CHEMISTRY

Democratizing synthesis by automation

Computer code directs interconnected modules that perform primary steps of synthesis

By **Anat Milo**

There is something particularly satisfying in watching an automaton as it impeccably performs its task, be it an Archimedean screw pump or a machine for making cookie cutters. In this vein, the videos accompanying the article on page 144 by Steiner *et al.* (*1*), depicting their self-driven synthetic chemistry setup—in effect, an automated chemical assembly line—are mesmerizing. The authors constructed a broad-purpose chemical synthesis system comprising interconnected modules coupled with a standardized computer code and architecture. Put in simpler terms, they built a fully equipped fume hood that functions without the need of a human operator. Validating their approach, the synthesizer autonomously prepared three pharmaceutical compounds—diphenhydramine hydrochloride, rufinamide, and sildenafil—according to tailored computer codes provided by the authors.

This work follows several recent efforts to build flexible automated machines that can execute full synthetic routes for a broad range of synthetic procedures (*2*–*5*). Machines perform the tasks of synthetic chemistry in ways that are repeatable, consistent, and fast, and in principle could outperform humans in reaction procedures that require accuracy, reproducibility, and reliability. Furthermore, automation fulfills the promise of technology disburdening people from drudgery and danger, and could free chemists to work on more creative aspects of chemistry. From an industrial perspective, it may lead to faster and more reliable processing.

Automation does not simply imply taking processes and using a robot to perform them as a human would. The process of automating a task inevitably changes the task, occasionally to its very core. In chemistry, synthetic methodologies often must be modified before they can be executed successfully by machines. For example, before the introduction of automated methods for peptide synthesis, robust peptide-coupling methodologies had to be accessible, and together these outcomes resulted in enormous advances in biological chemistry (*6*). As another example, to enable high-throughput screening at the nanoliter scale using a robotics system, a carbon-nitrogen coupling reaction had to be optimized to work in an unusual solvent, dimethyl sulfoxide (DMSO), that provided much greater solubility at room temperature (*7*). On-demand synthesis and purification of radiolabeled fluorinated compounds for positron emission tomography, which is dictated by the short half-life of ^{18}F, has been transformed by considering automation constraints at the earliest stages of reaction development (*8*–*11*). Flow chemistry relies on accurate control of reaction component ratios and mixing rates as well as mass and heat transfer, all of which are streamlined by automation. It is hardly surprising that an automated synthesis system capable of purifying several pharmaceuticals has already been enabled by an integrated continuous-flow strategy (*12*, *13*). More recently, by combining this strategy with in-line analytical capabilities, a reconfigurable continuous-flow system was designed for automated optimization of reaction conditions (*14*). These examples demonstrate how automation has already altered synthesis.

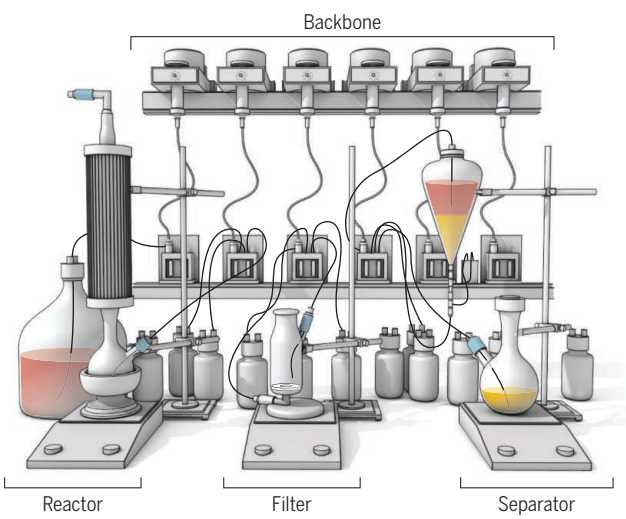
Synthetic chemists often take for granted the senses and skills required to perform simple lab operations. Steiner *et al.* mimicked many of these to construct their robotic system—for example, using an external magnet to capture the stirring bar after a reaction. In other cases, they had to adjust the way synthesis is performed. They struggled to find an automated means for detecting phase separations that would allow them to replicate a decision-based procedure that trained chemists would accomplish visually. Their solution consisted of a conductivity sensor to differentiate between the two phases, which is not only highly resourceful but could potentially outperform visual detection. Such scenarios, in which the machine applies strategies that distinguish it from a human counterpart, may be stepping stones toward superior performance on a particular task. In general, the advantage of using a machine is bolstered by endowing it with abilities that humans do not possess, such as the use of regions of the spectrum undetectable by the human eye or the use of electrical signals as sensors.

The rigorously designed synthetic platform presented by Steiner *et al.* is complemented by the approach they developed for controlling the modules. They first deconstructed and generalized the different steps common to most synthetic procedures and then broke these steps down into machine operations, designing drivers for each module to run these operations (see the figure). The physical connections between the modules are saved as graphs, allowing the system to be truly modular and easily reconfigured to include additional modules.

To bring the software and hardware together, Steiner *et al.* coded a compiler that

The synthesis engine under the fume hood

The main flow controller of the autonomous synthesis robot designed by Steiner *et al.* is illustrated. The modules shown include a reactor, a filter, and a separator, connected by a "backbone" of six-way valves and syringe pumps that move the reaction mixture between modules.



GRAPHIC: C. BICKEL/*SCIENCE*

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produces low-level commands for controlling the modules when given a graph of the physical setup and an input of high-level instructions. These instructions are coded with an extensible descriptive programming language (XDL) to allow users with no programming knowledge to easily encode machine- and human-readable synthetic protocols. This code is then transcribed into a machine-readable chemical assembly (ChASM) script that serves to break down the higher-level abstractions (e.g., reflux) into operations performed by the modules (e.g., stirring and heating). If widely accepted, this protocol could standardize the way procedures are reported and minimize ambiguity. Here again, automation may be responsible for a change in the very task it sought to automate. Ultimately, this type of approach could democratize the automation of synthetic protocols by allowing chemists to straightforwardly adapt and build upon the developed hardware and software.

In the short term, automation can lead to standardization and growth, but what are the long-term consequences of the changes that make a system amenable to automation? Would automation limit inventiveness in molecular sciences once many tasks are shaped into automatable categories? Would the application of automation in exploratory studies enable or hinder the identification of unexpected new phenomena? How would we fill the gap created by automation in the profession of making molecules? Would it ratchet us up to greater unforeseen progress, or would it facilitate indolence? These questions and many more need to be addressed in the coming years as the automation revolution unfolds. In this respect, the availability of technologies and computer codes heralds the automation of chemical synthesis and allows us to be involved in a process that will eventually redefine what it means to be a chemist. ■

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NEUROSCIENCE

Neuronal function of Alzheimer's protein

Cleavage products of an Alzheimer's disease protein are involved in synaptic homeostasis

By **Martin Korte**

For a long time, the huge importance of the cleavage product of the amyloid precursor protein (APP), amyloid-β (Aβ), in the etiology of Alzheimer's disease (AD) (*1*) has occluded the view of the physiological function of APP. But over the years, it has become clear that APP and its proteolytic products have important physiological functions (*2*) during brain development or in the adult brain in processes of activity-dependent synaptic plasticity (*3*) and possibly even protection against neurodegeneration (*4*, *5*). On page 143 of this issue, Rice *et al.* (*6*) discovered that a sushi-containing neurotransmitter receptor in the brain, $GABA_BR1a$ (γ-aminobutyric acid type B receptor subunit 1a), has a new interaction partner, APP. This provides important insights about the physiological function of APP and might open new avenues to treat AD—not only through targeting Aβ but also by strengthening alternate routes of cleaving APP and utilizing nonamylogenic pathways.

Unraveling APP functions and its binding partners has not been trivial, because APP undergoes complex processing, and this results in numerous fragments, which have different and sometimes opposing functional properties. Furthermore, APP functions are partially shared by APP-like protein 1 (APLP1) and APLP2, which confounds some experimental approaches. But what became clear is that one of the main APP proteolytic pathways leads to the secretion of large soluble ectodomains, called APPsα, APPsβ, and APPη (*2*). The main physiologically active agent seems to be APPsα, which is possibly neurotrophic and neuroprotective and may promote the strengthening of synapses, termed long-term potentiation (LTP), in the hippocampus (*3*), which is a cellular mechanism for learning and memory. Accumulating evidence suggests that AD symptoms are caused by both an overload of toxic substances, including Aβ, as well as deficits of protective molecules, such as low concentrations of APPsα.

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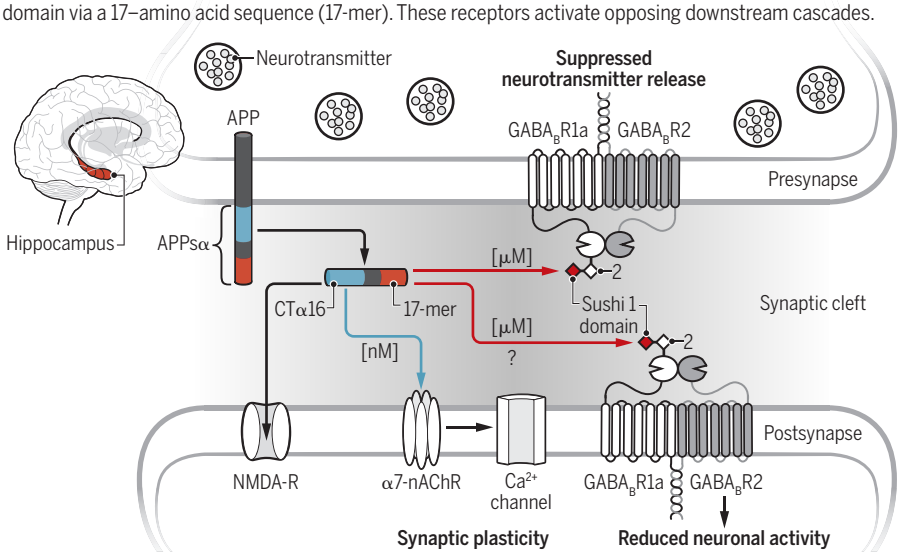
Cleavage products of APP regulate synaptic function

At nM concentration, APPsα is an allosteric activator of α7-nAChR, mediated by C-terminal 16 amino acids (CTα16). At μM concentrations, Rice *et al.* identified the $GABA_BR1a$ as a target of APPsα, binding the sushi 1 domain via a 17–amino acid sequence (17-mer). These receptors activate opposing downstream cascades.



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