ENGINEERING A BLUE LIGHT INDUCIBLE SPYCATCHER SYSTEM (BLISS) AS A TOOL FOR PROTEIN PHOTOPATTERNING AND OPTOGENETICS

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The SpyTag-SpyCatcher protein conjugation system has recently exploded in popularity due to its fast kinetics and high yield under biologically favorable conditions in both *in vitro* and intracellular settings. We imagine we can further expand the utility of this system by introducing the ability to spatially and temporally control the conjugation event. Taking inspiration from photoreceptor proteins in nature, we designed a method to integrate light dependency into the protein conjugation reaction. The light-oxygen-voltage 2 domain of *Avena sativa* (AsLOV2) undergoes a dramatic conformational change in response to blue light. We have thus genetically fused the SpyTag into the AsLOV2 domain to create a Blue Light Inducible SpyCatcher System (BLISS). In this design (Figure 1), the SpyTag is blocked from reacting with the SpyCatcher in the dark, but upon irradiation with blue light, the Jα-helix of the AsLOV2 undocks to expose the SpyTag. We screened several likely insertion points in the Jα-helix, and found a variant with desirable light switching behavior where after one hour of irradiation, the reaction is 80% complete, while only 10% of the AsLOV2-SpyTag protein reacted in the dark. This reaction can be quenched within minutes by returning the reaction to the dark. We demonstrated the spatial aspect of this light control mechanism through photopatterning proteins onto Ni-NTA coated slides. As our system is made solely from protein components, which can be genetically encoded, we can extend the same spatiotemporal control of proteins inside cells. We anticipate BLISS will be a strong tool for fabricating protein microassays, crafting biomaterial composition, as well as optically controlling enzyme activity and protein localization in cells.

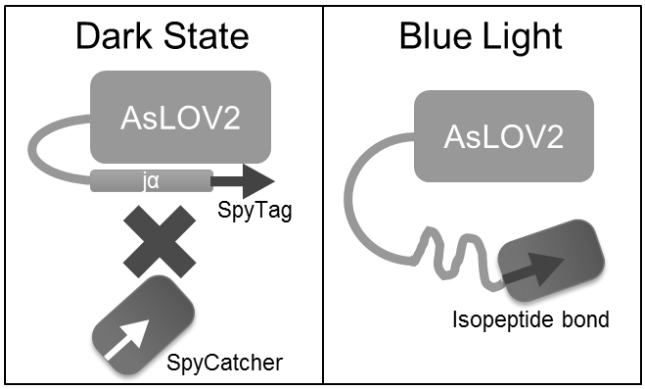

Figure 1 – BLISS design for photoactive protein conjugation