Enlightning the black box of nanocarrier formation inside of microfluidic mixing devices

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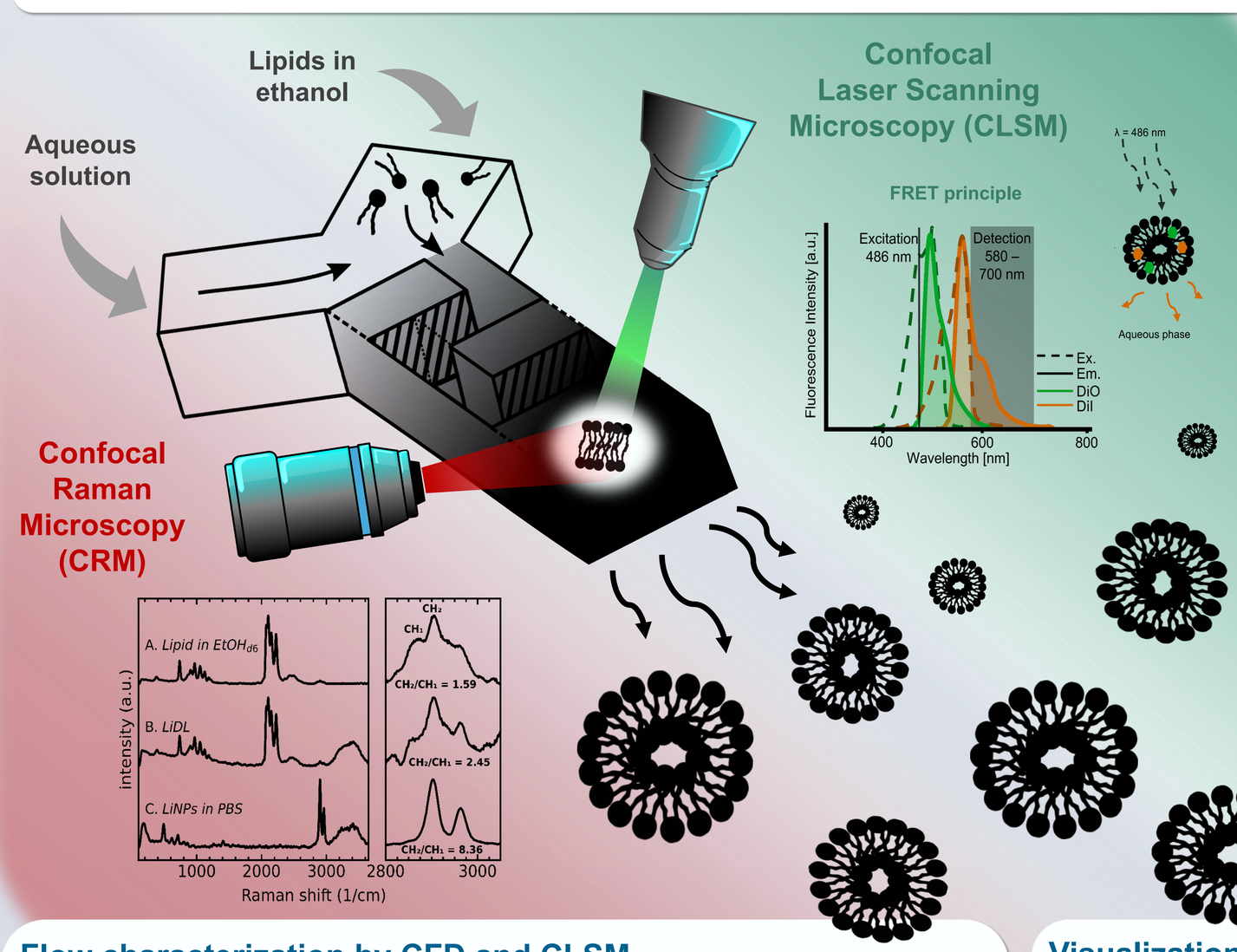
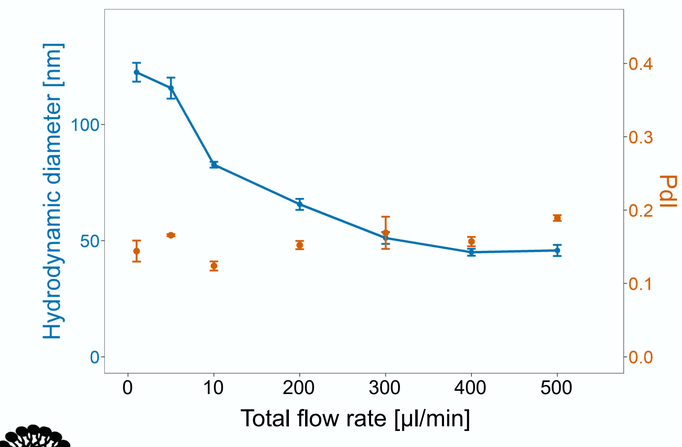
Introduction

Despite decades of research, the successful market authorization of nanocarrier formulations remains rare, primarily due to the lack of scalable and reproducible fabrication methods. Microfluidic-based manufacturing has emerged as a promising approach, particularly for lipid carriers. These processes typically rely on the controlled dilution of ethanolic lipid solutions with aqueous buffers. However, the dynamics of ethanol dilution and nanocarrier formation within microfluidic channels are poorly understood, limiting the optimization and reproducibility of these methods. This study establishes a robust analytical platform to investigate mixing efficiency and nanocarrier formation mechanisms within microfluidic chips using confocal laser scanning microscopy (CLSM) and confocal Raman microscopy (CRM) Studies were carried out in a baffle mixer made of polydimethylsiloxane (PDMS) with alternating baffles, decreasing the main channel width of 200 µm down to 50 µm.



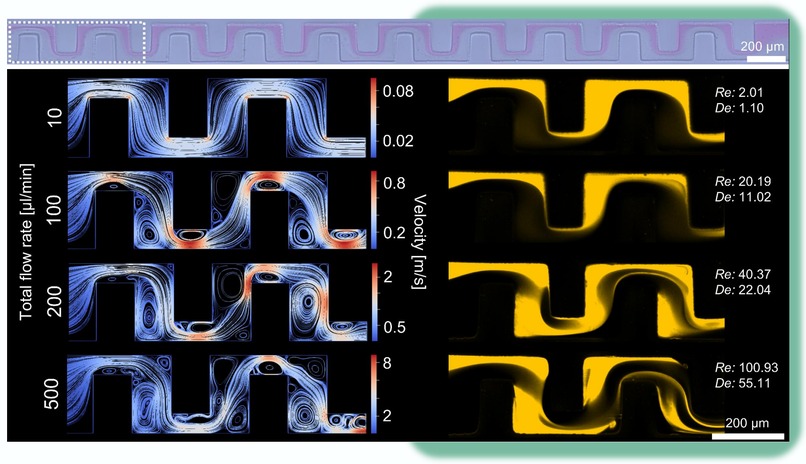
Influence of flow rate on nanocarrier size

Microfluidic-based fabrication enables precise control over carrier characteristics by adjustment of the total flow rate (TFR). Liposomes were prepared via nanoprecipitation using a 25 mM 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) solution in ethanol and phosphate-buffered saline at a flow rate ratio of 4. Whereas the polydispersity index (PdI) remained largely unaffected by the TFR, the hydrodynamic diameter substantially and continuously decreased with increasing flow rates. Between 50 and 200 µl/min, carrier size was reduced by approximately 50%, pointing to improved mixing capabilities and rapid lipid precipitation.



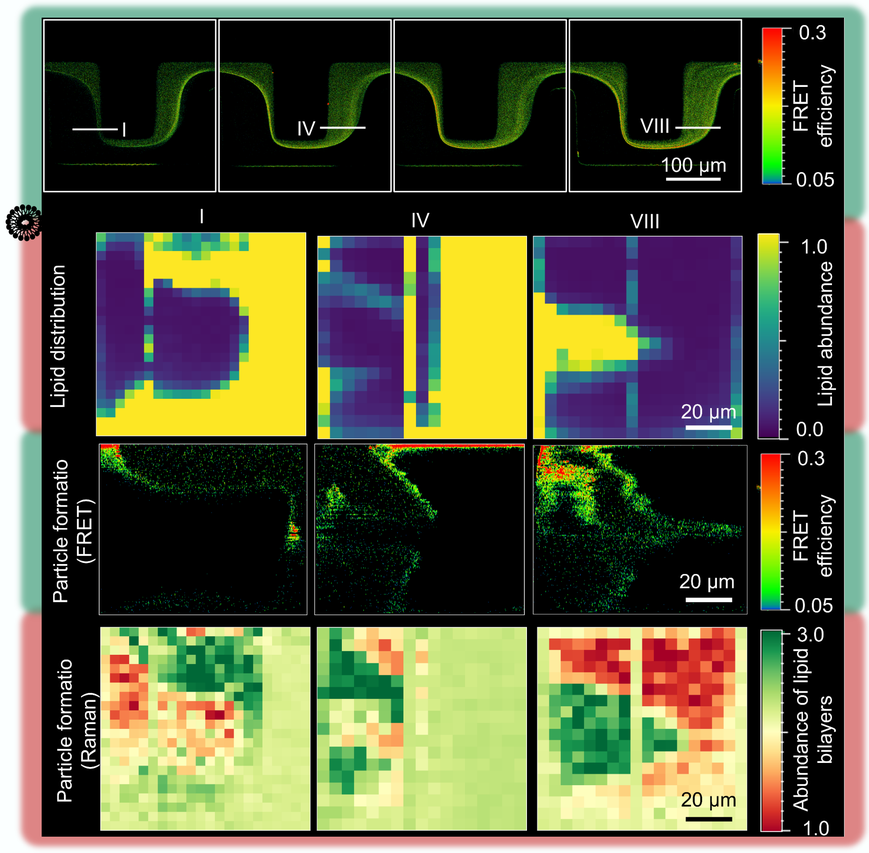
Flow characterization by CFD and CLSM

Flow profiles across the first four baffles were analyzed using computational fluid dynamics (CFD) and compared with CLSM images, using Rhodamine-labeling of the ethanolic phase. Alternating baffle positions induced centrifugal forces generating pressure gradients. Between 100 and 200 µl/min the flow dynamic shifted from laminar to disturbed. Notably, at 200 µl/min, the aqueous phase penetrated the ethanolic stream at the first baffle pointing to a significant disruption, although the overall flow profiles seemed similar.



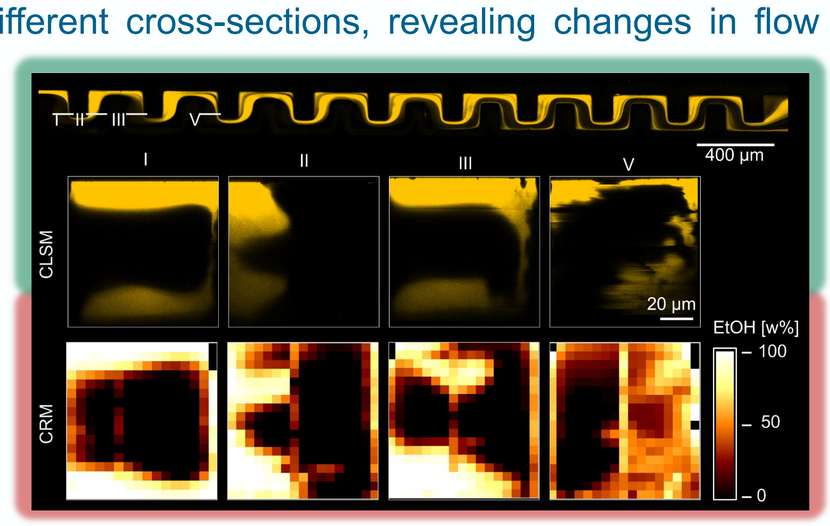
Visualization of carrier formation

In situ carrier formation was assessed with an ethanolic solution containing DOPC and two hydrophobic dyes at TFR 100 µl/min. Both dyes incorporate into lipid bilayers and can function as Foerster resonance energy transfer (FRET) pair when in critical proximity. FRET efficiency is increased following nanoprecipitation due to lipid bilayer formation, incorporation of dyes and their enhanced vicinity. CRM confirmed carrier formation via characteristic changes in C-H vibrations. Both methods generated highly comparable images, immensely strengthening the results. Carrier formation was most prominent at the aqueous-ethanolic interface. In the eighth baffle, delamination of both phases additionally created further regions of carrier formation.



Understanding ethanol dilution

Ethanol distribution within the baffles was analyzed via CLSM via Rhodamine labeling of the ethanolic phase or label-free CRM, respectively. Data of both methods showed comparable distribution patterns across different cross-sections, revealing changes in flow patterns at posterior baffles. Cross-sections at baffle V revealed phase delamination and formation of Dean vortices, further highlighting the importance of 3D flow characterization.



Conclusions and Outlook

- Ethanol dilution can be precisely tracked by CLSM and CRM
- Carrier formation can be visualized *in situ* using FRET dyes and CLSM or label-free by CRM
- Understanding of microfluidic mixing geometries can be increased tremendously, leading to better translation and reproducibility of nanocarrier fabrication

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