Chapter 2

Literature Review

2 Literature Review

Biopolymer-based nanomedicine besides the capability to exploit EPR, primarily utilizes either one or both of the following strategies to overcome the challenges of conventional therapy: Targeting the tumor microenvironment markers or cell-surface receptors on the cancer cells to deliver the therapeutics specifically to tumor sites and/or use of tumor microenvironment. The former exploits the over-expressed cell surface receptors on the cancer cells and abnormal cellular components of the tumor microenvironment to deliver the therapeutics to the targeted region actively. The latter uses tumor microenvironment stimuli like lower pH, presence of reactive oxygen species, redox imbalance, hypoxic conditions, enzymatic imbalance, etc., to selectively release the therapeutic payload, consequently enhancing the treatment's effectiveness while minimizing side effects. Also, mechanisms such as size shrinkage/expansion capability and surface charge switching behavior can be utilized to enhance tumor site accumulation. Amalgamating the aforementioned functional characteristics holds immense potential in improving the treatment efficacy, reducing systemic toxicity, achieving precise delivery, and overcoming treatment resistance by targeting microenvironment components promoting resistance.

Biopolymer-based TME-responsive nanomedicine provides numerous advantages like the use of biopolymers with tumor-responsive bonds results in safe, stable, biocompatible, and biodegradable nanocarriers with high tumor-targeting potential. In addition, it also allows integration with diagnostic components for real-time monitoring and delivery of multimodal therapeutics (chemotherapeutics, immunotherapy, photothermal agents, etc.,) specifically to the targeted region, forming a more comprehensive treatment approach. Therefore, biopolymer-based TME-responsive nanomedicine provides a universal approach for the design of safe and more effective drug delivery systems for precise cancer therapy.

2.1 Biopolymers with pH-responsive cleavage bonds

Biopolymers having an acid-cleavable linker may be hydrazone [1], imine [2], acetal/ketal [3], cis-acotinyl, orthoester, *β*-amino ester, or others can be used for the preparation of pH-responsive nanomedicine, where drug release is observed in acidic tumor microenvironment due to bond cleavage and subsequent degradation of nanoparticulate assembly (**Table 2.1**).

Ali Mohammadi et al., 2021 reported the use of dimethyl acrylamide-trimethyl chitosan, a modified polysaccharide of chitosan with imine linkage for the preparation of biocompatible, targeted, and pH-sensitive 60-carbon modified fullerenes (C60). C60 was capable of loading drug (DOX/PTX), delivery it tumor site and releasing in the acidic pH of cancerous tissue. The PAX/DOX-loaded nanomaterials protected the loaded drugs during circulation, and after reaching the target site, nanomaterials enter the cell through clathrin-mediated endocytosis where the drug is immediately released due to degradation or disassembly of a nanomaterial at acidic pH through the cleavage of imine bonds **(Figure 2.1)** [2].

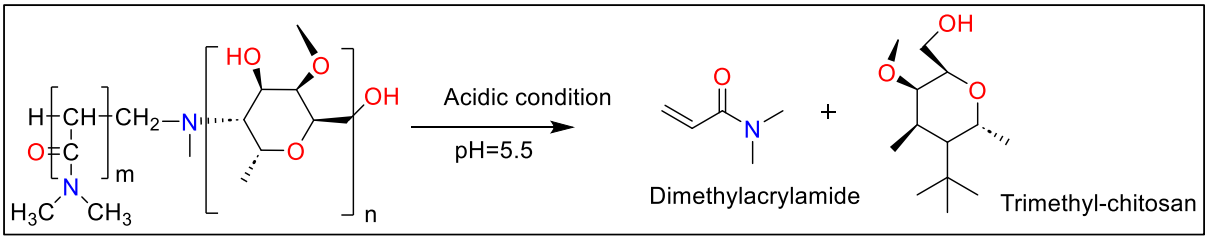

Figure 2.1 Mechanism of pH-responsive behavior of Dimethylacrylamide-Trimethyl chitosan; At acidic pH, DMMA-TCM exhibits imine bond cleavage resulting in dissociation of dimethylacrylamide and Trimethyl-chitosan [2]**.**

Manchun et al., 2015 prepared pH-responsive nanogels using dextrin for tumor-specific DOX delivery to colorectal cancer. Here, dextrin chains having were cross-linked by aldehyde cross-linkers to form a pH-sensitive acetal bond, due to reaction between

hydroxyl groups of dextrin molecules and carbonyl groups of glyoxal or formaldehyde. Prepared nanogel was found to significantly increase the DOX release in acidic pHs compared to physiological pH due to the hydrolysis of acetal bonds in acidic conditions, and subsequent destabilization of the structural integrity of nanosystems. Therefore, such a system can be utilized for drug release in acidic tumor microenvironments with pH 6.8 or at endosomal pH 5.0 [3].

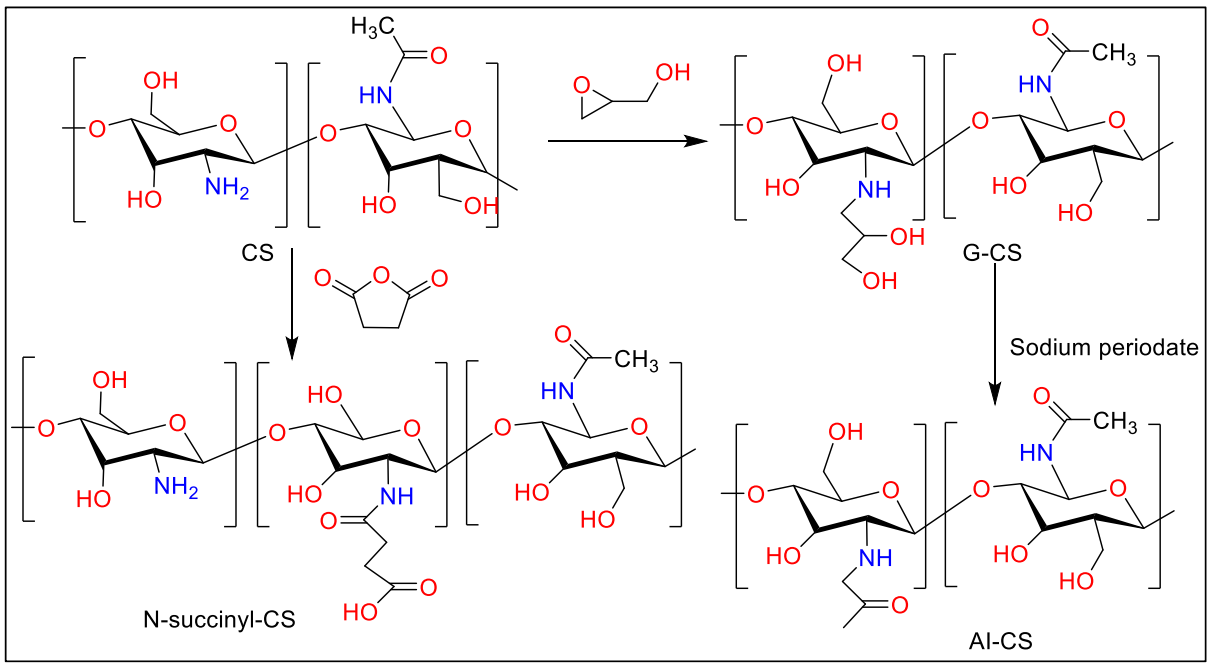

Figure 2.2 Synthetic route of N-succinyl-CS and Al-CS [4].

Chitosan can be modified to improve the properties of chitosan. N-succinyl-CS was prepared by using succinic anhydride to improve the solubility and mucoadhesive property while aldehyde-functionalized CS was obtained due to reaction of amine groups of chitosan with epoxide of glycidol to form chitosan with hydroxyl groups, further oxidized with sodium periodate to form aldehyde-CS **(Figure 2.2)**. Modified chitosan functionalized with free amine and aldehyde groups crosslinked to form injectable hydrogel via Schiff-base chemistry. The resultant system exhibited an acid-labile imine bond that cleaves via hydrolysis to degrade and release the loaded DOX at low pH

environments. The system was biocompatible and biodegradable. The degradation and swelling studies showed that the system highly pH-responsive, which could degrade faster at low pH [4].

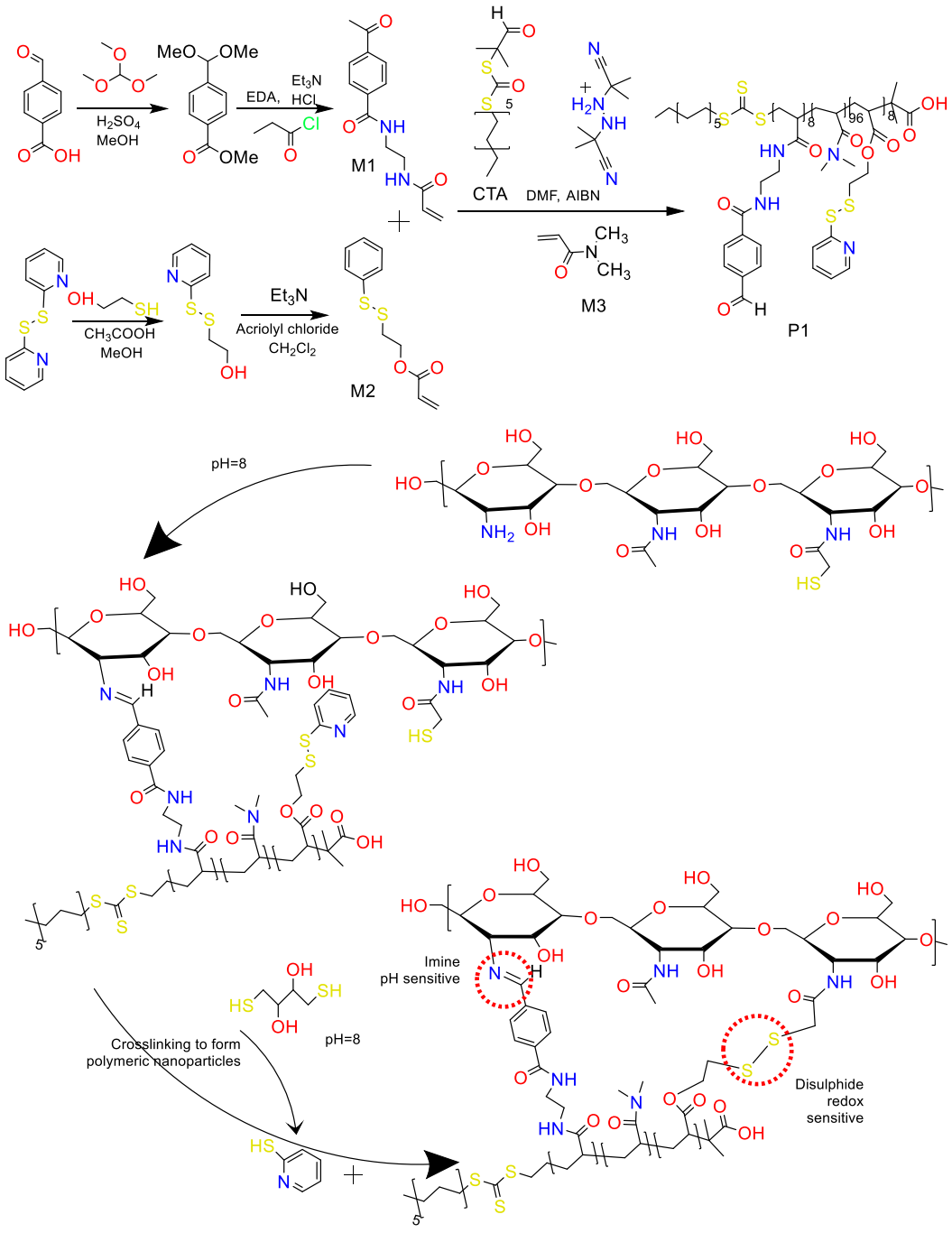

Figure 2.3 Synthesis of dual pH and redox-sensitive acrylic polymer conjugated thiolated chitosan [5].

Palacio et al., 2017 reported the use of a natural biopolymer, thiolated chitosan linked to acrylic polymer with imine and disulfide bonds for the preparation of stimuli-sensitive nanocarriers. Here, the primary amine groups on thiolated chitosan were coupled with aldehyde groups present in the pendant chains of the acrylic polymer to form the pH-sensitive imine bond **(Figure 2.3)**. Nanoparticles prepared using this polymer were of size around 136 nm. The imine bonds in nanoparticles were stable at normal physiological pH but easily broken at pH values around 5.5 commonly found in cancer cells. Also, the thiols groups of the polymer were linked with thiolated chitosan to form the disulfide bond. The nanoparticles prepared were of particle size of 85nm, studied for the effect of exposure to redox potential commonly found inside cancer cells. The stimuli resulted in the disassembly of the nanostructure [5]. ZnO quantum dots can also be used to develop pH-responsive nanomedicine. ZnO QDs are stable at physiological pH of 7.4 but rapidly break down into zinc ions at pH 5.5, triggering drug release in malignant cells and thereby minimizing premature drug release during storage and transit to the target location [6]**.**

2.2 Biopolymers with pH-responsive protonated chemical groups

The biopolymers used for such acidic responsiveness can be anionic or cationic. Gelatin is a peptide-based animal collagen derivative biopolymer that has numerous carboxyl, amine, and amide functional groups. Such functionality allows the gelatin to exhibit a pH-dependent cationic or anionic behavior, making it an ideal biopolymer for drug delivery application for pH-responsive controlled drug release [7]. Similarly, other biopolymers with such acid-sensitive behaviors in the tumor microenvironment may be used for the preparation of site-specific drug delivery systems for efficient cancer therapy.

2.3 Biopolymers with Cleavable ROS responsive bond

The level of H_2O_2 and GSHs is about a quarter fold higher in cancer cells compared to normal cells. Therefore, biopolymers with ROS/ GSH responsive moieties such as

disulfide [8], diselenide [9], ditelluride, thioether [10], thioacetal/thioketal [11,12], boronic acid/ester, can be used to design the ROS responsive DDS. These ROS-responsive polymers undergo accelerated carrier degradation and subsequent drug release in cancer cells with elevated H_2O_2/GSH levels, [9]. Thioacetals/thioketals, boronic acids, and oxalate esters undergo oxidative bond cleavage, while thioethers and selenoethers show transformation reaction to form sulfoxides/sulfones and selenoxides or selenones. Oxidative cleavage of thioacetals/thioketals may involve a substitution reaction, where ROS generated nucleophilic oxyanion sequentially substitutes thioether moieties [11].

Keratin is a redox-sensitive polymer with disulfide linkages that may be utilized to produce stimuli-sensitive nanoparticles. Keratin is a naturally occurring protein high in lysine and arginine that may be cleaved by trypsin protease, which is overexpressed in tumor tissues. Keratin-based nanoparticles have been found to have strong drug loading potential and may be employed for effective and safe delivery of loaded payload in tumor tissues via disulfide bond breaking. Ghaffari et al., 2018 used Pluronic and natural polymer keratin to create temperature and redox-sensitive nanoparticles loaded with curcumin. Prepared nanoparticles were discovered to influence drug release, with enhanced drug release up to nine times faster in the presence of both GSH and trypsin than in the control sample. [13]. Cheewatanakornkool et al., 2017 conjugated biopolymer thiolated-pectin to doxorubicin via reducible disulfide bonds. Drug delivery systems based on such biopolymer–drug conjugates have shown reduced responsiveness in tumor surroundings, which may be uncoupled by disulfide linker cleavage to release the DOX [8].

Jia et al., 2021 developed an incredibly sensitive reactive oxygen species (ROS)-responsive polymer (PCP) for the encapsulation of doxorubicin (DOX) and purpurin 18 (P18) to achieve photodynamic and chemotherapeutic synergy. To create MPEG-CD,

mono(6-amino-6-deoxy)-cyclodextrin and hydrophilic poly-mPEG-NHS are first polymerized. The MPEG-CD was then reacted with the molecule PHB-CDI, which had a borate bond, to produce the ROS responsive MPEG-CD-PHB (PCP). Prepared PCP-based ROS-sensitive micelles released loaded DOX and P18 after accumulation into tumor cells via increased permeability and retention (EPR) effect and endocytosis into the cell. The breakage of the borate link in PDP micelles in response to the elevated H_2O_2 concentration in the tumor microenvironment caused the release. PDP micelles were shown to have great stability, strong biocompatibility, quick phagocytosis, effective tumor permeability, remarkable biological safety, stimulus-responsive release, great anti-tumor activity, and little systemic toxicity [14].

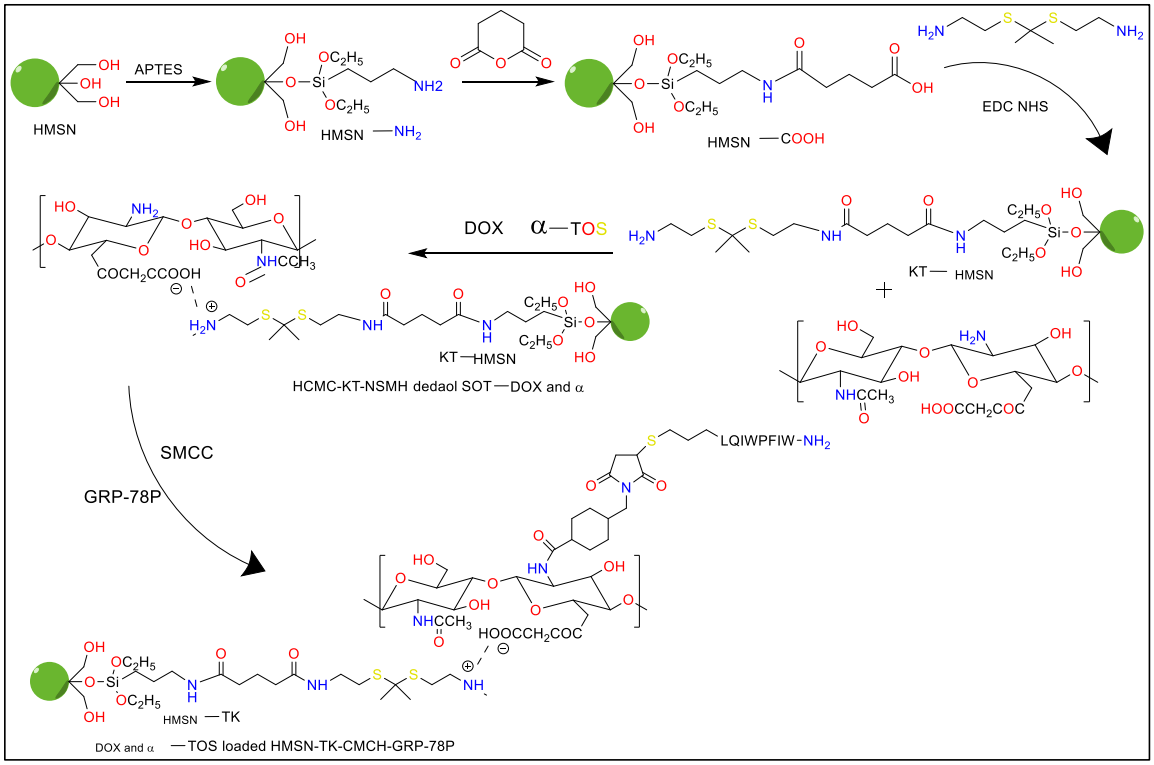

Figure 2.4 Synthesis of pH and redox-sensitive DOX/α-TOS-HMSN-TK-CMCH-GRP78P [15].

Soluble carboxymethyl chitin (CMCH) owing to good solubility, pH sensitivity, and gel-forming behavior was used by Ding et al., 2020 as a pH-sensitive shield for HMSN

nanocarriers encapsulating DOX and α-TOS, chemically modified with ROS-cleavable TK linker, and surface-anchored with GRP78P to give DOX/α-TOS-HMSN-TK-CMCH-GRP78P **(Figure 2.4)**. The more rapid release in acidic pH might be due to the protonation of carboxylic groups in HMSN-TKCMCH-GRP78P, which in turn change the zeta potential of electrostatically coated CMCH, resulting in the de-shelling of CMCH to promote the DOX diffusion from nanocarriers. The high H_2O_2 concentration further increased the DOX release due to the cleavage of TK bonds that results in CMCH peeling off and increased the release rate [15].

2.4 Biopolymers with amphiphilicity transition-ROS responsive bond

Biopolymers with hydrophobic mono-sulfide [16], selenium [17], tellurium [18], or ferrocene [19], may be used to formulate ROS responsive nanosystems. These may undergo a hydrophobic-to-hydrophilic transition in response to H_2O_2 to form the hydrophilic oxidative products. For example, hydrophobic mono-selenide can be converted to the relatively hydrophilic selenoxide or selenone under oxidative conditions [20]. Similarly, oxidation of tellurium in hyperbranched polymer to the higher oxidation states under the biologically relevant concentration of H_2O_2, and resulted in disruption of the amphiphilicity [18].

The destroyed balanced amphiphilicity of nanomedicine, in turn, leads to structural disassembly and, thus, subsequent drug release in TME. The decrease in size of nanoparticles may also be related to amphiphilicity transition in the presence of H_2O_2 stimuli, like hydrophobic sulfide to hydrophilic sulfoxide/sulfone transformation for size-switchable behavior of nanoparticles [16]. Yang et al., 2020 reported the synthesis of an amphiphilic polymer (ChS-g-PPS) based on chondroitin sulfate (ChS) with hydrophobic poly (propylene sulfide) (PPS) as the ROS-responsive moiety. The synthesis methods of ChS-g-PPS were based on EDC/NHS chemistry (for ChS derivatization), ring-opening

polymerization (for PPS copolymer), and thiol-ene click chemistry (for PPS grafting) **(Figure 2.5)**. The synthesized amphiphilic polymer can be used for developing ROS-triggered self-destructive NPs. H2O2 treatment caused the ChS-g-PPS NP suspension to completely clear, suggesting thioether oxidation. Also, the NPs exhibited size reduction on H2O2 treatment indicating superior hydrophilicity in H2O2 presence, as the peroxide can transform hydrophobic sulfide to hydrophilic sulfoxide/sulfone. The disassembly rate of ChS-g-PPS NP was also positively correlated to H2O2 concentration [16].

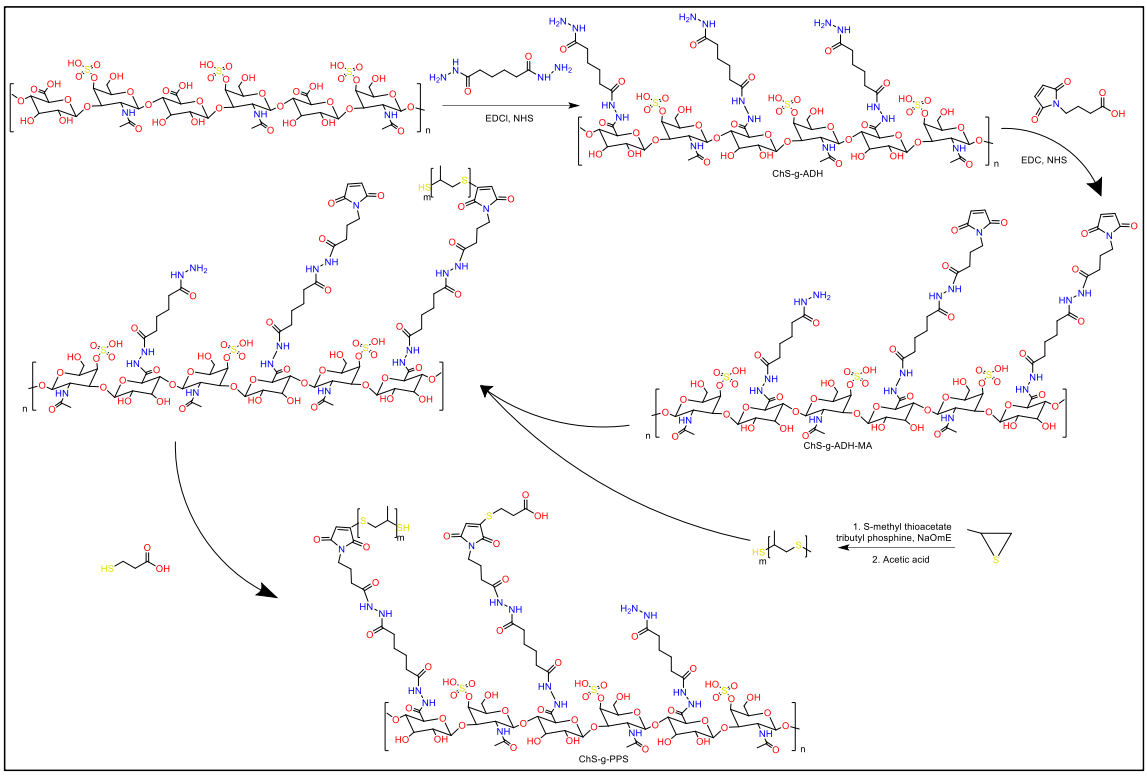

Figure 2.5 Synthesis of ROS-sensitive amphiphilic Poly(propylene sulfide) grafted Chondroitin sulfate (ChS) polymer ChS-g-PPS [16]**.**

Kim et al., 2019 also reported ROS-responsive thioether group-containing polymers as drug carriers for intracellular delivery of hydrophobic piperlongumine in cancer cells. After internalization into cancer cells, phase transformation of polymer-nanoparticles following exposure to ROS was observed owing to the oxidation of the hydrophobic sulfide groups to hydrophilic sulfoxide or sulfone moieties **(Figure 2.6)**. In response to

high levels of intracellular ROS in cancer cells, such hydrophobic-to-hydrophilic transition led to the rapid disintegration of the NPs and hence the subsequent release of the encapsulated drug into the cytoplasm [10].

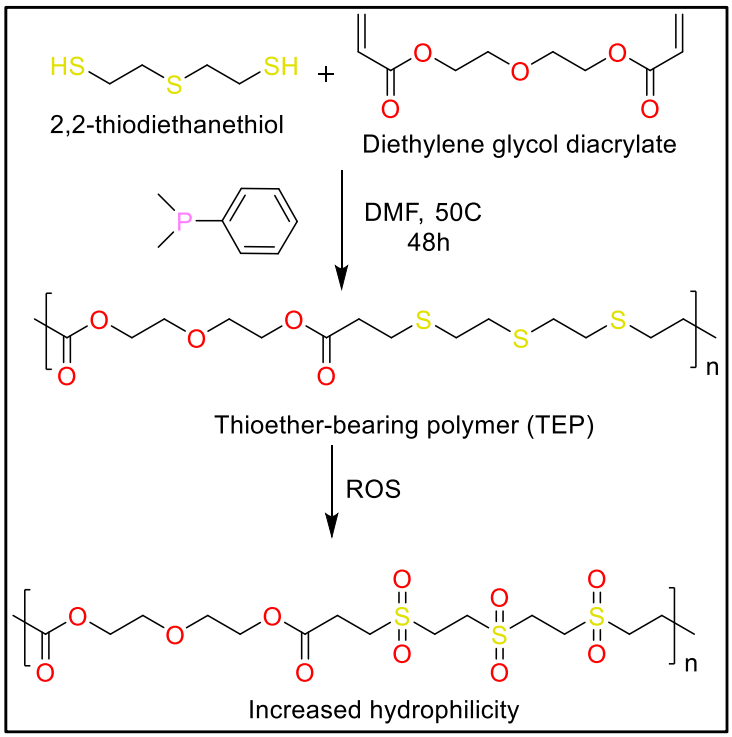

Figure 2.6 Synthesis of ROS-sensitive thioether-bearing polymer and mechanism of ROS mediated increased hydrophilicity [10]**.**

The modified biopolymers can also be synthesized for targeted and stimuli-responsive drug delivery. Oligomeric hyaluronic acid 2′-[propane-2,2-diyllbls(thio)] di-acetic acl-hydroxymethyl ferrocene, named HASF **(Figure 2.7)**, was synthesized for the preparation of novel dual ROS-sensitive (Thioketal linkages and Ferrocene) and CD44 receptors targeting amphiphilic carrier material. Here, the cleavage of thioketone bond due to 2′-[propane-2,2-diylbis (thio)] diacetic acl (TKL) and hydrophobic-hydrophilic transition of hydroxymethyl ferrocene resulted in the stimuli-responsive drug release at the target site. The size of drug-loaded nanocarriers based on synthesized HASF (HASF@Cur micelles) was of average size 150.8 nm with zeta potential, EE%, and DL% of -35.04 mV, 51.41 %,

and 4.95 %, respectively. The release rate of Cur was related to the concentration of H2O2 where the increase in H2O2 concentration, further increased the release rate of loaded drug [21].

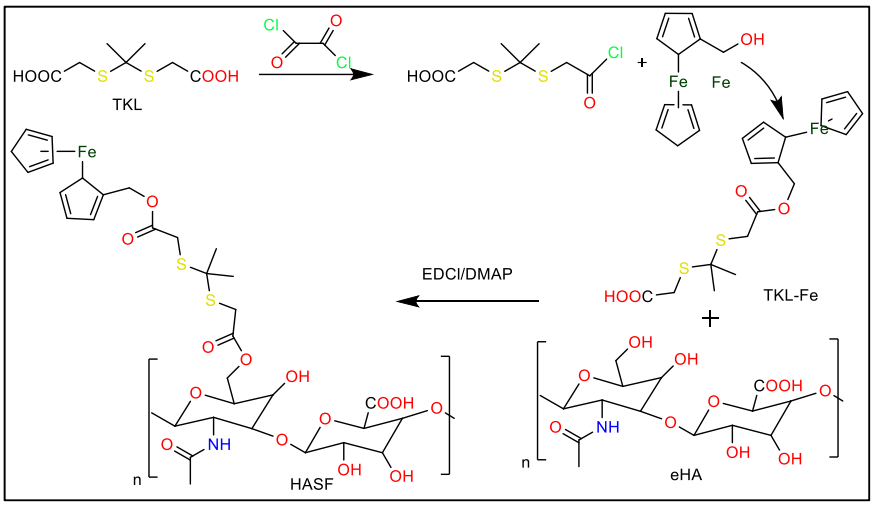

Figure 2.7 Synthesis of redox-sensitive and CD44 receptor-targeted HASF polymer [21]**.**

2.5 Biopolymers with enzyme responsive bonds

In the tumor microenvironment, tumor cells exhibit aberrant production of enzymes such as matrix metalloproteinases, cathepsins, phospholipases, oxidoreductases, and others. These enzymes can help nanoparticles detect tumor tissues more precisely, allowing for higher accumulation at primary tumor locations, where these enzymes exhibit catalytic activity for the enzymatic destruction of biomaterials containing enzyme responsive groups. Specific drug release, in turn, improves therapeutic efficacy while lowering systemic toxicity. Therefore, stimulus-responsive nanoscale delivery devices are a viable anticancer method for cancer therapy [22]**.** Huang et al., 2019 confirmed the increased expression of heparanase-1 in the tumor microenvironment. This enzyme can break down heparin, a biomaterial with high biocompatibility. Therefore, heparin-based enzyme-responsive nanoparticles may be designed for tumor subtypes with high heparanase-1 expression [23]. Over-expressed hyaluronidase (Hyal-1) in the tumor microenvironment might easily break down HA into low molecular weight fragments. As a way, HA might

also be employed as an enzyme-responsive group for drug release in tumors while limiting premature drug release in a physiological environment [6]**.** Hyaluronic acid (HA) is a polysaccharide that endows the HA-based nanosystems the stability, biocompatibility, and cell targeting capability. It provides selected delivery to cancer cells via CD44 receptor-mediated uptake and releases the drug inside the cancer cell on degradation by intracellular lysosomal hyaluronidase in cancer cells. Liu et al., 2016 reported the use of HA for the preparation of enzyme responsive nanosystems which improved the targeting and provided accelerated cytoplasmic drug release in cells. This significantly increased the therapeutic potency and specificity against cancer cells [24].

Xue et al. 2015 employed gelatin-based DDS for enzyme-responsive doxorubicin drug release in tumor tissues. The carriers were stable in physiological conditions and release drugs into the tumor microenvironment owing to gelatin hydrolysis in the presence of gelatinase, an endogenous proteolytic enzyme that is 7 to 8 times more abundant in tumor tissues than in normal tissues. Such enzyme-responsive drug release into tumors prevents non-specific burst drug release at non-target sites, resulting in tumor-specific drug release via increased permeability and retention (EPR) followed by enzymatic destruction of nanocarriers [25]. Chitin gel may be made by combining chitosan solution with acetic anhydride, which degrades in biological systems by lysozyme and fetal bovine serum [26]**.** Hydrophobic dodecyl glycidyl modified alginate (HMA) may self-assemble into micelles. A drug release study revealed that DOX was released at a faster rate in the tumor area. The release rate was considerably increased in the presence of Alpha-L-fucosidase, a lysosomal enzyme overexpressed in various cancerous tissues, notably hepatocellular carcinoma. In acid tumor microenvironments, the hydrophilicity of HMA may also be diminished due to protonation of most carboxyl groups in the HMA backbone chain and

stretching between the HMA and DOX increases, resulting in somewhat faster drug release. [27]**.**

Wu et al., 2019 described a zwitterionic stealth peptide (cell-penetrating Tat sequence) coating that responds to matrix metalloproteinase-9 (MMP-9), which is overexpressed in tumor microenvironments. [28]**.** Glycylphenylalanylleucylglycine tetrapeptide (GFLG) is another example of an enzyme-responsive substrate that is stable in blood. It is sensitive to tumor cellular environments, which contain secreted Cathepsin B, a cysteine protease that is up-regulated in tumor cell lysosomes. According to Zhang et al., 2017, GFLG may be employed as an enzyme-cleavable linker to create enzyme-responsive nanoparticles for intra-lysosomal drug release in cancer cells. Prepared nanoparticles (80–110 nm) had a longer intravascular half-life, a larger tumor accumulation potential via the EPR effect, and improved anticancer activity. It has been discovered that it is preferentially uptaken by cancer cells, releasing the GEM exclusively in the tumor environment. The resulting DDS dramatically reduced tumor sizes, giving about 2-fold more tumor growth suppression than GEMHCl while being physiologically safe to normal cells [29]**.**

2.6 Biopolymers with hypoxia activatable groups

Because of the uneven vascular networks that cause hypoxia in tumors, the deeper cells in the tumor mass continue to be deprived of oxygen. These cells multiply at a very slow rate in hypoxic environments and are less vulnerable to conventional anti-proliferative drugs. Thus, the intra-tumoral hypoxic microenvironment is significantly linked to increased tumor aggressiveness, lower therapeutic impact, and poor treatment prognosis. Many enzymes are involved in reduction processes or electron donation, including nitroreductase, azoreductase, inducible nitric synthase, methionine synthase reductase, diaphorase, and nicotinamide adenine dinucleotide phosphate (NADPH), which is increased in hypoxic cells. Following bioreduction, these enzymes may cause a change in

the physical or chemical characteristics of the carrier. Because of the advantages of the longer systemic circulation, high tumor accumulation, greater tumor penetration, and site-specific drug release in hypoxic tumors, particular bio-reductive groups can be employed to construct microenvironment-responsive nanoparticles. The azobenzene, nitroimidazole, and nitrobenzyl alcohol moieties are typical hypoxia-responsive groups that may be attached/linked to the biopolymer and then utilized to develop hypoxia-sensitive nanoparticles. Under hypoxic conditions, these moieties can be reduced by overexpressed enzymes, such as nitro groups, which are reduced by nitroreductase and NADPH into nitroso, hydroxyl amino, and finally, amino groups; and azobenzene, which is reduced by NADPH dehydrogenase (NQO1) and azoreductase into two separate aniline groups [30].

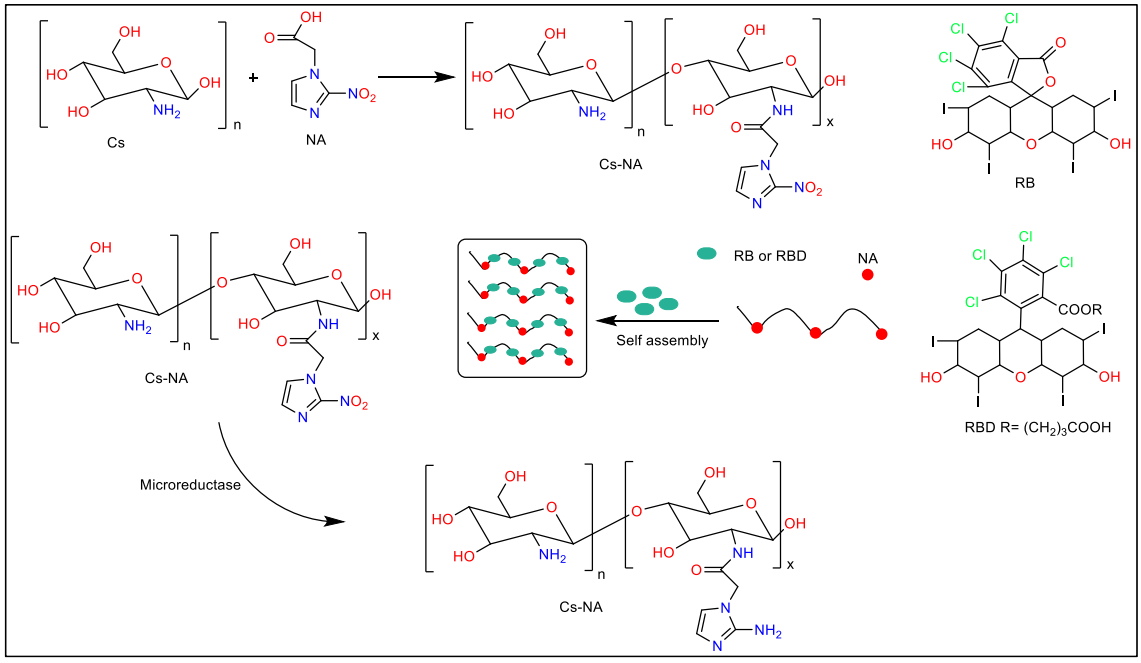

Figure 2.8 Synthesis of hypoxia-sensitive nitroimidazole modified chitosan (Cs-NA) [31].

To develop the hypoxia-sensitive biopolymer, 4-nitrobenzyl chloroformate was employed to modify the glycol chitosan. Hypoxia-responsive glycol chitosan nanoparticles feature a hydrophobic core of 4-nitrobenzyl chloroformate and a hydrophilic outer layer of glycol chitosan. Nanoparticles based on this type of biopolymer release the therapeutic payload precisely in the hypoxic tumor environment [32]. Another example of a hypoxia-sensitive

biopolymer is nitroimidazole modified chitosan (Cs-NA). Cs-NA was created by combining the carboxyl groups of NA with the primary amine groups of Cs through EDC/NHS condensation process **(Figure 2.8)** [31]**.**

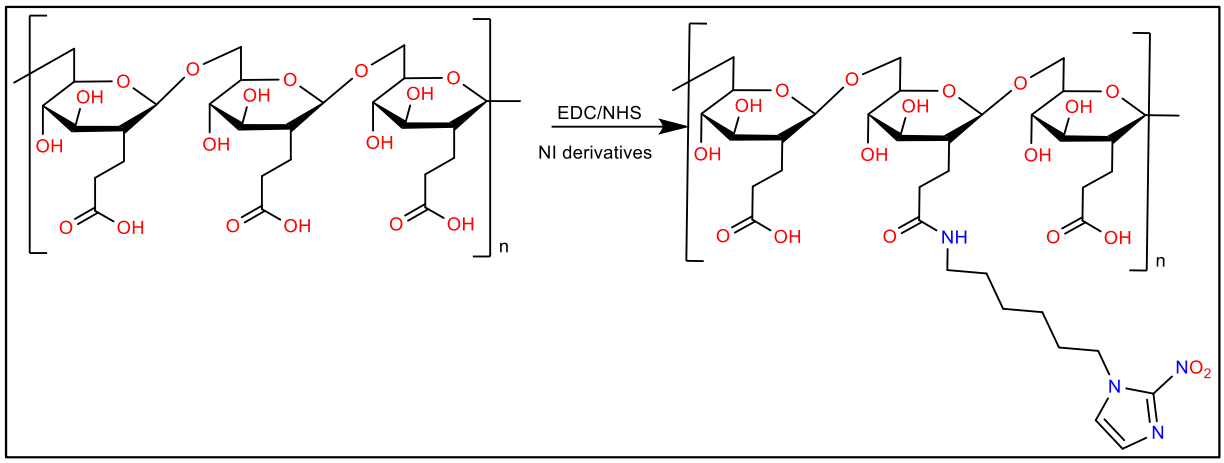

Figure 2.9 Synthesis of a 2-nitroimidazole modified carboxymethyl dextran by EDC/NHS mediated carbodiimide chemistry [33]**.**

Thambi et al., 2014 used a 2-nitroimidazole derivative to modify carboxymethyl dextran. The NI was first transformed to 6-(2-nitroimidazole)hexylamine before reacting with the carboxylic acids of CM-Dex. The NI derivative was then chemically attached to the water-soluble CM-Dex backbone through amide production **(Figure 2.9)**. The modified dextran derivative was utilized to make hypoxia-responsive nanoparticles. Doxorubicin, the model drug, was efficiently encapsulated within the NPs and released the drug at a higher rate when exposed to hypoxia. In physiological conditions, the nanocarriers were stable. The imaging results revealed that nanoparticles accumulated effectively at the tumor location. Under hypoxic environments, NPs were capable of selectively releasing the hydrophobic drug. In-vitro cytotoxicity experiments revealed that hypoxia cells had a more toxic response than normoxic cells [33].

Tumor microenvironment pH of extracellular tissue is about pH 6.5, comparatively lower than pH 7.4 reported for normal tissues [34]. The nanomedicine is rendered acid-

responsive via incorporation of acid-labile bonds or functional group in the polymeric matrix that responds to low pH environment of the tumor to release the therapeutics. Here, the drug may be attached to the nanoparticles through pH-responsive linkers or encapsulated in a carrier susceptible to degradation at acidic pH. These acid-sensitive linkages or carrier remain stable at physiological pH (pH 7.4) but undergo structural and/or chemical change in the acidic tumor microenvironment (pH 4.5–6.5) to ensure the rapid release of the drug for improved antitumor therapy. Chitosan is an example of a biopolymer that has a free amine group responsible for its pH responsive behavior and can be employed to design a pH responsive system for selective release of therapeutic payload in acidic conditions [35]. The acid-labile crosslinking via formation of acylhydrazone bond between Pectin hydrazide and oxidized carboxymethyl cellulose is also an example of tumor microenvironment pH responsive system [36]. Similarly, Carrageenan with PNIPAm forms nanocarrier via gamma irradiation method and show pH responsiveness due to sulfate groups in carrageenan [37].

Table 2.1 Biopolymer for tumor microenvironment responsiveness for site-specificity

Biopolymer	**Tumor microenvironment responsiveness**	**References**
Dextrin with aldehyde cross-linkers	Form a pH-sensitive acetal bond to release DOX in acidic pH	[3]
Thiolated chitosan linked to acrylic polymer	Form imine and disulfide bonds for pH and ROS responsiveness	[5]
N-succinyl-CS	pH-responsive degradation and swelling of nanogel	[4]
Dimethyl acrylamide-trimethyl chitosan	Imine bonds cleavage in acidic pH resulted in degradation or disassembly of nanomaterial to immediately release the drug.	[2]
Gelatin	pH-responsive	[7]

Keratin	GSH and trypsin protease induced drug release	[13]
thiolated-pectin	Form reducible disulfide bonds	[8]
Heparin	Heparanase-1 in the tumor microenvironment breakdown the heparin based NPs	[23]
Hyaluronic acid (HA)	Intracellular lysosomal hyaluronidase in cancer cells might easily breakdown HA into low molecular weight fragments to release drugs from HA-NPs	[24]
Gelatin	Gelatinase, an endogenous proteolytic enzyme overexpressed 7 to 8 times in tumor tissues hydrolyze the gelatin	[25]
Dodecyl-glycidyl modified alginate	Alpha-L-fucosidase mediated accelerated release	[27]
4-nitrobenzyl chloroformate modified glycol chitosan	Released the therapeutic payload in hypoxic tumors	[32]
Nitroimidazole modified chitosan	Hypoxia sensitive	[31]
2-nitroimidazole modified carboxymethyl dextran	NPs selectively released the drug in hypoxic environments	[33]
Bilirubin-grafted polylysine	The transition of bilirubin from hydrophobicity to hydrophilicity under ROS stimulus	[38]
N, N, N - Trimethyl chitosan	Dox release was triggered by ROS in tumor cells	[39]
Modified amphiphilic Dextran consisiting of peptides and hydrophobic deoxycholic acid (DOCA) into the backbone of dextran	The synthesized polymer was responsive to fibroblast activation protein alpha (FAP-α). On arriving tumor site, DPD NPs loaded with quercetin swiftly responded to FAP-α, cleaved and released drug around cancer-associated fibroblasts and remodeled the TME by diminishing fibrosis, normalizing vascularization,	[40]

(Dex-FAP-DOCA, DPD)	enhancing the hemoperfusion, relieving from hypoxia, and thus reinforced sequential treatment.	
Alginate grafted with 2-hydroxypropyl methacrylamide	pH-responsive amide bonds (-CONH-)	[41]
Amphiphilic conjugates of hydrophilic hyaluronic acid and hydrophobic O6-azobenzyloxycarbonyl (BG) group via amidation reaction	Contain azobenzene link that disrupts in hypoxic conditions of tumor tissues to release the payload.	[42]
Amphiphilic polymer consisting of Carboxymethyl chitosan (CMCS) and cystamine grafted oleic acid (OAss)	Contain reduction-sensitive hydrophobic segment of OAss having disulfide bonds (prepared by amidation reaction of cystamine and oleic acid). The carboxyl group of OAss easily react with amino group of CMCS to give amphiphilic polymers (CMssOA), used for preparation of pH/reduction-responsive nanoparticles	[43]

2.7 Cleavable-bond based targeting

Benzoic imine linkage is highly stable at the physiological pH but rapidly cleaves under tumor pH. Zhong et al., 2019 reported a transformable Dox-loaded hyaluronic acid supramolecular nano assembly with a benzoic imine linker, termed as Dox/HCVBP for tumor-targeted drug delivery. Here the pH-sensitive adamantane-PEG with benzoic imine (AD-B-PEG) **(Figure 2.10)** was used to modify the surface of the Dox-loaded HA nano assembly. AD-B-PEG was stable at pH 7.4 where NMT 10% of the polymer was only hydrolyzed up to 12 h but the hydrolysis of benzoic imine increased to 58.75% at pH 6.5 and 73.95% at pH 5.0 within 5 min. This revealed the highly pH-dependent nature of benzoic imine that was found to be very sensitive to the acidic environment. Dox/HCVBP

when injected intravenously found to keep maintain the stealth state in the blood, masking the HA by PEG shell and thereby reducing the interaction with the biosystem. The stealth layer degraded as a response to acidic stimuli, exposing HA at tumor microenvironment, responsible for recognizing the receptors on the surface of cancer cells state for cellular uptake. After HA-mediated endocytosis, the lower pH in the endo/lysosomes further accelerated the Dox release responsible for an enhanced antitumor effect. All this significantly increased the AUC by quarter fold and remarkably prolonged the $t_{1/2}$ by thrice compared to the control, Dox solution [44]. Hence, such pH-sensitive modification may effectively improve stability, prolong blood circulation, prevent prior drug loss, enhance intracellular drug delivery and provide rapid release of the payload within tumor cells.

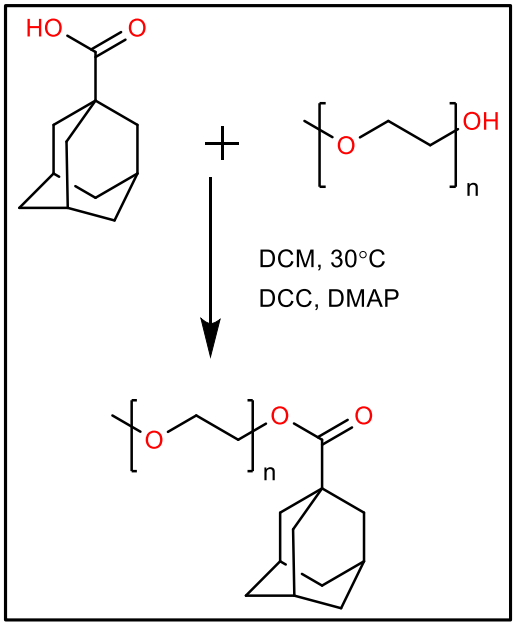

Figure 2.10 Synthesis of pH-sensitive adamantane-PEG

Lin et al., 2015 developed self-assembled nanogels using lysozyme-pectin for the pH-responsive delivery of the anticancer drug, methotrexate. The nanosystems showed good biocompatibility, low toxicity, decreased drug loss during blood circulation, improved intracellular drug release at tumor cells, and exhibited higher anticancer activity than free

MTX. The faster drug release in acidic environments than physiological pH was due to the pH responsiveness of the nanogels. The release was significantly increased at endo-/lysosomal pH 5.3, post endocytic uptake in HepG2 cells [45].

Pandey et al., 2020 reported the use of protein, biopolymer, and MOFs for the development of pH-responsive nanocarriers. Here, Lactoferrin (Lf) was employed as a protein matrix for encapsulation of titanocene, later enclosed with 5-FU in ZIF-8 framework to give ZIF-8@Lf-TC. ZIF-8 was prepared using 2-methyl imidazole as linker and Zinc ions having high pore volumes for loading drugs. The ZIF-8 was further coated with Lenalidomide-HA conjugate linked via hydrazone linkage (LND-HA@ZIF-8@Lf-TC). The resultant system demonstrated pH-responsive release of 5-FU and LND in a sustained manner due to the disintegration of the system in acidic media [1].

In a study conducted by Liu et al., 2017, a nanocapsule was fabricated by encapsulating stealthy cross-linked poly(2-methacryloyloxyethyl phosphorylcholine) and benzaldehyde groups around the protein bovine serum albumin. The benzaldehyde group into the nanocapsule further reacted with the amino group of doxorubicin to form a pH-responsive benzoic-imine bond. The bond was strong and stable under physiological conditions but cleave quickly in an acidic tumor microenvironment. The in-vitro results confirmed the acidic tumor microenvironment (pH ~6.5) responsive drug release to kill the HepG2 human liver cancer cells specifically. The half-life of Dox was significantly prolonged, and the area-under-curve was as much as 242-fold for nBSA-Dox compared to free Dox. Therefore, the systems exhibited a prolonged circulation time, improved tumor accumulation, and tumor microenvironment-responsive drug release, for better cancer therapy [46].

2.8 pH-dependent Protonation mediated targeting

The pH-responsive nanomedicine can also be prepared by using biopolymer with protonated chemical groups in nanosystems. These groups can accept or donate protons to undergo a pH-dependent conformational change responsible for on-demand drug release. In a study by Zou et al., 2013, gelatin, a proteinaceous biopolymer derived from the processing of animal collagen, was used to graft the surface of MSN. Gelatin-based capping layer on mesoporous silica nanoparticles (MSN@Gelatin) by temperature-induced gelation and subsequent glutaraldehyde mediated cross-linking resulted in a pH-responsive delivery system for targeted intracellular drug delivery. The gelatin capping layer and crosslinking prevented the dissolution of adsorbed gelatin, keeping the pores blocked at physiological pH and thus prohibited the drug release. While, in an acidic environment favored the release due to increased repulsion between the gelatin and MSN, subsequent uncapping and opening of MSN pores. The detachment of gelatin from MSN was attributed to the protonation of gelatin in acidic tumor pH. The zeta potential of MSN was -25.4 mV, which on capping changed to -0.226 mV. But the zeta potential was restored in acidic pH to -11 mV, due to protonation followed repulsion. The release study showed no release of doxorubicin in neutral pH and controlled release for over the 440 min period in mimicked environments of late endosome and lysosome, where the pH values were in the range 5.0–6.0. [7].

Within the acidic tumor microenvironment, chitosan may undergo a pH-sensitive structural change. The pH of cancer cells' endosomes and lysosomes is often in the 4-6 range. Drug release from CS-based nanocarriers can be triggered by the acidic intracellular environment. Furthermore, the addition of hydrophobic groups to it may boost its pH responsiveness even further. oleoyl-chitosan, for example, is sensitive to ambient pH fluctuations and may be utilized to encase DOX in nanospheres due to strong hydrophobic

contact between the oleoyl groups. The deprotonation of the amine groups at higher pH is responsible for minimum electrostatic repulsion between the CS polymeric chains (lowered zeta potential) and dominant hydrophobic interaction between the oleoyl chains, accounting for the smaller size of the nanospheres. However, at a lower pH, the amine groups were significantly protonated, resulting in charge repulsion between the oleoyl-CS chains, and polymer chain extension to increase the size of the nanospheres, and release the drug subsequently [47].

2.9 Size dynamic targeting

Nanoscale size is the basic necessity for achieving passive targeted drug delivery to tumor cells. The size of nanoparticles should be such that they effectively accumulate around leaky tumor cells based on EPR effects, and then diffuse into deeper tumor cells. Nanoparticles of about 100 nm diameter can achieve prolonged blood circulation and effective accumulation in tumor regions whereas NPs with a diameter smaller than 30 nm exhibit enhanced tumor penetration and cellular internalization [48]. Therefore, NPs with a larger diameter exert excellent EPR effects but low uptake. To obtain both enhanced tumor accumulation and efficient tumor penetration, either the NPs with smaller sizes are integrated into larger nanoparticles via encapsulation or surface conjugation, or the size shrinkage phenomenon as a response to extracellular tumor stimuli to form smaller sizes for deep tumor penetration was utilized.

2.10 Charge reversal targeting

The surface charge plays a vital role in tumor targeting. Carriers with negative or neutral charges avoid recognition and subsequent elimination by mononuclear phagocytic systems, thus possess prolonged circulation and relatively high accumulation in tumor tissues. In contrast, positive charge favors cellular internalization via electrostatic interactions with anionic cancer cell membranes as well as promotes endosomal escape

based on the “proton sponge” effect to avoid drug degradation in lysosomes. To address this issue, NPs with switchable surface charge emerged that reside negative or neutral charge while blood circulation to prevent non-specific uptake, and accumulate near the tumor. After arriving at tumor parenchyma, tumor signals trigger the surface charge conversion into a positive charge within few seconds (about 10s), which promotes the interaction of the NPs with the anionic membrane of cancer cells responsive for endocytic uptake [49]. Glycol chitosan (GC) is such a pH-sensitive polymer having the potential to alter surface charge from neutral to positive, which might enhance therapeutics accumulation within the tumor area. Zhu et al., 2020 demonstrated GC-based polymeric micelles that had a longer circulation period at physiological pH, a pH-stimulated charge transition in the tumor microenvironment, and enhanced cellular uptake and accumulation due to an increase in positive charge [50]. EPLYS (-Poly-L-lysine) is a naturally biodegradable homo poly(amino-acid) with acid labile -carboxylic acid groups and amino groups. EPLYS contain a positive charge owing to amine groups, which can be protected by converting their amines to -carboxylic amides. This leads to the production of a smart charge switchable polymeric molecule, the surface charge of which may be spontaneously reversed in certain pH ranges of the tumor microenvironment, and selectively accelerated the uptake by tumor cells. Guo et al., 2020 revealed PEG and EPLYS based pH responsive polymeric nanoparticles (DMMA-P-DOX/LAP), that can not only reverse their surface charge in tumor tissue microenvironment to improve cellular uptake but also specified the release of encapsulated drugs into tumor cells. DOX was introduced into the polymer side chain via an acid-cleavable imine bond to yield PEG-EPLYS-DOX as polymer-drug conjugate, and the residual amino groups in the EPLYS segment were amidated using 2,3-di-methylmaleic anhydride to shield the positive charge of the nanoparticles and prolong circulation time in blood. LAP was then physically

enclosed in order to create dual-drug-loaded polymeric nanoparticles (DMMA-P-DOX/LAP). Following systemic delivery, the nanoparticles were deposited in tumor tissue via the EPR effect, and their surface charge was ultra-sensitively reversed from negative to positive, boosting cellular uptake and increasing tumor penetration. Following that, the low intracellular pH could rapidly disintegrate the nanoparticles by initiating the breaking of the imine link, and the anticancer drugs were released into the cytoplasm to inhibit cell growth synergistically [51].

Li et al., 2021 disclosed charge reversible chitosan-polypyrrole-based nanogels (CH-PPy NGs) for improved tumor drug delivery. Chitosan was first grafted with PPy to create CH-PPy polymers, which were then cross-linked with glutaraldehyde to form CH-PPy NGs. The CH-PPy NGs (P-NGs) have a positive charge at pH 7.4 and pH 6.5, which is readily removed by RES during blood circulation. Treatment with a particular quantity of NaOH solution resulted in the preferential adsorption of OH on the Py rings due to which CH-PPy NGs converted to charge-reversible CH-PPy–OH–4 NGs (R-NGs) rendered with a negative charge. R-NGs with a negative charge at physiological pH extended the blood circulation period by about 22 times that of free DOX, and once in the tumor tissue, their surface charge converts to a positive at a slightly acidic pH of the tumor through rapid protonation of the Py ring, allowing for deep tumor penetration and cellular internalization. R-NGs had strong cytocompatibility, high doxorubicin (DOX) loading efficiency (87.58 percent to 89.63 percent), extended blood circulation, increased tumor accumulation, greater penetration, and tumor cellular internalization capability.

The rapid charge conversion of R-NGs from 11.3 mV to +10.4 mV happened when the solution pH was changed from 7.4 to 6.5, as indicated in the figure 2, and this process only took around 10 seconds. The charge conversion effect of R-NGs was shown to be stable over time where surface charges were sustained at about +11.0 mV at pH 6.5 and 11.5% at

pH 7.4 for up to 15 hours. DOX may be promptly released from R-NGs/DOX at pH 6.5; this pH-responsive release may be mediated by the proton sponge effect that occurs between cationic R-NGs and DOX. HCl in an acidic environment to allow for rapid DOX release via electrostatic repulsion between the NGs and DOX. DOX accumulation is 2.2 times greater in R-NG/DOX group than N-NG/DOX group [52].

2.11 Redox responsive nanomedicine

The oncogenic transformation in tumor cells results in the constant production of ROS at a higher level than in normal cells. These elevated ROS in tumors could be utilized for obtaining site-specific drug release. The characteristic groups like boronic ester, thioether/thioketal, selenide, and sulfide, are employed for the development of ROS-responsive nanomedicine [53,54]. The most common mechanism of nanoparticles dissociation is the cleavage of H_2O_2 labile bonds. This structural disruption results in the release of encapsulated drugs. For example, Li et al., 2019 reported ROS responsive micelles containing peroxalate ester as hydrogen peroxide (H2O2)-responsive bond and encapsulated with paclitaxel. Once the micelles were internalized by the tumor sites, the overexpression of H2O2 induced the deposition of micelles and triggered the drug release [55]. Yang et al., 2020 developed ROS-sensitive nanoparticles by combining an amphiphilic polymer based on chondroitin sulfate (ChS) with hydrophobic poly (propylene sulfide) (PPS) as the ROS-responsive moiety, chlorin e6 (Ce6) as the ROS-generator, and paclitaxel (PTX) as an anticancer drug. Developed CP/ChS-g-PPS NPs accumulated at tumor sites via increased permeability and retention (EPR) effect, and NIR laser irradiation of tumor foci triggered ROS generation and accelerated NPs self-destruction-based drug release. In tumor models, in vivo anticancer efficacy confirmed increased tumor inhibition rates. ROS-responsive self-destruction NPs were identified to spatiotemporally modulate drug release, limit off-target toxicity, and give a desired

therapeutic impact [16]. Viswanadh et al., 2021 synthesized glutathione-redox-sensitive thiolated vitamin-E-PEG1000-succinate (TPGH-SH) by conjugating TPGS with 4-amino thiophenol (4-ATP). Tumor microenvironment responsive drug release was obtained due to the presence of thiolated TPGS in the polymer backbone of redox-sensitive NP. In addition, surface modification with cetuximab further increased the drug delivery and subsequent drug release due to higher cellular uptake and NPs degradation guided intracellular release of loaded docetaxel, owing to breaking of disulfide bonds in the cancer microenvironment with elevated GSH concentrations [56]. Luo et al., 2021 utilized cathepsin B-responsive glycylphenylalanylleucylglycine (GFLG) functionalized polyHPMA for the development of redox-sensitive nanosystems. Herein, DOX was attached to the carrier through the GFLG linker as glutathione (GSH)-cleavable disulfide bond-bridge, resulting in polymeric prodrug (polyHPMA-DOX) that self-assembled into stimuli-responsive nanoparticles to encapsulate Ce6. The nanoparticles accumulated well in tumor cells, and their breakdown in the tumor microenvironment due to breakage of the disulfide link by high intracellular concentrations of GSH inside tumor cells resulted in the release of DOX. The released DOX, when coupled with the ROS produced by PDT, was discovered to impede the development of cancer cells [57].

Amphiphilicity transition is another mechanism of nanoparticle disassembly responsible for TME responsive drug release. As reported by Li et al., 2012, keratin-g-PEG copolymers carrying glutathione cleavable cross-links may be produced using thiol-ene click chemistry. The amphiphilicity and the thiol groups of such grafted copolymer can be utilized for the production of tumor-responsive DDS. Cross-linking of disulfide bonds with thiol groups on the keratin backbones can result in nanoparticles. The keratin-g-PEG copolymer nanoparticles were good doxorubicin carriers with GSH sensitive drug release. Moreover, trypsin promoted the release of the loaded DOX from the nanoparticles.

Experiments on in-vitro cellular uptake verified nanoparticles' effective absorption into cells and enhanced drug release into the cells [58]. In the same way, Poly(ethylene glycol)-poly(methionine) [PEG-P(Met)] can also be utilized to develop ROS-sensitive nanocarriers for drug delivery into cancer cells that are both safe and effective. PEG-P(Met) copolymers were discovered to generate micelles with hydrophobic PL by self-assembly. PEG-P(Met) promotes the intracellular release of loaded drugs into cancer cells. Nanoscale micelles were successfully accumulated into tumor tissues via the EPR effect and then ingested by cancer cells via endocytosis. Following cellular uptake, increased ROS levels in cancer cells caused a hydrophobic-to-hydrophilic transition of the polypeptide due to conversion of the P(Met)s hydrophobic thioether groups to hydrophilic sulfone or sulfoxide groups, resulting in destabilization of the synthesized polymer-based micelles and efficient PL release into cancer cells. PEG-P(Met) micelles increased apoptosis in MCF-7 human breast cancer cells as compared to free PL [59].

Amalgamation of redox-responsive inorganic system with biopolymers has also emerged in recent years in the development of redox responsive nanomedicine. Xia et al., 2024 designed a hybrid organic–inorganic nanoframework (LDH/HA/5-FU) by intercalation of 5-FU into the interlayer of copper–aluminum layered double hydroxide (CuAl-LDH) via ion exchange strategy and adsorption of hyaluronic acid (HA) on the surface of CuAl-LDH. LDH/HA/5-FU nanosheets exhibited superior anti-tumor potential in tumor microenvironment with low pH, endogenous H_2O_2 and high GSH level. HA renders the nanosheets selectivity to target CD44 receptors, CuAl-LDH undergoes pH-degradation to release 5-FU and Cu(II). The overexpressed GSH and H_2O_2 in tumor and cytosolic microenvironment induces in-situ reduction of Cu(II) ions to Cu(I). This Cu(I) catalyzes Fenton-like reactions to generate OH that disturbs copper homeostasis in tumor cells and causing cuproptosis. Since 5-FU release is pH dependent, the reduction of copper requires

ROS species, and Fenton-like reactions requires acidic conditions, the developed nanosheets exhibited selective activation in tumor microenvironment [60].

2.12 Enzyme responsive nanomedicine

Many enzymes have been found to get overexpressed in TME, mainly membrane metalloproteinases [61], hyaluronidases [62], lipases (phospholipase A2), or γ-glutamyl transpeptidase [63]. These enzymes can be used for receiving enzyme-responsive drug release in TME from nanoparticles incorporated with enzyme-specific moieties, selectively recognized and degraded by overexpressed enzymes. Xiaoan et al., 2014 used silsesquioxane with azobenzene gated on mesoporous silica nanocarriers as support for the development of a novel enzyme-responsive system. Reductive cleavage of azo bonds by azo-reductase resulted in gate opening and subsequent release of loaded drug at the target location [64]**.** In another study, Wang et al., 2017 linked the CDHA as a gate to dox encapsulated MSN using a disulfide linker. CDHA was synthesized using polymerizing reactions in which citric acid and ethylenediamine created a branching monomer that then reacted with the polymer hyaluronic acid. As a result, MSN-SS-CDHA was discovered to have improved photostability, great biocompatibility, and was specifically targeted to A549 cells overexpressing CD44 receptors. To avoid early drug leakage, CDHA entirely closed the pore (3 nm) of MSN nanoparticles. MSN-SS-CDHA nanocarriers were inactive in normal cells (without/or with low glutathione and HAase expression), but in tumor cells and tissues (with high concentrations of GSH and HAase), specific enzymatic cleavage of the disulfide bonds selectively removed the N-doped CDHA from the surface of MSN, resulting in the subsequent release of encapsulated DOX [65]**.** Kumar et al., 2017 used guar gum as a capping layer on a 5-fluorouracil-loaded mesoporous silica nanoparticle for enzyme-responsive colon targeted drug delivery. To achieve site-specific delivery, the drug release was activated by enzymatic breakdown of guar gum by colonic enzymes in the

simulated colonic microenvironment, preventing non-specific release in the simulated gastro-intestinal tract [66]. Kaur et al., 2023 utilized a partially oxidized hyaluronic acid dialdehyde (HADA) to conjugate amine containing cancer drugs via Schiff's reaction to prepare hyaluronidase enzyme responsive system. The high level of hyaluronidase enzyme in cancer microenvironment triggered accelerated disassembly HADA NPs via degradation of hyaluronic acid dialdehyde chains in nanoparticles while the imine bond formed between –NH2 of DOX and –CHO of HADA resulted in pH-dependent bond cleavage to release free DOX [62].

The intracellular overexpressed enzymes can also serve as triggers for size shrinkage or exposure of surface ligand to obtain more pronounced penetration and internalization into deep tumor cells. PTX-DOTAP@alloferon-1-heparin/protamine was proposed by Huang et al., 2019 as an enzyme-responsive size-reversible targeted nanoparticles drug delivery to cancer cells. In this case, particle size reduction and charge inversion enabled nanoparticle transportation to the depth of the tumor, with higher uptake by tumor cells with strong negative charges on the surface. The developed nanoparticle was observed to have a particle size of 106.1± 1.113 nm and a negative zeta-potential of -45.1 ±0.455 mV, allowing passive targeting to the tumor location. Heparin's negative charge resulted in strong circulatory system stability and a high concentration of nanoparticles at the tumor location. When the nanoparticles reached the heparanase-1 expressing extracellular matrix, they were quickly identified, and the heparin in the outer layer of the nanoparticle was degraded to release the alloferon-1 through ion diffusion in the tumor microenvironment. The released alloferon-1 could activate NK cells, reversing the immune system suppression caused by tumor cells. Furthermore, the dual action of enzymatic hydrolysis and ion diffusion resulted in the exposure of positively charged DOTAP core and eventual decrease in particle size to 59.30 ±0.783 nm and charge reversal to 25.4 ±0.257 mV. The

resulting nanoparticles with smaller particle size and a positive charge were easily endocytosed by strongly negatively charged tumor cells, increasing the therapeutic efficacy of the encapsulated chemotherapeutic drug [23]**.** Zhong et al., 2023 also reported tumor microenvironment responsive and size dynamic nanoparticles consisting of DOX encapsulated Laponite nanocrystals (LP) and Chitosan oligosaccharide (COS).

COS coating of LP endowed a tumor extracellular matrix responsiveness to the nanoparticles (LDC). LDC (size of ~100 nm) passes through the leaky tumor vasculature to enter tumor stroma where COS undergoes lysozyme mediated-degradation resulting in smaller LD nanoparticles (30 nm). This size reduction improved tumor penetration and provided higher tumor endocytosis [67]. Another tumor microenvironment, lysyl oxidase (LOX) responsive size-transforming nanoparticles were reported by Park et al., 2023, consisting of Imiquimod loaded polydopamine nanoparticles coated with collagen (CPN/IQ). The nanoparticles exhibited specific accumulation and prolonged retention in tumor microenvironment with excessive LOX. LOX oxidizes the collagen on CPN/IQ and collagen fibers in tumor microenvironment to the aldehyde groups that subsequently undergoes aldol condensation. This condensation anchors the nanoparticles covalently to the extracellular collagen matrix creating an artificial extracellular matrix near the tumor cells. The CPN/IQ anchored around the tumor when exposed to near infrared radiation results in strong anti-cancer effect due to prolonged and concentrated retention of CPN/IQ in the tumor microenvironment. [68].

2.13 Hypoxia responsive nanomedicine

Polymers that are sensitive to the hypoxic environment of a tumor can be used to develop nanoplatforms with hypoxia-guided drug release potential. Jang et al., 2020 developed hypoxia-responsive targeted glycol chitosan nanoparticles comprising of 4-nitrobenzyl chloroformate core, folic acid (HRGF) surface modification, and doxorubicin loading

(DOX). The prepared nanoparticles were shown to selectively bind FR- overexpressed cancer cells, where they quickly released the drug under hypoxic conditions due to NTR and NADPH cleavage of glycol chitosan-bound 4NC. Since the drug was only released in cancer tissue, it is likely to mask the therapeutics side effects [32].

Hypoxia responsive nanoparticles can be prepared using polymers with hypoxia cleavable linkage in the backbone of the polymer chain. Son et al., 2018 described a hypoxia-responsive polymer-conjugate combining carboxymethyl dextran (CMD) and an azo link carrying black hole quencher 3 (BHQ3) as the hypoxia-sensitive moiety. An amide bond was used to link the hydrophobic BHQ3 amine and the hydrophilic CMD. Because of its amphiphilicity, the prepared polymer self-assembled into nanoparticles (CMD-BHQ3 NPs). CMD-BHQ3 NPs loaded with doxorubicin (DOX) released the drug at a higher rate in a tumor hypoxic environment due to azo bond breakage. In tumor cells, azobenzene derivatives are reduced to aniline derivatives by numerous reductases in the presence of an electron donor, resulting in azo bond breakage via the electron transfer mechanism. In the hypoxic state, the NPs exhibited considerably more cytotoxicity than in the normoxic state [69]. Peng et al., 2021 reported a hypoxia-degradable zwitterionic poly(phosphorylcholine) nanogel with an azobenzene-contained crosslinker for tumor drug delivery. The nanogel formed had a prolonged blood circulation, a low immunological response, and increased tumor accumulation. Because of the degradation of the azo link, the prepared nanogel successfully decomposed into oligomers with low molecular weight in hypoxia conditions, demonstrating hypoxia-triggered drug release in tumor tissue regardless of the cell membrane barrier [70].

Nitric oxide is a TME modulator that dilates blood vessels in response to hypoxia and glutathione (GSH) levels in tumors. Zeng et al., 2023 reported Chitosan bridged octadecylamine (CO–SS–ODA) as an amphiphilic glycolipid-polymer containing disulfide

bond responsive to higher concentration of GSH in tumor cells. The polymer self-assemble to give GSH responsive nanomedicine (CO–SS–ODA/DOX) that release payload selectively in tumor cells via breakage of disulfide bond**.** *S*-Nitroso-*N*-acetylpenicillamine (S) was also linked to CO to render TSCO–SS–ODA/DOX Nitric oxide responsive behavior. TSCO–SS–ODA/DOX selectively internalizes in endothelial and cancer cells. In endothelial cells, TSCO–SS–ODA/DOX released NO and alleviated tumor hypoxia. It also decreased the infiltration of M2 macrophages in tumors and increased infiltration of M1 macrophages tumors, hence remodeling the TME. The disulfide bond was not sensitive to GSH concentration in endothelial cells showing less release of DOX. While at tumor site, the disulfide bond in TSCO–SS–ODA/DOX rapidly broke to release the encapsulated DOX indicating the selectivity of DOX delivery in cancer cells [71]. Another redox responsive nanomedicine was designed using an amphiphilic redox-sensitive alginate–SS–ibuprofen conjugate (LSA–SS–IBU) obtained by attaching hydrophobic ibuprofen (IBU) onto hydrophilic alginate through disulfide linkages. The conjugate can form stable micelles capable of retarding release of payload (DOX) in systemic circulation while providing glutathione (GSH) triggered release at target site [72].

2.14 Dual stimuli-responsive nanomedicine

Nanomedicine responsive to more than one or dual stimuli presents drugs in tumors more precisely to increase the efficacy of the drug. Xudong et al., 2020 created a hypoxia and pH dual-responsive drug delivery system using nitroimidazole-modified chitosan (Cs-NA). Cs-NA had a hypoxia-responsive nitroimidazole group and a pH-responsive feature due to the residual high number of amino groups in chitosan, which could be easily protonated in acidic solution. EPR properties allowed the passive accumulation of developed nanoparticles in tumor tissue. The hypoxic and acidic environment aided the

drug release post endocytosis. The structural shift of hydrophobic nitroimidazole to hydrophilic aminoimidazole was driven by the hypoxic environment via nitroreductase. When the stable structure of nanoparticles was disrupted, the drug was released, which was further accelerated by pH-responsive behavior [31]**.** Li et al., 2022 reported a dual-stimuli responsive system for precise drug delivery in tumor microenvironment. The nanoparticles formed due to the self-assembly of disulfide linker containing Podophyllotoxin and Hyaluronic acid conjugate. The nanoparticles efficiently accumulated at tumor site via CD44 receptor-mediated endocytosis and exhibited pH and redox responsive disassembly resulting in higher drug release and tumor inhibition [73]. Similarly, a nanomedicine exploiting the size dynamics and charge reversal in combination with TME-responsive behavior can provide a highly specific delivery system with superior efficiency. The size dynamics may improve tumor penetration, charge reversal may lead to endo/lysosomal escape and TME-responsive payload release may ensure selective disassembly of nanomedicine at the target site [74]. Some of the stimuli responsive nanoparticles are detailed in **Table 2.2**.

2.15 Stimuli activated surface ligand-mediated targeting

The strategy entails attaching particular molecules to the surface of nanocarriers that specifically target the receptors overexpressed by cancer cells, enabling cellular uptake of the loaded cargo via receptor-mediated endocytosis. These ligands are covered by a PEG shell during systemic circulation to inhibit nonspecific contact and clearance by the reticuloendothelial system (RES) and other non-cancerous cells. However, once arrived at the tumor location, PEG coating is loosed as a response to microenvironment stimuli, and the targeted ligand is exposed for greater cellular uptake in tumor cells via ligand-receptor interactions [75].

Table 2.2 Targeted nanomedicine responsive to the tumor microenvironment and their drug release mechanisms

Nanomedicine	Stimuli-responsive groups	Tumor microenvironment stimuli	Drug release mechanism	References
Dox-loaded hyaluronic acid-based transformable supramolecular nanoplatform	Benzoic imine linkage	Tumor acidic microenvironment	Higher Cellular uptake due to the direct exposure of HA and PEG shell detachment in the acidic environment	[44]
Doxorubicin conjugated nanocapsules through a pH-responsive group,	Benzaldehyde group into the nanocapsule react with the amino group of Dox to form an acid-responsive benzoic-imine bond	Acidic environment	Bond was very stable under physiological conditions, avoided undesired premature release, bond cleaved quickly in an acidic microenvironment to release the drug at the tumor site.	[46]
Nanomicelles, constructed by self-assembling of poly (ethylene glycol) (PEG)-stearamine (C18) conjugate with	Thioketal linker	ROS	Intracellular release of DOX and PhA, triggered by intrinsic endogenous ROS within cancer cells. In addition, laser irradiation enhanced the generation of singlet oxygen ($^1O_2$) by PhA, which further accelerated the cytoplasmic release of DOX through the rapid dissociation of nanomicelles. The local	[12]

a ROS-sensitive thioketal linker, and co-loaded with doxorubicin (DOX) and photosensitizer pheophorbide A (PhA)			ROS level was gradually increased due to light-activated PhA Provided enhanced locoregional chemo-photodynamic therapy.	
Nanoparticle,	N,N,N-Trimethyl chitosan (TMC) was used as the NPs framework, with 4-nitrophenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl carbonate (NBC), aryl boronic acid link	Reactive oxygen species (ROS)	Dox release was triggered by the oxidation of NBC by ROS in tumor cells and subsequent degradation of the NPs.	[39]
Nanosystems	hydrophobicity/hydrophilicity switchable bilirubin-grafted polylysine	ROS responsive	The transition of bilirubin from hydrophobicity to hydrophilicity under ROS stimulus	[38]
Nanocarriers composed of: MSNs,	Selenocysteine for ROS responsiveness and	ROS and redox	MSNs-SeSe-HA@DOX and MSNs-SS-HA@DOX exhibited a strong ROS and redox-responsive drug	[76]

responsive agent; hyaluronic acid as the coating/targeting agent, and cancer drugs.	dithiodipropionic for the redox responsiveness, to give diselenide/disulfide bonds between MSN and hyaluronic acid (MSNs-SeSe-HA or MSNs-SS-HA)		release. MSNs-SeSe-HA@DOX showed higher drug release in a medium with a high H2O2 concentration. (which mimics the cancerous cells), drug release from MSNs-SS-HA@DOX in a medium similar to intracellular conditions was also higher.	
Micelles	Methionine-polypeptides containing a thioether group responsive to ROS-phase transition and MMP-cleavable linkage.	ROS or protease	Exhibited MMP-sensitive cleavage and ROS-induced DOX release	[77]
Nanocapsule	Amphiphilic carboxylated ferrocene polymer, consisting of hydrophilic COOH segment and a hydrophobic ferrocenylmethyl methacrylate segment	ROS	on-demand release of paclitaxel	[19]
nanoparticles	poly(orthoester-thioether) (PSOE)	Hydrogen peroxide	Degraded rapidly due to hydrophobic-to-hydrophilic transition upon stimulation by hydrogen peroxide,	[78]

			rapid degradation of orthoester, accelerated release of doxorubicin from PSOE NPs under mildly acidic and ROS conditions.	
Nanogel	selenium-containing poly-phosphoesters	ROS	Selenide groups endowed the nanogels ROS responsiveness, drug-loaded PSeP nanogels displayed swollen behaviors to activate doxorubicin hydrochloride (DOX · HCl) release when subjected to the stimuli H_2O_2.	[79]
Nanoparticle	*S*-nitrosoglutathione (GSNO) conjugated PEG-poly(propylene sulfide) (PPS)	ROS and glutathione (GSH)	PPS block undergo oxidative conversion from hydrophobic sulfide to hydrophilic poly-sulfoxides/sulfones in the presence of ROS, showed GSH-responsive NO release from GSNO in cancer cells	[80]

Dysregulated pH is a common stimulus in tumors that may be utilized to design stimuli activated and ligand-focused drug delivery platforms. Sun et al. 2015 proposed an acid-sensitive bridged copolymer for the production of nanoparticles and tumor-targeted delivery of loaded siRNA. In this case, the poly(ethylene glycol) (PEG) corona provided physiological pH stability, shielding the nanovectors from RES clearance and allowing for longer circulation. When the pH sensitive connection breaks in an acidic tumor microenvironment, it promotes PEG detachment at tumor locations, exposing cell-penetration peptide on the surface of the nanovectors, facilitating cell targeting, efficient accumulation in tumor cells, and cellular uptake. As a result, cancer development was inhibited safely and effectively [81].

Similarly, other stimuli might be used to provide targeted distribution of nanomedicine with capabilities of cell-specific drug release in the cancer region. Yang, Zhang and Xu, 2018 demonstrated the use of hyaluronic acid for remodeling the surface of mesoporous silica nanoparticles (MSN) using carbodiimide chemistry to accomplish cancer cell targeting and hyaluronidases (Hyal)-induced intracellular drug release. The gelatin was then grafted onto the MSNHS to form a capping layer by glutaraldehyde-mediated cross-linking and PEG-shielded against unspecific cell uptake to extend the circulation period. The resulting MSN@HAgelatin-PEG (MHGP) encapsulated doxorubicin and examined drug release behaviors. An in-vitro investigation demonstrated that PEG chains shielded the targeted ligand against normal cells. PEG was removed from the outermost layer of the nanocarriers due to gelatin hydrolysis by MMP-2, resulting in exposure and activation of the HA function. After that, DOX-loaded nanocarriers were preferentially taken up by cancer cells via HA receptor-mediated endocytosis, with subsequent lysosomal enzyme hyaluronidase breakdown followed by DOX release. As a consequence, gelatin and hyaluronic acid may be employed to produce dual responsive DDS, where MMP-2-

triggered and hyaluronidases (Hyal)-catalyzed degradation of gelatin and hyaluronic acid (HA) led to drug release in the tumor microenvironment, respectively. Such DDS has dramatically increased anti-cancer activity [82].

PEGylated hyaluronic acid nanoparticles (HA-mPEG2k-DOX NPs) with tumor microenvironment sensitive properties were disclosed by Zhang et al 2020. The model drug DOX, which contains an amino group, was first covalently coupled with an aldehyde-containing compound through the Schiff base reaction to generate a pH-sensitive molecule containing an acid-sensitive imine. In-vivo, prepared NPs with particle sizes of 50 nm dramatically enhanced active targeting capacity to CD44 positive cells, increased cellular absorption efficiency, and extended systemic circulation duration by 12.5 times when compared to DOX HCl. The pH-responsive cleavable PEG was discovered to detach in moderately acidic tumor extracellular environments. Thereafter, exposed HA effectively promoted the cellular uptake of NPs via endocytosis and offered an escape from intracellular lysosomes. The antitumor results revealed that the NPs had the strongest anti-tumor impact while lowering the toxicity of the DOX. Overall, the pH-sensitive imine link and actively targeting HA behavior improved the cellular uptake and selective drug release in cancer cells [83].

Ligand targeted nanomedicine responsive to multiple stimulus of tumor microenvironment can be designed for more effective anticancer therapy. Xiao et al., 2023 reported a multi-stimulus responsive, receptor targeted size/charge switchable nanosystem for improving the tumor penetration and anti-tumor therapy. DOX-loaded nanocluster (DOX-icluster) was prepared using folic acid (FA) and dimethylmaleic anhydride (DMA) modified gelatin (FA-GelDMA) to coat mesoporous organosilicon nanoparticles (DOX-HMON-NH2). DOX-HMON-NH2 was prepared by HMON amination followed DOX loading. The electrostatic attraction between FA-GelDMA and DOX-HMON-NH2 resulted in formation

of DOX-icluster (about 200nm). Resultant nanoparticles specifically accumulated in tumor tissue where DMA from FA-GelDMA cleaved allowing matrix metalloproteinase (MMP-2) to degrade gelatin to unmask the cationic 50nm size DOX-HMON-NH2. The resultant smaller particles exhibited deeper tissue penetration and higher cellular internalization in 4T1 cells. DOX-HMON-NH2 was further degraded by intracellular glutathione (GSH) to release the loaded DOX [84]. Similarly, a dual receptor targeted self-assembled system using amphiphilic HA derivative was reported that utilizes Folate receptor (FA) and CD44 receptor-mediated targeted drug delivery to cancer cells. The hydrophilic HA was conjugated to the hydrophobic mangiferin (MA) and methotrexate (MTX) via ester linkage. Resultant HA-MA-MTX self-assembled and increased the accumulation of MTX in the tumor site and reduced the toxicity to normal tissues by MA. The targeted delivery was achieved due to MTX as a ligand for the folate receptor (FA) and HA for the CD44 receptor. After receptor-mediated cellular internalization in K7 tumor cells, the nanoparticles released the drug by disassembling the formed nanosystem. This improved the anti-tumor efficacy and reduced the nonspecific uptake toxicity [85]. These studies indicate the versatile and multifunctional properties of biopolymers, making them ideal for the development of ligand targeted stimuli responsive nanomedicine for effective and precise drug delivery to cancer cells [93].

2.16 Limitations

Despite numerous advantages to TME-responsive platforms over conventional therapies and huge strides have been made in developing TME-responsive platforms, it should be acknowledged that significant challenges exist to overcome. Such challenges include understanding complex tumor microenvironments and developing delivery platforms that can overcome the therapeutic barriers against all aspects of the microenvironment. Controlling the drug release in response to the tumor microenvironment, which is

heterogeneous by nature, is also a significant challenge as the stimuli involved in releasing therapeutics may vary with patient and tumor type. Another challenge is the delivery platform's ability to target specifically cancerous cells within the tumor precisely. Furthermore, the cancer acquires resistance to the treatments over time. Therefore, developing platforms adapting to such resistance is an imminent challenge. Similarly, considering the time and cost required for the assessment of biosafety and compatibility of novel nanomaterials before their use in therapeutic delivery, the requirement of extensive pre-clinical evaluations of the nanomedicine loaded with therapeutic payload for their performance, the scalability of these developed platforms, clinical translation, and regulatory approvals make these advanced technologies so costly that their accessibility to the patients becomes very limited.

2.17 Carriers for localized delivery of TME-Responsive nanomedicine

TME-responsive systems hold vast promise for making real improvements in cancer therapy by minimizing systemic toxicity and offering maximal therapeutic efficacy. The innovation is expected to be driven through further advancements in nanomaterial engineering, delivery systems, and TME biology. Localized delivery systems for TME-responsive nanomedicines have emerged as promising strategies to improve the efficacy and reduce off-target toxicity of cancer therapies. The new delivery platform utilizing microneedles can be applied to administer TME-responsive nanomedicine directly into tumors or localized tissues. Such processes share some benefits related to minimally invasive administration and localized drug release, with enhanced penetration capabilities of the stratum corneum or dense tumor stroma. The microneedles can deliver nanoparticles to the tumor environment, where they can penetrate more efficiently and achieve increased cellular internalization in cancer cells, thereby exerting a higher anticancer impact.

The microneedles have been employed for the delivery of anticancer therapeutics towards effective management of various cancers like skin melanoma, head and neck carcinoma, basal cell carcinoma, prostate, oral, and breast cancer [87,88]. Microneedles deliver payloads into the dermal layer by piercing the outermost skin layer as a barrier to the delivery. It overcomes systemic treatment side effects, avoids off-target distribution to other major organs of the body, circumvents premature degradation, and increases patient compliance by facilitating painless self-medication [89,90]. Nanoparticle-equipped microneedle systems can be utilized for effective cancer therapy due to their role in enabling loaded nanoparticles to pass the stratum corneum for localization at the tumor site, favoring high cellular internalization, apoptosis induction, and reduction in systemic toxicity and side effects. Lan et al., 2018 utilized microneedles for loading cisplatin encapsulated pH-responsive nanoparticles, offering high anticancer activity and reducing organ toxicity [91]. Various reports related to responsive nanoparticles loaded microneedles have been made that suggest the utility of this strategy as an effective way to target cancer therapy. The pH, enzyme, light, magnetic field, ultrasound, and multi-responsive nanoparticle in microneedles as advanced drug delivery systems offer high drug stability, targeting ability, controlled release, high cellular bioavailability, and more specific drug delivery [92].