

Thèse
pour l'obtention du

DIPLOME D'ETUDES SPECIALISEES
PHARMACIE INDUSTRIELLE ET BIOMEDICALE

DIPLOME D'ETAT DE DOCTEUR EN PHARMACIE

Soutenus le 2 juillet 2015

Par Alexis Courbet

Engineering next generation diagnostics **through synthetic biology**

JURY : President: Pr Pierre Antoine Bonnet
 Examiners: Pr Pascal Rathelot, Dr Franck Molina, Pr Eric Renard

Thesis prepared in **Sys2Diag** laboratory FRE3690 - **CNRS** / ALCEDIAG

Complex system modeling and engineering for diagnosis

©Copyright 2015 Alexis Courbet
All Rights Reserved

“Either one does not dream, or one does so interestingly. One should learn to spend one's waking life in the same way: not at all, or interestingly.”

Friedrich Nietzsche, *The Gay Science*

Acknowledgements

I would like to particularly thank my parents and my family, for supporting me and encouraging me in my decisions and commitments, and bringing me the love and intellectual stimulation that built me. Secondly and most importantly, my wonderful fiancée for her support, her comfort and her unconditional love.

I would like to thank Franck Molina, my PhD supervisor with thousand ideas as incredible as contagious, who offered me the chance to complete my goals, always supported me and my work, and opened me the doors to an extraordinary scientific world and intellectual horizons. I would also like to thank the entire Sys2Diag laboratory, staff and associates (Bio-Rad and Alcediag), Eric Renard's team at the University Hospital of Montpellier, and Drew Endy and his lab at Stanford.

I would also like to give special thanks to Jerome Bonnet, whom I am grateful to have met, without whose help much of my work would not have been possible, and for his help, his advice, his sympathy and his patience.

Many thanks to Patrick Amar for his sympathy, his sharp mind, his fresh ideas and broad scientific culture which did not fail to illuminate our passionate discussions.

Finally, I would like to thank all the scientists and professors of the University of Paris and Montpellier, as well as doctors and hospital practitioners who during my long studies communicated me their passion for biological and medical sciences. I also want to thank all the public health structures and research administration that support the advancement of knowledge and contribute to human, social and scientific progress: the CNRS, INSERM, the Public Hospital, the University and the structures that support young researchers, who made my studies and research possible.

Foreword

“La géométrie n'est pas vraie, elle est avantageuse.”

“Geometry is not true, it is advantageous.”

Henri Poincaré, *La science et l'hypothèse*

Biology has operated a natural evolution during the 20th century. Since the foundations of enzymology, through Jacques Monod and the advent of molecular biology and cybernetics, finally enriched by the holistic views of systems biology and quantitative biotechnologies of the 90's, this path finally resolved in the beginning of this century in a modern formulation: synthetic biology. It is constituted as an interdisciplinary approach focusing on the flow of matter, information and energy in living systems. Successor of molecular biology and genetic engineering, synthetic biology is synonymous to the paradigm shift in life sciences, effectively captured in the expression "understanding by building". The famous "What I cannot create, I do not understand" by Feynman, or to quote Stéphane Leduc, to analysis, succeeds "synthesis". This later unlikely visionary, proposed in 1912 that "Biology is a science like any other, (...) it must be successively descriptive, analytical and synthetic". This transition was evident with for instance the advent of synthetic chemistry. The interconnection between engineering (building) and science (understanding) is at the origin of predictive models in synthetic biology, enabling to fully exploit the nanoscale at which biological systems operate, fortified by billion years of optimization. Although synthetic biology applies engineering principles to living organisms (standardization, automation, *in silico* design...), the peculiarity of this discipline lies in its substrate, still widely misunderstood and untamed. For this reason it is perhaps one of the most ambitious modern scientific and human adventures, since synthetic biology seeks to understand and design off-balance systems, deconstruct emerging phenomena, read and rewrite the evolutionary history of life and its origins.

Living organisms can be regarded as nanomachines, which are themselves composed of the most effective nanocircuits to manipulate information, matter and energy at the molecular level. With the latter consideration and a biomedical perspective in mind, comes immediately an idea: exploiting living systems to treat. Medical practice has always used biological knowledge to move towards an ever more efficient practice, and as such synthetic biology as a new discipline finds its place: getting the most localized, fast, accurate, and intelligent medical procedure. Specifically, medical diagnosis is an exciting technological field of research that focuses on the most efficient modalities of extraction of physiological information to make it intelligible and meaningful on a clinical plan. In this sense, synthetic biology appears as a wonderful tool to probe patient's biology at the molecular level and interface it with clinical practice. In this thesis, I thus explored the potential synergy between this new discipline and emerging diagnostic technologies.

Finally, in a global perspective, synthetic biology is a new approach to tackle life sciences. It is a discipline that concentrates a large number of open scientific questions of the 20th century, and whose progress does not only provide an increased understanding of nature, but also new technological tools applicable to the living, including Humans and their health. The last decade has witnessed the rapid development of synthetic biology to full maturity. Fully grasping the biotechnology shift that is happening is, I believe, of the utmost importance to ensure the best biomedical progress as well as effective and fertile clinical translation.

Engineering next-generation diagnostics through synthetic biology

Abstract

Synthetic biology is an interdisciplinary field that uses engineering principles to systematically design and build devices, systems and biological organisms with specified functionalities via methodological assembly of catalogued and standardized biological building blocks. Although initially developed and used by fundamental researchers, the constant refinement of the synthetic biology tool box is on its way to translate to biomedical research, medicine and clinical practice. In the post-genomic time period, systems biology and now synthetic biology based approaches to medicine provide new ways to probe and understand molecular mechanisms of diseases and support biomarker discovery for the prediction and monitoring of various pathologies. A particularly tantalizing application of synthetic biology is to develop novel versatile programmable and *smart* diagnostic approaches closely interconnected with therapy. Here we discuss how this methodology can be employed to engineer next generation diagnostics, thus improving patient care and addressing global health issues, and we explore the technology readiness to answer the medical need arising from healthcare evolution.

Keywords: Synthetic biology, bioengineering, biomarkers, medical diagnosis, *in vitro* diagnostics, molecular diagnostics, personalized medicine, translational medicine, healthcare, biosensor

Table of contents

I.	Introduction	8
II.	Synthetic biology for exploring pathophysiology and discovery of new molecular targets	14
III.	Synthetic biology for the development of new diagnostic devices	17
	A. Top-down engineering of biological sensor systems <i>in vivo</i>	17
	Microbial systems.....	20
	Eukaryotic and prosthetic systems.....	25
	Viral systems.....	29
	B. Bottom-up engineering of biological sensor systems <i>ex vivo</i>	32
	Nucleic acids based systems.....	32
	Protein based systems.....	39
	Higher order functional assemblies and synthetic cell-like systems.....	46
	C. Engineering synthetic biological systems to support signal processing for medical diagnosis.....	53
	Synthetic circuits operating <i>in vivo</i>	55
	Synthetic circuits operating <i>ex vivo</i>	59
IV.	Enabling technologies	61
V.	Technology readiness, research agendas and future clinical challenges	64
	Selection of recent advances in synthetic biology of interest to medical diagnosis.....	73
	Bibliography.....	75

Introduction

Diagnosis of diseases remains a major technological problem of medical sciences, coping with medical evolution, clinicians' information overload, financial imperatives, health facility resources and capacity, as well as geographic and economic misdistribution¹. Moreover, longer life expectancy and an increasing number of risk factors lead to a global increase in infectious, metabolic, cancer and cardiovascular diseases. In this context, many pathologies thus require early diagnosis and systematic screening of populations at risk, using non-invasive methods in resource-limited settings².

Consequently the last decade witnessed important efforts to identify predictive biomarkers of these diseases, and to their discovery succeeded the need for their robust detection. These molecular signatures can be of various biochemical natures ranging from genetic and epigenetic markers to changes in complex evolution of proteome, genome or metabolome. Since individual biomarkers are limited in providing optimal diagnostic sensitivity and specificity, they cannot accurately account for complex molecular pathophenotypes and testing for multiple biomarkers at once can thus save time and resources while improving diagnostic accuracy³. Thus far, most diagnostic tools are either non-portable, high maintenance and costly devices, or molecular devices that are restricted to the detection of single molecules with mostly low sensitivity and specificity. Thus, the demand for versatile, simple, robust, multiplexed, portable and cost-effective diagnostics is constantly increasing.

The ever-increasing understanding of biological systems, as well as medical care evolution towards personalized solutions to diagnosis and therapy thus place evolving imperatives on medical bioanalytical technologies⁴. Convergence of precision medicine, diagnosis and therapy has led to the development of personalized medicine, companion diagnosis, and theranostic. So far, centralization of conventional *in vitro* diagnostics in clinical laboratories was required in order to match modern medical standards, achieve specific, sensitive, multiplexed or high-throughput measurements, and generate results with high robustness and reliability. However, this organization of diagnosis is time and resource-consuming and requires experienced personnel and bulky equipment. Most standard detection methods such as nucleic acid amplification, immunoassays or chromatography for example, are labor intensive and expensive. In comparison, recent technological advances in biosensors and related technologies such as microfluidics, lab-on-a-chip, microfabrication, and nanomaterials have been proposed to develop portable point-of-care diagnostics matching conventional standards concerning diagnostic accuracy, resource requirements, and rapidity^{5 6}. Such requirements have motivated the development of new assay formats, such as micrototal analysis

systems (μ TAS) and microfluidic paper based analytical devices (μ PADs) that can provide qualitative or quantitative analytical information⁷.

However, other clinical solutions to decentralize diagnostics from the biochemistry lab to the patient are likely to emerge. This would benefiting the individual as well as society, increasing convenience, improving therapies and reducing healthcare costs, while also benefiting regions with poor infrastructure. Consequently, the last decade showed increasing interest for the development of innovative diagnostic technologies, promising a new era of fast, versatile, easy-to-use, cost-effective and reliable point-of-care diagnostic tests^{8 9 6}. However, despite the clinical need, translation of new biodiagnostic technologies from research laboratories to the clinics has remained limited. The explosion of biosensor approaches integrated with information technologies and biology/electronic interfaces are likely to provide new solution for close patient monitoring. Thus, methods to engineer integrated, sensitive, selective, fast and low-cost diagnostic biosystems are of tremendous importance.

We envision that the future clinical practice is likely to be organized around new uses of diagnostic systems, either: (i) by the practitioner, (ii) under the direct supervision of a practitioner, (iii) directly by the patient. To achieve highest medical service, such diagnostic devices would enable to perform autonomous biodetection of pathological biomarkers, with high sensitivity, selectivity, robustness, rapidity, ease of operation, possibility of direct analysis of samples in complex matrices without preliminary sample treatment, and, last but not least, cost effectiveness. Moreover, to achieve highest value, future diagnostic devices would be implantable and passive, wirelessly connected to the clinician, while providing label free, near real time measurements new types of parameters and improved signal processing capabilities. The engineering of such stand-alone expert biosensing device at different scale for medical decision support, remains a critical challenge, with the biggest challenge to overcome being system integration.

We propose that these new capabilities can be brought by the emerging field of synthetic biology, benefiting from a constantly increasing capability to systematically inform and interface biology. Synthetic biology can serve the engineering of a novel generation of diagnostics with enhanced performance to augment clinicians' ability to monitor pathophysiological parameters. While this approach has so far yielded proves of concepts and a few real world applications, important efforts are slowly announcing the transition into clinical sensing applications.

The last decades can be regarded as the descriptive phase of molecular biology and functional genomic research that later permitted the advent of synthetic biology^{10 11 12 13}. Synthetic biology

applies engineering principles to biology, and as such has become a science of designing biological parts, devices, systems and organisms in a systematic and rational manner to create predictable, useful and novel biological functions. Systematically defining, cataloguing, engineering and standardizing modular biomolecular components based on always increasing amount of data, in easy accessible databases provide well-characterized and novel standard biological parts enabling hierarchical abstraction of biological functions^{14 15 16}, that can be assembled at systems level to provide new biological systems with user-defined functionalities (**Figure 1**). Combined with major technological improvements, synthetic devices and systems can be easily designed and simulated *in silico*, synthesized, transferred, and assembled in complex systems. Synthetic biology thus provides a method for systematic and rational assembly of synthetic parts into on-purpose systems, and as such can be defined as the science of structuring biological matter to achieve control on biological energy and information processing. For instance, the recent advances in synthetic genomics (i.e. writing and reading information stored in nucleic acid polymers) have permitted to gain control on living organisms with unprecedented precision. Although most synthetic biology labs do not focus their efforts on biomedical applications, fundamental advances in the design of new molecular devices prove useful for the future of biomedical synthetic biology and enhance translation into the clinics¹⁷. Maturation of the field and technological development enhances our ability to study and control bio-synthetic systems to be used for health applications^{18 19}. It is now slowly transitioning into the clinics and has already yielded successful biomedical applications, for example useful drugs^{20 21}, high value synthetic medical biomaterials²², or "smart" cell for therapeutic purposes^{23 24 25 26 27}. Although applications in the medical field remain limited as synthetic biology faces challenges toward human clinical applications^{28 24}, the research landscape is moving, as direct and effective application are becoming realities (**Figure 2**). Yet promising, biotechnologies attempting to bridge the gap between research and patient clinical care are still burdened with issues of reproducibility and standardization. However, we suspect that these issues can be addressed with the synthetic biology method to allow safe, robust and reliable clinical applications (**Figure 3**). We envision that medical diagnosis is a promising field to prove translational success of synthetic biology, and as such is already under extensive investigation.

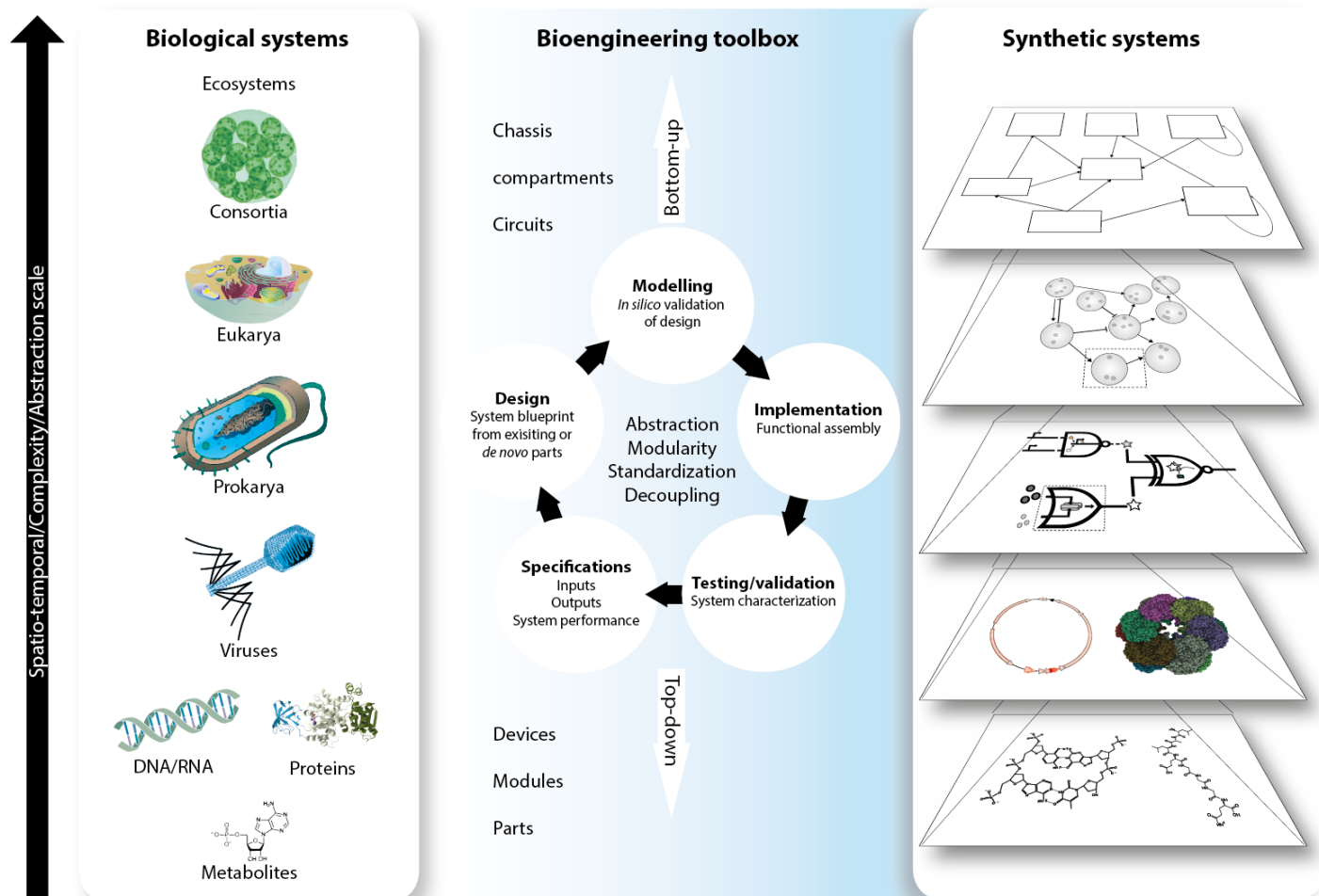


Figure 1: Synthetic biology: general framework that incorporates top-down and bottom-up perspectives in the synthetic biology design process.

The increasing knowledge of biological systems, their deconstruction, and the design of synthetic systems across different levels of complexity, is an iterative process that incorporates both top-down and bottom-up design considerations. The study of biological systems enables the accumulation of increasing amount of data that feed a “bioengineering toolbox” with standardized elements such as chassis, compartments, circuits, devices, modules and parts. Systematic engineering methods and mathematical tools constitute the conceptual framework by which synthetic biology operates. First, a design objective is formulated, taking into accounts functional constraints and specifications in terms of systems performance. Then, a synthetic biological system is designed by composing with well-characterized components with known properties, either *ab initio* (bottom-up) and/or combined with a larger biological context (top-down) and modeled *in silico* to identify potential modes of failures. The synthetic system is then constructed experimentally (implemented) and performance of the system is assessed. If the system fails to meet performance requirements, this new information can be used to refine the design and iterate the design process. This process constantly improves understanding of biology and reduces the number of iterations necessary to achieve a specific design objective. Knowledge based design infuses each levels of the abstract hierarchical scale of synthetic systems, which are in that sense parallel to “natural” biological systems.

Biosensing is indeed a mature application area of synthetic biology. IUPAC nomenclature defines a biosensor as “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds”²⁹. Applied to medical diagnosis, these devices combine biological molecules as the recognition and transducing elements to provide quantitative or semiquantitative analytical data corresponding to the concentration of a specific biomarker. Interestingly, biological systems are able to integrate various kinds of clinically relevant physical and chemical signals (nucleic acid, protein or lipid ligands, osmolarity, pH, temperature). This ability of biological systems to assess molecular pathophysiology by biorecognition of biomarker patterns is of great interest for the generation of diagnostic assays. Moreover, evolution has generated a vast natural repertoire that can be mined to retrieve useful biological functions, and synthetic biology provides tools and methods for their efficient re-engineering. In addition, biological systems have interesting characteristics for diagnostics, such as the ability to provide physiologically functional measurements, ability to perform ultrasensitive and specific response to input stimuli³⁰, and integration of complex signal processing abilities. They are also autonomous, auto-replicative and self-powered, miniaturizable, amenable for high throughput and can function in many different types of harsh environments. Additionally, biological systems are efficient problem-solving systems that use sensor and signal processing modules to analyze their environment relatively to their own state and compute phenotypic responses³¹. Indeed, the idea to engineer living organisms or their components as problem solving entities is not new^{32 33}, and molecular computers performing biological computation have been proposed for different purposes^{34 35 36}. Synthetic biosensors systems have an inherent modular architecture that provides high composability, in which 3 modules are exchangeable: sensor, processor, and reporter. The signal sensing event of biosensors can thus be associated to a computation process that can be engineered to integrate “compiled” medical knowledge in the form of a decision algorithm and computational versions of diagnosis using biological components have been proposed^{37 38 39}.

The diagnostic process attempts to classify patient conditions into distinct clinical categories that support medical decisions regarding treatment and prognosis⁴⁰. Medical diagnosis can thus be regarded as a logical problem, or an elementary computational process leading to medical decision making, considering attributes like symptoms or disease applied to patients formalized by Boolean functions⁴¹. In other words, the patient’s pathophysiological state is a function of molecular patterns. Considering *in vitro* diagnosis, this process formally implies: (i) medical knowledge: the relationship that exists between symptoms and disease that informs a decision algorithm (ii) the identification and molecular biosensing of biomarkers, (iii) a human readable signal corresponding to the final medical diagnosis. Since medical diagnosis identifies with the process of making decisions about the

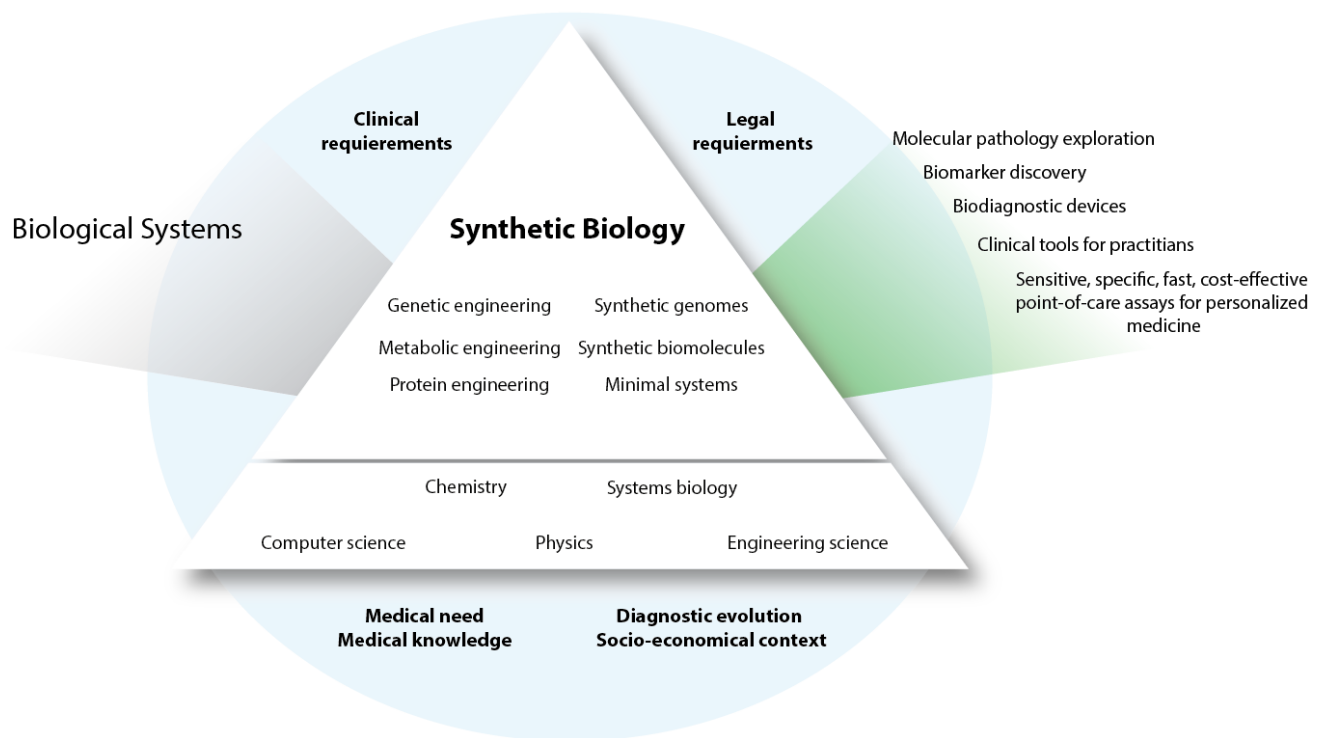


Figure 2: Synthetic biology to generate tools for medical diagnosis. The vast array of interdisciplinary methods and substrates that can be manipulated via synthetic biology enables the engineering of biological systems to develop diagnostic devices with increased design space. The capabilities offered by synthetic biology are likely to answer the medical need while solving issues arising from legal and socio-economical context.

state of human physiology, and biological systems can be used to implement the logical operations of medical diagnosis, it is possible to exploit the capabilities of biological systems for diagnostic applications, and synthetic biology enables the full integration into operational diagnostic devices (**Figure 4**).

Moreover, contrarily to conventional diagnosis in which pathological symptoms must appear in patients prior to clinical diagnosis, recent approaches have been proposed to improve this process. By nature biologically interfaceable, synthetic biological systems offers the possibility to develop implantable devices sensing pathological stimuli *in situ*, and immediately offering a therapeutic response (“sense-act-treat”, or theranostics *ex vivo*, or prosthetic circuits *in vivo*). This approach could prove extremely valuable in many clinical situations where therapeutic benefit is linked to the delay in analytical methods, clinical information management and interpretations, and effective patient care.

Diagnostic applications have thus recently attracted great interest from synthetic biologists. Here, we envision that synthetic biology most imminent medical impact is in the revolution of diagnostics, and its relation to personalized medicine and therapy through point-of-care and companion diagnostics. The aim of this review is (i) to demonstrate the importance of present and future synthetic biology approaches to medical diagnosis (ii) to map the landscape of novel biodiagnostic strategies and technologies emerging from synthetic biology (iii) to propose future orientations that could accommodate medical, socio-economical, industrial and legal requirements.

I. Synthetic biology for exploring pathophysiology and discovery of new molecular targets

A considerable need exists for improving understanding of diseases, and discovery of biomarkers for differential diagnosis, prognosis and monitoring of therapeutic interventions. Different strategies have thus been pursued to get insights on molecular pathophysiology, to unveil mechanisms and potential therapeutic targets, but also to discover predictive biomarkers of pathology development. As Richard Feynman said, “What I cannot create, I do not understand” (i.e. analysis-by-synthesis)⁴². In other words, the more we understand the complex behavior of biology, the more chance we will be able to engineer new diagnostic devices. In that perspective, synthetic biology represents a powerful approach towards new models and tools to explore and pathophenotypes. The rational and systematic reverse engineering of biosynthetic pathways, biological parts, synthetic genes and networks constitute valuable resources for the multi-level screening of disease mechanisms. It allows the iterative design and *in vivo* implementation of quantitative and dynamic models to test molecular hypotheses, and to perturb and probe biological networks topologies^{43 44}.

For instance, Yagi et al. recently shed new insights on breast cancer pathogenesis and approaches to diagnosis using a synthetic biology strategy to reconstitute G protein-regulated networks in breast cancer cells. They stably expressed an engineered G α_i -coupled GPCR, which had gained the ability to respond to a synthetic agonist, enabling them to probe the signaling pathways downstream of specific G proteins⁴⁵.

Synthetic biology recently also enabled the systematic synthesis of whole pathogens such as SARS or Influenza viruses^{46 47} or their components through complex DNA-gene synthesis and whole genome assembly techniques^{48 49}. This methodology offered fast access with low efforts to address pathogenicity mechanisms and provided new diagnostic targets. Novel immunoassays, as well as DNA arrays were developed for known or potential pathogens and newly described infectious

agents^{50 51 52}. For example, gene synthesis has recently been translated to clinical diagnosis with the discovery of Merkel Cell Polyomavirus (MCV) and its association with Merkel cell carcinoma, a rare human skin cancer⁵³. Systematic gene synthesis also enables synthetic codon sequence optimization of genes, and enhanced production of multi-epitope and chimeric antigens. Synthetic biology enables simplified screening and improved diagnostic performance via standardized and robust antigens, thus reducing assay variability and achieving high levels of sensitivity and specificity in serologic immunoassays of infectious agents⁵⁴ or autoimmune diseases⁵⁵. These strategies have been used to mimic specific epitopes from pathogens in many diagnostic systems. For example, a synthetic protein combining four different immunodominant epitopes from *Borrelia burgdorfi* generated an improved serological tests for the diagnosis and monitoring of Lyme disease⁵⁶. In the same way they provide more sensitive methods for detecting patient antibodies in diagnostic immunoassays, peptide synthesis through multi-epitope and chimeric genes can be valuable for the direct identification of new autoantigens⁵⁷. A method relying on synthetic representation of the proteome using phage display combined with high-throughput sequencing permitted to identify novel autoantigens in neurological syndromes⁵⁸. These synthetic approaches have also recently yielded comprehensive insights in human viral immunology. Xu et al. recently developed a high-throughput method to exhaustively explore the human virome relying on massively parallel DNA sequencing of a bacteriophage library displaying proteome-wide peptides from all human viruses⁵⁹.

While clinical management of complex diseases is increasingly relying on biomarkers, our ability to discover relevant ones remains limited by our dependence on endogenous molecules. The lack of specific, predictive or robust biomarkers still limits the diagnosis of many pathologies. Thus, recent attention has been given to the engineering of disease specific synthetic biomarkers. These exogenous agents are administered in the circulatory system where they record molecular events associated with pathological states. As such, they enable the non-invasive monitoring of non-classical parameters by producing new molecular signatures that can then be retrieved in clinical samples such as blood or urine. Several teams recently developed protease-sensitive biomarkers that respond to pathological enzymatic activities at diseases sites, and release reporters in circulation that are then concentrated in hosts' urine to be measured. The potential for early disease stage detection and monitoring compared to classical blood biomarkers has been reported with murine models of liver fibrosis, cancer and solid tumors, or cardiovascular diseases^{60 61 62 63}. These preliminary studies are important steps toward use of injectable synthetic biomarkers in the clinics, and could be generalized in a multiplex diagnostic platform and tailored for the diagnosis of various diseases.

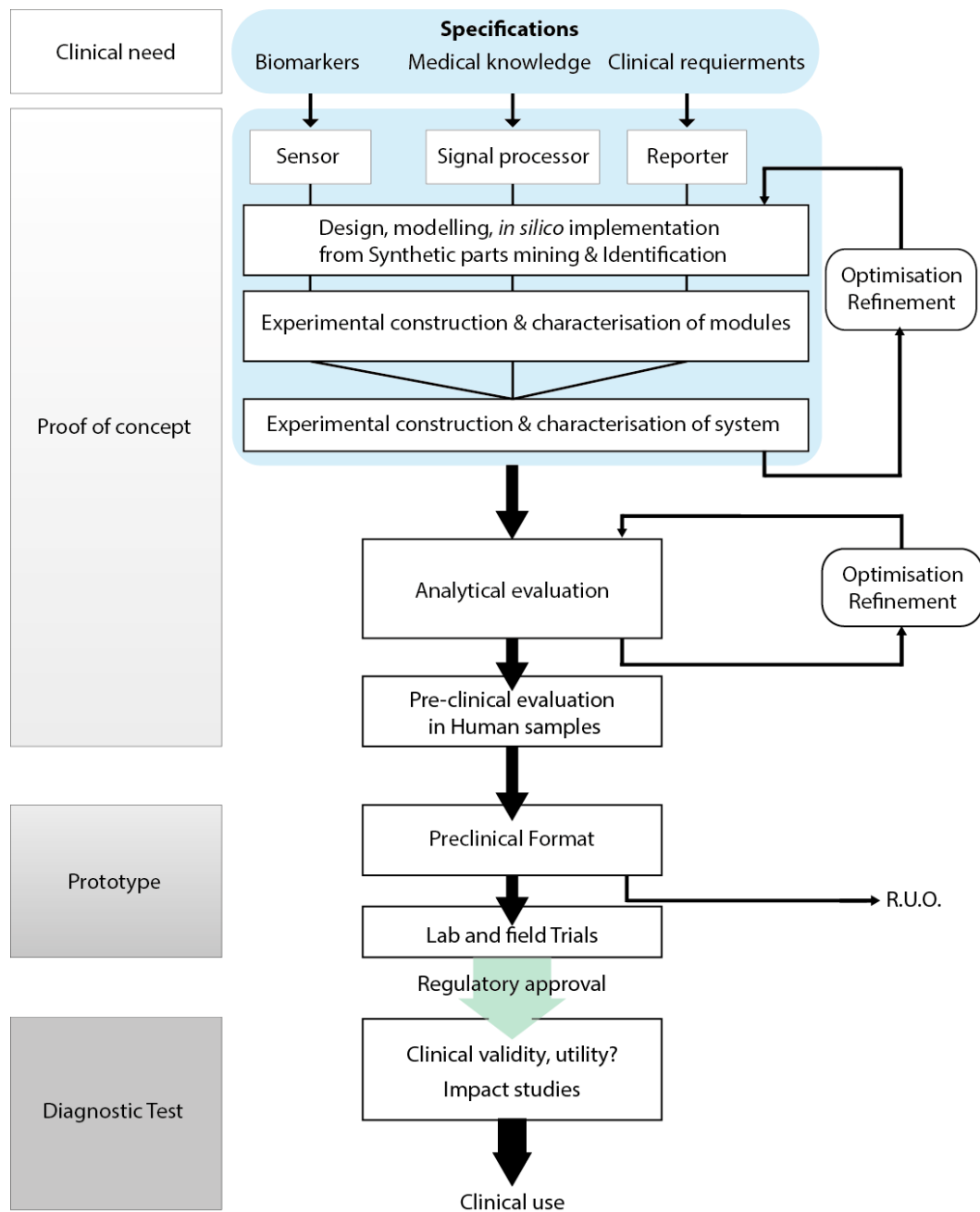


Figure 3: General framework for the conception of diagnostic devices, from bioengineering considerations to clinical use.

II. Synthetic biology for the development of new diagnostic devices

The framework considering biological entities as systems of interacting components capable of input detection, information processing, executing logical operations, and producing an output¹⁴, has led to the engineering of “intelligent” systems for biodetection purposes and as such be used for diagnostics applications. Such systems can be developed from the top-down perspective using modular biological parts assembled *in vivo* to generate useful synthetic phenotypes, or be assembled *ex-vivo* from a bottom-up perspectives for the monitoring of specific biomarkers (Figure 5, 6).

A. Top-down engineering of biological sensor systems *in vivo*:

The engineering of cell-based biosensing system has arisen as a major focus in the field of synthetic biology⁶⁴, and proved to be useful as a versatile and widely applicable method for detection and characterization of a wide range of analytes in biomedical analysis^{65 66 10 67 68}. These systems are capable of producing dose-dependent detectable signals in response to the presence of specific analytes in a given clinical sample. However, the first generation of cell-based biosensors mostly relied on cell native sensor modules without extra signal processing abilities, and thus can only detect isolated signals with low signal to noise ratio and poor robustness when used in complex matrices⁶⁹.

Consequently, synthetic biology efforts have focused on streamlining the construction of robust cell-based biosensors for biomedical applications. A wide range of modules have emerged through genetic engineering, and enhanced these systems in terms of modulation of sensitivity, specificity and dynamic range, near-real-time signal processing, multi-input (multiplexing) and logic operations, or toward the integration of orthogonal biological and electronic components^{70 71 72 73 74}. Cell based biosensors capable of multiplexing detection enable to classify complex conditions specified by combination of several signals, such as for example the cancerogenesis or the onset of chronic diseases. Many proofs of concept have highlighted the great advantage of *in vivo* integration of medical algorithm using biological logic circuits, in order to customize cell sensing and signaling into decision making systems, to be used for various clinical applications. In this way, sensor/reporter modules can be interfaced with fine signal processing such as digital logic and memory (see section 4) carried out *in vivo* by synthetic gene networks. This strategy enhances sensing specificity and accuracy of the output response (See section 4). In addition, engineering frameworks exist for the optimization of cell-based biosensors, such as directed evolution through MAGE or phage assisted continuous evolution. Even though synthetic gene circuits have been used for a decade to construct

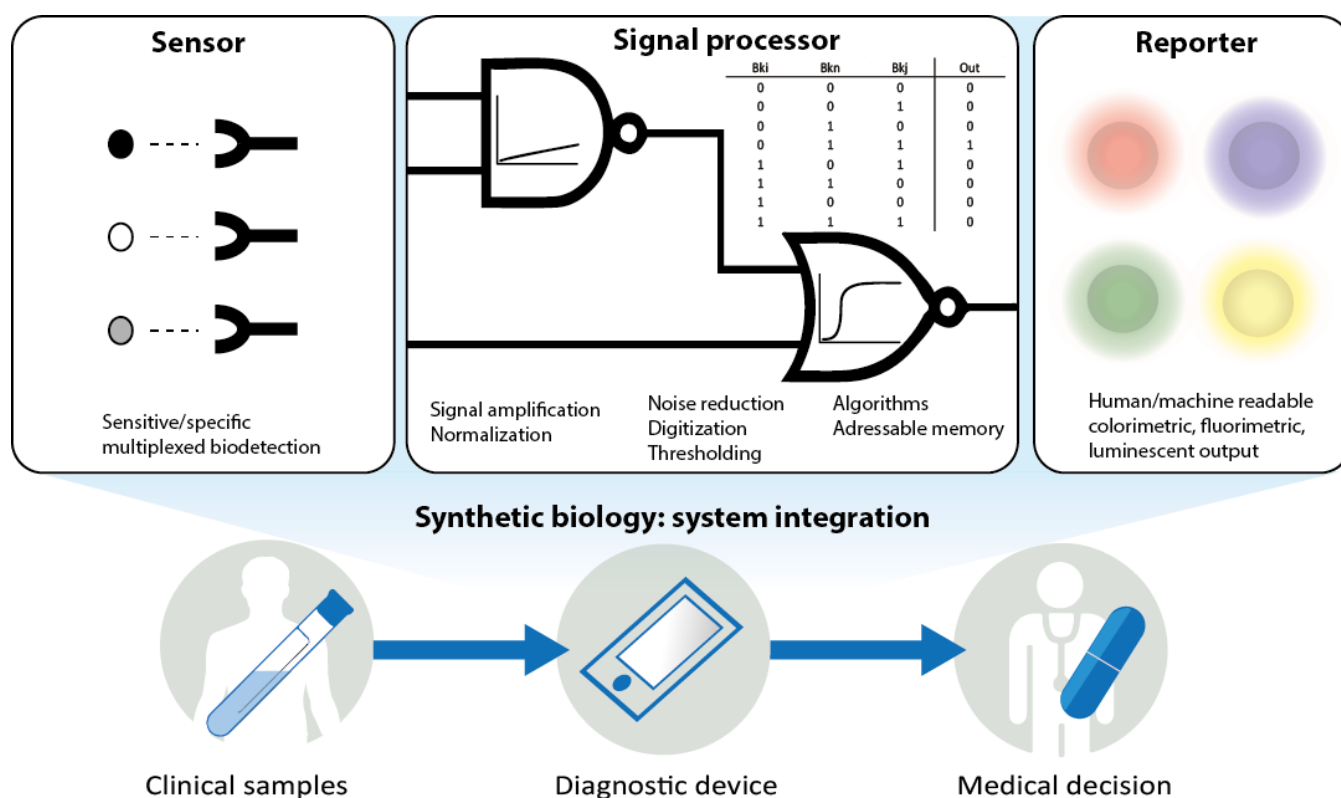


Figure 4: Synthetic biology enables system integration of diagnostic assays in biological devices.

Biological systems have evolved powerful molecular modules to sense and process biological signals and inform their phenotypes accordingly. Synthetic biology enables the re-engineering and composable assembly of such devices to develop novel, integrated diagnostic devices with user-defined specifications.

cells that respond to biological signals in a programmable fashion^{70 75 76}, current commercially available or proof of concept cell biosensors have so far been mostly used in contexts irrelevant of medical applications⁷⁷. In this perspective, we suspect that synthetic biology methods will enable a new era of robust, stand-alone and integrated smart biosensing devices for medical diagnosis.

These diagnostic devices reside within a chassis, or host cell, which supplies necessary resources for full functionality. The engineering of cell-based biosensor devices have been conducted in different cellular chassis, either plant⁷⁸, algae⁷⁹, mammal^{80 32}, yeast⁸¹, or a wide spectra of bacteria species⁸². Cell-based biosensors have been widely investigated for environmental and medical diagnosis because they enable cheap and simple large-scale field screening and measurements. However, they have other properties that make them interesting as diagnostic devices. They are relatively easy and inexpensive to prepare and store through cell culture, require low-cost reagents, and have evolved increased stability compared to biochemical probes (DNA, proteins) when exposed to perturbation (temperature, pH, ionic strength...). Moreover, cell-based assays are non-destructive, and provide more comprehensive and complex functional and physiological information than classical analytical methods, such as bioavailability^{65 76}. They can provide insights into the pathogenic mechanisms, potentially giving estimation of clinical risks associated with specific molecular events^{83 84}. Because of

the auto-replication of biological systems and self-powering a cell-based diagnostics system could be portable, and have reduced production costs compatible with systematic screening and widespread deployment. Last but not least, cell-based sensor systems can be implanted directly *in vivo*, thus permitting noninvasive detection of conditions in live cells or organisms over time, which can be particularly powerful for diagnostic applications.

Additionally, cell-based biosensors can be further integrated into high density devices to perform high through-put analyses and are amenable for miniaturization and incorporation into portable, μ TAS devices^{85 86 87 88 86 87 88 89} (**Figure 7**). In fact, micro-engineering, bioelectronics and microfluidic strategies (see section 5) enable the use of population of engineered cells, where a “cell-based chip” provides solid and fluidic support for long term maintenance and reagent/sample manipulation, acting simultaneously as a sensor, a processor analyzing complex data, and an output device that translates the detection of diseases into information intelligible to humans. For example, cell-based biosensors have been integrated “on-chip” with microelectrode arrays, photodiodes, field effect transistors, impedance or potentiometric sensors⁹⁰.

Commonly used reporter modules rely on colorimetric, fluorescent, or luminescent readouts, but can also be further interfaced electronic transducers such as acoustic detection, surface plasmon resonance, and electrochemical methods. Their choice mostly depends on assay specifications, in terms of sensitivities or technical resources. Importantly, colorimetric outputs are human readable, a property of interest for integration into low-cost, easy-to-use point of care devices, while luminescent signals offer ultrahigh sensitivities and wide dynamic range of detections. However, instead of measuring traditional end point signals, other biosensing frameworks exists, and can be achieved thanks to properties inherent to biological systems, where information processing capabilities of genetic networks *in vivo* can be exploited (see section 4). It is thus possible to define different modes of readout, such as linear, frequency, or threshold, or multivalued modes of detection. For example, a riboregulated transcriptional cascade counter that uses multiple regulatory layers, enables a cell-based biosensor to give an output that is the function of the number of successive time delayed input signal events. These “counting” systems could offer new modalities of biosensing where the output is the exact sum of signal triggers in time and not concentrations^{91 92}. Other authors have developed frequency-modulated cell-based biosensors, and suggested that oscillatory sensors could confer a number of advantages over traditional ones. Cell based biosensors relying on optical reporter can for example be improved by frequency measurement, which is less sensitive to environmental factors compared to bulk intensity measurements that require normalization and calibration^{93 94}.

Synthetic biology is thus advancing the design of genetically programmed cell-based biosensors by increasing the diversity of readout modes that can be implemented, the nature and complexity of molecular biomarker patterns that can be detected and processed.

Microbial systems

The first microbial systems designed for the detection of various molecular cues such as organic chemicals, heavy metals, drugs, or toxics were developed early and proved useful in many applications, such as the MicroTox (Modern Water) and BioTox (Aboatox) assays. In some cases they could operate in complex matrixes such as human serum⁹⁵ and urine⁹⁶ measuring biomarkers of toxic exposure, or *in vivo* where it was shown that exposure to antibiotics could be measured *in situ* in the rat gut, as well as other mammalian body fluids and tissues^{97 98 99} and on the field to assay complex foods¹⁰⁰ or soil samples¹⁰¹.

The microbial sensor module determines selectivity and sensitivity of detection of pathological signals, and is traditionally derived from bacterial sensory systems such as transcriptional regulators-inducible promoters from stress responses or degradation pathways. For this reason, natural systems used in first generation biosensors often lacked suitable selectivity/sensitivity required for biomedical applications, which motivated the increasing development of orthogonal sensing parts and devices through synthetic biology⁷⁵. The engineering of orthogonal sensing modules allowed more flexibility for tailoring detection specificities, sensitivity, and transfer functions. For example, the rational engineering of RNA riboswitches^{102 103}, or periplasmic binding protein^{104 105 106} enabled detection various small molecules ligands such as the drug theophylline¹⁰⁷, metal ions, nucleic acids, and proteins^{108 109} or extracellular biomarkers such as glucose, trinitrotoluene, L-lactate respectively. A growing repertoire of orthogonal synthetic parts dedicated to the engineering of biosensing systems is constantly emerging, such as ncRNAs^{110 111}, two-components systems, and intracellular protein transcriptional regulator-promoter pairs^{112 113}. Additionally, Synthetic biology provided methods for the proper assembly of complex genetic circuits to achieve reduction of expression noise and improve the signal-to-noise ratio, through the fine tuning of promoter strength^{114 115} to the integration of multiplexed inputs in single or multiple cell consortia⁷⁴.

Synthetic biology enables the straightforward engineering of gene networks that can be integrated in microbial cell to develop biological filters and amplifiers to enhance biosensing selectivity and sensitivity and to develop logic gated multi-input bacterial sensors. For example, we precedently developed a bacterial biosensor system we called Bactosensor, as an aid to diagnosis associated medical decision (Courbet et al.¹¹⁶) (**Figure 6: case 1**). This approach offers interesting advantages

that we believe could have consequences in medical practice. Bactosensors could provide simple use, cost-effectiveness, high sensibility and specificity, multiplexing and built in memory capacity, as well as embedding medical algorithms, while needing no clinical sample preparation⁷². Additionally, we proposed that encapsulation of bacteria in stable hydrogels could provide a disposable and portable format. We showed that bactosensors could operate in urine and serum, and demonstrated that their use could be of interest in the non-invasive screening for glycosuria and diabetes in urine samples. Although the use of bacterial biosensors in clinical samples had already been described^{97 117 95 96 99 100}, the robustness and reliability of living biosensors toward effective use in the clinic had not been addressed. Assaying complex “real world” samples is challenging because of the matrix effects of chemical mixtures on biosensor’s behavior. In our study, we thus proposed a systematic method to evaluate the operational robustness of bacterial biosensors for the clinics and optimization of biosensing, signal processing and readout synthetic modules.

However, classical approaches do not enable cell-based devices to sense all species of clinical relevance, such as protein biomarkers (albuminuria, antibodies, antigens...) which do not naturally enter bacterial cells. Cell based devices that interface robustly with host physiology necessitate the engineering of cell-surface sensors modules. Interestingly, bacteria are able to sense and respond to extracellular analytes via “two-component” systems, which constitute precious elements to implement new biosensing frameworks in prokaryotes. These receptors are intrinsically modular, and have already been successfully re-engineered for different biodetection purposes¹¹⁸. Moreover, programmable bacterial cells with alternative sensory modules such as mechanical, electrical and chemical systems to detect external stimuli via ion channel, or magnetosome for example, could be exploited for a variety of diagnostic applications^{119 120}.

Synthetic biology efforts also permitted to further advance the engineering of new microbial biosensor systems, through optimization of chassis¹²¹. Chassis can be engineered to behave appropriately in the desired environment, for example, a microorganism designed to operate in particularly physicochemical stress in harsh environments such as human serum. Moreover, a particular task or device may operate differently across chassis, and most laboratory strains of microorganism would not fit requirements for clinical applications. A promising solution is to develop synthetic streamlined chassis⁴⁸ with minimal functions required for its operation in clinical media. Most approaches made use of *Escherichia coli*, which still remains the model platform of choice for synthetic biology for its ease of use, vast biological knowledge and engineering experience. However, one drawback of using *E. coli* as a chassis is the limited repertoire of clinically relevant promoters to sense biomarkers. *Bacillus subtilis* is a promising and adaptable alternative chassis for synthetic

biology^{122 71} and could be of great interest for biosensing purposes as the number of parts and devices available increase, considering it offers interesting characteristics like genome minimisation, assembly of genome-scale heterologous DNA fragments, a wide range two-component and quorum-sensing systems, and the ability to sporulate after what it can be simply harvested and dried for long term storage and distribution. *B subtilis* is a promising chassis to develop bactosensors for its ability to engineer synthetic membrane receptors connected to orthogonal signal pathways to drive signal processing of pathological signals. In addition, biotechnological domestication of new chassis through synthetic biology, for example *pseudomonas*¹²³, is likely to promote the emergence of new, robust microbial platforms with interesting physiological and stress-endurance characteristics for biosensing in clinical conditions.

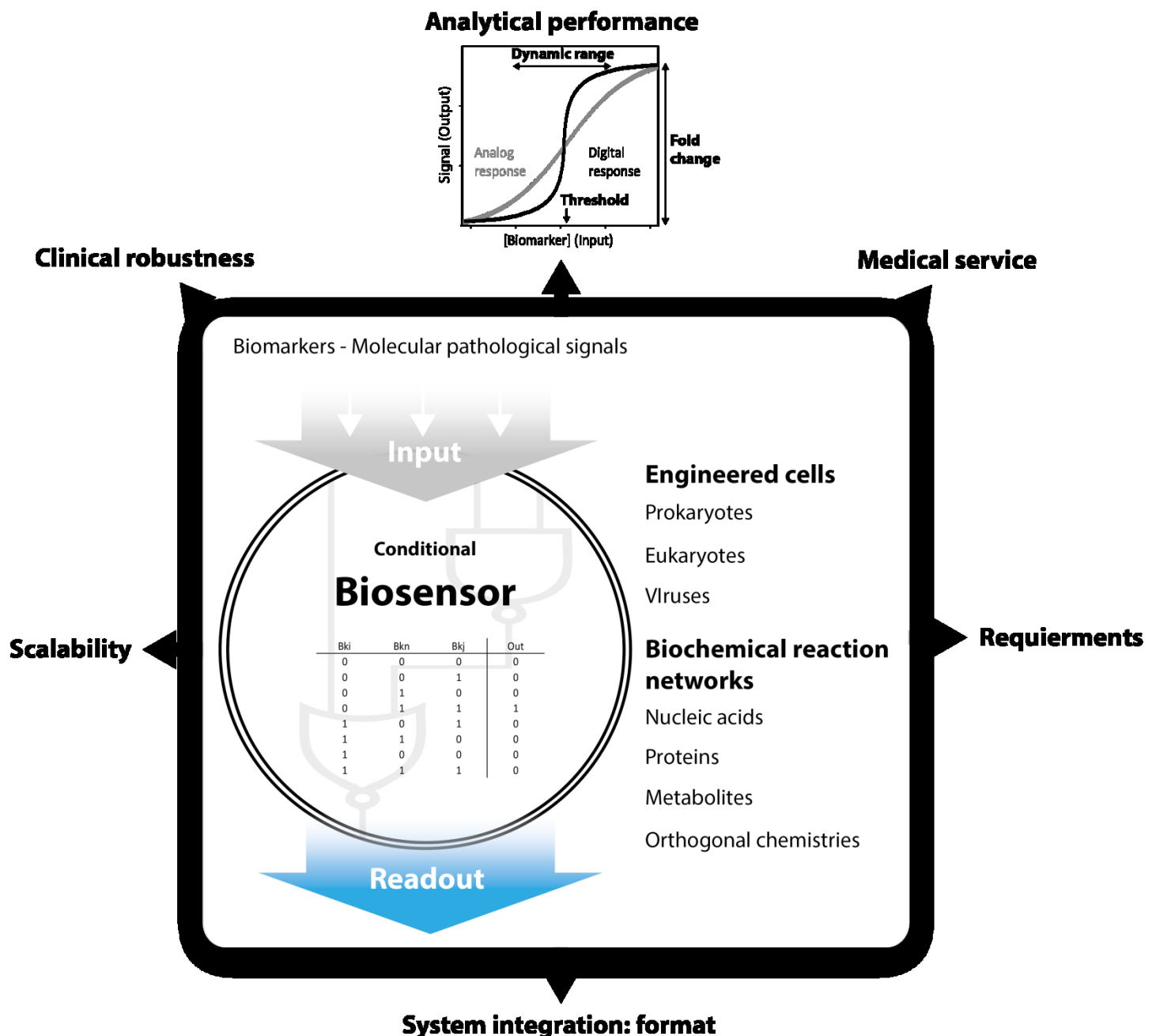


Figure 5: General considerations on constraints and architecture of synthetic biosensors for medical diagnosis. Biosensing devices conditionally generating a readable signal upon presence of specific patterns of pathological biomarkers can be synthesized using natural or synthetic components such as engineered cells, or biochemical reaction network. Such systems can be developed from the top-down perspective using modular biological parts assembled *in vivo* to generate useful synthetic phenotypes, or be assembled *ex-vivo* from a bottom-up perspectives. In order to obtain translational success, important constraints are to take into consideration in early design phases.

As a well-understood process, freeze drying of bacterial cells has been proposed as a convenient way for the long term storage and distribution of most bacterial species for biosensor assays. However, it adds an extra level of complexity and expense to the manufacturing process. In that regard, the properties of spores make their use interesting for the development of cell-based diagnostics. Sporulation enables stable storage format, handling and shipment of biosensors with extended shelf-life¹²⁴. Spores can be integrated in miniaturized portable devices where spore germination,

incubation with clinical samples, and signal detection are all integrated. For example, Date et al. have developed a μ TAS device for the detection of arsenite and zinc using engineered *B subtilis* spores. Germination of spores and quantitative response to the analyte could be obtained at room temperature in 2.5–3 h with detection limits of 1×10^{-7} M for arsenite and 1×10^{-6} M for zinc in serum samples¹²⁵. In another study, properties of spores themselves have been used to develop a real time biosensor, or label-free exponential signal-amplification system¹²⁶. The authors showed that this technique could be used to detect bacterial contamination in platelet concentrates with kinetics of the order of minutes.

Like formation of spores, immobilization of cells has received much attention. We propose that this strategy could improve the analytical performance, handling, storage and preservation of microbial biosensors without the need of continuous cultivation, and to make them suitable for integration into deployable and “ready to use” devices for unskilled personnel¹²⁷. Different strategies have been proposed as a way to obtain stable microbial biosensors encapsulation, covalent binding, adsorption, and cross-linking on various substrates. Although interesting formats have been proposed like paper strips¹²⁸ we suspect that the encapsulation of biosensors in hydrogel beads increase robustness and preserve viability and response characteristics of sensing cells under the harsh environmental conditions they are exposed to by protecting them, prevent their spread, and enable multiplexed biosensing as well as the combination of algorithmic operations in different population of beads at the same time.

Microbial cells thus offer a rich playground to engineer novel diagnostic tools, and we believe new biomedical technologies allowing novel usages are likely to emerge. For example, as natural commensal microbiome flora, prokaryotes could be used in the form of diagnostics probiotics to monitor for example gut pathologies *in situ*. A recent study showed that bacteria could be engineered to detect and record biological signals inside the mammalian gut in a programmable way¹⁹. More recently, Danino et al. engineered a probiotic *E. coli* strain as an orally administered diagnostics to noninvasively monitor liver cancerogenesis¹²⁹. Their microbial diagnostic platform was capable of recording signals arising from metastasis *in vivo* and generated an output signal measurable in the urine, for extended periods of time without deleterious health effects in mice.

Alternatively, other approaches to diagnostics development using engineered microbial cells are emerging. It has been recently demonstrated that microbial cells could be engineered to generate synthetic tunable multiscale nanomaterials (such as gold-particle patterning to create nanowires and

nanorods) that can be conjugated with target ligands and drug molecules for diagnostic applications¹³⁰.

Eukaryotic and prosthetic systems

Eukaryotic systems are physiologically closer to humans with a similar metabolism, and compared to prokaryotes benefit from a more sophisticated genome, proteome and cellular organization that increase the available bioengineering space. The extrapolation of biosensing measurements could thus be more informative, and of greater relevance for certain detection agendas. Although more complex and recent, the toolbox of biological parts and devices that operate in eukaryotic and more specifically mammalian cells is rapidly expanding^{131 132}.

Similarly to microbes, natural eukaryotic, or systematically prokaryote derived¹³³ nucleic acid and protein-based sensor modules were developed to detect diverse ligands such as small molecules: vitamins and metabolites^{134 135 136 137}, gases like acetaldehydes and nitric oxide^{138 139}, pH¹⁴⁰ or hypoxia¹⁴¹ or combinations of such input signals. Some recent mammalian biosensing systems made use of RNA-based sensors to detect clinically relevant biomarkers¹⁴². RNA aptamers are interesting as sensing modules because they can be easily engineered *de novo* to target either small molecules, proteins, or other RNAs inside live cells, through various selection strategies¹⁴³. For example, RNA aptamers could detect increased levels of intracellular protein inputs in the NF-κB- and Wnt-signaling pathways¹⁴⁴ by linking detection events to alternative splicing of an output gene.

Auslander et al. recently reported a mammalian cell-based biosensor capable of precise profiling of allergies in human whole-blood samples¹⁴⁵ (**Figure 6: Case 2**). This histamine sensor device consisted of a synthetic histamine-responsive signaling cascade in which the G protein coupled receptor HRH2 senses extracellular histamine levels and then triggers Gs-protein-mediated intracellular signaling and activation of a reporter gene. By exposing human patient's whole-blood samples to an array of allergens, basophil cells undergo an allergen-specific release of histamine which replicates the specific allergic reaction in the body. The serum is then isolated, and analyzed by designer cells that precisely score the allergen-triggered release of histamine, thereby integrating histamine levels with interesting sensitivity and dynamic range of response. This strategy proved very interesting, when current *in vivo* and *in vitro* diagnostic methods to determine the molecular etiology of allergic syndromes suffer from lack of reproducibility, patient discomfort, bulky experimentation, low dynamic range and poor correlation with clinical symptoms. This study emphasizes the interest of

such devices as it provides non-invasive, personalized allergy profiles, and pioneers the use of engineered cell-based biosensors for novel diagnostic methodologies.

Engineering of eukaryotic systems also enable the *intracellular* diagnostics approach, which involves genetically encoded noninvasive detection of combinations of small molecules, nucleic acids, and proteins in live cells over time. This strategy could allow the measurement of intracellular molecular and genomic markers, while taking into consideration the cellular context. For example, Instead of probing the chemical nature of a cell's genome, this new approaches can account for cell and genome and epigenome topology and regulatory organization *in situ*, which is known to be of functional physiological and pathological relevance^{146 147}. For instance, instead of measuring averaged signals of a cell population in the steady state, *in vivo* intracellular synthetic gene networks can give access to time and space resolution, while enabling the monitoring of the cell's gene-phenotype relationship, which is a fundamental challenge in human health.

Taking these considerations further, clinical synthetic biology has long been interested in the promise that engineering of mammalian cell-based biosensing devices could enable diagnosis of pathological states conjugated to therapeutic modulation of human physiology. Synthetic biologists are thus trying to develop and integrate *in vivo* synthetic gene networks that directly link the detection of molecular disease signals to targeted therapeutic activities, a strategy also known as *prosthetic network*. Prosthetic networks act as intracellular molecular prosthesis that sense, monitor and score disease-associated biomarkers and coordinate an adjusted diagnosis, and timely preventive or therapeutic responses for increased efficacy and safety. In the form of implantable devices, they could act as self-powered, autonomous sense-and-control circuits that trigger pharmacological systemic or *in situ* responses to restore deficient phenotypes. This recent strategy that has yet to prove applicable in the clinics, could offer potential applications in the long-term surveillance and intervention of cancerogenesis, but also infectious or chronic diseases, such as gout and diabetes.

The potential of medical prosthetic networks was demonstrated in a pioneering example reported by Kemmer et al.¹⁴⁸ Gout is associated with non-regulated, pathological levels of uric acid. The authors showed they could engineer a synthetic mammalian genetic circuit to sense, and maintain uric acid homeostasis in the bloodstream of mice. In their design, a modified *Deinococcus radiodurans*-derived protein that senses uric acid levels triggers a dose-dependent de-repression of a secretion-engineered *aspergillus flavus* urate oxidase that eliminates uric acid. The authors also showed they could insulate the circuit in transgenic cells by immunoprotective microencapsulation. Implantation of these designer cells could treat animals by reducing the levels of uric acid to subpathological

levels. Similar proves of concept have been demonstrated for metabolic diseases such as Diabetes¹⁴⁹, or diet induced obesity¹⁵⁰.

However, precise discrimination between clinical states is essential for such autonomous decision-making devices. Again, combination of multiple context-specific promoters has proved more efficient than single input approaches that suffer from linear responses and limited control of specificity and efficacy. In cancerology, more and more routine diagnoses are based on molecular signatures rather than anatomical anomalies. Nissim et al. thus engineered the mammalian two-hybrid system to act as an autonomous logical AND gate that integrates as inputs signals arising from cancer-related promoters and expresses a killer (or reporter) gene specifically in cancer cell lines. This approach provided increased response tunability and revealed a digital-like response of input amplification following a sharp activation threshold, providing robustness, minimizing input noise and false-positive identification of cell states¹⁵¹. In another key study, using the gene expression levels that clinicians commonly used to diagnose prostate cancer, Shapiro's group designed a computational DNA network that proceed to five yes/no molecular sensing events *in vivo* in order to detect biomarkers of prostate cancer. Briefly, this biomolecular computer was conditionally responsive to the presence of five positive biomarkers to generate a therapeutic output³⁸. In another example of intracellular prosthetic diagnostics, a platform that integrates logic and sensing could detect pathogenic patterns of miRNAs *in vivo*¹⁵². The authors generated a classifier system through straightforward engineering of nucleic acid hybridization reactions, which could assess whether the transient expression profile of six endogenous miRNAs matched a specific profile characteristic of cervical cancer. This genetic logic circuit could identify cancerous cells and triggers apoptosis in response. This approach could be in principle extended to the detection of complex molecular pathophenotype and connected to *in vivo* therapeutic actions. Synthetic gene network built using CRISPR-Cas9 technology in mammalian cells, also showed capable of integrating cellular pathophysiological information from two cancer specific promoters. Using these cancerous triggers as inputs, the system could then activate an output gene following a AND boolean operation. When using a luciferase output, the authors could detect bladder cancer cells or induce cell death using functional apoptotic genes as outputs¹⁵³. These studies brought promising proves of concept toward the clinical use of custom, personalized designer theranostic cells, which could be further engineered to produce different responses, such as the *in situ* production of imaging agents to aid the diagnostic of tumors and metastases, associated with an anticancer action.

However, physiologically relevant cues are often extracellular, and thus there require tools to sense various ligands and complex environment composed of cytokines, hormones, various proteins, pathogens, hypoxia, inflammation, or pH, while keeping high orthogonality in sensing components to

avoid modes of failure and interface robustly with the patient host. The engineering of such novel mammalian sensor systems can be achieved through different strategies: redirecting the output of natural receptors, or engineer existing transmembrane sensor proteins to recognize small molecule inputs or user specified antigens (reviewed in ¹⁵⁴). While the first approach in this direction showed successful demonstration to detect endogenous molecules via the rewiring of Notch, GPCRs or RTK signaling to elicit novel responses, diagnostic applications may require receptors that detect biomarkers for which there are no endogenous receptors¹⁵⁴. To address this need, some authors recently developed a technology they termed Modular Extracellular Sensor Architecture (MESA). It consists in a fully orthogonal architecture where independent, tunable protein receptor modules undergo ligand binding-induced dimerization, which further results in proteolytic trans-cleavage of the intracellular part, releasing a transcription factor previously sequestered at the plasma membrane. They developed a systematic platform for conditional transmembrane ligand detection that produces outputs in the form of either transcriptional regulation or reconstitution of enzymatic activity, and enable straightforward engineering for the detection of user defined ligands¹³⁴. Another interesting extracellular receptor that has received attention as a recognition element are G-protein coupled receptors (GPCRs). GPCRs represent the largest family of membrane receptors, are highly modular and their customization could benefit from a large range of natural binding repertoire ranging from small molecules to peptides and glycoproteins biomarkers. Moreover GPCR in cell-based biosensing can be connected to various cellular processes to be used as the sensor readout. For example, directed evolution of GPCRs permitted to obtain receptors with novel specificities for small molecules¹⁵⁵. This strategy has been employed in mice with good success and could be interesting for novel diagnostic or analytical purposes¹⁵⁶. In addition, the engineering of novel immune receptors, with the same modularity, diversity and selectivity as antibodies thus capable of sensing a wide range of disease-associated antigens, such as protein biomarkers of cancer, infections or cardiovascular risk, was achieved with chimeric antigen receptors (CARs). CARs are designed with single-chain antibodies (scFvs) that are fused to cytoplasmic regions of intracellular signaling elements (the CD3 zeta chain), which linkage leads to a novel modular input/output sensor that activates upon binding the target. These synthetic receptors also enabled the tailored reprogramming of T cells to respond to defined ligands, and proved clinically extremely promising for cancer immunotherapy^{157 158 159}. These synthetic receptors could open the way for novel cell-based biosensors for diagnostic applications.

While various eukaryotic chassis have been engineered into cell-based biosensors, mammalian cells have dominated synthetic biology medical proves of concepts. However, the yeast *Saccharomyces cerevisiae* also constitutes a potentially interesting chassis for biosensor development⁸¹, and can be

stored and distributed in a “dry active” state. As a model organism, many genetic engineering tools are available. Extracellular yeast mating peptide sensing systems are G-protein coupled receptors, Ste2 and Ste3, initiate an intracellular signaling pathway, and could be good target for the engineering of new biosensing devices. To date, *S. cerevisiae* remains an underexploited but promising platform for biosensor development.

Viral systems

The ability of viruses, and more specifically bacteriophages to specifically infect, and lyse their bacterial host has been exploited for many decades to reveal and identify bacterial species. Phage-based diagnostics have been recently further investigated as an emergent technology for the clinical diagnosis of infectious bacterial diseases, and synthetic biology approaches have already played a major role in the engineering of phage based technologies for the detection of human pathogens¹⁶⁰

¹⁶¹.

Near-real-time microbial diagnostics remain of critical interest in the clinics, where timely detection of pathogens and delivery of species specific evidence based therapy is a life-threatening issue¹⁶². Microbial diagnostics currently suffer from well-recognized shortcomings, since they requires an enrichment step during which pathogens are amplified over incubation times ranging from 10 to 48 h, or even more than 10 days for certain pathogens like *Mycobacterium tuberculosis*. Moreover, standard techniques such as microscopy lack sensitivity, nucleic-acid amplification tests such as PCR offer molecular specificity but have complex sample preparation and poor reliability (inhibition, false positives...), and immunoassays although highly sensitive, are labor intensive and challenging to implement multiplexed detection. To date, bacterial culture isolation remains the standard for species identification and confirmation. Consequently, there is a greater emphasis on the direct detection of pathogens from clinical specimens, without the need for tedious and slow isolation of pure bacterial cultures. Phage-based diagnostics can be regarded as a versatile, widely applicable and valuable solution to timely microbial diagnostics, and synthetic biology has already shown its potential to dramatically improve this technology (**Figure 6: Case 3**).

Natural phages can be engineered to deliver genetic information into specific bacterial species, thus exploiting their metabolism for the production of readable molecular signals (fluorescent, or luminescent proteins..)¹⁶⁰. Synthetic viruses can be rationally designed¹⁶³ or modified via directed evolution¹⁶⁴ and chemical and genetic modification can be used to generate numerous functionalities¹⁶⁵ and cell target specificity¹⁶⁶. Different phage-based assays formats and detection methods have been investigated: phage amplification with bacterial lysis¹⁶⁷ ¹⁶⁸, Phage/DNA

amplification followed by quantitative PCR (qPCR) to identify phage DNA amplification^{169 170}, dot blot assay¹⁷¹, phage-integrated colorimetric, fluorescent, and bioluminescent reporter genes^{172 173 174 175 176 177 178 179}, phage/protein amplification detection with phage-specific antibodies¹⁸⁰. More recent developments include quantum dot reporting, electrochemical and optoelectronic methods (for extensive review see¹⁸¹), or innovative biophysics methods¹⁸². Diagnostic sensitivities as low as 10 cells/mL with a response time of 1 hour in a clinical sample matrix have been described, and a number of proof of concept and commercial products showed a very good response time and sensitivity in medical context¹⁸¹. The utilization of cocktails of phages or the assembly of phage-derived recognition proteins has been proposed to specifically detect desired bacterial spectra. The advantage compared to other detection method like hybridization based assays, is that it doesn't require an enrichment step and sample pretreatment to achieve maximum specificity and sensitivity, and provides discrimination between living cells and dead cells. In addition, the wide bacterial selectivity range, host specificity, ease of use, straightforward production and extremely low reagent cost, seem to make phages ideal candidates to exploit as bacterial detectors in a variety of culture, food, water, clinical and environmental matrices¹⁸³. Phage diagnosis can also give information about the genetic nature of the host, and thus can be used for antibiotic susceptibility testing¹⁸⁴. For example, identification of *M. tuberculosis* by culture on solid or liquid media takes more than 10 days, requires specialized and costly equipment, and technical expertise and show poor sensitivity for identification. Mycobacteriophage amplification technology or reporter mycobacteriophage technology allows *M. tuberculosis* detection in less than 48 hours, along with providing antibiotic susceptibility testing¹⁸⁵. As another example, blood culture tests such as KeyPath™, allows for simultaneous identification of *S. aureus* and differentiation between MSSA and MRSA¹⁸⁰. Phage-based platforms are also currently clinically used for the detection of *Yersinia pestis*, *Bacillus anthracis*¹⁶⁰.

However, few prototypes have been fully translated from laboratory to the clinics and have been successfully commercialized. Key bioengineering advances provided by synthetic biology are required for full maturation of this technological field to achieve enrichment free, sensitive, specific, straightforward phage based diagnostic tests. High throughput and genetic engineering tools, libraries of robust and reliable devices and parts such as reporter genes, sensitive sensors and synthetic gene circuits may enabled the engineering of the huge natural phage “repertoire” chassis (over 10^{32}) at a much more higher pace than achieved so far^{186 187}.

Viral synthetic devices have also been shown to be useful for the rapid typing and monitoring of specific eukaryotic cell phenotypes. Until now, they have been extensively used for therapeutic purposes and virus-mediated delivery of effector genes and payloads¹⁸⁸. Similarly, prosthetic decision

making circuits embedding diagnostic algorithms can be delivered via viral vectors *in vivo* into mammalian cells, injecting molecular computers probing the internal state of a cell. As previously discussed, such payloads supported by synthetic gene circuits can then sense, score, monitor and store disease-relevant molecular information. For example it could contain cancer specific promoters^{189 190}, and an actuating device transmitting the cell's pathological state to human readable information.

These design principles have also extensively been investigated for *in vivo* imaging diagnostic strategies^{191 192}. It was applied for example for different imaging modalities: insertion of key genes in melanogenesis in a vaccinia virus vector allowed improved MRI and optoacoustic imaging, in a tumor specific manner¹⁹³. Another method for non-invasive optical imaging of tumors *in vivo* was successfully developed and uses engineered viruses that carry genes and probes to allow deep tissue molecular imaging¹⁹⁴ or further encodes enzymes (β -gal and glucuronidase) that can be monitored in the serum of tumor-bearing mice¹⁹⁵ as well as in the blood of humans with cancer¹⁹⁴.

Another interesting field of virus engineering research enabled by synthetic biology is the engineering of synthetic viral nanoparticles and their genome-free counterparts, virus-like particles. A broad range of genetic and chemical engineering methods have been developed to exploit virus nanoparticles as biomedical imaging diagnostics reagents, and the inclusion of peptide ligands on the particle surface permitted the improvement of current *in vitro* diagnostic assays based on the conventional enzyme linked immunosorbent assay¹⁶⁵. In such assays, the viral nanoparticle helps guiding the antibodies to achieve maximum capture of the biomarkers. In addition, high densities of antibodies on the surfaces of the nanoparticles lead to greater binding of biomarkers, which enhances detection sensitivities. For example, some authors showed that by combining viral nanoparticles, which are engineered to have dual affinity for troponin antibodies and nickel, they could detect troponin levels in human serum samples that are seven orders of magnitude lower than those detectable using conventional enzyme linked immunosorbent assays, exhibiting properties that could prove valuable in the early detection of the protein marker troponin I in patients with a higher risk of acute myocardial infarction^{196 197}. Other viral nanoparticles could perform similar highly sensitive diagnostic assays and could be implemented for a variety of biomarkers.

While phage based diagnostics technologies are maturing and transitioning to clinical microbiology, it is very likely that the further engineering of eukaryotic viruses will lead synthetic biologists to major medical developments toward the clinic¹⁶⁵. Viral nanotechnologies for diagnostic have now come of age and we believe that it will not be long before novel assays reach a prominent role in the clinic.

B. Bottom-up engineering of biological sensors systems *ex vivo*

Following the advances in the construction of programmable biosensing circuits in living organisms, *ex vivo* synthetic circuits assembled in minimal systems from the bottom-up constitute a viable platform for designing, understanding, and exploiting dynamic biochemical circuitry for biodetection and thus diagnostic purposes. Cell-based biosensing systems often rely on intracellular passive diffusion of analytes, or kinetics of transcriptional and translational processes that result in slow sensor responses. In addition, non-orthogonal gene networks constitute a load in engineered cells that can interact with chassis components and result in unpredictable and noisy response profiles. On the contrary, bottom-up synthetic systems that rely on nucleic acid, protein, or metabolites have temporal dynamics in the order of seconds or minutes. Released from unwieldy complexity, context dependencies, and unpredictability that burden the use of living systems, *ex vivo* systems allow researchers to directly access and manipulate modular biomolecular parts with unprecedented control and design space (**figure 6**). Advances in such bio-inspired functional systems¹⁹⁸ include diverse capabilities including: biosensing, algorithmics, memory, and various biological functionalities. Progress in this field demonstrated that cell-free synthetic biology is an promising field for the fundamental understanding of native biological systems but most importantly the engineering novel biotechnological tools for the clinics^{199 200}.

Nucleic acid based systems

Nucleic acids are versatile molecules capable of information processing and storage. They are governed by simple, predictable and programmable rules driven by watson-crick base-pairing interactions and strand displacement that enable their straightforward nanoscale synthesis and engineering with important design space²⁰¹. The past decades witnessed the development of complex *in vitro* nucleic acid circuits and devices highlighting the potential of using nucleobases and their polymers as building blocks to generate useful architectures^{202 203 204}. Nucleic acid based *in vitro* systems have made numerous contributions to biodiagnostic as well as biotechnology research, with the best example probably being the development of polymerase chain reaction. As signal detection, amplification and transduction depend on the programmability of waston-crick base pairing, nucleic acid circuits can be tuned and adapted to various applications compared to other biomolecular signal amplification reactions. Moreover, novel methods to select and amplify sequence-specific nucleic acids with specific recognition sites (aptamers) for low-molecular-weight analytes, macromolecules or whole cells and development of catalytic nucleic acids (DNAzymes or ribozymes) are promising and likely to provide new analytical tools²⁰⁵. Meanwhile, the field of DNA computing and molecular programming has taken an increasing importance for analytical applications²⁰⁶. The modularity of

nucleic acids, as well as their capacity to directly interact with a wide range of analytes, especially other nucleic acid biomarkers, enables the implementation of decision making circuits that are programmable functions between selected inputs and outputs, which are relevant to diagnostic applications²⁰⁴. A variety of sensing systems relying on nucleic acid devices have been developed during the past decade, with particular interest for riboswitches, aptamers, and catalytic nucleic acids (DNAzymes and ribozymes) coupled to more complex nucleic acid reaction networks.

Aptamers are synthetic single-stranded nucleic acids that selectively bind to a broad range of specific targets ranging from proteins to peptides, amino acids, drugs, metal ions, and even whole cells, and benefit from systematic and robust methods for their obtention through a combinatorial directed evolution method called SELEX²⁰⁷. They have demonstrated great promise in diagnostic biosensor development during the last decade, since they possess unique characteristics compared to antibodies or other biomimetic receptors, comparable or even better affinity, easy and cost-effective synthesis with high reproducibility and purity, simple and straightforward de novo design, engineering and chemical modification^{208 209}. Aptamers are thus powerful alternatives to antibodies or other biomimetic receptors for the development of diagnostics²¹⁰. They proved their value as diagnostic tools in several diagnostic applications and assay formats such as biomarker detection from cancer clinical testing to detection of infectious microorganisms and viruses (reviewed in²¹¹). For example, the possibility of using aptamers as an alternative molecular recognition element in ELISA has received great interest, which gave rise to an ELISA-derived assay called enzyme-linked apta-sorbent assay (ELASA)²¹². Taking the versatility of aptamers further, recent studies proposed to develop intelligent aptasensors that embed boolean logic. For instance, Zhou et al. engineered biocomputing systems with aptamer-based biochemical sensing controlling a self-powered biofuel cell that process the information. This proof-of-concept could detect patterns of thrombin and lysozyme inputs and generate an electrochemical output following a NAND truth table²¹³. Even though these logic biosensors were shown to operate effectively in complex physiological sample, they still require significant engineering efforts prior to a potential practical application. Moreover, while most diagnostics are still under the supremacy of immunoassays, further studies are needed to evaluate clinical robustness of aptasensors in clinical sample matrices and to provide new sensing formats (Reviewed in²¹⁴).

Similarly, the discovery of natural riboswitches has inspired application to ligand detection, exploiting the ability of RNA to recognize molecular targets and harnessing the ligand-dependent structural rearrangement of RNA to generate a measurable signal^{215 216 217}. Riboswitches are RNA aptameric elements in RNA devices that control gene expression, refolding, or allosteric ribozyme activities in

cis in response to a broad range of specific ligands²¹⁸. Riboswitches are integrated into RNA, and are mostly constituted of an internal ribosome entry site accessible for the ribosome only in the presence of a specific ligand, while it is inhibited in its absence. Because synthetic riboswitches make it possible to regulate any gene or RNA enzyme with an arbitrary molecule, they function as biosensors, in which the output is easily detectable protein expression or enzymatic activity that reflects the concentration of the corresponding ligand²¹⁹. Rational design strategies for constructing novel riboswitches that work in cell-free translation systems have been described, and their systematic engineering for different biosensing targets, such as FMN, tetracycline and sulforhodamine B have been demonstrated²²⁰. In another approach, Olea et al. described a general analytical method for the detection of target ligands based on self-replicating aptazymes. These “autocatalytic aptazymes” are constituted of an aptamer domain linked to the catalytic domain of a self-replicating RNA enzyme²²¹. Ligand-dependent self-replication of RNA proceeds in a self-sustained manner, undergoing isothermal and protein free exponential amplification. The rate of exponential amplification is a function of the concentration of the ligand, thus enabling quantitative ligand detection.

Catalytic nucleic acids, or DNAzymes, that can also be employed diagnostic reagents, and were extensively used as amplifying labels for optical and electrochemical sensing platforms. A vast repertoire of synthetic catalytic nucleic acids were recently engineered, such as metal-ion-dependent DNAzymes, aptamer inducible DNAzymes and cofactor-dependent DNAzymes that catalyzes cleavage or ligation of oligonucleotides or mimic native enzymatic functionalities. Furthermore, DNAzyme have been employed to trigger catalytic cascades and thus used for amplified autonomous sensing and DNA logic gate cascades and computing circuits. For instance, a method for the nanomolar detection of histidine was reported, using a L-histidine-dependent RNA-cleavage DNAzyme²²², or a HRP-mimicking DNAzyme cascade was engineered for the amplified aptamer mediated detection of PDGF²²³. DNAzymes also provided a colorimetric method to detect telomerase activity as a cancer specific cellular biomarker^{224 225}.

Other strategies recently developed, rely on the binding of single stranded DNA signals to a partially double-stranded complex by a single-stranded domain called a toehold, and then release the originally bound strand after branch migration has occurred. In this way, an output signal can be activated upon the arrival of an input signal, and the reaction rate can be controlled by the length and nature of the toehold. This concept permitted the development of many DNA strand displacement circuits’ strategies, resulting in a wide range of applications for *in vitro* biomedical diagnostics^{226 227}. For example, Chen et al. have recently developed a toehold exchange mechanism working with double-stranded nucleic acids, which they show can be used as a novel programmable

diagnostic device to detect single nucleotide polymorphism. They demonstrate that conditionally fluorescent DNA probes are capable of detecting variations of a single base in a target dsDNA, reliably over a wide range of conditions²²⁸. They then successfully apply this principle to diagnose individual point mutations in Rifampicine bacterial antibiotic resistance genes in E coli. This technology could prove interesting to screen extended genetic regions and multiplex SNP detection.

Moreover, toehold mediated strand displacement mechanism permitted to develop novel enzyme free nucleic acid amplification circuits for different diagnostic detection strategies, such as entropy-driven catalysis (EDC) circuits, seesaw gates, catalytic hairpin assembly (CHA) reactions and hybridization chain reactions (HCR)²²⁹. In such circuits, single-stranded nucleic acid inputs produce refolding of kinetically trapped substrates via exposed toeholds and strand exchange reactions, thus enabling conditional molecular interactions. Outputs of EDC, CHA and HCR are constituted of independent ssDNA, multiple duplexes and concatemers of increasing length respectively, and they can thus easily be coupled to different analytical modalities, with signal transduction characteristic that are suitable for diagnostics especially when the concentrations of input molecules are low. They include transduction to fluorescent, luminescent, electrochemical, enzymatic activity via DNAzymes, and colorimetric signals²³⁰. Researchers have already been able to use *in vitro* DNA circuits to amplify signals and detect RNA, proteins and small molecule analytes using different reporting methods combined in a plug-and-play way²³¹. This methods have provided new paradigms for the design of enzyme-free biosensors for point-of-care diagnostics²³⁰. CHA and HCR have been developed and adapted into novel diagnostic tools, where they showed improvement in sequence-specific detection of amplicons generated by enzymatic amplification²³². For example, CHA demonstrated improved signal-to-background ratio, while providing several hundred-fold amplification within a few hours detecting less than 10 copies/ μ l of a target sequence. Compared to conventional enzyme based amplification reactions, CHA provided high sequence specificity and false-positive signals arising from non-specific binding to templates was greatly suppressed. In another example, a non-nucleic acid small molecule analyte, lead, could be detected with sensitivities of 10–100 pM, which was 4 orders of magnitude better than the previously reported biosensors without amplification. In another study, CHA amplification reduced by more than 2 orders of magnitude the detection limit for thrombin aptamers to 20 pM, a sensitivity comparable to conventional ELISA. These nucleic acid circuits showed also capable of improving conventional immuno-assays methods. Immuno-HCR strategies notably increased the sensitivity of carcinoembryonic antigen detection cytokines and chemokines, as well as performing multiplex analysis²²⁹. HCR reactions can also be used for a detection of protein biomarkers²³³, as well as an imaging tool, and proved extremely useful to enhance signals from *in situ* hybridization and for imaging mRNA expression *in vivo*^{234 235}. These methods demonstrated high

sensitivity and specificity but also great versatility and could be readily programmed and adapted to different applications. In addition, some nonenzymatic or enzymatic cascades could stand as potential alternatives for polymerase chain reaction in terms of sensitivity. However, the timeframe in which these amplifier circuits generated an output is situated between 2 and 50h²²⁹, a delay that could still prevent usage in specific diagnostic set-ups. All these strategies can be coupled to develop complex biosensing modalities. For example, extensive efforts were directed to apply the enzymatic and nonenzymatic nucleic acid cascades for amplified sensing and gated detection of nucleic acids and aptamer substrate complexes. Analytical advantages of cascaded amplification and sensing include: isothermal conditions, no requirement in terms of special instrumentation, generation of human readable colorimetric signals, and increased versatility. They could thus be amenable for or point-of-care diagnosis or extended diagnostic modalities.

Integrating medical algorithms into DNA circuits for disease diagnosis has been achieved to tackle different real world pathologies, such as infectious diseases, cancer, or metabolic disorders. In order to be applied to medical diagnosis, clinically relevant biomarkers can be detected as inputs to nucleic acid circuits via riboswitches or aptamers that translate the recognition to DNA/RNA conformational change, which triggers a computation process following a diagnostic algorithm. Nucleic acid circuits originated from efforts to develop nucleic acid computation, and besides signal amplification they have other properties that prove useful in diagnostic assays. Nucleic acid circuits are particularly capable of implementing decision making algorithms by including logic gates, thresholding and bandpass elements, and as such be useful for background suppression and noise reduction, to provide novel diagnostic devices. For example, autonomous molecular computers have been engineered to distinguish pathological states, by integrating the detection of disease biomarkers such as mRNAs, miRNAs, proteins, and small molecules into a programmable detection algorithm²³⁶. In addition, the advantage of nucleic acid circuits is that they can be scaled up and extended to encompass basically any diagnostic agendas²²⁶, as highly complex sensing and computing circuit can be needed to assess complex pathophenotypes and achieve quantitative discrimination between healthy and disease states with high resolution. Such autonomous complex circuits with the capability to recognize patterns of molecular events, make decisions and respond to the environment have already been successfully developed, for example by mimicking neural network computation with considerable power²³⁷.

Cell types, both healthy and diseased, can be classified by inventories of their cell-surface markers using aptamers and nucleic acid circuits²³⁸. In a recent approach, You et al. developed DNA nanorobots for programmable analysis of multiple surface markers to enable the phenotype profiling on whole cells. They engineered a device combining structure-switching DNA aptamers with toehold-

mediated strand displacement reactions to perform autonomous Boolean logic-based analysis of multiple cancer cell-surface markers with production of a diagnostic signal, associated with a targeted therapeutic effect²³⁹. In a similar approach, Rudchenko et al. engineered a molecular automata capable of scanning lymphocyte surfaces using a combination of antibodies and DNA circuits to assess the presence or absence of cell surface markers on living human cells²⁴⁰.

Nucleic acid diagnostic devices have proved capable of operating in solution but also on solid surfaces such as paper^{241 242}. The use of transcriptionally generated RNA circuits along with post translational components as transducers might further simplify the production of nucleic acid circuits for point-of-care applications: instead of producing, purifying and storing multiple kinetically trapped nucleic acid substrates, double-stranded transcription templates could be used to generate these circuits *in situ*. For example, Pardee et al. recently developed toehold RNA switches integrated on paper-based biosensors that provide an alternate and versatile platform format for synthetic biologists (**Figure 6: Case 4**). This format enables the safe deployment of synthetic gene circuits beyond the laboratory. In this approach, they propose that commercially available cell-free systems freeze dried on paper could enable the inexpensive, sterile, and abiotic distribution of synthetic biology DNA-based biosensing technologies for the clinic. They demonstrate this technology with the detection of clinically relevant small-molecule and nucleic acids, rapid prototyping of complex gene circuits, and programmable *in vitro* diagnostics, including glucose sensors and strain-specific Ebola virus sensors²⁴³.

Moreover, synthetic nucleic acids can be also used as probes in higher order structure constituted of amplifying probes. For example, branched DNA assays, in which alkaline phosphatase labeled nucleotides bind branched DNA structures (bDNA) generating a chemiluminescent signal, have shown to increase the specificity of conventional assays, such as the VERSANT assay (Siemens healthcare, USA). The more accurate, automated, highly sensitive and broad dynamic range of bDNA assays, have proved them useful for the diagnosis, prognosis, monitoring of viral load, and effect of HIV, HCV and HBV antiviral therapy, when variability associated with the PCR assay made it less useful for monitoring patients on antiretroviral therapy^{244 245}.

Similarly, other architectures using synthetic nucleic acids probes have been described, such as DNA hydrogels biosensors: ssDNA sensing devices made of hybrid DNA-hydrogel respond to stimuli by altering shape and swelling properties after toehold-mediated DNA displacement reaction. This strategy has been implemented for the detection of various chemicals or proteins^{246 247}. Algorithmic control on assembly and operation of DNA nanostructures and machineries²⁴⁸, have also yielded

synthetic molecular machinery from DNA, or “nanomachines” that can be activated by interactions with specific molecular signals or by changes in their environment²⁴⁹. For example DNA origamis were proposed to be assembled into logic-controlled “Sense-Act-Treat” nanomachines capable of autonomous *in situ* diagnosis and therapy delivery^{250 251}, or stand-alone biocomputers capable of *in vitro* diagnosis²⁵². In the first example, switchable DNA nanocapsule closed by DNA strands hybridized to aptamer sequences could open upon recognition of certain cell surface proteins. More recently, following an *ex vivo* prototyping phase, this approach was successfully transitioned DNA origami robots operating in living cockroaches and is now being evaluated for patient use in a clinical trial²⁵³.

Orthogonal nucleic acid chemistries have also been proposed as new tools for diagnostics development. Novel synthetic nucleobases and their genetic polymers, known as XNA (xenonucleic acids) increase the chemical and structural diversity of nucleic acids, and open up the way for increased affinity and stability against enzymatic cleavage, expanded functionality such as enzymatic activity, and improved synthesis and selection procedures^{254 255}. For example, selection experiments against two human target proteins, VEGF and IFN- γ yielded XNA aptamers that bind with affinities that are >100-fold improved over those of aptamers containing only natural bases²⁵⁶. Other authors developed nanomolar to subnanomolar affinities to clinically relevant protein targets including PDGF and pro-inflammatory cytokine IL-6²⁵⁷, or small molecules such as camptothecin²⁵⁸. Recent studies also demonstrated the advantage of using XNAs detection probes in biological fluids, particularly because they permit to achieve significant improvement in stability by providing resistance to nucleases. For example, expanded nucleic acids aptamers showed promising properties as probes for *in vivo* tumor imaging. These authors developed a novel locked nucleic acid (LNA)/DNA chimeric aptamer probe that showed a great improvement in performance and serum stability compared to conventional aptamers²⁵⁹. These strategies showed that chemically expanded genetic alphabets can yield aptamers with greatly augmented affinities and stability, suggesting the potential of synthetic XNAs as a powerful tool for creating novel, highly functional nucleic acids. Orthogonal nuclease-resistant version of nucleic acids amplification reactions systems and probes, for example based on L-RNA molecules, were also described to have gained increased robustness²²¹. It constitutes an alternative approach that has been applied for example to the autocatalytic aptazymes to construct enzyme entirely from non-natural L-ribonucleotides²²¹. The mirror-image enzyme behaves identically as the D-RNA, but has gained complete resistance to ribonucleases.

Future advances in synthetic biology methodologies for the synthesis, characterization and evolution of synthetically augmented genetic polymers should help resolve numerous arising clinical questions, as well as providing fully programmable substrates for diagnostic and molecular computing. XNAs technology is also likely to provide a growing bioengineering toolbox of biochemical encoding and

manipulation of biological information, while also enabling to fully exploit their expanded range of physicochemical properties, orthogonality, and biostability. Additionally, *In vivo* circuits operation could further benefit from the use of orthogonal nucleic acid chemistries or even expanded nucleic acid alphabets.

Protein based systems

Proteins are versatile and modular tools that operate naturally as near real time effectors, and have been widely used in many biomedical applications. At the molecular level, many biological response functions are allosterically regulated protein activities that couple an input to an output function. Compared to nucleic acids that have limited diversity, and gene circuits that are inherently slow, the kinetic properties as well as the possibility to implement almost all biological functions: sensing, catalysis, signal processing, memory, among others, define polypeptides as powerful substrate for synthetic biology²⁶⁰. Post-translational tools defined as amino acids and their polymers offer a vast engineering playground for synthetic biologists^{261 198}. Thus, protein based biosensors provide attractive tools for the real time monitoring and control of molecular events in complex biological environments. However, their rational and systematic bottom-up engineering is often more delicate and error-prone than with nucleic acids. Although protein based strategies remain hindered by the difficulty to tailor signal transducers and receptors that can be readily compiled into defined diagnostic circuits, a true engineering approach for the design of protein sensors and circuit devices with standard functional and structural protein modules that sense, process, and amplify specific molecular signal of clinical interest, is recently emerging²⁶².

Protein–ligand interactions are part of almost every biological process and have tremendous importance in diagnostics. However, current protein based sensors are still largely based on single probes often isolated from naturally occurring proteins. Many synthetic biology approaches have thus tried to manipulate protein interfaces to enhance diagnostics performances and have enabled the development of new probes with improved capabilities in regard to straightforward integration in on-purpose formats, coupling of effector functions, robustness in biological samples, and specificity and sensitivity, among others.

Antibodies have been the long lasting paradigm of binding proteins with desired specificities and high affinities, but they have intrinsic limitations related to their molecular properties: large, bivalent, multidomain protein, dependence on disulphide bonds and complex glycosylation pattern, poor heat stability, and are difficult and expensive to manufacture. In recent years, engineered versions of antibodies and even orthogonal binding schemes have entered successfully translated towards

clinical application. In addition, new synthetic approaches for further improvements are likely to accelerate translation of novel protein probes and sensors. For example, the ability to conditionally direct antibodies could prove extremely useful. In a recent study, Gunnoo et al. showed that they could engineer antibodies displaying gated binding through site-specific, chemical phosphorylation of a recognition domain²⁶³. This gated binding could perform Boolean logic operations, such as induction in an enzyme-AND-antigen conditional manner. In this case the simultaneous expression of a cell surface antigen and secreted enzyme were used to conditionally generate binding function. This strategy permits to generate antibodies active only in the presence of specific biomarker inputs of different nature to enhance diagnostic precision.

Immunodetection can also be engineered to integrate environment cues, or also provide straightforward manipulation of sensor binding characteristics by the user. For example, pH gated antibodies have been recently developed by Strauch et al.²⁶⁴. They described a strategy to design pH-dependent protein interfaces and showed that they could design a protein that binds antibodies in a pH dependent way. This could prove extremely interesting for antibody affinity purification and certain diagnostic formats. This approach demonstrated how protein engineering can increase versatility and efficiency off conventional diagnostic reagents. Alternatively, manipulation of synthetic antibody genes could allow for the creation of new immunoglobulin devices for novel detection frameworks, such as multi-specific antibodies, that are already moving towards diagnostic applications²⁶⁵.

Directed evolution of proteins as enabled by synthetic biology, is a powerful and versatile bioengineering tool and solution for selecting proteins with desired functionalities²⁶⁶. Site-directed mutagenesis creates libraries of rationally designed protein variants that can be screened, to allow quick understanding of protein structure and its effects on function while looking for enhanced forms, all in one experiment. It has been extensively used, either alone or in combination with other methodologies such as computational design, to generate useful probes and diagnostic reagents²⁶⁷. For instance, a recent study presented a method they called antibody diagnostics via evolution of peptides (ADEPt) to evolve diagnostically efficient peptides for *de novo* discovery and detection of serum antibody biomarkers without knowledge of disease pathophysiology. As pathological antibodies repertoire are known to change in diverse diseases, this methods has proven useful to create diagnostics for early disease detection, stratification, and therapeutic monitoring, and enabled effective identification of a critical environmental agent involved in celiac disease²⁶⁸.

Meanwhile, a new generation of sensor proteins has been described, derived from small and robust non-immunoglobulin scaffolds that can be engineered with defined binding functions using the

methods of combinatorial protein design, and assembled with modular composability. As shape complementarity is an important part of molecular recognition, the capacity to precisely tune the shape of a binding scaffold to match a target of interest enables the generation of high-affinity protein based diagnostics²⁶⁹. Many protein scaffolds have been proposed and consolidated as smaller sets capable of multiple targeting and operation in different settings as diagnostic reagents, such as engineered affibodies, adnectins, anticalins, or DARPins²⁷⁰. They combine the binding properties of antibodies with improved properties such as small size, high stability, absence of cysteines, high yield bacterial expression and the possibility of building higher order and multispecific constructs.

Also described as interesting post-translational strategies for controlling the flow of information in biochemical reaction networks, synthetic protein scaffolds are particularly attractive because of the modular nature of the design, and permit spatial organization of enzymes, and have thus been employed to create orthogonal interaction domains for assembly of synthetic metabolons. They have been shown to improve biochemical reactions in multi-enzyme complexes through substrate channeling²⁷¹ and programmable fine-tuning of enzymatic reaction and yields²⁷².

Instead of relying on natural antibody production and associated tedious methods, manipulation of biomolecular recognition between ligands and proteins can also be performed *in silico*. Computational design of proteins has successfully been extended to new folds, new catalysts^{273 274}, on existing scaffolds²⁷⁵, and even non-natural reactions²⁷⁶ with defined specificities and affinities^{277 278}. Computational design of proteins enables the systematic engineering of binding sites, protein structure and function²⁷⁹. A decade ago, Looger et al. presented the first structure-based computational method to redesign protein ligand-binding specificities. Multiple soluble proteins receptors binding a number of small-molecule ligands with high selectivity and affinity, such as trinitrotoluene, L-lactate, serotonin, and the nerve agent pinacolylmethylphosphonic acid have been reportedly built in the periplasmic binding protein protein¹⁰⁶. These *de novo* engineered receptors can then be used as biosensors for their new ligands although the systematicity and reliability of the method has been questioned²⁸⁰. More recently, Tinberg et al. demonstrated an approach for designing *de novo* proteins that bind small molecules and use it to create specific binders for digoxigenin²⁸¹. The method relies on the design of highly energetically favorable, defined interactions with the ligand in customizable protein scaffolds. The binding-fitness was further mapped using library selections and deep sequencing, and enabled to optimize affinity to a picomolar level, comparable to conventional antibodies. Moreover, the selectivity for digoxigenin over the related steroids digitoxigenin, progesterone and 17 β -oestradiol, could be rationally programmed by manipulation of rational design of hydrogen-bonding interactions. The authors also found that these synthetic sensors had increased stability for extended periods at ambient temperatures, and could

be expressed at high levels in bacteria, properties that provide a more robust and cost-effective alternative compared to antibodies. Thus, these computational methods should enable the development of a new generation of biosensors and diagnostics for the detection of small molecule compounds. The computational design of protein-protein interaction, although suffering from shortcomings in current approaches, is now transitioning to reality, and recent successes show we could soon be capable of modulating, reengineering and designing on demand protein–protein interaction networks²⁸².

Protein switches are used in natural biological signal transduction systems, and enable cells to sense, integrate and respond to a variety of molecular signals. Consequently, the re-engineering of tailored protein switches could enable real time, *in situ* detection of clinically relevant inputs. Recent progress in constructing protein-based switches is likely to define a new generation of molecular diagnostics. For instance, the engineering of ligand binding protein sensor switches has led to many interesting devices. Protein switches and sensors can be built from simple, modular components, yet display highly complex signal-processing behavior²⁸³. Enzymes are of particular interest, as they can implement detection, signal processing and amplification and are amenable to modular engineering. Engineering of synthetic allosteric control in proteins, orthogonal protein building blocks, control of switchable protein-protein interactions or designing switchable enzyme are thus major fields of investigation²⁸⁴. In cells, kinases and phosphatases are inactive by default and get switched by specific signal to be processed. Modular autoinhibition is a natural occurring form of enzymatic regulation in which autoinhibitory domains conformationally inhibits the activity of another domain within the same molecule. Covalent modifications such as phosphorylation are then capable of relieving inhibition and confer a switch like behavior to enzymatic activity. For example, Dueber et al. in pioneering work^{285 286 287}, explored how modular domains can be assembled to build switches with nonlinear input/output function. They integrated the autoinhibitory interaction module of the yeast kinase N-WASP with several domain-peptide interactions from unrelated signaling proteins: Src homology 3 (SH3) and PDZ peptide-ligand interactions. These authors managed to fuse constitutively interacting domain–peptide pairs to generate a N-WASP protein responsive to peptide ligands, where different combinations of input modules could produce logic gated behaviors (AND, OR) and ultrasensitive, near-digital switching dynamics with signal amplification. The same domain fusion strategy was later also successfully applied to re-engineer guanine nucleotide exchange factors²⁸⁸.

Modular protein switches can also be engineered with orthogonal regulation processes. The synthetic coupling of overlapping protein domains, or domain fusion, so that small ligand, peptide or protein binding partners can then regulate allosteric activity of a enzymatic switch, have generated useful devices. For instance, ligand-sensing domains have been fused with dihydrofolate reductase,

β -lactamase^{289 290 289} and Src, p38, and focal adhesion kinase^{291 292} generating estrogen analogs, maltose or rapamycin inducible versions of these proteins respectively. Sallee et al. developed a method to systematically construct two-domain fusion proteins using naturally occurring sequence overlaps between interacting domains, which displayed mutually exclusive binding properties to ligands²⁹³. Although still suffering from lack of standardized protocols, issues with folding unpredictability and dynamics and relying on empirical optimization²⁸⁹, the coupling with screening strategies enable to fully exploit this approach, and in the future new tools could enable the straightforward engineering of such sensor systems.

Mutually exclusive binding interactions have also been used to develop protein sensors where ligand interacting fluorescent or bioluminescent modules modulate the efficiency of resonance energy transfer^{294 295}. Recently, an interesting and innovative approach was described by Griss et al., in which semisynthetic bioluminescent protein sensors with a new mechanism could be used for inexpensive point-of-care biosensors for companion diagnostics²⁹⁵ (**Figure 6: Case 5**). This technology also known as LUCIDs (Luciferase Based Indicators of Drugs) permitted precise quantification of specific drugs in patients serum by spotting drops of clinical sample on a paper format and recording the signal using a basic digital camera. LUCIDs have a modular design and consist of 3 basic blocks: a protein-based receptor, a luciferase and a synthetic part containing a fluorophore and a specific ligand. Upon ligand binding to the receptor module, the fluorophore is maintained in close contact with the luciferase permitting efficient bioluminescent resonance energy transfer. A competing specific analyte can displace the binding and hence abolish BRET efficiency. By measuring the ratio of light emitted from the luciferase and the synthetic fluorophore, one can quantify the concentration of the target analyte, in such a way that it doesn't depend on sensor concentration and signal intensity. These modular devices were integrated on paper format to generate portable devices, and engineered for the detection of a wide range of drugs: Methotrexate, Tacrolimus, Sirolimus, Cyclosporin, Topiramate, and Digoxin. They proved efficient and accurate with human samples, and promising for the development of new generations of portable companion diagnostic assays.

Similar sensors systems were developed that relied on complementation of luciferase fragments or on domain insertion within the luciferase structure permitting the monitoring of molecular physiology within living cells²⁹⁶. In another strategy, a β -lactamase fused to its inhibitor protein, and connected via a linker to a ligand receptor module, permitted to detect specific molecular cues via measurement of enzymatic activity^{297 298}. Additionally, Stein et al. recently reported a strategy for the construction of modular protein biosensors based on synthetic autoinhibited proteases whose activity can be modulated by specific proteolysis, ligand binding, or protein-protein interactions. They demonstrated that such protease-based ligand receptors and signal transducers could be

assembled into different types of integrated signal sensing and amplification circuits. They relied on structure-guided design and directed protein evolution to create signal transducers and also demonstrated the modular design of an allosterically regulated protease receptor following recombination with an affinity clamp peptide receptor. They engineered high functional plasticity in protein switches, not previously observed in naturally occurring receptor systems.

De novo design of synthetic protein networks can also mimic some of the basic logic functions of the more complex in biological networks, and integrate biosensing and signal processing capabilities²⁹⁹. Enzymes can also enable the construction of biochemical circuits where they are used to implement a “metabolic logic”, in which the inputs and the outputs are enzyme substrates and products^{300 301 302}. Such biomolecular logic systems for bioanalytical purposes can be designed to operate in a digital way, and process multiple biochemical information at once in cascades of biochemical reactions, to generate a final output in the form of a yes/no response, thus leading to high-fidelity decision making compared with traditional sensing devices operating in parallel. Biochemical reaction networks can thus be seen as the most direct and kinetically favorable way of coupling of the signal sensing with biochemical reporters. In such systems, biomarkers are biochemical entities that can interact and be processed by the enzyme network to generate a final colorimetric, fluorescent, luminescent or electrochemical output. The timely detection of complex patterns of multiple biomarkers with such biochemical systems could positively impact diagnosis and treatment of diseases³⁰³. This approach is fundamentally new regarding the sensor design and operation and careful attention to the biocomputing substrates and interface with other systems and electronic transducers have been explored. Enzyme-based reaction networks have further been interfaced with signal-responsive materials and electrodes and immobilization schemes have been reported for that purpose^{304 305 306 307 308}. A few examples of biochemical reaction networks of coupled enzymes implementing Boolean logic functions have been described as proof of concept to provide medical diagnostic solutions³⁰⁹. For example, biochemical reaction networks could detect complex patterns of pathophysiological biomarkers from liver, brain, hemorrhagic shock, oxidative stress, or abdominal trauma injury^{310 311 312 313 314}, or release a drug upon sensing and integrating pathological stimuli in a complex molecular algorithm^{315 316 317}. Moreover, in order to increase confidence level of such biosensors³¹⁸, the scaling up and concatenation of enzymatic boolean logic gates (e.g., AND, OR, XOR, NAND, NOR, etc.) in networks, information storage, or threshold filters have been implemented^{319 320}. Although such *de novo* strategies for the construction of tailored reaction networks still lack general robustness due to the small repertoire of enzyme and orthogonal functionality as well as the complexity and lack of knowledge on enzyme dynamics, extensive theoretical analysis has suggested ways of coping with noise and uncertainty in biochemical reaction networks^{321 322 323}, and computational tools for

automated design, analysis and model checking are more and more efficient and promising^{324 325 326 327}. Coupling protein- and nucleic acid-based devices can be achieved, and could generate useful devices in biological circuit engineering for diagnostic applications.

Similarly with nucleic acids, the genetic code expansion for synthesis of proteins containing non-canonical amino acids is a rapidly growing field in synthetic biology^{328 329}. Synthetic amino acids could enhance stability, activity³³⁰, and provide extended functionalities and overall operability of protein based diagnostic reagents. Already around 100 distinct non-canonical amino acids using orthogonal translation systems have been established, and enabled straightforward *in vivo* or *in vitro* production with synthetic post-translational modifications. This high control from synthetic genes to orthogonal post-translational machineries enables the fine design of novel protein probes with user defined properties. For instance, photocaged phospho-aminoacids have provided access to time-resolved *in vivo* measurements³³¹, and new possibilities in site-specific fluorescent labeling provided enhanced new protein probes. In another example, Wang et al. described a method relying on combination of unnatural amino acid mutagenesis and selective chemical modification that offered the possibility of integrating multiple designer fluorescent labels on polypeptides. This study described the first modular method to introduce multiple probes into proteins at any genetically controlled pair of sites in proteins at physiological temperature, pressure and pH³³². This preliminary work suggests that further expansion and applications are possible.

The increasing ability to rationally control synthetic genes and the sequence-structure relationships enable to use proteins as potential nanomaterials with a variety of sizes and shapes and functionalities. Protein devices and assemblies can now be engineered into highly homogeneous and precisely patterned nanostructures³³³, and offer advantages over traditional nanomaterials such as carbon nanotubes, silicon or metallic particles for their low cost and straightforward production, increased biocompatibility, functionalization and interfaceability³³⁴. The design of protein self-assembling nanostructures and protein nanomaterial has thus emerged as promising reagents with applications in medical diagnosis. Example involve protein nanowires, nanotubes, nanocapsules, nanopores, or hydrogels, that could show promising capabilities in biosensor design^{335 336 337 338}.

Although highly amenable for incorporation into integrated devices, protein based biosensors could have potential disadvantages regarding the storage capabilities, transport and shelf life. Translating these approaches towards mammalian cells and prokaryotes may open new avenues in protein-based biosensing and biocomputing for medical diagnosis. Moreover, direct coupling of biosensing and therapeutic activity in engineered proteins is paving the way for extremely interesting clinical applications, such as the recent synthetic glucose-responsive insulin³³⁹. Extensive research efforts

have so far yielded useful protein based sensor systems, but systematic methods for the engineering of novel devices will require further advances. Moreover, before the promises of synthetic biology approaches can be fully realized, the total connection between amino acid sequence and protein structure and function still remains to be elucidated.

Higher order functional assemblies & synthetic cell-like systems

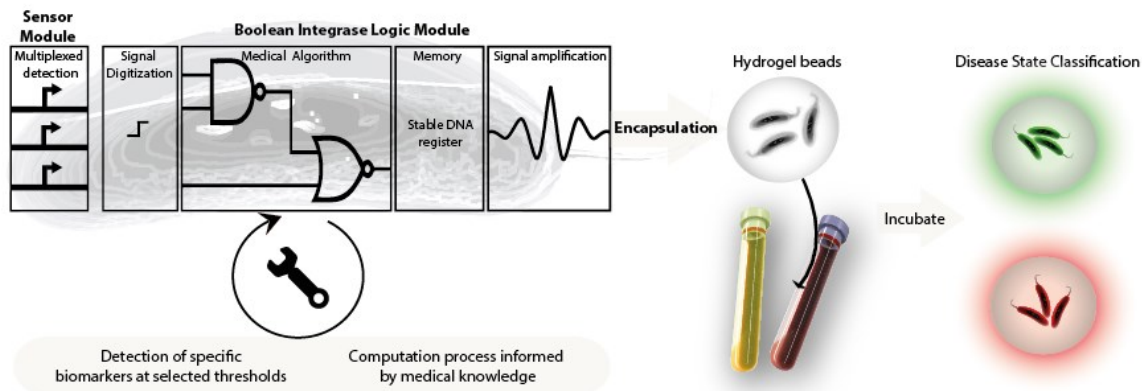
Living, emergent systems rely on regulatory processes as a central feature of their biological instructions. As we have seen, numerous strategies exploited a variety of their sensing mechanisms involving biochemical pathways, nucleic acids or proteins for the design of biomolecular logic gates *in vitro* or in living cells that can be further organized in biocomputing systems to develop intelligent diagnostics.

In vitro reaction networks can thus be designed for the sensing, processing and reporting of biomarkers, by exploiting biological species and their molecular functions. However, it is also possible to exploit the more complex architecture of living systems, which can be reassembled via bottom-up design in nonliving, on purpose systems^{340 341 342}. Although most reviewed diagnostic systems rely on simple architecture of few components, higher functional assembly of synthetic building blocks are possible, mimicking the natural architecture of living cells and giving access to complex features of living organisms.

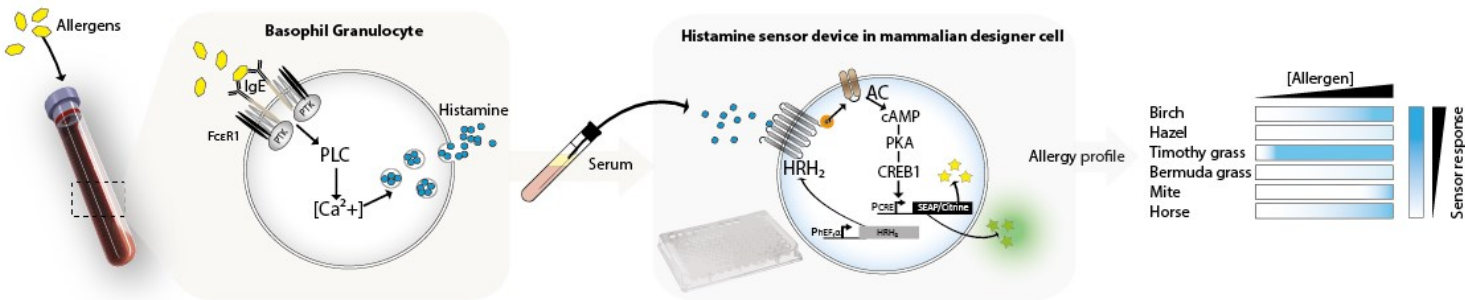
A key feature of biological systems is compartmentalization of information. Complex systems have evolved ways to cope with complexity of higher order architectures through the use of compartments. This strategy allows parallel chemical reactions and higher-level functions to be performed efficiently and simultaneously without loss of information content. New kinds of biotechnological supports arising from advances of synthetic biology and nanoscience give the opportunity to approach, interface, engineer, and assemble components and systems at the small working scale of biology, leading to the emergence of new strategies to diagnostics. The collusion of synthetic biology and nanomaterials will be key to realizing full potential³⁴³. Attempting to assemble synthetic parts in compartments approaching biological-scale functional density, such systems could prove capable of assuming near-cell like behavior³⁴⁴, efficient transduction of information and energy that permit complex molecular detection, signal processing, and biochemical actuation, while being autonomous and self-powered.

From the bioengineering perspective, this strategy has been extensively used in natural cells, where the host provides the compartment, building blocks and infrastructure to allow for the execution of instructions supported by the synthetic systems, but also mostly production, expression,

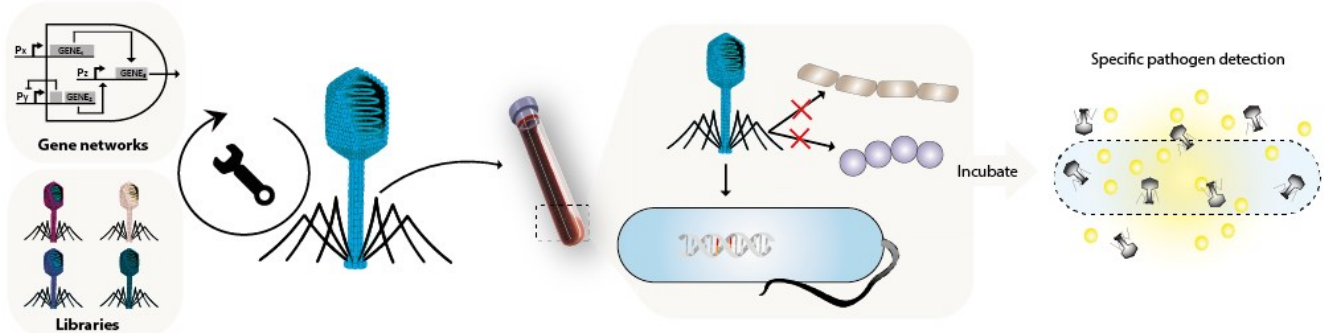
Case 1: Bacterial whole-cell biosensor



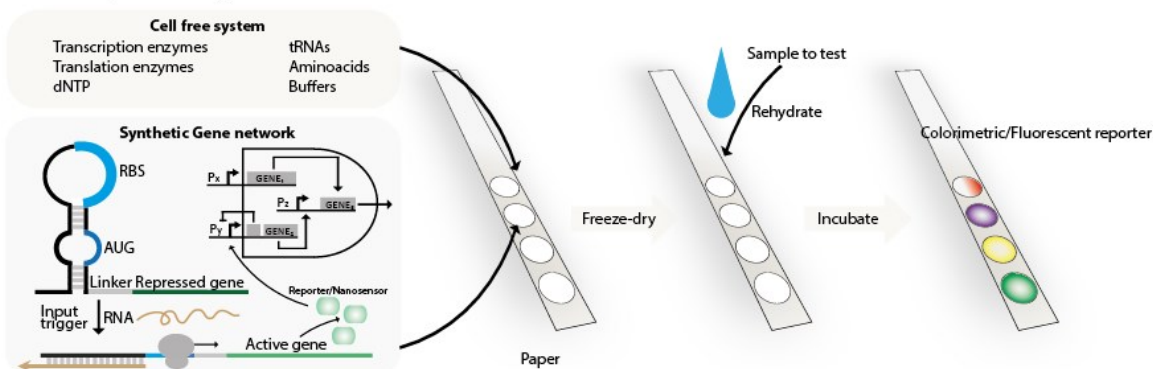
Case 2: mammalian whole-cell allergy profiler



Case 3: Bacteriophage engineering



Case 4: Paper-based gene networks



Case 5: Paper-based biochemical networks : luciferase-based indicators for companion diagnostics

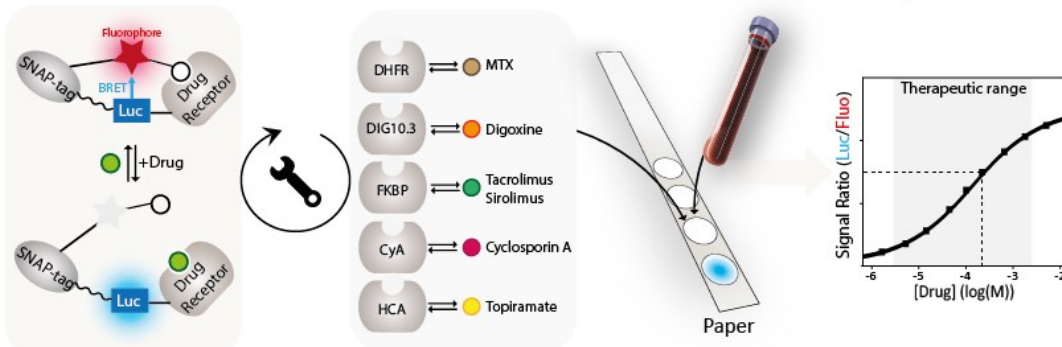


Figure 6: Case studies: recent synthetic biology research strategies to provide novel diagnostic tools. **Case 1:** next-generation bacterial biosensors for medical diagnosis detecting biomarkers in human clinical samples with a robust, programmable, and reliable behavior for clinical use (adapted from Courbet et al.¹¹⁶). **Case 2:** mammalian cell-based biosensors that score the allergen-triggered release of histamine from whole-blood-derived human basophils. A synthetic signaling cascade engineered within the allergy profiler rewires histamine input to the production of reporter protein, thereby integrating histamine levels in whole-blood samples (Adapted from Ausländer et al.¹⁴⁵). **Case 3:** Engineering bacteriophages as near-realtime microbial diagnostics by using them to transform target specific viable bacteria into factories for detectable molecules (adapted from Lu et al.¹⁸⁷). **Case 4:** Toehold RNA switches biosensors, *in vitro* paper-based platform that provides an alternate, versatile venue for synthetic biologists to operate and a medium for the safe deployment of engineered gene circuits beyond the lab. Commercially available cell-free systems are freeze dried onto paper, enabling the inexpensive, sterile, and abiotic distribution of synthetic-biology-based technologies for the clinic (adapted from Pardee et al.²⁴³). **Case 5:** Semisynthetic bioluminescent protein sensors approach proposed as an entirely new mechanism for inexpensive point-of-care biosensors. That permit quantification of specific drugs in patients samples by spotting minimal volumes on paper and recording the signal using a simple point-and-shoot camera (adapted from Griss et al.²⁹⁵).

maintenance and amplification. In the bottom-up design approach, however, compartmentalization only supports the user defined function without further energetic, metabolic, evolutionary, and regulatory cost, hence increasing the design space. The construction of fully multipurpose, conditional biosensing devices from biological components requires dealing with natural complexity emerging from biological systems. Tackling such challenges would thus require considering the design and engineering of organized, encapsulated systems from rationally assembled components³⁴⁵. These concepts have stressed the need for compartmentalization in bottom-up synthetic biology. Encapsulating complexity is an interesting framework for the conception of integrated systems with the ability to sense and transduce signals from their clinical environment and the ability to generate new biosensing devices with unprecedented control on re-programmability and versatility. These would be multicomponent, compartmentalized, non-replicating systems. This approach will necessarily require full expertise in design, engineering, and characterization of membrane systems and the modeling of complex systems. These approaches have been often captured under the concept of synthetic minimal cells, which potential for biosensing and biocomputing has been widely emphasized^{346 347 348 349}.

At the moment, synthetic vesicle-based systems of submicrometer scale, operating as high density intelligent biochemical sensor/effector systems have been proposed to perform diagnostic processes in physiological environments. Combining sensing and effector functionality at the nanoscale, they generate a conditional response that depends on environmental factors such as biomarker concentrations, pH or temperature at the target site³⁵⁰. They are basically composed of a carrier platform and a payload embedding circuitry for sensing, processing signal and reporting. Such stimuli-responsive hybrid nanostructured particles in a range of sizes from nanometers to a few micrometers include liposomes, polymerosomes, core-shell structures, nanogels, and more complex

architectures. The controlled assembly of synthetic polymer structures in vesicles is now possible with an unprecedented precision and modularity³⁵¹.

Synthetic vesicles have been extensively used for therapeutic strategies as drug nanocarriers, and proved efficient and successful in the treatment of diverse pathologies. Alternatively, they have also progressed toward analytical application as biosensors for bioanalysis for their ability to carry complex diagnostic reagents and electrochemical, fluorescent or chemiluminescent probes. Synthetic vesicles can also integrate synthetic biological parts such as engineered transmembrane and pore proteins, enzymes, nucleic acids or metabolites to integrate stimuli responsive behaviors³⁵². Encapsulation of hydrophilic compounds in their aqueous cavities and the insertion of fragile hydrophobic compounds in membranes offer protection and stabilization from harsh physiological conditions and allowed to act *in situ*³⁵³. Synthetic vesicles are known to enhance biochemical reactions, as thermodynamics of synthetic reactions are known to be favored by compartmentalization in picoscale volumes³⁵⁴, stabilize enzymatic processes, and provide signal amplification. The ability to functionalize vesicle surface to perform recognition functions, and targeting, selective transport and sensing is another important aspect of their use in bioanalysis³⁵⁵. Moreover, their small scale provides the opportunity to take advantage of patterns or multimodal molecular factors of the microenvironment *in situ*. Moreover, compartmentalize processes in different segregated spatial localizations can then be put under interactions with one another and create more complex biochemical networks³⁵⁶.

As the first described synthetic compartment, liposomes have been used for a wide spectrum of sensing modalities with a wide range of analytes. Many liposome-based assays have been reported such as liposome immunoassay (LIA), liposome immunolysis assay (LILA), liposome immunosorbent assay (LISA), flow-injection liposome immunoanalysis (FILIA), and cytolysin-mediated liposome immunoassay (CyMLIA), as well as chromatic polydiacetylene liposome based assays^{357 358}, providing low detection limits for analytes including hormones, viruses, bacteria, DNA/RNA segments, pesticides, tumor markers, proteins, antibodies and some drugs^{359 360} (reviewed in³⁶¹). Liposomes with engineered biological pores have also been extensively used for nanopore-based biosensing applications. Rational modifications by directed evolution or biochemistry have been carried out to reengineer mutant channels for desired biodetection purposes. For example, α -hemolysin, MspA or FhuA, and more recently phi29 derived synthetic nanopores have been engineered for sensing a wide range of analytes, from metal ions to organic molecules to DNA, RNA and peptides³⁶². Further efforts have been conducted to associate these architectures into point-of-care formats.

However, liposomes often suffer from poor lipidic membrane stability that could hinder their use. For that reason, important efforts have been conducted to engineer devices such as orthogonal polymeric vesicles with enhanced membrane properties for diagnostic, to protect reagents but allow them to interact *in situ*³⁶³. Polymeric vesicles structures similar to lipid vesicles can be engineered using synthetic block copolymers and stand as interesting candidates to develop orthogonal nanosystems for medical applications^{364 365}. They are more stable, more versatile, and less immunogenic than liposomes. Control over block copolymer chemistry enables tunable design of polymersome material properties. Optimization efforts allow scientists to design smart compartments encapsulating sensing and biocomputing biochemical networks made of nucleic acids, enzymes, and metabolites, and control on size, encapsulation of species, membrane properties and permeability to enhance sensing sensitivity and specificity, and allow insertion of membrane proteins³⁶⁶. Recent advances are shifting these active nanosystems systems towards smart-complex synthetic parts and polymer assemblies, like multi-compartment cascade reaction³⁶⁷.

Crucial to innovation in medical diagnosis is the development of new platforms that combine multifunctional compounds with stable, safe and implantable devices for close to patient strategies. As discussed before, theranostic strategies could decrease health burden of many pathologies by enabling the simultaneous detection and treatment of pathological events through interactions manipulated at the molecular level, by that mean achieving less side effects and timely delivering of therapy. Along with *in vitro* assays, synthetic vesicles based systems have been proposed to work as intelligent nanocarriers for theranostic. While surface functionalization enables selective targeting, theranostic nanocarriers could improve disease diagnostic and treatment because of their ability to execute conditional biological functions at targeted diseased sites³⁶⁸. Additionally, targeted nanodelivery systems would greatly benefit *in situ* imaging diagnosis³⁶⁹. Such injectable systems can process pathological signals and release *in situ* specific signals and/or drugs based on analysis of multiple signals. Several types of injectable diagnostics based on vesicle systems have been proposed, such as liposomes and synthetic polymeric systems. For instance, polymersomes have proven as excellent non-invasive intelligent fluorescent probes carrier for diagnostic imaging³⁷⁰. Another recent study obtained success in developing a platform based on polymeric artificial organelles to target specific cells for subcellular delivery of drugs, enzymes, nucleotides, and diagnostic agents³⁷¹.

Synthetic nanobiological assemblies have been exploited to construct new diagnostic assays with increased specificity and sensitivity. Assays relying on conventional assemblies can display important sensitivities for single molecular targets, whereas the engineering of multimodal nanoplatforms for sensing, imaging of biomarkers can prove capable of multiplexing input detection for a more

efficiently discrimination between complex disease phenotypes³⁷². Self-assembled nucleic acids nanostructures can provide templates for the spatial, ordered patterning of enzymes to develop high sensing efficiency and sensitivity of biocatalytic cascades for nanoscale devices. Such approaches have been used to develop for example glucose, ethanol or cocaine biosensing devices^{373 374 375 376 377}. Synthetic bionanoparticles can also perform Boolean logic operations using two proteolytic inputs associated with unique aspects of tumorigenesis³⁷⁸. Konry et al. also reported the integration of microarray sensor technology with algorithmic capability for the gated screening of proteins and DNA markers in a biological sample. The system they developed performed simple Boolean logic operations by coupling multiple molecular recognition inputs like IL-8 and specific genes to a fluorescence signal output³⁷⁹. Similarly, Janssen et al. recently developed synthetic antibodies for molecular diagnostics that are peptide DNA conjugates, enabling the control of antibody activity in a DNA based logic gated behavior³⁸⁰. In another study, hybrid biochemical reaction networks exploiting enzymes and oligonucleotides with a computing functionality were applied to the identification of bacteria exhibiting multi-drug resistance. This approach enabled the identification of the NDM-1-encoding gene and concurrently to screen, by a tailor-designed biomolecular logical gate, two genetic fragments encoding the active sites bound to carbapenem³⁸¹. A vast array of literature has covered the field of information-processing systems at the nanoscale to yield “smart” signal-responsive hybrid systems with built-in boolean logic^{382 316 383}.

Synthetic biopolymers have also been designed to act as biochemical stimuli responsive devices. In this approach, interaction of responsive polymers with molecular signals relies on the conjugation of polymers with biological molecules such as nucleic acids, enzymes, antibodies, and other proteins, or *de novo* molecularly imprinted polymers (reviewed in³⁸⁴) to yield diagnostic information or therapeutic activity *in vitro* or *in situ* upon systemic administration³⁸⁵. In this perspective, nanogels are likely to yield interesting diagnostic devices. Of polymeric nature, they can be tailored with a broad range of chemical modifications and entrap a large scope of biological molecules (nucleic acids, proteins and drugs). For instance, multi-functional core-shell nanogels combining magnetic regulation with biochemical sensing have been demonstrated³⁸⁶. Another approach relies on peptide-based or viral inspired self-assemblies for the design of hollow or solid peptidic nanostructures. For instance, Naskar et al demonstrated how multivesicular structures built from self-assembling peptides, could display calcium ions sensitivity. Such intelligent stimuli responsive behavior could enable approaches of medically relevant biodetection³⁸⁷. Expanding peptide-based nanostructures by exploiting rationally engineered peptide functions, receptor or enzymatic activity, is likely to lead to novel nanomaterials with complex sensing functionalities. Finally, synthetic biology could provide interesting approach for the integration, the production and functionalization of

metallic nanoparticles such as quantum dots or gold, which are of outstanding importance as diagnostic reagents. Synthetic biology is likely to provide ways to exploit new sensing and reporting mechanisms to create new tools by providing a biological interface to use metallic nanoparticles³⁸⁸.

Similarly, the engineering of so called “biofuel cells” have received much attention to develop autonomous, self-powered biodetection devices. Biofuel cells emerged from the effort to engineer an interface between electronics and biology, which could benefit bioanalysis^{389 390 391 392}. They display properties that defines them as robust *in vivo* power sources for bioelectronics, and could greatly benefit the development of implantable diagnostics, such as glucose biosensors, or more complex “smart” devices^{392 393 394}. For example, Zhou et al. developed aptamer biosensors based on biofuel cells, where power release was triggered by biochemical signals processed according to the boolean logic operations, to generate self-powered medical diagnostics “programmed” into a biocomputing system²¹³. Other advances have showed the coupling of a self-powered diagnostic operation with logic-activated drug release³⁹⁵. Combined with synthetic biology methods, such approaches could reveal valuable in producing novel tools.

Although still in its infancy, the opportunity to construct *de novo* increasingly complex processes and systems is emerging from the convergence of synthetic biology with new experimental and computational tools³⁹⁶. The ability to control the bottom-up design, synthesis and construction of synthetic systems by the direct assembly of synthetic nanoscale parts increases, likely to yield cell-like complexity and capabilities for tailored biodetection. We propose that new approaches exploiting synthetic compartments encapsulating biosensing, biocomputing and diagnostic reagents are likely to generate innovative medical devices in the future, and hold enormous potential as nanostructured biomaterials for future *in vivo* drug delivery and diagnostic imaging applications³⁹⁷. For some of such systems, clinical trials are in progress, but extensive clinical evidence of significant patient benefit will be further required³⁹⁸. The power of such systems can be realized with synthetic biology and bioengineering to generate functional devices for the clinics. Additionally, these approaches are likely to enhance our understanding and explore new ways of interfacing biological systems.

C. Engineering synthetic biological systems to support signal processing for medical diagnosis

Information processing occurs naturally across hierarchical levels ranging from molecules to cells, tissues, organisms and even ecosystems. Computation on biological signals thus ubiquitously takes place in biological systems¹⁸. Biological information is collected by sensing and signaling units, further processed and analyzed by organic matter, metabolites, proteins and gene circuits, and translated into specific molecular responses. Although biological processes are by nature noisy and use unreliable molecular devices interacting with analog and digital molecular signals, they manage to solve tasks precisely, in real time and energy-efficiently³⁹⁹. While trading a simple for a more complex design would be counterproductive, modular device oriented methodology with layered, standardized interface between sensing and reporter components can speed up the design, provide programmability and increase versatility and capabilities of engineered biosensing systems. The rationale behind such transmission devices, or signal processors, is to achieve signal integration from various sources, gain amplification, noise filtering, or logic operations⁴⁰⁰ and to connect various input sensors to reporting platforms for output multiplexing. Synthetic biology enables the construction of tailored signal processing by means of modular plug-and-play, and thus the reprogramming of natural information processing systems either *in vivo* or *in vitro*, into autonomous nanomedical devices that evaluate diagnostic rules *in situ*.

In the context of diagnostics, biological circuitry needs to be easily reprogrammed to integrate varying clinical constraints, different medical agendas and a vast range of pathologies. Moreover, it needs to support the improvement in system robustness and overall medical service. Additionally, time scale of biological processes is to take into account to engineer clinically compliant signal processing systems for appropriate diagnostic devices, as transcriptional and translational circuits dynamics occur over timescales of minutes to hours while biochemical processes occur in seconds or less²⁶⁰. Noise propagation in synthetic systems is also to take into account to obtain reliable behavior, which is dependent on systems dynamics and scale of processing circuits. Consequently, keeping faster and simpler systems would have fewer mode of failure and overall great chances of clinical success.

The need for novel health monitoring systems has progressively opened a new domain that results from the fusion of sensors and signal processing in synthetic biological systems. Properties such as ultra-low-power information processing capacities³⁹⁹, self-powering, compactness from micro to nanoscale, data storage, real-time signal processing and multi-sensor communication are all important advantage for synthetic biological systems to implement integrated medical diagnostic

devices. These properties enable the pre-processing and aggregating of low-level sensor physiological information to yield output signals intelligible by physicians, patients or researchers concerning diagnostically relevant events or biomarker patterns. Hence, we believe that the signal processing capabilities of synthetic systems can meet the challenge of developing portable autonomous health monitoring devices that can offer pragmatic solutions to achieve highest clinical impact, for developing countries or point of care, personalized medicine.

Critical parameters in the analytical performance of quantitative biosensing systems for diagnosis are the sensitivity (e.g. lowest analyte concentration that triggers a detectable response) and the dynamic range (DNAR, e.g. range of analyte concentrations where analyte concentration can be estimated based on the output signal), while optimizing the signal to noise ratio (response *fold change*). Quantitative systems provide analog signals which transfer function are ideally standardized response curve with wide DNR and low noise. Engineering biological analog detection can be performed using for example negative feedback loops. However, other qualitative or discrete, near-digital detection modalities are possible and can prove extremely valuable in specific context. For example, molecular ultrasensitive switches can provide digital behavior, providing an input detection threshold at which small changes in input biomarker concentration lead to large changes in output signal. Strategies involving positive feedback can be used to obtain digitization of signals. Cellular systems can also display fold-change detection, a response whose entire shape, including amplitude and duration, depends only on fold changes in input and not on absolute levels⁴⁰¹. A wide class of mechanisms has shown to display this response, which could prove useful for biodetection. Another property to consider when designing signal processing devices for diagnostic application is robustness, that is, the ability of a system to tolerate exogenous perturbations while limiting modes of failure in biodetection. Achieving modulation of transfer function of synthetic systems is thus of particular importance for the clinics (**Figure 5**).

Crosstalk between biological and synthetic circuitry must be in most cases prevented, while some signals need to be combined, added or compared to enable decision making. This requires computation processes to be implemented in synthetic devices. Biological information can be transformed through digital or analog processing or through a hybrid combination of both. A digital mode of operation has the advantage to enable the implementation of Boolean logic based decision making circuits. In that perspective, synthetic biology attempts to apply the digital paradigm of electronic engineering to develop algorithmic processes with biological components. Many examples of synthetic biological signal processing have been achieved using on digital information. Although more difficult, the rational design of analog based processing is appearing as extremely valuable to exploit the computational power of biology, as it could cope with more complex operations and

larger sets of variable in smaller circuits, and is closer to the natural mechanism of biological systems⁴⁰². Moreover, analog computation could for example enable pathological signal normalization, for instance directly computing ratios with physiological standard biomarkers like creatinine or albumin. We propose that an efficient and accurate signal processing approach to synthetic biological networks would integrate both analog and digital processing to achieve versatility, efficiency and reliability. Recent devices have been recently engineered in that direction, to perform analog to digital or digital to analog processing^{403 404 405 406 407}.

The aim of synthetic biology is to achieve systematical on purpose re-programation and tuning of these analytical characteristic for different biodetection agendas using modular signal processing circuit design. An increasing number of strategies have been developed for tuning the responses of biological systems. We believe it is on the way to enable the tuning of biological systems' transfer function the same way electrical signal processing is achieved.

Synthetic circuits operating *in vivo*

The first successful design and implementation of synthetic gene processing circuits were demonstrated with the genetic toggle switch and the repressilator^{408 409}. They proved that bioengineering-based methodology could enable the integration of user-defined information processing and computing capabilities in living cells. Following these studies, synthetic biologists have successfully established a repertoire of genetic components to engineer complex signal processing genetic circuits in living cells with a vast range of functionality, such as switches, oscillators, timers, memory, filters, logic gates, cell-cell communicators, or buffers^{410 411}. Since, synthetic gene networks have been extensively used to reprogram cells for useful task such as decision making for cell-based biosensors⁷⁰. Increasingly complex designer signal processing networks have been built in cellular systems to perform input-triggered genetic instructions with precision, robustness and computational logic. Moreover, the demonstration of the ability to rationally tune *in vivo* biological transfer functions in transcriptional, post transcriptional, and post-translational levels of regulation has been extensively described (review in⁴¹²).

For future medical and diagnostic agendas, complex processing circuits operating robustly in living cells may require new types of orthogonal parts with increase in orthogonality to host physiology, that offer control on dynamic range, digital or analog signal modes, low crosstalk, and design versatility. Next-generation gene networks for biodetection could as such comprise tunable filters and noise controllers, analog-to-digital and digital-to-analog converters, or even adaptive learning networks⁴¹¹. Moreover, the systematic design and quantification of genetic parts in context⁴¹³ is

leading to a new era of well-characterized regulatory synthetic genetic devices, such as bistronic RBS⁴¹⁴, ribozyme parts insulators⁴¹⁵, and synthetic terminators⁴¹⁶. Parts mining and computational design, and directed evolution are further expanding the number of regulators that can be used together within one cell⁴¹⁷.

Indeed, complexity of signal processing circuits *in vivo* is often limited to a few logic operations, because of unpredictable biochemical crosstalk occurring in the confined volume of the cell and the limited number of available parts, the size of signal processing circuits and composability has remain limited. Developing design strategy for the successful layering of orthogonal high performance parts or logic gates into large, integrated circuits in single cells remains a challenge. In a recent study, Moon et al. managed to overcome this challenge by applying part mining and directed evolution to build a set of orthogonal transcriptional AND gates in *Escherichia coli* that could then be concatenated into complex programs, such as 4-input AND gate that consists of 3 circuits that integrate 4 inducible systems, thus requiring 11 regulatory proteins. Optimizing, and refining the performance of individual gates was sufficient to predict the behavior of a complete program⁴¹⁸.

New design concepts have recently taken a new step with the development of digital recombinase based circuits. For example, in our previous work (Coubert et al.¹¹⁶) we found that promoters of clinical interest and control circuits that coordinate simple signal transduction showed inherent noisy and unpredictable responses with limited control over specificity and efficacy in host cells when operating in complex media. In fact, a known barrier to predictability in design is context⁴¹⁹. Synthetic gene circuits are often easily perturbed and their behavior altered by the environment they are exposed to⁴²⁰ and the host they are integrated into. Heterologous pathways have not had the advantage of long periods of co-evolution with other cellular substrates. Thus, their function often suffers from uncontrolled/unpredicted interactions with the surrounding cellular context and environment. Lack of robustness has limited the utility of engineered gene circuits for further medical applications and hinders advances in synthetic biology. In our recent work, we proposed that context sensitivity can be reduced by incorporating synthetic genetic tools precendently developed, while keeping few components for fewer modes of failure and increased safety and likelihood of approval of cell-based biosensors in medical setting⁴²¹. In order to buffer matrix effects and nonspecific environmental interferences, overcome variable part performance across changing complex media⁴²², and enables predictable and standardized translational coupling, we incorporated in our design (i) Expression Operating Unit (EOU)⁴¹⁴, (ii) a ribozyme insulator part, RiboJ⁴¹⁵ (iii) Digital gene switches and integrase logic gates⁴⁰⁶. Digitalizing along with amplifying and multiplexing input signals improves fidelity, sensitivity, mediate sharp response profiles and ensure robust biochemical processes. Bonnet et al. recently designed a new type of logic gates architectures which recapitulate

all conventional logic functions using integrases Bxb1 and TP901^{406 407}. These integrase logic gates enable truly digital and discrete response, compared to previous systems that produces intermediate expression levels^{151 152 36}. This property makes them highly relevant for medical applications, and particularly in diagnosis as it is often threshold based. This system also embeds a built in memory capacity which enables the recording of weak or transients signals while giving a constant amplified output. Compared to transcriptional switches¹⁹, this “true memory” has non-existent metabolic cost and is stably written in either chromosomal or plasmidic DNA, and could be addressed after extended periods of time and lysis of the bactosensor in clinical samples. These signal processing devices based on an engineered modular genetic logic gate have the advantage of high composability to be recombined for the programming of various medical algorithms. We suspect that these characteristics will be important to enable robust detection and computation in the context of intracellular and environmental fluctuations.

Taking synthetic parts improvement further, recombinases based systems have intrinsic properties that offer tremendous interest to develop cell-based biosensors: increase in scalability to larger networks by reducing their molecular payload, prevent cross-talk with off-target contrary to other DNA-binding proteins, and control on genetic circuit in time-dependent fashion⁴²³. Recently, Yang et al. extended the programmable memory capacity in a living cell to beyond 1 byte of information using 11 orthogonal integrases. A high number of events can thus be sensed, recorded and recalled at a later stage of the computation, thus increasing memory capacity could enable new type of biosensing to be performed in cells⁹².

Moreover, expanding the repertoire of available orthogonal genetic parts remains a challenge, particularly since digital logic requires many parts and will hinder the scalability of circuit design. Analogue circuits constitute an attractive alternative as they can compute high order non-boolean functions such as amplification, addition, multiplication and integration, and could be regarded as a promising way for future designs for *in vivo* computations systems applied to diagnosis. Along with digital circuits, synthetic analog gene circuits have been engineered to execute complex computational functions in living cells have been recently examined theoretically and experimentally¹⁸ and have recently demonstrated their value^{424 425 426 427}. Daniel et al. were capable of implementing analog circuits to straightforwardly compute arithmetic functions without necessitating layered digital logic gates. They demonstrated a wide dynamic range relying on positive feedback loops, which could perform or log-domain sensing, power law and addition or division of input molecular signals. Analog computation recently enabled the recording of sums of molecular events over a time period. Interestingly, ratiometric calculations are useful in diagnostic systems, because they enable the normalization of diagnostic threshold, comparisons between biomarker

levels and complex decisions. This approach could provide wide dynamic range biosensors for quantitative measurements of biomarkers along with a binary, digital readout approach. Farzadfard et al. demonstrated that genomic DNA could be used as a rewritable and flexible substrate to memorize analog information, such as the magnitude of an input signal, as a proportion of cells in a population. This platform could enable long-term cellular recorders for diagnostic applications⁷⁶.

Even though signal processing *in vivo* was first implemented with the use of synthetic gene circuits, fast kinetic events in biology are increasingly supported by protein-based signal processing systems. Beyond nucleic acid as a substrate for information in such circuits, protein-based synthetic systems have the potential to enable modular and efficient computation through post-translational mechanisms¹⁵⁴. Information processing can be supported by protein-protein interaction such as binding combined with activation or inhibition of catalytic activity like phosphorylation or proteolysis. Recently, intein splicing has received attention to construct synthetic protein circuits, as they support their own catalysis and subsequent excision followed by intein tagged protein fusion and function recovery. Interestingly, this event can be activated by small molecule ligands or protein scaffolds, and allows for spatial control, implementation of Boolean logic, or signal amplification via synthetic cascading^{428 429 430}. Protease degradation has also been described as a tool to engineer control signal processing in synthetic protein circuits⁴³¹. For example, Prindle et al. used protein degradation as a tool for rapid and tunable post-translational spatial and temporal control on gene expression⁴³². MAPK networks have also been successfully rationally engineered for synthetic cascading to generate modular, insulated, ultrasensitive and tunable signaling⁴³³. Other approaches have made use of chimeric regulatory proteins in synthetic signaling, exploiting for example two-component systems of bacteria, to achieve novel customized signaling⁴³⁴. Moreover, due to the fact that genetic circuits and proteins operate on different time scales, developing hybrid synthetic networks could prove valuable. For example, the output of protein-based information process could then be stored in recombinase-based memory register, or integrated via CRISPR-Cas9 or inteins splicing protein such as TALEs or ZFN^{435 436 437 438 439}.

Although cellular context can be assumed disruptive, it may also play supportive roles in the functioning of synthetic circuits and provide relative robustness, performance and maintenance that can be valuable and exploited in specific contexts. However, while the engineering of orthogonal biological parts and signal processing frameworks *in vivo* have proven valuable for synthetic biologists, potential discrepancies remain, such as high context and chassis dependency.

Synthetic circuits operating *ex vivo*

In cell-free systems, synthetic parts are exempt of adaptation and evolution and as a result can benefit from relatively more tunable and reproducible behavior. Efforts to reproduce the response capabilities of cellular circuits from the bottom-up approach have been reported with the assembly of synthetic biochemical reaction networks¹⁸. These synthetic systems involving biocatalytic reactions can be utilized for information processing or biocomputing. Extensive research has been conducted on *ex vivo* systems, greatly motivated by applications in biondiagnostic. Advances in biomolecular computing systems mimicking electronic substrates, has resulted in the development of novel synthetic biological signal processing framework. For example different biomolecular tools, including proteins/enzymes, and nucleic acids have been used to implement layered Boolean logic gates. While further scaling up the complexity of biochemical information processing systems had remained a challenge, recent results showed promises in that direction.

As we have precedently seen, nucleic acids are modular chemical building blocks with structural, mechanical and catalytic capabilities. Nucleic acid enzyme-based or enzyme-free computation systems, aptamers, ribozymes, circuits, origamis, and gels offer a wide repertoire for the design of biological signal sensors and processors⁴⁰⁰. DNA has been extensively and successfully used *in vitro* to implement networked logic operations, with an important scaling up in number of logic gates. Nucleic acids are capable of both carrying information and performing computations on that information. Circuits relying on nucleic acids as a substrate have few possible interactions and points of control making their quantitative design, simulation and description manageable. For example, Kim et al. showed how a synthetic nucleic acid circuit could be systematically designed to perform pulse generation, adaptation, and fold-change detection. This study demonstrated the programmability and ability of such circuits to obtain predictive dynamical systems in a cell-free environment for biosensing applications⁴⁴⁰. Chen et al. also reported a DNA-based architecture for implementing *in vitro* computational programs using the formalism of DNA reaction networks as a universal 'programming language' to implement any function that can be mathematically expressed. In this study, the formalization allows complex signal processing of intrinsically analogue biological and chemical inputs, and not only Boolean logic⁴⁴¹.

Proteins have also been used to make Boolean logic gates *in vitro*. During the last decade, numerous studies have pioneered the engineering of enzyme-based logic gates concatenated in information processing systems⁴⁴². Biochemical reaction networks can implement multi-signal Boolean logic or arithmetic operations such as addition or subtraction^{443 444 445}. Biomolecular circuits are also capable of implementing dynamic behaviors including pulsing, adaptation and fold-change detection⁴⁴⁶.

Novel cell-free biosensing concepts have capitalized on the idea of integrating multiple molecular inputs processed biochemically before transducing their output on “smart-material” interfaces such as functionalized electrodes or metallic nanoparticles, to give a hybrid bio/electronic signal processing. For instance, signal-responsive electrodes for signal readout have been coupled with biochemical logic gates^{316 447 448}. Moreover, taking technology further, future approaches could tend toward the full integration of biochemical and electronic processing⁴⁴⁹.

III. Enabling Technologies

In order to demonstrate true value in a global health perspective, we envision that novel synthetic biological systems for diagnosis would require technological support for effective clinical use and increased clinical compliance, as well as industrial scalability. Recently, the development of a vast range of satellite technologies synergistic with synthetic biology methods, showed great promise for the integration of medically applied synthetic systems into medical formats. Moreover, new technologies are constantly enlarging the bioengineering space, redefining the limits of biomedical synthetic biology.

For example, biotechnological innovation redefined nucleic acids writing and reading capabilities, with now the \$0.1/base and US\$1000/genome almost a reality, which then greatly influenced the synthetic biology field. While DNA synthesis evolution has enabled the genome scale engineering of biology to develop novel devices^{450 451}, sequencing nucleic acids covers a vast analytical and diagnostic landscape and it is a dynamic and promising area of research. Sequencing technologies now find application in medical diagnostics and pharmacogenomics and thus contribute to personalized medicine revolution. Moreover, cheap, portable and reliable sequencing equipment could be used to monitor and interface synthetic biological devices relying on nucleic acids, such as whole cell biosensor or biochemical reaction networks recording medical information in DNA.

However, new technological formats may be required to fully embrace possibilities offered by synthetic systems for new diagnostic modalities, in order to detect disease biomarkers from readily accessible bodily fluids with point-of-care devices that are inexpensive, noninvasive, accurate but do not require trained medical personnel (**Figure 7**).

Miniaturization is a way to achieve cost effective, scalable, and to easily implement synthetic biology approaches. The development of miniaturized point-of-care diagnostic tests may be enabled by chip-based technologies based on microfluidics such as μ TAS (Micro-total analysis systems) and μ PADs (Microfluidic Paper-Based Analytical Devices)^{452 7}. Microfluidics enables the manipulation of sub-picovolumes of diagnostic reagents and samples in microscale channels, where the microscale permits rapid detection by reducing the diffusion, mass and heat transport times and provides quasi-equilibrium state for the biochemical processes⁴⁵³. For instance, bridging of synthetic biology with droplet microfluidics has received tremendous attention to perform high-throughput sensitive assays. Manipulation and measurement of microscale diagnostic systems in droplets can be supported at kilohertz speeds while compartmentalization increases assay sensitivity and decreases the time required to reach detection thresholds⁴⁵⁴. For instance, cell-based biosensing systems have

	Format	Accuracy	Speed	Simplicity Handling	Portability Disposability	Through-put	Multiplexing	Implantable Theranostic	Integration Interfacing	Shelf life	Resource requirements	Expertise requirement	POC	Cost
Synthetic Biology	Conventional clinical laboratory	+++	+++	-	-	+++	+++		+++		-	-		
	μTAS	++	+++	+	+	+	+	-	++	++	+	+	+	+
	μPADs	+	+++	+++	+++	+	+	-	+	++	+++	++	+++	+++
	New formats?	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Figure 7: Considerations on clinical formats for integration of synthetic biology devices for the clinics. Here we compare potential formats that could accommodate synthetic biological components to develop novel diagnostics, in terms of analytical capabilities. (+), (++) , (+++) represent increasing advantages, (-) represents a clear disadvantage.

been largely integrated into microfluidic devices such as μTAS to facilitate their on-site application^{125 455}. Alternatively, we previously discussed the interest and promise of integrating synthetic systems on paper to develop novel diagnostic devices, such as freeze-dried synthetic gene networks²⁴³.

Coupling synthetic biology and microencapsulation technologies could also generate innovative cell-based biomedical applications, such as *in vitro* diagnostic formats or smart implantable theranostics⁴⁵⁶. Cell-based biosensors encapsulation and immobilization have been subject to promising technological evolutions enabling sealing of engineered prokaryotic or eukaryotic cells into portable, easy to handle formats, which provide suitable extracellular environment, semi-permeable and biocompatible microcapsule without the need of culture facilities. Microencapsulation can also be used to develop cell microarrays suitable for simultaneous measurement of a large number of samples. Various polymeric materials can be engineered at the nanoscale with control on biophysical properties and spatial patterning to enhance robustness and reliability of encapsulated synthetic cellular systems⁴⁵⁷. New nanofabrication technologies and synthetic biology approaches are likely to lead to new prospects for developing devices with tailored functionalities¹²⁷.

While nanobiotechnology has already been revolutionizing medical diagnostics, it is now having a profound impact on applied synthetic biology capabilities^{458 459 343}. Nanoscale biomaterials offer many avenues for progress such as molecular-scale bioelectronic interfaces that can be constructed using nanostructures, such as grapheme nanowires and nanotubes, and have capabilities defining them as excellent candidates for novel biosensors of high sensitivity, such as field effect enzymatic detection⁴⁶⁰. Nanobioelectronics is an emerging interdisciplinary technological field dealing with the interface between synthetic biology and nanomaterials and could enable enhanced integration and interrogation of biological systems. For example, the engineering of nanoelectronic/biological interfaces have the potential to produce breakthroughs in biodetection. Nanodevices can be engineered as ultrasensitive sensors with fine spatial resolution, and be integrated in live single or array of cells to probe complex physiological events⁴⁶¹. Nanoscale field effect transistors can be coupled with biological components to develop novel probes and sensing modalities, and even interface living tissues and organs (neurologic or cardiac diagnostic devices for instance). For

example, integrated sensory capability of the nano-bioelectronic scaffolds can enable real-time monitoring of the local electrical and pH changes of neural and cardiac smooth muscle tissue^{462 463}. Nanobioelectronics enables to build sophisticated, smart nanodevices with multifunctional capabilities, and promise a vast range of biomedical applications from *in vitro* diagnostics to implantable theranostics and novel synthetic systems for hybrid information processing^{463 464}. We suspect that several biomedical applications will result from this novel generation of biosensors for diagnostics.

Synthetic biological systems are currently engineered through iterative process of specification, design, and assembly. This method would often greatly benefit from stronger formalization of specification, architecture, and constraints. Synthetic biology, as a fusion with computer science has promised computational design of biological systems and laboratory automation for their systematic management⁴⁶⁵. Computer assisted automated design has been extensively used to enhance the design process. While still in its infancy, it would increase the predictability and reproducibility of experiments and lead to breakthroughs in the construction of new biological systems. This field of research is thus very likely to bring the next synthetic biology revolution⁴⁶⁶. For example, programming languages developed for synthetic biology provide standardized support for design and specification of parts and their properties, and their assembly into devices using rule-based constraints^{467 468}. Bioinformatic tools have also been developed for the *in silico* design of ribosome-binding sites, RNA based devices, protein switches or strong promoters that are insulated from contextual effects that enhance the robustness of translational applications, as well as automation of construction of genetic constructs and synthetic gene circuits^{469 470 471}, *de novo* design of proteins⁴⁷² biochemical pathways^{473 325 474}, or minimal systems⁴⁷⁵. The bottom-up engineering of synthetic systems will greatly benefits from *in silico* modeling for system prediction, model checking, sensitivity analysis, robustness assessment for bottom-up design of synthetic devices³²⁷.

Finally, the engineering of complex biological functions is now converging with the bottom-up construction of minimal systems/organisms through synthetic genomics. Synthetic genomics couples chemical synthesis of DNA with computational design, allowing the construction of novel genetic materials. For instance, the design and assembly of whole chromosomes, complex gene networks, and even whole genomes is now a reality. In the field of biosensing, it could for example confer the possibility of rapidly generating and tailoring cell-based biosensors. Moreover, optogenetic tools⁴⁷⁶, DNA nanotechnologies²⁰⁵, integrase based devices, genome editing tools such as ZFN, TALENs⁴⁷⁷ and more recently the CRISPR/Cas9 system⁴⁷⁸ allow ever finer and more precise engineering of genomes⁴⁷⁹.

IV. Technology readiness, research agendas and future clinical challenges

We propose that synthetic biology could be seen as a methodology to interface medical biology with clinicians. However, clinicians, medical biologists and health professional's expertise and implication will remain of outstanding importance. Moreover, novel bioengineering methods that we have discussed here and their associated application in the clinics are intended to be an aid for the clinician. Benefits may be gained from these more precise and reliable diagnostic tools, increasing readability and portability for resource poor settings, simplifying decision rules for clinicians, and thus reducing human error⁴⁸⁰ (**Figure 8**). While the field of mobile health and point-of-care is rapidly growing and likely to become wide spread reality through the use for example of connected devices such as smartphones^{481 482}, new supports may be required to achieve full potential, and synthetic biological systems stand as promising alternatives.

Indeed, diagnostics yield a great deal of information, which clinicians have to analyze and evaluate comprehensively in a short time. A few decades ago, computer sciences were first proposed to augment human reasoning in medicine⁴⁸³ and permitted to enhance medical care by improving decision-making capabilities of diagnostic systems and clinicians^{484 485}. For example, computer-aided detection and diagnosis is a procedure in medicine that assists doctors in the interpretation of imaging techniques. Similarly, new diagnostic possibilities permitted by synthetic biology could improve clinician's ability to assess pathological states and monitor diseases and their prognosis. Diagnosis strategies fall into the definition of computing, and synthetic biology provides a modular substrate for computation and interfacing. We suspect that the advances of synthetic biology could provide new expert biosensing diagnostic systems for the clinics as their effective use relies on bioengineering solutions ensuring robust and reliable behavior. Even Though computational versions of diagnosis using biological components have been proposed to date no biological computing system embedding a diagnostic algorithm following medical knowledge has been approved as a medical problem solving systems for clinical practice. There is still a long way to go until synthetic-biology-based biomedical devices become a wide spread clinical reality.

While the development of synthetic biosensors has increased in recent years, improvements in diagnostic accuracy, limits of detection, faster responses and miniaturization for improved medical service are yet to be demonstrated in clinical setting. Additionally, while most of the potentially clinically relevant bioanalytical platforms discussed here were implemented in "clean" environments, their operation and optimization in "real" biological samples, such as serum, urine, or saliva, is required.

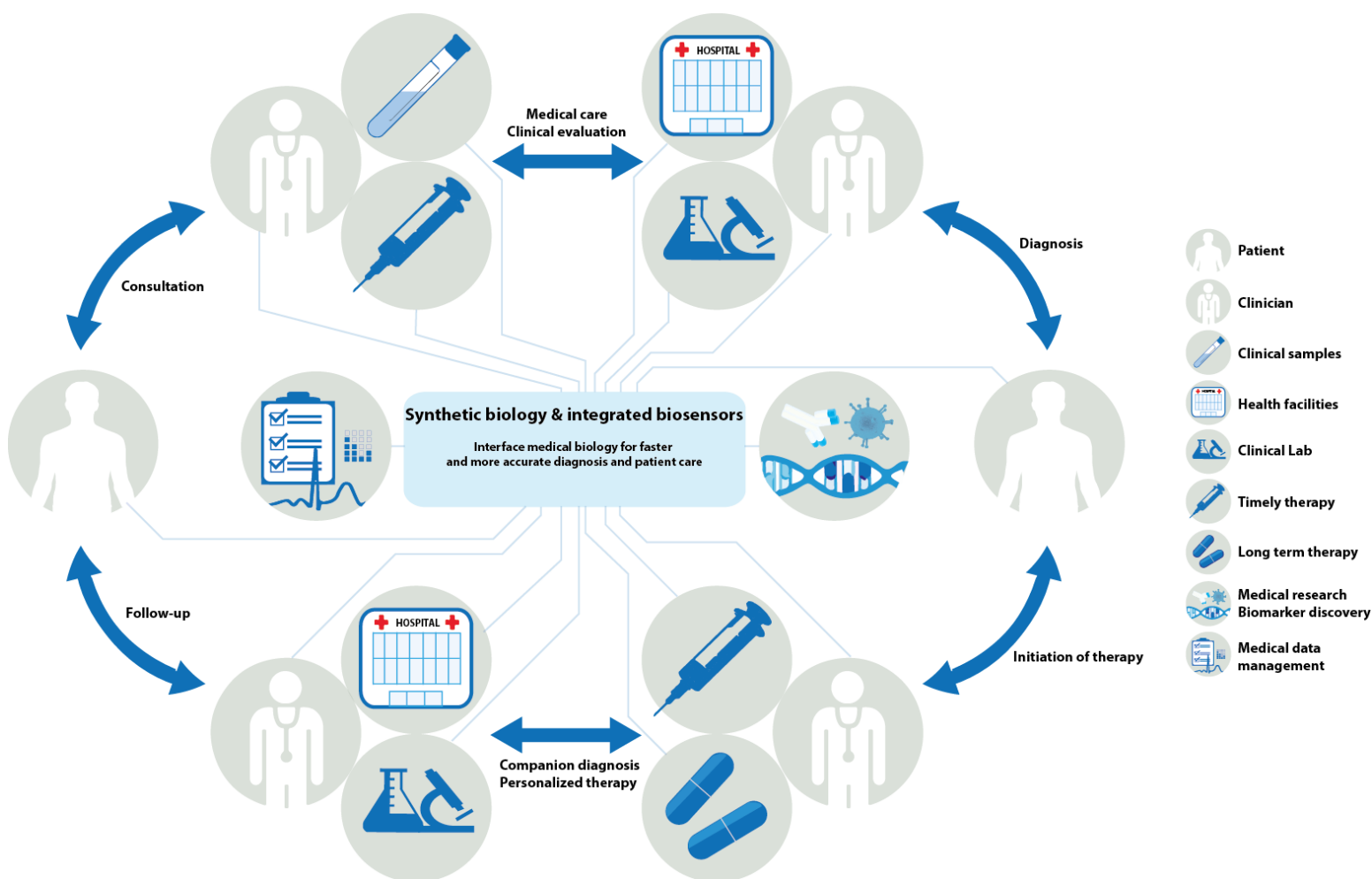


Figure 8: What can synthetic biology bring to medical diagnosis? Synthetic biology can be seen as a biotechnology enabling to interface patient's biology with health professionals for improved healthcare.

While proving extremely valuable in certain circumstances, and benefitting from constant refinement, increase in robustness and standardization, synthetic cell-based biosensors pose intrinsic limitations such as the evolutionary barrier^{486 69} or regulation issues that hinder the translation into the clinics. At the moment, biosafety and regulatory concerns of self-replicating genetically engineered cells forbid their use out of a controlled *in vitro* context⁴⁸⁷, and pose biohazard and risks of escape into open ecosystems⁴⁸⁸. However, Expanding as an important domain of research⁴⁸⁹, environmental and health risks could be contained by rigorous risk assessment and management, and potentially reduced by methodologies such as genome minimization, metabolite dependency, encapsulation, orthogonal systems or new genetic biocontainment strategies^{490 491}. Safety, harmonized regulatory regimes, standardization, as well as appropriate future risk assessment methods⁴⁹² are essential catalyst for effective commercialization and subsequent scaling up of the research⁴⁹³. In addition, public, market and regulatory structure may not be ideologically ready for

such dramatic change of concepts in medicine⁴⁹⁴. Although we addressed reliability and reproducibility issue arising from the use of engineered biological systems in clinical settings, most biosensors in development have yet to address safety and reglementary issues to start being used for medical applications. Commercial interest in bioreporter technology remains hampered by legislation controlling the application of genetically engineered bacteria, by the limited economic value of extensive but cheap market prospects and by the need to overcome technical problems inherent to living organisms⁷². In consequence, amongst the wide range of different biological substrates, cell-free devices have demonstrated the most clinical evidence. Between the two approaches to synthetic biology, top-down and bottom-up, the latter is probably more relevant to diagnostics as it provides more flexibility and highest control on properties and could more easily be cleared for regulatory approval (**Figure 9**).

The success of synthetic biology approaches is partly due to its exponential improvement in design capabilities while creating technological bridges with other emerging biological disciplines. Capitalizing on standardized biological parts and hierarchical abstraction of biological complexity enables bridging between medical and engineering disciplines (see, for example, the registry of standard biological Parts, or the JBEI-ICE). We envision that this approach will become more and more useful for synthetic biological biosensor engineering as multidisciplinary researcher groups become more familiar with the concept and more modular parts and devices become catalogued and standardized. Despite increasing complexity and highly innovative achievements in biological circuit design, synthetic biology mostly remains at a current clinical state of proof-of-concept. In order to progress more efficiently toward real world diagnostic application, we suggest that synthetic biology design should consider fields of exploration of high socio-economic burden where it could resolve real medical problems and prove highest medical benefits, such as companion diagnostics, infectiology, neurodegenerative and metabolic diseases, and developing world diagnostics issues, among others.

Probing and monitoring biomolecular and cellular networks instead of single components and biomarkers to predict the clinical outcomes of biological systems, has led to the promise of a systems biology based future medicine. While systems biology has started its translation into the medical field by unveiling dynamic individual patient–environment interactions, we suspect that this approach could be synergistic with synthetic biology since the later enables various modes and higher scale of measurements of biological parameters. Going toward personalized medical diagnosis, synthetic biology could provide us with the tools to allow synthesizing personalized biomarkers, or monitoring targeted therapies adapted to personal physiology. Such biomarkers will be adapted in their mode of action (for example via specific glycosylation patterns or epigenetic

markers), formulation, dosage, and release kinetics to the specific pathology of the patient. At the same time, theranostic and prosthetic strategies to diagnostics still pose challenges for implantation in humans to achieve an optimal benefit/risk. They could be solved with novel hybrid approaches, to obtain control on the implantable particle size, biocompatibility, immunological and pharmacokinetics properties if in blood circulation, target specificity for diagnosis, drug delivery or other functionality, controlled on programmable behaviors and mechanisms of stimuli responsiveness.

While the global value of synthetic biology market is expected to expand and reach \$16 billion by 2018, the market in synthetic diagnostics and pharmaceutical industry has been evaluated around \$5 billion in 2016, appearing as the most important industry driving innovation amongst chemicals, R&D, agriculture, and energy⁴⁹⁵. The market growth for biosensors is exploding , with medical sensors global market is expected to reach \$15 billion in 2019, with a growth of 6.3% from 2013 to 2019⁴⁹⁶. Additionally, the global market for theranostic nanomaterial was valued at \$112 billion in 2012 and is expected to reach \$188 billion by 2017⁴⁹⁷. However, managing intellectual property surrounding the use and construction of synthetic biology applications in biomedicine needs to be supported to promote innovation. While patent protected technologies are required to enable successful transitioning into the clinics, many of synthetic biology tools remain in the public domain or are subject to non-exclusive licensing, and access to some technology remain unclear⁴⁹⁸. We expect to see improvement in enabling intellectual property creation covering the field as it matures. Last but not least, economic consideration will play an increasingly important role in the biomedical context, if synthetic biology is to offer simpler, more elegant and least expensive solutions more likely to be clinically successful.

Top-Down approach: living cellular biosensors

- >Ethical/ regulatory/biosafety issues
- >Poor robustness
- >Tedious design, simulation and *in vitro* implementation
- >Complexity, Context and cell type dependency
- >Evolution of living systems
- >Slow kinetics
- >Possible lack of specificity
- >Low long-term stability and shelf-life

- >Low cost of production
- >Easy to produce and scale
- >Auto-replication
- >Multiplexing and highthroughput possibility
- >Functional/physiological information
- >Low resource requirements

Bottom-up approach: minimal biosensors

- >No ethical/regulatory/biosafety issues
- >Increased robustness
- >Facilitated design, simulation and *in vitro* implementation
- >No need for host cell, few components
- >No evolution
- >Fast response
- >Increased specificity
- >Increased stability and shelf-life

- >Possible high cost of production
- >Possibly more difficult to produce and scale?
- >Costly and tedious regeneration
- >Multiplexing and highthroughput possibility
- >No physiological information
- >Higher resource requirements

Figure 9: Advantages and drawbacks of top-down versus bottom-up approaches to synthetic biology for the developments of integrated biosensors for medical diagnosis.

An example of the most important synthetic biology initiative is the EraSynBio call for projects. Funded by the European Commission, it provides grants to European and American teams to build innovative transnational research. This call expects to support around 16 Million € for synthetic biology research. To obtain a recent and valuable overview of key drivers and technological ambitions of SB research, we analyzed projects sent at the ERAsynBio joint calls according to their applications (**Figure 10A**). It appeared that amongst 55 projects kept for review approximately one third was dedicated to fundamental research, another third to industrial and bio-production applications, and a last third to biomedical applications. Amongst these 21 biomedical projects, we could distinguish 15 dedicated to innovative biopharmaceutical production, 3 for the synthesis of medical biomaterials, and finally 3 projects dedicated to medical diagnosis of diseases. Attractiveness of diagnostic applications is further highlighted when looking at research trends in synthetic biology and bioengineering over the past decades (**Figure 10B**). This seemingly moderate transition to the industry could also be explained by the fact that although diagnosis market for POC technologies is blooming, they still remain to solve complex economic state of diagnostic market, the intellectual property problem, governance issue, and ethical, legal and social issues.

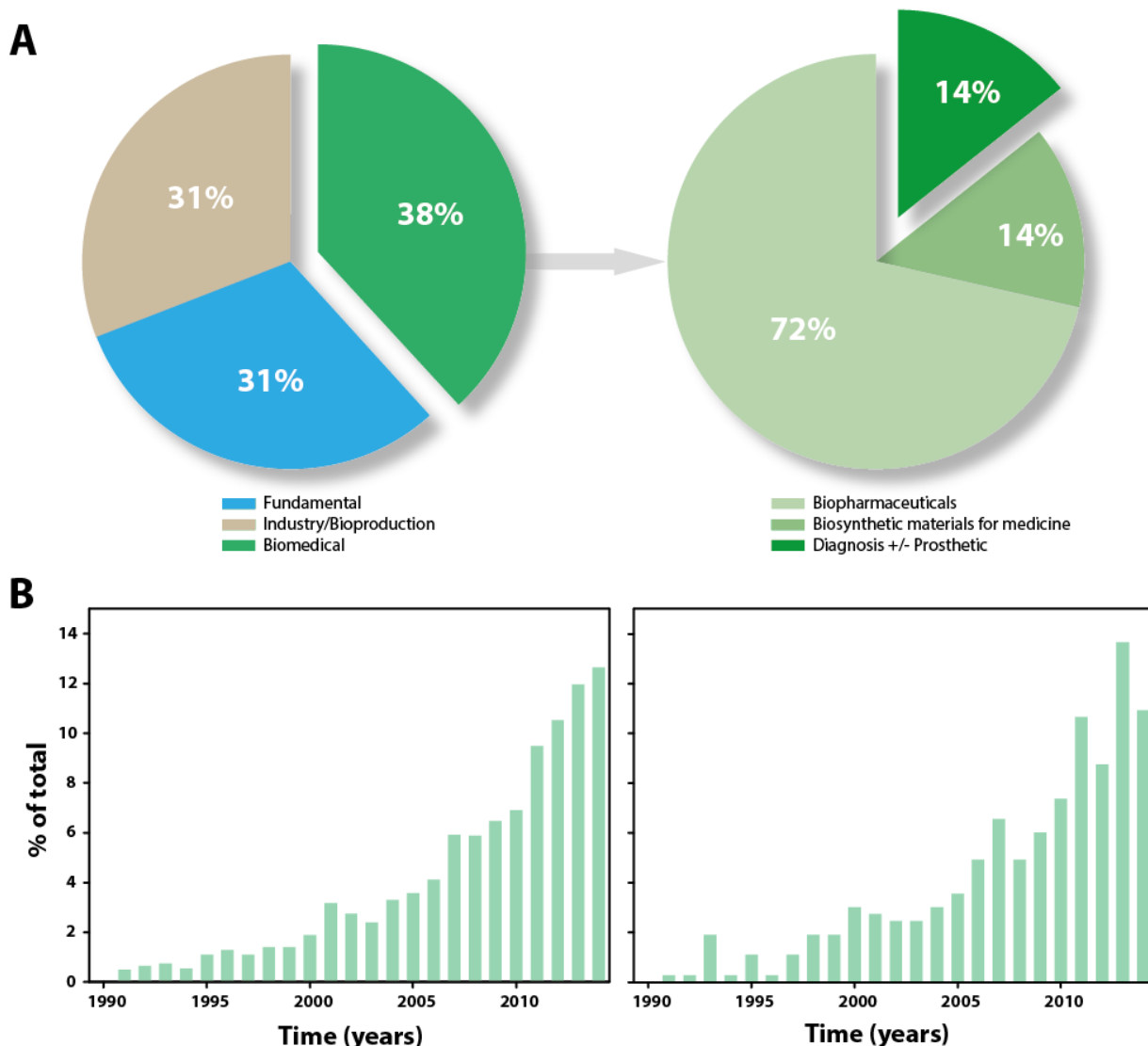


Figure 10: Synthetic biology and diagnosis: research trends. (A) We classified projects proposed to the ERASynBio call according to their fields of applications. Amongst 55 total projects selected for review, 21 were dedicated to the biomedical field. Amongst these 21 projects, 3 were focusing on medical diagnosis. (B) Evolution of research trends from the analysis of scientific publication contents. Left: bioengineering AND diagnosis, Right: synthetic biology AND diagnosis (Data was obtained through Web of Science)

In addition, recent new actors are investing the medical synthetic biology landscape. For instance the global company Google recently proposed a proof-of-concept technology relying on autonomous nanoparticles administered in systemic circulation to obtain personalized diagnostic information in real time⁴⁹⁹. Although appealing and still in its infancy, before becoming medical reality this technology needs to face societal and biological hurdles, but most of all, medical, toxicological and pharmacological uncertainties. Space exploration is also a stimulating field for medical synthetic biology. Government agencies such as NASA and ESA are looking for innovative biotechnological solutions to health monitoring, where very stringent space environments require autonomous, intelligent and integrated diagnostic systems with wireless distant monitoring, or implantable

theranostic functions^{500 501 502 503}. Last but not least, it is worth noticing the recent emergence of non-professional synthetic biology communities, such as DIY biology groups⁵⁰⁴. Although at modest embryonic stage, they have already produced proves of concept for local and cheap personalized medical diagnostic solutions, such as an open-source PCR diagnostic system tailored for fast and cheap malaria diagnosis⁵⁰⁵. Considering its increasing impact and possibilities of action, DIY synthetic biology may constitute in the future a significant source of innovation for global health.

Diagnostic devices with such novel capacities, even non-implantable, generate ethics concerns to take into consideration, namely security and data privacy⁵⁰⁶. Monitoring and recording data corresponding to health and medical parameters using decentralized synthetic biosensors that make autonomous decision, firstly involves medical confidentiality, and secondly clinical responsibility. Thus, there is a need to define boundaries for efficient patient protection from exploitation of meaningful physiological data by non-professionals, or in inadequate environment. Novel methods should preserve privacy and comfort of patients while ensuring reliable, secure and functional diagnostics to be made. We propose that minimal security requirements need to be formulated and integrated in early design phases. In addition, such emerging technologies displace established moral norms, and are likely to bring new issues to surface in the future, that will require extensive public debate⁵⁰⁷. While Synthetic biology is breaking established frontiers that were traditionally used for the governance of biotechnological research (e.g. medical, scientific and geopolitical authority and expertise regions), full transition into global health still requires global governance⁵⁰⁸.

These development costs, safety consideration and regulatory issues, combined with a few unsuccessful attempts to transition to the medical field, have often prevented synthetic biologist to tackle clinical problems. We envision that the true power of synthetic biology lies in the decoupling of the development of specific biodetection for targeted pathologies from the design of modular synthetic proteins, nucleic acids, cellular or cell-like devices could solve this challenge. Components optimized at different hierarchical layers could be systematically approved separately before assembly to speed up prototyping, by that mean easing the regulatory process, satisfying safety concerns, and lowering costs. Moreover, as the applications of synthetic systems in clinical medicine are ever more prominent we envision that the specific relevance and impact towards medicine will be realized through the bridging of biological devices like engineered cells, nucleic acids and proteins with non-biological materials such as nanomaterials.

Synthetic biology has grown and advanced enormously in the past few years. Robust methods now allow the assembly of engineered modular molecular and cellular devices with biosensing and information-processing capabilities. Researchers now begin to transition the engineering framework into the medical field, to ultimately realize intelligent, autonomous, programmable biosensors. We suspect that these advances are likely to announce a change of paradigm in diagnostics like the one next-generation sequencing technologies or antibodies in the development of immunosensors brought to medical diagnosis. The prospect for more complex synthetic devices to act as self-contained diagnostics is now established, and could evolve toward multipurpose nano-enabled implantable system for *in vivo* theranostics⁵⁰⁹. We envision that in the future biomolecular networks will make real-time, precise decisions to lead to enhanced health care. Indeed, as synthetic biology devices become ever more sophisticated and reliable, there will likely come a point at which diagnostic assays, methods, and platforms begin to be adapted to medical diagnosis rather than the other way around. A future in which medical synthetic biologists help to establish newer generations of analytical biological “hardware” synthetic devices, to improve clinical practice.

Selection of recent advances in synthetic biology of interest to medical diagnosis

•, ••, ••• indicate increasing study importance and medical relevance

Designation /Importance	Technology and approach	Input biomarkers	Output/Readout	Device format	Targeted pathology/indication	Clinics /Lab	Ref
VERSANT HCV, HBV, HIV-1 RNA 3.0 Assay (bDNA) •••	Target RNA is detected via hybridisation using a series of capture probes. The target RNA then complexes with a fluorescent label probe through a series of hybridisation events involving the target probe, preamplifier and amplifier nucleic acids. The use of non-canonical nucleosides in the amplifier, preamplifier and label probe increases the specificity of the assay, as non-target DNA present in the sample cannot nonspecifically hybridise with the artificial DNA.	Nucleic acids	Fluorescence	Microplate	Detect HCV in infected patients, measurement of HCV, HBV and HIV-1 viral loads during and after antiviral therapy	Clinics	510 511 245
Bactosensors as a programmable platform for cell-based diagnostics •••	Bacterial biosensors with genetically encoded digital amplifying genetic switches can detect clinically relevant biomarkers in human urine and serum. They perform signal digitization and amplification, multiplexed signal processing with the use of Boolean logic gates, and data storage. We also provide a framework to quantify robustness in clinical samples and a method for easily reprogramming the sensor module for distinct medical detection agendas. First demonstration that bactosensors can be used to detect pathological signals.	Any molecular signal sensed by bacteria	Fluorescent, colorimetric	Polymer beads	Diabetes	Lab	115
Programmable probiotics for detection of cancer in urine •••	First example of orally administered diagnostic <i>in vivo</i> that can noninvasively indicate the presence of cancerogenesis by producing easily detectable signals in urine. No deleterious health effects on the mice bearing engineered bacteria where detected. They demonstrate that probiotics can be programmed to safely and selectively deliver synthetic gene circuits to diseased tissue microenvironments <i>in vivo</i> .		LacZ reporter in urines, colorimetric	Orally administered probiotic	Liver cancer	Lab (mice)	128
Intelligent Logic via aptasensors based Biofuel cells ••	First example of controlled power release of biofuel cells by aptamer-based biochemical signals processed according to the Boolean logic operations, to generate self-powered smart medical diagnostics “programmed” into biocomputing systems.	Thrombin and lysozyme	Electrochemical	On-Chip (microfluidics)	Proof of concept	Lab	213
"Sense-Act-treat" Biofuel cell ••	Self-powered biocomputing logic-controlled intelligent integrated “Sense-Act-Treat” system based on a BFC	Lactic acid and lactate dehydrogenase (LDH)	Release of therapeutic drug (acetaminophen)		Abdominal Trauma	Lab	395
Boolean-format biocatalytic processing of enzyme biomarkers ••	Enzymatically-processed biochemical information presented in the form of a NAND truth table allowed for high-fidelity discrimination between normal (physiological) and abnormal (pathological)	Creatine kinase (CK) and lactate dehydrogenase (LDH)	Electrochemical	Point of care	soft tissue injury	Lab	512
Biocomputing enzyme logic system •	Biocomputing system composed of a combination of enzyme logic gates designed to process biochemical information related to pathophysiological conditions originating from various injuries.	lactate, norepinephrine and glucose	Optical and electrochemical		traumatic brain injury and hemorrhagic shock	Lab	513
Cell-based allergy profiler •••	Mammalian cell-based biosensors that scores the allergen-triggered release of histamine from whole-blood-derived human basophils. A synthetic signalling cascade engineered within the allergy profiler rewires histamine input to the production of reporter protein, thereby integrating histamine levels in whole-blood samples.	various allergens	Fluorescence /enzyme assay	Liquid phase	Allergic disorders	Lab	145
Programmed engineered genetic circuit in cells that respond to biological signals •	Modular design strategy to create Escherichia coli strains where a genetic toggle switch is interfaced with: (i) the SOS signaling pathway responding to DNA damage, and (ii) a transgenic quorum sensing signaling pathway from Vibrio fischeri.	DNA damage, QS molecules (AHL)	Fluorescent protein (GFP)		Proof-of-concept	Lab	70
Delivery of exogenous synthetic agents for noninvasive disease monitoring •••	Low-cost, non-invasive method that relies on nanoscale agents that are administered to reveal the presence of diseased tissues by producing a biomarker via proteolytic release, in the urine that can be detected using different methods	Synthetic biomarkers	Molecular signatures of biomarkers in blood and urine, readable my MS, single molecule array, or lateral flow assay		Cardiovascular diseases, liver fibrosis and cancer	Lab (mice)	60 61 62
Programmable autonomous biomolecular computing device •••	Context-sensing mechanism of a biomolecular automaton that can simultaneously sense different types of molecules	mRNAs, miRNAs, proteins, and small molecules	nucleotide quantification using PAGE analysis		Proof-of-concept	Lab	236
Paper Strip cell-base biosensors for detection of QS signals ••	Development of a fast, inexpensive, and portable filter-paper-based strip biosensor for the detection of bacterial quorum sensing signaling molecules, N-acylhomoserine lactones from Gram- mathogens in physiological samples.	AHLs	β-Galactosidase reporter: visual monitoring of a colorimetric signal	Paper strip	Gram- bacterial infetious diseases	Lab	128
Luciferase-based indicators of drugs (LUCIDs) •••	Semisynthetic bioluminescent protein sensors approach proposed as an entirely new mechanism for inexpensive point-of-care biosensors. That permits quantification of specific drugs in patient’s samples by spotting minimal volumes on paper and recording the signal using a simple point-and-shoot camera.	MTX, Tacrolimus, Sirolimus, cyclosporin, topiramate, digoxin	Luminescence signal recorded by a digital camera	Paper strip	Companion diagnostics	Lab	295

Designation /Importance	Technology and approach	Input biomarkers	Output/Readout	Device format	Targeted pathology/indication	Clinics /Lab	Ref
Paper-Based Synthetic Gene Networks ● ● ●	Toehold RNA switches biosensors, in vitro paper-based platform that provides an alternate, versatile venue for synthetic biologists to operate and a much-needed medium for the safe deployment of engineered gene circuits beyond the lab. Commercially available cell-free systems are freeze dried onto paper, enabling the inexpensive, sterile, and abiotic distribution of synthetic-biology-based technologies for the clinic, global health, industry, research, and education.	diverse small molecules analytes (glucose), nucleic acids (mRNA)	Colorimetric human readable signal	Paper strip	Wide range of pathologies, proof-of-concept for Ebola virus diagnosis	Lab	243
Conditionally fluorescent dsDNA probe ●	Double-stranded toehold exchange: novel programmable mechanism in which each single nucleotide polymorphism generates two thermodynamically destabilizing mismatch bubbles rather than the single mismatch formed during typical hybridization-based assays. Up to a 12,000-fold excess of a target that contains a single nucleotide polymorphism is required to generate the same fluorescence as one equivalent of the intended target, and detection works reliably over a wide range of conditions.	Small variations in nucleic acid sequences and point mutations	fluorescence		SNP, proof of concept with bacterial antibiotic resistance genes	Lab	228
Logic gates that respond to the presence of both protein and DNA in a sample ●	Microarray sensor technology with logic capability for screening combinations of proteins and DNA in a biological sample.	combinations of proteins and DNA	fluorescence		Chronic obstructive pulmonary disease (COPD)	Lab	379
Bacteriophage-based microbial diagnostics ● ● ●	Engineering bacteriophages as near-real time microbial diagnostics by using them to transform target specific viable bacteria into factories for detectable molecules	bacterial pathogens	fluorescence, luminescence, colorimetric signals, phage/protein amplification	Cultivation in complex clinical sample	Detection of B. anthracis, Y. pestis, M. tuberculosis, S. aureus, L monocytogenes, Salmonella, E coli, and antibiotic susceptibility	Clinics	160 181 187 161
Boolean gated antibodies for logic detection ● ●	Site-specific, chemical phosphorylation of a recognition domain creates boolean ‘gated’ antibodies. Binding is induced in an enzyme AND-antigen dependent manner. This ‘AND-Ab’ is active only in the presence of two biomarker inputs. Bivalent antibody–DNA conjugates as generic, noncovalent, and easily applicable molecular locks that allow the logic gated control of antibody activity using toehold-mediated strand displacement reactions.	Cell surface antigen and secreted enzyme, any epitope	Fluorescent/colorimetric output	Liquid phase	Immunoassays	Lab	263 380
Bacterial Quorum sensing biosensors for the clinics ● ●	Bacterial biosensing systems to evaluate QSMs in physiological samples (stool, saliva) of patients	QS molecules	Bioluminescence/colorimetric	Paper based	Inflammatory bowel disease, Ulcerative colitis, Crohn's disease	Lab	128 514
Microbial biosensor for <i>in vitro</i> pretreatment assessment of Cytarabine efficacy in leukemia ● ●	Microbial cell-based biosensor for the fast, in vitro prediction of leukemic cells response to the anticancerous drug Ara-C (cytosine arabinoside)	Ara-C	Bioluminescence	Liquid phase	Leukemia	Lab	515
Bacterial biosensing system to monitor Methyl mercury poisoning ●	Bacterial biosensing system that can rapidly detect bioavailable MeHg	MeHg	Bioluminescence	Liquid phase	Methylmercury poisoning	Lab	516
Engineered virus nanoparticles based immunoassays ● ●	The authors demonstrate that by combining viral nanoparticles, which are engineered to have dual affinity for troponin antibodies and nickel, with three-dimensional nanostructures they could detect troponin levels in human serum samples that are six to seven orders of magnitude lower than those detectable using conventional enzyme linked immunosorbent assays. The viral nanoparticle helps to orient the antibodies for maximum capture of biomarkers. High densities of antibodies on the surfaces of the nanoparticles lead to greater binding of the biomarkers, which enhances detection sensitivities.	Troponin I	Fluorescent, luminescent, electrochemical, enzymatic and colorimetric signals	Liquid phase	Acute myocardial infarction	Lab	196 197
Spore-based genetically engineered whole-cell sensing systems ● ●	Incorporated spore-based whole-cell sensing systems into	Zinc and arsenite	fluorescence, luminescence	miniaturized microfluidic format (μTAS)	Measurements of seric zinc and arsenite levels	Lab	125
Nucleic Acid Circuits ● ● ●	Toehold mediated strand displacement mechanism alone have permitted to develop novel enzyme free nucleic acid amplification circuits for different diagnostic detection strategies, such as entropy-driven catalysis (EDC) circuits, seesaw gates, catalytic hairpin assembly (CHA) reactions and hybridization chain reactions (HCR)	Wide rand of analytes	fluorescent, luminescent, electrochemical, enzymatic and colorimetric signals	Liquid or solid phase		Lab	229
Logic-Based Autonomous cell surface profiling ● ●	Cell types, both healthy and diseased, can be classified by inventories of their cell-surface markers using aptamers or antibodies. DNA nanorobots for programmable analysis of multiple surface markers to enable the clinical disease profile on whole cells. They engineered a device combining structure-switching DNA aptamers, or antibodies coupled with DNA devices with toehold-mediated strand displacement reactions to perform autonomous logic-based analysis of cell-surface markers.	Cell surface markers, Cluster of differentiation (CDs)	Fluorescence/targeted therapeutics	Liquid phase	human cancer cell models	Lab	239 240

Designation /Importance	Technology and approach	Input biomarkers	Output/Readout	Device format	Targeted pathology/indication	Clinics /Lab	Ref
Bioolecular logic gates that detect MDR bacteria ●●	Biochemical reaction networks exploiting enzymes and oligonucleotides with a computing functionality applied to the identification of bacteria exhibiting multi-drug resistance. This approach enables to identify the NDM-1-encoding gene (blaNDM-1) and concurrently to screen, by a tailor-designed biomolecular logical gate, two genetic fragments encoding the active sites bound to carbapenem.	Nucleic acids related to antibiotic resistance (NDM-1)	Electrochemical	Liquid phase/electrodes	MDR resistance of gram negative bacteria	Lab	381
Antibody diagnostics via evolution of peptides ●	Antibody diagnostics via evolution of peptides (ADEPt) to evolve diagnostically efficient peptides for de novo discovery and detection of antibody biomarkers without knowledge of disease pathophysiology. As pathological antibodies repertoire are known to change in diverse diseases, this methods has proven useful to create diagnostics for early disease detection, stratification, and therapeutic monitoring, and enabled effective identification of a critical environmental agent involved in celiac disease. Bacterial cell-displayed peptide libraries were quantitatively screened for binders to serum antibodies from patients with celiac disease.	Disease associated antibodies	Fluorescence	Liquid Phase	Celiac disease, theoretically many diseases	Lab	268
Synthetic genetic polymers XNA aptamers ●●	Novel synthetic nucleobases and their genetic polymers, known as XNA (xenonucleic acids) increase the chemical and structural diversity of nucleic acids, and open up the way for increased affinity and stability against enzymatic cleavage, expanded functionality such as enzymatic activity, and improved synthesis and selection procedures	PDGF, HIV RNA, Thrombin, Camptothecin, VEGF, Glucagon, IL-6, Cancerous cells	Various	Liquid phase	Various diseases	Lab	254
Prosthetic circuit to monitor and treat diet induced obesity ●●●	Mice transplanted with engineered cells bearing synthetic genetic circuit that constantly monitors blood fatty acid levels in the setting of diet-associated hyperlipidemia and coordinates reversible and adjustable expression of the clinically licensed appetite-suppressing peptide hormone.	fatty acid levels in blood	Appetite-suppressing peptide hormone Pramlintide	Microcapsule	Hyperlipidemia/Diet induced obesity	Lab	150
Biomolecular computer for diagnosis and therapy ●●●	Biomolecular computer performs in vitro the identification of a combination of cancer mRNA marker molecules at specific levels and generates a therapeutically active molecule	mRNAs	Therapeutic nucleic acid		models of small-cell lung cancer and prostate cancer	Lab	38
Bile acid-controlled prosthetic circuit ●●	Biosensor based on orthogonal synthetic gene switches that combine's bile acid-specific sensor capacity with dose-dependent expression of a specific transgene in mammalian cells and in mice.	pathological metabolites (Bile acids)	Therapeutic responses		Metabolic disorders	Lab	517
RNA control devices monitor signaling pathways and reprogram cellular fate ●	Protein-responsive RNAbased regulatory device integrating RNA aptamers that bind to disease associated protein ligands in key intronic locations of an alternatively spliced transcript linking intracellular protein concentrations to gene-expression events, and triggering apoptosis	Wnt and NF-kB pathway	Targeted apoptosis	intracellular RNA device	Cancer	Lab	144
Multi-input cancer cell classifier ●●●	Scalable synthetic genetic circuit works as a cell type classifier <i>in cellulo</i> by detecting customizable sets of endogenous pathological miRNAs and triggers apoptosis in HeLa cells	cancer specific endogenous miRNAs patterns	Apoptosis of cancer cells	intracellular genetic circuits	Cancer	Lab	152
Genetically Programmable platform to detect pathogens and trigger destruction ●	Proof-of-principle towards detection of <i>Pseudomonas aeruginosa</i> using quorum sensing signals and in situ destruction by an engineering E. coli secreting an engineered specific bacteriocin.	<i>P. aeruginosa</i> QS molecules (3OC12HSL)	Secretion of CoPy bacteriocin	in situ	Urinary tract and nosocomial infections	Lab	518
E. coli engineered into living diagnostics to probe the mammalian gut. ●●●	Engineered E coli that survive in mice gut gut and sense, remember, and report molecular signals thanks to a genetic circuits with a “trigger element” in which the lambda Cro gene is transcribed from a tetracycline-inducible promoter and a “memory element” derived from the cI/Cro region of phage lambda.	aTc	β-galactosidase reporter	Orally administered engineered bacterium, probiotic?	Proof-of-concept	Lab (mice)	19
Synthetic uric acid–responsive mammalian sensor circuit ●●●	Synthetic mammalian circuit to maintain uric acid homeostasis in the bloodstream. Modified <i>Deinococcus radiodurans</i> -derived protein that senses uric acids levels and triggers dose-dependent derepression of a secretion-engineered <i>Aspergillus flavus</i> urate oxidase that eliminates uric acid <i>in vivo</i> in mice	Uric acid	urate oxidase enzyme	Intraperitoneous implantation of microcapsules containing engineered cells	Proof-of-concept	Lab (mice)	148
Multifunctional Mammalian pH Sensor ●●●	The authors rewired the human proton-activated cell-surface receptor TDAG8 to chimeric promoters, creating a synthetic signaling cascade that monitors extracellular pH within the physiological range. The synthetic pH sensor was linked to production of insulin and implanted into type 1 diabetic mice developing diabetic ketoacidosis, creating a prosthetic network capable of automatically scoring acidic pH and coordinating an insulin expression response that corrected ketoacidosis.	pH, CO2	Fluorescence/ Insulin	Intraperitoneous implantation of microcapsules containing engineered cells	Proof-of-concept	Lab (mice)	140
Synthetic gene networks that detect bladder cancer cells ●●●	Synthetic gene network build using CRISPR-Cas9 technology in mammalian cells, that integrate cellular pathophysiological information from two cancer specific promoters as inputs and activate an output gene following a AND Boolean operation. When using a luciferase output, the authors could detect bladder cancer cells. The authors could also induce cell death using functional genes as outputs.	cancer specific intracellular transcriptional signals (human telomerase reverse transcriptase, human uroplakin II)	Luminescence, apoptosis	Intracellular gene circuits	Proof-of-concept	Lab	153
Protein switches that detect cancer and treats	The authors propose a strategy for designing protein therapeutics that link activation of a chosen therapeutic function to a specific cancer marker of choice. We demonstrate this strategy by creating a protein switch that renders cells susceptible to the in response to the cancer marker.	hypoxia-inducible factor 1α (HIF-1α)	Activation of the prodrug 5-fluorocytosine (5FC)	Intracellular protein switch	Human colon and breast cancer	Lab	141

Bibliography

1. Hay Burgess, D. C., Wasserman, J. & Dahl, C. A. Global health diagnostics. *Nature* **444**, 1–2 (2006).
2. Peeling, R. W., Smith, P. G. & Bossuyt, P. M. M. A guide for diagnostic evaluations. *Nat. Rev. Microbiol.* **6**, S2–S6 (2008).
3. Hood, L. Systems Biology and New Technologies Enable Predictive and Preventative Medicine. *Science* **306**, 640–643 (2004).
4. Walt, D. R. CHEMISTRY: Miniature Analytical Methods for Medical Diagnostics. *Science* **308**, 217–219 (2005).
5. Gubala, V., Harris, L. F., Ricco, A. J., Tan, M. X. & Williams, D. E. Point of Care Diagnostics: Status and Future. *Anal. Chem.* **84**, 487–515 (2012).
6. Yager, P., Domingo, G. J. & Gerdes, J. Point-of-Care Diagnostics for Global Health. *Annu. Rev. Biomed. Eng.* **10**, 107–144 (2008).
7. Lisowski, P. & Zarzycki, P. K. Microfluidic Paper-Based Analytical Devices (μ PADs) and Micro Total Analysis Systems (μ TAS): Development, Applications and Future Trends. *Chromatographia* **76**, 1201–1214 (2013).
8. Turner, A. P. F. Biosensors: sense and sensibility. *Chem. Soc. Rev.* **42**, 3184 (2013).
9. Giljohann, D. A. & Mirkin, C. A. Drivers of biodiagnostic development. *Nature* **462**, 461–464 (2009).
10. Weber, W. & Fussenegger, M. Emerging biomedical applications of synthetic biology. *Nat. Rev. Genet.* (2011). doi:10.1038/nrg3094
11. Arkin, A. P. & Schaffer, D. V. Network News: Innovations in 21st Century Systems Biology. *Cell* **144**, 844–849 (2011).
12. Smolke, C. D. & Silver, P. A. Informing Biological Design by Integration of Systems and Synthetic Biology. *Cell* **144**, 855–859 (2011).
13. Jasný, B. R. & Zahn, L. M. A Celebration of the Genome, Part I. *Science* **331**, 546–546 (2011).
14. Purnick, P. E. M. & Weiss, R. The second wave of synthetic biology: from modules to systems. *Nat. Rev. Mol. Cell Biol.* **10**, 410–422 (2009).
15. Canton, B., Labno, A. & Endy, D. Refinement and standardization of synthetic biological parts and devices. *Nat. Biotechnol.* **26**, 787–793 (2008).
16. Endy, D. Foundations for engineering biology. *Nature* **438**, 449–453 (2005).
17. Keret, O. Biomedical synthetic biology: an overview for physicians. *Isr. Med. Assoc. J. IMAJ* **15**, 308–312 (2013).
18. Benenson, Y. Biomolecular computing systems: principles, progress and potential. *Nat. Rev. Genet.* **13**, 455–468 (2012).
19. Kotula, J. W. *et al.* Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc. Natl. Acad. Sci.* **111**, 4838–4843 (2014).
20. Thaker, M. N. *et al.* Identifying producers of antibacterial compounds by screening for antibiotic resistance. *Nat. Biotechnol.* **31**, 922–927 (2013).
21. DeLoache, W. C. *et al.* An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. *Nat. Chem. Biol.* (2015). doi:10.1038/nchembio.1816
22. Bryksin, A. V., Brown, A. C., Baksh, M. M., Finn, M. G. & Barker, T. H. Learning from nature – Novel synthetic biology approaches for biomaterial design. *Acta Biomater.* **10**, 1761–1769 (2014).
23. Xie, M. & Fussenegger, M. Mammalian designer cells: Engineering principles and biomedical applications. *Biotechnol. J.* n/a–n/a (2015). doi:10.1002/biot.201400642
24. Ruder, W. C., Lu, T. & Collins, J. J. Synthetic Biology Moving into the Clinic. *Science* **333**, 1248–1252 (2011).
25. Nielsen, J. *et al.* Engineering synergy in biotechnology. *Nat. Chem. Biol.* **10**, 319–322 (2014).

26. Kis, Z., Pereira, H. S., Homma, T., Pedrigi, R. M. & Krams, R. Mammalian synthetic biology: emerging medical applications. *J. R. Soc. Interface* **12**, 20141000–20141000 (2015).
27. Heng, B. C. & Fussenegger, M. in *Encyclopedia of Molecular Cell Biology and Molecular Medicine* (ed. Meyers, R. A.) 1–17 (Wiley-VCH Verlag GmbH & Co. KGaA, 2014). at <<http://doi.wiley.com/10.1002/3527600906.mcb.20120067>>
28. Chen, Y. Y. & Smolke, C. D. From DNA to Targeted Therapeutics: Bringing Synthetic Biology to the Clinic. *Sci. Transl. Med.* **3**, 106ps42–106ps42 (2011).
29. in *IUPAC Compendium of Chemical Terminology* (eds. Nič, M., Jirát, J., Košata, B., Jenkins, A. & McNaught, A.) (IUPAC, 2009). at <<http://goldbook.iupac.org/B00663.html>>
30. Ferrell, J. E. & Ha, S. H. Ultrasensitivity part III: cascades, bistable switches, and oscillators. *Trends Biochem. Sci.* **39**, 612–618 (2014).
31. Monod, J. & Jacob, F. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harb. Symp. Quant. Biol.* **26**, 389–401 (1961).
32. Ausländer, S. & Fussenegger, M. From gene switches to mammalian designer cells: present and future prospects. *Trends Biotechnol.* **31**, 155–168 (2013).
33. Regev, A. & Shapiro, E. Cellular abstractions: Cells as computation. *Nature* **419**, 343–343 (2002).
34. Haynes, K. A. *et al.* Engineering bacteria to solve the Burnt Pancake Problem. *J. Biol. Eng.* **2**, 8 (2008).
35. Benenson, Y. *et al.* Programmable and autonomous computing machine made of biomolecules. *Nature* **414**, 430–434 (2001).
36. Ausländer, S., Ausländer, D., Müller, M., Wieland, M. & Fussenegger, M. Programmable single-cell mammalian biocomputers. *Nature* (2012). doi:10.1038/nature11149
37. *Biomolecular information processing: from logic systems to smart sensors and actuators*. (Wiley-VCH, 2012).
38. Benenson, Y., Gil, B., Ben-Dor, U., Adar, R. & Shapiro, E. An autonomous molecular computer for logical control of gene expression. *Nature* **429**, 423–429 (2004).
39. Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R. & Benenson, Y. Multi-Input RNAi-Based Logic Circuit for Identification of Specific Cancer Cells. *Science* **333**, 1307–1311 (2011).
40. *Fundamentals of clinical practice*. (Kluwer Academic/Plenum Publishers, 2002).
41. Ledley, R. S. & Lusted, L. B. Reasoning Foundations of Medical Diagnosis: Symbolic logic, probability, and value theory aid our understanding of how physicians reason. *Science* **130**, 9–21 (1959).
42. Elowitz, M. & Lim, W. A. Build life to understand it. *Nature* **468**, 889–890 (2010).
43. Bashor, C. J., Horwitz, A. A., Peisajovich, S. G. & Lim, W. A. Rewiring Cells: Synthetic Biology as a Tool to Interrogate the Organizational Principles of Living Systems. *Annu. Rev. Biophys.* **39**, 515–537 (2010).
44. Quo, C. F. *et al.* Reverse engineering biomolecular systems using -omic data: challenges, progress and opportunities. *Brief. Bioinform.* **13**, 430–445 (2012).
45. Yagi, H. *et al.* A Synthetic Biology Approach Reveals a CXCR4-G13-Rho Signaling Axis Driving Transendothelial Migration of Metastatic Breast Cancer Cells. *Sci. Signal.* **4**, ra60–ra60 (2011).
46. Tumpey, T. M. *et al.* Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* **310**, 77–80 (2005).
47. Becker, M. M. *et al.* Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. *Proc. Natl. Acad. Sci.* **105**, 19944–19949 (2008).
48. Gibson, D. G. *et al.* Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. *Science* **329**, 52–56 (2010).
49. Cello, J., Paul, A. V. & Wimmer, E. Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science* **297**, 1016–1018 (2002).
50. Berthet, N. *et al.* Reconstructed Ancestral Sequences Improve Pathogen Identification Using Resequencing DNA Microarrays. *PLoS ONE* **5**, e15243 (2010).
51. Burbelo, P. D., Ching, K. H., Bush, E. R., Han, B. L. & Iadarola, M. J. Antibody-profiling technologies for studying humoral responses to infectious agents. *Expert Rev. Vaccines* **9**, 567–578 (2010).

52. Burbelo, P. D. *et al.* LIPS arrays for simultaneous detection of antibodies against partial and whole proteomes of HCV, HIV and EBV. *Mol. Biosyst.* **7**, 1453 (2011).
53. Feng, H., Shuda, M., Chang, Y. & Moore, P. S. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* **319**, 1096–1100 (2008).
54. Burbelo, P. D., Ching, K. H., Bush, E. R., Han, B. L. & Iadarola, M. J. Antibody-profiling technologies for studying humoral responses to infectious agents. *Expert Rev. Vaccines* **9**, 567–578 (2010).
55. Gómara, M. J. & Haro, I. Synthetic peptides for the immunodiagnosis of human diseases. *Curr. Med. Chem.* **14**, 531–546 (2007).
56. Burbelo, P. D. *et al.* Rapid, Simple, Quantitative, and Highly Sensitive Antibody Detection for Lyme Disease. *Clin. Vaccine Immunol.* **17**, 904–909 (2010).
57. Meloen, R. H., Puijk, W. C., Langeveld, J. P. M., Langedijk, J. P. M. & Timmerman, P. Design of synthetic peptides for diagnostics. *Curr. Protein Pept. Sci.* **4**, 253–260 (2003).
58. Larman, H. B. *et al.* Autoantigen discovery with a synthetic human peptidome. *Nat. Biotechnol.* **29**, 535–541 (2011).
59. Xu, G. J. *et al.* Comprehensive serological profiling of human populations using a synthetic human virome. *Science* **348**, aaa0698–aaa0698 (2015).
60. Warren, A. D., Kwong, G. A., Wood, D. K., Lin, K. Y. & Bhatia, S. N. Point-of-care diagnostics for noncommunicable diseases using synthetic urinary biomarkers and paper microfluidics. *Proc. Natl. Acad. Sci.* **111**, 3671–3676 (2014).
61. Kwong, G. A. *et al.* Mass-encoded synthetic biomarkers for multiplexed urinary monitoring of disease. *Nat. Biotechnol.* **31**, 63–70 (2013).
62. Warren, A. D. *et al.* Disease detection by ultrasensitive quantification of microdosed synthetic urinary biomarkers. *J. Am. Chem. Soc.* **136**, 13709–13714 (2014).
63. Lin, K. Y., Kwong, G. A., Warren, A. D., Wood, D. K. & Bhatia, S. N. Nanoparticles That Sense Thrombin Activity As Synthetic Urinary Biomarkers of Thrombosis. *ACS Nano* **7**, 9001–9009 (2013).
64. Salis, H., Tamsir, A. & Voigt, C. Engineering bacterial signals and sensors. *Contrib. Microbiol.* **16**, 194–225 (2009).
65. Raut, N., O'Connor, G., Pasini, P. & Daunert, S. Engineered cells as biosensing systems in biomedical analysis. *Anal. Bioanal. Chem.* **402**, 3147–3159 (2012).
66. Marchisio, M. A. & Rudolf, F. Synthetic biosensing systems. *Int. J. Biochem. Cell Biol.* **43**, 310–319 (2011).
67. Yagi, K. Applications of whole-cell bacterial sensors in biotechnology and environmental science. *Appl. Microbiol. Biotechnol.* **73**, 1251–1258 (2007).
68. Liu, Q. *et al.* Cell-Based Biosensors and Their Application in Biomedicine. *Chem. Rev.* **114**, 6423–6461 (2014).
69. Michelini, E. *et al.* Field-deployable whole-cell bioluminescent biosensors: so near and yet so far. *Anal. Bioanal. Chem.* **405**, 6155–6163 (2013).
70. Kobayashi, H. *et al.* Programmable cells: interfacing natural and engineered gene networks. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 8414–8419 (2004).
71. *The science and applications of synthetic and systems biology: workshop summary.* (National Academies Press, 2011).
72. Van der Meer, J. R. & Belkin, S. Where microbiology meets microengineering: design and applications of reporter bacteria. *Nat. Rev. Microbiol.* **8**, 511–522 (2010).
73. Park, M., Tsai, S.-L. & Chen, W. Microbial Biosensors: Engineered Microorganisms as the Sensing Machinery. *Sensors* **13**, 5777–5795 (2013).
74. Wang, B., Barahona, M. & Buck, M. A modular cell-based biosensor using engineered genetic logic circuits to detect and integrate multiple environmental signals. *Biosens. Bioelectron.* **40**, 368–376 (2013).
75. Checa, S. K., Zurbriggen, M. D. & Soncini, F. C. Bacterial signaling systems as platforms for rational design of new generations of biosensors. *Curr. Opin. Biotechnol.* **23**, 766–772 (2012).

76. Daunert, S. *et al.* Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. *Chem. Rev.* **100**, 2705–2738 (2000).
77. Hernandez Espitia, C. A. & Osma, J. F. in *Biosensors: Recent advances and mathematical challenges* (eds. Osma, J. F. & Stoytcheva, M.) 51–96 (OmniaScience, 2014). at <<http://omniascience.com/monographs/index.php/monograficos/issue/view/15/showToc>>
78. Campàs, M., Carpentier, R. & Rouillon, R. Plant tissue- and photosynthesis-based biosensors. *Biotechnol. Adv.* **26**, 370–378 (2008).
79. Brayner, R., Couté, A., Livage, J., Perrette, C. & Sicard, C. Micro-algal biosensors. *Anal. Bioanal. Chem.* **401**, 581–597 (2011).
80. Banerjee, P., Franz, B. & Bhunia, A. K. in *Whole Cell Sensing Systems I* (eds. Belkin, S. & Gu, M. B.) 21–55 (Springer Berlin Heidelberg, 2010). at <http://link.springer.com/10.1007/10_2009_21>
81. Walmsley, R. M. & Keenan, P. The eukaryote alternative: Advantages of using yeasts in place of bacteria in microbial biosensor development. *Biotechnol. Bioprocess Eng.* **5**, 387–394 (2000).
82. Su, L., Jia, W., Hou, C. & Lei, Y. Microbial biosensors: A review. *Biosens. Bioelectron.* **26**, 1788–1799 (2011).
83. Stenger, D. A. *et al.* Detection of physiologically active compounds using cell-based biosensors. *Trends Biotechnol.* **19**, 304–309 (2001).
84. Banerjee, P. & Bhunia, A. K. Cell-based biosensor for rapid screening of pathogens and toxins. *Biosens. Bioelectron.* **26**, 99–106 (2010).
85. Hung, P. J., Lee, P. J., Sabounchi, P., Lin, R. & Lee, L. P. Continuous perfusion microfluidic cell culture array for high-throughput cell-based assays. *Biotechnol. Bioeng.* **89**, 1–8 (2005).
86. Tani, H., Maehana, K. & Kamidate, T. Chip-based bioassay using bacterial sensor strains immobilized in three-dimensional microfluidic network. *Anal. Chem.* **76**, 6693–6697 (2004).
87. El-Ali, J., Sorger, P. K. & Jensen, K. F. Cells on chips. *Nature* **442**, 403–411 (2006).
88. Melamed, S., Elad, T. & Belkin, S. Microbial sensor cell arrays. *Curr. Opin. Biotechnol.* **23**, 2–8 (2012).
89. Ben-Yoav, H., Melamed, S., Freeman, A., Shacham-Diamand, Y. & Belkin, S. Whole-cell biochips for bio-sensing: integration of live cells and inanimate surfaces. *Crit. Rev. Biotechnol.* **31**, 337–353 (2011).
90. *Cell-based biosensors: principles and applications.* (Artech House, 2009).
91. Friedland, A. E. *et al.* Synthetic Gene Networks That Count. *Science* **324**, 1199–1202 (2009).
92. Yang, L. *et al.* Permanent genetic memory with >1-byte capacity. *Nat. Methods* **11**, 1261–1266 (2014).
93. Prindle, A. *et al.* A sensing array of radically coupled genetic ‘biopixels’. *Nature* **481**, 39–44 (2011).
94. Gast, T. Sensors with oscillating elements. *J. Phys. [E]* **18**, 783–789 (1985).
95. Turner, K. *et al.* Hydroxylated polychlorinated biphenyl detection based on a genetically engineered bioluminescent whole-cell sensing system. *Anal. Chem.* **79**, 5740–5745 (2007).
96. Lewis, C. *et al.* Novel use of a whole cell *E. coli* bioreporter as a urinary exposure biomarker. *Environ. Sci. Technol.* **43**, 423–428 (2009).
97. Horswell, J. & Dickson, S. Use of biosensors to screen urine samples for potentially toxic chemicals. *J. Anal. Toxicol.* **27**, 372–376 (2003).
98. Bahl, M. I., Hansen, L. H., Licht, T. R. & Sørensen, S. J. In vivo detection and quantification of tetracycline by use of a whole-cell biosensor in the rat intestine. *Antimicrob. Agents Chemother.* **48**, 1112–1117 (2004).
99. Hansen, L. H., Aarestrup, F. & Sørensen, S. J. Quantification of bioavailable chlortetracycline in pig feces using a bacterial whole-cell biosensor. *Vet. Microbiol.* **87**, 51–57 (2002).
100. Kurittu, J., Lönnberg, S., Virta, M. & Karp, M. Qualitative detection of tetracycline residues in milk with a luminescence-based microbial method: the effect of milk composition and assay performance in relation to an immunoassay and a microbial inhibition assay. *J. Food Prot.* **63**, 953–957 (2000).

101. Paton, G. I., Reid, B. J. & Semple, K. T. Application of a luminescence-based biosensor for assessing naphthalene biodegradation in soils from a manufactured gas plant. *Environ. Pollut.* **157**, 1643–1648 (2009).
102. Green, A. A., Silver, P. A., Collins, J. J. & Yin, P. Toehold Switches: De-Novo-Designed Regulators of Gene Expression. *Cell* **159**, 925–939 (2014).
103. Muranaka, N., Sharma, V., Nomura, Y. & Yokobayashi, Y. Efficient Design Strategy for Whole-Cell and Cell-Free Biosensors based on Engineered Riboswitches. *Anal. Lett.* **42**, 108–122 (2009).
104. Marvin, J. S., Schreiter, E. R., Echevarría, I. M. & Looger, L. L. A genetically encoded, high-signal-to-noise maltose sensor. *Proteins Struct. Funct. Bioinforma.* **79**, 3025–3036 (2011).
105. Jeffery, C. J. Engineering periplasmic ligand binding proteins as glucose nanosensors. *Nano Rev.* **2**, (2011).
106. Looger, L. L., Dwyer, M. A., Smith, J. J. & Hellinga, H. W. Computational design of receptor and sensor proteins with novel functions. *Nature* **423**, 185–190 (2003).
107. Jo, J.-J. & Shin, J.-S. Construction of intragenic synthetic riboswitches for detection of a small molecule. *Biotechnol. Lett.* **31**, 1577–1581 (2009).
108. Win, M. N. & Smolke, C. D. A modular and extensible RNA-based gene-regulatory platform for engineering cellular function. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 14283–14288 (2007).
109. Baker, J. L. *et al.* Widespread Genetic Switches and Toxicity Resistance Proteins for Fluoride. *Science* **335**, 233–235 (2012).
110. Qi, L., Lucks, J. B., Liu, C. C., Mutalik, V. K. & Arkin, A. P. Engineering naturally occurring trans-acting non-coding RNAs to sense molecular signals. *Nucleic Acids Res.* **40**, 5775–5786 (2012).
111. Lucks, J. B., Qi, L., Mutalik, V. K., Wang, D. & Arkin, A. P. Versatile RNA-sensing transcriptional regulators for engineering genetic networks. *Proc. Natl. Acad. Sci.* **108**, 8617–8622 (2011).
112. De los Santos, E. L. C., Meyerowitz, J. T., Mayo, S. L. & Murray, R. M. *Engineering Transcriptional Regulator Effector Specificity using Computational Design and In Vitro Rapid Prototyping: Developing a Vanillin Sensor.* (2015). at <http://biorxiv.org/lookup/doi/10.1101/015438>
113. Wang, B., Barahona, M. & Buck, M. Amplification of small molecule-inducible gene expression via tuning of intracellular receptor densities. *Nucleic Acids Res.* **43**, 1955–1964 (2015).
114. Alper, H., Fischer, C., Nevoigt, E. & Stephanopoulos, G. Tuning genetic control through promoter engineering. *Proc. Natl. Acad. Sci.* **102**, 12678–12683 (2005).
115. Kudla, G., Murray, A. W., Tollervey, D. & Plotkin, J. B. Coding-Sequence Determinants of Gene Expression in Escherichia coli. *Science* **324**, 255–258 (2009).
116. Courbet, A., Endy, D., Renard, E., Molina, F. & Bonnet, J. Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Sci. Transl. Med.* **7**, 289ra83–289ra83 (2015).
117. Roda, A. *et al.* SENSITIVE DETERMINATION OF URINARY MERCURY(II) BY A BIOLUMINESCENT TRANSGENIC BACTERIA-BASED BIOSENSOR. *Anal. Lett.* **34**, 29–41 (2001).
118. Skerker, J. M. *et al.* Rewiring the specificity of two-component signal transduction systems. *Cell* **133**, 1043–1054 (2008).
119. Xu, J. & Lavan, D. A. Designing artificial cells to harness the biological ion concentration gradient. *Nat. Nanotechnol.* **3**, 666–670 (2008).
120. Booth, I. R., Edwards, M. D., Black, S., Schumann, U. & Miller, S. Mechanosensitive channels in bacteria: signs of closure? *Nat. Rev. Microbiol.* **5**, 431–440 (2007).
121. Danchin, A. Scaling up synthetic biology: Do not forget the chassis. *FEBS Lett.* **586**, 2129–2137 (2012).
122. Harwood, C. R., Pohl, S., Smith, W. & Wipat, A. in *Methods in Microbiology* **40**, 87–117 (Elsevier, 2013).
123. Nikel, P. I., Martínez-García, E. & de Lorenzo, V. Biotechnological domestication of pseudomonads using synthetic biology. *Nat. Rev. Microbiol.* **12**, 368–379 (2014).
124. Knecht, L. D., Pasini, P. & Daunert, S. Bacterial spores as platforms for bioanalytical and biomedical applications. *Anal. Bioanal. Chem.* **400**, 977–989 (2011).

125. Date, A., Pasini, P. & Daunert, S. Integration of spore-based genetically engineered whole-cell sensing systems into portable centrifugal microfluidic platforms. *Anal. Bioanal. Chem.* **398**, 349–356 (2010).
126. Rotman, B. & Cote, M. A. Application of a real-time biosensor to detect bacteria in platelet concentrates. *Biochem. Biophys. Res. Commun.* **300**, 197–200 (2003).
127. Michelini, E. & Roda, A. Staying alive: new perspectives on cell immobilization for biosensing purposes. *Anal. Bioanal. Chem.* **402**, 1785–1797 (2012).
128. Struss, A., Pasini, P., Ensor, C. M., Raut, N. & Daunert, S. Paper strip whole cell biosensors: a portable test for the semiquantitative detection of bacterial quorum signaling molecules. *Anal. Chem.* **82**, 4457–4463 (2010).
129. Danino, T. *et al.* Programmable probiotics for detection of cancer in urine. *Sci. Transl. Med.* **7**, 289ra84–289ra84 (2015).
130. Chen, A. Y. *et al.* Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* **13**, 515–523 (2014).
131. Bacchus, W., Aubel, D. & Fussenegger, M. Biomedically relevant circuit-design strategies in mammalian synthetic biology. *Mol. Syst. Biol.* **9**, 691–691 (2014).
132. Lienert, F., Lohmueller, J. J., Garg, A. & Silver, P. A. Synthetic biology in mammalian cells: next generation research tools and therapeutics. *Nat. Rev. Mol. Cell Biol.* **15**, 95–107 (2014).
133. Stanton, B. C. *et al.* Systematic Transfer of Prokaryotic Sensors and Circuits to Mammalian Cells. *ACS Synth. Biol.* **3**, 880–891 (2014).
134. Daringer, N. M., Dudek, R. M., Schwarz, K. A. & Leonard, J. N. Modular Extracellular Sensor Architecture for Engineering Mammalian Cell-based Devices. *ACS Synth. Biol.* **3**, 892–902 (2014).
135. Fung, E. *et al.* A synthetic gene-metabolic oscillator. *Nature* **435**, 118–122 (2005).
136. Weber, W., Lienhart, C., Daoud-El Baba, M. & Fussenegger, M. A biotin-triggered genetic switch in mammalian cells and mice. *Metab. Eng.* **11**, 117–124 (2009).
137. Weber, W., Bacchus, W., Daoud-El Baba, M. & Fussenegger, M. Vitamin H-regulated transgene expression in mammalian cells. *Nucleic Acids Res.* **35**, e116 (2007).
138. Weber, W., Daoud-El Baba, M. & Fussenegger, M. Synthetic ecosystems based on airborne inter- and intrakingdom communication. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 10435–10440 (2007).
139. Wang, W.-D., Chen, Z.-T., Kang, B.-G. & Li, R. Construction of an artificial intercellular communication network using the nitric oxide signaling elements in mammalian cells. *Exp. Cell Res.* **314**, 699–706 (2008).
140. Ausländer, D. *et al.* A synthetic multifunctional mammalian pH sensor and CO₂ transgene-control device. *Mol. Cell* **55**, 397–408 (2014).
141. Wright, C. M., Wright, R. C., Eshleman, J. R. & Ostermeier, M. A protein therapeutic modality founded on molecular regulation. *Proc. Natl. Acad. Sci.* **108**, 16206–16211 (2011).
142. Chang, A. L., Wolf, J. J. & Smolke, C. D. Synthetic RNA switches as a tool for temporal and spatial control over gene expression. *Curr. Opin. Biotechnol.* **23**, 679–688 (2012).
143. Ellington, A. D. & Szostak, J. W. In vitro selection of RNA molecules that bind specific ligands. *Nature* **346**, 818–822 (1990).
144. Culler, S. J., Hoff, K. G. & Smolke, C. D. Reprogramming Cellular Behavior with RNA Controllers Responsive to Endogenous Proteins. *Science* **330**, 1251–1255 (2010).
145. Ausländer, D. *et al.* A designer cell-based histamine-specific human allergy profiler. *Nat. Commun.* **5**, (2014).
146. Cavalli, G. & Misteli, T. Functional implications of genome topology. *Nat. Struct. Mol. Biol.* **20**, 290–299 (2013).
147. Lee, T. I. & Young, R. A. Transcriptional regulation and its misregulation in disease. *Cell* **152**, 1237–1251 (2013).
148. Kemmer, C. *et al.* Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat. Biotechnol.* **28**, 355–360 (2010).

149. Han, J., McLane, B., Kim, E.-H., Yoon, J.-W. & Jun, H.-S. Remission of Diabetes by Insulin Gene Therapy Using a Hepatocyte-specific and Glucose-responsive Synthetic Promoter. *Mol. Ther.* **19**, 470–478 (2011).
150. Rössger, K., Charpin-El-Hamri, G. & Fussenegger, M. A closed-loop synthetic gene circuit for the treatment of diet-induced obesity in mice. *Nat. Commun.* **4**, (2013).
151. Nissim, L. & Bar-Ziv, R. H. A tunable dual-promoter integrator for targeting of cancer cells. *Mol. Syst. Biol.* **6**, (2010).
152. Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R. & Benenson, Y. Multi-Input RNAi-Based Logic Circuit for Identification of Specific Cancer Cells. *Science* **333**, 1307–1311 (2011).
153. Liu, Y. *et al.* Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells. *Nat. Commun.* **5**, 5393 (2014).
154. Lim, W. A. Designing customized cell signalling circuits. *Nat. Rev. Mol. Cell Biol.* **11**, 393–403 (2010).
155. Conklin, B. R. *et al.* Engineering GPCR signaling pathways with RASSLs. *Nat. Methods* **5**, 673–678 (2008).
156. Heng, B. C., Aubel, D. & Fussenegger, M. G Protein–Coupled Receptors Revisited: Therapeutic Applications Inspired by Synthetic Biology. *Annu. Rev. Pharmacol. Toxicol.* **54**, 227–249 (2014).
157. Grupp, S. A. *et al.* Chimeric Antigen Receptor–Modified T Cells for Acute Lymphoid Leukemia. *N. Engl. J. Med.* **368**, 1509–1518 (2013).
158. Kalos, M. *et al.* T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia. *Sci. Transl. Med.* **3**, 95ra73–95ra73 (2011).
159. Brentjens, R. J. *et al.* CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia. *Sci. Transl. Med.* **5**, 177ra38–177ra38 (2013).
160. Schofield, D., Sharp, N. J. & Westwater, C. Phage-based platforms for the clinical detection of human bacterial pathogens. *Bacteriophage* **2**, (2012).
161. Citorik, R. J., Mimee, M. & Lu, T. K. Bacteriophage-based synthetic biology for the study of infectious diseases. *Curr. Opin. Microbiol.* **19**, 59–69 (2014).
162. Sin, M. L. Y., Mach, K. E., Wong, P. K. & Liao, J. C. Advances and challenges in biosensor-based diagnosis of infectious diseases. *Expert Rev. Mol. Diagn.* **14**, 225–244 (2014).
163. Miyata, K., Nishiyama, N. & Kataoka, K. Rational design of smart supramolecular assemblies for gene delivery: chemical challenges in the creation of artificial viruses. *Chem. Soc. Rev.* **41**, 2562–2574 (2012).
164. Bartel, M. A., Weinstein, J. R. & Schaffer, D. V. Directed evolution of novel adeno-associated viruses for therapeutic gene delivery. *Gene Ther.* **19**, 694–700 (2012).
165. Yildiz, I., Shukla, S. & Steinmetz, N. F. Applications of viral nanoparticles in medicine. *Curr. Opin. Biotechnol.* **22**, 901–908 (2011).
166. Märtsch, S., Huber, A., Hallek, M., Büning, H. & Perabo, L. A novel directed evolution method to enhance cell-type specificity of adeno-associated virus vectors. *Comb. Chem. High Throughput Screen.* **13**, 807–812 (2010).
167. Abshire, T. G., Brown, J. E. & Ezzell, J. W. Production and Validation of the Use of Gamma Phage for Identification of *Bacillus anthracis*. *J. Clin. Microbiol.* **43**, 4780–4788 (2005).
168. McNerney, R., Kambashi, B. S., Kinkese, J., Tembwe, R. & Godfrey-Faussett, P. Development of a Bacteriophage Phage Replication Assay for Diagnosis of Pulmonary Tuberculosis. *J. Clin. Microbiol.* **42**, 2115–2120 (2004).
169. Reiman, R. W., Atchley, D. H. & Voorhees, K. J. Indirect detection of *Bacillus anthracis* using real-time PCR to detect amplified gamma phage DNA. *J. Microbiol. Methods* **68**, 651–653 (2007).
170. Sergueev, K. V., He, Y., Borschel, R. H., Nikolich, M. P. & Filippov, A. A. Rapid and Sensitive Detection of *Yersinia pestis* Using Amplification of Plague Diagnostic Bacteriophages Monitored by Real-Time PCR. *PLoS ONE* **5**, e11337 (2010).

171. Fujinami, Y., Hirai, Y., Sakai, I., Yoshino, M. & Yasuda, J. Sensitive detection of *Bacillus anthracis* using a binding protein originating from gamma-phage. *Microbiol. Immunol.* **51**, 163–169 (2007).
172. Funatsu, T., Taniyama, T., Tajima, T., Tadakuma, H. & Namiki, H. Rapid and sensitive detection method of a bacterium by using a GFP reporter phage. *Microbiol. Immunol.* **46**, 365–369 (2002).
173. Waddell, T. E. & Poppe, C. Construction of mini-Tn10luxABcam/Ptac-ATS and its use for developing a bacteriophage that transduces bioluminescence to *Escherichia coli* O157:H7. *FEMS Microbiol. Lett.* **182**, 285–289 (2000).
174. Namura, M., Hijikata, T., Miyanaga, K. & Tanji, Y. Detection of *Escherichia coli* with Fluorescent Labeled Phages That Have a Broad Host Range to *E. coli* in Sewage Water. *Biotechnol. Prog.* **24**, 481–486 (2008).
175. Dusthacker, A. *et al.* Construction and evaluation of luciferase reporter phages for the detection of active and non-replicating tubercle bacilli. *J. Microbiol. Methods* **73**, 18–25 (2008).
176. Piuri, M., Jacobs, W. R. & Hatfull, G. F. Fluoromycobacteriophages for Rapid, Specific, and Sensitive Antibiotic Susceptibility Testing of *Mycobacterium tuberculosis*. *PLoS ONE* **4**, e4870 (2009).
177. Jacobs, W. R. *et al.* Rapid assessment of drug susceptibilities of *Mycobacterium tuberculosis* by means of luciferase reporter phages. *Science* **260**, 819–822 (1993).
178. Carrière, C. *et al.* Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **35**, 3232–3239 (1997).
179. Loessner, M. J., Rees, C. E., Stewart, G. S. & Scherer, S. Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable *Listeria* cells. *Appl. Environ. Microbiol.* **62**, 1133–1140 (1996).
180. Bhowmick, T. *et al.* Controlled Multicenter Evaluation of a Bacteriophage-Based Method for Rapid Detection of *Staphylococcus aureus* in Positive Blood Cultures. *J. Clin. Microbiol.* **51**, 1226–1230 (2013).
181. Smartt, A. E. *et al.* Pathogen detection using engineered bacteriophages. *Anal. Bioanal. Chem.* **402**, 3127–3146 (2012).
182. Pacheco-Gómez, R. *et al.* Detection of Pathogenic Bacteria Using a Homogeneous Immunoassay Based on Shear Alignment of Virus Particles and Linear Dichroism. *Anal. Chem.* **84**, 91–97 (2012).
183. Smartt, A. E. & Ripp, S. Bacteriophage reporter technology for sensing and detecting microbial targets. *Anal. Bioanal. Chem.* **400**, 991–1007 (2011).
184. Tawil, N., Sacher, E., Mandeville, R. & Meunier, M. Bacteriophages: biosensing tools for multi-drug resistant pathogens. *The Analyst* **139**, 1224 (2014).
185. Mani, V. *et al.* Emerging technologies for monitoring drug-resistant tuberculosis at the point-of-care. *Adv. Drug Deliv. Rev.* **78**, 105–117 (2014).
186. *Bacteriophages: biology and applications.* (CRC Press, 2005).
187. Lu, T. K., Bowers, J. & Koeris, M. S. Advancing bacteriophage-based microbial diagnostics with synthetic biology. *Trends Biotechnol.* **31**, 325–327 (2013).
188. Mastrobattista, E., van der Aa, M. A. E. M., Hennink, W. E. & Crommelin, D. J. A. Artificial viruses: a nanotechnological approach to gene delivery. *Nat. Rev. Drug Discov.* **5**, 115–121 (2006).
189. Kim, E. *et al.* Ad-mTERT-delta19, a conditional replication-competent adenovirus driven by the human telomerase promoter, selectively replicates in and elicits cytopathic effect in a cancer cell-specific manner. *Hum. Gene Ther.* **14**, 1415–1428 (2003).
190. Nettelbeck, D. M., Rivera, A. A., Balagué, C., Alemany, R. & Curiel, D. T. Novel oncolytic adenoviruses targeted to melanoma: specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter. *Cancer Res.* **62**, 4663–4670 (2002).

191. Manchester, M. & Singh, P. Virus-based nanoparticles (VNPs): platform technologies for diagnostic imaging. *Adv. Drug Deliv. Rev.* **58**, 1505–1522 (2006).
192. Adusumilli, P. S. *et al.* Intraoperative localization of lymph node metastases with a replication-competent herpes simplex virus. *J. Thorac. Cardiovasc. Surg.* **132**, 1179–1188 (2006).
193. Stritzker, J. *et al.* Vaccinia virus-mediated melanin production allows MR and optoacoustic deep tissue imaging and laser-induced thermotherapy of cancer. *Proc. Natl. Acad. Sci.* **110**, 3316–3320 (2013).
194. Haddad, D. & Fong, Y. Molecular imaging of oncolytic viral therapy. *Mol. Ther. — Oncolytics* **1**, 14007 (2015).
195. Hess, M. *et al.* Bacterial glucuronidase as general marker for oncolytic virotherapy or other biological therapies. *J. Transl. Med.* **9**, 172 (2011).
196. Park, J.-S. *et al.* A highly sensitive and selective diagnostic assay based on virus nanoparticles. *Nat. Nanotechnol.* **4**, 259–264 (2009).
197. Lee, J.-H. *et al.* A Three-Dimensional and Sensitive Bioassay Based on Nanostructured Quartz Combined with Viral Nanoparticles. *Adv. Funct. Mater.* **20**, 2004–2009 (2010).
198. Bromley, E. H. C., Channon, K., Moutevelis, E. & Woolfson, D. N. Peptide and Protein Building Blocks for Synthetic Biology: From Programming Biomolecules to Self-Organized Biomolecular Systems. *ACS Chem. Biol.* **3**, 38–50 (2008).
199. Hockenberry, A. J. & Jewett, M. C. Synthetic in vitro circuits. *Curr. Opin. Chem. Biol.* **16**, 253–259 (2012).
200. Hodgman, C. E. & Jewett, M. C. Cell-free synthetic biology: Thinking outside the cell. *Metab. Eng.* **14**, 261–269 (2012).
201. Breaker, R. R. & Joyce, G. F. The Expanding View of RNA and DNA Function. *Chem. Biol.* **21**, 1059–1065 (2014).
202. Linko, V. & Dietz, H. The enabled state of DNA nanotechnology. *Curr. Opin. Biotechnol.* **24**, 555–561 (2013).
203. Wang, F., Willner, B. & Willner, I. DNA nanotechnology with one-dimensional self-assembled nanostructures. *Curr. Opin. Biotechnol.* **24**, 562–574 (2013).
204. Padirac, A., Fujii, T. & Rondelez, Y. Nucleic acids for the rational design of reaction circuits. *Curr. Opin. Biotechnol.* **24**, 575–580 (2013).
205. Wang, F., Lu, C.-H. & Willner, I. From Cascaded Catalytic Nucleic Acids to Enzyme–DNA Nanostructures: Controlling Reactivity, Sensing, Logic Operations, and Assembly of Complex Structures. *Chem. Rev.* **114**, 2881–2941 (2014).
206. Seelig, G., Soloveichik, D., Zhang, D. Y. & Winfree, E. Enzyme-Free Nucleic Acid Logic Circuits. *Science* **314**, 1585–1588 (2006).
207. Famulok, M. & Mayer, G. Aptamers and SELEX in Chemistry & Biology. *Chem. Biol.* **21**, 1055–1058 (2014).
208. Yang, L. & Ellington, A. in *Fluorescence Sensors and Biosensors* (ed. Thompson, R.) 5–43 (CRC Press, 2005). at <<http://www.crcnetbase.com/doi/abs/10.1201/9781420028287.ch2>>
209. Famulok, M., Hartig, J. S. & Mayer, G. Functional Aptamers and Aptazymes in Biotechnology, Diagnostics, and Therapy. *Chem. Rev.* **107**, 3715–3743 (2007).
210. Ozer, A., Pagano, J. M. & Lis, J. T. New Technologies Provide Quantum Changes in the Scale, Speed, and Success of SELEX Methods and Aptamer Characterization. *Mol. Ther. Acids* **3**, e183 (2014).
211. Hong, P., Li, W. & Li, J. Applications of Aptasensors in Clinical Diagnostics. *Sensors* **12**, 1181–1193 (2012).
212. Toh, S. Y., Citartan, M., Gopinath, S. C. B. & Tang, T.-H. Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay. *Biosens. Bioelectron.* **64**, 392–403 (2015).
213. Zhou, M. *et al.* Aptamer-Controlled Biofuel Cells in Logic Systems and Used as Self-Powered and Intelligent Logic Aptasensors. *J. Am. Chem. Soc.* **132**, 2172–2174 (2010).
214. Zhou, W., Jimmy Huang, P.-J., Ding, J. & Liu, J. Aptamer-based biosensors for biomedical diagnostics. *The Analyst* **139**, 2627 (2014).

215. Serganov, A. & Nudler, E. A Decade of Riboswitches. *Cell* **152**, 17–24 (2013).
216. Tang, J. & Breaker, R. R. Rational design of allosteric ribozymes. *Chem. Biol.* **4**, 453–459 (1997).
217. Winkler, W. C., Nahvi, A., Roth, A., Collins, J. A. & Breaker, R. R. Control of gene expression by a natural metabolite-responsive ribozyme. *Nature* **428**, 281–286 (2004).
218. Frommer, J., Appel, B. & Müller, S. Ribozymes that can be regulated by external stimuli. *Curr. Opin. Biotechnol.* **31**, 35–41 (2015).
219. Berens, C. & Suess, B. Riboswitch engineering — making the all-important second and third steps. *Curr. Opin. Biotechnol.* **31**, 10–15 (2015).
220. Ogawa, A. Rational design of artificial riboswitches based on ligand-dependent modulation of internal ribosome entry in wheat germ extract and their applications as label-free biosensors. *RNA* **17**, 478–488 (2011).
221. Olea, C. & Joyce, G. F. in *Methods in Enzymology* **550**, 23–39 (Elsevier, 2015).
222. Kong, R.-M. *et al.* Unimolecular Catalytic DNA Biosensor for Amplified Detection of L - Histidine via an Enzymatic Recycling Cleavage Strategy. *Anal. Chem.* **83**, 7603–7607 (2011).
223. Tang, L. *et al.* Colorimetric and Ultrasensitive Bioassay Based on a Dual-Amplification System Using Aptamer and DNAzyme. *Anal. Chem.* **84**, 4711–4717 (2012).
224. Pavlov, V. *et al.* Amplified chemiluminescence surface detection of DNA and telomerase activity using catalytic nucleic acid labels. *Anal. Chem.* **76**, 2152–2156 (2004).
225. Freeman, R. *et al.* DNAzyme-Like Activity of Hemin-Telomeric G-Quadruplexes for the Optical Analysis of Telomerase and its Inhibitors. *ChemBioChem* **11**, 2362–2367 (2010).
226. Qian, L. & Winfree, E. Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades. *Science* **332**, 1196–1201 (2011).
227. Zhang, D. Y. & Seelig, G. Dynamic DNA nanotechnology using strand-displacement reactions. *Nat. Chem.* **3**, 103–113 (2011).
228. Chen, S. X., Zhang, D. Y. & Seelig, G. Conditionally fluorescent molecular probes for detecting single base changes in double-stranded DNA. *Nat. Chem.* **5**, 782–789 (2013).
229. Jung, C. & Ellington, A. D. Diagnostic Applications of Nucleic Acid Circuits. *Acc. Chem. Res.* **47**, 1825–1835 (2014).
230. Eckhoff, G., Codrea, V., Ellington, A. D. & Chen, X. Beyond allostery: Catalytic regulation of a deoxyribozyme through an entropy-driven DNA amplifier. *J. Syst. Chem.* **1**, 13 (2010).
231. Li, B., Ellington, A. D. & Chen, X. Rational, modular adaptation of enzyme-free DNA circuits to multiple detection methods. *Nucleic Acids Res.* **39**, e110–e110 (2011).
232. Li, B., Chen, X. & Ellington, A. D. Adapting Enzyme-Free DNA Circuits to the Detection of Loop-Mediated Isothermal Amplification Reactions. *Anal. Chem.* **84**, 8371–8377 (2012).
233. Zhang, B. *et al.* DNA-Based Hybridization Chain Reaction for Amplified Bioelectronic Signal and Ultrasensitive Detection of Proteins. *Anal. Chem.* **84**, 5392–5399 (2012).
234. Choi, H. M. T. *et al.* Programmable in situ amplification for multiplexed imaging of mRNA expression. *Nat. Biotechnol.* **28**, 1208–1212 (2010).
235. Choi, H. M. T., Beck, V. A. & Pierce, N. A. Next-Generation *in Situ* Hybridization Chain Reaction: Higher Gain, Lower Cost, Greater Durability. *ACS Nano* **8**, 4284–4294 (2014).
236. Gil, B., Kahan-Hanum, M., Skirtenko, N., Adar, R. & Shapiro, E. Detection of Multiple Disease Indicators by an Autonomous Biomolecular Computer. *Nano Lett.* **11**, 2989–2996 (2011).
237. Qian, L., Winfree, E. & Bruck, J. Neural network computation with DNA strand displacement cascades. *Nature* **475**, 368–372 (2011).
238. Zhou, G. *et al.* Multivalent Capture and Detection of Cancer Cells with DNA Nanostructured Biosensors and Multibranched Hybridization Chain Reaction Amplification. *Anal. Chem.* **86**, 7843–7848 (2014).
239. You, M. *et al.* DNA ‘Nano-Claw’: Logic-Based Autonomous Cancer Targeting and Therapy. *J. Am. Chem. Soc.* **136**, 1256–1259 (2014).
240. Rudchenko, M. *et al.* Autonomous molecular cascades for evaluation of cell surfaces. *Nat. Nanotechnol.* **8**, 580–586 (2013).

241. Allen, P. B., Arshad, S. A., Li, B., Chen, X. & Ellington, A. D. DNA circuits as amplifiers for the detection of nucleic acids on a paperfluidic platform. *Lab. Chip* **12**, 2951 (2012).
242. Chen, J., Zhou, X. & Zeng, L. Enzyme-free strip biosensor for amplified detection of Pb²⁺ based on a catalytic DNA circuit. *Chem Commun* **49**, 984–986 (2013).
243. Pardee, K. *et al.* Paper-Based Synthetic Gene Networks. *Cell* **159**, 940–954 (2014).
244. Elbeik, T. *et al.* Multicenter Evaluation of the Performance Characteristics of the Bayer VERSANT HCV RNA 3.0 Assay (bDNA). *J. Clin. Microbiol.* **42**, 563–569 (2004).
245. Elbeik, T. *et al.* Simultaneous runs of the Bayer VERSANT HIV-1 version 3.0 and HCV bDNA version 3.0 quantitative assays on the system 340 platform provide reliable quantitation and improved work flow. *J. Clin. Microbiol.* **42**, 3120–3127 (2004).
246. Campolongo, M. J., Tan, S. J., Xu, J. & Luo, D. DNA nanomedicine: Engineering DNA as a polymer for therapeutic and diagnostic applications. *Adv. Drug Deliv. Rev.* **62**, 606–616 (2010).
247. Zhang, L., Lei, J., Liu, L., Li, C. & Ju, H. Self-Assembled DNA Hydrogel as Switchable Material for Aptamer-Based Fluorescent Detection of Protein. *Anal. Chem.* **85**, 11077–11082 (2013).
248. *Algorithmic bioprocesses.* (Springer, 2009).
249. Bath, J. & Turberfield, A. J. DNA nanomachines. *Nat. Nanotechnol.* **2**, 275–284 (2007).
250. Douglas, S. M., Bachelet, I. & Church, G. M. A Logic-Gated Nanorobot for Targeted Transport of Molecular Payloads. *Science* **335**, 831–834 (2012).
251. Ben-Ishay, E., Abu-Horowitz, A. & Bachelet, I. Designing a Bio-responsive Robot from DNA Origami. *J. Vis. Exp.* (2013). doi:10.3791/50268
252. Wang, D. *et al.* Molecular Logic Gates on DNA Origami Nanostructures for MicroRNA Diagnostics. *Anal. Chem.* **86**, 1932–1936 (2014).
253. Amir, Y. *et al.* Universal computing by DNA origami robots in a living animal. *Nat. Nanotechnol.* **9**, 353–357 (2014).
254. Taylor, A. I., Arangundy-Franklin, S. & Holliger, P. Towards applications of synthetic genetic polymers in diagnosis and therapy. *Curr. Opin. Chem. Biol.* **22**, 79–84 (2014).
255. Pinheiro, V. B. *et al.* Synthetic Genetic Polymers Capable of Heredity and Evolution. *Science* **336**, 341–344 (2012).
256. Kimoto, M., Yamashige, R., Matsunaga, K., Yokoyama, S. & Hirao, I. Generation of high-affinity DNA aptamers using an expanded genetic alphabet. *Nat. Biotechnol.* **31**, 453–457 (2013).
257. Davies, D. R. *et al.* Unique motifs and hydrophobic interactions shape the binding of modified DNA ligands to protein targets. *Proc. Natl. Acad. Sci.* **109**, 19971–19976 (2012).
258. Imaizumi, Y. *et al.* Efficacy of Base-Modification on Target Binding of Small Molecule DNA Aptamers. *J. Am. Chem. Soc.* **135**, 9412–9419 (2013).
259. Shi, H. *et al.* Locked nucleic acid/DNA chimeric aptamer probe for tumor diagnosis with improved serum stability and extended imaging window in vivo. *Anal. Chim. Acta* **812**, 138–144 (2014).
260. Olson, E. J. & Tabor, J. J. Post-translational tools expand the scope of synthetic biology. *Curr. Opin. Chem. Biol.* **16**, 300–306 (2012).
261. Foo, J. L., Ching, C. B., Chang, M. W. & Leong, S. S. J. The imminent role of protein engineering in synthetic biology. *Biotechnol. Adv.* **30**, 541–549 (2012).
262. Grunberg, R. & Serrano, L. Strategies for protein synthetic biology. *Nucleic Acids Res.* **38**, 2663–2675 (2010).
263. Gunnoo, S. B. *et al.* Creation of a gated antibody as a conditionally functional synthetic protein. *Nat. Commun.* **5**, (2014).
264. Strauch, E.-M., Fleishman, S. J. & Baker, D. Computational design of a pH-sensitive IgG binding protein. *Proc. Natl. Acad. Sci.* **111**, 675–680 (2014).
265. Byrne, H., Conroy, P. J., Whisstock, J. C. & O’Kennedy, R. J. A tale of two specificities: bispecific antibodies for therapeutic and diagnostic applications. *Trends Biotechnol.* **31**, 621–632 (2013).

266. Currin, A., Swainston, N., Day, P. J. & Kell, D. B. Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. *Chem Soc Rev* **44**, 1172–1239 (2015).
267. Saito, K. *et al.* Luminescent proteins for high-speed single-cell and whole-body imaging. *Nat. Commun.* **3**, 1262 (2012).
268. Ballew, J. T. *et al.* Antibody biomarker discovery through in vitro directed evolution of consensus recognition epitopes. *Proc. Natl. Acad. Sci.* **110**, 19330–19335 (2013).
269. Gebauer, M. & Skerra, A. Engineered protein scaffolds as next-generation antibody therapeutics. *Curr. Opin. Chem. Biol.* **13**, 245–255 (2009).
270. Miao, Z., Levi, J. & Cheng, Z. Protein scaffold-based molecular probes for cancer molecular imaging. *Amino Acids* **41**, 1037–1047 (2011).
271. You, C. & Zhang, Y.-H. P. Self-Assembly of Synthetic Metabolons through Synthetic Protein Scaffolds: One-Step Purification, Co-immobilization, and Substrate Channeling. *ACS Synth. Biol.* **2**, 102–110 (2013).
272. Dueber, J. E. *et al.* Synthetic protein scaffolds provide modular control over metabolic flux. *Nat. Biotechnol.* **27**, 753–759 (2009).
273. Lu, Y., Yeung, N., Sieracki, N. & Marshall, N. M. Design of functional metalloproteins. *Nature* **460**, 855–862 (2009).
274. Kiss, G., Çelebi-Ölçüm, N., Moretti, R., Baker, D. & Houk, K. N. Computational Enzyme Design. *Angew. Chem. Int. Ed.* **52**, 5700–5725 (2013).
275. Kaplan, J. & DeGrado, W. F. De novo design of catalytic proteins. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 11566–11570 (2004).
276. Röthlisberger, D. *et al.* Kemp elimination catalysts by computational enzyme design. *Nature* **453**, 190–195 (2008).
277. Khoury, G. A., Smadbeck, J., Kieslich, C. A. & Floudas, C. A. Protein folding and de novo protein design for biotechnological applications. *Trends Biotechnol.* **32**, 99–109 (2014).
278. Feldmeier, K. & Höcker, B. Computational protein design of ligand binding and catalysis. *Curr. Opin. Chem. Biol.* **17**, 929–933 (2013).
279. Mandell, D. J. & Kortemme, T. Computer-aided design of functional protein interactions. *Nat. Chem. Biol.* **5**, 797–807 (2009).
280. Schreier, B., Stumpp, C., Wiesner, S. & Hocker, B. Computational design of ligand binding is not a solved problem. *Proc. Natl. Acad. Sci.* **106**, 18491–18496 (2009).
281. Tinberg, C. E. *et al.* Computational design of ligand-binding proteins with high affinity and selectivity. *Nature* **501**, 212–216 (2013).
282. Kortemme, T. & Baker, D. Computational design of protein-protein interactions. *Curr. Opin. Chem. Biol.* **8**, 91–97 (2004).
283. Stein, V. & Alexandrov, K. Synthetic protein switches: design principles and applications. *Trends Biotechnol.* **33**, 101–110 (2015).
284. Ostermeier, M. Designing switchable enzymes. *Curr. Opin. Struct. Biol.* **19**, 442–448 (2009).
285. Dueber, J. E., Yeh, B. J., Bhattacharyya, R. P. & Lim, W. A. Rewiring cell signaling: the logic and plasticity of eukaryotic protein circuitry. *Curr. Opin. Struct. Biol.* **14**, 690–699 (2004).
286. Dueber, J. E., Yeh, B. J., Chak, K. & Lim, W. A. Reprogramming control of an allosteric signaling switch through modular recombination. *Science* **301**, 1904–1908 (2003).
287. Dueber, J. E., Mirsky, E. A. & Lim, W. A. Engineering synthetic signaling proteins with ultrasensitive input/output control. *Nat. Biotechnol.* **25**, 660–662 (2007).
288. Yeh, B. J., Rutigliano, R. J., Deb, A., Bar-Sagi, D. & Lim, W. A. Rewiring cellular morphology pathways with synthetic guanine nucleotide exchange factors. *Nature* **447**, 596–600 (2007).
289. Guntas, G., Mansell, T. J., Kim, J. R. & Ostermeier, M. Directed evolution of protein switches and their application to the creation of ligand-binding proteins. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 11224–11229 (2005).
290. Guntas, G. & Ostermeier, M. Creation of an allosteric enzyme by domain insertion. *J. Mol. Biol.* **336**, 263–273 (2004).

291. Dagliyan, O. *et al.* Rational design of a ligand-controlled protein conformational switch. *Proc. Natl. Acad. Sci.* **110**, 6800–6804 (2013).
292. Karginov, A. V., Ding, F., Kota, P., Dokholyan, N. V. & Hahn, K. M. Engineered allosteric activation of kinases in living cells. *Nat. Biotechnol.* **28**, 743–747 (2010).
293. Sallee, N. A., Yeh, B. J. & Lim, W. A. Engineering modular protein interaction switches by sequence overlap. *J. Am. Chem. Soc.* **129**, 4606–4611 (2007).
294. Ibraheem, A. & Campbell, R. E. Designs and applications of fluorescent protein-based biosensors. *Curr. Opin. Chem. Biol.* **14**, 30–36 (2010).
295. Griss, R. *et al.* Bioluminescent sensor proteins for point-of-care therapeutic drug monitoring. *Nat. Chem. Biol.* **10**, 598–603 (2014).
296. Binkowski, B., Fan, F. & Wood, K. Engineered luciferases for molecular sensing in living cells. *Curr. Opin. Biotechnol.* **20**, 14–18 (2009).
297. Banala, S., Aper, S. J. A., Schalk, W. & Merckx, M. Switchable Reporter Enzymes Based on Mutually Exclusive Domain Interactions Allow Antibody Detection Directly in Solution. *ACS Chem. Biol.* **8**, 2127–2132 (2013).
298. Nirantar, S. R., Yeo, K. S., Chee, S., Lane, D. P. & Ghadessy, F. J. A generic scaffold for conversion of peptide ligands into homogenous biosensors. *Biosens. Bioelectron.* **47**, 421–428 (2013).
299. Ashkenasy, G. & Ghadiri, M. R. Boolean logic functions of a synthetic peptide network. *J. Am. Chem. Soc.* **126**, 11140–11141 (2004).
300. Niazov, T., Baron, R., Katz, E., Lioubashevski, O. & Willner, I. Concatenated logic gates using four coupled biocatalysts operating in series. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 17160–17163 (2006).
301. Sugita, M. Functional analysis of chemical systems in vivo using a logical circuit equivalent. II. The idea of a molecular automation. *J. Theor. Biol.* **4**, 179–192 (1963).
302. Arkin, A. & Ross, J. Computational functions in biochemical reaction networks. *Biophys. J.* **67**, 560–578 (1994).
303. Wang, J. & Katz, E. Digital biosensors with built-in logic for biomedical applications--biosensors based on a biocomputing concept. *Anal. Bioanal. Chem.* **398**, 1591–1603 (2010).
304. Katz, E., Minko, S., Halámek, J., MacVittie, K. & Yancey, K. Electrode interfaces switchable by physical and chemical signals for biosensing, biofuel, and biocomputing applications. *Anal. Bioanal. Chem.* **405**, 3659–3672 (2013).
305. Poghosian, A. *et al.* Integration of biomolecular logic gates with field-effect transducers. *Electrochimica Acta* **56**, 9661–9665 (2011).
306. Krämer, M. *et al.* Coupling of Biocomputing Systems with Electronic Chips: Electronic Interface for Transduction of Biochemical Information. *J. Phys. Chem. C* **113**, 2573–2579 (2009).
307. Katz, E. & Minko, S. Enzyme-based logic systems interfaced with signal-responsive materials and electrodes. *Chem Commun* **51**, 3493–3500 (2015).
308. Privman, M., Tam, T. K., Pita, M. & Katz, E. Switchable Electrode Controlled by Enzyme Logic Network System: Approaching Physiologically Regulated Bioelectronics. *J. Am. Chem. Soc.* **131**, 1314–1321 (2009).
309. Katz, E., Wang, J., Privman, M. & Halámek, J. Multianalyte Digital Enzyme Biosensors with Built-in Boolean Logic. *Anal. Chem.* **84**, 5463–5469 (2012).
310. Zhou, J., Halámek, J., Bocharova, V., Wang, J. & Katz, E. Bio-logic analysis of injury biomarker patterns in human serum samples. *Talanta* **83**, 955–959 (2011).
311. Halámek, J. *et al.* Multi-enzyme logic network architectures for assessing injuries: digital processing of biomarkers. *Mol. Biosyst.* **6**, 2554 (2010).
312. Pita, M. *et al.* Enzyme logic gates for assessing physiological conditions during an injury: Towards digital sensors and actuators. *Sens. Actuators B Chem.* **139**, 631–636 (2009).
313. Halámek, J. *et al.* Multiplexing of injury codes for the parallel operation of enzyme logic gates. *The Analyst* **135**, 2249 (2010).

314. Zhou, N. *et al.* Enzyme-based NAND gate for rapid electrochemical screening of traumatic brain injury in serum. *Anal. Chim. Acta* **703**, 94–100 (2011).
315. Mailloux, S., Halámek, J. & Katz, E. A model system for targeted drug release triggered by biomolecular signals logically processed through enzyme logic networks. *The Analyst* **139**, 982–986 (2014).
316. Tokarev, I. *et al.* Stimuli-responsive hydrogel membranes coupled with biocatalytic processes. *ACS Appl. Mater. Interfaces* **1**, 532–536 (2009).
317. Mailloux, S., Guz, N., Gamella Carballo, M., Pingarrón, J. M. & Katz, E. Model system for targeted drug release triggered by immune-specific signals. *Anal. Bioanal. Chem.* **406**, 4825–4829 (2014).
318. Guz, N., Halámek, J., Rusling, J. F. & Katz, E. A biocatalytic cascade with several output signals—towards biosensors with different levels of confidence. *Anal. Bioanal. Chem.* **406**, 3365–3370 (2014).
319. Privman, V., Domanskyi, S., Mailloux, S., Holade, Y. & Katz, E. Kinetic Model for a Threshold Filter in an Enzymatic System for Bioanalytical and Biocomputing Applications. *J. Phys. Chem. B* **118**, 12435–12443 (2014).
320. Bakshi, S., Zavalov, O., Halámek, J., Privman, V. & Katz, E. Modularity of Biochemical Filtering for Inducing Sigmoid Response in Both Inputs in an Enzymatic AND Gate. *J. Phys. Chem. B* **117**, 9857–9865 (2013).
321. Melnikov, D., Strack, G., Pita, M., Privman, V. & Katz, E. Analog Noise Reduction in Enzymatic Logic Gates. *J. Phys. Chem. B* **113**, 10472–10479 (2009).
322. Privman, V. *et al.* Biochemical Filter with Sigmoidal Response: Increasing the Complexity of Biomolecular Logic. *J. Phys. Chem. B* **114**, 14103–14109 (2010).
323. Privman, V., Strack, G., Solenov, D., Pita, M. & Katz, E. Optimization of Enzymatic Biochemical Logic for Noise Reduction and Scalability: How Many Biocomputing Gates Can Be Interconnected in a Circuit? *J. Phys. Chem. B* **112**, 11777–11784 (2008).
324. Hucka, M. *et al.* The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinforma. Oxf. Engl.* **19**, 524–531 (2003).
325. Rialle, S. *et al.* BioNetCAD: design, simulation and experimental validation of synthetic biochemical networks. *Bioinformatics* **26**, 2298–2304 (2010).
326. Amar, P. *et al.* A stochastic automaton shows how enzyme assemblies may contribute to metabolic efficiency. *BMC Syst. Biol.* **2**, 27 (2008).
327. Rizk, A., Batt, G., Fages, F. & Soliman, S. A general computational method for robustness analysis with applications to synthetic gene networks. *Bioinformatics* **25**, i169–i178 (2009).
328. Liu, C. C. & Schultz, P. G. Adding New Chemistries to the Genetic Code. *Annu. Rev. Biochem.* **79**, 413–444 (2010).
329. O'Donoghue, P., Ling, J., Wang, Y.-S. & Söll, D. Upgrading protein synthesis for synthetic biology. *Nat. Chem. Biol.* **9**, 594–598 (2013).
330. Acevedo-Rocha, C. G. *et al.* Non-canonical amino acids as a useful synthetic biological tool for lipase-catalysed reactions in hostile environments. *Catal. Sci. Technol.* **3**, 1198 (2013).
331. Lemke, E. A., Summerer, D., Geierstanger, B. H., Brittain, S. M. & Schultz, P. G. Control of protein phosphorylation with a genetically encoded photocaged amino acid. *Nat. Chem. Biol.* **3**, 769–772 (2007).
332. Wang, K. *et al.* Optimized orthogonal translation of unnatural amino acids enables spontaneous protein double-labelling and FRET. *Nat. Chem.* **6**, 393–403 (2014).
333. King, N. P. *et al.* Accurate design of co-assembling multi-component protein nanomaterials. *Nature* **510**, 103–108 (2014).
334. Castillo-León, J., Belotti, Y. & Svendsen, W. in *Nanomedicine in Diagnostics* (ed. Rozlosnik, N.) 50–67 (Science Publishers, 2012). at <<http://www.crcnetbase.com/doi/abs/10.1201/b11929-4>>
335. Men, D. *et al.* Seeding-Induced Self-assembling Protein Nanowires Dramatically Increase the Sensitivity of Immunoassays. *Nano Lett.* **9**, 2246–2250 (2009).

336. Leng, Y. *et al.* Integration of a Fluorescent Molecular Biosensor into Self-Assembled Protein Nanowires: A Large Sensitivity Enhancement. *Angew. Chem. Int. Ed.* **49**, 7243–7246 (2010).
337. Domigan, L. J. in *Protein Nanotechnology* (ed. Gerrard, J. A.) **996**, 131–152 (Humana Press, 2013).
338. Lee, J.-H. *et al.* Proteinticle Engineering for Accurate 3D Diagnosis. *ACS Nano* **7**, 10879–10886 (2013).
339. Chou, D. H.-C. *et al.* Glucose-responsive insulin activity by covalent modification with aliphatic phenylboronic acid conjugates. *Proc. Natl. Acad. Sci.* **112**, 2401–2406 (2015).
340. Matsubayashi, H. & Ueda, T. Purified cell-free systems as standard parts for synthetic biology. *Curr. Opin. Chem. Biol.* **22**, 158–162 (2014).
341. Jewett, M. C. & Forster, A. C. Update on designing and building minimal cells. *Curr. Opin. Biotechnol.* **21**, 697–703 (2010).
342. Stano, P. & Luisi, P. L. Semi-synthetic minimal cells: origin and recent developments. *Curr. Opin. Biotechnol.* **24**, 633–638 (2013).
343. Doktycz, M. J. & Simpson, M. L. Nano-enabled synthetic biology. *Mol. Syst. Biol.* **3**, 125 (2007).
344. Noireaux, V. & Libchaber, A. A vesicle bioreactor as a step toward an artificial cell assembly. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 17669–17674 (2004).
345. Wu, F. & Tan, C. The engineering of artificial cellular nanosystems using synthetic biology approaches: Artificial cellular nanosystems using synthetic biology approaches. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **6**, 369–383 (2014).
346. Stano, P. in *Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications* (ed. Carpi, A.) (InTech, 2011). at <http://www.intechopen.com/books/progress-in-molecular-and-environmental-bioengineering-from-analysis-and-modeling-to-technology-applications/advances-in-minimal-cell-models-a-new-approach-to-synthetic-biology-and-origin-of-life>
347. Zhang, Y., Ruder, W. C. & LeDuc, P. R. Artificial cells: building bioinspired systems using small-scale biology. *Trends Biotechnol.* **26**, 14–20 (2008).
348. Courbet, A., Molina, F. & Amar, P. Computing with Synthetic Protocells. *Acta Biotheor.* (2015). doi:10.1007/s10441-015-9258-8
349. Smaldon, J. *et al.* A computational study of liposome logic: towards cellular computing from the bottom up. *Syst. Synth. Biol.* **4**, 157–179 (2010).
350. Broz, P. *et al.* Toward intelligent nanosize bioreactors: a pH-switchable, channel-equipped, functional polymer nanocontainer. *Nano Lett.* **6**, 2349–2353 (2006).
351. Pasparakis, G., Krasnogor, N., Cronin, L., Davis, B. G. & Alexander, C. Controlled polymer synthesis—from biomimicry towards synthetic biology. *Chem Soc Rev* **39**, 286–300 (2010).
352. Christensen, S. M. & Stamou, D. G. Sensing-Applications of Surface-Based Single Vesicle Arrays. *Sensors* **10**, 11352–11368 (2010).
353. Tanner, P. *et al.* Polymeric vesicles: from drug carriers to nanoreactors and artificial organelles. *Acc. Chem. Res.* **44**, 1039–1049 (2011).
354. Fallah-Araghi, A. *et al.* Enhanced chemical synthesis at soft interfaces: a universal reaction-adsorption mechanism in microcompartments. *Phys. Rev. Lett.* **112**, 028301 (2014).
355. Moghimi, S. M., Hunter, A. C. & Murray, J. C. Nanomedicine: current status and future prospects. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **19**, 311–330 (2005).
356. Elani, Y., Law, R. V. & Ces, O. Vesicle-based artificial cells as chemical microreactors with spatially segregated reaction pathways. *Nat. Commun.* **5**, 5305 (2014).
357. Kim, J. P., Park, C. H. & Sim, S. J. Aptamer biosensors for label-free colorimetric detection of human IgE based on polydiacetylene (PDA) supramolecules. *J. Nanosci. Nanotechnol.* **11**, 4269–4274 (2011).
358. Zhu, C., Liu, L., Yang, Q., Lv, F. & Wang, S. Water-soluble conjugated polymers for imaging, diagnosis, and therapy. *Chem. Rev.* **112**, 4687–4735 (2012).

359. Ho, R. J., Rouse, B. T. & Huang, L. Interactions of target-sensitive immunoliposomes with herpes simplex virus. The foundation of a sensitive immunoliposome assay for the virus. *J. Biol. Chem.* **262**, 13979–13984 (1987).
360. Kolusheva, S., Molt, O., Herm, M., Schrader, T. & Jelinek, R. Selective detection of catecholamines by synthetic receptors embedded in chromatic polydiacetylene vesicles. *J. Am. Chem. Soc.* **127**, 10000–10001 (2005).
361. Liu, Q. & Boyd, B. J. Liposomes in biosensors. *The Analyst* **138**, 391–409 (2013).
362. Haque, F., Geng, J., Montemagno, C. & Guo, P. Incorporation of a viral DNA-packaging motor channel in lipid bilayers for real-time, single-molecule sensing of chemicals and double-stranded DNA. *Nat. Protoc.* **8**, 373–392 (2013).
363. Baumann, P., Tanner, P., Onaca, O. & Palivan, C. G. Bio-Decorated Polymer Membranes: A New Approach in Diagnostics and Therapeutics. *Polymers* **3**, 173–192 (2011).
364. Martino, C. *et al.* Protein expression, aggregation, and triggered release from polymersomes as artificial cell-like structures. *Angew. Chem. Int. Ed Engl.* **51**, 6416–6420 (2012).
365. Discher, D. E. Polymer Vesicles. *Science* **297**, 967–973 (2002).
366. Kamat, N. P., Katz, J. S. & Hammer, D. A. Engineering Polymersome Protocells. *J. Phys. Chem. Lett.* **2**, 1612–1623 (2011).
367. Peters, R. J. R. W. *et al.* Cascade Reactions in Multicompartmentalized Polymersomes. *Angew. Chem. Int. Ed.* **53**, 146–150 (2014).
368. Caldorera-Moore, M. E., Liechty, W. B. & Peppas, N. A. Responsive Theranostic Systems: Integration of Diagnostic Imaging Agents and Responsive Controlled Release Drug Delivery Carriers. *Acc. Chem. Res.* **44**, 1061–1070 (2011).
369. Phillips, M. A., Gran, M. L. & Peppas, N. A. Targeted nanodelivery of drugs and diagnostics. *Nano Today* **5**, 143–159 (2010).
370. Ren, T. *et al.* Multifunctional polymer vesicles for ultrasensitive magnetic resonance imaging and drug delivery. *J. Mater. Chem.* **22**, 12329 (2012).
371. Ben-Haim, N., Broz, P., Marsch, S., Meier, W. & Hunziker, P. Cell-Specific Integration of Artificial Organelles Based on Functionalized Polymer Vesicles. *Nano Lett.* **8**, 1368–1373 (2008).
372. Choi, J. *et al.* Biocompatible heterostructured nanoparticles for multimodal biological detection. *J. Am. Chem. Soc.* **128**, 15982–15983 (2006).
373. Wilner, O. I. *et al.* Enzyme cascades activated on topologically programmed DNA scaffolds. *Nat. Nanotechnol.* **4**, 249–254 (2009).
374. Fu, J., Liu, M., Liu, Y., Woodbury, N. W. & Yan, H. Interenzyme Substrate Diffusion for an Enzyme Cascade Organized on Spatially Addressable DNA Nanostructures. *J. Am. Chem. Soc.* **134**, 5516–5519 (2012).
375. Piperberg, G., Wilner, O. I., Yehezkeli, O., Tel-Vered, R. & Willner, I. Control of bioelectrocatalytic transformations on DNA scaffolds. *J. Am. Chem. Soc.* **131**, 8724–8725 (2009).
376. Wang, Z.-G., Wilner, O. I. & Willner, I. Self-Assembly of Aptamer–Circular DNA Nanostructures for Controlled Biocatalysis. *Nano Lett.* **9**, 4098–4102 (2009).
377. Golub, E., Freeman, R. & Willner, I. A hemin/G-quadruplex acts as an NADH oxidase and NADH peroxidase mimicking DNzyme. *Angew. Chem. Int. Ed Engl.* **50**, 11710–11714 (2011).
378. Von Maltzahn, G. *et al.* Nanoparticle self-assembly gated by logical proteolytic triggers. *J. Am. Chem. Soc.* **129**, 6064–6065 (2007).
379. Konry, T. & Walt, D. R. Intelligent Medical Diagnostics via Molecular Logic. *J. Am. Chem. Soc.* **131**, 13232–13233 (2009).
380. Janssen, B. M. G., van Rosmalen, M., van Beek, L. & Merckx, M. Antibody Activation using DNA-Based Logic Gates. *Angew. Chem. Int. Ed.* **54**, 2530–2533 (2015).
381. Lai, Y.-H. *et al.* Biomolecular logic gate for analysis of the New Delhi metallo- β -lactamase (NDM)-coding gene with concurrent determination of its drug resistance-encoding fragments. *Chem Commun* **50**, 12018–12021 (2014).
382. Motornov, M. *et al.* ‘Chemical Transformers’ from Nanoparticle Ensembles Operated with Logic. *Nano Lett.* **8**, 2993–2997 (2008).

383. Motornov, M. *et al.* An Integrated Multifunctional Nanosystem from Command Nanoparticles and Enzymes. *Small* **5**, 817–820 (2009).
384. Motornov, M., Roiter, Y., Tokarev, I. & Minko, S. Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. *Prog. Polym. Sci.* **35**, 174–211 (2010).
385. Lehner, R., Wang, X., Marsch, S. & Hunziker, P. Intelligent nanomaterials for medicine: Carrier platforms and targeting strategies in the context of clinical application. *Nanomedicine Nanotechnol. Biol. Med.* **9**, 742–757 (2013).
386. Wu, W. *et al.* Multi-functional core-shell hybrid nanogels for pH-dependent magnetic manipulation, fluorescent pH-sensing, and drug delivery. *Biomaterials* **32**, 9876–9887 (2011).
387. Naskar, J., Roy, S., Joardar, A., Das, S. & Banerjee, A. Self-assembling dipeptide-based nontoxic vesicles as carriers for drugs and other biologically important molecules. *Org. Biomol. Chem.* **9**, 6610 (2011).
388. Edmundson, M. C., Capeness, M. & Horsfall, L. Exploring the potential of metallic nanoparticles within synthetic biology. *New Biotechnol.* **31**, 572–578 (2014).
389. Zhou, M. & Dong, S. Bioelectrochemical Interface Engineering: Toward the Fabrication of Electrochemical Biosensors, Biofuel Cells, and Self-Powered Logic Biosensors. *Acc. Chem. Res.* **44**, 1232–1243 (2011).
390. Katz, E. & Pita, M. Biofuel Cells Controlled by Logically Processed Biochemical Signals: Towards Physiologically Regulated Bioelectronic Devices. *Chem. - Eur. J.* **15**, 12554–12564 (2009).
391. Tam, T. K., Pita, M., Ornatska, M. & Katz, E. Biofuel cell controlled by enzyme logic network — Approaching physiologically regulated devices. *Bioelectrochemistry* **76**, 4–9 (2009).
392. Zhou, M. & Wang, J. Biofuel Cells for Self-Powered Electrochemical Biosensing and Logic Biosensing: A Review. *Electroanalysis* **24**, 197–209 (2012).
393. Zebda, A. *et al.* Single Glucose Biofuel Cells Implanted in Rats Power Electronic Devices. *Sci. Rep.* **3**, (2013).
394. Falk, M., Andoralov, V., Silow, M., Toscano, M. D. & Shleev, S. Miniature Biofuel Cell as a Potential Power Source for Glucose-Sensing Contact Lenses. *Anal. Chem.* **85**, 6342–6348 (2013).
395. Zhou, M. *et al.* A Self-Powered ‘Sense-Act-Treat’ System that is Based on a Biofuel Cell and Controlled by Boolean Logic. *Angew. Chem. Int. Ed.* **51**, 2686–2689 (2012).
396. Liu, A. P. & Fletcher, D. A. Biology under construction: in vitro reconstitution of cellular function. *Nat. Rev. Mol. Cell Biol.* **10**, 644–650 (2009).
397. LeDuc, P. R. *et al.* Towards an in vivo biologically inspired nanofactory. *Nat. Nanotechnol.* **2**, 3–7 (2007).
398. Lehner, R., Wang, X., Wolf, M. & Hunziker, P. Designing switchable nanosystems for medical application. *J. Controlled Release* **161**, 307–316 (2012).
399. Sarpeshkar, R. *Ultra low power bioelectronics: fundamentals, biomedical applications, and bio-inspired systems.* (Cambridge University Press, 2010).
400. Miyamoto, T., Razavi, S., DeRose, R. & Inoue, T. Synthesizing Biomolecule-Based Boolean Logic Gates. *ACS Synth. Biol.* **2**, 72–82 (2013).
401. Shoval, O. *et al.* Fold-change detection and scalar symmetry of sensory input fields. *Proc. Natl. Acad. Sci.* **107**, 15995–16000 (2010).
402. Bradley, R. W. & Wang, B. Designer cell signal processing circuits for biotechnology. *New Biotechnol.* (2015). doi:10.1016/j.nbt.2014.12.009
403. Roquet, N. & Lu, T. K. Digital and analog gene circuits for biotechnology. *Biotechnol. J.* **9**, 597–608 (2014).
404. Bonnet, J. & Endy, D. Switches, Switches, Every Where, In Any Drop We Drink. *Mol. Cell* **49**, 232–233 (2013).
405. Siuti, P., Yazbek, J. & Lu, T. K. Synthetic circuits integrating logic and memory in living cells. *Nat. Biotechnol.* **31**, 448–452 (2013).
406. Bonnet, J., Yin, P., Ortiz, M. E., Subsoontorn, P. & Endy, D. Amplifying Genetic Logic Gates. *Science* **340**, 599–603 (2013).

407. Bonnet, J., Subsoontorn, P. & Endy, D. Rewritable digital data storage in live cells via engineered control of recombination directionality. *Proc. Natl. Acad. Sci.* **109**, 8884–8889 (2012).
408. Gardner, T. S., Cantor, C. R. & Collins, J. J. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403**, 339–342 (2000).
409. Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403**, 335–338 (2000).
410. Khalil, A. S. & Collins, J. J. Synthetic biology: applications come of age. *Nat. Rev. Genet.* **11**, 367–379 (2010).
411. Lu, T. K., Khalil, A. S. & Collins, J. J. Next-generation synthetic gene networks. *Nat. Biotechnol.* **27**, 1139–1150 (2009).
412. Ang, J., Harris, E., Hussey, B. J., Kil, R. & McMillen, D. R. Tuning Response Curves for Synthetic Biology. *ACS Synth. Biol.* **2**, 547–567 (2013).
413. Arkin, A. P. A wise consistency: engineering biology for conformity, reliability, predictability. *Curr. Opin. Chem. Biol.* **17**, 893–901 (2013).
414. Mutalik, V. K. *et al.* Precise and reliable gene expression via standard transcription and translation initiation elements. *Nat. Methods* **10**, 354–360 (2013).
415. Lou, C., Stanton, B., Chen, Y.-J., Munsky, B. & Voigt, C. A. Ribozyme-based insulator parts buffer synthetic circuits from genetic context. *Nat. Biotechnol.* **30**, 1137–1142 (2012).
416. Cambray, G. *et al.* Measurement and modeling of intrinsic transcription terminators. *Nucleic Acids Res.* **41**, 5139–5148 (2013).
417. Nielsen, A. A., Segall-Shapiro, T. H. & Voigt, C. A. Advances in genetic circuit design: novel biochemistries, deep part mining, and precision gene expression. *Curr. Opin. Chem. Biol.* **17**, 878–892 (2013).
418. Moon, T. S., Lou, C., Tamsir, A., Stanton, B. C. & Voigt, C. A. Genetic programs constructed from layered logic gates in single cells. *Nature* **491**, 249–253 (2012).
419. Cardinale, S. & Arkin, A. P. Contextualizing context for synthetic biology - identifying causes of failure of synthetic biological systems. *Biotechnol. J.* **7**, 856–866 (2012).
420. Mirasoli, M., Feliciano, J., Michelini, E., Daunert, S. & Roda, A. Internal response correction for fluorescent whole-cell biosensors. *Anal. Chem.* **74**, 5948–5953 (2002).
421. Bugaj, L. J. & Schaffer, D. V. Bringing next-generation therapeutics to the clinic through synthetic biology. *Curr. Opin. Chem. Biol.* **16**, 355–361 (2012).
422. Kwok, R. Five hard truths for synthetic biology. *Nature* **463**, 288–290 (2010).
423. Lapique, N. & Benenson, Y. Digital switching in a biosensor circuit via programmable timing of gene availability. *Nat. Chem. Biol.* **10**, 1020–1027 (2014).
424. Daniel, R., Rubens, J. R., Sarpeshkar, R. & Lu, T. K. Synthetic analog computation in living cells. *Nature* **497**, 619–623 (2013).
425. Sarpeshkar, R. Analog synthetic biology. *Philos. Trans. R. Soc. Math. Phys. Eng. Sci.* **372**, 20130110–20130110 (2014).
426. Zhang, H. *et al.* Rational design of a biosensor circuit with semi-log dose-response function in *Escherichia coli*. *Quant. Biol.* **1**, 209–220 (2013).
427. Purcell, O. & Lu, T. K. Synthetic analog and digital circuits for cellular computation and memory. *Curr. Opin. Biotechnol.* **29**, 146–155 (2014).
428. Mootz, H. D., Blum, E. S., Tyszkiewicz, A. B. & Muir, T. W. Conditional protein splicing: a new tool to control protein structure and function in vitro and in vivo. *J. Am. Chem. Soc.* **125**, 10561–10569 (2003).
429. Selgrade, D. F., Lohmueller, J. J., Lienert, F. & Silver, P. A. Protein Scaffold-Activated Protein Trans-Splicing in Mammalian Cells. *J. Am. Chem. Soc.* **135**, 7713–7719 (2013).
430. Park, S.-H., Zarrinpar, A. & Lim, W. A. Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. *Science* **299**, 1061–1064 (2003).
431. Bonger, K. M., Chen, L., Liu, C. W. & Wandless, T. J. Small-molecule displacement of a cryptic degron causes conditional protein degradation. *Nat. Chem. Biol.* **7**, 531–537 (2011).

432. Prindle, A. *et al.* Rapid and tunable post-translational coupling of genetic circuits. *Nature* **508**, 387–391 (2014).
433. O’Shaughnessy, E. C., Palani, S., Collins, J. J. & Sarkar, C. A. Tunable Signal Processing in Synthetic MAP Kinase Cascades. *Cell* **144**, 119–131 (2011).
434. Wang, B., Barahona, M., Buck, M. & Schumacher, J. Rewiring cell signalling through chimaeric regulatory protein engineering. *Biochem. Soc. Trans.* **41**, 1195–1200 (2013).
435. Lienert, F. *et al.* Two- and three-input TALE-based AND logic computation in embryonic stem cells. *Nucleic Acids Res.* **41**, 9967–9975 (2013).
436. Nielsen, A. A. & Voigt, C. A. Multi-input CRISPR/Cas genetic circuits that interface host regulatory networks. *Mol. Syst. Biol.* **10**, 763–763 (2014).
437. Lohmueller, J. J., Armel, T. Z. & Silver, P. A. A tunable zinc finger-based framework for Boolean logic computation in mammalian cells. *Nucleic Acids Res.* **40**, 5180–5187 (2012).
438. Zalatan, J. G. *et al.* Engineering Complex Synthetic Transcriptional Programs with CRISPR RNA Scaffolds. *Cell* **160**, 339–350 (2015).
439. Khalil, A. S. *et al.* A Synthetic Biology Framework for Programming Eukaryotic Transcription Functions. *Cell* **150**, 647–658 (2012).
440. Kim, J., Khetarpal, I., Sen, S. & Murray, R. M. Synthetic circuit for exact adaptation and fold-change detection. *Nucleic Acids Res.* **42**, 6078–6089 (2014).
441. Chen, Y.-J. *et al.* Programmable chemical controllers made from DNA. *Nat. Nanotechnol.* **8**, 755–762 (2013).
442. Katz, E. & Privman, V. Enzyme-based logic systems for information processing. *Chem. Soc. Rev.* **39**, 1835 (2010).
443. Pischel, U. Chemical approaches to molecular logic elements for addition and subtraction. *Angew. Chem. Int. Ed Engl.* **46**, 4026–4040 (2007).
444. Baron, R., Lioubashevski, O., Katz, E., Niazov, T. & Willner, I. Logic gates and elementary computing by enzymes. *J. Phys. Chem. A* **110**, 8548–8553 (2006).
445. Baron, R., Lioubashevski, O., Katz, E., Niazov, T. & Willner, I. Elementary arithmetic operations by enzymes: a model for metabolic pathway based computing. *Angew. Chem. Int. Ed Engl.* **45**, 1572–1576 (2006).
446. Hart, Y. & Alon, U. The Utility of Paradoxical Components in Biological Circuits. *Mol. Cell* **49**, 213–221 (2013).
447. Tam, T. K. *et al.* Biochemically Controlled Bioelectrocatalytic Interface. *J. Am. Chem. Soc.* **130**, 10888–10889 (2008).
448. Poghossian, A. *et al.* Interfacing of biocomputing systems with silicon chips: Enzyme logic gates based on field-effect devices. *Procedia Chem.* **1**, 682–685 (2009).
449. Wagler, P. F., Tangen, U., Maeke, T. & McCaskill, J. S. Field programmable chemistry: Integrated chemical and electronic processing of informational molecules towards electronic chemical cells. *Biosystems* **109**, 2–17 (2012).
450. Esvelt, K. M. & Wang, H. H. Genome-scale engineering for systems and synthetic biology. *Mol. Syst. Biol.* **9**, 641–641 (2014).
451. Gibson, D. G. Programming biological operating systems: genome design, assembly and activation. *Nat. Methods* **11**, 521–526 (2014).
452. Yetisen, A. K., Akram, M. S. & Lowe, C. R. Paper-based microfluidic point-of-care diagnostic devices. *Lab. Chip* **13**, 2210 (2013).
453. Huang, H. & Densmore, D. Integration of microfluidics into the synthetic biology design flow. *Lab. Chip* **14**, 3459 (2014).
454. Guo, M. T., Rotem, A., Heyman, J. A. & Weitz, D. A. Droplet microfluidics for high-throughput biological assays. *Lab. Chip* **12**, 2146 (2012).
455. Rivet, C., Lee, H., Hirsch, A., Hamilton, S. & Lu, H. Microfluidics for medical diagnostics and biosensors. *Chem. Eng. Sci.* **66**, 1490–1507 (2011).
456. Ausländer, S., Wieland, M. & Fussenegger, M. Smart medication through combination of synthetic biology and cell microencapsulation. *Metab. Eng.* **14**, 252–260 (2012).

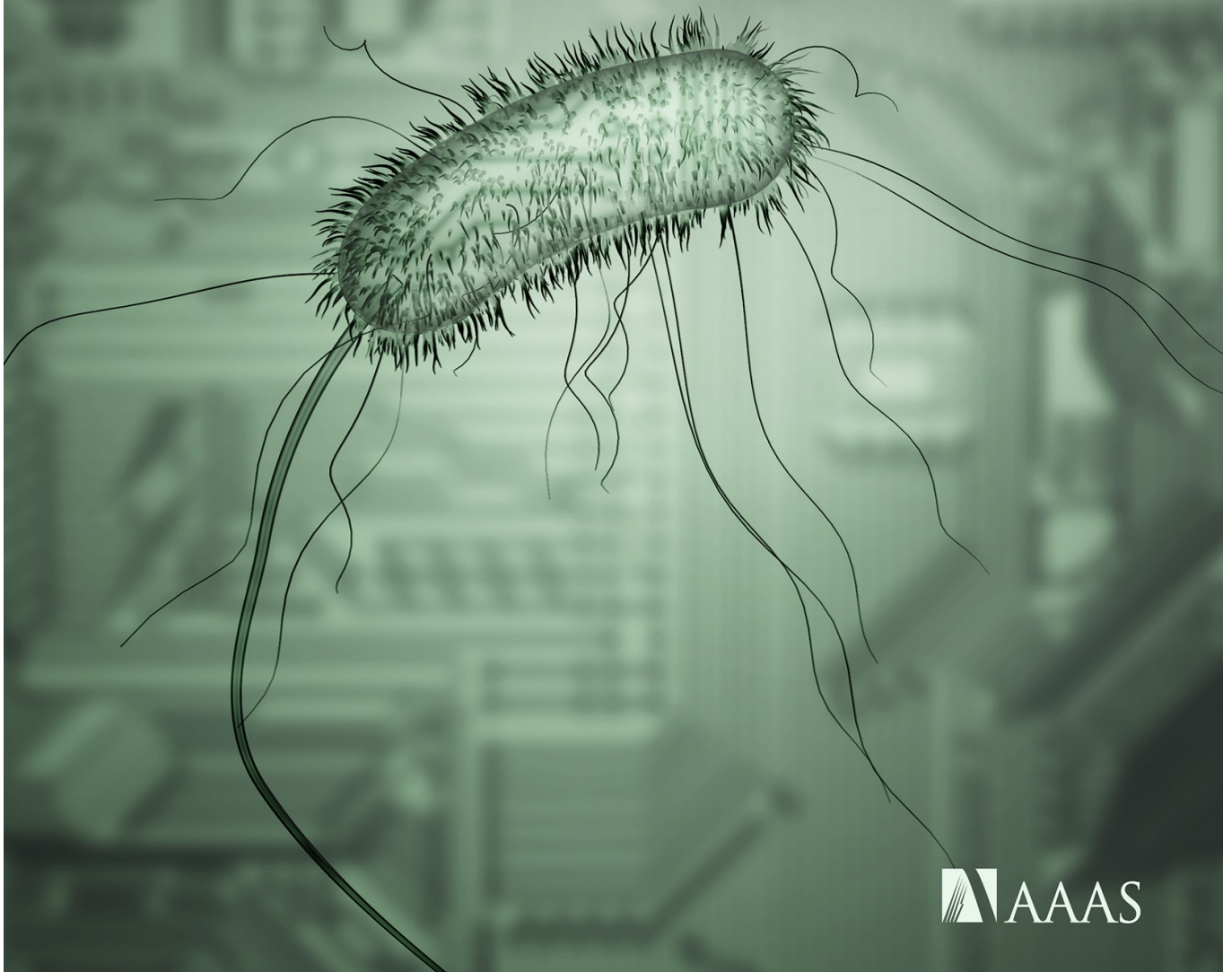
457. Seliktar, D. Designing Cell-Compatible Hydrogels for Biomedical Applications. *Science* **336**, 1124–1128 (2012).
458. Cohen-Karni, T., Langer, R. & Kohane, D. S. The Smartest Materials: The Future of Nanoelectronics in Medicine. *ACS Nano* **6**, 6541–6545 (2012).
459. Jewett, M. C. & Patolsky, F. Nanobiotechnology: synthetic biology meets materials science. *Curr. Opin. Biotechnol.* **24**, 551–554 (2013).
460. Choi, Y. & Yau, S.-T. Ultrasensitive biosensing on the zepto-molar level. *Biosens. Bioelectron.* **26**, 3386–3390 (2011).
461. Cohen-Karni, T. *et al.* Synthetically Encoded Ultrashort-Channel Nanowire Transistors for Fast, Pointlike Cellular Signal Detection. *Nano Lett.* **12**, 2639–2644 (2012).
462. Tian, B. *et al.* Macroporous nanowire nanoelectronic scaffolds for synthetic tissues. *Nat. Mater.* **11**, 986–994 (2012).
463. Tian, B. & Lieber, C. M. Synthetic Nanoelectronic Probes for Biological Cells and Tissues. *Annu. Rev. Anal. Chem.* **6**, 31–51 (2013).
464. Duan, X., Fu, T.-M., Liu, J. & Lieber, C. M. Nanoelectronics-biology frontier: From nanoscopic probes for action potential recording in live cells to three-dimensional cyborg tissues. *Nano Today* **8**, 351–373 (2013).
465. Densmore, D. M. & Bhatia, S. Bio-design automation: software + biology + robots. *Trends Biotechnol.* **32**, 111–113 (2014).
466. Linshiz, G., Goldberg, A., Konry, T. & Hillson, N. J. The Fusion of Biology, Computer Science, and Engineering: Towards Efficient and Successful Synthetic Biology. *Perspect. Biol. Med.* **55**, 503–520 (2012).
467. Galdzicki, M. *et al.* The Synthetic Biology Open Language (SBOL) provides a community standard for communicating designs in synthetic biology. *Nat. Biotechnol.* **32**, 545–550 (2014).
468. Bilitchenko, L. *et al.* Eugene – A Domain Specific Language for Specifying and Constraining Synthetic Biological Parts, Devices, and Systems. *PLoS ONE* **6**, e18882 (2011).
469. Hillson, N. J., Rosengarten, R. D. & Keasling, J. D. j5 DNA Assembly Design Automation Software. *ACS Synth. Biol.* **1**, 14–21 (2012).
470. Appleton, E., Tao, J., Haddock, T. & Densmore, D. Interactive assembly algorithms for molecular cloning. *Nat. Methods* **11**, 657–662 (2014).
471. Marchisio, M. A. & Stelling, J. Automatic Design of Digital Synthetic Gene Circuits. *PLoS Comput. Biol.* **7**, e1001083 (2011).
472. Baker, D. Protein folding, structure prediction and design. *Biochem. Soc. Trans.* **42**, 225–229 (2014).
473. Medema, M. H., van Raaphorst, R., Takano, E. & Breitling, R. Computational tools for the synthetic design of biochemical pathways. *Nat. Rev. Microbiol.* **10**, 191–202 (2012).
474. Beal, J. *et al.* An End-to-End Workflow for Engineering of Biological Networks from High-Level Specifications. *ACS Synth. Biol.* **1**, 317–331 (2012).
475. Lewis, D. D., Villarreal, F. D., Wu, F. & Tan, C. Synthetic Biology Outside the Cell: Linking Computational Tools to Cell-Free Systems. *Front. Bioeng. Biotechnol.* **2**, (2014).
476. Olson, E. J. & Tabor, J. J. Optogenetic characterization methods overcome key challenges in synthetic and systems biology. *Nat. Chem. Biol.* **10**, 502–511 (2014).
477. Sun, N. & Zhao, H. Transcription activator-like effector nucleases (TALENs): A highly efficient and versatile tool for genome editing. *Biotechnol. Bioeng.* **110**, 1811–1821 (2013).
478. Sander, J. D. & Joung, J. K. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat. Biotechnol.* **32**, 347–355 (2014).
479. Shalem, O. *et al.* Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. *Science* **343**, 84–87 (2014).
480. Scheff, T. J. Decision rules, types of error, and their consequences in medical diagnosis. *Behav. Sci.* **8**, 97–107 (2007).
481. Steinhubl, S. R., Muse, E. D. & Topol, E. J. The emerging field of mobile health. *Sci. Transl. Med.* **7**, 283rv3–283rv3 (2015).

482. Laksanasopin, T. *et al.* A smartphone dongle for diagnosis of infectious diseases at the point of care. *Sci. Transl. Med.* **7**, 273re1–273re1 (2015).
483. Schwartz, W. B. Medicine and the computer. The promise and problems of change. *N. Engl. J. Med.* **283**, 1257–1264 (1970).
484. Szolovits, P. & Pauker, S. G. Categorical and probabilistic reasoning in medical diagnosis☆. *Artif. Intell.* **11**, 115–144 (1978).
485. Weiss, S. M., Kulikowski, C. A., Amarel, S. & Safir, A. A model-based method for computer-aided medical decision-making. *Artif. Intell.* **11**, 145–172 (1978).
486. Zakeri, B. & Carr, P. A. The limits of synthetic biology. *Trends Biotechnol.* **33**, 57–58 (2015).
487. Konig, H., Frank, D., Heil, R. & Coenen, C. Synthetic Genomics and Synthetic Biology Applications Between Hopes and Concerns. *Curr. Genomics* **14**, 11–24 (2013).
488. Redford, K. H., Adams, W., Carlson, R., Mace, G. M. & Ceccarelli, B. Synthetic biology and the conservation of biodiversity. *Oryx* **48**, 330–336 (2014).
489. Dana, G. V., Kuiken, T., Rejeski, D. & Snow, A. A. Synthetic biology: Four steps to avoid a synthetic-biology disaster. *Nature* **483**, 29–29 (2012).
490. Mandell, D. J. *et al.* Biocontainment of genetically modified organisms by synthetic protein design. *Nature* **518**, 55–60 (2015).
491. Wright, O., Stan, G.-B. & Ellis, T. Building-in biosafety for synthetic biology. *Microbiology* **159**, 1221–1235 (2013).
492. Breitling, R., Takano, E. & Gardner, T. S. Judging synthetic biology risks. *Science* **347**, 107–107 (2015).
493. Archana Chugh, P. B. Synthetic Biology Based Biosensors and the Emerging Governance Issues. *Curr. Synth. Syst. Biol.* **01**, (2013).
494. Kelley, N. J. *et al.* Engineering Biology to Address Global Problems: Synthetic Biology Markets, Needs, and Applications. *Ind. Biotechnol.* **10**, 140–149 (2014).
495. Synthetic Biology Market - Global Industry Analysis, Size, Growth, Share And Forecast, 2012 - 2018. at http://www.researchandmarkets.com/reports/2225060/synthetic_biology_market_global_industry
496. Medical Sensors Market- Global Industry Analysis, Size, Share and Forecast 2013 - 2019. at <http://www.transparencymarketresearch.com/medical-sensors-market.html>
497. Nanomaterials in Personalized Medicine: Global Markets - HLC144A. at <http://www.bccresearch.com/market-research/healthcare/nanomaterials-thernostics-personalized-medicine-hlc144a.html>
498. Kahl, L. J. & Endy, D. A survey of enabling technologies in synthetic biology. *J. Biol. Eng.* **7**, 13 (2013).
499. Google's Nanoparticle Diagnostic Vision | November 24, 2014 Issue - Vol. 92 Issue 47 | Chemical & Engineering News. at <http://cen.acs.org/articles/92/i47/Googles-Nanoparticle-Diagnostic-Vision.html>
500. Menezes, A. A., Cumbers, J., Hogan, J. A. & Arkin, A. P. Towards synthetic biological approaches to resource utilization on space missions. *J. R. Soc. Interface* **12**, 20140715–20140715 (2014).
501. NASA Technical Reports Server (NTRS) - Nanotechnology Enabled Biological and Chemical Sensors. at <http://ntrs.nasa.gov/search.jsp?R=20120000087>
502. Terry, M. NASA, telemedicine, and endotronix: how NASA's research led to the creation of a cutting-edge telemedicine company. *Telemed. J. E-Health Off. J. Am. Telemed. Assoc.* **16**, 528–532 (2010).
503. Biomedical engineering / Space for health / Space Engineering & Technology / Our Activities / ESA. at http://www.esa.int/Our_Activities/Space_Engineering_Technology/Space_for_health/Biomedical_engineering

504. Landrain, T., Meyer, M., Perez, A. M. & Sussan, R. Do-it-yourself biology: challenges and promises for an open science and technology movement. *Syst. Synth. Biol.* **7**, 115–126 (2013).
505. Birth of the DIY Malaria Detector | Popular Science. at <<http://www.popsci.com/blog-network/biohackers/birth-diy-malaria-detector>>
506. Maisel, W. H. & Kohno, T. Improving the Security and Privacy of Implantable Medical Devices. *N. Engl. J. Med.* **362**, 1164–1166 (2010).
507. NEST-ethics in convergence : testing NEST-ethics in the debate on converging technologies for improving human performance - University of Twente Student Theses. at <<http://essay.utwente.nl/59321/>>
508. Douglas, C. M. W. & Stemerding, D. Governing synthetic biology for global health through responsible research and innovation. *Syst. Synth. Biol.* **7**, 139–150 (2013).
509. Juanola-Feliu, E. *et al.* Design of a Customized Multipurpose Nano-Enabled Implantable System for In-Vivo Theranostics. *Sensors* **14**, 19275–19306 (2014).
510. Sollis, K. A. *et al.* Systematic Review of the Performance of HIV Viral Load Technologies on Plasma Samples. *PLoS ONE* **9**, e85869 (2014).
511. Liu, C.-H. *et al.* Comparison of Abbott RealTime HCV Genotype II with Versant Line Probe Assay 2.0 for Hepatitis C Virus Genotyping. *J. Clin. Microbiol.* **53**, 1754–1757 (2015).
512. Windmiller, J. R. *et al.* Boolean-format biocatalytic processing of enzyme biomarkers for the diagnosis of soft tissue injury. *Sens. Actuators B Chem.* **150**, 285–290 (2010).
513. Manesh, K. M. *et al.* Enzyme logic gates for the digital analysis of physiological level upon injury. *Biosens. Bioelectron.* **24**, 3569–3574 (2009).
514. Kumari, A. *et al.* Biosensing systems for the detection of bacterial quorum signaling molecules. *Anal. Chem.* **78**, 7603–7609 (2006).
515. Alloush, H. M. *et al.* A bioluminescent microbial biosensor for in vitro pretreatment assessment of cytarabine efficacy in leukemia. *Clin. Chem.* **56**, 1862–1870 (2010).
516. Rantala, A. *et al.* Luminescent bacteria-based sensing method for methylmercury specific determination. *Anal. Bioanal. Chem.* **400**, 1041–1049 (2011).
517. Rössger, K., Charpin-El-Hamri, G. & Fussenegger, M. Bile acid-controlled transgene expression in mammalian cells and mice. *Metab. Eng.* **21**, 81–90 (2014).
518. Gupta, S., Bram, E. E. & Weiss, R. Genetically Programmable Pathogen Sense and Destroy. *ACS Synth. Biol.* **2**, 715–723 (2013).

Science Translational Medicine

27 May 2015



 AAAS

115. Courbet, A., Endy, D., Renard, E., Molina, F. & Bonnet, J. Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Sci. Transl. Med.* **7**, 289ra83–289ra83 (2015).