against the bacterial second messenger and vice versa. The virulence of a CdnP-disrupted *M. tb.* strain was substantially dampened, and mice lived 6 months longer when infected with this strain compared to when they are infected with the wild-type strain. Interestingly, while ENPP1 cleaves 2′3′-cGAMP at its 2′–5′ phosphodiester linkage, CdnP cleaves the 3′–5′ phosphodiester linkage. However, comparing K_m and k_{cat} values of the two enzymes toward different substrates[3,8,9] indicates that ENPP1 still seems to be the dominant hydrolase for 2′3′-cGAMP, and CdnP is likely to be mainly responsible for tuning down cyclic-di-AMP-mediated immunity (**Fig. 1**). This hypothesis can ultimately be tested by measuring the effect of CdnP knockout in *M. tb.* strains lacking the cyclic di-AMP synthase gene *disA*.

The authors proposed that CdnP could be targeted for *M. tb.* treatment, and tested this hypothesis using a linear analog of cyclic di-AMP, Ap(S)A, as a tool compound. They report that Ap(S)A inhibited both purified CdnP and ENPP1 and drastically boosted interferon production by synthetic cyclic di-AMP and 2′3′-cGAMP in macrophages with permeabilized membranes. However, it had only moderate effects in *M. tb.*–infected macrophages. It is possible that the compound cannot penetrate the host plasma membrane or the cell wall of *M. tb.* to reach CdnP, and the modest boosting effect is due to its inhibition effect on extracellular ENPP1. Future cell-permeable tool compounds are highly desirable. Finally, five human STING haplotypes have been identified so far, which are commonly referred to as the wild-type allele, the reference allele, HAQ, AQ, and Q alleles. Although cyclic di-AMP is as good as 2′3′-cGAMP in activating STING from laboratory mice strains, it does not activate the human reference and Q alleles[10]. Therefore, CdnP inhibitors are unlikely to exert effects in the human population harboring these STING alleles.

There is an interesting puzzle shared by the host ENPP1 and bacterial CdnP, both of which degrade intracellular cyclic dinucleotides but have been reported to be extracellular enzymes. In the case of CdnP, a recent study showed that CdnP in Group B *Streptococcus* is a cell-wall-anchored extracellular enzyme that degrades extracellular cyclic di-AMP[9]. Dey *et al.* also showed that CdnP is found only in the membrane fraction of *M. tb.*, although it is unclear which direction it faces on the *M. tb.* membrane. Strikingly, *M. tb.* mutants with disrupted *CdnP* have ~535-fold higher intracellular levels of cyclic di-AMP than wild-type *M. tb*. This result can be explained if a substantial portion of CdnP in *M. tb.* also faces inward. An alternative model is that CdnP is extracellular, but controls the intracellular level of cyclic di-AMP by driving export. Since the authors proposed CdnP as a new target for anti–*M. tb.* therapeutics, it is important to know its orientation on the cell wall of *M. tb.*, which has complex structures and poses a permeability barrier even for small-molecule drugs.

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Competing financial interests

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SYNTHETIC BIOLOGY

Sensing with modular receptors

Sensing and responding to diverse extracellular signals is a crucial aspect of cellular decision-making that is currently lacking in the synthetic biology toolkit. The development of modular receptor platforms allows for the rewiring of cellular input–output relationships.

Matthew Brenner, Jang Hwan Cho & Wilson W Wong

A key goal of synthetic biology is to engineer systems with programmable input–output (i/o) relationships. Essential to this process is the ability to couple an arbitrary input (e.g., an extracellular signal) to a user-defined output (e.g., a transcription program). Receptors capable of novel i/o relationships in mammalian cells can enable a host of applications, ranging from tissue patterning[1] to cancer immunotherapy[2]. For example, chimeric antigen receptors (CARs) can be used to redirect T-cell specificity toward cancer cells and have demonstrated promising results in clinical trials[3]. To endow cells with a greater repertoire of new functions, we will need modular receptors capable of achieving a broad array of i/o relationships and a set of design rules for creating these receptors. Recently, Schwarz *et al.* have developed a system that can address this challenge[4].

Some of the first efforts toward developing synthetic receptors have focused on reprogramming ligand specificity. Notably, receptors activated solely by synthetic ligands, or RASSLs, are G-protein-coupled receptors (GPCRs) that have been genetically engineered to respond to synthetic analogs of their natural ligands[5] (**Fig. 1**). Additionally, Tango GPCRs have been engineered to drive transcription and record GPCR activity, alongside their ability to activate endogenous GPCR signaling[6]. The development of CARs has allowed the rewiring of native immune signaling pathways toward new ligands[7]. Although these receptors have successfully rewired the cell-sensing pathways, they are neither modular nor general enough to allow the generation of *de novo* i/o pathways.

More recently, Lim and colleagues have developed orthogonal synthetic receptors built upon a minimal Notch receptor, aptly named synNotch, that are capable of programming both input and output via a proteolytic cleavage event to release an intracellular transcription factor from the receptor[1,8,9]. Both the extracellular ligand sensor and the intracellular transcription factor can be swapped out

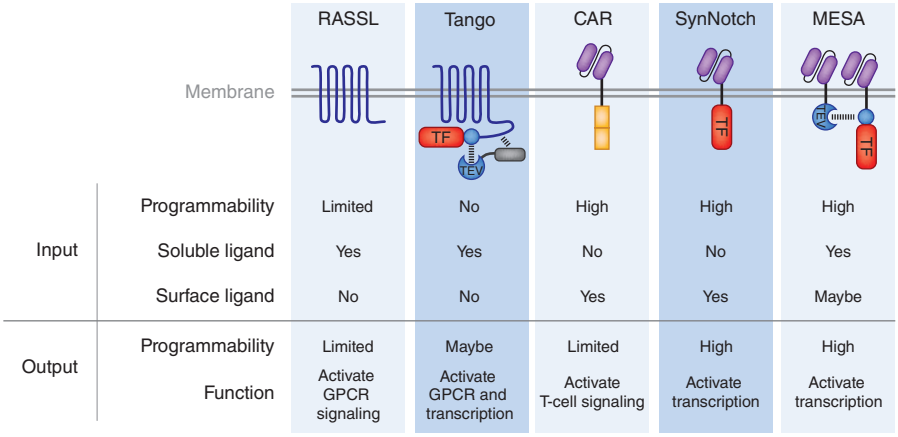

Figure 1 | Comparison of input–output properties for different synthetic receptor platforms in mammalian cells. Different synthetic receptors have varying input and output programmability, with synNotch and MESA being the most versatile. RASSL, receptor activated solely by synthetic ligand; CAR, chimeric antigen receptor; MESA, modular extracellular sensor architecture; TF, transcription factor; TEV, tobacco-etch virus protease; GPCR, G-protein-coupled receptor. The purple extracellular ovals on CAR, SynNotch, and MESA represent single-chain variable fragments from antibodies.

for other domains while maintaining the self-cleaving proteolytic core (**Fig. 1**). An impressive array of functionalities have been demonstrated using synNotch, including the reprogramming of human primary T-cell responses toward cancer and tissue patterning. Similarly to CARs, however, synNotch is not capable of detecting soluble ligands. Previously, Daringer *et al.* reported the development of a modular extracellular sensor architecture (MESA), which can respond to soluble ligands and produce transcriptional output in response[10]. However, this initial design was limited to the detection of small molecules, and therefore, the input was not fully modular. Thus, there exists a need for a broadly applicable synthetic receptor capable of detecting soluble ligands and driving a user-desired output.

The elegant work reported by Schwarz *et al.* further develops the MESA platform and enables more complex i/o programming of cellular response of human T cells for immunotherapy applications[4]. Specifically, they have engineered receptors to sense vascular endothelial growth factor (VEGF) and produce interleukin 2 (IL-2) in response. This non-native i/o pairing could potentially improve the anti-cancer function of engineered T cells in cellular immunotherapy by rewiring cells to produce an important T-cell cytokine in response to a factor responsible for the aberrant growth of tumors. The second-generation MESA system is comprised of two transmembrane chains, both bearing a single-chain variable fragment from a VEGF antibody: a protease chain (PC) and a target chain (TC) containing both a protease ligand and a transcription factor. Two receptors will dimerize when they bind the same extracellular ligand, and will result in cleavage of the transcription factor if the two receptors are a productive PC and TC pair. The newly released transcription factor initiates a user-dictated transcription cellular response. The authors systematically investigated several variables that could affect the dynamic range of the output, such as the length and composition of the extracellular linker, the expression level of each MESA chain, and input dose. Additionally, the authors showed that they could regulate endogenous gene expression by using a catalytically inactive Cas9 protein (dCas9) fused to a transcriptional activation domain (dCas9–TF) to drive the expression of IL-2. In this case, ligand binding will induce cleavage of dCas9–TF, which will then complex with a single-guide RNA (sgRNA) that is complementary to the target locus and will drive gene expression. In summary, the authors have developed a novel modular receptor system that could be readily implemented to engineer cells with customizable i/o responses.

The study from Schwarz *et al.* clearly shows how their modular MESA platform could be flexibly used to rewire cellular i/o relationships in a user-defined way. One of the future challenges is to apply MESA to therapeutically relevant cell types, such as human primary T cells, which often requires the stable expression of the receptors for long durations. Since the MESA platform demands a balanced expression of PC and TC for optimal performance, achieving such a balance stably will necessitate a separate synthetic biology design endeavor. This specific implementation of the MESA platform also required several non-trivial optimizations. If these optimizations are specific to the extracellular sensor domain, they may be more of a jumping-off point for future iterations than a set of general design rules. Lastly, it would be interesting to explore whether MESA receptors can also detect and respond to ligands that are expressed on the surface of cells. All in all, the MESA platform represents a powerful framework for designing novel modular and programmable receptors. These receptors could allow for non-traditional cell responses in fields such as cancer immunotherapy, in which the rewiring of T-cell activation to tumors holds great promise for recognizing and destroying tumor cells.

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