Journal of Computational Physics 232 (2013) 482–497

Contents lists available at SciVerse ScienceDirect

Journal of Computational Physics

journal homepage: www.elsevier.com/locate/jcp

From molecular dynamics to fluorescence anisotropy of fluorophores bound to oriented structures

Brian A. Mazzeo [a,*], David D. Busath [b]

[a] Department of Electrical and Computer Engineering, Brigham Young University, Provo, UT 84602, United States
[b] Department of Physiology and Developmental Biology, Brigham Young University, Provo, UT 84602, United States

ARTICLE INFO

Article history:
Received 10 April 2012
Received in revised form 19 July 2012
Accepted 21 August 2012
Available online 6 September 2012

Keywords:
Fluorescence anisotropy
Molecular dynamics

ABSTRACT

Molecular dynamics are often used to analyze and interpret fluorophore motions in relation to observed fluorescence anisotropy measurements. The Soleillet method allows computation of fluorescence anisotropy from molecular dynamics for isotropically oriented fluorophores, but not for oriented fluorophores, such as might be used to study oriented bacterial cultures, oriented, functionalized nanotubes, or oriented, stacked planar bilayers. A numerical approach to distribute molecular dynamics systems appropriately into a larger experimental frame context, allowing prediction of time-resolved and steady-state anisotropies for fluorophores distributed in the crystal-like arrays, is presented. The classical principles of absorption selectivity and motional effects on fluorescence anisotropy for isotropically distributed fluorophores are confirmed. Fluorescence anisotropy for fluorophores distributed on oriented cylinders are predicted to show a rich cylinder-angle dependence.

1. Introduction

Membranes are essential structures for life, regulating structure of, transport into, motion of, and communication between cells. Consequently, changes to cellular membranes can greatly influence the behavior of cells and whole organisms [1]. Reporter molecules can be embedded in membranes to infer membrane conditions [2,3]. In the laboratory, a simple model of a cell membrane, a vesicle, can be prepared synthetically. Lipid membranes are also found in primitive life structures such as bacteria and viruses. Membrane conditions are of interest in cases of foraging, phototaxis, magnetotaxis, or other phenomena resulting in oriented single cells [4–9]. Embedded fluorophores may be useful to analyze bacterial behavior near surfaces [10–12].

Molecular dynamics (MD) simulations are commonplace because of their versatility and ability to capture complex motions of membranes and proteins [13]. Great understanding of molecular processes is enabled by this computational technique. The dynamics of reporter molecules can be studied with exquisite precision [15], often at timescales and under conditions which are germane for fluorescence. The membrane conditions can also be altered and the effects on reporter molecules in the membranes can be computed. Experiments can then be done to confirm that the observed dynamics are indeed plausible and accurately capture the simulated phenomena.

For monitoring reporter molecules, fluorescence anisotropy (FA) is a well-established technique for characterizing the motion of free fluorophores in solutions as well as fluorophores bound to membranes and other molecules [2,16]. By using anisotropy measurements, it is possible to observe how physical properties of the dye solvent change and thus respond to perturbations such as drugs [17–21]. FA measurements are standard and use small volumes of fluid, thus conserving

* Corresponding author. Tel.: +1 801 422 1240.
E-mail address: bmazzeo@ee.byu.edu (B.A. Mazzeo).

0021-9991/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcp.2012.08.045

valuable proteins and drugs. Using both experimental FA and MD simulations allows crosschecking of assumptions and provides a better understanding of the molecular processes that are routinely observed in the laboratory [22–24].

Past theoretical approaches dealing with FA of fluorophores in oriented membranes have been concerned with using the fluorescence anisotropy measurements to deduce parameters for models of the fluorophore movement for cases based on spherical symmetry, such as fluorophores bound to free dissolved molecules that rotate rapidly on the fluorescence time scale or fluorophores bound to the surface of slowly rotating structures such as vesicles [2]. In the latter case, cylindrical symmetry in the molecular frame is occasionally exploited in an inverse problem approach [25]. Recent molecular dynamics simulations are now being used to predict the observed FA under isotropic conditions, a forward problem [26,27]. This paper extends the forward problem to the oriented membrane case.

Because of the small distance scales accessible, the molecule dynamics of a reporter molecule, such as a fluorophore, are often simulated in a planar arrangement such as a lipid bilayer. However, the associated experiments may use an interrogation strategy where the reporter molecule is bound to a membrane which itself may be a plane, vesicle, micelle, or an oriented rod [28]. *This paper addresses how to use planar bilayer MD simulations to calculate observed FA in experiments where reporter molecules are influenced by the orientation of their host membranes.* The basic concept is to consider the patch of planar bilayer from an MD simulation to represent a large ensemble of patches distributed uniformly over large geometric lipid objects that are bathed in a uniform, collimated beam of plane polarized light. The ensemble behavior is assessed numerically, representing integration over all possible configurations, but with the underlying statistical distribution of configurations governed by the assumption of a uniform distribution of tangential patches on the surfaces of the geometrical objects. The results can easily be extended to cases of oriented smectic or nematic crystals, detergent monolayers, oriented multilayers, or any other oriented system [29–31]. The reasonable assumptions will be made that fluorophore dynamics are much faster than re-orientation or tumbling of the membrane system and also that molecular translation is negligible. If significant, those dynamics could be included. This approach can be generalized to other cases, such as circular dichroism, where orientation-dependent excitation and emission are recorded.

The foundational principles that guide the development in this paper are:

- Normalization of the computed anisotropy due to photoselection because of the orientation-dependent absorption of the fluorophore in the experimental configurational ensemble.
- Calculation of the expected emission anistoropy due to fluorophore orientation changes between the absorption dipole and the emission dipole.

This general approach, which confirms and augments traditional theories [32,25,33,34,2,35], will allow researchers to examine complex systems using interrogation tools that are sensitive to initial and final orientations of molecular reporters. In particular, it is the first effort to allow evaluation of FA for fluorophores embedded in oriented cylindrical membranes such as oriented bacteria, filamentous viruses, lipidic nanotubes, etc.

2. Basic fluorescence anisotropy principles

The ensemble configuration for the geometry of this problem is shown in Fig. 1. Fluorophores are bound to membranes, which may be arbitrarily shaped. The fluorophores can move by translation in the molecular frame along the U and V axes. The fluorophore also can change its orientation relative to the molecular frame axes. In the absence of ordering forces, the fluorophores can be considered to be uniformly distributed over the vesicle surface.

In a FA experiment, polarized light illuminates the sample. Parallel and perpendicular polarizations of emitted light are recorded by the FA instrument [2]. The classical intensity of absorption is the square of the projected electric field onto the fluorophore absorption dipole. This intensity corresponds to the relative probability of absorption of polarized photons by the fluorophore. After a random period of time, the fluorophore then emits a photon with a polarization determined by the fluorophore emission dipole, which may be different from the absorption dipole, both due to modification of the electronic structure in the excited state and movement of the molecule since the arriving photon was absorbed.

As shown in Appendix A, Soleillet's principle allows the experimenter to predict the measured FA based on an assumption of isotropic distribution of the original fluorophores and independent, subsequent angular movements. A key result is that isotropically distributed fluorophores with no movement between absorption and emission will have a measured FA of 0.4, which is reduced from the maximal value of 1 for a perfectly oriented fluorophore crystal due to photoselective absorption, i.e. by the fact that absorption dipoles are randomly distributed with respect to the polarized beam.

3. Fixed fluorophore orientations

In the molecular frame, the angles θ_F and ϕ_F of the fluorophore relative to the membrane normal can be extracted from simulation as will be shown in Section 4. To compute the anisotropy expected for fluorophores oriented in a membrane structure and explore the different configurations in the associated ensemble space, the simulations are rotated to many different positions on the vesicle or cell surface. The rotation ρ reflects the rotational orientation of a patch on the vesicle or cell surface. θ and ϕ then reflect the angular positions of the membrane patch on the vesicle/cell surface. The rotations have