## Steady-State P800 Oxidation Kinetics Indicate That Heliobacterial Phototrophy is Light-Limited

Steven P. Romberger<sup>1,\*</sup>, Tayleigh Price<sup>2,3</sup>, Hallie Chavez<sup>1,4,5</sup>, Alysa Giudici<sup>1,2,6</sup>, Alexus Acton<sup>1,2,7</sup>, and Meaghan Stafford<sup>1,8</sup>

<sup>1</sup> Chemistry Department, Hiram College, Hiram, OH 44234, USA
<sup>2</sup> Biology Department, Hiram College, Hiram, OH 44234, USA
<sup>3</sup> Present address: Physician Assistant Program, Baldwin Wallace University, Berea, OH 44017,

USA

<sup>4</sup> Present address: Materials Science & Engineering Department, Case Western Reserve

University, Cleveland, OH 44106, USA

<sup>5</sup> Present address: ASM International, Novelty, OH 44073, USA

<sup>6</sup> Present address: College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

<sup>7</sup> Present address: Medpace Inc., Cincinnati, OH, 45277, USA

<sup>8</sup> Present address: Richard A. Gillespie College of Veterinary Medicine, Lincoln Memorial

University, Harrogate, TN 37752, USA

\*to whom correspondence should be sent: rombergersp@hiram.edu

The heliobacteria are a family of phototrophic bacteria known for their unique production of bacteriochlorophyll g and for their use of the simplest known Type I reaction center. In this work, we characterize P800 oxidation kinetics in whole cells of Heliomicrobium modesticaldum under the continuous illumination that is more consistent with in vivo conditions, an area of research that remains largely unexplored. When assayed at 800 nm, whole cells display a large bleaching immediately upon illumination by actinic light, corresponding to the production of P800<sup>+</sup>. The initial bleaching typically reaches a maximum intensity at 10 - 30 ms, at which point a slower, partial recovery leads to a steady-state that is smaller than the initial bleaching. The effects of charged redox reagents, in particular ferric ammonium citrate, and the cytochrome bc complex inhibitor azoxystrobin, demonstrate that this recovery phase is due to forward donation to  $P800^+$  from cytochrome c. A steady-state kinetics analysis comparing the effects of actinic intensity on the rate of P800 oxidation to that of P700 oxidation in spinach chloroplasts and whole cells of *Synechococcus* sp. PCC 7002, suggest that the heliobacterial reaction center is inherently light-limited. In support of a light-limited model, light saturation profiles of untreated cells compared to those treated with ferric ammonium citrate indicated that only 32% of the P800 pool is oxidized during continuous illumination. Taken together, these results indicate that, in stark contrast to all other known phototrophs, phototrophy in the heliobacteria is light-limited.