Probing Deuteration-Induced Phase Separation in Supported Lipid Monolayers using Tip-Enhanced Raman Spectroscopy

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Deuterium labelling is a common strategy to track the distribution of molecules in biological samples.[1][2] However, the alteration of biophysical properties caused by deuteration is not well understood yet. Conventional analytical tool lacks the sensitivity and spatial resolution to investigate this at the nanoscale. Herein, we demonstrate that hyperspectral tip-enhanced Raman spectroscopy (TERS) imaging is an effective tool to visualize deuteration-induced phase separation in a mixed dipalmitoylphosphatidylcholine monolayer (DPPC:d_{62}-DPPC at 1:1) supported on Au(111) surface. The DPPC:d_{62}-DPPC monolayer was transferred onto the Au(111) surface via the Langmuir Blodgett technique. Successful monolayer transfer was confirmed via topography measurements using atomic force microscopy. Since d_{62}-DPPC has a strong C-D stretching signal ($I_{C\text{-}D}$) in the silent region of the Raman spectrum from 2000 cm^{-1} to 2200 cm^{-1}, and DPPC has a strong C-H stretching signal ($I_{C\text{-}H}$) from 2800 cm^{-1} to 3000 cm^{-1}, the $I_{C\text{-}D}$ / $I_{C\text{-}H}$ ratio was used to visualize the phase separation. In TERS images, there were distinct DPPC-rich and d_{62}-DPPC-rich domains, which can be attributed to the differences in the molecular size and polar/non-polar interaction between the deuterated and non-deuterated chains of the d_{62}-DPPC and DPPC molecules. Finally, the size and polarity of d_{62}-DPPC and DPPC were quantitatively examined using LC-MS and correlated with their phase separation behaviour. The novel insights gained in this work expand our biophysical understanding of supported lipid membranes.

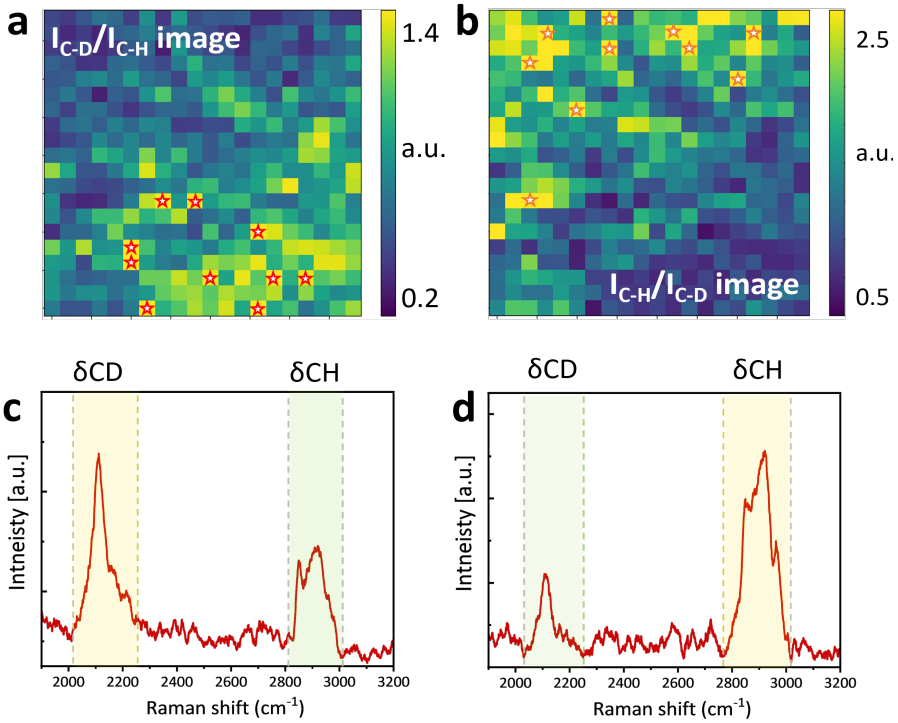

Figure 1. TERS images of the (a) $I_{C\text{-}D}/I_{C\text{-}H}$ and (b) $I_{C\text{-}H}/I_{C\text{-}D}$ ratio showing phase separation of the DPPC:d_{62}-DPPC domains. Averaged TERS spectra at the locations marked in Panels (c) a and (d) b.

[1] Navakauskas, Edvinas, Gediminas Niaura, and Simona Strazdaite. Colloids and Surfaces B: Biointerfaces, **2022**, 220, 112866.
[2] Atzrodt, J., Derdau, V., Kerr, W. J., & Reid, M. Angewandte chemie international edition, **2018**, 57 (7), 1758-1784.