

Rational Design of Cancer Nanomedicine: Nanoproperty Integration and Synchronization

Qihang Sun, Zhuxian Zhou, Nasha Qiu, and Youqing Shen*

Current cancer nanomedicines can only mitigate adverse effects but fail to enhance therapeutic efficacies of anticancer drugs. Rational design of next-generation cancer nanomedicines should aim to enhance their therapeutic efficacies. Taking this into account, this review first analyzes the typical cancer-drug-delivery process of an intravenously administered nanomedicine and concludes that the delivery involves a five-step CAPIR cascade and that high efficiency at every step is critical to guarantee high overall therapeutic efficiency. Further analysis shows that the nanoproperties needed in each step for a nanomedicine to maximize its efficiency are different and even opposing in different steps, particularly what the authors call the PEG, surface-charge, size and stability dilemmas. To resolve those dilemmas in order to integrate all needed nanoproperties into one nanomedicine, stability, surface and size nanoproperty transitions (3S transitions for short) are proposed and the reported strategies to realize these transitions are comprehensively summarized. Examples of nanomedicines capable of the 3S transitions are discussed, as are future research directions to design high-performance cancer nanomedicines and their clinical translations.

1. Introduction

Cancer nanomedicines have been designed to overcome the pharmacokinetic limitations associated with conventional drugs.^[1] The greatest advantage is their ability to preferentially accumulate in tumor tissues by exploiting tumors' enhanced permeability and retention (EPR) effect as result of their pathologically leaky vasculature and poor lymphatic drainage.^[2] In clinics, accumulated evidence show nanoparticle localization in solid human tumors after systemic administration to cancer patients.^[3] Several nanomedicines have entered clinical use including pegylated liposomal doxorubicin (Doxil),^[4] nano-particular albumin-bound, paclitaxel (Abraxane)^[5] and liposomal daunorubicin (DaunoXome),^[6] and many are in various stages of clinical trials.^[7] Compared with conventional chemotherapeutics, these nanomedicines have indeed shown many

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advantages, including improved drug solubility, prolonged circulation in the blood compartments, enhanced bioavailability, and much reduced adverse effects from the parent drugs, offering patients greatly improved quality of life. One such example is pegylated liposomal doxorubicin (Doxil), which can attenuate drug accumulation in the heart and thus overcome the main dose-limiting side effect of doxorubicin (DOX), cardiomyopathy. However, current cancer nanomedicines have failed to improve the therapeutic benefits—these nanomedicines cannot or only modestly improve patients' overall survival.^[8] For instance, Genexol-PM, which is a polymeric micelle composed of a diblock copolymer of monomethoxy PEG-block-poly(D,L-lactide) (mPEG-PDLLA), allows a much higher paclitaxel dose without increasing the toxicity, but the higher doses still fail to generate better therapeutic efficacy.^[9] Recently, the Phase

II clinical trial of BIND-014 has also proved disappointing. Detailed clinical analyses showed that these nanomedicines were indeed able to accumulate more in tumor tissues than free drugs with fewer side effects,^[10] but such higher concentrations of drugs did not lead to better therapeutic efficacy. Thus, how to engineer nanomedicines to make them not only effectively target tumor tissues, but also effectively exert the drugs' therapy is the key to developing new-generation nanomedicines of high therapeutic efficacy.

We previously concluded the overall requirements (i.e. 2R2SP requirements) for a nanomedicine to achieve high efficiencies in the corresponding steps.^[11] Along this line, this review analyzes the cancer drug delivery process and concludes that a typical cancer drug delivery of a nanomedicine from the intravenous injection site to the cytosol of a tumor cell is a cascade of at least five steps, a CAPIR cascade; to guarantee high overall therapeutic efficiency it is critical to ensure no step of low efficiency. It further analyzes the nanoproperties in terms of surface, size and stability needed for the 2R2SP requirements. We find that these needed nanoproperties are different and even opposing in different steps, most of which can be summarized as the PEG, charge, size and stability dilemmas. To integrate all the nanoproperties overcoming the four dilemmas into one nanomedicine, we further propose the stability, surface and size nanoproperty transitions (3S transitions for short) for the nanomedicine self-adaptive to the 2R2SP requirements during

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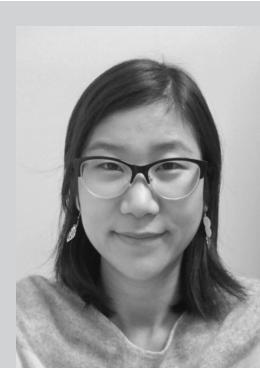
the CAPIR cascade. We comprehensively summarize the strategies that have been used to realize these transitions. At last, future research to design high-performance and the clinical translation of cancer nanomedicines is also discussed.

2. Nanomedicine Cancer Drug Delivery Process: The CAPIR cascade

The ultimate goal of cancer drug delivery is to ship drugs into cancer cells as free molecules to exert their pharmaceutical effects.^[12] Generally, an intravenously administered nanomedicine must go through a cascade of five steps:^[13] circulation in the blood compartments, accumulation in the tumor through its leaky, hastily built vasculature, subsequent penetration deep into the tumor tissue to reach tumor cells, internalization into those cells and, finally, intracellular drug release: the CAPIR cascade for short (**Figure 1a**). Thus, the overall delivery efficiency (Q), the percentage of the drug as free molecules inside targeted cells over the injected, of a system is a product of efficiencies of the five steps (Q_C, Q_A, Q_P, Q_I and Q_R). As a result, it is intuitive that while maximizing each efficiency is important to achieve high overall efficiency, it is more important to make sure none of the five efficiencies is close to zero; that would be the Achilles heel of the entire delivery cascade. This well explains the low therapeutic situation of Doxil in clinical use. It has been shown that Doxil has an expected circulation time longer than 30 h and effectively accumulated in tumors much more than free DOX, but its therapeutic efficacy was similar to that of DOX. Further analysis showed that a large amount of Doxil extravasated from the blood vessels in tumors but stayed around the periphery of the blood vessels.^[14] Thus, Doxil could not penetrate into the tumor to reach tumor cells, let alone the internalization and intracellular release.^[15]

3. Efficient Nanomedicine Needs: The 2R2SP Requirements and 3S transitions

For a nanomedicine, the CAPIR cascade is a long journey with a series of biological barriers and pitfalls. Immediately after intravenous injection, various plasma proteins may deposit onto the nanomedicine, causing opsonization and thereby elimination by the mononuclear phagocytic system (MPS).^[16] The reticuloendothelial system (RES), particularly liver and spleen, effectively sequesters foreign objects found in the blood.^[17] The physiological barriers (e.g., binding site barrier)^[18] in a solid tumor limiting intratumoral accumulation and distribution of nanomedicines are even more complicated.^[19] The poorly structured and heterogeneously distributed blood vessels make spatial distribution of nanomedicine heterogeneous in the tumor,^[20] while the dense extracellular matrix,^[21] tightly packed tumor cells^[22] and high interstitial fluid pressure^[23] make diffusion hard even for small molecules, let alone larger nanomedicines. The cellular membrane is a natural barrier that nanomedicines cannot diffuse through due to their large size; it also harbors P-glycoproteins as membrane-associated multidrug resistance to remove the released drugs,^[24] and the cytosol has genetic drug-resistance mechanisms^[25] such as drug metabolism.^[26]



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To overcome these barriers and successfully navigate the CAPIR cascade to finally ship drugs inside tumor cells as free molecules, a nanomedicine must have three capabilities in terms of drug carrying, surface and diffusivity.^[11] For drug carrying, it

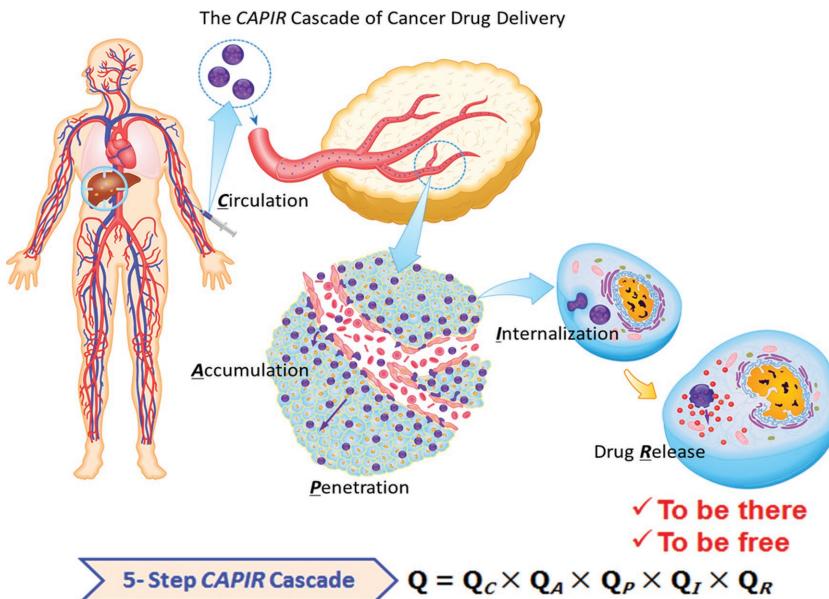


Figure 1. A sketch of the CAPIR cascade of a nanomedicine to deliver a free drug into cancer cells: circulation in the blood compartments, tumor accumulation and penetration, and subsequent cellular internalization and intracellular drug release. Reproduced with permission.^[13] The overall efficiency Q is the product of the five steps' efficiencies.

should first hold the drug tightly while en route to cancer cells (i.e. during the CAPI steps), but it must quickly release the drug once inside the cells (i.e., drug Retention vs. Release). For the nanomedicine surface, it should also be stealthy for a long blood circulation to give time for tumor accumulation (the C step) and slippery while in tumor tissues for tumor penetration (the P step); however, after reaching tumor cells, it must be able to interact with them (i.e., sticking to the cells) for efficient cellular uptake during the I step (i.e., surface Stealthy vs. Sticky). Furthermore, in the tumor tissue, the nanomedicine must have

good diffusivity to penetrate deep into the tumor to reach tumor cells remote from the blood vessels for cellular internalization (the P step). Thus, an effective nanomedicine must meet “drug Retention vs. Release (2R)”, “surface Stealthy vs. Sticky (2S)” and “tumor Penetration (P)” requirements, 2R2SP for short.^[11] Only if a nanomedicine is capable of simultaneously satisfying all of the 2R2SP requirements at the right time and in the right place would it go through the CAPIR cascade and bring free active drugs inside cells. These traits will produce overall high therapeutic efficacy and a good prognosis.

The corresponding nanoproperties needed for the 2R2SP requirements of a nanomedicine are shown in Figure 2. The 2R corresponds to the nanomedicine's stability transition. That is, the drug molecules are either firmly conjugated to or encapsulated in the carrier during the CAPI steps, but once inside cells, either the conjugation linker is cleaved or the carrier disassembles to release the drug. The 2S corresponds to the nanomedicine's surface-property transition, from being pegylated, neutral and hiding binding

groups (e.g. TAT groups) for a stealth surface during the CAP steps to being depegylated, positively charged and exposing binding groups for effective cell-membrane binding for internalization. The tumor-penetration ability of a nanomedicine is mainly determined by its size and charge. For effective P, the nanomedicine must be small, less than 30 nm.^[27] In contrast, larger sizes are reported to favor blood circulation and tumor accumulation.^[28] Recently, cationic charges were shown to improve tumor penetration,^[29] while neutral or a slightly negatively charged surface favors long blood circulation.^[30]

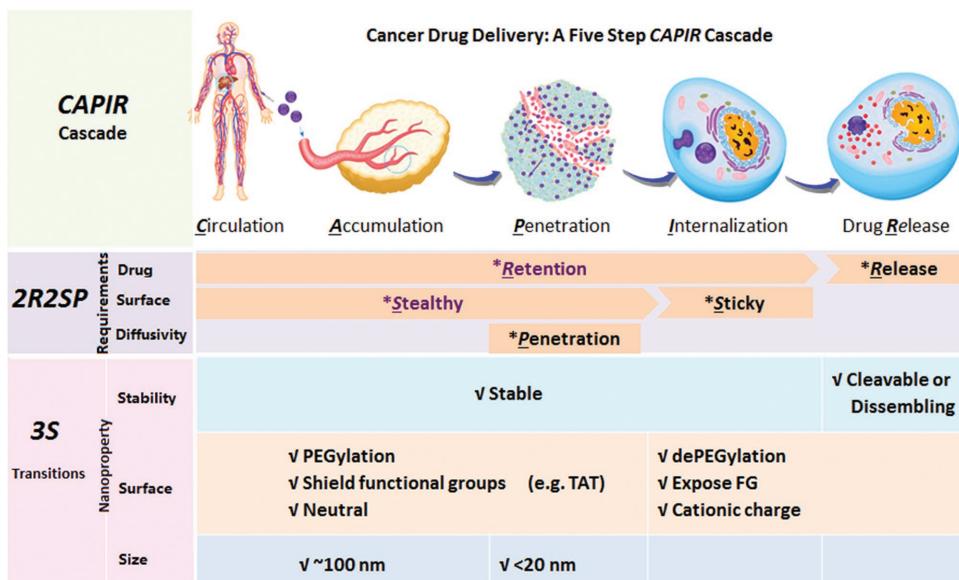


Figure 2. Summary of the 2R2SP requirements and the 3S transitions in the CAPIR cascade for a nanomedicine to have high overall drug-delivery efficiency.

As shown in Figure 2, these needed nanoproperties for the optimal efficiency of each CAPI step are different and even opposed, but they can be easily grouped into three transitions of the nanoproperties: stability (from being stable in the CAPI steps to being unstable or disassembling in the R step), surface (from being neutral/pegylated/shielding functional groups in the CAP steps to being cationic/de pegylated/exposing functional groups in the I step) and size (from being large in the CA steps to being small in the P step), the 3S transitions. Therefore, it can be concluded that a nanomedicine capable of the 3S transitions will meet the 2R2SP requirements and be able to efficiently traverse the whole CAPI cascade, efficiently delivering active drugs into tumor cells and giving rise to high therapeutic efficacy with few side effects.

4. Approaches to Achieve the 3S Transitions

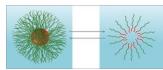
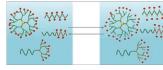
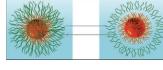
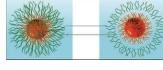
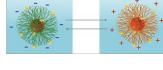
In the past decades, numerous so-called stimulus-responsive or smart nanomedicines have been designed to achieve some of the 3S transitions. In the following section we summarize these strategies in detail (Table 1).

4.1. Stability Transition

For cancer-drug delivery, intracellular drug release is optimal in most cases for several reasons. Premature drug release from nanocarriers in the blood compartment would not only reduce the amount of the drug arriving at tumors, but also cause systemic toxicity.^[31] Many free drugs can barely penetrate deep into tight tumor tissues due to their intermolecular interactions,^[32] while properly designed nanocarriers may penetrate well into tumors by reducing their interactions with the tumor matrix.^[13,33] Furthermore, endocytosis of nanomedicines and subsequent intracellular drug release would enable drugs to circumvent the membrane-associated multidrug-resistance mechanisms^[25a,34] and even intracellular drug resistance mechanisms if the release occurs inside the nucleus.^[35] However, once internalized, the nanomedicines must release the drugs; only free drugs can exert their pharmaceutical actions,^[36] and fast release leads to better efficacy^[33b,35a,37] compared to the diffusion-controlled slow drug release.^[38]

Therefore, an effective nanomedicine must stably retain drugs during the CAPI steps. Polymer-drug conjugates generally have no burst release problem as long as the linkers are stable in the blood.^[39] Micellar nanomedicines suffer most

Table 1. Strategies for 3S transitions.

3S transitions			
Transitions	Approaches	stimuli	Ref.
Stability transition	 <p>Stimuli-sensitive dissociation (micelles or liposomes)</p>	pH	[50,55]
		redox	[51]
		enzyme	[52,56]
		heat	[53,60]
		light	[54,61]
		ATP	[57,63]
Surface transition	 <p>Drug conjugate with reducible linker</p>	membrane fusion	[58]
		pH	[46a,b]
		redox	[47]
		enzyme	[48a]
		pH	[62b]
		redox	[62c,d]
Size transition	 <p>Capped with reducible stopper</p>	enzyme	[62e]
		light	[62f]
		pH	[87,90–94]
		redox	[88]
		enzyme	[89,95]
		pH	[87,90–94]
Surface transition	 <p>Detachable PEG shielding</p>	pH	[87,90–94]
		redox	[88]
		enzyme	[89,95]
		pH	[87a,103]
		enzyme	[89a,104,106]
		pH	[87a,103]
Size transition	 <p>Charge-conversional surface</p>	pH, β-carboxylic amides	[35a,114,169]
		pH, protonation/ deprotonation	[115,116]
		redox	[170]
		enzyme	[123c]
		light	[123a,b]
		enzyme	[124–126]
Size transition	 <p>Size-shrinkable nanocarrier delivery system</p>	mesoporous nanocarrier	[33a]
		multistage nanocarrier deliver system	[124–126]
		enzyme	[33a]
		mesoporous nanocarrier	[124–126]
		multistage nanocarrier deliver system	[33a]
		enzyme	[33a]
Size transition	 <p>"Cluster bomb"-like nanocarrier Delivery system</p>	PH	[171]
		membrane fusion	[13,172]
		PH	[171]
		membrane fusion	[13,172]
		PH	[171]
		membrane fusion	[13,172]

from the burst release problem because of drug adsorption on the micelle core/shell interface^[40] and micelle dissociation.^[41] Improving micelle stability by lowering the critical micelle concentration,^[42] loading drugs inside the core,^[40,43] covalently crosslinking the micelle cores^[44] and chemically conjugating drugs to the core^[45] can reduce or even eliminate premature drug release.

Once taken up into cancer cells, the nanomedicine should undergo a stability transition triggered by intracellular signals

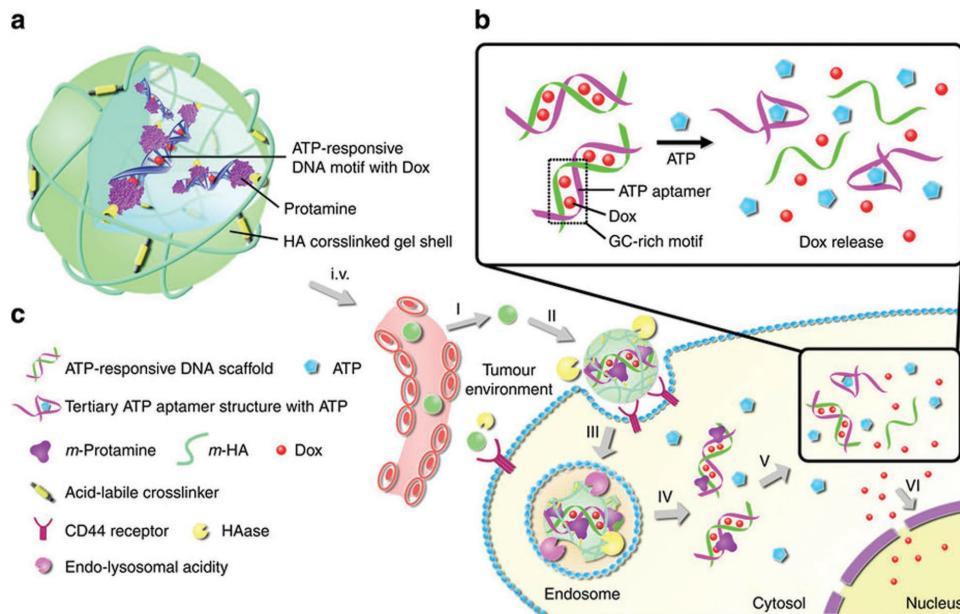


Figure 3. Scheme of the ATP-triggered Doxorubicin release system. Reproduced with permission.^[63] Copyright 2014, Nature Publishing Group.

to release the drugs. For polymer–drug conjugates, the key is to use blood stable but intracellular labile drug linkers including acid-labile bonds^[46] (such as hydrazone, oxime or acetals) responding to the lysosomal acidity, GSH-sensitive disulfide bonds^[47] responding to the elevated GSH level in tumor cells, or enzyme-cleavable bonds.^[48] For micelles, solubilizing the hydrophobic cores is a direct way to induce a stable-to-unstable transition for fast drug release.^[34b,37c] Stimuli-responsive micellar nanomedicines^[49] are designed to respond to such intracellular stimuli as lysosomal pH,^[50] redox gradients^[51] and enzymes,^[52] and external stimuli including temperature^[53] and light.^[54] Adenosine-5'-triphosphate (ATP), the energy molecule in the cells, has also been proposed as a trigger for the transition. Gu et al. demonstrated ATP-binding aptamer-incorporated DNA motif could trigger the release of intercalated doxorubicin through a conformational switch once the system located in an ATP-rich microenvironment (Figure 3). Similarly, liposomes can be triggered to dissociate by such intracellular triggers as pH change,^[55] enzymes,^[56] ATP^[57] and membrane fusion,^[58] and remote triggers^[59] including heat,^[60] ultrasound and light.^[61] Another type of nanomedicine is porous silica nanoparticles or nanocapsules, whose pores are capped with intracellular-labile stoppers; uncapping the pores is triggered by similar stimuli to release of the drugs.^[62]

The importance of the stable/unstable transition is even pronounced in cationic polymer-mediated gene delivery. Cationic polymers are generally used to condense DNA into polyplex nanoparticles to protect DNA from degradation and facilitate its cellular internalization and even nuclear localization.^[64] DNA/polymer complexes are thermodynamically stable and inherently resistant to dissociation due to the cooperative effects of multiple electrostatic interactions. Once inside the cells, such strong interaction and, thus, stability makes it difficult to release free DNA for transcription, the main barrier to efficient DNA transfection.^[65] Thus, the transition from extracellular

stable polyplexes to intracellular easily dissociating ones to release DNA is key to enhancing nonviral gene transcription efficiency.^[66] Several approaches have been used to design stimulus-responsive gene nanomedicines to create this stability transition. For example, long cationic polymers that can degrade into short segments triggered by intracellular signals—such as acid-labile polyethylenimine,^[67] GSH-cleavable disulfide-containing poly(amido amide),^[68] esterase-degradable poly(β -amino ester)s,^[69] and ROS-breakable poly(amino thioketal)^[70]—have improved transfection efficiency compared with unresponsive polycations because the intracellularly chopped short polymer chains cannot condense DNA and thus release the DNA for transcription. A polymer charge-reversal strategy from positive to neutral or negatively charged responding to intracellular signals can induce quick polymer/DNA dissociation to release the packed DNA for high transfection efficiency.^[66b,71]

4.2. Surface Transition

To achieve a stealthy-to-sticky transition, the nanomedicine's surface properties must undergo several changes including pegylation/depegylation, shielding/revealing targeting ligand and surface charge changes.

4.2.1. Depegylation

Poly(ethylene glycol) (PEG)^[72] along with other hydrophilic polymers including zwitterionic polymers,^[73] poly[N-(2-hydroxypropyl)methacrylamide] (HPMA),^[74] poly(2-oxazoline)^[75] and poly(L-glutamic acid)^[74–76] have been demonstrated to give nanomedicines a stealth property. These polymer chains can hinder adsorption of opsonic proteins and thus prevent scavenging by RES and MPS screening,^[17,77]

enabling nanomedicines to remain in blood circulation for long times, which has been recognized as essential for the nanomedicine's passive tumor targeting/accumulation by the EPR effect.^[78] Dense PEG shell also facilitates nanoparticle penetration in tumor. In nonviral gene delivery, the cationic surface of polyplexes must be modified with PEG^[79] or HPMA^[80] to make them resistant to serum and give long blood circulation times. Thus, pegylation (including introducing other hydrophilic polymers) is essential for a nanomedicine during the CA steps. However, for cellular internalization including endocytosis, fusion and direct membrane penetration, interaction of the nanomedicine with the cell membrane is essential.^[81] The hydrophilic stealth layer weakens this interaction due to the steric effect and water-cushion effect, and thereby slows the nanomedicines' cellular uptake.^[82] Furthermore, pegylated nanomedicines are generally endocytosed into lysosomes and cannot diffuse through or rupture the lysosomal membrane, and thus have a lysosomal trapping problem.^[83] In contrast, depegylated nanomedicines eliminating the interaction barrier are quickly internalized and can disrupt lysosomes to escape.^[49d,84]

The pegylation/de pegylation transition has thus been used to solve this so-called PEG dilemma,^[82a,85] in which the PEG layer is stable on the nanomedicine in blood circulation but it sheds the PEG shell at the extracellular site. The key to this sheddable PEG layer is the labile linker between the PEG chain and the hydrophobic chain, which should be cleavable in response to such stimuli^[86] as pH,^[87] reducing agents,^[88] or enzymes.^[89] Acid-triggered PEG-sheddable nanomedicines have been extensively investigated using various pH-labile linkages such as hydrazine,^[90] acetal,^[87b,91] β -thiopropionate,^[92] phosphoramidate bond^[93] and acid-labile amide.^[94] For instance, A PEG-cleavable lipid, via an acid-labile vinyl ether linker,^[87b] was used for pegylation/de pegylation of liposomes. At the acidic lysosomal pH, the vinyl ether linker hydrolyzed and the PEG layer was removed from the nanomedicine's surface, enabling to fuse with the lysosomal membrane for escape.^[87b] Kataoka et al.^[88a] designed a disulfide-linked block cationomer, PEG-SS-P[Asp(DET)], which formed a stable polyplex micelle with plasmid DNA. The PEG-shell detached in the intracellular high-GSH microenvironment. Enzyme-sensitive PEG deshielding systems have been developed using short peptides as the linker, which can be tumor-selectively cleaved by matrix metalloproteases (MMPs).^[89a,95] For instance, Torchilin et al. synthesized a PEG-peptide-PTX prodrug, where the peptide was a MMP2-cleavable octapeptide. The prodrug assembled with two kinds of phospholipids to create a MMP2-sensitive nanostructure. When the nanostructure extravasated to the tumor sites, the peptide was cleaved by the up-regulated extracellular MMP2, liberating the active drug and accomplishing the de pegylation.^[89a]

4.2.2. Shielding/Exposing Targeting Ligands or Functional Groups

Nanomedicines conjugated with cancer-specific targeting ligands (e.g., monoclonal antibodies, peptides, and small molecules) have been extensively explored to enhance tumor targeting.^[1a,12a] In particular, the ligands may enable the nanomedicines to recognize and stick to tumor cells having the corresponding receptors, triggering receptor-mediated endocytosis

of nanomedicines (I step). However, conjugation of targeting moieties may affect nanomedicines' stealth property and reduce their blood-circulation times due to the ligands' hydrophobicity or charges.^[96] For instance, grafting too many folic-acid ligands on the distal end of a PEG layer might induce the elimination of nanomedicines by the macrophages. Lu et al. prepared PEG-DSPE micelles with variable folate contents on the surface by adjusting the molar ratio of FA-PEG-DSPE and MPEG-DSPE.^[97] The micelles at a 1:100 molar ratio of FA-PEG-DSPE to MPEG-DSPE simultaneously avoided macrophages and had highly selective targeting ability. Furthermore, targeting groups may affect the penetration of the nanomedicines into tumor tissue. It has been reported that antibodies conjugated on nanomedicines retard penetration and cause a heterogeneous distribution of the nanomedicines due to binding-site barrier as result of antigen-antibody interaction.^[98] Many functional groups are needed for tumor penetration and cellular internalization. For instance, cell-membrane transduction peptides (CPPs) such as TATp ship nanomedicines into cells very efficiently and thus TATp-functionalized nanomedicines have very fast cellular internalization.^[99] However, CPPs are generally rich in arginine and lysine residues and thus carry positive charges, which cause nonselective interactions with cells or tissues; thus, CPP-functionalized nanomedicines distributed throughout the body when intravenously injected.^[100] Clearly, targeting ligands and other functional groups have great impacts on a nanomedicine's circulation, accumulation and penetration steps and the overall active targeting efficiency.^[101]

The density of targeting ligands can be adjusted to minimize their impact on the nanomedicine's blood circulation and tumor accumulation while increasing cellular uptake.^[102] Shielding the targeting/functional groups during blood circulation but exposing them once in tumors is the prevailing approach^[103] to this dilemma. Bae et al. formulated super pH-sensitive micelles with poly(L-histidine) (PHis) as a tumor extracellular pH-sensitive actuator for ligand exposure in the tumor.^[103a] For example, a mixture micelle was made from poly(L-lactic acid)-*b*-PEG-*b*-poly(L-histidine)(2kDa)-TAT (PLA-PEG-Phis-TAT) and PLA-PEG. At pH 7.4, nonionized water-insoluble PHis made the TAT stick to the hydrophobic PLA core surface hiding within the hydrophilic PEG corona, inactivating it.^[103b] However, at the weak acidic pH of 7.0 or 6.8, PHis was ionized, became soluble and extended, presenting the TAT moieties at the micelle shell surface and inducing fast internalization.^[103b] A similar strategy was used to expose a cell-interacting biotin ligand.^[103a] Recently, the exposure of targeting ligands/functional groups by de pegylation has also been realized.^[87a,89a,104] For example, a nanomedicine was pegylated through MMP-cleavable peptide while TATp was linked to the core using a short PEG chain with a stable linker. The TAT moieties were thus buried in the PEG bushes during blood circulation, but in the tumor microenvironment, once the peptide linker was cleaved by the up-regulated extracellular MMP, the PEG shell was shed and the hidden TATp was exposed.^[89a] Similarly, a pH-sensitive PEG-sheddable nanomedicine exposing CPP was reported by Wang et al.^[87a] Another strategy to expose active CPPs is to remove their deactivating patches. Cationic CPPs were linked with short anionic segments to shield the cationic charges by electrostatic interaction, thereby inhibiting their nonspecific

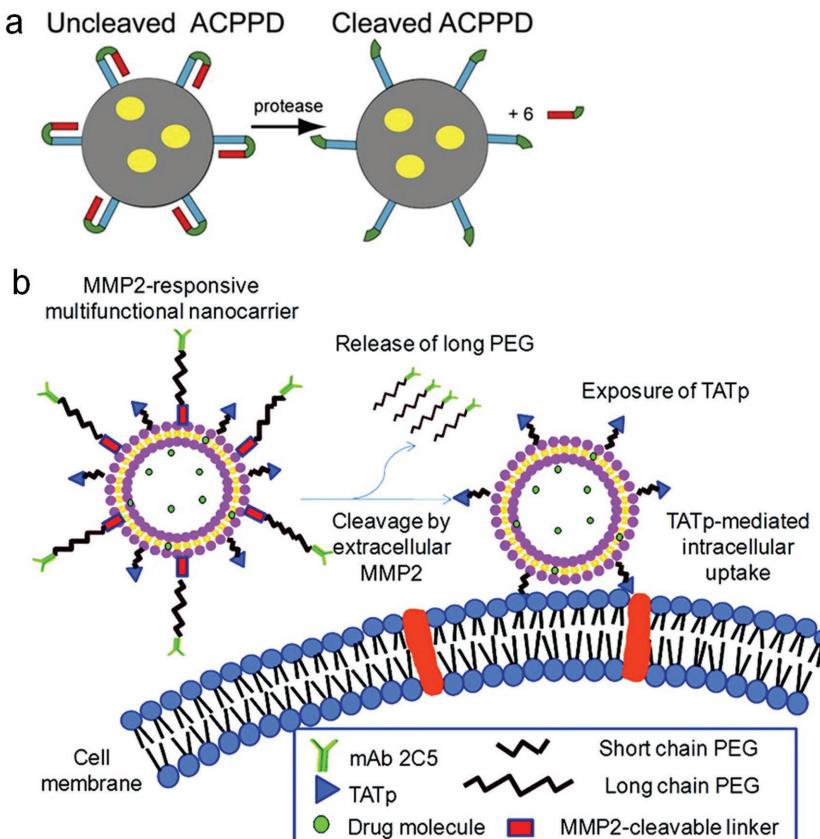


Figure 4. a) Schematic illustration of the exposure of active CPPs via removing the deactivating patches. Reproduced with permission.^[106a] Copyright 2010, National Academy of Sciences. b) Scheme of MMP2-responsive multifunctional liposomal nanocarrier and its drug delivery strategy. Reproduced with permission.^[89b] Copyright 2012, American Chemical Society.

interactions. The linkers were designed as acid- or enzyme-labile in the tumor extracellular microenvironment. The linker cleavage leads to the disassembly of the anionic patch and exposure of the CPPs.^[105] For example, Tsien et al. developed MMP2 activatable cell penetrating peptides in which the polyanions masked the polycation until MMP2 cut the linker, exposing TAT to adhere to cells (Figure 4a).^[106] Torchilin et al. also developed MMP2 sensitive multifunctional liposomal nanocarriers using MMP2-cleavable octapeptide as a linker to anchor PEG chains. The CPP was shielded by dense PEG chains during circulation but exposed in the tumor microenvironment once the MMP2 cleaved the linker (Figure 4b).^[89b]

4.2.3. Surface-Charge Reversal

Surface charge is another important feature that can manipulate the nanomedicine's stealth property in the bloodstream while enhancing cellular interaction once in the tumor.^[30a,35a,37b,107] Positively charged nanomedicines are quickly cleared from the bloodstream by MPS and thus have very short circulation times.^[108] A highly negatively charged surface (ζ -potential ≈ -40 mV) also induces MPS clearance compared to the neutral nanomedicines (ζ -potential $\approx \pm 10$ mV).^[109] Hence, it is important to first mask the positive or negative charges during blood circulation. Furthermore, nanomedicine surface charges also

influence their penetration in tumor tissues.^[110] Uncharged or stealthy particles can easily diffuse through tumors while those with either charge are inevitably trapped due to the electrostatic band pass formed by the dense extracellular matrix.^[111] After reaching tumor cells and for cellular internalization, positive charges are welcome because positive charges enable nanomedicines to stick to negatively charged cell membranes to trigger adsorption-mediated endocytosis.^[37a,b,112] Furthermore, the internalized nanomedicines are mostly transferred into lysosomes where a positively charged surface helps nanomedicines escape from the lysosomal trap.^[37b,113]

This surface-charge dilemma can be resolved by a charge-reversal strategy; that is, transition from being neutral during the CAP steps to positive in the I step enables nanomedicines to be stealthy in circulation for tumor accumulation and slippery for tumor penetration, but to internalize quickly, escape from the lysosome and target the nucleus. Such charge-reversal nanomedicines were developed using acid-labile β -carboxylic amides.^[35a,114] β -Carboxylic amides of primary or secondary amines are stable at basic pH but, at acidic pH, can undergo intramolecularly catalyzed hydrolysis to regenerate the corresponding amines, which are protonated and carry cationic charges in water.^[35a] The hydrolysis pH and rate of the amide depend on its structure^[113c,114a] (Figure 5). Introducing substituents or unsaturated double bonds makes

the amide to respond to the extracellular acidity (pH 6.5–7) or lysosomal pH (4–5).^[113c,114a] Our group^[35a,47a,52d,113c,114a,b] and others^[114d,115] have demonstrated that the polymers with acid-labile β -carboxylic amides have low interaction with blood components for long blood circulation, while regenerated amine groups promote cellular uptake or nuclear localization.^[35a] In addition, charge reversal can also be achieved through zwitterionic polymers by controlling the protonation/deprotonation ratio of protonizable moieties such as amino and carboxyl groups.^[115,116]

4.3. Size Transition

It is well recognized that the size of a nanomedicine is an important nanoproperty that affects its blood circulation time and tumor accumulation.^[117] Nanomedicines of about 100 nm have been found to have a longer blood circulation and exhibit better tumor accumulation due to the EPR effect,^[118] but this size is too big for the nanomedicines to diffuse into the tumor and thus accumulate at the blood-vessel extravasation sites with little penetration in tumor tissues,^[14,28,107,119] making them inaccessible to the cells remote from the blood vessels.^[120] Intuitively, small drug molecules would have the best diffusivity and the drug released at extravasation site would diffuse deeper and distribute more uniformly in the tumor tissue. However,

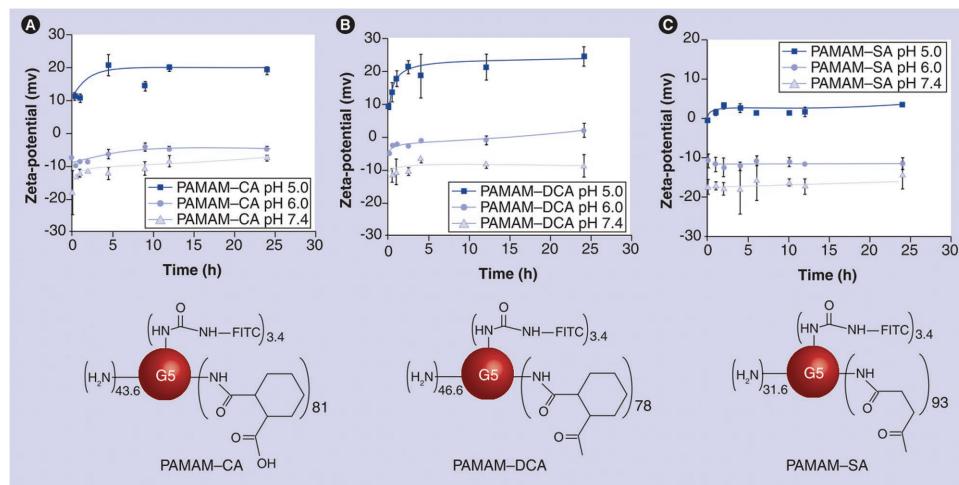


Figure 5. The charge-reversal kinetics of the amides of different structures at different acidities. Reproduced with permission.^[113c] Copyright 2010, Future Medicine Ltd.

many small-molecule drugs cannot diffuse easily due to avid drug binding to the dense extracellular matrix and tightly packed tumor cells close to the unevenly distributed blood vessels,^[32] not to mention their difficulty in getting into cells due to multidrug resistance at the cell membrane.^[25a,34a,121] In contrast, stealth nanomedicines smaller than 20 nm are capable of deep tumor penetration.^[118c,122] Unfortunately, such small nanomedicines are likely to be quickly cleared during blood circulation.^[118c]

Obviously, nanomedicine needs a size transition to address this size dilemma: It should be around 100 nm in the blood compartment for long circulation but sufficiently small in tumor tissue for penetration. Several approaches have been proposed to realize such a size transition.^[123] Tong and co-workers^[123a] demonstrated a photoswitching nanoparticle composed of spiropyran and lipid-PEG, which shranked from 103 to 49 nm upon irradiation at 365 nm, thereby enhancing tissue penetration in a subcutaneous HT-1080 mouse tumor model. A multistage delivery system^[124] was developed using mesoporous silicon particles (Stage 1) loaded with one or more types of small nanoparticles such as quantum dots or single-walled carbon nanotubes (Stage 2) to prevent the enzymatic degradation and RES uptake of the Stage 2 nanoparticles in blood circulation. This approach was then used to deliver small nanoparticles loaded with DNA or chemotherapeutic drugs.^[125] Recently, the Ferrari team reported an injectable nanoparticle generator consisting of nanoporous silicon particles packaged with poly(L-glutamic acid)-DOX conjugate which could self-assemble into small nanoparticles of 30 – 80 nm in tumor tissues^[126] (Figure 6). Another multistage nanoparticle-delivery system was a 100-nm gelatin nanoparticle encapsulated with 10-nm quantum dots; after extravasating from the leaky tumor vasculature, degradation of the gelatin in the tumor microenvironment released the quantum dots.^[33a] We reported a general strategy for the fabrication of dendrimer/lipid nanoassemblies; the 40 nm nanomedicine underwent tumor-triggered disassembly and release of small (several nanometers) dendrimers for tumor penetration.^[13,127]

4.4. Tumor Penetration

In addition to the size-transition discussed above,^[13,33b,123] other strategies have also been explored to improve tumor penetration ability of nanomedicines,^[128] including functionalizing nanomedicines with tumor penetrating peptides^[127,129] and modulating tumor extracellular matrix.^[130] Tumor-homing and -penetrating cyclic peptide iRGD,^[127,131] tLyP-1,^[132] PFVYLI (PFV)^[133] have been shown to enhance the tumor accumulation and penetration of nanomedicines, particularly tumor penetrating peptides containing a cryptic (R/K)XX(R/K) CendR element that must be C-terminally exposed to trigger neuropilin-1 (NRP-1) binding, cellular internalization and malignant tissue penetration.^[134]

Remodeling tumor microenvironments has been shown effective in enhancing nanomedicine penetration and thus efficacy.^[135] Recently, imatinib mesylate was found able to normalize the tumor microenvironment by inhibiting platelet-derived growth factor receptor-beta expression, tumor vessel normalization, and improving tumor perfusion.^[136] Disrupting tumor extracellular fibronectins by cyclopamine,^[137] depleting tumor collagen by losartan,^[137] inhibiting the expression of collagen I and TGF-beta by pirfenidone,^[136] inhibiting the TGF-beta signaling pathway by LY364947^[138] and reducing tumor extracellular matrix by collagenase^[139] have been shown to significantly improve tumor perfusion and thereby nanomedicine accumulation and intratumoral distribution. For instance, cancer-associated fibroblasts (CAFs), the major stromal cell type in the tumor microenvironment, play a key role in formation of the stromal barrier, leading to poor penetration for particulate therapeutics and even low perfusion for molecular drugs. Nie's group developed peptide-based nanomaterials targeting and depleting CAFs to overcome the problem (Figure 7). A dual-mode nanomaterial, active CAFs targeting via the mouse monoclonal antibody (mAb) combined with increased cellular uptake of peptide nanoparticles (PNP) coordinated by CPP and cholesterol, improved the tumor penetration of chemotherapeutic drug by depletion of CAFs and breakage of the stromal barrier for the treatment of CAF-rich solid tumors.^[140]

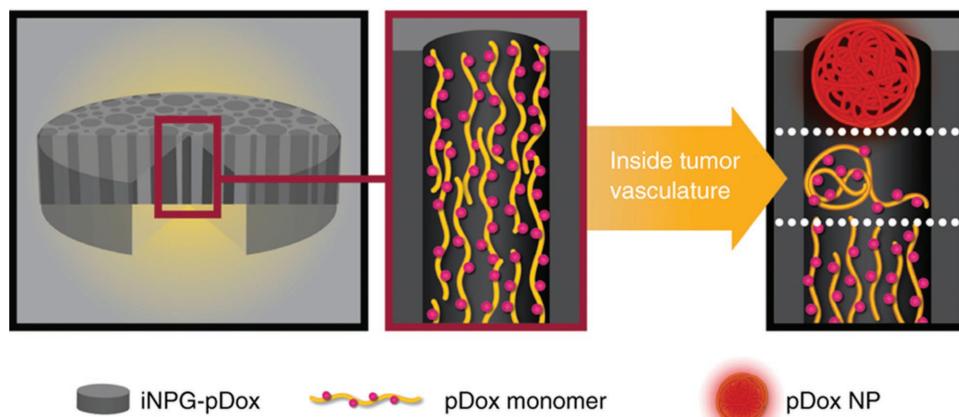


Figure 6. Scheme of an injectable nanoparticle generator. Nanoporous silicon particles were packaged with poly(L-glutamic acid)-DOX conjugate which could self-assemble into of 30–80 nm nanoparticles in tumor tissues. Reproduced with permission.^[126] Copyright 2016, Nature Publishing Group.

In other work, a CAF-targeting drug delivery nanosystem based on a cleavable amphiphilic peptide designed to be specifically responsive to FAP, a membrane-bound serine protease specifically expressed on CAFs.^[141] This nanomedicine transformed from self-assembled nanofibers to spherical nanoparticles could disrupt the stromal barrier, and enhance local drug accumulation. However, how to further improve the efficiency is still the key.

5. Problems in Current Nanomedicine Design

As discussed above, the needed nanomedicine properties for effective cancer-drug delivery have been extensively studied and various designs of nanomedicines including stimuli-responsive^[49c,86,115,142] or recent multistage nanosystems^[33a,124a,143] have been proposed to realize surface, stability or size transitions for better therapeutics. Yet,

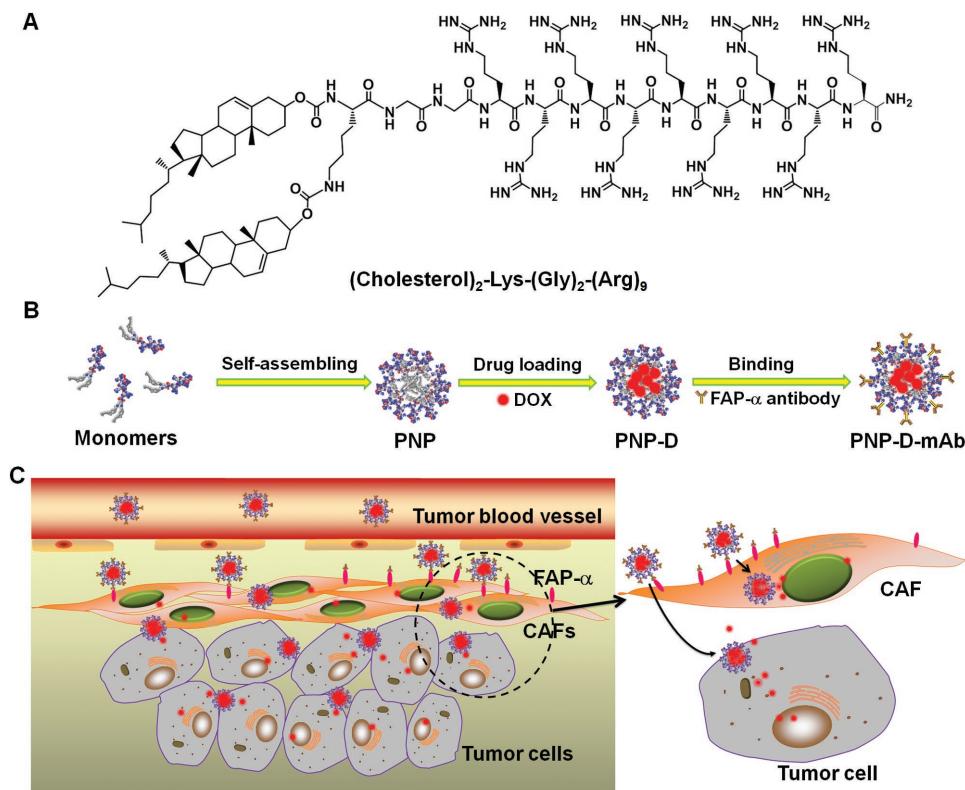


Figure 7. A dual-mode nanomedicine with the ability of cancer-associated fibroblasts (CAFs) targeting and efficient cell penetration (PNP-D-mAb). A) The structure of the cholesterol-modified CPP. B) Schematic illustration of the nanoparticle formation process including peptide assembling, drug loading, and mAb modification. C) The proposed mechanism of PNP-D-mAb in CAFs targeting and drug penetration. Reproduced with permission.^[140]

none of them simultaneously realizes all 3S transitions. Using our own work as an example, we developed a “charge-reversal” technique^[35a,47a,52d,113c,114a,b] to realize a nanomedicine’s surface charge transition (Figure 8). We converted the amines in the PEI chain of PCL-b-PEI to acid-labile β -carboxylic amides to shield the positive charges. The formed micelles were negatively charged or neutral at the physiological pH.^[35a] Once at acidic pH, the amides hydrolyzed and regenerated the amines carrying cationic charges. Thus, the nanoparticles were negatively charged or neutral in blood circulation but once in the tumor’s acidic extracellular medium, the nanoparticles became positively charged for fast cellular uptake, and highly positively charged in acidic lysosomes for lysosomal escape and nuclear localization. Similarly, the charge-reversal approach was used to modify TATp by amidizing its lysine residue amines to succinyl amides (^aTAT) to inhibit its nonspecific interactions in the bloodstream (Figure 8c).^[114e] Thus, ^aTAT-functionalized PEG-PCL micelles achieved long circulation in the blood compartments. Once in the acidic tumor microenvironment, the ^aTAT hydrolyzed and regenerated the active TAT, which helped the micelles get into cancer cells. The charge reversal provides a successful approach to realize the nano-carriers’ surface charge transition, helping nanomedicines complete the CAI steps and giving rise to enhanced antitumor activity. However, the design did not address the other two “S” transitions and thus did not perform well in the P and R steps.

Cell membrane coated nanomedicine is one of the recent focuses made a great success in the CA steps of the cascade, especially the circulation step (e.g., reducing opsonization, delaying phagocytic uptake, binding inflamed endothelium and facilitating transport across the endothelial layer).^[144] The novel strategy increases the accumulation of the nanomedicine at tumor sites by targeting the source cancer cells via a homotypic binding mechanism.^[145] However, attention should also be paid to the following steps including drug transport in the tumor (penetration) and inside tumor cells (internalization & release).

While it is generally accepted that small size favors nanomedicines’ tumor penetration, the optimal sizes for blood circulation/tumor accumulation vary according to the report. For instance, medium sized (60 nm), PEG-coated gold nanoparticles were found to accumulate more at the tumor sites than those of small (20 and 40 nm) and large (80

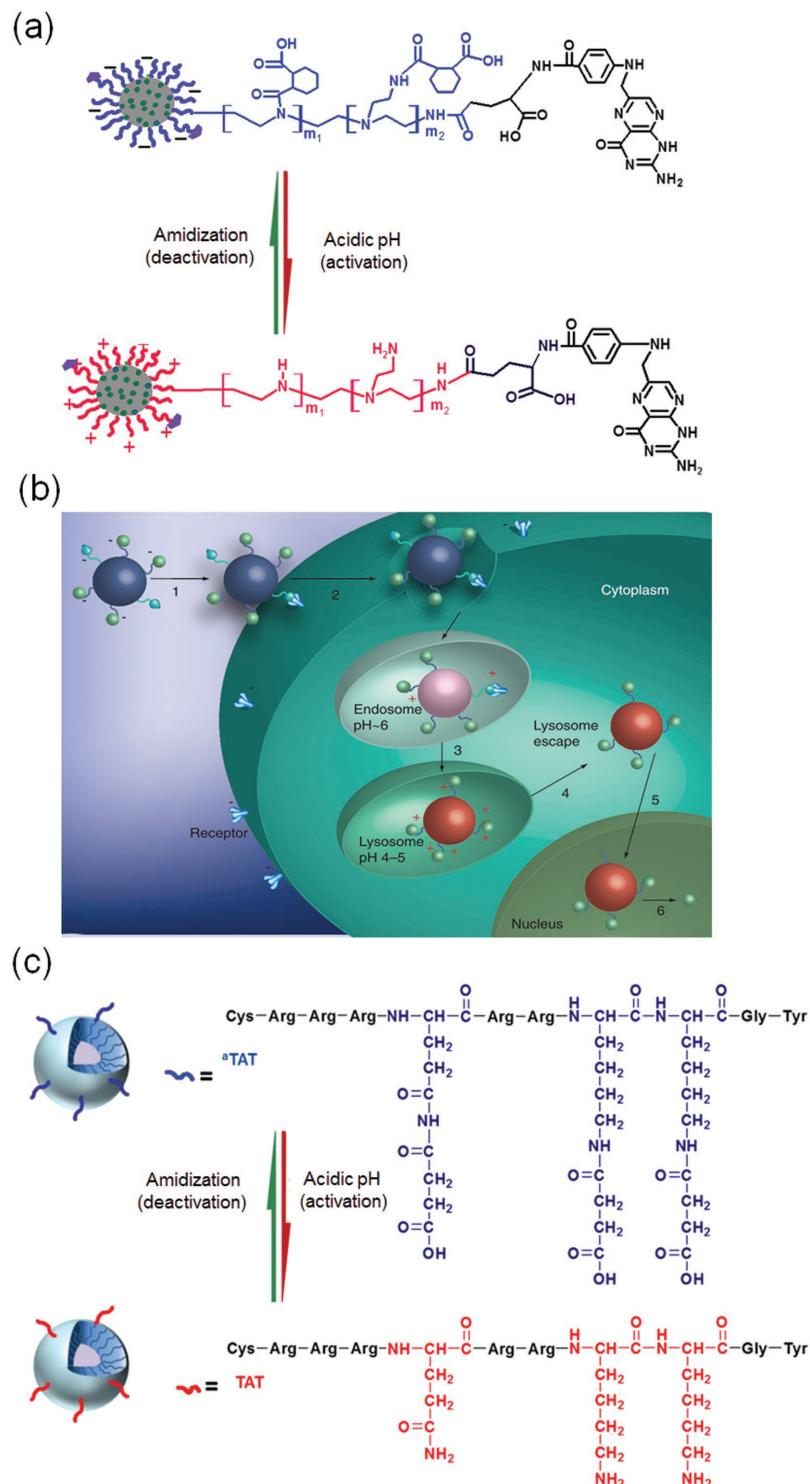


Figure 8. Charge-reversal technique to realize the surface transition. a,b) The chemical structure of a charge-reversible PCL-PEI/amide-FA nanoparticle and its charge reversal for drug delivery. Reproduced with permission.^[35a] c) The charge-reversal TAT. Reproduced with permission.^[114e] Copyright 2013, American Chemical Society.

and 100 nm) sizes;^[27] in contrast, ultrasmall (2 and 6 nm) tiopronin-coated gold nanoparticles were found circulating longer than small (15 nm) ones.^[146] While 25 nm micelles

were cleared more rapidly, and thus accumulated less than those of 60 nm,^[147] micelles with the size from 30 to 100 nm all had similar blood clearance, tumor accumulation and anti-cancer activity in hyperpermeable tumors.^[28] This controversy stems from that nanomedicines' pharmacokinetic properties are affected not only by their sizes, but also their chemical structures and physical properties. Nanomedicines with different sizes often also differentiate in their chemical block lengths and ratios,^[148] as well as stability, charge, mechanical properties, PEG-chain length or density, and even hydrophilic shell thickness.

Recently, we fabricated micelles with size as the only variable to elucidate the size effects on the CAPIR cascade^[118c] (**Figure 9**). The micelles were fabricated with sizes ranging from 20 to 300 nm from single-block amphiphilic copolymers of 7-ethyl-10-hydroxyl-camptothecin (SN38) prodrug to keep other properties the

same. The 100–160 nm micelles had longer blood circulation and better tumor accumulation than those of 20–30 nm. However, the 100–160 nm micelles penetrated only slightly into the tumor tissue, whereas those of 30 nm penetrated much more deeply, resulting in almost no difference in therapeutic efficacy. Therefore, tailoring the nanomedicine size to meet the requirements in each step of the CAPIR cascade is critical.

One exception that may not need the whole CAPIR cascade is the nanomedicines designed for photothermal therapy (PTT).^[149] PTT employs the photosensitizers to generate heat for thermal ablation of cancer cells upon NIR laser irradiation. Photosensitizer accumulation in tumor is sufficient and thus only the circulation and accumulation steps are needed. For PTT, the tumor-microenvironment-induced aggregation has been demonstrated as a strategy to improve the intratumoral accumulation due to assembly induced retention effect.^[150]

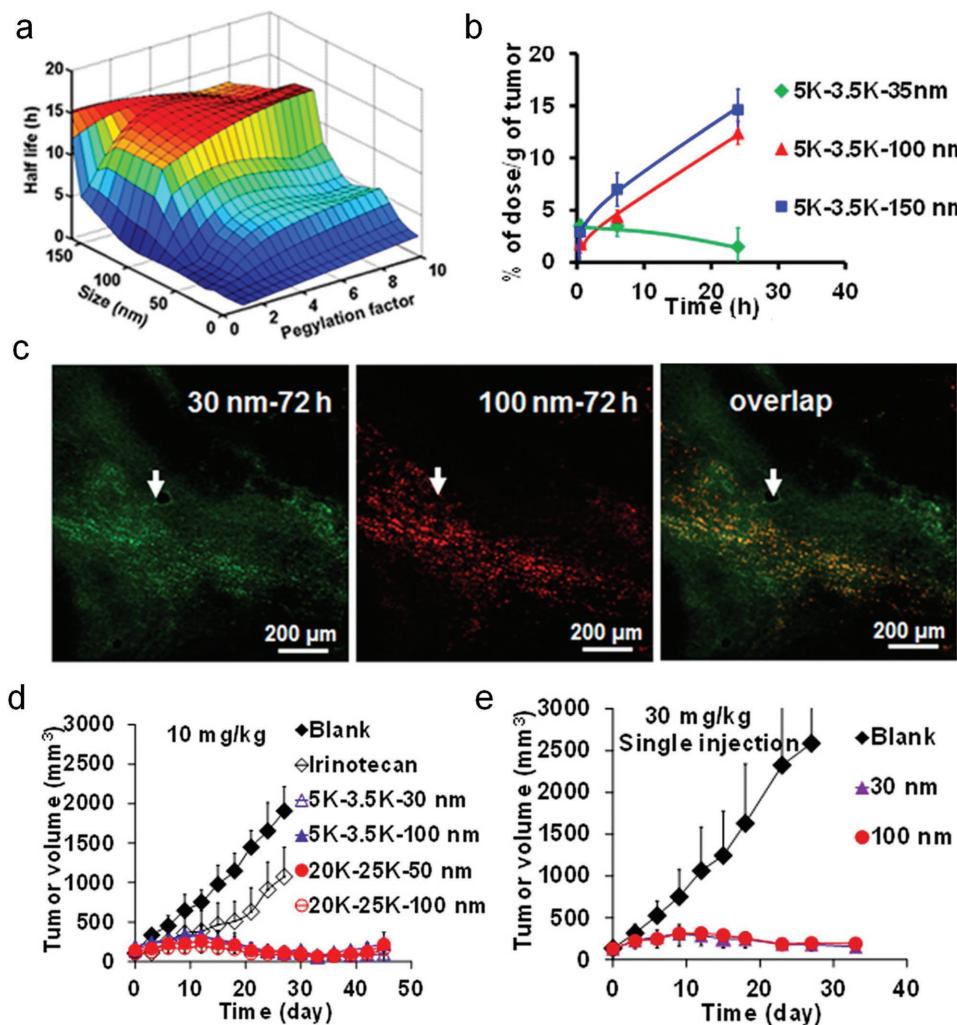
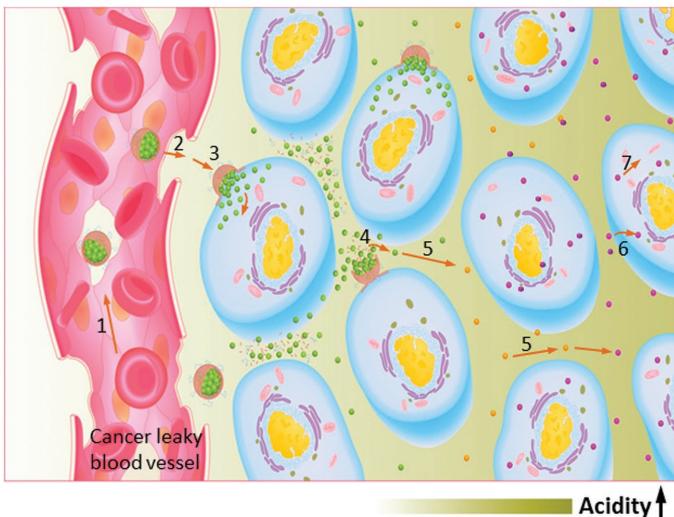


Figure 9. The calculated blood-circulation half-life of PEGx-P(HEMASN38) y micelles as functions of their size and pegylation factor (defined as PEG mass fraction in the block copolymer) (a). Tumor accumulation of intravenously injected micelles (b) and the histological imaging of intratumoral distribution of micelles with size of 30 or 100 nm (c). Anticancer activity of micelles tested on BCap37 xenografted tumors, tumor volume as a function of time of tumor-bearing mice treated with micelles (30 or 100 nm) of PEG5K-P(HEMASN38)3.5K or (50 or 100 nm) of PEG20K-P(HEMASN38)25K at an SN38-eq dose of 10 mg kg⁻¹ (every three days for five cycles) (d), or single intravenous injection of micelles (30 or 100 nm) of PEG5K-P(HEMASN38)3.5K at an SN38-eq dose of 30 mg kg⁻¹ (e). Reproduced with permission.^[118c] Copyright 2015, American Chemical Society.



- Dendrimer, neutral
- Dendrimer, slightly positively-charged
- Dendrimer, highly positively-charged
- 1 Circulation in the blood stream
- 2 Extravasation into tumor tissue
- 3 Fusion with cell membrane inducing intracellular dendrimer release
- 4 Fusion with cell membrane inducing extracellular dendrimer release
- 5 Dendrimers penetrating into tumor and becoming positively charged as the acidity increases
- 6 Positive charges inducing adsorption onto cell membrane and endocytosis
- 7 Intracellular drug release

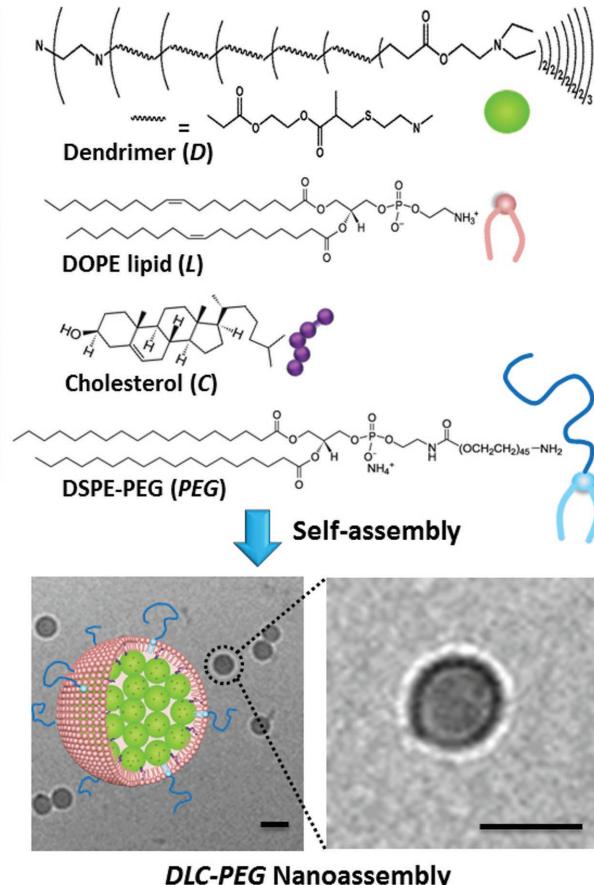


Figure 10. Sketch of the “cluster bomb”-like nanoassembly (left side) and its 3S transition and traversing the CAPIR cascade (right side): The nanoassembly was a pegylated lipid-coated assembly of 5 nm pH-responsive dendrimers. It circulated in the blood compartment and accumulated in the tumor via the EPR effect. Once in the tumor, fusion of the lipid layer with the cell membrane released the dendrimers intra- or extracellularly. The small and nearly neutral dendrimers in extracellular fluid further penetrated into the tumor tissue, where the extracellular pH is acidic (pH ≈ 6–7) and the dendrimer surface was then protonated and became positively charged, efficiently triggering fast cellular uptake. Once in the lysosomes, the dendrimers became soluble at the lysosomal pH and released the drug, bypassing the membrane-associated drug-resistance mechanisms. Reproduced with permission.^[13]

The similar treatment, photodynamic therapy (PDT) which uses photosensitizers to generate reactive oxygen species (ROS) to induce oxidation stress to kill cancer cells upon being illuminated with light,^[151] however, is different. Due to the extremely short life times of most ROS, the photosensitizers for PDT inside cancer cells and even inside cell nuclei can exert higher therapeutic activity.^[152] Thus, nanomedicines for PDT also need the CAPIR steps and thus 3S transitions.

6. Examples of 3S-Transition Nanomedicines

As discussed above, a nanomedicine must realize all 3S nano-property transitions to meet the 2R2SP requirements so as to efficiently traverse the CAPIR cascade for effective cancer-drug delivery. Manipulating nanoproperties to achieve the separate transition has been well demonstrated (Table 1); however, how to integrate all these needs into one system to simultaneously realize 3S transitions is the key to develop nanomedicines of high efficacy.

Our group recently engineered a dendrimer-lipid nanoassembly mimicking a “cluster-bomb” to achieve the 3S transitions^[13,153] (Figure 10). A sixth-generation nontoxic degradable polyaminoester dendrimer with a diameter of 5 nm was chosen as the “bomblet” for its ability to carry anticancer drugs^[154] and, more importantly, its pH-dependent 2-(*N,N*-diethylamino)ethyl termini. The dendrimer consisted of many tertiary amines in its backbone and was hydrophobic at the neutral pH but became soluble at pH around 6, and thus hydrophobic drugs could be encapsulated inside the dendrimer at the neutral pH, but released once at low pH. The lipid shell was composed of fusogenic DOPE phospholipid, pegylated DSPE-PEG lipid and cholesterol to keep the nanoassembly stable and stealthy in the blood compartment. The nanoassembly was around 45 nm and contained about twenty-seven 5 nm dendrimers in a pegylated lipid layer.

The nanoassembly was found to circulate in the blood similar to the well-known long-circulating PCL-PEG micelles (Figure 11a). Moreover, its tumor accumulation was 1.7 ($P = 0.038$) times that of PCL-PEG micelles (Figure 11b). In

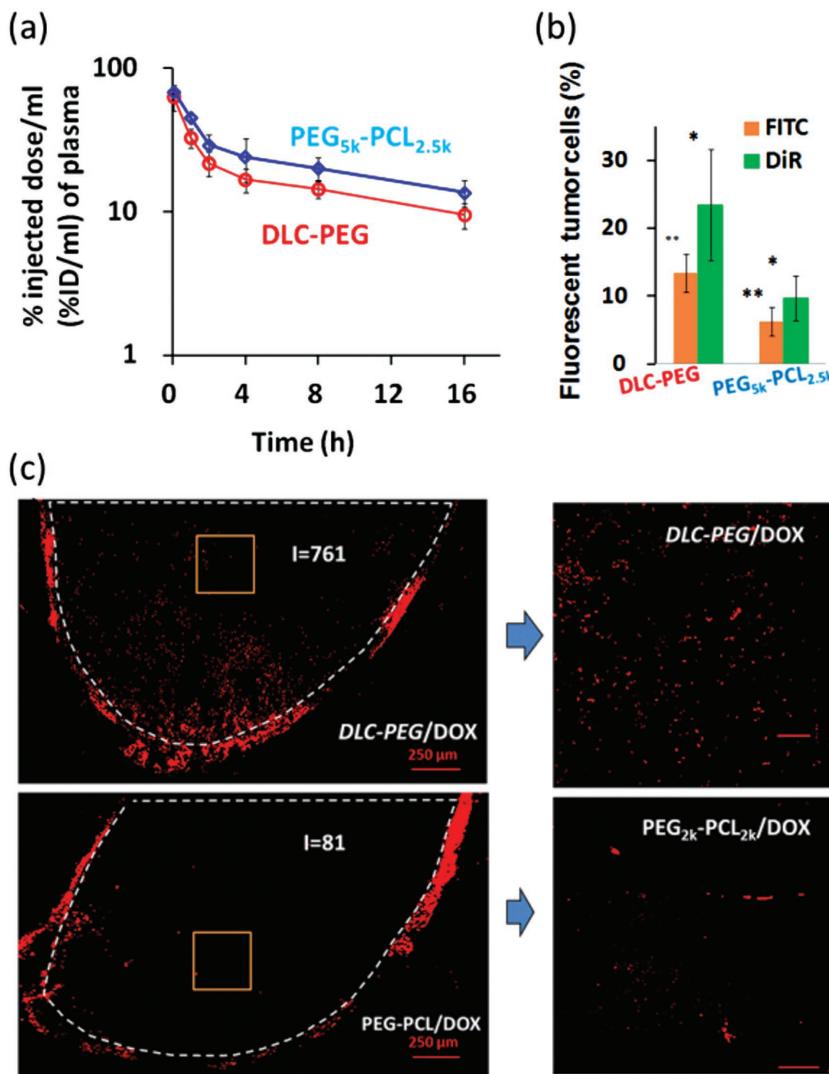


Figure 11. In vivo blood clearance (a) and tumor accumulation (b) of the DiR-loaded nanoassembly and PCL-PEG micelles (control) in female athymic mice after a single dose of $0.15 \text{ mg DiR kg}^{-1}$ body weight. c) Intratumoral DOX distribution of tumors treated with DOX-loaded nanoassembly or PCL-PEG micelles. Confocal images obtained by tile scan of the slides ($10\text{-}\mu\text{m}$ thick) sectioned from the treated BCAP-37 tumors. DOX is shown in red. Scale bar is $50\text{ }\mu\text{m}$. The DOX fluorescence intensity (I_{DOX}) inside the tumor excluding the edges (the area inside the dotted lines) was obtained in an arbitrary value by integration of the DOX fluorescence using MATLAB. Reproduced with permission.^[13]

tumors, the lipid-layer-cell-membrane fusion was designed as a trigger to strip off the lipid layer from the nanoassembly, as proven using a fluorescence-resonance energy-transfer (FRET) approach together with confocal microscopy observations and flow cytometry, to intracellularly and extracellularly release the small, nearly neutral, drug-carrying dendrimers for continue penetration. In BCAP-37 and MCF-7 mice tumor models, we found that DOX delivered by the nanoassembly was much more homogeneously distributed throughout the tumor tissue than that delivered by PCL_{2k}-PEG_{2k} micelles. The calculated DOX intensity (I_{DOX}) inside the tumor treated with this DOX-loaded nanoassembly was about nine times more than that treated with PCL_{2k}-PEG_{2k}/DOX (Figure 11c). Furthermore, the pH-dependent dendrimers gradually became positively charged

at the acidic tumor sites away from the blood vessels, triggering fast cellular internalization and thereby shipping drugs into the cell and circumventing the cells' multidrug resistance. Finally, inside the tumor cells, the DOX was released as a result of the dendrimer's pH-sensitive response. The nanoassembly's nanoproperties underwent the 3S transitions during the CAPIR cascade, leading to significantly better therapeutic efficacy than PEG-PCL/DOX micelles.

Similarly, another clustered nanoparticle possessing 3S property transitions to traverse the CAPIR cascade has just been reported by Wang et al.^[33b] The nanoparticle was prepared through the assembly of platinum (Pt) prodrug-conjugated poly(amidoamine)-graft-polycaprolactone (PCL-CDM-PAMAM/Pt) with PCL homopolymer and PEG-b-PCL copolymer. The PEG shell and initial size of 100 nm gave the nanoparticle long blood circulation and better tumor accumulation through the EPR effect, while the Pt prodrug-conjugated PAMAM dendrimer of 5 nm could be discharged at tumor sites triggered by the tumor extracellular acidity via the labile amide bond, for deep tumor penetration. At last, the active cisplatin was rapidly released from the PAMAM prodrugs in the reductive cytosol of tumor cells and led to robust antitumor efficacy.

7. Future Directions for Effective Nanomedicine Design

Up-to-date cancer nanomedicines have been developed for decades; however, those already in clinical uses and those under clinical trials have mainly focused on reducing adverse effects.^[155] It is the time to develop the next generation of nanomedicine with high therapeutic index for clinical use. As analyzed above, the CAPIR cascade is such

a complicated biological process, full of many traps, that it would be unrealistic to expect simply functionalized nanomedicines, for instance PEG-PLA micelles encapsulating drugs, to be able to effectively go through the whole CAPIR cascade and give high therapeutic efficacy. Using viruses for comparison, it is their ability to synchronize their functions to adapt their structures in each step of cell attachment, internalization, uncloaking, nuclear localization, replication, assembly and release that accounts for their high transfection efficiencies.^[156] Thus, next-generation nanomedicines should be self-adaptive to the CAPIR cascade. One feasible approach is to render the nanomedicine with 3S nanoproperty transitions adapting to the needs of each step in the CAPIR cascade to maximize the efficiency of each step, as demonstrated in reference.^[13] Thus,

when we design new nanomedicines, it is important to consider the whole CAPIR process and integrate the 3S nanoproperty transitions into one system.

However, we should point out that it would be impractical to expect a nanomedicine to have very high efficiency in every CAPIR step. Very importantly, we should focus on the major limiting steps to ensure their efficiencies are not too low. As the analysis of literature above shows, designs for long blood circulation and intracellular drug release and even internalization are already successful, but tumor accumulation and penetration seem to be the bottlenecks. Though controversial, detailed analysis of the reported tumor accumulation data of various nanomedicines found that only about 0.7% injected doses actually accumulated in the tumor,^[157] mainly because of the tumor's inherent pathological characteristics.^[158] In most cases, human tumors are small and only a small fraction of blood passing them, making large majority of the injected nanomedicine even never "visit" the tumor. Even worse, tumor vasculatures are unevenly distributed, poorly organized, leaky and compressed due to cancer cell hyperplasia.^[159] They therefore have reduced blood flow^[158,160] and reduced nanomedicine supply to the tumor.^[135a] The dynamic permeability of tumor blood vessels characterized by transient openings and closings at these leaky blood vessels^[161] may also limit the opportunity for nanomedicine extravasation. On the other hand, the poor or even nonexistent lymphatic drainage in tumor elevates interstitial fluid pressure (IFP),^[162] which in turn diminishes the fluid pressure gradient and thus the diffusion driving force between the tumor interstitium and intravascular space.^[163] Limited nanomedicine penetration and binding site barrier effect is also an important factor in causing such a low tumor accumulation: nanomedicines stuck in the periphery of the vasculature may lower vascular hyperpermeability and hamper continuous extravasation from the bloodstream. Further unfortunately, penetration of nanomedicines into the tumor is inherently difficult^[19] due to their large size compared to small molecules and the tumor's characteristically dense interstitial matrix with cross-linked matrix components.^[21b,135a,164] Thus, how to make more nanomedicines go through the tumor vasculature, extravasate out and further quickly diffuse away is current most important task.^[165]

Clinical translation is the ultimate goal of nanomedicine research. A 2R2SP nanocarrier with 3S nanoproperty transitions enable the nanomedicine effective in therapeutic efficacy; being clinical safety and production reproducibility are the other two basic requirements for a translational nanomedicine.^[166] These three clinical translation requirements are corresponding to the three key elements, CES, for a nanomedicine to be translational, i.e. nanocarrier 2R2SP capability (C), material excipientability (E, i.e., the feasibility for a material to be proven as excipient) and process scale-up ability (S)^[11] (**Figure 12**). Most of current research uses new materials and new approaches to obtain nanocarriers' 2R2SP capability to obtain high therapeutic index. However, when it comes to the design of a nanomedicine that is truly translational from the benchtop to the bedside, other two elements are indispensable to be comprehensively considered at early stage – that is to say, designs worthy of consideration for future work should include pairing nanocarrier capability with material excipientability and process scale-up ability.

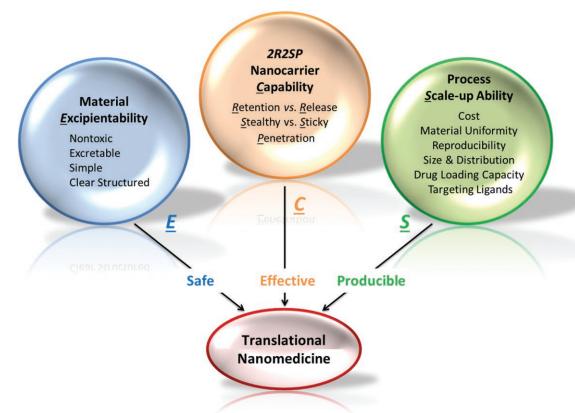


Figure 12. Three key CES elements, capability (C), material excipientability (E) and process scale-up ability (S) for designing translational nanomedicine.

A very discouraging saying haunting the nanomedicine research is that clinical translation and nanomedicine complexity are paradox—those multifunctionalized nanomedicines are too complicated to be translational. Indeed, complexity will make it more difficult for a system to enter clinical trials and do face challenges in commercial development. However, using approvals of clinical applications of viral vectors for comparison, we will find this is not true. Viral vectors are inherently complicated made of viral proteins, which are immunoactive and multifunctional and can change their structures responding to in vivo environments,^[156] not to mention their production complications and difficulties.^[167] However, because of their high efficiencies and therapeutic efficacies, viral vectors have been under extensive clinical trials for various diseases including cancer and now one is in clinical use in Europe.^[168] It is known that nonviral carrier materials can be made non-immunoactive and nontoxic using much less complicated processes than viral vectors. Thus, we should be confident that multifunctional nanomedicines with well-chosen materials and good manufacture practices^[11] must be more easily translational as long as they have sufficiently higher therapeutic efficacy than current therapies and great benefits outweigh the increasing cost of quality control and approval process.

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