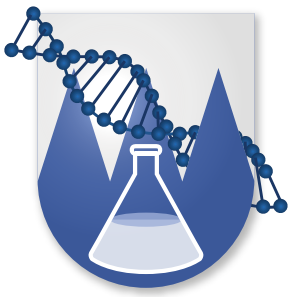

Spiez CONVERGENCE

Report on the sixth conference
29, 30 August and 8–11 September 2024

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Contents

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Executive Summary

Spiez CONVERGENCE 2024 was the sixth edition of this conference series hosted by Spiez Laboratory. The conference series is a Swiss "Science Diplomacy" initiative and part of the Swiss strategy for arms control and disarmament.[1] It provides a platform for the presentation and discussion of new developments in science and technology that may affect the regimes governing the prohibition of chemical and biological weapons. Before the actual conference, participants had the opportunity to partake in a virtual "ice breaker" event with two keynote presentations introducing the objectives of the conference. This report brings together the new developments that were presented and the trends and findings discussed in the plenary and the breakout sessions.

Four presentations focussed on the **Precise Editing of Molecules in Chemistry and Biology.**

Reprogramming the Genetic Code to incorporate non-natural amino acids in proteins greatly expands the theoretical number of possible proteins. Chemical polymers consist of one or very few building blocks and their chain length is generally difficult to control. The variety of polymers can grow with new monomers. In contrast, length and sequence in biological protein synthesis is easier to control but "only" 20 natural amino acids are available as monomers. Using non-natural amino acids greatly expands this number and allows the synthesis of polymers with different properties and functionalities. The physiological and environmental effects of such polymers and their potential risks are yet to be studied.

Skeletal Editing is a method that precisely edits the skeleton, i.e. the core structure of molecules. The ability to engineer single-atom changes in the skeleton of small molecules or polymers may allow finetuning of properties or remove unwanted structural features. The method can be utilised to greatly enhance the selectivity of chemical reactions. While many applications are beneficial, skeletal editing may open new synthesis pathways also for toxic chemicals.

Epigenetic Editing is a promising technique to modulate genome functions. The epigenome consists of chemical modifications of DNA and of proteins that can attach to DNA to turn genes on or off, and control the production of proteins. Changes to the epigenome are inheritable, yet reversible, the effect can be gene specific and it does not damage or change the DNA sequence. The method is less invasive than genome editing using e.g. CRISPR but similarly effective. The field for therapeutic applications for epigenetic editing is growing and biosafety as well as dual-use issues will have to be considered.

1 Swiss Federal Department of Foreign Affairs (FDFA), Arms Control and Disarmament Strategy 2022–2025, 02.02.2022

Editing RNA with Enzymes is a further editing technique to influence cellular regulatory mechanisms. Natural modifications of RNA act like a barcode and help the cells to distinguish its own RNA from that of other organisms. Editing RNA can affect the innate immunity, which, among others, recognises foreign RNA. If this editing takes place in coding regions for proteins, it can change the "interpretation of bases", which could lead to the production of a slightly different protein.

Digitalisation, Automation and Artificial Intelligence greatly influence today's advances in chemistry and biology and four presentations discussed relevant developments.

Computers need a *Language of Chemistry* in order to understand chemistry. Chemicals are defined by a molecular structure. The structure has to be turned into strings that a computer can read; atoms become letters and molecules words. With the help of such a language, machine learning tools can process vast amounts of data about chemical reactions: which precursor chemicals form what reaction product. Based on this data, software tools can make predictions about the outcome of chemical reactions or suggest pathways for retrosynthesis of a desired molecule. Todays' Large Language Models alone are not well suited for chemistry applications, but combined with a number of other new software tools, AI supported chemical synthesis planning is expected to automate chemical experiments.

Self-Driving Laboratories support the automation of synthesis and will greatly increase the productivity in chemical research and development. Researchers design an experiment, AI systems plan the implementation, robotic systems run the experiment, digital systems control the workflow and analytical tools assess the result. Experimental parameters can be optimised by a closed-loop *Bayesian* model. The method greatly reduces the time for reaction-optimisation, eliminates unsuccessful pathways and increases synthesis success rates.

The technology of Self-Driving Laboratories has also been used to *Autonomously navigate the Protein Fitness Landscape,* i.e. engineer proteins to assess the relationship between sequence and function of a protein. Such a laboratory system performs gene assembly, expression and biochemical characterisation. An AI tool then adjusts the sequence and repeats the experiment until it finds an optimal sequence for the "fittest" protein. It was demonstrated that an autonomous pipeline can greatly reduce the time needed for bioengineering an optimised protein.

Artificial Intelligence is used to reduce the Development Time of Vaccines for diseases with pandemic potential. The prefusion conformation of a spike protein e.g. SARS-CoV-2 is a good candidate for a vaccine. It needs to be stabilised so that the immune system develops antibodies against the prefusion but not the post-fusion conformation. Software tools are applied to predict the protein structure in its prefusion conformation and suggest ways how this structural conformation may be stabilised. An AI-driven pipeline has been designed and tested to develop vaccines against Arenaviruses.

In this year's conference three presentations were dedicated to advances in the **Manufacturing of Chemicals**.

Engineered Proteins in the form of enzymes *are used as Biocatalysts for Synthetic Applications* because they show great benefits for the synthesis of active pharmaceutical ingredients. Enzymes are proteins with catalytic activity and engineering and optimising them is a time-consuming task. It would be helpful, if software tools could predict how certain mutations would affect the properties of the enzyme. Predicting stabilising mutations is difficult, but it is possible to predict destabilising mutations. With bioinformatic tools, destabilising mutations can be filtered out and enzyme libraries can be created that contain information about the sequence space that should be explored for optimisation. This information helps to reduce the number of evolutionary cycles that are required to engineer a biocatalyst to its desired profile. Engineered biocatalysts have very beneficial applications but could theoretically also be used for the synthesis of toxic chemicals.

Click Chemistry are fast chemical reactions for a wide range of chemical and biomedical applications. Molecular building blocks "snap" together because of a high thermodynamic driving force, generally leading to a single reaction product. Click reactions have been used in living organisms by tagging certain proteins. This can allow the monitoring of biological processes. Click reactions can also be used as diagnostic tools for cancer imaging or as targeted release strategy. An example for a cancer therapy was presented, where an antibody is used to target a tumour, and through a click reaction, the drug is released.

Electrosynthesis is the addition or removal of electrons in chemical bonds at a cathode or an anode to form new compounds. The method opens up new synthetic approaches and has the potential to be a disruptive technology. The synthesis process can be optimised by the selection of materials for the electrodes and electrolytes and the design of the electrosynthesis cell. Machine learning is used to optimise the process and reaction parameters. Electrosynthesis has been successfully applied to a range of chemical reactions including cross-coupling reactions.

Another project presented under Manufacturing of Chemicals was the use of ionic liquids to desensitise the explosive triacetone triperoxide (TATP), without altering the olfactory properties in order to train sniffer dogs to detect improvised explosive devices. TATP dissolved in ionic liquids allows the safe storage of this otherwise highly sensitive substance.

New developments in **Therapeutic Applications and Drug Delivery** were addressed in three presentations.

3D Printing of Personalised Medicines is a technology that allows to compensate for person-to-person variability in the optimal therapeutic dose. It helps to improve the efficacy of treatment, reduce side effects and waste of therapeutics. 3D printed medicines "printlets" offer greater flexibility, including personalised dosage, the combination of different drugs in the same printlet, or a design that appeals to the patient. 3D printing of medicines is at the stage of pre-clinical trials and a GMP-compliant system is available for the production of printlets in a hospital environment.

In a *Novel Platform for Bacterial Anticancer Therapy* a bacterium has been genetically engineered for tumour targeting and protein delivery. After injection into the bloodstream, the bacterium *Yersinia enterocolitica* starts to grow rapidly inside tumours but is otherwise eliminated from the body. The bacteria can be engineered and used as a vessel to deliver bioactive proteins into a tumour. The method could become a promising new cancer treatment.

Engineered DNA Nanostructures for potential future Applications in Medicine and Biotechnology is a new application for DNA Origami. In earlier Spiez CONVERGENCE conferences the focus was on user-defined shapes of self-assembled single-stranded DNA nanoparticles. Now, the DNA surface of such nanostructures can be encoded with biological instructions. DNA origami shells are used as an antiviral trap. With the right size and shape, and containing virus-binding moieties, they trap virus particles inside them. Other applications include user-defined shapes that contain exposed gene-encoded structures in defined locations. An example was shown, where DNA subunits are designed as puzzle pieces. They only attach in a defined configuration and by that allow to control stoichiometry in gene expression. DNA origami has become a tool that may be used in many disciplines.

Threat-Agnostic Biodefence is a new concept to approach an emerging disease. Today's diagnostic testing is a slow process. It generally relies on a "rule-out" list-based approach and sequencing data to eliminate known causes of disease. The many advances in sequencing technology provide important information, but only about the potential of an organism and not about what it actually does at a point in time. In order to respond to a newly emerging or engineered pathogen or toxin, robust methods are required for detection, classification and characterisation, and they must provide information on "what it does". The number of possible microbes and toxins may be infinite. However, the ways they can infect or harm, and how a host can respond to it, are finite. Building a new diagnostic approach should focus on characterising those finite pathways as indicators for assessing a potential for harm.

The chapter on **Implications and Conclusions** is a summary of all the discussions during the conference, not only in the plenum but also in the smaller breakout groups.

What are the Major Trends – while the researcher remains at the centre of the scientific process, the trend towards "Self-Driving" Laboratories continues. Design, execution, evaluation and optimisation of experiments are assisted by computer algorithms and robotics. The search for and the optimisation of experimental parameters are performed with automated loops. Large Language Models combined with specialised machine learning tools lead to better predictions of chemical and biological mechanisms. Biological processes play a more important role in the manufacture of chemicals, pharmaceuticals and functional materials. Methods to edit chemical and biological structures in order to change their properties and functionalities are becoming more precise. New methods for drug delivery allow a more exact targeting and a personalised dosing of drugs. Most conference participants were of the view, that these scientific advances will in general be very beneficial and possibly strengthen a defence against a malicious use of chemical or biological agents. Established actors in industry can play an important role in self-regulating products or developments that could be causing concern. Scientific freedom and open access are seen as critical for good science. Early stages of research and scientific development should not be over-regulated and a balance must be found between openness and regulation.

Are there Risks and Potential Game Changers – There is misuse potential in these new scientific and technological advancements, should they intentionally be applied to the development or production of chemical or biological weapons. A risk assessment must take into consideration that new technologies have to compete with well-established methods. They have to demonstrate utility through testing and validation. This is particularly the case for chemical weapons, where effective agents and well-known methods for large scale production and dissemination exist. A promising new concept that was presented to support the defence against (new) toxins and pathogens is the "Threat-Agnostic-Approach". This approach focuses on the functionalities of what an agent does as indicators to assess the potential for an agent to cause harm e.g. toxicity or pathogenicity. This is different to today's list-based approach of looking for known agents that cause harm. Applying this principle to advances in science and technology in general by looking at the key features that they enable might prove useful to assess a potential of misuse. In addition to functionalities that characterise what an agent does, there are other functionalities such as ease of production, storage, dissemination, etc. that could be used as indicators.

A clear gap in the discussion about chemical or biological weapons is that animals and plants as targets of toxic chemicals or pathogens are often overlooked. Another big concern is, that mis- and disinformation undermine trust in the regimes, the credibility of the CWC and the OPCW, as well as the BWC. More effort is needed, also from the scientific community, to counter these threats. AI in combination with automation and robotics was considered a game changer at Spiez CONVERGENCE 2022. This year's conference confirms – the way how experiments are performed is changing.

What does all this mean for Arms Control – The research and development that is at the start of scientific and technological progress is expected to help solve many problems that confront us today. However, many of the fast-evolving technologies contain a potential for misuse. The question for regulators is, how can risks best be mitigated: by awareness-raising, by self-regulation, by government intervention, a combination of the three or neither? New technological capabilities do not equal new chemical or biological weapons. It is important to emphasise, utilising new developments in science and technology for a weapons program is not easy and generally out of reach for individual actors. It starts with an intention and requires a conscious decision to conduct a weapons research and development project. Therefore, the focus of risk mitigation for advances in science and technology should be state actors and not individuals or groups. The CWC and the BWC combined with national regulations provide a framework for the prevention of misuse. Unfortunately, treaties and policies for implementation cannot keep pace with today's rapid developments in science and technology. Self-regulation presents itself as obvious alternative, but industry driven self-regulation depends on awareness of dual-use risks and the willingness to address them. How can one deal with a misuse potential that is the result of a combination of advances in various disciplines? How can the need for transparency in science and the sharing of results be balanced with the necessity of preventing critical information from falling into the wrong hands? Governments, International Organisations, Academia and Industry must be partner in such deliberations. Spiez CONVERGENCE does not have all the answers, but will continue to offer a platform to raise and discuss relevant questions – as a Swiss contribution to "Science Diplomacy" – the next time in 2026.

Precise Editing in Chemistry and Biology

"Editing" molecular structures per se is not new. The Beckmann rearrangement, for example, was published first in 1886. Decades later it was employed in the synthesis of caprolactam, the monomer of nylon-6, and a precursor of the antibiotic azithromycin.

Precise and efficient editing of biomolecules is becoming a versatile tool that promises advances in diagnostics and therapies of diseases, the development of safe and effective vaccines, and the creation of novel materials with hitherto unknown or unachievable properties. However, genome editing or engineering has also been associated with security risks – in 2016, it was characterised as a global danger akin to weapons of mass destruction.[2] Previous Spiez CONVERGENCE conferences have looked at precision editing of large biomolecules. This year, four themes were discussed: reprogramming the genetic code using non-canonical amino acids; skeletal editing; epigenetic editing; and RNA editing.

Reprogramming the Genetic Code

The genetic information of most living organisms is encoded in their DNA using four "letters" – cytosine (C), guanine (G), adenine (A) and thymine (T). Arranged in three-letter "codons", these nucleotides spell out the identity and their sequence dictates the position of the amino acids within a protein. This information is transferred by messenger RNA (mRNA) to the cell's ribosomes, where proteins are synthesised using 20 natural ("canonical") amino acids.

Non-canonical amino acids expand the scope of the protein space and allow to construct polymers with different properties and functionalities.

This system can be tweaked to produce polymers with non-canonical amino acids. The result is a vast theoretical protein space of some 10^{130} entities. Most of these are of little interest, but some proteins may exhibit enhanced or unusual properties. Exploring such functionalities by producing and testing constructs that are not found in nature promises access to means of enhancing food security and environmental safety, novel materials, better and cheaper medicines and much more.

2 Daniel M. Gerstein: *How genetic editing became a national security threat*. Bull. Atomic Sci. (25 April 2016.)

Engineering biomolecules using non-canonical building blocks also overcomes certain limitations of chemical synthesis: in chemical polymerisation, the sequence of the building blocks forming a macromolecule is difficult to control. Also, chemical synthesis has a high energy demand. Biological synthesis, on the other hand, builds polymers with high precision and low energy consumption. Its scope is however limited by the natural amino acids used in proteins. Non-canonical amino acids expand the scope of the protein space and allow to construct polymers with different properties and functionalities.

The replacement of canonical tRNAs with non-canonical tRNAs makes the genetic code of modified organisms unreadable to the translation machinery of unmodified organisms and *vice versa*. Moreover, locking refactored codes into synthetic organisms completely blocks invasion by mobile genetic elements, including viruses.

To do so requires codons that encode the non-canonical amino acids, and a modification of the biological machinery that translates genetic information from RNA into proteins. To accomplish this, researchers exploit the redundancy in the translation system. In nature, most amino acids are coded by several codons. The choice of codons influences the abundance of the corresponding proteins. By repurposing some of them, codons and corresponding aminoacyl-tRNA synthetases can be re-assigned to new monomers. Repurposing raises the question: Which rules for codon redundancy need to be observed for the cell to remain functional?

To answer this question, computer-designed *E. coli* genome variants have been synthesised to test whether they function in living cells. From these experiments, they were able to extract rules for the repurposing of codons. The engineering of the translation machinery to enable it to incorporate non-canonical amino acids, makes it unable to read genetic code of unmodified organisms and *vice versa*. Moreover, locking refactored codes into synthetic organisms completely blocks invasion by mobile genetic elements, including viruses.

The result was a programmable system for the synthesis of polymers with a desired sequence using synthetic DNA with several reassigned codons and evolved orthogonal aminoacyl-tRNA synthetase/tRNA pairs.

The engineered cells are fully functional and retain the ability to replicate. They build pre-designed polymers integrating non-canonical amino acids. The behaviour of these synthetic polymers in the environment, their degradation or accumulation and possible physiological and environmental effects are yet to be studied.

Take-home points

- In nature, proteins are synthesised using the 20 natural ("canonical") amino acids. By tweaking this system, polymers with non-canonical amino acids can be produced.
- These modifications enable the creation of biomolecules with enhanced or unique properties and functionalities, paving the way for better treatments, novel materials or more efficient environmental solutions.
- Introducing non-canonical amino acids requires the repurposing of codons that encode those and a modification of the translation machinery that translates messenger RNA (mRNA) into proteins.
- The researchers designed synthetic organisms with an engineered code that block the invasion by mobile genetic elements, including viruses.
- The engineered cells function and synthesise pre-designed polymers from non-canonical amino acids, though further research is needed to understand their physiological and environmental effects.

Simply put...

Reprogramming the genetic code refers to a modification of the cell's system in how to read the genetic information to make proteins. In nature, the genetic code consists of 64 codons (sequence of three "letters") that encode the 20 natural amino acids, the building blocks of proteins. This system can be manipulated to incorporate non-natural amino acids into proteins, enabling the synthesis of new proteins with desired functionalities.

Skeletal Editing

A second theme was the precision editing of the skeleton of molecules. Most editing strategies change the composition of end groups or functional groups of molecules that are important for binding or reactivity. It is also possible, however, to engineer single-atom changes in the skeleton of small molecules or polymers to finetune properties, or remove unnecessary or undesirable structural features. Single atoms can be added, removed or replaced in ring structures or molecular skeletons. Editing small molecules can be used to achieve perfect site and stereo selectivity (important for the binding to target structures) or to replace single atoms in a ring structure. Editing the backbone of a molecule can also fine-tune the reactivity of groups close to the edit.

It is also possible, however, to engineer single-atom changes in the skeleton of small molecules or polymers to finetune properties, or remove unnecessary or undesirable structural features.

Skeletal editing has potential benefits in the discovery of drugs and agrochemicals, the design of toxin antidotes, the production of new functional materials, the breakdown of plastic waste or scrap tires, the diagnosis and treatment of diseases, and for the reduction of the weight of plastic for space missions. However, the technology may also open up novel pathways for the synthesis of chemical warfare agents, or allow the making of new agent variants through late-stage diversification, or it could lead to the diversification of low-molecular weight toxins.

Take-home points

- Skeletal editing allows changes in compounds, e.g. by adding or removing atoms on the skeleton of small molecules or polymers to finetune properties or remove undesired structural features.
- Skeletal editing could be beneficial in the discovery of drugs and agrochemicals, the design of toxin antidotes, the production of new materials, plastic waste breakdown, diagnosis and treatment of disease.
- Hypothetically, potential misuse of skeletal editing could include new methods of synthesis for known chemical warfare agents or new agents and toxins.

Simply put...

Molecules are composed of atoms linked together by bonds, forming their structure. Traditionally, changing the main structure of molecules (the "skeleton") was difficult and time-consuming. Scientists tended to change side groups or functional groups. Today, researchers cannot only make specific modifications to the outer parts of a molecule but also at the core or "skeleton" of a molecule. This unlocks new possibilities in fields such as drug discovery and polymer synthesis.

Epigenetic Editing

Another promising editing technique is epigenetic editing. The epigenome consists of the chemical modifications of DNA and proteins that bind to it, which direct actions such as turning genes on or off, thereby controlling the production of proteins. Epigenetic modifications modulate genome functions and determine the cell type. Changes in the epigenome are inheritable yet reversible, and are not encoded in the DNA sequence. Epigenomic players can be distinguished into writers, readers and erasers.

The epigenome consists of the chemical modifications of DNA and proteins that bind to it, which direct actions such as turning genes on or off, thereby controlling the production of proteins.

Histones (proteins that provide structural support for chromosomes) play a role in gene expression. If a histone has been "marked" near a gene, gene expression is repressed by DNA methylation (which closes the structure). Without methylation, the DNA is accessible for expression. Epigenetic marks can be rewritten, modulating the expression of a particular gene. The combination with catalytically inactive Cas9 (dCas9) allows the targeting of epigenetic editors for sustained modulation of DNA transcription, which whilst being equally effective, is less invasive than genome editing. This is advantageous for the treatment of genetic diseases. The effect is gene specific (targeted) and can be multiplexed; it is sustained; and it does not damage or change the genetic sequence.

Examples of current research are epigenetic silencing to reduce cholesterol levels in blood, and an epigenetic editor to treat prion disease and other neurodegenerative diseases. *In vivo* studies of epigenetic editing are multiplying, pharmaceutical companies are actively looking at clinical applications, and a first clinical trial started in 2022. Epigenetic editing is not (yet) fully predictable and epigenetic regulation needs to be better understood. Previously, there were doubts about whether epigenetic editing can be locus-specific, whether silenced genes are accessible, whether epigenetic marks cause gene expression, and whether editing will be sustainable. More research is needed, but the field of therapeutic application is widening. As it evolves, biosafety and dual-use issues will need to be considered.

Take-home points

- Epigenetic editing is a technique to alter gene expression by turning genes on or off without changing the DNA sequence, making it reversible.
- The epigenetic players, categorised into writers, erasers and readers, are involved in the modification of epigenetic marks such as DNA methylation or histone modification and can thus modulate gene expression.
- Epigenetic editing offers great potential for the treatment of certain diseases without changing the DNA and it already entered the first clinical trials in 2022.
- Though more research is needed and questions regarding biosafety and dual-use need to be addressed, the field of therapeutic applications is growing.

Simply put...

While the genome is the complete set of DNA (or RNA for some viruses), containing all the genes, the epigenome consists of chemical "tags" on the DNA that can regulate gene expression by switching genes on or off. Unlike the genome, that stays unchanged over time, the epigenome can change over time due to other factors such as stress, environment or diet. Through epigenetic editing, researchers can modify these chemical "tags" at a specific site, and therefore control if genes are turned on or off. New possibilities for treatments arise when genes involved in diseases can be turned on or off this way and regulate protein production.

RNA Editing

Another editing technique is RNA editing. It uses ADARs (adenosine deaminase acting on RNA)—enzymes that deaminate adenosine into inosine within double-stranded RNA (dsRNA). Inosine is functionally recognised by the cellular machineries as guanosine, which may change translation, splicing, and other regulatory mechanisms.

RNA modification acts as a kind of barcode to distinguish the cell's own RNA from that of other organisms. There are approximately 170 RNA modifications, some of which unique for specific life forms (eukarya, organelles, bacteria, archaea).

Inosine is functionally recognised by the cellular machineries as guanosine, which may change translation, splicing, and other regulatory mechanisms.

ADARs have a range of biological functions, including modification and targeting of small non-coding RNAs that play a role in gene silencing (miRNA), the functioning of the nervous system, the production of blood cells, many cancers, development, and innate (non-specific) immunity. ADAR1 is essential for maintaining a steady state in the organism (homeostasis) and for thresholding (balancing between the body's anti-inflammatory strategy and inflammation). An ADAR1 decrease overcomes resistance to so-called checkpoint inhibitors which block certain proteins from binding with surface proteins of tumour cells, allowing T cells to kill the cancer cells. Experiments with ADAR +/- mice have shown that these mutants are protected from malaria parasites, resulting from a boost of the animals' immune system.

RNA editing can be misused: it might be possible to engineer viruses or bacteria that the cellular defence system cannot recognise. However, size and biology set limits, and the effect of such modifications on the microbial genome could be detrimental. It is equally unlikely that the cellular machinery will be co-opted, since the cell has multiple lines of defence against foreign agents.

Take-home points

- RNA editing involves the use of ADARs, enzymes that convert adenosine into inosine within double-stranded RNA (dsRNA).
- The conversion of adenosine to inosine, read by the cellular machine as guanosine, allows to modulate translation, splicing, and other regulatory mechanisms.
- By editing dsRNA and thereby inserting inosine, ADARs help the cell to recognise "self" from "non-self" dsRNA, e.g. of viral origin.
- ADARs serve various biological functions and play a role in innate (non-specific) immunity. They could be used in cancer therapy.
- If the conversion happens in a coding region for a protein, another amino acid can be inserted which can alter the properties of the protein.

Simply put...

Different types of RNA (ribonucleic acid) exist and all of them are crucial for important biological processes in the cell, e.g., messenger RNA (mRNA) plays a pivotal role in the conversion of genetic information into proteins. RNA editing can occur either in non-coding or coding (coding for a protein) regions. If the editing happens in a protein-coding region, new variants of proteins could be generated. The most common type of RNA editing in mammals is the A-to-I editing (adenosine to inosine) with the help of ADAR enzymes.

Digitalisation, Automation and Artificial Intelligence

Digitalisation, automation and artificial intelligence have been reviewed at several Spiez CONVERGENCE conferences in the past. The report on Spiez CONVERGENCE 2022 concluded that it was the combined application of these technologies that could become a potential game changer. Therefore, Spiez CONVERGENCE 2024 looked again at these technologies.

AI-Accelerated Chemistry

If computers understood the "language of chemistry", they could predict chemical structures with desired properties, devise synthetic pathways and conditions to make them, test the properties of these chemicals, and optimise the design and synthetic pathway. This approach is data-driven, solution-focused, and accelerates chemical discovery. Different data sources can be exploited: chemical literature, experiments and simulations, and also patents. Patents contain data of millions of chemical reactions, and can be data-mined.

To use these data sources for machine learning, chemical structures and reactions need to be translated from structural representations into strings of symbols that a computer can read. This can be done using SMILES – the Simplified Molecular Input Line Entry System. The different atoms of a chemical structure are symbolised by letters and positioned along a string that follows the backbone of the molecule. Atoms are written as "letters", molecules as "words". Precursors (reactants) are "tokens" of a final product.

Today, ML tools can make accurate predictions of chemical reactions that have never been conducted, and their performance is better than that of rule- or graph-based approaches.

With this approach, machine learning (ML) tools can "translate" the data representing the precursors of a chemical reaction into data representing the reaction product. Such a "molecular transformer" has no rules of chemistry integrated, nor any chemical knowledge. It simply transforms input to output, similar to software that translates text from one language into another. It learns from processing vast amounts of reaction data, to determine which product would most likely result from a certain combination of precursors. Today, ML tools can make accurate predictions of chemical reactions that have never been conducted, and their performance is better than that of rule- or graph-based approaches.

This approach can be used to plan the retrosynthesis of a desired structure: the synthetic pathway is constructed step by step from a large set of known building blocks or templates (reactants), using optimisation tools such as Monte Carlo tree search and step-scoring. Molecular transformers can plan multistep syntheses using a combination of linguistic models and hyper-graph exploration strategies. Self-Driving Laboratories then combine computer designs of target structures with AI synthesis planning and robotic execution of synthesis and testing.

Models for chemical synthesis planning evolved from models with single modality, like retrosynthesis planning and prediction of reaction conditions to models with multiple modalities to predict synthesis procedures. A new trend includes fine-tuned Large Language Models (LLM), alone or augmented with chemical tools.

LLMs are decoder-only models: they predict the next "word" from all words given before. On their own, they are bad at chemistry. However, they can be augmented with existing chemistry tools. ChemCrow is a toolset that combines a range of molecular tools and public cheminformatics databases with general tools that support literature and web searches, interpret code, and interact with human experts. It integrates reaction tools developed for synthesis planning, as well as toxicity data and GHS classification. It is a tool kit to support user-defined scientific tasks. A task given in human language (example: plan and execute the synthesis of an insect repellent) prompts a process of reasoning and planning, searching literature to select a promising target compound, selecting the tools needed, and executing the task (for example in a cloud lab). This approach is modular and can be fully automated but human approval of physical experiments can be implemented. Safety tools can be integrated.

LLMs on their own are bad at chemistry. However, they can be augmented with existing chemistry tools.

Toolsets like ChemCrow allow the automated synthesis of novel molecules – requiring models that are able to design molecules with a desired property profile. Today's challenges are more modest: How best to mimic experimental performance, how to incorporate molecular dynamics, and how to design molecules that are synthetically accessible.

Take-home points

- The discovery of new chemicals is accelerated with data-driven and solution-focused tools. They suggest which chemicals to make and predict how to synthesise them.
- Using SMILES (readable strings of chemical structures) and machine learning tools such as "molecular transformer", accurate predictions on unknown chemical reactions can be made by translating data representing precursors into data representing the reaction product – similar to language translation software.
- Those tools can predict retrosynthetic pathways for a target molecule, and plan multi-step synthesis.
- Language models for synthesis started with models using a single type of data, followed by models including other types of data. A new trend leverages fine-tuned Large Language Models, alone or augmented with chemical tools.
- Large Language Models on their own are bad at chemistry, but they can be augmented with chemistry tools. One example is ChemCrow, an LLM chemistry agent, that combines a range of tools, and is able to plan and direct the execution of syntheses.

Simply put...

Large Language Models (LLMs) are machine learning models trained on large datasets, that can understand and predict the next word in a sentence. Trained on chemical data, they are able to predict reaction conditions, chemical properties and advise on synthetic pathways. This gives LLMs the possibility to be used in a variety of applications such as in drug discovery or in reaction and property predictions.

Self-Driving Laboratories

An important tool in the move towards automated synthesis are Self-Driving Laboratories (SDLs). Chemical research and development laboratories work in ways that are stuck in the past. Time to market measures in years if not decades, costs are high, and success rates low. Except for genome editing, the productivity of chemical R&D has declined exponentially since the 1950ies – a reversal of Moore's Law.

This illustrated how AI-driven R&D expands the chemical or parameter space screened for a solution, compresses the time needed for optimisation, avoids unsuccessful pathways, and consequently increases success rates.

SDLs aim to change this by combining AI with robotics and digital technologies. They allow closed-loop optimisation of lab processes. Researchers design experiments; the AI system decides how to implement the experimental design; robotic systems run the experiments; a digital system controls the workflow; and analytical tools assess whether the desired results have been achieved. If necessary, the experimental design is adjusted and the experiment run again.

To speed up the optimisation even further, a constrained *Bayesian* modelling was used that excluded certain areas from optimisation, replacing high-fidelity wet lab experiments by cheaper low-fidelity modelling approaches. To demonstrate the power of this approach, the researchers replicated the major development stages of a historic optimisation problem – the identification of a perfect catalyst to convert CO_2 into methanol. This optimisation involved 11 parameters and 7 constraints. It was completed within 6 weeks; historically, this had taken a century. This illustrated how AI-driven R&D expands the chemical or parameter space screened for a solution, compresses the time needed for optimisation, avoids unsuccessful pathways, and consequently increases success rates.

Take-home points

- Self-Driving Laboratories (SDLs) combine AI with robotics and digital technologies to accelerate the discovery of materials and molecules.
- SDLs accelerate experiments through closed-loop optimisation, involving researcher designed experiments, machine learning for decision-making, robotic execution, data analytics and continuous refinement of the process.
- AI-driven R&D is able to screen a larger chemical space and accelerate the discovery of high-potential candidates.

Simply put...

Self-Driving Laboratories are laboratories that integrate AI tools and robotics to autonomously conduct experiments or biological engineering tasks. Through a continuous feedback loop, they are able to test, conduct, analyse and optimise experiments, allowing for a faster discovery of potential candidates in drug discovery, chemical synthesis, material science or bioengineering.

Self-Driving Laboratories to autonomously navigate the Protein Fitness Landscape

SDL technology has also been used to autonomously explore the protein landscape by learning sequence-function relationships and designing proteins to test hypotheses. Protein structures are sent to a lab environment which performs fully automated gene assembly, protein expression and biochemical characterisation. The AI agent refines its understanding, adjusts the design and tasks the lab environment to run the next experiment. It has no prior knowledge of the protein fitness landscape, and autonomously decides which path to take to find fitness peaks. The system must choose between exploring

uncharted areas of the landscape or exploiting its current knowledge to identify an optimal sequence, or employ another approach. For example, the agent can use an upper-confidence bound algorithm to evaluate sequences with the largest predictive mean and confidence interval. It is also important to model inactive sequences ("holes" in the fitness landscape).

Protein structures are sent to a lab environment which performs fully automated gene assembly, protein expression and biochemical characterisation.

This technology was employed to benchmark cytochrome P450 thermostability. This is important for the conversion of biomass into biofuels and other bioproducts: activity and tolerance to high temperature are critical parameters for industrial applications. An experimental pipeline ("SAMPLE") was designed, consisting of a DNA assembly unit, a PCR unit, a setup for cell-free protein expression, and a biochemical characterisation assay. The agent would design a protein, which SAMPLE would synthesise and test, taking approximately 8 hours. The platform can search a large and diverse protein space using combinatorial synthesis and pre-synthesised DNA fragments.

In a first ever fully autonomous bioengineering system, four independent SAMPLE agents were deployed to optimise glycoside hydrolase thermostability. Each agent autonomously and independently learned, made decisions, designed proteins and experimentally tested the design. Although using different search paths, and although searching less than 2 percent of the full landscape, all agents discovered sequences that were at least 12 degrees more stable than the initial natural sequences. Catalytic efficiency also increased. This experiment demonstrated that an autonomous bioengineering pipeline such as SAMPLE can drastically reduce the time needed for bioengineering, and exploit multi-agent coordination.

Take-home points

- SDL technology can autonomously explore the protein landscape and engineer proteins.
- It consists of an intelligent agent, that learns protein sequence-function relationships, designs new proteins and sends them to a laboratory environment that performs gene assembly, protein expression and biochemical characterisation before sending the data back to the agent for refining.
- The time for biological engineering can drastically be reduced with such autonomous bioengineering pipelines.

Simply put...

The engineering of proteins with desired functions is traditionally a time-consuming and laborious process. However, Self-Driving Laboratories are now able to autonomously engineer and optimise proteins. An intelligent agent learns sequence-function relationships. It explores possible sequences of proteins, predicts and analyses their function(s). This enables the identification of the "fittest" proteins – those with top performing sequences – allowing researchers to efficiently design and test new proteins.

AI for Vaccine Development

Another example for the power of AI is the development of vaccines against diseases with pandemic potential. Computational protein design was critical for the development and delivery of RNA vaccines during the COVID-19 pandemic. But it still took 384 days to bring the vaccine into use. To prepare for the next pandemic, this time should be reduced to around 100 days. To this end machine learning will be crucial. An important concept to find immunogen candidates involves stabilising the prefusion conformation of proteins rather than the post-fusion stage. Antibodies against the post-fusion conformation would be ineffective as the prefusion conformation is the biologically relevant form to bind to cellular receptors. A suit of tools is used to predict protein structures. These predictions are then used to model the prefusion stabilisation, employing yet another suit of modelling tools. The final stage involves filtering, scoring, epitope-focussing and other strategies as well as antibody and *de novo* binder designs.

An important concept to find immunogen candidates involves stabilising the prefusion conformation of proteins rather than the post-fusion stage.

The objective is to implement a computational vaccine design pipeline. The first such pipeline has been designed and tested. It was developed for Arenaviruses, which include Lassa virus, Junin virus, Machupo virus, Guanarito virus, and Lymphocytic choriomeningitis virus. The full experimental structure of the virus envelope glycoprotein (GPC) and their antibody structure are available for only a few of them.

The pipeline was developed to:

- Predict the structures of the viral glycoproteins
- Design proteins to identify mutations that potentially stabilise the prefusion structure
- Redesign the interface to select the best mutations/combinations based on a total energy metric
- Integrate deep-learning tools in a Rosetta framework to use different tools side by side
- Experimentally validate the prefusion stabilisation.

When comparing AI based protocols and biophysical modelling techniques, AI tended to lead to higher polarity which may link to better solubility; AI was highly efficient in sampling the design space for relevant sequences and outperformed traditional methods; for scoring, however, biophysical modelling approaches still have an advantage. Whether AI methods can expand predictions beyond the observed protein structure space has yet to be assessed, and work on *de novo* mini binders has only begun.

A next challenge will be to improve, test and validate the pipeline on vaccine designs for Paramyxoviruses. The approach remains work in progress. Some of the tools used in the suite were underperforming, and the integration of AI and biophysical methods needs more work. However, computational structure-based design is expected to become an important aspect of vaccine design.

Take-home points

- To prepare for future pandemics, efforts focus on shortening vaccine development timelines to just 100 days with the help of machine learning.
- The researchers established a computational vaccine design pipeline to find appropriate immunogen candidates based on a variety of AI tools that predict the structure of proteins and model the prefusion stabilisation with a final step that involves filtering and scoring.
- The pipeline on vaccine designs has already been tested for Arenaviruses and the next challenge is to expand it to other viruses such as the Paramyxoviruses.

Simply put...

Computational vaccine design, with the integration of artificial intelligence tools, facilitates a faster identification of potential vaccine candidates. In vaccine design, researchers focus on finding and optimising the viral protein of interest (such as the spike protein in SARS-CoV-2) that triggers an immune response. They try to stabilise a conformation of this protein before it binds to a receptor, as this is the phase in which infection can be inhibited. Different computational tools exist for the prediction of protein structures and the modelling of stabilising conformations.

Manufacturing Chemicals

A number of innovative technologies for chemical manufacturing were discussed at previous Spiez CONVERGENCE conferences. This time, protein catalyst engineering, click chemistry and electrochemical synthesis were on the agenda.

Biocatalysis

Enzymes are used to make active pharmaceutical ingredients (APIs). They can reduce the number of necessary reaction steps, minimise functionalities that do not contribute to the physiological activity, minimise the use of solvent/water, and optimise the yield.

Biocatalysts can be isolated from nature, produced artificially, but also engineered. They have applications in drug synthesis, re- and upcycling, the synthesis of chemical products, and the design of enzyme cascades. To accelerate the engineering of enzymes with desired functionalities, accurate property prediction of mutational evolutionary paths is essential. It is difficult to predict which mutations are stabilising (improve fitness), but it is possible to predict destabilising (deleterious) mutations. LibGENiE is a publicly accessible tool that designs an optimised oligonucleotide library to be used in enzyme engineering experiments. This can be employed to reduce the enzyme sequence space to be explored for enzyme optimisation, and to design smart libraries enriched with custom information. Libraries constructed from oligo-pools that avoid destabilising mutations drastically reduce the number of evolutionary rounds needed to optimise the design of engineered biocatalysts with a desired profile.

Libraries constructed from oligo-pools that avoid destabilising mutations drastically reduce the number of evolutionary rounds needed to optimise the design of engineered biocatalysts with a desired profile.

This approach was used to engineer a ketoreductase (KRED). KREDs are used in the synthesis of a number of APIs, including a precursor of the experimental drug Ipatasertib, which targets certain types of breast and prostate cancers. A first step was a search for a KRED that catalyses a specific reaction step needed for the Ipatasertib synthesis. Several in-house and external sources were screened to select a candidate, and a KRED from *Sporidiobolus salmonicolor* exhibited promising conversion rates and stereoselectivity. This candidate was engineered using mutational scanning, substrate modelling and hot spot engineering. Several combinatorial libraries were constructed to generate a rich data set consisting of sequence and activity data for machine learning.

Using filtered libraries, the number of variants was reduced from 29,000 to 75. Catalytic conversion was increased by iterative mutagenesis on residues in or close to the active site of the enzyme. The conversion rates and product purity of hit variants were assessed; the substrate turnover of the wild type was 27 % and that of the variant 99 % or higher. This research showed that *in silico* predictions can "filter" a vast sequence space to increase screening speed and precision.

Engineered biocatalysts have a great potential for beneficial applications, but they could also be employed to manufacture known CW agents. However, the precursors required would be similar to those already controlled, and expensive protein engineering would be required. Biocatalysts could also be employed to increase the toxicity of known toxins. But many toxins are already extremely toxic, stable, and easy to produce. Protein engineering *might* be a tool to obfuscate the use of a toxin, by predicting sequences that fold into the same three-dimensional shape and with a similar or higher activity. Therefore, positive identification of a toxin should always include a determination of activity, sequence and structure.

Take-home points

- Biocatalysts, whether isolated from nature or engineered, are used to synthesise active pharmaceutical ingredients with fewer reaction steps and reduced waste.
- Biocatalysts have wide applications in drug synthesis, re-and upcycling, the chemical synthesis and the design of enzyme cascades.
- Bioinformatic tools, like LibGENiE, which filter out destabilising mutations, can accelerate the engineering of enzymes with desired functionalities.
- Enzyme engineering is advancing, due to in silico predictions that enable the filtering of sequence space, leading to faster and more targeted screening.
- Protein engineering *could* be misused to enhance or obfuscate toxins. Therefore, to accurately identify a toxin, not only sequence, but also activity and structure should be assessed.

Simply put...

Biocatalysts are natural or engineered enzymes that are able to accelerate (catalyse) chemical reactions. Compared to traditional chemical synthesis, biocatalysis can have advantages in selectivity, efficiency and sustainability. On an industrial scale, they have huge potential to synthesise bioactive and complex molecules. Researchers aim to expand the scope of natural accessible enzymes by designing and engineering new ones with desired properties. This discovery of new enzymes is accelerated by tools and advances in areas like bioinformatics or protein engineering.

Click Chemistry

Another technology discussed was click chemistry – a chemical synthesis where molecular building blocks snap together quickly and efficiently – and its application in biology. Click reactions must be modular, insensitive to solvent parameters, give high yields, be insensitive towards oxygen and water, regiospecific and stereospecific, and have a large thermodynamic driving force favouring a single reaction product (spring-loaded).

A first such reaction was the Cu(I) catalysed acid alkyne cycloaddition, which at room temperature readily forms triazole rings. It is completely regioselective. Triazoles mimic amide bonds and can be used to synthesise molecules such as glycopeptide-like constructs. However, this reaction requires a catalytic amount of Cu(I), and some products are explosive, posing challenges for safe handling and storage. This reaction as well as reactions using other nanometal catalysts are used in drug discovery, material science, polymer science, biomedical and biochemical research, and biophysical research.

Click chemistry – a chemical synthesis where molecular building blocks snap together quickly and efficiently

To be used in living systems, however, the reactions must be metal-free. Also, reactive groups must be non-native and non-perturbing; they must combine selectively, spontaneously and rapidly with each other; and they must not react with abundantly available functional groups of other biomolecules. But why use such reactions in living organisms? For example, to "monitor" biological processes: biomolecules of interest such as certain proteins are tagged. These "reporters" are subsequently conjugated with functional probes.

Click chemistry is being used as a diagnostic tool for cancer imaging or as targeted release strategy. Antibody-drug conjugates are designed to target specific receptors of cancer cells where a payload is released in high local concentration, causing cell death in the tumour. Antibody conjugates also allow pre-targeting and time-controlled release of drugs *in vivo*.

An example is the delivery of the anticancer drug doxorubicin in HeLa cells. Drug and antibody are covalently linked using trans-cyclooctenes (TCOs) and tetrazine. The antibody moiety enables pre-targeting whilst the click reaction causes the drug release. Usually, TCOs synthesis is tedious and difficult to scale, but a scalable continuous aqueous extraction process has been devised to make a wide range of TCOs.

Click chemistry has grown tremendously over the past 20 years, from pure chemical applications to *in vitro* and *in vivo* applications. Chemical reporter strategies have enhanced the understanding of biochemical processes, and clinical applications are used for drug targeting with antibodies, cell-penetrating peptides, activity-based probes, covalent inhibitors and more.

Take-home points

- Click chemistry involves chemical reactions, where molecular building blocks snap together efficiently.
- These reactions have valuable applications in different fields including drug discovery, material and polymer science, and biomedicine.
- Click chemistry can also be used in living organisms, such as in targeted release strategies or in diagnostic tools. However, the reactions need to be metal-free.
- Over the past two decades, click chemistry has grown significantly, moving from pure chemical applications to *in vitro* and *in vivo* applications, such as pre-targeting or controlled timing of drugs via *in vivo* click-release.

Simply put...

Click chemistry exists already as a concept for more than twenty years. It includes chemical reactions, where molecules are synthesised from building blocks that easily click together. Researchers sought to find a faster, more efficient and sustainable way for synthesising complex molecules. Several click reactions have since been developed. In addition to its use in chemical applications, click chemistry has found its way into living systems, where it can be used for various purposes.

Electrosynthesis

A final theme was electrosynthesis – a manufacturing strategy that supports the move towards green energy: the time-supply pattern of regenerative energy sources does not match the demand pattern. Hence, systems for efficient energy storage ("buffer systems") are needed, and a "power-to-chemistry®" approach can use energy during high supply times to synthesise fertilisers or other chemicals, or act as energy carrier. In this way, the decarbonisation of electricity production can at the same time enable industrial defossilation.

Electrosynthesis involves the addition (cathode) or removal (anode) of electrons in chemical bonds.

Electrosynthesis involves the addition (cathode) or removal (anode) of electrons in chemical bonds. It is inherently safe, saves metals and rare elements/resources, produces less waste, is reactively power adjustable, opens up new synthetic approaches and supports the green transformation.

In order of increasing selectivity and decreasing downstream processing requirements, applications include the manufacturing of synthetic fuels (hydrogen, methane, methanol, CO/hydrogen, higher alcohols), commodities (organic intermediates and precursors), and fine chemicals including fragrances, building blocks for pharmaceutical applications, active pharmaceutical ingredients (APIs), dyes and more. Examples of industrial electrochemical processes include the production of fragrances and certain medicines.

To optimise the processes, a wide range of materials for electrodes and electrolytes are being screened, cell designs vary between divided and undivided cells, and machine learning is employed to optimise process parameters.

Electrosynthesis can be employed to remediate contaminated soils. An example is the treatment of residues and soils contaminated with the insecticide Lindane, an isomer of hexachlorocyclohexane (HCH).

An example for the successful application of electrosynthesis is the Negishi cross-coupling reaction, a process widely used to stitch together two organic groups to make complex molecules. Products range from antibiotics to LEDs. A range of other interesting products have been synthesised by electrochemistry, such as 1*H*-1-hydroxyquinazoline-4-ones (a group that includes methaqualone and the cancer treatment Idelalisib), using carbon electrodes under sustainable electrolyte conditions with high yields. Another example are 4*H*-4-Hydroxybenzo[e]-1,2,4-thiadiazin-1,1-dioxide derivatives, some of which have potential as diagnostics or pharmaceutical drugs. 44 structures have been synthesised, the reactions are selective and high in yield, use metal free carbon electrodes, conduct simple constant electrolysis, are highly callable and use easy work-up protocols. Other product types include biologically active hydroxamic acids derived from biological feedstock, otherwise only accessible through complex extraction from biological sources. They have herbicidal, fungicidal and antibiotic properties. Another example is the electrochemical synthesis of periodate – a most versatile oxidiser in organic synthesis. Periodate can be used for levetiracetam (a treatment of epilepsy) synthesis.

Electrosynthesis can be employed in shuttle reactions (where a chemical entity of a donor molecule is transferred to an acceptor molecule). It can also be employed to remediate contaminated soils. An example is the treatment of residues and soils contaminated with the insecticide Lindane, an isomer of hexachlorocyclohexane (HCH). Globally, some 7 million tonnes of HCH waste remain in deposits such as landfills and will eventually need to be treated. Electrosynthesis offers an approach to upcycle the halogens contained in such materials.

In addition to applications of electrosynthesis, another project was presented, which was investigating the development of explosives simulants for the development of artificial noses. An explosive that is easy to make from common household chemicals is triacetone triperoxide (TATP). It is extremely sensitive to shock, friction and electrostatic ignition. It has a history of use in asymmetric threats and can result in high numbers of fatalities and severe injuries. Its characteristics pose challenges with regard to the treatment of TATP findings, which can lead to blast events, and destroy forensic evidence. TATP is difficult to handle for training purposes (sniffer dogs) or the calibration of instrumental detectors. But it dissolves in ionic solvents which are accessible through electrosynthesis. This effect can be used to prepare easy-to-handle pads that can be stored at ambient temperature. They are not sensitive to impact or friction, and safely simulate the olfactory properties of real improvised explosive devices (IEDs). The solubilising effect of ionic liquids in dichloromethane can also be used to desensitise and sample TATP findings; material so treated can be stored safely for several months and retains the full analytical response needed for forensics.

Take-home points

- Electrosynthesis, which involves the transfer of electrons (addition or removal) in chemical bonds, is considered a safe, waste-free, and green manufacturing strategy.
- Its applications range from the manufacturing of synthetic fuels and organic intermediates to fine chemicals like fragrances and dyes.
- A key example is the Negishi cross-coupling reaction, which enables the synthesis of complex molecules. Other products, such as biologically active hydroxamic acids or products which have potential as pharmaceutical drugs or diagnostics, have been synthesised electrochemically.
- Electrochemistry enables soil remediation such as treating soils contaminated with insecticides.
- Ionic liquids can dissolve explosives like triacetone triperoxide (TATP), offering safer methods for training (sniffer dogs) and detector calibration.

Simply put...

In electrosynthesis, an alternative to traditional chemical synthesis, compounds are synthesised with the use of electricity. Chemical reactions take place in an electrochemical cell containing usually two electrodes, one anode (loss of electrons) and one cathode (gain of electrons). Researchers can optimise reaction conditions to favour the production of a desired product. Electrosynthesis can be deployed at both laboratory and industrial scale.

Therapeutic Applications and Drug Delivery

Under this topic, novel approaches of targeted delivery of therapeutics and other advances in delivery were presented. Novel delivery methods allow to personalise the treatment and to target therapeutics to specific organs or cell types, which increases the therapeutic window and consequently reduces side effects. It is also relevant for engineering ways for toxic agents to cross biological barriers and reach their targets in the body.

3D Printing of Personalised Medicines

A technology used to personalise medicine uptake is 3D printing. It allows to deliver a dose calibrated to a particular patient's condition and therapeutic needs. Pills or capsules are manufactured at the hospital or a pharmacy close to the patient.

Standard medication is mass-manufactured efficiently at standardised doses. Yet the therapeutic dose that a particular patient actually needs varies from person to person, depending on a range of factors including age, gender, ethnicity, co-morbidities, body constitution, and other medicines that the patient takes. Accurate dose application is a challenge, and it has been estimated that 40 to 70 % of treatments are ineffective because of wrong dose application. A move towards personalised dosage would increase efficacy of treatments, decrease side effects and save costs.

Printlets allow flexible dosing, a combination of different drugs in the same printlet, the use of patient-centred designs and the preparation of unique dosage forms.

3D printing has been used in a variety of medical applications, including tissue engineering, the manufacturing of medical devices and prosthetics, in dentistry and for the manufacturing of printed tablets (printlets). Printlets allow flexible dosing, a combination of different drugs in the same printlet, the use of patient-centred designs and the preparation of unique dosage forms. Production can be automated, it is environmentally friendly, and it allows faster and cheaper batch manufacture in clinical trials. It can also synchronise identification of the therapeutical need, drug prescription, 3D printing of the medicine in personalised form, administration, and reassessment of the therapeutical needs. Such a loop requires software integration, the availability of pharma 3D printing software, printers and pharma ink, and diagnostics to monitor the drug concentration or another marker of treatment.

This concept has been tested successfully in the treatment of Maple Syrup Urine Disease (MSUD), a rare but serious inherited condition where the body cannot process certain amino acids. 3D printers were employed in a hospital setting to treat paediatric patients with personalised isoleucine dosages. Both compounded capsules and printlets were administered, and dose tailoring as well as patient acceptability were tested. 3D printed formulations were found to allow a tighter control within the target range.

Other applications of 3D printing are at the stage of pre-clinical and first-in-human trials. A wide range of 3D printing technologies are available today. Stereolithography combined with Vat polymerisation 3D printing as well as semisolid material extrusion were used in a trial to print chewable tablets for children with a rare metabolic disease. A GMP-compliant single-printhead 3D printer was developed that allows printlet production in a hospital environment in accordance with industry quality standards. A follow-up model uses a multi-nozzle printhead, allowing for higher throughput and greater automation. The printer uses FDA approved materials and proprietary software.

The regulatory framework for 3D printing at points of care or pharmacies is evolving, with regulations coming into effect in the UK, the EU and the USA. 3D printing of drugs is under consideration for veterinary use, clinical and pre-clinical studies, formulation optimisation, use in disaster response and emergency care, and even for space missions.

Take-home points

- There is a shift towards personalised medicine tailored to individual patients, which increases treatment effectiveness and reduces side effects.
- In addition to applications, such as tissue engineering and medical devices, 3D printing technology allows the manufacturing of tablets with personalised doses for patients. This offers advantages such as precise dosing, the combination of different drugs in one tablet, specification of design and preparation of unique dosage forms.
- 3D printers are already on the market, proving useful in hospitals close to the patient. They can be automated and require special software and pharmaceutical inks.
- The regulatory framework for 3D printing in healthcare is evolving.

Simply put...

It is already possible to use the 3D printing technology to tailor the dosage of drugs for individual patients which offers better control of the treatment. A pharmaceutical 3D printer equipped with a special software can rapidly design and print new personalised drugs using pharmaceutical inks. This leads to a shift from mass production to a decentralised, personalised production of medicine.

Engineered Bacteria for Cancer Therapy

Another technology discussed was a platform for bacterial anticancer therapy. Bacterial cancer therapy itself is not new. Bacillus Calmette-Guérin (BCG) therapy, for example, is used to treat certain bladder cancers. New is that genetically engineered bacteria are able to target tumours as well as deliver proteins. *Yersinia enterocolitica* has been developed as a vehicle for such treatments. This Gram-negative bacterium can cause mild gastroenteritis. Key to its virulence are bacterial proteins (effectors) that the bacterium injects through its Type 3 (T3) secretion system directly into the cell cytoplasm. This mechanism does not depend on specific receptors for interaction/uptake.

Y. enterocolitica is able to grow in a tumour environment, but is cleared everywhere else in the organism. Using virulence attenuated strains, which have been engineered to secrete proteins with anti-tumour activity, treatment of mice with tumours has been shown to be effective. Tumours are also colonised when bacteria are injected in one of two tumours in the same mouse. After four days, this single injection had resulted in the colonisation of the second, non-injected tumour.

The bacterium's T3 system can be used for intracellular delivery of bioactive (including human) proteins.

The bacterium's T3 system can be used for intracellular delivery of bioactive (including human) proteins. A bacterial signal sequence is linked to a protein or a part thereof (from peptides up to structures of 100,000 Da or larger), and injected through the T3 secretion system into human cells. Multiple proteins can be delivered by one bacterial strain; the strains are easy to generate and a large number of strains with different cargo proteins have been engineered.

Apoptosis (a form of programmed cell death) can be induced in eukaryotic cells by the bacterial delivery of certain proteins. This has limitations for the induction of an immune response against solid tumours. However, the platform technology holds significant promise in targeting multiple pathways engaging regulated cell death mechanisms to overcome these limitations.

Another cargo induces the production of Type I interferons (type-I INF). Type-1 INF have antiviral and immunostimulatory effects, and play an essential part in natural cancer immunosurveillance and response. The utility of the platform in cancer treatment was demonstrated in mouse cancer models using repeated intratumoral injections of different cargos prompting a type-I INF response, which resulted in suppression of tumour growth and in more than half of the cases tumour regression.

These preclinical tests have demonstrated the potential of using attenuated *Y. enterocolitica* as a platform for targeted delivery of therapeutic payloads to solid tumours. The engineered bacteria are cleared rapidly in most tissues but enrich in tumours and can be equipped with multiple payloads. In cancer cells, this mechanism can be used to induce cell death or cytokine release. The induction of a type-I INF response upholds the promise of being a potent way for cancer treatment and protecting from systemic metastases.

The utility of the platform in cancer treatment was demonstrated in mouse cancer models using repeated intratumoral injections of different cargos prompting a type-I INF response, which resulted in suppression of tumour growth and in more than half of the cases tumour regression.

Take-home points

- *Yersinia enterocolitica,* a bacterium causing mild gastroenteritis, has been engineered as a tool for targeted delivery of payloads to solid tumours through its Type 3 secretion system, functioning like a nano-syringe.
- When injected into mice, these attenuated engineered bacteria enrich in tumours but are rapidly eliminated everywhere else in the animal.
- The engineered bacteria can deliver single or multiple proteins (including human proteins) and can be equipped with various cargos capable of modulating eukaryotic cell biology, such as inducing anti-tumours immunity or cell death.

Simply put...

In cancer therapy, live bacteria can be engineered by removing their virulence factors making them a safe tool for the targeted delivery of bioactive proteins. Certain bacteria possess a secretion system that looks like a nano-syringe, which can be hijacked to deliver chosen proteins to the targeted cell. Here, the bacteria have been engineered in a way to target only tumours and not healthy cells, where they are eliminated relatively quickly from the body. This technology reveals new potential in cancer therapy.

DNA Origami

Another technology with growing application in biology is DNA origami. It was first discussed at Spiez CONVERGENCE 2016. Initially somewhat of a curiosity and at an exploratory stage, it was revisited at Spiez CONVERGENCE 2018, when its potential for targeted delivery of therapeutic (or toxic) cargoes had become clearer.

DNA can self-assemble to form nanoparticles. DNA origami relies on folding a long single-stranded (ss) DNA (the "scaffold") with hundreds of short ssDNAs (the "staples") using the base pairing mechanism of DNA. Staples can be designed to have multiple binding domains that bring together matching, otherwise distant domains of the scaffold. As a result, the scaffold folds in a user-defined manner.

Triangular DNA origami building blocks assemble into shells with user-defined geometries and apertures, which can engulf hepatitis B virus core particles and adeno-associated viruses.

In previous years, the focus of developing DNA origami has been on shape – designing nanomachines and sensors, embedding nanopores, creating frames for imaging, or building templates for synthesis. However, DNA is versatile – its surface can be modified and biological instructions can be encoded in it. A recent example for how these properties can be used was the development of a programmable virus trap. Triangular DNA origami building blocks assemble into shells with user-defined geometries and apertures, which can engulf hepatitis B virus core particles and adeno-associated viruses.

To develop antiviral drugs, a promising concept is the blockage of virus entry into host cells. Viruses are large and multivalent, and hence completely blocking their cell entry is difficult with small molecules. DNA origami shells can block virus-cell interactions and reduce viral load. The virus geometry is used as a guide to design pseudo-triangular DNA subunits that are similar to a protein subunit of a viral capsid. These subunits self-assemble into an icosahedral shell with the correct size and shape to encapsulate the virus. Other subunits form other geometries with different inner diameters.

These shells can be modified by introducing virus-binding moieties on their inside. For proof-of-concept experiments, such modifications were engineered using antibodies with lysines. The antibodies attached to the inner surfaces of the shells. Experiments with hepatitis B core particles demonstrated the ability of these shells to encapsulate the virus particles.

DNA origami objects can also be modified to encode biological instructions. Traditionally, scaffolds encodes for the M13 bacteriophage genome. The DNA sequence was of no importance as the objective was to fold DNA into user-defined shapes. To engineer DNA origami that encode for mammalian genes, the process of producing ssDNA scaffolds needs to be adjusted. This can be done in *E. coli* engineered to produce M13 phage ssDNA, but that limits the variable sequence space to around 2,000 base pairs. Splitting crucial M13 sequences between a helper plasmid and a phagemid increases this number to around 8,000. This allows to produce ssDNA scaffolds which are gene-encoded, and which can then be folded into structures that expose these genes in defined locations. These constructs can be inserted into cells by electroporation. It has yet to be understood, however, which design factors and scaffold or staple features influence gene expression.

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There are also suggestions to employ DNA origami for gene delivery in gene therapy. This technique faces efficiency issues for the transfer of foreign DNA/RNA into host cells, especially for large segments or when multiple segments need to be delivered simultaneously and the stoichiometry cannot be controlled. DNA origami can be folded to form a kind of puzzle, where DNA subunits can only attach to one another in a predefined configuration. This allows to control the stoichiometry of the gene expression.

A nuclear localisation signal can also be encoded in the DNA origami structure. The cell nucleus holds the cell's DNA blueprint, so all material inputs and exports need to be tightly controlled to protect cell integrity. That control is momentarily broken during cell division, and DNA origami nanoparticles can be incorporated into the nucleus using DNA nuclear targeting sequences (DTS). This has been demonstrated with the incorporation of mCherry encoding sequences into the cell nucleus (resulting in red fluorescence) using DTS.

DNA origami is quickly becoming a tool that can be used across many disciplines. It gives control over properties such as nanoparticle size and shape, and allows encoding for biological function and localisation. It can be used for the engineering of cell surfaces and interactions, the construction of DNA-based cytoskeletons in synthetic cells, the organisation of gene editing tools, genome integration, the development of DNA and RNA vaccines and chemotherapeutics, the construction of drug delivery systems that respond to stimuli such as pH change, the construction of logical gates, and the manufacturing of DNA hydrogels.

There still remain many unknowns when using DNA origami in cellular or *in vivo* systems:

- How long does DNA origami remain folded in cells, when does it denature and/or degrade, and can this be modulated and engineered?
- How does transcription from DNA origami occur, and can this be used to control or limit gene delivery for higher specificity?
- How do protein interactions change depending on the delivery method, and can protein interactions with the DNA sequences be used as "smart" delivery systems?

And what about RNA origami? RNA being more "flamboyant" than DNA, how does the 3D structure of RNA confer functionality and activity? Whatever the answers, the field of nuclear acid origami is certain to bring about more surprises and lead to practical applications.

Take-home points

- DNA origami is the nanoscale folding of a long single-stranded DNA (scaffold) with hundreds of designed short ssDNAs (staples), which bring the scaffold into a user-defined shape.
- DNA origami allows to control the size and shape of DNA nanoparticles, as demonstrated by their ability to self-assemble into DNA origami shells capable of encapsulating viruses.
- The surface of DNA can be modified and an example is the incorporation of antibodies to the inner surface of DNA origami shells to capture viruses inside.
- It is also possible to encode biological instructions into DNA origami; such as the encoding for mammalian genes or for nuclear localisation.
- DNA origami is a tool that can be used across many disciplines but there are still unknowns using DNA origami in cellular or *in vivo* systems.

Simply put...

DNA origami is the self-assembly of DNA into complex 3D structures. It is possible to control the size and shape of DNA origami objects, enabling the construction of many different DNA nanostructures. DNA origami has potential in a broad range of biotechnological applications such as in drug delivery or in biosensing. More recent developments include applications such as trapping viruses or encoding genes.

Threat-Agnostic Biodefence

Despite the progress discussed at Spiez CONVERGENCE 2024, there remains a fundamental challenge: biodefence builds on a gold standard (microbial culture and serial testing) that is slow and requires pre-existing knowledge of likely causes of an infection. Testing typically follows a "rule out" paradigm that eliminate causes of a disease one after another, and treatments are often based on symptoms rather than identification of the agent.

This paradigm failed at the start of the COVID-19 pandemic, and it will fail again with another emerging disease, or an engineered threat agent. Diagnostic tests rely on list-based approaches, which is retrospective. So is sequencing – it provides information about the potential of an organism, but not its activity at a point in time. Traits like pathogenicity and virulence are encoded by genome sequences, but realised by gene expression. Responding to emerging or engineered pathogens would benefit from robust threat-agnostic methods.

Such methods would need to be able to:

- Detect any known or previously unknown harmful microbe or biotoxin
- Classify an unknown, emerging, or engineered organism or biotoxin as harmful without knowing its name or sequence
- Characterise any sample, without any pre-existing information, and collect information on what it does, rather than having to cross-reference it to things seen before.

Such an approach is now becoming possible. Although the number of harmful microbes and biotoxins is potentially infinite, the number of ways they can infect or harm, and the number of ways a host can respond, are finite. If these pathways are characterised, it should be possible to determine the ability of some new material to cause harm. To this end, it is important to recall that stable biological systems are finely tuned organisms, and effective biological pathways have evolved to be highly conserved. To disrupt this equilibrium, pathogens and biotoxins only have a limited number of options. Building a diagnostic approach on that concept entails a move from serial testing and microbial culture to observing pathways. This is becoming possible thanks to significant advances in measurement and data analysis.

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A first challenge is to differentiate bacterial pathogens raised in a lab from those obtained in nature. After the analysis of hundreds of lab-adapted strains from repositories and wild-type strains of *Francisella tularensis* (rabbit fever), *Burkholderia pseudomallei* (glanders) and *Bacillus anthracis* (anthrax), remarkable differences in their function were characterised by proteomics despite significant DNA sequence similarity among them, indicating optimisation for their environment. For example, wild-type strains metabolise a large number of soil nutrients, while their lab-raised counterparts cannot. But the lab-raised counterparts survived better in anaerobic conditions. These patterns were consistent across different pathogens. By aggregating the protein expression data, a machine learning algorithm was able to differentiate wild-type from lab-adapted strains.

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Another example was a multi-omics analysis of time-series blood samples from Ebola patients in Sierra Leone, compared to samples of healthy individuals. This study identified markers present early in the infection that could explain why some patients did not recover whilst others did. Even at very early stages, these individuals showed neutrophil death—a sign of a dysregulated immune response—and leakage of pancreatic enzymes. Even a few days after infection, signatures in these samples showed significant similarity to signatures of sepsis – an example for pathogenicity as a function of the host response.

A first step in a threat-agnostic approach is to determine whether an agent is a pathogen. Virulence factors can be used as indicators. They:

- Enable adherence of a pathogen to the host cell – adhesins enabling biofilm formation (e.g., quorum sensing, interkingdom signalling, pili/fimbriae/fibrillae), or Glycan-binding proteins (e.g., hemagglutinin, spike, HIV gp120)
- Enable invasion of the host cells, such as the T3 system, nutritional scavengers (e.g., siderophores for iron, neuraminidase for carbohydrates), or local spread factors (neuraminidase)
- Increase severity, such as efflux pumps (e.g., to remove antibiotics from the bacterium cell to convey antibiotic resistance), toxin formation, or immune system evasion.

These virulence factors are conserved across pathogens. Whilst non-pathogenic organisms, too, carry the genes for these factors, they express fewer of them. The expression of multiple virulence factors contributes to pathogenicity and can be used as an indicator of pathogenicity.

The genes for these factors can be determined by DNA but some virulence factors require the presence of multiple genes, which may require long-read or whole-genome sequencing. In any case, gene sequencing determines capability but not activity.

Mass spectrometry-based omics can be used to directly detect the presence of virulence factors by measuring proteins or metabolites. These methods require multi-step preparation methods, and hundreds to thousands of molecules for confident detection.

The most sensitive assays are activity-based probes, which can be used directly to detect virulence factors. They require hundreds of microbes, and the presence of host cells to enable virus activity. These assays can also be used to enrich samples, resulting in sensitivities comparable to sequencing.

A next question is whether an agent is a biotoxin. To answer this, one needs to consider the way in which toxins impact host cells. Amongst others, toxins can disrupt ion channels, disrupt cell signalling, or inactivate protein synthesis. Here again, sequencing is useful but will indicate capability, not activity. Mass spectrometry can directly detect the toxin, but requires multistep workup and the presence of thousands of molecules. Activity-based assays are highly sensitive and can directly measure toxin activity (this may require the presence of host cells).

Amongst others, toxins can disrupt ion channels, disrupt cell signalling, or inactivate protein synthesis.

The final question is to determine how harmful the agent is. This is best done by functional assays that mimic host responses, such as organs-on-chip, cell-based functional assays, or component-based functional assays.

Organs on chips are three-dimensional representations of mammalian organs. They have several potential advantages as diagnostics because they may provide a better representation of a mammalian system than simple culture. However, they are often made from immortalised cell lines (cancerous cells or cells manipulated to be difficult to kill) so questions remain about whether they are capable of recapitulating a mammalian organ and, more importantly, what that actually means. Also, harvesting an organ-on-chip yields a very small sample (a few thousand proteins).

The alternative is to use mammalian cell co-cultures. They can be pooled to get larger volumes. Co-cultures approximate tissues in two dimensions, and are commonly used for functional assays to assess the impact of a pathogen or toxin on a mammalian cell system. A machine learning algorithm can be deployed to identify pathogenic mechanisms such as nutritional scavenging, disruption of the host cell metabolism, or programmed cell death.

Even when using co-cultures, the volumes are very small, which challenges detection limits. But there are ways to isolate or concentrate pathogens in samples prior to inoculation. These methods typically involve small molecules called activity-based probes which can detect virulence factors. They allow to identify cells with pathogenic activity present. If these cells can be grown in culture, a better product is achieved for analysis. But even if not, this approach still allows to eliminate the parts of the sample that are not of interest, thereby increasing analytical sensitivity.

When these enriched samples are inoculated into mammalian cell cultures, information on whether the sample is harmful or not can be gained, for example by using imaging techniques that make visible post-infection cell reactions such as cell deformations, compressed nuclei, or loss of cell adherence. In a recent experiment, a combinatorial evaluation of virulence factors and cell response correctly identified 30 out of 30 pathogens present in soil samples to genus and species level, without sequencing. In another experiment, permafrost soil samples were analysed, some of which contain bacteria that exhibit pathogenic signatures but do not have DNA sequences that correspond well to data in current databases.

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An alternative to culture-based assay is the use of what can be called component-based functional assays. This technique is still in an early stage, and tries to recreate the chemistry behind specific virulence factors or host cell responses in a cell-free format. An example is a device constructed by peeling the membranes off cells and placing them on transparent conducting polymers. When a toxin interferes with the immobilised membrane, a change in impedance occurs which can be measured. Such assays are shelf-stable and do not require human maintenance.

Approaches towards threat-agnostic biodefence are promising, although more research and development is necessary. The approach is multidisciplinary and will require collaboration across the relevant sciences and enabling technologies. More expertise is needed in identifying commonalities in pathogenic pathways; rapid functional assays need to be developed and validated; work is needed to develop bottom-up, standards-free identification and characterisation methods for proteins and metabolites; the tremendous progress in computation and analytics needs to be exploited; and a better understanding of the uncertainties underlying the entire process is needed. With high throughput functional analysis and standards-free identification and quantification, however, it should be possible to develop confident classifications, even for pathogens and toxins hitherto unseen.

Take-home points

- Current biodefence strategies rely on slow microbial culture and serial testing and require prior knowledge of likely causes of an infection.
- At present, biological threats are defined by lists or sequence data, but those methods are retrospective and can only identify known organisms or biotoxins.
- While sequencing informs about the potential of the organism, it does not capture activity at a point in time.
- It is possible to differentiate lab-adapted bacterial pathogens from those that are naturally occurring, despite similar DNA sequences, by looking at their function – the expression of proteins measured by proteomics.
- Threat-agnostic biodefence is going beyond name, sequence or list-based definitions of threats and will be robust for unknown, emerging or engineered pathogens.
- Threat-agnostic methods include questions whether an agent is a pathogen or a biotoxin and how harmful the agent is.
    - The number of ways a pathogen or a biotoxin can infect or impact a host is finite.
    - To identify an agent as a pathogen, it is possible to look at the presence of virulence factors and detection methods include sequencing, mass spectrometry-based omics; or functional assays that require the presence of host cells to enable virus activity.
    - To identify an agent as a biotoxin, it is possible to look how a toxin impacts its host and detection methods include sequencing, mass spectrometry and activity assays that may require the presence of host cells.
    - The final question looks at how harmful a pathogen or biotoxin is, by using assays that mimic host responses, such as organs-on-chips, cell-based functional assays or component-based functional assays.
- Organs-on-chips, three-dimensional representations of mammalian organs, are promising and actively researched, while cell-based functional assays using mammalian cell co-cultures can be easily run in multi-well formats and are already commonly used by researchers.
- Although component-based functional assays are in an early stage of development, they have great potential as they are shelf-stable and do not require human maintenance compared to cell-based assays.
- Threat-agnostic biodefence is a promising field but requires further research, development, expertise and collaboration.

Simply put...

A threat-agnostic approach aims to address and counter threats without having prior knowledge on them. Such an approach would enable the detection of unknown, emerging or engineered pathogens or biotoxins without relying on their identification but rather identifying communalities of pathogens. Currently, identifying a new, unknown organism is difficult. To be better prepared and react quickly in the event an unknown agent emerges, threat-agnostic methods are promising as they don't rely on lists to know what it is, but look at how harmful it is, e.g. by examining how a pathogen attacks its host.

Implications and Conclusions

What are the Major Trends

The participants observed again that AI/machine learning and enhanced computational power, combined with automation of research activity by using robotics and cloud labs, are enabling a more efficient and better targeted exploration of vast molecular spaces. The human researcher remains in the centre of the scientific process, but AI drastically helps to shorten the time needed to identify the most promising options and eliminate less successful pathways, thus increasing the success rate of research and development.

Researchers are combining Large Language Models with specialised bioinformatics machine learning tools to achieve more reliable predictions of chemical and biological mechanisms.

Search and optimisation loops are being automated and run faster. Researchers are combining Large Language Models with specialised bioinformatics machine learning tools to achieve more reliable predictions of chemical and biological mechanisms. Efforts are under way to develop concepts and methods that reach beyond list-based approaches that rely on past knowledge of which chemical or biological entities cause harm. Treatments that exploit innate immunity mechanisms, and functionality-based threat-agnostic pathogen and toxin identification methods are examples for complex multidisciplinary scientific approaches to overcome limitations in the response to chemical and biological threats.

The one-health concept requires new approaches for chemical and biological risk identification, assessment and mitigation. This will be important for strengthening a comprehensive approach towards health preparedness. At the same time, it should broaden the discussion around the norms governing chemical and biological arms control, which often overlook threats to animals and plants.

With regard to drug delivery, new methods to transport cargoes to specific targets in the human body are evolving, including methods that control release or help finer calibration of the dose. This allows better targeting of treatments and enables the personalisation of therapeutic interventions.

When it comes to manufacturing, the biological revolution is well underway. Biological processes and principles are being leveraged for the production of chemical and pharmaceutical products and complex functional materials. The ability of scientists to manipulate chemical structures in two and three dimensions, or to edit chemical and biological structures to create desired properties or functionalities, has gained in precision and predictability and is finding new applications. Research laboratories are becoming self-driving, designing, executing and evaluating experiments and optimising outcomes using digital and robotic systems under human supervision. In chemical synthesis, mechanisms that allow faster and more precisely controlled reactions or use alternative pathways are being explored.

The overriding sense of the conference was that science and technology is progressing generally in highly beneficial directions. This will also strengthen the defences against the malicious use of chemical and biological agents. Furthermore, as they move into wider application, scientific products and developments that cause concern will relate to tightly regulated markets and product cycles, mitigating the risks involved. Established actors in industry and research should be able to manage these risks. This may not be the case, however, with early-stage and small-scale endeavours (e.g., start-up companies and SMEs), who may lack the knowledge and capacity to implement necessary oversight frameworks.

At the same time, early stages of development must not be over-regulated – this would stifle innovation. Scientific freedom and open access are critical for good science, community building and collaborations, accountability, and integrity of the scientific enterprise. A balance needs to be struck between openness and regulatory control.

Are there Risks and Potential Game Changers

Many of the scientific and technological advances discussed in Spiez could possibly contribute to a growing risk potential: Gaps in understanding could pose biosafety risks (e.g., toxicity profile of novel proteins). Self-Driving Laboratories and other AI-enabled technologies raise concerns about accountability and incentives to apply appropriate governance mechanisms. Certain technologies may affect current (CWC) and possible future (BWC) verification systems and forensic capabilities (examples: epigenetic editing leaving virtually no traces, non-canonical amino acid technology being used to obfuscate toxin production). Techniques for designing, manufacturing and editing new chemical or biological entities could be misused for the development of novel agents or manufacturing processes.

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Current non-proliferation and arms control strategies reflect the threat from past programmes which is different from today's threat landscape. But science is not magic. Tools to design novel chemicals or biological entities, or new

manufacturing pathways, need to be experimentally validated, transferred into the real world of manufacturing, and compete against existing technologies and agents. In chemistry, there are proven ways to make comparatively cheap and effective warfare agents. In fact, recent CW uses involved agents known for more than a century. In biology, new creations have not yet evolved to survive and compete in an environment outside the laboratory; they may also be difficult to disseminate effectively. Dual-use risk assessments therefore need to remain clear-headed and avoid overhyping emerging capabilities.

When it comes to defences against toxic chemicals and pathogens, there were promising examples of generic approaches to diagnostics, detection and response such as threat-agnostic pathogen identification, vaccines that stimulate immune response, and the development of machine learning tools to speed up the time to develop and produce new vaccines or therapeutics. This reflects attempts to look beyond lists of known threat agents and instead focus on functionality – toxicity, pathogenicity, and also other properties such as ease of manufacturing, storage and dissemination, detectability and more. This mirrors both changing threat perceptions and the General-Purpose Criterion built into both the BWC and the CWC.

However, there remain gaps. Threats to plants and animals are often overlooked. There are societal thresholds to take certain countermeasures, gaps in funding and basic knowledge, and a lack of incentives for industry to manufacture products that have small profit margins or markets. It may be possible to exploit the BWC and CWC provisions on promoting international cooperation (Articles X and XI, respectively) to build stronger global capacity for disease management. It is also important to work on "old" challenges such as the mechanism of mustard agent poisoning or an effective nerve agent reactivator.

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There are other challenges: mis- and disinformation have become a major concern, undermining the trust in global regimes and institutions (e.g., disputes about chemical weapons uses and attribution), and in science (e.g., vaccine hesitancy). Scientists must play a role in countering these challenges, and more is needed to communicate effectively and in tailored ways with the public, with political leaders, and other actors.

Several technologies that deserve attention were not discussed this time, or only in passing: nanoparticles as drug delivery systems; methods to disseminate agents in operational environments, cell-free expression technology which has moved into large scale production; bioregulators combined with technologies that may make them more useful in the field and for functional enhancement of humans, RNA origami as a nascent technology with much potential; conjugation approaches to link proteins, viruses or other pathogenic/toxic material inside cells; systems biology. Concerns were also raised with regard to cybersecurity issues.

Spiez CONVERGENCE 2022 had concluded that AI and machine learning could become Game Changers, and that the combination of AI with synthetic biology, automation and robotics, Big Data, high-throughput synthesis and screening, leads to a context shift in how experiments are performed. Discussions at Spiez CONVERGENCE 2024 confirmed that AI was indeed lowering the bar and opening up new avenues. Its combined deployment together with other, more specialised machine learning and modelling tools and the connection of computerised design with the automation of experimental work and manufacturing is changing the science and technology landscape at the intersection of chemistry and biology.

What does all this mean for Arms Control

Fast scientific and technological advances at the intersection of chemistry and biology are expected to bring about vast benefits. They will make essential contributions to our responses to global warming, the need to secure adequate supplies of food and energy, and the threats to human, animal and plant health as well as biodiversity. They also strengthen countermeasures.

The very same advances also increase the potential for accidents occurring, or for misuse. Within the communities, awareness raising for how to assess and manage risks associated with fast-evolving technologies is essential. Regulators need to understand what these risks are and how they can best be mitigated – whether by regulation, or reliance on self-regulation in research and industry or awareness-raising, or (more likely) a combination of the three.

When it comes to nefarious uses of these advances for weapons purposes, it should first be understood that employing these scientific and technological tools is not "easy", nor is it imminent (testing, for example, remains a hurdle). There remains some time to reflect. The developments at the intersection of chemistry and biology are trans-disciplinary and require a systems approach to mitigate emerging risks. These emerging capabilities are beyond the reach of individual actors. Preventing malevolent use of these technologies for weapons purposes must remain focused on State actors and their intent. That requires further strengthening of the global CBW arms control framework. The CWC, the BWC and other non-proliferation initiatives/regimes, as well as national laws and regulations, create frameworks for managing these challenges. But treaties alone will not suffice, and are slow in response to the still-accelerating pace of science and technology. Even policies – more pliable than laws and treaties – nearly always lag behind technological progress.

Regulators need to understand what these risks are and how they can best be mitigated – whether by regulation, or reliance on self-regulation in research and industry or awareness-raising, or (more likely) a combination of the three.

Self-regulation is important, but how effective is it: do corporate compliance systems in industry need more third-party evaluation? How to address the often-mentioned lack of awareness and education? How to manage the tension between transparency (open science) and the need to prevent critical information ending up in the hands of malicious actors? How to develop oversight mechanisms in the multidisciplinary environment that is characteristic for chemical and biological convergence? How to engage with new actors such as self-driving or cloud labs?

These are not easy questions. Governments and International Organisations, but equally scientists and arms control organisations and experts have a role to play in identifying challenges, developing solutions, devising guidance and enabling stakeholder engagement. They also have a key responsibility in upholding the norms enshrined in the arms control treaties, even or perhaps in particular in times of geopolitical pressure. Spiez CONVERGENCE remains a place where such exchanges can take place in an open, well-informed and congenial manner.

Take-home points

- The integration of AI and machine learning with automation (e.g. robotics and cloud labs) accelerates research and development, allowing the exploration of larger molecular spaces.
- To overcome limitations in the response to chemical and biological threats, threat-agnostic methods are being developed, focusing on functionality rather than on list-based approaches to identify pathogens and toxins.
- In the context of a one-health approach, the norms governing chemical and biological threats should include often overlooked threats to plants and animals.
- New methods in targeted drug delivery and control of dosing support better targeting of treatments and personalised medicine.
- In manufacturing, biological processes and principles are used for the production of chemicals and complex materials with greater precision, with the aid of Self-Driving research Laboratories automating processes.
- A careful balance between scientific freedom/openness and regulatory control is needed; also, the early stages of development should not be over-regulated.
- Some of the discussed techniques could pose potential risks, such as accountability concerns for Self-Driving Laboratories, technologies that may affect current and possible future verification systems and forensic capabilities; or techniques that could be misused for developing novel agents or manufacturing processes.
- While new advances in science and technology are changing the threat landscape, emerging capabilities should not be overhyped when it comes to the assessments of dual-use risks. For instance, new scientific tools still need to be validated and already proven ways to make effective warfare agents exist.
- The progress of science and technology strengthens defences against the malicious use of chemical and biological agents, with examples like threat-agnostic pathogen identification or the use of AI to speed up vaccine development.
- Mis-and disinformation have become a major challenge, requiring scientists to actively counter these and communicate effectively with political leaders and other stakeholders.
- While advances in science and technology bring vast benefits and may provide new countermeasures; they also increase the potential for risks, emphasising the need for awareness raising and mitigation strategies such as regulation or self-regulation.
- Treaties or policies, which often lag behind the science and technology progress, will not be enough to manage these challenges.
- Given the complexity of new scientific and technological tools, their malevolent use requires significant resources, and they are more likely found in state-supported activities. This emphasises the importance of further strengthening the effectiveness of control measures and of upholding the norms of the global CBW arms control framework.

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