# Nanoscale

## Mini Review

# Advances in targeting strategies for nanoparticles in cancer imaging and therapy

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In the past decade, nanoparticles have offered great advances in diagnostic imaging and targeted drug delivery. In particular, nanoparticles have provided remarkable progress in cancer imaging and therapy based on the material science and biochemical engineering technology. Researchers constantly attempted to develop the nanoparticles which can deliver drugs more specifically to cancer, and these efforts brought the advances in targeting strategy of nanoparticles. This minireview will discuss the progress in targeting strategies for nanoparticles focused on the recent innovative works for nanomedicine.

## 1. Introduction

Targeted drug delivery has been one of the critical issues for efficient therapy. Since Ehrlich's 'magic bullet concept', constant attempts have been made to deliver drugs straight to the intended target tissues.[1, 2]  Based on these efforts, significant progress has been achieved in pharmaceutics with improved pharmacokinetics and therapeutic effect. In particular, nanotechnology has offered great advances in drug delivery systems. Nanoparticles generally have properties of prolonged blood circulation and disease-specific accumulation *in vivo* so that medical application of nanoparticles is expected to realize target-specific drug delivery. In practice, a variety of nano-formulated drugs demonstrated highly targeted delivery to tumors and emerged as a powerful tool as carriers for anti-cancer agents.[3, 4]  In addition, nanoparticles have also been used for diagnostic imaging of cancers with labeling of probes, such as chemical/fluorescent dyes, radioactive isotopes, and other imaging contrast agents.[4-6] In consideration of their performances, nanoparticles have a great potential as an innovative cancer 'theranostic' strategy, a simultaneous therapy and diagnosis using a single formulation.

In early days of cancer theranostics using nanoparticles, physiochemical property of the particles was considered as the first priority. Basically, size of particles is one of the important factors to determine the behavior of the nanoparticles.[7, 8] Size-dependent properties of nanoparticle involve in the biodistribution and clearance of the particles, and smaller nanoparticles tend to indicate slower removal from the blood circulation than larger ones.[8-10] However, too small nanoparticles (< 6 nm of a hydrodynamic diameter) are rapidly eliminated via urine.[11, 12] Consequently, drug-loaded polymeric micelles are usually tested with 10 to 100 nm sizes for desired pharmacokinetics,[8, 13, 14] and the proposed cut-off size of nanoparticles for disease-specific drug delivery is known to be under 380 nm.[8]

Although nanoparticles and size-controlled drug formulations themselves have shown effective tumor-targeted delivery, constant attempts to achieve efficient drug delivery brought the advances in targeting strategy of nanoparticles. Indeed, advanced functional materials resulted in a remarkable progress in targeting strategy of nanoparticles. Nanoparticles have been engineered to incorporate cancer cell-specific ligands including antibodies, peptides, and aptamers on their surface.[15-18] The targeting moieties significantly increased binding affinity of nanoparticles to cancer cells, and ligand-grafted nanoparticles could be highly internalized into the target cells.[19, 20]

More recently, new targeting strategies considering multiple biological factors were suggested. Cancer microenvironment and biological characteristics of cancer cells would exert a strong influence on the efficiency of drug delivery,[8, 21] and various cancer environmental factors including local blood flow, pH, and distribution of extracellular matrix and immunocytes are now intensively studied to achieve more efficient delivery of nanoparticles. In this minireview, the progress in targeting strategies for nanoparticles will be discussed focused on the recent noticeable strategies. New concepts and approaches to overcome the limitation of conventional targeting methods will be introduced with innovative works for nanomedicine.

## 2. Nanoparticles as a targeted drug delivery system

### Nanoparticles for targeted drug delivery

Ideal targeted drug delivery systems should satisfy several requirements including 1) the ability to escape from the blood vessels in the pathological lesions, 2) the possibility to retain in the disease site, and 3) selective cellular uptake of the drug-carrier to the targeted cells.[8, 22] On account of the aforementioned requirements, nanoparticles have great potentials as an effective drug carrier system. Nanoparticles highly accumulate in the site of disease due to their small size. The abnormal blood vessels in diseases, such as tumor angiogenic vessels, often show coarse structures that nano-sized (<1 μm of a hydrodynamic diameter) particles can easily penetrate the large pores of the blood vessels.[20, 23] In addition,

solid tumors also have poorly organized lymphatic systems which cause low drainage and the local retention of the nanoparticles. Based on these enhanced vascular permeability and retention (EPR) effects in tumors (Fig. 1A),[24] nanoparticles have been intensively studied as a carrier system for tumor-targeted drug delivery.
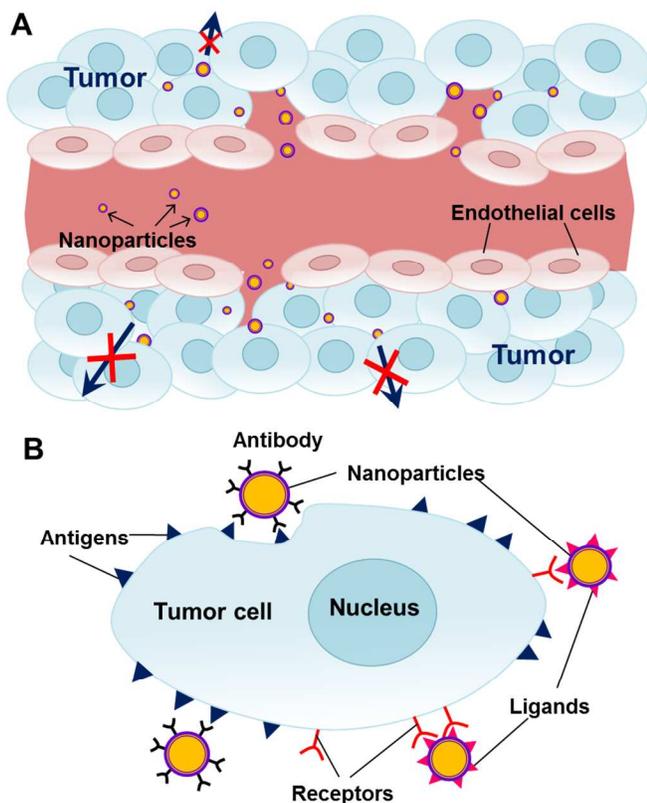


**Fig. 1** (A) Enhanced permeability and retention effects of typical nanoparticles. (B) Interaction of particle with cell surface antigens and receptors.

The size-related properties of nanoparticle have influences on its pharmacokinetics,[3, 7-9, 11, 25] depth of penetration,[26] and cellular responses in tissues.[27] To maximize the target-specific distribution and deep tissue penetration, nano-formulated drugs are generally designed to be around 100 nm sized particles.[8, 13, 14] However, other physiochemical properties, such as surface charge, shape, and structural flexibility, are also important to determine the in vivo behavior of nanoparticles.[28, 29] Consequently, more wide ranges of nano-formulations are practically tested to achieve the best performance, depending on the purpose of applications.

Although nanoparticles are fundamentally effective in tumor specific delivery, the size dependent EPR effects based approaches alone usually suffer from the limitations of insufficiency in targeting efficiency. In practice, EPR effects may vary according to the tumor vascularization.[20, 21] Moreover, reaching to the target site and internalization of the nanoparticles to the target cells may be different matter. To address this problem, researchers attempted to develop more smart nanocarriers by increasing the selectivity of the cellular uptake of nanoparticles to the targeted cells (Fig. 1B).

## Advances in nanoparticles for more accurate delivery: Ligand-receptor interaction

A variety of factors can be involved in the binding and recognition of nanoparticles. In general, cellular internalization mechanisms of small drug and typical nanoparticles include clathrin-mediated, caveolae-mediated endocytosis, and macropinocytosis which are not so specific.[30-32] To increase the internalization of nanoparticles to the specific cells, nanoparticles should show higher binding affinity to target cells than other cells. Accordingly, additional targeting motifs have been grafted on the surface of nanoparticles.

To achieve successful ligand-mediated targeting strategy, target cells should express overwhelming amount of specific receptors binding to the grafted moieties. Cancer cells usually overexpress certain kinds of receptors or antigens which are closely related to growth hormone or cell metabolism. Overexpression of vascular endothelial growth factor receptor (VEGFR), αvβ3 integrin, transferrin receptors, or folate receptor was widely exploited for ligand-mediated tumor targeting strategy. Researchers conjugated relevant targeting biomolecules to the surface of nanoparticles to improve the targeting efficiency. Other biological ligands, such as antibody, fragment antigen-binding (Fab fragment), and aptamer, also have been popular materials to modify the surface of nanoparticles (Fig. 2A).
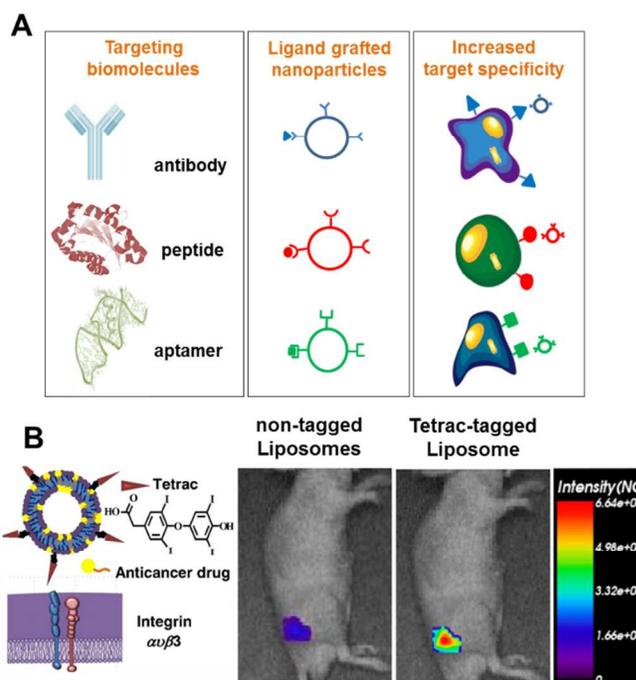


**Fig. 2** (A) Various targeting biomolecules and surface modified nanoparticles. (B) Scheme of Tetrac tagged liposome and enhanced delivery by ligand-mediated targeting strategy. (B) Reprinted with permission from ref. 34. Copyright (2006) Elsevier

Surface modification does not always increase the EPR effects of nanoparticles, but it is definitely effective in target cell-specific endocytosis.[33] However, sometimes, increased target cell internalization in tumor tissues directly involved in the enhanced delivery of nanoparticles.[34-36] For instance, tetraiodothyroacetic acid (tetrac) could enhance the tumor-

targeting ability of PEGylated liposome (Fig. 2B).[34] The terac, small molecule which binds to integrin αvβ3, was used for surface modification of liposomes. Although physical properties of liposomes were not significantly changed, tetrac-tagged liposomes (TPL) showed improved delivery in integrin αvβ3 overexpressing A375 cells and A375 tumors. TPLs showed significantly higher cancer cell localization than unmodified PEGylated liposome, and tumor growth was effectively retarded using edelfosine formulated in TPL. Ligand-mediated targeting strategy could provide better therapeutic effects with more accurate delivery of nanoparticles.

Ligand-mediated targeting has evolved over a period time. To overcome the limitation of incomplete targeting of single ligand, dual ligand-grafted nanoparticles have been developed.[37-42] Since transferrin, folic acid, Arginine-Glycine-Aspartic acid (RGD) peptide, and Fab fragment have their own mechanism to increase binding affinity to cancer cells,[19, 43-45] use a combination of targeting moieties has been tried to expect synergistic effects in tumor targeting. The dual-ligand targeting strategy indeed provided more chances to be internalized into target cells.[37-42] In addition, it allows effective targeting to the tumor which expresses single receptor proteins, heterogeneously. To create a reliable synergy effect of dual-ligand targeting system, the pair and the ratio of targeting ligands should be carefully selected and optimized.

## 3. More recent targeting strategies for enhanced delivery of nanoparticles

### Limitations of conventional targeting strategies and biological issues

In spite of the remarkable progress in nanoparticles as drug carriers, only a few nano- formulated drugs are in clinical use.[46, 47] Although many of nanoparticles demonstrated the feasibility of targeted drug delivery system, most of them showed disappointing results in the phases of clinical research. The challenges in clinical use of nanoparticles can be overcome through the understanding the present limitations of nanoparticle approaches and maximizing the targeting efficiency of developed nanoparticle based formulations.[48, 49] The current targeting approaches using nanoparticles, in fact, have been biased towards engineering of nanoparticles. However, more recent studies suggested that understanding of biological factors and tissue microenvironment of diseases would be a key for clinical use of nanoparticles.[21, 23, 47] In practice, local blood flow, pH condition, amounts of extracellular matrix (ECM) and its organization may have critical influences on the EPR effect and biodistribution of nanoparticles in vivo. Cancer cell heterogeneity is also a problem to cause cancer recurrence or incomplete cure of cancer. To address these problems, researchers are trying to create new perspectives on nanoparticles. The alternative targeting strategies with better understanding of disease now provide the clues new concept of nanoparticle targeting.

### Tumor microenvironment responsive systems

One of the common conditions of cancer microenvironment is low pH. Most of tumors have acidic extracellular pH (< pH 6.8), which is contrary to surrounding normal tissues ($\fallingdotseq$ pH 7.4).[50, 51] The acidity of tumor interstitium is mainly attributed to the high rate of glycolysis which may increase proton production in cancer cells.[52] In addition to the increased proton production, elevated interstitial fluid pressure and poor lymphatic drainage also may contribute lower the local pH by reduced proton clearance.[53] Accumulation of cancer cell metabolite and protons could result in acidic microenvironment, and it offers low pH-responsive systems for enhancing tumor targeting.
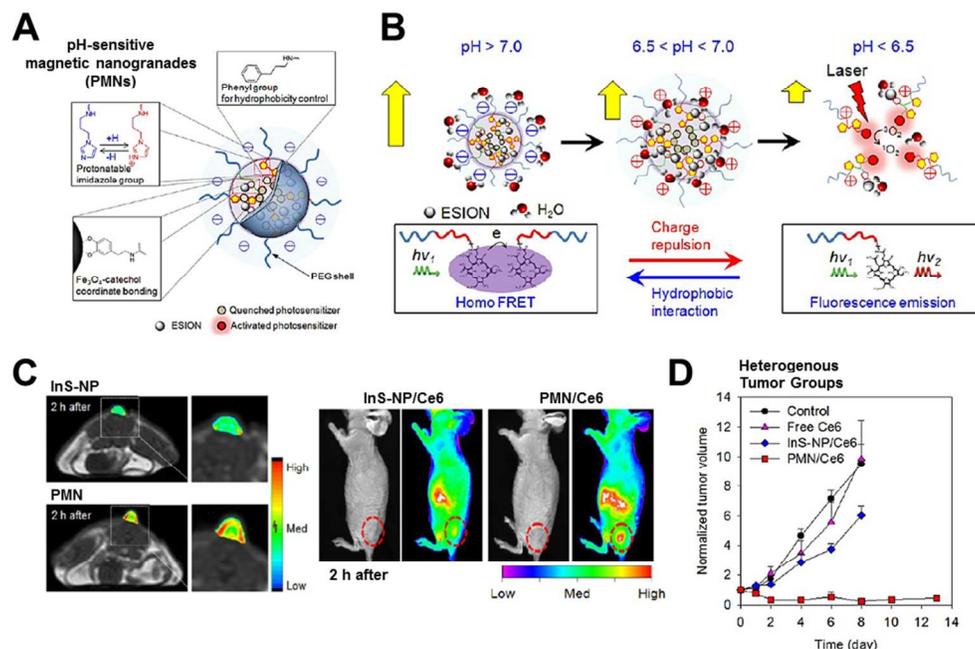


**Fig.3** (A) Design of pH-sensitive magnetic nanogrenades (PMNs) (B) Structural changes of PMNs depending on pH and laser. (C) In vivo T1-weighted MR images and NIR fluorescence images of HCT116 tumors after intravenous injection of PMNs (InS-NPs, pH-insensitive nanoparticles as a control group). (D) In vivo tumor photodynamic therapy using PMNs. Group 1, saline; group 2,

free Ce6; group 3, InS-NPs; and group 4, PMNs treated (group 2, 3, and 4 included equivalent to 2 mg/kg body of Ce6). Reprinted with permission from ref. 54. Copyright (2014) American Chemical Society.

Recently, Hyeon *et al.* have described the tumor pH-sensitive self-assembled nanoparticles which include pH-responsive polymeric ligands, MR imaging contrast agent, and photodynamic agent. The nanoparticles were termed as pH-sensitive magnetic nanogrenades (PMNs) that they showed ultrasensitive MR/optical bimodal imaging and photodynamic therapy of heterogeneous tumors *in vivo* (Fig. 3A).[54] The PMNs have two different kinds of poly(ethylene glycol)−poly(β-benzyl-L-aspartate) (PEG−PBLA) based receptor-binding modules. One of the components includes 1-(3-aminopropyl) imidazole (API) and dopamine, and the other one has API and 3-phenyl-1-propylamine (PPA). The catechol groups of former components facilitated self-assembly of nanoparticles, acting as high-affinity anchors for iron oxide.[55] By contrast, PPA of the latter one involve in the phase transition of PMNs to give pH responsive properties. Based on the combination of these receptor-binding modules, the PMNs could be primarily swollen at tumor tissues by imidazole ionization to enhance their payload delivery. Then, they were secondarily dissociated in endosome (pH < 5.5−6.0) for further ionization. It helps PMNs to be delivered into cancer cells to release optical/MR contrast and photodynamic agents for theranostics through surface-charge switching from negative to positive at the acidic tumor condition (Fig. 3B). The unique pH-sensitive PMNs enabled successful MR and optical imaging of tumors with less than 3 mm of diameter (Fig. 3C). Furthermore, PMN-based targeted photodynamic therapy was highly effective, especially in heterogeneous tumor xenografts.

## Communicating control system

Another new targeting strategy is integrating two different functional nanoparticle systems for communicating to each other. Bhatia *et al.* were inspired by biological communication to improve targeting abilities, and they designed communicating nanoparticle system using signaling and receiving modules.[56] Gold nanorods (NRs) and tumor-targeted tissue factor (tTF) are 'signaling' modules as coagulation cascade-activating factors in tumor, and magnetofluorescent iron oxide nanoworms (NWs) and doxorubicin-loaded liposomes (LPs) are clot-targeted 'receiving' nanoparticles (Fig. 4). Two 'signaling' components locally activated the coagulation cascade in tumors. The NRs passively accumulated in tumors to initiate coagulation cascade activation through photothermal conversion, and tTF induced extrinsic coagulation cascade.[57] The receiving nanoparticles targeted coagulation region of tumors. Both of the NW, prototypical imaging agent, and drug-loaded LPs expose fibrin- or coagulation transglutaminase factor XIII (FXIII)-binding peptides on their surface. The signaling and receiving modules could amplify tumor targeting by transmission of information as artificial inputs and outputs. Autonomously communicating system through coagulation process improved targeting efficiency by up to 40-fold higher tumor accumulation of receiving modules than those without communication, leading to an effective tumor imaging and therapy.
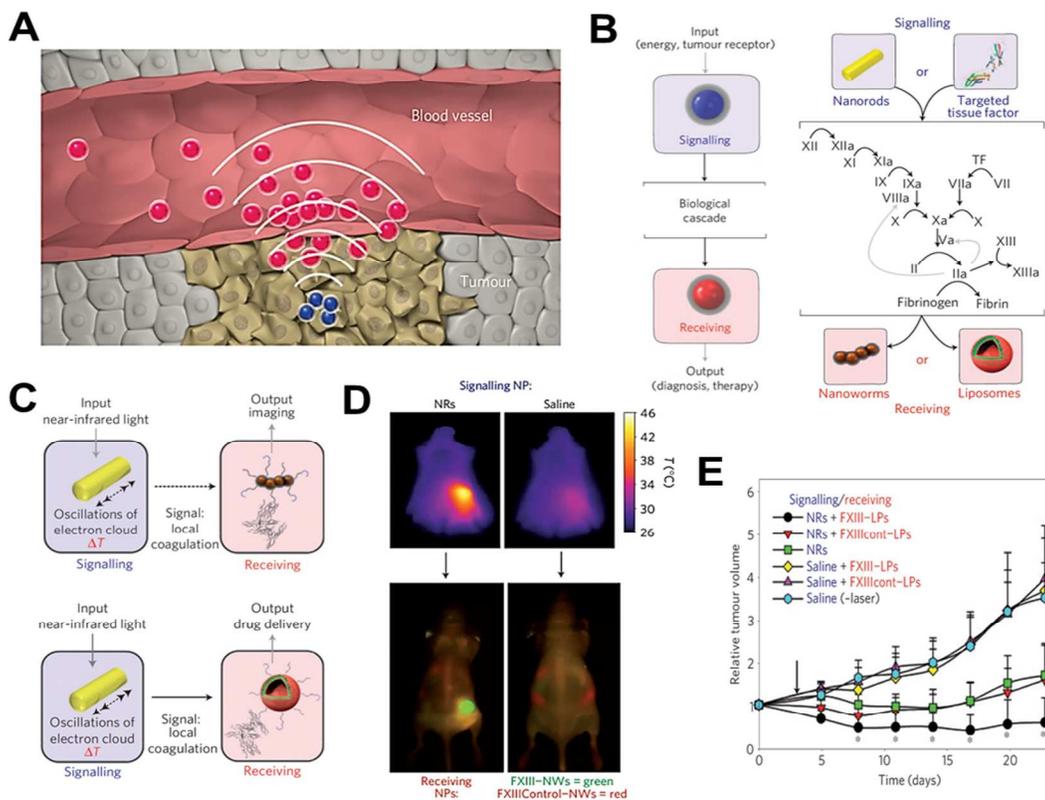


**Fig. 4** (A) Schematic representation of communication between system components for amplified tumor targeting. (B) Coagulation cascade pathway between the signaling and receiving components. (C) Schematic representation of an amplified diagnostic or therapeutic system of communicating NPs. (D) Thermographic images of the photothermal NR heating (top) and whole body fluorescence images (bottom) showing the distribution of the coagulation-targeted receiving NPs. (E) Amplified therapeutic

efficacy of communicating NP systems. Tumor growth curve after a single treatment. Reprinted with permission from ref. 56. Copyright (2011) Nature Publishing Group.
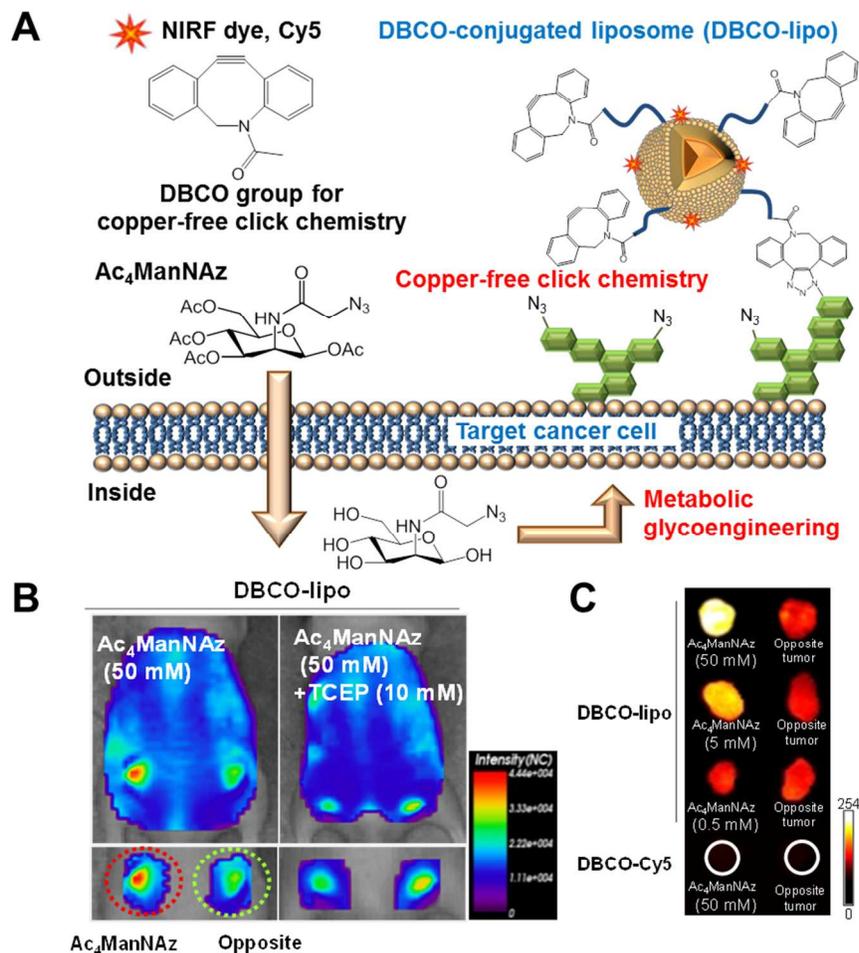


**Fig. 5** (A) Schematic representation of in vivo chemical tumor-targeting strategy for nanoparticles based on metabolic glycoengineering and bioorthogonal copper-free click chemistry. (B) In vivo biodistribution of dibenzyl cyclooctyne-conjugated liposomes (DBCO-lipo) in mice bearing two tumors with and without Ac$_4$ManNAz pre-treatment (left, Ac$_4$ManNAz-treated; right, saline-treated). (C) Tumor accumulation of DBCO-lipo dependent on the dose of pre-treated Ac$_4$ManNAz (5 h post-injection of DBCO-lipo). Reprinted with permission from ref. 65. Copyright (2014) American Chemical Society.

### Chemical targeting systems using bioorthogonal click chemistry

Bioorthogonal chemistry also inspired targeting strategies of nanoparticles. Bioorthogonal chemistry is diverse chemical reactions which occur in living organisms without interfering of other biochemical reacitions, by unique unnatural functional groups.[58] It has led to many novel innovations in the chemical and biological field, based on its high specificity.[58-61] In particular, its application in biological fields with metabolic glycoengineering holds great promise for specific labelling of target cells. Unnatural aminosugars could have chemical groups which are absent from natural living cells, and the cells can expose the unique chemical groups on their surface after the uptake and metabolic process of these unnatural aminosugars.[62-65] Therefore, the introduced chemical groups may play roles as

a highly specific artificial chemical receptor to develop a new targeting strategy.

Kim *et al.* designed a new *in vivo* tumor-targeting strategy for nanoparticles through the bioorthogonal copper-free click chemistry and metabolic engineering (Fig. 5).[65] Unnatural glycan, tetraacetylated N-azidoacetyl-D-mannosmine (Ac$_4$ManNAz), was treated to cancer cells that the cells expressed artificial sialic acids with azide on their surface. The generated azide groups on cell surface were confirmed using relevant chemical phosphine, and azide group was expressed on the cytoplasmic membrane in a dose-dependent manner. Importantly, various cancer cells including A549, U87MG, MCF-7, and KB cells exhibited homogenous azide groups on the cytoplasmic membrane. For *in vivo* studies, bilateral A549 tumor bearing mice were prepared, and Ac$_4$ManNAz was intratumorally injected in only left side of tumor. Dibenzyl

cyclooctyne conjugated liposomes (DBCO-lipo) were intravenously injected, and the copper-free click chemistry between DBCO on liposomes and azide groups on tumor tissues resulted in improved tumor-targeting ability of DBCO-lipo in the left side of the tumor. In addition, DBCO-lipo accumulation in Ac$_4$ManNAz treated tumor was impeded by pretreatment of tris(2-carboxyethyl)-phosphine (TCEP), a chemical for blocking azide groups. These results all suggested that the bioorthogonal copper-free click chemistry can provide more accurate delivery of nanoparticles.

An extended study was carried out using a similar chemical tumor-targeting of strategy.[66] To introduce artificial receptor-like molecules on cell surface, Ac$_4$ManNAz was delivered to cancer cells using tumor-homing glycol chitosan nanoparticles.[23, 66] The unnatural glycan was successfully delivered to cancer cells *in vitro* and *in vivo* to induce azide groups on cancer cell surface. The two-step *in vivo* tumor targeting enabled delivery of large amounts of drug containing nanoparticles in the second step, and it allowed the therapeutic application of bioorthogonal copper-free click chemistry based nanoparticles. In practice, Ce6 loaded bicyclo[6.1.0]nonyne (BCN)-modified nanoparticles showed significantly higher tumor-accumulation than unmodified one, and they indicated better therapeutic effect by photodynamic therapy.[64] The bioorthogonal copper-free click chemistry based nanoparticles are expected to be a new treatment strategy for cancer treatment. In particular, induction of artificial cell surface marker may play key roles to overcome the incomplete cure of cancer with heterogeneity-related problems

## 4. Conclusion and outlook

As a drug carrier system, nanoparticles have offered great advances in imaging and therapy for cancers. The size-related physical properties and EPR effects of nanoparticles fundamentally lead to their tumor-targeted delivery, but constant efforts to accomplish more tumor-specific drug delivery have made great progress in targeting strategy of nanoparticles. Several recent innovative studies in particular suggested highly targeted delivery of nanoparticles with improved understanding of cancer biology. Multivalent ligands targeting strategy, microenvironment responsive system, communicating control system, and chemical targeting using bioorthogonal click chemistry all carefully considered multiple biological factors to overcome the current limitations in targeted drug delivery. Besides previous achievements in development of nano-biomaterials, current efforts to exploit cancer biology may provide opportunities for nanoparticles to go one step further in their clinical use. New approaches to targeting strategies for nanoparticles will serve as a foundation for the development of ideal drug delivery systems in the future.

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## Notes and references

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