Vesicle Photonics

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Abstract

Amphiphiles, under appropriate conditions, can self-assemble into nanoscale thin membrane vessels (vesicles) that encapsulate and hence protect and transport molecular payloads. Vesicles assemble naturally within cells but can also be artificially synthesized. In this article, we review the mechanisms and applications of light-field interactions with vesicles. By being associated with light-emitting entities (e.g., dyes, fluorescent proteins, or quantum dots), vesicles can act as imaging agents in addition to cargo carriers. Vesicles can also be optically probed on the basis of their nonlinear response, typically from the vesicle membrane. Light fields can be employed to transport vesicles by using optical tweezers (photon momentum) or can directly perturb the stability of vesicles and hence trigger the delivery of the encapsulated payload (photon energy). We conclude with emerging vesicle applications in biology and photochemical microreactors.

INTRODUCTION

We define a vesicle as any quasi-spherical membrane that encapsulates a liquid-filled reservoir. This general definition does not specify the composition of the membrane, and a diverse range of materials has been used to provide vesicles with properties specific for different applications. Here, we focus on vesicles designed for photonic applications by exploiting optical processes occurring either at the membrane or in the liquid interior (**Figure 1*a***). The photonic properties and applications we discuss focus on light emission from vesicles, including fluorescence and nonlinear properties, as well as optical methods to manipulate vesicles, such as optical tweezers and vesicle destabilization.

Materials used to form such vesicles can be divided largely into two groups: (*a*) materials that serve primarily as carriers for other, more photoresponsive molecules, consisting primarily of natural and synthetic lipids (1–3) and polymers (4, 5), and (*b*) materials that possess intrinsic conducting or plasmonic properties, such as metals (6–9) and conducting polymers (intrinsically conductive polymers or conjugated polymers) (10, 11). Certain lipids (1), surfactants (12), and block copolymers (13) can be used to form vesicles of the first group, referred to as liposomes, niosomes (for nonionic surfactants), and polymersomes, respectively. The self-assembly of the amphiphilic molecular component of vesicles is a result of the aggregation of hydrophobic domains in response to a polar environment. This interaction can lead to many stable aggregate structures, such as spherical vesicles, micelles, rods, worms, and sponge phases (14). Different methods promote the formation of particular vesicular structures, as was recently reviewed elsewhere (1).

Hydration of thin multilayer (lamellar) films is one of the most common methods for synthesizing vesicles. This process can result in a concentration-dependent reorganization of the lamellae and is described by phase diagrams such as the one shown in **Figure 1*b*** (14). The final vesicular conformation is stabilized by a balance of forces stemming from the hydration of the water-soluble component, the surface tension between the hydrophobic domain and water, and the stretching of the hydrophobic aggregate that composes the membrane interior (15). All these

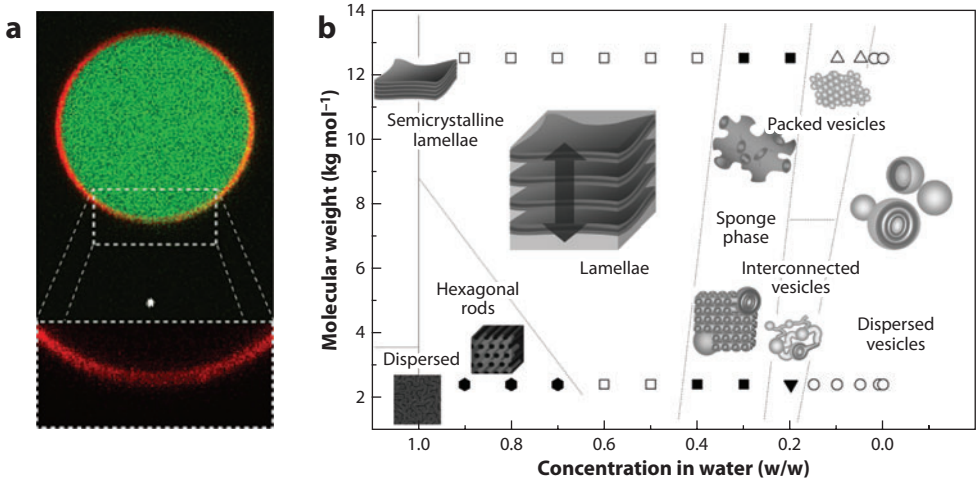

Figure 1

(*a*) Fluorescence confocal microscope image of a giant unilamellar vesicle with a red fluorescent membrane and a green fluorescent cargo. Adapted from Reference 166. (*b*) Aggregate structures as a function of block copolymer molecular weight and concentration. Adapted from Reference 14.

forces can be influenced by the size of the hydrophilic and hydrophobic domains; this size ratio is particularly critical for polymersomes, which use higher-molecular-weight domains to provide increased stability over liposomes (13). A more recent and versatile method of vesicle formation with high control over composition and size is the generation of double emulsions within microfluidic channels (16–18). This method provides enhanced control over the composition of the aqueous interior of the vesicles but is limited by a slow rate of vesicle production.

The interaction of light with vesicles can be due to the intrinsic optical properties of the vesicles' membranes. In addition, vesicles may serve as templates for loading photoresponsive molecules. The inclusion of molecules in the aqueous phase during vesicle formation can result in the encapsulation of these molecules within the aqueous core of the vesicle. Loading of amphiphilic and hydrophobic molecules can be achieved by dissolving them along with the assembling amphiphile in a mutual solvent prior to thin-film formation or dispersion into an aqueous solution. Although the encapsulation of hydrophilic molecules can be challenging (19), amphiphilic and hydrophobic molecules can be readily retained within the vesicle membrane with high efficiencies. For example, we have found the loading of hydrophobic photo-oxidizer ethyl eosin into the membranes of poly(ethylene glycol)-*bl*-poly(propylene sulfide) (PEG-*b*-PPS) vesicles to occur with a 93% encapsulation efficiency (5).

Photosensitive vesicles with metallic walls, often referred to as hollow nanoshells, demonstrate geometry-dependent plasmon resonances (20) that can provide advantages compared with solid-core alternatives of the same composition (21). Depending on the thickness of the wall, the plasmon peak for gold nanoshells can be tuned to be within the near-infrared (NIR) regime (6, 22), and thus these particles have been used frequently for in vivo photothermal ablation and imaging (23, 24). Photonic applications of hollow nanoshells are not reviewed here, but their material properties and synthesis are included below for completeness. Metallic nanoshells have been fabricated primarily by using template-based methods that employ silica (25), cobalt (26, 27), silver (7), or polymer (28) nanoparticles. The template core can then be removed through dissolution or a galvanic replacement to leave behind a hollow shell (6, 7, 29). Investigators have developed several new methods that avoid the often harsh temperature and chemical conditions required for template removal (8, 9, 22).

LIGHT EMISSION FROM VESICLES

Light emission from vesicles can take place by integrating a light-emitting entity within either the vesicle or its membrane. Light emission from vesicles can also take place via nonlinear processes at the vesicle membrane interface, such as second harmonic scattering (SHS), coherent Raman scattering, and sum frequency scattering (SFS). Nonlinear effects originate both due to the membranes' inherent nonlinear responses and due to the incorporation of a photoactive chromophore. Because of the possibility of biocompatibility through the choice of appropriate materials, light-emitting vesicles have found many applications in bioimaging, such as highly sensitive and specific biomarkers; the most recent application is in imaging-guided therapy. Nonlinear vesicle optics have found applications in studies of vesicle membrane composition and electrostatic properties (30, 31). Alternative imaging methods (e.g., MRI) are also possible by using vesicles; however, such techniques are not discussed in this section, which focuses instead more on the fluorescence and nonlinear optical properties of vesicles, as well as on the methods and applications of linear or nonlinear light emission from vesicles.

Fluorescent Vesicles

In 2004 Nie and colleagues (32) reported the encapsulation of quantum dots within an amphiphilic film. Although strictly the amphiphilic film did not self-assemble into a vesicle to encapsulate the

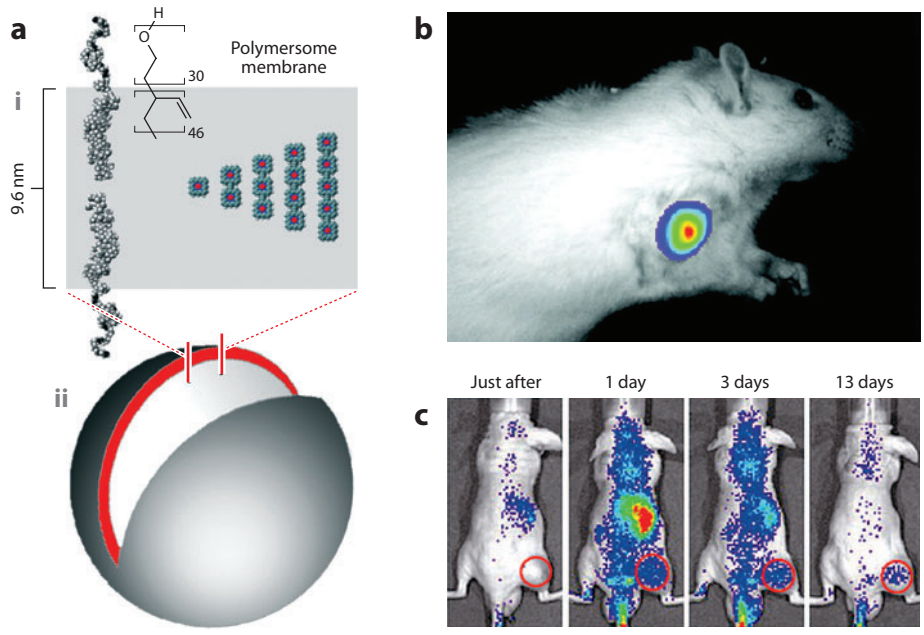

Figure 2

(*a*) A schematic of a light-emitting polymersome (*i*). In the amphiphilic diblock copolymer PEO_{30}-PBD_{46}, the hydrophobic PBD tail orients from end to end to form bilayer membranes. In the illustrated polymersome (*ii*), an excised cross-sectional slice illustrates a bilayer PBD membrane (*gray*) containing hydrophobic PZn-based NIRFs (*red*). (*b*) In vivo imaging of NIR-emitting polymersomes targeting tumors in a rat. Adapted from Reference 33. (*c*) NIR tomographic images of tumor-bearing mice at different time points after the injection of light-emitting vesicles. Abbreviations: PBD, polybutadiene; PEO, polyethylene oxide; NIR, near-infrared; NIRF, near-infrared fluorophores. Adapted from Reference 37.

quantum dots, the report pioneered the simultaneous targeting and imaging of specific cell types in which light emanates from encapsulated emitters. Diagnostic applications of polymersomes were reported on the basis of NIR-emitting porphyrin fluorophores inserted into the hydrophobic block of the membrane, where the light emission was strong enough to detect tumors in vivo in mice (**Figure 2**) (33). More recently, the more stable Alexa dyes (34) and quantum dots (35) were conjugated and encapsulated, respectively, within vesicles for the same purposes.

An exciting application of light-emitting vesicles is the possibility of localizing the vesicles themselves. Such localization methods enable the monitoring of vesicle-mediated cellular processes or the delivery tracking of the coencapsulated biomolecules without the need to sacrifice the organisms (36). Such methods have been demonstrated in animals (**Figure 2**), illustrating a 13-day-long tracking of drug-loaded vesicles (37) in whole animals, as well as in single cells (38). Regarding cellular processes, light-emitting vesicles have been applied to image endocytosis or exocytosis in cells of the nervous and circulatory systems. Such processes enable the transport of chemical messengers that are commonly encapsulated in naturally self-assembled vesicles in cells (39). In these studies, the vesicle becomes fluorescent either (*a*) by encapsulating dyes (40) or associating them in their membrane (41) or (*b*) by fusing transgenic fluorescent proteins to selected vesicle proteins (42). Encapsulated fluorophores within vesicles with plasmonic properties (also termed plasmon-coupled fluorescent probes) may also experience enhanced photoresistance and consequently prolonged emission duration before bleaching. One such approach was reported in

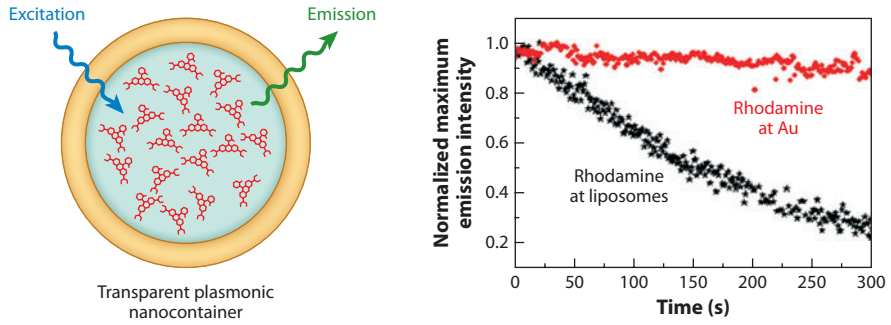

Figure 3

(*Left*) The encapsulation of fluorescent molecules in metallic wall vesicles can lead to their increased photoresistance while maintaining their brightness. (*Right*) Localization of fluorophores near metallic surfaces decreases the lifetime of their excited state, lowering the probability of the triplet state transition and thus reducing oxygen photodegradation. Adapted from Reference 43.

metallic wall vesicles, in which plasmonic walls were engineered to promote transmission of the excitation and emission from the fluorophores and, more importantly, to shorten the fluorescence lifetime of the fluorophores, hence minimizing their photobleaching (**Figure 3**) (43). Metallic shells also substantially narrow the emission bandwidth from the enclosed fluorophores due to similar coupling to surface plasmon modes (44).

Cancer phototherapy is another area where light-emitting vesicles have found applications. In one of the most recent reports, vesicles were self-assembled from porphyrin bilayers [thus termed porphyrosomes (45)]. Porphyrosomes exhibited the capacity not only to target tumors in vivo, but also to photothermally ablate the cancer cells under optical excitation, similarly to the plasmon-based phototherapies that are discussed in a later section (44, 46). Fluorescent vesicles have also found in vitro applications, for example, in protein microarray (47) and DNA hybridization (48) assays. In this case, the clear advantage of fluorescent vesicles over conventional labeling methods is the substantially stronger emission due to the larger number of contained fluorophores in a vesicle.

Fluorescent vesicle applications can also involve optical sensing, in which photosensitive compounds are associated within the vesicle and, upon environmental changes, their optical response is altered. Typical examples involve oxygen (49) and ion concentration (50) sensors, in which the emission intensity of the incorporated fluorophores changes depending on the state of the surrounding environment. Also possible is colorimetric sensing, in which environmental changes are manifested in changes in emission wavelength. Temperature- and pH-dependent changes in the backbone conformation of amphiphilic polydiacetylene (PDA) polymers can shift their light absorbance from 650 nm to 550 nm (10). Their utility as sensors is limited because the addition of PDA to a solution under investigation can alter the solution properties. Lee et al. (11) addressed this issue by encapsulating PDA vesicles within larger capsules with semipermeable membranes, allowing small molecules to enter and influence the PDA but preventing the PDA from exiting. Lee et al. conjugated guanine-rich single-stranded DNA potassium probes onto PDA liposomes, which selectively shifted their light absorbance in the presence of potassium ions, even with a physiologically 30-fold-higher background concentration of sodium ions (51). An ultrasensitive Förster resonance energy transfer (FRET)-based pH probe was demonstrated by Zhang et al. (52), who retained a water-soluble energy donor within a vesicle composed of an energy-accepting membrane. Water-soluble, bispyrene-based donor molecules with pH-sensitive fluorescence were loaded into the interior of vesicles composed of cross-linkable perylene bisimide

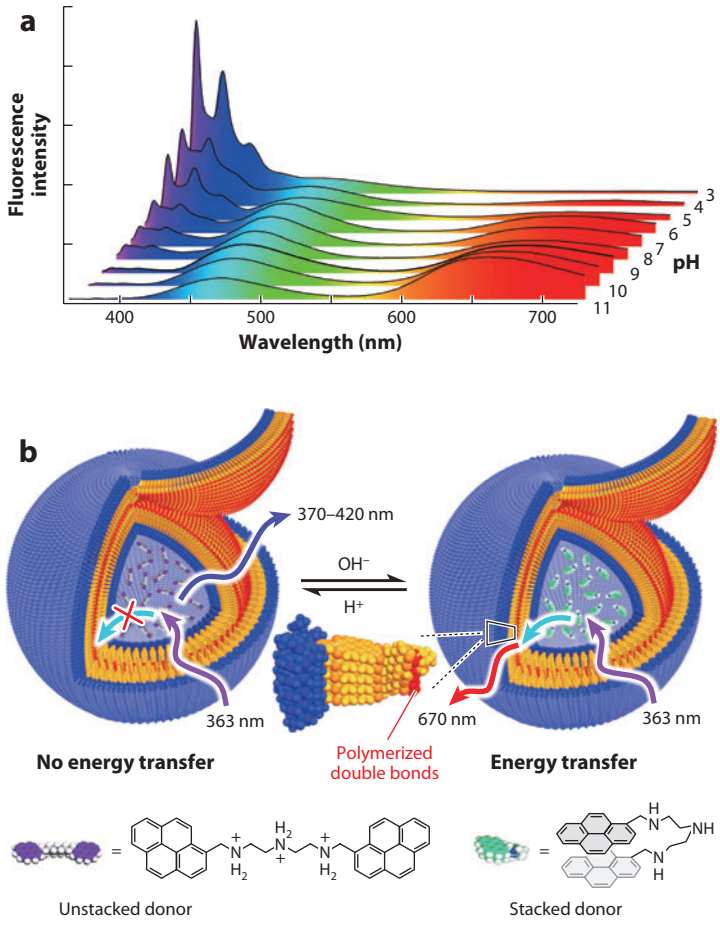

Figure 4

Donor-loaded polymerized vesicles with bilayer pyrene acceptor membranes function as fluorescent pH sensors. (*a*) Fluorescence emission spectra of vesicles in aqueous solutions at pH 3.0–11.0. (*b*) Schematic of vesicles with pH-tunable energy transfer. Adapted from Reference 52.

amphiphilic acceptors. The degree of spectral overlap between the donor emission and acceptor absorption increased as the pH of the surrounding solution increased, allowing for a gradual shift in fluorescence across the entire visible light range from a purple-blue 370–420-nm fluorescence below a pH of 4 to a white emission at pH 9.0 to a red 670-nm emission at pH 10.4 (**Figure 4**).

Nonlinear Optics of Vesicles

The vesicles' second-order nonlinear optical response gives rise to SHS and sum frequency generation (SFG) (53–55). The second-order response originates from spatial noncentrosymmetry of the vesicle membrane. Because interfaces are by definition noncentrosymmetric, SHG (second harmonic generation) and SFG are specific only to the (membrane) interface and can be used to measure (*a*) (changes in) the structural composition of the membrane leaflets and (*b*) the electrostatic potential on the membrane.

SHS was used for the first time in 1998 to follow the transport of SHS active markers, such as styryl and malachite green (MG^+) dyes, through a liposome membrane (53). Upon mixing of

liposomes and SH marker solutions, a strong SH signal appears, which originates from increasing asymmetry that follows the attachment of a marker to the outer leaflet (53, 56–61). As the marker molecules cross the membrane, the spatial asymmetry is decreased so that a time-dependent decay of the SHS signal is observed. The effect of liquid-crystalline vesicles versus that of gel membrane vesicles on bilayer transport of MG^+ was investigated by using SHS. In contrast to liquid crystalline liposomes, gel membrane liposomes do not facilitate membrane transport, as witnessed by a time-constant SHS signal. The tight molecular packing conditions in the gel phase inhibit membrane transport. Cholesterol retards the rate of transport by as much as a factor of 6 in liposomes composed of a 1:1 ratio of DOPG (dioleoylphosphatidylglycerol) and cholesterol (59). Similar experiments also showed a complex dependence of MG^+ transport with respect to the use of electrolytes (60). By using the same MG^+ cation, the efficiency of different ionophores was investigated; a channel former, a lyophilic cyclopeptide, and a weak acid were compared. For each ionophore, different transport kinetics were observed (61). SHG and SFG were used fairly recently to label-free probe the surface structure of membranes (55, 62). These methods generally exhibit relatively weak optical response. However, a scheme was very recently proposed to increase the detection efficiency significantly (63).

Vibrationally resonant SFS measurements were used to target the head group of one of the components in the vesicle bilayer. In such experiments, the pulse pair consists of an infrared pulse and a visible pulse. The infrared pulse can be tuned to an infrared resonance so that a detailed chemical structure can be obtained label free, allowing one to investigate chemical interactions, molecular orientation, and ordering (64). The SFS measurements on vesicle solutions showed that the vesicle response was nonvanishing. The results, depicted in **Figure 5**, suggest that the negative constituents do not partition equally to both leaflets. The calculated scattering patterns

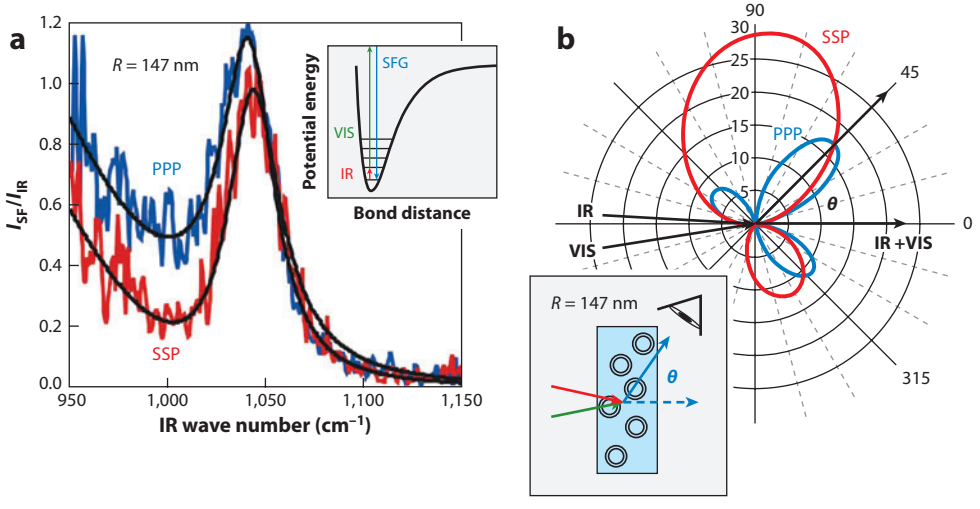

Figure 5

SFS scattering data from a vesicle solution. The spectra (*a*) display the vibrational modes of the head groups of the constituents (in this case the sulfate stretch mode of the anionic surfactant). The response originates from a combined infrared and Raman excitation and results in a scattering pattern (*b*), which is calculated for a single 147-nm membrane. As the vesicles have no positional correlations, the scattering pattern consists of an incoherent sum of the separate vesicle contributions. Abbreviations: IR, infrared; SF, sum frequency; SFG, sum frequency generation; VIS, visible. PPP and SPP refer to polarization state notation. Adapted from Reference 55.

are also displayed in **Figure 5** and demonstrate the absence of scattering in the forward (phase-matched) direction, which is common for second-order light scattering (65). Thus, at least on the timescale of picoseconds, the membranes of the inner and outer leaflet are not identical (55). These results also indicate that many membrane processes are influenced by short-timescale statistical fluctuations in the membrane structure, which may have important consequences for the way in which membranes mediate processes such as protein uptake and ion transport.

The membrane surface potential can be determined as well (62, 66–68). In 2001, Liu et al. (62) worked out a method based on the Gouy-Chapman model to derive the membrane surface potential of the outer membrane of POPG (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol) liposomes from SHS experiments. Although out of the scope of this review, the ability to measure surface potentials with SHS was explored with the help of amphiphilic dyes so that membrane potentials in cells could be measured with essentially a membrane resolution (69).

Inelastic photon scattering can also take place in vesicles, making their Raman spectroscopic analysis possible. One of the first reports, published in 1978, was on the effects of transmembrane ion gradients, surrounding pH, and temperature on the membranes of erythrocytes (70). More recently, micro-Raman microscopy (71), optical trapping (72), and coherent Raman [especially coherent anti-Stokes Raman scattering (CARS)] (73) have enabled enhanced sensitivity down to the single-vesicle level. Optical trapping is employed to immobilize the vesicles and permit their observation for prolonged periods. This method has found applications in mapping the kinetics of hydrolysis (74) and in polymerization of phospholipid vesicles (75), as well as in the payload distribution within liposomes in drug delivery applications (76). CARS has been applied mostly in quantitative chemical imaging, such as that detecting the coexistence of different lipid-phase domains in liposomes (77, 78). Such studies are impossible otherwise due to the absence of specific fluorescent probes for different lipid domains.

OPTICAL MANIPULATION OF VESICLES

The encapsulated payload in a vesicle is protected by the vesicle membrane, which can be additionally functionalized to enable targeting of the vesicle to specific locations. Transport to a specific location can be either through passive diffusion or through the transport of the surrounding medium. However, vesicle manipulation, i.e., transport and release through membrane destabilization, can also take place actively, for example, under the action of electrostatic forces. The dynamic manipulation and transport of vesicles have found many applications, ranging from studying the mechanical properties of vesicles to the control of the boundary conditions of chemical reactions. Trapping can be achieved by exerting electrical (79) or mechanical (13) nanoscale forces on the vesicles. Photons can also exert forces on microscale objects and hence transport or immobilize them (80). This principle, frequently referred to as optical trapping, has had a significant impact on molecular and cell biology (81) and has found many applications in vesicle microrheology. In this section, we discuss recent advances in vesicle trapping and destabilization using optical fields.

Optical Trapping of Vesicles

In early studies, laser tweezers were employed to immobilize vesicles and to explore the effect of laser illumination on them, giving rise to interesting observations of unconventional vesicle shape transformations, such as peristaltic pearl formation along cylindrical tubes (82), the unbinding of locally pinched membranes (83), vesicle elongation (84), and even the expulsion of smaller vesicles from larger ones (85). Such phase transformations occur through the energy transfer from the

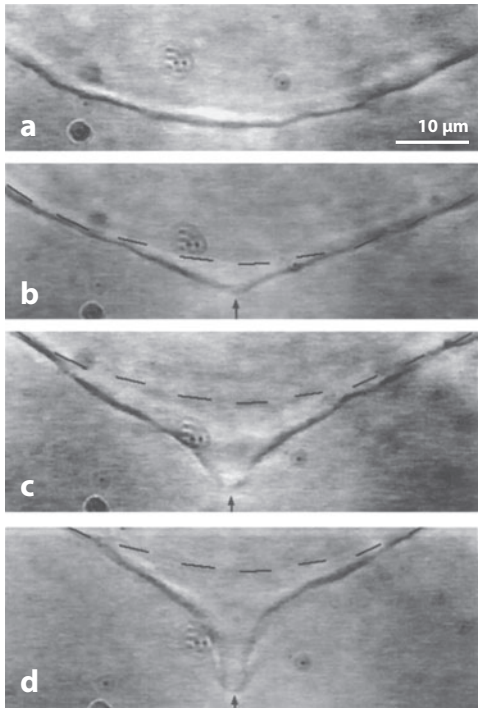

Figure 6

Mechanical manipulation of a liposome membrane using optical tweezers over the course of a few hours. *a*, *b*, *c*, and *d* denote frames at different times. The dark line denotes part of the vesicle membrane; the vesicle extends beyond the image frame. A laser beam is focused where the arrow is pointing (in the *middle of the image*). In the focus location, the membrane deformation takes place over a few hours. Adapted from Reference 86.

trapping photons to the vesicle membrane. Due to the tight focusing conditions of the trapping light, an intense electric field is created; this field can generate novel photochemical species on the vesicle membrane. This membrane modification in turn modifies the membrane surface tension (86), leading to shape transformations of the vesicle (**Figure 6**). Similar shape transformations have been independently observed by inducing chemical perturbations to the vesicle membrane (87). Shape transformations can also exhibit a dynamic character due to competing effects of the laser action and the opposing hydrodynamic forces (88).

Contrary to the conventional optical trapping of solid dielectric microspheres, stable vesicle trapping is challenging due to the low refractive index contrast of the vesicle with respect to that of the surrounding buffers. To address this, one approach involves decorating the vesicle interior or its membrane with dielectric particles and trapping these added particles instead (89). In this way, the exerted optical forces on the dielectric particles can be transferred to the vesicle. By using this strategy, a dual optical trap of two endovesicle particles enabled quantitative single-liposome force spectroscopy and hence precision measurement of the vesicle's surface rigidity and tension (90). Optical manipulation of surface-tethered beads has been applied to microrheology studies of vesicles coated with actin filament (91), revealing the added shear modulus on the lipid membrane due to the presence of this filament network (92).

An alternative approach to stable vesicle trapping is to enhance the refractive index contrast of the vesicle with the surrounding medium by encapsulating fluids of higher refractive indices.

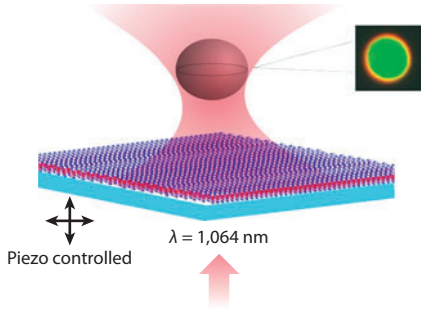

Figure 7

An illustration of optical trapping of liposomes using a 1,064-nm laser beam. By infilling the vesicles with a sucrose solution, vesicles as small as 50 nm can be trapped. The inset shows a confocal image of a vesicle whose membrane is labeled with a red chromophore and encapsulated in a green-emitting label. Adapted from Reference 94.

For example, a membrane-impermeable sucrose solution can be encapsulated. This technique has been employed in a complex multitrap geometry (93) to control the vesicle shape via multitrapping locations on a single vesicle, as well as in vesicle immobilization or transport, and was recently demonstrated to stably trap liposomes as small as 50 nm (**Figure 7**) (94). Optical immobilization is critical for sensitive vesicle detection, such as incoherent (95) or coherent (96) Raman analysis, as well as for differential contrast imaging of membrane thermal fluctuations (97). The transport of stably trapped vesicles and its sudden halt have also been applied to giant-unilamellar-vesicle relaxation dynamics, indicating that membrane rigidity is the dominant factor in the associated relaxation kinetics (98).

Beyond investigation of basic vesicle properties, another unique application of optical tweezers is light-induced transport of vesicles. This type of light-vesicle interaction was applied by bringing two liposomes in contact and subsequently fusing them under illumination with UV radiation. This process gave rise to rapid mixing of the liposomes' contents, forming a microscale reactor (99). In another embodiment, optical tweezers transported vesicles within microfluidics and immobilized them in a chamber where the effect of environmental osmotic pressure changes on the vesicles could be determined (100).

Optical Vesicle Destabilization

Vesicle destabilization and payload release can be triggered through chemical or physical means. Chemical means usually refer to changes in the surrounding environment [e.g., pH changes, hydrolysis (101–103)], whereas physical ones involve the application of external fields that force the vesicles to destabilize at a controllable rate. Among other applications, remotely triggered vesicle release has found substantial attention in drug delivery, in which the ability to spatiotemporally control the profile of the drug administration can enhance the therapeutic efficiency and minimize unwanted effects (104–106).

External fields that can trigger release from vesicles can be magnetic (107), optical (108), thermal (109), or even mechanical in the form of ultrasound waves (110). Herein, we focus on the use of optical fields in vesicle destabilization and release of hydrophilic or hydrophobic compounds (**Figure 8**). Light is an attractive trigger source due to the widespread use of lasers in modern biosciences (e.g., microscopy) and therapy [e.g., photodynamic therapy (PDT)]. Excellent reviews on drug delivery using external triggers have been previously reported (111–113); our focus is

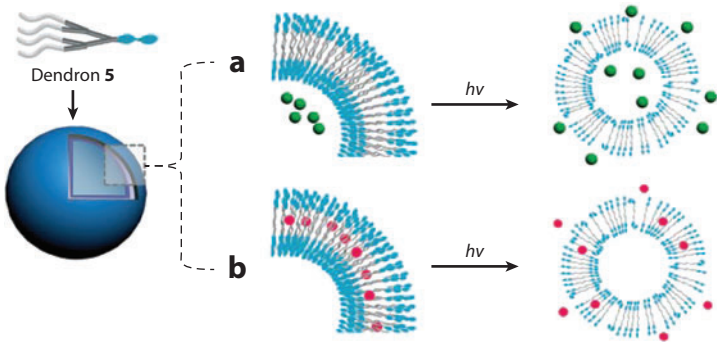

Figure 8

A schematic illustration of optical vesicle destabilization under optical illumination. The destabilization can lead to the release of either (*a*) hydrophilic compounds (*green spheres*) encapsulated within the vesicle or (*b*) hydrophobic compounds (*red spheres*) that reside within the vesicle membrane. The destabilization mechanism is due to photolytic cleavage. *hv* denotes optical illumination. Adapted from Reference 117.

specifically on the underlying optical mechanisms and most recent reports on light-driven vesicle destabilization, as well as on the kinetics of release of the different methods.

The type of light-vesicle interaction that can lead to vesicle destabilization depends on the architecture of the membrane. However, in most cases, the vesicles need to encapsulate or be associated with a photosensitive compound that acts as an optical transducer (optode) converting optical energy to a chemical perturbation to the vesicle stability. Vesicle destabilization methods that do not contain an optode are not as rapid or efficient and necessitate long exposures with intense light fields; these methods are discussed in more detail in the above section of laser tweezers, which offer this prolonged illumination capability.

Various types of interactions between photons and vesicles leading to destabilization can take place. Such interactions can be based on changes induced to the vesicle membrane, such as perturbations to its polarity based on cleavage and oxidation, as well as optothermal mechanisms based on plasmonic nanoparticles. Photoisomerization, a method to alter the shape of light-sensitive molecules, can also induce polarity changes in the vesicle membrane; however, we discuss this mechanism separately below, as the result of such processes can be reversible.

Polarity perturbations. The stability of amphiphile-based vesicles is highly dependent on the link and balance between the hydrophobic and hydrophilic components of their membrane. Consequently, one method for perturbing vesicle stability is to alter the polarity of one of the amphiphile constituents or to separate them through cleavage. These can be achieved with optical means, such as photocleavage and photo-oxidation, enabling triggered release from the vesicle. Specific to photocleavage, due to certain structural similarities between vesicles and micelles, the latter are included, highlighting specifically key methods to control the destabilization rate as well as the possibility of employing two-photon and upconversion optical effects.

Cleavage. In this type of interaction, light removes a photochromic group, usually attached to the hydrophobic block of the vesicle membrane. The basic principle of optically removing a photosensitive group has also been applied to the optical activation of biomolecules, such as small interfering RNA caged by phosphate groups (114) and DNA-dendron conjugates (115), as well as to the disassembly of high-order virus complexes (116). More specific to vesicles or micelles,

however, these carrier systems are usually designed such that the removal of the chromophore increases the polarity of the hydrophobic block or cleaves the amphiphile itself.

Vesicles (117, 118) have also been designed to contain a nitrobenzyl moiety as the photolabile group, whereas alternative photochromic groups, such as compounds based on naphthoquinone (119) and nitrophenylalanine (121), were more recently reported in vesicles and micelles. Jiang et al. (122) reported micelles with a hydrophobic block bearing a pyrene moiety, which upon UV irradiation detaches, converting the hydrophobic block to a hydrophilic one and perturbing the micelle stability.

To enhance the carrier photodegradation rate, Han et al. (123) incorporated multiple photobreakable moieties along the hydrophobic block chain. With this method, an average micelle lifetime of 20–30 s under optical excitation was possible. These data, however, were measured in suspension, which masks transport kinetics, as well as sample heterogeneity. Most reports based on photocleavage employ destabilizing radiation in the UV spectral range. Vesicles or micelles with a redshifted photoresponse are more attractive in biological applications because photodamage and skin absorption are reduced at longer wavelengths (124). This has so far been addressed by using photochromic groups with a high two-photon absorption cross section or by integrating upconverting nanoparticles, thus enabling NIR vesicle photosensitivity (125, 126).

Oxidation. Since Raab's (127) observation in 1900 that sunlight through his lab window killed bacteria immersed in specific dye solutions, photosensitized oxidation has considerably enhanced the photobiology toolbox. One widespread example is PDT, in which diseased tissue is irreversibly destroyed via the illumination of sensitizers that are localized on the cells or the tissue (128), with applications in cancer or antimicrobial treatment (129, 130). In this section, we focus on light interacting with sensitized vesicles and micelles in the presence of oxygen. Two processes (or their superposition) may be involved in photo-oxidation (131). The type I process refers to the interaction of the chromophore with the substrate; in the type II process, the optical excitation is transferred to molecular oxygen, generating singlet oxygen ($^1O_2$).

In vesicle or micelle destabilization, the substrate undergoing oxidation is the self-assembling amphiphile, and although both types I and II are possible, singlet oxygen is the most common mechanism. Ion transport through rhodopsin-recombinant membranes under illumination was one of the earliest reports on membrane photo-oxidation (132). Early vesicle studies involved liposomes (133–135) and ghost erythrocytes (136), using sensitizers such as porphyrins (137, 138) and methylene blue (139). More recently, liposome rupture based on the osmotic pressure increase through photo-oxidation was demonstrated (140). Rapid polymersome rupture was most recently demonstrated by using an oxidation-sensitive membrane (103); at the single-particle level, the kinetics of release were revealed to be significantly faster than those of passive diffusion (5).

Vesicle destabilization through oxidation can take place through paths other than polarity perturbations. Photosensitized polymersome illumination leading to vesicle shape changes and rupture due to heating was also reported (141, 142). Similarly, by using the micropipette aspiration technique (13), the photochemical effects on an optically excited sensitized polymersome were found to involve osmotic swelling, deflation, and membrane cross-linking (143).

One type of PDT also involves the photo-oxidation of endocytic vesicles. In this type of interaction, also frequently termed photochemical internalization, biomolecules and sensitizers are passively transported from the extracellular environment to the cell endosome. Under optical excitation, the sensitizers generate reactive oxygen species that disrupt the endosomal membrane, hence enabling cytoplasmic biomolecule release (**Figure 9**) (144, 145). Investigators recently reported that this technique was used in rapid DNA delivery (146) and gene therapy (147) in rat tissues.

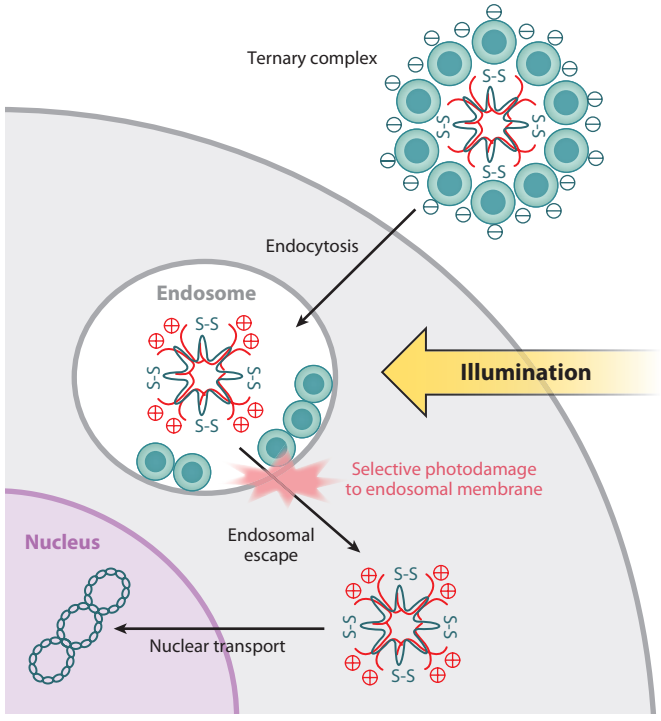

Figure 9

The itinerary of biomolecule intracellular delivery in photochemical internalization. Initially, the complex is passively transferred to the cell endosome through endocytosis. Under optical excitation, the sensitizer ruptures the endosomal membrane, and the complex is released in the cell cytosol. Adapted from Reference 147.

Contrary to cleavage that usually necessitates damaging UV radiation, vesicle photo-oxidation is commonly driven by visible radiation. As a result, vesicle photo-oxidation has already found biophysical and biomedical applications, such as the biochemical modulation of the cellular surrounding environment (148, 149), intracellular delivery (5, 150, 151), ophthalmological treatment (152), and antimicrobial chemotherapy (153). Photosensitive vesicles were also recently investigated in the context of studies of the origin of life (154).

Plasmonic nanoparticles. Optical absorption can increase the local temperature in the environment surrounding the absorbents. Metallic nanostructures exhibit strong light absorption in a wavelength-selective manner on the basis of their morphology and size (155). This type of optical resonance, referred to as surface plasmon resonance, occurs due to the coherent interaction of incident photons with the metal's free electrons (156). Plasmons have found biomedical applications such as sensors and nanoscale optothermal sources for hydrogel manipulation (157) and cancer therapy (44).

Plasmonic nanoparticles have also been integrated with vesicles, thus creating a photosensitive complex, the stability of which can be controlled by optothermal means. Such examples involve the decoration of polymeric nanocapsules with plasmonic nanoparticles (**Figure 10**), which under laser illumination lead to capsule deformation, rupture, and release (158, 159); release is also possible in a direction-specific manner (160). These biomaterial systems have found applications

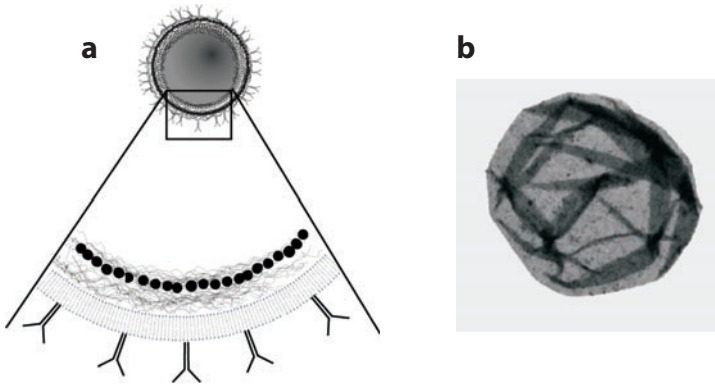

Figure 10

(*a*) A schematic of an optically addressable nanocapsule. The inset shows a detail of the capsule's membrane comprising the nanoparticles (*dark spheres*), the multiple polyelectrolyte layers that form the capsule (*dark fibers*), and an outer lipid layer functionalized with receptor molecules. From Reference 158.
(*b*) Transmission electron microscopy image of a capsule, decorated with silver nanoparticles. The vesicle diameter is approximately 1 μm. Adapted from Reference 162.

in intracellular delivery of chromophores (161, 162) as well as in peptides for enhancing antigen presentation in mammalian cells (163).

In phospholipid membranes (see Introduction section), surface-anchored plasmonic nanoparticles can raise the local temperature and hence induce gel-fluid transitions at a nanoscale similar to the plasmonic particle size (164). This mechanism has been applied to liposomal destabilization and release, for which wavelength dependence was additionally achieved by incorporating nanoparticles with different spectral responses (2). The distance between the liposomes and the plasmonic nanoparticles affects the temperature increase experienced by the liposome and thus plays an important role in vesicle destabilization. DNA linkers were employed to control this distance, hence promoting or inhibiting liposomal release (165). In an alternative approach, optical tweezers were employed to vary the distance of a nanoparticle from the phospholipid membrane, enabling in this way the measurement of the temperature profile of the illuminated nanoparticle simply by monitoring the rupture and release from the membranes themselves (166, 167).

Highly absorbing carbon black nanoparticles in the vicinity of cell membranes were recently reported to achieve enhanced transfection results (168). Even though the origins of transfection are photothermal, the exact mechanism is different from the reported phase transitions in plasmonic nanoparticles and involves the generation and collapse of cavitation bubbles (169, 170), a concept yet to be demonstrated in simpler vesicle systems.

Reversible rupture. The aforementioned types of light-driven vesicle destabilization are irreversible because the vesicle or micelle has undergone extensive damage and cannot self-assemble back to its initial state under typical conditions. Reversible vesicle destabilization can occur via photoisomerization, in which reversibility is possible either by thermal relaxation or by the employment of radiation at a different wavelength. Photoisomerization necessitates the incorporation of a moiety that under optical excitation can undergo a shape change, specifically a transition from its *cis* isomer to its *trans* isomer (**Figure 11**) (171). Isomerization leads to vesicle destabilization because the *trans* isomer exhibits substantially lower surface tension than the *cis* isomer, a transition that effectively disrupts vesicle stability (172).

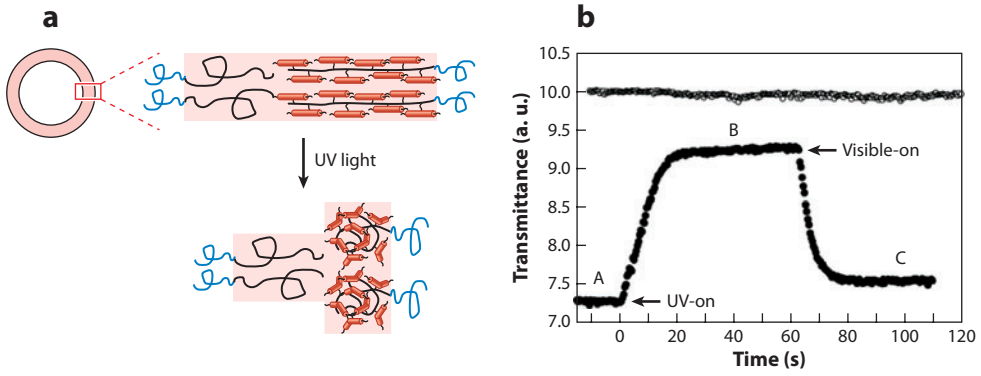

Figure 11

(*a*) A cartoon illustrating the copolymer conformation within the membrane of a polymersome containing an azobenzene compound. Under UV illumination, the hydrophobic compound (polybutadiene in this instance), which is in a nematic state, isomerizes and undergoes a conformational change to a disordered isotropic state. Adapted from Reference 175. (*b*) The optical transmittance through a polymersome suspension changes upon UV or visible radiation illumination. Vesicle rupture with UV light leads to a smaller average vesicle diameter and hence to lower turbidity (*A* and *C*). The inverse occurs under visible excitation (*B*). Adapted from Reference 173.

Reversible polymersome destabilization occurs by employing a hydrophobic block based on an azobenzene-containing liquid crystal polymer. UV illumination leads to rupture, and visible illumination induces reassembly of the vesicles (**Figure 11**) (173–175). Azobenzene is the most common isomerization chromophore, which can also be electrostatically associated with the vesicle membrane (176), although alternatives such as spiropyran-merocyanine isomerization have recently emerged (177). Intracellular delivery of DNA using this technique leads to enhanced expression profiles of the intracellular enhanced green fluorescent protein (178). Azobenzene molecules have also been applied in mesoporous silica, where they act as nanomechanical impellers, regulating in this way the fluidic resistance experienced by the payload and controlling payload release from the structures (179, 180).

CONCLUSIONS

Vesicles self-assemble naturally or artificially via a variety of different methods, from a wide range of materials, and with sizes that range from the nanoscale to the microscale. The vesicle composition can be engineered with a variety of optically active compounds, either at their membrane or in the water-filled core. Due to these properties, vesicles form a unique class of optical particles that can simultaneously protect or transport molecular cargoes, as well as exhibit unique application-specific optical properties. In this article, we review vesicle properties and applications in imaging, nonlinear optics, and sensing, as well as in transporting, destabilizing, and rupturing vesicles by using light fields.

The aforementioned developments in vesicle photonics open up the way for further investigations, especially those due to vesicle resemblance to cells and their membranes. Such examples include protein-mediated membrane transport; the membrane's role in disease proliferation (e.g., Alzheimer's and Parkinson's diseases); and self-assembly, uptake, and release mechanisms. Additionally, metabolic pathways in living organisms are compartmentalized by membranes and vesicles [e.g., photosynthesis (181)], and investigations of cellular mimetic photoreactors have attracted substantial interest to this end (182).

DISCLOSURE STATEMENT

The authors are 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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