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 effects[1] (*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 kidneys[11] – 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]

110 **1.3 The PEG dilemma**

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

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

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

138

139 **2. Physical dePEGylation**

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

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

152 In a study of three different lipid-PEG conjugates, no lipid-PEG exchange was observed for long chain,
153 saturated lipid anchors 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG, C18:0 – 2 x 18
154 carbon FA chain; no double bonds) whereas exchange occurred in the time frame of hours for shorter
155 saturated lipids 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG; C14:0) or long chain,
156 unsaturated lipids 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE-PEG; C18:1 – 2 x 18 carbon
157 FA chain; each 1 double bond, ω9).[33] This time frame enabled efficient accumulation of liposomes in
158 tumor sites *via* the EPR effect (prior to dePEGylation) coupled with increased cellular uptake within the
159 tumor (following dePEGylation). Conversely, a similar study found that only in the case of DSPE-PEG
160 were circulation times prolonged enough to see efficient passive accumulation of nanoparticles within
161 the tumor.[34] These conflicting results highlight the fine balance required to achieve efficient passive
162 accumulation within target tumors and subsequent dePEGylation *via* physical desorption of lipid-PEG
163 reagents. The propensity for non-specific PEG exchange with biological membranes *in vivo,* prior to
164 reaching the target tumor, has likely limited the widespread application of these approaches.

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

172

173 **3. Chemical dePEGylation strategies**

174 By far the most common method to achieve *extracellular* dePEGylation of nanoparticle surfaces, within
175 the tumor microenvironment, is through chemical approaches. In these cases, PEG is grafted to the
176 nanoparticle *via* a stimuli-responsive covalent chemical bond (Table 1 and 2).[40] Stimuli can be both
177 endogenous and exogenous. In the case of endogenous stimuli, intrinsic differences in the
178 pathophysiology of tumor and healthy tissues are exploited, namely the low pH,[41] reducing[42] and matrix
179 metalloprotease (MMP)-rich environment[43] of certain solid tumors. Exogenous stimuli, including light
180 and heat, have the benefit of being under complete user control in both time and space.[44] In a clinical
181 setting however, these approaches rely on the ability to efficiently deliver stimuli (*e.g.* light) to tissues

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

184

185 ### 3.1 pH-sensitive dePEGylation

186 The mildly acidic (pH 6.5-7.2) extracellular environment of hypoxic tumors – a result of increased
187 glucose catabolism and efflux of H^+ by cancer cells – has been exploited to trigger extracellular
188 dePEGylation of nanoparticle surfaces.[45] For this, chemical functionalities stable at physiological pH (pH
189 7.4) but labile at lower pH are required. The most commonly used acid labile chemical groups are vinyl
190 ethers,[46-50] hydrazones,[51-61] acetals,[62-69] β-thiopropionates,[70] ortho esters[71-73] and benzoic imines[74-82]. Here
191 however, it is important to differentiate between the mildly acidic *extracellular* pH within the tumor
192 microenvironment (pH 6.5-7) and the strongly acidic *intracellular* pH within late endosomes/lysosomes
193 (pH 4.5-5.5) and to stress that optimal sensitivity (and subsequent dePEGylation efficiency) of these
194 acid-labile functionalities is typically at pH 5-5.5. Therefore all these pH-sensitive systems demonstrate
195 inefficient/sluggish acidolytic dePEGylation within the *extracellular* tumor microenvironment. This can
196 be exploited to achieve prolonged and sustained drug release within the tumor and/or partial
197 dePEGylation may still generate the desired outcome. For example, Gu *et al*. reported pH dependent
198 dePEGylation of polycationic micelles through grafting of PEG, *via* benzoic imine linkages, to
199 poly-L-lysine(PLL)/cholic acid co-polymers.[82] By measuring changes in surface charge (zeta potential),
200 the authors were able to show colloidal stability at physiological pH as well as increasing rates of
201 dePEGylation with decreasing pH (complete acidolyis at pH 5.5 within 10 min). At pH 6.5-7 (*i.e.* pH of
202 the extracellular tumor microenvironment) only partial dePEGylation was observed, however this was
203 accompanied by a significant increase in hemolytic activity suggesting partial dePEGylation was
204 sufficient to endow these particles with the desired function. As this system was not tested in cancer
205 models *in vivo*, it remains to be seen whether this slow rate of acidolysis will adversely affect function
206 and efficacy. Indeed, the individual successes of pH responsive dePEGylation systems ultimately
207 depends on the ability to deliver therapeutically relevant drug doses to cancer cells above and beyond
208 those of the administered free drug alone. It is worth noting, however, these technologies – as with any
209 system exploiting endogenous stimuli – will likely demonstrate significant variations in efficacy due to
210 patient-to-patient heterogeneity of tumor pathologies.[83, 84]

211

212 ### 3.2 Redox-sensitive dePEGylation

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

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

226

227 **3.3 Protease-sensitive dePEGylation**

228 Within the tumor microenvironment, there are high levels of *extracellular*
229 matrix metalloproteinases (MMPs). These lytic enzymes are secreted at high levels by tumor cells to
230 degrade the extracellular matrix (ECM) and aid cancer cell migration.[135, 136] Short peptides containing
231 enzyme-consensus sequences, linking PEG to a nanoparticle surface, have been effectively used to
232 dePEGylate nanoparticles within the tumor microenvironment.[137-161] Torchilin *et al.* have reported two
233 elegant examples of MMP-triggered dePEGylation. The first employed a multifunctional liposomal
234 formulation comprising longer, MMP-cleavable lipid-PEG_{3400} constructs and shorter, non-cleavable
235 TAT-functionalised lipid-PEG_{2000} constructs.[141] In the absence of MMPs, longer PEG_{3400} chains
236 effectively shielded the cell penetrating function of the underlying TAT peptide and liposomes were
237 sparingly taken up by cells. Upon MMP-mediated dePEGylation however, the newly revealed
238 TAT-functionalised liposomes were avidly taken up by 4T1 breast cancer cells. Going one step further,
239 the same group reported a similar strategy of exploiting MMP-mediated dePEGylation to reveal newly
240 functional drug polymer micelles.[146] Crucially in this approach, dePEGylation did not destroy the
241 integrity of the underlying drug-filled micelle leading to efficient stimuli responsive, *intracellular* drug
242 delivery to cancer cells, as demonstrated in mice models.

243 It is worth noting here that both cathepsin B (protease)[162] and esterases[138] have also been exploited to
244 trigger dePEGylation of nanomedicines. However, cathepsin B is only found at high levels within
245 (*intracellular*) cancer cells, while esterases are widely distributed in plasma and healthy tissues and not
246 therefore specific to the tumor microenvironment. In our opinion, MMP-mediated dePEGylation of
247 nanoparticles within the tumor microenvironment represents the most selective and efficient strategy to
248 enhance the efficacy of cancer nanomedicines exploiting *endogenous* stimulus.

249

250 **3.4 Light-sensitive dePEGylation**

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

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

256 For light triggered dePEGylation of potential cancer nanomedicnes, *o*-nitrobenzyl (*o*-Nb),[63, 163-166]
257 platinum-azide complexes[167] and azobenze[168] functionalities have all been explored.[169] In the case of
258 *o*-Nb functionalities, non-hydrolytic photolysis proceeds through a cyclic intermediate followed by the
259 release of the desired alcohol and a nitroso by-product.[170] To increase biological compatibility, methoxy
260 substitution of the aryl ring results in reduced toxicity of nitroso byproducts.[171] We have recently
261 reported two separate strategies in which light triggered dePEGylation was successfully used to initiate
262 efficient drug delivery to target cancer cells. In the first example, we created 100 nm, loose core shell
263 micelles composed exclusively of photolabile doxorubicin-PEG_{2000} reagents.[165] In the absence of light,
264 micelles were stable, non-toxic (*i.e.* not taken up by cells *in vitro*) and no doxorubicin release was
265 observed over time. Upon light (365 nm) activation triggered dePEGylation, micelle destabilisation and
266 subsequent burst drug release resulted in *in vitro* cytotoxicity comparable to free doxorubicin. In
267 addition, we were able to demonstrate precise spatiotemporal control of doxorubicin delivery to cells *in*
268 *vitro* through light templated activation. We are currently assessing this system *in vivo* to determine
269 circulation lifetimes and tumor accumulation of PEGylated doxorubicin prodrug micelles prior to light
270 triggered dePEGylation.

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

281 The use of light does, of course, raise valid concerns going forward into the clinic. In all reported
282 examples of light triggered dePEGylation, systems are most sensitive to high energy UV-A light (<400
283 nm). Short wavelength UV light suffers from poor tissue penetration (100-200 μm) and, following
284 prolonged exposure, can elicit significant photocytotoxicity.[173] Only for polymeric systems containing
285 platinum-azide complexes[167] was photolytic dePEGylation investigated using visible light irradiation.
286 Here, decreasing photolytic efficiency correlated with longer light wavelengths. Here however, it is
287 important to note that photodynamic therapies,[174] combining chemical photosensitizers and light
288 activation, are already widely used in the clinic to treat a range of medical conditions, including acne,
289 atherosclerosis and cancer.[175] Furthermore, advances in fibre optic technologies (to deliver UV light deep

290 within tissue),[176] the development of photolabile chemical bonds sensitive to longer wavelength light[177]
291 and the optimization of photosensitive chemical functionality to minimize light exposure, will only
292 further the clinical applicability of light. One promising development has been photolabile chemical
293 groups sensitive to two photon light,[178] to not only increasing tissue penetration (>1 cm) of light and
294 minimising induced photocytotoxicity but, by restricting light activation to the focal point of two photon
295 beams, enabling activation volumes in patients of <1 femtolitre. In this vein, we and others have also
296 shown it is possible to cleave *o*-Nb groups using 2-photon light.[179] There are currently no examples of
297 responsive dePEGylation of nanoparticles using alternative external stimuli (*e.g.* heat or ultrasound).

298

299 **4. Conclusion**

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

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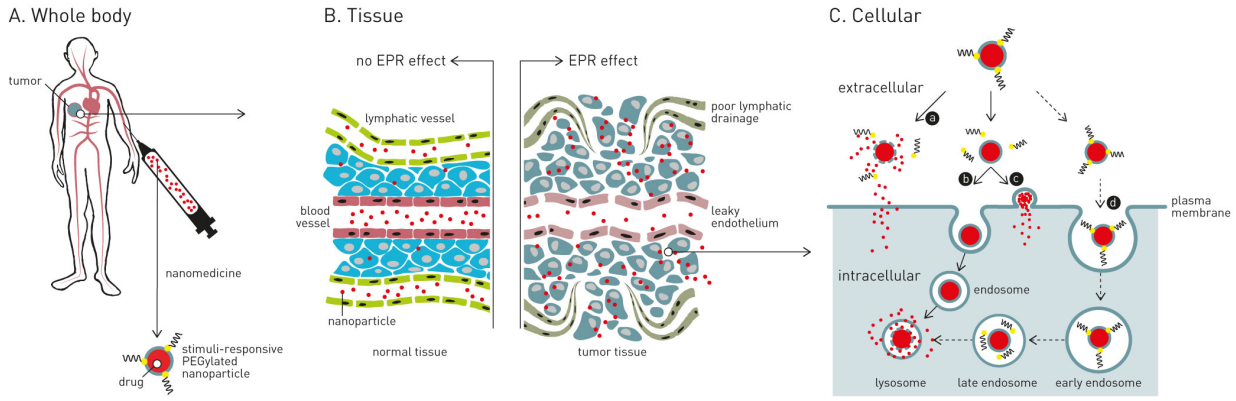


Figure 1. Following passive targeting of solid tumors *via* the enhanced permeability and retention (EPR) effect, stimuli-responsive dePEGylation of cancer nanomedicines can lead to various routes of enhanced drug delivery: **route a** – extracellular dePEGylation, nanocarrier rupture and extracellular 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.

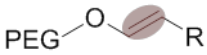
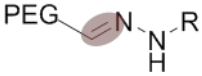
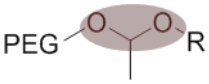
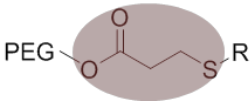
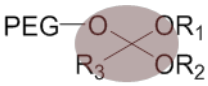
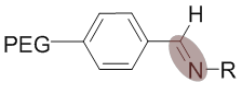
Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [*refs*]	
				Intracellular	Extracellular
Intracellular*	**Low pH** Late endosome (pH <6.5) Lysosome (pH 5.5-6.5)	 Vinylether	Liposomes	*[46-49]*	
			Micelles	*[50]*	
		 Hydrazone	Liposomes	*[52-54]*	
			Micelles	*[55-61]*	
		 Acetal	Liposomes	*[62]*	
			Micelles	*[63-69]*	
		 β-thiopropionate	Micelles	*[70]*	
		 Ortho ester	Liposomes	*[71-73]*	
		 Benzoic-imine	Micelles	*[74-76]*	
	Reduction Glutathione (GSH) (2-10 mM)		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.

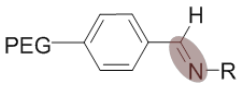
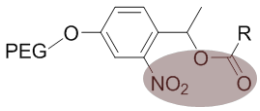
Site of dePEGylation within tumor	Stimulus	Example chemical structure	Nanocarrier	Drug release [*refs*]	
				Intracellular	Extracellular
Extracellular	**Low pH** (pH 6.5-7)	 Benzoic-imine	Polymeric Nanoparticles	*[77]*	
			Magnetic nanoparticles		*[78-80]*
			Micelles	*[81]*	*[82]*
	Reduction Glutathione (GSH) (4-80 µM)	 Disulfide	Liposomes		*[88-92]*
	Enzymatic (non-specific) Esterase		Liposomes	*[137, 138]*	
	Enzymatic (specific) Matrix metallo-proteinases (MMPs)	 (*peptide consensus sequence*)	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. Kaths, J. B. Conneely, B. A. Alman, M. Selzner, M. A. Ostrowski, O.
378 A. 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. Chimenti, 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. Balcı and A. Top, *J. Polym. Res.*, 2018, **25**, 104-115.

62. J. A. Boomer, H. D. Inerowicz, Z. Y. Zhang, N. Bergstrand, K. Edwards, J. M. Kim and D. H. Thompson, *Langmuir*, 2003, **19**, 6408-6415.
63. N. Kalva, N. Parekh and A. V. Ambade, *Polym. Chem.*, 2015, **6**, 6826-6835.
64. J. A. Boomer, H. D. Inerowicz, Z.-Y. Zhang, N. Bergstrand, K. Edwards, J.-M. Kim and D. H. Thompson, *Langmuir*, 2003, **19**, 6408-6415.
65. H. Wang, J. He, M. Zhang, Y. Tao, F. Li, K. C. Tam and P. Ni, *J Mater Chem B*, 2013, **1**, 6596-6607.
66. J. Hu, J. He, M. Zhang and P. Ni, *Polym. Chem.*, 2015, **6**, 1553-1566.
67. S. Zhang, J. Xu, H. Chen, Z. Song, Y. Wu, X. Dai and J. Kong, *Macromol. Biosci.*, 2017, **17**,1600258-1600267.
68. L. Xiao, L. Huang, F. Moingeon, M. Gauthier and G. Yang, *Biomacromolecules*, 2017, **18**, 2711-2722.
69. A. M. Jazani and J. K. Oh, *Macromolecules*, 2017, **50**, 9427-9436.
70. M. Oishi, F. Nagatsugi, S. Sasaki, Y. Nagasaki and K. Kataoka, *Chembiochem*, 2005, **6**, 718-725.
71. X. Guo, J. A. MacKay and F. C. Szoka, Jr., *Biophys. J.*, 2003, **84**, 1784-1795.
72. W. Li, Z. Huang, J. A. MacKay, S. Grube and F. C. Szoka, Jr., *J. Gene. Med.*, 2005, **7**, 67-79.
73. J. S. Choi, J. A. MacKay and F. C. Szoka, Jr., *Bioconjug. Chem.*, 2003, **14**, 420-429.
74. H. Rongbin, X. Lei, L. Ying, D. Xiangping, C. Xuan, L. Lanfang, Y. Cuiyun, C. Yanming and T. Guotao, *J. Pharm. Pharmacol.*, 2016, **68**, 751-761.
75. S. Wu, L. Zheng, C. Li, Y. Xiao, S. Huo and B. Zhang, *J. Polym. Sci. Pol. Chem.*, 2017, **55**, 2036-2046.
76. S. Yang, F. Zhu, Q. Wang, F. Liang, X. Qu, Z. Gan and Z. Yang, *J. Mater. Chem. B*, 2015, **3**, 4043-4051.
77. Y. Guan, H. Lu, W. Li, Y. Zheng, Z. Jiang, J. Zou and H. Gao, *ACS Appl. Mater. Interfaces*, 2017, **9**, 26731-26739.
78. J. Wang, C. Gong, Y. Wang and G. Wu, *Colloids Surf. B Biointerfaces*, 2014, **118**, 218-225.
79. J. Wang, C. Gong, Y. Wang and G. Wu, *RSC Adv.*, 2014, **4**, 15856-15863.
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. Mater. Chem. B*, 2016, **4**, 3387-3397.
81. X. Guan, Z. Guo, L. Lin, J. Chen, H. Tian and X. Chen, *Nano Lett.*, 2016, **16**, 6823-6831.
82. W.-P. C. Jingxia Gu, Xiaozhong Qu, Jiguang Liu, Sum-Yee Lo and Zhenzhong Yang, *Biomacromolecules*, 2008, **9**, 255–262.
83. M. W. Dewhirst and T. W. Secomb, *Nat. Rev. Cancer*, 2017, **17**, 738-750.
84. J. I. Hare, T. Lammers, M. B. Ashford, S. Puri, G. Storm and S. T. Barry, *Adv. Drug Deliver. Rev.*, 2017, **108**, 25-38.
85. T. Sun, A. Morger, B. Castagner and J. C. Leroux, *Chem. Commun.*, 2015, **51**, 5721-5724.
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. Inter.*, 2015, **7**, 28656-28664.
87. P. Kuppusamy, H. Q. Li, G. Ilangovan, A. J. Cardounel, J. L. Zweier, K. Yamada, M. C. Krishna and J. B. Mitchell, *Cancer Res.*, 2002, **62**, 307-312.

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

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

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

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