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Real-Time, Nonlinear Optical Probe of Molecular Transport across Living *Escherichia coli* Cell Membranes JIA ZENG, HEATHER ECKENRODE, HAI-LUNG DAI, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323 — We will demonstrate for the first time that a nonlinear optical technique- Second Harmonic Generation- can be used to monitor, with real time resolution, the transport of a molecule across the membranes of a living cell. The transport of the hydrophobic ionic dye molecule malachite green (MG) through both membranes of the gram-negative bacteria *Escherichia coli*, the outer membrane and the cytoplasmic membrane, has been studied. A kinetic model, assuming that the MG molecules penetrate the bacteria outer membrane through classic porin channels while transport across the cytoplasmic membrane is by diffusion through the phospholipid bilayer, is proposed to account for experimental observations. Analysis of the SHG data enables quantitative determination of the transport rate constants and the adsorption equilibrium constants for the *Escherichia coli* cells living in different environments.

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