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Label-free and charge-sensitive second harmonic imaging of giant vesicle hydration

A biological membrane forms a dynamic and complex barrier between compartments of the living cell and its environment. However, its *in vivo* studies are difficult because it consists of a high variety of lipids and proteins and is continuously reorganized by the cell. Giant unilamellar vesicles (GUVs) are a powerful model system of the cell membrane due to their comparable size and membrane curvature. The majority of studies carried out on GUVs utilize fluorescence microscopy in combination with fluorescent markers. However, these methods of membrane imaging typically neglect molecular level details. As a consequence, there is virtually no knowledge on the role of membrane hydration, even though it is clear that without water lipid bilayer membranes cannot exist. A recent improvement in imaging throughput has resulted in the construction of a second harmonic imaging device that can non-resonantly and dynamically image interfacial water molecules [1,2]. This microscope was subsequently used to image the hydration of macroscopic free-floating membranes in aqueous solutions [3]. Here, we envision to extend our approach to SH image the interfacial hydration of GUVs. By varying the ionic strength of the adjacent solutions and lipid composition of the vesicles, we show that the non-resonant SH response of water molecules aligned by charge-dipole interactions with charged lipids can also be used as a label-free probe of membrane structure of GUVs.

[1] Carlos Macias-Romero et al., *Optics express*, 2014, 22 (25), 31102–31112.

[2] Carlos Macias-Romero et al., *Science*, 2017, 357 (6353), 784–88.

[3] Orly B. Tarun et al., *PNAS*, 2018, 115 (16), 4081–4086.

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