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Review Article

The Yin and Yang of the protein corona on the delivery journey of nanoparticles

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ABSTRACT

Nanoparticles-based drug delivery systems have attracted significant attention in biomedical fields because they can deliver loaded cargoes to the target site in a controlled manner. However, tremendous challenges must still be overcome to reach the expected targeting and therapeutic efficacy *in vivo*. These challenges mainly arise because the interaction between nanoparticles and biological systems is complex and dynamic and is influenced by the physicochemical properties of the nanoparticles and the heterogeneity of biological systems. Importantly, once the nanoparticles are injected into the blood, a protein corona will inevitably form on the surface. The protein corona creates a new biological identity which plays a vital role in mediating the bio–nano interaction and determining the ultimate results. Thus, it is essential to understand how the protein corona affects the delivery journey of nanoparticles *in vivo* and what we can do to exploit the protein corona for better delivery efficiency. In this review, we first summarize the fundamental impact of the protein corona on the delivery journey of nanoparticles. Next, we emphasize the strategies that have been developed for tailoring and exploiting the protein corona to improve the transportation behavior of nanoparticles *in vivo*. Finally, we highlight what we need to do as a next step towards better understanding and exploitation of the protein corona. We hope these insights into the "Yin and Yang" effect of the protein corona will have profound implications for understanding the role of the protein corona in a wide range of nanoparticles.

KEYWORDS

protein corona, nanoparticles, biological identity, drug delivery, bio–nano interaction

1 Introduction

Nanomedicine is an interdisciplinary field integrating nanoscience, nanoengineering, and nanotechnology [1, 2]. Thanks to the unique physicochemical properties at the nanoscale, different nanoparticles (NPs) are widely designed and developed to diagnose and treat diseases [3–5]. For example, the NPs-based approaches are widely and successfully used to diagnose, treat, and prevent COVID-19 [6]. Compared with the conventional therapeutic methods, NPs have an ideal size, a modifiable surface, and good biocompatibility; thus, the great potential of NPs lies in their targeting ability and controlled release of their loaded cargoes [7–13]. Numerous NPs have been designed for cancer therapy based on passive and active targeting strategies. The core theory underlying passive targeting is the enhanced permeability and retention (EPR) effect. Due to the abnormal architecture of tumor blood vessels, NPs can passively accumulate at the tumor site [14, 15]. Active targeting usually involves the interaction between specific ligands (e.g., antibodies, peptides, chemical linkers, and proteins) on the surface of NPs and overexpressed receptors on tumor cells or in the microenvironment [16, 17]. However, less than 1% of injected NPs reach the tumor region, which indicates that the targeting strategy does not perform as well as expected [18, 19]. So far, only a handful of NPs have been approved as nanomedicines for clinical application. Thus, it is essential to fundamentally understand the interaction between NPs and the biological system (bio–nano interaction) *in vivo*.

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After administration, NPs must undergo an epic journey and overcome many complex biological obstacles before exerting its therapeutic effect. Mainly, NPs must overcome opsonization and subsequent recognition by the mononuclear phagocyte system (MPS) to achieve prolonged circulation time, accumulate at the target site via passive/active targeting, penetrate deeply into the target tissue, and then undergo cellular internalization and controlled release (Fig. 1(a)) [20–22]. However, the formation of the protein corona (PC) in the blood once NPs are injected is the first clear indication of bio–nano interaction. PC, which K. A Dawson first named in 2007, is a new protein layer on the surface of NPs through non-selectively adsorbing proteins and other biomolecules in complex biological fluids due to their high surface free energy (Fig. 1(b)) [23, 24]. The PC confers a new biological identity by altering the aggregation state, size, and interfacial properties of the NPs, and thus plays a vital role in bridging the bio–nano interaction and determines the ultimate biological results [25–27]. However, we must recognize that the role of PC on the NP's delivery journey has its pros and cons. At the beginning, PC was recognized as a barrier to hinder the *in vivo* delivery and badly affect the final fate of NPs; thus, how to avoid PC formation is a critical topic for the application of NPs *in vivo* [28]. With further understanding on the PC-mediated bio–nano interaction, it is now widely accepted that complete prevention of PC formation meets difficulty, but PC holds great potential to improve the delivery journey of NPs in turn.

The Yin and Yang describes how opposite objects, such as negative–positive, may be interdependent, interconnected, or complementary in the natural world. Like Yin and Yang, the PC effect on the NP's delivery is not changeless but can be dynamically regulated and employed in a positive way for different biological applications. Thus, systematically understanding the impact of PC on the NP's delivery and the interactive conversion will accelerate the translational process of NPs into nanomedicines. In this review, we first summarize the adverse effect (Yin effect) of the PC on the transportation behavior of NPs at each stage of their journey *in vivo*. Next, we emphasize the strategies that have been developed for tailoring and exploiting the PC (Yang effect) to improve the transportation behavior of NPs *in vivo* (Fig. 2). Lastly, we propose the mission for our young generation to finish in the future and the perspectives to develop better functional and clinically translatable targeted nanomedicines.

2 Yin of the PC on the delivery journey of NPs

Endowed with a new biological identity after being injected into the blood, the exogenous or artificial NPs will (in most cases) lose its original identity. On the one hand, the formation of PC will "cover" the unique function of the NP itself, such as reducing the release rate of loaded cargoes and the biodegradability of NPs. On the other hand, the formation of PC changes (destroys) the designed recognition interface between the NP and the biological target, while simultaneously introducing a new recognition interface with unexpected consequences. Meanwhile, aggregates of injected NPs mediated by the adsorbed proteins in the biological environment are also responsible for the actual bio–nano interaction and biological outcome [29–31]. In this section, we will discuss the Yin effect of the PC on the rapid clearance, targeting ability, cellular internalization, controlled cargo release, metabolism, and excretion of NPs (Table 1).

2.1 Increasing clearance by the MPS and reducing the circulation time

Once the NP is injected into the bloodstream, a prolonged circulation time is the fundamental guarantee for effective delivery of the NP into the target tissue. However, the rapid clearance of NPs by the MPS, which consists of phagocytic cells (chiefly macrophages) in the liver, spleen, and lymph nodes, is a significant barrier to long circulation. Among the organs involved in eliminating NPs, the liver plays a vital role because most injected NPs accumulate there. Kupffer cells are liver-resident macrophages which are responsible for the recognition of foreign bodies through multiple cell membrane receptors, including the scavenger receptor, Fc receptor, Toll-like receptor, C-type lectin receptor, node-like receptor, and so on [32, 33].

Among the proteins on the surface of NPs, opsonins act as signals for recognition by the surface receptors on macrophages, leading to macrophage activation and increased NP uptake. Thus, opsonins will exacerbate the clearance of NPs from the blood circulation [34]. So far, several opsonins are known to be related to the clearance of NPs, including high-density lipoproteins (HDL),

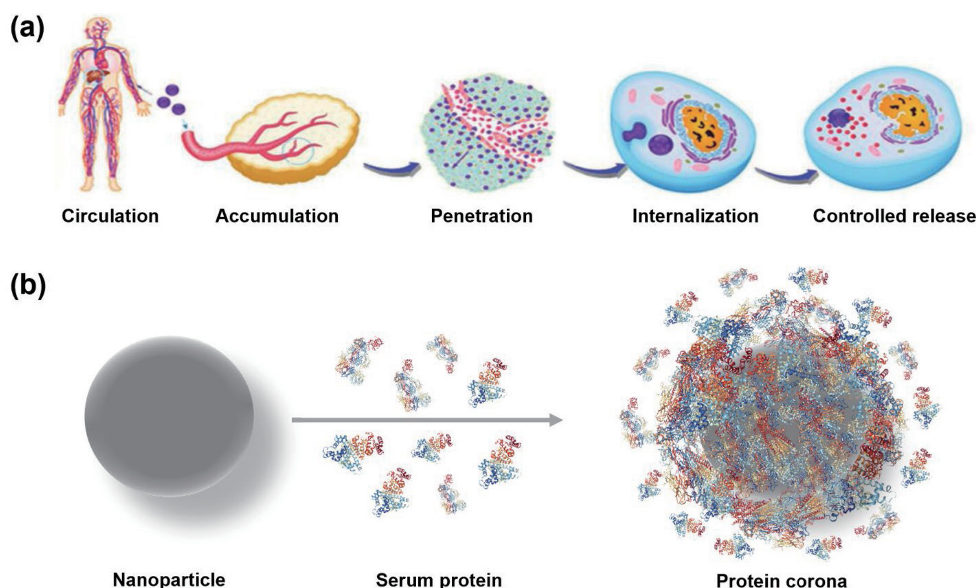

Figure 1 The delivery journey of NPs *in vivo*. (a) Different biological processes are involved in the delivery journey of a NP, including overcoming opsonization and subsequent detection by the mononuclear phagocyte system, prolonging the circulation time, accumulation via passive/active targeting, penetration into the deep tissue, cellular internalization, and controlled release. Adapted with permission from [20], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2014. (b) Schematic of the formation of PC on the NPs surface.

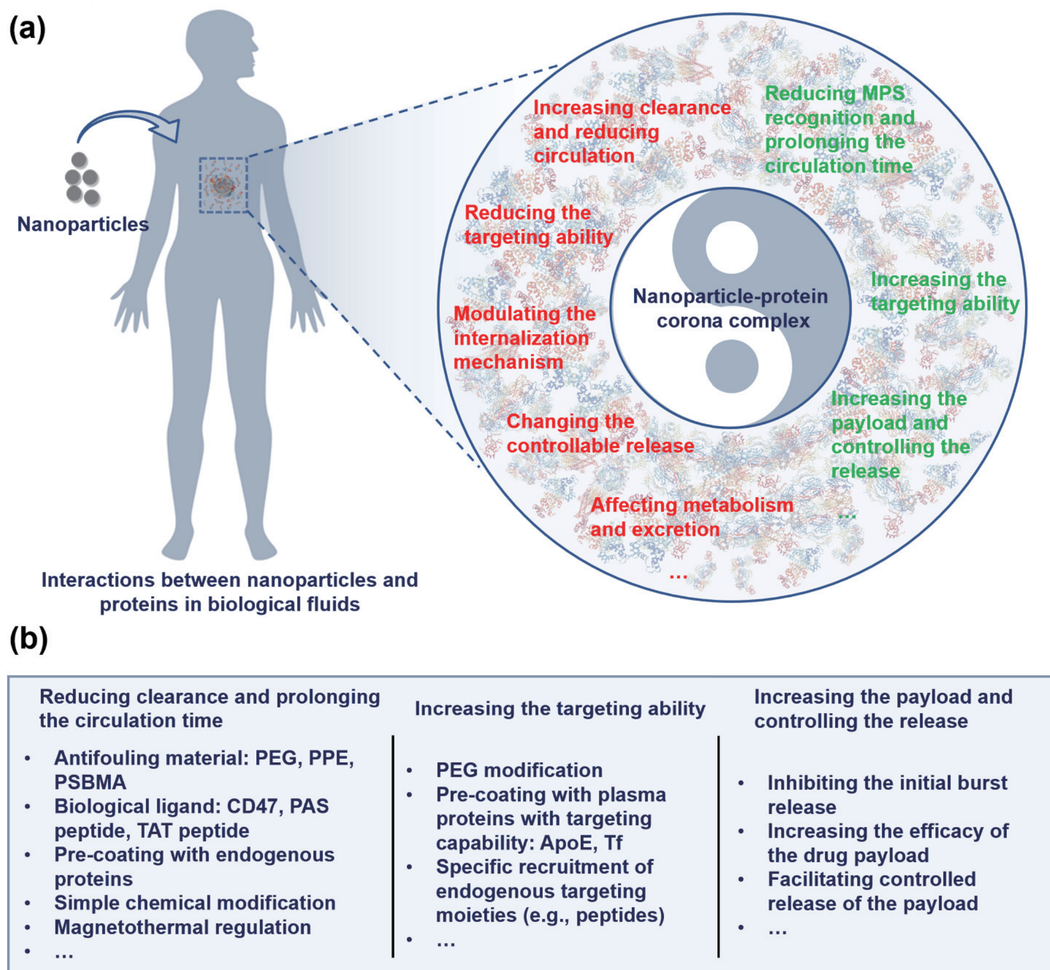

Figure 2 Overview of the Yin and Yang effect of the PC on the delivery journey of NPs. (a) The Yin and Yang effect of the PC on the transportation behavior of NPs. (b) The developed strategies for tailoring and exploiting the PC to improve the transportation behavior *in vivo*.

complement component 1q, properdin, immunoglobulins (Ig), and surfactant protein A and D [35]. For instance, immunoglobulins (e.g., IgM and IgG) on the surface of NP-PC complexes can act as epitopes to trigger phagocytosis by immune cells. In addition, fibrin promotes macrophage phagocytosis through receptor-mediated internalization to accelerate blood clearance [36]. As part of the innate immune system, complement proteins can help eliminate foreign substances. When exogenous NPs enter the body, they activate the complement cascade, which is composed of more than 30 proteins [28]. Rotello and colleagues found that specific complement proteins play critical roles in recognition of gold NPs (GNPs) by macrophages [37]. However, Farokhzad and colleagues found that complement activation is not responsible for the difference in the clearance speed of polymer NPs [38]. To better understand the impact of the PC on NP clearance, the authors synthesized a library of polymer NPs by tuning the sizes and polyethylene glycol (PEG) densities (Fig. 3(a)). Interestingly, they found that the density of 20 PEG chains per 100 nm^2 is a threshold and the blood clearance is rapid if the PEG density of NPs is below this critical point (Fig. 3(b)). When complement protein 3 (C3) knockout mice were compared with normal controls, the circulation time of polymer NPs was not increased, which suggests that the C3 is not important for NP clearance (Fig. 3(c)). Considering the enrichment of apolipoproteins (Apos) in the hard corona, the authors further studied the roles of lipid trafficking pathways in the clearance of NPs. In animals deficient in Apo E ($ApoE^{-/-}$), there was increased clearance of NPs below the threshold of PEG density, which suggests that ApoE on the surface of NPs plays a dysopsonic role (Fig. 3(d)). The relative abundance of ApoE in NPs above the threshold of PEG density is lower and the particles are cleared more slowly; thus, the effect of ApoE on the clearance of NPs above the threshold is weaker. Similarly, the circulation time is also increased when NPs with lower PEG density are pre-coated with another Apo, clusterin. However, clusterin has no effect on NPs with higher PEG density. This suggests that clusterin acts as a dysopsonin on the surface of NPs only if the PEG density is below the threshold (Fig. 3(e)). Furthermore, from the viewpoint of biology, the authors found that the low-density lipoprotein receptor (LDLR) plays a vital role in recognizing NPs, but it needs to be clarified whether Apo is essential for the recognition (Fig. 3(f)). In another study on opsonin-induced clearance, the authors found that compared with polyacrylamide-grafted zinc oxide (ZnO) NPs, bare ZnO NPs had a higher opsonin protein content and thus showed early clearance and short circulation [39]. Another lipoprotein, HDL, also promotes the internalization of silicon dioxide (SiO_2) NPs by human macrophages but not human monocytes [40].

Conversely, dysopsonins, including albumin and certain Apos (such as ApoA4, ApoC3, and ApoE as mentioned above), help NPs escape recognition and uptake by phagocytes; thus, the circulation time in the bloodstream and the accumulation efficiency of NPs in other organs and tissues would be increased. We will discuss in Section 3 how dysopsonins improve the transportation behavior of NPs. Together, these studies of the role of Apos and other proteins on transportation behavior have led to a complex view of the PC effect.

We highlight here that understanding the biological composition and spatial structure of the PC is essential to reduce the rapid clearance and ultimately enhance the efficacy of NPs in biomedical applications *in vivo*. It is now understood that the

Table 1 Summary of Yin of the PC on the delivery journey of NPs[a]

Delivery journey	NPs	Key proteins/serum	Effect of PC	Ref.
Clearance and circulation	GNPs	C4BPA F2	Specific complement proteins play critical roles in recognition of GNPs by macrophages.	[37]
	ZnO NPs	IgG C3 C1q MBP	Compared with polyacrylamide-grafted ZnO NPs, bare ZnO NPs are more vulnerable recognized by the receptor of macrophage and immune effector cells, resulting in an early clearance and shorter circulation time.	[39]
	SiO_2 NPs	HDL	PC promotes the internalization of SiO_2 NPs by human macrophages but not by human monocytes.	[40]
Targeting ability	SiO_2 NPs functionalized with Tf	FBS	PC reduces the targeting ability of SiO_2 NPs functionalized with Tf.	[52]
	PS NPs functionalized with Tf	Cerebrospinal fluid	PC reduces the targeting ability of PS NPs functionalized with Tf.	[53]
	Nanodiamonds functionalized with TPP	FBS Human plasma	PC reduces the mitochondrial targeting specificity.	[54]
Cellular internalization	GNPs	BSA	PC decreases the internalization efficiency.	[58]
	PS NPs	FBS		[59]
	IONPs	FBS		[60]
	SiO_2 NPs	FBS		[61]
	Cationic nanoliposomes	FBS Human serum	PC affects the internalization pathway of cationic nanoliposomes and the subcellular distribution of the loaded cargo.	[62]
	Liposomes SiO_2 NPs	FBS at different concentrations	NPs uptake is lower at higher serum concentrations and PC composition can also affects the internalization pathway.	[63]
Controlled cargo release	TTSL	CD-1 mouse plasma	The release of DOX from TTSL in full plasma is slow and incomplete no matter *in vitro* incubation or recovery from the blood.	[68]
	TSL	Mouse plasma Human plasma Rat plasma FBS	The PC formed in mouse plasma reduces the release temperature of TSL, but the effect in human plasma, rat plasma, and FBS is negligible.	[69]
	Negatively charged liposomes	FBS Human LDL BSA	Negatively charged liposomes leak loaded cargo extensively at nanomolar lipid concentrations when in the presence of serum.	[70]
	DNA nanostructures	FBS	PC inhibits the endosomal escape of DNA nanostructures.	[73]
Metabolism and excretion	NPs@PMAO NPs@Glc NPs@PEG	Serum Albumin Fibrinogen	PC inhibits NPs@PMAO degradation in lysosomal mimicking medium; corona composition affects the degradation rate: NPs@PEG (enrich albumin) is faster than NPs@Glc (enrich fibrinogen).	[77]

[a]C4BPA: complement component 4 binding protein alpha; F2: coagulation factor II; C1q: complement component 1q; MBP: mannose-binding protein.

traditional concept of receptor–ligand interaction (at least in some cases) is not suitable to explain the recognition of multiple motifs on the surface of NPs. For instance, Yan et al. reported that most of the known ligands of the macrophage receptor with collagenous structure (MARCO) fail to compete for the binding of NPs with the MARCO. This suggests that NPs have a distinctive mode of binding to the receptor [41]. Therefore, we believe that a fundamental understanding of the molecular recognition between the PC surface and the biological surface is the top priority for developing strategies to reduce the liver clearance and enhance the circulation time.

2.2 Reducing the targeting ability of NPs

Targeting properties are one of the unique benefits of NP. Targeted delivery can reduce the side effects of the loaded drug and increase the concentration in disease sites. For passive targeting, NPs with appropriate physicochemical characteristics circulate in the bloodstream for a long time and thus accumulate in disease sites due to the EPR effect [42, 43]. Thus, passive targeting can be affected by the upstream events of the NP's journey, that is, the circulation time and the speed of clearance [44]. Also, the efficiency of passive targeting depends on the NP's physicochemical properties [45, 46]. We hypothesize that the penetration of a NP into the disease site will be affected by PC formation, because the physicochemical properties of the NPs are already changed by PC.

Active targeting strategies rely on utilizing specific ligands to functionalize NPs for recognition by their receptors at the disease site [47]. Thus, exposure of the correct epitope is the precondition for recognition of the NP by the overexpressed receptors at the disease site. However, the epitope will be covered by the PC, and numerous studies have reported that PC formation strongly affects

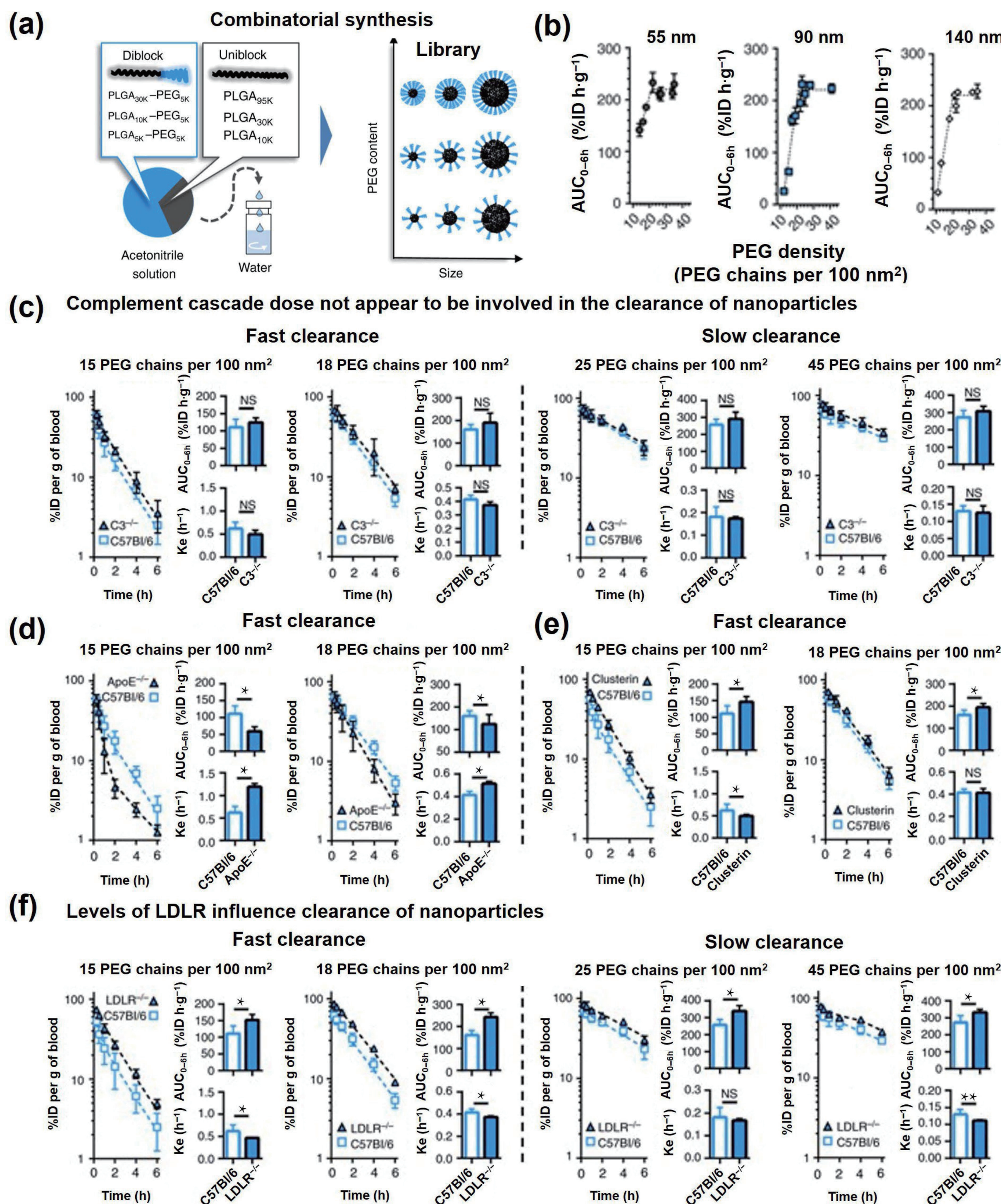

Figure 3 Understanding the *in vivo* PC effect on pharmacokinetics. (a) The library of polymer NPs with different PEG densities and sizes. (b) Pharmacokinetic analysis of the library of NPs. (c) Circulation analysis of different NPs in wildtype and C3$^{-/-}$ mice. (d) Circulation analysis of NPs with the PEG density below the threshold in wildtype and ApoE$^{-/-}$ mice. (e) Circulation analysis of NPs with lower PEG density pre-coated with clusterin. (f) Circulation profiles of different NPs in wildtype and LDLR$^{-/-}$ mice. Adapted with permission from [38], © Bertrand, N. et al. 2017.

the active targeting ability of NPs [48–51]. For example, Dawson and his colleagues first demonstrated that the targeting ability of silica NPs functionalized with transferrin (Tf) was reduced when the PC formed after incubation with serum (Figs. 4(a) and 4(b)) [52]. Wang et al. confirmed this phenomenon using polystyrene (PS) NPs coated with a cerebrospinal fluid PC (Fig. 4(c)) [53]. Zou et al. also found that the PC can affect the intracellular targeting ability of nanodiamonds (NDs) functionalized with the targeting agent triphenylphosphine (TPP). After modifying the polyglycerol-functionalized ND with TPP at a low and high density, the authors found that large numbers of proteins were recruited onto the surface of ND with high TPP density. The PC caused the NDs to be retained in the endosomal and lysosomal compartments, thus reducing the mitochondrial targeting specificity [54].

The physicochemical properties of NPs can affect the PC formation; thus, the physicochemical properties will indirectly affect the active targeting ability of NPs mediated by PC [34]. In general, hydrophobic or electrically charged NPs adsorb more proteins than hydrophilic or neutral NPs; therefore, the targeting ability of the two former types is more susceptible to compromise [55]. The size and surface charge of the NPs can also affect the type and the relative number of proteins. Interestingly, Vali and

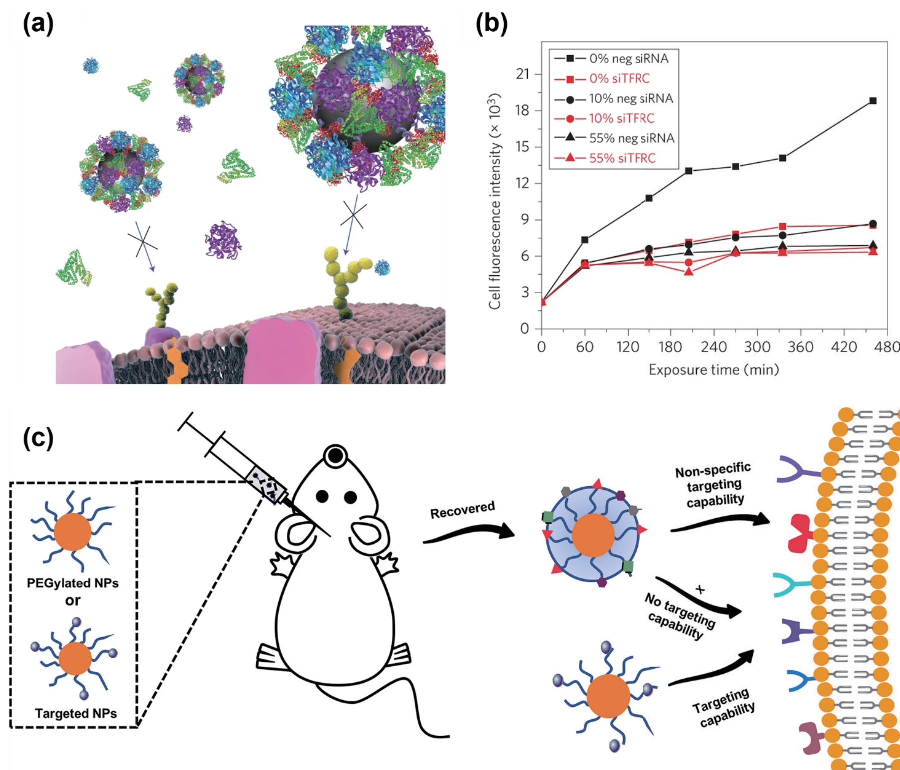

Figure 4 PC formation reduces the targeting ability of NPs. (a) Schematic showing loss of the targeting ability of Tf-functionalized NPs after PC formation. (b) The median cell fluorescence intensity of A549 cells exposed to Tf-functionalized NPs in minimum essential medium (MEM) with 0%, 10%, and 55% serum. The uptake of Tf-functionalized NPs decreased with the rise in serum content. Adapted with permission from [52], © Nature Publishing Group 2013. (c) The formation of a cerebrospinal fluid PC can lead to the loss of active targeting specificity. Adapted with permission from [53], © Elsevier B.V. 2021.

colleagues found that the molecular weight of adsorbed proteins is positively related to the size of NPs. The larger NP tends to adsorb larger proteins, and smaller NPs tend to adsorb smaller proteins [56]. Therefore, the surface properties of NPs themselves play a crucial role in tailoring the PC and affecting the targeting ability.

Taking all these examples together, it is clear that the Yin effect of the PC on the targeting efficiency of NPs is an important critical problem for the clinical application. Undoubtedly, there is a great need for us to uncover the molecular and biophysical mechanisms underlying formation of the PC, and to further develop appropriate methods to regulate the PC to promote the accumulation of NPs at the desired site.

2.3 Modulating the internalization mechanism

After the NP arrives at the disease site, efficient uptake is essential for effective intracellular drug delivery. Cellular internalization pathways will determine the intracellular localization and fate of the internalized NPs. NPs can be internalized by the recipient cell through several different endocytosis pathways, such as receptor-dependent/independent endocytosis, macropinocytosis, and phagocytosis [57]. It is now recognized that the endocytosis pathways employed by cells depend on a NP's physicochemical properties. Again, the PC can change "what can be seen by cells" and influence the downstream pathways.

It is widely observed that the uptake of NPs by cells in the absence of the PC is higher than that in the presence of the PC [58–60]. However, the underlying mechanism is unclear. Salvati et al. found that the adhesion of inorganic silica NPs to the cell membrane is more robust when serum is absent, and thus the internalization efficiency is also higher in the absence of serum [61]. Our previous work found that in the absence of serum, cationic nanoliposomes can interact with the cell membrane by electrostatic interaction and then fuse with it. However, the cell can take up the cationic nanoliposomes through energy-dependent endocytosis after incubation with 10% fetal bovine serum (FBS) or human serum (Figs. 5(a) and 5(b)) [62]. Interestingly, the subcellular distribution of the model drug doxorubicin (DOX) also changed in different serum conditions. We found that free DOX was mainly delivered into the cell nucleus and some into lysosomes (Fig. 5(c), upper row). Like the free DOX, the DOX loaded into the cationic nanoliposomes was mainly transported into the nucleus in the absence of serum (Fig. 5(c), middle row). However, the DOX loaded into the cationic nanoliposomes was localized in the lysosomes after incubation with 10% FBS (Fig. 5(c), lower row). This work highlights that the PC is an essential factor affecting the endocytosis pathways and subcellular distribution of loaded cargoes. Salvati et al. also found that the NPs can be internalized via different endocytosis pathways when incubated with different serum concentrations, which suggests that the PC composition can also affect the recognition and internalization pathways [63]. This work highlights that the recognition and internalization pathways employed by cells are highly related to the composition and spatial structure of the PC.

Again, the adsorption of biomolecules onto the surface of NPs cannot be avoided after injection into the bloodstream, and it is believed that the molecular composition of the PC formed *in vivo* is different from that formed *in vitro* [64]. We propose that the internalization mechanism of the same NP by the same type of cell would be different under *in vivo* and *in vitro* conditions. An important goal is to identify the internalization mechanism of NPs in an actual animal model.

2.4 Changing the controllable release

Controllable release is another characteristic of NPs that can reduce the number of injections needed and decrease the potential toxicity to other tissues. Depending on the functional properties of NPs, the release of loaded cargoes can be activated by external or

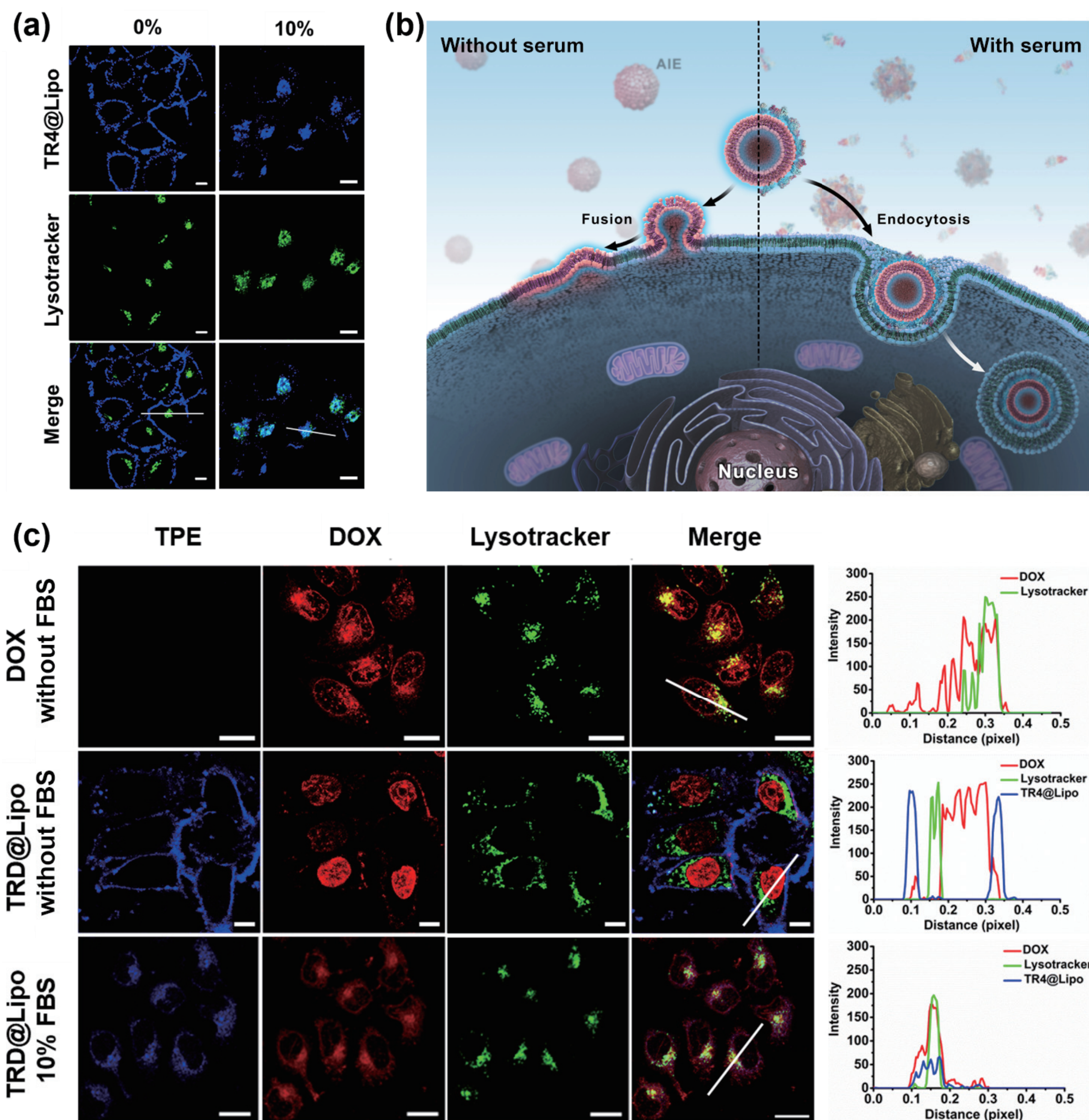

Figure 5 The PC affects the cellular internalization mechanism of cationic nanoliposomes. (a) Fluorescence images of living cells treated with cationic nanoliposomes with or without 10% FBS. (b) Schematic showing how PC formation changes the cellular uptake mechanism of cationic nanoliposomes. (c) The subcellular distributions of free DOX (upper row), and DOX loaded into cationic nanoliposomes without FBS (middle row) and with 10% FBS (lower row). Adapted with permission from [62], © Wang, Y. F. et al. 2021. TR4: a cell membrane probe comprising four arginine residues, a palmitic acid tail, and tetraphenylethylene (TPE); TR4@Lipo: TR4-containing liposomes; TRD@Lipo: DOX-loaded TR4@Lipo.

internal stimuli. When NPs enter a complex *in vivo* environment, protein adsorption may interfere with drug release mechanisms or cause aggregation of the NPs, which may limit the ability of the nanocarrier to release drugs [65–67]. For example, Kostarelos and colleagues studied the drug release profile of DOX encapsulated within temperature-sensitive liposomes (TSL) in different conditions (Fig. 6(a)) [68]. The authors prepared traditional TSL (TTSL) and lysolipid-containing TSL (LTSL) to study the PC effect on the thermosensitive release. As shown in Figs. 6(b) and 6(c), compared with TTSL, the loaded DOX can be quickly released from the LTSL, and the release rate can reach 100% after incubation in HBS buffer and mouse plasma at 42 °C. For the TTSL system, only < 10% of loaded DOX was released in the HBS buffer. Next, the authors compared the release profile of LTSL and TTSL after recovery from the blood. Interestingly, the loaded DOX can be quickly and wholly released from purified LTSL and TTSL at 42 °C in HBS buffer (Fig. 6(d)). In full plasma, DOX was still rapidly released from LTSL. However, the release of DOX from TTSL was slow and incomplete no matter *in vitro* incubation or recovery from the blood (Figs. 6(c) and 6(e)). This is due to adsorption of the free protein in the full plasma onto the surface of TTSL, which blocks the drug release. Further, Rädler and colleagues found that the PC formed in mouse plasma can reduce the release temperature of TSL, but the effect of the corona formed in human plasma, rat plasma, and FBS is negligible [69]. Liposomes are soft nanomaterials, and they can be easily damaged after adsorption of proteins onto their surface. Thus, the fast leakage of loaded cargoes from liposomes caused by the PC is another key challenge when using a controllable release system [70, 71].

The loaded cargoes of NPs can be activated and released by factors in the endogenous microenvironment, such as overexpressed enzymes, redox status, and pH [72]. However, the PC may cause steric hindrance to prevent adequate recognition and response at the target site. For example, Lunoc and colleagues reported that the PC could inhibit the endosomal escape of DNA nanostructures [73]. The DNA nanostructures were coated with an endosome escape peptide, aurein 1.2. The researchers found that the PC blocked the electrostatic interaction between the membranes and the peptide, displacing the lipids and altering the

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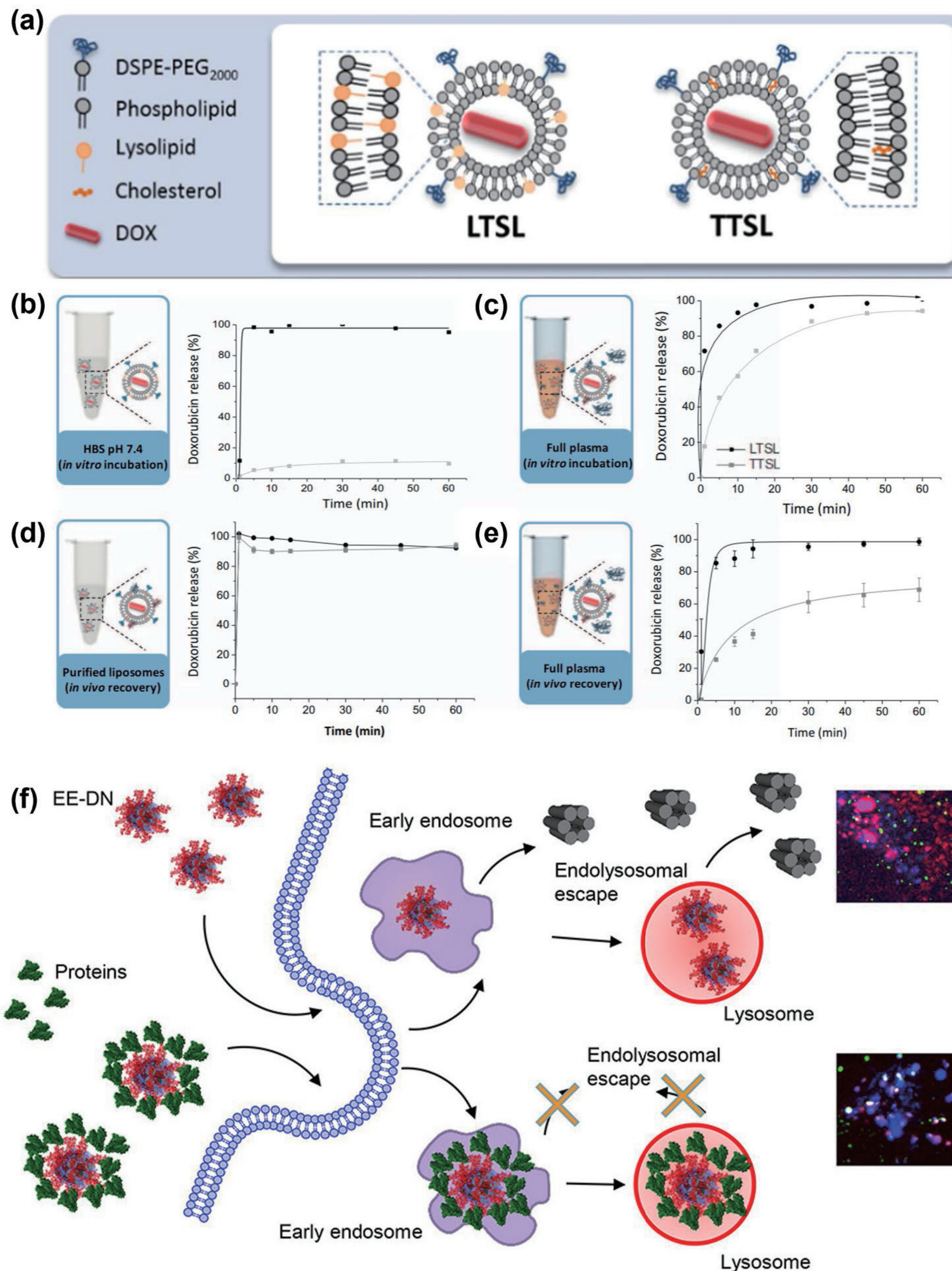

Figure 6 The PC changes the controllable release of the payload. (a) Schematic of LTSL and TTSL. The release rate of DOX from the two kinds of liposomes, TTSL and LTSL, after incubation with (b) HBS buffer and (c) mouse plasma. The release rate of DOX from the *in vivo* recovered liposomes in (d) the absence of free proteins and (e) total plasma. Adapted with permission from [68], © Elsevier B.V. 2018. (f) Schematic showing how PC formation on DNA nanostructures inhibits endosomal escape. Adapted with permission from [73], © American Chemical Society 2021.

membrane structures. Thus, endosomal escape of DNA nanostructures was inhibited (Fig. 6(f)). In addition, enzyme-responsive NPs have been widely reported for drug delivery [74–76]. However, the PC effect on the response activities is poorly understood. Together, these examples show that the effect of the PC on controllable release is highly dependent on the type of NPs, the loaded cargoes, the response-release mechanism, and so on.

2.5 Effect on the metabolism and excretion of NPs

Metabolism and excretion are essential for avoiding the long-term toxicity of NPs. However, little is known about the metabolism and excretion of NPs itself, so the effect of the PC on these processes is also poorly understood. Generally, once the NPs accumulate in the organs, they undergo a biotransformation or biodegradation process. The PC on their surface has undesirable effects, such as changing the degradation rate of the NPs, which will affect the release of the drug cargo or cause long-term accumulation of drugs in the body.

Studies have found that iron oxide NPs (IONPs) modified with poly(maleic anhydride-alt-1-octadecene) (NPs@PMAO) degrade more slowly with PC *in vitro* than without PC, which indicates that the PC inhibits NPs@PMAO degradation [77]. Next, the authors studied the *in vivo* degradation of IONPs modified with glucose (NPs@Glc) and PEG (NPs@PEG). Interestingly, they found that the degradation rate is affected not only by the

presence of the PC but also by its composition. Compared to the degradation rates of NPs@PEG and NPs@Glc, the core of NPs@PEG degraded faster in the body than the core of NPs@Glc. The PC of NPs@Glc was rich in fibrinogen, while the PC formed on NPs@PEG contained more albumin. The presence of albumin induces faster degradation of NPs@PEG in the liver and spleen. Chen et al. found that naked molybdenum disulfide nanodots were oxidized entirely after 30 days. In contrast, nanodots modified with human serum albumin (HSA) and mouse plasma proteins protected the molybdenum disulfide from oxidation and resulted in 62.3% and 60.9% molybdenum retention, respectively, after 30 days [78]. These results indicate that the presence of HSA can protect the exposed surface of molybdenum disulfide from oxygen, oxidants, and hydrolysis and hinder the oxidation of Mo^{4+} and S^{2-} in the nanodots.

These essential works imply that the presence of the PC can inhibit or extend the biodegradation and biotransformation process, but the long-term retention of NPs in the body maybe can cause toxicity problems. We appeal to researchers to focus more on a deeper understanding of the long-term fate of NPs (that is, metabolism and excretion), and the impact of the PC in this process should be considered.

3 Yang of the PC on the delivery journey of NPs

As discussed in Section 2, the new biological identity of NP creates a new challenge for the *in vivo* applications of NPs. Fortunately, by acquiring a detailed and fundamental understanding of the PC, we will be able to develop advanced strategies to reconstruct the NPs surface and control the formation and composition of the PC. This will allow us to transform the Yin effect into the Yang effect. The goal is to develop highly efficacious and safe NPs that can efficiently overcome the discussed biological barriers. The exploration and tailoring of the PC is a very cutting-edge research topic, and it provides an excellent opportunity to resolve the application difficulties of NPs. As this topic is in the early stages of development, in this section we mainly focus on advanced strategies to reduce liver clearance, increase the targeting ability, and control the payload release by regulating the PC (Table 2).

3.1 Reducing MPS recognition and prolonging the circulation time

As mentioned above, NPs coated with a PC, especially a corona containing opsonins, are often rapidly cleared by the MPS. Thus, rational decoration of the surface of NPs to reduce the adsorption of opsonin proteins or increase the adsorption of dysopsonins is an effective strategy to prolong the systemic circulation time.

Unlike the strategy for regulating the adsorption of specific proteins onto the surface of NPs, PEG, as a classic antifouling material, is widely used to prolong the circulation time and reduce MPS recognition by generally inhibiting the adsorption of proteins [79–81]. However, the antifouling performance of PEG cannot wholly block the adsorption of proteins onto NPs. For optimal performance, scientists have to adjust the density and length of PEG. Theoretically, the backfill length of PEG should be shorter than the length of the targeting ligand linker to ensure binding between the targeting moiety and its corresponding cellular receptor (Fig. 7(a)) [82]. Furthermore, in order to form a better barrier against adsorption, the density of PEG must be sufficient to form a "brush" conformation instead of a "mushroom" conformation (Fig. 7(b)) [27, 80, 83]. Although it is generally believed that NPs coated with antifouling polymers (such as PEG) have no or low immunogenicity, PEGylated NPs can still trigger the phenomenon of "accelerated blood clearance" (ABC) upon repeat administration [84–86]. Polyphosphoesters (PPEs), which possess controllable hydrophilicity, biodegradability, and additional chemical functionality, would be appropriate PEG substitutes [87]. For example, Simon et al. revealed that the PPEylation of NPs could reduce the non-specific adsorption of proteins while maintaining the original targeting ability (Fig. 7(c)) [88]. When mannosylated PPEs were noncovalently adsorbed onto the surface of the NPs, the NPs still had a high uptake into targeted monocyte-derived dendritic cells and low uptake into nontargeted monocytes after incubation in human blood plasma (Fig. 7(d)). Another class of stealth polymers is zwitterionic polymers, which contain cationic and anionic charges in the same chain. It has been validated that zwitterionic polymers can reduce protein adsorption and have low immunogenicity [89, 90]. For example, fibre rods modified with poly(sulfobetaine methacrylate) (PSBMA) can effectively prevent protein opsonization and reduce capture by macrophages [89]. In addition, a coating of amphoteric natural starch on polymer NPs reduces PC formation and prolongs circulation time [91].

Besides antifouling polymers, peptides and proteins have also been applied as stealth materials. The integral membrane glycoprotein cluster of differentiation 47 (CD47) is a member of the immunoglobulin superfamily. It is expressed in various human and mouse cells, especially hematopoietic cells, vascular endothelial cells, and tumor cells [92]. CD47 can interact with the transmembrane protein signal regulatory protein α (SIRPα) on phagocytes as a marker of self [93]. This CD47-SIRPα interaction suppresses phagocytic engulfment by producing a "do not eat me" signal [94]. Belhadj et al. designed a novel combined "eat me/

Table 2 Summary of Yang of the PC on the delivery journey of NPs[a]

Delivery journey	NPs	Strategy	Key proteins/serum	Effect of PC	Ref.
Circulation and clearance	EVs	Surface modified with PEG	Mouse plasma	Decrease clearance and prolong circulation time	[79]
	PRINT NPs		BSA		[80]
	PS NP	Surface coated with PPE	Clusterin	Enrich the dysopsonin clusterin and reduce phagocytes uptake	[87]
	PMMA NP				
	HES NPs				
	PS NP		Human plasma		[88]
	PMMA NPs				
	Fiber rods	Surface coated zwitterionic polymers PSBMA	BSA	Reduce the adsorption of proteins and prolong circulation time	[89]
	SCS NPs	Surface coated with amphoteric natural starch	FBS	Reduce the adsorption of proteins even in high levels of serum medium	[91]
	SCMM NPs				
	SCMMA NPs				

(Continued)

Delivery journey	NPs	Strategy	Key proteins/serum	Effect of PC	Ref.
Circulation and clearance	Exosomes-hybrid vesicles	CD47-expressing exosomes	CD47	Artificial corona containing CD47 as a don't eat me signal to minimize recognition and phagocytosis of NPs by MPS	[95]
	RBC-Gd-PLGA NPs	CD47-expressing RBC-M fused onto PLGA NPs			[96]
	Ferritin NPs	Surface coated with PAS peptides	Clusterin	Decrease *in vivo* C3 activation and PC formation	[97]
	Liposomes	Modified with TAT	Mouse serum	TAT seems to adsorb dysopsonins rather than opsonins and protects the liposome from clearance.	[103]
	PLGA NPs	Decorated with BSA	BSA	The artificial BSA corona enables the NPs to evade recognition and engulfment by MPS and avoids targeting shielding by plasma protein.	[105]
	PLGA NPs	Maleimide-decorated NPs selectively anchor endogenous albumin	Albumin	*In situ* albumin-enriched corona protects NPs from phagocytosis in the bloodstream and prevents the ABC phenomenon.	[106]
	PLGA NPs	Incubated with BSA	BSA	The pre-coated BSA corona prolongs NPs blood circulation time *in vivo*.	[108]
	Pt NPs	Spontaneously formation of HSA corona	HSA	Prolong NPs blood circulation time *in vivo*	[109]
	Liposomes	Incubated with human plasma proteins	Human plasma proteins	Artificial corona formed at high protein concentration significantly decreases the capture of liposomes by circulating leukocytes in whole blood.	[110]
	Graphene GNP IONPs	Simple chemical modification and pre-coated with ApoE	ApoE	The hydroxyl groups can decrease the adsorption of the opsonin proteins; ApoE pre-adsorption enhances the blood circulation time.	[111]
	IONPs	Modulating the composition via magnetothermal effect	ApoE Clusterin Hrg Albumin	Magnetothermal regulation inhibits NPs opsonins absorption and facilitates dysopsonins absorption; the circulation time of IONPs is enhanced.	[114]
Targeting ability	GNPs	Modulating the molecular weight of PEG	Albumin Tf	GNPs-550 adsorbs more albumin and Tf than GNP-350 and GNP-1000 and enhances the targeting ability and antitumor efficacy of GNPs-550.	[115]
	LNPs	Regulating the chain lengths and molar ratios of PEG conjugated lipid in LNPs	ApoE	ApoE-enriched PC promotes the internalization of LNPs by HepG2.	[116]
	LNPs	Regulating the chain length and amount of PEG conjugated lipid in LNPs	Apo	Apo-enriched corona promotes LNPs accumulation in tumor.	[117]
	LNPs	Pre-coated with ApoE4	ApoE4	ApoE4 corona increases NPs translocation into brain parenchyma and improves brain NPs accumulation.	[119]
	Tf-NPs	Pre-coated Tf-NPs with PC derived from healthy mice	Healthy mice serum	Pre-coated Tf-NPs with healthy mice serum significantly enhance the active targeting capacity.	[120]
	GSH-MSN	Pre-coated GSH-MSN with recombinant protein GST-HER2-Afb	HER2-binding affibody	The recombinant PC prevents the clearance of NPs by macrophages and ensures systematic targeting functions *in vitro* and *in vivo*.	[121]
	PPD NPs	Decorated NPs with DHA to enable to specifically recruit endogenous ApoE	ApoE	The ApoE-anchored corona prolongs PPD NPs blood circulation time and facilitates NPs accumulating in tumor.	[123]
	Liposomes	Regulating lipid composition to recruit special proteins	Apos	Apos-rich PC enables liposomes brain targeting capability.	[124]
	Liposomes	Modifying liposomal surface with a short nontoxic peptide	ApoA1 ApoE ApoJ	Apos-rich PC enhances the brain-targeted delivery.	[125]
	CTB-NPs	Surface modified with CTB	Mouse plasma	PC does not affect the targeting capacity of CTB-NPs.	[126]
	COF NPs	Modified with T_{10} to realize the specific binding with Tf	Tf	Tf corona confers COF NPs the capability to overcome BBB and effective and precise brain tumor therapy.	[127]
Controlled cargo release	Abraxane SPIONs	Incubated with human serum or FBS	Human plasma FBS	PC reduces the release rate of the loaded drugs and significantly reduces the burst release.	[130]
	GNRs	Incubated with ES	ES	PC on GNRs serves as a functional platform to carry DNA and DOX.	[131]
	GNRs GNBs CNTs	Incubated with human serum or HSA	HSA	The local environment PC regulates the release kinetics of the payload.	[132]
	PCNV	—	FBS	PC promotes antigen cytosolic controlled release.	[135]

[a]HES: hydroxyethyl starch; SCS: starch-coated polystyrene; SCMM: starch-coated poly(methyl methacrylate); SCMMA: starch-coated poly(methyl methacrylate-co-acrylic acid); RBC: red blood cell; RBC-M: red blood cell membrane; RBC-Gd-PLGA: Gd-PLGA NPs core coated with RBC-M; MPS: mononuclear phagocytic system; Pt: platinum; Hrg: histidine-rich glycoprotein; Tf-NPs: transferrin-modified NPs; PPD: PLGA-PEG2000-DHA; Aβ: amyloid beta; GNBs: gold nanobones; CNTs: carbon nanotubes; PCNV: PC-driven nanovaccine; PMMA: poly(methyl methacrylate).

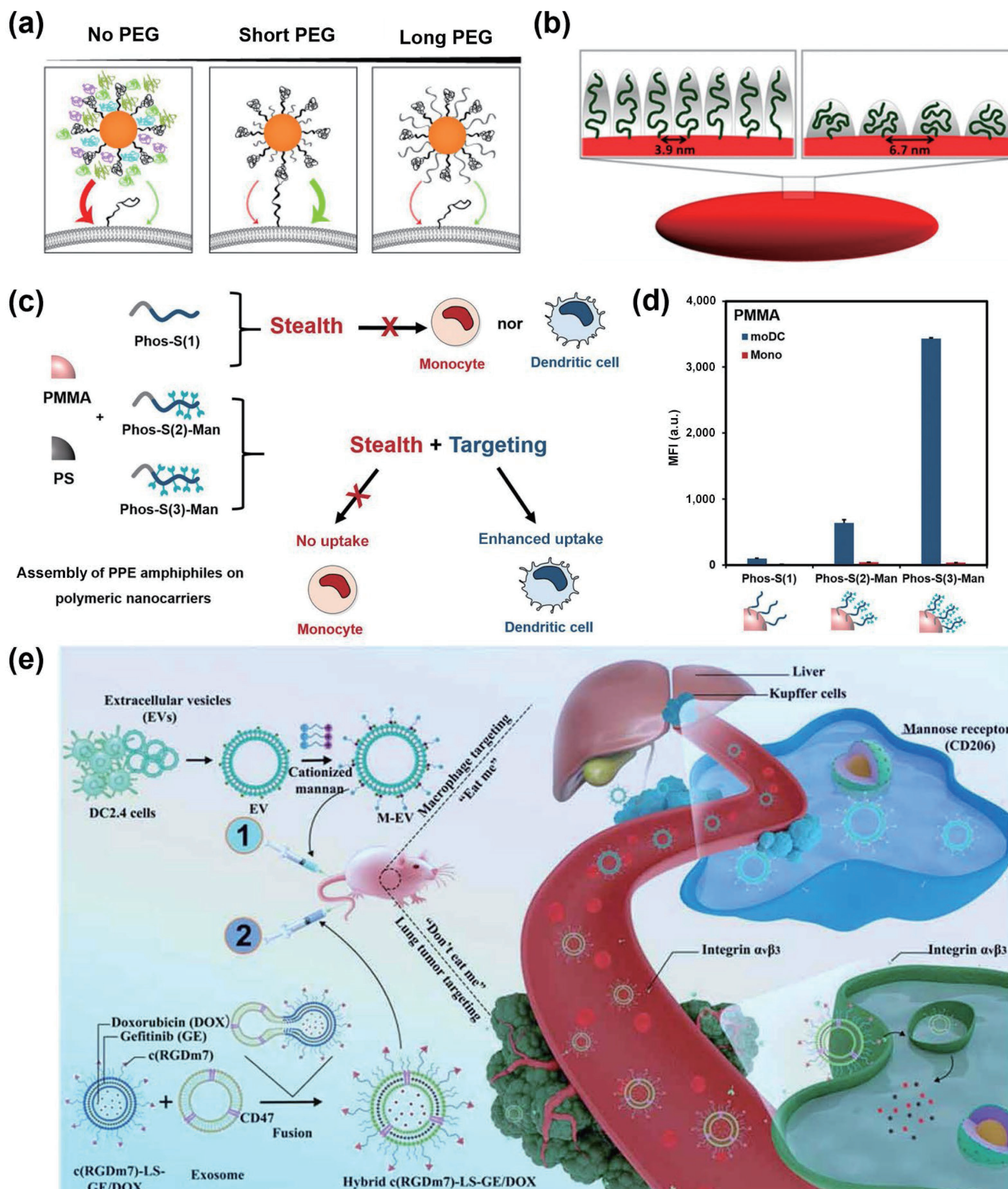

Figure 7 The strategies for reducing MPS recognition and prolonging the circulation time. (a) The molecular weight of the surface PEG affects the targeting specificity of the NPs. Adapted with permission from [82], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2014. (b) Schematic of PRINT hydrogel NPs with functionalized PEG in brush (left) and mushroom (right) conformations. Adapted with permission from [80], © American Chemical Society 2012. (c) Schematic of the assembly of PPE amphiphiles on polymer NPs to confer stealth and targeting properties. (d) The uptake of NPs with mannosylated PPE on the surface into targeted monocyte-derived dendritic cells (moDC) and nontargeted monocytes (Mono) after incubation in human blood plasma. Adapted with permission from [88], © Simon, J. et al. 2019. (e) Schematic of the "eat me/don't eat me" strategy. The mannan-modified EVs are first injected into the mouse as an "eat me" signal to saturate macrophages. Then the exosome-hybrid targeted vesicles are injected into the mouse as a "don't eat me" signal to prolong the circulation time and increase tumor accumulation. Adapted with permission from [95], © Belhadj, Z. et al. 2020.

don't eat me" tactic with the dual effects of targeting lung tumors and minimizing NP clearance by MPS organs (liver, spleen) [95]. First, they injected extracellular vesicles (EVs) functionalized with cationized mannan to target and saturate the receptors of macrophages as an "eat me" signal. Then, nanocarriers modified with c(RGDm7) were fused with CD47-enriched exosomes to escape the MPS as a "don't eat me" signal (Fig. 7(e)). The results indicated that both the circulation time and accumulation at the tumor site of nanocarriers were increased. In a similar vein, CD47-enriched erythrocyte membranes were used as a concealment layer to coat gadolinium-loaded polymeric NPs. This biomimetic strategy achieved long circulation time and effective contrast-enhanced magnetic resonance imaging [96]. In another study, non-immunogenic PAS peptides (formed by proline (P), alanine (A), and serine (S)) were conjugated onto the surface of NPs. This strategy effectively reduced PC formation and impeded the activation of complement protein C3 *in vivo* [97]. In addition, transactivator of transcription (TAT) is a cell-penetrating peptide

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that can generate pores on the cell membrane [98, 99]. The simplicity of its sequence, ready availability, biodegradability, and especially its bioactivity make the TAT peptide an excellent candidate for ligand modification of NPs [100–102]. It has been reported that NPs modified with TAT peptide tend to recruit dysopsonins rather than opsonins, thus protecting the NPs from clearance [103].

Another effective strategy is decorating the NPs with endogenous plasma proteins, such as the dysopsonins albumin and clusterin, to adjust the bio–nano interaction [104]. For example, Palanikumar and co-workers cross-linked a bovine serum albumin (BSA) corona on poly(lactic-co-glycolic acid) (PLGA) NPs to avoid opsonization [105]. More interestingly, Li and colleagues constructed maleimide-coated PLGA NPs, which were able to selectively anchor albumin in their PC. The maleimide group specifically conjugated with the cysteine-34 residue of albumin [106]. This strategy helps avoid the ABC phenomenon following the second administration and facilitates accumulation of NPs at the disease site. These results suggest that an *in-vitro* pre-coated artificial albumin corona [105, 107, 108] or an *in situ* albumin-enriched corona [106, 109] can shield NPs from phagocytosis in the bloodstream. Compared to modification with a specific protein, pre-coating with plasma proteins is an alternative and simple way to avoid MPS recognition. For instance, Caracciolo and colleagues found that pre-coating liposomes with artificial corona can reduce capture by circulating immune cells and prolong the circulation time *in vivo* [110].

Corona formation is affected by the physicochemical properties of the NP surface; thus, simple chemical modification of the NP surface can provide an easy way to tailor the PC composition. For example, Lu et al. investigated the impact of the surface hydrophilicity on the PC formation [111]. They used simple chemical methods to modify the surface of graphene with hydroxyl groups, and then they compared protein adsorption onto fully hydroxylated, partly hydroxylated, and unmodified graphene (Fig. 8(a)). The result of simulations showed that the number of residues contacting the graphene sheet decreased with the increased level of hydroxyl groups (Fig. 8(b), using HSA as a model). When the contact surface area (CSA) was calculated between the graphene sheet and proteins, the results suggested fewer opsonin proteins (HSA and IgE) were adsorbed onto fully hydroxylated and partly hydroxylated graphene than the pure unmodified graphene, while the adsorption of ApoE was similar for all three graphene (Fig. 8(c)). This work demonstrates that the introduction of hydroxyl groups (to change the physicochemical properties of the surface) can decrease the adsorption of “bad” proteins while having a negligible effect on “good” proteins with a protective role.

The physical regulation of the PC has also been validated as a potential approach to prolong circulation. Magnetic NPs have

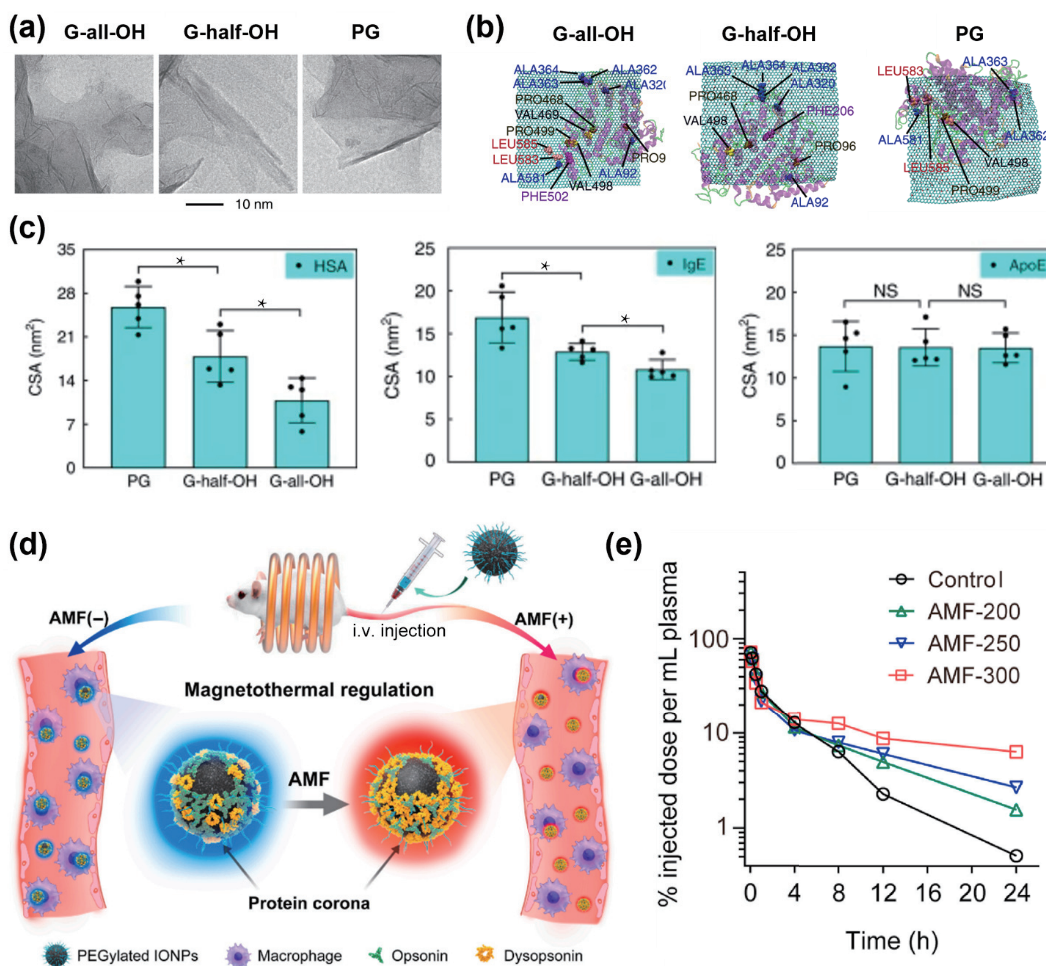

Figure 8 Physical and chemical strategies for regulating the PC to prolong circulation. (a) Transmission electron microscopy (TEM) images of fully hydroxylated, partly hydroxylated, and unmodified graphene sheets. (b) Snapshots of the computer simulation of the interaction between HSA and graphene sheets modified with different groups. (c) The contact surface area between graphene with the different surface modifications and HSA (left), IgE (middle), and ApoE (right). Adapted with permission from [111], © Lu, X. et al. 2019. (d) Schematic of magnetothermal regulation of the PC on IONPs. *In situ* exposure to an AMF induces IONPs to generate localized heat and changes in the composition of the opsonin and dysopsonin content of the PC. (e) Concentrations of IONPs in the bloodstream over time with or without exposure to different AMF. Adapted with permission from [114], © Elsevier Ltd. 2021. G-all-OH: fully hydroxylated graphene; G-half-OH: partly hydroxylated graphene; PG: pure graphene.

been widely used in preclinical and clinical applications as delivery carriers, imaging agents, iron supplements, etc. [112, 113]. Fan and colleagues were the first to tailor the PC of IONPs through magnetothermal regulation [114]. They found that the level of opsonins was reduced and the level of dysopsonins was increased in the PC of IONPs after treatment with an alternating magnetic field (AMF) *in vitro* and *in vivo* (Fig. 8(d)). Notably, the circulation time of IONPs was enhanced after magnetothermal regulation compared to untreated IONPs, and the increased circulation time was dependent on the AMF (Fig. 8(e)). This work is a great model to inspire new innovative approaches for PC regulation *in vivo*.

3.2 Increasing the targeting ability

As discussed in Section 3.1, PEG is mainly used to avoid MPS clearance based on the stealth effect. A few studies have reported that PEG can also increase the targeting ability of NPs. For instance, Cui et al. manipulated the PC by conjugating three different types of low molecular weight PEG onto the surface of GNPs. The molecular weight of PEG was 350, 550, and 1,000 Da. The results showed that PEG550-coated GNPs (GNP-550) adsorbed more albumin with dysopsonin function and Tf with tumor-targeting ability than GNP-350 and GNP-1000. When DOX was loaded between the GNPs and the PEG molecules, GNP-550 showed the highest uptake by HepG2 hepatocellular carcinoma cells and caused the highest cytotoxicity. *In vivo* studies also showed that DOX-loaded GNP-550 had the best targeting ability and antitumor efficacy [115]. In addition, Chen et al. adjusted the surface charge, PEG length, and PEG content of lipid NPs (LNPs) to recruit endogenous ApoE in the circulatory system and achieve targeted delivery of small interfering RNA to HepG2 cells [116, 117].

An alternative strategy to increase the targeting ability is to pre-coat the NPs with specific plasma proteins with inherent targeting capabilities. The primary function of Apos is to transport lipids, but ApoA1 and ApoE can cross the blood-brain barrier (BBB) to transport NPs into the brain via receptor-mediated transcytosis [118]. Thus, Dal Magro et al. pre-coated ApoE4 onto polysorbate 80-stabilized lipid NPs to produce an artificial Apo corona, which effectively facilitated the passage of the NPs through the BBB and promoted their accumulation in the brain parenchyma [119]. Yu et al. developed Tf-precoated PEGylated PS NPs through Tf/Tf receptor-mediated interactions for enhanced cancer-targeted therapy [120]. In another study, Oh and colleagues designed PC shield NPs (PCSNs) by pre-coating glutathione (GSH)-modified mesoporous silica NP (GSH-MSN) with the recombinant protein [121]. The recombinant protein comprises a human epidermal growth factor receptor 2 (HER2)-binding affibody (Afb) and GSH-S-transferase (GST) (GST-HER2-Afb)(Fig. 9(a)). The recombinant protein GST-HER2-Afb can coat on the surface of PCSNs via the supramolecular interaction between GSH and GST. As shown in Fig. 9(b), the obtained PCSNs evaded internalization by macrophages due to reduced serum protein adsorption. PCSNs also effectively targeted and accumulated in HER2-overexpressing SK-BR3 cells through interactions with the receptor ErbB2 (Fig. 9(c)).

As explained earlier, non-specific protein adsorption can shield the targeting moieties of NPs. Thus, specific recruitment of endogenous targeting moieties would be an alternative way to overcome this barrier [53, 122]. For example, Li et al. constructed a novel PLGA-PEG2000 NPs decorated with dihydroartemisinin (DHA) which specifically recruits endogenous ApoE onto the surface of the NPs [123]. The corona-anchored ApoE prolonged the blood circulation and facilitated the accumulation of NPs in the tumor through the EPR effect and the active transport mediated by the LDLR. In another example, Arcella et al. constructed brain-targeted cationic liposomes consisting of the cationic lipid DOTAP and the neutral lipid DOPE by adsorbing the Apos in the plasma to form a PC [124].

Different peptides have also been developed for the more specific recruitment and presentation of targeting proteins. For example, Zhan et al. constructed bio-inspired liposomes (SP-sLip) by functionalizing them with peptide $A\beta_{25\text{-}35}$ [125]. The peptide $A\beta_{25\text{-}35}$ can specifically recognise with the lipid-binding domain of exchangeable Apos. After being introduced into the bloodstream, SP-sLip specifically adsorbed Apos, including ApoA1, ApoE, and ApoJ, to achieve brain-targeted delivery (Figs. 9(d) and 9(e)). Compared with DOX-loaded plain liposomes, DOX-loaded SP-sLip showed obvious enhancement of brain targeting and treatment effect (Fig. 9(f)). In addition, the authors also used the peptides strategy in PLGA NPs for brain-targeted delivery. Zhan et al. functionalized the surface of PEGylated PLGA NPs with cholera toxin subunit B (CTB-NP), which recognizes and binds with GM1, a glycosphingolipid expressed on brain capillary endothelial cells. The results demonstrated that CTB-NP overcame the shielding effect of the PC and facilitated *in vivo* glioma-targeted drug delivery [126]. Moreover, Huo et al. constructed covalent organic framework (COF) NPs and then covalently modified them with T_{10} peptide [127]. T_{10} peptide can specifically recruit Tf from the bloodstream to form a Tf corona on the surface of COF NPs. The Tf corona COF NPs were able to cross the BBB and achieve targeted drug delivery to gliomas.

3.3 Increasing the payload and controlling the release

Controllable drug release is an essential feature of targeted NPs, but it still presents many significant challenges. The PC endows the NP with a new biological and physicochemical interface; thus, the proteins in the corona can potentially be exploited to control payload release. When NPs enter the bloodstream, the carrier may quickly release its drug cargo. This effect is called the initial burst release. Good nanomedicine candidates must have the ability to inhibit this burst release and instead facilitate controlled drug release [128, 129]. Mahmoudi and his colleagues studied how the PC changes the release of paclitaxel from Abraxane [130]. They found that the formation of PC reduced the release rate of the loaded drugs and significantly reduced burst release. In addition, the authors also showed that the formation of the PC reduced the burst release effect of superparamagnetic IONPs (SPIONs), but the impact on polymeric nanocapsules is very slight. Therefore, the formation of PC on specific types of NPs acts as a brake to benefit the drug release kinetics.

Apart from inhibiting burst release, the PC also increases the efficacy of the drug payload and facilitates its controlled release. For example, Hamad-Schifferli and colleagues found that the PC structure can be exploited as a functional platform to carry small molecular therapeutics [131]. Using gold nanorods (GNRs) as a model, the PC formed in equine serum (ES) on the surface of nanorods has an excellent capacity for DNA and DOX loading compared with covalent attachment strategies (Fig. 10(a)). Benefiting from the optical properties of nanorods, the payload can be controllably released from the corona complexes of nanorods by heat or ultrafast laser excitation. Further, the release kinetics of the payload also can be tuned by regulating the local environment of the PC [132]. If the corona-nanorods complexes were incubated with both soft and hard corona proteins, the payload was easily released with the exchange of free proteins and proteins from the hard corona. However, the release rate was slower if the corona-nanorods complexes were incubated only with soft corona proteins (Fig. 10(b)). Thus, regulating the corona composition of the corona-nanorods complex is potentially a valuable and simple way to control the payload release *in vivo*.

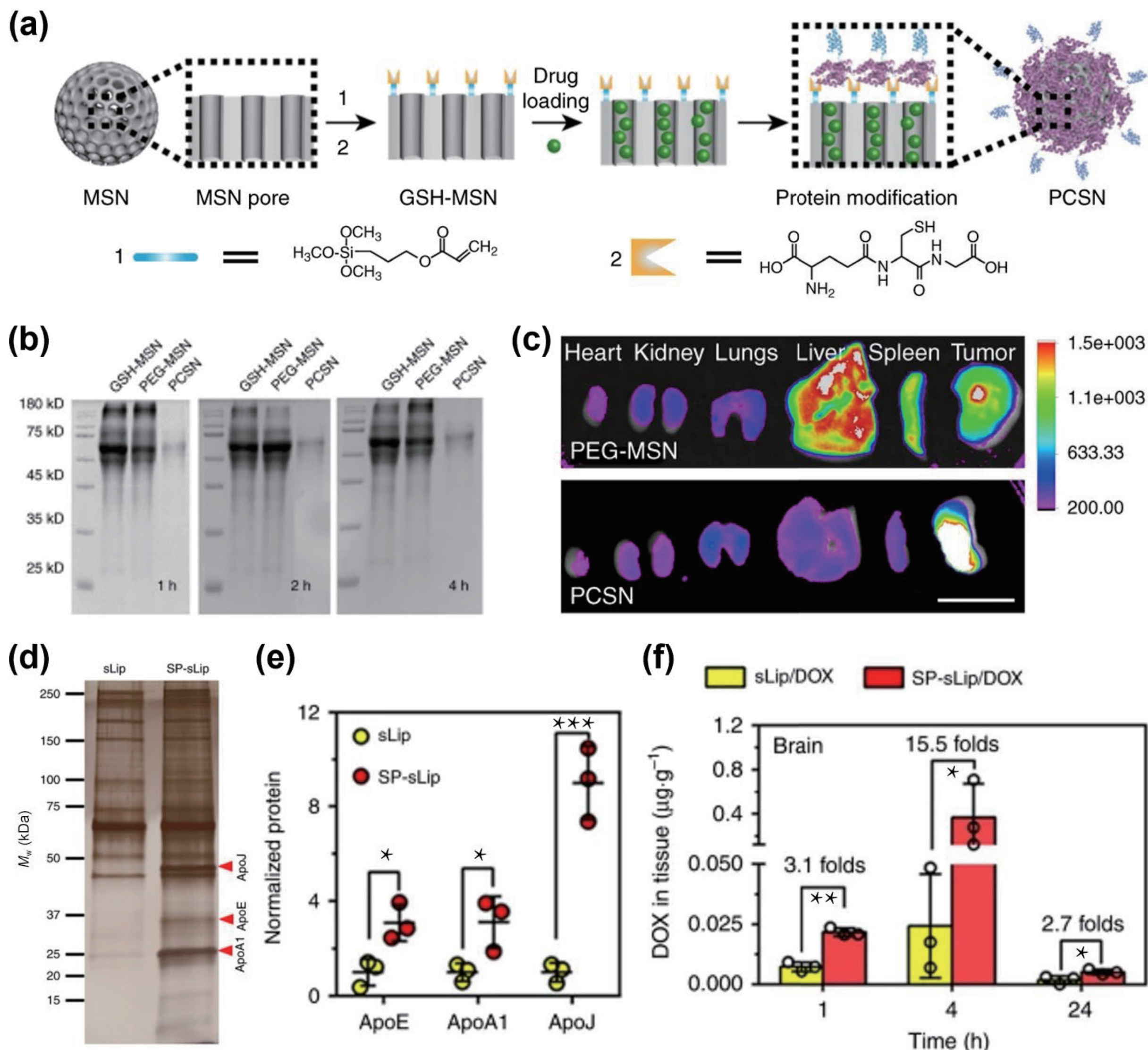

Figure 9 Regulation strategies to increase the targeting ability of NPs. (a) Schematic showing how pre-coating of targeting NPs with a corona reduces the adsorption of proteins and maintains targeting ability. (b) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of serum proteins attached to different NPs treated with 55% serum for different time. (c) Biodistribution of two different injected NPs in mice organs. Adapted with permission from [121], © Oh, J. Y. et al. 2018. (d) Analysis of the PC of sLip and SP-sLip after incubation with mouse plasma for 1 h. (e) Quantitative analysis of ApoE, ApoA1, and ApoJ on the surface of sLip and SP-sLip after *in vivo* adsorption. (f) Quantitative analysis of DOX in the brain of mice treated with SP-sLip/DOX and sLip/DOX. Adapted with permission from [125], © Zhang, Z. et al. 2019. SP-sLip: $A\beta_{25\text{-}35}$ with amide on the C-terminal methionine (SP)-modified liposomes.

Immunotherapy provides tremendous promise for improving tumor treatment. As a robust delivery platform, NPs have been widely used to deliver antigen, adjuvant, and therapeutics for tumor immunotherapy [133, 134]. Recently, Liu et al. reported a PC-driven nanovaccine to controlled release the antigen and adjuvant CpG [135]. The nanovaccines were constructed by the assembly of cleavable antigen-CpG conjugate and fluorinated dendrigraft-poly-lysine. The nucleic acid molecule CpG can firmly anchor with the fluorinated dendrigraft-poly-lysine thanks to the fluorine effect. Thus, the conjugated antigen can be protected against the unexpected release with PC formation on nanovaccines before they enter the cells. However, when the cleavable disulfide bond is broken under the reductive condition inside the cells, the existing PC can trigger the effective release of antigen from nanovaccines to the cytoplasm for efficient antigen presentation.

Beyond the exploitation of the PC to improve the transportation behavior of NPs, we must stress that the PC is a promising platform for other biomedical applications. For example, the PC has been used to detect diseases, such as Alzheimer's disease [136] and other central nervous system diseases [137], pancreatic cancer [138–141], and breast cancer [142]. The exploitation of the PC is still in the early stage, and we believe that scientists will develop more intelligent and less complicated strategies as our understanding of the PC improves. Here, we highlight again that understanding the spatial orientation and structure of PC is crucial for us to achieve novel strategies for biological control and advanced therapeutics.

4 Conclusions and future perspectives

There has been a massive amount of research into NPs in the last 30 years. NPs, as promising new-generation platforms, have been applied to disease treatment and diagnosis. However, the performance of actively targeted NPs is still not as perfect as we expected. It has also been widely realized that the artificially endowed unique characteristics of the surface of NPs are not steady but change as the surrounding environment changes. The formation of a PC creates a new biological identity, which is a fundamental problem for the development of nanomedicines. Now, we partly understand the potential effect of the PC on the delivery journey of NPs. This helps us to exploit the PC as a

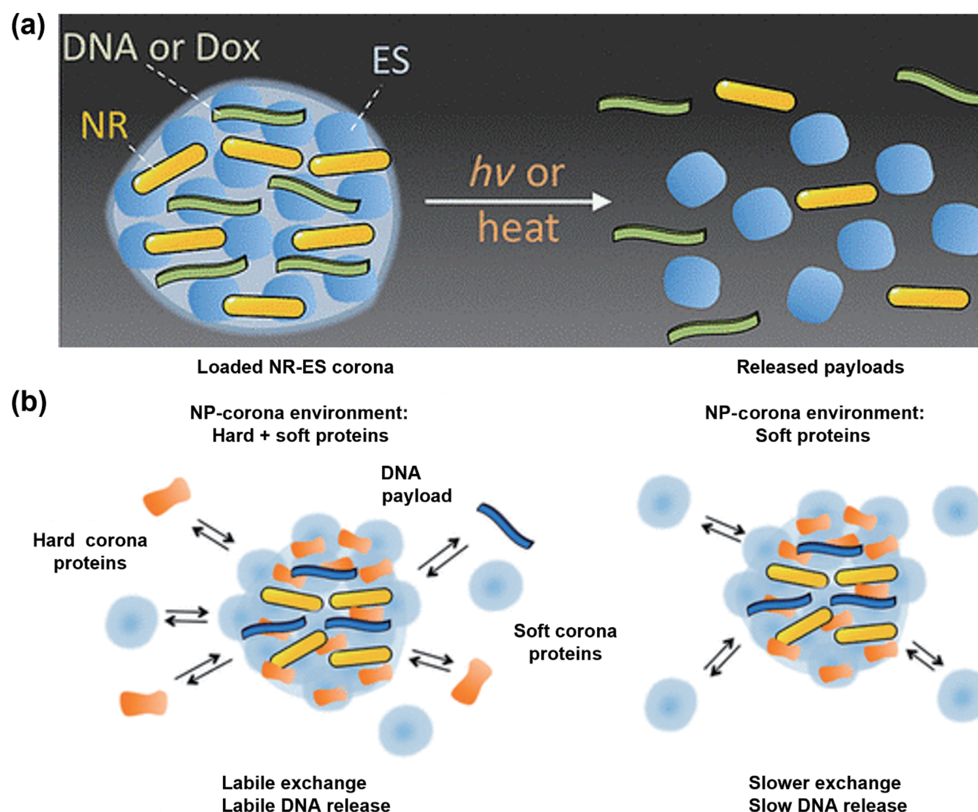

Figure 10 Effect of the PC on controlling the payload release. (a) Schematic of the PC on NR loaded with DNA and DOX. The payload can be controllably released from the corona complexes by laser excitation or heat. Adapted with permission from [131], © American Chemical Society 2012. (b) Schematic of the PC in different local environments. Corona-NR complexes loaded with DNA in the presence of hard and soft corona proteins can release the payload in an easy way. The release rate of the payload is slower if the corona-NR complexes are incubated with soft corona proteins. Adapted with permission from [132], © American Chemical Society 2013.

functional and versatile surface to design safe and efficient new-generation targeted NPs. Considering the application of advanced technology has a hysteresis quality compared with the development of technology, we believe that targeted NPs (just like mRNA vaccines which have become widely used for the prevention of COVID-19 in these past two years) will become a significant component of precision medicine. For better practical application, the following suggestions need to be considered in the future.

Firstly, we emphasize the importance of methodology for *in vivo* PC research. When isolating the PC for analysis from *in vivo* environment, two critical problems are contamination and composition change. The existing methods for analysis of the PC, such as centrifugation and chromatographic and magnetic separation, can cause loss of weakly bound proteins by dissociation from the NP-PC complexes [143]. Meanwhile, endogenous biological NPs, such as protein aggregates, can be co-isolated with the NP-PC complexes; thus, to some degree, the composition information is misleading for downstream research and applications [144]. Here, we stress the urgent development of *in situ* methodology for *in vivo* PC research field [145, 146]. The new methodology should focus on the dynamic adsorption and evolution of the PC with high temporal and spatial resolution *in vitro* and *in vivo*.

Secondly, we emphasize the importance of understanding the basic theory of bio–nano interaction to exploit the PC *in vivo*. As mentioned above, although different strategies have been developed to reverse the adverse effects of the PC for *in vivo* application, we must recognize that our fundamental understanding of the bio–nano interaction mediated by the PC is minimal and biased. If we are enthusiastic about developing different delivery strategies but ignore the fundamental mechanism of these interactions, we will go back to the old ways as we did in the last 30 years. As the Chinese proverb says, sharpening your axe will not delay your job of chopping wood. Here, we are appealing to the community to focus on the fundamental scientific questions about the PC, including: (1) the formation mechanism at the level of single-molecules; (2) the composition and architecture of the PC *in vivo*; (3) the effect of the PC on bio–nano interface recognition and interaction at the molecular level *in vivo*; (4) the correlation between the biological identity and the biological function and outcomes.

Thirdly, we emphasize the systematic nature of the PC effect on delivery journey of NPs. With our increasing understanding of bio–nano recognition, there is no doubt that the PC, as a functional and versatile platform, plays a central role in the clinical translation of NPs. The effects of PC on the whole delivery journey of NPs are manifold; thus, the overall effectiveness of NPs will be most severely compromised by the most critical obstacles in the whole journey (that is, the bucket effect). Thus, in future studies, it will be important to systematically evaluate the PC effects in the whole journey.

Last, but most importantly, we emphasize the unity of the Yin and Yang effect of the PC. A robust delivery system is directly related to the internal balance between Yin and Yang effect. Our initial goal is to exploit the PC to improve the Yang effect (such as targeting efficiency) and reduce the Yin effect (such as liver clearance). However, if the Yang effect is a hundred percent (that is, no liver clearance) for NPs, maybe it cannot be metabolized, and the harmful effect could be greater than the therapeutic effect. Thus, we highlight that we should not pursue the highest Yang effect; instead, our scientific aim is to find the balance window of the Yin and Yang effect of PC in the human body, and our technical aim is to adjust the balance between the Yin and Yang effect in the window with advanced nanotechnology. In the

balance window mediated by PC, NPs would produce the desired efficacy and safety is guaranteed.

Overall, with recent technological advances and understanding on the underlying mechanics of bio–nano interaction, we believe that the PC will play a central and positive role in the development of nanomedicines.

Acknowledgements

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