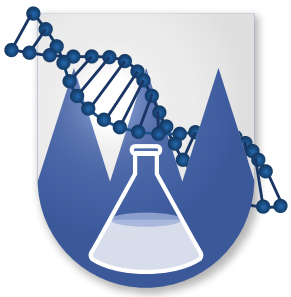

Spiez CONVERGENCE

Report on the second workshop
5–8 September 2016

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Office for Civil Protection FOCP
SPIEZ LABORATORY

CSS
ETH Zurich

Federal Department of Defence, Civil Protection and Sports DDPS
Federal Office for Civil Protection FOCP

SPIEZ LABORATORY
The Swiss Federal Institute for NBC-Protection,
in collaboration with the Center for Security Studies, Swiss Federal Institute of Technology ETHZ

CH-3700 Spiez
Tel. +41 (0) 58 468 14 01
Fax +41 (0) 58 468 14 02
labor-spiez@babs.admin.ch
www.labor-spiez.ch

www.spiezconvergence.com

Contents

Acknowledgements 4

Executive Summary 6

Introduction 11

Chemical synthesis, chemical modification and large molecules 13

Additive manufacturing 19

Genome editing – CRISPR technology 21

Big Data / Omics 26

DNA memory and programming 28

DNA origami 30

Tacit knowledge 32

Conclusions – implications of convergence between chemistry and biology for arms control 34

Participants 38

Acknowledgements

Spiez Laboratory, the Swiss Federal Institute for NBC-Protection, is responsible for the content of the report. It does not reflect an official Swiss position.

Financial and logistical support of the following organisations made the workshop and this report possible:

- Swiss Federal Department of Foreign Affairs, Division for Security Policy
- Swiss Federal Department of Defence, Civil Protection and Sports, International Relations Defence
- Swiss Federal Institute of Technology Zurich (ETHZ), Center for Security Studies (CSS)

The report has been prepared by the following conference rapporteurs, session chairs and workshop organisers:[1]

Rapporteurs:

Michelle Dover Ploughshares Fund

Amanda Moodie Center for the Study of Weapons of Mass Destruction

James Revill Harvard Sussex Program

Session Chairs:

Stefan Mogl Spiez Laboratory

Ralf Trapp CBW arms control and disarmament consultant

Mark Smith Wilton Park

John Walker UK Foreign and Commonwealth Office

1. For full affiliation, see participants list, p. 38

Speakers:

Thomas Barnes Intellia Therapeutics, Inc.

Stan Brouns Delft University of Technology

Marc Cadisch Spiez Laboratory, Federal Office for Civil Protection

Carlos Castro Ohio State University

Jonathan Forman Organisation for the Prohibition of Chemical Weapons (OPCW)

Vadim Govorun Federal Research and Clinical Center of Physical – Chemical Medicine of the Federal Medical Biological Agency

Robert Grass Swiss Federal Institute of Technology Zurich (ETHZ)

Frank Grütter Division for Security Policy, Federal Department of Foreign Affairs[2]

Matthias Halter International Relations Defence, Federal Department of Defence, Civil Protection and Sports[2]

Patrik Hoffmann Swiss Federal Laboratories for Materials Science and Technology (EMPA)

Farren Isaacs Yale University

Laura Na Liu Max Planck Institute for Intelligent Systems

Alain Marx Lonza AG

Andrew May Caribou Biosciences, Inc.

Tony Nolan Imperial College London

Eugene Ong Insitute for Research in Molecular Medicine

Samuel Perli Massachusetts Institute of Technology (MIT)

James Revill Harvard Sussex Program

Markus Rimann Zurich University of Applied Science

Peter Seeberger Max Planck Institute of Colloids and Interfaces

Michael Studer Bern University of Applied Sciences

Ahmet Üzümcü Organisation for the Prohibition of Chemical Weapons (OPCW)[2]

We thank the speakers for their contributions

Workshop Programme and Organising Team:

Lisa Brüggemann, Cédric Invernizzi, Franziska Mala, Stefan Mogl, Beat Schmidt, Marc Strasser Spiez Laboratory

Claudia Otto, Oliver Thränert Center for Security Studies (CSS), Swiss Federal Institute of Technology Zurich (ETHZ)

Ralf Trapp CBW arms control and disarmament consultant

2. Official opening speaker

Executive Summary

In 2014, Switzerland started a new workshop series to discuss the convergence in chemistry and biology, and how advances in science and technology (S+T) may affect the Chemical and Biological Weapons Conventions. This workshop series is a Swiss contribution to an active and substantive S+T review process. Ambassador Üzümcü, the OPCW Director-General, opened Spiez CONVERGENCE 2016 by reflecting on chemical weapons use in the Syrian conflict and the report of the Joint Investigative Mechanism, which had just been released. The very sophisticated technological advances discussed under the concept of convergence stand in stark contrast to the use of crude chemical weapons technology described in these investigations. This chemical weapons use serves as a troubling reminder of how important it is to uphold the provisions of the CWC and BWC, and to ensure they remain effective in view of today's technological advancements.

The program for Spiez CONVERGENCE 2016 was compiled through literature screening looking at developments in basic research, industry applications and issues raised in the arms control community. In sum, the workshop discussed 'hot-science' topics of *possible* relevance to both the CWC and the BWC.

Chemical synthesis, chemical modification and large molecules were discussed to review developments in the biologically mediated manufacturing of chemicals. The market share of such processes has been negatively affected by a drop in crude oil prices. Nevertheless technologies for converting biomass – sugars, starch, lignocelluloses – into chemical products continue to be developed. While the biochemical conversion of sugars is a well-established industrial process, conversion of starch and celluloses is more challenging. Because sugars and starch are food sources and therefore less desirable as raw materials, lignocelluloses, that are very abundant, offer a valuable alternative. Lignocelluloses are not easy to convert – opening the cell structure to allow access to these polysaccharides requires sophisticated methodologies – but new strategies exist in the form of consolidated bioprocessing. Some progress has been made towards industrial application but for now, sugar and starch remain the dominant feedstock for industrial production of chemical products from biomass. The opposite approach to the breaking down of natural bio-materials is the synthesis of complex carbohydrates from small building blocks. Automated carbohydrate synthesis is becoming commercially available, permitting the synthesis of biomolecules with ever greater complexity; what in the past took months, can be achieved today in hours. The technology offers many possibilities and may make new biomaterials available. This includes new vaccines based on carbohydrate conjugates, for which clinical trials are expected to start soon. A trend in the pharmaceutical industry is to move towards using

highly active pharmaceutical ingredients (HAPI) – a third of novel drugs developed fall into this category. HAPI production plants are technologically highly complex and containment standards are similar to high-safety biological facilities. In many aspects they resemble a larger-scale CWC Schedule 1 facility but because of their production profile they remain below the CWC declaration threshold for discrete organic chemicals (DOC)-producing facilities. A further approach for synthesizing chemicals is engineering the genome with the aim to convert cells into chemical factories. Recent advances in gene editing enable a shift from reading to writing and editing genomes, and reprogramming cells. The technology is costly and faces many challenges for industrial applications including a time to market of about a decade. Nevertheless, advances in gene editing are revolutionizing the field at laboratory scale. A technology on the horizon is the development of a new genetic code using non-standard amino acids, which will expand the chemicals available for protein synthesis. This technology could lead to novel compounds with profound differences in their characteristics compared to the proteins we know from nature.

Additive manufacturing or 3D printing of specialized equipment has in recent years and during Spiez CONVERGENCE 2014 been discussed as a development with potential security risks. The technology is maturing and using powder bed (metal, metal alloys, ceramics) melted with electron or laser beam appear to be the most promising approaches for future applications. With the technology maturing, its limitations also become more apparent. As the powder bed process employs continuous welding, it carries an inherent risk of welding defects that could cause material fatigue. Such defects are difficult to detect and cannot be predicted. The melting speed for the layers of powder bed furthermore sets a limit to productivity. This process is an excellent tool for fast prototyping and producing repairs but less suitable for large scale industrial manufacturing of critical high performance pieces. Promising developments were discussed with regard to 3D printing with biological materials. The technology is based on a layer by layer printing of a bio-ink in a sterile environment using laser printing or inkjet. Future applications include the fabrication of living tissue or the development of tissue models. The goal of reproducing biological function in the concept of 'organ printing' remains a big challenge. 3D printing of biological materials is today a research tool utilized to model tissue functions. It is currently a single use process and reproducibility is a weakness. A key development for future success is that standardized bio-inks of good quality are becoming commercially available.

Genome editing technologies have been included on a US threat list this year and therefore received much attention from an arms control perspective. Site-directed genome engineering aims at specific modifications of cellular properties – a further step in technological advancement. This became possible after the lowering of cost in sequencing technology has led to large databases and a better understanding of biological systems. CRISPR/Cas9 is a new gene editing technology that is derived from a bacterial defense mechanism against viruses. It permits introducing changes to DNA within cells and it provides the ability to edit genetic code accurately and precisely. Gene editing has been possible for many years using other techniques (i.e. Zinc finger proteins, TALE nucleases) but CRISPR/Cas9 is simpler and more easily accessible. There

are two different types of known CRISPR systems and research is shifting today from understanding this technology to applications. The technology is utilized for the development of a broad range of new bio-based products, including therapeutics, antimicrobials, animal health products and crop genetics. Recent developments include in-vivo and ex-vivo drug treatments, but there remain significant challenges in this area. For the delivery of drugs to patients, lipid nanoparticles are promising tools. One particular application of CRISPR/Cas9 based genome editing are *gene drives*. Gene drives create an inheritance bias for themselves and can force a genetic modification through an entire population, provided, the species has a short generation time and a high population turnover. The technology is expected to work well with insects and is discussed as a form of vector eradication to fight malaria. Current research focuses on spreading genetic sterility. Another approach could be interfering with parasite development in the mosquito. The approach to eradicate an entire population however, poses a whole range of questions in relation to safety and security as well as ethics. With regards to weapons relevance, the implications of gene editing technologies are probably modest. But should a biological weapons program be started today, these technologies would likely become part of it. CRISPR represents a transformational technology that will yield many beneficial applications. And, it demonstrates that risk assessment must not only be based on scientific potential – contextual analysis and effective communication are required to avoid disproportionate reactions.

Omics technologies have moved from genomics to transcriptomics to proteomics and metabolomics. Biologists' focus turned from reading the program of biological systems (DNA) to understanding the systems' functioning. Large sequence databases were accumulated but efforts to better understand biological systems meet unanticipated complexity. Research shows that the proteome organization is not explainable by the genome organization alone and that there are further regulatory processes. Another problem is data reliability. For the development of practical applications, industry depends on reliable databases and a significant portion of academic data is not reproducible. It has therefore been suggested that more effort be put into curating databases instead of engaging in further sequencing.

The memory and programming capability of biological systems is researched to look for alternative solutions because of shortcomings in existing technology. For example, conventional data storage media are limited in capacity and deteriorate over a relatively short timeframe. Because of the drop in cost in DNA sequencing and synthesis technology, storing data in DNA could be an interesting option. Long term storage of large amounts of data in DNA encapsulated in glass is today technically feasible, but due to the cost of about USD 1,000 per 1 MB still too expensive. Another interesting application of this technology is to use it for 'barcoding' of materials such as chemicals, intermediates or food products. While recording in living cells is not suitable for long term storage, genomically encoded cells could be used for computing functions. In a layered approach, DNA codes can be programmed and with programmed DNA, devices can be built. Circuits are created from such devices followed by modules based on the circuits etc. This research is increasingly linked to synthetic biology and can be combined with gene editing tools such as CRISPR.

DNA origami is today a technology at the stage of fundamental research with little practical application so far. But nevertheless it offers interesting concepts. DNA origami uses DNA for building nanostructures. Single, double or bundled DNA strands are used to modulate mechanical stiffness of a structure. By combining such structures with nanoparticles (e.g. gold), researchers succeeded in creating three dimensional behavior of nanoparticles imbedded in the structure, which could be triggered by an external factor (e.g. light). Such dynamic systems could be developed into sensors. The goal of this research is to create molecular structures which interact in a type of 'nanofactory' or to develop molecular robotics.

Tacit knowledge is essential to make something that is possible into something that actually works. Therefore, tacit knowledge is indispensable for successfully integrating new scientific discoveries into the design of new products and also new weapons. This remains true despite the trend of 'deskilling' in the life sciences due to new technologies such as CRISPR. Tacit knowledge is a hindrance for non-state actors but experience from the past shows, it can also be a critical success factor in weapons development programs. However, the lack of tacit knowledge does not prevent the use of improvised or crude weapons systems like chlorine barrel bombs. Such examples show that context is important and that scenario and context must be part of a risk assessment.

This report ends with a summary of the *policy discussion* on the last day of the workshop. Convergence may affect the CWC and the BWC at the level of scope as well as with regard to implementation. In terms of scope, assessing how developments like nanomachinery fall under the General Purpose Criterion of the treaties requires a good understanding of the new technology and a continuous review of future developments. When assessing implications of advances in S+T, a general approach is more important than singling out an individual development; doing so carries the risk of missing others. Many of the science trends reviewed under the concept of convergence affect mainly treaty implementation and not treaty scope. A good example are how changes in production technologies affect CWC declarations. Biologically mediated processes have already been addressed by the OPCW SAB, and they affect declaration, as well as implementation – material balance control for verification purposes is not likely to be possible in biotechnology facilities. Another challenge are chemical production plants that are comparable in relevance to Schedule 1 facilities. The problem is not new, but in the past was of little relevance because only few such industry facilities existed. The CWC contains provisions to address this issue but this requires political will. There are several examples of overstating the impact of new scientific discoveries – sometimes by the scientist themselves. Policy responses must be proportional and must consider what is likely to result in applications. 3D printing of process equipment is a good example. The risk of this technology was overestimated in the past. The same may apply to gene editing. New gene editing technologies have added functionality but their limitations as well as practical implications are not yet known. It will be important to follow the development of gene drives. The concept for it was in place but convenient gene editing tools were missing. CRISPR opened the door here for large progress.

The S+T developments described in this report offer promising benefits for society, but their exploitation for harmful purposes by various actors in the context of their dual use potential cannot be disregarded. A point mentioned many times during Spiez CONVERGENCE 2016 was the importance of effective communication – scientists and their work are part of the solution and not part of the problem. A conversation about security threats should be multi-disciplinary, while at the same time recognizing that the same thing may be understood differently depending on the community or the context. We hope, that this report will serve as valuable contribution for developing common understandings in policy discussions in various fora.

Introduction

Scientific and technological advances emerging from convergence of biology and chemistry bring enormous benefits but also unknown safety and security threats for society. Potential implications for existing arms control regimes need to be discussed between all involved communities. The second edition of Spiez CONVERGENCE provided a unique platform for effective communication between experts from academia, industry and policy making as well as experts involved with the implementation of related arms control requirements.

This report summarises the discussions held at the second Spiez CONVERGENCE Workshop organized by Spiez Laboratory that took place from 5–8 September 2016. The workshop series is part of a broader effort by Switzerland aimed at strengthening the Biological Weapons Convention (BWC) and the Chemical Weapons Convention (CWC) by discussing significant developments in science and technology and their potential bearing on both Conventions. Spiez CONVERGENCE is a Swiss contribution to the preparations of the Eighth Review Conference of the BWC in 2016 as well as the upcoming Fourth Review Conference of the CWC in 2018.

Both Conventions comprehensively prohibit an entire category of weapons of mass destruction, and they overlap in the area of toxins and bioregulators. Their implementation systems, however, differ profoundly with regard to international verification, institutionalisation and methodologies used to review and assess the impact of advances in science and technology. On the other hand, as a result of convergence in the life sciences, they share a growing part of the world of science, technology and industry. Hence, there is good reason for also sharing information and assessments between the two treaty communities – how are changes in research and development affecting industrial-scale applications? And what do these changes mean for preventing the re-emergence of chemical and biological weapons, for protection against such weapons, for national implementation as well as for the fostering of international cooperation and assistance, and, in the case of the CWC, for verification?

The Conventions are snapshots of the world of science and technology at the time when they were first negotiated. This world of science and technology is dynamic and fast changing.

Both Conventions are science-based and work on the principle that science and technology should enhance human life, but science and technology also have misuse potential. The Conventions are snapshots of the world of science and technology at the time when they were first negotiated. This world of science and technology is dynamic and fast changing. Our knowledge base is constantly expanding, not merely with regard to chemistry and biology but also with regard to enabling technologies. Information technology, imaging software, simulation and modelling, the use of mobile devices, new methods of drug delivery, the use of drones for agent delivery and surveillance are examples of new applications and capabilities that are all relevant to CBW arms control. Some of these developments expand existing knowledge and capabilities, others may create hitherto unknown opportunities. The treaty communities have discussed many of the issues for years – for example the biologically mediated production of chemicals – but changes in the industrial environment may now call for practical steps. Other developments might create new capabilities, and it will be important to make accurate assessments of their impact. The arms control community needs to stay informed about progress in science, technology and industrial application, screen the horizon for what is happening, and try to understand how these developments may change the implementation environment of the two Conventions. In doing so, it will be essential to distinguish between what is possible in the world of research (where people are exploring new ideas with a high degree of flexibility), and what will actually manifest itself in practice (where industry is looking for reliability and profitability). Technological progress does not mutate automatically into new weapons, and the evaluation of advances in science and technology needs to be informed by the emerging potential in science and technology but also the context within which these new discoveries will be used.

IT WILL BE ESSENTIAL TO DISTINGUISH BETWEEN WHAT IS POSSIBLE IN THE WORLD OF RESEARCH (WHERE PEOPLE ARE LOOKING FOR NEW IDEAS AND FLEXIBILITY), AND WHAT WILL ACTUALLY MANIFEST ITSELF IN PRACTICE (WHERE INDUSTRY IS LOOKING FOR RELIABILITY AND PROFITABILITY).

Such types of assessment require effective communication between the communities of science, industry, diplomacy and security. This workshop intended to provide a platform for such conversation.

Chemical synthesis, chemical modification and large molecules

Biologically mediated manufacturing of chemicals is of growing importance in research and industry. This is a rapidly expanding area with diverse applications as well as a prominent example for the increasing impact of convergence in biology and chemistry.

The first Spiez CONVERGENCE Workshop in 2014 as well as the OPCW SAB in its recent report on convergence pointed to the growing importance of biologically mediated manufacturing of chemicals. Advances in this direction continue even though earlier prognoses about the expected market share of biologically mediated chemicals manufacturing were dampened somewhat given the drop in crude oil prices. More generally speaking, science, technology and industry are moving from the era of electronics and information into the age of biotechnology. The workshop looked at four distinct subjects under this topic: converting biomass into platform chemicals, synthesizing complex biomolecules (polysaccharides), the manufacturing of highly active pharmaceutical ingredients, and editing genomes to re-programme cells.

Biomass is the only renewable carbon source available other than carbon dioxide, and therefore remains an attractive source for the manufacturing of chemicals.

Biomass is the only renewable carbon source available other than carbon dioxide, and therefore remains an attractive source for the manufacturing of chemicals. It can be used for making a variety of interesting materials ranging from biofuels to vaccines and diagnostics.

There are a number of routes for converting biomass into chemical products, starting from simple sugars to starchy polymeric biomass and lignocelluloses. The biochemical conversion of sugars is a well-established industrial process involving sugar, water and microorganisms. Bioconversion of starchy biomass and celluloses is more challenging. Starches first need to be converted into sugars by depolymerisation through enzymatic hydrolysis. Bioconversion of celluloses requires pre-treatment followed by fermentation.

The bioconversion of sugars yields a variety of platform chemicals, including ethanol (which can be further converted to ethylene, ethyl esters, butadiene), succinic acid (which can be used to manufacture 1,4-butanediol, gamma butyrolactone, tetrahydrofuran), 3-hydroxy propionic acid (a starting point for

1,3-propandiol and acrylic acid), itaconic acid (leading to polymers and methyl methacrylate), lactic acid (a starting point for polymers and acrylic acid), and hydrocarbons used as jet and diesel fuels or as synthetic rubber.

However, both sugars and starch are food sources and hence not desirable as starting points for the manufacturing of chemical products. Lignocellulose, on the other hand, is a most abundant raw material. But it is not easy to convert, and therefore requires pre-treatment to open up the cell structure and allow access to the polysaccharides. Such pre-treatment may involve steam explosion, sulphuric acid treatment, or pre-treatment using ammonia or lime. Lignocellulose, furthermore, is a dirty biomass that contains sand and soil, which can lead to abrasions of process equipment. Its bioconversion results in a variety of sugars at low concentrations still carrying significant lignin ballast. The enzymatic hydrolysis process is difficult to scale up and economically not attractive given the costs of feedstock, enzymes and equipment.

A way of overcoming the difficulties of conversion is consolidated bioprocessing, i.e. integrating cellulose production, hydrolysis and fermentation. It leads to lower costs and a much more simplified process flow that avoids solid-liquid separation. Several strategies exist for such an integrated single-step conversion: a 'native' strategy using a strain that can produce its own enzyme and is modified to create the target product, a recombinant strategy using a strain that is designed to produce the target product but not its own enzymes, co-cultures of the two, a genetically modified 'superbug' with high enzyme efficiency and that is adapted to the target chemical, and a microbial consortium of aerobic and anaerobic strains.

A WAY OF OVERCOMING THE DIFFICULTIES OF CONVERSION IS CONSOLIDATED BIOPROCESSING, I.E. INTEGRATING CELLULOSE PRODUCTION, HYDROLYSIS AND FERMENTATION.

But whilst some progress has been made towards an industrial application of these processes, sugar and starch still remain the dominant feedstock for industrial production of chemical products from biomass. Bioconversion of lignocelluloses into ethanol, on the other hand, is close to commercial production.

The antipode to breaking down natural biological materials to manufacture platform chemicals is the synthesis of complex biomolecules from small building blocks. Past studies have looked at the synthesis of DNA and peptides. This edition of Spiez CONVERGENCE took a closer look at the automated synthesis of carbohydrates.

Carbohydrates are important biomolecules that have structural functions, are used in signalling and have many other interesting biological properties. But they are not easy to synthesise. The automated synthesis of proteins (1984) and nucleic acids (1980) has been used for some time; automated carbohydrate synthesis is only now becoming reality. The main reason is that carbohydrate synthesis is more complex, involving some 50 different monomers. Researchers have now created automated synthesis platforms and are using tools to better understand biological processes and structures

BIOCONVERSION OF LIGNOCELLULOSES INTO ETHANOL, ON THE OTHER HAND, IS CLOSE TO COMMERCIAL PRODUCTION.

of glycans. This is expected to lead into solutions for unmet medical needs and it may help create novel materials. Commercial synthesizers and monomers for automated carbohydrate synthesis are becoming available leading to the synthesis of many more glycans with ever-greater complexity.

Automation has led to significant time compression. When in previous years, the synthesis of a glycan antigen took 12–18 months, the same can be achieved today in 19 hours. Also, the technology is beginning to spread globally.

The global biomass production today measures 105 billion tonnes, 80% of which are carbohydrates. They mostly play structural roles (chitin, cellulose) but automated synthesis is expected to make new biomaterials available. Some fundamental questions still need to be addressed: what biomaterials would result from combining chitin and celluloses and what would their properties be; what is the exact relationship between structure and folding and which forces are involved; how do the enzymes actually work; is there a way of creating origami with carbohydrates similar to DNA?

When in previous years, the synthesis of a glycan antigen took 12–18 months, the same can be achieved today in 19 hours.

Carbohydrate synthesis clearly has potential, including nanotechnology using polysaccharides as building blocks. There also are possibilities for making new vaccines based on carbohydrate conjugates, which are expected to be cost effective and of therapeutic interest for treating hospital-acquired resistant infections. Clinical trials for such new vaccines are expected to start soon. Other applications include new materials, microarrays for diagnostics tests (e.g., for toxoplasmosis), and monoclonal antibodies as diagnostics and treatments.

Carbohydrates are important biomolecules that have structural functions, are used in signalling and have many other interesting biological properties. But they are not easy to synthesise.

One of the drivers is market pressure. In the past, the industry target for moving a new product from approval to market was between 4 and 6 years; today this time is in many cases shorter. In the pharmaceutical industry, fast track designation, breakthrough therapy designation, accelerated drug approval and priority reviews are increasingly applied to shorten time to market. In 2014, 2 of 3 new drugs were designated in one of these fast track categories.

Another trend in the pharmaceutical industry is the move towards using highly active pharmaceutical ingredients (HAPI). More than a third of all novel drugs fall into this category. This has led to a significant change in the footprint of the production plants that develop and manufacture active ingredients. Such plants are technologically highly complex, operate against short timelines, and require strong managerial, technical, safety and environmental standards. Effective management systems need to ensure flexibility and effective risk management. Each step in the technological scheme is reviewed, and risk management strategies are applied such as primary and secondary

containments, decontamination, regular leakage checks, extensive cleaning procedures, monitoring systems concerning health, safety and the environment, air monitoring, and waste management. Workforce performance and a strong safety culture, compliance with regulatory and company-internal requirements and commitment to safety are critical. As a consequence, so is regular training and re-training – initial training measures can take up to 6 months. Companies use strategies to ensure continuous improvement and involvement of the entire workforce.

Another trend in the pharmaceutical industry is the move towards using highly active pharmaceutical ingredients (HAPI). More than a third of all novel drugs fall into this category.

For safety and security reasons, companies control access to critical areas at the manufacturing site, organise processes in ways that minimise transports on site, and apply devices for electronic tracking of people and key materials as well as for batch controls. A broad assets base enables them to swiftly scale up from development to production. Containment standards are similar to what is traditionally used in high-safety biology facilities. These facilities in many respects resemble a larger-scale Schedule 1 facility. But given their production profile they tend to remain below the declaration threshold for discrete organic chemicals (DOC) facilities.

A final synthetic approach that was discussed aims at expanding nature's repertoire by engineering (re-programming) genomes. Recent advances in gene editing have enabled the shift from reading to writing and editing genomes and reprogramming cells for useful purposes. Novel gene editing tools increase in throughput and a sharp drop in cost have led to a renaissance in genomics with genome wide assessments and unprecedented access to structure at the level of individuals (personalized genome sequencing). Combined with experience from engineering metabolic pathways this now allows for converting cells into a chemical factory.

Recent advances in gene editing have enabled the shift from reading to writing and editing genomes and reprogramming cells for useful purposes.

With regard to industry applications, it was noted that time to market remains at around a decade, and the technology is costly and faces many challenges. Biology is (very) complex: cells have a large number of regulatory mechanisms many of which we have yet to understand. Engineering new biological functions into cells means bearing in mind effects of evolution. Then there are the challenges of transferring such engineered systems into an industrial production environment – the technology still remains inefficient, laborious and expensive, and there are technical limitations to engineer organisms. A key challenge is contamination (of the product as well as the process) which can lead to unsafe products or instabilities in the manufacturing process.

Nevertheless, advances in gene editing are revolutionising the field, at least at the laboratory bench. An alternative approach to CRISPR is Multiplex Automated Genome Engineering (MAGE) which introduces a library of synthetic

DNA into a large cell pool, resulting in combinatorial diversity across the entire genome and different pathways. The chromosomes of living cells are used as templates for rapid genome editing and evolution – this approach cuts short development time – it allows optimisation of strains and pathways within days and can easily be applied to the production of a wide range of target compounds. An example is the use of *E. coli* for the production of the antioxidant lycopene, which is currently being commercialised.

On the horizon is the development of a new genetic code using non-standard amino acids, thereby expanding the chemical repertoire available for protein synthesis.

On the horizon is the development of a new genetic code using non-standard amino acids, thereby expanding the chemical repertoire available for protein synthesis. This could result in novel compounds with profound differences in structure, stability, activity, and binding as compared to the proteins we know from nature. In the laboratory, the recoding of genomes by completely replacing a natural by an artificial codon has already been accomplished. One experiment used *E. coli* to completely recode all 321 UAG Stop-codons to UAA. Such experiments are undertaken to test the malleability of the genetic code, to expand the chemistry and functionality of proteins and could lead to new materials with properties different from the accessible 20 amino acids known from nature. The hope is to fine-tune the properties of these novel materials for new applications as nanostructures, therapeutics, industrial enzymes, sensors, in drug delivery, or to engineer genetic isolation and virus resistance. This could allow more stable bio-manufacturing, or the development of intrinsic biosafety containment allowing the safe use of GMOs in open systems (for example as medicine or in agriculture). Such engineered organisms could be recoded so they cannot grow outside the laboratory, as they depend on synthetic amino acids that are not available in nature.

Take-home points

- Biologically mediated manufacturing of chemicals gains in importance
- Biomass can be used for generating a variety of materials like biofuels, vaccines and diagnostics
- Lignocelluloses are attractive as a starting material because contrary to sugars and starch it is not a food source
- Automated synthesis of complex biomolecules (nucleic acids, proteins, carbohydrates) is commercially applied, e.g. to generate novel pharmaceuticals and biomaterials
- Advances in gene editing enabled the shift from reading to writing and editing genomes to reprogramming and converting cells for commercial purposes
- Development of a new genetic code using non-standard amino acids is promising to expand the chemical repertoire available for protein synthesis
- Technical problems and also societal acceptance – especially GMOs – are obstacles to overcome

This may indicate a move from new research tools (MAGE, CRISPR) to new applications (genome recoding), resulting in a new orthogonal biology and from there may emerge new solutions that would help to address such issues as virus resistance or biological containment. But in addition to the technical hurdles that this technology needs to overcome, there also is the issue of public perceptions. Experiences with GMOs have clearly shown that societal acceptance requires early engagement with the public and a participatory communications strategy.

Additive manufacturing

Additive manufacturing or '3D printing' was discussed because of its potential impact on design and methods of production for a wide variety of different products offering new capabilities.

Additive manufacturing has been discussed in recent years as a new technology that could pose security risks with regard to the manufacturing of specialized equipment. It therefore appeared to have relevance for export controls of specialized equipment suitable for CBW manufacturing and use.

The technology has further matured with regard to the energy beams used (photon and electron beams), the materials available (metals, metal alloys, ceramics) and the manner in which the material is supplied (vapour, powder, powder bed, wire). Powder bed and laser or electron beam appear to be the most promising approaches for future applications, but as the technology matures, its limitations are becoming clear. 3D printing with powder bed is a continuous welding process – the speed of melting therefore sets a limit to productivity. Previous predictions that 'this is the method of the future, only 10 times faster and 10 times more precise' are technically no longer supportable. There also is a speed-quality relationship related to the properties of the powder used, which limits the ability to increase productivity. It has now become clear that additive manufacturing is an excellent tool for fast prototyping and repair, but not necessarily for large-scale industrial manufacturing of critical pieces where high performance standards are essential.

It has now become clear that additive manufacturing is an excellent tool for fast prototyping and repair, but not for large-scale industrial manufacturing of critical pieces where high performance standards are essential.

Because the process is a continuous welding, there will always be defects in the printed piece, and they are not easy to predict or detect. The result is that there is an unknown potential for material fatigue. Each 3D-printed piece is unique so quality assurance cannot rely on testing a certain number of manufactured pieces from a particular batch – each piece would have to be assessed (and even this would not guarantee that a given piece was free from error). Finally, there is the issue of manufacturing safety: there is explosion hazard associated with the handling of large amounts of fine powder, which calls for sophisticated filtration systems, affecting the costs of industrial scale manu-

facturing. In short, it appears that the technology, whilst retaining its niche utility for fast prototyping and repair, is unlikely to ever be used for industrial-scale manufacturing.

More promising developments do relate or are related to 3D printing with biological materials. Bio-inks can be printed onto a structure or as a scaffold, which has led to (albeit overstated) concepts of 'organ printing'. Future applications could include synthetic organ transplants, biofabrication of living tissue, or the development of tissue models for biological research or pharmacological testing. The technology involves layer-by-layer printing with bio-inks (biological polymerised material, bio-gels) followed by incubation and maturation. Different printing techniques include laser printing and inkjet printing encased in sterile containment. Diverse layers of biological materials can be combined to add complexity.

Take-home points

- Additive manufacturing is a suitable tool for fast prototyping and repair
- At present it is not suitable for large-scale industrial manufacturing of critical pieces
- 3D printing of biological materials is still a research tool, used to model tissue functions
- Standardized and commercially available bio-inks are drivers for future success in this research area

The goal of reproducing biological function remains a big challenge. Biological systems are far more complex than what can be 3D-printed today. There has been work on attempting to print muscle-like materials, or tubular structures to mimic vascular structures such as blood vessels. But it remains difficult to 'focus' bio-ink printing to achieve higher resolution, and it is not clear what resolution is needed for a viable product. There are also challenges with regard to printing implants; these need to remain in the position where they were implanted, remain viable and integrate within the body. Nevertheless 3D printing has certain advantages over amyloid cultures: the latter are difficult to control with respect to their size and also their properties may not be as clear because they are derived from cancer cells.

Finally, reproducibility remains an issue. 3D printing of biological materials at this stage is a research tool used to model tissue functions. It is a single-use process followed by extensive cleaning and sterilisation, and its reproducibility is low. Its most likely application will be in the field of drug development and toxicity testing – for example as liver model using human cells to get native functionality; skin models for testing cosmetics or skin toxicity; or lung models. The bio-inks are key for future success, and they are now commercially available and standardized at constant quality, so research is no longer dependent on in-house manufacturing.

3D printing of biological materials at this stage is a research tool used to model tissue functions. Its most likely application will be in the field of drug development and toxicity testing.

Genome editing – CRISPR technology

CRISPR is an affordable and easy to use genome editing technique that since its discovery has rapidly found applications in research as well as industry. With 'gene drives' genome editing is reaching a new level.

Over the last 20 years, significant improvements have been made in sequencing technology, resulting in a far better understanding of the genomic world. Moreover the cost of genome sequencing has dramatically decreased, leading to vast amounts of data that could be used to gain understanding of biological systems.

The next step in this process has been gene editing. Site-directed genome engineering enables specific modification of cellular properties and drives a series of important functions that include knockout, replacement, activation, modulation, repression and deletion of cellular properties. CRISPR/Cas9 has received much attention, but genome editing already started before this technology emerged. There has been an explosion of scientific activity since the 1990s when it was discovered how to engineer naturally occurring nuclease that would target specific sequences. Engineered meganucleases, and subsequently specific binding proteins (Zinc Finger Proteins) were developed. In early 2010 a somewhat analogous family of TALE Nucleases were generated. These techniques are all still available, but CRISPR/Cas9 is conceptually much simpler and hence more easily accessible. And the field is not static so new advances are expected.

CRISPR is one of many bacterial defence mechanisms against invaders (viruses, bacteriophages). Others include uptake prevention, abortive infection by cell suicide, or restriction modification cleaving incoming DNA. CRISPR is a form of acquired immunity where the bacterium cell incorporates invader DNA sequences into its chromosome; it then uses single guide RNA to screen for new invasions and a specific protein to induce cleavage of that invader DNA. This is a flexible, simple, effective tool to enable the introduction of changes to DNA within cells from virtually every organism it has been tested in. It provides the ability to edit the genetic code accurately and precisely.

This is a flexible, simple, effective tool to enable the introduction of changes to DNA within cells from virtually every organism it has been tested in. It provides the ability to edit the genetic code accurately and precisely.

There are two types of CRISPR systems. Type I CRISPR encodes multiple Cas genes; it works through adaptation, expression and interference. In adaptation a new spacer is acquired and integrated the CRISPR array; in the expression /

RNA maturation phase, a cascade protein complex takes RNA made from the CRISPR array and cleaves it into individual units that remain bound to the complex and guide the complex to the target viral DNA; during interference, the protein complex binds to the DNA and recruits other factors to cleave the viral DNA leading to its inactivation.

There are known off-target effects in nature, which respond to virus mutations and provide a degree of flexibility in the defence. Also, the degradation of the viral DNA leads to fragments that can be incorporated into the memory for future attack responses.

Type II CRISPR systems encode a limited number of genes: key example of Type II today is Cas9. Enzymes likely to gain attention in the future include Cpf1 and C2c2.

- Cpf1 leaves no blunt ends and provides a staggered cut, which may lead to interesting applications; moreover Cpf1 has the advantage of using shorter oligos, which can be made synthetically.
- C2c2 is a protein with 1400 amino acids that is able to cleave complementary RNA, and while cleaving target RNA it becomes highly active and destroys all RNA around. There could be some uses for this particular function, for example as a mechanism for aborting an infection.

Research is now moving from understanding CRISPR to its application. The technology is being deployed for the development of a broad range of new bio-based products including therapeutics, antimicrobials, animal health products and in crop genetics, as well as for research in areas such as cell engineering, phenotypic screening and genomics, and industrial biotechnology (microbial fermentation, therapeutics). With 5,000 Mendelian disorders understood at the genetic level, there is now the potential to correct the genetic mutation in somatic cells with gene therapy; with 40 crop species with reference genome sequences, precision breeding can be utilized to develop crops with improved performance; and with 3,400 complete microbial genome sequences, harmful microbes can be selectively killed whilst 'friendly' microbes are preserved.

Research is now moving from understanding CRISPR to its application.

In a more recent development, both *in vivo* and *ex vivo* drug treatments are being pursued. There remain serious challenges and risk mitigation approaches are being used. Progress is piggybacking on other fields, in particular with regard to drug delivery into the body. A second challenge is the gene editing itself. What really matters for the therapeutic effect is what happens after the cleavage, when the cells take over and changes are fundamentally driven by DNA repair and the distribution of DNA repair outcomes. Specificity, too, is an issue – genome wide screens have a high tolerance for off-targeting while human therapeutics for non-terminal diseases have a very low tolerance.

In a more recent development, both *in vivo* and *ex vivo* drug treatments are being pursued.

The selection of treatable diseases is driven by an understanding of how genome editing works and what gene edits lead to. The list of potentially treatable diseases is long, but the disease must be reversible (i.e. it cannot be used for defects that affect neurons). In practice, RNA guides needed to be ranked in order of what works best (high on-target, low off-target); sites are identified using bioinformatics tools and next generation sequencing, and then evaluated in a tedious process.

The list of potentially treatable diseases is long, but the disease must be reversible.

For the delivery of these treatments to patients, animal experiments have shown that lipid nanoparticles (LNP) have several advantages: they are biodegradable, show low immunogenicity, can carry a larger load than viruses, and are stable in circulation. LNPs were distributed systemically and gene edits remained permanent. *Ex vivo* electroporation also has been tested and is now undergoing clinical trials. Nevertheless, the repair after cleavage needs to be better understood for scaling the technology to use in humans.

Other interesting developments relate to increasing the yield and reducing cost of vaccine production, e.g. the polio vaccine. Yet another upcoming field for the application of CRISPR technology is agriculture. Gene editing offers potential for increasing protein production, for example by breeding pigs with a gene knockout that removes the receptor for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) – a method currently at the proof of concept stage.

In sum, gene editing technology is getting simpler, and its wider application may have a catalytic effect that enables progress in other fields. Many gaps, nevertheless, remain in understanding CRISPR and its effects.

One particular application that genome editing has opened up are gene drives. In 'normal' gene modifications, a genetic modification remains at a fairly low frequency in the affected population, and, if it imposes a loss of fitness, will be eliminated over several generations. A gene drive forces a genetic modification through the population by creating a bias in inheritance for itself. The gene to be edited must be present in the germ line. If it affects fertility, it also should be a recessive gene so the female needs both copies of the disrupted gene to become infertile. In this way the disrupted gene will spread through the population. Short generation time and high population turnover are needed for a gene drive to be efficient, which is why it is expected to perform well in insects.

A gene drive forces a genetic modification through the population by creating a bias in inheritance for itself.

Gene drives are currently discussed as a form of vector eradication to fight malaria. Anopheles is the only vector for the transmission of the disease to humans, and efforts have been made over decades to control this mosquito. But tradi-

tional methods have serious drawbacks (resistance to insecticides, difficulty of access to certain habitats, toxicity, lack of specificity, or only being effective under certain circumstances).

There are, in general, two approaches to using a gene drive to fight malaria: either spreading genetic sterility in the mosquito population or interrupting the parasite development in the mosquito. So far, the discussion has focused on the first approach, using either suppression (disrupting an essential mosquito gene) or replacement (using a cargo that imparts a phenotype). Such a gene drive requires only a small number of engineered insects to be released; the engineered insects themselves do the hard job of spreading the faulty gene. The approach is relatively self-sustained and species-specific.

There are, in general, two approaches to using a gene drive to fight malaria: either spreading genetic sterility in the mosquito population or interrupting the parasite development in the mosquito.

A possible approach is the use of site-specific Homing Endonuclease Genes (HEGs) as a template. There are several options available to reengineer site-specific endonucleases, CRISPR/Cas9 embryo injection was used in one series of experiments as gene knockout, creating a recessive female fertility phenotype. Modelling and cage experiments have confirmed that the gene drive spreads despite causing negative fitness. Of course nature will counter-steer and resistance is likely to develop, but multiplexing to target multiple sites can reduce this resistance potential, also widespread sampling can be used to find the least genetic diversity.

The potential that gene drives could have for species eradication is huge. So a first question was – is this easy to do? The answer is 'no'. It requires more than understanding and using CRISPR, and depends on a range of further knowledge, skills and capacities relating to the host and its biology. Also, one must get the genetics right. From an arms control perspective, the implications are probably modest. Nevertheless, the concept of gene drives and CRISPR technology has received much attention recently, and gene editing has been classified in 2016 by the US as a global threat. However, with regard to weapons relevance, the impact of gene editing remains limited. Gene editing likely would be part of any current CBW program at the research and development stage. But there are many steps that have to be taken from proof of concept to a useful weapon, delivery being just one example. Easier ways of biological warfare already exist, and so do options for protection. Furthermore, knowledge of anti-CRISPR proteins could be used to neutralise Cas9-based weapons systems. The risks associated with gene editing need to be carefully analysed and understood within the context in which they have emerged.

Also, with regard to gene drives, once released into nature they cannot be called back. The results may not always be what research expected or predicted, but they can affect very large numbers of organisms.

But there are other risks associated with the potential to wipe out an entire insect population – the consequences of creating a niche and thus opportunity for other species in an ecosystem are not well understood. So even in the case of fighting malaria, one should ask whether it would not be safer to focus on knocking out the host's receptor that associates with the parasite. This receptor, unfortunately, is not known at this stage.

In addition to safety and security concerns, ethical issues are also likely to emerge. This will in part depend on which species are being used (insect versus human cell lines, for example). Also, with regard to gene drives, once released into nature they cannot be called back. The results may not always be what research expected or predicted, but they can affect very large numbers of organisms. Careful risk assessment and a step-by-step approach are therefore essential. The research community is aware of the inherent risks and is advancing cautiously.

Take-home points

- Gene editing technologies become simpler and cheaper
- CRISPR has a wide set of applications that may have a catalytic effect on the progress in other research fields
- It is used for the development of new bio-based products and industrial biotechnology
- Many gaps remain in understanding CRISPR and its potential future impact, e.g. how to modulate cellular repair mechanism after DNA cleavage for improved therapeutic outcome, or CRISPR application for generating gene drives
- The risks associated with gene editing need to be carefully analyzed and understood within the context in which they have emerged

Gene editing and gene drives can be expected to yield many beneficial applications, and CRISPR is an example for a transformational technology that effective horizon screening should identify, but it also illustrates that risk assessments must not be based solely on scientific potential, but require a contextual analysis to avoid disproportionate reactions.

Effective, participatory and timely communication, again, will be critical and public perception will ultimately determine societal acceptance. Language is important: if not used properly, it can create anxiety and misconceptions or even misinformation. Biology is inherently complex and the dialogue between scientists and society is not always easy, nor is it something the scientific world has addressed sufficiently.

Big Data / Omics

Omics technologies are of increasing importance for providing and analysing biological data, especially for extensive screenings. The aim of Omics is a holistic description of an organism at a particular point in time under defined conditions. The technologies render information about structure, function as well as dynamic behaviour.

The impact of advances in the 'Omics' on the chemical and biological arms control regime has been discussed for several years now. Further progress has been made in systems biology and bioinformatics with regard to extracting from and understanding large sets of biological data. The Omics have moved on from genomics to transcriptomics and more recently proteomics and metabolomics, and vast numbers of data have been accumulated. Biologists are moving from being able to read the program (DNA) to understanding the functioning of the machinery that decodes DNA, and the complexities of how this information is used for the production of biomolecules and for metabolism.

Nevertheless, biology remains messy and working from first principles remains for the time being out of reach. There have been huge efforts towards a more holistic approach and large datasets have been accumulated but these efforts are met by unanticipated complexity and errors or inconsistencies in the data generated by different experimental platforms. Researchers spend a lot of time validating and integrating existing datasets. Also, there remain gaps in the understanding of transcription (certain effects cannot be explained by the predicted eight different structural groups of transcription factors), and research has shown that the proteome organization is not explainable by the genome organization, indicating the existence of other regulatory processes. There are also regulatory processes without an actual regulator, where the structure of the promoter appears to act as regulator or where regulation information is contained in the genome rather than expressed through the transcriptome.

Biologists are moving from being able to read the program (DNA) to understanding the functioning of the machinery that decodes DNA, and the complexities of how this information is used for the production of biomolecules and for metabolism.

Another area of work that can bring new insights relates to small Open Reading Frames (sORFs) encoded proteins and their functions; this involves searching for encoded peptides to define their possible functions as bioregulators. Experimental methods and bioinformatics approaches in the search for biologically active sORF-encoded peptides in *Physcomitrella* patens moss have resulted in a three-step process of prediction, analysis and estimating biological functions. The hope is that this knowledge could eventually be applied

to other plants, animals and humans. This is of particular interest as sORFs have been found in all genomes and there are only a few known examples of sORF-encoded bioactive peptides.

But there is a practical aspect to this theoretical work: attempts have been made to estimate the structure in the binding process with a view to designing an antibody-based nerve agent scavenger. In that research, phage-display libraries (immunization and screening *in vitro*) and autoimmune repertoires *(in vivo)* were combined, leading to a process of reactive immunization and kinetic selection. This is a combinatorial approach to create a novel artificial scavenger / biocatalyst – essentially a 'reactibody' that combines screening for bindings with the conversion of single chain variable fragment (scFV) into whole size human antibody.

Industry (which depends on reliable data) has realized that many academic data do not replicate and consequently has taken to re-measuring essential data even if published data exists.

What has become clear is that the integration of data and data reliability remain problematic. A lot of time is spent developing and using sophisticated models but without the necessary data storage and quality controls in place. Instead of engaging in further sequencing, there are growing suggestions that it may make more sense to critically analyse the existing data and purify databases to eliminate mistakes. A similar conclusion has been drawn in discussions about chemical as well as microbial forensics, where it has become apparent that there was a need for well-curated databases and reference standards. Industry (which depends on reliable data) has realized that many academic data do not replicate and consequently has taken to re-measuring essential data even if published data exists.

Take-home points

- Omics technologies have moved on from genomics to transcriptomics, proteomics and metabolomics
- Databases with large amounts of data have been generated but data reliability remains problematic
- There is an increasing need for cleaning up databases and generate reliable reference standards
- Omics has high potential for screening applications in industry and medicine

DNA memory and programming

Large amounts of data and increasing limitations in resources create a growing demand for reliable high capacity data storage. New ways of long term DNA storage, DNA as barcodes as well as programming living organisms with engineered DNA reach for new avenues.

Another theme that has come up in many recent discussions is the relationship between biology and information. For example, DNA can be used to store information. Also, progress is being made in biological computing.

Conventional data storage media have limits in capacity and deteriorate over time so that recorded information is lost or degrades. DNA can store huge amounts of data and might serve as an alternative, but it also decays, and it has a high error rate. But fossil records show that DNA can survive for very long periods of time if properly encapsulated: over 700,000 years. The drop in the cost of DNA sequencing and synthesis is beginning to render DNA data storage as an interesting option. Living cells would not be a suitable medium – they are error prone. 'Synthetic fossils', on the other hand, in which DNA is encapsulated in glass are a possibility for data storage. Long term and large volume storage is already technically feasible; it needs to be combined with a coding system and an error correction process. Today, the main limiting factor is cost rather than technology: 1 MB DNA data storage would cost in excess of USD 1,000.

DNA can store huge amounts of data and might serve as an alternative, but it also decays, and it has a high error rate. But fossil records show that DNA can survive for very long periods of time if properly encapsulated: over 700,000 years.

The technology can also be used to 'barcode' materials such as chemical products, intermediates or food products. Nanoparticles carrying a DNA barcode can be combined with digital PCR 'reading' so that very small particle numbers were needed. Other applications include photo detectors using caged DNA. Whichever the application, multiple times reading is possible, and informatics tools for coding and organizing information are state of the art.

Recording information in living cells is not suitable for long-term storage but can be used in computing, in the form of cells acting as distributed, genomically encoded memory. If cells can be engineered to sense certain signals, they can also be used as parts of computing devices in the same way as computer systems are used to compute abstract models. In

If cells can be engineered to sense certain signals, they can also be used as parts of computing devices in the same way as computer systems are used to compute abstract models.

a layered approach, DNA codes can be programmed; with programmed DNA, devices can be built and with these devices circuits followed by modules, networks, and entire systems. Today researchers already can build modules that 'do interesting things'.

Computation and memory have their roots in electronics, but are today increasingly linked with synthetic biology. Traditional digital synthetic gene circuits and logic models or games have been designed for a couple of decades already. It is possible to turn genes on and off, but creating more complex multiple input-output signalling processes to allow higher-level computations is more complicated and requires integrating logic and memory. For example, pairs of recombinases can be used to implement logic gates, essentially building machines in living cells that sense a state using DNA changes. Furthermore, attempts are under way to implement analogue biological circuits in *E. coli* or human cells, as well as circuits that can switch between an analogue and a digital stage.

ATTEMPTS ARE UNDER WAY TO IMPLEMENT ANALOGUE BIOLOGICAL CIRCUITS IN *E. COLI* OR HUMAN CELLS, AS WELL AS CIRCUITS THAT CAN SWITCH BETWEEN AN ANALOGUE AND A DIGITAL STAGE.

These approaches can be combined with gene editing tools such as CRISPR to leverage human biology to build an integrated approach in which a DNA modified enzyme would be used to encode analogue information. That research inspired the question whether cells could be engineered so that Cas looks for perfect homology. Through multiple cycles of self-targeting mutation it was possible to encode multiple mutations, which indicates how long the system has been active. Another possibility is that DNA could be used to record biological events *in vivo,* memorising both intensity and duration of exposure. Future applications of such systems could include cellular barcoding and lineage tracing.

Areas of future medical application may include the treatment of infectious diseases, cancer and developmental and genetic diseases; they may also find applications in biosensing and material foundries.

Take-home points

- Long term and large volume DNA data storage with 'synthetic fossils' is technically feasible
- Storage needs to be combined with a coding system and an error correction process
- Costs are still high but are expected to be reduced exponentially with future technological advances (e.g. sequencing and synthesis)
- DNA memory can be used as 'barcode' for materials and products
- DNA codes are programmable: recording information in living cells can be used in computing in the form of cells acting as distributed, genomically encoded memory with possible application as sensors
- DNA programming progresses toward simulating with digitally functioning biological circuits analogue biological systems

DNA origami

DNA is a fascinating versatile molecule that can be structurally formed and turned into nanotools with diverse functions other than encoding information – DNA origami. The three dimensional (mechanical) properties of DNA origami can be directed by interaction with environmental factors.

DNA origami uses single-stranded DNA molecules, adding hundreds of DNA strands for folding, as a template for building nanostructures and patterns. To these templates, functionality can be added by DNA capture strands that enable specific interactions. DNA is an interesting material for performing this kind of research – it can be easily sequenced and synthesized in automated fashion. The rules for DNA self-assembly and folding are well understood, it is easy to manage, well defined, flexible, and its mechanical stiffness can be tuned by combining single-stranded DNA (very flexible) with double-stranded DNA (fairly flexible) and bundled DNA (stiff). It can also be combined with nanoparticles (e.g. gold).

In this way, researchers can create three-dimensional behaviour of nanoparticles under the influence of external factors such as light, e.g. in the form of DNA controlled motion, three-dimensional reconfiguration, or distinct optical behaviour caused by interaction with left or right handed polarized light.

In this way, researchers can create three-dimensional behaviour of nanoparticles under the influence of external factors such as light, e.g. in the form of DNA controlled motion, three-dimensional reconfiguration, or distinct optical behaviour caused by interaction with left or right handed polarized light.

Examples shown at the workshop included a 'plasmonic walker', and systems that formed structures that open and close. In addition to light, other environmental factors such as changes in pH can also be used as triggers.

Other examples that were discussed concerned the engineering of complex motion and dynamics. The design approach is top-down: the desired shape is approximated as cylinders, a DNA scaffold is woven into the structure, and single and double-stranded DNA segments are used to modulate stiffness as required (creating 'hinges' and folds). In this way, it is possible to design complex dynamics (e.g., thermally driven, conformational ensembles, or systems that are highly responsive to small changes in the local environment). It has become possible to mimic complex shapes as well as motions, leading to the design of nanoscale tools such as callipers or cutting devices.

It has become possible to mimic complex shapes as well as motions, leading to the design of nanoscale tools such as callipers or cutting devices.

Take-home points

- DNA origami applications are still at the stage of fundamental research
- Future use of DNA origami in drug delivery as nanocontainers and external release triggers
- DNA origami could be integrated with DNA computing
- DNA origami design may be automated in the future
- DNA origami nanotools may be constructed that eventually could be assembled into nanofactories
- At present, fabrication is slow and small scale and cost is a limiting factor

Such dynamic systems can be developed into sensors, but the ultimate goal of these experiments is to make them work in ensemble, perform different functions, and eventually these can be assembled into nanofactories. Future research is directed at actuation (higher speed, better control, and more complex motions), the design of multi-scaffolding structures, hybrid nanosystems incorporating other materials (e.g., enzymes) and elements that can position them precisely. This may be useful for drug delivery using nanocontainers and external release triggers. Also, DNA origami may be integrated with DNA computing.

Today, such applications are at the stage of fundamental research, and there have been very few publications concerning application *in vivo*. The assessment is that many years will need to pass before practical applications such as molecular robotics become feasible. Major challenges remain: design based on structure is a bottleneck but could perhaps be automated in the future; fabrication remains slow and small scale; and cost is a limiting factor.

Tacit knowledge

Although an increasing democratization in the life sciences can be observed, tacit knowledge – the expert 'know how' required to conduct scientific and technical processes successfully – remains an important aspect when evaluating the practical impact of scientific advances on arms control.

Tacit knowledge is an important aspect that affects the evaluation of advances in S+T – it helps to understand what hurdles remain before new scientific discovery may be taken into the design of new weapons. It comes in various forms: 'weak' tacit knowledge – know-how that simply has not been recorded; somatic – developed by extensive practicing; communal – tacit knowledge that depends on team interaction. It is essential for changing what is possible into what actually works, but it can fade over time if not used, or disappear with individuals leaving the scene.

Tacit knowledge is essential for changing what is possible into what actually works, but it can fade over time if not used, or disappear with individuals leaving the scene.

Despite the 'deskilling' and democratization that have been noted as trends in the life sciences, tacit knowledge remains important. Applying scientific discovery in practice takes time and experience, and the experience from past weapons programmes has shown that it can be critical for success. This remains true despite such transitional technologies as CRISPR – often portrayed as an ingredient for the synthesis of dangerous pathogens but in reality it is simply a new tool.

For State programmes, tacit knowledge is only a modest hurdle to overcome, although even there history has examples to show that lack of tacit knowledge can delay progress considerably. But the lack of tacit knowledge can clearly be a hindrance for non-State actors, and they will more often than not opt for types of weaponry they understand and where they already have the tools at hand. The science community is increasingly aware of the risks and is addressing issues such as self-controls and responsible science conduct. Informal communities that engage in life science projects outside established academic structures have begun taking measures to raise awareness, and they make use of advice on biosafety.

Context is important in assessing the risks associated with existing as well as emerging technologies, and the appreciation of context and scenario must therefore be part of risk assessments.

The lack of tacit knowledge does not prevent terrorists or criminals to opt for 'scruffy' improvised chemical or biological weapons such as the barrel bombs releasing chlorine that were used in Syria. Context is important in assessing the risks associated with existing as well as emerging technologies, and the appreciation of context and scenario must therefore be part of risk assessments. It is important, however, to avoid overstating the threats associated with emerging science and technology, as this may have significant negative repercussions for beneficial uses and hamper scientific progress and exchange.

Take-home points

- Tacit knowledge remains an important factor for evaluation and implementation of advances in science and technology
- It can be of huge value for certain actors outside the academic environment
- Increasing awareness in the scientific community for security risks is of high importance
- Issues such as self-control and responsible science conduct need to be addressed in academia and industry environments
- It is necessary to avoid exaggeration of threats associated with emerging science and technology to avoid negative effects on beneficial uses of scientific progress and exchange

Conclusions – implications of convergence between chemistry and biology for arms control

There was no attempt to formally agree on any conclusions at the workshop, but these are the themes that the final round of discussion highlighted:

Convergence has the potential to affect the CB arms control regimes at the level of scope, as well as with regard to implementation. With regard to the scope of prohibitions, the general consensus was that all new discoveries are well covered by the General Purpose Criterion built into both Conventions. Nevertheless, several issues have arisen:

- In general, are nanosystems that mimic biological systems covered?
- More specifically: if the mechanisms exploited by such nanosystems are mechanical, but they have an effect on life processes, can they be considered toxic or infectious agents, or are they delivery systems?
- But also, how realistic is the threat by such systems with regard to their probable hostile use as weapons?

Nanomachinery of course is not a human invention. DNA records information, nanomolecules transcribe genetic information, living cells are biochemical nanofactories, vaccines and virus-like particles (VLPs) self-assemble; cytolysine protein penetrates cell walls and allows to inject toxin to hijack the host cell. Using similar mechanisms, VLPs (which resemble viruses, but do not contain viral genetic material and are therefore non-infectious) have been engineered and can be loaded with drugs or toxins. Second and third generation VLPs are already undergoing clinical trials for vaccine research.

It is important to maintain a general approach rather than single out individual developments at the possible expense of missing others and thus implying uncertainty where there is none.

To cover these developments under both the CWC and the BWC, the General Purpose Criterion is crucial. But how does it apply and how can it be implemented? These are not trivial questions, and they underscore the need to keep reviewing S+T and make sure advances are well understood, their coverage under the treaties is clarified, and the results of these assessments are clearly communicated. At the same time, it is important to maintain a general approach rather than single out individual developments at the possible expense of missing others and thus implying uncertainty where there is none.

Many trends subsumed under the concept of convergence, however, affect implementation rather than treaty scope. A striking example is the impact of new manufacturing technologies on CWC declarations and verification: bio-mediated processes have already been highlighted by the OPCW Scientific Advisory Board (SAB) – this technology is now well established and despite adverse economic pressures may further grow in market share. This does already pose questions with regard to unequal CWC declarations; it also affects verification because material balance-based control systems are likely to fail in biotechnology.

Small, highly flexible plants that manufacture highly active compounds, often using multipurpose equipment and equipped with safety features that in the past were indicative of chemical agent production, challenge the design assumptions underlying the CWC Schedules as well as production thresholds.

But there are new challenges related to chemical plants with 'intrinsic CW capability', a concept that has bedevilled negotiators for decades. Small, highly flexible plants that manufacture highly active compounds, often using multipurpose equipment and equipped with safety features that in the past were indicative of chemical agent production, challenge the design assumptions underlying the CWC Schedules as well as production thresholds. The current CWC system may miss certain facilities that may be of comparable relevance to some Schedule 1 facilities. The issue is not new, but what was of little consequence in the past is today not uncommon in industry. Strengthening national implementation and linking safety and security more closely can help respond to this change but the CWC verification system at this stage does not adequately cover such facilities, given their characteristics and size. This issue needs to be addressed from a legal and policy perspective. The CWC contains all the tools needed to adapt implementation systems when necessary, but it will require political will of the States Parties to agree on them.

The workshop also illustrated that such responses must be proportional to the risks, and appropriate. Adaptations and new regulations need to be informed not merely by what is scientifically possible but what is likely to result in practical applications. There have been many examples of overstating the impact of new scientific discoveries – driven by a desire to advertise science, the need to generate funding, or simply because the limitations of new developments have yet to be understood. Risk and context analysis, therefore, are important throughout the cycle from scientific discovery to application. The application of emerging technology is influenced by many factors (economic, policies, regulatory, competition with mature technologies). Impact analysis therefore needs to remain sensible and avoid over-reactions. An example that became apparent during the workshop was 3D printing of process equipment: the security risks of this technology were likely overestimated in the past.

Adaptations and new regulations need to be informed not merely by what is scientifically possible but what is likely to result in practical applications. There have been many examples of overstating the impact of new scientific discoveries.

Similarly, it needs to be seen how big the risks associated with genome editing are today. CRISPR and C2c2 show great potential and will enlarge and simplify significantly the gene editing toolbox. What the implications of this technological advancement are from a CWC/BWC perspective remains less clear. A large number of agents have been looked at in the past, and technologies such as RNA interference, for example, are already widely used. New technologies such as C2c2 add functionality, but their utility as well as limitations are yet to be fully understood.

But there is room for surprise: CRISPR was able to fill a gap. The ground work for gene drives was already in place but a convenient tool for genome editing was missing, creating frustration and obstructing progress. With the discovery of CRISPR, this was overcome, opening the door for non-linear progress. After only a few years of research, gene drives are almost ready to be released into the environment. The impact of such releases, on the other hand, is way ahead of our understanding, and continuing research needs to include careful security risk mitigation strategies.

After only a few years of research, gene drives are almost ready to be released into the environment. The impact of such releases, on the other hand, is way ahead of our understanding, and continuing research needs to include careful security risk mitigation strategies.

These are examples for challenges posed by basic research to arms control – there is a misuse potential in chemistry and biology, and serendipity effects can occur. This should however not lead to attempts to curtail scientific progress, the benefits of which by far normally outweigh these risks. For effective risk management, context is important – terrorists, for example, are not likely to exploit basic research as this tends to be too complicated, time consuming and uncertain in effect compared to other means of violence that are already available.

It is important that the risk mitigation strategies combine efforts of the security community with those of the research and industry communities, building on raising awareness.

It is important that the risk mitigation strategies combine efforts of the security community with those of the research and industry communities, building on raising awareness. Much has already been done to this effect but sustainable impact needs a critical mass and the embedding of these principles in the professional culture of the communities concerned.

For a balanced approach, it is equally important to also focus on the benefits. Advances in the Omics – an area of strong science convergence – are expected to have a significant impact on our technical capabilities for investigations, surveillance, and forensics. For this to happen, well-curated and comprehensive databases are needed. Data generation is still moving very fast, and analysis and quality of data are still lagging behind. But it is increasingly recognized that the quality of the analysis depends on the quality of the data and efforts are under way to address this problem.

The workshop also underlined the importance of effective communication strategies – not as one-directional information transfer but an up-stream and participatory educational involvement. It is important to ensure that scientists are seen as part of the solution rather than the problem. In this dialogue, it will be important to deal with uncertainties and ambiguity. This requires a multidisciplinary environment where different communities are connected, and it requires appreciation that the same thing may mean different things to different communities, or in different contexts.

A final thought was this: it is critical to embed safety, security, compliance and ethical conduct into the culture of the professionals working with chemical and biological matters – to make these issues part of mainstream thinking within the community. Only in this way can a construct as complicated as the General Purpose Criterion be filled with life. The Hague Guidelines for responsible science for chemistry professionals, developed by the OPCW SAB and supported by IUPAC, are an important initiative for promoting cultural change in the S+T community that could serve as an example for how to approach this matter.

It is critical to embed safety, security, compliance and ethical conduct into the culture of the professionals working with chemical and biological matters – to make these issues part of mainstream thinking within the community.

Participants

Bankoul Sergei Swiss Armed Forces Logistics, Medical Services Directorate, *Head of Medical CBRN Defense,* CH

Barnes Thomas Intellia Therapeutics, Inc., *Chief Scientific Officer,* US

Bohm Elizabeth The Royal Society, *Senior Policy Adviser,* UK

Bonin Sergio Arms Control and Disarmament Section, Federal Department of Foreign Affairs, *Political Affairs Officer,* CH

Boon-Huan Tan DSO National Laboratories, *Program Director,* SG

Brooks Matthew Defence Science and Technology Laboratory (DSTL), *Principal Scientist,* UK

Brouns Stan Delft University of Technology, *Associate Professor Molecular Microbiology,* NL

Cadisch Marc Spiez Laboratory, Federal Office for Civil Protection, *Director,* CH

Castro Carlos The Ohio State University, OH, *Assistant Professor,* US

Coates David Defence Science and Technology Laboratory (DSTL), *Fellow in CB Non-Proliferation,* UK

Corriveau Joseph L. US Army Edgewood Chemical Biological Center, *Director,* US

Curty Christophe Organisation for the Prohibition of Chemical Weapons, *SAB member,* CH

Dennison Genevieve Defence Science Technology Group, *Defence Scientist,* AU

Dover Michelle Ploughshares Fund, *Program Officer,* US

Faukal Katharina Austrian Armed Forces, *Veterinary Officer,* AT

Flanagan Meg U.S. Department of State, *Microbiologist,* US

Forman Jonathan Organisation for the Prohibition of Chemical Weapons, *Science Policy Adviser,* OPCW

Govorun Vadim Federal Research and Clinical Center of Physical – Chemical Medicine of Federal Medical Biological Agency, *Director,* RU

Grass Robert Swiss Federal Institute of Technology Zurich (ETHZ), *Senior Scientist,* CH

Grütter Frank Division for Security Policy, Federal Department of Foreign Affairs, *Ambassador,* CH

Halter Matthias International Relations Defence, Federal Department of Defence, Civil Protection and Sports, *Head Arms Control and Disarmament,* CH

Hart John Stockholm International Peace Research Institute (SIPRI), *Senior Researcher,* SE

Hoffmann Patrik EMPA, Swiss Federal Laboratories for Materials Science and Technology, *Head of Laboratory,* CH,

Invernizzi Cédric Spiez Laboratory, Federal Office for Civil Protection, *Head of Arms Control Biology,* CH

Isaacs Farren Yale University, *Assistant Professor,* US

Jordi Daniel Spiez Laboratory, Federal Office for Civil Protection, *Head of NBC Protection Division,* CH

Lentzos Filippa Kings College London, *Senior Research Fellow,* UK

Li Hua Institute of Pharmacology and Toxicology, AMMS, *Professor,* CN

Liu Laura Na Max Planck Institute for Intelligent Systems, *Professor,* DE

Marx Alain Lonza AG, *Plant Manager,* CH

May Andrew Caribou Biosciences, Inc., *Chief Scientific Officer,* US

McLeish Caitriona The Harvard Sussex Program, *Senior Fellow,* UK

Mikulak Robert U.S. Department of State, *Expert,* US

Mogl Stefan Spiez Laboratory, Federal Office for Civil Protection, *Head of Chemistry Division,* CH

Moodie Amanda Center for the Study of Weapons of Mass Destruction, *Research Associate,* US

Nolan Tony Imperial College London, *Senior Research Fellow,* UK

Ong Eugene Insitute for Research in Molecular Medicine, *Lecturer/Researcher,* MY

Otto Claudia Swiss Federal Institute of Technology Zurich (ETHZ), *Senior Researcher,* CH

Perli Samuel Massachusetts Institute of Technology (MIT), *Post Doctoral Associate,* US

Rath Johannes Faculty Life Sciences, University Vienna, *Head DNA Laboratory,* AT

Revill James Harvard Sussex Program, *Research Fellow,* UK

Rimann Markus Zurich University of Applied Science, *Group Leader 3D Tissues and Biofabrication,* CH

Schmidt Beat Spiez Laboratory, Federal Office for Civil Protection, *Head of Arms Control Chemistry,* CH

Seeberger Peter Max Planck Institute of Colloids and Interfaces, *Professor & Director,* DE

Seyfried-Doering Britta Bundesamt für Wirtschaft und Ausfuhr, *Biologist,* DE

Smith Mark Wilton Park, *Programme Director, Defence & Security,* UK

Strasser Marc Spiez Laboratory, Federal Office for Civil Protection, *Head of Biology Division,* CH

Studer Michael Bern University of Applied Sciences, *Professor,* CH

Tang Cheng Ministry of National Defence, *Deputy Chief / Office of Disposal of JACW,* CN

Thränert Oliver Center for Security Studies, ETH Zürich, *Head of Think Tank,* CH

Timperley Christopher Organisation for the Prohibition of Chemical Weapons, *Chairperson, OPCW Scientific Advisory Board,* UK

Trapp Ralf Independent, *International Disarmament Consultant,* FR

Üzümcü Ahmet Organisation for the Prohibition of Chemical Weapons, *Director-General,* OPCW

Vanninen Paula VERIFIN, University of Helsinki, *Director,* FI

van Straten Francois Organisation for the Prohibition of Chemical Weapons, *SAB member,* ZA

Walker John Foreign and Commonwealth Office, *Head, Arms Control and Disarmament Research Unit,* UK

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Spiez CONVERGENCE
5 – 8 September 2016, Spiez, Switzerland