**Title** Stimuli-responsive pro-drug chemistries for drug delivery

*Author(s), and Corresponding Author(s)** Vincenzo Taresco, Cameron Alexander, Nishant Singh* and Amanda K. Pearce*.

Dr Vincenzo Taresco, Prof. Cameron Alexander, Dr Nishant Singh, Dr Amanda K. Pearce. School of Pharmacy, University of Nottingham, NG7 2RD, UK. E-mail: amanda.pearce@nottingham.ac.uk, nishant.singh@nottingham.ac.uk; Tel: +44 (0)115 846 7678.

**Abstract**

Research into advanced therapeutic materials is of growing importance worldwide, particulary in the disease areas of infection, neurodegeneration and oncology. Advances have been made in treating all these diverse pathologies but there still remain many areas of challenge. Amongst the most difficult are those involving highly potent and/or cytotoxic agents which present the inherent problem of adverse off-target effects. Of key importance is to widen the therapeutic window for such agents by reducing access to non-diseased cells and enhancing release at targeted sites. Spatiotemporal controlled release can be achieved by exploiting physical, chemical or biological stimuli present at the specific diseased area. A crucial strategy involves drug-carrier linkages able to respond to physiological or biochemical stimuli present in the disease region, and there is now significant literature on (polymeric) pro-drugs based on the drug+carrier+cleavable linker philosophy, predominantly for cancer applications. We have therefore focused this mini-review mainly on single/multi-stimuli-responsive pro-drugs for cancer therapies, covering some of the leading examples of pro-drug chemistries used to endow polymers with controlled and site-specific drug delivery properties. Additionally, we have emphasized the possibilities for exploiting similar approaches to disease-associated stimuli

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present in infections, both bacterial and viral, in inflammatory and immune diseases, and in degenerative disorders.

## 1. Introduction

The requirement to deliver bio-active agents selectively to target sites in the body, for diagnosis and therapy, is a major focus for research in the physical and biomedical sciences.[1] While there have been important advances in recent years,[2] with a number of academic papers describing successful targeted therapies in small-to- medium scale trials,[3] there remain significant unmet needs in drug delivery and complex scientific and clinical challenges to overcome.[4]

There are multiple disease areas in which targeted drug delivery could be transformative, but the most extensive research has been carried out in cancer drug delivery.[5] This is because many, though by no means all, anti-cancer agents are designed to be highly cytotoxic, and thus have the inherent problem of adverse off-target effects. Accordingly, the focus for many researchers in the field has been to widen the therapeutic window for these agents, by reducing their access to non-diseased cells and enhancing their release at the targeted sites. The mechanisms by which this strategy can be accomplished include the use of physical, chemical or biological stimuli at the points where drug delivery systems spatially encounter the diseased regions.[6] Of particular importance have been methods to encode chemistries in the drug-carrier linkage which are activated by physiological or biochemical cues present in the disease region. This pro-drug approach has been very widely trialed in more conventional therapeutics, and indeed many drugs that are taken routinely by patients, such as aspirin, are in fact pro-drugs. The anti-cancer drug tamoxifen can also be considered as a pro-drug, since a number of its metabolites such as 4-hydroxytamoxifen are more potent in certain patients than the parent drug. There is now a wide-spread literature on pro-drugs and it is not surprising that some of

the pioneering examples of polymer therapeutics for cancer were based on a drug+carrier+cleavable linker philosophy.[7]

We have therefore focused this mini-review primarily on stimuli-responsive pro-drugs for cancer therapies, but emphasize that similar approaches can exploit disease-associated stimuli present in infections, both bacterial and viral, in inflammatory and immune disorders, and in degenerative disorders.[8] The specific stimuli include altered redox states, secretion of cytokines and other signaling molecules, so in principle can be exploited by many of the mechanisms already used to activate anti-cancer systems. We thus outline below the main classes of activating chemistries used in oncological drug delivery while alerting the reader to the many possibilities for other therapeutic areas which may be addressed in similar ways.

**Importance of responsive linker pro-drug chemistry for drug delivery**

The development of nanoparticles which can be injected into a patient and which circulate for prolonged time periods to effect therapy is a demonstrable achievement of drug delivery research.[9] Further optimization of active and passive targeting mechanisms have allowed for the delivery of nanoparticles specifically to disease sites of interest. With many targets and targeting ligands available to enhance selective tumor accumulation of nanoparticles, the development pathway for anti-cancer drug carriers has often focused on specific ligand functionalization to improve the delivery process. However, in order to maximize delivery of therapeutics to sites of interest while minimizing adverse side effects due to non-specific toxicity, it is important not just to take the carrier to the target site, but to ensure that the therapeutic agent is stable within the delivery system and only released when the disease region is reached. This outcome is harder to achieve for drugs physically entrapped within a carrier, hence the drive for pro-drug mechanisms of in situ drug release.
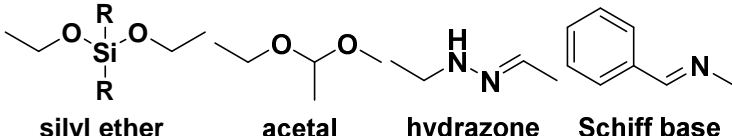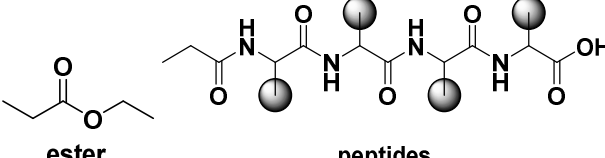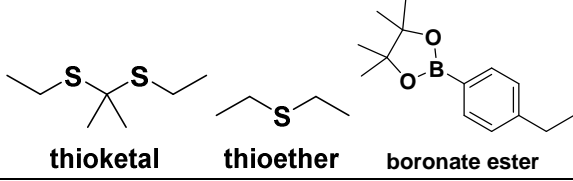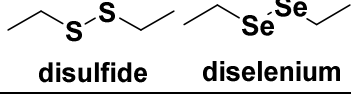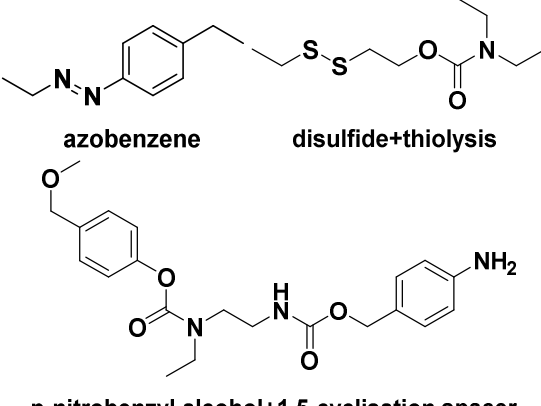
2. **Methods of stimuli-responsive drug delivery**

There are significant hurdles in the intravenous administration of therapeutic drugs, largely due to the poor water solubility of many commonly used drugs, as well as the short biological half-lives typical for small molecules *in vivo*. This results in a low bioavailability of the drug in vivo and high doses that need to be administered to counteract this. Additionally, it is well known that systemic delivery of small molecule drugs can result in non-specific toxicity in other areas of the body, potentially resulting in severe side-effects, which are exacerbated by the high doses needed for a therapeutic effect. Physical encapsulation of therapeutics within the core of stealth nanoparticles such as liposomes or vesicles was an initial approach to overcome these drawbacks. This method however can display variable loading efficiencies of the therapeutic, where a dependence on particle material, drug solubility, drug-particle interactions, functional groups of both the drug and the particle material and loading conditions heavily dictate the successful inclusion of the therapeutic within the nanocarrier.[10] Additionally, physically entrapped drugs typically display less controlled release rates, with burst release commonly seen.[11]

An alternative approach is to attach instead the drug through a covalent linkage, forming a prodrug, which contributes numerous advantages to a drug delivery system when used in conjunction with both passive and active targeting. While covalent attachment requires more sophisticated synthesis requirements, the resultant system will typically be more stable to unwanted burst release of the drug, can facilitate a higher loading efficiency, and provides additional control over both the rate of the release and the site of drug release in vivo.[12] Pathological sites such as tumor tissues and inflamed wounds when compared to the healthy tissues exhibit abnormalities such a low pH, hypoxia, high temperature, over-expressed proteins and enzymes and elevated levels of reactive small molecules such as metabolites and Reactive Oxygen Species (ROS). Such characteristic properties of disease sites have therefore been used to design functional prodrug carriers to obtain site specific delivery and controlled release of therapeutic payloads (Table 1). These programmed prodrug carriers can thus undergo specific

physio-chemical transformations such as molecular structural rearrangements, disassembly, inversion or disassociation into sub units triggered by these bio-relevant cues/abnormalities at targeted sites for site specific release of the drug load.[13]

**Table 1.** Examples of responsive linkers adopted for stimuli-driven prodrug controlled release

| Stimuli | Responsive linkers | References |
|---|---|---|
| pH | <br>silyl ether     acetal     hydrazone    Schiff base | 16-21, 65, 67 |
| enzyme | <br>ester     peptides | 23-29, 64-66 |
| oxidative | <br>thioketal    thioether   boronate ester | 31-38, 58, 59, 62, 69 |
| redox | <br>disulfide    diselenium | 41-47, 62, 64, 66-69 |
| self-immolative | <br>azobenzene    disulfide+thiolysis<br><br><br>p-nitrobenzyl alcohol+1,5-cyclisation spacer | 50-57, 68 |

Typical endogenous stimuli that are exploited for this purpose are pH, concentrations of enzymes, redox potential and oxidative conditions, as well as additional exogenous stimuli such as light, infra-red light (IR), ultrasound and magnetic field, which will not be discussed here.[6b, 14] Degradation in these specific environments can be controlled through the specific linkage used for attachment of the drug to the nanoparticle carrier, and therefore this component

is of particular importance. This review summarizes the current methods of stimuli-responsive drug delivery achieved through covalent prodrugs, as well as offering insight and a reference point for further development of these systems in the future.

**Endogenous stimuli-responsive drug delivery**

### pH responsive systems

Covalent linkages that are acid-labile can be employed to exploit both the acidic extracellular environment of tumors (~pH 6.5) due to the irregular angiogenesis in fast-growing tumors, as well as the acidic environment of endosomal and lysosomal vesicles within a cell (~pH 5).[15] Nanoparticles internalized through receptor-mediated endocytosis will enter the cell in vesicles, which acidify causing a local environment of pH ~5-6, as well as fusion with lysosomes (pH ~4-5). This can be exploited through degradable covalent drug conjugations, such as acid-sensitive acetal, ester, Schiff-base, silyl ether and hydrazone bonds.

Acetal-based prodrug delivery systems have several advantages, namely fast degradation in acidic conditions and an absence of acidic degradation products. Gu and coworkers used this strategy to prepare acetal-linked paclitaxel (PTX) prodrug nanoparticles for delivery to various cancer cell lines.[16] They attached PTX onto block copolymers of poly(ethylene glycol)-b-poly(acrylic acid) (PEG-PAA) via an acid-labile acetal bond to the PAA block, and the prodrugs could self-assemble into micelles. They showed pH-dependent drug release, with minimal release of the drug in physiological conditions and accelerated release under acidic pH conditions. The micelles could impart toxicity in vitro in KB and HeLa cancer cells, as well as paclitaxel-resistant A549 cells, which was attributed to local release of the drug, as well as hydrolysis of the acetal linkage resulting in release of paclitaxel in its native state, ensuring maximum efficacy of the drug.

Parrott et al.[17] described a pH-responsive drug delivery system based on acid-labile silyl ether functionalized prodrugs of gemcitabine (GEM). The prodrug could be polymerized into a

nanoparticle for additional protection of the drug during circulation, and they varied the side groups of the silyl ether in order to investigate the effect on rate of drug release. They were able to show that by increasing the steric bulk of the substituents on the silyl group, they could tune the release of the drug in an acidic environment to be in hours, days, or months, and that this effect was enhanced when the particles were in an acidic environment, such as that within endosomes. Finally, they showed that the nanoparticles could impart toxicity on LNCaP tumor cells through hydrolysis of the silyl ether, resulting in local delivery of gemcitabine. This approach was also explored by Yan and coworkers, who utilized silyl ether prodrugs of the chemotherapeutic camptothecin to achieve controlled release from mesoporous silica nanoparticles (MSN).[18] They synthesized both trimethyl silyl ether and triethyl silyl ether derivatives of the drug, which were then covalently bonded to the surface of the nanoparticles. Their conjugates showed minimal drug release in physiological pH 7.4, but in an acidic environment showed accelerated hydrolysis and release of the drugs, with the methyl derivative demonstrating a faster release profile than the ethyl derivative due to steric bulk. Both conjugates were able to impart toxicity to cells at a similar levels to that of the free drug.



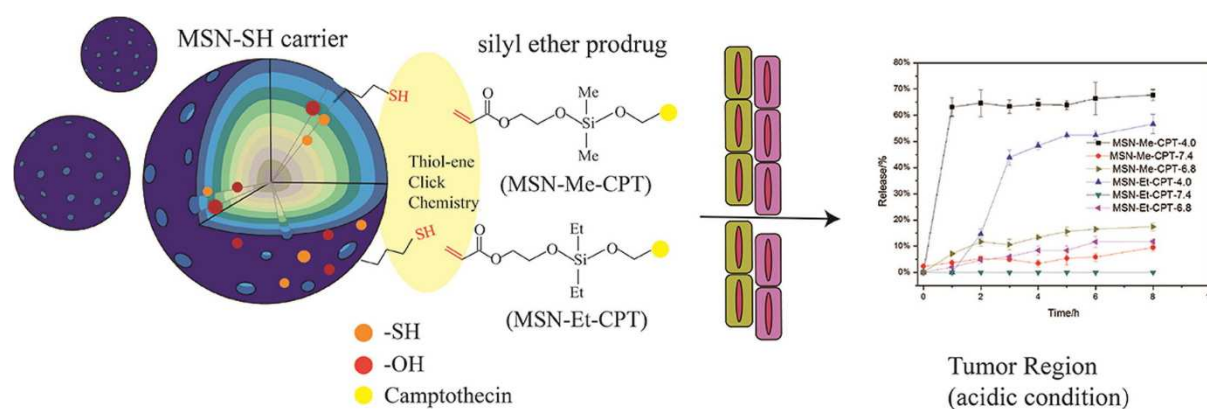**Figure 1. Illustration of an acid-responsive silyl ether prodrug being released from an MSN-SH nanocarrier (MSN-mesoporous silica, SH-thiol, Me-methyl, Et-ethyl, CPT-Camptothecin). Reprinted with permission from ref 18. Copyright 2017 Elsevier.**

Zhou et al.[19] developed drug delivery particles from the self-assembly of amphiphilic N-(2-Hydroxypropyl) methacrylamide (HPMA) copolymers, featuring prodrugs of doxorubicin and

β-sitosterol attached to the polymer backbone through acid-labile hydrazone linkages. Following self-assembly to form micelles, the polymers were further crosslinked via hydrazone bonds for physiological stability. The micelles remained stable at pH 7.4 with minimal drug release observed, however at pH 5.0 the micelles were able to release approximately 80% of the drugs after 8 hours. While in vitro IC50 values in Hep G2 and A549 cell lines were similar for both the crosslinked and non-crosslinked micelles, in an in vivo mouse xenograft hepatocarcinoma model, the crosslinked micelles showed higher tumor accumulation and an improved anti-tumor effect over the non-crosslinked micelles.

Similarly, Thurecht and coworkers synthesized acid-labile prodrugs of doxorubicin (DOX) by attaching the drug to PEGMA [poly(ethylene glycol methacrylate]-based hyperbranched polymers through hydrazone bonds.[20] The polymers were stable at pH 7.4 with less than 5 % drug release, but showed controlled release of the drug in acidic pH conditions. The polymers were able to be localized within tumor cells in vitro, and demonstrated comparable cytotoxicity to free drug. Hydrolysis of the hydrazone linkage in vitro was confirmed through confocal imaging, where the drug could be seen to overlay with polymer signal initially in endosomes, followed by transport into the nucleus alone, as observed through the innate fluorescence of doxorubicin. Finally, the conjugate was able to induce a therapeutic effect on prostate cancer xenograft tumors in vivo, with a significant reduction in tumor volume compared to free drug and controls.

Jia et al.[21] reported an anticancer acid-labile prodrug of doxorubicin, where they utilised pH-sensitive Schiff-base linkages to achieve stimuli-responsive drug delivery. The prodrug comprised unimolecular micelles of star-like amphiphilic copolymers, synthesized from benzoaldehyde and hydrophilic poly(ethylene glycol methyl ether methacrylate), with the doxorubicin attached to the copolymer benzoaldehyde groups via Schiff-base. The micelles showed stability in physiological conditions, with an increase in drug release observed over time in a pH 5.0 environment, as well as improved control over release kinetics as compared to

physically encapsulated micelles of DOX. In vitro experiments confirmed that the unimolecular micelles could be internalised by human cervical cancer HeLa cells and impart toxicity following hydrolysis of the Schiff-base.

**Enzyme-responsive systems**

Enzyme dysregulation is observed in many disease-associated microenvironments, and thus has been exploited as a powerful tool in the nanomedicine field for the development of enzyme-responsive nanomaterials, which are able to specifically target the affected site and thereby regulate drug release. Several more broad overviews covering the developments in enzyme-responsive liposomes and polymeric linkers/nanoparticles, as well as their adoption into the controlled drug-delivery field are discussed from different perspectives and degree of detail in these recent suggested reviews.[22]

A preliminary example involving physical encapsulation of drugs is described by Aluri et al.,[23] who developed a novel class of enzyme-responsive comb-like poly(ester-urethane)s based on naturally occurring L-Tyrosine amino acids. The amine and carboxyl moieties were converted into dual function ester-urethane functionalities, and then subjected to solvent-free melt polycondensation, adopting PEG chains as spacer units. The amphiphilicity of the materials was optimized by tuning the length of the hydrophilic PEG chains and the nature of the alkyl side chains anchored to the L-Tyrosine phenolic residue. Stable nanoparticles of 200 nm were formed through self-assembly of the materials in an aqueous environment, and clinically relevant drugs such as DOX and Camptothecin were successfully encapsulated, with the drug-loaded nanoparticles demonstrating good stability in simulated extracellular conditions. However, in an environment of esterase enzymes, the nanoparticles underwent rapid biodegradation with subsequent cargo-release, exclusively in the intracellular conditions. The drug-loaded nanoparticles demonstrated improved cytotoxicity and selectivity, as well as higher cellular internalization compared to the free drugs.
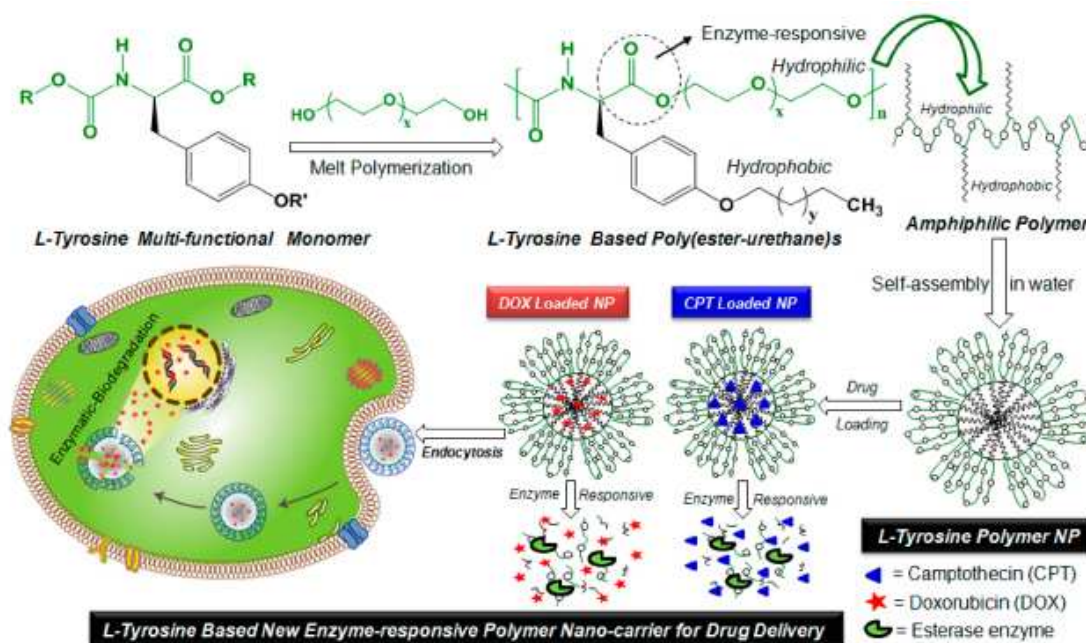
**Figure 2. Designing of new classes of L-tyrosine based amiphiphilic poly(ester-urethane)s and employ their enzyme-responsive self-assembled nanoparticles as multiple anticancer drugs in cancer cells. Reprinted with permission from ref 23. Copyright 2017 American Chemical Society.**

Gianneschi's group developed a library of novel polymers used as a nanoparticle platform with high targeting recognition of matrix metalloproteinases (MMPs), which are over-expressed in an array of cancer types and present as catalytic, extracellular or membrane-bound tumor markers.[24] In an elegant attempt to apply this smart, enzyme-sensitive nanoparticle platform as a chemotherapeutic delivery system, two monomers were designed and synthesized as norbornene derivatives to be polymerized via ring opening metathesis polymerizations (ROMP).[25] A hydrophobic monomer was achieved through direct biodegradable ester-linkage of the norbornene scaffolds with the potent anticancer drug paclitaxel (PTX-up to 63 % drug loading in the final micelles), while a hydrophilic monomer was decorated with a specific peptide sequence to endow the formed micelles with a motif for MMP recognition. Upon exposure to the enzymes, the micelles underwent a significant morphology change from well-defined 20 nm particles to microscale structures. The safety and efficacy of the materials were assessed through a variety of in vivo proof-of-concept studies. The nanoparticles demonstrated enhanced selectivity for the tumor site, and minimal off-target toxicity was observed for the

drug-loaded nanoparticles, as well as no observed adverse effects from the non-responsive nanoparticles. The resultant nano to micro size transition achieved in the tumor environment allowed for enhanced nanoparticle accumulation and controlled drug release. By varying the nature of the peptide sequence and the nature of the hydrophobic core, the same group has since developed a series of innovative enzyme-responsive polynorbornene platforms for the efficient targeting of ischemic tissues,[26] as well as a unique non-invasive delivery of a material scaffold to acutely infarcted myocardium.[27]

In addition to anticancer therapies, there is also a growing application of enzyme-responsive materials for bacterial-strain targeted delivery of antimicrobial agents.[28] In this regard, Li et al.[29] developed polymeric vesicles that undergo self-immolative degradation in response to enzymes such as penicillin G amidase and β-lactamase. The degradative action of these enzymatic systems, which are closely related to drug-resistant bacterial strains, led to the controlled and sustained release of the payload antibiotics.

**ROS-responsive systems**

It has been shown that, compared with their normal counterparts, many types of tumor cells consistently produce a high level of ROS, due to their accelerated aerobic metabolism, such as superoxides ($O_2^-$), hydroxyl radicals ($\cdot OH$), hypochlorite ions ($OCl–$), hydrogen peroxides ($H_2O_2$), and singlet oxygen species ($^1O_2$).[30] Therefore, these increased levels of ROS can be exploited as endogenous stimuli for specific drug release through the use of oxidative-sensitive linkages. There are many types of ROS-responsive materials explored in drug delivery applications, including those containing characteristic groups such as thioether, selenium/tellurium, thioketal, boronic ester, sulfide and ferrocene groups.

Hagen et al.[31] described an aminoferrocene-based polymer prodrug, targeted towards specific tumoricidal behaviour in the enhanced ROS environment of cancer cells. The ROS-responsive ferrocene groups were activated from their non-toxic dormant state by the oxidative conditions, producing a toxic quinone methide species, as well as an efficient catalyst for further ROS

production. Their organometallic complexes exhibited enhanced anticancer activity in cellular assays, and targeted cancer cells selectively over normal cells. The activation reaction proceeded autocatalytically, which led to the generation of large quantities of ROS in cancer cells causing selective toxicity towards human promyelocytic leukemia and human glioblastoma-astrocytoma, but were non-toxic towards representative nonmalignant cells.

Aryl boronic acids and their esters are well-known to be cleaved by $H_2O_2$.[32] Based on this, as well as the nontoxicity of boronic acids, esters, and the end product boric acid, Kuang et al.[33] designed and synthesized boronate prodrugs of nitrogen mustards and investigated their inducible reactivities in cancer cells. In the absence of $H_2O_2$, the prodrug remained nontoxic to cells, indicating that the prodrug complex masked the toxicity of the nitrogen mustard mechlorethamine, however in the presence of $H_2O_2$ the boronic ester could selectively react with $H_2O_2$ to form a boronate intermediate that rapidly hydrolyzed causing the drug to be released. The active drug could impart toxicity to the cells through its mechanism of causing DNA interstrand cross-links, resulting in cell death by preventing cell replication and transcription. In a further study,[34] they investigated more efficient boronic ester prodrugs based on quinone methide, that could additionally be coupled with multiple potent effectors to maximise the ROS-inducible cytotoxicity of prodrugs. The anticancer prodrugs were activated with different functional leaving groups, and thus under tumor-specific conditions (high level of ROS), the arylboronic esters were readily cleaved by the $H_2O_2$ to release 2,5-bis(trimethylammonium)-benzyl-1,4-diol, which generated biquinone methide in situ, as well as two additional effectors that promoted increased quinone methide production.

Boronate ester-based ROS sensitivity was also exploited by Daniel et al.,[35] where they designed a block copolymer prodrug system that could self-assemble in aqueous conditions to form particles. The prodrug consisted of an inhibitor for matrix metalloproteinases (MMPs), which are commonly found in areas of increased ROS concentration, tethered to the polymer backbone through an aryl boronic ester moiety. The stimuli-responsive linker was stable under

normal physiological conditions, however could undergo nucleophilic attack by $H_2O_2$, resulting in the expulsion of a phenolate intermediate, which could spontaneously release the MMP inhibitor. In a similar approach, Zhang *et al.*[36] synthesized a polymer-drug conjugate of camptothecin containing an ROS trigger-responsive domain. In their work, camptothecin was conjugated to a polymer carrier through carbamate linkages attached to a central ROS-responsive boronic ester group. Exposure to increased levels of $H_2O_2$ caused the boronic ester group to be cleaved, resulting in a cascade self-immolative degradation of the polymer prodrug resulting in drug release. The conjugates demonstrated significantly increased cellular apoptosis of cancer cells both *in vitro* and *in vivo* when exposed to elevated levels of ROS, compared to the same polymer in normal conditions.

The group of Farokhzad utilized a thioketal as the ROS-responsive prodrug linkage to develop nanoparticles for the delivery of the anticancer drug mitoxantrone (MTO).[37] The drug was transformed into a polyprodrug through the use of an ROS-cleavable thioketal-containing linker, and self-assembled with lipid-polyethylene glycol to form polyprodrug nanoparticles (polyMTO NP). Upon exposure to a high concentration of ROS, the nanoparticles could undergo an ROS-responsive elimination reaction, inducing chain-breakage release of intact drug molecules leading to significant inhibition of tumor cell growth both in vitro and in vivo. In comparison, the nanoparticles remained nontoxic in non-oxidative conditions.
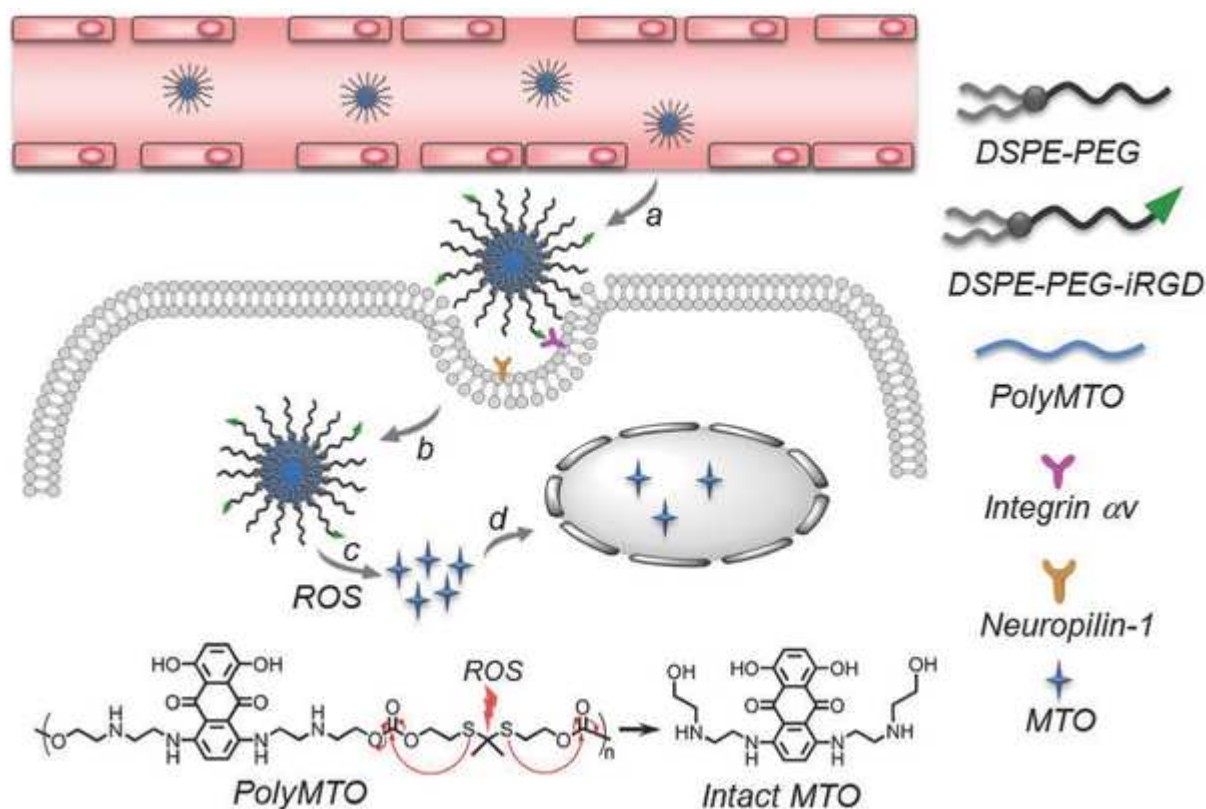
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**Figure 3. Schematic illustration of the poly mitoxantrone (MTO)-based nanoparticle platform for targeted and deeply penetrating cancer therapy. a, b) After intravenous injection, the internalising RGD (iRGD)-mediated targeting strategy facilitates the tumor tissue penetration and tumor cell uptake of the nanoparticles. c) Subsequently, the high level of ROS in cancer cells can break thioketal bond in the polyMTO to induce chain-breakage patterned release of intact MTO for d) disrupting DNA synthesis and efficient cancer therapy. DSPE-PEG-[(1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)], mitoxantrone (MTO). Reprinted with permission from ref 37. Copyright 2017 Wiley.**

A thioether-based ROS-responsive prodrug system was investigated by Liu et al.[38] They designed hyperbranched polymer micelle prodrugs of the anticancer drug SN38, utilizing a thioether linkage for conjugation of the drug to the polymer backbone. In the presence of $H_2O_2$, the thioether linkage could be oxidized into sulfones or sulfoxides, resulting in hydrolysis and subsequent release of the SN38. Additionally, the micelles encapsulated cinnamaldehyde (CA), which could induce further intracellular ROS production, thereby accelerating the release of the anticancer drug. The prodrug system with encapsulated CA demonstrated increased cytotoxicity when compared to the free drug, and was selective for an ROS environment.

**Redox-responsive systems**

Overexpression of the cell protectant glutathione (GSH) occurs in some tumor tissues, and the resultant increased concentration of this compound has been exploited by nanoparticles endowed with GSH sensitive functionalities for intracellular drug delivery. In general, GSH is responsible for regulating the cellular reductive microenvironment, and is found at approximately 100-1000 times higher levels in intracellular compartments than in human plasma and blood. In addition, some tumor cells express cytosolic GSH to levels above 4 times higher (2-10mM) than normal cells, allowing for cancer-specific intracellular therapeutic delivery after cellular uptake.[39] Some select examples are discussed below, with a more comprehensive overview to be found in other recent reviews.[40]

Suna et al.[41] recently demonstrated that a prodrug polymer with a redox responsive disulfide bridge had higher drug release and consequent antitumor activity when compared to its redox insensitive polymer counterpart, both in vitro and in vivo. Through reversible addition fragmentation chain transfer (RAFT) polymerization, they synthesized two prodrug block copolymers using hydrophilic poly(oligo(ethylene glycol) methacrylate (POEG) with hydrophobic N-methacryloyl-N-(tert-butoxycarbonyl)aminohexyl methacrylamide (MBA, redox-insensitive) or N-methacryloyl-N'-(tert-butoxycarbonyl)cystamine (MBC, redox-sensitive). Dasatinib (Das-an oncogenic tyrosine kinase inhibitor) was then attached covalently to the hydrophobic block of the block copolymers to attain the final redox responsive or non-responsive prodrug polymers (POEG-b-PSSDas and POEG-b-PCCDas respectively). These polymers were then additionally loaded with Doxorubicin to form self-assembling micellar structures with doxorubicin (DOX) in the core and Dasatinib attached to the polymer chains forming a dual drug delivery system. Owing to the high GSH concentration in tumor cells, the DOX loaded POEG-b-PSSDas showed triggered release of both drugs with an enhanced antitumor effect and prolonged survival rate in an aggressive murine breast cancer model (4T1.2) when compared to DOX or DOX+Das loaded POEG-b-PCCDas micelles.

In an interesting example by Tappertzhofen et al.,[42] cationic block copolymers containing disulfide bonds were used to form polyplexes with negatively charged pDNA for gene delivery. A family of cationic block polymers, in this case polylysine-b-p[HPMA] with different ratios of HPMA and lysine, were synthesized using RAFT polymerization, as well as a similar set of polymers with a disulfide bridge between the two blocks, i.e. polylysine-S-S-b-p[HPMA] to endow the carriers with bioreductive responsiveness. Two selected cationic polymers, with and without a disulfide bridge, and with HPMA: Lysine ratio 7:1, were used to form polyplexes with pDNA by physical mixing to give polyplex micelles. Both polymer complexes showed successful internalization by HEK-293T cells, however the transfection abilities of the polymers differed greatly. As a negative control, pDNA alone showed no detectable transfection, as well as a very low transfection efficiency for the polyplex without the disulfide bridge. On the other hand, the pDNA-polymer complex with the disulfide linker mediated a high transfection frequency of the EGFP+ cells in a dose dependent manner without inducing toxicity. Similarly, Tai et al.[43] designed and synthesized arginine, histidine and stearyl containing polypeptides crosslinked via disulfide linkers (SHRss) to act as bioreducible carriers of siRNA. Arginine was chosen for its ionic interactions with the negatively charged RNA, histidine for its ability to act as a proton sponge and raise the pH in the endosome for endosomal escape of the polymer-RNA complex and stearyl moieties for enhanced cellular uptake and endosomal escape. The zeta potential and particle size of SHRss/siRNA complexes were found to be dependent on the N/P ratio, with optimal values for cellular uptake (>200nm, 25mV) noted for N/P values >5. Cellular uptake of the SHRss/siRNA complexes into Luc-HeLa cells was found to be higher than the nonreducible controls, and in Luc-HeLa and mCherry-HEK293 cells, SHRss groups showed higher gene silencing than nonreducible SHR groups at all N/P ratios, which was attributed to the combined effect of the presence of the stearyl and disulfide moieties. Subcellular trafficking and localization of the siRNA in the cytoplasm was demonstrated, with SHRss2/Cy3-siRNA transfected cells showing enhanced endosomal escape

and uniform distribution in the cytoplasm when compared to LF2000-positive vector and nonreducible SHR/siRNA complex controls. In vivo studies showed that the accumulation of SHRss2/Cy5-siRNA complexes within Luc-HeLa xenograft tumors was ~ 6-fold higher than that of Cy5-siRNA treatment, in which the gene silencing effect of redox-responsive SHRss2/siLuc complexes measured by luciferase gene silencing showed weakened tumor luminescence post treatment, while SHRss2/NC-siRNA treatment showed no change.

As well as achieving disulfide bridge reduction, redox conditions can also act as a trigger for other kinds of responsive functional groups or linkers. To this end, Shen et al.[44] recently designed a strategy for combined release of Paclitaxel and Cisplatin from an injectable thermoresponsive hydrogel system. The hydrophobic ends of two diblock mPEG-PLGA (Polyethylene glycol - PEG, Poly(lactic-co-glycolic acid) - PLGA) polymers were covalently linked to a Pt(IV) prodrug, which could be converted to the active cisplatin drug in the reductive intracellular environment. PTX was also encapsulated in the hydrophobic core of the self-assembled core-shell structure of the amphiphilic polymers. Due to the thermoresponsive behaviour, at temperatures greater than 37°C, the system underwent a sol-gel transition, as well as demonstrating efficient tumor inhibition efficacy and sustained release for up to 2.5 months owing to the bioreducible nature of the carrier. A similar approach was described by Xiao et al.[45] in which a Cisplatin(IV) prodrug and Rhodamine B were attached independently to mPEG-b-PCL-b-PLL (PEG-polyethylene glycol, PCL-polycaprolactone, PLL-poly-L-lysine) backbone, and the mixed prodrug micelles could achieve efficient drug release and antitumor efficiency due to the low pH and reductive intracellular environment.
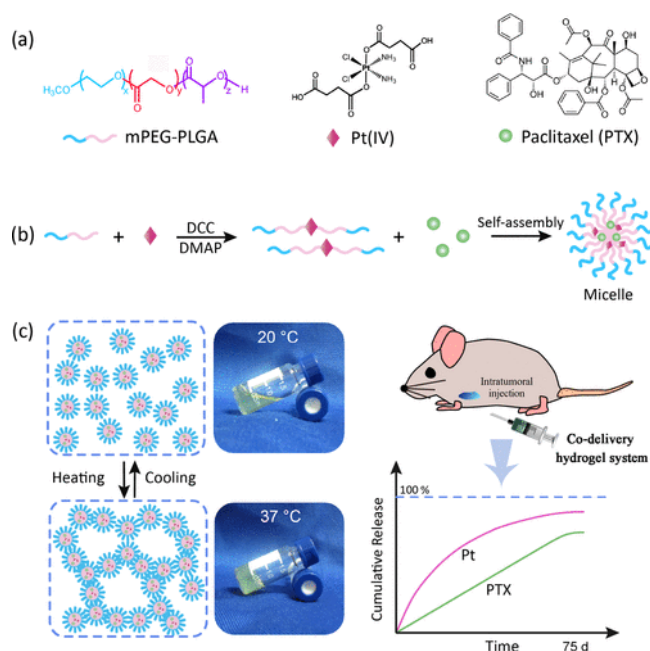
**Figure 4. Design of hydrogel formulation for the combination delivery of cisplatin and PTX. (a) Molecular structures of the mPEG-PLGA diblock copolymer, Pt(IV) prodrug, and PTX. (b) Cartoon representation of mPEG-PLGA–Pt(IV) polymer prodrug conjugate and its self-assembly with PTX. (c) Thermo-responsive sol-gel transition by the polymer-prodrug conjugate loaded with PTX and redox sensitive co-delivery of PTX and cisplatin by the drug carrier gel. Reprinted with permission from ref 44. Copyright 2017 American Chemical Society.**

Finally, it is worth mentioning an example of a bioreducible carrier featuring diselenium bonds to achieve redox-responsive drug delivery. The Se-Se bond has a lower bond disassociation energy (172 kJ/mol) compared to that of the S-S link (240kJ/mol), and thus if properly shielded against nonspecific release in non-reductive conditions, the diselenide linker has the potential for more efficient and rapid drug delivery in cancer cells.[46] In this context, Wei et al.[47] recently demonstrated that a diselenide appended poly(ester urethane) triblock copolymer (PAUR-SeSe) was able to release more drug when compared to the S-S containing poly(ester urethane) triblock copolymer (PAUR-S-S). Under similar GSH concentrations (30 mM), PAUR-SeSe micelles released 85 % of the encapsulated DOX, whereas 67 % of the drug was released by PAUR-S-S micelles in 48 hours. In subsequent in vitro experiments, HN30 cells were treated with free DOX (negative control), PAUR-S-S-DOX (positive control) and PAUR-SeSe-DOX, and both micelles showed enhanced anticancer efficiency when compared to free DOX. Furthermore, DOX loaded PAUR-SeSe demonstrated six-fold higher antitumor effect than the S-S analogue, due to the faster cleavage and enhanced drug release of the diselenium

bond in the reductive intracellular environment. While this example is most correctly described as a system in which the drug is encapsulated rather than covalently bound as a pro-drug, it nevertheless has strong analogies to polymer pro-drug strategies in that the overall macromolecular carrier uses diselenium bonds in a stimulus-responsive manner, but more extensive in vivo characterization will be needed before these chemistries can be considered for possible clinical use.

**Stimuli responsive self-immolative materials**

An emerging area of materials research involves the use of endogenous and /or exogenous stimuli to catalyze the controlled deconstruction of macromolecular structures and in so doing, to accelerate drug release.[48] These so-called 'self-immolative' materials, usually designed around dendrimer, oligomer and linear polymer structures, are encoded to respond to external stimuli through the cleavage of a trigger, which leads to a cascade of intramolecular chemical reactions resulting finally in the complete degradation of the polymer into small molecules which are easily cleared. In this section we report some of the most recent proof-of-concept studies highlighting the potential of self-immolative materials for drug delivery and other biomedical applications. For further discussions of the concepts underlying self-immolative materials, including aspects such as strategies for their synthesis, architectures, chemical nature of the linkers and deconstruction profiles, we refer the reader to more specialist reviews.[14g, 14i, 48-49]

Azobenzene derivatives have been intensely exploited in the context of stimuli-responsive materials for their reversible trans-cis photo-isomerization that occurs upon exposure to UV-vis light. However, it been demonstrated only recently[50] that in addition to the tendency to photo-isomerize, azobenzene derivatives can also undergo chemical reduction with subsequent 1,6-elimination, triggering the de-polymerization of a self-immolative polymer.[51] Eom et al.[52] designed a graft copolymer bearing an azobenzene motif for the redox-activated delivery of

DOX in the colon. In this way, they could also exploit the fact that azobenzene moieties can be cleaved by azo-reductase enzymes present in the intestinal microbial flora. The copolymer was prepared through atom transfer radical polymerization, and was initially of neutral charge, however, upon redox-azobenzene-cleavage, a cascade of events released a free ammonium cation on the polymeric side chains. Once activated, the positively charged DOX-polymer conjugate could be taken up by HT-29 cells, and showed comparable cytotoxicity to the free drug, while the neutral non-activated copolymer did not show any appreciable toxicity. It is important to note that the azo-bond can be also reduced/cleaved under hypoxia conditions, which further opens up new avenues for the development of drug delivery systems.

Xie et al.[53] developed a simple and elegant self-immolative polymeric nanoparticle system to act as a dual-function vector for delivery of therapeutic miRNA and targeting of dysregulated polyamine metabolism in cancer. The nanoparticles were based on a biodegradable polycation prodrug, and included GSH-reducible disulfide bridges within the backbone of the disulfide-bis(ethylnorspermine) (DSS-BEN) polymer, which were able to induce selective triggering of nanoparticle degradation within the cytoplasm of cells. The nanoparticles were able to demonstrate effective miRNA release and depletion of natural cellular polyamine levels. Finally, the concomitant miR-34a expression and polyamine metabolism regulation demonstrated enhanced cell killing in vitro in HCT116 human cancer cells, as well as superior antitumor activity in an in vivo tumor model.
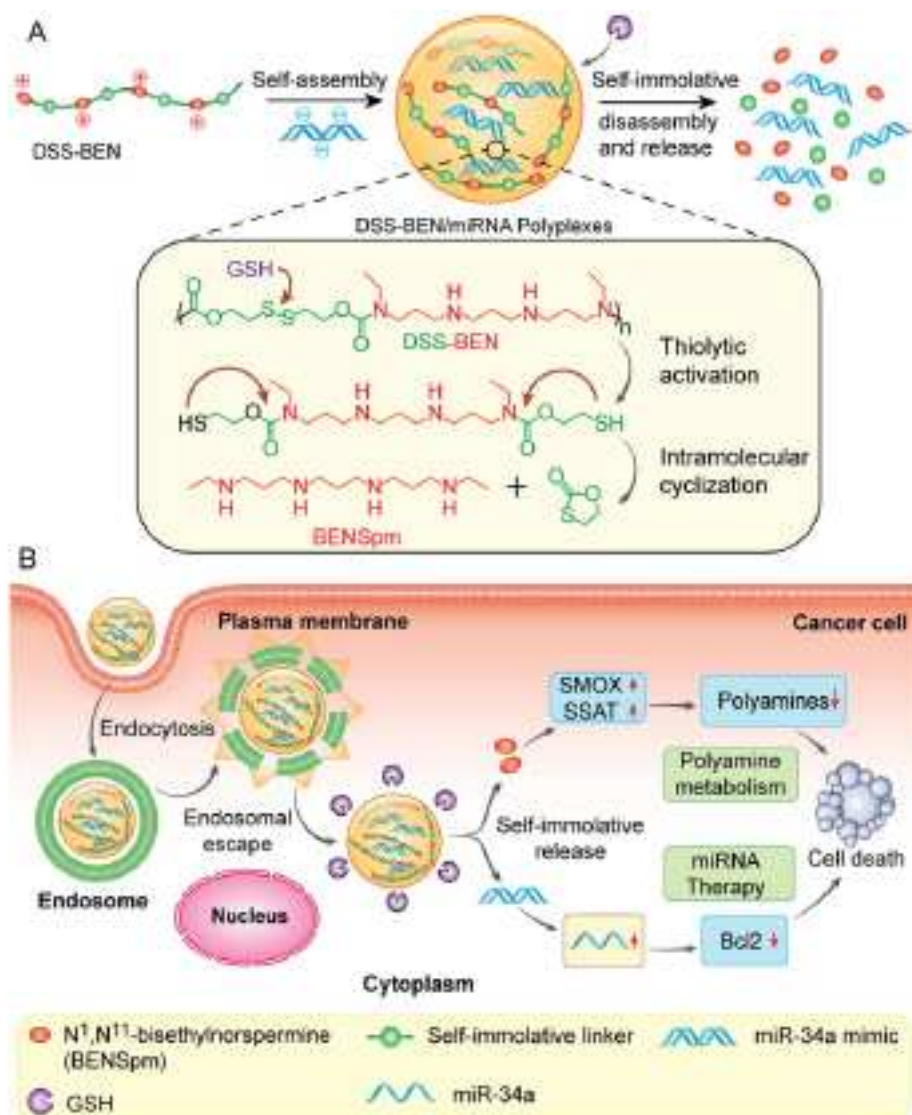
**Figure 5. Mechanism of action of disulfide-bis(ethylnorspermine) (DSS-BEN)/miR-34a nanoparticles. (A) DSS-BEN condenses miRNA into nanoparticles by electrostatic interactions. (only linear form of DSS-BEN is shown but branched forms are also present) (B) Upon endocytosis and endosomal escape, the particles are subjected to cytoplasmic reduction by GSH, followed by disassembly and release of both BENSpm and miR-34a mimic. BENSpm induces expression of enzymes involved in polyamine catabolism, which reduces intracellular polyamine levels. MiR-34a mimic increases cellular miR-34a levels, which leads to Bcl-2 downregulation. Reprinted with permission from ref 53. Copyright 2017 Elsevier.**

A novel biodegradable polyurethane bearing a pendant redox-trigger (p-nitrobenzyl alcohol/ p-NBA), a 1,5-cyclisation spacer (N-2-(hydroxyethyl)ethylene diamine) and a self-immolative linker (p-hydroxylbenzyl alcohol) was prepared by C.-H. Whang and collaborators.[54] The incorporation of p-NBA in the polyurethane backbone allows for potential enzymatic reduction, such as by nitroreductases expressed in pathological bacteria, as well as demonstrating an alternative functionality for redox degradation rather than the typical disulfide bridges. Self-immolative polymer degradation through alternating 1,6-elimination decarboxylation and 1,5-

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intramolecular cyclisation was assessed, as well as reductive triggered release of paclitaxel from the nanoparticles. The redox-triggered polymer disassembly enabled paclitaxel release three times faster than that of the untreated nanoparticles.

The stimuli-responsive self-immolative polymer concept has been used to develop sacrificial layer materials for the controlled release of actives in the presence of triggering conditions.[55] Ergene et al.[56] reported the first example of a self-immolative polycation exerting a fast and broad-spectrum antimicrobial activity. The polycation comprised cysteamine-functionalized poly (benzyl ethers) with silyl end-capping, and retained antibacterial activity upon depolymerization after fluorine specific treatment. The unzipping of the polymeric backbone released molecules with higher solubility and lower hemolytic toxicity compared to the original polymer. Han et al.[57] described the first example of self-immolative electrospun nanofiber membranes based on a self-immolative polymer and polyacrylonitrile, which showed depolymerization 25 times faster than that of a cast film of the same blend. This led to a drastic change in surface properties from highly hydrophobic (110°) to hydrophilic (0°). The same blend was also coaxially electrospun in the presence of polyvinylpyrrolidone/dye as a loaded core, in order to assess the triggered release of the model dye. The nanofibers showed almost no release of the encapsulated material in non-triggering conditions, while immediate dye release was observed in triggering solutions. This approach can be considered as a proof-of-concept for the customisation of on-demand release of components embedded into fibers.

### Combination therapies

In order to enhance the performance of stimuli-responsive prodrugs, it has increasingly become common to design systems that respond to more than one stimuli, resulting in an enhancement of efficacy or a construct that responds over a range of varying conditions. While drug delivery through exogenous stimuli was not covered in the present review, the examples discussed below introduce the potential for combination approaches utilizing either two endogenous stimuli, or a combination of endogenous and exogenous stimuli to improve treatment efficacy.

In one example, Liu et al.[58] prepared a multifunctional prodrug comprising gemcitabine conjugated to a photosensitizer, meso-tetraphenylporphyrin (TPP) through a reactive oxygen species cleavable thioketal linker. Upon irradiation by red light, the TPP generated singlet oxygen species, which not only caused cell damage directly but also which was able to cleave the ROS-responsive thioketal linkage of the prodrug, resulting in gemcitabine release and further enhanced cell damage. This approach thus demonstrated the feasibility of a multifunctional prodrug with on-demand remote spatiotemporal control of drug release. While increased levels of intracellular ROS are typical for cancer cells, this external approach reduces the chance of biological variability, and generates a more effective concentration of ROS. A similar approach was demonstrated by Zhou et al.,[59] who developed a ROS-responsive prodrug vesicles for on-demand delivery of doxorubicin in Triple Negative Breast Cancer. Their vesicles were assembled from an ROS-activatable Dox prodrug, a poly(ethylene glycol) (PEG)-modified photosensitizer pyropheophorbide-a (PPa), an unsaturated phospholipid and cholesterol. Upon laser irradiation, ROS were generated within the vesicles, thereby activating the DOX prodrug through cleavage of a thioketal spacer, resulting in delivery at the tumor site for combined local-regional chemotherapy and PDT. Additionally, the produced ROS could oxidize the unsaturated lipids, causing an increase in the permeability of the lipid membrane, triggering ultrafast release of the drug.

Bio et al.[60] implemented a combined PDT/chemotherapy approach for the delivery of an aminoacrylate prodrug of combretastatin A-4 (CA4) to tumor cells. Far-red light, such as that utilized during PDT, was used activate the photosensitizer phthalocyanine to produce a high local concentration of singlet oxygen species, which subsequently cleaved the aminoacrylate prodrug linker to locally release CA4.[61] The released CA4 resulted in toxic effects on cancer cells both in vitro and in vivo in tumor models, in comparison to a non-degradable prodrug control that showed no drug release or toxicity in vivo.

In an example of a combination approach using two endogenous stimuli, Gu and co-workers developed a nanocarrier carrying a prodrug of the anticancer drug SN38 that was responsive to both a reducing and oxidative environment.[62] The rationale for this work was the inherent heterogeneity characteristic of many tumor types, resulting in potential areas of enhanced intracellular glutathione (GSH) co-existing with regions of overproduced ROS, either through different tumors, different regions within a tumor, or even one tumor cell at different stages.[63] Based on this, they designed their nanocarrier to contain a prodrug of the phenol ester of SN38 conjugated to an oligomeric ethylene glycol carrier through a thioether-ester moiety. In a hydrophobic environment, the phenol ester was designed to remain stable, however in a hydrophilic environment, the phenol ester was intended to cleave rapidly, initiated through ROS-mediated oxidation of the thioether to a hydrophilic sulfone or sulfoxide. Gu et al observed that the phenol ester could undergo glutathione-triggered thiolysis, and therefore their system was able to decompose and quickly release the drug when triggered by either GSH or ROS, or the two in combination. Their nanocarrier successfully released SN38 in in vitro assays, and further showed efficacy in in vivo tumor model, where mice implanted with xenograft breast tumors showed significantly higher SN38 concentration in tumor tissues compared to control. Further in vivo experiments in a colorectal model showed significantly improved survival rates and tumor growth inhibition for the mice receiving the degradable prodrug treatment compared to free drug.

Zhang et al.[64] synthesized a novel drug-delivery enzyme and redox dual-responsive polymeric nanocarrier platform with active targeting abilities, in order to achieve rapid intracellular cargo release for cancer treatment (see Figure 6). The dual-responsive targeting polymeric micelles were produced through the self-assembly of a mixture of two polymeric materials. The first material was a polymeric-prodrug of camptothecin, synthesized through conjugation of the drug (Camptothecin-CPT) to monomethyl poly (ethylene glycol) (PEG) via a redox-responsive linker (s-s) (mPEG-ss-CPT). The second material was an amphiphilic block copolymer

synthesised through the conjugation of a hydrophobic polycaprolactone (PCL) block to a hydrophilic PEG via an azobenzene spacer (Azo) for enzyme-responsiveness. The PEG chains were further decorated with phenylboronic acid (PBA) to provide the final micelles with active targeting features (PBA-PEG-Azo-PCL). In vitro assays in simulated tumor cell microenvironment conditions confirmed that the micelles could be disrupted in the presence of azoreductase, resulting in rapid release of camptothecin. In vivo experiments confirmed that the micelles had enhanced specificity towards subcutaneous hepatoma carcinoma cells through the active targeting, and demonstrated remarkable therapeutic activity to liver H22 tumors with low toxicity to normal tissues, resulting in a survival rate of approximately 100 % after 160 days of treatment.



**Figure 6. Schematic design of enzyme and redox dual-triggered intracellular release from actively targeted polymeric micelles to enhance cancer treatment. Abbreviations: monomethyl poly (ethylene glycol) (PEG), redox-responsive linker (s-s), camptothecins (CPT), polycaprolactone (PCL), azobenzene spacer (Azo) phenylboronic acid (PBA). Reprinted with permission from ref 64. Copyright 2017 American Chemical Society.**

A facile approach for preparing multi-stimuli responsive branched DOX-conjugate-copolymers with high molecular weight (around 165 kDa) was recently reported by Wei et al.[65] The branched DOX-conjugated materials were based on poly N-(2-hydroxylpropyl)methacrylamide), obtained through a one-pot RAFT copolymerization with enzyme-sensitive (papain or cathepsin B) cross-linkers. DOX was coupled to the branched

polymer backbone post-polymerization through pH-sensitive hydrazone bonds, and release of DOX in a pH-dependent manner was observed over a reasonable timeframe from the pre-formed nanoparticles. The self-assembled nanoparticles showed rapid stimuli-responsive breakdown, releasing fragments with low molecular weights that could be easily cleared by the body. The particles were approximately 100 nm in size, with a negative surface charge to ensure good in vivo stability. Finally, the nanoparticles were observed to accumulate in tumor tissue to a higher degree than the free drug control, leading to an enhanced anti-tumor effect against 4T1 tumor models, with no adverse side effects observed in vivo.

Li et al.[66] demonstrated enhanced delivery of PTX to tumor cells using enzyme and redox dual-responsive carriers, by conjugating PTX to hydroxyethyl starch (HES) using a disulfide linker (HES-SS-PTX). The HES-SS-PTX conjugate self-assembled in water to form redox responsive nanoparticles with a diameter of 150nm with ~ 6 % PTX loading. The α-1,4 glycosidic bond of HES was cleavable by α-amylase, endowing the NPs with dual responsiveness. The resulting particles showed an increased half-life and higher accumulation in the tumor site when compared to a commercially available PTX formulation. In a reductive cancer cell environment, cleavage of the disulfide bonds triggered collapse of the nanoparticles and burst release of the drug, while in parallel the HES shell degradation by α-amylase enzymes allowed deeper penetration of the particles into the tumor site. Finally, HES-SS-PTX demonstrated improved in vivo anti-tumor efficacy (64 %) and lower cytotoxicity when compared to Taxol (52 %) in 4T1 tumor- bearing mice.

Mavuso et al.[67] recently described a redox and pH dual-responsive copper-ligand nanoliposome bioactive complex for the treatment of chronic inflammation. Pathological sites are prone to an oxidative imbalance due to formation of ROS and other metabolites, which triggers a counteractive upregulation of antioxidants such as GSH, and thus the authors targeted the presence of upregulated GSH, as well as the low pH in inflamed tissues to achieve drug delivery. For this purpose, Cu(II) was used to link predinosolone succinate (PS) in the presence

of a glyglycine (glygly) ligand to yield a copper ligand bioactive complex [Cu(glygly)(PS)]. This bioactive complex was then loaded into a cystamine-appended cationic Eudragit E100 polymer (EuE100-Cyst) with a phospholipid bilayer to give pH and redox responsive nanolipids (NLs). The [Cu(glygly)(PS)] complex demonstrated improved inflammatory and oxidant inhibitory activity when compared to free PS drug, and the free radical scavenging activity (60 %) and lipoxygenase (LOX-5) inhibitory effect (37 % ) of the complex was also found to be higher than for the free drug (4 % and  6 % respectively). The low pH also had significant impact on the drug release, with > 75 % released at pH 5 in 6 hours, while only 23 % release was recorded at pH 7.4. These results suggest the potential of such pH and redox responsive systems for the treatment of inflammatory conditions.

Self-immolative polymersomes have been produced from the self-assembly in water of copolymers with a hydrophilic block based on poly (N,N-dimethylacrylamide), and a self-immolative hydrophobic polycarbonate block caged with perylen-3-yl, 2-nitrobenzyl or disulfide moieties.[68] Upon removal of the caging moiety triggered by either UV-vis light or reductive stimulus, the block copolymer degraded into water-soluble small molecules. Triggered drug co-release and controllable access toward proton, oxygen, and enzymatic substrates could be achieved for the guest-loaded self-immolative-polymersomes. Logic gate combined applications of triggers (OR, AND and XOR-type) in programmed enzymatic catalysis were also demonstrated.

The Gillies group designed a library of UV, $H_2O_2$ and thiol multi-responsive end-cap linkers, which were subsequently used to prepare a set of amphiphilic poly(ethyl glyoxylate)-poly (ethylene oxide) copolymers.[69] These materials were able to self-assemble into nanoparticles with sizes of less than 100 nm, and their depolymerization could be triggered at concentrations of DTT and $H_2O_2$ less than 0.01 equivalents relative to monomer, confirming an amplified cleavage of the multi-responsive end-cap linkers leading to disruption of the nanoparticles. Finally, doxorubicin, coumarin and Nile Red specific release were modulated by selectively

tuning the external stimulus. The facile variability of the polymer-drug linkers makes these materials appealing as multi-responsive platforms, suggesting further possibilities as their uses as drug-delivery carriers.

**Conclusions**

This review has aimed to cover some of the leading examples of pro-drug chemistries used to endow polymers with controlled and site-specific drug delivery properties. The heterogeneous nature of pathogenic sites with respect to normal cell environments, and with with inherent associated conditions such high reduction potential, hypoxia, low pH, overexpressed enzymes and high ROS, has been the primary inspiration for the design of prodrug systems responsive to these specific changes acting as stimuli. Accordingly, the chemistries that have been shown to be effective for controlled delivery of therapeutics in response to these particular disease environments should be capable of translation across different types of disease. For example, in the treatment of infectious diseases, there are many problems in common with cancer therapies such as systemic exposure of cytotoxic agents, sub-therapeutic dosing in cases of narrow therapeutic window agents and the development of resistance. Polymer therapeutics with site-specific release induced by local biological cues or orthogonal stimuli can therefore be readily used in acute and chronic infections, and some promising examples have recently emerged.[70] Oxidative stress is also an important component of certain cardiovascular disorders, and nanoparticles which are responsive to ROS have shown efficacy in myocardial ischaemia models.[71] It is also worth noting that combination systems, both in terms of combinations of stimuli, and involving combinations of active molecules, show potential in multiple therapeutic areas. Regenerative medicine, in which diverse cell populations require different cytokines and growth factors at different times and in defined spatial regions during growth, is perhaps one of the most exciting areas for responsive polymer pro-drugs. It is our perspective that the advances made in the specific release of drugs as shown in this article will

strongly apply to delivery strategies in fields other than oncology, and we hope that this view encourages researchers into new, and as yet unexplored, clinical challenges.

However, for any clinical applications of polymer pro-drug materials there remain numerous scientific and practical barriers which must be overcome. Safety and efficacy are the most important criteria for any therapeutic and the complexities of polymer therapeutics make the establishment of full safety profiles time-consuming and expensive. For regulatory approval, pro-drugs of any type must be evaluated for all breakdown products, and the pharmacokinetic and pharmacodynamics properties of most synthetic polymers are highly complicated even without consideration of the multiple fragments which can be produced on biodegradation. For orally dosed formulations, the requirements are less severe, as excretion of large molar mass fragments is more rapid, but nevertheless, the quantitative release of drugs from polymer pro-drug carriers still needs to be established, and with multiple drug-linker-carrier breakdown products this adds significant difficulty to a full safety and efficacy study. Potential re-uptake of low molar mass species may occur following oral therapy, and effects on gut microflora may also be significant for the patient's health. The issues for injectable polymer formulations are even more formidable, owing to the requirements for absolute sterility over prolonged storage and administration, multiple organ exposure once injected, and uncertainties in circulatory dynamics for different molar mass fragments and breakdown products. The common assumption that all the low molar mass breakdown products will be rapidly cleared is also questionable for injectable formulations as multiple re-uptake and processing pathways may exist for fatty acid type fragments emerging from polymer degradation. Nevertheless, there is now a considerable clinical database for injected nanomedicines over > 20 years, and a host of powerful new pre-clinical models to evaluate investigational therapeutics. New imaging modalities are enabling the detection of nanomaterials, fragments and biomarkers at ever-increasing sensitivity, and longitudinal studies as well as large-scale genomic data are providing patient information for many more indications beyond oncology

Accordingly, if research into the advanced therapeutics outlined in this article continues to advance, and if the regulatory and complexity issues can be solved at affordable cost, then there are many possibilities for polymer pro-drugs to address successfully some of the most pressing current medical needs.

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Amanda Pearce completed her PhD in 2015 under the supervision of Assoc. Prof. Kristofer Thurecht and Prof. Andrew Whittaker at the University of Queensland, Australia, focusing on the synthesis of functional polymeric materials for cancer therapeutics. She then moved to the group of Prof. Cameron Alexander in 2016 to carry out post-doctoral research as a part of the EPSRC funded 'Next Generation Biomaterials Discovery' grant, where she is furthering her research on the synthesis of polymeric drug delivery carriers, with an interest in how size and architecture affects *in vivo* performance.

Nishant Singh obtained his PhD as a 'Marie Curie Early Stage Researcher' with *Sobresaliente Cum Laude* in 2016 from Universitat Jaume I, Spain under the supervision of Prof. Beatriu Escuder. He moved to the group of Prof. Cameron Alexander as a postdoc in 2017 shifting his focus of research from catalytic self-assembling hydrogels to drug delivery. Currently, as a part of EPSRC funded 'Next Generation Biomaterials Discovery' grant he is working on stimuli responsive drug carriers for treatment of biofilms and related infections

Vincenzo Taresco defended his PhD in 2013 at the University of Rome La Sapienza, with a thesis aimed at the development of different medical-platforms based on novel antimicrobial polymers. From 2014 he has been working at the School of Pharmacy of Nottingham. In this period, he has divided the core of his research in the design and synthesis of (multi) responsive polymers, their use in the development of smart drug/gene delivery carriers and their screening through automated high-throughput methodologies

Cameron Alexander is Professor of Polymer Therapeutics, an Engineering and Physical Sciences Research Council (EPSRC) Impact Fellow and a Royal Society Wolfson Research Merit Fellow at the School of Pharmacy, University of Nottingham, UK.

Professor Alexander received degrees (BSc, PhD) in Chemistry from the University of Durham, UK, and carried out post-doctoral research at the Melville Laboratory for Polymer Synthesis, University of Cambridge. He is a Fellow of the Royal Society of Chemistry, a recent (2009-2014) EPSRC Leadership Fellow, and has published nearly 200 refereed articles. He received the Royal Society of Chemistry Macro Group Medal in 2014 for contributions to polymer science. Professor Alexander has been fortunate to work with scientists from more than 20 countries in his research group.

**Table of contents entry**

**The delivery of active agents to target regions in the body can be addressed by exploiting differences in biology and physiology at disease sites.** This review evaluates some of the most significant advances in pro-drug chemistries used in polymer therapeutics.

**Keywords -** disease targeting, drug delivery, responsive materials, pro-drugs, polymer therapeutics

Vincenzo Taresco, Cameron Alexander, Nishant Singh* and Amanda K. Pearce*.

**Title: Stimuli-responsive pro-drug chemistries for drug delivery**

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