Ultrafast spectroscopy and advanced data analysis of Oxyluciferin and its synthetic derivatives to understand the molecular mechanism of Bioluminescence

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The research interest in the amazing natural phenomenon bioluminescence – light emission from chemically produced excited states observed with bacteria, fungi and invertebrates, has been revived recently due to its immense potentials for application to novel ultrasensitive bioanalytical techniques, including in vivo bioluminescence imaging, gene expression and trace ATP analysis. One of the most studied bioluminescent reaction systems currently are bioluminescence beetles, particularly fireflies. Fireflies utilize the ground state of the heterocyclic acid D-luciferin as a substrate for enzymatic (protein luciferase) multistep oxidation to generate oxyluciferin ($OxyLH_2$) in its first excited state. Subsequent deexcitation produces visible photon, which is observed as bioluminescence. Due to alleged instability and lifetime about few nanoseconds, the spectrochemistry and structure of $OxyLH_2$ within luciferase in its exited state have still remained unexplored.

To understand dynamics of the emitter we undertook thoroughly stationary and ultrafast time resolved (fluorescence, absorption, infrared) studies of oxyluciferin and its derivatives in different environment and different state (solution and crystals). We also developed a strategy based on a powerful mathematical procedure (combining careful selection of model compounds with multiset data analysis) to disentangle individual spectra of the components of different chemical forms of oxyluciferin. For the first time, the different species (ground state and excited state) of the oxyluciferin system in aqueous solution were mathematically extracted from an extensive set of pH-dependent measurements [1]. Obtained results open also the perspective for purposefully designed emitters exhibiting optimized performance of the polarity-dependent emission and being applied as fluorescence probe in vitro and in cells.

References

[1] M. Rebarz et al., Chem. Sci., DOI: 10.1039/C3SC50715G (2013)