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Conformational Analysis and Molecular Dynamics Study: 2-Arachidonoyl-sn-glycero-3-phosphoinositol (2-AGPI) in a DOPC Bilayer

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GPR55 is a Class A G protein-coupled receptor that has been shown to be activated by cannabinoids (Henstridge et al. FASEB J. 2008). The receptor is expressed in several mammalian tissues including several regions of the brain. The Sugiura group has reported that GPR55 is also activated by lysophosphatidylinositol (LPI) found in rat brain (Oka et al. BBRC, 2007), with 2-AGPI having the highest actvity (Sugiura et al. ICRS Abstract 9, 2008). Since 2-AGPI is found in lipid and is shown to interact with the membrane-embedded GPR55 receptor, we undertook a study of the location and conformations that 2-AGPI can adopt in a phospholipid bilayer. Initial study of 2-AGPI necessitated the development of parameters for a P-O-C-C torsion angle. *Ab initio* calculations were performed using standard 6-31G* basis set at both Hartree-Fock (HF) and Møller-Plesset (MP2) levels of theory to determine the rotational profile and molecular geometry of a model fragment of 2-AGPI containing the P-O-C-C linkage. This study revealed a broad global minimum at 260° and a shallower minimum at 50°. A Monte Carlo/simulated annealing (Conformational Memories) study of 2-AGPI in a low dielectric was then undertaken. The study produced 105 conformers which had the same general shape, a tight U, and contained several internal hydrogen bonds between the ring hydroxyls and the phosphate and glycerol moieties. Finally, 2-AGPI was added to a fully hydrated, pre-equilibarted DOPC bilayer (28 waters/lipid; 72 lipid molecules with 36 in each leaflet) and the behavior of 2-AGPI in DOPC was studied using the NAMD2 molecular dynamics software package (NPAT ensemble; P = 1atm, T=310K) with the CHARMM27 parameter set including data for polyunsaturated lipids, along with the TIP3P model for water. [Support: NIH DA023266 and DA021358].

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A Novel Analysis Technique For Determining Area Per Lipid And Electron Density Profiles From Large Lipid Bilayer Molecular Dynamics Simulations

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Determination of the area per lipid (A_L) and electron density profiles (EDP) from molecular dynamics simulations of lipid bilayers is complicated in large systems where mesoscopic undulations develop. Typically, A_L is determined by a projection onto the *xy*-plane (often using the periodic box dimensions or Voronoi tessellation). However, this approach underestimates A_L by not accounting for the out-of-plane undulations. As an alternative, we have used interpolated surfaces to more accurately characterize the simulated A_L. We apply a 2-dimensional spatial filter with a frequency response optimized to a characteristic wave-number, q_0, as determined by Lindahl and Edholm. In so doing, the interpolated bilayer surface captures the desired low-*q* modes of undulation while damping the undesirable high-*q* protrusions. A_L values from our filtered trajectories yield an increase of 1-3 Å^2 over that of the traditional calculations. Regarding EDPs, current algorithms parse the atoms into bins orthogonal to the normal of the bilayer. In large bilayer simulations, undulations introduce heterogeneous sampling in the z-slices. This heterogeneity convolves an averaging function with the electron density, resulting in a smoothed profile, an artifact of the calculation. Subsets of the simulated bilayer, however, with lengths that are less than the characteristic wavelength corresponding to q_0, are locally flat. By transforming each local patch to a coordinate frame whose z-axis is parallel to the normal of the plane of that patch, we can implement homogeneous z-slicing to accurately determine the EDP.

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Undriven Bead Diffusion Through Extracellular Matrix

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The dense interwoven laminin and collagen sheets that make up the extracellular matrix (ECM) can be a difficult medium for various drug delivery methods to move through efficiently and consistently. We employ rhodamine-conjugated dextran molecules of various molecular weights as in vitro probes and present data indicating how probe diameter affects the effective diffusion constant, Deff, of the molecule. Polydimethylsiloxane wells create a controlled geometry for the diffusion experiments. We optically observe the florescent bead front diffuse through ECM and take time-lapse images of the diffusion in action. This image sequence is used to characterize the medium's diffusion properties; fluorescence intensity is plotted with respect to time over the dimensions of the channel. The goal of this research is to understand how bead diameter alters Deff and determine if there is a cut-off diameter at which diffusion constant increases drastically.

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Image Correlation Spectroscopy Reveals Global Dynamics of Wound Healing

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We apply techniques based on correlation spectroscopy to quantify cell migration during wound re-epithelialization in an axolotl wound healing model. We show that cell hypertrophy (about 37% in volume) is present from the time of injury and continues throughout re-epithelialization. Our combined imaging techniques (transmission and laser scanning fluorescence) microscopy and analysis algorithms (Image Correlation Spectroscopy and Spatio-Temporal Image Correlation Spectroscopy) allow us to determine this complex sequence of events from the point of injury until the re-epithelialization is complete. Using non-invasive optical sectioning, we determine that the basal keratinocytes migrate into the wound bed faster than the suprabasal keratinocytes. Additionally, Image Correlation Spectroscopy (ICS) reveals cell hypertrophy as there is an increase in width of the spatial autocorrelation function as a function. Using camera based transmission microscopy, we observe that the enlarged cells produce a point of dislocation that foreshadows and dictates the initial direction of the migrating cells. Globally, the cells follow a concerted vortex motion that is maintained after the wound is fully re-epithelialized. Using Spatio-Temporal Image Correlation Spectroscopy (STICS), we quantify the velocities of the cells undergoing this spiral motion. Closer examination reveals that there is a transition from a chaotic state to a highly organized cohesive motion. This transition is seen in as little as 1 hour post injury. Our results suggest that geometrical constraints combined with the observed cell hypertrophy may dictate the mechanism by which cells repopulate the wound bed.

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Studying Molecular Dynamics And Interactions In Living Zebrafish Embryos By Fluorescence Correlation Spectroscopy

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Fluorescence Correlation Spectroscopy (FCS) is a powerful technique to assess molecule dynamics and interactions on a single molecule level. It has been routinely used to harvest biomedical information both *in vitro* and *in vivo*. Numerous intracellular measurements have been reported in cytoplasm, nucleoplasm and plasma membrane. However, it is now generally accepted that the Petri-Dish based cell culture system cannot represent the essential physiological environment of a living organism and 3D cultures are only a partial solution. Here, we adapted single wavelength fluorescence cross-correlation spectroscopy (SW-FCCS) to work in a living animal model. We chose zebrafish as the optical transparency of early stage zebrafish embryo makes microscopic techniques suitable and established genetic tools enable one to easily express foreign genes within the embryo. First we investigated the penetration depth in the embryo body as tissue tends to cause light scattering and decrease signal to noise ratio if working deep beneath skin. We practiced one- and two-photon excitation and obtained FCS curves up to 80 μm and 200 μm, respectively. Then we characterized the diffusion coefficients of genetically expressed EGFP in muscle fibers and motor neurons and also investigated the mobility of a membrane expressed G protein coupled receptor-CXCR4b. Finally, we measured the dissociation constant (K_D) between a small RhoGTPase and an actin-binding scaffolding protein, in living zebrafish embryos and the results were compared with data obtain from CHO-K1 cultured cells. We showed that molecular dynamics and interactions can be studied in small living animals that provide a genuine physiological environment and questions of developmental biology on a single molecule level are directly accessible by FCS.