TRANSCRIPTION

# Synthetic transcriptional synergy

## Synthetic transcription factors can mimic their naturally cooperative counterparts

*By* **Andrew H. Ng** [1,2,3] *and* **Hana El-Samad** [1,4]

The architecture of transcription factors (TFs) is surprisingly modular (*1*). Each factor broadly consists of a DNA binding domain and an activation domain that recruits the cell's transcription apparatus. These domains can be combined in a plug-and-play manner to build synthetic TFs (synTFs). Such synTFs have been successfully used to program activation or repression of a gene of interest (*2*–*4*), a functionality that has proven essential for many studies in molecular biology and applications in biotechnology. On page 593 of this issue, Bashor *et al.* (*5*) present a successful approach for engineering cooperative synTF assemblies. These assemblies bring engineered control of gene expression closer to achieving the richness of behaviors exhibited by naturally cooperative TFs.

When a synTF is designed to have one-to-one binding to its cognate DNA target (the promoter region of a particular gene), the dose response of gene expression is largely graded (until saturation), typically fitted by a Michaelis-Menten saturation curve. As a result, the types of gene expression regulation that can be programmed with synTFs in this manner occupy only a fraction of the capability of natural TFs.

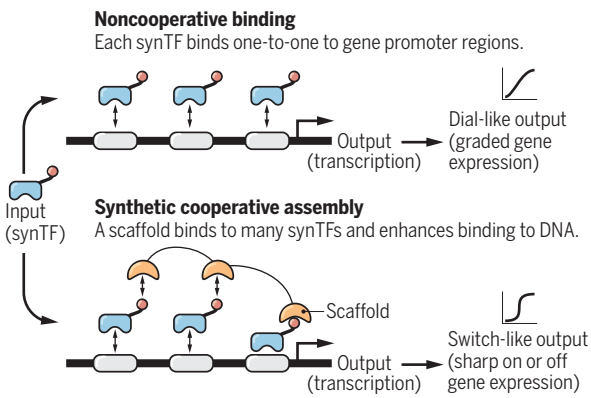
By contrast, the dose-response relationship between many naturally occurring TFs and their promoters is sigmoidal, a result that has often been ascribed to the phenomenon of cooperativity. Cooperativity is a general process in which one molecule (e.g., a TF) can bind another (e.g., DNA) at multiple sites, with binding at one site affected (e.g., enhanced) by occupancy at other sites. It is broadly accepted based on studies of bacterial gene regulation (*6*) that cooperativity of TF binding can increase the "sharpness" of gene expression. Sharp (or switch-like) regulation of transcription allows a gene to switch decisively "on" in an all-or-none manner, at a precise and narrow concentration range of its inducer. Switch-like gene regulation is essential for many biological processes, most notably in developmental contexts (*7*).

Previous studies have reported (*3*, *8*) synTFs (those with a zinc finger structural motif) that interact with a single binding motif on a promoter to initiate transcription. To generate a cooperative response, Bashor *et al.* used a scaffold of covalently linked protein domains (called PDZ domains) that bind to a PDZ domain–interacting ligand fused to a particular synTF. In the presence of the PDZ scaffold, binding of a single synTF-PDZ ligand fusion to DNA increases the probability that another synTF-PDZ ligand fusion will bind to an adjacent DNA binding motif, forming a complex of synthetic transcriptional activators (see the figure). The formation of this cooperative assembly is highly tunable; varying the number of DNA binding motifs in the promoter and the number of PDZ domains, as well as the affinity of the PDZ ligand and the affinity of the DNA binding domain, enables programmable dose responses with customizable shape and sharpness characteristics. Using a thermodynamic model of their system, Bashor *et al.* identified the parameters necessary to implement OR and AND logic on a system with inputs consisting of two orthogonal synTFs. By testing several variants of assemblies of these two synTFs, the authors demonstrated experimentally that cooperative assembly of synTFs is required for Boolean-like behavior. Finding that cooperative synTF assembly also delays activation and hastens deactivation of transcription, the authors constructed circuits that could perform complex dynamic signal processing, such as a persistence filtering device, in which gene expression ensues only for long inputs but not short pulses, and a decoding device that responds preferentially to certain input frequencies. Overall, these rationally designed synthetic transcriptional cooperative assemblies will enable a large array of applications that rely on switch-like gene expression control.

Cooperativity is a highly nuanced phenomenon (*9*) that plays a complex role in transcriptional regulation. For example, in addition to configurational cooperativity, cells make use of allosteric cooperativity. In the context of transcription, allosteric cooperativity would stabilize a modified conformation of a transcriptional regulator or DNA after a binding event, therefore altering binding of additional molecules. Both types of cooperativity may be at play in the formation of large multisubunit transcriptional complexes that involve interactions between the DNA and its structural components, the general transcriptional machinery, and coactivator or co-repressor molecules. This is perhaps best exemplified by the interferon-β enhanceosome, an intricate protein-DNA complex that regulates gene expression in response to viral infection. High cooperativity in this structure transforms weak interactions between individual molecules into a tight and functional assembly (*10*). Further, recent theoretical studies suggest that pairwise cooperative binding of TFs is not sufficient to explain the sharpness observed in the regulation of certain eukaryotic genes such *bicoid* in *Drosophila melanogaster*. Higher-order cooperativity from pioneer factors (transcription factors that target DNA sites at silent genes) and chromatin remodeling can generate a sharper response than that expected from pairwise cooperativity, but the assump-

### Engineered control

Synthetic transcription factors (synTFs) can bind to DNA in two ways.

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tion of thermodynamic equilibrium still limits what is achievable. Greater sharpness can be achieved by expending energy to maintain the system away from equilibrium (*11*). Furthermore, formation of phase-separated superenhancers by cooperative interactions has been implicated in transcriptional regulation (*12*). The ordered assembly of these components and the contribution of cooperativity to gene expression characteristics remain unclear.

Cells harness cooperativity in a variety of contexts that extend beyond transcriptional control. One example is T cell activation, which depends on the cooperative clustering of the T cell receptor with costimulatory molecules in an ordered immunological synapse (*13*). T cells can also be synthetically activated with chimeric antigen receptors (CARs), which are fusions of motifs from the T cell receptor and costimulatory molecules. CARs have demonstrated therapeutic success, but they do not form an ordered immunological synapse (*14*). It is therefore possible that reengineering a CAR to include multisubunit cooperativity could enhance its function and further its application in engineered cell therapy (*15*). A modular framework for constructing synthetic cooperative responses could be a major advance for the field of synthetic biology, boosting the ability to dissect the requirements, constraints, principles, and properties of cooperative processes. ■

***"...synthetic cooperative responses could be a major advance for the field of synthetic biology..."***


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QUANTUM INFORMATION

# *Overcoming quantum decoherence with plasmonics*

## The use of nanoscale plasmonic metamaterials can optimize photon-matter interactions

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Photons occupy a special place as carriers of quantum information because they propagate information at the speed of light, with almost zero cross-talk, and interact relatively weakly with matter. They are primary candidates for implementing quantum networks (*1*), which are essential for both secure communication and transmission of quantum information. Nonclassical states of light (such as squeezed states) are also used in quantum simulation and emerging quantum sensing approaches. However, the robustness of photons as carriers of quantum information is a double-edged sword. In order to produce single photons or make them interact with each other, light must couple with matter. Photonic technologies, especially those implemented with nanoscale plasmonic metamaterials, can enable these interactions and help realize the full potential of photons in quantum information technology.

Unlike the strong interactions of electrons in solids, the weak interactions of photons with matter cause substantial difficulties in generating single photons and performing quantum operations at practical rates, exacerbating the effect of propagation losses. For example, in satellite-based quantum communication experiments (*2*), the demonstrated secure data transfer rates are in the kilohertz range or less. The probabilistic nature of linear optical quantum gates and heralded single-photon sources (pairs of correlated photons) (*3*) reduces the rate of successful multiphoton operations that are needed for most quantum information applications. Deterministic sources of indistinguishable single photons can be realized with quantum emitters (*4*), but they usually operate at low temperatures and their intrinsic operation speed is dictated by the spontaneous emission rate, which is typically less than 1 GHz.

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Most photonic quantum technologies used so far face these issues of slow operation and require a targeted and strong enhancement of light-matter interactions. Optical resonators achieve such an enhancement, proportionally to the ratio of the resonator's quality factor $Q$ (a measure of the photon storage time in the cavity) to the volume $V$ in which the light is confined. In traditional dielectric photonic cavities (see the figure, left), $Q$ can be very large, but the degree to which $V$ can be reduced is restricted by the diffraction-limited volume $V_0$. Moreover, additional efforts to increase $Q$ ultimately hinder high-speed performance (*5*).

In contrast, relatively low-$Q$ plasmonic metamaterials enhance light-matter coupling by using highly localized electromagnetic modes of metallic nanostructures (see the figure, right). The performance improvement comes mainly from the nanoscale confinement of light, which decreases $V$ by many orders of magnitude compared to dielectric cavities. Relatively high radiative losses of these low-$Q$ plasmonic cavities enable broadband operation at much faster speeds (*6*) than can be achieved with high-$Q$ dielectric resonators. Also, light-matter interaction in such broadband and ultrafast plasmonic cavities can be sped up so that it outpaces fast quantum decoherence rates in matter.

This plasmonic speed-up approach strongly contrasts with the conventional pursuit of longer matter coherence time through the use of low temperatures, low pressures, and other ways of increasing and protecting the coherence. The plasmonics-based strategy could enable, for example, a room-temperature on-demand source of indistinguishable photons operating at terahertz rates (*7*). Producing single indistinguishable photons at such high rates and at room temperature would strongly expedite long-distance quantum communication with portable chip-scale devices. Despite a relatively low $Q$, the light-confinement properties of plasmonic cavities can drive them into the strong coupling regime with single quantum emitters (*8*, *9*). This achievement is one of the important steps needed for ultrafast and deterministic multiphoton operations. Plas-

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