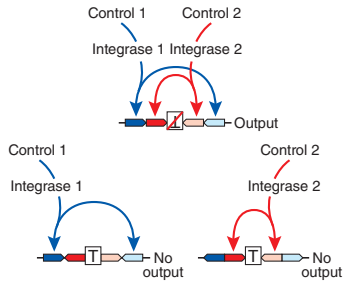
SYNTHETIC BIOLOGY

Complex logic in a single layer

Two independently controlled regulators modulate the output in amplifying Boolean logic gates.

Boolean logic allows one to better understand a complicated system, such as a computer or a living cell, by breaking it down into simple functions: combinations of present and absent input affect the output in a predictable way. But compared to electrical engineers, who can string a large number of logic gates together to carry out complex operations, synthetic biologists looking to program a cellular function are more limited in their tools.



An XNOR gate allows transcription only if both control signals are present in equal concentrations.

Certain types of functions cannot be implemented directly. For example, a two-input exclusive OR gate (XOR)—a gate for which the output is true only if two inputs are different—has classically required four different gates. "Some functions are complicated," says Drew Endy from Stanford University. "In electronics you get away with it, but when people implement logic gates with transcription factors, they need more molecules, and we don't have that many under control yet. The set is quite limited."

One of Endy's goals has been to simplify gate architecture and implement logic gates in a single layer, rather than having to layer one gate upon another, using the same regulatory molecules. His team members based their design on a transistor, the cornerstone of all modern electronics, a three-terminal device in which a control signal manipulates the flux across a wire; they call its biological counterpart a "transcriptor." But unlike a transistor, which can make only one control connection between input and output, the transcriptor can integrate multiple control signals at a single position in a DNA strand.

Two independent control signals, here two molecules that induce the transcription of two integrases, control the advance of a polymerase across logic elements along the DNA. By

CHEMISTRY

CRYSTALLOGRAPHY WITHOUT CRYSTALS

Researchers use networked porous metal complexes as crystalline 'sponges' that absorb and orient small molecules for X-ray crystallography.

X-ray crystallography, widely regarded as the most reliable method for structural determination of small organic molecules, provides researchers with direct structure information at atomic resolution. But the technique comes with a tall experimental hurdle: molecules must be crystallized. This process can require a substantial amount of time, necessitates trial and error and is challenging for limited sample quantities. Additionally, not all molecules can be coaxed into forming crystals.

Makoto Fujita of the University of Tokyo and his colleagues think they have a good solution to this experimental challenge. They recently showed that networked porous metal complexes can act as crystalline sponges, absorbing small-molecule 'guests' from solution into their pores. The sponges take the place of a crystal, presenting the small molecules in a regularly ordered, repeating arrangement that is needed for X-ray diffraction.

"We had often experienced the X-ray observation of guest molecules which penetrated into the pores," says Fujita. They had thought that this phenomenon was common to networked porous metal complexes but eventually realized that it was a unique property of their particular sponges made from tris(4-pyridyl)-1,3,5-triazine and a metal salt, either $Co(NCS)_2$ or ZnI_2. "A secret of the strong host-guest interaction in our complexes lies in the triazine-cored ligand, which attracts various guests onto its electron-deficient π plane," says Fujita. "After realizing the very specific feature of our complexes, we had an idea to generalize the phenomena into the X-ray observation of common organic molecules."

The team's unique crystalline sponges exhibit a three-dimensional cage framework, with pores filled with solvent. The solvent in the pores can be exchanged for a liquid guest molecule

manipulating the logic elements, the integrases switch the gate and cause the polymerase to either advance or terminate, thus producing or blocking output, respectively.

The logic elements are composed of terminators flanked by asymmetric recognition sites for the integrases, and the integrases either invert or excise the terminators depending on the orientation of the sites. This design allowed the researchers to implement each of the six Boolean gates (AND, NAND, OR, NOR, XOR and XNOR) in a single layer in *Escherichia coli*. For example, for the XNOR gate (where output occurs only if the two control signals are equal, either present or absent), which had previously never been realized in single cells, the design featured a logic element with an inverse terminator. The inverse terminator allowed transcription if neither integrase was active or if both integrases were active (in which case one integrase would flip the terminator into an active conformation and the other would flip it back to being inactive). If only one integrase were present, the terminator would be flipped into the active state and block transcription.

The theoretical design was only the first step: Endy's team also needed to have a reliable way to describe performance. "If you are interested in a 0 or 1 Boolean output, you have to set thresholds to define what is low or high," says Endy. "Traditional genetic logic gates often rely on continuously varying signals, and there is no obvious threshold a priori." The researchers instead quantified gate switching in response to increasing levels of integrase inducer concentrations until they found combinations of integrase levels that triggered both digital switching and output (GFP) signal amplification.

Being able to amplify the input signals is important for more complex circuits composed of more than one gate. "If the output signals vary only over a very modest range," explains Endy, "I will not be able to hook it up to another gate because the output of the first gate is not strong enough to switch the second; but if I can increase the dynamic range of the output, I can compose gates upon gates upon gates."

Several groups are already using the transcriptor for applications as diverse as controlling synthetic pathways with multiple inputs and following the colonization of gnotobiotic mice. Endy's gates are in the public domain, waiting to be used.

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RESEARCH PAPERS

Bonnet, J. *et al.* Amplifying genetic logic gates. *Science* **340**, 599–603 (2013).

simply by placing a drop of the guest on the crystalline sponge. Through a slow diffusion process, the system eventually reaches equilibrium such that the guest molecules are regularly ordered. As with a single crystal, the ordered molecules in the crystalline sponge scatter X-rays into amplified beams, producing an X-ray diffraction pattern.

The technique is highly sensitive: 80 nanograms of guest was sufficient in the case of the compound guaiazulene for structure determination by X-ray diffraction. The method makes it possible to analyze liquid compounds and even highly volatile compounds, such as isoprene, without any special treatment. It can be used to unambiguously assign the absolute conformation of chiral molecules, as the team showed for the compound santonin, which contains four chiral centers. The approach can be combined with liquid chromatography to rapidly read out the structures of compounds in mixtures, as the researchers demonstrated by analyzing polymethoxyflavones in orange peel.

Fujita has been surprised at the very positive response to his group's work. "Within only a few weeks after publication, I received more than 100 e-mails, including just appreciations, collaboration offers and sample requests," he says.

Although the researchers stress the immediate practicality of the method for many applications in basic chemistry research and in industry—given that these crystalline sponges can be easily prepared from readily available starting materials—they are also working to improve the method. "Our method is currently not applicable to very large molecules, highly flexible molecules and water-soluble molecules," says Fujita. "We are developing new porous hosts that can accommodate these guests at fixed geometries." Perhaps in the future, even the field of protein crystallography will benefit from the use of crystalline sponges.

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RESEARCH PAPERS

Inokuma, Y. *et al.* X-ray analysis on the nanogram to microgram scale using porous complexes. *Nature* **495**, 461–466 (2013).