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ID-11-P-3357 Determination of Orientational Distributions of Fluorescent Molecules in Phospholipid Membranes by Polarization Fluorescence Microscopy

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Orientation of fluorescent molecules (fluorescent dyes or fluorescent proteins) associated with a biological membrane can report on the molecular environment within the membrane, but also on molecular processes involving membrane proteins. In our other work we have demonstrated that changes in fluorescent molecule orientation, detected by two-photon polarization microscopy (2PPM), can be used to report on binding of ligands to G protein coupled receptors (GPCRs), interactions between GPCRs and G proteins, G protein activation, changes in the cell membrane voltage, changes in intracellular calcium concentration, and other molecular processes taking place in living cells. Apart from simply reporting a non-specified change in molecular orientation, 2PPM and related techniques should also be able to yield detailed, quantitative information on molecular orientation and its changes, and therefore provide quantitative insights into membrane protein structure, directly in living cells. In order to develop the quantitative structural capabilities of 2PPM, we have now investigated simple model systems that allow conclusions drawn from our microscopic observations to be independently verified by computational molecular dynamics simulations. Here we present the results of our microscopic observations and molecular dynamics simulations of simple fluorescent dyes and fluorescent protein constructs associated with artificial lipid membranes, demonstrating the quantitative capabilities of 2PPM and related techniques.

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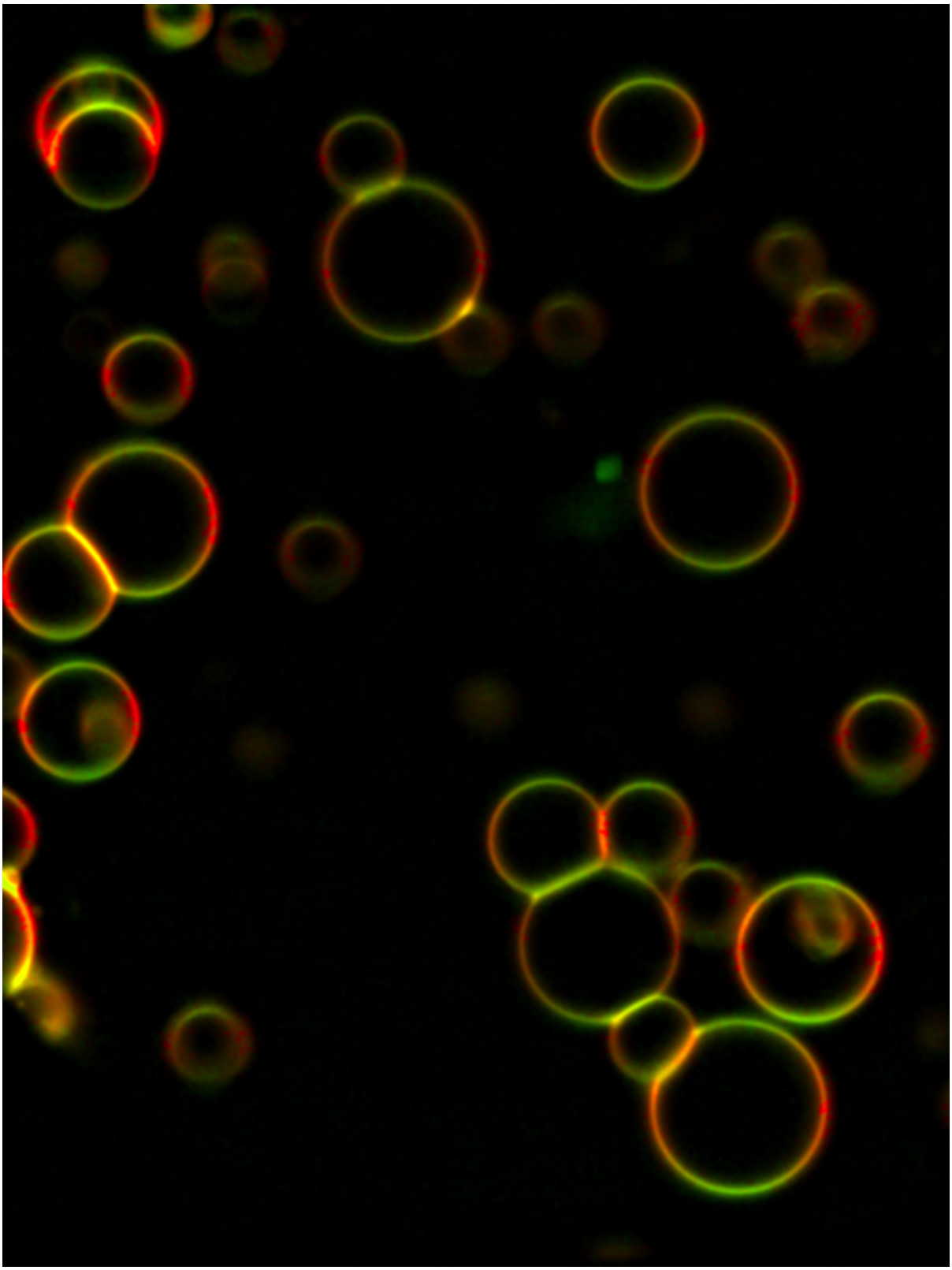

Fig. 1: Giant unilamellar phospholipid vesicles stained with a fluorescent dye (F2N12S), imaged by two-photon polarization microscopy. Fluorescence excited by light polarized horizontally and vertically is colored red and green, respectively. The red-green pattern is indicative of the dye molecules being present in a restricted orientational distribution