Phototherapeutic release of carboxyfluorescein from liposomal carriers

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Introduction

Background Light targeted drug delivery allows for increased control of the locus, timing, and dosage of delivered treatment, making it safer and more affective than systematically administered drugs[2,3]. This protocol uses a photolytic trigger attached to the surface of liposomes[1]. Upon illumination by 525 nm light, the lytic agent, Melittin, is released from the blocking segment to release the contained carboxyfluorescein to measure phototherapeutic release. Optimization of this protocol will allow for tPA-loaded liposomes to be assayed in an *in vitro* setting.

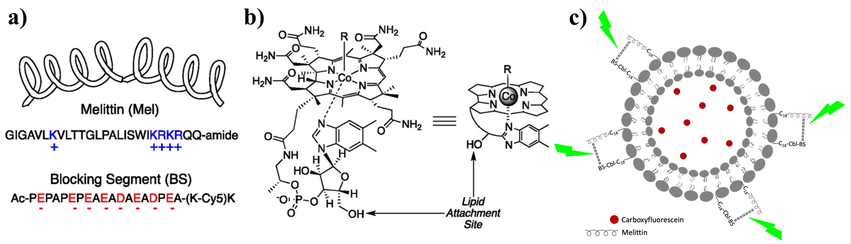
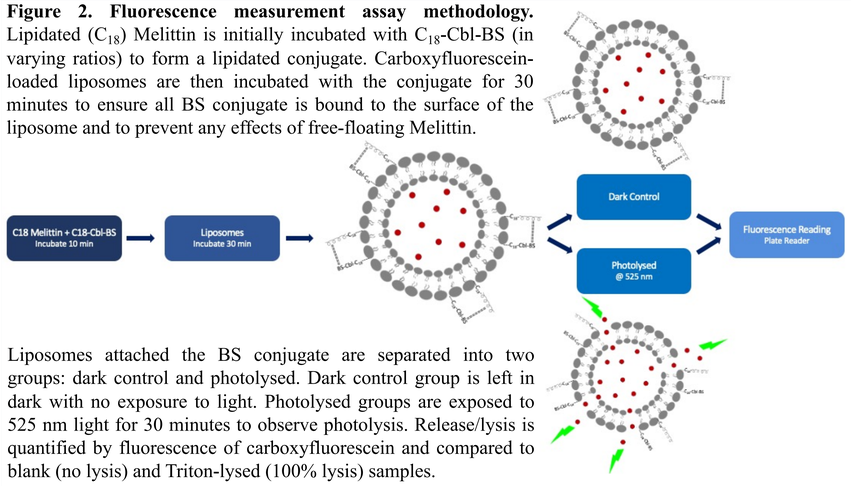


Figure 1. Elements of photorelease system[1]. (a) Biochemical composition of lytic agent, Melittin, and blocking segment (BS). **(b)** Cobalamin (Cbl) synthetically modified with lipid and BS peptide appended as photocleavable ligand to Co. **(c)** Liposomal attachment of C_{18}-Cbl-BS conjugate allows for photolytic control of liposomes by Melittin upon illumination by 525 nm light by release of the BS from the Cbl core, resulting in cleavage of liposomes by Melittin.

Methodology

Figure 2. Fluorescence measurement assay methodology. Lipidated (C_{18}) Melittin is initially incubated with C_{18}-Cbl-BS (in varying ratios) to form a lipidated conjugate. Carboxyfluorescein-loaded liposomes are then incubated with the conjugate for 30 minutes to ensure all BS conjugate is bound to the surface of the liposome and to prevent any effects of free-floating Melittin.



Liposomes attached the BS conjugate are separated into two groups: dark control and photolysed. Dark control group is left in dark with no exposure to light. Photolysed groups are exposed to 525 nm light for 30 minutes to observe photolysis. Release/lysis is quantified by fluorescence of carboxyfluorescein and compared to blank (no lysis) and Triton-lysed (100% lysis) samples.

C_{18}-Mel:C_{18}-Cbl-BS Dependency

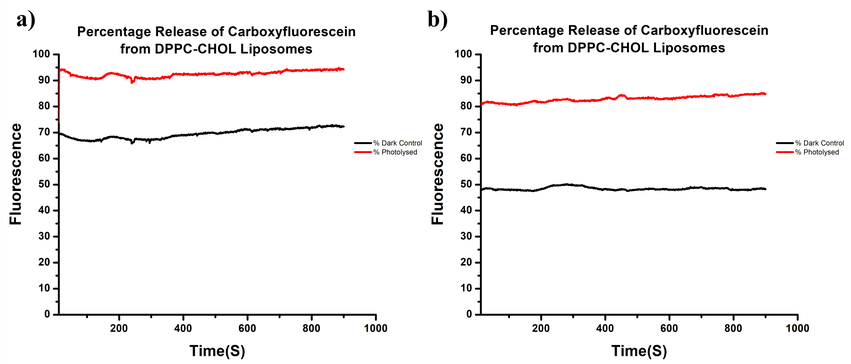


Figure 3. Percent lysis in varied ratios of lipidated Melittin to Cbl BS. (a) 1:2 ratio of C_{18}-Mel:C_{18}-Cbl-BS with final concentrations of 5 uM to 10 uM. **(b)** 1:3 ratio of C_{18}-Mel:C_{18}-Cbl-BS with final concentrations of 5 uM to 15 uM. 1:3 ratio of C_{18}-Mel:C_{18}-Cbl-BS exhibits larger difference in percent lysis between dark control and photolysed sample due to decreased activity of free-floating C_{18}-Melittin in solution.

DMSO and 525 nm Light Dependency

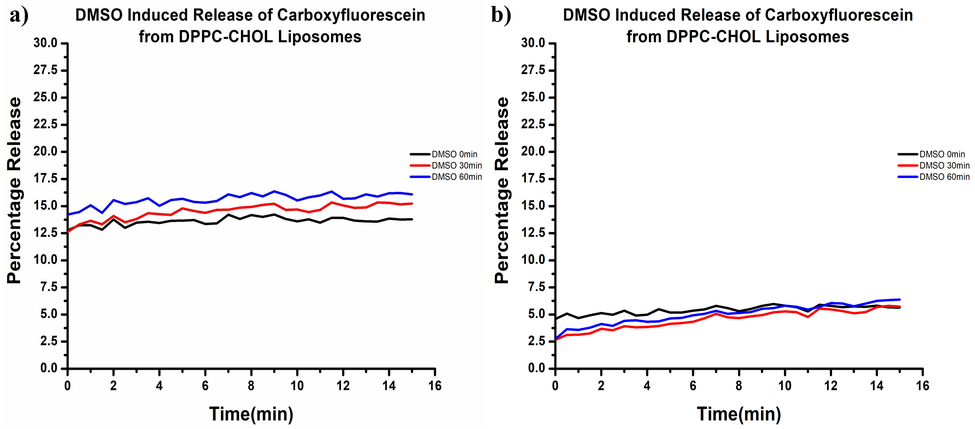


Figure 4. DMSO induced lysis at varying concentrations and timepoints. a) DMSO induced lysis of DPPC-Chol liposomes at 0-, 30-, and 60-minute time point at [DMSO] = 15%. **b)** DMSO induced lysis of DPPC-Chol liposomes at 0-, 30-, and 60-minute time points at [DMSO] = 5%. [DMSO] = 5% was determined as maximum concentration of DMSO to match blank lysis levels.

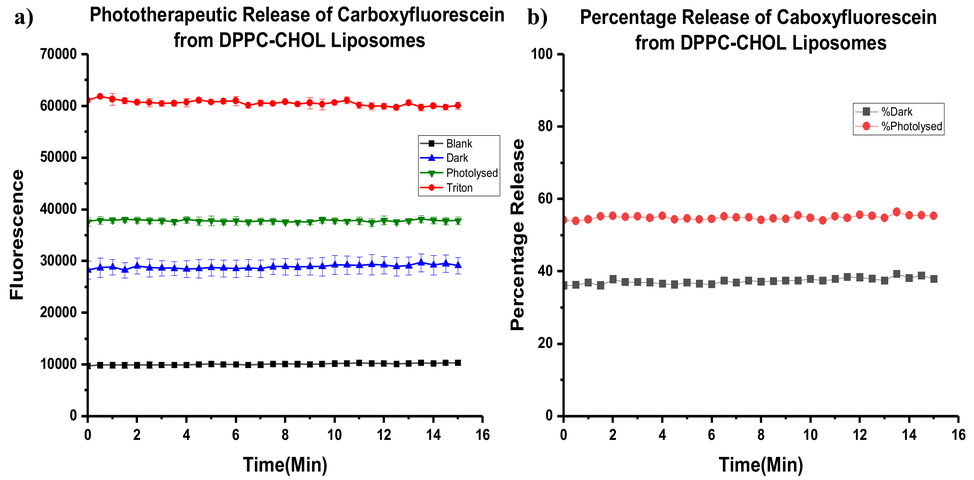


Figure 5. Phototherapeutic release of carboxyfluorescein at a 1:2 ratio of C_{18}-Mel:C_{18}-Cbl-BS with 2.5% DMSO. (a) Time dependent release of blank (no lysis) and Triton-lysed (100% lysis) compared to dark control (no light exposure) and photolysed (exposed to 525 nm light) liposome samples. **(b)** Percent release from dark control and photolysed liposome samples calculated by subtracting blank lysis from both samples and determine the percent out of Triton lysis.

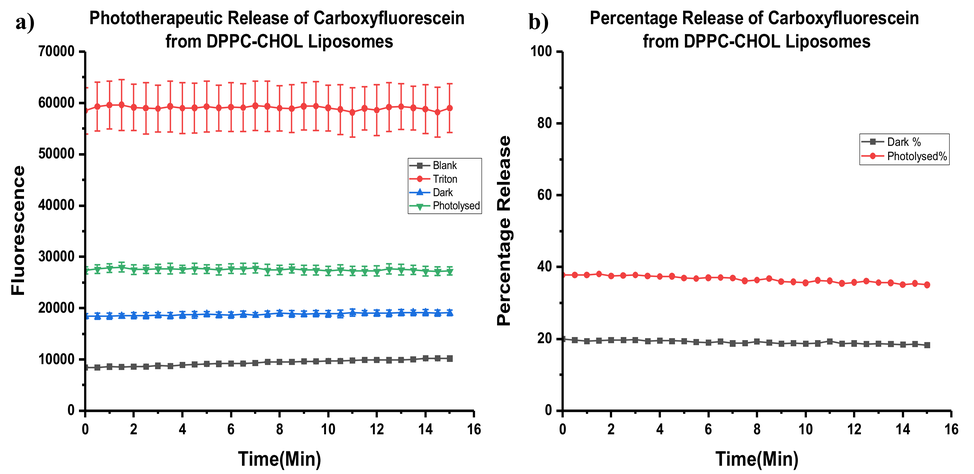


Figure 6. Phototherapeutic release of carboxyfluorescein at a 1:3 ratio of C_{18}-Mel:C_{18}-Cbl-BS with 2.5% DMSO. (a) Time dependent release of blank (no lysis) and Triton-lysed (100% lysis) compared to dark control (no light exposure) and photolysed (exposed to 525 nm light) liposome samples. **(b)** Percent release from dark control and photolysed liposome samples. Lysis is likely less than that of Figure 5 due to lower amount of free-floating C_{18}-Melittin.

Dark and Photolytic Conditions

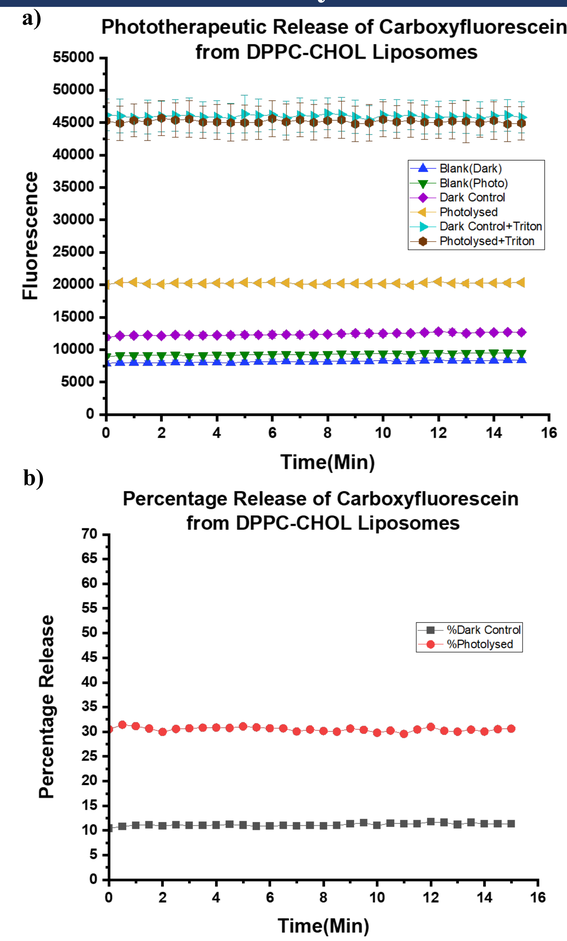


Figure 7. Dark- and light- controlled release of carboxyfluorescein; 1:3 ratio of C_{18}-Mel:C_{18}-Cbl-BS with final concentration of 5 uM to 15 uM. (a) *Blank (Dark)*, blank sample kept in the dark; *Blank (Photo)*, blank exposed to 525 nm light; *Dark Control*, liposomes with photo-trigger kept in the dark; *Photolysed*, liposomes with photo-trigger exposed to 525 nm light for 30 min; *Dark Control + Triton*, sample treated identical to Dark control and lysed with Triton; *Photolysed + Triton*, sample treated identical to Photolysed and lysed with Triton. **(b)** Percent release of carboxyfluorescein indicates ~20% difference in lysis between *Dark Control* and *Photolysed* liposome samples.

Conclusions and Future Research

Major Conclusions:

(1) [DMSO] ≤ 5% is ideal to prevent DMSO-induced lysis of liposomes

(2) A 1:3 ratio of C_{18}-Mel:C_{18}-Cbl-BS yields 30% photolysis of liposomes while achieving a dark control with almost identical lysis to initial blank liposome samples

Future Research:

(1) Further optimize liposome formulation that yields best stability (least passive release) and best release when treated with phototherapeutic agents and light

(2) Use tPA-loaded liposomes in an *in vitro* assay to mitigate induced thrombolytic clots

Acknowledgements

1. Vickerman, B. M.; O'Banion, C. P.; Tan, X.; Lawrence, D. S. Light-Controlled Release of Therapeutic Proteins from Red Blood Cells. *ACS Cent. Sci.* **2021**, 7, 93-103, DOI: 10.1021/acscentsci.0c01151
2. Zamanlu M, Farhoudi M, Eskandani M, Mahmoudi J, Barar J, Rafi M, Omidi Y. Recent advances in targeted delivery of tissue plasminogen activator for enhanced thrombolysis in ischaemic stroke. J Drug Target. **2018** ;26(2):95-109. Epub 2017/08/11. doi: 10.1080/1061186X.2017.1365874. PubMed PMID: 28796540.
3. Koudelka S, Mikulik R, Masek J, Raska M, Turanek Knotigova P, Miller AD, Turanek J. Liposomal nanocarriers for plasminogen activators. J Control Release. **2016**; 227:45-57. Epub 2016/02/16. doi: 10.1016/j.jconrel.2016.02.019. PubMed PMID: 26876783.