than did engrafted neurons from control organoids, as well as different synaptic connections with other neurons and modified electrical activity. These characteristics of Timothy syndrome can be explored only in matured transplanted tissues.

Perhaps the biggest advance in the study comes from the demonstration that the human neurons extended axonal projections into the rat brain tissue, and formed synapses with rat neurons. Furthermore, Revah and colleagues showed — using a technique called optogenetics — that the human neurons could influence the animals' behaviour. In optogenetics, neurons are genetically engineered to express light-sensitive proteins called channelrhodopsins. Blue light activates these proteins, and so activates the neuron that expresses them. The authors used this approach to stimulate human neurons in rats that were being trained to lick a spout to get a water reward. They found that stimulating the neurons prompted the rat to lick, whereas red light or application of blue light to rats that had not received the transplant did not. Thus, the human neurons are involved in the rats' reward–response learning process (Fig. 1).

Human neurons are different from those of all other species, and discrepancies in the rate at which rat and human neurons develop will limit how well human-to-rodent xenografts can mirror human brain function. Nevertheless, the ability to produce mature human neural tissues that integrate with their host at the circuit level provides exciting opportunities for studying the development and basic biology of human neural circuitry, as well as representing a new system for testing therapies for human neurological diseases.

A future area of research could be to incorporate other parts of the human brain into developing rats (both cortical and striatal tissues, for instance), to achieve more-complex human circuitry. However, these experiments pose key ethical questions — related, for instance, to how such research should be overseen, and to the procurement of human biomaterials and donor consent. Other factors to take into account include the potential of the work to lead to therapies for human diseases, and the benefits in terms of reducing the overall amount of animal research needed to find such therapies. Finally, crucial questions surround whether an organoid can have consciousness and moral status. Active discourse between researchers, bioethicists, regulators and the public are required to develop frameworks and boundaries for research that uses organoids to model the circuitry of the human brain[12,13].

J. Gray Camp is at the Roche Institute for Translational Bioengineering, Roche Pharma Research and Early Development, Roche Innovation Center, Basel 4070, Switzerland. **Barbara Treutlein** is in the Department of Biosystems Science and Engineering, ETH Zurich, Basel 4058, Switzerland.
e-mails: jarrettgrayson.camp@unibas.ch; barbara.treutlein@bsse.ethz.ch

1. Eiraku, M. *et al. Cell Stem Cell* **3**, 519–532 (2008).
2. Eiraku, M. *et al. Nature* **472**, 51–56 (2011).
3. Lancaster, M. A. & Knoblich, J. A. *Science* **345**, 1247125 (2014).
4. Camp, J. G. & Treutlein, B. *Nature* **545**, 39–40 (2017).
5. Kelley, K. W. & Pașca, S. P. *Cell* **185**, 42–61 (2022).
6. Revah, O. *et al. Nature* **610**, 319–326 (2022).
7. Spence, J. R. *et al. Nature* **470**, 105–109 (2011).
8. Yu, Q. *et al. Cell* **184**, 3281–3298 (2021).
9. Linaro, D. *et al. Neuron* **104**, 972–986 (2019).
10. Espuny-Camacho, I. *et al. Neuron* **77**, 440–456 (2013).
11. Mansour, A. A. *et al. Nature Biotechnol.* **36**, 432–441 (2018).
12. Hyun, I., Scharf-Deering, J. C. & Lunshof, J. E. *Brain Res.* **1732**, 146653 (2020).
13. Hyun, I. *EMBO J.* **38**, e103331 (2019).

The authors declare no competing interests.

Organic chemistry

Machine assembly of long carbohydrates

Hanchao Cheng & Peng George Wang

Efforts to probe the biological functions of carbohydrates have long been limited by the lack of such molecules with well-defined structures. An automated carbohydrate synthesizer has been developed that could remedy this.

Carbohydrates not only are a biochemical source of energy, but also have key roles in biological-signal transmission, cell recognition and as structural components of living organisms[1]. Naturally occurring carbohydrates are often structurally undefined — they consist of mixtures of molecules, complicating studies of their biological activity. Structurally well-defined carbohydrates (which consist of just one type of molecule) are therefore in great demand for biological studies, but have been difficult to synthesize, especially when the molecules are large. Writing in *Nature Synthesis*, Yao *et al.*[2] report an impressive solution to this long-standing problem: an automated carbohydrate synthesizer that greatly streamlines the preparation of large carbohydrates in solution by reducing the number of steps in which intermediate compounds must be isolated.

Composed of building blocks known as monosaccharides, carbohydrates are one of the four most important families of substances that make up complex organisms, along with nucleic acids, proteins and lipids. But, unlike nucleic acids and proteins, which are linear polymers, each monosaccharide can connect to any one of several positions on other monosaccharides. Carbohydrates produced by biological systems therefore inevitably have structural variation, making it hard to obtain structurally well-defined carbohydrates from natural sources.

This problem can, in principle, be overcome by synthesizing carbohydrates. However, conventional approaches for doing this have been limited to molecules with no more than about 100 monosaccharides[3], because they involve many manual steps, such as difficult isolations of intermediate compounds (Fig. 1a). Automated systems for carbohydrate synthesis have been reported, including enzyme-mediated methods[4,5] and electrochemical assembly in solution[6], but have been unable to produce carbohydrates containing more than about 150 monosaccharides[7].

Yao and colleagues have now solved this problem. Their automated solution-phase synthesizer has three key sections: the synthesis system, an online monitoring system (consisting of sensors that track the progress of reactions) and control software that allows the hardware to be programmed. In the synthesis system, a reactor is equipped with a magnetic stirrer and thermostatic controls (which can vary the reaction temperature between −80 °C and 100 °C). A lamp is also provided to enable visible-light-induced reactions, which offer a 'green', sustainable approach for carbohydrate synthesis[8]. An automatic injector system controls the delivery of the reactants, reagents and solvents to the reactor, using a self-correcting algorithm to carry out accurate injections of up to 18 different reaction components. This system cleans itself using fresh solvent between injections, to prevent cross-contamination of the solution-carrying channels.

The process of constructing carbohydrates on the synthesizer is similar to playing the classic video game *Snake*. In the game, players direct the eponymous reptile to pick up food — the more food it eats, the longer it grows. Similarly, users of Yao and

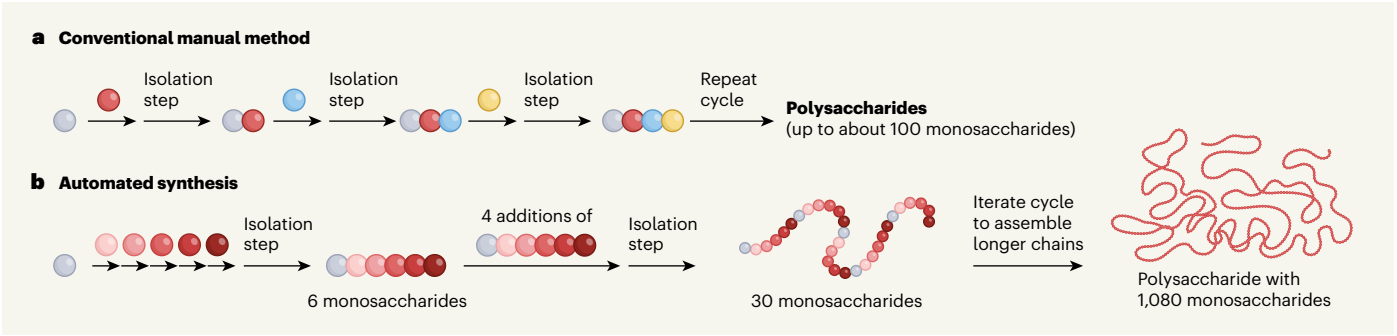

Figure 1 | Increasing the efficiency of carbohydrate synthesis. a, In the conventional strategy for carbohydrate synthesis, target molecules are assembled by the sequential addition of monosaccharides (spheres) to a growing chain. Each addition, and the subsequent isolation of the resulting product, is carried out manually, limiting the size of polysaccharides that can be assembled. **b**, Yao *et al.*[2] report an automated 'multiplicative' strategy carried out by machine. The chemistry is optimized so that several additions of monosaccharides (or larger building blocks) can occur in one reaction vessel before the resulting product is isolated, greatly reducing the number of isolation steps. The product of each cycle of the process is used as the building block in the next cycle. In this way, the authors prepared a polysaccharide containing 1,080 monosaccharides.

colleagues' carbohydrate synthesizer use a computer to feed reagents into a reactor, directing the growth of a long 'snake' built from monosaccharides.

The synthetic process used on the authors' machine works as follows. First, in the presence of either a chemical promoter or a photoactivator (a compound that uses light to trigger a reaction), equal quantities of two monosaccharide-containing building blocks (the glycosyl donor and the glycosyl acceptor) react to produce a structurally defined disaccharide. This disaccharide can then act as a glycosyl acceptor in a reaction with another glycosyl donor, extending the chain to form a trisaccharide. If this cycle is repeated multiple times, a wide range of structurally well-defined carbohydrates can, in principle, be made – from short chains (oligosaccharides) to long chains (polysaccharides).

The synthesizer speeds up the preparation of polysaccharides by using an alternative method called automated multiplicative synthesis, in which chains of saccharides, rather than only monosaccharides, are used as the building blocks (Fig. 1b). In this way, Yao *et al.* constructed a library of well-defined polysaccharides. Impressively, the authors prepared a polysaccharide containing 1,080 monomers – possibly the longest polysaccharide with a well-defined structure to have been made so far.

Yao and colleagues' system has advantages that save time and resources compared with manual carbohydrate synthesis and previously reported automated carbohydrate synthesizers. The online monitoring system analyses reactions, providing feedback that allows decisions to be made about whether to proceed with the next step in the synthesis. For example, it can check that accurate volumes of solutions have been added to the reactor, thereby avoiding possible side reactions and the associated waste of valuable starting materials.

Perhaps the biggest advantage of the synthesizer is that it avoids the use of protecting groups – chemical groups that are used in lengthy syntheses to mask reactive parts of a molecule so that modifications can be made elsewhere. Many different protecting groups are often used in conventional syntheses of complex molecules, each of which usually adds two steps to the synthetic route (one to add the protecting group to the molecule, and another to take it off again when it is no longer needed). Each of these steps produces an intermediate compound that typically must be isolated before it can be used in the next step of the synthesis.

Yao and colleagues' automated protocols can bypass these steps, resulting in better yields of complex carbohydrates than obtained with manual multistep methods. Furthermore, the synthesizer removes the need for a highly skilled workforce to carry out manual synthetic reactions – biologists who need structurally defined carbohydrates for their studies could make them for themselves. The machine also saves time for chemists, who can now use it to synthesize valuable polysaccharides while they are enjoying a coffee break.

The question now arises of whether this strategy can be broadened to synthesize glycoproteins[9,10] – molecules consisting of a protein with attached carbohydrates. Glycoproteins are of interest because they have diverse biological functions, such as in reproduction and the immune system. If Yao and colleagues' synthesizer can combine chemical and enzymatic synthetic methods to prepare structurally well-defined glycoproteins, it will aid studies of the molecules' biological roles and mechanisms of action.

Another potential application is in the field of antibody–drug conjugates[11] (ADCs), which consist of a protein (the antibody) attached to an anticancer drug. ADCs hold great promise for the targeted killing of tumour cells, and have several potential advantages over small-molecule anticancer chemotherapies – such as improved pharmacokinetics (properties that quantify how a compound is absorbed, distributed, metabolized and eliminated by the body), *in vivo* stability and safety profiles. There is much interest in attaching carbohydrates to antibodies in ADCs, because this can greatly increase ADC toxicity to cancer cells. A method that can synthesize libraries of antibodies with attached carbohydrates would therefore aid ADC development.

Yao and colleagues' research emphasizes the value of machine-driven chemical synthesis of carbohydrates. By enabling the automated preparation of gram-scale quantities of diverse, structurally well-defined carbohydrates, such technology should promote rapid advances in glycobiology and medicinal chemistry, and even in areas such as materials science. The authors' work is also a superb example of how expertise and persistence can solve long-standing scientific problems – in this case, how to prepare complicated carbohydrates simply and efficiently.

Hanchao Cheng and **Peng George Wang** are in the Department of Pharmacology, School of Medicine, Southern University of Science and Technology, Shenzhen 518000, People's Republic of China.
e-mail: wangp6@sustech.edu.cn

1. Varki, A. *Nature* **446**, 1023–1029 (2007).
2. Yao, W. *et al. Nature Synth.* https://doi.org/10.1038/s44160-022-00171-9 (2022).
3. Wu, Y., Xiong, D.-C., Chen, S.-C., Wang, Y.-S. & Ye, X.-S. *Nature Commun.* **8**, 14851 (2017).
4. Zhang, J. *et al. Angew. Chem. Int. Edn* **57**, 16638–16642 (2018).
5. Li, T. *et al. Nature Chem.* **11**, 229–236 (2019).
6. Nokami, T. *et al. Org. Lett.* **15**, 4520–4523 (2013).
7. Joseph, A. A., Pardo-Vargas, A. & Seeberger, P. H. *J. Am. Chem. Soc.* **142**, 8561–8564 (2020).
8. Wang, Y., Carder, H. M. & Wendlandt, A. E. *Nature* **578**, 403–408 (2020).
9. Sears, P. & Wong, C.-H. *Science* **291**, 2344–2350 (2001).
10. Li, C. & Wang, L.-X. *Chem. Rev.* **118**, 8359–8413 (2018).
11. Khongorzul, P., Ling, C. J., Khan, F. U., Ihsan, A. U. & Zhang, J. *Mol. Cancer Res.* **18**, 3–19 (2020).

The authors declare no competing interests.
This article was published online on 29 September 2022.