Disruption of the FMN-A524 interaction cascade and Glu513 induced collapse of the hydrophobic barrier promotes light induced Jα-helix unfolding in AsLOV2

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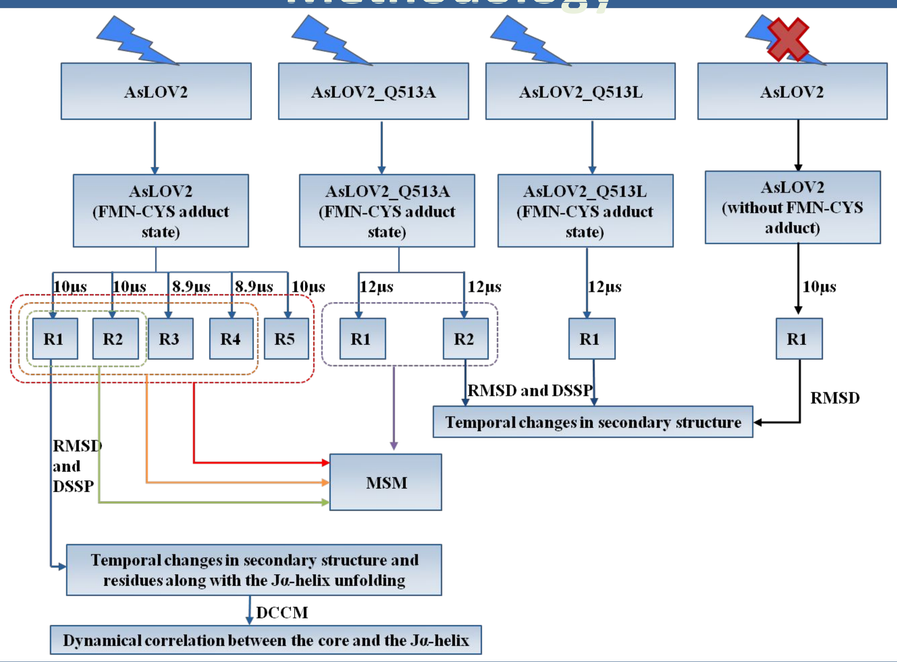
Abstract

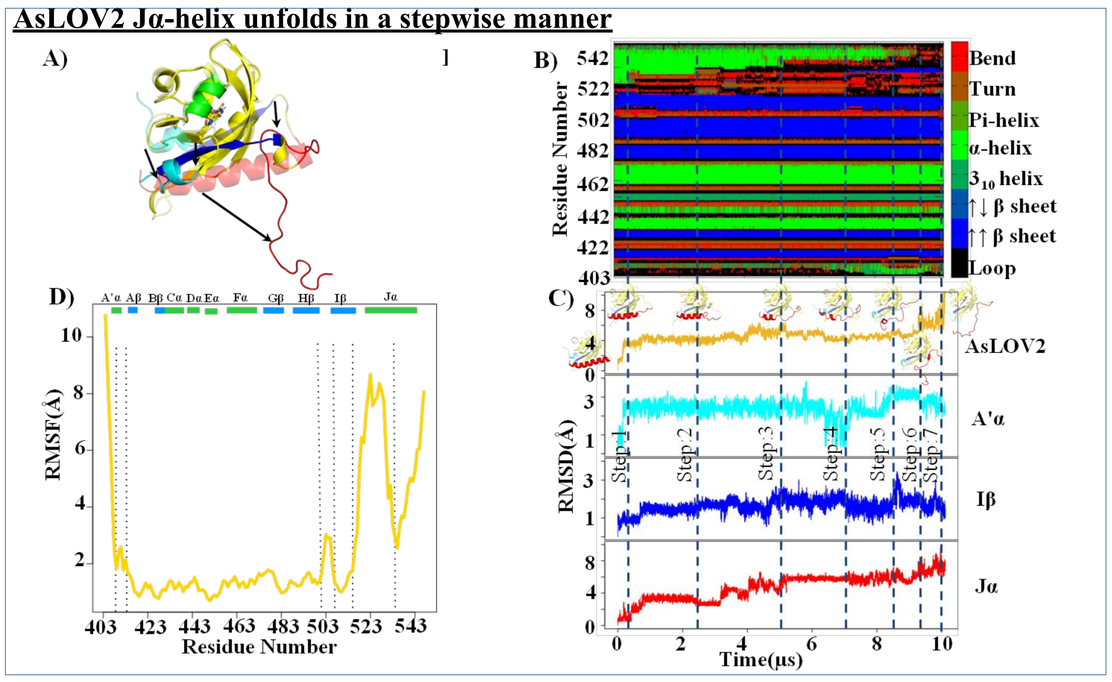
The C terminal Jα-helix of the *Avena Sativa*'s Light Oxygen and Voltage (AsLOV2) protein, unfolds on exposure to blue light. This characteristic seeks relevance in applications related to engineering novel biological photoswitches. Using Molecular Dynamics (MD) simulations and Markov State Modeling (MSM) approach we provide the mechanism that explains the stepwise unfolding of the Jα-helix. The unfolding was resolved into seven steps represented by the structurally distinguishable states distributed over the initiation and the post initiation phases. Wherein, the initiation phase occurs due to the collapse of the interaction cascade FMN-Q513-N492-L480-W491-Q479-V520-A524, the onset of the post initiation phase is marked by breaking of the hydrophobic interactions between Jα-helix and Iβ-strand. This study indicates that the displacement of N492 out of the FMN binding pocket, not necessarily requiring Q513, is essential for the initiation of the Jα-helix unfolding. Rather, the structural reorientation of Q513 activates the protein to cross the hydrophobic barrier and enter the post initiation phase. Similarly, the structural deviations in N482, rather than its integral role in unfolding, could enhance the unfolding rates. Further, the MSM studies on the wild type and the Q513 mutant, provide the spatio-temporal roadmap that layout the possible pathways of structural transition between the dark and the light states of the protein. Overall, the study provides insights useful to enhance the performance of AsLOV2 based photoswitches.

Methodology

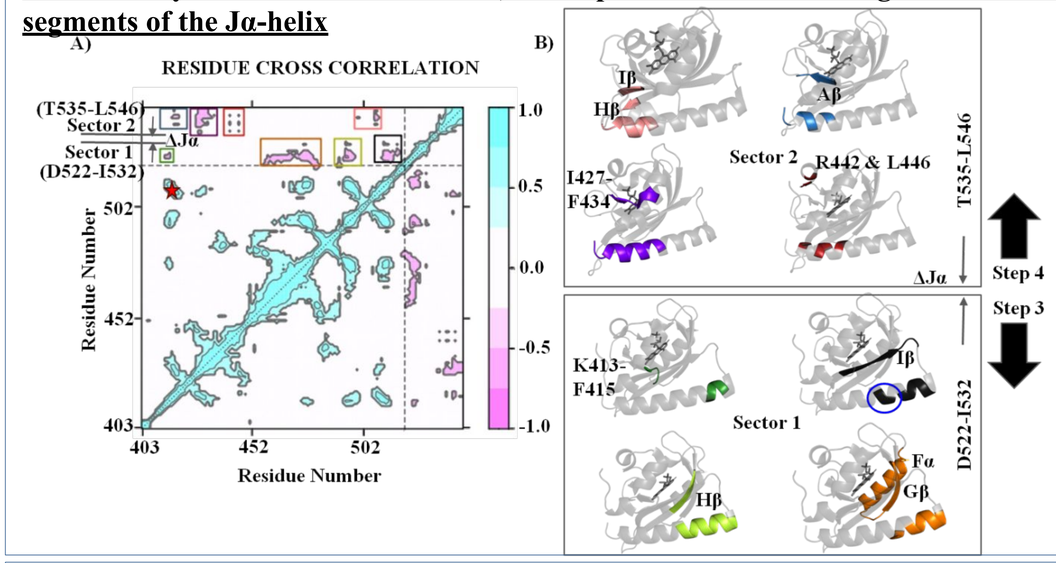


Results

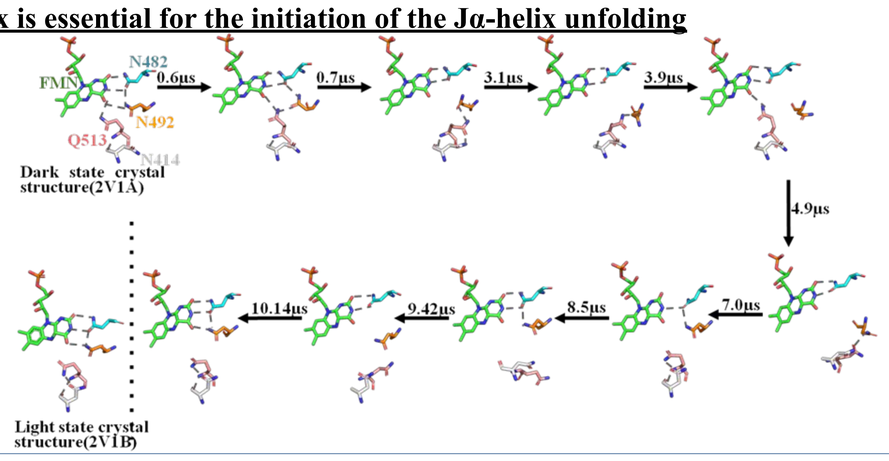
AsLOV2 Jα-helix unfolds in a stepwise manner



Localized dynamics in AsLOV2 core, corresponds to the unfolding of the different segments of the Jα-helix

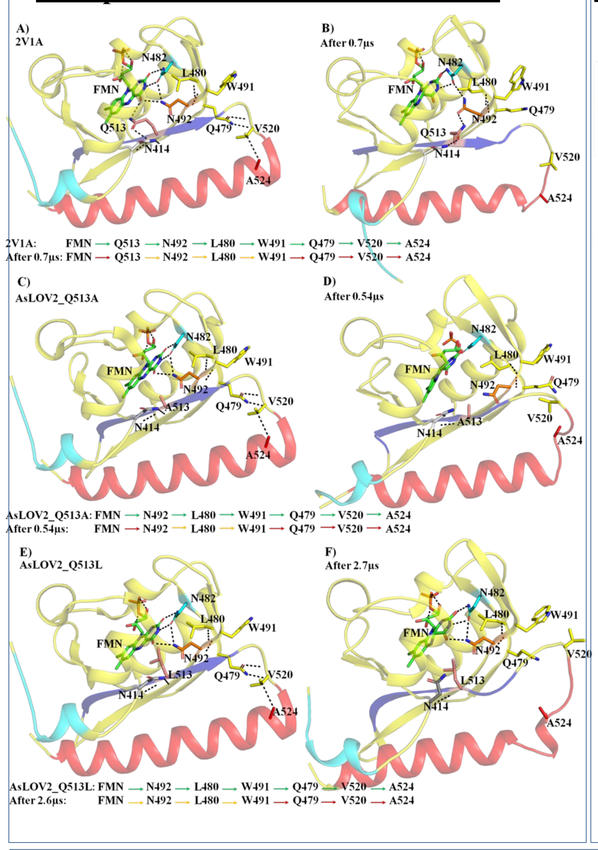


Disruption of the interaction cascade that links the FMN and the N terminal of the Jα-helix is essential for the initiation of the Jα-helix unfolding

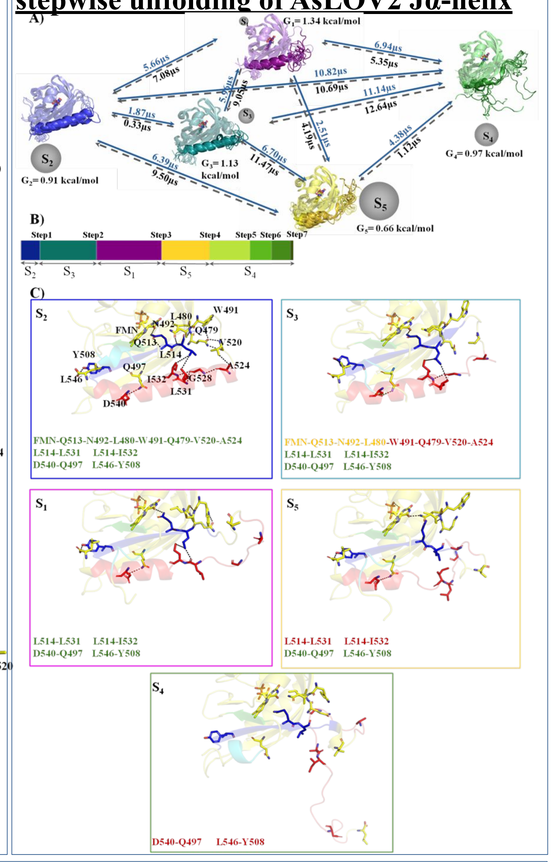


Results

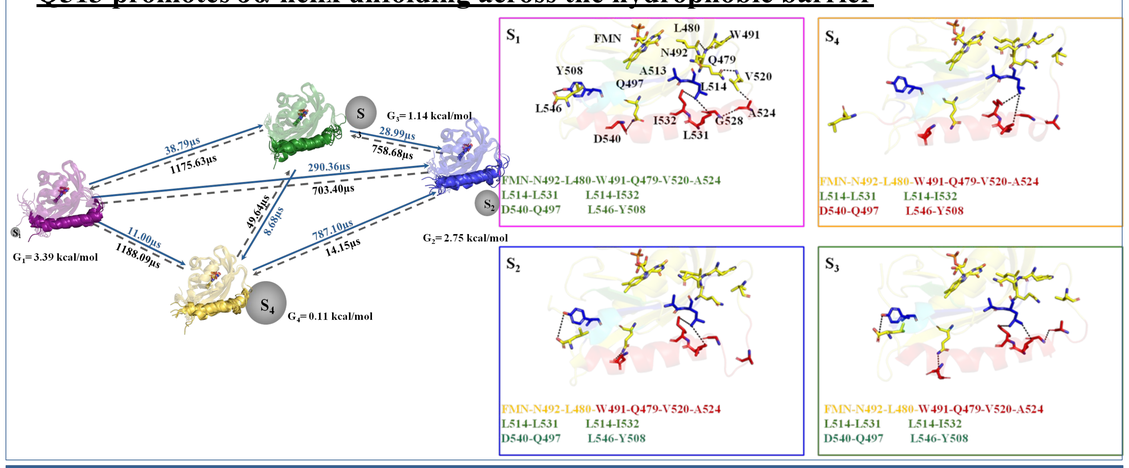
N492's transient displacement, irrespective of Q513 is sufficient for the disruption of the interaction cascade



Spatio-temporal road map showing pathways that lead to light activated stepwise unfolding of AsLOV2 Jα-helix



Q513 promotes Jα-helix unfolding across the hydrophobic barrier



Conclusions

Overall, the study provides the spatio-temporal roadmap for the light induced Jα-helix unfolding of AsLOV2. This work highlights two potential features (i) the disruption of the interaction cascade L480-W491-Q479-V520-A524 between the FMN and the $J\alpha_N$ segment is essential for initiation of unfolding and (ii) Q513 reorientation promotes efficient unfolding through activating the protein to bypass the hydrophobic barrier. The structural insights discussed here are imperative and useful due to the wider role of LOV2 domains in development of efficient photoswitches and its applications in optogenetics (1-8).

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References

1. Gil, A.A., C. Carrasco-López, L. Zhu, E.M. Zhao, P.T. Ravindran, M.Z. Wilson, A.G. Goglia, J.L. Avalos, and J.E. Toettcher. 2020. Optogenetic control of protein binding using light-switchable nanobodies. *Nat Commun*. 11:4044.
2. Hartzell, E.J., J. Terr, and W. Chen. 2021. Engineering a Blue Light Inducible SpyTag System (BLISS). *J. Am. Chem. Soc.* 143:8572–8577.
3. Losi, A., K.H. Gardner, and A. Möglich. 2018. Blue-Light Receptors for Optogenetics. *Chem Rev*. 118:10659–10709.
4. Lungu, O.I., R.A. Hallett, E.J. Choi, M.J. Aiken, K.M. Hahn, and B. Kuhlman. 2012. Designing photoswitchable peptides using the AsLOV2 domain. *Chem Biol*. 19:507–517.
5. Niopek, D., D. Benzinger, J. Roensch, T. Draebing, P. Wehler, R. Eils, and B. Di Ventura. 2014. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. *Nat Commun*. 5:4404.
6. Niopek, D., P. Wehler, J. Roensch, R. Eils, and B. Di Ventura. 2016. Optogenetic control of nuclear protein export. *Nat Commun*. 7:10624.
7. Polstein, L.R., and C.A. Gersbach. 2012. Light-inducible spatiotemporal control of gene activation by customizable zinc finger transcription factors. *J Am Chem Soc*. 134:16480–16483.
8. Strickland, D., K. Moffat, and T.R. Sosnick. 2008. Light-activated DNA binding in a designed allosteric protein. *Proceedings of the National Academy of Sciences*. 105:10709–10714.