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When robotics met fluidics

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High-throughput fluidic technologies have increased the speed and accuracy of fluid processing to the extent that unlocking further gains will require replacing the human operator with a robotic counterpart. Recent advances in chemistry and biology, such as gene editing, have further exacerbated the need for smart, high-throughput experimentation. A growing number of innovations at the intersection of robotics and fluidics illustrate the tremendous opportunity in achieving fully self-driving fluid systems. We envision that the fields of synthetic chemistry and synthetic biology will be the first beneficiaries of AI-directed robotic and fluidic systems, and largely fall within two modalities: complex integrated centralized facilities that produce data, and distributed systems that synthesize products and conduct disease surveillance.

Introduction

State-of-the-art advancements in automation are poised to revolutionize the field of fluidics. Experiments that do not require flow are increasingly performed in vials with fluid handling robots.[1] These high-throughput capabilities are often being combined with artificial intelligence (AI)-driven experiment planning and optimization for target-driven chemical discovery.[2] A notable example is ChemOS, a versatile software package that orchestrates and synchronizes automated instrumentation that interacts with state-of-the-art AI algorithms[3] and facilitates communication between researchers and robotic systems. Such an approach can also be applied to biological systems, as pioneered by Zymergen, to accelerate genomic research,[4] or to emerging efforts in directed evolution.[5]

Many chemical and biological synthesis processes – owing to their stoichiometric requirements – either require flow or benefit from a flow environment that cannot be achieved in batch systems. In particular, chemical reactions that include multiple phases (solid, liquid, gas), extreme conditions (temperature, pressure), or photo/electro-chemistry often require a flow environment.[6] Similar needs exist for biomolecular processes where a flux of substrates and products can fuel biosynthesis (*e.g.* proteins and DNA) or provide turnover to otherwise static systems.[7] Microfluidics – a modality that provides small-scale flow – can provide high surface-to-volume ratios that enable precise control over reaction conditions, optical and electrical access, and rapid mass and heat transfer (and is used here interchangeably here with the term fluidics). In addition, flow systems allow for high-throughput reactions with in-line testing, enabling continuous monitoring and control that is suited to closed-loop machine learning optimization.[8]

Despite the clear synergies between robotic and fluidic systems, these communities have traditionally functioned in a disparate fashion, and platforms that effectively pair these technologies are rare. Established robotic and fluidic systems include digital microfluidics,[9] printers,[10] fluid handling robots,[11] robot-controlled microneedles,[12] and robot-ready electroporation devices that deliver genetic payloads.[13] Achieving robotic control over the more complex fluidic functions associated with lab-on-a-chip technologies will require new research and developments (Fig. 1). One approach is to embed all complex fluid components within a device module, and task an AI-controlled robot to manipulate, serve, run and store the modules – a robot as lab-on-a-chip-lab-manager. Modules could be reordered and interchanged as needed to control flow kinetics throughout a chemical process, and then robotically connected to supporting infrastructure. Additionally, robotic arms could embody all fluid delivery and testing components (pumps, optics, electrodes, sensors), and thus remove the bulky supporting infrastructure that often limits lab-on-a-chip technologies. Finally, AI could be used as a tool for meta-analysis of the information derived from distributed fluidic devices, such as

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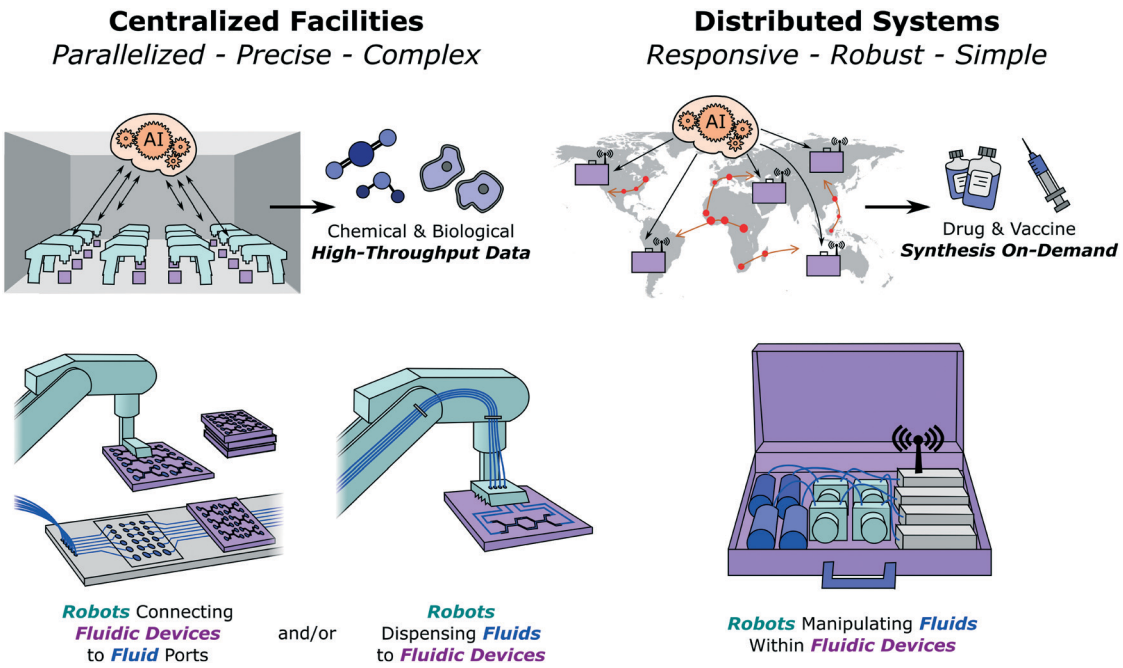

Fig. 1 Two modes for robotic and fluidic partnership: robotics and fluidics in centralized facilities that provide high-throughput chemical and biological experimental data; and robotics and fluidics in integrated portable systems for distributed on-demand drug and vaccine production based on AI driven disease path forecasting.

diagnostic information that indicates the spread of disease in rural areas.

Two paths to fluidics-robotics integration

In a majority of potential robotics and fluidics applications, data is the ultimate product. In many of these cases, centralized facilities that take advantage of economies of scale have an advantage (Fig. 1 - left). Such facilities can perform assays that require only small input volumes (chemical and biological) and the distribution of outputs (data) is facile. However, such Fig. 1. Two modes for robotic and fluidic partnership: robotics and fluidics in centralized facilities that provide high-throughput chemical and biological experimental data; and robotics and fluidics in integrated portable systems for distributed on-demand drug and vaccine production based on AI driven disease path forecasting facilities require a large footprint, and must be supported by significant resources which incur capital costs and require higher power consumption.

With automation, these facilities can operate "lights-out", without human operators, save for minimal oversight and periodic maintenance. Such automation of bench lab efforts has the potential to improve data quality through reduced variability introduced by, for instance, manual pipetting, and more broadly could help to address issues of data reproducibility.[14] Likewise, such standardization brings benefits to the design, synthesis and assembly of DNA, where – in combination with software – automation is an increasingly powerful tool in scaling up design and test cycles in synthetic biology. The success of such robotic-fluidic facilities will require design for, and fidelity to, data as the product. While it may be possible to generate small samples of an emergent product or a high-performance fluid blend, production must necessarily be scaled separately through existing chemical, biological and pharmaceutical production routes.

In applications in which the product is a high-value and low-volume fluid, much simpler automated systems can be effective when distributed at the point-of-need – for instance in conducting vaccine synthesis, disease surveillance, and personalized medicine on-site and on-demand (Fig. 1 - right). In contrast to the high-capital cost and complexity of centralized facilities, this application area presents a low-cost ceiling and low threshold for user complexity. Recent efforts in miniaturization have led to significant gains in this area, including an integrated refrigerator-sized system that can produce certifiable pharmaceuticals at 4500 doses per day,[15] as well as a suitcase platform that provides even greater mobility and reach – which has been employed for on-site manufacturing of protein therapeutics and biomolecules.[16] In addition, advancements in cell-free transcription and translation promise on-demand biomolecular manufacturing from freeze-dried ingredients that can be stored for long periods of time without climate control.[17]

To date, automated fluidics typically work in a dead-reckoning regime that is prescribed ahead of time by a human user. We envision AI accelerating these distributed technologies at two levels: first optimizing centralized facility outputs *via* analysis of requisite demand, and second, by facilitating a meta-analysis of data derived from on-site devices.

In the following sections, we delve into how robotics and fluidics have begun to impact research and development in a variety of applications.

Centralized data-driven fluidic systems

Self-driving laboratories

Recently, there has been significant progress in combining AI with automated experimentation and synthesis. Many research labs across different fields have been using automation and AI to accelerate discovery. In biology, the early automatic experiments are in functional genomics.[18,19] Pioneering works used Bayesian analysis of decision tree learning and the automated Robot Scientist "Adam" to test the functional genomics hypotheses in yeast. In later works,[20] the Robot Scientist is improved (as "Eve") to screen a huge library of compounds for drug targets, with the screening guided by an active learning algorithm. Subsequent progress led to significant breakthroughs, such as the discovery of an anti-cancer drug. The Robot Scientist (Adam and Eve) marks a significant early milestone in combining machine learning with automated experimental systems. Another example is an automated system with diversity-oriented target-focused synthesis (as "DOTS").[21] The DOTS workflow is as follows: (i) DOTS selects a chemical reaction with available building blocks, (ii) the new molecule is virtually screened and the top docking molecules are selected, and (iii) the molecule is synthesized in Chemspeed and characterized in Labcyte. This workflow has allowed successful optimization of an important selective inhibitor of bromodomain.[22]

In addition to biology, many AI-regulated systems have also been developed for automated material discoveries and chemical reactions more generally. One example is the autonomous research system (ARES)[24,25] optimizing the growth of single wall carbon nanotubes (SWCNT). The ARES uses a laser to heat individual pillars that grow SWCNTs in a chemical vapor deposition chamber. The laser is simultaneously used for *in situ* Raman spectroscopy. The intensity of the G band from Raman spectra corresponds to the SWCNTs' yield. The random forest algorithm is used to optimize the SWCNTs' synthesis conditions for the highest growth rate based on the Raman spectra. A more general platform is ChemOS which was developed to orchestrate and

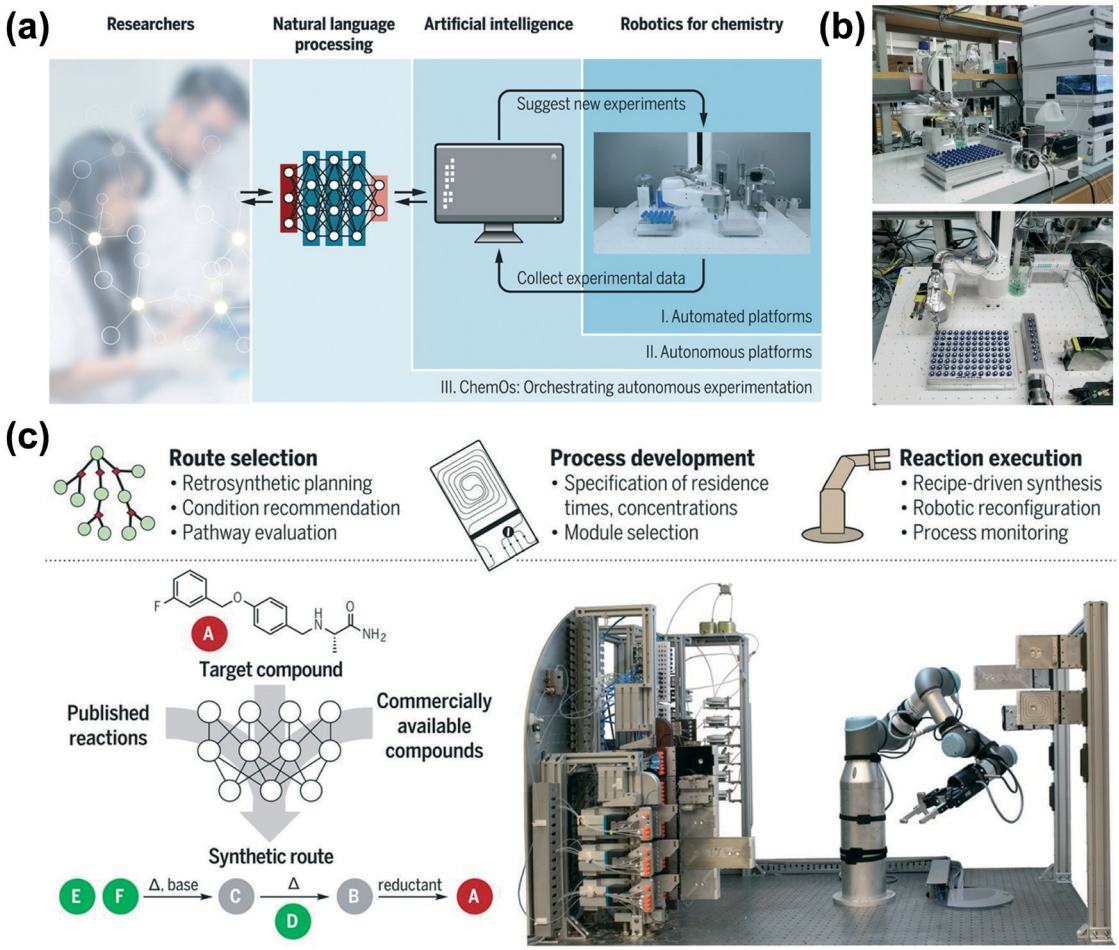

Fig. 2 (a) Flowchart of machine-learning based automatic batch chemical synthesis, reproduced with permission from AAAS, copyright 2018,[2] with (b) as the experimental system. (c) Reconfigurable module-based robotic chemical synthesis system for flow chemistry, reproduced with permission from AAAS, copyright 2019.[23]

synchronize heterogeneous automated instrumentation.[2] It interacts with AI algorithms, and facilitates the communication between researchers and robotics (Fig. 2a and b). ChemOS has been applied to optimize the density of polymer blends, the pH of solutions, and the stability of organic photovoltaics,[26] with real-time reaction monitoring and feedback. Various AI algorithm modules have been included in ChemOS, such as Phoenics and Chimera,[27,28] which are particularly well-suited for self-driving laboratories[29] by enabling the exploitation of parallelized robotics infrastructure for high-dimensional condition space.

While the traditional batch synthesis approach is the mainstream in these centralized self-driving laboratories, the experimental throughput could be notably improved with flow synthesis in many cases. Achieving fully automatic flow synthesis is challenging, as it requires the system to both identify flow synthesis recipes and manipulate fluidic platforms automatically – software and hardware that are now still human-reliant. Recently, AI has been used to plan chemical synthetic routes suitable for flow chemistry, through massive learning from the published chemical reaction database (*e.g.*, Reaxys). The synthetic route refined by AI can either offer significant yield improvement compared to conventional routes (*e.g.*, improving BRD 7/9 inhibitor synthetic yield by 6–8 times) or address unknown paths towards chemical species that have never been artificially synthesized before (*e.g.*, engelheptanoxide C).[30] AI is getting ever closer to replacing humans in configuring synthetic conditions/recipes.

In terms of hardware, the complex chemical reactions performed with flow chemistry can often be divided into multiple connected and straightforward reaction steps (*e.g.*, constant temperature condition, gas/liquid separation). The flow chemistry is thus well-suited to a module-based fluidic system design.[31] Recently, it has been demonstrated that manipulating fluidic modules and assembling them into a functional flow synthesis system can be performed by a robotic platform without human input (Fig. 2c).[23] The robot can precisely arrange, replace, connect and clean the fluidic modules according to the synthetic recipe generated by a central AI. Together, the AI-guided control and the flexible robot for flow chemistry sets an important milestone towards a fully autonomous and high-throughput chemical/biological synthesis future.

What's next in centralized robotics and fluids?

Recent demonstrations of centralized robotic and fluidic integration are highly automated; however, they are not highly autonomous. The level of autonomy in a process can be assessed with the thoughtful taxonomy of Jenson *et al.*[32] With the most advanced systems demonstrating impressive automation of chemical processes, we expect rapid advancement in increasing autonomous control. Particularly with much of the hardware in place, the software can advance quickly.

We introduced recent progress on centralized robotics and fluidics systems for biological and chemical synthesis. We expect this successful model will also inspire the development of physical working fluids, specifically the automated development of fluids with an optimization set of physical properties. For instance, the development of efficient refrigerants with low greenhouse gas intensity could benefit from automated synthesis and physical property testing, where a combination of fluid properties, such as viscosity, heat capacity, heat conductivity and coefficient of performance were to be optimized. These thermal properties, in addition to safety (*e.g.*, flammability) and environmental factors (*e.g.*, global warming potential) are key to advancing this sector. A previous study has applied thermodynamic and environmental criteria to find and screen potential refrigerants *via* simulation, and found 31 viable options for single component refrigerants.[33] Blends of these 31 options may promise performance improvements but present a vast experimental parameter space. Screening a refrigerant fluid blend is an example of an industrial application expected to benefit from high-throughput experimentation, boosted by Bayesian optimization to most efficiently determine the global optimum of a predefined combination of safety, environmental, and thermal performance levels.

In the current centralized robotics and fluidics systems, the data throughput is still limited, or the testing data is not a key output. For example, for the automated flow chemistry system in synthesizing small organic molecules (Fig. 2c), the synthesis recipes are configured through previously published databases with AI, and experimental data during the synthesis is not essential.[23] However, for synthesizing working fluids with particular physical properties (*e.g.*, refrigerant blends), the available fluid data is sparse compared to chemical reaction databases. We expect microfluidics to play a significant role here to feed data-hungry AI algorithms, by providing direct measurement (i) of different fluid physical properties,[34] (ii) at extreme fluid conditions (*e.g.*, temperatures up to 573 K and pressures up to 50 MPa),[35,36] and (iii) at a orders of magnitude higher throughput compared to conventional systems.[37]

It is also important to realize the limitations of a centralized automated fluidic system. Specifically, when certain steps in a synthesis process cannot be fully automated, those steps will act as a bottleneck, for example the clinical trials for drug development. In addition, there are some scientific fields that are not well-suited for centralized automated fluidic systems, as currently these systems often aim to address optimization subjects within specific parameter spaces, and thus are limited to given optimization goals. For instance, understanding the functionalities of molecules (*e.g.*, proteins) in complex biological/chemical processes requires theoretical explanations based on experimental results, and there is no optimum to be defined. There certainly is excellent progress being made recently,[38] but for now limitations remain. The mathematics behind an efficient machine learning algorithm

(*e.g.*, hidden layers of a deep neural network) will not necessarily reflect the scientific details or reasons of a given biological/chemical process. Such tasks, where fundamental details are of more importance compared to the efficiency of production or optimum, should be left to an experienced researcher.

Distributed fluidic systems

Responsive and on-demand fluids

The conventional production of protein therapeutics relies on cell-based recombinant expression and has revolutionized the access to drugs such as insulin, making the biologics category of therapeutics possible. While tremendously successful at advancing food and health security, these protein products are disproportionately available in well-resourced, urban centres. These drugs often require a constant distribution cold chain, adding significant cost, and their centralized production limits access, especially in remote settings (*e.g.* rural communities, global health) and during public health emergencies (*e.g.* disease pandemics).

The marriage of fluidics and robotics represents a natural next step to address these unmet needs in drug delivery. With precise mechanical control and systems that can run without intervention, the de-centralized production of therapeutics could have tremendous impact on the accessibility of drugs. Not only could automated and de-centralized systems help to improve global access to biologics in such scenarios, they hold the potential to manage the prohibitive cost of personalized medicine. As we will discuss, efforts are already underway to bring this concept into practice.

Automation of fluidics for synthetic chemistry has shown that this approach is possible for small molecule-based therapeutics,[15] and semi-automated systems have also begun to take up the challenge for biologics. Housed within a portable microfluidic platform, cells (*e.g. Pichia pastoris*) engineered to secrete protein-based therapeutics have been demonstrated to produce single therapeutic doses of recombinant human growth hormone and interferon-α2b.[39] Other efforts, while less integrated, have scaled this concept into an automated system capable of generating hundreds to thousands of clinical-grade doses of these same therapeutics.[40]

A practical challenge to a truly distributed network of therapeutic production is the cell-based nature of

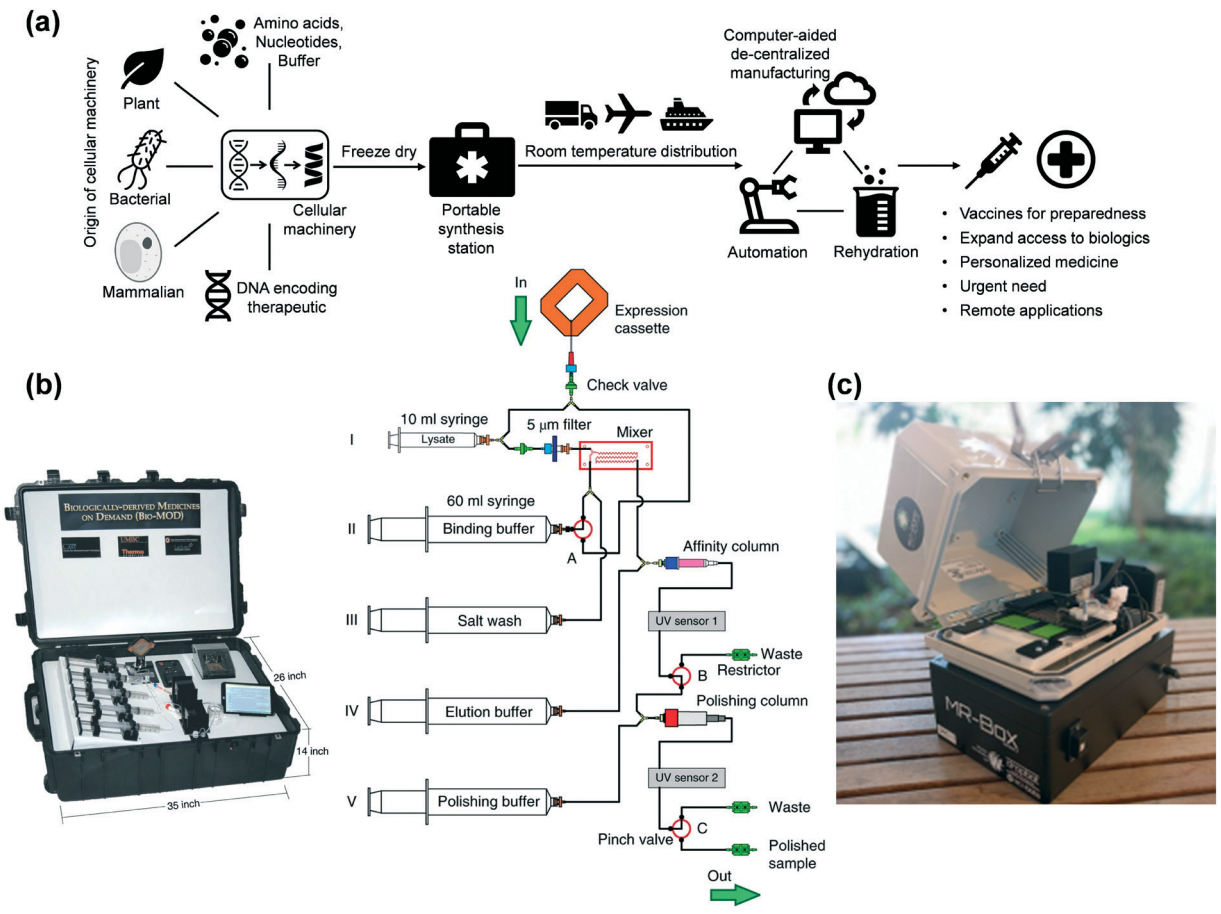

Fig. 3 (a) Conceptual diagram of portable fluidic platform in synthesizing biomolecules (*e.g.*, vaccines) with cell-free method.[17] (b) Point-of-care portable station for automatically synthesising proteins in need, reproduced with permission for Springer Nature, copyright 2018.[16] (c) Picture of a prototype, portable digital microfluidic disease diagnostic system developed for use in field-work (photo credit: Ryan Fobel).

conventional production. Cells are the workhorse of modern biotechnology, but outside of the laboratory setting their capacity to self-replicate poses concerns over biosafety, as does the practicality of maintaining cells. To address this challenge, parallel efforts have begun to develop cell-free systems for the synthesis of protein-based therapeutics.[41–44] These biochemical systems, which reconstitute the cellular processes of transcription and translation, yet are sterile and abiotic, have been used to synthesize vaccines, antibodies and a host of other biologics.[45] Importantly, by freeze-drying the cell-free enzymes, this manufacturing capacity can be distributed to the point of need at room temperature.[17,46,47]

Efforts are becoming ever more sophisticated, including eukaryotic cell-free systems,[48–51] and have demonstrated the synthesis of proteins with post-translational modifications, such as glycosylation and disulphide bond formation.[52–55] As with cell-based synthesis, groups have also begun to develop fluidic systems to de-skill the manufacturing process (Fig. 3a and b).[16] While these systems are not yet truly automated, the incorporation of fluidics and machine learning to biology is making for exciting advancements in this interdisciplinary space. Looking to a not so distant future where low-cost DNA synthesis is readily available, the de-centralized production of the drugs themselves seems increasingly possible. Likewise systems may soon be capable of receiving sequence information electronically for the synthesis of both DNA and protein,[56] enabling the distribution of new therapeutics with the click of a button.

Smart digital microfluidics

Another technology that represents the vanguard of distributed robotic/microfluidic systems is digital microfluidics (DMF). Digital microfluidic systems allow for manipulation of liquid reagent droplets on an open surface, often driven by electromechanical forces (either wetting[9,57,58] or dewetting[59]). Importantly, unlike the conventional microfluidic paradigm in which function is prescribed by channel-geometry and arrangement by the designer, DMF systems can process assays with wildly different fluid-handling requirements – all without physical modification of the chip. Because (in its most common implementation) DMF relies only on the application of voltage for droplet locomotion, it lends itself perfectly to computer-control, in which different assay protocols can simply be programmed for use with the same hardware. Simultaneously, DMF platforms are highly amenable to miniaturization because they effectively have no moving parts – other than the liquid that is to be moved.

The primary advantage of DMF is reconfigurability – for example, applications that vary widely, such as chemical synthesis,[60,61] chemometric process-optimization,[62,63] and automated cell culture and analysis.[64,65] – can be implemented in systems that are nearly identical, with no changes to the (generic) format. Centralized robotic systems also have the advantage of generic architecture and reconfigurability, but in contrast to centralized systems, the modest footprint of DMF systems allows deployment in difficult-to-reach settings – for example, as a field laboratory at a refugee camp in a remote region in northwest Kenya[66] or within the miniscule space within the bore of the magnet in a nuclear magnetic resonance spectrometer.[67] Fig. 3c shows a portable digital microfluidics system capable of performing assays anywhere in the world with fidelity.

What's next in distributed robotics and fluidics?

Smart designs of microfluidic features have pushed portable point-of-care diagnostics towards a specialist operator-free scenario. These automated microfluidic devices integrate reagents and manipulations all within one chip for a predefined assay recipe[68] to reduce the sample cost and device footprint. Meanwhile, the development of autonomous microrobotics (*e.g.*, microswimmers)[69,70] for manipulating molecules/nanoparticles in a small-scale fluid environment indicates another important way in integrating robotics and fluidics for distribution, especially for precise drug synthesis.

A brave new world is emerging for distributed robotics and fluidics. We see automation and AI coalescing with in-field systems to yield a level of disease-informatics that is unprecedented. It has been shown that artificial intelligence working with derivative information can predict the spread of critical diseases (Fig. 1).[71,72] Pairing such capabilities with portable diagnostic systems[66] that are geo-located will provide disease monitoring organizations with information that predicts the "warpath" of a disease with meteorological accuracy, or better. Working with such information could allow these organizations to combat outbreaks, quell perennial endemic illness, or institute vaccination campaigns that eradicate these diseases before they spread.

Deployable and de-centralized facilities may ultimately enable a more nimble, customized supply chain, with just-in-time and just-on-site manufacturing of perishable, high-value products such as protein-based drugs. There may be opportunities for distributed, automated production of high complexity-small batch items that would be difficult to achieve at low-cost in a centralized production model, such as small batches of specifically radio-labelled chemicals and personalized medicines. There is also potential for a distributed manufacturing model to reduce carbon emissions associated with the current supply chain for specialized products.

The practicality and applicability of distributed automated fluidic synthesis systems may be limited by the complexity of a synthesis recipe, or a dialysis assay, including the number of steps and requirements for synthesizing conditions. Current distributed fluidics systems permit <10 steps of a predefined process, and are restricted to room temperature and near-atmospheric pressures. Addressing these limitations by integrating more complex capabilities and more modules, will increase cost and weight – both of which are highly constrained in distributed synthesis applications. The

solution to this trade-off will be application specific, with each system incorporating only the essential complexity required at site.

Conclusions

From the perspective of chemistry and biology, the marriage of robotics and fluidics provides a route to smart, high-throughput synthesis and testing – a physical, chemical, and biological manifestation of increasingly powerful artificial intelligence.

Conflicts of interest

There are no conflicts of interest to declare.

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