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A biomolecular circuit for high-performance in vivo biosensing: monitoring of copper levels in Wilson Disease

Copper is an essential trace element tightly regulated within organisms, particularly in hepatocytes, where the ATPase Copper Transporting Beta protein (ATP7B) maintains homeostasis. Mutations in ATP7B result in Wilson disease (WD), causing harmful copper accumulation and organ dysfunction. Despite its importance, accurately measuring copper concentrations remains challenging due to low levels, with no techniques available for in vivo intracellular measurement. Thus, developing a biomolecular copper biosensor could revolutionize drug screening and gene therapy monitoring in animal models. Here, we applied theoretical results from Control Engineering to design a biomolecular circuit that yielded a high-performance copper biosensor in terms of minimal leakiness, and significant fold change. Building on our previous work [https://doi.org/10.1101/2023.09.20.558637], we engineered an adenoviral vector carrying a synthetic gene circuit. This circuit included an artificial transcription factor (rtTA) controlled by a Metal Responsive Elements promoter. The rtTA drives the expression of Secreted Alkaline Phosphatase (SEAP), modified at its 3'UTR with a Direct Repeat sequence recognized by the CRISPR-Cas endoribonuclease CasRx that is under the control of a CMV/TO promoter. We transduced Hek293T cells with the adenoviral vector and confirmed that the SEAP concentration in the growth medium is proportional to the copper concentration, exhibiting extremely low leakiness in the absence of copper and about two orders of magnitude fold induction. We subsequently transduced the livers of both 6-week-old Atp7b knockout and wild-type mouses through systemic injections and are currently collecting blood samples over time up to 24 weeks to measure SEAP levels and assess the biosensor's ability to accurately measure copper accumulation in hepatocytes. Our work provides a unique solution for monitoring copper levels in animal models, thus aiding the preclinical studies of gene therapy approaches to WD. This could also be applied to animal models of different diseases by changing the copper-sensitive promoter to another metal-responsive one.

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