



Spectral Shift FRET Studies of Photosensitive Membrane Proteins

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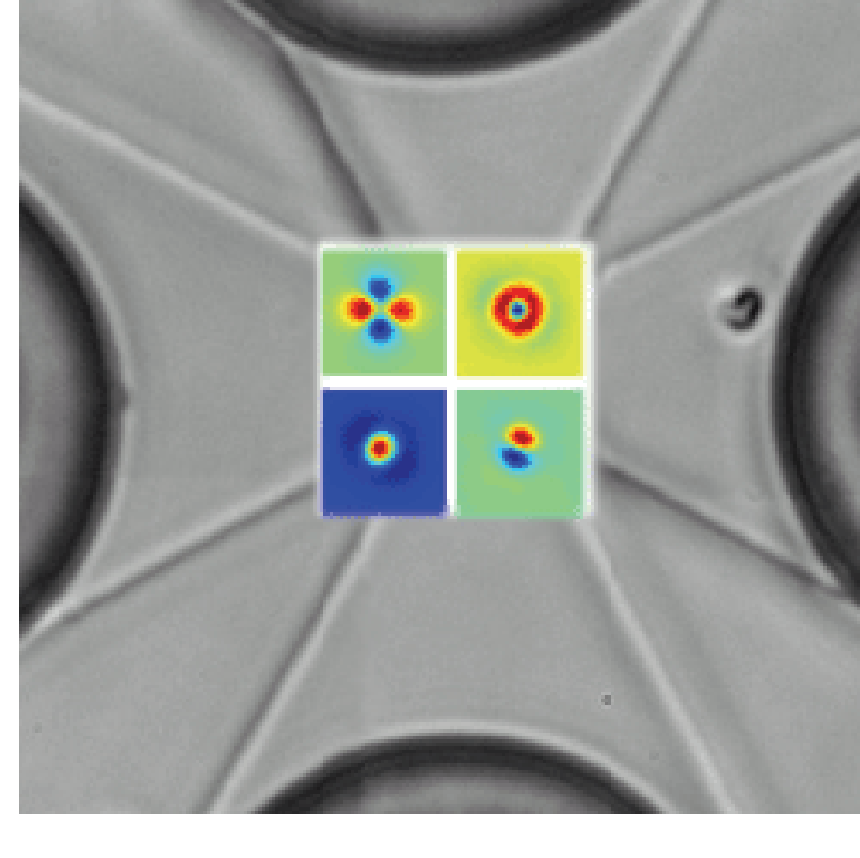
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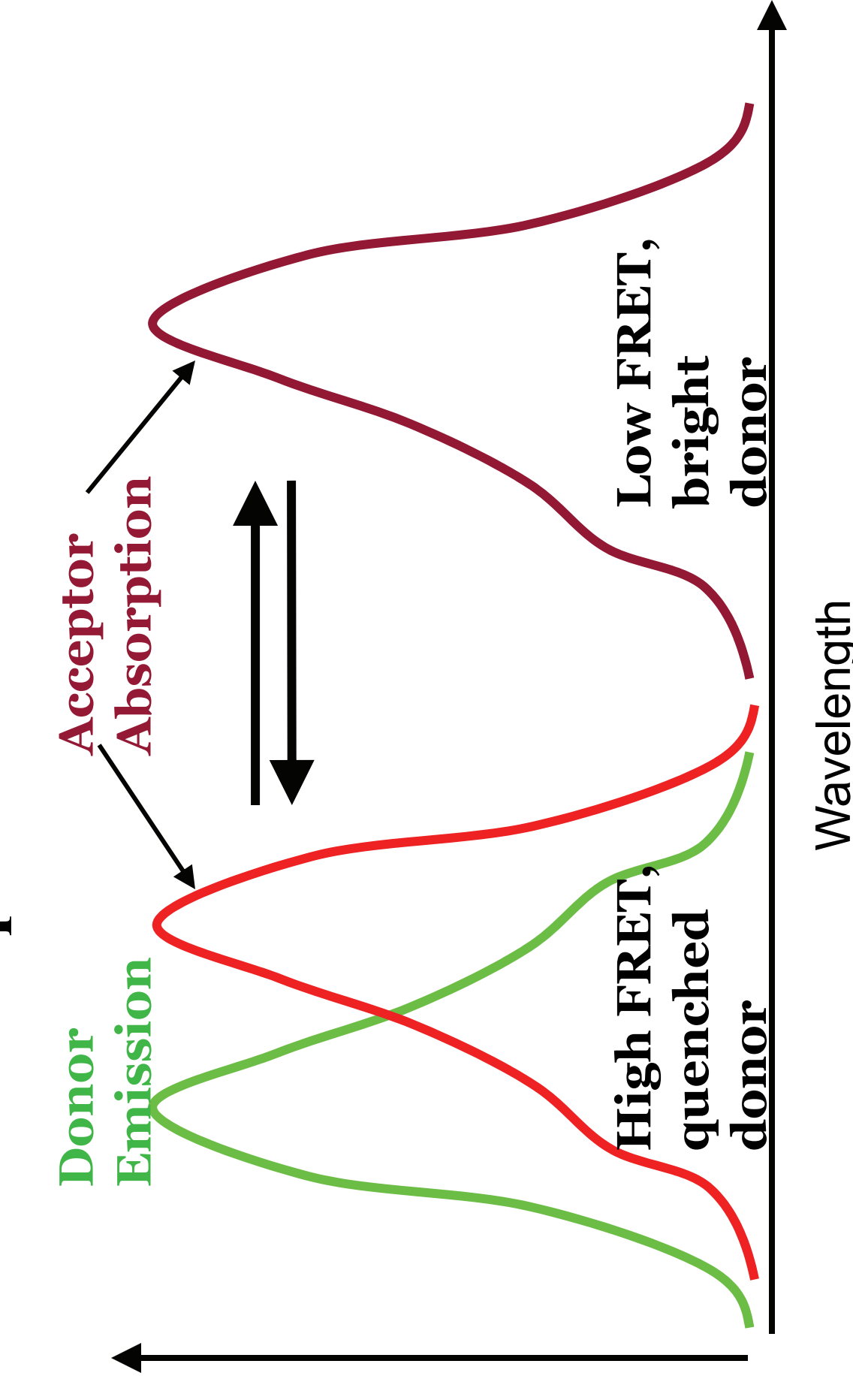
Cohen Lab

Abstract

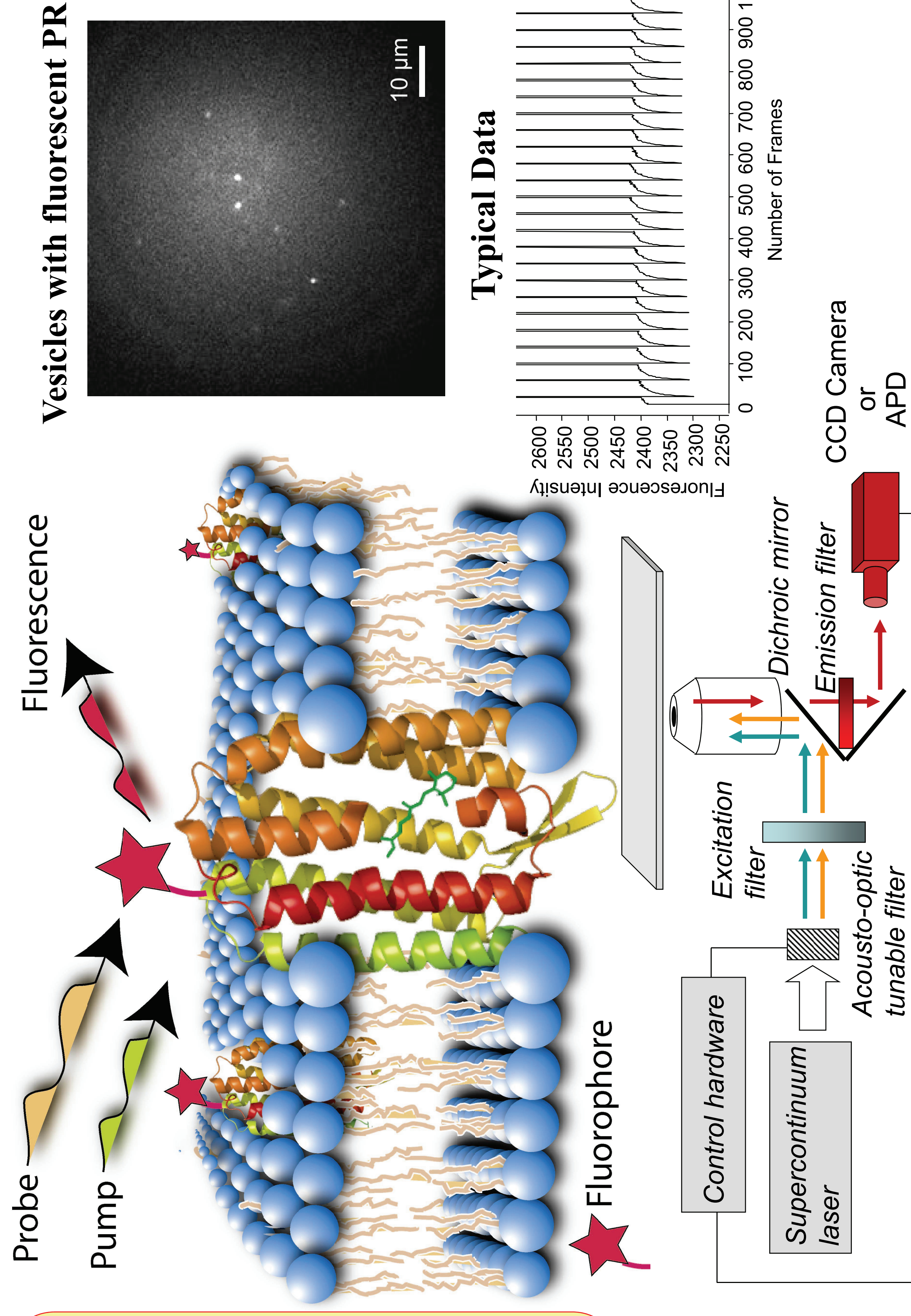
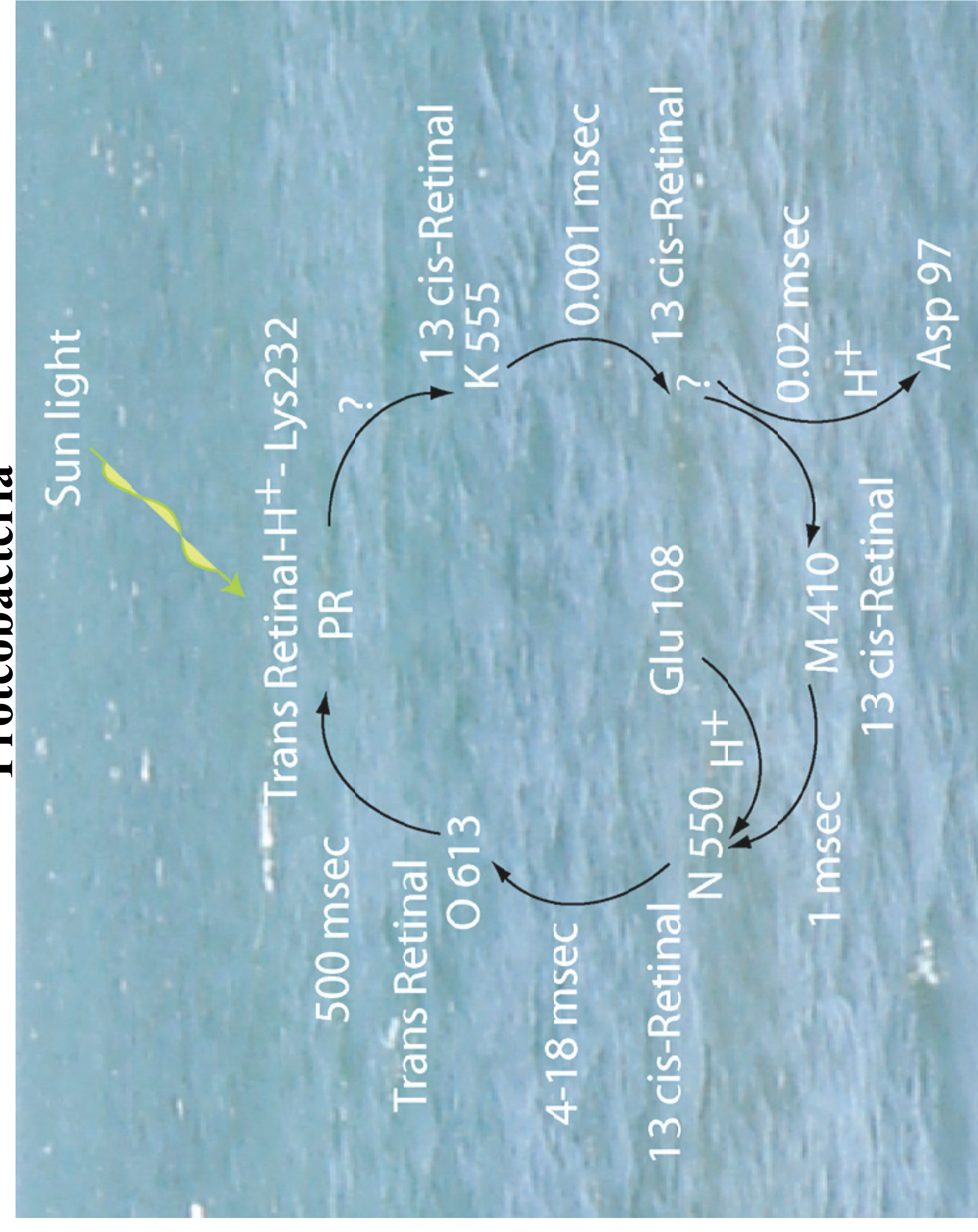
We present a highly sensitive method of studying the dynamics of photosensitive membrane proteins by using Spectral Shift FRET. In normal FRET, the rate of energy transfer depends on the spatial separation of donor and acceptor. In Spectral Shift FRET, the rate of energy transfer depends on the spectral separation of a donor and an environmentally sensitive acceptor. Our method is particularly suited to macromolecules that contain an endogenous chromophore that undergoes a chromatic shift, in which case only a single fluorescent label is required. The label serves as a fluorescence donor, and the endogenous chromophore serves as an environmentally sensitive quencher.

Proteorhodopsin (PR) and Sensory Rhodopsin II (SRII), found in marine plankton, are light sensitive membrane proteins that convert light into chemical energy by creating a motive force across the membrane. The retinal chromophore undergoes dramatic spectral shifts during the photocycle. Alexa dyes were incorporated into PR and SRII. The photocycle was initiated by a flash at the activation wavelength, and the ensuing dynamics were probed by measuring the fluorescence quantum yield of the Alexa dyes. 100 nm size vesicle containing a few copies of membrane proteins were sufficient to monitor the dynamics of the photocycle, thereby providing a highly sensitive method to monitor bacterial rhodopsins. The technique of Spectral Shift FRET provides an important new tool for studies of photosensitive proteins.

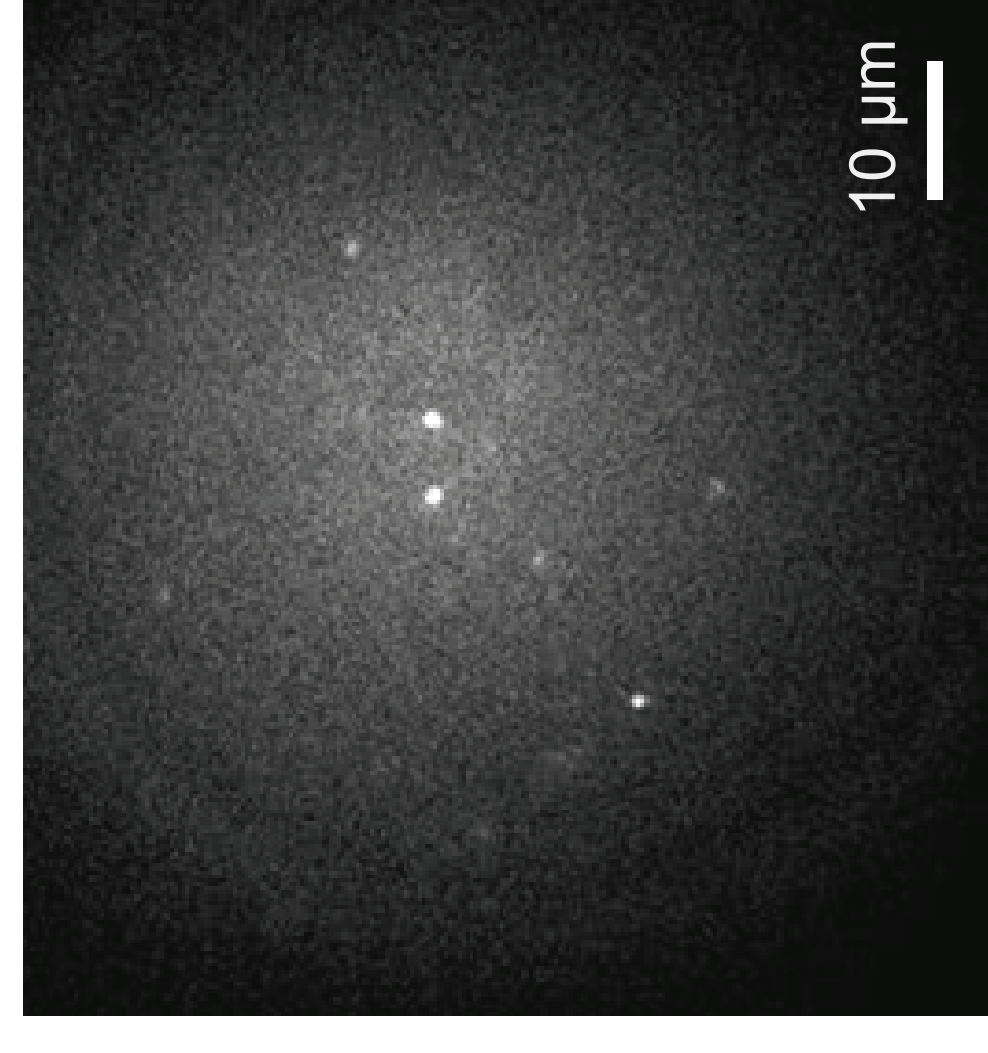
Spectral Shift FRET



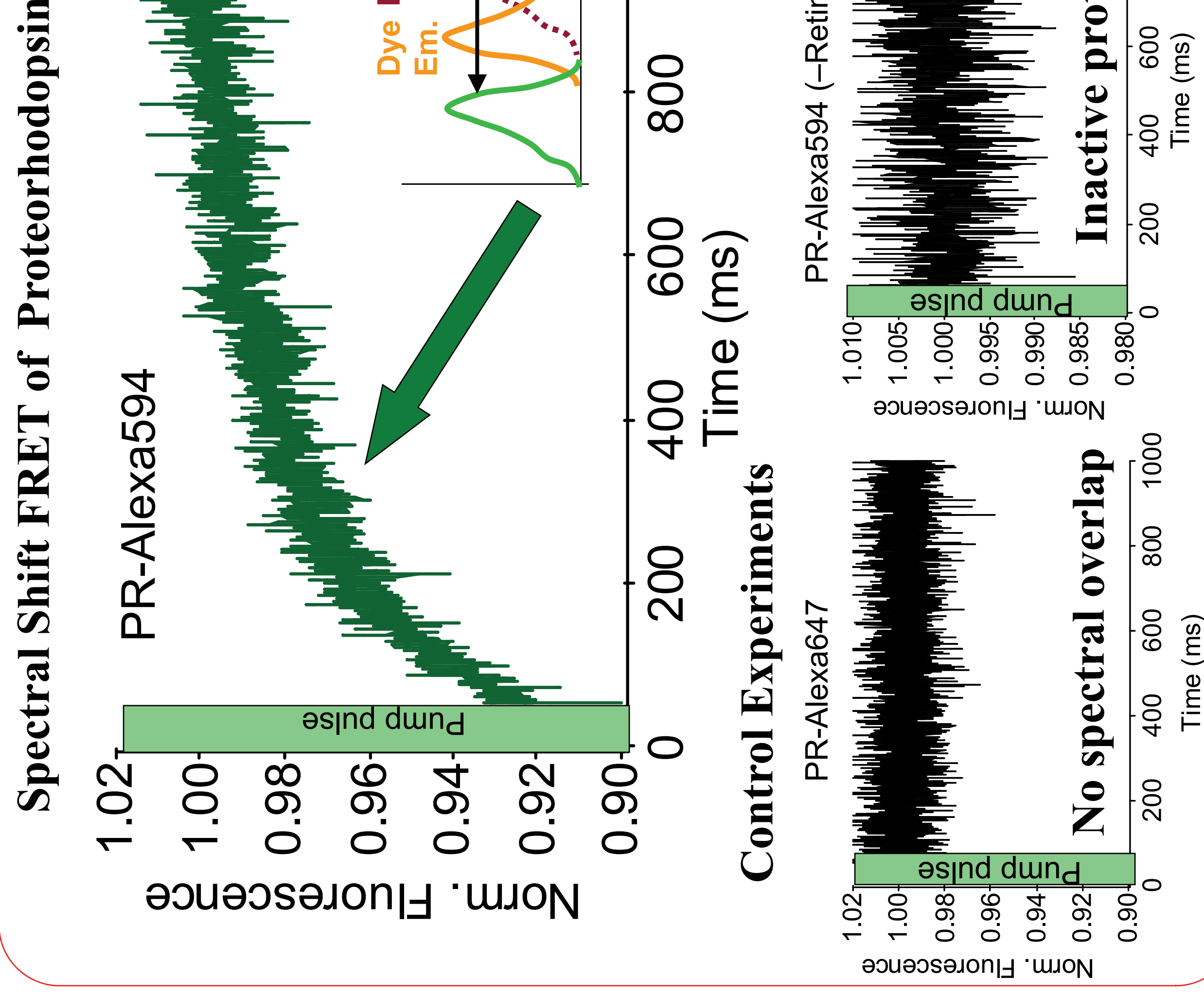
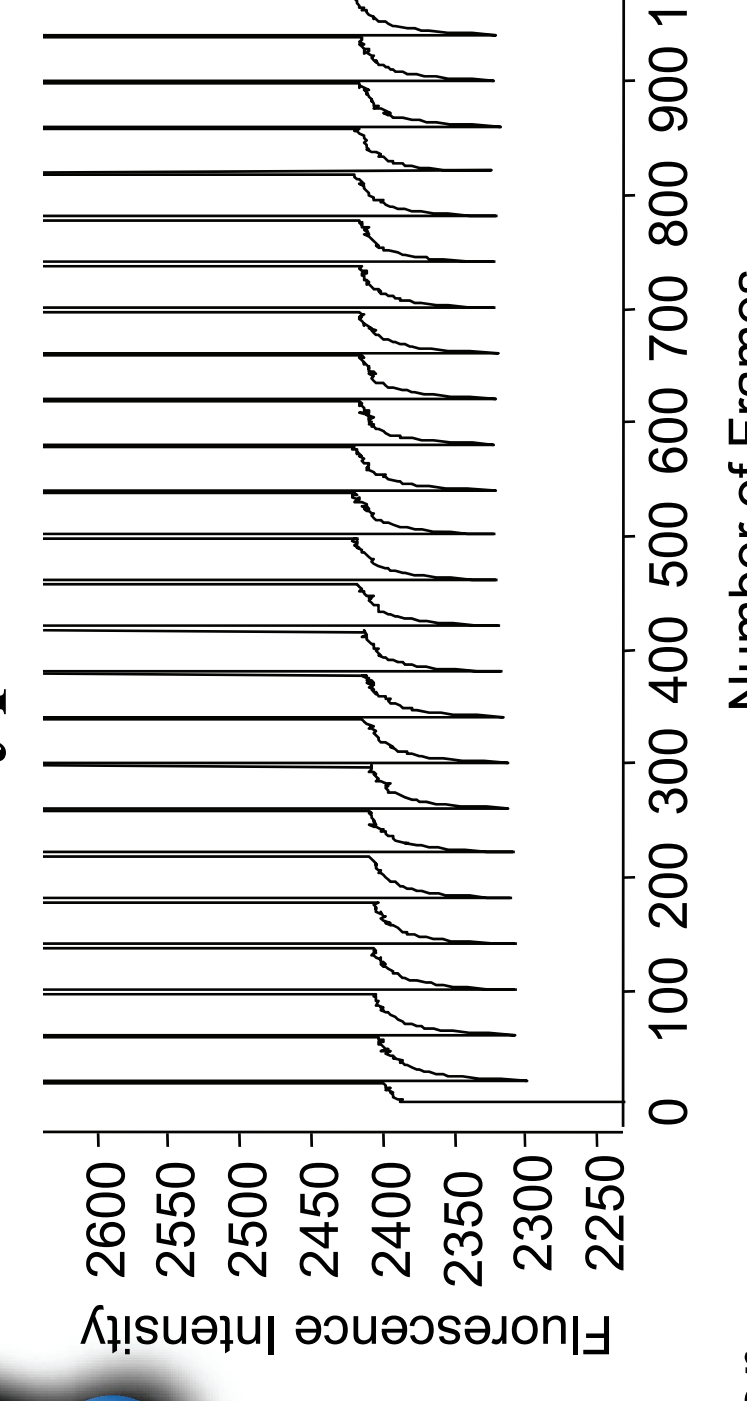
Energy Conversion in Oceans: Photocycle of PR in Proteobacteria



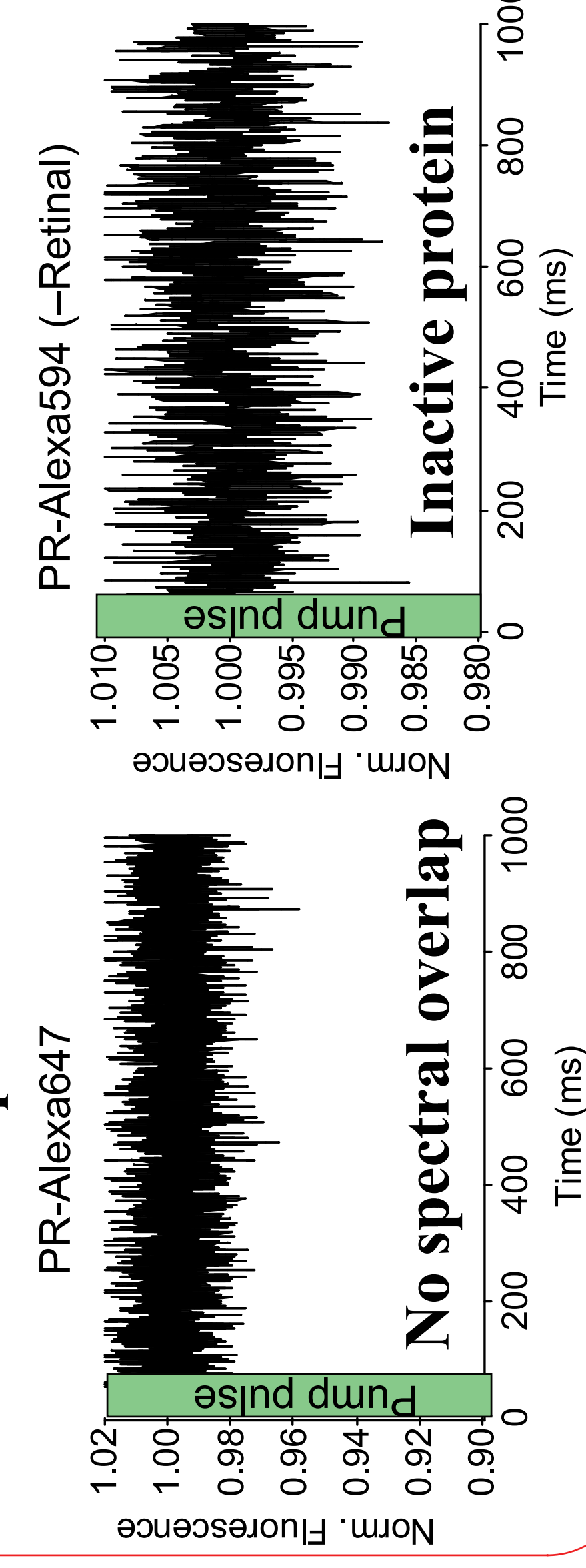
Vesicles with fluorescent PR



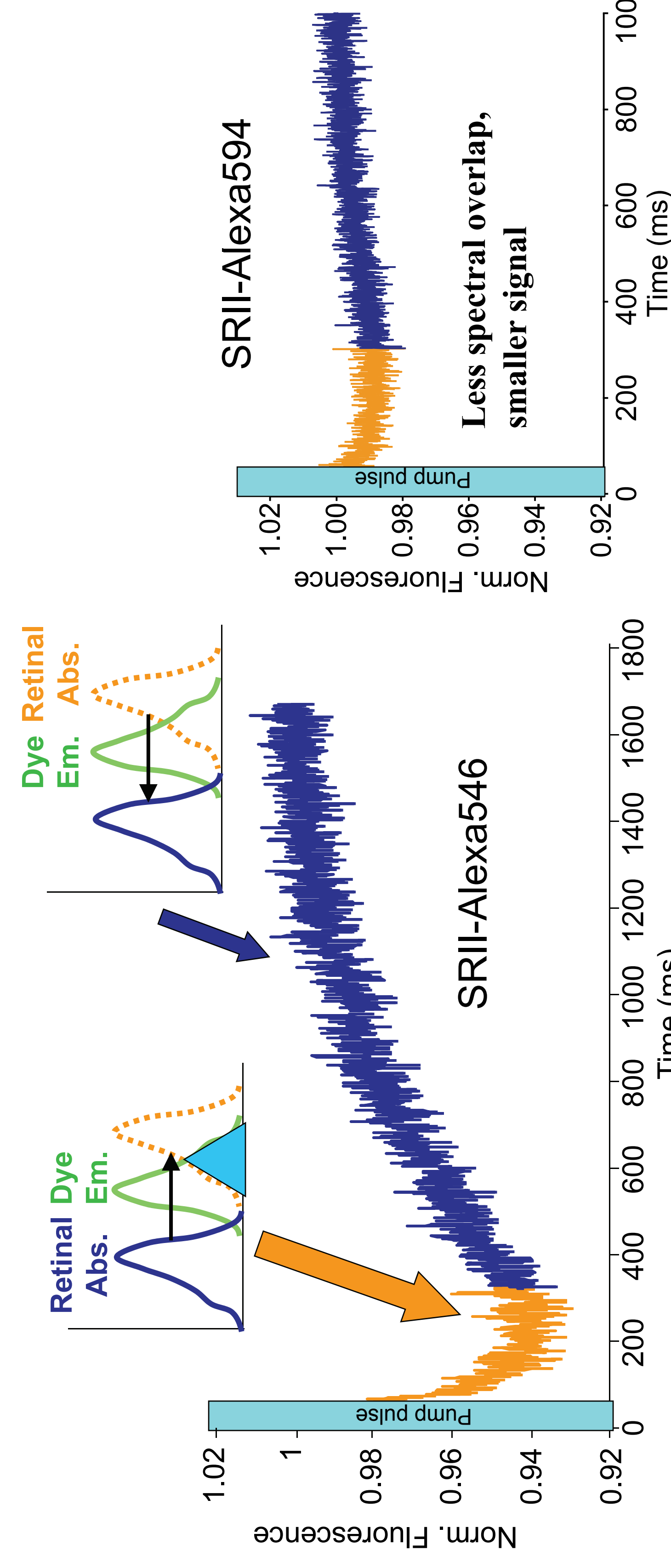
Typical Data



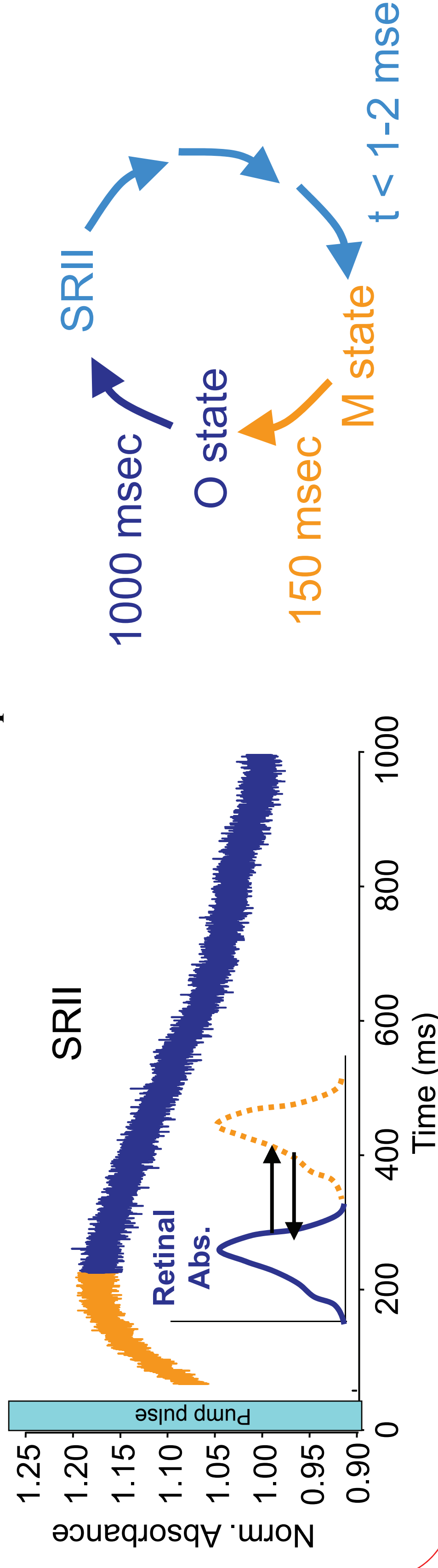
Control Experiments



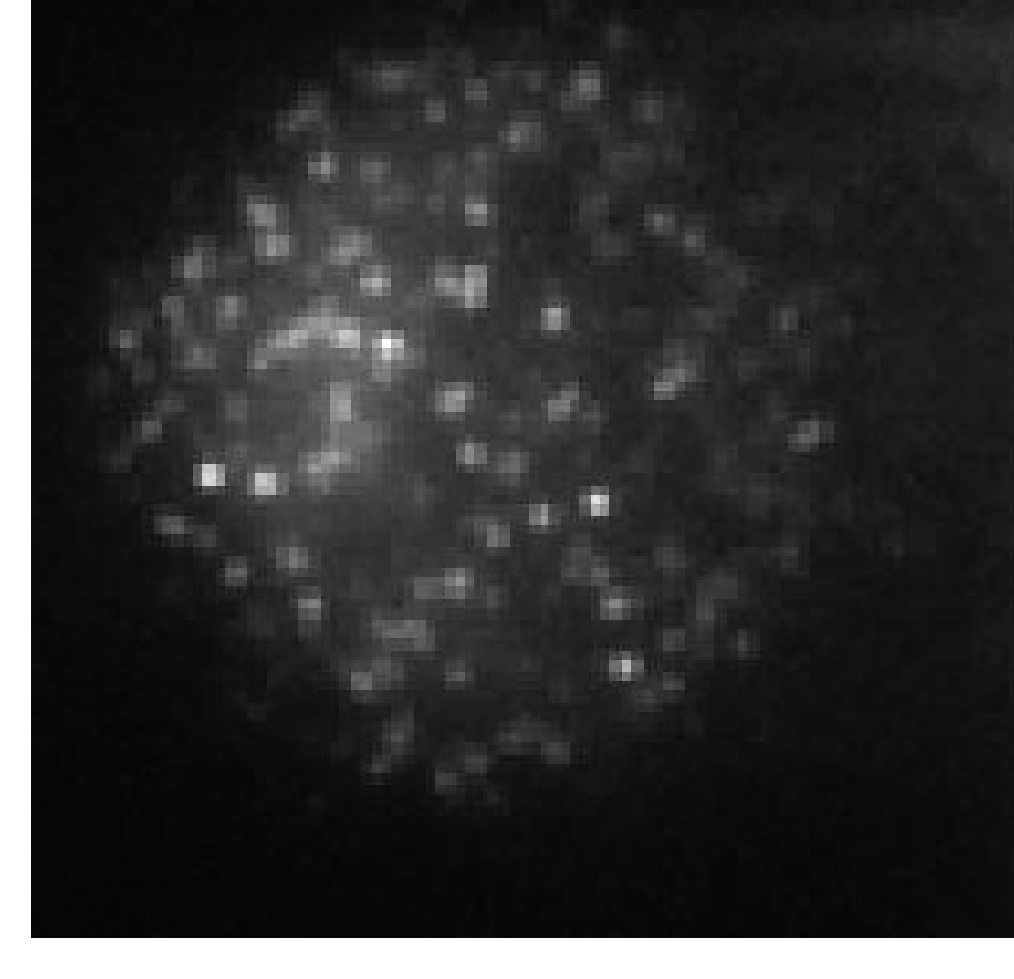
Spectral Shift FRET of Sensory Rhodopsin II



Conventional Transient Absorption Probed at 532 nm



Towards single-molecule dynamics...



Single-molecule image of Proteorhodopsin in lipid bilayer

Future work

1. Single molecule dynamics of photosensitive membrane proteins.
2. Studying the fluctuations in the dynamics of photosensitive proteins by using Anti-Brownian Electrokinetic Trap (ABEL trap).

Acknowledgments

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