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# 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 H<sub>2</sub>O 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*-[PtCl<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] (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 1978<sup>1</sup>. 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 crosslinks<sup>2</sup>. 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<sup>3–8</sup>, enzyme inhibitors<sup>9–11</sup>, photosensitizers in photodynamic therapy<sup>12–15</sup>, and immunosuppressants<sup>16</sup>, and in biology as

36 luminescent probes (for imaging and the detection of biomolecules and cellular compartments<sup>17-20</sup>).  
37 Although the cytotoxicity of Ru complexes has been known since the mid-1950s<sup>21</sup>, the therapeutic  
38 potential of Ru complexes was only recognized in the mid-1980s following the publication of Clarke's  
39 'activation by reduction' hypothesis for the molecular mechanism of Ru(III) complexes<sup>22</sup>. Under  
40 physiological conditions, Ru can exist in +II, +III and/or +IV oxidation states,<sup>23</sup> each of which is  
41 stabilized by different coordination environments. The most common forms are 18e<sup>-</sup> Ru(II) species —  
42 either pseudo-octahedral complexes or pseudo-tetrahedral complexes featuring an η<sup>6</sup>-arene and three  
43 other donors. Ru(IV) complexes are typically highly soluble in H<sub>2</sub>O and often feature oxo, carboxylatoor  
44 sulfido ligands. In general, Ru(III) complexes appear to be less toxic than Ru(II) and Ru(IV) complexes<sup>24</sup>.  
45 Nevertheless, the potentials of the Ru redox couples are sensitive to the ligand environment and are  
46 readily accessed under physiological conditions, such that interconversion between the different  
47 oxidation states can be fast in vivo. Indeed, Ru(IV) or Ru(III) can be reduced to Ru(II) by biological  
48 reductants (such as ascorbate, glutathione and single-electron-transfer proteins), and Ru(II) can be  
49 oxidized to Ru(III) or Ru(IV) by biological oxidants (such as O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> and cytochrome oxidase)<sup>24,25</sup>.

51 The major strategy used in the development of Ru-based anticancer drug candidates is to administer a  
52 relatively inert high-valent compound that undergoes reduction in vivo to its active reduced form. This  
53 'activation by reduction' is most commonly seen with "biologically inactive" Ru(III) prodrugs that must  
54 be reduced to their Ru(II) form to be biologically active (for example, **NAMI-A** or **KP-1339** complexes;  
55 FIG. 1), a process analogous to the activation of Pt(IV) prodrugs to (relatively) labile Pt(II) species. This  
56 is possible in the presence of high levels of biological reductants (for example, glutathione)<sup>26</sup>, such as  
57 in the hypoxic regions typically found in solid tumours<sup>27,28</sup>. If the active Ru(II) drug candidate leaves the  
58 tumour cell and moves to a more oxygenated environment, such as in healthy tissue, biological oxidants  
59 convert the Ru(II) complex to its inactive Ru(III) form<sup>24</sup>. Mitochondrial and microsomal single electron  
60 transfer proteins can also reduce Ru(III) to Ru(II)<sup>29</sup>. However this Ru reduction can be also performed  
61 by transmembrane electron transport systems enabling the development of metallo-prodrug candidates  
62 with anticancer properties independent of the cell entry mechanism<sup>23</sup>. These Ru(II) complexes then  
63 undergo aquation, typically with displacement of one or two weakly bound donors such as halido ligands.  
64 For example, the Ru(II) arenes [Ru(η<sup>6</sup>-*p*-cymene)Cl<sub>2</sub>(pta)] (**RAPTA-C**; pta = 1,3,5-triaza-7-  
65 phosphadamantane; FIG. 1) and [Ru(η<sup>6</sup>-toluene)Cl<sub>2</sub>(pta)] (**RAPTA-T**)<sup>30-32</sup>, as well as the diamine-  
66 arene species [Ru(η<sup>6</sup>-4-methylbiphenyl)(1,2-diaminoethane)Cl]PF<sub>6</sub> (**RM-175**) and their derivatives<sup>33,34</sup>  
67 each have Cl<sup>-</sup> ligands that can be displaced by H<sub>2</sub>O. Substitution at a Ru(III) centre is also possible, and  
68 can afford Ru(III) aquation products and polynuclear species<sup>35-37</sup>. In both the reduction and aquation  
69 strategies to yield labile species, the medicinal efficacy of the ruthenium complex is directly related to  
70 the inherent reactivity of the Ru centre. Another strategy in drug design with Ru centres is to construct  
71 building blocks with three-dimension structures that have relative kinetic inertness for ligand exchange.

72 Here, the presence of Ru centre enable 3D geometries not available with organic chemistry. For example,  
73 the Ru(II) complex **DW1** and its enantiomer **DW2** mimic the shape of the alkaloid staurosporine, and  
74 complexes thus also exhibit impressive kinase inhibition activity (targeting glycogen synthase kinase 3  
75 (GSK3) signalling) and cytotoxicity in human melanoma cancer cells<sup>38</sup>.

76  
77 The biological properties of Ru complexes — such as bioactivity, cellular uptake and intracellular  
78 distribution — depend, among others, on chemical properties such as electronegativity, chemical  
79 hardness, stereochemistry and net charge of the Ru centre. Ru drug candidates often feature strongly  
80 bound ligands, such as amine, phosphine,  $\pi$ -bound arenes and cyclopentadienyl derivatives. These are  
81 complemented by weakly bound ligands such as  $\text{Cl}^-$  or  $\text{RCO}_2^-$ , which can be displaced by chelators or  
82 simply when an excess of competitive ligands is present. As is the case for reduced Pt derivatives, the  
83 lower-valent Ru(II) and Ru(III) complexes have favourable ligand exchange kinetics with O-donor and  
84 N-donor ligands. Ru complexes have several other advantages for biological and medicinal applications,  
85 including their usual low (or zero) toxicity to healthy tissues and their distinct mode of action. Indeed,  
86 they operate through different pathways to most Pt-based drugs, which typically only interact with DNA  
87 (BOX 1). Ru(II) and Ru(III) complexes have similar ligand exchange kinetics to the Pt(II) complexes  
88 used as antineoplastic drugs<sup>39</sup>. For small ligands such as  $\text{H}_2\text{O}$ , the ligand exchange rate on the Ru centre  
89 is on the order of hours, which is similar to the timescale of cell division in many cell types<sup>40</sup>.

90  
91 Furthermore, Ru can bind biomolecules responsible for Fe solubilization and transport in plasma,  
92 including serum transferrin and albumin. Rapidly dividing cells, such as cancer cells, require more Fe,  
93 which leads to an upregulation of the number of transferrin receptors at the cell surface<sup>41</sup>. Consequently,  
94 Ru complexes have been proposed to preferentially target cancer cells over healthy cells<sup>42</sup>, which might  
95 explain their fairly low toxicity to the latter. This hypothesis is still the subject of debate<sup>43</sup> because Ru  
96 complexes can enter cells by both transferrin-dependent and transferrin-independent mechanisms<sup>44-45</sup>.

97  
98 To date, no Ru drug candidates have been commercialized, although four Ru complexes are in various  
99 stages of clinical trials<sup>46</sup>. Trials of two well-known Ru-based drug candidates, *trans*-  
100  $\text{ImH}[\text{Ru}^{\text{III}}(\text{Im})(\text{Me}_2\text{SO})\text{Cl}_4]$  (**NAMI-A**; FIG. 1; Im = imidazole) and *trans*-indazolium $[\text{Ru}^{\text{III}}(1H$ -  
101  $\text{indazole})_2\text{Cl}_4]$  (**KP1019**) have been halted since **NAMI-A** showed limited efficacy in a phase II clinical  
102 trial<sup>47</sup> and **KP1019** exhibited poor solubility under physiological conditions in a phase I clinical trial<sup>48</sup>-  
103 <sup>49</sup>. However, (pre-)clinical tests are being conducted on other Ru complexes: the *trans*- $\text{Na}[\text{Ru}^{\text{III}}(1H$ -  
104  $\text{indazole})_2\text{Cl}_4]$  (**KP1339**), the Ru(II) polypyridyl **TLD-1433** and **RAPTA-C** (FIG. 1). We note that by  
105 simply converting the indazolium salt **KP1019** to its  $\text{Na}^+$  salt **KP1339** affords a complex with greater  
106  $\text{H}_2\text{O}$  solubility that is in a phase IIA trial at the time of writing<sup>50-51</sup>. **TLD-1433** just completed phase I  
107 clinical trial as a photosensitizer for photodynamic therapy (PDT) to treat bladder cancer<sup>52-53</sup>. **RAPTA-**

108 C (FIG. 1) is currently in pre-clinical evaluation<sup>12</sup>. The testing of different Ru complexes as anticancer  
109 drug candidates in clinical studies is encouraging news for those wishing to develop Ru anticancer drugs  
110 as replacements for Pt-based drugs.

111  
112 Although the above strategies for Ru drug candidate design are sound, the poor solubility and/or low  
113 stability of many Ru complexes in aqueous solution under physiological conditions and/or  
114 metabolic/biodistribution profile limit their intravenous administration, and thus limits the amount of  
115 the complex that reaches the target tissue. Most Ru complexes studied to date have a short half-life in  
116 the circulation<sup>23,24</sup> and a high overall clearance rate. Thus, although Ru complexes are undoubtedly  
117 promising drug candidates<sup>25</sup>, new drug delivery methods<sup>54-55</sup> are needed in order for their therapeutic  
118 potential to be realized.

119  
120 [H1] Polymeric carriers for drug delivery

121 The aim of nanomedicine is to create more effective and safer medicines through the identification of  
122 new drugs with novel mechanisms of action and the development of innovative drug formulations and  
123 delivery methods. Of the various nanomaterials available for drug delivery, macromolecules have  
124 attracted increased interest<sup>56</sup>. Our definition of macromolecules includes biomacromolecules, as well as  
125 synthetic polymers or dendrimers.. The properties of these macromolecules depend on the choice of  
126 repeating unit(s) (in particular, its structure), the ratio between hydrophilic and hydrophobic moieties in  
127 the polymer, and the length or size (molecular weight and molecular weight distribution) of the polymer  
128 and the resulting phase morphology. Encapsulation of a drug within a polymer is based on a 'bottom  
129 up' approach, in which the properties of each building blocks and Ru moiety are combined to prepare  
130 nanoscale materials. Self-assembling macromolecules provide several advantages for drug delivery.  
131 Although most of these macromolecules are typically not bioactive, the final biological application will  
132 determine the choice of polymer and, in particular, its degradation kinetics, which is directly linked to  
133 its biodegradability. Biocompatible polymers are by definition non-toxic and do not induce an immune  
134 response. Among biocompatible polymers, biodegradable polymers<sup>57</sup> are mainly used for drug delivery,  
135 whereas non-biodegradable polymers as best suited for other applications, such as in bioimaging. A  
136 polymer is considered to be biodegradable if degradation occurs due to environmental action, which  
137 includes biological processes such as hydrolysis, oxidation and UV irradiation and/or biocatalytic  
138 processes involving, for example, bacteria, fungi and algae<sup>58</sup>. The definition of biodegradability that is  
139 used depends on the application of the polymer, such as biomedical or environmental applications. For  
140 example, biodegradation has been defined as enzymatic degradation and/or chemical decomposition  
141 associated with living organisms and their secretion products<sup>59</sup>. Biodegradable polymers for controlled  
142 drug delivery are typically degraded through hydrolysis by action of H<sub>2</sub>O molecules<sup>60</sup> and/or by enzyme

143 cleavage<sup>61</sup>. Several factors influence the degradation rate of a polymer<sup>61</sup>, but the most important  
144 parameter is the kinetics of drug release<sup>62</sup>.

145  
146 Polymer architecture (for example, the presence of hydrolytic linkages, chain branching,  
147 stereochemistry), molecular weight and morphology (for example, the length of the repeating units,  
148 degree of crystallinity, degree of chain flexibility, and surface area) have a major effect on the observed  
149 degradation rate, as well as the surrounding conditions (for example, pH and temperature)<sup>61</sup>. For a  
150 medical device, the size, geometry and porosity of the polymer are also important factors<sup>63</sup>.

151  
152 The chemical functionalities in the repeating unit or units and the corresponding polymer may confer  
153 responsiveness to different stimuli, such as physical stimuli (for example, ionic strength), chemical  
154 stimuli (for example, hydrolysis or pH), biochemical stimuli (for example, enzymes) or environmental  
155 stimuli (for example, light or temperature), which can lead to the triggered release of a biologically  
156 active compound.

157  
158 Hierarchically ordered and complex architectures can be obtained according to the ratio of hydrophilic  
159 and hydrophobic moieties, as well as the presence of directional short and/or large non-covalent  
160 interactions. Monomers and their corresponding self-assembled polymers can form various 1D, 2D or  
161 3D architectures, such as rods (linear); thin films (lamellar); nanoparticles, microparticles, micelles,  
162 vesicles and polymer–lipid hybrids (spherical); and worm-like micelles, nanotubes or hydrogels (3D-  
163 crosslinked networks). Self-assembly is driven by multiple types of interactions such as strong  
164 hydrophobic or electrostatic interactions, hydrogen bonding,  $\pi$ – $\pi$  stacking, metal–ligand coordination  
165 and stereocomplexation, or their synergistic associations. In contrast to covalent polymers,  
166 supramolecular polymers assemble by non-covalent and dynamic interactions. The self-assembled  
167 polymers in smart materials can self-heal or adapt their architecture in response to a small change of  
168 environment. The dynamic nature of self-assembly is responsible for the reversible nature of self-  
169 assembly and stimuli-responsiveness. In the case of delivery systems, the biologically active compound  
170 can be delivered in a triggered<sup>64-65</sup> and controlled<sup>66-67</sup> way that reduces adverse effects. By limiting the  
171 drug dose and ensuring the drug efficiently targets specific organelles, it can be possible to restrict drug  
172 resistance<sup>68-69</sup>. The principle challenge in the design and construction of self-assembled macromolecular  
173 systems is ensuring their stability, a task that requires precise control of the balance between attractive  
174 and repulsive forces.

175  
176 Finally, the surface of the polymer can be decorated with recognition motifs (such as small biomolecules  
177 or peptides) to increase the specificity of targeting to organelles or diseased tissues. The effect of  
178 vectorization of macromolecular delivery systems is not described in detail in this Review. Instead, we

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

181

182 Ru complexes used in medicinal applications often have low H<sub>2</sub>O solubility on account of their  
183 hydrophobic organic ligands. This and other inherent drawbacks can be addressed by encapsulating the  
184 complexes in delivery systems — a promising approach that differs from the traditional solution that  
185 involves formulating a drug with diluents (referred to as excipients). Macromolecules physically protect  
186 Ru complexes from biological degradation (by hydrolysis or reaction with proteolytic enzymes, radicals,  
187 reductants or nucleophilic species) and/or photodegradation (UV solar light). Furthermore, the  
188 polymeric matrix limits the exposure of healthy tissues to the drug and shields the Ru complex from the  
189 immune system, thereby preventing its elimination through renal excretion<sup>70</sup>.

190

191 A polymer carrier increases the targeted delivery of a small molecule drug by selectively accumulating  
192 in diseased tissues because of the enhanced permeability and retention (EPR) effect<sup>71-73</sup>. This effect  
193 results from the leaky, highly permeable vasculature and poor lymphatic drainage of these tissues (BOX  
194 2), resulting in the efficient extravasation of nanocarriers from the tumour vasculature and their retention  
195 in the tumour interstitium. The EPR effect also leads to the encapsulated drug having a greater half-life  
196 than the free drug in the bloodstream. In mice, the plasma half-life of most small-molecule drugs is less  
197 than 3 min, while drugs subject to the EPR effect have a half-life of 6 h or more<sup>74</sup>. A drug that exhibits  
198 an EPR effect in mice does not always also behave this way in humans<sup>75</sup>. In mice, tumour growth is  
199 limited to several weeks, whereas in humans' tumours can grow over several years, such that a long  
200 time period is available for angiogenesis. Furthermore, the EPR effect varies with the type of tumour<sup>76</sup>,  
201 and new tools need to be developed to improve the efficiency of nanocarriers for the delivery of  
202 anticancer drugs<sup>76</sup>.

203

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

214

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

228

#### 229 [H1] Physical encapsulation

230 Physical encapsulation of Ru complexes in polymeric carriers is similar to the encapsulation of organic  
231 biologically active compounds<sup>78</sup>. When designing polymers to serve as efficient Ru carriers, one must  
232 consider several features of the polymer, including size, charge, structure and degradation kinetics.

233

#### 234 [H2] Polymer size

235 The size of a nanocarrier directly affects the efficiency with which Ru complexes target diseased tissues.  
236 Therapeutically-relevant Ru complexes are small and can passively diffuse out of capillaries into the  
237 interstitial fluid, leading to undesired adverse effects. Tumour tissues are structurally distinct from  
238 healthy tissues (BOX 2), and these differences have been exploited to design efficient macromolecular  
239 delivery systems. Although the diameter of a carrier intended for biological applications is typically in  
240 the range 10 – 1000 nm, the carrier's diameter should ideally not exceed 300 nm in order to enable the  
241 EPR effect (BOX 2) to ensure efficient passive targeting of tumour tissues<sup>79</sup>. Directly linked to carrier  
242 size, the molecular weight of individual solvated biodegradable polymer chains should be >40 kDa to  
243 ensure an efficient EPR effect and a long circulation time without renal excretion<sup>70</sup>. In the case of  
244 individual solvated non-biodegradable polymer chains,<sup>80</sup> the molecular weight is limited to 40 kDa to  
245 ensure renal elimination. However, for nanocarriers based on the assembly of polymer chains (such as  
246 micelles or liposomes), it is not possible to estimate the minimum molecular weight for individual  
247 polymer chains owing to the variety of polymer structures and assemblies.

248

#### 249 [H2] Polymer charge



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

254

255 [H2] Polymer chemical structure

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

267

268 [H2] Polymer biodegradability

269 Depending on the biological application, the biodegradability of the polymer as well as its composition  
270 must be controlled to regulate the disintegration of the polymeric nanocarrier and the subsequent release  
271 of the Ru complex. Except for a few applications, such as bioimaging or the controlled release of a  
272 therapeutic gas, most delivery systems require biodegradable polymers. The use of non-biodegradable  
273 polymers is not always linked to the problem of accumulation. These polymers must be eliminated by a  
274 furtive way. The use of non-biodegradable copolymers allows one to ameliorate polymer properties  
275 (such as hydrophilicity, stability), but the elimination of these species can occur without the release of  
276 a pharmaceutically active compound. In the case of biodegradable polymers, the initial hydrolysis of  
277 cleavable links leads to the release of surface-adsorbed Ru complexes, after which the residual polymer  
278 biodegrades slowly and releases the remaining payload in a controlled fashion over the course of weeks  
279 to years<sup>82</sup>. This fast initial release is termed the ‘burst effect’ and should be minimized in order to  
280 maximise targeting efficiency.<sup>83-84</sup> The rate at which a Ru complex is released from a biocompatible  
281 polymers depends on multiple factors, such as the concentration gradient of the Ru complex in the  
282 polymeric matrix, the mobility and diffusion of the Ru complex in the nanocarrier, the polymer  
283 degradation rate (which is related to polymer properties such as the composition, molecular weight,  
284 molecular-weight distribution, composition, crystallinity and chemical structure). Ru complexes should  
285 be released by diffusion and/or through biochemical degradation of biodegradable polymers. Depending

286 on the structure of the cleavable functionality, biodegradation can be catalysed in the acidic environment  
287 characteristic of diseased tissues. Different degradation kinetic profiles can be obtained depending on  
288 the regioregularity or stereoregularity of the polymer sequence in the copolymers<sup>85</sup>. The overall rate of  
289 degradation for alternating copolymers is slower than for random counterparts and alternating  
290 copolymers degrade with a uniform linear degradation profile, which gives access to a homogeneous  
291 mixture after the first burst stage leads to an initial rapid drop in weight.

292  
293 The principal advantage of physical entrapment is that the integrity of the Ru complex (that is, the  
294 geometry, oxidation state and stereochemistry of the Ru centre) is conserved. However, the stability of  
295 the nanocarrier and its preservation for prolonged periods after synthesis are important challenges  
296 associated with using physical encapsulation. A balance must be found between a carrier that is too  
297 stable (and does not correctly release the Ru complex) and one that is too unstable (and does not reach  
298 the biological target or prematurely disassembles).

299

### 300 **[H1] Polymer micelles**

301 Polymer micelles were first used as drug delivery carriers in the 1980s<sup>86-87</sup>. Polymeric micelles are self-  
302 assembled nanosize colloidal particles that are obtained from amphiphilic block copolymers, usually in  
303 aqueous medium, and are generally larger than dendrimers and liposomes. Spherical polymeric micelles  
304 are typically 10 – 100 nm in diameter<sup>88</sup> and have an extremely narrow size distribution. This size can  
305 increase when serum proteins adsorb, sometimes rendering the particles too large for renal excretion<sup>89</sup>.  
306 In addition to the archetypal spherical shapes, these micelles can self-assemble into cylindrical and  
307 flexible structures (~40 nm in width and 20 – 40  $\mu\text{m}$  in length)<sup>90-91</sup>.

308  
309 Above the critical micelle concentration (CMC), single chains of amphiphilic polymers self-assemble  
310 into micelles to minimize the contact between the hydrophobic block and the aqueous medium<sup>92</sup>. The  
311 CMC is an important parameter in evaluating the stability of micelles. In general, the CMC for polymers  
312 ( $1-10 \mu\text{M}^{-1}$ ) is lower than that for low molecular weight surfactants ( $\text{mM}^{-1}$  range)<sup>88</sup>. A low CMC induces  
313 greater thermodynamic and kinetic stability in polymer micelles than in surfactant-based micelles. In  
314 principle, the formation of micelles is driven by a decrease in free energy. Hydrogen bonding between  
315  $\text{H}_2\text{O}$  molecules and the hydrophilic segment, as well as minimization of contact between the  
316 hydrophobic segment and the aqueous medium, enable the formation of a core-shell micelle by entropy-  
317 driven microphase separation.

318  
319 Diblock copolymers are usually used to obtain core-shell micelle architectures. The outer shell is  
320 composed of the hydrophilic blocks to enable stable dispersion in aqueous environments. The  
321 hydrophilic shell protects the encapsulated Ru complex by minimizing adsorption of biocomponents

322 (such as protein) onto the surface of the micelle during circulation in the bloodstream or interaction with  
323 cellular membranes. The inner core is formed from the hydrophobic block and is stabilized by  
324 hydrophobic interactions. In the core-shell architecture, the hydrophobic segment provides a space,  
325 termed a reservoir, for the physical encapsulation of hydrophobic Ru complexes. Longer hydrophilic  
326 segments are used in amphiphilic diblock copolymers to obtain spherical micelles. The limited kinetic  
327 stability of micelles can be a problem because there is a dynamic equilibrium between the self-assembled  
328 micelle and the bulk phase<sup>93</sup>. This equilibrium depends on temperature, polymer concentration, the pH  
329 and ionic strength of the biological medium. For biological applications, micelles must be stable during  
330 transport to the biological target but the equilibrium must shift when reaching the target region, so as to  
331 ensure efficient release of the Ru complex. In addition, the stability of micelles is highly dependent on  
332 the glass transition temperature ( $T_g$ ) of the hydrophobic polymer block that constitutes the core of the  
333 micelle.

334

335 The ratio of hydrophilic to hydrophobic block size can be controlled during synthesis to obtain micelles  
336 of a desired size. The physicochemical properties of the amphiphilic polymer determine the choice of  
337 polymer micelle preparation method, which include emulsion-solvent evaporation<sup>94</sup>,  
338 nanoprecipitation<sup>95</sup>, dialysis<sup>96</sup> and a thin-film method<sup>97</sup>. The size and morphology of micelles can be  
339 modulated by the choice of copolymer molecular weight, block length, composition and the preparation  
340 method. The maximum achievable drug loading depends on the chemical affinity between the Ru  
341 complex and the hydrophobic polymer block as well as the preparation method<sup>98</sup>.

342

343 Physical studies of dendrimers indicate that these highly branched monodisperse macromolecules exist  
344 in solution as unimolecular micelles — motifs that can be used as potential delivery systems<sup>99-100</sup>. In  
345 contrast to linear, cross-linked or low-branched polymers with a narrow molecular weight distribution,  
346 dendrimers have a precise molecular weight that is optimal for reproducible pharmacokinetic studies.  
347 The high density of functional groups on the surface of dendrimers allows higher drug loading and well-  
348 defined structures to be obtained.

349

350 [H1] Microparticles and nanoparticles

351 Spherical polymeric microparticles and nanoparticles (FIG. 2) are defined as matrix-type solid colloidal  
352 particles in which biological species are dissolved, entrapped, encapsulated or absorbed to the polymer  
353 matrix<sup>101</sup>. These particles are typically larger than micelles (usually 100–200 nm in diameter).  
354 Homopolymers, such as poly( $\epsilon$ -caprolactone) (PCL), poly(lactic acid) (PLA) and poly(glycolic acid)  
355 (PGA) and the corresponding copolymer poly(lactid-*co*-glycolic)acid (PLGA), are used to prepare solid  
356 polymer nanoparticles<sup>102</sup>. Self-assembly of these polymers is based on hydrophobic or electrostatic  
357 interactions; for example, in ionotropic gels, the cations or anions link the different polymer strands.

358 However, the hydrophobic surfaces of these particles are not suitable for long-term circulation in the  
359 blood (BOX 2). For this reason, the surface of these particles must be coated with hydrophilic polymers  
360 to ensure that they are invisible to the reticuloendothelial system (RES). Another solution is the use of  
361 an amphiphilic diblock copolymer containing a polyethylene glycol (PEG) segment<sup>102</sup> (BOX 2).  
362 Nanoparticles can be prepared using several preparation methods, although nanoprecipitation remains  
363 the most popular method<sup>102</sup>.

364

### 365 **[H2] Biodegradable polymers**

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

371

372 The range of natural biodegradable polymers suitable for the encapsulation of Ru complexes is  
373 unfortunately rather small because of limitations regarding their preparation. In addition, the presence  
374 of several identical functional groups in the lateral pendants of these polymers makes selective  
375 functionalization difficult. However, naturally occurring polysaccharides are interesting candidates as  
376 drug carriers because they mimic the extracellular matrix, which is involved in tissue regrowth and  
377 repair. For example, chitosan, a component of crustacean exoskeletons, is a polycationic polysaccharide  
378 that consists mostly of  $\beta$ -(1 $\rightarrow$ 4)-linked D-glucosamine units (FIG. 3). Chitosan is H<sub>2</sub>O-soluble in mildly  
379 acidic solutions (pH < 6.3) because its amine groups become protonated. In addition to its antimicrobial  
380 properties<sup>103-104</sup>, chitosan has low O<sub>2</sub> and CO<sub>2</sub> permeability and acts as a temporary barrier against  
381 photoluminescence quenchers. Tris(2,2'-bipyridyl)ruthenium(II) ([Ru(bpy)<sub>3</sub>]<sup>2+</sup>) embedded in a  
382 homogeneous and transparent thin film of chitosan has been used as a temperature-sensitive  
383 luminescence sensor<sup>105</sup>. The biocompatibility of chitosan and the red emission ( $\lambda_{\text{ex}} = 455$  nm,  
384  $\lambda_{\text{em}} = 605$  nm) of the Ru complex, at which the maximum penetration of biological tissue by light is  
385 possible, makes this a promising sensor for biological sensing applications.

386

387 Alginate is a polysaccharide consisting of (1 $\rightarrow$ 4)-linked residues of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-  
388 glucuronic acid in varying proportions and sequences along the chain (FIG. 3). Alginate has been used in  
389 various therapies<sup>106</sup>, such as chemotherapy or cell-based therapy (the use of living cells as therapeutic  
390 agents<sup>107-108</sup>). For example, deprotonating the carboxylic acids on the polymer and introducing divalent  
391 cations lead to ionotropic gelification and the formation of alginate beads. The polyanion can  
392 encapsulate cationic Ru-nitrosyl complexes such as {Ru[2-(4-chlorobenzylideneamino)-4-

393 nitrophenol] $(\text{PPh}_3)_2(\text{NO})\}^+$ , a photolabile species that controllably releases of NO *in vitro* after  
394 irradiation with visible light<sup>109</sup>.

395

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

407

408 Aliphatic polyesters, such as PLA, PGA and PLGA (FIG. 3), are the most commonly used biodegradable  
409 polymers in biological applications, owing to their controlled degradation (weeks to years) by hydrolysis  
410 *in vitro* and *in vivo*<sup>110-112</sup>. The biocompatible degradation products of these polyesters — lactic acid and  
411 glycolic acid — are non-toxic and are metabolized to give CO<sub>2</sub> and H<sub>2</sub>O as benign by-products<sup>86,113</sup>. All  
412 these polymers [have been approved as therapeutic drug carriers by the US Food and Drug  
413 Administration (FDA)<sup>114</sup>. However, these polymers are hydrophobic, such that the resulting polymeric  
414 nanoparticles must be stabilized in aqueous medium by the addition of amphiphilic polymers as  
415 surfactants in single H<sub>2</sub>O–oil emulsion.

416 For example, **KP1019** has been encapsulated in PLA nanoparticles (~164 nm in diameter) in the  
417 presence of a non-ionic surfactant such as the poloxamer (a type of polyether, see below) Pluronic<sup>®</sup> F-  
418 68 or a polysorbate (an ethoxylated sorbitan esterified with hydrophobic acid) such as Tween 80. The  
419 entire assembly is prepared by single oil-in-H<sub>2</sub>O emulsion<sup>115</sup>, and in contrast to Pluronic<sup>®</sup> F-68, Tween  
420 80 prevents drug precipitation at drug doses that are necessary for *in vivo* use. It is thought that **KP1019**  
421 conjugation to Tween 80 involves substitution of a Cl<sup>−</sup> ligands for a moderately basic oxygenic group  
422 in Tween 80 atom, with the resulting linkage being more stable than is the case for Pluronic<sup>®</sup> F-68, which  
423 only has ethereal O atoms. On binding Tween 80, **KP1019** complexes not only undergoes ligand  
424 substitution but also reduction, with electron paramagnetic resonance spectroscopic data being  
425 consistent with the paramagnetic Ru(III) centres being converted to diamagnetic Ru(II) sites, whose exact  
426 structure is unknown. Indeed, the autoxidation of polysorbates such as Tween 80 in the presence of  
427 transition metals is accompanied by the simultaneous reduction of the metal ion<sup>116</sup>. Reduction of the  
428 Ru(III) centre is accompanied by a colour change (from brown to deep green) and is associated with 20-

429 fold higher cytotoxicity compared with free **KP1019**<sup>116</sup>. Of note, this present biodegradable nanoparticle  
430 approach has been applied to Ru-mediated PDT. The method was enabled by the efficient loading (~50–  
431 60%), by nanoprecipitation, of a  $[\text{Ru}^{\text{II}}(1,10\text{-phenanthroline})_3]^{2+}$  photosensitizer, in which the ligands are  
432 decorated with hydrophilic triethyleneglycol-functionalized-5-fluorene groups, into PLGA  
433 nanoparticles (100 nm) in the presence of Pluronic<sup>®</sup> P-188<sup>117</sup>. The resulting nanoparticle was stable and  
434 less toxic than the free photosensitizer, releasing only a small amount of photosensitizer while in the  
435 dark under physiological conditions. After irradiation with 740 nm light, more of the two-photon-excited  
436 photosensitizer was released, such that a substantial level of singlet oxygen (<sup>1</sup>O<sub>2</sub>) could be generated to  
437 kill C6 glioma cells.

438  
439 H<sub>2</sub>O-soluble Ru–NO complexes have also been encapsulated in PLGA nanoparticles and microparticles  
440 using a double emulsion preparation (H<sub>2</sub>O–oil–H<sub>2</sub>O). Thus, Ru nitrosyls featuring amine co-ligands such  
441 as NH<sub>3</sub>, cyclam or *N,N,N',N'*-ethylenediaminetetraacetate, can be encapsulated in PLGA  
442 microparticles<sup>118</sup> (up to 1,600 nm in diameter) or nanoparticles<sup>119</sup> (220–840 nm in diameter). The  
443 primary H<sub>2</sub>O–oil emulsion, consisting of the NO-donor Ru complex solubilized in aqueous polyvinyl  
444 alcohol (PVA) aqueous solution and emulsified in an organic phase containing PLGA, was transferred  
445 into an aqueous PVA solution to produce the final H<sub>2</sub>O/oil/H<sub>2</sub>O emulsion. In these cases, a low loading  
446 efficiency (25–32%) was observed and the PLGA matrix absorbed light in the same spectral region as  
447 the embedded Ru–NO complexes, which are thus less liable to undergo photoactivation and exert their  
448 biological action. Despite the lower phototoxicity of encapsulated versus free complexes, the latter show  
449 improved targeting of tumour cells. *Trans*-[Ru(NO)(cyclam)Cl](PF<sub>6</sub>)<sub>2</sub> in PLGA nanoparticles killed  
450  $53.8 \pm 6.2\%$  of cells and *trans*-[Ru(NO)(NH<sub>3</sub>)<sub>4</sub>(py)](BF<sub>4</sub>)<sub>3</sub> in PLGA microparticles killed  $63 \pm 3\%$  of  
451 cells. The size of the PLGA particles apparently does not influence the drug release profile, which  
452 features an initial burst in the first 24 h followed by a slow release due to the hydrolytic degradation of  
453 PLGA, which resulted in the gradual release of NO through pores in the particle surface. Although the  
454 time required for complete degradation of the PLGA matrix was not reported, it is likely that  
455 microparticles had longer release times than nanoparticles. A double emulsion preparation was also used  
456 to efficiently encapsulate the related complex *trans*-[RuCl([15]aneN<sub>4</sub>)NO]Cl<sub>2</sub>, which could be loaded at  
457 a level of  $51.0 \pm 5.0\%$  in spherical PLGA nanoparticles ( $830 \pm 18$  nm in diameter)<sup>120</sup>.

458  
459 Unconventional inverse non-aqueous emulsion was necessary to encapsulate the polar NO complex  
460  $\{[(N,N'-1,2\text{-phenylene})\text{bis}(1\text{-methyl-}1H\text{-imidazole-}2\text{-carboxamide})]\text{Ru}(\text{NO})\text{Cl}\}$ , which is poorly  
461 soluble in H<sub>2</sub>O and organic solvents. The complex was hosted in nanoparticles consisting of gelatin,  
462 PLA, poly(vinyl formal) (PVF) and poly(ethyleneterephthalate) (PET) using amphiphilic  
463 poly[(butylene-*co*-ethylene)-*b*-(ethylene oxide)] as a surfactant and hexafluoroisopropanol as a  
464 solvent<sup>121</sup>. Localized in the PVF domain, the Ru complex released NO only slowly after photo-

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

470

471 It is also possible to encapsulate a Ru complex, such as [(*N,N'*-(1,2-phenylene)bis(1-methyl-1*H*-  
472 imidazole-2-carboxamido)Ru(NO)Cl)], by electrospinning poly(L-lactide-*co*-D/L-lactide) to afford a  
473 nanofibrous non-woven composite<sup>122</sup>. What resulted was a bimodal distribution: ~650 nm diameter  
474 fibres (10wt% complex loading) and ~950 nm fibres (25wt% complex loading). After exposure to low-  
475 intensity UV-A light, a low continuous amount ( $0.08 \pm 0.02\%$ ) of NO was immediately released,  
476 with only a small amount of NO being leached ( $0.26 \pm 0.10\%$ ) after five days<sup>122</sup>. Relative to polyesters,  
477 polyamides are degraded more slowly because amides are generally more resistant to hydrolysis.  
478 Polyamides have been used as protective shells for a Ru(II) photosensitizer bearing three disulfonated  
479 4,7-diphenyl-1,10-phenanthroline ligands. The nanoparticles (hydrodynamic radius 20–25 nm) were  
480 constructed from polyacrylamide (PAA) or amine-functionalized polyacrylamide (AF-PAA)<sup>123</sup> matrices  
481 that prevent quenching of photogenerated <sup>1</sup>O<sub>2</sub>, thereby allowing gas diffusion in the exterior of the  
482 nanoparticle. The Ru photosensitizer leached from the AF-PAA-based nanoparticles only very slowly  
483 (over a period of days) owing to the electrostatic interactions between ammonium groups on the host  
484 and the sulfonates on the Ru complex. This system appears promising for the targeted production of <sup>1</sup>O<sub>2</sub>  
485 in PDT applications.

486

487 [H2] Non-biodegradable or slowly-biodegradable polymers

488 Biocompatible non-biodegradable polymers have only few biological applications, including  
489 bioimaging, theranostics and the controlled release of a biologically active gas (CO or <sup>1</sup>O<sub>2</sub> for PDT).  
490 These applications have mostly used commercially available (or easy-to-prepare) diblock or triblock  
491 copolymers that serve as a protective shell against biological species (for encapsulated gas-donor Ru  
492 complexes), light, <sup>1</sup>O<sub>2</sub> or radicals (for encapsulated photosensitizers or phosphorescent Ru complexes).  
493 Most of these copolymers contain one polymer segment that is hydrophilic on account of carboxylic  
494 acid groups. The gas permeability and the photophysics of the polymeric matrices seem to be the  
495 principal properties to control for the efficient design of these delivery systems.

496

497 The CO-releasing molecule CORM-2 (4.1wt%, FIG. 1) was encapsulated in styrene–maleic acid  
498 copolymer micelles with a hydrodynamic diameter of 165.3 nm. These nanomicelles were bioactive for  
499 longer *in vivo* than the free Ru complex CORM-2, had a 35-fold longer half-life in circulation after  
500 intravenous injection in mice and showed selective accumulation in inflamed tissues<sup>124</sup>. In small doses,

501 CO has anti-oxidative and anti-inflammatory effects, and the slow release of CO here is desirable such  
502 that these micelles are drug candidates for diseases including ischaemia–reperfusion injury, bacterial  
503 and viral infections, hypertension and diseases (including inflammatory bowel disease) caused by  
504 reactive oxygen species (ROS) such as  $^1\text{O}_2$  and oxyl radicals. To improve the efficiency of  
505 photoactivable Ru complexes, they have been encapsulated in cross-linked polymer nanoassemblies and  
506 the influence of factors such as photoactivation, hydrophobicity, and solution ionic strength and pH, on  
507 complex loading and release studied. Cross-linked polymeric nanoassemblies based on  
508 poly(ethyleneglycol)–poly(aspartate) block copolymer (PEG–PASP) were also used to encapsulate  
509 three different cationic Ru complexes through electrostatic interaction with the carboxylate acid groups  
510 of the ASP core block<sup>125</sup>. The rate of Ru release from the PEG–PASP nanoassembly is highly dependent  
511 on the hydrophobicity of the complex and the solution ionic strength but surprisingly independent of  
512 pH. The Ru(II) polypyridyl {Ru(bipy)<sub>2</sub>[7-(hydroxymethyl)dipyrido[3,2-a:2',3'-c]phenazine]}<sup>2+</sup> is a PDT  
513 photosensitizer that has been encapsulated in block copolymers consisting of poly(*N,N*-  
514 dimethylaminoethyl methacrylate) (PDMAEMA) as one segment and either poly(methyl methacrylate)  
515 (PMMA) or a statistical copolymer (PDMAEMA-*co*-PMMA) as a second segment.<sup>126</sup> No release of the  
516 Ru complex from micelles with PDMAEMA-*b*-PMMA ( occurs in response to ultrasound, whereas Ru  
517 release from micelles with the block copolymer featuring PDMAEMA and PDMAEMA-*co*-PMMA  
518 segments occurs in response to ultrasound, reflecting the different core–shell structures of the different  
519 particles.

520 Poloxamers are biocompatible non-ionic triblock copolymers with an ABA structure, comprising a  
521 hydrophobic central B block flanked by hydrophilic lateral A blocks<sup>127-129</sup>. The A block is poly(ethylene  
522 oxide) and the B block is poly(propylene oxide), and one can adjust the molar ratio (from 1:9 to 8:2)  
523 and molecular weight to tune the physicochemical properties of amphiphilic poloxamers, in particular  
524 gelation temperature and *in vivo* properties, such as their interaction with cells and membranes. The  
525 commercial poloxamers Pluronic<sup>®</sup> P-123 and Pluronic F-127 can self-assemble into spherical micelles.  
526 A bioprobe for high-resolution two-photon quantitative imaging of oxygen in aqueous media was  
527 obtained by encapsulating the commercial hydrophobic phosphorescent dye [Ru(4,7-diphenyl-1,10-  
528 phenanthroline)<sub>3</sub>]<sup>2+</sup> in the core of Pluronic<sup>®</sup> F-127 nanomicelles using hydrophobic interactions<sup>130</sup>. Based  
529 on the ‘collisional quenching’ process, the collision between O<sub>2</sub> and an excited luminophore provides a  
530 non-radiative pathway for highly electronically excited species to relax, decreasing its phosphorescence  
531 intensity and lifetime<sup>131</sup>. The hydrophobic core of each nanomicelle permits the diffusion of O<sub>2</sub> and hosts  
532 the phosphorescent dye, with the aqueous solubility of the assembly being dependent on the length of  
533 the hydrophilic tails. After self-assembly, it is possible to isolate by filtration only the nanomicelles  
534 larger than 5 nm, with small assemblies being less desirable because they can diffuse across the  
535 vasculature endothelium. The nanomicelle probes are stable for several months in H<sub>2</sub>O and for several  
536 hours in biological medium — long enough to make a two-photon quantification of O<sub>2</sub> and perform



537 multiphoton microscopy. Furthermore, these nanomicelle probes are at least twice as sensitive as the  
538 free phosphorescent dye in H<sub>2</sub>O, although the O<sub>2</sub>-sensing response of the probes is dependent on  
539 temperature and solvent, such that calibrations are required for *in vivo* testing. The hydrophobic Ru  
540 dithiolate [Ru(*p*-cymene)(1,2-dicarba-*closo*-dodecarborane-1,2-dithiolato)] (FIG. 1), a complex  
541 investigated in boron neutron capture therapy (BNCT), has been encapsulated in the core of Pluronic<sup>®</sup>  
542 P123 core-shell nanomicelles to increase its H<sub>2</sub>O solubility and targeting of cancer cells<sup>132</sup>. The molar  
543 ratio A:B of the hydrophilic to hydrophobic domains for Pluronic<sup>®</sup> P123 is lower than for Pluronic<sup>®</sup> F-  
544 127, such that the former has a larger nanomicelle core, with its estimated hydrodynamic diameter being  
545 7.8–21.4 nm and dispersity 0.04 according to dynamic light scattering measurements. The Ru complex  
546 has a lower anticancer activity after encapsulation, but the accumulation of the encapsulated Ru complex  
547 in A2780 (human ovarian) and A2780cisR (cisplatin-resistant human ovarian) cancer cell lines is 2.5-  
548 fold higher than that of the free Ru complex. In A2780cisR cells, the encapsulated Ru complex remains  
549 sensitive to neutron irradiation, with the antiproliferative activity of these nanomicelles, at micromolar  
550 concentrations of Ru, being 1.4-fold higher than that of non-irradiated nanomicelles.

551

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

553 Phospholipids and other low-molecular weight surfactants can self-assemble in aqueous medium to  
554 afford spherical vesicles consisting of one or more phospholipid bilayers. These vesicles are examples  
555 of liposomes and feature amphiphilic phospholipids consisting of a hydrophobic tail and a hydrophilic  
556 head group. Spherical liposomes contain a aqueous lumen delimited by a hydrophobic membrane.  
557 Hydrophilic biologically active compounds can be enclosed in the internal aqueous volume inside the  
558 spherical liposome, whereas lipophilic biologically active compounds can be incorporated in the lipid  
559 bilayer. Liposomes have been the most common type of nanocarriers for the targeted delivery of  
560 amphiphilic drugs for more than 50 years<sup>133</sup>. Polymer-decorated liposomes are obtained by coating  
561 preformed Ru complex-loaded liposomes with hydrophilic polymers, whereas polymer-lipid hybrids  
562 are obtained by inserting polymer chains (with a covalently bound Ru complex) into the lipid bilayer  
563 during the self-assembly of phospholipids<sup>134-135</sup> (FIG. 2). We now describe the role of the polymer in  
564 polymer-decorated liposomes and polymer-lipid hybrids.

565

566 [H2] Spherical polymer-decorated liposomes

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

573 the Ru complex. Absorption and fluorescence quenching studies confirmed that hydrophobic  
574 [Zn(phthalocyanine)] can be incorporated into the slightly deformed lipid bilayer whereas the Ru  
575 complex can move between the internal and external liposome environments. The different localizations  
576 of the active compounds can be explained in terms of the electrostatic interactions between the cationic  
577 Ru complex and the anionic phospholipid head groups. In contrast, charge-neutral [Zn(phthalocyanine)]  
578 predominantly engages in hydrophobic interactions with the organic tails. After photoactivation, these  
579 stealth liposomes reduce the viability of mouse B16-F10 melanoma cells by ~95% in vitro. Using the  
580 same guests, one can form ultradeformable liposomes by adding the non-ionic surfactant  
581 polyoxoethylene (20) sorbitan monolaurate (polysorbate 20; also known as Tween 20) to the surface of  
582 liposomes<sup>137-138</sup>.

583

#### 584 [H2] Polymer–lipid hybrids

585 It is possible that not all Ru complexes correctly enter in liposomes, for example because the complexes  
586 instead interact favourably with the surface of the polymer–lipid hybrid. To address this, the surface of  
587 liposomes or polymer–lipid hybrids can be coated with amphiphilic Ru complexes. The complexes must  
588 be functionalized with a hydrophobic segment to be correctly incorporated in the lipid membrane during  
589 the self-assembly of phospholipids. A library of nucleolipid nanovectors consisting of uridine or  
590 thymidine nucleobases was designed and synthesized to encapsulate amphiphilic Ru complexes<sup>139</sup> (FIG.  
591 S1a). One or two oleic acid residues attached to the secondary OH of ribose serve as a motif for insertion  
592 into the lipid monolayer or multi-layer in aqueous solution, and a hydrophilic oligoethylene glycol chain  
593 at the 5' end contributes to the stealth properties of these nanovectors. A pyridine motif was inserted in  
594 order to give a complex analogous to **Azi-Ru** (FIG. 1), a species similar to the anticancer drug candidates  
595 **NAMI-A** and **KP1019**. The Ru complexes remain hidden among the phospholipid head groups at the  
596 surface of the liposomes (FIG. S1a). The position of the Ru complexes as well as the liposome membrane  
597 composition (consisting of the zwitterion palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) or the  
598 cationic lipid 1,2-dioleoyl-3-trimethylammoniumpropane chloride (DOTAP)) effectively slow the  
599 hydrolysis by retarding the ligand exchange process<sup>140-141</sup>, which is consistent with the high kinetic  
600 stability of these formulations, which persist for months. Incorporating Ru complexes into a  
601 phospholipid membrane at levels below the loading limit induces only a small deformation in the  
602 membrane and only minimally affects the morphology of the liposomes. After encapsulation of a  
603 uridine-based nucleolipid **HoUrRu** (FIG. S1a) in POPC-based or DOTAP-based liposomes, the  
604 assembly has higher antiproliferative activity than free **Azi-Ru** (IC<sub>50</sub> ~10 μM versus ~300 μM in MCF7  
605 cells and IC<sub>50</sub> 10–20 μM versus ~440 μM in WiDr cells)<sup>142</sup>. A triplet–triplet annihilation upconversion  
606 (TTA-UC) system for the activation of a [Ru(bpy)<sub>2</sub>(1,3-bis(methylsulfido)-2-dodecyloxypropane)]<sup>2+</sup>  
607 anticancer prodrug candidate has been reported<sup>143</sup> (FIG. S1b). The stealth property of liposomes  
608 consisting of the neutral phosphatidylcholine lipids 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC)

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

625

#### 626 [H1] Covalent conjugation

627 The major drawbacks of physical encapsulation include the uncontrolled release of a therapeutic agent,  
628 especially the ‘burst’ release (except in the case of polymeric carriers based on pH-responsive or photo-  
629 responsive polymers), and the often low amount of Ru complexes that can be loaded into the host. An  
630 alternative strategy involves covalent conjugation of Ru complexes to a polymer to give metallopolymer  
631 prodrug candidates<sup>144</sup>. The first clinical trials of prodrugs developed using this strategy involved using  
632 the polymer–protein conjugates PEG-L-asparaginase (Oncaspar) and styrene maleic anhydride-  
633 neocarzinostatin (Zinostatin Stimalmer) to treat leukaemia or hepatocellular carcinoma, respectively<sup>145</sup>.  
634 As with physical encapsulation, the Ru complex is temporarily protected by the polymer against reactive  
635 species in the biological medium. This approach enables controlled high loading of Ru complexes into  
636 the metallopolymer and ensures the prolonged release of the therapeutic agent during polymer  
637 biodegradation. Both Ru(II) and Ru(III) complexes have been covalently conjugated to polymers.  
638 Biodegradation can involve degradation of the polymer backbone or first the activation of the Ru(III)  
639 centre (by reduction to Ru(II)) followed by degradation of the polymer backbone. Most of the  
640 metallopolymer candidates discussed above were developed as prodrug candidates that target cancer  
641 cells<sup>146-147</sup>. Similarly, the Ru complexes physically encapsulated in polymer carriers are principally  
642 investigated in view of cancer therapy. Standard metallopolymer prodrug candidates usually have a  
643 ‘polymer–linker–therapeutic agent’ architecture, but additional functionalities can be added to achieve  
644 specific targeting or to create combined therapies. Although not discussed in detail in this Review,

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

649  
650 In addition to size, surface charge and biodegradability, the structure of the metallopolymer is an  
651 important consideration in nanocarrier design. First, a suitable conjugation method is one that ensures  
652 that the covalent link between the polymer and the Ru complex remains intact during the transport of  
653 the carrier but is cleaved in the biological medium for delivery to the target site. The cleavage of the  
654 covalent link activates or reactivates the Ru complex after chemical degradation of the metallopolymer.  
655 For this reason, the use of biodegradable polymers is essential in the preparation of polymer-based  
656 metalloprodrugs, with the degradation rate of the metallopolymer controlling the drug delivery kinetics.  
657 A Ru centre can bind polymers containing N-donor ligands, such as amines or pyridines, or O-donors,  
658 such as carboxylates, in different positions, including at the polymer main chain, terminal groups or  
659 pendant groups. Depending on the number of vacant coordination sites (or sites at which a weakly basic  
660 ligand is present) on a Ru complex, the polymer can be a monodentate or bidentate (or, more rarely,  
661 terdentate) ligand, such that the Ru centre can crosslink polymers. Second, the loading rate and  
662 physicochemical properties of the Ru complex must be carefully selected because covalent conjugation  
663 of the Ru complex with the polymer can affect the complex's physicochemical properties such as H<sub>2</sub>O  
664 solubility and/or hydrophilic/hydrophobic balance. Third, functionalities sensitive to external stimuli  
665 can be inserted in the polymer backbone and or pendants or in the Ru centre. Photoresponsive  
666 functionalities are the most popular because they enable precise spatial and temporal control of active  
667 compound release. Various polymeric architectures can be obtained, namely linear or brush  
668 (co)polymers, branched polymers (dendrimers) and supramolecular polymers. Depending on where the  
669 Ru centre is conjugated to the polymer (the backbone, a lateral pendant or the centre of a star polymer),  
670 different synthetic strategies are available and these are covered in the following discussion.

671

672 [H1] Linear and brush metallopolymer prodrugs

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

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

682

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

684 Ru(II) complexes that release diatomic therapeutic gases are the major type of Ru complexes that form  
685 metallopolymers by strong Ru–L<sub>polymer</sub> linkages. The complexes can be activated chemically<sup>148-150</sup> or  
686 photochemically<sup>136</sup>, and the gas molecules are liberated without the complete degradation of the polymer  
687 backbone of a brush copolymer. The ligands for the Ru coordination can be inserted during the monomer  
688 preparation before polymerization. For example, up to 2500 equivalents of a CO-releasing Ru complex  
689 are stored in every micelle of a triblock copolymer featuring a hydrophilic PEG block, a poly(orthinine  
690 acrylamide) block bearing [Ru(CO)<sub>3</sub>Cl(orthinoate)] moieties and a hydrophobic poly(*n*-  
691 butylacrylamide) block<sup>148</sup> (FIG. 5a). The incorporation of CO donors (with 15–37 Ru-based units per  
692 polymer, 4.7–10.3wt% Ru) into micelles with a hydrodynamic diameter of 29–44 nm makes the Ru  
693 complex less susceptible to attack from thiols (such as cysteamine and glutathione) than is free  
694 [Ru(CO)<sub>3</sub>Cl(glycinato)] (**CORM-3**, FIG. 1), such that the Ru complexes in the polymer release CO  
695 more slowly. Thus, there is a substantial reduction in the cytotoxicity of the [Ru(CO)<sub>3</sub>Cl(amido  
696 acidate)] moiety due to the stealth properties of the PEG block. Moreover, in contrast to free  
697 [Ru(CO)<sub>3</sub>Cl(glycinato)], the CO-releasing micelles efficiently attenuated lipopolysaccharide-induced  
698 nuclear factor-κB activation in THP-1 Blue cells derived from human monocytes (which are linked to  
699 inflammation)<sup>148</sup>. In a subsequent study, a pyridine ligand was inserted in the hydrophobic polyvinyl  
700 block to give P(OEGA)-P(4VP-CORM-2) (FIG. 5a)<sup>150</sup>, a polymer variant of **CORM-2**. Whereas CO  
701 release was spontaneous in the earlier study, in this study the rate of CO release could be controlled by  
702 changing pH. CO release was rapid in acidic conditions and slower at neutral pH, where the polymer  
703 micelles may form a compact structure in which the CO-donor Ru complexes are more protected inside  
704 the micelle core, preventing fast CO release. Conversely, at acidic pH, the pyridine groups not bound to  
705 Ru tend to be protonated, resulting in a less compact structure in which the Ru centres are more  
706 accessible and liable to release CO. This approach of using a polyvinylpyridine block to construct a  
707 polymeric version of **CORM-2** can also be applied to making a polymeric NO-releasing agent featuring  
708 *trans*-{Ru[1,2-bis(pyridine-2-carboximido)-4,5-dimethylbenzene](L<sub>polymer</sub>)NO} fragments. The  
709 polyvinylpyridine also features cross-linked poly(2-hydroxyethyl methacrylate) (pHEMA) in a  
710 copolymer formed from HEMA and ethyleneglycol dimethacrylate (EDGMA) (synthesized by radical-  
711 induced copolymerization)<sup>151</sup> (FIG. 5b). In contrast to carriers designed for CO-releasing Ru complexes,  
712 the rapid release of NO is strictly dependent on exposure to UV light (even with a low intensity of 5–  
713 10 mW), such that NO release is more controllable<sup>151, 150</sup>.

714

715 Functionalized tpy complexes were used as a linker for crosslinking of diblock copolymers of  
716 pentafluorophenyl acrylate (PFA) and 2-(2',3',4',6'-tetra-O-acetyl- $\beta$ -D-glucosyloxy)ethyl methacrylate  
717 (AcGlcEMA) prepared by reversible addition–fragmentation chain-transfer (RAFT) polymerization<sup>152</sup>  
718 (FIG. 6). After deacylation, nanoparticles with an average size of 60 nm had a core–shell structure with  
719 the Ru(II) complex as the core and glycopolymers as the shell. The nanoparticles were H<sub>2</sub>O-soluble  
720 owing to the hydrophilic corona of glycopolymers and showed low cytotoxicity in KB cells.) These  
721 nanoparticles might therefore be useful as a cellular label for one-photon and two-photon fluorescence  
722 bioimaging. Coordination of Ru on labile ligands

723 A polymer decorated with weakly basic ligands can serve as a scaffold for a triggered release or triggered  
724 activation of a metallopolymer prodrug, wherein the weak Ru–L<sub>polymer</sub> bonds are readily cleaved in a  
725 controlled manner. The activation can be effected by various stimuli, such as a change in the biological  
726 medium, hydrolysis or light.

727  
728 As described above for physical encapsulation, natural biodegradable polymers have also been used as  
729 delivery vehicles for Ru complexes, whose release is activated by hydrolysis of the polymer in the  
730 biological medium<sup>153-154</sup>. The linear polysaccharide chitosan is one such biodegradable polymer, and the  
731 primary amine in each monomer can serve as a functional handle to which a Ru centre can be appended.  
732 For example, the amine can be decorated with caffeic acid as a ligand or undergo a Schiff base  
733 condensation to afford an imine ligand<sup>153-154</sup> (FIG. 6a). The conjugation of caffeic acid-modified chitosan  
734 to the {Ru(*p*-cym)Cl}<sup>+</sup> fragment induces a change of backbone chitosan structure<sup>152</sup> to give 30–120 nm  
735 particles. Anticancer activity may be realized if hydrolysis occurs to release the active dimer {[ $\eta^6$ -  
736 arene)Ru]<sub>2</sub>( $\eta^2$ -OH)<sub>3</sub>}<sup>+</sup>. Likewise, the Schiff base is also not a strong ligand for Ru(III), which can be  
737 released so as to afford antibacterial activity against Gram-positive bacteria, such as *B. subtilis*, *S.*  
738 *aureus*, and Gram-negative bacteria, such as *E. coli*, *K. pneumoniae* and *P. aeruginosa*<sup>154</sup>.

739  
740 In photoactivated chemotherapy (PACT), a non-toxic or minimally toxic prodrug candidate becomes  
741 more toxic after light irradiation. This photo-release strategy is based on masking the functional groups  
742 involved in the toxicity using a photo-cleavable motif. As in photodynamic therapy (PDT), the use of  
743 photo-responsive cages as carriers is of great interest owing to the spatial and temporal control of  
744 activation.

745  
746 Inspired by studies of Ru(II) polypyridyl photocages,<sup>155-156</sup> two studies reported the preparation of  
747 different photoresponsive platforms based on metalloblock copolymers for combined PACT and PDT.  
748 Both studies made use of an active Ru fragment (FIG. 7a) bound to the polymer through a weakly  
749 coordinating 4-[(6-hydroxyhexyl)oxy]benzotrile residue<sup>157-158</sup>, such that red light cleaves the Ru–  
750 L<sub>polymer</sub> bond to release the anticancer drug candidate and generate <sup>1</sup>O<sub>2</sub> to inhibit cancer cell growth. In

751 the first study<sup>157</sup>,  $\{\text{Ru}(2,2'\text{-biquinoline})_2\}^{2+}$  binds up to two nitriles, each being at the end of a long  
752 organic chain terminated by a PEG chain that confers stealth properties. Here, red light irradiation causes  
753 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  
754 photolabile Ru complex was anchored in the main chain on the block copolymer.<sup>158</sup> Depending on the  
755 size of each of its constituent blocks, the amphiphile can assume different mesostructures: micelle,  
756 hollow sphere and large compound micelle. It is the micelles, of diameter 12 nm (41wt% Ru loading)  
757 that had the highest cellular uptake and anticancer activity,<sup>158</sup> decreasing HeLa cell viability by 73%  
758 after red-light irradiation.

759

760

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

765

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

775

776 [H1] Linear and star metallopolymer prodrugs

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

781

782 [H2] Convergent strategy

783 The convergent strategy for the preparation of star metallopolymer drugs is based on the prior synthesis  
784 of polymers as macroligands followed by the coordination of the Ru centre by ligand exchange. For  
785 example, bpy ligands are readily functionalized with biodegradable polyesters<sup>160-162</sup> using ring-opening  
786 polymerization of cyclic ester monomers. PCL (FIG. 8A) is amenable for incorporation into a drug

787 carrier owing to its low degradation rate and its permeability to small molecules studies<sup>160-161</sup>. The  
788 polymerization of  $\epsilon$ -caprolactone by subcritical CO<sub>2</sub> processing gives biodegradable open cell foams,  
789 which contrasts the otherwise semicrystalline microstructure of poly( $\epsilon$ -caprolactone) and enabled its use  
790 for culturing cells<sup>161</sup>. The same convergent strategy was also used to develop a polymer-containing  
791 analog of the anticancer drug candidate **TM34** ([Ru(C<sub>5</sub>H<sub>5</sub>)(bpy)(PPh<sub>3</sub>)]OTf) (FIG. 8A). The polymer  
792 drug had an IC<sub>50</sub> in the micromolar range in human MCF-7 and MDA-MB-231 breast cancer cell lines  
793 and the A2780 ovarian adenocarcinoma cell line<sup>162</sup>.

794

795

## 796 **[H2] Divergent strategy**

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

800

801 Luminescent [Ru(bpy)<sub>3</sub>]<sup>2+</sup>-centred star block copolymers with a hydrophobic PLA core and a  
802 hydrophilic poly(acrylic acid) corona (FIG. 8b) have served as a preliminary model for imaging  
803 probes<sup>163</sup>. In particular, a Ru complex was used as a metaloinitiator for 4-(dimethylamino)pyridine-  
804 catalysed ring-opening polymerization of lactide, followed by atom transfer radical polymerization to  
805 introduce poly(*tert*-butyl)acrylate chains. The esters were finally hydrolysed using Me<sub>3</sub>SiI to give a  
806 H<sub>2</sub>O-soluble poly(acrylate) corona. The same divergent strategy was used to synthesise a star-shaped  
807 [Ru(bpy)<sub>3</sub>]<sup>2+</sup>-centred poly(ethyleneimine), where the polycationic PEI polymer is used for gene  
808 delivery<sup>164-165</sup> by electrostatic binding of the carrier with DNA, thereby protecting it against cleavage by  
809 nucleases<sup>166</sup>. As an alternative to PEG, a biocompatible polyoxazoline was used to confer stealth  
810 properties to the metallopolymer drug. The living cationic ring-opening polymerization of 2-ethyl-2-  
811 oxazoline followed by an acid hydrolysis of amide groups provided protection for the hexafunctional  
812 [Ru(bpy)(CH<sub>2</sub>Cl)<sub>2</sub>]<sub>3</sub><sup>2+</sup> core (FIG. 8b). The system seems to be limited for imaging properties and was  
813 first tested for the transfection of the LNCaP prostate cancer cell line. The entrapment of DNA in the  
814 polyoxazoline was comparable to that by Ru-free linear cationic polymers of a higher molecular weight  
815 and was more efficient than that by Ru-free branched PEI of a similar molecular weight.

816

817

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



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

824

825 [H1] Ru centre-rich metallopolymers

826 In the synthesis of metallopolymers, it is sometimes difficult or impossible to control the loading of the  
827 Ru complexes, except in the case of a star-shaped architecture with a Ru-based central core. One method  
828 involves covalent conjugation of Ru complexes to well-defined architecturally branched polymers. The  
829 globular or spherical structure of these polymers — known as dendrimers — represent an alternative to  
830 using polymeric nanoparticles in delivery systems. However, most dendrimer backbones are not easily  
831 biodegradable, such that Ru complexes are almost always covalently conjugated to the end of the arms  
832 constituting the dendrimer shell and not to the dendrimer core (only one example exists of physical  
833 encapsulation of a Ru complex in a dendrimer)<sup>168</sup>. The charge on Ru complexes conjugated to a  
834 dendrimer shell determines the conjugate's H<sub>2</sub>O solubility and can be adjusted to induce selective  
835 accumulation in cellular compartments and thereby tune toxicity. In most cases, the strategies used to  
836 synthesize standard polymers have also been used for the conjugation of Ru complexes. The ligand units  
837 are inserted as terminal groups on branched chains of the dendrimer shell, after which they bind Ru  
838 centres by displacing weakly bound ligands<sup>169-174</sup>.

839

840 In contrast to linear polymers or those with few crosslinks, assemblies based on dendrimers have a dense  
841 architecture, which probably slows the release of encapsulated Ru complexes substantially. However, a  
842 direct relationship between chemical structure and the bioactivity of dendrimers has yet to be  
843 rationalized. Dendrimers are among the smallest drug delivery carriers, with sizes in the range of tens  
844 of nanometers, although a direct size comparison between various types of dendrimers is difficult owing  
845 to different molecular packing. Hydrogen bonding in polyamide dendrimers increases the density of  
846 globular dendrimers with higher generations, whereas polyamine chains maintain greater flexibility  
847 leading to less dense globular dendrimers. To study the effects of dendrimer size on cytotoxicity, a large  
848 library of first- to fourth-generation Ru(II) arene complexes based on the diaminobutane (DAB)-  
849 poly(propyleneimine) dendrimer scaffold was generated<sup>169-172</sup> (FIG. S2). Dendrimer toxicity generally  
850 increased as a function of its size<sup>175</sup> but, surprisingly, increased cytotoxicity was observed for each group  
851 of larger dendrimers (third and fourth generations), demonstrating that cytotoxicity is clearly linked to  
852 metallodendrimer size. Each drug candidate featured a terminal aromatic group conjugated to an imine  
853 functionality. The aromatic substituents minimize and counterbalance the effect of the imine groups,  
854 which were susceptible to hydrolysis in the final products  $\{[\text{Ru}(\text{arene})\text{Cl}]_n(\text{dendrimer ligand})\}^{m+}$ . In each  
855 case, replacing the Cl<sup>-</sup> ligands with PTA affords cationic complexes with lower IC<sub>50</sub> values in A2780  
856 and A2780 cisR cell lines, owing to the interaction of PTA with DNA<sup>172</sup>. The 4<sup>th</sup> generation compound  
857 with the *N,O* bidentate neutral ligand and *p*-cymene ligand (FIG. S2) with 32 arms had the highest

858 cytotoxicity ( $IC_{50} = 0.8 \mu\text{M}$  in A2780 cells,  $IC_{50} = 2.7 \mu\text{M}$  in A2780cisR cells and  $IC_{50} = 2.6 \mu\text{M}$  in  
859 healthy HEK cells. Preliminary biological studies showed that the insertion of ferrocenyl group  
860 conjugated on the Ru complex (FIG. S2) reduced the proliferation of A2780 and A2780cis R cells by  
861  $>50\%$  ( $IC_{50} < 5 \mu\text{M}$  at equi-iron concentrations of  $5 \mu\text{M}$ ) and substantially reduced the proliferation of  
862 SISO human cervix cancer cells, LCLC-103H human lung cancer cells and 5637 bladder cancer cells in  
863 vitro<sup>172</sup>.

864 A dendritic system based on a pentaerythritol core bearing the pendant tpy (for the coordination of Ru  
865 centre) and functionalized with 1,2-dicarba-*closo*-dodecaborane units (FIG. S2) has been used in  
866 BNCT<sup>176-177</sup>. This promising dendrimer has yet to be biologically tested. [

867

868 [H1] Concluding remarks

869 The use of Ru complexes in medicinal chemistry has increased substantially in the past decade,  
870 encouraged by clinical trials of **NAMI-A**, **KP1039** and **TLD-1433**. Most Ru complexes discussed in  
871 this Review are envisioned for the treatment of various cancers using chemotherapy and/or other  
872 therapies, such as PDT or BNCT. However, research on the encapsulation of Ru complexes in polymeric  
873 carriers is still in its infancy and must compete with research on the encapsulation of Pt-based drugs<sup>196-</sup>  
874 <sup>197</sup>, which were discovered earlier and dominate the chemotherapeutics market. In contrast to Pt-based  
875 drugs, two strategies, developed in parallel, exist for the encapsulation of Ru complexes — physical  
876 encapsulation and covalent conjugation. Commercially available or easily accessible polymers are  
877 typically chosen for the encapsulation of Ru compounds. Although the synthetic approaches are clear,  
878 the relationship between the properties of the polymer and the properties of the final polymer-  
879 encapsulated complex has rarely been clarified or linked to the delivery properties of these complexes  
880 in biological media. Physical encapsulation remains the most common strategy to deliver Ru complexes  
881 because it is simpler and faster than covalent conjugation. However, the structure of these physically or  
882 covalently bound species advances towards more sophisticated architectures comprising a time and space  
883 controlled release of biological active Ru complex using an external stimulus.

884

885 For the physical encapsulated Ru complex delivery systems, the stability of the carrier is an important  
886 consideration for improved drug delivery and prolonged storage of nanocarriers. The effect of  
887 nanocarrier shape on the efficiency of drug delivery has been only lightly studied<sup>198</sup>. Nanocarriers used  
888 for the physical encapsulation of Ru complexes are most often spherical; tubular nanocarriers that can  
889 deliver metal complexes and specifically target cellular compartments are rare. The functionalization of  
890 inorganic nanotubes (such as carbon nanotubes) is difficult, such that biodegradable organic nanotubes  
891 derived from alginate and chitosan<sup>199</sup> appear better vehicles for the delivery of biologically active Ru  
892 complexes. Another emerging approach is the preparation of delivery systems containing two anticancer

893 drugs for combination therapy, such as chemotherapy combined with PDT or BNCT. Ru complexes  
894 have already been incorporated on the surface of Au nanomaterials or C nanotubes as photothermal  
895 agents<sup>55</sup>. Physical encapsulation of Ru complexes in biocompatible photothermic polymers<sup>200</sup>, such as  
896 polyaniline or melanine derivatives, is another potential delivery system for combined photothermal  
897 therapy and chemotherapy.

898

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

904

905

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1375

1376 [H1] Competing interests

1377 The authors declare no competing interests.

1378

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1386

1387 **Display items**

1388 Box 1| **The mechanisms of action of Ru complexes**

1389

1390 **DNA damage**

1391 Cellular dysfunction in disease or cancer can result from DNA damage (such as autoxidation in air,  
1392 disproportionation or hydrolysis). The proliferation rate in cancer cells is high and is regulated by the  
1393 cell cycle and DNA replication. Therefore, damaged DNA is a key target for Ru-based anticancer drug



1394 candidates and is the major mechanism by which many FDA-approved metallo-therapeutics (such as  
1395 Pt-based drugs) and organic oncology drugs (such as doxorubicin and gemcitabine) operate. Ru  
1396 complexes with planar aromatic ligands selectively bind to DNA by covalent and/or non-covalent  
1397 modes. Covalent binding is irreversible and results in the formation of DNA–Ru complex adducts that  
1398 distort the DNA backbone and disrupt replication and transcription, leading to cell death (as observed  
1399 for ( $\eta^6$ -arene)Ru<sup>II</sup> complexes, such as **RM-175** (FIG. 1)). Non-covalent binding is usually reversible and  
1400 includes interactions such as electrostatic binding, intercalation and groove binding, as observed for  
1401 **RAPTA-C** (FIG. 1). Most cationic Ru<sup>II</sup> complexes have one or more planar aromatic ligands, which can  
1402 intercalate between adjacent base pairs in DNA. Distortion of the double helix caused by these ligands  
1403 can inhibit or completely block transcription and replication.

1404

#### 1405 *DNA metabolism*

1406 A set of proteins are responsible for genome integrity. Topoisomerase II is the principal enzyme in the  
1407 cell nucleus involved in DNA replication, transcription, recombination and sister chromatid segregation  
1408 during mitosis<sup>179</sup>. Selective inhibition of topoisomerase II can inhibit neoplastic cell proliferation and  
1409 possibly induces apoptosis through DNA fragmentation by the accumulation of permanent double strand  
1410 breaks. For example, [RuCl<sub>2</sub>(C<sub>6</sub>H<sub>6</sub>)(Me<sub>2</sub>SO)] is a topoisomerase II inhibitor that covalently binds to  
1411 DNA by ionic interaction of the Ru<sup>II</sup> centre with DNA nucleobases<sup>180</sup>. **KP1019** (FIG. 1) localizes in the  
1412 cytoplasm and binds efficiently to serum proteins<sup>181</sup> and interferes with the P-glycoprotein<sup>182</sup>.

1413

1414 The antioxidant glutathione (GSH) protects cells from reactive oxygen species (ROS), UV radiation and  
1415 heavy metal toxicity. In its reduced form, GSH is readily oxidized to glutathione disulfide (GSSG) by  
1416 the enzyme glutathione *S*-transferase (GST). Overexpression of GST by tumour cells is implicated in  
1417 drug resistance, and an organometallic (*p*-cymene)Ru<sup>II</sup> complex conjugated to ethacrynic acid, a GST  
1418 inhibitor, efficiently inhibits GST and is toxic to a GST-overexpressing cancer cell line<sup>183</sup>.

#### 1419 *Protein kinases*

1420 Protein kinases are phosphorylating enzymes that have important roles in signalling pathways  
1421 associated with intercellular uptake and cell proliferation. The identification of novel kinase inhibitors  
1422 is important for the development of new anticancer therapies. The major strategy for the design of new  
1423 Ru complexes that target protein kinases involves the use of substitutionally inert Ru<sup>II</sup> centres  
1424 coordinated to a bioactive ligand. The resulting rigid, stable 3D structures are extremely selective  
1425 protein kinase inhibitors<sup>184</sup> (**DW1/2**, FIG. 1).

#### 1426 **Box 2 | Methods of targeting tumours**

1427 The pH in cancerous or inflamed tissues is usually abnormally low (pH = 5–6 in the extracellular  
1428 medium) and local hyperthermia (around 42 °C). Many tumours have a leaky vasculature and lack or  
1429 have altered lymphatic drainage. The vascular endothelium in tumours proliferates rapidly and

1430 discontinuously, which results in a disorganized vasculature with a large number of ‘open’ intercellular  
1431 junctions (0.2–1.2  $\mu\text{m}$  in diameter, compared with  $<10\text{ nm}$  in normal vessels). These biological  
1432 characteristics are exploited to design efficient drug delivery carriers, such as anticancer prodrugs. High  
1433 molecular weight molecules, such as polymers and particles of diameter  $\sim 20\text{--}500\text{ nm}$  (except for  
1434 ‘stealth’ carriers), passively accumulate in the tumour area. This ‘passive targeting’, termed the  
1435 enhanced permeability and retention (EPR) effect<sup>71-73</sup>, is possible because these molecules can cross the  
1436 tumour endothelial barrier through the open junctions of the leaky vasculature and accumulate because  
1437 of insufficient lymphatic drainage from the tumour. Of note, ‘targeting’ in this context does not refer to  
1438 a specific interaction between a target molecule and a cellular receptor but instead refers to the selective  
1439 accumulation of the nanocarrier in tumour tissues rather than in healthy tissues — the drug concentration  
1440 in tumour tissues can be up to 10–100-fold higher than in healthy tissues<sup>185</sup>. The EPR effect allows the  
1441 use of a lower drug dose and increases the efficacy of the biologically active compound, thereby limiting  
1442 adverse effects. The diameter of polymer particles does not remain constant during the delivery process  
1443 and tends to decrease over time. Consequently, the design of fairly large nanocarriers (final diameter  
1444  $<100\text{ nm}$  to avoid removal from the blood and accumulation in the liver and spleen) with high Ru loading  
1445 is preferred<sup>186</sup>. Nanoparticles of intermediate diameter (10–100 nm) avoid renal clearance and have  
1446 prolonged circulatory times compared with nanoparticles  $<10\text{ nm}$  in diameter), owing to their slow  
1447 transport across the endothelium<sup>187</sup>. Despite their small size, nanoparticles  $<20\text{ nm}$  in diameter show  
1448 better tumour penetration<sup>188-190</sup> because they can pass through the leaky capillary walls in tumour tissue  
1449 and also return to the bloodstream by diffusion. It is hypothesized that nanoparticles 10–20 nm in  
1450 diameter can re-enter the tumour tissue without being eliminated by any clearance mechanisms<sup>191</sup>, which  
1451 might explain their efficient accumulation in tumour tissues. The contradictory results in these studies  
1452 suggest that the accumulation of nanoparticles in the tumour is not only dependent on their size.

1453  
1454 To obtain an efficient delivery system, the physicochemical properties of a nanocarrier, such as the  
1455 biocompatibility, hydrophilicity/hydrophobicity balance, carrier size and surface charge of the polymer  
1456 can be adjusted during nanocarrier preparation. All of these features affect the non-specific interactions  
1457 of polymers with cells of reticuloendothelial system (RES) in the circulation and interactions of  
1458 polymers with various components inside tumour tissues. The uptake of nanocarriers, especially those  
1459 that are hydrophobic, by the RES is predominantly through phagocytosis by macrophages, which is  
1460 initiated by the adsorption of various serum proteins termed opsonins. The EPR effect is time-dependent  
1461 and requires adequate circulation stability for at least several hours to be efficient. To increase their  
1462 circulation time and protect them from opsonization, carriers can be coated with a sufficiently thick and  
1463 dense layer of hydrophilic polymers, such as PEG or other polymeric glycols, or synthetic glucuronic  
1464 acid derivatives or polyphosphoramides to form ‘stealth carriers’<sup>191-194</sup>. Optimization of the  
1465 hydrophilic/hydrophobic balance of a nanocarrier is the primary factor affecting the efficiency of drug

1466 delivery to tumour tissues. Generally recognized as safe by the FDA, PEG is the standard polymer for  
1467 the preparation of stealth carriers owing to its biocompatibility<sup>91</sup> and low toxicity<sup>195</sup>. The targeting of  
1468 nanocarriers to tumour tissues is not only limited to the EPR effect but can also involve pH-dependent  
1469 hydrolytic activation in the tumour tissue or redox processes in the cytosol. Furthermore, molecules such  
1470 as peptides, antibodies or carbohydrate units, can be attached to the surface of nanocarriers to increase  
1471 tumour specificity. The choice of molecule depends on the mechanism of the transported drug; for  
1472 example, chemotherapeutic agents have various mechanisms of action, such as DNA alkylation  
1473 (cisplatin) or intercalation (doxorubicin), or inhibition of microtubules (taxanes), metabolic processes  
1474 (gemcitabine) or angiogenesis (endostatin).

1475

1476 Figure 1 | **Representative Ru complexes with medicinal potential.** A variety of Ru species — from  
1477 Ru<sup>II</sup> to Ru<sup>IV</sup>, cationic to anionic — have been designed for medicinal applications. The complexes were  
1478 intended as agents for chemotherapy<sup>3-11</sup> and photodynamic therapy<sup>12-15</sup>, as well miscellaneous based on  
1479 their vasodilation, antioxidant, anti-inflammatory, anti-microbial and anti-malarial properties.<sup>16,38, 119, 124,</sup>  
1480 <sup>136, 149-150</sup>

1481 Figure 2 | **Methods to encapsulate Ru complexes in polymers.** Ru complexes can have different  
1482 charges and are usually incorporated into polymers using either covalent conjugation or physical  
1483 encapsulation. The former is irreversible, while the latter process is typically reversible such that the Ru  
1484 species can often leach from the assemblies.

1485

1486 Figure 3 | **Some prominent biodegradable polymers.** Chitosan is a linear polymer of an aminosugar,  
1487 which can be protonated under physiological conditions. In contrast, alginate is also a sugar derivative  
1488 but is an anionic polymer. Poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and the corresponding  
1489 copolymer poly(lactid-co-glycolic)acid (PLGA), as well as poly( $\epsilon$ -caprolactone) (PCL) are all  
1490 biodegradable polyesters.

1491

1492 Figure 4 | **Synthetic strategies for Ru-conjugated polymer prodrug candidates.** **a** | The convergent  
1493 synthesis approach involves coordinating Ru to a preformed macromolecular organic ligand. **b** |  
1494 Alternatively, the divergent strategy uses a Ru complex with strongly bound ligands as a metalloinitiator  
1495 for polymerization.

1496

1497 Figure 5 | **Photoactivable nanocarriers.** **a** | Ru<sup>II</sup>(CO)<sub>3</sub> fragments serve as sources of CO in hydrolyzable  
1498 polymers that are activation by hydrolysis. **b** | Light activation can cause a Ru–NO bond to break and  
1499 release NO or release an anticancer Ru drug that generates <sup>1</sup>O<sub>2</sub><sup>157</sup>. The toxicity of this metalloprodrug  
1500 principally comes from the photoactivation of Ru drug candidate remaining coordinated in the polymer  
1501 chain.

1502

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  $\{[(\eta^6-$   
1505  $\text{arene})\text{Ru}]_2(\eta^2\text{-OH})_3\}^+$ . **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  $\text{H}_2\text{O}$  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.

1512

1513 Figure 8 | **Synthesis of Ru complexes bearing ligands with two (up to six) polymer chains. A** | The  
1514 convergent synthesis method can afford  $\text{Ru}^{\text{II}}$  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.

1518