Abstract Submitted
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Conformational Dynamics of the Receptor Protein Galactose/Glucose Binding Protein[1] TROY MESSINA, DAVID TALAGA, Rutgers, the State University of New Jersey, Department of Chemistry and Chemical Biology, Pistataway, NJ 08854 — We have performed time-correlated single photon counting (TCSPC) anisotropy and Stokes Shift measurements on bulk solutions of galactose/glucose binding protein. Site-directed mutagenesis was used to provide a single cysteine amino acid near the sugar-binding center of the protein (glutamine 26 to cysteine – Q26C). The cysteine was covalently labeled with the environmentally-sensitive fluorophore acrylodan, and a long-lived ruthenium complex was covalently attached to the N-terminus to provide a fluorescent reference. The TCSPC data were analyzed using global convolute-and-compare fitting routines over the entire glucose titration and temperature range to provide minimal reduced chi-squared values and the highest time resolution possible. Using a standard ligand-binding model, the resulting distributions show that the closed (ligand-bound) conformation exists even at zero glucose concentration. At 20°C, the relative abundance of this conformation is as high as 40%. The temperature dependence of this conformational study will be discussed and related to the ligand-binding free energy surface.

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