OXYGEN DIFFUSION PATHWAYS IN MUTATED FORMS OF A LOV PHOTORECEPTOR FROM *METHYLOBACTERIUM RADIOTOLERANS*: A MOLECULAR DYNAMICS STUDY

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*Mr*4511 LOV (Light, Oxygen and Voltage) protein is a blue light sensing photoreceptor from *Methylobacterium radiotolerans*, binding flavin mononucleotide (FMN) as chromophore. Blue light activation of LOV domains triggers the reversible formation of a FMN-cysteine adduct by a photocycle that goes through the FMN excited triplet state. LOV domains can be engineered as fluorescent sensors and actuators for optogenetics and photomedicine [1].
First experimental data on *Mr*4511 LOV protein [2] indicate its high potential as a photosensitizer for singlet oxygen (SO) the cytotoxic reactive excited state of molecular oxygen, produced by diffusion limited energy transfer from the FMN triplet state. This feature is obtained after the single mutation of reactive cysteine C71, a change that prevents formation of the photoproduct. In addition, the lack of a tryptophan, conserved in ca. 75% of LOV domains and shown to strongly quench the FMN triplet lifetime (τ_T) in LOV proteins, allows for *Mr*4511 LOV a longer τ_T than for other LOV domains in C71S and C71G variants [2].
After an homology modeling of *Mr*4511 LOV, that has lead to a dimeric protein stabilized by the presence of a strong leucine zipper in the C-terminal helices, a mutation of the photocycle substrate cysteine into serine (C71S) has been introduced *in silico* to make it a SO photosensitizer, and the mutated form stability was tested by MD simulations.
Afterwards, both transient and persistent oxygen channels were detected and analysed both in the wt and in the mutated protein. Molecular oxygen was then placed both outer and into the chromophore cavity and potential diffusion pathways were explored with MD simulations, showing a high accessibility of the binding cavity and a high persistence of oxygen inside.
Mutations that might favor SO generation were designed based on their position with respect to the FMN and the oxygen channels, taking into account the ability of certain amino acids to quench FMN triplet state and SO. Therefore, C71S/Y61T and C71S/Y61S double mutants were generated *in silico* and their stability was checked. The analysis of their oxygen diffusion pathways showed an increased diffusion and persistence of oxygen molecules inside the binding cavity, indicating a promising model for SO photosensing and its biomedical and biophysical applications.

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[2] E. Consiglieri, et al., Photochem. Photobiol. Sci. 18 (2019): 2657–2660.

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