Abstract citation ID: bvac150.1505

Steroid Hormones and Receptors

RF03 | PMON286

Engineering Steroid Receptors to Respond to Chemicals of Choice

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In synthetic biology, small molecules are commonly used for altering gene expression through the (in)activation of gene switches. Current switches, however, have a narrow species range, are non-titratable, or use non-inert inducers which evoke important side effects. Universal gene switches (XRs) that have dynamic ranges without interference with the host cell physiology will overcome these limitations. Ideal starting points for developing such XRs are the steroid receptors (SRs). The SRs are frequently applied as regulator domain or gene switch in organisms in which they are not naturally expressed, like microbes and plants. Several SRs, such as the estrogen receptor alpha (ERα), have been redesigned to respond to synthetic effectors but these variants require inducer amounts that are detrimental to the host or affect endogenous SRs.

By combining in silico modelling with directed evolution in the yeast Saccharomyces cerevisiae, we developed a platform and strategy for evolving SRs towards a chemical of

choice. Validation of this method was achieved by first evolving the ERα to employ tamoxifen as an agonist, yielding the preTERRA variant. Subsequently, activation by estradiol was removed, rendering a tamoxifen receptor (TERRA). We show that TERRA gets activated by subtoxic tamoxifen amounts while remaining insensitive for physiological estradiol levels. Further evaluation demonstrated TERRA to be transferable to mammalian cells. Ongoing research aims at converting the TERRA receptor into a regulatory domain for controlling nuclear translocation of Cre recombinases and Cas9 proteins in both cell lines and mouse models.

The same platform was employed for adapting ERα towards the plasticizer Bisphenol A. This yielded an ERα variant that is no longer inducible by estradiol but is responsive to lower concentrations of Bisphenol in yeast. The transferal to cell lines will provide us with an additional regulatory domain. The ability to engineer any SR towards a synthetic ligand of choice not only expands the synthetic biology toolbox but can find applications in landscaping SR-related drug resistance and ligand-inducibility.

Presentation: Saturday, June 11, 2022 1:48 p.m. - 1:53 p.m., Monday, June 13, 2022 12:30 p.m. - 2:30 p.m.