'SucCilators': A synthetic biology-based toolbox for light-controlled metabolic regulation

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The coordination of metabolic and bioenergetics processes between different cells and tissues is quintessential for proper function of the organs, especially under changing physiological conditions that alter the metabolic demand. Therefore, metabolic information needs to be exchanged between tissues and cells via networks of senso-regulatory proteins that orchestrate cellular metabolic functions in a process that is termed metabolic communication. Recent studies indicate that the Krebs cycle metabolites, succinate and citrate, act as key metabolites that are utilized by epithelial cells, immune cells and the gut microbiota for communication purposes. Specifically, in macrophages, Krebs cycle re-programming results in elevated succinate that shifts the cells to a pro-inflammatory state. Indeed, recent studies found high levels of microbiota metabolized succinate in inflammatory bowel disease (IBD) patients and showed that elevated succinate uptake into macrophages leads to chronic inflammation. Hence, clearance of high succinate from the gut lumen is a potential therapeutic target for IBD and other inflammatory diseases. We aim to engineer light responsive polypeptides focusing on encoding and fine-tuning light-responsive behavior in metabolic enzymes to control the homeostasis of specific metabolites. One of the approaches for engineering light-responsive behavior is by the incorporation of a photo-switchable group. Among these, azobenzenes – a class of molecules known to undergo reversible light-based. Upon irradiation with light of the appropriate wavelength (λtrans->cis), the unsubstituted azobenzene molecule undergoes a dramatic switch from the trans to the cis configuration. Importantly, this process is reversible. To develop therapeutic and research tools for manipulating pro inflammatory metabolites, we propose to incorporate azobenzene-based light responsive sAAs into the sequence of the succinate binding enzyme, succinate dehydrogenase (SDHA), with the goal of manipulating the enzymatic activity and substrate binding ability with light. A published structure of SDHA (pdb 6VAX) is used to decide the position and identity of amino acid residues for azobenzene-sAA incorporation. 6 constructs of different mutations were tested. Although all the 6 constructs were able to express, only 3 mutated SDHA were light responsive. In these mutated SDHA constructs, irradiation with UV (cis configuration) caused a decrease in the SDHA activity.