GDCh Communication Angewandte International Edition Chemie www.angewandte.org

How to cite: *Angew. Chem. Int. Ed.* **2025**, *64*, e202500824
doi.org/10.1002/anie.202500824

Phospholipid Vesicles

Enzyme Controlled Transient Phospholipid Vesicles for Regulated Cargo Release

*Akhil Venugopal, Subhadip Ghosh, Annalisa Calò, Gian Marco Tuveri, Giuseppe Battaglia, and Mohit Kumar**

Abstract: Metabolism in biological systems involves the continuous formation and breakdown of chemical and structural components, driven by chemical energy. In specific, metabolic processes on cellular membranes result in in situ formation and degradation of the constituent phospholipid molecules, by consuming fuel, to dynamically regulate the properties. Synthetic analogs of such chemically fueled phospholipid vesicles have been challenging. Here we report a bio-inspired approach for the in situ formation of phospholipids, from water soluble precursors, and their fuel driven self-assembly into vesicles. We show that the kinetic competition between anabolic and catabolic-like reactions leads to the formation and enzymatic degradation of the double-tailed, vesicle-forming phospholipid. Spectroscopic and microscopic analysis demonstrate the formation of transient vesicles whose lifetime can be easily tuned from minutes to hours. Importantly, our design results in the formation of uniform sized (65 nm) vesicles simply by mixing the precursors, thus avoiding the traditional complex methods. Finally, our sub-100 nm vesicles are of the right size for application in drug delivery. We have demonstrated that the release kinetics of the incorporated cargo molecules can be dynamically regulated for potential applications in adaptive nanomedicine.

Metabolic processes are essential functions of living systems, where chemical and supramolecular structures are cyclically synthesized (anabolism), by consuming chemical energy, and degraded (catabolism) using chemical reaction networks. Such processes enable the hallmark features of life such as adaptability and spatiotemporal control. Inspired by nature, synthetic supramolecular systems have been developed that continuously consume (chemical-) energy to sustain their structures. This energy input transforms simple precursor molecules into self-assembling components which are inherently unstable and revert to their original forms once the energy input (fuel) ceases, giving them a transient existence and thus temporally controlled. This has resulted in transient self-assembled nanomaterials[1–2] using various fuels,[3] for application in enhanced catalysis, self-erasing inks, transient electronics, multiple helical states etc.[2h,3g,4] However, chemical fuel driven lipid vesicles are rarely investigated, and it would be of considerable interest for active material research and nanomedicine in particular.

The self-assembly of phospholipids (PhLs) into bilayers is the primary process in forming cell membranes. These bilayers are responsible for compartmentalizing chemical potential pools to sustain metabolism and ultimately support life. Synthetic analogs of PhLs have mostly utilized self-assembly of pre-formed PhLs as protocell models, delivery vehicles etc.[5] In contrast, PhLs are formed in situ in cells which provide the dynamic and adaptable nature to cellular membranes. In cells, an anabolic process consumes chemical energy like ATP to form PhLs, whereas the same PhLs are catabolically degraded by the membrane-bound enzymes like lipase.[6] Such metabolic processing of PhLs provides cells with dynamic, spatiotemporal characteristics. In this regard, Fletcher and co-workers have demonstrated the in situ formation of autocatalytic lipid micelles and vesicles,[7] composed mostly of synthetic thioalkanes and not PhLs. Devaraj and co-workers, along with others have reported in situ PhLs synthesis, which finally forms stable vesicles under equilibrium. However, they are not chemically fueled, thus

[*] A. Venugopal, Dr. S. Ghosh, Prof. A. Calò, G. M. Tuveri, Prof. G. Battaglia, Dr. M. Kumar
Institute for Bioengineering of Catalonia (IBEC), Calle Baldiri Reixac 10–12, 08028 Barcelona (Spain)
E-mail: mohit.kumar@ub.edu

A. Venugopal, Prof. A. Calò
Department of Electronic and Biomedical Engineering, University of Barcelona, Calle Marti i Fraquès 1–11, 08028 Barcelona (Spain)

G. M. Tuveri
Department of Condensed Matter Physics, University of Barcelona, Barcelona (Spain)

Prof. G. Battaglia
Catalan Institution for Research and Advanced Studies (ICREA), Barcelona (Spain)

Dr. M. Kumar
Department of Inorganic and Organic Chemistry, University of Barcelona, Calle Marti i Fraquès 1–11, 08028 Barcelona (Spain).

Dr. M. Kumar
Institute de Química Teòrica i Computacional, University of Barcelona, 08028 Barcelona (Spain)

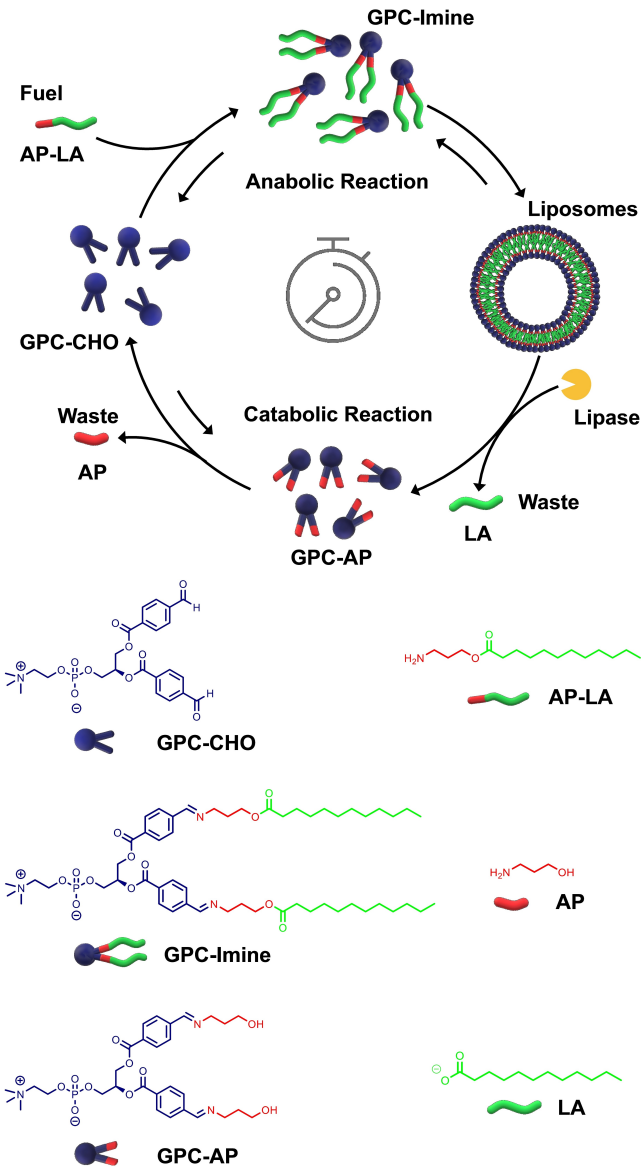
are not cyclically formed and degraded and hence lack temporal regulation.[8–9] They have recently reported an example of fueled, temporally controlled PhL vesicles that demonstrate biological membrane plasticity.[10] However, it requires ester-forming coupling agents, and the catabolic hydrolysis is spontaneous and thus lacks temporal tunability. Hence, there is a need of novel design for the in situ formation of PhLs fueled by chemical energy, to form biomimetic lipid vesicles which can dynamically regulate cargo delivery.

We present an in situ synthesis of biomimetic PhL and their chemical fuel driven self-assembly under physiological conditions, resulting in vesicles with a programmable lifetime. The chemical design introduces an ester bond to form the PhL (anabolic reaction), which spontaneously self-assembles into a vesicle. Furthermore, the simultaneous presence of lipase results in the hydrolysis of the same ester (catabolic reaction) leading to disassembly (Scheme 1). However, in situ ester formation is challenging and usually requires coupling agents,[11] which can have side reactions in the biological milieu. We have avoided it by using the design of amino-ester fuel.[1e] Here, the hydrophilic phosphorylcholine aldehyde (GPC-CHO) reacts with hydrophobic amino-ester fuel (AP-LA) to form an imine bond and thus results in a double tail PhL GPC-Imine containing ester unit (Scheme 1). Furthermore, like in a living cell, the presence of lipase hydrolyses the lipid GPC-Imine to disintegrate the vesicle and regenerate GPC-CHO, and a new cycle starts by utilizing fresh fuel. Additionally, we present a temporal control over the vesicle lifetime where the release kinetics of incorporated cargoes can be dynamically regulated. Enzymatic reaction assisted transient vesicles have been reported by Maiti et al., however, it is with a completely different chemical design of synthetic surfactant, rather than biomimetic PhL.[2h] Thus, we present a novel design of in situ formation of PhLs, from completely water-soluble precursors, and catabolic degradation of PhLs, as a biomimetic approach to form chemically fueled transient vesicles.

The hydrophilic aldehyde derivative GPC-CHO and the aminopropanol ester derivative of lauric acid (AP-LA) as hydrophobic fuel (Scheme 1) were synthesized as described in Scheme S1–S2. The chemical design of fuel was to ensure structural resemblance with biological lipids and to retain water solubility. The amine of the fuel AP-LA reacts with the aldehyde of GPC-CHO to form amphiphilic GPC-Imine that self-assembles into vesicles. Additionally, the presence of lipase hydrolyses the ester bonds of the GPC-Imine, leading to the disassembly of vesicles along with waste formation, resulting in transient PhL vesicles (Scheme 1).

The kinetics of GPC-Imine formation (anabolic) was investigated by adding AP-LA to GPC-CHO at physiological conditions. The initial transparent reaction mixture became turbid within minutes after adding AP-LA, indicating the formation and self-assembly of GPC-Imine (Figure S1). The time-dependent high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis confirmed the formation of double-tailed GPC-Imine with almost complete conversion within 20 minutes along with 5 percent of single-tailed intermediate (Figure 1a, Figure S2–S7). Furthermore, time-dependent ^{1}H NMR showed the disappearance of the aldehyde signal at 10.02 ppm, indicating complete consumption of GPC-CHO within 20 minutes leading to the formation of self-assembled GPC-Imine (Figure 1b). Thus, NMR and HPLC analysis confirmed an almost complete conversion of GPC-CHO into GPC-Imine.

Since HPLC and NMR are time point measurements, we looked at the UV/Vis spectral changes to probe reaction in real time. The absorption spectra of GPC-CHO showed a maximum at 254 nm which upon conversion to GPC-Imine red shifted to 260 nm along with a shoulder at 282 nm (Figure 1c). Thus, a plot of time-dependent absorbance changes at 282 nm showed a gradual increase and plateauing of GPC-Imine concentration within 20 minutes, further confirming the HPLC and NMR analysis (Figure 1d, b).



Scheme 1. Schematic representation of the in situ PhL formation and their transient vesicle assembly along with various chemical structures.

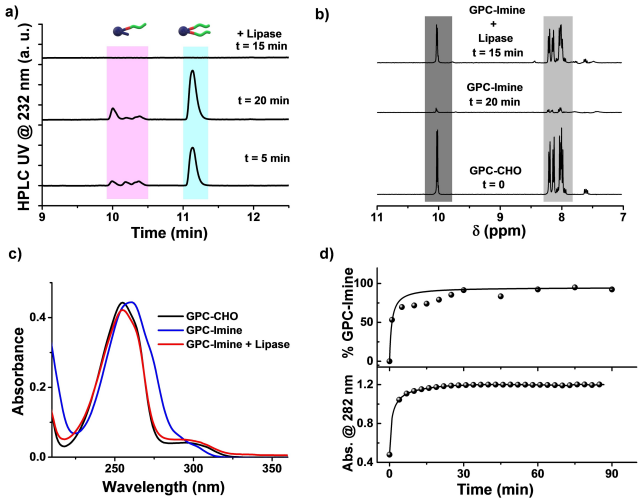


Figure 1. a) Time dependent HPLC analysis of GPC-Imine before and after lipase addition. Multiple peaks for single tailed lipid at ≈10.19 minutes correspond to chemically different possibilities (Figure S4). For HPLC measurement, the GPC-Imine were reduced to their corresponding secondary amine using $NaBH_3CN$. Thus, HPLC detects only the amine form and not the imine. b) time dependent ^1H NMR showing the disappearance and reappearance of GPC-CHO (5 mM GPC-CHO, 15 mM AP-LA). c) Absorption spectra of GPC-CHO, GPC-Imine before and after hydrolysis with lipase, 0.1 mm cuvette. d) Kinetics of GPC-Imine formation obtained from HPLC peak area (top) and UV/Vis absorbance changes at λ = 282 nm (bottom, 1 mm cuvette). Conditions: 1 mM GPC-CHO, 3 mM AP-LA, excess lipase, pH 7.4 HEPES buffer.

Having described the formation kinetics, we probed the degradation (catabolic) reaction through lipase-catalyzed ester hydrolysis of GPC-Imine. The addition of an excess of lipase to preformed GPC-Imine resulted in the disappearance of the GPC- Imine and the reappearance of the GPC-CHO as confirmed by HPLC and NMR (Figure 1a–b). Absorption spectra after lipase addition were also very similar to GPC-CHO (Figure 1c), indicating ester hydrolysis of the GPC-Imine and regeneration of GPC-CHO. Interestingly, we did not observe the GPC-AP intermediate due to the spontaneous conversion of GPC-AP to GPC-CHO via imine hydrolysis (Scheme 1, Figure S8). Therefore, we have confirmed that lipase hydrolysis of the GPC-Imine regenerates GPC-CHO and produces waste.

To further probe the supramolecular transformation, dynamic light scattering (DLS) measurements were performed. The AP-LA solution showed small aggregates below 10 nm (Figure 2a, Figure S9). However, the in situ formation of GPC-Imine resulted in around 65 nm nanostructure, with a very good correlation function, indicating uniform size (PDI = 0.221). Furthermore, lipase-catalyzed degradation of GPC-Imine led to the loss of 65 nm DLS peak, indicating disassembly and the appearance of large aggregates (approx. 3.5 μm). These large aggregates were confirmed to be the crystals of lauric acid (LA) waste generated during hydrolysis (Figure S10). The transmission electron microscopy (TEM) micrographs of self-assembled GPC-Imine confirmed the formation of well-defined vesicles of around 60 nm in diameter. However, the AP-LA alone showed no observable morphology (Figure 2b–c, Figure S11). Moreover, after hydrolysis by lipase, large crystal-

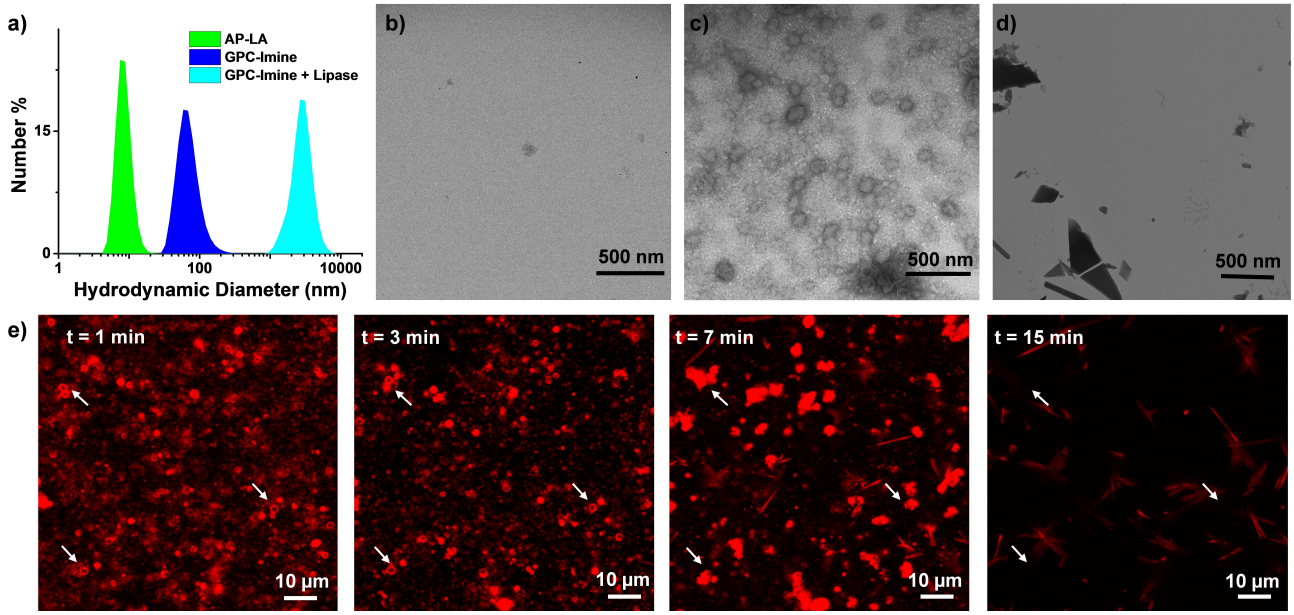


Figure 2. a) DLS data showing the change in hydrodynamic diameter when 5 mM GPC-CHO and 15 mM AP-LA react to form GPC-Imine vesicle and upon addition of 600 U/mL lipase (1 hr of incubation after adding AP-LA and lipase). TEM images of b) Fuel AP-LA; c) GPC-Imine vesicles; d) After addition of lipase to the vesicles. e) Snapshots of confocal video imaging of lipase induced disintegration of vesicles with 2000 U/mL lipase. The white arrows highlight a few vesicles that transform over time (over the 4 images). Conditions: 5 mM GPC-CHO and 15 mM AP-LA.

line structures of waste LA were observed, indicating the complete disintegration of vesicles (Figure 2d).

To understand the supramolecular organization, atomic force microscopy (AFM) was used. Lipid vesicles are known to open into bilayer membranes on mica surface.[12] We observed the formation of ~5 nm thick bilayer membrane (Figure S12), similar to the thickness of biological membranes.[13] The energy minimized structure of GPC-Imine showed a molecular length of ~2.5 nm (Figure S13), indicating that 5 nm is indeed from a bilayer structure. In solution and real-time visualization of these structures using confocal fluorescence microscopy showed complete disintegration of individual vesicles into crystalline structures within 15 minutes. It confirms that initially GPC-Imine forms vesicles that degrade upon enzymatic hydrolysis to generate crystalline LA as waste (Figure 2e, Supporting Information video). Although confocal is limited to the visualization of large structures, it confirms the structural transformation in solution.

We have, thus, demonstrated two independent processes: a) anabolic formation of GPC-Imine vesicle, and b) catabolic lipase-catalyzed hydrolysis and disintegration of GPC-Imine vesicle. Next, for simultaneous formation and disintegration of the vesicle, GPC-CHO, AP-LA, and lipase were premixed initially (Figure 3a, Figure S14–S15). E.g., with 400 U/mL of lipase, we observed a fast formation of GPC-Imine within 10 min, followed by a slow hydrolysis in 100 min. This confirms the autonomous formation and degradation of GPC-Imine, resulting in transient lipid vesicles with a specific lifetime. Furthermore, increasing the concentration of lipase resulted in shorter lifetime of the vesicles (>2 hrs to 20 min for complete hydrolysis), resulting in temporal control (Figure S16).

Finally, we reactivated the system by adding a fresh batch of AP-LA fuel after the first cycle of formation and degradation (60 min), which resulted in regeneration of the GPC-Imine followed by hydrolysis with lipase (Figure 3b, Figure S17). However, damping was observed with each cycle, presumably due to the accumulation of waste, which is common in such closed systems. DLS and TEM analysis demonstrated the formation of vesicles upon refueling. However, these formed multilamellar vesicles due to the co-assembly of hydrophobic waste LA (Figure S18–S22). Altogether, we have demonstrated the formation of GPC-Imine transient vesicles whose lifetime can be tuned by changing enzyme concentration and the self-assembly can be re-activated by adding fresh fuel. It's worth noting that since lipase naturally resides within the hydrophobic membrane,[14] it neither reacts with the fuel nor with the GPC-CHO and the selective hydrolysis of the GPC-Imine is facilitated by the formation of vesicle (Figure S23). We have also utilized the reactivation capability of our lipid vesicles to temporally control the catalytic hydrolysis of a model hydrophobic ester by lipase (Figure S24). Such a reaction is otherwise not possible in aqueous condition and thus highlights the system's potential for transient catalysis.

One of the features of biology is to maintain their non-equilibrium structures by continuous metabolism and by

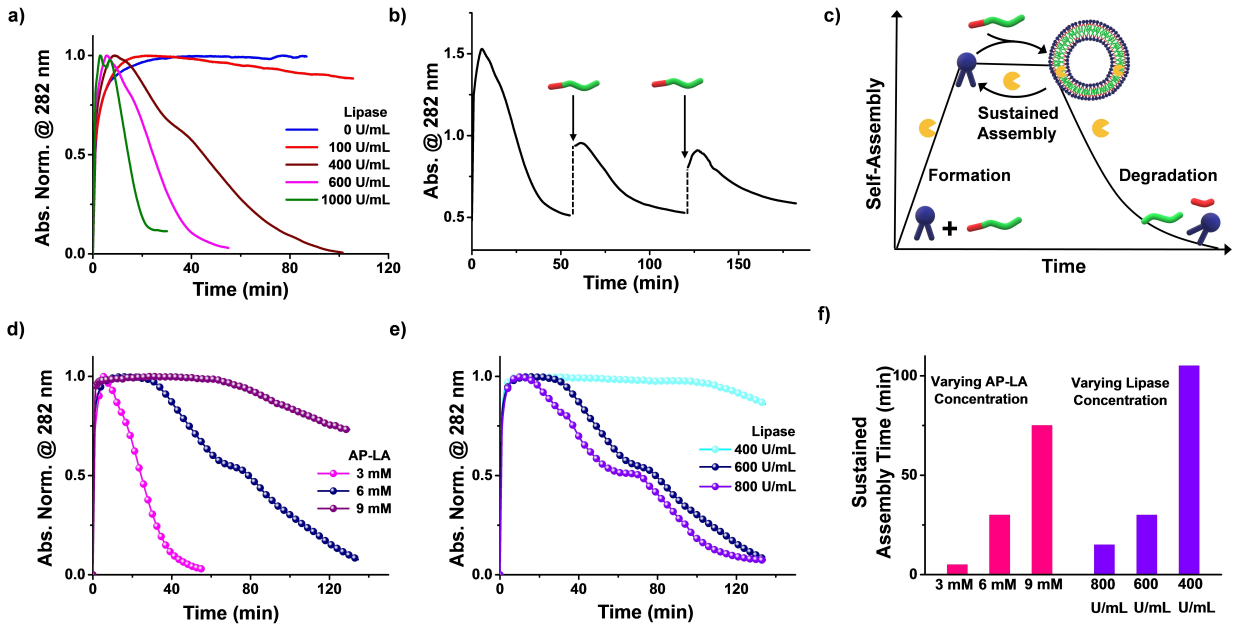


Figure 3. a) Time-dependent absorbance changes in presence of lipase showing the autonomous formation and degradation of GPC-Imine resulting in transient lipid vesicles. b) Refueling the system with fresh batch of AP-LA fuel after first cycle with 1 mM GPC-CHO, 3 mM AP-LA, 600 U/mL lipase. c) Schematic showing the need of constant fuel for sustaining GPC-Imine concentration. Absorbance changes showing sustained GPC-Imine concentration d) by varying AP-LA and fixed lipase concentration at 600 U/mL, e) by varying lipase and fixed AP-LA concentration (6 mM). f) Histogram showing the regulation of time over which GPC-Imine vesicle can be sustained (as obtained from d, e). Conditions: 1 mM GPC-CHO. Small shoulder observed in b) (40 min), d) (70 min), e) (70 min) are due to precipitation of lauric acid crystals (Figure S26). The data in a), d, e) are normalized between 0 and 1 for clear comparison.

constantly consuming fuel. However, synthetic analogs of non-equilibrium steady state (NESS) are seldom reported due to their chemical complexity.[3e,15] Although NESS requires an open system, we aimed to sustain the vesicle by maintaining the concentration of GPC-Imine over time by initially adding an excess of fuel (Figure 3c). Thus, we probed the change in the concentration of GPC-Imine using absorbance over time (Figure 3d, AP-LA 9 mM). We observed a plateau for up to 75 min, indicating a steady concentration. During this period, the concentration of GPC-Imine and, consequently, the self-assembly is maintained even if the individual lipid (GPC-Imine) is constantly degraded and replaced by new ones. Additionally, increasing the fuel concentration at fixed lipase concentration and vice versa resulted in controlling the time over which vesicle could be sustained from 5 min to 75 minutes and 105 min to 10 min, respectively (Figure 3d–e, Figure S25). Thus, we have demonstrated that our chemically fueled vesicles can be sustained over time with easily controllable lifetime (Figure 3f).

It is to be highlighted that in our design, the vesicles are formed by simply mixing the water-soluble precursors (GPC-CHO + AP-LA), leading to the in situ formation of PhL GPC-Imine that self-assembles into vesicles of controlled dispersity (PDI = 0.221). Additionally, our nanovesicles, with an average size of approx. 65 nm, are optimal for drug delivery applications. In contrast, traditionally, vesicles formed with PhL require complex methods like film rehydration, solvent switch, etc., to result in polydisperse, multilamellar vesicles that need further extrusion procedures to obtain desirable size.[16] Thus, our vesicle design is highly advantageous which could be due to the in situ formation of the PhL GPC-Imine, which self-assembles into uniform vesicles. Therefore, we aimed to demonstrate the application of our chemically fueled vesicles for dynamically regulated cargo release. We have chosen Nile red as a model hydrophobic cargo, which was localized within the hydrophobic bilayer of the GPC-Imine vesicle by simple mixing (Figure S27).

The localization of Nile red on the vesicle membrane was characterized by enhanced fluorescence, and the release was monitored by probing the decrease in fluorescence.[17] We observed that GPC-Imine vesicles in their equilibrium state (i.e., without lipase) did not release the cargo, as seen by no change in fluorescence, confirming the impermeable nature of the vesicle (Figure 4). However, in the presence of lipase, the chemically fueled GPC-Imine vesicle showed a continuous decrease in fluorescence intensity, indicating cargo (Nile red) release. Furthermore, increasing the lipase concentration from 400 to 1000 U/mL resulted in faster release kinetics. Thus, we have demonstrated that the fuel-driven vesicles can be used to release the hydrophobic dye, where the release kinetics can be regulated by the rate of enzymatic reaction. Furthermore, our in vitro cargo release experiments demonstrated that GPC-Imine vesicles facilitate controlled release of cargo (Nile red) to HeLa cells without significant cytotoxicity (Figure S28). Finally, we have also encapsulated hydrophilic dye, pyranine, within the lumen of the GPC-Imine vesicles and examined its release.

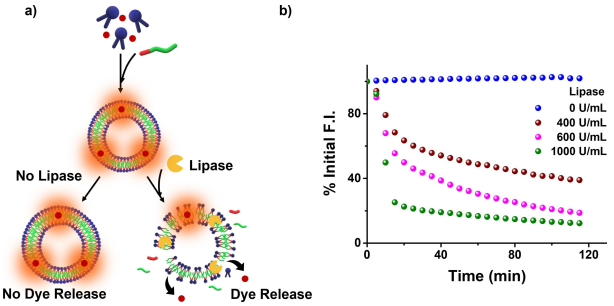


Figure 4. a) Schematic showing the localization of hydrophobic cargo, Nile Red, and its release in presence and absence of lipase. b) Release profile of hydrophobic Nile Red from the chemically fueled vesicles. Conditions: 1 mM GPC-CHO, 3 mM AP-LA and 0.25 mM Nile red.

Our results demonstrate that hydrophilic cargos can also be encapsulated within our vesicles, and their release kinetics can be controlled by varying the concentration of lipase (Figure S29). Taken together, these results demonstrate the potential of our system for temporally regulated drug delivery in nanomedicine.

In summary, we have presented a fuel-driven dissipative self-assembly of PhL into transient vesicles with a controllable lifetime. In situ formation of PhL through an imine bond with fuel and lipase-catalyzed ester hydrolysis led to the anabolic formation and subsequent catabolic degradation of vesicles, respectively. This resulted in transient vesicles whose lifetime could be easily tuned by varying the concentration of the fuel or the lipase. Most interestingly, the uniformly sized ~65 nm vesicles were formed autonomously by simply mixing the precursors, therefore avoiding the traditional lengthy process of film rehydration, membrane extrusion etc.[16] These optimally sized vesicles were used to encapsulate model cargoes and the release kinetics could be dynamically regulated by the enzyme concentration. Thus, our biomimetic design of metabolic PhL into uniform vesicles, combined with the ease of vesicle formation and controlled cargo release, makes it an ideal candidate for adaptive nanomedicine. Furthermore, since lipase is overexpressed in many inflammatory diseases, our lipase-regulated cargo release will be suitable for targeted therapy.

Supporting Information

The authors have cited additional references within the Supporting Information.[18–19]

Acknowledgements

MK acknowledges funding from “la Caixa” Foundation (ID 100010434, fellowship code LCF/BQ/PI21/11830035) and the Marie Skłodowska-Curie grant agreement No 847648 and Ramon y Cajal fellowship (RYC2021-035016-I) from the Spanish ministry of science and innovation (MICIU). Additional funding from Proyectos de Generación de Conoci-

miento grant (PID2021-126244NA-I00: PI-MK, PID2020-119914RB-I00: PI-GB), the Agencia Estatal de Investigación for the María de Maeztu CEX2021-001202-M. GB thanks the ERC-CoG CheSSTAG #769798 and PoC-MAIN #101123468 to partially support AV salary and research. GMT thanks the MICIU for FPI studentship (PID2020-119914RB-I00). We thank the Agencia de Gestión de Ayudas Universitarias y de Investigación (AGAUR) for the grant SGR 01538 and for SG fellowship (2022 BP 00214). AC acknowledges the Foundation "Bartolo Longo III Millennio" for financial support. We thank B.V.V.S Pavan Kumar for useful discussion.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: supramolecular chemistry · phospholipids · vesicles · systems chemistry · transient assembly

[1] a) J. Boekhoven, A. M. Brizard, K. N. Kowlgi, G. J. Koper, R. Eelkema, J. H. van Esch, *Angew. Chem. Int. Ed.* **2010**, *49*, 4825–4828; b) J. Boekhoven, W. E. Hendriksen, G. J. M. Koper, R. Eelkema, J. H. van Esch, *Science* **2015**, *349*, 1075–1079; c) R. Klajn, P. J. Wesson, K. J. Bishop, B. A. Grzybowski, *Angew. Chem. Int. Ed.* **2009**, *48*, 7035–7039; d) I. Lagzi, B. Kowalczyk, D. Wang, B. A. Grzybowski, *Angew. Chem. Int. Ed.* **2010**, *49*, 8616–8619; e) A. Jain, S. Dhiman, A. Dhayani, P. K. Vemula, S. J. George, *Nat. Commun.* **2019**, *10*, 450; f) M. Sawczyk, R. Klajn, *J. Am. Chem. Soc.* **2017**, *139*, 17973–17978; g) S. P. Afrose, S. Bal, A. Chatterjee, K. Das, D. Das, *Angew. Chem. Int. Ed.* **2019**, *58*, 15783–15787; h) N. Singh, A. Lopez-Acosta, G. J. M. Formon, T. M. Hermans, *J. Am. Chem. Soc.* **2022**, *144*, 410–415.

[2] a) T. M. Hermans, N. Singh, *Angew. Chem. Int. Ed.* **2023**, *62*, e202301529; b) S. Yu, S. Xian, Z. Ye, I. Pramudya, M. J. Webber, *J. Am. Chem. Soc.* **2021**, *143*, 12578–12589; c) B. Su, T. Chi, Z. Ye, Y. Xiang, P. Dong, D. Liu, C. J. Addonizio, M. J. Webber, *Angew. Chem. Int. Ed.* **2023**, *62*, e202216537; d) C. Donau, F. Spath, M. Sosson, B. A. K. Kriebisch, F. Schnitter, M. Tena-Solsona, H. S. Kang, E. Salibi, M. Sattler, H. Mutschler, J. Boekhoven, *Nat. Commun.* **2020**, *11*, 5167; e) C. Wanzke, M. Tena-Solsona, B. Rieß, L. Tebcharani, J. Boekhoven, *Mater. Horiz.* **2020**, *7*, 1397–1403; f) S. Debnath, S. Roy, R. V. Ulijn, *J. Am. Chem. Soc.* **2013**, *135*, 16789–16792; g) M. Kumar, D. Sementa, V. Narang, E. Riedo, R. V. Ulijn, *Chem. Eur. J.* **2020**, *26*, 8372–8376; h) S. Maiti, I. Fortunati, C. Ferrante, P. Scrimin, L. J. Prins, *Nat. Chem.* **2016**, *8*, 725–731.

[3] a) L. Heinen, T. Heuser, A. Steinschulte, A. Walther, *Nano Lett.* **2017**, *17*, 4989–4995; b) G. Fusi, D. Del Giudice, O. Skarsetz, S. Di Stefano, A. Walther, *Adv. Mater.* **2023**, *35*, e2209870; c) B. Klemm, R. W. Lewis, I. Piergentili, R. Eelkema, *Nat. Commun.* **2022**, *13*, 6242; d) A. Joseph, C. Contini, D. Cecchin, S. Nyberg, L. Ruiz-Perez, J. Gaitzsch, G. Fullstone, X. Tian, J. Azizi, J. Preston, G. Volpe, G. Battaglia, *Sci. Adv.* **2017**, *3*, e1700362; e) A. Sorrenti, J. Leira-Iglesias, A. Sato, T. M. Hermans, *Nat. Commun.* **2017**, *8*, 15899; f) D. Barpuzary, P. J. Hurst, J. P. Patterson, Z. Guan, *J. Am. Chem. Soc.* **2023**, *145*, 3727–3735; g) K. Liu, A. Blokhuis, C. van Ewijk, A. Kiani, J. Wu, W. H. Roos, S. Otto, *Nat. Chem.* **2024**, *16*, 79–88.

[4] a) R. Chen, S. Neri, L. J. Prins, *Nat. Nanotechnol.* **2020**, *15*, 868–874; b) S. Pal, A. Reja, S. Bal, B. Tikader, D. Das, *Angew. Chem. Int. Ed.* **2022**, *61*, e202111857; c) M. Kumar, N. L. Ing, V. Narang, N. K. Wijerathne, A. I. Hochbaum, R. V. Ulijn, *Nat. Chem.* **2018**, *10*, 696–703; d) S. Bal, C. Ghosh, T. Ghosh, R. K. Vijayaraghavan, D. Das, *Angew. Chem. Int. Ed.* **2020**, *59*, 13506–13510; e) S. Yang, G. Schaeffer, E. Mattia, O. Markovitch, K. Liu, A. S. Hussain, J. Ottele, A. Sood, S. Otto, *Angew. Chem. Int. Ed.* **2021**, *60*, 11344–11349; f) S. Dhiman, A. Jain, S. J. George, *Angew. Chem. Int. Ed.* **2017**, *56*, 1329–1333; g) S. Dhiman, A. Jain, M. Kumar, S. J. George, *J. Am. Chem. Soc.* **2017**, *139*, 16568–16575; h) H. Che, S. Cao, J. C. M. van Hest, *J. Am. Chem. Soc.* **2018**, *140*, 5356–5359; i) E. Del Grosso, G. Ragazzon, L. J. Prins, F. Ricci, *Angew. Chem. Int. Ed.* **2019**, *58*, 5582–5586; j) E. Del Grosso, E. Franco, L. J. Prins, F. Ricci, *Nat. Chem.* **2022**, *14*, 600–613.

[5] a) K. A. Podolsky, N. K. Devaraj, *Nat. Chem. Rev.* **2021**, *5*, 676–694; b) I. A. Chen, P. Walde, *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a002170; c) A. M. Wagner, H. Eto, A. Joseph, S. Kohyama, T. Haraszti, R. A. Zamora, M. Vorobii, M. I. Giannotti, P. Schwille, C. Rodriguez-Emmenegger, *Adv. Mater.* **2022**, *34*, e2202364; d) J. Lou, J. A. Schuster, F. N. Barrera, M. D. Best, *J. Am. Chem. Soc.* **2022**, *144*, 3746–3756; e) C. Pernpeintner, J. A. Frank, P. Urban, C. R. Roeske, S. D. Pritzl, D. Trauner, T. Lohmuller, *Langmuir* **2017**, *33*, 4083–4089; f) A. Joseph, A. M. Wagner, M. Garay-Sarmiento, M. Aleksanyan, T. Haraszti, D. Soder, V. N. Georgiev, R. Dimova, V. Percec, C. Rodriguez-Emmenegger, *Adv. Mater.* **2022**, *34*, e2206288.

[6] R. A. Coleman, D. G. Mashek, *Chem. Rev.* **2011**, *111*, 6359–6386.

[7] a) I. Colomer, A. Borissov, S. P. Fletcher, *Nat. Commun.* **2020**, *11*, 176; b) A. H. J. Engwerda, J. Southworth, M. A. Lebedeva, R. J. H. Scanes, P. Kukura, S. P. Fletcher, *Angew. Chem. Int. Ed.* **2020**, *59*, 20361–20366; c) M. G. Howlett, A. H. J. Engwerda, R. J. H. Scanes, S. P. Fletcher, *Nat. Chem.* **2022**, *14*, 805–810; d) M. G. Howlett, S. P. Fletcher, *Nat. Chem. Rev.* **2023**, *7*, 673–691; e) E. A. J. Post, S. P. Fletcher, *Chem. Sci.* **2020**, *11*, 9434–9442.

[8] a) J. Flores, R. J. Brea, A. Lamas, A. Fracassi, M. Salvador-Castell, C. Xu, C. R. Baiz, S. K. Sinha, N. K. Devaraj, *Angew. Chem. Int. Ed.* **2022**, *61*, e202200549; b) A. Seoane, R. J. Brea, A. Fuertes, K. A. Podolsky, N. K. Devaraj, *J. Am. Chem. Soc.* **2018**, *140*, 8388–8391.

[9] Y. Zhou, H. Yang, C. Wang, Y. Xue, X. Wang, C. Bao, L. Zhu, *Chem. Sci.* **2021**, *12*, 3627–3632.

[10] A. Fracassi, A. Seoane, R. Brea, N. Devaraj, *ChemRxiv.* **2024**, 10.26434/chemrxiv-22024-x26437rkm.

[11] a) P. S. Schwarz, M. Tena-Solsona, K. Dai, J. Boekhoven, *Chem. Commun.* **2022**, *58*, 1284–1297; b) C. Wanzke, A. Jussupow, F. Kohler, H. Dietz, V. R. I. Kaila, J. Boekhoven, *ChemSystemsChem* **2019**, *2*, e1900044.

[12] Z. Lv, S. Banerjee, K. Zagorski, Y. L. Lyubchenko, in *Nanoscale Imaging: Methods and Protocols* (Ed.: Y. L. Lyubchenko), Springer New York, New York, NY, **2018**, pp. 129–143.

[13] O. G. Mouritsen, M. Bloom, *Biophys. J.* **1984**, *46*, 141–153.

[14] P. Reis, K. Holmberg, H. Watzke, M. E. Leser, R. Miller, *Adv. Colloid Interface Sci.* **2009**, *147–148*, 237–250.

[15] A. M. Bergmann, J. Bauermann, G. Bartolucci, C. Donau, M. Stasi, A. L. Holtmannspotter, F. Julicher, C. A. Weber, J. Boekhoven, *Nat. Commun.* **2023**, *14*, 6552.

[16] E. Rideau, F. R. Wurm, K. Landfester, *Adv. Biosys.* **2019**, *3*, e1800324.
[17] A. M. Bayer, S. Alam, S. I. Mattern-Schain, M. D. Best, *Chem. Eur. J.* **2014**, *20*, 3350–3357.
[18] a) C. B. Minkenberg, F. Li, P. van Rijn, L. Florusse, J. Boekhoven, M. C. Stuart, G. J. Koper, R. Eelkema, J. H. van Esch, *Angew. Chem. Int. Ed.* **2011**, *50*, 3421–3424; b) Y. Yamano, K. Tsuboi, Y. Hozaki, K. Takahashi, X. H. Jin, N. Ueda, A. Wada, *Bioorg. Med. Chem.* **2012**, *20*, 3658–3665; c) A. Calò, M. Sanmartí-Espinal, P. Iavicoli, M.-A. Persuy, E. Pajot-Augy, G. Gomila, J. Samitier, *Soft Matter* **2012**, *8*, 11632; d) A. Venugopal, L. Ruiz-Perez, K. Swamynathan, C. Kulkarni, A. Calo', M. Kumar, *Angew. Chem. Int. Ed.* **2023**, *62*, e202208681.
[19] a) A. D. Becke, *J. Chem. Phys.* **1992**, *96*, 2155–2160; b) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789; c) P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, *J. Phys. Chem.* **1994**, *98*, 11623–11627; d) F. Weigend, R. Ahlrichs, *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297–3305.

Manuscript received: January 10, 2025
Accepted manuscript online: February 12, 2025
Version of record online: February 21, 2025

[Correction added on 11 March 2025, after first online publication: Figure 4 has been updated, Supporting Video has been insert.]