# Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

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Item 6 of the provisional agenda
**Standing agenda item: review of developments in the field of science and technology related to the Convention**

## Advances in science and technology related to the Convention

*Summary*

The Seventh Review Conference decided that the 2012 to 2015 intersessional programme would include a Standing Agenda Item on review of developments in the field of science and technology related to the Convention. This paper expands upon and updates the background information document on advances in scientific and technology prepared by the ISU for the 2013 Meeting of Experts (BWC/MSP/2013/MX/INF.01/Rev.1), the background paper on advances that have potential benefits for the Convention prepared for the 2012 Meeting of Experts (BWC/MSP/2012/MX/INF.3), and the overview of advances in enabling technologies provided to the 2012 Meeting of Experts (BWC/MSP/2012/MX/INF.1).

# I. Advances in science and technology

## A. Introduction

1. The Seventh Review Conference decided that under the Standing Agenda Item reviewing developments in the field of science and technology related to the Convention States parties will consider:

"(a) new science and technology developments that have potential for uses contrary to the provisions of the Convention;

(b) new science and technology developments that have potential benefits for the Convention, including those of special relevance to disease surveillance, diagnosis and mitigation;

(c) possible measures for strengthening national biological risk management, as appropriate, in research and development involving new science and technology developments of relevance to the Convention;

(d) voluntary codes of conduct and other measures to encourage responsible conduct by scientists, academia and industry;

(e) education and awareness-raising about risks and benefits of life sciences and biotechnology.

(f) science- and technology-related developments relevant to the activities of multilateral organizations such as the WHO, OIE, FAO, IPPC and OPCW;

(g) any other science and technology developments of relevance to the Convention."[1]

2. The Seventh Review Conference also decided that "the following topical scientific subjects will be considered in the years indicated:

"(a) advances in enabling technologies, including high-throughput systems for sequencing, synthesizing and analyzing DNA; bioinformatics and computational tools; and systems biology (to be considered in 2012);

(b) advances in technologies for surveillance, detection, diagnosis and mitigation of infectious diseases, and similar occurrences caused by toxins in humans, animals and plants (to be considered in 2013).

(c) advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues (to be considered in 2014);

(d) advances in production, dispersal and delivery technologies of biological agents and toxins (to be considered in 2015)."[2]

3. This background information document provides an overview of:

(a) advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues;

(b) advances related to dealing with disease, updating background information provided in 2013 (BWC/MSP/2013/MX/Rev.1) and in 2012 on advances with potential benefits for the Convention (BWC/MSP/2012/MX/INF.3);

(c) advances in enabling technologies, updating background information provided in 2012 (BWC/MSP/2012/MX/INF.1)

## B. General Trends

4. The background document on advances in science and technology compiled by the ISU for the Seventh Review Conference identified six trends: convergence between disciplines; increasing understanding of the underlying principles and mechanisms of the life sciences; shifting focus of priority areas within commercial biotechnology; a greater geographical distribution of capacity; open science; and media, perceptions and interactions with society. The 2013 ISU background document on the same topic noted a seventh trend: the increased use of research collaborations.[3] These trends all remain extant. Some updates

[1] BWC/CONF.VII/7, part III, paragraph 22.
[2] BWC/CONF.VII/7, part III, paragraph 23.
[3] BWC/MSP/2013/MX/INF.1/Rev.1

include the continued shift away from small molecule drugs to biotechnology products for the pharmaceutical industry;[4] continued R&D efforts for hundreds of new vaccines and drugs;[5] and ongoing initiatives to make vaccines and drugs available to all, noticeable for instance through the growth in the biosimilars market, estimated to reach $1.95 billion by 2018, and the continued expansion of the Developing Countries Vaccine Manufacturers Network.[6]

5. An eighth trend may now be added: the growing tacit knowledge requirement for life science work.[7] Researchers attempting to replicate experiments raised the alarm on the growing difficulty of reproducing research; this issue has become so severe that those seeking to replicate results obtained at another lab are now encouraged to do so through joint work.[8] This trend is, in part, driving the seventh trend: research collaborations are set up to bring together the "barrage of high-end equipment that no one can afford,"[9] but also to pool the tacit knowledge required to effectively employ these pieces of sensitive equipment. The security implications of tacit knowledge have also been considered in the context of: synthetic biology;[10] and for the regime addressing the threat posed by biological weapons.[11]

# II. Advances in the understanding of diseases, including pathogenicity, virulence, toxicology, and immunology

## A. Transmissibility

6. There is an increased understanding in the nature of mutations necessary to make certain diseases air-transmissible, sometimes without causing loss of pathogenicity. This has been achieved in part through controversial gain-of-function research. Of note were

[4] Kevin Grogan, "Tufts report confirms domination of biotech products," PharmaTimes, 14 November 2013, http://www.pharmatimes.com/article/13-11-14/Tufts_report_confirms_domination_of_biotech_products.aspx.

[5] "Medicines in Development for Vaccines 2013," PhRMA, http://www.phrma.org/medicines-in-development-for-vaccines-2013.
Lynne Taylor, "US Biopharma: 452 drugs for rare diseases now in R&D," PharmaTimes, 9 October 2013, http://www.pharmatimes.com/article/13-10-09/US_biopharma_452_drugs_for_rare_diseases_now_in_R_D.aspx.

[6] Alaric Daerment, "Biosimilars Market to reach $1.95 billion by 2018, study finds," MedTech, November 25, 2013, https://www.medtech.org/news/global.aspx?recid=4207 ;
Sonia Pagliusi et al., "Developing Countries Vaccines Manufacturers Network: Doing good by making high-quality vaccines available to all," Vaccine (18 April 2013), http://www.ncbi.nlm.nih.gov/pubmed/23598479.

[7] In this paper, the term tacit knowledge is being used to refer to knowledge that is obtained through hands-on experience, as opposed to through written instructions and other forms of passive learning.

[8] Mina Bissell, "Reproducibility: The Risks of the Replication Drive," Nature, Vol. 503 (20 November 2013): 333-334, http://www.nature.com/news/reproducibility-the-risks-of-the-replication-drive-1.14184.

[9] Ewen Callaway, "Structural biologists share their toys," Nature, Vol. 483 (1 March 2012): 15-16, http://www.nature.com/news/structural-biologists-share-their-toys-1.10122.

[10] Catheribe Jefferson et al, "Synthetic Biology and Biosecurity: How scared should we be?", Kings College London, May 2014, http://www.kcl.ac.uk/sspp/departments/sshm/research/Research-Labs/CSynBI@KCL-PDFs/Jefferson-et-al-(2014)-Synthetic-Biology-and-Biosecurity.pdf

[11] James Revill and Catherine Jefferson, "Tacit knowledge and the biological weapons regime", Science and Public Policy, 2013, pp1-14, http://spp.oxfordjournals.org/content/early/2013/12/11/scipol.sct090.abstract

- two 2013 studies which produced a series of hybrids of the H5N1 and H1N1 avian influenza viruses capable of spread via respiratory droplets. The research teams used a similar methodology as employed in the 2013 H5N1 papers but employed high-throughput approaches, such as directed evolution;[12]
- the announcement by a collection of leading researchers of the intent to undertake gain-of-function research on H7N9 avian influenza viruses. The authors proposed and discussed the ethics of their planned studies before carrying out the research; and[13]
- a 2014 study in which a research group undertook gain-of-function research with the H7N1 avian influenza virus and were able to demonstrate that this virus was able to spread in ferrets (a mammal) without a loss of virulence.[14]

## B. Pathogenicity and virulence

7. There is new data and analysis on inhalational variants of diseases caused by pathogens that might be relevant to the Convention, including: a new hypothesis for the mechanism by which inhalational anthrax kills;[15] and a more detailed understanding of primary pneumonic plague.[16]

8. There is a growing knowledge base on *strain-specific* disease virulence and pathogenicity, including: why certain influenza strains are more adept than others at growing in human lung tissue;[17] the discovery of a virulence plasmid in some Non-

[12] Robert Roos, "Study: Lab-made H5N1-H1N1 viruses spread in guinea pigs," University of Minnesota, Center for Infectious Disease Research and Policy, 2 May 2013, http://www.cidrap.umn.edu/news-perspective/2013/05/study-lab-made-h5n1-h1n1-viruses-spread-guinea-pigs;
Ying Zhang et al., "H5N1 Hybrid Viruses Bearing 2009/H1N1 Virus Genes Transmit in Guinea Pigs by Respiratory Droplet," *Science*, Vol. 340, no. 6139 (21 June 2013): 1459-1463, http://www.sciencemag.org/content/340/6139/1459.abstract;
Wei Zhang et al., "An Airborne Transmissible Avian Influenza H5 Hemagglutinin Seen at the Atomic Level," *Science*, Vol. 340, no. 6139 (21 June 2013): 1463-1467, http://www.sciencemag.org/content/340/6139/1463.abstract.

[13] Ron A. M. Fouchier, Yoshihiro Kawaoka, et al., "Avian Flu: Gain-of-function experiments on H7N9," *Nature* Vol. 500 (8 August 2013): 150-151, http://www.nature.com/nature/journal/v500/n7461/full/500150a.html.

[14] Troy C. Sutton et al., "Airborne Transmission of Highly Pathogenic H7N1 Influenza in Ferrets," Journal of Virology (2 April 2014), http://jvi.asm.org/content/early/2014/03/27/JVI.02765-13.abstract

[15] Kenneth Mark Coggeshall et al., "The sepsis model: an emerging hypothesis for the lethality of inhalation anthrax, "*Journal of Cellular and Molecular Medicine*, Vol 17, no. 7 (17 July 2013): 914-920, http://www.ncbi.nlm.nih.gov/pubmed/23742651.

[16] Roger D. Pechous et al., "Early Host Cell Targets of Yersinia pestis during Primary Pneumonic Plague" *PLOS Pathogens* (3 October 2013), http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003679.

[17] Jessica Knepper et al., "The Novel Human Influenza A(H7N9) Virus is Naturally Adapted to Efficient Growth in Human Lung Tissue," *mBio*, Vol. 4, no. 5 (8 October 2013), http://mbio.asm.org/content/4/5/e00601-13.abstract.

Typhoidal Salmonella strains;[18] and how strain differences lead to different innate immune responses, which in turn impacts mortality rates.[19]

9. There have been advances in the understanding of plant-pathogens interaction, including the structure and function of virulence proteins.[20]

10. There is a better understanding of the role played by some cytokines, which are small proteins involved in cell signalling, during the course of diseases, for example the detected cytokine response in A(H1N1)pdm09 influenza patients.[21]

11. There has been progress in understanding how bacteria and viruses spoof or hijack host machinery to regulate their own proliferation,[22] for example for the bacteria *Streptococcus pyogenes*.[23]

## C. Toxicology

12. A new botulinum toxin serotype, Type H, was discovered in 2013, over 40 years after the last serotype discovery.[24] There are now eight known serotypes of this toxin. The

[18] Christina Bronowski et al., "Genomic Characterisation of Invasive Non-Typhoidal Salmonella enterica Subspecies enterica Serovar Bovismorbificans Isolates from Malawi," *PLOS Neglected Tropical Diseases* (14 November 2013), http://www.plosntds.org/article/info%3Adoi%2F10.1371%2Fjournal.pntd.0002557.

[19] Jian Wu et al., "Strain-specific innate immune signaling pathways determine malaria parasitemia dynamics and host mortality," *PNAS*, Vol. 111, no. 4 (28 January 2014): E511-20, http://www.ncbi.nlm.nih.gov/pubmed/24474800.

[20] Lennart Wirthmueller et al., "On the front line: structural insights into plant-pathogen interactions," Nature Reviews Microbiology, Vol.11, no.11 (November 2013): 761-776, http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3118.html.

[21] A. Bradley-Steward et al., "Cytokine responses in patients with mild of severe influenza A(H1N1) pdm09," The Journal of Clinical Virology, Vol. 58, no. 1 (September 2013): 100-107, http://www.ncbi.nlm.nih.gov/pubmed/23790455.

[22] Vanderbilt University Medical Center, Department of Pathology, Microbiology, and Immunology, "Microbial Pathogenesis and Host Interactions Journal Club," 2 May 2014, http://www.mc.vanderbilt.edu/root/vumc.php?site=vmcpathology&doc=35941 ;

[23] Moshe Baruch et al., "An Extracellular Bacterial Pathogen Modulates Host Metabolism to Regulate Its Own Sensing and Proliferation," *Cell*, Vol. 156, no. 1-2 (19 January 2014): 97-108, http://www.cell.com/cell/abstract/S0092-8674%2813%2901539-0.

[24] The two original papers are:
Jason R. Barash, Stephen S. Arnon, "A Novel Strain of Clostridium botulinum That Produces Type B and Type H Botulinum Toxins," *The Journal of Infectious Diseases*, Vol. 209, no. 2 (7 October 2013): 183-191, http://jid.oxfordjournals.org/content/early/2013/10/07/infdis.jit449.short.
Nir Dover et al., "Molecular Characterization of a Novel Botulinum Neurotoxin Type H Gene," The Journal of Infectious Diseases, Vol. 209, no. 2 (7 October 2013): 192-202, http://jid.oxfordjournals.org/content/209/2/192.
Several discussion papers have also been published:
David C. Hooper, Martin S. Hirsch, "Novel Clostridium botulinum Toxin and Dual Use Research of Concern Issues," The Journal of Infectious Diseases, Vol. 209, no. 2 (7 October 2013): 167, http://jid.oxfordjournals.org/content/209/2/167;
Michel R. Popoff, "Botulinum Neurotoxins: More and More Diverse and Fascinating Toxic Proteins," *The Journal of Infectious Diseases*, Vol. 209, no. 2 (7 October 2013): 168-169, http://jid.oxfordjournals.org/content/209/2/168;
David A. Relman, "Inconvenient Truths" in the Pursuit of Scientific Knowledge and Public Health," *The Journal of Infectious Diseases*, Vol. 209, No. 2 (7 October 2013): 170-172, http://cisac.stanford.edu/publications/inconvenient_truths_in_the_pursuit_of_scientific_knowledge_and_public_health/.

genetic sequence was voluntarily withheld by the researchers, at least until an antitoxin can be developed.

13. There is a greater understanding of ricin toxin including: work on neutralizing monoclonal antibodies for ricin, with vaccine potential;[25] and the creation of a ricin genetic interaction map, with therapy potential.[26]

14. New details of toxin evolution has been uncovered thanks to the genome sequencing of toxins; for example, the dynamic predator-prey evolution of cobra toxin has been analysed using this technique.[27]

15. Efforts have been made to improve the efficacy of testing the toxicity of chemicals, including through the use of living human tissues grown through 3D bioprinting.[28]

## D. Resistance

16. There has been progress in identifying resistance genes, in identifying mutations which lead to these resistance genes, and in knowing whether mutations for resistance impact replication and pathogenicity. Some examples include: the identification of malaria resistance genes in a species of mosquito;[29] the partial identification of H1N1 mutations leading to Tamiflu resistance;[30] and the finding that oseltamivir resistance gain in H7N9 can occur without substantially altering its virulence and pathogenicity.[31]

[25] Anastasiya Yermakova, David J. Vance, Nicholas J. Mantis, "Sub-Domains of Ricin's B Subunit as Targets of Toxin Neutralizing and Non-Neutralizing Monoclonal Antibodies," *PLoS One*, Vol. 7, no. 9 (September 11, 2012), http://www.ncbi.nlm.nih.gov/pubmed/22984492.

[26] Bassick MC et al., "A systematic mammalian genetic interaction map reveals pathways underlying ricin susceptibility," *Cell*, Vol. 152, no. 4 (8 February 2013): 909-922, http://www.ncbi.nlm.nih.gov/pubmed/23394947.

[27] Freek J. Vonk et al., "The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system," *PNAS* (22 October 2013) http://www.pnas.org/content/early/2013/11/27/1314702110.abstract.

[28] Signe Brewster, "NIH partners with Organovo to test new drugs on 3D printed living tissue," GIGAOM, January 14, 2014, https://gigaom.com/2014/01/14/nih-partners-with-organovo-to-test-new-drugs-on-3d-printed-living-tissue/;
Matthew D. Segall, Chris Barber, "Addressing Toxicity Risk when Designing and Selecting Compounds in Early Drug Discovery," *Drug Discovery Today* (19 January 2014), http://www.ncbi.nlm.nih.gov/pubmed/24451294.

[29] Jun Li et al., "Genome-block expression-assisted association studies discover malaria resistance genes in Anopheles gambiae," *PNAS* (11 November 2013), http://www.pnas.org/content/early/2013/11/27/1321024110.short?rss=1.

[30] Nicholas Renzette, "Evolution of the Influenza A Virus Genome during Development of Oseltamivir Resistance *In Vitro*," *Journal of Virology*, Vol. 88, no. 1 (October 2013): 272-281, http://jvi.asm.org/content/88/1/272.

[31] Rong Hai et al., "Influenza A(H7N9) virus gains neuramidnidase inhibitor resistance without loss of in vivo virulence or transmissibility," Nature Communications, Vol. 4 (June 23, 2013), http://www.nature.com/ncomms/2013/131210/ncomms3854/full/ncomms3854.html.

## E. Immunology and host response avoidance

17. Progress has been made in identifying immune system evasion mechanisms used by a number of pathogens, including: those which use RNA structural motifs;[32] the evasion role of surface structures of flaviviruses;[33] and a new hypothesis which holds that the primary effect of the *B. anthracis* toxin in inhalational anthrax cases is to assist in host response evasion.[34]

18. Progress has also been made in understanding pathogen - host response dynamics, including: the interplay between *Mycobacterium tuberculosis* and the host macrophage;[35] better understanding of MicroRNSas and their role in host antimicrobial defense;[36] and an increased recognition of the crucial role of miRNA in regulating antimicrobial defense.[37]

19. Investigation into the persistence of immune response in humans to disease has continued, including that of persistent immune responses following Ebola Virus infection.[38]

20. A "generic" antibody-binding protein has been found, and which may be used by certain bacteria for host immune response evasion.[39] The protein is capable of binding with a broad array of antibodies, preventing the latter from functioning. Previously, antibody-binding proteins had been highly specific to a particular antibody. Research on potential applications is ongoing, including for novel anti-bacterial therapeutics.

## F. How pathogens evolve and spread

21. Advances in enabling technologies, such as genome sequencing, continue to reveal valuable information on a number of pathogens. Some examples of new findings include

[32] Jennifer L. Hyde et al., "A Viral RNA Structural Element Alters Host Recognition of Nonself RNA," Science, Vol. 343, no. 6172 (February 14, 2014): 783-787, http://www.sciencemag.org/content/343/6172/783.abstract.

[33] David L. Akey, "Flavivirus NS1 Structures Reveal Surfaces for Associations with Membranes and the Immune System," *Science*, Vol. 343, no. 6173 (21 February 2014): 881-885, http://www.sciencemag.org/content/343/6173/881.abstract.

[34] Kenneth Mark Coggeshall et al., "The sepsis model: an emerging hypothesis for the lethality of inhalation anthrax," *The Journal of Cellular and Molecular Medicine*, Vol. 17, no. 7 (July 2013): 914-920, http://www.ncbi.nlm.nih.gov/pubmed/23742651.

[35] Manikuntala Kundu, Joyoti Basu, "Mycobacterium tuberculosis and the host macrophage: maintaining homeostatis or battling for survival?" *Current Science*, Vol. 105, no. 5 (September 2013): 617, http://connection.ebscohost.com/c/articles/90546356/mycobacterium-tuberculosis-host-macrophage-maintaining-homeostasis-battling-survival.

[36] Katherine J. Siddle et al., "A Genomic Portrait of the Genetic Architecture and Regulatory Impact of microRNA expression in response to infection," Genome Research (2014), http://genome.cshlp.org/content/early/2014/01/30/gr.161471.113.

[37] Cristel Archambaud et al., "The Intestinal Microbiota Interferes with the microRNA Response upon Oral Listeria Infection," mBio, Vol. 4, no. 6 (10 December 2013), http://mbio.asm.org/content/4/6/e00707-13.

[38] Ariel Sobarzo et al., "Persistent Immune Responses after Ebola Virus Infection," The New England Journal of Medicine, Vol. 369 (2013): 492-493, http://www.nejm.org/doi/full/10.1056/NEJMc1300266.

[39] The Scripps Research Institute, "The Ultimate Decoy: Scientist Find Unique Protein that Misdirects Immune System," *News & Views*, Vol. 14, Issue 7 (3 March 2014), www.scripps.edu/newsandviews/e_20140303/lerner.html;
Rajesh K. Grover et al., "A Structurally Distinct Human Mycoplasma Protein that Generically Blocks Antigen-Antibody Union," *Science*, 343, no. 6171 (7 February 2014): 656-661, https://www.sciencemag.org/content/343/6171/656/suppl/DC1.

the formulation of a hypothesis to explain Ebola virus evolution based on an analysis of 27 Ebola virus strains from 5 species;[40] increased evidence that bats are a natural reservoir for Ebola and Marburg viruses based on the complete viral genome sequence studies of four viral hemorrhagic fever outbreaks;[41] and the discovery based on genomic analysis that the *Y. pestis* strain responsible for the first known human plague pandemic is a novel branch in the *Y. pestis* phylogeny.[42]

22. Progress continues on building a better understanding of influenza, in particular its capability for mutation, including: details of reassortments between H7N9 and H9N2 strains;[43] and the determination of actual substitution positions in H3N2, which revealed that H3N2 mutations were more predictable than thought.[44]

# III. Advances in enabling technologies

## A. Characterizing biological systems and networks

23. DNA sequencing costs have continued to decrease: in January 2014, the price for sequencing a genome was down to roughly $4000, from just over $5000 in October 2013.[45] Similarly, the cost of human gene mapping has steadily gone down.[46]

24. Developments in gene sequencing are also illustrating the importance of non-coded regulatory control, for example, there has been progress in mapping the epigenetic basis of complex traits.[47]

25. Proteomics (the study of all the proteins in an organism) has continued to benefit from technology developments and new techniques. For instance, there has been progress in rapidly determining the biological functions of a proteome by application of Proteomics Expansion Pipeline (PEP) technology;[48] and a new method called High-Resolution IsoElectric Focusing (HiRIEF) Liquid Chromatography–Mass Spectrometry (LC-MS)

[40] Y. H. Li, S. P. Chen, "Evolutionary History of Ebola Virus," Epidemiology and Infection, Vol. 142, no. 6 (June 2014): 1138-1145, http://www.ncbi.nlm.nih.gov/pubmed/24040779.

[41] C.G. Albarino, "Genomic analysis of filoviruses associated with four viral hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo in 2012," *Virology*, Vol. 442, no. 2 (1 August 2013): 97-100, http://www.sciencedirect.com/science/article/pii/S0042682213002237.

[42] David M. Wagner et al., "Yersinia pestis and the Plague of Justinian 541-543 AD: a genomic analysis," *The Lancet Infectious Diseases*, Vol. 14, no. 4 (April 2014): 319-326, http://www.thelancet.com/journals/laninf/article/PIIS1473-3099%2813%2970323-2/abstract.

[43] Z. Meng et al., "Possible Pandemic Threat From New Reassortment of Influenza A(H7N9) Virus in China," *Eurosurveillance* 19, no. 6 (February 2014).

[44] Björn F. Koel et al., "Substitutions Near the Receptor Binding Site Determine Major Antigenic Change During Influenza Virus Evolution", *Science*, Vol. 342, no. 6161 (22 November 2013): 976-979, https://www.sciencemag.org/content/342/6161/976.abstract.

[45] National Human Genome Research Institute, "DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP)," 8 April 2014, http://www.genome.gov/27541954.

[46] Ashlee Vance, "Human Gene Mapping Price to Drop to $1,000, Illumina Says," *Bloomberg*, 15 January 2014, http://www.bloomberg.com/news/2014-01-15/human-gene-mapping-price-to-drop-to-1-000-illumina-says.html.

[47] Sandra Cortijo et al., "Mapping the Epigenetic Basis of Complex Traits," *Science*, Vol. 343, no. 6175 (7 March 2014): 1145-1148, https://www.sciencemag.org/content/343/6175/1145.abstract.

[48] Xing Wang, Michael Davies, David Wang, "New Tool Facilitates Functional Proteomics: PEP Technology Can Also Be Used to Speed Protein Purification," *Genetic Engineering & Biotechnology* News, Vol. 34, no. 3 (1 February 2014), http://www.genengnews.com/gen-articles/new-tool-facilitates-functional-proteomics/5126/.

enables deeper proteome coverage and unbiased proteogenomics, enabling identification of previously unknown protein-coding loci.[49]

26. Advances in metabolomics (the study of all the biochemical processes or metabolism of an organism) have potential application to microbial forensics. For example, unique strain-specific metabolic processes can increasingly be matched with particular environmental niches, allowing for greater specificity in the origin of a pathogen.[50]

27. A genetic interaction mapping system for mammals has been designed. The technique is based on combinatorial RNA interference, and allows one to map genetic pathways of different phenotypes.[51]

28. Experimental-computational techniques for inferring network models of cell response to perturbations has continued to mature.[52] These models can be used, for example, to estimate drug effects on particular cell lines.

## B. Engineering biological systems and networks

29. It is now possible to design organisms with an expanded genetic alphabet.[53] That is, one can add new unnatural base pairs to expand on the standard two-base-pair genetic alphabet (A-T and G-C) in DNA.

30. It is now increasingly possible to employ DNA manipulation and gene circuit engineering on mammalian cells, in part through the use of the CRISPER-Cas9 system. For example, gene-modified monkeys have been engineered using this method.[54]

31. The capability to genetically modify insects is increasingly harnessed; for instance, the use of genetically modified mosquitoes has been approved in Brazil in an attempt to fight dengue fever.[55]

32. The synthesis of a eukaryotic chromosome has been achieved.[56] Progress continues in the quest to synthesize an entire yeast genome and is being pursued by an intercontinental research group.

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[49] Rui M M Branca et al., "HiRIEF LC-MS enables deep proteome coverage and unbiased proteogenomics," *Nature Methods*, Vol. 11 (2014): 59-62, http://www.nature.com/nmeth/journal/v11/n1/full/nmeth.2732.html.

[50] Jonathan M. Monk et al., "Genome-scale metabolic reconstructions of multiple Escherichia coli strains highlight strain-specific adaptations to nutritional environments," *PNAS*, (25 November 2013), http://www.pnas.org/content/early/2013/11/20/1307797110.abstract.

[51] Bassick MC et al., "A systematic mammalian genetic interaction map reveals pathways underlying ricin susceptibility," *Cell*, Vol. 154, no. 4 (14 February 2013): 909-922, http://www.ncbi.nlm.nih.gov/pubmed/23394947.

[52] Evan J. Molinelli et al., "Perturbation Biology: Inferring Signaling Networks in Cellular Systems," *PLOS Computational Biology* (19 December 2013), http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1003290.

[53] Denis A. Malyshev et al., "A Semi-synthetic Organism with an Expanded Genetic Alphabet," *Nature*, Vol. 509 (15 May 2014): 385-388, http://www.nature.com/nature/journal/v509/n7500/full/nature13314.html.

[54] Yuyu Niu et al., "Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated gene Targeting in One-Cell Embryos," *Cell*, Vol. 156, no. 4 (13 February 2014): 836-843, http://www.cell.com/abstract/S0092-8674%2814%2900079-8.

[55] "Brazil approves use of genetically modified mosquitoes," New Scientist, 23 April 2014, http://www.newscientist.com/article/dn25457-brazil-approves-use-of-genetically-modified-mosquitoes.html#.U3Cgclfokak..

33. There is an increased capacity for the design and employment of biological scaffolds. For example, there is a new understanding of bacteria growth patterns, which can be used to create synthetic gene circuits for biological scaffolds.[57]

34. It is now possible to "write" membranes onto graphene surfaces through the use of Lipid Dip-Pen Nanolithography, which can be applied to biosensor design.[58]

35. The capability to "reprogram" killer T lymphocyte cells, for instance to specifically target cancer cells or HIV, is now well established.[59] This research provides an avenue for novel disease treatment.[60]

36. There is progress in achieving better control of production processes. For instance, there is now a better understanding of noise propagation, which can cause significant variation in gene expression and phenotype in genetically identical cells exposed to the same environment.[61] This has applications for handling noise in synthetic circuits.

37. Complementing lab work developments, there have also been developments in prediction and planning software, including: generating effective RNA molecule designs through a successful crowd-sourced-with-expert-feedback project;[62] and progress in computational enzyme design.[63]

## C. Gathering and manipulating biological information

38. The development of enhanced algorithms to assist with gathering and manipulating biological information and meta-information is becoming increasingly important. For example, DNA sequencing algorithms often limit the speed of sequencing, rather than the device itself.[64] Meta-information analysis, where software is used to scan and make

[56] Narayana Annaluru et al., "Total Synthesis of a Functional Designer Eukaryotic Chromosome," Science, Vol. 344, no. 6179 (April 4, 2014): 55-58, http://www.sciencemag.org/content/344/6179/55.abstract ;
Elizabeth Pennisi, "Building the Ultimate Yeast Genome," *Science*, Vol. 343, no. 6178 (28 March 2014): 1426-1429, https://www.sciencemag.org/content/343/6178/1426.summary.

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[59] Raul Vizcardo et al., "Regeneration of human tumor antigen-specific T cells from iPS cells derived from mature CD8+ T cells," *Cell Stem Cell*, Vol. 12, no. 1 (3 January 2013): 31-36, http://www.cell.com/cell-stem-cell/abstract/S1934-5909%2812%2900711-4 ;
Nishimura T. et al., "Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation," *Cell Stem Cell*, Vol. 12, no. 1 (3 January 2013): 114-126, http://www.cell.com/cell-stem-cell/abstract/S1934-5909%2812%2900636-4.

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[63] Gert Kiss et al., "Computational enzyme design," *Angewandte Chemie*, Vol. 52, no. 22 (25 March 2013): 5700-5725, http://onlinelibrary.wiley.com/doi/10.1002/anie.201204077/abstract.

[64] Michael C. Schatz, Ben Langmead, "The DNA Data Deluge," *IEEE Spectrum*, 27 June 2013, http://spectrum.ieee.org/biomedical/devices/the-dna-data-deluge.

predictions based on scientific literature, has shown success in harnessing the increasingly vast worldwide life science output.[65]

39. Improvements have been made in modelling and predicting the impacts of overexpressing genes. This is an approach common in research to determine gene function and in biotechnology to increase yields.[66]

40. There is continuing interest in improving the ability to convert biological material into data and back.[67] The idea is as follows: an organism (or part of an organism) is sequenced in one place, the sequenced data is transmitted electronically to a second location, and the sequence data is then used to recreate the organism. A recent paper detailing a platform that could be used in this manner for vaccine development has been published.[68]

## D. Generic enabling technologies

41. There is a better understanding of genome dynamics during evolution, more specifically in how the rate and nature of genotypic changes relate to phenotypic changes.[69]

42. There have been improvements in the capability of separating microbial DNA from host DNA, which will lead to improvements in microbial genome databases and a better understanding of how these are linked to organism functions.[70]

43. The CRISPER-Cas9 system continues to be refined, allowing for better genome editing abilities, based on: a better understanding of the Cas9 enzyme family;[71] and new guide RNAs for the system.[72]

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[70] Fiona Stewart et al., "Selective Enrichment of Microbial DNA: Separation by Differential Methylation Density Reduces Whole Microbiome DNA Sequencing Cost," *Genetic Engineering & Biotechnology News*, Vol. 34, no. 3 (1 February 2014), http://www.genengnews.com/keywordsandtools/print/1/33754/.

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[72] Yanfang Fu et al., "Improving CRISPR-Cas nuclease specificity using truncated guide RNAs," Nature Biotechnology, Vol. 32 (2014): 279-284, http://www.nature.com/nbt/journal/v32/n3/full/nbt.2808.html.

# IV. Dealing with diseases and toxins

## A. Detection

44. Progress continues on toxin detection, including: enhanced quality assurance for the detection of biological toxins;[73] the development of biochips capable of detecting several different toxins;[74] progress in ricin detection equipment;[75] progress in saxitoxin detection without the use of antibodies or animals;[76] and a biosensor for the detection of paralytic shellfish toxins in marine algae.[77]

45. Simultaneous multiple bacteria fingerprinting technology has progressed, in part through applications of nanotechnology and photonics. For instance, a system to detect meningitis has been developed where silver nanoparticles are combined with a sample, a laser beam is shone onto it, and the shift in wavelength is used to identify bacteria.[78]

46. DNA sample analysis continues to gain speed, with multiple detection applications.[79]

## B. Prevention and prophylaxis

47. The technique of genetically engineering live vaccines continues to mature.[80]

48. A wide range of vaccines based on a number of innovative techniques are currently in development. For instance, there have been: attempts at bivalent vaccines based on

[73] Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk, http://equatox.net/

[74] Chirstopher Pöhlmann, Thomas Elßner, "Fully-Automated Electrochemical Biochip Platform for Rapid, Simultaneous and Sensitive Bioagent Detection," http://www.foi.se/Global/V%C3%A5ra%20tj%C3%A4nster/Konferenser%20och%20seminarier/CBW%20symposium/Proceedings/Elssner.pdf.

[75] Anderson GP et al., "Single domain antibody-quantum dot conjugates for ricin detection by both fluoroimmunoassay and surface plasmon resonance," *Analytica Chimica Acta*, Vol. 786 (5 July 2013): 132-138, http://www.sciencedirect.com/science/article/pii/S0003267013006594 ; Huebner M et al., "A Glyco-chip for the Detection of Ricin by an Automated Chemiluminescence Read-out System," *Analytical Sciences*, Vol. 29, no. 4 (2013): 461-466.

[76] Sara M. Handy et al., "First report of the use of a saxitoxin-protein conjugate to develop a DNA aptamer to a small molecule toxin," *Toxicon*, Vol. 61 (January 2013): 30-37.

[77] Julie P. Meneely et al., "Development and validation of an ultrasensitive fluorescence planar waveguide biosensor for the detection of paralytic shellfish toxins in marine algae," *Biosensors and Bioelectronics*, Vol. 41 (15 March 2013): 691-697.

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antibodies, for example an Ebola virus – Rabies Virus dual vaccine;[81] and attempts at a pan H-1 influenza vaccine.[82]

49. Finally, innovative research has been conducted on how a building's architectural design affects bacterial communities.[83] On top of wide-ranging public health and biosafety applications, this has potential security applications in designing buildings to be more attack-resistant and easier to decontaminate.

## C. Therapeutics

50. Antibodies are also being harnessed for therapeutics; for example, progress towards an Ebola virus treatment based on antibodies that could be effective following the onset of symptoms.[84]

51. There have also been progress in the use of biopharming; for instance, the aforementioned experimental antibody therapeutics for Ebola virus disease is produced by a bioengineered tobacco plant.

52. mRNA therapeutics has seen continued commercialization.[85] It is hoped that the technique will speed up development and manufacture of treatments for rare diseases.[86]

53. Deep-sea marine ecosystems are increasingly tapped for their potential to provide novel sources of chemicals for therapeutics; for instance a potential anthrax antibiotic has been developed based on a marine microorganism product.[87]

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[81] Joseph E. Blaney, "Antibody Quality and Protection from Lethal Ebola Virus Challenge in Nonhuman Primates Immunized with Rabies Virus Based Bivalent Vaccine," *PLOS Pathogens* (May 30, 2013), http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003389.

[82] Florian Kramer et al., "Assessment of influenza virus hemagglutinin stalk-based immunity in ferrets," *Journal of Virology*, Vol. 88, no. 6 (January 8, 2014): 3432-3442, http://www.ncbi.nlm.nih.gov/pubmed/24403585.

[83] Steven W. Kembel et al., "Architectural Design Derives the Biogeography of Indoor Bacterial Communities," PLOS ONE (January 29, 2014), http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0087093.

[84] James Pettitt et al., "Therapeutic Intervention of Ebola Virus Infection in Rhesus Macaques with the MB-003 Monoclonal Antibody Cocktail," *Science Translational Medicine*, Vol. 5, no. 199 (21 August 2013): 199ra133, http://stm.sciencemag.org/content/5/199/199ra113.

[85] For more information on such approaches see: R. Scott McIvor, "Therapeutic Delivery of mRNA: The Medium Is the Message," *Molecular Therapy*, Vol. 19, no. 5 (2011): 822-823, http://www.nature.com/mt/journal/v19/n5/full/mt201167a.html.

[86] "Alexion, Moderna to Develop Rare Disease-Fighting mRNA Therapeutics," GEN, 13 January 2014, http://www.genengnews.com/gen-news-highlights/alexion-moderna-to-develop-rare-disease-fighting-mrna-therapeutics/81249355/.

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