Review

Self-immolative polymers in biomedicine

Yue Xiao,[a,1] Xuyu Tan,[b,1] Zhaohui Li[a]* and Ke Zhang[b]*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Self-immolative polymers (SIPs) have been under development for over a decade, and efforts in application followed shortly after their inception. One main area of application is biomedicine, where SIPs are used to construct devices and biosensors, to develop new biotechnology abilities, or to directly interface with the living system. Where traditional polymers are stable at room temperature, SIPs undergo rapid degradation when a labile capping group is removed, allowing SIPs to offer a highly unusual degradation profile compared with traditional polymers. This review summarizes recent efforts to leverage the unique properties of SIPs for biomedical purposes, which are categorized into sensors, drug delivery, and biotechnology. By doing so, this review aims to stimulate future studies in this rapidly growing and promising area.

Introduction

Biodegradable polymers not only have emerged as environmentally friendly alternatives to commodity plastics, but are increasing finding their adoption in the rapidly growing field of biomedicine.[1-4] Traditional biodegradable polymers such as polysaccharides[5] and polyesters[6] typically consist of hydrolyzable bonds in the polymer backbone. Because every such bond can undergo hydrolysis, a gradual, diffusion-limited degradation profile starting from the surface of the materials is often associated with this type of polymer.[7] For many applications in biomedicine, however, materials that can maintain their integrity under physiological conditions, but can undergo rapid degradation on demand, are desired.[8]

Self-immolative polymers (SIPs) offer exactly such an unusual degradation profile. In a typical SIP, cleavage of the capping group by a specific chemical or biological agent initiates a cascade of irreversible, intramolecular fragmentation reactions, leading to the complete disintegration of the polymer into small molecule components.[9-14] Thus, a single scission event can lead to multiple degradation reactions. The development of SIPs can be traced back to self-immolative spacers,[9, 15-17] which were originally developed for prodrug chemistry in 1981.[18] SIPs consist of multiple repeating self-immolative spacers, and were first reported by three groups in 2003 in the form of dendrimers.[19-21] In 2008, the first linear SIP was reported by Shabat and coworkers.[22] Since then, new self-immolative chemistries,[10, 11, 13] polymer architectures,[12, 23] and applications[12, 24, 25] have been extensively explored. There has been a review article about self-immolative structures roughly every two years since 2012, with topics spanning molecular amplification, self-immolative chemistry, and trigger-controlled decapping.[9-14] The current review assumes familiarity with self-immolative chemistries,[13, 25] and instead focuses on a subset of SIPs that are either chemically and biologically compatible with living systems or can be adopted for other biomedical fields such as sensor development and biotechnology, which do not require directly interfacing the SIP with living systems.

Challenges for SIPs in Biomedicine

While essentially all polymers are thermodynamically unstable at sufficiently high temperatures, SIPs can rapidly degrade at room temperature once the SIP end-cap is removed by an external trigger, which can include pH, temperature, redox conditions, ions, enzymes, light, among others.[9-14] The exposed reactive chain-end undergoes a series of head-to-tail, domino-like depolymerization reactions (typically sequential elimination or cyclization), which completely convert the SIP into small molecule fragments. Because each de-capping reaction produces many molecules of the fragment, SIPs have the potential to provide a high degree of amplification, be it chemical signals or drug release.[26] In addition, the SIPs can be used to form a bulk material or higher-order assemblies such as micelles, vesicles, and nanoparticles. Upon degradation, the structural integrity of the material on a mesoscopic or macroscopic level is rapidly compromised, leading to a steep change in their properties.

Several distinct classes of SIPs have been explored to date, including (thio)carbamates, (thio)carbonates, phthalaldehydes, benzyl ethers/esters, and glyoxylates (Fig. 1).[13] However, not all of these chemistries are immediately suitable for biomedical applications. At least three factors must be considered: 1) compatibility between the degradation chemistry with aqueous media, 2) rates of hydrolytic vs. triggered degradation, and 3) toxicity of the polymer and degraded fragments. For example, linear poly(benzyl ethers) (PBEs), first developed by Phillips and

[a] College of Chemistry, Green Catalysis Center, Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, Zhengzhou University, Zhengzhou 450001, P. R.
[b] Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115, United States
[1] These authors contributed equally

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

co-workers, is a highly versatile class of SIPs with a hydrolytically stable polymer backbone, facile chain-growth synthesis, and possibility for side-chain initiated depolymerization.[23, 27-32] However, because the reactive chain-end facilitating degradation is a phenoxide anion (conjugate acid pKa of ~10), protonation in aqueous media at neutral or acidic conditions would cap the phenolate and dramatically slow down degradation. Polyphthalaldehydes (PPAs), on the other hand, are rarely used with biological systems because of the relatively toxic phthalaldehyde degradation products. The majority of application for PPA is found in lithographic patterning due to their rapid degradation rate.[24] While the biocompatibility of these polymers remains a concern, they are well suited for the construction of devices, tests, etc that do not interface directly with living systems. In contrast, polyglyoxylates (PGs), which Gillies and co-workers recently developed,[33-36] is compatible with aqueous buffers, and the degradation products are alcohols and glyoxylic acid hydrate, which are nontoxic at low concentrations.[37] These properties makes PGs an exemplative SIP for *in vitro* or *in vivo* use cases, which have yet to populate the literature.

Sensory Materials

SIP can be beneficial for sensor designs by increasing detection sensitivity through signal amplification. By incorporating a suitable end-cap group, SIPs can be used to detect a variety of chemical and biological activities. Current SIP-based sensory platforms are mainly designed for *in silico* usage, for which toxicity and even compatibility with aqueous media may not be a concern. However, for future probes designed for long-term *in vitro* monitoring or *in vivo* studies, these and other imitating factors cannot be ignored.

Currently, the majority of sensory SIPs are based on the dendritic structure, wherein the sensing group is located at the focal point of the polymer.[12, 38] In principle, dendrimers can provide faster signal amplification, because each degradation cycle produces two or more reactive chain ends, leading to an exponential growth in overall rate. However, due to the intramolecular steric hindrance, the stepwise synthesis is increasingly difficult with increasing generation numbers. Dendritic SIPs with a generation number higher than three is not often reported.[19] As a result, dendritic SIPs often lead to less total amplification than linear SIPs having a high degree of polymerization.[10, 22, 26]

While typical sensory SIPs are based on chain end-initiated degradation, SIPs may be designed to undergo side chain-initiated self-immolation reactions. Termed chain-shattering, this mechanism allows materials to spontaneously degrade along the main chain with a triggering event occurring at each of the monomer units.[39, 40] In principle, this type of degradation cannot achieve the same level of signal amplification as end-capped SIPs, because not all triggering events produce a full degradation, which would require a near-stoichiometric amount of the de-capping reaction. However, given a sufficient analyte concentration, these chain-shattering SIPs can achieve a faster degradation rates than end-capped SIPs due to the higher concentration of potential cleavage sites. Of note, no sensors to date have been constructed using chain-shattering SIPs, which presents an opportunity for exploration.

Fluorescent/luminescent polymers

Polycarbamates derived from 4-aminobenzyl alcohol are a promising SIP for sensor design, which produces amplified fluorescence signal output. Shabat and coworkers designed and synthesized a linear self-immolative polycarbamate based on 4-aminobenzyl alcohol monomers modified with an *o*-acrylic acid moiety (Fig. 2A).[22, 41] The free monomer acts as a push-pull system, producing a fluorescence emission at 510 nm. The fluorescence is quenched in the polymer form by masking of the aniline through a carbamate bond. Cleavage of the trigger releases the fluorogenic building blocks and generates strong fluorescence. Another key element of the SIP involves the use of pendant carboxylic acid groups, which gives the polymer excellent water solubility under physiological conditions.

In addition to using aniline dyes to impart fluorescence on-off switching to SIPs, chemiluminescence has also been explored as an output signal (Fig. 2B).[42] Amplified by the SIP, chemiluminescence can produce superior signal-to-noise ratios via long-lasting light emissions. Recently, Shabat and coworkers reported a novel chemiexcitation turn-on mechanism that can be incorporated into SIPs, allowing for amplified chemiluminescence output. This design takes advantage of Schaap's adamantylidene-dioxetane, which is conjugated to a quinone methide monomer. Upon triggered degradation, a phenolate-dioxetane species is generated, which spontaneously decomposes through a chemiexcitation reaction (chemically initiated electron-exchange luminescence, or CIEEL) to generate an excited state benzoate and adamantanone. Emission of blue light (499 nm) occurs as the benzoate decays to the ground state. The system successfully responded to three model analytes (F^-, Pd(0)/Pd(II), and H_2O_2). This CIEEL-based signal amplification strategy may prove to be highly useful for the detection of low-abundance analytes. However, broadening the analyte library to include more biomedically relevant species and a robust mechanism linking the detection event to the complete degradation of the polymer remain challenges.

Point-of-care assay platforms

Point-of-care (POC) and point-of-use assays are critical for identifying and measuring analytes in a variety of non-laboratory environments. While many qualitative POC assays are available in the form of dipsticks and lateral-flow tests, quantitative assays are much more challenging to develop.[43] The ideal POC assay not only should be inexpensive, straightforward to operate, and provide rapid, quantitative, and reproducible results, but also should do so without the use of an external readout system. "Reader-less" quantitative POC assay still is a formidable scientific and technological challenge.

Phillips and coworkers were the first to adapt the self-immolative chemistry to the construction of a quantitative POC assay platform (Fig. 3).[44] In one example (Fig. 3B), a self-immolative polycarbamate oligomer was designed as a phase-switching reagent. Upon reaction with hydrogen peroxide (a

model analyte), water-insoluble oligomers are converted to water-soluble products. This switching reaction changes the wettability of the surface of a paper-based microfluidic device, allowing a sample to wick through the three-dimensional device more quickly. By measuring the flow-through time, the quantities of the analyte can be extrapolated down to low nanomolar concentrations. Instead of depending on the degree of polymerization to achieve signal amplification, this clever design leverages the rapid degradation rate of SIPs. Oligomers as short as octamers enable quantitative detection. In a second-generation design (Fig. 3C), this general strategy was modified to include a reference region, which eliminates the influence of environmental factors such as temperature, humidity, and sample viscosity.[45] The number of channels were further increased to allow simultaneous detection of Pb^{2+} and Hg^{2+} using enzyme-conjugated DNAzyme-aptamers complex, which specifically recognize these ions and release glucose oxidase (GOX) that in turn generate SIP-activating H_2O_2.[46] The time-based detection assays require a single step by the user, yet accounts for variations in sample volume, assay temperature, humidity, and contaminants that would otherwise require controlled assay condition and multiple processing procedures. In addition, the assay was able to reach low to mid femtomolar detection range with measurement times ranging from ~30 s to ~15 min. Overall, these remarkable devices are strongly linked to the rapid response characteristics of SIPs, and are very difficult to achieve using traditional degradable polymers.

Overall, the examples listed here demonstrate how the rapid responsiveness and signal-amplifying properties of SIPs can be employed to create sensors of outstanding performance. Undoubtedly, SIP sensors are still a rapidly emerging field worthy of further ingenuity and investigation.

Drug Delivery

Drug delivery is by far the most explored area for the biomedical use of SIPs. Compared with most drug delivery platforms, SIPs promise two key advantages: 1) a diverse range of stimuli that can be used to induce depolymerization, and 2) rapid, amplified response to a low concentration of such stimulus. Challenges for implementation lie in the realization (through new chemistry) and well-crafted use of these promises. One possibility is to formulate SIPs into higher-order assemblies such as capsules, polymeric micelles, vesicles, which can then encapsulate drug molecules through hydrophobic interactions. Another possibility is to generate SIP-based, fully covalent polymeric prodrugs. Currently, the main SIP chemistries adopted are carbamates and glyoxylates, which are water-compatible and produces relatively non-toxic fragments. In terms of polymer architecture, linear SIPs, chain-shattering SIPs, as well as branched SIPs have all been reported. Despite a relatively large number of reported efforts, however, the majority of SIP systems are still in early stages of technology validation and have not entered into advanced preclinical and clinical studies.

Microparticles and microcapsules

In recent years, a number of microparticles with self-immolative elements have been developed.[47] SIPs within microparticles exhibit similar degradation characteristics as bulk materials until substantial degradation has taken place, which would introduce more polymer-solvent interactions. Moore and coworkers reported the first pH-responsive microcapsules consisting of self-immolative polycarbamates (Fig. 4A, **a**),[48] which were further cross-linked under interfacial polymerization conditions. Removal of end caps (Boc or Fmoc) initiated a cascade of 1,6-elimination and decarboxylation, which stimulated the release of the microcapsule contents via depolymerization and subsequent capsule rupture. Although this early study was not specifically targeted for drug delivery, it serves as an important proof of concept for subsequent work. A similar approach was adopted by Almutairi to construct UV- and two-photon NIR-responsive polycarbamate particles, which were able to realize a burst-release profile (Fig. 4A, **b-2, b-5**).[49] The same group also developed microcapsules based on chain-shattering SIPs (Fig. 4C, **d-1, d-4, d-6**), which incorporate 2,6-bis(hydroxymethyl)-4-methylphenol monomers to achieve greater sensitivity to external triggers (H_2O_2, UV, and near-IR light).[50-52] While these self-immolative elements are based on the phenolate anion as the reactive degrading species, which in principle can be rendered inactive by protonation in water, their apparent effectiveness suggests that the local hydrophobic environment of the microcapsule reduces water accessibility and allows the elimination reactions to occur much faster than protonation. Cheng et al improved this chemistry by using 2,6-bis(hydroxymethyl)aniline as the co-monomer (Fig. 4C, **e-3**).[39] Protonation of the aromatic amine occurs in all but very acidic media (pKa of PhN^+H_3 is ~4.6), thus the polymer is able to degrade more fully (at least in principle) than the phenol-based SIPs in the presence of water, although a direct comparison is not available.

More recently, Gillies and coworkers prepared particles from polymer blends of PG and poly(lactic acid) (PLA) via emulsion to realize stimulated drug release (Fig. 4B).[53] The blend led to a two-stage release profile: an initial rapid release upon triggered depolymerization of the PG domain, and a slower diffusion-based release from the PLA. The extent of initial release by UV light or dithiothreitol [DTT] increased with an increasing PG:PLA ratio, demonstrating that the release profile can be tuned according to the particle composition. Of note, while SIPs derived from 2,6-bis(hydroxymethyl)phenol/aniline can undergo 1,4-elimination reactions twice, leading to chain scission and loss of the self-immolation fragment, a single elimination reaction is sufficient for chain scission. Representative chemical designs include the use of 1-(4-aminophenyl) ethane-1,2-diol (Fig. 4C, **f-3**)[54] and 4-hydroxybenzaldehyde,[55] although both of these studies were not specifically used for drug delivery.

Common to the self-immolation reactions discussed thus far is the reliance on nucleophilic oxygen or nitrogen species to initiate aromatic elimination. These reactions produce quinone methide or imine methide intermediates, which are potential alkylating agents and increase safety concerns for *in vivo* use of the SIPs at high concentrations. To improve biocompatibility,

Almutairi et al designed chain-shattering SIPs based on poly(caprolactone)s[56]and poly(ester amide)s (Fig. 4D).[57] Instead of degrading through aromatic elimination, these polymers carry masked amine groups adjacent to the ester linkages. Once de-masked, intramolecular cyclization leads to the scission of the polymer backbone, producing much more biocompatible fragments (amino acids, cyclic lactams) and no alkylating species.

With substantial advances in SIP chemistry, *in vitro* studies of SIP-based drug carriers are beginning to emerge. Štěpánek and coworkers recently studied a reactive oxygen species (ROS)-sensitive SIP drug carrier based on 2,6-bis-(hydroxymethyl)-p-cresol in several cell lines (PC-3, HeLa, DLD1) (Fig. 5).[58] The particles were successful in specifically releasing their drug cargos (Nile red and paclitaxel) when ROS was introduced at biologically relevant concentrations for tumors and inflamed tissues. In addition, there was a statistically significant level of selectivity towards cancer cells (high ROS) compared to normal cells (normal ROS). However, in cell viability assays, the ROS-responsive particles did not substantially outperform the ROS-nonresponsive particles, suggesting that there is still a significant knowledge gap between the chemical designs and *in vitro* performance that must be closed.

Micelles, nanoparticles, and vesicles

Small particles with large surface area are more exposed to the solvent, and thus SIPs formulated into these small structures face additional design requirements, for example hydrolytic stability (to prevent immature degradation) and facile degradation chemistry in water. In an early study, Gillies and coworkers developed an SIP consisting of N,N'-dimethylethylenediamine and 4-hydroxybenzyl alcohol linked by carbamate linkages, which is end-capped with poly(ethylene glycol) (PEG) via an ester linkage (Fig. 6A, **a-1**).[59] The resulting amphiphilic block copolymer self-assembled into a micellar form, which was capable of encapsulating and subsequently releasing a fluorescent dye in aqueous solution through the hydrolysis of the ester linkage. Recently, the same group replaced PEG with the thermo-responsive polymer, poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), as the hydrophilic block, and adopted a UV-responsive linker as the end-cap for the SIP block (Fig. 6A, **a-10**).[60] The resulting material exhibits dual stimuli-responsiveness. Interestingly, at a temperature (65 °C) above the lowest critical solution temperature (LCST) of PDMAEMA, the systems underwent more rapid depolymerization even for a non-UV-cleavable control. These data suggest that the depolymerization reaction itself is temperature-sensitive in the temperature range studied, and that there is substantial hydrolytic chain scission causing non-specific depolymerization. In addition to micelles, SIP-based vesicular assemblies have also been studied. Liu and co-workers reported a polycarbamate-*b*-poly(N,N-dimethylacrylamide) (PDMA) block copolymer, which self-assembled to form polymersomes (Fig. 6B, **d-3, d-5, d-6**).[61] Termed SIPsomes, these structures work exceptionally well in releasing encapsulated payloads (camptothecin [CPT], doxorubicin [Dox], eosin Y, and enzymes) in response to environmental stimuli (e.g. enzyme, UV/visible light, and reductive environment predetermined by the chosen end-cap).

In recent years, PGs have been identified as an attractive material for drug delivery due to the benign degradation product, glyoxylic acid hydrate, a metabolic intermediate. The chemistry was introduced by Gillies and coworkers, who reported a range of PG homopolymers and a PEG-*b*-PG-*b*-PEG triblock copolymers (Fig. 6A, **b-7**).[33] The block copolymers underwent self-assembly to form micelles in an aqueous solution, which were able to undergo UV-initiated depolymerization and disintegration. The approach was later expanded to include additional end-caps which are responsive to reducing thiols and H_2O_2 (Fig. 6A, **b-2, b-8, b-9**),[62] species that are intrinsically present in the body and associated with inflammation and cancer. The micelles were shown to encapsulate Nile red, Dox, and curcumin. All payloads were rapidly released in the presence of low concentrations of the triggering agent, suggesting an amplification effect. The same team also explored different pendant ester groups and other aldehyde monomers to tune the micelle core properties in terms of stability, hydrophobicity, and their ability to load hydrophobic drugs (e.g. celecoxib).[63] All systems released celecoxib more rapidly when UV light was applied as a stimulus than when not triggered. Toxicity studies *in vitro* using MDA-MB-231 cells showed that the toxicity depended on the structure of the polymer and whether degradation had been triggered. Thus, PGs-based micelles represent a highly tunable system with an excellent safety profile, making them a promising candidate as SIP-based drug carriers to move forward with.

Since the degradation of benzyl esters/ethers can be retarded by the protonation of the propagating phenolate, systems involving these functionalities must be carefully designed for use in water. Li and co-workers addressed this challenge by incorporating the self-immolating units into a chain-shattering triblock copolymer, PEG-*b*-poly(benzyl ester)-*b*-PEG, with each repeating benzyl ester unit capable of sensing H_2O_2. The high concentration of the stimuli-sensing units makes up for low reactivity of the phenol, allowing the system to function in water (Fig. 6A, **c-4**).[64] Still, for complete loss of the benzyl esters, more than 10 days is required in a D_2O/acetone nitrile mixture, which is relatively slow for SIP degradation. For this model system, drug delivery was contemplated but not tested.

Another role for SIPs in drug delivery is for them to serve as gatekeepers to enable controlled drug release. Mesoporous silica nanoparticles (MSNs) are a classic drug carrier, where drug molecules with a size under the particle pore size can be efficiently loaded into the pores.[65] Manzano and coworkers developed an acid-responsive SIP coating for the MSNs (Fig. 6C).[66] At physiological pH, the SIP blocks the drug molecules from diffusing out of the pore. Upon triggering by low pH, the SIP disintegrates, opening the pores and allowing the cargo to escape. The benefit of this strategy is that a small amount of the SIP can be used to control the release of a large quantity of drugs, which makes the deleterious side effects of the SIP

fragments more manageable, although this potential has not been carefully studied.

Polymeric prodrugs

While SIPs can serve as a hydrophobic reservoir to encapsulate drug payloads, it is also feasible to utilize pendant functional groups of the drug molecule to covalently incorporate the drug with the SIP to form polymeric prodrugs. Using the chain-shattering polymer design, Cheng and coworkers integrated 10-hydroxycamptothecin and 9-aminocamptothecin directly into the backbone of SIPs using 2,6-bis(hydroxymethyl)aniline as a co-monomer to form polycarbonates and polycarbamates, respectively.[67, 68] Because a step-growth mechanism is involved in the polymerization, the bifunctional derivatives of CPT are required for this strategy to work (Fig. 7). Once the photo-capping agent (*o*-nitrobenzyl) on the aromatic amine is removed, the linkages on both sides of the self-immolative units rapidly degrade, facilitating the release of free, unmodified drug molecules. Because the prodrug polymer is completely hydrophobic, a PEG-*b*-poly(L-lactide) micelle was used to carry the polymer in the micelle core. Remarkably, these prodrug formulations exhibited relatively low toxicity until the UV activation, which restored the toxicity of the drug to nearly the same level as the free drug.[67] The same group later adapted this type of materials to sense intracellular reductive environments using disulfide-capped self-immolative units.[68] Along this line, our group have reported oligonucleotide-linked self-immolative prodrugs. The drug component (CPT, paclitaxel) was either connected onto a self-immolative core (Fig. 8A),[69] or tethered onto a non-degradable polymer as pendants via self-immolative linkers (Fig. 8B).[70] The prodrug polymers were then covalently conjugated with a single strand of antisense DNA. Again, when the corresponding stimuli were provided (UV, intracellular thiols), the toxicities of the conjugated drugs were recovered almost completely. The key feature of these systems is that the DNA-polymer amphiphiles form a micellar structure in aqueous solutions. These micelles are structurally analogous to "spherical nucleic acids", or SNAs, which are known to enter cells in large quantities (free nucleic acid typically does not enter cells). Thus, the poor solubility of the drug is transformed into an advantage by the prodrug polymer, which enables the co-delivery of DNA and drug using nothing but payloads themselves.

Recently, Oupický and coworkers designed a chain-shattering polycarbamate containing a bioreductively cleavable disulfide monomer and a cationic drug co-monomer, N^1,N^{11}-bisethylnorspermine (BENSpm), which is a polyamine analogue (Fig. 8C).[71] The polymer is polycationic due to the drug component, and thus was capable of forming a polyplex with a miRNA-34a mimic. Once inside the cell, the nanoparticles underwent breakage into their small molecule fragments, releasing BENSpm and the miRNA-34a mimic in their free form. The team performed a careful biochemical analysis of the polyplex *in vitro*, showing upregulation of intracellular miR-34a and downregulation of Bcl-2 (one of the downstream targets of miR-34a). Simultaneously, the released BENSpm induced the expression of rate-limiting enzymes in poly- amine catabolism and depleted natural cellular polyamines. The synergy between the two components not only enhanced cancer cell killing *in vitro*, but also enhanced antitumor efficacy *in vivo* in a subcutaneous xenograft mouse model (HCT116, human colorectal carcinoma). This important work is one of very few studies that bring an original SIP design together with careful *in vitro* and in vivo analyses, and validated the feasibility of SIPs as a powerful delivery vector for combination cancer therapy. As the self-immolation chemistry systems matures and the SIP family expands, it is anticipated that future efforts will gradually bifurcate to include both fundamental chemical research and studies interfacing SIPs with real biological conditions, using appropriate cellular and animal models.

SIPS for Biotechnology

Polymer materials are widely used in biotechnology, such as coatings, biomimetic actuators, bio-separation, chemical valves, immobilization of biocatalysts, among others.[72, 73] As such, the possibility for SIPs in biotechnology is almost limitless. However, efforts to adopt SIPs for biotechnological applications is only at a very early stage. Here, we highlight two distinct applications of SIPs that have been recently reported.

Antimicrobial polymers

The majority of the synthetic antimicrobial polymers are non-degradable. However, one can imagine that under certain scenarios it is desirable for the polymer to be rapidly removed once its purpose has been served. For example, an antimicrobial polymer coating can be applied to implanted medical devices to prevent biofilm formation. However, should inflammatory responses develop, it is desirable to rapidly remove the polymer coating with an external or internal trigger. While biodegradable antimicrobial polymers based on polyesters, polyurethanes, polycarbonates have been reported, they typically do not possess the rapid response characters that the SIPs exhibit.[74]

Ergene and Palermo developed the first self-immolative antibacterial polymers (Fig. 9A).[31] These SIPs are based on PBEs containing pendant primary ammonium groups and silyl ether end-caps (responsive to fluoride ions). Being polycationic, these PBEs exerted potent, rapid, and broad-spectrum antibacterial activity, but were also highly haemolytic. In follow-up work, the same group grafted varying ratios of PEG in addition to the primary ammonium to the PBEs to modulate their hydrophobic-hydrophilic balance and the strength of the antibacterial activity.[32] With 25-50% (mol%) of 800 kDa PEG, antibacterial activities were largely retained, while haemolytic activities were tolerable. When activated by fluoride ion, the SIPs were able to degrade into small molecule fragments in methanol or dimethylformamide. It is unclear, however, whether these polymers can be activated for degradation in a neutral aqueous buffer due to the use of the PBE chemistry. Overall, these two studies represent an initial effort to leverage the properties of SIP chemistry for a yet unexplored area.

Protein Labeling

The development of tools that allow labelling of proteins *in vitro* and *in vivo* is a highly active and interdisciplinary area of research. Nagano and co-workers developed the first enzyme activity-based labelling probe based on a 4-hydroxybenzyl alcohol-type self-immolative spacer, which generates a quinone methide species from the catalytic activity of an enzyme.[75] Quinone methides are electrophiles that rapidly alkylate nucleophilic sites such as amines, thiols, or hydroxyls, which makes them useful for chemical modification or labelling of cellular components such as nucleotides and proteins.[76-81] Similar to quinone methides, azaquinone methides also reacts rapidly with nucleophiles, and can be generated from a degrading polycarbamate SIP. Shabat and co-workers designed an activity-linked fluorescent labelling SIP probe that responds to penicillin G-amidase (PGA) and the catalytic antibody Ab38C2 (Fig. 9B).[82] Removal of the capping groups by the enzymes produces azaquinone methide species, which then labels the enzyme. Interestingly, this process is also fluorogenic, as the resulting aniline nitrogen allows for extended pi conjugation but not the carbamate nitrogen. This convenient feature makes it possible to use fluorescence as the readout for the labelling[22] in addition to other techniques such as mass spectrometry. However, it is likely required that the labelled protein solution be purified before fluorescence intensity is measured, as the fraction of azaquinone methides that does not label the protein will react with water to produce the same fluorescence. This is a potential shortfall that future designs should address.

Summary and Outlook

While the chemistries for SIPs have been studied for over a decade, the exploration of their use in biomedical research is only at its infancy. Currently, the majority of the biomedical use cases for SIPs center around therapeutic delivery, with some initial explorations in sensor design and biotechnology. For SIPs to keep moving forward in biomedicine, there must be continued quest to increase the degree of polymerization, increase the control of the polymerization to produce desired polydispersities and chain-end functionalities, and to adopt more biocompatible chemistries. Furthermore, to develop a successful biomedical application of SIPs, an appropriate chemistry must be carefully selected to navigate around the limitations posed by the environment in which the SIP will operate and the reactivity/toxicity of the degradation fragments, and to leverage the unique degradation characteristics of SIPs over conventional polymers, namely rapid degradation and triggered activation. This not only requires familiarity of the chemical intricacies of the various SIP classes, but also clever ways of using them. The work by Philips in the construction of time-based detection systems, the development by Gillies of the biocompatible SIPs based on polyglyoxylates, and the effort by Oupický in the testing of a self-immolative prodrug dual-delivery system are the prime examples of SIPs' success in biomedicine. These studies provide a glimpse into the immense possibilities and unexplored opportunities represented by SIP-based materials in biomedicine.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

KZ acknowledges support by the National Institutes of Health (the National Institute of General Medical Sciences Award Number 1R01GM121612-01) and the National Science Foundation (CAREER Award Number 1453255). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

Notes and references

1. M. J. L. Tschan, E. Brulé, P. Haquette and C. M. Thomas, *Polymer Chemistry*, 2012, **3**, 836-851.
2. H. Tian, Z. Tang, X. Zhuang, X. Chen and X. Jing, *Prog Polym Sci*, 2012, **37**, 237-280.
3. R. A. Gross and B. Kalra, *Science*, 2002, **297**, 803-807.
4. L. S. Nair and C. T. Laurencin, *Prog Polym Sci*, 2007, **32**, 762-798.
5. J. Pushpamalar, A. K. Veeramachineni, C. Owh and X. J. Loh, *ChemPlusChem*, 2016, **81**, 504-514.
6. B. Tyler, D. Gullotti, A. Mangraviti, T. Utsuki and H. Brem, *Adv Drug Deliver Rev*, 2016, **107**, 163-175.
7. M. Vert, *Biomacromolecules*, 2005, **6**, 538-546.
8. F. Seidi, R. Jenjob and D. Crespy, *Chem Rev*, 2018, **118**, 3965-4036.
9. A. D. Wong, M. A. DeWit and E. R. Gillies, *Adv Drug Deliver Rev*, 2012, **64**, 1031-1045.
10. G. I. Peterson, M. B. Larsen and A. J. Boydston, *Macromolecules*, 2012, **45**, 7317-7328.
11. S. T. Phillips and A. M. DiLauro, *ACS Macro Letters*, 2014, **3**, 298-304.
12. M. E. Roth, O. Green, S. Gnaim and D. Shabat, *Chemical Reviews* 2016, **116**, 1309-1352.
13. R. E. Yardley, A. R. Kenaree and E. R. Gillies, *Macromolecules*, 2019, **52**, 6342-6360.
14. S. T. Phillips, J. S. Robbins, A. M. DiLauro and M. G. Olah, *J Appl Polym Sci*, 2014, **131**, 40992.
15. I. Tranoy-Opalinski, A. Fernandes, M. Thomas, J. P. Gesson and S. Papot, *Anti-Cancer Agents in Medicinal Chemistry*, 2008, **8**, 618-637.
16. C. A. Blencowe, A. T. Russell, F. Greco, W. Hayes and D. W. Thornthwaite, *Polymer Chemistry*, 2011, **2**, 773-790.
17. A. Alouane, R. Labruere, T. Le Saux, F. Schmidt and L. Jullien, *Angew Chem Int Ed Engl*, 2015, **54**, 7492-7509.
18. P. L. Carl, P. K. Chakravarty and J. A. Katzenellenbogen, *J Med Chem*, 1981, **24**, 479-480.
19. R. J. Amir, N. Pessah, M. Shamis and D. Shabat, *Angewandte Chemie International Edition*, 2003, **42**, 4494-4499.
20. F. M. H. de Groot, C. Albrecht, R. Koekkoek, P. H. Beusker and H. W. Scheeren, *Angewandte Chemie International Edition*, 2003, **42**, 4490-4494.

21. S. Li, M. L. Szalai, R. M. Kevwitch and D. V. McGrath, *J Am Chem Soc*, 2003, **125**, 10516-10517.
22. A. Sagi, R. Weinstain, N. Karton and D. Shabat, *J Am Chem Soc*, 2008, **130**, 5434-5435.
23. Y. Xiao, H. Li, B. H. Zhang, Z. H. Cheng, Y. Li, X. Y. Tan and K. Zhang, *Macromolecules*, 2018, **51**, 2899-2905.
24. F. Wang and C. E. Diesendruck, *Macromol Rapid Comm*, 2018, **39**, 1700519.
25. M. Gisbert-Garzaran, M. Manzano and M. Vallet-Regi, *Chem Eng J*, 2018, **340**, 24-31.
26. R. Weinstain, A. Sagi, N. Karton and D. Shabat, *Chemistry – A European Journal*, 2008, **14**, 6857-6861.
27. M. G. Olah, J. S. Robbins, M. S. Baker and S. T. Phillips, *Macromolecules*, 2013, **46**, 5924-5928.
28. M. S. Baker, H. Kim, M. G. Olah, G. G. Lewis and S. T. Phillips, *Green Chem*, 2015, **17**, 4541-4545.
29. K. Yeung, H. Kim, H. Mohapatra and S. T. Phillips, *J Am Chem Soc*, 2015, **137**, 5324-5327.
30. Y. Xiao, Y. Li, B. Zhang, H. Li, Z. Cheng, J. Shi, J. Xiong, Y. Bai and K. Zhang, *ACS Macro Letters*, 2019, **8**, 399-402.
31. C. Ergene and E. F. Palermo, *Biomacromolecules*, 2017, **18**, 3400-3409.
32. C. Ergene and E. F. Palermo, *J. Mat. Chem. B*, 2018, **6**, 7217-7229.
33. B. Fan, J. F. Trant, A. D. Wong and E. R. Gillies, *J Am Chem Soc*, 2014, **136**, 10116-10123.
34. B. Fan, J. F. Trant and E. R. Gillies, *Macromolecules*, 2016, **49**, 9309-9319.
35. A. R. Kenaree and E. R. Gillies, *Macromolecules*, 2018, **51**, 5501-5510.
36. Q. E. A. Sirianni, A. R. Kenaree and E. R. Gillies, *Macromolecules*, 2019, **52**, 262-270.
37. C. H. Brachais, J. Huguet and C. Bunel, *Polymer*, 1997, **38**, 4959-4964.
38. S. Gnaim and D. Shabat, *Acc Chem Res*, 2014, **47**, 2970-2984.
39. Y. Zhang, L. Ma, X. Deng and J. Cheng, *Polymer Chemistry*, 2013, **4**, 224-228.
40. K. A. Miller, E. G. Morado, S. R. Samanta, B. A. Walker, A. Z. Nelson, S. Sen, D. T. Tran, D. J. Whitaker, R. H. Ewoldt, P. V. Braun and S. C. Zimmerman, *J Am Chem Soc*, 2019, **141**, 2838-2842.
41. W. Wang and C. Alexander, *Angewandte Chemie International Edition*, 2008, **47**, 7804-7806.
42. S. Gnaim and D. Shabat, *J Am Chem Soc*, 2017, **139**, 10002-10008.
43. P. Yager, G. J. Domingo and J. Gerdes, *Annu Rev Biomed Eng*, 2008, **10**, 107-144.
44. G. G. Lewis, J. S. Robbins and S. T. Phillips, *Macromolecules*, 2013, **46**, 5177-5183.
45. G. G. Lewis, J. S. Robbins and S. T. Phillips, *Anal Chem*, 2013, **85**, 10432-10439.
46. G. G. Lewis, J. S. Robbins and S. T. Phillips, *Chem Commun*, 2014, **50**, 5352-5354.
47. A. P. Esser-Kahn, S. A. Odom, N. R. Sottos, S. R. White and J. S. Moore, *Macromolecules*, 2011, **44**, 5539-5553.
48. A. P. Esser-Kahn, N. R. Sottos, S. R. White and J. S. Moore, *J Am Chem Soc*, 2010, **132**, 10266-10268.
49. C. de Gracia Lux, C. L. McFearin, S. Joshi-Barr, J. Sankaranarayanan, N. Fomina and A. Almutairi, *ACS Macro Letters*, 2012, **1**, 922-926.
50. N. Fomina, C. McFearin, M. Sermsakdi, O. Edigin and A. Almutairi, *J Am Chem Soc*, 2010, **132**, 9540-9542.
51. N. Fomina, C. L. McFearin, M. Sermsakdi, J. M. Morachis and A. Almutairi, *Macromolecules*, 2011, **44**, 8590-8597.
52. C. de Gracia Lux, S. Joshi-Barr, T. Nguyen, E. Mahmoud, E. Schopf, N. Fomina and A. Almutairi, *J Am Chem Soc*, 2012, **134**, 15758-15764.
53. M. T. Gambles, B. Fan, A. Borecki and E. R. Gillies, *ACS Omega*, 2018, **3**, 5002-5011.
54. L. Ma, R. Baumgartner, Y. Zhang, Z. Song, K. Cai and J. Cheng, *Journal of Polymer Science Part A: Polymer Chemistry*, 2015, **53**, 1161-1168.
55. Y. Cui, M. Zhang, F.-S. Du and Z.-C. Li, *ACS Macro Letters*, 2017, **6**, 11-15.
56. C. de Gracia Lux and A. Almutairi, *ACS Macro Letters*, 2013, **2**, 432-435.
57. C. de Gracia Lux, J. Olejniczak, N. Fomina, M. L. Viger and A. Almutairi, *Journal of Polymer Science Part A: Polymer Chemistry*, 2013, **51**, 3783-3790.
58. E. Jäger, A. Höcherl, O. Janoušková, A. Jäger, M. Hrubý, R. Konefał, M. Netopilik, J. Pánek, M. Šlouf, K. Ulbrich and P. Štěpánek, *Nanoscale*, 2016, **8**, 6958-6963.
59. M. A. DeWit and E. R. Gillies, *J Am Chem Soc*, 2009, **131**, 18327-18334.
60. R. E. Yardley and E. R. Gillies, *Journal of Polymer Science Part A: Polymer Chemistry* 2018, **56**, 1868-1877.
61. G. Liu, X. Wang, J. Hu, G. Zhang and S. Liu, *J Am Chem Soc*, 2014, **136**, 7492-7497.
62. B. Fan and E. R. Gillies, *Mol Pharm*, 2017, **14**, 2548-2559.
63. B. Fan, R. E. Yardley, J. F. Trant, A. Borecki and E. R. Gillies, *Polymer Chemistry*, 2018, **9**, 2601-2610.
64. C.-C. Song, R. Ji, F.-S. Du and Z.-C. Li, *Macromolecules*, 2013, **46**, 8416-8425.
65. A. Baeza, M. Manzano, M. Colilla and M. Vallet-Regí, *Biomaterials Science*, 2016, **4**, 803-813.
66. M. Gisbert-Garzaran, D. Lozano, M. Vallet-Regi and M. Manzano, *Rsc Advances*, 2017, **7**, 132-136.
67. Y. Zhang, Q. Yin, L. Yin, L. Ma, L. Tang and J. Cheng, *Angewandte Chemie International Edition*, 2013, **52**, 6435-6439.
68. K. Cai, J. Yen, Q. Yin, Y. Liu, Z. Song, S. Lezmi, Y. Zhang, X. Yang, W. G. Helferich and J. Cheng, *Biomaterials Science*, 2015, **3**, 1061-1065.
69. X. Tan, B. B. Li, X. Lu, F. Jia, C. Santori, P. Menon, H. Li, B. Zhang, J. J. Zhao and K. Zhang, *J Am Chem Soc*, 2015, **137**, 6112-6115.
70. X. Tan, X. Lu, F. Jia, X. Liu, Y. Sun, J. K. Logan and K. Zhang, *J Am Chem Soc*, 2016, **138**, 10834-10837.
71. Y. Xie, T. Murray-Stewart, Y. Wang, F. Yu, J. Li, L. J. Marton, R. A. Casero and D. Oupický, *J Control Release*, 2017, **246**, 110-119.
72. I. Y. Galaev and B. Mattiasson, *Trends Biotechnol*, 1999, **17**, 335-340.
73. R. Freitag, *Synthetic Polymers for Biotechnology and Medicine.*, Boca Raton: CRC Press, 2002.
74. C. Ergene, K. Yasuhara and E. F. Palermo, *Polymer Chemistry*, 2018, **9**, 2407-2427.
75. T. Komatsu, K. Kikuchi, H. Takakusa, K. Hanaoka, T. Ueno, M. Kamiya, Y. Urano and T. Nagano, *J Am Chem Soc*, 2006, **128**, 15946-15947.
76. S. Halazy, C. Danzin, A. Ehrhard and F. Gerhart, *J Am Chem Soc*, 1989, **111**, 3484-3485.

77. Q. P. Wang, U. Dechert, F. Jirik and S. G. Withers, *Biochem Bioph Res Co*, 1994, **200**, 577-583.
78. K. D. Janda, L.-C. Lo, C.-H. L. Lo, M.-M. Sim, R. Wang, C.-H. Wong and R. A. Lerner, *Science*, 1997, **275**, 945-948.
79. M. Kurogochi, S.-I. Nishimura and Y. C. Lee, *J Biol Chem*, 2004, **279**, 44704-44712.
80. Y. Liu and S. E. Rokita, *Biochemistry-us*, 2012, **51**, 1020-1027.
81. C. Huang, Y. Liu and S. E. Rokita, *Signal Transduction and Targeted Therapy*, 2016, **1**, 16009.
82. R. Weinstain, P. S. Baran and D. Shabat, *Bioconjugate Chem*, 2009, **20**, 1783-1791.

Figures

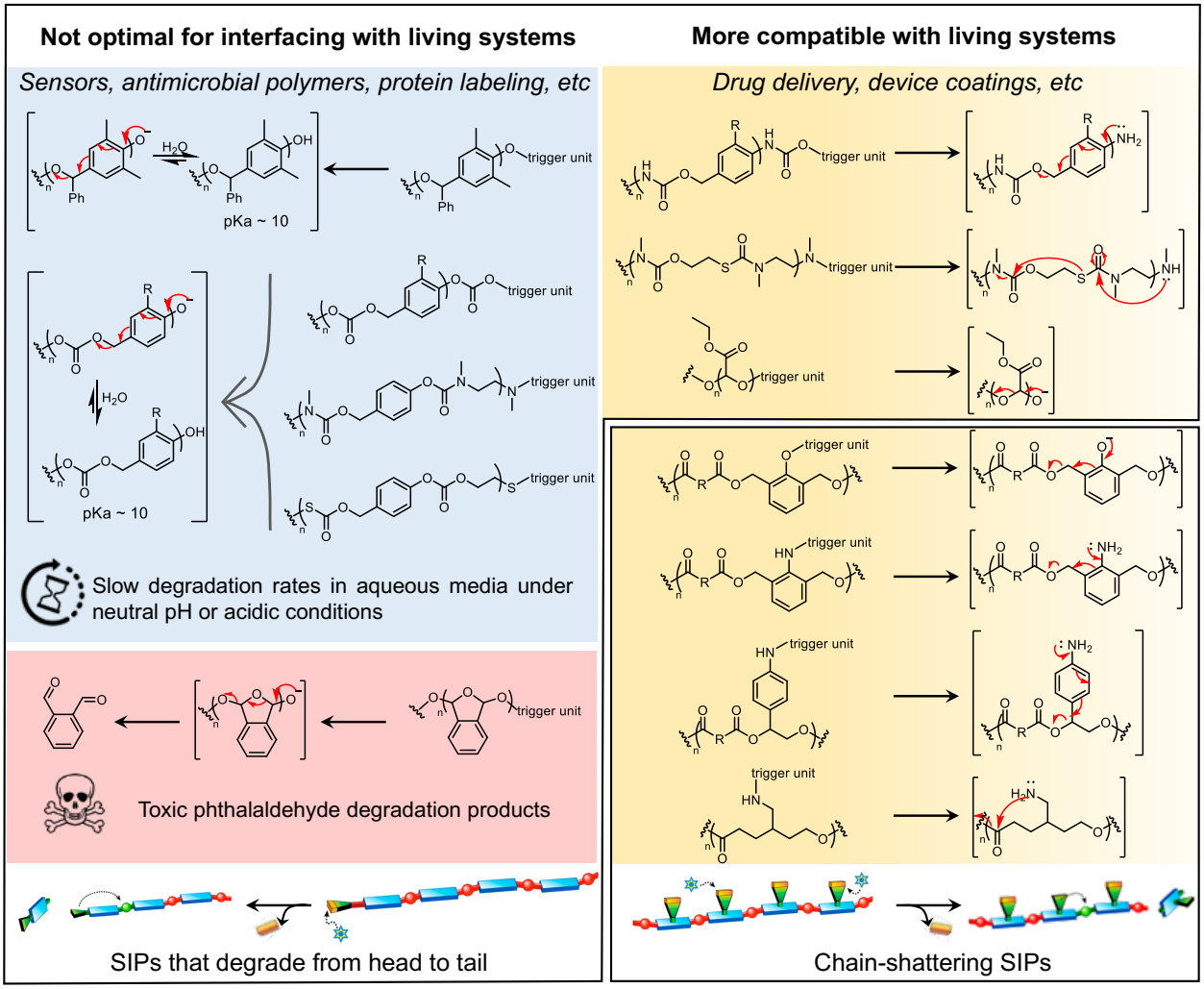

Figure 1. Self-immolative chemistries grouped into their compatibility with living systems. The cartoons of SIPs in this figure were reproduced with permission from ref 40. Copyright 2019, American Chemical Society.

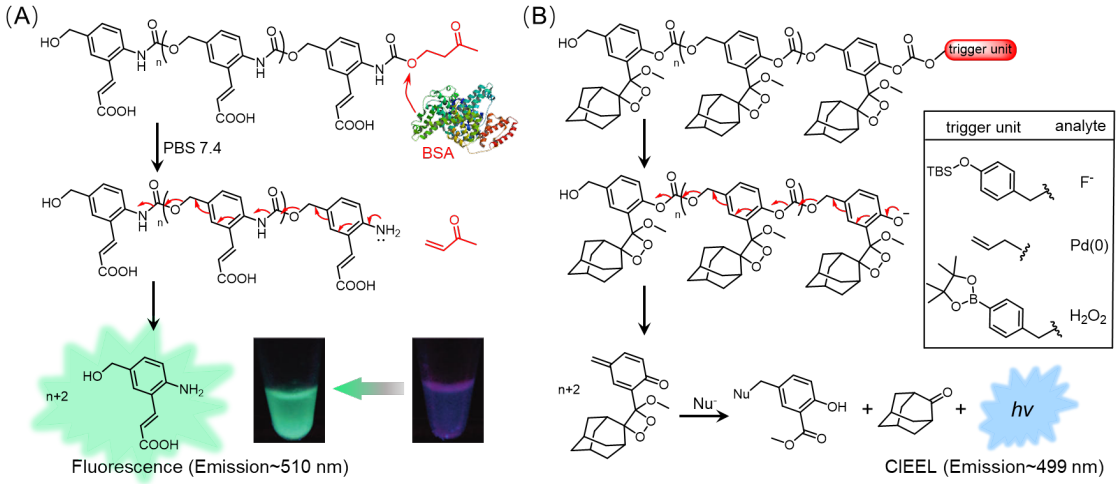

Figure 2. A) Fluorescent SIP based on 4-aminobenzyl alcohol derivative. Reproduced with permission from ref. 41. Copyright 2008, Wiley-VCH. (B) Chemiluminescent SIPs based on 4-hydroxybenzyl alcohol derivative. Reproduced with permission from ref. 42. Copyright 2017, American Chemical Society.

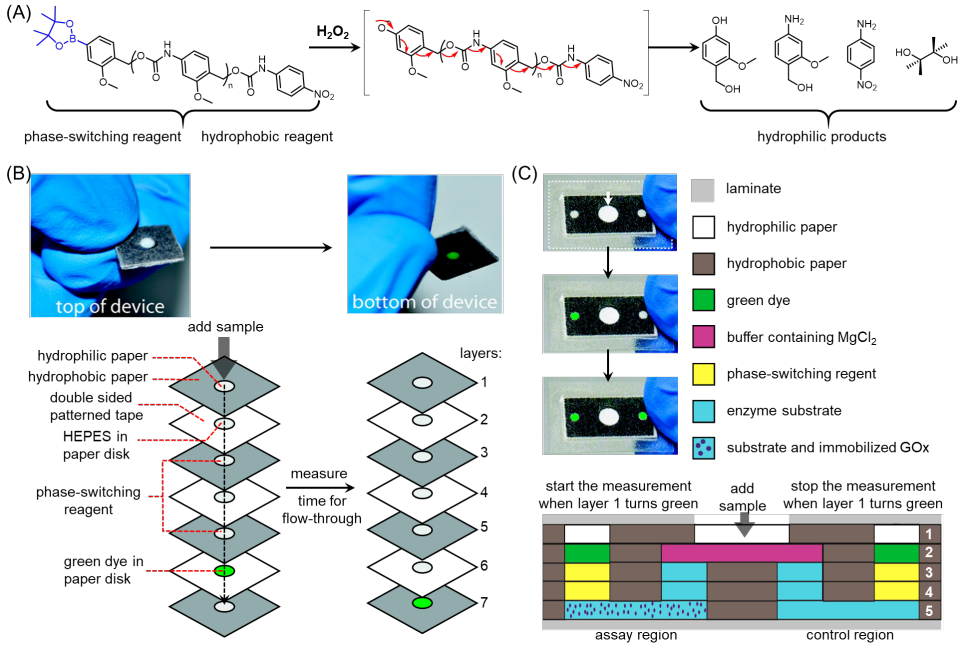

Figure 3. Polycarbamate-based POC assay platform. A) Phase-switching reagent design and the depolymerization mechanism; (B) Polycarbamate-based single-channel POC assay platform, which can directly detect H_2O_2. Reproduced with permission from ref. 44. Copyright 2013, American Chemical Society; (C) Polycarbamate-based dual-channel POC assay platform, which can detect enzymes respectively (alkaline phosphatase, β-galactosidase). Reproduced with permission from ref. 45. Copyright 2013, American Chemical Society.

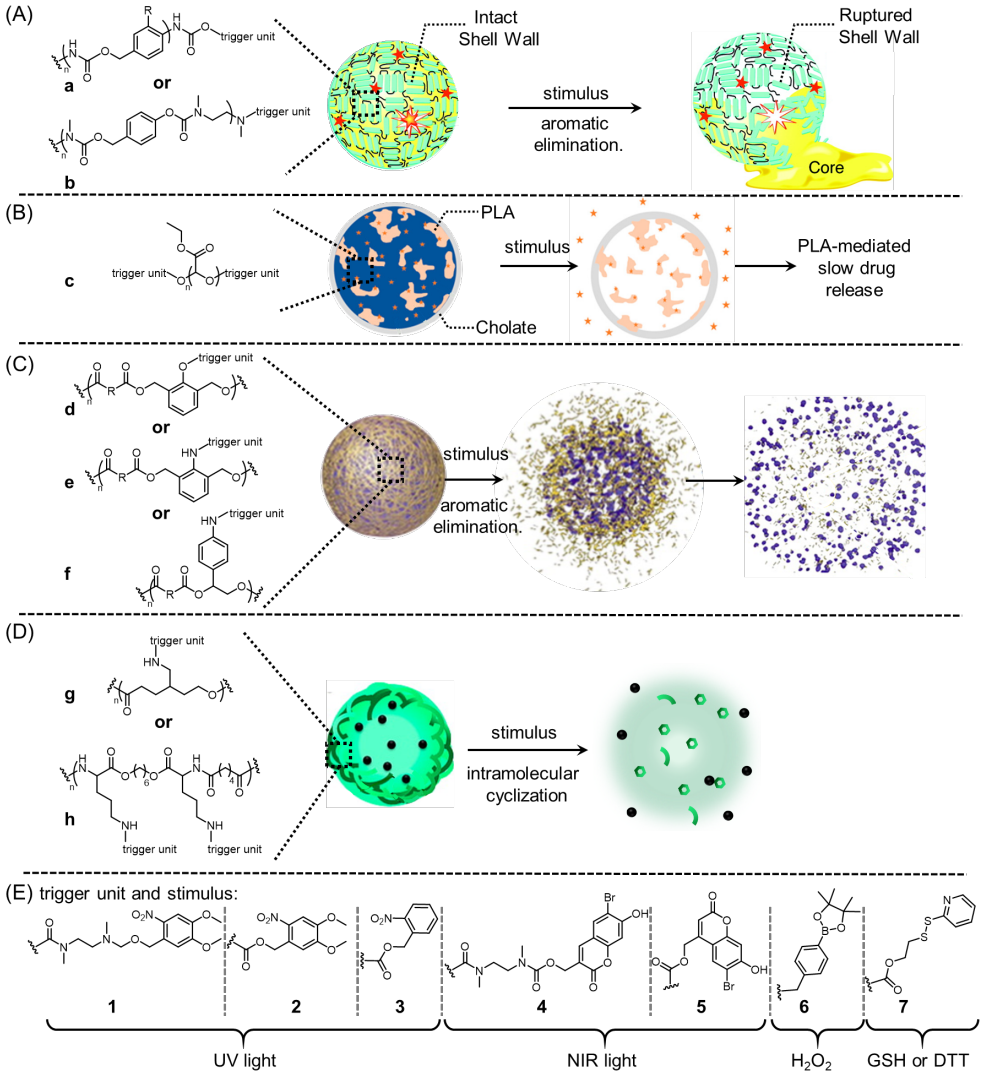

Figure 4. Self-immolative microparticles or microcapsules. A) Particles that consist of 4-aminobenzyl alcohol or 4-hydroxybenzyl alcohol-based polycarbamate. Reproduced with permission from ref. 48. Copyright 2010, American Chemical Society. B) Particles from polymer blends of PG and PLA. Reproduced with permission from ref. 53. Copyright 2018, American Chemical Society. C) Particles based on aromatic elimination-type chain-shattering SIPs. Reproduced with permission from ref. 52. Copyright 2012, American Chemical Society. D) Particles based on intramolecular cyclization-type chain-shattering SIPs. Reproduced with permission from ref. 56. Copyright 2013, American Chemical Society. E) Stimuli-sensing trigger units used in various SIPs.

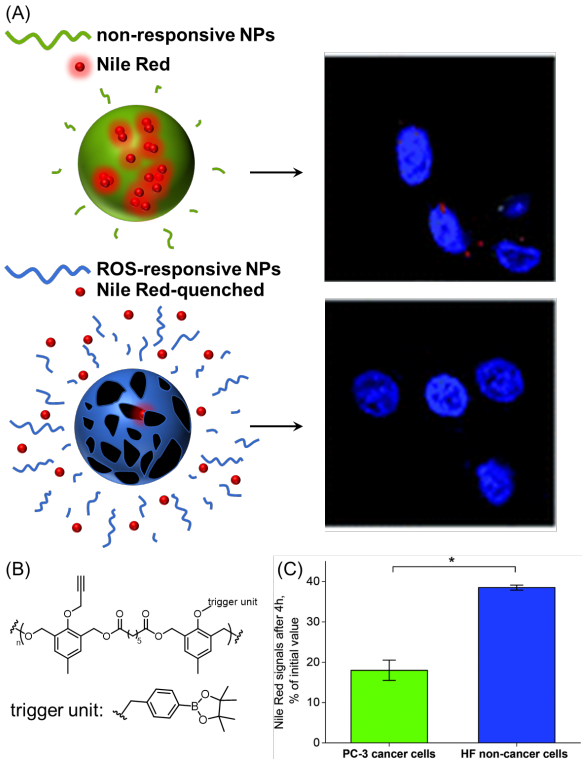

Figure 5. ROS-sensitive SIP drug carrier based on 2,6-bis-(hydroxymethyl)-p-cresol. A) Schematics of ROS-responsive and control particles for Nile red release. B) The structure of the chain-shattering SIP used in this work. C) Quantification of Nile red release from NR-loaded particles in PC-3 and HF cells. Reproduced with permission from ref. 58. Copyright 2016, Royal Society of Chemistry.

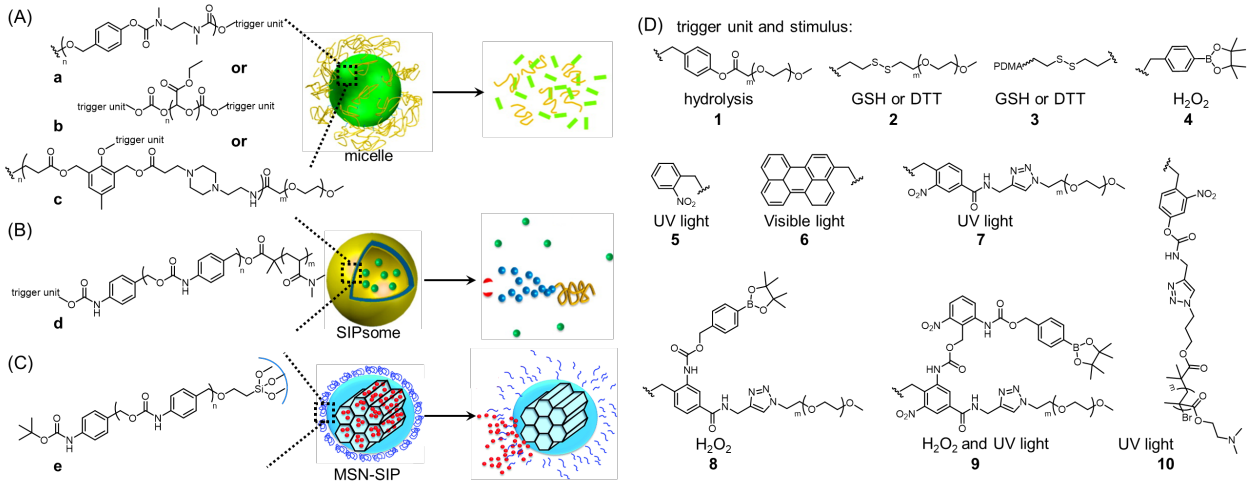

Figure 6. SIPs-based micelles, polysomes, and vesicles drug delivery. A) SIPs-based micelles. Reproduced with permission from ref 59. Copyright 2009, American Chemical Society. B) SIPs-based polysomes. Reproduced with permission from ref 61. Copyright 2014, American Chemical Society. C) SIPs as gatekeepers for MSN. Reproduced with permission from ref. 66. Copyright 2017, Royal Society of Chemistry. D) Stimuli-sensing trigger units.

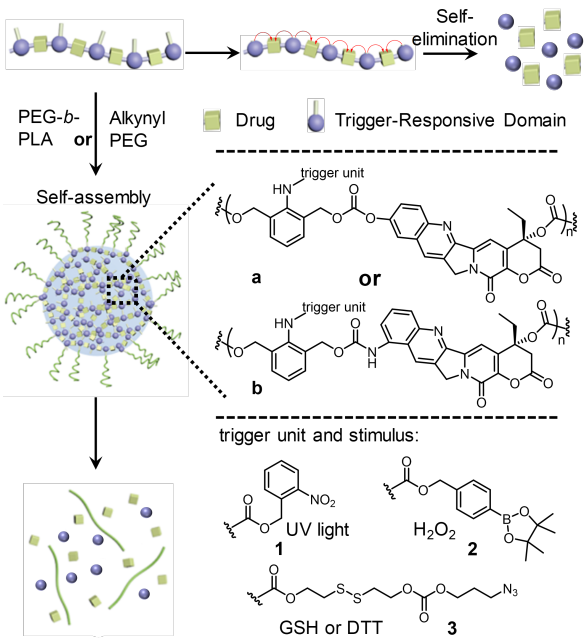

Figure 7. Chain-shattering SIP-based polymeric prodrug. Reproduced with permission from ref. 68. Copyright 2015, Royal Society of Chemistry.

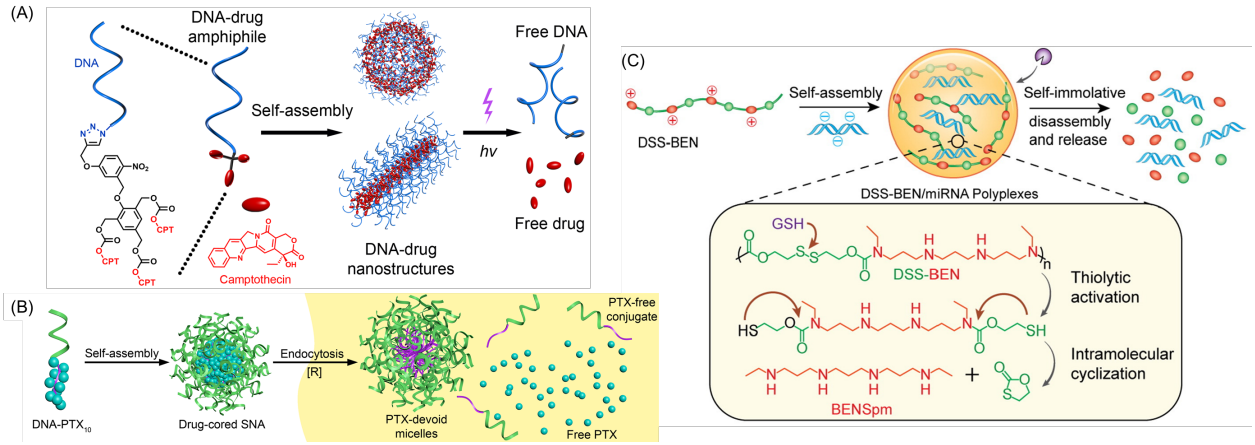

Figure 8. Self-immolative polymeric prodrugs in the co-delivery of oligonucleotides and drugs. A-B) Oligonucleotide-linked self-delivering systems. The drug component (CPT, PTX) was either connected onto a self-immolative core or tethered onto a non-degradable polymer as pendants via self-immolative linkers. Reproduced with permission from ref. 69-70. Copyright 2015, 2016, American Chemical Society. C) Chain-shattering polycarbamate containing a bioreductively cleavable disulfide monomer and a cationic drug co-monomer for miRNA/drug co-delivery. Reproduced with permission from ref. 71. Copyright 2016, Elsevier B.V.

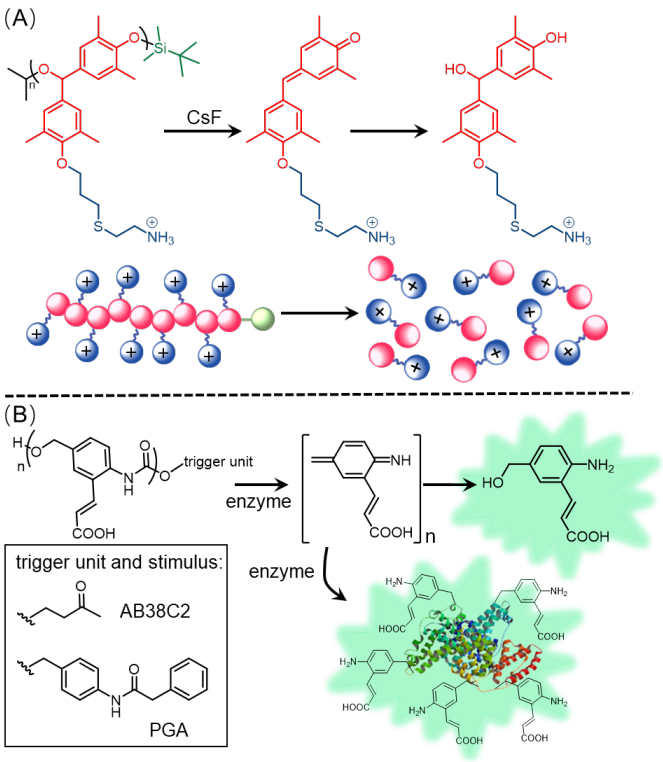

Figure 9. A) PBE-based self-immolative antimicrobial polymers. Reproduced with permission from ref. 31. Copyright 2017, American Chemical Society. B) Polycarbamate-based protein labeling probe. Reproduced with permission from ref. 82. Copyright 2009, American Chemical Society.