Redox-Responsive Delivery Systems

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Abstract

Stimuli-responsive systems for the transport and delivery of materials to a given location at a specific time are highly valuable in numerous applications. The characteristics of the delivery system are dictated by the requirements of a particular application, which include the nature of the stimulus for actuation of the delivery process. Electron transfer has moved to the forefront as a stimulus for responsive delivery systems, particularly for those used in drug and reagent delivery, and for analyte transport/separation avenues. Interest in redox-activated delivery of materials arises from the abundance of redox-active stimuli that can be used to make delivery occur, the often simple chemical nature of the activation process, and the ease of constructing delivery vehicles with an integrated redox-responsive trigger group. This review is focused on vesicle- and micelle-based vehicles whose contents can be delivered by a redox stimulus due to their potential to meet the needs of key applications.

1. INTRODUCTION

The potential use of molecular assemblies for the containment and subsequent stimulated release of contained guests in a given location or at a selected time dates to the early vesicle/liposome and micelle literature. Triggered release of secured molecular cargo from a container made of assembled, monomeric molecular species was suggested in many early works, but not until the 1980s was the stimulated release of cargo from micelles, vesicles, and liposomes shown to be feasible (1). The vast majority of the work in this area has been driven by the substantial need for molecular containment systems that can protect drugs (guests), and allow for their transport to and release at a given site in the body, in a temporally dependent manner—that is, drug-delivery systems (2). Within the past 15 years, the development of several prominent analytical science applications, such as immobilized liposome chromatography, liposome capillary electrophoresis, flow-injection liposome immunoassays, liposome-amplified sensors, and microfluidic analytical devices, led to the requirement of new analyte transduction modalities or analyte/reagent delivery routes that could be achieved by stimuli-responsive containment and release systems (3, 4). Interest in molecular assembly containment systems for these application areas is based on their intrinsic characteristics combined with the diverse nature and number of possible stimuli triggering scenarios for contents release.

Initial interest in stimuli-responsive, molecular assembly–based containment and release systems that respond to electron-transfer (redox) stimuli was virtually coincidental with founding work on pH-triggerable phospholipid vesicles (liposomes). In a brief 1980 report, Baumgartner & Fuhrhop (5) made the first redox-active vesicles based on bipyridinium amphiphiles. Although their work demonstrated that nonpolar molecules can be solubilized or hosted by the redox-active bipyridinium vesicles and that the redox moieties of the vesicles can be reduced with a chemical reducing agent, there was no indication that the solubilized guests could be released upon redox activation. Two years later, Fendler's group (6) reported the synthesis of a large number of bipyridinium surfactants that were readily made into micelles and vesicles; the nature of the assembly (aggregate type) depended on the structure of the surfactant. Vesicles constructed from these redox-active surfactants could contain and retain 2-aminopyridine, but there was no discussion of redox-induced release of the guests. This is not surprising, as most initial efforts in the area of redox-active assemblies were spurred on by ways in which energy transduction in naturally occurring membranes could be modeled and mimicked with synthetic membrane systems. Nonetheless, these studies by Baumgartner & Fuhrhop and the Fendler group led to a developing field focused on molecule-delivery systems whose contents can be released, in many cases reversibly, as a result of the application of a redox stimulus.

The guiding forces that led early investigators in the field of redox-active delivery systems are still essential to the development of these systems for a given application today, and they can be summed up in one universal question: What are the desired characteristics of the delivery system? This obvious and apparently singular question is of course built upon questions that address (*a*) the environment in which the delivery system must be maintained and that, in turn, may be different from the one in which the system encounters the stimulus for contents delivery; (*b*) the mechanism of action for the redox stimulus that will be used to trigger delivery; (*c*) the attributes of the material to be delivered; and (*d*) the rate of contents delivery. These questions ultimately lead to the choice of the physical nature of the delivery system, that is, the aggregate structure.

This review focuses on molecular assembly–based delivery systems that respond to an electron-transfer stimulus. Such systems have benefited greatly from the lessons learned from redox-active polymers (7), dendrimers (8), and supported monolayers (9) applied to molecule capture and/or release. In particular, I discuss delivery systems composed of aggregated amphiphilic monomer

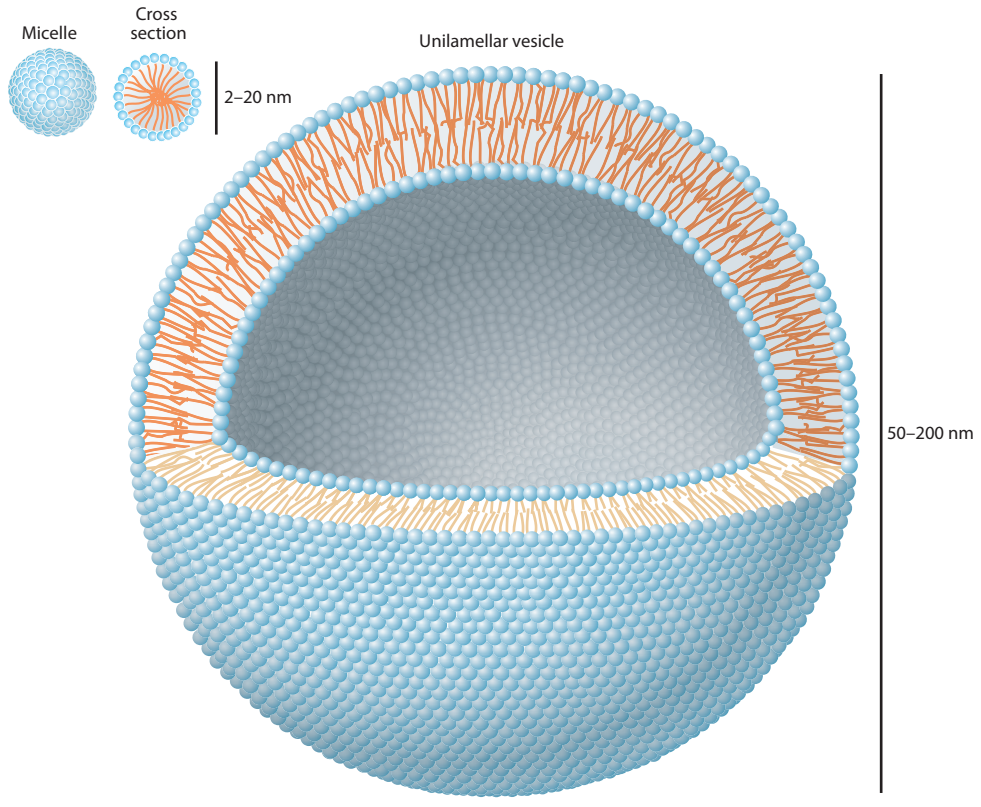


Figure 1

Two specific types of molecular aggregates based on surfactant monomers.

species in the form of micelles or vesicles (**Figure 1**). The crux of research on redox-stimulated delivery of contents from molecular assemblies/aggregates is the disassembly of the aggregate structure into another form, such as individual amphiphile monomers, to expose the contents to the surrounding medium. For traditional micelles, this redox-induced transformation is typically achieved as a result of the increased solubility of the monomer amphiphile via a change in charge and/or hydrophilicity of the amphiphile, such that the critical concentration of amphiphiles needed to create an aggregate structure is increased. For micelles or vesicles stabilized via cross-links, removal of the cross-linking structure leads to disassembly of the monomer units as a result of decreased intermolecular interactions. In addition to these modes of action, new directions include those wherein electron-transfer reactions can be used to remove chemical groups from the polar heads of amphiphiles and lipids so as to cause a phase transition of the amphiphile/lipid system. These redox-induced head group removal routes for the destabilization of molecular assemblies are quite powerful and show much promise for the development of a new class of sophisticated delivery agents with tunable delivery characteristics.

In 2005, my laboratory began to study the behavior of surfactants with a quinone head group that can be cleaved after redox activation (10). This new research endeavor was initiated as a result of our work with redox-active dendrimer encapsulation systems (11–13). Our efforts turned toward use of the redox-removable quinone propionic acid head group as part of a phospholipid

that can be fashioned into unilamellar vesicles (liposomes) whose contents can be released upon redox activation. These triggerable quinone liposomes and their derivatives show much promise for applications in microanalysis devices and drug delivery.

CMC: critical micelle concentration

Below, I give an overview of the work of the pioneers, and those of us who have followed, in the field of molecular assembly–based delivery systems that respond to an electron-transfer stimulus. In particular, I review their fundamental hosting and release behavior, as well as their reported use in analytical science and drug-delivery applications.

2. MICELLES AND MICELLE-LIKE AGGREGATES

2.1. Disaggregation Caused by Charge and Polarity Changes

Disruption of micelles and micelle-like aggregates can be achieved by altering either the charge on or the relative polarity of the individual surfactant monomers that constitute the aggregate. As a result, the surfactant monomer concentration at which the surfactant aggregate forms is no longer the same.

2.1.1. Change in the charge of amphiphiles. Saji et al. (14, 15) are credited with creating and demonstrating the original redox-responsive delivery systems in 1985. They recognized that the ability to change the charge state of a surfactant by an electron-transfer stimulus would have a significant impact on its ability to form molecular aggregates, such as micelles, at a given concentration and temperature. Knowing that oxidized ferrocenes are relatively stable in aqueous media, Saji et al. investigated two ferrocene-based, single–aliphatic chain surfactants whose critical micelle concentration (CMC) could be altered by oxidation of the neutral ferrocene substituent to the cationic ferrocenium (**Figure 2**). The first system was based on (11-ferrocenyl-undecyl)trimethylammonium bromide (FTMA), with the ferrocene group in the tail of the surfactant (14); the other molecular aggregate, based on ferrocenyl-dimethylundecylammonium bromide (FUTA) (15), was composed of monomers with ferrocene attached to the polar head of an ammonium-based *n*-dodecane surfactant, $FcCH_2N^+(CH_3)_2C_{12}H_{25}$ (**Figure 2**). In both cases, the native (reduced) version of the surfactants, in aqueous media, formed micelles containing 90–230 surfactant monomers, and they could solubilize (host) a relatively water-insoluble azo dye. Upon oxidation of the micelles with electrochemical or chemical methods, the CMC of the surfactants increased dramatically, which demonstrated that the more highly charged monomers are more soluble. As a result of micelle oxidation, the hosted dye precipitated from solution; this process was highly reversible, for resolubilization of the dye occurred upon reduction of the solution.

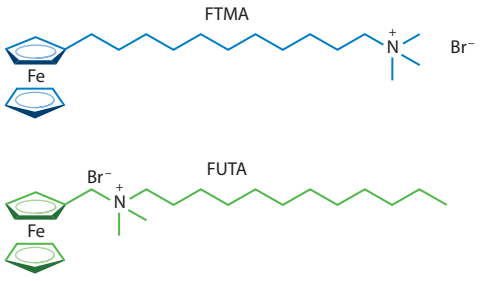


Figure 2

The structures of (11-ferrocenyl-undecyl)trimethylammonium bromide (FTMA) and ferrocenyl-dimethylundecylammonium bromide (FUTA).

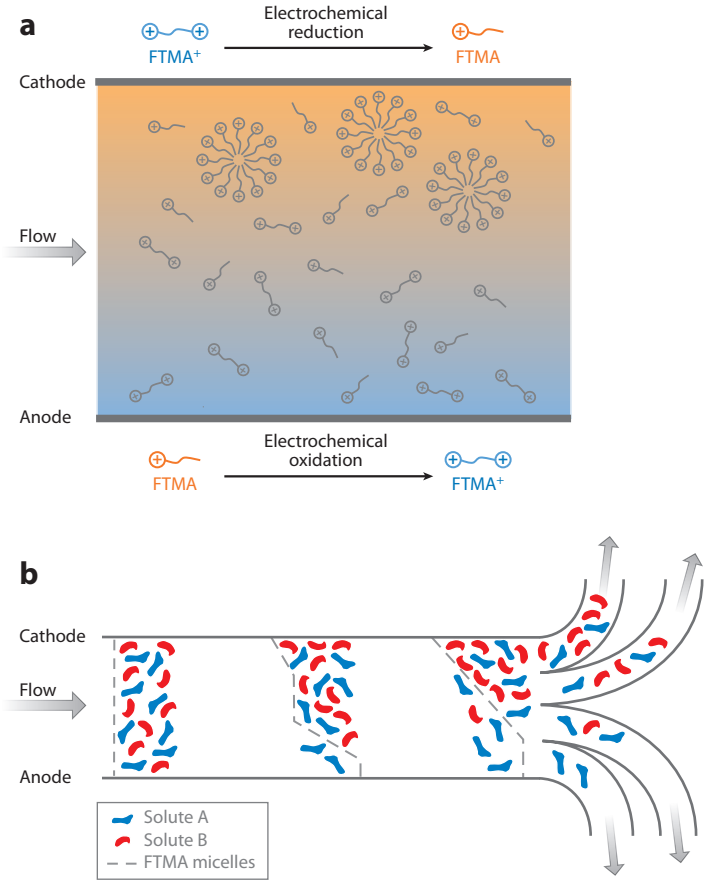


Figure 3

(*a*) An electrochemically generated gradient in a concentration of (11-ferrocenyl-undecyl) trimethylammonium bromide (FTMA) across a microfluidic channel. (*b*) Differential transport of two species, A and B, in a microfluidic channel that has opposing electrodes and contains an aqueous solution of FTMA.

The Saji group and others (16–20) later showed that this effect is not limited to ferrocenes by successfully switching the aggregate state of surfactants to which other redox-active groups were appended, using the redox-active surfactant either alone or in conjunction with other surfactants. The authors used various redox groups, including azophenols (16), anthraquinones (17, 18), phenothiazine (19), and tetrathiafulvalene (20). However, in only two of the reports were successful hosting and release of a guest demonstrated (16, 20).

Building on this research, Abe and colleagues (21) found that the viscoelasticity of solutions containing FTMA and salicylate could be drastically changed by oxidation of the FTMA/salicylate mixtures, which created a new class of electrorheological fluids whose physical properties could be altered by using very low voltages. The large change in viscoelasticity observed upon oxidation was a result of the destruction of micrometer-long, worm-like micelle structures that existed for the native FTMA/salicylate solutions. In addition, these materials are envisioned as redox-responsive hosting/release systems for dyes and perfumes.

Liu & Abbott (22) recently reported a new continuous-flow separation methodology based on the production of a concentration gradient of FTMA monomers and micelles (**Figure 3**). The

basis for the separation is the partitioning of analyte into FTMA micelles; the concentration of the latter is controlled across the flow path of a microfluidic device. The separation of analytes occurs as a result of the differing degree of analyte interaction with the FTMA micelles; the magnitude of the interaction depends on analyte properties. A major advantage of this method is that the gradient of FTMA concentration is controlled by applied electrode potential, so the separation of analytes with slightly different characteristics, including those that are sparingly soluble in water, can be readily achieved. This electrochemical field-flow fractionation method is attractive for the separation and purification of species in field-deployable microfluidic devices because (*a*) it requires the application of voltages much lower than those used in the traditional electric field-flow fractionation method and (*b*) it can separate samples in high–ionic strength solutions.

2.1.2. Polarity changes of amphiphiles. Micelles in aqueous media can be constructed from amphiphiles that are diblock copolymers with a hydrophilic block and a hydrophobic block; the hydrophobic portion forms the core of the micelle, and the micelle shell or corona consists of the hydrophilic polymer block. Variations in the chemical nature of the two different blocks can be used to create micelle structures with stabilities that are typically much higher than those of micelles made from traditional low-molecular-weight amphiphiles, as reflected by their lower critical aggregation concentrations and increased time for dissociation to the monomeric state upon dilution. In addition, hosting levels of hydrophobic materials in the core of polymeric micelles can be enhanced through the selection of a liquid-like core polymer. Furthermore, responsive moieties can be placed in either block to allow for micelle collapse and release of the guest upon an encounter with a specific type of stimulus. Thus, the architecture of polymeric micelles can be tuned to improve their ability to retain guest material in a given environment and to subsequently release the guest upon endogenous or exogenous cues.

Recently, Hubbell and colleagues (23) showed that redox-responsive micelles can be constructed from amphiphiles based on a diblock copolymer composed of poly(ethylene oxide) (PEO)–poly(propylene sulfide) with different numbers of propylene sulfide monomer repeats, denoted EO_{44}-PS_x. Loading of the sparingly water-soluble drug cyclosporin A into the poly(propylene sulfide) hydrophobic core is a function of the propylene sulfide monomer repeat number x; the weight percent of loaded cyclosporin A ranges from 3.1% to 19% for $x = 10$–40. Such high values of guest loading are a direct consequence of the micelle design that leads to the presence of the amorphous poly(propylene sulfide) block and its relative proportion to the hydrophilic PEO block. Upon environmental (air) oxidation of the poly(propylene sulfide) core to the corresponding poly(propylene sulfone), which is hydrophilic, slow and steady release of the cyclosporin A occurred. Previous work from Hubbell's group (24, 25) showed that oxidation of poly(propylene sulfide) to poly(propylene sulfone) can be achieved with various oxidants. Near-quantitative, linear (nonburst) cyclosporin A release was achieved within 9 to 12 days under aerobic conditions at 37°C; the 12-day release was a function of the length of the poly(propylene sulfide) block. The EO_{44}-PS_{10} released all of its contents within 9 days, whereas complete release required 12 days for the EO_{44}-PS_{20} and EO_{44}-PS_{40} micelles. To date, these are the only known polymeric micelles whose contents can be delivered by a change in the hydrophilicity of one of the blocks as a result of a redox process.

2.2. Disconnections That Lead to Disaggregation

Although vesicles stabilized by disulfide linkages of the amphiphiles that made up the vesicles were reported in 1991 (26), not until 2000 were thiol-responsive micelles shown to be feasible

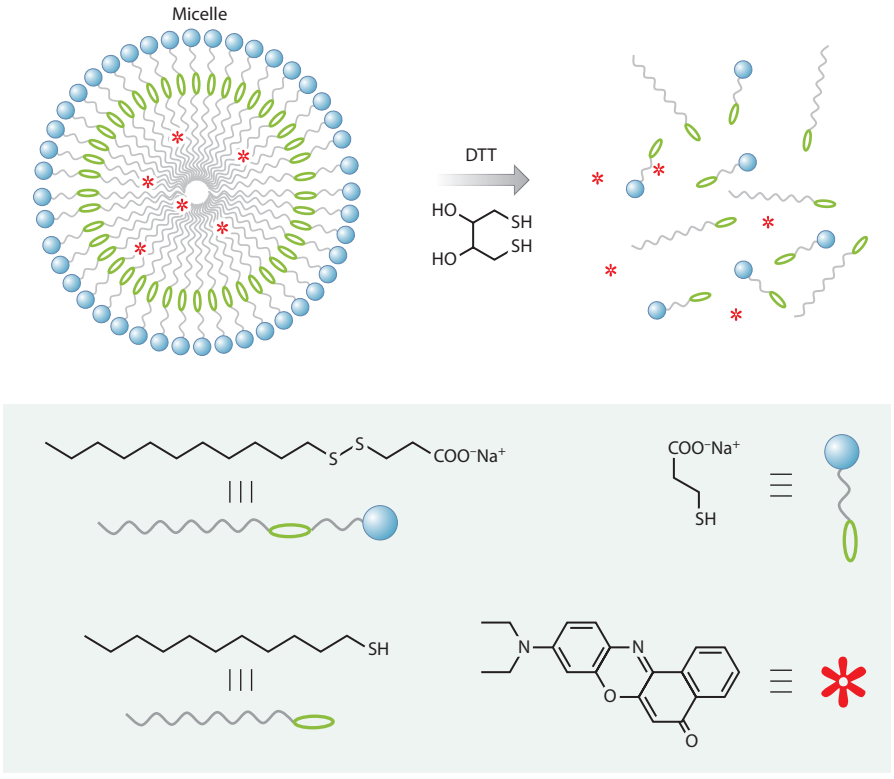


Figure 4

Schematic depiction of dithiothreitol (DTT)-induced disruption of micelles composed of sodium 3–(undecyldisulfanyl)propanoate and containing the solvatochromic dye Nile red (*red asterisks*).

(27). Further, in 2007, micelles containing disulfide linkages were found to release their contents through the reduction of the disulfide bond within the amphiphiles (28). Thayumanavan and colleagues (28) demonstrated that a two-component amphiphile, consisting of an anionic head group linked to a neutral, nonpolar alkane tail by a disulfide, forms micelles in aqueous media. Following the addition of the powerful thiolytic reducing agent dithiothreitol [DTT; $E_{1/2} = -0.33$ V versus a standard hydrogen electrode (SHE) (29)], the disulfide bond of the monomeric surfactant was cleaved to yield a much more soluble amphiphile, thereby diminishing the source of surfactant in equilibrium with the micellar species. As a result, micelles that contained the relatively insoluble dye Nile red released the dye within 95 min of exposure to DTT (**Figure 4**).

Thayumanavan and colleagues (30) later showed that polymeric micelles based on cleavable disulfide-linked block copolymers release all of their hydrophobic cargo within three days of exposure to 1.6×10^{-2} M glutathione, which is present in certain cancer cells at levels greater than 1×10^{-2} M (31). McCormick and colleagues (32) used a similar approach for shell cross-linked block copolymer–based micelles; these authors used DTT as the reducing agent. Furthermore, the Zhang group (33) made micelles from block copolymers containing diselenide bonds that can be broken up by glutathione reduction and hydrogen peroxide oxidation.

DTT: dithiothreitol

SHE: standard hydrogen electrode

3. VESICLE, LIPOSOME, AND POLYMERSOME ASSEMBLIES

3.1. Charge and Polarity Changes That Result in Destabilization of Assemblies

Electron-transfer reactions can be used to change either the charge or the polarity of assemblies that have an amphiphile bilayer structure. The result is conversion to another aggregate state, such as a micelle. The amphiphiles can be made of simple organic lipids, phospholipids, or polymers.

3.1.1. Changes in charge. Gokel and colleagues (34–36) recognized the importance and potential of redox-active amphiphiles that could be used to make switchable, vesicular containment systems; these authors moved the field forward from the initial studies by Baumgartner & Fuhrhop and the Fendler group by creating various amphiphiles whose properties could be altered via charge-state changes resulting from electron-transfer reactions. These amphiphiles included surfactants based on ferrocene and metal cation-ligand complexes; the redox moiety was attached to either aliphatic chains or cholesterol. The cationic versions of these amphiphiles existed as vesicles that, upon chemical reduction to less-charged species, were destroyed, as observed by electron microscopy and light-scattering data. For example, in its oxidized (ferrocenium) state, a ferrocenylmethanol–cholesterol ether derivative formed large vesicles, whereas the neutral ferrocene derivative did not (34). However, these reports made no mention of contents release.

Further work with ferrocene-based surfactants by the Abe group (37–39) clearly indicated that the oxidation state of the ferrocene group dictates whether the surfactant forms vesicles or micelles in aqueous media, in either the presence or absence of other amphiphiles. Although these studies reported glucose-encapsulation efficiencies, they did not directly address the release of contents. Interestingly, Abbott and colleagues (40–42) later showed that one of the ferrocene amphiphiles developed by Abe and coworkers, namely *bis*-(*n*-ferrocenyl-alkyl)dimethylammonium bromide (BFDMA), forms multilamellar, liquid-crystalline complexes with DNA (lipoplexes) in both its oxidized and reduced states. The oxidized BFDMA/DNA lipoplexes provided low levels of cell transfection, but DNA delivery into cells was highly efficient with the reduced BFDMA/DNA lipoplexes. Importantly, the charge state of ferrocene in poly(ferrocenylsilane) diblock copolymer electrolytes may be used for control of the electrolyte's aggregate state and, therefore, the ability to deliver contents from their vesicular form (43, 44).

In a clever twist on the work of the Abe and Abbott groups, Yuan and colleagues (45) showed that redox switching of the ferrocene group in PEO–ferrocene surfactant/poly(styrene)–β-cyclodextrin surfactant (PEO-Fc/PS-β-CD) assemblies can lead to the formation and destruction of vesicles (**Figure 5**). By taking advantage of the well-known high binding constant of neutral ferrocene (as compared with the low affinity of ferrocenium) for β-CD, these authors found that the dye Rhodamine B can be retained within vesicles of PEO-Fc/PS-β-CD. However, upon electrochemical oxidation to the ferrocenium species, the PEO-Fc/PS-β-CD monomers of the ~100-nm-diameter vesicles dissociate from one another, thereby causing the vesicles to disintegrate as a result of increased amphiphile solubility in aqueous milieu; the process is reversible in nature. By varying the strength of the applied external potential, the authors could tune the onset of burst-phase Rhodamine B release over a broad range; earlier onset of release occurred with higher applied potential.

Park and colleagues (46) have taken advantage of the characteristics of poly(aniline) in its various redox states to construct switchable vesicles from an oligo(aniline)–tailed nonionic surfactant. The tetraaniline-PEO amphiphile in its reduced leucoemeraldine base form formed ~120-nm-diameter unilamellar vesicles that retained a fluorescein derivative upon dialysis treatment. On the basis of light-scattering, electron microscopy, and visible spectroscopy results,

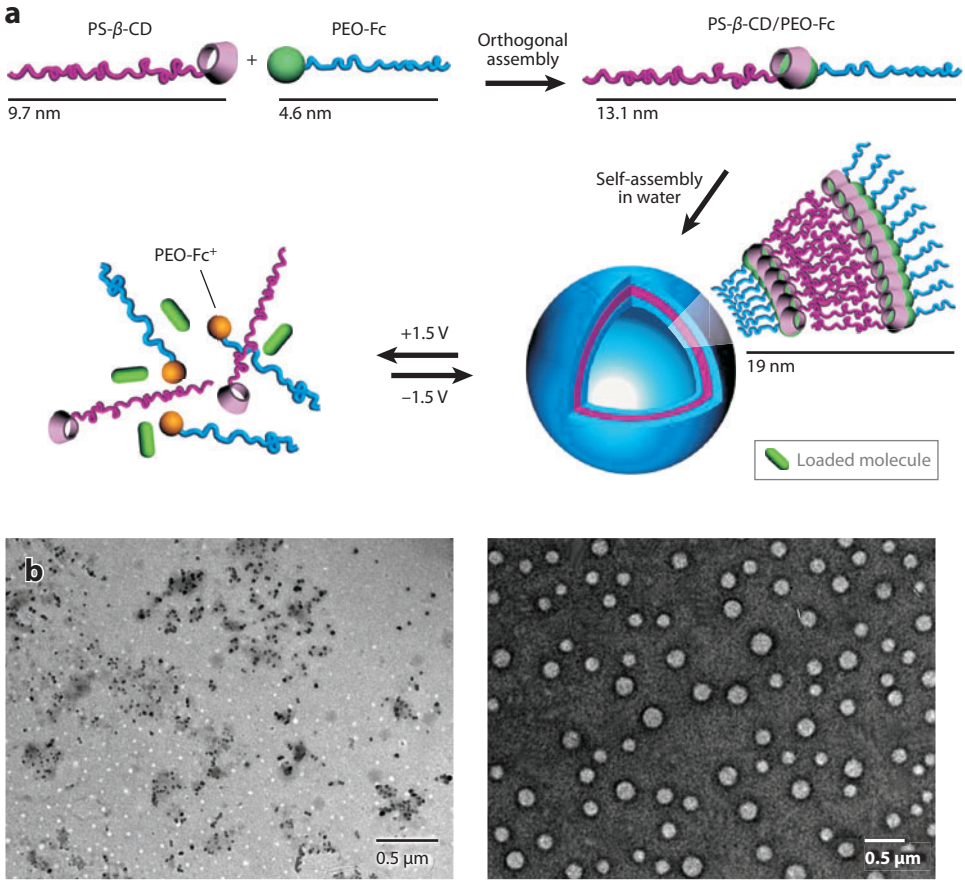


Figure 5

(*a*) Structures of poly(styrene)–β-cyclodextrin (PS-β-CD) and poly(ethylene oxide)–carboxylate (PEO-Fc) and schematic of the voltage-responsive controlled assembly and disassembly of PS-β-CD/PEO-Fc supramolecular vesicles. (*b*) Transmission electron micrographs of the PS-β-CD/PEO-Fc aggregates upon electrochemical treatment at (*left*) +1.5 V or (*right*) -1.5 V.

electrochemical oxidation of the tetraaniline-PEO amphiphile to its emeraldine base form led to disassembly of the vesicles, yielding puck-like micelles and free fluorescein dye. This change, which is highly reversible in nature, was rationalized by the changes in packing density of the hydrophobic tetraaniline units when moving from the leucoemeraldine base form to the emeraldine base form; lateral shrinkage of the tetraaniline core upon oxidation to the emeraldine base state causes membrane destabilization and rupture to form the puck-like micelles.

3.1.2. Polarity change. Stimuli-responsive vesicles made from block polymer–based amphiphiles, also known as polymersomes, are becoming popular due to the wide variety of functionalities that can be designed into their structure. By combining hydrophilic block polymers with others that are hydrophobic in nature, one can create an amphiphile that forms stable vesicles. In addition, similar to the scenario described above with micelle-like aggregates formed from diblock copolymer amphiphiles, activation of a stimuli-responsive unit incorporated into the hydrophobic

block can cause destruction of the vesicle as a result of changes in the charge or polarity of the amphiphile.

In an elegant study by Hubbell and colleagues (25), an amphiphile based on an ABA triblock copolymer composed of EO_{16}-PS_{50}-EO_{16} was used to construct polymersomes. Oxidation of the EO_{16}-PS_{50}-EO_{16} polymersomes by hydrogen peroxide—either directly added as a reagent or produced intravesicularly by glucose oxidase (24)—led to the formation of worm-like micelles. This transition resulted from oxidation of the hydrophobic poly(propylene sulfide) block to the hydrophilic poly(propylene sulfone), an event that takes place within 250 h for 0.03% by volume hydrogen peroxide. This study did not discuss the release of contents from the EO_{16}-PS_{50}-EO_{16} polymersomes upon redox-activated destabilization.

3.2. Disconnections Caused by Redox Reactions

The release of contents from amphiphile bilayer–based structures, vesicles, and liposomes can result from either a phase transition to an amphiphile monolayer–based micelle state (rapid burst release) or an enhanced permeability of the bilayer membrane (release by leakage). Such a release can be accomplished by electron transfer–induced cleavage of amphiphile moieties, typically at the polar head of the amphiphile.

3.2.1. Disruption of stabilizing disulfide cross-links. The original report of a vesicle stabilized with an amphiphile possessing a pendant, cleavable functionality was made in 1991 by Ishigami and coworkers (26). This inaugural work showed that stable liposomes can be made from a cystine amphiphile and mixtures of lecithin and cholesterol. The responsive lipid was composed of two fatty acid chains (lauroyl) covalently attached to the amine functionalities of cystine. The authors hypothesized that direct addition of a reducing agent or an enzymatically produced reducing agent of sufficient potency would lead to cleavage of the disulfide bond in the head group of the cystine lipid, thereby causing liposome contents release. Leakage of liposome contents—not rapid release in a burst fashion—was demonstrated through the addition of ascorbic acid; the permeability rate of the entrapped liposome contents was a function of the ascorbic acid–to–lipid ratio. Approximately 75% of the contents leaked out of the liposomes within 5 h when a 9.5:1 ratio of ascorbic acid to cystine lipid was used (40% cystine lipid, 20% cholesterol, and 40% lecithin lipid).

In an exciting sensing application of the cystine lipid–based vesicles, Ishigami and colleagues (26) showed that the reducing agent reduced flavin adenine dinucleotide ($FADH_2$), produced via a coupled enzymatic reaction, causes release of a fluorescent dye from the interior of liposomes. In this study, the $FADH_2$ was produced from FAD (flavin adenine dinucleotide) as a result of the latter's reduction by reduced NADPH (nicotinamide adenine dinucleotide phosphate), which was generated by the enzymatic consumption (oxidation) of glucose by glucose dehydrogenase. Thus, the presence of glucose was transduced into a fluorescence signal via dehydrogenase action. However, a significant delay in the onset of the release of the calcein dye after the addition of 4×10^{-3} M glucose to the liposomal/enzyme system pointed to a strong dependence of the contents release on redox agent ($FADH_2$) concentration, as was observed with the ascorbic acid reducing agent. This observation indicated that attempts to use this system for analyte detection would lead to a lack of sensitivity and, thus, applicability. Nonetheless, this first report of redox-induced release of liposome contents became the cornerstone for subsequent investigations that have yielded liposomes that permit significantly faster contents release for drug-delivery and analytical applications.

Hubbell and colleagues (47) demonstrated the viability of thiolytically reduction-sensitive polymersomes. Through the incorporation of a stimuli-responsive cleavable linker between the two

polymer blocks, it is possible to make vesicles whose contents can be liberated upon application of an appropriate stimulus. In this study, a block copolymer composed of PEO and poly(propylene sulfide) connected together by a disulfide (EO_{17}-SS-PS_{30}) could be readily made into stable vesicles. The authors reported that the liposomes could be opened upon addition of either cysteine or glutathione, which cleaved the disulfide of the EO_{17}-SS-PS_{30}, as observed in the case of cysteine by the release of entrapped dye from the vesicles. The rate of contents delivery was such that roughly 30% of the total contents were freed within 15 min of the addition of cysteine; complete release required multiple additions of the thiol reducing agent. Cellular uptake of the EO_{17}-SS-PS_{30} polymersomes by mouse macrophage cells was achieved, and apparent release of contents by thiolytic reduction in the endosome occurred. Interestingly, either the EO_{17}-SH or HS-PS_{30} products from polymersome reduction caused endosomal disruption, thereby causing the release of calcein into the cytoplasm of the macrophage cells. In a recent report by the Wang group (48), similar polymersome contents release was achieved with vesicles composed of PEO linked to poly(lactic acid) by a disulfide.

PE: phosphoethanolamine

3.2.2. Head group removal—disulfide cleavage. Zalipsky and coworkers (49, 50) proved that reductive removal of a protecting group from the head of potentially fusogenic lipids in liposomes leads to aggregation of the deprotected liposomes and concomitant release of liposome contents. The first-generation lipids were based on 1,2-distearoyl-*sn*-glycero-3-phosphorylethanolamine (DSPE), one of many phosphoethanolamine (PE) lipids that cannot sustain a lamellar form at pH values below ~8.5, the pKa of the ethanolamine moiety. As a result, liposomes cannot be formed from PE lipids under typical physiological pH conditions. However, Zalipsky and coworkers recognized that attachment of a protecting group onto the amine group of the ethanolamine functionality yields a lipid with both the geometric and charge requirements needed to form stable liposomes (lamellar phase) at various pH values. These authors proposed that removal of the protecting group would then lead to liposome destabilization and fusion, causing the release of liposome contents. They chose a disulfide protecting group for the DSPE lipid; this group was composed of a 2,000-molecular-weight PEO attached to dithiodipropionic acid. Stable liposomes were made from the protected DSPE lipid, and their thiolytic reduction by 1×10^{-2} M DTT resulted in destabilization of the liposomes and contents release caused by cleavage of the disulfide bond of the dithiodipropionic acid linker. Fluorescent lipid mixing assays indicated that lipid bilayer fusion is the key to contents release; this observation was consistent with the original hypothesis. Reductive removal of the bulky PEO groups from the liposomes led to release of 100% of the entrapped dye within 2 h. However, the requirement that the potent reductant DTT [$E_{1/2} = -0.33$ V versus SHE (29)] had to be used to liberate liposome contents, and the formation of the resulting modified lipid (3-mercaptopropionyl–DSPE) after reduction, made this system unattractive for drug-delivery applications.

As a result, Zalipsky et al. (51) implemented a cleverly designed cleavable group to ensure its complete removal from PE lipids upon exposure to biologically relevant thiols. In this case, the bulky PEO group was attached to DSPE via a dithiobenzylcarbamate (DTB) linker because full removal of this linker from amine groups had been demonstrated in prodrugs (52). Upon thiolytic reduction in aqueous media, this linker cleaves via an electronic cascade elimination route (53) to form carbon dioxide and (4-mercaptophenyl)methanol, thereby releasing the amine group to which it was originally attached (**Figure 6**). Stable PEO-DTB-DSPE liposomes were successfully formed for both the *ortho*-DTB and *para*-DTB linkers, as noted from lack of contents leakage in pH-7.2 buffer. However, upon exposure of PEO-DTB-DSPE liposomes to micromolar and larger concentrations of cysteine [$E_{1/2} = -0.22$ V versus SHE (54)] at 37°C, release of 100% of the contents could be achieved in 20 to 30 min. Interestingly, the rate of contents release

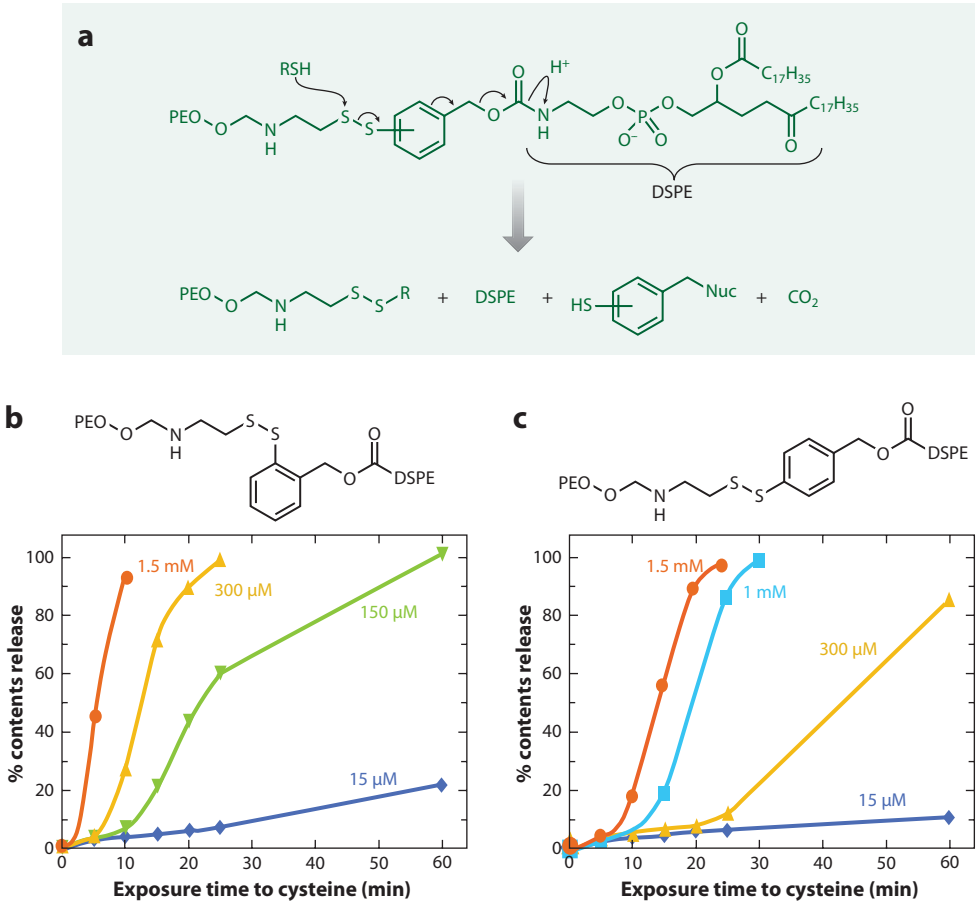


Figure 6

(*a*) Schematic of thiol-induced cleavage of poly(ethylene oxide) (PEO) from 1,2-distearoyl-*sn*-glycero-3-phosphorylethanolamine (DSPE) lipid linked by the dithiobenzylcarbamate (DTB) group. (*b*,*c*) Temporally dependent contents release from PEO-DTB-DSPE liposomes with either (*b*) the *ortho*-DTB linker or (*c*) the *para*-DTB linker as a function of the concentration of the cysteine reducing agent.

was sensitive to the DTB linker connectivity; liposomes made from PEO-DTB-DSPE lipids containing the *ortho*-DTB linker released their contents twice as fast as those liposomes made from lipids containing the *para*-DTB linker. This finding stands in contrast to the results from the original study of prodrugs using the DTB linker, wherein release of the drug from the DTB moiety was four times faster in the case of the *para*-DTB linker versus that of the *ortho* isomer. These outcomes point to differences in DTB elimination chemistry that may result from different chemical environments for the DTB in the lipid bilayer assembly compared with free solution.

The Arbuzova group (55) recently showed that liposomes stabilized by an amphiphile with a cleavable, bulky, cationic head group can be made to release their contents upon thiolytic reductive cleavage of the bulky head group from the amphiphile. The new amphiphile may have a more cylindrical shape, which would stabilize liposomes containing a large amount of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). Upon thiolytic reductive removal of the bulky head group by cysteine and glutathione, the resulting amphiphile purportedly takes on an inverted

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine

cone shape more similar to that of DOPE, thereby destabilizing the liposomes and making them amenable to fusion with other liposomes or membranes. Interestingly, cellular uptake for this thiol-sensitive liposome is comparable to that for its noncleavable analog, but the amount of contents release within macrophages was 10 times greater for the thiol-sensitive liposomes.

3.2.3. Head group removal—cyclization of reduced quinone propionic acids. Our group has been actively pursuing liposomes composed of phospholipids stabilized by the presence of a quinone propionic acid attached to the head group of a lipid that would normally be unable to form stable liposomes in aqueous media under physiological pH conditions (56). Through removal of the quinone protecting groups from the liposomes via quinone group reduction, the resulting PE-rich liposomes can approach one another and aggregate, then release their contents (**Figure 7**).

The synthetic lipids we have been investigating are composed of commercially available DOPE lipids covalently linked to substituted quinone propionic acids through the amine functionality of the lipid ethanolamine head group (**Figure 7**). As mentioned above, attachment of protecting groups to the amine of PE lipids can create phospholipids that are able to form stable lamellar-phase structures if the necessary geometric and charge requirements are met; removal of the protecting group renders the PE lipid incapable of sustaining a stable lamellar phase. The quinone propionic acid groups, either present as the free acid or attached to an amine (amide) or alcohol (ester), can undergo two-electron reduction by chemical agents to yield the respective hydroquinone propionic acid. Formation of the hydroquinone allows for an intramolecular cyclization reaction wherein the phenol hydroxyl group (immediately adjacent to the propionic acid) attacks the carbonyl of the acid side chain, which leads to cleavage of any group linked through the carboxyl functionality and formation of the cyclized product (lactone). Previous work with free *ortho*-hydroxyhydrocinnamic acids yielded the observation that the rate of the intramolecular cyclization reaction (lactonization) for those compounds is greatly increased if the quinone propionic acid has a trimethyl-lock motif, that is, two methyls at the geminal position and a third *ortho* to the site of attachment for the propionic acid side chain (57). Such a trimethyl-lock scenario for the *ortho*-hydroxyhydrocinnamic acids results in cyclizations that have kinetic half-lives of minutes. Similar kinetic half-life values have been observed for a select group of reduced quinone propionic acids attached to simple amines (58). As a result, the trimethyl-locked quinone propionic acid protecting group has been targeted for synthetic strategies (59) and in the development of potential prodrugs (60, 61).

Liposomes prepared with DOPE derivatized with the trimethyl-locked quinone propionic acid ($R_1 = R_2 = R_3 = R_4 = CH_3-$) can be readily opened to dispense their contents upon addition of a reducing agent with a sufficiently negative reduction potential. The release of calcein dye occurs rapidly upon addition of sodium dithionite [$E_{1/2} = -0.7$ V (62)] to ~100-nm-diameter liposomes composed of the trimethyl-locked quinone lipid Q-DOPE (**Figure 8**). Prior to reductant addition, the liposomes are stable with regard to contents release. The presence of the trimethyl-lock motif causes the release of liposome contents, as has been observed in control experiments with liposomes constructed from a quinone that does not possess the trimethyl lock ($R_4 = H-$) (**Figure 8**). Thus, upon exposure of a sufficient and critical number of ethanolamine head groups on the Q-DOPE liposome surface as the result of lactone formation, the now-PE-rich liposomes can approach and contact each other such that their contents can be released.

Most liposome-based (and other) delivery systems respond to either a very specific stimulus or a general one. As a result, almost all systems suffer from a lack of tunability, which arises either from the stringent synthetic design that went into their making or from the lack of existing chemistries that would provide a response to a given type of stimulus. This situation is similar to

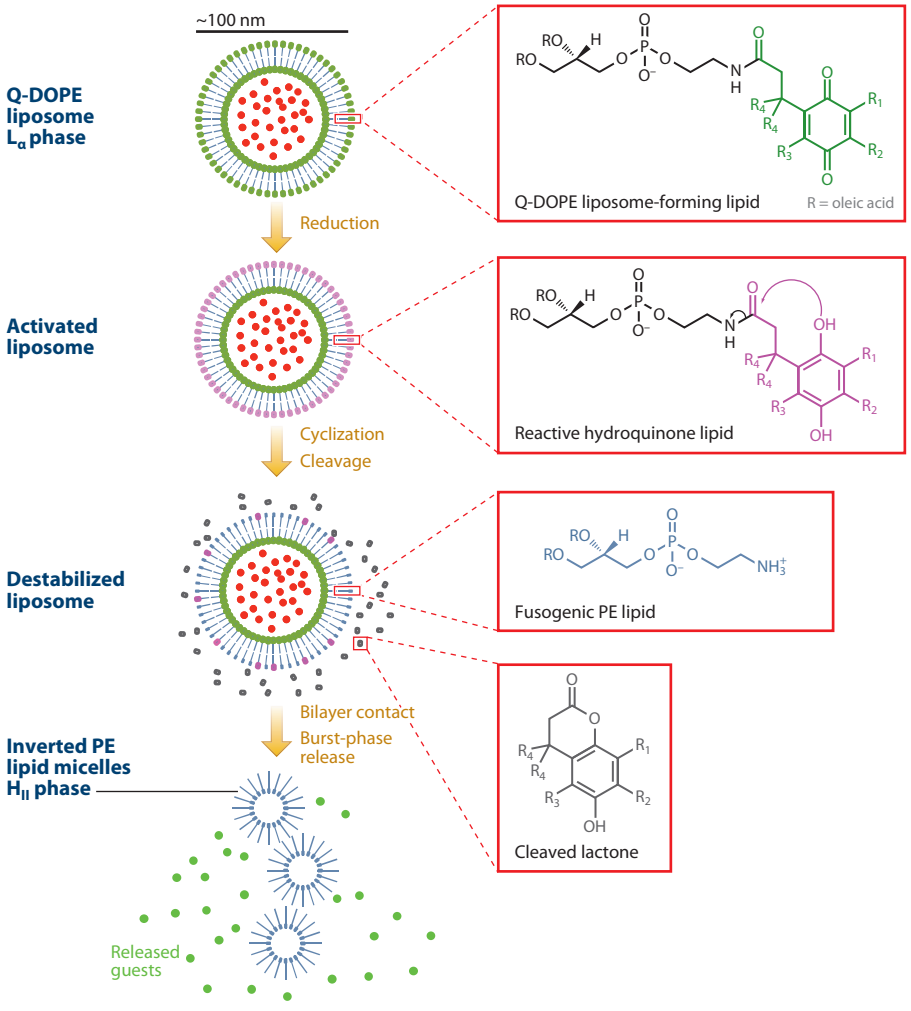


Figure 7

Schematic of reduction-induced removal of quinone propionic acid units from quinone propionic acid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine [Q(R_1)-DOPE] liposomes and subsequent release of contents upon a lamellar-inverted hexagonal-phase transition of the DOPE lipids. Abbreviation: PE, phosphoethanolamine.

that of an analytical methodology with a characteristic high selectivity but low sensitivity, or one that possesses a high sensitivity but a low selectivity.

An extremely important aspect of quinone propionic acid DOPE [Q(R_1)-DOPE] liposomes is their potential for tunable contents release; the triggering of release should be exquisitely influenced by the electronic nature of the quinone trigger group. In other words, the proper choice of quinone ring substituents, say, at position R_1, is expected to dictate the rate of protecting group removal from the lipid and, thus, the rate of contents delivery. A unique characteristic of such a tunable system is that the type of stimulus required for contents release remains the same, so the synthetic approach does too, which greatly simplifies evaluation and implementation of the tunable system. Achieving such a level of control over the release of materials is highly valuable

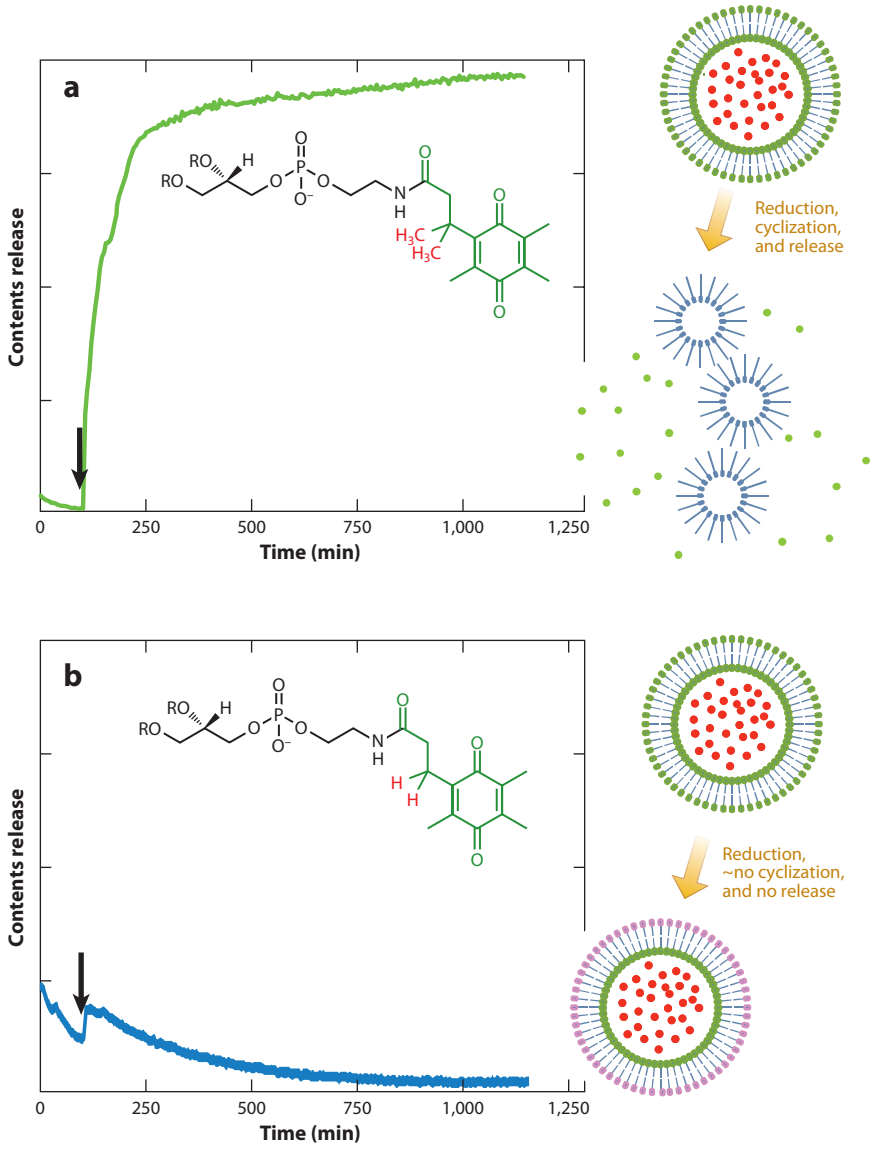


Figure 8

Temporally dependent contents release from Q-DOPE (quinone 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) liposomes made from (*a*) Q(R_1)-DOPE (quinone propionic acid DOPE) lipids possessing the trimethyl lock and (*b*) those not possessing the trimethyl lock. The black arrows indicate initiation by dithionite.

to their use in providing reagents in microfluidic devices or transduction of analyte presence, or for the delivery of therapeutics, at given times or locations.

Our group recently showed that a high degree of control over the rate of contents release from Q(R_1)-DOPE liposomes can be achieved through simple variation of the nature of the substituent on the quinone ring at R_1. Electron-withdrawing substituents at R_1 were initially thought to accelerate the rate of lactone formation–based removal of the quinone propionic acid protecting

group from the DOPE lipids of the liposomes and thereby increase the rate of contents delivery, whereas electron-donating substituents were thought to lead to a slower rate. This hypothesis is based on (*a*) the nature of the key species involved in the rate-determining step of the lactonization reaction, namely the tetrahedral intermediate resulting from phenol attack on the propionic acid carbonyl, and (*b*) the solution conditions used; higher rate constants are favored by those species whose negative charge can be stabilized by ring substituents (63). However, the rate of the overall lactonization reaction is also affected by the amount of tetrahedral intermediate formed from phenol attack on the propionic acid carbonyl, an amount that is increased by species with increased electron density in the ring. Furthermore, steric interactions between R_1 and the quinone ring substituents are also expected to influence the rate. Thus, the overall rate of reduced quinone protecting group removal was anticipated to be a complex function of the nature of the substituent at R_1.

We found that contents release for a group of Q(R_1)-DOPE liposomes is indeed heavily influenced by the presence of different substituents at R_1 (**Table 1**) (64). Q(R_1)-DOPE liposomes with R_1 = Br release their contents faster than liposomes with R_1 = *n*-propyl-NH, which in turn release their contents faster than liposomes with CH_3 and H substituents. The time required for Q(Br)-DOPE liposomes to release 50% of their contents is significantly less (32–38%) than for the Q(*n*-propyl-NH)-DOPE and Q(CH_3)-DOPE liposomes and 72% less than for Q(H)-DOPE liposomes. The order of the time required for contents release (Br < *n*-propyl-NH < CH_3 < H) is in qualitative agreement with decreasing order of the rate constant for the conversion of the reduced quinone (hydroquinone) to the lactone: Br > CH_3 > *n*-propyl-NH > H. These rate constants were obtained from cyclic voltammograms of the free acids via the analysis method employed by Nicholson & Shain (65) for an electrochemical step-chemical step scenario. Although the more electron-withdrawing Br substituent yields a faster rate constant for consumption of the reduced quinone and a faster rate for liposome opening versus the more electron-donating substituents, there is no clear trend in the rates for the electron-donating capability of the R_1 substituent, as expected.

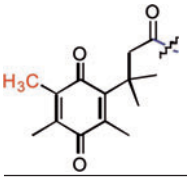
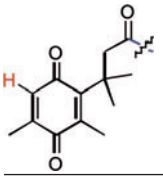
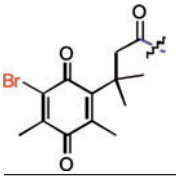
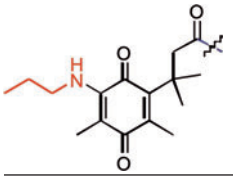
4. FUTURE CHALLENGES

4.1. Development of Tunable Redox-Active Trigger Groups

An area that requires investigation is the development of tunable trigger groups that respond to the same triggering stimulus such that the rate of contents delivery from the systems to which the trigger groups are appended is a function of the structure of the tunable trigger group. Delivery systems based on such tunable trigger groups will be highly valuable in drug- and reagent-delivery applications that demand that different rates of contents be provided at a given location. For example, such systems may be used to deliver multiple drugs to diseased sites so that one drug is provided faster or slower than another to provide the needed pharmacodynamics for successful treatment. Similarly, providing two different reagents in a chemical analysis (or synthesis) system at two different rates is desirable in many cases.

In light of these requirements, the significance of our observations with Q(R_1)-DOPE liposomes cannot be overstated with regard to the tunable nature of liposome contents release, which is achieved with a chemically similar trigger group and an identical type of stimulus for triggering (**Table 1**). Such an outcome is important for the development of delivery systems specifically selected for a given stimulus because the flexibility and simplicity of the synthetic route for the trigger group are great. Currently, the only two systems that can achieve such a tunable contents release with a given triggering stimulus are the Q(R_1)-DOPE liposomes and those used by

Table 1 Time required for the release of 50% ($t_{50\%}$) of liposomal contents from liposomes composed of substituted quinone propionic acid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine [Q(R_1)-DOPE] lipids

Q(R_1)-DOPE	$t_{50\%}$ (min)
	23.0 ± 6.5
	33.9 ± 4.4
	37.0 ± 2.1
	80.7 ± 10.5

Zalipsky et al. (51) (discussed in Section 3.2.2), which consist of the *ortho*- and *para*- DTB trigger groups attached to DSPE. In the Zalipsky group's work, removal of the DTB trigger group is very sensitive to the isomer of the DTB, whereas with the Q(R_1)-DOPE liposomes, the contents release is a function of the substituent at R_1. Thus, it will be valuable to examine contents release from liposomes with quinone propionic acid trigger groups wherein the varied substituent is placed at R_2 versus R_1. In addition, the use of the *ortho*-quinone isomer of the quinone propionic acid trigger is worthy of investigation, given that the uncatalyzed NADH reduction of substituted *ortho*-benzoquinones is 100-fold faster than that for the corresponding *para*-benzoquinones (66); our group is currently performing studies in this area.

Another valuable direction for future study is that of delivery systems with a chosen trigger group that selectively and sensitively responds to individual triggers in a given class of stimuli such that delivery of contents is rapid for one type of triggering stimulus of a given class but effectively nonexistent for another type of triggering stimulus in the same class. For example, triggers could possess different reducing powers (reduction potentials), such that only triggers with a potent-enough reducing potential would cause activation of a particular trigger group on a delivery vehicle. We recently reported that creation of such a trigger is possible with the quinone propionic acid trigger group when the R_1 substituent is a methyl group (67); only reducing agents with a sufficiently negative reduction potential can liberate the quinone propionic acid trigger group.

4.2. Increased Diversity of Redox-Active Trigger Groups

Research efforts with stimuli-responsive molecular delivery vehicles that can be activated by electron-transfer events will continue to grow, given the wide variety of triggering events that are present in biological systems and that can be designed to occur at solid electrodes and from chemical and photogenerated species. Given the potential diversity of synthetic chemistries, the development of many trigger groups and their use in delivery systems will become an intense area of research.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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