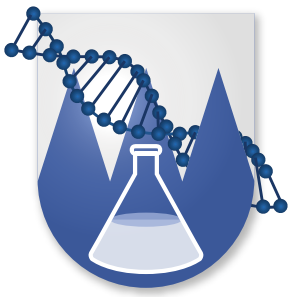

Spiez CONVERGENCE

Report on the third workshop
11–14 September 2018

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Executive Summary

In 2014, the Spiez CONVERGENCE workshop series set out to identify advances in Science and Technology (S&T) that may pose a challenge to the regimes governing the prohibition of chemical and biological weapons. The main focus of the series is on proofs of concept, technological breakthroughs, or scientific game changers that may affect our understanding and perception of such weapons. Spiez CONVERGENCE 2018 was the third meeting to review developments in S&T at the crossroads of chemistry and biology with specialists from academia, the chemical and biotech industries, and the arms control and security community.

The past two editions covered a wide range of subjects and some of these were addressed again this year because of their continued relevance. Revisiting particular subjects leads to a deeper understanding of their state of maturity. On the one hand it shows how fast progress is made, on the other hand it uncovers that not all S&T promises may become reality. By revisiting earlier assessments, better predictions for the short or medium term period can be made.

CRISPR technology has made genome editing easier, faster and more accessible. The ability to recode the guide RNA made it possible to turn CRISPR into a tool that could target any gene. The rich diversity of CRISPR systems can be combined with selected biocatalysts that cause a variety of desired genome modifications and work towards practical applications is under way. An example presented at the workshop is the reversal of antibiotic resistance in bacteria or the development of diagnostic techniques based on DNA and RNA sequence recognition. Yet, many CRISPR applications are still at the proof-of-concept stage and practical challenges such as delivery and off-target effects need to be overcome to reach clinical application for CRISPR based therapeutics. Furthermore, ethical issues have arisen for clinical applications in particular with regard to gene editing in the germ line.

Today, industry manufactures a number of complex biomolecules using **Synthetic Biology**. Complex *in vitro* designs are attractive as they provide access to interesting products (oligosaccharides, proteins, assays) while expanding the space of biotechnology. However, moving from *in vitro* to *in vivo* systems requires a change from engineering design to evolution. Targeted mutagenesis offers a fast, high-throughput tool to analyse pathogen-specific resistance processes including resistance processes that do not exist in nature yet, which could be integrated into drug development. A practical application of synthetic and systems biology is the development of rapid diagnostic tests for the emergence of antibiotic resistance in bacteria. But despite all the

progress made, there remain clear limitations to the engineering of biological systems, and tacit knowledge remains important. The introduction of cloud laboratories for synthetic biology promises to increase the speed of synthesis and agent characterisation because they provide a reproducible environment using standardised protocols. However, cloud laboratories raise security related questions: e.g. their potential utilisation for malevolent purposes and the risk of them becoming targets for remote attacks.

Continuous processes have shown distinct advantages over batch production in **chemical manufacturing** and are, with the exception of the production of pharmaceuticals, widely applied today. Continuous processes would come with many advantages for pharmaceutical manufacturing if well mastered. For bio-processes however, working with living organisms poses challenges with regard to process variability and disturbances. Supervisory Control And Data Acquisition (SCADA) systems are common in the chemical industry but need adaptation for bio-process monitoring and control. A process optimisation system that was presented at the workshop is specific for a particular production process and cell line. In other words, each target molecule requires a dedicated process. Work is under way to develop continuous bio-manufacturing processes for a range of pharmaceutical products but target specificity is a serious obstacle. What is needed, is a combination of the versatility and interchangeability of flow reactors with efficient iterative process unit modules. A solution presented at the workshop was a radial synthesiser – a fully automated, remotely controlled, modular assembly system in radial configuration that can manufacture several small molecules using the same hardware. A radial synthesiser is suitable for multistep syntheses as well as the generation of big data libraries. Such synthesisers centralise chemical synthesis and may be operated anywhere. They indicate a shift in the way chemical synthesis is performed and allow wet experiments to be outsourced to remote automated systems.

In the discussion on **nanomaterials** during Spiez CONVERGENCE 2016, DNA origami was characterised as early-stage exploratory research. Today, first experiments with DNA objects as cancer therapeutics are being conducted in laboratory animals. Triangular DNA origami structures can be stacked to form capsid-like structures up to 200 nm in diameter. They are rigid and could be developed as targeted drug delivery vehicles. The stability of DNA origami structures *in vivo* however remains problematic and practical applications in medicine or industry would require mass production, for which manufacturing costs will have to be reduced. The construction of nanocarriers for the purpose as future drug delivery means and molecular bio-sensors to deliver membrane-active antimicrobial peptides is a promising area of research and also related to the development of "functional food". Research in human breast milk showed nanostructure formation by self-organisation through biological systems that are present in the body during digestion. Such food and digestion inspired nanostructures may be suitable as carriers for drugs that are not stable in aqueous environment and that would normally degrade in the stomach. Nanocarriers in general can improve the efficacy of drugs and can be used to design stimuli-responsive drug delivery systems. A wide range of chemotherapeutics, cancer treatments, nucleic acids, proteins, and other biomolecules

use different types of graphene oxides (GO) for their application and delivery. GO are two-dimensional nano-scale carbon structures. Different derivatives of GO are of particular interest as they are biocompatible, easy to functionalise, suitable as efficient drug loading structures, scalable, and inexpensive. Biological effects as well as toxicity of GO nanoparticles depend on their particle size, oxidation groups, and functionalisation as well as the type of cell. In a CBW context, it is possible that such nanoparticles could be delivered as aerosols and inhaled into the lungs for uptake through the blood brain barrier. They may therefore be suitable for the targeted delivery of high amounts of toxins or bioregulators.

Additive Manufacturing (AM) has been a topic at all three workshops since 2014 and the industry continues to grow rapidly. AM gives the end user the control over the product design and has the ability to disrupt conventional supply chains. Different processes are available today that include powder bed fusion, directed energy deposition, material extrusion, Vat photo-polymerisation, binder jetting, material jetting, or sheet lamination to print 3D objects using a range of materials, such as metals, polymers, ceramics, foundry sand, waxes, or paper. While AM is faster than traditional manufacturing it cannot compete with sheet metal fabrication unless it provides other advantages. AM of complex parts to high performance standards remains a challenge. Of particular interest in the context of CBW arms control are AM processes to build 3D objects that can withstand high temperature, pressure steam sterilisation, or highly corrosive chemicals. Today, only industrial AM systems are able to produce such high-quality parts to a standard that can compete with other current industrial processes. Such industrial systems require professional know-how and technical competence. It is unlikely that 3D printers capable of manufacturing corrosion resistant parts or equipment would be available to individuals or consumers soon. Over the next 5 years the list of printable materials is likely to grow significantly and AM is expected to be adopted across multiple industries as well as enter into education. Regulatory standards for 3D printing and processing will have to be developed.

In the field of **Bioinformatics, Omics, and Big Data**, advances in genomics and transcriptomics are driven by next-generation sequencing (NGS) techniques whilst work in the other fields relies heavily on mass spectrometry. NGS techniques have been very successful, partly because they rely on the biological design principles of DNA and RNA as information carriers that can easily be written and read. Today, researchers can perform parallel read operations of 10 billion molecules in a single experiment and experimental precision has increased to enable single molecule manipulations. A combination of factors (the right molecules for study, parallelisation, low cost and high sensitivity) has lead into what some call an NGS revolution. However, individual genomes carry information about the individual concerned and the analysis of individual genome data could be used to link individuals to specific genetic characteristics. This could compromise privacy rights. As a consequence, multiomics projects pose challenges with regard to the protection of patient-specific data. Current privacy-preserving data mining technologies therefore need to be improved with regard to performance, secure access and data protection. The field of Machine Learning (ML) has progressed to neural networks and neural

network architectures with multiple layers and nodes that permit better predictions. Deep Learning (DL) requires access to Big Data as well as huge computational power. Artificial intelligence (AI) using (deep) machine learning offers a way forward to managing and making sense of the vast amounts of data. Recent examples of the predictive power of AI (optimisation of organic syntheses, accurate predictions of reaction performances and yields) have raised questions about whether AI may replace chemists. Such DL has overcome many limitations of physics-based models. It is able to build models from simple representations of chemical and biological entities to suggest synthesisable structures with improved properties. Combined with automation algorithms this integration of chemical and biological synthesis with AI expands the number of materials that can be synthesised, tested, and optimised. There is a fundamental question today of whether humans can control the evolution of AI but at the same time, pressure to move ahead with AI is strong. Reliable safeguards need to be defined and implemented as the technology evolves.

Spiez CONVERGENCE always closes with a **policy discussion** about the impact that advances in S&T may have on the treaties and regimes related to CBW. Since 2014 the focus of discussions has shifted from materials and equipment, to information, automation and remote manufacturing. This shift from sensitive materials and equipment to information can also provide new opportunities for oversight, compliance monitoring, and verification. In the case of additive manufacturing, production is moving closer to the point of use. If radial synthesisers that centralise chemical synthesis become industry standard for bio-manufacturing of pharmaceuticals, their location could be anywhere. In a similar manner, the introduction of cloud laboratories for synthetic biology centralises laboratory work and separates the scientists from the actual laboratory experiment. As a result of this, the role of the end-user or actors in the process is changing and access to data as well as intangible transfers are becoming more relevant from a regulatory and control perspective. These changes would also affect potential CBW programmes. Novel CBW production facilities would have a smaller footprint and different technological features compared to what is known from past state programmes. In the case of non-state actors attempting to acquire CBW capabilities, such attempts are likely to remain opportunistic and constraints continue to exist regarding access to critical materials and equipment, methods for effective dissemina-

tion of agents, programme related costs, and importantly also, tacit knowledge. For a state actor it is difficult to assess how new materials and methods discussed in this workshop would fit into a contemporary CBW programme. Such an assessment would have to differentiate between CB weapons developed as WMD and those developed for small-scale purposes such as sabotage or assassinations. The question that must be asked in this context is: are implementation systems adopted by the States Parties of the two Conventions as well as national export control measures still effective in the changing environment? Many of the S&T advances reviewed in this workshop series call for multi-stakeholder approaches between the research community, industry, and National Authorities to develop partnerships and governance systems. Our evaluations may have a short and a longer-term perspective: e.g., 3D printers that use polyfluorinated polymers that could undermine export controls require a swift response to manage emerging risks, whereas the use of cloud services in chemical and biological manufacturing may affect implementation over time.

With the findings presented in this report, Spiez CONVERGENCE aims to support the stakeholder communities of the Chemical Weapons Convention, the Biological Weapons Convention, and related regimes in their assessment of the impact of S&T advances on CB disarmament and security.

Introduction

“Convergence” is a characteristic feature of today’s life sciences and affects the regimes governing the prohibition of chemical and biological weapons. Spiez CONVERGENCE 2018 was the third workshop of our series to review developments in Science and Technology (S&T) at the crossroads of chemistry and biology. It again brought together specialists from academia, from the chemical and biotech industries, and from the arms control and security community.

The aim of the workshop series is to strengthen both the Chemical Weapons Convention (CWC) as well as the Biological Weapons Convention (BWC) by facilitating an informed conversation between different stakeholder communities about the impact that S&T advances may have on the functioning of the two treaties. Both conventions provide for a comprehensive prohibition of, respectively, chemical and biological weapons.

Although the two Conventions overlap in the prohibition of toxin weapons, they differ in other key features. The BWC relies on implementation by the States Parties, supported by a small (three staff) Implementation Support Unit (ISU). The CWC is implemented through the Organisation for the Prohibition of Chemical Weapons (OPCW), by means of national implementation measures adopted by States Parties as well as OPCW programmes ranging from verification of compliance to assistance and protection, and the fostering of international cooperation in the chemical field.

Both conventions are anchored in the past of former state weapons programmes but at the same time, they are of unlimited duration. To adapt to change – in particular to developments in S&T – they rely on a general-purpose criterion that links prohibitions to the purpose of a chemical or biological agent. Both treaties require States to “integrate” the norms by building them back into their legislative, regulatory, and administrative systems. The CWC, in addition, provides for international verification. National implementation, international verification, and other provisions of both Conventions have to keep pace with advances in S&T.

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A key purpose of reviewing S&T developments at the crossroads of chemistry and biology is to identify and assess any risks they may pose to the arms control regimes. Risk assessment must be sufficiently granular to take account of different scenarios and actors, from naturally occurring and re-emerging diseases, to unintended consequences of legitimate chemical and biological activities, and to the deliberate use of such materials for hostile purposes. Consciousness about intents and capabilities of different actors, including "lone wolfs", organised crime, terrorism, and states or state sponsored groups is necessary. Other contextual factors also influence perceptions about the relevance and strength of the norms. Examples are the use of chemical weapons in Syria, recent assassination attempts using chemical agents, or natural disease outbreaks as reminders of their destructive and disruptive potential. An increasingly multipolar world with challenges to the current international rules-system, the changing nature of armed conflict as well as heavy defence investment in the life sciences may raise questions about the harnessing of biotechnology for nefarious purposes. In addition to evaluating the abuse potential that advances may create, we must recognise the benefits advances may offer to societal demands such as managing climate change, overcoming poverty, ensuring access to food and safe water, or improving public health.

Risk assessment must be sufficiently granular to take account of different scenarios and actors, from naturally occurring and re-emerging diseases, to unintended consequences of legitimate chemical and biological activities, and to the deliberate use of such materials for hostile purposes.

Evaluating the impact of convergence must therefore be interdisciplinary and inclusive. This is why exchanges between academia, the industrial community and arms control experts are important. Diverse actors and scenarios lead to the consideration of different types of risk, involving more (or less) sophistication in weapons design, and may call for various types of responses. This workshop focused mainly on risks associated with state and state sponsored activity – professional scientists working in interdisciplinary teams with sustained funding that could be channeled into developing sophisticated biochemical weapons. Such risks differ from those posed by other (non-state) actors. Participants set out to explore which proofs-of-concept, technological breakthroughs, or scientific game changers, by themselves or together, might shift or flip concepts and perceptions of chemical and biological warfare in ways that might challenge the assumptions underlying CBW arms control.

Evaluating the impact of convergence must therefore be interdisciplinary and inclusive.

Findings from previous Workshops

Discussions in 2014 and 2016[2] covered a wide range of subjects: from the synthesis of physiologically active molecules including (highly) active pharmaceutical ingredients (HAPI / API) to large molecules such as DNA, proteins and carbohydrates; from chemical and biological synthesis at laboratory scale to industrial-scale chemical and bio-manufacturing; from additive manufacturing of metal components to bio-printing of structures mimicking organic tissue; from genome editing using CRISPR technology to the application of gene drives to fight malaria; from OMICs and big data to the use of DNA for information storage and computing; from patchy particles to DNA origami with potential for the design of nanoparticles for drug delivery or as nanomachines.

Previous workshops recognised convergence as an integrative and collaborative approach in the life sciences that brings together theoretical concepts, experimental techniques and knowledge of different science and engineering disciplines at the intersection of chemistry and biology. The maturity of the technologies reviewed ranged from fundamental research to manufacturing techniques used at industrial scale and distributed globally.

These past discussions underscored the importance of understanding the state of maturity of a given technology when evaluating its impact on arms control and security. Not everything that S&T promise will become reality: tacit knowledge remains an important modulator of practical applications, new scientific discovery does not equal new weapons, and the context within which scientific discovery is taken forward from the lab bench to practical application is important.

Spiez CONVERGENCE 2018 looked back at previous findings, reviewed the current state of S&T, and attempted to identify trends that may affect the two regimes in terms of both benefits and risks.

Spiez CONVERGENCE 2018 looked back at previous findings, reviewed the current state of S&T, and attempted to identify trends that may affect the two regimes in terms of both benefits and risks. It ended with a general discussion of potential implications for the CWC and the BWC and was structured around the following themes:

- CRISPR genome editing
- Synthetic biology
- Synthetic and analytical chemistry
- Material sciences including nanotechnology
- Additive manufacturing
- Bioinformatics, OMICs and big data

2 Link to previous reports

CRISPR Genome Editing

Previous workshops concluded that CRISPR technology has begun to transform genome editing, making it less cumbersome, less time consuming, and less expensive, while broadening its potential for practical application.

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats (CRISPR) were first observed in 1987, but their role as an adaptive bacterial immune system was not immediately recognised. In 2002, CRISPR associated (Cas) genes were identified – invariably located adjacent to a CRISPR locus, which suggested that they were functionally related. In 2005, the spacer sequences were recognised as matching foreign (virus) DNA, and by 2007 it was established that CRISPR provided acquired resistance in prokaryotes against virus infections.

The ability to recode the guide RNA then made it possible to turn CRISPR into a tool that could target *any* gene. Once targeted, the system can be used to perform a variety of functions including the introduction of point mutations, gene insertions, deletions of DNA segments, silencing or modulation of gene expression, the identification of essential genes, the localisation of DNA and the tracking of RNA in living cells, and the repair of gene defects. Even recoding an entire *E. coli* genome with amino acids not found in nature has been accomplished.

Even recoding an entire *E. coli* genome with amino acids not found in nature has been accomplished.

CRISPR was not the first method available to edit the genome; many of the concepts and knowledge used in genome editing predate CRISPR. However, it has made genome editing easier, faster and more accessible.

So far, much of this work involved a particular CRISPR-Cas system (Cas-9). Nevertheless, CRISPR-Cas is highly diverse, with two classes, six types and multiple variants thereof. This diversity has only recently been exploited for more finely-tuned genetic interventions, including base editing without cutting the DNA, relying on donor templates or homology-directed repairs. This rich diversity can furthermore be combined with selecting biocatalysts that cause a variety of desired genome modifications.

An example is Cas13.A, which cuts RNA instead of DNA. Under certain conditions, it goes into "overdrive" and indiscriminately cuts RNA in the vicinity of its RNA target. This "off-target" effect has been exploited as a diagnostic platform called SHERLOCK (**S**pecific **H**igh Sensitivity **E**nzymatic **R**eporter Un**LOCK**ing), which detects RNA and DNA and other genetic signatures of interest in minute amounts, for example for cancer diagnosis.

Work towards practical CRISPR applications is under way, for example the reversal of antibiotic resistance in bacteria, the development of diagnostic techniques based on DNA and RNA sequence recognition, and gene therapy. Yet, many CRISPR applications are still at the proof-of-concept stage and practical challenges such as delivery and off-target effects need to be overcome for CRISPR based therapeutics to reach clinical application.

As the technology moves closer towards clinical applications, ethical issues have arisen, in particular with regard to gene editing in the germ line. There is an active discussion among practitioners of governance principles such as the promotion of well-being, transparency, due care, respect of science, respect of the patient, fairness, and transnational cooperation. International human genome editing conferences provide platforms for these discussions. At the same time, the existing regulatory framework does provide quality and safety standards that apply to genome editing, and companies have taken self-regulatory steps to align approaches within the industry.

Yet, many CRISPR applications are still at the proof-of-concept stage and practical challenges such as delivery and off-target effects need to be overcome for CRISPR based therapeutics to reach clinical application.

Take-home points

- The ability to recode the guide RNA made it possible to turn CRISPR into a tool that could target *any* gene.
- CRISPR has made genome editing easier, faster and more accessible.
- The rich diversity of CRISPR systems can be combined with selecting biocatalysts that cause a variety of desired genome modifications.
- Work towards practical applications is under way, for example the reversal of antibiotic resistance in bacteria or the development of diagnostic techniques based on DNA and RNA sequence recognition.
- Ethical issues for clinical applications have arisen, in particular with regard to gene editing in the germ line.

Synthetic Biology

Synthetic biology is a fast-evolving discipline with great transformational potential: biology is increasingly described as an information science; biotechnology has moved from crude tools to targeted approaches; the costs of synthesis and assaying have dropped dramatically resulting in wider diffusion of the technologies; scientists have become experts in non-templated synthesis of information-carrying large molecules.

Despite important advances in synthetic biology in recent years, the traditional cycle from idea to product remains slow and expensive. The observation of effects still remains a bottleneck. Efficiency and reproducibility can be improved drastically by combining virtual simulations with automation, parallel processing, and advanced models. Multi-tenant, fully robotic, modular cloud laboratories allow users to interact through computing devices with remote laboratory modules that can translate their "experimental intent" into experimental work. Cloud laboratories promise to increase the speed of synthesis and agent characterisation, facilitate collaborations, improve utilisation of investment capital and augment data quality and accessibility. Customisation through virtualisation is expected to enhance flexibility while helping to aggregate and centralise infrastructure and applying methods that are capable of abstraction. Cloud labs can speed up the process from idea to data, and provide a reproducible environment using standardised protocols.[3]

Multi-tenant, fully robotic, modular cloud laboratories allow users to interact through computing devices with remote laboratory modules that can translate their "experimental intent" into experimental work.

In the past, global collaborations have evolved around the sharing of data, whilst the laboratory environment itself has changed little. Cloud laboratories are an indicator of a new form of collaboration in cyberspace, for wet chemical / biological experimentation. This will create new opportunities, and it may enable the use of artificial intelligence in drug development.

But the emergence of cloud laboratories in synthetic biology also raises questions about how to ensure that users do not misuse the technology for malevolent purposes (e.g. the synthesis of CB agents or illicit drugs). In addition, such laboratories themselves could become targets of remote attacks. The industry is actively addressing these problems, and applies customer-screening

3
For an open standard for specifying experimental protocols for biological research in a way that is precise, unambiguous, and understandable to both humans and computers see http://autoprotocol.org

protocols, screens molecular structures and synthetic routes using models for contextual understanding, implements reagent access controls, and relies on trusted user networks and firewalls. This mirrors approaches taken by companies that provide DNA synthesis services.

How, then, have advances in synthetic biology manifested themselves in industrial production? A number of complex biomolecules are manufactured using synthetic biology. Examples include artemisinine, the manufacturing of isoprene from glucose, sterile insect technique, and drug screenings. The Synthetic Biology Centre of the Woodrow Wilson Centre[4] lists 116 products as either being on the horizon or on (or close to) the market.

There remain hurdles before synthetic biology can become competitive in an industrial environment, while ensuring reliability and reproducibility.

Nevertheless, even natural products, such as vitamins, that appear to be prime candidates for production by fermentation are still mostly produced by chemical synthesis. There remain hurdles before synthetic biology can become competitive in an industrial environment, while ensuring reliability and reproducibility.

A first challenge is "real engineering". Process engineering requires modularity and the integration of different parts of a process model to identify the optimal operating point. In biological systems, complexity is a challenge: multiple enzymes interacting with substrates have to be modelled in the same system, taking account of various feedbacks and numerous conversions.

Nevertheless, complex *in vitro* designs are attractive as they provide access to interesting products (oligosaccharides, proteins, assays) and expand the space of biotechnology. Also, there is no need to keep cells alive, intermediate substrate / product toxicity and mass transfer issues are absent, reaction conditions can easily be controlled, and the process is highly selective.

To manage the complexities of such systems, experimental techniques are being developed to increase data density (information content) and quality. Modelling an *in vitro* system that contained eleven enzymes has shown that merely combining enzyme kinetics one by one was not sufficient. Aspects of chemical systems theory as well as an additional layer of complexity had to be incorporated into the model as well. This took several years of work and was accomplished using existing theoretical concepts and equations.

However, the model was not transferable to *in vivo* systems. Moving from *in vitro* to *in vivo* requires a change from engineering design to evolution. Scientists do not really understand what enzymes actually do in a cell, and how they interact with the cell's "chassis". Using an iterative process (designing variants and testing them to find the best-performing variant as a starting point for a next iteration) is not practical given that the number of pathways and variants grows exponentially. Researchers therefore try to find models that allow them to rationally reduce data libraries. Instead of engineering design, researchers use combinatorial optimisation of pathways. Much progress has been made with regard to enzyme evolution, data gathering, converting data into DNA se-

4 www.synbioproject.org/cpi

quences, and combinatorial synthesis. The same applies to integrating physical models with biology, improving hardware and automation.

Despite all this, researchers only get it right 80 % of the time. They don't quite understand why this is so, but there remain clear limitations to the engineering of biological systems, and tacit knowledge remains important.

A practical example of the application of synthetic and systems biology is the development of rapid diagnostic tests for the emergence of antibiotic resistance in bacteria. A comparison of the introduction of antibiotics with the emergence of resistance (from the sulphonamides of the early 1930ies to Dactomycin in 2004) suggests that antibiotic resistance is inevitable. Analysing resistance processes however can provide insights into the mode of action of a drug, reveal its binding sites, map resistance-conferring mutations, and help forecasting how quickly bacteria are likely to develop resistance. Also, once resistance-conferring variants are known, their clinical detection can be used to signal the emergence of resistance, and the cross-resistance pattern of such mutations can drive the selection of alternative therapies.

A practical example of the application of synthetic and systems biology is the development of rapid diagnostic tests for the emergence of antibiotic resistance in bacteria.

A classical test for the emergence of resistance is the fluctuation test (frequency of resistance assay). A huge number of cells (10 billion) are incubated on agar plates for 3 days, followed by analysis of the frequency of resistant variants. An alternative test is the 60-days passaging experiment in which bacteria are gradually adapted to a given antibiotic. Both tests depend on natural mutations, and hence are slow and low in throughput. Resistance evolution can be accelerated using a plug-and-play engineering system called pORTMAGE in which a single plasmid mutagenises pathogens using randomised DNA oligomers. These oligos carry mutations of interest; they are synthesised by an error-prone DNA synthesis that mimics natural mutagenic processes and results in libraries of mutant cells that show a great genetic diversity. This process can be repeated many times to further increase the rate of mutation. The same workflow can also be applied to evolve cells for the production of novel proteins, or to evolve novel phenotypes.

Resistance evolution can be accelerated using a plug-and-play engineering system called pORTMAGE in which a single plasmid mutagenises pathogens using randomised DNA oligomers.

But do pathogenic and non-pathogenic strains develop resistance in similar ways? To study this question, experiments were conducted using two strains of *E. coli* (pathogenic *E. coli* CFT073 (UPEC)) and non-pathogenic *E. coli* K-12). Single step mutations that are known to confer Trimethoprim resistance were delivered into the genes of the two strains, the cell libraries selected on agar plates, and the mutations determined with next-generation sequencing (NGS), showing strain specific differences in mutation patterns. This means that resistance analysis using non-pathogenic strains may sometimes be misleading and that resistance should be analysed directly in clinical isolates.

The same approach was used to investigate resistance evolution in topoisomerase targeting drugs, using 130 oligomers to deliver mutations to each position in four target genes, followed by standard fluctuation assays and characterisation by NGS. The test setup was used to identify known binding sites of Ciprofloxacin (to confirm the validity of the test), and subsequently applied to Gepotidacin, a compound currently in Phase II clinical trials. Numerous variants were identified that conferred high levels of resistance, and further analysis suggests strongly that two particular mutations interfere with the interaction of the drug with two binding sites. Thus, it seems that the drug candidate is only two mutations away from pathogens acquiring resistance, a conclusion that was consistent with the findings of the clinical trial.

These studies have shown that targeted mutagenesis offers a fast, high-throughput tool to analyse pathogen-specific resistance processes, including resistance processes that don't exist yet in nature.

These studies have shown that targeted mutagenesis offers a fast, high-throughput tool to analyse pathogen-specific resistance processes, including resistance processes that don't exist yet in nature. Such tests can be integrated into drug development.

Take-home points

- Cloud laboratories promise to increase the speed of synthesis and agent characterisation, and provide a reproducible environment using standardised protocols.
- Cloud laboratories for synthetic biology raise questions about their utilisation for malevolent purposes and becoming targets of remote attacks.
- Today, a number of complex biomolecules are manufactured industrially using synthetic biology.
- Complex *in vitro* designs are attractive as they provide access to interesting products and expand the field of biotechnology.
- Moving from *in vitro* to *in vivo* requires a change from engineering design to evolution.
- Despite much progress, there remain clear limitations to the engineering of biological systems, and tacit knowledge remains important.
- A practical application of synthetic and systems biology is the development of rapid diagnostic tests for the emergence of antibiotic resistance in bacteria.
- Targeted mutagenesis offers a fast, high-throughput tool to analyse pathogen-specific resistance processes and can be integrated into drug development.

Synthetic and Analytical Chemistry

Continuous biomanufacturing is a relatively new approach in the pharmaceutical industry. Continuous processes have distinct advantages over batch production: higher volume productivity and greatly improved economy; short residence time of the product in the equipment and thus better product quality and process stability; continuous product output over longer periods of time (30–40 days); elimination of certain process steps; possibility of process optimising for high yield *and* high purity; increased safety because of lower amounts present within the equipment at any moment; and reduced environmental impact.

With the exception of pharmaceuticals, today most low-profit-margin products are manufactured by continuous processes. Pharmaceuticals continue to be produced in batch, which is both costly and wasteful. Continuous pharmaceutical manufacturing would create many advantages. However, working with living organisms poses challenges with regards to process variability and disturbances, and the interaction between different process units is not well understood. Different process steps require different residence times, and times to respond to process abnormalities are short. Also, unlike chemical and food processing industries, continuous pharmaceutical manufacturing lacks integration of monitoring and process control, and smart control systems are missing.

SCADA systems are common in the chemical industry, but usually limited to data management and protection. For bio-process monitoring and control, the system needed to integrate data in formal, sequential and historical ways, combining process know-how with bioprocess modelling and digitalisation to better understand the relationship between process inputs and outputs, and to enhance the process model by learning.

One way of addressing these issues was to use Raman spectroscopy for process monitoring. The overlap of peaks of the different sample constituents (glucose, ammonium, amino acids, etc.) requires multivariate data analysis and validated predictive models. This approach was able to predict several quality attributes including most of the amino acids. Integrating this tool with downstream processes to monitor target proteins and DNA posed several problems, and required a supervisory control and data acquisition (SCADA) network to manage the vast data volumes. SCADA systems are common in the chemical industry, but usually limited to data management and protection. For bio-process monitoring and control, the system needed to integrate data in formal, sequential and historical ways, combining process know-how with bioprocess modelling and digitalisation to better understand the relationship between process inputs and outputs, and to enhance the process model by learning. Such a system also allows process optimisation and predictive maintenance. It holistically integrates process monitoring, control, and high-level optimisation.

What is needed is a combination of the versatility and interchangeability of flow reactors with efficient iterative process unit modules.

The system presented at the workshop uses a hierarchical control structure starting from a regulatory layer (bottom layer, time scale in seconds) through a supervisory layer (minutes to months), an optimisation layer (weeks to months) and an enterprise resource-planning layer (months to years). Integrating all levels remains difficult.

Such a system is specific for a particular production process and cell line. Each new target molecule requires a dedicated process. The proposed control and monitoring system is highly complex, which for the moment also creates certain instabilities. At the same time, the system creates a detailed record of process parameters and analytical data, which could be used for regulatory monitoring.

Work is under way to develop continuous bio-manufacturing processes for a range of pharmaceutical products. However, the target specificity of continuous processing described above is a serious obstacle. What is needed is a combination of the versatility and interchangeability of flow reactors with efficient iterative process unit modules. A solution presented at the workshop was a radial synthesiser – a fully automated, remotely controlled, modular assembly system in radial configuration that can manufacture several small molecules using the same hardware. While traditional continuous flow systems perform all process steps simultaneously, a radial configuration allows running them sequentially. This limits the output volume but allows running reactions at different flow rates and temperatures within the same hardware without equipment reconfiguration. The system is suitable for multistep syntheses and the generation of big data libraries. It ensures reproducibility and supports generating, capturing, storing and sharing data. Furthermore, it can operate in any location worldwide which could help to overcome current resource limitations for advanced chemical research in certain regions and countries. Except for reactions that require very high temperatures or pressures, the system setup is fast, as no reconfiguration is required. At the same time, if one centralises chemicals synthesis in such facilities, regulatory oversight and controls can be facilitated.

All this indicates a shift in the way chemistry is done, freeing chemists from laboratory work by outsourcing to remote automated systems, and refocusing their role on working with data rather than conducting wet experiments. There remain some technical limitations: solids (such as catalysts) in flow systems remain a major problem, and the reliability of pumps is still insufficient. Flow rates are a limiting factor. At the moment, production outputs measure in the hundreds of grams per day. This would need to be scaled up for industrial-scale manufacturing. There also is the question of achieving real control over the system based on models that identify all relevant control parameters (concentrations, reaction times, temperatures, mass transfer rates, flow rates, etc.) and interface with systems that monitor these parameters. Better interaction between chemists and engineers can help to overcome these issues, and there are high expectations that the way complex molecules are being synthesised will change.

There remain some technical limitations: solids (such as catalysts) in flow systems remain a major problem, and the reliability of pumps is still insufficient.

Take-home points

- Working with living organisms poses challenges for continuous pharmaceutical manufacturing.
- Raman spectroscopy combined with multivariate data analysis and validated predictive models looks promising for process monitoring.
- A process optimisation system presented at the workshop is specific for a particular production process and cell line, i.e. each target molecule requires a dedicated process.
- Work is under way to develop continuous bio-manufacturing processes for a range of pharmaceutical products but target specificity is a serious obstacle.
- A radial synthesiser is a fully automated, remotely controlled, modular assembly system to manufacture several small molecules using the same hardware. It is suitable for multistep syntheses and the generation of big data libraries.
- By centralising chemical synthesis and through their ability to be operated anywhere, synthesisers indicate a shift in the way chemistry is done – they allow wet experiments to be outsourced to remote automated systems.

Material Sciences including Nanotechnology

Previous workshops drew attention to advanced materials designed for drug delivery, or those that can mimic biological functions for research or reconstructive medicine. A field that drew attention was DNA origami, which exploits the self-organisation of DNA to "self-construct" nanostructures. The size, shape and other properties of such nanostructures depend on the nucleic acid sequence of the scaffold DNA strands, as well as the sequences of the DNA staple strands used to lock the scaffold strands into the desired shape. A long-term vision of this work is to fabricate artificial nanomachines that mimic the functions of biological systems.

An example was the assembly of capsid-like structures from triangular DNA origami structures. Using weak non-covalent interactions between terminal bases, such triangular structures can be stacked (similar to Lego bricks) to form capsid-like structures up to 200 nm in diameter. The structures are rigid and in principle could be developed as targeted drug delivery vehicles.

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Other structures have been developed to mimic rotary mechanisms – similar to those found in certain biocatalysts. It was possible to design origami with undirected rotary motion, but to mimic biological function, a better understanding of atomic accuracy in three dimensions is needed.

The stability of such structures remains problematic: they quickly disassemble under *in vivo* conditions. Coating their surface with polymers or peptides may mitigate this effect. In any event, at this stage it is not clear how to design catalytically active centres and how to integrate them into larger objects.

Practical applications of DNA origami structures in medicine or industry would require mass production. This requires economising the manufacturing of the staple strands. These are manufactured today by solid-state synthesis, at a price of around € 200,000 per gram DNA origami. A cost-cutting option could be to design a pseudo-gene with staples interlaced with self-cutting DNAzyme cassettes, which cut the DNA on either side of the staple in the presence of Zinc. This pseudo-gene can be inserted into a phage backbone, multiplied in *E. coli*, and Zinc ions are added after harvest to induce self-cleavage. Tests in 2-litre bioreactors have shown that the price of the DNA origami can be reduced tenfold. Up-scaling to an 800 litre bioreactor is expected to reduce the price of DNA origami to € 200 per gram, larger industrial applications in 10,000 L bioreactors would lower the price further.

Two years ago, DNA origami was characterised as early-stage exploratory research. Today, first experiments with rigid DNA objects are being conducted on laboratory animals. Furthermore, *in vitro* experiments have designed DNA origami "boxes" with lids on either side that can be opened and closed, a phenomenon that could be used to selectively release cancer treatments.

These advances pose the question of how far scientists are from creating "artificial life". Despite the remarkable progress, the bottom-up *de novo* assembly of completely artificial cells from DNA objects is still a long distance away. DNA origami is a young experimental technique, but the hope is that experimentation will help researchers understand more how biological machines function. This may lead to new drug delivery methods.

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A different approach to future drug delivery as well as molecular bio-sensing is the use of nanocarriers to deliver membrane-active antimicrobial peptides; this research relates to the development of "functional food".

Research in human breast milk showed the importance of nanostructure formation. The digestion of breast milk was simulated *in vitro* by mixing it with digestive juice, and the formation of structures was observed using small-angle X-ray scattering (SAXS) and transmission electron cryomicroscopy (Cryo-TEM). The digestion process is highly sophisticated, moving from a kinetically stabilised emulsion through flocculation and coalescence to the self-assembly of highly organised structures such as lipid crystals. These structures could function as carriers for hydrophobic molecules (e.g., building blocks used in the nervous system, or vitamins). Similar experiments were conducted to simulate egg digestion – this, too, showed the transformation of lipids into highly ordered liquid crystalline structures. These self-organisation effects can be used as blueprints for food and digestion inspired nanostructures that are suitable as carriers for antimicrobial peptides or other drugs that are not stable in aqueous environment and that would normally be degraded in the stomach.

A different approach to future drug delivery as well as molecular bio-sensing is the use of nanocarriers to deliver membrane-active antimicrobial peptides; this research relates to the development of "functional food".

Highly organised cubic nanostructures have been synthesised, loaded with peptides, and tested for antimicrobial activity. Peptides would normally degrade in the stomach but are protected by the nanostructures. A change in pH results in structural changes which switches antimicrobial activity on and off on demand. Other possible applications could include antimicrobial surfaces utilising immobilised nanocarriers, and nanocellulose-based biocomposites with antimicrobial activity. Nanocarriers can improve the efficacy of drugs and can be used to design stimuli-responsive drug delivery systems.

So far, these experiments have been conducted *in vitro*, but *in vivo* studies are in preparation. These nanostructures are biological systems that are present in the body during digestion, so there should be fewer hurdles when moving towards

clinical trials. At the same time, it is possible that such nanoparticles could be delivered as aerosols and inhaled through the lungs for uptake through the blood brain barrier. Such nanocarriers would be suitable for the targeted delivery of high amounts of toxins or bioregulators. Practical applications would require control over the loading of the structures with peptides / toxin and optimisation using different fatty acids and monoglycerides – artificial intelligence (AI) might help speeding up such complex optimisations.

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Finally in this section, the potential of using graphene oxide nanoparticles for drug delivery was discussed. Graphenes are two-dimensional nano-scale sheet-like carbon structures that can be formed into various shapes depending on the intended use. Discovered in 2004, they have numerous applications in research as well as the manufacturing of nanodevices, batteries, composite materials and other products. Work on biomedical applications began in 2008 and the field is still growing.

Among the various graphene derivatives, graphene oxides (GO) are of particular interest as they are biocompatible, easy to functionalise, suitable as efficient drug loading structures, scalable and inexpensive. GO are prepared by oxidising graphite using potassium permanganate (modified Hummers method), and subsequently reducing it to (highly) reduced GO. Different GO applications require different particle sizes (3–10 nm for quantum dots, 10-50 nm for *in vivo* drug delivery, 100–200 nm for *in vitro* drug delivery), and the preparation of mono-dispersed GO remains a challenge.

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Biological effect as well as toxicity (and hence safety) of GO nanoparticles depend on particle size, oxidation groups, functionalisation and cell type. Functionalisation methods for biomedical application include amongst others PEGylation, PEI modification (for gene delivery), and bioconjugation (peptides, antibodies, aptamer). GO interacts with cells at the plasma membrane, during cell uptake at the nuclear membrane, and in the cytoplasm where it may biodegrade. Cellular uptake of GO particles has been shown to involve clathrin mediated energy-dependent endocytosis.

A wide range of chemotherapeutics, cancer treatments, nucleic acids, proteins and other biomolecules have been delivered using different types of GO. Drug loading is achieved by, amongst others, physisorption, electrostatic interactions, and chemical conjugation. GO can be used for the sequential or combined delivery of multiple drugs to enhance therapeutic efficacy, for example in certain cancer treatments.

Graphene oxides can also be used for regenerative medicine applications. Nanofibers doped with GO promote the differentiation of stem cells towards

osteoblasts; in animal models, certain GO-doped nanofibrous membranes promote tendon-to-bone integration, which is utilised in injury repair.

Challenges to the biomedical application of GO nanostructures include the development of easy, cost effective and environmentally friendly synthesis and functionalisation; drug delivery beyond cancer treatment; controlling biological effects and safety in large animals and humans; and the development of quality standards and regulations.

GO can be used for the sequential or combined delivery of multiple drugs to enhance therapeutic efficacy, for example in certain cancer treatments.

Take-home points

- DNA origami is a young experimental technique that may assist researchers deepen their understanding of how biological machines function.
- Two years ago, DNA origami was characterised as early-stage exploratory research. Today, first experiments with DNA objects are being conducted in laboratory animals.
- Triangular DNA origami structures can be stacked to form capsid-like structures up to 200 nm in diameter, and could be developed as targeted drug delivery vehicles; their stability *in vivo* remains problematic.
- Practical applications of DNA origami structures in medicine or industry demand mass production, which requires economising their manufacturing.
- Food and digestion inspired nanostructures may be suitable as carriers for drugs that are not stable in aqueous environment and that would normally degrade in the stomach.
- Nanocarriers can improve the efficacy of drugs and can be used to design stimuli-responsive drug delivery systems.
- It is possible that nanoparticles could be delivered as aerosols and inhaled through the lungs for uptake through the blood brain barrier. Such nanocarriers would be suitable for the targeted delivery of high amounts of toxins or bioregulators.
- Graphene oxides (GO) are two-dimensional nano-scale sheet-like carbon structures with the potential for drug delivery.
- Different derivatives, are of particular interest as they are biocompatible, easy to functionalise, suitable as efficient drug loading structures, scalable and inexpensive.
- A wide range of chemotherapeutics, cancer treatments, nucleic acids, proteins and other biomolecules have been delivered using different types of GO.

Additive Manufacturing

Discussions of additive manufacturing (AM or "3D printing") in previous workshops shifted from initial high expectations combined with concerns about implications for export controls of specialised equipment, to recognition of process limitations that would stand in the way of using AM for the routine production of parts that have to withstand high performance challenges including highly corrosive chemicals.

The technology is still evolving. AM is faster than traditional (subtractive) manufacturing but cannot compete with sheet metal fabrication unless it provides other advantages (high complexity, small numbers of items manufactured to specification, personalised design, fast prototyping, redesign of parts to reduce weight while preserving strength). The interest in AM also emanates from its ability to disrupt conventional supply chains, giving end users control over the product design. This may go as far as users "printing their lab in the field", but there remain limitations (power supply, meeting performance standards). Despite its advantages, AM is unlikely to replace conventional manufacturing such as injection moulding, casting or machining, except when complexity is high and the production numbers are low.

The interest in AM also emanates from its ability to disrupt conventional supply chains, giving end users control over the product design.

The AM industry continues to grow rapidly: in the year 2000, the global market was estimated at € 0.5 billion; today, it has grown to € 4.5 billion and it is estimated to grow to € 7.7 billion over the next five years. The number of 3D printer manufacturers is still expanding, and so is the number of AM service companies. Industrial applications include aerospace (engine components and feature addition to existing parts), medicine (devices and implants), the marine, oil and gas industries (corrosion-resistant and wear-resistant layers of wellheads, valves, and drilling tools), precision engineering (rapid tooling, tool and die repair and modification), and cars (prototyping, repairs, manufacturing of valves and shafts).

Initial concerns were about the possible spread of high performance 3D printers, whilst today the trend is towards concentrating high-end AM systems (high resolution, large format, high degree of complexity, special materials) in service centres. Only industrial AM systems can supply high-quality parts to a competitive standard with incumbent industrial processes.

Different processes can be used to print 3D objects: powder bed fusion, directed energy deposition, material extrusion, Vat photo-polymerisation, binder jetting, material jetting and sheet lamination. Depending on the printing technology used, the materials available include metals, polymers, ceramics, foundry sand, waxes, and paper.

Of particular interest in the context of CBW arms control are AM processes that build 3D objects that can withstand high temperature and pressure steam sterilisation (for biological equipment), or corrosion caused by high temperatures, concentrations and ionic strength of reactants (for chemical equipment). High performance materials that meet such requirements have been subjected to export controls, such as Ni and high-Ni alloys, certain metals (Ti, Ta, Zr, Nb) and their alloys, ferrosilicon, flouropolymers, glass and glass-lined equipment, graphite or carbon graphite, carbides of Si, and oxides of Ti, Al and Zr. Many of these materials are used today in additive manufacturing. Recent additions were polytetrafluoroethylene (PTFE) and polyvinylidine fluoride (PVDF); the former is at present offered only via AM service centres whilst the latter is compatible with commercially available 3D printers. Little information is available on the corrosion resistance of parts printed with these materials.

Of particular interest in the context of CBW arms control are AM processes that build 3D objects that can withstand high temperature and pressure steam sterilisation (for biological equipment), or corrosion caused by high temperatures, concentrations and ionic strength of reactants (for chemical equipment).

Corrosion resistant metal parts can be 3D printed using powder bed fusion and binder jetting. Both processes require know-how, technical competence and a thorough process understanding. Powder bed fusion uses either a laser beam or an electron beam to melt layers of metal to form sintered parts. Binder jetting uses a binder to “glue” layers of material together (metals, ceramics, minerals and other materials) to build the parts. Subsequently, the printed parts need to be cleaned to remove excess powder (using vibration, gravity, compressed air, or water pressure). Powder residues will sinter during subsequent heat treatment. Binder jetting uses solvents, which must be removed thermally; this can lead to carbon contamination and cracking. The parts also need thermal densification, and shrinkage is difficult to predict for larger parts. The high cooling rate of powder bed fusion, too, can lead to build failure, distortions and cracking. The parts therefore need to be stress relieved by heat. To achieve a proper microstructure and mechanical properties, parts are subjected to solution (or solution plus precipitation) heat treatment in both processes. Finally, critical features for assembly are added back and imprecisions corrected.

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A range of surface finishing methods is available to ensure high performance, including corrosion resistance (metal plating, sanding / grinding, grit blasting / shot peening, vibration and tumbling, heat treatment, vapour smoothing

and solvent dipping). These techniques work well for external and certain internal surfaces. For complex internal geometries, it is not clear whether this can always be achieved. Finally, the parts are quality controlled using a range of methods (fluorescent penetrant inspection, radiographic inspections, CT / VCT scanning). In short: AM of complex parts to high performance standards remains a challenge.

Laser aided AM can be used to enhance the properties of alloys: powders or wires are fed through the centre of a laser beam using specially designed nozzles, to print single-layer, multilayer or functionally graded objects. A wide range of metals can be used as base and additive materials. Process control (e.g., using IR sensors) is critical, as is post-printing machining to ensure quality.

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Another way of enhancing performance is the addition of carbide particles during AM. Given the mismatch in thermal and metallurgical properties, and the high percentage of carbide needed, there are problems when using micron-size carbide participles. Carbide nanoparticles have been utilised instead, but challenges with regard to homogenous distribution, thermal stability and dissolution of the nanoparticles in the metal matrix have yet to be overcome. Special powder preparation methods were tested, and the composites showed a significant increase in wear and corrosion resistance, tensile strength and hardness.

In general, there still remain certain knowledge gaps in AM to achieve the ultimate goal of 100 % reproducibility (between printing rounds and also between different printers). There are gaps in understanding of what happens when feedstock is heated to melt and then cooled to solidify. X-ray imaging techniques are employed to fill these gaps. A better grasp of these processes could reduce the need for post printing processing and improve corrosion resistance.

At this stage, AM technology is driven by software and open innovation. “The intelligence resides in the cloud”, hardware and tools are increasingly available or accessible, and engineering is reduced to operating a computer. In this way, AM fits neatly into the “industry 4.0” model in which data is gathered from all points of a manufacturing process, evaluated and used to control production. Production processes react in real-time using sensors and next-generation robots.

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3D printing is a key enabler in this model. Expectations remain high: over the coming two years, industrial uses of AM are expected to increase and 3D printing will become larger, faster, of higher precision, and of higher quality. Over the next 5 years, the list of printable materials is expected to grow significantly, 3D printing will penetrate education, processing and regulatory standards will be developed, and AM is expected to be adopted across multiple industries. Within 10 years, AM is thought to become a commonplace alternative to conventional manufacturing that will seamlessly integrate with other technologies, and production service centres should be available globally.

At the same time, the production of corrosion resistant metal parts will continue to require robust industrial processes, and it is unlikely that 3D printers capable of manufacturing highly corrosion resistant parts or equipment would be available to individuals or consumers soon.

3D printing will penetrate education, processing and regulatory standards will be developed, and AM is expected to be adopted across multiple industries.

Take-home points

- The AM industry continues to grow rapidly and has the ability to disrupt conventional supply chains, giving end users control over the product design.
- Different processes (powder bed fusion, directed energy deposition, material extrusion, Vat photo-polymerisation, binder jetting, material jetting, sheet lamination) and materials (metals, polymers, ceramics, foundry sand, waxes, paper) can be used to print 3D objects.
- AM is faster than traditional manufacturing but cannot compete with sheet metal fabrication unless it provides other advantages, and AM of complex parts to high performance standards remains a challenge.
- In the context of CBW arms control AM processes building 3D objects that can withstand high temperature and pressure steam sterilisation or corrosion are of particular interest.
- Only industrial AM systems can supply such high-quality parts to a standard competitive with incumbent industrial processes.
- Professional know-how and technical competence is required and it is therefore unlikely that 3D printers capable of manufacturing highly corrosion resistant parts or equipment would be available to individuals or consumers soon.
- Over the next 5 years, the list of printable materials is expected to grow significantly, 3D printing will penetrate education, processing and regulatory standards will be developed, and AM is expected to be adopted across multiple industries.

Bioinformatics, Omics, and Big Data

Historically, OMICs started with the study of the genome and expanded gradually to include the transcriptome, the proteome, the lipidome, the glycome and the metabolome. Advances in genomics and transcriptomics are driven by next-generation sequencing (NGS) techniques, whilst work in the other fields relies heavily on mass spectrometry.

NGS techniques have been very successful, partly because they rely on the biological design principles of the molecules they study: DNA and RNA have evolved over millions of years as information carriers that can be written and read easily. Parallelisation has increased the capacity of sequencing drastically. Today, researchers can perform parallel read operations of 10 billion molecules in a single experiment. Also, precision has increased to enable single molecule manipulations. At the same time, this molecular simplicity enables a broad range of applications, such as ribosome profiling to identify which RNA molecules are currently transcribed at which position, identification of how parts of a chromosome interact with one another, or screens to determine which genes are associated with which phenotype.

Today, researchers can perform parallel read operations of 10 billion molecules in a single experiment. Also, precision has increased to enable single molecule manipulations.

Sensitivity has increased to a point where single cells can be studied. Traditional methods used grinded tissue. This provides an average of what happens, e.g. in a cell culture. The analysis of a single cell provides a much more granular picture instead. A vast range of single-cell protocols have been developed, enabled by the dramatic fall in the sequencing cost and a drastically reduced error rate of NGS.

Sensitivity has increased to a point where single cells can be studied.

This combination of factors (the right molecules for study, parallelisation, low cost and high sensitivity) has lead into what some call a NGS revolution. But it cannot replace good experimental design. The creativity of researchers still determines the value of experiments, or the limits of insight they can achieve. This idea was behind the setting up of a functional genomics support centre at the ETH Zurich and the University of Zurich to provide OMICs support services to address:

- The fast speed at which protocols and applications are evolving;
- The continuing fast advances in sequencing technology;
- The need to ensure proper documentation and reproducibility;
- Protection of the confidentiality of samples and data;
- Data storage;
- Demands in computation; and
- The diversity of applications.

These services include, for example, the detection and correction of specimen mislabelling in multiomics data sets, the integration of data management processes at different levels (administration and management; support for lab work and instrument- as well as sample-specific processes; data processing, analysis and integration), and references and annotation management.

Multiomics projects pose challenges with regard to the protection of patient-specific data.

Furthermore, multiomics projects pose challenges with regard to the protection of patient-specific data. Individual genomes carry information about the individual concerned. Hence, the analysis of individual genome data could be utilised to link individuals to specific genetic characteristics, thereby compromising privacy rights. This problem can be mitigated by controlling access to data that would limit the use of available information, through policies (challenging as institutions with diverse backgrounds are involved), or privacy-preserving data mining technologies. The latter could include secure multiparty computations such as homomorphic encryption (very secure but at high computational costs), SGX hardware (vulnerable to side-channel attacks), or differential privacy (adding noise to the data which reduces data quality). In short, personal genomics data create unique challenges in terms of privacy protection. Exposure of such data carries the risk of identity leakage and leaks of sensitive phenotype information. Current privacy-preserving data mining technologies need to be improved with regard to performance and security. There also is the challenge that multiple databases may hold information pertaining to the same individual – privacy protection may still be technically possible but would become very costly.

To deep machine learning, which – combined with big data and automation - allows to integrate chemical and biological synthesis with artificial intelligence (AI).

Finally, the workshop turned to deep machine learning, which – combined with big data and automation – allows to integrate chemical and biological synthesis with artificial intelligence (AI). Several factors drive this convergence:

- A recognition of the potentially enormous "chemical space", with almost infinite numbers of possible new molecules and materials[5];
- The immense possibilities offered by bespoke molecules and materials;
- Moore's law of increase in computing capabilities; and
- A better understanding of the interactions of molecules and materials with biology.

This enormous chemical space may be largely "empty" with chemical structures of little or no utility. Finding "islands of utility" with materials that have useful properties cannot simply be achieved by "working faster", but the combination of deep learning, automation, and evolutionary methods may accomplish this.

An example is the development of personalised cancer treatments using systems biology as a framework to bring together different OMICs to develop strategies to discover relevant biomarkers. Combinatorial chemistry methods, microarray techniques, and high throughput as well as high content automated tests can then provide the tools to apply these strategies in practice.

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$4^{3,000,000,000}$ theoretically possible variants of DNA, 20^{200} possible variants of proteins, 10^{100} of materials – compared to 10^{80} particles of matter present in the observable universe.

Material science has taken the lead in developing new drug candidates. Tools and techniques today allow up to 10,000 variants of materials to be produced, characterised and tested in a single day. Chemical mark-up language is being developed to enable digitisation and communication between human researchers and machines. Artificial intelligence (AI) using (deep) machine learning offers a way forward to managing the vast amounts of data. Machine learning (ML) has progressed from simple decision trees and nearest neighbours algorithms to neural networks and neural network architectures with multiple layers and nodes to improve predictions. Deep learning (DL) requires access to big data and, most importantly, huge computational power.

Recent examples of the predictive power of AI (optimisation of organic syntheses, accurate predictions of reaction performances and yields) have raised questions about whether AI may replace chemists. DL has overcome many limitations of physics-based models: it is able to work with massive data sets, it can access open data sources, it builds models from very simple representations of chemical and biological entities, and it can suggest synthesisable structures with improved properties. Available platform technologies can be applied to a wide range of systems and AI can apply evolutionary methods to generate autonomous systems for synthesis of biologically active molecules and materials. Data quality is important but AI is surprisingly tolerant to noisy data and can identify outliers in large data sets, suggest re-measurements, and revise models.

Deep learning builds models from very simple representations of chemical and biological entities, and it can suggest synthesisable structures with improved properties.

DL has been applied recently to quantum mechanical calculations. The rate-determining factor is computational speed and power. Neuromorphic chips or optical diffractive deep neural networks may be game changers, allowing to move from evolutionary algorithms to adaptive autonomous systems. The convergence of automation with evolutionary algorithms allows the number of materials that can be synthesised, tested and optimised to vastly expand. Synthesis and property optimisation problems can be formulated using variables such as reagents, catalysts, process conditions, solvents, and variables related to molecules and materials. Optimisation targets can be defined as product yields, enantiomeric excess, catalytic activity, electromagnetic, mechanical and thermodynamic properties, or molecular binding constants.

DL also can accelerate the rate of optimisation of evolutionary processes *in silico*. These are effective tools in materials discovery, and the combination of automation, experimental design, machine learning and evolutionary algorithms allows exploring large material spaces in the search for new materials. Finally, the loop has been closed from robotic synthesis to encoding structures, automated testing, model building, predicting improvements, and decoding improvements into new structures for new robotic synthesis – a way towards the optimisation of new biomaterials.

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Such an approach is no longer science fiction: a first AI robot for independent drug development (called "Eve") is operational. But this is only the beginning and new opportunities may include:

- Mining of data lakes and use of evolutionary methods for rapid discovery and design of bespoke molecules and materials;
- Simulation of complex materials such as proteins;
- Greatly enhanced ability to predict viable chemical reaction conditions and yields;
- Adaptive autonomous (bio)chemical production systems with greatly reduced costs;
- Standardisation and mark-up languages, open access to DL methods and access to cloud resources; and
- Bespoke evolved robotic systems for specific applications.

There is, however, the fundamental question of whether humans can control the evolution of AI. There have been warnings about the inability of humans to compete with fast-evolving AI. At the same time, pressures to move ahead with AI are strong. It will be important to find reliable safeguards as the technology evolves.

It will be important to find reliable safeguards as the technology evolves.

Take-home points

- Researchers can perform parallel read operations of 10 billion nucleic acid molecules in a single experiment and experimental precision has increased to enable single molecule manipulations.
- Multiomics projects pose challenges with regard to the privacy protection of patient-specific data because the analysis of individual genome data could be used to link individuals to specific genetic characteristics.
- Current privacy-preserving data mining technologies need to be improved with regard to performance and security.
- Machine learning has progressed to neural networks and neural network architectures that allow better predictions.
- Deep Learning has overcome many limitations of physics-based models, e.g. it is able to build models from simple representations of chemical and biological entities, and can suggest synthesisable structures with improved properties.
- Deep Learning requires access to big data, huge computational power, and combined with automation enables integration of chemical and biological synthesis with artificial intelligence, thus expanding the number of materials that can be synthesised, tested, and optimised.
- There is a fundamental question of whether humans can control the evolution of AI and reliable safeguards need to be found and implemented as the technology evolves.

Summary and Conclusions

This workshop series had set out to facilitate an informed conversation between different stakeholder communities of the CWC and the BWC about the impact that advances in science and technology have on the two treaties and their implementation. Science and technology affect these regimes in different ways: they can challenge the scope of their prohibitions, change their implementation environment calling for adaptations in national implementation and/or verification conduct, offer new verification tools and techniques, and provide new defences against chemical and biological weapons. They can also affect perceptions and incentives regarding CB weapons.

Yet, the life sciences and related technology are not driven by CB weapons objectives, but by drivers from within the science and technology enterprise and by societal demands and expectations for enhanced international cooperation. This broadens the context within which risk mitigation strategies are developed: they must not obstruct scientific and technological progress but help to steer its application away from misuse.

So what has changed since the first convergence workshop in Spiez in 2014? In a nutshell: the focus of discussions has shifted from materials and equipment, to information, automation and remote manufacturing. Whilst not completely new, these trends now seem to characterise a broader domain of chemistry and biology, and their practical applications are leading to faster and better-quality manufacturing of more complex chemical and biological products and solutions. With manufacturing moving closer to the point of use, the role of the end-user is changing. Also, remote and distributed production of the components of complex items can be directed towards different service providers, or distributed manufacturing can be combined with *in situ* manufacturing using devices such as 3D printers. As a consequence, access to data as well as intangible transfers are becoming more relevant from a regulatory and control perspective.

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At the same time, traditional mainstream technologies are evolving. The way in which new technologies will manifest themselves in industry, and the pace with which they will spread, depends on a host of economic and other factors, including how well they can compete with mature technologies.

Nevertheless, the advances in S&T are creating a potential that can be misused for CB warfare purposes: the development of strategies for the design or evolution of novel CBW agents, new (cheaper and safer) ways of manufacturing known agents, more effective methods of delivering CBW agents to target, and technological change also may make it more problematic to recognise malign activity. Novel CBW production facilities would have a smaller footprint and different technological features compared to what is known from past state programmes.

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How S&T advances may (or may not) translate into CBW threats also depends on the actors involved. Non-state actors may be able to take advantage of some of these advances, but many of the obstacles they faced in the past still persist. Their attempts to acquire CBW capabilities are likely to remain opportunistic, and experience has shown that constraints continue to exist with regard to costs, difficulties to effectively disseminate agents in the field, and access to critical materials, equipment, and tacit knowledge. If non-state actors are to use improvised CB weapons, these would have to become part of the means of how they currently fight their wars.

State actors pose different issues. Risk assessments are not simply about new molecules, dissemination techniques, or production methods, but more importantly about how such new materials and methods would fit into a contemporary CBW programme. At the same time, one would have to ask what the incentives were to open such a new programme, and which institutional and funding frameworks it would require. Additionally, in today's security context one would not only have to think in terms of WMD utility but also consider smaller-scale uses for sabotage or assassinations.

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Then, there is the question of whether the implementation systems adopted by the States Parties of the two Conventions, as well as measures taken to prevent the spread of CBW capabilities such as export controls, continue to function in the changing environment. Questions that countries need to ask themselves are: is there a need to change existing or adopt additional regulations; are the systems used to identify facilities and activities for CWC declarations still adequate or are certain aspects of industrial activity under the radar screen; do transfer controls related to materials, equipment and intangibles still provide the desired level of assurance against CBW proliferation?

Many of these issues need to be addressed by National Authorities, but to do so they need to grasp what the advances in S&T mean for implementation. That poses the question of S&T literacy of National Authorities, which competencies they need to hold and how they can access information, training for their staff, and technical support (including from the OPCW or through the ISU).

Many of the S&T advances will call for multi-stakeholder governance approaches. Reaching out to the research community and industry will be important for National Authorities to develop partnerships and governance systems that avoid the pitfalls of trying to regulate within a rapidly changing domain of industrial and scientific activity. There is a risk that new regulations become obsolete when they are adopted, or create undesirable obstacles to beneficial applications.

At the same time, National Authorities cannot simply rely on industry taking its own governance steps. They need to reach out to partners in research and industry to help them understand the security implications of S&T, and develop awareness for the need to manage the risks associated with these advances.

The advances in synthetic biology or the emergence of cloud manufacturing, for example, point to the need to address responsibility issues especially with regard to security. Many companies adhere to and apply codes of responsible conduct, and although the focus is often on safety, security issues can, and need to be built into these mechanisms.

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When industry is a partner in mitigation strategies, it can roll out solutions through its national and international trade associates and company relations. Examples in the chemical industry (Responsible Care®) or self-regulations adopted by DNA synthesising companies can be used to inspire similar measures in other industries, such as additive manufacturing, synthetic biology, or cloud manufacturing.

Such an outreach would at the same time facilitate a dialogue between the stakeholders that could help National Authorities to better understand where new regulations are needed, and what kind of regulations would be appropriate.

Advances in S&T will not only affect chemical weapons disarmament and related security risks, but they are equally relevant to other societal issues (illicit drugs, safety, environmental protection, cyber security). Hence, National Authorities also need to consider how they interact with other competent authorities, share information and synchronise regulations and enforcement.

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The shift from sensitive materials and equipment to information can also provide new opportunities for oversight, compliance monitoring and verification. It would require a transition from controlling / verifying materials and equipment (which will remain important for traditional CBW threats) to

capturing and analysing data sets and pathways. The strength of AI and deep learning in detecting and analysing patterns could be utilised for addressing compliance concerns, or in forensic investigations. This cannot replace human evaluation but it could strengthen the chain of evidence in support of verification, or lead to new investigation leads.

These advances do, however, pose the question whether verification systems are still asking the right questions. If materials and equipment are less relevant as triggers of verification activities or indicators of the nature of activities, what other signals can be applied in verification? Options may include the analysis of investment streams, of trends in the patent literature, or of applications rather than technologies. Current national compliance assessment systems may be able to adopt such approaches, but what about international verification measures or export controls? These are questions that deserve to be studied in the medium-term, as answers are unlikely to come easy.

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Finally, it is important to stress that the risks that may emanate from advances in S&T must not be overstated. Exaggeration can lead to over-reactions, for example regulations that become a hindrance for progress and beneficial applications, or by creating political and public perceptions that obstruct investment into beneficial scientific work.

This workshop has shown how enabling the synthesis in small amounts in different ways and at faster speed is changing chemistry. However, for risk assessments at the state level, size matters. CRISPR is an example: it is a research tool with significant potential for improving diagnostics and treatments. It also has weapons potential. But to assess the impact of CRISPR, one needs to appreciate the significant roadblocks before its wider application beyond research, such as effective delivery. Whilst it is conceivable that CRISPR could be used to modify BW agents leading to higher virulence or antibiotic resistance, it is probably still easier to find such new agents in nature.

In a state programme, these multiple requirements create significant counterincentives. This may be different in the context of alternative, smaller-scale use scenarios.

For weapons purposes, the discovery of a new toxic chemical or infectious agent is only a first step. The introduction of a new CB weapon needs to satisfy many criteria: predictable and reproducible effect, field utility, stability for storage and in the environment, cheap to manufacture, ability to be integrated into operational systems, successful in testing to meet military acceptance criteria, compatible with existing military doctrines, to name a few. Also, high-tech solutions have to appreciate that well over 90 % of a disseminated agent never reaches the intended target. As an example, even if peptide-loaded nanoparticles were engineered for easy aerosolisation and crossing the blood brain barrier, how attractive would a weapon be when almost all the material

would be dispersed off-target under field conditions? In a state programme, these multiple requirements create significant counterincentives. This may be different in the context of alternative, smaller-scale use scenarios.

In sum, the workshop confirmed the usefulness of continuing to screen the S&T horizon to recognise and assess developments that may affect the CWC and the BWC. Such evaluations have a short and a longer-term perspective – there are advances that require a swift response to manage emerging risks (for example the availability of 3D printers that use polyfluorinated polymers which could undermine export controls and may therefore call for swift regulatory adaptation). Others may affect implementation over time (such as the use of cloud manufacturing services in chemical and biological manufacturing) or simply need to be taken into account in the implementation processes. The participants confirmed the usefulness of continuing regular exchanges among the different stakeholder communities of the CWC and the BWC about the impact of S&T advances on CB disarmament and security.

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Take-home points

- Since 2014 the focus of discussions has shifted from materials and equipment, to information, automation, and remote manufacturing.
- This shift from sensitive materials and equipment to information can also provide new opportunities for oversight, compliance monitoring, and verification.
- With manufacturing moving closer to the point of use, the role of the end-user is changing; access to data as well as intangibles transfers are becoming more relevant from a regulatory and control perspective.
- Novel CBW production facilities would have a smaller footprint and different technological features compared to what is known from past state programmes.
- Non-state actors' attempts to acquire CBW capabilities are likely to remain opportunistic and constraints continue to exist with regard to costs, effective dissemination of agents and access to critical materials, equipment and tacit knowledge.
- How would new materials and methods fit into a contemporary state actors' CBW programme; not only as WMD utility but also for smaller-scale uses for sabotage or assassinations?
- Are implementation systems adopted by the States Parties of the two Conventions, as well as export control measures still effective in the changing environment?
- Many of the S&T advances will call for multi-stakeholder approaches between the research community, industry and National Authorities to develop partnerships and governance systems.
- Spiez CONVERGENCE evaluations may have a short and a longer-term perspective: 3D printers that use polyfluorinated polymers which could undermine export controls require a swift response to manage emerging risks, whereas the use of cloud services in chemical and biological manufacturing may affect implementation over time.

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