Life as We Don't Know It

Protocell experiment during the *Making_Life II* workshop 2014. Photo by Erich Berger.

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Alternative Biofacts – Life as we don't (yet) know it

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Abstract

Life as we know it, the result of more than 3.5 billion years of evolution, has a remarkably unique and uniform biochemistry and genetic information processing. Science is now going beyond these uniform structures and therefore creating new-to-nature forms of life. Here, we discuss some important (yet often neglected) concepts, ideas and empirical works that will essentially contribute to our deeper understanding of life as we know it, and open up the possibilities to understand, anticipate and engineer new forms of life. In this context, we describe the field of xenobiology and explain its aims to expand the natural framework of scaffolds, chemistries and building blocks to achieve new-to-nature biodiversity. The molecules, molecular complexes and processes along the flow of genetic information ("central dogma") are particularly attractive targets for xenobiology. For example, the development of alternative nucleic acids (xenonucleic acids, XNAs) or permutating the genetic code from its current form via systematic introduction of non-canonical amino acids are promising routes towards biocontained synthetic cells. Technologies derived from these scientific achievements are expected to (a) design, construct and evolve microbes with novel metabolic capabilities; (b) produce useful chemicals and materials with novel characteristics; (c) propagate synthetic eco-systems and food-chains; and (d) might assist in recovering from the ongoing mass extinction. Much needs to be understood about new-to-nature life forms, but we suggest that it will be of great interest not only for science but also for the art-science community.

Life as unity

The ancient Greeks, including Aristotle, believed in *generatio spontanea*, the idea that life could suddenly come into being from non-living matter on an everyday basis. Pioneering empirical examinations of Pasteur in the 19th century, however, demonstrated that life in contemporary Earth is not generated spontaneously from non-living matter, but that *omne vivum ex ovo*, all life comes from life (Pasteur 1922). With this matter settled for once, it remained unclear of what kind of components life is made of. In this way, Pasteur provided a solid experimental basis for what we know today as

inheritance, or vertical gene transfer (VGT). Since Pasteur, our knowledge about basic genetics (especially on genetic code and horizontal gene transfer) expanded and latest at the beginning of the 21 century it becomes clear that the genetic code can be referred to as the "lingua franca" of life on earth, which enables the maintenance of universal biochemistry (Kubyshkin, Acevedo-Rocha et al. 2018). This establishes the basis for the transfer of genetic information (VGT) from one to the next generation in the frame of one species or population but also dissemination of biological novelty through horizontal gene transfer (HGT) between different species and populations.

Ideas about the interconnectedness of life on our planet came e.g. from Austrian Geologist Eduard Suess, who coined the term "biosphere" in 1875. The Russian/Ukrainian geologist V.I. Vernadsky published a book in 1926 entitled "The Biosphere" were these ideas were intuitively anticipated and expressed (Vernadsky 1998)[2]. Vernadsky captured all essential components that were described as "Gaia Hypothesis" in the 1970s which postulates that the chemical composition of the Earth is unique compared to other planets and similar cosmic bodies due to the life processes (Lovelock and Margulis 1974). Vernadsky proposed the hypothesis that all living matter can be considered as a single entity – a (super) organism that spans the entire surface of the earth – a biosphere. It is a unique system that stores chemical energy by converting (mainly) solar radiation into mechanical, molecular and chemical energy.

Today, we know that Vernadsky was intuitively right: although there are species barriers in the production of offspring (VGT), there are no geographical limits to HGT in all habitats where bacteria, eukaryotes, archaea and virus particles thrive – from deep-sea hydrothermal wells to Siberian permafrost (Pawluk 2017, Reche, D'Orta et al. 2018).

Chemical composition and organization of life's unity

Scientists used a large part of the 20th century to reveal that the conjecture of "The Biosphere" and the Gaia hypothesis prove to be correct up to the molecular level. It turned out that the *basic* chemical constitution of all living organisms consists of a limited number of small molecules and polymers. The building blocks of these molecules consist predominantly of only six atoms, summarized in the acronym CHNOPS, which stands for Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorus and Sulfur. Carbohydrates are molecules consisting of carbon and hydrogen atoms that are fundamental to all life forms on Earth as they play an essential role in all aspects of biology, e.g. they can store energy (e.g. as sugar molecules), provide structural support (as polysaccharides), and play an important role in proteins and information storage (such as DNA). Nitrogen is an essential component of amino acids that make up proteins and enzymes, some of the most important building blocks of life, but is also part of DNA and enables photosynthesis in chlorophyll. Oxygen is most relevant for the energy flow and breathing. Phosphorus in combination with carbon and hydrogen form lipids that include fats, oils, and waxes to store energy or protect the organism. Lipids are indispensable to cells as they make up the cell membrane, a thin layer of molecules that define the inner and outer space of the cell. Phosphorus is also essential in the formation of the backbone

2 The book remained largely unknown until its recent English translation.

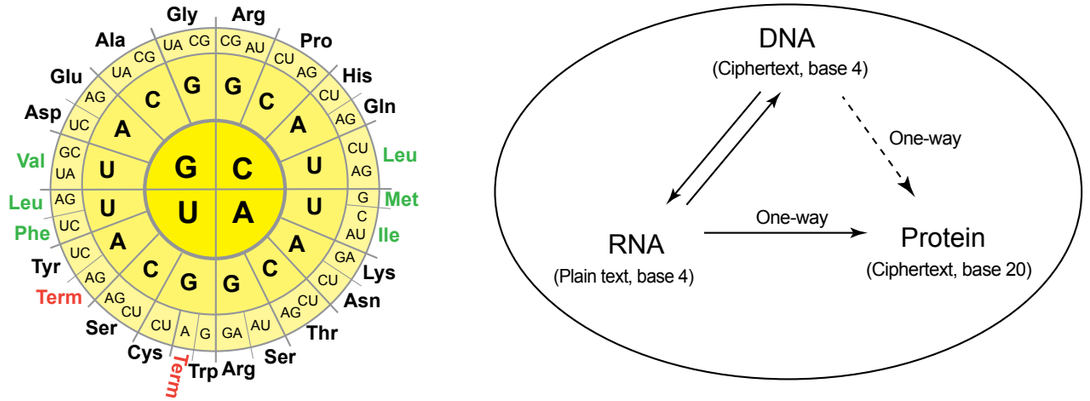

Figure 1 (Left) Circular depiction of the genetic code (Kubyshkin, Acevedo-Rocha et al. 2018) (Right). "Central dogma" of molecular biology describes essentially the unidirectional flow of genetic information in life (Crick 1970). That means, once "information" has passed into protein it cannot get out again. Information inherited as DNA is *transcribed* to RNA (as both are nucleic acids, consisting of 4 building blocks or bases) and then *translated* to proteins (that consist of 20 different amino acids). While information can be directly transcribed back and forth between RNA and DNA, information flow from RNA to proteins is a one-way street. In this figure the term base stands for information system on the basis of 4 or 20 building blocks, not the chemical base. In RNA and DNA the chemical and informational term happens both to be called base.

structure of DNA. The final letter S stands for sulfur, an essential component of some amino acids.

While CHNOPS describes the building blocks of life on the atomic level, it is actually the molecular level that sustains life. There are basically four categories of molecules that are paramount for all living beings: proteins, linear polymers such as proteins, and nucleic acids (e.g. DNA) and large molecules such as carbohydrates and lipids (See: Cooper and Sunderland 2000 for more details on the chemistry of life). Proteins and nucleic acids, as well as some carbohydrates and to a certain extent lipid, are macromolecules, meaning that they consist of a limited set of similar building blocks. In the nucleic acid DNA, only four building blocks (A, T, G, C) make up for example the entire human genome, which totals about three billion of those four building blocks. While chemists know more than 700 amino acids, proteins are made up of no more than 20 (+2) so called "proteinogenic" amino acid building blocks. It can be seen as one of the greatest insights of the 20th century that life consists of a very specific and small fraction of all theoretically possible CHNOPS containing molecules. A set of molecules widely considered as "canonical" (Cooper 2000)

From the vampire squid in the abyss of the ocean, to the highest trees of the rainforest, to bacteria living in our guts, to extremophile archaebacteria that prefer hot springs or acidic rivers, to ourselves the human species, all forms of life we know so far are made up of these specific molecules of life.

But not only do they share a common, one could also say normative, biochemistry, they also show a remarkable lack of diversity in the way information is transferred from one type of biomolecule to the other (see figure 1).

DNA and RNA are made up of 4 building blocks or bases, in case of DNA it is ATGC, while in RNA it is AUGC. Even though T and U are different, the transcription from one to the other is bijective, as A matches with T (or U) and G with C. So no encoding is necessary. Only when a text based on 4 letters is translated to a text with 20 letters, a *code* is needed. In other words a code is the key to translate an input to an output when there is more than one possibility to do so. Extant biology without exception uses a system where three nucleic building blocks, a so-called triplet, define one amino acid. Since we have four building blocks, times three we have a total of 4 × 4 × 4 = 64 triplets coding for 20 amino acids and the stop signal (21 in total). The importance of the code becomes even more clear when the total number of possible codes that code for 20 amino acids and one stop codon is calculated, resulting in the enormous number of 418,000, 000,000,000,000,000,000,000,000,000,000,000 ,000,000,000,000,000,000,000,000,000,000,000 ,000,000,000,000 possibilities ($21^{64} = 4{,}18 \times 10^{84}$) (Schmidt 2019). This number is higher than the total estimated number of elementary particles in the observable universe 10^{78} (Silk 2005). Contemplating this number, it becomes clear that evolution would never have been able to generate and select all possible genetic codes. There are plausible theories to why the genetic code became the way it is (Hartman and Smith 2014, Wong, Ng et al. 2016), one of the (many) constraints is the robustness of the code. In other words, the genetic code is exceptionally tolerant to DNA mutations and will produce the same or very similar proteins despite changes in the composition of nucleic acids (Freeland and Hurst 1998).

The genetic code is also called standard genetic code, because it is implemented in all but a few organisms (or organelles, subcellular bodies such as mitochondria). Besides the standard code, so far 25 slightly different codes have been discovered in nature (see https://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi). Some more will probably be discovered in the future, but it remains absolutely clear that a vast majority of all 1,5 million known and 10 million estimated species on Earth (Mora,

Tittensor et al. 2011) use exactly the same genetic code. The code-normativity of life of Earth, the tremendous lack of diversity in interpreting genetic information, is overwhelmingly clear. Evolutionary biologists consider this knowledge a strong indication that all living beings are related to one another, in the sense that we might all share an unknown last universal common ancestor (LUCA) that populated the Earth billions of years ago (Acevedo-Rocha, Fang et al. 2013).

From Analysis to Synthesis

While in the 20th century biology was mainly seen as an analytical science, some visionaries, such as James Danielli (1911–1984) were able to glimpse into the future of life. As Danielli wrote in 1972 in his landmark article "Artificial Synthesis of New Life Forms", all sciences eventually undergo three phases, namely the phase of (1) description, (2) analysis and (3) synthesis. While physics and chemistry had all arrived in the stage of synthesis, biology in the 1970s was still an overwhelmingly analytical science (with the exception of a few recombinant genetic experiments). Since the beginning of the 21st century there are clear indications and outright declarations to convert biology into a real synthetic discipline. Not surprisingly, the third phase of biology, for a lack of a better term, was baptised synthetic biology (although the term itself goes back to the beginning of the 20th century, see (Le Duc 1910).

For the last 15–20 years synthetic biology has attempted to redesign natural systems and to make biology easer to engineer. The field of synthetic biology, however, is less homogenous than one might guess, as many different approaches, methodologies and strategies are used to carry out a number of different goals. One of the most prominent approaches deals with top-down metabolic engineering, in other words, the capacity of (mostly) microbes to convert input (such as sugar or methane) to a desired output (such as fuel or medicine) by redesigning their genetic pathways. This approach uses existing organisms (e.g. yeast, the gut bacterium *E. coli*) and tinkers with selected genes to alter their physiological functionality. It is very much application oriented and may aim to support the bio-economy.

Another approach is the definition of a minimal cell, that is the reduction of the complexity of extant living cells to the point where it can barely survive. These minimal cells would then represent the most basic possible form of life, and could answer the question what life is and what minimal level of complexity is needed to sustain life. An example is the bacteria and parasitic pathogen Mycoplasma that has one of the smallest genomes (about 500,000 base pairs). Scientists, for example, currently try to further cut down the size of the genome of Mycoplasma (Acevedo-Rocha, Fang et al. 2012).

While metabolic engineering and the minimal cell approach both require extant cells as a starting point, the proto- or synthetic cell community wants to create life from scratch. For this bottom-up approach it is necessary to create an empty cell that is then filled with a number of functional biomolecules (Powell 2018).

Animating the bio-imaginary: xenophile biology

Yet another objective of synthetic biology is to try to change the chemical compositions of living cells, i.e. to create an artificial biological diversity (Schmidt 2010). This objective, in turn, fosters a new sub-field of synthetic biology called xenobiology. In ancient Greek, *xenos* meant a stranger or foreigner usually (if not an attacker) to be treated friendly. (The term xenophobic describes an indiscriminate aversion against strangers regardless if they come in peace and good spirits or if they come to conquer and destroy. Xenophilic on the other hand describes the love for strangers.) Since biology is the science of living things, xenobiology describes life forms that are unfamiliar to us.

One of the most striking attempts of xenobiology is to alter the chemical building blocks of nucleic acids (DNA, RNA), the molecules that store most of the hereditary information.

While in all known living beings, genetic information storage and processing rely on just two polymers, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), it is unclear whether their role reflects evolutionary "accidents" or fundamental functional (e.g. chemical or biological) constraints. Using polymerase evolution and design it was shown that genetic information can be stored in and recovered from various alternative genetic polymers[3] collectively called XNA for xeno nucleic acids) not found in Nature (Pinheiro, Taylor et al. 2012). Beyond heredity, specific XNAs have the capacity for Darwinian evolution. This means that heredity and evolution, two hallmarks of life, are not limited to DNA and RNA but are likely to be emergent properties of more than two polymers capable of information storage.

Xenobiologists have also enlarged the genetic alphabet of DNA with unnatural base pairs that led for example to a genetic code that has 6 bases ATGCPZ instead of 4 bases ATGC (Benner and Sismour 2005). So far at least 60 candidate bases (that means hypothetical 3,600 base pairs) were tested for possible incorporation in the DNA (Leconte, Hwang et al. 2008). In a few cases the novel base pairs were introduced to living systems and have been reproduced inside plasmids (a circular form of DNA) in bacteria (Zhang, Lamb et al. 2017). This means the genetic code has been modified by the expansion of the genetic alphabet (Dien, Morris et al. 2018).

Given that over 700 amino acids are known from Nature and only 20 (+2) are used in the genetic code, it probably doesn't come as a surprise that the expanded nucleic acid alphabet is met with an expanded amino acid alphabet, where non-canonical amino acids are used to make polypepdites and proteins (Hoesl and Budisa 2011, Hirose, Tsiamantas et al. 2019). It even seems plausible that not just a few amino acids are replaced, but that they are all replaced by others belonging to an entirely different group of amino acid, undoing an evolutionary "decision". Recently Budisa and Kubyshkin provided a solid argumentation that original development of the polypeptide biosynthesis seems more a random walk rather than a 'choice' or a physical-chemically imposed solution, and Nature simply recruited the available components, in this particular case – a set of canonical amino acids encoded in genes (Kubyshkin and Budisa, 2019). They also provided a long-term perspective by creating another scaffold capable to allow a functional proteome based on different building blocks and underlying principles

3 The nucleic acids were: HNA (1,5 anhydrohexitol nucleic acids), CeNA (cyclohexenyl nucleic acids), LNA (2'-O,4'-C-methylene-ß-D-ribonucleic acids; locked nucleic acids), ANA (arabinonucleic acids), FANA (2™-fluoro-arabinonucleic acid) and TNA (α-L-threofuranosyl nucleic acids)

of protein folding than those that we know (Kubyshkin, Grage et al. 2018).

In many cases the incorporation of non-canonical amino acids is combined with a different form of nano-performativity[4]. A few examples are known where the genetic code itself was changed. To change the code, one strategy is to first select an amino acid or stop codon that is encoded by more than one triplet. The natural redundancy is important here, because by carefully editing the genome it is possible to replace one triplet that codes for amino acid X or a stop codon with another triplet coding for the same amino acid or stop codon. When this has been achieved, the corresponding tRNA (the molecule that mediates the code) can be modified without harming the organism, and a different amino acid can be linked to the tRNA (Lajoie, Rovner et al. 2013, Kubyshkin and Budisa 2017). In one case a bacteria was reprogrammed so it would only use 57 instead of 64 triplets (Ostrov, Landon et al. 2016).

One of the keystones of Darwinian evolution is the fact that geographically (and hence genetically) isolated species tend to evolve unique and heritable changes over time. The classical example is Darwin's finches, which illustrates the way gene pools of the finch have adapted to take advantage of different food constrains. What is true for Darwin's finches also applies for cells in general. Through man-made, directed evolution of life-forms we can attempt to achieve the implementation of new and sophisticated chemistries (elements, reactions, metabolic pathways) into the protoplasm of desired life forms (Wiltschi and Budisa 2007). Xenobiology is the attempt to learn if the chemical standard composition of life forms (invariant for around four billion years) can be changed and whether we could open the door to possible parallel biological worlds, that were not (and could not have been) explored by natural evolution (Hoesl, Oehm et al. 2015).

From chemical to biological synthesis

In the first years of today's ubiquitous synthetic chemistry, the synthesis of complex substances, originally produced from plants and animals, was assumed as an impossible task. Additionally, a lot of physiological conditions were experimentally inaccessible in those days. This left space for the appearance of metaphysical concepts like the idea that organic compounds were just formed in presence of a special, vital power ("*vis vitalis*"), acting exclusively in creatures. Accordingly, metaphysical concepts were used as main criteria to decide between animate and inanimate matter (Church and Regis 2012, Venter 2013). Yet in the beginning of the 19th century, this metaphysical viewpoint was proven wrong by chemical synthesis of organic molecules (e.g. urea Woehler's *Harnstoffsynthese* in 1828) (Wöhler 1828, Multhauf 1966). Although this was not the first milestone for the synthesis of naturally occurring, organic compounds, starting from then, the awareness of the accessibility of natural, organic molecules increased. Complex compounds could be manufactured starting from simple structures in a stepwise and controlled manner. Less than 50 years later, organic synthetic chemistry has turned into an engineering discipline with the ambition to synthesize all naturally occurring, organic substances (Fisher 1907), and even substances that do not occur in Nature. The complete chemical synthesis of any molecule (a natural or artificial product), from simple, commercially available precursors is called

4 Nano-performativity describes human actions on the nanometer level.

"Total synthesis" (Nicolaou, Vourloumis et al. 2000). It is one of the goals in the life sciences to achieve an equivalent success with biological systems (Erb, Jones et al. 2017).

Nature sans frontiere: CHNOPS welcomes FRuSiCl

The fundamental characteristics of wild, synthetic and xenobiology is that in wild and synthetic biology living systems are restructured via exchange and combination of (evolutionary or technically) standardised parts (genes, modules, biobricks), either through horizontal gene transfer or via genetic modification. In contrast, xenobiology uses non-canonical) molecules to create chemically modified organisms (CMOs) (Acevedo-Rocha and Budisa 2011). These CMOs will manage to use other permutations of CHNOPS but also combine non-CHNOPS chemical elements, such as fluorine (F), ruthenium (Ru), silicon (SI) and chlorine (Cl) (Acevedo-Rocha and Schulze-Makuch 2015).

Fluorine (atomic number 17), for example, is the most electronegative element in the periodic table, and its reactive chemistry is beyond the catalytic scope of the vast majority of the conventional enzymes (O'Hagan 2008). So far only one natural enzyme called fluorinase has been found in Nature (in a *Streptomyces* species), that is able to incorporate fluoride (F–) into organic compounds (Dong, Huang et al. 2004), by attaching F to carbon atoms in living cells. Although fluorinase has been characterized in detail (O'Hagan, Schaffrath et al. 2002, Zhu, Robinson et al. 2007), its biotechnological applications are so far limited to a narrow spectrum of small molecules produced *in vitro* (Walker and Chang 2014).

Nature did not use fluorine significantly as a building block for organic matter since it is largely insoluble contained within inorganic substances on Earth (Berger, Voller et al. 2017). While chlorine- or bromine-containing organohalogens were efficiently used by living beings for billions of years of evolution, biotransformation of organoflurine compounds is rather limited due to the exceptional strength of the carbon-fluorine bond. Organofluorine compounds nowadays are rather seen as environmental stressors that generally induce significant biological effects on individual cells and whole populations by enabling inhibition of enzymes, cell-cell communication, membrane transport, and processes for energy generation (Merkel and Budisa 2012). On the other hand, being almost exclusively synthetized by humans (e.g. advanced materials, fine chemicals, drugs or pesticides) there was not sufficiently long evolutionary time for microbial populations to invent and spread resistance mechanisms against such toxic substances (Biava and Budisa 2014).

Therefore, the intense research in this direction is inevitable as organoflurine compounds (which are massively used in human industrial, agricultural and household activities are also known as "inert" substances) will have a strong tendency to accumulate and persist in soil and water, and are therefore will be extremely difficult to remediate. On the other hand, the use of organofluorine compounds to produce biomass or cells with altered metabolism has a great future.

Furthermore, *Streptomyces* is not an ideal host for metabolic engineering of reactions involving fluorine, as it displays high fluorine-sensitivity, slow growth and low yield of fluorinated compounds (Deng, O'Hagan et al. 2004). The EC H2020 research and innovation project SinFonia, aims to transfer fluorinase to a soil bacterium called *Pseudomonas putida* that is also a model organism for industrial biotechnology especially in processes for biopolymer production. SinFonia engineers the metabolism of *P. putida* to execute bio-fluorination reactions leading to new-to-nature fluoropolymers from renewable substrates.

We can even think about the most prominent example of synthetic fluorine containing organic compounds of anthropogenic origin Teflon – a highly fluorinated polymer used in everyday life. Would the biosynthesis of "Teflon-proteins" be a realistic prospect (Budisa, Pipitone et al. 2004)? Given the case that living beings never adopted fluorine as biogenic element, its accommodation into the chemistry of life as we know it is still a formidable challenge. Living organisms would have to be able to survive adaption on fluorine through massive modifications of their enzymes and proteins that are originally evolved on a hydrocarbon basis. This certainly requires the rewriting of their entire genomic text by the accumulation of different types of mutations and their combinations. Given the recent success in the laboratory evolution of the chemical composition of proteins or nucleic acids, we believe that design of artificial cells with fluorine chemistry is a very challenging but achievable goal (Budisa, Kubyshkin and Schulze-Makuch, 2014).

There should be no doubt, that microorganisms and especially bacteria which possess an exceptional capacity to develop fast metabolic or genetic responses to chemical stresses will be used to evolve and proliferate by using exclusively the toxic fluorine containing compounds for growth. Such "fluorous-life" will consist of biocontained microbial strains extremely important for the emerging problems of environmental biosafety. Being reliant on the exclusive presence of the xeno-nutrients for survival and proliferation, these evolved microbial stains are promising platforms for creating fully synthetic life. The engineering of the genetic code allows us to add fluorinated non-canonical amino acids to the existing repertoire of the 20 canonical amino acids prescribed by the genetic code (Budisa 2004).

Fluorine, however, is not the only novel element of interest, in fact there are a number of non-biogenic elements with high enzymatic potential. The metathesis reaction, for example, was exclusively used in synthetic chemistry, but with support from the European Commission (EC) FP7 research project METACODE, it was successfully transferred to the metabolism of bacteria by designing and evolving artificial metalloenzymes. Metalloenzymes are enzymes that contain at least one metal atom that enhances its catalytic power. This is why metathesis is now also possible in vivo, using enzymes that have been designed to incorporate the chemical element **ruthenium** (a rare transition metal with atomic number 44) into an enzyme (Jeschek, Reuter et al. 2016).

Very recently, a paper published by the 2018 Nobel prize winner Frances H. Arnold, showed that an enzyme that catalyzes **silicon** (Si) carbon (C) bonds was evolved, providing a first step toward engineering the biotechnological production of organo-silicon compounds, in other words the direct merging of the carbon and the silicon world (Kan, Lewis et al. 2016). The EC H2020 Future and Emerging Technology project MADONNA is currently investigating the full potential of these new-to-nature organo-silicon compounds.

In a tour de force biochemical experiment, a French-German collaboration showed for the first time that the element **chlorine** (Cl) can be incorporated into one of the most essential building blocks, namely the DNA base T (as in ATGC). In a directed evolution experiment the thymine was replaced by 5`chloro-uracil (Marliere, Patrouix et al. 2011).

These European projects, by the way, demonstrate a form of chemical emancipation[5] from Nature and probably only possible when science does not stop at national borders.

Novel molecular building blocks and codes

The number of potentially novel building blocks for protein biosynthesis is virtually unlimited as organic chemistry can provide a great diversity of non-canonical amino acids, nucleobases and unnatural cofactors that can be used to produce synthetic life either by experimental evolution or de novo chemical syntheses. To achieve these goals, we need first conceptual tools that question/ challenge our current concepts, wisdom and logic behind the amino acid repertoire establishment in evolution and the "frozen" code and conservation of the basic life chemistry (Kubyshkin and Budisa 2017). With such understanding in mind, we would be able to propose a possible scenario ("chemical worlds") for basic building blocks of structural and functional diversification as a starting point for attempts to create alternative life structures (and technologies derived thereof) from the first principles (Acevedo-Rocha and Schulze-Makuch 2015). This is plausible, since *in vitro* works have demonstrated that the creation of a totally new genetic code set is possible. Numerous experiments in microfluidic devices or *in vitro* platforms show that many alternative components of life can be controlled and manipulated (Kubyshkin and Budisa, 2019).

The same can be said for non-canonical DNA bases that have been developed into diagnostic tools for infectious diseases (Benner and Sismour 2005). The unnatural base pair system consists of an expanded genetic alphabet that is built into oligo nucleotide fragments on specific sites, or via enzymatic incorporation of extra, functional components into nucleic acids. These fragments containing unnatural base pairs can be obtained via PCR amplifications. Diagnostic molecular beacons with fluorescent dye linked to the unnatural bases can serve as molecular diagnostic tools, e.g. to target infectious diseases of interest (Kimoto, Cox et al. 2011). Furthermore, aptamers (nucleotide or peptide molecules that bind to a specific target molecule) containing unnatural bases, due to their unique features in affinity, thermo stability and resistance to nucleases, are considered valuable for pharmaceutical applications (Matsunaga, Kimoto et al. 2015).

It should always be kept in mind that life can not be reduced entirely to chemistry nor physics (Figure 2). Life is not just information flow, neither is it only energy flow. It is also not a mere self-organisation with catalytically-driven chemical supercycles. Life is more, it is the organisation (unity) of all these phenomena. Thus, to create synthetic life with an expanded, reduced or altered genetic code, ongoing work should be combined with system bio-engineering work on self-assembled bio-orthogonal compartments and devices, along with alternative energy sources (other than chemo-osmotic gradients), novel types of information transduction pathways and alternative metabolic cycles with new to

5 Emancipation: The act of setting something free from something else. See: http://www.businessdictionary.com/definition/emancipation.html

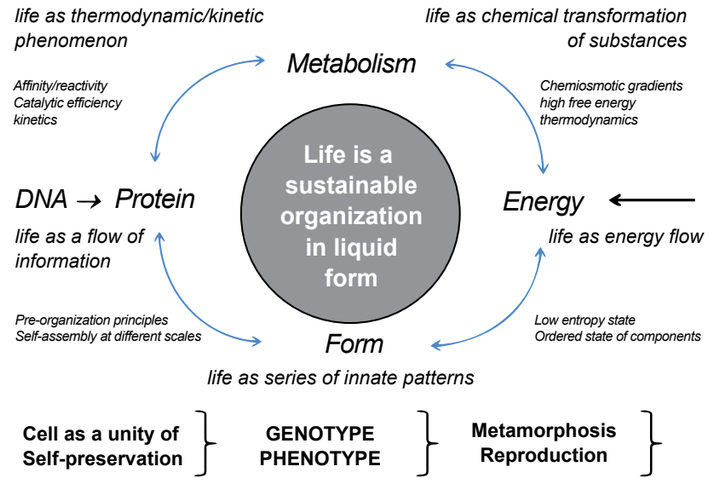

Figure 2 A conceptual view of life with minimal requirements ("minimal cell") as defined by Gànti and others (Gànti 2003). Such a system regulates and controls metabolism, energy supply, and distinct forms/ patterns. It contains at least one subsystem acting as an information carrier; the information contained is fundamental to the entire system (genetic information). It enables maintenance, self-preservation, metamorphosis and reproduction via a complex set of genotype/ phenotype interactions and processes. Prepared according to Diwo and Budisa (2019).

nature catalytic cascades and molecular machines (Agostini, Voller et al. 2017).

This makes a completely new biological world conceivable and plausible. The design of genetically modified organisms (in the context of classical genetics) is only the beginning of a long road in search of reliable methods for the evolution and development of artificial biodiversity while preserving the old natural world. An important task for xenobiology, therefore, is to pursue chemically-diverse artificial evolution of viable and robust cells that can grow and replicate in isolation from natural species (Schmidt 2010, Schmidt and de Lorenzo 2012, Acevedo-Rocha and Budisa 2016, Schmidt and de Lorenzo 2016).

If we accomplish to change the way the genetic code is read in a living organism as well as to add new "letters" or building blocks, the corresponding cell will constitute an informational enclave since the genetic exchange (called horizontal gene transfer or HGT) with natural cells is impaired. This could be an important aspect in regards to biological safety, because the risk of horizontal gene transfer to natural cells is supposed to be strongly reduced (Acevedo-Rocha and Budisa 2011, Wright, Stan et al. 2013, Budisa 2014, SCHER, SCENIHR et al. 2014, SCHER, SCENIHR et al. 2015, Wright, Delmans et al. 2015). Therefore, xenobiology seeks for conditions in which the cells can be cultivated in the laboratory or released into the environment, but stay genetically isolated from naturally occurring species (Schmidt 2013). These conditions might also include e.g. supercritical fluids that have different properties compared to regular fluids and could play a role as life-sustaining solvents for alien life forms (Budisa and Schulze-Makuch, 2014).

Negotiating a responsible use of xenobiology

Synthetic chemistry has without doubt been a major factor in improving the lives of billions of people. Synthetic chemistry is so ubiquitous that we hardly recognise how important it is to support our (post) modern lifestyles, supplying materials, pharmaceuticals, textiles, fuel, building materials etc. Chemistry, however, was also responsible for a number of problems (such as persistent organic pollutants or POPs, toxins, endocrine disruptors among others). (Synthetic) chemistry is a doubled-edged sword with the power to do good and bad, and is therefore regulated in most parts of the world. In Europe

> REACH (EC 1907/2006) aims to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. This is done by the four processes of REACH, namely the registration, evaluation, authorisation and restriction of chemicals. REACH also aims to enhance innovation and competitiveness of the EU chemicals industry. "No data no market": the REACH Regulation places responsibility on industry to manage the risks from chemicals and to provide safety information on the substances.[6]

Other even more stringent regulations apply to specific industries, such as the pharmaceutical industry. Since the mid 1970's regulations are also in place for the production and use of genetically modified organisms, aiming to avoid unintended consequences and intentional misuse by rogue actors. So far xenobiology is supposed to be covered by either REACH (on the chemical level) or the GMOs regulations (on the biological level).

Optimise diversity

Further expansion of the capabilities to create biochemical diversity with xenobiology will raise questions to which extent the existing guidelines, codes of conduct, practices and regulations are sufficient to cover novel forms of life. The current technical capabilities of xenobiology are still rather modest, mostly restricted to proof of concepts with few applications available, but they show the pathway to a future where multilayered radical diversification is the norm and not the exception. One could say the time has come when the central dogma of biology, the DNA-RNA-proteinogenic amino acid-"normativity", is challenged by alternative life forms and biochemical arrangements. Should natural life forms be privileged over currently unknown, yet unborn and evolutionary marginalised versions of life?

It is clear that life can manifest itself in a number of different forms. Up to now most biologists have quickly assumed that natural forms of life have evolved because no other forms of life are as fit. By beginning to understand that Nature, for a number of reasons, did not have the chance to test and select all possible variants of life supporting molecules and codes, we start to see more clearly the limitations of evolutionary processes when it comes to the exploration of the animated combinatorial space.

Mankind is responsible for the latest, the sixth, mass extinction of life on Earth. Even if all human

6 http://ec.europa.eu/environment/chemicals/reach/reach_en.htm

induced extinction factors (mainly land use change and agriculture) would suddenly disappear, it would take millions of years for biodiversity to recover (Ceballos and Ehrlich 2018, Davis, Faurby et al. 2018).

Synthetic biology might be used for conservation of wildlife (Redford, Adams et al. 2013), it has also offered (our bad conscience) the option of de-extinction, to bring back life forms that once populated the Earth (Jennings 2017), or other ways to reduce biodiversity loss (Piaggio, Segelbacher et al. 2017) or reverse ecosystem degradation (Maestre, Sole et al. 2017). Contrary to these conservative views, synthetic and xenobiology might actually add novelty to ecosystems (Fuentes 2018). If we are allowed to dream big, maybe it can even enable the recovery from the sixth mass extinction, supporting the next explosive radiation of biodiversity, see e.g. (Sahney and Benton 2008).

In the past, visions of future life forms and ecologies – as shown for example in the epic book *After Man: A Zoology of the Future* (Dixon 1981) – extrapolated canonic evolutionary principles to the far future.

With tools such as synthetic and xenobiology, however, humans could attempt to start a bioremediation on a global scale (de Lorenzo, Marliere et al. 2016). Before we focus on this huge task, however, we obviously need to better understand the challenges and opportunities of designed and novel ecosystems (Higgs 2017, Sole, Montanez et al. 2018).

Speculative artworks[7] both communicate the challenge and start to explore ways to respond. Designing the recovery from the contemporary mass extinction could indeed be a very tempting topic not only for science but also for the art-science community (Harrower et al. 2018).

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7 See for example "Designing for the sixth extinction" by Daisy Ginsberg. https://www.daisyginsberg.com/work/designing-for-the-sixth-extinction

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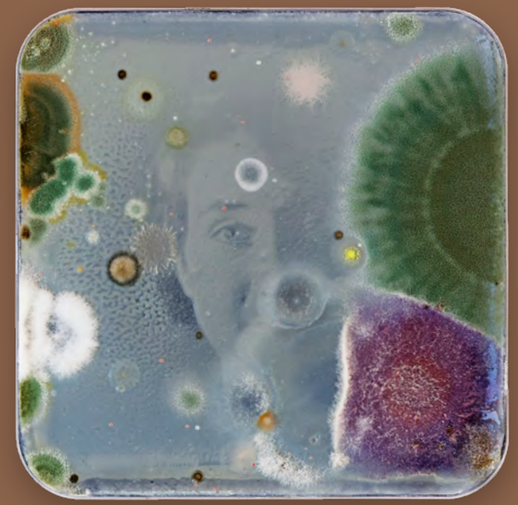

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