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DePEGylation strategies to increase cancer nanomedicine efficacy

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Abstract: To maximize drug targeting to solid tumors, cancer nanomedicines with prolonged circulation times are required. To this end, poly(ethylene glycol) (PEG) has been widely used as a steric shield of nanomedicine surfaces to minimize serum protein absorption (opsonisation) and subsequent recognition and clearance by cells of the mononuclear phagocyte system (MPS). However, PEG also inhibits interactions of nanomedicines with target cancer cells, limiting the effective drug dose that can be reached within the target tumor. To overcome this dilemma, nanomedicines with stimuli-responsive cleavable PEG functionality have been developed. These benefit from both long circulation lifetimes en route to the targeted tumor as well as efficient drug delivery to target cancer cells. In this review, various stimuli-responsive strategies to dePEGylate nanomedicines within the tumor microenvironment will be critically reviewed.

Keywords: nanomedicine, cancer, stimuli responsive, dePEGylation, EPR effect

1. Introduction

In the treatment of cancer, the main challenge is how to deliver cytotoxic drugs to cancer cells while minimizing off-target toxicity in healthy cells and tissue. Patients currently undergoing cancer chemotherapy will typically experience debilitating side effects¹ (e.g. impaired immune system, nausea, cardiomyopathy, hair loss), and in many cases, the cumulative lifetime dose of an anti-cancer drug (e.g. doxorubicin; 550 mg/kg) must be limited, irrespective of therapeutic success, to avoid permanent bodily damage.² Efforts have therefore been made to develop nanomedicines capable of delivering drugs specifically to cancer cells.³

Over the past 30 years, two clinically effective *targeted* cancer therapies have emerged: antibody-drug conjugates (ADCs) and nanoparticle-based systems. Currently, 4 ADCs and 7 distinct nanoparticle-based drug delivery systems (DDS), targeted against a variety of human cancers, have received market approval.^{4, 5} For ADCs, active targeting of cancer cells is achieved through antibody recognition of (over-)expressed receptors (tumor-associated antigens).⁶ Once bound, ADCs are endocytosed, the conjugated drug released and the cell destroyed. Although effective, ADCs are costly to manufacture, can elicit adverse immunogenic responses (limiting repeat dosing) and are largely restricted to the delivery of small molecule (and serum stable) drugs.⁷ In the case of nanoparticle-based DDS, drugs are encapsulated within a self-assembled nanoparticle, hidden and protected from the *in vivo* environment. Pharmacokinetic (PK) profiles are dictated by the nanoparticle and, in theory, it is possible to deliver almost any therapeutic cargos, from small molecule drugs to plasmid DNA, to target cells and tissue within the body. An enormous variety of nanoparticle-based DDS have been reported, however the most widely investigated, and the majority approved for clinical application, are liposomes.⁸ In the targeted treatment of cancer, all clinically approved nanoparticle-based nanomedicines are liposomes designed to passively target tumors *via* the enhanced permeability and retention (EPR) effect.^{9, 10}

1.1 The enhanced permeability and retention (EPR) effect

Following administration to the body, small molecule drugs freely diffuse into tissue and away from the site of injection. In contrast, intravenously (*i.v.*) injected nanoparticles are restricted to the circulating blood flow, unable to cross the tightly packed endothelium due to their larger size. For optimal biodistribution, nanoparticles should be larger than 10 nm in diameter – below which they are filtered from the body *via* the kidneys¹¹ – and smaller than 200 nm in diameter – above which they are rapidly recognized and phagocytosed by blood resident macrophages (principle cells of the mononuclear phagocyte system, MPS), within the liver and spleen, and are cleared from the body.¹²

The EPR effect is a phenomena characterized by the ill-defined ('leaky') vasculature and poor lymphatic drainage of tumors that arises as a result of rapid angiogenesis (blood vessel growth) within tumor tissue.¹³ Circulating nanoparticles circulating through the tumor vasculature can therefore passively diffuse across gaps in the endothelium, accumulate within the tumor and remain there for extended

periods of time. Once within the tumor, nanoparticle encapsulated drugs either passively diffuse from the nanoparticle or an endogenous or exogenous stimulus can be exploited to trigger release.

To maximize passive targeting of nanomedicines to solid tumors *via* the EPR effect, nanoparticles with long circulation lifetimes are sought. Put simply, the more times nanoparticles pass through the tumor vasculature, the more will accumulate there. Care must therefore be taken to minimize drug leakage from the nanoparticle *en route* to the tumor while ensuring therapeutically relevant concentrations of drugs are released once there. In the case of liposome-drug formulations, this involves careful choice of lipid reagents (*e.g.* cholesterol to rigidify fluid lipid membranes) to fine tune drug retention/release profiles while at the same time maximizing circulation lifetimes.¹⁴

1.2 Polyethylene glycol (PEG)

To achieve long circulation lifetimes, the principal biological barrier a nanoparticle must overcome is recognition and clearance by cells of the mononuclear phagocyte system (MPS).¹⁵ The principle organ of the MPS is the liver where hepatic macrophages – Kupffer cells – are highly proficient at recognizing and removing macromolecular, colloidal and pathogenic waste from circulation.^{16, 17} Without any surface modification, up to 99% of systemically administered nanoparticles are cleared by the liver.¹⁸ In most cases, it is believed rapid adsorption of blood proteins to the surface of nanoparticles, (a process known as opsonisation), acts as the recognition beacon for MPS cells.¹⁹ For this reason, sterically shielding nanoparticle surfaces with biocompatible polymers, such as polyethylene glycol (PEG), has been effectively employed to minimize opsonisation and prolong blood circulation times of nanoparticles *in vivo*.²⁰

PEG is a synthetic polymer of repeating ethylene glycol units. Used as a reagent or additive in a wide range of biological, chemical and industrial settings,^{21, 22} it is commercially available in a range of geometries (linear, branched, star, comb), molecular weights (from 300 Da – 6-7 repeating units – up to 10 MDa - >200,000 repeating units) and can be easily functionalized. PEGylation of nanoparticle surfaces has been shown to decrease serum protein adsorption, reduce nanoparticle uptake in the liver and prolong circulation lifetimes.²³ Recently, reports have emerged to suggest PEG can elicit an immunogenic response in mammals.²⁴ However, the extent of this response, caused by binding of anti-PEG antibodies, remains unclear.²⁵ PEG remains an FDA approved polymer and is still the most widely used polymeric coating of nanomedicines, both in academic and industrial research. In terms of cancer nanomedicines, PEGylated liposomal-doxorubicin (Doxil®) has been used clinically for over 20 years in the treatment of select breast and ovarian cancers, multiple myeloma and AIDS-related Kaposi's sarcoma.²²

1.3 The PEG dilemma

While PEGylation prolongs circulation lifetimes, it also limits the cellular uptake of nanoparticles and therefore effective drug delivery to target cancer cells.²⁶ This so-called ‘PEG dilemma’ has proved a major obstacle in the effective delivery of therapeutic cargos to cancer cells, particularly those that must be actively transported across the target cellular membrane (*e.g.* proteins and oligonucleotides).²⁷ For instance, in the delivery of oligonucleotides (ODNs) or small interfering RNAs (siRNAs), significantly lower transfection/transduction efficiencies were observed for PEGylated vs. non-PEGylated DDS.²⁸ To overcome this dilemma, strategies have been proposed to trigger the *extracellular* shedding of PEG (*i.e.* dePEGylation) from a nanoparticle surface upon reaching the target tumor. This leads to one of three scenarios (Figure 1): 1) rupture of the nanoparticle and extracellular drug release; 2) cellular uptake (endocytosis) of the intact nanoparticle-drug complex or 3) in the case of liposomes, fusion with the target cellular membrane and drug release directly to the cell cytoplasm, crucially avoiding degradative endocytotic liposome uptake.

In a significant number of reported dePEGylation strategies, it is required that PEGylated nanoparticles are first taken up by target cancer cells, whereupon the low pH, reductive and protease-rich environment of the late endosome/lysosome can be effectively exploited to trigger *intracellular* dePEGylation and drug release. However, these systems *do not* overcome the “PEG dilemma” and the very limited uptake of PEGylated nanoparticles remains a major drawback. As such, these systems will not be further discussed in this review but are included in the comprehensive summary of dePEGylation strategies presented in Table 1.

For strategies involving *extracellular* dePEGylation within the target tumor, a key difference is whether dePEGylation causes destabilization of the nanocarrier and *extracellular* drug release (*i.e.* burst release), or intact nanocarrier internalization by target cancer cells and *intracellular* drug release. In the case of *extracellular* drug release, only drugs able to passively diffuse (or be actively transported) across target cancer cell membranes (*e.g.* membrane permeable doxorubicin) can be used. In the case of *intracellular* drug release, the delivery of membrane impermeable therapeutics (*e.g.* proteins, oligonucleotides) is possible. In either scenario, it is essential cancer cells are exposed to therapeutically relevant doses of cytotoxic drugs if improved therapeutic indices are to be achieved.

2. Physical dePEGylation

Two physical approaches to dePEGylate nanoparticle surfaces within target tissues have been investigated. The first, most relevant for liposomal nanomedicines, relies on the exchange of PEGylated lipids from a liposome membrane to a target membrane sink (*e.g.* target cancer cell membranes).²⁹ Here, the rate at which exchange occurs is heavily dependent on the lipid anchor tethering PEG to the liposome membrane (*i.e.* how strongly it is held within the liposome membrane).³⁰ The length and saturation of (phospho)lipid fatty acid (FA) chains determines both the thickness and rigidity of a lipid membrane. FA

chain lengths within biological membranes typically vary between C12 and C30 – the number of carbon atoms.³¹ FA chains can be saturated (no double bonds) or unsaturated (1 or more double bond). Saturated FAs pack closely together to form rigid lipid membranes (gel state), whereas unsaturated FAs loosely pack to form fluid membranes liquid crystalline state).³² In addition, the shorter the FA chains, the more fluid the membrane. This is reflected in the liquid crystalline-to-gel transition temperatures (T_m) of individual (phospho)lipids.

In a study of three different lipid-PEG conjugates, no lipid-PEG exchange was observed for long chain, saturated lipid anchors 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG, C18:0 – 2 x 18 carbon FA chain; no double bonds) whereas exchange occurred in the time frame of hours for shorter saturated lipids 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG; C14:0) or long chain, unsaturated lipids 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE-PEG; C18:1 – 2 x 18 carbon FA chain; each 1 double bond, $\omega 9$).³³ This time frame enabled efficient accumulation of liposomes in tumor sites *via* the EPR effect (prior to dePEGylation) coupled with increased cellular uptake within the tumor (following dePEGylation). Conversely, a similar study found that only in the case of DSPE-PEG were circulation times prolonged enough to see efficient passive accumulation of nanoparticles within the tumor.³⁴ These conflicting results highlight the fine balance required to achieve efficient passive accumulation within target tumors and subsequent dePEGylation *via* physical desorption of lipid-PEG reagents. The propensity for non-specific PEG exchange with biological membranes *in vivo*, prior to reaching the target tumor, has likely limited the widespread application of these approaches.

The second physical approach relies on non-covalent adsorption of PEG to a nanoparticle surface.³⁵⁻³⁹ For example, carboxylate-functionalized PEG adsorbed to a cationic nanoparticle surface.³⁷ In this case, partial protonation of carboxylate groups within the acidic (pH 6.5-7) extracellular tumor microenvironment leads to dePEGylation and subsequent cellular nanoparticle uptake. While this approach is conceptually simple, the stability of the absorbed PEG corona in serum and the propensity of premature dePEGylation under physiological conditions (*e.g.* high salt) and/or through competition from other serum components has likely limited the widespread investigation of this approach.

3. Chemical dePEGylation strategies

By far the most common method to achieve *extracellular* dePEGylation of nanoparticle surfaces, within the tumor microenvironment, is through chemical approaches. In these cases, PEG is grafted to the nanoparticle *via* a stimuli-responsive covalent chemical bond (Table 1 and 2).⁴⁰ Stimuli can be both endogenous and exogenous. In the case of endogenous stimuli, intrinsic differences in the pathophysiology of tumor and healthy tissues are exploited, namely the low pH,⁴¹ reducing⁴² and matrix metalloprotease (MMP)-rich environment⁴³ of certain solid tumors. Exogenous stimuli, including light and heat, have the benefit of being under complete user control in both time and space.⁴⁴ In a clinical setting however, these approaches rely on the ability to efficiently deliver stimuli (*e.g.* light) to tissues

often deep within the body. The various stimuli-responsive chemistries commonly used in both the *intra-* and *extracellular* dePEGylation of nanoparticles are summarized in Table 1 and 2.

3.1 pH-sensitive dePEGylation

The mildly acidic (pH 6.5-7.2) extracellular environment of hypoxic tumors – a result of increased glucose catabolism and efflux of H⁺ by cancer cells – has been exploited to trigger extracellular dePEGylation of nanoparticle surfaces.⁴⁵ For this, chemical functionalities stable at physiological pH (pH 7.4) but labile at lower pH are required. The most commonly used acid labile chemical groups are vinyl ethers,⁴⁶⁻⁵⁰ hydrazones,⁵¹⁻⁶¹ acetals,⁶²⁻⁶⁹ β-thiopropionates,⁷⁰ ortho esters⁷¹⁻⁷³ and benzoic imines⁷⁴⁻⁸². Here however, it is important to differentiate between the mildly acidic *extracellular* pH within the tumor microenvironment (pH 6.5-7) and the strongly acidic *intracellular* pH within late endosomes/lysosomes (pH 4.5-5.5) and to stress that optimal sensitivity (and subsequent dePEGylation efficiency) of these acid-labile functionalities is typically at pH 5-5.5. Therefore all these pH-sensitive systems demonstrate inefficient/sluggish acidolytic dePEGylation within the *extracellular* tumor microenvironment. This can be exploited to achieve prolonged and sustained drug release within the tumor and/or partial dePEGylation may still generate the desired outcome. For example, Gu *et al.* reported pH dependent dePEGylation of polycationic micelles through grafting of PEG, *via* benzoic imine linkages, to poly-L-lysine(PLL)/cholic acid co-polymers.⁸² By measuring changes in surface charge (zeta potential), the authors were able to show colloidal stability at physiological pH as well as increasing rates of dePEGylation with decreasing pH (complete acidolysis at pH 5.5 within 10 min). At pH 6.5-7 (*i.e.* pH of the extracellular tumor microenvironment) only partial dePEGylation was observed, however this was accompanied by a significant increase in hemolytic activity suggesting partial dePEGylation was sufficient to endow these particles with the desired function. As this system was not tested in cancer models *in vivo*, it remains to be seen whether this slow rate of acidolysis will adversely affect function and efficacy. Indeed, the individual successes of pH responsive dePEGylation systems ultimately depends on the ability to deliver therapeutically relevant drug doses to cancer cells above and beyond those of the administered free drug alone. It is worth noting, however, these technologies – as with any system exploiting endogenous stimuli – will likely demonstrate significant variations in efficacy due to patient-to-patient heterogeneity of tumor pathologies.^{83, 84}

3.2 Redox-sensitive dePEGylation

Glutathione (GSH), is an abundant reducing agent (2-10 mM) in most mammalian cells, including cancer cells.⁸⁵ Extracellular GSH concentrations in healthy tissue are approximately 1000x lower (2–20 μM),⁸⁶ however this value can increase up to 4-fold (4-80 μM) within the tumor microenvironment.⁸⁷ There are conflicting reports as to whether this small differential in extracellular GSH concentrations can indeed be exploited to trigger *extracellular* dePEGylation. While a small number of studies report (partial)

extracellular cleavage of disulfide linked PEG constructs within the tumor microenvironment,⁸⁸⁻⁹² most exploit GSH as an *intracellular* trigger only.⁹³⁻¹³⁴ In these cases, the very large differential between *extra-* and *intracellular* GSH concentrations is a readily exploitable endogenous trigger. Indeed, for systems designed to exploit *intracellular* GSH levels, *extracellular* stability (*i.e.* very limited reduction) of disulfide-PEG constructs is often reported as a key feature in maintaining colloidal stability of nanoparticles in circulation and *en route* to the target tumor. In our critical opinion, exploiting the marginally elevated *extracellular* GSH levels of the tumor microenvironment is an ineffective strategy to overcome the ‘PEG dilemma’.

3.3 Protease-sensitive dePEGylation

Within the tumor microenvironment, there are high levels of *extracellular* matrix metalloproteinases (MMPs). These lytic enzymes are secreted at high levels by tumor cells to degrade the extracellular matrix (ECM) and aid cancer cell migration.^{135, 136} Short peptides containing enzyme-consensus sequences, linking PEG to a nanoparticle surface, have been effectively used to dePEGylate nanoparticles within the tumor microenvironment.¹³⁷⁻¹⁶¹ Torchilin *et al.* have reported two elegant examples of MMP-triggered dePEGylation. The first employed a multifunctional liposomal formulation comprising longer, MMP-cleavable lipid-PEG₃₄₀₀ constructs and shorter, non-cleavable TAT-functionalised lipid-PEG₂₀₀₀ constructs.¹⁴¹ In the absence of MMPs, longer PEG₃₄₀₀ chains effectively shielded the cell penetrating function of the underlying TAT peptide and liposomes were sparingly taken up by cells. Upon MMP-mediated dePEGylation however, the newly revealed TAT-functionalised liposomes were avidly taken up by 4T1 breast cancer cells. Going one step further, the same group reported a similar strategy of exploiting MMP-mediated dePEGylation to reveal newly functional drug polymer micelles.¹⁴⁶ Crucially in this approach, dePEGylation did not destroy the integrity of the underlying drug-filled micelle leading to efficient stimuli responsive, *intracellular* drug delivery to cancer cells, as demonstrated in mice models.

It is worth noting here that both cathepsin B (protease)¹⁶² and esterases¹³⁸ have also been exploited to trigger dePEGylation of nanomedicines. However, cathepsin B is only found at high levels within (*intracellular*) cancer cells, while esterases are widely distributed in plasma and healthy tissues and not therefore specific to the tumor microenvironment. In our opinion, MMP-mediated dePEGylation of nanoparticles within the tumor microenvironment represents the most selective and efficient strategy to enhance the efficacy of cancer nanomedicines exploiting *endogenous* stimulus.

3.4 Light-sensitive dePEGylation

Photolabile chemical bonds have been extensively used, in both chemistry and biological contexts, to precisely control where and when new functionality is revealed. Unlike endogenous stimuli such as pH,

redox and enzymatic cleavage, the application of light can be precisely controlled in both time, space and intensity (*i.e.* is user defined) and requires no other reactive species (other than, in some cases, water). In addition, photolysis is generally rapid (few seconds, pulsed laser), quantitative and clean.

For light triggered dePEGylation of potential cancer nanomedicines, *o*-nitrobenzyl (*o*-Nb),^{63, 163-166} platinum-azide complexes¹⁶⁷ and azobenzene¹⁶⁸ functionalities have all been explored.¹⁶⁹ In the case of *o*-Nb functionalities, non-hydrolytic photolysis proceeds through a cyclic intermediate followed by the release of the desired alcohol and a nitroso by-product.¹⁷⁰ To increase biological compatibility, methoxy substitution of the aryl ring results in reduced toxicity of nitroso byproducts.¹⁷¹ We have recently reported two separate strategies in which light triggered dePEGylation was successfully used to initiate efficient drug delivery to target cancer cells. In the first example, we created 100 nm, loose core shell micelles composed exclusively of photolabile doxorubicin-PEG₂₀₀₀ reagents.¹⁶⁵ In the absence of light, micelles were stable, non-toxic (*i.e.* not taken up by cells *in vitro*) and no doxorubicin release was observed over time. Upon light (365 nm) activation triggered dePEGylation, micelle destabilisation and subsequent burst drug release resulted in *in vitro* cytotoxicity comparable to free doxorubicin. In addition, we were able to demonstrate precise spatiotemporal control of doxorubicin delivery to cells *in vitro* through light templated activation. We are currently assessing this system *in vivo* to determine circulation lifetimes and tumor accumulation of PEGylated doxorubicin prodrug micelles prior to light triggered dePEGylation.

In the second example, light triggered dePEGylation was used to precisely control, in time and space, the function of a simplified membrane fusion system. This system comprises two complementary peptides – peptide E and K – displayed from opposing lipid membranes (either liposome-liposome or liposome-cell).¹⁶³ In this case, PEGylation (*via* a photolabile cholesterol-PEG construct) of one lipid membrane effectively shielded the interaction between complementary peptides. However, upon light triggered dePEGylation regain of fusion function was instantaneous. We have subsequently shown our simplified membrane fusion system can be used to selectively deliver liposome-encapsulated cargos, *via* membrane fusion, to target (xenografted) cancer cells *in vivo* (zebrafish larvae).¹⁷² Extending this approach to include light triggered dePEGylation, to enable precise user control of drug delivery, is the subject of current investigations in the group.

The use of light does, of course, raise valid concerns going forward into the clinic. In all reported examples of light triggered dePEGylation, systems are most sensitive to high energy UV-A light (<400 nm). Short wavelength UV light suffers from poor tissue penetration (100-200 μ m) and, following prolonged exposure, can elicit significant photocytotoxicity.¹⁷³ Only for polymeric systems containing platinum-azide complexes¹⁶⁷ was photolytic dePEGylation investigated using visible light irradiation. Here, decreasing photolytic efficiency correlated with longer light wavelengths. Here however, it is important to note that photodynamic therapies,¹⁷⁴ combining chemical photosensitizers and light activation, are already widely used in the clinic to treat a range of medical conditions, including acne, atherosclerosis and cancer.¹⁷⁵ Furthermore, advances in fibre optic technologies (to deliver UV light deep

within tissue),¹⁷⁶ the development of photolabile chemical bonds sensitive to longer wavelength light¹⁷⁷ and the optimization of photosensitive chemical functionality to minimize light exposure, will only further the clinical applicability of light. One promising development has been photolabile chemical groups sensitive to two photon light,¹⁷⁸ to not only increasing tissue penetration (>1 cm) of light and minimising induced photocytotoxicity but, by restricting light activation to the focal point of two photon beams, enabling activation volumes in patients of <1 femtolitre. In this vein, we and others have also shown it is possible to cleave *o*-Nb groups using 2-photon light.¹⁷⁹ There are currently no examples of responsive dePEGylation of nanoparticles using alternative external stimuli (*e.g.* heat or ultrasound).

4. Conclusion

Stimuli-responsive dePEGylation is a proven strategy to increase the efficacy of cancer nanomedicines passively targeting solid tumors *via* the EPR effect. This approach has the dual advantage of both extended circulation lifetimes of PEGylated nanoparticles (to enhance passive targeting efficiency to tumors) as well as enhanced drug delivery profiles of non-PEGylated (or ruptured) nanoparticles within the tumor microenvironment. To achieve maximal effect, nanomedicines must remain PEGylated *en route* to the tumor (*i.e.* are serum stable) and be efficiently dePEGylated within the *extracellular* tumor microenvironment. Given the very low cellular uptake of PEGylated nanoparticles, strategies that report stimuli-responsive *intracellular* dePEGylation should not be considered effective. In our view, the most promising stimuli-responsive nanomedicines to date have exploited the MMP-rich microenvironment of solid tumors to trigger targeted and *extracellular* dePEGylation. However, by exploiting endogenous pathophysiological differences between healthy and diseased tissue, such as differences in MMP concentrations, the efficacy of these stimuli-responsive systems in patients will likely vary due to patient-to-tumor tumor heterogeneity.⁸³ In contrast, dePEGylation triggered by external stimuli, such as light, is exclusively determined by the user. While these approaches negate potential differences in efficacy driven by tumor heterogeneity, the current technological limitations of delivering external stimuli to site specific locations in patients remains a major drawback. However, the continued advance and optimisation of fibre-optic technologies as well more advanced photolabile chemical groups will only promote further the future application of light triggered cancer nanomedicines.

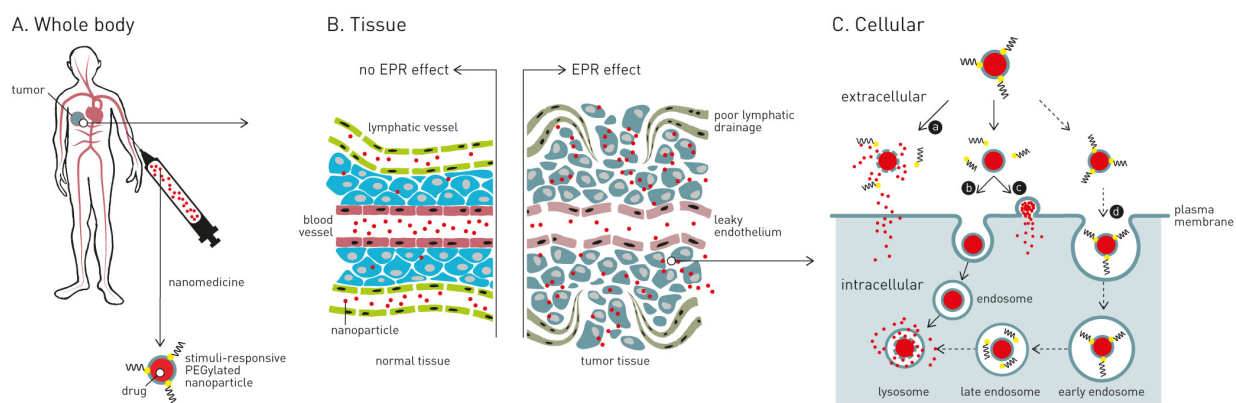


Figure 1. Following passive targeting of solid tumors *via* the enhanced permeability and retention (EPR) effect, stimuli-responsive dePEGylation of cancer nanomedicines can lead to various routes of enhanced drug delivery: **route a** – extracellular dePEGylation, nanocarrier rupture and extracellular drug delivery; **route b** – extracellular dePEGylation, endocytotic nanocarrier uptake and intracellular drug delivery; **route c** – extracellular dePEGylation, nanocarrier fusion with cancer cell membrane and direct cytosolic drug delivery (most relevant for liposomal nanomedicines); **route d*** – endocytotic nanocarrier uptake, intracellular dePEGylation and intracellular drug delivery. * this route *does not* overcome the “PEG dilemma” and the very limited uptake of PEGylated nanoparticles is a major drawback of these systems.

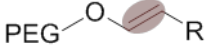
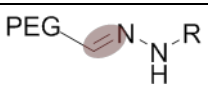
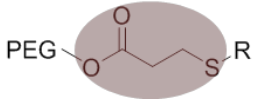
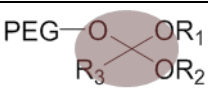
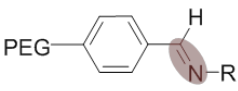
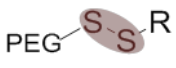
Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [refs]	
				Intracellular	Extracellular
Intracellular*	Low pH Late endosome (pH <6.5) Lysosome (pH 5.5-6.5)	 Vinylether	Liposomes	[46-49]	
			Micelles	[50]	
		 Hydrazone	Liposomes	[52-54]	
			Micelles	[55-61]	
		 Acetal	Liposomes	[62]	
			Micelles	[63-69]	
		 β -thiopropionate	Micelles	[70]	
	Reduction Glutathione (GSH) (2-10 mM)	 Ortho ester	Liposomes	[71-73]	
		 Benzoic-imine	Micelles	[74-76]	
			Liposomes	[93]	
			Polymersomes	[94, 95]	
			Micelles	[96-121, 133]	
			Graphene Oxide	[122-124]	
			Mesoporous silica nanoparticles (MSN)	[125-132]	
			Magnetic nanoparticles	[134]	
	Enzymatic Cathepsin B	 (peptide consensus sequence)	Liposomes	[162]	

Table 1. Various stimuli responsive chemical functionality used to trigger intracellular dePEGylation of cancer nanomedicines within the tumor microenvironment. *given the very limited uptake of PEGylated nanoparticles, systems reliant on intracellular triggers do not overcome the ‘PEG dilemma’ and are not further discussed in this review.

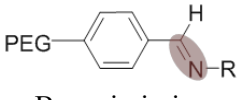
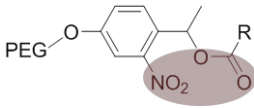
Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [refs]	
				Intracellular	Extracellular
Extracellular	Low pH (pH 6.5-7)	 Benzoic-imine	Polymeric Nanoparticles	[77]	
			Magnetic nanoparticles		[78-80]
			Micelles	[81]	[82]
	Reduction Glutathione (GSH) (4-80 μ M)	 Disulfide	Liposomes		[88-92]
	Enzymatic (non-specific) Esterase	 	Liposomes	[137, 138]	
	Enzymatic (specific) Matrix metallo-proteinases (MMPs)	 <i>(peptide consensus sequence)</i>	Liposomes	[139-145]	
			Quantum dots (QDs)	[157]	
			Polymersomes	[158]	
			Magnetic nanoparticles	[159]	
			Micelles	[146-156]	[160, 161]
	External Light	 	Liposomes	[163]	
			MSN	[164]	
			Micelles	[168]	[63, 165-167, 169]

Table 2. Various stimuli responsive chemical functionality used to trigger extracellular dePEGylation of cancer nanomedicines within the tumor microenvironment.

355 References

- 356 1. R. M. McQuade, V. Stojanovska, R. Abalo, J. C. Bornstein and K. Nurgali, *Front. Pharmacol.*,
357 2016, **7**, 414-427.
- 358 2. A. M. Rahman, S. W. Yusuf and M. S. Ewer, *Int. J. Nanomedicine*, 2007, **2**, 567-583.
- 359 3. Y. H. Bae and K. Park, *J. Control. Release*, 2011, **153**, 198-205.
- 360 4. A. Beck, L. Goetsch, C. Dumontet and N. Corvaia, *Nat. Rev. Drug Discov.*, 2017, **16**, 315-337.
- 361 5. H. I. Chang and M. K. Yeh, *Int. J. Nanomedicine*, 2012, **7**, 49-60.
- 362 6. S. C. Alley, N. M. Okeley and P. D. Senter, *Curr. Opin. Chem. Biol.*, 2010, **14**, 529-537.
- 363 7. H. L. Perez, P. M. Cardarelli, S. Deshpande, S. Gangwar, G. M. Schroeder, G. D. Vite and R. M.
364 Borzilleri, *Drug Discov. Today*, 2014, **19**, 869-881.
- 365 8. J. Shi, P. W. Kantoff, R. Wooster and O. C. Farokhzad, *Nat. Rev. Cancer*, 2017, **17**, 20-37.
- 366 9. H. Maeda, J. Wu, T. Sawa, Y. Matsumura and K. Hori, *J. Control. Release*, 2000, **65**, 271-284.
- 367 10. K. Greish, *Methods Mol. Biol.*, 2010, **624**, 25-37.
- 368 11. M. Longmire, P. L. Choyke and H. Kobayashi, *Nanomedicine*, 2008, **3**, 703-717.
- 369 12. E. Blanco, H. Shen and M. Ferrari, *Nat. Biotechnol.*, 2015, **33**, 941-951.
- 370 13. H. Maeda, H. Nakamura and J. Fang, *Adv. Drug Deliv. Rev.*, 2013, **65**, 71-79.
- 371 14. A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifehpour, M.
372 Samiei, M. Kouhi and K. Nejati-Koshki, *Nanoscale Res. Lett.*, 2013, **8**, 102-110.
- 373 15. D. A. Hume, *Curr. Opin. Immunol.*, 2006, **18**, 49-53.
- 374 16. A. J. Tavares, W. Poon, Y. N. Zhang, Q. Dai, R. Besla, D. Ding, B. Ouyang, A. Li, J. Chen, G.
375 Zheng, C. Robbins and W. C. W. Chan, *P. Natl. Acad. Sci. USA*, 2017, **114**, 10871-10880.
- 376 17. K. M. Tsoi, S. A. MacParland, X. Z. Ma, V. N. Spetzler, J. Echeverri, B. Ouyang, S. M. Fadel, E. A.
377 Sykes, N. Goldaracena, J. M. Kathis, J. B. Conneely, B. A. Alman, M. Selzner, M. A. Ostrowski, O.
378 Adeyi, A. Zilman, I. D. McGilvray and W. C. Chan, *Nat. Mater.*, 2016, **15**, 1212-1221.
- 379 18. Y. N. Zhang, W. Poon, A. J. Tavares, I. D. McGilvray and W. C. W. Chan, *J. Control. Release*,
380 2016, **240**, 332-348.
- 381 19. P. Aggarwal, J. B. Hall, C. B. McLeland, M. A. Dobrovolskaia and S. E. McNeil, *Adv. Drug Deliv.*
382 *Rev.*, 2009, **61**, 428-437.
- 383 20. J. S. Suk, Q. G. Xu, N. Kim, J. Hanes and L. M. Ensign, *Adv. Drug Deliver. Rev.*, 2016, **99**, 28-51.
- 384 21. G. Pasut and F. M. Veronese, *J. Control. Release*, 2012, **161**, 461-472.
- 385 22. P. L. Turecek, M. J. Bossard, F. Schoetens and I. A. Ivens, *J. Pharm. Sci.*, 2016, **105**, 460-475.
- 386 23. Y. Maitani, *J. Drug Deliv. Sci. Tec.*, 2011, **21**, 27-34.
- 387 24. R. P. Garay, R. El-Gewely, J. K. Armstrong, G. Garratty and P. Richette, *Expert Opin. Drug Del.*,
388 2012, **9**, 1319-1323.
- 389 25. H. Schellekens, W. E. Hennink and V. Brinks, *Pharm. Res-Dordr.*, 2013, **30**, 1729-1734.
- 390 26. H. Hatakeyama, H. Akita and H. Harashima, *Biol. Pharm. Bull.*, 2013, **36**, 892-899.
- 391 27. H. Hatakeyama, H. Akita and H. Harashima, *Adv. Drug Deliv. Rev.*, 2011, **63**, 152-160.
- 392 28. H. Y. Xue, P. Guo, W. C. Wen and H. L. Wong, *Curr. Pharm. Design*, 2015, **21**, 3140-3147.
- 393 29. J. W. Holland, C. Hui, P. R. Cullis and T. D. Madden, *Biochemistry*, 1996, **35**, 2618-2624.
- 394 30. J. R. S. a. M. J. Zuckermann, *Biochemistry*, 1993, **32**, 3153-3161.
- 395 31. A. S. Janoff, *Lab Invest.*, 1992, **66**, 655-658.
- 396 32. S. Leekumjorn, H. J. Cho, Y. F. Wu, N. T. Wright, A. K. Sum and C. Chan, *Bba-Biomembranes*,
397 2009, **1788**, 1508-1516.

398 33. W. M. Li, L. Xue, L. D. Mayer and M. B. Bally, *Bba-Biomembranes*, 2001, **1513**, 193-206.

399 34. G. Adlakha-Hutcheon, M. B. Bally, C. R. Shew and T. D. Madden, *Nat. Biotechnol.*, 1999, **17**,

400 775-779.

401 35. F. Fan, Y. Yu, F. Zhong, M. Gao, T. Sun, J. Liu, H. Zhang, H. Qian, W. Tao and X. Yang,

402 *Theranostics*, 2017, **7**, 1290-1302.

403 36. M. Barattin, A. Mattarei, A. Balasso, C. Paradisi, L. Cantu, E. Del Favero, T. Viitala, F.

404 Mastrotto, P. Caliceti and S. Salmaso, *ACS Appl. Mater. Interfaces*, 2018, **10**, 17646-17661.

405 37. C. Zhao, L. Shao, J. Lu, X. Deng and Y. Wu, *ACS Appl. Mater. Interfaces*, 2016, **8**, 6400-6410.

406 38. M. Fan, Y. Zeng, H. Ruan, Z. Zhang, T. Gong and X. Sun, *Mol. Pharm.*, 2017, **14**, 3152-3163.

407 39. A. Pourjavadi, Z. M. Tehrani and C. Bennett, *Int. J. Polym. Mater. Po.*, 2015, **64**, 570-577.

408 40. B. Romberg, W. E. Hennink and G. Storm, *Pharm. Res.*, 2008, **25**, 55-71.

409 41. B. A. Webb, M. Chimentì, M. P. Jacobson and D. L. Barber, *Nat. Rev. Cancer*, 2011, **11**,

410 671-677.

411 42. G. Ilangovan, H. Q. Li, J. L. Zweier and P. Kuppusamy, *Mol. Cell Biochem.*, 2002, **234**,

412 393-398.

413 43. C. Mehner, A. Hockla, E. Miller, S. Ran, D. C. Radisky and E. S. Radisky, *Oncotarget*, 2014, **5**,

414 2736-2749.

415 44. S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, 2013, **12**, 991-1003.

416 45. M. Meyer and E. Wagner, *Expert Opin. Drug Deliv.*, 2006, **3**, 563-571.

417 46. H. K. Kim, J. Van den Bossche, S. H. Hyun and D. H. Thompson, *Bioconjug. Chem.*, 2012, **23**,

418 2071-2077.

419 47. J. Shin, *J. Control. Release*, 2003, **91**, 187-200.

420 48. N. Bergstrand, M. C. Arfvidsson, J. M. Kim, D. H. Thompson and K. Edwards, *Biophys. Chem.*,

421 2003, **104**, 361-379.

422 49. J. A. Boomer, M. M. Qualls, H. D. Inerowicz, R. H. Haynes, V. S. Patri, J. M. Kim and D. H.

423 Thompson, *Bioconjug. Chem.*, 2009, **20**, 47-59.

424 50. Z. Xu, W. Gu, L. Chen, Y. Gao, Z. Zhang and Y. Li, *Biomacromolecules*, 2008, **9**, 3119-3126.

425 51. M. Kanamala, B. D. Palmer, W. R. Wilson and Z. Wu, *Int. J. Pharm.*, 2018, **548**, 288-296.

426 52. C. L. Chan, R. N. Majzoub, R. S. Shirazi, K. K. Ewert, Y. J. Chen, K. S. Liang and C. R. Safinya,

427 *Biomaterials*, 2012, **33**, 4928-4935.

428 53. A. Apte, E. Koren, A. Koshkaryev and V. P. Torchilin, *Cancer Biol Ther*, 2014, **15**, 69-80.

429 54. L. Zhang, Y. Wang, Y. Yang, Y. Liu, S. Ruan, Q. Zhang, X. Tai, J. Chen, T. Xia, Y. Qiu, H. Gao and

430 Q. He, *ACS Appl Mater Interfaces*, 2015, **7**, 9691-9701.

431 55. G. F. Walker, C. Fella, J. Pelisek, J. Fahrmeir, S. Boeckle, M. Ogris and E. Wagner, *Mol Ther*,

432 2005, **11**, 418-425.

433 56. F. Li, J. He, M. Zhang, K. C. Tam and P. Ni, *RSC Adv.*, 2015, **5**, 54658-54666.

434 57. F. Li, J. He, M. Zhang and P. Ni, *Polym. Chem.*, 2015, **6**, 5009-5014.

435 58. D. Chen, Q. Tang, J. Zou, X. Yang, W. Huang, Q. Zhang, J. Shao and X. Dong, *Adv. Healthc.*

436 *Mater.*, 2018, **7**, 1701272-1701281.

437 59. M. Yang, L. Yu, R. Guo, A. Dong, C. Lin and J. Zhang, *Nanomaterials (Basel)*, 2018, **8**, 167-184.

438 60. N. Sun, C. Zhao, R. Cheng, Z. Liu, X. Li, A. Lu, Z. Tian and Z. Yang, *Mol. Pharm.*, 2018, **15**,

439 3343-3355.

440 61. B. Balci and A. Top, *J. Polym. Res.*, 2018, **25**, 104-115.

441 62. J. A. Boomer, H. D. Inerowicz, Z. Y. Zhang, N. Bergstrand, K. Edwards, J. M. Kim and D. H.
442 Thompson, *Langmuir*, 2003, **19**, 6408-6415.

443 63. N. Kalva, N. Parekh and A. V. Ambade, *Polym. Chem.*, 2015, **6**, 6826-6835.

444 64. J. A. Boomer, H. D. Inerowicz, Z.-Y. Zhang, N. Bergstrand, K. Edwards, J.-M. Kim and D. H.
445 Thompson, *Langmuir*, 2003, **19**, 6408-6415.

446 65. H. Wang, J. He, M. Zhang, Y. Tao, F. Li, K. C. Tam and P. Ni, *J Mater Chem B*, 2013, **1**,
447 6596-6607.

448 66. J. Hu, J. He, M. Zhang and P. Ni, *Polym. Chem.*, 2015, **6**, 1553-1566.

449 67. S. Zhang, J. Xu, H. Chen, Z. Song, Y. Wu, X. Dai and J. Kong, *Macromol. Biosci.*, 2017,
450 **17**,1600258-1600267.

451 68. L. Xiao, L. Huang, F. Moingeon, M. Gauthier and G. Yang, *Biomacromolecules*, 2017, **18**,
452 2711-2722.

453 69. A. M. Jazani and J. K. Oh, *Macromolecules*, 2017, **50**, 9427-9436.

454 70. M. Oishi, F. Nagatsugi, S. Sasaki, Y. Nagasaki and K. Kataoka, *Chembiochem*, 2005, **6**,
455 718-725.

456 71. X. Guo, J. A. MacKay and F. C. Szoka, Jr., *Biophys. J.*, 2003, **84**, 1784-1795.

457 72. W. Li, Z. Huang, J. A. MacKay, S. Grube and F. C. Szoka, Jr., *J. Gene. Med.*, 2005, **7**, 67-79.

458 73. J. S. Choi, J. A. MacKay and F. C. Szoka, Jr., *Bioconjug. Chem.*, 2003, **14**, 420-429.

459 74. H. Rongbin, X. Lei, L. Ying, D. Xiangping, C. Xuan, L. Lanfang, Y. Cuiyun, C. Yanming and T.
460 Guotao, *J. Pharm. Pharmacol.*, 2016, **68**, 751-761.

461 75. S. Wu, L. Zheng, C. Li, Y. Xiao, S. Huo and B. Zhang, *J. Polym. Sci. Pol. Chem.*, 2017, **55**,
462 2036-2046.

463 76. S. Yang, F. Zhu, Q. Wang, F. Liang, X. Qu, Z. Gan and Z. Yang, *J. Mater. Chem. B*, 2015, **3**,
464 4043-4051.

465 77. Y. Guan, H. Lu, W. Li, Y. Zheng, Z. Jiang, J. Zou and H. Gao, *ACS Appl. Mater. Interfaces*, 2017,
466 **9**, 26731-26739.

467 78. J. Wang, C. Gong, Y. Wang and G. Wu, *Colloids Surf. B Biointerfaces*, 2014, **118**, 218-225.

468 79. J. Wang, C. Gong, Y. Wang and G. Wu, *RSC Adv.*, 2014, **4**, 15856-15863.

469 80. M. Zhang, J. Liu, Y. Kuang, Q. Li, H. Chen, H. Ye, L. Guo, Y. Xu, X. Chen, C. Li and B. Jiang, *J.*
470 *Mater. Chem. B*, 2016, **4**, 3387-3397.

471 81. X. Guan, Z. Guo, L. Lin, J. Chen, H. Tian and X. Chen, *Nano Lett.*, 2016, **16**, 6823-6831.

472 82. W.-P. C. Jingxia Gu, Xiaozhong Qu, Jiguang Liu, Sum-Yee Lo and Zhenzhong Yang,
473 *Biomacromolecules*, 2008, **9**, 255-262.

474 83. M. W. Dewhirst and T. W. Secomb, *Nat. Rev. Cancer*, 2017, **17**, 738-750.

475 84. J. I. Hare, T. Lammers, M. B. Ashford, S. Puri, G. Storm and S. T. Barry, *Adv. Drug Deliver. Rev.*,
476 2017, **108**, 25-38.

477 85. T. Sun, A. Morger, B. Castagner and J. C. Leroux, *Chem. Commun.*, 2015, **51**, 5721-5724.

478 86. Q. L. Li, S. H. Xu, H. Zhou, X. Wang, B. A. Dong, H. Gao, J. Tang and Y. W. Yang, *Acs Appl. Mater.*
479 *Inter.*, 2015, **7**, 28656-28664.

480 87. P. Kuppusamy, H. Q. Li, G. Ilangoan, A. J. Cardounel, J. L. Zweier, K. Yamada, M. C. Krishna
481 and J. B. Mitchell, *Cancer Res.*, 2002, **62**, 307-312.

-
- 482 88. P. S. Kulkarni, M. K. Haldar, R. R. Nahire, P. Katti, A. H. Ambre, W. W. Muhonen, J. B. Shabb, S.
483 K. Padi, R. K. Singh, P. P. Borowicz, D. K. Shrivastava, K. S. Katti, K. Reindl, B. Guo and S.
484 Mallik, *Mol. Pharm.*, 2014, **11**, 2390-2399.
- 485 89. K. M. McNeeley, E. Karathanasis, A. V. Annapragada and R. V. Bellamkonda, *Biomaterials*,
486 2009, **30**, 3986-3995.
- 487 90. W. Y. Rui Kuai, Yao Qin, Huali Chen, Jie Tang, Mingqing Yuan, Zhirong Zhang, and Qin He,
488 *Mol. Pharmaceut.*, 2010, **7**, 1816-1826.
- 489 91. L. Mei, L. Fu, K. Shi, Q. Zhang, Y. Liu, J. Tang, H. Gao, Z. Zhang and Q. He, *Int. J. Pharm.*, 2014,
490 **468**, 26-38.
- 491 92. J. Tang, H. Fu, Q. Kuang, L. Zhang, Q. Zhang, Y. Liu, R. Ran, H. Gao, Z. Zhang and Q. He, *J. Drug*
492 *Target*, 2014, **22**, 313-326.
- 493 93. J. Tang, L. Zhang, H. Gao, Y. Liu, Q. Zhang, R. Ran, Z. Zhang and Q. He, *Drug Deliv.*, 2016, **23**,
494 1130-1143.
- 495 94. L. Jia, D. Cui, J. Bignon, A. Di Cicco, J. Wdzieczak-Bakala, J. Liu and M. H. Li,
496 *Biomacromolecules*, 2014, **15**, 2206-2217.
- 497 95. T. Ren, W. Wu, M. Jia, H. Dong, Y. Li and Z. Ou, *ACS Appl. Mater. Interfaces*, 2013, **5**,
498 10721-10730.
- 499 96. W. Hou, F. Xia, C. S. Alves, X. Qian, Y. Yang and D. Cui, *ACS Appl. Mater. Interfaces*, 2016, **8**,
500 1447-1457.
- 501 97. H. Sun, B. Guo, R. Cheng, F. Meng, H. Liu and Z. Zhong, *Biomaterials*, 2009, **30**, 6358-6366.
- 502 98. X. Q. Li, H. Y. Wen, H. Q. Dong, W. M. Xue, G. M. Pauletti, X. J. Cai, W. J. Xia, D. Shi and Y. Y. Li,
503 *Chem. Commun.*, 2011, **47**, 8647-8649.
- 504 99. X.-J. Cai, H.-Q. Dong, W.-J. Xia, H.-Y. Wen, X.-Q. Li, J.-H. Yu, Y.-Y. Li and D.-L. Shi, *J. Mater.*
505 *Chem.*, 2011, **21**, 14639-14645.
- 506 100. T.-B. Ren, W.-J. Xia, H.-Q. Dong and Y.-Y. Li, *Polymer*, 2011, **52**, 3580-3586.
- 507 101. H. Y. Wen, H. Q. Dong, W. J. Xie, Y. Y. Li, K. Wang, G. M. Pauletti and D. L. Shi, *Chem. Commun.*,
508 2011, **47**, 3550-3552.
- 509 102. Q. Guo, P. Luo, Y. Luo, F. Du, W. Lu, S. Liu, J. Huang and J. Yu, *Colloids Surf B Biointerfaces*,
510 2012, **100**, 138-145.
- 511 103. Y. Zhong, W. Yang, H. Sun, R. Cheng, F. Meng, C. Deng and Z. Zhong, *Biomacromolecules*,
512 2013, **14**, 3723-3730.
- 513 104. X. Wang, H. Sun, F. Meng, R. Cheng, C. Deng and Z. Zhong, *Biomacromolecules*, 2013, **14**,
514 2873-2882.
- 515 105. C. Cui, Y. N. Xue, M. Wu, Y. Zhang, P. Yu, L. Liu, R. X. Zhuo and S. W. Huang, *Biomaterials*,
516 2013, **34**, 3858-3869.
- 517 106. Y. Ping, Q. Hu, G. Tang and J. Li, *Biomaterials*, 2013, **34**, 6482-6494.
- 518 107. J. Ding, J. Chen, D. Li, C. Xiao, J. Zhang, C. He, X. Zhuang and X. Chen, *J. Mater. Chem. B*, 2013,
519 **1**, 69-81.
- 520 108. T. Thambi, G. Saravanakumar, J.-U. Chu, R. Heo, H. Ko, V. G. Deepagan, J.-H. Kim and J. H.
521 Park, *Macromol. Res.*, 2012, **21**, 100-107.
- 522 109. L. Jia, Z. Li, D. Zhang, Q. Zhang, J. Shen, H. Guo, X. Tian, G. Liu, D. Zheng and L. Qi, *Polym.*
523 *Chem.*, 2013, **4**, 156-165.

-
- 524 110. K. Wang, Y. Liu, W.-J. Yi, C. Li, Y.-Y. Li, R.-X. Zhuo and X.-Z. Zhang, *Soft Matter*, 2013, **9**,
525 692-699.
- 526 111. H. Zhu, C. Dong, H. Dong, T. Ren, X. Wen, J. Su and Y. Li, *ACS Appl. Mater. Interfaces*, 2014, **6**,
527 10393-10407.
- 528 112. X. Ai, J. Sun, L. Zhong, C. Wu, H. Niu, T. Xu, H. Lian, X. Han, G. Ren, W. Ding, J. Wang, X. Pu and
529 Z. He, *Macromol. Biosci.*, 2014, **14**, 1415-1428.
- 530 113. H. Dong, C. Dong, W. Xia, Y. Li and T. Ren, *Med. Chem. Commun.*, 2014, **5**, 147-152.
- 531 114. C. Cui, P. Yu, M. Wu, Y. Zhang, L. Liu, B. Wu, C. X. Wang, R. X. Zhuo and S. W. Huang, *Colloids*
532 *Surf. B Biointerfaces*, 2015, **129**, 137-145.
- 533 115. H. Wen, H. Dong, J. Liu, A. Shen, Y. Li and D. Shi, *J. Mater. Chem. B*, 2016, **4**, 7859-7869.
- 534 116. Y. Zhu, X. Wang, J. Zhang, F. Meng, C. Deng, R. Cheng, J. Feijen and Z. Zhong, *J. Control.*
535 *Release*, 2017, **250**, 9-19.
- 536 117. H. Fan, Y. Li, J. Yang and X. Ye, *J. Phys. Chem. B*, 2017, **121**, 9708-9717.
- 537 118. J. Li, Y. J. Ma, Y. Wang, B. Z. Chen, X. D. Guo and C. Y. Zhang, *Chem. Eng. J.*, 2018, **341**,
538 450-461.
- 539 119. H. Wang, M. Sun, D. Li, X. Yang, C. Han and W. Pan, *Artif. Cells Nanomed. Biotechnol.*, 2018,
540 **46**, 313-322.
- 541 120. W. Chen, P. Zhong, F. Meng, R. Cheng, C. Deng, J. Feijen and Z. Zhong, *J. Control. Release*,
542 2013, **169**, 171-179.
- 543 121. Y. Cao, J. Zhao, Y. Zhang, J. Liu, J. Liu, A. Dong and L. Deng, *RSC Adv.*, 2015, **5**, 28060-28069.
- 544 122. Y. Li, Z. Wu, D. Du, H. Dong, D. Shi and Y. Li, *RSC Adv.*, 2016, **6**, 6516-6522.
- 545 123. H. Xiong, Z. Guo, W. Zhang, H. Zhong, S. Liu and Y. Ji, *J. Photochem. Photobiol. B*, 2014, **138**,
546 191-201.
- 547 124. H. Wen, C. Dong, H. Dong, A. Shen, W. Xia, X. Cai, Y. Song, X. Li, Y. Li and D. Shi, *Small*, 2012,
548 **8**, 760-769.
- 549 125. J. Jiao, X. Li, S. Zhang, J. Liu, D. Di, Y. Zhang, Q. Zhao and S. Wang, *Mater. Sci. Eng. C Mater.*
550 *Biol. Appl.*, 2016, **67**, 26-33.
- 551 126. Y. Wang, N. Han, Q. Zhao, L. Bai, J. Li, T. Jiang and S. Wang, *Eur. J. Pharm. Sci.*, 2015, **72**,
552 12-20.
- 553 127. H. M. Gong, Z. F. Xie, M. X. Liu, H. H. Sun, H. D. Zhu and H. L. Guo, *Colloid Polym. Sci.*, 2015,
554 **293**, 2121-2128.
- 555 128. L. Chen, Z. Zheng, J. Wang and X. Wang, *Microporous Mesoporous Mater.*, 2014, **185**, 7-15.
- 556 129. H. He, H. Kuang, L. Yan, F. Meng, Z. Xie, X. Jing and Y. Huang, *Phys. Chem. Chem. Phys.*, 2013,
557 **15**, 14210-14218.
- 558 130. Y. Cui, H. Dong, X. Cai, D. Wang and Y. Li, *ACS Appl. Mater. Interfaces*, 2012, **4**, 3177-3183.
- 559 131. H. Kim, S. Kim, C. Park, H. Lee, H. J. Park and C. Kim, *Adv Mater.*, 2010, **22**, 4280-4283.
- 560 132. H. Gong, Z. Xie, M. Liu, H. Zhu and H. Sun, *RSC Adv.*, 2015, **5**, 59576-59582.
- 561 133. Y. Dong, X. Ma, H. Huo, Q. Zhang, F. Qu and F. Chen, *J. Appl. Polym. Sci.*, 2018, **135**,
562 46675-46685.
- 563 134. J. Yu, X. Li, Y. Luo, W. Lu, J. Huang and S. Liu, *Colloids Surf. B Biointerfaces*, 2013, **107**,
564 213-219.
- 565 135. R. K. E. Charles C. Pak, Patrick L. Ahl, Andrew S. Janoj, Paul Meers, *Biochim. Biophys. Acta*,
566 1999, **1419** 111-126.

-
- 567 136. L. L. H. Benjamin E.Turk, Elizabeth T. Piro, and Lewis C. Cantley, *Nat. Biotechnol.*, 2001, **19**,
568 661-667.
- 569 137. D. Chen, W. Liu, Y. Shen, H. Mu, Y. Zhang, R. Liang, A. Wang, K. Sun and F. Fu, *Int. J.*
570 *Nanomedicine*, 2011, **6**, 2053-2061.
- 571 138. H. Xu, Y. Deng, D. Chen, W. Hong, Y. Lu and X. Dong, *J. Control. Release*, 2008, **130**, 238-245.
- 572 139. T. Terada, M. Iwai, S. Kawakami, F. Yamashita and M. Hashida, *J. Control. Release*, 2006, **111**,
573 333-342.
- 574 140. F. Zhou, B. Feng, T. Wang, D. Wang, Q. Meng, J. Zeng, Z. Zhang, S. Wang, H. Yu and Y. Li, *Adv.*
575 *Func. Mater.*, 2017, **27**, 1606530-1606541.
- 576 141. P. K. Lin Zhu, and Vladimir P. Torchilin, *ACS Nano*, 2012, **6**, 3491-3498.
- 577 142. M. R. Gordon, B. Zhao, F. Anson, A. Fernandez, K. Singh, C. Homyak, M. Canakci, R. W. Vachet
578 and S. Thayumanavan, *Biomacromolecules*, 2018, **19**, 860-871.
- 579 143. F. Guo, J. Wu, W. Wu, D. Huang, Q. Yan, Q. Yang, Y. Gao and G. Yang, *J. Nanobiotechnology*,
580 2018, **16**, 57-69.
- 581 144. P. Yingyuad, M. Mevel, C. Prata, S. Furegati, C. Kontogiorgis, M. Thanou and A. D. Miller,
582 *Bioconjug. Chem.*, 2013, **24**, 343-362.
- 583 145. H. Hatakeyama, H. Akita, K. Kogure, M. Oishi, Y. Nagasaki, Y. Kihira, M. Ueno, H. Kobayashi,
584 H. Kikuchi and H. Harashima, *Gene Ther.*, 2007, **14**, 68-77.
- 585 146. L. Zhu, T. Wang, F. Perche, A. Taigind and V. P. Torchilin, *Proc. Natl. Acad. Sci. U. S. A.*, 2013,
586 **110**, 17047-17052.
- 587 147. L. Zhu, F. Perche, T. Wang and V. P. Torchilin, *Biomaterials*, 2014, **35**, 4213-4222.
- 588 148. K. L. Veiman, K. Kunnapuu, T. Lehto, K. Kiisholts, K. Parn, U. Langel and K. Kurrikoff, *J.*
589 *Control. Release*, 2015, **209**, 238-247.
- 590 149. H. Zhou, H. Sun, S. Lv, D. Zhang, X. Zhang, Z. Tang and X. Chen, *Acta Biomater.*, 2017, **54**,
591 227-238.
- 592 150. Y. Tu and L. Zhu, *J. Control. Release*, 2015, **212**, 94-102.
- 593 151. W. Ke, J. Li, K. Zhao, Z. Zha, Y. Han, Y. Wang, W. Yin, P. Zhang and Z. Ge, *Biomacromolecules*,
594 2016, **17**, 3268-3276.
- 595 152. G. Salzano, D. F. Costa, C. Sarisozen, E. Luther, G. Mattheolabakis, P. P. Dhargalkar and V. P.
596 Torchilin, *Small*, 2016, **12**, 4837-4848.
- 597 153. J. Yoo, N. Sanoj Rejinold, D. Lee, S. Jon and Y. C. Kim, *J. Control. Release*, 2017, **264**, 89-101.
- 598 154. Z. Dai, Y. Tu and L. Zhu, *J Biomed Nanotechnol*, 2016, **12**, 1199-1210.
- 599 155. Y. Zeng, Z. Zhou, M. Fan, T. Gong, Z. Zhang and X. Sun, *Mol. Pharm.*, 2017, **14**, 81-92.
- 600 156. J. M. Shin, S. J. Oh, S. Kwon, V. G. Deepagan, M. Lee, S. H. Song, H. J. Lee, S. Kim, K. H. Song, T.
601 W. Kim and J. H. Park, *J. Control. Release*, 2017, **267**, 181-190.
- 602 157. H. Han, D. Valdeperez, Q. Jin, B. Yang, Z. Li, Y. Wu, B. Pelaz, W. J. Parak and J. Ji, *ACS Nano*,
603 2017, **11**, 1281-1291.
- 604 158. J. Li, S. Xiao, Y. Xu, S. Zuo, Z. Zha, W. Ke, C. He and Z. Ge, *ACS Appl. Mater. Interfaces*, 2017, **9**,
605 17727-17735.
- 606 159. C. Nazli, G. S. Demirer, Y. Yar, H. Y. Acar and S. Kizilel, *Colloids Surf. B Biointerfaces*, 2014,
607 **122**, 674-683.
- 608 160. Z. Dai, Q. Yao and L. Zhu, *ACS Appl. Mater. Interfaces*, 2016, **8**, 12661-12673.

-
- 609 161. D. Guarnieri, M. Biondi, H. Yu, V. Belli, A. P. Falanga, M. Cantisani, S. Galdiero and P. A. Netti,
610 *Biotechnol. Bioeng.*, 2015, **112**, 601-611.
- 611 162. J. X. Zhang, S. Zalipsky, N. Mullah, M. Pechar and T. M. Allen, *Pharmacol. Res.*, 2004, **49**,
612 185-198.
- 613 163. L. Kong, S. H. C. Askes, S. Bonnet, A. Kros and F. Campbell, *Angew. Chem. Int. Edit.*, 2016, **55**,
614 1396-1400.
- 615 164. F. Zhang, L. Kong, D. Liu, W. Li, E. Mäkilä, A. Correia, R. Lindgren, J. Salonen, J. J. Hirvonen, H.
616 Zhang, A. Kros and H. A. Santos, *Adv. Therap.*, 2018, **1**, 1800013-1800024.
- 617 165. L. Kong, D. Poulcharidis, G. F. Schneider, F. Campbell and A. Kros, *Int. J. Mol. Sci.*, 2017, **18**,
618 2033-2040.
- 619 166. Q. Jin, T. Cai, H. Han, H. Wang, Y. Wang and J. Ji, *Macromol. Rapid Commun.*, 2014, **35**,
620 1372-1378.
- 621 167. D. Zhou, J. Guo, G. B. Kim, J. Li, X. Chen, J. Yang and Y. Huang, *Adv. Healthc. Mater.*, 2016, **5**,
622 2493-2499.
- 623 168. J. Wang, Y. Ouyang, S. Li, X. Wang and Y. He, *RSC Adv.*, 2016, **6**, 57227-57231.
- 624 169. G. Saravanakumar, H. Park, J. Kim, D. Park, S. Pramanick, D. H. Kim and W. J. Kim,
625 *Biomacromolecules*, 2018, **19**, 2202-2213.
- 626 170. Y. V. Il'ichev, M. A. Schworer and J. Wirz, *J. Am. Chem. Soc.*, 2004, **126**, 4581-4595.
- 627 171. Michael P. Hay, Bridget M. Sykes, W. A. Denny and C. J. O'Connor, *J. Chem. Soc., Perkin*
628 *Trans.*, 1999, **1**, 2759-2770.
- 629 172. J. Yang, Y. Shimada, R. C. L. Olsthoorn, B. E. Snaar-Jagalska, H. P. Spaink and A. Kros, *ACS*
630 *Nano*, 2016, **10**, 7428-7435.
- 631 173. T. J. McMillan, E. Leatherman, A. Ridley, J. Shorrocks, S. E. Tobi and J. R. Whiteside, *J. Pharm.*
632 *Pharmacol.*, 2008, **60**, 969-976.
- 633 174. A. P. Castano, P. Mroz and M. R. Hamblin, *Nat. Rev. Cancer*, 2006, **6**, 535-545.
- 634 175. C. M. Allen, W. M. Sharman and J. E. Van Lier, *J. Porphyr. Phthalocya.*, 2001, **5**, 161-169.
- 635 176. S. H. Yun and S. J. J. Kwok, *Nat. Biomed. Eng.*, 2017, **1**, 8-15.
- 636 177. L. Fournier, C. Gauron, L. Xu, I. Aujard, T. Le Saux, N. Gagey-Eilstein, S. Maurin, S. Dubruille,
637 J. B. Baudin, D. Bensimon, M. Volovitch, S. Vríz and L. Jullien, *ACS Chem. Biol.*, 2013, **8**,
638 1528-1536.
- 639 178. X. M. M. Weyel, M. A. H. Fichte and A. Heckel, *ACS Chem. Biol.*, 2017, **12**, 2183-2190.
- 640 179. K. Peng, I. Tomatsu, B. van den Broek, C. Cui, A. V. Korobko, J. van Noort, A. H. Meijer, H. P.
641 Spaink and A. Kros, *Soft Matter*, 2011, **7**, 4881-4887.