Plant hormone sensors as scaffolds for biosensor design

Most plant hormone sensors, including the abscisic acid receptor PYR1, function through chemically induced dimerization. Using computationally designed libraries of PYR1, we created high-affinity receptors for 21 structurally diverse ligands, setting the stage for large-scale small-molecule biosensor development

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The problem

Biosensors are at the core of many biotechnologies — from drug and metabolite diagnostics to inducible genetic circuits and cellular imaging technologies. To enable these applications, small-molecule biosensors have typically been co-opted from nature (including, for example, bacterial allosteric transcription factors[1] and G-protein-coupled receptors[2]) or designed from scratch (for example, computationally designed proteins[3]). Although impressive progress has been made in this area, creating biosensors for user-defined molecules is still a challenging and time-consuming process. Ideally, biotechnologists would be able to design new small-molecule biosensors in a matter of weeks to months, as in antibody development timelines.

One part of solving the biosensor problem is to identify versatile sensing scaffolds that can be easily reprogrammed to recognize new ligands and convert binding events into biological or chemical outputs. In chemically induced dimerization (CID), a ligand triggers the formation of a receptor–ligand–effector complex. CID systems are attractive as biosensors because they couple sensing and actuation.

The solution

As plants use several CID systems for signaling[4], we explored whether one specific module — the system that senses the plant stress hormone abscisic acid (ABA) — can be used as a scaffold for biosensor construction. In this system, ABA binds to the PYR1 receptor, triggering an allosteric change, which leads to the formation of a ternary complex between ABA–PYR1 and the phosphatase HAB1. Our previous work showed that ligand recognition by PYR1 could be reprogrammed with a small number of mutations;[5] however, the question of how broadly applicable this scaffold is for biosensor development had not been explored.

To investigate the programmability of PYR1, we computationally designed a library of PYR1 receptors harboring large numbers of mutations in binding pocket residues (Fig. 1a). The library was expressed in yeast and new sensors were isolated using yeast two-hybrid methods, which enable the growth-based selection of ligand-responsive receptors (Fig. 1b). The library was screened against 38 diverse natural and synthetic cannabinoids and organophosphate pesticides, which are important toxicological and environmental targets. These efforts identified 18 distinct mutant PYR1s that responded to 21 different chemicals. Seven of these PYR1 mutants were further mutated into high-affinity (in the nanomolar range) sensors and subsequently linked by molecular cloning to split transcription factors or enzymes to engineer cell-based and in vitro sensing applications. In addition, an ELISA-like diagnostic assay was built using a computationally optimized HAB1 variant and mutant PYR1 receptors. These systems enabled detection of analytes at picomolar to nanomolar concentrations (Fig. 1c). These data show that PYR1-derived biosensors are broadly compatible with various output modalities and establish PYR1 as a versatile new scaffold for biosensor design.

Future directions

The ability to design new biosensors for any molecule of interest would be truly transformative. For example, new drug tests could be rapidly developed and deployed in response to emergent chemical threats. Similarly, biosensing sentinel organisms (microbes or plants) could be rapidly designed to detect environmental contaminants. Isolating 21 small-molecule biosensors with relative ease from a single screen is a meaningful step towards creating a universal biosensor scaffold. However, a few key questions still need to be answered: most importantly, how broadly applicable the PYR1 scaffold is for different ligand classes. Large-scale screens of chemically diverse libraries should help to answer this question. Data from such screens will help to define the molecular features that work best with this new scaffold and, when combined with computational predictions, have the potential to empower the predictive design of biosensors for user-defined molecules.

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EXPERT OPINION

"The authors describe a new approach and demonstrate its ability to rapidly create a wide array of biosensors compatible with a diverse set of molecules. These biosensors have the potential to be the foundation for sensors of significant importance in the biomedical and agricultural sectors. Of potentially broader impact is this clearly documented approach combining computational and synthetic biology techniques, which could further expand on the molecules and biological systems described here." **Justin Siegel, University of California, Davis, CA, USA**

FIGURE

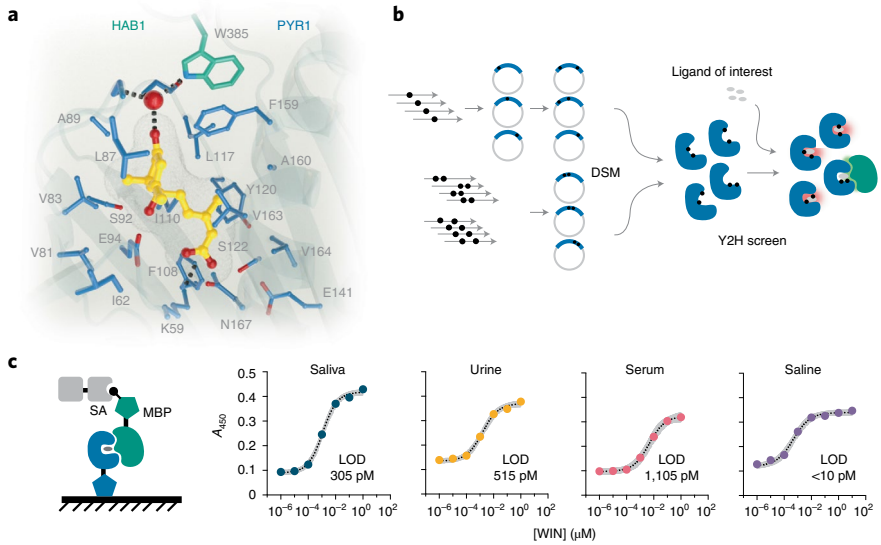

Fig. 1 | PYR1-based biosensors. a, The side chains of residues in the binding pocket of PYR1 that were targeted for mutagenesis, along with ABA (yellow) and the tryptophan residue (W385, green) of HAB1 that 'locks' HAB1 to PYR1. **b**, Sensor evolution pipeline. Binding with HAB1 (green) indicates that a specific mutant PYR1 (blue) binds the ligand of interest. DSM, double-site mutant; Y2H, yeast two-hybrid. **c**, ELISA-like assay using an engineered PYR1 mutant that recognizes the synthetic cannabinoid WIN 55,212-2. Assays were conducted in five-fold dilutions of saliva, urine, serum and saline. LOD, lower limit of detection; A_{450}, absorbance at 450 nm; SA, streptavidin; MBP, maltose binding protein.

BEHIND THE PAPER

On the basis of past work from the Cutler laboratory, we had known for some time that the PYR1 receptor could be engineered to respond to new ligands — specifically, the agrochemical oomyceticide mandipropamid and others[5]. We were initially unsure how far we could push molecular recognition with this CID system; we were skeptical about the odds of success with organophosphate ligands because of their relatively small size. We were pleasantly surprised by the number of new sensors isolated in our pilot screens, and this result emboldened us to develop the platform further. **I.W.**

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FROM THE EDITOR

"Several biomolecule families have been adapted as biosensors designed to bind to the target protein with high specificity, but each typically has a limited spectrum of ligand-binding possibilities and lacks flexibility in output modalities. The authors describe here how the plant hormone receptor PYR1 can be engineered with sensor–response functions that can be ported to a wide range of sensor modalities, providing a new scaffold for biosensor development." **Joao Duarte, Senior Editor, *Nature Biotechnology***