Interfacial water probing by CARS spectroscopy on biological samples exposed to intense pulsed electric fields

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The main consequence of the interaction between cells and pulsed electric fields is the destabilization of the plasma membrane leading to its permeabilization. Previous vibrational spectroscopy studies (S.M. Gruenbaum et al., *J. Chem. Phys.,* **135**, 2011) have shown that interfacial water is highly organized around the phospholipids of the membrane. Moreover, various numerical studies (such as Molecular Dynamics simulations) have shown that the interfacial water is highly disturbed by the pulsed electric field. We detail here a study of the behavior of the interfacial water around biological objects, before and after exposition to intense pulsed electric fields, by Coherent Anti-stokes Raman Scattering (CARS) spectroscopy.

The home-made wide-field CARS system (A. Silve et al., *J. Raman Spectroscopy.*, **43**, 2012, pp.644-650) allows sensitive spectroscopy to be performed in liquid water without distortion caused by non resonant contributions to the signal. The study is performed on two model systems: Giant Unilamellar Vesicles (GUV) made of phospholipids, which are a simple mimic of the plasma membrane, and DC-3F cells (Chinese hamster lung fibroblasts). The biological samples were exposed to high amplitude, 10 nanoseconds electric pulses leading to the electroporation of the membranes.

CARS spectrum of interfacial water observed on GUVs and living cells were found to be rather similar, and different from that of pure water. Two main water bands were observed and assigned to the presence of two different types of hydrogen bonds in liquid water, namely the "lipid associated" and the "bulk-like" hydrogen bonds. They characterize two different organizations of the interfacial water close to the membrane. After the delivery of the electric pulses, the relative intensities of these spectral components changed: the "lipid associated" band became dominant, whereas the "bulk-like" bands decreased (figure 1). The changes in the bands pattern are attributed to the alteration of the "bulk-like" hydrogen bonds and thus of the organization of the interfacial water molecules which are actually the first to be affected by the electric pulses delivery, as expected from the molecular dynamics simulations. These results reinforce the mechanistic details in the numerical models and provide experimental information about the behavior of the interfacial water after electroporation.

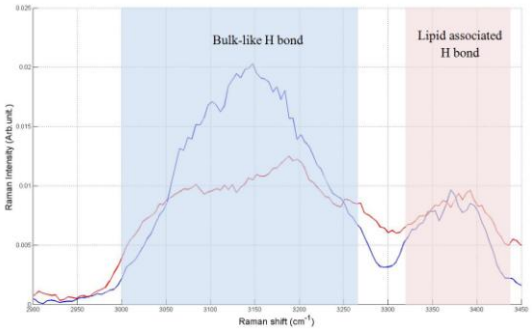

Figure 1: CARS spectrum of interfacial water around DC-3F cells before (blue curve) and after (red curve) electroporation.