ELECTRONIC SUPPORTING INFORMATION

Fluorescence lifetime microscopy reveals the biologically-related photophysical heterogeneity of oxyblepharismin in light-adapted (blue) *Blepharisma japonicum* cells

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ABSTRACT: The step-up photophobic response of the heterotrich ciliate *Blepharisma japonicum* is mediated by a hypericinic pigment, blepharismin, which is not present in any of the known six families of photoreceptors, namely rhodopsins, phytochromes, xanthopsins, cryptochromes, phototropins, and BLUF proteins. Upon irradiation, native cells become light-adapted (blue) by converting blepharismin into the photochemically stable oxyblepharismin (OxyBP). So far, OxyBP has been investigated mainly from the photophysical point of view *in vitro*, either alone or complexed with proteins. In this work, we exploit the vivid fluorescence of OxyBP to characterize its lifetime emission in blue *B. Japonicum* cells, on account of the recognized role of fluorescence lifetime to provide physicochemical insights on the fluorophore environment at nanoscale. In the biological context, OxyBP modifies its emission lifetime as compared to isotropic media. The phasor approach to fluorescence lifetime microscopy in confocal mode highlights that fluorescence originates from two excited states whose relative balance changes throughout the cell body. Additionally, Cilia and kinetids, i.e. the organelles involved in photomovement, display lifetime asymmetry between the anterior and posterior part of the cell. From these data, some hypotheses on the phototransduction mechanism are proposed.

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1. FIGURES

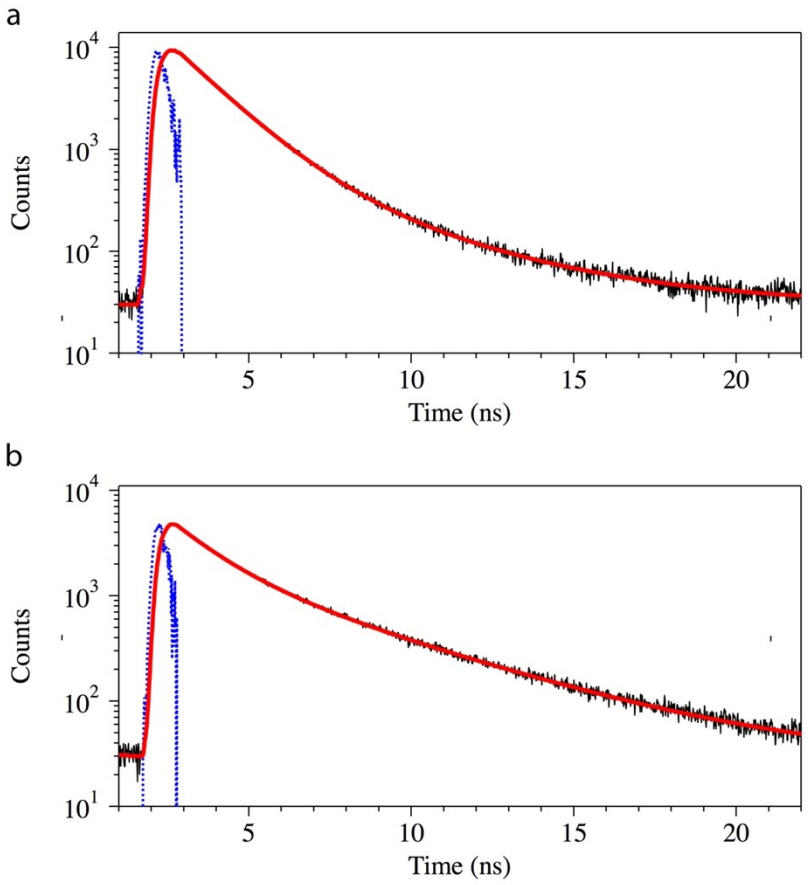

Figure S1. Time-correlated single photon counting (TCSPC) measurement of fluorescence emission decay of OxyBP in (a) CH_3OH and (b) bBJ cells. Black trace: TCSPC data; blue trace: instrument response function; red trace: bi-exponential fit.