SYNTHETIC BIOLOGY

Tuning by degradation

A library of degrons allows tuning of half-life and expression levels of proteins in genetic circuits.

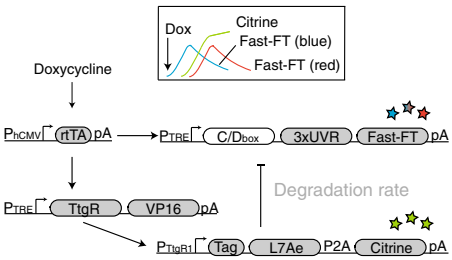
Martin Fussenegger at ETH Zurich is not a newcomer to synthetic biology. He has worked on designer cells, cells endowed with specific circuits that can sense and respond to stimuli, for many years with great success. But he came up against the same limitation time and again. "We were working on control switches for decades and we were always changing promoters and tuning operators, tandem repeats and so on, and all we got at the end was always the same dynamic range." Fussenegger said that they were resigned to the fact that high expression came with higher leakiness and therefore expression levels needed to be lowered to achieve tight control, but the dynamic range of the components was not tunable.

For some applications, such tuning is essential; for example, to create a genetic oscillator, a system that rhythmically turns on and off, one needs to precisely regulate the half-lives of its proteins. To achieve such precise regulation, Hélène Chassin, a PhD student in the Fussenegger lab, considered destabilizing tags (degrons). These short peptides carry signals that destine a protein for degradation, with the speed of degradation depending on the nature of the tag.

Chassin created a library of 30 degrons, each characterized by a fusion to a tetracycline (Tet)-dependent transactivator (tTA) and the expression of a reporter protein controlled by a Tet response element. She could modulate the dose–response curves of the Tet system up to 18-fold. Because the degrons depend only on the endogenous degradation machinery of a cell, their performance does not change with the protein they are fused to, which makes this a robust system that can be transferred to any protein of choice. The team is making the degrons, together with models predicting their dynamic range, available to interested users.

To showcase the power of the tools, the team designed pulse generators. These networks respond to an input, in this case doxycycline, by activating a reporter and a repressor for the reporter, both tagged with different degrons. Depending on the strength of the degrons, the pulse length and level of the reporter can be fine-tuned.

This ability is likely to also allow improvements to the recent designer cells that have come out of the Fussenegger lab. Among these are aroma cells to treat chronic pain (*Nat. Biomed. Eng.* **2**, 114–123; 2018). These cells initiate a signaling cascade that responds to spearmint aroma with the production of an analgesic peptide that blocks a voltage-gated sodium channel that triggers pain. Another set of designer cells mimic immune cells and express a genetic circuit that senses bacterial components and, in response, expresses an enzyme that lyses the bacteria (*Cell* **174**, 259–270; 2018). Yet another example is biomedical tattoos, cells that sense increased levels of calcium, common in the early stages of many cancers, and respond with the expression of melanin, which becomes visible as a 'synthetic mole' (*Sci. Transl. Med.* **10**, eaap8562; 2018).



A pulse generator tuned with degron tags. Adapted with permission from Chassin et al. (2019), Springer Nature.

All these applications were assembled with known components, and expression levels were adjusted through the modification of genetic components. Fussenegger predicts that incorporating degrons into designer cells and thereby changing their dynamic behavior will open more applications: "now we can really start to tune designer cells to provide the dynamics we require for specific therapeutic impact, for example, to have them record disturbances over long periods of time without producing false positives."

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Research papers

Chassin, H. et al. A modular degron library for synthetic circuits in mammalian cells. *Nat. Commun.* **10**, 2013 (2019).