

Abstract Submitted  
for the MAR07 Meeting of  
The American Physical Society

**High-throughput biophysics of functional tuning in photoactive yellow protein**<sup>1</sup> WOUTER HOFF, Dept of Microbiology and Molecular Genetics, Oklahoma State University, ANDREW PHILIP, GEORGE PAPADANTONAKIS, Dept of Biochemistry and Molecular Biology, University of Chicago, OSU TEAM, UOFC TEAM — The relationship between the structure of a protein and its function is a central unresolved problem in biology. We use photoactive yellow protein (PYP) to develop quantitative high-throughput methods to study this problem. PYP is a small bacterial photoreceptor with rhodopsin-like photochemistry based on its p-coumaric acid (pCA) chromophore. The absorbance maximum and pKa of the pCA in the active site of native PYP are shifted from 400 nm and 9.0 in water to 446 nm and 2.8 in the protein. Thus, PYP offers a unique model system to probe protein-ligand interactions. Here we show that high-throughput microscale methods can be used for quantitative biophysical studies of the absorbance spectrum PYP, its fluorescence quantum yield, apparent pKa of the pCA, protein stability against chemical denaturation, and kinetics of the last PYP photocycle step. A wide range of properties was observed among the mutants, and structural features that tune functional properties were identified. These results open the way for high-throughput quantitative biophysical studies of PYP.

<sup>1</sup>WDH is supported by NIH grant MG063805

Wouter Hoff  
Dept of Microbiology and Molecular Genetics, Oklahoma State University

Date submitted: 20 Nov 2006

Electronic form version 1.4