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Nonlinear Imaging of Lipid Membrane Alterations Elicited by Nanosecond Electric Pulses

Pulsed Power Research Group

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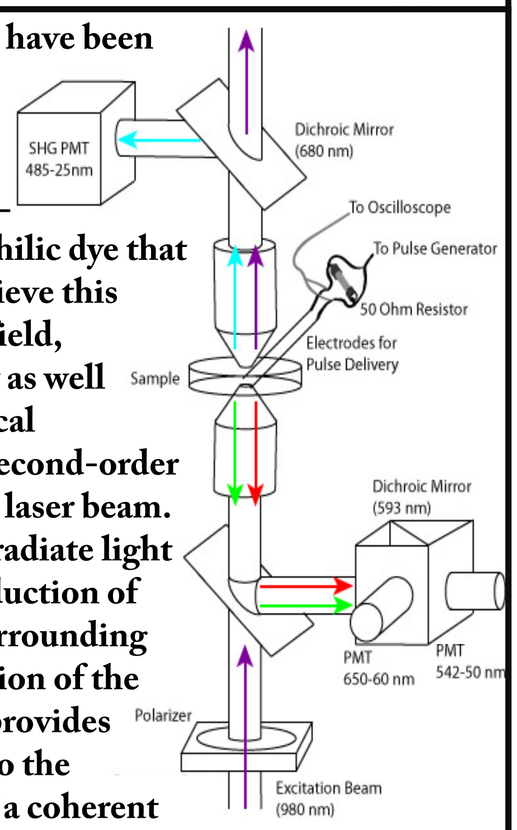
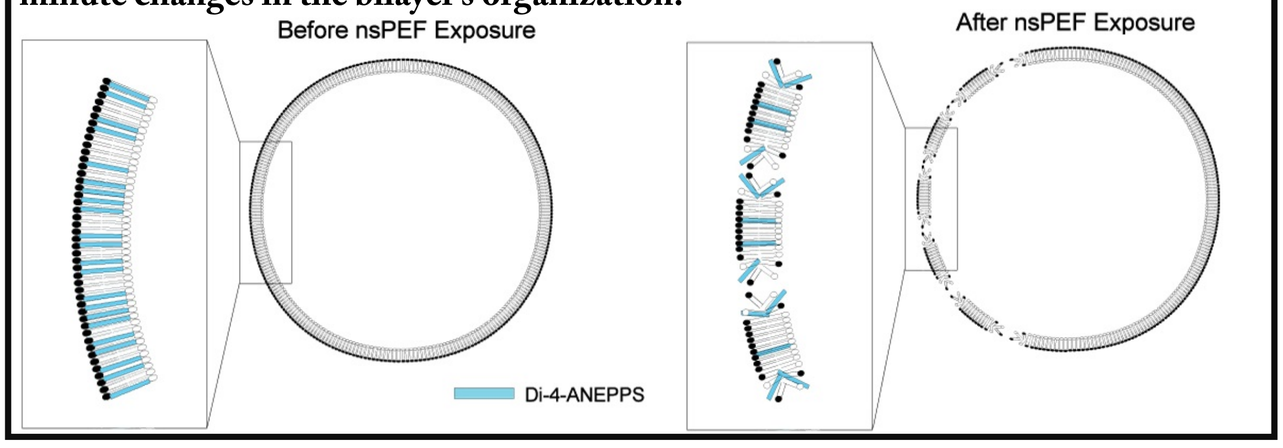
Background: Can Biology Be Tuned by Pulsed Power?

Over the past 30 years or so, researchers have explored electroporation - where electric fields are used to create holes in the lipid bilayer membrane of cells. This method has been used to great success for gene transfection and drug delivery. More recently, however, groups have begun investigating the use of intense (~MV/m), ultra-brief (<μs) electric pulses to increase membrane permeability and alter cell activity. Unfortunately, the root causes for these responses are still unclear. Molecular Dynamic (MD) simulations posit that the increase in membrane permeability is the result of aqueous pore development akin to those seen at much lower, electroporation voltages, but this hasn't yet been proven experimentally. As the lipid membrane is the epicenter for many cellular functions, it is critical to gain a better a understanding of the physics at play resulting from exposure to nanosecond electric pulses (nsEP). Our research centers on a new methodology developed to observe subtle membrane perturbation *in vitro* using second harmonic generation (SHG). Not only can it can provide experimental evidence of nanopore (aqueous pores <2nm diameter) formation, but holds promise as a tool for any experiment studying the order of symmetry-breaking interfaces.

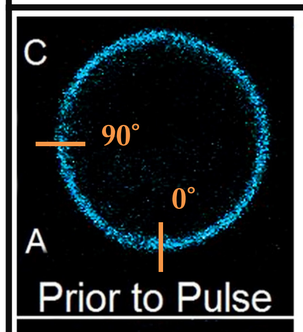
Experimental Setup and Methodology

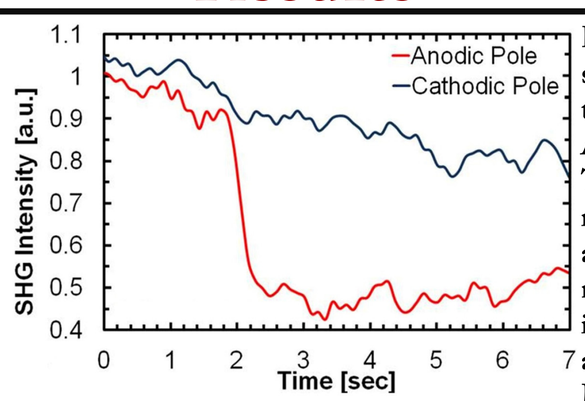
External forces acting upon the plasma membrane have been shown to cause rapid disturbances. To understand this interaction, a minimally invasive, highly sensitive imaging technique that enables monitor--ing the structure of the plasma membrane is need--ed. We chose Di-4-ANEPPDHQ (Di-4), a lipophilic dye that embeds itself into lipid membranes, to help us achieve this goal. Di-4 is sensitive to the surrounding electric field, allowing it to report changes in membrane fluidity as well as voltage. It is, however, the probe's inherent optical nonlinearities that prove most useful. In SHG, a second-order nonlinear polarization is induced with an incident laser beam. This generates oscillating dipole moments that reradiate light at twice the energy of the excitation beam. The induction of this dipole is sensitive to the static electric field surrounding the probe and the steady-state molecular polarization of the probe molecule. The cell membrane, meanwhile, provides a substrate for the dyes to be aligned with respect to the interface. This alignment allows the generation of a coherent SHG signal from the membrane, which we exploit to detect minute changes in the bilayer's organization.

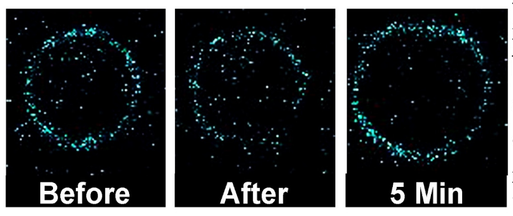




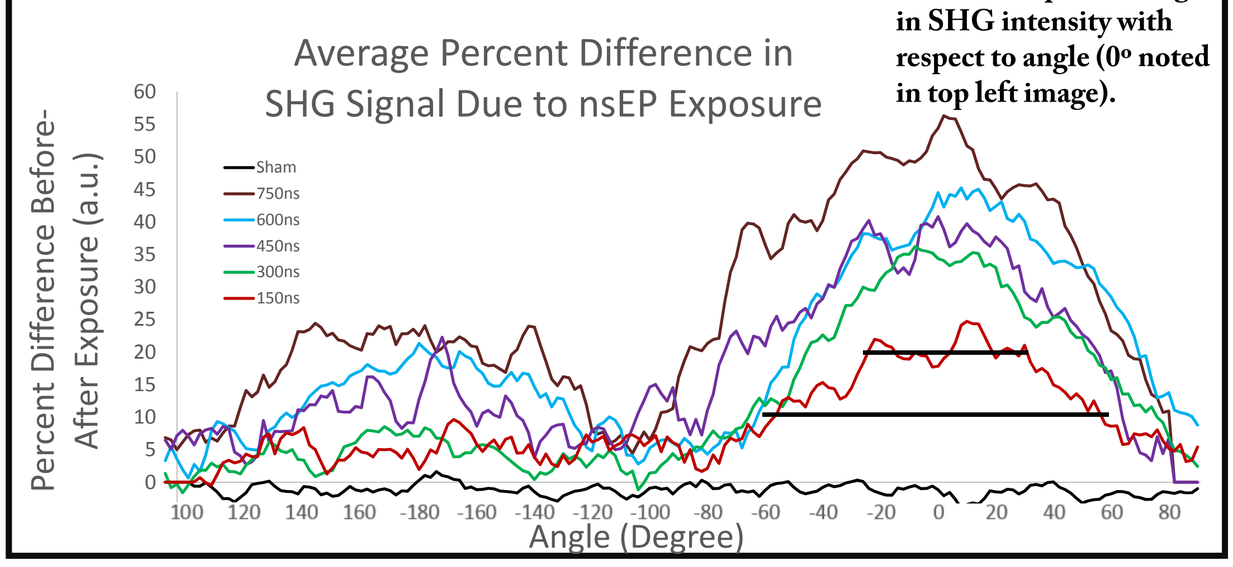
Results





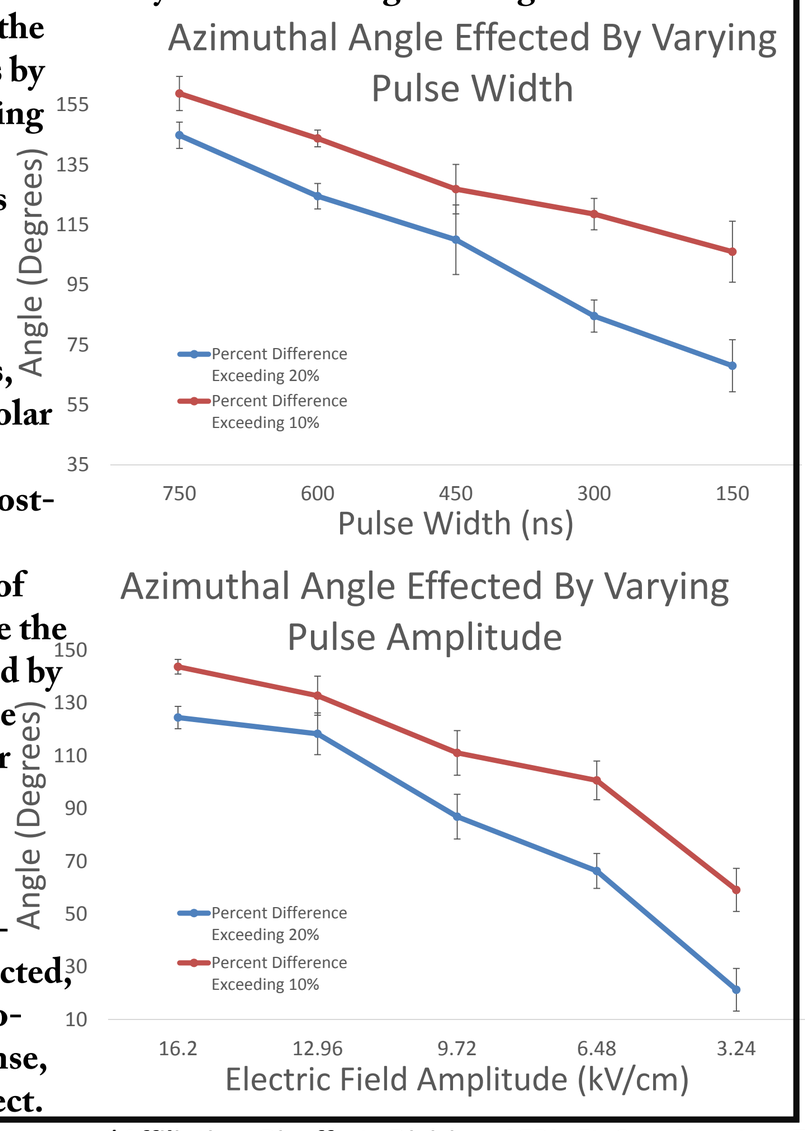


Left: SHG images showing drop in signal on the anodic pole (marked A) of the cell. Top Right: Time trace of SHG response with nsEP applied at 2s that shows near-instantaneous drop in SHG signal at the anodic pole of the cell. Middle Right: SHG images immediately before and after exposure as well as 5 minutes post-exposure showing recovery of SHG signal. Below: Graph of change in SHG intensity with respect to angle (0° noted in top left image).



Discussion

To test whether Di-4 would report rapid structural changes in membrane organization, we applied a single 600 ns-duration, 16.2 kV/cm electric pulse to the labeled cell. Before the pulse, the intensity of the SHG signal is high at each of these poles. Immediately after the pulse, the SHG intensity drops by ~50% on the side of the cell facing the anodic electrode. The response is near-instantaneous with little recovery in the 5 s post-exposure. The response matches the previously observed effect of this stimulus, where ion uptake displayed a polar dependence and persisted for minutes. Images taken 5 min post-exposure confirm the eventual recovery of the cell and return of SHG signal. In order to explore the extent of the membrane affected by the pulse, the percent difference in SHG signal (before and after nsEP exposure) is recorded angularly with respect to the applied electric field. Varying pulse width reveals a linear cor--relation with angular area affected, while scaling the amplitude pro--duces a more nonlinear response, pointing to a voltage-gated effect.



Conclusions and Future Work

By taking advantage of the selection criteria of SHG, we were able to use Di-4 to monitor rapid disruption of the plasma membrane. Because SHG can only be generated when the probes are aligned in the plasma membrane, the SHG signal diminishes significantly upon disruption. This technique holds tremendous potential for use in the study of how external stimuli interact with and change the orientation of biological membranes. We are currently expanding on this work by applying it to artificial membranes. This research will enhance our understanding by limiting biological confounders. In addition, we are exploring the electrophoretic processes at play with bipolar pulses and MD simulations.

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ORISE Oak Ridge Institute for Science and Education

ARCS Advancing Science in America

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