# Engineering New Molecules for Probing and Programming Cellular Systems

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Systems biology emphasizes the study of whole biological ensembles, rather than isolated parts, in order to build a predictive biological understanding of a defined system's behavior. Synthetic biology is an emerging field that emphasizes the application of engineering design principles to the construction of biological systems that exhibit complex dynamical or logical behavior. There is exciting potential in the intersection between these two fields to significantly advance design-based biological engineering efforts toward solving pressing human needs in the areas of health and medicine, energy, materials, and improving our understanding of the natural living world. Therefore, a core component of synthetic biology research is the ability to construct precisely regulated, complex, synthetic networks that are optimally interfaced with existing networks in the cell. This requires an understanding of the biological ensembles that govern a system's behavior and the sophisticated biological components that comprise the regulatory architecture of these circuits, as well as technologies that enable such components to be constructed in a tailor-made, application-specific fashion.

Information flow through cellular networks is responsible for regulating cellular function at both the single cell and multi-cellular systems levels. The ability to temporally and spatially monitor and regulate this information is critical to the understanding and programming of complex cellular behavior. Despite significant advances in the elucidation of the regulatory and metabolic networks that control cellular function, relatively little is understood about the dynamic fluctuations in protein expression, post-translational modifications, or metabolite concentrations associated with these networks. One of the key limitations to understanding dynamic fluctuations in intracellular biomolecule concentrations is the lack of enabling technologies that allow for user-specified probing and programming of these cellular events.

Cells employ a variety of sensor biomolecules to dynamically evaluate their environments and trigger appropriate cellular responses. These naturally-occurring sensor molecules have evolved over many years to be optimized for particular cellular sensing events and as such do not generally represent flexible platforms that can be reprogrammed for different input/output detection events. However, engineered platforms for the design of tailor-made molecules that can sense structural and chemical events will rapidly advance current capabilities to program cell behavior and is a critical technology for many of the challenges in synthetic biology and biological engineering research.

I will discuss recent advances that have been made in developing the molecular design and cellular engineering strategies for the construction of tailor-made sensor platforms that can temporally and spatially monitor and regulate information flow through diverse cellular networks. The construction of sensor platforms based on allosteric regulation of non-coding RNA (ncRNA) activity will be presented, where molecular recognition of a ligand-binding event is coupled to a conformational change in the RNA molecule. This regulated conformational change may be linked to an

appropriate readout signal by controlling a diverse set of ncRNA gene regulatory activities. Research that has demonstrated the modularity, design predictability, and specificity inherent in these molecules for cellular control will be highlighted and its implications for synthetic and systems biology research will be discussed. In addition, the flexibility of these sensor platforms enables these molecules to be incorporated into larger circuits based on molecular computation strategies to construct sensor sets that will perform higher-level signal processing toward complex systems analysis and cellular programming strategies. The application of these molecular sensors to studying cellular systems through monitoring *in vivo* fluctuations in biomolecule levels and to regulating dynamic cellular behavior will be discussed. In particular, the following cellular engineering research areas will be addressed: optimizing metabolic engineering platforms for the production of valuable chemicals and the design of 'intelligent' therapeutic molecules.

**Keywords (3-5):**
molecular sensors
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**Definitions:**

*systems biology* – a field that emphasizes the study of whole biological ensembles, rather than isolated parts, in order to build a predictive biological understanding of a defined system's behavior.

*synthetic biology* - a field that emphasizes the application of engineering design principles to the construction of biological systems that exhibit complex dynamical or logical behavior.

*biological ensembles* – the entire set of biomolecules and their dynamic interactions that result in a particular system's behavior.

*regulatory architecture* – the control systems and design plans for assembly of the structural and functional components of a biological system.

*molecular sensor* – a molecule (for the purposes of this presentation, typically a protein or nucleic acid) that has the ability to detect a specific analyte through a binding event and subsequently translate that binding event into a detectable signal.

*allosteric regulation* - the regulation of the activity of a biomolecule (i.e., protein or nucleic acid) by the binding of an effector molecule at a site on the biomolecule distinct from its active site.

*signal processing* – the processing, amplification and interpretation of diverse biological input signals (typically biomolecule levels), including the analysis and manipulation of such signals.

*biomolecule* – a general term for any molecule that may be present in a cell, including metabolites, proteins, and RNA molecules.

*metabolic engineering* – a field that emphasizes the redirection of cellular metabolism for the efficient or improved production of useful or novel chemicals.

*'intelligent' therapeutic* – a therapeutic molecule that has the ability to self-regulate its activity in a temporal, spatial, or cell-type dependent fashion, resulting in targeting capabilities and thereby more safe and effective therapies.