Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

Journal of

Materials Chemistry B

RSCPublishing

FEATURE ARTICLE

Biomolecules-conjugated nanomaterials for targeted cancer therapy

Cite this: DOI: 10.1039/x0xx00000x

Jian Wang, Ting Ting Wang, Peng Fei Gao and Cheng Zhi Huang*

Received ooth January 2014,
Accepted ooth January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

Biomolecules perform vital functions in biology. These functional biomolecules with diverse modifications hold great promise for further applications in bioanalysis and cancer therapy. However, these functional biomolecules face challenges, especially in the field of drug delivery for cancer therapy. For example, functional biomolecules are typically unstable when uptaken by cells, which are easy digested by enzymes. To address this obstacle, nanomaterials have been employed as drug carriers or cargos, which are powerful nanoplatforms for imaging and cancer treatment. Multifunctionality of these nanoplatforms offers great amount advantages over conventional reagents, including targeting to a diseased site to minimize systemic toxicity, the ability to solubilize hydrophobic or labile drugs to improved pharmacokinetics. In this review, we summarized typical functional biomolecules-conjugated nanomaterials for targeting drug delivery. Under proper conditions, the targeted drug delivery can be achieved from a high density of biomolecules that are bound to the surface of nanomaterials, resulting in a high affinity for targets. The high density of biomolecules then leads to a high local concentration, being able to prevent the degradation by enzyme. Furthermore, biomolecule-nanomaterial conjugates have been identified to enter cells more easily than free biomolecules, and controllable drug release then could be obtained by the stimuli-responsive mechanism, such as redox, pH, light, thermal, enzyme-trigged strategies. Now and in the future, with the development of artificial biomolecules as well as nanomaterials, the targeted drug delivery based on the elegant biomolecules-nanomaterials conjugation approaches are expected to achieve great versatility, additional functions, and more advances.

1 Introduction

Over the last few decades, more and more diseases have caused great threat on human health.[1, 2] Especially, cancer is considered as an as one of leading causes of mortality, touching every region and socioeconomic level, which accounts for one in every eight deaths worldwide. Just in 2012, there were an estimated 14.1 million cases of cancer diagnosed and 8.2 million deaths around the world. It is estimated that cancers will account for 1,665,540 new cases and 29,720 deaths in the United States during 2014.[3] Nowadays, to treat a wide variety of deadly cancers, e.g., leukemia,[4] lung cancer,[5] breast cancer,[6] colon cancer[7] and prostate cancer,[8] clinicians have made increasing use of anticancer drugs. However, most anticancer drugs in clinical use are limited due to their general toxicity to proliferating cells, including some normal cells,[9] resulting in the unwanted side effects. Inefficient delivery and poor penetration of these therapeutic drugs into tumors hamper the therapeutic efficacy.[10] Furthermore, the substantial risk of systemic toxicity of most small-molecule drugs originating from poor pharmacokinetic profiles and solubility can not be ignored. Then, targeted and effective drug delivery systems that could alter the pharmacokinetic profile of drugs, lower off-target toxicity and enhance the therapeutic index, would be highly desirable.[11]

The common drug delivery materials including liposomes,[12] micelles,[13] organic polymer,[14] dendrimers,[15] cyclodextrins,[16] have been developed. On one hand, these materials have addressed some problems that caused by small-molecule drugs in some degree. On the other hand, the accumulation of these materials within malignant lesions is generally attributed to passive targeting as a consequence of enhanced permeability and retention (EPR).[17] It was found that EPR can lead to more than 50-fold accumulation within tumors as compared to the healthy tissues in animals.[18] However, the passive process accumulation in the tumor sites, which are not specifically designed toward a biological receptor,

requires a long circulation half-life to obtain sufficient drug delivery,[19] greatly depending on the size and surface charge.[20] Thus, passive targeting is sometimes considered as the "non-targeting",[21] due to that the drug-materials complexes distribute through the leaky vasculature, which is determined by the biological environment, rather than the active recognition by the tumors.

Active recognition refers to the specific interactions between drug carrier and the target cells, which is usually achieved by specific ligand-receptor recognition, including aptamer-target,[22] and antibody-antigen,[23] folic acid-folate receptor,[24] *etc*. The ligand-receptor interactions between biomolecules occurs when they are in close proximity (<0.5 nm),[21] which supplies the opportunity to bind target sites. In addition, it has been believed that the active targeting would prolong the blood circulation time compared with the passive one to enhance the therapeutic efficacy. However, it is questionable whether the tumor-targeting ligand will enhance efficacy for cancer therapy, because some biomolecules are not stable during storage[25] or in serum nuclease-catalyzed degradation,[26, 27] leading to the low bioavailability, which is the major challenge for these biomolecules such as DNA or RNA. Other limitations are the poor penetration, immunogenicity,[28] and the charge-dependent non-specific attachment. [29]

To address these issues, Minkin group did a lot of helpful work and found that DNA-gold nanoparticle (AuNPs) conjugates could efficiently enhance binding affinity, and improve the ability to enter cells and resist enzymatic degradation. Furthermore, they quantified the enhanced stability of DNA-AuNPs conjugates with respect to enzyme-catalyzed hydrolysis of DNA, which presented the evidence that the negatively charged surfaces of AuNPs and resultant high local salt concentrations were responsible for enhanced stability.[26] Following, they investigated the stability of siRNA on the surface of AuNPs. The result indicated that the rapid endonuclease hydrolysis at specific sites near the AuNP-facing terminus of siRNA were different from those free siRNA in solution, suggesting that the chemical environment of siRNA on a nanoparticle surface can alter the recognition of siRNA by serum nucleases and change the inherent stability of the nucleic acid.[27]

Importantly, Liang group determined the size-dependent penetration ability of AuNPs and the potential application of ultrasmall AuNPs for intranucleus delivery and therapy.[30] It was found that AuNPs smaller than 10 nm (2 and 6 nm) could enter the nucleus, whereas larger ones (10 and 16 nm) were just in the cytoplasm. They also investigated the possibility of using 2 nm AuNPs as carriers for nuclear delivery of a triplex-forming oligonucleotide (TFO) that bound to the c-myc promoter. Compared to free TFO, AuNPs-conjugated TFO was more effective for delivering c-myc RNA and c-myc protein, resulting in the decrease of cell viability, which demonstrated that the entry of AuNPs into the cell nucleus was critically size-dependent. Then, guidelines were provided to choose appropriate nanocarriers for different biomedical purposes.

Recently, to satisfy these requirements for drug delivery, such as good biocompatibility, easy to modify, high loading efficiency, no prerelease, and facile to monitor the release process, *etc*,[31] more and more biomolecules-nanomaterials conjugates have developed for cancer therapy.[22, 32-34] It is commonly believed that biomolecules-nanomaterials conjugates facilitate the active targeting after extravagating out of the vasculatures and then migrating into the malignant cells. Namely, active targeting could facilitate the "in situ" uptake of random distributed biomolecules-nanomaterials conjugates in the blood vessels. Farokhzad and coworkers divided the active targeting in a more specific way, including vascular targeting and tumor targeting.[35] In the first case, the biomolecules bound to endothelial cell-surface receptors, which could facilitate the accumulation of conjugates in the vascular tissues to eradicate the tumor blood vessels to block nutrient and oxygen to the tumors, leading to the collapse of tumors. While in the latter one, the biomolecules usually bound to the specific receptors, increasing the accumulation and cell uptake of conjugates, then leading to the enhancement of therapeutic efficacy after triggers, such as redox-activation,[36] pH-response,[37] enzyme,[38] heating,[39] light,[40] ultrasound,[41] electric field,[42] or magnetic field.[43]

The above fundamental researches demonstrate that nanomaterials have improved the properties of biomolecules, and the conjugates also have helped us to control the physico-chemical, toxicological and pharmacological properties of nanomaterials, as well as supply the possibility for highly efficiently targeted drug delivery. Nanocarriers functionalized with biomolecules or targeting ligands are promising platforms for cancer therapy, due to their ability to circulate in the bloodstream for longer period and their selectivity for tumor cells, preventing healthy tissues from damage.[44] In addition, those functional nanomaterials are able to perform multiple functions simultaneously. In details, some multifunctional nanomaterials that can absorb light with high efficiency are especially useful for therapeutical applications, which not only can reveal the molecule-specific signatures of cancer with high sensitivity and high spatial resolution when used as imaging reagents with the assistance of photoacoustic instruments, but also mediate the selective killing of cancer cells *via* photothermal ablation and heating-triggered drug release. For example, gold nanorods (AuNRs) could serve as drug carrier, fluorescence quencher and photothermal reagent for cancer treatment,[45] as well as the imaging reagent under dark-filed microscope.[46]

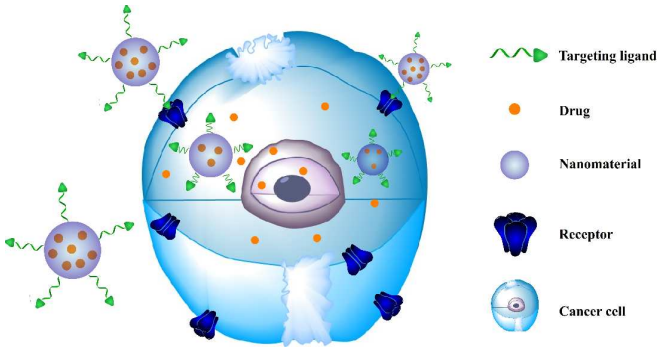


Fig. 1 Schematic of targeting ligand-conjugated nanomaterials for drug delivery and release.

In this review, the representative examples of functional biomolecules-conjugated nanomaterials and their applications in drug delivery for cancer therapy will be summarized. As shown in Fig.1, functional nanomaterials can act as cargoes to deliver targeting ligands and drugs to diseased sites. After the

biorecognition between ligand and receptor, the conjugates could be internalized to release drugs upon stimulation. Herein, the common targeting ligands mainly refer nucleic acid, proteins, peptide, folic acid, and other molecules. Meanwhile, various materials such as metals, semiconductors, and carbon will be introduced to better understand the mechanism of cancer treatment.

2 Targeting ligands

2.1 Nucleic acid

Nucleic acids, the most basic molecules for the storage of genetic information, can be divided into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), which has been considered as important building blocks by virtue of the remarkable molecular self-recognition capabilities and unique structural motif. In the last two decades, the applications of nucleic acids have been further expanded by the introduction of a type of molecular probe termed aptamers. [47]

Aptamers are single-stranded oligonucleic acid that can bind to various molecular targets and viewed as chemical antibodies, which have found wide applications in many areas, such as bio-sensing, medicine, pharmacology, and cancer treatment.[25] In this review, we focus on the application of aptamers in the field of cancer therapy. Coupled with the ability to recognition a variety of targets from small metal ions to cancer cells,[48-51] as well as several advantages including facile and reproducible synthesis, easy and controllable modification, long-term stability, sustain reversible denaturation, lack of immunogenicity, high specificity, nontoxicity and fast tissue penetration, aptamers have been developed into ideal probes in molecular medicine to elucidate the molecular foundation of diseases and treat cancers, and a few of them are either approved by the U.S. Food and Drug Administration or are currently in clinical trials.[52]

2.1.1 DNA and DNA aptamers

Yuan *et al*. constructed a photonmanipulated mesoporous release system based upon azobenzene-modified DNA (Fig. 2).[53] Herein, using mesoporous silica nanoparticles (MSNPs) for fabricating the drug carriers was by virtue of the advantages of MSNPs, including high chemical and thermal stability, tunable pore sizes, high surface area, modifiable surface properties, zero premature release, targeted delivery, and controllable release.[54] Moreover, MSNs are noncytotoxic and undergo cellular uptake into acidic lysosomes by endocytosis, which make them a promising candidate for intracellular stimuli-responsive drug delivery.[55]

In this drug carrier, the azobenzene incorporated DNA double strands were immobilized at the pore mouth of MSNPs, and anticancer drug doxorubicin (Dox) was loaded into pores. The photoisomerization of azobenzene induced-hybridization/ dehybridization switch of complementary DNA resulted in the capping/uncapping of pore gates to control the release of Dox. In details, visible irradiation at 450 nm (*trans*-azobenzene) led to the hybridization of the linker and the complementary DNA arm, capping the pore gates to keep Dox in the pores. While irradiation with UV (365 nm) converted azobenzene to the *cis*- form, resulting in the dehybridization and pore opening to release Dox. These DNA/mesoporous nanoplatform permitted to be explored as carriers for cancer chemotherapy due to its light wavelength-responsive property as well as good biocompatibility. It is envisioned that this photo-controlled drug release system could find potential applications in cancer therapy. [53]

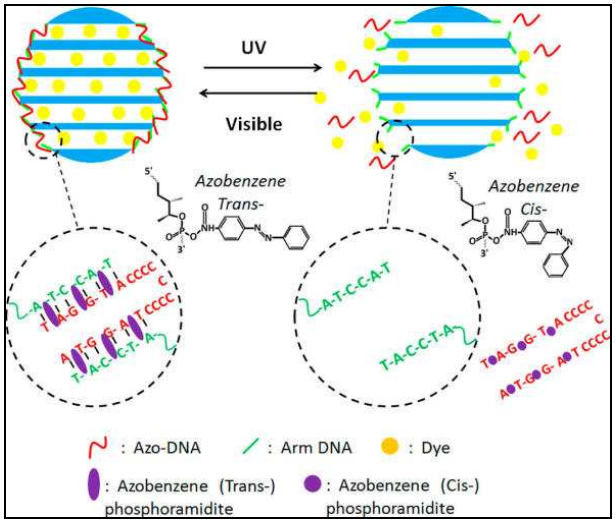


Fig. 2 Schematic of azobezene-modified DNA-controlled reversible release system.[53]

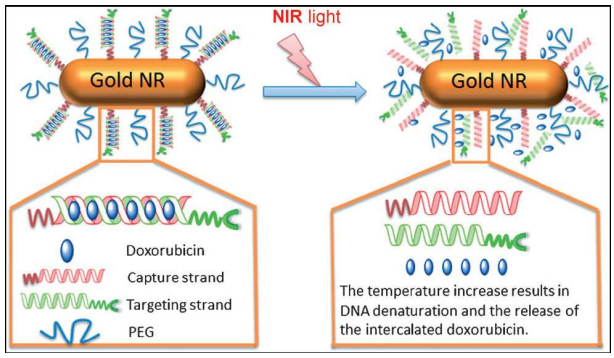


Fig. 3 DNA assembly of a targeted NIR-responsive drug delivery platform.[56]

Farokhzad group designed a DNA assembly for targeted NIR-responsive cancer therapy using AuNRs as platform (Fig.3).[56] AuNRs, a kind of elongated AuNPs, have gained great popularity from their first synthesis in the 1990s, and have found promising applications in the fields of drug delivery and photothermal therapy, which arising from the nanorods' unique optical and photothermal properties such as the availability of tunable size and shape, the ability to surface modification and drugs/molecules conjugation, and the relative biocompatibility.[57] As illustrated in Fig. 3, this platform comprised three distinct functional components: complementary DNA strands, AuNRs and a polyethylene glycol (PEG) layer. The DNA strands consisted of sequential CG base pairs to provide sites for loading chemotherapeutic drug Dox. In addition to serving as drug-loading scaffold, one strand of the DNA (named capture strand) was thiolated to AuNRs surface, and the complementary strand (termed targeting strand) was pre-conjugated with ligands for cells specific targeting. AuNRs served as the drug cargos and thermal reagent both for cancer thermal therapy and for denaturing the DNA double helix upon NIR irradiation, leading to the triggered release of Dox at target site for chemotherapy. The PEG layer allows AuNRs to avoid recognition by the immune system to prolong the

circulation half-life in the blood-vessels. Most notably, this DNA-assembled platform could facilitate the extravasation out of circulation at the tumor site and diffusion in the tumor extracellular space, thus enhancing the antitumor efficacy.

Huang[58] and Jon[59] achieved the targeted therapy for human acute lymphoblastic leukemia and prostate cancer through the similar drug-loaded aptamer-AuNPs bioconjugates, respectively. While Qu group[60] and Tang group[61] fabricated nanocarriers for intracellular controlled release to kill breast cancer cells with SiO_2@AuNRs nanocomposites that capped with reversible single-stranded DNA valves, which were manipulated by NIR light-triggered switching between the laser on/off states. These nanocarriers could deliver drug molecules accurately in a controlled manner, which is indispensable for the treatment of some diseases with precise dosage at a desired time in a specified area. Moreover, therapeutic effect could be enhanced due to the synergistic treatment of thermal therapy from AuNRs and chemotherapy from anticancer drug.

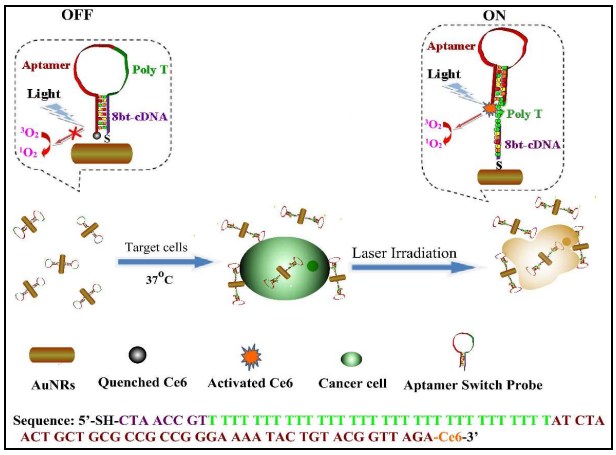


Fig. 4 Schematic representation of ASP-photosensitizer-AuNRs for PTT and PDT.[45]

Taking the advantages of AuNRs, our group designed a multimodal therapy for human acute lymphoblastic leukemia.[45] To obtain this goal, an aptamer switch probe (ASP) coupled with a photosensitizer molecule chlorin e6 (Ce6) was linked to the surface of AuNRs for target cancer cells for photodynamic therapy (PDT) and photothermal therapy (PTT). In the presence of target cancer cells, the ASP transferred its conformation to drive Ce6 away from gold surface, thereby producing singlet oxygen for PDT upon light exposure. Since each AuNR was modified with many ASP-Ce6 molecules, the AuNR-ASP-Ce6 conjugate yielded enhanced binding affinity[62] and therapeutic effect by the additional ability to carry many photosensitizers. Moreover, absorption of radiation by AuNRs enabled further cell destruction by the photothermal effect. Herein, AuNRs were not only useful for photothermal therapy but are also helpful for the delivery Ce6 and controlling the PDT of Ce6 by quenching/recovery of fluorescence, and even activate PDT with additional effect through the thermal-triggered dehybridization of DNA. Consequently, this multimodal conjugate offered a remarkably improved and synergistic therapeutic effect compared to PTT or PDT alone, providing high specificity and therapeutic efficiency.

Recently, more and more aptamer-conjugated nanomaterials have been developed for specific cancer cell recognition and targeted cancer therapy.[63] We also proposed another targeted cancer therapy by one interchain switchable aptamer-based photosensitizer-AuNR platform for targeted multimodal therapy, using leukemia as a model cancer for proof of concept, which provided a highly specific and enhanced therapeutic outcome from the synergistic treatment of PPT and PDT.[64] Furthermore, we manufactured a five-function composite of aptamer conjugated Fe_3O_4@Au nanorose for cancer cell targeting, magnetic resonance imaging, optical imaging, photothermal and chemotherapy.[65] Sgc8 aptamers were conjugated on nanoroses surface for targeting CCRF-CEM cancer cells and the extended GC base pairs were for loading anticancer drug (Dox), which was specifically delivered into cancer cells for chemotherapy and optical imaging. The inner Fe_3O_4 cores functioned as an MRI reagent since they can be conjugated with bioactive molecules and have the ability to form a magnetic field gradient under an external magnetic field.[66] Au shells have been considered as one promising candidate for photothermal ablation because of the resonance absorption in them NIR region, which can be tuned by varying the relative size of the core and shell.[67] In this work, the photothermal effect was achieved through NIR absorption by the rose-shaped gold shell, causing a rapid rise in temperature and also resulting in a facilitated release of the anticancer drug doxorubicin carried by the nanoroses. The five-function-embedded nanoroses showed great advantages in multimodality.

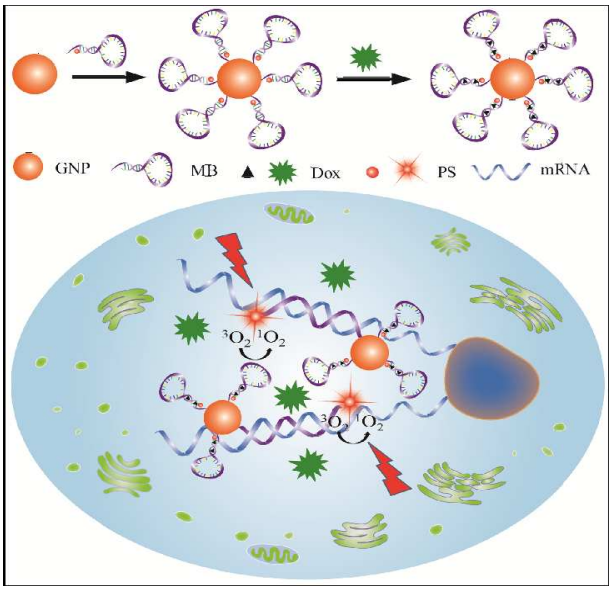


Fig. 5 Schematic illustration of the design and intracellular release of tumour mRNA-triggered nanocarrier.[68]

Tang group found that the DNA-conjugated cargoes could be controlled with RNA for drug delivery and release.[68-70] They constructed an AuNPs platform as drug nanocarrier for cancer therapy (Fig. 5). Here, AuNPs possess vivid optical properties with strong optical resonances associated with their surface plasmons, which are controlled by the geometry and size of the nanoparticle. AuNPs have the additional advantages of biocompatibility, as well as the robust chemistries for binding a range of molecules and functional materials to AuNP surface.[67] In their work, molecular

beacon (MB), which consisted of a stem-loop structure, was employed as the drug carrier for activated release. The loop portion of the MB was designed with a complementary sequence to target nucleic acid and could recognize specifically target mRNA. The stem region of MB was formed by complementary arm sequences to intercalate the chemotherapeutic reagent Dox. One arm of the stem linked with photosensitizer molecule Ce6 and the other arm attached to AuNPs surface, which presented strong surface plasmon absorption in visible region to effectively quench the fluorescence of the photosensitizer and Dox. When in the presence of the complementary targets, more stable duplexes were formed by the hybridization of MB and target sequences, causing the opening of MB to release of Dox and Ce6 molecules. The fluorescence and cytotoxicity of Dox and Ce6 were recovered upon laser irradiation. Then this tumour mRNA-triggered nanocarrier could be used for chemotherapy and PDT, enabling the activated release of chemotherapeutic reagents and photosensitizers in cancer cells.

2.1.2 RNA and RNA aptamers

Like DNA, RNA and its conjugates could be constructed for cancer therapy as well. Furthermore, RNA interference (RNAi) has emerged as a powerful strategy for suppressing gene expression, offering the potential to dramatically accelerate *in vivo* drug target validation, as well as the promise to create novel therapeutic approaches if it can be effectively applied *in vivo*.[71]

Gong and Chen developed a multifunctional AuNRs-based nanocarrier capable of co-delivering small interfering RNA (siRNA) against achaete-scute complex-like 1 (ASCL1) and an anticancer drug Dox specifically to neuroendocrine (NE) cancer cells for combined chemotherapy and siRNA-mediated gene silencing. The octreotide-conjugated AuNR-based nanocarriers (Au-Dox-OCT, targeted) exhibited a much higher cellular uptake in a human carcinoid cell line than non-targeted AuNR-based nanocarriers (Au-Dox). Moreover, Au-Dox-OCT-ASCL1 siRNA resulted in significantly higher gene silencing in NE cancer cells than non-targeted Au-Dox-ASCL1 siRNA complex as measured by an immunoblot analysis. Thus, combined chemotherapy and RNA silencing using NE tumor-targeting Au nanocarriers could potentially enhance the therapeutic outcomes in treating NE cancers.[72]

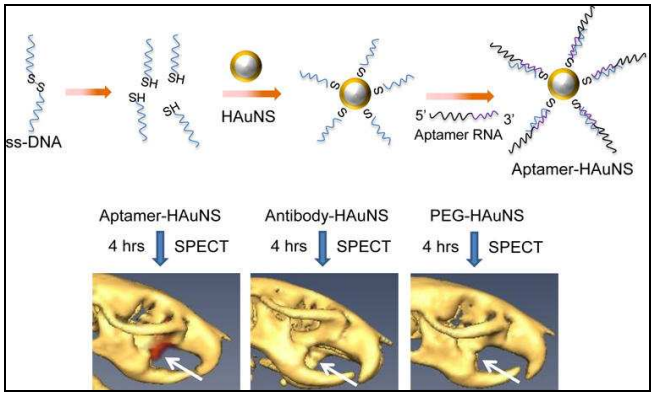


Fig. 6 Comparison of CT mice images after intravenous injection of aptamer-HAuNS, antibody-HAuNS, or PEG-HAuNS. Arrows: tumor. [73]

Li group compared the binding affinity and selective targeting of RNA aptamer-, antibody- and PEG coated hollow gold nanospheres (HAuNS) to epidermal growth factor receptors (EGFR).[73] The selective binding imaging confirmed that there was more tumor uptake of aptamer-HAuNS than antibody-HAuNS (Fig. 6), indicating that surface coating of HAuNS did significantly alter the physicochemical properties or pharmacokinetics of the nanoparticles. Besides, aptamer directed to EGFR was a promising ligand for targeted delivery of HAuNS for selective thermal ablation of head and neck cancers overexpressing EGFR.

Farokhzad *et al.* designed the quantum dots (QDs)-RNA aptamer-Dox conjugate,[74] which could be served as platform for the targeted cancer imaging and therapy. This multifunctional conjugates can be uptaken by target prostate cancer cell through prostate specific membrane antigen (PSMA) mediate endocytosis. In lysosome, Dox released from conjugates to kill cancer cells following the possible mechanisms including physical dissociation of Dox from the conjugates and biodegradation of PSMA aptamer by lysosomal enzymes in the lysosomes. Moreover, the release of Dox from the QD-Apt(Dox) conjugates caused the recovery of fluorescence from both QD and Dox ("ON" state), thereby sensing the intracellular delivery of Dox and enabling the synchronous fluorescent localization and killing of cancer cells.[74] Here, QDs, known as semiconductor nanocrystals, have been increasingly employed as biological imaging and labeling probes because of their unique optical properties, including high quantum yield, broad absorption with narrow photoluminescence spectra, low photo-bleaching, and resistance to chemical degradation. Moreover, the facile surface modification of QDs with biomolecules has resulted in the development of sensitive and specific targeted imaging and diagnostic modalities for *in vitro* and *in vivo* applications.

Jo and coworkers developed novel and valuable therapeutic complexes, namely, dual aptamers (both RNA and peptide aptamer) modified to gold nanostars (AuNS) for the targeting and treating of prostate cancers, including PSMA(+) and PSMA(-) cells, which exhibited both high selectivity and efficiency.[22] Importantly, the homogeneously well-fabricated star-shaped AuNPs presented a great deal of advantages, such as tunable plasmon bands, a tremendously high two-photon action cross section, and a large absorption-to-scattering ratio in response to NIR radiation. This superiority makes AuNS one of the most powerful photothermolysis probes applicable to diverse *in vitro* and *in vivo* hyperthermia therapies.

2.2 Proteins

Proteins are the most abundant biomolecules within cells and participate in essentially all processes. Dissecting these processes in many cases requires the ability to label proteins in the context of living cells.[75] A wide variety of proteins have been found different functions, which can be classified into antibody, enzyme, and so on.

2.2.1 Antibody

Monoclonal antibodies have demonstrated considerable utility in the clinically treating cancers, but unmodified antibodies are rarely curative, especially when used as single reagents. Thus, it is considerably interest to conjugate antibodies to improve their

potency and selectivity, and to increase activity at the tumour site while sparing normal tissues.[76] Nowadays, antibody-conjugated nanomaterials have been used for the targeting and treating specific cancer by biomarkers overexpressed on the surface membrane of cancer cells.[77]

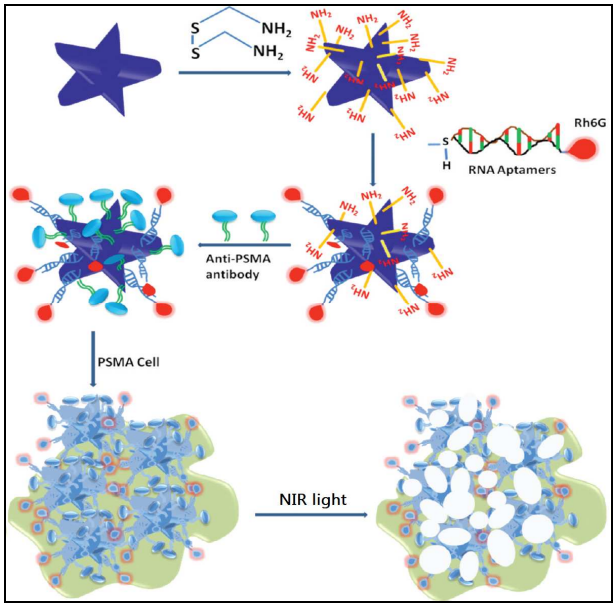


Fig.7 Schematic representation of multifunctional popcorn-shaped gold nanoparticle-based sensing of an LNCaP breast cancer cell line.[78]

Ray reported a multifunctional gold nanopopcorn-based surface-enhanced Raman scattering (SERS) assay for targeted sensing, therapy treatment, and in situ monitoring of photothermal therapy response (Fig. 7).[78] The results showed that, in the presence of LNCaP human prostate cancer cells, multifunctional popcorn-shaped AuNPs form several hot spots and provide a significant enhancement of the Raman signal intensity, which could recognize human prostate cancer cells at the 50-cells level, indicating that the localized heating that occurs during near infrared irradiation could cause irreparable cellular damage to the prostate cancer cells with high efficacy. To selectively sensing, therapy, and monitoring of therapy progress, both anti-PSMA antibody and A9 RNA anti-PSMA aptamers were conjugated with gold nanopopcorn. In nanopopcorn, the central sphere acts as an electron reservoir, while the tips are capable of focusing the field at their apexes, which will provide a sufficient field of enhancement. As a result, popcorn-shaped AuNPs could provide enhancement of Raman signals as well as functions as the hyperthermal reagent for cancer treatment.

Some similar strategies based on the antibody-antigen or ligand-receptor interaction have been designed for targeted cancer therapy.[79] El-Sayed group developed thermal therapy using anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies conjugated AuNRs, which bound specifically to the surface of the malignant type cells with a much higher affinity due to the overexpressed EGFR on the cytoplasmic membrane of the malignant cells.[46] Drezek and coauthors demonstrated a novel nanoshell-based platform for integrating cancer imaging and therapy applications. HER2, a clinically relevant cancer biomarker, were conjugated to nanoshells, to scatter light in the NIR to enable optical molecular cancer imaging, and to absorb light to allow selectively destruct targeted carcinoma cells that overexpress HER2 through photothermal therapy.[80]

2.2.2 Enzyme

Enzymes are a kind biomolecule with biological catalytic function and most of them are proteins, which could improve the efficiency in almost all of the cellular activities processes. Directed enzyme prodrug therapy is an extensive area of research in cancer chemotherapy.[81] Although very promising, the current directed approaches are still hampered by inefficient enzyme expression and tumor targeting. Gwenin and coauthors investigated the viability of using AuNPs as a novel delivery vehicle for prodrug-activating enzymes. The resulting AuNPs-enzyme conjugates showed excellent stability in changing proton and sodium chloride environments. Their work provided the foundation for attaching prodrug-activating enzymes to metal nanoparticles for future use in directed enzyme prodrug therapy.[82]

2.2.3 Transferrin

Transferrin (Tf) is as an effective ligand for actively targeting malignant cells to deliver drugs, proteins and genes,[83] which is essential for normal cell growth and maintenance. Acute hepatitis, anemia, and pregnancy are associated with the symptoms of the increased levels of Tf, while nephritic syndrome, rheumatism, cirrhosis, malignant tumor, and acute leukemia are characterized by the decrease of Tf.[84]

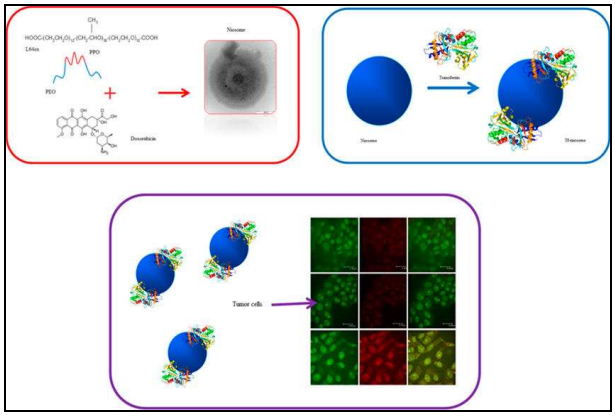


Fig. 8 The preparation and confocal microscopic analysis of uptake of Tf niosomes in human breast cancer cells. [85]

An efficient tumor-targeted niosomal delivery system for the vehiculation of Dox was designed by Muzzalupo group (Fig. 8).[85] Niosomes were prepared from a mixture of an opportunely modified Pluronic L64 surfactant and cholesterol as a membrane additive, which were then conjugated to Tf to produce Tf-niosomes. The specific uptake of Tf-niosomes into cells was evaluated *via* incubation of MCF-7 and MDA-MB-231 cells. The results showed that Dox can be easily encapsulated into niosomes. Moreover, Tf-niosomes conjugates demonstrated far greater extents of cellular uptake by MCF-7 and MDA-MB-231 cells, suggesting that the uptake was mainly controlled by transferrin receptor-mediated endocytosis, which could be potentially useful as a target Dox delivery system in anticancer therapy.

2.2.4 Other proteins

Other proteins, such as human serum albumin (HSA), [86] bovine serum albumin (BSA)[87], concanavalin A,[88] protamine,[89] E-selectin[90] and avidin,[91] *etc*, are promising candidates for targeted drug delivery. For instance, Quan group constructed a smart aptamer conjugated and protein (avidin)-capped mesoporous nanovalve for drug delivery. Due to selective targeting ability of the aptamers on the surface of MSN, the loaded nanocarriers can specifically target and then enter cancer cells through receptor-mediated endocytosis. Since the pores of MSN-avidin-aptamer were capped, there was less premature leakage of drugs during the delivery process. Upon entering the target cancer cells, MSN-avidin-aptamer would be uncapped by intracellular vitamin H (biotin), accelerating the release of drugs from nanocarriers and the killing of target cancer cells. [91]

As displayed in Fig. 9, Cheon group provided an easy and simple method to obtain inorganic nanoparticles that could penetrate the blood-brain barrier (BBB).[92] To enhance the brain transportation ability *via* adsorptive-transcytosis, serum albumin on MNPs' surface was partially cationized *via* cross-linking process**.** Herein, BBB, the protective system of central nervous system from harmful xenobiotics and endogenous molecules, precludes the entrance of drugs and imaging reagents into the brain. BBB permeable inorganic nanoparticles can be useful for imaging and therapeutics for **c**entral nervous system.

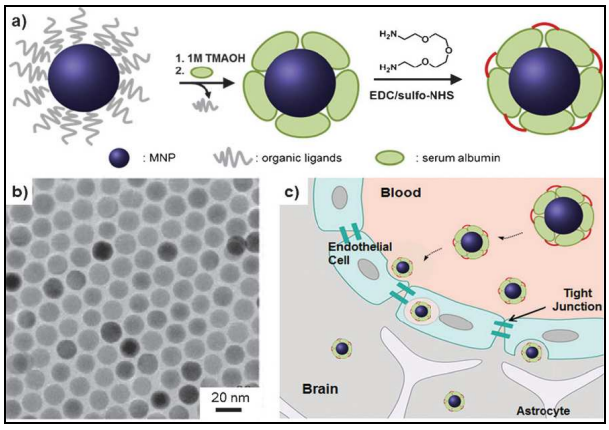


Fig. 9 Serum albumin coated MNPs (SA-MNPs). (a) Surface modification of SA-MNPs. (b) TEM image of MNPs. (c) Schematic of the BBB penetration of SA-MNPs.[92]

2.3 Peptide

Peptides are formed by joining amino acids together through peptide bonds, which are usually composed of less amino acid than proteins. Peptides and specifically short peptides are very attractive due to their good biocompatibility, ease of synthesis, function ability as well as tunable bioactivity along with the availability of rich chemistry for fine-tuning the structure.[93]

Cell-penetrating peptides (CPPs) such as transactivator of transcription (TAT) peptide have been explored for promoting *in vitro* cell penetration and nuclear targeting of various cargos, but their positive charges could cause strong nonspecific interactions, making them inapplicable for *in vivo* applications. As shown in Fig. 10, TAT was used to demonstrate a molecular modification approach for inhibiting nonspecific interactions of CPPs in the bloodstream while reactivating their functions in the targeted tissues or cells. The TAT lysine residues' amines were linked to succinyl amides, completely inhibiting TAT's nonspecific interactions in the blood compartment; once in the acidic tumor interstitium or internalized into cell endo/lysosomes, the succinyl amides were quickly hydrolyzed, fully restoring TAT's functions. Thus, TAT-functionalized micelles achieved long circulation in the blood compartment and efficiently accumulated and delivered Dox to tumor tissues, giving rise to high antitumor activity and low cardiotoxicity. [94]

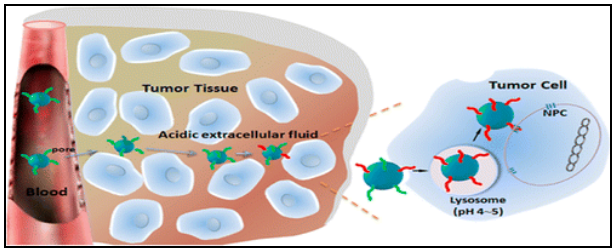


Fig. 10 Acid-active cell-penetrating peptides for *in vivo* tumor-targeted drug delivery.[94]

Moreover, Zhang group designed a new smart peptide-functionalized MSN with a plug-gate nanovalve (PGN) for targeted drug release in cancer cells.[95] They also found that the peptides were able to grow from homogeneous solution into fibroid structure and the gelation was attributed to the entanglement of fibers or the formation of network structures and entrapment of solvent molecules *via* surface tension.[96]

RGD peptide, which is composed of arginine, glycine and aspartic acid, could preferentially bind $\alpha_v\beta_3$ integrin receptors that overpressed on the surface of many cancer cells. Tang group have developed strategies with RGD peptide functionalized Au-Fe_2O_3 for cancer cell-specific apoptosis and real-time imaging,[97] and RGD peptide modified Au-ZnO hybrid nanoparticle for catalyzing lysosomal ROS production, enabling the real-time monitoring of ROS-induced apoptosis of cancer cells.[98]

Cytokines are a kind typical peptide with board biological activity, which are produced by the immune cells (such as monocytes, macrophages, T cells, B cells, NK cells) and some non-immune cells (endothelial cells, epithelial cells, fibroblasts and so on) after stimulation. Shenoi and coworkers found tumor necrosis factor α (TNF-α), a representative cytokine, is potential to improve tumor specific action in combination with locally applied thermal therapy in prostate cancer when conjugated to AuNPs,[99] which could be achieved in the absence of neutrophil recruitment, suggesting that immune/inflammatory regulation is not central to its power as part of a multimodal approach. It is anticipated that more potent tumor vasculature specific combinations of vascular disrupting reagents and nanoparticles with the goal of transitioning optimal regimens into clinical trials will be directly investigated.

2.4 Vitamin

Vitamins such as vitamin B9 (folic acid) and vitamin H (or vitamin B7, biotin) could be employed for cancer targeting as well.

Folic acid (FA), a low-molecular-weight vitamin, plays an essential role in cell survival and binds with high affinity to the folate receptor (FR), a membrane-anchored protein that overexpressed in many of human cancerous cells.[100] The bound folate and folate conjugates enter cells through receptor-mediated endocytosis, which has been attractive for targeted cancer imaging and therapy. What's more, FA has the advantages of low cost, nonimmunogenic character and ability to conjugate with a wide variety of NPs.[70, 101]

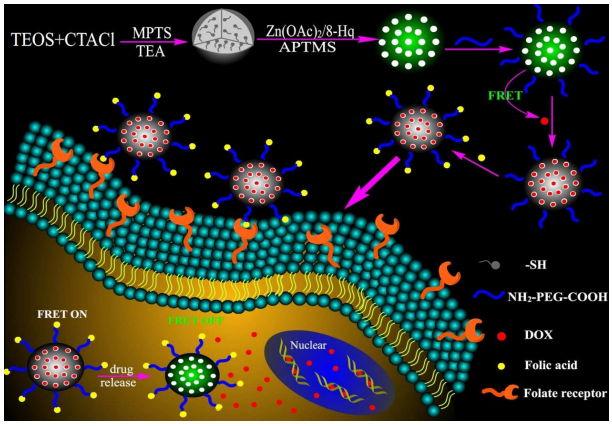


Fig. 11 A diagram showing the synthesis of Dox-FMSN-PEG-FA and sensing of the drug release process by FRET in cancer cell.[102]

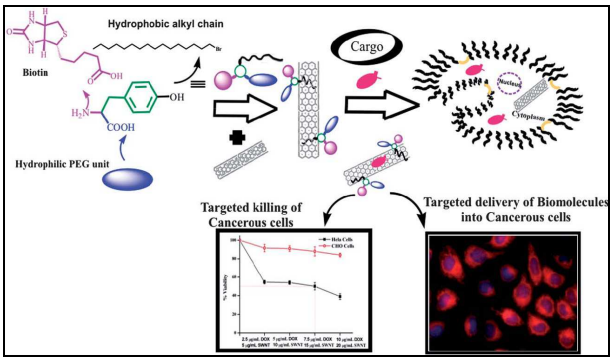


Fig. 12 Pictorial representation of the specific delivery of drug and biomolecule loaded SWNT-biotinylated amphiphile nanoconjugate into cancer cells.[34]

Our group designed pH-sensitive drug delivery systems using coordination polymer spheres and fluorescent mesoporous silica nanoparticles,[102-104] resepectively.

In Fig. 11, a targeted cancer therapy imaging and sensing system was designed based on Dox-loaded green fluorescent mesoporous silica nanoparticles (FMSN) conjugated with FA, by linking with α-amine-ω-propionic acid hexaethylene glycol (NH_2-PEG-COOH). It was found that the FA-grafted and PEGlated nanocomposite displayed excellent biocompatibility towards Hep2 cells. However, under the same conditions, FA modified Dox-FMSN presented higher cytotoxicity towards folate-receptor-rich Hep2 cancer cells than the Dox-FMSN composites without FA. When the resultant composites entered into the cells, the green fluorescence of FMSN gradually recovered along with the release of Dox, to achieve the purpose of real-time monitoring of intracellular drug release and cancer therapy.

Based on the specific binding between folic acid and folate-receptor, Shin and coworkers develpoed ternary conjugate heparin-folic acid-paclitaxel for specific drug delivery into human head and neck cancer cells.[105] Herein, paclitaxel, another most widely used chemotherapeutic agent in clinic, was used for chemotherapy of cancer cells. Furthermore, as commonly used carrier for growth factor delivery, heparin was used to increase the therapeutic specificity and efficacy of paclitaxel.

Biotin is also named vitamin H or vitamin B7, is a CO_2-carrier for several important biological reactions, including gluconeogenesis, the synthesis of fatty acids, and the metabolism of amino acids. As an aid in maintaining blood sugar levels in people, biotin promotes the health of sweat glands, nerve tissue, bone marrow, male sex glands, blood cells, skin, and hair. Notably, biotin levels might be correlated with diseases such as diabetes mellitus, liver and skin disorders, immunological and neurological abnormalities, and epilepsy.[106]

A specific targeting of cancerous cells using a non-covalently water dispersed nanoconjugate of biotinylated amphiphile-single walled carbon nanotube (CNTs) was reported by Brahmachari and coworkers.[34] The fundamental approach involved incorporation of the biotin into the architecture of CNTs dispersing reagent to develop a multifaceted delivery vehicle with high stability, substantial cell viability and targeted specificity towards cancer cells.[34] Importantly, the biotinylated amphiphile-CNTs dispersion successfully transported the fluorescently labeled Cy3-oligoneucleotide (loaded on CNTs surface) into the cancerous HeLa and HepG2 cells after 3 h of incubation, in contrast to CHO and HEK-293 cells (devoid of overexpressed biotin receptors). The presence of the biotin moiety in the cellular transporters facilitated the internalization of cargo due to the overexpressed biotin receptors in the cancer cells. Herein, CNTs were employed to construct platform because of their unique physical, chemical, electrical, and mechanical, which can induce proliferation and differentiation of neurons and osteoblasts and serve as drug and vaccine delivery vehicles for cancer treatment.[107]

2.5 Other targeting ligands

Other targeting ligands such as, hyaluronic acid, chitosan, dopamine, and heparin[108] could be designed as drug carriers for specific drug delivery as well, based on the targeting ligand-receptor interaction.

2.5.1 Hyaluronic acid

Hyaluronic acid (HA), as a major extracellular constituent of connective tissues, is an anionic, naturally occurring polysaccharide. HA can bind to CD44, a receptor that is overexpressed on various tumor cells, leading to recent studies focused on pharmaceutical applications of HA for the development of anticancer therapeutics.[109]

In Fig. 13, our group synthesized BSA-stabilized Au_{20} nanoclusters (Au_{20}NCs) with high fluorescence quantum yield and successfully applied to active tumor-targeted imaging *in vitro* and *in vivo* by conjugating HA.[110] Furthermore, the advantages of the

as-prepared Au_{20}NCs, such as small sizes, high quantum yield, excellent photostability, non-toxicity, made them ideal candidates as luminescent probes for optical imaging *in vitro* and *in vivo*. Compared with that by passive targeting, the uptake of HA-conjugated Au_{20}NCs by both cancer cells and tumor-bearing nude mice could be greatly improved by receptor-mediated endocytosis. Because of their selective accumulation at the tumor sites, the HA functionalized-Au_{20}NCs probes can be used as potential indicators for cancer diagnosis.

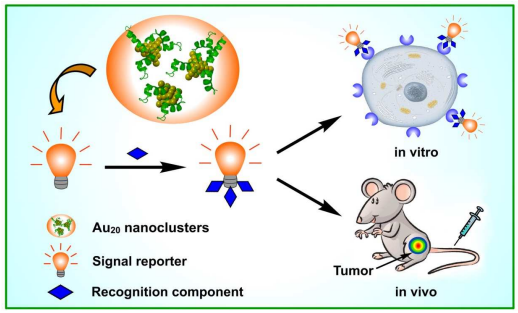


Fig. 13 Highly luminescent and stable gold nanoclusters for active tumor-targeted imaging.[110]

Additionally, HA-nanographene oxide conjugates (NGO-HA) could be designed for photothermal ablation therapy of melanoma skin cancer using a NIR laser with HA as an effective transdermal delivery carrier of chemical drugs and biopharmaceuticals. Notably, nanographene oxide, a two-dimensional sp^2 bonded carbon sheet with intriguing electrical, mechanical, optical, and chemical properties, has attracted tremendous interests in a wide range of research fields such as biomedicine[111] and especially thermal therapy owing to the high NIR absorption.[112]

2.5.2 Chitosan

Chitosan is a well-known biopolymer that offers distinctive advantages such as good biocompatibility, remarkable affinity to proteins, and has already proved successful use in nanomedicine in delivering therapeutic drugs, proteins, and genes.[113]

The multifunctional chitosan nanospheres which co-carried AuNRs and cisplatin were prepared by Jiang group to enhance cytotoxicity of some chemotherapeutic reagents at temperature rise. Due to the enhanced cytotoxicity of cisplatin at elevated temperatures, cisplatin-loaded hybrid nanospheres showed about one-second lower IC50 values than hybrid nanospheres alone *in vitro* and almost complete tumor growth inhibition *in vivo*. Compared with chemotherapy or photothermal treatment alone, the combined photothermal therapy and chemotherapy had a significantly synergistic effect and improved the therapeutic efficacy, which was supported by immuno-fluorescence staining and *in vivo* apoptosis imaging.[114]

Similarly, chitosan-coated chalcogenides, such as hollow CuS[115] and MoS_2 nanosheets,[116] have been developed as chemotherapeutic drug nanocarriers for NIR photothermal-triggered drug delivery, facilitating the combination of chemotherapy and photothermal therapy for cancer therapy.

2.5.3 Dopamine

The catecholamine, dopamine, is produced *in vivo* in different parts of the peripheral and central nervous system as well as in the medulla of adrenal glands. It is involved in a wide range of physiological processes, including hormonal regulation, cardiovascular and renal functions, as well as control of motor functions. In the central nervous system, dopamine functions as a neurotransmitter, being released by presynaptic neurons upon arrival of an action potential and received by the G-protein coupled receptors of the post synaptic neurons, initiating a cascade of biochemical processes.[117]

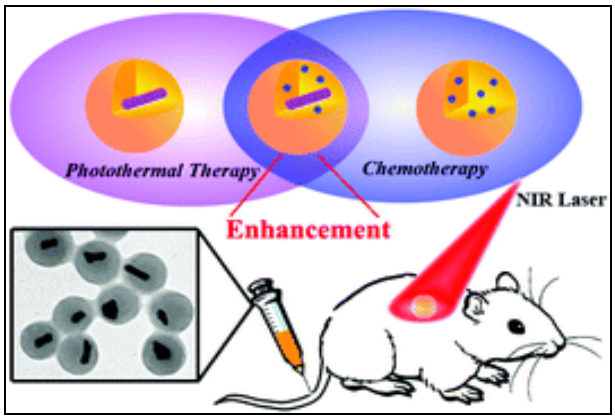


Fig. 14 Combined near-IR photothermal therapy and chemotherapy using AuNRs/chitosan hybrid nanospheres to enhance the antitumor effect.[114]

A new photothermal therapeutic reagent based on biopolymer dopamine-melanin colloidal nanospheres was described by Lu group.[118] Benefitting from their naturally wide distribution in humans, dopamine-melanin colloidal nanospheres exhibited robust biocompatibility and biodegradability, and provided up to 40% photothermal conversion efficiency. After administration, they could efficiently damage tumors at low power density and short irradiation time without damaging healthy tissues.[118]

In addition to the above mentioned targeting ligands, there are also others that could be developed for specific cancer therapy. Targeting ligand conjugated-nanomaterials could be applied to the highly efficient and specific drug loading and release, which can supply great therapeutics outcomes.

Table 1 summarizes the common strategies using MSNs and AuNRs as nanocarriers. On one hand, these nanomaterials could further form nanocomposites with other materialsto process more unique properties for the potential application. For instance, the nanocomposites of Fe_3O_4@SiO_2@AuNRs@mSiO_2 presented excellent magnetic and thermal ability, which also could be employed for drug loading in silica pores. On the other hand, these nanocomposites could be functionalized with various targeting ligands, which are very powerful for chemotherapy, PTT, PDT or imaging. [119]

3 Conclusions and outlook

This review is intended to provide an introduction to newcomers. Some common targeting ligands, including nucleic acid, proteins, peptide, vitamin, were cnjugated to nanomaterials such as metallic, magnetic, mesoporous silica nanoparticles, semiconductor

Table 1 The common strategies of biomolecules-conjugated MSNs and AuNRs for targeted cancer therapy

Targeting ligands	Targets	Nanomaterials	Drugs	Application	Ref.
Azobenzene DNA	UV/VIS light	MSNs	Dox	Chemotherapy	Ref. [53]
siRNA	P-glycoprotein	MSNs	Dox	siRNA delivery, chemotherapy	Ref. [120]
Avdin	Vitamin H	MSNs	Dox	Chemotherapy	Ref. [91]
TRC105 antibody	CD105/endoglin	MSNs	Dox	Chemotherapy, imaging	Ref. [79]
Peptide (SP94) Therapeutic RNA	endosomolytic peptide hepatocellular carcinoma	MSNs	Dox	Gene Silencing and chemotherapy	Ref.[121]
Glucose oxidase	Glucose	MSNs	Dox	chemotherapy	Ref. [122]
K8 peptide	K8(RGD)2	MSNs	Dox	chemotherapy	Ref. [95]
Peptide (IP)	interleukin 13	MSNs-GO	Dox	Chemotherapy, PTT	Ref. [123]
aptamer AS1411	Nucleolin	AuNR@MSNs	Dox	chemotherapy, PTT, imaging	Ref. [60]
Folic acid	Folate receptor	MSNs	Dox, $TMPyP_4$*	PDT, chemotherapy	Ref.[124]
Sgc8 apatmer	PTK7 membrane proteins	AuNRs	Ce6*	PDT, PTT	Ref. [45, 64]
Octreotide/siRNA	somatostatin receptors	AuNRs	Dox	Chemotherapy, Gene Silencing, PTT	Ref. [72]
Peptide	matrix metalloprotease	AuNRs	PPa*	Bioimaging, PDT	Ref. [125]
Folic acid	Folate receptor	AuNRs	^{125}I	SPECT/CT imaging, PTT	Ref. [126]
anti-EGFR antibody	EGFR	AuNRs	/	PTT	Ref. [46]
Hyaluronic acid	hyaluronan receptor	AuNRs-GO	Dox	Chemophotothermal combined therapy	Ref. [127]
RGD peptide	$\alpha_v\beta_3$ integrin receptors	AuNRs@SiO_2	Dox	Chemotherapy, PTT	Ref. [128]
Folic acid	Folate receptor	AuNRs@SiO_2	/	X-ray/CT imaging radiation therapy, PTT	Ref. [129]
Folic acid	Folate receptor	AuNRs@mSiO_2@ QDs	/	CT and fluorescence imaging , PTT	Ref. [101]
Folic acid	Folate receptor	Fe_3O_4@SiO_2@Au NRs@mSiO_2	/	MRI, PTT	Ref. [119]

Note: * means photosensitiser; / represents no drug loading; SiO_2 means silica coating; mSiO_2 represents mesoporous silica coating.

quantum dots, carbon and chalcogenides, accordingly formulate them for applications in drug delivery, gene silencing chemotherapy, photodynamic therapy and photothermal therapy.

By summarizing recent progress in integrating targeting ligands with various types of nanomaterials, we have demonstrated that these novel targeting ligands-conjugated nanomaterials benefit cancer therapy through increased specificity and efficacy as well as reduced toxicity. More importantly, the multifunctional conjugates own especial properties and targeting ligands-conjugated nanomaterials with diverse characteristics exhibit additional capability for cancer therapy. For instance, nanorods,[45] nanostar,[22] nanoshells,[80] nanopopcorn,[78] graphene[112] and chalcogenides[116] could function as hyperthermal reagents. The development of such multifunctional nanosystems combined with targeting ligands, drugs and nanomaterials will become popular strategies of novel platforms for targeted therapy. Recently, enormous efforts have been made toward multimodal therapies. The chemotherapy/PTT,[65] PTT/PDT[45, 64] and chemotherapy/RNA silencing[72] have been designed for multimodal therapies with enhanced therapeutics outcomes.

It should be noted that some targeting ligands-conjugated nanomaterials have mainly been performed *in vitro*, which is necessary to perform more stringent *in vivo* testing to demonstrate the effectiveness of these systems. Prior to application to human clinical application, the animal models to evaluate the safety and efficacy should be continued.

Even although much progress have been made by researchers, some obstacles in nanoconjugates-mediated cancer therapy are that certain materials such as metal-organic frameworks[130] and carbon dots[131] are still difficult to modify for targeted cancer therapy. Furthermore, the drugs adopted the previous reports are mainly restricted to Dox,[132] paclitaxel,[133] Ce6,[64] or cisplatin, [134] which require to further expand more medicine to load for targeted cancer therapy.

Acknowledgements

All authors herein are grateful to the financial support from the Natural Science Foundation Project of China (No. 21035005 and 21405123), Chongqing (cstc2014jcyjA50006) and SWU (SWU112092) as well as the Cultivation Plan of Chongqing Science & Technology Commission for 100 Outstanding Science and Technology Leading Talents.

Notes and references

Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, PR China. Fax: +86-23-68367257; Tel: +86-23-68254659; E-mail: chengzhi@swu.edu.cn.

1 A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu and M. J. Thun, *CA-Cancer J. Clin.*, 2007, **57**, 43-66.

2 A. Jemal, R. Siegel, J. Xu and E. Ward, *CA-Cancer J. Clin.*, 2010, **60**, 277-300.

3 *American Cancer Society (ACS), Cancer Facts and Figures, 2014 (Accessed Oct. 5th, 2014, at http://www.cancer.org/).*
4 M. V. Blagosklonny, *Leukemia*, 2006, **20**, 385-391.
5 H. W. Chen, C. D. Medley, K. Sefah, D. Shangguan, Z. Tang, L. Meng, J. E. Smith and W. Tan, *ChemMedChem*, 2008, **3**, 991-1001.
6 E. C. Dreaden, S. C. Mwakwari, L. A. Austin, M. J. Kieffer, A. K. Oyelere and M. A. El-Sayed, *Small*, 2012, **8**, 2819-2822.
7 E. H. Huang and M. S. Wicha, *Trends Mol. Med.*, 2008, **14**, 503-509.
8 J. Wang, K. Sefah, M. B. Altman, T. Chen, M. You, Z. Zhao, C. Z. Huang and W. Tan, *Chem. Asian J.*, 2013, **8**, 2417-2422.
9 R. V. J. Chari, *Acc. Chem. Res.*, 2008, **41**, 98-107.
10 Y. Cheng, J. D. Meyers, A.-M. Broome, a. E. Kenney, J. P. Basilion and C. Burda, *J. Am. Chem. Soc.*, 2011, **133**, 2583-2591.
11 Z. Cheng, A. A. Zaki, J. Z. Hui, V. R. Muzykantov and A. Tsourkas, *Science*, 2012, **338**, 903-910.
12 W. T. Al-Jamal and K. Kostarelos, *Acc. Chem. Res.*, 2011, **44**, 1094-1104.
13 Y. Ma, X. Liang, S. Tong, G. Bao, Q. Ren and Z. Dai, *Adv. Funct. Mater.*, 2013, **23**, 815-822.
14 H. Yang, P. F. Gao, W. B. Wu, X. X. Yang, Q. L. Zeng, C. Li and C. Z. Huang, *Polym. Chem.*, 2014, **5**, 1965-1975.
15 A. K. Patri, J. F. Kukowska-Latallo and J. R. Baker Jr, *Adv. Drug Deliver. Rev.*, 2005, **57**, 2203-2214.
16 T. Sun, Q. Guo, C. Zhang, J. Hao, P. Xing, J. Su, S. Li, A. Hao and G. Liu, *Langmuir*, 2012, **28**, 8625-8636.
17 H. Meng, M. Xue, T. Xia, Z. Ji, D. Y. Tarn, J. I. Zink and A. E. Nel, *ACS Nano*, 2011, **5**, 4131-4144.
18 D. Peer, J. Karp, S. Hong, O. Farokhzad, R. Margalit and R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751-760.
19 R. J. Holmberg, S. Bolduc, D. Beauchemin, G. Jerkiewicz, H. Schulz, U. Kohlhaas and H. Strzelecki, *ACS Appl. Mater. Interfaces*, 2012, **4**, 6487-6498.
20 L. Zhang, Y. Li and J. C. Yu, *J. Mater. Chem. B*, 2014, **2**, 452-470.
21 Y. H. Bae and K. Park, *J. Control. Release*, 2011, **153**, 198-205.
22 H. Jo, H. Youn, S. Lee and C. Ban, *J. Mater. Chem. B*, 2014, **2**, 4862-4867.
23 I. H. El-Sayed, X. Huang and M. A. El-Sayed, *Nano Lett.*, 2005, **5**, 829-834.
24 A. C. Antony and P. S. Low, *Adv. Drug Deliver. Rev.*, 2004, **56**, 1055-1058.
25 X. Fang and W. Tan, *Acc. Chem. Res.*, 2010, **43**, 48-57.
26 D. S. Seferos, A. E. Prigodich, D. A. Giljohann, P. C. Patel and C. A. Mirkin, *Nano Lett.*, 2008, **9**, 308-311.
27 S. N. Barnaby, A. Lee and C. A. Mirkin, *Proc. Nati. Acad. Sci. USA*, 2014, **111**, 9739-9744.
28 J. S. Rudra, T. Sun, K. C. Bird, M. D. Daniels, J. Z. Gasiorowski, A. S. Chong and J. H. Collier, *ACS Nano*, 2012, **6**, 1557-1564.
29 Y.-E. L. Koo, G. R. Reddy, M. Bhojani, R. Schneider, M. A. Philbert, A. Rehemtulla, B. D. Ross and R. Kopelman, *Adv. Drug Deliver. Rev.*, 2006, **58**, 1556-1577.
30 S. Huo, S. Jin, X. Ma, X. Xue, K. Yang, A. Kumar, P. C. Wang, J. Zhang, Z. Hu and X.-J. Liang, *ACS Nano*, 2014, **8**, 5852-5862.
31 Y. L. Colson and M. W. Grinstaff, *Adv. Mater.*, 2012, **24**, 3878-3886.
32 K.-T. Yong, I. Roy, M. T. Swihart and P. N. Prasad, *J. Mater. Chem.*, 2009, **19**, 4655-4672.
33 I. Fratoddi, I. Venditti, C. Cametti and M. V. Russo, *J. Mater. Chem. B*, 2014, **2**, 4204-4220.
34 S. Brahmachari, M. Ghosh, S. Dutta and P. K. Das, *J. Mater. Chem. B*, 2014, **2**, 1160-1173.
35 O. C. Farokhzad and R. Langer, *ACS Nano*, 2009, **3**, 16-20.
36 Z. Luo, K. Cai, Y. Hu, L. Zhao, P. Liu, L. Duan and W. Yang, *Angew. Chem. Int. Ed.*, 2011, **50**, 640-643.
37 J. Liu, C. Detrembleur, A. Debuigne, M.-C. De Pauw-Gillet, S. Mornet, L. Vander Elst, S. Laurent, E. Duguet and C. Jerome, *J. Mater. Chem. B*, 2014, **2**, 1009-1023.
38 Y. Yang, J. Aw, K. Chen, F. Liu, P. Padmanabhan, Y. Hou, Z. Cheng and B. Xing, *Chem. Asian J.*, 2011, **6**, 1381-1389.
39 J. Liu, C. Detrembleur, M.-C. De Pauw-Gillet, S. Mornet, L. V. Elst, S. Laurent, C. Jerome and E. Duguet, *J. Mater. Chem. B*, 2014, **2**, 59-70.
40 S. Yang, N. Li, D. Chen, X. Qi, Y.-J. Xu, Y. Xu, Q. Xu, H. Li and J.-M. Lu, *J. Mater. Chem. B*, 2013, **1**, 4628-4636.
41 N. Huebsch, C. J. Kearney, X. Zhao, J. Kim, C. A. Cezar, Z. Suo and D. J. Mooney, *Proc. Nati. Acad. Sci. USA*, 2014, **111**, 9762-9767.
42 J. Ge, E. Neofytou, T. J. Cahill, R. E. Beygui and R. N. Zare, *ACS Nano*, 2011, **6**, 227-233.
43 M. S. Aw, J. Addai-Mensah and D. Losic, *J. Mater. Chem.*, 2012, **22**, 6561-6563.
44 M. Iafisco, J. M. Delgado-Lopez, E. M. Varoni, A. Tampieri, L. Rimondini, J. Gomez-Morales and M. Prat, *Small*, 2013, **9**, 3834-3844.
45 J. Wang, G. Zhu, M. You, E. Song, M. I. Shukoor, K. Zhang, M. O. D. Altman, Y. Chen, Z. Zhu, C. Z. Huang and W. Tan, *ACS Nano*, 2012, **6**, 5070-5077.
46 X. H. Huang, I. H. El-Sayed, W. Qian and M. A. El-Sayed, *J. Am. Chem. Soc.*, 2006, **128**, 2115-2120.
47 M. Famulok, J. S. Hartig and G. Mayer, *Chem. Rev.*, 2007, **107**, 3715-3737.
48 L. Q. Chen, S. J. Xiao, P. P. Hu, L. Peng, J. Ma, L. F. Luo, Y. F. Li and C. Z. Huang, *Anal. Chem.*, 2012, **84**, 3099-3110.
49 S. J. Zhen, L. Q. Chen, S. J. Xiao, Y. F. Li, P. P. Hu, L. Zhan, L. Peng, E. Q. Song and C. Z. Huang, *Anal. Chem.*, 2010, **82**, 8432-8437.
50 S. J. Xiao, P. P. Hu, Y. F. Li, C. Z. Huang, T. Huang and G. F. Xiao, *Talanta*, 2009, **79**, 1283-1286.
51 J. Wang, P. Zhang, J. Y. Li, L. Q. Chen, C. Z. Huang and Y. F. Li, *Analyst*, 2010, **135**, 2826-2831.
52 G. Zhu, M. J. Donovan, Z. Zhao, M. Ye and W. Tan, *Chem. Commun.*, 2012, **48**, 10472-10480.
53 Q. Yuan, Y. Zhang, T. Chen, D. Lu, Z. Zhao, X. Zhang, Z. Li, C.-H. Yan and W. Tan, *ACS Nano*, 2012, **6**, 6337-6344.
54 P. Zhang, F. Cheng, R. Zhou, J. Cao, J. Li, C. Burda, Q. Min and J.-J. Zhu, *Angew. Chem. Int. Ed.*, 2014, **53**, 2371-2375.
55 D. He, X. He, K. Wang, M. Chen, Y. Zhao and Z. Zou, *J. Mater. Chem. B*, 2013, **1**, 1552-1560.
56 Z. Xiao, C. Ji, J. Shi, E. M. Pridgen, J. Frieder, J. Wu and O. C. Farokhzad, *Angew. Chem. Int. Ed.*, 2012, **51**, 11853-11857.
57 A. M. Alkilany, L. B. Thompson, S. P. Boulos, P. N. Sisco and C. J. Murphy, *Adv. Drug Deliver. Rev.*, 2012, **64**, 190-199.

58 Y.-L. Luo, Y.-S. Shiao and Y.-F. Huang, *ACS Nano*, 2011, **5**, 7796-7804.
59 D. Kim, Y. Y. Jeong and S. Jon, *ACS Nano*, 2010, **4**, 3689-3696.
60 X. Yang, X. Liu, Z. Liu, F. Pu, J. Ren and X. Qu, *Adv. Mater.*, 2012, **24**, 2890-2895.
61 N. Li, Z. Yu, W. Pan, Y. Han, T. Zhang and B. Tang, *Adv. Funct. Mater.*, 2013, **23**, 2255-2262.
62 Y.-F. Huang, K. Sefah, S. Bamrungsap, H.-T. Chang and W. Tan, *Langmuir*, 2008, **24**, 11860-11865.
63 Q. Liu, C. Jin, Y. Wang, X. Fang, X. Zhang, Z. Chen and W. Tan, *NPG Asia Mater.*, 2014, **6**, e95.
64 J. Wang, M. You, G. Zhu, M. I. Shukoor, Z. Chen, Z. Zhao, M. B. Altman, Q. Yuan, Z. Zhu, Y. Chen, C. Z. Huang and W. Tan, *Small*, 2013, **9**, 3678-3684.
65 C. Li, T. Chen, I. Ocsoy, G. Zhu, E. Yasun, M. You, C. Wu, J. Zheng, E. Song, C. Z. Huang and W. Tan, *Adv. Funct. Mater.*, 2014, **24**, 1772-1780.
66 E. Kim, K. Lee, Y.-M. Huh and S. Haam, *J. Mater. Chem. B*, 2013, **1**, 729-739.
67 R. Bardhan, S. Lal, A. Joshi and N. J. Halas, *Acc. Chem. Res.*, 2011, **44**, 936-946.
68 N. Li, H. Yang, W. Pan, W. Diao and B. Tang, *Chem. Commun.*, 2014, **50**, 7473-7476.
69 G. Qiao, L. Zhuo, Y. Gao, L. Yu, N. Li and B. Tang, *Chem. Commun.*, 2011, **47**, 7458-7460.
70 W. Pan, H. Yang, T. Zhang, Y. Li, N. Li and B. Tang, *Anal. Chem.*, 2013, **85**, 6930-6935.
71 D. Peer, E. J. Park, Y. Morishita, C. V. Carman and M. Shimaoka, *Science*, 2008, **319**, 627-630.
72 Y. Xiao, R. Jaskula-Sztul, A. Javadi, W. Xu, J. Eide, A. Dammalapati, M. Kunnimalaiyaan, H. Chen and S. Gong, *Nanoscale*, 2012, **4**, 7185-7193.
73 M. P. Melancon, M. Zhou, R. Zhang, C. Xiong, P. Allen, X. Wen, Q. Huang, M. Wallace, J. N. Myers, R. J. Stafford, D. Liang, A. D. Ellington and C. Li, *ACS Nano*, 2014, **8**, 4530-4538.
74 V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. W. Kantoff, R. Langer and O. C. Farokhzad, *Nano Lett.*, 2007, **7**, 3065-3070.
75 Z. HAO, S. HONG, X. CHEN and P. R. CHEN, *Acc. Chem. Res.*, 2011, **44**, 742-751.
76 G. J. L. Bernardes, G. Casi, S. Trüssel, I. Hartmann, K. Schwager, J. Scheuermann and D. Neri, *Angew. Chem. Int. Ed.*, 2012, **51**, 941-944.
77 H. Hong, K. Yang, Y. Zhang, J. W. Engle, L. Feng, Y. Yang, T. R. Nayak, S. Goel, J. Bean, C. P. Theuer, T. E. Barnhart, Z. Liu and W. Cai, *ACS Nano*, 2012, **6**, 2361-2370.
78 W. Lu, A. K. Singh, S. A. Khan, D. Senapati, H. Yu and P. C. Ray, *J. Am. Chem. Soc.*, 2010, **132**, 18103-18114.
79 F. Chen, H. Hong, Y. Zhang, H. F. Valdovinos, S. Shi, G. S. Kwon, C. P. Theuer, T. E. Barnhart and W. Cai, *ACS Nano*, 2013, **7**, 9027-9039.
80 C. Loo, A. Lowery, N. Halas, J. West and R. Drezek, *Nano Lett.*, 2005, **5**, 709-711.
81 C. Li, M.-F. Penet, F. Wildes, T. Takagi, Z. Chen, P. T. Winnard, D. Artemov and Z. M. Bhujwalla, *ACS Nano*, 2010, **4**, 6707-6716.
82 V. V. Gwenin, C. D. Gwenin and M. Kalaji, *Langmuir*, 2011, **27**, 14300-14307.
83 J. L. Li, D. Day and M. Gu, *Adv. Mater.*, 2008, **20**, 3866-3871.
84 J. Zhang, Y. Sun, B. Xu, H. Zhang, Y. Gao, H. Zhang and D. Song, *Biosens. Bioelectron.*, 2013, **45**, 230-236.
85 L. Tavano, R. Muzzalupo, L. Mauro, M. Pellegrino, S. Andò and N. Picci, *Langmuir*, 2013, **29**, 12638-12646.
86 Q. Quan, J. Xie, H. Gao, M. Yang, F. Zhang, G. Liu, X. Lin, A. Wang, H. S. Eden, S. Lee, G. Zhang and X. Chen, *Mol. Pharmaceut.*, 2011, **8**, 1669-1676.
87 Y. Wang, J. Chen and J. Irudayaraj, *ACS Nano*, 2011, **5**, 9718-9725.
88 N. Smiljanic, V. Moreau, D. Yockot, J. M. Benito, J. M. García Fernández and F. Djedaïni-Pilard, *Angew. Chem. Int. Ed.*, 2006, **45**, 5465-5468.
89 C. Wang, X. Ma, S. Ye, L. Cheng, K. Yang, L. Guo, C. Li, Y. Li and Z. Liu, *Adv. Funct. Mater.*, 2012, **22**, 2363-2375.
90 K. Rana, C. A. Reinhart-King and M. R. King, *Mol. Pharmaceutics*, 2012, **9**, 2219-2227.
91 L.-L. Li, M. Xie, J. Wang, X. Li, C. Wang, Q. Yuan, D. Pang, Y. Lu and W. Tan, *Chem. Commun.*, 2013, **49**, 5823-5825.
92 Y. S. Yim, J.-s. Choi, G. T. Kim, C. H. Kim, T.-H. Shin, D. G. Kim and J. Cheon, *Chem. Commun.*, 2012, **48**, 61-63.
93 J. J. Panda and V. S. Chauhan, *Polym. Chem.*, 2014, **5**, 4418-4436.
94 E. Jin, B. Zhang, X. Sun, Z. Zhou, X. Ma, Q. Sun, J. Tang, Y. Shen, E. Van Kirk, W. J. Murdoch and M. Radosz, *J. Am. Chem. Soc.*, 2013, **135**, 933-940.
95 G.-F. Luo, W.-H. Chen, Y. Liu, J. Zhang, S.-X. Cheng, R.-X. Zhuo and X.-Z. Zhang, *J. Mater. Chem. B*, 2013, **1**, 5723-5732.
96 X.-D. Xu, L. Liang, C.-S. Chen, B. Lu, N.-l. Wang, F.-G. Jiang, X.-Z. Zhang and R.-X. Zhuo, *ACS Appl. Mater. Interfaces*, 2010, **2**, 2663-2671.
97 W. Gao, L. Ji, L. Li, G. Cui, K. Xu, P. Li and B. Tang, *Biomaterials*, 2012, **33**, 3710-3718.
98 W. Gao, W. Cao, H. Zhang, P. Li, K. Xu and B. Tang, *Chem. Commun.*, 2014, **50**, 8117-8120.
99 M. M. Shenoi, I. Iltis, J. Choi, N. A. Koonce, G. J. Metzger, R. J. Griffin and J. C. Bischof, *Mol. Pharmaceutics*, 2013, **10**, 1683-1694.
100 P. S. Low, W. A. Henne and D. D. Doorneweerd, *Acc. Chem. Res.*, 2008, **41**, 120-129.
101 H.-X. Xia, X.-Q. Yang, J.-T. Song, J. Chen, M.-Z. Zhang, D.-M. Yan, L. Zhang, M.-Y. Qin, L.-Y. Bai, Z.-Y. Ma and Y.-D. Zhao, *J. Mater. Chem. B*, 2014, **2**, 1945-1953.
102 J. Wang, P. F. Gao, X. X. Yang, T. T. Wang, J. Wang and C. Z. Huang, *J. Mater. Chem. B*, 2014, **2**, 4379-4386.
103 P. F. Gao, L. L. Zheng, L. J. Liang, X. X. Yang, Y. F. Li and C. Z. Huang, *J. Mater. Chem. B*, 2013, **1**, 3202-3208.
104 J. Wang, H. Liu, F. Leng, L. Zheng, J. Yang, W. Wang and C. Z. Huang, *Micropor. Mesopor. Mater.*, 2014, **186**, 187-193.
105 X. Wang, J. Li, Y. Wang, K. J. Cho, G. Kim, A. Gjyrezi, L. Koenig, P. Giannakakou, H. J. C. Shin, M. Tighiouart, S. Nie, Z. Chen and D. M. Shin, *ACS Nano*, 2009, **3**, 3165-3174.
106 J.-a. A. Ho and C.-H. Hung, *Anal. Chem.*, 2008, **80**, 6405-6409.
107 Y. Liu, Y. Zhao, B. Sun and C. Chen, *Acc. Chem. Res.*, 2012, **46**, 702-713.

108 N. Vasimalai and S. A. John, *J. Mater. Chem. B*, 2013, **1**, 5620-5627.
109 K. Y. Choi, H. Y. Yoon, J.-H. Kim, S. M. Bae, R.-W. Park, Y. M. Kang, I.-S. Kim, I. C. Kwon, K. Choi, S. Jeong, K. Kim and J. H. Park, *ACS Nano*, 2011, **5**, 8591-8599.
110 P. Zhang, X. X. Yang, Y. Wang, N. W. Zhao, Z. H. Xiong and C. Z. Huang, *Nanoscale*, 2014, **6**, 2261-2269.
111 K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva and A. A. Firsov, *Science*, 2004, **306**, 666-669.
112 Y.-W. Wang, Y.-Y. Fu, Q. Peng, S.-S. Guo, G. Liu, J. Li, H.-H. Yang and G.-N. Chen, *J. Mater. Chem. B*, 2013, **1**, 5762-5767.
113 S. C. Boca, M. Potara, A.-M. Gabudean, A. Juhem, P. L. Baldeck and S. Astilean, *Cancer Lett.*, 2011, **311**, 131-140.
114 R. Chen, X. Zheng, H. Qian, X. Wang, J. Wang and X. Jiang, *Biomater. Sci.*, 2013, **1**, 285-293.
115 L. Guo, D. D. Yan, D. Yang, Y. Li, X. Wang, O. Zalewski, B. Yan and W. Lu, *ACS Nano*, 2014, **8**, 5670-5681.
116 W. Yin, L. Yan, J. Yu, G. Tian, L. Zhou, X. Zheng, X. Zhang, Y. Yong, J. Li, Z. Gu and Y. Zhao, *ACS Nano*, 2014, **8**, 6922-6933.
117 L. Sasso, A. Heiskanen, F. Diazzi, M. Dimaki, J. Castillo, M. Vergani, E. Landini, R. Raiteri, G. Ferrari, M. Carminati, M. Sampietro, W. E. Svendsen and J. Emneus, *Analyst*, 2013, **138**, 3651-3659.
118 Y. Liu, K. Ai, J. Liu, M. Deng, Y. He and L. Lu, *Adv. Mater.*, 2013, **25**, 1353-1359.
119 D.-W. Wang, X.-M. Zhu, S.-F. Lee, H.-M. Chan, H.-W. Li, S. K. Kong, J. C. Yu, C. H. K. Cheng, Y.-X. J. Wang and K. C.-F. Leung, *J. Mater. Chem. B*, 2013, **1**, 2934-2942.
120 H. Meng, W. X. Mai, H. Zhang, M. Xue, T. Xia, S. Lin, X. Wang, Y. Zhao, Z. Ji, J. I. Zink and A. E. Nel, *ACS Nano*, 2013, **7**, 994-1005.
121 C. E. Ashley, E. C. Carnes, K. E. Epler, D. P. Padilla, G. K. Phillips, R. E. Castillo, D. C. Wilkinson, B. S. Wilkinson, C. A. Burgard, R. M. Kalinich, J. L. Townson, B. Chackerian, C. L. Willman, D. S. Peabody, W. Wharton and C. J. Brinker, *ACS Nano*, 2012, **6**, 2174-2188.
122 F. Tang, L. Li and D. Chen, *Adv. Mater.*, 2012, **24**, 1504-1534.
123 Y. Wang, K. Wang, J. Zhao, X. Liu, J. Bu, X. Yan and R. Huang, *J. Am. Chem. Soc.*, 2013, **135**, 4799-4804.
124 C. Chen, L. Zhou, J. Geng, J. Ren and X. Qu, *Small*, 2013, **9**, 2793-2800.
125 B. Jang and Y. Choi, *Theranostics*, 2012, **2**, 190-197.
126 B. Jang, S. Park, S. H. Kang, J. K. Kim, S.-K. Kim, I.-H. Kim and Y. Choi, *Quant. Imag. Med. Surg.*, 2012, **2**, 1-11.
127 C. Xu, D. Yang, L. Mei, Q. Li, H. Zhu and T. Wang, *ACS Appl. Mater. Interfaces*, 2013, **5**, 12911-12920.
128 S. Shen, H. Tang, X. Zhang, J. Ren, Z. Pang, D. Wang, H. Gao, Y. Qian, X. Jiang and W. Yang, *Biomaterials*, 2013, **34**, 3150-3158.
129 P. Huang, L. Bao, C. Zhang, J. Lin, T. Luo, D. Yang, M. He, Z. Li, G. Gao, B. Gao, S. Fu and D. Cui, *Biomaterials*, 2011, **32**, 9796-9809.
130 M. C. Bernini, D. Fairen-Jimenez, M. Pasinetti, A. J. Ramirez-Pastor and R. Q. Snurr, *J. Mater. Chem. B*, 2014, **2**, 766-774.
131 Z. L. Wu, P. Zhang, M. X. Gao, C. F. Liu, W. Wang, F. Leng and C. Z. Huang, *J. Mater. Chem. B*, 2013, **1**, 2868-2873.
132 R. Venkatesan, A. Pichaimani, K. Hari, P. K. Balasubramanian, J. Kulandaivel and K. Premkumar, *J. Mater. Chem. B*, 2013, **1**, 1010-1018.
133 F. Ren, S. Bhana, D. D. Norman, J. Johnson, L. Xu, D. L. Baker, A. L. Parrill and X. Huang, *Bioconjugate Chem.*, 2013, **24**, 376-386.
134 B. Ahn, J. Park, K. Singha, H. Park and W. J. Kim, *J. Mater. Chem. B*, 2013, **1**, 2829-2836.

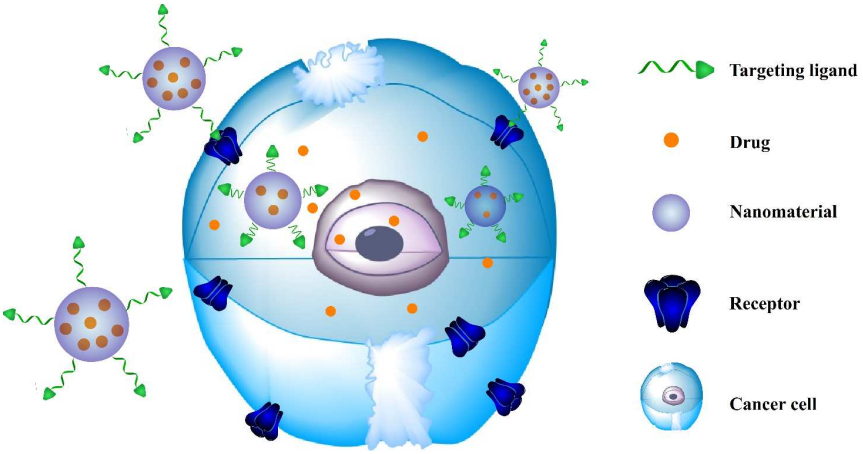
Graphic abstract

for

Biomolecules-conjugated nanomaterials for targeted cancer therapy

Jian Wang, Ting Ting Wang, Peng Fei Gao and Cheng Zhi Huang*

Now and in the future, with the development of artificial biomolecules as well as nanomaterials, the targeted drug delivery based on the elegant biomolecules-nanomaterials conjugation approaches have been developed to achieve great versatility, additional functions, and more advances.



Schematic of targeting ligand-conjugated nanomaterials for drug delivery and release.