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Synthetic biology and biotechnology for military medicine and chemical and biological defence

– current state and future perspectives

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Summary

This report presents a horizon scan of potential capabilities within military medicine and chemical and biological (CB) defence based on technological developments in synthetic biology and biotechnology. It is based on a review of existing literature from open sources and NATO reports.

Synthetic biology and biotechnology have gained increasing attention by NATO and defence research institutes, and they are highlighted in the NATO Science and Technology Trend Report 2023-2043 as an Emerging and Disruptive Technology (EDT). Synthetic biology includes technologies that enable design and modification of organisms and biological systems to perform new tasks. Rapid advances within synthetic biology and biotechnology enable improvements in areas such as medicine, materials, sustainability, and energy production, potentially benefitting the defence sector in the form of strategic technological advantages and enhanced preparedness. However, improved access outside regulated labs raises concerns due to open-source pathogen genomics, de-skilling in biology, and affordable DNA synthesis. This underscores the need to stay informed about advancements to effectively counter potential malevolent applications.

Our report highlights the potential benefits of synthetic biology while reviewing its core technologies, like DNA synthesis and sequencing, genetic engineering, stem cell technology, and biosensors. We explore how these advancements can benefit both military medical services and CB defence, as well as yield benefits for civilian preparedness and public health. Our report places particular emphasis on medical countermeasures and detection applications, including efforts to address the critical issue of antibiotic resistance. Furthermore, we explore treatments for physical trauma, including innovative methods like regenerative medicine, which incorporates advanced technologies like stem cells and 3D-printing. In addition, we highlight the potential of gene sequencing for detection and identification purposes (DIM), disease prevention, treatment, and soldier enhancement, including ethical and privacy concerns as key barriers to its advancement.

Our general recommendations include allocating significant resources to research, development, testing, and evaluation (RDT&E) and fostering collaboration with academia and private enterprises. However, ethical framework and governance must be addressed as soon as possible. The security risks are another challenge posed by the convergence of biotechnology and digital technologies, which require innovative solutions to safeguard sensitive data and research.

Our report also presents capability-specific suggestions. First, the development of DIM methods for genetically engineered microbes within the realm of CB defence should be encouraged. Additionally, biotechnological approaches that enable rapid, on-site production of medical countermeasures should be explored. Attention should also be given to regulatory frameworks concerning human enhancement, encompassing genetic modifications of humans and genetically modified organisms (GMOs) for both medical and non-medical applications. Lastly, it is essential to adopt and adapt civilian applications of synthetic biology and biotechnologies, leveraging them for DIM capabilities, the development of new antibiotics and vaccines, and the advancement of technology aimed at combating antimicrobial-resistant microbes.

Sammendrag

I denne rapporten presenterer vi en oversikt over militære kapabiliteter innen medisin og kjemisk og biologisk (CB) vern som kan dra nytte av utviklingen innenfor syntetisk biologi og bioteknologi. Rapporten er basert på eksisterende litteratur fra åpne kilder og NATO-rapporter.

Syntetisk biologi og bioteknologi har fått økt oppmerksomhet i NATO og fra ulike forsvarsforskningsinstitutter. I NATO Science and Technology Trend Report 2023-2043 blir de sett på som nye og banebrytende teknologier (*emerging and disruptive technologies*). Syntetisk biologi omfatter teknologier som gjør det mulig å designe og modifisere organismer og biologiske systemer til å utføre nye, ønskede oppgaver. Syntetisk biologi og bioteknologi er i rask utvikling og gir enorme muligheter innen blant annet medisin, materialer, bærekraft og energiproduksjon, som kan ha stor nytteverdi for forsvarssektoren. Samtidig gir utviklingen økt bekymring for ondsinnet bruk av disse teknologiene på grunn av åpen tilgang til patogeners genomsekvenser, fordi det trengs mindre ferdigheter for å få til biologisk manipulasjon (*de-skilling* i biologien), og på grunn av lavere syntesekostnader. Dette understreker hvor viktig det er å holde seg oppdatert på teknologiske fremskritt for å effektivt motvirke potensielle ondsinnede anvendelser.

I denne rapporten fremhever vi fordelene og mulighetene innenfor syntetisk biologi, og vi går gjennom sentrale teknologier bak utviklingen, blant annet DNA-syntese og -sekvensering, genredigering, stamcelleteknologi og biosensorer. Vi utforsker hvordan disse teknologiske fremskrittene kan være til fordel for forsvarssektoren og for sivil beredskap og folkehelse. Vi legger særlig vekt på medisinske mottiltak og deteksjon, blant annet problemstillingene rundt og mulige løsninger på antibiotikaresistens. Videre tar vi for oss fremtidens behandlinger av fysisk traume ved hjelp av regenerativ medisin der bruk av stamceller og 3D-printing kan spille en viktig rolle. Vi fremhever potensialet for gensekvensering i å forebygge og behandle sykdommer, i deteksjon og identifikasjon (DIM) og i forbedring av soldater. Vi tar også for oss etiske og personvernsmessige bekymringer som sentrale hindringer for utviklingen.

De generelle anbefalingene fra arbeidet med denne rapporten inkluderer at betydelige ressurser bør tildeles forskning, utvikling, testing og evaluering, og at samarbeidet med akademia og private aktører bør fremmes. Samtidig må utviklingen av etiske rammeverk og styring settes på dagsordenen. Sikkerhetsrisikoene som oppstår når bioteknologi og digitale teknologier konvergerer, er også en utfordring, og det kreves innovative løsninger for å sikre sensitive data og forskning.

Vi har også noen kapabilitetsspesifikke forslag i tillegg til de generelle anbefalingene. For det første bør det legges vekt på å utvikle DIM-metoder for genetisk endrede mikroorganismer som en del av CB-vernet. I tillegg bør det utforskes teknologier som gjør rask, lokal produksjon av medisinske mottiltak mulig. Videre bør det etableres regulatoriske rammeverk knyttet til menneskelig forbedring, som omfatter genetisk modifikasjon av mennesket og genetisk endrende organismer (GMO), til både medisinsk og ikke-medisinsk bruk. Til slutt er det vesentlig å få frem at det er viktig å omfavne sivile anvendelser av syntetisk biologi og bioteknologi, inkludert DIM-kapabiliteter, nye antibiotika, vaksiner og teknologi som bidrar til å bekjempe antibiotikaresistens.

Contents

Abbreviations

AI	Artificial intelligence
AMP	Antimicrobial peptide
BTWC	Biological and Toxin Weapons Convention
Cas	CRISPR associated protein
CB	Chemical and biological
CBRN	Chemical, biological, radiological and nuclear
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CWC	Chemical Weapons Convention
DNA	Deoxyribonucleic acid
DoD	US Department of Defense
EDT	Emerging and disruptive technologies
FDA	Food and drug administration
G6PD	Glucose-6-phosphatase dehydrogenase
GMO	Genetically modified organism
Hb	Haemoglobin
HBOCs	Haemoglobin-based oxygen carriers
HDR	Homology-directed repair
HIV	Human immunodeficiency viruses
iPSC	Induced pluripotent stem cells
LFA	Lateral flow assay
ML	Machine learning

MSC	Mesenchymal stem cell
NASA	National Aeronautics and Space Administration
NHEJ	Non-homologous end joining
PCR	Polymerase-chain reaction
PFC	Perfluorocarbons
RBC	Red blood cell
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
sgRNA	Single guide RNA
TALENs	Transcriptional activator-like effector nucleases
TNO	Netherlands Organisation for Applied Scientific Research
TRL	Technology readiness level
WBC	White blood cell
ZFN	Zink finger nucleases

Definitions

Bactericidal	Kills bacteria.
Bacteriostatic	Prevents growth of bacteria.
Epigenome	The chemical changes to the DNA and histone proteins that regulate the expression of the genes.
Genome	All genetic information of an organism.
Genotype	An organism's hereditary information
Microbiome	The community of microorganisms found living together in the same habitat.
Phenotype	An organism's observed properties
Proteome	The entire set of proteins that can be expressed by a genome, organism, cell or tissue at a certain time.
Transcriptome	The set of all RNA-transcripts, including coding and non-coding, in an individual.

1 Introduction

Synthetic biology is the science of designing and modifying organisms and biological systems to perform new tasks. Synthetic biology uses our understanding of how deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) dictate functional properties of proteins, and thus cells, as well as our ability to synthesise novel DNA strands, to engineer novel biological systems that can theoretically adopt any function. Reengineering of biological machinery has applications in medicine, industry, energy, agriculture and environmental protection; however, it can also be used for malignant purposes and may become a significant security risk. Synthetic biology research has the ability to boost defensive capabilities in a range of sectors and a deeper understanding of the science is necessary to ensure responsible development and adoption, combat future threats, and maximize the benefit for the Defence sector and rest of society.

Synthetic biology is a term that dates back to 1980, but that remained an obscure field of science until around 2004. Fueled by the sequencing era of biological research, together with recent advances in genome editing and DNA synthesis capabilities, the number of synthetic biology, primary article publications grew exponentially during the early 2010s (see Figure 1.1). These and other technologies within medicine and chemical and biological (CB) defence are described in the next chapter (Table 2.1 and Section 2).

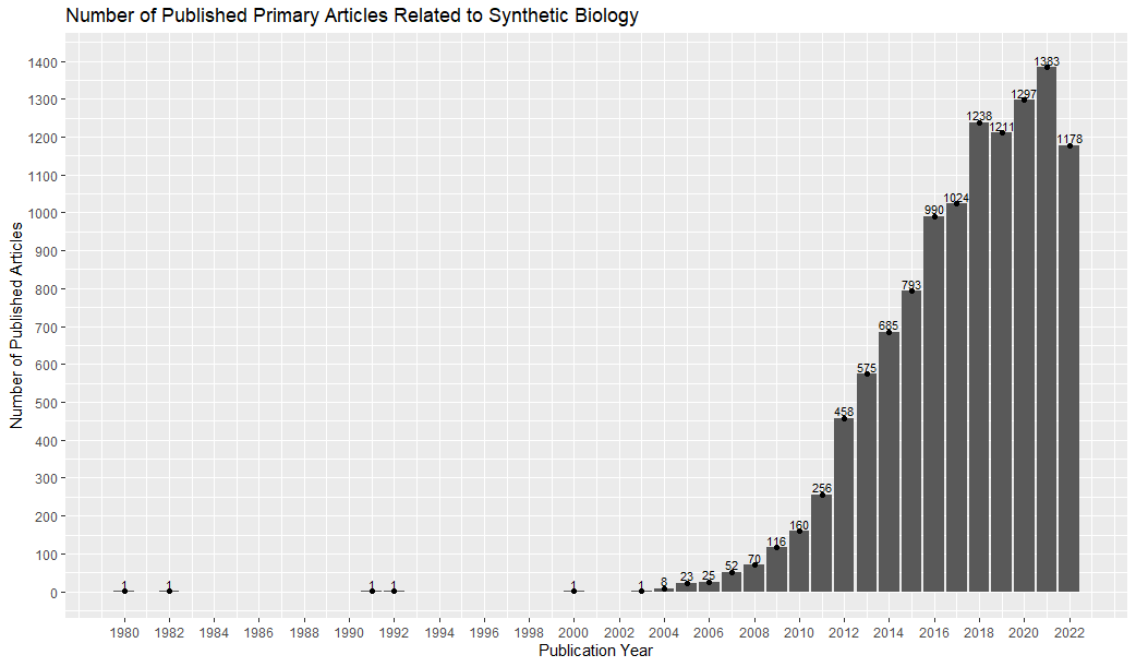

Figure 1.1 The number of published primary articles each year mentioning "Synthetic biology" in the title, abstract or keywords obtained from the Web of Science bibliographic database (retrieved 07.12.2022).

Besides applications in military medicine, synthetic biology may be impactful in many other areas that can increase military resilience and readiness. One such area is energy storage and generation. Sustainable energy production solutions based on synthetic biology may meet both military and civilian needs, by providing persistent power supplies to remote areas, produced in environmentally friendly ways. Synthetic biology may also be applicable for agricultural purposes, making plants more resistant to infections and increasing the production yield [1].

The growth in synthetic biology research has greatly benefited from, and contributed to public databases of genetic information that now flourish online, e.g. GenBank [2] and UniProt [3]. These databases contain a vast amount of annotated genetic sequences, protein-protein interactions, disease-related mutations, and regulatory pathways, all contributing to an expanding toolbox of useful biological processes. As all genetic information is encoded in the same format of nucleotide sequences, which can now be synthetically synthesized, modified and added to existing DNA, the databases provide instructions for creating modules of biological function [4]. Increasingly, synthetic biologists are designing biological mechanisms and organisms using a plug-and-play method, where the modules from different sources can be combined to create de-novo synthetic biology [1].

As research continues to expand the synthetic biology toolbox, industrial applications are now being realized. The synthetic biology market value is expected to grow from around 10 billion USD in 2022 to approximately 70 billion USD in 2030 (see Figure 1.2). This indicates a moderately rapid adoption of recent scientific advances by the industry. Furthermore, McKinsey global institute predicts that synthetic biology will have a direct yearly economic impact of up to 4 trillion USD over the next 10 to 20 years [5]. Synthetic biology and biotechnology are also expected to have enormous impact on global challenges, including climate change and pandemics. More and more nations are including bioeconomy to their economic strategies where synthetic biology and biotechnology are enablers of the global transition to a more bio-based economy. This transition is necessary to sustainably fulfil the food, health, and energy demands of a growing population. The already scarce natural resources are challenged by constrains of climate change [6], and the lack of access to such resources is historically a major reason for international conflicts. Sustainable production through the integration of synthetic biology and biotechnology platforms, coupled with the utilization of previously discarded resources, raw materials, waste, and residual materials, while minimizing waste streams, may significantly reduce environmental impacts and is fundamental to the future of a circular economy [1, 7].

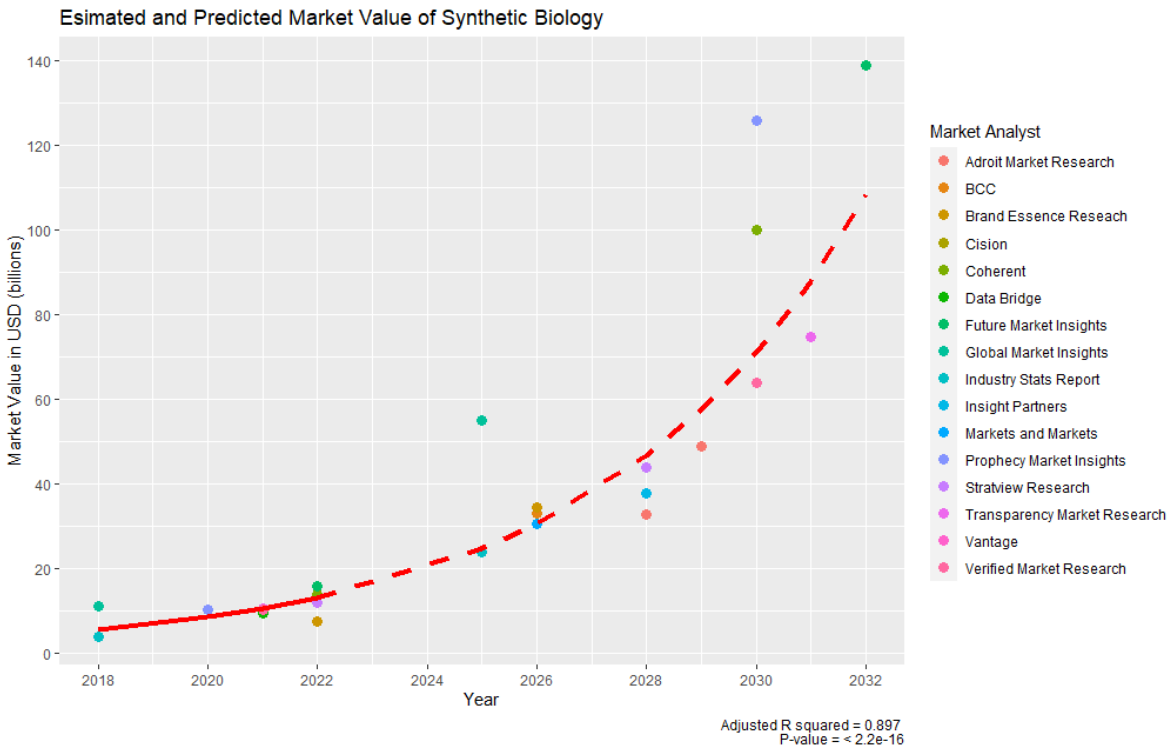

Figure 1.2 Meta-analysis of the estimated and predicted market value of synthetic biology according to 16 institutes of market research. Linear modelling of the log market value was used to calculate a cumulative predicted value with an adjusted R^2 value of 0.897.

Large-scale production of industrial biomolecules is likely to be the main driver of the synthetic biology market [8]. Mass implementation of medical applications such as drug synthesis, vaccines, diagnostic tools and genetically modified cells and organs will presumably take longer as testing requirements are more vigorous with regard to health and safety. However, several medical products with a synthetic biology origin are already in use, such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) messenger RNA (mRNA) vaccine [9]. This vaccine has proved that synthetic biology is a powerful tool in combating emerging diseases, and the technique will hopefully fuel production of new, and improved vaccines for other diseases.

Synthetic biology is rapidly redefining our understanding of how we can utilize biological systems to perform tasks beyond what nature intended. Capitalizing on this emerging technology can boost defensive capabilities through enhanced healthcare and detection, identification and monitoring (DIM) tools. The use of synthetic organisms for biomolecule production could enable hospitals, healthcare centres and research institutions to produce drugs, vaccines and diagnostic tests without ultra-specific facilities and instruments. This could shift the current pharmaceutical industry towards a more self-sufficient model, which would provide increased national security during turbulent times.

Over all, synthetic biology and biotechnology are enabling human society to exploit the highly effective, automated and diverse machinery of biological organisms for new and exciting purposes. The technologies and capabilities discussed in this report are limited to a small subset of the potentials synthetic biology and biotechnology represent. The main technologies driving synthetic biology and biotechnology research are highlighted, before the applications and opportunities these key technologies represent for military forces including medical services are delved deeper into. These technologies are currently primarily developed by the civilian industry or public health sector, but are also highly relevant to military medicine and CB defence.

1.1 Current state

Synthetic biology and biotechnology have gained increasing attention by defence research institutes within NATO in the last couple of decades, with several reports from e.g. the NATO Science and Technology Organization (STO), Norwegian Defence Research Establishment (FFI), Netherlands Organisation for Applied Scientific Research (TNO) and US Department of Defense (DoD). One recent example of biotechnology being brought to attention is in the 2023 NATO Summit in Vilnius where the leaders agreed to develop new strategies for key Emerging and Disruptive Technologies (EDTs), including biotechnology and human enhancement [10]. In the aftermath of the Covid-19 pandemic, biological risks and biosecurity have become a topic of high priority across nations. As an illustration, the UK government's 2023 Biological Security Strategy aims to enhance resilience against biological (B) threats, including the development of a real-time Biothreats Radar [11].

Analyses of technology trends and the associated process of technology watch are crucial steps to identify new, important Defence-relevant technologies and communicate the potential impact of these technologies. In the NATO Science and Technology Trend report 2023-2043, synthetic biology and biotechnology were highlighted as technology areas of importance for defence and security, including rapid gene sequencing technologies and applications of synthetic biology within CB-countermeasures, biosensors and bio-manufacturing of e.g. consumables such as organic fuels, energetics and drugs [12]. The U.S. DoD also states that synthetic biology is critically important for the development of medical countermeasures, materials for protective equipment and detection technology [13]. The NATO STO's Long-Term Scientific Study on CBRN Defence report also underscores the importance of synthetic biology, with a specific focus on CB medical countermeasures and DIM. There is unanimous consensus that the field of synthetic biology must be extensively explored to secure strategic technological advantages and enhance readiness [14].

NATO STO's research group HFM-305 "Synthetic Biology in Defence: opportunities and challenges" focused in their final report on the advanced applications of synthetic biology in medicine; human performance augmentation; CB defence; sensing; agriculture; environmental management; materials; energetics, propellants, and plasticizers; energy storage and generation;

and coding and computers [1]. Each of these applications and opportunities, and advantages enabled by their implementation are further described in the HFM-305 report.

The HFM-335 Specialist Team "Biotechnology, Human Enhancement & Human Augmentation" within NATO STO, recently published a report [15] illustrating how biotechnology and synthetic biology will among others improve diagnostics (to counter CB threats), and provide advances in medicine that enable improved diagnosis of illness and injury and subsequent recovery [15]. Biotechnology is a game-changer which has significant implications for military capabilities. Human enhancement is also a technological trend emphasized by NATO [12].

To better understand and exploit key EDTs is an important task for NATO STO. The efforts directed to biotechnology, human enhancement and synthetic biology by NATO STO, including reports mentioned above [1, 12, 14, 15], are assessed and further recommendations are given in the recently published note by NATO's Chief Scientist on behalf of the NATO Science and Technology Board (STB). The three main recommendations were to 1) maintain significant resources toward research and development and foster collaboration with academia and private enterprises, 2) establish ethical framework and governance, and 3) enhance security in biotechnology as the convergence of biotechnology and digital technologies poses particular risks [16].

Synthetic biology and biotechnology are being utilized in various degrees in military medicine and CB defence today. As will be discussed further in Section 5.1.4, pharmacogenomics is being used to predict soldiers' response to certain medications, which is one example of how DNA sequencing and bioinformatics are well-established technologies. Further, DIM of CB agents utilize different biotechnologies, such as lateral flow assays (LFAs), that employs antigen-antibody binding to detect certain substances, and molecular methods such as polymerase chain reaction (PCR) and DNA sequencing. In addition, different CB programs of the US DoD use synthetic biology in their research and development of CB medical countermeasures, one example being Filovirus vaccines and treatments [13]. Moreover, there do not currently exist specific treatments for many conventional CB threats, such as sulphur mustard, which are only treated symptomatically. Also, the mechanisms of long-term effects after several chemical exposures are not completely understood, and more research on this is needed [14]. The use of synthetic biology can provide some answers. Regarding B threats, many of the infections caused by B agents are only treatable with antibiotics. With the emerging antibiotic crisis, with increasing resistance among bacteria, development of new antibiotics or alternative treatment strategies are crucial. This topic is further addressed in Section 5.1.1.

With respect to CB decontamination, several needs must be met, potentially by the use of synthetic biology. The issue of new or altered CB agents is also relevant for decontaminants. Moreover, current decontaminants are not environmentally friendly and quite aggressive toward equipment. Milder and more effective decontaminants should be developed in order to meet the need for proper decontamination of sensitive equipment, such as optics and electronics, and the interior of vehicles, in addition to being more environmental friendly. Decontaminants that are effective against a wider range of agents and in less amounts would also be favourable as it will ease the logistical burden [14].

As previously mentioned, improvements in DIM of CB agents are of great interest to military organisations. Equipment for detecting both modified B agents and new B agents generated by synthetic biology are essential as synthetic biology methods get more accessible for state and non-state actors. Platforms providing rapid and unambiguous results can be developed and leveraged through synthetic biology, and thereby improve CB defence.

1.2 Dual-use concerns

Emerging synthetic biology technologies and platforms represent security and ethical concerns due to the dual-use potential of many of the involved technologies that can threaten human health and wellbeing as well as the environment. One concern is that microorganisms can be made more harmful and dangerous. Reproductive and resistant microbes can be engineered and accidentally or intentionally released and spread. These can also be programmed to escape our current methods for DIM and treatment, and they can be given traits making them more robust, contagious and with different modes of transmission [1]. Synthetic biology technologies have enabled de novo construction of bacterial and viral genomes in laboratories, e.g. the synthesis of horsepox virus [17] and the recreation of the 1918 Spanish flu virus [18]. This, combined with commercial developments in DNA synthesis and DNA assembly technologies and their reduced costs can result in do-it-yourself biology and garage laboratories creating novel or known pathogens with increased ability to spread and cause disease [19]. Another potential concern is the use of synthetic biology and biotechnology to create more potent chemicals, biochemicals and toxins for malicious use. Synthetic biology is not only applicable on microbes, but also on human beings. It may be possible to modulate human physiology in novel ways, e.g. by engineering the microbiome, genome or immune system [20]. Different treaties and tools such as the Chemical Weapons Convention (CWC) [21], Biological and Toxin Weapons Convention (BTWC) [22], Australia Group [23], and Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons (UNSGM) [24] aim to limit the development, production, stock-piling and spread of CB agents. These can be challenged by developments in synthetic biology, e.g. by the creation of novel B agents and constructing microorganisms able to produce precursors for chemical weapons [1, 25].

1.3 Aim of this report

Over the next 20 years, biotechnology and synthetic biology may significantly contribute with new knowledge and technologies important for military capabilities. The Defence sector needs to keep up with these technologies and consider how they best can be applied for military purposes. According to NATO, there are five enabling components underpinning CB defence activity [26]:

1) DIM

2) Knowledge management

3) Physical protection

4) Hazard management

5) Medical countermeasures and casualty care

In this report, we address research and development crucial for military medical services and two of the fundamental components of CB defence; medical countermeasures and DIM. The report includes examples of research on new medical countermeasures, precision medicine, human enhancement, regenerative medicine, and DIM with a focus on sequencing technologies and biosensors.

2 Technologies

The following technologies, presented in Table 2.1, are described in this chapter.

Table 2.1 Overview of important technologies within synthetic biology and biotechnology.

Technologies	Brief description	Examples of applications
DNA synthesis	Synthetically generate DNA molecules by adding nucleotides in the desired order.	PCR, genetic cloning and other molecular biology techniques, vaccine production.
DNA sequencing	The determination of the exact sequence of nucleotides in a DNA molecule.	Diagnosis of genetic and infectious diseases, forensics, paternity testing, evolutionary biology.
Genetic engineering	Modification/manipulation of an organism's genes.	Gene therapy, biomolecule production, increase tolerance to disease in plants.
Stem cells	Undifferentiated or partly differentiated cells	Treatment of different diseases (e.g. leukaemia, lymphoma), tissue regeneration
Biosensors	Analytical device that combines a biological component with a physiochemical detector	Biomolecule monitoring, CB hazards
AI/ML	Perceiving, synthesising and inferring information by machines	Drug development, computer-aided diagnosis, research and development within numerous fields

2.1 DNA synthesis

The basis of synthetic biology is the discovery of DNA as the unit of heredity and the development of the central dogma of molecular biology in the 1950s; that genetic information goes from DNA to RNA to protein products (see Figure 2.1) [27]. DNA stores the information and RNA carries

and translates the information to proteins that are responsible for a wide range of functions in the cell.

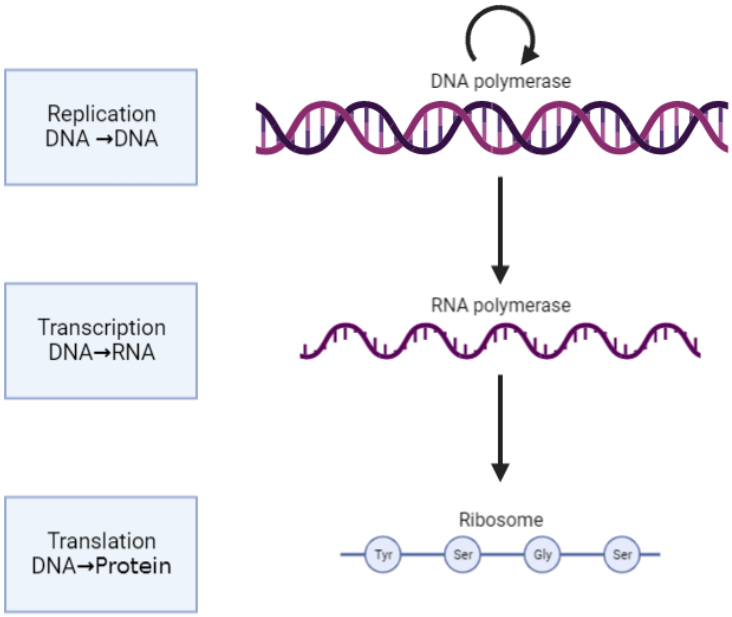

Figure 2.1 Figure illustrating the central dogma of molecular biology. DNA is replicated by DNA polymerase prior to cell division. RNA polymerase transcribes the DNA to RNA, that later is translated to chains of amino acids that form three dimensional proteins.

About the same time as the central dogma was stated, DNA synthesis was discovered. DNA synthesis is the process of combining deoxynucleic acids (adenine, cytosine, guanine, and thymine) by using DNA polymerase to form DNA. DNA synthesis can refer to different processes, from naturally occurring replication of DNA within living cells, to synthetic construction of genes in vitro. Today DNA synthesis makes up the basis of most techniques and approaches within the synthetic biology field. This way, designed DNA sequences can be produced for different molecular purposes, such as DNA amplification, editing and mutagenesis [25].

2.2 DNA sequencing

Since the first sequenced human genome in 2003 [28], the role of genetic information in biomedical research has vastly increased. The genetic code, the DNA, serves as a blueprint for all biological processes and is therefore central in understanding life at the most fundamental level.

As sequencing technology evolved the major challenge went from acquiring an accurate DNA sequence to deciphering the acquired code [29].

DNA encodes the proteins that make up our cellular machinery and work together to sustain life. The functional properties of proteins, however, are determined by more than the DNA sequence that encodes it, such as its three-dimensional structure, post-translational modifications and interactions with other proteins. As such, predicting protein function based purely on genetic information is not a trivial task. Furthermore, proteins often perform a range of tasks and their activity and function are regulated by other proteins, molecules and environmental factors. Extensive experimental research has resulted in vast databases of functional annotations for the different identified genes, resulting in an ever-increasing culmination of genetic understanding.

The recent increase in computational power together with the availability of large publicly available datasets of genetic information and functional annotation has facilitated a growth in computational biology [30]. Machine learning algorithms can now be trained on databases of largely experimentally acquired genetic understanding to predict function, regulation, structure and interactions of genes and proteins, exponentially reducing manual labour and costs [31]. As more and more genetic information becomes available, algorithms will become better at predicting function from nucleotide sequences, further increasing our ability to redesign and modify biology to our needs.

Today, genetic information is incorporated into a large fraction of biomedical research and used in applied sciences for organism identification and characterization as well as diagnosis and personalized medicine. Furthermore, by understanding the fundamental building blocks of life, progress has been made in creating synthetic biological tools and indeed wholly synthetic organisms. These tools and organisms can technically be developed to perform any biological task such as production of biological compounds, specific reactions to their immediate environment, breakdown of specific chemicals and identification of other organisms based on some unique signature.

2.3 Genetic engineering

Our ability to sequence DNA and RNA gives us insight into gene function and combined with other molecular tools, such as restriction enzymes and DNA cloning, researchers today can do changes to the DNA in cells. Different terms are being used interchangeably regarding modifying DNA, but the term genetic engineering will be used throughout this report. Genetic engineering is one of the key groups of techniques in synthetic biology and enables understanding of basic biology underlying disease as well as discovery of novel therapeutic targets, by exploring the connection between genotype and phenotype [32]. A set of technologies are utilized, enabling changing of single base pairs, adding new segments of DNA, deleting regions of DNA and regulating gene expression. One example of genetic engineering is the transfer of genetic material between different species [33]. Specific genes with desired functions are first identified before

the DNA is either isolated and copied by recombinant DNA techniques or artificially synthesized. The DNA construct is then incorporated into a plasmid vector which then is inserted into a host organism, in many cases a bacterium. The plasmid and the gene of interest will be amplified as the bacterium divides. Other organisms such as yeast and insects are more suitable as host organisms for certain genes [34]. As the host organism divides, it produces proteins coded by the genetic material in the genome and in plasmids, including the genetic material incorporated in vitro. This is also called heterologous protein expression. In this way we can use microorganisms as biofactories of important molecules, e.g., for research purposes or in industry (further discussed in Section 5.1).

2.3.1 Somatic and germline cells

Genetic engineering in multicellular organisms can be within either somatic or germline cells. Somatic cells are non-reproductive cells, and genetic changes will not be transferred to the progeny of the organism. In germline cells on the other hand, the genetic material will be passed on to next generations, so genetic changes to these cells will not only affect the organism going through the gene editing, but also its decedents [35]. This raises ethical concerns that must be taken into account alongside the technical and safety challenges associated with gene editing.

2.3.2 Examples of editing tools

Within genetic engineering, one finds editing tools such as clustered regularly interspaced short palindromic repeat/CRISPR-associated nuclease (CRISPR/Cas), transcriptional activator-like effector nucleases (TALENs) and Zink finger nucleases (ZFNs). These methods are based on the use of engineered enzymes that bind and cleave or occupy specific sites on the DNA, where DNA sequences can be removed, added, replaced or blocked. The double-stranded breaks in the DNA created by some of these nucleases are repaired by the cell's repair systems, either by non-homologous end joining (NHEJ) or homology-directed repair (HDR), which results in changes to the DNA (see Figure 2.2) [36]. Genetic engineering tools can be utilized in numerous areas, such as basic research on gene function and structure, production of useful proteins in industry, generation of transgenic plants and animals, and diagnostics and treatment [34].

CRISPR/Cas, derived from prokaryotic defence systems, has been of particular interest since its discovery as a gene editing tool. It has been used in numerous studies with aims such as identification of drug targets, biomarkers and mechanisms of drug resistance [37]. CRISPR/Cas has become a powerful tool and has revolutionized genome editing and engineering in eukaryotic cells (e.g. plant, animals, yeast), and has a potential of being used in both diagnostics and therapeutically (see Section 5.1, 5.4 and 5.5). The best-known example is the CRISPR/Cas9 system which is illustrated in Figure 2.2. It consists of two main components, the enzyme Cas9 and a designable single guide RNA (sgRNA). sgRNA guides the enzyme to the target sequence of the DNA, where Cas9 performs a double-stranded break. CRISPR/Cas is a simple, fast, cheap and accurate gene editing tool compared to older methods such as ZFNs and TALENs. One of the reasons is the use of the ~20 nucleotide sgRNA, which is the only component that must be modified to target different sequences. The CRISPR/Cas system can be used in all organisms to regulate gene expression or to target and study specific DNA sequences [38]. A Cas enzyme able

to cut RNA has also been identified, Cas13, enabling genetic alterations of RNA molecules expressed by a cell or organisms, including RNA viruses [39].

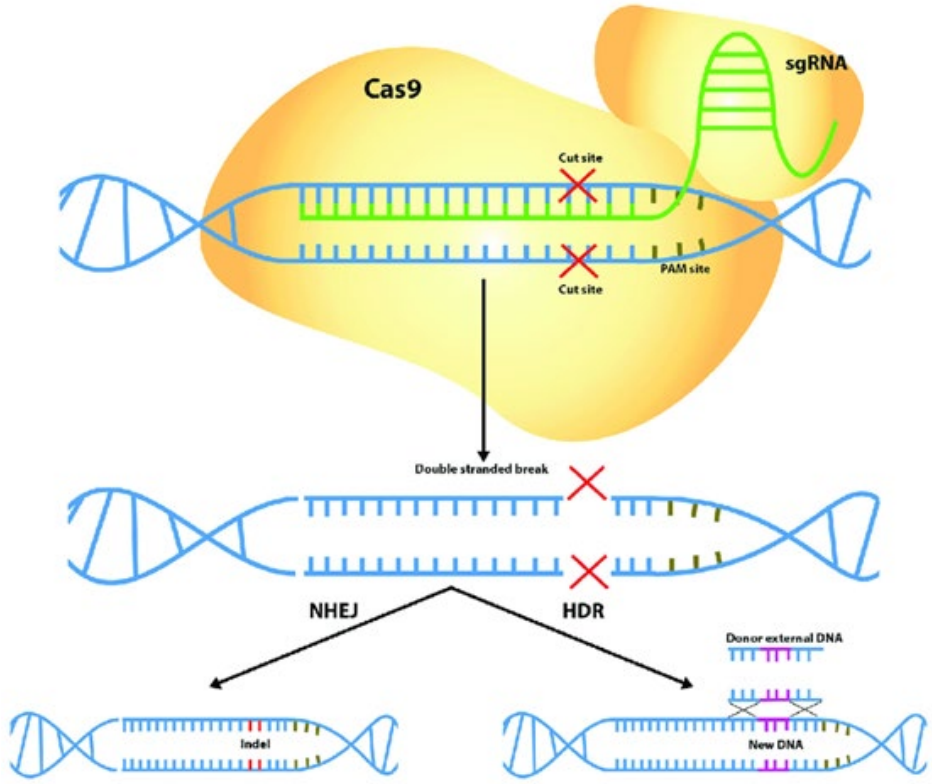

Figure 2.2 Illustration of the mechanisms of CRISPR/Cas9. The sgRNA is designed to target a specific DNA strand and guides the Cas9 to the site where a double-stranded cut is conducted. By the cell's own repair system the cut is repaired, leading to DNA changes. Adapted from Cribbs and Perera [40].

2.4 Stem cell technology

Stem cells are undifferentiated cells that can differentiate into various types of cells. These are of interest as they can give insight into important biological properties of the human body (e.g. for cancer research) and show promise as a treatment for diseases/injuries some of which are not curable today. Stem cells are to be found in adult body tissue and embryos. Scientists have also developed methods to make stem cells, induced pluripotent stem cells (iPSCs), from fully differentiated cells (somatic cells), by reprogramming them. Stem cells are classified based on their potency, from the most potent totipotent stem cells, to the least potent unipotent stem cells.

Stem cells can differentiate and proliferate into various cell types and potentially regenerate entire organs [41]. Stem cells are currently used in treating some hematopoietic disorders such as leukaemia and lymphoma. Hematopoietic stem cells are collected from a donor's bone marrow or peripheral blood or umbilical cord blood. The patient receives chemotherapy that kills the patient's dysfunctional cells, before healthy stem cells are introduced [42]. New applications of stem cells have been investigated for decades, and are further discussed in Section 5.4.

2.5 Biosensors

Biosensors come in a wide range of formats but in this report they are referred to as biological systems that recognize an input signal and generate an output that can drive cellular change or be detected and measured (illustrated in Figure 2.3) [43]. Biomolecular scaffolds identified and characterized from both prokaryote and eukaryote cells have been employed to perform this task, such as nucleic acids, enzymes, antibodies and transcription factors. As more and more molecular processes have been defined and understood the toolbox of recognition elements has grown and allowed researchers to create recognition elements that can react to a wide array of input signals like small molecules, organic and inorganic compounds, proteins and even light. This growing toolbox also provides a wide range of output signals such as fluorescence, enzymatic activity, gene expression, protein production and changes in electrochemical currents. These output signals are coupled with a transducer element that senses a change in a physical, chemical or optical properties, and then signals that to the user in a detectable format such as changes in absorbance, fluorescence, colour or conductance that can be visualized and measured for downstream application [43].

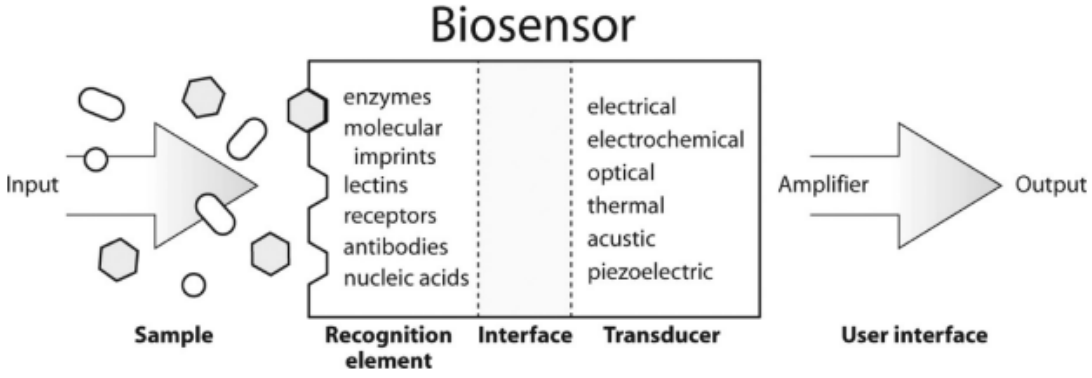

Figure 2.3 Components of biosensors. The input signal is recognized by a biological system that is coupled to a transducer element. The transducer relays the signal in which it becomes detectable and possible to measure [44]. Reprinted from Chambers et al. [44].

2.5.1 Protein-based recognition

Most biosensors have either protein-based or nucleic acid-based recognition elements. Protein-based biosensors utilizes proteins such as antibodies, enzymes and receptors to detect analytes [43]. Researchers now use a combination of computational prediction, together with natural selection techniques to produce new proteins with specific ligand binding properties. One such technique is selection-based platforms, which employ libraries of DNA with computationally predicted binding activity that are coupled with the peptide it encodes and displayed to the target molecule of interest. The peptides bind to the ligand and are then washed leaving only strongly binding proteins. These can then be sequenced, and new libraries of peptide sequences can be made based on the first iteration to improve binding activity, robustness and other attributes [45]. This evolutionary approach allows the synthetic production of brand new proteins with specific binding abilities that can be used in a range of applications including biosensor production. Techniques such as selection-based platforms and self-assembly peptides have now been used to create biosensor systems that can detect explosives such as TNT [46] and chemical warfare agents [47].

2.5.2 Nucleic acid based-recognition

In addition to protein-based recognition, biosensors can be based on recognition by nucleic acids. These are based on complementary base pairing interactions, and if the target nucleic acid sequence is known complementary sequences can be synthesized, labelled and immobilized in the sensor. This approach come with some benefits and limitations compared to protein-based biosensors. Nucleic acids are more stable than proteins as they rely predominantly on primary structure rather than the tertiary structure of protein folding that can be disrupted and degraded without appropriate handling. Additionally, antibodies and their related functional analogues do not guarantee high-affinity binding toward every desired epitope every time, thus rely on considerable effort in selection and improvement. On the downside, nucleic acid based-recognition require additional extraction to properly access genetic material, and thus increases the number of purification and capture steps [43].

2.5.3 Whole-cell-based and cell-free biosensors

Biosensors can be whole-cell-based or cell-free. Both come with their advantages and limitations. Whole-cell systems are better suited for long-term continuous sensing due to their ability to regenerate over time. Cell-free systems on the other hand is better suited for simpler analyte detection [48]. Biosensors with genetically modified living cells also have limited sensitivity, as the analyte needs to cross the cell membrane to activate the protein synthesis machinery. Recent advances in cell-free protein synthesis have increased the safety, sensitivity and response time of protein synthesis based biosensors [49]. By extracting the process from the cells and producing the fluorescent proteins directly in solution the detection range of the biosensors is greatly enlarged while simultaneously preventing deleterious mutations in the organism and the need to keep the cells viable [49]. Furthermore, the shelf life of these cell-free protein synthesis solutions has been radically increased by freeze-drying technology, which removes up to 95% of the water in the solution, resulting in room temperature shelf life of 3-12 months [50]. Freeze-drying cell-

free protein synthesis solutions still face some limitations, such as reduced protein activity, and researchers are now working to improve the technology by liposome encapsulation [51] and using sugar solutions to protect the components during the drying process [52].

2.5.4 Readout systems

When the analyte is detected by the recognition system, the signal can be read out in different ways such as colour changes, fluorescence, luminescence, or electrochemically. Colorimetric based biosensors produce a signal that is detected as a colour change, usually achieved by translation of an enzyme that reacts with a substrate resulting in colour change. Detecting colour change requires no complex equipment and minimal operator expertise, while still producing a measurable quantitative signal, making them ideal for point-of-care diagnostics and compound identification. The second readout system is based on fluorescence, a widely used phenomena in traditional diagnostics, such as PCR. The reporter consists of a fluorophore and a quencher linked together by nucleic acids, and fluorescent energy is emitted upon cleavage [53].

2.6 Artificial intelligence and machine learning

The increase in computational power (mentioned in Section 2.2) and technical advances lead to large amount of data, and it is likely that artificial intelligence (AI) and machine learning (ML) will aid in processing, analysing and sorting these large amounts of diverse data types. AI and ML are related but different. While AI is a branch of computer science attempting to build machines capable of intelligent behaviour, ML is the science of getting computers to act without being explicitly programmed. AI and ML are not synthetic biology or biotechnology technologies themselves, but have the potential to catalyse and speed up synthetic biology and biotechnology capability developments. It can be used to process sensing data from different platforms and aid in decision-making, understanding foreign spoken languages, setting medical diagnoses, perceiving obstacles and threats, hazard prediction etc. [14].

3 Technology Readiness Level (TRL)

The maturity level of a particular technology can be expressed in terms of technology readiness level (TRL). The scale (Figure 3.1) was developed by the National Aeronautics and Space Administration (NASA) to compare the maturity between different types of technologies and it has been adopted by other agencies and departments such as the US DoD and the European Space Agency [54-56]. In the TRL scale technologies range from being observed basic principles (TRL 1) to "flight proven" (TRL 9) [56]. Later in this paper, as different synthetic biology and biotechnology capabilities are explored, the criteria presented in Figure 3.1 are utilized to determine TRLs and provide insight into the estimated timeframes for these technologies. The capabilities vary in TRLs, from being on the level of basic research and concept formulation (< TRL 3) to fully proven and implemented concepts (TRL 9), including those applicable to commercial and industrial settings, thus showcasing the diverse potential for near-market and industrial applications. One example of a capability of high maturity is the use of bacteriophages as antibiotics (Section 5.1.1.1). In some countries phages are already being used to treat infections that do not respond to common antibiotics, and in the United States a phage therapy has recently been approved for clinical trials. Though being relatively mature, this technology also has some barriers to overcome, which can be solved by the use of synthetic biology. The same applies to precision medicine (Section 5.2). Genetic information is already being used in diagnostics of some diseases, e.g., as a screening tool of foetal chromosomal abnormalities, and in pharmacogenomics. At the same time, much knowledge has the potential of being generated when it comes to genomic approaches to diagnosis, prevention, treatment and prognostication [57]. An example of a technology of low maturity is 3D printing of complex organs such as hearts (Section 5.4.1.2). Advancements have been made in this area of research, with the successful printing and transplantation of a stem cell-based bioprinted ear, but before printing more complex body parts there is a long way to go. It is difficult to state the expected timeframe of complete usable systems, such as wearable biosensors for pathogen detection and in-field bioreactors for therapeutics productions, as these capabilities are combinations of multiple technologies with different level of maturity. The suggestions made at the end of each capability chapter of this report are based on literature found on the topic and how far development had reached on the TRL scale (Figure 3.1) at the time of publication.

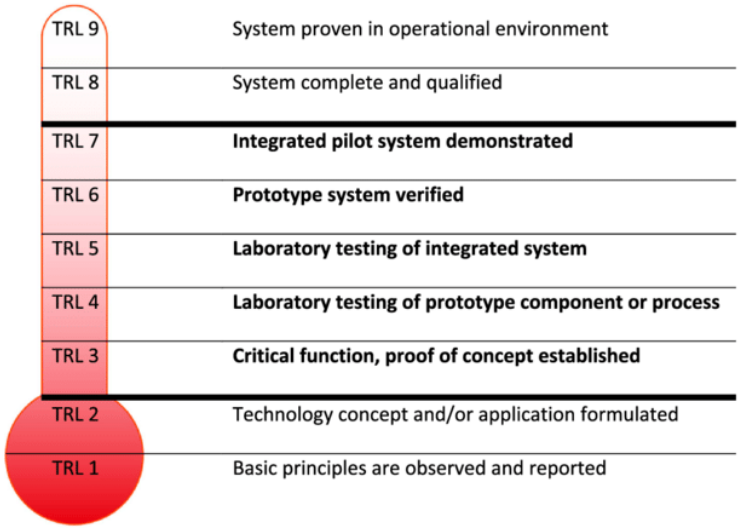

Figure 3.1 Technology readiness levels (TRLs) chart with descriptions. Adapted from Fasterholdt et al. [58], based on early NASA model.

5 Capabilities for military medicine and CB defence enabled by synthetic biology and biotechnology

5.1 Medical countermeasures

Advances within the field of synthetic biology have accelerated the ability to engineer existing organisms and potentially create novel ones. This could be beneficial within fields such as medicine, enabling discovery and production of new CB countermeasures that may improve our preparedness. Medical countermeasures and protective measures include biological products, drugs and devices that prevent, treat or ameliorate illness in the event of an emergency caused by an infectious agent, toxin or chemical, either natural or manmade. Examples could be various sensors imbedded in the fabric of personal protective equipment, vaccines, antibiotics, antivirals and antitoxins [20]. Biological exposure can be a result of B weapons or naturally occurring epi- or pandemic situations, e.g., the recent SARS-CoV-2 pandemic.

Despite intensive medical research in the past, significant medical countermeasure gaps are still evident and must be addressed to meet CB defence needs. This includes all aspects of medical countermeasures, from prophylaxis and pre-exposure treatments, to diagnostics, acute therapy and long-term follow-up [14]. New countermeasures developed by the use of synthetic biology could be antibiotics and vaccines resilient to the resistant mechanisms seen in microorganisms today, or neutralizing therapeutics, e.g. antibodies, that bind threat agents with high affinity to neutralize them [1]. Synthetic biology can also contribute to the fundamental understanding of molecular toxicology, e.g. by knock-out mouse models that can reveal molecular targets of CB agents or new antidotes or antimicrobials [14]. Although holding great promise, it is also possible to imagine malicious uses that could threaten civilians and military personnel [20]. This again may trigger the need for new countermeasures and protective measures [1].

5.1.1 Antimicrobials

An important research area for both public health and military medicine is the development of novel antibiotics. Antibiotics are essential not only when treating infectious diseases, but are also routinely administrated prior to, during and after surgery, cancer treatments, organ transplantations etc. Therefore, increased occurrence of antibiotic resistance is of great concern. Bacteria are either intrinsically resistant towards antibiotics, by lacking the target of the antibiotic, or they can become resistant by genomic mutations or horizontal gene transfer. Today, only a few antibiotics are capable of combating the most resistant bacteria strains [59]. Synthetic biology technologies have raised significant concerns regarding deliberate genomic alterations in microbes, potentially making microbes causing treatable or preventable infections resistant to current antimicrobials, antivirals and vaccines [25].

The use of synthetic biology has given insight into antibiotic resistance mechanisms among different species, such as *Escherichia coli* and *Staphylococcus aureus*, to some antibiotics [60]. Examples of tools that often are used for this purpose are recombinant DNA technology, DNA

sequencing, CRISPR/Cas and bioinformatics. Genes involved in susceptibility to certain antibiotics have been revealed, making it possible to do further research on drugs targeting proteins coded by these genes. Adjuvants that inhibit specific resistance mechanisms can then be administrated together with conventional antibiotics.

Microbes such as bacteria and yeast are being used to produce different types of molecules, e.g., in the pharmaceutical industry. The organism is either naturally producing the molecule of interest or is genetically modified, as discussed in Section 2.3. The best-known example of this is the use of recombinant DNA technology to produce human insulin in *E. coli*. Microorganisms such as *E. coli* have the ability to divide and grow fast, enabling large-scale production of biochemical compounds. This also includes bactericidal or bacteriostatic ingredients in antibiotics. A potential strategy for production of novel antibiotics and other therapeutics is, therefore, to use genetic engineering tools to engineer new biosynthetic pathways in microbial hosts as resistance mechanisms are revealed and novel therapeutic components are discovered [61]. Additionally, many of the natural antibacterial products produced by bacteria, comes from a few easy-to-cultivate soil organisms. Species that are harder to cultivate and that originate from other environments, have yet not been fully investigated. This combined with new and improved molecular tools enables discovery of new antimicrobial compounds, with low levels of resistance [62].

5.1.1.1 Bacteriophages

Another approach to the antibiotic crisis, is to look for other ways to kill or neutralize the organisms or to be ahead of potential infections by protective measures. These could be narrow-spectrum drugs, antimicrobial peptides, bacteriophages, monoclonal antibodies, vaccines and probiotics, combined with more efficient diagnostics [63]. Bacteriophages, also termed phages, are natural bacterial predators and kill bacteria efficiently, also those resistant to antibiotics. Natural phages do have limited host ranges, and some bacteria are resistant. To overcome this genetic engineering together with ML and AI can be utilized to make bacteriophages selectively target the pathogens and their resistant genes [64]. Phages are already being used to treat some infections or in combination with conventional antibiotics in a few countries. Nevertheless, phage therapy should be further investigated, as it holds great potential as an alternative to narrow-spectrum antibiotics, with little resistance and few adverse effects. When new resistant bacterial strains are identified, they could be quickly sequenced to determine their target genes and resistant mechanism genes, and this information could then be used in developing a new antibacterial drug, based on bacteriophages which could be given separately or combined with conventional antibiotics.

5.1.1.2 Antimicrobial peptides

Antimicrobial peptides (AMP) are small bioactive molecules produced by all cells as first line of defence against microbes in eukaryotes or as competitive strategy to inhibit competing cells in prokaryotes (bacteria). Compared to conventional antibiotics, AMPs are less prone to resistance and kill microbes more efficiently, even those being multi-drug resistant. They can also activate the innate immune system to improve the body's defence against resistant microbes. AMPs are

microbe-specific, avoiding disruption of the normal microflora. Unfortunately, AMPs also have some unwanted traits, for example being toxic to eukaryotes and their susceptibility to bacterial proteolysis. These hurdles must be addressed to fully utilize AMPs' great antimicrobial potential, through genome editing and chemical alterations [65].

5.1.1.3 CRISPR/Cas systems

CRISPR/Cas systems have the potential for treating a range of diseases, including cancer, genetic, infectious and immunological diseases [37]. This section will focus on the use of CRISPR/Cas for treating infections as an alternative to antibiotics. A CRISPR/Cas system can either work as an antibacterial itself, by targeting the bacterial chromosome to induce cell death, or be used in addition to conventional antibiotics by disrupting plasmids carrying resistance genes. To increase the pathogen's susceptibility to the antibiotic, CRISPR/Cas systems can be designed in a way that specific genes giving traits such as antibiotic resistance, biofilm formation and other virulence factors can be removed or blocked, thereby increase the antibiotic's effectiveness [65].

CRISPR/Cas systems are also attractive and flexible potential antivirals, as their sgRNA is easily designed to target the desired DNA or RNA. As Cas-proteins targeting both RNA and DNA have been identified, CRISPR/Cas systems can directly cleave RNA and DNA genomes and thereby execute antiviral activity on both RNA and DNA viruses. One example of an RNA virus is SARS-CoV-2, and the majority of viruses that infects human are in fact RNA viruses [66]. CRISPR/Cas could be one strategy to fight the many viral infections that are of global health concern, but the current state of development is limited to testing in animal models [66, 67].

Several factors concerning safety, accuracy and efficiency must be enhanced to fully exploit CRISPR/Cas as a therapeutic tool. Examples of areas that need improvement are delivery methods, Cas efficacy, sgRNA specificity, off-target effects and immunogenicity [37].

5.1.2 Vaccines and preventive measures

The ability to develop and distribute vaccines quickly and accurately is of great importance to minimize the effects of newly emerging viruses and bacterial infections. Traditional vaccine development has been time-consuming and complex, typically taking 10-15 years. By comparison, the process of developing a Covid-19 vaccine took 12-24 months after the SARS-CoV-2 genomic sequence was known [68]. Different vaccine platforms exist, but the mRNA vaccine, not fully proven until the SARS-CoV-2 outbreak, shows great promise for the future, being flexible and faster and easier to produce. This section will not put large emphasis on the current vaccine platforms, but delve into other promising medical preventive measures.

5.1.2.1 Engineered microbes as probiotics

Microbes can be used as preventive measures against B agents. One example is to modify benign microbes to express toxin receptor mimics on their surface that will work as competitive inhibitors for pathogenic toxins [69]. Living engineered organisms can also be utilized by controlling the release and localization of specific molecules under certain conditions, for instance at the offset of an infection. Engineered microbes can also be able to sense and respond to pathogenic

infections inside the body, by quorum sensing. Cell-to-cell communication between the pathogen and the engineered probiotic microbe will regulate metabolism and physiological activities within the probiotic, leading to e.g., secretion of antitoxins or AMPs specifically targeting the pathogen. One example is *E. coli* which have been engineered to detect infectious agents as *Vibrio cholera* and *Pseudomonas aeruginosa* [69, 70]. So far, this approach has shown best effects when the probiotics are given prior to the pathogen exposure, suggesting this to be a preventive measure against possible infectious agents. Such engineered microbes could be part of nutritional additives given to soldiers prior to and during deployment, in order to maintain a stable and balanced gut health (discussed in Section 5.3.2).

5.1.2.2 Use of phages for immunization

Phages do not only show potential as antimicrobials but can also be utilized in vaccination, in the form of either DNA phage vaccines or phage-displayed vaccines. For DNA phage vaccines to accomplish immunization, they must be engineered to be non-lytic and carry virus epitope genes. For instance, upon oral administration, the phage will infect harmless microbes in the gut, e.g., *E. coli*. The viral epitope will then be displayed by the bacteria leading to enhanced immunization and protection against the infectious agent, without killing the host bacteria. Naturally occurring phages only infect bacteria cells, but they can also be engineered to infect mammalian cells. Upon infection, they can transfer engineered viral DNA/RNA into the cell and cause expression of harmless viral proteins by the human cell and thereby cause immunization, similar to the mechanism of mRNA vaccines. In a phage-displayed vaccine, phages can present viral-like proteins on their surface and cause immune responses without infecting commensal bacteria or mammalian cells [70].

5.1.2.3 Universal vaccines

All vaccines produced today target specific viruses, e.g., influenza virus or SARS-CoV-2. The hunt for universal vaccines is ongoing for both influenza and coronaviruses and in the future novel technologies might enable development of broad-range vaccines against whole pathogen families [14]. Several different influenza viruses seasonally circulate in the human population. Some common viruses are being closely monitored, but we cannot accurately predict which subtype that will cause the next pandemic. Several attempts in creating multi-targeting influenza vaccines have been conducted with traditional vaccine technology, but up to date influenza vaccines are only targeting the subtype that is most likely to circulate the given season. Recently, researchers have developed an mRNA vaccine able to elicit antibodies in mice and ferrets that reacted to all 20 encoded antigens (derived from the 20 subtypes of Influenza A and B). This approach can also be used for other viruses, such as corona- and rhinoviruses, with the former targeting multiple spike components. Additional studies are necessary to determine the maximum number of antigens being delivered simultaneously. Perhaps one vaccine could cover all viral infections, or at least all respiratory ones, in the future [71].

5.1.3 Bioreactors

Producing small molecules by using biotechnology in host organisms is well-established in laboratories and industries, one example being the development of recombinant insulin back in the 1980s [72]. Large-scale technologies enabling this are not as well established under field conditions. Engineered whole-cell and cell-free platforms may be used under field conditions to rapidly produce therapeutic and prophylactic molecules on-demand in developing countries as well as during remote military and space missions, where cold-chain requirements cannot be met and only small-scale production is necessary.

Whole-cell platforms are best suited for applications where large quantities of product are needed, but they have several limitations regarding robustness. Therefore, efforts enhancing organism resilience to metabolic and environmental stresses are necessary. Harsh environmental conditions over long periods lead to large amount of mutational stress on the organism. Genetic stability should therefore be enhanced by designing synthetic DNA circuits resistant to mutations to improve platform hardiness. Also, developing methods for a more diverse range of host organisms known to be more stress-resilient, e.g. *Bacillus* spores, could lead to deployment of living cells in variable climates [48].

Cell-free platforms are easier made shelf-stable and can yield faster production times, with smaller product quantities. As an example, platforms under current development include a freeze-dried paper-based platform where cell-free systems and DNA constructs are rehydrated in order to activate production of functional proteins by multi-enzyme pathways. Such platforms can be stored for over a year at room temperature, and be activated when needed [73]. Also microfluidics platforms are being developed, enabling continuous-flow production of antimicrobial peptides. These platforms must also be combined with purification capabilities to produce ready-to-use therapeutics [48].

During military missions or in developing countries the settings are often extreme, with many limitations such as no or limited power supply. This limitation can be by-passed by coupling the platforms with microbial fuel cells, where living organisms can be used to convert chemical energy to electrical power. Systems are also reliant on input of pure oxygen, which is problematic with low accessibility to resources. To overcome this, the organisms could be rewired to lower their oxygen demand, so that operation from air instead of pure oxygen is possible, or use organisms that thrive at naturally occurring oxygen levels.

What is important for platforms to be used under field conditions, is that they are compact and portable, can be stored at room temperature and activated or rehydrated when the need for a certain compound arises. A fully purified, ready-to-use product should ideally be ready to use within hours. This also eases the need for stockpiling of inventory at locations with limited storage capabilities [48].

For war zone or field applications specifically, or even in low resource areas, on-demand production of therapeutic drugs, medical countermeasures and vaccines would overcome huge limitations regarding transportation and storage. In the long run, newly detected pathogens with

epidemic potential, could be investigated on-site, and based on findings (e.g. DNA sequences) new drugs or vaccines targeting these pathogens specifically could be developed on-demand. This would minimize the outbreak caused by the pathogen, hindering the spread.

Box 4.1 – Summary of synthetic biology and biotechnology applications within medical countermeasures

Medical countermeasures are essential for both CB threats we are currently facing and novel threats we can meet in the future, both naturally occuring and those manmade by the use of synthetic biology. One especially alarming issue is the growing occurence of antimicrobial resistance. Synthetic biology and biotechnology technologies are essential in the discovery, development and production of new therapeutics and preventive measures such as vaccines and prophylactic treatments. Microbes are currently utilized in laboratories and industries for biomolecule production where resources are practically unlimited. For future applications, development in bioreactor technologies enables usage under field conditions, to make therapeutics on-demand on-site.

5.1.4 TRL

Table 5.1 Estimated TRLs for development of new synthetic biology/biotechnology-driven medical countermeasure capabilities for military purposes.

Capability	Today	TRL 9
Bacteriophage-based antibiotics*	TRL 9	---
CRISPR/Cas as antibiotic/antiviral	TRL 3	2040
Microbioreactors	TRL 3	2040
Universal vaccines	TRL 4	2040

* Already partially established, these concepts hold the potential for further development and utilization.

5.2 Precision medicine

We are moving from a reactive, one-solution-fits-all approach to medicine toward being more proactive and personalized. Precision and personalized medicine, two terms often used interchangeable, have as a major goal to identify diseases at an early stage and to treat them as efficiently as possible, with minimal toxicity and adverse effects [74]. The diseases range from cancers and auto-immune diseases to infections. An important term within this field is omics data. This includes a wide range of data concerning our genes and our microbiome such as genomics, epigenetics, transcriptomics and microbiomics. This, in addition to environmental and lifestyle factors, affects health and disease progression, and is important information within precision medicine. Such information helps fit patients into subgroups that define their treatment plans and improves patient outcomes [74]. Different molecular and computational tools are used to gain this information, among them DNA sequencing, bioinformatic tools and computational power that can process large quantities of data (Big Data). This way one can identify genetic mutations to screen for when trying to determine an individual's risk of developing certain disorders, and to determine which treatments best target specific mutations [74]. One example of precision medicine used on service members in the US military today is the use of glucose-6-phosphatase dehydrogenase (G6PD) assays to predict whether a service member will experience haemolysis when prescribed a certain anti-malarial therapy or not, and which medication might be the best suitable alternative [74]. This example belongs to the area of pharmacogenomics, where the genome's role, especially the liver enzyme genotype, in medicine response is identified. Another example is the screening for sickle cell trait (the presence of one sickle cell gene, *HbS*) which is performed prior to military accession in the US military. This is due to increased morbidity among service members carrying this trait. These recruits will be disqualified from certain services and counselled on how to exercise and rest to avoid complications [75].

5.2.1 Sequencing for prediction and prevention

In the long run, all soldiers' genomes could be sequenced, and genetic markers to predict susceptibility to different diseases and how different exposures impact the soldiers are identified. In this way genetic information could be used in selection of soldiers for different military operations or tasks, to set up personalized training programs, outline treatment plans for most probable health incidences, such as CB exposure, infections (e.g., as done today with anti-malarial therapy), trauma, and to customize disease-prevention strategies. In other words, precision medicine is not only applicable when treating diseases, it could also be used in soldier enhancement. Another example is using genetic information of gut microbiota to compose probiotic treatments to ensure stable gut conditions, as our gut wellbeing affects our physical performance and cognitive function. This information together with the soldier's genetic information could also be used in personalized nutrition plans (nutrigenomics). Further, the introduced prebiotic organisms could be engineered to sense levels of different compounds in the gut, including presence of pathogens, and to supplement or counteract them when needed [76]. More on this topic is discussed in Sections 5.1.2.1 and 5.3.2. Other drugs for enhanced performance can also be developed based on omics information, e.g., drugs for increased stamina and concentration.

5.2.1.1 CB exposure

Taking genetic and biological factors into account, e.g., age, gender, enzyme deficiencies etc., can be beneficial when treating soldiers for CB exposures, as the response to an exposure is often individual. This information can be used to make current countermeasures more efficient through more personalized dosage and way of administration and in the development of new medical countermeasures. With omics databases of soldiers on the battlefield, drugs could be produced on-demand on-site based on the information of the affected soldier and the agent. The genetic information of a pathogen can be utilized when deciding the type of countermeasure, and how to prevent an outbreak from spreading. Lab-on-a-chip devices are beneficial for these purposes, those that analyse pathogens and those that can produce pharmaceuticals on-site (further discussed in Section 5.1.3).

5.2.1.2 Retired personnel/veterans

Genetic and biological information may also lead to more adequate treatments of chronic diseases and mental disorders in future veterans. With increased databases and computational power, including AI and ML, the detection of abnormal and disease-causing mutations or genetic variants will be more effective, leading to faster diagnostics and therapy initiation. This will reduce time, cost and risk of adverse effects, providing soldiers with a better post-service life [74].

5.2.2 Ethical concerns regarding precision medicine

The use of precision medicine, especially whole genome sequencing and collection of personal data, addresses some ethical concerns that must be considered. The most prominent one is storage and sharing of sensitive data, but other issues appear as technology and capabilities evolve. Currently, obtained data belong to a small fraction of the population and needs to be more diverse to give a thorough and trustworthy understanding of any person's traits. Furthermore, genetic testing can result in incidental findings, placing the responsibility on the physician requesting the test to determine whether this information should be disclosed to the person being tested. Such information not only affects the tested individual but also extends to their family members who share genetic risks [77].

Box 4.2 – Summary of synthetic biology and biotechnology applications within precision medicin

Genetic markers are already being used to some extent for diagnostics and treatment of diseases, e.g. some cancers. Additionally, screening of genetic markers in US soldiers is performed to predetermine possible malarial treatments and to reveal predisposure to genetic diseases. Screening of genetic markers serves great potential for optimization of soldier performance and treatment of a wide range of diseases.

5.2.3 TRL

Table 5.2 Estimated TRLs for development of new synthetic biology/biotechnology-driven precision medicine capabilities for military purposes.

Capability	Today	TRL 9
Pharmacogenomics*	TRL 9	---
Diagnostics*	TRL 9	---

* Already partially established, these concepts hold the potential for further development and utilization.

5.3 Human enhancement

Synthetic biology and biotechnology have the capacity to enhance human performance directly or indirectly, as discussed in recent NATO STO reports [1, 15]. Direct enhancement can be achieved by increasing robustness and overall health of individuals by genetic engineering, while DNA sequencing and genetic functional understanding can uncover underlying diseases as well as desired characteristics that can aid in selecting personnel for military operations. An increased capacity for physiological monitoring can also improve human performance as biomarkers for debilitating conditions such as fatigue, dehydration and malnutrition could be detected early. This could aid individuals as well as health personnel to prevent cognitive impairment, as well as provide key personnel with information about soldier condition and capacity.

5.3.1 Selection

As our understanding of molecular function increases, researchers are now connecting genetic information to phenotypic traits displayed by individuals. As most phenotypic traits are a

culmination of many genetic factors, identifying gene signatures that correspond to certain characteristics is difficult. Understanding the genetic elements behind phenotypes is based on sequencing data from many individuals that share a certain trait, which is then compared to reveal similar genetic signatures. Overrepresented mutations can then be investigated further to determine whether they are likely to be related to the phenotype in question. A recent study used this technique to identify mutations related to the superior high altitude tolerance and increased exercise capacity seen in the Sherpa population [78].

As sequencing costs decrease, and databases of genetic information grow, the ability to identify individuals that are likely to display a desired phenotype expands. Genetic information can therefore provide a method of selecting military personnel that have higher exercise capacity, physical and mental robustness while simultaneously identifying underlying diseases. Furthermore, genetic information can then be used to generate personalized exercise and nutrition programs, treatment in response to trauma and injury, and disease prevention strategies.

5.3.2 Microbiome modification

Drug factory probiotics is a relatively new field of research that can potentially provide a new approach to treating a range of diseases [79]. By engineering gut microbiota to produce therapeutic molecules, delivery and dosage could potentially be regulated automatically by the bacteria themselves. Biomarkers of disease could be used as initiators of therapeutic biomolecule production, thus activated only when needed, directly at the site of infection. Drug factory probiotics could potentially be used to combat common diarrheal diseases in military personnel stationed abroad by introducing the bacteria before deployment. There are some regulatory barriers and concerns regarding the introduction of genetic engineered organisms to the human body, and considerable research is needed before introducing engineered microbiota into the human gut. A more complete understanding of the long-term genetic stability is needed as the modified bacteria will not only be exposed to the human body but pass into the environment through faecal material. However, the importance of our microbiome is gaining traction and research in this area is likely to gain more momentum.

Box 4.3 – Summary of synthetic biology and biotechnology applications within human enhancement

Advancements in synthetic biology and biotechnology have opened up possibilities for human enhancement. Direct enhancement involves improving individuals' overall health and robustness through various means, including genetic engineering and personalized treatment strategies. Indirect enhancement focuses on physiological monitoring and early detection of biomarkers related to conditions like fatigue and dehydration, enabling timely intervention. Genetic information can aid in selecting personnel for specific tasks and identifying underlying diseases. Another area of exploration is modification of the human microbiome, with the potential use of engineered microbiota to produce therapeutic molecules for targeted treatment. However, ethical considerations and long-term effects on genetic stability need to be thoroughly addressed before implementing such advancements.

5.3.3 TRL

Table 5.3 Estimated TRLs for development of new synthetic biology/biotechnology-driven human enhancement capabilities for military purposes.

Technology	Today	TRL 9
Genetic selection*	TRL 9	---
Microbiome modification	TRL 4	2040

* Already partially established, these concepts hold the potential for further development and utilization.

5.4 Regenerative medicine

An area within medicine in which synthetic biology has great potential for military operations is regenerative medicine. A definition commonly used is that regenerative medicine is the “repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and aging” [80]. Regenerative medicine is of especially interest within military medicine, for instance in treating injuries sustained in military operations or accidents, saving lives, facilitation of return to duty, and veteran health care. Regenerative medicine does not exclusively concern replacement of damaged tissue and

organs, but also incorporates research on self-healing. The body's innate healing response to injury can be aided by introducing foreign materials that promote regeneration [81].

5.4.1 Tissue engineering

An important field within regenerative medicine is tissue engineering. This field combines engineering, material science and biology to assemble scaffolds, cells and biologically active molecules into functional tissues in order to restore, maintain or improve damaged tissues or organs. Examples of engineered tissues approved by the US Food and Drugs Administration (FDA) are artificial skin and cartilage [82]. Today, organ and tissue failure and loss are often treated with transplantation of donated organs and tissues, which is problematic due to the lack of donors and severe immune complications due to poor biocompatibility [81]. These issues are also highly relevant for military personnel injured on the battlefield, or with acquired organ or tissue diseases.

5.4.1.1 Stem cells

Stem cells are of immense interest in the tissue regeneration research area. As described in Section 2.4, stem cells are undifferentiated cells that can differentiate into almost any kind of cell if exposed to different factors. In addition to proliferating and differentiating into mature cells, they do also secrete cytokines, antimicrobial peptides, immunomodulatory molecules and growth factors themselves that facilitate the body's regeneration processes [83]. Injection of stem cells could be a routine procedure in treating injuries and wounds in the future [84]. Synthetic biology techniques can enable control of stem cell gene expression patterns in order to drive cell fate outcomes. This may improve tissue regeneration with stem cells. Examples of tissues that potentially can be regenerated by stem cell therapy are bone, liver, nerve, muscle and skin tissue [85]. For instance, bone tissue regeneration after an injury by the use of stem cells could be a better option to grafting autologous bone tissue from the patient due to limited supply of autologous bone, risk of complication during bone grafting and disruption to the bone donor site.

5.4.1.2 3D bioprinting

Stem cells need an environment (a scaffold) with access to the right nutrition to grow into new tissue. To create this scaffold, additional factors and cells can be organized by 3D bioprinting. The accessibility of bioprinting as a tool has expanded lately, from being limited to specialized researchers building the printers from scratch to being used by a wider range of research groups who now can buy the hardware off-the-shelf. In vitro production of biocomponents has two main applications; as a research tool for investigation of healthy and diseased tissue and fabrication of replacement tissue [86]. 3D bioprinting could potentially solve requirements in medical research in areas such as drug delivery, regenerative medicine and functional organ and tissue replacement, being more time- and cost-effective than today, not relying on animal models [87]. In 2022, the first successful clinical trial of transplantation of a 3D printed ear was performed, as the first of its kind. An external ear was created by the use of the patient's stem cells, and used to treat microtia, a deformity where the external ear is underdeveloped [88]. Although extremely promising, bioprinting strategies often suffer trade-offs in terms of appropriate materials,

resolution, cell viability, and creation of vascular networks. These are important areas of research in order to excel in bioprinting technologies [81]. In the long run the field of bioprinting could advance to the point of producing personalized organs and tissues on demand, solving the shortage of organ donors and saving the lives of patients with organ defects and injuries. The fact that the organs will be personalized also makes the transplantation safer, with reduced risk of organ rejection.

In the future, when biotechnology advances to the stage of printing complete organs, military personnel can undergo comprehensive scans to create precise computational models of their organs. In the event of an injury where transplantation becomes the optimal treatment, a bioprinter can be programmed to produce a perfect replica of the patient's original organ requiring replacement. Within a short span, the newly printed organ is ready for transplantation. Moreover, this remarkable technology can also extend its capabilities to repair damaged tissues like skin, bone, and muscles resulting from injuries such as blasts, burns, or chemical exposure. By precisely mapping the injury and the surrounding healthy tissues, bioprinting offers a revolutionary solution for tissue replacement.

Bioprinted organs can also be utilized in drug research and development. Different organs' response to a range of substrates could be tested in vitro, making the drug development process faster, cheaper, more animal friendly and safer [14].

5.4.2 Animal-grown organs

Another solution to the shortcoming of donated organs, is to grow human-like organs in large livestock. This way, livestock such as pigs and cattle could be used as incubators for livers, kidneys, lungs and hearts. Until now, several attempts of xenotransplantations, transplantation of organs, tissues or cells between different species, have been performed. One of the latest, the transplantation of a genetically modified pig heart to human was somewhat successful, but the patient's condition worsened after surgery, and he died two months later. The biggest issue to overcome is the strong immune responses to foreign organs, which is especially prominent when the organ originates from a different species. To solve this a number of genetic modifications must be done, e.g., by tools such as CRISPR/Cas, including knocking down immune rejecting-related genes, insertion of human genes and growth genes for controlling the size of the organ. Also, animal-derived diseases, remnants of virus DNA in the animal DNA, and different lifespans between humans and animals must be taken into consideration [89].

Another way to grow organs in animals is to introduce human stem cells to an animal embryo, that later is implanted into a surrogate animal, e.g., a sow, where it will grow to full size. The stem cells could either come from the patient in need of organ transplant, or in advance from donors. In an early pig embryo, the animal genes responsible for the organ of interest are removed by using gene editing tools before the human stem cells are injected. The pig will then develop organs consisting of both pig and human cells, except the organ of interest which will only contain human cells. This could for example be a pancreas that when fully grown could be transplanted into a human. So far creation of human-pig and human-monkey chimeras have been reported. In these cases, human stem cells have been injected into pig and monkey embryos, that later were

discarded. One of the greatest limitation for this approach is the ethical concerns. Many are concerned that the boundary between human and animal gets blurred by mixing up genes and cells [90].

There is no doubt that the lack of organs for transplantation is a huge issue for both military personnel and civilians. The different approaches mentioned in this section could be possible solutions to this problem. The use of animal organ donors and xenotransplantation are widely debated and lead to prominent ethical concerns, especially where human genes or cells are incorporated into the animal, but also regarding animal wellbeing. Animal protection organizations express huge scepticism and want to limit the research in these fields. Organizations such as Dyrevernalliansen and NOAH both want to prohibit all research on animals, especially the use of animals as organ factories for humans [91, 92]. The same ethical concerns are not as prominent when creating organs from scratch in the laboratory. Even being very complex, creating organs from scratch seems like the best and potentially the safest option when overcoming technical barriers.

5.4.3 Synthetic blood

Another example of an application of synthetic biology that holds potential of impacting both military and civilian medicine is synthesizing artificial blood. The accessibility of blood products is limited in hospitals and especially in war zones and less developed areas due to a lack of donors, biocompatibility and storage requirements. Since the beginning of the 1800s blood has been transfused from healthy people for treating patients with blood loss and diseases [93]. Research on the area of blood substitutes expanded due to shortcomings of blood during the Vietnam War. Also, the discovery of Human Immunodeficiency Virus (HIV) and other blood transmittable diseases motivated this field of research [94]. Today blood transfusion is a common medical practice as part of surgical procedures, treatment of certain diseases, giving birth and so on. Donated blood is traditionally separated into three parts; red blood cells (RBCs, for oxygen transport), plasma (scaffold with proteins and solutes), and the third part consist of platelets (coagulation) and white blood cells (WBCs, immune system). These different blood products have different storage requirements, from room temperature and agitation to frozen [95]. The limited shelf-lives of blood products lead to high turnover numbers and dependencies on frequent donations. In remote areas and when treating acute massive bleedings outside of hospitals, whole blood is preferred, since it contains all components lost in a bleeding and is easier stored than three different components (up to 42 days when kept refrigerated). When treating diseases such as sickle cell anaemia on the other hand, only the RBCs are necessary to transfuse, and in other cases the patient needs platelets specifically.

Artificial blood must meet certain requirements; one being compatibility to (all) human bodies and second, it must be able to take up, carry and release oxygen where it is necessary. Also, it must be shelf stable, enabling cost- and labour-effective storage over a long period of time, in contrast to natural blood products. So far, two types of synthetic blood products to replace RBCs have shown potential; haemoglobin-based oxygen carrying agents (HBOCs) and perfluorocarbons (PFC), the former showing most promise. HBOCs are either isolated haemoglobin (Hb), e.g., from cows, or synthetically produced. To synthesize Hb engineered

microbes (e.g. *E. coli*) able to produce Hb are grown in bioreactors before the Hb is isolated [94]. The advantage of using cell-free Hb is that the problems of blood typing and compatibility that come with the red blood cells are eliminated. Currently, there are limited synthetic blood products for humans on the European market, but several products show promising results. One example is Hemopure, which is available in South Africa and Russia, but yet has not been fully approved for clinical use in the US or EU due to safety concerns. Hemopure is stabilized, cross-linked bovine Hb in salt solution, which can be stored at room temperature for up to three years [96]. Freeze-dried encapsulated human Hb has also been developed, but has yet been not tested on humans. The Hb powder is stored in bags at room temperature and does only need to dissolve in sterile water prior to use. Then the paramedic on the battlefield or in the ambulance can give the Hb substitute immediately without the need to check the blood type. In the future this Hb could be produced by genetically engineered yeast or bacteria [97].

A cell-dependent approach that could take the place of donated blood, is producing the different blood cells in bioreactors. Platelets, WBCs, and RBCs are then derived from pluripotent stem cells. These can be genetically manipulated (e.g., using recombinant DNA technology and CRISPR/Cas) to make them universal blood products, compatible to all humans. In red blood cells, for instance, the genes encoding blood type antigens (e.g., ABO, Rhesus, Kell etc.) are knocked out, before the cells undergo differentiation into mature blood cells that can be transfused to the patient. These bioreactors must be compact and mobile, making it possible to produce the cells in military hospitals on site. Large-scale production of blood cells and platelets ex vivo from stem cells would obviate the overall shortage of blood products [98].

Box 4.4 – Summary of synthetic biology and biotechnology applications within regenerative medicine

Replacement, or in this case regeneration, of injured or diseased tissues and organs could be vital for battlefield-injured soldiers. By the use of synthetic biology and biotechnology we are one step closer to a better understanding of the tissue healing process, and, in the long run, laboratory production of tissue and organ replacements. Synthetic biology and biotechnology together with material science and engineering could lead to capabilities such as personalized 3D-printed tissues and organs, production of biocompatible body fluids and tissues in laboratories and stem cell-based wound treatments. This could make tissue and organ replacement safer, with fewer complications and inconvenience for the patient and donors.

5.4.4 TRL

Table 5.4 Estimated TRLs for development of new synthetic biology/biotechnology-driven regenerative medicine capabilities for military purposes.

Capability	Today	TRL 9
3D bioprinting organs	TRL 3	2045
Xenotransplantation of organs	TRL 6	2035
Freeze-dried haemoglobin	TRL 7	2030
Lab/industrial grown blood cells	TRL 6	2035

5.5 Detection, Identification and Monitoring capabilities of biosensors

The "gold standard" in the rapid bio-detection and -identification context is PCR. Today this technology enable quite fast and accurate identification of most organisms based on their nucleic acids, with bench top-sized instrumentation [99]. Sequencing can also be used for pathogen identification, and has the unique advantage of being able to identify all pathogens present including genetic modified organisms, in contrast to PCR, which requires pathogen specific primers (explained in Section 2.2). Recent advances in sequencing technologies, such as Nanopore sequencing [100], now enable the user to sequence samples in the field with limited resources rather than being limited to an advanced laboratory setting. Furthermore, the price of sequencing per nucleotide, as well as the price of the device has dropped dramatically making sequencing for DIM purposes more reasonable. Both PCR and sequencing provide high sensitivity and accuracy but they also require specific sample preparation and trained operators [14]

As discussed earlier, a potential future threat might be the creation of altered pathogen by the use of synthetic biology. These might have altered protein expression or modified nucleic acid sequences, making them able to evade our current DIM methods, especially nucleic acid based methods such as PCR. Therefore, it is crucial to develop non-targeted DIM methods able to provide confirmed and unambiguous identification even for modified organisms. Biosensors are very suitable for DIM applications for both biological and non-biological analytes. Synthetic biology now allows researchers to develop biosensors with novel receptors that can bind to molecules that there are no natural cellular receptors with affinity for. The large toolbox of bioreceptors and transducers now available can be combined to produce sensors with different characteristics such as high sensitivity and/or specificity, as well as sensors that require minimal expertise and simple equipment.

5.5.1 Medical monitoring

The development within biosensor technology has gained tremendous interest within the fields of biomedicine and healthcare. Biosensors' potential of monitoring hard-to-access environments of the body, such as the gut, and long-term and remote monitoring can potentially transform the way diagnoses and treatments are managed and offer opportunities for new discoveries.

One example is the use of engineered cells combined with microelectronics which have resulted in ingestible micro-bio-electronics for in situ gastrointestinal biomarker detection, where the ingestible device communicates with an external one [48]. The biomarkers could be haemoglobin, which indicates gastrointestinal bleeding, or other biomedical markers, or even pathogens such as *Bacillus cereus*. As already discussed, gut health is connected to physical performance and cognitive function. To be able to diagnose gastrointestinal irregularities fast and noninvasively is beneficial to both military personnel and civilians as the risks of complications are reduced and the time to treatment onset is shortened.

Cellular biomedical tattoos have also been tested for biosensing purposes. These tattoos can potentially monitor long-term blood concentrations of different biomolecules, such as calsium [101]. This enables early warning of conditions that progress asymptomatically, and thereby allow for an earlier intervention.

5.5.2 Hazard detection

Simple biosensors are already being used to large extent in detection of pathogens, such as the SARS-CoV-2 rapid tests (LFA) that are based on antigen-antibody-binding. Other pathogens can also be detected by LFAs such as ricin, *Bacillus anthracis*, botulium toxin etc., and synthetic biology offer powerful tools in the development of such tests. These have low demands regarding equipment and trained personnel, and offer a diagnosis in just a few minutes.

An example of a less mature application of synthetic biology in diagnostics is the use of CRISPR/Cas-based biosensors. The CRISPR/Cas-based techniques SHERLOCK (specific high sensitivity enzymatic reporter unlocking) and DETECTR (DNA endonuclease-targeted CRISPR trans reporter) have both been tested on Zika virus, dengue virus and bacterial pathogens and show great promise. The use of CRISPR/Cas enables programmable detectors of high specificity and sensitivity. When binding of target nucleic acid occurs, the cleaving enzymes (Cas) degrades nearby nucleic acids in addition to the target. These nucleic acids can be RNA probes labelled with molecules that when cleaved can be visualized by e.g. fluorescence [102, 103]. CRISPR/Cas technologies can be combined with LFAs in small point-of-care rapid tests [104] for detection of B agents from different samples, such as human body fluids and environmental samples (see Figure 5.1).

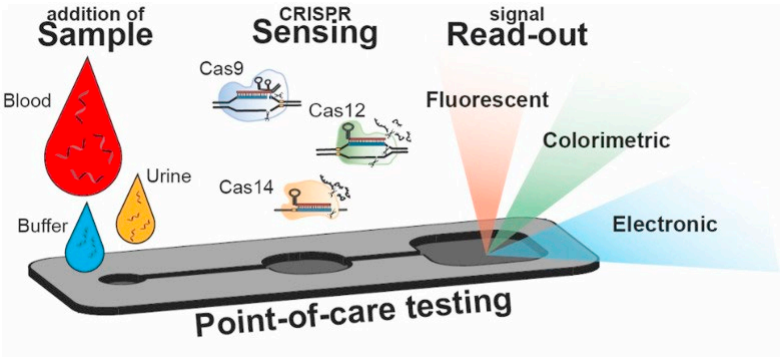

Figure 5.1 Illustration of CRISPR/Cas systems for point-of-care testing. Adapted from van Dongen, et al. [105].

In addition, biosensors can potentially be engineered to detect antimicrobial resistance, either resistance genes or resistance mechanisms such as efflux pumps and degrading enzymes. Being able to rapidly determine restistance among infectious agents makes a helpful tool in deciding correct treatments of bacterial infections, leading to reduced overall antimicrobial resistance development and more effective treatments [48].

Besides of the development of cell-free platforms for detection of B agents, whole-cell platforms for explosive detection have shown great promise. When these platforms are exposed to vapour or leakage from undetonated explosives, engineered bacteria begin to fluoresce. The fluorescent signal is detected by exposing the bacteria with UV-radiation [106]. Similarly, plants can be genetically modified to respond to leakages from explosives through e.g. morphological changes [107]. This could potentially ease the burden of demining and detection of explosives which today is a dangerous and resource demanding task.

5.5.3 Integrated systems

Synthetic biology can create biosensors for different purposes. Biosensors may improve defensive capabilities through a number of applications such as CB threat detection, monitoring of perishable supplies such as food, integrated soldier physiology monitoring, treatment monitoring and in support of biomedical research [12]. Biosensors offer a range of characteristics that can be tailored to the system to which it is integrated. Fabrics such as uniforms and CB protective equipment can be outfitted with biosensors that detect CB hazards, food packages can have integrated sensors that can detect spoilage and implantable sensors can monitor soldier physiology through specific biomarkers.

The ability of biosensors to be combined with electronic signals provide opportunities for each sensor to report their results to central systems that can deliver information to key personnel [12, 14]. Furthermore, the biosensors ability to be coupled with electronic detectors that can be monitored remotely can provide significant defensive advantages. Remote monitoring of

foodstuff condition, military personnel physiology and CB detection can give superior informational overview to key personnel while simultaneously reducing the total workload.

The DIM capabilities of biosensors have the potential to change the way to perform point-of-care diagnostics with their ability to selectively recognize biological analytes such as DNA/RNA with minimal equipment. This is especially important in situations where there are limited medical infrastructure and personnel, e.g., in less developed countries or in remote area military operations. The recent pandemic proved the need to have rapid diagnostic systems that can be produced locally, with easily adjustable recognition systems and with the ability to detect strain variants at low concentrations. It is not unlikely that several, if not all, of these capabilities will be needed to control and defend against a biological attack in the age of synthetic biology. Biosensors, combined with improved bioinformatics, AI for rapid diagnostics, remote monitoring and novel materials have the potential to significantly enhance combat casualty care and operational readiness and civilian preparedness [12].

Box 4.5 – Summary of synthetic biology and biotechnology applications within DIM

PCR and sequencing are currently the “gold standard” methods for rapid pathogen identification, but biosensors offer advantages such as the ability to detect a wide range of pathogens, including modified organisms, and their potential for point-of-care diagnostics. Recent advancements in synthetic biology have enabled the development of biosensors with novel receptors and transducers. Biosensors have the potential to revolutionize diagnostics and enhance defence against B threats in various settings. In medical monitoring, biosensors offer the potential for early detection and remote monitoring of conditions. They can be used to monitor biomarkers in hard-to-access areas of the body, enabling timely interventions. In hazard detection, biosensors enable rapid and reliable identification of pathogens, including using CRISPR/Cas-based techniques. Biosensors can also be engineered to detect antimicrobial resistance and respond to explosives. Integrated systems incorporating biosensors have various applications, from CB threat detection to monitoring perishable supplies and soldier physiology. Biosensors can transmit data to central systems, enabling real-time monitoring and remote analysis. Overall, biosensors have the potential to revolutionize bio-detection, enhance combat casualty care, and improve defence against synthetic biology-based threats.

5.5.4 TRL

Table 5.5 Estimated TRLs for development of new synthetic biology/biotechnology-driven DIM capabilities for military purposes.

Capability	Today	TRL 9
Ingestible bio-electronic devices	TRL 4	2035
Biomedical tattoos	TRL 3	2035
CRISPR/Cas-based biosensors	TRL 6	2030
Whole-cell explosive detection	TRL 6	2030

7 Conclusions and recommendations

Synthetic biology and biotechnology are emerging areas of research with great potential within military, industry and medicine while simultaneously being a potential threat to biosecurity. Being at the forefront of synthetic biology research therefore serves as both a security measure and an opportunity to boost defensive capabilities. Synthetic biology and biotechnology have also significantly expanded and will continue to expand the bioeconomy by enabling sustainable production of valuable biological resources and fostering innovation across various industries.

The use of synthetic biology to engineer and manipulate biology have beneficial effects from the defence perspective including the development of new countermeasures and protective measures to maintain operational readiness in challenging conditions. Synthetic biology is therefore attracting a lot of attention from NATO and its allies as an EDT, both regarding utilization of the technologies and biosecurity. Emerging synthetic biology technologies and platforms may deliver capabilities that can dramatically improve the capabilities of the NATO alliance. The convergence of synthetic biology, Big Data and AI is expected to contribute to the design of new drugs, purposeful genetic modifications, direct manipulation of biochemical reactions, and living sensors.

Advances within synthetic biology have the potential to make us more resilient to naturally occurring, accidental and intentional biological and chemical events by providing more effective medical countermeasures. Synthetic biology-based medical countermeasures can greatly benefit less developed areas as well as military operations, where storage and laboratory infrastructure are limited.

There is no doubt that the use of genetic markers and gene variants as indicators have a great impact on our understanding of human physiology and pathophysiology. Genetic information, biological factors and computational power are combined to shift medicine towards a personalized approach, where individual factors can be taken into account. This do also have applications in military medicine and human enhancement as this can enable prediction of diseases and treatment outcomes, in addition to being a helpful tool in soldier selection, where some traits are more preferable than others.

The convergence of synthetic biology and biotechnologies, with AI, ML and/or adaptive manufacturing may revolutionize regenerative medicine. In the future, it would not be unthinkable to replace injured or unhealthy organs and tissue with 3D-printed organs and tissue grown in a laboratory. This, together with advances in stem cell engineering may also have applications in basic research, e.g., organ-on-a-chip platforms.

The use of sequencing for identification purposes already has revolutionized modern microbiology, enabling fast and unambiguous identification of unknown microbes (DIM technologies). DIM has gotten an essential role especially as a military capability. The next step would be to develop biosensors for point-of-care use. Biosensors are ideal for DIM applications, detecting both biological and non-biological analytes. Synthetic biology enables the creation of

biosensors with novel receptors, providing high sensitivity and specificity. They are user-friendly, requiring minimal expertise and simple equipment.

The TRL vary between the different capabilities arising from technological breakthroughs and development in synthetic biology and biotechnology and is difficult to pinpoint accurately. Furthermore, predicting the full-scale implementation of the relevant capabilities is no trivial task, and prospects can change dramatically with new discoveries. As the industry is adopting synthetic biology and biotechnology, we expect several of the capabilities discussed in this report to get a boost towards a TRL of 9, e.g., different usages of CRISPR/Cas and the production of biomolecules. How fast this occurs depends largely on what the market deems most valuable. Combining synthetic biology and biotechnology with other EDTs, such as AI, ML, Big Data and additive manufacturing, will accelerate the advancement of nascent technologies with vast untapped potential, e.g., cell-free protein synthesis, and 3D-bioprinting.

As technologies such as genetic engineering develops, new ethical concerns emerge. A number of technologies have reached high maturity without being on the market or in everyday use. In numerous cases, it is not the technological hurdles that impose constraints; rather, the primary limitations in most areas addressed in this report stem from ethical challenges. E.g., genetic engineering of humans is no longer a far-fetched concept, and the ethical as well as security aspects of this need to be carefully considered. As genetic engineering tools for humans approach approval, concerns emerge regarding their accessibility and affordability for all individuals. Furthermore, defining the boundary between addressing health-related issues and pursuing desired physiological traits becomes a pivotal question. New policies, regulations, strategies and regulatory framework must be updated or developed to address these ethical concerns, ensuring reasonable use of new technologies. Ethical barriers vary significantly among different parties (countries, corporations, research groups etc.), reflecting diverse cultural, regulatory, and economic perspectives. Some may prioritize stringent ethical considerations such as the potential for genetic manipulation to infringe upon human rights or environmental impacts, while others may focus more on economic competitiveness and scientific advancement, potentially overlooking ethical concerns.

The majority of investments in synthetic biology and biotechnology will reside in the civilian area. Therefore, applications by the Defence sector must focus on adapting civilian and commercial biomedical applications to bridge gaps in military capabilities.

The three general recommendations outlined by NATO STB [16], presented in section 1.1, hold significant relevance for the Norwegian Armed Forces: Civil-military collaboration is essential.

1) It is imperative to allocate substantial resources for research and development, while fostering collaborations with academia and private enterprises.

2) The establishment of an ethical framework and governance is essential.

3) Enhancing security measures in biotechnology is crucial, especially given the unique risks associated with the convergence of biotechnology and digital technologies.

Furthermore, the following recommendations are specific to capabilities outlined in this report:

- CB defence: It is essential to prioritize the advancement of techniques for DIM of genetically engineered microbes.

- Medical countermeasures: the use of biotechnologies to enable on-demand and rapid manufacturing of countermeasures should be further examined.

- Human enhancement: regulatory frameworks are of especial importance within this field. This applies both to genetic modification of humans and genetically modified organisms (GMOs) used for medical and non-medical purposes.

- Overall, it will be important to efficiently adopt and adapt civilian applications of synthetic biology and biotechnologies for military purposes, from DIM capabilities to new antibiotics and vaccines and technology to combat antimicrobial resistant microbes.

References

1. HFM-TR-305, *Synthetic Biology for Defence*. 2022, NATO Science and Technology Organization.
2. *GenBank*. Available from: https://www.ncbi.nlm.nih.gov/genbank/.
3. *UniProt*. Available from: https://www.uniprot.org/.
4. Mehmood, M.A., U. Sehar, and N. Ahmad, *Use of bioinformatics tools in different spheres of life sciences.* Journal of Data Mining in Genomics & Proteomics, 2014. **5**(2): p. 1.
5. Chui, M., et al., *The Bio Revolution Innovations transforming economies, societies, and our lives*. 2020, McKinsey Global Institute.
6. Bueso, Y.F. and M. Tangney, *Synthetic biology in the driving seat of the bioeconomy.* Trends in biotechnology, 2017. **35**(5): p. 373-378.
7. Clarke, L. and R. Kitney, *Developing synthetic biology for industrial biotechnology applications.* Biochemical Society Transactions, 2020. **48**(1): p. 113-122.
8. Otero-Muras, I. and P. Carbonell, *Automated engineering of synthetic metabolic pathways for efficient biomanufacturing.* Metabolic Engineering, 2021. **63**: p. 61-80.
9. Jackson, L.A., et al., *An mRNA vaccine against SARS-CoV-2—preliminary report.* New England journal of medicine, 2020.
10. Wells, B., *2023 NATO Summit Outcomes - Note by the Chair*. 2023, NATO Science & Technology Board (STB).
11. Government, U., *UK Biological Security Strategy* 2023.
12. Reding, D.F., et al., *Science & Technology Trends 2023-2043: Across the Physical, Biological, and Information Domains*. 2023, NATO Science & Technology Organization.
13. *Department of Defense 2017 Annual Report to Congress on Chemical and Biological Defense Program.* 2017.
14. HFM-273, *Long-term Scientific Study on CBRN Defence*. 2021, NATO Science and Technology Organization.
15. HFM-TR-335, *Biotechnology, Human Enhancement and Human Augmentation: A Comprehensive Overview of its Topical Content*. 2022, NATO Science and Technology Organization.
16. Wells, B., *NATO Science & Technology Organization (STO) contributions to the Understand phase report on Biotechnologies to the DPRC*. 2023, NATO Science & Technology Board (STB).
17. Noyce, R.S., S. Lederman, and D.H. Evans, *Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments.* PloS one, 2018. **13**(1): p. e0188453.
18. Tumpey, T.M., et al., *Characterization of the reconstructed 1918 Spanish influenza pandemic virus.* science, 2005. **310**(5745): p. 77-80.
19. Jefferson, C., F. Lentzos, and C. Marris, *Synthetic biology and biosecurity: challenging the "myths".* Frontiers in Public Health, 2014. **2**: p. 115.
20. National Academies of Sciences, E. and Medicine, *Biodefense in the age of synthetic biology.* 2018.
21. Organisation for the Prohibition of Chemical Weapons. *Chemical Weapons Convention*. Available from: https://www.opcw.org/chemical-weapons-convention.
22. United Nations Office for Disarment Affairs. *Biological Weapons Convention*. Available from: https://disarmament.unoda.org/biological-weapons/.

23. *The Australia Group*. Available from: https://www.dfat.gov.au/publications/minisite/theaustraliagroupnet/site/en/index.html.
24. United Nations Office for Disarmament Affairs. *Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons (UNSGM)*. Available from: https://disarmament.unoda.org/wmd/secretary-general-mechanism/.
25. Berendsen, E.M., et al., *Synthetic biology - A dual use technology of concern* 2017(FFI-Rapport 17/16604).
26. NATO, *CBRN Defence on Operations.* 2022(ATP-3.8.1 Volume 1).
27. Shapiro, J.A., *Revisiting the central dogma in the 21st century.* Annals of the New York Academy of Sciences, 2009. **1178**(1): p. 6-28.
28. Collins, F.S., M. Morgan, and A. Patrinos, *The Human Genome Project: lessons from large-scale biology.* Science, 2003. **300**(5617): p. 286-290.
29. Shendure, J. and E.L. Aiden, *The expanding scope of DNA sequencing.* Nature biotechnology, 2012. **30**(11): p. 1084-1094.
30. Kuhlman, B. and P. Bradley, *Advances in protein structure prediction and design.* Nature Reviews Molecular Cell Biology, 2019. **20**(11): p. 681-697.
31. AlQuraishi, M., *Machine learning in protein structure prediction.* Current opinion in chemical biology, 2021. **65**: p. 1-8.
32. Lanigan, T.M., H.C. Kopera, and T.L. Saunders, *Principles of genetic engineering.* Genes, 2020. **11**(3): p. 291.
33. Smith, M. *Genetic Engineering*. 2022 December 8, 2022; Available from: https://www.genome.gov/genetics-glossary/Genetic-Engineering.
34. Nicholl, D.S., *An introduction to genetic engineering*. 2008: Cambridge University Press.
35. Evans, J.H., *Setting ethical limits on human gene editing after the fall of the somatic/germline barrier.* Proceedings of the National Academy of Sciences, 2021. **118**(22): p. e2004837117.
36. Gaj, T., C.A. Gersbach, and C.F. Barbas III, *ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering.* Trends in biotechnology, 2013. **31**(7): p. 397-405.
37. Liu, W., et al., *Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics.* Precision Clinical Medicine, 2021. **4**(3): p. 179-191.
38. Harrison, M.M., et al., *A CRISPR view of development.* Genes & development, 2014. **28**(17): p. 1859-1872.
39. Abudayyeh, O.O., et al., *RNA targeting with CRISPR–Cas13.* Nature, 2017. **550**(7675): p. 280-284.
40. Cribbs, A.P. and S.M. Perera, *Focus: Genome editing: Science and bioethics of CRISPR-Cas9 gene editing: An analysis towards separating facts and fiction.* The Yale journal of biology and medicine, 2017. **90**(4): p. 625.
41. Murell, D. and Y. Brazier. *What are stem cells, and what do they do?* 2018 October 19, 2018; Available from: https://www.medicalnewstoday.com/articles/323343.
42. Lennard, A. and G. Jackson, *Stem cell transplantation.* Bmj, 2000. **321**(7258): p. 433-437.
43. Walper, S.A., et al., *Detecting biothreat agents: From current diagnostics to developing sensor technologies.* ACS sensors, 2018. **3**(10): p. 1894-2024.
44. Chambers, J.P., et al., *Biosensor recognition elements.* Current issues in molecular biology, 2008. **10**(1-2): p. 1-12.
45. Cui, Y., et al., *Biomimetic peptide nanosensors.* Accounts of chemical research, 2012. **45**(5): p. 696-704.

46. Zhang, Q., et al., *Graphene oxide-based optical biosensor functionalized with peptides for explosive detection.* Biosensors and Bioelectronics, 2015. **68**: p. 494-499.
47. Kim, K., et al., *Destruction and detection of chemical warfare agents.* Chemical reviews, 2011. **111**(9): p. 5345-5403.
48. Brooks, S.M. and H.S. Alper, *Applications, challenges, and needs for employing synthetic biology beyond the lab.* Nature Communications, 2021. **12**(1): p. 1-16.
49. Zhang, L., W. Guo, and Y. Lu, *Advances in cell-free biosensors: principle, mechanism, and applications.* Biotechnology Journal, 2020. **15**(9): p. 2000187.
50. Smith, M.T., et al., *Lyophilized Escherichia coli-based cell-free systems for robust, high-density, long-term storage.* Biotechniques, 2014. **56**(4): p. 186-193.
51. Schwarz-Schilling, M., et al., *Chemical communication between bacteria and cell-free gene expression systems within linear chains of emulsion droplets.* Integrative Biology, 2016. **8**(4): p. 564-570.
52. Karig, D.K., et al., *Preservation of protein expression systems at elevated temperatures for portable therapeutic production.* Journal of The Royal Society Interface, 2017. **14**(129): p. 20161039.
53. Chen, K., et al., *Research progress of CRISPR-based biosensors and bioassays for molecular diagnosis.* Frontiers in Bioengineering and Biotechnology, 2022. **10**: p. 1581.
54. Sauser, B., et al., *A systems approach to expanding the technology readiness level within defense acquisition*. 2009, STEVENS INST OF TECH HOBOKEN NJ SCHOOL OF SYSTEMS AND ENTERPRISES.
55. TEC-SHS, E., *Technology readiness levels handbook for space applications*. 2008, TECSHS/5551/MG/ap Iss1 Rev6.
56. Mankins, J.C., *Technology readiness levels.* White Paper, April, 1995. **6**(1995): p. 1995.
57. Franks, P.W., et al., *Technological readiness and implementation of genomic-driven precision medicine for complex diseases.* Journal of Internal Medicine, 2021. **290**(3): p. 602-620.
58. Fasterholdt, I., et al., *A qualitative exploration of early assessment of innovative medical technologies.* BMC health services research, 2018. **18**(1): p. 1-11.
59. Uddin, T.M., et al., *Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects.* Journal of infection and public health, 2021. **14**(12): p. 1750-1766.
60. Rousset, F., et al., *Genome-wide CRISPR-dCas9 screens in E. coli identify essential genes and phage host factors.* PLoS genetics, 2018. **14**(11): p. e1007749.
61. Krishnamurthy, M., et al., *Bacterial genome engineering and synthetic biology: combating pathogens.* BMC microbiology, 2016. **16**(1): p. 1-11.
62. Hutchings, M.I., A.W. Truman, and B. Wilkinson, *Antibiotics: past, present and future.* Current opinion in microbiology, 2019. **51**: p. 72-80.
63. Cook, M.A. and G.D. Wright, *The past, present, and future of antibiotics.* Science Translational Medicine, 2022. **14**(657): p. eabo7793.
64. Parsons, C., P. Brown, and S. Kathariou, *Use of bacteriophage amended with CRISPR-Cas systems to combat antimicrobial resistance in the bacterial foodborne pathogen Listeria monocytogenes.* Antibiotics, 2021. **10**(3): p. 308.
65. Getahun, Y.A., et al., *Multidrug-Resistant Microbial Therapy Using Antimicrobial Peptides and the CRISPR/Cas9 System.* Veterinary Medicine: Research and Reports, 2022: p. 173-190.
66. Baddeley, H.J. and M. Isalan, *The Application of CRISPR/Cas Systems for Antiviral Therapy.* Frontiers in Genome Editing, 2021: p. 28.

67. Freije, C.A. and P.C. Sabeti, *Detect and destroy: CRISPR-based technologies for the response against viruses.* Cell host & microbe, 2021. **29**(5): p. 689-703.
68. Kashte, S., et al., *COVID-19 vaccines: rapid development, implications, challenges and future prospects.* Human cell, 2021. **34**(3): p. 711-733.
69. Braff, D., D. Shis, and J.J. Collins, *Synthetic biology platform technologies for antimicrobial applications.* Advanced drug delivery reviews, 2016. **105**: p. 35-43.
70. Khan, A., et al., *Combating Infectious Diseases with Synthetic Biology.* ACS Synthetic Biology, 2022. **11**(2): p. 528-537.
71. Arevalo, C.P., et al., *A multivalent nucleoside-modified mRNA vaccine against all known influenza virus subtypes.* Science, 2022. **378**(6622): p. 899-904.
72. Vajo, Z., J. Fawcett, and W.C. Duckworth, *Recombinant DNA technology in the treatment of diabetes: insulin analogs.* Endocrine reviews, 2001. **22**(5): p. 706-717.
73. Pardee, K., et al., *Portable, on-demand biomolecular manufacturing.* Cell, 2016. **167**(1): p. 248-259. e12.
74. Poon, L., et al., *A Review of Genome-Based Precision Medicine Efforts Within the Department of Defense.* Military medicine, 2022. **187**(Supplement_1): p. 25-31.
75. De Castro, M., et al., *Genomic medicine in the military.* NPJ Genomic Medicine, 2016. **1**(1): p. 1-4.
76. Intelligence, O.o.T., *Technical Assessment: Synthetic Biology*. 2015.
77. Korngiebel, D.M., K.E. Thummel, and W. Burke, *Implementing precision medicine: the ethical challenges.* Trends in pharmacological sciences, 2017. **38**(1): p. 8-14.
78. Bhandari, S. and G.L. Cavalleri, *Population history and altitude-related adaptation in the Sherpa.* Frontiers in physiology, 2019. **10**: p. 1116.
79. Landry, B.P. and J.J. Tabor, *Engineering diagnostic and therapeutic gut bacteria.* Bugs as Drugs: Therapeutic Microbes for the Prevention and Treatment of Disease, 2018: p. 331-361.
80. Mason, C. and P. Dunnill, *A brief definition of regenerative medicine.* 2008.
81. Mao, A.S. and D.J. Mooney, *Regenerative medicine: current therapies and future directions.* Proceedings of the National Academy of Sciences, 2015. **112**(47): p. 14452-14459.
82. *Tissue Engineering and Regenerative Medicine*. 2022.
83. Hsu, M.-N., et al., *CRISPR technologies for stem cell engineering and regenerative medicine.* Biotechnology advances, 2019. **37**(8): p. 107447.
84. Ude, C.C., et al., *Application of stem cells in tissue engineering for defense medicine.* Military Medical Research, 2018. **5**(1): p. 1-18.
85. Han, Y., et al., *Mesenchymal stem cells for regenerative medicine.* Cells, 2019. **8**(8): p. 886.
86. Betz, J.F., V.B. Ho, and J.D. Gaston, *3D Bioprinting and its application to military medicine.* Military Medicine, 2020. **185**(9-10): p. e1510-e1519.
87. Vanaei, S., et al., *An overview on materials and techniques in 3D bioprinting toward biomedical application.* Engineered Regeneration, 2021. **2**: p. 1-18.
88. Nutt, D., *Cornellian-founded company implants 3D-bioprinted ear*. 2022, Cornell Chronicle.
89. Wang, W., et al., *First pig-to-human heart transplantation.* The Innovation, 2022. **3**(2): p. 100223.
90. Servick, K., *Embryo Experiments Take 'Baby Steps' Toward Growing Human Organs in Livestock.* Science Magazine, 2019.
91. Lybæk, S. *Xenotransplantasjon er et villspor*. 2014 21.03.2014; Available from: https://dyrevern.no/article/xenotransplantasjon-er-et-villspor/.

92. NOAH. *Ikke stjel dyrs organer - si nei til xenotransplantasjon*. 2007 04.10.2007; Available from: https://www.dyrsrettigheter.no/forsok/ikke-stjel-dyrs-organer-si-nei-til-xenotransplantasjon/.
93. Heier, H.E., *Fra livsfarlige eksperimenter til livreddende transfusjoner.* Bioingeniøren, 2021(1).
94. Sarkar, S., *Artificial blood.* Indian J Crit Care Med, 2008. **12**(3): p. 140-4.
95. Feuerstein, S.J., et al., *Freeze-dried plasma in major haemorrhage: a systematic review.* Vox sanguinis, 2020. **115**(4): p. 263-274.
96. Jahr, J.S., M. Moallempour, and J.C. Lim, *HBOC-201, hemoglobin glutamer-250 (bovine), Hemopure®(Biopure Corporation).* Expert opinion on biological therapy, 2008. **8**(9): p. 1425-1433.
97. Pan, D., et al., *Erythromer (EM), a nanoscale bio-synthetic artificial red cell: proof of concept and in vivo efficacy results.* Blood, 2016. **128**(22): p. 1027.
98. Spear, A.M., et al., *Regenerative medicine and war: a front-line focus for UK defence.* NPJ Regenerative medicine, 2018. **3**(1): p. 1-9.
99. Zhu, H., et al., *PCR past, present and future.* Biotechniques, 2020. **69**(4): p. 317-325.
100. Wang, Y., et al., *Nanopore sequencing technology, bioinformatics and applications.* Nature biotechnology, 2021. **39**(11): p. 1348-1365.
101. Tastanova, A., et al., *Synthetic biology-based cellular biomedical tattoo for detection of hypercalcemia associated with cancer.* Science translational medicine, 2018. **10**(437): p. eaap8562.
102. Tan, X., et al., *Synthetic biology in the clinic: engineering vaccines, diagnostics, and therapeutics.* Cell, 2021. **184**(4): p. 881-898.
103. Bogers, J.F., et al., *Bright fluorescent nucleic acid detection with CRISPR-Cas12a and poly (thymine) templated copper nanoparticles.* Biology Methods and Protocols, 2021. **6**(1): p. bpaa020.
104. Berghuis, N.F., et al., *Combining CRISPR–Cas12a with terminal deoxynucleotidyl transferase dependent reporter elongation for pathogen detection using lateral flow test strips.* Biology Methods and Protocols, 2022. **7**(1): p. bpac015.
105. van Dongen, J.E., et al., *Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities.* Biosensors and Bioelectronics, 2020. **166**: p. 112445.
106. Habib, M.K., *Controlled biological and biomimetic systems for landmine detection.* Biosensors and Bioelectronics, 2007. **23**(1): p. 1-18.
107. Deyholos, M., et al. *Feasibility of landmine detection using transgenic plants*. in *Detection and Remediation Technologies for Mines and Minelike Targets XI*. 2006. SPIE.

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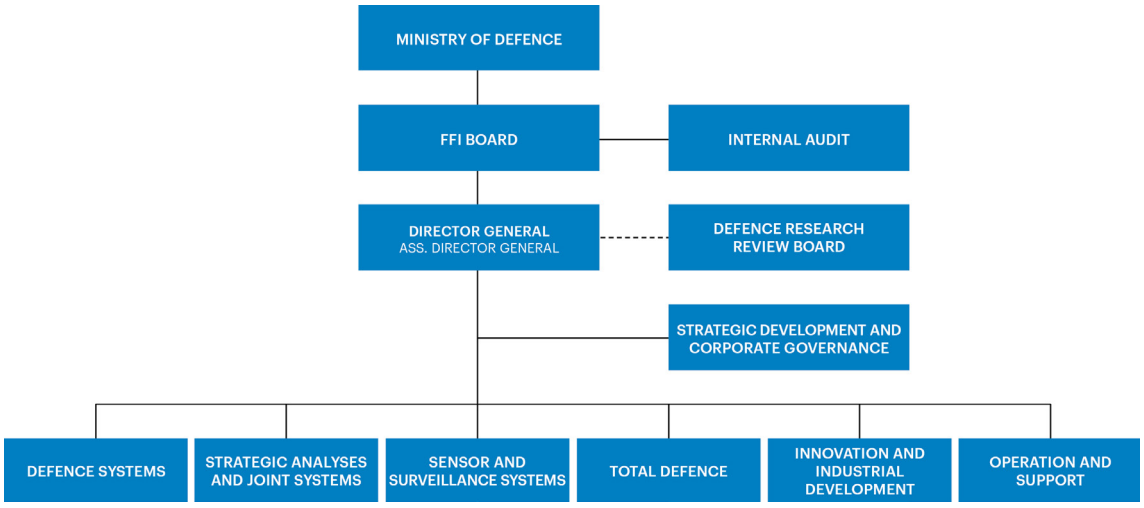

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