Responsive Polymeric Nanoparticles for Controlled Drug Delivery

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Abstract

Small molecule drugs often have limited solubility or display rapid clearance showing poor selectivity that leads to undesired side-effects. Although prodrug strategies can improve solubility and lower toxicity, activation "on demand" as well as targeted transport of prodrugs remains a challenge in drug delivery. Responsive polymeric nanoparticles can help obliterate these challenges with the encapsulation or conjugation of drugs, allowing release at the target site upon triggering by an internal or external stimulus. The adaptable design of polymeric nanoparticles allows them to play a vital role in achieving the specific and desired response following application of a specific stimulus. Here, the most recent progress in responsive polymeric nanoparticles is reviewed with a focus on the chemical properties of the utilised polymers.

APPENDIX: POLYMER ABBREVIATIONS

bPEI	branched polyethyleneimine
PAGE	Poly(allyl glycidyl ether)
PBzMA	Poly(benzyl methacrylate)
PDAMAAm	Poly(*N,N*-dimethylaminopropyl acrylamide)
PDMS	Poly(dimethylsiloxane)
PDLLA	Poly(*DL*-lactic acid)
PDMAEMA	Poly(dimethylaminoethyl methacrylate)
PEG	Poly(ethylene glycol)
PGMA	Poly(glycidyl methacrylate)
PHPMA	Poly[*N*-(2-hydroxypropyl)methacrylamide]
PLA	Poly(lactic acid)
PLGA	Poly(lactide-*co*-glycolid)
PNAAAm	Poly(*N*-alkylacrylamide)
PNBMA	Poly(*n*-butyl methacrylate)
PNIPAAm	Poly(*N*-isopropyl acrylamide)
PNCA	Poly(norborene cholic acid)
PNOEG	Poly[norborene oligo(ethylene glycol)]
PU	Polyurethane
PVCL	Poly(*N*-vinyl caprolactam)

PVFC	Poly(vinyl ferrocene)
P2F	Poly[*N*-(2,2-difluoroethyl)acrylamide]

Introduction

The last decade has seen significant progress in the development of novel and more efficient therapeutics; however, their safe and controlled delivery to the target site, such as tumors, and poor pharmacokinetics properties remain a challenge that is often associated with undesirable side-effects.[1,2]

Nanoparticles are an attractive approach for the delivery of a variety of therapeutic agents (*e.g*. small molecules, nucleic acids and peptides/proteins) due to their biocompatibility and the way in which they can impact the transport abilities of small molecule therapeutics, an approach that has resulted in clinically applied polymeric vesicles (Doxil®[3] and Daunoxome®[4]) and micelles (Genexol-PM®[5]). Nanoparticles in this review are structures with dimensions typically between 10–1000 nm that can absorb, encapsulate or contain conjugated cargos. Polymeric nanoparticles (PNPs) are formed by the self-assembly and/or crosslinking of amphiphilic polymers with the most common structures including micelles, vesicles, and dendrimers. Vesicles are of particular interest as they can encapsulate both hydrophilic and hydrophobic drugs either in their hydrophilic core or hydrophobic “membrane”, respectively. Among the various types of nanoparticles, PNPs have gained special attention because of ease of synthesis, while offering a variety of well-defined structures.

The most common methods to form self-assembled PNPs include nanoprecipitation[6,7] and solvent evaporation.[8,9,10] Recent innovations include nanoprecipitation using electrospray[11] and hydrodynamic flow through microchannels[12] to give narrow size distributions with optimization of morphology of

the nanoparticles. PNPs are also accessible *via* the method of "salting-out"[13,14] and supercritical anti-solvent approaches.[15,16] In addition to the "bottom-up" methods, PNPs have been synthesized by "top-down methods" such as microemulsion polymerization.[17,18]

Drugs incorporated into PNPs are protected from the biological environment and often result in lower toxicity,[19] offering enhanced half-lives and blood circulation times[20,21] while allowing targeting either passively (EPR effect) or actively by functionalization with "homing" molecules (*e.g.* folic acid, peptides and antibodies).[22,23,24,25] Despite these opportunities, controlled "on demand" drug release from PNPs at the target site remains a challenge. Nanoparticles that respond to an external trigger resulting in the release of drugs offer a promising approach for the controlled delivery of drugs or imaging probes (Figure 1). Multiple triggers exist and can be classified as either internal (e.g. enzymes, pH) or external triggers (e.g. light, temperature, magnetic field or small molecule). This review focuses on recent advances in responsive nanoparticles and their application to drug delivery (Table 1).

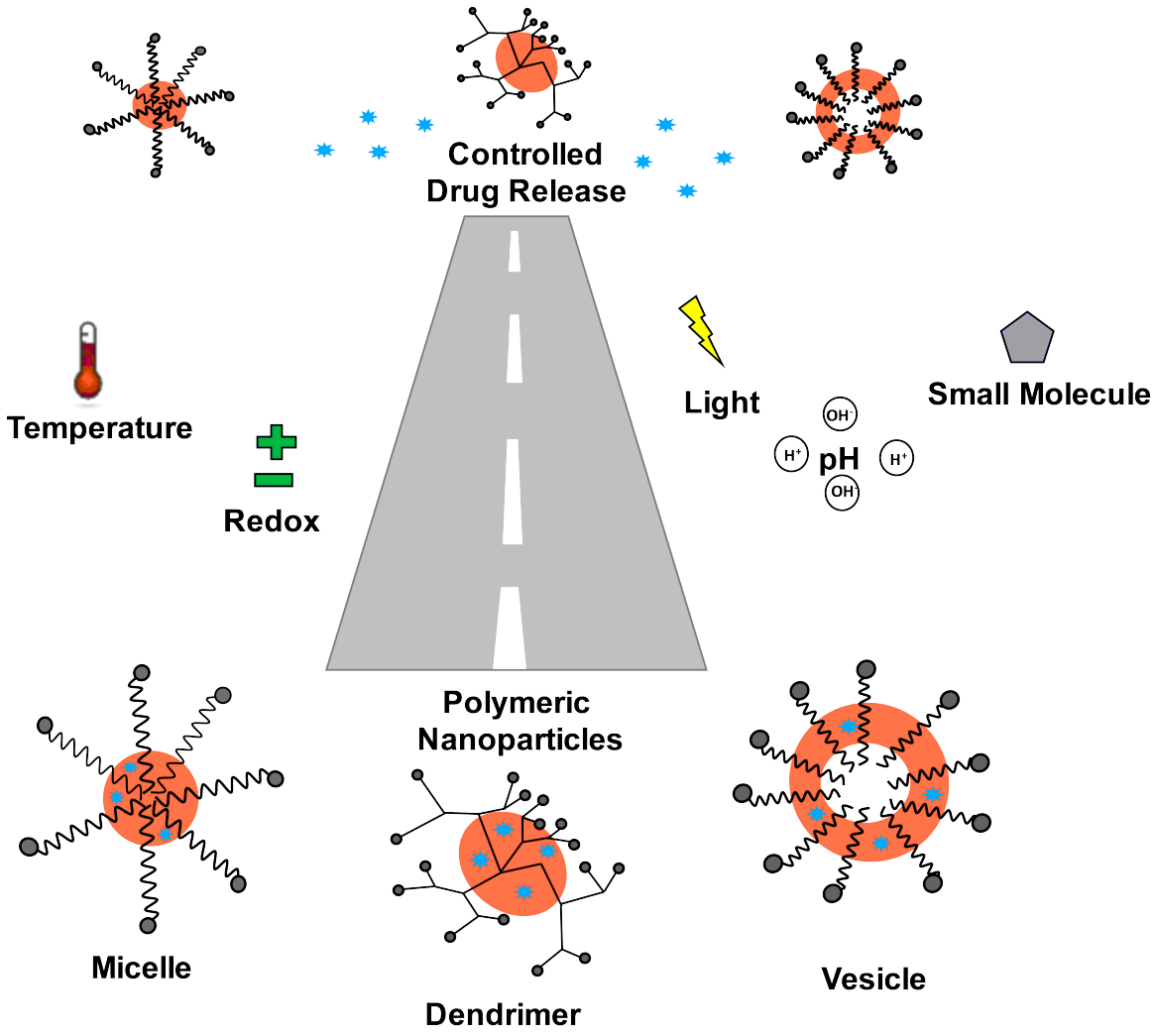

Figure 1 Structures of representative polymeric nanoparticles (micelles, dendrimers and vesicles) with the hydrophobic area highlighted in orange and the most common stimuli (light, pH, small molecules, temperature and redox) used to enable the controlled drug release on demand. Often combinations of different stimuli are used to increase the sensitivity and selectivity. Drug cargos are highlighted in blue.

Thermoresponsive Polymeric Nanoparticles

Temperature is a widely applied trigger for responsive PNPs in drug delivery. Although a range of polymers show thermoresponsive behavior, only a few polymers display biologically relevant lower and upper critical solubility temperatures (LCST and UCST, respectively) with examples including PNAAAm (LCST 32–33 °C) and PVCL (LCST 33 °C) (Table 2).[26] These polymers show good solubility and swelling in water at temperatures below their LCST due to a high degree of hydration, whereas the hydrophobic interactions between polymers dominate at temperatures above the LCST resulting, often, in an aqueous insoluble polymer. A rapid, temperature-dependent switch in physical properties can be used to control the size, drug loading and hydrophilicity of thermoresponsive PNPs, with triggering the release of encapsulated cargos on demand.

Block-*co*-polymers based on PNIPAAm are the most common materials for thermoresponsive PNPs because of their adjustable LCST and relative ease of synthesis (radical polymerization). Hiruta et al[27] showed that fluorescein conjugated PNIPAAm-*co*-PDAMAAm micelles, with a transition phase temperature of 37.4 °C, could be triggered by increasing the temperature by only one degree, resulting in significant swelling and enhanced uptake of these PNPs in murine RAW264.7 macrophages. Abulateefeh et al[28] reported improved cellular uptake of PLGA micelles encapsulating paclitaxel, with an increase in temperature from 37 to 40 °C leading to enhanced cytotoxicity in human MCF-7 breast cancer cells. To extend the scope of thermoresponsive polymers, there has been an increasing interest in finding thermoresponsive fragments that can be used to decorate non-thermoresponsive polymer backbones. Oligo(ethyleneglycol) was recently reported as a promising alternative to PNIPAAm due to reduced immunogenicity[29] and has

been *co*-polymerized (*via* ring opening metathesis) as both block and random *co*-polymers with cholic acid pendants bearing norborene.[30] Both types of *co*-polymer based micelles showed temperature responses with similar cloud points and demonstrated successful release of encapsulated paclitaxel in human SKOV-3 ovarian cancer cell based cytotoxicity assays. Despite their similar thermoresponsiveness and release profiles, the micelles formed from the block *co*-polymer were significant bigger in size (65 nm) compared to the micelles formed from the random *co*-polymer (20 nm). Triblock *co*-polymers, as an alternative to diblock *co*-polymers, offer accessibility to new structures. PVCL-PDMS-PVCL formed stable vesicles at room temperature with encapsulated doxorubicin. PVCL is known to have thermoresponsive behaviour that enabled shrinking of the vesicles by increasing the temperature to 40 °C, resulting in drug release “on demand” and cytotoxicity on human HeLa cervical cancer cells.[31]

An increasing number of thermoresponsive PNPs consist of, or incorporate, peptides and carbohydrates.[32,33] Polypeptides offer an attractive approach for the formation of defined polymer structures with functionalization of amino acid side chains allowing subtle control of the polymer features. Gu et al[34] functionalized the side chains of polyaspartamide with both an isopropylamide group as a temperature responsive component and hydroxy alkyl chains generating a hydrophobic segment, resulting in defined PNPs with an average size of 55 nm at 25 °C with release of encapsulated doxorubicin following a temperature increase from 25 to 55 °C. Chitosan has been functionalized with hydroxybutyl groups to generate a thermoresponsive natural polymer.[35] Hydroxybutyl chitosan has been decorated with hydrophobic deoxycholic acid to allow generation of an amphiphilic structure that was able to encapsulate

doxorubicin.[36] The drug loaded PNPs were non-toxic at 37 °C with drug release and toxicity triggered at 43 °C.

Experiments *in vivo* have shown that tumor tissues are sensitive to externally applied heat in the range between 40 and 43 °C, which is believed to be related to the higher level of reactive oxygen species and a lower pH.[37] In addition, tumor tissues also show abnormal temperature gradients caused by several parameters, such as abnormal blood flow and inflammation, that can be used as a natural temperature trigger, offering thermal release selectivity.[38] Guo et al[39] investigated the active uptake by endocytosis of folic acid functionalized PLA micelles, showing that doxorubicin was released at hyperthermia (40°C) inside cells (no release observed at normothermia).

Reverse micelles formed from amphiphilic PEG-PU, have been reported as temperature sensitive PNPs showing up to 40 % decrease in size when heated from 20 °C to 35 °C.[40] The reported PEG-PU PNPs have shown full release of encapsulated BSA after 10 hours in ethyl oleate. However, for potential use in drug delivery, reverse micelles must be water-soluble, which has been only realised for hydrolysis triggered cargo release from reverse micelles.[41,42]

Despite the progress in thermoresponsive PNPs, major challenges remain. Encapsulated cargos typically generate an initial burst release and display leakage of cargo, whereas conjugated cargos require an additional trigger. In addition, PVCL, P2F and PNIPAAm show crosslinking upon electron beam radiation (0–20 kGy), which is often used in conjunction with thermal anticancer treatment.[43] A vital issue that needs to be resolved is the storage and shipping of these materials, which carries practical concerns if they are to be used clinically.

Light responsive PNPs

Light as a physical stimulus offers a unique "clean" approach to trigger materials which can be localized in time and space.[44] Although, in theory, the whole spectrum of visible light can be used, near infrared (NIR) light has been favored over UV light because of its deeper tissue penetration depth and lower cytotoxicity.[45] Light responsive PNPs have been formed from amphiphilic polymers that comprise of chromophores with the ability to change their polarity upon exposure to light, or from polymer-drug conjugates linked *via* a light sensitive moiety that undergoes bond cleavage and subsequent drug release following illumination. Chemical moieties that change their structure and polarity reversibly upon irradiation are called "photoswitches" and are of special interest due to their on/off abilities. Here, the focus will be on the most recent developments with respect to design and application of these photoswitches.

Kohane used photoswitchable PNPs formed from spiropyran and DSPE-PEG based lipids for the delivery of doxorubicin into HeLa cells. Spiropyran exhibits a significant change in polarity when exposed to UV light, shrinking the PNPs from 103 nm to 49 nm (Figure 2).[46] The same PNPs were able to deliver docetaxel *in vivo,* showing inhibition of human HT-1080 derived fibrosarcoma in nude mice.[47]

Azobenzenes have received great interest due to their reversible photo-triggered switching between the apolar *trans*- (dipole of ~0 D) and the polar *cis*-isomer (dipole of ~4.4 D).[48] A recent example was reported by Blasco et al[49] who developed a

azobenzene-containing “miktoarm” star AB_3 polymer that formed vesicles in an aqueous environment with the ability to encapsulate hydrophilic and hydrophobic payloads. Irradiation with light (350–400 nm) lead to disruption of the self-assembled polymer and consequent cargo release. Donor−Acceptor Stenhouse Adducts (DASA) are a relatively new class of organic photoswitches that were reported by Read de Alaniz and Hawker.[50,51] Conjugation of hydrophobic alkynes and a hydrophilic PEG on the DASA formed micelles that showed disassembly upon irradiation (with visible light) due to an increase in hydrophilicity. Successful release of paclitaxel in MCF-7 cell culture showed the potential of this new chromophore for drug delivery systems.[52]

Park et al[53] developed light responsive PNPs by physical incorporation of 2-(4-methoxystryryl)-4,6-bis(trichloromethyl)-1,3,5-triazene into acetylated dextrans. The triazene derivate generates HCl upon irradiation with 345 nm light, which leads to degradation of the acetylated dextran, resulting in degradation of PNPs. This approach was used to release encapsulated anticancer drug irinotecan in human HT-29 colon cancer cells upon irradiation.

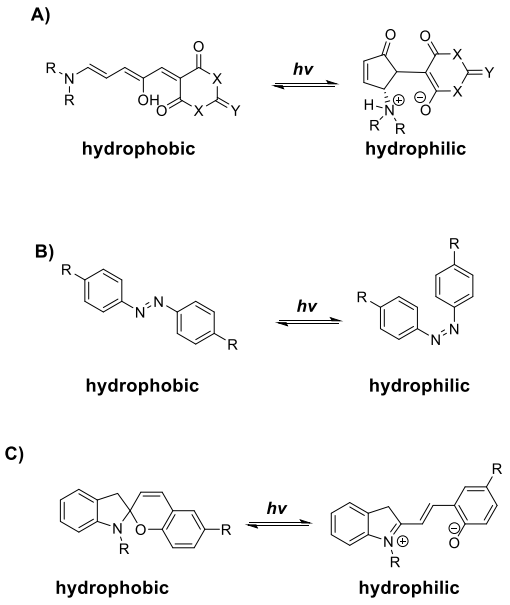

Figure 2 Chemical structures of photoswitches. A) Donor−Acceptor Stenhouse Adducts (*hv* = visible light), B) Azobenzene (*hv* = 300 – 400 nm), C) Spiropyran (*hv* = UV light).

pH responsive PNPs

pH regulation is an essential process in living systems. This finely tuned biochemical balance is easily affected by various pathologies and therefore an ideal trigger for the targeting of abnormal conditions such as tumor tissue that typically has a more acidic environment (pH ~ 6.5–7.2) compared to healthy tissues (pH 7.4), which is caused by insufficient supply of oxygen and nutrients, leading to glycolytic metabolism and acidic by-products. Similarly, intracellular organelles, such as endosomes (early endosomes pH 6.0–6.5, late endosomes 5.0–6.0) and lysosomes (pH 4.5–5.0), exhibit low intracellular pH “hot spots”.[54,55] pH responsive PNPs mainly utilize amphiphilic *co*-polymers with ionizable groups (*e.g.* carboxy, amino) or acid labile covalent bonds, which result in disassembly of the nanoparticles or cleavage of

a conjugated drug, respectively, upon a change in pH. Several chemical bonds have defined pH ranges where hydrolysis occurs resulting in bond cleavage; however, to utilize this for drug delivery, hydrolysis needs to happen in a biologically relevant pH range along with fast reaction rates. Historically, esters have been one of the most used acid labile moieties, but have lost attractiveness due to their instability towards enzymatic degradation. In this review, the focus is on the most promising approaches to pH responsive PNPs reported in the last 10 years.

Amphiphilic polymers bearing ionizable groups with the ability to form charged species are often classified as acid or basic sensitive polymers, and have been extensively applied in the design of pH responsive PNPs.[56] Recently, “charge switching” moieties that undergo a transition upon pH triggering have been developed. This concept was realized by capping amines with an anhydride (*e.g.* 2,3-dimethylmaleic anhydride) to form a negatively charged β-carboxylic amide at pH 7.4, whereas lowering the pH (< 6.5) triggers a chemical reaction that alters the overall charge of the PNPs.[57,58] Dual-pH responsive polypeptide micelles, PLLeu-PLL(DMA)-Tat(SA), were reported bearing two different anhydride capping moieties with different reaction rates (Figure 3), first, in the extracellular tumor environment (pH 6.5). The transition from negatively to positively charged was suggested by the author, to be the result of elimination of dimethylmaleic anhydride; however, the formation of succinimides, is a known reaction in peptide chemistry and offers another explanation for the pH responsiveness, namely cyclisation to give the succimide or maleicimide (the maleicimide is much faster), which needs to be addressed. The removal of the maleicimide enabled enhanced cellular uptake of the particles *via* endocytosis, where the more acidic endosomes (pH 5.0) could trigger the decapping of the succinamide masked lysine, which exposed the nucleus

targeting peptide, enabling subsequent delivery of encapsulated doxorubicin into the nucleus of HeLa cells.[59] Sun et al[60] reported a dimethylmaleic amide bridged amphiphilic block-*co*-polymer PEG-*co*-PDLLA that loses its PEG shell at extracellular tumor pH, exposing the amino functionalized PDLLA, resulting in an improved cellular uptake in human MDA-MB-231 breast cancer cells. When encapsulating docetaxel, these PNPs inhibited the growth of a MDA-MB-231 tumor xenograft.

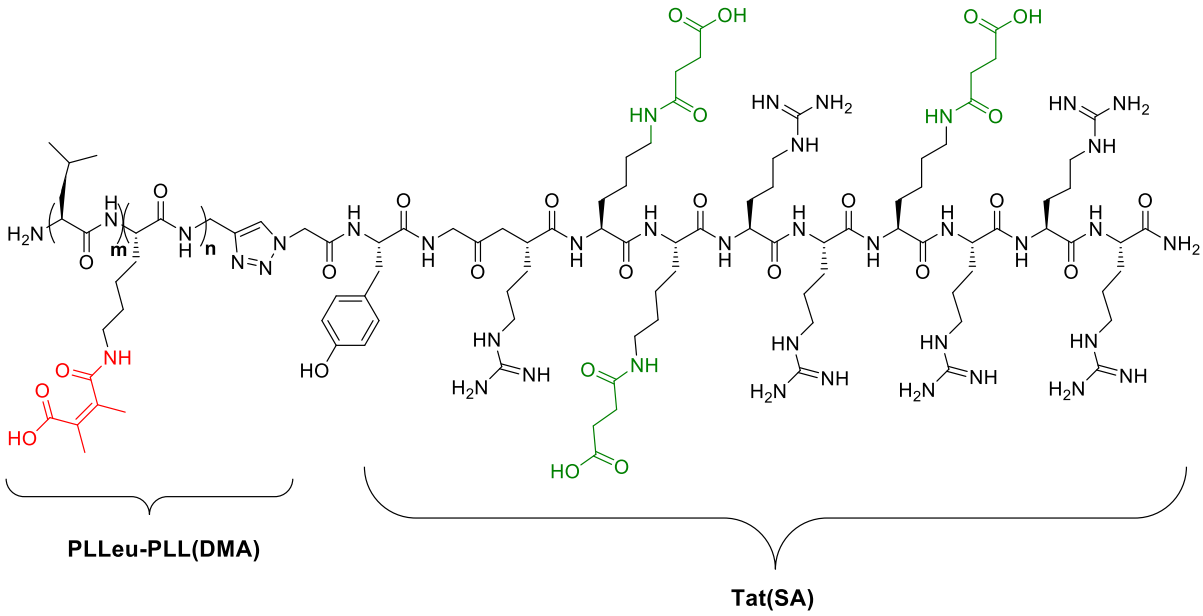

Figure 3 PLLeu-PLL(DMA)-Tat(SA) that forms micelles in aqueous conditions. In red 2,3-dimethylmaleic capped PLL (cleavable at pH 6.5) and in green succinyl capped Tet (cleavable at pH 5.0).

Hydrazones exhibit a sharp pH dependent degradation profile and have been investigated extensively as a pH responsive moiety to conjugate drugs to polymers (Figure 4).[61] Aryal et al[62] demonstrated the successful conjugation of levulinic acid modified cisplatin to the hydrophobic part of PEG-PLA PNPs *via* a hydrazine bond with an excellent control over drug loading and particle size distribution. At pH 5,

cisplatin is cleaved and cytotoxicity is switched on. This cisplatin–polymer conjugate showed significantly higher cytotoxicity than free cisplatin against human A2780 ovarian cancer cells, most likely due to an enhanced cellular uptake of the PNPs.

Acetylated dextrans (Ac-Dex) have been widely investigated as pH responsive systems because of their ease of synthesis and biocompatibility. Zhang et al[63] reported acetylated (Ac) PEG-*b*-Ac-Dex PNPs encapsulating doxorubicin, which showed no significant drug release at pH 7.4. An enhanced cellular uptake *via* endocytosis was observed after partial deacetylation at pH 5.5, which the authors relates to an increase in PNP size, whereas the more acidic environment in the endosomes triggered the disassembly of the PNPs and doxorubicin release.

Ortho esters are another acid labile chemical moiety for pH responsive PNPs. Ji et al[64] reported 6-OH ortho ester-modified β-cyclodextrin (β-CD) that formed PNPs with adamantane-modified PEG as a host-guest molecule, with 50% of the ortho-esters hydrolysed at pH 6.4 after 10 h, showing response to minute changes in pH.

Recently, phopshoramidites have gained attention as an acid labile group with the possibility of incorporation into a polymer backbone. Wang et al[65] reported an oxazaphospholidine monomer that was polymerized to give polyphosphoramidates; however, a low pH (3–4) was required to cleave these bonds, which currently limits the application of phopshoramidites based PNPs for drug delivery to acidic regions (such as the stomach).

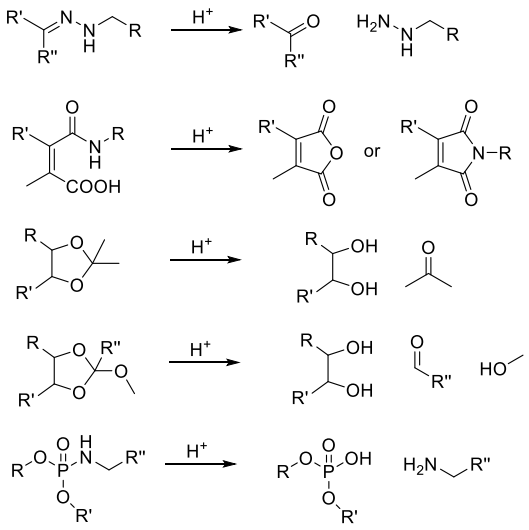

Figure 4 Commonly used acid labile covalent bonds in pH responsive PNPs.

Redox Responsive PNPs

Redox responsive PNPs are of special interest because of the variety of oxidative and reductive potentials in living systems. The most dominating naturally occurring redox trigger is the reducing agent glutathione that is present in a significant higher intracellular (~ 2–10 mM) than extracellular (2–10 μM) concentration. Reactive oxygen species (ROS), which are strong oxidizing reagents, are found in a significantly higher concentration within tumor tissues.[66,67,68] Over the last decade, a variety of elements with redox potential across the periodic table have been incorporated into PNPs, with boron,[69] iron,[70] platinum[71] and elements of group (VI)[72,73,74] being the most common. The redox responsive group can be located at the block junctions to separate the hydrophilic and hydrophobic parts,[75] followed by

PNPs degradation and release of cargo.[76] Alternatively, drugs can be conjugated to PNPs by a redox responsive linker.[77,78]

Disulfide bond is a well-established redox sensitive group and probably the most utilized one in drug delivery. Several methods have been developed to incorporate disulfides into polymers, including the use of a pyridyl disulfide-terminated RAFT initiator[79] and monomers, as well as thiol exchange reactions for post polymer modification (reviewed in detail elsewhere).[80] Wu et al[81], for example, used disulfide linked L-cysteine and fatty diacids with tuneable hydrophobicity to form biocompatible PNPs. These particles were triggered by intracellular glutathione, resulting in nanoparticle degradation and release of doxorubicin, which was inhibited by addition of the strong 1,4-acceptor *N*-ethylmaleimide. Recently, Yin et al[82] demonstrated the successful *in vitro* and *in vivo co*-delivery of two therapeutics, hydrophobic paclitaxel and hydrophilic AURKA-specific siRNA, with a hyaluronic acid based amphiphilic conjugate incorporating disulfide bonds. PGMA is a remarkable platform for redox responsive polymers (among others) because of synthetic ease and possibility of modification by ring opening of the epoxide.[83,84] Armes investigated extensively polymerization-induced self-assembly of PNPs using PGMA and their use for biomedical applications.[85,86] For example, by polymerizing disulfide containing PGMA with HPMA under mild RAFT conditions, redox sensitive worm like structures have been formed with promising properties for drug delivery applications.[87]

Diselenides have gained increasing attention and found numerous applications in controlled drug release from PNPs. Selenium has a lower homolytic Se–Se bond energy (172 kJ mol^{-1}) compared to S–S bond (251 kJ mol^{-1}), which offers an interesting new platform for redox chemistry in a biological environment. Although

the incorporation of diselenium into a polymer is challenging, significant process was achieved, *e.g*. with a selenium-containing RAFT initiator, leading to well defined polymers with a narrow PDI.[88] Gao et al[89] demonstrated that these selenium–RAFT terminated polymers could be transformed into diselenide containing polymers by aminolysis with hexylamine, followed by spontaneous oxidation coupling reaction.

Recently, azobenzene containing vesicles have been reported as alternative redox sensitive PNPs.[90] Vesicles with a diameter of 200 nm were formed by non-covalently crosslinking PGMA with β-cyclodextrin (host) and azobenzene (guest), with the vesicles showing high thermal, light and acid stability. Reduction of the azo-moiety with sodium dithionite, mimicking redox environment in the colon, to the corresponding aniline resulted in controlled cargo release *in vitro*.

Amphiphilic polymers that are sensitive towards oxidation offer an additional tool to form redox responsive PNPs. Here, metallopolymers and boronate containing polymers gained special attention due to their high sensitivity at low ROS concentrations. Shi et al[91] showed that ferrocene containing ABC triblock *co*-polymer PDMAEMA-*b*-PBzMA-*b*-PVFC formed vesicles with a permeable membrane that could be reversibly switched on and off by redox triggering. Although these systems have not yet been applied in a biological environment, they are a promising approach in the development of smart host–guest systems. Recently, vesicles with different arylboronate capping moieties were used to form vesicles with the ability to respond to the oxidative milieu.[92] Upon hydrolysis of the boronate ester a self-immolative linker lead to further decapping of an amine functionality and crosslinking of the remaining polymer backbone.

Small Molecule Responsive PNPs

A relatively new approach to trigger the controlled release of a drug is to utilize small molecules. These can be applied either as an endogenous stimulus (*e.g.* glucose) or an external synthetic small molecule stimulus, and take advantage of highly selective reactions that can take place in the challenging biological environment. Although the reaction between glutathione and a Michael acceptor, such as an acrylate, can be considered as a small molecule triggered reaction, it has only found limited applications due to poor selectivity with other biological nucleophiles.

In 1994, Okano was the first to report synthetic glucose responsive material made from phenylboronic acid polymer that formed macrogels, which were able to bind gluconic acid modified insulin (GA-Ins). High concentration of free glucose lead to an exchange with GA-Ins from the gels and a controlled release of insulin.[93,94] The first generation glucose responsive materials, however, faced major challenges; with the materials typically exhibiting decreased efficiency at physiological pH and the hydrogels, having a long response time (> 1h).[95] Benzoboroxoles are an interesting alternative to phenyl boronic acid based glucose acceptors due to their capability to bind carbohydrates in water at pH 7.[96] To improve the binding affinity towards specific carbohydrates, Gunasekara et al[97] reported "carbohydrate imprinted micelles" by crosslinking boroxole-functionalized monomers that showed high binding affinity to several carbohydrates, including glucose, which is a promising approach to "synthetic lectins" for drug delivery.

Bradley was first to report an externally applied small molecule trigger for PNPs by applying a tetrazine mediated inverse electron demand Diels Alder reaction (DA_{INV}). Biocompatible vesicles that showed high drug loading were formed by the interaction

of hydrophobic allyl ethers (a dienophile for DA_{INV})[88] and underwent DA_{INV} with a hydrophilic tetrazine under biologically relevant conditions, resulting in a change in their morphology (Figure 5).[99] Thus, doxorubicin could be released on demand by triggering PNPs with a biocompatible tetrazine. As an alternative approach, Neumann et al[100] conjugated doxorubicin to PEGylated PNPs *via* a carbamate linkage (Figure 4). Here, a vinylether dienophile underwent a DA_{INV} followed by an oxidation that resulted in a 1,6-elimination and drug release. The carbamate-conjugated doxorubicin PNPs showed no initial burst release and no cytotoxicity, with the ability to release the drug on demand by addition of the tetrazine, the first time that PNPs were triggered by a synthetic small molecule external stimulus, resulting in a switch on of cytotoxicity as determined in human PC3 prostate cancer cell assays.

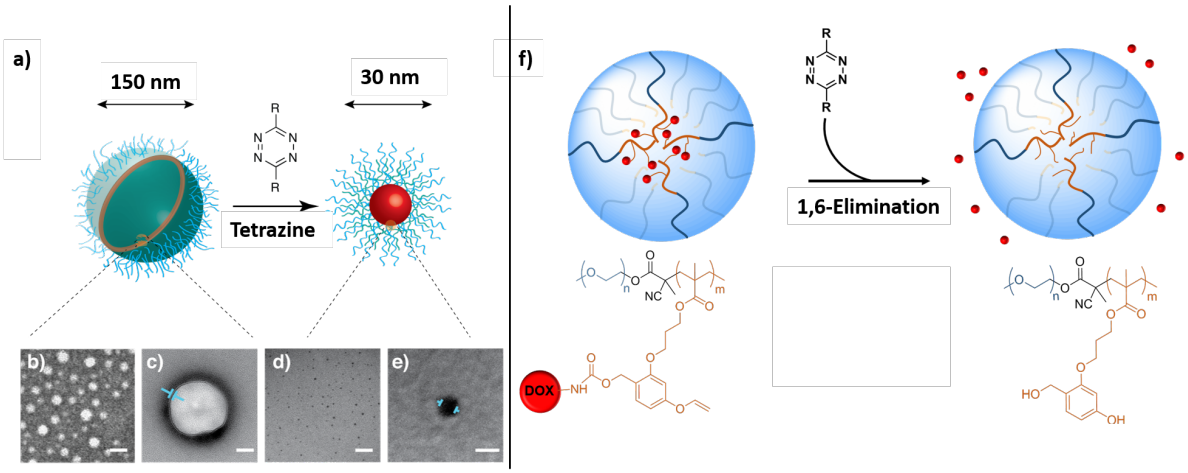

Figure 5 a) Modification of PEG-*b*-PAGE (Mn = 5.8 kDa; PDI = 1.03) vesicles with a cationic tetrazine through a DA_{INV} reaction resulted in the generation of micelles (30 nm) reduced in size compared to the original vesicles (150 nm). The morphology of the PNPs was confirmed by TEM (uranyl acetate staining) showing b) vesicles in the absence of tetrazine (scale bar 200 nm), c) a single vesicle with ~4 nm membrane (thickness indicated by arrows) (scale bar 50 nm), d) the micelles

after treatment with tetrazine (scale bar 200 nm), e) a single micelle (diameter ~30 nm, scale bar 50 nm) (reproduced with permission from ref. 89. f) Tetrazine triggered release of doxorubicin from PEG-P(DOX) based micelles showing anticancer properties in PC3 cell culture assays (reproduced with permission from ref. 100).

Outlook

Nanoparticles have been of great interest since the approval of Doxil® for anticancer treatment, with several nanoparticles currently in clinical trials. Responsive polymeric nanoparticles are a powerful tool in the field of drug delivery due to their chemical versatility, flexibility and broad spectrum of physical properties. The use of responsive polymeric nanoparticles offer new approaches in nanomedicine, taking advantage of the targeting ability of nanoparticles, the on demand activation of prodrugs by application of an internal or external trigger and their ability to stabilize/ solubilize drugs of choice. By using a dual or multi-stimuli responsive polymeric nanoparticle system, the sensitivity towards biological conditions in cancer cells can be improved.

To date, however, most of the commonly used responsive PNPs rely on *in vitro* experiments and suffer from a lack of *in vivo* data that would prove their full potential. The environment in biological systems is far more complex and challenging than in simple cell culture and, therefore, the transition from cell culture to human continues to be one of the major challenges to overcome.

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Table 1 Examples of responsive polymeric nanoparticles with the corresponding stimulus.

Structure	Polymer	Stimulus	Cargo	Size [nm]	Ref
Micelle	PNPAAm-fluorescein	thermal	fluorescein	400	27
Micelle	PLGA-*b*-(PEGMEMA-*co*-PPGMA)	thermal	paclitaxel	26	28
Micelle	PNOEG-*co*-PNCA	thermal	paclitaxel	65	29
Vesicle	PVCL-*b*-PDMS-*b*-PVCL	thermal	doxorubicin	210	31

n/a	P(Asp)-co-P(alkylamide)	thermal	doxorubicin	55	33
Micelle	Folic acid-PLA	thermal	doxorubicin	72	39
Reverse Micelle	PEG-*b*-PU	thermal	BSA	300	40
n/a	DSPE-PEG	light	doxetaxel	103	46,47
Vesicle	PEG-*b*-PEZO	light	Nile Red	640	49
Micelle	PEG-*b*-DASA	light	paclitaxel	n/a	52
n/a	Ac-Dex	light	irinotecan	520	53
Micelle	PLLeu-PLL(DMA)-Tat(SA)	pH	doxorubicin	20	59
Micelle	PEG-*b*-PDLLA	pH	docetaxel	100	60
n/a	PEG-*b*-PLA	pH	cis-platin	86	62
Micelle	PEG-*b*-AC-Dex	pH	doxorubicin	72	63
Vesicle	PEG-β-cyclodextrin	pH	-	200	64
n/a	Cys-PDSA	redox	doxorubicin	n/a	81
Micelle	HA-ss-(OA-g-bPEI)	redox	paclitaxel	220	82
Vesicle	PGMA-β-cyclodextrin	redox	RhB	200	85
Worm-Gels	PGMA-*b*-PHPMA	redox	-	-	90
Vesicle	PDMAEMA-*b*-PBzMA-*b*-PVFC	redox	-	500	91
Vesicles	PEO-*b*-PNBMA	redox	PTX and Dox	520	92
Macrogel	PBA	glucose	GA-Ins	1-4k	93,94
Vesicle	PEG-*b*-PAGE	tetrazine	doxorubicin	150	99
Micelle	PEG-*b*-Dox	tetrazine	doxorubicin	35	100

Table 2 Recently reported thermoresponsive PNPs with their cargos and LCST.

Polymer	Structure	Size [nm]	LCST [°C]	Cargo	Ref
PNPAAm-fluorescein	micelles	400	37.4	fluorescein	26
PLGA-*b*-(PEGMEMA-*co*-PPGMA)	micelles	26	39	paclitaxel	27
PNOEG-*co*-PNCA	micelles	65	37	paclitaxel	29
PVCL-*b*-PDMS-*b*-PVCL	vesicle	210	37–42	doxorubicin	30
P(Asp)-*co*-P(alkylamide)	n/a	55	37	doxorubicin	33
Hydroxbutyl chitosan	nanogel	350	38.2	doxorubicin	35

Folic acid-PLA	micelles	72	39.2	doxorubicin	38