Understanding response of lipid nanoparticles' structure to Phopsholipase D activity

Marco Fornasier [a,b], Sem Brinkman [a], Mauricio Lopez Crespo [a] and Hanna Barriga [a,b]

[a] Division of NanoBiotechnology, Department of Protein Science, SciLifeLab, KTH Royal Institute of Technology, Stockholm 17165, Sweden

[b] Department of Neuroscience, Karolinska Institute, Tomtebodavägen 18A, Solna 17176, Sweden

marcofo@kth.se

Aberrant regulation of certain enzymes has been implicated in the pathogenesis of various diseases. Specifically, Phospholipase D (PLD), a pivotal enzyme responsible for hydrolyzing phospholipids to remove choline headgroups, is overexpressed in breast cancer **[1]** . Given the emerging importance of lipid nanoparticles (LNPs) as drug and gene vectors, understanding how LNP structure may be related to their biological performance in complex environments is needed. If, for example, PLD can discriminate between LNPs with different structures, it may be feasible to design enzyme-responsive carriers and tune their properties for cancer therapy. Recently, the group **[2]** showed that PLD degrades the lipid substrate at different rates in cubosomes and vesicles, hinting to a specific role of the structure of the carrier. In this work, we address how surface coverage (stabilizer concentration), inner structure and morphology of different LNPs, *e.g.* , cubosomes, hexosomes, vesicles and clinically relevant LNPs may influence their response to PLD. LNPs were prepared using clinically relevant lipid compositions (e.g., Pfizer, Onpattro, Moderna vaccines) and lipid compositions yielding non-lamellar structures studied by the group **[2,3]** . Employing NTA, DLS, zeta potential measurements and a fluorescence assay, we monitor the enzymatic conversion of DOPC and DSPC (both substrates of the enzyme) to free choline in solution, and how this affects the properties of the LNPs in terms of size, surface charge, and concentration. Overall, structured LNPs present a higher activity respect to the lamellar counterparts and clinically relevant formulations; in addition, the surface coverage of the PEGylated lipids in LNPs slows down the kinetics of the enzymatic degradation of the substrate. Different endpoints are reached in the fluorescence assay depending on the LNP structure and composition, highlighting a different accessibility of the substrate within the LNP. SAXS experiments are performed on the LNPs incubated with PLD in the same conditions, showing that the enzymatic reaction decreases the order of the structure and induces phase transitions. Preliminary SANS characterization shows that the addition of PLD to structured LNPs induced a change in the lipid redistribution, most likely due to accumulation of high anionic charge in the membrane via PC cleavage. This study aids in further structure-function correlations of LNPs for cancer therapy and in design of smater drug carriers.

Keywords:
structure-function, responsive carriers, scattering, drug delivery, bilayers

References:

[1] Cho et al. Molecules and Cells. 40, 805. **2017** .
[2] Barriga et al. Advanced Materials. 34, 2200839. **2022** .
[3] Ojansivu et al. Advanced Materials. *37, 2419538.* **2025** .