pH-triggered cancer-targeting polymers: From extracellular accumulation to intracellular release

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ABSTRACT

Stimuli-responsive polymers are promising to achieve targeted delivery, improved stability during circulation, and controlled release of therapeutic and diagnostic agents. Among them, pH-responsive polymeric nanocarriers have attracted significant attention as pH varies in different body fluids (e.g., stomach, intestine, and colon) and intracellular organelles (e.g., endosome, lysosome, and mitochondria) to maintain homeostasis, while distinctive pH changes are also found in certain pathological states. For example, the extracellular environment of the tumor is acidic, which can be employed to drive selective delivery. During the internalization process, since most nanocarriers enter cells upon endocytosis where a drop of pH from 6.5 to 5.0 can occur from endosome to lysosome, pH-sensitive groups have been developed for enhanced cargo release. In this review, both non-covalent and covalent interactions responsive to pH changes are introduced, with a focus on the structure–property relationship and their applications in cancer targeting and endosomal escape.

KEYWORDS

cancer targeting, pH-responsive, structure–property relationship, charge shifting, acid-labile linkage

1 Introduction

Conventional drug delivery systems (DDSs) are developed to optimize the drug behavior *in vivo*, such as allowing the delivery of poorly soluble compounds, increasing drug stability during the circulation, and increasing the bioavailability. In addition to that, an ideal DDS is expected to further improve the therapeutic efficacy as well as to minimize the toxicity, which is particularly important in cancer therapy. Therefore, continuous efforts have been made to develop DDSs which combine targeted delivery with controlled release (i.e., on-demand release exclusively at the target site). In this context, the intrinsic features of tumor microenvironment (TME) different from normal tissues have been identified and employed for targeted therapy and precise detection. Characteristic signals within TME including acidic pH, hypoxia, high-level reactive oxygen species (ROS), and some over-expressed enzymes, can be used to construct targeted DDSs to tumor sites. Among these, the mildly acidic pH is frequently utilized, which is distinguishable from the blood and health tissues. More importantly, the pH retains constantly across different tumor types (often in the range of 6.5–6.8) [1], making it a universal trigger for various cancers. Meanwhile, progress made in chemistry and macromolecular synthesis methods allows pH-dependent regulation of the cellular uptake and controlled release of DDSs.

As a popular member of DDSs, polymers have the potential to improve the delivery efficiency of various cargoes due to their tunable stability, favorable loading capacity, ideal pharmacokinetics, targeting ability, and biosafety. The almost infinite diversity of polymers in view of the carrier size, drug-polymer interaction (i.e., covalent or non-covalent), multifunctionalities (e.g., attachment of specific ligands, degradability), etc. have paved the way to deliver cargoes from small-molecule drugs to macro biomolecules (e.g., nucleic acids and proteins) [2–5]. However, many biological barriers exist such as destruction from the immune system, non-specific distribution, difficulty in the cellular entrance, and entrapment inside endo-lysosomes [6–8]. Although significant progress has been made to overcome these barriers, only limited examples have been used in clinics [9–11] and fewer have been used to fight against cancer. By taking advantages of TME features [1, 12, 13], stimuli-responsive polymers have thus been developed as promising tools to carry therapeutic drugs or imaging sensors to the tumor site [14–19], and these polymers can undergo physicochemical changes upon exposure to internal (e.g., pH, redox) or external (e.g., light, magnetic field) triggers [17, 18, 20, 21]. Among these various stimuli, pH sensitivity has been widely explored for targeted delivery due to two types of pH difference: (a) pathological versus normal tissues (e.g., lower pH in the TME, as a result of the increase in lactate production produced by the Warburg effect [1, 22]) and (b) acidic membrane-bound vesicles within the cell including endosome and lysosome (as most nanosystems enter cells via endocytosis). The extracellular mild acidic pH value and an even more acidic intracellular environment [23] make it possible to design smart nanosystems for cancer-selective delivery, enhanced cellular uptake, and efficient endo-/lysosomal escape followed by payload release [24].

Accordingly, pH-responsive polymers have been extensively

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explored due to their (i) synthetic flexibility (with a wide range of available pH-sensitive groups/linkers for polymer fabrication or cargo loading) [25–27], (ii) tunable pK_a values corresponding to specific biological pH (e.g., bloodstream pH ≈ 7.4, TME pH ≈ 6.5–6.8, early endosome pH ≈ 5.9–6.2, and lysosome pH ≈ 5.0–5.5 [28, 29]) for improved delivery performance such as high loading, stable formulation, targeted accumulation, high cellular uptake, fast endosomal escape, and sufficient release [30, 31], and (iii) rapid response towards the stimuli [26]. Thus, significant progress has been made in the development of pH-responsive polymers [27, 32–34], providing useful tools for cancer diagnosis and therapy [26, 35–37].

In this review we summarize available pH-responsive groups (charge reversal upon protonation/deprotonation) and acid-labile linkages, illustrating their structure–property relationship and applications in cargo loading/release, cancer targeting, and endosomal escape. This review introduces the recent progress of pH-triggered cancer-targeting strategies, in the hope to facilitate the development of desired carriers and promote the clinical translation of suitable delivery systems shortly.

2   Architectures of pH-responsive polymers

Polymer architecture is an important feature to determine polymers' properties including the size, loading capacity, release behavior, circulation time, and biodistribution [38, 39]. pH-responsive polymers are extensively explored from the simplest linear chain to a wide range of more complicated architectures including graft polymers [40, 41], branched/hyper-branched polymers [26, 35], and dendrimers [42] (Fig. 1(a)). Polymers have

been reported to self-assemble or fabricate into various morphological forms for a wide range of applications (Fig. 1(b)) [43, 44]. For efficient delivery, the polymers should keep stable during circulation and accumulate in larger amounts in the tumor interstitial space for enhanced intracellular delivery, which largely depends on polymers' charge, shape, and size as well as the tumor type [45, 46]. For example, polymeric nanoparticles with a neutral or slightly positive charge and a size range of 12–50 nm in elongated shape would be ideal for transport within solid tumors [46]. In addition, nanoparticles are required to be biocompatible for delivery applications [47].

pH-responsive polymers have two main categories: (i) The responsive units are based on ionizable functional groups, in which pH-induced charge shifting plays an important role in disturbing the hydrophilic–hydrophobic equilibrium inside the architecture, resulting in disassembly or swelling of the nanomaterial to release the cargo or turn on fluorescence; (ii) the polymer contains acid-labile linkages (e.g., acetal, ketal, imine, and hydrazine) which will degrade upon pH decreases (Fig. 1(c)). We will discuss these pH-responsive reactions and their chemical tunability in details with following sections.

3   Classification of pH-responsive polymers based on ionization

According to the charge distribution under physiological condition (pH ≈ 7.4), pH-responsive polymers can be divided into three categories: (a) anionic polymers [24, 48], (b) cationic polymers [24, 48, 49], and (c) zwitterionic polymers [50–52] (Fig. 2).
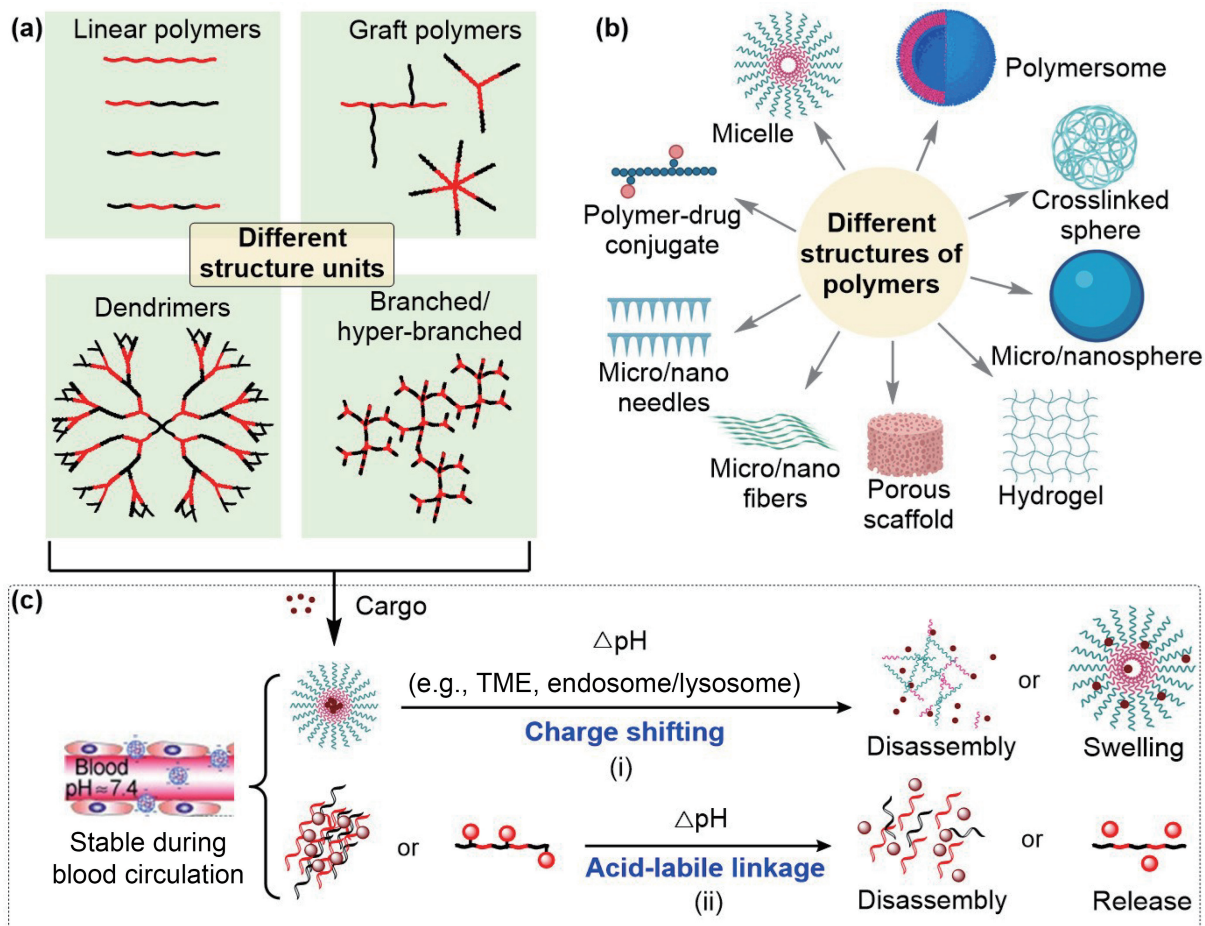

Figure 1   pH-responsive polymers with (a) different structure units and (b) in different morphological structures which may undergo (c) cargo release either via (i) charge shifting (leading to polymer disassembly or swelling) or (ii) breakage of the acid-labile linkage within the polymer or between the polymer and cargo to cause polymer disassembly or drug detachment from the polymer.
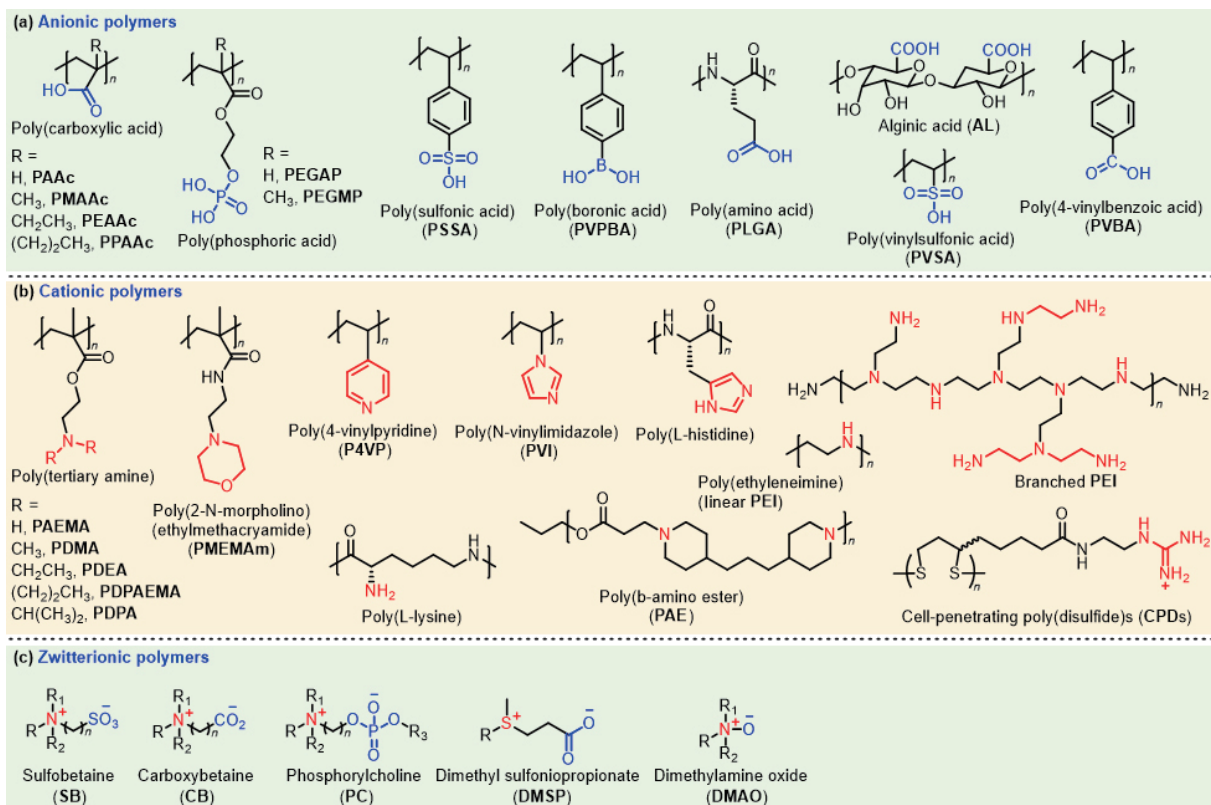
Figure 2 Classification of pH-responsive polymers. (a) Anionic polymers containing side chains include carboxylic acid, phosphoric acid, sulfonic acid, or boronic acid. (b) Cationic polymers fabricated with primary/secondary/tertiary amine, morpholine, pyridine, imidazole, or guanidine, which will be protonated under acidic conditions. (c) Zwitterionic polymers made from structural units containing both cationic and anionic groups.

3.1 Anionic polymers

Usually, anionic polymers have acidic pendant groups, which release protons at pH 7.4 and become hydrophilic, whereas in acidic conditions they get protonated and become hydrophobic [48, 53]. Such transition results in deformation (via swelling or collapse) of the nanostructure or a change in its interaction with the cargo, leading to the cargo release. Representative examples of anionic polymers are polyacids, which are shown in Fig. 2(a) [25–27]. These polymers have wide applicability in delivering catanionic cargoes [54]. For example, the anticancer drug, doxorubicin (DOX), positively charged at physiological pH, can form stable formulations with polyacids during blood circulation while getting released selectively within the acidic TME due to a decrease of electrostatic interaction upon protonation of the polyacids [53].

3.2 Cationic polymers

Cationic polymers with pH sensitivity have ionizable polyamines, that are usually hydrophobic at physiological pH and get protonated in acidic conditions to become hydrophilic (Fig. 2(b)). The pK_a (which is defined as the pH with an equal concentration of the protonated and deprotonated forms) of cationic polymers can be adjusted by chemical modification of the side amine groups [55–58]. These cationic polymers have the advantages of enhanced cellular uptake and endosomal escape. For example, poly(ethylene imine) (PEI) and poly(arginine)s possessing positive charges are widely utilized for gene delivery [59]. They can also be used in combination with anionic polymers to tune the overall charge for better pharmacokinetics. To be noted, biodegradable backbones are warmly welcome these days to minimize the side effects on biological systems. Polymers containing disulfide bonds and polyorganophosphazenes (POPs) have attracted increasing attention as they can undergo spontaneous degradation for efficient cargo release with minimal inherent cytotoxicity [60–65],

which is particularly important for detection and modulation purposes. It is now possible to prepare a polymer with the desired pK_a value between 1 and 14 by using a wide range of pH-responsive groups [26]. Among these, tertiary amine-based polymers have been widely studied due to their finely tunable hydrophobicity and pK_a via different substitutions (Fig. 3(a)) [28, 58, 66]. This strategy has been well documented not only for therapeutic cargo delivery but also for diagnostic detection, where small pH changes (0.21 pH units) can be visualized [28, 29, 67].

Pioneered by Gao et al., ultra pH-sensitive (UPS) nanoparticles based on tertiary amines with exactly measured hydrophobic substituents as ionizable blocks have been developed to meet fast temporal response and high sensitivity to narrow pH variations [29]. By using poly(ethylene oxide) as the hydrophilic component and two different tertiary amines as ionizable hydrophobic blocks, two ultra pH-sensitive nanoprobes (UPS$_e$ and UPS$_i$) embedded with hydrophobic fluorophores (e.g., cyanine) were prepared, to achieve their ionizable block copolymer micelles upon self-assembly [28]. And the inherent fluorescence was quenched in this supramolecular nanoparticle form due to photoinduced electron transfer (Pet) and homo-fluorescence resonance energy transfer (FRET) effects [29], which can be restored by acidic pH (pH < pK_a) within TME (for UPS$_e$) and endo-/lysosomes (for UPS$_i$), respectively, as charged ammonium groups were formed to promote micelle dissociation along with fluorescence emission (Fig. 3(b)). The developed UPS imaging platforms can facilitate to depict a high-resolution imaging of primary and metastatic tumors, promising to contribute to entire tumor resections during surgery.

A more recent study showed that above-mentioned UPS system can be generalized to the entire physiological pH range of 5.0–7.4 for precise fluorescent imaging of endosome maturation and cancer identification. By copolymerization of tertiary amine-containing monomers with a series of non-ionizable monomers, the pH transitions of resulting nanoparticles can be fine-tuned,
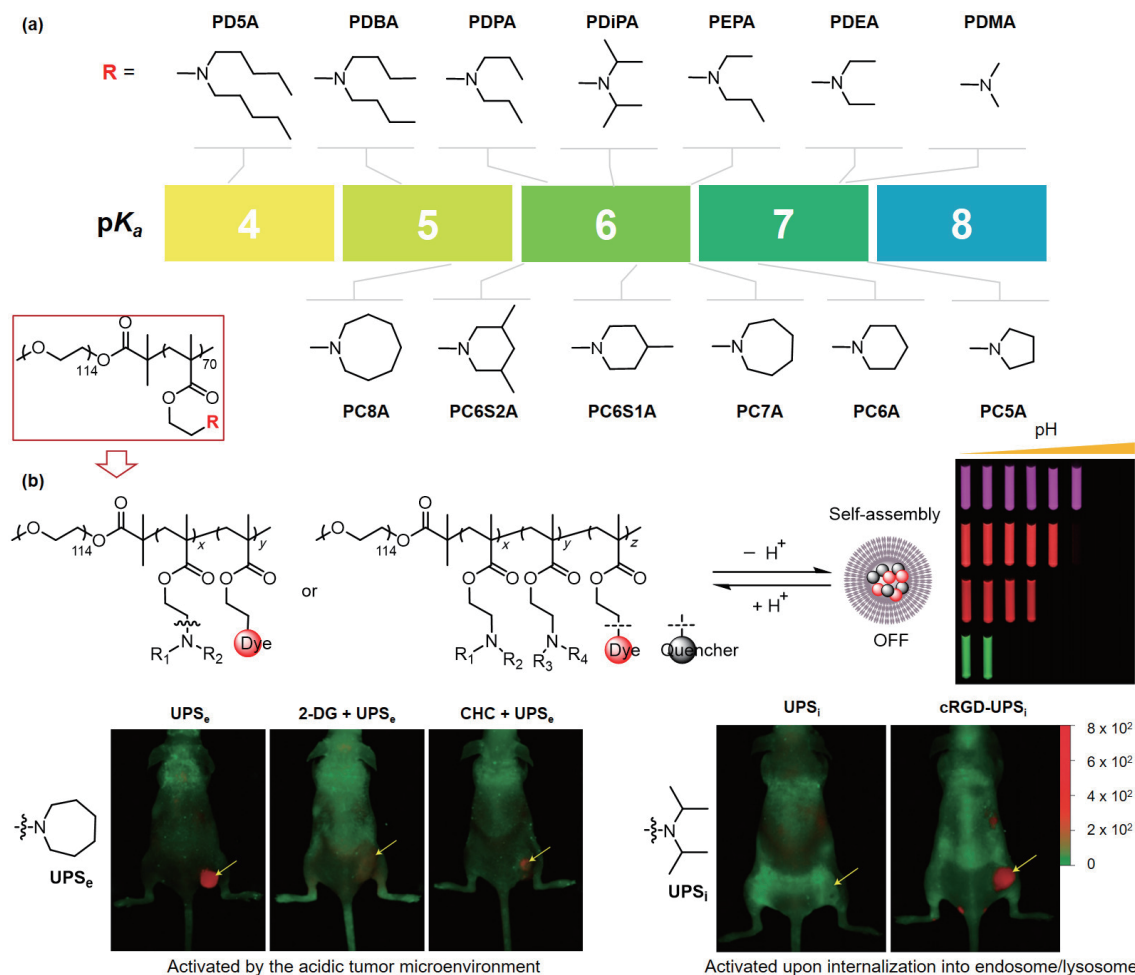
Figure 3 Tertiary amine-based polymers with tunable pK_a. (a) Variations in pK_a of polymers via structural changes in tertiary amine chemistry. (b) Schematic illustration of the self-assembled UPS nanoparticles with quenched fluorescence via Pet and homo-FRET effects of the dye or assisted by the quencher (up), which will be activated to liberate fluorescence upon pH changes. Chemical structures of nanoprobes, UPS$_e$ and UPS$_i$, and their use to selectively visualize the TME or acidic endocytic organelles (down). 2-DG: 2-deoxy-d-glucose, competitively inhibits glucose uptake. CHC, α-cyano-4-hydroxycinnamate, a suicide inhibitor of monocarboxylate transporter that prevents the secretion of lactic acid from cancer cells. cRGD: cyclic RGD targeting tumor cells. Reprinted with permission from Ref. [28], © Nature Publishing Group 2013.

which might be a powerful toolkit for biological studies and cancer theranostics to precisely monitor the subtle pH fluctuation during biological events [68]. Furthermore, this strategy has also been used for monitoring the cytosolic entry of cell-penetrating peptides [69], digitizing organelle pH after receptor-mediated endocytosis in tumor cells [58], and boosting anti-tumor immunity for cancer immunotherapy [34, 70].

3.3 Zwitterionic polymers

Zwitterionic polymers have been employed for constructing charge-reversal noncarriers sensitive to pH changes by tuning the molar ratios between cationic and anionic groups [19]. Those that contain an identical number of cationic and anionic groups on each monomer throughout the polymeric backbone are also called amphoteric polymers, which keep highly hydrophilic with reduced nonspecific protein adsorption in an overall neutral charged status [71–73]. Examples are given in Fig. 2(c), which have been widely explored for diverse biomedical applications such as drug delivery, antifouling coatings of biomedical implants, and protein stabilization [51, 52, 74–78], largely due to their negligible immunogenicity, low cytotoxicity, systematic stability, and long circulation time [74].

Moreover, these polymers are capable to exhibit pH-responsive charge conversion, simply by changing the ratio of anionic and cationic groups to adjust their isoelectric points [33, 75]. In addition, more flexibility can be introduced by incorporating

specific pH-responsive groups. For example, Mizuhara et al. [79] engineered a zwitterionic-surface nanosystem by using the acylsulfonamide derivatives, which compose trimethyulammonium termini and an aryl acylsulfonamide (Fig. 4(a)). The resulting nanoparticle showed a sharp charge shift from neutral to cationic within TME, which exhibited high cellular uptake due to effective electrostatic interactions with negatively charged plasma membrane and/or stronger disruptive influence on the bilayers [80]. In contrast, when the aryl acylsulfonamide was replaced by an alkyl analogue, the nanoparticle kept the zwitterionic surface even at acidic pH, consistent with the lower pK_a of alkyl acylsulfonamides. By taking advantages of such zwitterionic-to-cationic charge shift for targeted cancer delivery, a zwitterionic poly(carboxybetaine) (PCB)-derived nanomedicine was reported recently by Wang et al. [81]. To achieve efficient charge conversion from neutral to positive within TME, an acid-labile group was introduced (Fig. 4(b)), showing promising opportunities to deal with a broad range of cargoes due to their low systemic toxicity and high efficiency.

4   Interactions between the cargo and polymer

For pH-sensitive polymers, different strategies have been reported for cargo loading, ranging from physical adsorption via different non-covalent interactions such as (a) hydrophobic interaction [55], (b) electrostatic interaction [82], and (c) π–π interaction [83, 84] to (d) covalent linkage [85] (Fig. 5).
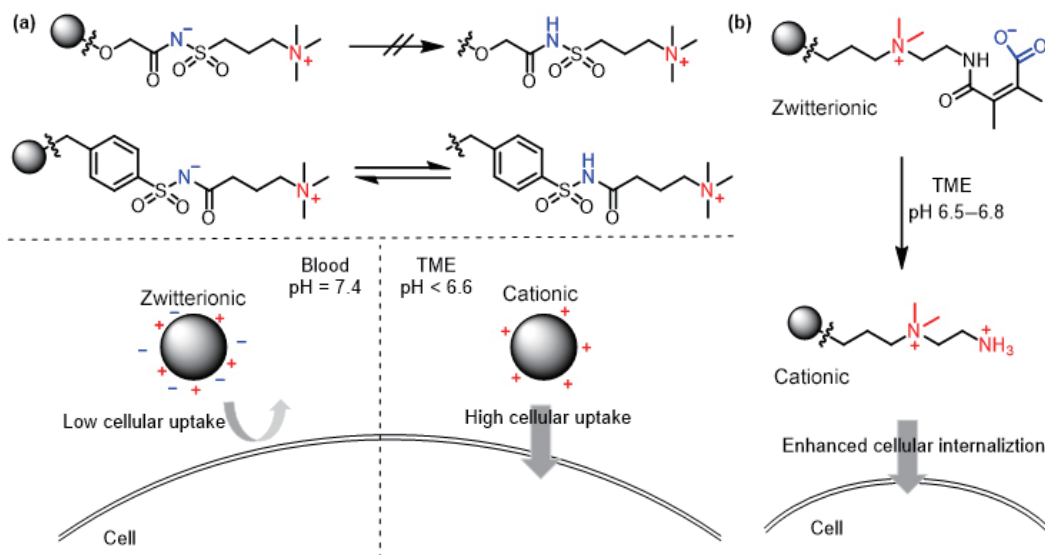
Figure 4 Schematic illustration of pH-sensitive zwitterionic polymers developed for cancer targeting. (a) Aryl acylsulfonamide-functionalized zwitterionic carrier for enhanced cellular uptake at acidic TME, in sharp contrast to its alkyl analogue which did not show such charge shift at acidic pH [79]. (b) Zwitterionic-to-cationic charge shift system for cancer-selective delivery by using acid-labile caging groups [81].

In general, the relative strength of non-covalent interactions is in the sequence of "electrostatic interaction > hydrogen bond > π–π interaction" [86]. Different from non-covalent loading, covalent binding is stoichiometric and more controllable. Very recently our group reported the preparation of pH-responsive tumor-targeting cell-penetrating poly(disulfide)s (CPDs) for direct cytosolic delivery of antibodies by taking advantage of the protonation of imidazole moieties within the acidic TME [85]. This newly, easily-operable, and direct cytosolic delivery strategy bypassing endocytosis is promising for *in vivo* applications, and the neutral charge of the polymer minimized non-specific binding and improved stability during blood circulation.

Polymeric nanoparticles which exhibited pH-responsive charge shifting properties have been widely studied due to their capabilities to deliver therapeutic agents selectively to tumor sites and accelerated release from the endo-/lysosome trapping. These nanoparticles with a nearly neutral or negative charge in the blood circulation prolonged the circulation time as required for nanoparticles to reach the targeted tumor sites sufficiently, while their zeta potential switched into a positively charged form in the acidic environment of tumor, enhancing cell internalization for targeted drug delivery. Although significant progress has been made in this area, the surface charge shifting from negative to positive takes from several minutes to several hours, and the efficiency should be taken into consideration in practical applications [87].

5 Covalent interactions: acid-labile bonds

5.1 Summary of acid-labile linkers

Not only non-covalent interactions have been employed in the design of pH-responsive polymers, but acid-labile covalent linkage has also been brought in. Acid-labile linkers including maleic acid amide (MAA) and benzoic amines, which can exhibit cleavage in a slightly acidic environment, are widely exploited to create targeted delivery platforms. By design, these delivery systems exhibit stable formulation and enough stability during systemic circulation before reaching the targeted tumor site. After reaching the acidic TME or the endo-lysosome, the acid-sensitive linkers are cleaved by hydrolysis, resulting in disassembly or release of the payloads. The general structures of commonly used acid-labile bonds and the corresponding hydrolyzed products are summarized in Fig. 6 [11, 26, 27, 32]. Usually, the acid-labile bond is introduced either in the polymer structure (incorporated into the polymer backbone or the side chain) or as the linkage between the polymer and cargo [88].

The incorporation of acid-labile bonds at the junction of hydrophilic and hydrophobic blocks achieves shell-sheddable micelles, which have interesting implications in intracellular delivery of biomacromolecules [78–80] or co-delivery of toxins deeper into a solid tumor for combination therapy [81] or even targeting the cellular organelles such as mitochondria [82]. Since some pH labile bonds (e.g., ortho ester, hydrazone) hydrolyze rapidly only at lower pH (< 6.0), they are mainly used for intracellular drug release after endocytosis, not suitable for tumor extracellular matrix (pH 6.5–6.8) [26]. Therefore, special attention is given to those bonds with tunable pH sensitivity or responding to mild acidic conditions such as the TME.

5.2 Maleic acid amide bonds

Amide bonds are supposed to be stable under most conditions (i.e., degrade at pH < 1 or pH > 13), however those β-carboxylic acid amides (β-CAs) containing *cis*-α,β-double bond (also called MAA) can be degraded into corresponding amine derivatives and anhydrides/dicarboxylic acids under weakly acidic conditions [89]. With a negative charge at neutral pH due to the free carboxyl groups, these MAA derivatives can hydrolyze to release the positively charged amine groups under acidic conditions, causing a negative-to-positive charge switching [90]. Such pH-sensitive charge conversion is of particular interest and has been extensively studied for the effective delivery of small-molecule drugs, nucleic acids, and proteins with selective and enhanced cellular uptake at TME [91]. Furthermore, these pH-sensitive linkers can also help in manipulating the size of nanoparticles to cope with the challenges such as having a big enough size to evade the renal clearance during blood circulation while changing to a smaller size within the target TME for enhanced retention and penetration [91, 92].

The pH sensitivity of MAA is due to the internal attack of the amide carbonyl group by the β-carboxylate [93]. More interestingly, it was found that their pH sensitivity can be fine-tuned to degrade at relatively mild acidic conditions by altering the functional groups attached onto the *cis* double bonds [24, 90]. Studies have revealed that the greater the number of substituents on the *cis* double bonds is, the more sensitive to acidic pH the
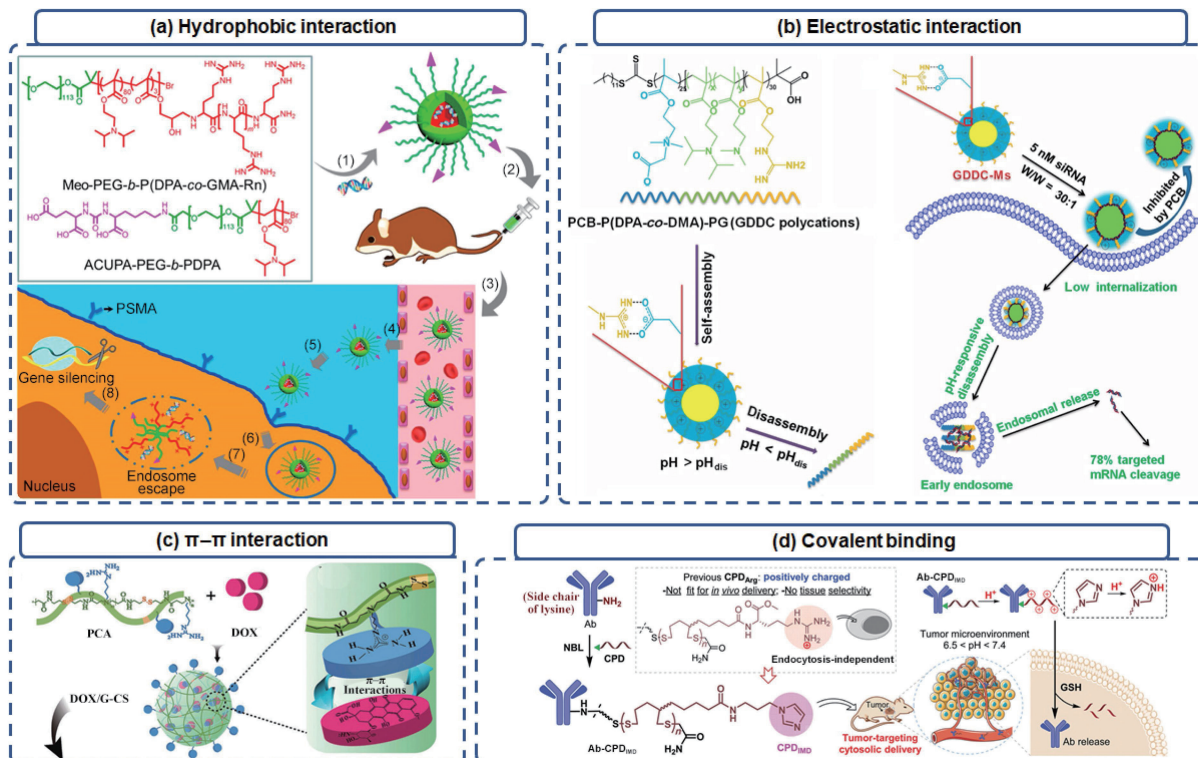
Figure 5 Cargo loading strategies with pH-sensitive polymers. (a) Hydrophobic interaction. Reprinted with permission from Ref. [55], © American Chemical Society 2017. (b) Electrostatic interaction. Reprinted with permission from Ref. [82], © American Chemical Society 2016. (c) π–π interaction. Reprinted with permission from Ref. [83], © The Royal society of chemistry 2018. (d) Covalent binding. Reprinted with permission from Ref. [85], © The Royal society of chemistry 2022.
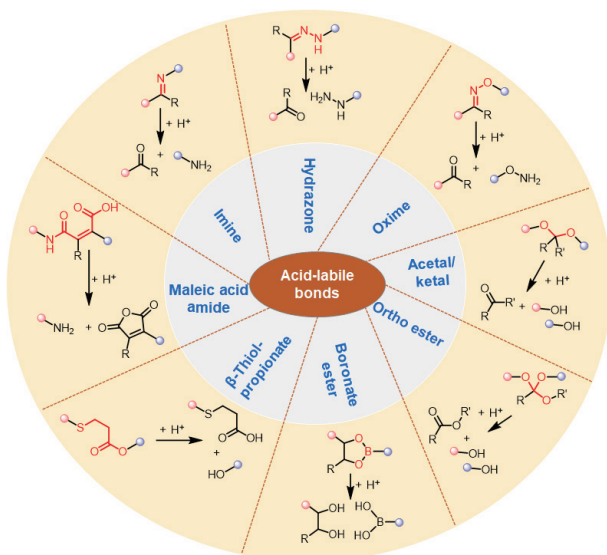

Figure 6 Summary of currently available acid-labile bonds including imine, hydrazone, oxime, acetal/ketal, ortho ester, boronate ester, β-thiol-propionate, and maleic acid. The corresponding hydrolyzed products at acidic pH are presented as well.

MAA derivative is. Figure 7(a) shows a list of representative MAA derivatives and their pH sensitivity [76, 93–95].

As 2,3-dimethylmaleic amide (DMMA) has a relatively fast charge-reversal rate at pH 6.8, Du et al. [96] employed it in preparing the charge-conversional nanogel for intracellular release of DOX as triggered by acidic TME. This nanogel furnished with DMMA side chains exhibited high loading of DOX due to electrostatic interactions of positively charged DOX and negatively charged carboxylate groups, along with high stability at physiological pH. The slightly acidic TME resulted in the cleavage of the amide bond of DMMA to liberate positively charged amines, accelerating the cellular uptake of DOX (Fig. 7(b)).

In another study, Su et al. [97] took advantage of the different pH sensitivity of two MAA derivatives (i.e., MCM sensitive to $pH_e = 6.5–6.8$ and Cit sensitive to $pH_i = 5.0–6.0$, respectively) to construct a tandem pH-triggered nanogel system (denoted as CDM-MA-NG) by using cargo protein as a crosslinker (Fig. 7(c)). CDM-MA-NG was fabricated by *in situ* crosslinking of proteins (amine groups on protein surface) with PEG containing mono-substituted maleic anhydride (MA) to give Cit, followed by surface functionalization with bis-substituted maleic anhydride (CDM) to form MCM linkage.

The obtained nanogel showed significantly high protein loading efficiency (71%) with high stability under normal physiological environments and effective protection of the crosslinked cargo protein from serum fouling, proteolytic and thermal degradation. Systemic administration of therapeutic proteins-embedded CDM-MA-NG showed the great potential to suppress the tumor growth and hence, extended the survival rate.

5.3 Imine bonds

Among the acid-labile linkers, the most frequently used are amide linkers, followed by the imine linkers [24]. Imine (C=N) and its family of benzoic imine, hydrazone, and oxime with acid-labile linkages have been widely discovered as effective intracellular delivery vehicles. The carbon-nitrogen double bond is formed via condensation reaction between aldehydes/ketones and primary amines (also called "Schiff base reaction"). Additionally, this reaction is reversible.

The acid-catalyzed hydrolysis rate of the imine bond depends on pH and temperature. Usually, acidic pH and increased temperature accelerate the hydrolysis rate, and some researchers agree that imine bonds produced with alkyl amines and alkyl aldehydes are not stable enough even at physiological environments (i.e., pH 7.4 at 37 °C) [32]. Thus, it has been proposed that stability can be improved by conjugating with the π-bond to form benzoic-imine bonds. For instance, Su et al. [98] synthesized a copolymer (mPEG-C=N-PAsp(MEA)-CA)
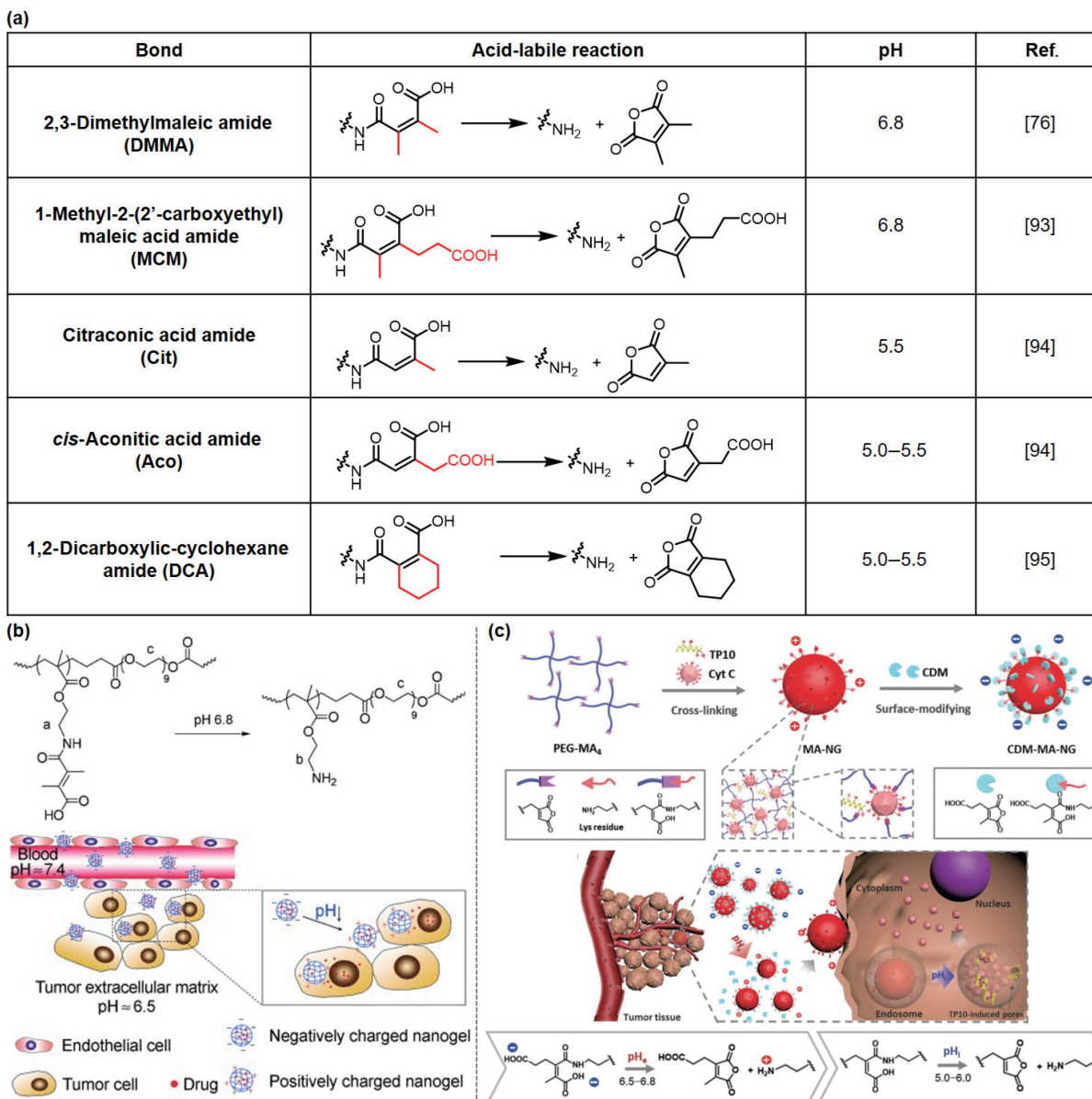
Figure 7 (a) pH-sensitive hydrolysis of maleic acid amide derivatives [76, 93–95]. (b) Illustration of pH-responsive and charge-conversional nanogel containing DMMA in the TME which enhanced cellular uptake by tumor cells. Reprinted with permission from Ref. [96], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2010. (c) Design and synthesis of the tandem pH-responsive nanogel CDM-MA-NG. Reprinted with permission from Ref. [97], © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2018.

containing mPEG, polyaspartic acid (PAsp), cholic acid (CA), thiol groups (aminoethanethiol (MEA)), and imine linkage (Fig. 8(a)). Acid labile benzoic imide bond was introduced between mPEG and PAsp in the backbone, which exhibited charge reversible property, and thiol groups were incorporated in the side chain to form a disulfide crosslinked interlayer to minimize the premature drug leakage in the blood circulation.

The self-assembled micelle showed good loading capability of hydrophobic anticancer drug DOX and a hydrophobic near-infrared fluorescence (NIRF) dye (DiR) by providing the micelle core to encapsulate hydrophobic drugs via hydrophobic interaction [99], with faster degradation and enhanced cell adherence and proliferation due to CA [100]. Most importantly, the cleavage of the benzoic imide bond within acidic TME resulted in efficient cellular uptake followed by GSH-dependent release of DOX in cancer cells. Overall, this pH and reductive responsive micelle might provide an effective delivery system with improved therapeutic efficacy and reduced non-specific toxicity.

Furthermore, the hydrolysis rate of the imine bond can be adjusted by changing the substituents linked to imine bonds via their inductive, resonance, and steric effects [88, 101]. In general, electron-withdrawing groups attached to the imine bond result in better hydrolysis stability. For example, imines substituted with electron-negative O and N atoms (C=N–O, oximes; or C=N–N, hydrazones), considerably increase the stability of hydrolysis which is due to a decrease in electrophilicity by the mesomeric effect [88]. Hydrazone bonds can be formed upon the condensation of hydrazide groups (from the polymers) with ketone/aldehyde groups (from the cargoes), while the approval of their development and regulation is hard to be realized in practice. Moreover, the cationic polymer residues generated after hydrazone bond degradation, may bring some cytotoxicity, which limits their further applications. Oximes are found to be more stable toward hydrolysis as compared to hydrazones, largely due to their resistance against protonation by the stronger inductive effect of the more electron-negative oxygen atom. For example, Kalia et al. showed that oxime degraded much more slowly than most simple hydrazones (except the trialkylhydrazonium ion) at acidic conditions [102]. The general order of hydrolytic stability is given in Fig. 8(b). Overall, the unique imine chemistry with easy
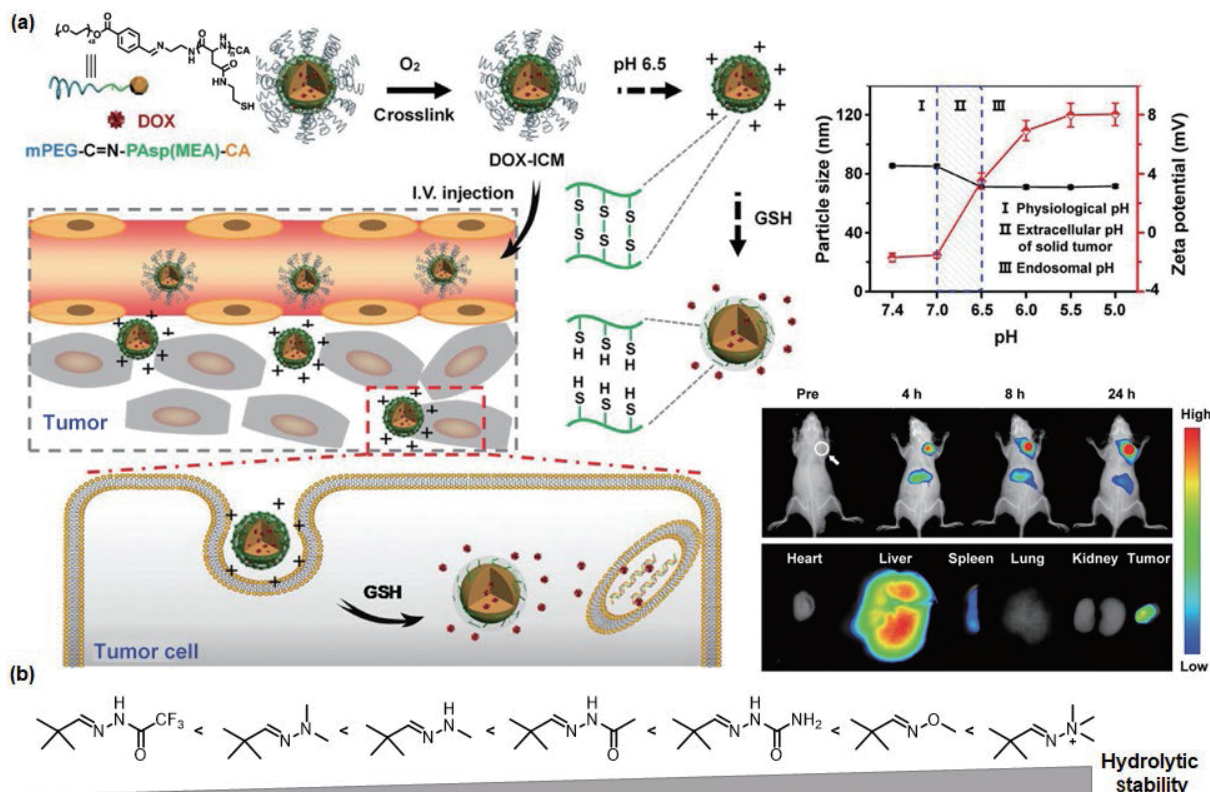
Figure 8 (a) Preparation of the DOX-loaded pH- and GSH-dual responsive micelle (DOX-ICM) employing benzoic-imide linkage, and its responsiveness *ex vivo* and *in vivo* towards pH change. Reprinted with permission from Ref. [98], © The Royal society of chemistry 2019. (b) The general order of hydrolytic stability of different hydrazones.

synthesis and tunable hydrolysis reactivity makes it promising for intracellular delivery applications while special attention needs to be paid in choosing the proper structure [88].

5.4 Acetal/ketal linkers

Among various pH-responsive linkers, acetals and ketals have also been widely explored as they can liberate neutral and non-toxic byproducts upon hydrolysis. Furthermore, their facile synthesis makes it possible to fine-tune the degradation behavior to the desired range of pH [103]. For instance, Liu et al. conducted a detailed mechanistic study by changing the substitutions of acetal- and ketal-based linkers to investigate their effect on pH-dependent degradation in six different series of molecules (a total of 18 molecules) [103]. Polymeric nanogels containing these acetal and ketal linkers were prepared to investigate the correlations in host–guest characteristics with pH variations (Fig. 9(a)). The relative hydrolysis rate at pH 5 was tabulated by using the unsubstituted benzylidene acetal 13 as the standard (whose reaction rate was set as 1) to understand the reaction kinetics of these acetal/ketal molecules. As illustrated by the results, an increase in distance between the electron-withdrawing moiety (amide) and the ketal moiety resulted in a decrease in half-life (molecules 1 and 3 containing linker length of 2 and 6 carbons have a half-life of 32 h and 24 min, respectively), attributed to a decrease in stability of ketal moiety and an increase in stability of carboxonium ion intermediate by lowering the transition energy. This detailed mechanistic study of structure-property correlations provides a comprehensive overview of structural variations to alter the pH-sensitivity of acetal- and ketal-based linkers, offering a wide range of polymeric materials containing acetal/ketal linkers for effective delivery with desired release patterns for applications not limited to drug delivery, tissue engineering, and diagnostic applications.

In another example, Chauhan et al. [104] constructed a diverse

library of acid-cleavable polymers via high-throughput combinatorial chemistry by acetal exchange or polycondensation reactions between polyol and acetal or between polyol and vinyl ether monomers, into a library of unique polyacetals (Figs. 9(b)–9(d)). A high-throughput fluorometric pH-sensitivity assay was conducted using fluorescence quenching of Nile red upon release from the particles into an aqueous buffer (Fig. 9(e)). In this study, the acetals were found to be hydrolyzed faster than the ketals at acidic pH (e.g., E3C1 vs. E1C1), whereas ketals obtained from cyclic ketone monomers usually exhibited slower hydrolysis rates than those from acyclic monomers (e.g., E2O1 vs. E1O1). Furthermore, the polyacetal (T4-VE3) obtained from the reaction of 1,1,1-tris(hydroxymethyl)ethane (T4) and di(ethylene glycol) divinyl ether (VE3) was identified as the most selective towards tumor pH against physiological pH. By chemically linking angiotensin receptor blocker (ARB) drugs to this T4-VE3 polyacetal through an ester linkage, the resulting TMA-ARBs were inactive during blood circulation and significantly accumulated in tumors via enhanced permeability and retention effect (EPR), wherein they degraded in response to the slightly acidic environment to release active ARBs. With this design, significantly enhanced responses to immune-checkpoint inhibitors in mice bearing primary and metastatic breast cancers were achieved.

Moreover, since the acetal protecting group strategies are useful tools for temporary blockage of specific diols, dextrans made from polysaccharides have been widely explored to prepare acetalated dextran (Ac-Dex) as a potential candidate for drug delivery applications [105, 106] as well as theranostic purposes [107].

5.5 Other pH-sensitive linkers

Recent progress in organic chemistry and nanotechnology provides promising opportunities to achieve new pH-sensitive linkers. We list here some interesting pH-sensitive linkers such as (a) silyl ether linkers [108–111], (b) 1,2-cyclohexanecarboxylic acid
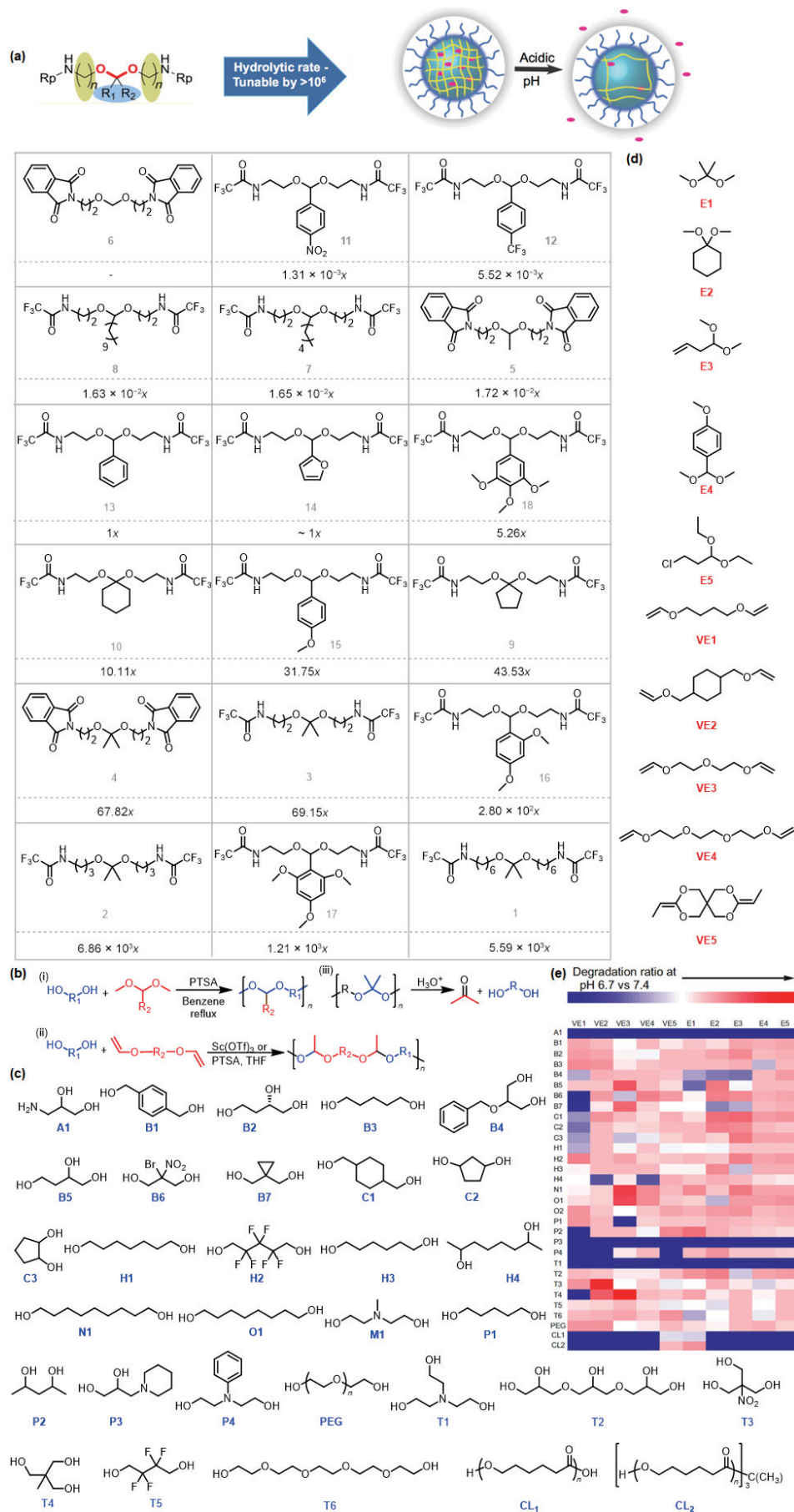
Figure 9 (a) Scheme of drug encapsulated polymeric nanogels containing pH-cleavable acetal/ketals cross-linkers, and relative hydrolysis rates tabulated of different acetal/ketals containing nanogels. Reprinted with permission from Ref. [103], © American Chemical Society 2017. (b) Modular acetal exchange (i) and polycondensation (ii) reactions to form polyacetals and their hydrolysis in acidic conditions (iii). Sets of (c) 31 polyols and (d) 5 acetals together with 5 vinyl ethers for polyacetal synthesis. (e) High-throughput fluorometric assay to investigate pH-sensitivity of newly assembled polyacetals. Reprinted with permission from Ref. [104], @ Chauhan, V. P. et al. 2019.

amides [112, 113], (c) phosphoramidate-based linkers [114], and (d) triazole-based self-immolative linkers [115] (Fig. 10). To be specific, silyl ethers are widely used as protecting groups for alcohols with tunable deprotection rate under acidic conditions by alternation of the alkyl substituents on the silicon atom. To further increase the water solubility of polymers containing silyl ether groups before and after the bond cleavage, an asymmetric bifunctional silyl ether was reported (Fig. 10(a)) [108], whose hydrolysis rate increased as the steric bulk of the substituent on the silicon atom decreased. This chemistry was further applied to pH-dependent release of parental drugs from nanocarriers [109, 110] as well as antibody-drug conjugates [111].

Other pH-sensitive linkers based on amines have also been substantially explored. Amides with neighboring carboxylic acids also show pH-dependent hydrolysis, among which amides made from PEI and *cis*-1,2-cyclohexanedicarboxylic anhydride were reported to hydrolyze at pH < 6 (Fig. 10(b)). The resulting PEI demonstrated a negative-to-positive charge reversal, promising for selective drug uptake and release in cancerous tissues with reduced non-specific binding during blood circulation [112]. In addition, Berkman et al. reported a series of pH-responsive phosphoramidate-based linkers capable to release amine-containing drugs at various pH values (ranging from 7.4–3.0) (Fig. 10(c)) [114]. The hydrolysis is dependent on the pK_a of the leaving amine (e.g., primary, secondary, and aniline) and can be tuned by altering the distance between a neighboring weakly acidic moiety (e.g., carboxylic acid or pyridinium) and the phosphorus center. The wide range of stability at different pH values makes

phosphoramidate promising in the design of selective bond cleavage within the TME or inside the cellular endocytic cycle. More recently, a self-immolative triazole-based system was developed in which cleavage of the triazole intermediate was switchable depending on the environmental pH (Fig. 10(d)) [115]. Different from other acid-labile linkers, this tirazole linker was significantly stable under acidic conditions, while releasing the payload in basic conditions/upon deprotonation. As conveniently prepared using the alkyne-azide cycloaddition reaction, this system shed light on the development of new stimuli-responsive systems for controlled-release applications.

6 Conclusion and future remarks

The tumor microenvironment has distinctive features compared to normal tissues, such as slightly acidic environment of pH (6.5–6.8), hypoxia, and elevated levels of ROS and certain enzymes, providing opportunities to develop smart stimuli-responsive polymers to deliver various therapeutic or diagnostic agents to the tumor site. In particular, pH-responsive polymers have attracted great attention due to a number of advantages such as tunable sensitivity towards a narrow range of pH change, controllable drug release via charge shift or bond cleavage, and enhanced endosomal escape, thus highly promising for precise cancer diagnosis and therapy. Many sophisticated strategies have been adopted to design pH-responsive polymeric nanomaterials by using pH-responsive charge reversal groups or acid-labile linkers for delivering therapeutic and diagnostic agents. These
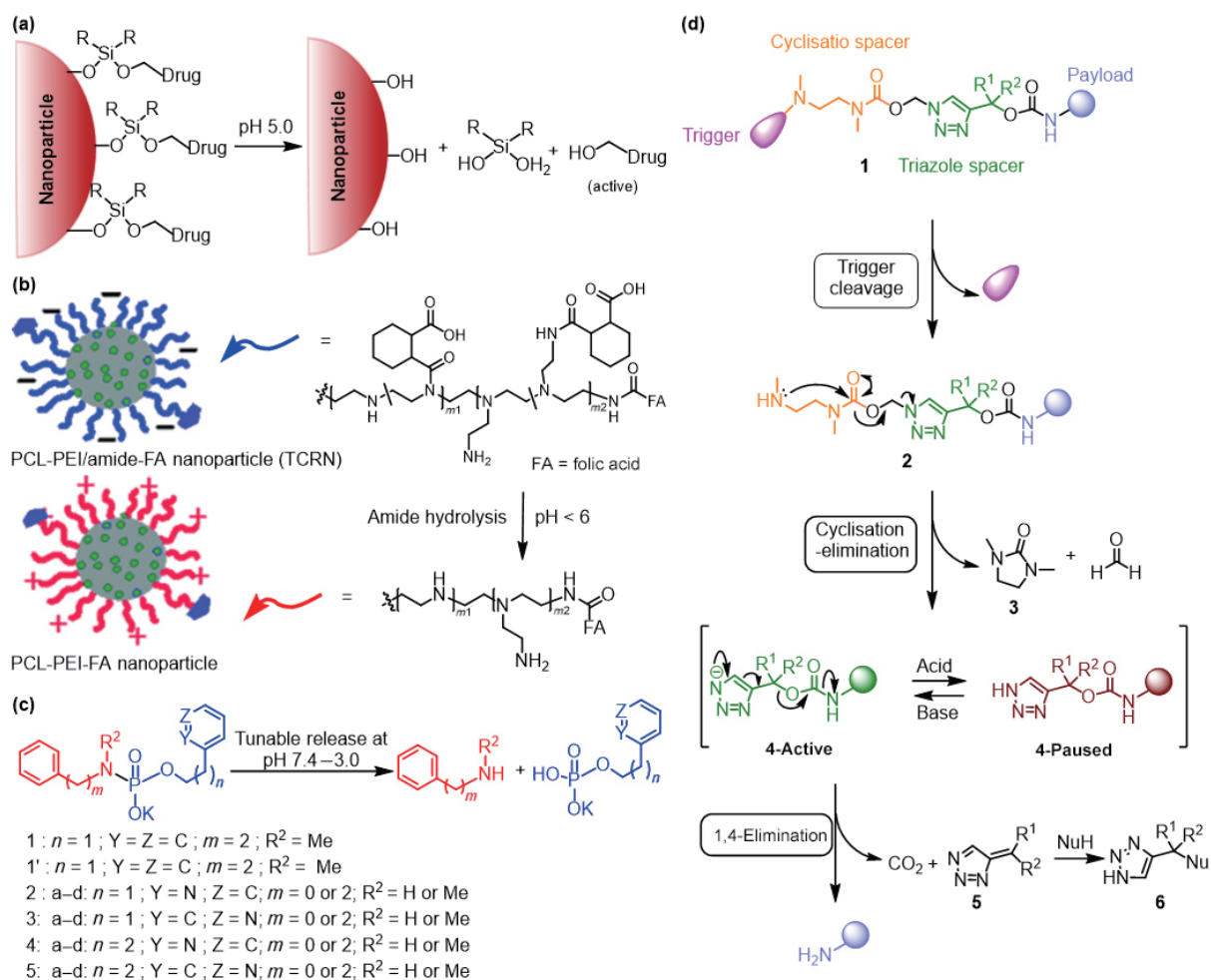

Figure 10 Other pH-sensitive linkers, including (a) silyl linker whose hydrolysis rate increased as the steric bulk of the substituent on the silicon atom decreased [108], (b) 1,2-cyclohexanecarboxylic acid amide cleavable at pH < 6.0 [112], (c) phosphoramide based linker with tunable bond stability at a wide pH range (from pH 7.4 to 3.0) [114], and (d) triazole-based self-immolative linker used for controlled drug release at basic pH [115].

strategies enable the polymers to selectively deliver the cargo specifically in response to changes in pH of tumor extracellular fluid or after endocytosis in acidic compartments such as endosomes or lysosomes, showing significant achievements to overcome the delivery challenges such as non-specific binding, low cellular uptake, endosomal entrapment and subsequent lysosomal degradation.

To be noted, there are still many issues waiting to be addressed before the successful clinical translation of pH-responsive polymers. Namely the biosafety issues related to premature release of the drug and long-term toxicity of the delivery vehicles, uncertainty of the exact release efficiency of drugs/reagents in the tumor site/inside the tumor cells, and effects on the environment (e.g., pH change, accumulation of byproducts from polymer degradation) due to the pH stimulated polymer change, large-scale manufacturing difficulties, and the non-specific accumulation of polymers in normal organs like the liver. For example, some reported studies demonstrated endosomal escape, but the efficiency remains low, which is even more serious for intracellular delivery of nucleic acids and proteins that may lose the activity during this process. These challenges invite the researchers to develop new pH-responsive polymeric nanomaterials with trackable release to better evaluate the delivery outcome [116, 117], bypassing the endocytosis via direct cytosolic delivery to facilitate the intracellular delivery of nucleic acid/protein cargoes, etc. [85, 118]. In addition to single pH-responsiveness, further incorporation of other stimuli to get dual- and multi-responsiveness can significantly increase the selectivity and overall efficiency/biosafety [5].

Acknowledgements

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