Liposomal Delivery of the STING Agonist diABZI: Formulation, Characterization, and Induction of Type I Interferon Responses in Mouse and Human Tumor-Immune Models

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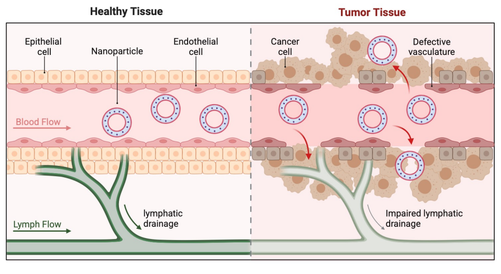
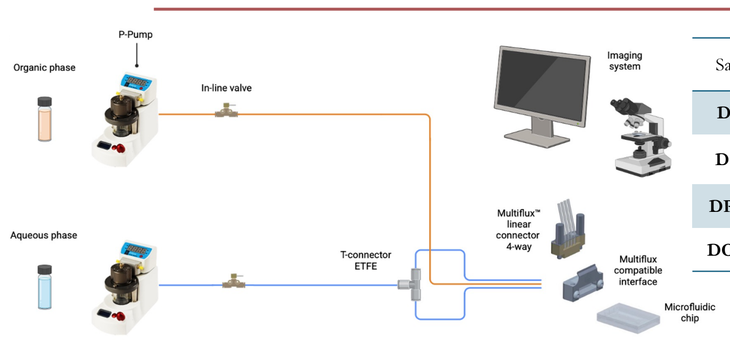
INTRODUCTION



diABZI (compound-3) is a selective agonist of the Stimulator of Interferon Genes (STING), capable of activating innate immune responses through the induction of type I interferons and pro-inflammatory cytokines, thereby promoting antitumor immunity[1]. Although STING agonists have emerged as promising immunotherapeutic agents for both hematological and solid malignancies their clinical application is limited by suboptimal tumor delivery and systemic adverse effects[2]. To overcome these liminations, diABZI was encapsulated into both DPPC- and pH-sensitive DOPE-based liposomal formulations.

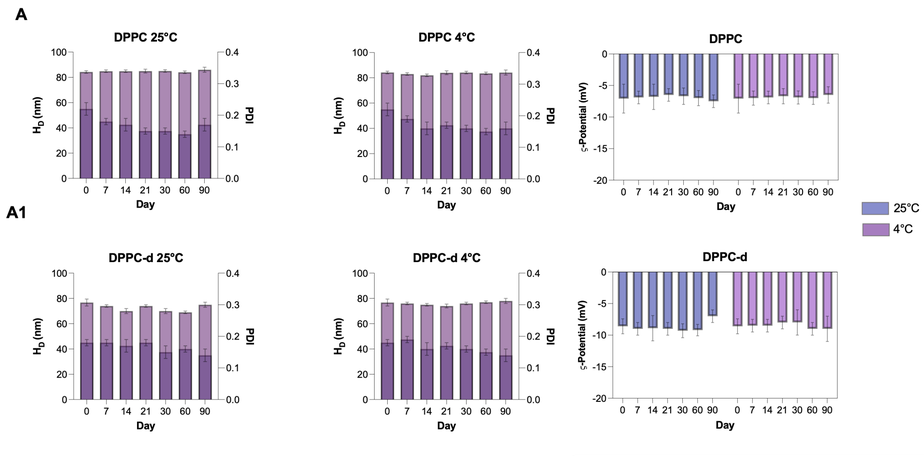
METHODS

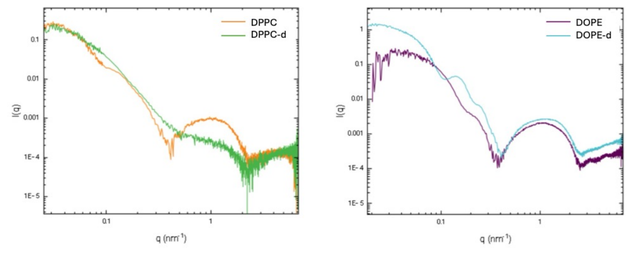


Sample	H_D (nm) ± SD	PDI	ζ-Potential (mV) ± SD	EE (%)
DPPC	84 ± 1	0.22	-7 ± 2	-
DOPE	91 ±	0.17	-5 ± 1	-
DPPC-d	113 ± 1	0.11	-9 ± 1	100%
DOPE-d	77 ± 3	0.18	-13 ± 4	50%

RESULTS

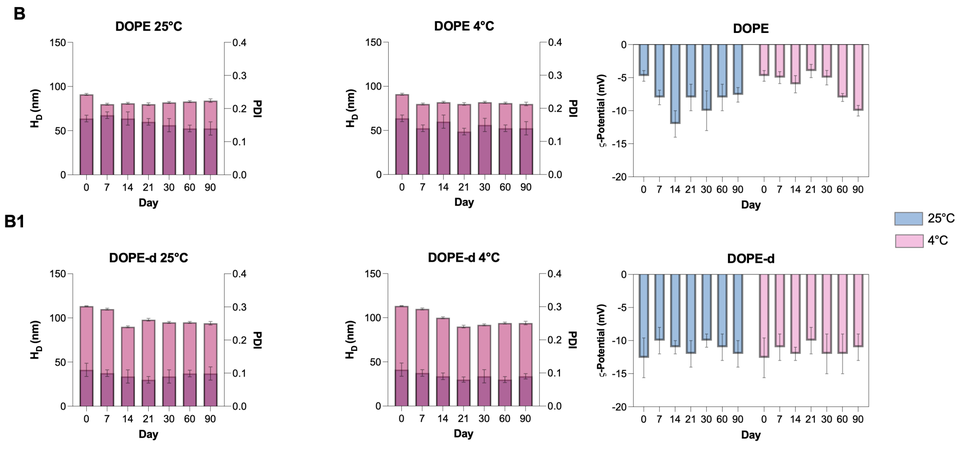
Stability Studies and SAXS analysis





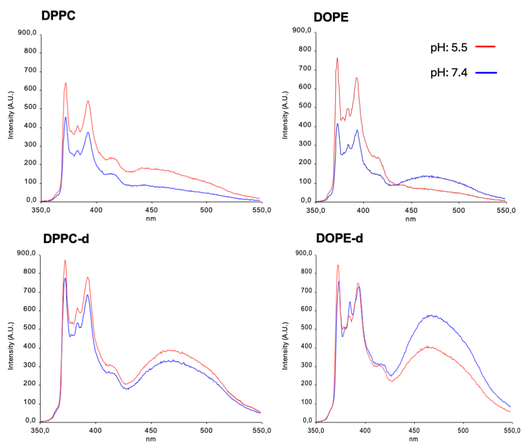
diABZI effect:

- **DPPC-d**: more evident changes in the 0.4–2 nm^{-1} range → possible changes in electron-density contrast and/or slight membrane reorganization
- **DOPE-d**: minor impact on membrane architecture



pH-sensitivity Evaluation

Bilayer features

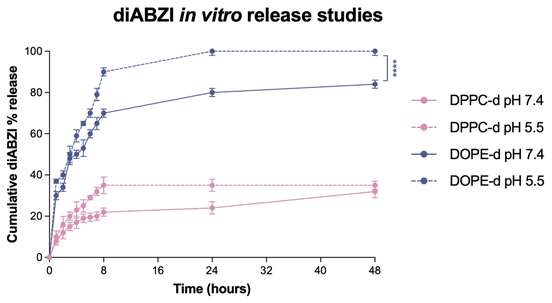


Sample	pH	Anisotropy (∝1/Fluidity)
DPPC	7.4	0.29
	5.5	0.31
DOPE	7.4	0.20
	5.5	0.18
DPPC-d	7.4	0.32
	5.5	0.29
DOPE-d	7.4	0.24
	5.5	0.18

DPPC-d → ≈ unchanged microviscosity
Increased vesicle size in DOPE-d may provide additional space for diABZI and pyrene accommodation.

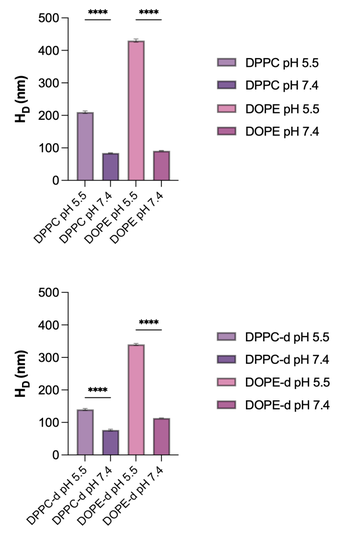
DOPE-d → ↑ microviscosity
Acidic pH induces spectral changes in DOPE and DOPE-d, consistent with the fusogenic behavior of DOPE.

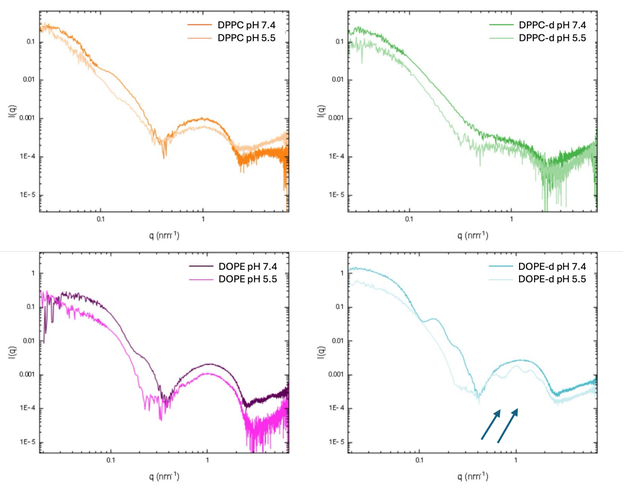
diABZI *in vitro* release studies



The release profile shows a clear pH-dependent behavior of DOPE-d formulation. Under acidic conditions, a rapid increase in release was observed, followed by a plateau, whereas under neutral conditions, the release remained substantially lower.

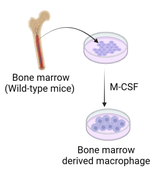
Effect of buffer pH on vesicle size





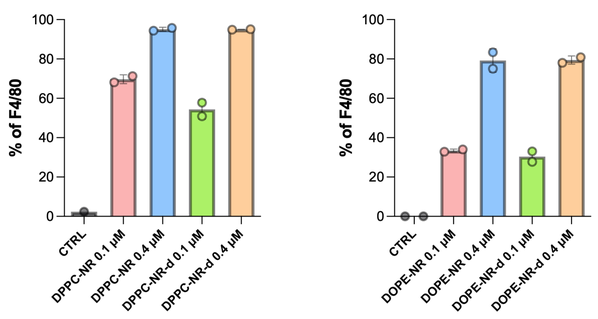
pH has a limited effect on DPPC and DPPC-d. It induces only minor changes in the local bilayer organization of DOPE, potentially affecting the electron density distribution, membrane hydration, or compound–membrane interactions, without disrupting the overall liposomal architecture. The drug-loaded system is therefore more responsive to acidification.

In vitro evaluation



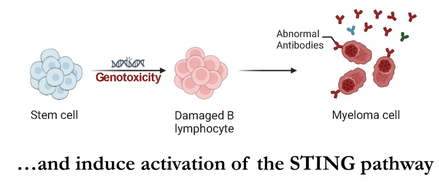
1 Bone Marrow Derived Macrophages (BMDMs)

Evaluation of the incorporation of Nile Red (NR)-loaded vesicles after 6 h incubation

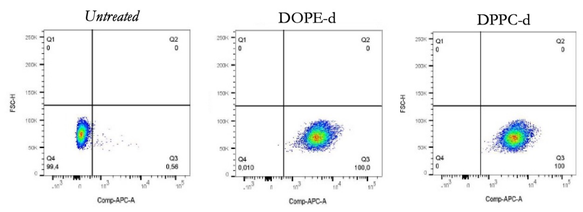


NR incorporation increased with increasing concentration, with DPPC-based formulation showing higher NR incorporation than DOPE-based ones, particularly at 0.1 µM. At 0.4 µM, both formulations showed high incorporation levels, with DPPC-based formulation and DOPE-based formulation reaching ~95% and ~80%, respectively.

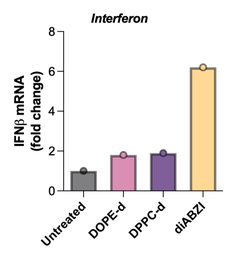
2 Multiple Myeloma (MM) and THP1 cells



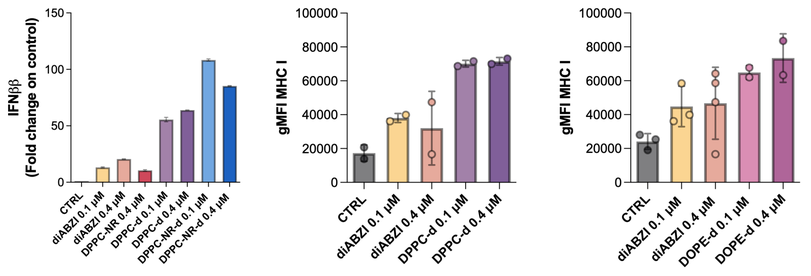
DOPE and DPPC containing diABZI are taken up by MM cells



…and induce activation of the STING pathway

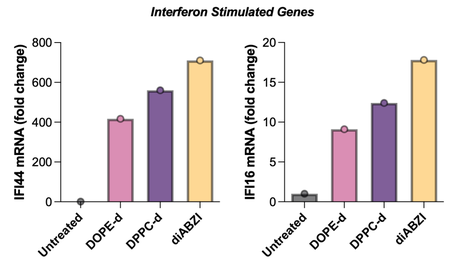


BMDMs upregulate MHC class I cell surface expression and induce type I IFN transcription upon stimulation with liposome-encapsulated diABZI



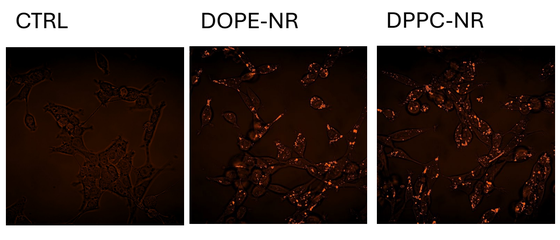
Liposomal formulations strongly enhanced MHC-I expression and IFN-β production compared with diABZI.

Flow cytometry analysis showed a marked treatment-induced shift toward higher APC-A fluorescence. While control cells were almost exclusively APC-A negative, treatment resulted in cells falling within the APC-A-positive gate. This pronounced shift indicates induction of the APC-associated marker across the cell population. Moreover, the tested formulations efficiently activated the interferon response, resulting in induction of interferon signaling.



3 Preliminary studies on mouse CRC cells SL4

Qualitative accumulation of NR-loaded vesicles in mouse CRC cells SL4 after 30' incubation



Both vesicles are rapidly internalized after 5' incubation.

CONCLUSIONS

This study investigated the structural and immunological properties of DPPC- and DOPE-based liposomes as potential carriers for diABZI delivery. SAXS analysis revealed that DPPC liposomes largely maintained their bilayer organization upon acidification, whereas DOPE-based liposomes underwent pronounced pH-dependent structural rearrangements, particularly following diABZI encapsulation. These findings are consistent with the fusogenic properties of DOPE and were further supported by fluorescence spectroscopy. Both formulations were efficiently internalized by mouse and human tumor cell lines and macrophages. Moreover, diABZI-loaded liposomes induced a type I interferon response comparable to that elicited by free diABZI. Overall, these results highlight the potential of DOPE-based liposomes as pH-responsive intracellular delivery systems for diABZI. Further studies are needed to evaluate the *in vivo* efficacy, safety, and biodistribution of diABZI-loaded liposomes.

REFERENCES

[1]Broeckel R, Browne A, Sucoloski S, et al. STING Agonist Induced Innate Immune Responses Drive Anti-Respiratory Virus Activity *In Vitro* with Limited Antiviral Efficacy *In Vivo*. *ACS Infect Dis*. 2024;10(9):3392-3407. doi:10.1021/acsinfecdis.4c00504
[2]Wang J, Meng F, Yeo Y. Delivery of STING agonists for cancer immunotherapy. *Curr Opin Biotechnol*. 2024;87:103105. doi:10.1016/j.copbio.2024.103105