Contribution ID: 1734 Type: Oral

## Functional studies of archaeal cysteine-less LOV domain

Wednesday 30 October 2024 17:00 (15 minutes)

## Functional studies of archaeal cysteine-less LOV domain

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LOV (Light Oxygen Voltage) domains are sensor modules of photosensitive proteins widespread in bacteria, archaea and eukaryotes. Flavin-binding fluorescent proteins (FbFPs), derived from LOV domains with site directed mutagenesis, can be used as genetically encoded reporters in optogenetics [1] and microscopy [2]. Moreover, oxygen-independent fluorescence of FbFPs and their smaller size represent substantial advantages over green fluorescent protein (GFP). Consequently, FbFPs inclusion in the fluorescence reporter toolbox can be beneficial for imaging in anaerobic biological systems [3].

Conserved cysteine, that forms a covalent adduct with flavin chromophore, plays a crucial role in LOV domains signal transduction [4]. Hence, cysteine knock-out mutants are often used as negative controls in LOV photoreceptor studies. However, recent research [5] specifies that there are biologically active native cysteine-less LOV domains and their signaling mechanism is based on flavin chromophore reduction to the neutral semiquinone radical state.

In this work previously uncharacterized natural cysteine-less LOV domain of BAT (bacterio-opsin activator) protein from thermophilic haloarchaea *Halanaeroarchaeum sulfurireducens* was successfully cloned and expressed in *E.coli*, purified and loaded with excess of chromophore. Eventually we studied the photophysics of this LOV domain and verified photoinduced one electron reduction of flavin cofactor to the neutral semiquinone radical state.

## Literature list

- 1. Blue-light receptors for optogenetics / A. Losi, K. H. Gardner, A. Möglich // Chemical reviews. –2018. –Vol. 118.
- 2. LOV-based reporters for fluorescence imaging / A. M. Buckley et al. // Current opinion in chemical biology. –2015. –Vol. 27.
- 3. Flavin-based fluorescent proteins: emerging paradigms in biological imaging / A. Mukherjee, C. M. Schroeder // Current opinion in biotechnology. –2015. –Vol. 31.
- 4. Primary reactions of the LOV2 domain of phototropin studied with ultrafast mid-infrared spectroscopy and quantum chemistry / M. T. A. Alexandre et al. // Biophysical journal. -2009. -Vol. 97.
- $5. \ Signal\ transduction\ in\ light-oxygen-voltage\ receptors\ lacking\ the\ adduct-forming\ cysteine\ residue\ /\ Yee\ E.$
- F. et al. // Nature communications. -2015. -Vol. 6.

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Session Classification: Life Science

Track Classification: Life Science