N. Ji, Z.-W. Mao, *Inorg. Chem. Front.* **2019**, *6*, 2817.
[121] J. E. Reed, A. A. Arnal, S. Neidle, R. Vilar, *J. Am. Chem. Soc.* **2006**, *128*, 5992.
[122] A. Arola-Arnal, J. Benet-Buchholz, S. Neidle, R. Vilar, *Inorg. Chem.* **2008**, *47*, 11910.
[123] N. H. Abd Karim, O. Mendoza, A. Shivalingam, A. J. Thompson, S. Ghosh, M. K. Kuimova, R. Vilar, *RSC Adv.* **2014**, *4*, 3355.
[124] A. Ali, M. Kamra, S. Roy, K. Muniyappa, S. Bhattacharya, *Bioconjugate Chem.* **2017**, *28*, 341.
[125] A. K. Saini, V. Sharma, P. Mathur, M. M. Shaikh, *Sci Rep* **2016**, *6*, 34807.
[126] N. Busto, M. C. Carrión, S. Montanaro, B. Díaz De Greñu, T. Biver, F. A. Jalón, B. R. Manzano, B. García, *Dalton Trans.* **2020**, *49*, 13372.
[127] X. Tian, Q. Zhang, M. Zhang, K. Uvdal, Q. Wang, J. Chen, W. Du, B. Huang, J. Wu, Y. Tian, *Chem. Sci.* **2017**, *8*, 142.
[128] D. Sun, Y. Liu, D. Liu, R. Zhang, X. Yang, J. Liu, *Chem. Eur. J.* **2012**, *18*, 4285.
[129] D. Sun, Y. Liu, Q. Yu, D. Liu, Y. Zhou, J. Liu, *Journal of Inorganic Biochemistry* **2015**, *150*, 90.
[130] E. J. New, D. Parker, *Org. Biomol. Chem.* **2009**, *7*, 851.
[131] E. J. New, D. Parker, D. G. Smith, J. W. Walton, *Current Opinion in Chemical Biology* **2010**, *14*, 238.
[132] J. Yu, D. Parker, R. Pal, R. A. Poole, M. J. Cann, *J. Am. Chem. Soc.* **2006**, *128*, 2294.
[133] R. Pal, D. Parker, *Org. Biomol. Chem.* **2008**, *6*, 1020.
[134] G.-L. Law, C. Man, D. Parker, J. W. Walton, *Chem. Commun.* **2010**, *46*, 2391.
[135] K. E. Prosser, D. Xie, A. Chu, G. A. MacNeil, B. R. Varju, R. T. Kadakia, E. L. Que, C. J. Walsby, *Chem. Eur. J.* **2021**, *27*, 9839.
[136] Q. Cao, J. Yang, H. Zhang, L. Hao, G.-G. Yang, L.-N. Ji, Z.-W. Mao, *Chem. Commun.* **2019**, *55*, 7852.
[137] D. Chao, S. Ni, *Sci Rep* **2016**, *6*, 26477.
[138] C. A. Puckett, J. K. Barton, *Biochemistry* **2008**, *47*, 11711.
[139] B.-Z. Zhu, X.-J. Chao, C.-H. Huang, Y. Li, *Chem. Sci.* **2016**, *7*, 4016.
[140] X.-J. Chao, M. Tang, R. Huang, C.-H. Huang, J. Shao, Z.-Y. Yan, B.-Z. Zhu, *Nucleic Acids Research* **2019**, *47*, 10520.
[141] C. Li, M. Yu, Y. Sun, Y. Wu, C. Huang, F. Li, *J. Am. Chem. Soc.* **2011**, *133*, 11231.
[142] C. Li, Y. Liu, Y. Wu, Y. Sun, F. Li, *Biomaterials* **2013**, *34*, 1223.
[143] J. Chen, Z. Luo, Z. Zhao, L. Xie, W. Zheng, T. Chen, *Biomaterials* **2015**, *71*, 168.
[144] M. Matson, F. R. Svensson, B. Nordén, P. Lincoln, *J. Phys. Chem. B* **2011**, *115*, 1706.
[145] L. C. Greaves, A. K. Reeve, R. W. Taylor, D. M. Turnbull, *The Journal of Pathology* **2012**, *226*, 274.
[146] J. S. Armstrong, *British J Pharmacology* **2006**, *147*, 239.
[147] L. E. Enslin, K. Purkait, M. D. Pozza, B. Saubamea, P. Mesdom, H. G. Visser, G. Gasser, M. Schutte-Smith, *Inorg. Chem.* **2023**, *62*, 12237.
[148] J. Li, L. Guo, Z. Tian, S. Zhang, Z. Xu, Y. Han, R. Li, Y. Li, Z. Liu, *Inorg. Chem.* **2018**, *57*, 13552.
[149] S. Mahadevan, M. Palaniandavar, *Bioconjugate Chem.* **1996**, *7*, 138.
[150] F. R. Svensson, J. Andersson, H. L. Åmand, P. Lincoln, *J Biol Inorg Chem* **2012**, *17*, 565.
[151] Z.-P. Zeng, Q. Wu, F.-Y. Sun, K.-D. Zheng, W.-J. Mei, *Inorg. Chem.* **2016**, *55*, 5710.
[152] X.-J. Chao, C.-H. Huang, M. Tang, Z.-Y. Yan, R. Huang, Y. Li, B.-Z. Zhu, *Nucleic Acids Research* **2023**, *51*, 3041.
[153] S. Gao, S. Wang, R. Fan, J. Hu, *Biomedicine & Pharmacotherapy* **2020**, *127*, 110114.

[154] L. A. Jelen, A. H. Young, J. M. Stone, *J Psychopharmacol* **2021**, *35*, 109.
[155] F. L. Thorp-Greenwood, V. Fernández-Moreira, C. O. Millet, C. F. Williams, J. Cable, J. B. Court, A. J. Hayes, D. Lloyd, M. P. Coogan, *Chem. Commun.* **2011**, *47*, 3096.
[156] K. Wang, L. Liu, G. Qi, X. Chao, W. Ma, Z. Yu, Q. Pan, Z. Mao, B. Liu, *Adv. Sci.* **2021**, *8*, 2004379.
[157] J. Gong, A. Chehrazi-Raffle, S. Reddi, R. Salgia, *j. immunotherapy cancer* **2018**, *6*, 8.
[158] F. Okano, T. Saito, Y. Minamida, S. Kobayashi, T. Ido, Y. Miyauchi, U. Wasai, D. Akazawa, M. Kume, M. Ishibashi, K. Jiang, A. Aicher, C. Heeschen, T. Yonehara, *Cancer Research Communications* **2023**, *3*, 640.
[159] D. Kryza, J. Wischhusen, M. Richaud, M. Hervieu, J. Sidi Boumedine, J. Delcros, S. Besse, T. Baudier, P. Laval, S. Breusa, E. Boutault, H. Clermidy, N. Rama, B. Ducarouge, M. Devouassoux-Shisheboran, J. Chezal, A. Giraudet, T. Walter, P. Mehlen, D. Sarrut, B. Gibert, *EMBO Mol Med* **2023**, *15*, e16732.
[160] S. Jiang, M. Richaud, P. Vieugué, N. Rama, J. Delcros, M. Siouda, M. Sanada, A. Redavid, B. Ducarouge, M. Hervieu, S. Breusa, A. Manceau, C. Gattolliat, N. Gadot, V. Combaret, D. Neves, S. Ortiz-Cuaran, P. Saintigny, O. Meurette, T. Walter, I. Janoueix-Lerosey, P. Hofman, P. Mulligan, D. Goldshneider, P. Mehlen, B. Gibert, *EMBO Mol Med* **2021**, *13*, e12878.
[161] M. Lelle, S. Kaloyanova, C. Freidel, M. Theodoropoulou, M. Musheev, C. Niehrs, G. Stalla, K. Peneva, *Mol. Pharmaceutics* **2015**, *12*, 4290.
[162] E. Figueras, A. Martins, A. Borbély, V. Le Joncour, P. Cordella, R. Perego, D. Modena, P. Pagani, S. Esposito, G. Auciello, M. Frese, P. Gallinari, P. Laakkonen, C. Steinkühler, N. Sewald, *Pharmaceutics* **2019**, *11*, 220.
[163] Z. Xu, D. Guo, Z. Jiang, R. Tong, P. Jiang, L. Bai, L. Chen, Y. Zhu, C. Guo, J. Shi, D. Yu, *European Journal of Medicinal Chemistry* **2019**, *183*, 111682.
[164] E. Rassy, L. Rached, B. Pistilli, *The Breast* **2022**, *66*, 217.
[165] T. Esteves, F. Marques, A. Paulo, J. Rino, P. Nanda, C. J. Smith, I. Santos, *J Biol Inorg Chem* **2011**, *16*, 1141.
[166] F. Vultos, C. Fernandes, F. Mendes, F. Marques, J. D. G. Correia, I. Santos, L. Gano, *ChemMedChem* **2017**, *12*, 1103.
[167] C. Fernandes, E. Palma, F. Silva, A. Belchior, C. I. G. Pinto, J. F. Guerreiro, H. M. Botelho, F. Mendes, P. Raposinho, A. Paulo, *IJMS* **2022**, *23*, 7238.
[168] G. Rádis-Baptista, *Toxins* **2021**, *13*, 147.
[169] M. Lindgren, M. Hällbrink, A. Prochiantz, Ü. Langel, *Trends in Pharmacological Sciences* **2000**, *21*, 99.
[170] E. Koren, V. P. Torchilin, *Trends in Molecular Medicine* **2012**, *18*, 385.
[171] C. A. Puckett, J. K. Barton, *J. Am. Chem. Soc.* **2009**, *131*, 8738.
[172] L. Cosgrave, M. Devocelle, R. J. Forster, T. E. Keyes, *Chem. Commun.* **2010**, *46*, 103.
[173] C. Dolan, R. D. Moriarty, E. Lestini, M. Devocelle, R. J. Forster, T. E. Keyes, *Journal of Inorganic Biochemistry* **2013**, *119*, 65.
[174] J. Brunner, J. K. Barton, *Biochemistry* **2006**, *45*, 12295.
[175] S. H. van Rijt, H. Kostrhunova, V. Brabec, P. J. Sadler, *Bioconjugate Chem.* **2011**, *22*, 218.
[176] C. A. Puckett, J. K. Barton, *Bioorganic & Medicinal Chemistry* **2010**, *18*, 3564.
[177] J. B. Rothbard, T. C. Jessop, R. S. Lewis, B. A. Murray, P. A. Wender, *J. Am. Chem. Soc.* **2004**, *126*, 9506.
[178] I. Neundorf, J. Hoyer, K. Splith, R. Rennert, H. W. Peindy N'Dongo, U. Schatzschneider, *Chem. Commun.* **2008**, 5604.
[179] P. Zhang, H. Huang, S. Banerjee, G. J. Clarkson, C. Ge, C. Imberti, P. J. Sadler, *Angew. Chem. Int. Ed.* **2019**, *58*, 2350.

[180] M. Marfori, A. Mynott, J. J. Ellis, A. M. Mehdi, N. F. W. Saunders, P. M. Curmi, J. K. Forwood, M. Bodén, B. Kobe, *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2011**, *1813*, 1562.
[181] A. Mahipal, M. Malafa, *Pharmacology & Therapeutics* **2016**, *164*, 135.
[182] B. J. Lee, A. E. Cansizoglu, K. E. Süel, T. H. Louis, Z. Zhang, Y. M. Chook, *Cell* **2006**, *126*, 543.
[183] B. Martoglio, B. Dobberstein, *Trends in Cell Biology* **1998**, *8*, 410.
[184] S. A. Adam, T. J. Lobl, M. A. Mitchell, L. Gerace, *Nature* **1989**, *337*, 276.
[185] Y. Yoneda, T. Semba, Y. Kaneda, R. L. Noble, Y. Matsuoka, T. Kurihara, Y. Okada, N. Imamoto, *Experimental Cell Research* **1992**, *201*, 313.
[186] P. Haefliger, N. Agorastos, A. Renard, G. Giambonini-Brugnoli, C. Marty, R. Alberto, *Bioconjugate Chem.* **2005**, *16*, 582.
[187] F. Noor, A. Wüstholz, R. Kinscherf, N. Metzler-Nolte, *Angewandte Chemie International Edition* **2005**, *44*, 2429.
[188] F. Noor, R. Kinscherf, G. A. Bonaterra, S. Walczak, S. Wölfl, N. Metzler-Nolte, *ChemBioChem* **2009**, *10*, 493.
[189] S. I. Kirin, I. Ott, R. Gust, W. Mier, T. Weyhermüller, N. Metzler-Nolte, *Angew. Chem. Int. Ed.* **2008**, *47*, 955.
[190] M. Ginj, K. Hinni, S. Tschumi, S. Schulz, H. R. Maecke, *J Nucl Med* **2005**, *46*, 2097.
[191] P. Chen, J. Wang, K. Hope, L. Jin, J. Dick, R. Cameron, J. Brandwein, M. Minden, R. M. Reilly, *J Nucl Med* **2006**, *47*, 827.
[192] D. L. Costantini, C. Chan, Z. Cai, K. A. Vallis, R. M. Reilly, *Journal of Nuclear Medicine* **2007**, *48*, 1357.
[193] D. L. Costantini, K. McLarty, H. Lee, S. J. Done, K. A. Vallis, R. M. Reilly, *J Nucl Med* **2010**, *51*, 1084.
[194] C. Chan, Z. Cai, R. Su, R. M. Reilly, *Nuclear Medicine and Biology* **2010**, *37*, 105.
[195] S. Barrabés, I. Ng-Choi, M. Á. Martínez, B. R. Manzano, F. A. Jalón, G. Espino, L. Feliu, M. Planas, R. de Llorens, A. Massaguer, *Journal of Inorganic Biochemistry* **2020**, *212*, 111214.
[196] S. Heckl, U. Vogel, *J Pharmacol Exp Ther* **2006**, *319*, 657.
[197] C. L. Santos-Cuevas, G. Ferro-Flores, E. L. Rojas-Calderón, R. García-Becerra, D. Ordaz-Rosado, C. Arteaga De Murphy, M. Pedraza-López, *Nuclear Medicine Communications* **2011**, *32*, 303.
[198] L. Blackmore, R. Moriarty, C. Dolan, K. Adamson, R. J. Forster, M. Devocelle, T. E. Keyes, *Chem. Commun.* **2013**, *49*, 2658.
[199] C. S. Burke, A. Byrne, Tia. E. Keyes, *J. Am. Chem. Soc.* **2018**, *140*, 6945.
[200] A. H. Day, M. H. Übler, H. L. Best, E. Lloyd-Evans, R. J. Mart, I. A. Fallis, R. K. Allemann, E. A. H. Al-Wattar, N. I. Keymer, N. J. Buurma, S. J. A. Pope, *Chem. Sci.* **2020**, *11*, 1599.
[201] A. Leonidova, V. Pierroz, R. Rubbiani, J. Heier, S. Ferrari, G. Gasser, *Dalton Trans.* **2014**, *43*, 4287.
[202] A. Leonidova, V. Pierroz, R. Rubbiani, Y. Lan, A. G. Schmitz, A. Kaech, R. K. O. Sigel, S. Ferrari, G. Gasser, *Chem. Sci.* **2014**, *5*, 4044.
[203] C. Mari, V. Pierroz, A. Leonidova, S. Ferrari, G. Gasser, *Eur. J. Inorg. Chem.* **2015**, *2015*, 3879.
[204] M. Sibrian-Vazquez, J. Ortiz, I. V. Nesterova, F. Fernández-Lázaro, A. Sastre-Santos, S. A. Soper, M. G. H. Vicente, *Bioconjugate Chem.* **2007**, *18*, 410.
[205] O. Aronov, A. T. Horowitz, A. Gabizon, M. A. Fuertes, J. M. Pérez, D. Gibson, *Bioconjugate Chem.* **2004**, *15*, 814.
[206] E. S. Kawasaki, A. Player, *Nanomedicine: Nanotechnology, Biology and Medicine* **2005**, *1*, 101.

[207] S. M. Moghimi, A. C. Hunter, J. C. Murray, *FASEB j.* **2005**, *19*, 311.
[208] K. Riehemann, S. W. Schneider, T. A. Luger, B. Godin, M. Ferrari, H. Fuchs, *Angew Chem Int Ed* **2009**, *48*, 872.
[209] J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad, *Nat Rev Cancer* **2017**, *17*, 20.
[210] R. Van Der Meel, E. Sulheim, Y. Shi, F. Kiessling, W. J. M. Mulder, T. Lammers, *Nat. Nanotechnol.* **2019**, *14*, 1007.
[211] C. Zhang, L. Yan, X. Wang, S. Zhu, C. Chen, Z. Gu, Y. Zhao, *Nano Today* **2020**, *35*, 101008.
[212] A. K. Iyer, G. Khaled, J. Fang, H. Maeda, *Drug Discovery Today* **2006**, *11*, 812.
[213] D. Kalyane, N. Raval, R. Maheshwari, V. Tambe, K. Kalia, R. K. Tekade, *Materials Science and Engineering: C* **2019**, *98*, 1252.
[214] J. Chen, Z. Guo, H. Tian, X. Chen, *Molecular Therapy - Methods & Clinical Development* **2016**, *3*, 16023.
[215] Y. (Chezy) Barenholz, *Journal of Controlled Release* **2012**, *160*, 117.
[216] J. M. Caster, A. N. Patel, T. Zhang, A. Wang, *WIREs Nanomed Nanobiotechnol* **2017**, *9*, e1416.
[217] A. Parodi, E. P. Kolesova, M. V. Voronina, A. S. Frolova, D. Kostyushev, D. B. Trushina, R. Akasov, T. Pallaeva, A. A. Zamyatnin, *IJMS* **2022**, *23*, 13368.
[218] S. N. Tammam, H. Me. Azzazy, H. G. Breitinger, A. Lamprecht, *Mol. Pharmaceutics* **2015**, *12*, 4277.
[219] L. Xiong, X. Du, F. Kleitz, S. Z. Qiao, *Small* **2015**, *11*, 5919.
[220] L. Pan, Q. He, J. Liu, Y. Chen, M. Ma, L. Zhang, J. Shi, *J. Am. Chem. Soc.* **2012**, *134*, 5722.
[221] L. Pan, J. Liu, J. Shi, *Chem. Soc. Rev.* **2018**, *47*, 6930.
[222] A. S. Walsh, H. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, *ACS Nano* **2011**, *5*, 5427.
[223] Y. Huang, W. Huang, L. Chan, B. Zhou, T. Chen, *Biomaterials* **2016**, *103*, 183.
[224] Y. Tian, Y. Huang, P. Gao, T. Chen, *Chem. Commun.* **2018**, *54*, 9394.
[225] L. Liu, X. Li, S. Hou, Y. Xue, Y. Yao, Y. Ma, X. Feng, S. He, Y. Lu, Y. Wang, X. Zeng, *Chem. Commun.* **2009**, 6759.
[226] P. Van De Wetering, E. E. Moret, N. M. E. Schuurmans-Nieuwenbroek, M. J. Van Steenbergen, W. E. Hennink, *Bioconjugate Chem.* **1999**, *10*, 589.
[227] M. R. Gill, J. U. Menon, P. J. Jarman, J. Owen, I. Skaripa-Koukelli, S. Able, J. A. Thomas, R. Carlisle, K. A. Vallis, *Nanoscale* **2018**, *10*, 10596.
[228] D. Wei, Y. Huang, B. Wang, L. Ma, J. Karges, H. Xiao, *Angew Chem Int Ed* **2022**, *61*, DOI 10.1002/anie.202201486.
[229] Y. Fan, C. Li, F. Li, D. Chen, *Biomaterials* **2016**, *85*, 30.
[230] J. Chen, C. Ning, Z. Zhou, P. Yu, Y. Zhu, G. Tan, C. Mao, *Progress in Materials Science* **2019**, *99*, 1.
[231] H. S. Han, K. Y. Choi, *Biomedicines* **2021**, *9*, 305.
[232] S. E. Skrabalak, L. Au, X. Lu, X. Li, Y. Xia, *Nanomedicine* **2007**, *2*, 657.
[233] A. Oyelere, *NSA* **2008**, *Volume 1*, 45.
[234] S. E. Skrabalak, J. Chen, Y. Sun, X. Lu, L. Au, C. M. Cobley, Y. Xia, *Acc. Chem. Res.* **2008**, *41*, 1587.
[235] D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, C. A. Mirkin, *Angewandte Chemie International Edition* **2010**, *49*, 3280.
[236] Y. Xia, W. Li, C. M. Cobley, J. Chen, X. Xia, Q. Zhang, M. Yang, E. C. Cho, P. K. Brown, *Acc. Chem. Res.* **2011**, *44*, 914.
[237] N. R. S. Sibuyi, K. L. Moabelo, A. O. Fadaka, S. Meyer, M. O. Onani, A. M. Madiehe, M. Meyer, *Nanoscale Res Lett* **2021**, *16*, 174.

[238] R. Shukla, V. Bansal, M. Chaudhary, A. Basu, R. R. Bhonde, M. Sastry, *Langmuir* **2005**, *21*, 10644.
[239] M. Kodiha, E. Hutter, S. Boridy, M. Juhas, D. Maysinger, U. Stochaj, *Cell. Mol. Life Sci.* **2014**, *71*, 4259.
[240] E. Hinde, K. Thammasiraphop, H. T. T. Duong, J. Yeow, B. Karagoz, C. Boyer, J. J. Gooding, K. Gaus, *Nature Nanotech* **2017**, *12*, 81.
[241] K. Huang, H. Ma, J. Liu, S. Huo, A. Kumar, T. Wei, X. Zhang, S. Jin, Y. Gan, P. C. Wang, S. He, X. Zhang, X.-J. Liang, *ACS Nano* **2012**, *6*, 4483.
[242] V. Sokolova, G. Nzou, S. B. van der Meer, T. Ruks, M. Heggen, K. Loza, N. Hagemann, F. Murke, B. Giebel, D. M. Hermann, A. J. Atala, M. Epple, *Acta Biomaterialia* **2020**, *111*, 349.
[243] J. M. de la Fuente, C. C. Berry, *Bioconjugate Chem.* **2005**, *16*, 1176.
[244] W. Xie, L. Wang, Y. Zhang, L. Su, A. Shen, J. Tan, J. Hu, *Bioconjugate Chem.* **2009**, *20*, 768.
[245] A. K. Oyelere, P. C. Chen, X. Huang, I. H. El-Sayed, M. A. El-Sayed, *Bioconjugate Chem.* **2007**, *18*, 1490.
[246] R. Vankayala, C.-L. Kuo, K. Nuthalapati, C.-S. Chiang, K. C. Hwang, *Adv. Funct. Mater.* **2015**, *25*, 5934.
[247] L. Pan, J. Liu, J. Shi, *ACS Appl. Mater. Interfaces* **2017**, *9*, 15952.
[248] D. B. Chithrani, *Insciences J.* **2011**, 115.
[249] G. Ajnai, A. Chiu, T. Kan, C.-C. Cheng, T.-H. Tsai, J. Chang, *Journal of Experimental & Clinical Medicine* **2014**, *6*, 172.
[250] S. Salatin, S. Maleki Dizaj, A. Yari Khosroushahi, *Cell Biology International* **2015**, *39*, 881.
[251] S. D. Brown, P. Nativo, J.-A. Smith, D. Stirling, P. R. Edwards, B. Venugopal, D. J. Flint, J. A. Plumb, D. Graham, N. J. Wheate, *J. Am. Chem. Soc.* **2010**, *132*, 4678.
[252] N. Jiménez-Mancilla, G. Ferro-Flores, C. Santos-Cuevas, B. Ocampo-García, M. Luna-Gutiérrez, E. Azorín-Vega, K. Isaac-Olivé, M. Camacho-López, E. Torres-García, *J. Label Compd. Radiopharm* **2013**, *56*, 663.
[253] T. Paunesku, S. Vogt, B. Lai, J. Maser, N. Stojićević, K. T. Thurn, C. Osipo, H. Liu, D. Legnini, Z. Wang, C. Lee, G. E. Woloschak, *Nano Lett.* **2007**, *7*, 596.
[254] W. Ling, M. Wang, C. Xiong, D. Xie, Q. Chen, X. Chu, X. Qiu, Y. Li, X. Xiao, *J. Mater. Res.* **2019**, *34*, 1828.
[255] A. Abdelkawi, A. Slim, Z. Zinoune, Y. Pathak, *Coatings* **2023**, *13*, 1660.
[256] I. A. Cree, P. Charlton, *BMC Cancer* **2017**, *17*, 10.
[257] S. Santhosh, P. Kumar, V. Ramprasad, A. Chaudhuri, *Future Oncology* **2015**, *11*, 279.
[258] Q. Cao, Y. Li, E. Freisinger, P. Z. Qin, R. K. O. Sigel, Z.-W. Mao, *Inorg. Chem. Front.* **2017**, *4*, 10.
[259] J. M. Woynarowski, *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2002**, *1587*, 300.
[260] M. P. Knauert, *Human Molecular Genetics* **2001**, *10*, 2243.
[261] M. Dalla Pozza, A. Abdullrahman, C. J. Cardin, G. Gasser, J. P. Hall, *Chem. Sci.* **2022**, *13*, 10193.
[262] L. R. Devi, M. K. Raza, D. Musib, V. Ramu, J. Devi, M. Roy, *Inorganica Chimica Acta* **2020**, *500*, 119208.
[263] N. Agorastos, L. Borsig, A. Renard, P. Antoni, G. Viola, B. Spingler, P. Kurz, R. Alberto, *Chem. Eur. J.* **2007**, *13*, 3842.
[264] I. Amarsy, S. Papot, G. Gasser, *Angew Chem Int Ed* **2022**, *61*, e202205900.
[265] A. Nel, E. Ruoslahti, H. Meng, *ACS Nano* **2017**, *11*, 9567.
[266] A. A. Natfji, D. Ravishankar, H. M. I. Osborn, F. Greco, *Journal of Pharmaceutical Sciences* **2017**, *106*, 3179.

[267] K. Greish, H. Nehoff, N. Parayath, L. Domanovitch, S. Taurin, *IJN* **2014**, 2539.
[268] J. Wolfram, M. Zhu, Y. Yang, J. Shen, E. Gentile, D. Paolino, M. Fresta, G. Nie, C. Chen, H. Shen, M. Ferrari, Y. Zhao, *CDT* **2015**, *16*, 1671.
[269] B. Wang, J.-J. Yin, X. Zhou, I. Kurash, Z. Chai, Y. Zhao, W. Feng, *J. Phys. Chem. C* **2013**, *117*, 383.
[270] H. Xiao, R. Qi, S. Liu, X. Hu, T. Duan, Y. Zheng, Y. Huang, X. Jing, *Biomaterials* **2011**, *32*, 7732.
[271] Y. Wang, Y. Jiang, D. Wei, P. Singh, Y. Yu, T. Lee, L. Zhang, H. K. Mandl, A. S. Piotrowski-Daspit, X. Chen, F. Li, X. Li, Y. Cheng, A. Josowitz, F. Yang, Y. Zhao, F. Wang, Z. Zhao, A. Huttner, R. S. Bindra, H. Xiao, W. Mark Saltzman, *Nat Biomed Eng* **2021**, *5*, 1048.
[272] D. Tang, H. Zhou, M. Cui, G. Liang, H. Zhang, H. Xiao, *Advanced Materials* **2023**, *35*, 2300048.
[273] Y.-X. Zhu, H.-R. Jia, G.-Y. Pan, N. W. Ulrich, Z. Chen, F.-G. Wu, *J. Am. Chem. Soc.* **2018**, *140*, 4062.
[274] A. Ku, V. J. Facca, Z. Cai, R. M. Reilly, *EJNMMI radiopharm. chem.* **2019**, *4*, 27.
[275] Targeted Alpha Therapy Working Group, C. Parker, V. Lewington, N. Shore, C. Kratochwil, M. Levy, O. Lindén, W. Noordzij, J. Park, F. Saad, *JAMA Oncol* **2018**, *4*, 1765.
[276] U. Hennrich, K. Kopka, *Pharmaceuticals* **2019**, *12*, 114.
[277] L. Urso, A. Nieri, L. Uccelli, A. Castello, P. Artioli, C. Cittanti, M. C. Marzola, L. Florimonte, M. Castellani, S. Bissoli, F. Porto, A. Boschi, L. Evangelista, M. Bartolomei, *Pharmaceutics* **2023**, *15*, 1110.
[278] K. Liepe, A. Shinto, *Ther Adv Med Oncol* **2016**, *8*, 294.
[279] E. Deshayes, M. Roumiguie, C. Thibault, P. Beuzeboc, F. Cachin, C. Hennequin, D. Huglo, F. Rozet, D. Kassab-Chahmi, X. Rebillard, N. Houédé, *DDDT* **2017**, *Volume 11*, 2643.
[280] S. M. Nimjee, B. A. Sullenger, *CMC* **2020**, *27*, 4181.
[281] D. W. Hwang, H. Y. Ko, J. H. Lee, H. Kang, S. H. Ryu, I. C. Song, D. S. Lee, S. Kim, *J Nucl Med* **2010**, *51*, 98.
[282] F. Ghahremani, D. Shahbazi-Gahrouei, A. Kefayat, H. Motaghi, M. A. Mehrgardi, S. H. Javanmard, *RSC Adv.* **2018**, *8*, 4249.
[283] R. E. Wang, H. Wu, Y. Niu, J. Cai, *CMC* **2011**, *18*, 4126.
[284] C. Kratschmer, M. Levy, *Nucleic Acid Therapeutics* **2017**, *27*, 335.
[285] R. Tang, M. Wang, M. Ray, Y. Jiang, Z. Jiang, Q. Xu, V. M. Rotello, *J. Am. Chem. Soc.* **2017**, *139*, 8547.
[286] A. Shahryari, M. Saghaeian Jazi, S. Mohammadi, H. Razavi Nikoo, Z. Nazari, E. S. Hosseini, I. Burtscher, S. J. Mowla, H. Lickert, *Front. Genet.* **2019**, *10*, 868.
[287] D. Cross, J. K. Burmester, *Clinical Medicine & Research* **2006**, *4*, 218.
[288] A. El-Aneed, *Journal of Controlled Release* **2004**, *94*, 1.
[289] M. Foldvari, D. W. Chen, N. Nafissi, D. Calderon, L. Narsineni, A. Rafiee, *Journal of Controlled Release* **2016**, *240*, 165.
[290] E. Musatkina, H. Amouri, M. Lamoureux, T. Chepurnykh, C. Cordier, *Journal of Inorganic Biochemistry* **2007**, *101*, 1086.
[291] S. S. Bhat, A. S. Kumbhar, A. A. Kumbhar, A. Khan, P. Lönnecke, E. Hey-Hawkins, *Chem. Commun.* **2011**, *47*, 11068.
[292] J. Yin, X. Meng, S. Zhang, D. Zhang, L. Wang, C. Liu, *Biomaterials* **2012**, *33*, 7884.
[293] B. Yu, Y. Chen, C. Ouyang, H. Huang, L. Ji, H. Chao, *Chem. Commun.* **2013**, *49*, 810.
[294] J. Yao, Y. Fan, Y. Li, L. Huang, *Journal of Drug Targeting* **2013**, *21*, 926.
[295] H. Zu, D. Gao, *AAPS J* **2021**, *23*, 78.
[296] C. M. Berger, X. Gaume, P. Bouvet, *Biochimie* **2015**, *113*, 78.

[297] S. Romano, N. Fonseca, S. Simões, J. Gonçalves, J. N. Moreira, *Drug Discovery Today* **2019**, *24*, 1985.
[298] B. Ferrara, S. Belbekhouche, D. Habert, C. Houppe, B. Vallée, S. Bourgoin-Voillard, J. L. Cohen, I. Cascone, J. Courty, *Nanotechnology* **2021**, *32*, 322001.
[299] F. Madani, S. Lindberg, Ü. Langel, S. Futaki, A. Gräslund, *Journal of Biophysics* **2011**, *2011*, 1.
[300] A. K. Varkouhi, M. Scholte, G. Storm, H. J. Haisma, *Journal of Controlled Release* **2011**, *151*, 220.
[301] P. Lönn, A. D. Kacsinta, X.-S. Cui, A. S. Hamil, M. Kaulich, K. Gogoi, S. F. Dowdy, *Sci Rep* **2016**, *6*, 32301.
[302] S. A. Smith, L. I. Selby, A. P. R. Johnston, G. K. Such, *Bioconjugate Chem.* **2019**, *30*, 263.
[303] S. Soares, J. Sousa, A. Pais, C. Vitorino, *Front. Chem.* **2018**, *6*, 360.
[304] P. Heffeter, U. Jungwirth, M. Jakupec, C. Hartinger, M. S. Galanski, L. Elbling, M. Micksche, B. Keppler, W. Berger, *Drug Resistance Updates* **2008**, *11*, 1.

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