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Understanding Pathogenicity: Providing Information about Advances in Science and Technology to the Biological Weapons Convention

Under award N00244-14-1-0039, discussions on relevant advances in science and technology and their potential implications, aligned with the Biological Weapons Convention’s (BWC)’s 2014-2015 intersessional program of work, were held in association with BWC Meetings of Experts in Geneva, Switzerland. Two complementary summaries resulted from these discussions, produced and disseminated under the auspices of the Biosecurity Working Group of IAP: The Global Network of Science Academies. The meetings brought together academic and industry scientists with scientific, technical, and policy experts from BWC States Parties and civil society organizations. The discussions reflected the engagement of members of the scientific community in considering potential biosecurity concerns and they explored a possible model for providing independent input to the BWC in a way that aligns with the interests and needs of scientific and technical experts as well as the diplomatic and policy community. The Eighth BWC Review Conference will take place in November 2016. The topic of models by which the BWC forum can keep abreast of rapid scientific and technical developments is anticipated to be one of the items considered during Review Conference preparations and discussions.
Understanding Pathogenicity: A Workshop for the BWC Meeting of Experts

Workshop Summary
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Prepared under the auspices of the IAP Biosecurity Working Group

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CONVENING ORGANIZATION AND SPONSORS

The workshop and summary were produced under the auspices of the Biosecurity Working Group of IAP: The Global Network of Science Academies. IAP, formerly known as the InterAcademy Panel on International Issues, is a network of 107 of the world’s academies of science. Its primary goal is to help member academies work together to advise citizens and public officials on the scientific aspects of critical global issues. The IAP Biosecurity Working Group was established in 2004 to undertake IAP’s work at the intersection of biosciences and security. The Working Group now includes the academies of Australia, China, Cuba, Egypt, India, Nigeria, Pakistan, Poland (chair), Russia, United States, and United Kingdom and concentrates on two issues: a) education about dual-use issues in the context of responsible conduct of science, and b) implications of trends in science and technology (S&T) for the operation of the Biological Weapons Convention (BWC) and other nonproliferation treaties.

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SUMMARY

A workshop was held in August 2014 under the auspices of the IAP Biosecurity Working Group to discuss understanding and modulating pathogen virulence mechanisms and host immune responses. The workshop focused on two complementary strategies for combating infectious diseases: targeting pathogen virulence factors and modifying a host’s immune responses. These issues were directly relevant to the 2014 intersessional focus of the Biological Weapons Convention (BWC). An understanding of pathogenicity and immunology has the potential to be misapplied to create pathogens with increased virulence or to decrease the effectiveness of responses to infection. Alternatively, advances in this understanding offer promising new strategies in disease treatment. The workshop brought together approximately 35 scientists from academia and industry, scientific and technical experts from BWC delegations, and members of stakeholder communities interested in BWC issues. The workshop did not attempt to arrive at consensus conclusions, although several points were made by multiple participants, including the caution that novel approaches to alter host and pathogen responses are possible but enormously complex. The methods discussed present interesting opportunities, and would likely be used as additional lines of defense in concert with traditional therapeutics. The presentations at the workshop also raised the point that lines of research may have unexpected positive, as well as potential negative results for other fields of study. As a result, many participants highlighted the need for continuing communication between scientists and policy-makers and for members of the scientific community to be aware of how they present the findings and implications of their work. An open question of significant interest remains the issue of how to evaluate the risks and benefits of certain areas of research and the control of resulting information: who should determine whether the research is conducted, how the results are distributed, and based on what criteria?
INTRODUCTION
Advances in science and technology can have profound implications for non-proliferation regimes such as the Biological Weapons Convention (BWC). As part of its annual program of intersessional meetings and five year review conferences, the BWC considers relevant scientific developments in order to ensure that the treaty keeps pace with a changing landscape and takes into account the impact of developments on treaty goals and implementation. In 2014, the scientific focus of the BWC is on “advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues.” These issues are at the very heart of the BWC in that understanding pathogenicity might have the potential to be misapplied to create pathogens with increased virulence or to decrease the effectiveness of responses to infection, but advances may also offer promising new strategies in disease response.

A workshop preceding the 2014 Meeting of Experts organized under the auspices of IAP: The Global Network of Science Academies brought together approximately 35 scientists from academia and industry, scientific and technical experts from BWC delegations, and members of stakeholder communities interested in BWC issues. The workshop focused on two complementary strategies for combating infectious diseases: targeting pathogen virulence factors and modifying a host’s immune responses. In addition to presentations, the meeting discussed potential applications and implications of this field of discovery for the BWC and the biosecurity community (see Appendix for the workshop agenda, discussion questions, and participant list). This report summarizes the presentations and discussions that occurred.

Overview of host-pathogen interactions
Kenneth Berns, University of Florida
The first workshop speaker, Kenneth Berns, provided background on the immune response to pathogenic organisms. He began by pointing out that infectious diseases have been the major cause of human mortality throughout recorded history. As we have become more proficient in preventing and treating infections, life spans have increased, causing other major causes of death, such as cancer and cardiovascular disease, to become more common. Three advances in particular have been important in preventing and treating disease: the provision of clean water, immunization, and the development of antibiotics. Despite these successes, various infectious diseases, such as AIDS/HIV, tuberculosis, malaria, measles, influenza, dengue, Ebola and Chikungunya remain major threats to human health and a reminder that we live in a milieu in which microbes are ubiquitous. In fact, not only do we live in close proximity to multitudes of microbes, but they are an inherent component of the human body. Nevertheless, Berns suggested that we survive because we have developed sophisticated systems of defense in the form of the immune system.

The immune system is complex and has two parts: first, the innate immune system, which recognizes general molecular patterns and affords an immediate or short-term
response which can slow or inhibit infections. These early activities buy time for the second part of the immune system, the adaptive response, to become activated and trigger antibody production, allowing the cell mediated immune response to deal with extracellular and intracellular pathogens, respectively (Figure 1). However, just as humans have evolved sophisticated immune systems to deal with microbes, microbes too have adapted to elude human host defences. The microbes use strategies including changing surface proteins to avoid recognition, synthesising decoys to fool the immune system, or inactivating the protective pathways of the host. In some cases, this process is well understood; in other cases, the cause of damage at the molecular and/or cellular level has not been resolved.

![Figure 1 Components of the immune system. A. The innate and adaptive immune responses act in synergy to respond to microbial infection. B. The adaptive immune system has two arms—one that leads to the production of antibodies largely targeted against extracellular pathogens, and one that leads to the destruction cells that are infected with intracellular pathogens. SOURCE: A. Figure courtesy of Stefanie N. Vogel, University of Maryland School of Medicine. B. Institute of Medicine. 2001. Multiple Sclerosis: Current Status and Strategies for the Future. Washington, DC: National Academies Press.](image)

Berns went on to suggest that vaccines represent one of the great success stories of medicine, although many pathogens remain for which we have not yet been successful in developing effective vaccines. The second great achievement in the war against infectious diseases has been the development of antibiotics and antivirals. With increasing knowledge of the molecular mechanisms underlying replication, it was possible to develop effective antivirals against such diseases as influenza, AIDS, and hepatitis C infection. The development of such drugs remains particularly important in the maintenance of public health defenses.

For all the benefits of advances in biology, it is also apparent that there has been a history of biology being misapplied for hostile purposes. Indeed, most biological research can be classified as dual-use. However, it is so-called “dual-use research of concern” (DURC) that is a particular worry, with seven types of experiments defined as DURC by the U.S. National Science Advisory Board for Biosecurity (NSABB). One classic
example of DURC is the work done in Australia on the mousepox virus and cytokine IL-4. This type of research raised two key questions:

1) To what extent, if any, should some types of experiments be prohibited or conducted under circumstances where dissemination of the results should be limited?

2) Should some information be redacted, and, if so, who should determine those who might have access?

Berns noted that there have been a number of recent cases when authors and journal editors mutually agreed to redact some details, but this may not always be the case. It would be preferable to deal with such questions early on, ideally prior to the commencement of research, an approach pursued in the United States, and something which could be considered at the international level. In the ensuing discussion, participants queried whether papers had been turned down because of potential biosecurity or biosafety concerns. Although the speaker was not aware of any papers which had been rejected outright on these grounds, he was aware of examples where editors worked with authors to come to voluntary agreements to redact and revise manuscripts prior to publication. Attempting to revise papers after the fact, however, remains problematic.

**Introduction to the workshop’s focus**

*Nancy Connell, Rutgers New Jersey Medical School*

Following Kenneth Berns’s introduction to the immune system and to potential scientific and policy concerns that may arise from dual use biological research, the workshop chair, Nancy Connell introduced the specific topics of the day’s agenda.

Connell introduced the focus of the meeting by making reference to Article X of the BWC, which obligates states to avoid hampering economic and technical developments and encourages the fullest possible exchange and cooperation in biological sciences. She also drew attention to Article XII and the importance of reviewing science and technology as part of the established five year review conference process, particularly as the BWC was created in 1972, a time when scientists were just beginning to clone genes. Since then, there have been remarkable developments in the life sciences. To keep abreast of such developments, a new process emerged from the 7th Review Conference in 2011 to address specific topics during the 2012-2015 intersessional process. Developments in pathogenicity and their implications for the Convention was the topic under discussion at the 2014 Meeting of Experts and was the focus of this workshop.

The workshop’s morning and afternoon sessions discussed areas of active research that rely on novel strategies to combat infectious microorganisms. Most traditional strategies to control diseases rely on boosting production of antibodies directed against a bacterial pathogen through vaccination, or on delivering drugs designed to kill a
pathogen by disrupting key components of its replication and growth cycle. However, selective pressure can rapidly lead to the emergence of antibiotic resistance, which has become a global problem. An alternative approach to controlling infection targets virulence mechanisms a pathogen uses to overcome host defence systems. Such virulence strategies can include production of bacterial toxins, secretion of factors that alter the host’s immune reaction, formation of protective biofilms, and many others. Over the past few years, advances have continued to be made in understanding the complex interplay that occurs between host and pathogen following infection and interest has grown in exploring alternative control strategies. For example, by not directly killing the pathogen, anti-virulence approaches may be subject to less selective pressure to develop resistance and may help preserve the body’s normal microbial flora.

TARGETING PATHOGEN VIRULENCE FACTORS
Fredrik Almqvist, Umeå University
The first speaker of the session, Fredrik Almqvist, focused on “pilicides” and “curlicides” and the role of chemistry-based screening approaches to explore how molecules can be exploited to improve health. The speaker highlighted the significance of antibiotics and the importance of the discovery of penicillin. However, he suggested that there had been limited developments in the field of antibiotics since WWII and that antibiotic resistance was on the rise, with good bacteria (commensal strains) potentially spreading resistance and creating problems in the future.

Almqvist indicated that bacteria have a system of communication that enables them to exploit weakened immune systems and attach to the host, sometimes invading in order to replicate. The formation of biofilms of aggregates of microorganisms and secreted matrix molecules, which are assembled by multiple species, are also of significant concern as they can be capable of withstanding antibiotics. Furthermore, location of bacterial arrival in the body can be important. For example, *Escherichia coli* (*E. coli*) bacteria in the stomach are relatively unproblematic, but *E. coli* in the urinary tract may attach and replicate. The infection can be difficult to control, with the bacteria invading deep into host cells where they can lay dormant, causing recurrent infections later.

Almqvist pointed to the importance of understanding the biology behind such processes, specifically the role of adhesive fibers, or “pili,” which enable some bacteria to attach to hosts cells. Building on such an understanding, Almqvist’s team developed a class of pilicide compounds that can block the chaperone-usher molecular pathways used by the bacteria and inhibit assembly of the pili, thereby slowing down the process of bacterial infection. His team has screened numerous compounds for their ability to inhibit pilus formation, with the intention of making a battery of chemical variations to control the activity of pili (Figure 2). This strategy may have implications for dealing with disease. For example, the only current treatment available for tuberculosis is a cocktail of antibiotics, and the use of pilicides offers an alternative means of targeting bacterial virulence.
Abigail Male, University of Southampton

The second presentation in the morning session focused on protein-protein interactions. Such interactions control most cellular processes, but they remain underexplored and targeting these interactions with conventional drug discovery methodologies remains a challenging, unexplored territory. Abigail Male stated that high-throughput screening has yielded some success, but that a general method for rapid screening of very large libraries of molecules would be of great value. She has approached this challenge using genetic selection, which has several advantages over traditional methods for drug discovery. In combination with a large molecular library, it can become a powerful method for uncovering inhibitors of protein-protein interactions. In Male’s research, she has been able to screen around 100 million cyclic peptides for inhibitors of a chosen protein-protein interaction. Using this method, developing an active peptide sequence which can inhibit selected protein-protein inhibitors is feasible within the space of two months.

The bacterium *Bacillus anthracis*, the causative agent of anthrax, is able to infect both humans and animals and can be produced in vitro and used as a biological weapon. Male reviewed information on anthrax pathogenesis, specifically drawing attention to the binding of anthrax Protective Antigen (PA) protein to the human CMG2 receptor on cells (Figure 3). An inhibitor of this protein-protein interaction could disrupt the cell signalling cascade initiated by anthrax protein-cell receptor binding, potentially mitigating or preventing anthrax toxicity. To explore whether this presented a viable therapeutic option, Male and her team created a “reverse two-hybrid system” to study the interaction of CMG2 with PA with a view to screening via a SICLOPPS (Split-Intein Circular Ligation of Peptides and Proteins) library, which can be used for the synthesis of a large number of cyclic peptides. Male’s team has so far screened four cyclic peptide libraries and identified several sequences of potential utility. They had further tested their compounds in vitro to explore whether they were able to prevent anthrax lethal
factor from entering into cells, indicating they had managed to disrupt the protein interaction.

**FIGURE 3 Process of anthrax infection and disease progression.** The binding of anthrax toxin PA (protective antigen) to the cellular receptor CMG2/TEM8 is essential for the internalization of anthrax toxins LF (lethal factor) and EF (edema factor), which lead to many of the downstream symptoms of infection. Both Dr. Male and Dr. Posillico discussed strategies that block the interaction of anthrax PA with CMG2/TEM8. Dr. Male discussed the use of high throughput genetic screening techniques to identify protein molecules that disrupt the interaction. Dr. Posillico discussed the development of an antibody that binds to PA and prevents it from binding to the cellular receptor. SOURCE: Figure courtesy of Elusys Therapeutics, Inc.

Male concluded by noting that her team is working on second-generation inhibitors and had found molecules that were potentially more effective in interfering with the targeted protein-protein interaction. During the discussion, questions were raised about the normal function of CMG2, a transmembrane protein that is induced during capillary morphogenesis and which is hijacked by anthrax PA. Male indicated that the protein’s normal function was not yet clear. She also indicated that the approach of disrupting such protein-protein interactions could have potential therapeutic application not just for infectious diseases, but also for non-infectious ones.

*Elizabeth G. Posillico, Elusys Therapeutics, Inc.*

The third speaker discussed an antibody-based approach to targeting bacterial virulence factors, focusing also on those expressed by *Bacillus anthracis*. Elizabeth G. Posillico indicated that this approach had much potential, but took time to come to fruition and meet the necessary safety requirements. She first reviewed the process of anthrax infection: after inhalation, spores are taken up by host phagocytic cells, such as alveolar macrophages and dendritic cells, and transported to lymph nodes where they
germinate, secreting toxins. In its early stages, anthrax disease is asymptomatic and early detection remains difficult – however, once anthrax becomes systemic, the toxins contribute to a shock-like syndrome, leading to death.

The mailing of letters containing anthrax spores that occurred in the United States in 2001 (“Amerithrax”) demonstrated the limitations of available antibiotics, which were only able to help half of those who contracted inhalation anthrax as a result of exposure. The incident raised interest in finding alternative public health responses to anthrax. In response, Elusys Therapeutics explored the process of infection from pre-exposure to treatment through both intravenous and intramuscular routes. Posillico also drew attention to the importance of anthrax protective antigen (PA) because of its role in the conduit into the cell for anthrax toxins lethal factor (LF) and edema factor (EF), which subsequently interfere with immune defenses. She outlined how Elusys Therapeutics had been developing an antitoxin, ETI-204, designed to neutralize PA and prevent downstream toxin formation and immune cell apoptosis. Animal trials with the drug demonstrated that PA levels in untreated primates were higher than in ETI-204-treated primates, indicating that the drug shows promise in neutralizing the toxin quickly at low levels. The brain, spleen, liver, and lymph nodes of animals prophylaxed with ETI-204 were examined after 56 days with no bacteria detected.

In conclusion, Posillico stated that the ETI-204 antitoxin neutralizes the protective antigen of *B. anthracis* when administered during active infection. Moreover the antitoxin limits dissemination of vegetative *B. anthracis* to blood and peripheral organs without undermining innate immune cell functions. In the discussion that followed, participants enquired as to the efficacy of using ETI-204 in conjunction with antibiotics, such as levofloxacin, ciprofloxacin and doxycycline; in response, it was suggested that this would be useful to consider further. Others asked about the circulating half-life of ETI-204, which is 21 days.

*Michael Wong, Sarepta Therapeutics*

In the final presentation of the morning session, Michael Wong began by discussing the effects of antibacterial agents on the broader microbiome, and the unintended ripple effects that can be generated by interventions. The research Wong presented focused on the use of a Phosphorodiamidate Morpholino Oligomer (PMO) platform to modify gene expression. PMO alters gene expression by binding to RNA and blocking the cellular step in which an RNA molecule is translated into a protein. The PMO platform is able to target mRNA in vivo and it offers the possibility of high specificity, good stability, and broad versatility. Wong indicated that PMO could have applications in the modulation of host responses to active infection, such as through modification of the expression and production of pro-inflammatory cytokines or pathways.

The PMO platform has been successfully tested in animal models in response to viral threats such as a West Nile virus outbreak in penguins (2002) and concerns over the
emergence of pandemic influenza (2009) and dengue virus (2010), with efficacy for flu and dengue shown in mouse and ferret models. In the case of pandemic flu, for example, the Sarepta Therapeutics team responded to a U.S. Department of Defense (DOD) Transformational Medical Technology request for rapid response capabilities, and moved from concept to compound in seven days. Several Investigational New Drug (IND) submissions have been filed with the U.S. Food and Drug Administration (FDA). More recently, the platform has been tested in response to bacterial agents, such as a drug-resistant Acinetobacter.

The Sarepta team has also been involved in developing medical countermeasures against Ebola and Marburg viruses under the Food and Drug Administration’s (FDA’s) animal rule, for the purpose of protecting warfighters in areas where these viruses are endemic. These filoviruses have a 21-day incubation period and high mortality rates, and to date, no therapeutics are available although vaccines are being developed. The PMO platform is being used to target key viral genes understood to subvert host immune responses and combinations of different PMOs targeting different key genes can be used. Significant increases in survival rates in primates infected with Ebola or Marburg have been observed. Wong’s team applied deep-sequencing techniques to samples of the virus recovered from infected animals to check for evidence of mutations in the binding site they had targeted with the PMO treatment, but none were found. Wong concluded that the PMO approach had been proven safe and effective in the short-term in animal models and in initial Phase I clinical trials in healthy human adults.

In the discussion, participants asked when Sarepta Therapeutics would be able to start further human trials. Wong indicated that interest in the PMO platform had been growing and that the DOD and the FDA had been helpful in streamlining the process where possible. A further question was asked regarding the shelf life of the Sarepta Ebola virus therapeutics; Wong indicated that it was good for at least three years and currently appeared to be stable. Others asked about the practical implications of the technology and how the therapy would be utilized. Wong replied that the approach was originally designed for warfighters, not mass prophylaxis. However, it could be useful in post-exposure treatment of health workers and those that had come into contact with Ebola victims. It was still unclear whether it would be possible to achieve widespread treatment for Ebola with the current technology.

**DISCUSSION OF IMPLICATIONS AND RELEVANCE TO THE BWC FORUM**

During the discussion session that followed the morning’s presentations, participants drew attention to the convergence between biology and chemistry, which was reflected in many of the research areas discussed and is a topic of growing interest for both the BWC and the Chemical Weapons Convention. The importance of drawing out further implications of the research covered in the session for the BWC was raised. It was suggested that all participants should think beyond Article I and the potential for misuse or hostile applications of new research. The positive implications of developments in
science and technology for the Convention should also be highlighted, such as the role developments could play in the provision of assistance in the event of a violation of the Convention.

Other participants drew attention to challenges in the timelines for new drug development and regulatory approval, as well as costs. Drug development frequently proves more complex than anticipated and participants queried whether more could be done on the supply side to facilitate the development of medical countermeasures. The lack of a commercial market for countermeasures to diseases such as anthrax was raised, in that companies may be entirely dependent on government funding in this area. This necessitates a significant commitment on the part of states, but also on the part of private companies, who are required to justify their selection of research areas to investors and shareholders. Still other participants raised the issue of patents, noting that exciting research in academia can be patented too early from an industry perspective, because of the pressure to publish. It was suggested that changes to the patent system or measures such as extending the lifetime of a patent can be helpful to stimulate some areas of infectious disease research.

MODIFYING HOST IMMUNE RESPONSES

The afternoon session focused on modulating the immune responses that result from infection with a pathogen. As Kenneth Berns discussed at the start of the meeting, the host immune response is a complex interplay of cells and signaling molecules. A key question is whether these defense mechanisms can be transiently and safely modulated to overcome the virulence strategies of infectious agents.

Diane Williamson, Defence Science and Technology Laboratory (DSTL)

The first speaker in the afternoon session addressed the topic of manipulating the host immune response for therapeutic benefit. Diane Williamson began by reiterating how pathogens have evolved to evade aspects of host immunity and that difficulty and delays in diagnosis remain, with treatment sometimes required without a complete knowledge of the causative pathogen. Accordingly, there remains a need for therapies with a wide spectrum of action. One avenue for the development of novel solutions can be based on understanding pathogen and host interactions and developing means of identifying opportunities to manipulate host immune responses.

One way to build such an understanding is to compare RNAs expressed in the organs of infected and unexposed mice, using bioinformatics to aid in the interpretation of the data. This aids researchers in the identification of potential targets in the host and builds a better understanding of the disease process. Such an approach provides a wealth of output data on, for example, inflammatory response and granulocyte adhesion. Williamson illustrated the utility of this approach by describing the characterization of lung epithelium in mice infected with the bacterium Francisella tularensis, showing how this knowledge can be used to identify potential therapies that inhibit pathogenesis and protect the lung.
Williamson proceeded to outline how the human immune response can overcompensate when dealing with certain infections, resulting in a “cytokine storm,” a process whereby too many immune cells become stimulated, potentially leading to sepsis, organ failure, and death (Figure 4). Intervening in this process through vaccines, anti-microbials, or anti-inflammatories to promote enhanced microbial clearance is important in restoring the balance and returning the host to a state of well-being. Anti-inflammatories are one particularly useful approach that researchers at DSTL have successfully used in conjunction with antibiotics to improve survival rates of infected mice. DSTL researchers have also investigated strategies for enhanced microbial clearance by activating dendritic cells (cells that stimulate an adaptive immune response) ex vivo and transferring these cells into mice infected with the bacterium *Burkholderia pseudomallei*. The animals treated with the activated dendritic cells showed significantly reduced bacterial loads.

![FIGURE 4 Activation of the immune system.](image)

Williamson concluded by stating that we need to understand host cell responses in order to manipulate such responses for benefit, either by blocking or activating host cell targets depending on the context. Accomplishing these manipulations is difficult because the host response is highly complex and interactive. Nevertheless, certain kinds of manipulation can demonstrate a significant impact on microbial clearance and survival.

In the discussion, participants asked whether the beneficial applications of this research could be reversed and used for hostile purposes. Williamson indicated that the
complexity of the research made it difficult to reverse the direction of application for hostile purposes. Other participants queried whether it was possible to handle immune suppression early on, to which Williamson responded that it was difficult to stop and that intervening too early can have additional repercussions, which are difficult to anticipate and control.

Alan Cross, University of Maryland

The second speaker of the afternoon session began by reiterating that there is increasing antimicrobial resistance among clinical isolates of bacteria and that the antimicrobial pipeline has been drying up. These problems are compounded by the fact that new bacterial challenges are emerging, suggesting that alternative approaches to antimicrobial therapy must be considered. One such approach is through “non-antibiotics” that block pathogen virulence factors, inhibit triggers for biofilm induction and maintenance, or temporarily block host factors required for bacteria to replicate or cause disease. One of the problems with targeting virulence is that these approaches are all largely pathogen-specific.

Cross accordingly presented two examples of potential host-directed therapies. One approach would be to stimulate host innate immunity in order to promote broad-based antimicrobial activity. This approach has the advantage of enabling early treatment before the causative agent is identified, but runs the risk of causing inappropriate activation of the immune system or tissue damage. A second host-directed strategy would be to inhibit a pathologic host response, essentially controlling the cytokine storm and facilitating a return to immunologic homeostasis. This approach points to the need for new classes of therapeutics that dampen inflammation while allowing pathogen elimination to continue, and underscores the importance of broad-spectrum, host-directed drugs that are effective against multiple pathogens.

Cross presented an example from the Kaempfer laboratory, which developed peptide antagonists that can be used to mimic inhibitors of pro-inflammatory cytokines, essentially manipulating the elements that trigger a host cytokine storm. Using animal models, the researchers demonstrated that intravenous delivery of an inhibitory peptide decreased cytokine storms and protected mice from a lethal challenge with the bacterium Streptococcus pyogenes in the absence of antibiotics, even when administered as late as five hours after infection. The researchers achieved similar success in protecting mice from a lethal challenge with E. coli. Cross concluded by stating that host-oriented therapy for infectious diseases have the potential to provide broad-based antimicrobial activity. Such a host-focused approach can enable early treatment of suspected infections before the causative organism is identified and potentially reduces the risk of selecting for antimicrobial resistance. However, inappropriate innate immune modulation could lead to tissue damage or immunosuppression and, accordingly, the work of identifying and understanding the mechanisms of suitable therapeutic agents is still in the early stages.
Daniel Kalman, Emory University
The final speaker focused on host-targeted chemotherapeutics for infectious diseases. Daniel Kalman described studies of how pathogens move into cells, survive within them, then exit, and the resulting immune response the cells launch against the pathogens. Researchers are using such knowledge to identify means to interfere with the process and to produce broad-spectrum anti-pathogen therapeutics. Kalman suggested that a starting point was the convergence of microbiology and oncology because pathogens utilize many of the same cellular and biochemical signalling pathways that are disregulated in cancer. More specifically, Kalman and his research team are addressing the question of whether pathogens utilize tyrosine kinases, similar to those expressed in the host, in pathways associated with motility and whether anticancer drugs that target these kinases could be employed to inhibit microbial pathogenesis. Several bacterial and viral pathogens have been investigated, including pathogenic *E. coli* (EPEC, EHEC); poxvirus (vaccinia, variola); Filoviruses (Ebola, Marburg); *Mycobacterium tuberculosis* and *Franciscella tularensis*, among others.

Kalman noted that poxviruses and enteropathogenic *E. coli* (EPEC) bacteria have similarities in how the pathogen exploits the host’s signaling systems for actin motility, which involve tyrosine kinase enzymes (Figure 5). Using the tyrosine kinase inhibitor drug Gleevec, which was originally designed for cancer treatment, researchers were able to limit poxvirus motility and reduce viral spread within mice, allowing them to survive an otherwise lethal dose. Kalman identified a number of other microbes that employ related tyrosine kinases, against which Gleevec was likewise effective. He also noted that Gleevec reduced intracellular survival of *Mycobacterium tuberculosis* in infected macrophages. Gleevec appeared to be useful even against antibiotic-resistant strains of tuberculosis, something that is being further explored along with studies co-administering Gleevec and antibiotic therapies.

**FIGURE 5** A. The pathogen EPEC can make use of host cellular pathways associated with actin motility. B. The drug STI-571 (also referred to as imatinib mesylate or Gleevec) can affect this actin pathway and represents a potential host-directed approach to reduce pathogen motility. For example, treatment with Gleevec increases survival in mice infected with vaccinia virus. SOURCE: A. Figure courtesy of Daniel Kalman, Emory University School of Medicine. B. Reprinted by permission from Macmillan Publishers Ltd.
Kalman indicated that dosing matters significantly to the effectiveness of the Gleevec therapy and is working to determine the optimum dose. The basis for this dosing effect appears to be stimulation of the production of myeloid cells in the bone marrow (which play roles in innate immunity) at a dose lower than that used to treat cancer. This effect mimics a normal response to infection. Once dosing in humans is better understood, Kalman’s team plans to conduct human safety trials of the drug co-administered with antibiotics in patients with antibiotic-resistant tuberculosis. Kalman concluded by stating that host-directed therapeutics can be used to treat infections caused by an array of pathogens, because many pathogens use conserved biochemical pathways. Some of the pathways used by pathogens are disregulated in cancer, so potentially useful drugs may already be on hand. He also noted that lower dosages of drugs such as Gleevec, which stimulates a myelopoietic response, may be useful in treating infections, including those arising from pathogens that don’t utilize the specific pathways targeted (e.g., the bacterial genus *Franciscella*). Finally, he remarked that this work is rooted in observations about pathogen interactions with the host, illustrating the continued necessity of supporting basic science efforts.

In the discussion, participants asked about the response of Gleevec to granulocyte-colony stimulating factor (GCSF). Kalman indicated that the effect of GCSF is fairly limited in terms of the myeloid cell population and is virtually ineffective against infective diseases. He added that the drug works to induce all of myeloid cells and that his team had results showing that there is no discernible effect on the specific activities of the myeloid cells.

**DISCUSSION OF IMPLICATIONS AND RELEVANCE TO THE BWC FORUM AND IDENTIFICATION OF KEY MESSAGES**

In the final session, participants again turned their attention to the implications of the developments discussed during the workshop and their relevance for the BWC. Participants discussed how the examples of modulating host responses could be exploited for hostile purposes, for example by manipulating the host’s immune response or facilitating pathogenesis. It was suggested that this was effectively the thrust of the research on mousepox virus and IL-4, noted in the first talk of the day. As a result, the possibility of making potentially negative modulations to a host immune response exists, although other participants indicated that it can be difficult to switch the objectives and directions of complex research from peaceful to hostile purposes.

Participants also drew attention to the complexity of such research, highlighting the initial difficulties faced by those without significant expertise in understanding pathways and therapies discussed over the course of the workshop. In this regard, one message for the policy community that could support more informed discussion of the risks of dual-use research would be to highlight the complexity of many of the areas being
studied. Similarly, other participants indicated that access to research data alone would not be enough to enable broad-scale misuse of developments, and that natural threats remained a greater concern. Additional participants highlighted the importance of stressing the positive benefits of such potentially dual-use research to policy actors in the BWC forum, and the role such research could play in, for instance, the provision of medical countermeasures assistance in the event of a violation of the convention.

Drawing on the recent discussions around influenza “gain-of-function” research, several participants raised the importance of independent risk-benefit analyses to inform how to move forward. Some participants at the workshop suggested that a consideration of the risks should be done at an early stage; however, other participants noted that claims over both benefits and risks remain contestable and uncertain, even after the fact, and would be even more ambiguous at the beginning of the research process. In this sense, a discussion of risks and benefits at an early stage, while likely preferable, would also be inherently more uncertain and problematic. This point was reinforced by the unforeseen potential of some research to have unexpected positive results for other fields of study, like how oncology research had informed tests of existing anti-cancer drugs against pathogens utilizing similar biochemical pathways. Still other participants suggested a need for greater media acumen when presenting aspects of complex and potentially dual use science. It was suggested that some offhand remarks had been unhelpful in the discussions around gain-of-function research and had threatened to undermine efforts for a nuanced debate around such studies. Others drew attention to the diversity of H5N1 research, with different researchers taking different levels and types of precautions in their approaches.

Finally, the discussion raised the difficult issue of who should have responsibility for deciding on the risks and benefits of potential lines of research and, in cases where there are real concerns, who should determine how the results are distributed and based upon what criteria. Participants remarked that evaluations conducted by small groups or behind closed doors could be particularly problematic, as could unnecessary censorship or restriction of access to research.

CONCLUDING REMARKS
The workshop brought together members of the scientific community and scientific and technical experts participating in the BWC forum to explore fields of research linked to the 2014 BWC intersessional program of work. In addition to exploring new research findings, the meeting discussed whether such fields have relevance to the implementation of the Convention. The workshop did not attempt to arrive at formal, consensus-based conclusions. However, several key points were raised by multiple participants over the course of the discussions and are provided here to help inform ongoing discussions:

• Novel approaches to targeting infection are theoretically possible but enormously complex: new methods will likely be used as additional lines of defense in concert with traditional therapeutics.
• There is the potential for research to have unexpected positive results for other fields of study, and vice versa, through convergence of biological fields of study.
• Greater media acumen in presenting aspects of dual-use science could help inform reasonable discussions and debates about the conduct of such research.
• Evaluation of risks and benefits and control of information remains an area of interest and debate: who should determine how research results are distributed? Upon what criteria should such decisions be made?
• Concern remains over mechanisms supporting continuing sustainability of advances, particularly in areas such as the development of anti-pathogen therapeutics where large commercial markets are not anticipated.
• The value of regular communication between scientists and policy-makers continues to be highlighted as a valuable part of nuanced discussions over the implications of scientific advances in fields that may have dual use potential.
APPENDIX

Agenda

Chair: Nancy Connell, Rutgers New Jersey Medical School

10:00 Welcome and Introduction

Overview of host-pathogen interactions
Kenneth Berns, University of Florida

Introduction to workshop focus
Nancy Connell, Rutgers New Jersey Medical School

10:45 Targeting pathogen virulence factors
Fredrik Almqvist, Umeå University
Abigail Male, University of Southampton
Elizabeth G. Posillico, Elusys Therapeutics, Inc.
Michael Wong, Sarepta Therapeutics

12:20 Discussion of implications and relevance to the BWC forum

12:45 Lunch

14:00 Modifying host immune responses
Diane Williamson, Defence Science and Technology Laboratory (Dstl)
Alan Cross, University of Maryland
Daniel Kalman, Emory University (remotely)

15:00 Discussion of implications and relevance to the BWC forum

15:30 Identifying key messages and concluding remarks
Nancy Connell, Rutgers New Jersey Medical School

16:00 Adjourn

Discussion Questions

1. What are the most significant ways in which understanding and altering pathogen virulence mechanisms and host-pathogen interactions can contribute to improved disease treatments, defense against biological weapons, and other beneficial uses?

2. What key technical and policy barriers must be overcome to enable this field to advance effectively?

3. In what ways does research on understanding and altering pathogen virulence mechanisms and host-pathogen interactions raise potential dual-use concerns and what strategies might be useful in helping to mitigate potential concerns that arise?
4. When considering the risks and benefits of undertaking scientific investigations in this field and designing experiments, what key questions or issues do you think about?

5. What message would you most want to convey from the science community researching pathogenicity to the policy community concerned about the BWC and biosecurity issues (and in parallel, from the policy community to the scientific community)

Participants

Fredrik Almqvist
Umea University

Naser Al-Ansari
Hamad Medical Corporation, Qatar

Kenneth Berns
University of Florida

Katherine Bowman
U.S. National Academy of Sciences

Zbigniew Ciołek
Mission of Poland, Geneva

John Clements
Tulane University

Nancy Connell
Rutgers New Jersey Medical School

Alan Cross
University of Maryland

Karen Fang
U.S. Department of State

Julie Fisher
George Washington University

Meg Flanagan
U.S. Department of State

Elizabeth Frithz
Sweden Defense Research Agency

Grzegorz Graniak
Military Institute of Hygiene and Epidemiology, Warsaw

Rita Guenther
U.S. National Academy of Sciences

C. Andrew Halliday
Foreign Affairs, Trade and Development, Canada

Jo L. Husbands
U.S. National Academy of Sciences

Britt Johnson
U.S. Department of State

Jeannette Macey
Public Health Agency Canada

Abigail Male
University of Southampton

Alemka Markotic
University Hospital of Infectious Diseases, Zagreb

Lorna Miller
Dstl Porton Down

Piers Millet
Woodrow Wilson Center for International Scholars

Tatyana Novossiolova
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Kenneth Oye  
Massachusetts Institute of Technology  

Sonia Pagliusi  
Developing Countries Vaccine Manufacturers Network (DCVMN)  

James Revill  
University of Sussex  

Elizabeth Posillico  
Elusys Therapeutics, Inc.  

Fran Sharples  
U.S. National Academy of Sciences  

Paul Sheives  
Biotechnology Industry Organization (BIO)  

Ryszard Slomski  
Polish Academy of Sciences  

Ralf Trapp  
Independent consultant  

Andrea Wilkinson  
MedImmune LLC  

Diane Williamson  
Dstl Porton Down  

Michael Wong  
Sarepta Therapeutics
Advances in Design and Use of Microbial Production Systems: A Workshop for the BWC Community

Workshop Summary
Sarah Mueller and Katherine Bowman, U.S. National Academies of Sciences, Engineering, and Medicine, Rapporteurs

Prepared under the auspices of the IAP Biosecurity Working Group

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Strategies for Reducing Design and Development Barriers 8
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CONVENING ORGANIZATION AND SPONSORS

The workshop and summary were produced under the auspices of the Biosecurity Working Group of IAP: The Global Network of Science Academies. IAP, formerly known as the InterAcademy Panel on International Issues, is a network of 111 of the world’s academies of sciences. Its primary goal is to help member academies work together to advise citizens and public officials on the scientific aspects of critical global issues. The IAP Biosecurity Working Group was established in 2004 to undertake IAP’s work at the intersection of biosciences and security. The Working Group now includes the academies of Australia, China, Cuba, Egypt, India, Nigeria, Pakistan, Poland (chair), Russia, United States, and United Kingdom and concentrates on two issues: a) education about dual-use issues in the context of responsible conduct of science, and b) implications of trends in science and technology (S&T) for the operation of the Biological Weapons Convention (BWC) and other nonproliferation treaties.

The project was supported by the Naval Postgraduate School’s Project on Advanced Systems and Concepts for Countering Weapons of Mass Destruction (PASCC) via Assistance Grant/Agreement No. GRANT N00244-14-1-0039 awarded by the NAVSUP Fleet Logistics Center San Diego (NAVSUP FLC San Diego) and by internal support from the U.S. National Academy of Sciences and IAP: The Global Network of Science Academies. The views expressed in written materials or publications, and/or made by speakers, moderators, and presenters, do not necessarily reflect the official policies of the Naval Postgraduate School nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the organizations or agencies that provided support for the project.
SUMMARY
A workshop was held in August 2015 under the auspices of the IAP Biosecurity Working Group to discuss trends in microbial and biologically-based (bio-based) production. The meeting examined how the design and scale-up of such systems is changing the nature of producing biological and chemical products, what factors are driving this expansion, and what implications these developments may have for the implementation of the Biological Weapons Convention (BWC).

Microbial systems can be used to produce therapeutic proteins, as well as chemical molecules such as drugs and biofuels. Microbial production of high value and feedstock chemicals is becoming an increasingly attractive option as factors involved in global supply and demand have changed in response to scientific and technical developments, current economic conditions, and national biotechnology investments. The possible applications of bio-based production continue to expand, enabled by advances in the ability to manipulate genes and metabolic pathways through synthetic biology. The meeting explored examples of companies at the forefront of these trends, including in the creation of design tools and platform technologies that are accelerating the progress of microbial and bio-based production and increasing its reliability, fidelity, and simplicity.

INTRODUCTION AND EXAMPLES IN ACTION
The bioeconomy is built upon advances in the application of science and technology to living organisms and a number of factors are driving a growing interest in it, including availability of feedstock resources, environmental considerations, and financial investments and incentives. The first session of the workshop provided an overview of bio-based production and explored changes to the chemical and biotechnology industries through examples of activities in several countries. As the presentations highlighted, industry is global in scope and speakers noted a number of international partnerships in the development of bio-based production systems. Biological industry is not a monolithic entity, however. Drivers and challenges vary in different sectors, for example for higher-margin, smaller batch specialty products versus bulk chemicals such as biofuels.

Industrial use of bio-based production systems: drivers and challenges
Detlef Männig, Evonik Industries

The bioeconomy, which encompasses the production of renewable biological resources and the conversion of these resources and waste streams into value-added products including food, feed, other industrial and medical products, and energy, makes up a major portion of economic activity across the globe, promoting a more resource-efficient and sustainable economy. Evonik, a German specialty chemicals company, is a prime example of the integration of biotechnology into a traditional chemical company. Advances in metabolic engineering, fermentation, and biocatalysis are allowing Evonik to shift production of a number of specialty chemicals, polymers, and amino acids to bio-based methods (Figure 1). Evonik draws on both in-house research and development (R&D) and venture capital investments to advance its bio-based portfolio, with 10% of its internal R&D budget and 1/3 of its total venture capital budget going towards biotechnology research.
Many factors influence the development of the bioeconomy within companies and externally. Government and industry regulation can dramatically impact adoption and implementation – more than 20 countries have adopted national bioeconomy strategies, shaping prices and influencing investment decisions. Regulations based on renewable energy and sustainability also factor in pushing the bioeconomy forward. Other significant external drivers include energy costs (fertilizers, transportation, and fuel used to grow and harvest feedstocks used in bio-based production), the availability and reliability of raw materials, and new biotechnologies.

Within a company, a primary factor influencing the adoption of bio-based production is profitability. Customers may not be willing to pay a premium for bio-based products versus ones produced by conventional methods. Companies must demonstrate a clear advantage during life cycle analysis to justify a new production method, including economic and/or ecologic benefits. Maintaining quality, consistency, and year-round availability can also be a challenge when developing bio-based production methods compared to chemical synthesis. As with any new and developing technology, it may take years of research before a product or process becomes profitable, so companies must be willing to make long-term investment decisions and take on risk in order to reap benefits in the future. As expertise from a wider range of disciplines becomes increasingly relevant, companies also need to consider how to incorporate new areas of knowledge, tools, and career paths.

**FIGURE 1** A biological feedstock streamlines the process for producing polyamide 12, which is used in packing materials, bags, and films. SOURCE: Presentation to the workshop by Detlef Männig, Evonik Industries, used with permission.

**Developments in biofuels and green chemicals in Brazil**
*Alfred Szwarc, Brazilian Sugarcane Industry Association (UNICA)*

Sucrose derived from sugarcane and other sources serves as an important feedstock material for the production of a number of industrial and commercial products. New processing techniques
are emerging that use sugarcane biomass for the production of fuels, cosmetics, biopolymers, and household products (Figure 2). Brazil is a leader in the application and utilization of bio-based energy sources and sugarcane bi-products harvested from biomass, i.e. trash leaves and tops from the sugarcane plants, and bagasse material remaining after sugar extraction, produce 3.3% of Brazilian electricity.

![Sugarcane Mill To Biorefinary](image)

**FIGURE 2** Sugarcane and sugarcane-derived materials can be used to produce a number of valuable commercial products and fuels. SOURCE: Presentation to the workshop by Alfred Szwarc, UNICA, used with permission.

A number of international partnerships are helping the Brazilian sugarcane industry take advantage of technologies such as new strains of genetically modified yeast and bacteria, and new enzymes. Through a partnership with Solazyme, for example, sugar is converted by microalgae to BioOil, used by cosmetics and personal care companies. Through a partnership with Amyris Biotechnologies, sugarcane juice is fermented by engineered microbes to produce drop-in bio-diesel and bio-jet fuel. In 2012 the Brazilian airline Azul flew a plane from Campinas to Rio de Janeiro on 50% bio-jet fuel, and in 2014 the Brazilian airline GOL flew a Boeing 737 on 10% bio-jet blend from Orlando, Florida to Sao Paulo.

The strong biotechnology sector in Brazil is aided by federal programs that promote biotechnology in the sugarcane industry. PAI SS is a government program that provides low cost financing and grants for innovation and technology related to sugar cane, for example for 2nd generation ethanol production and sugarcane biomass utilization. BIOEN, through Sao Paulo State, provides grants for R&D. Additionally, in 2015 the import tariff was reduced from 14% to 2% for all enzyme-based products to be used in 2nd generation ethanol production or bio-derived chemical products.

Although there is significant institutional support for biotechnology in Brazil, low oil prices are currently limiting interest in renewable energy alternatives. For bio-fuel and other sugarcane-based energy sources to replace fossil fuels, market competitiveness must be demonstrated. As
stated in the previous presentation, consumers may not accept paying a premium for these technologies. In order to increase market competitiveness, further regulatory interventions to incentivize investment can be a helpful strategy – for example, a mandate on the minimum content of ethanol in gasoline.

**Advancing the Malaysian bioeconomy through production of next generation oleochemicals**

*Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation*

A particular focus for Malaysian industry has been biomass conversion from the oil palm tree, which has the highest yield of plant oils per unit area and produces fruit for 20-25 years. The oil palm currently produces two types of oils from its fruit, crude palm oil and crude palm kernel oil, and there is potential for additional feedstock utilization from cellulosic sugar and lignin from palm fronds and empty fruit bunches, as well as bio-gas from palm oil mill effluent. These bio-feedstocks are generally converted to value-added chemicals via fermentation, enzymatic processing, catalysis, and/or thermochemical reactions (Figure 3). Additional R&D is being conducted to investigate other potential bio-feedstocks, including cassava, sugarcane, paddy, tea, and rubber, all native to ASEAN countries.

**FIGURE 3** A number of pathways lead to the production of biochemicals and biomaterials from the oil palm tree. SOURCE: Presentation to the workshop by Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation, used with permission.

Malaysia has significant biological resources as well as infrastructure and engineering expertise to support bio-based production, although challenges remain. In order to take advantage of developments in biotechnology and synthetic biology, including scale-up expertise, partnerships are a common strategy employed by companies in the Malaysian bioeconomy. The Malaysian government has also made significant investments in promoting the bioeconomy ecosystem including the establishment of dedicated industrial parks and infrastructure for biotechnology R&D. At Bio-Xcell in Johor, for example, companies are making dodecanedioic acid, adipic acid, and sebacic acid via fermentation, using glycerin as a fermentation feedstock in the production of lactic acid and bio-isoprene, and producing insulin and other pharmaceuticals via biomass
fermentation. Building shared facilities decreases the capital investments needed by companies to participate, promoting further development of these specialty chemical industries. In addition to infrastructure investments, the Malaysian government has introduced a variety of regulatory incentives for companies participating in the bioeconomy. Incentives include tax deductions for technology acquisition, import tax exemptions, industrial building allowances, and unrestricted employment of foreign scientists, allowing companies to recruit talent from around the world.

**Advances in design and use of microbial production systems**
*Ryszard Slomski, Poznań University of Life Sciences, Polish Academy of Sciences*

A number of advances in chemical and biological production are being explored in Poland, and the presentation provided information on several ongoing projects. The National Centre for Research and Development in Poland and the University of Life Sciences in Poznań, for example, are working together to develop a technique for the production of 2nd generation ethanol from the biomass of sorghum and miscanthus. Other collaborative research efforts include the development of cannabinoids with low THC content for cancer patient treatment. In addition, research is being undertaken to study the bio-conversion of glycerol to polyols and dicarboxylic acids and into 1,3-propanediol, a product used in materials such as polyurethanes, polyesters, and resins. Components of the metabolic pathway to convert glycerol to 1,3-propanediol were transferred from pathogenic bacteria such as *Klebsiella pneumoniae*, which are good natural producers of these molecules, into the nonpathogenic bacterium *Escherichia coli*, demonstrating the utility of synthetic biology to improve industrial biotechnology processes.

Such biotechnology advances in Poland and across the world have been accelerated by the rapid decrease in the price and time needed for genome sequencing, as well as a rapid increase in computing capability (Figure 4).

![Sequencing the Human Genome](image)

**FIGURE 4** The cost and time to sequence genomic DNA has fallen dramatically since the turn of the 21st century, enabling a number of biotechnology advances. SOURCE: Presentation to the workshop by Ryszard Slomski, Poznań University of Life Sciences, used with permission.
STRATEGIES FOR REDUCING DESIGN AND DEVELOPMENT BARRIERS
Progress in a number of areas supports the growth of microbial and biologically-based production, including advances in fields such as synthetic biology and efforts to make biological design and development processes easier, faster, less expensive, and more reliable. The afternoon session provided examples of research institutes and companies that are tackling challenges such as improved design tools, rapid implementation and automation platforms, and the creation of special-purpose engineered organisms.

Developing an integrated design tool: Recent progress and remaining challenges
Markus Herrgard, Technical University of Denmark

The Novo Nordisk Foundation Center for Biosustainability (CFB) at the Technical University of Denmark is a non-profit center exploring the use of bacterial and yeast strains for the production of bulk and fine chemicals and food ingredients, and the use of mammalian cell lines for the production of protein-based therapeutics. To support translational efforts in microbial cell factory design and production, CFB utilizes a strategy called iLoop (Iterative Cell Factory Development Loop), in which specialized teams support different phases of product development: designing, building, testing, and analyzing the organisms involved.

The design of microorganisms can take place at a variety of biological levels – DNA, protein, pathways, organisms, communities, processes, and industries (Figure 5). At most levels, rational design is difficult because of the large numbers of possibilities to test. Achieving predictable control is also challenging for reasons including context-sensitivity of genetic “parts”, inability to fully predict key aspects such as enzyme-substrate specificity, unanticipated side effects, variation in the way strains respond to changes in processing conditions, and others. Although empirical data are required for optimization, a key advantage of design tools is that they allow researchers to reduce the search space for screening new organisms with desired properties. Although investigators cannot predict whether or not a gene construct or engineered organism will behave as expected, by limiting the search space, they can avoid iterations that would not be worth pursuing.

**Design complexity**

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<th>Number of choices</th>
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<td>1</td>
<td>10000</td>
<td>Chemical product</td>
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</table>

**FIGURE 5** There are a number of biological levels at which design can occur, from genes to industrial scale production. SOURCE: Presentation to the workshop by Markus Herrgard, Technical University of Denmark, used with permission.
A number of tools are increasingly available at all levels of design - examples include software programs to model the effects of gene modifications and databases to aid in predicting enzyme function based on sequence similarity to known proteins. Efforts are underway to integrate methodologies between levels. Design exchange standards are also emerging, allowing for improved data sharing and more rapid implementation of in vivo testing. The establishment of best practices and a systematic investigation of experimental validation methods will further aid in supporting the adoption of bio-based production.

**Streamlining laboratory workflows and data analysis**

*Sean Ward, Synthace Ltd.*

The bioeconomy continues to grow and industrial revenues to increase in sectors such as biofuels, food and agriculture, biologics feedstocks, and biochemicals. At the same time, the price per base of DNA sequencing and synthesis has fallen dramatically. However, the number of drugs produced per unit of R&D investment is falling – there is now less than 1 new drug produced per $1 billion of R&D funds. Dr. Ward referred to this as Erooms’s Law (the inverse of Moore’s Law).

To address these increasing R&D costs, Synthace is working to transform biological experimentation from an almost “artisanal” experience to a more uniform, automated process. Variables associated with a test organism and other experimental conditions can be broken into discreet operations. For example, an experiment may be divided into components such as feedstock, temperature, time, aeration, culture medium, and others. Factors associated with an experimental organism might be expressed in terms of the genetic expression cassette used in the study, locations of genomic integration, and chaperone molecules present. By separating experimental dimensions into discrete variables, an automated system can more systematically establish correlations among many factors being changed at once. Synthace manages this design and data collection through a software program called Antha, which allows researchers to select experimental conditions, repeat and modify previous runs, and keep an electronic log of results that can be analyzed to determine the sets of conditions that yielded desired outcomes. The experiments are carried out using automated robots, reducing user error. The Antha platform is hardware agnostic so that it is interoperable and transferrable among users at different labs. Scalability can also be included in the design of experiments, allowing for easier transfer from lab-scale to industrial-scale applications. By automating biology in this manner, Synthace seeks to decrease costs, increase experimental iterations, and enable data to be shared more effectively across multiple researchers, increasing the possibility of breakthrough discoveries (Figure 6).
FIGURE 6  The use of software platforms to manage experimental design and the incorporation of control robotic handling increase the numbers of measurements that can be made and decrease the average costs to create a new genetic construct. SOURCE: Presentation to the workshop by Sean Ward, Synthace Ltd., used with permission.

Identifying disruptive technologies in biotechnology

Jason Kelly, Ginkgo BioWorks

The patent on recombinant DNA was awarded in 1974 and Dr. Kelly pointed to it as the first disruptive biotechnology. While there was early recognition of the significance of recombinant DNA technology, it was not possible to predict the many ways in which its value would be realized. Over time, major research programs and well-known companies became established to manipulate DNA in the development of protein therapeutics, agricultural products, industrial enzymes, diagnostics, oil cleanup strategies, vitamins, animal feed, brewing, dyes, and many other applications.

Dr. Kelly posed the question, is bioengineering a similar disruptive technology? The database of gene sequences is doubling every 18 months and costs are continuing to fall – suppliers can fabricate DNA sequences up to 5,000 base pairs in less than 90 days. Researchers are developing more standardized systems of biological parts and devices, making biology easier and more reproducible to manipulate. Ginkgo BioWorks is harnessing these technologies to create engineered microbes to be used in fermentation processes, turning feedstocks into high value chemicals and ingredients. As “the organism company” Ginkgo partners with others, for example with chemical companies, to design microbial strains for specific purposes and to integrate them into a company’s workflow. Ginkgo BioWorks has thus established itself as an organism foundry integrating software, robotics, and laboratory personnel with a vision of functioning like an organism manufacturing facility (Figure 7). A goal for this kind of centralized organism development resource is to expand the capabilities of companies interested in biotechnology and to further increase the opportunities for bio-based production of goods.
DISCUSSION

What are the Implications for the BWC?

Piers Millett, Biosecure Ltd.

Dr. Millett introduced the discussion session on potential implications of advances in biotechnology, synthetic biology, and biological design for the scope and operation of the BWC. As the workshop presentations illustrated, academic and industrial researchers from across the globe are developing microbial-based and bio-based production systems for biologics and chemicals. In some cases, these resources require significant investments (such as organism foundries) and may remain centralized in nature. In other cases, the development of cheaper benchtop-size and drop-in automated equipment may lead to wider distribution of capabilities. Which models, centralized versus distributed, will be followed by different types of technical advances over time remains an open question.

Advances in areas such as tool and platform development, automation, and experimental analysis are leading to progress on multiple fronts in design and development of biological production processes. However, the field is not yet at a stage in which a researcher could simply enter a desired end product into a software package, have the system map out the metabolic pathways, and robotically conduct the experiments necessary to achieve the desired result. A significant role remains for tacit knowledge and specialized resources. Practical challenges also remain in scale-up from laboratory to industrial-scale production of relevant microorganisms. Complex system aspects must be controlled, making it difficult for someone to switch from one route of production to another, whether that would entail use of a new organism, feeding an organism a new feedstock, or trying to produce a new end product. Each synthetic scheme would require intense optimization to achieve robustness and cost-effectiveness.

The discussion touched on the level of skill that would be required to take a typical laboratory organism and modify it to produce a chemical or biologic agent of concern. Speakers noted the
existence of simple kits for the expression of common genes such as green fluorescent protein
in standard microbes such as *E. coli*, but indicated that modifying an organism into a pathogen
remains difficult. Obtaining the biological parts, DNA sequences, plasmids, and other
components is likely to present a major hurdle, as most would not be sold on the open market.
The changing nature of biological production systems may also have implications for the types
of evidence that might be used to identify an illicit BW program. It is hard to fully eliminate gene
fragments, even with autoclaving. For the BWC, this means that there may be additional
possible strategies to identify an attempted manufacture of a new pathogen.

Several positive implications of advances in bio-based production technology were also noted by
participants. Technologies could be used to proactively mitigate risks associated with products
that have off-target uses. For example, scientists could produce castor beans without ricin toxin
to reduce the potential for production of ricin from the crop. Emerging biotechnologies can also
be used to promote population defense against human and natural threats on a shorter
timescale of days, rather than weeks or months. Significant time can be saved in vaccine
production, for example, when a new pathogen can be sequenced and the data emailed to a
vaccine production facility rather than shipping the pathogen itself around the globe.

Finally, participants noted the value of using data and concrete examples rather than
abstractions when discussing potential biosecurity concerns, governance, and the BWC with
academic and industrial communities. One strategy to promote ethical use of technologies is the
establishment of norms within the scientific community for responsible conduct of science, safe
laboratory practices that support biosafety/security, and awareness of prohibitions under the
BWC and CWC. Self-governance mechanisms to counter potential risks can help demonstrate
that scientists are willing to address safety and security concerns, with parallels being drawn to
cybersecurity and community engagement in responsible practices. A number of suggestions
were raised to foster engagement with industry, with one suggestion being as small as adding
“We support the BWC” to company websites to bring the Convention into the conversation.
Another participant noted that no company wants to be connected to potential BW risks and
that companies would be wary of bringing attention to the topic. However, it was suggested
that the issue could be framed as a part of corporate responsibility. The important role of
champions in discussing biosecurity topics and of finding ways to encourage or incentivize
companies to participate in such discussions was highlighted.

Continuing to engage academic and industrial communities in the BWC remains important and
various models for achieving this goal could be considered. Several participants noted that
bringing scientists and diplomats together is good in theory, but the scientific presentations can
be complex and it is difficult to delve significantly into the implications for the BWC. Options to
help address these challenges include use of working groups to consider issues presented by
experts with the goal of presenting a digested version of the information to diplomats in a user-
friendly and BWC-specific format. An additional strategy used by OPCW has been to host
“Science for Diplomats” lectures on topics related to articles of the CWC, held over lunchtime
between Convention sessions. Topics started at the level of “What is an atom? What is a
molecule?” and now deal with S&T topics of more direct relevance to the CWC. Through the
sessions, scientists and diplomats were able to build further trust and shared understanding.
Opportunities for ongoing communication among academic and industrial scientists and policy-
makers may help support nuanced discussions on the implications of scientific advances
relevant to the work of the Biological Weapons Convention.
APPENDIX

AGENDA

10:30 Welcome
Katherine Bowman, U.S. National Academies of Sciences, Engineering, and Medicine

Introduction and Examples in Action
Detlef Männig, Evonik Industries
Alfred Szwarc, Brazilian Sugarcane Industry Association (UNICA)
Muhammad Farish Kamaludin, Malaysian Biotechnology Corporation

12:15 Lunch

13:45 Reconvene
Ryszard Slomski, Poznań University of Life Sciences

Strategies for Reducing Design and Development Barriers
Markus Herrgard, Technical University of Denmark
Sean Ward, Synthace Ltd.
Jason Kelly, Ginkgo BioWorks

What are the Implications for the BWC?
Piers Millett, Biosecure Ltd.
Discussion

16:30 Adjourn

DISCUSSION QUESTIONS

1. Why are diverse companies investing in advanced bio-based production capabilities for biological and chemical molecules – what is driving this trend forward and how it is changing the industry landscape (particularly for systems that are microorganism-based)?

2. How are new tools and systems making it easier to design and create microorganisms that produce specific biological or chemical molecules of interest?

3. What are the benefits of bio-based production technologies for the Biological Weapons Convention – for example, in responding faster or more flexibly to disease outbreaks or in developing therapeutics against emerging diseases?

4. Are any potential risks posed by these advances – for example, in making it easier to develop or produce a pathogen or toxin, or by increasing the numbers of people around the world skilled in implementing these techniques?

5. As universities and companies expand efforts to design microbial systems, how are the norms of responsible scientific conduct being communicated and promoted?
   a. Who should be responsible for communicating these norms?
   b. Are there mechanisms for assessing whether or not proposed research and development activities raise potential biosecurity concerns?

6. How can BWC policy makers keep abreast of developments in science and technology and learn about the views of academic and industry scientists?
   a. What system(s) do you think would work most effectively?
   b. For academic/industry scientists – what concerns would you want to convey to BWC policy makers (for example, in not unduly stifling innovation)?
   c. For policy makers – what concerns would you have for academic/industry participants (for example, in ensuring responsible development for beneficial purposes)?