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### Title

Exploring transient binding and diffusion of molecules on bilayer surfaces with two-photon fluorescence microscopy

### Permalink

<https://escholarship.org/uc/item/7st7f03w>

### Journal

BIOPHYSICAL JOURNAL, 82(1)

### ISSN

0006-3495

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### Publication Date

2002

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Peer reviewed

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**Exploring transient binding and diffusion of molecules on bilayer surfaces with two-photon fluorescence microscopy.**

46th Annual Meeting of the Biophysical Society, San Francisco, California, 2002.

*Biophys J.* 2002; 82(1): 546c.

**Abstract**

We are examining the utility of two-photon imaging techniques in the study of the association of molecules, small fluorophores and fluorophore-labeled proteins, with a bilayer surface. Giant unilamellar vesicles are being used as the model membrane surface and a variety of fluorescent materials are being presented to the bilayer surface via microinjector or passive diffusion from a resting, material-loaded capillary. We are presently optimizing the hardware and experimental design to improve our sensitivity and image time-resolution. Preliminary data on small uncharged fluorophores indicate a rapid initial binding step followed by diffusion across the membrane surface. The spreading of the material on the surface of the bilayer appears to be too fast to be described by simple membrane diffusion of the dye in the membrane. We are developing a diffusion model to explain the observed spreading in terms of a rapid solution-membrane partitioning which then increases membrane diffusion through the introduction of a strong interfacial diffusion parameter. Support: NIH, PHS P41 5 RR03155.