



# Physical stimuli-responsive vesicles in drug delivery: Beyond liposomes and polymersomes

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## ABSTRACT

Over the past few decades, a range of vesicle-based drug delivery systems have entered clinical practice and several others are in various stages of clinical translation. While most of these vesicle constructs are lipid-based (liposomes), or polymer-based (polymersomes), recently new classes of vesicles have emerged that defy easy classification. Examples include assemblies with small molecule amphiphiles, biologically derived membranes, hybrid vesicles with two or more classes of amphiphiles, or more complex hierarchical structures such as vesicles incorporating gas bubbles or nanoparticulates in the lumen or membrane. In this review, we explore these recent advances and emerging trends at the edge and just beyond the research fields of conventional liposomes and polymersomes. A focus of this review is the distinct behaviors observed for these classes of vesicles when exposed to physical stimuli - such as ultrasound, heat, light and mechanical triggers - and we discuss the resulting potential for new types of drug delivery, with a special emphasis on current challenges and opportunities.

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**Abbreviations:** ctDNA, circulating tumor DNA; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; DLPV, doxorubicin-loaded polymer vesicles; EPR effect, enhanced permeability and retention effect; EV, extracellular vesicle; GCVs, giant cluster-like vesicles; HMME, hematoporphyrin monomethyl ether; MB, microbubble; CMB, classical MB; FCMB, folate-conjugated MB; NIR, near infra-red; PEG, polyethylene glycol; PDT, photo-dynamic therapy; PGPR, polyglycerine polyricinoleate; PLA, polylactic acid; PNIPAM, poly(*N*-isopropylacrylamide); ROS, reactive oxygen species; <sup>1</sup>O<sub>2</sub>, singlet oxygen; T<sub>m</sub>, transition melting temperature (of lipids); TSL, thermosensitive liposome; UCNp, light upconversion nanoparticle; UCST and LCST, upper critical solution temperature and lower critical solution temperature (of polymersomes); US, ultrasound.

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## 1. Introduction

The aim of drug delivery is to increase therapeutic efficacy. Drug delivery vesicles can aid in this pursuit by enhancing on-target effects while reducing off-target toxicity [1,2]. For example, by increasing local drug concentration in diseased tissues while reducing accumulation in other tissues and organs (e.g. the liver or kidneys), or by releasing a drug in response to a biological stimulus (e.g. the release of insulin in response to a change in blood glucose levels in a diabetic patient). The central challenge is thus endowing drug delivery vesicles with the means to influence the spatiotemporal distribution of therapeutics.

A range of bio-responsive nanomaterials and vesicles have been developed towards addressing this challenge. This includes materials that respond to physiological changes in pH, redox environment, and the presence of enzymes (e.g. upon endocytosis by cells) [3,4]. A complementary route towards enhanced spatiotemporal control of therapeutics is using drug delivery vesicles that can be triggered by physical stimuli (e.g. ultrasound or light) instead—or in addition to—physiological stimuli [5]. The design and development of drug delivery vesicles integrating responsive and triggering functionalities has therefore received increasing attention.

Liposomes are often considered the archetype of many drug delivery vesicles, originally described half-a-century ago [6] and widely considered the first “nanoscale drug” approved for clinical use [7,8]. Polymersomes are closely related vesicles, assembled from polymers instead of lipids [9] and first reported in the mid-1990s [10,11]. Liposomes and polymersomes have received intense research interest since their introduction - especially in biomedical applications - as recently reviewed by Pattni and co-workers [12] and Hu and co-workers [13].

Excitingly, in the last few years a range of new vesicle-based drug delivery systems have emerged that defy easy classification as they go beyond many of the conventional strategies and materials typically associated with liposomes and polymersomes (Fig. 1). These developments are fueled by recent advances in materials science and engineering, and the resulting vesicles typically fall at the edge or between main themes of liposome and polymersome research. The potential of these emerging vesicle systems to help drive a step-change in drug delivery responsive to physical stimuli is the subject of intense research, and the focus of this review.

In this review, we explore emerging approaches incorporating non-conventional strategies and materials (e.g. hybrid materials, gases and emulsions) of various origins (e.g. synthetic or biological) for the assembly of diverse classes of stimuli-responsive vesicles (Fig. 1). Following a short primer on triggers, amphiphiles and hierarchical structures, the discussion is structured around the targeted physical stimuli: (i) ultrasound, (ii) heat, (iii) light, and (iv) mechanical stimuli. We conclude by providing an outlook on current challenges and opportunities, and

explore potential research directions towards accelerating the development and translation of the next generation of vesicle-based drug delivery systems.

## 2. Triggered drug delivery using vesicles

### 2.1. Endogenous and exogenous triggers

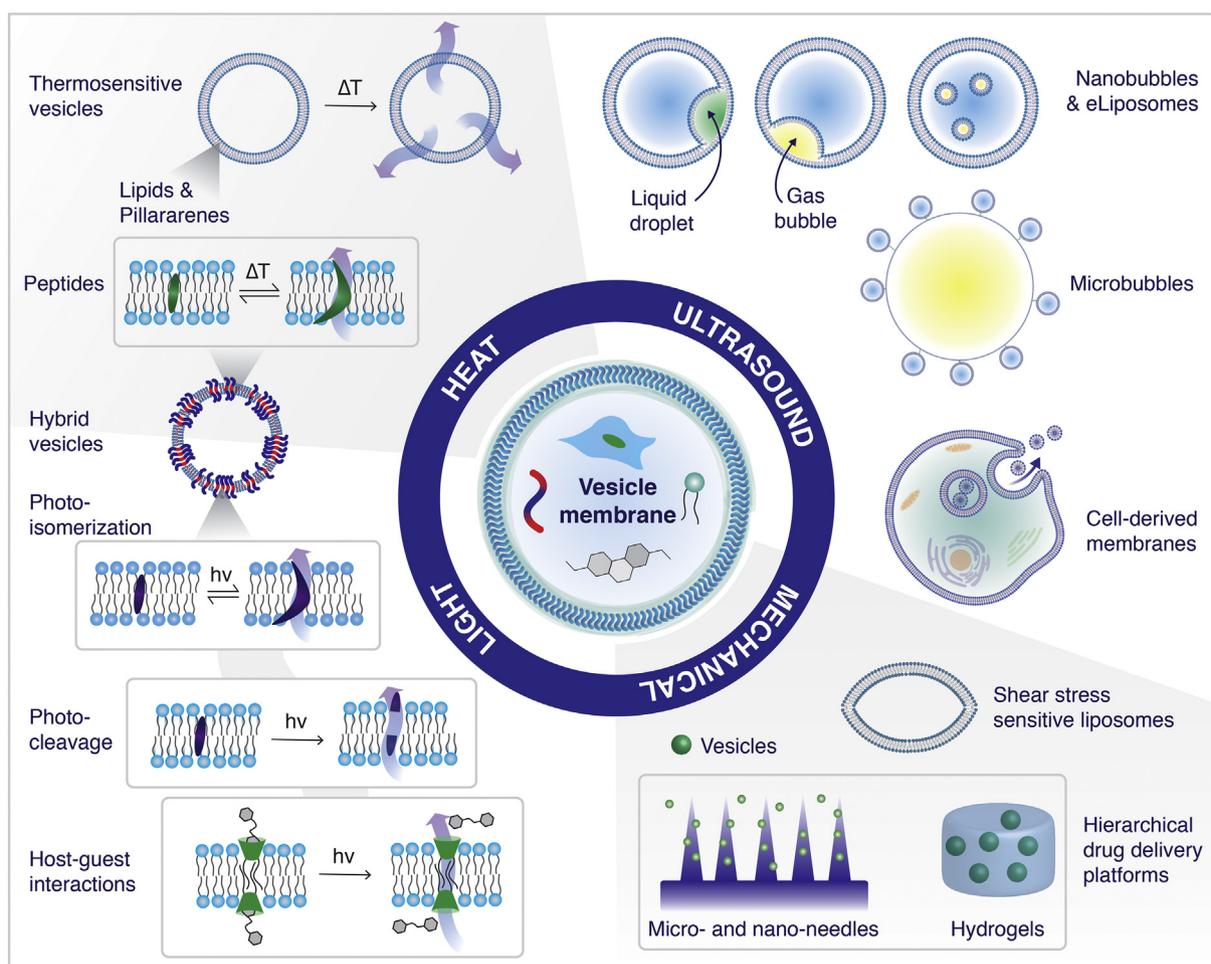
Stimuli-responsive systems can roughly be divided into two groups according to whether they react to endogenous or exogenous stimuli [14]. Nanomaterials that react upon endogenous triggers take advantage of the physiological differences between diseased and healthy tissue, such as chemical differences including pH [15], hypoxia [16,17], redox [18] or enzymes [19,20], and physical differences including mechanical triggers [21] such as shear stress-mediated release [22,23] and temperature changes [24]. Altered environmental changes can be found for example in tumors, infections, ischemia and inflammatory diseases. Exogenously triggered nanomaterials can react to external physical stimuli like light [25,26], ultrasound [27,28] and magnetic fields [29], as well as mechanical triggers [21] such as shear thinning upon injection [30]. Within this review we focus on physically triggered vesicular systems, specifically intrinsic mechanical and temperature changes and extrinsic light, ultrasound and mechanical stimuli. As the nomenclature around responsiveness, triggering, and targeting can sometimes be confusing, we here largely follow the definitions and terminology described by Wang and Kohane [5].

### 2.2. Conventional vesicle-based nanocarrier systems

Vesicles are an extensively studied and varied class of nanocarriers that consist of an amphiphilic membrane bilayer enclosing an aqueous core. These supramolecular structures can be formulated from a wide range of amphiphilic molecules including lipids, polymers, and organic and inorganic small molecules. Their formulation is driven primarily by thermodynamically favorable clustering of hydrophobic moieties (e.g. lipid tails) while hydrophilic moieties (e.g. phospholipid head groups) face the aqueous solution [31]. The geometry of the constituent amphiphilic building blocks dictates the self-assembled morphology and is often described by the packing parameter,  $p$ , defined as:

$$p = \frac{v}{a_0 l_c}$$

where,  $v$  is the volume of the hydrophobic chains,  $a_0$  is the optimal head-group area and  $l_c$  is the hydrophobic tail length [32]. In general, vesicles form when  $p \approx 1$ , spherical micelles form when  $p \leq 0.33$ , cylindrical micelles when  $0.33 \leq p \leq 0.5$ , and inverted micelle structures such



**Fig. 1.** Examples of vesicle drug delivery systems including non-conventional amphiphiles or hierarchical assemblies, which deliver a payload in response to heat, light, ultrasound and mechanical forces.

as the micellar cubic phases at  $p > 1$  [33]. Similar approximations can be applied to dendrimer self-assemblies [34] and block co-polymers [35]. Thanks to impressive progress in materials science there are plenty of examples of vesicle-based delivery vectors, predominantly comprising lipid [7,8,36–38] and polymer-based [13,39–42] vesicles typically prepared through self-assembly approaches such as thin film hydration or template-based assembly [43,44]. Some liposome formulations are already approved by the US Food and Drug Administration and in clinical use, including Doxil® [45], AmBisome® and their derivatives [46]. A range of others are in translational status towards clinical use [46].

### 2.3. Vesicles comprising alternative amphiphiles and/or hierarchical structures

There is a plethora of examples of well-established, conventional liposomal and polymersome-based drug delivery systems [12,13]. Emerging at the edge of these research fields are vesicle-based constructs formulated using alternative amphiphiles and/or higher hierarchical structures for physically triggered drug delivery—although some examples have already reached more advanced stages (notable examples include vesicle-bubble [27] and vesicle-emulsion constructs which have been successful in the field of ultrasound-mediated drug delivery and imaging [47]). These more exotic vesicle constructs have been used to transport a range of active cargos including small molecule pharmaceuticals, enzymes, nucleic acids [48,49] and imaging agents [50], using optimization techniques for specific therapeutic activities commonly used in the design of more conventional liposome and

polymersome systems. These emerging approaches can offer new avenues in the preparation of vesicles, with tailored surface chemistry, size, shape and architecture, which may improve their drug release behavior, targeting properties, and biodistribution profiles [51,52]. Vesicle surfaces can further be functionalized with “stealth” or targeting moieties (e.g. polyethylene glycol and/or antibodies) [53]. The addition of polyethylene glycol (PEG) chains to the outer surface increases steric repulsion and results in longer blood circulation times [54]. In this review, we present an up-to-date summary of vesicle delivery systems responsive to diverse physical triggers. The focus is on vesicle-based constructs comprising “alternative” amphiphiles and/or higher hierarchical structures, with an emphasis on contributions over the last few years.

## 3. Ultrasound

Clinical ultrasound (US) is one of the largest application areas of exogenously triggered physical release from vesicles, and is a non-invasive, inexpensive, readily available, and well-established tool for both clinical imaging and therapeutics. Microcarriers for US contrast, typically containing low molecular weight perfluorinated gases, or mixtures of these gases with air, have been used in the clinic since the 1990s [55]. In recent years, US-sensitive constructs comprising gas-filled bubbles and vesicles have been extensively studied for imaging and drug delivery [56]. The gas bubble may be micrometer-sized and have vesicles tethered to its surface, or may be nanometer-sized and incorporated into the vesicle membrane or in the internal aqueous compartment. The possibility of using perfluorocarbons in liquid state at physiological

temperature has also been investigated, and the term eLiposomes has been coined to describe liposomes containing such emulsion droplets [57–59,67,68]. Here, we discuss the developments over the last few years in the field of vesicle–bubble and vesicle–droplet constructs for US-mediated drug delivery, along with recent applications using biologically-derived membranes (Table 1).

### 3.1. Microbubbles

Constructs combining micrometer-sized, gas-filled bubbles (microbubbles, MB) and drug-loaded vesicles are promising US-mediated delivery vectors. The pressure wave induced during US can cause these bubbles to oscillate and collapse during a process called inertial cavitation [74]. Depending on the US frequency applied, this process can induce transient permeation or irreversible damage to the membranes of neighboring cells and artificial vesicle constructs, leading to vesicle rupture and release of encapsulated contents. Typically, gas bubbles comprising mixtures of air and perfluorinated gas are stabilized using a monolayer of phospholipids or other amphiphiles including polymers and surfactants [75], and vesicles containing therapeutics can be tethered to their surface. MB examples stabilized by silica shells [60] and organic/inorganic hybrids [61] have also been reported. The release of small molecules and enzymes from liposomes conjugated to lipid-stabilized MBs is well reported in the literature, for example in the release of thrombin to accelerate localized blood clotting [62]. *In vivo* studies have also shown that low frequency ultrasound can be used to increase the permeability of the blood–tumor barrier in a rat model [76]. This effect has been exploited by Fan and co-workers who formulated folate-conjugated, DNA-loaded cationic MBs which form vesicles *in situ* under focused US (FUS) [63]. These vesicles contain the same DNA payload as the parent bubbles and *in vivo* studies showed they could be used to target tumors (Fig. 2) [63].

### 3.2. Vesicles containing microbubbles and nanobubbles

MBs have also been encapsulated into vesicles and used to deliver, for example, chemotherapeutic agents [77]. Wrenn and co-workers found that vesicles formulated from various amphiphiles can be tailored for specific applications [64]. Specifically, microcapsules formed from polylactic acid (PLA) have been used for imaging because they can prevent gas escape into the bulk (increasing bubble longevity), inhibit

inertial cavitation and absorb the energy of inertial cavitation where it does not occur (improving safety). Liposomes on the other hand, specifically large unilamellar vesicles, have shown improved release profiles versus polymersomes in US-mediated drug delivery, and by varying the lipid composition it is possible to tune bilayer phase behavior. While there are several microbubble formulations on the market [78], the production of nanometer-scale bubbles, which can be incorporated into the lumen or membrane of vesicles, is also a highly active area of research. Bubbles as small as around 100 nm have been shown to survive up to 100-fold longer than their micron-sized counterparts under US [79]. Their smaller size can also make them more permeable to capillaries in the tumor vasculature and they could therefore penetrate tissues more easily compared to their larger counterparts [80]. While the junctions in epithelial cells are usually smaller than 8 nm, tumor tissues can on the other hand have a “leaky” vasculature allowing nanoparticles on the order of 100 nm to enter and be retained because of the defective lymphatic drainage system in what is often referred to as the enhanced permeability and retention (EPR) effect [81]. It is important however to note that the EPR effect is highly tumor specific, and is not present in all tumors [82].

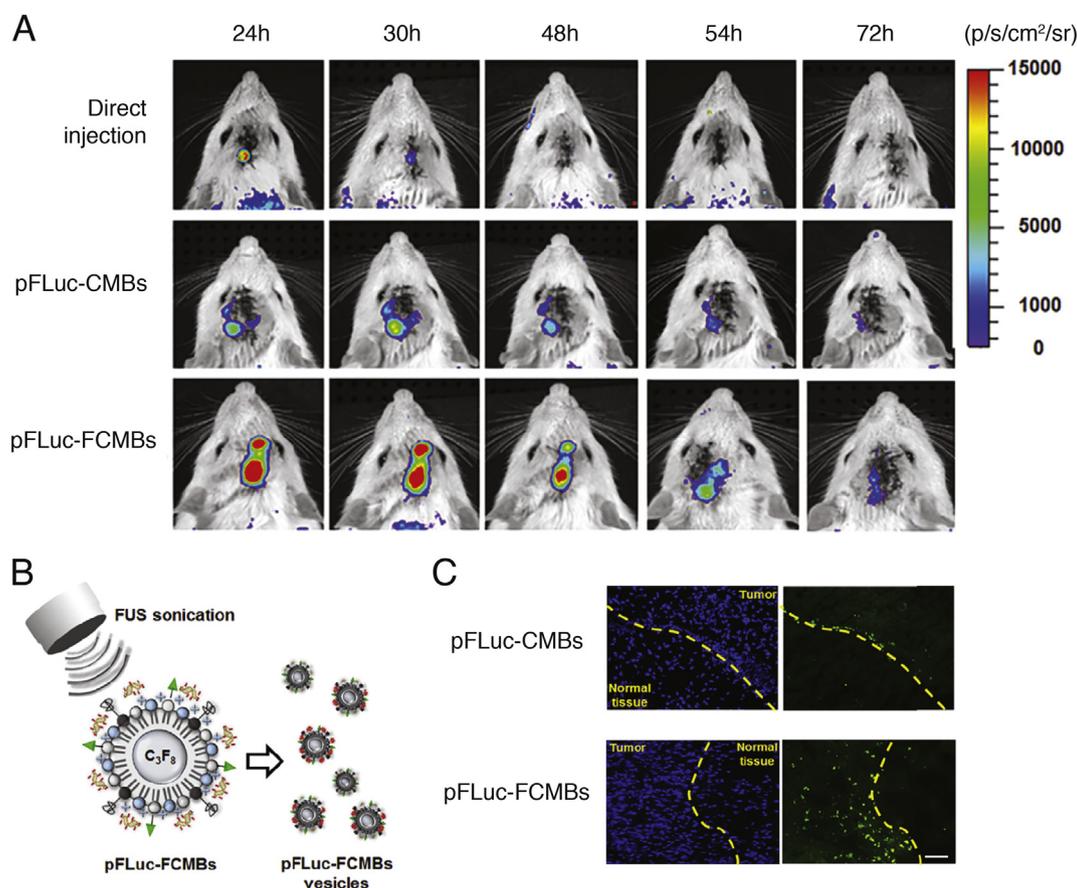
Nanometer-scale bubbles can be formulated with narrower size distributions than MBs, and because of their smaller size they can more easily be included in the aqueous core of vesicles [83]. A nanobubble–vesicle construct, where lipid-stabilized nanobubbles are incorporated into the aqueous compartment of liposomes, has been investigated as a treatment for stroke. The authors could deliver plasmin *in vitro* to human whole blood clots, leading to clot lysis [84]. Negishi and co-workers have also reported DNA-coated cationic liposomes of around 650 nm diameter containing nanometer-sized, phospholipid-stabilized perfluoropropane bubbles. They could deliver bFGF-expressing pDNA locally using focused US, which led to the induction of angiogenic factors and improved blood flow [66].

### 3.3. Vesicles containing emulsion droplets

In addition to perfluorinated gases, there has been increasing interest in using amphiphile-stabilized droplets of US-sensitive perfluorinated liquids. The term eLiposomes has been coined to describe liposomes containing such emulsion droplets [57], which have been used for drug delivery and gene transfection *in vitro* [58]. Lattin and co-workers have shown that droplets formed from

**Table 1**  
Ultrasound-responsive vesicles listed by triggered species, including architectural details on example delivery systems and amphiphiles used.

Triggered Species	Example Delivery System	Vesicle Amphiphiles	Ref
Perfluorinated gases/air	Microbubbles (MBs)	Lipids	[63,65]
	Vesicles tethered to MBs	Lipids	[62]
	MBs encapsulated in vesicles	Lipids	[77]
	Nanobubbles	Polymers/lipids	[72,73,79,80]
Emulsion droplets	Nanobubbles encapsulated in vesicles	Lipids	[66,84]
	Nanodroplets with membrane-embedded iron oxide particles	Polymer	[61]
Emulsion droplets in vesicle lumen	Folate decorated emulsion droplets	Lipids	[83]
	Vesicles encapsulating emulsion droplets	Lipids	[57–59,67,68,85]
	Vesicles encapsulating hydrogen peroxide in lumen and iron oxide in membrane	Polymer	[70]
Emulsion droplets in vesicle membrane	Giant cluster vesicles containing X-ray contrast agent Lipiodol®	Lipids	[69]
	Vesicles with PFOB emulsion droplets in membranes	Polymer	[71]
Sonosensitizers in vesicle membrane	Vesicles with the sonosensitizer HMME in membranes	Polymers/Lipids	[72,86]
Direct US on biological membranes	Low frequency US increases permeability of blood–tumour barrier in rats	Cell membrane	[76]
	Nanobubbles co-delivered with doxorubicin-loaded polymer vesicles in monocytes	Polymer, cell membrane	[91]
	US in presence of MBs induces formation of transient and permeant structures at the membrane level associated with caveolae-dependent endocytosis	Lipids, cell membrane	[92]
	Red blood cell membrane-derived microdroplets	Red blood cell membrane	[93]
	Genetically modified gas vesicles expressed intracellularly in some microbes	Protein	[94]



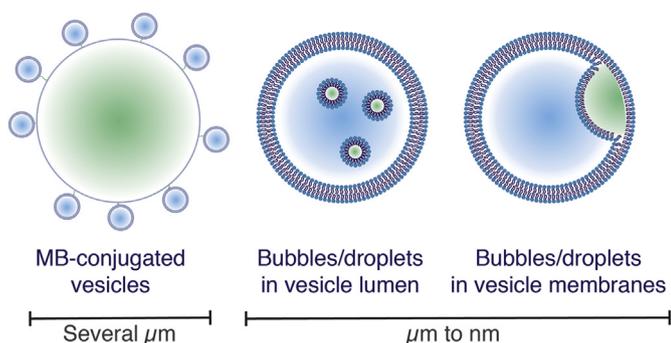
**Fig. 2.** *In vivo* gene transfection using classical (CMB) and folate-conjugated (FCMB) MBs loaded with plasmid encoding the red firefly luciferase gene (pFLuc). (A) *In vivo* gene transfection on rat brain tumor model with 700 kPa. (B) Outline of technique in which vesicles are formed *in situ* during US. (C) The distribution of pFLuc-CMBs vesicles or pFLuc-FCMBs vesicles within brain tissue after treatment. The vesicles of pFLuc-FCMBs only largely accumulated in the tumor location, suggesting that folate conjugated onto pFLuc-FCMBs shells might promote uptake of MB-derived vesicles into tumor tissue from cerebral vessels. Green spots: MB-derived vesicles. Scale bar corresponds to 50  $\mu$ m. Adapted from [63] with permission from Elsevier.

perfluorocarbons (e.g. perfluoropentane or perfluorohexane) can be stabilized with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and extruded to uniform size [57]. Liposomes, also formed from DPPC, are then assembled around the droplets using the interdigitation method and extrusion, leading to droplets encapsulated in the aqueous core. US-mediated release of encapsulated calcein dye was increased when the droplet size [67] and overall vesicle construct [68] was bigger. However, although 200 nm eLiposome constructs released less encapsulated dye than their larger 800 nm counterparts, this smaller size has the advantage of more easily being endocytosed (by non-phagocytic cells) for cytosolic delivery applications. For example, folate-conjugated eLiposomes with diameters from 200 to 600 nm have been successfully used to deliver a model drug to HeLa cells *in vitro* [59]. It has further been shown that both free emulsion droplets and eLiposomes loaded with a model drug and labeled with folate can be internalized into the endosome of folate receptor-expressing HeLa cells. Vaporization of the endosome by US led to release of the contents of the fractured endosome into the cytosol, including drugs delivered to the extracellular matrix [85]. This process provides a route to delivering extracellular drugs and drugs encapsulated in eLiposomes to the cytosol. The authors also observed that the perfluorocarbon used has an effect, with perfluoropentane proving more effective than perfluorohexane in delivering the model drug to the cytosol. Efforts have also been made to improve efficacy of commercially available contrast agents. For example, giant cluster-like vesicles (GCVs) have been loaded with the commercially available X-ray contrast agent Lipiodol® and fluorescent dye. They are destroyed by an US activated device, commonly used for cutting and sealing tissue in laparoscopic surgeries [69].

This technique could also be applied to delivering therapeutics at the site of US-mediated tissue incision.

In a move away from perfluorinated hydrocarbons, Li and co-workers have reported a hydrogen peroxide filled PLGA polymersome including iron oxide nanoparticles in the membrane [70]. Here, the hydrogen peroxide in the encapsulated solution provides oxygen which acts as the echogenic source. On US-mediated disruption of the membrane the hydrogen peroxide encounters the membrane-bound iron oxide nanoparticles and by the Fenton reaction forms hydroxyl free radicals which act as the therapeutic moiety. It was observed that this non-thermal process eradicated malignant tumors in a nude mouse model.

In the above examples, the emulsion is incorporated into the vesicle lumen, however there are examples of emulsion droplets in the membrane bilayer as well (Fig. 3). Polymer-based vesicles allow transport of perfluorinated contrast agents in the liquid state, such as perfluorooctyl bromide (PFOB), which has a boiling point of 144 °C. While lipid-based vesicles have been investigated for this use, polymer-based vesicles have been found to be a potentially more promising avenue since the interaction between the phospholipids and the PFOB is not strong enough to easily form vesicles that are stable on the nano-scale. Li and co-workers have reported a vesicle formulated from poly(ethylene glycol) methyl ether-*b*-poly(*D,L* lactic acid) block copolymers that encapsulates PFOB in the membrane bilayer, which provides good US contrast in blood pool imaging in *in vivo* studies [71]. Sonodynamic therapy is a technique that uses US and a sonosensitizer encapsulated in vesicles that, upon US treatment, is released and forms reactive oxygen species. Hematoporphyrin monomethyl ether (HMME) is a hydrophobic liquid and a known sonosensitizer and can be easily



**Fig. 3.** Schematic representation of different kinds of echogenic liposomes. Left, microbubbles with vesicles tethered to their surface. Middle, bubbles or emulsions encapsulated within vesicles. Right, gas or emulsion-in-bilayer vesicles. Blue denotes aqueous encapsulated contents and green denotes perfluorocarbon and/or air.

incorporated into polymer membranes [72] and liposome bilayers. Chen and co-workers have shown in *in vitro* studies that liposomes labelled with the mitochondria-targeting triphenylphosphonium bromide, with HMME loaded into the lipid membrane, can lead to around 60% cell death upon 3 minutes of US, versus 20% in the liposome-only control [86]. Localized heating by high-intensity focused ultrasound can be used as an external trigger to release content from temperature-sensitive liposomes (TSLs, see section 4.1.1) and polymersomes, in the absence of perfluorinated bubbles or emulsions. This is used to induce thermal ablation of tumors at around 57 °C [87], and more commonly, to induce non-destructive hyperthermia in the region of 39–42 °C [88,89]. Extrapolating from this success with conventional techniques, non-lipid or polymer-based vesicles with US sensitivity are another avenue for future investigation. However, so far there is only one report, by Song and Zheng, of non-liposome or non-polymer-based constructs with similar properties [90]. They described tetraphenylethylene macrocyclic compounds that can self-assemble into hollow spheres. When triggered with US, these constructs rearrange to form “bird nest” structures composed of nanorods with potential applications in controlled release of anti-cancer drugs.

### 3.4. Ultrasound and biological membranes

Recently there have been examples of US-triggered constructs including biologically derived membranes. Specifically, loading MB-vesicle mixtures into cells for intracellular drug transport makes for a Trojan horse-type delivery. For example, Huang *et al.* found in mouse studies that monocytes could be loaded with polymeric nanobubbles and subjected to FUS in the presence of doxorubicin-loaded polymer vesicles (DLPV). This enabled the vesicles to target tumors more specifically than if they were directly injected intravenously [91]. Specifically, in the monocyte delivery system 60% was delivered to the tumor vs. 80% delivery to the liver in the case of MBs and DLPVs alone.

Sonoporation uses low intensity US to induce cavitation of MBs. This leads to enhanced endocytosis and formation of transient membrane

pores in neighboring cells [92], and has been shown to trigger the release of extracellular vesicles (EVs) [65]. Paproski and co-workers first reported that the release of EVs from cancer cells could be stimulated using US and lipid-coated nanobubbles [73]. This led to a 100-fold increase in the circulating tumor DNA (ctDNA). Analysis of ctDNA is a powerful tool for liquid biopsies and profiling tumor genetics and the authors could characterize the tumor phenotype, including its aggressiveness, based on the protein and nucleic acids presented in these EVs produced under US triggered MB cavitation. The identification of aggressive tumors before they metastasize is critical to improve patient outcomes, as the vast majority of cancer deaths are due to metastatic cancer [82].

In an example using droplets, red blood cell ghosts formulated into microdroplets with perfluoropentane have been proposed for anti-cancer theranostics. When loaded with a chemotherapeutic agent and triggered with US, they led to around 70% cell death in *in vitro* experiments with a HeLa cell model [93].

At smaller length scales, gas vesicles (GVs) are nanometer-sized anisotropic vesicles with a 2 nm thick protein shell. Expressed intracellularly in some bacteria and archaea, these gas-permeable vesicles regulate cell buoyancy in aqueous environments. Lakshmanan and co-workers have recently shown that these vesicles can be genetically engineered to modify their harmonic response, surface charge, collapse pressure, targeting specificity, and fluorescent properties. *In vivo* imaging studies in mice showed that the harmonic contrast was greatly improved in genetically modified GV vs. the wild-type, and *in vitro* studies of GV modified to include several fluorescent dyes showed that cellular targeting was possible [94]. Further discussion of such vesicles and their applications can be found in a review by Aw and Paniwnyk [95].

## 4. Heat

Temperature regulated processes play a vital role in biology, and many biomedical applications of vesicles have taken inspiration from biology to pursue heat-triggerable mechanisms (Table 2). Localized hyperthermia has by itself become an effective tool for the treatment of solid tumors [96,97]. In this method, tumors are heated to temperatures exceeding 50 °C which has a cytotoxic effect and leads to coagulated necrosis. However, this treatment method has the drawback of inhomogeneous heating causing incomplete destruction of tumors as well ineffective targeting of deeply seated tumors and disperse metastases. The combination of hyperthermia with thermoresponsive vesicles can improve its efficacy due to accumulation of nanocarriers within the tumor site *via* passive or active targeting. The success of a thermoresponsive system depends on the availability of a sensitive carrier that readily reacts to small temperature changes.

### 4.1. Conventional thermoresponsive vesicles

#### 4.1.1. Liposomes

Almost all thermoresponsive vesicles described in the literature are based on liposomes and polymersomes. The thermoresponsiveness of liposomes can be modulated by tuning the lipid composition to alter the phase transition temperature ( $T_m$ ), around which conformational

**Table 2**  
Thermoresponsive vesicles listed by type of triggered vesicle, including details on amphiphile type and triggered mechanism.

Type of vesicle	Type of amphiphile	Triggered mechanism	Ref
TSL	Lipids	Rearrangement of fatty acid chains above $T_m$ of lipids leads to permeable membranes, can be ultrasound mediated	[88,98–101]
Liposomes encapsulating $\text{NaCO}_2$	Lipids	Decomposition of $\text{NaCO}_2$ loaded into liposomes, dissociation of vesicles	[102,103]
Polymersomes	Polymers	Change of amphiphile hydrophilicity upon LCST or UCST, dissociation of vesicles	[13,42,104–108]
Hybrid vesicle	Mixture of amphiphiles (lipids, polymers, peptides, etc.)	Rearrangement of component mostly peptides, permeable membranes	[109–111]
Macrocycle vesicles	Functionalized pillararene macrocycles	Dissociation of vesicles	[112,113]

changes within the lipid bilayer are triggered. Around the melting temperature of the lipid, fatty acid chains rearrange from a gel state to a liquid crystal state. This can either happen instantaneously at the melting temperature or it can be a more progressive process that begins at the melting temperature and completes at a higher temperature. In both cases, this process leads to a more fluid and leaky membrane and results in drug release [98–100], with a significant increase in the membrane permeability around the transition melting temperature. These conventional thermosensitive liposomes (TSLs) were first introduced in the 1970s by Yatvin *et al.* [101], and within the last decades a large body of research has been generated in their development all the way towards clinical testing [99]. Other thermoresponsive mechanisms have also been investigated. For example, sodium bicarbonate can be loaded into liposomes to facilitate a pH gradient for doxorubicin, but also to trigger release upon heating [102,103]. Hyperthermia causes the sodium bicarbonate to decompose into ammonia and carbon dioxide bubbles, resulting in liposomal membrane defects and therefore release of the loaded drug. The authors demonstrated high biostability of their nanocarrier after intravenous injection into mice and showed effective tumor-selective chemotherapy upon heat trigger. Combinations of various triggers can often lead to even greater efficacy and targeting ability. For example, Kheirloom and co-workers used US-mediated hyperthermia to release a pH-sensitive doxorubicin–copper complex from long-circulating TSLs that gathered in tumors due to the EPR effect. Intravascular release of the drug was achieved by applying US to the tumor for a total of 25 minutes, including 5 minutes prior to injection of the therapeutic. In *in vivo* experiments with DL-tumor bearing mice, after twice weekly treatments over 28 days, the tumors had disappeared. The tumors were still undetectable eight months after treatment [98].

#### 4.1.2. Polymersomes

Polymers are commonly used as thermoresponsive materials, with examples including poloxamers, poly(*N*-substituted acrylamide)s, poly(*N*-vinylcaprolactam)s and chitosans [13,104–106]. These react reversibly to temperature changes in a very narrow temperature window.

The lower critical solution temperature (LCST) describes the temperature at which, upon heating, a hydrophilic polymeric structure changes to a hydrophobic state and becomes insoluble in water. On the other hand, when a polymer becomes soluble upon heating they are described by the upper critical solution temperature (UCST) [42]. For example, the most investigated example of polymeric amphiphiles, poly(*N*-isopropylacrylamide) (PNIPAM), has a LCST of 32 °C which is close to normal physiological temperatures. For both liposomes and polymersomes, plenty of thermoresponsive examples can be found in the literature and both have been thoroughly reviewed [2,13,14,107,108]. Here we instead focus on emerging candidates including hybrid vesicles and supramolecular amphiphiles.

#### 4.2. Thermal rearrangement of peptides embedded in vesicle membranes

Hybrid vesicles are formed from two or more different types of amphiphiles, for example phospholipids, polymers or small molecules. For example, Al-Amahdy *et al.* reported on temperature-responsive hybrid vesicles [109,110] that are comprised of lipids and a leucine-zipper peptide imparting thermoresponsiveness. This peptide increases the thermostability of the overall lipid vesicles at 42 °C by decreasing its membrane fluidity. It is known that the peptide forms a super helix coiled-coil structure at lower temperatures and that this self-assembled structure is dissociated at increased temperatures, which is the proposed mechanism for the thermoresponsive drug release behavior. The authors showed loading of the chemotherapeutic doxorubicin and demonstrated successful delivery into tumor tissue after intravenous injection into mice. The payload was then released upon heat trigger causing local cytotoxicity and tumor regression.

In another hybrid example, vesicles formulated from lipids and an elastin-like polypeptide showed thermally responsive phase transition behavior (Fig. 4). Kim and co-workers showed that functionalizing these nanocarriers with cyclic arginine-glycine-aspartic acid (cRGD) ligands resulted in 8-fold and 10-fold higher uptake into  $\alpha_v\beta_3$  integrin overexpressing U87MG and HUVEC cells respectively, and efficient cytotoxicity was demonstrated upon application of heat [111]. The

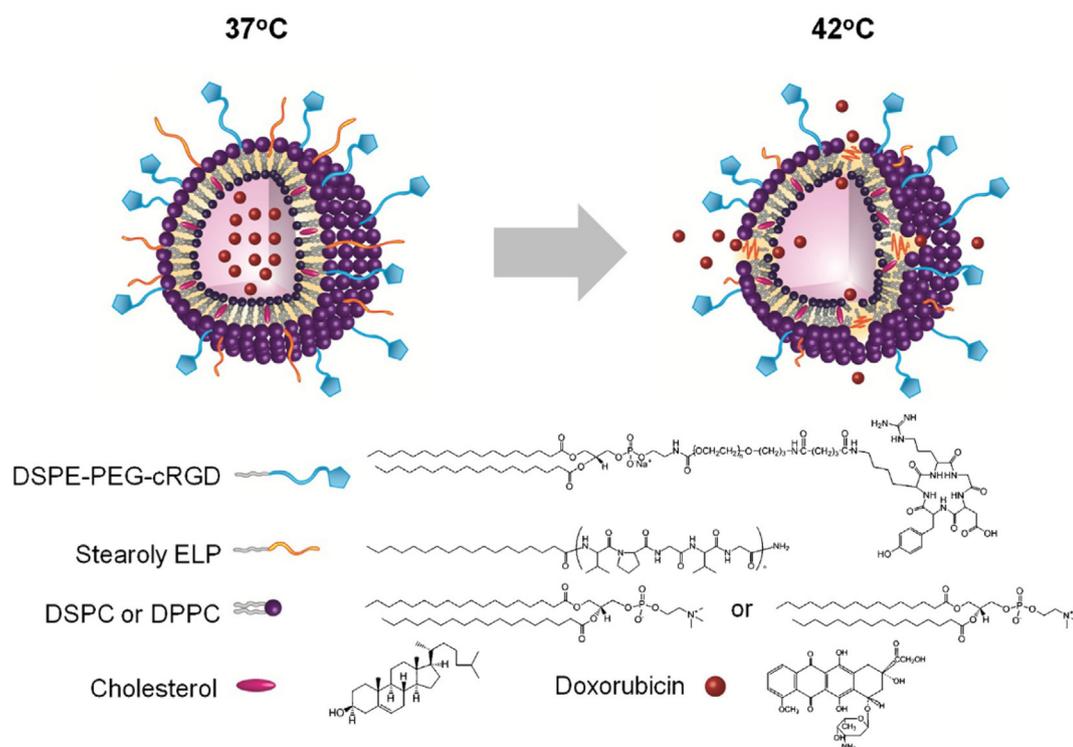


Fig. 4. Hybrid vesicles consisting of lipids, a targeting unit and elastin-like peptide for mild hyperthermic release of doxorubicin. Adapted from [111] with permission from Elsevier.

vesicles showed good loading and release profiles for doxorubicin and thermo-responsive properties were maintained for up to 12 h, with payload release triggered under mild hyperthermia. Moreover, the nanocarrier showed an approximately 5-fold higher accumulation in tumor tissue compared to non-targeted vesicles after intravenous injection into mice.

#### 4.3. Pillararenes

Zhou *et al.* reported vesicles with thermo-responsive properties constructed by glycol chain functionalized amphiphilic pillar[5]arenes [112]. These macrocycle amphiphiles can self-assemble into 120 nm sized bilayer vesicles that collapse upon heating. Interestingly, this process is reversible as the vesicles reform upon cooling. Moreover, the authors established magnetic responsiveness by incorporating magnetic nanoparticles within the bilayer. Co-encapsulated fluorescent calcein was then released upon application of a magnetic field due to deformation of the vesicles into irregular structures.

Doxorubicin-loaded, supramolecular pillar[6]arene-based vesicles were reported by Cao and co-workers (Fig. 5) [113]. As well as being thermo-responsive, these supramolecular amphiphile vesicles also showed responsive behavior to changes in pH and the addition of  $\text{Ca}^{2+}$ . The authors showed *in vitro* experiments with MCF-7 cancer cells and NIH3T3 cells that their system does not reduce the toxicity of doxorubicin towards cancer cells. Moreover, they demonstrated that heat could trigger cytotoxicity, and that cytotoxicity without heat

trigger towards NIH3T3 cells was reduced compared to treatment with free doxorubicin.

## 5. Light

Photoresponsive vesicles allow for spatial as well as temporal control with non-invasive tissue penetration (Table 3), and most examples have a specific wavelength for illumination in the UV range [114]. UV light is often used because of its high energy, which can induce conformational changes or the breaking of chemical bonds. However, a major drawback in using UV light is the low penetration depth because of scattering in biological tissue. Recent examples have therefore focused on light in the near infrared region (NIR), which shows low absorbance by skin and tissue enabling a penetration from hundreds of micrometers (for UV light) to centimeters. Two photon technologies can increase the efficacy of NIR nanocarriers even further and utilizes pulsed lasers which provide a high density of photons in short time pulses (nanosecond to femtosecond). These are absorbed by photolabile groups with two photon cross sections, for example coumarin derivatives.

### 5.1. Vesicles for light-responsive drug release

Phototriggerable nanomedicines can usually be categorized into three groups: (i) light-responsive drug-releasing therapeutics, (ii) photothermal therapeutics, and (iii) photodynamic therapeutics. Light-sensitive drug release is usually facilitated by implementing light-cleavable linkers, light-induced crosslinking, light-induced

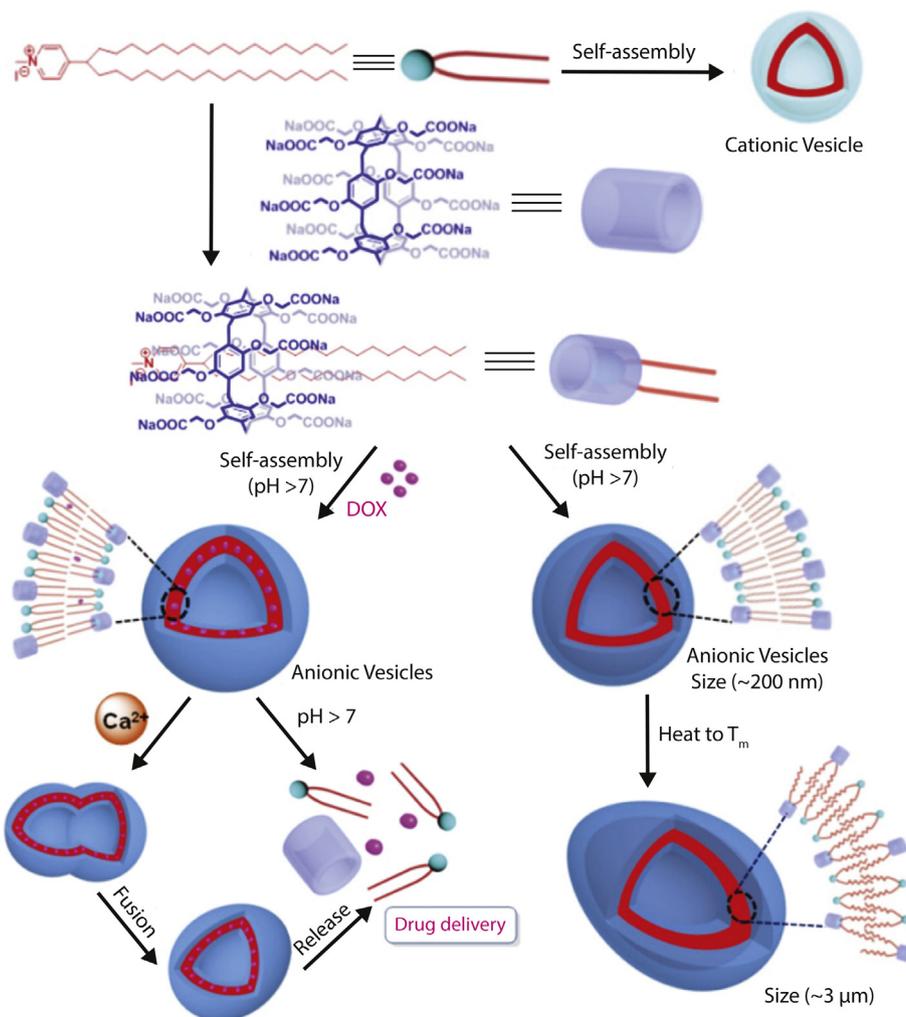


Fig. 5. Multi-responsive pillar[6]arenes. Adapted with permission from [113]. Copyright (2014) American Chemical Society.

**Table 3**

Photoresponsive vesicles listed by type of light triggered mechanism including details on mechanism and architectural composition of example delivery systems.

Category	Mechanism (triggered molecule)	Example delivery system	Ref	
Light responsive drug release	Cleavable linker (coumarin and nitrobenzyl) Photoisomerization (spiropyran, azobenzene, azopyrazole)	Dendrimersomes with nitrobenzyl units	[115]	
		Cationic azobenzene amphiphiles forming vesicles	[116]	
		Amphiphilic cyclodextrin vesicles with surface immobilized azobenzene-conjugated cargo	[48,117–121]	
		Azobenzene functionalized amphiphilic cyclodextrin	[122]	
		Supra-amphiphiles <i>via</i> host guest interaction of pillararene and azobenzene	[123,124]	
	Light Upconversion Nanoparticles (UCNPs)	Supra-amphiphiles <i>via</i> host guest interaction of cucurbituril, methylviologen and azobenzene	Amphiphilic cyclodextrin vesicles with surface immobilized arylazopyrazole photoswitch	[136]
			Lipids with spiropyran units	[151,152]
			UCNPs embedded in hybrid vesicle (lipids and amphiphilic azobenzene units)	[125]
			Exosomes with membrane embedded gold nanorods	[126]
			Cell derived nanovesicles with membrane embedded DiR	[127]
Photothermal therapeutic	Heating (gold nanorods, DiR)	Vesicles with membrane embedded photosensitizer	[50]	
		Amphiphilic cyclodextrin vesicles with surface-immobilized squaraine	[128]	
		Amphiphilic cyclodextrin vesicles with surface immobilized phthalocyanine	[129]	

degradation, or light-responsive conformational changes. Well-known examples of light-triggerable components that can directly react with light are azobenzenes or spiropyrans that react *via* photoisomerization [130,131] and nitrobenzyl or coumarin groups that react *via* cleavage [132,133] (Fig. 6).

#### 5.1.1. Vesicles with photoisomerizable or photocleavable units

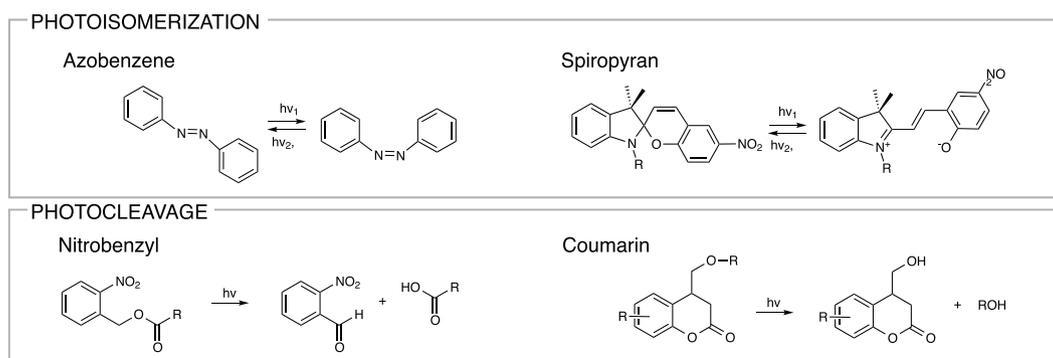
Azobenzene derivatives are among the most investigated examples in the UV-range due to their *trans*-to-*cis* isomerization when exposed to wavelengths around 300–400 nm [134]. Transition back to the more thermodynamically stable *trans* form can be obtained by either irradiation in the visible range or thermal relaxation. The isomerization is accompanied by physical changes (like polarity) which is often used to control the self-assembly of its nanocarrier. Despite their extensive use, azobenzenes have two major drawbacks. As already mentioned, UV light has a high scattering rate in biological tissue that can be damaging to healthy tissue, and the *cis*-isomer has low thermodynamic stability [135]. As a result, current research efforts are more directed towards the functionalization of azobenzenes (*e.g.* fluorinated azobenzenes) to shift the wavelength for isomerization into the visible or even NIR region and to obtain more stable *cis*-isomers [136–138]. However, examples for visible or NIR region *trans* to *cis* switching of azobenzene derivatives have to our knowledge not yet been reported in vesicles. The following examples for azobenzenes focus therefore on recent and innovative examples of UV light-responsive switches.

The synthesis of a new cationic azobenzene (CAB) derivative that self-assembles into vesicles when mixed with anionic SDS in aqueous

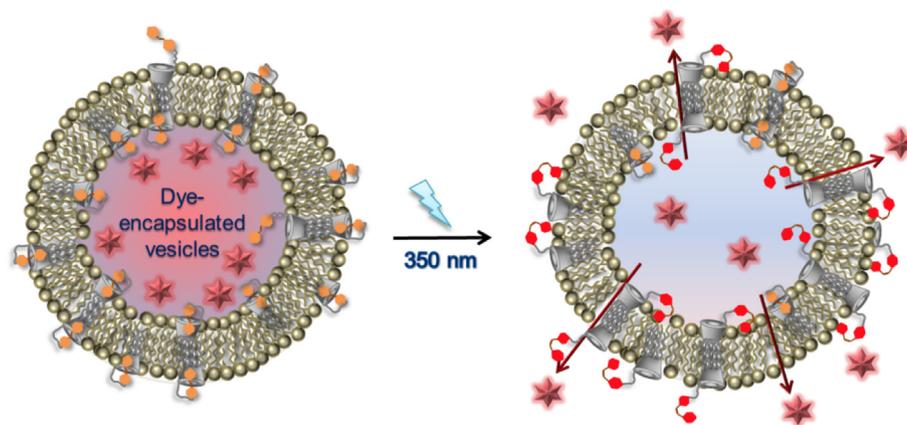
solution was recently reported by Geng *et al.* [116]. The authors show that these CAB/SDS vesicles undergo photoresponsive drug release *via* a *cis*-*trans* isomerization upon irradiation of the azobenzene derivative, and that irradiated doxorubicin-loaded CAB/SDS vesicles exhibit a higher cytotoxicity than non-irradiated ones.

In another example, amphiphilic cyclodextrin molecules have been utilized to form vesicles that can recognize photoresponsive azobenzene units *via* host-guest inclusion [139]. These cyclodextrin vesicles are biocompatible, non-toxic, versatile and easy to functionalize, with guest molecules bound *via* reversible host-guest recognition to their surface [139,140]. Ravoo and co-workers have shown reversible recognition processes on vesicles emulating biological systems [118–120,141,142] as well as drug release and targeting with a variety of different biomolecules including carbohydrates [143–146], peptides [147–149], proteins [48,121] and nucleotides [48,49,117]. Building on this, Kauscher *et al.* have synthesized an amphiphilic  $\beta$ -cyclodextrin that is covalently labelled with azobenzene [122] and embedded into a liposomal membrane consisting of DOPE, DOPC and cholesterol. Here, azobenzene binds into the cyclodextrin cavity in its *trans* form, but once it is isomerized to its *cis* form the inclusion complex dissociates. The authors showed that this light-responsive switching can be used to control the vesicle membrane permeability, where UV light irradiation was followed by content release (Fig. 7).

Stricker and colleagues enhanced the photoswitching ability of their cyclodextrin vesicles by introducing a new arylazopyrazole photoswitch, including water-soluble carboxylated derivatives [136]. While in azobenzene molecules the absorbance spectra of the *cis* and



**Fig. 6.** Chemical structures of photosensitive small molecules that can undergo photoisomerization and photocleavage.



**Fig. 7.** Amphiphilic cyclodextrin with covalent azobenzene side functionalization for phototriggered drug release from hybrid vesicles. Reproduced with permission from [122], Royal Society of Chemistry.

*trans* isomers overlap, arylazopyrazole derivatives can be isomerized from *cis* to *trans* with green light, and the absorbances of the *cis* and *trans* isomers do not overlap. As a result the authors show nearly quantitative isomerization with UV (*trans* to *cis*) and green light (*cis* to *trans*).

Photoresponsive host–guest recognition-based vesicles comprising pillar[6]arenes and azobenzenes were first formulated into vesicles by Yu *et al.* in 2012 [123], and are a good alternative to the more established cyclodextrin–azobenzene systems. This group has since presented azobenzene-conjugated polymers that form amphiphiles with pillar[6]arenes [124]. Under UV irradiation they dissociate and the azobenzene-functionalized polymers self-assemble into micelles. Visible light irradiation reverses the formation of micelles back to vesicles. Giant supramolecular vesicles have also been observed when the host molecule cucurbit[8]uril is mixed with maleimide-modified methylviologen and hydrophobic 3,4,5-tris(*n*-dodecyloxy) benzoylamide with an azobenzene moiety [150]. Again, azobenzene isomerization causes the supra-amphiphile to dissociate leading to release of an encapsulated drug. The maleimide moiety allows for easy functionalization with target molecules like the RGD peptide or bovine serum albumin and authors demonstrated cellular uptake by three different human cancer cell lines as well as stability and drug delivery abilities of the nanocarrier.

Spiropyran units have been incorporated into liposomes as early as 1998 by Ohya *et al.* [151]. They synthesized a single chain lipid containing the spiropyran unit at its hydrophobic terminus, which could release a dye on UV irradiation. Recently, Kwangmettamat and Kudernac reported the synthesis of a small amphiphile bearing two spiropyran moieties and an ethyleneglycol backbone [152]. These amphiphiles assemble into vesicles in water, and expand to double their size upon irradiation and resulting photoisomerization. Interestingly, this behavior is reversible and as soon as irradiation is stopped the vesicles shrink back to their initial size. The authors envision incorporation of their synthesized amphiphile into a range of self-assembled structures which can undergo complex shape transformations, due to regions of bilayer phase separation.

Finally, dendrimer-based vesicles, termed dendrimersomes, have been designed to contain photodegradable hydrophobic blocks with *o*-nitrobenzyl units throughout their backbones. Upon exposure to UV light, the dendrimers undergo complete photolytic backbone cleavage, causing dissociation of the dendrimersome self-assemblies and release of both hydrophilic and hydrophobic payloads [115].

### 5.1.2. Upconversion of NIR light

Light can also be used for indirect photoactivation *via* light upconversion [153]. Light upconversion describes the absorption of NIR light by the carrier into more energetic visible or UV light. The

upconverted light then causes the cleavage or isomerization of the phototriggerable functionality [154,155]. In one example, Yao *et al.* loaded upconversion nanoparticles (UCNPs) into hybrid vesicles consisting of lipids and an amphiphilic azobenzene-molecule [125]. NIR irradiation at 980 nm is upconverted to UV light by the encapsulated UCNPs, which is in turn absorbed by the azobenzene moieties within the vesicle membrane. The azobenzene units then undergo a *trans*-to-*cis* isomerization, allowing the release of encapsulated drugs. Simultaneous irradiation with visible light causes a continuous rotation-inversion mechanism that results in leaky membranes. The authors showed how vesicles loaded with the cancer drug doxorubicin caused tumor growth inhibition after intravenous injection into nude mice bearing MCF-7/ADR tumors and NIR irradiation of the tumor tissue.

### 5.2. Photothermal therapy

Photothermal therapy describes the transformation of NIR into heat and the subsequent treatment and ablation of diseased tissue, mostly cancer tissue [156–158]. Given the high spatial and temporal selectivity, and minimal toxicity to healthy tissue, this technique exhibits some unique advantages compared to conventional therapeutic modalities [25,159]. Since heat can be used to change the fluidity of bilayer membranes, combination of photothermal agents with vesicles is often used to create responsive drug delivery systems.

Vesicles derived from natural membranes have been investigated for this purpose. In one example, Wang and co-workers engineered exosomes to be nanoplatforams for chemo- and photothermal tumor therapy [126]. Exosomes are naturally derived vesicles shed from many cell types [160,161]. In their work [126], the authors cultured donor cells with RGD peptides and sulphhydryl groups in their membranes, and exosomes collected from these cells showed the same surface modification. Gold nanorods were immobilized *via* Au–S bonds on the surface of these exosomes and further covalent modification with tumor targeting moiety folic acid was carried out. The authors demonstrated accumulation of the exosomes in tumor tissue after intravenous injection into mice, due the dual targeting of folic acid and RGD peptides, and enhanced drug release in response to the heat generated by gold nanorods in response to NIR irradiation.

The near infrared dye DiR has also been used to transform NIR into heat by Wu *et al.* in a naturally derived vesicle system [127]. They used cell-based nanovesicles obtained *via* extrusion of DC2.4 cells. Several extrusion steps with diminishing extrusion pore sizes resulted in vesicles with a diameter of about 100 nm. By co-extruding the cells with doxorubicin in solution, doxorubicin-loaded vesicles were obtained, and DiR was subsequently inserted into the vesicle membrane by adding an ethanol solution of the dye to the vesicle suspension. The authors report that NIR irradiation causes heat that eradicates tumor

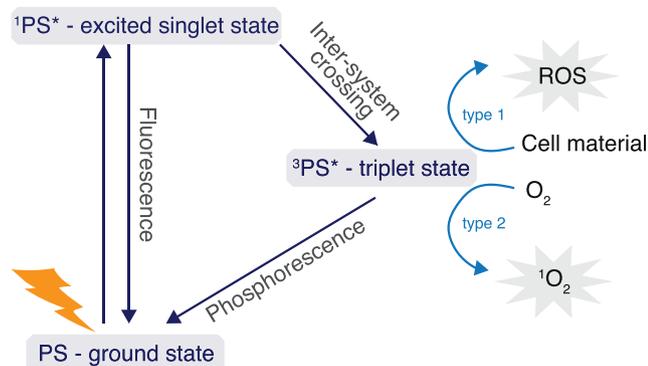
cells but also destroys the nanovesicle membrane resulting in doxorubicin release. *In vitro* tumor cytotoxicity and *in vivo* tumor growth inhibition in a murine model was demonstrated.

### 5.3. Photodynamic therapy

Photodynamic therapy (PDT) is the non-invasive treatment of cancerous or diseased tissue, where toxic species that can cause cell death are produced via the irradiation of a photosensitizer [162,163]. Upon irradiation, the photosensitizer is excited to its singlet state and reaches a triplet state via intersystem crossing (Fig. 8). Upon relaxation, electrons are transferred either to the surrounding cell material causing the production of radicals, or singlet oxygen is generated via the interaction of the photosensitizer with oxygen. Most PDT nanosystems are based on the photosensitizer porphyrin with Photofrin being the first sensitizer approved for treating cancer in the 1990s [164]. However, porphyrin absorbs mostly in the visible region, which is a disadvantage because of the low penetration depths of visible light in biological tissue [165,166]. Research has therefore been directed to photosensitizers that absorb in the visible or near infrared region and has included examples of squaraine [167], porphyrin, cyanin, bodipy and phthalocyanine [168] derivatives [169]. Most of these photosensitizers are macrocyclic compounds with poor water solubility [170]. Due to their extended conjugated  $\pi$ -system they tend to form H-aggregates, which reduce their ability to produce singlet oxygen. Combination of these photosensitizers with a delivery platform has been shown to reduce aggregation and to increase their photosensitizing ability [171,172].

Vesicles have been used to transport photosensitizers or have been directly constructed out of photosensitizers. Porphysomes, vesicles consisting of porphyrin-functionalized lipids, have been reported by Lovell *et al.* [50]. These photo-therapeutic vesicles can also act as imaging agents, as quenched porphysomes allow for visualization of the lymphatic system by photoacoustic tomography. NIR fluorescence was triggered by degradation of enzymatic linkers allowing for low-background fluorescence imaging. The photo-therapeutic application was demonstrated by systemic administration, upon which the porphysomes accumulated within tumor tissue. The tumor was then successfully ablated via laser irradiation.

Kauscher and Ravoo synthesized a squaraine molecule covalently equipped with two adamantane molecules [128]. This symmetric molecule could be bound via host-guest interaction to cyclodextrin vesicles, where it generated radicals on their surface upon irradiation with NIR light. This approach has potential for medical application due to the use of NIR and since target molecules can be attached to the vesicle surface in a host-guest-driven manner. The same group reported cyclodextrin-based vesicles decorated with phthalocyanine-derivatives (Fig. 9) [129,173]. Phthalocyanines are macrocycles with outstanding photosensitizing properties due to their high absorption values within



**Fig. 8.** Jablonski diagram illustrating photosensitizer excitation and relaxation in photodynamic therapy. Upon relaxation of the photosensitizer from the triplet to ground state, reactive oxygen species (ROS) or singlet oxygen ( $^1O_2$ ) is formed.

the near infrared region, their high intersystem crossing yields, long triplet state lifetimes, and high singlet oxygen quantum yields [174–176]. Within a study by Galstyan *et al.*, phthalocyanines were functionalized with adamantane groups, which bind via host-guest inclusion into cyclodextrin cavities [129]. The authors showed that the immobilization of the photosensitizer on the surface of cyclodextrin-based vesicles prohibits inactivation via aggregation and enhances the phototherapeutic action of the phthalocyanines. The system was then improved by changing the zinc(II) phthalocyanine core to a Si(IV) core for higher phototherapeutic performance, and changing their symmetric functionalization with adamantane units to an asymmetric one for axial immobilization on cyclodextrin vesicles, which further prohibits undesired inactivation via H-aggregation in water. *In vitro* phototoxicity testing against methicillin-resistant *Staphylococcus Aureus* bacteria showed almost complete inactivation.

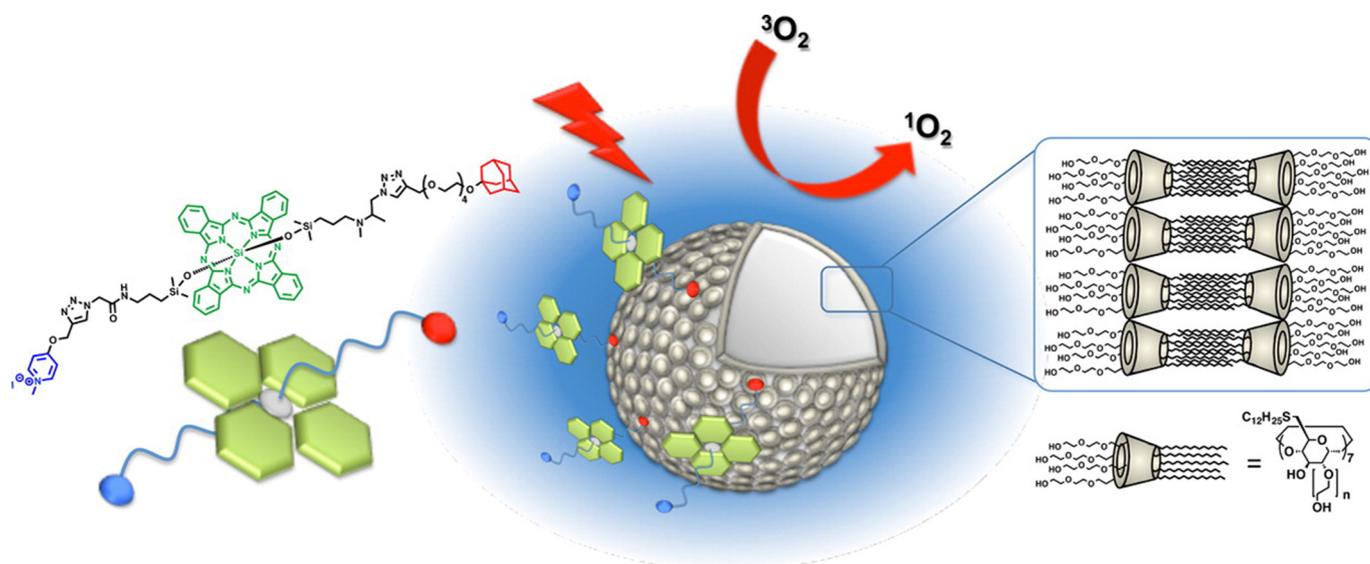
In an example of multi-stimuli responsive vesicles, Corato and co-workers used magnetically induced hyperthermia combined with photodynamic therapy for tumor ablation [177]. Their hybrid liposomes encapsulate iron oxide nanoparticles inside their hydrophilic core and exhibit the photosensitizer mTHPC payload within their membranes. The authors first showed *in vitro* that each treatment individually (hyperthermia and PDT) resulted in tumor cell death. Combined methods resulted in complete cell destruction with synergistic apoptotic signaling pathways. *In vivo* studies showed tumor regression with single treatments and complete eradication with combined treatment.

## 6. Mechanical triggers

Characteristic mechanical environments within the body can be harnessed as endogenous triggers for drug delivery for a range of self-assembled constructs, as reviewed recently by Zhang and co-workers [21]. Mechanical stimuli, despite their interesting applications, remain one of the least commonly studied response triggers, with few examples including vesicles in the literature. In this area, new mechano-responsive materials and innovative combinations of vesicles within more complex delivery systems may have an important role to play (Table 4).

### 6.1. Shear stress as an endogenous trigger

The increased endogenous shear stress observed in arteries of patients with cardiovascular disease has been investigated as a triggering mechanism for localized delivery of *e.g.* vasodilators [184] or anti-thrombotic drugs [22]. The shear stresses in healthy vessels range from 0.1–7 Pa depending on the type and location [185]. However, flow simulations estimate that local shear stresses in critically constricted coronary arteries are as high as 20 Pa [178]. Holme and co-workers were the first to report vesicles sensitive to the shear stresses commonly seen in the diseased heart [23]. They formulated liposomes made from an artificial 1,3-diamidophospholipid, Pad-PC-Pad, which are stable in low shear stress but release their payload at elevated shear stress (Fig. 10). Using a model cardiovascular system based on X-ray tomography images of diseased human coronary arteries [178], they showed that these circulating vesicles released 25% more of their encapsulated cargo after only one pass through a constricted model artery, versus vesicles that passed through a “healthy” model artery control. This unusual property was attributed to the vesicles’ lenticular morphology, which is due to a preferential destabilization in the lipid bilayer along the equator. The vasodilator nitroglycerine was successfully incorporated into these liposomes with 20–50% encapsulation efficiency, and complement activation in human sera and whole pigs showed the constructs to be non-toxic [179,180]. Holme *et al.* also reported that long-circulating liposomes formulated from a natural mixture of phospholipids extracted from chicken egg, EggPC, with a proportion of the surfactant Brij® S10 showed increased release over time in the high shear stress, diseased artery model versus liposomes formulated from solely EggPC [23].



**Fig. 9.** Cyclodextrin vesicles with immobilized asymmetric functionalized Si(IV)phthalocyanine via host-guest interaction. Reprinted with permission from [129]. Copyright 2016 American Chemical Society.

### 6.2. Compressive forces in osteoarthritis

In the treatment of osteoarthritis, compressive forces between bones across joints can act as a trigger for release. To this end, mechanically-responsive liposome-hydrogel constructs have been reported by Stalder and Zumbuehl [181]. The authors chemically tethered liposomes comprising a mixture of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) and DPPC to an oxidized dextran hydrogel and observed the release of vesicle contents in response to hydrogel compression. Here, the mechanosensitive release is due to the synergy of the hydrogel and vesicles. The DPPE in the vesicle formulation was found to be crucial for release, with hydrogel-vesicle constructs comprising only DPPC showing no release upon compression.

### 6.3. Dermal and transdermal drug delivery

Encapsulating hydrophilic therapeutics within vesicles for increased uptake is a cornerstone of dermal and transdermal vesicular delivery systems. Many vesicle constructs have been proposed for this purpose, including ethosomes (phospholipid and ethanol-based vesicles), niosomes (non-ionic surfactant based vesicles), pro-niosomes and vesosomes (nested liposomal structures) [186]. These predominantly rely on enhanced passive diffusion through the skin compared to conventional liposomes and polymersomes. Vesicle permeability can be vastly improved by physical perturbation of the skin *via*.g. microneedles [187,188], low frequency ultrasound, electroporation or iontophoresis [189]. These exogenous delivery methods can allow vesicles and other

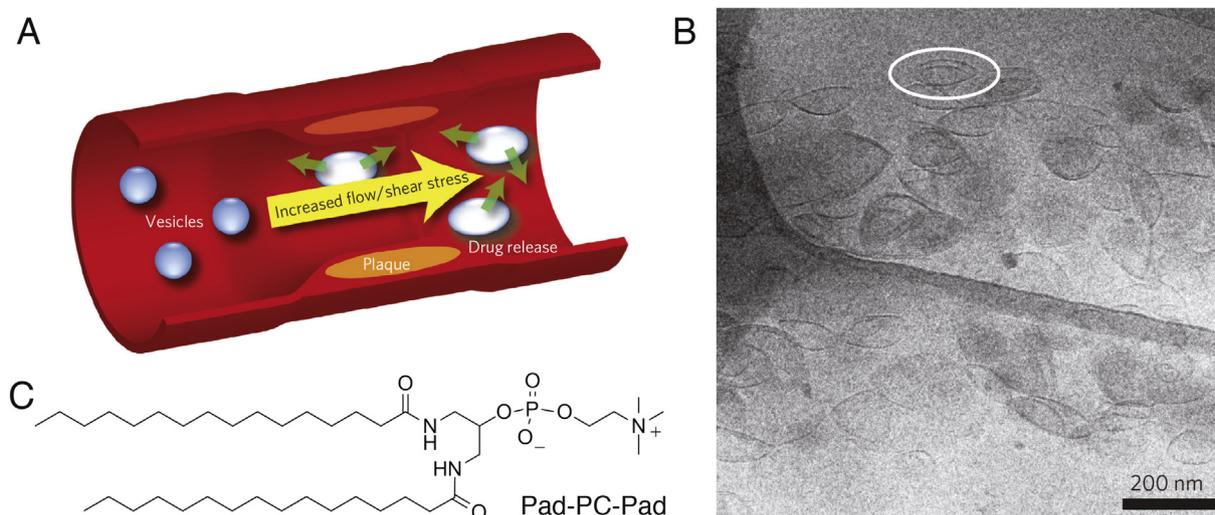
active therapeutics to traverse the otherwise impenetrable outermost layer of dead skin (the stratum corneum) and facilitate, for example, transcutaneous immunization [190]. Pamornpathomkul *et al.* have recently reported cationic niosomes loaded into hollow microneedles as a vector for transcutaneous immunization, which showed higher immune response in comparison with a subcutaneous injection control and no infection or bleeding in the skin [182]. Microneedle patches have also been integrated with hypoxia-sensitive hyaluronic acid vesicles encapsulating insulin and glucose oxidase, for the fast and glucose-dependent delivery of insulin to treat diabetes. The vesicles in this “smart insulin patch” dissociate under hypoxic conditions found in hypoglycemia, releasing insulin. In a mouse model, the patch was capable of regulating blood glucose levels to within normal values [191]. On the single cell level, recent work by Chiappini *et al.* has studied the feasibility of needles loaded with a therapeutic moiety for delivery across the cell membrane. Specifically, they showed that mesoporous silicon nanoneedles are able to deliver nucleic acids [192] and quantum dots [193] into the cytosol with negligible impact on cell viability. Micro- and nanoneedle platforms for delivering vesicle constructs are an interesting avenue for future research in both transdermal and transmembrane delivery.

### 6.4. Shear-thinning injectables

Mechanical forces can be used as a tool during administration by means of shear-thinning injectables. Himmelein *et al.* presented a hydrogel using cyclodextrin vesicles as multivalent junctions between cellulose fibers [183]. The fibers are equipped with adamantane functions that can interact *via* host-guest inclusion with the cyclodextrin cavities

**Table 4**  
Vesicle systems with mechanical triggers listed by type of mechanical trigger including information on treated disease as well as details on example delivery systems and their triggered mechanism.

Mechanical trigger	Type of delivery system	Triggered mechanism	Ref
Shear stress in diseased heart	1,3-diamidophospholipid vesicles	Lenticular vesicles destabilized under shear stress, content release	[23]
Compressive forces across joints in osteoarthritis	Oxidized dextran hydrogel with tethered DPPC/DPPE liposomes	Content release due to mechanosensitive synergy of vesicles and hydrogel	[181]
Physical perturbation of skin	Hollow microneedles with cationic niosomes, microneedle patches integrated with hypoxia-sensitive hyaluronic acid vesicles	Vesicles delivered into skin due to perturbation of stratum corneum	[182,191]
Shear force	Cyclodextrin vesicles as crosslinker for adamantane functionalized cellulose fibers	Host guest complex disassembles and gel dissociates	[183]



**Fig. 10.** Shear stress delivery from a synthetic, mechanosensitive liposome. A, concept overview of a stenosed artery. B, Cryo-TEM of Pad-PC-Pad vesicles showing lenticular morphology. C, Pad-PC-Pad chemical structure. A and B reproduced with permission from [23].

on the surface of the vesicles. Due to the reversibility of the inclusion complexes, the gel shows shear-thinning and self-healing properties. This work could be extended to other hydrogels such as hyaluronic acid hydrogels, which are of interest due to their biocompatibility. Recently Loebel *et al.* have published a protocol for preparing HA hydrogels that are stabilized using the same adamantine–cyclodextrin host–guest interactions, albeit without vesicle moieties [30].

## 7. Summary and outlook

Vesicle constructs for drug delivery have seen significant advances over the last few years. Examples include constructs prepared with bubbles or perfluorinated emulsions which can be combined with clinical ultrasound, and constructs assembled from biologically-derived membranes. While the possibility of leveraging external actuation such as ultrasound or electromagnetic radiation is of great interest scientifically, additional consideration is needed when considering these emerging types of vesicle constructs for clinical translation.

Consider a vesicle engineered to release an anti-cancer drug upon irradiation with light. Upon the design of such a system one should ask what the additional benefit is compared to directly treating the tumor with ionizing radiation (i.e. the well-established clinical practice of radiotherapy [194]). Potential answers could involve the possibility of leveraging synergistic drug–radiotherapy responses [195], or using vesicles that enhance the “abscopal effect” to improve patient outcomes during immunotherapy [196]. A related challenge when using light-activated vesicles is the depth dependency which often becomes an issue when moving from small animal models to large animal models (and eventually patients), although recent reports show that nanophotosensitizers combined with Cerenkov radiation can potentially address this challenge [197].

When designing new types of vesicles for drug delivery and translational purposes there is always an inherent challenge of balancing new (and often exciting) functionality with simplicity and (often less exciting) tried-and-true approaches. This includes the biocompatibility of the vesicles (which involves different considerations for drug delivery systems compared to other biomaterial-based devices [198]) and performing a careful cost–benefit analysis before adding functionality to any vesicles intended for clinical translation [199]. These are multifaceted challenges which are best addressed through inter- and multidisciplinary approaches, for example through “convergent science” [200,201].

Exploring strategies for bridging the gap between more fundamental bio–nano science and translational research remains one of the most

challenging—but also potentially one of the most rewarding—research directions in the field [82]. For example, there is currently intense debate around the concept and clinical relevance of “targeting” [202,203], and the strengths and weaknesses associated with many commonly used animal models [82]. Vesicles that respond to physical stimuli may provide a complementary avenue for exploring these and similar controversial topics. Many of the external triggers associated with these vesicles (e.g. ultrasound and electromagnetic radiation) can enable precise, clinically-relevant triggering mechanisms with very high spatial and temporal resolution, compared to vesicles that are triggered in response to endogenous cues (such as changes in pH or enzyme concentrations) which may display substantial heterogeneity between different tissues, patients and patient populations. Using externally actuated vesicles could therefore potentially help decouple some of the compounding effects that arise due to this biological heterogeneity. As a result, these emerging vesicle technologies may prove instrumental for furthering the development of property–performance relationships and predictive design rules, for advancing fundamental understanding in bio–nano science, and for guiding the development of the next-generation of drug delivery vesicles.

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