Protein-based artificial nanosystems in cancer therapy

Zhang, Nan; Mei, Kun; Guan, Ping; Hu, Xiaoling; Zhao, Yanli

2020

Zhang, N., Mei, K., Guan, P., Hu, X. & Zhao, Y. (2020). Protein-based artificial nanosystems in cancer therapy. *Small*, 16(23), 1907256-. <https://dx.doi.org/10.1002/smll.201907256>

<https://hdl.handle.net/10356/148134>

<https://doi.org/10.1002/smll.201907256>

This is the peer reviewed version of the following article: Zhang, N., Mei, K., Guan, P., Hu, X. & Zhao, Y. (2020). Protein-based artificial nanosystems in cancer therapy. *Small*, 16(23), 1907256-. <https://dx.doi.org/10.1002/smll.201907256>, which has been published in final form at <https://doi.org/10.1002/smll.201907256>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

Downloaded on 26 Apr 2023 22:23:53 SGT

Protein-Based Artificial Nanosystems in Cancer Therapy

Nan Zhang, Kun Mei, Ping Guan, Xiaoling Hu,* Yanli Zhao*

N. Zhang, K. Mei, P. Guan, Prof. X. Hu

School of Natural and Applied Sciences, Northwestern Polytechnical University, Xi'an, 710072,
P. R. China. Email: huxl@nwpu.edu.cn

N. Zhang, Prof. Y. L. Zhao

Division of Chemistry and Biological Chemistry, School of Physical and Mathematical
Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371. Email:
zhaoyanli@ntu.edu.sg

Prof. Y. L. Zhao

School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang
Avenue, Singapore 639798

Abstract

Proteins, like “actors,” play different roles in specific applications. In the past decade, significant achievements have been made with protein-engineered biomedicine for cancer therapy. Certain proteins such as human serum albumin, working as carriers for drug/photosensitizer delivery, have entered clinical uses due to their long half-life, biocompatibility, biodegradability, and inherent non-immunogenicity. Proteins with catalytic abilities are promising as adjuvant agents for other therapeutic modalities or as anticancer drugs themselves. These catalytic proteins are usually defined as enzymes with high biological activity and substrate specificity. However, clinical applications of these kinds of proteins remain rare due to protease-induced denaturation and weak cellular permeability. Based on the characteristics of different proteins, tailor-made protein-based nanosystems could make up for

their individual deficiencies. Therefore, this review summarizes elaborately designed protein-based nanosystems, where proteins serve as drug carriers, adjuvant agents, or therapeutic drugs to make full use of their intrinsic advantages in cancer therapy. It intends to provide up-to-date progress on research in the field of protein-based nanomedicine.

Keywords: cancer therapy, catalytic proteins, drug carriers, nanomedicine, protein-based nanosystems

1. Introduction

Protein-based therapeutic modality is of great value as 70% of popular drugs are protein-related biologics.^[1] To date, more than 200 proteins have been approved for the treatment of cancers, protein deficiencies, and genetic disorders.^[2] Despite great contributions made by protein therapy, there are still numerous limitations present in clinical applications because the structure and property of each protein are unique.^[3]

Plasma proteins, such as human serum albumin (HSA) and ferritin (FRT), are in great abundance. Since paclitaxel-albumin conjugate has been approved as a commercially available anticancer drug, the potential of albumin as the carrier for therapeutic agents has been well explored.^[4] As a plasma protein, albumin without immunogenicity could escape from the degradation and clearance *via* physiological mechanisms.^[5] Moreover, albumin possesses a variety of functional groups for chemical modifications and metal ion chelation, while its hydrophobic region can be employed as a pocket for the delivery of small organic drugs or photosensitizers.^[6] Therefore, albumin-engineered nanosystems do not need additional decorations of polyethylene glycol (PEG) or other biocompatible molecules for achieving longer blood circulation and reduced immunogenicity. However, the size of native albumin is less than 10 nm. Considering cancer therapy, nanoparticles with larger sizes (about 100-200 nm) perform better as compared to their ultrasmall counterparts (<10 nm) in terms of blood circulation half-time, pharmacokinetics, and tumor accumulation by enhanced permeability and retention (EPR) effect.^[7] Conversely, small nanoparticles show stronger tumor penetration capability than larger ones once entering into tumor sites.^[8] To rationally design albumin carriers, size-switchable properties under tumor microenvironment stimuli (such as mild acid, hypoxia, and redox) was integrated.^[9] Another type of plasma protein, FRT, is a typically cage-

like protein.^[10] Similarly, FRT is resistant to nonspecific plasma protein adsorption, and could survive from systemic degradation and inactivation.^[11] Ferritin has an inner cavity of 8 nm, showing great potential for being exploited as efficient loading and transferring carriers and combined with other functional motifs to construct FRT-engineered nanosystems for cancer therapy.^[12]

Catalytic proteins, known as enzymes, can be classified as cytotoxic and non-cytotoxic (Scheme 1). They are susceptible to the opsonization of nonspecific plasma protein adsorption and can be rapidly degraded or cleared by human body.^[13] Among non-cytotoxic enzymes, catalase can decompose endogenous H_2O_2 into O_2 and relieve tumor hypoxia^[14] while hyaluronidase (HAase) can digest the hyaluronic acid network of tumor interstitium and loose extracellular matrix.^[15] Thus, they are usually employed as adjuvant agents to modulate tumor microenvironments and improve the therapeutic efficiency of other modalities. However, because of their immunogenicity, protease-induced denaturation, and weak cellular permeability,^[16] suitable carriers were developed to protect them from the following three perspectives. 1) Organic systems such as liposomes, prodrug-based vesicles, polymersomes, and polyion complexes are used to wrap enzymes.^[17] They show potential for spatiotemporal delivery of enzymes into tumor sites. 2) Some typical inorganic materials such as silica; metal oxides, nanosheets, and nanotubes; and quantum dots are employed for enzyme loading.^[18] They are usually robust and could be prepared on a large scale. 3) The enzyme surface is modified with polymers.^[16b,19] Conjugations with PEG or other biocompatible linear polymers *via* a “graft-to” or “graft-from” approach are used to reduce the immunogenicity of enzymes and enhance their blood circulation and cellular uptake. Polymer networks, except for linear polymers, are also employed for enzyme protection; cross-linkers are needed to generate a compact sheath for this.^[20] Above all, no matter which type of nanosystem is used, a high loading efficiency, biocompatible and degradable components, and the good retention and smart release of enzymes at a specified location should be taken into consideration. For cytotoxic enzymes such as glucose oxidase (GOx), ribonuclease A (RNase A), and caspase-3, nanosystems designed to carry them would need to be more sophisticated than those used for non-cytotoxic enzymes. This means that: 1) the bioactivity of therapeutic enzymes could be switched off during the circulation to weaken immune response caused by the leakage, and 2)

the “on-demand” release of enzymes regulated by the tumor microenvironment should be fully developed with the reactivation of their pharmacokinetics.^[21]

Scheme 1. Protein-based nanosystems for cancer treatment.

Proteins play an important role in cancer therapy and there have been several excellent reviews on protein nanotherapeutics recently.^[4,22] For single-protein based systems, Liu et al. chose HSA as a typical model for the design, screening, and clinical applications of HSA-based drugs.^[22a] Fu et al. discussed GOx-based catalytic systems with applications in biosensors as well as cancer screening and mortality.^[22c] Cheng et al. focused on rationally designed nanovehicles as methods of intracellular protein delivery; among the former, nanogels and polymersomes appeared effective for overcoming multiple barriers for native proteins.^[22e] Lv et al. put a particular focus was on polymer-based cytosolic protein delivery.^[22f] Liu et al. classified therapeutic proteins as a large category, with differences in carriers of nanoparticles, hydrogels, and scaffolds ranging from nanoscale to macroscale,^[22b] in terms of the multimodal applications of protein-engineered materials. From our perspective, each protein has its own unique structure and properties. When employed in cancer therapy, these proteins have capacities as drugs/photosensitizer carriers, adjuvant agents to promote the efficiency of other therapy modalities, and therapeutic drugs themselves. In some specific conditions, natural enzymes could be replaced by nanozymes with similar catalytic properties and superior tolerance. Based on these features, we herein review rational design and tailor-made nanosystems based on different proteins for cancer treatment.

2 Proteins as carriers of drugs/photosensitizers

As discussed above, plasma proteins including HSA and FRT are in large abundance. They are promising carriers for drugs and photosensitizers attributed to their inherent non-immunogenicity, biocompatibility, and biodegradability.

2.1 FRT

Ferritin, a protein nanocage, is a major iron storage protein of 450 kDa existing in most cell types. It is a globular arrangement composed of a 24 polypeptide subunit shell of 12 nm

and iron oxide core of 8 nm.^[10,23]

2.1.1 Properties of FRT

Ferritin and its derivatives are ideal candidates for photosensitizer and drug delivery. 1) Ferritin could be found in human beings with biosafety and non-immunogenicity. Compared to other biocompatible materials, FRT has quite monodisperse and small size, good for intracellular accumulation.^[23] 2) Diverse surfaces including exterior, interior, and interface between polypeptide subunits endow FRT with multiple reactivities. Its exterior surface could be genetically or chemically modified to introduce polymers or targeting molecules, followed by the alteration of solubility and specificity.^[24] The iron core (about 4500 Fe³⁺) could be removed from the inner cavity of FRT and replaced by other metal involving substrates, whereas functional groups located on the interior surface would facilitate the formation of corresponding nanocomposites.^[25] Furthermore, since FRT is an assembly of 24 subunits with a high degree of symmetry, the interface is used to manipulate chimeric assembly and symmetry-broken reassembly.^[26] 3) The nanostructure of FRT is flexible, and loading photosensitizers/drugs into FRT could be carried out by two ways. One is to make use of its pH-responsive disassembly/reassembly behavior. Unlike their rigidity under high temperature up to 85 °C and pH value of 8.5–9.0, the nanocage would be broken down and transformed into individual subunits when exposed to an acidic condition (pH = 2), and subunits could be regenerated into an intact cage structure when pH is tuned back to neutral. Such a pH regulated disassembly/reassembly behavior is beneficial for the construction of FRT-based hybrid systems and drug loading into the FRT interior.^[27] The other is the penetration mode. There are some channels in FRT to connect the interior/exterior environment, between which threefold and fourfold channels play different roles. Metal cations are usually transferred in and out through hydrophilic threefold channels, with a potential gradient direction toward the cavity. In the opposite direction, fourfold channels are used to expel substrates from the interior.^[28]

Figure 1. (A) Schematic illustration for the formation and working mechanism of P-RFRTs, and photographs of P-RFRTs and free ZnF₁₆Pc solutions under different conditions. Reproduced with permission.^[12b] Copyright 2013, American Chemical Society. (B) Therapy studies performed on U87MG tumor-bearing nude mice (n = 5/group), and time dependent

uptake of the nanosystem by U87MG cells. Scale bar, 50 μm . Reproduced with permission.^[29]
Copyright 2013, American Chemical Society.

2.1.2 Drug/photosensitizer loading

As shown in Figure 1, Zhen et al. reported that arginylglycylaspartic acid (RGD) peptide-modified FRT (P-RFRTs) could achieve photosensitizer and drug delivery in a tumor site-specific manner.^[12b,29] In the photosensitizer-loaded system, the FRT surface was genetically modified by RGD to generate in an iron-free condition for obtaining a hollow structure. The RGD is a kind of peptide with binding affinity to integrin $\alpha_v\beta_3$ overexpressed on tumor endothelial cells, contributing to a higher tumor accumulation rate of 26.82 as compared to that of two in most systems without targeting components. Then, a photosensitizer was loaded into the FRT interior by the penetration mode without breaking down the protein cage structure. A good tumor inhibition rate of 83.64% on day 12 was achieved. Similarly, doxorubicin could be loaded into FRT with high efficiency above 70% after forming a pre-complex with copper ions. Huang et al. employed IR820 dye as an imaging and photothermal conversion agent.^[12a] The encapsulation of IR820 dye was carried out by pH-manipulated “opening and closing” behavior of FRT with loading efficiency of 17.32%. They found that 550 nm light was suitable for fluorescence imaging, while 808 nm light was good for photoacoustic imaging and photothermal therapy with 100% tumor elimination in vivo.

2.1.3 In situ conversion of iron core

Apoferitin, a product of FRT when genetically expressed in an iron free environment or the ferric hydroxide-phosphate core, is removed by chemical reduction and often employed for biomedical applications.^[30] Notably, the preparation of apoferritin is not very easy; it requires a lot of both time and raw materials. Therefore, the question of how to use the iron oxide core of FRT directly is attracting significant attention. Prussian blue (PB), composed of Fe^{3+} and a hexacyanoferrate complex of $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$, shows a photothermal response upon near-infrared (NIR) light irradiation; therefore, it is capable of use in photothermal therapy (PTT).^[31] Thus, the interior of FRT provides a suitable place to generate PB with existing reactants and a protective protein shell. Zhang et al. reported the synthesis of a nano-sized PB-encapsulated structure using FRT as the reactor where ferrocyanide and ferric ion are mixed together.^[32]

While they developed this material as a mimetic peroxidase for glucose detection, the special structure of PB-encapsulated FRT should also have the capacity of using photothermal conversion for PTT.

2.1.4 FRT Hybrids

In addition to serving as reaction vessels for the synthesis of size-constrained nanomaterials, protein cages could also act as nanoscale building blocks for higher order assemblies.^[10,33] An interesting structure is a Janus nanoparticle containing the protein cage. As shown in Figure 2, Douglas et al. used a DNA binding protein cage (LiDps) to construct toposelectively modified streptavidin nanoplatform.^[34] 1) A tetrapeptide added to C-termini of 12 protein subunits could introduce sulfhydryl group for one-side S-S bond immobilization on beads of LiDps. 2) Exposed surface sulfhydryl group of LiDps was then modified by biotin *via* a spacer, followed by streptavidin functionalization *via* strong biotin-avidin affinity. 3) Protein cages could be released from beads by the reduction of the disulfide linkage, leaving nonbiotinylated surface available for other functionalization. They demonstrated that streptavidin functionalized LiDps coupled with a biotinylated monoclonal antibody could target a microbial pathogen. Moreover, fluorescent tags could be conjugated onto the nonbiotinylated surface of LiDps *via* maleimide-sulfhydryl reaction to image this targeting event.

Figure 2. (A) Cells targeted with fluorescently tagged cage nanoplatform. (B) Surface-masking approach used to toposelectively biotinylate LiDps to the Janus nanoparticle. (C) Structure of functionalized nanoplatform observed by transmission electron microscopy (TEM). Shapes that would be expected for two orthogonal projections of a heterodimer cross section are indicated. Scale bar is 20 nm. Reproduced with permission.^[34b] Copyright 2009, American Chemical Society.

This kind of Janus-like protein cage possesses unique structure and properties for cancer therapy. An LiDps is a member of the FRT superfamily. Developing Janus-FRT based therapeutic systems might guarantee that photosensitizers or drugs could be loaded into the FRT inner cavity as we discussed above, asymmetrically modified targeting tags could control the orientation of the nanosystem when targeting to the cell surface, more sophisticated asymmetric

supramolecular assembly could be achieved, multiple functions without extensive crosslinking or aggregates are expected, and further coupling with other functional proteins would lead to synergistic or cascade therapeutic effect.

2.2 HSA

Human serum albumin, the most abundant plasma protein found in human blood, is produced in the liver.^[4] The HSA itself does not exhibit obvious therapeutic effects, but HSA-related drugs have been extensively explored as potential systems in cancer therapy. Abraxane, paclitaxel-HSA conjugated nanoparticle, has been approved by the U.S. FDA as an anticancer chemotherapy drug. This is attributed to intrinsic biocompatibility, no immunogenicity and multiple reactivity of HSA. Inspired by the mechanism of biomineralization, HSA is able to sequester inorganic ions to directly generate protein shell protected metal oxide nanoclusters in alkaline conditions.^[35] Notably, major features of such HSA-based systems are to increase their size during blood circulation for enhanced tumor accumulation, and also to decompose them into individual counterparts when arriving at tumor sites for stronger tumor permeability.

2.2.1 HSA-based nanosystems with deformable/soft structures

As shown in Figure 3A, Xu et al. developed a kind of flexible hollow capsule (defined as MoS₂/HSA) with a nanoscale size of 280 nm; it consists of a MoS₂ inner layer and HSA outer shell.^[36] Compared to its counterparts, the MoS₂/HSA with a lower Young's modulus of 222 ± 20 MPa showed enhanced tumor accumulation and superior therapeutic efficacy attributed to its deformable structure. Further combination with chemicals used in drugs led to a high drug loading capacity and synergistic thermal/chemotherapy. However, unlike the superior photothermal property achieved by intact MoS₂ nanosheets, the photodynamic performance of some organic photosensitizers would be impaired once they reached an aggregated state. Ren et al. developed a core-shell system to avoid the condensed state of photosensitizers encapsulated in the inner cavity of carriers *via* π - π stacking interaction with reduced photodynamic therapy (PDT) efficacy (Figure 3B).^[37] In this system, HSA could enable IR780 dye to keep distance from each other. The loading efficiency (70% w/w) of IR780 dye was quite high. The core was formed from perfluorotributylamine (PFTBA), which was beneficial to the dissolution and duration of action of ¹O₂. Employing HSA to disperse IR780 dye has two merits: one is higher loading rate than commercially available lipids and the other is that HSA can

prolong the triplet-state lifetime of the dye and generation time of the $^1\text{O}_2$.

Figure 3. (A) (a) Schematic illustration for the construction process of the MoS_2/HSA hollow nanocapsule, (b,c) TEM images of the $\text{SiO}_2\text{-MoS}_2$ nanospheres, and (d,e) TEM images of the flexible MoS_2/HSA hollow nanocapsule. Reproduced with permission.^[36] Copyright 2018, Wiley-VCH. (B) Design and structure of triple effect core-shell system (Nano-PDT), and TEM images of (left) IR780@HSA and (right) IR780@PFTBA@HSA . Reproduced with permission.^[37] Copyright 2017, American Chemical Society.

2.2.2 HSA-based nanosystems with a switchable size

Chen et al. modified HSA with the photosensitizer chlorin e6 (Ce6) and cis-platinum prodrug as biomineralized templates to generate MnO_2 nanoclusters.^[8b] The obtained nanosystem possessed PDT, chemotherapy, and $\text{H}_2\text{O}_2/\text{pH}$ dual responsiveness. Integrated materials with larger size compared to their components made them advantageous to a longer blood circulation half-time and higher resistance against clearance. Because of the H_2O_2 decomposition into O_2 by MnO_2 , PDT efficiency could be well-promoted. Meanwhile, these integrated HSA- MnO_2 nanoparticles could be decomposed into individual complexes with the size below 10 nm attributed to the reaction between MnO_2 and H^+ , leading to a stronger intratumoral penetration ability.^[38] In another work, Yang and coworkers used azobenzene derivatives as hypoxia-responsive crosslinkers to connect Ce6-conjugated HSA and prodrug-conjugated HSA.^[39] Once accumulated at tumor sites, the nanocomplexes with a size of around 100-150 nm could be cleaved into their ultrasmall counterparts in hypoxia conditions. Individual Ce6-conjugated HSA showed better therapeutic efficiency as compared to their aggregated counterparts.^[40] Similarly, Liu et al. made use of tamoxifen (TAM), an antiestrogenic drug with hydrophobic properties, to induce the self-assembly of Ce6-conjugated HSA with a whole particle size of 130 nm.^[8a] Tamofixin possesses dual characteristics of pH responsiveness and hypoxia relief capability. Tamofixin underwent the transition from a hydrophobic to hydrophilic property once the pH condition of microenvironment changed from neutral to mildly acidic, leading to the dissociation of whole materials into individual units with the size of 10 nm. Moreover, TAM could downregulate the O_2 consumption of cancer cells by

the inhibition of the mitochondrial electron transport chain at complex I, thus resulting in hypoxia attenuation and improved PDT efficacy.^[41]

2.2.3 HSA-polymer conjugates

Kuan et al. employed polyamidoamine (PAMAM) dendrimers to modify native HSA.^[42] Highly branched structures of dendrimers make them appealing as drug carriers, as they provide versatile functional groups and sufficient internal space for accommodating drugs.^[43] The PAMAM dendrimer family could further promote cellular uptake attributed to a positively charged amino group. The intrinsic cytotoxicity of PAMAM could be manipulated by the dendrimer generation. The authors demonstrated that G2-dendrimer modified HSA showed higher drug loading and moderate cytotoxicity as compared to the protein modified by other generation dendrimers. Han et al. designed a kind of proteinosome.^[44] Proteinosomes generated from covalently conjugated protein-polymer complexes are different from others because they employ supramolecular chemistry to produce amphiphilic proteins, for which adamantane-terminated poly(N-isopropylacrylamide) chain and cyclodextrin-modified bovine serum albumin (BSA) could assemble into micelles *via* host-guest inclusion. After the BSA corona was cross-linked, the polymer chain in the core could be removed by excess free cyclodextrin, and nanoscale proteinosomes were finally prepared. They demonstrated that this type of proteinosome was an ideal candidate for biomedical applications owing to the intact protein corona shell and inner cavity for drug loading.

Figure 4. (A) Schematic illustration of LMWP-modified albumin nanoparticles *via* a green synthesis method. Reproduced with permission.^[45] Copyright 2016, American Chemical Society. (B) (a,b) BBB composition and main alterations found in pathological conditions, (c) BBB transport mechanisms for brain delivery of nanoparticles, and (d) main nanoparticle features influencing systemic delivery and BBB passage. Reproduced with permission.^[46] Copyright 2016, Elsevier.

2.2.4 HSA-binding receptor pathway to overcome blood-brain barrier

As shown in Figure 4, Lin et al. applied albumin noncovalently coated drugs to penetrate

the blood-brain barrier (BBB).^[45] The BBB determines which kind of molecules could enter the brain, where tight junction proteins occupy endothelial cells and lead to poor transcellular endocytosis.^[46] For drug delivery to the brain, nutrient transporters^[47] whose receptors located inside the brain could be potential candidates. Except for transferrin, GLUT-1, and LAT-1 receptors, albumin receptors and gp60 were found to be responsible for albumin intake into brain tumor tissues.^[48] To make use of the hydrophobic characteristics of drugs including paclitaxel (PTX) and fenretinide (4-HPR), Lin et al. employed a cell-penetrating peptide (i.e., low molecular weight protamine (LMWP)) to modify albumin for co-encapsulating these two drugs *via* hydrophobic interaction. They demonstrated that this drug delivery platform toward a brain tumor showed significant efficiency.

2.2.5 HSA for metal ion chelation and in situ adsorption

Zhang and coworkers conjugated maleimide modified octahedral ligand-iridium(III) complex (Ir1) to albumin.^[49] Human serum albumin has abundant histidine residues for metal ion chelation and a free thiol group at cysteine-34 for chemical modification. The obtained Ir1-HSA showed favorable phosphorescence enhancement, promoting $^1\text{O}_2$ quantum yield as compared to that of an unconjugated one. Moreover, Ir1-HSA tended to accumulate in the nucleus of cancer cells to exhibit remarkable photocytotoxicity. As discussed before, HSA is the most abundant plasma protein found in human blood with a concentration of 40 mg mL⁻¹. Jin et al.^[50] designed a lipid-modified floxuridine homomeric oligonucleotide (LFU20). The hydrophobic tail of a lipid could insert into the albumin core *via* hydrophobic interaction to form albumin protected LFU20 complex once LFU20 was intravenously injected, leading to better inhibition toward the tumor proliferation.

3 Catalytic proteins with non-cytotoxicity as adjuvant agents

For non-cytotoxic enzymes such as catalase and HAase, they usually suffer from poor stability toward protease digestion and low cellular permeability. To maximize their potential as adjuvant agents to modulate tumor microenvironment and improve therapeutic efficiency of other modalities, a rational design of nanosystems to protect and deliver them is quite important.

3.1 Oxygen-dependent therapeutic modalities

Rapid proliferation and malformed blood vessels render solid tumors with a hypoxic

microenvironment.^[51] Oxygen-dependent therapeutic modes are therefore greatly limited. Photodynamic therapy is a noninvasive method with spatiotemporal selectivity, in which cytotoxic reactive oxygen species (ROS), especially singlet oxygen ($^1\text{O}_2$), are generated *via* photo-activating photosensitizers in the presence of molecular oxygen.^[52] For radiation therapy, DNA damages would occur when treated with ionizing radiation, and the generated oxygen radicals could induce cell apoptosis.^[53] The efficacy of radiation therapy is highly oxygen concentration-dependent. One feasible mechanism is that DNA damages after ionizing radiation could be stabilized by O_2 molecules so that the DNA repair by cells themselves could be inhibited. To enhance photo- and radio-related therapy, hypoxia-associated resistance should be resolved by oxygen generation in a hypoxic tumor microenvironment.

3.1.1 Catalytic property of catalase

To overcome hypoxia, delivering O_2 to tumor sites by nanocarriers is a promising strategy.^[54] However, direct delivery of O_2 by O_2 -dependent PDT or chemotherapy systems may threaten normal tissues. In addition, spontaneous exchange between O_2 and CO_2 usually provides an insufficient O_2 supply when delivered by red blood cells.^[17a] Therefore, in situ generation of O_2 in tumor sites to enhance O_2 -dependent PDT or chemotherapy is of great value. As it is well known, endogenous H_2O_2 content is much higher in tumor cells than that in normal cells.^[55] Therefore, decomposing H_2O_2 into H_2O and O_2 by bio/artificial catalysts (catalase or metal oxides) should be a useful approach.^[14,56] Catalase, an antioxidant enzyme, shows quite high enzyme activity toward the H_2O_2 decomposition ($2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}$) at a ratio of 1 catalase to 1,000,000 H_2O_2 molecules. Catalase is a tetramer composed of four equal subunits with a total molecular weight of 250 kDa. The bioactivity of catalase is constant over the pH range of 4.0-8.5, protecting cells against oxidative damage to nucleic acids, lipids and proteins.^[57]

3.1.2 Catalase-involved organic systems

As shown in Figure 5A, Zhang et al. constructed a liposomal system including catalase and cisplatin prodrug conjugated phospholipid for enhanced cancer chemo-radiotherapy.^[58] They used Pt(IV) conjugated 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) and other commercially available lipids to build liposomes, in which catalase was loaded subsequently and thus the catalytic activity was well retained. The comparison of the catalase-Pt(IV), Pt(IV),

and catalase liposomes indicates that the catalase-Pt(IV) liposome was most effective for indicating the DNA damage of tumor cells in hypoxic conditions under X-ray irradiation. Liposomes with a watery lumen show potential for spatiotemporal delivery of enzymes into tumor sites, but usually with low efficiency. This is induced by the hydrophilic nature of most enzymes and there is a lack of driving force for proteins to enter into the hydrophilic cavities of these carriers. Researchers found that polymersomes, composed of amphiphilic copolymers, could improve the loading efficiency of proteins when functional groups locating preferentially in the lumen are designed to offer multiple interactions with proteins. As compared to liposomes, polymersomes could be more easily designed and prepared. Some polymersome-related studies discussed below show cytosolic delivery of enzymes, although catalase is not the model in these systems. Qiu et al. designed a kind of triblock copolymers of poly(ethylene glycol)-b-poly(α -aminopalmitic acid)-b-poly(L-aspartic acid).^[59] Cyclic RGD peptide decoration and protein toxin loading provided remarkably improved treatment efficiency toward an orthotopically xenografted A549 lung tumor. As different components could bring out different functions, a series of copolymers were prepared, where spermine,^[60] polyethylenimine (PEI),^[61] poly(acrylic acid),^[62] and poly(2-(diethylamino)ethyl methacrylate)^[63] segments served as polyelectrolytes at the inner shell, poly(trimethylene carbonate-co-dithiolane trimethylene carbonate), and poly(N-2-hydroxypropyl methacrylamide-g-lipoic acid) segments as reduction-responsive motifs. Further decoration of the outer shell with angiopep-2 or apolipoprotein E peptide could achieve BBB penetration and a highly efficient anticancer therapy for glioblastoma.^[64]

Zhao et al. reported using host-guest system to achieve tumor cell targeting, hypoxia relief, and enhanced PDT efficiency (Figure 5B).^[65] They prepared cyclodextrin modified hyaluronic acid as the framework to specifically target the CD44 receptor overexpressed on some tumor cells. Adamantane-modified Chlorin e6 (aCe6) could be loaded into the framework *via* host-guest interaction and catalase was coupled onto the framework. Supramolecular assembly enabled an ordered arrangement and precise ratio control between different components in a synergistic system. The results showed that a catalase-integrated supramolecular system could provide a high accumulation rate and improved PDT efficacy at tumor sites. Chen et al. used poly(lactic-co-glycolic) acid (PLGA) to encapsulate catalase for efficient radiotherapy (Figure

5C). A toll-like-receptor-7 agonist R837 was also loaded within the PLGA shell to modulate the immune-suppressive tumor microenvironment. In this system, long term immunological memory was built against tumor recurrence as an immune-enhanced radiotherapy method.^[66]

Figure 5. (A) A scheme showing the preparation of catalase-Pt(IV) liposome for tumor hypoxia relieved cancer chemo-radiotherapy. Reproduced with permission.^[58] Copyright 2017, Elsevier. (B) Conjugation of cyclodextrin-modified hyaluronic acid with catalase, followed by supramolecular encapsulation of aCe6. Reproduced with permission.^[65] Copyright 2019, American Chemical Society. (C) Schematic illustration for the mechanism of antitumor immune response induced by PLGA-based radiotherapy in combination with checkpoint-blockade to inhibit cancer metastases and recurrence. Reproduced with permission.^[66] Copyright 2019, Wiley-VCH. (D) (a) A scheme showing the in situ encapsulation of catalase into hollow nanospheres and subsequent PEGylation, and (b) TEM image of the final nanosystem. Reproduced with permission.^[69] Copyright 2016, Wiley-VCH. (E) Synthesis of multifunctional theranostic nanoplatform for dual-mode radical and NIR photothermal therapy. Reproduced with permission.^[70] Copyright 2017, Springer.

3.1.3 Catalase-involved inorganic systems

The preparation of inorganic nanomaterials usually requires harsh conditions—including extreme pH or temperature, organic solvents, and lengthy procedures—which are incompatible with proteins.^[67] However, inorganic nanoparticles have the merits of adjustable size, designable morphology, multiple reactivity, and—sometimes—a porous structure. Furthermore, they can be internalized by cells through the EPR effect. Making use of these merits to develop protein-inorganic hybrid therapeutic platforms is becoming an intense research topic for which compatibility is a prerequisite.^[68] As shown in Figure 5D, Song et al. prepared catalase-encapsulated hollow tantalum oxide (TaOx) *via* one-step synthesis.^[69] A TaOx nanoshell was obtained *via* the sol-gel reaction of tantalum ethoxide in a water-ethanol mixture, while catalase could be in situ loaded into hollow TaOx just by pre-adding catalase into tantalum precursor solution. The PEG modification on the outer surface of TaOx was then carried out with layer-by-layer technology. Since tantalum is known to be a nontoxic radio-sensitizing element,

catalase loaded inside the nanoshell could further enhance the efficacy of radiotherapy. Moreover, TaOx possesses a mesoporous structure to allow the diffusion of H₂O₂ into the cavity and protect the loaded catalase from protease digestion with well-maintained bioactivity.

In contrast to existing ideas of encapsulating catalase in protective materials and reserving the catalytic activity to decompose H₂O₂ into O₂ and relieve tumor hypoxia, Chen et al. developed catalase-imprinted nanoparticles composed of an Fe₃O₄/Fe nanorod core as a Fenton agent and fibrous SiO₂/polydopamine (SiO₂/PDA) shell as an imprinted layer (Figure 5E).^[70] Based on the discovery that H₂O₂ concentrations increases markedly from 10⁻⁸ to 10⁻⁴ M when the catalytic activity of catalase is blocked, their elaborately designed system generated an imprinted shell specifically to catch catalase and inhibit its bioactivity in tumor cells. Within the imprinted shell, fibrous SiO₂ was to increase the adsorption capacity toward catalase protein and polydopamine was to offer the biocompatibility and NIR photothermal effect. Simultaneously, the conversion from H₂O₂ to more toxic •OH *via* the Fenton reaction of Fe₃O₄/Fe would trigger the tumor cell apoptosis. In vitro experiments showed the ability and superiority of this imprinted nanoplatform at capturing and inhibiting catalase at the cellular level while •OH was generated to kill cancer cells effectively. Another similar work was reported by Lee et al.,^[71] who used an irreversible inhibitor 3-amino-1,2,4-triazole to exhaust antioxidant catalase; the inhibitor was co-loaded with copper ions into mesoporous silica to achieve an enhanced Fenton reaction.

3.1.4 Catalase-mimic inorganic nanomaterials

With regard to two aspects of catalase-involved nanosystems, employing nanozymes to replace natural enzymes to conduct mimicry roles under some conditions is useful in practical applications.^[72] Nanozymes are defined as synthetic nanomaterials with the following intrinsic enzyme-like activities: 1) They have very efficient activity despite structural fragility. Their large size, varying surface charge, and the flexible structure of catalase make them sensitive to chemical modifications. Therefore, catalase-encapsulated materials often suffer from a certain degree of loss from catalytic activities; 2) They are widespread, but often with a low serum stability. Native proteins are susceptible to plasma protein adsorption defined as opsonization. However, catalase-appended materials often degradation easily and have a short half-life. Unlike natural enzymes, nanozymes are usually robust, prepared on the large scale, and can be

internalized into cells *via* endocytosis without encapsulation assistance. Moreover, nanozymes normally show moderate enzyme-like bioactivity for which the burst reaction occurring between released natural enzymes and substrates could be avoided. When considering catalase, its catalytic effect might significantly increase the amount of O_2 locally, thus resulting in O_2 toxicity.^[73]

As shown in Figure 6A, Liu et al. discovered that PAMAM dendrimer conjugated gold nanoclusters (AuNCs-NH₂) could show intrinsic catalase mimic activity over a broad pH range.^[74] As we know, the catalytic activity of nanozymes is pH-dependent.^[75] Homolysis of H_2O_2 to more toxic $\bullet OH$ is a kind of peroxidase-like activity, which often occurs under acidic conditions. Catalase-like activity of decomposing H_2O_2 to H_2O and O_2 mostly takes place under neutral or alkaline conditions. The development of catalase mimics is greatly limited when trapped inside endosomes (pH $\approx$ 5.5) or lysosomes (pH $\approx$ 4.8) of cells. Liu and coworkers verified that AuNCs-NH₂ did not behave peroxidase- or superoxide dismutase (SOD)-like activity, attributed to the existence of tertiary amines. Superoxide dismutase is an antioxidant metal enzyme present in organisms, which can catalyze superoxide anion radical disproportionation to produce oxygen and hydrogen peroxide. Therefore, the cytotoxicity arising from $\bullet OH$ generation and the depletion of $O_2^{\bullet -}$ by the PDT process were inhibited. They also demonstrated that treating with AuNCs-NH₂ and photosensitizers sequentially could bring out enhanced PDT efficacy, and these materials were harmless in the dark and to normal cells.

Integrating O_2 generator and photosensitizer into one carrier would be sometimes complex. As shown in Figure 6B, Yao et al. reported a simplified one-for-all system based on cerium oxide upconversion nanoparticles (Ce-UCNPs), that could work as an O_2 generator like catalase and as a photocatalyst to realize NIR triggered PDT.^[76] In their system, Ce-UCNPs were prepared by a hard template method to obtain a hollow and porous structure. Cerium oxide could generate O_2 from H_2O_2 effectively even at weakly acidic conditions, while doped UCNPs could convert NIR light to UV emission to trigger photocatalytic ability of cerium oxide, accompanied with the production of cytotoxic $O_2^{\bullet -}$ and $\bullet OH$ from H_2O and O_2 . These Ce-UCNPs were quite photostable as compared to organic photosensitizers. Further combination with RGD peptide and doxorubicin was beneficial to achieving superior therapy efficiency.

In another study (Figure 6C), Chen et al. developed a hafnium-involved manganoporphyrin

metal-organic framework (MOF) to implement hypoxia relief (with catalase-like manganese ions) and enhanced radiotherapy (with a hafnium cluster-based radiosensitizer).^[77] Zhen et al.^[78] conducted one-step biomineralization of BSA-iridium oxide hybrid nanoparticles (BSA-IrO₂). As shown in Figure 6D, this simple integration enabled the multiple functions of photoacoustic, computed tomography, and thermal imaging; photothermal conversion; and photocatalytic activities. The catalase-like activity of BSA-IrO₂ could protect normal tissues from H₂O₂-related inflammation, overcome hypoxia to improve phototherapy, and enhance photoacoustic imaging. Liang et al. integrated noble metals, semiconductors, and sonosensitizers into a one-for-all system (Figure 6E).^[79] First they constructed Janus-like Pt-CuS nanohybrids with a hollow structure, where Pt was used as the nanozyme and CuS as photothermal conversion accelerator. Next the sonosensitizer was loaded into the cavity of the nanohybrids and coated with a temperature-sensitive polymer. Interestingly, the polymer brushes acting as gatekeepers can block active sites on the nanozyme at a temperature lower than the lower critical solution temperature (LCST) while its activity accelerated significantly once the temperature was increased beyond the LCST, resulting in hypoxia relief and enhanced sonodynamic therapy. In addition to H₂O₂, glutathione (GSH), known as a free radical scavenger, is also overexpressed in many types of tumors. As shown in Figure 6F, Yin et al. developed a MnFe₂O₄@MOF complex^[80] that exhibited dual enzyme-like activities of catalase and GSH peroxidase. The GSH peroxidase is an important peroxidase widely found in the human body. Selenium is a component of GSH peroxidase, which can catalyze the transformation of GSH into a disulfide compound (GSSG) and reduce toxic peroxides to non-toxic hydroxyl compounds. The corresponding PDT efficacy of the MOF was enhanced and the depletion of ROS by GSH decreased.

Figure 6. (A) (a) Catalase-like activities of AuNCs-NH₂ and (b) a simple strategy of conventional PDT combined with self-supplied O₂. Reproduced with permission.^[74] Copyright 2017, Wiley-VCH. (B) Schematic illustration for the synthesis of Yb³⁺/Tm³⁺ codoped mesoporous cerium oxide hollow nanoparticles (Ce-UCNPs). Reproduced with permission.^[76] Copyright 2018, Wiley-VCH. (C) Schematic illustration for synthesizing the nanosensitizer. Reproduced with permission.^[77] Copyright 2019, Royal Society of Chemistry. (D) Schematic

illustration for synthetic process and therapeutic mechanism of BSA-IrO₂ nanoparticles. Reproduced with permission.^[78] Copyright 2018, Wiley-VCH. (E) Schematic illustration for the synthesis procedures and antitumor mechanism of Pt-CuS nanohybrids. Reproduced with permission.^[79] Copyright 2019, American Chemical Society. (F) Schematic illustration of MnFe₂O₄@MOF for persistently providing O₂ and consuming GSH to efficiently enhance PDT. Reproduced with permission.^[80] Copyright 2019, Wiley-VCH.

3.2 HAase

Protein-based nanosystems with switchable size present useful functions. Larger size is beneficial to prolonging blood circulation and more effective tumor accumulation, while smaller size contributes to enhanced intratumoral interstitial penetration. A main reason restricting the penetration of the nanosystems is due to densely stacked extracellular matrix in tumor interstitium, composed of a variety of fibrous structural proteins such as collagen, polysaccharide (i.e., hyaluronic acid), and glycoproteins (i.e., glycosaminoglycans).^[81] This matrix greatly hinders the nanosystem perfusion. As HAase and collagenase are general enzymes for hyaluronic acid/collagen digestion, they could be used to increase the permeability of nanosystems into tumor interstitium.^[82]

As shown in Figure 7A, Zhu et al.^[83] modified the hyaluronic acid chain with methacrylate and cholesteryl groups, among which the methacrylate group was for radical-triggered chemical crosslinking and the cholesteryl group afforded the affinity for protein encapsulation. This hyaluronic acid based nanosystem was an ideal carrier for intracellular protein delivery, where deoxyribonuclease I (DNase I) was selected as a therapeutic protein to induce tumor cell death *via* the DNA digestion. In addition to DNase I, activity blocked HAase was co-loaded for nanosystem degradation, and therapeutic protein release. The amino group of HAase was reacted by citraconic anhydride, and thus the activity of HAase was shielded. Once accumulated at the mildly acidic condition of tumor sites, the introduced citraconic anhydride could be quickly cleaved. The reactivation of HAase could gradually break down the hyaluronic acid shell and tumor interstitium, and promote the intracellular release of DNase I to kill cancer cells. In a similar report (Figure 7B), dextran (DEX) was used to modify native HAase *via* a pH-responsive linker.^[84] Compared to free HAase, DEX conjugation could enhance the stability of

HAase against protease digestion, and corresponding immunogenicity induced by original HAase was inhibited (Figures 7C, and D).^[15,85] When the DEX-HAase conjugate was pre-injected intravenously, it showed prolonged blood circulation. After the injection and passive accumulation at tumor sites, densely stacked hyaluronic acid network in tumor interstitium could be broken down by the released HAase, and thereby a loose extracellular matrix was generated. Subsequent injection of liposome-encapsulated photosensitizers or anti-PD-L1 led to better PDT and immune checkpoint blockade therapy efficiency, benefited from enhanced intratumoral penetration of oxygen and therapeutic agents using activity-manipulated HAase as adjuvant nanomedicine.

Figure 7. (A) Schematic presentation for the self-assembly and tumor-specific self-degradation of crosslinked hyaluronic acid nanosystem. Reproduced with permission.^[83] Copyright 2018, Wiley-VCH. (B) Synthesis of DEX-HAase and the tumor microenvironment triggered HAase release to induce hyaluronic acid breakdown. Reproduced with permission.^[84] Copyright 2019, Wiley-VCH. (C) Scheme showing the effect of HAase on the modulation of tumor microenvironment. Reproduced with permission.^[15] Copyright 2016, American Chemical Society. (D) Preparation and characterization of HAase-modified nanocarriers. Reproduced with permission.^[85b] Copyright 2016, American Chemical Society.

4 Catalytic proteins with cytotoxicity as therapeutic agents

Cytotoxic proteins, such as GO_x, RNase A, and caspase-3, show high bioactivity even at low concentrations, enabling them to exhibit great potential in pharmaceuticals. However, they suffer from similar issues as non-cytotoxic proteins including immunogenicity, protease-induced denaturation and weak cellular permeability. To deliver these proteins, suitable carriers were developed with the consideration of improving the cytotoxicity.

4.1 GO_x

Glucose oxidase is a kind of oxido-reductase that is very common in living organisms, and when purified from *Aspergillus niger* it is a dimeric glycoprotein consisting of two identical subunits with a total molecular weight of 160 kDa.^[22c]

4.1.1 GO_x as a chemotherapy agent

To catalyze the oxidation of glucose into gluconic acid and H_2O_2 by using O_2 as an electron acceptor, each subunit of GO_x contains one flavin adenine dinucleotide (FAD) and one iron ion, while FAD works as the cofactor. Initially, glucose is oxidized to D-glucono- δ -lactone, which can be further hydrolyzed to gluconic acid. Meanwhile, FAD as an electron acceptor is reduced to FADH_2 . Next, FADH_2 is reoxidized to FAD by O_2 with higher reduction potential, while O_2 itself is reduced to H_2O_2 .^[86] The reaction equation is shown as: $\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{Gluconic Acid}$. Taking substrates and products of glucose oxidation into consideration, GO_x has four aspects of potentials to be used in cancer therapy: 1) Cutting off energy supply – glucose exhaustion, 2) Hypoxia activated chemotherapy – hypoxia-based toxic drugs, 3) pH-responsive drug delivery system – enhanced acidity by gluconic acid, and 4) High level of oxidative stress – conversion of H_2O_2 to more toxic $\bullet\text{OH}$.

4.1.2 GO_x -involved nanosystems with improved enzyme activity

GO_x -mediated starvation therapy^[21b,87] is related to the anaerobic glycolysis (the Warburg effect) of cancer cells. The proliferation of cancer cells is highly dependent on glucose levels.^[88] Hao et al. specially modified the N-terminus of GO_x with a cationic polymer of poly(N,N'-dimethylamino-2-ethyl methacrylate) to modulate the generation of H_2O_2 .^[89] Native GO_x has an isoelectric point of 4.2 and shows electronegativity in physical conditions. Using a cationic polymer with a pK_a of 7.8 to modify the surface of native GO_x made it positively charged in a weakly acidic tumor microenvironment. In cell experiments, the GO_x -polymer conjugate was more toxic than native GO_x , with a 1.5-fold lower half-maximal inhibitory concentration, 4-fold higher uptake efficiency, and 1.8-fold higher level of H_2O_2 generation. For in vivo studies, the tumor retention was increased 2-fold and tolerability was enhanced 5-fold. Zhang et al. (Figure 8A) introduced MnO_2 as a motor to generate O_2 for glucose oxidation and downregulate the side effects of accumulated H_2O_2 , acid, and hypoxia.^[90] During the catalytic process of glucose oxidation by GO_x , O_2 consumption would aggravate tumor hypoxia and further limit glucose depletion, while the H_2O_2 and acid generated would promote tumor metastasis and prolong the survival time of the tumor *via* induced autophagy. Furthermore, glucose transporter 1 (Glut1) would be overexpressed when correlated with tumor proliferation and metastasis. This integrated system enabled the cyclic utilization of O_2 through the decomposition of H_2O_2 catalyzed by MnO_2 in an acidic environment. Restrained Glut1 expression also contributed to

more effective therapy.

4.1.3 Nanosystems for co-loading of GO_x and Fenton agents

Although GO_x starvation therapy shows great potential, cancer cells could upregulate their adaptability with parallel energy supplies and therefore limit therapeutic efficacy of this method. A combination of starvation therapy with other therapeutic modes has been suggested to achieve a better therapy performance.

In regard to the Fenton reaction, metal ions including Fe, Mn, Co, Ag, and Cu are usually employed for the conversion from H₂O₂ to more toxic •OH.^[91] Integrating metal ion-based inorganic materials with GO_x should be feasibly designed. To easily implement the goal, Qiao and coworkers employed hemoglobin as a Fenton agent.^[92] Hemoglobin is an oxygen-transport protein in red blood cells, composed of four heme groups with an iron ion at each core. These iron ions can be released to trigger Fenton reaction when in contact with H₂O₂. In their study, hemoglobin and GO_x were co-encapsulated in acid-decomposable MOF *via* a simple step, and the obtained nanosystem showed noticeable cancer cell toxicity. As shown in Figure 8B, Ke et al. constructed a polymersome nanoreactor, for which GO_x was encapsulated in the inner cavity of poly-prodrug, while ultra small Fe₃O₄ nanoparticles were incorporated in membranes.^[93] Pendant (2-(pentamethyleneimino) ethyl methacrylate) segment of the poly-prodrug could be protonated at a tumor microenvironment of pH 6.5 to increase the membrane permeability, allowing glucose and O₂ transportation and catalysis by loaded GO_x. Cascade glucose oxidation by loaded GO_x and Fenton reaction by Fe₃O₄ nanoparticles could generate a large amount of •OH *in situ*^[94] and allow chemotherapeutic drugs to be released from the poly-prodrug through the cleavage of ROS-responsive thioketal linker. This type of nanoreactor could achieve satisfactory cancer therapy outcome.

In another system composed of GO_x and Fe₃O₄, Liu et al. introduced mild, multiwalled carbon nanotube-based PTT to elevate the kinetics of Fenton reaction and improve the efficiency of chemodynamic therapy.^[95] Hu et al also demonstrated that the catalytic activity of GO_x could be enhanced by increasing the temperature in the range of 43–60 °C.^[96] The combination of PTT and chemodynamic therapy using GO_x and inorganic nanoparticles presents a promising application potential. Mild hyperthermia not only contributes to the enhanced bioactivity of enzymes and accelerated kinetics of inorganic nanoparticles, but also

strengthens blood flow to relieve hypoxia and supply abundant O₂. In turn, tumor cells are usually thermo-resistant during the PTT process due to the synthesis of heat shock protein (HSP) dependent on the adenosine triphosphate (ATP) level. In the starvation therapy stage, both ATP and HSP levels could be downregulated, eventually leading to more sensitive tumor cells and improved PTT efficacy.^[97]

4.1.4 Nanosystems for co-loading of GO_x and hypoxia activated drugs

Several hypoxia-activated drug systems have been developed. Zhang et al. employed hyaluronic acid-modified and acid-decomposable calcium carbonate (CaCO₃) for co-loading GO_x and triapazamine (TPZ) pro-drug, achieving cancer therapy in both a spatial and temporal manner.^[98] When accumulated at tumor sites, the decomposition of CaCO₃ and the release of TPZ occurred due to glucose oxidation by GO_x and elevated acidity by corresponding gluconic acid. Meanwhile, exacerbated hypoxia could activate TPZ to its toxic state. Similarly, Yang et al. (Figure 8C) designed yolk-shell organosilica with dual porosity and degradability as a suitable carrier for GO_x and TPZ.^[99] Yolk core had a small pore structure while the shell possessed dendritic large pores, where a tetrasulfide bond bridged framework could deplete GSH and preferentially release GO_x. The TPZ could be activated later to induce the cancer cell apoptosis.

Figure 8. (A) Schematic illustration of the MnO₂ motor effect in the nanosystem for starving therapy with interfering Glut1 expression. Reproduced with permission.^[90] Copyright 2018, American Chemical Society. (B) Preparation of polymersome nanoreactor for cooperative cancer therapy *via* systemic administration. Reproduced with permission.^[93] Copyright 2019, American Chemical Society. (C) (a,b) Schematic illustration of the cascade delivery system, and (c) TEM image of the delivery system and Bio-TEM images of the system after the incubation with 4T1 cells for different days, illustrating a shell-to-yolk stepwise degradability. Scale bar: 200 nm. Reproduced with permission.^[99] Copyright 2018, Wiley-VCH.

4.1.5 Nanosystems for co-loading of GO_x and gasotransmitter donors

Carbon monoxide (CO), known as a kind of gasotransmitter, could sensitize tumor cells for chemotherapeutic drugs as well as induce mitochondria dysfunction to produce ROS, leading

to the proliferation inhibition of tumor cells.^[100] To avoid systemic toxicity of direct CO delivery, it was discovered that CO could be released in situ in the form of manganese carbonyls (MnCO) when triggered by excess H₂O₂.^[101] Wang et al. employed poly(lactic-co-glycolic acid) to co-encapsulate GO_x and MnCO, followed by the coating of a red blood cell membrane for enhanced circulation and accumulation.^[102] Nitric oxide (NO), a member of the gasotransmitter family, could cause mitochondria and DNA nitrosation and kill cancer cells at a concentration higher than 1 μM.^[103] Fan et al. used L-arginine as the NO donor, which could release NO when treated by inducible NO synthase enzyme or H₂O₂.^[104] In the case of H₂O₂, more biocidal peroxynitrite species would be produced to further improve starvation-gas therapy efficacy.

4.1.6 Nanosystems as GO_x analogues

Liu et al. reported dual nanozyme-incorporated porphyrin MOFs for synergic cancer therapy.^[105] Herein, platinum nanoparticles acted as catalase mimics, while gold nanoparticles with an ultrasmall size worked for GO_x mimics. Supported porphyrin framework played roles of photosensitizer for PDT and fluorescence imaging agent. Within this system, Pt, Au, and porphyrin cooperated with each other to achieve multiple functions. The decomposition of H₂O₂ into O₂ by platinum nanoparticles contributed to enhanced glucose depletion and PDT efficacy, while gold nanoparticles could work effectively as GO_x to starve cancer cells.

4.2 RNase A

RNase A is a kind of nuclease that could hydrolyze RNA into small molecules, resulting in cytotoxicity when captured by cells.^[106] RNase A as a therapeutic protein has entered clinical stage to supplement some conventional cancer treatment modalities including surgery, phototherapy, chemotherapy, and radiotherapy.^[107] Unlike GO_x, the substrate of which is glucose, RNase A could play roles only in the case where it enters into cells.

4.2.1 Nanosystems for loading of activity-blocked RNase A

It was reported that the activity of RNase A was highly correlated with its lysine residues.^[108] As shown in Figure 9A, Wang et al. modified RNase A with an aryl boronic ester molecule (NBC). The NBC could react with the amino group of RNase A to generate a carbamate linker. This modification was believed to temporarily inhibit the bioactivity of RNase A.^[109] Once triggered by intracellular ROS at tumor sites, the NBC group could be cleaved to release RNase A in its free state. In addition to the capacity to reversibly modulate the bio-

function of RNase A, the NBC conjugation also resulted in decreased isoelectric point of RNase A and therefore stronger affinity with positively charged lipids when encapsulated for delivery. This tumor proliferation-inhibited nanosystem could avoid systemic toxicity and achieve spatiotemporal regulation on the bio-function of therapeutic protein. He et al. introduced hematoporphyrin as a photosensitizer to achieve photo-manipulated activity of RNase A (Figure 9B).^[110] The supported substrate was constructed by ketal cross-linked PEI, which could be degraded in an acidic microenvironment. The NBC-modified RNase A was then adsorbed onto this substrate *via* electronic interaction. Finally, the whole nanosystem was coated by hematoporphyrin-modified hyaluronic acid. Compared to the PEG corona, hyaluronic acid could facilitate active targeting of the nanosystem toward tumor cells. Upon light irradiation, more ROS was generated *via* the PDT effect in a spatiotemporal manner. Integrating all functions in one nanoplatform was a useful strategy to achieve selective killing efficiency, longer blood circulation, endosomal escape *via* PEI-induced “proton sponge” effect, and acid triggered degradability.

4.2.2 Nanosystems for encapsulation of RNase A

The encapsulation of cytotoxic proteins by nanogels is another feasible method instead of reversibly blocking the active sites of proteins with small molecules. As shown in Figure 9C, Liu et al. constructed hierarchical polymer gels.^[111] RNase A was first encapsulated into the polymer network containing positively charged monomers and reduction-responsive cross-linkers. Subsequently, doxycycline drug was co-loaded with the protein into an acid-labile polymer nanogel. Finally, the hyaluronic acid shell was chemically introduced onto the protein/drug-loaded composites. Nanocarriers designed to pass through multiple physiological barriers *in vivo* should meet two prerequisites. To prolong the blood circulation, avoid the opsonization of non-specific plasma protein adsorption, accumulate at tumor sites *via* EPR effect, and escape from renal clearance, nanocarriers with relatively large size (about 100-200 nm) and negatively charged surface were proven to be appropriate. For subsequent penetration through tumor tissues and internalization by tumor cells, however, nanocarriers with smaller size (around 10 nm) and positively charged surface seem superior. In this case, Liu et al. took the size conversion and charge reversal into consideration simultaneously. Under the stimuli of a tumor microenvironment, large/negative particles could transform into small/positive

counterparts for achieving better cancer killing efficiency.

While several nanogel systems developed by Zhong and co-workers did not refer to RNase, these nanosystems for intracellular delivery of other anticancer proteins showed great potential. Granzyme B (GrB) holds the activity of proliferative inhibition to cancer cells. To increase the delivery of GrB into ovarian and breast cancer cells, a dual-targeted nanogel was developed.^[112] Hyaluronic acid separately conjugated with tetrazole/GE11 peptide and cystamine methacrylamide was crosslinked to wrap GrB by combining nanoprecipitation with a photoclick reaction. Hyaluronic acid shows an affinity to the CD44 receptor, and GE11 peptide targets to the epidermal growth factor receptor (EGFR). Enhanced delivery and uptake efficiency of GrB was achieved by the CD44/EGFR dual-targeted nanogel, and the versatility of this nanosystem was verified in their other work.^[113] They used the same nanogel for the inhibition of metastatic breast cancer. The therapeutic protein was saporin, which could block the protein synthesis in cells. It should be mentioned that the preparation of these nanogels usually needs organic solvents and photoirradiation. Taking safety and efficiency into account, Ding and co-workers used E3/K3 coiled-coil peptide as the crosslinking agent.^[114] This coiled-coil motif is pH-sensitive, exhibiting the self-assembly stability at neutral conditions and the unfolding behavior to release cargoes under a mild acid condition. The incorporation of coiled-coil peptide into the nanogel enables efficient intracellular protein release and delivery. The nanogels prepared from nanoprecipitation often show modest size control and distribution. As nanogels with tunable and uniform size would perform better, Huang et al. employed the microfluidics technique to prepare smaller nanogels with a narrow size distribution.^[115] In this case, they used a 30 amino acid synthetic peptide named as GALA peptide. GALA shows the conformation conversion from random coil to α -helix when pH varies from neutral to slightly acidic, thus facilitating endosomal escape of the nanogels.

For native RNase delivery, inorganic materials with large pores are also ideal carriers. As shown in Figure 9D, Shao et al. designed a kind of biodegradable mesoporous silica to carry RNase A.^[116] By incorporating diselenide bridged organosilica precursor into the framework of mesoporous silica, dual responsiveness was achieved. Under oxidative conditions, diselenide bond was cleaved into seleninic acid. This hybrid silica possessed large pores (3.8 nm $\times$ 2.8 nm $\times$ 2.2 nm) for the loading of RNase A. Moreover, the cancer cell membrane coating could confer

the nanosystem with targeting and immune-evading properties.

Figure 9. (A) RNase A modifications with (a) NBC for ROS-responsive release, and (b) hematoporphyrin for nonresponsive protein engineering, and (c) encapsulation of RNase A–NBC into cationic lipid-like nanoparticles for intracellular protein delivery. Reproduced with permission.^[109] Copyright 2014, Wiley-VCH. (B) Schematic illustration of light-controlled protein delivery assisted by the nanocomplex toward synergistic cancer therapy. Reproduced with permission.^[110] Copyright 2018, Wiley-VCH. (C) Schematic illustration for hierarchical assembly and adaptive disassembly of RNase A-loaded polymer system. Reproduced with permission.^[111] Copyright 2018, American Chemical Society. (D) Schematic illustration for the synthesis procedure of biodegradable diselenide-bridged mesoporous silica and its application for dual-responsive, cancer cell-membrane-mimetic protein delivery. TEM images and reconstructed 3D models of different types of mesoporous silica. Reproduced with permission.^[116] Copyright 2018, Wiley-VCH.

4.3 Caspase-3

Caspase-3, a cysteine-aspartic acid protease of the “executioner” family, is known to induce cell apoptosis even at low levels. The active site of cysteine residue could be modified to modulate the bioactivity of caspase-3.^[117] Thayumanavan et al. (Figure 10A) designed a polymer chain to reversibly silence the killer protein *via* disulfide bond in caspase-3.^[118] Having the polymer, proteins could be separated and protected from protease digestion. Moreover, their activity was switched off during the delivery, which could weaken harmful consequences caused by off-target effects.

Figure 10. (A) Preparation of nanogel-caspase-3 conjugate through the disulfide linkage. Reproduced with permission.^[118] Copyright 2015, American Chemical Society. (B) Chemical structures of polymers and nanoassembly, and protein release in the presence of a reducing agent. Reproduced with permission.^[119] Copyright 2017, American Chemical Society. (C) Illustration of the chemistry for protein-polymer nanoassembly formation and stimulus-induced release. Reproduced with permission.^[120] Copyright 2019, American Chemical Society.

While another two similar systems developed by Thayumanavan et al. did not refer to caspase-3, they were useful for designing nanosystems for intracellular delivery of toxic proteins. Unlike conventionally used polyelectrolyte adsorption and lipid encapsulation, covalent arrangement of polymer networks around the surface of proteins could enable the loading of proteins with neutral surface charge and high fidelity.^[119] A polymer with pendant functional group of p-nitrophenylcarbonates (NPCs) could react with lysine moiety of proteins (Figure 10B). The remaining NPCs were blocked by diamine cross-linker to generate an integral polymer sheath around proteins. The disulfide bond located at the β -position next to carbamate oxygen could trigger the cascade release under reducing conditions. In regard to the polymer sheath, the disulfide bond could be cleaved to break the polymer network, while the following carbamate cleavage could further release the original amine and native proteins. The protein encapsulation, release and activity retention tests of cytochrome c, lysozyme, and RNase A indicates the versatility of this kind of polymer.

Unlike small organic molecules, proteins possess heterogeneously distributed functional groups, and protein-related reactions usually take place in a low concentration. The polymer design by taking the size and charge of different proteins into consideration is urgently needed. Another study used the “salicylhydroxamate–arylboronic acid” click reaction (Figure 10C).^[120] The lysine residues of proteins were modified by arylboronic acid, which could be degraded under oxidative conditions of H_2O_2 . The salicylhydroxamate functional group of the designed polymer showed high reaction efficiency toward the arylboronic acid group. The experimental results indicated that the click reaction between arylboronic acid-conjugated proteins and salicylhydroxamate-modified polymer chains was quite fast and quantitative even at a low concentration of proteins. A variety of proteins with different sizes (from -14 to -400 kDa) and surface charges were tested to verify the universal usage of this kind of lysine modification method and polymer type. In addition to the H_2O_2 -responsive boronic acid group, introducing other stimulus-responsive functional groups together with boronic acid could expand the sensitivity of protein-polymer assemblies. Moreover, guanidium-containing polymers and fluoropolymers were also verified to be highly effective. Natural and synthetic guanidium-containing compounds showed strong biological activity. Multiple interactions including salt

bridges and hydrogen bonding interactions between glutamic acid/aspartic acid on proteins and guanidium groups of polymer chains were quite strong and stable. Fluoropolymers are known for their hydrophobic, lipophobic, and anti-fouling properties, as these properties contribute to high self-assembly and intracellular delivery tendency as well as reduced protein denaturation. Several detailed examples about guanidium-containing polymers and fluoropolymers were summarized in an excellent review by Lv and co-workers.^[22f]

5. Summary of different protein-based nanosystems

Plasma proteins are quite stable in their native states during blood circulation. Biocompatibility, biodegradability, and specific receptors make them appealing as carriers for drugs and photosensitizers.

Ferritin, a cage-like protein, works as a pocket for drugs/photosensitizers and biomineralization. The decoration of FRT with tumor-targeting ligands could further enhance the delivery efficiency. Ferritin-based nanosystems usually require separation and purification to have cargo-loaded capability, and thus large-scale production is somehow limited. Human serum albumin, the most abundant protein in plasma, serves as a “Trojan Horse” for several compounds. No matter what kind of structure is developed to address concerns on both tumor accumulation and permeability, fundamental understanding of the interactions between HSA and its cargo is a prerequisite. Human serum albumin-templated biomineralization and physical adsorption/chemical conjugation of HSA corona are two common ways to obtain HSA-inorganic hybrid materials, having the merits of designable morphology, adjustable size, and facile preparation. However, biocompatibility-related issues still need to be improved when considering the combination of inorganic materials and fragile proteins, such as hemolysis and biodegradability. In the case of HSA-based organic systems, when drug molecules are covalently coupled to HSA, the effect on physiological behavior of HSA is hard to evaluate. When they bind to each other noncovalently, chemical screening and computational simulation of binding sites as well as binding affinity of HSA to drug molecules/linkers should be systematically evaluated.

For catalytic proteins, whether cytotoxic or non-cytotoxic, they usually suffer from poor stability toward protease digestion and low cellular permeability. A series of rationally designed

nanocarriers have been developed to protect these proteins from the degradation and deliver them to desired locations, while avoiding immune response and systemic toxicity. Both organic polymer sheaths and inorganic nanoparticles discussed above could meet these requirements.

These nanocarriers could be categorized into different nanosystems based on the proteins used. For proteins as adjuvant agents, the main therapeutic effects are exhibited by nanovehicles, while proteins work synergistically. In some cases, there is no need to release proteins, since their substrates and products could freely go in/out *via* the pores of nanocarriers. Whether they are released or not, their catalytic ability should be activated in tumor sites and the nanocarriers should be biocompatible and biodegradable. Therapeutic proteins show high specificity and activity. Among the nanovehicles, liposomes, nanogels, nanocapsules, polyion complexes, and polymersomes are similar, as they have watery cavities and network structures. They show great promise for intracellular delivery of therapeutic proteins.

Herein, we introduce two more novel protein-based nanotherapeutics. One is antigen protein delivery for cancer immunotherapy.^[121] Cancer-specific immune responses would be activated once these antigens are efficiently delivered to dendritic cells and other antigen-presenting cells.^[122] Cytotoxic T lymphocytes generated could attack and eliminate the target cells. Moreover, T cell activation could be conducted when motifs with adjuvant functions of activating dendritic cells are integrated into nanosystems. Liposome- and polymer-based nanosystems for the delivery of ovalbumin, a tumor-specific antigenic protein, have been rapidly developed.^[123] Another one is the CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeat and CRISPR-associated protein 9) for gene editing.^[124] Direct delivery of Cas9 protein and single guide RNA (sgRNA) has been regarded as a promising alternative to show better control on decreasing the risks of over-dosing and off-target effects than that of DNA component encoding Cas9 and sgRNA.^[125] Strong negative charge and poor stability toward endonuclease somehow limit the application of RNA therapeutics. Therefore, developing appropriate carriers to protect RNA and proteins from degradation and deliver them to the cell nucleus is urgently required.^[126]

6 Conclusions and perspectives

Proteins, naturally existing species, exhibit a great promise for cancer theranostics. For

proteins, structure determines property. Proteins take different responsibilities in living systems due to their varied structures. Plasma proteins with inherent biocompatibility are suitable vehicles for drug delivery. Catalytic proteins are adjuvant agents or therapeutic drugs by themselves. While different protein-based nanosystems have shown great potential to inhibit and eliminate tumors, protein nanotherapeutics are still in infancy.

For plasma proteins, they possess multiple binding sites for conjugation chemistry. A systematic databank should be established to show suitable binding sites, binding affinity, and conjugation methods for various binders. Moreover, the conjugation methods should be more straightforward, facile, and specific. Bio-orthogonal reactions are in great need to be fully explored for improved efficiency and conjugation selectivity.

For catalytic proteins, when delivered by rationally designed nanovehicles, their therapeutic efficiency could be maximized. More accurate and timely information should be provided, including the distribution of therapeutic proteins in tumor cells and normal cells, and whether or not full activation of their pharmacokinetics could be achieved at desired locations. For clinical translation, more sophisticated tumor models should be established, and more in vivo long-term safety tests need to be conducted. We believe that, as more therapeutic proteins will be discovered and smarter nanovehicles will be designed, protein-based nanosystems for cancer therapy will show increased value.

Acknowledgement

The authors are grateful for the financial support of China Scholarship Council, the National Nature Science Foundation of China (No. 51433008), the Singapore Agency for Science, Technology and Research (A*STAR) AME IRG grant (No. A1883c0005), and the Singapore National Research Foundation Investigatorship (No. NRF-NRFI2018-03).

References

- [1] a) G. Walsh, *Nat. Biotechnol.* **2014**, 32, 992; b) A. M. Scott, J. D. Wolchok, L. J. Old, *Nat. Rev. Cancer* **2012**, 12, 278.
- [2] a) B. Leader, Q. J. Baca, D. E. Golan, *Nat. Rev. Drug Discovery* **2008**, 7, 21; b) G. Walsh, *Nat. Biotechnol.* **2010**, 28, 917; c) Y. Luo, Y. Song, M. Wang, T. Jian, S. Ding, P. Mu, Z. Liao, Q. Shi, X. Cai, H. Jin, *Small*

- 2019**, *15*, 1902485.
- [3] M. Yan, J. Du, Z. Gu, M. Liang, Y. Hu, W. Zhang, S. Priceman, L. Wu, Z. H. Zhou, Z. Liu, T. Segura, Y. Tang, Y. Lu, *Nat. Nanotechnol.* **2009**, *5*, 48.
  - [4] E. N. Hoogenboezem, C. L. Duvall, *Adv. Drug Delivery Rev.* **2018**, *130*, 73.
  - [5] D. Sleep, J. Cameron, L. R. Evans, *Biochim. Biophys. Acta, Gen. Subj.* **2013**, *1830*, 5526.
  - [6] a) U. Kragh-Hansen, *Pharmacol. Rev.* **1981**, *33*, 17; b) S. Curry, H. Mandelkow, P. Brick, N. Franks, *Nat. Struct. Biol.* **1998**, *5*, 827.
  - [7] G. Yang, H. Gong, T. Liu, X. Sun, L. Cheng, Z. Liu, *Biomaterials* **2015**, *60*, 62.
  - [8] a) Z. Yang, Q. Chen, J. Chen, Z. Dong, R. Zhang, J. Liu, Z. Liu, *Small* **2018**, *14*, 1803262; b) Q. Chen, L. Feng, J. Liu, W. Zhu, Z. Dong, Y. Wu, Z. Liu, *Adv. Mater.* **2016**, *28*, 7129; c) S. Ruan, X. Cao, X. Cun, G. Hu, Y. Zhou, Y. Zhang, L. Lu, Q. He, H. Gao, *Biomaterials* **2015**, *60*, 100; d) K. Huang, H. Ma, J. Liu, S. Huo, A. Kumar, T. Wei, X. Zhang, S. Jin, Y. Gan, P. C. Wang, S. He, X. Zhang, X.-J. Liang, *ACS Nano* **2012**, *6*, 4483; e) S. Ruan, Q. He, H. Gao, *Nanoscale* **2015**, *7*, 9487.
  - [9] a) Z. Sheng, D. Hu, M. Zheng, P. Zhao, H. Liu, D. Gao, P. Gong, G. Gao, P. Zhang, Y. Ma, L. Cai, *ACS Nano* **2014**, *8*, 12310; b) Y. Peng, Z. Zhao, T. Liu, X. Li, X. Hu, X. Wei, X. Zhang, W. Tan, *Angew. Chem., Int. Ed.* **2017**, *56*, 10845; c) Q. Chen, J. Chen, C. Liang, L. Feng, Z. Dong, X. Song, G. Song, Z. Liu, *J. Controlled Release* **2017**, *263*, 79.
  - [10] Y. Hu, D. Chen, S. Park, T. Emrick, T. P. Russell, *Adv. Mater.* **2010**, *22*, 2583.
  - [11] L. Li, C. J. Fang, J. C. Ryan, E. C. Niemi, J. A. Lebrón, P. J. Björkman, H. Arase, F. M. Torti, S. V. Torti, M. C. Nakamura, W. E. Seaman, *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 3505.
  - [12] a) P. Huang, P. Rong, A. Jin, X. Yan, M. G. Zhang, J. Lin, H. Hu, Z. Wang, X. Yue, W. Li, G. Niu, W. Zeng, W. Wang, K. Zhou, X. Chen, *Adv. Mater.* **2014**, *26*, 6401; b) Z. Zhen, W. Tang, C. Guo, H. Chen, X. Lin, G. Liu, B. Fei, X. Chen, B. Xu, J. Xie, *ACS Nano* **2013**, *7*, 6988; c) G. Jutz, P. van Rijn, B. Santos Miranda, A. Böker, *Chem. Rev.* **2015**, *115*, 1653; d) A. A. Alkhateeb, J. R. Connor, *Biochim. Biophys. Acta Rev. Cancer* **2013**, *1836*, 245; e) Z. Wang, P. Huang, O. Jacobson, Z. Wang, Y. Liu, L. Lin, J. Lin, N. Lu, H. Zhang, R. Tian, G. Niu, G. Liu, X. Chen, *ACS Nano* **2016**, *10*, 3453.
  - [13] a) Y. Lu, W. Sun, Z. Gu, *J. Controlled Release* **2014**, *194*, 1; b) J. E. Gagner, W. Kim, E. L. Chaikof, *Acta Biomater.* **2014**, *10*, 1542; c) R. Vaishya, V. Khurana, S. Patel, A. K. Mitra, *Expert Opin. Drug Delivery* **2015**, *12*, 415; d) C. Wang, Y. Ye, Q. Hu, A. Bellotti, Z. Gu, *Adv. Mater.* **2017**, *29*, 1606036.
  - [14] H. Wang, Y. Chao, J. Liu, W. Zhu, G. Wang, L. Xu, Z. Liu, *Biomaterials* **2018**, *181*, 310.

- [15] H. Gong, Y. Chao, J. Xiang, X. Han, G. Song, L. Feng, J. Liu, G. Yang, Q. Chen, Z. Liu, *Nano Lett.* **2016**, *16*, 2512.
- [16] a) X. Zhang, K. Zhang, R. Haag, *Biomater. Sci.* **2015**, *3*, 1487; b) E. M. Pelegri-O'Day, E.-W. Lin, H. D. Maynard, *J. Am. Chem. Soc.* **2014**, *136*, 14323.
- [17] a) X. Song, J. Xu, C. Liang, Y. Chao, Q. Jin, C. Wang, M. Chen, Z. Liu, *Nano Lett.* **2018**, *18*, 6360; b) H. Chen, J. Tian, W. He, Z. Guo, *J. Am. Chem. Soc.* **2015**, *137*, 1539; c) D. Sueyoshi, Y. Anraku, T. Komatsu, Y. Urano, K. Kataoka, *Biomacromolecules* **2017**, *18*, 1189; d) Y. Anraku, A. Kishimura, M. Kamiya, S. Tanaka, T. Nomoto, K. Toh, Y. Matsumoto, S. Fukushima, D. Sueyoshi, M. R. Kano, Y. Urano, N. Nishiyama, K. Kataoka, *Angew. Chem., Int. Ed.* **2016**, *55*, 560; e) J. Li, Y. Li, Y. Wang, W. Ke, W. Chen, W. Wang, Z. Ge, *Nano Lett.* **2017**, *17*, 6983.
- [18] a) I. Ortac, D. Simberg, Y.-s. Yeh, J. Yang, B. Messmer, W. C. Trogler, R. Y. Tsien, S. Esener, *Nano Lett.* **2014**, *14*, 3023; b) Y. Zhang, T.-T. Shen, A. M. Kirillov, W.-S. Liu, Y. Tang, *Chem. Commun.* **2016**, *52*, 7939; c) G. Shim, M.-G. Kim, J. Y. Park, Y.-K. Oh, *Adv. Drug Delivery Rev.* **2016**, *105*, 205; d) A. Castro Nava, M. Cojoc, C. Peitzsch, G. Cirillo, I. Kurth, S. Fuessel, K. Erdmann, D. Kunhardt, O. Vittorio, S. Hampel, A. Dubrovskaya, *Int. J. Cancer* **2015**, *137*, 2492; e) J. Y. Lee, J. S. Kim, J. C. Park, Y. S. Nam, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2016**, *8*, 178; f) S. Ding, Z. Li, Y. Cheng, C. Du, J. Gao, Y.-W. Zhang, N. Zhang, Z. Li, N. Chang, X. Hu, *Nanotechnology* **2018**, *29*, 375604; g) S. Ding, Z. Lyu, X. Niu, Y. Zhou, D. Liu, M. Falahati, D. Du, Y. Lin, *Biosens. Bioelectron.* **2019**, 111830.
- [19] J. H. Ko, H. D. Maynard, *Chem. Soc. Rev.* **2018**, *47*, 8998.
- [20] a) W. Chen, M. Zheng, F. Meng, R. Cheng, C. Deng, J. Feijen, Z. Zhong, *Biomacromolecules* **2013**, *14*, 1214; b) D. Li, C. F. van Nostrum, E. Mastrobattista, T. Vermonden, W. E. Hennink, *J. Controlled Release* **2017**, *259*, 16.
- [21] a) Z. Gu, M. Yan, B. Hu, K.-I. Joo, A. Biswas, Y. Huang, Y. Lu, P. Wang, Y. Tang, *Nano Lett.* **2009**, *9*, 4533; b) W. Zhao, J. Hu, W. Gao, *ACS Appl. Mater. Interfaces* **2017**, *9*, 23528; c) C. Wang, Y. Ye, G. M. Hochu, H. Sadeghifar, Z. Gu, *Nano Lett.* **2016**, *16*, 2334; d) M. Zhao, A. Biswas, B. Hu, K.-I. Joo, P. Wang, Z. Gu, Y. Tang, *Biomaterials* **2011**, *32*, 5223.
- [22] a) Z. Liu, X. Chen, *Chem. Soc. Rev.* **2016**, *45*, 1432; b) X. Liu, C. Wang, Z. Liu, *Adv. Healthcare Mater.* **2018**, *7*, 1800913; c) L.-H. Fu, C. Qi, J. Lin, P. Huang, *Chem. Soc. Rev.* **2018**, *47*, 6454; d) D. Jiang, D. Ni, Z. T. Rosenkrans, P. Huang, X. Yan, W. Cai, *Chem. Soc. Rev.* **2019**, *48*, 3683; e) L. Cheng, L. Yang, F. Meng, Z. Zhong, *Adv. Healthcare Mater.* **2018**, *7*, 1800685; f) J. Lv, Q. Fan, H. Wang, Y. Cheng, *Biomaterials* **2019**,

218, 119358.

- [23] Y. Hu, D. Samanta, S. S. Parelkar, S. W. Hong, Q. Wang, T. P. Russell, T. Emrick, *Adv. Funct. Mater.* **2010**, *20*, 3603.
- [24] a) E. Falvo, E. Tremante, R. Fraioli, C. Leonetti, C. Zamparelli, A. Boffi, V. Morea, P. Ceci, P. Giacomini, *Nanoscale* **2013**, *5*, 12278; b) X. Lin, J. Xie, L. Zhu, S. Lee, G. Niu, Y. Ma, K. Kim, X. Chen, *Angew. Chem., Int. Ed.* **2011**, *50*, 1569; c) Y. J. Kang, H. J. Yang, S. Jeon, Y.-S. Kang, Y. Do, S. Y. Hong, S. Kang, *Macromol. Biosci.* **2014**, *14*, 619.
- [25] a) D. He, J. Marles-Wright, *New Biotechnol.* **2015**, *32*, 651; b) I. Yamashita, K. Iwahori, S. Kumagai, *Biochim. Biophys. Acta Gen. Subj.* **2010**, *1800*, 846.
- [26] Z. Wang, Y. Dai, Z. Wang, O. Jacobson, F. Zhang, B. C. Yung, P. Zhang, H. Gao, G. Niu, G. Liu, X. Chen, *Nanoscale* **2018**, *10*, 1135.
- [27] a) P. Arosio, R. Ingrassia, P. Cavadini, *Biochim. Biophys. Acta Gen. Subj.* **2009**, *1790*, 589; b) M. Bellini, S. Mazzucchelli, E. Galbiati, S. Sommaruga, L. Fiandra, M. Truffi, M. A. Rizzuto, M. Colombo, P. Tortora, F. Corsi, D. Prospero, *J. Controlled Release* **2014**, *196*, 184; c) M. Kim, Y. Rho, K. S. Jin, B. Ahn, S. Jung, H. Kim, M. Ree, *Biomacromolecules* **2011**, *12*, 1629.
- [28] N. Surguladze, S. Patton, A. Cozzi, M. G. Fried, J. R. Connor, *Biochem. J.* **2005**, *388*, 731.
- [29] Z. Zhen, W. Tang, H. Chen, X. Lin, T. Todd, G. Wang, T. Cowger, X. Chen, J. Xie, *ACS Nano* **2013**, *7*, 4830.
- [30] a) X. Jiang, C. Sun, Y. Guo, G. Nie, L. Xu, *Biosens. Bioelectron.* **2015**, *64*, 165; b) B. Hennequin, L. Turyanska, T. Ben, A. M. Beltrán, S. I. Molina, M. Li, S. Mann, A. Patané, N. R. Thomas, *Adv. Mater.* **2008**, *20*, 3592.
- [31] a) J. Wu, X. Wang, Q. Wang, Z. Lou, S. Li, Y. Zhu, L. Qin, H. Wei, *Chem. Soc. Rev.* **2019**, *48*, 1004; b) W. Chen, K. Zeng, H. Liu, J. Ouyang, L. Wang, Y. Liu, H. Wang, L. Deng, Y.-N. Liu, *Adv. Funct. Mater.* **2017**, *27*, 1605795; c) X. Jia, X. Cai, Y. Chen, S. Wang, H. Xu, K. Zhang, M. Ma, H. Wu, J. Shi, H. Chen, *ACS Appl. Mater. Interfaces* **2015**, *7*, 4579.
- [32] W. Zhang, Y. Zhang, Y. Chen, S. Li, N. Gu, S. Hu, Y. Sun, X. Chen, Q. Li, *J. Nanosci. Nanotechnol.* **2013**, *13*, 60.
- [33] P. van Rijn, N. C. Mougín, D. Franke, H. Park, A. Böker, *Chem. Commun.* **2011**, *47*, 8376.
- [34] a) S. Kang, P. A. Suci, C. C. Broomell, K. Iwahori, M. Kobayashi, I. Yamashita, M. Young, T. Douglas, *Nano Lett.* **2009**, *9*, 2360; b) P. A. Suci, S. Kang, M. Young, T. Douglas, *J. Am. Chem. Soc.* **2009**, *131*, 9164.
- [35] a) Y. Wang, T. Yang, H. Ke, A. Zhu, Y. Wang, J. Wang, J. Shen, G. Liu, C. Chen, Y. Zhao, H. Chen, *Adv.*

- Mater.* **2015**, *27*, 3874; b) Z. Yang, S. Luo, Y. Zeng, C. Shi, R. Li, *ACS Appl. Mater. Interfaces* **2017**, *9*, 6839;
- c) J. Zhang, G. Hao, C. Yao, J. Yu, J. Wang, W. Yang, C. Hu, B. Zhang, *ACS Appl. Mater. Interfaces* **2016**, *8*, 16612.
- [36] C. Xu, Z. Teng, Y. Zhang, L. Yuwen, Q. Zhang, X. Su, M. Dang, Y. Tian, J. Tao, L. Bao, B. Yang, G. Lu, J. Zhu, *Adv. Funct. Mater.* **2018**, *28*, 1804081.
- [37] H. Ren, J. Liu, F. Su, S. Ge, A. Yuan, W. Dai, J. Wu, Y. Hu, *ACS Appl. Mater. Interfaces* **2017**, *9*, 3463.
- [38] Z. Zhou, Y. Liu, M. Zhang, C. Li, R. Yang, J. Li, C. Qian, M. Sun, *Adv. Funct. Mater.* **2019**, *29*, 1904144.
- [39] G. Yang, S. Z. F. Phua, W. Q. Lim, R. Zhang, L. Feng, G. Liu, H. Wu, A. K. Bindra, D. Jana, Z. Liu, Y. Zhao, *Adv. Mater.* **2019**, *31*, 1901513.
- [40] a) W. Z. Yuan, P. Lu, S. Chen, J. W. Y. Lam, Z. Wang, Y. Liu, H. S. Kwok, Y. Ma, B. Z. Tang, *Adv. Mater.* **2010**, *22*, 2159; b) M. Li, Y. Gao, Y. Yuan, Y. Wu, Z. Song, B. Z. Tang, B. Liu, Q. C. Zheng, *ACS Nano* **2017**, *11*, 3922.
- [41] Y. M. Woo, Y. Shin, E. J. Lee, S. Lee, S. H. Jeong, H. K. Kong, E. Y. Park, H. K. Kim, J. Han, M. Chang, J.-H. Park, *PLoS One* **2015**, *10*, e0132285.
- [42] S. L. Kuan, B. Stöckle, J. Reichenwallner, D. Y. W. Ng, Y. Wu, M. Doroshenko, K. Koynov, D. Hinderberger, K. Müllen, T. Weil, *Biomacromolecules* **2013**, *14*, 367.
- [43] a) L. Palmerston Mendes, J. Pan, P. V. Torchilin, *Molecules* **2017**, *22*, 1401; b) Y. Dong, T. Yu, L. Ding, E. Laurini, Y. Huang, M. Zhang, Y. Weng, S. Lin, P. Chen, D. Marson, Y. Jiang, S. Giorgio, S. Pricl, X. Liu, P. Rocchi, L. Peng, *J. Am. Chem. Soc.* **2018**, *140*, 16264; c) T. Wei, C. Chen, J. Liu, C. Liu, P. Posocco, X. Liu, Q. Cheng, S. Huo, Z. Liang, M. Fermeglia, S. Pricl, X.-J. Liang, P. Rocchi, L. Peng, *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 2978.
- [44] G. Han, J.-T. Wang, X. Ji, L. Liu, H. Zhao, *Bioconjugate Chem.* **2017**, *28*, 636.
- [45] T. Lin, P. Zhao, Y. Jiang, Y. Tang, H. Jin, Z. Pan, H. He, V. C. Yang, Y. Huang, *ACS Nano* **2016**, *10*, 9999.
- [46] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, *J. Controlled Release* **2016**, *235*, 34.
- [47] W. J. Geldenhuys, A. S. Mohammad, C. E. Adkins, P. R. Lockman, *Ther. Delivery* **2015**, *6*, 961.
- [48] a) R. K. Mittapalli, V. K. Manda, C. E. Adkins, W. J. Geldenhuys, P. R. Lockman, *Ther. Delivery* **2010**, *1*, 775; b) A. M. Merlot, D. S. Kalinowski, D. R. Richardson, *Front. Physiol.* **2014**, *5*, 299.
- [49] P. Zhang, H. Huang, S. Banerjee, G. J. Clarkson, C. Ge, C. Imberti, P. J. Sadler, *Angew. Chem., Int. Ed.* **2019**, *58*, 2350.
- [50] C. Jin, H. Zhang, J. Zou, Y. Liu, L. Zhang, F. Li, R. Wang, W. Xuan, M. Ye, W. Tan, *Angew. Chem., Int. Ed.*

2018, *57*, 8994.

- [51] a) Y. Lou, P. C. McDonald, A. Oloumi, S. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U. auf dem Keller, S. Leung, D. Huntsman, B. Clarke, B. W. Sutherland, D. Waterhouse, M. Bally, C. Roskelley, C. M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.-Y. Winum, C. T. Supuran, S. Dedhar, *Cancer Res.* **2011**, *71*, 3364; b) R. K. Jain, *Cancer Cell* **2014**, *26*, 605.
- [52] a) P. Agostinis, K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. O. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B. C. Wilson, J. Golab, *Cancer J. Clin.* **2011**, *61*, 250; b) N. M. Idris, M. K. Gnanasammandhan, J. Zhang, P. C. Ho, R. Mahendran, Y. Zhang, *Nat. Med.* **2012**, *18*, 1580; c) B. Tian, C. Wang, S. Zhang, L. Feng, Z. Liu, *ACS Nano* **2011**, *5*, 7000.
- [53] a) R. Baskar, K. A. Lee, R. Yeo, K.-W. Yeoh, *Int. J. Med. Sci.* **2012**, *9*, 193; b) X.-D. Zhang, D. Wu, X. Shen, J. Chen, Y.-M. Sun, P.-X. Liu, X.-J. Liang, *Biomaterials* **2012**, *33*, 6408; c) G. Le Duc, I. Miladi, C. Alric, P. Mowat, E. Bräuer-Krisch, A. Bouchet, E. Khalil, C. Billotey, M. Janier, F. Lux, T. Epicier, P. Perriat, S. Roux, O. Tillement, *ACS Nano* **2011**, *5*, 9566.
- [54] a) M. Gao, C. Liang, X. Song, Q. Chen, Q. Jin, C. Wang, Z. Liu, *Adv. Mater.* **2017**, *29*, 1701429; b) G. Song, C. Liang, X. Yi, Q. Zhao, L. Cheng, K. Yang, Z. Liu, *Adv. Mater.* **2016**, *28*, 2716; c) Z. Luo, M. Zheng, P. Zhao, Z. Chen, F. Siu, P. Gong, G. Gao, Z. Sheng, C. Zheng, Y. Ma, L. Cai, *Sci. Rep.* **2016**, *6*, 23393; d) W. Tang, Z. Zhen, M. Wang, H. Wang, Y.-J. Chuang, W. Zhang, G. D. Wang, T. Todd, T. Cowger, H. Chen, L. Liu, Z. Li, J. Xie, *Adv. Funct. Mater.* **2016**, *26*, 1757; e) F. B. Jensen, *J. Exp. Biol.* **2009**, *212*, 3387.
- [55] a) M. López-Lázaro, *Cancer Lett.* **2007**, *252*, 1; b) A. R. Lippert, G. C. Van de Bittner, C. J. Chang, *Acc. Chem. Res.* **2011**, *44*, 793.
- [56] a) J. Kim, H. R. Cho, H. Jeon, D. Kim, C. Song, N. Lee, S. H. Choi, T. Hyeon, *J. Am. Chem. Soc.* **2017**, *139*, 10992; b) W. Zhu, Z. Dong, T. Fu, J. Liu, Q. Chen, Y. Li, R. Zhu, L. Xu, Z. Liu, *Adv. Funct. Mater.* **2016**, *26*, 5490.
- [57] C. Glorieux, B. Calderon Pedro, *Biol. Chem.* **2017**, *398*, 1095.
- [58] R. Zhang, X. Song, C. Liang, X. Yi, G. Song, Y. Chao, Y. Yang, K. Yang, L. Feng, Z. Liu, *Biomaterials* **2017**, *138*, 13.
- [59] M. Qiu, Z. Zhang, Y. Wei, H. Sun, F. Meng, C. Deng, Z. Zhong, *Chem. Mater.* **2018**, *30*, 6831.
- [60] P. Yao, Y. Zhang, H. Meng, H. Sun, Z. Zhong, *Biomacromolecules* **2018**, *20*, 184.
- [61] W. Yang, Y. Wei, L. Yang, J. Zhang, Z. Zhong, G. Storm, F. Meng, *J. Controlled Release* **2018**, *290*, 141.

- [62] W. Yang, Y. Xia, Y. Zou, F. Meng, J. Zhang, Z. Zhong, *Chem. Mater.* **2017**, *29*, 8757.
- [63] G. Liu, S. Ma, S. Li, R. Cheng, F. Meng, H. Liu, Z. Zhong, *Biomaterials* **2010**, *31*, 7575.
- [64] a) Y. Jiang, W. Yang, J. Zhang, F. Meng, Z. Zhong, *Adv. Mater.* **2018**, *30*, 1800316; b) Y. Jiang, J. Zhang, F. Meng, Z. Zhong, *ACS Nano* **2018**, *12*, 11070.
- [65] a) S. Z. F. Phua, G. Yang, W. Q. Lim, A. Verma, H. Chen, T. Thanabalu, Y. L. Zhao, *ACS Nano* **2019**, *13*, 4742.
- [66] Q. Chen, J. Chen, Z. Yang, J. Xu, L. Xu, C. Liang, X. Han, Z. Liu, *Adv. Mater.* **2019**, *31*, 1802228.
- [67] a) I. Bilecka, M. Niederberger, *Nanoscale* **2010**, *2*, 1358; b) C. N. R. Rao, S. R. C. Vivekchand, K. Biswas, A. Govindaraj, *Dalton Trans.* **2007**, 3728.
- [68] M. Malmsten, *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 468.
- [69] G. Song, Y. Chen, C. Liang, X. Yi, J. Liu, X. Sun, S. Shen, K. Yang, Z. Liu, *Adv. Mater.* **2016**, *28*, 7143.
- [70] J. Chen, S. Lei, K. Zeng, M. Wang, A. Asif, X. Ge, *Nano Res.* **2017**, *10*, 2351.
- [71] R. K. Kankala, Y. Kuthati, C.-L. Liu, C.-Y. Mou, C.-H. Lee, *RSC Adv.* **2015**, *5*, 86072.
- [72] a) H. Wei, E. Wang, *Chem. Soc. Rev.* **2013**, *42*, 6060; b) X. Wang, Y. Hu, H. Wei, *Inorg. Chem. Front.* **2016**, *3*, 41.
- [73] a) Y. Tao, E. Ju, J. Ren, X. Qu, *Adv. Mater.* **2015**, *27*, 1097; b) W. He, Y.-T. Zhou, W. G. Wamer, X. Hu, X. Wu, Z. Zheng, M. D. Boudreau, J.-J. Yin, *Biomaterials* **2013**, *34*, 765.
- [74] C.-P. Liu, T.-H. Wu, C.-Y. Liu, K.-C. Chen, Y.-X. Chen, G.-S. Chen, S.-Y. Lin, *Small* **2017**, *13*, 1700278.
- [75] a) Z. Chen, J.-J. Yin, Y.-T. Zhou, Y. Zhang, L. Song, M. Song, S. Hu, N. Gu, *ACS Nano* **2012**, *6*, 4001; b) J. Li, W. Liu, X. Wu, X. Gao, *Biomaterials* **2015**, *48*, 37.
- [76] C. Yao, W. Wang, P. Wang, M. Zhao, X. Li, F. Zhang, *Adv. Mater.* **2018**, *30*, 1704833.
- [77] Y. Chen, H. Zhong, J. Wang, X. Wan, Y. Li, W. Pan, N. Li, B. Tang, *Chem. Sci.* **2019**, *10*, 5773.
- [78] W. Zhen, Y. Liu, L. Lin, J. Bai, X. Jia, H. Tian, X. Jiang, *Angew. Chem., Int. Ed.* **2018**, *57*, 10309.
- [79] S. Liang, X. Deng, Y. Chang, C. Sun, S. Shao, Z. Xie, X. Xiao, P. a. Ma, H. Zhang, Z. Cheng, J. Lin, *Nano Lett.* **2019**, *19*, 4134.
- [80] S.-Y. Yin, G. Song, Y. Yang, Y. Zhao, P. Wang, L.-M. Zhu, X. Yin, X.-B. Zhang, *Adv. Funct. Mater.* **2019**, *29*, 1901417.
- [81] a) G. Kong, R. D. Braun, M. W. Dewhirst, *Cancer Res.* **2000**, *60*, 4440; b) W. Jiang, Y. Huang, Y. An, B. Y. S. Kim, *ACS Nano* **2015**, *9*, 8689.
- [82] a) B. A. Buhren, H. Schrumpf, N.-P. Hoff, E. Bölke, S. Hilton, P. A. Gerber, *Eur. J. Med. Res.* **2016**, *21*, 5; b)

- H. Alipour, A. Raz, S. Zakeri, N. Dinparast Djadid, *Asian Pac. J. Trop. Biomed.* **2016**, *6*, 975.
- [83] Q. Zhu, X. Chen, X. Xu, Y. Zhang, C. Zhang, R. Mo, *Adv. Funct. Mater.* **2018**, *28*, 1707371.
- [84] H. Wang, X. Han, Z. Dong, J. Xu, J. Wang, Z. Liu, *Adv. Funct. Mater.* **2019**, *29*, 1902440.
- [85] a) K. Xu, F. Lee, S. Gao, M.-H. Tan, M. Kurisawa, *J. Controlled Release* **2015**, *216*, 47; b) H. Zhou, Z. Fan, J. Deng, P. K. Lemons, D. C. Arhontoulis, W. B. Bowne, H. Cheng, *Nano Lett.* **2016**, *16*, 3268.
- [86] S. B. Bankar, M. V. Bule, R. S. Singhal, L. Ananthanarayan, *Biotechnol. Adv.* **2009**, *27*, 489.
- [87] a) S.-Y. Li, H. Cheng, B.-R. Xie, W.-X. Qiu, J.-Y. Zeng, C.-X. Li, S.-S. Wan, L. Zhang, W.-L. Liu, X.-Z. Zhang, *ACS Nano* **2017**, *11*, 7006; b) Z. Yu, P. Zhou, W. Pan, N. Li, B. Tang, *Nat. Commun.* **2018**, *9*, 5044.
- [88] a) M. V. Liberti, J. W. Locasale, *Trends Biochem. Sci.* **2016**, *41*, 211; b) Z. Chen, W. Lu, C. Garcia-Prieto, P. Huang, *J. Bioenerg. Biomembr.* **2007**, *39*, 267; c) S. J. Bensinger, H. R. Christofk, *Semin. Cell Dev. Biol.* **2012**, *23*, 352.
- [89] H. Hao, M. Sun, P. Li, J. Sun, X. Liu, W. Gao, *ACS Appl. Mater. Interfaces* **2019**, *11*, 9756.
- [90] Y.-H. Zhang, W.-X. Qiu, M. Zhang, L. Zhang, X.-Z. Zhang, *ACS Appl. Mater. Interfaces* **2018**, *10*, 15030.
- [91] C. Zhang, W. Bu, D. Ni, S. Zhang, Q. Li, Z. Yao, J. Zhang, H. Yao, Z. Wang, J. Shi, *Angew. Chem., Int. Ed.* **2016**, *55*, 2101.
- [92] H. Ranji-Burachaloo, A. Reyhani, P. A. Gurr, D. E. Dunstan, G. G. Qiao, *Nanoscale* **2019**, *11*, 5705.
- [93] W. Ke, J. Li, F. Mohammed, Y. Wang, K. Tou, X. Liu, P. Wen, H. Kinoh, Y. Anraku, H. Chen, K. Kataoka, Z. Ge, *ACS Nano* **2019**, *13*, 2357.
- [94] M. Huo, L. Wang, Y. Chen, J. Shi, *Nat. Commun.* **2017**, *8*, 357.
- [95] X. Liu, Y. Liu, J. Wang, T. Wei, Z. Dai, *ACS Appl. Mater. Interfaces* **2019**, *11*, 23065.
- [96] J.-J. Hu, M.-D. Liu, F. Gao, Y. Chen, S.-Y. Peng, Z.-H. Li, H. Cheng, X.-Z. Zhang, *Biomaterials* **2019**, *217*, 119303.
- [97] a) W.-H. Chen, G.-F. Luo, Q. Lei, S. Hong, W.-X. Qiu, L.-H. Liu, S.-X. Cheng, X.-Z. Zhang, *ACS Nano* **2017**, *11*, 1419; b) J. Zhou, M. Li, Y. Hou, Z. Luo, Q. Chen, H. Cao, R. Huo, C. Xue, L. Sutrisno, L. Hao, Y. Cao, H. Ran, L. Lu, K. Li, K. Cai, *ACS Nano* **2018**, *12*, 2858.
- [98] M.-K. Zhang, C.-X. Li, S.-B. Wang, T. Liu, X.-L. Song, X.-Q. Yang, J. Feng, X.-Z. Zhang, *Small* **2018**, *14*, 1803602.
- [99] Y. Yang, Y. Lu, P. L. Abbaraju, I. Azimi, C. Lei, J. Tang, M. Jambhrunkar, J. Fu, M. Zhang, Y. Liu, C. Liu, C. Yu, *Adv. Funct. Mater.* **2018**, *28*, 1800706.
- [100] S. García-Gallego, G. J. L. Bernardes, *Angew. Chem., Int. Ed.* **2014**, *53*, 9712.

- [101] a) A. C. Kautz, P. C. Kunz, C. Janiak, *Dalton Trans.* **2016**, 45, 18045; b) P. C. Kunz, H. Meyer, J. Barthel, S. Sollazzo, A. M. Schmidt, C. Janiak, *Chem. Commun.* **2013**, 49, 4896.
- [102] Y. Wang, Z. Liu, H. Wang, Z. Meng, Y. Wang, W. Miao, X. Li, H. Ren, *Acta Biomater.* **2019**, 92, 241.
- [103] a) X. Zhang, G. Tian, W. Yin, L. Wang, X. Zheng, L. Yan, J. Li, H. Su, C. Chen, Z. Gu, Y. Zhao, *Adv. Funct. Mater.* **2015**, 25, 3049; b) J. Xu, F. Zeng, H. Wu, C. Hu, C. Yu, S. Wu, *Small* **2014**, 10, 3750.
- [104] W. Fan, N. Lu, P. Huang, Y. Liu, Z. Yang, S. Wang, G. Yu, Y. Liu, J. Hu, Q. He, J. Qu, T. Wang, X. Chen, *Angew. Chem., Int. Ed.* **2017**, 56, 1229.
- [105] C. Liu, J. Xing, O. U. Akakuru, L. Luo, S. Sun, R. Zou, Z. Yu, Q. Fang, A. Wu, *Nano Lett.* **2019**, 19, 5674.
- [106] N. Kordalivand, D. Li, N. Beztsinna, J. Sastre Torano, E. Mastrobattista, C. F. van Nostrum, W. E. Hennink, T. Vermonden, *Chem. Eng. J.* **2018**, 340, 32.
- [107] a) W. Ardelt, B. Ardelt, Z. Darzynkiewicz, *Eur. J. Pharmacol.* **2009**, 625, 181; b) P. A. Leland, R. T. Raines, *Chem. Biol.* **2001**, 8, 405.
- [108] R. T. Raines, *Chem. Rev.* **1998**, 98, 1045.
- [109] M. Wang, S. Sun, C. I. Neufeld, B. Perez-Ramirez, Q. Xu, *Angew. Chem., Int. Ed.* **2014**, 53, 13444.
- [110] H. He, Y. Chen, Y. Li, Z. Song, Y. Zhong, R. Zhu, J. Cheng, L. Yin, *Adv. Funct. Mater.* **2018**, 28, 1706710.
- [111] M. Liu, S. Shen, D. Wen, M. Li, T. Li, X. Chen, Z. Gu, R. Mo, *Nano Lett.* **2018**, 18, 2294.
- [112] J. Chen, J. Ouyang, Q. Chen, C. Deng, F. Meng, J. Zhang, R. Cheng, Q. Lan, Z. Zhong, *ACS Appl. Mater. Interfaces* **2017**, 9, 24140.
- [113] J. Chen, H. He, C. Deng, L. Yin, Z. Zhong, *Int. J. Pharm.* **2019**, 560, 57.
- [114] L. Ding, Y. Jiang, J. Zhang, H.-A. Klok, Z. Zhong, *Biomacromolecules* **2018**, 19, 555.
- [115] K. Huang, Y. He, Z. Zhu, J. Guo, G. Wang, C. Deng, Z. Zhong, *ACS Appl. Mater. Interfaces* **2019**, 11, 22171.
- [116] D. Shao, M. Li, Z. Wang, X. Zheng, Y.-H. Lao, Z. Chang, F. Zhang, M. Lu, J. Yue, H. Hu, H. Yan, L. Chen, W.-f. Dong, K. W. Leong, *Adv. Mater.* **2018**, 30, 1801198.
- [117] a) A. G. Porter, R. U. Jänicke, *Cell Death Differ.* **1999**, 6, 99; b) D. C. González-Toro, J.-H. Ryu, R. T. Chacko, J. Zhuang, S. Thayumanavan, *J. Am. Chem. Soc.* **2012**, 134, 6964; c) R. T. Chacko, J. Ventura, J. Zhuang, S. Thayumanavan, *Adv. Drug Delivery Rev.* **2012**, 64, 836; d) J.-H. Ryu, R. T. Chacko, S. Jiwpanich, S. Bickerton, R. P. Babu, S. Thayumanavan, *J. Am. Chem. Soc.* **2010**, 132, 17227.
- [118] J. Ventura, S. J. Eron, D. C. González-Toro, K. Raghupathi, F. Wang, J. A. Hardy, S. Thayumanavan, *Biomacromolecules* **2015**, 16, 3161.
- [119] K. Dutta, D. Hu, B. Zhao, A. E. Ribbe, J. Zhuang, S. Thayumanavan, *J. Am. Chem. Soc.* **2017**, 139, 5676.

- [120] B. Liu, M. Ianosi-Irimie, S. Thayumanavan, *ACS Nano* **2019**, *13*, 9408.
- [121] K. Palucka, J. Banchereau, *Nat. Rev. Cancer* **2012**, *12*, 265.
- [122] J. Banchereau, A. K. Palucka, *Nat. Rev. Immunol.* **2005**, *5*, 296.
- [123] a) Y. Yoshizaki, E. Yuba, N. Sakaguchi, K. Koiwai, A. Harada, K. Kono, *Biomaterials* **2014**, *35*, 8186; b) E. Yuba, A. Harada, Y. Sakanishi, S. Watarai, K. Kono, *Biomaterials* **2013**, *34*, 3042.
- [124] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, E. Charpentier, *Science* **2012**, *337*, 816.
- [125] a) R. Rouet, B. A. Thuma, M. D. Roy, N. G. Lintner, D. M. Rubitski, J. E. Finley, H. M. Wisniewska, R. Mendonsa, A. Hirsh, L. de Oñate, *J. Am. Chem. Soc.* **2018**, *140*, 6596; b) W. Zhou, H. Cui, L. Ying, X. F. Yu, *Angew. Chem., Int. Ed.* **2018**, *57*, 10268.
- [126] W. Sun, W. Ji, J. M. Hall, Q. Hu, C. Wang, C. L. Beisel, Z. Gu, *Angew. Chem., Int. Ed.* **2015**, *54*, 12029.

Table of Contents Graphic

Recent significant development of elaborately designed protein-based artificial nanosystems as drug carriers, adjuvant agents and therapeutic species is summarized, aiming to stimulate this important research area toward more practical applications.

Author Biography:

Nan Zhang earned her B.Sc. degree from the School of Natural and Applied Sciences, Northwestern Polytechnical University, China. In 2015, she joined Prof. Xiaoling Hu's group as a Ph.D. candidate in the same university. Sponsored by the China Scholarship Council (CSC), she has spent two years (August 2017–August 2019) as a visiting student in Prof. Yanli Zhao's group at Nanyang Technological University. She is currently working on protein-based nanomaterials for bio-related applications.

Xiaoling Hu received her Ph.D. degree in material physics and chemistry from the School of Materials, Northwestern Polytechnical University, China in 2005. Now, she is a professor in Department of Applied Chemistry from the School of Natural and Applied Sciences, Northwestern Polytechnical University. Her current research interests involve the design and preparation of functional polymer materials used for biomolecule recognition, as well as the computational modeling and simulation regarding the interaction mechanism between disease protein and carbon-based nanomaterials. She has published more than 30 journal papers in the leading journals.

Yanli Zhao is currently a Professor at Nanyang Technological University. He received his B.Sc. degree in chemistry from Nankai University and his Ph.D. degree in physical chemistry there under the supervision of Professor Yu Liu. He was a postdoctoral scholar with Professor Sir Fraser Stoddart at University of California Los Angeles and subsequently at Northwestern University. He also conducted his postdoctoral research with Professor Jeffrey Zink at University of California Los Angeles. His current research focuses on integrated nanoparticles for theranostics, and porous materials for energy storage and catalysis.

Scheme 1. Protein-based nanosystems for cancer treatment.

Figure 1. (A) Schematic illustration for the formation and working mechanism of P-RFRTs, and photographs of P-RFRTs and free ZnF_{16}Pc solutions under different conditions. Reproduced with permission.^[12b] Copyright 2013, American Chemical Society. (B) Therapy studies performed on U87MG tumor-bearing nude mice ($n = 5/\text{group}$), and time dependent uptake of the nanosystem by U87MG cells. Scale bar, 50 μm . Reproduced with permission.^[29] Copyright 2013, American Chemical Society.

Figure 2. (A) Cells targeted with fluorescently tagged cage nanoplatform. (B) Surface-masking approach used to toposelectively biotinylate LiDps to the Janus nanoparticle. (C) Structure of functionalized nanoplatform observed by transmission electron microscopy (TEM). Shapes that would be expected for two orthogonal projections of a heterodimer cross section are indicated. Scale bar is 20 nm. Reproduced with permission.^[34b] Copyright 2009, American Chemical Society.

Figure 3. (A) (a) Schematic illustration for the construction process of the MoS₂/HSA hollow nanocapsule, (b,c) TEM images of the SiO₂-MoS₂ nanospheres, and (d,e) TEM images of the flexible MoS₂/HSA hollow nanocapsule. Reproduced with permission.^[36] Copyright 2018, Wiley-VCH. (B) Design and structure of triple effect core-shell system (Nano-PDT), and TEM images of (left) IR780@HSA and (right) IR780@PFTBA@HSA. Reproduced with permission.^[37] Copyright 2017, American Chemical Society.

Figure 4. (A) Schematic illustration of LMWP-modified albumin nanoparticles *via* a green synthesis method. Reproduced with permission.^[45] Copyright 2016, American Chemical Society. (B) (a,b) BBB composition and main alterations found in pathological conditions, (c) BBB transport mechanisms for brain delivery of nanoparticles, and (d) main nanoparticle features influencing systemic delivery and BBB passage. Reproduced with permission.^[46] Copyright 2016, Elsevier.

Figure 6. (A) (a) Catalase-like activities of AuNCs-NH₂ and (b) a simple strategy of conventional PDT combined with self-supplied O₂. Reproduced with permission.^[74] Copyright 2017, Wiley-VCH. (B) Schematic illustration for the synthesis of Yb³⁺/Tm³⁺ codoped mesoporous cerium oxide hollow nanoparticles (Ce-UCNPs). Reproduced with permission.^[76] Copyright 2018, Wiley-VCH. (C) Schematic illustration for synthesizing the nanosensitizer. Reproduced with permission.^[77] Copyright 2019, Royal Society of Chemistry. (D) Schematic illustration for synthetic process and therapeutic mechanism of BSA-IrO₂ nanoparticles. Reproduced with permission.^[78] Copyright 2018, Wiley-VCH. (E) Schematic illustration for the synthesis procedures and antitumor mechanism of Pt-CuS nanohybrids. Reproduced with permission.^[79] Copyright 2019, American Chemical Society. (F) Schematic illustration of MnFe₂O₄@MOF for persistently providing O₂ and consuming GSH to efficiently enhance PDT. Reproduced with permission.^[80] Copyright 2019, Wiley-VCH.

Figure 7. (A) Schematic presentation for the self-assembly and tumor-specific self-degradation of crosslinked hyaluronic acid nanosystem. Reproduced with permission.^[83] Copyright 2018, Wiley-VCH. (B) Synthesis of DEX-HAase and the tumor microenvironment triggered HAase release to induce hyaluronic acid breakdown. Reproduced with permission.^[84] Copyright 2019, Wiley-VCH. (C) Scheme showing the effect of HAase on the modulation of tumor microenvironment. Reproduced with permission.^[15] Copyright 2016, American Chemical Society. (D) Preparation and characterization of HAase-modified nanocarriers. Reproduced with permission.^[85b] Copyright 2016, American Chemical Society.

Figure 8. (A) Schematic illustration of the MnO_2 motor effect in the nanosystem for starving therapy with interfering Glut1 expression. Reproduced with permission.^[90] Copyright 2018, American Chemical Society. (B) Preparation of polymersome nanoreactor for cooperative cancer therapy *via* systemic administration. Reproduced with permission.^[93] Copyright 2019, American Chemical Society. (C) (a,b) Schematic illustration of the cascade delivery system, and (c) TEM image of the delivery system and Bio-TEM images of the system after the incubation with 4T1 cells for different days, illustrating a shell-to-yolk stepwise degradability. Scale bar: 200 nm. Reproduced with permission.^[99] Copyright 2018, Wiley-VCH.

Figure 9. (A) RNase A modifications with (a) NBC for ROS-responsive release, and (b) hematoporphyrin for nonresponsive protein engineering, and (c) encapsulation of RNase A–NBC into cationic lipid-like nanoparticles for intracellular protein delivery. Reproduced with permission.^[109] Copyright 2014, Wiley-VCH. (B) Schematic illustration of light-controlled protein delivery assisted by the nanocomplex toward synergistic cancer therapy. Reproduced with permission.^[110] Copyright 2018, Wiley-VCH. (C) Schematic illustration for hierarchical assembly and adaptive disassembly of RNase A-loaded polymer system. Reproduced with permission.^[111] Copyright 2018, American Chemical Society. (D) Schematic illustration for the synthesis procedure of biodegradable diselenide-bridged mesoporous silica and its application for dual-responsive, cancer cell-membrane-mimetic protein delivery. TEM images and reconstructed 3D models of different types of mesoporous silica. Reproduced with permission.^[116] Copyright 2018, Wiley-VCH.

Figure 10. (A) Preparation of nanogel-caspase-3 conjugate through the disulfide linkage. Reproduced with permission.^[118] Copyright 2015, American Chemical Society. (B) Chemical structures of polymers and nanoassembly, and protein release in the presence of a reducing agent. Reproduced with permission.^[119] Copyright 2017, American Chemical Society. (C) Illustration of the chemistry for protein-polymer nanoassembly formation and stimulus-induced release. Reproduced with permission.^[120] Copyright 2019, American Chemical Society.

Table of Contents Graphic

Recent significant development of elaborately designed protein-based artificial nanosystems as drug carriers, adjuvant agents and therapeutic species is summarized, aiming to stimulate this important research area toward more practical applications.