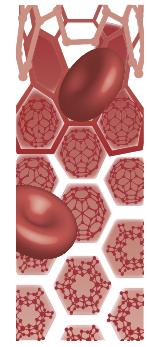
Review

For reprint orders, please contact: reprints@futuremedicine.com

Nanomedicine



Copper-induced tumor cell death mechanisms and antitumor theragnostic applications of copper complexes

Yicheng Jiang[1], Zhiyi Huo[1], Xiaole Qi[1,2], Tongmei Zuo**[,2] & Zhenghong Wu*[,1]

[1]Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, Nanjing, 210009, PR China

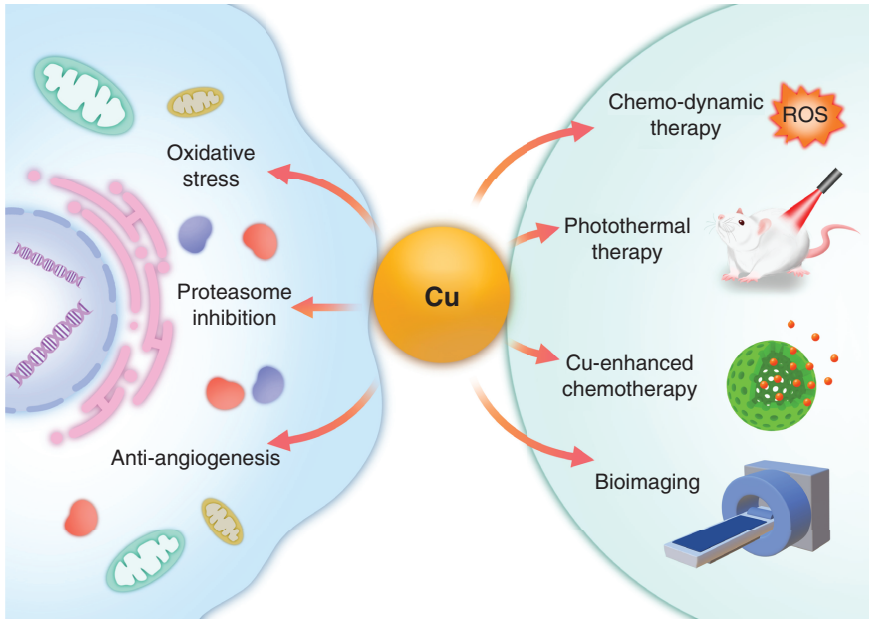
[2]Industrial Technology Innovation Platform, Zhejiang Center for Safety Study of Drug Substances, Hangzhou, 310018, China

*Author for correspondence: Tel.: +86 150 6220 8341; zhenghongwu66@cpu.edu.cn

**Author for correspondence: zuotongmei@163.com

Recent studies found that unbalanced copper homeostasis affect tumor growth, causing irreversible damage. Copper can induce multiple forms of cell death, including apoptosis and autophagy, through various mechanisms, including reactive oxygen species accumulation, proteasome inhibition, and antiangiogenesis. Hence, copper *in vivo* has attracted tremendous attention and is in the research spotlight in the field of tumor treatment. This review first highlights three typical forms of copper's antitumor mechanisms. Then, the development of diverse biomaterials and nanotechnology allowing copper to be fabricated into diverse structures to realize its theragnostic action is discussed. Novel copper complexes and their clinical applications are subsequently described.

Graphical abstract:



First draft submitted: 11 October 2021; Accepted for publication: 6 January 2022; Published online: 21 January 2022

Keywords: antitumor • bioimaging • chemodynamic therapy • copper • Fenton reaction • phototherapy • reactive oxygen species

Copper, a transition metal and a vital trace element, plays an essential role in every living organism. In 1928, scientists first took the importance of physiological copper seriously [1]. In the 1960s, the antitumor activity of Cu-TSC (thiosemicarbazones) complexes was described, introducing a new era of cancer therapies using copper [2,3].

Future Medicine

Researchers then became aware of copper's ability to covalently combine with certain anticancer drugs (e.g., camptothecin [CPT] and doxorubicin [DOX]) to promote DNA cleavage, designing a series of Cu-drug compounds for enhanced cytotoxicity [4,5]. Other fascinating features of Cu, such as superoxide dismutase-like activity and proangiogenic effects, also attracted researchers to determine their value and apply them in clinical trials [6,7]. Into the 21st century, inspired by the Fe-induced Fenton reaction, the Cu-based Fenton reaction gradually proved a hot topic and further enriched the studies on reactive oxygen species (ROS)-mediated oxidative stress, elevating the stature of Cu in cancer treatments [8,9].

Copper ions are absorbed from the diet in the intestine. Copper bound with serum albumin in the bloodstream is then delivered to all organs in human bodies, primarily deposited in the liver; it is ultimately excreted via the biliary pathway [10–12]. Cu is involved in catalyzing redox reactions as a catalytic cofactor in the cell because of the accessible conversion between Cu^{+} and Cu^{2+} two valance states. Many critical intracellular proteins and enzymes, including superoxide dismutase (SOD), cytochrome c oxidase (CCO) and lysyl oxidase (LOX), also harness this property to achieve their physiological functions [13].

In tumor cells, the critical role of Cu ions should not be ignored. Many studies have demonstrated that Cu levels in serum and tumor tissue of cancer patients are higher than in healthy subjects [14]. Cu is involved in three essential processes of cancer progression: cell proliferation, angiogenesis and metastasis [15,16]. Hence, abnormal Cu levels become a new target for cancer treatment. There are two main strategies for treating tumors by affecting Cu homeostasis *in vivo* [17]: increasing the concentration of Cu in tumor cells via Cu-containing compounds or Cu ionophores [18] and decreasing the Cu concentration in tumor cells by Cu chelators [19,20], such as tetrathiomolybdate (TM) [21], D-penicillamine (D-pen) [22] and trientine [23].

Disturbance of Cu homeostasis can induce cell death through multiple approaches, including Cu-induced apoptosis, autography and paraptosis, among others. Of note, Cu-induced cell death through ROS accumulation and mitochondrial dysfunction is different from ferroptosis. Ferroptosis is an iron-dependent, nonapoptotic form of cell death controlled by system X_c^--glutathione (GSH)-GPX4-dependent antioxidant defense pathway [24–26]. In contrast, Cu-induced cell death capitalizes on the Fenton reaction to generate ROS and then upgrade the expression of apoptosis-related genes or cause mitochondrial dysfunction to induce the apoptosis pathway.

Here, we surveyed and summarized several typical Cu-induced tumor cell death mechanisms. The first of these is oxidative stress: Cu-mediated Fenton reaction or antioxidant molecule depletion leads to elevated ROS levels, which in turn causes mitochondrial dysfunction and accelerates apoptosis. Second, we considered proteasome inhibition: Cu binds with proteasome subunits, bringing about ubiquitinated protein accumulation. Third, we surveyed antiangiogenesis: Cu depletion will inhibit the formation of new vessels and further cut off the nutrient supply to the tumor tissue. These anticancer mechanisms all lead to inevitable tumor growth inhibition and even cell death. With the advantages of Cu in cancer therapies, the potential biomedical applications of Cu deserve to be widely exploited. Therefore, we subsequently highlighted the applications of Cu in the field of chemodynamic therapy (CDT), chemotherapy (CT), phototherapy (PT) (Table 1) and bioimaging. The graphical summary of this review is shown in Figure 1.

Antitumor mechanisms of copper

Oxidative stress

As an effective tumor-killing method, increasing Cu concentration in malignant tissues produces its tumoricidal effect mainly depending on oxidative stress triggered by excess Cu ions. Oxidative stress can be defined as disturbance in the oxidant–antioxidant balance, manifested by steady-state ROS concentration is transiently or chronically enhanced. ROS are the by-product of normal oxygen metabolism and are chemical species with one unpaired electron derived from molecule oxygen [53,54]. Although a low or medium level of ROS serves many essential cellular functions, high concentrations of ROS are detrimental to both normal and tumor cells [55]. Therefore, elevating malignant tissue cellular ROS can be a practical approach to treat disparate cancers. Fenton reaction is one of the most common metal-mediated catalysis reactions to produce ROS. The metal catalysts in this reaction usually are transition metals, such as iron (II), Cu (I), chromium (III), cobalt (II) and nickel (II). Cupric (Cu^{2+}) is also vulnerable to reduction to cuprous (Cu^{+}) by Haber–Weiss reaction, which offers Cu the ability to mediate the Fenton reaction to produce the most active hydroxyl radicals (OH·). In particular, the Cu-based Fenton reaction can react over a broader pH range, and its reaction rate dramatically increases ($k = 1.0 \times 10^4\ M^{-1}\ s^{-1}$) compared

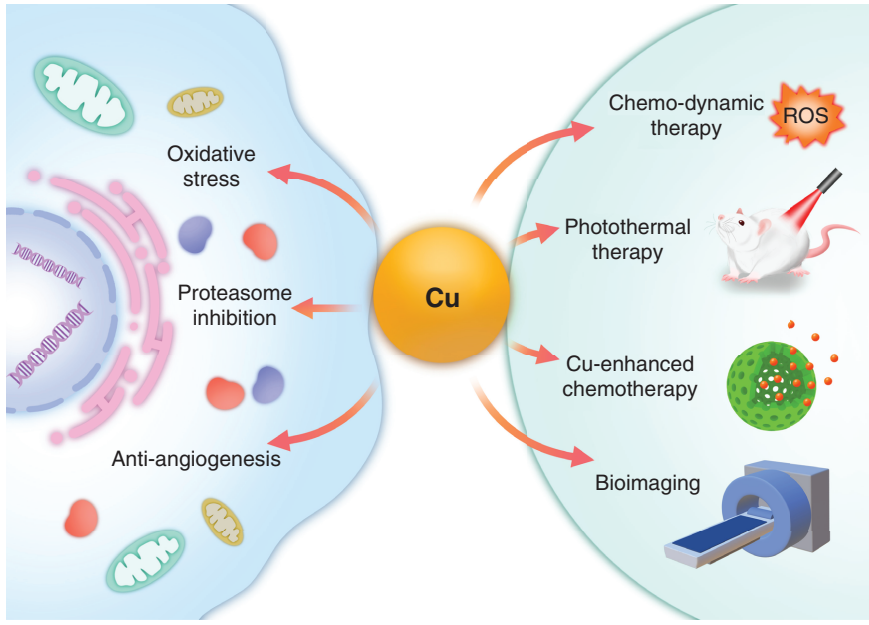


Figure 1. Schematic of the antitumor mechanisms and applications of copper.
ROS: Reactive oxygen species.

with other metal-based Fenton reactions [56,57].

$$\text{Haber} - \text{Weiss reaction} \quad Cu^{2+} + \cdot O_2 \rightarrow Cu^{+} + O_2$$

$$\text{Cu} - \text{based Fenton reaction} \quad Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH\cdot$$

Apart from the Fenton reaction, oxidative stress can also be triggered via the depletion of antioxidants. GSH, a γ-glutamyl-cysteinyl-glycine tripeptide, is one of the specific antioxidants. GSH related antioxidant defense system (ADS) exhibits a potent scavenging effect on the highly reactive hydroxyl to protect cells against injurious agents [58]. Remarkably, copper can oxidize reduced GSH to oxidized glutathione disulfide (GSSG) to achieve GSH depletion, further interfering with ADS to make cells more sensitive to harmful stimuli [59], while this reaction will not harm normal cells because of the commonly higher GSH concentration in the tumor milieu [60].

$$Cu^{2+} + \text{GSH} \rightarrow Cu^{+} + GSSG$$

A high concentration of ROS induced by Cu is also lethal to mitochondria, the power station of the cell in the maintenance of respiration and ATP production. The anticancer mechanism of the clinical antitumor drug elesclomol has been demonstrated to be related to selective transport of Cu to mitochondria and an increase in their local ROS [61]. Moreover, Liu *et al.* [62] also proved that baicalein, a natural flavonoid derived from traditional Chinese herbs, exerts its anticancer effect by affecting redox recycling of Cu ions to provoke OH production and induce mitochondrial apoptosis. Further, Cu can alter mitochondrial membrane permeability, regulate various gene levels involved in apoptosis and cause DNA cleavage, thereby realizing mitochondrial-mediated apoptosis [63–65]. In addition, Cu homeostasis is crucial because Cu is a vital cofactor in mitochondrial respiratory-chain-related enzymes [66]. Cui *et al.* [67] proved depletion of mitochondrial Cu could alter cell respiration patterns, giving rise to depressed oxidative phosphorylation (OXPHOS) and increased glycolysis, which is lethal to some cancer types that are dependent on OXPHOS (e.g., triple-negative breast cancer) because of insufficient power supply. Shao *et al.* [68] found that vital regulator p53 was overexpressed under Cu-induced oxidative stress stimuli and caused a series of proapoptosis processes. In addition, the Cu complexes ([Cu(ttpy-tpp)Br_2]Br, (CTB) they designed can also activate mitochondrial fission-related Drp1 protein, which is in the mitochondrial outside the membrane

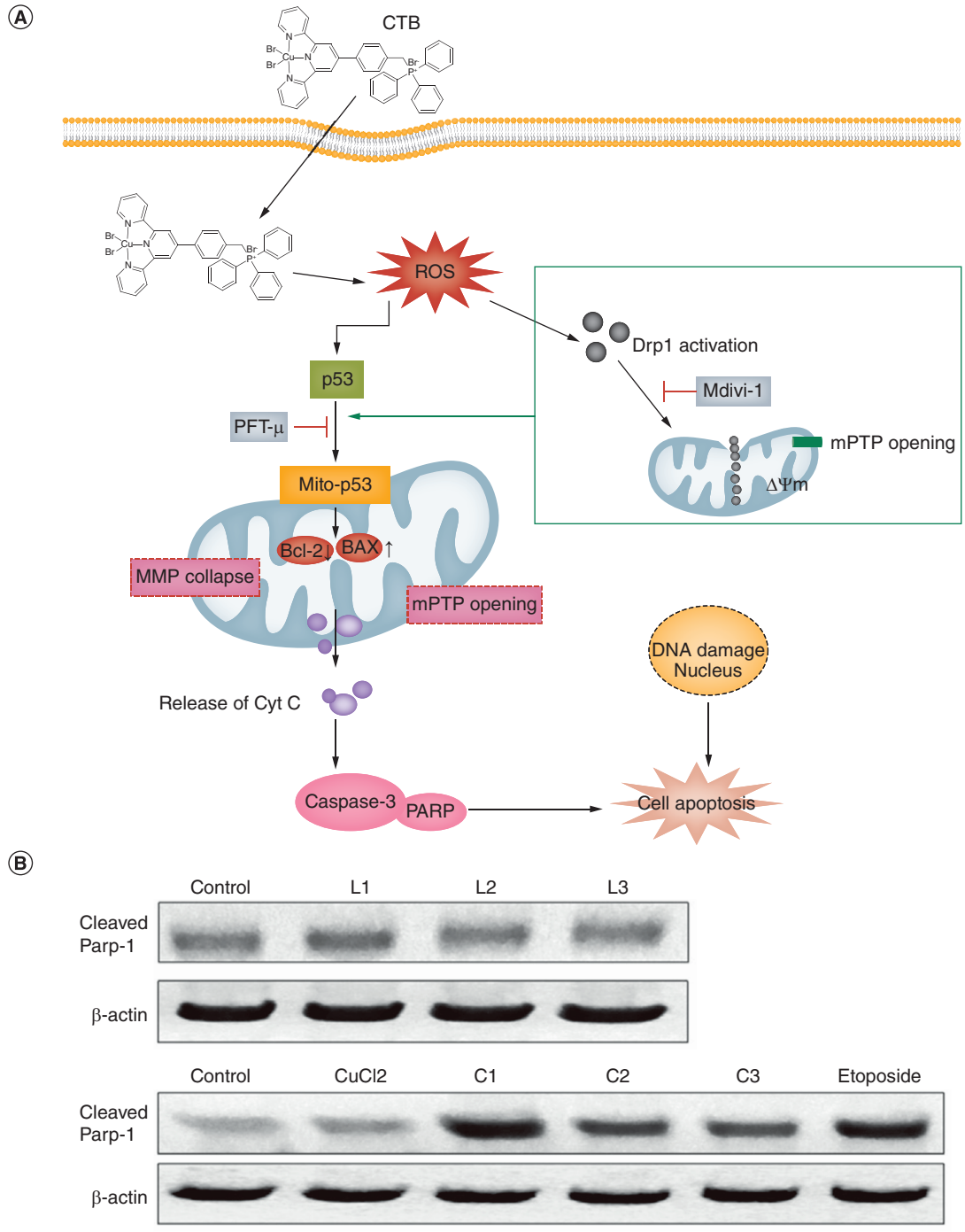


Figure 2. Copper-induced oxidative stress causes damage to mitochondria and DNA. (A) Illustration of the mechanism of CTB subunit-induced apoptosis in SMMC-7721 cells. **(B)** Western blot analysis of cleaved Parp-1 after incubated with 1 mM of ligands (L1, L2 and L3) and Cu (II) complexes (C1, C2 and C3) in HeLa cells for 24 h. Cu (II) complexes can increase cleaved Parp-1; however, ligands treatment cannot change the cleaved Parp-1.
CTB: [Cu(ttpy-tpp)Br2]Br; ROS: Reactive oxygen species.
(A) Reproduced with permission from [68], licensed with CC BY 4.0. **(B)** Reproduced with permission from [69], © 2018 Elsevier Masson SAS.

and can interact with Bax or Bak to disturb the mitochondrial out membrane potential (MOMP), prompting mitochondrial dysfunction (Figure 2A).

Studies have revealed that Cu complexes can cause DNA damage by inserting into DNA and then using the hydroxyl radicals generated by the Cu-based Fenton reaction to oxidize and decompose the DNA helix [70,71]. Additionally, Qi *et al.* [69] discovered that after incubating HeLa cells with Cu complexes, the expression of cleaved Parp-1 was significantly increased, implying that the DNA repair process has been hindered, which can expedite the apoptosis (Figure 2B). Further, Cu-induced oxidative stress can activate oncogenes and a large amount of harmful lipid peroxides generation, which can produce toxic secondary messengers and biomacromolecules damage (e.g., DNA and proteins), contributing to multiple pathological phenomena and apoptosis [72,73].

Except for apoptosis (type I programmed cell death [PCD]), Cu and Cu-induced ROS are also active participants in activation of the cell autophagy pathway (type II PCD) and necrosis [74,75]. Tsang *et al.* [76] proved that Cu could directly interact with autophagic kinases ULK1 and ULK2 to activate ULK1/2-dependent signaling and the formation of autophagosome complexes. For those tumor types that rely on autophagy to sustain tumor growth, for instance, $KRAS^{G12D}$-driven lung adenocarcinoma, Cu-chelation therapy will be an effective way. Moreover, Cu complexes could evoke the upregulation of autophagy-assisted proteins such as LC3 and p62 to promote autophagy-dependent cell death [77]. Meanwhile, Kang *et al.* [78] suggested that Cu complexes could simultaneously induce apoptosis and autophagy via oxidative-stress-mediated mitochondrial dysfunction. Although these results highlighted the pivotal role of Cu complexes in cancer treatment, the cell death pathway mediated by the Cu complex is different for diverse cancer cells. Further research is needed to better understand Cu-induced cell death.

Proteasome inhibition

The proteasome is a multiprotein complex located in the cytosol and nucleus. It mainly plays a role in degrading redundant or useless proteins and regulating cellular processes such as proliferation, apoptosis and metastasis. Research has confirmed that proteasome inhibition could inspire cytochrome c into cytosol and activate the caspase cascade, further provoking apoptosis in the tumor [79]. Interestingly, studies showed cancer cells are more dependent on proteasome for their rapid proliferation, which means they are more sensitive [80]. Moreover, studies have verified that some complexes containing transition metals, such as Cu, Mn and Au, showed excellent ability to inhibit proteasome [81]. Santoro *et al.* [82] confirmed that Cu (II) ions could exert the function of inhibiting proteasome by directly binding and partial redox effects in cell-free conditions or HeLa cells. More recently, except for specific proteasome inhibitors (PIs) such as bortezomib and carfilzomib, many Cu complexes were developed as PIs for cancer treatment, including clioquinol (CQ)–Cu, 8-hydroxyquinoline (8-OHQ)–Cu, disulfiram (DSF)–Cu and Schiff Base–Cu complexes [83].

In eukaryotic cells, the 26S proteasome consists of one 20S core particle and two 19S regulatory particles. Further, the 20S proteasome core exerts caspase-like, trypsin-like and chymotrypsin-like (CT-like) activities, consisting of β1, β2 and β5 subunits. In particular, the β5 subunit is regarded as pivotal apoptosis-inducing inhibitory sites, and only the inhibition of CT-like activities is adequate stimulus for apoptosis. Cater *et al.* [84] delivered bis(thiosemicarbazonato)–Cu complexes into prostate cancer cells and confirmed that increasing intracellular bioavailable Cu concentration could effectively kill the cancerous cells by inhibiting proteasomal CT-like activity mechanistically. Oliveri *et al.* [85] demonstrated a 22-fold increase in cytotoxicity and proteasome inhibition ability of 5-aminomethyl-8-hydroquinoline in the presence of Cu, which will be an excellent proteasome inhibitor, overcoming tumor cell's resistance to bortezomib. Zhou *et al.* [86] clarified that the Schiff-Base–Cu complex they synthesized, Cu(a4s1), can selectively inhibit the β2 subunit from achieving proteasome inhibition. They confirmed that Cu(a4s1) could be buried deeper in the β2 subunit structure than in the β5 subunit. Further, the amounts of hydrogen bind between Cu(a4s1) and β2 subunits are more than that in β5 subunits.

Proteasome inhibition and ubiquitinated protein accumulation can further cause activation of endoplasmic reticulum (ER) stress. Chen *et al.* [87] synthesized Cu diethyldithiocarbamate ($Cu(DDC)_2$) nanoparticles (NPs) for treating drug-resistant prostate cancers. To further study the anticancer mechanism of $Cu(DDC)_2$ NPs, they used a bright-field microscope to observe the morphology of DU145-TXR cells treated by $Cu(DDC)_2$ NPs (Figure 3A). Clearly, some cytoplasmic vacuoles originated in the ER. This result demonstrated that $Cu(DDC)_2$ NPs can cause paraptosis, a PCD mechanism featured in cytoplasmic vacuolation.

Antiangiogenesis

Angiogenesis is the initial process of both tumor proliferation and metastasis. A mass of nutrients supplied from blood vessels can feed an expanding tumor larger than 1–2 mm. Tumor tissues are generated on or around an existing vessel to form a perivascular cuff [89]. Angiogenesis depends on the action of multifarious small molecules,

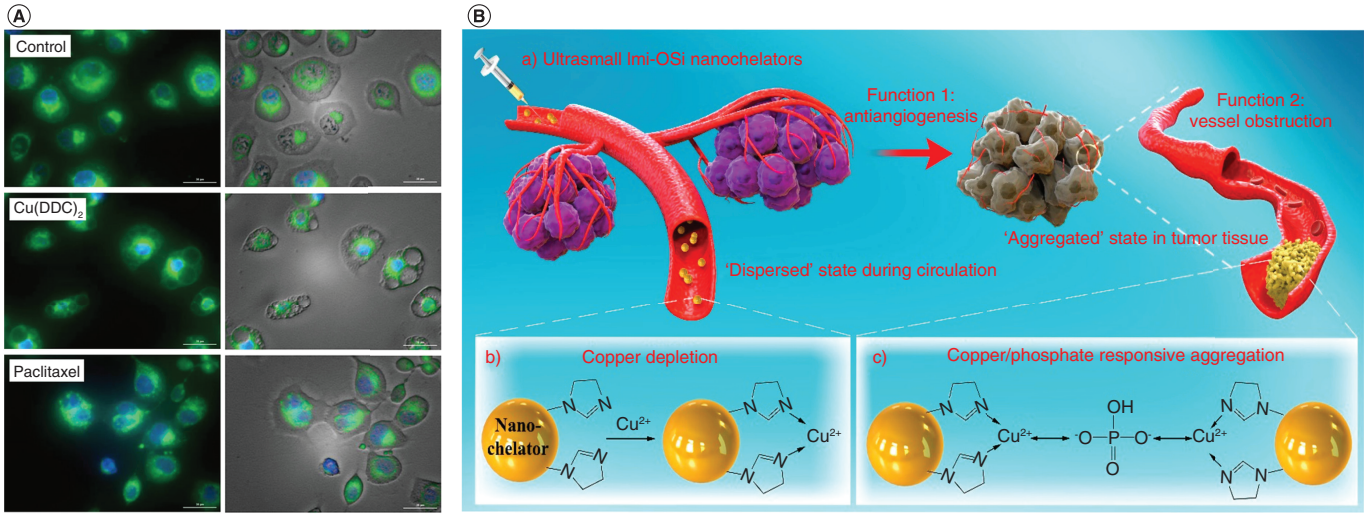


Figure 3. Copper is a vital element during proteasome inhibition and angiogenesis. (A) $Cu(DDC)_2$ nanoparticles (NPs) induced paraptosis manifested by cytoplasmic vacuolation resulted from dilated endoplasmic reticulum (ER). (DU145-TXR cells were treated with blank polyethylene glycol–polylactic acid, polyethylene glycol–polylactic acid/$Cu(DDC)_2$ NPs, and paclitaxel for 8 h and stained with ER-ID green dye and Hoechst 33342. Right panel: Merged fluorescence and bright field images. **(B)** Scheme of the antiangiogenesis functions of Imi-Osi (imidazole and organosilica) nano-chelators.
$Cu(DDC)_2$: Cu diethyldithiocarbamate.
(A) Reproduced with permission from [87], © 2018 American Chemical Society. **(B)** Reproduced with permission from [88], © 2019 American Chemical Society.

including VEGF, essential bFGF and IL-1. Studies have reported that Cu is a pivotal element that can promote angiogenesis and can not only link to HIF-1 to activate these crucial factors mediating angiogenesis, but also binds to angiogenin to stimulate the formation of blood vessels and influence endothelial progenitor cells to foster angiogenesis. Furthermore, Cu has been shown to directly motivate endothelial cell migration and proliferation and fibronectin synthesis, both essential in angiogenesis [90,91]. Copper depletion can turn off the 'angiogenic switch,' block the endothelial cell proliferation process and halt the cell cycle in the G_0 phase. In addition to direct capture of cellular Cu ions, inhibition of Cu transporters or chaperones (e.g., ATOX1 and CTR-1) is another way to realize Cu imbalance and antiangiogenesis [92–94].

Several traditional Cu chelators are used to suppress angiogenesis by decreasing the Cu concentration and downregulating the factors associated with vascular growth, such as penicillamine, tetrathiomolybdate (TM), captopril and trientine [95]. Among these, TM has been used in Wilson's disease as a Cu depletion agent for decades [96]; recently, its antineoplastic efficacy was explored and studied in early clinical trials as a novel cancer therapy for metastatic cancers [97,98]. Today, the research and development cost of new anticancer drugs is prohibitive. The concept of 'new uses for old drugs' reduces the investment in drugs dramatically and brings new inspiration for treating disease. The anticancer mechanism of TM can be explained by the formation of a stable tripartite complex consisting of TM, Cu and proteins that trigger out-of-balance Cu homeostasis in tumor cells, further suppressing VEGF and other growth factors, resulting in obstruction of the angiogenesis pathway [99].

The Cu-mediated antiangiogenesis effect is a substantial leap forward. Still, when combined with other cancer therapies or new vessel-targeting mechanisms, the tumoricidal impact can be further amplified. For example, immunotherapy has shown potent anticancer effects, but its implementation is mired by a short action time and it is only effective for a minority of patients. With the help of Cu's antiangiogenesis property, immunotherapeutic drugs combined with Cu chelators will be a novel curative system for cancer [100]. In addition, Sun *et al.* [101] designed a micellar carrier CPLP-X for codelivery of DOX and Probe X (as a Cu chelator and tracer) to achieve a synergistic effect containing antiangiogenesis and CT. CPLP-X affects angiogenesis by chelating Cu ions like crabs grabbing food. On the other hand, the disequilibrium of Cu homeostasis will also destroy other basic cell growth processes and antioxidant defense systems, which can enhance DOX-involved chemotherapeutic effects.

Certainly, vessel-targeting therapy involves not only antiangiogenesis but blocking the vessels with 'blood clots' to cut off the nutrient supply for tumor tissues. For example, a dual-function nanoscale Cu chelator, Imi-Osi,

which consists of imidazole and organosilica, can exert excellent Cu-capturing ability (Figure 3B). After chelating with Cu ions to cause Cu deficiency and vessel inhibition, these NPs transition between 'dispersed' and 'aggregated' states. Thrombi that are formed will lead to vessel obstruction under the stimulation of elevated Cu and phosphate levels to reinforce tumor vasculature-targeted therapy [88].

Antitumor applications of copper complexes

Chemodynamic therapy

With the overwhelming upsurge of nano-catalysts, CDT, owing to its tumor-specificity and relative safety, has received considerable attention in the field of tumor therapy. Cu-based CDT induces apoptosis by using the Fenton reaction, producing a high concentration of toxic ROS and further increasing oxidative stress and biomacromolecules damage [102]. For instance, self-assembled Cu–amino acid mercaptide NPs (Cu-Cys NPs) could react with local GSH and reduce Cu^{2+} to Cu^{+}. Subsequently, the generated Cu^{+} would react with H_2O_2 to increase ROS level. Overloaded ROS would activate a continuous oxidative stress response leading to cell apoptosis [27]. Copper–thiosemicarbazones (Cu–TSC) complexes are another famous Cu-containing family to achieve ROS accumulation and enhanced cytotoxicity of TSC for treating cancers [103]. It is worth mentioning that inflammation in the body can induce carcinogenesis and promote cancer metastasis. Hence, some Cu (II) complexes containing nonsteroidal anti-inflammatory drugs (NSAIDs) are emerging to treat inflammation for preventing and treating cancer. Boodram *et al.* [104] proved Cu–NSAID complexes could induce ROS accumulation, DNA damage and COX-2 activity inhibition in breast cancer stem cells (CSC)-like cells.

Moreover, Cu complexes with subcellular targeting properties can engender more precise strikes to combat cancer cells. Kaur *et al.* [105] reported that a Cu (II) complex with a Schiff base ligand and a polypyridyl ligand could enter the ER and elevate the ROS level *in situ* to induce ER stress, further evoking immunogenic cell death mode in breast CSCs. Given the elevated Cu levels and redox vulnerability in cancer cells, Cu-involved pro-oxidative anticancer agents (PAA) are developed to target this particular feature. The 3-hydroxyflavone-inspired Cu pro-ionophore (PHF) is the effective PAA, which can simultaneously influence GSH, Cu and ROS levels to target the vulnerable redox balance of cancer cells [106].

However, although the CDT of Cu-involved nano-catalysts has been widely tested and made noticeable advances in cancer therapies, it also faces many challenges that can impair the CDT performance, such as insufficient endogenous H_2O_2 level, overexpressed GSH and the hypoxia tumor environment [107,108]. Despite the concentration of H_2O_2 in tumor cells surpassing that in normal cells, it cannot meet the requirement for producing enough toxic ROS *in situ* [109]. To address this dilemma, many novel approaches have been considered, and many other therapeutic strategies have been integrated into synergistic therapies to enhance the efficacy of Cu-mediated CDT [110,111]. Wang *et al.* [28] found that the Fenton-like catalytic performance of copper sulfide (CuS) is relevant to the surface reaction sites. Thus, they compared the CDT efficacy of hollow and solid Cu_9S_8 NPs by evaluating hydroxyl radicals' generation by methylene blue (MB) assay and ROS fluorescence probe test. Under the same temperature condition (298 and 316 K), hollow Cu_9S_8 NPs can reach 46 and 95% MB degradation separately, whereas solid Cu_9S_8 NPs only obtained 37 and 67% degradation rates (Figure 4A & B). This result is consistent with the 4T1 cell viability test that indicated hollow Cu_9S_8 NPs exhibit better CDT effects. Currently, metal-organic frameworks (MOFs), with their high porosity, large surface area and tunable functionality, are emerging carriers for Cu delivery [112–114]. Tian *et al.* [29] used trimesic acid to synthesize Vk3@MOF-199, which integrated Cu ions and Vk3. Vk3 can use the high expression of NQO1 to achieve the tumor-specific H_2O_2 amplification, and the elevated H_2O_2 level can enhance Cu-based Fenton reaction-induced CDT (Figure 4C).

Except for copper ions, other transition metals also possess Fenton or Fenton-like catalytic ability. On this basis, combining Cu and other metal complexes will improve single Cu-mediated CDT. Wang *et al.* [30] designed an innovative nanostructure, PCN-224(Cu)-GOD@MnO_2. The addition of MnO_2 can aid in the production and accumulation of H_2O_2. Meanwhile, Cu^{2+}-TCPP can deplete excess GSH to reduce antioxidant activity. Those two mechanisms of PCN-224(Cu)-GOD@MnO_2 improve the CDT effect. Moreover, although as the classical Fenton reaction catalyst, the reaction pH range of Fe-mediated reaction is too narrow to match the actual weak acidic condition of the tumor. Thus, for sufficient ROS generation, Cu ferrite nanospheres (CFNs), which have two redox repairs (Fe^{2+}/Fe^{3+} and Cu^{+}/Cu^{2+}), were designed, and this CFN showed good tumor inhibition performance with the help of near IR (NIR) irritation [31]. Surprisingly, the chemotherapeutic drug DOX also has the ability to increase the efficiency of Fenton's reaction. Cao *et al.* [32] co-loaded DOX in the bovine albumin (BSA)–Cu structure to form DOX@BSA-Cu. They confirmed that DOX at low concentration is nontoxic to cells but can reduce O_2 to

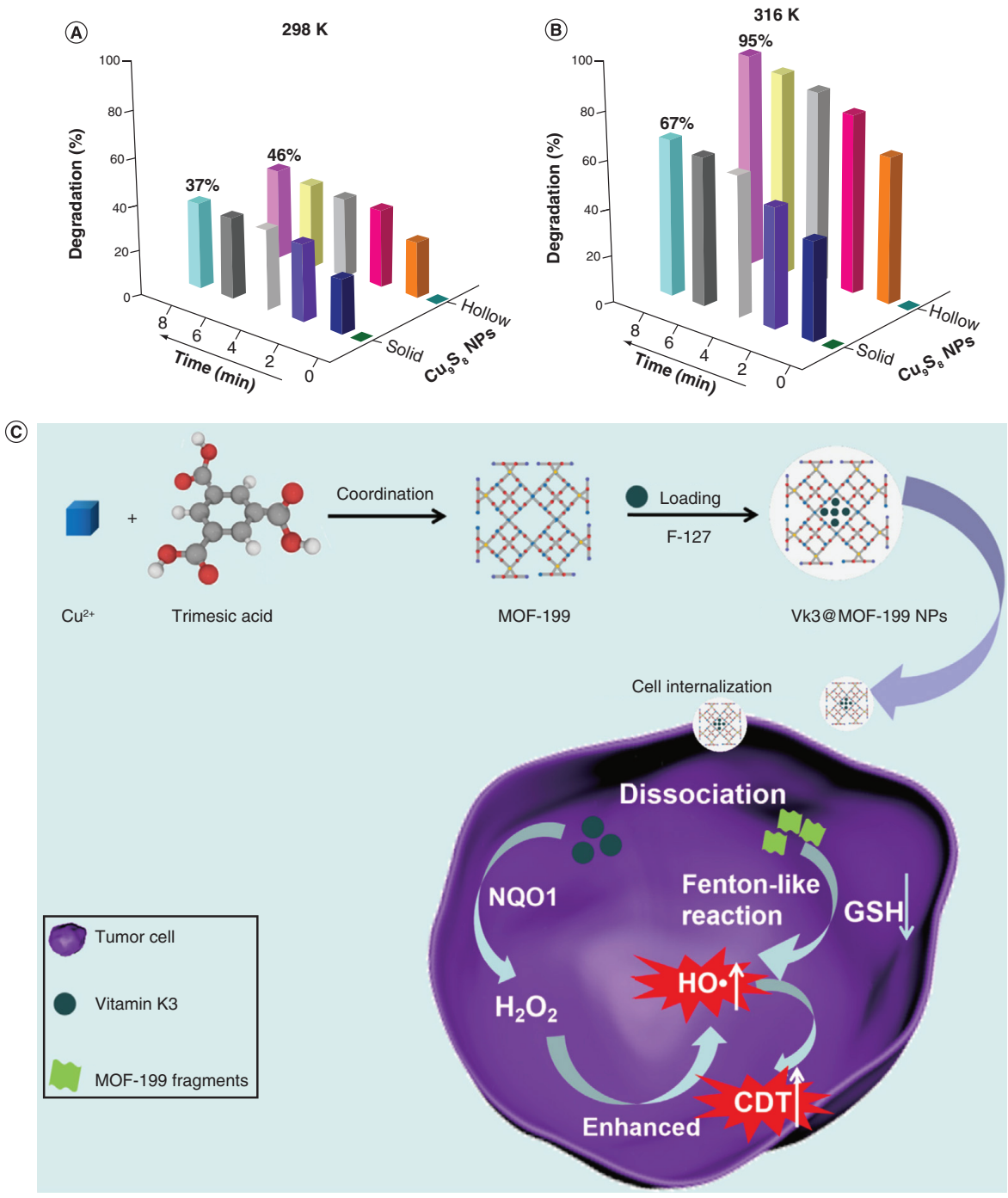


Figure 4. The chemodynamic therapy effects induced by copper complexes. (A & B) Colorimetric analysis of the hollow and solid Cu_9S_8 Fenton-like reaction of methylene blue decolorization at 298 K and 316 K separately at 1–9 min under dark conditions. **(C)** Scheme of the synthesis of Vk3@MOF-199 NPs and the chemo-dynamic therapy. GSH: Glutathione; MOF: Metal-organic framework; NP: Nanoparticle.
(A & B) Reproduced with permission from [28], © 2019 Elsevier B.V. **(C)** Reproduced with permission from [29], © 2020 Elsevier Inc.

O_2^- through electrons transfers between anthracene and semiquinone structures and ultimately increase the content of H_2O_2 for enhanced CDT.

In addition to enhancing the efficacy of the Fenton reaction, Cu complexes can also be fabricated into carriers or combined with other therapies for advanced anticancer effects. Pinho *et al.* [33] proved that Cu(II)-phenanthroline

complexes loaded in pH-sensitive long-circulating liposomes can amplify their anticancer activity in colon cancer treatment. Copper/manganese silicate nanospheres (CMSNs) were coated by MCF-7 cancer cell membranes (designated as mCMSNs) for improving the biocompatibility and homotypic targeting ability of nanoparticles. This well-defined Cu silicate could achieve CDT and release O_2, which will relieve oxygen deficiency in PDT. Meanwhile, light stimuli such as UV-Visible (UV-Vis) or NIR laser irradiation could also improve ROS generation efficiency in Fenton reactions [115]. These mechanisms work together to impair the antioxidant defense of cancer cells and make the cells more sensitive to ROS, resulting in excellent inhibition effect on tumors.

Phototherapy

Most common phototherapies contain photodynamic therapy (PDT) and photothermal therapy (PTT). PDT is a noninvasive therapy for premalignant and neoplastic diseases. High spatiotemporal precision, controllability and tumor targeting have helped promote PDT in various cancer treatments. Three factors are indispensable for realizing PDT: photosensitizer (PS), light source and molecular oxygen. Cu and other transition metal complexes possess outstanding photophysical characteristics, making them ideal PS candidates [116]. Cu (I) bis-phenanthroline complex displayed *in vitro* photocytotoxicity and DNA damage ability in A549 cancer cells upon blue light excitation [117]. However, the visible light is not highly penetrating. Veeranarayanan *et al.* [34] fabricated a bimetal chalcogenide nanocrystal, Cu_3BiS_3 NC, which can respond to ultra-low NIR laser power; this led to complete tumor regression without damage to the skin. Except for UV-Vis light, due to the high penetration rate of x-ray, it is also used for photosensitizer activation. Cu–cysteamine nanoparticles (Cu–Cys NPs) conjugated with a pH-low insertion peptide (pHLIP) could be excited by x-ray for enhancing the PDT efficacy and tumor treatment [35].

PTT is another kind of anticancer treatment regimen using light. It has aroused broad research interest, exhibiting unique advantages including noninvasiveness, high specificity, short treatment time and intense tissue penetration. PTT realizes tumor irradiation through hyperthermic ablation irradiated by an NIR laser. Hyperthermia heats tumor cell temperature to exceed the cytotoxic threshold (42.5°C), inducing cell death in tumor tissue and ensuring the survival of surrounding normal tissues [118]. Coincidently, Cu chalcogenides have strong absorption in the NIR wavelength range, so they are often used as typical photothermal agents [119,120]. Cu@CPP-800, comprising porous carbon polyhedral with Cu incorporated, showed excellent photothermal conversion properties. Under 808-nm NIR irradiation, the photothermal conversion efficiencies (η) of Cu@CPP-800 reached 48.5%, and the temperature in the tumor site reached 64.6°C after 10-min irradiation following intravenous injection of Cu@CPP-800 (Figure 5A) [36]. Although Cu-mediated PTT has shown excellent irradiation effects on tumors, residual tumor cells will trigger tumor recurrence after insufficient ablation [121]. More precise or organelle targeting Cu-mediated PTT effects are needed. For instance, nucleus-targeting moiety helps Cu complexes destroy genetic substances, strengthening the Cu-mediated PTT efficacy and avoiding overheating normal tissues [122]. PDA is a widely explored photothermal conversion agent, and it can be used as a coating for Cu complexes to enhance hyperthermia because of its excellent adhesion property [37]. In addition, after PTT treatment, tumor cells may release antigens to surrounding tissue. Wang *et al.* [38] thus synthesized a Cu-doped hybrid nano-enzyme that exhibited immunotherapy, starvation and photothermal functions to effectively inhibit tumor growth and metastasis.

However, a single phototherapy cannot completely eliminate the tumor. An important factor contributing to the efficacy of PDT is sufficient oxygen concentration to generate enough ROS, whereas the tumor hypoxia microenvironment cannot satisfy this condition. Further, inadequate PTT will promote tumor metastasis [123,124]. Hence, to avoid these side effects and improve the efficiency of anticancer treatment, many studies combined PDT with PTT to achieve tumor eradication and metastasis inhibition. Black phosphorus (BP) nanosheets possess a high surface-to-volume ratio so that it can be used as a novel drug-delivery platform and is itself a next-generation water-dispersible inorganic photosensitizer. CuS nanodots and folic acid (FA) can be modified in the BP nanosheets to fabricate high biocompatibility phototherapy agents designed as BP–CuS–FA (Figure 5B), which have promising potential for synergistic PDT–PTT cancer treatment [39]. Curcio *et al.* [40] combined iron oxide nanoflower-mediated magnetic hyperthermia (MHT) with CuS-mediated PDT–PTT to achieve an image-guided tritherapeutic tumor strategy. Usually, however, the effects of PDT and PTT are often activated by two different lasers, respectively. Girma *et al.* [41] synthesized BSA-functionalized chalcopyrite $CuFeS_2$, which can have an antitumor role under a single 671-nm laser irradiation to address this dilemma and simplify the treatment procedure. Some studies also clarified that noble metal materials could enhance the photothermal performance of CuS NPs based on the localized surface plasmon resonance effect. For instance, under NIR irradiation, Au–CuS yolk-shell nanoparticles could inhibit

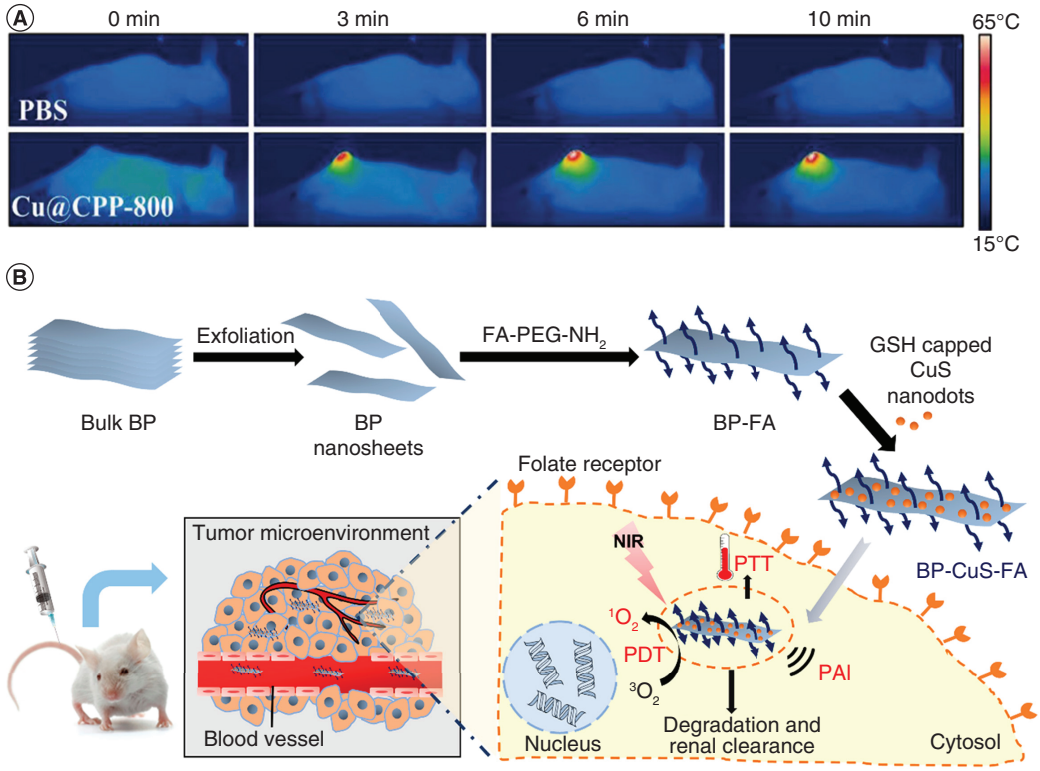


Figure 5. Copper complexes exhibit remarkable photothermal therapy and photodynamic therapy effects. (A) Near IR (NIR) thermal images of HeLa tumor-bearing mice at other time points 6 h after intravenous injection of a Cu@CPP-800 dispersion or PBS under NIR irradiation (808 nm). **(B)** Schematic illustration of the preparation of BP–CuS–FA nanoconjugate for photoacoustic-imaging-guided and tumor-targeted synergistic photodynamic–photothermal therapy in cancer treatment.
BP: Black phosphorus; FA: Folic acid; GSH: Glutathione; PBS: Phosphate-buffered saline.
(A) Reproduced with permission from [36], © 2019 WILEY-VCH Verlag GmbH & Co. **(B)** Reproduced with permission from [39], © 2020 American Chemical Society.

markedly tumor growth, further expanding the feasibility and application of CuS-induced phototherapy in tumor inhibition [42].

Cu-enhanced CT

Clinical CT is defined as using one or chemotherapeutic drugs to treat cancer. These drugs are considered a 'double-edged sword' because they all have large cytotoxicity to normal as well as malignant cells, resulting in unexcepted side effects such as inhibited growth of hair, bone marrow and gastrointestinal tract cells. Therefore, CT possesses the most effective therapeutic effect among various therapies, but its applications remain limited [125].

As early as 1983, Kimito *et al.* [126] proved that Cu complexes could increase the antitumor effect of ascorbate; these authors obtained a good survival rate in an Ehrlich ascites tumor mice model after intraperitoneal administration of ascorbate and Cu glycylglycylhistidine complexes. Given that Cu and its complexes can be molded or integrated into other nanoplatforms to form various shapes quickly, Cu complexes have received much attention as CT drug carriers. The structures of Cu-involved drug vectors can be divided into three main types. First, Cu complexes with a center hole can provide a reservoir for drug molecules. For example, hollow CuS (HCuS) has a mesoporous structure with a large surface area and high internal cavity for anticancer drug-loading. Furthermore, it is also an excellent phototherapy and imaging contrast agent [127]. Second, Cu complexes are hybridized into other hollow or porous frameworks for drug-loading. Third, Cu can be combined with CT drugs to form infinite co-ordination polymer or MOF to achieve synergistic anticancer effects.

Dong *et al.* [43] used hollow CuS nanoparticles as drug-delivery systems to confer high stability, distinctive photothermal conversion and hydrophilicity to load the hydrophobic anticancer drug CPT to increase the low solubility of CPT and enhance 980-nm laser-triggered tumoricidal efficacy with a lower drug dose. Further, because

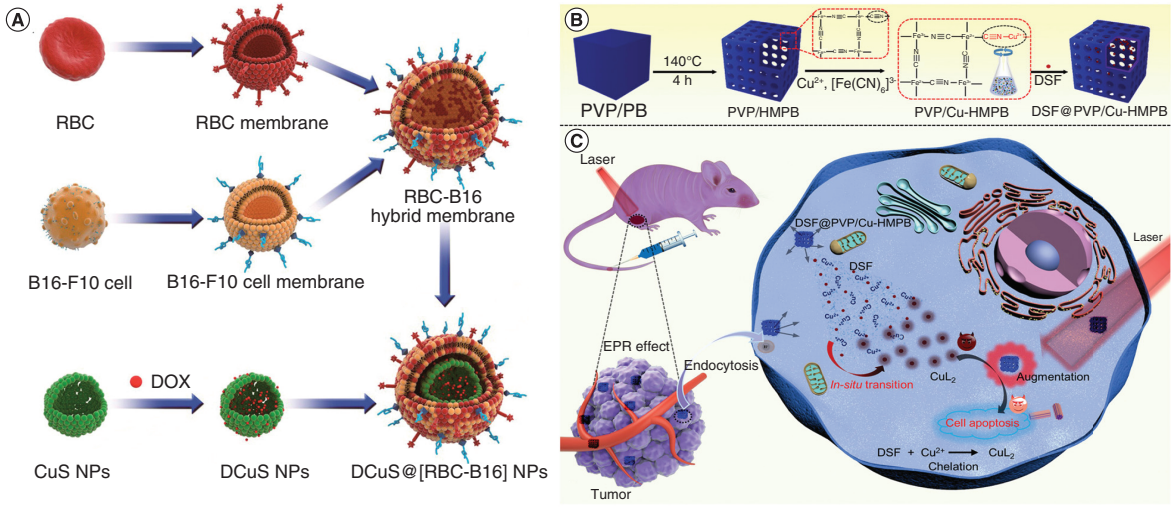


Figure 6. Copper complexes can act as a chemotherapeutic drug carrier. (A) Scheme of membrane encapsulation process of DCuS@[RBC-B16] NPs. **(B)** Construction process of DSF@PVP/Cu-HMPB nanomedicine. **(C)** Illustration of the tumor-specific *in situ* transition of DSF into CuL_2 by DSF-Cu^{2+} chelating reaction and augmentation generated by photothermal effect under near IR laser irradiation.
DSF: Disulfiram; EPR: Enhanced permeability and retention; HMPB: Hollow mesoporous prussian blue; NP: Nanoparticle; PB: Prussian blue; PVP: Polyvinylpyrrolidone; RBC: Red blood cell.
(A) Reproduced with permission from [45], © 2018 American Chemical Society. **(B & C)** Reproduced with permission from [52], © 2020 Wiley-VCH Verlag GmbH & Co.

Cu^{2+} and CT drugs must be accurately accumulated in tumor cells to avoid toxic side effects, Feng *et al.* [44] used FA to augment hollow mesoporous CuS NPs loaded with the anticancer drug bleomycin (BLM). They chose L-menthol (LM) as the drug leak-blocking agent. Because CuS NPs can absorb light and change it to heat, temperature rise during NIR irradiation will activate the 'solid–liquid' phase transition of LM. Meanwhile, benefiting from the tumor-targeted feature of FA, this intelligent NIR-responsive nanostructure can realize controlled drug release *in situ*. To promote the specific targeting ability of drug-loaded Cu complexes and avoid the immune system, cell-membrane-coated technology is a feasible and emerging approach to endow NPs with good biocompatibility. Hence, after encapsulating DOX with hollow CuS, using hybrid membrane consisting of red blood cells (RBCs) and melanoma cells (B16-F10 cells) to camouflage drug-loaded nanoparticles and finally compose DCuS@[RBC-B16] NPs (Figure 6A). These membrane camouflaged NPs can interfere with melanoma tumor growth, with a nearly 100% inhibition rate and enhanced homogeneous targeting ability, improving the PTT efficacy of CuS and reducing damage to surrounding tissue [45].

In addition to the hollow structure of the Cu complex itself, Cu can be integrated into other hybrids. For instance, Zhang *et al.* [46] connected CuS NPs and mesoporous silica platform loaded DOX with two complementary DNA sequences to construct a NIR/thermal-triggered drug-delivery system. Recently, Cu-doped layered double hydroxide (LDH) nanosystem as a multimodal delivery carrier came up views. Liu *et al.* [47] introduced a novel Cu-based nanoplatforms for skin-tumor treatment consisting of three components: Cu-doped LDH as the primary scaffold, indocyanine green as the PTT/PDT agent along with Cu and BSA-DOX as the acid-responsive prodrug. This multifunctional nanodrug could combine PDT, PTT and CT to lower the doses of CT drugs and systemic toxicity.

Moreover, because Cu can form coordination bonds with drugs or various donor ligands and formed Cu coordination complexes are also biologically active agents in disease therapies [128]. For instance, metformin (Met), is an antidiabetes agent; Cu coordination complex Cu–Met NPs can be used as a novel nanoscale vehicle to load DOX for fabricating DOX@Cu-Met NPs. Cu^{2+} offers the Met-based drug-delivery system stability to prevent premature drug leakage. Moreover, Met and DOX in this system as mitochondrial respiration inhibitors and CT drugs, respectively, can release from nanostructures under GSH and acid stimuli to relieve the intratumoral hypoxia and promote H_2O_2 accumulation together. Consequently, Cu-induced CDT efficacy can be enhanced [48]. Shen *et al.* [49] were inspired by this property and designed a dual-drug-loaded carrier-free nanomedicine. They chose CT drugs AQ4N and gossypol and used their plentiful hydroxyl and aldehyde group to coordinate with Cu^{2+} to

synthesize HA@AQ4N-Cu(II)-gossypol NPs. This versatile carrier-free nanomedicine could be accumulated at the tumor site through three targeting properties, and Cu^{2+} in this structure can not only serve as a connecting agent but also enhance the antitumor effect. This nanomedicine could significantly decrease the dose of CT drugs with the use of only one-fiftieth of AQ4N and half the gossypol of general clinical doses.

Beyond traditional CT drugs, some marketed drugs can also combine with Cu-induced CDT to achieve previously unattainable anticancer activity. DSF is commonly used as an anti-alcoholism drug but has become a hot topic recently due to its surprising anticancer activity. DSF–Cu can augment ROS levels and arrest cell cycle progression at the G_0/G_1 phase [129]. Studies have clarified that this unexpected tumor-lethality activity of DSF–Cu comes from the Cu-DDC complex consisting of DSF's metabolites DDC and Cu [130]. Hence, delivering the $Cu(DDC)_2$ directly to the tumor site becomes a feasible strategy to enhance DSF–Cu-mediated anticancer efficacy. Said Suliman *et al.* [50] chose cyclodextrin to encapsulate $Cu(DDC)_2$ for increased apparent solubility and stability against the chemo-resistant triple-negative cancer cells. Hartwig *et al.* [51] tested the antitumor activity of liposomal $Cu(DDC)_2$ in both 2D and 3D cell models. Especially in neuroblastoma 3D spheroids, $Cu(DDC)_2$ liposomes showed significant cytotoxicity, making its *in vivo* antitumor effect more credible. In addition, the application of Cu^{2+}-enriched hollow mesoporous Prussian blue (HMPB) as a drug carrier is gradually emerging. It can also be used as a pH-responsive carrier to load DSF. An acidic environment can destroy this nanostructure, and DSF and Cu^{2+} are released simultaneously to form $Cu(DDC)_2$ via DSF-Cu^{2+} chelating reaction (Figure 6B & C). Further, the PTT effect induced by Cu-HMPBs upon NIR irradiation will augment the anticancer effects of DSF–Cu. The weight of the tumor after DSF@PVP/Cu-HMPB and laser treatment is obviously smaller than other groups, indicating that DSF@PVP/Cu-HMPB offers a promising capability for cancer treatment [52].

Bioimaging

Magnetic resonance imaging

MRI is an essential imaging method to detect cancer due to its excellent soft-tissue contrast and unlimited penetration depth. MRI uses radiofrequency pulse in an external magnetic field to excite the spin of protons in human bodies and generate NMR signals to form images at the cellular and subcellular levels [131]. Because of unpaired electrons in its outermost orbital, the Cu complex is regarded as an alternative MR contrast agent [132,133]. Liu *et al.* [134] invented an intriguing Cu (I) phosphide nanocrystal (CP NCs) as an *in situ*-generated MRI contrast agent by using a redox reaction between diamagnetic Cu (I) and paramagnetic Cu (II). The CP NCs can avoid the poor target-to-background MRI ratios. Nowadays, scientists coalesce Cu with other transition metals to assemble hybrid coimaging agents for enhanced MRI intensity. For example, bimetallic gold/copper nanostructured composites were synthesized for contrast-enhanced dual-modal x-ray computed tomography (CT) and MRI, which can increase flexibility and replaceability of radiotherapy system [135]. Wang *et al.* [136] constructed ternary chalcogenide $Cu_xFe_yS_z$ with Cu and Fe, which takes advantage of the excellent NIR photoabsorption property of FeS_2 and photothermal effect of CuS. Finally, they chose Cu_5FeS_4 with the optimal Cu/Fe ratio for MR imaging, and the MRI scans showed strong ability to locate tumors (Figure 7A). Sang *et al.* [137] synthesized $Ni_3S_2/Cu_{1.8}S$ nano-heterostructures and found Ni and Cu ions in this system are commonly used in MR images due to the spin single electrons and the paramagnetic ability. Undoubtedly, $Ni_3S_2/Cu_{1.8}S$ showed considerable capability in therapy and real-time monitored imaging *in vivo*. Radiotherapy (RT) was also found to help enhance Cu-mediated MRI sensitivity. Fan *et al.* [138] used low-dose RT treatment 3 days before intravenous injection of G5.NHAc-Pyr/Cu (II) complexes. The results of a quantitative analysis of tumor MR signal-to-noise ratio (SNR) at different time points (Figure 7B) indicate that the treatment with RT had significantly higher SNR than that without RT, indicating that RT treatment does enhance MRI intensity and prolong imaging time of Cu complexes.

Photoacoustic imaging

Photoacoustic imaging (PAI) is a novel technology that uses optical excitation and ultrasonic detection. PAI uses a nanosecond-pulsed laser beam to irradiate biological tissues; after molecules *in vivo* absorb light, thermal energy induces pressure jump, which can launch ultrasonic waves. Acoustic detectors then receive these ultrasonic impulse signals and form images [141].

In oncology, some specific overexpressed endogenous molecules (e.g., GSH) can aid in tumor diagnosis. Detection of those biomarkers by PAI requires the PAI agent to recognize abnormal signals and then switch to a detectable state. To address this issue, an Fe-Cu@PANI (iron-copper co-doped polyaniline) nanoparticle was synthesized. Cu^{2+} in this system can react with GSH to produce protonated PANI, which can generate heat under NIR

Table 1. Summary of copper complexes and their therapeutic applications.

Copper complexes	Therapy	Tested model	Remark	Ref.
Cu-Cys NPs	CDT	MCF-7R-bearing NOD-SCID mice	*In situ* glutathione-activated and H_2O_2-reinforced CDT	[27]
Hollow Cu_9S_8 NPs	CDT	4T1 tumor-bearing mice	Enhanced CDT due to the increased number of active sites and photothermal performance compared to solid Cu_9S_8 NPs	[28]
Vk3@MOF-199	CDT	4T1 tumor-bearing mice	NQO1 catalyze Vk3 to produce sufficient H_2O_2 for amplified CDT	[29]
PCN-224(Cu)-GOD@MnO_2	CDT	U14 tumor-bearing mice	O_2 and H_2O_2 supplied by MnO_2 and GOD enhance the Cu-mediated Fenton reaction efficacy	[30]
CFNs	CDT/PDT/PTT	U14 tumor-bearing mice	Photo-enhanced CDT with TME-modulating capacity	[31]
DOX@BSA-Cu	CDT	4T1 cells and MCF-7 cells	DOX improves the H_2O_2 content and hydroxyl radical generation for enhanced CDT	[32]
mCMSNs	CDT/PDT	MCF-7 tumor-bearing mice	Target-cell-specific GSH depletion-enhanced CDT; hypoxia-relieved PDT	[33]
Cu_3BiS_3 NCs	PDT	MCF-7 tumor xenograft-bearing mice	Cu_3BiS_3 nanocrystals realize complete tumor regression by using an ultra-low dose NIR laser	[34]
pHLIP-Cu-Cy	PDT	CRL-2116 tumor-bearing mice	Cu-Cy NP-induced PDT can be activated by X-ray leads to improved penetration depth and tumor treatment effect	[35]
Cu@CPP-800	PTT	HeLa tumor-bearing mice	Cu@CPP-800 possess outstanding photothermal conversion efficiency of 48.5% at 808-nm laser irritation and excellent tumor ablating ability	[36]
CuO-NPs@PLGA/PDA/PEG	PTT	Cal-33 cells	PDA shells enhance the NIR absorption of CuO NPs	[37]
CuCo(O)/GOx@PCNs	PTT/IMT/starvation therapy	4T1 tumor-bearing mice	Oxygen supply, glucose consumption and photothermal ablation can be achieved by CuCo(O)/GOx@PCNs; immune response effect can further inhibit metastasis and recurrence of tumor	[38]
BP-CuS-FA	PDT/PTT	4T1 tumor-bearing mice	A biocompatible and photodegradable CuS carrier can achieve a single laser-activated PDT/PTT process	[39]
IONF@CuS nanohybrid	PDT/PTT/MHT	PC-3 tumor bearing nude mouse	This all-in-one nanohybrid provides a cumulative heating and novel tri-therapeutic tumor strategy	[40]
Ce6:$CuFeS_2$@BSA-FA	PDT/PTT	HeLa and HepG2 cells	Synergistic PDT–PTT of Ce6:$CuFeS_2$@BSA-FA can be induced by a single laser	[41]
Au-CuS YSNPs	CDT/PDT/PTT	4T1 tumor-bearing mice	Au-CuS YSNPs enhance the PDT/PTT efficacy due to localized surface plasmon resonance effect	[42]
CPT@CuS	CT	H22 cells-bearing mice	CuS NPs improve the solubility and increase the biocompatibility of CPT; Cu-induced PTT further increase synergistic performance	[43]
FA-HM$Cu_{2-x}S$/BLM/LM	CDT/PDT/PTT/CT	MCF-7 tumor-bearing mice	NIR-response drug release and further initiated BLM activation to induce DNA cleavage	[44]
DCuS@[RBC-B16] NPs	CDT/PTT/CT	melanoma-bearing mice	Membrane camouflages prolong the lifetime and enhance the homogeneous targeting ability of DCuS@[RBC-B16] NPs	[45]
MSN-DNA-CuS	PTT/CT	HeLa and MCF-7 cells	Photothermal controllable and GSH response drug release	[46]
ICG/Cu-LDH@BSA-DOX	PDT/PTT/CT	B16F0 tumor-bearing mice	pH sensitive drug release and achieve high tumor elimination rate with a low dose	[47]
Dox@Cu-Met NPs	CDT/CT	Breast-tumor-bearing mice	pH and GSH in the TME dual-stimuli responsive drug release and mutually enhanced CT/CDT	[48]
HA@AQ4N-Cu(II)-gossypol NPs	CT	PC-3-tumor-bearing nude mouse	Ultra-high drug loading content; multiple tumor targeting ability; excellent synergistic antitumor effect; negligible side effects	[49]
Cyclodextrin DDC-Cu inclusion complexes	CT	MDA-MB-231 cells	Cyclodextrin increase the solubility and toxic effect of DDC-Cu	[50]
$Cu(DDC)_2$ liposome	CT	Kelly and SH-SY5Y cells	$Cu(DDC)_2$ liposome showed effective cytotoxicity in both 2D and 3D neuroblastoma cell models	[51]
DSF@PVP/Cu-HMPB	CT	4T1 tumor-bearing mice	TME-triggered Cu^{2+} release for in situ anti-cancer complex CuL_2 generation; NIR irradiation further augment the anti-cancer activity	[52]

BLM: Bleomycin; BP: Black phosphorus; BSA: Bovine serum albumin; CDT: Chemodynamic therapy; CFN: Copper ferrite nanosphere; CPT: Camptothecin; CT: Chemotherapy; DDC: Diethyldithiocarbamate; DSF: Disulfiram; DOX: Doxorubicin; FA: Folic acid; GOD: Glucose oxidase; GSH: Glutathione; HMPB: Hollow mesoporous prussian blue; mCMSN: Mesoporous copper/manganese silicate nanosphere; MOF: Metal-organic framework; NIR: Near infrared; NP: Nanoparticle; pHLIP: pH-low insertion peptide; PT: Phototherapy; RBC: Red blood cell; TME: Tumor microenvironment; YSNP: Yolk-shell nanoparticle.

laser irradiation, offering NPs the possibility to achieve tumor-microenvironment-responsive PA imaging [142]. CuS itself is also a characteristic PAI contrast agent applied to detect tumor proliferation and metastasis [143]. Bindra *et al.* [144] developed a self-assembled nanosystem (SCP-CS) consisting of a semiconducting polymer (SCP) and CuS nanoparticles. With the help of CuS, the PA signal of SCP-CS in the tumor was 5.2-fold higher than the pure SCP-treated group. To obtain a sufficient SNR of CuS-mediated PAI, Santiesteban *et al.* [145] chose

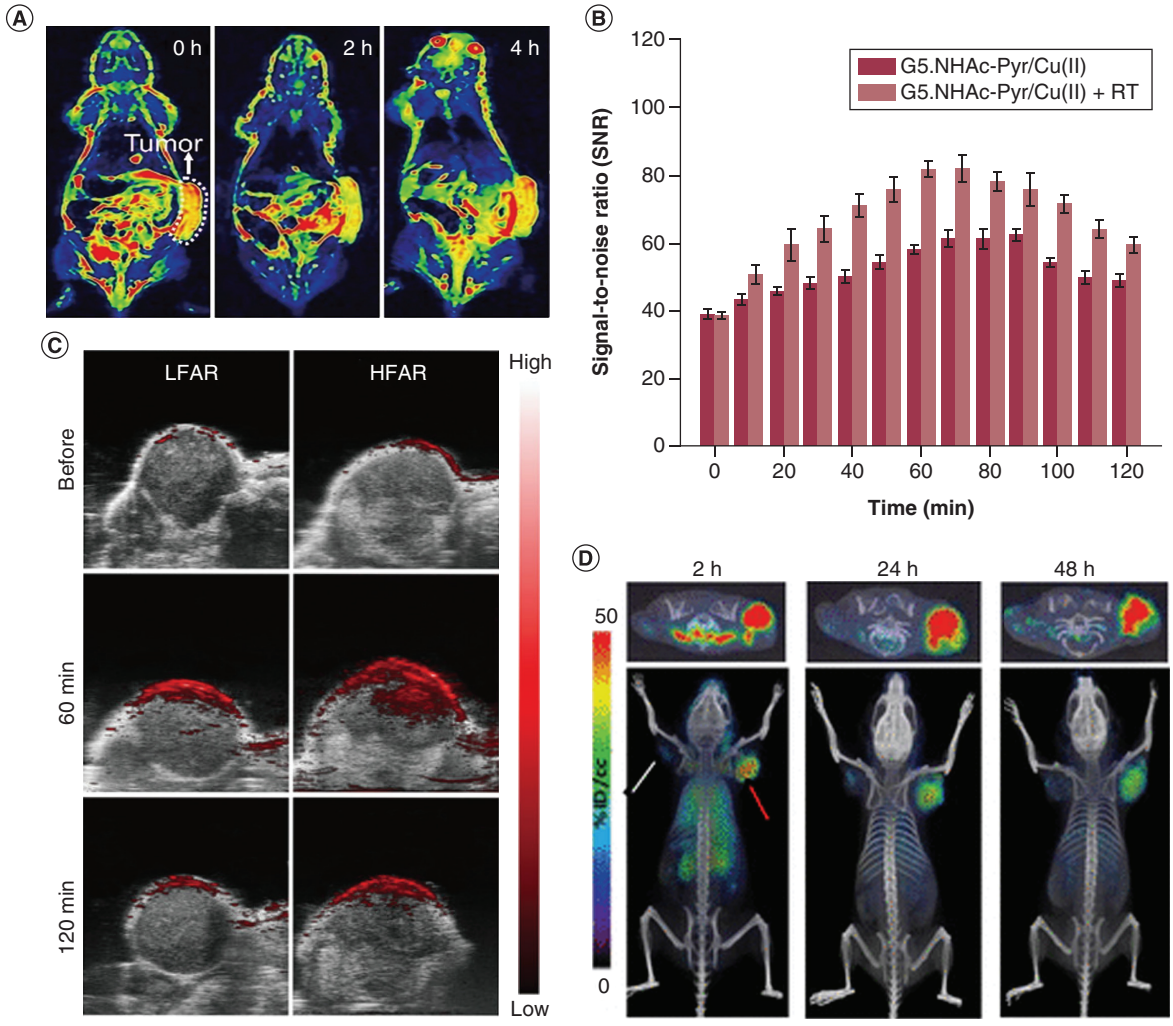


Figure 7. Imaging capabilities of copper complexes. (A) MR images of mice after intravenous injection of Cu_5FeS_4. **(B)** Quantitative analysis of tumor MR signal-noise ratio at different time points after injection of G5.NHAc-Pyr/Cu (II) complexes with and without radiotherapy treatment. **(C)** Photoacoustic imaging of the KB-HFAR and KB-LFAR after injection of the Gd/CuS@PEI-FA-PS NGs before and at 60 and 120 min. **(D)** Transaxial (top panel) and volume-rendered (bottom panel) positron-emission tomography–computed tomography images of [^{64}Cu]atezolizumab uptake in CHO-hPD-L1 (red arrow, high PD-L1 expression) and CHO (white arrow, low PD-L1 expression) tumors (n = 3) confirm PD-L1-mediated uptake of the radiotracer.
KB-HFAR: KB-tumors with high-level folic acid receptor expression; KB-LFAR: KB-tumors with low-level folic acid receptor expression.
(A) Reproduced with permission from [136], © Elsevier Inc. **(B)** Reproduced with permission from [138], © 2019 American Chemical Society. **(C)** Reproduced with permission from [139], © 2020 American Chemical Society. **(D)** Reproduced with permission from [140], © 2016 American Chemical Society.

perfluorocarbon nanodroplets (PFCnDs) to enhance the photoacoustic contrast of CuS. Combining the two PS agents miraculously compensated for each other's limitations and together provided an encouraging development for clinical tumor diagnostic applications. Zhang *et al.* [139] developed a novel nanogel (NG) Gd/CuS@PEI-FA-PS for MRI/PAI-guided tumor-targeted PTT. After a 60-min intravenous injection of Gd/CuS@PEI-FA-PS NGs, the PA signals reached the maximum value in the transplanted KB tumor model, demonstrating this nanogel has tumor-specific distribution characteristics and good retention capacity (Figure 7C). Although PAI has great value in clinical practice, most PAI employs a 2D imaging modality. The biodistribution and anatomic information of the NIR probe *in vivo* cannot be fully understood. Hence, NIR-II 3D PAI is a necessary stage for developing and optimizing PAI technology. Tests show CuS NPs can retain their superior imaging performance in NIR-II 3D PAI as a contrast agent [146].

Positron emission tomography imaging

Positron emission tomography (PET) is a topical imaging method that relies on collisions of the positron and a negative electrons in tissues. Currently, Cu-based radionuclides have received attention as positron-emitting radionuclides because of their positron energies, long half-life and excellent coordination chemistry. ^{64}Cu (half-life: $T_{1/2}$ = 12.7 h; decay characteristics: β^+, 0.653 MeV[17.8%]; β^-, 0.579 MeV[38.4%]) make it an ideal PET tracer and a novel drug component in RT [147].

^{64}Cu is often integrated into various NPs or biomaterials via different chelators to form PET imaging agents applied to detect the biodistribution of nanomedicine or precise tumor imaging [148]. However, the physicochemical properties, pharmacokinetics and biodistribution of these Cu NPs conjugated with chelators were distinct from those of unchelated Cu NPs, thus making the data obtained useless. Therefore, Shi *et al.* [149] developed ^{64}Cu-PPF (^{64}Cu-porphyrin-peptide-folate) and successfully gained PET images of tumors. Surprisingly, they found PPF, as the Cu chelator moiety, did not affect pharmacokinetics and biodistribution, providing a promising application as PET imaging probes for cancer theragnostics. Furthermore, some studies also verified that ^{64}Cu its ionic form $^{64}Cu^{2+}$ is a considerable PET agent without high cost and complicated radiolabeling steps for diagnosing [150]. Zhou *et al.* [151] tried to integrate positron emitter ^{64}Cu into the core structural component to form radioactive [^{64}Cu]-CuS NPs, which is more suitable for PET imaging. In the PET images, the tumor is clearly visible, proving PEG-[^{64}Cu]-CuS NPs are excellent PET agents. This will help determine how the drug accumulates at the tumor site. Further, the deposition of radioactivity in the bladder and gastrointestinal tract also reflects that Cu complexes possess biosafety and capability, which could be excreted by renal and hepatobiliary tract. Moreover, $^{64}CuCl_2$-PET is another approach to assess intracranial and other extrahepatic metastasis of hepatocellular carcinoma and improve the prognosis of patients with this cancer by tracking Cu metabolism [152].

^{64}Cu-labeled probes without chelators have been extensively studied due to their unique PET imaging property and more realistic pharmacokinetics parameters *in vivo*. Lesniak *et al.* [140] used ^{64}Cu-labeled atezolizumab for quantifying the dynamic expression of PD-L1 in tumors to evaluate the response to immune modulation therapies. PET–CT images illustrated that ^{64}Cu-labeled atezolizumab could significantly accumulate in the CHO-hPD-L1 tumors (high PD-L1 expression) compared with CHO tumors (low PD-L1 expression) and other peripheral tissues (Figure 7D). Detection and dynamic monitoring at the cellular level can be achieved by labeling molecules that can be specifically recognized by cells with ^{64}Cu. Kim *et al.* [153] capitalized on ^{64}Cu-labeled polyglucose nanoparticles (Macrin) for quantitative PET imaging of tumor-associated macrophages (TAMs). The retention rate of therapeutic nanoparticles in TAM-rich tumors is more than 700 times that of TAM-deficient tumors. Visualization of TAMs through ^{64}Cu-mediated PET will act as a guide for patient selection into nanomedicine trials and exert therapeutic outcomes prediction effect for prognosis of solid cancers. Shi *et al.* [154] labeled graphene nanomaterials with ^{64}Cu by the electron transfer between $^{64}Cu^{2+}$ and π-bond on the surface of graphene nanosheets (RGO). Then PEG was decorated on the ^{64}Cu-RGO for prolonged blood circulation time. Compared with the chelator-loaded ^{64}Cu PET contrast agent (^{64}Cu-NOTA-RGO-PEG), this chelator-free contrast agent ^{64}Cu-RGO-PEG showed a superb tumor uptake and excellent *in vivo* radiostability with negligible ^{64}Cu detachment in 4T1 tumor-bearing mice.

Conclusion

As one of the most crucial microelements in our bodies, Cu not only exerts essential functions, but, as ongoing research shows, it is also a novel target and a new drug in anticancer therapies. Scientists have found Cu-mediation can significantly increase ROS levels via the Fenton reaction. This discovery solved the inadequate Fe-based Fenton efficacy and has created a new field for Cu complexes. The imbalance of Cu homeostasis causes devastating damage to cancer cells, such as insufficient nutrient and proteasome inhibition. Moreover, abnormal Cu levels cause organelle damage, as seen with Cu-induced mitochondrial dysfunction. These results indicate that Cu treatment is a potential anticancer strategy; with the help of emerging biomaterials, Cu has increased functions and properties to efficiently kill cancer cells. In addition, excellent imaging properties and photothermal conversion abilities made Cu complexes excellent imaging and PT agents for diagnosing tumors and preventing tumor relapse. In summary, Cu-involved cancer application is an open field and worthy of further exploration.

Future perspective

With rising concern about metal drugs, multifunctional Cu complexes have become appealing therapeutic and diagnostic tools that have gradually become substitutes for the common transition metal complexes, such as iron or manganese complexes, in cancer therapies. However, a new idea is usually accompanied by premature theories

and preliminary experiments. As a novel anticancer strategy, the first challenge Cu-involved therapies face is biocompatibility and biosafety. Although research has proved the tumoricidal ability of Cu complexes, long-term stability and biosafety tests are indispensable steps in drug development. In this review, we have discussed four typical anticancer mechanisms of Cu complexes. However, different cancer types possess diverse characteristics, which means scientists should consider the pertinence and specificity in the drug design process. Further, there are still many Cu transporters, metallochaperones and cupric enzymes as new anticancer targets that have not been explored. Moving forward, when Cu complexes enter the scale-up stage, whether their nanostructures remain original properties depends on more intensive study. It is responsible to believe that Cu and its complexes will bring about a new era for cancer treatment, with strong theragnostic ability and fewer side effects.

Executive summary

Background

- The copper (Cu) level in the tumor cells is higher than in normal cells. Hence, disturbance of copper homeostasis is a novel strategy for cancer treatment. Both increasing and decreasing the Cu level can lead to tumor cell damage.
- Cu can cause multiple tumor cell death forms, including apoptosis, autophagy and necrosis, through various mechanisms, including reactive oxygen species (ROS) accumulation, mitochondrial dysfunction, proteasome inhibition and antiangiogenesis.
- Cu can be involved in diverse cancer therapies, such as chemodynamic therapy, phototherapy and chemotherapy. It can also be used as an imaging agent for theragnostic applications.

Mechanisms

- Cu can mediate the Fenton reaction to generate ROS, thus increasing ROS levels in tumor cells and further inducing oxidative stress, mitochondrial dysfunction and DNA cleavage.
- Cu can bind with proteasome to inhibit their activities and further induce apoptosis or paraptosis.
- Cu is a vital element for promoting angiogenesis. Hence, its depletion will interfere with angiogenesis and block the proliferation of endothelial cells.

Applications

- Cu induces chemodynamic therapy through the Fenton reaction, which can produce a high concentration of toxic ROS.
- Due to the excellent photophysical and photothermal conversion properties of Cu chalcogenides, they can respond to light stimuli and cause ROS accumulation or hyperthermic ablation, resulting in tumor damage.
- Cu complexes can be shaped or integrated into other nanostructures as drug carriers to load chemotherapeutic drugs and enhance the toxicity of chemotherapy.
- Cu and its complexes possess great imaging characteristics, allowing for various imaging technologies, such as MRI, photoacoustic imaging and positron emission tomography, for tumor visualization and location.

Author contributions

Y Jiang wrote the manuscript. X Qi and Z Huo contributed to the conception of the review. T Zuo and Z Wu contributed significantly to the guidance and revision of the manuscript.

Financial & competing interests disclosure

This work was supported by Double First-Class Innovation Team of China Pharmaceutical University (CPU2018GY40, China), National Mega-project for Innovative Drugs (2019ZX09721001, China). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Reference

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Saverio Tardito LM. Copper compounds in anti-cancer strategies. *Curr. Med. Chem.* 16(11), 1325–1348 (2009).
2. Booth BA, Sartorelli AC. Synergistic interaction of kethoxal bis(thiosemicarbazone) and cupric ions in sarcoma 180. *Nature* 210(5031), 104–105 (1966).
3. Cappuccino JG, Banks S, Brown G *et al.* The effect of copper and other metal ions on the anti-tumor activity of pyruvaldehyde bis(thiosemicarbazone). *Cancer Res.* 27(5), 968–973 (1967).
4. Kuwahara J, Suzuki T, Sugiura Y. Studies on anti-tumor drugs targeting DNA: photosensitive DNA cleavage of copper-camptothecin. *Nucleic Acids Symp. Ser.* 16, 201–204 (1985).

5. Cova D, Sassano M, Monti E, Piccinini F. DNA damage induced by doxorubicin, 4′-epidoxorubicin and their copper(II) complexes. *Arch. Toxicol.* 64(7), 597–598 (1990).
6. Huber KR, Sridhar R, Griffith EH *et al*. Superoxide dismutase-like activities of copper(II) complexes tested in serum. *Biochim. Biophys. Acta* 915(2), 267–276 (1987).
7. Kargozar S, Mozafari M, Ghodrat S *et al*. Copper-containing bioactive glasses and glass-ceramics: from tissue regeneration to cancer therapeutic strategies. *Mater. Sci. Engin. C Mater. Biol. Appl.* 121, 111741 (2021).
8. Sulce A, Bulke F, Schowalter M *et al*. Reactive oxygen species (ROS) formation ability and stability of small copper (Cu) nanoparticles (NPs). *RSC Adv.* 6(80), 76980–76988 (2016).
9. Singh P, Youden B, Yang Y *et al.* Synergistic multimodal cancer therapy using glucose oxidase@CuS nanocomposites. *ACS Appl. Mater. Interfaces* 13(35), 41464–41472 (2021).
10. Linder MC, Hazeghazam M. Copper biochemistry and molecular biology. *Am. J. Clin. Nutr.* 63(5), 797–811 (1996).
11. Ge EJ, Bush AI, Casini A *et al.* Connecting copper and cancer: from transition metal signalling to metalloplasia. *Nat. Rev. Cancer* doi:10.1038/s41568-021-00417-2 (2021) (Epub ahead of print).

•• **Systematically elucidates the cellular pathways in which copper ions are involved in cancer cells and provides insights into new targets for cancer therapy. In addition, cancer treatment strategies based on copper homeostasis disruption are introduced in detail.**

12. Rodriguez-Tomas E, Baiges-Gaya G, Castane H *et al.* Trace elements under the spotlight: a powerful nutritional tool in cancer. *J. Trace Elem. Med. Biol.* 68, 126858 (2021).
13. Denoyer D, Masaldan S, La Fontaine S, Cater MA. Targeting copper in cancer therapy: 'copper that cancer'. *Metallomics* 7(11), 1459–1476 (2015).
14. Lelievre P, Sancey L, Coll J-L *et al*. The multifaceted roles of copper in cancer: a trace metal element with dysregulated metabolism, but also a target or a bullet for therapy. *Cancers* 12(12), 3594 (2020).
15. De Luca A, Barile A, Arciello M, Rossi L. Copper homeostasis as target of both consolidated and innovative strategies of anti-tumor therapy. *J. Trace Elem. Med. Biol.* 55, 204–213 (2019).
16. Silva DaD, De Luca A, Squitti R *et al.* Copper in tumors and the use of copper-based compounds in cancer treatment. *J. Inorg. Biochem.* 226, 111634 (2022).
17. Steinbrueck A, Sedgwick AC, Brewster JT Ii *et al.* Transition metal chelators, pro-chelators, and ionophores as small molecule cancer chemotherapeutic agents. *Chem. Soc. Rev.* 49(12), 3726–3747 (2020).
18. Oliveri V. Biomedical applications of copper ionophores. *Coord. Chem. Rev.* 422, 213474 (2020).
19. Baldari S, Di Rocco G, Heffern MC *et al*. Effects of copper chelation on BRAF(V600E) positive colon carcinoma cells. *Cancers* 11(5), 659 (2019).
20. Heuberger DM, Harankhedkar S, Morgan T *et al.* High-affinity Cu(I) chelator PSP-2 as potential anti-angiogenic agent. *Sci. Rep.* 9, 14055 (2019).
21. Maiti BK, Moura JJG. Diverse biological roles of the tetrathiomolybdate anion. *Coord. Chem. Rev.* 429, 213635 (2021).
22. Sciegienka SJ, Solst SR, Falls KC *et al.* D-penicillamine combined with inhibitors of hydroperoxide metabolism enhances lung and breast cancer cell responses to radiation and carboplatin via H_2O_2-mediated oxidative stress. *Free Radic. Biol. Med.* 108, 354–361 (2017).
23. Lopez J, Ramchandani D, Vahdat L. Copper depletion as a therapeutic strategy in cancer. *Met. Ions Life Sci.* 19, 303–330 (2019).
24. Doll S, Freitas FP, Shah R *et al.* FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 575(7784), 693 (2019).
25. Angeli JPF, Krysko DV, Conrad M. Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nat. Rev. Cancer* 19(7), 405–414 (2019).
26. Stockwell BR, Jiang X, Gu W. Emerging mechanisms and disease relevance of ferroptosis. *Trends Cell Biol.* 30(6), 478–490 (2020).
27. Ma B, Wang S, Liu F *et al.* Self-assembled copper-amino acid nanoparticles for in situ glutathione "AND" H_2O_2 sequentially triggered chemodynamic therapy. *J. Am. Chem. Soc.* 141(2), 849–857 (2019).
28. Wang YR, An L, Lin JM *et al*. A hollow Cu_9S_8 theranostic nanoplatform based on a combination of increased active sites and photothermal performance in enhanced chemodynamic therapy. *Chem. Eng. J.* 385, 123925 (2020).
29. Tian H, Zhang M, Jin G, Jiang Y, Luan Y. Cu-MOF chemodynamic nanoplatform via modulating glutathione and H_2O_2 in tumor microenvironment for amplified cancer therapy. *J. Colloid Interface Sci.* 587, 358–366 (2021).
30. Wang Z, Liu B, Sun Q *et al.* Fusiform-like copper(II)-based metal-organic framework through relief hypoxia and GSH-depletion co-enhanced starvation and chemodynamic synergetic cancer therapy. *ACS Appl. Mater. Interfaces* 12(15), 17254–17267 (2020).
31. Liu Y, Zhen W, Jin L *et al.* All-in-one theranostic nanoagent with enhanced reactive oxygen species generation and modulating tumor microenvironment ability for effective tumor eradication. *ACS Nano* 12(5), 4886–4893 (2018).
32. Cao R, Sun W, Zhang Z *et al.* Protein nanoparticles containing Cu(II) and DOX for efficient chemodynamic therapy via self-generation of H_2O_2. *Chin. Chem. Lett.* 31(12), 3127–3130 (2020).

33. Pinho JO, Da Silva IV, Amaral JD *et al.* Therapeutic potential of a copper complex loaded in pH-sensitive long circulating liposomes for colon cancer management. *Int. J. Pharm.* 599(Pt 2), 120463 (2021).
34. Veeranarayanan S, Mohamed MS, Poulose AC *et al.* Photodynamic therapy at ultra-low NIR laser power and x-ray imaging using Cu(3)BiS(3) nanocrystals. *Theranostics* 8(19), 5231–5245 (2018).
35. Shrestha S, Wu J, Sah B *et al.* X-ray induced photodynamic therapy with copper–cysteamine nanoparticles in mice tumors. *Proc. Natl. Acad. Sci. U. S. A.* 116(34), 16823–16828 (2019).
36. Weng Y, Guan S, Wang L *et al.* Defective porous carbon polyhedra decorated with copper nanoparticles for enhanced NIR-driven photothermal cancer therapy. *Small* 16(1), 1905184 (2020).

•• **The Cu@CPP samples they synthesized shows a remarkable photothermal conversion efficiency and tumor ablation ability.**

37. Maor I, Asadi S, Korganbayev S *et al.* Laser-induced thermal response and controlled release of copper oxide nanoparticles from multi-functional polymeric nanocarriers. *Sci. Technol. Adv. Mater.* 22(1), 218–233 (2021).
38. Wang Q, Niu D, Shi J, Wang L. A three-in-one ZIFs-derived CuCo(O)/GOx@PCNs hybrid cascade nanozyme for immunotherapy/enhanced starvation/photothermal therapy. *ACS Appl. Mater. Interfaces* 13(10), 11683–11695 (2021).
39. Jana D, Jia S, Bindra AK *et al.* Clearable black phosphorus nanoconjugate for targeted cancer phototheranostics. *ACS Appl. Mater. Interfaces* 12(16), 18342–18351 (2020).
40. Curcio A, Silva AKA, Cabana S *et al.* Iron oxide nanoflowers @ CuS hybrids for cancer tri-therapy: interplay of photothermal therapy, magnetic hyperthermia and photodynamic therapy. *Theranostics* 9(5), 1288–1302 (2019).
41. Girma WM, Dehvari K, Ling Y-C, Chang J-Y. Albumin-functionalized $CuFeS_2$/photosensitizer nanohybrid for single-laser-induced folate receptor-targeted photothermal and photodynamic therapy. *Mater. Sci. Eng. C-Mat. Biol. Appl.* 101, 179–189 (2019).
42. Chang Y, Cheng Y, Feng Y *et al.* Resonance energy transfer-promoted photothermal and photodynamic performance of gold-copper sulfide yolk-shell nanoparticles for chemophototherapy of cancer. *Nano Lett.* 18(2), 886–897 (2018).
43. Dong K, Liu Z, Li Z *et al*. Hydrophobic anti-cancer drug delivery by a 980 nm laser-driven photothermal vehicle for efficient synergistic therapy of cancer cells *in vivo*. *Adv. Mater.* 25(32), 4452–4458 (2013).
44. Feng Q, Zhang W, Li Y *et al.* An intelligent NIR-responsive chelate copper-based anti-cancer nanoplatform for synergistic tumor targeted chemo-phototherapy. *Nanoscale* 9(40), 15685–15695 (2017).
45. Wang DD, Dong HF, Li M *et al.* Erythrocyte-cancer hybrid membrane camouflaged hollow copper sulfide nanoparticles for prolonged circulation life and homotypic-targeting photothermal/chemotherapy of melanoma. *ACS Nano* 12(6), 5241–5252 (2018).
46. Zhang L, Li Y, Jin Z *et al*. An NIR-triggered and thermally responsive drug delivery platform through DNA/copper sulfide gates. *Nanoscale* 7(29), 12614–12624 (2015).
47. Liu J, Sun L, Li L *et al*. Synergistic cancer photochemotherapy via layered double hydroxide-based trimodal nanomedicine at very low therapeutic doses. *ACS Appl. Mater. Interfaces* 13(6), 7115–7126 (2021).
48. Meng X, Zhang X, Lei Y *et al*. Biodegradable copper-metformin nanoscale coordination polymers for enhanced chemo/chemodynamic synergistic therapy by reducing oxygen consumption to promote H_2O_2 accumulation. *J. Mater. Chem. B* 9(8), 1988–2000 (2021).
49. Shen S, Wu Y, Li K *et al.* Versatile hyaluronic acid modified AQ4N-Cu(II)-gossypol infinite coordination polymer nanoparticles: Multiple tumor targeting, highly efficient synergistic chemotherapy, and real-time self-monitoring. *Biomaterials* 154, 197–212 (2018).
50. Said Suliman A, Khoder M, Tolaymat I *et al.* Cyclodextrin diethyldithiocarbamate copper II inclusion complexes: a promising chemotherapeutic delivery system against chemo-resistant triple negative breast cancer cell lines. *Pharmaceutics* 13(1), 84 (2021).
51. Hartwig F, Koell-Weber M, Suess R. Preclinical *in vitro* studies with 3D spheroids to evaluate Cu(DDC)(2) containing liposomes for the treatment of neuroblastoma. *Pharmaceutics* 13(6), 894 (2021).
52. Wu W, Yu L, Pu Y *et al.* Copper-enriched Prussian blue nanomedicine for in situ disulfiram toxification and photothermal anti-tumor amplification. *Adv. Mater.* 32(17), e2000542 (2020).

•• **This research successfully combined DSF/Cu and PTT, showing an outstanding synergistic effect on tumor elimination on both subcutaneous and orthotopic tumor-bearing models.**

53. Chio IIC, Tuveson DA. ROS in cancer: the burning question. *Trends Mol. Med.* 23(5), 411–429 (2017).
54. Reczek CR, Chandel NS. The two faces of reactive oxygen species in cancer. In: *Annual Review of Cancer Biology, Vol. 1*. Jacks T, Sawyers CL (Eds). Palo Alto Annual Reviews, 79–98 (2017).
55. Aboelella NS, Brandle C, Kim T *et al*. Oxidative stress in the tumor microenvironment and its relevance to cancer immunotherapy. *Cancers* 13(5), 986 (2021).
56. Bokare AD, Choi W. Review of iron-free Fenton-like systems for activating H_2O_2 in advanced oxidation processes. *J. Hazard. Mater.* 275, 121–135 (2014).
57. Hao YN, Zhang WX, Gao YR *et al*. State-of-the-art advances of copper-based nanostructures in the enhancement of chemodynamic therapy. *J. Mater. Chem. B* 9(2), 250–266 (2021).
58. Bansal A, Simon MC. Glutathione metabolism in cancer progression and treatment resistance. *J. Cell Biol.* 217(7), 2291–2298 (2018).

59. Ngamchuea K, Batchelor-McAuley C, Compton RG. The copper(II)-catalyzed oxidation of glutathione. *Chemistry* 22(44), 15937–15944 (2016).

60. Mo R, Gu Z. Tumor microenvironment and intracellular signal-activated nanomaterials for anti-cancer drug delivery. *Mater. Today* 19(5), 274–283 (2016).

61. Nagai M, Vo NH, Shin Ogawa L *et al.* The oncology drug elesclomol selectively transports copper to the mitochondria to induce oxidative stress in cancer cells. *Free Radic. Biol. Med.* 52(10), 2142–2150 (2012).

62. Liu ZH, Yang CX, Zhang L *et al*. Baicalein, as a prooxidant, triggers mitochondrial apoptosis in MCF-7 human breast cancer cells through mobilization of intracellular copper and reactive oxygen species generation. *OncoTargets. Ther.* 12, 10749–10761 (2019).

63. Slator C, Barron N, Howe O, Kelleet A. Cu(o-phthalate)(phenanthroline) exhibits unique superoxide-mediated NCI-60 chemotherapeutic action through genomic DNA damage and mitochondrial dysfunction. *ACS Chem. Biol.* 11(1), 159–171 (2016).

64. Rochford G, Molphy Z, Kavanagh K *et al.* Cu(ii) phenanthroline-phenazine complexes dysregulate mitochondrial function and stimulate apoptosis. *Metallomics* 12(1), 65–78 (2020).

65. Laws K, Bineva-Todd G, Eskandari A *et al*. A copper(II) phenanthroline metallopeptide that targets and disrupts mitochondrial function in breast cancer stem cells. *Angew. Chem. Int. Ed.* 57(1), 287–291 (2018).

66. Cobine PA, Moore SA, Leary SC. Getting out what you put in: copper in mitochondria and its impacts on human disease. *Biochim. Biophys. Acta, Mol. Cell Res.* 1868(1), 118867 (2021).

67. Cui L, Gouw AM, Lagory EL *et al.* Mitochondrial copper depletion suppresses triple-negative breast cancer in mice. *Nat. Biotechnol.* 39(3), 357–+ (2021).

• **Mitochondiral copper depletion will change the respiration patterns of triple-negative breast cancer cells, which causes decrease of ATP and results in apoptosis.**

68. Shao J, Li M, Guo Z *et al.* TPP-related mitochondrial targeting copper (II) complex induces p53-dependent apoptosis in hepatoma cells through ROS-mediated activation of Drp1. *Cell. Commun. Signal.* 17(1), 149–167 (2019).

69. Qi J, Yao Q, Tian L, Wang Y. Piperidylthiosemicarbazones Cu(II) complexes with a high anti-cancer activity by catalyzing hydrogen peroxide to degrade DNA and promote apoptosis. *Eur. J. Med. Chem.* 158, 853–862 (2018).

70. Angele-Martinez C, Nguyen KVT, Ameer FS *et al*. Reactive oxygen species generation by copper(II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy. *Nanotoxicology* 11(2), 278–288 (2017).

71. Jomova K, Hudecova L, Lauro P *et al.* The effect of luteolin on DNA damage mediated by a copper catalyzed Fenton reaction. *J. Inorg. Biochem.* 226, 111635 (2022).

72. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* 482(3), 419–425 (2017).

73. Greenwood HE, Witney TH. Latest advances in imaging oxidative stress in cancer. *J. Nucl. Med.* 62(11), 1506–1510 (2021).

74. Perillo B, Di Donato M, Pezone A *et al.* ROS in cancer therapy: the bright side of the moon. *Exp. Mol. Med.* 52(2), 192–203 (2020).

75. Periasamy VS, Riyasdeen A, Rajendiran V *et al.* Induction of redox-mediated cell death in ER-positive and ER-negative breast cancer cells by a copper(II)-phenolate complex: an *in vitro* and *in silico* study. *Molecules* 25(19), 4504 (2020).

76. Tsang T, Posimo JM, Gudiel AA *et al*. Copper is an essential regulator of the autophagic kinases ULK1/2 to drive lung adenocarcinoma. *Nat. Cell Biol.* 22(4), 412–424 (2020).

77. Xiong K, Zhou Y, Karges J *et al.* Autophagy-dependent apoptosis induced by apoferritin-Cu(II) nanoparticles in multidrug-resistant colon cancer cells. *ACS Appl. Mater. Interfaces* 13(33), 38959–38968 (2021).

78. Kang Z, Qiao N, Liu G *et al*. Copper-induced apoptosis and autophagy through oxidative stress-mediated mitochondrial dysfunction in male germ cells. *Toxicol. In Vitro* 61, 104639 (2019).

79. Galczynska K, Drulis-Kawa Z, Arabski M. Antitumor activity of Pt(II), Ru(III) and Cu(II) complexes. *Molecules* 25(15), 3492 (2020).

80. Santini C, Pellei M, Gandin V *et al*. Advances in copper complexes as anticancer agents. *Chem. Rev.* 114(1), 815–862 (2013).

•• **Thoroughly introduces diverse copper complex structures and their powerful tumor-killing effects.**

81. Shagufta, Ahmad I. Transition metal complexes as proteasome inhibitors for cancer treatment. *Inorg. Chim. Acta* 506, 119521 (2020).

82. Santoro AM, Monaco I, Attanasio F *et al.* Copper(II) ions affect the gating dynamics of the 20S proteasome: a molecular and in cell study. *Sci. Rep.* 6(1), 33444 (2016).

83. Zhang Z, Wang H, Yan M *et al*. Novel copper complexes as potential proteasome inhibitors for cancer treatment (Review). *Mol. Med. Rep* 15(1), 3–11 (2017).

84. Cater MA, Pearson HB, Wolyniec K *et al.* Increasing intracellular bioavailable copper selectively targets prostate cancer cells. *ACS Chem. Biol.* 8(7), 1621–1631 (2013).

85. Oliveri V, Lanza V, Milardi D *et al.* Amino- and chloro-8-hydroxyquinolines and their copper complexes as proteasome inhibitors and antiproliferative agents. *Metallomics* 9(10), 1439–1446 (2017).

86. Zhou T, Cai Y, Liang L *et al.* Discovery of a potent and highly specific beta2 proteasome inhibitor from a library of copper complexes. *Bioorg. Med. Chem. Lett.* 26(23), 5780–5784 (2016).

87. Chen W, Yang W, Chen P *et al.* Disulfiram copper nanoparticles prepared with a stabilized metal ion ligand complex method for treating drug-resistant prostate cancers. *ACS Appl. Mater. Interfaces* 10(48), 41118–41128 (2018).
88. Yang Y, Tang J, Zhang M *et al.* Responsively aggregatable Sub-6 nm nanochelators induce simultaneous antiangiogenesis and vascular obstruction for enhanced tumor vasculature targeted therapy. *Nano Lett.* 19(11), 7750–7759 (2019).
89. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 407(6801), 249–257 (2000).
90. Park KC, Fouani L, Jansson PJ *et al.* Copper and conquer: copper complexes of di-2-pyridylketone thiosemicarbazones as novel anti-cancer therapeutics. *Metallomics* 8(9), 874–886 (2016).
91. Baldari S, Di Rocco G, Toietta G. Current biomedical use of copper chelation therapy. *Int. J. Mol. Sci.* 21(3), 1069 (2020).
92. Narayanan IG, Natarajan SK. Peptides derived from histidine and methionine-rich regions of copper transporter 1 exhibit anti-angiogenic property by chelating extracellular Cu. *Chem. Biol. Drug Des.* 91(3), 797–804 (2018).
93. Yee EMH, Brandl MB, Pasquier E *et al.* Dextran-catechin inhibits angiogenesis by disrupting copper homeostasis in endothelial cells. *Sci. Rep.* 7(1), 7638 (2017).
94. Karginova O, Weekley CM, Raoul A *et al.* Inhibition of copper transport induces apoptosis in triple-negative breast cancer cells and suppresses tumor angiogenesis. *Mol. Cancer Ther.* 18(5), 873–885 (2019).
95. Nasulewicz A, Mazur A, Opolski A. Role of copper in tumour angiogenesis – clinical implications. *J. Trace Elem. Med. Biol.* 18(1), 1–8 (2004).
96. Czlonkowska A, Litwin T, Dusek P *et al.* Wilson disease. *Nat. Rev. Dis. Primers* 4(1), 21 (2018).
97. Denoyer D, Clatworthy SaS, Cater MA. Copper complexes in cancer therapy. *Met. Ions Life Sci.* 18, 470–496 (2018).
98. Stupple A. Tetrathiomolybdate inhibits mitochondrial complex IV and mediates degradation of hypoxia-inducible factor-1α in cancer cells. *Sci. Rep.* 5, 14296 (2015).
99. Hassouneh B, Islam M, Nagel T *et al.* Tetrathiomolybdate promotes tumor necrosis and prevents distant metastases by suppressing angiogenesis in head and neck cancer. *Mol. Cancer Ther.* 6(3), 1039–1045 (2007).
100. Zhou P, Qin J, Zhou C *et al.* Multi-functional nanoparticles based on a polymeric copper chelator for combination treatment of metastatic breast cancer. *Biomaterials* 195, 86–99 (2019).
101. Sun T, Zhang G, Guo Z *et al.* Co-delivery of Cu(I) chelator and chemotherapeutics as a new strategy for tumor theranostic. *J. Controlled Release* 321, 483–496 (2020).

- **Antiangiogenesis can be achieved by using copper chelator CPLP-X, and this Cu^{+} capture events can be monitored in real time.**

102. Getachew G, Korupalli C, Rasal AS, Chang J-Y. ROS generation/scavenging modulation of carbon dots as phototherapeutic candidates and peroxidase mimetics to integrate with polydopamine nanoparticles/GOx towards cooperative cancer therapy. *Composites Part B Engineer.* 226, 109364 (2021).
103. Sirbu A, Palamarciuc O, Babak MV *et al.* Copper(ii) thiosemicarbazone complexes induce marked ROS accumulation and promote nrf2-mediated anti-oxidant response in highly resistant breast cancer cells. *Dalton Trans.* 46(12), 3833–3847 (2017).
104. Boodram JN, Mcgregor IJ, Bruno PM *et al.* Breast cancer stem cell potent copper(II)-nonsteroidal anti-inflammatory drug complexes. *Angew. Chem. Int. Ed.* 55(8), 2845–2850 (2016).
105. Kaur P, Johnson A, Northcote-Smith J *et al.* Immunogenic cell death of breast cancer stem cells induced by an endoplasmic reticulum-targeting copper(II) complex. *Chembiochem* 21(24), 3618–3624 (2020).
106. Bao XZ, Dai F, Li XR, Zhou B. Targeting redox vulnerability of cancer cells by prooxidative intervention of a glutathione-activated Cu(II) pro-ionophore: hitting three birds with one stone. *Free Radic. Biol. Med.* 124, 342–352 (2018).
107. Zhang L, Wan SS, Li CX *et al.* An adenosine triphosphate-responsive autocatalytic Fenton nanoparticle for tumor ablation with self-supplied H_2O_2 and acceleration of Fe(III)/Fe(II) conversion. *Nano Lett.* 18(12), 7609–7618 (2018).
108. Wang T, Zhang H, Liu H *et al.* Boosting H_2O_2-guided chemodynamic therapy of cancer by enhancing reaction kinetics through versatile biomimetic Fenton nanocatalysts and the second near-infrared light irradiation. *Adv. Funct. Mater.* 30(3), 1906128 (2020).
109. Lin LS, Huang T, Song J *et al.* Synthesis of copper peroxide nanodots for H_2O_2 self-supplying chemodynamic therapy. *J. Am. Chem. Soc.* 141(25), 9937–9945 (2019).
110. Chang M, Wang M, Wang M *et al.* A Multi-functional cascade bioreactor based on hollow-structured Cu_2MoS_4 for synergetic cancer chemo-dynamic therapy/starvation therapy/phototherapy/immunotherapy with remarkably enhanced efficacy. *Adv. Mater.* 31(51), 1905271 (2019).
111. Sun S, Chen Q, Tang Z *et al.* Tumor microenvironment stimuli-responsive fluorescence imaging and synergistic cancer therapy by carbon-Dot-Cu(2+)nanoassemblies. *Angew. Chem. Int. Ed.* 59(47), 21041–21048 (2020).
112. Kaneti YV, Tang J, Salunkhe RR *et al.* Nanoarchitectured design of porous materials and nanocomposites from metal-organic frameworks. *Adv. Mater.* 29(12), 1604898 (2017).
113. Velasquez-Hernandez MDJ, Linares-Moreau M, Astria E *et al.* Towards applications of bioentities@MOFs in biomedicine. *Coord. Chem. Rev.* 429, 213651 (2021).

114. Simon-Yarza T, Mielcarek A, Couvreur P, Serre C. Nanoparticles of metal-organic frameworks: on the road to *in vivo* efficacy in biomedicine. *Adv. Mater.* 30(37), 1707365 (2018).

115. Liu C, Wang D, Zhang S *et al.* Biodegradable biomimic copper/manganese silicate nanospheres for chemodynamic/photodynamic synergistic therapy with simultaneous glutathione depletion and hypoxia relief. *ACS Nano* 13(4), 4267–4277 (2019).

116. Mckenzie LK, Sazanovich IV, Baggaley E *et al.* Metal complexes for two-photon photodynamic therapy: a cyclometallated iridium complex induces two-photon photosensitization of cancer cells under Near-IR light. *Chem-Eur. J.* 23(2), 234–238 (2017).

117. Al Hageh C, Al Assaad M, El Masri Z *et al.* A long-lived cuprous bis-phenanthroline complex for the photodynamic therapy of cancer. *Dalton Trans.* 47(14), 4959–4967 (2018).

118. Goel S, Chen F, Cai W. Synthesis and biomedical applications of copper sulfide nanoparticles: from sensors to theranostics. *Small* 10(4), 631–645 (2014).

119. Coughlan C, Ibanez M, Dobrozhan O *et al.* Compound copper chalcogenide nanocrystals. *Chem. Rev.* 117(9), 5865–6109 (2017).

120. Nikam AN, Pandey A, Fernandes G *et al.* Copper sulphide based heterogeneous nanoplatforms for multimodal therapy and imaging of cancer: recent advances and toxicological perspectives. *Coord. Chem. Rev.* 419, 213356 (2020).

121. Hu JJ, Cheng YJ, Zhang XZ. Recent advances in nanomaterials for enhanced photothermal therapy of tumors. *Nanoscale* 10(48), 22657–22672 (2018).

122. Li N, Sun Q, Yu Z *et al.* Nuclear-targeted photothermal therapy prevents cancer recurrence with near-infrared triggered copper sulfide nanoparticles. *ACS Nano* 12(6), 5197–5206 (2018).

123. Cheng L, Wang C, Feng L *et al.* Functional nanomaterials for phototherapies of cancer. *Chem. Rev.* 114(21), 10869–10939 (2014).

124. Zhao Y, Chen B-Q, Kankala RK *et al.* Recent advances in combination of copper chalcogenide-based photothermal and reactive oxygen species-related therapies. *ACS Biomater. Sci. Eng.* 6(9), 4799–4815 (2020).

125. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy. *Eur. J. Pharm. Biopharm.* 93, 52–79 (2015).

126. Kimoto E, Tanaka H, Gyotoku J *et al.* Enhancement of anti-tumor activity of ascorbate against Ehrlich ascites tumor cells by the copper:glycylglycylhistidine complex. *Cancer Res.* 43(2), 824–828 (1983).

127. Curcio A, De Walle AV, Benassai E *et al.* Massive intracellular remodeling of CuS nanomaterials produces nontoxic bioengineered structures with preserved photothermal potential. *ACS Nano* 15(6), 9782–9795 (2021).

128. Krasnovskaya O, Naumov A, Guk D *et al.* Copper Coordination compounds as biologically active agents. *Int. J. Mol. Sci.* 21(11), 3965 (2020).

129. Hassani S, Ghaffari P, Chahardouli B *et al.* Disulfiram/copper causes ROS levels alteration, cell cycle inhibition, and apoptosis in acute myeloid leukaemia cell lines with modulation in the expression of related genes. *Biomed. Pharmacother.* 99, 561–569 (2018).

130. Mcmahon A, Chen W, Li F. Old wine in new bottles: advanced drug delivery systems for disulfiram-based cancer therapy. *J. Controlled Release* 319, 352–359 (2020).

131. Sosnovik DE, Weissleder R. Emerging concepts in molecular MRI. *Curr. Opin. Biotechnol.* 18(1), 4–10 (2007).

132. Zhou M, Tian M, Li C. Copper-based nanomaterials for cancer imaging and therapy. *Bioconjugate. Chem.* 27(5), 1188–1199 (2016).

133. Yuan H, Zhao Y, Yang C *et al.* Copper-thioguanine metallodrug with self-reinforcing circular catalysis for activatable MRI imaging and amplifying specificity of cancer therapy. *Sci. China. Chem.* 63(7), 924–935 (2020).

134. Liu Y, Wu JD, Jin YH *et al.* Copper(I) phosphide nanocrystals for in situ self-generation magnetic resonance imaging-guided photothermal-enhanced chemodynamic synergetic therapy resisting deep-seated tumor. *Adv. Funct. Mater.* 29(50), 1904678 (2019).

135. Perlman O, Borodetsky A, Kauffmann Y *et al.* Gold/copper@polydopamine nanocomposite for contrast-enhanced dual modal computed tomography–magnetic resonance imaging. *Acs Applied Nano Materials* 2(10), 6124–6134 (2019).

136. Wang Z, Wang Y, Guo H *et al.* Synthesis of one-for-all type Cu_5FeS_4 nanocrystals with improved near infrared photothermal and Fenton effects for simultaneous imaging and therapy of tumor. *J. Colloid Interface Sci.* 592, 116–126 (2021).

137. Sang D, Wang K, Sun X *et al.* NIR-driven intracellular photocatalytic O_2 evolution on Z-scheme Ni_3S_2/Cu1.8S@HA for hypoxic tumor therapy. *ACS Appl. Mater. Interfaces* 13(8), 9604–9619 (2021).

138. Fan Y, Zhang J, Shi M *et al.* Poly(amidoamine) dendrimer-coordinated copper(II) complexes as a theragnostic nanoplatform for the radiotherapy–enhanced magnetic resonance imaging and chemotherapy of tumors and tumor metastasis. *Nano Lett.* 19(2), 1216–1226 (2019).

139. Zhang C, Sun W, Wang Y *et al.* Gd-/CuS-loaded functional nanogels for MR/PA imaging-guided tumor-targeted photothermal therapy. *ACS Appl. Mater. Interfaces* 12(8), 9107–9117 (2020).

140. Lesniak WG, Chatterjee S, Gabrielson M *et al.* PD-L1 detection in tumors using Cu-64 Atezolizumab with PET. *Bioconjugate. Chem.* 27(9), 2103–2110 (2016).

141. Wang LV, Hu S. Photoacoustic tomography: *in vivo* imaging from organelles to organs. *Science* 335(6075), 1458–1462 (2012).

142. Wang S, Zhang L, Zhao J *et al*. A tumor microenvironment-induced absorption red-shifted polymer nanoparticle for simultaneously activated photoacoustic imaging and photothermal therapy. *Sci. Adv.* 7(12), eabe3588 (2021).

143. Bharathiraja S, Manivasagan P, Moorthy MS *et al*. Chlorin e6 conjugated copper sulfide nanoparticles for photodynamic combined photothermal therapy. *Photodiagnosis Photodyn. Ther.* 19, 128–134 (2017).

144. Bindra AK, Wang D, Zheng Z *et al.* Self-assembled semiconducting polymer based hybrid nanoagents for synergistic tumor treatment. *Biomaterials* 279, 121188 (2021).

145. Santiesteban DY, Dumani DS, Profili D, Emelianov SY. Copper Sulfide perfluorocarbon nanodroplets as clinically relevant photoacoustic/ultrasound imaging agents. *Nano Lett.* 17(10), 5984–5989 (2017).

146. Zhou HC, Ren J, Lin Y *et al.* Intravital NIR-II three-dimensional photoacoustic imaging of biomineralized copper sulfide nanoprobes. *J. Mater. Chem. B* 9(13), 3005–3014 (2021).

147. Brandt M, Cardinale J, Aulsebrook ML *et al*. An overview of PET radiochemistry, part 2: radiometals. *J. Nucl. Med.* 59(10), 1500–1506 (2018).

148. Lee I, Lim I, Byun BH *et al.* A preliminary clinical trial to evaluate (64)Cu-NOTA-Trastuzumab as a positron emission tomography imaging agent in patients with breast cancer. *EJNMMI Res.* 11(1), 8 (2021).

149. Shi J, Liu TWB, Chen J *et al.* Transforming a targeted porphyrin theranostic agent into a PET imaging probe for cancer. *Theranostics* 1, 363–370 (2011).

150. Chakravarty R, Chakraborty S, Dash A. (64)Cu(2+) ions as PET probe: an emerging paradigm in molecular imaging of cancer. *Mol. Pharm.* 13(11), 3601–3612 (2016).

151. Zhou M, Zhang R, Huang M *et al.* A chelator-free multi-functional [64Cu]CuS nanoparticle platform for simultaneous micro-PET/CT imaging and photothermal ablation therapy. *J. Am. Chem. Soc.* 132(43), 15351–15358 (2010).

152. Wachsmann J, Peng F. Molecular imaging and therapy targeting copper metabolism in hepatocellular carcinoma. *World J. Gastroenterol.* 22(1), 221–231 (2016).

153. Kim H-Y, Li R, Ng TSC *et al.* Quantitative imaging of tumor-associated macrophages and their response to therapy using Cu-64-labeled macrin. *ACS Nano* 12(12), 12015–12029 (2018).

154. Shi S, Xu C, Yang K *et al.* Chelator-free radiolabeling of nanographene: breaking the stereotype of chelation. *Angew. Chem., Int. Ed.* 56(11), 2889–2892 (2017).