A modular receptor platform to expand the sensing repertoire of bacteria

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Abstract

Living cells can sense and process myriad signals in order to survive and reproduce. Using the tools of synthetic biology, researchers have started to modify cells to perform sophisticated biosensing, diagnostics or targeted therapies. However, these approaches are currently limited by the difficulty to detect molecules for which no receptor is found in nature.

Here we use ligand-induced dimerization of monomeric DNA-Binding Domains (split-DBDs) fused to an artificial Ligand-Binding Domain (LBD) to engineer modular receptors operating in bacteria. As a versatile LBD scaffold, we used VHH camelid antibodies (1), which can be engineered to detect many ligands of interest. We designed both cytosolic and transmembrane receptors and highlighted several principles that can be used to improve receptor behavior. Finally, using L-form bacteria (2) with an deficient outer membrane, we demonstrate that our transmembrane receptor can detect extracellular proteins and trigger downstream intracellular signaling.

Because of the versatility of antibody-based detection and the number of existing transcriptional regulators, we anticipate that our platform could be tailored to derive orthogonal receptors detecting many ligands of interest. Scalable detection systems using split-DBDs could be combined with existing sensors to support many applications including diagnostics, environmental monitoring, or cellular therapeutics.

Engineering of synthetic cytosolic biosensor

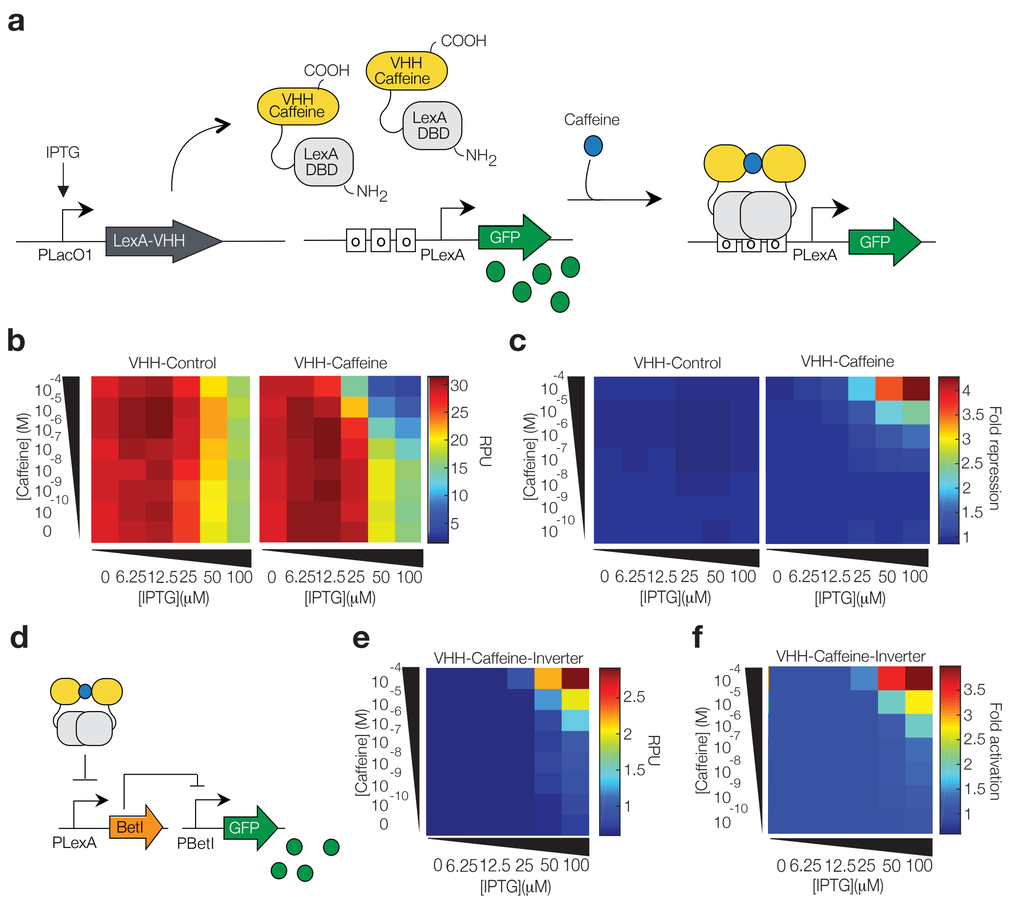

Figure 1. Engineering a synthetic cytosolic biosensor using a split DNA-binding domain. (a) Overview of the split-repressor system. LexA DBD was fused to a camelid VHH antibody that recognizes caffeine (VHH-Caffeine). The monomeric chimeric receptor is expressed in the cytosol upon IPTG induction. In the presence of caffeine, the chimeric receptor dimerizes and binds to the LexA operator, blocking expression of the reporter gene. (b) Response of cells containing the artificial receptors LexA-VHH-Caffeine and LexA-VHH-Control to increasing concentrations of caffeine at different expression levels. (c) Repression fold of the two LexA-VHH fusions. For each IPTG concentration, fold changes were calculated from (B) relatively to cells grown without caffeine (lower row). (d) General architecture of LexA-VHH-Caffeine connected to the BetI inverter. LexA-VHH-Caffeine controls BetI expression, which controls the expression of the reporter gene. (e) Caffeine response of cells containing the LexA-VHH-Caffeine receptor connected to the BetI inverter. (f) Fold activation of the LexA-VHH-Caffeine/BetI inverter circuit. Calculated as in (C). **These results demonstrate that split-DBDs can be activated via ligand-induced dimerization of antibody-based LBD.**

Engineering of synthetic transmembrane receptor

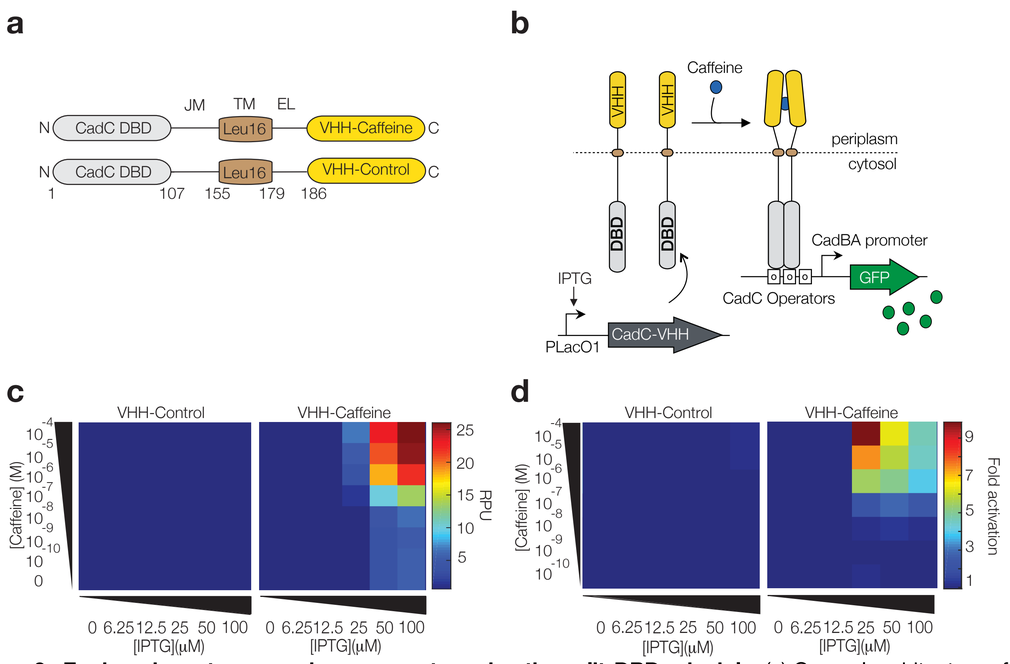

Figure 2. Engineering a transmembrane receptor using the split-DBD principle. (a) General architecture of transmembrane split-DBD system. The DBD and Juxtamembrane of the CadC transcriptional activator (3) were fused to an Leu(16)TM, an external linker, and VHH-Caffeine or VHH-Control as the LBD. (b) Principle of transmembrane receptor activation. Genes encoding CadC-VHH fusions are placed under the control of the pLacO1 promoter. The N-terminal CadC DBD is located in the cytosol and the C-terminal VHH in the periplasm. In the presence of caffeine, the chimeric receptor CadC-VHH-Caffeine undergo ligand-induced dimerization andactivates downstream reporter gene expression. (c) Response of CadC-VHH-Caffeine and CadC-VHH-Control to increasing concentrations of caffeine at different expression level. (d) Activation fold of the two CadC-VHH fusions. Fold changes were calculated from (C) as in Fig. 1. **These results demonstrate that a synthetic transmembrane receptor can be engineered by fusing split-DBDs with a periplasmic VHH scaffold.**

Tuning the Signal to Noise ratio of synthetic transmembrane receptor

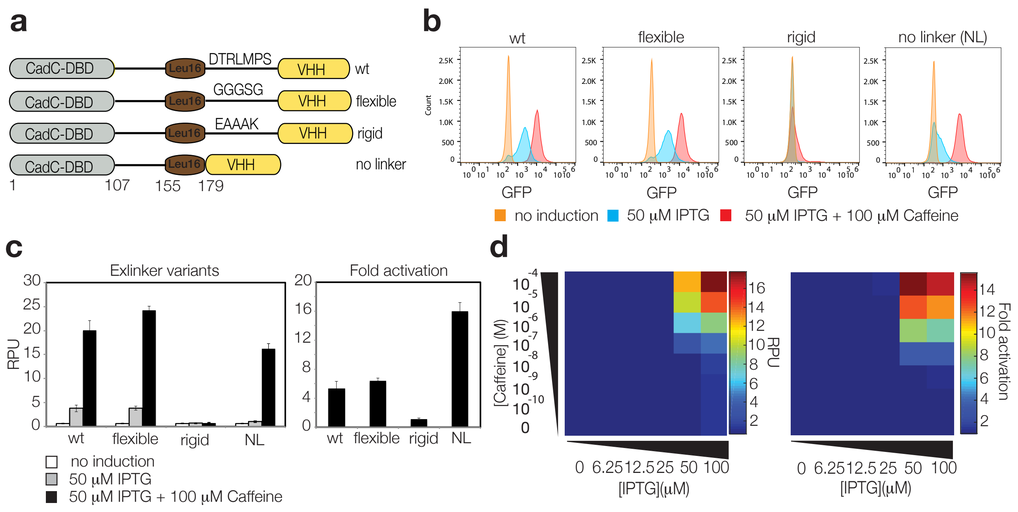

Figure 3. Optimizing transmembrane receptor signal-to-noise ratio through linker engineering. (a) Schematic diagram of transmembrane split-DBD receptor with different external linkers. (b) Flow-cytometry data from cells containing the different variants of transmembrane split-activators. (c) Quantification of the response to caffeine at different IPTG concentrations of cells containing different variants with RPUs (left panel) and fold change (right panel). (d) Response of the CadC-VHH-Caffeine NL variant to caffeine at different expression levels (left panel). Fold changes were calculated as in Fig. 1 (right panel). **These results demonstrate that chimeric transmembrane receptor response can be tuned by optimizing interdomain linker sequences.**

Detection of protein ligand with cell wall deficient L-form bacteria

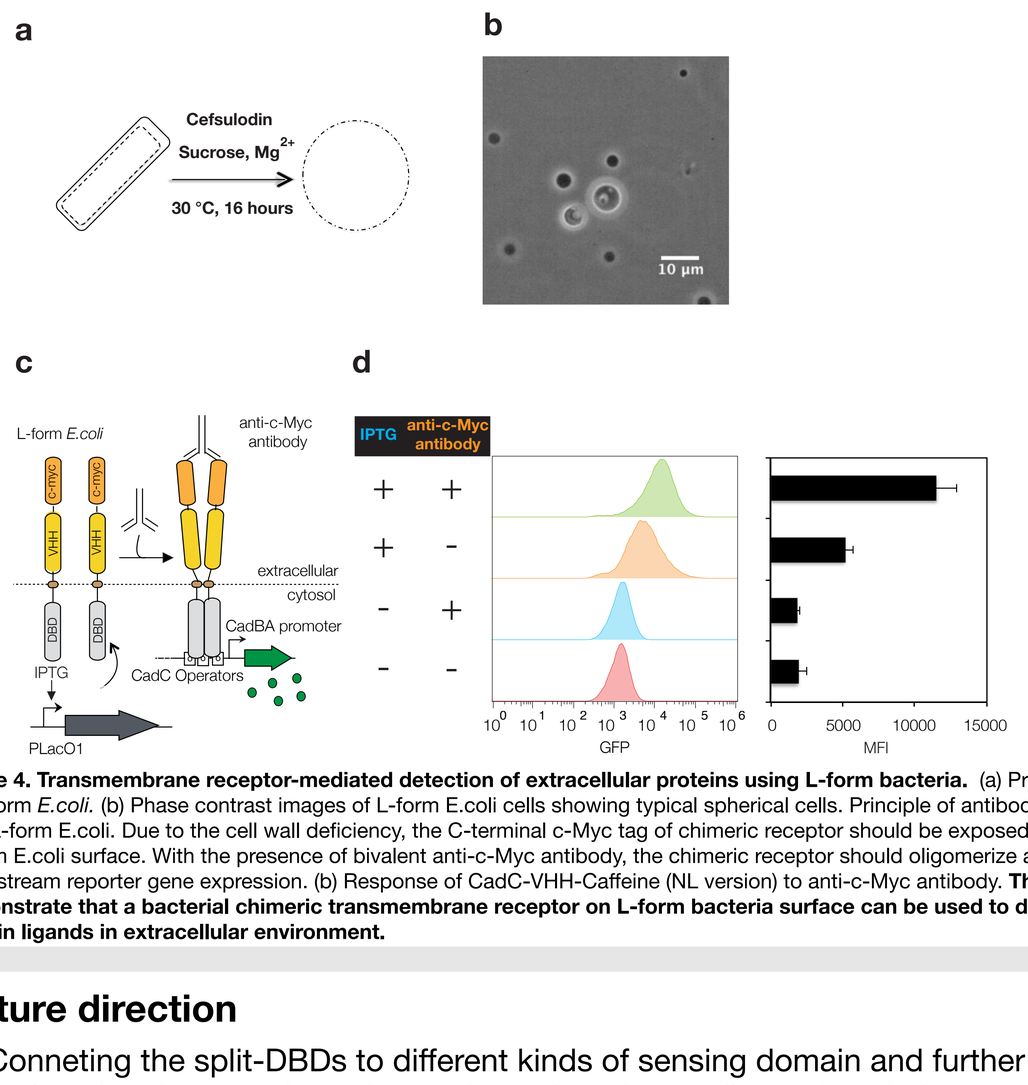

Figure 4. Transmembrane receptor-mediated detection of extracellular proteins using L-form bacteria. (a) Preparation of L-form *E.coli*. (b) Phase contrast images of L-form E.coli cells showing typical spherical cells. Principle of antibody detection with L-form E.coli. Due to the cell wall deficiency, the C-terminal c-Myc tag of chimeric receptor should be exposed on the L-form E.coli surface. With the presence of bivalent anti-c-Myc antibody, the chimeric receptor should oligomerize and trigger downstream reporter gene expression. (b) Response of CadC-VHH-Caffeine (NL version) to anti-c-Myc antibody. **These results demonstrate that a bacterial chimeric transmembrane receptor on L-form bacteria surface can be used to detect protein ligands in extracellular environment.**

Future direction

1. Conneting the split-DBDs to different kinds of sensing domain and further fine-tuning signal output through protein engineering or directed evolution methods.
2. Perform high-order signal processing of modular biosensor through genetic logic or signal amplification (4).
3. Development of portable device with L-form bacteria by encapsulation and stabilization in hydrogels (5).

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