Tunable Thermal Bioswitches as a Control Modality for Next Generation Therapeutics

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ABSTRACT

Synthetic biology is rapidly contributing to the field of therapeutic development to create increasingly potent agents for the treatment of a variety of diseases. These living "designer therapeutics" are capable of integrating multiple sensory inputs into decision making processes to unleash an array of powerful signaling and effector responses. Included in the great therapeutic potential of these agents, however, is a cognate risk of severe toxicity resulting from runaway on-target or erroneously induced off-target activity. The ability to remotely control engineered therapeutic cells after deployment into patient tissue would drastically reduce the potential dangers of such interventions. However, among existing biological control methods, systemic chemical administration typically lacks the spatial precision needed to modulate activity at specific anatomical locations, while optical approaches suffer from poor light penetration into biological tissue. On the other hand, temperature can be controlled both globally and locally — at depth — using technologies such as focused ultrasound, infrared light and magnetic particle hyperthermia. In addition, body temperature can serve as an indicator of the patient's condition. Overall, temperature is a versatile signal which can provide a handle to actuate a biological response for the control of therapeutic agents.

In this thesis, a tunable and modular system is developed to respond to thermal perturbations in cellular environments and affect a biological response. At the core of this system is a pair of single-component thermosensing proteins whose dimerization is strongly and sharply coupled to their thermal environment. These domains are first utilized in their native context as negative regulators of transcription in prokaryotes, wherein they are integrated into genetic circuits to control expression of reporter genes. These gene circuits show strong and sharp thermal activation and can be utilized in multiplex to affect higher order logical operations. Cells imbued with these circuits demonstrate transcriptional activation upon global thermal elevation within the host animal within which they reside (fever) or upon a spatiotemporally localized temperature shift imparted by focused ultrasound hyperthermia. In subsequent work, one of these bioswitches is introduced into mammalian cells where it functions as a modular Protein-Protein Interaction (PPI) domain, conferring temperature-dependent protein localization.

The work conducted in this thesis demonstrates the feasibility of utilizing temperature as a stimulus for biological activity. This technology can be harnessed to regulate therapeutically relevant processes in bacterial and mammalian cells such as transcriptional regulation and protein localization, and potentially broader protein function. The thermal bioswitches described herein could be utilized to engineer an array of research tools and biological therapies with actuation driven by spatiotemporally precise noninvasively applied stimuli or by real-time sensing of host conditions.

PUBLISHED CONTENT AND CONTRIBUTIONS

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D.P. participated in the conception of the project, constructed and tested genetic circuits in vitro and in vivo, prepared the data, and participated in the writing of the manuscript.

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D.P. participated in the writing of the manuscript.

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