Review Article

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# Protein-Caged Nanoparticles: A Promising Nanomedicine Against Cancer

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Cancer is a severe threat to human wellness. A broad range of nanoparticles (NPs) have been developed to treat cancer. Given their safety profile, natural biomolecules such as protein-based NPs (PNPs) are promising substitutes for synthetic NPs that are currently used in drug delivery systems. In particular, PNPs have diverse characteristics and are monodisperse, chemically and genetically changeable, biodegradable, and biocompatible. To promote their application in clinical settings, PNPs must be precisely fabricated to fully exploit their advantages. This review highlights the different types of proteins that can be used to produce PNPs. Additionally, the recent applications of these nanomedicines and their therapeutic benefits against cancer are explored. Several future research directions that can facilitate the clinical application of PNPs are suggested.

**Key Words:** *Nanomedicine; Drug Delivery Systems; Nanoparticles; Phototherapy*



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## INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality globally and remains challenging to treat despite substantial trials and efforts devoted to identifying suitable treatment methods. The commonly used therapeutics approaches include radiation therapy, chemotherapy, and surgery. However, each of these approaches involve significant risks. Surgical intervention and high-intensity radiation may irreversibly damage healthy tissue. Similarly, insufficiently targeted drug accumulation and low pharmacokinetics of chemotherapeutics drugs lead to susceptibility to multi-drug resistance and limit the therapeutic potency against cancer. Thus, an efficient selective drug delivery system (DDS) must be identified to address the limitations of traditional therapies.

Advancements in nanotechnology have driven the development of nanomedicines (NMs) that can overcome the limitations of conventional medicines and are expected to have notable therapeutic benefits against cancer. NMs allow the accumulation of therapeutic drugs over a specific cancer area through enhanced permeability and retention (EPR). Additionally, NMs can simultaneously diffuse more than one therapeutic and imaging agent for effective synergistic therapy. Nanoparticles (NPs) are promising for application in the medicinal field, especially for drug delivery, systemic vaccine development, imaging, and diagnosis.[1-3] Many nanocarriers such as metal-oxide frameworks, polymeric micelles, liposomes, polymeric NPs, inorganic NPs, and proteins have been applied to treat cancer.[4] Specifically, in recent decades, the use of several NPs with distinct nanostructures, components, physicochemical properties, and functionalities has been explored for treating cancer. These NP platforms are characterized by various material arrangements such as polymeric micelles, dendrimers, liposomes, exosomes, gold NPs, organosilica/mesoporous silica NPs, magnetic NPs, proteins, metal-organic framework NPs, two-dimensional nanomaterials, and aggregation-induced emission dots.[5-10] Protein-based NP systems are promising naturally derived DDSs that are highly biodegradable and biocompatible and exhibit functionalities suitable for different biomedical applications, specifically as cancer NMs.[11-14]

This review highlights different types of proteins that are commonly used to produce protein-based NMs along with their characteristics and advantages. Additionally, we discuss their diverse biomedical applications and ther-

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apeutic benefits against cancer.

## ADVANTAGES OF PROTEIN NPs

Proteins are natural biopolymers with distinctive functionalities and advantages in materials and biomedical sciences. Proteins are suitable for NP formulation owing to their amphiphilicity that enables interplay with both the solvent and drug. Protein-based NPs (PNPs) exhibit the following advantages: (i) Natural biological origin: Natural proteins are metabolizable and biodegradable and amenable to surface modifications to allow the conjugation/connection of targeting ligands and therapeutic drugs; (ii) abundant species; (iii) biodegradability and biocompatibility; (iv) excellent biological functionality, for instance, enzyme catalysis and antibody immunity; (v) distinct three-dimensional configuration; (vi) high amphiphilicity that promotes interaction with hydrophobic and hydrophilic solvents or molecules; and (vii) innate carboxyl, hydroxyl, and amino groups for chemical conjugations. Furthermore, PNPs involve gentle synthesis processes that do not involve high temperatures or harmful organic solvents. Moreover, the synthesized NMs can dissolve in the physiological milieu without further surface alternations. Compared with other NPs, PNPs have been extensively applied in radiation, optics, immunity, catalysis, magnetism, ultrasound, and gene-related therapeutics modalities to address the limitations of conventional therapeutic protocols. Compared with other NM techniques, PNPs have emerged as promising protein-based DDSs to treat cancer. Fig. 1 shows biomedical application of different PNPs.

## TYPES OF PROTEINS USED IN NMs

Several proteins have been used to design NMs for treating cancer, such as water-soluble proteins such as albumin and transferrin, insoluble proteins (gliadin and zein), soy proteins (glycine), milk protein (β-lactoglobulin), collagen protein (gelatin), metal-protein complex (ferritin), lipoproteins (Lipoprotein), and others (hemoglobin (Hab), fibrin, and fibrinogen (Fib)).

### 1. Albumin

Albumin (Ab) is a widely used serum protein that can be acquired from different sources such as bovine serum (bovine serum Ab, BSA), egg white (ovalbumin, OVA), human serum (human serum Ab, HSA), rat serum (rat serum Ab, RSA). Albumin with nanocarriers acts as a highly concentrated protein in the circulatory system and promotes the retention of osmotic pressure to facilitate the transportation and binding of nutrients to cancer cells.[15,16] Several endogenous molecules and therapeutic drugs have been explored for binding Ab to be used as a storage center and transporter of serum protein. These serum proteins are highly water soluble and can be freely diluted with salt solvents. Given the maximum solubility of Ab until 40% W/V at a pH of 7.4, it is an ideal macromolecular DDS that can carry a variety of drugs. Ab is highly stable over a pH

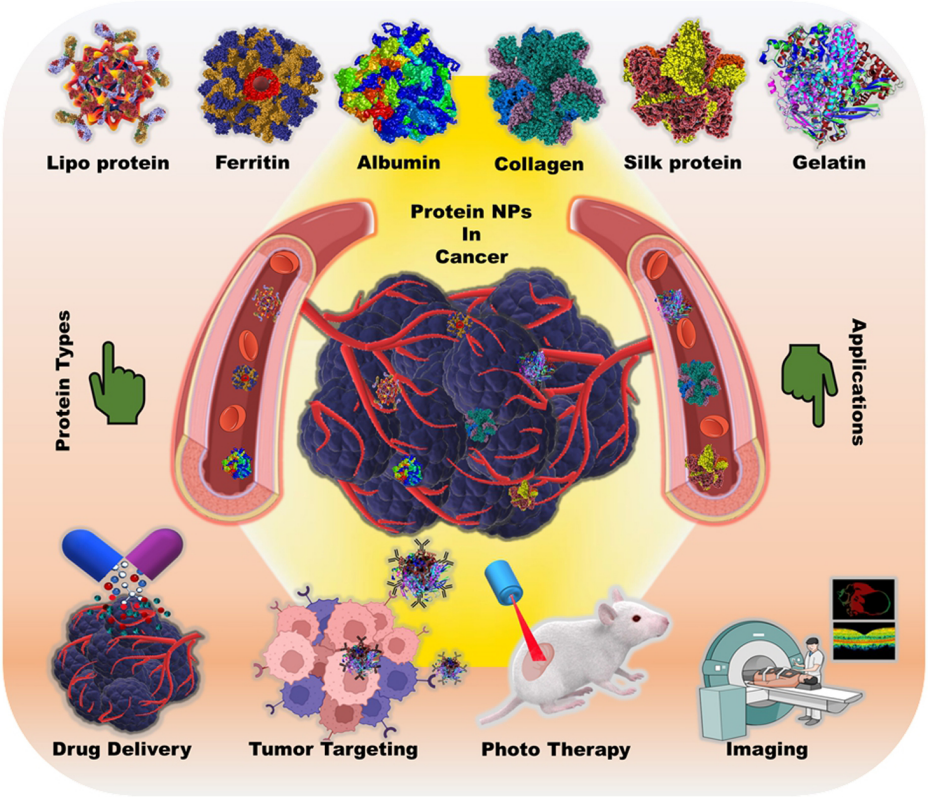


**FIG. 1.** Schematic of different types of protein nanoparticles and their applications.

range of 4-9 and can be heated at 600℃ for 10-12 h without deteriorating its original properties.[17] Among different types of Ab, BSA and HAS have attracted the highest approval for cancer therapeutics and diagnostics owing to their low toxicity, abundance, appropriate drug delivery properties, easy purification, and low cost. Owing to these properties and their excellent biodegradability and immunogenicity, Ab is a model DDS for cancer treatments.

**2. Ferritin**

Ferritin is an iron-binding spherical protein with a molecular weight (MW) of 474 kDa, which consists of 24 subunits with internal and external diameters of 8 and 12 nm, respectively. The hollow core interior of ferritin can accumulate 4500 iron atoms ($Fe^{3+}$). Ferritin proteins protect cells from oxidative damage induced by iron-based Fenton-like reactions. In addition, ferritin is present in both intracellular and extracellular environments including the mitochondria, nucleus, and cytosol. The ferritin nanocage consists of an H-chain (21 kDa, 10-20%) and L-chain (19 kDa, 80-90%), and the remaining 55% of the sequence is the same as that of the H-chain.[18] The H-chain consists of a dinuclear ferroxidases site that contributes to the $Fe^{2+}$ to $Fe^{3+}$ oxidation reaction in ferritin. In contrast, the L-chain consists of additional glutamate residue on the inner protein shell, which boosts the mineralization and oxidation reaction of $Fe^{2+}$ to $Fe^{3+}$ at the H-chain. Two of the L- and H-chains can self-assemble into a cage termed a ferritin nanocage. Owing to their biochemical and structural traits, ferritins have been extensively used in bio-nanotechnology applications. The external layer of ferritin can be functionalized by targeting ligands via chemical modification, and the hollow internal layer can be packed with various metals, small molecules, imaging agents, and therapeutic drugs. Ferritin is highly thermostable and can resist temperatures of up to 800℃ for 10 min.[16] Additionally, ferritin NPs can be reversibly disassembled in extremely acidic (pH 2-3) or basic (pH 9-12) environments. The H-chains of the ferritin nanocage can target Tf receptors (TfRs) that are overexpressed in the cell membrane of many solid tumors.

**3. Lipoproteins**

Lipoproteins are special natural particles composed of complexes of lipids and proteins that transport fats inside the body. Lipoprotein NPs consist of an inner hydrophobic core involving cholesterol ester and triglyceride molecules that are enclosed by a water-compatible phospholipid layer that is embedded with apolipoproteins and free cholesterol. The apoprotein shell enhances the water solubility of Lipoprotein NPs and enables active targeting toward overexpressed apoprotein receptors of cancer cells. Lipoproteins vary in terms of the size, lipoprotein-lipid composition, and density in aqueous environments, for example, low-density Lipoprotein (LD Lipoprotein, 22-27 nm), high-density Lipoprotein (HD Lipoprotein, 7-13 nm), intermediate-density Lipoprotein (ID Lipoprotein, 27-30 nm), and very low-density Lipoprotein (VLD Lipoprotein, 35-80 nm).[19] Among these varieties, LD Lipoprotein and HD Lipoprotein have been used for the delivery of anticancer cargo. Both these Lipoproteins exhibit natural targeting properties: HD Lipoprotein exhibits two surface apolipoproteins and apoproteins, AI (Apo A-I), which can directly bind to scavenger receptor class B type-1 (SR-B1), a multifunctional HD Lipoprotein receptor found on a variety of cell surfaces. Similarly, LD Lipoprotein has apolipoprotein B100 (ApoB-100) on its external surface, which acts as the binding domain for LD Lipoprotein receptors. Lipoprotein NPs exhibit high biocompatibility, biodegradability, drug entrapping, and immune escape properties. Moreover, they are characterized by enhanced targeted tumor accumulation and penetration.[20]

**4. Collagen**

Collagens are a large family of proteins that are distributed in different tissues inside the body. Specifically, 30% of collagens consist of several sets of proteins with simple, repetitive amino acid sequences that determine the specifying signature for the proteins. The intrinsic properties of amino acid sequences drive the spontaneous self-assembly of three identical polypeptide chains into a unique triple-helical structure. Collagen is the main crucial component of the extracellular matrix (ECM).[21] Moreover, collagen is the most abundant protein in animals with an MW of 300 kDa. Collagen-based PNPs can reduce drug systemic toxicity and enhance the NPs internalization by cells. Moreover, they are thermally stable.

**5. Gelatin**

Gelatin is a proteinaceous material that can be applied to produce PNPs. Reversible hydrolysis of collagen yields gelatin.[22] As a medicinal drug, gelatin has been recognized as a biodegradable material since the initial periods of drug production.[23] Gelatin can be divided into two types (A and B) based on the process of collagen hydrolysis.[24] Type A gelatin is cationic with an isoelectric point (IEP) of 7-9, whereas type B gelatin is anionic with an IEP of 4.8-5. Gelatin consists of both anionic and cationic groups, hydrophobic groups, and a triple helix structure with repeating sequences of proline, glycine, and alanine.[25] The key characteristics of gelatin depend on the high contents of these repeating sequence molecules, for example, (i) gelatin is nonpyrogenic and can be sterilized, (ii) it is inexpensive, and (iii) it exhibits low antigenicity. Gelatin proteins have different ionizable functional groups such as phenol, imidazole, guanidine, amine, and carboxylic group (COOH), which promote chemical modifications/conjugation and surface functionalization. The introduction of crosslinking agents such as glutaraldehyde into the gelatin protein can enhance the size, shape, stability, and in vivo circulation time compared with those of unmodified gelatin.[26] The drug release behaviors depend on the degree of crosslinking. Overall, gelatin PNPs are highly efficient protein-based DDSs.

**6. Other proteins**

Proteins such as fibrin/fibrinogen, globulins, myoglobin, Tf, legumin, glycine, and zein have also been exploited as protein-based DDSs. Fibrin is a natural serum protein resulting from fibrinogen within blood coagulation.[27] Fibrin is biodegradable and biocompatible and results in a promising DDS for sustained therapeutic drug release kinetics over a prolonged period. Viale et al.[28] prepared doxorubicin (DOX)-loaded fibrin gel to realize antitumor activity in human neuroblastoma. Intravenously injected DOX-loaded fibrin gel led to 90% tumor inhibition compared with that in the control groups.

Fib is a soluble and third-most abundant serum protein (340 kDa) that contributes to the protein corona formation of several types of NPs.[29] Fib acts like a precursor to fibrin that is produced by the liver at a rate of 1.7-5 g/d. Fibrin-associated protein (FREP) such as Fib-like protein-1 (FGL-1) and Fib-like protein-2 (FGL-2) can help treat cancer by regulating the invasion, proliferation, and migration of tumor cells.[30] Moreover, these proteins can regulate the actions of immune cells in TME.[30] Fib can target inflammation sites ensuring the disruption of physical or chemical tissue, tumor, microbe, and atherosclerotic plaque. Owing to these advantages and its prolonged circulation in blood (half-life of 3-5 d), Fib is an attractive DDS for treating and imaging solid tumors. Zhang et al.[31] prepared fibrin hydrogel for the co-delivery of two therapeutic loads: cyclophosphamide (CTX) and a PDL1 antibody. CTX promoted the infiltration of T-effector cells, and the combined checkpoint blockade inhibition by PDL1 helped reverse the tumor immunosuppressive microenvironment in both breast and ovarian cancer models. Rejinold et al.[32] used Fib as a thermo-responsive nanogel grafted with poly (N-vinyl caprolactone) for the delivery of 5-fluorouracil (5-FU) combined with megestrol acetate. The prepared Fib nanogel helped in controlled drug release with enhanced cell toxicity against human breast cancer (MCF-7) cells.[32] In addition, the advantages and disadvantages of different proteins are discussed

**TABLE 1.** Advantages and disadvantages of different protein nanoparticles

| Material | Advantages | Disadvantages |
|---|---|---|
| Human serum albumin | ▸ High stability<br>▸ High solubility in physiological fluids<br>▸ Biodegradability<br>▸ Non-immunogenicity<br>▸ Non-toxicity<br>▸ Availability and readiness | ▸ High cost |
| Gliadin | ▸ Biocompatibility<br>▸ Biodegradability<br>▸ Non-immunogenicity<br>▸ Non-toxicity<br>▸ High stability | ▸ Large particle size<br>▸ Rapid degradation |
| Gelatin | ▸ Biocompatibility<br>▸ Biodegradability<br>▸ Easy bridging<br>▸ Safety | ▸ Low mechanical strength<br>▸ Rapid degradation |
| Silk protein fibroin | ▸ High stability<br>▸ Flexibility with high mechanical strength<br>▸ Suitable for various machining conditions<br>▸ Low immunogenicity<br>▸ Biodegradability<br>▸ Biocompatibility | ▸ Sericin may cause immunogenicreactions<br>▸ Slow degradation of silk II crystalline antiparallel β-sheet domain |
| Legumin | ▸ Bio-adhesive nature<br>▸ Large surface area<br>▸ Small particle size<br>▸ Low immunogenicity<br>▸ High stability | ▸ Low yield |
| Lipoprotein | ▸ Non-immunogenicity<br>▸ Biodegradability<br>▸ Biocompatibility<br>▸ Long circulation half-life<br>▸ Naturally targeting property | ▸ Difficult to separate native LDL |
| Ferritin | ▸ High stability<br>▸ pH stability<br>▸ Thermal stability<br>▸ Biodegradability | ▸ High cost |

in Table 1.

Hab has a diameter and an MW of 5.5 nm and 64.5 kDa, respectively, and two pairs of subunits (α and β). Each subunit is enclosed with a heme group in its hydrophobic area. Hab characteristics depend on two states: a) the tense (T) or oxygenated state, with low affinity toward $O_2$, b) the relaxed (R) state or deoxygenated state, with high affinity toward $O_2$, corresponding to the physiological characteristics of oxygen transport and binding. Hab is frequently used as an oxygen supplier for supporting reactive oxygen species (ROS) in TME. However, its high renal toxicity and short blood circulation limit its biological applications. Notably, Hab can be decorated and modified into several biocompatible NPs to improve its drug delivery applications. For example, Hab NPs modified with polyethylene glycol (PEG-Hab) through a covalent conjugation reaction enhanced the long circulation, biocompatibility, and delivery of paclitaxel (PTX) drug for cancer chemotherapy.[33]

## APPLICATION IN CANCER NMs

### 1. Delivery of therapeutic drugs

PNPs have been used as DDSs to supply several types of therapeutic cargoes such as vaccines, growth factors, nucleic acids, and chemotherapeutic drugs, to eliminate the adverse effects of releasing therapeutic cargoes and boost their effectiveness inside the tumor microenvironment (TME). The therapeutic is loaded onto the PNPs either through diffusion or by modification of the protein surface to achieve covalent or non-covalent binding. The loading efficiencies depend on the NP size, drug solubility, and media material (pH). Huang et al.[34] investigated the loading ability of curcumin using glycosylated modified BSA protein NPs. The BSA protein corresponding to a loading content of 55.47%±0.45% increased the bioavailability of curcumin.[34] Moreover, cationic BSA (CBSA) has been studied as an effective delivery system of siRNA toward lung metastatic cancer.[35] The optimal cationic modification of BSA enabled siRNA binding and targeted delivery of siRNA into the lungs. Moreover, CBSA promoted the formation of stable nanosized particles and prevented the degradation of siRNA.[35] Similarly, Wang et al.[36] prepared a calcium ($Ca^{2+}$)-siRNA nanocomplex with BSA to improve its biocompatibility and toxicity of ligament stem cells. The hollow structures of PNPs can be used to carry hydrophilic and hydrophobic drugs. The cytotoxic chemo drug Auoxo3, a gold-based compound can deliver therapeutics toward tumorigenic cells through the ferritin nanocage.[37] According to the cell toxicity results, Auoxo3 drug with the ferritin nanocage was more effective than the control.[37] Wang et al.[38] exploited the surface charge of silk proteins and mixed silica NPs with the nanocomplex for drug delivery. Sericin and silk fibroin (SF) have a negative zeta potential with high drug entrap efficiency. NPs encapsulated with DOX retain 33% of their efficiency after mixing for 24 h.[39] The porous nature of the sericin-hydroxyapatite complex results in sustained release at the desired site.[39]

Drug release from PNPs can be realized through protein degradation or erosion, migration of drugs via pores, and release from polymer surfaces through internal stimuli (ROS, glutathione, or pH) and external stimuli such as photo, magnetic, or sonic field application. Similarly, silk sericin (SS) conjugated to polylactide (PLA) forms an amphiphilic polymer-protein conjugation via bis-aryl hydrazone linkage, as shown in Fig. 2. Self-assembled DOX-loaded SS-PLA PNPs exhibit 70% DOX re..lease under pH 5.0.[40] Amphiphilic PNPs are potential DDSs for liver carcinoma.[40]

Moreover, PNPs can be used to deliver multiple drugs to the cancer site. Duclairoir et al.[41] quantified the release of numerous polar drugs from gliadin NPs. The outcomes suggested that hydrophobic drugs are released slowly compared with hydrophilic drugs present in the gliadin NPs. Nevertheless, the developed synthetic protein coated with transcytotic peptide iRGD enhanced the delivery of AMD3100, (CXCR4 antagonist) to block the CXCL2/CXCR4 pathway in glioblastoma through systemic delivery.[42] The synthetic protein enabled controlled release and improved AMD3100 bioavailability for clinical applications.[42]

### 2. Tumor targeting

Most of the existing tumor-targeting chemo drugs realized passive targeting based on the EPR effect.[43] NPs and nano drugs actively target numerous tumors through high-

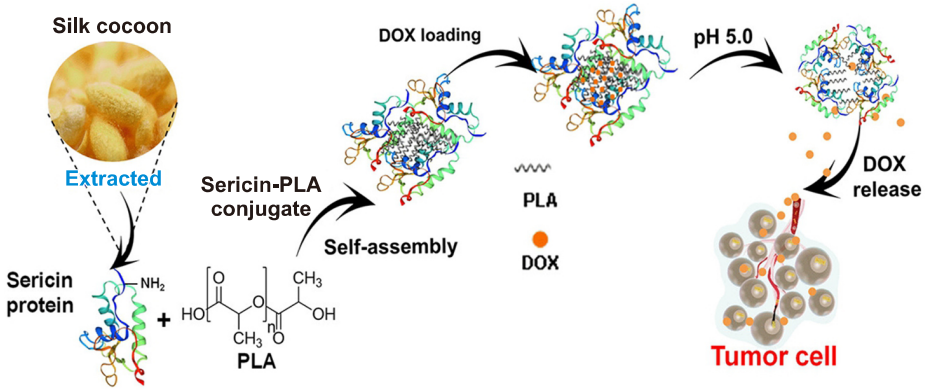


**FIG. 2.** Silk sericin, a hydrophilic protein conjugated with hydrophobic polylactide (PLA) to deliver DOX to liver carcinoma. DOX is released from the NPs under intracellular tumor pH as pH-stimuli responsive PNPs. Reproduced with permission from 40. Copyright ©2020, American Chemical Society.

ly expressed cell surface receptors present in tumor cells or TMEs including fibroblasts and blood vessels.[4] Nutrient transporters have been prepared for biomimetic delivery and glioma targeting. Recently, Lin et al.[44] proved that gp60 and SPARC receptors are overexpressed in tumor endothelium and glioma cell surfaces. These receptors can attach to Ab via SPARC- and gp60-mediated biomimetic transport mechanisms. Lin et al.[44] prepared low-MW protamine-modified albumin NPs that were encapsulated with fenretinide and paclitaxel, resulting in tumor targeting with improved drug accumulation.

Similar to Ab, ferritin NPs are commonly used PNPs that can simultaneously function as both drug carriers and targeting molecules. Ferritin can bind overexpressed TfRs that can internalize NPs via receptor-mediated endocytosis. Wang et al.[45] fused Ab and ferritin protein complexes that are able to incorporate both β-lapachone and manganese ($Mn^{2+}$) through a spatiotemporal coordination mechanism.

$Mn^{2+}$ secured by mannose-decorated Ab and β-lapachone captured by ferritin can be crosslinked to form a hybrid nano assembly that dissociates protein units in TME. Functionalized with mannose (Man), the moieties act as an active targeting ligand to target immune dendritic cells (DC), with ferritin binding to TfRs on the tumor cell surface. After cellular internalization, the release of β-lapachone drives immunogenic cell death and releases plenteous dsDNA into the TME, whereas $Mn^{2+}$ elevates the sensitivity of the cGAS-dsDNA signaling pathway to trigger downregulation of immunostimulatory signals, as shown in Fig. 3.[45] The smart cGAS-dsDNA nano antagonist improves T-cell immunity against solid tumors in vivo and thus represents an intelligent technique for cancer immunotherapy.

Li et al.[46] constructed a ZnF16Pc encapsulated ferritin nanocage. ZnF16Pc was used as a near-infrared photosensitizer that generates toxic gas upon laser irradiation. The authors coupled fibroblast activation protein (FAP), known as single-chain variable fragment (scFv), to the outer surface of NPs. Given that the FAP was found to be highly expressed in over 90% of cancer-associated fibroblasts, the PNPs could target the murine 4T1 breast cancer tumor model.[46] In addition to direct protein targeting, PNPs can effectively target tumors by implementing targeting peptides on the tumor cell surface. For example, integrin αvβ3 and αvβ are overexpressed in many cancers cell surfaces. Bari et al.[47] developed SF NPs and decorated them with cyclopentapeptides (cRGDs) on the protein surface, which exhibit a strong affinity toward integrin αvβ3 and αvβ5. The use of cRGD peptide improved the particle uptake into human urinary bladder cancer cells (ECV). Similarly,

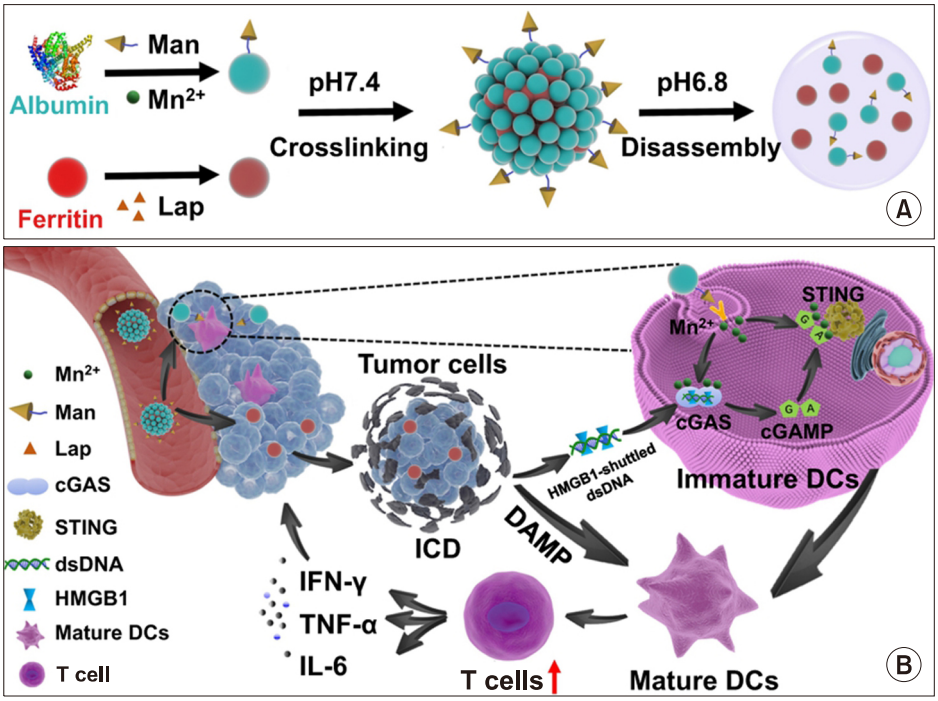


**FIG. 3.** (A) BSA/ferritin-based protein assembly encapsulating mannose-modified Mn and β-lapachone that dissociate under tumor pH 6.8. (B) Systemically administered BSA-Man@$Mn^{2+}$-Ft@Lap nano assembly rapidly disintegrates in the perivascular region of solid tumors into highly diffusive BSA-Man@$Mn^{2+}$ and Ft@Lap components, enabling enhanced penetration into the tumor interior. Ft@Lap molecules bind to the TfRs on tumor cells for targeted Lap delivery to elicit efficient immunogenic apoptosis, leading to the release of abundant DAMPs such as HMGB1 and dsDNA. Additionally, BSA-Man@$Mn^{2+}$ molecules can be selectively internalized by immature DCs to enhance the dsDNA sensitivity of the cGAS-STING axis. These activities cooperate to promote DC maturation and cross-priming, thereby enhancing the infiltration and effector functions of tumor-specific T cells for efficient immunotherapy. Reproduced with permission from 45. Copyright ©2022, Nature Communications.

Mullapudi et al.[48] targeted bladder cancer through a cluster of differentiation (CD47)-targeted Ab nanoparticles. In general, CD47 receptors are highly expressed on the surfaces of bladder cancer cells. The authors used the amino acid sequence CkRFYVVMWKk (k=D analog of lysine, denoted as txCD47) in the targeting peptide to target the CD47 receptor and doing so could release gemcitabine into a tumor cell in a controlled manner.[49]

## 3. Phototherapy

Phototherapy is a reliable, secure, and dose-controlled light-mediated therapy for destroying cancer cells. Photochemical interactions in the form of photodynamic therapy (PDT) and photothermal interactions represent the key mechanisms for the direct eradication of cancer.[5,50] Photothermal therapy (PTT) uses photothermal agents or metal NPs to alter the absorbed light energy into heat energy to increase the temperature of a tumor when irradiated with near-infrared (NIR) external light. Wang et al.[51] used *Bombyx More* derived SF protein to prepare self-assembled nanofibers by using positively charged Au NPs to achieve enhanced PTT. The SF/Au PNPs exhibited a red-shifted absorption band in the NIR range and converted light energy to heat under 808 nm laser irradiation. In contrast, He et al.[52] used IR1061, a second near-infrared dye to inhibit tumor growth by applying a NIR-II laser. The author used apoferritin (AFn) protein to capture PTX and IR1061 through protein chemical reactions. The pH sensitivity advantage of AFn promoted the delivery of therapeutic cargoes as compared with the control particles, resulting in effective chemo-phototherapy to treat breast cancer.[52]

PNPs play a synergistic role in natural protein performance enhancement and toxicity reduction against ovarian cancer. Shen et al.[53] fabricated RHMH18, a recombinant protein integrating histidine, HSA, enzyme restriction site, and arginine-glycine-aspartic acid (RGD) by genetic engineering. Ultrasmall Au NPs and docetaxel (DTX) were used as the thermal agent and chemotherapeutic drug, respectively. The RGD in the fused protein could effectively enter the tumor site via active integrin receptor targeting and enhanced the thermal effect in the extracellular matrix, as shown in Fig. 4. Laser-induced release of DTX was noted to be effective against MMP-2. This co-chemo/phototherapy approach was noted to be a promising cancer treatment strategy.[53]

Through its biomineralization and metal binding abilities, proteins can bind not only to Au but also many other metals such as copper (Cu), selenium (Se), iron (Fe), platinum (Pt), and Mn. Recently, BSA protein was used to bind Mn-doped Cu-Se (Mn:CuSe) NPs through one-pot synthesis.[54] Furthermore, folic acid (FA) and chlorine e6 (Ce6) were conjugated onto the BSA protein corona to create Mn:CuSe@BSA-FA-Ce6 multifunctional PNPs. Through folate receptor-mediated endocytosis, the NPs internalized inside the tumor. Upon laser treatment, Mn:CuSe enabled magnetic resonance imaging (MRI) and elevated heat generation in the TME, as shown in Fig. 5. Photosensitizer Ce6 resulted in improved photodynamic application upon 670 nm laser irradiation. Overall, Mn:CuSe@BSA-FA-Ce6 PNPs is a promising DDS for image-guided synergistic PTT/PDT-activated therapy against cervical cancer.[54]

## 4. Bioimaging

Timely identification and diagnosis are important clinical procedures, especially for cancer treatment. Biomedical imaging technologies, such as computed tomography (CT), X-ray radiography, MRI, fluorescence imaging, positron emission tomography (PET) imaging, and ultrasound imaging, are essential for the early identification and therapeutic progress of several cancers. To obtain more accurate functional and anatomical information, imaging contrast agents are used to differentiate normal and abnormal

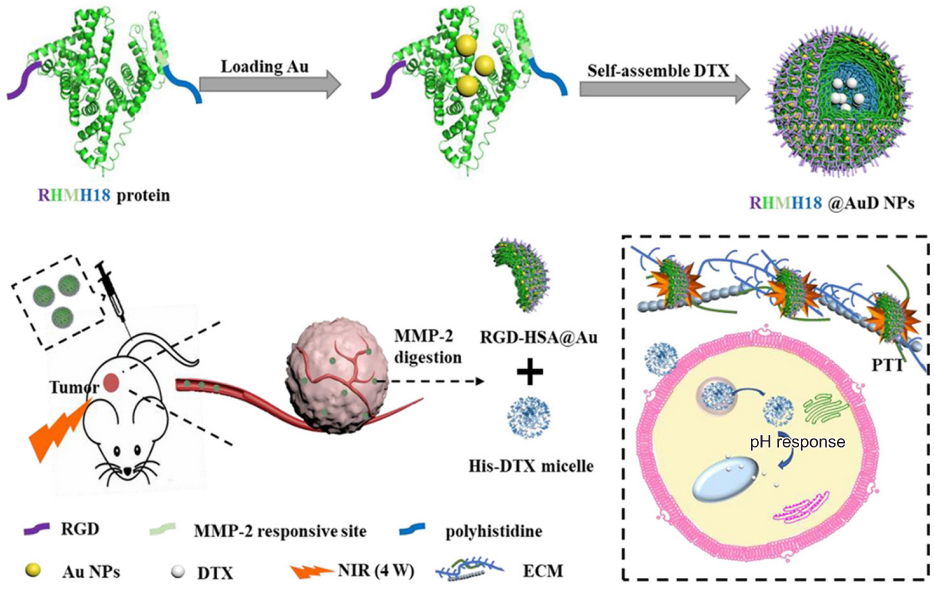


**FIG. 4.** Fabrication of HSA fused protein NPs with loaded gold (Au) and docetaxel (DTX) (RHMH18@AuD) NPs and their synergistic chemo-photothermal application in the treatment of human ovarian cancer. Reproduced with permission from 53. Copyright ©2022, American Chemical Society.

tissues. An et al.[55] created a dye, ultrasmall Cy5-red fluorescent protein (smURFP), and mixed it with BSA to formulate a protein composite. This nanocomposite could be intravenously provided to mice to target tumors via the EPR effect. The fluorescence protein enabled fluorescence imaging with deep tissue penetration. Indocyanine green (ICG) can be used as a photo contrast imaging agent for imaging tumors.[56] PNPs enable targeted imaging via targeted ligand decoration on the nanoparticle surface.[57] H-ferritin was used as a carrier to link luciferin to construct Luclinker@HFn NPs via a glutathione-responsive linker. The protein complex could release luciferin into the cytoplasm and enable bioluminescence imaging (BLI) through the luciferase-luciferin binding system. The in vitro and in vivo BLI indicated that cancer cells can readily uptake PNPs, thereby decreasing the background noise.[57] Therefore, protein nanocomposites are potent carriers for contrast agents in luciferase tumor models.

Metallic NPs are well-known imaging agents in cancer research. Copper sulfide (CuS) NPs loaded with ferritin nanocage were used as an imaging carrier system. Because of the excellent NIR absorbance of the CuS-Fn complex, it was implemented in in vivo photoacoustic imaging (PA).[58]

In addition, Cu ions can serve as a PET imaging agent, as shown in Fig. 6. Thus, the multifunctional CuS-Fn complex can enable highly sensitive, non-invasive, and quantitative dual-modal in vivo imaging for cancer therapeutics and diagnosis.[58]

PNPs are typically used in MRI imaging through magnetic NPs or MRI imaging agents. Tao et al.[59] constructed iron oxide NPs with BSA and (poly (acrylic acid)- poly (methacrylic acid), PMAA-PTTM) macromolecules to enable MRI. After NP administration via a vein, $T_1$-weighted MRI imaging resulted in a dark kidney and liver, indicating $Fe_3O_4$-BSA was a more suitable $T_2$-weighted contrast agent.[59] However, $Fe_3O_4$-PMAA-PTTM revealed the opposite results, which indicated that the targeting ligand considerably influenced the biomedical imaging results.

Zhang et al.[60] used AFn to create a unique DDS for diagnosis and treatment. DOX was loaded inside the ferritin hollow structure and bismuth sulfide ($Bi_2S_3$), a radiation sensitizer, was inserted into the protein shell. $Bi_2S_3$ enabled radiation sensitization because of the high attenuation coefficient (X-ray) of $Bi^{3+}$ ions. CT imaging suggested a high accumulation of Dox@AFBS NPs at the target site.[60] Overall, combined chemo/radiotherapy can successfully decrease the size of a cervical tumor. PNPs nanoparticles not only successfully reduced cervical tumors but also actively help in various tumor inhibition that is provided in Table 2.[61-73]

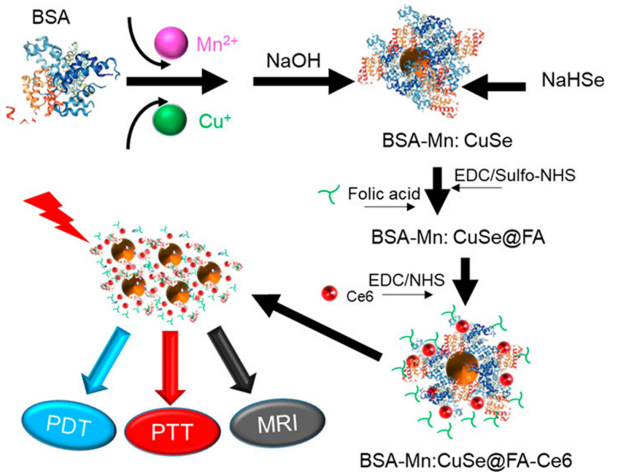


**FIG. 5.** Multifunctional PNPs. BSA is used to bind Mn-doped Cu–Se (Mn: CuSe) NPs. Moreover, folic acid (FA) and chlorine e6 (Ce6) are conjugated onto the BSA protein corona to create Mn:CuSe@BSA-FA-Ce6 NPs. Through folate receptor-mediated endocytosis, the NPs internalized inside the tumor. Upon laser treatment, Mn:CuSe@BSA-FA-Ce6 NPs enabled magnetic resonance imaging (MRI) in addition to elevated heat generation and photodynamic application in the TME. Reproduced with permission from 54. Copyright ©2019, American Chemical Society.

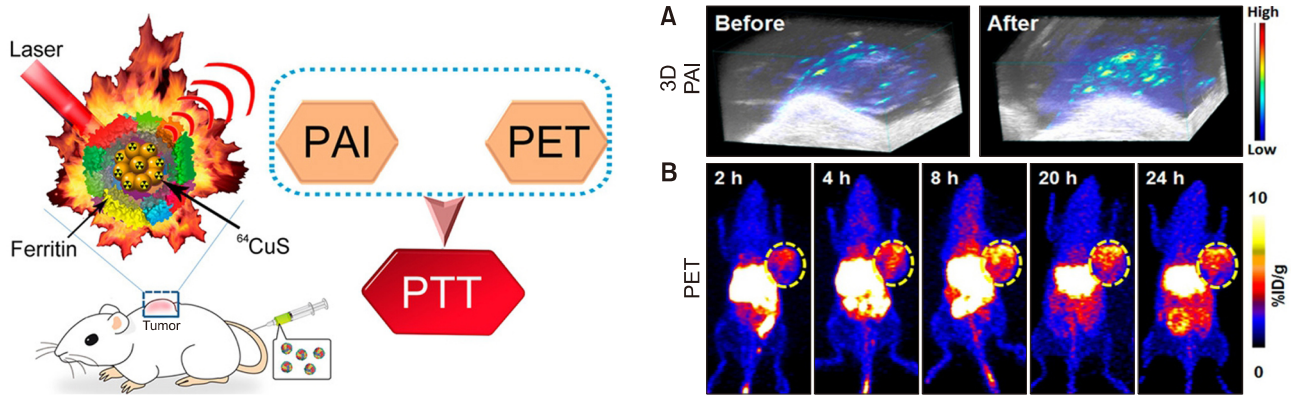


**FIG. 6.** Copper sulfide loaded ferritin nanocage (CuS–Fn nanocomposites) for dual-modal image-guided PTT therapy. (A) Photoacoustic images of CuS–Fn NPs in U87 MG tumor after post-injection. (B) Time-dependent positron emission tomography (PET) images of CuS–Fn NPs. Reproduced with permission from 58. Copyright ©2016, American Chemical Society.

**TABLE 2.** Various PNPs and its role in different cancer treatments

| Protein Types | Therapeutic load/ Surface modification | Cancer Model | Role | Reference |
|---|---|---|---|---|
| Ferritin | Lapatinib/ Pseudolaric acid B | Triple negative breast cancer | EGFR inhibition, Ferroptosis Induction | 61 |
| | Chlorin e6, ferritin-homing peptide HKN15 modification | Breast cancer | Targeting of HKN15 peptide, PDT amplify ROS production, Ferroptosis inhibition | 62 |
| | Epirubicin, mucin 1 (MUC1) aptamer modification | Colon cancer | Site specific drug delivery, MRI imaging, high therapeutic effect, and diagnosis | |
| | ZnF16Pc (photosensitizer), Fibroblast-activation protein (FAP) modification | Breast cancer, Lung cancer | Anti-CAFs immunity, Site specific PDT treatment | 63 |
| Albumin | Paclitaxel, Indocyanine green, hyaluronidase | Pancreatic cancer | Hyperthermia induction under laser, Breakdown extracellular matrix, chemotherapeutic effect | 64 |
| | Indocyanine green, Human epidermal growth factor receptor 2 (HER2) affibody modification | Metastatic breast cancer, non-small-cell lung cancer | Excellent photothermal effect, HER2 specific targeting ability, developed therapeutic value | 65 |
| | Paclitaxel, programmed cell death-ligand 1 (PD-L1) modification | EMT-6 xenografted breast cancer | PDL1 targeting improves tumor sensitivity, synergistic chemo-immunotherapy, immunomodulation | 66 |
| Silk Protein (Sericin, fibroin) | Iron oxide NPs, ROR1 siRNA, silk MS1Fe1 functionalization | Triple negative breast cancer | Active Her2(+) cancer targeting, hyperthermia/ gene therapy | 67 |
| | Anticancer agent ASC-J9, Magnetic NPs, G3 peptide modification | Colorectal cancer | Hela/colorectal cancer specific targeting, magnetic field induction, high ASC-J9 drug delivery | 68 |
| Lipoprotein | Pyrimidine heterocyclic anticancer agent | Breast cancer, Prostrate cancer | Enhanced cytotoxic activity, LDL receptor internalization ability, cancer tubulin inhibition | 69 |
| | Radio-sensitizing miR-34a RNA, ApoA-1 mimetic peptide | Head and neck cancer | Metabolic activity reduction, improved apoptotic activity, DNA damage | 70 |
| Gelatin | Melanin, $Mn^{2+}$, PEG 5000 modification | Breast cancer | Improved renal clearance, PA/MRI dual imaging ability | 71 |
| Collagen | Glutaraldehyde & PEG co-modified fluorinated chitosan, Pirabucin, Interleukin-12 agent | Bladder cancer | A long-term immune memory cell effect, higher intravesical dose, Intravesical instillation therapeutic response | 72 |
| | EGFR siRNA, Au NPs | Lung cancer | EGFR inhibition, 70% tumor reduction, ROS production, PA imaging | 73 |

## CLINICAL APPLICATIONS

Ab-based NPs have been used for treating cancer in clinical settings, according to government clinical websites. Abraxane (Celgene) has been commonly used in clinical trials to treat different types of cancer. Abraxane represents Ab-bound PTX NPs that can be used to treat pancreatic cancer, NSCLC, and breast cancer owing to the biocompatibility and targeted ability of Abraxane.[74] Ontak (Eisai) represents Denileukin diftitox (synthesized protein combining L-2 and diphtheria toxin) NPs that are used to treat T-cell lymphoma owing to their excellent lysosomal escape properties and T-cell-specific targeting ability.[75] In the case of cells that limit or are associated with caveolae, additional ligands must be used to expeditiously deliver Ab NPs loaded with chemotherapeutics into the desired sites of a cell. Additionally, maintenance of the drug activity is challenging because the protein cargo may be disabled throughout the NP preparation process. At present, only the above-mentioned two drugs have been used in clinical applications. Although many Ab-based NPs have been used in clinical trials, PNP development remains in a nascent stage. Future research must be aimed at exploring the safety, biodistribution, pharmacokinetics properties, and synthesis techniques of PNPs to establish a promising DDS for clinical applications.

## CONCLUDING REMARKS

A broad range of PNPs have been used in imaging/diagnostic and therapeutic applications. PNPs are highly stable NPs that can be easily produced, compared with other colloidal DDSs. In addition, they can be extensively applied in vivo as various proteins can be incorporated through an eco-friendly, cost-effective, and easy synthesis process that involves only a few chemicals

and is thus associated with high reproducibility and quality. Future attempts at designing PNPs for cancer NMs must focus on reducing immunogenicity, improving drug pharmacokinetics, and promoting lyso/endosomal escape. Instead of using traditional drugs such as PTX, DOX, and DTX, we recommend the use of more effective therapeutics in early clinical trials. To promote the shift from the ideation of PNPs to clinical application, they must be extensively explored in cancer research. With such advancements, PNPs are expected to usher in a new era in cancer treatment.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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