# HIGH-RESOLUTION SPECTRAL AND FLUORESCENCE LIFETIME IMAGING OF YET UNKNOWN LIGHT-SENSING PROTEIN: SUBCELLULAR HETEROGENEITY IN LIGHT-ADAPTED (BLUE) *BLEPHARISMA JAPONICUM*

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In billion years, Nature has evolved various molecular solutions to transduce light into a biological signal. Most of the known light-sensing proteins belong to one of the following six families: rhodopsins, phytochromes, xanthopsins, cryptochromes, phototropins, and BLUF proteins [1]. Yet, the chromoprotein thought to be responsible for the step-up photophobic response of *Blepharisma japonicum* nevertheless belongs to a different and ignote family. It is solely known that the photobehavior is driven by the benzodianthronic molecule blepharismin, which is distributed all over the cell body either free or confined in pigment granules, coloring the ciliate in red [2]. Notably, upon continuous irradiation with white light of moderate intensity (3-30 $W/m^2$), BJ photoconverts into a blue, light-adapted form (bBJ). The blue color stems from the photo-oxidation of blepharismin into the naftodianthronic molecule oxyblepharismin (OxyBP) [3]. Blue cells have the same photophobic response as native red cells and action spectroscopy experiments demonstrated that OxyBP works analogously as BP for eliciting the photoresponse [4].
Owing to the complete lack of knowledge about the chromoprotein, in our work we addressed one major question: are the nanoenviroments of OxyBP spatially homogenous or heterogenous in bBJ cells? The relevancy of this question stems from the hypothetical multiple functions of the chromophore in the ciliate cell [1]. Practically, we assessed whether the spectral and fluorescence lifetime features of OxyBP were homogeneously or heterogeneously distributed throughout the cell. Remarkably, we overcame the main drawback of conventional FLIM, i.e. the low number of photons per pixel, by the *phasor approach*, a fit-free graphic method [5] that we have extensively applied to nanoenvironmental analysis of living eukaryotic cells [6]. Our results show that OxyBP sees different nanoenvironments associated to distinguishable biological structures of the ciliate cell. At the same time OxyBP is embedded in physicochemical contexts quite dissimilar from isotropic media of different polarity and viscosity. These findings point towards specialized and organelle-related photochemical roles of OxyBP associated to one or more proteins.

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