CHEMICALLY INDUCED DIMERIZATION MODULES AS A PLATFORM FOR PLANT BIOSENSOR ENGINEERING

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Key Words: Protein engineering, biosensors, plant biotechnology

Protein biosensors for small molecules have important applications in agriculture, medicine, and security, but it remains difficult to rapidly produce a high-affinity sensor for a given ligand. This is partly due to two major challenges. First, most small molecule ligands have only a small number of residues with which a protein can make energetically favorable contacts, making it difficult to engineer high-affinity binding. Second, even if a high-affinity binding protein is engineered, it is difficult to transduce the binding event into an output.

The majority of plant hormone perception occurs by chemically induced dimerization, where binding of the hormone to a soluble receptor causes a conformational change that allows the receptor to form a heterodimer with an interaction partner. These CID modules make an ideal platform for engineering small molecule biosensors because they naturally address the two primary challenges above: their unique architecture allows sensitive biosensors to be constructed from low-affinity receptors and protein dimerization provides a natural method of ligand binding transduction. The ability to engineer CID modules would lead directly to *in planta* biosensors and would also have broader applications to biosensor design in other biological systems.

Here we describe the development of a general biosensor engineering platform using the abscisic acid receptor PYR1 of *Arabidopsis thaliana*, which was previously engineered to sense the agrochemical mandipropamid.[1] We combine comprehensive mutagenesis[2,3], high-throughput screening, deep sequencing, and machine learning to rapidly construct a model of the fitness landscape for binding of PYR1 to a specific ligand. We then use this model to design a targeted library to screen for higher affinity sensors. For high-throughput screening, we use both an established yeast two-hybrid (Y2H) screen and a novel yeast surface display (YSD) system. These techniques offer complementary advantages: Y2H is straightforward to implement and requires no purified protein, while YSD offers higher throughput and more stringent quantification of protein-protein interactions. Finally, we describe early development of two additional CID modules from the gibberellin and strigolactone sensing networks of *A. thaliana*.

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