Bortezomib-Encapsulated CuS/Carbon Dots
Nanocomposites for Enhanced Photothermal Therapy
via Stabilization of Polyubiquitinated Substrates in
the Proteasomal Degradation Pathway

Ying Yu[†], Meiyu Song[†], Cailing Chen[‡], Yangyang Du[†], Chunguang Li[†], Yu Han[‡], Fei Yan^{†}, Zhan
Shi^{*†} and Shouhua Feng[†]*

[†] State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, International Joint
Research Laboratory of Nano-Micro Architecture Chemistry (NMAC), International Research
Center for Chemistry-Medicine Joint Innovation, College of Chemistry, Jilin University, 2699
Qianjin Street, Changchun 130012, China

[‡]Advanced Membranes and Porous Materials Center, Physical Sciences and Engineering Division,
King Abdullah University of Science and Technology, Thuwal, 23955-6900, Saudi Arabia

ABSTRACT

Photothermal therapy (PTT) is an emerging therapeutic strategy in the treatment of cancer; however, a critical challenge remains in the rational design of synergistic nanoparticles as potential photothermal transduction agent that can effectively enhance the therapeutic outcome of PTT for tumor ablation. Herein, we rationally designed, developed, and characterized hollow-structured CuS nanoparticles composited with carbon dots (CuSCD), which demonstrated excellent photothermal conversion efficiency under 808 nm laser irradiation with enhanced biocompatibility and reduced toxicity. Following coating macrophage membrane hybridized with T7 peptide on the surface of proteasome inhibitor loaded CuSCD, CuSCDB@MMT7 exhibited targeted specificity to cancer cells with the characteristics of immune escaping and enhanced transferrin receptor-mediated endocytosis. Predominantly, CuSCDB@MMT7-triggered PTT exhibited the accumulation of polyubiquitinated tumor suppressor protein that is heat stabilized under NIR induced hyperthermia, facilitating augmented tumor cell apoptosis and the attenuated metastasis. This study provides a proof-of-concept for the proteasome inhibitor-loaded CuS/carbon dots nanocomposites-PTT strategy, and highlights a promising therapeutic strategy for realizing enhanced therapeutic outcomes for effective clinical cancer therapy.

KEYWORDS: photothermal therapy, carbon dots, CuS nanoparticles, bortezomib, polyubiquitinated proteins

Photothermal therapy (PTT) is a minimally invasive and potentially effective therapeutic modality that is capable of eliminating a variety of cancer types.¹ PTT is essentially the ablation of tumor through localized hyperthermia achieved by utilizing photothermal effect of photothermal transduction agents (PTAs), which converts optical energy into thermal energy upon irradiation

with light.^{2,3} However, the major challenge to PTT is the limited photothermal conversion efficiency (PCE) of photothermal transduction agents (PTAs), which may lead to inefficient and incomplete ablation of the entire tumors on irradiation.^{4,5} In particular, a barrier is that the minimum laser power intensity for effective PTT is still significantly higher than the conservative limit for human skin exposure. Improving the PCE and reducing the laser power density has become a direction for the development of PTT. Owing to the high PCE, enhanced photothermal stability, versatility, and ease of synthesis and surface modification, inorganic or its hybrid materials have been extensively used as the PTAs.⁶ However, in addition to the reported potential toxicity of some inorganic agents, their poor stability in the physiological condition, low drug loading capacity, low biodegradability, and compositions complexity may impede their metabolism resulting in their long-time accumulation in the body, thus these drawbacks have limited their clinical applications.⁷ To address these limitations, studies have been devoted by various research groups for the investigation of numerous inorganic materials including, carbon-based materials.⁸ Of note, carbon dots with broad optical absorptions, reasonable photothermal properties, degradability, and biocompatibility properties, received increased attention.⁹

Despite their high PCE, the applications of these PTAs in clinical trials remain limited, predominantly due to overheating of the tumor area, which potentially causes no specific damage to normal tissues. Besides, substantially low delivery efficiency of PTAs in tumors, relatively poor specificity toward malignant tissues, and development of resistance to PTT due to the aberrant protein expression remains challenging.¹⁰ Recently, chemo-photothermal combination therapy has presented a great prospect with regards to overcoming cross-resistance and achieving synergistically enhanced therapeutic outcome without potential toxicities and exhibiting ability to treat tumor metastases outside the scope of irradiation,^{11,12} however, clinical use of chemo-photothermal

1
2
3 combination therapy encountered several challenges including *in vivo* instability, unfavorable
4 pharmacokinetics, biodistribution characteristics, and multiple drug resistance to cancer cells.¹³
5
6 Fundamentally, abnormalities in the development of cancer are the continual unregulated
7 proliferation of cancer cells with the ability to invade and metastasize, which are attributed due to
8 aberrant gene expression, regulation or modifications. Although these strategies promoted PTT
9 efficacy, there has been only limited success in the exploration of highly effective approaches to
10 achieve complete tumor ablation without tumor regrowth. Consequently, it is highly desirable to
11 develop some more effective materials with high PCE and molecule inhibitors that are able to
12 maximize PTT-based anticancer efficacy. However, a critical challenge remains in the rational
13 design of synergistic nanoparticles that can effectively promote phototherapy and other therapeutic
14 modality in a mutually favorable manner.¹⁴⁻¹⁶
15
16
17
18
19
20
21
22
23
24
25
26
27
28

29 The ubiquitin-proteasome system is the principal pathway that degrades individual proteins in a
30 tightly regulated manner and is responsible for the clearance of short-lived, misfolded, damaged,
31 or unwanted cellular proteins.¹⁷ Besides, many cancers rely on finely tuned proteasome activity
32 for degradation of tumor suppressors and cell cycle checkpoint inhibitors essential for rapid cell
33 division. The ubiquitin is a heat-stable polypeptide¹⁸ and the polyubiquitinated protein can be
34 recognized by the 26S proteasome and degraded or processed.¹⁹ Proteasome inhibitors could
35 protect various tumor suppressor proteins, including I κ B α , p27, p21, p53 *et al.*,²⁰⁻²³ from
36 degradation caused by ubiquitin-proteasome system. Bortezomib (Velcade; Millennium
37 Pharmaceuticals, Inc., Cambridge, NJ), the first proteasome inhibitor approved by the U.S. Food
38 and Drug Administration (FDA) has been shown to exhibit promising antitumor activity in
39 multiple tumors.^{19, 24} However, some severe toxic side effects and poor blood stability due to boric
40 acid groups associated with bortezomib that chelated with certain reactive functional groups
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

present in plasma proteins *in vivo* have critically limited its usefulness in the clinic.^{25,26} To overcome these limitations and enhance treatment outcome, a nanomedicine-based strategy with improved systemic stability and reduced toxicity to bortezomib, which could contribute to expanding the scope of clinical oncology treatment of bortezomib is urgently needed.²⁷

Herein, we designed and synthesized a synergetic anticancer system that integrated PTT and bortezomib utilizing hollow-structured CuS/carbon dots nanocomposites as multifunctional drug carriers. This hybrid nanostructure not only improved the PCE upon 808 nm laser irradiation at a lower power density but also exhibited enhanced biocompatibility and low toxicity. After coating macrophage membrane hybridized with T7 peptide on the surface of bortezomib loaded CuSCD, as-fabricated CuSCDB@MMT7 demonstrated targeted specificity to tumor cells with the characteristics of immune escaping and enhanced cell endocytosis. More importantly, the combination of PTT and bortezomib exhibited synergistic effects and significantly attenuated the growth of tumor cell and metastasis *in vitro* and *in vivo* via prevention from degradation caused by proteasome system particularly photothermally induced hyperthermia (Figure 1). This study provided a proof-of-concept for the proteasome inhibitor-loaded CuS/carbon dots nanocomposites as potential PTA for targeted PTT and anticipate that it will serve as a podium for the translation of the technology into clinical application.

RESULTS AND DISCUSSION

CuS/carbon dots nanocomposites (CuSCD) was synthesized through a solvothermal reaction between CuS and carbon dots (CDs). The X-ray diffraction (XRD) analysis confirmed the formation of CuS and CuSCD. The XRD patterns of CuS and CuSCD were consistent with the standard data for hexagonal phase CuS (JCPDS No. 06-0464) and carbon dots ($2\theta = 24.9^\circ$),

respectively (Figure S1).^{28,29} The Raman spectrum of CuSCD exhibited a G band and D band at 1559 and 1373 cm^{-1} , respectively, and a sharp peak at 471 cm^{-1} attributed to the presence of CuS, which was consistent with the previous result (Figure S2).^{30,31} FTIR spectroscopy was carried out to identify the components of the nanocomposites (Figure S3). The vibration peaks at 3316 cm^{-1} was ascribed to the O–H stretching vibration, the bands at 3100–2800 cm^{-1} can be assigned to $\nu(\text{C–H})$, and the characteristic peak at 1663 cm^{-1} can be attributed to the C=O stretching vibration. The peak at 1543 cm^{-1} was assigned to the stretching vibration of aromatic C=C and the broad bands at 1070 cm^{-1} were attributed to C–S.^{32–34} For magnetically decorated CuSCD, the intensities of the oxygen functional groups (1663 cm^{-1}) were reduced to a significant extent, indicating that most of oxygen functional groups were removed and the stretching vibration of aromatic C=C peak at 1535 cm^{-1} nearly disappeared. The enhanced peak in the C–S (1070 cm^{-1}) confirmed the formation of the CuSCD. A survey spectrum exhibited peaks corresponding to C 1s (~ 284.7 eV), N 1s (410.7 eV), O 1s (530.9 eV), Cu 2p (932 eV), and S 2p (164 eV). The Cu 2p spectrum (Figure S4) indicated two prominent peaks at ~ 932 and 951 eV confirming the presence of Cu(I) and Cu(II) in CuSCD. The ratio for Cu^{2+} and Cu^{+} within CuS is 1.51 (Figure S5). The microstructure was characterized by transmission electron microscopy (TEM).^{35,36} The result indicated that the CuS nanoparticles exhibited a hollow structure with an average diameter of approximately 200 nm. The spherical carbon dots were uniformly deposited on the surface of CuS to form the nanoshell/satellite morphology (Figure 2a,b). The N_2 adsorption/desorption isotherms of CuSCD were illustrated in Figure S6. The typical Type-III isotherm and H3 hysteresis loops indicated the presence of pores in the CuSCD. The Brunauer–Emmett–Teller (BET) surface area and total pore volume of CuSCD were calculated to be 15.0638 m^2g^{-1} and 0.072 cm^3g^{-1} .

The deposition density of carbon dots was found to be dependent on the concentration of carbon dots during the synthetic process (Figure S7). Therefore, to obtain the optimum PCE, the CuSCD sample with 1 ml of carbon dots solution was selected for the following investigations. The content of carbon dots onto CuS was measured using thermogravimetric analysis (TGA). The results indicated that the CuS samples exhibited a weight loss of 7.98% with raising temperature to 800 °C (Figure S8). Fluorescence quantitative analysis further confirmed the loading of CD on CuS is 9.88% (m/m) (Figure S9). Taken together, these results indicated that the CuSCD nanohybrid was effectively synthesized. The components of CuSCD were further analyzed through elemental mapping and energy-dispersive spectroscopy analysis, which collectively indicated the presence of Cu, S, and C (Figure S10).

From the UV–vis absorption spectra, CuSCD exhibited broad absorption bands from 500 to 900 nm (Figure S11). The thermal properties of CuSCD or CuS aqueous solution were investigated with 808 nm laser irradiation. As presented in Figure 2c, the temperature of CuSCD solution (100 µg/ml) could increase to 65.6 (42.2 °C increased) with the irradiation of 0.5 Wcm⁻² for 7 min, while the temperature of CuS and water could only increase to 47.5°C (24.1 °C increased) and 25.8 °C (2.4°C increased), respectively. The corresponding PCE (η) of CuSCD could reach to 39.7%, which was 1.9-fold higher than CuS (21%) (Figure S12). Moreover, the high photothermal efficiency was dependent on the concentration of the CuSCD and laser power density (Figure S13), respectively. The temperature of the CuSCD solution (100 µg/ml) rapidly reached 90.3°C after irradiation with 808 nm laser (1 Wcm⁻²) for 7 min. Additionally, the heat-generation ability of CuSCD remained stable after three cycles of heating and cooling (heating 5 min and cooling 5 min for one cycle, Figure 2d). Besides, CuSCD's structure remained intact with 808 nm laser irradiation of 1 Wcm⁻² for 10 min (Figure S14). This enhanced PCE of CuSCD was further

confirmed with a theoretical model which calculated the electronic structures of CuS supported CD using vienna *ab-initio* simulation package (Figure S15). The results demonstrated that the excited electron from the CuSx can efficiently transferred to the graphite, indicating an efficient energy transfer between these two materials and favorable improvement in the photothermal effect of CuSCD.

Next, the NIR photothermal effect of CuSCD in 4T1-induced tumor model was investigated. Following 1 h of tail vein injection of CuS or CuSCD (5mg/Kg and 100 μ L, respectively), the mice were anesthetized. Considering the maximum permissible exposure (MPE) of skin (0.33 W cm^{-2} for 808 nm laser), the tumors were irradiated with NIR light at the power density of 0.3 W cm^{-2} for 6 min. The change of temperature at the tumor site was monitored using a thermal imaging camera. As presented in Figure 2e, it was observed that the CuS group exhibited a temperature increment of $15.1 \pm 0.9 \text{ }^{\circ}\text{C}$ at the tumor site, while the CuSCD groups exhibited a temperature increment of $17.4 \pm 0.8 \text{ }^{\circ}\text{C}$, suggesting that CuSCD could accumulate in the tumor through a passive targeting strategy and had higher NIR-photothermal effect than CuS *in vivo* for thermal therapy of cancer. It should be noted that although the CuSCD could accumulate in the tumor and had only $39.3 \pm 1.4 \text{ }^{\circ}\text{C}$ of temperature in 6 min, the photothermal effect remained poor due to the poor biocompatibility, targeting ability, and immune escaping ability of bare CuSCD. We thus developed a biomimetic nanotechnology by coating macrophage membrane hybridized with T7 peptide on the surface of CuSCD (CuSCDB@MMT7) to substantially enhance the photothermal effect.

The synthesis of CuSCDB@MMT7 was carried out as described previously involving.^{37,38} 1) isolation of macrophage membrane; 2) preparation of T7 peptide-conjugated PEGylated phospholipids (T7-PEG-DSPE). The T7 peptide (HAIYPRH) could specifically target the

transferrin receptor, which is overexpressed on the surface of cancer cells,^{39,40} 3) formulation of bortezomib loaded CuSCD nanoparticle core. Because bortezomib could not be transported to tumor tissues effectively for solid tumor, and the dosage cannot be increased to overcome poor tissue penetration due to an increased risk for toxic side effects. Thus, switching to a nanodrug formulation will offer the potential to alter the pharmacokinetics and provide a more favorable efficacy/toxicity profile for bortezomib in human trials. Especially, developing a controlled release formulation, such as NIR-Triggered bortezomib release nano-platform, can potentially lead to reducing off-target toxicity and a higher therapeutic index. 4) fusion of membrane-T7-PEG-DSPE vesicles on the surface of bortezomib-loaded CuSCD cores. The average hydrodynamic size of the CuSCDB@MMT7 was 222.5 ± 20.1 nm (PDI = 0.129) with a ζ -potential of $-18. \pm 1.8$ mV, which were slightly larger and less charged than those of the bare CuSCD (205.3 ± 20.4 nm, PDI = 0.087, -8.3 ± 1.3 mV) due to the presence of macrophage membrane envelop (Figure 3a to c). The characteristic membrane proteins of macrophage including CD44, CD135, and CD309, were well expressed on cell lysate, cell membrane, and CuSCDB@MMT7, as evidenced by SDS-PAGE analysis and Western-blot analysis (Figure 3d,e). However, no protein signal was detected in the CuSCD. The stability of the CuSCDB@MMT7 in PBS containing 10% fetal bovine serum at 37 °C was monitored using dynamic light scattering and only negligible changes in size was observed, demonstrating the high stability of CuSCDB@MMT7 in biological milieu (Figure S16). The drug loading and the encapsulation efficiencies of CuSCDB@MMT7 were $20.5 \pm 0.6\%$ and $81.9 \pm 2.5\%$ (Figure S17), respectively. The relatively high drug loading capacity can be attributed to the large cavity of the hollow structure of the CuSCD and the negative surface potential, both of which facilitate the storage and absorbance of positive bortezomib molecules.

The photothermal conversion experiments were performed to characterize the photothermal effects of CuSCDB@MMT7. The CuSCD and CuSCDB@MMT7 both exhibited similar temperature increment profiles, indicating that the macrophage membrane/T7 peptide coating and the loading drug had negligible effects on the photothermal properties of CuSCDB@MMT7. The temperature of CuSCDB@MMT7 could rapidly rise up to 65.3 °C with the NIR laser irradiation of 808 nm at a power density of 0.5 Wcm⁻² for 7 min, whereas the temperature of H₂O alone maintained 23.1 °C (Figure 3f). Next, the bortezomib release behaviors in PBS were investigated. The drug release rate of CuSCDB@MMT7 did not exhibit considerable difference at different values of pH (5.5, 6.5 and 7.4) indicating that pH-independent release of the drug. Approximately, 17.5% of bortezomib in CuSCD exhibited a burst release during the first 6 h (pH 7.4), which should be predominantly attributed to the premature drug leakage. After coating CuSCD with cell membranes, the drug release was apparently sustained with cumulative release of only 5.2% within 6 h, indicating the sustained coating efficiency and excellent stability of CuSCDB@MMT7 under the physiological conditions. Upon 808 nm irradiation, the 65.5% of drug release rate was recorded, which was 12.5-fold higher than no NIR light stimulation (Figure S18).

To test the targeting effect of the CuSCDB@MMT7 to cancer cells, the intracellular uptake of human or mouse macrophage membrane-coated CuSCDB@MMT7 (the liposome-coated CuSCD, CuSCD@LP, as the control)⁴¹ were investigated in mouse or human macrophage (RAW and Thp-1 cells), normal cells (L929 and LO2 cells), cancer cells (4T1 and MCF7 cells) qualitatively and quantitatively using confocal microscopy and Inductively coupled plasma atomic emission spectroscopy (ICP-AES), respectively. Compared to the intensity of the red fluorescence in the CuSCD@LP treated group the red fluorescence intensity in cells treated with CuSCDB@MMT7 was higher, indicating that the cell membrane with T7 peptide on the outer shell of the

CuSCDB@MMT7 increased the cellular uptake to cancer cells. Next, to test the specificity of nanoparticles, we treated the LO2 and L929 cells (normal cells with negative transferrin receptor expression) with the CuSCDB@MMT7 or CuSCD@LP. Both nanoparticles exhibited weaker signaling indicating higher selectivity of CuSCDB@MMT7 for targeting cancer cells. To verify the capability of escape from the macrophage phagocytosis of CuSCDB@MMT7, the nanoparticles were tested in the mouse or human macrophages. Compared with the CuSCD@LP, the CuSCDB@MMT7 showed low fluorescence intensity in RAW 264.7 and Thp1 cells. All these results were also confirmed with ICP-AES (Figure 3g,h and Figure S19) in 4T1, Raw or L929 cells treated with various nanoparticles (CuSCDB with a control 7 peptide with/without macrophage membrane coating as the control), and the results suggested that CuSCDB@MMT7 was specifically targeted to tumor cells, and exhibited immune escape capability from immune cells.

To further confirm the function of T7 peptides onto CuSCDB@MMT7 as the targeting agent, 4T1 cells were treated with different concentrations of DSPE-PEG-T7 coated CuSCD, subsequently performed the ICP-AES for Cu^{2+} quantification. As shown in Figure S20, a significantly higher intracellular Cu^{2+} content was found in CuSCD coated with T7 peptide group than with no functional 7 peptide control, indicating that T7 peptide modified nanoparticles could promote cellular uptake through a receptor-mediated pathway owing to the distinct affinity for transferrin receptors overexpressed in cancer cells. To further elucidate the underlying transmembrane mechanisms of CuSCDB@MMT7, siRNAs were used as specifically knockdown transferrin receptor (TFR) expression to inhibit TFR-mediated endocytosis. As presented in Figure S21, when the cells were treated with free T7 peptides or transferrin receptors siRNA, no significant variation in Cu^{2+} content was observed from increased concentration of CuSCDB@MMT7-treated cells

1
2
3 compared to their control group. All these results further validated the successful modification of
4 T7 peptide on the surface of CuSCDB@MMT7 and suggested that the internalization of
5 CuSCDB@MMT7 in cancer cells involves transferrin receptor-mediated endocytosis.
6
7

8
9
10
11 Given the targeted specificity to cancer cells with the characteristics of immune escaping and
12 enhanced cell endocytosis of the CuSCDB@MMT7 demonstrated *in vitro*, it was appealing to
13 investigate the targeting ability of the CuSCDB@MMT7 to the tumor *in vivo*. The NIR
14 photothermal effect of CuSCD in the 4T1 tumor model was performed. Following 1 h of tail vein
15 injection with CuSCD, CuSCD@MM or CuSCD@MMT7 (5mg/Kg, 100 μ L), the mice were
16 anesthetized, and then the tumors were irradiated with NIR light at the power density of 0.3 Wcm⁻²
17 for 6 min. The temperature changes at the tumor location were monitored by the infrared thermal
18 camera as shown in Figure 3i, j. It was deduced that CuSCD@MMT7 exhibited higher NIR-
19 photothermal effect than CuSCD or CuSCD@MM suggesting promising targeting-tumor
20 capabilities were benefited from the outer layer coating with macrophage membrane and T7
21 peptide, and the temperature increment was adequate to damage the cancer cell with
22 photothermally induced hyperthermia to improve the efficiency of PTT.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 To explore the NIR-triggered photothermal effect and the enhanced cancer-cell-killing ability, *in*
40 *vitro* functional studies were performed. The cytotoxicity of CuSCDB@MMT7 against 4T1,
41 MCF-7, L929, and LO2 cell lines were evaluated using CCK-8 assay (Figure S22 and Figure S23).
42 The drug-free CuSCD@MMT7 exhibited negligible dark cytotoxicity at the indicated
43 concentrations against 4T1, MCF-7, L929, and LO2 cells, demonstrating the good
44 biocompatibility of the carriers. NIR laser evidently improved the photothermal/targeting
45 therapeutic efficacy of CuSCD@MMT7 against 4T1 and MCF7 cells in contrast to the control
46 sample. The equal concentration of CuSCDB@MMT7 exhibited distinctly lower cytotoxicity than
47
48
49
50
51
52
53
54
55
56
57
58
59
60

free bortezomib, which can be attributed to the slow-release of bortezomib loaded in nanocomposites. Notably, significant antiproliferative effects were observed in the cells treated with CuSCDB@MMT7 with irradiation, which could result from the accelerated release of bortezomib from the nanocarriers through laser-induced hyperthermia and the triggered phototoxicity of CuSCDB@MMT7. The NIR irradiation-induced phototoxicity was further evaluated using LIVE/DEAD Viability/Cytotoxicity assay (Figure 4a), colony assay (Figure 4b,c) and wound healing study (Figure 4d,e). Upon NIR irradiation, cell death, colony-forming ability, and migration were most significantly suppressed in CuSCDB@MMT7 with irradiation treated cells, which was consistent with the findings of antiproliferative results. To further prove the advantages for the combined bortezomib–photothermal therapy, we evaluated the half-maximal inhibitory concentration (IC₅₀) in MCF7 and 4T1 cells treated with different concentrations of the CuSCDB@MMT7 and CuSCD@MMT7 under 808 nm laser irradiation for 5 min (0.5 Wcm⁻²). As shown in Figure 4f, the CuSCDB@MMT7 inhibited cell proliferation in MCF7 and 4T1 cells with IC₅₀ values of approximately 51.6 and 51.9 µg/ml, which were significantly lower than CuSCD@MMT7 (123.4 and 138.9 µg/ml). We also treated both cell line using the 100 µg/ml of nanoparticles with different irradiation power density of laser for 5min. It can be seen that the CuSCDB@MMT7 significantly blocked the proliferation of both cell line with lower IC₅₀ values of irradiation power density than CuSCD@MMT7 (Figure 4g). Chou-Talalay combination index displays a highly synergistic effect between PTT and bortezomib in 4T1 and MCF7 cells (Figure S24). Collectively, these *in vitro* results indicated that the PTT therapy combined with bortezomib holds potential promises to achieve the synergistic effect and improved treatment performances.

The ubiquitin-proteasome pathway is involved in a myriad of biological functions and plays a crucial role in tumorigenesis as well as in cancer progression and aggressiveness. The ubiquitin is

a heat-stable polypeptide¹⁸ and the polyubiquitinated substrate protein can be recognized by the 26S proteasome and degraded or processed.¹⁷ Proteasome inhibitors could protect various tumor suppressor proteins involved in apoptosis and cell cycle, including I κ B α ,²⁰ p27,²¹ and p21,²² from degradation caused by ubiquitin-proteasome system. Moreover, several reports have emphasized that the recombinant ubiquitin-labeled substrate can be stabilized at high temperature (≈ 85 °C) because fusion of the substrate to ubiquitin does not alter the heat resistance of ubiquitin molecule.^{42,43} Based on these findings, we hypothesized that proteasome inhibitors could prevent protein from degradation caused by proteasome system and photothermally induced hyperthermia. To elucidate the molecular mechanisms underlying NIR-triggered photothermal therapeutic effect of CuSCDB@MMT7 on cancer cells, we first assessed the levels of polyubiquitinated proteins among different treatments of 4T1 or MCF7 cells. As presented in Figure 5a,b, we determined that upon the NIR irradiation, CuSCDB@MMT7 significantly promoted accumulation of polyubiquitinated proteins, facilitating inhibition of ubiquitination-mediated protein degradation. To further assess if the polyubiquitinated proteins will be stabilized under the NIR-triggered therapeutic effect of CuSCDB@MMT7, we performed Western blot analysis to detect ubiquitin and its substrates expression in cells treated with different concentrations of CuSCDB@MMT7 or CuSCD@MMT7 under 808 nm laser irradiation for 0 to 7 min (0.3 W cm^{-2}). As expected, the CuSCDB@MMT7 significantly increased polyubiquitinated proteins leading to enhanced activity of I κ B α , p27, and p21 at dose- or time- course-dependent manner (Figure 5c to f), whereas the I κ B α , p27, and p21 showed rapid degradation in cells treated with CuSCD@MMT7 under the NIR irradiation. To further confirm the role of bortezomib as the crucial player in protecting protein from damage caused by CuSCDB@MMT7-based photothermally induced hyperthermia, we used CuSCD@MMT7 loaded doxorubicin-treated cells under the NIR irradiation as a control and

investigated the expressions of polyubiquitinated protein including I κ B α , p27, and p21. Doxorubicin is a cytotoxic chemotherapy drug used to treat various cancers but with a different mechanism. The primary mechanism of action of doxorubicin involves the drug's ability to slow or stop the growth of cancer cells by blocking DNA replication and ultimately inhibiting protein synthesis. As illustrated in Figure 5 c,e, the I κ B α , p27, and p21 exhibited the similar rapid degradation as CuSCD@MMT7 group indicating an unexpected impairment in combination therapy with PTT and chemotherapy, which may lead to the tumor cell survival and development of resistance to PTT due to the inactivation of these tumor suppressors. Together, CuSCDB@MMT7-based enhanced photothermal therapeutic effect *via* heat-stabilizing polyubiquitinated substrates of proteasome involved in cell proliferation and survival achieved promising therapeutic outcomes (Figure 5g).

Next, we evaluated systematically toxicology of CuSCDB@MMT7 *in vivo* to ensure their safe clinical translation. The liver function was evaluated by measuring the levels of ALT, AST, ALB, GLB, and TP. Whilst kidney function was assessed by measuring BUN and CREA markers. No significant difference between CuSCDB@MMT7-treated groups and PBS control group was observed at day 60 (Figure S25), elucidating that nanoparticles exhibited little toxicity to liver and kidney. The haematological markers were also determined. Compared with PBS group, all the parameters in the CuSCDB@MMT7-treated groups were found to be normal, indicating no systemic infection and inflammation were caused during the entire evaluation period (Figure S26). Further histological analyses of major organs confirmed the good biocompatibility of CuSCDB@MMT7 (Figure S27). Moreover, the injection of CuSCDB@MMT7 had negligible influence on the growth of mice (Figure S28). These preliminary *in vivo* biosafety findings indicated that CuSCDB@MMT7 is nontoxic, which supports their further clinical implications.

Furthermore, the biodistribution of CuSCDB@MMT7 was also evaluated to assess their behaviors *in vivo*. After systemic intravenous injection (i.v.) of 4T1 tumor-bearing mice, the biodistribution analysis of CuSCDB@MMT7 (CuSCDB, CuSCDB@T7, CuSCDB@CP, CuSCDB@MM, CuSCDB@MMCP as control) at different time points was initially investigated. As illustrated in Figure S29, the CuSCDB@MMT7 predominantly accumulated at the relative low level in liver and spleen due to the immune escaping ability from reticuloendothelial system. More importantly, the ICP-AES results demonstrated that CuSCDB@MMT7 accumulated efficiently at tumor sites during the first 24 h, which gradually increased with time, validating that CuSCDB@MMT7 could accumulate at tumor sites through EPR and active targeting effect.

Encouraged by the excellent photothermal effect, high tumor accumulation, and good biocompatibility of CuSCDB@MMT7, *in vivo* photothermal therapeutic efficacy was also further evaluated in the 4T1 breast tumor xenograft model (Figure 6a). 4T1 tumor-bearing mice were randomly divided into six groups ($n = 6/\text{group}$) and formulated with PBS, PBS+NIR, bortezomib, bortezomib+NIR, CuSCD@MMT7, CuSCD@MMT7+NIR, CuSCDB@MMT7, and CuSCDB@MMT7+NIR, respectively. As shown in Figure 6b, the two control groups (PBS and PBS+NIR) exhibited rapid tumor growth and all tumors in these groups increased by six-fold within 60 days. Compared with control groups, negligible antitumor efficacy was monitored for the mice treated with CuSCD@MMT7 or CuSCDB@MMT7 alone. However, anti-tumor effect was achieved for the mice receiving PTT (CuSCD@MMT7+NIR) with only $51.1 \pm 6.7\%$ reduction in tumor volume, because PTT alone could not completely eliminate all the cancer cells at the site of the tumor. A moderate inhibition in tumor was observed in bortezomib treated group ($36.1 \pm 5.1\%$ reduction), confirming that single drug therapy could barely suppress the tumor growth. Notably, the combination of chemotherapy and PTT exhibited superior anti-tumor efficacy

with a $93.1 \pm 9.9\%$ reduction in tumor volume. The mice were euthanized and the excised tumors were photographed and weighed. The average tumor weight was 834.1 ± 74.6 mg, 548.3 ± 131.5 mg, 810.3 ± 113.4 mg, 761.4 ± 110.1 mg, 788.1 ± 54.5 mg, 511.2 ± 68.4 mg, 492.8 ± 103.6 mg and 60.9 ± 30.1 mg for the mice treated with PBS, bortezomib, CuSCD@MMT7, CuSCDB@MMT7, PBS+NIR, bortezomib+NIR, CuSCD@MMT7+NIR, and CuSCDB@MMT7+NIR, respectively (Figure 6c). Analysis of the H&E stained tumor sections confirmed considerably enhanced necrosis in the CuSCDB@MMT7+NIR group, indicating that the combination of bortezomib and PTT was most effective in suppressing tumor growth (Figure 6d). Consistently, inhibition of the tumor proliferation was also confirmed by the PCNA staining (Figure S30). Besides, no weight loss was detected for the mice injected with CuSCDB@MMT7 followed by irradiation, confirming low systemic toxicity of the CuSCDB@MMT7 (Figure 6e). Due to the excellent anti-tumor performance and negligible systemic toxicity of CuSCDB@MMT7, the overall survival was significantly augmented, in contrast with the mice receiving bortezomib or PTT alone (Figure 6f).

To evaluate the anti-metastatic efficacy of the CuSCDB@MMT7, the lung, liver, and spleen were visualized and the numbers of pulmonary metastatic nodules and spleen weight were quantified. As shown in Figure S31 and Figure 6g, lung and liver metastatic nodules in CuSCDB@MMT7 + NIR group was significantly less in numbers than that of other groups (lung: PBS, 21.8 ± 3.3 ; bortezomib, 12.8 ± 3.9 ; CuSCD@MMT7, 19 ± 3.9 ; CuSCDB@MMT7, 17.7 ± 3.5 ; PBS + NIR, 21.2 ± 2.8 ; bortezomib + NIR, 14 ± 4.7 ; CuSCD@MMT7 + NIR, 12.8 ± 3.1 ; CuSCDB@MMT7 + NIR, 3.8 ± 2.5 ; liver: PBS, 20 ± 2.8 ; bortezomib, 10.8 ± 3.3 ; CuSCD@MMT7, 17.2 ± 3.4 ; CuSCDB@MMT7, 14.3 ± 3.8 ; PBS + NIR, 18.3 ± 2.6 ; bortezomib + NIR, 10.8 ± 3.1 ; CuSCD@MMT7 + NIR, 9.3 ± 1.6 ; CuSCDB@MMT7 + NIR, 3 ± 1.4). H&E staining further revealed a significant reduction in the number and size of visible metastatic nodules in

CuSCDB@MMT7 + NIR group. Moreover, the PBS control group displayed an increased infiltration of tumor cells into the spleens, leading to considerable damage to red pulp and white pulp of the spleen with splenomegaly. CuSCDB@MMT7 + NIR treatment markedly suppressed the metastatic tumor cells in the spleen with reversed the splenomegaly (PBS, 768.5 ± 85.2 mg; bortezomib, 586 ± 67.9 mg; CuSCD@MMT7, 841.3 ± 104.6 mg; CuSCDB@MMT7, 685.7 ± 93.3 ; PBS + NIR, 839.7 ± 90 mg; bortezomib + NIR, 556.7 ± 108.2 mg; CuSCD@MMT7 + NIR, 561 ± 59.6 mg; CuSCDB@MMT7 + NIR, 280.7 ± 69.9 mg). Heart and kidney did not show any pathological alterations (Figure S32). Mechanistic investigation showed that tumor cells from CuSCDB@MMT7 + NIR group exhibited the highest protein levels of I κ B α , p21 and p27 (Figure S33). Finally, to further prove the advantage for the strategy of combined bortezomib–photothermal therapy, we compared the efficacy of CuSCDB@MMT7 and CuSCD@MMT7 *in vivo* independently. As shown in Figure S34, PTT alone (CuSCD@MMT7) could achieve the same therapeutic efficacy to CuSCDB@MMT7 in tumor-bearing mice at overdose of CuSCD@MMT7 (≈ 15 mg/Kg) with high systemic toxicity. Collectively, these results revealed that synergistic therapy of CuSCDB@MMT7 could not only undermine the local tumor but can also produce significant antitumor effect to prevent the tumor metastasis with low systemic toxicity for enhanced photothermal therapy *via* stabilization of heat-stabilizing polyubiquitinated substrates of proteasome. Further analysis of the combination of PTT with other proteasome inhibitors (including carfilzomib⁴⁴ and ixazomib)⁴⁵ or deubiquitinase inhibitors^{46,47} is warranted, in order to achieve optimal photothermal effect with minimal toxicity. Conceivably, the cancer cell may develop thermoresistance to the PTT due to the activation of heat shock proteins (HSPs).^{48,49} Thus, it would be interesting to consider and analyze a combination strategy of co-carrying proteasome inhibitors and HSP inhibitors in PTT for enhanced therapeutic outcomes.

CONCLUSIONS

In summary, we rationally designed and developed bortezomib-loaded biomimetic nanocomposites with the macrophage membrane and T7 peptide co-coating for targeted tumor therapy. The CuSCDB@MMT7 provides advantages in terms of 1) enhanced PCE through hybridization of CuS with carbon dots; 2) increased specificity for cancer cell targeting and favorable immune evasion; 3) enhanced anti-tumor efficacy, which not only inhibited the tumor growth but also suppressed the metastasis; 4) increased heat-stability of various substrates in the ubiquitin-dependent proteasomal degradation pathway involved in cell proliferation and survival, which realized promising treatment outcomes. Furthermore, this study improves our understanding of molecular mechanism underlying CuSCDB@MMT7-triggered cell death upon NIR exposure involves the accumulation of polyubiquitinated I κ B α , p21, and p27 protein that are heat stabilization under NIR induced hyperthermia, thereby resulting in the disruption of downstream aberrant signaling pathway that favors cell survival, promoting cell apoptosis and preventing cell metastasis. 5) design of nanocomplexes as the clinically potential agent for combination therapy is very promising strategy for cancer therapy. The major advantages in this combination therapy is the excellent synergistic therapeutic efficacy achieved at a relatively low drug dose and laser irradiation compared to the monotherapies by using similar types of nanoparticles. Overall, this work highlights a promising therapeutic strategy for the treatment of advanced cancer, supports their further evaluation, and provides a framework for realizing improved therapeutic outcomes for potent clinical cancer therapy.

EXPERIMENTAL SECTION

Materials: All chemical used were commercially available organic reagents and were of analytical grade. Copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) and sodium sulfide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) were procured from Acros Co., Ltd. Polyvinylpyrrolidone (PVP-K30) was purchased from Sigma-Aldrich, Ltd. Sodium hydroxide (NaOH) and hydrazine anhydrous solution ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$) were purchased from Beijing Chemical Reagent Co. Ltd. Ethylenediamine was purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd., China. Saccharomyces was purchased from Shandong Sukahan Co. Ltd. Dialysis bags were purchased from Shanghai Yuanye Bio-Technology Co. Ltd., China.

Characterization: The powder X-ray diffraction (PXRD) patterns were recorded on a Rigaku D/Max 2550 X-ray diffractometer with $\text{Cu K}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) radiation (40 kV, 40 mA). Transmission electron microscopy (TEM) and energy-dispersion X-ray (EDX) elemental mapping analysis of CuSCD were conducted on an FEI Tecnai G2 S-Twin microscope equipped with a field emission gun operating at an accelerating voltage of 200 kV. The infrared (IR) spectra were recorded with KBr pellets in the $400\text{--}4000 \text{ cm}^{-1}$ region on a Bruker IFS 66v/S FT-IR spectrometer. Low-pressure N_2 gas sorption experiments at 77 K were carried out on a Micro metrics ASAP 2020 volumetric gas sorption instrument. Before gas adsorption measurements, the samples were heated to $100 \text{ }^\circ\text{C}$ to completely remove the solvent molecules. Ultraviolet-visible (UV-vis) absorption spectroscopy was conducted on a Shimadzu UV-2450 UV-vis spectrophotometer. X-ray photoelectron spectroscopy (XPS) analysis was carried out using an ESCALAB 250 spectrometer with a monochromatic $\text{Mg-K}\alpha$ as X-ray source (1486.6 eV). Raman spectra were obtained by Laser Raman spectrometer JY-T64000 (HORIBA Jobin Yvon), using radiation at 514 nm. Thermogravimetric measurement was performed on pre-weighed samples using a Netzsch STA 449C apparatus by heating from -from room temperature to $800 \text{ }^\circ\text{C}$ at a heating rate of $10 \text{ }^\circ\text{C min}^{-1}$ under N_2 atmosphere.

Synthesis of carbon dots: Previously, we have demonstrated that microwave-assisted hydrothermal conditions facilitate the growth of N-doped carbon dots (CDs). Briefly, saccharomycetes (0.4000 g) as carbon source and ethylenediamine (5 ml) as nitrogen resource were dissolved in deionized water (15 mL). Subsequently, the mixture was stirred and transferred to a microwave oven (ETHOS ONE, ITALY), and heated up to and held at 200 °C for 2 h. After naturally cooling down to room temperature, raw products were obtained. Thereafter, the product was centrifuged and then dialyzed against deionized water through a dialysis membrane (MW = 500-1000) for 24 h, the CDs solution was obtained. The dialyzed solution was subjected to rotary evaporation to obtain 5 ml of a concentrated solution of CDs. The CDs made from saccharomyces exhibited excellent photoluminescence properties and a narrow size distribution range.

Synthesis of hollow CuS and CuSCD nanospheres: CuS nanospheres were synthesized according to a previously reported method with slight modification.⁵⁰ Briefly, 100 μ L CuCl₂ solution (0.5 M) was dissolved in 25 mL deionized water containing 0.25 g PVP-K30 using magnetic stirring at room temperature. Subsequently, 25 mL of NaOH solution (pH = 9.0) was added, followed by addition of 4 μ L N₂H₄·H₂O (85 %) to form a bright-yellow suspension of Cu₂O spheres. After 10 min, 200 μ L Na₂S aqueous solution (0.32g mL⁻¹) was added to the suspension. For CuSCD, 1 ml of the concentrated solution of CDs prepared above was injected under vigorous stirring. The reaction solution was shaken at a rotation rate of 200 rpm using an incubator shaker at 60 °C for 2 h. Lastly, CuSCDs nanospheres were centrifuged at 11000 rpm for 15 min and washed three times with deionized water and ethanol, then, the CuSCDs nanospheres were dispersed in 10 mL ethanol.

CuSCDB@MMT7 Preparation: For macrophage membrane isolation, the cell membrane was derived from RAW 264.7 or Thp1 using previously reported protocols.⁵¹ T7-PEG-DSPE was prepared as reported previously.⁵² To prepare of bortezomib-loaded CuSCD, 2 mg free drug was

dissolved in 0.2 mL DMSO and then added into 100 $\mu\text{g/mL}$ dispersion of CuSCD (mixture total volume 20 mL) at 24°C for 24h with magnetic stirring. The excess un-loaded bortezomib from the resultant product (CuSCDB) was redispersed and washed away by 10 mL PBS (pH 7.4) with 1% DMSO through centrifugation (10000 rpm, 10 min) for several times. The supernate was collected to determine the total bortezomib content in supernatants by using the HPLC or UV absorbance according to a predetermined standard curve. To prepare of macrophage membrane with T7 peptides vesicles, membrane and T7-DSPE-PEG were physically extruded through a 220 nm polycarbonate membrane for several passes. Vesicles then conjugated to bortezomib-loaded CuSCD cores were fused through extrusion with an Avanti mini-extruder (Avanti Polar Lipids). We set up various nanoparticles as the control: CuSCDB modified with the control 7 peptide (CuSCDB@CP); CuSCDB modified with T7 peptide (CuSCDB@T7); CuSCDB coated with macrophage membrane and control peptide (CuSCDB@MMCP); CuSCDB coated with macrophage membrane (CuSCDB@MM); CuSCD coated with liposome (CuSCD@LP).

Theoretical model: The DFT calculations were performed using Vienna *ab-initio* simulation package (VASP). Based on the XPS results, the CuS_x structure comprised of both the Cu⁺ and Cu²⁺ cations. The prototypical CuS_x model was constructed using the CuS structure by randomly removing 27% of the S atoms. The model contained 3*5*6 unit cells, with the (001) facet pointing to the vacuum. There was over 3 nm vacuum to separate the slabs. The structural optimization of the CuS_x was performed using the Perdew-Burke-Ernzerh (PBE) level of the density functional theory (DFT). The electrons were described using the projected-augmented plane-wave (PAW) method with a 400-eV energy cutoff implemented in the VASP package. The Brillouin zone of the slab was described using 4*4*1 k-points. Both the coordinates and the cell parameters were optimized with the criteria of the force difference of smaller than 0.0001 eV/nm. The density of

states (DOS) was obtained using the optimized structure with a 520 eV as energy cutoff. Subsequently, the band structure between the CuSx and graphite was aligned by assuming the vacuum potential as the zero points. The vacuum energy was obtained by averaging the electrostatic potential over planes parallel to the CuSx or graphite surfaces. Both the CBM and VBM of the CuSx and the fermi level of the graphite was aligned based on this energy level.

Photothermal Effect of Nanoparticle Composites in Solution: To examine the photothermal behavior of nanoparticle composites in solution, different concentrations were irradiated with 808 nm NIR light. Briefly, 100 μ L of the aqueous solutions of nanoparticle composites with different concentrations (100, 25, 12.5, 6.25, and 0 mg/ml) were deposited into the Eppendorf tube, respectively. The tube containing solution was irradiated with an 808 nm continuous-wave laser at a power density of 0.5, 0.8, and 1.0 Wcm⁻², respectively, with the different exposure time. Pre- and post- irradiated temperature changes were recorded using an infrared thermal imaging camera.

Calculation of the photothermal conversion efficiency (PCE): The PCE of CuSCD composites with different CDs contents was determined according to the previous method.²⁸ Briefly, CuSCD with different concentrations of CDs (0, 0.2, 0.5, 1 and 2 ml) were deposited into a 96-well plate. The wells were irradiated with an 808 nm laser (0.5 W cm⁻²) at different exposure time. Temperature were recorded using an infrared thermal imaging camera (FOTRIC).

The PCE, η , was calculated using Equation 4. The detailed calculation was performed as follows:

Based on the total energy balance for this system:

$$\sum_i m_i c_{p,i} \frac{dT}{dt} = Q_{NP_s} + Q_s - Q_{loss} \quad (1)$$

where m and C_p refer to the mass and heat capacity of solvent (water), respectively. T is the temperature of the solution. Q_{NP} is the photothermal energy input by CuSCD, Q_s is the heat associated with the light absorbance of the solvent, and it is calculated to be approximately equal to 0 mW, and Q_{loss} is thermal energy lost to the surroundings.

$$Q_{NP} = I(1 - 10^{-A_\lambda})\eta \quad (2)$$

$$Q_{loss} = hA\Delta T \quad (3)$$

Based on Eq.1, Eq.2, and Eq.3, the PCE (η) can be determined as

$$\eta = \frac{hs\Delta T_{max} - Q_s}{I(1 - 10^{-A_\lambda})} \quad (4)$$

where I is the laser power, A_λ is the absorbance of CuSCD at 808 nm, and η is the PCE, h is the heat transfer coefficient, A is the surface area of the container, and ΔT is the temperature change, which is defined as $T - T_{surr}$. The hs was calculated from Figure S12. The hs was calculated using the following eq 5.

$$t = \frac{\sum_i m_i c_{p,i}}{hs} \theta \quad (5)$$

$$\theta = \frac{\Delta T}{\Delta T_{max}} \quad (6)$$

Substituting hA value into Eq.4, the PCE (η) of CuSCD can be calculated and listed in the table.

V_{CDs}	0 ml	0.2 ml	0.5 ml	1 ml	2 ml
-----------	------	--------	--------	------	------

hs	0.00405	0.00387	0.00404	0.00404	0.00398
$\Delta T_{\max}$	18.9°C	23.6°C	31.3°C	42.2°C	34.7°C
$A_{\lambda 808}$	0.56	0.58	0.61	0.85	0.66
η	21.0%	24.7%	33.7%	39.7%	35.4%

Bortezomib release in vitro: To evaluate the release kinetics of bortezomib, CuSCDB@MMT7 were incubated with different amount of bortezomib (0 to 2 mg), and analyzed by using high-performance liquid chromatography (HPLC) as described previously.⁵³ Briefly, the sample or standard (0 to 20 mg/ml of bortezomib) were injected into the HPLC column in the mobile phase. The absorbance of the samples were measured at 255 nm. The standard curve was used to determine the concentration of bortezomib. The loading efficiency (LE) was calculated as follows:

$LE = (M_{\text{total}} - M_{\text{un}}) / M_{\text{total}}$, where M_{total} is the amount of total bortezomib and M_{un} that of unloaded bortezomib).

Bortezomib release from the nanoparticles was measured after incubating CuSCDB@MMT7 (100 $\mu\text{g/ml}$) in different pH values of PBS with/without 808 nm irradiation (0.5 Wcm^{-2} , 5 min). The supernatant was collected and the amount of bortezomib released was determined using the HPLC.

Cell membrane protein characterization: To determine whether RAW 264.7 cell membrane, cell lysate and CuSCDB@MMT7 have the same protein profile, the membrane protein was characterized by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method as described previously.⁵³ The protein signals were measured by the gel imaging system.

Evaluation of the cellular uptake of CuSCDB@MMT7: The cellular uptake was evaluated as previous report.⁵¹ Briefly, Nile red-labeled CuSCDB@MMT7 were incubated with cells for 30

1
2
3 min. The cells were washed and fixed with 4% formaldehyde. After 15 min, cells were observed
4
5 under a confocal fluorescent microscope.
6

7
8 To evaluate the uptake of CuSCDB@MMT7 quantitatively, different concentrations of
9
10 nanoparticles were incubated with cells for 30 min. The cells were collected, washed, and
11
12 dissolved using nitric acid overnight. Then, the sample was diluted to 500 μ l using deionized water.
13
14 A Perkin-Elmer Optima 3300DV ICP spectrometer was used to analyze the concentration of Cu^{2+} .
15
16

17
18 *Cell culture and transfection:* MCF-7, 4T-1, L929, Thp-1 and RAW 264.7 were procured from
19
20 American Tissue Culture Collection. LO2 cell line was obtained from the Institute of Basic
21
22 Medical Sciences, Chinese Academy of Medical Sciences. All cells were cultured in Dulbecco's
23
24 modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Life Technology)
25
26 at 37 °C in a humidified incubator with 5% CO_2 . Transfection study was performed as described
27
28 previously.^{54,55} Briefly, cells (1×10^6) were seeded into 6-well plates overnight before transfection.
29
30 The transferrin receptor siRNA (100 nM) and scramble (Sangon Biotech) were introduced into
31
32 cells using Lipofectamine™ RNAiMAX (Thermo Fisher Scientific).
33
34
35
36

37
38 *Western blotting:* The Western blot analysis was performed as previously described.⁵⁶ The
39
40 following antibodies obtained from Santa Cruz Biotechnology: Anti- β -actin (sc-47778), anti-
41
42 ubiquitin (sc-166553) and anti-CD44 (sc-13131). Anti-p27 (3686S), anti-p21 (2947S), anti-I κ B α
43
44 (4814S), anti-CD135 (3464S) and anti-CD309 (2478S) from Cell Signaling Technology.
45
46

47
48 *Cell proliferation assay:* Cell proliferation assays were performed using Cell Counting Kit-8
49
50 (CCK8, Dojindo Molecular Technologies) following manufacturer's instructions. Briefly, cells
51
52 were seeded in 96-well plates at the density of 1×10^5 per well, and cultured for 24 h. The medium
53
54 was then replaced with that containing nanoparticles or nanoparticles loaded with different
55
56
57
58
59
60

concentrations of bortezomib. After a 2 h incubation, wells were irradiated (0.5 Wcm^{-2} , 5 min), and the cells were cultured for 24 or 48 h. The absorbance was measured by a microplate reader (BioTek Epoch) at 450 nm. All the experiments were performed in triplicate.

Live Dead Cell Experiment: Calcein AM and PI co-staining were used to assess cell viability following manufacturer's instructions (Dojindo Molecular Technologies). Briefly, cells were grown in 24-well plates at a density of 1×10^4 cells per well. After incubation for 16 hours, the cells with different treatment were cultured overnight. The cells were washed and stained with calcein AM (live cells) and sodium propidium iodide (dead cells), respectively, and observed under a Nikon confocal fluorescence microscope.

Colony-forming assays: Colony-forming assays were performed as previously described.⁵⁷ Briefly, 1×10^4 cells were seeded in 24-well plates. Cells with different treatment (short time treatment and the cells were not apoptotic) were cultured for additional 48 h. Cells were fixed and stained by Giemsa staining following the manufacturer's instructions (BASO).

Wound-healing assays: Wound-healing assays were performed as previously described.^{54, 57} The migration of cells towards the wound was photographed under a Nikon fluorescence microscope and cell migration rate was quantified using CorelDRAWX8 Software (Corel Corporation).

Animal studies: Balb/c mice (female, 4-6 weeks old) were purchased from Charles River Company and maintained in a pathogen-free environment at ambient temperature under the guidelines approved by the ethics committee of Jilin University. All animal procedures were performed according to the research protocol approved by the Animal Care and Use Committee of Jilin University. To develop a tumor model, 4T1 cells (1×10^6) were subcutaneously injected into the right flank mammary gland of each mouse. When the tumor volume reached $\approx 100 \text{ mm}^3$, mice were

divided randomly into 8 groups (6 mice in each group): (1) PBS, (2) bortezomib (0.5 mg/Kg, equivalent to the dose loaded in CuSCDB@MMT7), (3) CuSCD@MMT7 (5 mg/Kg), (4) CuSCDB@MMT7 (5mg/Kg), (5) PBS with laser irradiation, (6) bortezomib (0.5 mg/Kg) with laser irradiation, (7) CuSCD@MMT7 (5 mg/Kg) with laser irradiation, (8) CuSCDB@MMT7 (5mg/Kg) with laser irradiation. The dosage of bortezomib is within the range of clinically attainable levels. The mice were intravenously injected with 100 μ l of the respective formulations every two days, and irradiated for 5 min with an NIR laser (808 nm, 0.3 Wcm⁻²) 4 h after each injection as appropriate (Six injections were administered in total). The tumor volume and mouse body weight were measured every two days. Tumor diameters were measured with a digital caliper and tumor volume was calculated using the formula: tumor volume (mm³) = (length $\times$ width $\times$ height $\times$ Π /6). After 60 days, all the mice were euthanized and the major organs and tumors were collected for H&E staining and IHC staining. The weight of the spleen and the number of metastasis nodules are determined at the end of each experiment. For the tumor nodules counting, the liver or lung of each tumor-bearing mouse was harvested, then the lesions (tumors) $\geq$ 0.5 mm in a single dimension were counted, mapped, measured and photographed. The H&E staining was performed to confirm the results. The lesion counting and H&E-stained slides were independently reviewed by 3 pathologists in a blinded fashion.

Immunohistochemical (IHC) staining and Hematoxylin and Eosin (H&E): The IHC and H&E staining was performed as previously described.⁵⁴ The following antibodies were used for IHC staining: anti-PCNA (Cell Signaling Technology, #12727), anti-p21 (Cell Signaling Technology, #2947S), anti-p27 (Cell Signaling Technology, #3686S) and anti-I κ B α (Cell Signaling Technology, #4814S). The images were captured using a Nikon confocal fluorescence microscope and analyzed using Image-Pro Plus software.

1
2
3 *In vivo biocompatibility of CuSCDB@MMT7*: Male Balb/c mice (4-6 weeks old, n=5) were
4 intravenously administrated with CuSCDB@MMT7 (10 mg/Kg, 100 μ l) or PBS. After 60 days,
5 the mice were sacrificed, complete blood investigation and serum biochemistry assays were carried
6 out by collecting 0.5 mL blood from the mice. The major organs (heart, liver, spleen, lung, and
7 kidney) were harvested, stained with hematoxylin and eosin, and examined under digital
8 microscope. The *in vivo* toxicology was determined using hematological, biochemical, and
9 histological analyses. The white blood cells (WBS), red blood cells (RBC), mean corpuscular
10 volume (MCV), platelets (PLT), mean corpuscular hemoglobin (MCH), and hemoglobin
11 concentration (MCHC) were measured. Blood biochemical examination parameters included
12 alanine transaminase (ALT), aspartate transaminase (AST), total protein (TP), blood urea nitrogen
13 (BUN), albumin (ALB), alkaline phosphatase (ALP), direct bilirubin (DBIL), and glucose (GLU).
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

29 *In vivo biodistribution of CuSCDB@MMT7*: The quantitative biodistribution analysis were
30 performed as previously described.⁵⁵ 4T1 tumor-bearing mice (≈ 200 mm³ volume of tumor) were
31 intravenously injected with various nanoparticles (20 mg/Kg, 100 μ l) or PBS (for normalization
32 of endogenous Cu) and randomly divided into three groups (n=5). At 24 h post-administration, the
33 tissues or organs (tumor, heart, spleen, kidney, liver, lung and blood) were dissected and weighed.
34 The tissues digested in aquaregia (80 $^{\circ}$ C) to analyze the content of copper using ICP-AES (Perkin-
35 Elmer Optima 3300DV ICP spectrometer).
36
37
38
39
40
41
42
43
44
45

46 *Statistical analysis*: For *in vitro* experiments, such as CCK-8 assay, western blot, clonogenic
47 assays, wound healing studies, *etc.* were repeated at least three times. The statistical analysis was
48 performed using one-way analysis of variance (ANOVA) with Tukey's test. Survival curves were
49 generated using the Kaplan–Meier method (log-rank test). All analyses were performed using the
50
51
52
53
54
55
56
57
58
59
60

GraphPad Prism 8 Software. $p < 0.05$ was considered statistically significant. All p values were two-tailed.

Figure 1. Schematic illustration of the generation of proteasome inhibitor-encapsulated CuS/carbon dots nanocomposites for enhanced photothermal therapy *via* heat-stabilization of various substrates in the ubiquitin-dependent proteasomal degradation pathway

Figure 2. Characterization of CuSCD. a) Representative TEM images of CuS, CD and CuSCD. b) Dark-field TEM image (i), TEM/EDS Cu–K map (ii), TEM/EDS S–K map (iii) and TEM/EDS C–K map (iv) of the CuSCD. c) Photothermal-heating curves and represented thermal images of

different concentrations of (100 $\mu\text{g/ml}$) CuS and CuSCD under 808 nm laser irradiation at a power density of 0.5 $\text{W}\cdot\text{cm}^{-2}$ for 7 min. d) On-off curves of CuSCD (100 $\mu\text{g/ml}$) under 808 nm laser irradiation at 0.5 $\text{W}\cdot\text{cm}^{-2}$ for 3 cycles. e) Representative photo-thermal images of 4T1 tumor-bearing mice treated with CuS and CuSCD (100 $\mu\text{g/mL}$) with/out 808 nm laser irradiation at 0.3 $\text{W}\cdot\text{cm}^{-2}$ for 6 min with the corresponding temperature curves.

Figure 3. Characterization of CuSCDB@MMT7. a) Representative TEM image. b) Particle size and c) zeta potential of nanoparticles by DLS. d) Representative image of SDS gel showing protein bands of different nanoparticles and whole cells. e) Western blot analysis of cell membrane markers for CuSCD, cell lysate, macrophage membrane and CuSCDB@MMT7. f) Photothermal-heating curves and represented thermal images of H₂O, CuSCD or CuSCD@MMT7 (100 μ g/ml)

1
2
3 under 808 nm laser irradiation at a power density of 0.5 Wcm^{-2} for 7 min. g) Representative
4 fluorescence images of cellular uptake of Nile Red-labeled CuSCD@LP or CuSCD@MMT7 (red
5 fluorescence) in 4T1, L929, or RAW 264.7 cells after 30 min of incubation. The nucleus was
6 stained with DAPI (blue). Scale bar = $50 \mu\text{m}$. h) Cu^{2+} content was measured by ICP-AES in 4T1,
7 Raw or L929 cells treated with CuSCD@LP or CuSCD@MMT7 at various concentrations for 30
8 min. i) Representative photo-thermal images of 4T1 tumor-bearing mice treated with PBS, CuSCD,
9 CuSCD@MM or CuSCD@MMT7 ($100 \mu\text{g/ml}$) with/out 808 nm laser irradiation at $0.3 \text{ W}\cdot\text{cm}^{-2}$
10 for 6 min with the corresponding j) temperature curves.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 4. *In vitro* anti-tumor activity of CuSCDB@MMT7. a) Representative fluorescence images of differentially-treated MCF7 or 4T1 cells (as indicated) after live/dead staining. Scale bar = 50 μm . b) Representative pictures of colonies formed by MCF7 or 4T1 cells treated as indicated. c) Quantification of colony numbers in the differentially-treated groups. d) Representative images of cell mobility in MCF7 or 4T1 cells treated as indicated. e) Quantification of wound-healing assays. Viability of MCF7 or 4T1 cells treated with CuSCD@MMT7 or CuSCDB@MMT7 at f) the indicated concentrations of nanoparticles or g) power density of laser (100 $\mu\text{g}/\text{ml}$ nanoparticles).

The data are represented as mean $\pm$ S.D. from three independent experiments, $*p < 0.05$, $**p < 0.01$. C@M7, CuSCD@MMT7; CB@M7, CuSCDB@MMT7.

Figure 5. The molecular mechanism underlying the PTT effects of CuSCDB@MMT7. a) Western blot and b) quantification analysis for ubiquitin, IκBα, p21 or p27 in MCF7 and 4T1 cells treated with PBS, bortezomib, CuSCD or CuSCDB@MMT7 with/out 808 nm laser irradiation (0.5 Wcm⁻², 5 min) for 24 h. c-f) The effects of CuSCDB@MMT7 on photo-thermal stabilization of polyubiquitinated proteins. Representative western blot (c) and quantification analysis (d) for ubiquitin, IκBα, p27 and p21 in 4T1 cells treated with CuSCD@MMT7, CuSCD-DOX@MMT7 or CuSCDB@MMT7 PBS under 808 nm laser irradiation (0.3 Wcm⁻², 5 min) at dose-dependent manner for 24 h. Representative western blot (e) and quantification analysis (f) for ubiquitin, IκBα, p27 and p21 in 4T1 cells treated with 50 μg/ml of CuSCD@MMT7, CuSCD-DOX@MMT7 or

1
2
3 CuSCDB@MMT7 PBS under 808 nm laser irradiation (0.3 Wcm^{-2}) at time-course-dependent
4 manner for 24 h. The results are derived from three biological replicates. g Schematic illustrations
5 of molecular mechanism underlying the PTT effects of CuSCDB@MMT7. Data are mean $\pm$ SD;
6
7
8
9
10 $*p < 0.05$, $**p < 0.01$. Image J was used for quantification and β -actin was the internal control.
11
12 C@M7, CuSCD@MMT7; CB@M7, CuSCDB@MMT7; CD@M7, CuSCD-Dox@MMT7.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 6. *In vivo* anti-tumor therapeutic effect of CuSCDB@MMT7. a) Schematic illustration of 4T1 tumor model establishment and the therapeutic regimen. b) Tumor growth curves. c) Tumor weight. d) Representative images of H&E-stained tumors from the different treatment groups. Scale bar = $100\text{ }\mu\text{m}$. e) Body weight of the mice in different groups. f) The survival curve of mice calculated by Kaplan-Meier estimate for 60 days. g) Number of tumor nodules on the lung (left), liver (middle), and spleen weight (right) in the different groups. The data are represented as mean $\pm$ S.D. $n = 6$ mice per group. Data are mean $\pm$ SD; $**p < 0.01$. C@M7, CuSCD@MMT7; CB@M7, CuSCDB@MMT7.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge *via* the Internet at <http://pubs.acs.org>.

Additional experimental details and data, including XRD pattern, Raman spectra, FT–IR spectra, TEM image, XPS spectrum, TGA curves, UV–vis spectrum, fluorescence spectrum, photothermal conversion efficiency assay, photothermal-heating curves, theoretical model, stability of CuSCDB@MMT7, drug loading, encapsulation efficiencies, *in vitro* bortezomib-release, ICP-AES, cell viability, synergy assay, blood biochemical assay, Haematological analysis, IHC images, biodistribution assay, body weight curves and H&E staining images (PDF)

The authors declare no competing financial interest.

AUTHOR INFORMATION

Corresponding Author

*E-mail: feiyang@jlu.edu.cn (F. Yan)

*E-mail: zshi@mail.jlu.edu.cn (Z. Shi)

ORCID

Yu Han: 0000-0003-1462-1118

Fei Yan: 0000-0003-4750-1200

Zhan Shi: 0000-0001-9717-1487

Shouhua Feng: 0000-0002-6967-0155

Author Contributions

F. Y. and Z. S. designed and guided the overall research project. Y.Y., M.S., C.C., Y.D., C.L. performed the experiments. Y.Y., C.C. and C.L. carried out the preparation and characterization of the nanoparticles. M.S. and Y. D. performed cell and animal experiments. Y.Y., M.S., Y. H., F. Y., Z. S. and S.F. analyzed data and prepared the manuscript. F. Y. and Z. S. conceived ideas and supervised the entire research project. All authors discussed the results and commented on the manuscript.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (NSFC; nos. 81870117, 21771077, 21771084 and 21621001), the National Key Research and Development Program of China (no. 2016YFB0701100), the 111 project (no. B17020), and the Jilin Province Science and Technology Development Plan (20190201252JC). The authors also gratefully acknowledge the financial support by Program for JLU Science and Technology Innovative Research Team (JLUSTIRT).

REFERENCES

- (1) Liu, Y. J.; Bhattarai, P.; Dai, Z. F.; Chen, X. Y. Photothermal Therapy and Photoacoustic Imaging *via* Nanotheranostics in Fighting Cancer. *Chem. Soc. Rev.* **2019**, *48*, 2053-2108.
- (2) Li, J. C.; Pu, K. Y. Semiconducting Polymer Nanomaterials as Near-Infrared Photoactivatable Protherapeutics for Cancer. *Acc. Chem. Res.* **2020**, *53*, 752-762.
- (3) Zhen, X.; Xie, C.; Jiang, Y. Y.; Ai, X. Z.; Xing, B. G.; Pu, K. Y. Semiconducting Photothermal Nanoagonist for Remote-Controlled Specific Cancer Therapy. *Nano Lett.* **2018**, *18*, 1498-1505.
- (4) Hu, W. B.; Miao, X. F.; Tao, H. J.; Baev, A.; Ren, C.; Fan, Q. L.; He, T. C.; Huang, W.; Prasad, P. N. Manipulating Nonradiative Decay Channel by Intermolecular Charge Transfer for Exceptionally Improved Photothermal Conversion. *ACS Nano* **2019**, *13*, 12006-12014.
- (5) Gu, W. G.; Zhang, T.; Gao, J. S.; Wang, Y.; Li, D. J.; Zhao, Z. W.; Jiang, B.; Dong, Z. W.; Li,

H. Albumin-Bioinspired Iridium Oxide Nanoplatfrom with High Photothermal Conversion Efficiency for Synergistic Chemo-Photothermal of Osteosarcoma. *Drug Deliv.* **2019**, *26*, 918-927.

(6) Liang, S.; Deng, X. R.; Chang, Y.; Sun, C. Q.; Shao, S.; Xie, Z. X.; Xiao, X.; Ma, P. A.; Zhang, H. Y.; Cheng, Z. Y.; Lin, J. Intelligent Hollow Pt-CuS Janus Architecture for Synergistic Catalysis-Enhanced Sonodynamic and Photothermal Cancer Therapy. *Nano Lett.* **2019**, *19*, 4134-4145.

(7) Ulbrich, K.; Hola, K.; Subr, V.; Bakandritsos, A.; Tucek, J.; Zboril, R. Targeted Drug Delivery with Polymers and Magnetic Nanoparticles: Covalent and Noncovalent Approaches, Release Control, and Clinical Studies. *Chem. Rev.* **2016**, *116*, 5338-5431.

(8) Panwar, N.; Soehartono, A. M.; Chan, K. K.; Zeng, S. W.; Xu, G. X.; Qu, J. L.; Coquet, P.; Yong, K. T.; Chen, X. Y. Nanocarbons for Biology and Medicine: Sensing, Imaging, and Drug Delivery. *Chem. Rev.* **2019**, *119*, 9559-9656.

(9) Du, J. J.; Xu, N.; Fan, J. L.; Sun, W.; Peng, X. J. Carbon Dots for *In Vivo* Bioimaging and Theranostics. *Small* **2019**, *15*, e1805087.

(10) Hu, J. J.; Liu, M. D.; Gao, F.; Chen, Y.; Peng, S. Y.; Li, Z. H.; Cheng, H.; Zhang, X. Z. Photo-Controlled Liquid Metal Nanoparticle-Enzyme for Starvation/Photothermal Therapy of Tumor by Win-Win Cooperation. *Biomaterials* **2019**, *217*, 119303.

(11) Wang, D. D.; Dong, H. F.; Li, M.; Cao, Y.; Yang, F.; Zhang, K.; Dai, W. H.; Wang, C. T.; Zhang, X. J. Erythrocyte-Cancer Hybrid Membrane Camouflaged Hollow Copper Sulfide Nanoparticles for Prolonged Circulation Life and Homotypic-Targeting Photothermal/Chemotherapy of Melanoma. *ACS Nano* **2018**, *12*, 5241-5252.

(12) Zhai, Y. H.; Ran, W.; Su, J. H.; Lang, T. Q.; Meng, J.; Wang, G. R.; Zhang, P. C.; Li, Y. P. Traceable Bioinspired Nanoparticle for the Treatment of Metastatic Breast Cancer *via* NIR-Triggered Intracellular Delivery of Methylene Blue and Cisplatin. *Adv. Mater.* **2018**, *30*, e1802378.

(13) Deng, R.; Ji, B.; Yu, H. L.; Bao, W.; Yang, Z. Q.; Yu, Y.; Cui, Y. H.; Du, Y. Y.; Song, M. Y.; Liu, S. J.; Meguellati, K.; Yan, F. Multifunctional Gold Nanoparticles Overcome MicroRNA Regulatory Network Mediated-Multidrug Resistant Leukemia. *Sci. Rep.* **2019**, *9*, 5348.

(14) Zhang, Z. W.; Wang, H.; Tan, T.; Li, J.; Wang, Z. W.; Li, Y. P. Rational Design of

Nanoparticles with Deep Tumor Penetration for Effective Treatment of Tumor Metastasis. *Adv. Funct. Mater.* **2018**, *28*, 1801840.

(15) Li, J. C.; Cui, D.; Jiang, Y. Y.; Huang, J. G.; Cheng, P. H.; Pu, K. Y. Near-Infrared Photoactivatable Semiconducting Polymer Nanoblockaders for Metastasis-Inhibited Combination Cancer Therapy. *Adv. Mater.* **2019**, *31*, e1905091..

(16) Li, J. C.; Cui, D.; Huang, J. G.; He, S. S.; Yang, Z. B.; Zhang, Y.; Luo, Y.; Pu, K. Y. Organic Semiconducting Pro-Nanostimulants for Near-Infrared Photoactivatable Cancer Immunotherapy. *Angew. Chem., Int. Ed.* **2019**, *58*, 12680-12687.

(17) Thibaut, T. A.; Smith, D. M. A Practical Review of Proteasome Pharmacology. *Pharmacol. Rev.* **2019**, *71*, 170-197.

(18) Ciechanover, A.; Hod, Y.; Hershko, A. A Heat-Stable Polypeptide Component of an Atp-Dependent Proteolytic System from Reticulocytes. *Biochem. Bioph. Res. Co.* **1978**, *81*, 1100-5.

(19) Borissenko, L.; Groll, M. 20s Proteasome and Its Inhibitors: Crystallographic Knowledge for Drug Development. *Chem. Rev.* **2007**, *107*, 687-717.

(20) Shembade, N.; Ma, A.; Harhaj, E. W. Inhibition of NF-Kappa B Signaling by A20 Through Disruption of Ubiquitin Enzyme Complexes. *Science* **2010**, *327*, 1135-139.

(21) Pagano, M.; Tam, S. W.; Theodoras, A. M.; Beer-Romero, P.; Del Sal, G.; Chau, V.; Yew, P. R.; Draetta, G. F.; Rolfe, M. Role of the Ubiquitin-Proteasome Pathway in Regulating Abundance of the Cyclin-Dependent Kinase Inhibitor P27. *Science* **1995**, *269*, 682-5.

(22) Zhang, X.; Schulz, R.; Edmunds, S.; Kruger, E.; Markert, E.; Gaedcke, J.; Cormet-Boyaka, E.; Ghadimi, M.; Beissbarth, T.; Levine, A. J.; Moll, U. M.; Dobbelsstein, M. MicroRNA-101 Suppresses Tumor Cell Proliferation by Acting as an Endogenous Proteasome Inhibitor via Targeting the Proteasome Assembly Factor POMP. *Mol. Cell* **2015**, *59*, 243-57.

(23) Grossman, S. R.; Deato, M. E.; Brignone, C.; Chan, H. M.; Kung, A. L.; Tagami, H.; Nakatani, Y.; Livingston, D. M. Polyubiquitination of P53 by a Ubiquitin Ligase Activity of P300. *Science* **2003**, *300*, 342-4.

- (24) Paramore, A.; Frantz, S. Bortezomib. *Nat. Rev. Drug Discov.* **2003**, *2*, 611-612.
- (25) Chanan-Khan, A. A.; Kaufman, J. L.; Mehta, J.; Richardson, P. G.; Miller, K. C.; Lonial, S.; Munshi, N. C.; Schlossman, R.; Tariman, J.; Singhal, S. Activity and Safety of Bortezomib in Multiple Myeloma Patients with Advanced Renal Failure: A Multicenter Retrospective Study. *Blood* **2007**, *109*, 2604-6.
- (26) Bross, P. F.; Kane, R.; Farrell, A. T.; Abraham, S.; Benson, K.; Brower, M. E.; Bradley, S.; Gobburu, J. V.; Goheer, A.; Lee, S. L.; Leighton, J.; Liang, C. Y.; Lostritto, R. T.; McGuinn, W. D.; Morse, D. E.; Rahman, A.; Rosario, L. A.; Verbois, S. L.; Williams, G.; Wang, Y. C., *et al.* Approval Summary for Bortezomib for Injection in the Treatment of Multiple Myeloma. *Clin. Cancer Res.* **2004**, *10*, 3954-3964.
- (27) Hu, X. F.; Chai, Z. L.; Lu, L. W.; Ruan, H. T.; Wang, R. F.; Zhan, C. Y.; Xie, C.; Pan, J.; Liu, M.; Wang, H.; Lu, W. Y. Bortezomib Dendrimer Prodrug-Based Nanoparticle System. *Adv. Funct. Mater.* **2019**, *29*, 1807941.
- (28) Deng, X. R.; Li, K.; Cai, X. C.; Liu, B.; Wei, Y.; Deng, K. R.; Xie, Z. X.; Wu, Z. J.; Ma, P. A.; Hou, Z. Y.; Cheng, Z. Y.; Lin, J. A Hollow-Structured CuS@Cu₂S@Au Nanohybrid: Synergistically Enhanced Photothermal Efficiency and Photoswitchable Targeting Effect for Cancer Theranostics. *Adv. Mater.* **2017**, *29*, 1701266.
- (29) Yu, Y.; Li, C. G.; Chen, C. L.; Huang, H.; Liang, C.; Lou, Y.; Chen, X. B.; Shi, Z.; Feng, S. H. Saccharomyces-Derived Carbon Dots for Biosensing pH and Vitamin B₁₂. *Talanta* **2019**, *195*, 117-126.
- (30) Milekhin, A. G.; Yeryukov, N. A.; Sveshnikova, L. L.; Duda, T. A.; Rodyakina, E. E.; Gridchin, V. A.; Sheremet, E. S.; Zahn, D. R. T. Combination of Surface- and Interference-Enhanced Raman Scattering by Cus Nanocrystals on Nanopatterned Au Structures. *Beilstein J. Nanotech.* **2015**, *6*, 749-754.
- (31) Ge, J. C.; Lan, M. H.; Zhou, B. J.; Liu, W. M.; Guo, L.; Wang, H.; Jia, Q. Y.; Niu, G. L.; Huang, X.; Zhou, H. Y.; Meng, X. M.; Wang, P. F.; Lee, C. S.; Zhang, W. J.; Han, X. D. A Graphene Quantum Dot Photodynamic Therapy Agent with High Singlet Oxygen Generation. *Nat. Commun.* **2014**, *5*, 4596.

- (32) Wang, J.; Wang, C. F.; Chen, S. Amphiphilic Egg-Derived Carbon Dots: Rapid Plasma Fabrication, Pyrolysis Process, and Multicolor Printing Patterns. *Angew. Chem. Int. Ed.* **2012**, *51*, 9297-9301.
- (33) Wu, H.; Chen, Y.; Dai, X. Y.; Li, P. Y.; Stoddart, J. F.; Liu, Y. *In Situ* Photoconversion of Multicolor Luminescence and Pure White Light Emission Based on Carbon Dot-Supported Supramolecular Assembly. *J. Am. Chem. Soc.* **2019**, *141*, 6583-6591.
- (34) Wang, Y.; Liu, L. W.; Wang, Q.; Hu, S. Y.; Zou, P.; Shi, J.; Zhang, X. H. Optimization of the Aqueous Synthesis of Cu₂S Quantum Dots with Different Surface Ligands. *Nanotechnology* **2016**, *27*, 015705.
- (35) Xie, Y.; Riedinger, A.; Prato, M.; Casu, A.; Genovese, A.; Guardia, P.; Sottini, S.; Sangregorio, C.; Misztal, K.; Ghosh, S.; Pellegrino, T.; Manna, L. Copper Sulfide Nanocrystals with Tunable Composition by Reduction of Covellite Nanocrystals with Cu⁺ Ions. *J. Am. Chem. Soc.* **2013**, *135*, 17630-7.
- (36) Ludwig, J.; An, L.; Pattengale, B.; Kong, Q. Y.; Zhang, X. Y.; Xi, P. X.; Huang, J. E. Ultrafast Hole Trapping and Relaxation Dynamics in *p*-Type CuS Nanodisks. *J Phys. Chem. Lett.* **2015**, *6*, 2671-5.
- (37) Chen, Z.; Zhao, P. F.; Luo, Z. Y.; Zheng, M. B.; Tian, H.; Gong, P.; Gao, G. H.; Pan, H.; Liu, L. L.; Ma, A. Q.; Cui, H. D.; Ma, Y. F.; Cai, L. T. Cancer Cell Membrane-Biomimetic Nanoparticles for Homologous-Targeting Dual-Modal Imaging and Photothermal Therapy. *ACS Nano* **2016**, *10*, 10049-10057.
- (38) Hu, C. M. J.; Zhang, L.; Aryal, S.; Cheung, C.; Fang, R. H.; Zhang, L. F. Erythrocyte Membrane-Camouflaged Polymeric Nanoparticles as a Biomimetic Delivery Platform. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 10980-10985.
- (39) Cheng, X.; Yu, D.; Cheng, G.; Yung, B. C.; Liu, Y.; Li, H.; Kang, C.; Fang, X.; Tian, S.; Zhou, X.; Liu, Q.; Lee, R. J. T7 Peptide-Conjugated Lipid Nanoparticles for Dual Modulation of Bcl-2 and Akt-1 in Lung and Cervical Carcinomas. *Mol. Pharm.* **2018**, *15*, 4722-4732.
- (40) Gao, L. Y.; Liu, X. Y.; Chen, C. J.; Wang, J. C.; Feng, Q.; Yu, M. Z.; Ma, X. F.; Pei, X. W.;

Niu, Y. J.; Qiu, C.; Pang, W. H.; Zhang, Q. Core-Shell Type Lipid/Rpaa-Chol Polymer Hybrid Nanoparticles for *In Vivo* Sima Delivery. *Biomaterials* **2014**, *35*, 2066-78.

(41) Sun, H. P.; Su, J. H.; Meng, Q. S.; Yin, Q.; Chen, L. L.; Gu, W. W.; Zhang, P. C.; Zhang, Z. W.; Yu, H. J.; Wang, S. L.; Li, Y. P. Cancer-Cell-Biomimetic Nanoparticles for Targeted Therapy of Homotypic Tumors. *Adv. Mater.* **2016**, *28*, 9581-9588.

(42) Woo, S. K.; Lee, J. I.; Park, I. K.; Yoo, Y. J.; Cho, C. M.; Kang, M. S.; Ha, D. B.; Tanaka, K.; Chung, C. H. Multiple Ubiquitin C-Terminal Hydrolases from Chick Skeletal Muscle. *J. Biol. Chem.* **1995**, *270*, 18766-73.

(43) Lee, J. I.; Woo, S. K.; Kim, K. I.; Park, K. C.; Baek, S. H.; Yoo, Y. J.; Chung, C. H. A Method for Assaying Deubiquitinating Enzymes. *Biol. Proced. Online* **1998**, *1*, 92-99.

(44) Stewart, A. K.; Rajkumar, S. V.; Dimopoulos, M. A.; Masszi, T.; Spicka, I.; Oriol, A.; Hajek, R.; Rosinol, L.; Siegel, D. S.; Mihaylov, G. G.; Goranova-Marinova, V.; Rajnics, P.; Suvorov, A.; Niesvizky, R.; Jakubowiak, A. J.; San-Miguel, J. F.; Ludwig, H.; Wang, M.; Maisnar, V.; Minarik, J., *et al.* Carfilzomib, Lenalidomide, and Dexamethasone for Relapsed Multiple Myeloma. *N. Engl. J. Med.* **2015**, *372*, 142-52.

(45) Dimopoulos, M. A.; Gay, F.; Schjesvold, F.; Beksac, M.; Hajek, R.; Weisel, K. C.; Goldschmidt, H.; Maisnar, V.; Moreau, P.; Min, C. K.; Pluta, A.; Chng, W. J.; Kaiser, M.; Zweegman, S.; Mateos, M. V.; Spencer, A.; Iida, S.; Morgan, G.; Suryanarayan, K.; Teng, Z., *et al.* Oral Ixazomib Maintenance Following Autologous Stem Cell Transplantation (Tourmaline-Mm3): A Double-Blind, Randomised, Placebo-Controlled Phase 3 Trial. *Lancet* **2019**, *393*, 253-264.

(46) Turnbull, A. P.; Ioannidis, S.; Krajewski, W. W.; Pinto-Fernandez, A.; Heride, C.; Martin, A. C. L.; Tonkin, L. M.; Townsend, E. C.; Buker, S. M.; Lancia, D. R.; Caravella, J. A.; Toms, A. V.; Charlton, T. M.; Lahdenranta, J.; Wilker, E.; Follows, B. C.; Evans, N. J.; Stead, L.; Alli, C.; Zarayskiy, V. V., *et al.* Molecular Basis of Usp7 Inhibition by Selective Small-Molecule Inhibitors. *Nature* **2017**, *550*, 481-486.

(47) Kategaya, L.; Di Lello, P.; Rouge, L.; Pastor, R.; Clark, K. R.; Drummond, J.; Kleinheinz, T.; Lin, E.; Upton, J. P.; Prakash, S.; Heideker, J.; McClelland, M.; Ritorto, M. S.; Alessi, D. R.; Trost,

M.; Bainbridge, T. W.; Kwok, M. C. M.; Ma, T. P.; Stiffler, Z.; Brasher, B., *et al.* Usp7 Small-Molecule Inhibitors Interfere with Ubiquitin Binding. *Nature* **2017**, *550*, 534-538.

(48) Cheng, L.; Zhang, F. R.; Wang, S. H.; Pan, X. T.; Han, S. C.; Liu, S.; Ma, J. J.; Wang, H. Y.; Shen, H. Y.; Liu, H. Y.; Yuan, Q. P. Activation of Prodrugs by Nir-Triggered Release of Exogenous Enzymes for Locoregional Chemo-Photothermal Therapy. *Angew. Chem. Int. Ed.* **2019**, *58*, 7728-7732.

(49) Yang, G. G.; Zhou, D. J.; Pan, Z. Y.; Yang, J.; Zhang, D. Y.; Cao, Q.; Ji, L. N.; Mao, Z. W. Multifunctional Low-Temperature Photothermal Nanodrug with *In Vivo* Clearance, ROS-Scavenging and Anti-Inflammatory Abilities. *Biomaterials* **2019**, *216*, 119280.

(50) Liu, Y. L.; Ai, K. L.; Liu, J. H.; Deng, M.; He, Y. Y.; Lu, L. H. Dopamine-Melanin Colloidal Nanospheres: An Efficient Near-Infrared Photothermal Therapeutic Agent for *In Vivo* Cancer Therapy. *Adv. Mater.* **2013**, *25*, 1353-9.

(51) Zhang, Q. Z.; Dehaini, D.; Zhang, Y.; Zhou, J. L.; Chen, X. Y.; Zhang, L. F.; Fang, R. H.; Gao, W. W.; Zhang, L. F. Neutrophil Membrane-Coated Nanoparticles Inhibit Synovial Inflammation and Alleviate Joint Damage in Inflammatory Arthritis. *Nat. Nanotechnol.* **2018**, *13*, 1182-1190.

(52) Cheng, Y. R.; Yang, F.; Zhang, K.; Zhang, Y. Y.; Cao, Y.; Liu, C. H.; Lu, H. T.; Dong, H. F.; Zhang, X. J. Non-Fenton-Type Hydroxyl Radical Generation and Photothermal Effect by Mitochondria-Targeted WSSe/MnO₂ Nanocomposite Loaded with Isoniazid for Synergistic Anticancer Treatment. *Adv. Funct. Mater.* **2019**, *29*, 1903850.

(53) Sun, K. J.; Yu, W. J.; Ji, B.; Chen, C. B.; Yang, H. M.; Du, Y. Y.; Song, M. Y.; Cai, H. Q.; Yan, F.; Su, R. Saikosaponin D Loaded Macrophage Membrane-Biomimetic Nanoparticles Target Angiogenic Signaling for Breast Cancer Therapy. *Appl. Mater. Today.* **2020**, *18*, 100505 .

(54) Yan, F.; Shen, N.; Pang, J.; Zhao, N.; Deng, B.; Li, B.; Yang, Y.; Yang, P.; Molina, J. R.; Liu, S. A Regulatory Circuit Composed of DNA Methyltransferases and Receptor Tyrosine Kinases Controls Lung Cancer Cell Aggressiveness. *Oncogene* **2017**, *36*, 6919-6928.

(55) Deng, R.; Shen, N.; Yang, Y.; Yu, H.; Xu, S.; Yang, Y. W.; Liu, S.; Meguellati, K.; Yan, F.

Targeting Epigenetic Pathway with Gold Nanoparticles for Acute Myeloid Leukemia Therapy. *Biomaterials* **2018**, *167*, 80-90.

(56) Yan, F.; Shen, N.; Pang, J. X.; Zhao, N.; Zhang, Y. W.; Bode, A. M.; Al-Kali, A.; Litzow, M. R.; Li, B.; Liu, S. J. A Vicious Loop of Fatty Acid-Binding Protein 4 and DNA Methyltransferase 1 Promotes Acute Myeloid Leukemia and Acts as a Therapeutic Target. *Leukemia* **2018**, *32*, 865-873.

(57) Yan, F.; Shen, N.; Pang, J.; Xie, D.; Deng, B.; Molina, J. R.; Yang, P.; Liu, S. Restoration of Mir-101 Suppresses Lung Tumorigenesis through Inhibition of Dnmt3a-Dependent DNA Methylation. *Cell Death Dis.* **2014**, *5*, e1413.

For Table of Contents Only