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Light-induced molecular rotation triggers on-demand release from liposomes†

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Controllable molecular release from delivery vehicles is essential to successfully reduce drug toxicity and improve therapeutic efficacy. Light-powered hydrophobic molecular motors were therefore incorporated in liposomes to use molecular rotation to facilitate on-demand release. The extent of the release was precisely controlled by irradiation times, providing a simple yet sophisticated responsive molecular nanocarrier.

Within the field of nanomedicine, specialized approaches to transport pharmaceutically active compounds to target sites by means of nanostructures is one of the main goals.1 The use of specialized nanocarriers (NCs) are considered to be highly promising in treating various diseases including combating infections, inflammation, fibrosis, and cancer.2,3 Many nanoparticle systems have been developed over the years for diagnosis and therapy of diseases, which includes solid inorganic particle systems, polymeric nanospheres, polymeric micelles and polymersomes, protein nanoparticles, and lipid-based nanoparticles such as liposomes and lipid nanoparticles (LNPs).2,4–8 A key aspect of NCs is not only to accommodate the drugs but particularly to release them on demand in order to increase local drug concentrations to achieve therapeutic effectiveness, while preventing side effects.5

For on demand drug release from NCs, external triggers or local factors are often envisioned. Local factors that can be exploited are e.g. a change in pH (such as in tumors) or alterations in temperature and pH due to inflammation of the tissue.10 Many stimuli-responsive systems that have been developed are polymer-based, because of the good control over their composition, which is necessary to fine-tune their response to specific stimuli and highly promising drug delivery systems have been developed based on these polymers.11,12

Lipid-based systems are also extremely attractive for triggered release because of ease of preparation, flexibility of design, low immune response, and capability of containing large payloads which facilitates clinical translation.13 Liposomes are often employed for delivery of genetic material (gene delivery),14–16 anti-cancer drugs,17,18 hormones,19 and for imaging purposes.20,21 The lipid-delivery systems can also rely on controlled/triggered-release taking advantage of local factors of the microenvironment in diseased tissue, which is not desirable in case of inter-patient and intra-patient heterogeneity. Hence, triggers that are not purely related to the local environment are being used in the development of nanocarriers, including magnetic fields,22 ultrasound,23 and light.24

Particularly the use of photo-responsive delivery approaches is highly desirable, as these will enable on demand release. Photo-responsive polymersomes have been developed to release molecular payloads,25 including light-triggered nitric oxide release for corneal wound healing.26 Light-dependent release has the disadvantage of potential phototoxicity,27 but if the system allows short enough exposure, phototoxicity can be prevented or reduced. Another disadvantage is that most release mechanisms induce membrane destabilization and membrane stability cannot be recuperated.

As an alternative, we propose a molecular motor (MM) liposome complex that enables light-triggered release through mechanical action without inducing phototoxicity, and allows for controlled step-wise release through reversibility of molecular motion. Most formulations that have been approved in the clinic and are historically much longer investigated, are phospholipid-based structures.4 Previously, light-triggered release using amphiphilic phthalocyanine in conventional liposomes have been designed to release payload using near-infra...
liposomes upon irradiation. Recently, such molecular motor was used to direct stem cell fate. These unidirectional rotary motors function in mixed molecular systems to induce motion. The molecular motor (MM) is hydrophobic and similar to other small hydrophobic components can be stored inside the hydrophobic domain of a phospholipid membrane. Upon irradiation with UV-light, the MM undergoes a photoisomerization around the central double bond that is followed by a thermal relaxation step. Two cycles are needed to complete a full rotation (Supp. Info 1, ESI†) and have been shown to function in mixed molecular systems to induce motion. Liposomes (single unilamellar vesicles, SUVs) with MM were made at phospholipid:MM weight ratios of 50:1 (MM1) and 25:2 (MM2), by means of lipid film rehydration and subsequent extrusion (ESI,† materials and methods). Dynamic light scattering analysis, indicated that all liposomes displayed similar sizes with slightly altered distribution (Fig. 1A). However, the zeta-potential of the non-loaded liposomes and MM1 liposomes was similar while MM2 liposomes are more negatively charged (Fig. 1B), indicating that the molecular motor resides more at the surface due to the deprotonated carboxylates and generates a more negative surface.

The liposomes were loaded with calcein as a model compound as it is very suitable to analyze calcein release from liposomes based on the self-quenching fluorescent properties of calcein. Liposomes were loaded with a calcein solution at a concentration above the self-quenching concentration, which is maintained inside the liposome. Upon release of calcein into its environment as would occur after destabilization of the loaded liposomes, its concentration will decrease causing an increase in fluorescence. Therefore, released calcein will become clearly measurable and distinguishable from the non-released calcein. By measuring the fluorescence intensity over time and comparing it with full release (complete liposome dissolution using detergents), the release (%) over time was determined.

Both control liposomes and MM1 liposomes were subjected to the same treatments and calcein release over time (70 min) was assessed in the absence and presence of irradiation (Fig. 2). MM2 liposomes were not included in the studies as for MM2 without UV-irradiation high leakage was identified (Suppl. Info 2, ESI†) while no significant release was detected for the control liposomes and MM1 liposomes. These results indicate that the low abundance of MM inside the lipid bilayer does not compromise the low permeability of the lipid membrane. Subsequently, calcein-loaded liposomes were irradiated for 30 s at a wavelength of 365 nm. UV
irradiation causes the molecular motors to rotate and induce molecular motion.\textsuperscript{36}

With a single irradiation period of 30 s, the irradiated MM1 liposomes displayed enhanced release compared to the control liposomes. A calcein release up to 10\% was observed within 60–70 min. after which the release leveled off. The partial release indicates that moderate release is facilitated rather than that all content is liberated at once (burst release), which in turn indicates that the molecular motion of the motor inside the lipid bilayer does not result in destruction of the membrane. Restoration of the membrane integrity allowed the liposome to regain its initial stability. The liposomes without MM that were subjected to UV-irradiation did not display any calcein release, indicating that the liposome integrity was not compromised due to the high energetic irradiation (Fig. 2). Therefore, UV-induced local increase in temperature or molecular alterations to the lipid membrane did not induce the calcein release, but it can be attributed to the rotation of the molecular motor.

The quantified release at intermediate time points further exemplify the difference between MM1 liposomes and control liposomes (Supp. Info 3, ESI\textsuperscript{†}). It is clearly distinguishable that most of the release occurs shortly after the irradiation-step and only for the liposomes that have the molecular motor incorporated into the lipid membrane. Release of 1\% is detected for liposomes without the MM inside the membrane. This result indicates that there is a very minor amount of unspecific release, which was not detected in the samples without irradiation. This release indicates that the UV-irradiation influence the system, most like due to (local) heating that facilitates slight permeabilization of the liposome membrane. However, in none of the cases did the UV-irradiation alter the size of the liposomes (Supp. Info 4, ESI\textsuperscript{†}).

In order to identify the extent of control over the calcein release from MM-containing liposomes, also a higher irradiation time was investigated. By increasing the irradiation dosage, either by time or intensity, the molecular motors would provide enhanced molecular rotatory motion. Liposomes from

![Fig. 2 Liposomes without and with MM (1:50, MM1) without irradiation and with irradiation for 30 s of which the calcein release was studied using fluorescence spectroscopy. Measurements are average ± SD of three independent experiments.](image1)

For 30 and 60 s of which the calcein release was studied using fluorescence spectroscopy.

![Fig. 3 Liposomes with MM (1:50) without irradiation and with irradiation for 30 and 60 s of which the calcein release was studied using fluorescence spectroscopy.](image2)
that small molecules (calcein) are released. This release only occurs in the presence of the molecular motor and combined with UV-irradiation. Without either the molecular motor or the UV-irradiation no significant release was found. An increase in irradiation dose produced enhanced calcein release, which indicates that with this relatively simple approach a high degree of control over drug release can be obtained. The incorporation of such an approach is not limited to phospholipid systems but is also envisioned to be compatible with polymer-based NCs. Recently, both two photon excitation using Near-IR irradiation\(^{12}\) and responsiveness to visible light\(^{19}\) have been explored which will improve the biocompatibility of the NCs.

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Conflicts of interest

The authors declare the following competing financial interest(s):

P. V. R is co-founder/scientific advisor/shareholder of BiomACS BV. There are no other conflicts to declare.

Notes and references