Polymer encapsulation of ruthenium complexes for biological and medicinal applications

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Abstract | Some Ru complexes have extremely promising anticancer or antibacterial properties, but their poor H₂O solubility and/or low stability of many Ru complexes in aqueous solution under physiological conditions and/or metabolic/biodistribution profile prevent their therapeutic use. To overcome these drawbacks, various strategies have been developed to improve the delivery of these compounds to their target tissues. The first strategy is based on physical encapsulation of Ru complexes in carriers, such as polymeric micelles, microparticles, nanoparticles and polymer–lipid hybrids, which enabled the delivery and controlled release of the active Ru drug candidate. The second strategy involves covalent conjugation of the ruthenium complex to a polymer to give a prodrug that can be converted to the active drug at a more controllable rate. In this Review, we provide an overview of recent developments in polymer encapsulation of Ru complexes for biological and medicinal applications, and place particular emphasis on the role of the polymer in the delivery carriers.

[H1] Introduction

Platinum-based drugs have dominated the field of medicinal inorganic chemistry since the discovery of the biological activity of *cis*-[PtCl₂(NH₃)₂] (cisplatin) in the late 1960s and its approval for the treatment of some types of cancer by the US Food and Drug Administration (FDA) in 1978¹. Large doses of cisplatin are required to induce a therapeutic effect against different types of cancers. The dosage leads to various adverse effects for patients because cisplatin exhibits low selectivity towards cancer cells and has a short half-life in the blood. Moreover, some cancer cells are intrinsically resistant to cisplatin or can acquire resistance through an increased rate of repair of DNA intrastrand crosslinks². Other Pt-based drugs, such as oxaliplatin and carboplatin, have been approved for therapeutic use, but have adverse effects (such as neurotoxicity and/or ototoxicity) similar to those of cisplatin. Alternative (metal-based) compounds with new mechanisms of action are therefore needed to avoid the problem of resistance to Pt-based drugs. Over the past two decades, Ru(II) and Ru(III) complexes have been intensively investigated in medicinal chemistry as anticancer and antibacterial agents^{3–8}, enzyme inhibitors^{9–11}, photosensitizers in photodynamic therapy^{12–15}, and immunosuppressants¹⁶, and in biology as

luminescent probes (for imaging and the detection of biomolecules and cellular compartments^{17–20}). Although the cytotoxicity of Ru complexes has been known since the mid-1950s²¹, the therapeutic potential of Ru complexes was only recognized in the mid-1980s following the publication of Clarke's 'activation by reduction' hypothesis for the molecular mechanism of Ru(III) complexes²². Under physiological conditions, Ru can exist in +II, +III and/or +IV oxidation states,²³ each of which is stabilized by different coordination environments. The most common forms are 18e[−] Ru(II) species — either pseudo-octahedral complexes or pseudo-tetrahedral complexes featuring an η⁶-arene and three other donors. Ru(IV) complexes are typically highly soluble in H₂O and often feature oxo, carboxylato or sulfido ligands. In general, Ru(III) complexes appear to be less toxic than Ru(II) and Ru(IV) complexes²⁴. Nevertheless, the potentials of the Ru redox couples are sensitive to the ligand environment and are readily accessed under physiological conditions, such that interconversion between the different oxidation states can be fast in vivo. Indeed, Ru(IV) or Ru(III) can be reduced to Ru(II) by biological reductants (such as ascorbate, glutathione and single-electron-transfer proteins), and Ru(II) can be oxidized to Ru(III) or Ru(IV) by biological oxidants (such as O₂, H₂O₂ and cytochrome oxidase)^{24,25}.

The major strategy used in the development of Ru-based anticancer drug candidates is to administer a relatively inert high-valent compound that undergoes reduction in vivo to its active reduced form. This 'activation by reduction' is most commonly seen with "biologically inactive" Ru(III) prodrugs that must be reduced to their Ru(II) form to be biologically active (for example, **NAMI-A** or **KP-1339** complexes; FIG. 1), a process analogous to the activation of Pt(IV) prodrugs to (relatively) labile Pt(II) species. This is possible in the presence of high levels of biological reductants (for example, glutathione)²⁶, such as in the hypoxic regions typically found in solid tumours^{27,28}. If the active Ru(II) drug candidate leaves the tumour cell and moves to a more oxygenated environment, such as in healthy tissue, biological oxidants convert the Ru(II) complex to its inactive Ru(III) form²⁴. Mitochondrial and microsomal single electron transfer proteins can also reduce Ru(III) to Ru(II)²⁹. However this Ru reduction can be also performed by transmembrane electron transport systems enabling the development of metallo-prodrug candidates with anticancer properties independent of the cell entry mechanism²³. These Ru(II) complexes then undergo aquation, typically with displacement of one or two weakly bound donors such as halido ligands. For example, the Ru(II) arenes [Ru(η⁶-*p*-cymene)Cl₂(pta)] (**RAPTA-C**; pta = 1,3,5-triaza-7-phosphaadamantane; FIG. 1) and [Ru(η⁶-toluene)Cl₂(pta)] (**RAPTA-T**)^{30–32}, as well as the diamine-arene species [Ru(η⁶-4-methylbiphenyl)(1,2-diaminoethane)Cl]PF₆ (**RM-175**) and their derivatives^{33,34} each have Cl[−] ligands that can be displaced by H₂O. Substitution at a Ru(III) centre is also possible, and can afford Ru(III) aquation products and polynuclear species^{35–37}. In both the reduction and aquation strategies to yield labile species, the medicinal efficacy of the ruthenium complex is directly related to the inherent reactivity of the Ru centre. Another strategy in drug design with Ru centres is to construct building blocks with three-dimension structures that have relative kinetic inertness for ligand exchange.

Here, the presence of Ru centre enable 3D geometries not available with organic chemistry. For example, the Ru(II) complex **DW1** and its enantiomer **DW2** mimic the shape of the alkaloid staurosporine, and complexes thus also exhibit impressive kinase inhibition activity (targeting glycogen synthase kinase 3 (GSK3) signalling) and cytotoxicity in human melanoma cancer cells³⁸.

The biological properties of Ru complexes — such as bioactivity, cellular uptake and intracellular distribution — depend, among others, on chemical properties such as electronegativity, chemical hardness, stereochemistry and net charge of the Ru centre. Ru drug candidates often feature strongly bound ligands, such as amine, phosphine, π -bound arenes and cyclopentadienyl derivatives. These are complemented by weakly bound ligands such as Cl^- or RCO_2^- , which can be displaced by chelators or simply when an excess of competitive ligands is present. As is the case for reduced Pt derivatives, the lower-valent Ru(II) and Ru(III) complexes have favourable ligand exchange kinetics with O-donor and N-donor ligands. Ru complexes have several other advantages for biological and medicinal applications, including their usual low (or zero) toxicity to healthy tissues and their distinct mode of action. Indeed, they operate through different pathways to most Pt-based drugs, which typically only interact with DNA (BOX 1). Ru(II) and Ru(III) complexes have similar ligand exchange kinetics to the Pt(II) complexes used as antineoplastic drugs³⁹. For small ligands such as H_2O , the ligand exchange rate on the Ru centre is on the order of hours, which is similar to the timescale of cell division in many cell types⁴⁰.

Furthermore, Ru can bind biomolecules responsible for Fe solubilization and transport in plasma, including serum transferrin and albumin. Rapidly dividing cells, such as cancer cells, require more Fe, which leads to an upregulation of the number of transferrin receptors at the cell surface⁴¹. Consequently, Ru complexes have been proposed to preferentially target cancer cells over healthy cells⁴², which might explain their fairly low toxicity to the latter. This hypothesis is still the subject of debate⁴³ because Ru complexes can enter cells by both transferrin-dependent and transferrin-independent mechanisms⁴⁴⁻⁴⁵.

To date, no Ru drug candidates have been commercialized, although four Ru complexes are in various stages of clinical trials⁴⁶. Trials of two well-known Ru-based drug candidates, *trans*- $\text{ImH}[\text{Ru}^{\text{III}}(\text{Im})(\text{Me}_2\text{SO})\text{Cl}_4]$ (**NAMI-A**; FIG. 1; Im = imidazole) and *trans*-indazolium $[\text{Ru}^{\text{III}}(1H\text{-indazole})_2\text{Cl}_4]$ (**KP1019**) have been halted since **NAMI-A** showed limited efficacy in a phase II clinical trial⁴⁷ and **KP1019** exhibited poor solubility under physiological conditions in a phase I clinical trial⁴⁸⁻⁴⁹. However, (pre-)clinical tests are being conducted on other Ru complexes: the *trans*- $\text{Na}[\text{Ru}^{\text{III}}(1H\text{-indazole})_2\text{Cl}_4]$ (**KP1339**), the Ru(II) polypyridyl **TLD-1433** and **RAPTA-C** (FIG. 1). We note that by simply converting the indazolium salt **KP1019** to its Na^+ salt **KP1339** affords a complex with greater H_2O solubility that is in a phase IIA trial at the time of writing⁵⁰⁻⁵¹. **TLD-1433** just completed phase I clinical trial as a photosensitizer for photodynamic therapy (PDT) to treat bladder cancer⁵²⁻⁵³. **RAPTA-**

C (FIG. 1) is currently in pre-clinical evaluation¹². The testing of different Ru complexes as anticancer drug candidates in clinical studies is encouraging news for those wishing to develop Ru anticancer drugs as replacements for Pt-based drugs.

Although the above strategies for Ru drug candidate design are sound, the poor solubility and/or low stability of many Ru complexes in aqueous solution under physiological conditions and/or metabolic/biodistribution profile limit their intravenous administration, and thus limits the amount of the complex that reaches the target tissue. Most Ru complexes studied to date have a short half-life in the circulation^{23,24} and a high overall clearance rate. Thus, although Ru complexes are undoubtedly promising drug candidates²⁵, new drug delivery methods⁵⁴⁻⁵⁵ are needed in order for their therapeutic potential to be realized.

[H1] Polymeric carriers for drug delivery

The aim of nanomedicine is to create more effective and safer medicines through the identification of new drugs with novel mechanisms of action and the development of innovative drug formulations and delivery methods. Of the various nanomaterials available for drug delivery, macromolecules have attracted increased interest⁵⁶. Our definition of macromolecules includes biomacromolecules, as well as synthetic polymers or dendrimers.. The properties of these macromolecules depend on the choice of repeating unit(s) (in particular, its structure), the ratio between hydrophilic and hydrophobic moieties in the polymer, and the length or size (molecular weight and molecular weight distribution) of the polymer and the resulting phase morphology. Encapsulation of a drug within a polymer is based on a 'bottom up' approach, in which the properties of each building blocks and Ru moiety are combined to prepare nanoscale materials. Self-assembling macromolecules provide several advantages for drug delivery. Although most of these macromolecules are typically not bioactive, the final biological application will determine the choice of polymer and, in particular, its degradation kinetics, which is directly linked to its biodegradability. Biocompatible polymers are by definition non-toxic and do not induce an immune response. Among biocompatible polymers, biodegradable polymers⁵⁷ are mainly used for drug delivery, whereas non-biodegradable polymers as best suited for other applications, such as in bioimaging. A polymer is considered to be biodegradable if degradation occurs due to environmental action, which includes biological processes such as hydrolysis, oxidation and UV irradiation and/or biocatalytic processes involving, for example, bacteria, fungi and algae⁵⁸. The definition of biodegradability that is used depends on the application of the polymer, such as biomedical or environmental applications. For example, biodegradation has been defined as enzymatic degradation and/or chemical decomposition associated with living organisms and their secretion products⁵⁹. Biodegradable polymers for controlled drug delivery are typically degraded through hydrolysis by action of H₂O molecules⁶⁰ and/or by enzyme

cleavage⁶¹. Several factors influence the degradation rate of a polymer⁶¹, but the most important parameter is the kinetics of drug release⁶².

Polymer architecture (for example, the presence of hydrolytic linkages, chain branching, stereochemistry), molecular weight and morphology (for example, the length of the repeating units, degree of crystallinity, degree of chain flexibility, and surface area) have a major effect on the observed degradation rate, as well as the surrounding conditions (for example, pH and temperature)⁶¹. For a medical device, the size, geometry and porosity of the polymer are also important factors⁶³.

The chemical functionalities in the repeating unit or units and the corresponding polymer may confer responsiveness to different stimuli, such as physical stimuli (for example, ionic strength), chemical stimuli (for example, hydrolysis or pH), biochemical stimuli (for example, enzymes) or environmental stimuli (for example, light or temperature), which can lead to the triggered release of a biologically active compound.

Hierarchically ordered and complex architectures can be obtained according to the ratio of hydrophilic and hydrophobic moieties, as well as the presence of directional short and/or large non-covalent interactions. Monomers and their corresponding self-assembled polymers can form various 1D, 2D or 3D architectures, such as rods (linear); thin films (lamellar); nanoparticles, microparticles, micelles, vesicles and polymer–lipid hybrids (spherical); and worm-like micelles, nanotubes or hydrogels (3D-crosslinked networks). Self-assembly is driven by multiple types of interactions such as strong hydrophobic or electrostatic interactions, hydrogen bonding, π – π stacking, metal–ligand coordination and stereocomplexation, or their synergistic associations. In contrast to covalent polymers, supramolecular polymers assemble by non-covalent and dynamic interactions. The self-assembled polymers in smart materials can self-heal or adapt their architecture in response to a small change of environment. The dynamic nature of self-assembly is responsible for the reversible nature of self-assembly and stimuli-responsiveness. In the case of delivery systems, the biologically active compound can be delivered in a triggered⁶⁴⁻⁶⁵ and controlled⁶⁶⁻⁶⁷ way that reduces adverse effects. By limiting the drug dose and ensuring the drug efficiently targets specific organelles, it can be possible to restrict drug resistance⁶⁸⁻⁶⁹. The principle challenge in the design and construction of self-assembled macromolecular systems is ensuring their stability, a task that requires precise control of the balance between attractive and repulsive forces.

Finally, the surface of the polymer can be decorated with recognition motifs (such as small biomolecules or peptides) to increase the specificity of targeting to organelles or diseased tissues. The effect of vectorization of macromolecular delivery systems is not described in detail in this Review. Instead, we

focus our discussion on the effect of the polymer in macromolecular carriers on the passive targeting of diseased tissues (BOX 2).

Ru complexes used in medicinal applications often have low H₂O solubility on account of their hydrophobic organic ligands. This and other inherent drawbacks can be addressed by encapsulating the complexes in delivery systems — a promising approach that differs from the traditional solution that involves formulating a drug with diluents (referred to as excipients). Macromolecules physically protect Ru complexes from biological degradation (by hydrolysis or reaction with proteolytic enzymes, radicals, reductants or nucleophilic species) and/or photodegradation (UV solar light). Furthermore, the polymeric matrix limits the exposure of healthy tissues to the drug and shields the Ru complex from the immune system, thereby preventing its elimination through renal excretion⁷⁰.

A polymer carrier increases the targeted delivery of a small molecule drug by selectively accumulating in diseased tissues because of the enhanced permeability and retention (EPR) effect⁷¹⁻⁷³. This effect results from the leaky, highly permeable vasculature and poor lymphatic drainage of these tissues (BOX 2), resulting in the efficient extravasation of nanocarriers from the tumour vasculature and their retention in the tumour interstitium. The EPR effect also leads to the encapsulated drug having a greater half-life than the free drug in the bloodstream. In mice, the plasma half-life of most small-molecule drugs is less than 3 min, while drugs subject to the EPR effect have a half-life of 6 h or more⁷⁴. A drug that exhibits an EPR effect in mice does not always also behave this way in humans⁷⁵. In mice, tumour growth is limited to several weeks, whereas in humans' tumours can grow over several years, such that a long time period is available for angiogenesis. Furthermore, the EPR effect varies with the type of tumour⁷⁶, and new tools need to be developed to improve the efficiency of nanocarriers for the delivery of anticancer drugs⁷⁶.

Using a polymeric carrier should improve the efficacy and the targeting efficiency of a biologically active compound by increasing its availability, which could potentially alleviate the adverse effects of Ru complexes. Furthermore, macromolecular delivery systems incorporating multiple compounds enable multi-action and multi-target delivery or the possibility to combine therapeutic and diagnostic tools to afford theranostic agents. For example, two Ru compounds for chemotherapy and PDT can be delivered simultaneously. Alternatively, as diagnostic tools, a bioactive compound can be delivered in association with a Ru dye for imaging. Despite the numerous advantages of macromolecular delivery systems, the nanoarchitectures constructed from polymers are not always completely biocompatible. Indeed, the polymer and/or its degradation product(s) can be mildly toxic, such that extensive *in vitro* and *in vivo* testing is required to minimize this toxicity.

Two major strategies have been used to encapsulate Ru complexes in macromolecular systems — physical encapsulation and covalent conjugation (FIG. 2). Physical encapsulation relies on non-covalent interactions between the Ru complex and the polymeric matrix. Covalent conjugation of the Ru complex to the polymer affords a well-defined metallopolymer prodrug. In this Review, we provide an overview of polymers used to encapsulate Ru complexes for biomedical applications. We discuss the importance of the relationship between the properties of the polymer and the final Ru-containing polymeric carriers. The polymeric systems are classified on the basis of the type of interaction between the Ru complex and the polymer (physical entrapment or covalent conjugation) and according to the structure of the resulting microparticles or nanoparticles. Of note, Ru-containing nanohybrids, which are defined here as hybrids comprising Ru complexes and a non-polymer inorganic nanomaterial (such as Au nanostructures), porous nanostructures (such as zeolites), SiO₂ nanostructures, quantum dots and C nanotubes are not covered in this Review. In such species, the polymer only has a minor effect on the final properties of nanohybrids, which are in any case reviewed elsewhere^{55,77}.

[H1] Physical encapsulation

Physical encapsulation of Ru complexes in polymeric carriers is similar to the encapsulation of organic biologically active compounds⁷⁸. When designing polymers to serve as efficient Ru carriers, one must consider several features of the polymer, including size, charge, structure and degradation kinetics.

[H2] Polymer size

The size of a nanocarrier directly affects the efficiency with which Ru complexes target diseased tissues. Therapeutically-relevant Ru complexes are small and can passively diffuse out of capillaries into the interstitial fluid, leading to undesired adverse effects. Tumour tissues are structurally distinct from healthy tissues (BOX 2), and these differences have been exploited to design efficient macromolecular delivery systems. Although the diameter of a carrier intended for biological applications is typically in the range 10 – 1000 nm, the carrier's diameter should ideally not exceed 300 nm in order to enable the EPR effect (BOX 2) to ensure efficient passive targeting of tumour tissues⁷⁹. Directly linked to carrier size, the molecular weight of individual solvated biodegradable polymer chains should be >40 kDa to ensure an efficient EPR effect and a long circulation time without renal excretion⁷⁰. In the case of individual solvated non-biodegradable polymer chains,⁸⁰ the molecular weight is limited to 40 kDa to ensure renal elimination. However, for nanocarriers based on the assembly of polymer chains (such as micelles or liposomes), it is not possible to estimate the minimum molecular weight for individual polymer chains owing to the variety of polymer structures and assemblies.

[H2] Polymer charge

The surface charge of nanocarriers affects their stability and targeting efficiency. Although a positively charged surface favours cell adhesion for rapid endocytosis, a neutral or negative surface charge is preferred because of lower non-specific adsorption of proteins and non-specific phagocytosis by cells of the reticuloendothelial system (RES)⁸¹.

[H2] Polymer chemical structure

Depending on the solubility of the Ru complex in the polymeric matrix, the chemical structure of a biocompatible polymer should be chosen to ensure chemical and physical compatibility between the polymeric carrier and the Ru complex. Furthermore, a suitable preparation method should be selected to ensure sufficient loading of the Ru complex. The carrier should ideally have a well-defined structure to avoid phase separation, which can lead to inhomogeneity and lower biological efficacy. The integrity of the carrier nanostructure must also be maintained after encapsulation of the Ru complex. Because many medicinally-relevant Ru complexes have a charge, it is necessary to control the physical interactions (mostly hydrophobic or electrostatic interactions) between the polymeric matrix and the Ru complex. A judicious choice of nanocarrier architecture must be made, and this involves selecting a suitable ratio of hydrophobic and hydrophilic domains and appropriately functionalizing the polymer with polarisable residues.

[H2] Polymer biodegradability

Depending on the biological application, the biodegradability of the polymer as well as its composition must be controlled to regulate the disintegration of the polymeric nanocarrier and the subsequent release of the Ru complex. Except for a few applications, such as bioimaging or the controlled release of a therapeutic gas, most delivery systems require biodegradable polymers. The use of non-biodegradable polymers is not always linked to the problem of accumulation. These polymers must be eliminated by a furtive way. The use of non-biodegradable copolymers allows one to ameliorate polymer properties (such as hydrophilicity, stability), but the elimination of these species can occur without the release of a pharmaceutically active compound. In the case of biodegradable polymers, the initial hydrolysis of cleavable links leads to the release of surface-adsorbed Ru complexes, after which the residual polymer biodegrades slowly and releases the remaining payload in a controlled fashion over the course of weeks to years⁸². This fast initial release is termed the ‘burst effect’ and should be minimized in order to maximise targeting efficiency.⁸³⁻⁸⁴ The rate at which a Ru complex is released from a biocompatible polymers depends on multiple factors, such as the concentration gradient of the Ru complex in the polymeric matrix, the mobility and diffusion of the Ru complex in the nanocarrier, the polymer degradation rate (which is related to polymer properties such as the composition, molecular weight, molecular-weight distribution, composition, crystallinity and chemical structure). Ru complexes should be released by diffusion and/or through biochemical degradation of biodegradable polymers. Depending

on the structure of the cleavable functionality, biodegradation can be catalysed in the acidic environment characteristic of diseased tissues. Different degradation kinetic profiles can be obtained depending on the regioregularity or stereoregularity of the polymer sequence in the copolymers⁸⁵. The overall rate of degradation for alternating copolymers is slower than for random counterparts and alternating copolymers degrade with a uniform linear degradation profile, which gives access to a homogeneous mixture after the first burst stage leads to an initial rapid drop in weight.

The principal advantage of physical entrapment is that the integrity of the Ru complex (that is, the geometry, oxidation state and stereochemistry of the Ru centre) is conserved. However, the stability of the nanocarrier and its preservation for prolonged periods after synthesis are important challenges associated with using physical encapsulation. A balance must be found between a carrier that is too stable (and does not correctly release the Ru complex) and one that is too unstable (and does not reach the biological target or prematurely disassembles).

[H1] Polymer micelles

Polymer micelles were first used as drug delivery carriers in the 1980s⁸⁶⁻⁸⁷. Polymeric micelles are self-assembled nanosize colloidal particles that are obtained from amphiphilic block copolymers, usually in aqueous medium, and are generally larger than dendrimers and liposomes. Spherical polymeric micelles are typically 10 – 100 nm in diameter⁸⁸ and have an extremely narrow size distribution. This size can increase when serum proteins adsorb, sometimes rendering the particles too large for renal excretion⁸⁹. In addition to the archetypal spherical shapes, these micelles can self-assemble into cylindrical and flexible structures (~40 nm in width and 20 – 40 μ m in length)⁹⁰⁻⁹¹.

Above the critical micelle concentration (CMC), single chains of amphiphilic polymers self-assemble into micelles to minimize the contact between the hydrophobic block and the aqueous medium⁹². The CMC is an important parameter in evaluating the stability of micelles. In general, the CMC for polymers (1–10 μ M⁻¹) is lower than that for low molecular weight surfactants (mM⁻¹ range)⁸⁸. A low CMC induces greater thermodynamic and kinetic stability in polymer micelles than in surfactant-based micelles. In principle, the formation of micelles is driven by a decrease in free energy. Hydrogen bonding between H₂O molecules and the hydrophilic segment, as well as minimization of contact between the hydrophobic segment and the aqueous medium, enable the formation of a core-shell micelle by entropy-driven microphase separation.

Diblock copolymers are usually used to obtain core-shell micelle architectures. The outer shell is composed of the hydrophilic blocks to enable stable dispersion in aqueous environments. The hydrophilic shell protects the encapsulated Ru complex by minimizing adsorption of biocomponents

(such as protein) onto the surface of the micelle during circulation in the bloodstream or interaction with cellular membranes. The inner core is formed from the hydrophobic block and is stabilized by hydrophobic interactions. In the core-shell architecture, the hydrophobic segment provides a space, termed a reservoir, for the physical encapsulation of hydrophobic Ru complexes. Longer hydrophilic segments are used in amphiphilic diblock copolymers to obtain spherical micelles. The limited kinetic stability of micelles can be a problem because there is a dynamic equilibrium between the self-assembled micelle and the bulk phase⁹³. This equilibrium depends on temperature, polymer concentration, the pH and ionic strength of the biological medium. For biological applications, micelles must be stable during transport to the biological target but the equilibrium must shift when reaching the target region, so as to ensure efficient release of the Ru complex. In addition, the stability of micelles is highly dependent on the glass transition temperature (T_g) of the hydrophobic polymer block that constitutes the core of the micelle.

The ratio of hydrophilic to hydrophobic block size can be controlled during synthesis to obtain micelles of a desired size. The physicochemical properties of the amphiphilic polymer determine the choice of polymer micelle preparation method, which include emulsion-solvent evaporation⁹⁴, nanoprecipitation⁹⁵, dialysis⁹⁶ and a thin-film method⁹⁷. The size and morphology of micelles can be modulated by the choice of copolymer molecular weight, block length, composition and the preparation method. The maximum achievable drug loading depends on the chemical affinity between the Ru complex and the hydrophobic polymer block as well as the preparation method⁹⁸.

Physical studies of dendrimers indicate that these highly branched monodisperse macromolecules exist in solution as unimolecular micelles — motifs that can be used as potential delivery systems⁹⁹⁻¹⁰⁰. In contrast to linear, cross-linked or low-branched polymers with a narrow molecular weight distribution, dendrimers have a precise molecular weight that is optimal for reproducible pharmacokinetic studies. The high density of functional groups on the surface of dendrimers allows higher drug loading and well-defined structures to be obtained.

[H1] Microparticles and nanoparticles

Spherical polymeric microparticles and nanoparticles (FIG. 2) are defined as matrix-type solid colloidal particles in which biological species are dissolved, entrapped, encapsulated or absorbed to the polymer matrix¹⁰¹. These particles are typically larger than micelles (usually 100–200 nm in diameter). Homopolymers, such as poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) and the corresponding copolymer poly(lactid-*co*-glycolic)acid (PLGA), are used to prepare solid polymer nanoparticles¹⁰². Self-assembly of these polymers is based on hydrophobic or electrostatic interactions; for example, in ionotropic gels, the cations or anions link the different polymer strands.

However, the hydrophobic surfaces of these particles are not suitable for long-term circulation in the blood (BOX 2). For this reason, the surface of these particles must be coated with hydrophilic polymers to ensure that they are invisible to the reticuloendothelial system (RES). Another solution is the use of an amphiphilic diblock copolymer containing a polyethylene glycol (PEG) segment¹⁰² (BOX 2). Nanoparticles can be prepared using several preparation methods, although nanoprecipitation remains the most popular method¹⁰².

[H2] Biodegradable polymers

All biodegradable polymers have hydrolytically or proteolytically labile bonds in their backbone and/or in their crosslinker (FIG. 3). No additional functionalization is necessary for the use of these polymers for delivery applications. They break down into smaller polymer fragments (which can sometimes even be common metabolites in the body) that can be readily metabolized and cleared from the body, ensuring that they are non-toxic and non-immunogenic.

The range of natural biodegradable polymers suitable for the encapsulation of Ru complexes is unfortunately rather small because of limitations regarding their preparation. In addition, the presence of several identical functional groups in the lateral pendants of these polymers makes selective functionalization difficult. However, naturally occurring polysaccharides are interesting candidates as drug carriers because they mimic the extracellular matrix, which is involved in tissue regrowth and repair. For example, chitosan, a component of crustacean exoskeletons, is a polycationic polysaccharide that consists mostly of β -(1 $\rightarrow$ 4)-linked D-glucosamine units (FIG. 3). Chitosan is H₂O-soluble in mildly acidic solutions (pH < 6.3) because its amine groups become protonated. In addition to its antimicrobial properties¹⁰³⁻¹⁰⁴, chitosan has low O₂ and CO₂ permeability and acts as a temporary barrier against photoluminescence quenchers. Tris(2,2'-bipyridyl)ruthenium(II) ([Ru(bpy)₃]²⁺) embedded in a homogeneous and transparent thin film of chitosan has been used as a temperature-sensitive luminescence sensor¹⁰⁵. The biocompatibility of chitosan and the red emission ($\lambda_{\text{ex}} = 455$ nm, $\lambda_{\text{em}} = 605$ nm) of the Ru complex, at which the maximum penetration of biological tissue by light is possible, makes this a promising sensor for biological sensing applications.

Alginate is a polysaccharide consisting of (1 $\rightarrow$ 4)-linked residues of β -D-mannuronic acid and α -L-gluronic acid in varying proportions and sequences along the chain (FIG. 3). Alginate has been used in various therapies¹⁰⁶, such as chemotherapy or cell-based therapy (the use of living cells as therapeutic agents¹⁰⁷⁻¹⁰⁸). For example, deprotonating the carboxylic acids on the polymer and introducing divalent cations lead to ionotropic gelification and the formation of alginate beads. The polyanion can encapsulate cationic Ru-nitrosyl complexes such as {Ru[2-(4-chlorobenzylideneamino)-4-

nitrophenol](PPh₃)₂(NO)}⁺, a photolabile species that controllably releases of NO *in vitro* after irradiation with visible light¹⁰⁹.

Despite being non-toxic, the limited available functionalities and high cost of natural biodegradable polymers have resulted in synthetic polymers being more frequently used as delivery carriers. The biodegrading of most synthetic polymers proceeds through the hydrolytic cleavage of ester bonds, as is the case for poly(lactic acid) (PLA, FIG. 3), poly(glycolic acid) (PGA) and the corresponding copolymers poly(lactid-*co*-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL). However, the presence of other chemical functionalities, such as anhydrides, ortho-esters, phosphoesters, phosphazenes and cyanoacrylates (containing a C–C bond that is hydrolytically unstable due to the proximity of cyano electron withdrawing groups) also make a polymer degradable. The degradation of PLA and PLGA affords acidic products that catalyse further degradation of the polymers. In contrast, hydrolysis of the semicrystalline polymer PCL does not afford acidic by-products, such that PCL has a low degradation rate and is useful for the preparation of long-term devices.

Aliphatic polyesters, such as PLA, PGA and PLGA (FIG. 3), are the most commonly used biodegradable polymers in biological applications, owing to their controlled degradation (weeks to years) by hydrolysis *in vitro* and *in vivo*¹¹⁰⁻¹¹². The biocompatible degradation products of these polyesters — lactic acid and glycolic acid — are non-toxic and are metabolized to give CO₂ and H₂O as benign by-products^{86, 113}. All these polymers have been approved as therapeutic drug carriers by the US Food and Drug Administration (FDA)¹¹⁴. However, these polymers are hydrophobic, such that the resulting polymeric nanoparticles must be stabilized in aqueous medium by the addition of amphiphilic polymers as surfactants in single H₂O–oil emulsion.

For example, **KP1019** has been encapsulated in PLA nanoparticles (~164 nm in diameter) in the presence of a non-ionic surfactant such as the poloxamer (a type of polyether, see below) Pluronic® F-68 or a polysorbate (an ethoxylated sorbitan esterified with hydrophobic acid) such as Tween 80. The entire assembly is prepared by single oil-in-H₂O emulsion¹¹⁵, and in contrast to Pluronic® F-68, Tween 80 prevents drug precipitation at drug doses that are necessary for *in vivo* use. It is thought that **KP1019** conjugation to Tween 80 involves substitution of a Cl[–] ligands for a moderately basic oxygenic group in Tween 80 atom, with the resulting linkage being more stable than is the case for Pluronic® F-68, which only has ethereal O atoms. On binding Tween 80, **KP1019** complexes not only undergoes ligand substitution but also reduction, with electron paramagnetic resonance spectroscopic data being consistent with the paramagnetic Ru(III) centres being converted to diamagnetic Ru(II) sites, whose exact structure is unknown. Indeed, the autoxidation of polysorbates such as Tween 80 in the presence of transition metals is accompanied by the simultaneous reduction of the metal ion¹¹⁶. Reduction of the Ru(III) centre is accompanied by a colour change (from brown to deep green) and is associated with 20-

fold higher cytotoxicity compared with free **KP1019**¹¹⁶. Of note, this present biodegradable nanoparticle approach has been applied to Ru-mediated PDT. The method was enabled by the efficient loading (~50–60%), by nanoprecipitation, of a $[\text{Ru}^{\text{II}}(1,10\text{-phenanthroline})_3]^{2+}$ photosensitizer, in which the ligands are decorated with hydrophilic triethyleneglycol-functionalized-5-fluorene groups, into PLGA nanoparticles (100 nm) in the presence of Pluronic® P-188¹¹⁷. The resulting nanoparticle was stable and less toxic than the free photosensitizer, releasing only a small amount of photosensitizer while in the dark under physiological conditions. After irradiation with 740 nm light, more of the two-photon-excited photosensitizer was released, such that a substantial level of singlet oxygen ($^1\text{O}_2$) could be generated to kill C6 glioma cells.

H_2O -soluble Ru–NO complexes have also been encapsulated in PLGA nanoparticles and microparticles using a double emulsion preparation (H_2O –oil– H_2O). Thus, Ru nitrosyls featuring amine co-ligands such as NH_3 , cyclam or N,N,N',N' -ethylenediaminetetraacetate, can be encapsulated in PLGA microparticles¹¹⁸ (up to 1,600 nm in diameter) or nanoparticles¹¹⁹ (220–840 nm in diameter). The primary H_2O –oil emulsion, consisting of the NO-donor Ru complex solubilized in aqueous polyvinyl alcohol (PVA) aqueous solution and emulsified in an organic phase containing PLGA, was transferred into an aqueous PVA solution to produce the final H_2O /oil/ H_2O emulsion. In these cases, a low loading efficiency (25–32%) was observed and the PLGA matrix absorbed light in the same spectral region as the embedded Ru–NO complexes, which are thus less liable to undergo photoactivation and exert their biological action. Despite the lower phototoxicity of encapsulated versus free complexes, the latter show improved targeting of tumour cells. *Trans*- $[\text{Ru}(\text{NO})(\text{cyclam})\text{Cl}](\text{PF}_6)_2$ in PLGA nanoparticles killed $53.8 \pm 6.2\%$ of cells and *trans*- $[\text{Ru}(\text{NO})(\text{NH}_3)_4(\text{py})](\text{BF}_4)_3$ in PLGA microparticles killed $63 \pm 3\%$ of cells. The size of the PLGA particles apparently does not influence the drug release profile, which features an initial burst in the first 24 h followed by a slow release due to the hydrolytic degradation of PLGA, which resulted in the gradual release of NO through pores in the particle surface. Although the time required for complete degradation of the PLGA matrix was not reported, it is likely that microparticles had longer release times than nanoparticles. A double emulsion preparation was also used to efficiently encapsulate the related complex *trans*- $[\text{RuCl}([15]\text{aneN}_4)\text{NO}]\text{Cl}_2$, which could be loaded at a level of $51.0 \pm 5.0\%$ in spherical PLGA nanoparticles (830 ± 18 nm in diameter)¹²⁰.

Unconventional inverse non-aqueous emulsion was necessary to encapsulate the polar NO complex $\{[(N,N'-1,2\text{-phenylene})\text{bis}(1\text{-methyl-}1H\text{-imidazole-2-carboxamide})]\text{Ru}(\text{NO})\text{Cl}\}$, which is poorly soluble in H_2O and organic solvents. The complex was hosted in nanoparticles consisting of gelatin, PLA, poly(vinyl formal) (PVF) and poly(ethyleneterephthalate) (PET) using amphiphilic poly[(butylene-*co*-ethylene)-*b*-(ethylene oxide)] as a surfactant and hexafluoroisopropanol as a solvent¹²¹. Localized in the PVF domain, the Ru complex released NO only slowly after photo-

irradiation, because the host absorbs light access and mass transfer within the polymeric matrix is hindered. It is thought that using hexafluoroisopropanol (a dense liquid) as the solvent affords densely packed polymeric nanoparticles (with diameters < 300 nm), from which the Ru complex cannot escape, even after NO photorelease. The use of such unconventional emulsion preparations could be interesting for the preparation of nanocarriers with slow release of encapsulated biological compounds.

It is also possible to encapsulate a Ru complex, such as $[(N,N'-(1,2\text{-phenylene})\text{bis}(1\text{-methyl-}1H\text{-imidazole-2-carboxamido)Ru(NO)Cl)]$, by electrospinning poly(L-lactide-*co*-D/L-lactide) to afford a nanofibrous non-woven composite¹²². What resulted was a bimodal distribution: ~650 nm diameter fibres (10wt% complex loading) and ~950 nm fibres (25wt% complex loading). After exposure to low-intensity UV-A light, a low continuous amount ($0.08 \pm 0.02\%$) of NO was immediately released, with only a small amount of NO being leached ($0.26 \pm 0.10\%$) after five days¹²². Relative to polyesters, polyamides are degraded more slowly because amides are generally more resistant to hydrolysis. Polyamides have been used as protective shells for a Ru(II) photosensitizer bearing three disulfonated 4,7-diphenyl-1,10-phenanthroline ligands. The nanoparticles (hydrodynamic radius 20–25 nm) were constructed from polyacrylamide (PAA) or amine-functionalized polyacrylamide (AF-PAA)¹²³ matrices that prevent quenching of photogenerated $^1\text{O}_2$, thereby allowing gas diffusion in the exterior of the nanoparticle. The Ru photosensitizer leached from the AF-PAA-based nanoparticles only very slowly (over a period of days) owing to the electrostatic interactions between ammonium groups on the host and the sulfonates on the Ru complex. This system appears promising for the targeted production of $^1\text{O}_2$ in PDT applications.

[H2] Non-biodegradable or slowly-biodegradable polymers

Biocompatible non-biodegradable polymers have only few biological applications, including bioimaging, theranostics and the controlled release of a biologically active gas (CO or $^1\text{O}_2$ for PDT). These applications have mostly used commercially available (or easy-to-prepare) diblock or triblock copolymers that serve as a protective shell against biological species (for encapsulated gas-donor Ru complexes), light, $^1\text{O}_2$ or radicals (for encapsulated photosensitizers or phosphorescent Ru complexes). Most of these copolymers contain one polymer segment that is hydrophilic on account of carboxylic acid groups. The gas permeability and the photophysics of the polymeric matrices seem to be the principal properties to control for the efficient design of these delivery systems.

The CO-releasing molecule CORM-2 (4.1wt%, FIG. 1) was encapsulated in styrene–maleic acid copolymer micelles with a hydrodynamic diameter of 165.3 nm. These nanomicelles were bioactive for longer *in vivo* than the free Ru complex CORM-2, had a 35-fold longer half-life in circulation after intravenous injection in mice and showed selective accumulation in inflamed tissues¹²⁴. In small doses,

CO has anti-oxidative and anti-inflammatory effects, and the slow release of CO here is desirable such that these micelles are drug candidates for diseases including ischaemia–reperfusion injury, bacterial and viral infections, hypertension and diseases (including inflammatory bowel disease) caused by reactive oxygen species (ROS) such as $^1\text{O}_2$ and oxyl radicals. To improve the efficiency of photoactivable Ru complexes, they have been encapsulated in cross-linked polymer nanoassemblies and the influence of factors such as photoactivation, hydrophobicity, and solution ionic strength and pH, on complex loading and release studied. Cross-linked polymeric nanoassemblies based on poly(ethyleneglycol)–poly(aspartate) block copolymer (PEG–PASP) were also used to encapsulate three different cationic Ru complexes through electrostatic interaction with the carboxylate acid groups of the ASP core block¹²⁵. The rate of Ru release from the PEG–PASP nanoassembly is highly dependent on the hydrophobicity of the complex and the solution ionic strength but surprisingly independent of pH. The Ru(II) polypyridyl $\{\text{Ru}(\text{bipy})_2[7\text{-(hydroxymethyl)dipyrido}[3,2\text{-}a:2',3'\text{-}c]\text{phenazine}]\}^{2+}$ is a PDT photosensitizer that has been encapsulated in block copolymers consisting of poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) as one segment and either poly(methyl methacrylate) (PMMA) or a statistical copolymer (PDMAEMA-*co*-PMMA) as a second segment.¹²⁶ No release of the Ru complex from micelles with PDMAEMA-*b*-PMMA (occurs in response to ultrasound, whereas Ru release from micelles with the block copolymer featuring PDMAEMA and PDMAEMA-*co*-PMMA segments occurs in response to ultrasound, reflecting the different core–shell structures of the different particles.

Poloxamers are biocompatible non-ionic triblock copolymers with an ABA structure, comprising a hydrophobic central B block flanked by hydrophilic lateral A blocks^{127–129}. The A block is poly(ethylene oxide) and the B block is poly(propylene oxide), and one can adjust the molar ratio (from 1:9 to 8:2) and molecular weight to tune the physicochemical properties of amphiphilic poloxamers, in particular gelation temperature and *in vivo* properties, such as their interaction with cells and membranes. The commercial poloxamers Pluronic® P-123 and Pluronic F-127 can self-assemble into spherical micelles. A bioprobe for high-resolution two-photon quantitative imaging of oxygen in aqueous media was obtained by encapsulating the commercial hydrophobic phosphorescent dye $[\text{Ru}(4,7\text{-diphenyl-1,10-phenanthroline})_3]^{2+}$ in the core of Pluronic® F-127 nanomicelles using hydrophobic interactions¹³⁰. Based on the ‘collisional quenching’ process, the collision between O_2 and an excited luminophore provides a non-radiative pathway for highly electronically excited species to relax, decreasing its phosphorescence intensity and lifetime¹³¹. The hydrophobic core of each nanomicelle permits the diffusion of O_2 and hosts the phosphorescent dye, with the aqueous solubility of the assembly being dependent on the length of the hydrophilic tails. After self-assembly, it is possible to isolate by filtration only the nanomicelles larger than 5 nm, with small assemblies being less desirable because they can diffuse across the vasculature endothelium. The nanomicelle probes are stable for several months in H_2O and for several hours in biological medium — long enough to make a two-photon quantification of O_2 and perform

multiphoton microscopy. Furthermore, these nanomicelle probes are at least twice as sensitive as the free phosphorescent dye in H₂O, although the O₂-sensing response of the probes is dependent on temperature and solvent, such that calibrations are required for *in vivo* testing. The hydrophobic Ru dithiolate [Ru(*p*-cymene)(1,2-dicarba-*c**lo**so*-dodecarborane-1,2-dithiolato)] (FIG. 1), a complex investigated in boron neutron capture therapy (BNCT), has been encapsulated in the core of Pluronic® P123 core-shell nanomicelles to increase its H₂O solubility and targeting of cancer cells¹³². The molar ratio A:B of the hydrophilic to hydrophobic domains for Pluronic® P123 is lower than for Pluronic® F-127, such that the former has a larger nanomicelle core, with its estimated hydrodynamic diameter being 7.8–21.4 nm and dispersity 0.04 according to dynamic light scattering measurements. The Ru complex has a lower anticancer activity after encapsulation, but the accumulation of the encapsulated Ru complex in A2780 (human ovarian) and A2780cisR (cisplatin-resistant human ovarian) cancer cell lines is 2.5-fold higher than that of the free Ru complex. In A2780cisR cells, the encapsulated Ru complex remains sensitive to neutron irradiation, with the antiproliferative activity of these nanomicelles, at micromolar concentrations of Ru, being 1.4-fold higher than that of non-irradiated nanomicelles.

[H1] Polymer-decorated liposomes and polymer-lipid hybrids

Phospholipids and other low-molecular weight surfactants can self-assemble in aqueous medium to afford spherical vesicles consisting of one or more phospholipid bilayers. These vesicles are examples of liposomes and feature amphiphilic phospholipids consisting of a hydrophobic tail and a hydrophilic head group. Spherical liposomes contain a aqueous lumen delimited by a hydrophobic membrane. Hydrophilic biologically active compounds can be enclosed in the internal aqueous volume inside the spherical liposome, whereas lipophilic biologically active compounds can be incorporated in the lipid bilayer. Liposomes have been the most common type of nanocarriers for the targeted delivery of amphiphilic drugs for more than 50 years¹³³. Polymer-decorated liposomes are obtained by coating preformed Ru complex-loaded liposomes with hydrophilic polymers, whereas polymer-lipid hybrids are obtained by inserting polymer chains (with a covalently bound Ru complex) into the lipid bilayer during the self-assembly of phospholipids¹³⁴⁻¹³⁵ (FIG. 2). We now describe the role of the polymer in polymer-decorated liposomes and polymer-lipid hybrids.

[H2] Spherical polymer-decorated liposomes

A drug can spend a longer time in circulation if loaded in a liposome coating with hydrophilic polymers, which confer ‘stealth’ on the liposome (BOX 2) by protecting it from normal clearance mechanisms. For example, the PDT photosensitizer [Zn(phthalocyanine)] and NO donor [Ru(tpy)(α -diimine)NO]³⁺ (tpy = 2,2':6',2''-terpyridine) were encapsulated in 82 nm stealth liposomes consisting of L- α -dipalmitoyl phosphatidylcholine (DPPC) and cholesterol coated with PEG2000 (50–60% loading)¹³⁶. On irradiation with visible light, [Zn(phthalocyanine)] induces ROS formation and NO is simultaneously released from

the Ru complex. Absorption and fluorescence quenching studies confirmed that hydrophobic [Zn(phthalocyanine)] can be incorporated into the slightly deformed lipid bilayer whereas the Ru complex can move between the internal and external liposome environments. The different localizations of the active compounds can be explained in terms of the electrostatic interactions between the cationic Ru complex and the anionic phospholipid head groups. In contrast, charge-neutral [Zn(phthalocyanine)] predominantly engages in hydrophobic interactions with the organic tails. After photoactivation, these stealth liposomes reduce the viability of mouse B16-F10 melanoma cells by ~95% in vitro. Using the same guests, one can form ultradeformable liposomes by adding the non-ionic surfactant polyoxoethylene (20) sorbitan monolaurate (polysorbate 20; also known as Tween 20) to the surface of liposomes¹³⁷⁻¹³⁸.

[H2] Polymer–lipid hybrids

It is possible that not all Ru complexes correctly enter in liposomes, for example because the complexes instead interact favourably with the surface of the polymer–lipid hybrid. To address this, the surface of liposomes or polymer–lipid hybrids can be coated with amphiphilic Ru complexes. The complexes must be functionalized with a hydrophobic segment to be correctly incorporated in the lipid membrane during the self-assembly of phospholipids. A library of nucleolipid nanovectors consisting of uridine or thymidine nucleobases was designed and synthesized to encapsulate amphiphilic Ru complexes¹³⁹ (FIG. S1a). One or two oleic acid residues attached to the secondary OH of ribose serve as a motif for insertion into the lipid monolayer or multi-layer in aqueous solution, and a hydrophilic oligoethylene glycol chain at the 5' end contributes to the stealth properties of these nanovectors. A pyridine motif was inserted in order to give a complex analogous to **Azi-Ru** (FIG. 1), a species similar to the anticancer drug candidates **NAMI-A** and **KP1019**. The Ru complexes remain hidden among the phospholipid head groups at the surface of the liposomes (FIG. S1a). The position of the Ru complexes as well as the liposome membrane composition (consisting of the zwitterion palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) or the cationic lipid 1,2-dioleoyl-3-trimethylammoniumpropane chloride (DOTAP)) effectively slow the hydrolysis by retarding the ligand exchange process¹⁴⁰⁻¹⁴¹, which is consistent with the high kinetic stability of these formulations, which persist for months. Incorporating Ru complexes into a phospholipid membrane at levels below the loading limit induces only a small deformation in the membrane and only minimally affects the morphology of the liposomes. After encapsulation of a uridine-based nucleolipid **HoUrRu** (FIG. S1a) in POPC-based or DOTAP-based liposomes, the assembly has higher antiproliferative activity than free **Azi-Ru** (IC₅₀ ~10 μM versus ~300 μM in MCF7 cells and IC₅₀ 10–20 μM versus ~440 μM in WiDr cells)¹⁴². A triplet–triplet annihilation upconversion (TTA-UC) system for the activation of a [Ru(bpy)₂(1,3-bis(methylsulfido)-2-dodecyloxypropane)]²⁺ anticancer prodrug candidate has been reported¹⁴³ (FIG. S1b). The stealth property of liposomes consisting of the neutral phosphatidylcholine lipids 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC)

or 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 4 mol% sodium *N*-(carbonyl-methoxy polyethyleneglycol-2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (MPEG-2000-DSPE)) is conferred by the PEG moiety. Both the triplet photosensitizer/donor [Pd(tetraphenyltetrabenzoporphyrinato)] and the triplet annihilator perylene (or 2,5,8,11-tetra(*tert*-butyl)perylene) are hydrophobic and localize in the lipid bilayer. After irradiation with red light in the phototherapeutic window, triplet–triplet energy transfer occurs between the sensitizer/donor and annihilator, followed by a TTA-UC between two triplet annihilators in their triplet state. This process allows for the incident red light to induce emission of blue light from the perylene derivatives. Furthermore, the photoactivable Ru(II) prodrug is located at the surface of the polymer–lipid hybrid, because the dodecyl chain in the bis(thioether) ligand allows for its incorporation into the lipid membrane, leading to the surface being decorated with the Ru(II) complex. After the non-radiative Förster resonance energy transfer (FRET), the blue light emission does not cause tissue damage but does induce the loss of the bis(thioether) ligand to afford [Ru(bpy)₂(OH₂)₂]²⁺, which has anticancer activity. Of note, this liposome nanomaterial was tested in the practical conditions of a phototherapeutic operation; the upconverted blue light can be generated by TTA-UC even through >10 mm of chicken or pig tissue.

[H1] Covalent conjugation

The major drawbacks of physical encapsulation include the uncontrolled release of a therapeutic agent, especially the ‘burst’ release (except in the case of polymeric carriers based on pH-responsive or photo-responsive polymers), and the often low amount of Ru complexes that can be loaded into the host. An alternative strategy involves covalent conjugation of Ru complexes to a polymer to give metallopolymer prodrug candidates¹⁴⁴. The first clinical trials of prodrugs developed using this strategy involved using the polymer–protein conjugates PEG-L-asparaginase (Oncaspar) and styrene maleic anhydride-neocarzinostatin (Zinostatin Stimalmer) to treat leukaemia or hepatocellular carcinoma, respectively¹⁴⁵. As with physical encapsulation, the Ru complex is temporarily protected by the polymer against reactive species in the biological medium. This approach enables controlled high loading of Ru complexes into the metallopolymer and ensures the prolonged release of the therapeutic agent during polymer biodegradation. Both Ru(II) and Ru(III) complexes have been covalently conjugated to polymers. Biodegradation can involve degradation of the polymer backbone or first the activation of the Ru(III) centre (by reduction to Ru(II)) followed by degradation of the polymer backbone. Most of the metallopolymer candidates discussed above were developed as prodrug candidates that target cancer cells^{146–147}. Similarly, the Ru complexes physically encapsulated in polymer carriers are principally investigated in view of cancer therapy. Standard metallopolymer prodrug candidates usually have a ‘polymer–linker–therapeutic agent’ architecture, but additional functionalities can be added to achieve specific targeting or to create combined therapies. Although not discussed in detail in this Review,

vectorization of Ru complexes can be achieved by conjugation to biomolecules (such as proteins), an approach that avoids loss of the Ru complex before the target is reached. Moreover, it provides better control of the release and makes the stability of the metallopolymer in the circulation higher than that of physically encapsulated Ru complexes.

In addition to size, surface charge and biodegradability, the structure of the metallopolymer is an important consideration in nanocarrier design. First, a suitable conjugation method is one that ensures that the covalent link between the polymer and the Ru complex remains intact during the transport of the carrier but is cleaved in the biological medium for delivery to the target site. The cleavage of the covalent link activates or reactivates the Ru complex after chemical degradation of the metallopolymer. For this reason, the use of biodegradable polymers is essential in the preparation of polymer-based metalloprodrugs, with the degradation rate of the metallopolymer controlling the drug delivery kinetics. A Ru centre can bind polymers containing N-donor ligands, such as amines or pyridines, or O-donors, such as carboxylates, in different positions, including at the polymer main chain, terminal groups or pendant groups. Depending on the number of vacant coordination sites (or sites at which a weakly basic ligand is present) on a Ru complex, the polymer can be a monodentate or bidentate (or, more rarely, terdentate) ligand, such that the Ru centre can crosslink polymers. Second, the loading rate and physicochemical properties of the Ru complex must be carefully selected because covalent conjugation of the Ru complex with the polymer can affect the complex's physicochemical properties such as H₂O solubility and/or hydrophilic/hydrophobic balance. Third, functionalities sensitive to external stimuli can be inserted in the polymer backbone and or pendants or in the Ru centre. Photoresponsive functionalities are the most popular because they enable precise spatial and temporal control of active compound release. Various polymeric architectures can be obtained, namely linear or brush (co)polymers, branched polymers (dendrimers) and supramolecular polymers. Depending on where the Ru centre is conjugated to the polymer (the backbone, a lateral pendant or the centre of a star polymer), different synthetic strategies are available and these are covered in the following discussion.

[H1] Linear and brush metallopolymer prodrugs

The synthesis of linear or brush metallopolymers requires the preparation of functionalized polymers as macroligands before coordination of the therapeutic Ru agents, which bind the polymer through ligand exchange or by presenting a reactive functional group on their periphery (FIG. 5). To ensure efficient self-assembly and to protect the conjugated Ru complex from the biological medium, block copolymers with hydrophilic and hydrophobic segments are most commonly used for the preparation of micelles. Therapeutically-relevant Ru agents have been covalently conjugated to the hydrophobic segment, allowing their placement in the core of the micelle. The functional groups for the coordination of the Ru

complexes are usually inserted as the final groups in the lateral chains of brush copolymers. These ligand groups can be strong or weak ligands for Ru.

[H2] Coordination of Ru to strong donor sites on a polymer

Ru(II) complexes that release diatomic therapeutic gases are the major type of Ru complexes that form metallopolymers by strong Ru–L_{polymer} linkages. The complexes can be activated chemically¹⁴⁸⁻¹⁵⁰ or photochemically¹³⁶, and the gas molecules are liberated without the complete degradation of the polymer backbone of a brush copolymer. The ligands for the Ru coordination can be inserted during the monomer preparation before polymerization. For example, up to 2500 equivalents of a CO-releasing Ru complex are stored in every micelle of a triblock copolymer featuring a hydrophilic PEG block, a poly(orthinine acrylamide) block bearing [Ru(CO)₃Cl(orthinoate)] moieties and a hydrophobic poly(*n*-butylacrylamide) block¹⁴⁸ (FIG. 5a). The incorporation of CO donors (with 15–37 Ru-based units per polymer, 4.7–10.3wt% Ru) into micelles with a hydrodynamic diameter of 29–44 nm makes the Ru complex less susceptible to attack from thiols (such as cysteamine and glutathione) than is free [Ru(CO)₃Cl(glycinato)] (**CORM-3**, FIG. 1), such that the Ru complexes in the polymer release CO more slowly. Thus, there is a substantial reduction in the cytotoxicity of the [Ru(CO)₃Cl(amido acidate)] moiety due to the stealth properties of the PEG block. Moreover, in contrast to free [Ru(CO)₃Cl(glycinato)], the CO-releasing micelles efficiently attenuated lipopolysaccharide-induced nuclear factor-κB activation in THP-1 Blue cells derived from human monocytes (which are linked to inflammation)¹⁴⁸. In a subsequent study, a pyridine ligand was inserted in the hydrophobic polyvinyl block to give P(OEGA)-P(4VP-CORM-2) (FIG. 5a)¹⁵⁰, a polymer variant of **CORM-2**. Whereas CO release was spontaneous in the earlier study, in this study the rate of CO release could be controlled by changing pH. CO release was rapid in acidic conditions and slower at neutral pH, where the polymer micelles may form a compact structure in which the CO-donor Ru complexes are more protected inside the micelle core, preventing fast CO release. Conversely, at acidic pH, the pyridine groups not bound to Ru tend to be protonated, resulting in a less compact structure in which the Ru centres are more accessible and liable to release CO. This approach of using a polyvinylpyridine block to construct a polymeric version of **CORM-2** can also be applied to making a polymeric NO-releasing agent featuring *trans*-{Ru[1,2-bis(pyridine-2-carboximido)-4,5-dimethylbenzene](L_{polymer})NO} fragments. The polyvinylpyridine also features cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA) in a copolymer formed from HEMA and ethyleneglycol dimethacrylate (EDGMA) (synthesized by radical-induced copolymerization)¹⁵¹ (FIG. 5b). In contrast to carriers designed for CO-releasing Ru complexes, the rapid release of NO is strictly dependent on exposure to UV light (even with a low intensity of 5–10 mW), such that NO release is more controllable^{151, 150}.

Functionalized tpy complexes were used as a linker for crosslinking of diblock copolymers of pentafluorophenyl acrylate (PFA) and 2-(2',3',4',6'-tetra-O-acetyl- β -D-glucosyloxy)ethyl methacrylate (AcGlcEMA) prepared by reversible addition–fragmentation chain-transfer (RAFT) polymerization¹⁵² (FIG. 6). After deacylation, nanoparticles with an average size of 60 nm had a core–shell structure with the Ru(II) complex as the core and glycopolymers as the shell. The nanoparticles were H₂O-soluble owing to the hydrophilic corona of glycopolymers and showed low cytotoxicity in KB cells.) These nanoparticles might therefore be useful as a cellular label for one-photon and two-photon fluorescence bioimaging. Coordination of Ru on labile ligands

A polymer decorated with weakly basic ligands can serve as a scaffold for a triggered release or triggered activation of a metallopolymer prodrug, wherein the weak Ru–L_{polymer} bonds are readily cleaved in a controlled manner. The activation can be effected by various stimuli, such as a change in the biological medium, hydrolysis or light.

As described above for physical encapsulation, natural biodegradable polymers have also been used as delivery vehicles for Ru complexes, whose release is activated by hydrolysis of the polymer in the biological medium^{153–154}. The linear polysaccharide chitosan is one such biodegradable polymer, and the primary amine in each monomer can serve as a functional handle to which a Ru centre can be appended. For example, the amine can be decorated with caffeic acid as a ligand or undergo a Schiff base condensation to afford an imine ligand^{153–154} (FIG. 6a). The conjugation of caffeic acid-modified chitosan to the {Ru(*p*-cym)Cl}⁺ fragment induces a change of backbone chitosan structure¹⁵² to give 30–120 nm particles. Anticancer activity may be realized if hydrolysis occurs to release the active dimer {[(η^6 -arene)Ru]₂(η^2 -OH)₃}⁺. Likewise, the Schiff base is also not a strong ligand for Ru(III), which can be released so as to afford antibacterial activity against Gram-positive bacteria, such as *B. subtilis*, *S. aureus*, and Gram-negative bacteria, such as *E. coli*, *K. pneumoniae* and *P. aeruginosa*¹⁵⁴.

In photoactivated chemotherapy (PACT), a non-toxic or minimally toxic prodrug candidate becomes more toxic after light irradiation. This photo-release strategy is based on masking the functional groups involved in the toxicity using a photo-cleavable motif. As in photodynamic therapy (PDT), the use of photo-responsive cages as carriers is of great interest owing to the spatial and temporal control of activation.

Inspired by studies of Ru(II) polypyridyl photocages,^{155–156} two studies reported the preparation of different photoresponsive platforms based on metalloblock copolymers for combined PACT and PDT. Both studies made use of an active Ru fragment (FIG. 7a) bound to the polymer through a weakly coordinating 4-[(6-hydroxyhexyl)oxy]benzonitrile residue^{157–158}, such that red light cleaves the Ru–L_{polymer} bond to release the anticancer drug candidate and generate ¹O₂ to inhibit cancer cell growth. In

the first study¹⁵⁷, $\{\text{Ru}(2,2'\text{-biquinoline})_2\}^{2+}$ binds up to two nitriles, each being at the end of a long organic chain terminated by a PEG chain that confers stealth properties. Here, red light irradiation causes loss of the nitrile to generate $\{\text{Ru}(2,2'\text{-biquinoline})_2(\text{OH}_2)_2\}^{2+}$. In the second study, a single hydrophobic photolabile Ru complex was anchored in the main chain on the block copolymer.¹⁵⁸ Depending on the size of each of its constituent blocks, the amphiphile can assume different mesostructures: micelle, hollow sphere and large compound micelle. It is the micelles, of diameter 12 nm (41wt% Ru loading) that had the highest cellular uptake and anticancer activity,¹⁵⁸ decreasing HeLa cell viability by 73% after red-light irradiation.

The position of the coordination of the Ru complex in the polymer main chain or lateral chains, as well as its molecular weight, were crucial for the structure and the size of the nanoparticles as well as cellular uptake. For these two metallopolymer, the Ru complexes were not only used as photoactivators but were also responsible for the bioactivity of the metallopolymer.

Related to the above design is a hydrogel based on biocompatible hyaluronic acid hydrazide polymer chains, which can be crosslinked with $[\text{Ru}(\text{bpy})_2(3\text{-pyridinaldehyde})_2]^{2+}$ in a Schiff base condensation that affords a photodegradable linkage (FIG. 7b)¹⁵⁹. The Ru complex function primarily as a photoactivator and remains linked to one polymer chain after photoactivation. This metallopolymer gel can be loaded with bacterial β -lactamase TEM1 as a model protein cargo, the release of which occurs after photodissociation of a Ru–N_{pyridine} bond. The photodelivery of TEM1 from microgel particles (average diameter $74 \pm 6 \mu\text{m}$) can occur even with low doses of visible light, with complete degradation of the particles occurring over 60 s at 10 mW cm^{-2} . Moreover, the resulting metallopolymer containing the imine of $[\text{Ru}(\text{bpy})_2(3\text{-pyridinaldehyde})(\text{OH}_2)_2]^{2+}$ could act as a ROS photogenerator.

[H1] Linear and star metallopolymer prodrugs

Ru(II) polypyridyls have been used as the central core in the preparation of linear or star metallopolymer, the choice of which depends on the number of functionalized ligands on the Ru centre. The two strategies for the synthesis of linear and star metallopolymer developed so far, convergent and divergent, are now described (FIG. 8).

[H2] Convergent strategy

The convergent strategy for the preparation of star metallopolymer drugs is based on the prior synthesis of polymers as macroligands followed by the coordination of the Ru centre by ligand exchange. For example, bpy ligands are readily functionalized with biodegradable polyesters¹⁶⁰⁻¹⁶² using ring-opening polymerization of cyclic ester monomers. PCL (FIG. 8A) is amenable for incorporation into a drug

carrier owing to its low degradation rate and its permeability to small molecules studies¹⁶⁰⁻¹⁶¹. The polymerization of ϵ -caprolactone by subcritical CO₂ processing gives biodegradable open cell foams, which contrasts the otherwise semicrystalline microstructure of poly(ϵ -caprolactone) and enabled its use for culturing cells¹⁶¹. The same convergent strategy was also used to develop a polymer-containing analog of the anticancer drug candidate **TM34** ([Ru(C₅H₅)(bpy)(PPh₃)]OTf) (FIG. 8A). The polymer drug had an IC₅₀ in the micromolar range in human MCF-7 and MDA-MB-231 breast cancer cell lines and the A2780 ovarian adenocarcinoma cell line¹⁶².

[H2] Divergent strategy

The divergent strategy uses a Ru complex as a metaloinitiator for polymerization. Polymerization by ring-opening polymerization or radical polymerization is carried out after pre-assembly of the Ru complexes (FIG. 8b), which have end reactive groups that enable the initiation of polymerization.

Luminescent [Ru(bpy)₃]²⁺-centred star block copolymers with a hydrophobic PLA core and a hydrophilic poly(acrylic acid) corona (FIG. 8b) have served as a preliminary model for imaging probes¹⁶³. In particular, a Ru complex was used as a metaloinitiator for 4-(dimethylamino)pyridine-catalysed ring-opening polymerization of lactide, followed by atom transfer radical polymerization to introduce poly(*tert*-butyl)acrylate chains. The esters were finally hydrolysed using Me₃SiI to give a H₂O-soluble poly(acrylate) corona. The same divergent strategy was used to synthesise a star-shaped [Ru(bpy)₃]²⁺-centred poly(ethyleneimine), where the polycationic PEI polymer is used for gene delivery¹⁶⁴⁻¹⁶⁵ by electrostatic binding of the carrier with DNA, thereby protecting it against cleavage by nucleases¹⁶⁶. As an alternative to PEG, a biocompatible polyoxazoline was used to confer stealth properties to the metallopolymer drug. The living cationic ring-opening polymerization of 2-ethyl-2-oxazoline followed by an acid hydrolysis of amide groups provided protection for the hexafunctional [Ru(bpy)(CH₂Cl)₂]²⁺ core (FIG. 8b). The system seems to be limited for imaging properties and was first tested for the transfection of the LNCaP prostate cancer cell line. The entrapment of DNA in the polyoxazoline was comparable to that by Ru-free linear cationic polymers of a higher molecular weight and was more efficient than that by Ru-free branched PEI of a similar molecular weight.

A NO-donor salen ligand Ru complex functionalized with two terminal styrene pendants were incorporated into the main polymer chain during the radical polymerisation of ethyleneglycoldimethacrylate, in the presence of azobisisobutyronitrile as radical initiator, for the preparation of porous particles (75 μ m or 125 μ m in diameter and an average pore diameter of 60 μ m

(FIG. 8Bb).¹⁶⁷ After photoirradiation at 370 nm, this porous material released NO to proteins during *in vitro* tests on equine skeletal muscle myoglobin.

[H1] Ru centre-rich metallopolymers

In the synthesis of metallopolymers, it is sometimes difficult or impossible to control the loading of the Ru complexes, except in the case of a star-shaped architecture with a Ru-based central core. One method involves covalent conjugation of Ru complexes to well-defined architecturally branched polymers. The globular or spherical structure of these polymers — known as dendrimers — represent an alternative to using polymeric nanoparticles in delivery systems. However, most dendrimer backbones are not easily biodegradable, such that Ru complexes are almost always covalently conjugated to the end of the arms constituting the dendrimer shell and not to the dendrimer core (only one example exists of physical encapsulation of a Ru complex in a dendrimer)¹⁶⁸. The charge on Ru complexes conjugated to a dendrimer shell determines the conjugate's H₂O solubility and can be adjusted to induce selective accumulation in cellular compartments and thereby tune toxicity. In most cases, the strategies used to synthesize standard polymers have also been used for the conjugation of Ru complexes. The ligand units are inserted as terminal groups on branched chains of the dendrimer shell, after which they bind Ru centres by displacing weakly bound ligands¹⁶⁹⁻¹⁷⁴.

In contrast to linear polymers or those with few crosslinks, assemblies based on dendrimers have a dense architecture, which probably slows the release of encapsulated Ru complexes substantially. However, a direct relationship between chemical structure and the bioactivity of dendrimers has yet to be rationalized. Dendrimers are among the smallest drug delivery carriers, with sizes in the range of tens of nanometers, although a direct size comparison between various types of dendrimers is difficult owing to different molecular packing. Hydrogen bonding in polyamide dendrimers increases the density of globular dendrimers with higher generations, whereas polyamine chains maintain greater flexibility leading to less dense globular dendrimers. To study the effects of dendrimer size on cytotoxicity, a large library of first- to fourth-generation Ru(II) arene complexes based on the diaminobutane (DAB)-poly(propyleneimine) dendrimer scaffold was generated¹⁶⁹⁻¹⁷² (FIG. S2). Dendrimer toxicity generally increased as a function of its size¹⁷⁵ but, surprisingly, increased cytotoxicity was observed for each group of larger dendrimers (third and fourth generations), demonstrating that cytotoxicity is clearly linked to metallodendrimer size. Each drug candidate featured a terminal aromatic group conjugated to an imine functionality. The aromatic substituents minimize and counterbalance the effect of the imine groups, which were susceptible to hydrolysis in the final products {[Ru(arene)Cl]_n(dendrimer ligand)}^{m+}. In each case, replacing the Cl⁻ ligands with PTA affords cationic complexes with lower IC₅₀ values in A2780 and A2780 cisR cell lines, owing to the interaction of PTA with DNA¹⁷². The 4th generation compound with the *N,O* bidentate neutral ligand and *p*-cymene ligand (FIG. S2) with 32 arms had the highest

cytotoxicity ($IC_{50} = 0.8 \mu M$ in A2780 cells, $IC_{50} = 2.7 \mu M$ in A2780cisR cells and $IC_{50} = 2.6 \mu M$ in healthy HEK cells. Preliminary biological studies showed that the insertion of ferrocenyl group conjugated on the Ru complex (FIG. S2) reduced the proliferation of A2780 and A2780cis R cells by >50% ($IC_{50} < 5 \mu M$ at equi-iron concentrations of $5 \mu M$) and substantially reduced the proliferation of SISO human cervix cancer cells, LCLC-103H human lung cancer cells and 5637 bladder cancer cells in vitro¹⁷².

A dendritic system based on a pentaerythritol core bearing the pendant tpy (for the coordination of Ru centre) and functionalized with 1,2-dicarba-*closo*-dodecaborane units (FIG. S2) has been used in BNCT¹⁷⁶⁻¹⁷⁷. This promising dendrimer has yet to be biologically tested. [

[H1] Concluding remarks

The use of Ru complexes in medicinal chemistry has increased substantially in the past decade, encouraged by clinical trials of **NAMI-A**, **KP1039** and **TLD-1433**. Most Ru complexes discussed in this Review are envisioned for the treatment of various cancers using chemotherapy and/or other therapies, such as PDT or BNCT. However, research on the encapsulation of Ru complexes in polymeric carriers is still in its infancy and must compete with research on the encapsulation of Pt-based drugs¹⁹⁶⁻¹⁹⁷, which were discovered earlier and dominate the chemotherapeutics market. In contrast to Pt-based drugs, two strategies, developed in parallel, exist for the encapsulation of Ru complexes — physical encapsulation and covalent conjugation. Commercially available or easily accessible polymers are typically chosen for the encapsulation of Ru compounds. Although the synthetic approaches are clear, the relationship between the properties of the polymer and the properties of the final polymer-encapsulated complex has rarely been clarified or linked to the delivery properties of these complexes in biological media. Physical encapsulation remains the most common strategy to deliver Ru complexes because it is simpler and faster than covalent conjugation. However, the structure of these physically or covalently bound species advances towards more sophisticated architectures comprising a time and space controlled release of biological active Ru complex using an external stimulus.

For the physical encapsulated Ru complex delivery systems, the stability of the carrier is an important consideration for improved drug delivery and prolonged storage of nanocarriers. The effect of nanocarrier shape on the efficiency of drug delivery has been only lightly studied¹⁹⁸. Nanocarriers used for the physical encapsulation of Ru complexes are most often spherical; tubular nanocarriers that can deliver metal complexes and specifically target cellular compartments are rare. The functionalization of inorganic nanotubes (such as carbon nanotubes) is difficult, such that biodegradable organic nanotubes derived from alginate and chitosan¹⁹⁹ appear better vehicles for the delivery of biologically active Ru complexes. Another emerging approach is the preparation of delivery systems containing two anticancer

drugs for combination therapy, such as chemotherapy combined with PDT or BNCT. Ru complexes have already been incorporated on the surface of Au nanomaterials or C nanotubes as photothermal agents⁵⁵. Physical encapsulation of Ru complexes in biocompatible photothermic polymers²⁰⁰, such as polyaniline or melanine derivatives, is another potential delivery system for combined photothermal therapy and chemotherapy.

Overall, research into the encapsulation of Ru complexes will undoubtedly continue to develop in the future with the expected increase in the number of FDA-approved Ru-based drugs. Owing to the structural variety of polymers and Ru complexes, new delivery platforms will certainly be developed in the coming years for various biological applications beyond their conventional use as anticancer therapies.

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[H1] Competing interests

The authors declare no competing interests.

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Display items

Box 1| The mechanisms of action of Ru complexes

DNA damage

Cellular dysfunction in disease or cancer can result from DNA damage (such as autoxidation in air, disproportionation or hydrolysis). The proliferation rate in cancer cells is high and is regulated by the cell cycle and DNA replication. Therefore, damaged DNA is a key target for Ru-based anticancer drug

candidates and is the major mechanism by which many FDA-approved metallo-therapeutics (such as Pt-based drugs) and organic oncology drugs (such as doxorubicin and gemcitabine) operate. Ru complexes with planar aromatic ligands selectively bind to DNA by covalent and/or non-covalent modes. Covalent binding is irreversible and results in the formation of DNA–Ru complex adducts that distort the DNA backbone and disrupt replication and transcription, leading to cell death (as observed for (η^6 -arene)Ru^{II} complexes, such as **RM-175** (FIG. 1)). Non-covalent binding is usually reversible and includes interactions such as electrostatic binding, intercalation and groove binding, as observed for **RAPTA-C** (FIG. 1). Most cationic Ru^{II} complexes have one or more planar aromatic ligands, which can intercalate between adjacent base pairs in DNA. Distortion of the double helix caused by these ligands can inhibit or completely block transcription and replication.

DNA metabolism

A set of proteins are responsible for genome integrity. Topoisomerase II is the principal enzyme in the cell nucleus involved in DNA replication, transcription, recombination and sister chromatid segregation during mitosis¹⁷⁹. Selective inhibition of topoisomerase II can inhibit neoplastic cell proliferation and possibly induces apoptosis through DNA fragmentation by the accumulation of permanent double strand breaks. For example, [RuCl₂(C₆H₆)(Me₂SO)] is a topoisomerase II inhibitor that covalently binds to DNA by ionic interaction of the Ru^{II} centre with DNA nucleobases¹⁸⁰. **KP1019** (FIG. 1) localizes in the cytoplasm and binds efficiently to serum proteins¹⁸¹ and interferes with the P-glycoprotein¹⁸².

The antioxidant glutathione (GSH) protects cells from reactive oxygen species (ROS), UV radiation and heavy metal toxicity. In its reduced form, GSH is readily oxidized to glutathione disulfide (GSSG) by the enzyme glutathione *S*-transferase (GST). Overexpression of GST by tumour cells is implicated in drug resistance, and an organometallic (*p*-cymene)Ru^{II} complex conjugated to ethacrynic acid, a GST inhibitor, efficiently inhibits GST and is toxic to a GST-overexpressing cancer cell line¹⁸³.

Protein kinases

Protein kinases are phosphorylating enzymes that have important roles in signalling pathways associated with intercellular uptake and cell proliferation. The identification of novel kinase inhibitors is important for the development of new anticancer therapies. The major strategy for the design of new Ru complexes that target protein kinases involves the use of substitutionally inert Ru^{II} centres coordinated to a bioactive ligand. The resulting rigid, stable 3D structures are extremely selective protein kinase inhibitors¹⁸⁴ (**DW1/2**, FIG. 1).

Box 2 | Methods of targeting tumours

The pH in cancerous or inflamed tissues is usually abnormally low (pH = 5–6 in the extracellular medium) and local hyperthermia (around 42 °C). Many tumours have a leaky vasculature and lack or have altered lymphatic drainage. The vascular endothelium in tumours proliferates rapidly and

discontinuously, which results in a disorganized vasculature with a large number of ‘open’ intercellular junctions (0.2–1.2 μm in diameter, compared with $<10\text{ nm}$ in normal vessels). These biological characteristics are exploited to design efficient drug delivery carriers, such as anticancer prodrugs. High molecular weight molecules, such as polymers and particles of diameter $\sim 20\text{--}500\text{ nm}$ (except for ‘stealth’ carriers), passively accumulate in the tumour area. This ‘passive targeting’, termed the enhanced permeability and retention (EPR) effect⁷¹⁻⁷³, is possible because these molecules can cross the tumour endothelial barrier through the open junctions of the leaky vasculature and accumulate because of insufficient lymphatic drainage from the tumour. Of note, ‘targeting’ in this context does not refer to a specific interaction between a target molecule and a cellular receptor but instead refers to the selective accumulation of the nanocarrier in tumour tissues rather than in healthy tissues — the drug concentration in tumour tissues can be up to 10–100-fold higher than in healthy tissues¹⁸⁵. The EPR effect allows the use of a lower drug dose and increases the efficacy of the biologically active compound, thereby limiting adverse effects. The diameter of polymer particles does not remain constant during the delivery process and tends to decrease over time. Consequently, the design of fairly large nanocarriers (final diameter $<100\text{ nm}$ to avoid removal from the blood and accumulation in the liver and spleen) with high Ru loading is preferred¹⁸⁶. Nanoparticles of intermediate diameter (10–100 nm) avoid renal clearance and have prolonged circulatory times compared with nanoparticles $<10\text{ nm}$ in diameter), owing to their slow transport across the endothelium¹⁸⁷. Despite their small size, nanoparticles $<20\text{ nm}$ in diameter show better tumour penetration¹⁸⁸⁻¹⁹⁰ because they can pass through the leaky capillary walls in tumour tissue and also return to the bloodstream by diffusion. It is hypothesized that nanoparticles 10–20 nm in diameter can re-enter the tumour tissue without being eliminated by any clearance mechanisms¹⁹¹, which might explain their efficient accumulation in tumour tissues. The contradictory results in these studies suggest that the accumulation of nanoparticles in the tumour is not only dependent on their size.

To obtain an efficient delivery system, the physicochemical properties of a nanocarrier, such as the biocompatibility, hydrophilicity/hydrophobicity balance, carrier size and surface charge of the polymer can be adjusted during nanocarrier preparation. All of these features affect the non-specific interactions of polymers with cells of reticuloendothelial system (RES) in the circulation and interactions of polymers with various components inside tumour tissues. The uptake of nanocarriers, especially those that are hydrophobic, by the RES is predominantly through phagocytosis by macrophages, which is initiated by the adsorption of various serum proteins termed opsonins. The EPR effect is time-dependent and requires adequate circulation stability for at least several hours to be efficient. To increase their circulation time and protect them from opsonization, carriers can be coated with a sufficiently thick and dense layer of hydrophilic polymers, such as PEG or other polymeric glycols, or synthetic glucuronic acid derivatives or polyphosphoramides to form ‘stealth carriers’¹⁹¹⁻¹⁹⁴. Optimization of the hydrophilic/hydrophobic balance of a nanocarrier is the primary factor affecting the efficiency of drug

delivery to tumour tissues. Generally recognized as safe by the FDA, PEG is the standard polymer for the preparation of stealth carriers owing to its biocompatibility⁹¹ and low toxicity¹⁹⁵. The targeting of nanocarriers to tumour tissues is not only limited to the EPR effect but can also involve pH-dependent hydrolytic activation in the tumour tissue or redox processes in the cytosol. Furthermore, molecules such as peptides, antibodies or carbohydrate units, can be attached to the surface of nanocarriers to increase tumour specificity. The choice of molecule depends on the mechanism of the transported drug; for example, chemotherapeutic agents have various mechanisms of action, such as DNA alkylation (cisplatin) or intercalation (doxorubicin), or inhibition of microtubules (taxanes), metabolic processes (gemcitabine) or angiogenesis (endostatin).

Figure 1 | Representative Ru complexes with medicinal potential. A variety of Ru species — from Ru^{II} to Ru^{IV}, cationic to anionic — have been designed for medicinal applications. The complexes were intended as agents for chemotherapy³⁻¹¹ and photodynamic therapy¹²⁻¹⁵, as well miscellaneous based on their vasodilation, antioxidant, anti-inflammatory, anti-microbial and anti-malarial properties.^{16,38, 119, 124, 136, 149-150}

Figure 2 | Methods to encapsulate Ru complexes in polymers. Ru complexes can have different charges and are usually incorporated into polymers using either covalent conjugation or physical encapsulation. The former is irreversible, while the latter process is typically reversible such that the Ru species can often leach from the assemblies.

Figure 3 | Some prominent biodegradable polymers. Chitosan is a linear polymer of an aminosugar, which can be protonated under physiological conditions. In contrast, alginate is also a sugar derivative but is an anionic polymer. Poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and the corresponding copolymer poly(lactid-co-glycolic)acid (PLGA), as well as poly(ϵ -caprolactone) (PCL) are all biodegradable polyesters.

Figure 4 | Synthetic strategies for Ru-conjugated polymer prodrug candidates. **a** | The convergent synthesis approach involves coordinating Ru to a preformed macromolecular organic ligand. **b** | Alternatively, the divergent strategy uses a Ru complex with strongly bound ligands as a metalloinitiator for polymerization.

Figure 5 | Photoactivable nanocarriers. **a** | Ru^{II}(CO)₃ fragments serve as sources of CO in hydrolyzable polymers that are activation by hydrolysis. **b** | Light activation can cause a Ru–NO bond to break and release NO or release an anticancer Ru drug that generates ¹O₂¹⁵⁷. The toxicity of this metalloprodrug principally comes from the photoactivation of Ru drug candidate remaining coordinated in the polymer chain.

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1503 Figure 6 | **Ru conjugates of chitosan.** **a** | Caffeic acid-modified chitosan can bind Ru through its
1504 catechololate moiety. The Ru moiety can undergo hydrolysis to afford the bioactive dimer $\{[(\eta^6\text{-arene})\text{Ru}]_2(\eta^2\text{-OH})_3\}^+$. **b** | A chitosan Schiff base can serve as a ligand for Ru in a polymer drug
1506 candidate with antibacterial activity.

1507
1508 Figure 7 | **Photo-triggered aquation of Ru–polymer conjugates unmasks reactive moieties.** **a** |
1509 Displacement of nitrile ligands with H_2O affords reactive Ru polypyridyl aquos that can sensitize the
1510 formation of $^1\text{O}_2$. **b** | Here, the Ru serves as a photolabile cross-link between polymer chains in a gel.
1511 Breaking this link can enable the release of cargo such as proteins.

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1513 Figure 8 | **Synthesis of Ru complexes bearing ligands with two (up to six) polymer chains.** **A** | The
1514 convergent synthesis method can afford Ru^{II} species with bpy ligands difunctionalized with polymer
1515 chains. **Ba** | Ru complexes bearing reactive groups at their periphery can serve as initiators from which
1516 ring-opening polymerization can occur. **Bb** | A $[\text{Ru}(\text{salen})(\text{NO})\text{Cl}]$ derivative with pendant vinyl groups
1517 is an initiator for radical polymerization.

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