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Spotlight

Engineered membrane receptors with customizable input and output functions

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Morsut *et al.* reported a synthetic receptor system, based on the natural Notch receptor, with customizable input and output functions. Their work on advanced receptor design expands the reach of synthetic receptor systems. Incorporating new protein design tools with better-understood membrane biophysics will create the next generation of engineered receptors.

Cells can sense, compute, and respond to many different environmental stimuli. Over the past 60 years, the field of synthetic biology has developed methodology and tools to co-opt and manipulate cellular systems with precision, with the goal of reprogramming cellular parts to perform 'programs' like a computer. The ability to harness biological machinery to assemble new proteins and perform new functions has led to advances in diagnostics, therapeutics, and bioproduction processes.

Over the past 20 years, synthetic biology has made great strides towards this goal. Applying the principles of computer science and engineering to biology, genetic parts have been assembled to produce transcriptional switches and oscillators, to ultimately create cellular biosensors [1]. These principles were quickly adapted and applied to engineer custom protein receptors, offering a route to control how cells sense and respond to their environment [2].

In 2016, Morsut and colleagues reported the development of a synthetic receptor system (SynNotch), based on the natural Notch receptor, with customizable input and output functions. SynNotch receptors are composed of an extracellular binding domain, the native Notch transmembrane domain, and an intracellular transcription factor. When the engineered extracellular domain binds to a defined ligand, the transmembrane is cleaved by a protease, releasing the transcription factor and initiating programmed gene expression. In this way, the detection of specific extracellular molecules can be transduced into the biosynthesis of specific genetic polymers and proteins. Because of the modularity of this system, different extracellular binding domains to various ligands and transcription factors can be easily installed, allowing for user-defined and customizable sensing and response programs. Further, the diversity of genetically encoded outputs allows multiple orthogonal SynNotch receptors to be used in tandem to sense and respond to multiple ligands with a single cell [2]. This platform has been used to 'daisy chain' multiple receptors together in engineered T cells, creating cells that can more precisely recognize cancer cells [3]. More recently, SynNotch has been expanded to utilize domains from natural receptors, other than the original mouse Notch receptor [4].

In parallel with the development of SynNotch, other receptor systems have been developed to allow cells to sense a wide array of stimuli and respond in more nuanced and complex ways [5,6]. The modular extracellular sensor architecture (MESA) was developed to sense soluble ligands. Like SynNotch, the MESA system allows for the customization of ligand binding and transcription factors, enabling the sensing and response functions to be precisely tuned. Instead of a single chain, however, MESA leverages two proteins, which dimerize upon ligand binding, causing the cleavage and release of a transcription factor, subsequently initiating an engineered signaling cascade. MESA has been further improved by systematically characterizing how different parts of the sensor affect performance, guided by computational guided protein design [5,7]. Like MESA, the generalized extracellular molecule sensor platform (GEMS) has been developed to sense soluble ligands. This system of engineered signaling proteins also relies on protein dimerization in response to ligand binding, but triggers natural signaling cascades. The GEMS system has been integrated into high throughput workflows to develop cellular receptors against new soluble targets, offering a route to engineer receptor systems to sense a soluble ligand of choice [6]. Through the development of these receptor systems, work has gone into engineering receptor scaffolds on either side of the membrane to diversify both the type of molecules that can be sensed and the genetic response [4–7].

Investigating how other parts of the sensor, such as the transmembrane domain, influence receptor signaling could expand the membrane receptor toolbox. Although the transmembrane domain has been demonstrated to affect signaling [4,7], transmembrane designs have not yet been explored to the extent to which the soluble protein components on either side of the membrane have. Modifying the transmembrane domain, and its subsequent interactions with the lipid membrane, should impact the sensitivity and performance of the designed receptor as a whole. Cellular membranes are laterally organized, which enables interactions between different molecules, such as proteins and lipids, to be precisely controlled and proteins to function properly. Despite their importance in cellular function, it is still not clear how membrane protein–lipid interactions could be used to modulate membrane protein conformation or interactions with other proteins, ultimately altering membrane protein function. The ability to design and organize proteins

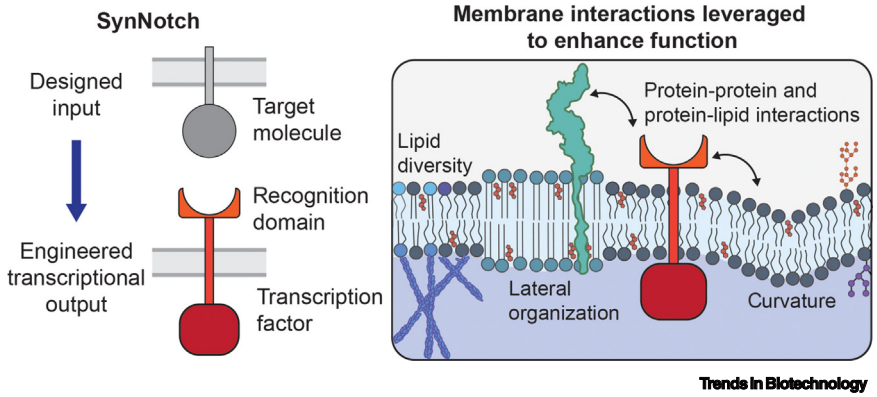

Figure 1. Membrane biophysical features could be used to enhance the capabilities of engineered receptor systems. (Left) SynNotch enables cells to sense new inputs and respond to them in novel ways. (Right) By considering membrane biophysical features, such as lipid–protein and protein–protein interactions, in the design of next generation transmembrane sensors, expanded sensing capabilities may be achieved.

within cellular membranes could allow us to engineer cellular receptors with enhanced function (Figure 1). For example, the spatial distribution of membrane lipids and membrane-associated proteins can greatly affect processes such as phagocytosis and T cell signaling [8,9]. Further, altering the organization of transmembrane proteins, either by engineering the spacing of soluble components from the membrane or enhancing interactions between the hydrophobic transmembrane domain and other membrane components such as lipids, has led to improved CAR-T and SynNotch signaling [10,11]. Separately, the spatial organization of proteins in synthetic membranes has been shown to control protein–protein interactions and protein activity and tune cellular signaling processes [12,13]. Together, these studies highlight the potential impact of engineering the transmembrane domain of receptors and tuning membrane-protein interactions could have on the capabilities of engineered receptors. Incorporating new protein design tools [14] and an understanding of membrane biophysics into the design of the next generation of engineered receptors will expand the sensing and therapeutic potential of engineered receptor systems.

Declaration of interests

No interests are declared.

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