



Max-Planck-Institut für Polymerforschung  
Max Planck Institute for Polymer Research



# **Self-Assembled Nanocarriers Based on Amphiphilic Ruthenium Complexes for Anticancer Phototherapy**

**Dissertation**

Zur Erlangung des Grades

**„Doktor der Naturwissenschaften“**

im Promotionsfach Chemie

Eingereicht am

Fachbereich Chemie, Pharmazie und Geowissenschaften

der Johannes Gutenberg-Universität Mainz

Von

**Xiaolong Zeng**

Mainz, 2021



JOHANNES GUTENBERG  
UNIVERSITÄT MAINZ



---

Die vorliegende Arbeit wurde im Zeitraum von März 2017 bis Juli 2021 am Max-Planck-Institut für Polymerforschung in Mainz im Arbeitskreis von Prof. Dr. Hans-Jürgen Butt und Prof. Dr. Si Wu angefertigt.

Dekan:

Prodekan:

Gutachter 1:

Gutachter 2:

Date of oral examination:





---

## Table of Contents

Abstract.....	1
Zusammenfassung.....	3
Motivation.....	5
Chapter 1: Introduction.....	6
1.1 Nanocarriers to overcome drug resistance in anticancer therapy.....	6
1.1.1 Mechanisms of drug resistance in cancer treatment.....	6
1.1.2 Strategies for reversing drug resistance.....	8
1.1.3 Nanocarriers to overcome drug resistance.....	9
1.2 Nanocarriers with favorable properties to enhance delivery efficiency.....	10
1.2.1 Five-step delivery cascade of nanocarriers.....	10
1.2.2 Physicochemical properties of nanocarriers.....	13
1.3 Nanocarriers based on ruthenium complexes for anticancer treatment.....	14
1.3.1 Ru complexes in cancer therapy.....	14
1.3.2 Nanocarriers based on Ru complexes for cancer therapy.....	23
1.3.3 Challenges of nanocarriers based on Ru complexes in cancer therapy.....	28
1.4 References.....	29
Chapter 2: Light-cleavable Ru(II) complexes with tunable properties for enhanced delivering efficiency in phototherapy.....	38
2.1 Statement of contribution.....	39
2.2 Introduction.....	40
2.3 Results and discussion.....	42

---

2.3.1 Synthesis of Ru-PEG .....	42
2.3.2 Self-assembly of Ru-PEG.....	43
2.3.3 Light-cleavable Ru-PEG with tunable properties.....	44
2.3.4 Tumor penetration ability of Ru-PEG .....	46
2.3.5 Cellular uptake and in vitro cytotoxicity assessment .....	49
2.3.6 Lysosomal damage and apoptosis mechanisms.....	51
2.3.7 In vivo anticancer assessment.....	53
2.4 Conclusions .....	55
2.5 References .....	55
2.6 Supporting information .....	59
Chapter 3: Fighting against drug-resistant tumors using a dual-responsive	
Pt(IV)/Ru(II) bimetallic polymer .....	86
3.1 Statement of contribution .....	87
3.2 Introduction .....	88
3.3 Result and discussion .....	91
3.3.1 Synthesis of PolyPt/Ru .....	91
3.3.2 Self-assembly of PolyPt/Ru.....	92
3.3.3 Dual-responsiveness of PolyPt/Ru .....	92
3.3.4 In vitro cytotoxicity against drug resistant cancer cells .....	96
3.3.5 Cellular uptake and in vitro cytotoxicity assessment .....	98
3.3.6 In vivo anticancer assessment.....	100
3.4 Conclusions .....	104

---

3.5 References .....	105
3.6 Supporting Information .....	108
Chapter 4 Summary and Outlook .....	146
4.1 Summary .....	146
4.2 Outlook.....	147
Publications.....	148
Acknowledgment .....	149

## Abstract

Photoresponsive ruthenium (Ru) complexes have promising anticancer properties. Self-assembled nanocarriers have been used to deliver Ru complexes for anticancer phototherapy. Comparing with small molecule, nanocarriers based on Ru complexes exhibit improved bioavailability and extended blood circulation. However, conventional self-assembled nanocarriers suffer low delivery efficiency and lack of combined therapy. The inefficient nanocarriers limit their anticancer efficacy due to the low Ru concentration at target sites. Single therapy suffer limitations to combat tumor complexity. A major goal in this field is to develop nanocarriers with high delivery efficiency, and further overcome the complexity of tumor, such as drug resistance. In this thesis, we reported novel nanocarriers based on photoresponsive Ru complexes to address these issues.

In Chapter 2, we designed a light-cleavable Ru nanocarriers (Ru-PEG) with tunable properties to enhance the delivery efficiency. Ru-PEG consist a hydrophobic Ru moiety and a hydrophilic PEG moiety. Ru-PEG assembled into vesicles. Light irradiation of Ru-PEG induces the morphological transformation, changing from vesicles to large compound micelles (LCMs). Light irradiation also caused the release of PEG, leading to the increment of surface charge of Ru nanocarriers. The formed LCMs had a smaller diameter, which improved the penetration depth at tumor sites. The increased positive charged Ru nanocarriers enabled efficient internalization. Both of them improved the delivery efficiency of Ru complexes.

In chapter 3, we reported a dual-responsive Ru-containing nanocarriers (PolyPt/Ru) to reverse drug resistance. PolyPt/Ru is a triblock polymer with two hydrophilic PEG moieties and one hydrophobic Pt/Ru bimetallic moiety. PolyPt/Ru self-assembled into

nanoparticles. Irradiation of PolyPt/Ru with red light generated  $^1\text{O}_2$ , induced nanocarrier degradation, and triggered the release of Ru(II) anticancer agents. The anticancer drug, cisplatin, was released in the intracellular environment via the reduction of Pt(IV) moieties. The released Ru(II) anticancer agent, cisplatin, and the generated  $^1\text{O}_2$  have different anticancer mechanisms. Their synergistic effects inhibited the growth of resistant tumors.

## **Zusammenfassung**

Photoresponsive Ruthenium (Ru)-Komplexe haben vielversprechende anticancerogene Eigenschaften. Selbstorganisierte Nanoträger wurden verwendet, um Ru-Komplexe für die Phototherapie gegen Krebs herzustellen. Im Vergleich zu kleinen Molekülen weisen Nanoträger auf Basis von Ru-Komplexen eine verbesserte Bioverfügbarkeit und Zirkulation im Blutkreislauf auf. Herkömmliche selbstorganisierte Nanoträger leiden jedoch unter einer geringen Abgabeffizienz und einem Mangel an kombinierter Therapie. Die ineffizienten Nanoträger haben eine begrenzte anticancerogene Wirkung aufgrund der geringen Ru-Konzentration am Wirkort. Die Einzeltherapie leidet unter Einschränkungen bei der Bekämpfung der Tumorkomplexität. Ein Hauptziel auf diesem Gebiet ist die Entwicklung von Nanoträgern mit hoher Abgabeffizienz und die weitere Überwindung der Komplexität von Tumoren, wie z. B. der Arzneimittelresistenz. In dieser Arbeit berichteten wir über neuartige Nanoträger, die auf photoresponsiven Ru-Komplexen basieren, um diese Probleme anzugehen.

In Kapitel 2 haben wir einen lichtspaltbaren Ru-Nanoträger (Ru-PEG) mit einstellbaren Eigenschaften entwickelt, um die Abgabeffizienz zu verbessern. Ru-PEG besteht aus einer hydrophoben Ru-Einheit und einer hydrophilen PEG-Einheit. Ru-PEG organisiert sich selbst zu Vesikeln. Die Bestrahlung von Ru-PEG mit Licht induziert die morphologische Transformation von Vesikeln zu großen zusammengesetzten Mizellen (LCMs). Gleichzeitig wird PEG freigesetzt, was zu einer Erhöhung der Oberflächenladung der Ru-Nanoträger führt. Die gebildeten LCMs hatten einen kleineren Durchmesser, was zu einer erhöhten Eindringtiefe in die Tumore führte. Die stärker positiv geladenen Ru-Nanoträger ermöglichten eine effiziente Internalisierung. Beides führte zu einer verbesserten Abgabeffizienz von Ru-Komplexen.

In Kapitel 3 berichteten wir über einen doppelt ansprechenden Ru-haltigen Nanoträger (PolyPt/Ru), der Arzneimittelresistenzen umgeht. PolyPt/Ru ist ein Triblockpolymer mit zwei hydrophilen PEG-Einheiten und einer hydrophoben Pt/Ru-Bimetalleinheit und organisiert sich selbst zu Nanopartikeln. Die Bestrahlung von PolyPt / Ru mit rotem Licht erzeugte  $^1\text{O}_2$ , induzierte den Abbau von Nanoträgern und löste die Freisetzung von Ru(II)-Zytostatika aus. Das Zytostatikum Cisplatin wurde in der intrazellulären Umgebung über die Reduktion von Pt(IV)-Einheiten freigesetzt. Das freigesetzte Ru(II) - Zytostatikum Cisplatin und das erzeugte  $^1\text{O}_2$  weisen unterschiedliche anticancerogene Mechanismen auf. Ihre synergistischen Wirkungen hemmten das Wachstum resistenter Tumoren.

## **Motivation**

Self-assembled nanocarriers have been applied to deliver Ru complexes towards cancer cells in cancer therapy. To date, several strategies have been developed to optimize the delivery efficiency. For example, PEGylated nanocarriers are used to prolong blood circulation. Charged nanocarriers enable efficient internalization by cancer cells. However, these strategies suffer antagonistic effects. In general, PEGylation is negatively related to cellular internalization. Positive charged nanocarriers absorb proteins during circulation, which lead to be cleared by macrophage. Nanocarriers with tunable properties may address these problems.

However, such smart nanocarriers based on Ru complexes do not exist. To achieve this goal, I made use of a light-cleavable Ru complex to construct nanocarriers with tunable properties. I expect the PEGylated Ru-containing nanocarriers can reduce specific protein absorption during circulation. After accumulating at tumor sites and reaching cancer cells, I expect that light-induced the release of PEG can improve cellular internalization. I wish the adjustable nanocarriers based on light-cleavable Ru complexes can achieve high delivery efficiency.

Another issue for nanocarriers based on Ru complexes is to reverse the drug resistance of tumor. Most chemotherapeutic drugs become insensitive to cancer cells after using for several times. Using anticancer drugs with different mechanisms of action may solve this problem. To achieve this goal, I developed a dual-responsive bimetallic polymer, which contained Ru anticancer agents and cisplatin anticancer drugs. The photoresponsive Ru complexes and cisplatin had different anticancer mechanisms. I expect the combination of Ru complexes and cisplatin can reverse drug resistance.



## **Chapter 1: Introduction**

### **1.1 Nanocarriers to overcome drug resistance in anticancer therapy**

#### **1.1.1 Mechanisms of drug resistance in cancer treatment**

Cancer is a major burden of death over the world. Cancer is responsible for nearly 10 million deaths every year.<sup>1</sup> About one in six deaths accounts for cancer globally.<sup>2</sup> Typically, cancer treatment involves surgery, radiotherapy and chemotherapy.<sup>3</sup> Chemotherapy has been most widely applied for cancer treatment.<sup>4</sup> However, cancer patients may be resistant to anticancer drugs after receiving chemotherapy several times.<sup>4</sup> Drug resistance results in therapeutic failure and eventually cause patients death.

The mechanism of drug resistance falls into several categories (Figure 1).<sup>5</sup> Firstly, the decrement of cellular uptake causes drug resistance.<sup>6,7</sup> The cellular uptake of anticancer drugs influences the therapeutic performance of chemotherapy.<sup>6</sup> Cancer cells take up drugs either via receptors/transporter, endocytosis or diffusion across the plasma membrane.<sup>8</sup> However, the alteration or mutation of receptors/transporter in resistant cancer cells reduces the cellular uptake efficiency, thus decreasing the intracellular drug concentrations.<sup>9</sup> This results in cancer cells insensitivity to drugs. The reduced uptake of toxic drugs is the most major causes of drug resistance.

Secondly, the active drug efflux causes drug resistance.<sup>10,11</sup> The efflux pump, such as P-gp protein, which is overexpressed on the membrane of resistant cancer cells.<sup>10, 12</sup> They can not only inhibit the drug uptake but also pump drugs out of cells.<sup>12</sup> Drug efflux decreases intracellular drug concentration. The up-regulated overexpression of P-gp proteins results in a significant resistance to chemotherapy.

Thirdly, the enhanced DNA repair capacity causes drug resistance.<sup>13, 14</sup> Many anticancer drugs cause damage to DNA.<sup>15</sup> This is responsible for the cytotoxicity of

these drugs. Resistant cancer cells repair DNA when it becomes damaged. When the magnitude of damage is reduced, DNA repair confers resistance to anticancer drugs.

The other mechanism of drug resistance is due to the inactivation of drugs.<sup>16</sup> Some intracellular biomolecules strongly bind and sequester the specific drugs.<sup>17</sup> For example, glutathione (GSH) has been implicated in resistance to several drugs, including platinum-containing compounds,<sup>16</sup> and alkylating agents such as melphalan.<sup>18</sup> Similarly, cytochrome P450 enzymes causes the inactivation of anticancer drug, irinotecan.<sup>19</sup> These biomolecules are prior binding to drugs, decrease their toxicities to cancer cells, and cause drug resistance. Moreover, when administered intravenously, various proteins in blood may also bind to anticancer drugs, leading to the inactivation.<sup>9</sup>

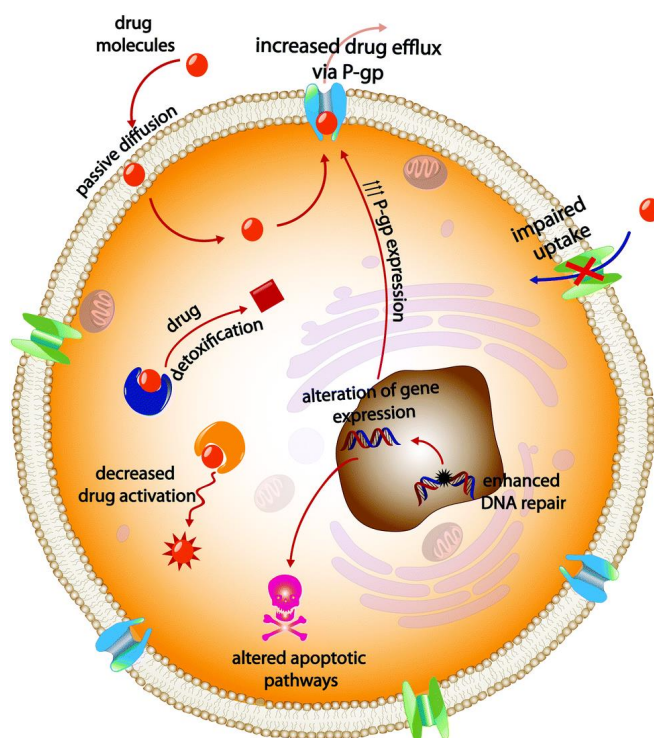


Figure 1. Schematic illustration of nanocarriers to overcome drug resistance. Adapted with permission from ref 5. Copyright 2016 Royal Society of Chemistry.

### 1.1.2 Strategies for reversing drug resistance

Several strategies have been proposed to combat drug resistance in cancer treatment.<sup>4, 20, 21</sup> Modification of targeting molecules can overcome drug resistance that is caused by reduced cellular uptake.<sup>22</sup> Therefore, anticancer drugs are conjugated with specific molecules such as antibody or targeting peptides.<sup>23</sup> These molecules can specifically target the receptors which are overexpressed on the cell membrane. The biochemical reaction between targeting molecules and receptors activates specific signals, which further promote the internalization of drugs into cancer cells.<sup>24</sup> The enhanced internalization reverses drug resistance.

Synchronous administration of anticancer drugs along with protein inhibitors, such as P-gp protein inhibitors or DNA repair protein inhibitors, may reverse drug resistance.<sup>25, 26</sup> P-gp inhibitors possess greater affinity against P-gp proteins with high specificity.<sup>27</sup> This subsequently decreases the activity of P-gp proteins, leading to the inhibition of drug efflux, and thus achieving the reversal of drug resistance.

Depletion of intracellular GSH may modulate drug resistance caused by the inactivation of drugs.<sup>28, 29</sup> Some small molecules, such as buthionine sulfoximine (BSO), are applied to deplete GSH levels.<sup>30</sup> BSO can inhibit the gamma-glutamylcysteine synthetase, which involves the first step of GSH bio-synthesis, thus decreasing the intracellular GSH levels.<sup>30</sup> BSO can also increase the sensitivity of anticancer drugs to cancer cells,<sup>31</sup> as a result, drug resistance is reversed.

Although the above-mentioned strategies have been developed, it is challenging to overcome multiple resistant pathways. The current strategies still suffer limitations in clinic. For example, antibody may be rapidly degraded by enzyme or cleared from the bloodstream.<sup>32</sup> Administration of anticancer drugs and protein inhibitors is limited to their different circulation times. The different components may not reach the target sites

simultaneously, leading to non-ideal effect.<sup>33</sup> BSO always suffer from poor solubility in aqueous solution and poor selectivity towards tumor. Thus, reversing drug resistance still poses a challenge.

### **1.1.3 Nanocarriers to overcome drug resistance**

Recently, nanotechnology has been developed to provide a promising alternative, compared to the conventional small-molecule chemotherapeutics. Nanotechnology circumvents drug resistance by encapsulating, attaching, and conjugating therapeutic biological agents to nanocarriers. Nanocarriers hold several advantages for reversing drug resistance in cancer treatment (Figure 2).<sup>34</sup>

Firstly, nanocarriers can deliver multiple therapeutic payloads in one system, such as P-gp inhibitors and anticancer drugs, acting as “all-in-one” strategy.<sup>25</sup> Different therapeutic agents that are encapsulated/conjugated into nanocarriers have the same pharmacokinetic profiles. Their similar bio-distribution exhibit the synergistics effect at the target sites.

Secondly, nanocarriers can be easily achieved surface modification.<sup>35</sup> Various functional molecules can be simultaneously attached onto the surface of nanocarriers. Modification by antibodies provides active targeting ability towards cancer cells.<sup>36</sup> Modification by poly (ethylene glycol) (PEG) achieves stealthy effect during circulation,<sup>37</sup> which prevent the inactivation of loaded cargoes from blood clearance and enzyme degradation.

Furthermore, the internalization category of nanocarriers is different with free drugs. Most free drugs enter into cancer cells by diffusion across membranes,<sup>38</sup> which is easily recognized by P-gp proteins that pumping drugs out from cancer cells. However, nanocarriers, such as gold nanoparticles or silica nanoparticles, effectively enter into

cancer cells through endocytosis, with bypass of drug efflux pumps.<sup>39, 40</sup> These advantages endow nanocarriers with the capacity to address complicated and combined mechanisms of drug resistance.

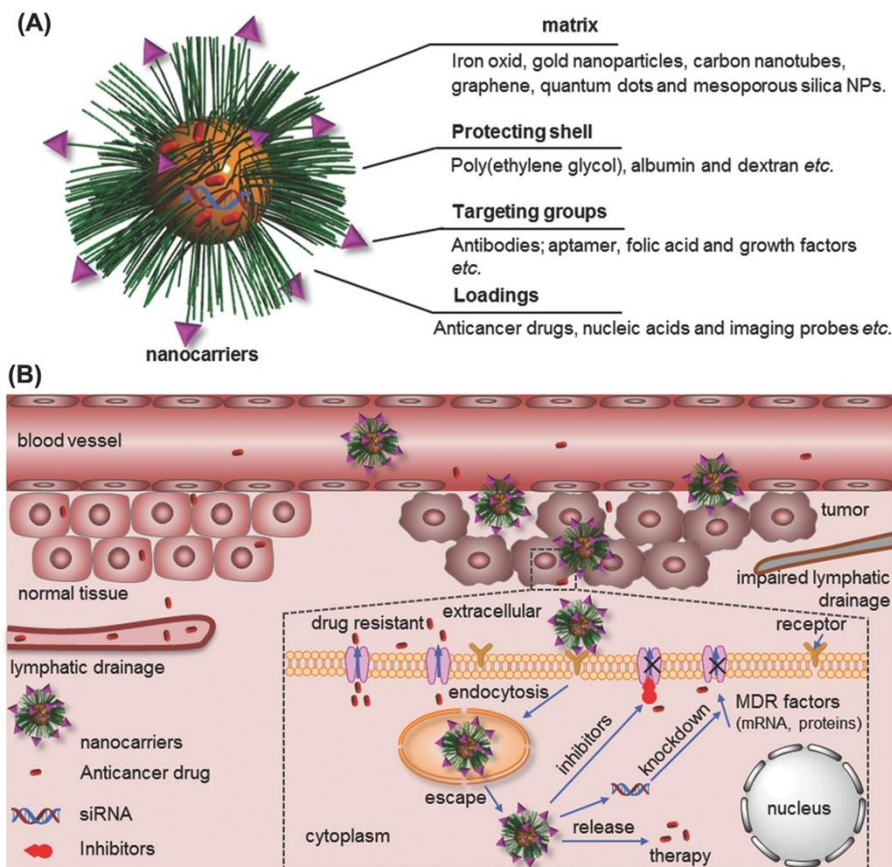


Figure 2. Schematic illustration of nanocarriers to overcome drug resistance. Adapted with permission from ref 34. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA.

## 1.2 Nanocarriers with favorable properties to enhance delivery efficiency

### 1.2.1 Five-step delivery cascade of nanocarriers

The ultimate goal of nanocarriers is to deliver drugs into cancer cells as free molecules, to exert their pharmaceutical effects. Typically, intravenously administered nanocarriers go through a cascade of five steps: 1) circulation in blood; 2) accumulation at tumor sites; 3) penetration into deep tumor tissues; 4) internalization into cancer cells; and 5) intracellular drug release (Figure 3).<sup>41</sup> The anticancer efficacy of loaded drugs is

determined by the entire delivery efficiency of nanocarriers.<sup>41</sup> The cascade involves the above-mentioned five steps. Thus, optimizing each step is crucial for achieving high total efficiency. It is more important to ensure that none of efficiency of each step approaches zero. Otherwise, this will be the Achilles heel of the entire delivery cascade.

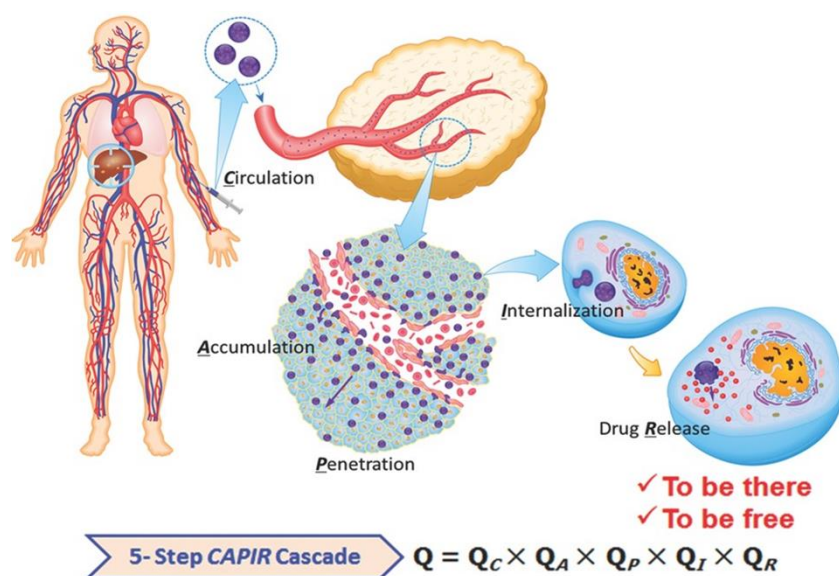


Figure 3. Five-step cascade of nanocarriers. Adapted with permission from ref 41. Copyright 2017 WILEY-VCH Verlag GmbH & Co. KGaA.

**Circulation in blood.** Proteins are easily absorbed onto nanocarriers in blood.<sup>42</sup> The complement system and opsonizing proteins tag nanocarriers as foreign substance.<sup>42</sup> Subsequently the tagged nanocarriers are cleared by macrophage and thus have short circulation time. Introducing of poly(ethylene glycol) (PEG) or its alternatives, such as polysaccharides,<sup>42</sup> poly(2-oxazoline)s,<sup>43</sup> and polyphosphoesters,<sup>44</sup> can prolong circulation time. This is due to the surface modification of PEG, forming a hydrated barrier, thus hindering the interaction between nanocarriers and specific proteins. The formed barrier reduce protein adsorption and prolong circulation.

**Accumulation at tumor sites.** There are two basic strategies to enhance the accumulation of nanocarriers at tumor sites, either by passive accumulation (passive

targeting) or tumor-targeting vectors (active targeting). Passive targeting can be achieved through the enhanced permeability and retention (EPR) effect of nanocarriers.<sup>45</sup> Whereas, active targeting realize tumor accumulation through the conjugation of specific targeting ligands.<sup>23</sup>

**Penetration into deep tumor tissues.** The heterogeneity of tumor tissues have a number of biological obstacles, such as cancer-associated fibroblasts and high interstitial pressure.<sup>46</sup> These obstacles hinder the deep penetration of nanocarriers, thus obstructing the therapeutic efficacy of drug-loaded nanocarriers. Researchers have developed recombinant human hyaluronidase (PH20), onto the surface of nanocarriers to enhance the drug penetration in tumors.<sup>47</sup> PH20 could degrade the tumor extracellular matrix to facilitate interstitial diffusion, thus enhancing the penetration depth of nanocarriers.<sup>47</sup>

**Internalization into cancer cells.** Internalization refers a cellular process that nanocarriers are taken up into cells. Surface modification of specific ligands provides high internalization of nanocarriers. The ligands bind to the receptors which are expressed on the surface of cancer cells. The specific ligand-receptor recognition could activate the intracellular signal and promote the cell internalization of nanocarriers.<sup>23</sup>

**Intracellular drug release.** Stimuli-responsive nanocarriers trigger the on-demand drug release. These stimuli could be endogenous owing to the difference between tumor and normal tissues, including pH, redox potential, reactive oxygen species level and enzyme.<sup>48</sup> Drug release can be also triggered via exogenous stimuli such as light, magnetic field, ultrasound and electric field.<sup>48</sup> In comparison to those non-responsive nanocarriers in which drugs are released by uncontrollable spontaneous diffusion, stimuli-responsive nanocarriers enable for high release behavior.

### 1.2.2 Physicochemical properties of nanocarriers

The delivery efficiency is influenced by the physicochemical properties of nanocarriers.<sup>49</sup> The size, surface charge and shape of nanocarriers are the major determination of actions in biological environments.

**Size.** Size directly determines the surface area of nanocarriers.<sup>50</sup> The surface area significantly influences the protein absorption during circulation. Smaller nanocarriers was found to absorb more lipoproteins, such as clusterin.<sup>51</sup> Whereas, larger nanocarriers attracted higher affinity towards complement related proteins, such as prothrombin and gelsolin.<sup>51</sup> The specific absorbed protein can be further recognized by macrophage, which then lead to eliminate nanocarriers. Different types of absorbed proteins result in varying internalization of macrophage.<sup>52</sup> As a result, the smaller nanocarriers similarly exhibited slower elimination rate, comparing with the much faster elimination of larger nanocarriers.

The size of nanocarriers is also crucial for accumulation and penetration at tumor sites.<sup>50</sup> Because most nanocarriers accumulate at tumor sites via EPR effect. Nanocarriers with smaller size are easy to permeate from blood vessel to tumors. Thus, decreasing the size affect their vascular permeation positively, increasing the accumulation of nanocarriers. Moreover, smaller nanocarriers are obviously beneficial for penetrating deeper through the tumor matrix, while larger nanocarriers only stayed near the vasculature.<sup>40</sup>

**Surface charge.** Surface charge affects the protein absorption during circulation.<sup>50</sup> Positively charged nanocarriers adsorb more proteins than those with negatively charged. Neutral charged nanocarriers exhibited significantly reduced protein absorption.<sup>50</sup> Abundant protein absorption results in higher recognition by macrophage,



which contribute to blood clearance. Therefore, nanocarriers with neutral or negative charge show lower clearance rate, achieving long circulation time.

In addition, cell internalization process is also influenced by surface charge of nanocarriers.<sup>53</sup> Biological cell membrane exhibits negative charge. Positively charged nanocarriers are generally efficiently taken up than negatively charged ones. Similarly, charged nanocarriers are taken up better than their uncharged counterparts.

**Shape.** Shape is another important physicochemical property that determines the blood circulation.<sup>50</sup> Various shapes of nanocarriers can be taken up by macrophages at different rates, resulting in varying circulation times. Specifically, non-spherical nanocarriers have deviating hydrodynamic behaviors compared to the spherical ones. Previous studies indicated that non-spherical shape like filomicelles, nanorods, nanowires, and nanodisks have extended circulation time compared to their spherical counterparts.<sup>50</sup>

The shape of nanocarriers also have impact on the accumulation at tumor sites. Non-spherical nanocarriers have distinct motions that include tumbling and rolling, resulting in distinct margination dynamics.<sup>54</sup> Elongated nanocarriers, on the other hand, typically have less drag and larger surface areas than spheres.<sup>54</sup> Therefore, defined non-spherical nanocarriers can strongly adhere to the endothelial walls due to enhanced multivalent bonding, showing lower accumulation rates.

### **1.3 Nanocarriers based on ruthenium complexes for anticancer treatment**

#### **1.3.1 Ru complexes in cancer therapy**

Recently, metallodrugs have attracted interest for cancer treatment.<sup>16,55-57</sup> Among them, ruthenium (Ru) complexes have drawn highly attention because of their unique properties.<sup>58,59</sup> The nature and the numbers of ligands, that coordinated to Ru(II) centers,

can be varied to tune the physicochemical properties of these complexes. These characteristics makes them attractive in biomedical field, for example, as new classes of anticancer agents for chemotherapy, photosensitizers for photodynamic therapy (PDT) and photoactivatable prodrugs for photoactivated chemotherapy (PACT).<sup>59</sup>

### 1.3.1.1 Ru complexes as anticancer agents for chemotherapy

Various Ru complexes have been developed and found to exhibit anticancer activities. Till now, three of them have entered in clinical trial, including KP1019,<sup>60</sup> KP1339,<sup>61</sup> and NAMI-A (Figure 4).<sup>62</sup> Ru complexes hold efficient cellular uptake against cancer cells. After taken up by cancer cells, they can interact with DNA, blocking the synthesis of DNA or protein, thus achieving the cell death.<sup>58</sup> These results encourage researchers to design more Ru complexes with desirable activities for cancer treatment.

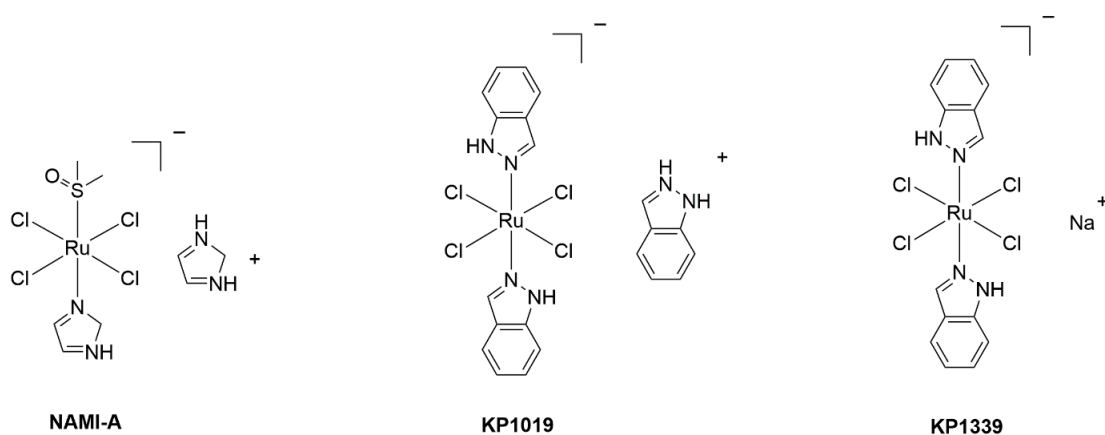


Figure 4. Chemical structure of anticancer agents, NAMI-A, KP1019 and KP1339.

Zeng and coworkers developed chiral structural Ru complexes **1a** and **1b**, which their toxicity depends on the cellular localization (Figure 5).<sup>63</sup> They found that these complexes inhibited the cell growth when they located in the cell nucleus. However, when they mainly enriched in the cytoplasm, the cytotoxicity dramatically decreased and showed negligible anticancer performance.

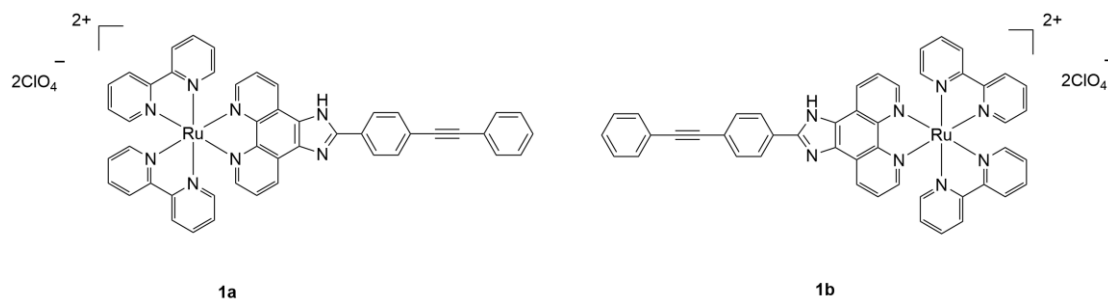


Figure 5. Chemical structure of Ru complex **1a-b**.<sup>63</sup>

Since the toxicity caused by Ru complexes strongly depends on their cellular localization. Afterwards, both Liu's and Chen's groups designed a series of Ru complexes **2a-2e**, which can target mitochondria (Figure 6).<sup>64-69</sup> Mitochondria acts as an important targeting organelle for anticancer drug in chemotherapy. They found all these Ru complexes caused cell apoptosis efficiently via a mitochondrial pathway.

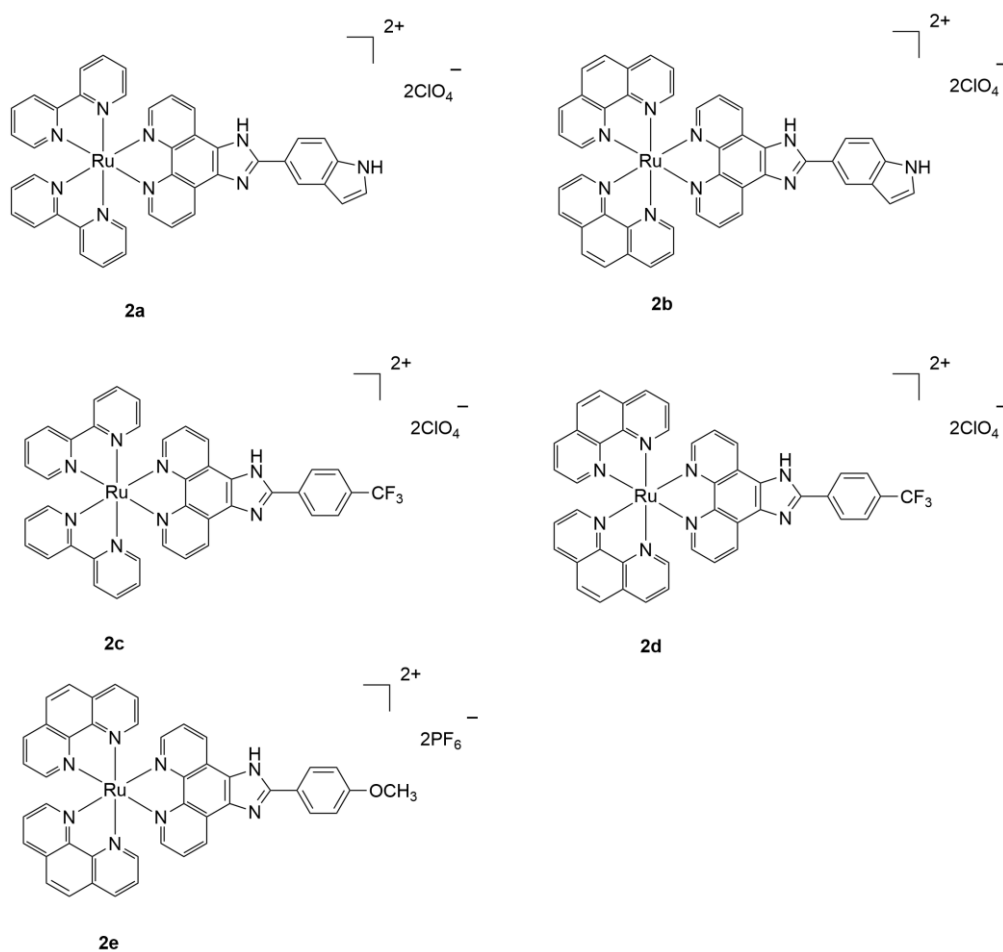


Figure 6. Chemical structure of Ru complexes **2a-e**.<sup>64-69</sup>

### 1.3.1.2 Ru complexes as photosensitizers for photodynamic therapy

Ru complexes used in PDT rely on the generation of reactive oxygen species (ROS), to cause cell damage. Upon light irradiation, Ru complexes transform from their ground states (singlet state) into relatively long-lived excited states (triplet state).<sup>70</sup> The excited triplet state then enables the production of ROS by two pathways. First, it can directly react with a substrate, such as the cell membrane or a molecule, and transfers an electron to form radicals. These radicals interact with oxygen to generate oxygenated products (type I PDT pathway).<sup>71, 72</sup> Alternatively, the excited Ru complexes can transfer their energy to oxygen, to form singlet oxygen ( $^1\text{O}_2$ ) (type II PDT pathway).<sup>71, 72</sup>  $^1\text{O}_2$  is a toxic species, which further causes cell damage. The ROS species generated from two pathways can both kill cancer cells.

As a pioneer, McFarland and coworker designed a series of Ru complexes **3a-3d**, with variable lengths of polythiophene chains, used in PDT (Figure 7).<sup>73</sup> By introducing the polythiophene chain, these complexes hold low-lying intraligand ( $^3\text{IL}$ ) excited states, exhibiting high  $^1\text{O}_2$  production abilities. The  $^1\text{O}_2$  quantum yields increased with the increment of polythiophene chain. The toxicities of these complexes were very low in the dark, whereas can be enhanced 200-fold upon light irradiation. Worth of note, **3c** in this family is currently undergoing human phase IB clinical trials, which is the well-known photosensitizer, TLD-1433.

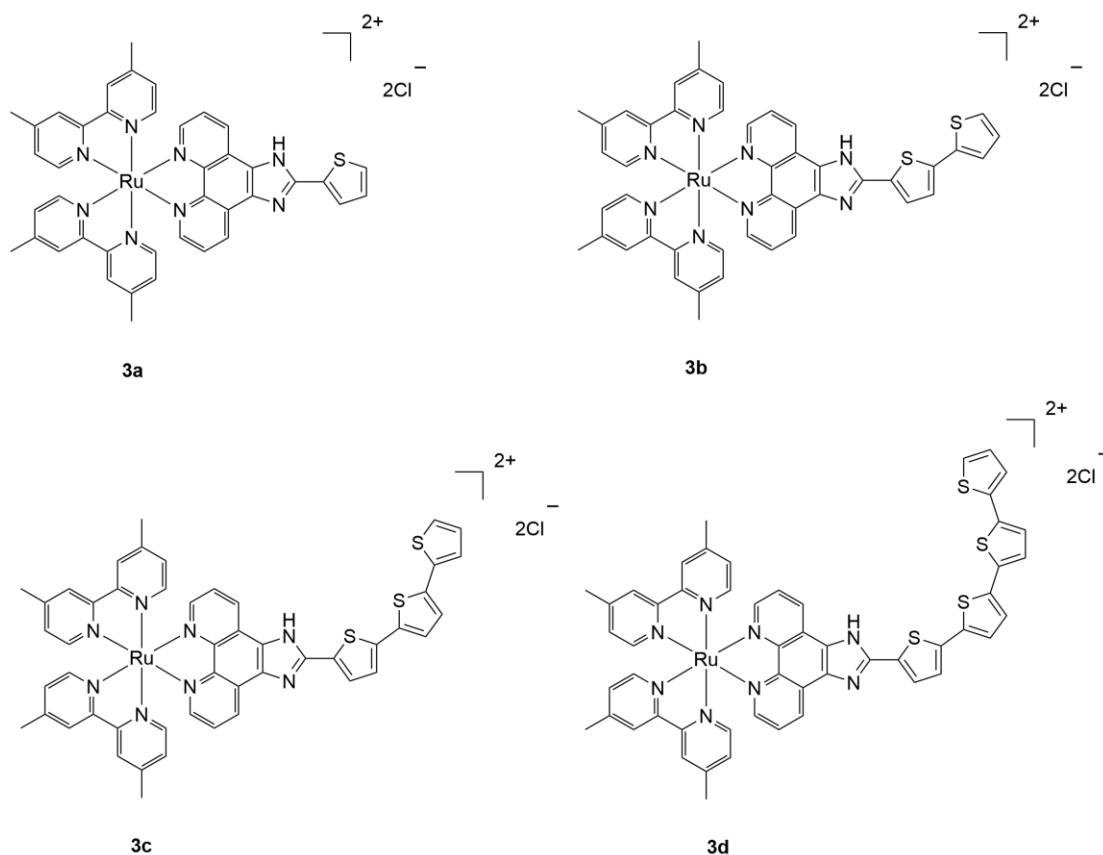


Figure 7. Chemical structure of Ru complexes **3a-d**.<sup>73</sup>

The same group later reported another Ru complexes **4**, which also possessed the properties of low-energy and long-lived <sup>3</sup>IL excited states (Figure 8).<sup>74</sup> **4** contains a  $\pi$ -expansive dppn ligand (dppn = benzo[i]dipyrido[3,2- a:2',3'-c]phenazine), which proved to be extremely sensitive to trace amounts of oxygen, leading to efficient PDT even under hypoxic conditions.

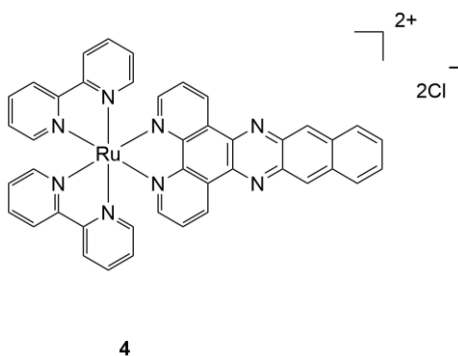


Figure 8. Chemical structure of Ru complexes **4**.<sup>74</sup>

Recently, a series of highly charged Ru complexes **5a-5c** was developed by Chao and coworkers.<sup>75</sup> In all these complexes,  $[\text{Ru}(\text{bpy})_3]^{2+}$  (bpy = 4,4'-bipyridine) was chosen as the architecture because of its excellent two-photon absorption (TPA) properties. Tertiary ammonium groups were modified on the surface, to increase the water solubility and the binding affinity towards cell membrane (Figure 9). Moreover, **5a-5c** were found to localize in lysosome, which is a favorable location for PDT. Lysosomes are associated with cell apoptosis and necrosis. As a result, upon irradiation, the generation of  $^1\text{O}_2$  possessed significant anticancer performance.

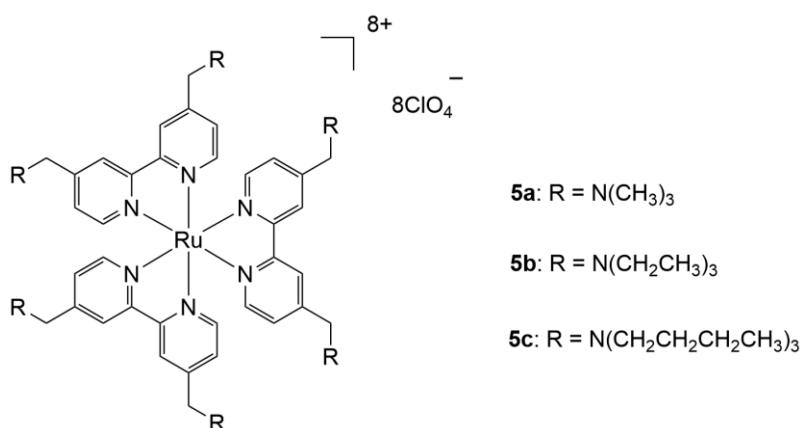


Figure 9. Chemical structure of Ru complexes **5a-5c**.<sup>75</sup>

### 1.3.1.3 Ru complexes as prodrugs for photoactivated chemotherapy

Similar to PDT, photoactivatable Ru complexes in PACT are also poorly toxic in darkness but become highly toxic after light irradiation.<sup>76</sup> However, the toxic species are the released components instead of the generated  $^1\text{O}_2$ .<sup>77</sup> Chemically speaking, photoactivatable Ru complexes possess an activity-inhibited therapeutic moiety and a photolytic bond.<sup>76</sup> The non-irradiated Ru complexes are inactive to biological environment. Upon light irradiation, the photolytic bond are cleaved and release the toxic moieties, which further interact with DNA, lipids or protein of cancer cells.

The mechanism of action of photolytic Ru complexes is based on their  $d^6$ -metal structure.<sup>77</sup> The photochemical behavior of these complexes have the nature with lowest lying triplet state. Upon light irradiation, the triplet excited states of metal-to-ligand character ( $^3\text{MLCT}$ ) interconvert into low-lying triplet metal-centered ( $^3\text{MC}$ ) states or d-d states (Figure 10).<sup>78</sup> This characteristic offer a strong dissociation ability, leading to a ligand substitution and resulting in the release of leaving groups such as the toxic moiety.

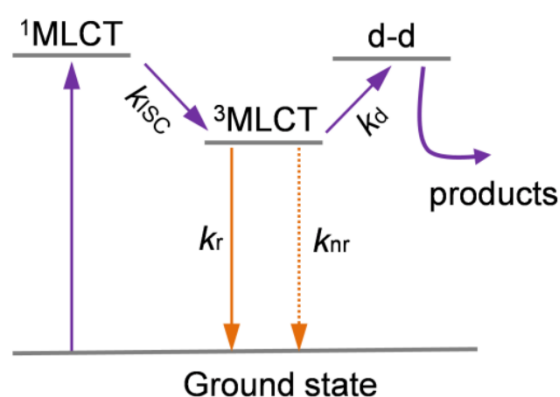


Figure 10. The mechanism of action of photolytic Ru complexes.

The toxic moiety can be anticancer drugs. Ru complexes act as caging moieties for the photo-induced release of anticancer drugs. In 2015, Renfrew and coworkers developed a Ru complex **6** which was used for releasing imidazole-based drug, econazole (Figure 11).<sup>79</sup> Econazole is used for oral and intravenous applications in cancer treatment.<sup>80</sup> When caged by Ru complex, it showed negligible toxicity against several kinds of cancer cells. However, upon green light irradiation, **6** caused the release of econazole drug, which then resulted in a 34-fold increment in cytotoxicity against cancer cells.

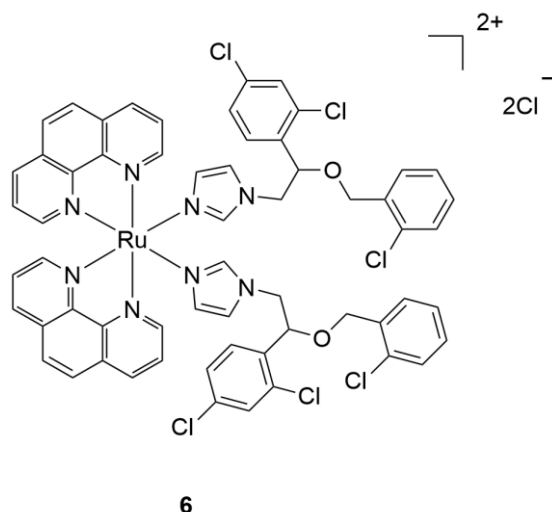


Figure 11. Chemical structure of Ru complexes **6**.<sup>79</sup>

Recently, Bonnet and coworkers designed two Ru complexes **7a-7b**, for the photo-induced releasing a toxic nicotinamide phosphoribosyltransferase (NAMPT) inhibitor STF-31 (Figure 12).<sup>81</sup> The inhibition of NAMPT can induce apoptosis of cancer cells. When STF-31 coordinated to Ru center, the toxicity was restricted via complexation. Upon light irradiation, both **7a** and **7b** uncaged the inhibitor STF-31, exhibiting enhanced toxicity against cancer cells under normoxia and even hypoxia conditions.

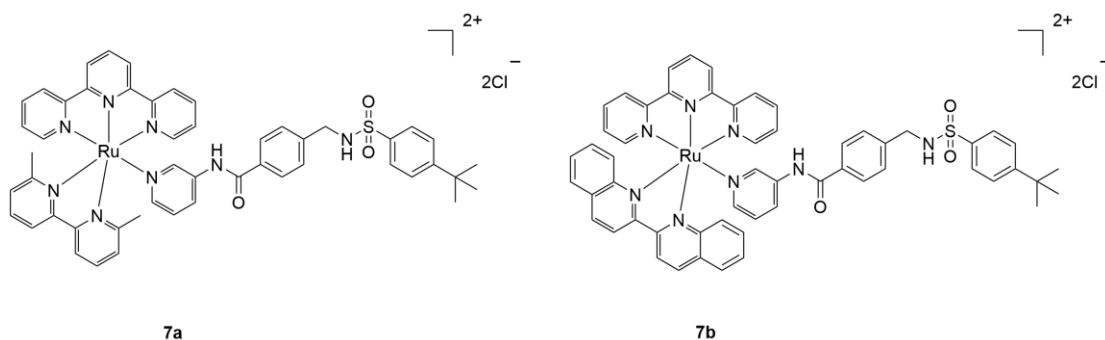


Figure 12. Chemical structure of Ru complexes **7a-b**.<sup>81</sup>

The toxic moiety can also be the photolytic Ru complexes. Ru complexes were in parallel used as the photo-released drug candidates. Glazer and coworkers reported two photolytic Ru complexes **8a-8b** with sterically clashing ligands (Figure 13).<sup>82</sup> Light



irradiation of **8a** caused the release of dm-bpy (dm-bpy = 4,4'-dimethyl-2,2'-dipyridyl) ligand, whereas **8b** released dm-dpq (dm-dpq = 2,9-dimethyl-dipyrido[3,2-f:2',3'-h]quinoxaline) ligand. Both of them formed toxic  $[\text{Ru}(\text{bpy})_2]^{2+}$  complex. The toxic Ru complexes bound cellular DNA and killed cancer cells. Moreover, the researchers also found that the photoreaction kinetics was 30-fold faster for **8a** than for **8b**, due to the rigidity of dm-dpq ligand. This further caused the photo-induced cytotoxicity of **8a** was higher than **8b**, thus killing cancer cells more efficiently.

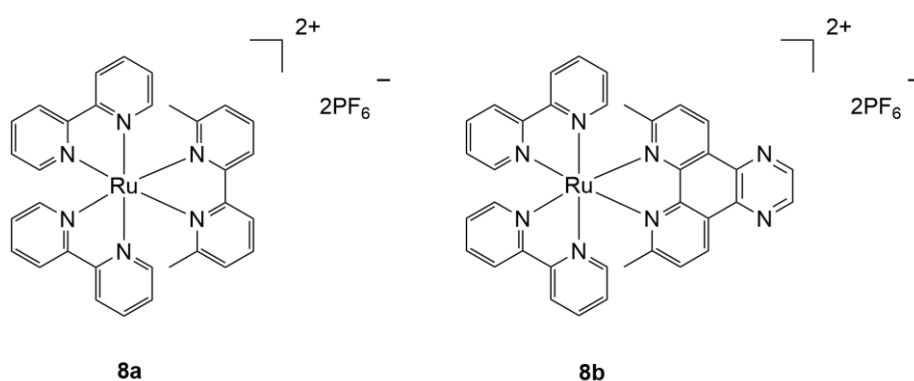


Figure 13. Chemical structure of Ru complexes **8a-b**.<sup>82</sup>

Recently, Turro and coworkers synthesized a novel cyclometalated Ru complexes **9**, for photo-induced ligand dissociation (Figure 14).<sup>83</sup> Such cyclometalated ligand resulted in geometric distortion of Ru complex and thus red-shifted the absorption wavelength. They found that **9** can be cleaved at 690 nm light irradiation, which was located in the therapeutic window for biomedical applications. After irradiation, the released toxic Ru complex further covalently bound to DNA, resulting in an enhancement of 14-fold toxicity compared with that in the dark.

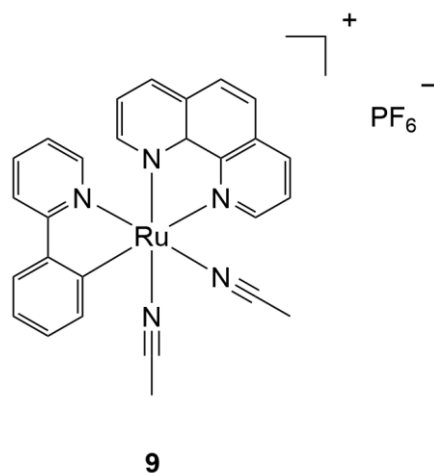


Figure 14. Chemical structure of Ru complexes **9**.<sup>83</sup>

### 1.3.2 Nanocarriers based on Ru complexes for cancer therapy

Although Ru complexes hold great potential for cancer therapy, they still suffer limitations due to the poor aqueous solubility. Moreover, most Ru complexes have short half-life and are easily eliminated during blood circulation. They also lack targeting ability, which limit the amount of complexes that reach the tumor tissues. Therefore, nanocarriers are highly required for delivering Ru complexes to address these issues.<sup>84</sup>

Nanocarriers-mediated Ru complexes for cancer treatment have several advantages. Typically, PEGylated nanocarriers prolong blood circulation. Besides, nanocarriers-mediated Ru complexes enable the accumulation at tumor sites via EPR effect. Light irradiation activates the Ru complexes only at the tumor sites, achieving selective tumor killing. There are two main strategies for nanocarriers-mediated Ru complexes: 1) physical encapsulation of Ru complexes within nanocarriers; 2) covalent conjugation of Ru complexes to nanocarriers.<sup>84</sup>

### 1.3.2.1 Physical encapsulation of Ru complexes within nanocarriers

Physical encapsulation relies on the non-covalent interaction between Ru complexes and nanocarriers matrix. Physical encapsulation restricts the exposure of Ru complexes to healthy tissues and shields them from clearance during circulation. Ru complexes are encapsulated into nanocarriers such as micelles, liposomes or nanoparticles.

Glazer and coworkers reported the encapsulation of three Ru complexes into nanoassembled micelles (Figure 15).<sup>85</sup> These Ru complexes were varied with different hydrophobicity. They found that the increased hydrophobicity decreased the total release amount, and slowed down release rate of Ru complexes. The release behaviors were also influenced by ionic strength of the solution, but variations in pH had negligible effect. The released complexes from nanoassembled micelles were further used for treating A549 cancer cells, which showed improved pharmacological properties.

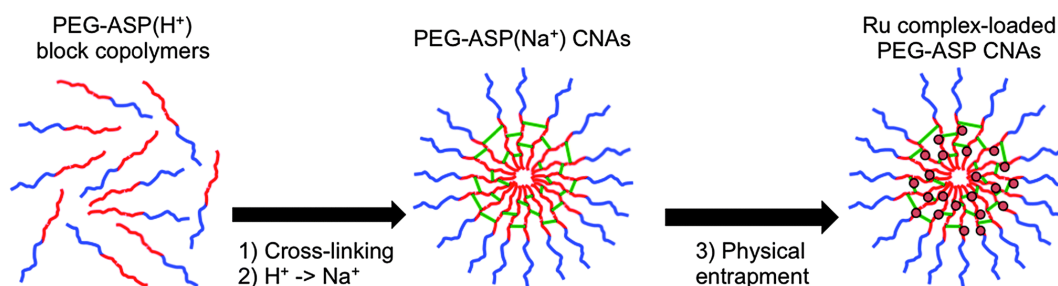


Figure 15. Ru complexes were encapsulated in nanoassembled micelles. Adapted with permission from ref 85. Copyright 2016 Royal Society of Chemistry.

Keppler and coworkers prepared a nanoparticle formulation for encapsulating Ru complex, KP1019.<sup>86</sup> Poly(lactic acid) (PLA) nanoparticles with two different surfactants (Pluronic F68 and Tween 80) were prepared using oil-in-water emulsion. Comparing with Pluronic F68, Tween 80 was able to prevent the precipitation of KP1019 and allow reproducible preparation of KP1019-containing nanoparticles. The

choice of surfactant directly influences the encapsulation efficiency and nanoparticle stability of Ru complexes.

### **1.3.2.2 Covalent conjugation of Ru complexes to nanocarriers**

The main drawbacks of physical encapsulation are the uncontrollable release of Ru complexes, which is so-called “burst” release.<sup>84, 87</sup> Physical encapsulation also suffers limitation due to the limited encapsulation amount. Covalent conjugation strategy may address these issues. Covalent conjugation using stimuli-responsive bonds enables controlled release behavior of Ru complexes.<sup>88</sup> Covalent conjugation can achieve high Ru loading amount by a well-defined chemical structure. Moreover, our group found that conjugating photo-responsive Ru complexes into polymeric nanocarriers, were more stable than free Ru complexes under similar conditions.<sup>89</sup> Photo-responsive Ru complexes can be also covalently developed for switchable surfaces,<sup>90</sup> and polymer gels,<sup>91</sup> by proper structural design. Recently, Ru complexes can be covalently conjugated to polymeric nanocarriers, or onto protein nanocarriers.

Our group designed and synthesized three block copolymers nanocarriers with red light-responsive Ru complexes in their side chains (Figure 16).<sup>92</sup> Each polymer contained a hydrophilic PEG block and a hydrophobic Ru-containing block. By proper design of the different hydrophilic/hydrophobic block ratio, these copolymers self-assembled into micelles, vesicles, and large compound micelles. All of them can be taken up by cancer cells, whereas the micelles exhibited the highest cellular uptake efficiency. Red light irradiation induced the release of Ru complexes and the generation of  $^1\text{O}_2$ , both of them synergistically killed the cancer cells.

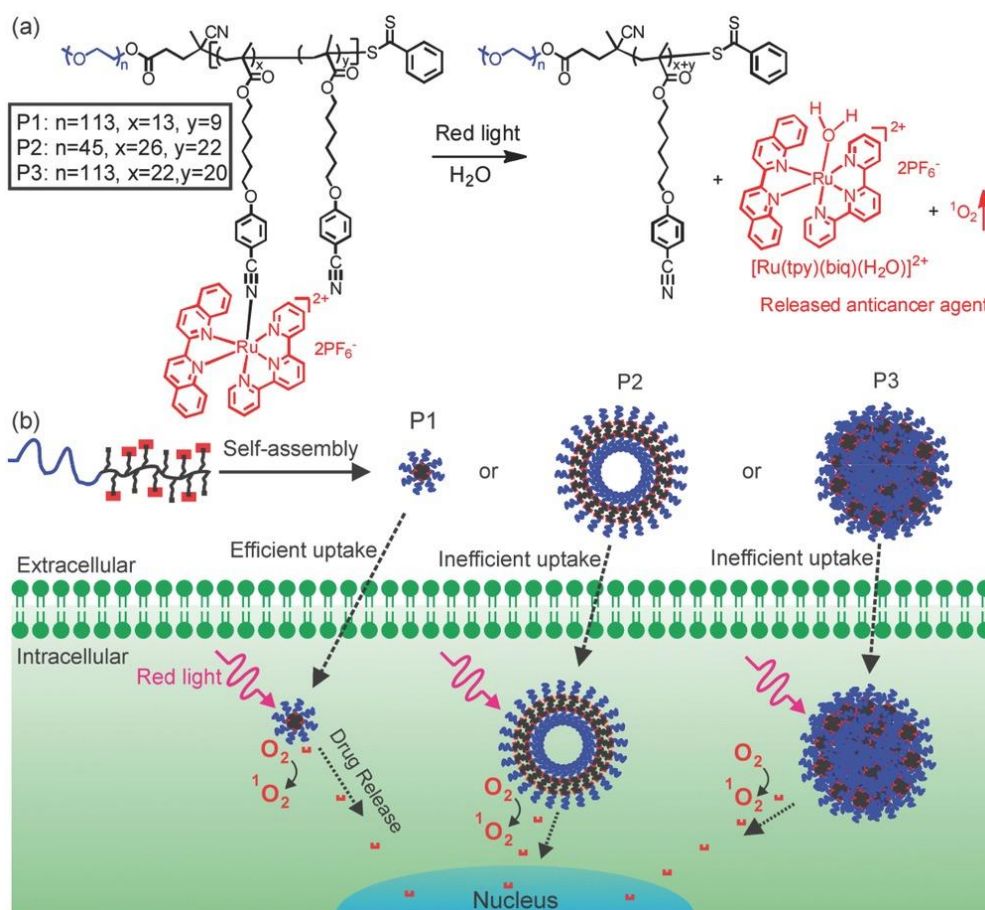


Figure 16. Ru complexes were covalently conjugated into polymer side chains. Adapted with permission from ref 92. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA.

Our group later introduced Ru complexes into the main-chain of a polymeric nanocarriers (Figure 17).<sup>93</sup> The Ru-containing polymer possessed two hydrophilic PEG block and a hydrophobic Ru block, which further self-assembled into nanoparticles (PolyRu). PolyRu nanoparticles accumulated at tumor sites via EPR effect. Moreover, in this well-defined polymeric structure, Ru complexes achieved more than 50% drug loading efficiency. Ru complexes can be controllably released from PolyRu upon red light irradiation. The released toxic Ru complexes caused the inhibition of tumor growth, with the help of generated <sup>1</sup>O<sub>2</sub>.

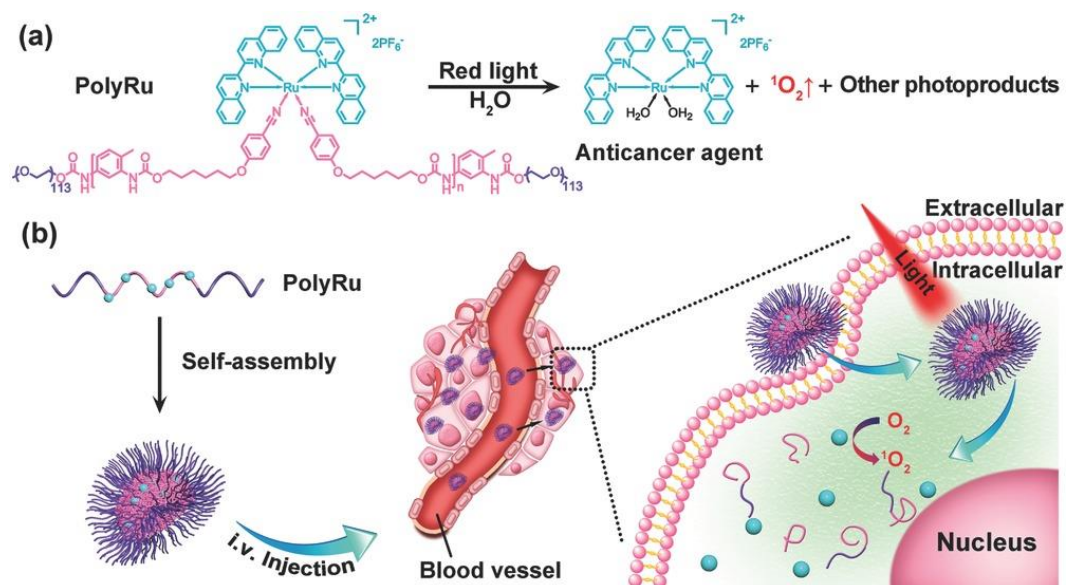


Figure 17. Ru complexes were covalently conjugated into polymer main chains. Adapted with permission from ref 93. Copyright 2017 WILEY-VCH Verlag GmbH & Co. KGaA.

Ru complexes are also covalently conjugated onto natural protein nanocarriers. Tanja and coworkers reported the use of blood plasma protein, serum albumin, for delivering Ru complexes (Figure 18).<sup>94</sup> They also introduced triphenylphosphine group on the protein surface, which offered the nanocarriers with mitochondria targeting ability. Irradiation of this nanocarriers can produce <sup>1</sup>O<sub>2</sub> due to the photosensitized Ru complexes. Attaching multiple Ru complexes into one protein achieved extremely high toxicity under light irradiation, with an enhancement of 220-fold toxicity comparing with that in the dark.

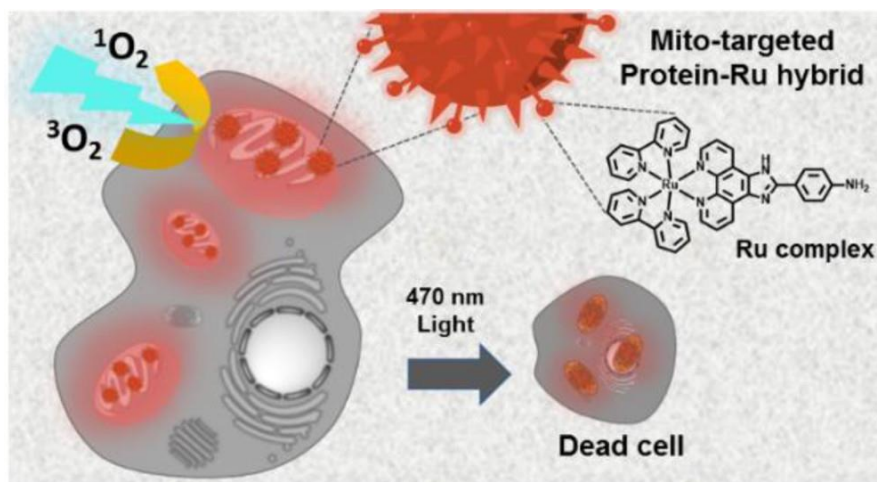


Figure 18. Ru complexes were covalently conjugated onto the surface of natural proteins. Adapted with permission from ref 94. Copyright 2017 American Chemical Society.

### 1.3.3 Challenges of nanocarriers based on Ru complexes in cancer therapy

A variety of nanocarriers for delivering Ru complexes have been developed in cancer therapy. However, they still suffer several limitations to reach their full potential. First, the current delivery efficiency of nanocarriers is still need to be improved. Desirable development of nanocarriers with high delivery efficiency is appreciated. Second, considering to the highly complexity of tumor, such as the resistance to anticancer drug, multifunctional nanocarriers based on Ru complexes for reversing drug resistance are emergently required. Thus, the major challenges of nanocarriers based on Ru complexes are: 1) how to enhance the delivery efficiency of these complexes, 2) and further overcome drug resistance in cancer treatment. In chapter 2, I will show the development of light-induced morphological change and positive charge increase, based on the light-cleavable Ru complexes, for improving the tumor penetration depth and cellular uptake efficiency in cancer treatment. To combat the complexity of tumor, in chapter 3, I am going to present my contribution to reverse drug resistance by

applying Ru complexes with combined chemotherapy, photodynamic therapy and photoactivated chemotherapy.

#### 1.4 References

- [1] Jha, P., Avoidable global cancer deaths and total deaths from smoking. *Nature Reviews Cancer* **2009**, 9 (9), 655-664.
- [2] Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians* **2018**, 68 (6), 394-424.
- [3] Herskovic, A.; Martz, K.; Al-Sarraf, M.; Leichman, L.; Brindle, J.; Vaitkevicius, V.; Cooper, J.; Byhardt, R.; Davis, L.; Emami, B., Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. *New England Journal of Medicine* **1992**, 326 (24), 1593-1598.
- [4] Vasan, N.; Baselga, J.; Hyman, D. M., A view on drug resistance in cancer. *Nature* **2019**, 575 (7782), 299-309.
- [5] Sobot, D.; Mura, S.; Couvreur, P., How can nanomedicines overcome cellular-based anticancer drug resistance? *Journal of Materials Chemistry B* **2016**, 4 (30), 5078-5100.
- [6] Pisco, A. O.; Jackson, D. A.; Huang, S., Reduced intracellular drug accumulation in drug-resistant leukemia cells is not only solely due to MDR-mediated efflux but also to decreased uptake. *Frontiers in oncology* **2014**, 4, 306.
- [7] Muley, H.; Fadó, R.; Rodríguez-Rodríguez, R.; Casals, N., Drug uptake-based chemoresistance in breast cancer treatment. *Biochemical pharmacology* **2020**, 113959.
- [8] Yang, N. J.; Hinner, M. J., Getting across the cell membrane: an overview for small molecules, peptides, and proteins. *Site-specific protein labeling* **2015**, 29-53.
- [9] Mansoori, B.; Mohammadi, A.; Davudian, S.; Shirjang, S.; Baradaran, B., The different mechanisms of cancer drug resistance: a brief review. *Advanced pharmaceutical bulletin* **2017**, 7 (3), 339.
- [10] Fletcher, J. I.; Haber, M.; Henderson, M. J.; Norris, M. D., ABC transporters in cancer: more than just drug efflux pumps. *Nature Reviews Cancer* **2010**, 10 (2), 147-156.



- [11] Löscher, W.; Potschka, H., Drug resistance in brain diseases and the role of drug efflux transporters. *Nature Reviews Neuroscience* **2005**, *6* (8), 591-602.
- [12] Smyth, M. J.; Krasovskis, E.; Sutton, V. R.; Johnstone, R. W., The drug efflux protein, P-glycoprotein, additionally protects drug-resistant tumor cells from multiple forms of caspase-dependent apoptosis. *Proceedings of the National Academy of Sciences* **1998**, *95* (12), 7024-7029.
- [13] Helleday, T.; Petermann, E.; Lundin, C.; Hodgson, B.; Sharma, R. A., DNA repair pathways as targets for cancer therapy. *Nature Reviews Cancer* **2008**, *8* (3), 193-204.
- [14] Zhang, S.; Zhong, X.; Yuan, H.; Guo, Y.; Song, D.; Qi, F.; Zhu, Z.; Wang, X.; Guo, Z., Interfering in apoptosis and DNA repair of cancer cells to conquer cisplatin resistance by platinum (IV) prodrugs. *Chemical Science* **2020**, *11* (15), 3829-3835.
- [15] Hurley, L. H., DNA and its associated processes as targets for cancer therapy. *Nature Reviews Cancer* **2002**, *2* (3), 188-200.
- [16] Johnstone, T. C.; Suntharalingam, K.; Lippard, S. J., The next generation of platinum drugs: targeted Pt (II) agents, nanoparticle delivery, and Pt (IV) prodrugs. *Chemical reviews* **2016**, *116* (5), 3436-3486.
- [17] Genovese, I.; Fiorillo, A.; Ilari, A.; Masciarelli, S.; Fazi, F.; Colotti, G., Binding of doxorubicin to Sorcin impairs cell death and increases drug resistance in cancer cells. *Cell death & disease* **2017**, *8* (7), e2950-e2950.
- [18] Damia, G.; D'Incalci, M., Mechanisms of resistance to alkylating agents. *Cytotechnology* **1998**, *27* (1), 165-173.
- [19] Rodriguez-Antona, C.; Ingelman-Sundberg, M., Cytochrome P 450 pharmacogenetics and cancer. *Oncogene* **2006**, *25* (11), 1679-1691.
- [20] Wang, X.; Zhang, H.; Chen, X., Drug resistance and combating drug resistance in cancer. *Cancer Drug Resistance* **2019**, *2* (2), 141-160.
- [21] Saraswathy, M.; Gong, S., Different strategies to overcome multidrug resistance in cancer. *Biotechnology advances* **2013**, *31* (8), 1397-1407.
- [22] Loganzo, F.; Sung, M.; Gerber, H.-P., Mechanisms of resistance to antibody–drug conjugates. *Molecular cancer therapeutics* **2016**, *15* (12), 2825-2834.
- [23] Zolot, R. S.; Basu, S.; Million, R. P., Antibody–drug conjugates. *Nature Reviews Drug Discovery* **2013**, *12* (4), 259-260.

- [24] Worm, D. J.; Els - Heindl, S.; Beck - Sickinger, A. G., Targeting of peptide - binding receptors on cancer cells with peptide - drug conjugates. *Peptide Science* **2020**, *112* (3), e24171.
- [25] Tang, J.; Zhang, L.; Gao, H.; Liu, Y.; Zhang, Q.; Ran, R.; Zhang, Z.; He, Q., Co-delivery of doxorubicin and P-gp inhibitor by a reduction-sensitive liposome to overcome multidrug resistance, enhance anti-tumor efficiency and reduce toxicity. *Drug Delivery* **2016**, *23* (4), 1130-1143.
- [26] Zhang, M.; Hagan IV, C. T.; Min, Y.; Foley, H.; Tian, X.; Yang, F.; Mi, Y.; Au, K. M.; Medik, Y.; Roche, K., Nanoparticle co-delivery of wortmannin and cisplatin synergistically enhances chemoradiotherapy and reverses platinum resistance in ovarian cancer models. *Biomaterials* **2018**, *169*, 1-10.
- [27] Kim, R. B., Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug metabolism reviews* **2002**, *34* (1-2), 47-54.
- [28] Xiao, X.; Wang, K.; Zong, Q.; Tu, Y.; Dong, Y.; Yuan, Y., Polyprodrug with glutathione depletion and cascade drug activation for multi-drug resistance reversal. *Biomaterials* **2021**, *270*, 120649.
- [29] Han, Y.; Yin, W.; Li, J.; Zhao, H.; Zha, Z.; Ke, W.; Wang, Y.; He, C.; Ge, Z., Intracellular glutathione-depleting polymeric micelles for cisplatin prodrug delivery to overcome cisplatin resistance of cancers. *Journal of Controlled Release* **2018**, *273*, 30-39.
- [30] Drew, R.; Miners, J. O., The effects of buthionine sulphoximine (BSO) on glutathione depletion and xenobiotic biotransformation. *Biochemical pharmacology* **1984**, *33* (19), 2989-2994.
- [31] Li, Q.; Yin, X.; Wang, W.; Zhan, M.; Zhao, B.; Hou, Z.; Wang, J., The effects of buthionine sulfoximine on the proliferation and apoptosis of biliary tract cancer cells induced by cisplatin and gemcitabine. *Oncology letters* **2016**, *11* (1), 474-480.
- [32] Wang, N.; Cheng, X.; Li, N.; Wang, H.; Chen, H., Nanocarriers and their loading strategies. *Advanced healthcare materials* **2019**, *8* (6), 1801002.
- [33] Karges, J.; Yempala, T.; Tharaud, M.; Gibson, D.; Gasser, G., A Multi - action and Multi - target RuII–PtIV Conjugate Combining Cancer - Activated Chemotherapy and Photodynamic Therapy to Overcome Drug Resistant Cancers. *Angewandte Chemie International Edition* **2020**, *59* (18), 7069-7075.

- [34] Lin, G.; Mi, P.; Chu, C.; Zhang, J.; Liu, G., Inorganic nanocarriers overcoming multidrug resistance for cancer theranostics. *Advanced science* **2016**, *3* (11), 1600134.
- [35] Li, Z.; Shan, X.; Chen, Z.; Gao, N.; Zeng, W.; Zeng, X.; Mei, L., Applications of Surface Modification Technologies in Nanomedicine for Deep Tumor Penetration. *Advanced Science* **2021**, *8* (1), 2002589.
- [36] Goddard, Z. R.; Marín, M. J.; Russell, D. A.; Searcey, M., Active targeting of gold nanoparticles as cancer therapeutics. *Chemical Society Reviews* **2020**.
- [37] Schöttler, S.; Becker, G.; Winzen, S.; Steinbach, T.; Mohr, K.; Landfester, K.; Mailänder, V.; Wurm, F. R., Protein adsorption is required for stealth effect of poly (ethylene glycol)-and poly (phosphoester)-coated nanocarriers. *Nature nanotechnology* **2016**, *11* (4), 372-377.
- [38] Dewhirst, M. W.; Secomb, T. W., Transport of drugs from blood vessels to tumour tissue. *Nat Rev Cancer* **2017**, *17* (12), 738-750.
- [39] Torney, F.; Trewyn, B. G.; Lin, V. S.; Wang, K., Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat Nanotechnol* **2007**, *2* (5), 295-300.
- [40] Shan, Y.; Ma, S.; Nie, L.; Shang, X.; Hao, X.; Tang, Z.; Wang, H., Size-dependent endocytosis of single gold nanoparticles. *Chem Commun (Camb)* **2011**, *47* (28), 8091-3.
- [41] Sun, Q.; Zhou, Z.; Qiu, N.; Shen, Y., Rational design of cancer nanomedicine: nanoproperty integration and synchronization. *Advanced materials* **2017**, *29* (14), 1606628.
- [42] Schöttler, S.; Landfester, K.; Mailänder, V., Controlling the stealth effect of nanocarriers through understanding the protein corona. *Angewandte Chemie International Edition* **2016**, *55* (31), 8806-8815.
- [43] Sedlacek, O.; Monnery, B. D.; Filippov, S. K.; Hoogenboom, R.; Hruby, M., Poly (2 - Oxazoline) s–Are They More Advantageous for Biomedical Applications Than Other Polymers? *Macromolecular rapid communications* **2012**, *33* (19), 1648-1662.
- [44] Simon, J.; Wolf, T.; Klein, K.; Landfester, K.; Wurm, F. R.; Mailänder, V., Hydrophilicity Regulates the Stealth Properties of Polyphosphoester - Coated Nanocarriers. *Angewandte Chemie International Edition* **2018**, *57* (19), 5548-5553.
- [45] Torchilin, V., Tumor delivery of macromolecular drugs based on the EPR effect. *Advanced drug delivery reviews* **2011**, *63* (3), 131-135.

- [46] Liu, T.; Han, C.; Wang, S.; Fang, P.; Ma, Z.; Xu, L.; Yin, R., Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. *Journal of hematology & oncology* **2019**, *12* (1), 1-15.
- [47] Hong, Y.; Nam, G. H.; Koh, E.; Jeon, S.; Kim, G. B.; Jeong, C.; Kim, D. H.; Yang, Y.; Kim, I. S., Exosome as a vehicle for delivery of membrane protein therapeutics, PH20, for enhanced tumor penetration and antitumor efficacy. *Advanced Functional Materials* **2018**, *28* (5), 1703074.
- [48] Mura, S.; Nicolas, J.; Couvreur, P., Stimuli-responsive nanocarriers for drug delivery. *Nature materials* **2013**, *12* (11), 991-1003.
- [49] Li, Z.; Xiao, C.; Yong, T.; Li, Z.; Gan, L.; Yang, X., Influence of nanomedicine mechanical properties on tumor targeting delivery. *Chemical Society Reviews* **2020**, *49* (8), 2273-2290.
- [50] Jo, D. H.; Kim, J. H.; Lee, T. G.; Kim, J. H., Size, surface charge, and shape determine therapeutic effects of nanoparticles on brain and retinal diseases. *Nanomedicine: Nanotechnology, Biology and Medicine* **2015**, *11* (7), 1603-1611.
- [51] Zhao, Z.; Ukidve, A.; Krishnan, V.; Mitragotri, S., Effect of physicochemical and surface properties on in vivo fate of drug nanocarriers. *Advanced drug delivery reviews* **2019**, *143*, 3-21.
- [52] Behzadi, S.; Serpooshan, V.; Tao, W.; Hamaly, M. A.; Alkawareek, M. Y.; Dreaden, E. C.; Brown, D.; Alkilany, A. M.; Farokhzad, O. C.; Mahmoudi, M., Cellular uptake of nanoparticles: journey inside the cell. *Chemical Society Reviews* **2017**, *46* (14), 4218-4244.
- [53] Calatayud, M. P.; Sanz, B.; Raffa, V.; Riggio, C.; Ibarra, M. R.; Goya, G. F., The effect of surface charge of functionalized Fe<sub>3</sub>O<sub>4</sub> nanoparticles on protein adsorption and cell uptake. *Biomaterials* **2014**, *35* (24), 6389-6399.
- [54] Ta, H. T.; Truong, N. P.; Whittaker, A. K.; Davis, T. P.; Peter, K., The effects of particle size, shape, density and flow characteristics on particle margination to vascular walls in cardiovascular diseases. *Expert opinion on drug delivery* **2018**, *15* (1), 33-45.
- [55] Cisnetti, F.; Gautier, A., Metal/N - Heterocyclic Carbene Complexes: Opportunities for the Development of Anticancer Metallodrugs. *Angewandte Chemie International Edition* **2013**, *52* (46), 11976-11978.
- [56] Wang, X.; Wang, X.; Jin, S.; Muhammad, N.; Guo, Z., Stimuli-responsive therapeutic metallodrugs. *Chemical reviews* **2018**, *119* (2), 1138-1192.

- [57] Mjos, K. D.; Orvig, C., Metallodrugs in medicinal inorganic chemistry. *Chemical reviews* **2014**, *114* (8), 4540-4563.
- [58] Zeng, L.; Gupta, P.; Chen, Y.; Wang, E.; Ji, L.; Chao, H.; Chen, Z.-S., The development of anticancer ruthenium (II) complexes: from single molecule compounds to nanomaterials. *Chemical Society Reviews* **2017**, *46* (19), 5771-5804.
- [59] Mari, C.; Pierroz, V.; Ferrari, S.; Gasser, G., Combination of Ru (II) complexes and light: new frontiers in cancer therapy. *Chemical science* **2015**, *6* (5), 2660-2686.
- [60] Hartinger, C. G.; Jakupec, M. A.; Zorbas - Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K., KP1019, a new redox - active anticancer agent—Preclinical development and results of a clinical phase I study in tumor patients. *Chemistry & biodiversity* **2008**, *5* (10), 2140-2155.
- [61] Heffeter, P.; Atil, B.; Kryeziu, K.; Groza, D.; Koellensperger, G.; Körner, W.; Jungwirth, U.; Mohr, T.; Keppler, B. K.; Berger, W., The ruthenium compound KP1339 potentiates the anticancer activity of sorafenib in vitro and in vivo. *European Journal of Cancer* **2013**, *49* (15), 3366-3375.
- [62] Sava, G.; Zorzet, S.; Turrin, C.; Vita, F.; Soranzo, M.; Zabucchi, G.; Cocchietto, M.; Bergamo, A.; DiGiovine, S.; Pezzoni, G., Dual action of NAMI-A in inhibition of solid tumor metastasis: selective targeting of metastatic cells and binding to collagen. *Clinical Cancer Research* **2003**, *9* (5), 1898-1905.
- [63] Zeng, Z.-P.; Wu, Q.; Sun, F.-Y.; Zheng, K.-D.; Mei, W.-J., Imaging Nuclei of MDA-MB-231 Breast Cancer Cells by Chiral Ruthenium (II) Complex Coordinated by 2-(4-Phenyacetylenophenyl)-1 H-imidazo [4, 5 f][1, 10] phenanthroline. *Inorganic chemistry* **2016**, *55* (11), 5710-5718.
- [64] Yu, Q.; Liu, Y.; Xu, L.; Zheng, C.; Le, F.; Qin, X.; Liu, Y.; Liu, J., Ruthenium (II) polypyridyl complexes: Cellular uptake, cell image and apoptosis of HeLa cancer cells induced by double targets. *European journal of medicinal chemistry* **2014**, *82*, 82-95.
- [65] Li, L.; Wong, Y.-S.; Chen, T.; Fan, C.; Zheng, W., Ruthenium complexes containing bis-benzimidazole derivatives as a new class of apoptosis inducers. *Dalton transactions* **2012**, *41* (4), 1138-1141.

- [66] Chen, T.; Mei, W.-J.; Wong, Y.-S.; Liu, J.; Liu, Y.; Xie, H.-S.; Zheng, W.-J., Chiral ruthenium polypyridyl complexes as mitochondria-targeted apoptosis inducers. *MedChemComm* **2010**, *1* (1), 73-75.
- [67] Zhao, Z.; Luo, Z.; Wu, Q.; Zheng, W.; Feng, Y.; Chen, T., Mixed-ligand ruthenium polypyridyl complexes as apoptosis inducers in cancer cells, the cellular translocation and the important role of ROS-mediated signaling. *Dalton Transactions* **2014**, *43* (45), 17017-17028.
- [68] Du, Y.; Fu, X.; Li, H.; Chen, B.; Guo, Y.; Su, G.; Zhang, H.; Ning, F.; Lin, Y.; Mei, W., Mitochondrial fragmentation is an important cellular event induced by ruthenium (II) polypyridyl complexes in osteosarcoma cells. *ChemMedChem* **2014**, *9* (4), 714-718.
- [69] Chen, T.; Liu, Y.; Zheng, W.-J.; Liu, J.; Wong, Y.-S., Ruthenium polypyridyl complexes that induce mitochondria-mediated apoptosis in cancer cells. *Inorganic chemistry* **2010**, *49* (14), 6366-6368.
- [70] Heindl, M.; Hongyan, J.; Hua, S.-A.; Oelschlegel, M.; Meyer, F.; Schwarzer, D.; González, L., Excited-State Dynamics of [Ru (S-Sbpy)(bpy) 2] 2+ to Form Long-Lived Localized Triplet States. *Inorganic chemistry* **2021**, *60* (3), 1672-1682.
- [71] Felsher, D. W., Cancer revoked: oncogenes as therapeutic targets. *Nature Reviews Cancer* **2003**, *3* (5), 375-379.
- [72] Li, X.; Lovell, J. F.; Yoon, J.; Chen, X., Clinical development and potential of photothermal and photodynamic therapies for cancer. *Nature Reviews Clinical Oncology* **2020**, *17* (11), 657-674.
- [73] Shi, G.; Monro, S.; Hennigar, R.; Colpitts, J.; Fong, J.; Kasimova, K.; Yin, H.; DeCoste, R.; Spencer, C.; Chamberlain, L., Ru (II) dyads derived from  $\alpha$ -oligothiophenes: a new class of potent and versatile photosensitizers for PDT. *Coordination Chemistry Reviews* **2015**, *282*, 127-138.
- [74] Yin, H.; Stephenson, M.; Gibson, J.; Sampson, E.; Shi, G.; Sainuddin, T.; Monro, S.; McFarland, S. A., In vitro multiwavelength PDT with 3IL states: Teaching old molecules new tricks. *Inorganic chemistry* **2014**, *53* (9), 4548-4559.
- [75] Huang, H.; Yu, B.; Zhang, P.; Huang, J.; Chen, Y.; Gasser, G.; Ji, L.; Chao, H., Highly charged ruthenium (II) polypyridyl complexes as lysosome - localized photosensitizers for two - photon photodynamic therapy. *Angewandte Chemie International Edition* **2015**, *54* (47), 14049-14052.

- [76] Bonnet, S., Why develop photoactivated chemotherapy? *Dalton Transactions* **2018**, 47 (31), 10330-10343.
- [77] Farrer, N. J.; Salassa, L.; Sadler, P. J., Photoactivated chemotherapy (PACT): the potential of excited-state d-block metals in medicine. *Dalton Transactions* **2009**, (48), 10690-10701.
- [78] Zayat, L.; Filevich, O.; Baraldo, L. M.; Etchenique, R., Ruthenium polypyridyl phototriggers: from beginnings to perspectives. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2013**, 371 (1995), 20120330.
- [79] Karaoun, N.; Renfrew, A. K., A luminescent ruthenium (II) complex for light-triggered drug release and live cell imaging. *Chemical Communications* **2015**, 51 (74), 14038-14041.
- [80] Choi, E. K.; Park, E. J.; Phan, T. T.; Kim, H. D.; Hoe, K.-L.; Kim, D.-U., Econazole Induces p53-Dependent Apoptosis and Decreases Metastasis Ability in Gastric Cancer Cells. *Biomolecules & therapeutics* **2020**, 28 (4), 370.
- [81] Lameijer, L. N.; Ernst, D.; Hopkins, S. L.; Meijer, M. S.; Askes, S. H.; Le Dévédec, S. E.; Bonnet, S., A red - light - activated ruthenium - caged NAMPT inhibitor remains phototoxic in hypoxic cancer cells. *Angewandte Chemie* **2017**, 129 (38), 11707-11711.
- [82] Howerton, B. S.; Heidary, D. K.; Glazer, E. C., Strained ruthenium complexes are potent light-activated anticancer agents. *Journal of the American Chemical Society* **2012**, 134 (20), 8324-8327.
- [83] Palmer, A. M.; Peña, B.; Sears, R. B.; Chen, O.; El Ojaimi, M.; Thummel, R. P.; Dunbar, K. R.; Turro, C., Cytotoxicity of cyclometallated ruthenium complexes: the role of ligand exchange on the activity. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2013**, 371 (1995), 20120135.
- [84] Villemin, E.; Ong, Y. C.; Thomas, C. M.; Gasser, G., Polymer encapsulation of ruthenium complexes for biological and medicinal applications. *Nature Reviews Chemistry* **2019**, 3 (4), 261-282.
- [85] Dickerson, M.; Howerton, B.; Bae, Y.; Glazer, E. C., Light-sensitive ruthenium complex-loaded cross-linked polymeric nanoassemblies for the treatment of cancer. *Journal of Materials Chemistry B* **2016**, 4 (3), 394-408.

- [86] Fischer, B.; Heffeter, P.; Kryeziu, K.; Gille, L.; Meier, S. M.; Berger, W.; Kowol, C. R.; Keppler, B. K., Poly (lactic acid) nanoparticles of the lead anticancer ruthenium compound KP1019 and its surfactant-mediated activation. *Dalton Transactions* **2014**, 43 (3), 1096-1104.
- [87] Yoo, J.; Won, Y.-Y., Phenomenology of the initial burst release of drugs from PLGA Microparticles. *ACS Biomaterials Science & Engineering* **2020**, 6 (11), 6053-6062.
- [88] Zhou, H.; Chen, M.; Liu, Y.; Wu, S., Stimuli - Responsive Ruthenium - Containing Polymers. *Macromolecular rapid communications* **2018**, 39 (22), 1800372.
- [89] Chen, M.; Sun, W.; Kretzschmann, A.; Butt, H.-J.; Wu, S., Nanostructured polymer assemblies stabilize photoactivatable anticancer ruthenium complexes under physiological conditions. *Journal of inorganic biochemistry* **2020**, 207, 111052.
- [90] Xie, C.; Sun, W.; Lu, H.; Kretzschmann, A.; Liu, J.; Wagner, M.; Butt, H.-J.; Deng, X.; Wu, S., Reconfiguring surface functions using visible-light-controlled metal-ligand coordination. *Nature communications* **2018**, 9 (1), 1-9.
- [91] Liu, J.; Xie, C.; Kretzschmann, A.; Koynov, K.; Butt, H. J.; Wu, S., Metallopolymer Organohydrogels with Photo - Controlled Coordination Crosslinks Work Properly Below 0° C. *Advanced Materials* **2020**, 32 (14), 1908324.
- [92] Sun, W.; Parowatkin, M.; Steffen, W.; Butt, H. J.; Mailänder, V.; Wu, S., Ruthenium - containing block copolymer assemblies: red - light - responsive metallopolymers with tunable nanostructures for enhanced cellular uptake and anticancer phototherapy. *Advanced healthcare materials* **2016**, 5 (4), 467-473.
- [93] Sun, W.; Li, S.; Häupler, B.; Liu, J.; Jin, S.; Steffen, W.; Schubert, U. S.; Butt, H. J.; Liang, X. J.; Wu, S., An amphiphilic ruthenium polymetallodrug for combined photodynamic therapy and photochemotherapy in vivo. *Advanced Materials* **2017**, 29 (6), 1603702.
- [94] Chakraborty, S.; Agrawalla, B. K.; Stumper, A.; Vegi, N. M.; Fischer, S.; Reichardt, C.; Kögler, M.; Dietzek, B.; Feuring-Buske, M.; Buske, C., Mitochondria targeted protein-ruthenium photosensitizer for efficient photodynamic applications. *Journal of the American Chemical Society* **2017**, 139 (6), 2512-2519.



**Chapter 2: Light-cleavable Ru(II) complexes with tunable properties  
for enhanced delivering efficiency in phototherapy**

*Xiaolong Zeng, Yufei Wang, Jianxiong Han, Wen Sun, Hans-Jürgen Butt, Xing-Jie  
Liang\*, and Si Wu\**

X. Zeng, J. Han, Prof. S. Wu

CAS Key Laboratory of Soft Matter Chemistry

Hefei National Laboratory for Physical Sciences at the Microscale

Department of Polymer Science and Engineering

University of Science and Technology of China

Hefei 230026, China

E-mail: siwu@ustc.edu.cn

Y. Wang, Prof. X.-J. Liang

Chinese Academy of Sciences (CAS) Key Laboratory for Biomedical Effects of

Nanomaterials and Nanosafety

CAS Center for Excellence in Nanoscience

National Center for Nanoscience and Technology

Beijing 100190, China

E-mail: liangxj@nanoctr.cn

Y. Wang, Prof. X.-J. Liang

University of Chinese Academy of Sciences

Beijing 100049, China

X. Zeng, J. Han, Prof. H.-J. Butt, Prof. S. Wu

Max Planck Institute for Polymer Research

Ackermannweg 10, 55128 Mainz, Germany

Prof. Dr. W. Sun

State Key Laboratory of Fine Chemicals

Dalian University of Technology

2 Linggong Road, Hi-Tech Zone, Dalian 116024, China

Keywords: metallodrugs, photo-responsive, morphological transformation, tumor penetration, zeta potential

The work in this chapter is based on the manuscript 'Light-cleavable Ru(II) complexes with tunable properties for enhanced delivering efficiency in phototherapy' which has been in preparation for submission.

## **2.1 Statement of contribution**

Si Wu convinced the idea.

Si Wu, Xing-Jie Liang and Hans-Jürgen Butt led the project.

Xiaolong Zeng prepared the materials and did the synthesis.

Xiaolong Zeng and Yufei Wang did the biological experiments.

Jianxiong Han contributed to TEM measurement.

Wen Sun contributed to ESI-MS measurement.

Xiaolong Zeng and Yufei Wang contributed equally to this work.

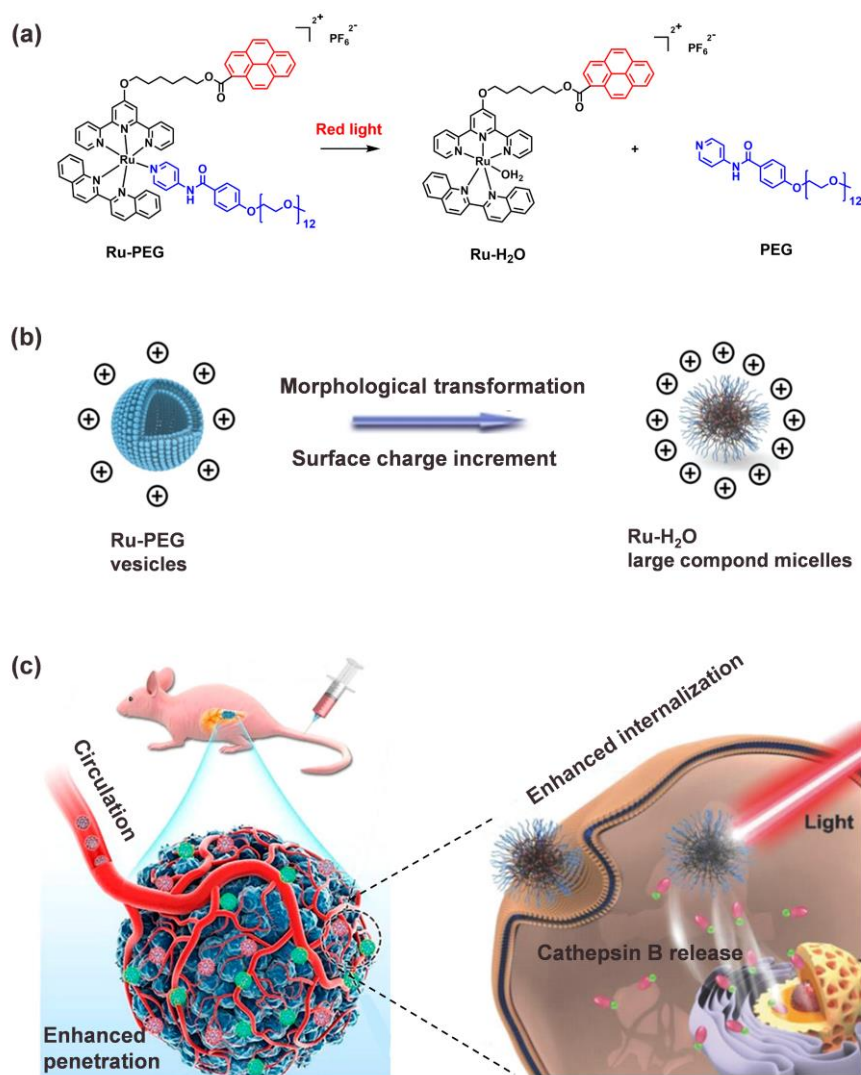
## 2.2 Introduction

Metal complexes, such as platinum (Pt) complexes, have been widely used for cancer therapy in clinic.<sup>1,2</sup> Recently, ruthenium (Ru) complexes are promising alternatives for Pt drugs.<sup>3</sup> In particular, photoresponsive Ru complexes have been applied in phototherapy with improved selectivity.<sup>4</sup> These complexes are generally nontoxic in dark but become highly toxic at irradiated sites.<sup>5</sup> Most photoresponsive Ru complexes are lacking of aqueous solubility.<sup>6-10</sup> Moreover, these complexes are highly charged, which easily form protein corona during circulation.<sup>11-13</sup> Modification of poly(ethylene glycol) (PEG) has been widely used to address these issues. PEGylation can not only improve water solubility of metallodrugs,<sup>14</sup> but also reduce the specific protein absorption.<sup>15</sup> However, PEGylation suffered limitations due to the competed effect. For example, PEGylation typically increases the size of metallodrugs, which hinder the tumor penetration depth.<sup>16</sup> Moreover, PEGylation offsets the surface charge of metallodrugs, which is antagonistic to efficient cellular internalization.<sup>17</sup> Thus, PEGylated metallodrugs still possess challenge.

In addition, the anticancer mechanisms of action of photoresponsive Ru complexes are still unclear. Most Ru complexes target DNA or specific proteins.<sup>18-22</sup> Those have critical roles in determining cellular activity.<sup>23-25</sup> However, photoresponsive Ru complexes are typically non-fluorescent,<sup>26-29</sup> which hinder the exploration of the mechanisms of using bio-imaging.

Herein, a photo-cleavable Ru complex (Ru-PEG), with dePEGylation and fluorescence properties, is reported. Ru-PEG is an amphiphilic compound. A hydrophobic Ru moiety was covalently linked to a fluorescent pyrene moiety. A hydrophilic pyridine-containing PEG ligand was coordinated to Ru center. The hydrophobic Ru moiety was toxic because some Ru complexes showed anticancer

activities. The hydrophilic PEG moiety was biocompatible because it prolongs blood circulation and suppress nonspecific adsorption of proteins. Ru-PEG self-assembled into vesicles, which accumulated at tumor sites via EPR effect. Irradiation of Ru-PEG induced the release of PEG, underwent morphological transformation to form Ru-H<sub>2</sub>O large compound micelles (LCMs). These LCMs had smaller size, which lead to a deeper tumor penetration depth. LCMs also hold higher surface charge, which is beneficial to cellular internalization. Photo-induced the PEG release, the transformation to small size LCMs, as well as the increment of surface charge, made a combination effect to deliver toxic Ru towards cancer cells. Moreover, irradiation of Ru-PEG with red light generated <sup>1</sup>O<sub>2</sub> for photodynamic therapy. With the conjugation of fluorescent pyrene, Ru-PEG simultaneously served as imaging agents for spatiotemporal visualization, which helped us to better understand the anticancer mechanisms of action in intracellular environment.



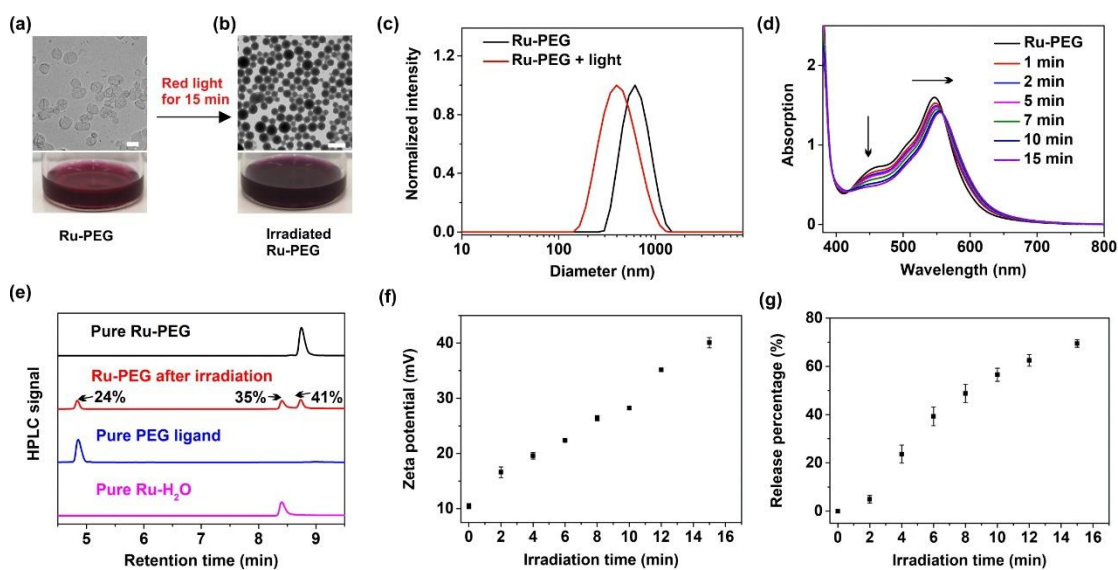
**Figure 1.** (a) Structure and photoreaction of the amphiphilic Ru-PEG. Red light irradiation induced the release of the anticancer agent Ru-H<sub>2</sub>O and the generation of <sup>1</sup>O<sub>2</sub>. (b) Self-assembly of Ru-PEG and its morphology transformation upon red light irradiation. (c) Illustration of Ru-PEG and Ru-H<sub>2</sub>O during blood circulation, tumor penetration and cell internalization.

## 2.3 Results and discussion

### 2.3.1 Synthesis of Ru-PEG

To prepare Ru-PEG, we first synthesized the pyridine-containing ligand PEG (Figure S1) and ruthenium (Ru) aquar complex Ru-H<sub>2</sub>O (Figure S2) via multi-step routes. All intermediates were fully characterized using nuclear magnetic resonance (NMR)

spectroscopy (Figure S5-11). Afterwards, the pyridine-containing ligand was coordinated with the Ru-H<sub>2</sub>O via complexation (Figure S3). NMR spectroscopy and mass spectrometry (MS) demonstrated that Ru-PEG was successfully synthesized (Figure S12-S14). As a comparison, a similar Ru complex without pyrene unit was synthesized to investigate the contribution of pyrene moiety for self-assembly (Figure S15-S17).



**Figure 2.** TEM images of Ru-PEG vesicles before (a) and after (b) light irradiation. Scale bar: 500 nm. (c) The diameter of Ru-PEG vesicles before and after light irradiation measured by dynamic light scattering (DLS). (d) UV-vis absorption spectra of Ru-PEG vesicles upon red light irradiation ( $25 \text{ mW cm}^{-2}$ , 15 min) over time. (e) Photosubstitution of Ru-PEG studied by high-performance liquid chromatography (HPLC). Ru-PEG was irradiated using 656 nm light ( $25 \text{ mW cm}^{-2}$ , 15 min). (f) Zeta potential of Ru-PEG vesicles upon light irradiation ( $25 \text{ mW cm}^{-2}$ , 15 min). (g) The release percentage of Ru-H<sub>2</sub>O from Ru-PEG vesicles under red light irradiation ( $25 \text{ mW cm}^{-2}$ , 15 min).

### 2.3.2 Self-assembly of Ru-PEG

We prepared the Ru-PEG vesicles via self-assembly. Ru-PEG was dissolved in THF. Water was added dropwise to the mixture to trigger the self-assembly. Subsequently, Ru-PEG in an aqueous solution were obtained by removing THF via dialysis against

water. Transmission electron microscopy (TEM) showed that Ru-PEG self-assembled into vesicles with an average diameter of 460 nm (**Figure 2a**). Dynamic light scattering (DLS) results showed that the Ru-PEG vesicles had an average hydrodynamic diameter of 520 nm (Figure 2c), which was comparable to the TEM result. The Ru-PEG vesicles in aqueous solution were stable for 36 h in the dark (Figure S19). However, Ru-PEG without pyrene unit cannot form any defined nanostructure (Figure S21). This was attributed to the lack of pyrene unit, which contributed to  $\pi$ - $\pi$  stacking and hydrophobic effect.<sup>30</sup>

### 2.3.3 Light-cleavable Ru-PEG with tunable properties

The Ru moiety is light-responsive, making Ru-PEG vesicles transformable (**Figure 1**). We studied the transformation of Ru-PEG vesicles induced by red light irradiation (Figure 2b). After red light irradiation, the morphology of vesicles changed to LCMs. DLS showed that LCMs had a smaller diameter with 290 nm (Figure 2c). We also performed UV-vis absorption spectroscopy, to study the photoreaction of Ru-PEG vesicles (Figure 2d). Light irradiation of Ru-PEG vesicles red-shifted the metal-to-ligand charge transfer (MLCT) band from 530 nm ( $\lambda_{\text{max}}$  of Ru-PEG) to 554 nm ( $\lambda_{\text{max}}$  of Ru-py-H<sub>2</sub>O). The spectral change was achieved within 15 min, suggesting the photosubstitution of Ru-PEG.

The TEM, DLS and UV-vis results demonstrated the qualitative transformation of Ru-PEG. High-performance liquid chromatography (HPLC) was then performed to quantify the photosubstitution of Ru-PEG (Figure 2e). Ru-PEG is represented by only one signal. After 656 nm light irradiation (30 mW cm<sup>-2</sup>, 15 min), two new peaks, the photoproducts, appeared. Comparing the retention time of the photoproducts with pure PEG ligand and Ru-H<sub>2</sub>O confirmed that the two peaks were assigned to PEG and Ru-H<sub>2</sub>O, respectively. This was further demonstrated by UV-vis absorption spectroscopy

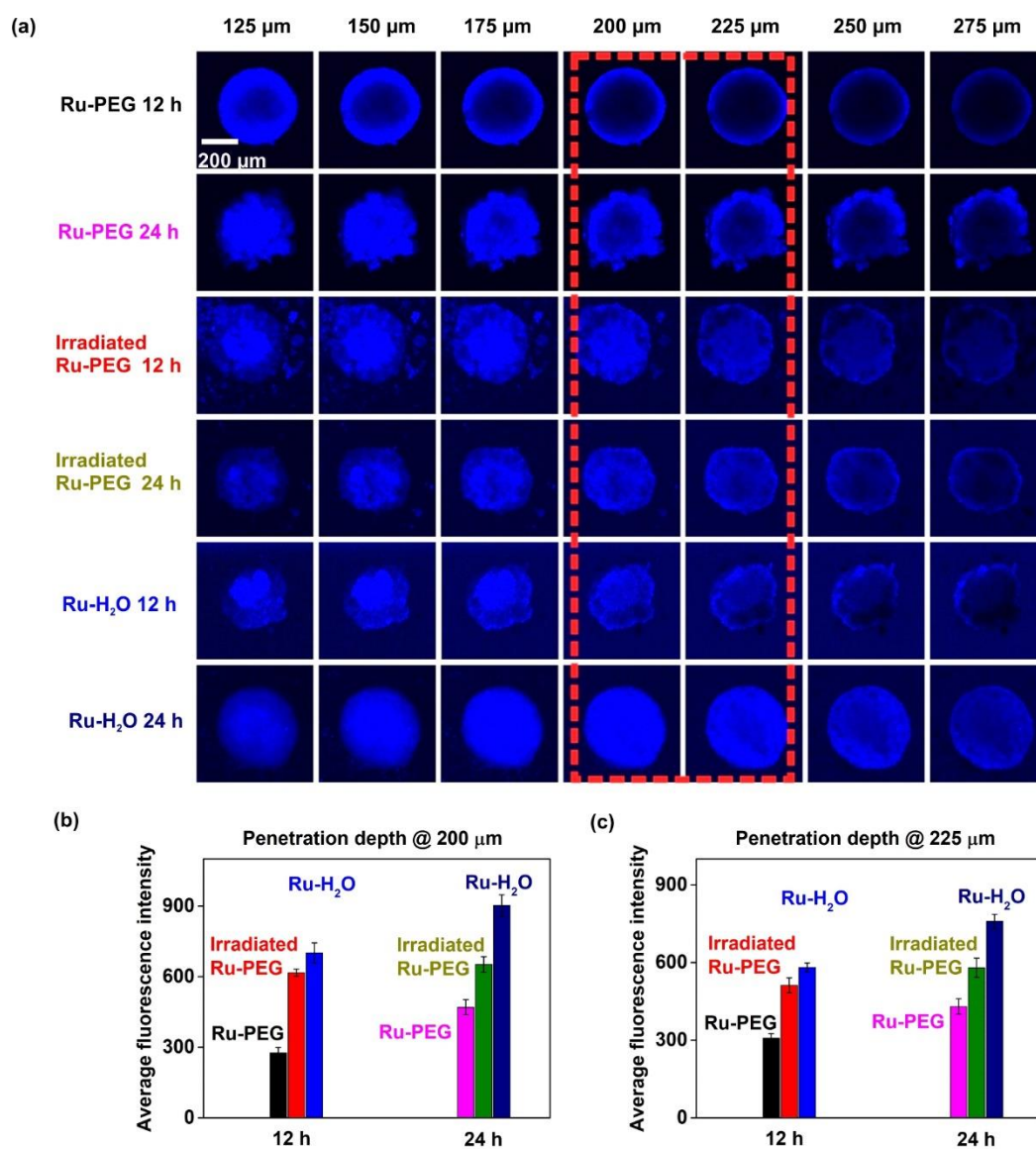
detector in the HPLC system (Figure S22). The integration ratio of PEG ligand, Ru-H<sub>2</sub>O and residual Ru-PEG was 24%, 35% and 41%, respectively. This result showed the release of PEG ligand from Ru-PEG via red light irradiation.

Light induced the release of neutral charged PEG, leading to increment of zeta potential (Figure 2f). Before irradiation, the zeta potential of Ru-PEG vesicles was +11 mV. Zeta potential gradually increased to +41 mV when the irradiation time was prolonged to 15 min. The released PEG lead to the expose of highly positive charged Ru-H<sub>2</sub>O, which enhanced the zeta potential in solution.

Light irradiation also caused the release of Ru-H<sub>2</sub>O from Ru-PEG vesicles (Figure 2g). The released profiles was investigated by HPLC. Up to 40% was released at first 6 min irradiation. The released percentage achieved 70% when the irradiation time was prolonged to 15 min, indicating the efficient release of Ru-H<sub>2</sub>O.

The released Ru-H<sub>2</sub>O also formed nanostructure. We directly triggered the self-assembly of Ru-H<sub>2</sub>O. TEM image showed Ru-H<sub>2</sub>O self-assembled into LCMs (Figure S20a), which was consistent with irradiated Ru-PEG vesicles (Figure 2b). However, Ru-H<sub>2</sub>O LCMs were not stable in aqueous solution, forming large aggregates after 24 h (Figure S20b). DLS results showed that the Ru-H<sub>2</sub>O LCMs trend to aggregate, with a larger hydrodynamic diameter of 5625 nm (Figure S20c). This was comparable to the TEM observations. Furthermore, Ru-H<sub>2</sub>O LCMs hold extremely high surface charge (Figure S20d), which was not ideal for nanocarriers during blood circulation due to the specific protein absorption.<sup>23</sup>



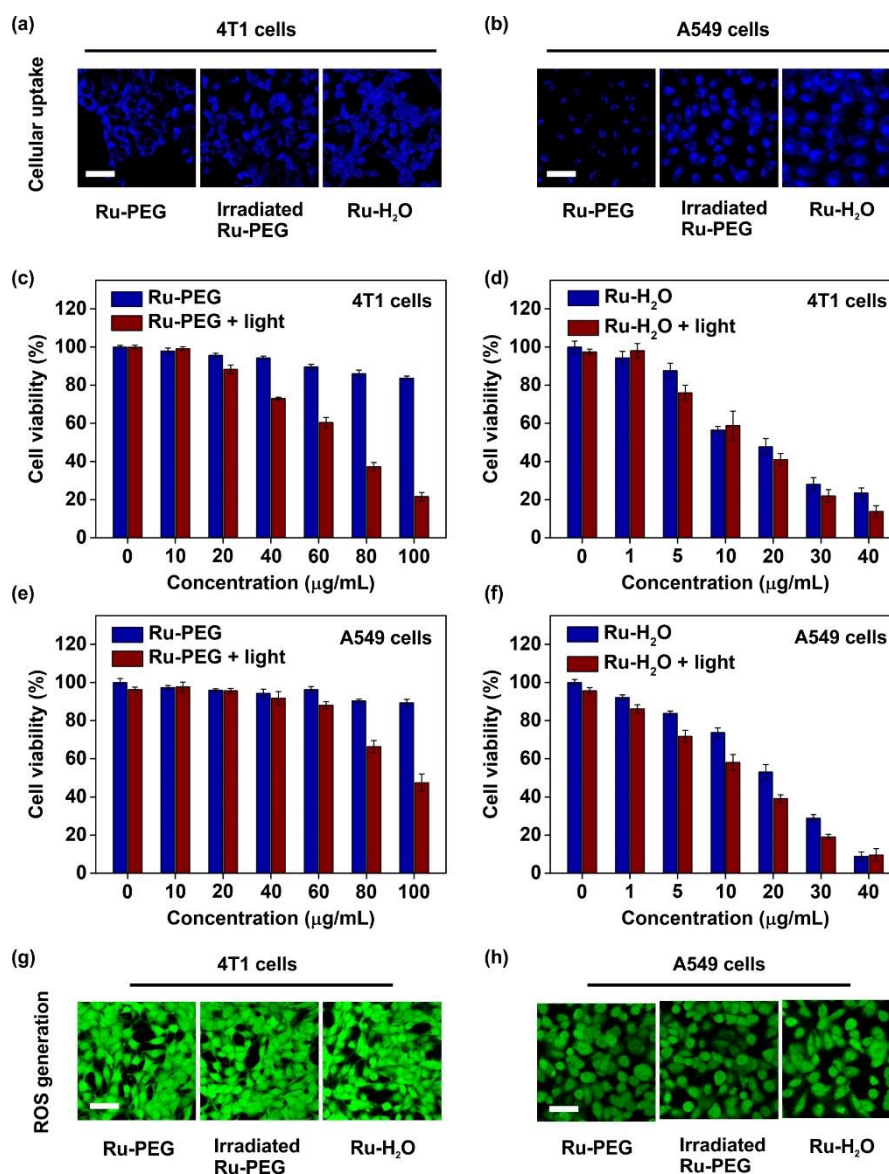


**Figure 3.** (a) In vitro tumor penetration of Ru-PEG vesicles, irradiated Ru-PEG vesicles and Ru-H<sub>2</sub>O large compound micelles (LCMs) in 4T1 multicellular tumor spheroids (MTS). Ru-PEG and Ru-H<sub>2</sub>O were excited at 405 nm. The MTS were visualized using CLSM in Z-stacks with 25  $\mu\text{m}$  intervals. Scale bar = 200  $\mu\text{m}$ . (b) Average fluorescence intensity at 200  $\mu\text{m}$  penetration depth calculated by ImageJ software. (c) Average fluorescence intensity at 225  $\mu\text{m}$  penetration depth calculated by ImageJ software.

### 2.3.4 Tumor penetration ability of Ru-PEG

As nanocarriers, Ru-PEG vesicles can prolong blood circulation, accumulate at tumor and then penetrate into deep tumor tissues. We next investigated the tumor penetration ability of Ru-PEG vesicles in a 4T1 multicellular tumor spheroids (MTS) model

**(Figure 3a).** Confocal laser scanning microscopy (CLSM) was performed using Z-stack at 25  $\mu\text{m}$  intervals from apex to equator. After incubating with Ru-PEG for 12 h, the fluorescence was only detected around the periphery of MTS but not the interior. Ru-PEG penetrated deeper when incubating with 24 h. In contrast, when pre-treated with vesicles for 15 min and then irradiated for 10 min, followed by further incubating to 12 h and 24 h, the irradiated Ru-PEG could penetrate throughout the spheroids and reach into the interior. This was due to the morphology transformation from vesicles to nanoparticles, with the decreasing of nanostructure size. As a comparison, Ru-H<sub>2</sub>O LCMs were directly incubated with MTS, they mainly located in the center of the spheroids and distributed throughout the whole spheroids, which was consistent with the irradiated Ru-PEG. The fluorescence intensities of 200 and 225  $\mu\text{m}$  penetration depth were further calculated using ImageJ software (Figure 3b and c). These results indicated the deeper penetration depth of Ru-PEG vesicles after light irradiation, with the aid of light-induced morphology transformation.



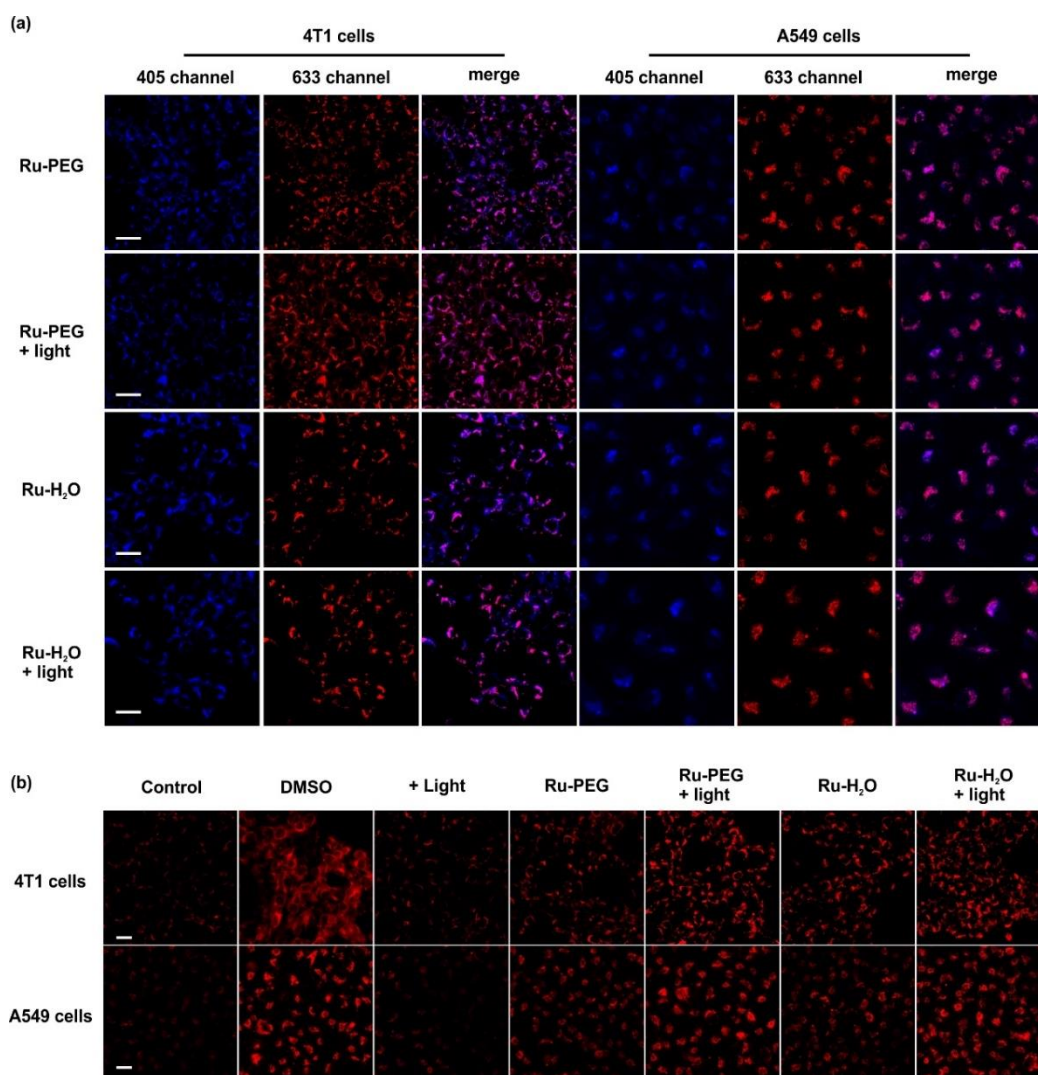
**Figure 4.** (a) Confocal laser scanning microscopy (CLSM) images of Ru-PEG vesicles, irradiated Ru-PEG vesicles and Ru-H<sub>2</sub>O LCMs after incubation with 4T1 and A549 cancer cells for 2 h. Ru-containing nanostructures were excited with a 405 nm laser (blue). Scale bars: 25 μm. (b) and (c) Viability of 4T1 (c) and A549 (d) cancer cells treated with various concentration of Ru-PEG vesicles in the dark and under light irradiation. The cells were irradiated with 671 nm red light (25 mW cm<sup>-2</sup>, 10 min) after incubation with Ru-PEG for 2 h. Cell viability was tested after the cells were further incubated in the dark for 24 h. (d) and (e) Viability of 4T1 (d) and A549 (e) cancer cells treated with various concentration of Ru-H<sub>2</sub>O LCMs in the dark and under light irradiation. The cells were irradiated with 671 nm red light (25 mW cm<sup>-2</sup>, 10 min) after incubation with Ru-PEG or Ru-H<sub>2</sub>O for 2 h. Cell viability was tested after the cells were further incubated in the dark for 24 h. (f) Generation of intracellular <sup>1</sup>O<sub>2</sub> in 4T1 and A549 cells observed by CLSM. <sup>1</sup>O<sub>2</sub> was detected using the indicator DCFH-DA (green). Scale bars: 50 μm.

### 2.3.5 Cellular uptake and in vitro cytotoxicity assessment

After penetrated into the deep tumor tissues and reached tumor cells, Ru-PEG was then taken up by these cells. We investigated the cellular uptake against 4T1 and A549 cancer cells (**Figure 4a** and **b**). Both cell lines were incubated with vesicles for 2 h in the dark. Subsequently, CLSM images were taken to observe the blue fluorescence excited from Ru-PEG vesicles. The observation of blue fluorescence suggested that Ru-PEG vesicles can be taken up by both cancer cells. The penetrated Ru-PEG may also be photo-cleaved by light before taken up by cancer cells. We found that the irradiated Ru-PEG could enhance the cellular uptake. This was assigned to the photo-induced increment of zeta potential, leading to a higher uptake efficiency. Ru-H<sub>2</sub>O LCMs as comparison to further demonstrate this observations. Flow cytometry was used to quantitatively analyze the uptake efficiency of Ru-PEG vesicles under different conditions (**Figure S24**). After incubation for 2 h, the uptake efficiencies of Ru-PEG and irradiated Ru-PEG for 4T1 cells were 43% and 77%, and for A549 cells were 41% and 89%, respectively. These results demonstrated that photo-induced the increment of zeta potential contributed a higher cellular uptake efficiency.

We then investigated the anticancer performance of Ru-PEG against 4T1 and A549 cancer cells. In the dark, the cell viabilities had negligible influence when increasing the concentration of Ru-PEG. However, after light irradiation, when concentration of Ru-PEG was 100  $\mu\text{g/mL}$ , the cell viabilities decreased to 21% and 48% for the 4T1 and A549 cells, respectively (**Figure 4c, e**). We infer that the decrease of cell viability occurred because light trigger the release of Ru -H<sub>2</sub>O, which caused toxicity to cancer cells. To further illustrate this point, the toxicity of Ru-H<sub>2</sub>O was investigated against 4T1 and A549 cells. The cell viabilities significantly decreased when incubating with Ru-H<sub>2</sub>O in the dark. Moreover, the viabilities of 4T1 and A549 cells further decreased

to 18% and 16% when incubating with 40  $\mu\text{g/mL}$  upon light irradiation (Figure 3d, f). The enhanced cytotoxicity was attributed to the generated  $^1\text{O}_2$ . The generation of  $^1\text{O}_2$  in 4T1 and A549 cells was detected using a green fluorescent probe (Figure 4g, h). Green fluorescence was observed when the cells irradiated with Ru-PEG or Ru-H<sub>2</sub>O in combination with the probe. Light induced the release of Ru-H<sub>2</sub>O and the generation of  $^1\text{O}_2$  achieved efficiently tumor killing.



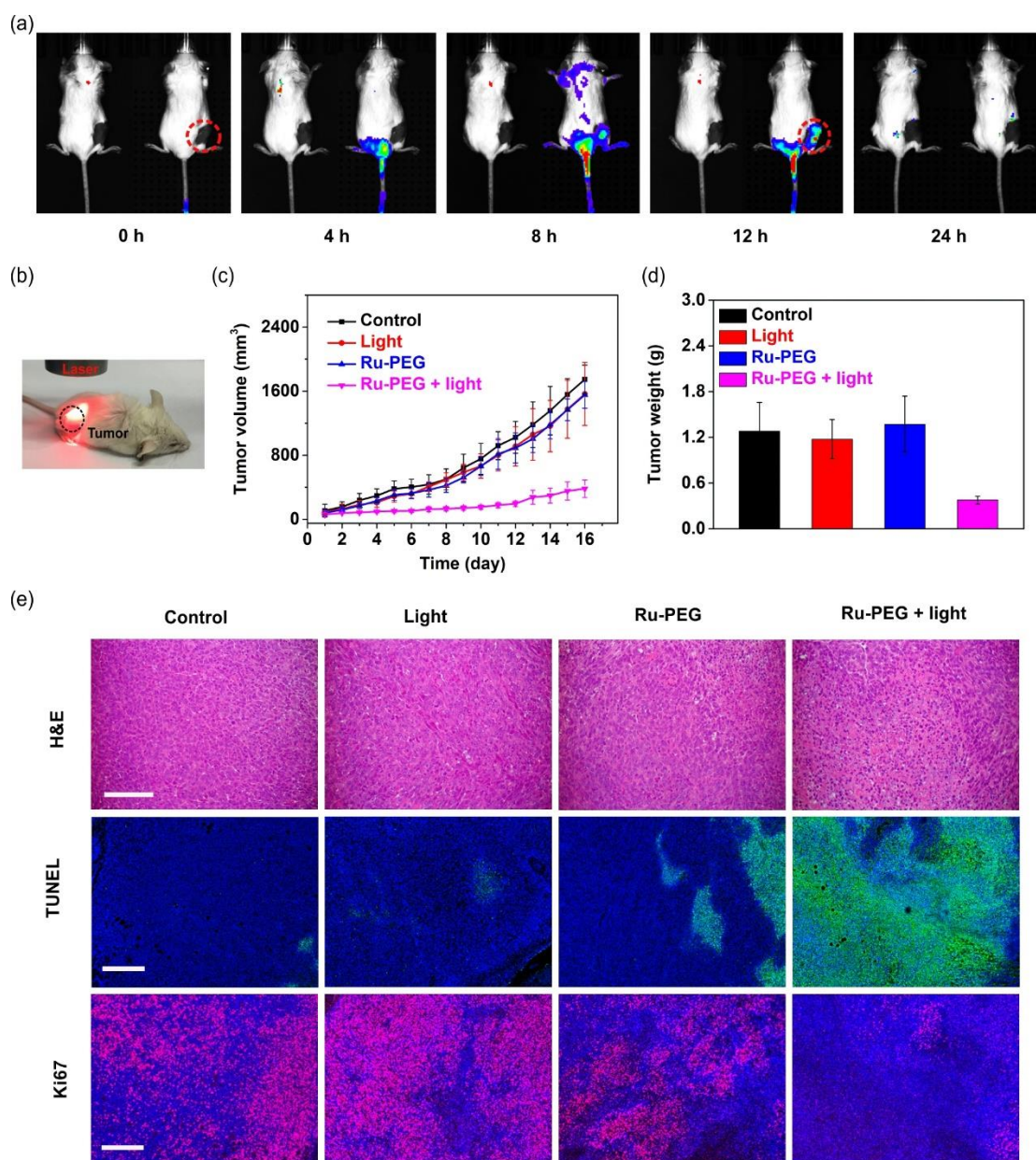
**Figure 5.** (a) CLSM images of colocalization with lysosome of Ru-PEG vesicles and Ru-H<sub>2</sub>O LCMs incubated before and after irradiation. Ru-containing nanostructures were excited with a 405 nm laser (blue). Lysosome was labelled using LysoTracker deep red (red). Scale bars: 50  $\mu\text{m}$ . (b) Observation of cathepsin B released from lysosomes to the cytosol induced under different conditions. DMSO was set as a positive control. Cathepsin B was labelled with Magic Red and excited at 633 nm. Scale bars: 50  $\mu\text{m}$ .

### 2.3.6 Lysosomal damage and apoptosis mechanisms

The generated  $^1\text{O}_2$  can damage the biological membrane.<sup>31</sup> The membrane of lysosome is an important target site for hydrophobic photosensitizers in photodynamic therapy.<sup>32</sup> Thus, the delivery of Ru-PEG and Ru-H<sub>2</sub>O to lysosome was then investigated (**Figure 5a**). The weak blue fluorescence was from Ru-PEG or Ru-H<sub>2</sub>O. Lysosome was stained using LysoTracker dyes. CLSM images showed that before light irradiation, the blue fluorescence preferably overlapped with the red emission in the lysosome. After light irradiation, only a part of Ru-PEG or Ru-H<sub>2</sub>O migrated from lysosome, and the colocalization coefficient negligibly decreased, indicating a lysosome-targeting ability of these Ru complexes.

The generation of  $^1\text{O}_2$  within lysosome result in lysosomal permeability via massive peroxidation of membrane lipids.<sup>31</sup> The disruption of lysosomal membrane further cause the release of lysosomal proteases, such as cathepsin B, which induces apoptosis of cancer cells.<sup>33</sup> As Ru-PEG or Ru-H<sub>2</sub>O located within lysosome, the release of cathepsin B caused by  $^1\text{O}_2$  was detected using fluorescent Magic Red dye (Figure 5b). The control group display red dotlike fluorescence mostly located in lysosome. After incubated with Ru-PEG or Ru-H<sub>2</sub>O followed by red light irradiation, the diffusion of red fluorescence from lysosome to cytosol was observed, which was similar to the positive group caused by incubation with DMSO. These results demonstrated the cathepsin B was released from lysosome to cytosol after  $^1\text{O}_2$ -induced lysosomal damage, which further caused apoptosis of cancer cells.





**Figure 6.** (a) Fluorescence images of 4T1 tumor-bearing mice after intravenous injection of saline (left, control) and Ru-PEG vesicles (right). Images were taken after injection for 0, 4, 8, 12 and 24 h. The dashed circle indicates the tumor. (b) A photograph shows red light irradiation on a mouse model. (c) Tumor volumes of 4T1 tumor-bearing mice during different treatments. (d) Average tumor weights at day 17 after different treatments. (e) H&E, TUNEL, and Ki67 staining of tumor sections isolated from the mice on day 17. The damaged DNA strands were labelled by FITC (green). The proliferated cells were labelled by Ki67 (red). Cell nuclei were stained with DAPI (blue). Scale bars: 100  $\mu\text{m}$ .

### 2.3.7 In vivo anticancer assessment

Encouraged by the anticancer efficacy of Ru-PEG *in vitro*, we studied the anticancer efficacy of Ru-PEG *in vivo*. Hemolysis analysis indicated that Ru-PEG have good blood compatibility (Figure S25). We then investigated the anticancer performance of Ru-PEG using a 4T1 tumor-bearing mouse model.

Ru-PEG accumulated at tumor sites when administered intravenously (**Figure 6a**). Fluorescence images of the mouse model (right) and a control mouse (left) were taken over time after injection. A weak fluorescence signal was observed in the mouse model at first 4 h. The fluorescence from dye-loaded Ru-PEG at the tumor site reached a maximum at 12 h, which indicated that Ru-PEG efficiently accumulated at the tumor site. The fluorescence intensity became weaker after 24 h, suggesting the vesicles were cleared through metabolism. In contrast, the control mouse injected with saline showed no fluorescence, which proved that the fluorescence was produced by the injected vesicles. These results demonstrated that Ru-PEG nanovesicles accumulate at the tumor site, owing to the EPR effect.

Twelve hours after Ru-PEG nanovesicles were intravenously injected into the mouse model, the tumor was irradiated with a 671 nm laser for 15 min (Ru-PEG + light group) (Figure 6b). Three additional experiments were conducted for comparison: 1) 4T1 tumor-bearing mice were injected with saline (Control Group); 2) 4T1 tumor-bearing mice were irradiated with light (Light Group); and 3) 4T1 tumor-bearing mice were injected with Ru-PEG (Ru-PEG Group). We compared the anticancer efficacy by monitoring the tumor volumes of each group over 16 days (Figure 6c). The tumor volume in the control group increased by ~17 times. The result of the light group was similar to that of the control group, indicating that mild light irradiation does not have an inhibitory effect (Figure S26). Ru-PEG treatment caused a negligible tumor



inhibition, since no photo-cleavage was conducted. Compared to the three groups mentioned above, tumor growth in the Ru-PEG + light group was notably inhibited. This result suggests that the released Ru-H<sub>2</sub>O and generated <sup>1</sup>O<sub>2</sub> improved the anticancer performance.

Subsequently, the mice were euthanized, and the tumors were isolated for analysis. The average tumor weight in the Ru-PEG + light group was much lighter than that in the other three groups (Figure 6d). Immunohistochemical analyses were also performed to illustrate the mechanism of the anticancer activity using Ru-PEG vesicles (Figure 6e). A hematoxylin and eosin (H&E) staining assay showed large areas of apoptosis and necrosis, which suggested that the Ru-PEG + light group treatment exhibited considerable tumor inhibition. Similar results were observed in TUNEL staining images. In addition, Ki67 staining assay offered additional evidence for the anticancer mechanism. Ki67 is a sensitive marker for cell proliferation. Comparing the fluorescence of Ki67 at tumor sites, we found that sparse fluorescence area in the Ru-PEG + light group. We hypothesized that the released Ru-H<sub>2</sub>O and generated <sup>1</sup>O<sub>2</sub> induced DNA damage, which increased genomic instability and apoptosis. This caused the decrement of Ki67 protein marker.

We also investigated the systemic toxicity during the treatments by comparing the H&E staining images of the main organs (heart, liver, lung, spleen, and kidney) (Figure S27). Negligible pathological alteration of the organs was observed, indicating the low systemic toxicity of Ru-PEG. The body weights of the mice did not change significantly during the treatments (Figure S28), which suggests that the treatments had minimal side effects. The combined results demonstrate that the use of Ru-PEG can treat 4T1 tumors with improved efficacy and minimized systemic toxicity.

## 2.4 Conclusions

In conclusion, we synthesized a photo-responsive Ru-PEG vesicles to enhance delivering efficiency for solid tumor treatment. Ru-PEG exhibited transformable properties upon light irradiation due to the design of the molecular structure. Highly delivering efficiency in a mouse model was achieved using Ru-PEG during the penetration and internalization process. Our study revealed that the favorable design of nanocarriers with tunable properties is a new strategy to enhance the antitumor efficacy. We believe that more multi-functional nanocarriers with transformable properties can be developed for effective cancer therapy in both research and clinic.

## 2.5 References

- [1] Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R., Nanocarriers as an emerging platform for cancer therapy. *Nature nanotechnology* **2007**, *2* (12), 751-760.
- [2] Villemin, E.; Ong, Y. C.; Thomas, C. M.; Gasser, G., Polymer encapsulation of ruthenium complexes for biological and medicinal applications. *Nature Reviews Chemistry* **2019**, *3* (4), 261-282.
- [3] Abbina, S.; Takeuchi, L. E.; Anilkumar, P.; Yu, K.; Rogalski, J. C.; Shenoi, R. A.; Constantinescu, I.; Kizhakkedathu, J. N., Blood circulation of soft nanomaterials is governed by dynamic remodeling of protein opsonins at nano-biointerface. *Nature communications* **2020**, *11* (1), 1-12.
- [4] Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K., Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of controlled release* **2000**, *65* (1-2), 271-284.
- [5] Chen, S.; Zhong, Y.; Fan, W.; Xiang, J.; Wang, G.; Zhou, Q.; Wang, J.; Geng, Y.; Sun, R.; Zhang, Z., Enhanced tumour penetration and prolonged circulation in blood of polyzwitterion–drug conjugates with cell-membrane affinity. *Nature Biomedical Engineering* **2021**, 1-19.

- [6] Zhou, Q.; Shao, S.; Wang, J.; Xu, C.; Xiang, J.; Piao, Y.; Zhou, Z.; Yu, Q.; Tang, J.; Liu, X., Enzyme-activatable polymer–drug conjugate augments tumour penetration and treatment efficacy. *Nature nanotechnology* **2019**, *14* (8), 799-809.
- [7] Behzadi, S.; Serpooshan, V.; Tao, W.; Hamaly, M. A.; Alkawareek, M. Y.; Dreaden, E. C.; Brown, D.; Alkilany, A. M.; Farokhzad, O. C.; Mahmoudi, M., Cellular uptake of nanoparticles: journey inside the cell. *Chemical Society Reviews* **2017**, *46* (14), 4218-4244.
- [8] Meng, F.; Cheng, R.; Deng, C.; Zhong, Z., Intracellular drug release nanosystems. *Materials today* **2012**, *15* (10), 436-442.
- [9] Wang, W.; Cheng, D.; Gong, F.; Miao, X.; Shuai, X., Design of multifunctional micelle for tumor - targeted intracellular drug release and fluorescent imaging. *Advanced Materials* **2012**, *24* (1), 115-120.
- [10] Sun, Q.; Zhou, Z.; Qiu, N.; Shen, Y., Rational design of cancer nanomedicine: nanoproperty integration and synchronization. *Advanced materials* **2017**, *29* (14), 1606628.
- [11] Shi, C.; Guo, D.; Xiao, K.; Wang, X.; Wang, L.; Luo, J., A drug-specific nanocarrier design for efficient anticancer therapy. *Nature communications* **2015**, *6* (1), 1-14.
- [12] Suk, J. S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L. M., PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced drug delivery reviews* **2016**, *99*, 28-51.
- [13] Prencipe, G.; Tabakman, S. M.; Welsher, K.; Liu, Z.; Goodwin, A. P.; Zhang, L.; Henry, J.; Dai, H., PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation. *Journal of the American Chemical Society* **2009**, *131* (13), 4783-4787.
- [14] Sun, Q.; Ojha, T.; Kiessling, F.; Lammers, T.; Shi, Y., Enhancing tumor penetration of nanomedicines. *Biomacromolecules* **2017**, *18* (5), 1449-1459.
- [15] Tang, L.; Yang, X.; Yin, Q.; Cai, K.; Wang, H.; Chaudhury, I.; Yao, C.; Zhou, Q.; Kwon, M.; Hartman, J. A., Investigating the optimal size of anticancer nanomedicine. *Proceedings of the National Academy of Sciences* **2014**, *111* (43), 15344-15349.

- [16] Yue, Z.-G.; Wei, W.; Lv, P.-P.; Yue, H.; Wang, L.-Y.; Su, Z.-G.; Ma, G.-H., Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles. *Biomacromolecules* **2011**, *12* (7), 2440-2446.
- [17] Mura, S.; Nicolas, J.; Couvreur, P., Stimuli-responsive nanocarriers for drug delivery. *Nature materials* **2013**, *12* (11), 991-1003.
- [18] Rosenblum, D.; Joshi, N.; Tao, W.; Karp, J. M.; Peer, D., Progress and challenges towards targeted delivery of cancer therapeutics. *Nature communications* **2018**, *9* (1), 1-12.
- [19] Jo, D. H.; Kim, J. H.; Lee, T. G.; Kim, J. H., Size, surface charge, and shape determine therapeutic effects of nanoparticles on brain and retinal diseases. *Nanomedicine: Nanotechnology, Biology and Medicine* **2015**, *11* (7), 1603-1611.
- [20] Fang, Y.; Xue, J.; Gao, S.; Lu, A.; Yang, D.; Jiang, H.; He, Y.; Shi, K., Cleavable PEGylation: a strategy for overcoming the “PEG dilemma” in efficient drug delivery. *Drug Delivery* **2017**, *24* (2), 22-32.
- [21] Zhu, Y.; Chen, C.; Cao, Z.; Shen, S.; Li, L.; Li, D.; Wang, J.; Yang, X., On-demand PEGylation and dePEGylation of PLA-based nanocarriers via amphiphilic mPEG-TK-Ce6 for nanoenabled cancer chemotherapy. *Theranostics* **2019**, *9* (26), 8312.
- [22] Hu, Q.; Chen, Q.; Gu, Z., Advances in transformable drug delivery systems. *Biomaterials* **2018**, *178*, 546-558.
- [23] Lundqvist, M.; Stigler, J.; Elia, G.; Lynch, I.; Cedervall, T.; Dawson, K. A., Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proceedings of the National Academy of Sciences* **2008**, *105* (38), 14265-14270.
- [24] Mari, C.; Pierroz, V.; Ferrari, S.; Gasser, G., Combination of Ru (II) complexes and light: new frontiers in cancer therapy. *Chemical science* **2015**, *6* (5), 2660-2686.
- [25] Zeng, L.; Gupta, P.; Chen, Y.; Wang, E.; Ji, L.; Chao, H.; Chen, Z.-S., The development of anticancer ruthenium (II) complexes: from single molecule compounds to nanomaterials. *Chemical Society Reviews* **2017**, *46* (19), 5771-5804.
- [26] Hartinger, C. G.; Jakupec, M. A.; Zorbas - Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K., KP1019, a new redox - active anticancer agent—Preclinical development and results of a clinical phase I study in tumor patients. *Chemistry & biodiversity* **2008**, *5* (10), 2140-2155.

- [27] Heffeter, P.; Atil, B.; Kryeziu, K.; Groza, D.; Koellensperger, G.; Körner, W.; Jungwirth, U.; Mohr, T.; Keppler, B. K.; Berger, W., The ruthenium compound KP1339 potentiates the anticancer activity of sorafenib in vitro and in vivo. *European Journal of Cancer* **2013**, *49* (15), 3366-3375.
- [28] Bonnet, S., Why develop photoactivated chemotherapy? *Dalton Transactions* **2018**, *47* (31), 10330-10343.
- [29] Schöttler, S.; Becker, G.; Winzen, S.; Steinbach, T.; Mohr, K.; Landfester, K.; Mailänder, V.; Wurm, F. R., Protein adsorption is required for stealth effect of poly (ethylene glycol)-and poly (phosphoester)-coated nanocarriers. *Nature nanotechnology* **2016**, *11* (4), 372-377.
- [30] Liu, K.; Xing, R.; Zou, Q.; Ma, G.; Möhwald, H.; Yan, X., Simple peptide - tuned self - assembly of photosensitizers towards anticancer photodynamic therapy. *Angewandte Chemie* **2016**, *128* (9), 3088-3091.
- [31] Zhou, Z.; Liu, J.; Rees, T. W.; Wang, H.; Li, X.; Chao, H.; Stang, P. J., Heterometallic Ru–Pt metallacycle for two-photon photodynamic therapy. *Proceedings of the National Academy of Sciences* **2018**, *115* (22), 5664-5669.
- [32] He, G.; Xu, N.; Ge, H.; Lu, Y.; Wang, R.; Wang, H.; Du, J.; Fan, J.; Sun, W.; Peng, X., Red-Light-Responsive Ru Complex Photosensitizer for Lysosome Localization Photodynamic Therapy. *ACS Applied Materials & Interfaces* **2021**.
- [33] Oberle, C.; Huai, J.; Reinheckel, T.; Tacke, M.; Rassner, M.; Ekert, P. G.; Buellesbach, J.; Borner, C., Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocytes. *Cell Death & Differentiation* **2010**, *17* (7), 1167-1178.

## 2.6 Supporting information

### Materials

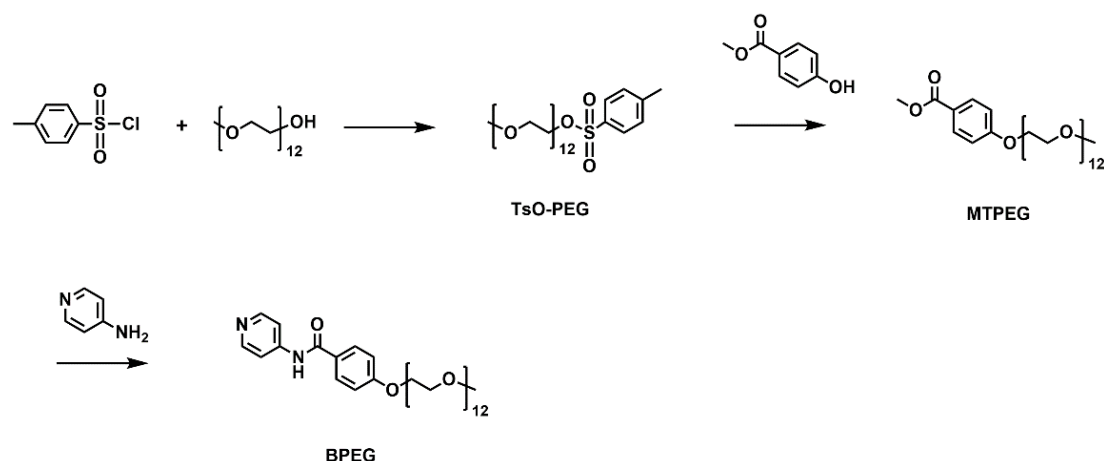
RuCl<sub>3</sub>·xH<sub>2</sub>O (99.9%), 2,2'-biquinoline (98%), silver hexafluorophosphate (98%), potassium hexafluorophosphate (99%), *p*-toluolsulfonylchlorid (99%), 1-pyrenecarboxylic acid (97%), methyl 4-hydroxybenzoate (99%), 4-aminopyridine (98%), potassium carbonate (99%), potassium *tert*-butoxide (98%), methoxy-PEG12-hydroxyl (95%) were purchased from Sigma-Aldrich. 1,6-dihydroxyhexane (99%), N,N'-dicyclohexylcarbodiimide (DCC) (99%), triethylamine (99%) were purchased from Alfa Aesar. THF (1 mL, technical grade, stabilizer free) was purchased from Acros Organics. 4'-Chloro-2,2',6',2''-terpyridine (>98%) was purchased from AEchem Scientific Corporation. 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD fluorescent dye) was purchased from Thermo Fisher Scientific. PBS with a pH of 7.4 (10×) was purchased from Life Technologies. All other solvents (HPLC grade) were purchased from Sigma-Aldrich or Fisher Scientific. Milli-Q water with a resistivity of 18.2 MΩ·cm was used in this study. Dialysis tubing (500 MWCO) was purchased from SERVA Electrophoresis GmbH, Germany.

### Instruments and Characterization

<sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H NMR) was recorded on a 250-MHz Bruker Spectrospin NMR spectrometer at 25 °C. <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C NMR) and <sup>1</sup>H - <sup>1</sup>H correlation spectroscopy (COSY) were recorded on a 300-MHz Bruker Spectrospin NMR spectrometer at 25 °C. Mass spectrometric (MS) data were carried out using LTQ Orbit rap XL instruments at Dalian University of Technology, Dalian, China. HPLC analysis was performed on an Agilent HPLC system equipped with a

1100 Series Quaternary pump, a 1200 Series diode detector, and a Merck Chromolith Performance RP18e 100-3 mm HPLC column. UV-Vis detector in the HPLC system was set at 260 nm for analysis. UV-Vis absorption spectra were measured on a Lambda 900 spectrometer (Perkin Elmer). Fluorescence spectra were recorded on a TIDAS II spectrometer (J&M). Transmission electron microscopy (TEM) images were taken on a Hitachi HT7700 Transmission Electron Microscope. The diameters of the nanoparticles were determined by dynamic light scattering (DLS) on a Nano ZS90 particle size analyzer, Malvern (UK). A DPSS laser with a wavelength of  $\lambda = 671$  nm (CNI-671-200-LN-AC-3, Laser 2000 GmbH, Germany) was used to induce photoreactions of  $[\text{Ru}(\text{Ptpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  and  $[\text{Ru}(\text{Ptpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  vesicles. The laser was equipped with a thermoelectric cooling system. The output power of the laser was controlled by a tabletop laser driver (PSU-III-FDA, Changchun New Industries Optoelectronics Technology Co., Ltd., China) and measured using an optical power meter (model 407A, Spectra-Physics Corporation). A laser at 671 nm was employed as the light source for *in vitro* and *in vivo* experiments. The output power of the laser was controlled by a fiber coupled laser system (FC-671-1W, Changchun New Industries Optoelectronics Technology) and measured by a power meter (LP100/TS15, Changchun New Industries Optoelectronics Technology).

## Synthesis



**Figure S1.** Synthetic route of BPEG.

**Synthesis of 3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxaheptatriacontan-1-ol, 1-(4-methylbenzenesulfonate) (TsO-PEG) (Figure S1):**

NaOH (34.0 mg, 0.85 mmol) was dissolved in water (1 mL) and cooled to 0°C, and subsequently added to a solution of methoxy-PEG12-hydroxyl (280.0 mg, 0.5 mmol) in 3 mL THF under constant stirring. The reaction mixture was kept at 0 °C and p-toluenesulfonyl chloride (118.0 mg, 0.62 mmol) dissolved in THF, was carefully added. The reaction mixture was allowed to heat to room temperature and stirred overnight. The crude reaction mixture was concentrated in vacuo featuring a water-bath temperature of 30°C and subsequently two times extracted with DCM. The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and all volatiles were evaporated in vacuo. TsO-TEG was yielded as a pure colorless viscous liquid (315.3 mg, 0.44 mmol, 88%).



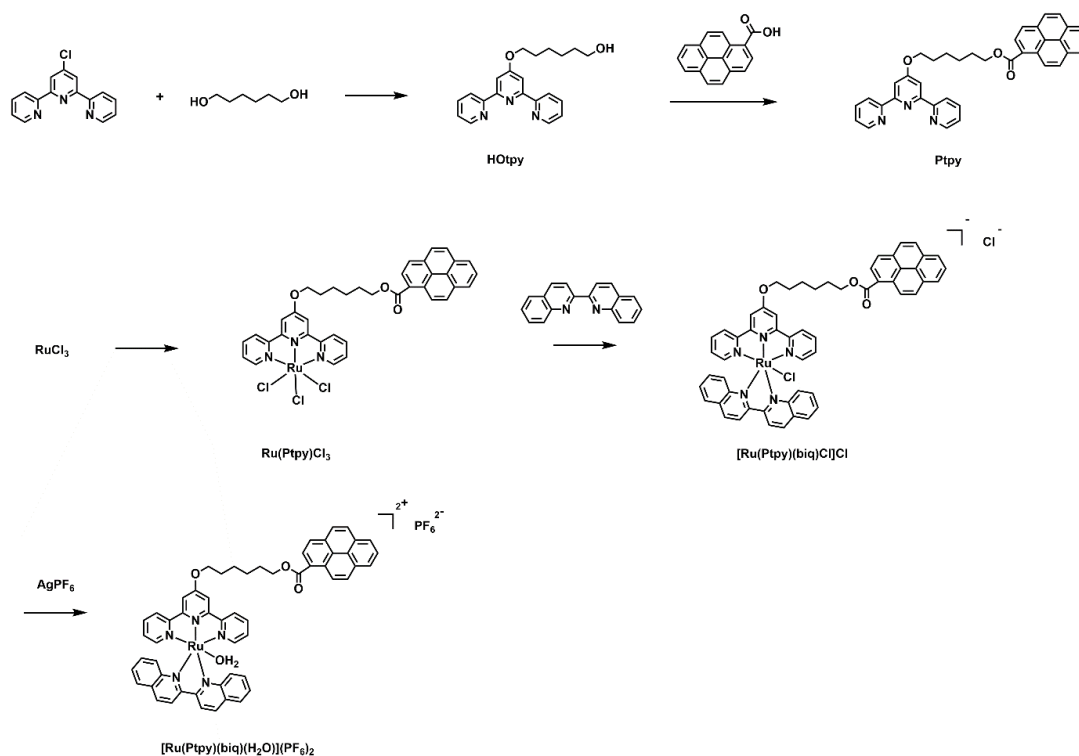
---

**Synthesis of methyl 4-[(26-hydroxy-3,6,9,12,15,18,21,24,27,30,33,36-octaoxahexacos-1-yl)oxy]benzoate (MTPEG) (Figure S1):**

TsO-TEG (300.0 mg, 0.42 mmol) was dissolved together with 4-hydroxybenzoate (77.0 mg, 0.5 mmol) and K<sub>2</sub>CO<sub>3</sub> (116.0 mg, 0.84 mmol) in dry DMF (5 mL) and heated to 70°C for 20 h in a nitrogen atmosphere. The solution displayed an intense brownish color shortly after launch. Water was added and the crude reaction mixture was four times extracted with DCM. The organic layers were combined and washed with water as well as brine and subsequently dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation of the solvents, the crude product was subjected to column chromatography (eluent: methanol/dichloromethane =1:20). MTPEG was yielded as a pure colorless viscous liquid (225.2 mg, 0.32 mmol, 76%).

**Synthesis of benzamide, N-4-pyridinyl-4-[(26-hydroxy-3,6,9,12,15,18,21,24,27,30,33,36-octaoxahexacos-1-yl)oxy]- (BPEG) (Figure S1):**

MTPEG (69.4 mg, 0.1 mmol) was dissolved together with 4-aminopyridine (10.34 mg, 0.11 mmol) in THF (1 mL, technical grade, stabilizer free). A solution of potassium *tert*-butoxide in THF (2M, 0.1 mL) was slowly added to the reaction mixture. The mixture was stirred uncapped for 2 h, and full conversion was proven by TLC. The solvents were evaporated and the crude product subject to column chromatography (eluent: methanol/dichloromethane = from 1:20 to 1:10) yielding a slight yellow liquid (60.3 mg, 0.079 mmol, 79%).



**Figure S2.** Synthetic route of  $[\text{Ru(Ptpy)(biq)(H}_2\text{O)}] (\text{PF}_6)_2$  (defined as Ru-H<sub>2</sub>O).

### Synthesis of 4'-hydroxyhexyloxy-2,2':6',2''-terpyridine (HOtpy) (Figure S2):

KOH (2.10 g, 37.5 mmol) were suspended in 32 mL dry DMSO under argon atmosphere. 1,6-dihydroxyhexane (4.41 g, 37.3 mmol) was added and the mixture was stirred for 20 min at 80 °C. Then 4'-chloro-2,2':6',2''-terpyridine (1.83 g, 6.45 mmol) was added and the mixture was stirred for 4 h at 80 °C. The mixture was then poured into water (250 mL) and extracted with ethylacetate. The solvent was then removed by rotary evaporation and the resulting yellow oil was purified by column chromatography using ethyl acetate as the eluent (1.72 g, 4.9 mmol, 76%).

**Synthesis of 4-hydroxyhexyloxy-2,2':6',2''-terpyridine ester of pyrenecarboxylic acid (Ptpy) (Figure S2):**

1-pyrenecarboxylic acid (246.0 mg, 1 mmol), HOtpy (418.0 mg, 1.19 mmol) and DMAP (219.0 mg, 1.79 mmol) were dissolved in 25 ml dry DCM, DCC (309.0 mg, 1.5 mmol) in 8 ml DCM was added on an ice bath and then stirred at room temperature for 24h. After filtration, the solution was diluted with DCM then washed with water and dried with MgSO<sub>4</sub>. The solvent was then removed by rotary evaporation and the resulting yellow oil was purified by column chromatography (eluent: ethyl acetate/petroleum ether = from 1:2 to 1:1). Ptpy was obtained as a light yellow solid (387.4 mg, 0.67 mmol, 67%).

**Synthesis of Ru(Ptpy)Cl<sub>3</sub> (Figure S2):**

RuCl<sub>3</sub>·xH<sub>2</sub>O (58.0 mg, 0.28 mmol) and Ptpy (162.0 mg, 0.28 mmol) were mixed in absolute ethanol (40 mL). The mixture was heated at reflux under argon for 4 h with vigorous magnetic stirring. The mixture was then cooled to room temperature. Fine brown yellow powders appeared and were filtered from the reddish yellow solution. The product Ru(Ptpy)Cl<sub>3</sub> was washed with ethanol and diethyl ether, and air-dried. The product (218.2 mg, 0.27 mmol, 98%) was used directly for the next step.

**Synthesis of [Ru(Ptpy)(biq)Cl]Cl (Figure S2):**

Ru(Ptpy)Cl<sub>3</sub> (78.4 mg, 0.1 mmol) and 2,2'-biquinoline (25.6 mg, 0.1 mmol) were mixed in 1:1 ethanol/H<sub>2</sub>O mixture (14 mL) and the solution was bubbled with argon for 5 min. Then, trimethylamine (15 μL) was added to the mixture. The reaction mixture was refluxed under argon for 24 h in the dark. After that, the mixture was filtered hot





weight.  $[\text{Ru}(\text{tpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  was obtained as a pink solid (72.9 mg, 0.044 mmol, 81%).

### **Sample preparation**

#### **Preparation of Ru-PEG vesicles:**

To prepare Ru-PEG vesicles, 1.6 mL distilled water was added dropwise into a 0.4 mL THF containing 2 mg Ru-PEG and stirred in the dark for 20 min. The stirring rate was 500 rpm and dropwise speed was set as 2.5 sec/drop. The obtained vesicles dispersion was kept still in the dark for 4 h. Afterwards the dispersion was dialyzed against 5 L Milli-Q water for 48 h using a dialysis tube (MW cutoff, 1 kDa). Milli-Q water was replaced every 12 h.

As a comparison, similar procedure was carried out to trigger the self-assembly of Ru-PEG without pyrene moiety. Specifically, distilled water was added dropwise into a THF solution containing 2 mg Ru-PEG without pyrene moiety and stirred in the dark for 20 min. The final concentration was maintained at 1 mg/mL. The obtained solution was kept still in the dark overnight. Afterwards the solution was dialyzed against 5 L Milli-Q water for 48 h using a dialysis tube (MW cutoff, 1 kDa). Milli-Q water was replaced every 12 h.

#### **Preparation of DiD dye loaded $[\text{Ru}(\text{Ptpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$ vesicles:**

To prepare DiD dye loaded Ru-PEG vesicles, 50  $\mu\text{L}$  DiD solution (1 mg/mL in THF) was added into 0.15 mL THF containing 2 mg Ru-PEG. The solution was stirred for 20 min with stirring rate at 500 rpm. After that, 1.8 mL distilled water was added dropwise

into the solution and kept still for another 4 h. The DiD dye loaded vesicles dispersion was dialyzed against 5 L Milli-Q water for 48 h using a dialysis tube (MW cutoff, 1 kDa). Milli-Q water was replaced every 12 h.

## **Cell experiments**

### **Cell culture**

Mouse mammary carcinoma cell line 4T1 was cultured RPMI-1640 medium. Human lung cancer cell line A549 was cultured in DMEM medium. All cell lines were complemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% penicillin/streptomycin (Life Technologies, USA) and maintained in humidified atmosphere at 37 °C and 5% of CO<sub>2</sub>. Treatment with trypsin (0.25%) (Life technologies, USA) for 5 minutes was employed to detach the cells for further assays.

### **Cell viability assay**

All cell viability tests were evaluated using a standard Cell Counting Kit-8 (CCK-8) assay kit (Solarbio, China). Typically, cancer cells were incubated with DMEM/ RPMI-1640 medium in 96-well plates at a density of  $5.0 \times 10^3$  cells per well overnight. The medium was then replaced by DMEM/ RPMI-1640 containing different compounds or Ru-PEG vesicles in the dark. To investigate the cell viability of Ru-H<sub>2</sub>O or Ru-PEG vesicles under irradiation, each sample was added to the cell medium and cultured for 4 h, followed by exposure to 671 nm red light irradiation for 10 min. Then, cells were incubated for 24 h. CCK-8 working solution was added as 10 μL per well, followed by another incubation for 2 h. Cell viability was assessed by the measurement of the

absorbance at the wavelength of 450 nm on the microplate reader (EnSpire, Perkin Elmer). Treatment procedures were replicated five times for each cell line.

### **Cell imaging by confocal laser scanning microscopy (CLSM)**

For the cellular uptake experiments,  $1 \times 10^5$  cells per milliliter were seeded in 35 mm diameter  $\mu$ -dishes and cultured overnight in supplemented medium. The medium was replaced by fresh medium containing 30  $\mu\text{g}/\text{mL}$  Ru-PEG vesicles. The cells were incubated for 2 h. Ru-PEG vesicles were further removed by washing with PBS three times. Live cells images were taken using CLSM (LSM710, Carl Zeiss). Ru-PEG vesicles were excited with a 405 nm laser and detected in the range from 425 to 475 nm.

For the intracellular singlet oxygen ( $^1\text{O}_2$ ) detection assay, the fluorescent probe DCFH-DA (Solarbio, China) was used according to the manufacture's instruction. Specifically,  $1 \times 10^5$  cells per milliliter were seeded in 35 mm diameter  $\mu$ -dishes and cultured overnight in supplemented medium. The cells were divided into five groups: (1) Control group; (2) + Light group; (3) + Ru-PEG vesicles + light group; (4) + Ru-PEG vesicles + probe group; (5) + Ru-PEG vesicles + light + probe group. Then medium or 30  $\mu\text{g}/\text{mL}$  Ru-PEG vesicles were incubated with the cells for 2 h. Afterwards, serum-free DCFH-DA solution (15  $\mu\text{M}$ ) was incubated with cells in (4) and (5) groups for 20 min. Cells in group (2), (3), and (5) were then irradiated with 671 nm red light for 1 min at an intensity of  $125 \text{ mWcm}^{-2}$ . Live cells were further imaged using CLSM (LSM710, Carl Zeiss). DCFH-DA was excited with a 488 nm laser, detected in the range from 500 to 550 nm. The vesicles were excited with a 405 nm laser and detected in the range from 425 to 475 nm.



## **Animal experiments**

### **Animal use and tumor model establishment**

BALB/c mice (female, 18-20 g, 4-6 weeks) were purchased from Vital River Laboratory Animal Center (Beijing, China). All mice were maintained under specific pathogen-free conditions and had free access to food and water throughout all the experiments. All protocols for animal studies conformed to the Guide for the Care and Use of Laboratory Animals.

The tumor model was established by inoculating  $1.2 \times 10^6$  4T1 cells in the flank region of each mouse. Tumor volume was measured using a vernier caliper and calculated as  $V=L*W^2/2$ , where L and W were the length and width of the tumor, respectively.

### **Hemolysis analysis**

The fresh whole blood was obtained from BALB/c mice using sodium citrate as an anti-coagulant with a blood/ anticoagulant ratio of 9:1. The whole blood was subsequently centrifuged at  $1000 \times g$  for 5 min and then the plasma and buffy coat layer were removed. The obtained red blood cells (RBCs) were collected and washed with saline for three times, followed by suspending in 15 mL saline. The RBCs suspension was incubated with saline containing different concentrations of Ru-PEG vesicles with the ratio of 1:1 (v/v). The final concentration of Ru-PEG vesicles was ranging from 100 to 500  $\mu\text{g/mL}$ . The positive hemolysis control was induced by replacing saline with same volume of water and the negative hemolysis control was saline without any Ru-PEG vesicles. Each sample was repeated three times. All samples were incubated at 37 °C for 24 h. After incubation, the RBCs were centrifuged at  $1000 \times g$  for 5 min and the absorbance of supernatants at 540 nm was measured by microplate reader. The

hemolysis percentage was calculated by measuring the optical density (OD) as the following formula:

$$\text{Hemolysis (\%)} = \frac{[(\text{OD of sample absorbance} - \text{OD of background absorbance}) / (\text{OD of positive control} - \text{OD of negative control})] \times 100\%.$$

### ***In vivo* fluorescence imaging**

The 4T1 tumor-bearing mice at a tumor size approximately 100 mm<sup>3</sup> were treated with saline as control group or DiD-loaded Ru-PEG vesicles through intravenous injection via tail vein. The dosage of Ru-PEG vesicles was 16 mg/kg. After 0, 4, 8, 12, 24 h, the mice were anesthetized with isoflurane and imaged under the *in vivo* imaging system (IVIS Spectrum, PerkinElmer). The excitation wavelength was fixed at 633 nm.

### ***In vivo* anticancer efficacy evaluation**

The anticancer efficacy was evaluated by monitoring the tumor volumes using 4T1 tumor models. The tumor nodules were allowed to reach approximately 100 mm<sup>3</sup> before initial treatment. All the mice were numbered using ear tags and the initial tumor volume and body weight were recorded. Subsequently, the mice were randomly divided into four groups: (1) a group of 4T1 tumor-bearing mice was injected with saline (control group); (2) a group of 4T1 tumor-bearing mice was irradiated with light only (light group); (3) a group of 4T1 tumor-bearing mice was injected with Ru-PEG vesicles (Ru-PEG group); (4) a group of 4T1 tumor-bearing mice was injected with Ru-PEG vesicles (Ru-PEG + light group). The number of mice in each group was five. Injections were performed on the first, third and fifth day during the treatments. Mice

in group (3) and (4) were treated with equivalent dosage of 4 mg/kg Ru-PEG nanovesicles. 671 nm light irradiation ( $125 \text{ mWcm}^{-2}$ , 10 min) in group (4) was performed after 12 h i.v. injection of Ru-PEG vesicles. To exclude the effect by light irradiation, Mice in group (2) were set as a negative control. The body weight and tumor volume of each mouse were measured every day. At day 17, the mice from each group were sacrificed and tumor were isolated for weighing.

### **Histological analyses**

At the end of tumor inhibition experiments, the mice from each group were sacrificed and tumor were collected. Main organs including heart, liver, lung, kidney and spleen were also collected and fixed in 4% formaldehyde solution followed by paraffin embedding. The treated tumors and organs were then cut into pieces with thickness of 2  $\mu\text{m}$ , and stained with hematoxylin and eosin (H&E) to assess histological alterations by microscope. Embedding and H&E staining were done by Wuhan Servicebio Technology Co., Ltd.

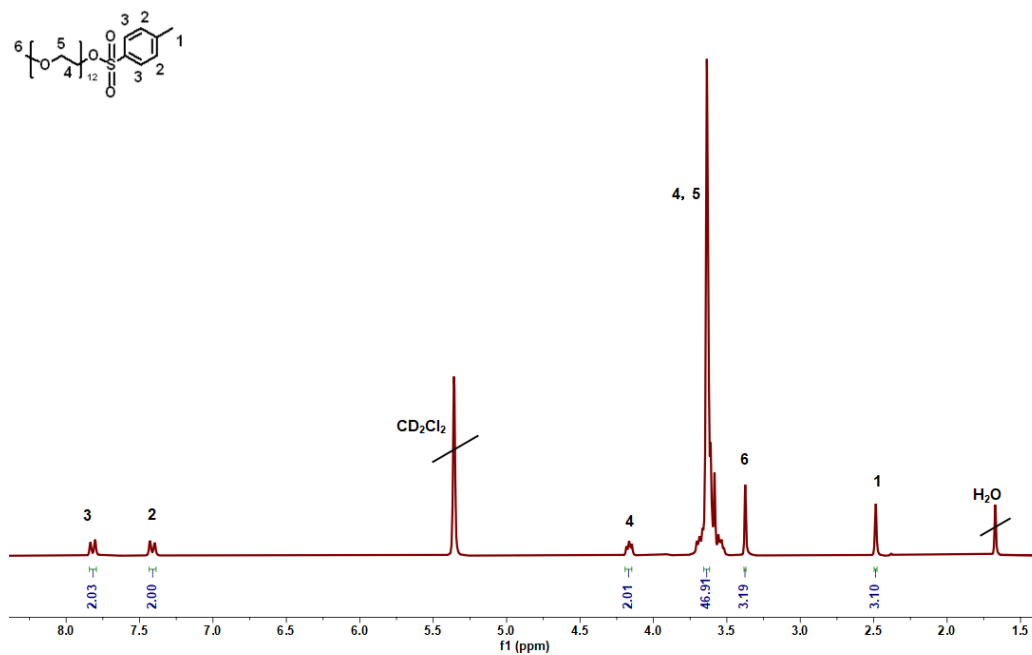
### **TUNEL and Ki67 assay**

The isolated tumors were fixed in 4% formaldehyde solution followed by paraffin embedding and TUNEL/Ki67 staining. Typically, the paraffin embedded tumors were cut into approximately 8.0  $\mu\text{m}$  by a rotary microtome (YD-1508A) and stained with 0.25% toluidine blue O. The DNA fragmentation was labelled using a TUNEL apoptosis detection kit. The cell proliferation indicator Ki67 staining assays were performed according to the standard protocol. Embedding and TUNEL/Ki67 staining

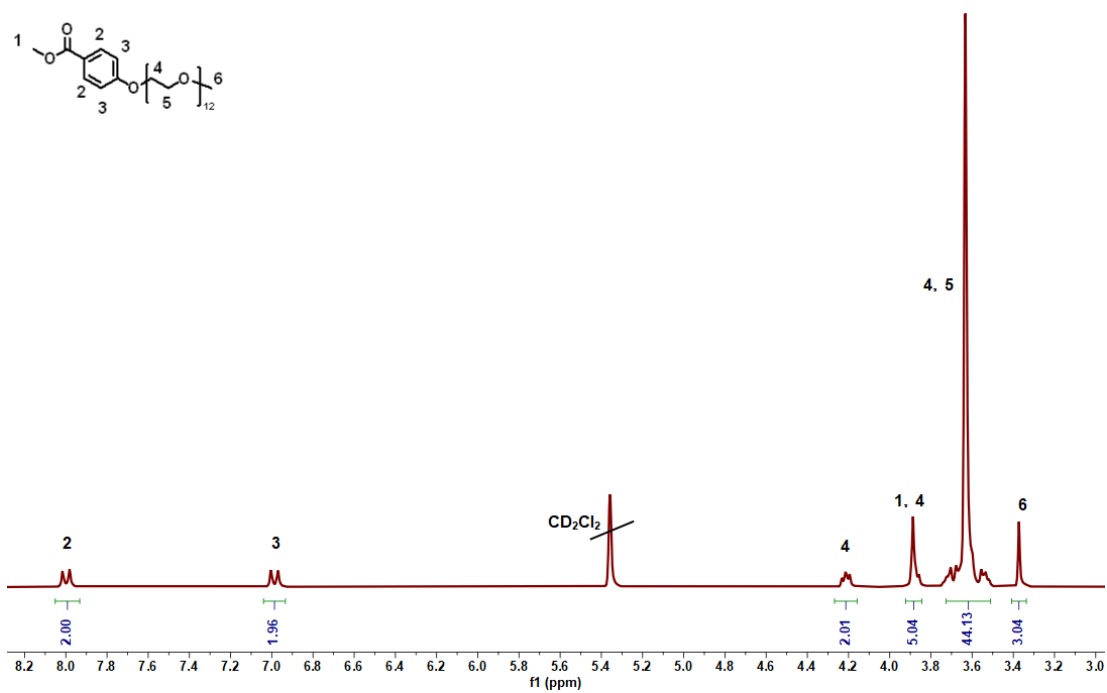
were done by Wuhan Servicebio Technology Co., Ltd. Antibodies were purchased from Wuhan Servicebio Technology Co., Ltd. Samples were observed by CLSM.

### **Statistical analysis**

One-way ANOVA with Tukey's multiple comparison analysis was used to determine the significance of differences between different groups. Data were calculated and appropriate statistical results were marked as \* $p < 0.05$  and \*\*\* $p < 0.001$ .



**Figure S5.**  $^1\text{H}$  NMR of TsO-PEG (250 MHz,  $\text{CD}_2\text{Cl}_2$ ).



**Figure S6.**  $^1\text{H}$  NMR spectrum of PCE (250 MHz,  $\text{CD}_2\text{Cl}_2$ ).

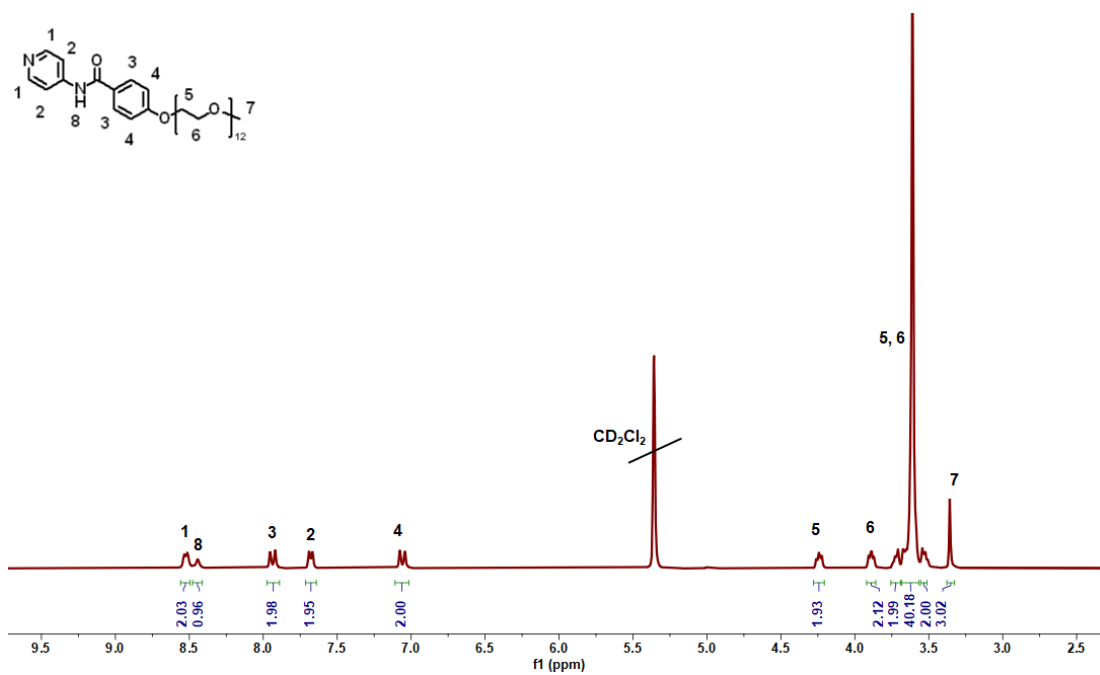


Figure S7. <sup>1</sup>H NMR of BPEG (250 MHz, CD<sub>2</sub>Cl<sub>2</sub>).

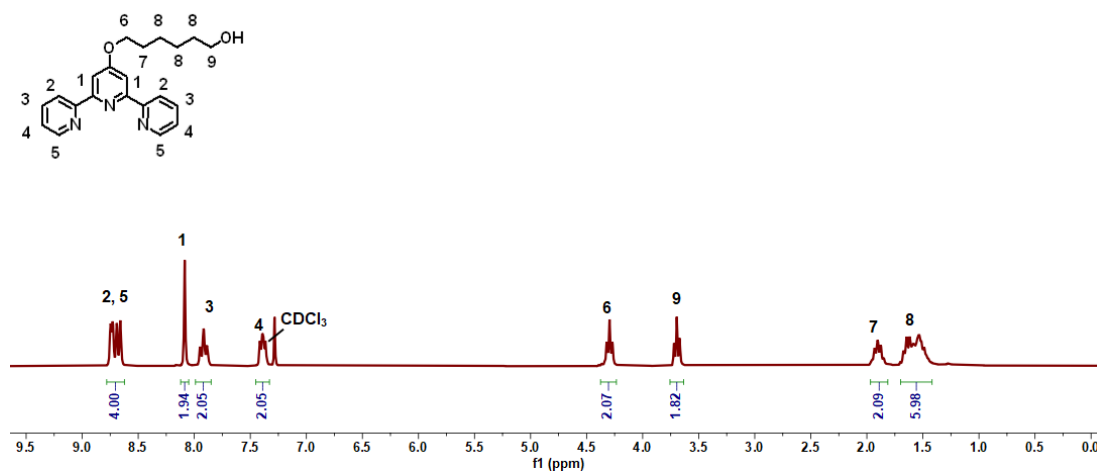


Figure S8. <sup>1</sup>H NMR of HOtpy (250 MHz, CDCl<sub>3</sub>).

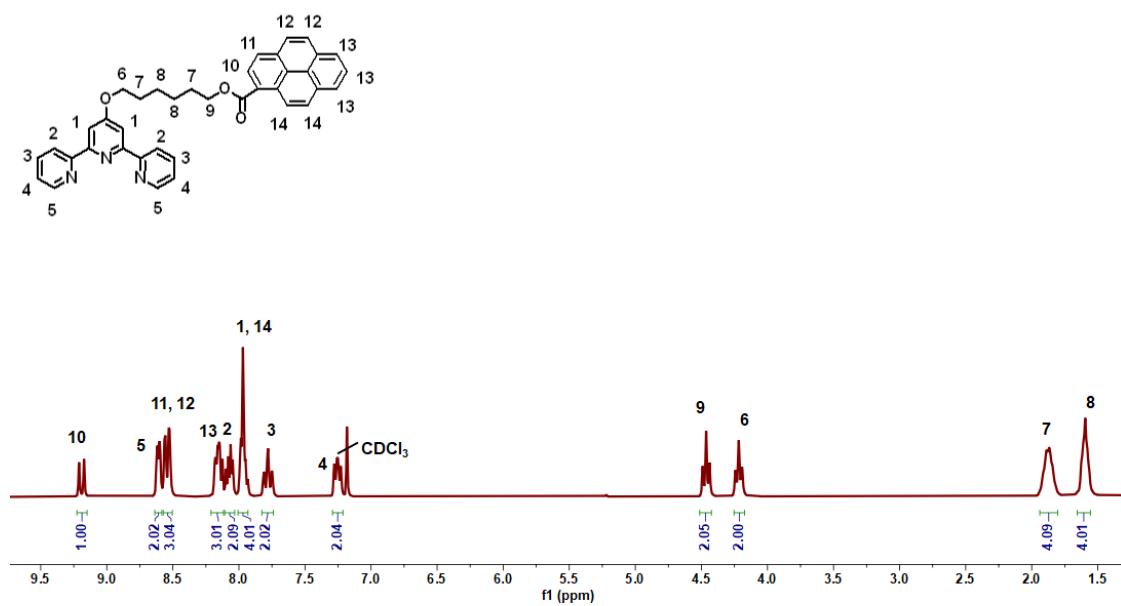


Figure S9.  $^1\text{H}$  NMR of Ptpy (250 MHz,  $\text{CDCl}_3$ ).

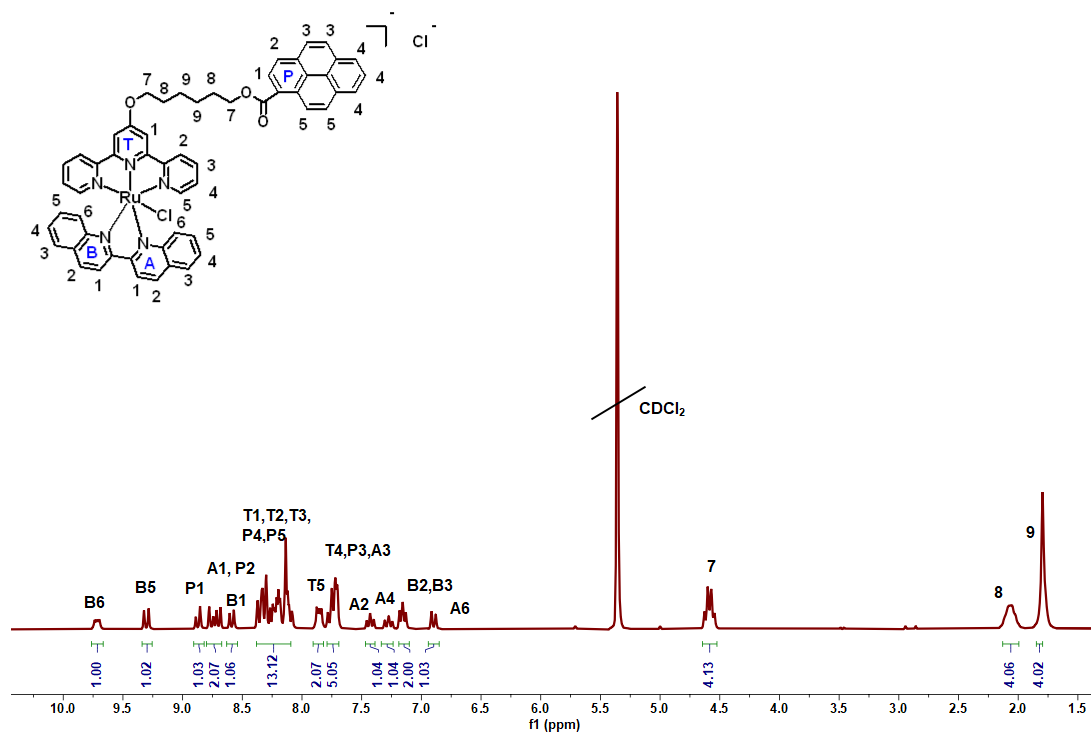
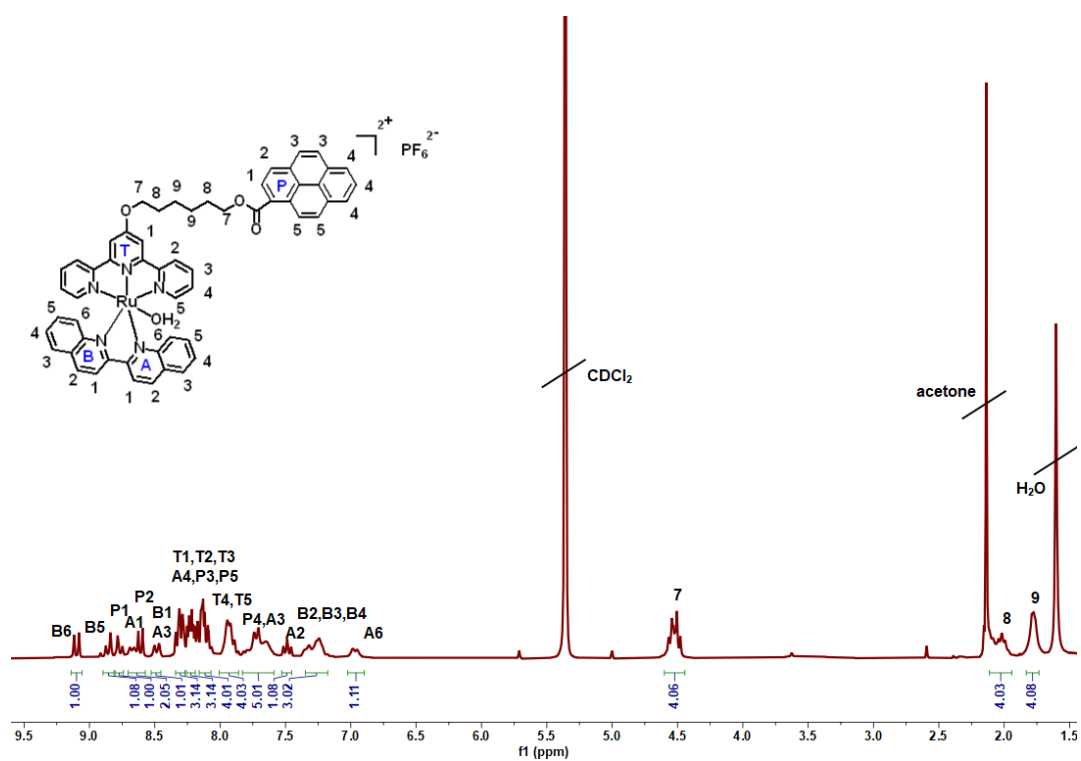
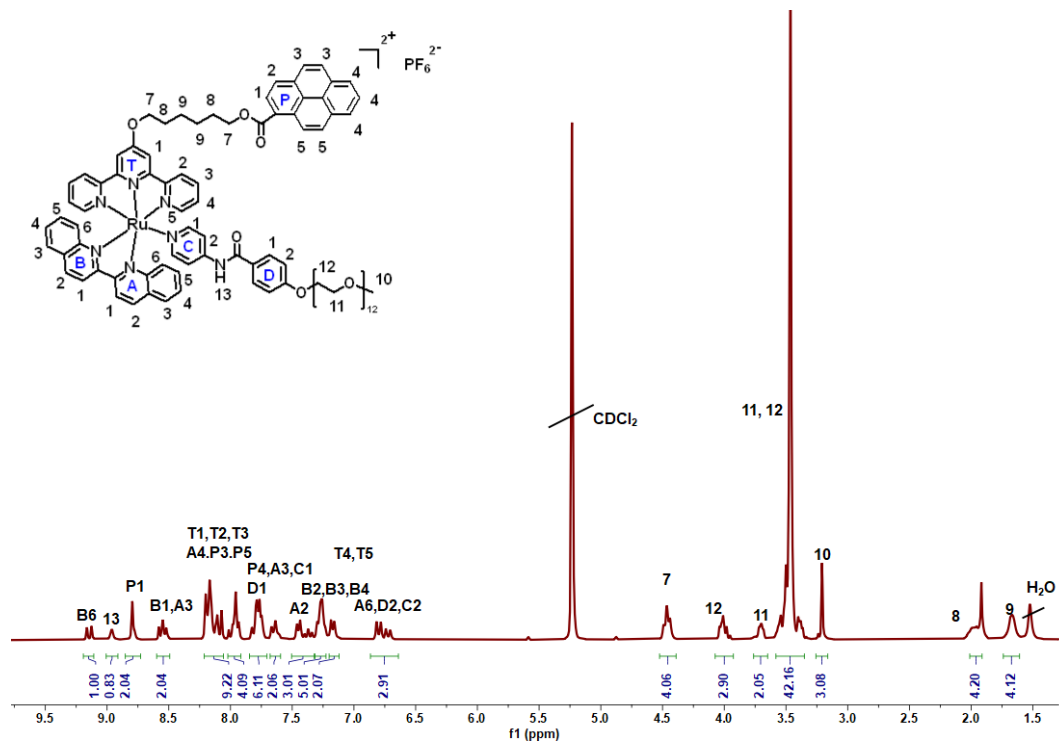


Figure S10.  $^1\text{H}$  NMR of  $[\text{Ru}(\text{Ptpy})(\text{biq})\text{Cl}]\text{Cl}$  (250 MHz,  $\text{CD}_2\text{Cl}_2$ ).

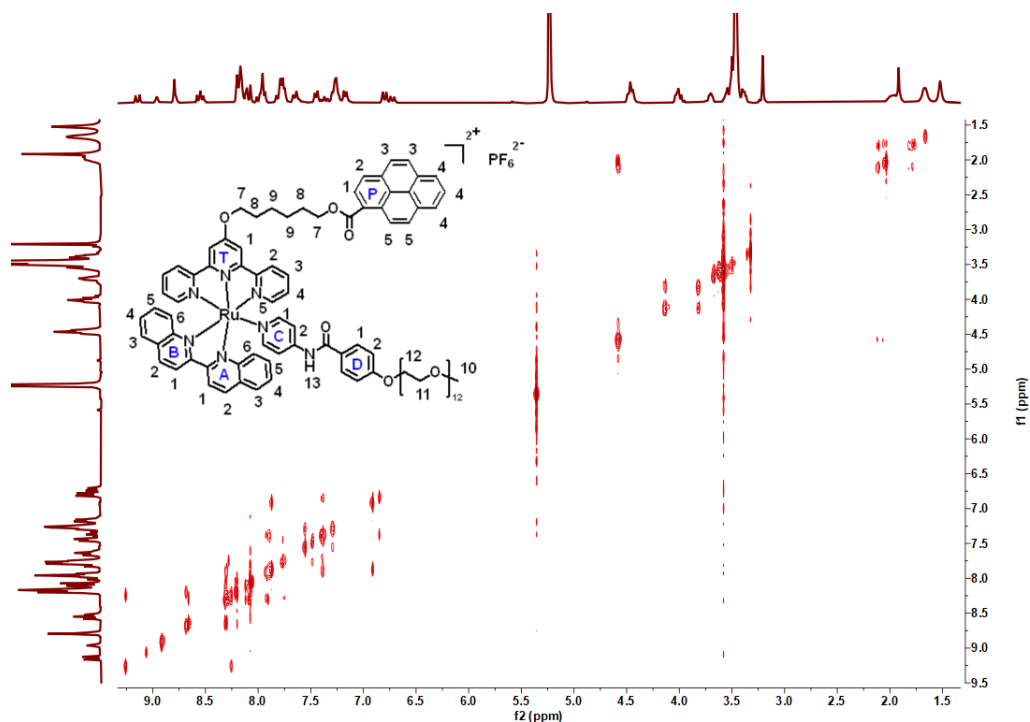


**Figure S11.** <sup>1</sup>H NMR of [Ru(Ptpy)(biq)(H<sub>2</sub>O)](PF<sub>6</sub>)<sub>2</sub> (250 MHz, CD<sub>2</sub>Cl<sub>2</sub>).

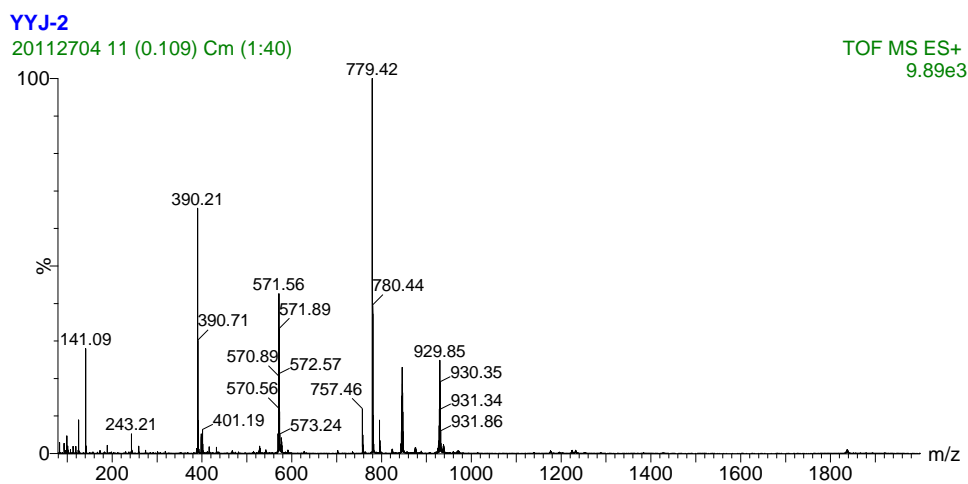


**Figure S12.** <sup>1</sup>H NMR of [Ru(Ptpy)(biq)(BPEG)](PF<sub>6</sub>)<sub>2</sub> (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>).





**Figure S13.** COSY of  $[\text{Ru}(\text{tpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).



**Figure S14.** ESI-MS of  $[\text{Ru}(\text{Ptpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  ( $M = [\text{Ru}(\text{tpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$ ,  $L = \text{BPEG}$ ).  $m/z = 929.8$  is assigned to  $[\text{M} + \text{Na} - \text{PF}_6]^{2+}$ ,  $m/z = 571.5$  is assigned to  $[\text{M} + \text{Na} - 2\text{PF}_6]^{3+}$ ,  $m/z = 779.5$  is assigned to  $[\text{L} + \text{Na}]^+$ ,  $m/z = 390.2$  is assigned to  $[\text{L} + \text{H} + \text{Na}]^{2+}$ .

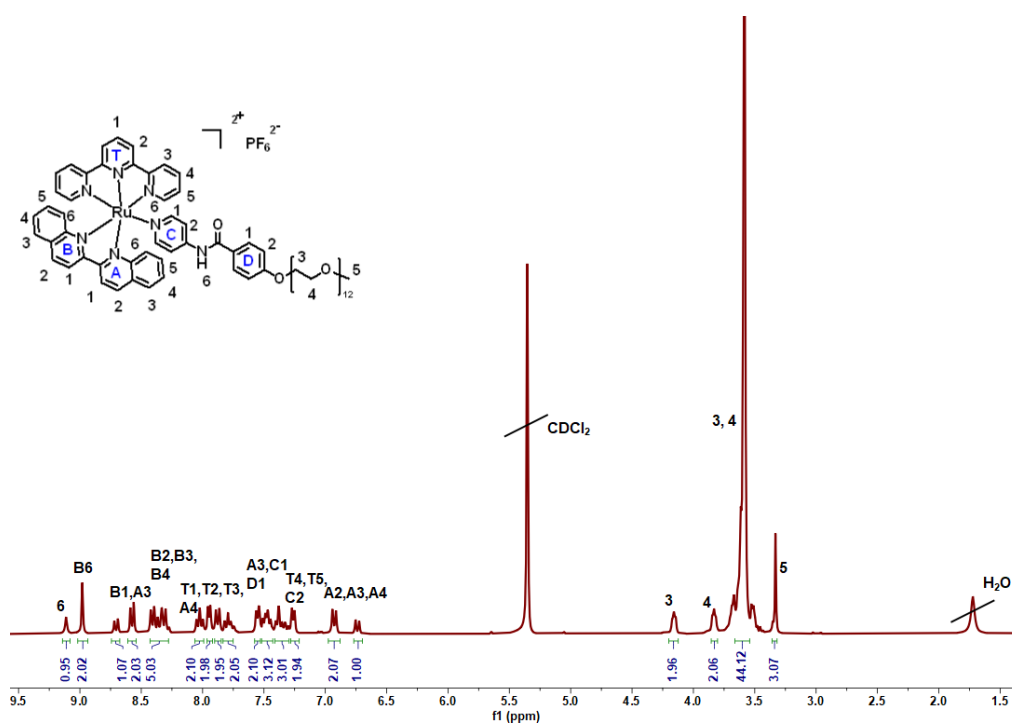


Figure S15.  $^1\text{H}$  NMR of  $[\text{Ru}(\text{tpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).

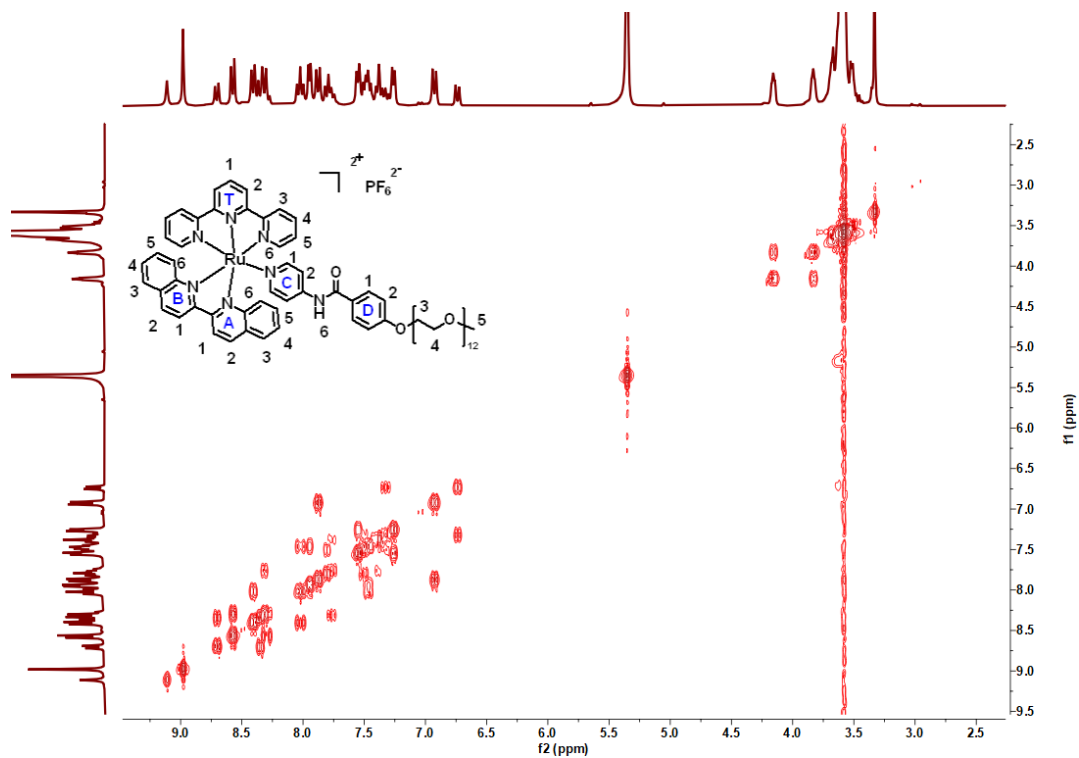
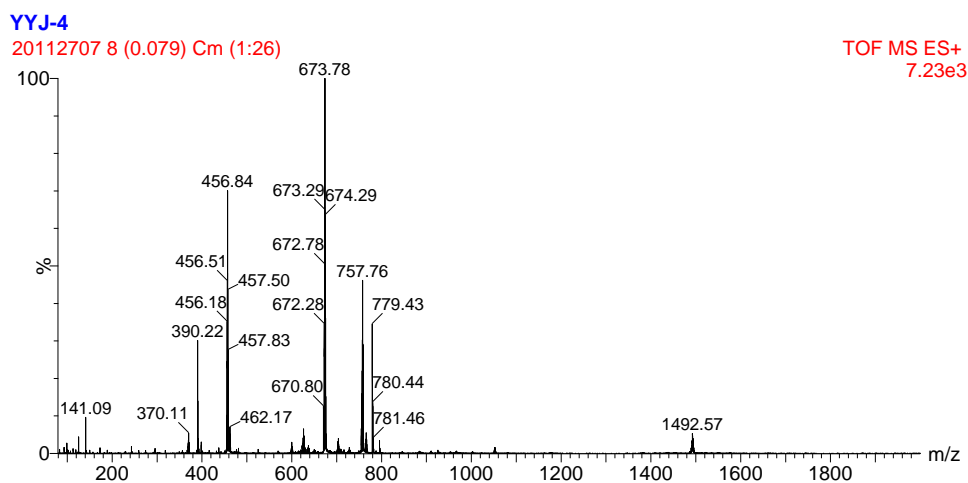
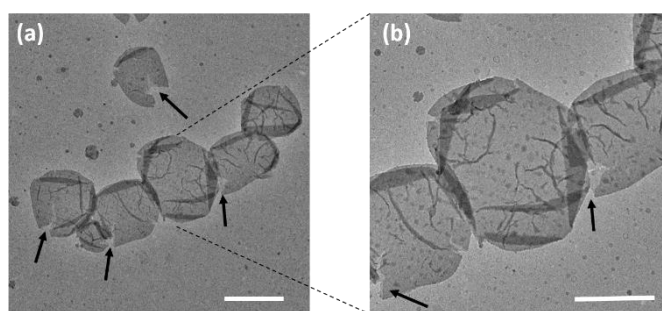


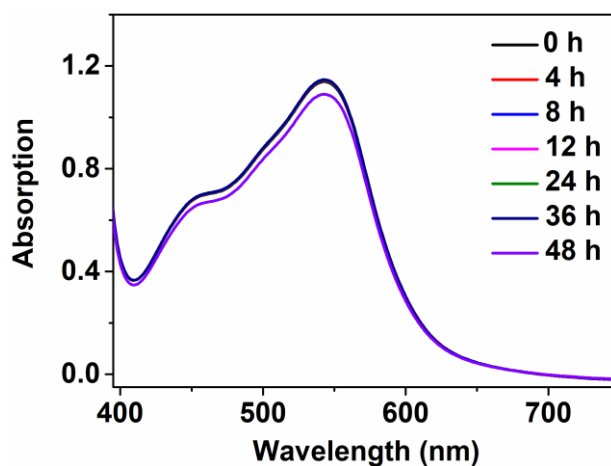
Figure S16. COSY of  $[\text{Ru}(\text{tpy})(\text{biq})(\text{BPEG})](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).



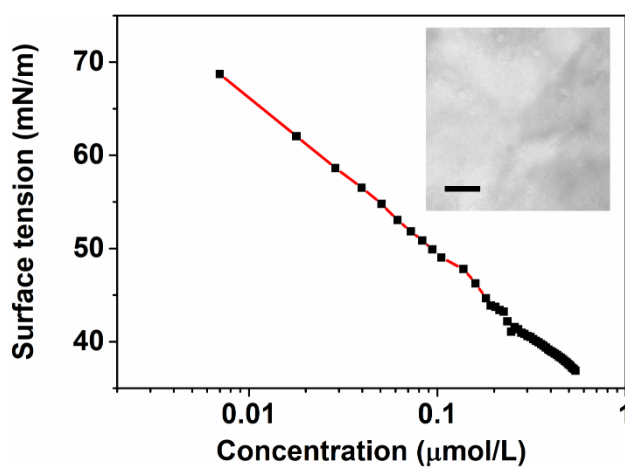
**Figure S17.** ESI-MS of  $[\text{Ru}(\text{tpy})(\text{biq})(\text{PEG})](\text{PF}_6)_2$  ( $M = [\text{Ru}(\text{tpy})(\text{biq})(\text{PEG})](\text{PF}_6)_2$ ,  $L = \text{PEG}$ ).  $m/z = 456.84$  is assigned to  $[\text{M} + \text{Na}_2\text{PF}_6]^{3+}$ ,  $m/z = 673.78$  is assigned to  $[\text{M} - 2\text{PF}_6]^{2+}$ ,  $m/z = 757.76$  is assigned to  $[\text{M} + \text{Na} - \text{PF}_6]^{2+}$ ,  $m/z = 390.22$  is assigned to  $[\text{L} + \text{Na} + \text{H}]^{2+}$ .



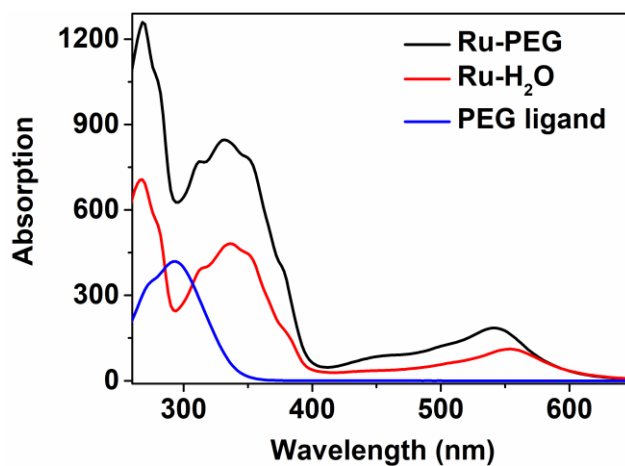
**Figure S18.** (a) TEM image of self-assembled Ru-PEG. Scale bar: 500 nm. (b) TEM image of self-assembled Ru-PEG in the selected area. Scale bar: 200 nm. Some nano-objects with open mouths (indicated as black arrows) illustrated that these self-assembled Ru-PEG are hollow and vesicular.<sup>1</sup>



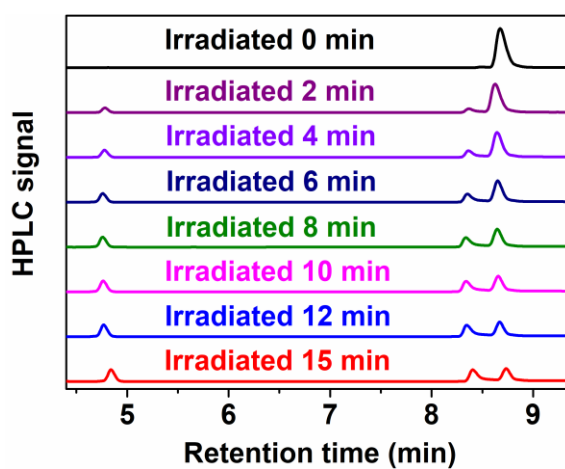
**Figure S19.** Stability of Ru-PEG vesicles over 48 h studied by UV-vis. The UV-vis spectra showed negligible change after 48 h, indicating good stability of Ru-PEG vesicles.



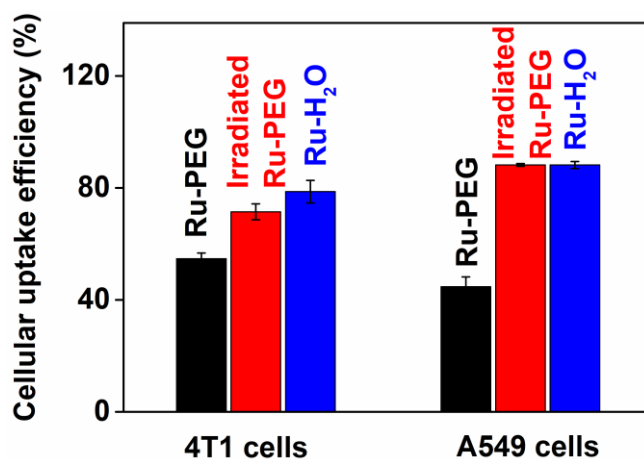
**Figure S20.** Surface tension of Ru-PEG upon adding of water. The inserted figure shows the TEM image of self-assembly of Ru-PEG without pyrene moiety. Comparing with the self-assembled nanostructure of Ru-PEG in Figure 2 in the main manuscript, Ru-PEG showed random aggregates reminiscent of a precipitate. These results clearly showed Ru-PEG were able to self-assemble into vesicles most probably due to the  $\pi$ - $\pi$  stacking and hydrophobic effect of pyrene moiety.



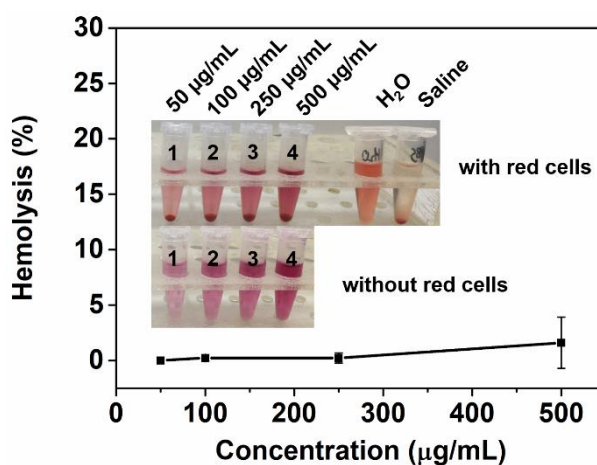
**Figure S21.** UV-vis absorption spectra Ru-PEG and the photoproducts in HPLC measurements in Figure 2 in the main manuscript. The spectra were measured by the UV-vis detector in the HPLC system.



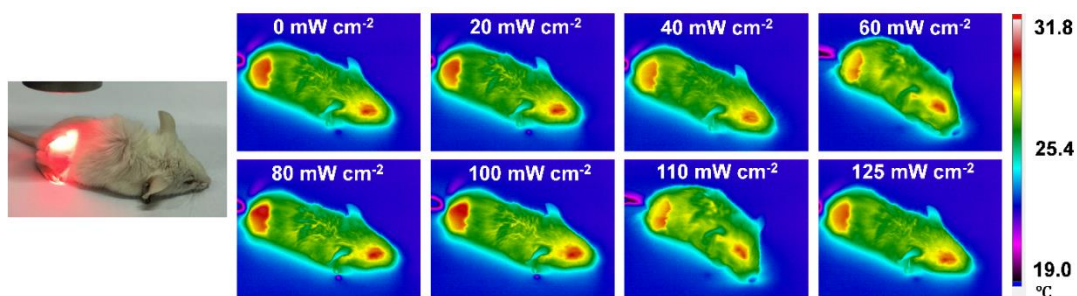
**Figure S22.** The released behavior of Ru-PEG vesicles upon light irradiation studied by HPLC.



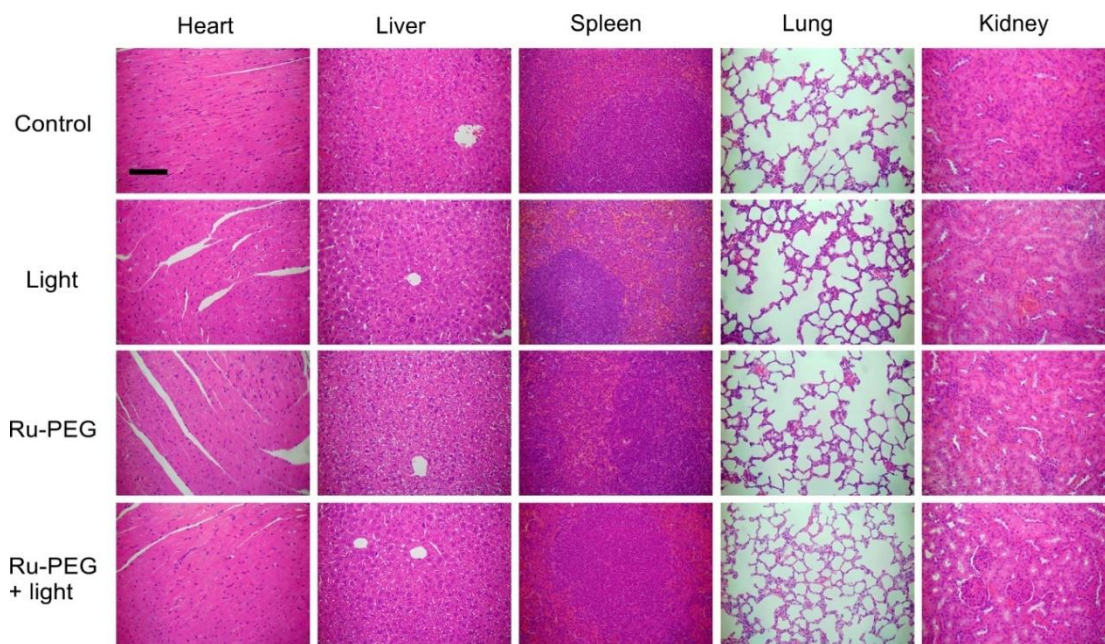
**Figure S23.** Ru-PEG, irradiated Ru-PEG and Ru-H<sub>2</sub>O taken up by 4T1 and A549 cells after 2h incubation determined by flow cytometry.



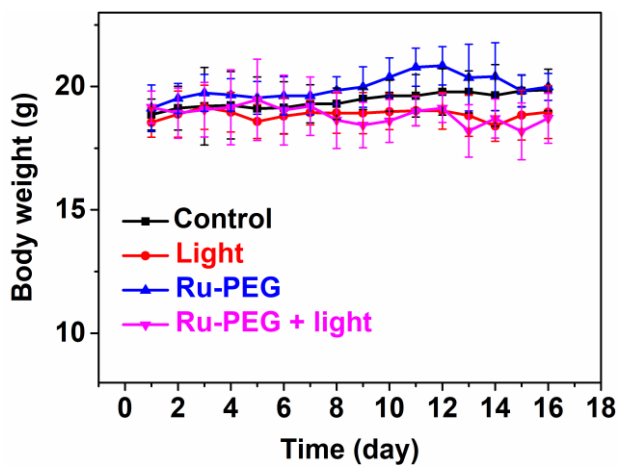
**Figure S24.** Blood hemolysis treated with Ru-PEG vesicles at various concentrations from 50 µg/mL to 500 µg/mL. Water was set as a positive control and saline was set as a negative control. Even after incubation for 24 h, these nanoparticles exhibited non-hemolytic with hemolysis lower than the permissible level of 5%. Ru-PEG vesicles were compatible with red blood cells.



**Figure S25.** Thermal IR imaging of a 4T1 tumor-bearing mouse exposed to 671 nm red light irradiation at various dosages. Light irradiation did not cause overheating problem.



**Figure S26.** Histopathological analysis by H&E staining of tissue sections isolated from 4T1 tumor-bearing mice. Scale bar: 100 μm.



**Figure S27.** Body weight changes of mice during treatments (n = 5).

### Reference

[1] Chen, L.; Xiang, J.; Zhao, Y.; Yan, Q., Reversible self-assembly of supramolecular vesicles and nanofibers driven by chalcogen-bonding interactions. *Journal of the American Chemical Society* **2018**, *140* (23), 7079-7082.



**Chapter 3: Fighting against drug-resistant tumors using a dual-responsive Pt(IV)/Ru(II) bimetallic polymer**

*Xiaolong Zeng, Yufei Wang, Jianxiong Han, Wen Sun, Hans-Jürgen Butt, Xing-Jie Liang\*, and Si Wu\**

X. Zeng, J. Han, Prof. S. Wu

CAS Key Laboratory of Soft Matter Chemistry

Hefei National Laboratory for Physical Sciences at the Microscale

Department of Polymer Science and Engineering

University of Science and Technology of China

Hefei 230026, China

E-mail: siwu@ustc.edu.cn

Y. Wang, Prof. X.-J. Liang

Chinese Academy of Sciences (CAS) Key Laboratory for Biomedical Effects of

Nanomaterials and Nanosafety

CAS Center for Excellence in Nanoscience

National Center for Nanoscience and Technology

Beijing 100190, China

E-mail: liangxj@nanoctr.cn

Y. Wang, Prof. X.-J. Liang

University of Chinese Academy of Sciences

Beijing 100049, China

X. Zeng, J. Han, Prof. H.-J. Butt, Prof. S. Wu

Max Planck Institute for Polymer Research

Ackermannweg 10, 55128 Mainz, Germany

Prof. Dr. W. Sun

State Key Laboratory of Fine Chemicals

Dalian University of Technology

2 Linggong Road, Hi-Tech Zone, Dalian 116024, China

Keywords: metallodrugs, photo-responsive, morphological transformation, tumor penetration, zeta potential

Published in *Advanced Materials*, 2020, 32(43): 2004766.

Reproduced from *Advanced Materials*, 2020, 32(43): 2004766 with permission from the WILEY-VCH Verlag GmbH & Co. KGaA.

### **3.1 Statement of contribution**

Si Wu convinced the idea.

Si Wu, Xing-Jie Liang and Hans-Jürgen Butt led the project.

Xiaolong Zeng prepared the materials and did the synthesis.

Xiaolong Zeng and Yufei Wang did the biological experiments.

Jianxiong Han contributed to TEM measurement.

Wen Sun contributed to ESI-MS measurement.

Xiaolong Zeng and Yufei Wang contributed equally to this work.

### 3.2 Introduction

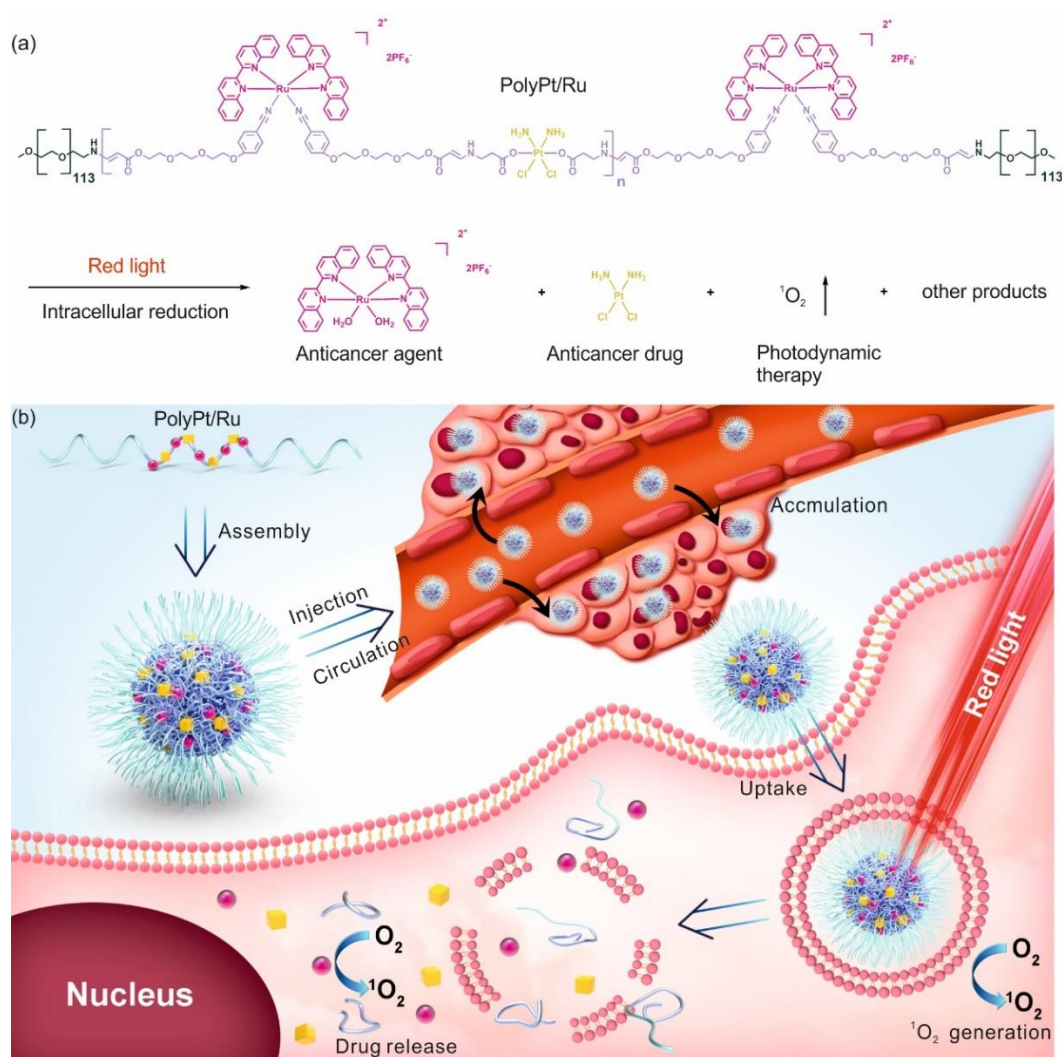
Cisplatin and various other Pt(II) anticancer drugs are administered to the majority of patients undergoing chemotherapy.<sup>[1, 2]</sup> The development of drug resistance during chemotherapy treatment is a major problem; cisplatin becomes inefficient in patients after extended use.<sup>[3, 4]</sup> Cisplatin resistance is due to multiple deactivation pathways that render the treatment inefficient.<sup>[1]</sup> First, proteins such as serum albumin in blood deactivate cisplatin.<sup>[5]</sup> Second, cisplatin cannot be efficiently taken up by cisplatin-resistant cancer cells.<sup>[6]</sup> Third, intracellular biomolecules such as metallothionein (MT) and glutathione (GSH) may strongly bind and sequester cisplatin.<sup>[7]</sup> Fourth, the DNA of cancer cells that are damaged by cisplatin, can be repaired by proteins.<sup>[8]</sup> All these deactivation pathways hinder the curative effects of cisplatin.

Some strategies have been developed to overcome the above-mentioned deactivation pathways. For example, Pt(IV) prodrugs, which release cisplatin in cancer cells, have been developed.<sup>[9-12]</sup> Pt(IV) prodrugs are more resistant to ligand substitution reactions than cisplatin because Pt(IV) centers are saturated and kinetically more inert.<sup>[1]</sup> Thus, Pt(IV) can minimize unwanted side reactions with biomolecules prior to DNA binding. Another strategy to overcome deactivation is to combine cisplatin with other anticancer agents such as paclitaxel, 5-fluorouracil, gemcitabine or ruthenium complexes.<sup>[13-16]</sup> Mixtures of anticancer agents possess multiple targets and actions; this strategy strengthens the therapeutic effects via the different anticancer mechanisms of the different agents.<sup>[14]</sup> A third strategy to overcome deactivation is to use nano-carriers for the delivery of cisplatin.<sup>[17, 18]</sup> Some nano-carriers can circulate longer in the bloodstream, accumulate at tumor tissue via the enhanced permeation and retention (EPR) effect, be taken up by tumor cells efficiently, and release Pt drugs on demand.<sup>[19-22]</sup> These properties of nano-carriers can prevent deactivation pathways. Although the

above-mentioned strategies prevented certain deactivation pathways for cisplatin, it is difficult to overcome multiple deactivation pathways. Thus, reversing cisplatin resistance poses a challenge.

Herein, the design of a dual-responsive Pt(IV)/Ru(II) bimetallic polymer PolyPt/Ru to overcome multiple deactivation pathways for cisplatin is reported. We demonstrated the use of PolyPt/Ru for the treatment of cisplatin-resistant tumors in a patient-derived xenograft (PDX) mouse model (**Figure 1**). PolyPt/Ru is an ABA-type triblock polymer with a hydrophobic Pt(IV)/Ru(II) bimetallic block and two hydrophilic poly(ethylene glycol) (PEG) blocks. The Pt(IV) moieties in the hydrophobic block are prodrugs that produce cisplatin in intracellular reduction environments. The Ru(II) moieties were incorporated in the hydrophobic block because some Ru(II) complexes show anticancer activity and have entered clinical trials.<sup>[23, 24]</sup> In particular, some Ru(II) complexes can generate  $^1\text{O}_2$  and undergo ligand substitution under light irradiation.<sup>[25-30]</sup> Photocaged Ru(II) complexes are usually nontoxic to nonirradiated tissues and can become toxic in cancer cells through photoactivation.<sup>[24, 29]</sup> This photoactivation strategy can improve selectivity in cancer treatment. The Ru(II) moieties in PolyPt/Ru can generate  $^1\text{O}_2$  for photodynamic therapy (PDT) and release anticancer Ru(II) complexes for photoactivated chemotherapy (PACT). Additionally, we used the hydrophilic and biocompatible PEG block because it can prolong blood circulation and suppress nonspecific adsorption of proteins that deactivate metallodrugs.<sup>[31-33]</sup> PolyPt/Ru self-assembles into nanoparticles, which accumulate at tumor sites and are taken up by cisplatin-resistant cancer cells, where it releases cisplatin in the reductive microenvironments. Irradiating PolyPt/Ru with red light generates  $^1\text{O}_2$  and induces degradation of the Ru(II) moieties, and the release of anticancer Ru(II) complexes. The damage caused by the released Ru(II) complexes and  $^1\text{O}_2$  are different from those

caused by cisplatin, which can eliminate the deactivation pathway via repair.<sup>[34]</sup> The combination of the released cisplatin and Ru(II) complexes as well as the generated  $^1\text{O}_2$  has a synergistic effect against cisplatin-resistant tumors. Therefore, PolyPt/Ru can overcome multiple deactivation pathways and reverse cisplatin resistance. Photoactivation of PolyPt/Ru only occurs at the irradiated tumor tissue, which further improves the selectivity of the cancer treatment.



**Figure 1.** (a) Structure of the amphiphilic triblock copolymer PolyPt/Ru. Red light irradiation and intracellular reduction induced degradation of the polymer, generation of  $^1\text{O}_2$ , and release of the anticancer drug cisplatin and the anticancer agent  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  (biq = 2,2'-biquinoline). (b) Schematic illustration of self-

assembly, extracellular and intracellular processes for anticancer therapy using PolyPt/Ru.

### 3.3 Result and discussion

#### 3.3.1 Synthesis of PolyPt/Ru

To prepare PolyPt/Ru, we synthesized the Ru(II)-containing monomer  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (biq = 2,2'-biquinoline, PCE = propiolic acid 2-[2-[2-(4-cyanophenoxy)ethoxy]ethoxy]ethyl ester) (Figure S1) and Pt(IV)-containing monomer precursor  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  (AAE = 3-aminopropionic acid ester, TFA = trifluoroacetic acid) (Figure S2) via multi-step routes. The monomers and intermediates were fully characterized using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) (Figure S4-S15). Subsequently, the Pt(IV)-containing monomer precursor was deprotonated and polymerized with the Ru(II)-containing monomer via spontaneous amino-yne click polymerization (Figure S3, S16). Finally, PolyPt/Ru was synthesized by terminating the polymer with poly(ethylene glycol) methyl ether amine (mPEG5k-NH<sub>2</sub>) (Figure S3). NMR spectroscopy and gel permeation chromatography (GPC) demonstrated that PolyPt/Ru was successfully synthesized (Figure S17 and S18). The molar mass of PolyPt/Ru measured by NMR spectroscopy was  $17 \text{ kg mol}^{-1}$ , which was comparable to that measured by GPC ( $18 \text{ kg mol}^{-1}$ ). The weight fraction of the Pt(IV)/Ru(II) block was ~42%, indicating that PolyPt/Ru has a high content of anticancer Pt(IV) and Ru(II) moieties.

### 3.3.2 Self-assembly of PolyPt/Ru

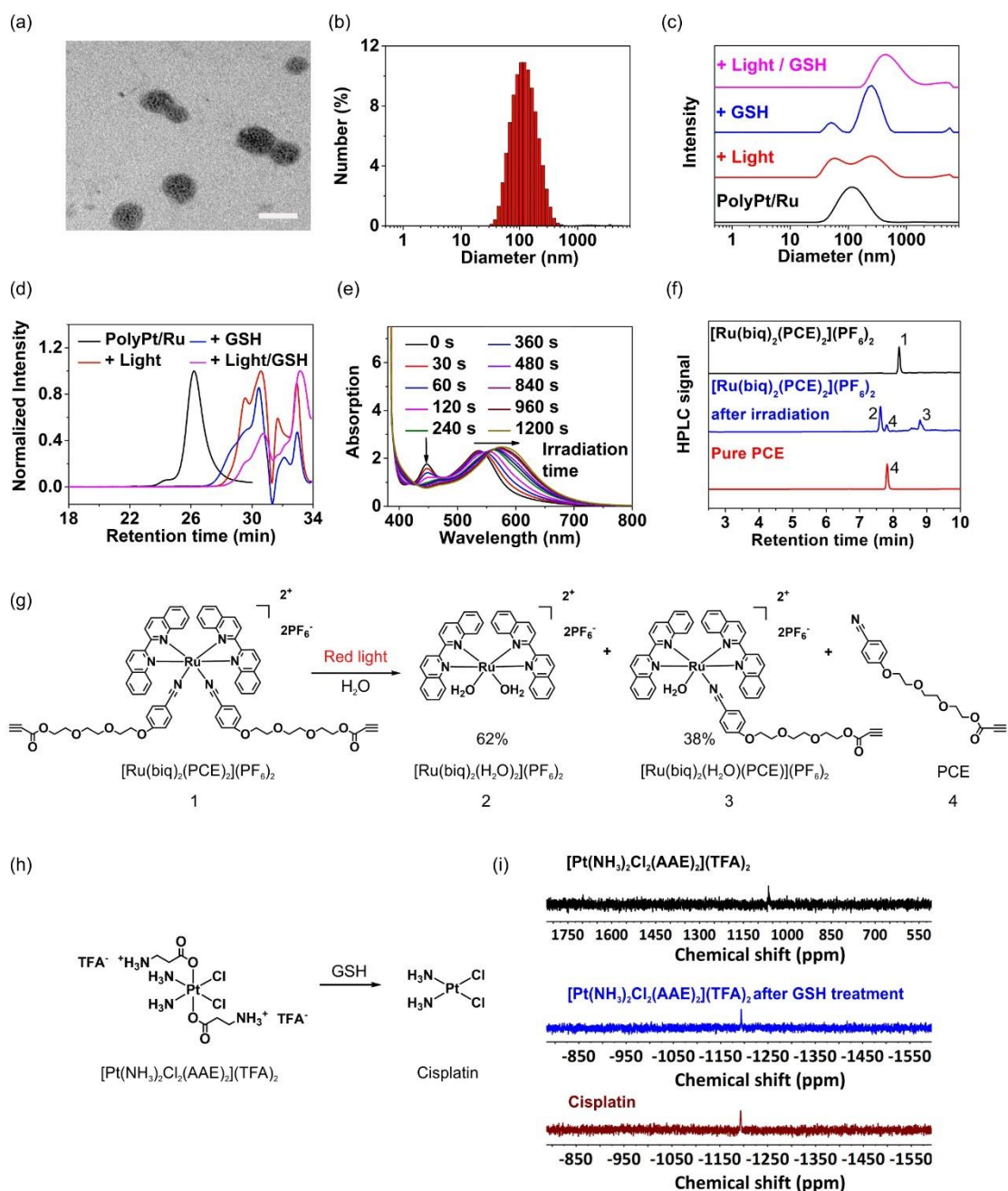
We prepared the PolyPt/Ru nanoparticles via self-assembly. PolyPt/Ru was dissolved in a THF/DMF mixture. Water was added dropwise to the mixture to trigger the self-assembly. Subsequently, PolyPt/Ru nanoparticles in an aqueous solution were obtained by removing the THF and DMF via dialysis against water. Transmission electron microscopy (TEM) showed that PolyPt/Ru self-assembled into nanoparticles with an average diameter of 90 nm (**Figure 2a**). Dynamic light scattering (DLS) results showed that the PolyPt/Ru nanoparticles had an average hydrodynamic diameter of 111 nm (**Figure 2b**), which was comparable to the TEM result. The PolyPt/Ru nanoparticles in aqueous solution were stable for 24 h in the dark (**Figure S19**).

### 3.3.3 Dual-responsiveness of PolyPt/Ru

The Ru(II) and Pt (IV) moieties in PolyPt/Ru are light- and reduction-responsive, making PolyPt/Ru nanoparticles degradable (**Figure 1**). We studied the degradation of PolyPt/Ru nanoparticles induced by red light irradiation, GSH treatment, and the combination of red light and GSH treatment using DLS (**Figure 2c**). The average diameter of the original PolyPt/Ru nanoparticles was 111 nm. After red light irradiation, photoproducts with diameters of 56 nm and 269 nm appeared. This observation suggested that the PolyPt/Ru nanoparticles degraded into smaller-sized fragments, and some larger-sized hydrophobic aggregates of photoproducts. Similar DLS signals were observed when PolyPt/Ru nanoparticles were treated with GSH. When PolyPt/Ru nanoparticles were treated with a combination of light and GSH, the smaller fragments disappeared, and larger aggregates were formed. TEM observations further confirmed that light or GSH treatments resulted in morphological changes (**Figure S20**).

We also performed GPC measurements in a good solvent (DMF), to analyze the degraded products (Figure 2d). The hydrophobic products were easily dissolved in DMF to detect the degraded fragments through GPC. The PolyPt/Ru polymer eluted earlier than the relative degradation products. The delay in the retention time of the products indicated the formation of a series of low-molecular-weight fragments, which confirmed that light and GSH degraded PolyPt/Ru. In addition, UV-vis absorption spectroscopy showed that photosubstitution of the Ru(II) moieties occurred when the PolyPt/Ru nanoparticles were irradiated with red light in the presence or absence of GSH (Figure S21). Irradiating PolyPt/Ru nanoparticles in the presence of GSH resulted in a faster reaction. This observation indicated that the degradation of Pt(IV) moieties using GSH promoted the photoreactivity of the Ru(II) moieties in the PolyPt/Ru nanoparticles (Figure S22).





**Figure 2.** (a) A TEM image of PolyPt/Ru nanoparticles. Scale bar: 100 nm. (b) The diameter of PolyPt/Ru nanoparticles measured by dynamic light scattering (DLS). (c) DLS measurements of PolyPt/Ru nanoparticles. + Light: PolyPt/Ru nanoparticles after light irradiation (671 nm, 125 mW cm<sup>-2</sup>, 20 min); + GSH (glutathione): PolyPt/Ru nanoparticles after GSH treatment (5.0 mM, 4 h); + Light/GSH: PolyPt/Ru nanoparticles after light irradiation (671 nm, 125 mW cm<sup>-2</sup>, 20 min) with GSH treatment (5.0 mM, 4 h). (d) GPC traces of PolyPt/Ru. + Light: PolyPt/Ru after light irradiation (671 nm, 125 mW cm<sup>-2</sup>, 20 min); + GSH: PolyPt/Ru after GSH treatment (5.0 mM, 4 h); + Light/GSH: PolyPt/Ru after light irradiation (671 nm, 125 mW cm<sup>-2</sup>, 20 min) with GSH treatment (5.0 mM, 4 h). (e) UV-vis absorption spectra of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  under light irradiation (671 nm, 125 mW cm<sup>-2</sup>) over time. (f) Photosubstitution of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  studied by high-performance liquid

chromatography (HPLC).  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  was irradiated using 671 nm light ( $125 \text{ mW cm}^{-2}$ , 20 min). (g) Scheme for the photosubstitution of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$ . (h) Scheme for the reduction of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ . (i)  $^{195}\text{Pt}$  NMR spectra of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ ,  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  after GSH treatment (5.0 mM, 4 h), and pure cisplatin.

The TEM, DLS, GPC and UV-vis absorption spectroscopy results demonstrated the qualitative degradation of PolyPt/Ru. PolyPt/Ru degradation is difficult to quantify because the products are complex mixtures. Therefore,  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  and  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  were used as model compounds to investigate the reaction mechanism. UV-vis spectroscopy was used to study the photosubstitution of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (Figure 2e). Red light irradiation of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  red-shifted the metal-to-ligand charge transfer (MLCT) band from 535 nm ( $\lambda_{\text{max}}$  of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$ ) to 586 nm ( $\lambda_{\text{max}}$  of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$ ). The spectral change was achieved within 20 min, suggesting the efficient photosubstitution of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$ . High-performance liquid chromatography (HPLC) was then performed to quantify the photosubstitution of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (Figure 2f).  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  is represented by the signal peak, **1**. After 671 nm light irradiation ( $125 \text{ mW cm}^{-2}$ , 20 min), peak **1** disappeared, and three new peaks, the photoproducts, appeared. Comparing the retention time of the photoproducts with pure PCE ligand confirmed that peak **4** was the PCE. Furthermore, according to the UV-vis absorption spectroscopy detector in the HPLC system, peaks **2** and **3** could be assigned to  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  and  $[\text{Ru}(\text{biq})_2(\text{PCE})(\text{H}_2\text{O})](\text{PF}_6)_2$ , respectively (Figure S23). The contents of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  and  $[\text{Ru}(\text{biq})_2(\text{PCE})(\text{H}_2\text{O})](\text{PF}_6)_2$  were 62% and 38%, which showed that most of the PCE ligands were cleaved from  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  via red light irradiation (Figure 2g).

The reduction-responsiveness mechanism of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  was investigated using  $^{195}\text{Pt}$  NMR spectroscopy (Figure 2h, i). The chemical shift of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  was located at 1067.1 ppm. The chemical shifts of both cisplatin and the reductive product were located at  $-1194.7$  ppm, suggesting that the reductive product was cisplatin. The  $^{195}\text{Pt}$  NMR results showed that  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  produced cisplatin via GSH treatment.

### 3.3.4 In vitro cytotoxicity against drug resistant cancer cells

Cisplatin released from PolyPt/Ru can inhibit cancer cell growth. The half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) values of cisplatin for the sensitive A549 and BEL-7404 cancer cells were as low as 16.10 and 5.68  $\mu\text{g}/\text{mL}$ , respectively (Table 1). These results showed that cisplatin efficiently inhibited the growth of sensitive cancer cells. However, the  $\text{IC}_{50}$  values of cisplatin for the resistant A549-DDP and 7404-CP20 cancer cells were an order of magnitude higher (Table 1), which indicates that cisplatin is ineffective against resistant cancer cells.

Furthermore, we studied the anticancer performance of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ , which can produce cisplatin under intracellular reduction environments. The  $\text{IC}_{50}$  values of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  for the sensitive A549 and BEL-7404 cells, and the resistant A549-DDP and 7404-CP20 cells were 41%, 14%, 58%, and 54% lower than those of cisplatin (Table 1), respectively. This indicates that  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  can inhibit both sensitive and resistant cancer cells more efficiently than cisplatin.

**Table 1.** Half-maximal inhibitory concentration (IC<sub>50</sub>) values of cisplatin, [Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(AAE)<sub>2</sub>](TFA)<sub>2</sub> and [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> (Figure S24).

Cell line	Cisplatin	Pt(NH <sub>3</sub> ) <sub>2</sub> Cl <sub>2</sub> (AAE) <sub>2</sub> (TFA) <sub>2</sub>	[Ru(biq) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ](PF <sub>6</sub> ) <sub>2</sub>		
	IC <sub>50</sub> <sup>a)</sup>	IC <sub>50</sub>	IC <sub>50</sub> , dark	IC <sub>50</sub> , light	PI <sup>c)</sup>
sensitive A549	16.10 (0.92)	9.38 (0.99) <sup>d)</sup>	3.66 (1.02)	2.72 (0.93)	1.34
sensitive BEL-7404	5.68 (0.86)	4.89 (0.93)	2.97 (0.93)	0.68 (0.19)	4.36
cisplatin-resistant A549-DDP <sup>b)</sup>	100.1 (3.46)	41.80 (0.72)	19.70 (0.94)	5.99 (0.97)	3.28
cisplatin-resistant 7404-CP20 <sup>b)</sup>	77.30 (0.96)	35.10 (1.00)	28.70 (1.11)	8.87 (1.09)	3.23

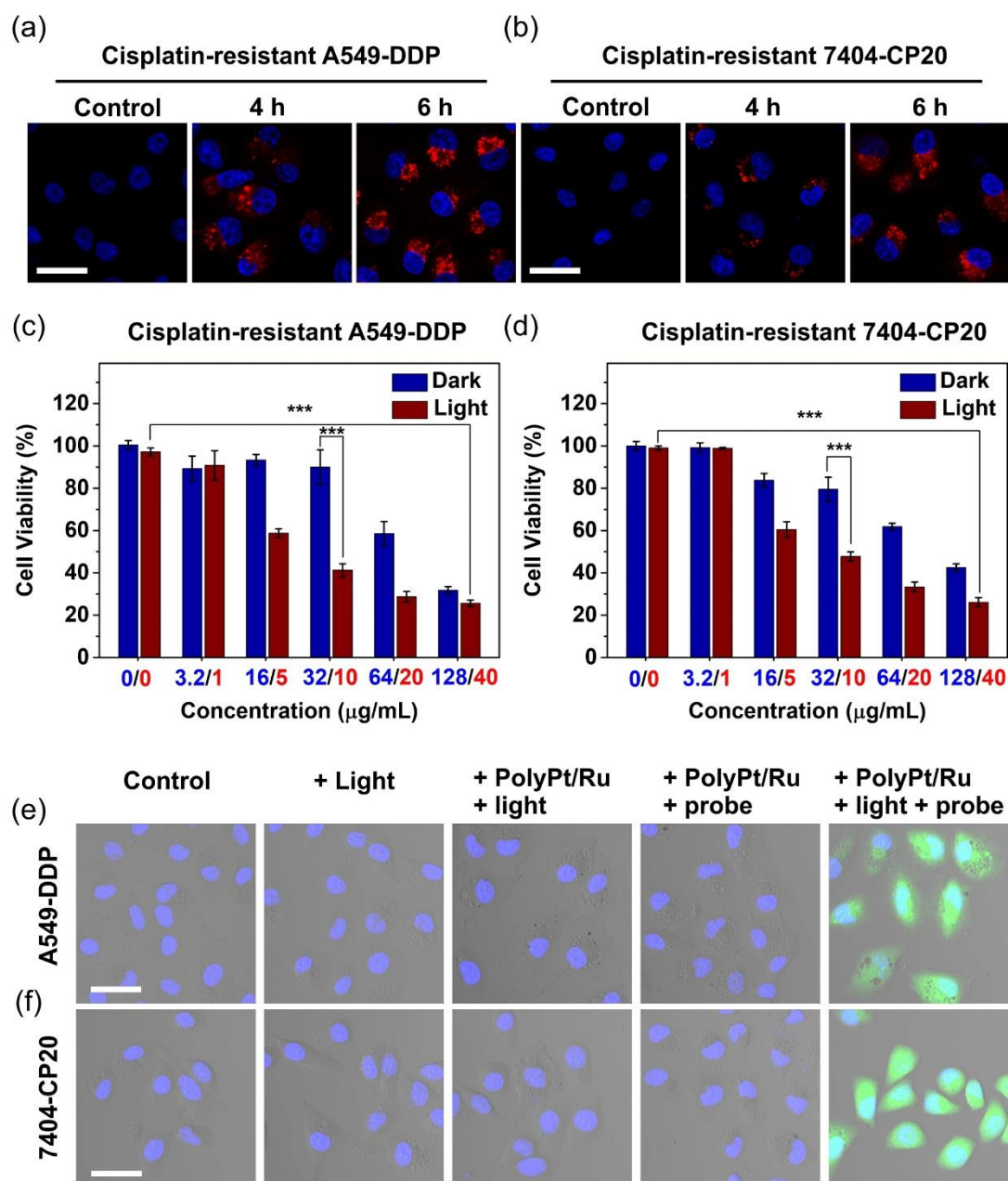
<sup>a)</sup> The IC<sub>50</sub> unit is µg/mL. <sup>b)</sup> Resistance factor (RF) against cisplatin, defined as IC<sub>50</sub> (resistant cell) against cisplatin / IC<sub>50</sub> (sensitive cell) against cisplatin. The RF of A549-DDP and 7404-CP20 cells was 6.21 and 13.60, respectively. <sup>c)</sup> Phototoxicity index (PI), defined as IC<sub>50</sub>, dark / IC<sub>50</sub>, light. <sup>d)</sup> Standard error of each independent experiment.

[Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> released from PolyPt/Ru has anticancer activity against sensitive cancer cells.<sup>[35]</sup> Its anticancer activity is enhanced via photoirradiation because of <sup>1</sup>O<sub>2</sub> generation (Figure S25).<sup>[35]</sup> Here, we compared its activity against sensitive and resistant cells. In the dark, the IC<sub>50</sub> values of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> for the sensitive A549 and BEL-7404 cells were 3.66 and 2.97 µg/mL, respectively (Table 1). Upon red light irradiation, the IC<sub>50</sub> values of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> for the sensitive cells were as low as 2.72 and 0.68 µg/mL; the anticancer efficacy of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> against the sensitive cells was enhanced via photoirradiation because of <sup>1</sup>O<sub>2</sub> generation. Furthermore, the IC<sub>50</sub> values of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> for the resistant A549-DDP and 7404-CP20 cells were 19.70 and 28.70 µg/mL in the dark (Table 1). Upon red light irradiation, the IC<sub>50</sub> values of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> for the resistant cells were as low as 5.99 and 8.87 µg/mL, respectively. The IC<sub>50</sub> values of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> for A549-DDP and 7404-CP20 after photoirradiation were 70% and 69% lower than those

in the dark, respectively. This demonstrated that  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  exhibited enhanced anticancer activity against resistant cancer cells.

### 3.3.5 Cellular uptake and in vitro cytotoxicity assessment

The anticancer efficacy of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  and  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  encouraged us to deliver PolyPt/Ru nanoparticles into cisplatin-resistant cancer cells. We studied the uptake of PolyPt/Ru nanoparticles by cisplatin-resistant cancer cells. Both resistant A549-DDP and 7404-CP20 cells were incubated with PolyPt/Ru nanoparticles for 6 h in the dark. Subsequently, confocal laser scanning microscopy (CLSM) images were taken at 4 h and 6 h. The observation of red fluorescence indicated that PolyPt/Ru nanoparticles were efficiently taken up by both resistant cancer cells (**Figure 3a, b** and **Figure S27, 28**). Flow cytometry was used to quantitatively analyze the uptake efficiency of PolyPt/Ru nanoparticles (**Figure S26**). After incubation for 6 h, the uptake efficiencies for A549-DDP and 7404-CP20 cells were 76.4% and 54.4%, respectively. These results demonstrated that PolyPt/Ru nanoparticles were efficiently taken up by the cisplatin-resistant cells.



**Figure 3.** (a) and (b) Confocal laser scanning microscopy (CLSM) images of PolyPt/Ru nanoparticles (red) after incubation with cisplatin-resistant A549-DDP (a) and 7404-CP20 (b) cancer cells for different time periods. Nuclei were stained with Hoechst 33343 (blue). Scale bars: 25  $\mu\text{m}$ . (c) and (d) Viability of cisplatin-resistant A549-DDP (c) and 7404-CP20 (d) cancer cells treated with equivalent Ru(II) and Pt(IV) concentrations in the nanoparticles in the dark and under light irradiation. The equivalent Ru(II) concentration was shown in blue color and the equivalent Pt(IV) concentration was shown in red color. The cells were irradiated with 671 nm red light ( $125 \text{ mW cm}^{-2}$ , 20 min) after incubation with PolyPt/Ru nanoparticles for 6 h. Cell viability was tested after the cells were further incubated in the dark for 24 h. (e) and (f) Generation of intracellular  $^1\text{O}_2$  in A549-DDP and 7404-CP20 cells as observed by CLSM. The cells were divided into five groups: control group; + light group: cancer cells irradiated with light (671 nm light,  $125 \text{ mW cm}^{-2}$ , 1 min); +PolyPt/Ru + light

group: cancer cells with PolyPt/Ru nanoparticles and light irradiation (671 nm light, 125 mW cm<sup>-2</sup>, 1 min); +PolyPt/Ru + probe group: cancer cells with PolyPt/Ru nanoparticles and the <sup>1</sup>O<sub>2</sub> probe; +PolyPt/Ru + light + probe group: cancer cells with PolyPt/Ru nanoparticles, <sup>1</sup>O<sub>2</sub> probe, and light irradiation (671 nm light, 125 mW cm<sup>-2</sup>, 1 min). Nuclei were stained with DAPI (blue). <sup>1</sup>O<sub>2</sub> was detected using the indicator DCFH-DA (green). Scale bars: 50 μm.

We then investigated the anticancer performance of PolyPt/Ru nanoparticles against cisplatin-resistant cells (Figure 3c, d). In the dark, the cell viabilities decreased as the concentration of the nanoparticles increased. When the equivalent Ru(II) and Pt(IV) concentrations in the nanoparticles were 128 and 40 μg/mL, the cell viabilities decreased to 31% and 42% for the A549-DDP and 7404-CP20 cells, respectively. We infer that the decrease in cell viability occurred because cisplatin was produced via the reduction of the Pt(IV) moieties in PolyPt/Ru. Moreover, light irradiation further decreased the viability of the cancer cells (Figure 3c, d). The enhanced cytotoxicity was attributed to <sup>1</sup>O<sub>2</sub> generated and [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> released (Figure S28). The generation of <sup>1</sup>O<sub>2</sub> in resistant A549-DDP and 7404-CP20 cells was detected using a green fluorescent probe (Figure 3e, f). No green fluorescence was observed in the absence of PolyPt/Ru nanoparticles, the probe, or light irradiation. However, green fluorescence was observed when the cells with PolyPt/Ru nanoparticles and the probe were irradiated with red light. Additionally, the cytotoxicity caused by the release of cisplatin and [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub>, along with the generated <sup>1</sup>O<sub>2</sub> was notable (Figure S29). Their synergistic effects reversed cisplatin resistance (Figure S30, 31).

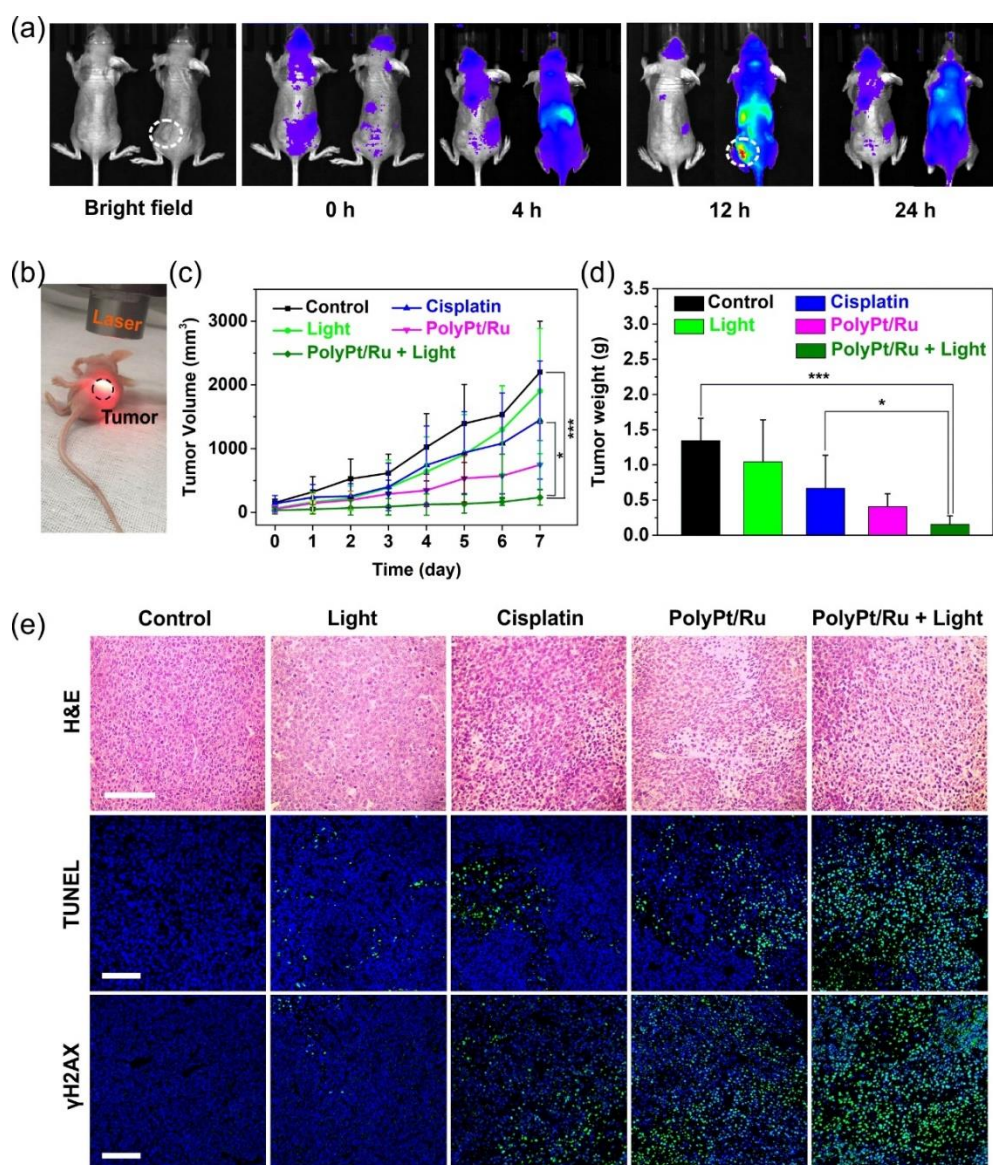
### 3.3.6 In vivo anticancer assessment

Encouraged by the anticancer efficacy of PolyPt/Ru nanoparticles *in vitro*, we studied the anticancer efficacy of PolyPt/Ru nanoparticles *in vivo*. Hemolysis analysis indicated

that PolyPt/Ru nanoparticles have good blood compatibility (Figure S32). We then investigated the anticancer performance of PolyPt/Ru nanoparticles using a hepatic patient-derived cancer xenograft (PDX) mouse model. PDX models are vastly similar to patient tumors because the tumor tissue is taken from patients and grown in physiologically relevant tumor microenvironments.<sup>[36, 37]</sup> PDX models have been applied in molecular and genetic investigations of drug resistance in previous studies.<sup>[38-40]</sup>

To study the accumulation of nanoparticles, a PDX tumor-bearing mouse was intravenously injected with PolyPt/Ru nanoparticles from the tail vein. Fluorescence images of the mouse model (right) and a control mouse (left) were taken over time after injection (**Figure 4a**). No signal and a weak signal were observed at 0 and 4 h in the mouse model, respectively. The fluorescence from PolyPt/Ru nanoparticles at the tumor site reached a maximum at 12 h, which indicated that PolyPt/Ru nanoparticles efficiently accumulated at the tumor site. The fluorescence intensity at 24 h became weaker, suggesting that the nanoparticles were cleared through metabolism. In contrast, the control mouse injected with saline showed no fluorescence, which proved that the fluorescence was produced by the injected nanoparticles. These experiments demonstrated that PolyPt/Ru nanoparticles accumulate at the tumor site, which we deduced was due to the well-known EPR effect.





**Figure 4.** (a) Fluorescence images of PDX tumor-bearing mice after intravenous injection of saline (left, control) and PolyPt/Ru nanoparticles (right). Images were taken after injection for 0, 4, 12 and 24 h. The dashed circle indicates the tumor. (b) A photograph shows red light irradiation on a mouse model. (c) Tumor volumes of PDX tumor-bearing mice during different treatments. (d) Average tumor weights at day 7 after different treatments. (e) H&E, TUNEL, and  $\gamma$ H2AX staining of tumor sections isolated from the mice on day 7. The damaged DNA strands and  $\gamma$ H2AX were labelled by FITC (green). Cell nuclei were stained with DAPI (blue). Scale bars: 100  $\mu$ m.

Twelve hours after PolyPt/Ru nanoparticles were intravenously injected into the mouse model, the tumor was irradiated with a 671 nm laser for 20 min (PolyPt/Ru + light group). Four additional experiments were conducted for comparison: 1) PDX

tumor-bearing mice were injected with saline (Control Group); 2) PDX tumor-bearing mice were irradiated with light (Light Group); 3) PDX tumor-bearing mice were injected with an equivalent dosage of cisplatin (Cisplatin Group), and 4) PDX tumor-bearing mice were injected with PolyPt/Ru nanoparticles (PolyPt/Ru Group). We compared the anticancer efficacy by monitoring the tumor volumes of each group over 7 days (Figure 4c). The tumor volume in the control group increased by ~30 times. The result of the light group was similar to that of the control group, indicating that mild light irradiation does not have an inhibitory effect (Figure S33, 34). Cisplatin treatment caused a tumor growth of ~13 times, since PDX tumors are resistant to cisplatin. For the PolyPt/Ru group, the increase in the tumor volume was smaller (~8 times) than those in the previous groups. This result indicated that the Pt(IV) moieties in PolyPt/Ru contributed to tumor inhibition. Compared to the four groups mentioned above, tumor growth in the PolyPt/Ru + light group was notably inhibited. This result suggests that the synergistic effects of this treatment improved the anticancer performance.

Subsequently, the mice were euthanized, and the tumors were isolated for analysis (Figure S36). The average tumor weight in the PolyPt/Ru + light group was much lighter than that in the other four groups (Figure 4d). Immunohistochemical analyses were also performed to illustrate the mechanism of the anticancer activity using PolyPt/Ru nanoparticles (Figure 4e). A hematoxylin and eosin (H&E) staining assay showed large areas of apoptosis and necrosis, which suggested that the PolyPt/Ru + light group treatment exhibited considerable tumor inhibition. Similar results were observed in TUNEL staining images. Furthermore, the  $\gamma$ H2AX staining assay provided more insight into the anticancer mechanism since  $\gamma$ H2AX is a sensitive marker for DNA damage (Figure S35). Comparing  $\gamma$ H2AX formation at tumor sites, we found that enhanced genomic DNA damage appeared in the PolyPt/Ru + light group. We

hypothesized that the released anticancer agents induced DNA damage, which increased genomic instability and apoptosis. Once DNA damage occurs,  $\gamma$ H2AX forms clusters near the impaired DNA strands.

We also investigated the systemic toxicity during the treatments by comparing the H&E staining images of the main organs (heart, liver, lung, spleen, and kidney) (Figure S38). Negligible pathological alteration of the organs was observed, indicating the low systemic toxicity of PolyPt/Ru. The body weights of the mice did not change significantly during the treatments (Figure S39), which suggests that the treatments had minimal side effects. The combined results demonstrate that the use of PolyPt/Ru can treat PDX tumors with improved efficacy and minimized systemic toxicity.

### **3.4 Conclusions**

In conclusion, we synthesized a dual-responsive, bimetallic polymer, PolyPt/Ru, to overcome multiple deactivation pathways for cisplatin. PolyPt/Ru showed excellent performance against cisplatin-resistant tumors due to the design of the polymer structure, and the synergistic effects of the bimetallic moieties. Cisplatin resistance in a PDX model was reversed using PolyPt/Ru. Our study revealed that the design of multi-metallic polymers with multi-responsiveness is a new strategy to treat drug-resistant cancers. Since many bioactive metal complexes exist, the design principles reported here provide a foundation for the design of multi-metallic polymers for biomedical applications. We anticipate that more multi-metallic polymers with adjustable functions can be developed for personalized nanomedicine and enhanced clinical effectiveness.

### 3.5 References

- [1] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, *116*, 3436.
- [2] W. Zhang, J. Shen, H. Su, G. Mu, J.-H. Sun, C.-P. Tan, X.-J. Liang, L.-N. Ji, Z.-W. Mao, *ACS Appl. Mater. Interfaces* **2016**, *8*, 13332.
- [3] X.-J. Liang, H. Meng, Y. Wang, H. He, J. Meng, J. Lu, P. C. Wang, Y. Zhao, X. Gao, B. Sun, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 7449.
- [4] M. D. Hall, M. Okabe, D.-W. Shen, X.-J. Liang, M. M. Gottesman, *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 495.
- [5] G. Ferraro, L. Massai, L. Messori, A. Merlino, *Chem. Commun.* **2015**, *51*, 9436.
- [6] S. Li, C. Li, S. Jin, J. Liu, X. Xue, A. S. Eltahan, J. Sun, J. Tan, J. Dong, X.-J. Liang, *Biomaterials* **2017**, *144*, 119.
- [7] Y. Min, C. Q. Mao, S. Chen, G. Ma, J. Wang, Y. Liu, *Angew. Chem., Int. Ed.* **2012**, *124*, 6846.
- [8] J. Hu, J. D. Lieb, A. Sancar, S. Adar, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 11507.
- [9] Z. Wang, Z. Xu, G. Zhu, *Angew. Chem., Int. Ed.* **2016**, *55*, 15564.
- [10] W. H. Ang, I. Khalaila, C. S. Allardyce, L. Juillerat-Jeanneret, P. J. Dyson, *J. Am. Chem. Soc.* **2005**, *127*, 1382.
- [11] X. Ling, X. Chen, I. A. Riddell, W. Tao, J. Wang, G. Hollett, S. J. Lippard, O. C. Farokhzad, J. Shi, J. Wu, *Nano Lett.* **2018**, *18*, 4618.
- [12] M. Stilgenbauer, A. M. Jayawardhana, P. Datta, Z. Yue, M. Gray, F. Nielsen, D. J. Bowers, H. Xiao, Y.-R. Zheng, *Chem. Commun.* **2019**, *55*, 6106.
- [13] Y. H. Kim, S. W. Shin, B. S. Kim, J. H. Kim, J. G. Kim, Y. J. Mok, C. S. Kim, H. S. Rhyu, J. H. Hyun, J. S. Kim, *Cancer* **1999**, *85*, 295.
- [14] J. Karges, T. Yempala, M. Tharaud, D. Gibson, G. Gasser, *Angew. Chem., Int. Ed.* **2020**, *59*, 7069.
- [15] M. Michalak, M. S. Lach, M. Antoszczak, A. Huczyński, W. M. Suchorska, *Molecules* **2020**, *25*, 537.
- [16] Y. Zheng, D. Y. Zhang, H. Zhang, J. J. Cao, C. P. Tan, L. N. Ji, Z. W. Mao, *Chem. Eur. J.* **2018**, *24*, 18971.
- [17] R. K. Pathak, S. Dhar, *J. Am. Chem. Soc.* **2015**, *137*, 8324.
- [18] Z.-T. Cao, Z.-Y. Chen, C.-Y. Sun, H.-J. Li, H.-X. Wang, Q.-Q. Cheng, Z.-Q. Zuo, J.-L. Wang, Y.-Z. Liu, Y.-C. Wang, *Biomaterials* **2016**, *94*, 9.

- [19] R. Xing, Q. Zou, C. Yuan, L. Zhao, R. Chang, X. Yan, *Adv. Mater.* **2019**, *31*, 1900822.
- [20] J. Li, D. Cui, Y. Jiang, J. Huang, P. Cheng, K. Pu, *Adv. Mater.* **2019**, *31*, 1905091.
- [21] M. Abbas, Q. Zou, S. Li, X. Yan, *Adv. Mater.* **2017**, *29*, 1605021.
- [22] X. Xu, P. E. Saw, W. Tao, Y. Li, X. Ji, S. Bhasin, Y. Liu, D. Ayyash, J. Rasmussen, M. Huo, *Adv. Mater.* **2017**, *29*, 1700141.
- [23] L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao, Z.-S. Chen, *Chem. Soc. Rev.* **2017**, *46*, 5771.
- [24] C. Mari, V. Pierroz, S. Ferrari, G. Gasser, *Chem. Sci.* **2015**, *6*, 2660.
- [25] L. N. Lameijer, T. G. Brevé, V. H. van Rixel, S. H. Askes, M. Siegler, S. Bonnet, *Chem. Eur. J.* **2018**, *24*, 2709.
- [26] W. Sun, R. Thiramanas, L. D. Slep, X. Zeng, V. Mailänder, S. Wu, *Chem. Eur. J.* **2017**, *23*, 10832.
- [27] G. Carrone, L. Zayat, L. D. Slep, R. Etchenique, *Phys. Chem. Chem. Phys.* **2017**, *19*, 2140.
- [28] L. Zayat, C. Calero, P. Alborés, L. Baraldo, R. Etchenique, *J. Am. Chem. Soc.* **2003**, *125*, 882.
- [29] L. N. Lameijer, D. Ernst, S. L. Hopkins, M. S. Meijer, S. H. Askes, S. E. Le Dévédec, S. Bonnet, *Angew. Chem., Int. Ed.* **2017**, *129*, 11707.
- [30] M. H. Al-Afyouni, T. N. Rohrabough, K. F. Al-Afyouni, C. Turro, *Chem. Sci.* **2018**, *9*, 6711.
- [31] P. del Pino, F. Yang, B. Pelaz, Q. Zhang, K. Kantner, R. Hartmann, N. Martinez de Baroja, M. Gallego, M. Möller, B. B. Manshian, *Angew. Chem., Int. Ed.* **2016**, *128*, 5573.
- [32] A. Salvati, A. S. Pitek, M. P. Monopoli, K. Prapainop, F. B. Bombelli, D. R. Hristov, P. M. Kelly, C. Åberg, E. Mahon, K. A. Dawson, *Nat. Nanotechnol.* **2013**, *8*, 137.
- [33] J. Chen, J. Ding, Y. Wang, J. Cheng, S. Ji, X. Zhuang, X. Chen, *Adv. Mater.* **2017**, *29*, 1701170.
- [34] B. S. Howerton, D. K. Heidary, E. C. Glazer, *J. Am. Chem. Soc.* **2012**, *134*, 8324.
- [35] W. Sun, S. Li, B. Häupler, J. Liu, S. Jin, W. Steffen, U. S. Schubert, H. J. Butt, X. J. Liang, S. Wu, *Adv. Mater.* **2017**, *29*, 1603702.
- [36] Y. Cong, H. Xiao, H. Xiong, Z. Wang, J. Ding, C. Li, X. Chen, X. J. Liang, D. Zhou, Y. Huang, *Adv. Mater.* **2018**, *30*, 1706220.

- [37] L. Rao, G. T. Yu, Q. F. Meng, L. L. Bu, R. Tian, L. S. Lin, H. Deng, W. Yang, M. Zan, J. Ding, *Adv. Funct. Mater.* **2019**, *29*, 1905671.
- [38] K.-T. Kim, H. W. Lee, H.-O. Lee, S. C. Kim, Y. J. Seo, W. Chung, H. H. Eum, D.-H. Nam, J. Kim, K. M. Joo, *Genome Biol.* **2015**, *16*, 127.
- [39] C. A. Stewart, C. M. Gay, Y. Xi, S. Sivajothi, V. Sivakamasundari, J. Fujimoto, M. Bolisetty, P. M. Hartsfield, V. Balasubramaniyan, M. D. Chalisehar, *Nat. Cancer* **2020**, *1*, 423.
- [40] O. Poirion, X. Zhu, T. Ching, L. X. Garmire, *Nat. Commun.* **2018**, *9*, 4892.

### 3.6 Supporting Information

#### Materials

RuCl<sub>3</sub>·xH<sub>2</sub>O (99.9%), 2,2'-biquinoline (98%), cis-diammineplatinum(II) dichloride (95%), 4-hydroxybenzotrile (98%), 2-[2-(2-chloroethoxy)ethoxy]ethanol (96%), propiolic acid (95%), potassium tetrachloroplatinate(II) (98%), sodium hexachloroplatinate(IV) hexahydrate (98%), 1,3-diphenyliso benzofuran (DPBF) (97%) were purchased from Sigma-Aldrich. Boc-β-alanine (99%), N,N'-dicyclohexylcarbodiimide (DCC) (99%) , potassium hexafluorophosphate (98%), silver hexafluorophosphate (98%) were purchased from Alfa Aesar. Poly(ethylene glycol) methyl ether amine (mPEG5k-NH<sub>2</sub>) was purchased from Creative PEGWorks. 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD fluorescent dye) was purchased from Thermo Fisher Scientific. PBS buffer with a pH of 7.4 was purchased from Life Technologies. All other solvents (HPLC grade) were purchased from Sigma-Aldrich or Fisher Scientific. Milli-Q water with a resistivity of 18.2 MΩ·cm was used in this study. Dialysis tubing (3.5K MWCO) was purchased from SERVA Electrophoresis GmbH, Germany.

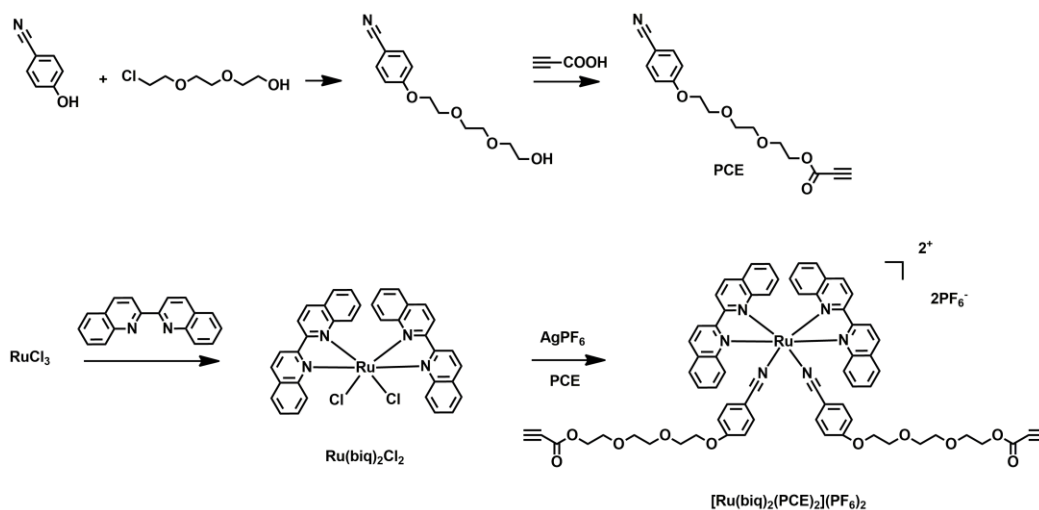
#### Instruments and Characterization

<sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H NMR), <sup>13</sup>C nuclear magnetic resonance (<sup>13</sup>C NMR) and <sup>1</sup>H -<sup>1</sup>H correlation spectroscopy (COSY) were recorded on a 250 or 300 MHz Bruker Spectrospin NMR spectrometer at 25 °C. <sup>195</sup>Pt nuclear magnetic resonance (<sup>195</sup>Pt NMR) spectra were recorded on a 500 MHz Bruker Spectrospin NMR spectrometer at 25 °C. The chemical shift (δ) was externally referenced using K<sub>2</sub>PtCl<sub>4</sub> for Pt(II) complexes and Na<sub>2</sub>PtCl<sub>6</sub> for Pt(IV) complexes. The molecular weights and molecular

weight distributions of the polymers were determined using a PSS-WinGPC (pump: SECcurity) equipped with UV and RI detectors running in dimethylformamide (DMF) with 1g/L LiBr at 50 °C calibrated against polystyrene standards. HPLC analysis was performed on an Agilent HPLC system equipped with a 1100 Series Quaternary pump, a 1200 Series diode detector, and a Merck Chromolith Performance RP18e 100-3 mm HPLC column. UV-vis detector in the HPLC system was set at 260 nm for analysis. UV-vis absorption spectra were measured on a Lambda 900 spectrometer (Perkin Elmer). Fluorescence spectra were recorded on a TIDAS II spectrometer (J&M). Transmission electron microscopy (TEM) images were taken on a Hitachi HT7700 Transmission Electron Microscope. The diameters of the nanoparticles were determined by dynamic light scattering (DLS) on a Nano ZS90 particle size analyzer, Malvern (UK). A DPSS laser with a wavelength of  $\lambda = 671$  nm (CNI-671-200-LN-AC-3, Laser 2000 GmbH, Germany) was used to induce photoreactions of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  and PolyPt/Ru nanoparticles. The laser was equipped with a thermoelectric cooling system. The output power of the laser was controlled by a tabletop laser driver (PSU-III-FDA, Changchun New Industries Optoelectronics Technology Co., Ltd., China) and measured using an optical power meter (model 407A, Spectra-Physics Corporation). A laser at 671 nm was employed as the light source for *in vitro* and *in vivo* experiments. The output power of the laser was controlled by a fiber coupled laser system (FC-671-1W, Changchun New Industries Optoelectronics Technology) and measured by a power meter (LP100/TS15, Changchun New Industries Optoelectronics Technology).



## Synthesis



**Figure S1.** Synthetic route of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$ .

### Synthesis of [2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]benzonitrile (Figure S1)

A DMF (20 mL) solution containing 4-hydroxybenzonitrile (1.19 g, 10.00 mmol),  $\text{K}_2\text{CO}_3$  (1.38 g, 10.00 mmol) and KI (100 mg, 0.60 mmol) was heating to 110 °C for 30 min. Subsequently, 2-[2-(2-chloroethoxy)ethoxy]ethanol (1.85 g, 11.00 mmol) in 10 mL DMF was added dropwise to the aforementioned mixture and stirred at 110 °C for 24 h. After cooling to room temperature, the solvent was evaporated. The crude product was purified through column chromatography using ethyl acetate/petroleum ether (from 1/1 to 2/1) as the eluent. [2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]benzonitrile was obtained as a colorless oil (2.12 g, 84.4%).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.66 – 7.57 (m, 2H), 7.05 – 6.98 (m, 2H), 4.19 (dd,  $J = 5.6, 3.6$  Hz, 2H), 3.89 – 3.84 (m, 2H), 3.73 – 3.65 (m, 6H), 3.58 (dd,  $J = 5.5, 3.5$  Hz, 2H).

**Synthesis of propiolic acid 2-[2-[2-(4-cyanophenoxy)ethoxy]ethoxy]ethyl ester****(PCE, Figure S1)**

Propiolic acid (700 mg, 10.00 mmol), [2-[2-(2-hydroxyethoxy)ethoxy]ethoxy] benzonitrile (2.51 g, 10.00 mmol) and 4-dimethylaminopyridine (122 mg, 1.00 mmol) were dissolved in 20 mL dry dichloromethane (DCM) under argon atmosphere. Dicyclohexylcarbodiimide (DCC) (2.06 g, 10.00 mmol) in 10 mL DCM was added dropwise in an ice bath. After stirring at room temperature for 24 h, the solution was filtrated and evaporated. The crude product was purified through column chromatography using ethyl acetate/petroleum ether (from 1/2 to 1/1) as the eluent. PCE was obtained as a light yellow oil (2.64 g, 87.1%).  $^1\text{H NMR}$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ )  $\delta$  7.70 – 7.59 (m, 2H), 7.06 – 6.98 (m, 2H), 4.38 – 4.31 (m, 2H), 4.20 (dd,  $J = 5.7, 3.5$  Hz, 2H), 3.88 (dd,  $J = 5.7, 3.6$  Hz, 2H), 3.71 (pd,  $J = 4.9, 2.3$  Hz, 6H), 3.02 (s, 1H).

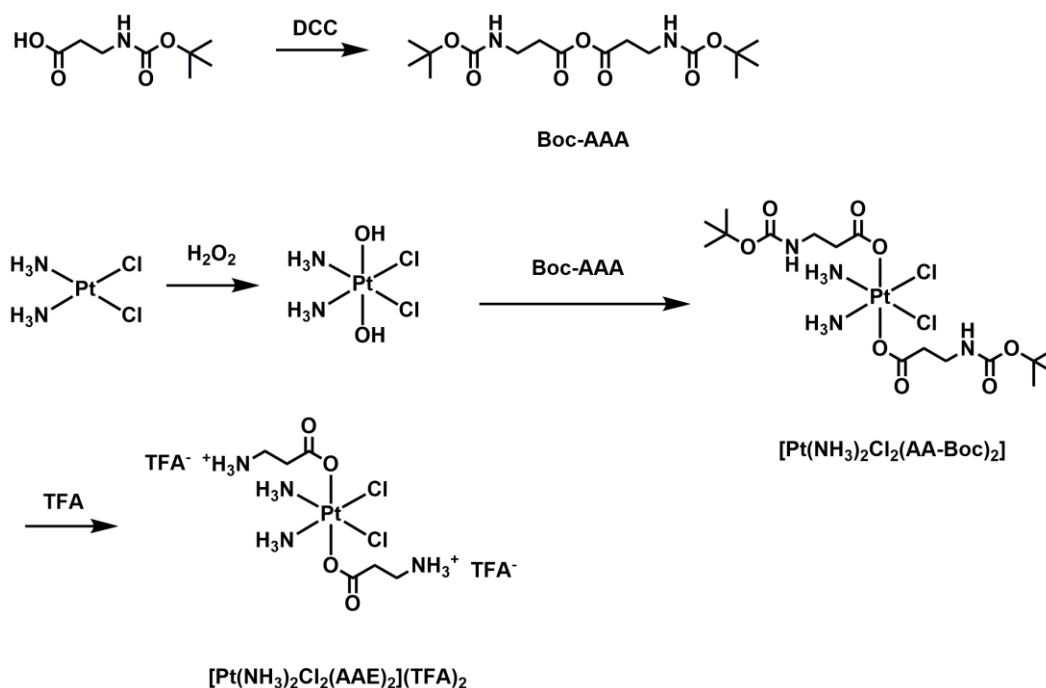
**Synthesis of  $\text{Ru}(\text{biq})_2\text{Cl}_2$  (Figure S1)**

$\text{Ru}(\text{biq})_2\text{Cl}_2$  was synthesized according to the literature.<sup>[1]</sup>

**Synthesis of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (Figure S1)**

$\text{Ru}(\text{biq})_2\text{Cl}_2$  (200 mg, 0.28 mmol) and  $\text{AgPF}_6$  (92 mg, 0.58 mmol) were dissolved in 1:1 ethanol/ $\text{H}_2\text{O}$  mixture (10 mL). The mixture was degassed and refluxed at 80 °C overnight in an argon (Ar) atmosphere. After cooling to room temperature,  $\text{AgCl}$  was removed and the solution was reduced to 5 mL. Subsequently, saturated  $\text{KPF}_6$  solution was added. The precipitate was filtered, followed by washing with a small amount of  $\text{H}_2\text{O}$ , and dried to obtained  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$ . Afterwards,

[Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> (206 mg, 0.22 mmol) and PCE (139 mg, 0.46 mmol) were dissolved in 15 mL acetone. The solution was degassed and refluxed at 55 °C in the dark for 12 h. After cooling to room temperature, the solvent was evaporated and the crude product was purified through column chromatography using methanol/DCM (from 1/10 to 1/8) as the eluent. [Ru(biq)<sub>2</sub>(PCE)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> was obtained as a red solid (154 mg, 46.3%). <sup>1</sup>H NMR (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 9.51 (d, J = 8.8 Hz, 2H), 8.68 (d, J = 8.7 Hz, 2H), 8.34 (t, J = 9.1 Hz, 6H), 8.06 (d, J = 7.6 Hz, 2H), 7.93 (d, J = 8.6 Hz, 4H), 7.83 (d, J = 8.7 Hz, 2H), 7.56 (t, J = 7.4 Hz, 2H), 7.46 (d, J = 8.6 Hz, 4H), 6.96 (d, J = 8.7 Hz, 6H), 6.83 (d, J = 8.7 Hz, 2H), 4.33 – 4.24 (m, 4H), 4.16 (t, J = 4.6 Hz, 4H), 3.82 (t, J = 4.5 Hz, 4H), 3.71 – 3.60 (m, 12H), 3.00 (s, 2H). <sup>13</sup>C NMR (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>) δ 163.77, 160.34, 160.11, 152.43, 150.66, 149.60, 132.76, 129.46, 128.94, 101.06, 74.96, 74.34, 70.64, 70.54, 69.14, 68.45, 68.18, 65.64, 65.22. ESI-MS: [M-2PF<sub>6</sub>]<sup>2+</sup>: calcd. 610.1629; obsd. 610.1635. Δ = 0.9 ppm.



**Figure S2.** Synthetic route of [Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(AAE)<sub>2</sub>](TFA)<sub>2</sub>.

**Synthesis of N-Boc aminopropanoic acid anhydride (Boc-AAA, Figure S2)**

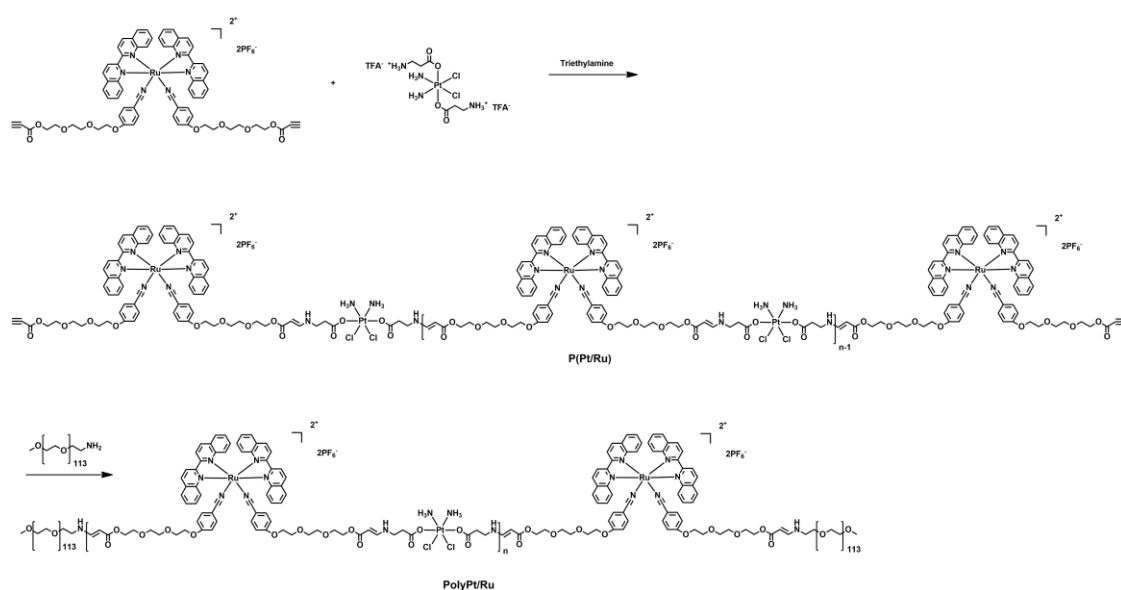
Boc- $\beta$ -alanine (600 mg, 3.20 mmol) was dissolved in dry 5 mL DCM. The solution was cooling down to 0 °C in an ice bath. DCC (320 mg, 1.58 mmol) in 5 mL DCM was added dropwise into the above solution. After stirring at room temperature for 24 h, the solution was filtrated and evaporated. The crude product was taken up by small amount of toluene and stored in refrigerator for 4 h. The precipitation was removed and the solvent was evaporated. Boc-AA was obtained as a colorless oil without any further purification (1.02 g, 89.7%).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.09 (s, 2H), 3.45 (q, J = 6.0 Hz, 4H), 2.70 (t, J = 5.9 Hz, 4H), 1.45 (s, 18H).

**Synthesis of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AA-Boc})_2]$  (Figure S2)**

To obtain  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AA-Boc})_2]$ , first cisplatin (169 mg, 0.56 mmol) was oxidized with  $\text{H}_2\text{O}_2$  to afford  $[\text{PtCl}_2(\text{NH}_3)_2(\text{OH})_2]$ , then followed by mixing with Boc-AAA (1.02 g, 2.82 mmol) in DMF. The mixture was kept at 50 °C overnight. Subsequently, DMF was removed under high vacuum, a small amount of methanol was added into the resulting yellow oil. The solution was added dropwise into diethyl ether and the forming precipitate was collected by centrifugation.  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AA-Boc})_2]$  was obtained as white solid after washing with water and diethyl ether (178 mg, 43.9%).  $^1\text{H NMR}$  (300 MHz, MeOD)  $\delta$  3.29 (dd, J = 6.4, 4.4 Hz, 4H), 2.51 (dd, J = 8.6, 4.3 Hz, 4H), 1.45 (s, 18H).

### Synthesis of $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ (Figure S2)

$[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AA-Boc})_2]$  (100 mg, 0.27 mmol) was dissolved in 2 mL DCM/TFA (1:1, v/v) mixture. The solution was stirred at room temperature for 30 min. Afterwards, the solvent was removed under a stream of Ar atmosphere. A small amount of DCM was added to re-dissolve the crude product. The formed solution was added dropwise in a cold diethyl ether. The resulting precipitate was collected by centrifugation.  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  was obtained as white solid (95.6 mg, 74.9%).  $^1\text{H}$  NMR (250 MHz,  $\text{D}_2\text{O}$ )  $\delta$  3.10 (t,  $J = 6.4$  Hz, 4H), 2.72 (t,  $J = 6.4$  Hz, 4H). ESI-MS:  $[\text{M}+\text{H}]^+$ : calcd. 477.0498; obsd. 477.0414.  $\Delta = 7$  ppm.



**Figure S3.** Synthetic route of P(Pt/Ru) and PolyPt/Ru.

**Synthesis of P(Pt/Ru) (Figure S3)**

Polymerization was performed via a spontaneous amino-yne click reaction according to the reported literature.<sup>[2,3]</sup> To start with,  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (151 mg, 0.10 mmol) was added into a 5 mL Schlenk tube equipped with a mini magnetic stir bar. The tube was degassed and flushed with Ar atmosphere. 200  $\mu\text{L}$  DMF containing  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  (54 mg, 0.08 mmol) along with 20  $\mu\text{L}$  DIPEA was injected dropwise into the tube and stirred at room temperature in the dark overnight. After reaction, the mixture was poured into cold diethyl ether and stored in  $-20\text{ }^\circ\text{C}$  overnight. The precipitates were filtered and washed with acetone three times, followed by drying in vacuum to a constant weight. P(Pt/Ru) was obtained as a light red solid (124 mg).

**Synthesis of PEG-*b*-P(Pt/Ru)-*b*-PEG (PolyPt/Ru, Figure S3)**

To obtain PolyPt/Ru, poly(ethylene glycol) methyl ether amine (mPEG5k-NH<sub>2</sub>) with a primary amine was used to terminate the ethynyl groups.<sup>[4]</sup> Typically, 100 mg P(Pt/Ru) was added into a 5 mL Schlenk tube equipped with a mini magnetic stir bar. The tube was degassed and flushed with Ar atmosphere. mPEG5k-NH<sub>2</sub> (110 mg, 0.022 mmol) in 1 mL DMF was injected into the tube by syringe, and then stirred at room temperature for 12 h. Subsequently, the mixture was poured into cold diethyl ether. The resulting precipitates were filtered and washed with a small amount of water, followed by redissolving in THF and precipitating from diethyl ether. The precipitates were washed with water and diethyl ether to obtain PolyPt/Ru as a pink solid (57 mg).

## Sample preparation

### Preparation of PolyPt/Ru nanoparticles

To prepare PolyPt/Ru nanoparticles, 1.8 mL distilled water was added dropwise into a 0.2 mL THF/DMF mixture (7/1, v/v) containing 2 mg PolyPt/Ru and stirred in the dark for 20 min. The stirring rate was 300 rpm. Afterwards, the nanoparticle dispersion was dialyzed against 5 L Milli-Q water for 48 h using a dialysis tube (MW cutoff, 3.5 kDa). Milli-Q water was replaced every 12 h.

### Drug release profiles

PolyPt/Ru nanoparticles or  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  were prepared in Milli-Q water with or without 5.0 mM GSH, respectively. The solution was placed into an end-sealed dialysis bag and immersed in aforementioned solution before incubating in a continuous shaker at 37 °C. 1 mL of the dialysate was withdrawn at specified time intervals and then fresh solution (1 mL) was added to the dialysate. The released drug content was calculated using UV-Vis absorption spectroscopy.

### Preparation of DiD dye loaded PolyPt/Ru nanoparticles

To prepare DiD dye loaded PolyPt/Ru nanoparticles, 50  $\mu\text{L}$  DiD solution (1 mg/mL in DMF) was added into 0.2 mL THF/DMF (7/1, v/v) containing 2 mg PolyPt/Ru. The solution was stirred for 20 min with stirring rate at 300 rpm. After that, 1.8 mL distilled water was added dropwise into the solution and kept stirring for another 30 min. The DiD dye loaded nanoparticles dispersion was dialyzed against 5 L Milli-Q water for 48 h using a dialysis tube (MW cutoff, 3.5 kDa). Milli-Q water was replaced every 12 h.

## **Cell experiments**

### **Cell culture**

Human lung cancer cell line A549 and its cisplatin-resistant cell line A549-DDP, human liver cancer cell line BEL-7404 and its cisplatin-resistant cell line BEL-7404-CP20 (7404-CP20) were generously gifted by Michael M. Gottesman's laboratory at National Cancer Institute, National Institutes of Health, USA. A549 and cisplatin-resistant A549-DDP cell lines were cultured in DMEM medium. BEL-7404 and cisplatin-resistant 7404-CP20 cell lines were cultured in RPMI-1640 medium. The resistance of A549-DDP and 7402-CP20 was maintained by cisplatin-containing medium (1  $\mu\text{g}/\text{mL}$ ). The cells were used in the assays after one week from the end of the treatment to avoid interferences. All cell lines were complemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% penicillin/streptomycin (Life Technologies, USA) and maintained in humidified atmosphere at 37 °C and 5% of CO<sub>2</sub>. Treatment with trypsin (0.25%) (Life technologies, USA) for 5 minutes was employed to detach the cells for further assays.

### **Cell viability assay**

All cell viability tests were evaluated using a standard Cell Counting Kit-8 (CCK-8) assay kit (Solarbio, China). Typically, cancer cells were incubated with DMEM/ RPMI-1640 medium in 96-well plates at a density of  $5.0 \times 10^3$  cells per well overnight. The medium was then replaced by DMEM/ RPMI-1640 containing different compounds or PolyPt/Ru nanoparticles in the dark. To investigate the cell viability of [Ru(biq)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> or PolyPt/Ru nanoparticles under irradiation, each sample was added to the cell medium and cultured for 6 h, followed by exposure to 671 nm red light



irradiation for 20 min. Then, cells were incubated for 24 h. CCK-8 working solution was added as 10  $\mu$ L per well, followed by another incubation for 4 h. Cell viability was assessed by the measurement of the absorbance at the wavelength of 450 nm on the microplate reader (EnSpire, Perkin Elmer). Treatment procedures were replicated three times for each cell line.

### **Combination effect analysis**

The combination index (CI) was calculated according to the median-effect analysis.<sup>[5]</sup>

<sup>6]</sup> CI was determined with the following equation:  $CI = (D)_1/(D_{50})_1 + (D)_2/(D_{50})_2$ , where  $(D_{50})_1$  is the dose of agent 1 required to produce 50% effect alone, and  $(D)_1$  is the dose of agent 1 required to produce the same 50% effect in combination with  $(D)_2$ . Similarly,  $(D_{50})_2$  is the dose of agent 2 required to produce 50% effect alone, and  $(D)_2$  is the dose required to produce the same effect in combination with  $(D)_1$ . This mathematical algorithm generates CI value  $<0.9$  when the combinations are synergistic,  $0.9-1.1$  when they are additive, and  $>1.1$  when they are antagonistic.

### **Cell imaging by confocal laser scanning microscopy (CLSM)**

For the cellular uptake experiments,  $1 \times 10^5$  cells per milliliter were seeded in 35 mm diameter  $\mu$ -dishes and cultured overnight in supplemented medium. The medium was replaced by fresh medium containing 50  $\mu$ g/mL DiD-loaded PolyPt/Ru nanoparticles. The cells were incubated with different time periods. Subsequently, the nuclei were stained with Hoechst 33342 (0.5  $\mu$ g/mL, Sigma, USA). PolyPt/Ru nanoparticles were further removed by washing with PBS three times. Live cells images were taken using CLSM (LSM710, Carl Zeiss). DiD-loaded PolyPt/Ru nanoparticles were excited with

a 633 nm laser and detected in the range from 650 to 750 nm. The cell nuclei were excited with a 405 nm laser and detected in the range from 425 to 475 nm.

For the intracellular singlet oxygen ( $^1\text{O}_2$ ) detection assay, the fluorescent probe DCFH-DA (Solarbio, China) was used according to the manufacture's instruction. Specifically,  $1 \times 10^5$  cells per milliliter were seeded in 35 mm diameter  $\mu$ -dishes and cultured overnight in supplemented medium. The cells were divided into five groups: (1) Control group; (2) + Light group; (3) +PolyPt/Ru + light group; (4) +PolyPt/Ru + probe group; (5) +PolyPt/Ru + light + probe group. Then medium or 50  $\mu\text{g}/\text{mL}$  PolyPt/Ru nanoparticles were incubated with the cells for 6 h. Afterwards, serum-free DCFH-DA solution (15  $\mu\text{M}$ ) was incubated with cells in (4) and (5) groups for 20 min. Cells in group (2), (3), and (5) were then irradiated with 671 nm red light for 1 min at an intensity of  $125 \text{ mWcm}^{-2}$ . All the cell nuclei were stained with Hoechst 33342 (0.5  $\mu\text{g}/\text{mL}$ , Sigma, USA). Live cells were further imaged using CLSM (LSM710, Carl Zeiss). DCFH-DA was excited with a 488 nm laser, detected in the range from 500 to 550 nm. The cell nuclei were excited with a 405 nm laser and detected in the range from 425 to 475 nm.

### **Cellular uptake efficiency by flow cytometer**

$1 \times 10^4$  cells per well were seeded onto 24-well culture plates and cultured overnight in supplemented medium. After that, the medium was replaced by fresh prepared medium containing 50  $\mu\text{g}/\text{mL}$  DiD-loaded PolyPt/Ru nanoparticles. The cells were incubated for different time, followed by washing with PBS buffer three times to remove the surface-bound nanoparticles. After the cells were digested by trypsinase and washed by PBS, the uptake efficiency was calculated by dividing the number of fluorescent cells

by the number of total cells. The fluorescent intensity was recorded by a flow cytometer (BD Accuri C6).

## **Animal experiments**

### **Animal use and tumor model establishment**

BALB/c nude mice (female, 18-20 g, 4-6 weeks) were purchased from Vital River Laboratory Animal Center (Beijing, China). All mice were maintained under specific pathogen-free conditions and had free access to food and water throughout all the experiments.

The patient-derived xenograft (PDX) models were established as the previous report.<sup>[7]</sup> Typically, the PDX tumor sample was cut into approximately  $2 \times 2 \times 2$  mm<sup>3</sup>/fragment. Each fragment was subcutaneously inoculated into the shoulders of the mice. Tumor volume was measured using a vernier caliper and calculated as  $V=L*W^2/2$ , where L and W were the length and width of the tumor, respectively. The established PDX model was called passage 1 (P1). When the tumor size of P1 reached approximately 750 mm<sup>3</sup>, the tumor was isolated and sliced into small approximately  $2 \times 2 \times 2$  mm<sup>3</sup>/fragment and re-inoculated into mice to obtain the subsequent passage called P2. The similar procedure was conducted to obtain the subsequent passage called P3. The tumor from P3 was performed in the further *in vivo* experiments.

### **Hemolysis analysis**

The fresh whole blood was obtained from BALB/c nude mice using sodium citrate as an anti-coagulant with a blood/ anticoagulant ratio of 9:1. The whole blood was subsequently centrifuged at  $1000 \times g$  for 5 min and then the plasma and buffy coat layer were removed. The obtained red blood cells (RBCs) were collected and washed with saline for three times, followed by suspending in 15 mL saline. The RBCs suspension

was incubated with saline containing different concentrations of PolyPt/Ru nanoparticles with the ratio of 1:1 (v/v). The final concentration of PolyPt/Ru nanoparticles was ranging from 200 to 1000  $\mu\text{g/mL}$ . The positive hemolysis control was induced by replacing saline with same volume of water and the negative hemolysis control was saline without any PolyPt/Ru nanoparticles. Each sample was repeated three times. All samples were incubated at 37 °C for 24 h. After incubation, the RBCs were centrifuged at  $1000 \times g$  for 5 min and the absorbance of supernatants at 540 nm was measured by microplate reader. The hemolysis percentage was calculated by measuring the optical density (OD) as the following formula:

$$\text{Hemolysis (\%)} = [(\text{OD of sample absorbance} - \text{OD of background absorbance}) / (\text{OD of positive control} - \text{OD of negative control})] \times 100\%.$$

### ***In vivo* fluorescence imaging**

The PDX tumor-bearing mice at a tumor size approximately  $100 \text{ mm}^3$  were treated with saline as control group or DiD-loaded PolyPt/Ru nanoparticles through intravenous injection via tail vein. The dosage of PolyPt/Ru nanoparticles was 10 mg/kg. After 0, 4, 12, 24 h, the mice were anesthetized with isoflurane and imaged under the *in vivo* imaging system (IVIS Spectrum, PerkinElmer). The excitation wavelength was fixed at 633 nm.

### ***In vivo* anticancer efficacy evaluation**

The anticancer efficacy was evaluated by monitoring the tumor volumes using PDX models. The tumor nodules were allowed to reach approximately  $100 \text{ mm}^3$  before initial

treatment. All the mice were numbered using ear tags and the initial tumor volume and body weight were recorded. Subsequently, the mice were randomly divided into five groups: (1) a group of PDX tumor-bearing mice was injected with saline (control group); (2) a group of PDX tumor-bearing mice was irradiated with light only (light group); (3) a group of PDX tumor-bearing mice was injected with equivalent dosage of cisplatin (cisplatin group); (4) a group of PDX-bearing mice was injected with PolyPt/Ru nanoparticles (PolyPt/Ru group); (5) a group of PDX tumor-bearing mice was injected with PolyPt/Ru nanoparticles (PolyPt/Ru + light group). The number of mice in each group was 5. Injections were performed on the first and third day during the treatments. Mice in group (3), (4), (5) were treated with equivalent dosage of 2 mg/kg cisplatin. 671 nm light irradiation ( $125 \text{ mWcm}^{-2}$ , 20 min) in group (5) was performed after 12 h i.v. injection of PolyPt/Ru nanoparticles. To exclude the effect by light irradiation, Mice in group (2) were set as a negative control. The body weight and tumor volume of each mouse were measured every day. At day 7, the mice from each group were sacrificed and tumor were isolated for weighing.

### **Histological analyses**

At the end of tumor inhibition experiments, the mice from each group were sacrificed and tumor were collected. Main organs including heart, liver, lung, kidney and spleen were also collected and fixed in 4% formaldehyde solution followed by paraffin embedding. The treated tumors and organs were then cut into pieces with thickness of 2  $\mu\text{m}$ , and stained with hematoxylin and eosin (H&E) to assess histological alterations by microscope. Embedding and H&E staining were done by Wuhan Servicebio Technology Co., Ltd.

### **TUNEL assay**

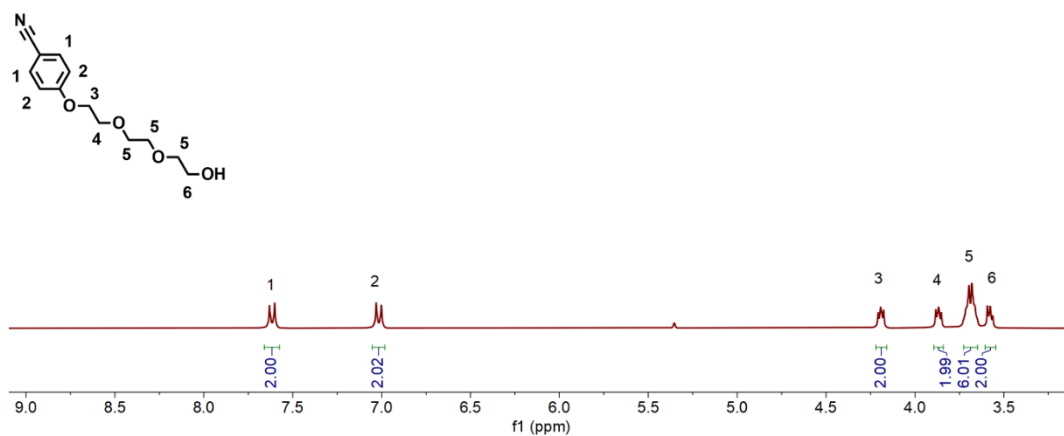
The isolated tumors were fixed in 4% formaldehyde solution followed by paraffin embedding and TUNEL staining. Typically, the paraffin embedded tumors were cut into approximately 8.0  $\mu\text{m}$  by a rotary microtome (YD-1508A) and stained with 0.25% toluidine blue O. The DNA fragmentation was labelled using a TUNEL apoptosis detection kit and then observed by CLSM. Embedding and TUNEL staining were done by Wuhan Servicebio Technology Co., Ltd.

### **Immunohistochemical analysis**

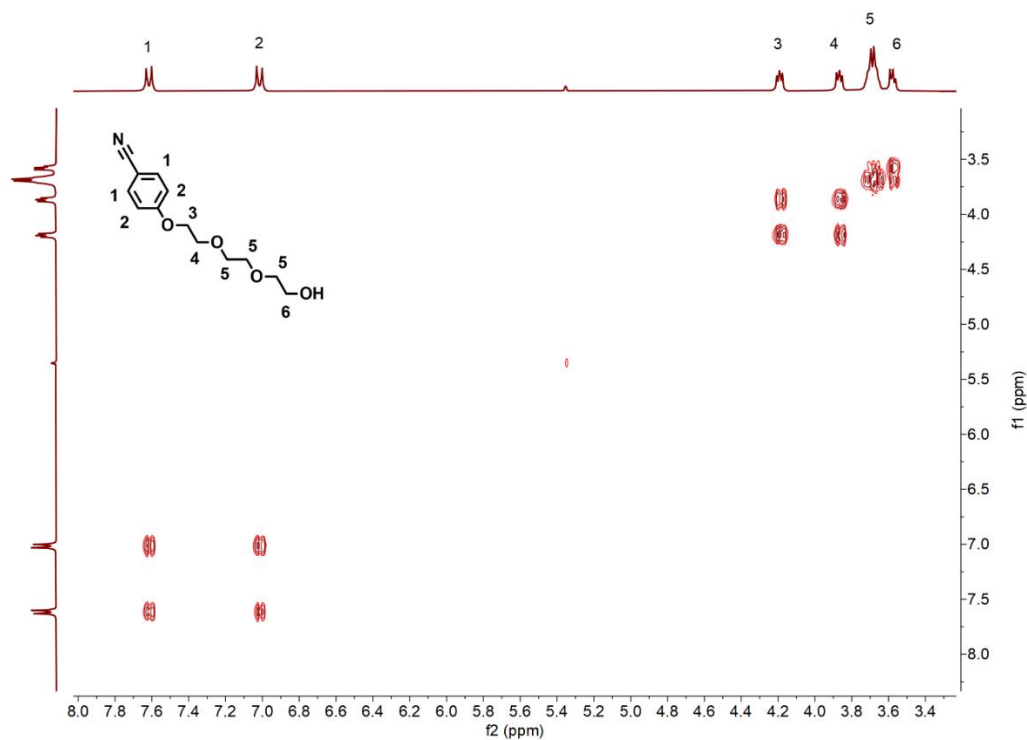
The isolated tumors were fixed in 4% formaldehyde solution at room temperature for 15 min, followed by washing with PBS and incubating with 1% BSA in phosphate buffered saline tween-20 (PBST) for 30 min to block unspecific binding of antibodies. Then, the tumor sections were incubated with antibody  $\gamma\text{H2AX}$  (1:800 diluted in 1% BSA in PBST) in a humidified chamber for 1 h at 37 °C, followed by incubation with a secondary antibody in 1% BSA for 1 h at 37 °C in the dark. Antibodies were purchased from Wuhan Servicebio Technology Co., Ltd. Samples were observed by CLSM.

### **Statistical analysis**

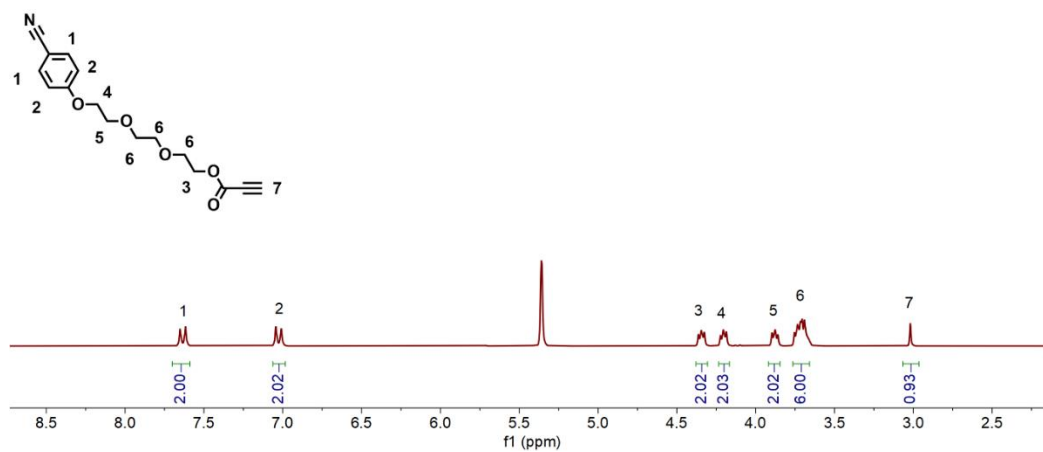
One-way ANOVA with Tukey's multiple comparison analysis was used to determine the significance of differences between different groups. Data were calculated and appropriate statistical results were marked as \* $p < 0.05$  and \*\*\* $p < 0.001$ .



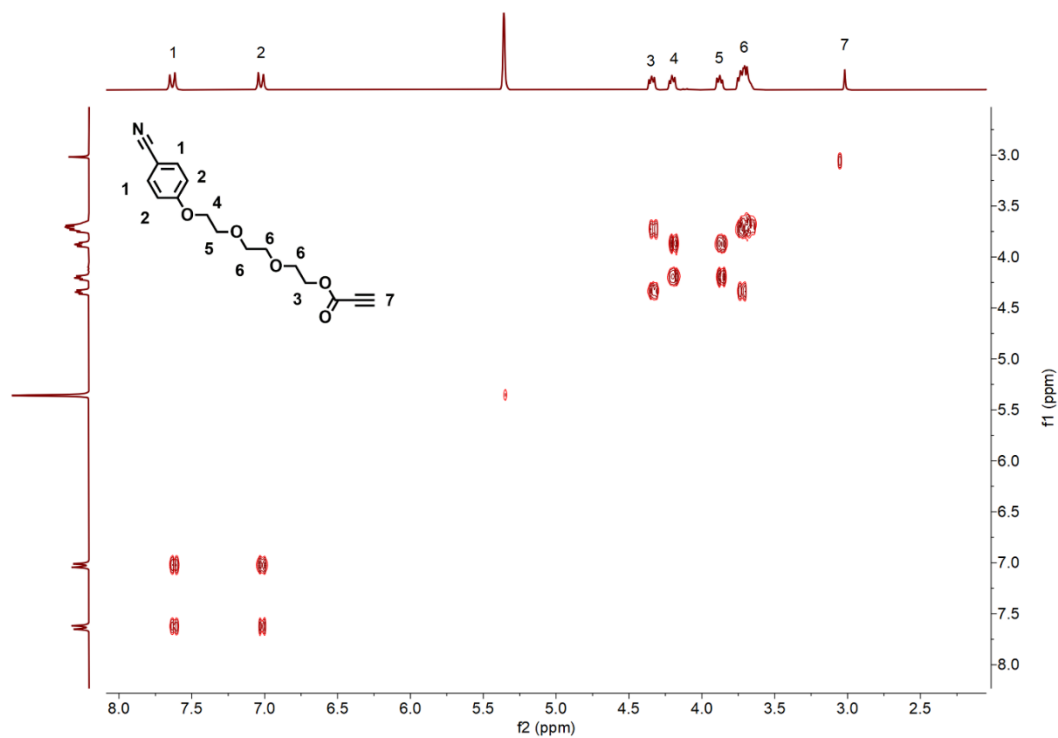
**Figure S4.** <sup>1</sup>H NMR spectrum of [2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]benzonitrile (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>).



**Figure S5.** COSY spectrum of [2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]benzonitrile (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>).



**Figure S6.** <sup>1</sup>H NMR spectrum of PCE (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>).



**Figure S7.** COSY spectrum of PCE (300 MHz, CD<sub>2</sub>Cl<sub>2</sub>).



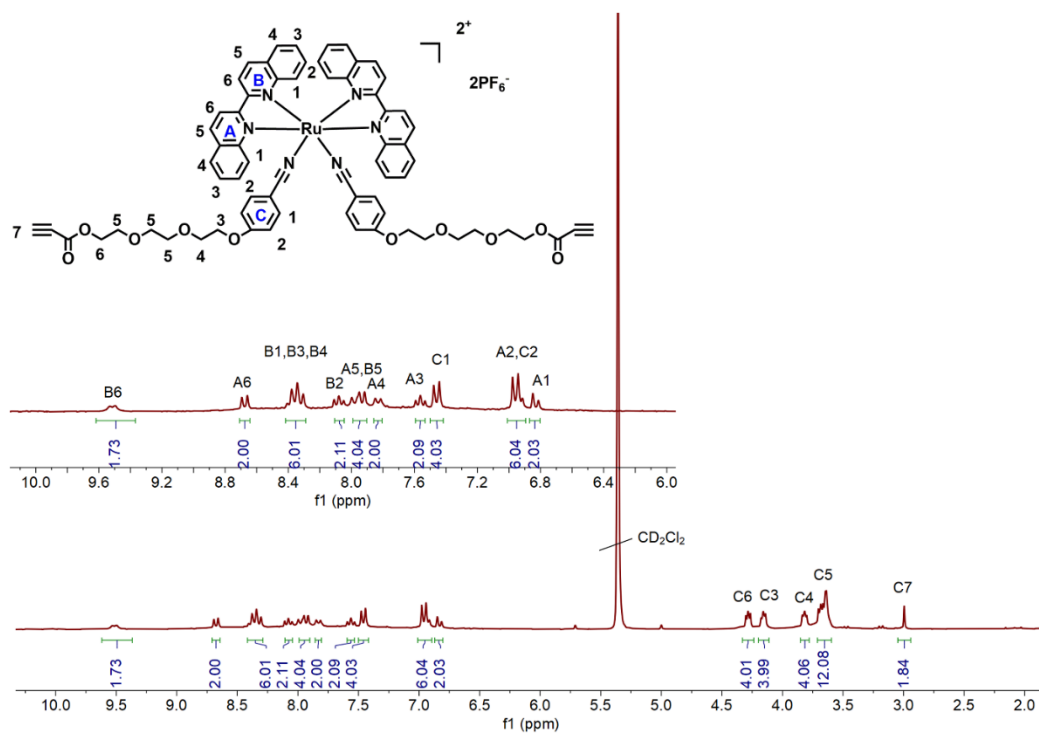


Figure S8.  $^1\text{H}$  NMR spectrum of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).

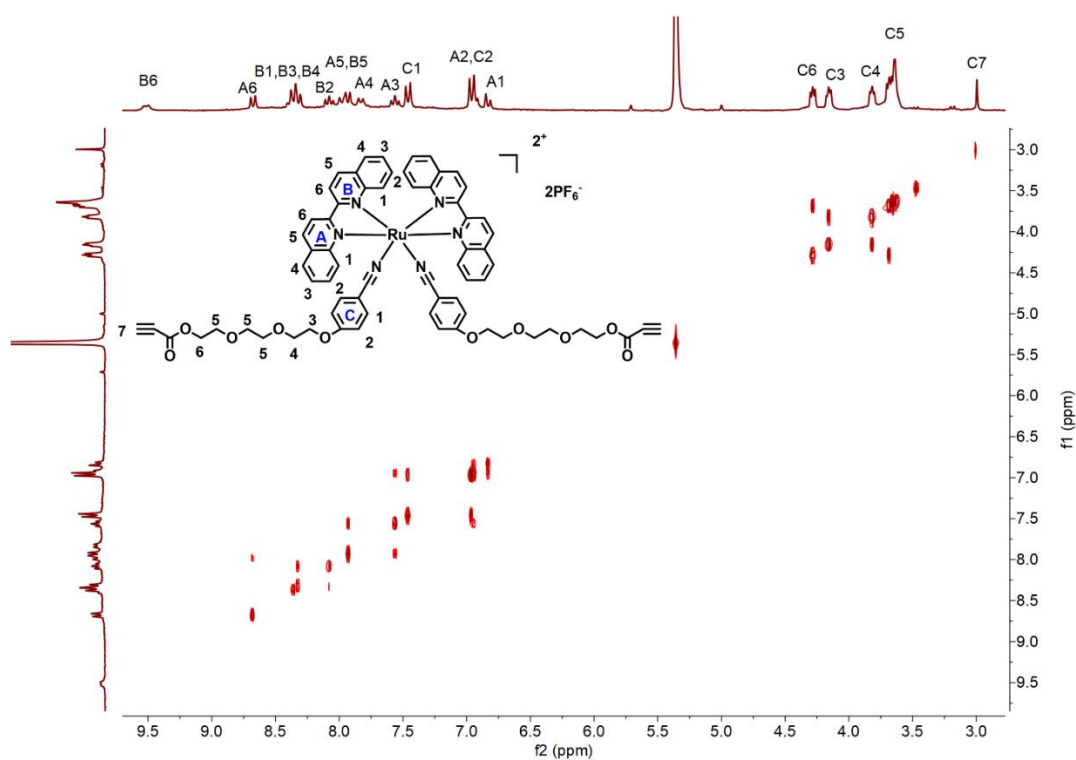
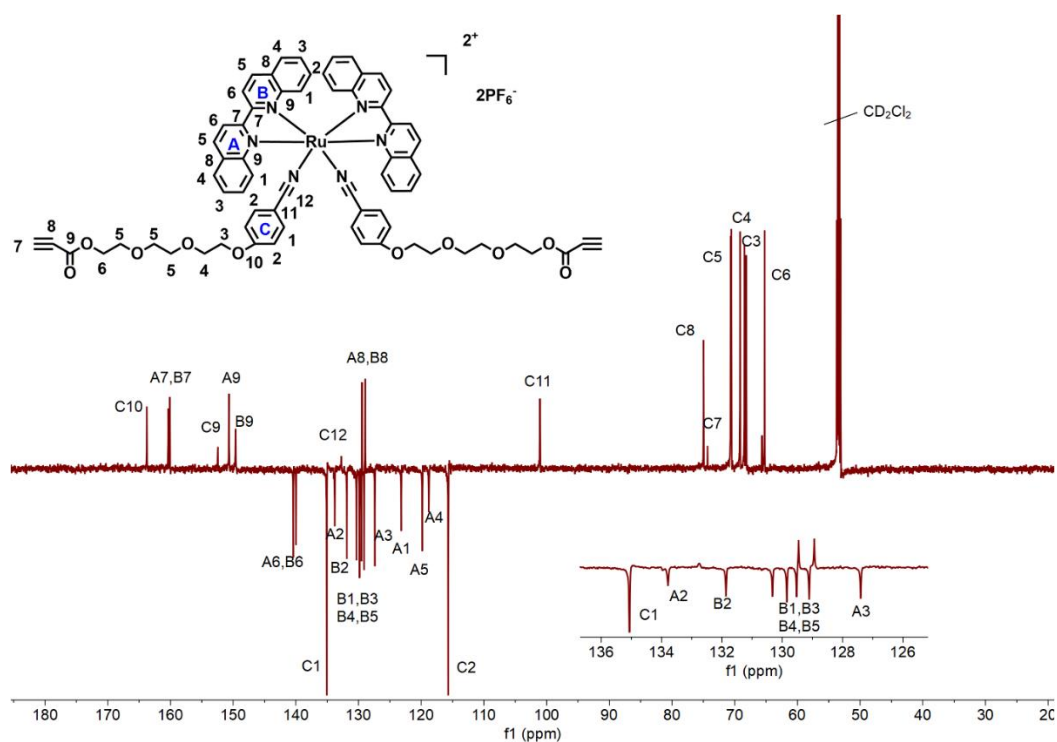
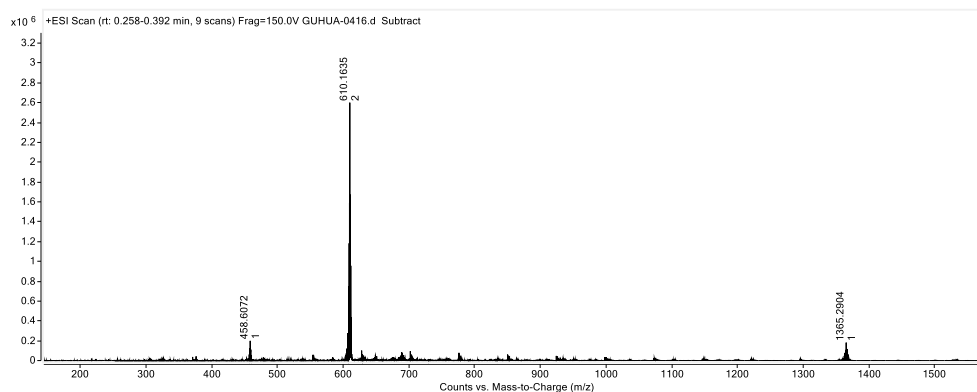


Figure S9. COSY spectrum of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).

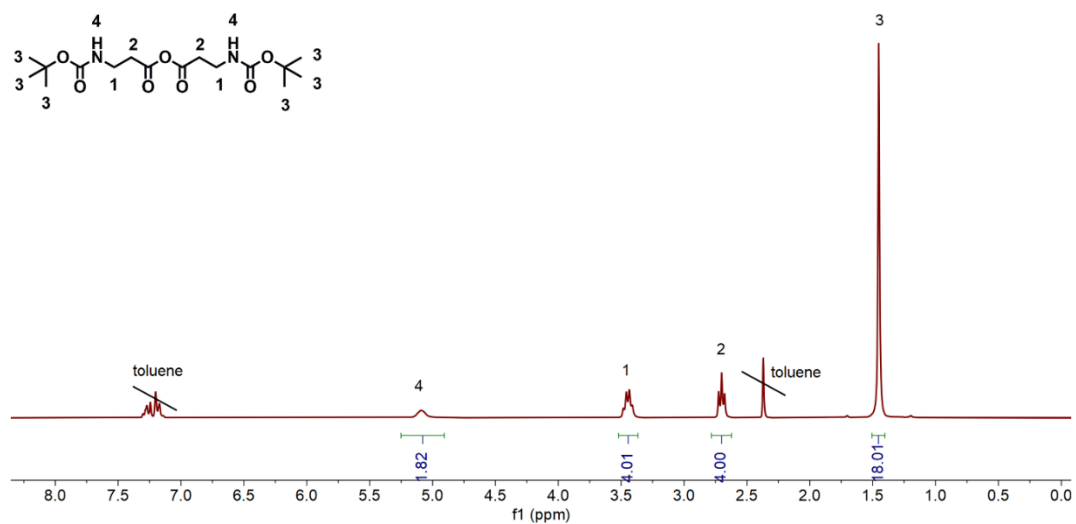


**Figure S10.**  $^{13}\text{C}$  NMR spectrum of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  (300 MHz,  $\text{CD}_2\text{Cl}_2$ ).

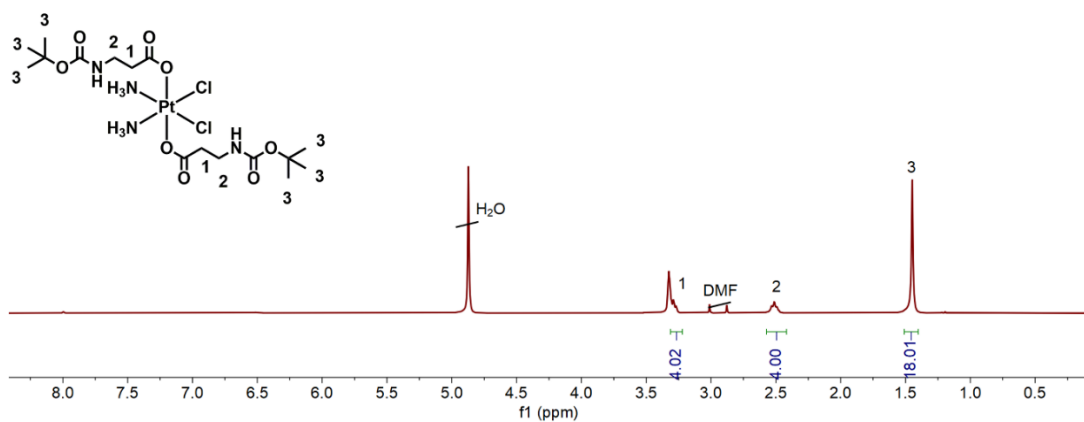


**Figure S11.** ESI-MS spectrum of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$ .  $[\text{M}-2\text{PF}_6]^{2+}$ : calcd.

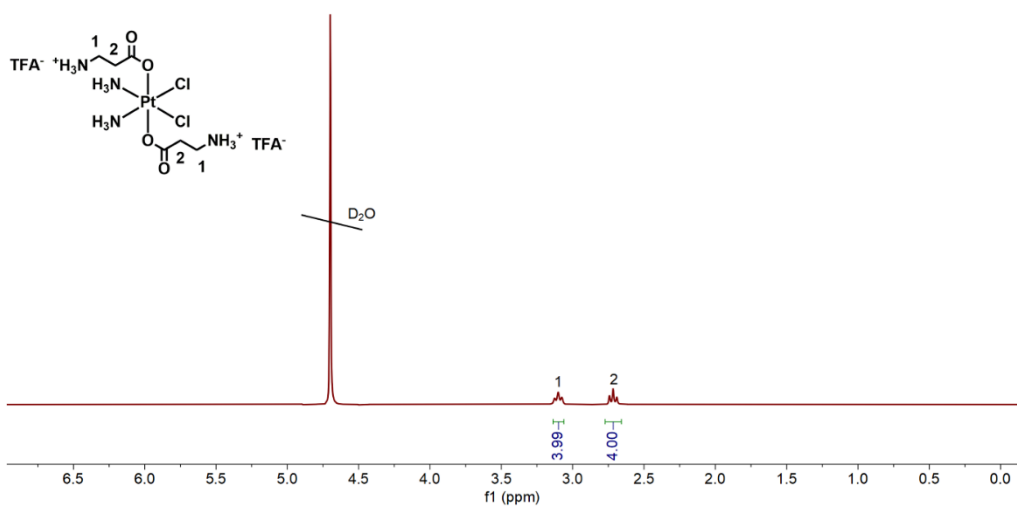
610.1629; obsd. 610.1635.  $\Delta = 0.9$  ppm.



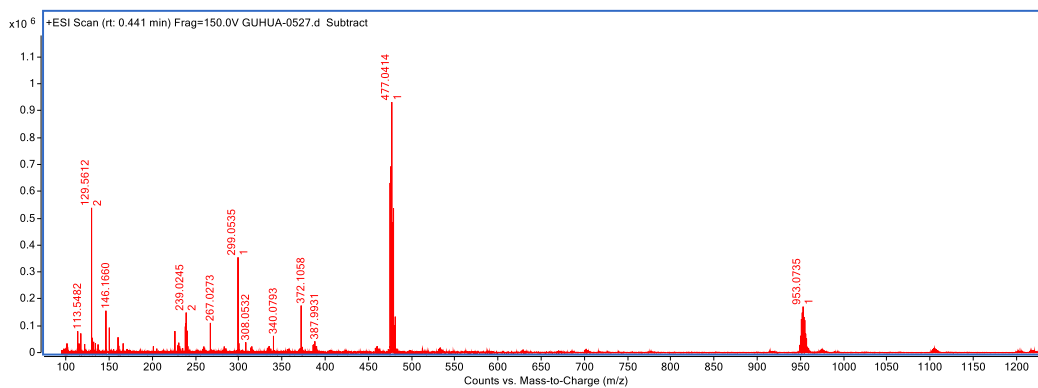
**Figure S12.** <sup>1</sup>H NMR spectrum of Boc-AAA (300 MHz, CDCl<sub>3</sub>).



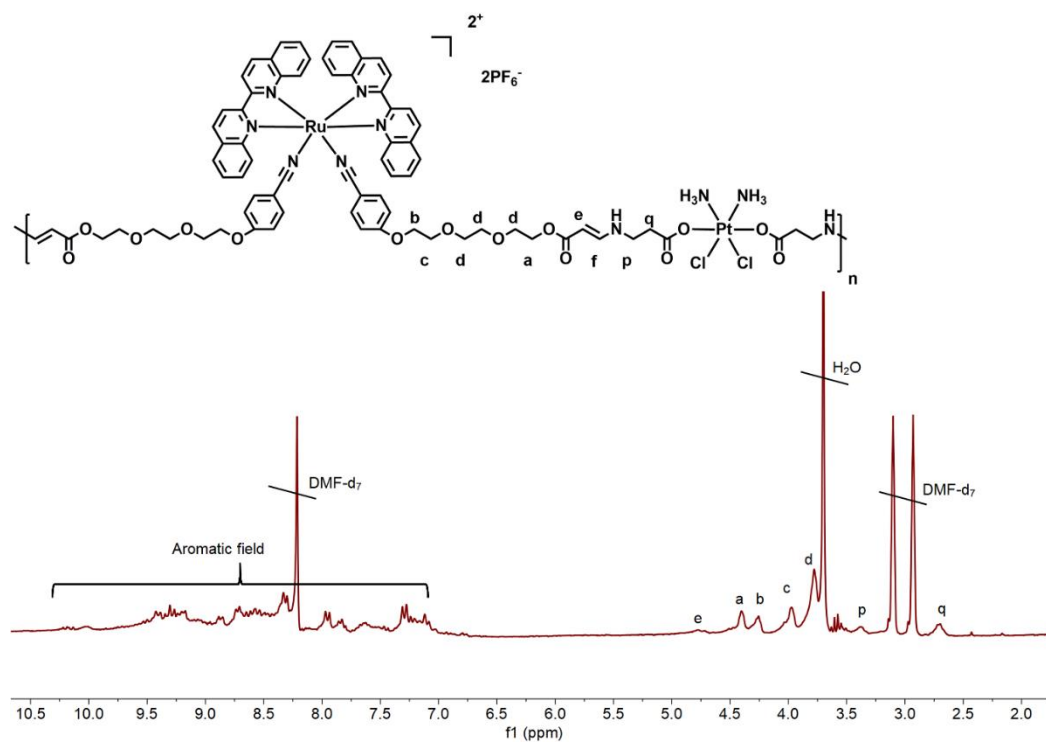
**Figure S13.** <sup>1</sup>H NMR spectrum of [Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>(AA-Boc)<sub>2</sub>](TFA)<sub>2</sub> (300 MHz, MeOH-*d*<sub>6</sub>).



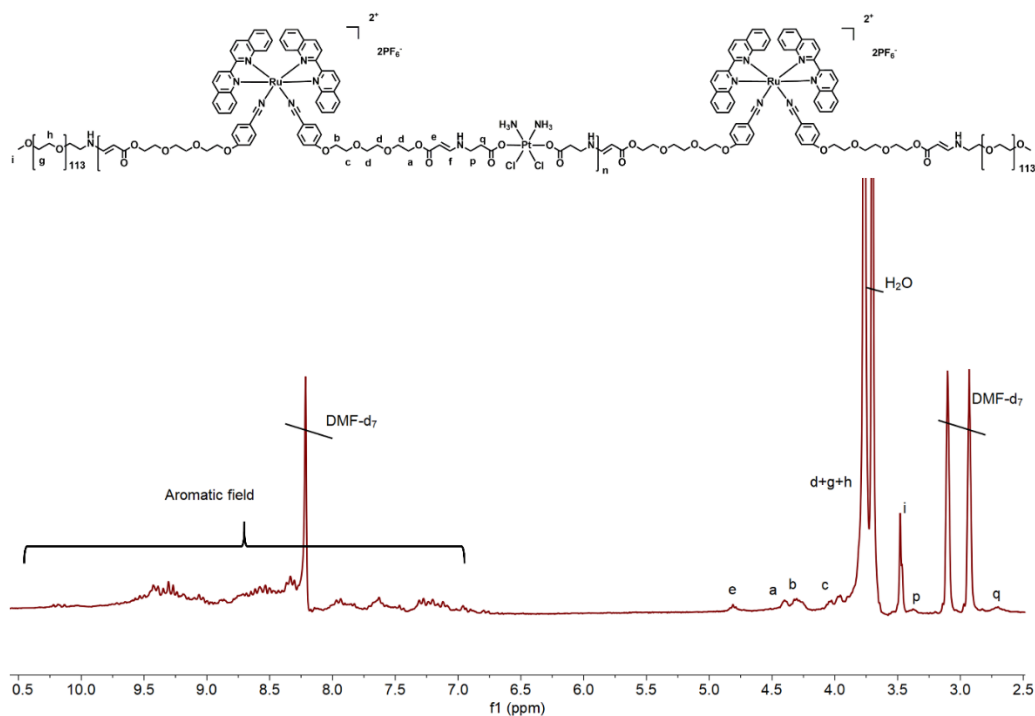
**Figure S14.**  $^1\text{H}$  NMR spectrum of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ . (250 MHz,  $\text{D}_2\text{O}$ ).



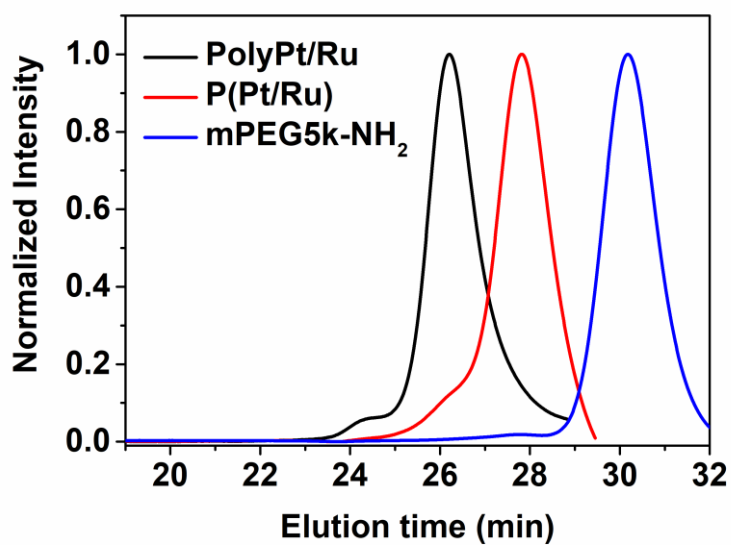
**Figure S15.** ESI-MS spectrum of  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ .  $[\text{M}+\text{H}]^+$ : calcd. 477.0498; obsd. 477.0414.  $\Delta = 7$  ppm.



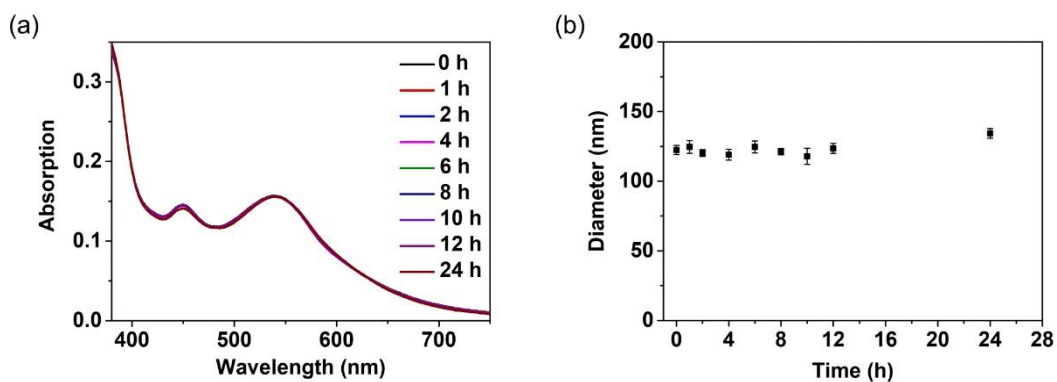
**Figure S16.**  $^1\text{H}$  NMR spectrum of P(Pt/Ru) (300 MHz, DMF- $d_7$ ).



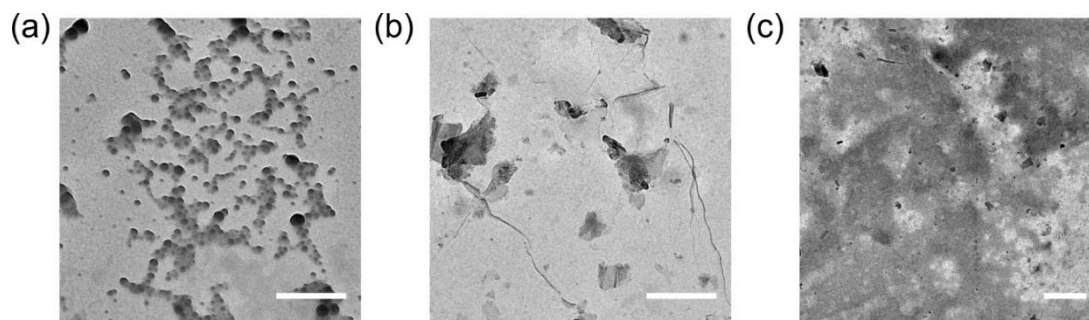
**Figure S17.**  $^1\text{H}$  NMR spectrum of PolyPt/Ru (300 MHz,  $\text{DMF-d}_7$ ). NMR provided details about the structure of PolyPt/Ru. The ethynyl proton of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  at 2.98 ppm could not be observed in the spectra of PolyPt/Ru. In contrast, a new peak at 4.75 ppm appeared, which was assigned to one of the  $\text{HC}=\text{CH}$  signal next to the nitrogen atom of PolyPt/Ru. The other proton signal of  $\text{HC}=\text{CH}$  group located in the aromatic region, which was overlapped with those of the biq ligand. Moreover, the peaks of aromatic area of PolyPt/Ru were broad and overlapped with adjacent signals, which are typical for polymers. The molecular weight of PolyPt/Ru was estimated according to the integral ratio of the peaks at 4.75 ppm and 3.41 ppm in the  $^1\text{H}$  NMR spectrum. The molar mass of PolyPt/Ru measured by  $^1\text{H}$  NMR spectrum was 17 kg/mol. The actual loading contents of Ru(II) and Pt(IV) repeat units were 32% and 10%, respectively.



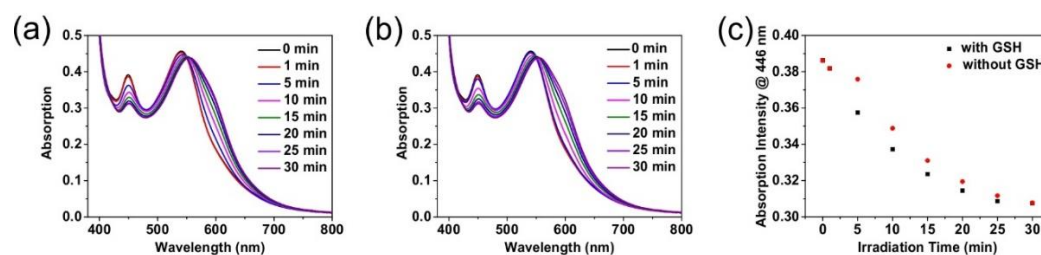
**Figure S18.** GPC traces of PolyPt/Ru, P(Pt/Ru), mPEG5k-NH<sub>2</sub>.



**Figure S19.** (a) Stability of PolyPt/Ru nanoparticles in PBS buffer studied by UV-vis absorption spectroscopy. (b) Stability of PolyPt/Ru nanoparticles in PBS buffer studied by DLS. Results showed that PolyPt/Ru nanoparticles were stable.

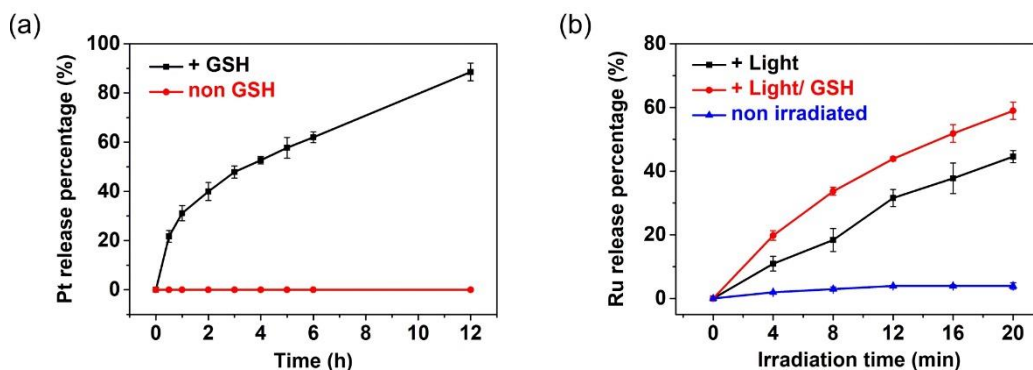


**Figure S20.** (a) TEM of PolyPt/Ru nanoparticles after 671 nm red light irradiation ( $125 \text{ mW cm}^{-2}$ , 30 min); (b) TEM of PolyPt/Ru nanoparticles after 5.0 mM GSH treatment; (c) TEM of PolyPt/Ru nanoparticles after 671 nm red light irradiation ( $125 \text{ mW cm}^{-2}$ , 30 min) with 5.0 mM GSH treatment. Scale bars: 500 nm.

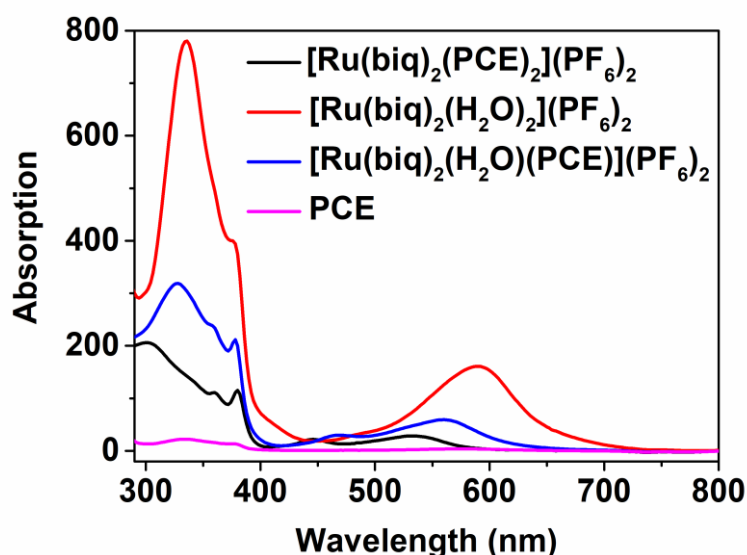


**Figure S21.** Change of UV-Vis absorption spectrum of PolyPt/Ru nanoparticles under red light irradiation ( $125 \text{ mW cm}^{-2}$ , 30 min) (a) in the presence of 5.0 mM GSH and (b) in the absence of 5.0 mM GSH. (c) Relative absorption changes at 446 nm of PolyPt/Ru nanoparticles. PolyPt/Ru nanoparticles exhibited a broad  $^1\text{MLCT}$  band ranging from 450 nm tailing to 800 nm. Upon 671 nm light irradiation for 30 min, the  $^1\text{MLCT}$  band of PolyPt/Ru nanoparticles was both red-shifted in the presence or absence of GSH. By comparing the absorption intensity at 446 nm, light irradiation in the presence of GSH showed slightly faster photoreaction rate. This is because GSH induced reduction of Pt(IV) moieties and partial degradation of PolyPt/Ru nanoparticles. So, water molecules had a higher probability to enter PolyPt/Ru nanoparticles, causing faster photosubstitution of Ru(II) moieties.

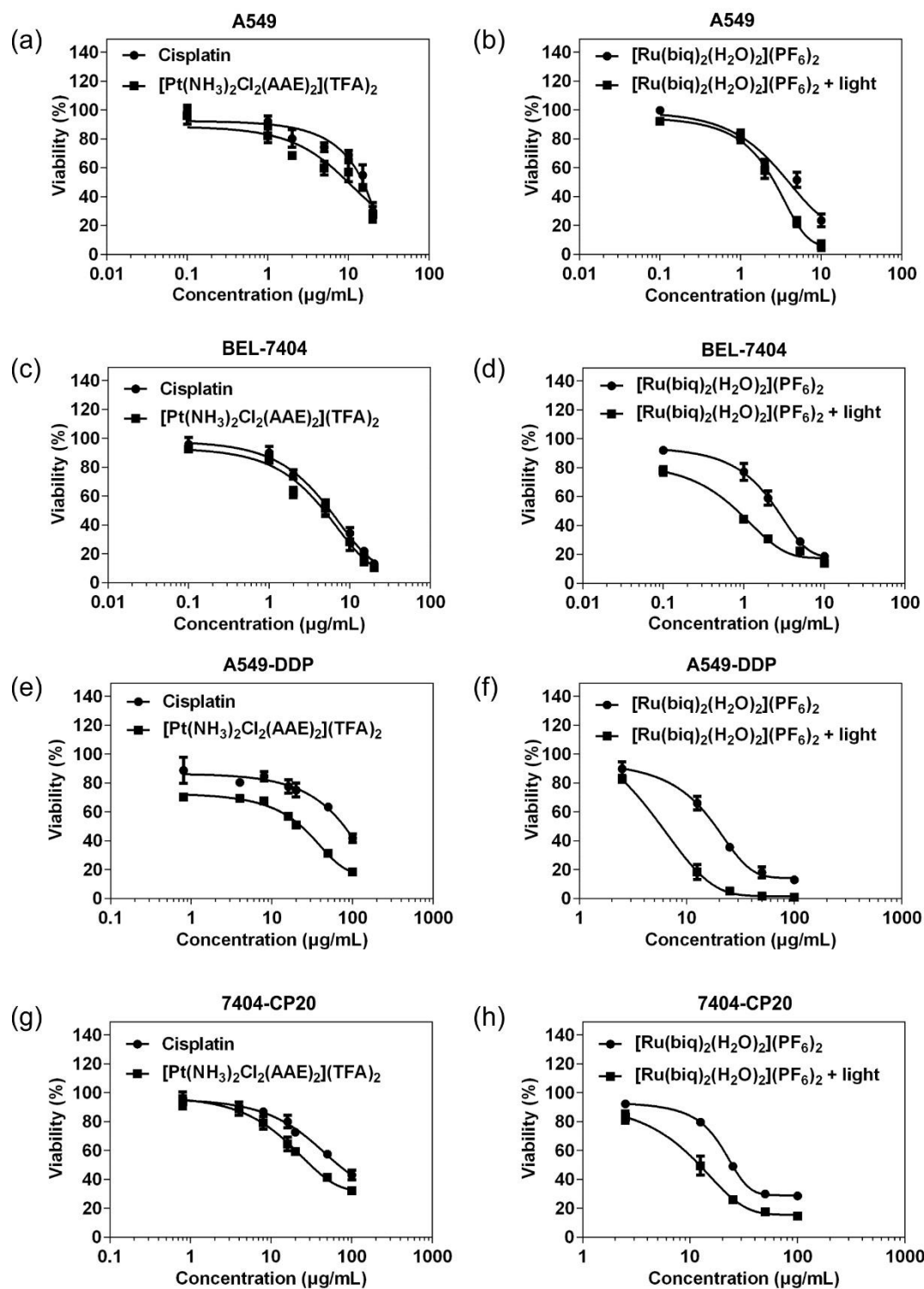




**Figure S22.** (a) The released percentage of cisplatin from  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  under 5.0 mM GSH. (b) The released percentage of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  from PolyPt/Ru nanoparticles under red light irradiation ( $25 \text{ mW cm}^{-2}$ , 20 min) and red light irradiation ( $25 \text{ mW cm}^{-2}$ , 20 min) with 5.0 mM GSH. Up to 45% of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  was released under light irradiation. Furthermore, 60% of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  was released under light irradiation in the presence of GSH, indicating that the degradation of Pt(IV) moieties promoted the release of the Ru(II) moieties in the PolyPt/Ru nanoparticles.

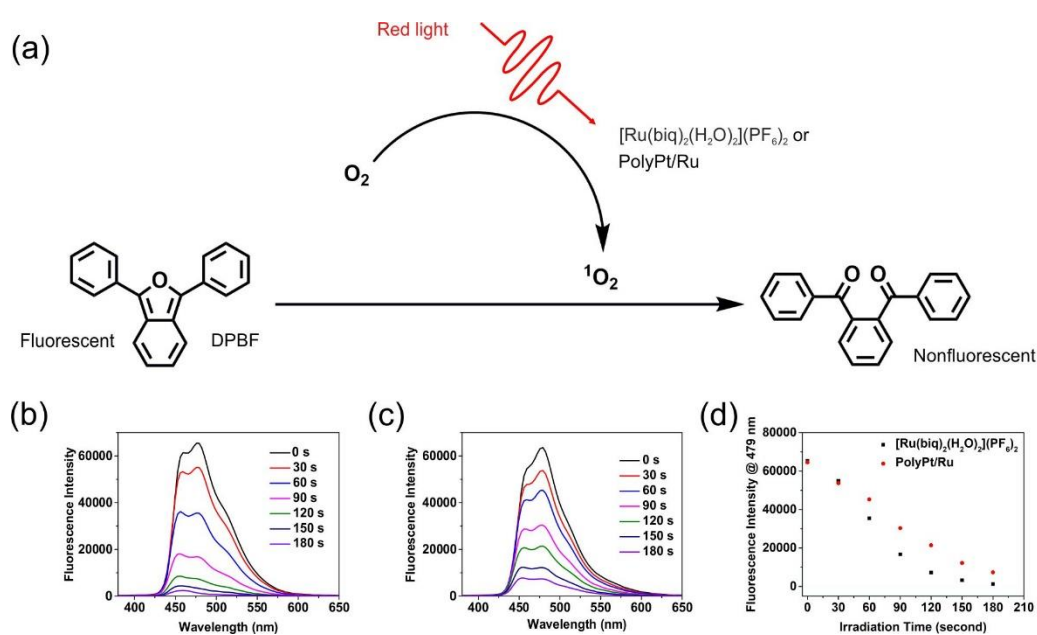


**Figure S23.** UV-vis absorption spectra of  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  and the photoproducts in HPLC measurements in Figure 2 in the main manuscript. The spectra were measured by the UV-vis detector in the HPLC system.

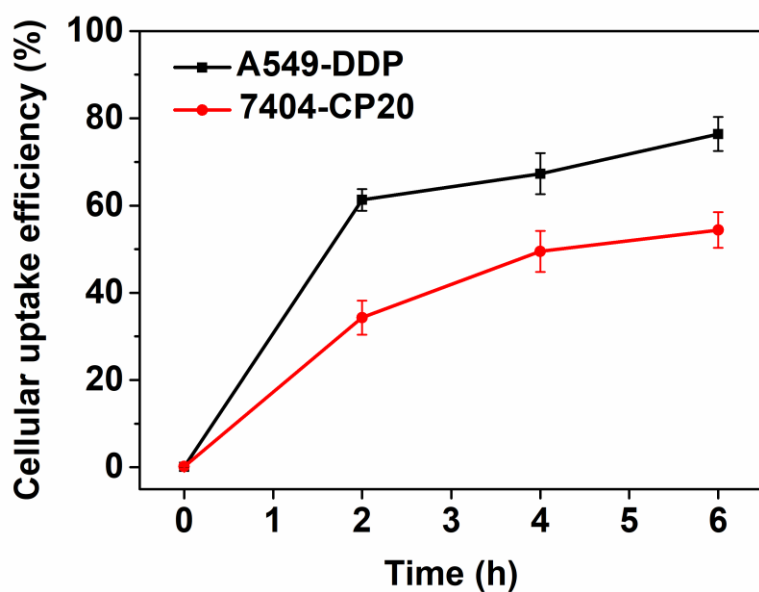


**Figure S24.** Viability of A549 (a), BEL-7404 (c), A549-DDP (e), 7402-CP20 (g) cells incubated with cisplatin or  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$  with different concentrations for 24 h. Viability of A549 (b), BEL-7404 (d), A549-DDP (f), 7402-CP20 (h) cells incubated with  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  in the dark or under 671 nm red light irradiation

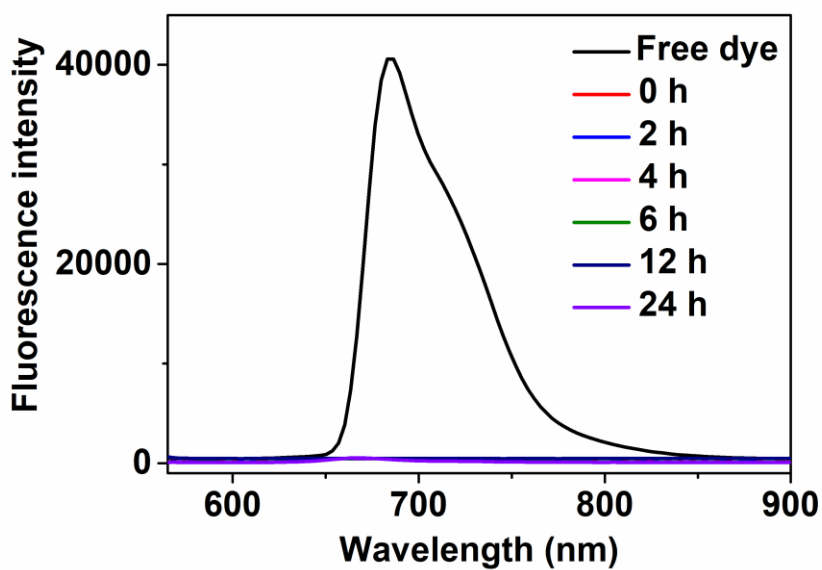
with different concentrations for 24 h. Light irradiation ( $125 \text{ mW cm}^{-2}$ , 20 min) was given after 6 h incubation and cell viability was assessed after further incubation of 24 h.



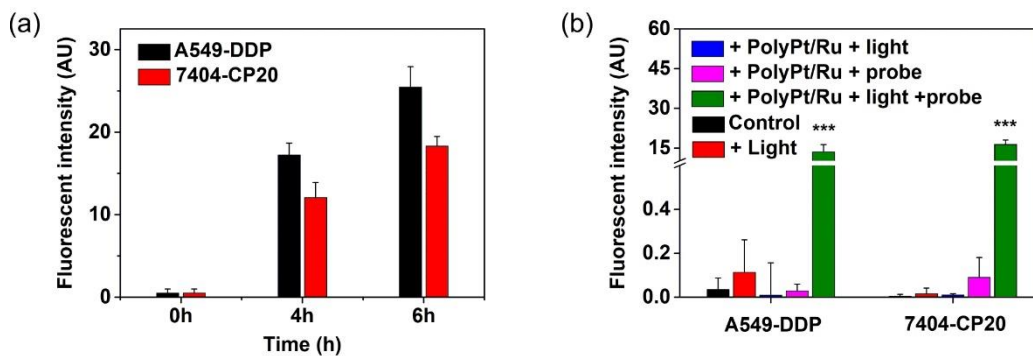
**Figure S25.** (a) Mechanism of using 1, 3-diphenylisobenzofuran (DPBF) to detect singlet oxygen ( $^1\text{O}_2$ ) generated by  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  or PolyPt/Ru under red light irradiation. (b) Fluorescence spectrum of DPBF (150  $\mu\text{M}$ ) in the presence of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  (200  $\mu\text{g/mL}$ ) under 671 nm light irradiation (50  $\text{mW/cm}^2$ ) in a water/methanol mixture. (c) Fluorescence spectrum of DPBF (150  $\mu\text{M}$ ) in the presence of PolyPt/Ru (200  $\mu\text{g/mL}$ ) under 671 nm light irradiation (50  $\text{mW/cm}^2$ ) in a water/methanol mixture. (d) Relative fluorescence intensity at 479 nm of DPBF (150  $\mu\text{M}$ ) in the presence of  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  or PolyPt/Ru under light irradiation.



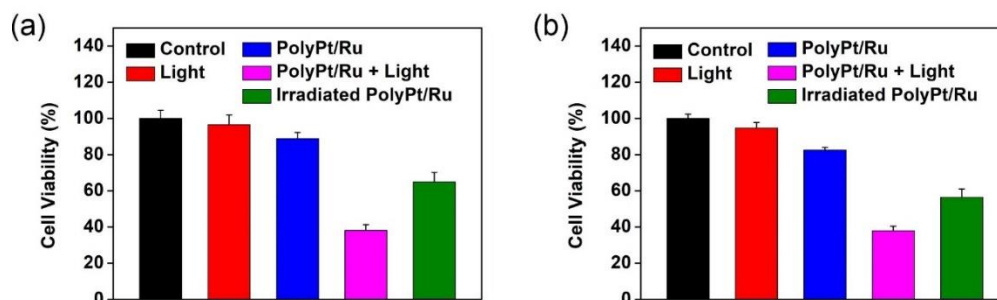
**Figure S26.** Cellular uptake of PolyPt/Ru nanoparticles by A549-DDP and 7404-CP20 cells determined by flow cytometry.



**Figure S27.** Fluorescence spectrum of the free dye (black line) and fluorescence spectra of dialysate after dialysis of the dye-loaded PolyPt/Ru nanoparticles in the cell culture medium after different time periods. No fluorescence was observed from the dialysate, indicating that there was no leakage of the dye.



**Figure S28.** Fluorescence quantitative analyses of (a) cellular uptake and (b)  $^1\text{O}_2$  generation in Figure 3. The fluorescence intensities were calculated using ImageJ software.

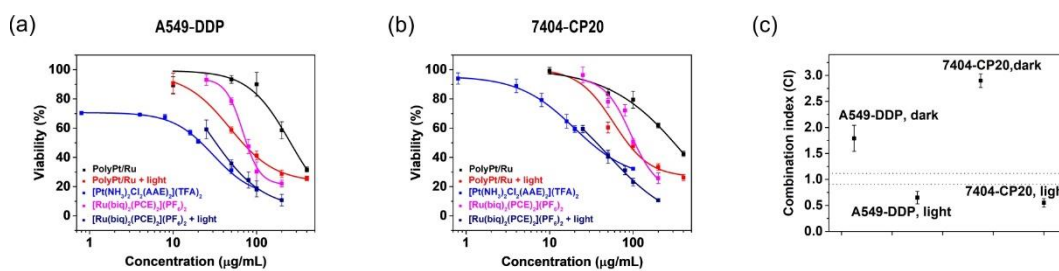


**Figure S29.** Viability of A549-DDP (a), and 7402-CP20 (b) cells treated under different conditions. These experiments differentiated the contribution of chemotherapy and photodynamic therapy (PDT). The control group exhibited the viability of cells without any treatment. The light group exhibited the viability of cells with 671 nm red light irradiation ( $125 \text{ mW cm}^{-2}$ , 20 min) in the absence of PolyPt/Ru nanoparticles. The PolyPt/Ru nanoparticles group exhibited the viability of cells in the presence of PolyPt/Ru nanoparticles ( $100 \mu\text{g/mL}$ ) in the dark, which showed the cytotoxicity caused by released cisplatin. The PolyPt/Ru nanoparticles + light group exhibited the viability of cells with 671 nm red light irradiation ( $125 \text{ mW cm}^{-2}$ , 20 min) in the presence of PolyPt/Ru nanoparticles ( $100 \mu\text{g/mL}$ ), which showed the cytotoxicity

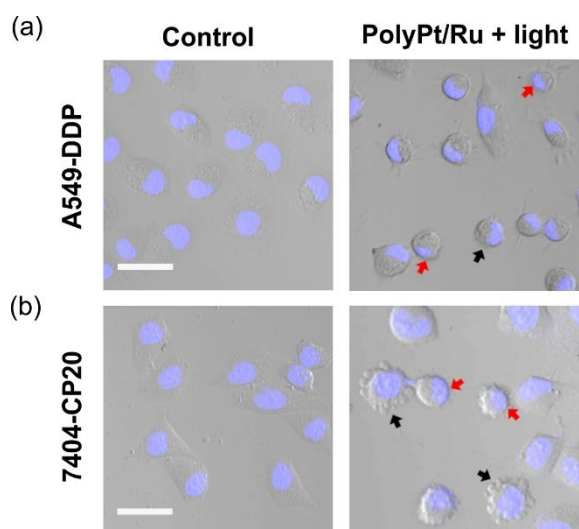
caused by released cisplatin and released  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  in combination with generated  $^1\text{O}_2$ .

For the irradiated PolyPt/Ru nanoparticles group, PolyPt/Ru nanoparticles ( $100 \mu\text{g/mL}$ ) were irradiated ( $125 \text{ mW cm}^{-2}$ , 20 min) in the absence of cells. Light irradiation degraded nanoparticles, released  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  and generated  $^1\text{O}_2$ .  $^1\text{O}_2$  has a short life time and disappeared when light is switched off. Then, PolyPt/Ru nanoparticles after irradiation was incubated with cells for viability test. In this case, this group only showed the effect of chemotherapy without any effect of  $^1\text{O}_2$ .

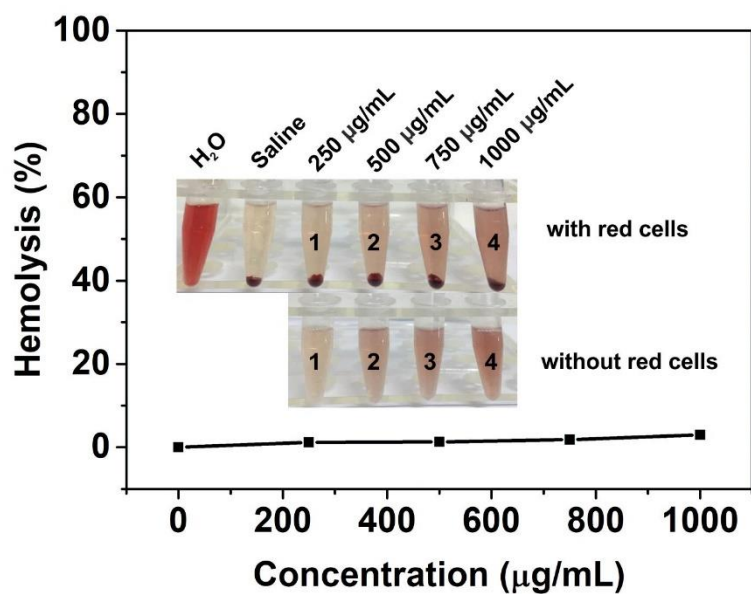
According to these results, the cytotoxicity caused by released cisplatin to A549-DDP was 12%, caused by released  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  to A549-DDP was 23% and caused by PDT to A549-DDP was 27%. Similarly, the cytotoxicity of released cisplatin to 7402-CP20 was 18%, the cytotoxicity of released  $[\text{Ru}(\text{biq})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$  to 7402-CP20 was 26% and the PDT effect was 21%.



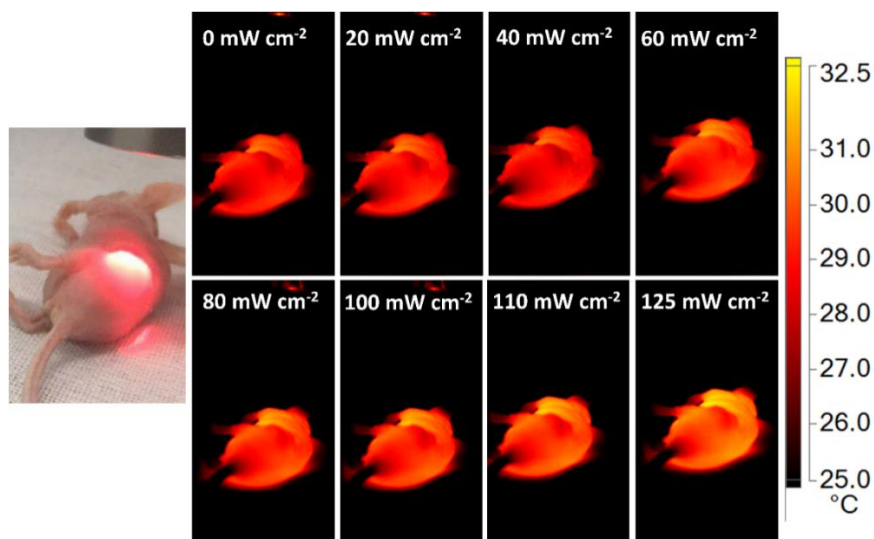
**Figure S30.** In vitro cytotoxicity of PolyPt/Ru nanoparticles, PolyPt/Ru nanoparticles with red light irradiation ( $671 \text{ nm}$ ,  $125 \text{ mW cm}^{-2}$ , 20 min),  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{AAE})_2](\text{TFA})_2$ ,  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  and  $[\text{Ru}(\text{biq})_2(\text{PCE})_2](\text{PF}_6)_2$  with red light irradiation ( $671 \text{ nm}$ ,  $125 \text{ mW cm}^{-2}$ , 20 min) against (a) cisplatin-resistant A549-DDP and (b) cisplatin-resistant 7404-CP20 cancer cells after 24 h incubation. (c) Combination index (CI) against both A549-DDP and 7404-CP20 cells under different conditions.



**Figure S31.** CLSM images of live/dead cells after incubation with PolyPt/Ru nanoparticles, and irradiating with red light. We compared the cell morphology before and after the treatment with PolyPt/Ru + light. In the control groups, A549-DDP and 7404-CP20 cells were uniformly adherent and spindle-shaped. After the cells were incubated with PolyPt/Ru and irradiated with red light, the cells showed inferior adherence, shrunken and changed to round shapes. In addition, nucleus condensation (red arrows) and apoptotic bodies (black arrows) were observed as biomarkers of cell apoptosis. Therefore, these changes indicate that red light irradiation in the presence of PolyPt/Ru caused cytotoxicity in both A549-DDP and 7404-CP20 cells. Scale bars: 50  $\mu\text{m}$ .

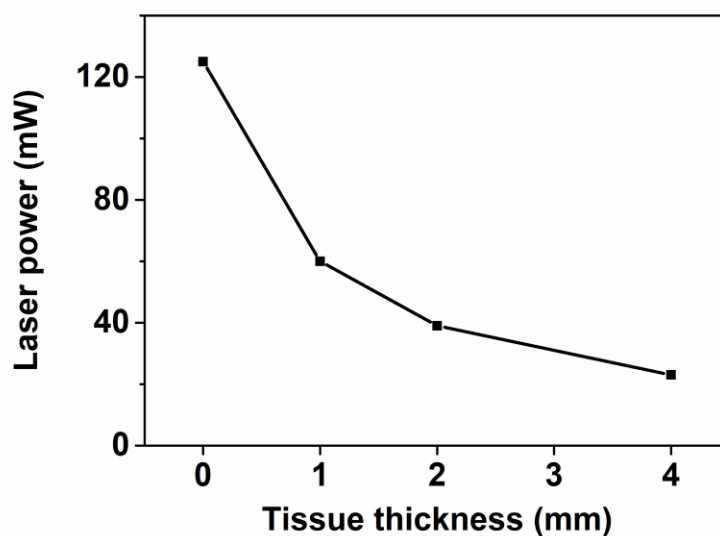


**Figure S32.** Blood hemolysis treated with PolyPt/Ru nanoparticles at various concentrations from 250 µg/mL to 1000 µg/mL. Water was set as a positive control and saline was set as a negative control. Even after incubation for 24 h, these nanoparticles exhibited non-hemolytic with hemolysis lower than the permissible level of 5%. PolyPt/Ru nanoparticles were compatible with red blood cells.

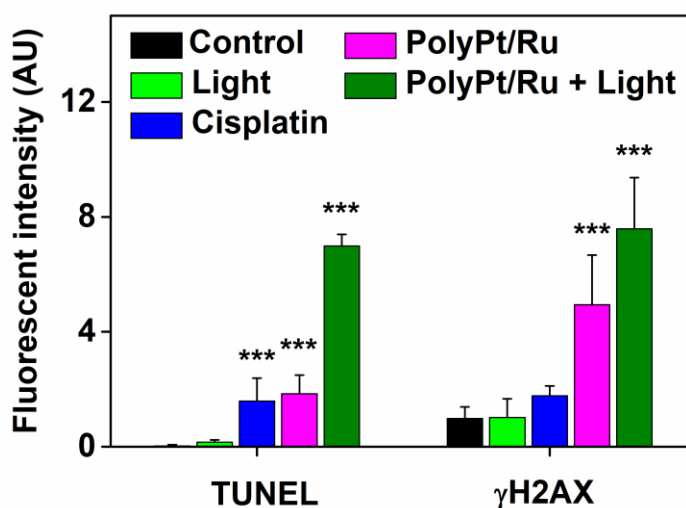


**Figure S33.** Thermal IR imaging of a tumor-bearing mouse exposed to 671 nm red light irradiation at various dosages. Light irradiation did not cause overheating problem.

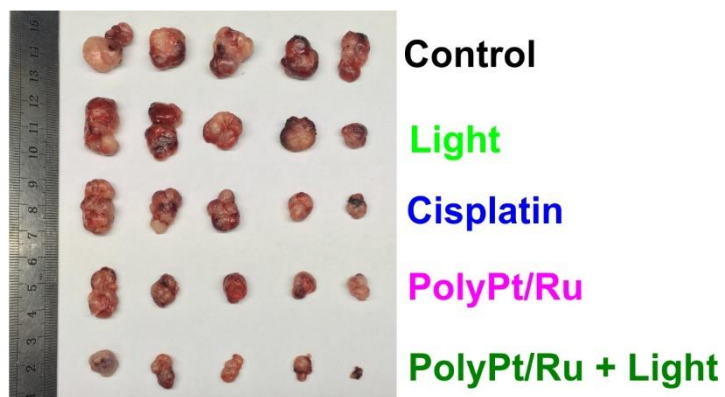




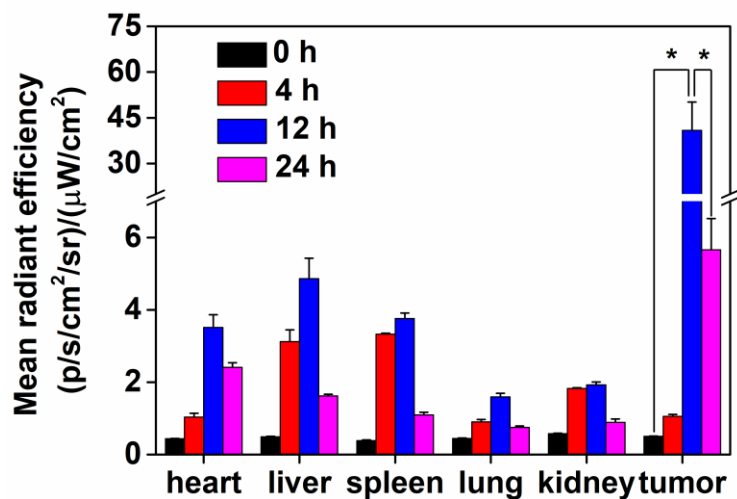
**Figure S34.** Laser power before and after passing through 1-, 2- and 4-mm-thick tissues. The light intensity after passing through 4-mm-thick tissue was ~20 % of the original intensity (without tissue). Red light with the intensity of  $25 \text{ mW cm}^{-2}$  was sufficient to efficiently induce drug release. PolyPt/Ru nanoparticles can be activated at such a light intensity.



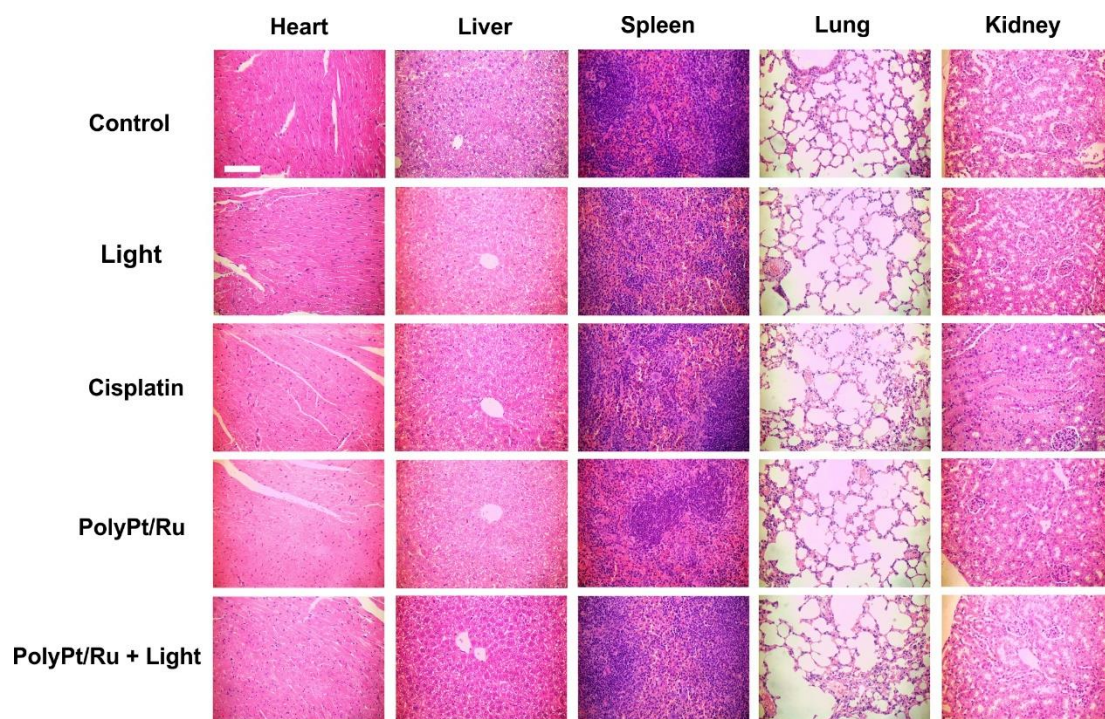
**Figure S35.** Fluorescence quantitative analyses of TUNEL and  $\gamma$ H2AX staining in Figure 4e. The fluorescence intensities were quantified using ImageJ software.



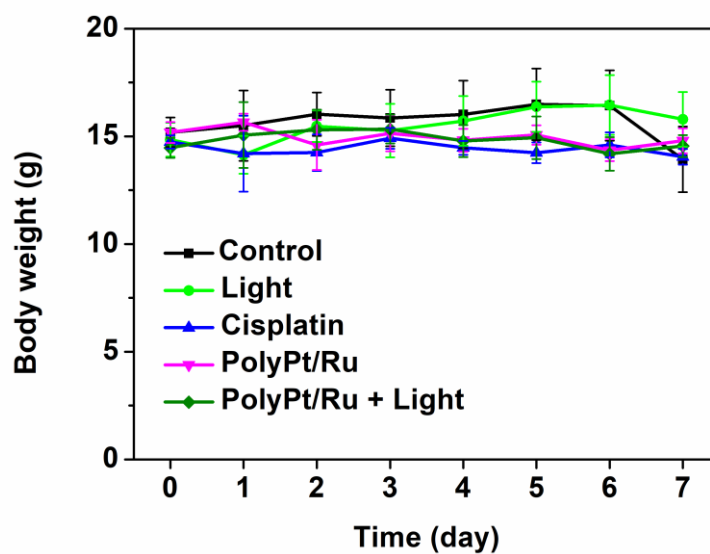
**Figure S36.** Photo of PDX tumors isolated from the mice on day 7.



**Figure S37.** Mean radiant efficiency of different organs and the tumors from mice after injection of dye-loaded PolyPt/Ru nanoparticles for different time periods.



**Figure S38.** Histopathological analysis by H&E staining of tissue sections isolated from PDX tumor-bearing mice. Scale bar: 100  $\mu\text{m}$ .



**Figure S39.** Body weight changes of mice during treatments (n = 5).

**Reference**

- [1] W. Sun, S. Li, B. Häupler, J. Liu, S. Jin, W. Steffen, U. S. Schubert, H.-J. Butt, X.-J. Liang, S. Wu, *Adv. Mater.* **2017**, *29*, 1603702.
- [2] X. Chen, R. Hu, C. Qi, X. Fu, J. Wang, B. He, D. Huang, A. Qin, B. Z. Tang, *Macromolecules* **2019**, *52*, 4526.
- [3] B. He, H. Su, T. Bai, Y. Wu, S. Li, M. Gao, R. Hu, Z. Zhao, A. Qin, J. Ling, B. Z. Tang, *J. Am. Chem. Soc.* **2017**, *139*, 5437.
- [4] O. S. Fenton, J. L. Andresen, M. Paolini, R. Langer, *Angew. Chem., Int. Ed.* **2018**, *130*, 16258.
- [5] Z. Ren, S. Sun, R. Sun, G. Cui, L. Hong, B. Rao, A. Li, Z. Yu, Q. Kan, Z. Mao, *Adv. Mater.* **2020**, *32*, 1906024.
- [6] R. Zhang, J. Yang, M. Sima, Y. Zhou, J. Kopecek, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12181.
- [7] M. He, L. Yu, Y. Yang, B. Zou, W. Ma, M. Yu, J. Lu, G. Xiong, Z. Yu, A. Li, *Chin. Chem. Lett.* **2020**, DOI: 10.1016/j.ccllet.2020.05.034.

## Chapter 4 Summary and Outlook

### 4.1 Summary

We successfully developed photoresponsive nanocarriers Ru-PEG, based on light-cleavable Ru complexes, and applied them in anticancer therapy. Ru-PEG was demonstrated to have tunable properties by proper design. The adjustable Ru-PEG improved the delivery efficiency of toxic Ru towards cancer cells, to exert their maximum anticancer performance *in vitro* and *in vivo*. The transformable nanocarriers are expected to keep their morphology and related functions in the original stage, and to further transform for achieving improved function to subsequently enhance delivery effectiveness in the following stage. The transformable Ru-PEG nanocarriers provides guidance and concept for developing smart nanocarriers with tunable properties/functionality, representing a promising strategy to enhance the anticancer treatment efficacy.

We also successfully prepared a dual-responsive bimetallic nanocarrier PolyPt/Ru to overcome the drug resistance of tumor. Due to the configuration of the polymer structure and the synergistic effects of the bimetallic moieties, PolyPt/Ru was demonstrated to reverse cisplatin-resistant tumors. We strongly believe that this novel polymer and the approach of combining a Pt complex as chemotherapeutic drug and a Ru complex as photoactivatable prodrugs has great potential for further development. The multi-metallic polymers with multi-responsiveness is a new strategy to treat drug-resistant cancers. Given the abundance of bioactive metal complexes, the design concepts presented here serve as a basis for the development of multi-metallic polymers for biomedical applications.

## 4.2 Outlook

There are still several open questions and challenges regarding to the Ru-based nanocarriers. First, the activated wavelength of Ru complexes need to be red-shifted. The suitable wavelength for biomedical applications is at the near-infrared (NIR) region. Although Ru complexes can be activated using NIR light via two-photon process or upconverting nanoparticle (UCNP)-assisted photochemistry. Both of them are based on inefficient non-linear optical processes. Direct red-shifting the absorption wavelength of Ru complexes by proper structural design is highly desirable. The use of fluorinated aromatic rings or cyclometalated ligands can be taken into consideration.

Second, the complete anticancer mechanisms of action of Ru complexes need to be further investigated. The intracellular biomolecules have special function, especially DNA and proteins. How Ru complexes interact with these biomolecules and then cause cell death are need to be explored. This helps us understand the structure-activity relationship of Ru complexes, and further select the suitable Ru for selective and effective anticancer therapy.

Third, the long-term biocompatibility of Ru-based nanocarriers need to be improved. Current systems are typically based on synthetic polymers. The building blocks of the polymers and photoproducts from the polymers may cause toxicity. The use of biocompatible natural nanocarriers, such as peptides, or proteins is good alternatives.

Forth, considering the complexity of tumor, more multiple functional nanocarriers should be designed. For example, the combination of Ru complexes with other therapeutic modalities may provide opportunities to combat the complexity of tumor. The combination of Ru-based nanocarriers with bio-imaging agents can achieve the imaging-guiding cancer therapy.

## Publications

1. **Xiaolong Zeng**<sup>#</sup>, Yufei Wang<sup>#</sup>, Jianxiong Han, Wen Sun, Hans-Jürgen Butt, Xing-Jie Liang<sup>\*</sup>, Si Wu<sup>\*</sup>. Fighting against drug-resistant tumors using a dual-responsive Pt(IV)/Ru(II) bimetallic polymer. *Advanced Materials*, 2020, 2004766.
2. **Xiaolong Zeng**, Xuechang Zhou<sup>\*</sup>, Si Wu<sup>\*</sup>. Red and near-infrared light-cleavable polymers. *Macromolecular Rapid Communications*, 2018, 39(14): 1800034.
3. **Xiaolong Zeng**<sup>#</sup>, Yufei Wang<sup>#</sup>, Jianxiong Han, Wen Sun, Hans-Jürgen Butt, Xing-Jie Liang<sup>\*</sup>, Si Wu<sup>\*</sup>. Light-cleavable Ru(II) complexes with tunable properties for enhanced delivering efficiency in phototherapy. *In preparation*.
4. Wen Sun<sup>#</sup>, Raweevan Thiramanas<sup>#</sup>, Leonardo D. Slep, **Xiaolong Zeng**, Volker Mailänder<sup>\*</sup>, Si Wu<sup>\*</sup>. Photoactivation of anticancer Ru complexes in deep tissue: how deep can we go? *Chemistry—A European Journal*, 2017, 23(45): 10832-10837.
5. Wen Sun, **Xiaolong Zeng**, Si Wu<sup>\*</sup>. Photoresponsive ruthenium-containing polymers: potential polymeric metallodrugs for anticancer phototherapy. *Dalton Transactions*, 2018, 47(2): 283-286.

(<sup>#</sup> contributed equally)

## **Acknowledgment**

First of all, I would like to thank my supervisor Prof. Dr. Hans-Jürgen Butt for offering me the opportunity as a Ph.D. student in his group. He is really nice, helpful and patient to us. I sincerely appreciate his unwavering support and kind assistance during my PhD period. His encouragement inspire me a lot not only in scientific research but also in my daily life.

Then, I would like to express thank to my project leader Prof. Dr. Si Wu for his constructive guidance. He is always helping me to solve problems. Without his suggestions and discussions, I cannot complete such a challenging research area. He gave me numerous critical suggestions for improving my research to a better level than that I cannot reach by myself.

Additionally, I would also like to thank Prof. Dr. Xing-Jie Liang in the National Center for Nanoscience and Technology, who offered me the chance for biological experiments in his group. With his kind help and fruitful discussions, I benefit a lot on the scientific research and biological field.

In the meantime, I would also like to show my appreciation to Yufei Wang, without her assistance on the biological experiments, I cannot finish these projects. I also want to thank the people I met at National Center for Nanoscience and Technology, Dr. Fangzhou Li, Dr. Ningqiang Gong, Dr. Shuyi Li, Dr. Junge Chen, Dr. Mei Lu, Lu Liu, Yuxuan Zhang, Guangchao Qing, Jingjing Wang, Yongchao Wang and Jie Zhong. I'll always miss the good time we had together.

I would also like to express my sincere gratitude my colleague and friend, Prof. Dr. Wen Sun, for his help on the compound synthesis, and his encouragement and suggestions for my project. I'm grateful to his warm caring for all the time.



## Acknowledgment

---

Besides, I want to thank Dr. Manfred Wagner for the helpful NMR measurements and discussions, Gunnar Kircher for his help on organic synthesis and discussions, Best Andreas for his help on laser setup, and Beate Müller for HPLC measurement and discussions. I can finish my project with their kindly assistance.

I would also like thank the members of our sub-group, Dr. Philipp Weis, Dr. Chaoming Xie, Annika Kretzschmann, Yannick Nyquist, Jianxiong Han, Jiahui Liu and Yazhi Liu. They have given me a lot of enjoyment over the years. I also want to thank many people I met at Max Planck Institute for Polymer Research, Dr. Xuelin Yao, Dr. Aiting Gao, for their kindly help and discussions.

Finally, I would also like to thank my girlfriend, Dr. Mengyi Li, for her support and giving me courage to pass through the tough moments in my study and life.

Last but not the least, my most sincere thanks go to my parents. They have been supporting and motivating me to be a better man. When I feel depressed, they were always by my side.