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Published in:
Trends in Biotechnology

Link to article, DOI:
10.1016/j.tibtech.2022.06.004

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

Trends in Biotechnology

CellPress OPEN ACCESS

Feature Review

Challenges and opportunities in bringing nonbiological atoms to life with synthetic metabolism

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The relatively narrow spectrum of chemical elements within the microbial 'biochemical palate' limits the reach of biotechnology, because several added-value compounds can only be produced with traditional organic chemistry. Synthetic biology offers enabling tools to tackle this issue by facilitating 'biologization' of non-canonical chemical atoms. The interplay between xenobiology and synthetic metabolism multiplies routes for incorporating nonbiological atoms into engineered microbes. In this review, we survey natural assimilation routes for elements beyond the essential biology atoms [i.e., carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), and sulfur (S)], discussing how these mechanisms could be repurposed for biotechnology. Furthermore, we propose a computational framework to identify chemical elements amenable to biologization, ranking reactions suitable to build synthetic metabolism. When combined and deployed in robust microbial hosts, these approaches will offer sustainable alternatives for smart chemical production.

Highlights

A few non-canonical chemical elements are already present in living systems, and their assimilation routes have been characterized; however, this represents only a fraction of the atoms that could be incorporated in biology.

Nature holds a treasure trove of novel, sometimes elusive, mechanisms for blending non-canonical chemical elements into living cells through synthetic metabolism.

Methylation and *S-adenosyl-L-methionine (SAM)*-mediated incorporation are only two examples of reactions that can be harnessed for introducing new-to-Nature elements for biotechnological purposes.

Synthetic metabolism and enzyme engineering are promising techniques to update the periodic table of Life.

Thermodynamic calculations based on bond-formation free-energy values and analysis of chemical reaction databases help identify element-and-organic substrate pairs suitable for biologization.

The predominant (and outdated) view of the chemical composition of a cell

Despite the existence of 118 chemical elements in the periodic table, all life forms on Earth are principally built on the same set of six essential chemical elements. C, H, N, O, P, and S represent the bulk elemental building blocks that form macromolecules (i.e., DNA, RNA, proteins, and lipids) and low-molecular-weight metabolites. Inorganic ions of the alkali group [Group 1; e.g., potassium (K) and sodium (Na)], earth-alkali group [Group 2; e.g., calcium (Ca) and magnesium (Mg)], and halogens [Group 17; mostly chlorine (Cl), bromine (Br), and iodine (I)] comprise the majority of soluble intracellular ions and, together with some trace minerals [e.g., iron (Fe), manganese (Mn), copper (Cu), molybdenum (Mo), and zinc (Zn)], complete the chemical environment of the cell. Over the years, microbial engineering has enabled the bioproduction of many high value-added compounds from this somewhat limited set of chemical elements, including essential medicines (e.g., insulin [1], artemisinin [2], and antibiotics [3]), diverse natural products [4], chemical building blocks (e.g., in the form of organic acids; glycolic acid [5], lactic acid, itaconic acid, and citric acid [6]), or biopolymers [e.g., polyhydroxyalkanoates [7–9], poly(lactic acid) [10], or poly(glycolic acid) [11]]. Currently, many high value-added goods and materials contain **nonbiological elements** [e.g., fluorine (F), silicon (Si), and boron (B); see Glossary] that a cell cannot synthesize or only uses limitedly for anabolic reactions; therefore, such nonbiological atoms are produced via traditional chemical synthesis. At the same time, the market contribution from biotechnological production in the USA in 2018 was estimated at US$ 2.6 trillion (~10% of the gross domestic product [12]), and this figure continues to expand [13]. Biotechnology is now a thriving economic sector internationally, and a critical driver for employment and economic growth [14,15]. Facing a changing climate, sustainable production of value-added goods is becoming more important than ever [16], and the economic

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contribution of biotechnology is expected to increase proportionally fast. Therefore, to ensure a smooth transition to a sustainable bioeconomy in which biotechnological production has an essential role, it is necessary to expand the chemical palette for the biosynthesis of new products [17]. However, how can we bridge the gap between the molecules that can be produced by living cells and the demands of an expanding biotechnological market?

In this review, we explore **non-canonical** chemical elements (i.e., beyond C, H, N, O, P, and S) identified in organisms and their potential roles in their metabolic networks. This assessment provides an overview of natural availability and the possibility of engineering metabolic assimilation, as well as identifying potentially untapped genetic resources that could serve as a starting point for bioproduction strategies. We also describe past and current biotechnological efforts deployed for incorporating novel atoms into biology. We highlight recent advances in **synthetic metabolism** and metabolic engineering efforts toward the biosynthesis of **new-to-Nature** molecules, as well as disclosing the associated challenges (Figure 1). We conclude with a theoretical framework that identifies chemical elements that, due to thermodynamic bonding preferences, represent promising atoms amenable to '**biologization**'.

Organisms naturally incorporate non-canonical chemical elements, but not all routes for assimilation have been described

Although the prevalent view of biochemistry is based solely on the presence of the six essential elements mentioned previously (i.e., C, H, N, O, P, and S), mounting evidence gathered over the past few years supports the notion that cells take up and (to some extent) metabolize a plethora of non-canonical chemical species. This occurrence is especially true for microorganisms and plants, which display considerable growth dependence on their environments and need to adapt to their chemical surroundings (i.e., environmental niches) to survive [18]. The elements, molecules, and identified route(s) of adsorption are discussed in the following section; Table 1 provides a summary of the non-canonical atoms most commonly found in biology and their potential for biotechnological applications.

Transmembrane transport of nonbiological chemical elements

Transport of non-canonical elements into the cytoplasm is the first hurdle that microbial cells (and metabolic engineers) have to overcome before they can be assimilated into metabolism. Membrane transport can be accomplished via several mechanisms, depending on the type and chemical state of the element in question (e.g., whether it is oxidized or reduced). For example, there are three known ways of B uptake (i.e., passive and facilitated diffusion, and active transport [19]; see the section 'Arsenic, selenium and boron as representative metalloids and nonmetals'). Fluoride and other halogens are transported actively due to their negatively charged nature in aqueous solutions (which also poses a hurdle for chemical activation of the cognate anions as nucleophiles, since they are highly hydrated [20]). Here, transporter specificity is mainly defined by the charge of the chemical species and less so by the ionic radius, following the general trend that the bigger radii, the more selective the transporter [21,22]. Another mechanism for the uptake of non-canonical elements can occur via siderophore secretion, which is a relevant strategy deployed by microbial cells for capturing metals. In this case, small molecules are secreted by the cell to bind and chelate the metal, with different specificities depending on the siderophore; examples include members of the structurally diverse families of catecholates, hydroxamates, and carboxylates. This chelation process increases the solubility of the metallic ion, neutralizes its charge, and allows for efficient uptake of elements that are typically scarce in some natural environments [23]. In general, the transport of nonbiological elements into microbial cells has not been described in detail, but it appears likely that the corresponding uptake mechanisms are aligned

Glossary

Adaptive laboratory evolution: sequential process to generate adapted/evolved microbial strains with desired traits based on natural selection as the driving force.
Biologization: introduction of nonbiological elements or molecules into biology (e.g., in a microbial host) via a combination of rational and evolutionary engineering.
Cell factory: engineered living (usually, microbial) cell with an optimized metabolism to produce energy or chemicals of industrial interest.
K_M and k_{cat}: substrate concentration needed to reach half-maximum reaction velocity of an enzymatic reaction, and turnover number (substrate molecules processed per time unit). These are the critical parameters used to assess the efficiency of any given biochemical pathway.
New-to-biology/-Nature: elements or reactions currently not present in biological systems.
Non-canonical/nonbiological elements: chemical elements either not currently present in biological systems or found only in traces.
Synthetic metabolism: set of metabolic reactions and biochemical pathways (some of which stem from *de novo* design) implanted in suitable host organisms to either boost product biosynthesis or to obtain altogether new-to-Nature molecules.
Xenobiology: branch of synthetic biology dealing with non-natural chemical elements, especially in terms of alternative life forms based on non-canonical biochemical processes.

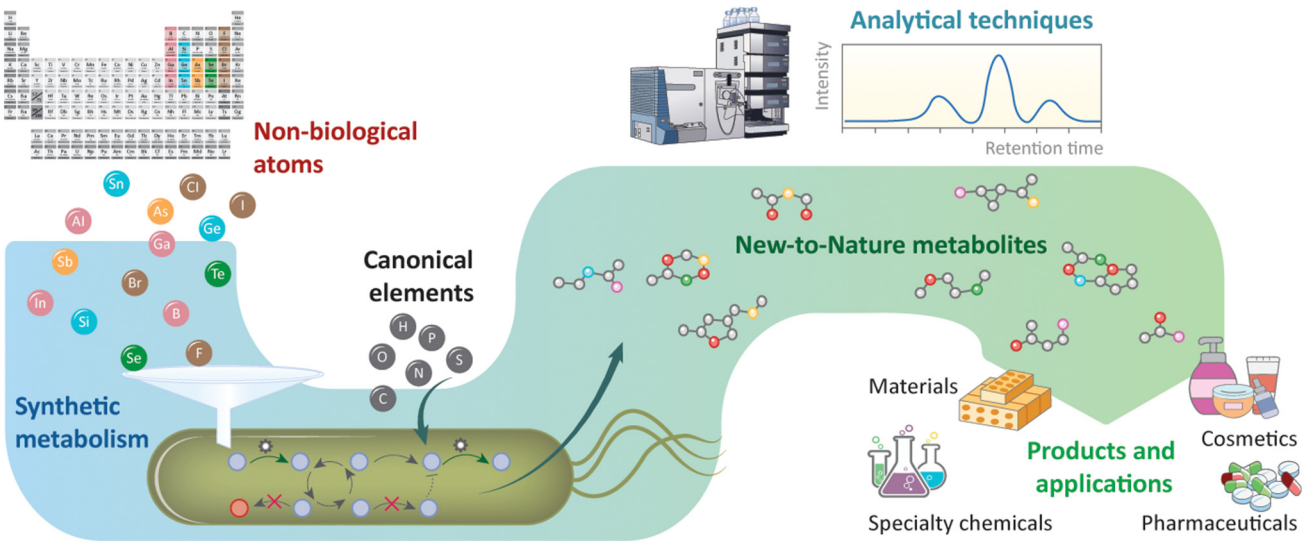

Figure 1. Synthetic biology drives the production of novel, value-added molecules containing nonbiological chemical elements. Synthetic metabolism is an emerging strategy to blend non-canonical atoms into microbial biochemistry by establishing an interplay with the canonical elements of all living cells. Engineering novel pathways for nonbiological atom incorporation, together with tailoring cell factories for efficient bioproduction, and developing analytical methodologies to detect new-to-Nature metabolites, form core efforts toward upgrading the periodic table of Life. Abbreviations: Al, aluminium; As, arsenic; B, boron; Br, bromine; Cl, chlorine; F, fluorine; Ga, gallium; Ge, germanium; I, iodine; In, indium; Sb, antimony; Se, selenium; Si, silicon; Sn, tin; Te, tellurium.

with their biological counterparts through substrate promiscuity. Here, we focus on the fate of non-canonical elements once they are transported into the cell cytoplasm.

Methylation is a common strategy for detoxification of nonbiological metalloids and metals

A simple way for organisms to incorporate nonbiological elements into organic compounds and assimilate them in their metabolic network is via attachment of a methyl group, a widespread, unspecific strategy for detoxification of reactive chemicals. This biochemical mechanism appears to be particularly relevant for elements mainly belonging to the transition metal and metalloid groups, since direct methylation toward metabolite biosynthesis has been shown, for example, for nonradioactive halogen elements [24,25]. Methylation emerged as a general detoxification strategy because methylated species are often far less reactive than the original compound (or ion). Thus, methyl-activated molecules impose a lesser metabolic burden on the cell compared with the parental chemical species [26]. Such is the case for metals and metalloids, including arsenic (As), chromium (Cr), mercury (Hg), cadmium (Cd), cobalt (Co), and lead (Pb), which are known to be considerably toxic for living cells in their elemental form. These heavy metals and metalloids tend to bind to proteins, lipids, and small metabolites, thereby causing an imbalance in central carbon metabolism and energy homeostasis, and directly interfering with enzymatic function(s) and DNA modification mechanisms [27,28]. For example, the preferential targeting of sulfhydryl (–SH) groups by these reactive elements affects protein folding and the overall redox state of the cell, with the concomitant production of damaging reactive oxygen species [29], and methylation has been shown to counteract some of these deleterious effects. Methylation is not the only way in which a cell can detoxify chemical elements, and several redox enzymes perform similar tasks [30–33]. However, these redox-dependent detoxification mechanisms (for a given chemical species Y) do not encompass the formation of a C–Y bond.

Methylation-dependent mechanisms offer possibilities for establishing synthetic metabolism, for both biosynthesis and biodegradation. For example, bacteria, algae, fungi, plants, and mammals

Table 1. Overview of the importance of non-canonical elements in biology and examples of technical applications involving metabolites containing these chemical elements

Element	Biological	Details[a]	Biotechnological interest	Biotechnological implementation
B	Yes	Small molecules, antibiotics, quorum sensing	Biopolymers, precursors for chemical synthesis	Enzymatic C–B bond formation
Aluminium (Al)	Yes	Chelates of phosphate or citrate found in human urine	Some Al-containing fungicides	
Gallium	No	Not fully elucidated	Chemotherapy, diagnostics	
Indium (in)	No	Not fully elucidated	^{111}In used as radiotracer	
Si	Yes	Skeleton of diatoms; organosilicates (SiO_4)	Plant biology, supplement in agriculture	Enzymatic C–Si bond formation
Germanium	Yes	Methylated species	Medicines, organogermanes act as antioxidants	
Tin (Sn)	Yes	Methylated species (mainly anthropogenic origin)	Some Sn-containing anticancer agents	
Arsenic	Yes	Methylated species, small metabolites of marine species; polyarsenic molecules	Pesticides, herbicides, medicine, bioremediation	
Antimony	Yes	Methylated species	Some medicines; antiprotozoan drugs; catalysts for chemical synthesis	
Selenium	Yes	Amino acids, proteins	Medicinal chemistry, nanoparticles, bioremediation, wastewater treatment	
Tellurium (Te)	Yes	Methylated species	Bioremediation	Te-containing amino acids and proteins
F	Yes	*Streptomyces cattleya* (fluorinase) and cognate catabolic routes	Medicinal compounds, polymers	Heterologous expression of natural fluorination enzymes in different microbial hosts
Cl	Yes	Natural products	Polymers (e.g., polyvinyl chloride), antibiotics, medicinal compounds	
Br	Yes	Natural products	Medicinal compounds	
I	Yes	Some small metabolites	Medicinal compounds	

[a]Only covalently-bound atoms are considered in the examples.

readily methylate inorganic As to form monomethylarsonic acid [MMA, $CH_3AsO(OH)_2$] and dimethylarsinic acid [DMA, $(CH_3)_2AsO_2H$],two chemical species that are thought to be less toxic than inorganic arsenite (AsO_3^{3-}) [34,35]. Recent investigations on As metabolism established toxicity profiles for both MMA and DMA, indicating that there is a hierarchy of the impact of As chemical species on biological processes [35,36]. Extending the aryl group could be an interesting way to not only detoxify As, but also mobilize the metalloid into novel organic compounds. As (V) salts, which are similarly toxic, undergo a different fate from that of arsenite: arsenate (AsO_4^{3-}) can be directly processed through Embden–Meyerhof–Parnas glycolysis to yield 1-arseno-3-phosphoglycerate [37]. Although this molecule is believed to be relatively unstable in an aqueous milieu (e.g., the cell cytoplasm), this transformation could be exploited as an entry point for the target incorporation of As into biochemistry, provided that other reactions in such a synthetic metabolism can maintain the C–As bond. These mechanisms offer novel methodologies for As mobilization from contaminated environments, yielding organic arsenocompounds (some of which can be used for specific applications, e.g., drugs). Additionally, the use of As-containing pesticides has been largely disputed due to the risk of ground water contamination. In this case, synthetic metabolism can be harnessed for sequestering As species from polluted water,

fixing the metalloid in its organic form(s) in engineered microorganisms for cleansing and remediation purposes [38].

Other chemical elements have also been identified in a methylated state in environmental samples and, indeed, global methylation cycles for metallic and metalloid elements exist in nature. Lewis and colleagues [39] showed that organogermanium is actively generated during freshwater methanogenesis, from which germanium (Ge)-containing molecules are transported into the open ocean and remain largely unreactive. Not only mono-, but also di- and trimethylated Ge compounds have been detected in some aquatic environments, suggesting that downstream metabolic routes for Ge assimilation exist in biological systems [39,40]. However, the biochemical origin of di- and trimethyl-Ge has not yet been identified and a chemical or anthropogenic origin cannot be excluded. The methylation cycle concludes in marine anoxic environments that favor demethylation and releases elemental Ge again [41]. Ge is advocated as a nontoxic alternative to toxic organotin reagents, and organogermanium compounds produced biologically could be an interesting alternative to the expensive chemical methods needed to obtain precursors for GeO_2 chemical vapor deposition. Some organogermanium compounds are of medical interest because they have promising anticancer properties and are used for the treatment of several other medical conditions, such as compound GEE-132 {repagermanium, *bis*[2-carboxyethylgermanium(IV) sesquioxide]} [42]. Other Ge-containing organic compounds of interest include germatranes, which are cyclic molecules stabilized with a hypervalent Ge atom [43,44]. Halogen-substituted germatranes have shown efficacy in improving a variety of neurological and oncological conditions (i.e., improved memory and antitumor activity) [45]. In addition, Ge-substituted sugars, such as 6-O-[3-(trimethylgermyl)propyl]-β-D-glucopyranoside, or trialkylgermyl-substituted trifluoroacetylfuranes, also show promising antitumor and immunomodulatory activity with generally higher water solubility than comparable agents [45].

The molecular mechanisms and genes involved in methylation processes are not yet comprehensively elucidated, but methyl donors, such as *S*-adenosyl-L-methionine (SAM) and methylcobalamin, have been implicated in these transformations [46]. In fact, SAM also acts in biochemical mechanisms involving many other metallic and metalloid species, including As, gold (Au), Cr, Pb, palladium (Pd), platinum (Pt), selenium (Se), tin (Sn), and tellurium (Te), suggesting a major role for SAM in general methylation-dependent detoxification [46]. As discussed for As, methylation of metallic elements does not always lead to a less toxic species. For example, organotin compounds generally show higher toxicity compared with their non-organic counterparts [47]. Although organic Sn species in the environment appear to be of anthropogenic origin, biotic methylcobalamin (vitamin B_{12})-mediated Sn methylation has been identified [47], highlighting unidentified metabolic routes for metal incorporation into metabolites. Indeed, some *Pseudomonas* species accumulate up to 2% (w/w) in organotin compounds [48], and Sn incorporation could represent another example of misrecognition or metabolic 'mistakes' [49]. These high Sn accumulation capabilities offer interesting possibilities for bioremediation. Currently, the most widely used methods to this end involve O or nutrient supply to contaminated soil (passive bioremediation) or aeration of soil by piling, periodic turning, and irrigation to favor the growth of specific microorganisms (active bioremediation) [38,50]. The isolation of natural variants of soil or marine bacteria endowed with high-metal uptake profiles or metabolic engineering to increase (organo-) metal uptake could become an efficient alternative for bioremediation and contaminant mobilization. Importantly, the use of metagenomics could provide an efficient way for the identification of novel enzymes acting on nonbiological elements from environmental samples and nonculturable microbes [51–54].

Given that methylation is a major regulatory mechanism for many cellular processes, because it controls gene transcription, drug metabolism, or DNA repair, among others [55,56], it does not

come as a surprise that the Enzyme Commission (EC) currently annotates more than 400 classes of (putative) methyltransferases, many of which are specific for the methylation of metals and metalloids. We argue that the abundance of annotated methyltransferases represents a treasure trove for biotechnological applications, and suggests that attachment of a methyl group is a potential entry point for introducing novel atoms into low-molecular-weight metabolites, and microbial metabolism in general. The promiscuity of methyltransferases is key to this idea, and cataloging the substrate range of these enzymes would be a first step in their application in synthetic metabolism. Metagenomics has successfully been used to identify novel and known methyltransferases that act on non-canonical elements [57–61]. These studies illustrate the value of metagenomics as a feasible method for the identification of genes (and mechanisms encoded by them) that could be harnessed for the assimilation of novel chemical entities. While this biochemical entry point for non-canonical chemical elements appears to be rooted in the lack of specificity of some enzymes, other biological processes appear to be atom dependent. Here, we describe key findings on natural assimilation of nonbiological elements into small, low-molecular-weight metabolites, and discuss the importance and abundance of certain selected elements in their biological context.

Organisms harbor a range of small metabolites containing nonbiological atoms

Based on a growing body of experimental evidence, it is now clear that many nonbiological chemical elements do in fact enter the cell and become converted into organic compounds and metabolites in several species. The relative abundance of non-canonical atoms in metabolites, in turn, could provide cues to both their importance in biological systems and ease of assimilation for biotechnological purposes. To provide a general framework, we catalogued the chemical elements that constitute the 308 035 metabolites stored in the ZINC small-molecule database [62]. The ZINC database (recently updated as ZINC20 [63]) is a curated collection of commercially available chemical compounds prepared especially for virtual screening and, therefore, perfectly suited for this purpose. Furthermore, this database includes primary and secondary metabolites from bacteria, eukaryotes, and archaea. Although the catalog is in no way exhaustive and might not reflect real distributions in nature, ZINC provides good coverage across domains in the Tree of Life. We established a hierarchy in the abundance of single atoms in all the database compounds, which led to the identification of C, O, N, P, and S as the most abundant structural elements (as expected, due to their role in maintaining life), directly followed by the nonradioactive halogens (Cl, Br, F, and I) and B (Figure 2A), together with As and Se. Figure 2B shows examples of naturally occurring metabolites containing nonbiological chemical elements that have been identified from environmental isolates; these are discussed in the following section according to this hierarchical analysis.

Fluorine is a unique element among the halogen group with enormous potential for incorporation through synthetic metabolism

The halogens are located in Group 17 of the periodic table and have essential roles in cellular processes. For example, chloride not only functions as a major intracellular anion, but is also found as a component of secondary metabolites. Chloride and bromide are not discussed in depth herein, but the reader is directed to a series of comprehensive reviews on the topic [64–68]. By contrast, F deserves special consideration due to its chemical characteristics as the most electronegative element in the periodic table and its enormous industrial importance. Owing to its relative small size (50 pm atom radius, similar to the 25 pm radius of H), and its high electronegativity (3.98 on the Pauling scale), the addition of F to an organic molecule significantly affects its electronic properties without disturbing its spatial structure. The establishment of organofluorine synthesis or, in particular, the creation of C–F bonds, the strongest single bond known in organic chemistry, is often only possible under extreme conditions and with certain preferred substrates [69]. We

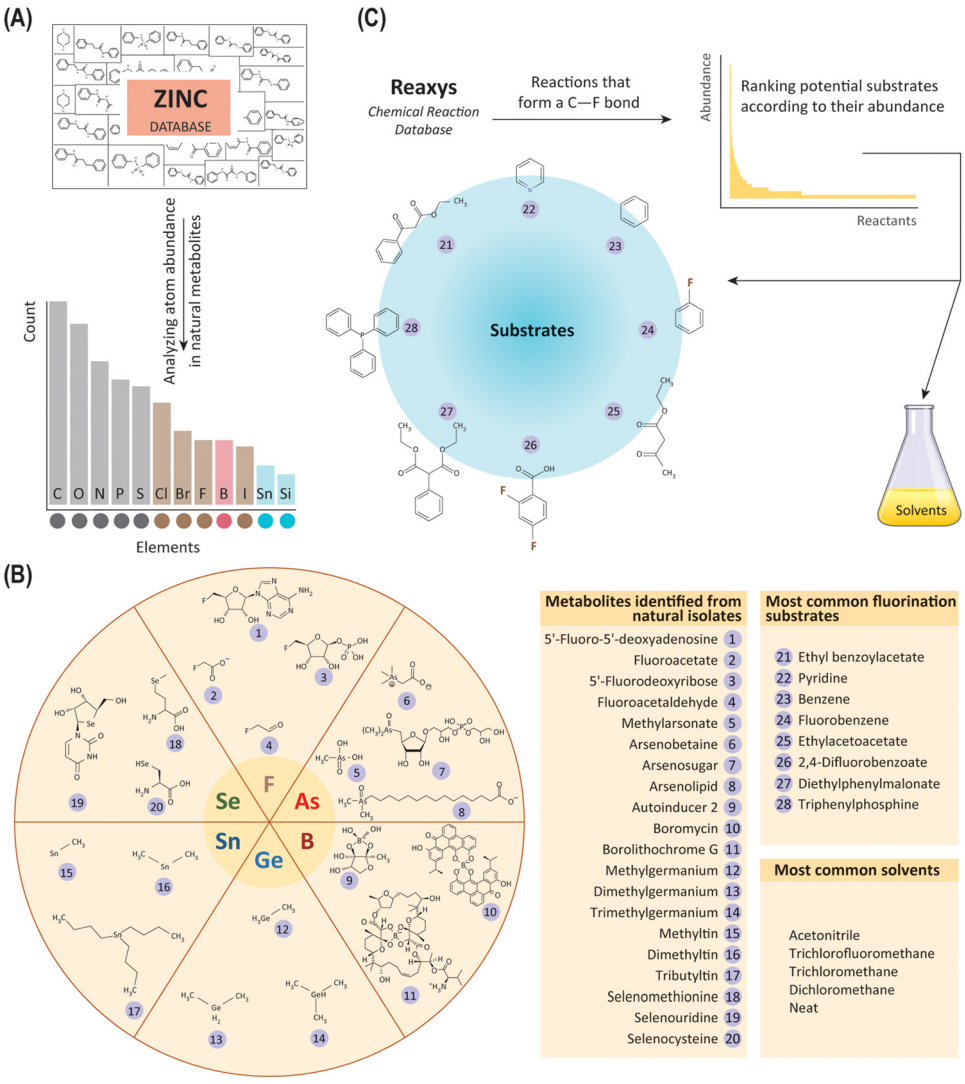

Figure 2. Identifying non-canonical chemical elements found in metabolites and substrates for *de novo* fluorination. (A) Overview of the computational approach proposed in this study to identify chemical elements present in biology. The core aspect of this approach is the quantification of metabolites annotated and stored in the ZINC chemical database [62], which yields a ranking of 'canonical' and 'non-canonical' atoms according to their abundance in natural systems. (B) Examples of important metabolites that contain 'nonbiological' elements [i.e., fluorine (F), arsenic (As), boron (B), germanium (Ge), tin (Sn), and selenium (Se)] as identified in various environmental sources (e.g., microbial isolates). An extended description of some of these metabolites can be found in the main text. (C) A theoretical workflow developed for the identification and ranking of suitable fluorination substrates [where a carbon (C) atom can form a C–F bond], based on reported chemical reactions stored in the Reaxys Chemical Reaction Database (www.elsevier.com/solutions/reaxys). The chemical structures around the blue-shaded circle represent the top-eight fluorination reactants based on analysis of this database. Abbreviations: Br, bromine; Cl, chlorine; I, iodine; O, oxygen; N, nitrogen; P, phosphorus; S, sulfur; Si, silicon.

attempted to classify the breadth of fluorination substrates and reactions involved in *de novo* C–F bonding with the view of establishing synthetic metabolism. The Reaxys chemical reaction database[I] was harnessed to quantitatively rank reactions involving the formation of C–F bonds, identifying the most commonly used reaction conditions, substrates, and solvents in chemical fluorination (Figure 2C). The most frequently fluorinated substrates in the Reaxys database

were ethyl benzoylacetate (21), followed by pyridine (22), benzene (23), fluorobenzene (24), ethylacetoacetate (25), 2,4-difluorobenzoate (26), diphenylmalonate (27), and triphenylphosphine (28). Some of these chemicals are of interest for the production of value-added organofluorines that include, but are not limited to, aromatic anticancer agents from (21) and (22), aniline (a commodity chemical) from (23), cyclohexenones from (24), or chalcones and derivatives as flavonoid precursors from (26) [70,71]. Interestingly, compounds (21), (23), (27), and (28) are insoluble or only marginally soluble in water, which is in line with our finding that the solvents predominantly used in chemical fluorination are aprotic (Figure 2C). Aprotic solvents favor nucleophilic fluorinations because, unlike aqueous solutions, F (or, most often, fluoride, F^-) does not become 'trapped' in a hydration shell, which would otherwise render it largely unreactive [72].

From the analysis in the preceding text, it became clear that the conditions and reagents identified as amenable for direct fluorination of organic structures would prove challenging to implement in living cells. The main reason preventing the biologization of F through some of the top reactions identified in our *in silico* analysis is that they would be minimally effective in an aqueous environment under physiological conditions, although aromatic compounds (22–24) could be incorporated in synthetic metabolism, such as by harnessing monooxygenases as the biocatalyst [73]. Nonetheless, and because of these constraints, the identification some 20 years ago of an enzymatically catalyzed fluorination reaction that functions in ambient environments was unexpected [74]. O'Hagan and colleagues [24] performed *in vitro* conversion assays of free F^- and SAM with partially purified protein extracts obtained from the soil actinobacterium *Streptomyces cattleya*. This analysis led to the identification of 5'-fluoro-5'-deoxyadenosine (5'-FDA) as the direct halogenated product of the fluorinase enzyme (Figures 2B and 4A). Not surprisingly, desolvation of the F^- ion appears to be a major thermodynamic barrier, as suggested in our reaction screening (Figure 2C), a hurdle that fluorinase has to overcome, allowing F^- to become a good nucleophile [75]. One of the consequences of this feature is that S_N2 halogenases are very slow-acting enzymes [76]. Pardo and colleagues recently identified the fastest-acting fluorinase in Nature known so far [77]. Surprisingly, *in silico* screening pinpointed an archaeal enzyme that does not display the features typical of fluorinases isolated from *Actinomycetes*; when the fluorinase from *Methanosaeta* sp. was introduced into a *Pseudomonas* **cell factory**, it mediated the highest accumulation known of fluorometabolites, in either natural or synthetic organisms.

The canonical biosynthetic pathway for fluorometabolites includes the conversion of 5'-FDA to fluoroacetate and 4-fluorothreonine in an enzymatic sequence that involves phosphorylation of 5'-FDA, release of the adenine moiety, followed by isomerization to yield (3*R*,4*S*)-5'-fluoro-5'-deoxy-D-ribulose-1-phosphate [78]. An aldolase then splits off fluoroacetaldehyde, which is subsequently oxidized to fluoroacetate or converted into fluorothreonine [24,79]. Fluoroacetate is a well-known toxin enriched in plants of the genus *Dichapetalum* (up to 8000 ppm), in which it is hypothesized to act as a defense mechanism against grazing by herbivores [80,81] and effectively so: the lethal dose of fluoroacetate for mammals is around 5–30 mg kg^{-1}. Specifically, the shrub *Dichapetalum toxicarum* also synthesizes and accumulates fluorinated fatty acids, most prominently 18-flurooleic and 16-fluoropalmitic acids, which are believed to serve a similar defensive purpose [81]. The pathway leading to these fluorometabolites is essentially the same as for their nonfluorinated counterparts, involving the promiscuous action of traditional fatty acid biosynthesis enzymes, and starting with fluoroacetyl-coenzyme A (CoA) as the monomer for sequential extension [81]. Unlike bacteria, plants compartmentalize the metabolism of fluorinated carboxylic and fatty acids to reduce their potential toxicity. Although it appears plausible that F uptake and assimilation into metabolites also serves as a defense mechanism in bacteria, the underlying evolutionary reason has not yet been identified [82]. Several detoxification

mechanisms for fluorinated molecules have been identified in bacteria, such as through dehalogenation [83] or deacylation of tRNAs loaded with fluorinated amino acid moieties that would otherwise end up in proteins [84]. As discussed in the preceding text, most biochemical mechanisms involving F incorporation are probably based on misrecognition, due to the small atomic size of this halogen.

Arsenic, selenium and boron as representative metalloids and non-metals

As has been discussed as an alternative life-supporting element in astrobiology and synthetic biology owing to its potential to act as a phosphorous mimic. A bacterium of the *Halomonadaceae*, isolated from the Mono Lake in California, was reported to grow with As salts instead of P [85]. Strain GFAJ-1 appeared to assimilate AsO_4^{3-} over phosphates, and As atoms were (erroneously) indicated to be part of the DNA backbone. These findings could not be replicated by any other research group and remain controversial [86]. A study published in 2012 concluded that arsenate does not contribute to growth of strain GFAJ-1 when phosphate is limiting, and DNA purified from GFAJ-1 cells incubated with limiting amounts of PO_4^{3-} and abundant AsO_4^{3-} did not exhibit the spontaneous hydrolysis expected for arsenate ester bonds [87]. Similarly, Basturea and colleagues [88] found that arsenate causes massive ribosomal breakdown in strain GFAJ-1, providing the phosphates necessary for growth. Another study confirmed the essentiality of P and also showed that several As-containing compounds, most notably hexoses, were formed abiotically when the bacterium was incubated in the presence of high AsO_4^{3-} concentrations [89].

Although it can be safely excluded that As can sustain life *in lieu* of P, the idea remains exciting that microbes could make use of a toxic element, as well as what its fate might be *in vivo*. Inorganic As is readily methylated (see 'Methylation is a common strategy for detoxification') and more than 25 species of organo-arsenicals have been identified in marine organisms [90]. Besides methylated and oxidized As species, marine algae have been found to contain arsenocholine, arsenobetaine, and more than 15 different arsenosugars, in which the As is attached to the 5'-carbon of a 5'-deoxyribose moiety. Although the biosynthesis pathway is not fully understood, it has been proposed to be dependent on the 5'-adenosylradical of SAM [91]. Abiotic formation of arsenosugars could also be possible [89]. Additionally, other metabolites, such as terminal As-containing long-chain fatty acids, arsenosugar-phospholipids, and even a polyarsenic organic compound (arsenicin A; 2,4,6-trioxa-1,3,5,7-tetraarsaadamantane) have been isolated from algae, fish, and the marine sponge *Echinochalina bargibanti* [92–94]. Although their origins and functions remain to be elucidated, these arsenocompounds are speculated to comprise defense mechanism, given that arsenicin A has potent bactericidal and fungicidal activities. That they are simply a product of enzyme promiscuity is another possibility that cannot be ruled out [93,94]. Interestingly, however, these observations show some striking similarity to the assimilation of F and the end-metabolites produced by *Streptomyces*. As has a relatively minor importance from a biotechnological perspective, although both naturally occurring and synthetic organo-arsenicals exhibit promising antimicrobial and anticancerous properties [95,96].

Se is another interesting non-canonical trace element in biology that, although found in all three domains of life, is not universally used, occurring only in trace quantities. Given that seleno-biology has been discussed in depth in recent publications [97–99], will only briefly mention the most important aspects here. Se is incorporated into the traditional sulfur-amino acids cysteine and methionine instead of sulfur atoms. Se takes a special place among the non-canonical chemical elements because it is the only atom known to date that is actively integrated into amino acids and proteins without any deleterious effects. Interestingly, an entire set of highly

specific molecular machinery and specialized enzymes exists that incorporates SeH_2 into amino acids and, subsequently, into proteins [100]. In some cases, unexpectedly, Se can also end up in other metabolites, such as nucleosides, thereby forming 5'-methylaminomethyl-2'-selenouridine [101] or selenouridine (Figure 2B). It is speculated that these molecules and pathways are remnants from the time of the big oxygenation event ~2.3 billion years ago, when cells needed to counteract oxidative damage caused by the sudden spike in environmental molecular O. Se, acting as a powerful antioxidant, could have provided this service by hijacking an already existing biochemistry for its incorporation [100].

The substitution of Se for S in cysteine and methionine can have profound effects on enzyme kinetics and chemical properties of the resulting selenoprotein. Activity assays showed a 300-fold increase in catalytic activity (k_{cat}) and a threefold increase in the K_M of a selenocysteine-containing formate dehydrogenase from *Escherichia coli* compared with the native variant, which only has cysteine [99]. Other selenoproteins, such as glutathione peroxidase or glycine reductase, show similar trends of increased activity when Se is used instead of sulfur [99]. It is tempting to speculate that the reason for this dramatic change in enzyme activity stems from not only overall structural rearrangements caused by the presence of the new atom, but also the change in chemical characteristics it brings about, such as electron configuration, hydrophobicity, or pK_a. One possible mechanism comes from the study of MSRB1 protein, a selenomethionine- and selenocysteine-containing methionine-*R*-sulfoxide reductase that reduces oxidized methionine [102]. When Se is present, it binds to the sulfoxide O, and electrons are transferred to a neighboring cysteine, consequently releasing the O atom as water. Finally, thioredoxin catalyzes the breakdown of Se–S bonds between amino acids. In the presence of sulfur, which directly binds O, the release of O is much slower. Hence, it appears that Se oxidation has a higher degree of reversibility compared with S, ultimately leading to a higher catalytic activity [103]. These examples illustrate how the incorporation of a nonbiological atom (in this case, a 'bystander' chemical element) can have profound effects on cell biochemistry.

Yet another important xeno-element that deserves separate attention is B. The biology of B is relatively well known: B uptake is mediated by three different transport systems that have been identified so far: (i) passive diffusion of boric acid through the cell membrane; (ii) facilitated diffusion of boric acid through ion channels; and (iii) active transport of the borate (BO_3^{3-}) anion [19]. Due to its high pK_a of 9.2, most B (96%) is present intracellularly as boric acid (BO_3H_3) and, to a minor extent, as borate anion [104]. Yet, what is the fate of B in terms of incorporation into metabolites? Several B-containing complex metabolites have been identified thus far (Figure 2B), most notably macrolide antibiotics, such as boromycin (the first metabolite identified to contain B [105]) and structurally related tartrolons [106,107]. In addition, the 'universal', interspecies quorum-sensing molecule Autoinducer 2 (AI-2) has been shown to contain B in the form of furanosyl borate diester [108]. B assimilation by microorganisms has a long history, given that B-containing pigments (borolithochromes) were isolated and structurally assessed from the fossilized calcareous red alga *Solenopora jurassica* [109]. Notably, hydrated borate is connected via an ester bond to the C scaffold in all the previously-mentioned metabolites, and no covalent C–B bond has been identified as a natural occurrence to date. B is becoming ever more important in medicinal chemistry for developing new therapeutics, with drugs often containing one or several C–B bonds [110]. A vacant *p*-orbital endows B with special characteristics as an electrophile that readily forms dative bonds with nucleophiles, changing its uncharged, trigonal-planar structure to an anionic, tetrahedral structure [110]. Several B therapeutics have been developed in recent years via organic chemistry, including the renowned proteasome inhibitor 'Velcade' and inhibitors of arginase, serine proteases, or fatty acid biosynthesis [111–115]. Thus, it is not surprising that synthetic biology aims to find solutions for the effective incorporation of B into a C structure [116].

Biotechnological efforts toward the incorporation of non-canonical chemical elements into microbial metabolism

The design and implementation efforts to incorporate nonbiological elements for the biosynthesis of new molecules has only recently been gaining momentum. Fueled by early studies on **xenobiology**, the scope of metabolic reactions and mechanisms that can be harnessed for the biologization of non-canonical atoms is expanding to provide biosynthetic routes that will surely contribute to broaden the scope of bio-based production. Here, we describe strategies rooted in enzyme and metabolic engineering that have successfully been used to bring novel chemical elements to life as well as potential host organisms to carry out novel biochemistries (Figure 3).

The importance of selecting a suitable microbial host to engineer synthetic metabolism

Introducing novel elements into the biochemistry of production organisms is likely to causes disturbances such as metabolic homeostasis, toxicity, growth retardation, enzyme inefficiency or promiscuity, and, as a consequence, low product yields. To overcome these foreseeable issues, it is vital to choose an appropriate host. Depending on the desired product and elemental feedstock available, both traditional (e.g., *E. coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, *Pseudomonas putida*, *Streptomyces coelicolor*, and *Saccharomyces cerevisiae*) and nontraditional hosts (e.g., *Trichoderma reesei*, *Aspergillus oryzae*, *Komagataella phaffii*, *Kluyveromyces lactis*, and *Vibrio natriegens*) are available. Additional selection criteria, including growth efficiency, tool availability, ease of handling, physicochemical tolerance, and metabolic adaptability, should be considered when choosing a host. Blombach and colleagues [117] provide a superb overview of selection criteria for no-traditional microbial hosts. For an in-depth review of industrially relevant chassis (both established and emerging), the reader is referred to a series of recent publications on the topic [118–124].

Assembling synthetic pathways for addition of nonbiological elements into metabolism

Fluorinated compounds are of special interest for biotechnology because they are vital for our everyday lives. Approximately 25% of the most commonly used pharmaceuticals contain a F atom,

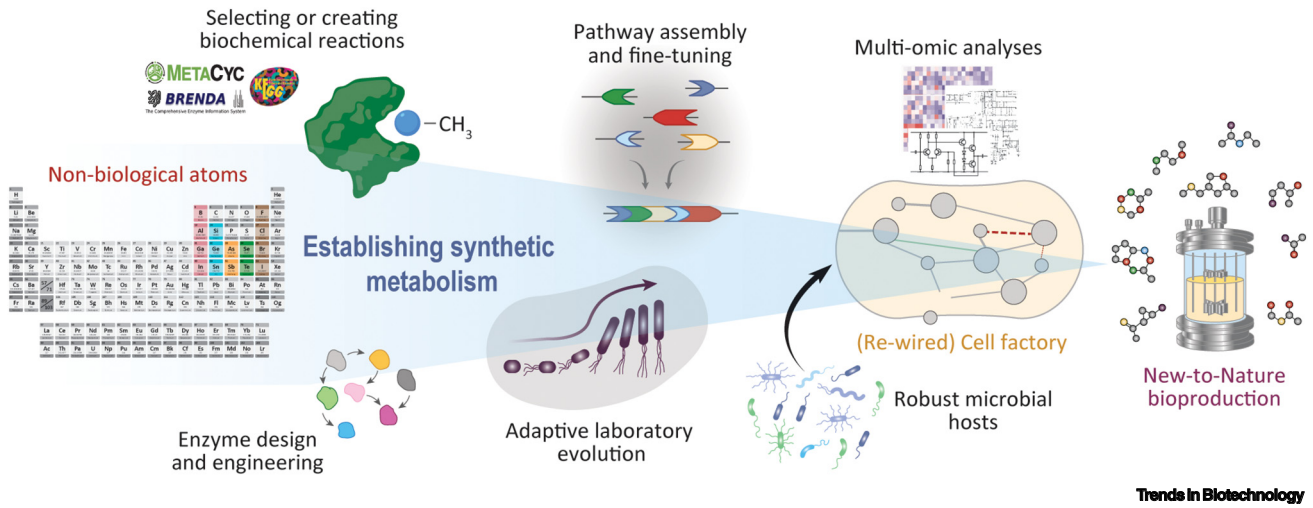

Figure 3. A pipeline for incorporation of nonbiological elements into engineered organisms based on synthetic metabolism. Enzymatic reactions for creating novel carbon (C)–X bonds (where X is a non-canonical atom) can be designed *de novo*; methyltransferases provide a possible starting point for incorporating chemical elements in cases where natural assimilation pathways are unknown or evolving new reactions proves difficult. Enzyme and metabolic engineering (e.g., heterologous pathway assembly, gene knock-in and knock-out, and fine-tuning of transcriptional response with synthetic devices), in combination with adaptive laboratory evolution, are implemented to build cell factories based on nontraditional microbial hosts to incorporate nonbiological elements into new-to-Nature compounds. Multi-omic analyses are incorporated to characterize the behavior of metabolically rewired cell factories, and provide clues for further optimization toward a consolidated bioprocess.

which often enhances their mechanism of action and allows for improved uptake rates due to increased hydrophobicity [125,126]. In addition, a plethora of compounds for agrochemical industries, diagnostics, or material sciences is based on fluorochemistry. This includes polymers, such as polytetrafluoroethylene (PTFE, 'Teflon') or polyvinyl fluoride (PVF), marked through their high temperature resistance and nonstick/friction-reducing properties for both hydrophobic and hydrophilic substances. Biotechnological efforts are undertaken that aim to biologically incorporate F into organic molecules [75,127]. However, the *de novo* creation of C–F bonds remains challenging, although synthetic metabolism for halogenation based on feeding fluorinated moieties that can be processed by engineered hosts appears to be a plausible route to biosynthesis [128]. Using fluoroacetate as a starting point, Walker and colleagues [129] demonstrated the assimilation into fluoromalonyl-CoA and fluorinated polyketides thereof in engineered *E. coli* cells. By overexpressing the genes encoding a malonyl-CoA synthase, MatB, an acetoacetyl-CoA synthase NphT7, and *R*-hydroxyl-forming acetoacetyl-CoA reductase PhaB, the authors established a flux through fluoromalonyl-CoA that reached product concentrations between 0.1 and 1 mM, enough for the use by intracellularly produced polyketide synthases. Other engineering approaches to introduce F into metabolism include the heterologous expression of the gene encoding fluorinase in *E. coli* with co-expression of the gene encoding a SAM transporter that enabled *de novo* 5'-FDA biosynthesis [130]. A prerequisite for success was eliminating the major fluoride exporter CrcB to increase intracellular F^- levels. In a recent study, the biosynthesis of fluorometabolites was engineered in *P. putida* by expressing optimized fluorinase and phosphorylase genes under control of a fluoride-responsive riboswitch [131]. In these engineered strains, the addition of mineral F^- to the culture medium triggered the expression of genes encoding the first two enzymes in the biofluorination pathway, thereby circumventing the need for expensive additional chemical inducers to afford fluorosugar and fluoronucleotide production.

Thus, a combined approach of metabolic and enzyme engineering proves useful for biotechnological applications of non-canonical elements (Figure 3). This requires an enzyme toolkit as a starting point, and looking at Nature for hints of novel mechanisms for the incorporation of non-biological chemical elements. As indicated previously, plants from several genera (e.g., *Acacia*, *Agropyron*, *Dichapetalum*, *Gastrolobium*, *Glycine*, *Sorghum*, and *Mimosa*) are known to synthesize fluoroacetate to different extents, and the mechanism of C–F bond formation is based on the action of pyridoxal 5'-phosphate (P5P)-dependent enzymes [132]. Although the precise reaction mechanism has not yet been fully elucidated, it is proposed that promiscuous reactions of β-cyanoalanine synthase promote the activation of alanine, serine, *O*-acetylserine, phosphoserine, or cysteine via P5P, which in turn allows for a nucleophilic attack of F^- on the generated carbenium ion [132]. The mechanisms of action of fluorinase and the P5P enzyme are compared in Figure 4. These are examples of the potential for synthetic biology and metabolic and protein engineering to establish bonds between biological and nonbiological atoms. Enzyme-engineering efforts for novel element incorporation are discussed in more detail below.

Enzyme and evolutionary engineering for creating novel C–X bonds

Protein engineering is a powerful methodology that enables changes in enzyme kinetics (K_M, k_{cat}), substrate, or product specificities and, importantly, the creation of new-to-Nature biochemistries. Genetic methods for the creation of variations in protein sequence are diverse, covering various strategies ranging from rational design to targeted and random mutagenesis, sexual recombination, and directed evolution. Directed evolution has been used on several occasions to perform novel biochemical reactions, such as carbene transfer to alkenes, two-carbene transfers to form cyclopropanes or cytochrome P450 Fe-catalyzed C–H amination [133–135]. Unnatural C–Si bond formation (and even production of chiral organoboranes) has been established in *E. coli* through the implementation of heterologous, engineered P450 enzymes, and became

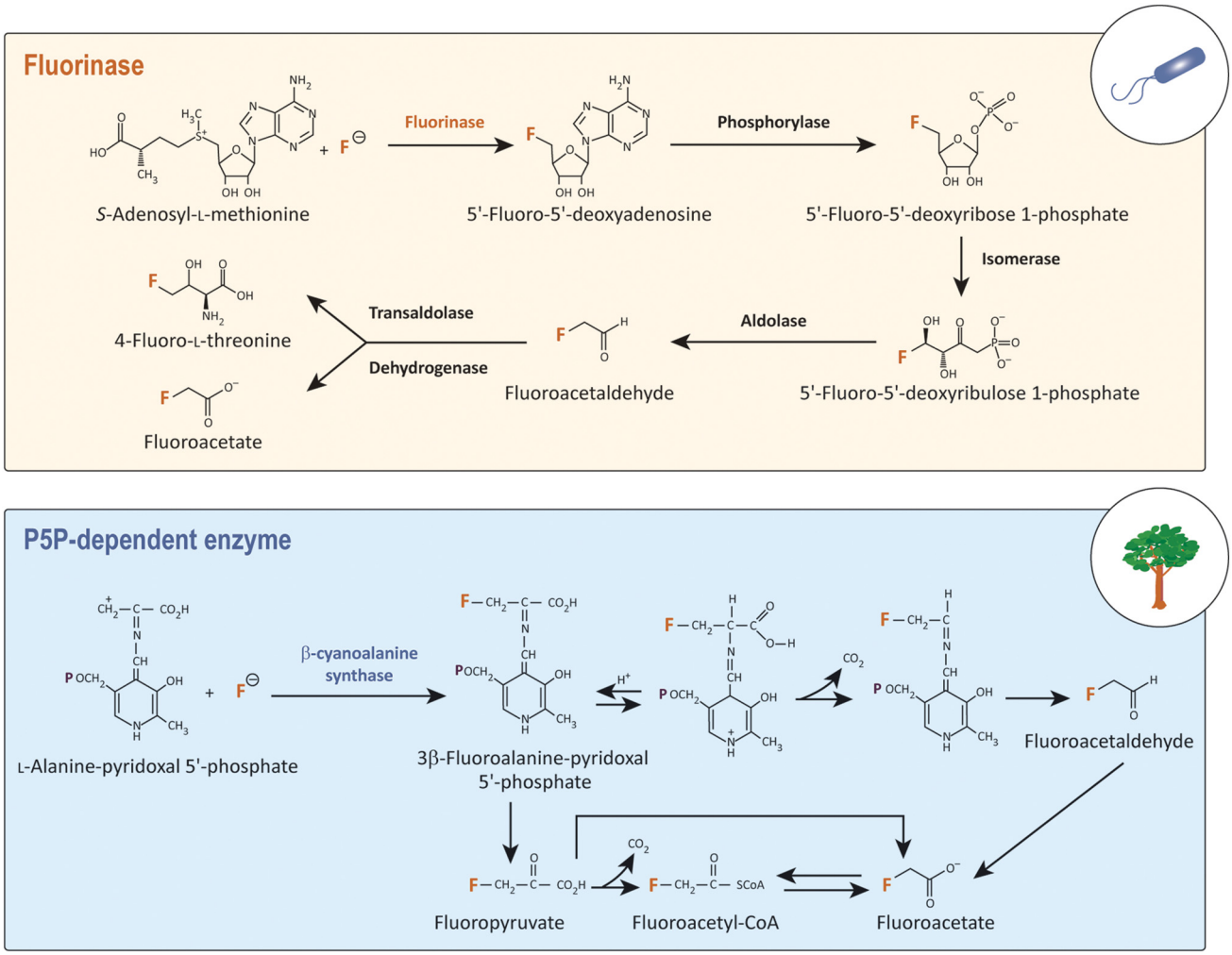

Figure 4. Two reaction mechanisms of enzymatic fluorination. (A) Biofluorination of *S*-adenosyl-L-methionine (SAM) using mineral fluoride (F^-) by fluorinase from *Streptomyces cattleya* (and related species) to form 5'-fluoro-5'-deoxyadenosine, followed by its step-wise conversion to fluoroacetate and 4-fluoro-L-threonine. The enzymes involved in each reaction are indicated in bold type. (B) Promiscuous F^- addition to L-alanine-pyridoxal 5'-phosphate (P5P) by a P5P-dependent β-cyanoalanine synthase and proposed reactions yielding fluoroacetate from either 3-fluoropyruvate or fluoroacetaldehyde or in plants. The phosphate groups in P5P and its derivatives are indicated by a purple P to simplify the scheme.

the first example of the rational introduction of a new chemical element into biology [116,136]. These examples are the *crème de la crème* of biochemical engineering, and represent a huge leap forward for both xenobiology and new-to-Nature bioproduction. In this sense, directed evolution constitutes a powerful strategy for optimizing 'promiscuous' reactions inherent to the enzyme(s) under investigation. Certain chemical transformations can be catalyzed by enzymes under specific physicochemical conditions [137], yet these activities are rather secondary and highly restrained, and their potential can be unleashed with the help of rational mutagenesis and appropriate selection schemes [138]. Such a strategy represents another powerful untapped resource for establishing the assimilation of novel chemical elements [128].

To 'biologize' chemical reactions beyond the optimization of promiscuous enzymatic activities, chemists also use novel cofactors in artificial metalloenzymes (ArMs). Notably, iridium and

rhodium (as replacement for Fe in myoglobin and P450 enzymes) catalyze C–H shifts, cyclopropanation of inactivated alkenes, and intramolecular nitrene C–H insertions, chemical reactions unknown to biology [139–142]. Although there are no known examples of novel cofactor-catalyzed biochemical reactions for introducing nonbiological atoms into the core biochemistry of microbial hosts, exploiting chemical characteristics of elements to tailor desired enzyme functionalities is an appealing feature to be explored [143]. A recent publication described an additional technique for inserting non-canonical atoms into biological systems. Here, the Cu-mediated chemical introduction of B into alanine, followed by the incorporation of this boro-amino acid into a variety of proteins, afforded novel diol binding to histones as well as selective engineering of thermo- and proteolytic stability [144]. This practical example represents a new level of enzyme engineering with nonbiological elements that might afford the generation of synthetic metabolism.

After the initial protein-engineering program has been deployed, the resulting optimized enzymes (and the novel reactions they catalyze) should be integrated into engineered microbial hosts, which then serve as **cell factories** for bioproduction. Due to the substantial biochemical differences between *in vitro* and *in vivo* conditions, this step is frequently accompanied by poor *in vivo* functionality of the engineered enzymes, at least upon direct selection in the host of interest. Additionally, bringing novel (bio-) chemistries into a living cell might disturb its endogenous metabolic network, creating stress or toxicity effects that will ultimately lead to reduced growth and production titers. To overcome potential genetic and biochemical limitations (including toxicity issues), **adaptive laboratory evolution** (ALE) has proven to be a powerful approach to 'nest' novel reactions in surrogate hosts [145,146]. Here, a modified strain is subjected to an evolutionary selection pressure for an extended period of time based on the desired phenotype. Naturally occurring mutations allow for fitness increases (i.e., growth rate, production titer, or stress resistance [147]) that enable the mutants to outcompete the remaining, nonevolved cells. As an untargeted, unbiased methodology, ALE facilitates biochemical optimizations in an often unpredictable manner [148–150]. To successfully engineer novel functionalities in living cells, selection strains are often engineered such that the novel reactions can complement a synthetic auxotrophy, thereby providing a direct force for evolution via enhanced bacterial growth [138,151]. Expanding on these general approaches for engineering new-to-Nature chemistries in living cells, here we describe a theoretical strategy to identify non-canonical atoms amenable to biologization, based on bonding preferences to biologically used elements.

A theoretical framework to identify chemical elements amenable to biologization

To identify atoms that could be engineered into living cells toward updating the periodic table of Life, we propose an unbiased selection criterion based on molecular thermodynamics of chemical bonds between biological and nonbiological elements. We gathered free energy (ΔG_0) values for single, double, and triple bonds between the main set of biological elements (i.e., C, N, O, P, and S) [152], and expanded the list to include all atoms from Groups 14–17, periods 2–5, 16 elements in total (Figure 5A). Energy values for these chemical bonds (in a pairwise X–Y configuration) were obtained from a web interface, based on molecular dynamics calculations adopted to optimize energetic approximations [152]. Our calculations take the specific geometry for each bonding type into account, although they might not reflect the conditions of the intracellular milieu. The choice of atoms for this analysis was determined by: (i) the chemical similarities between the novel and biological elements due to their position in the periodic table of elements; and (ii) their ability to form covalent bonds with C explored via a systematic thermodynamics exploration of their intrinsic reactivity.

Figure 5B shows a correlation plot between and bond enthalpy (i.e., the bond strength) and the Gibbs free energy of bond formation. This graphical representation identifies the chemical

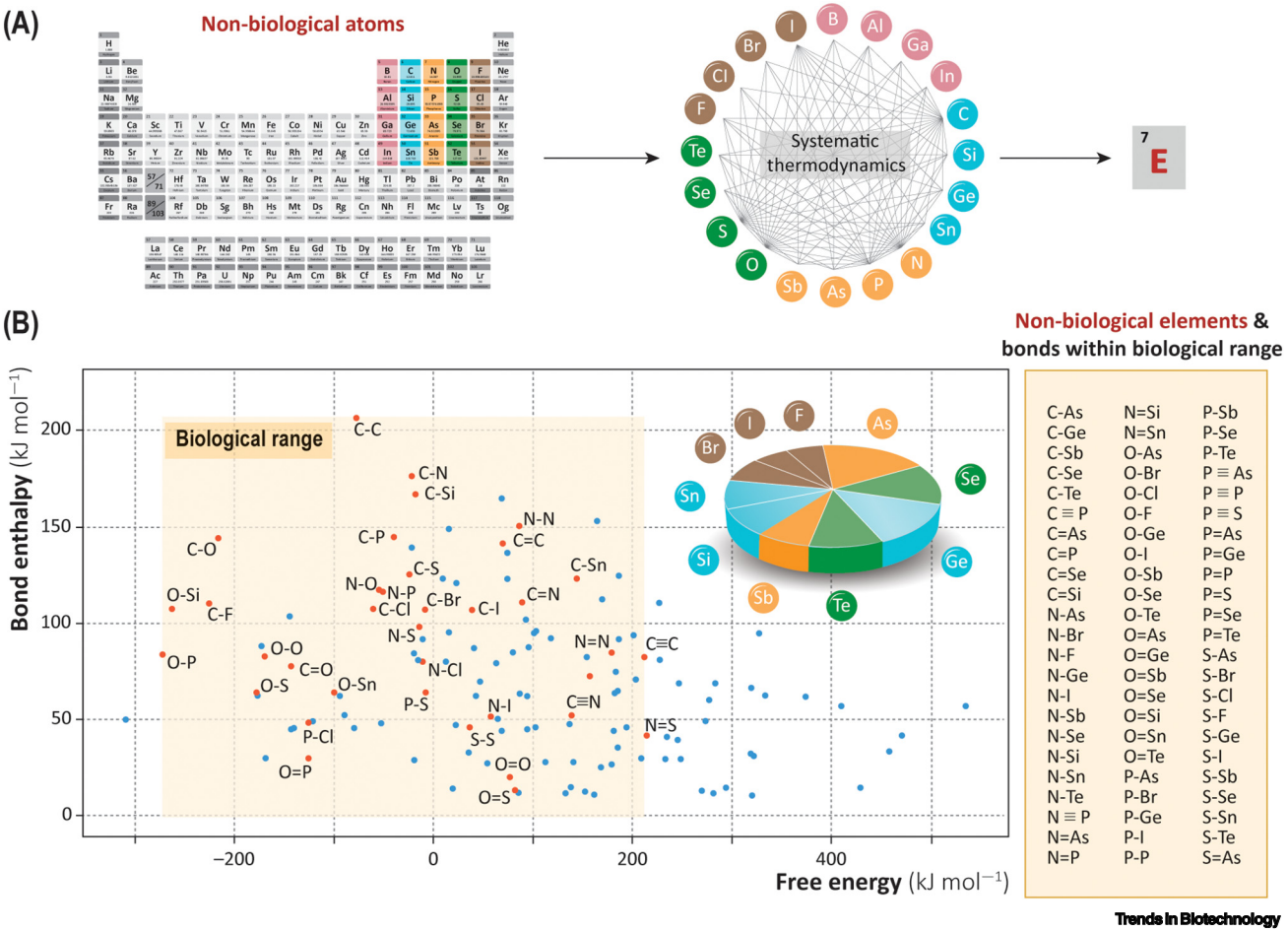

Figure 5. A systematic approach for selecting nonbiological chemical elements for xenobiology based on thermodynamics. (A) Schematic of a systematic pairing between biological and nonbiological elements chosen for thermodynamic-based analysis toward 'biologization'. (B) Correlation plot of bond enthalpy and Gibbs free-energy, which defines a range for biological elements (shaded by a light-yellow box) and their bonding preferences (red dots) and nonbiological elements (blue dots). The inset highlights nonbiological elements within the biological range of Gibbs free-energy, ranked by relative abundance. The box to the right identifies chemical bonds that fall within the biological range of Gibbs free-energy (both canonical and non-canonical elements) but have not yet been reported in biological systems. Thermodynamic constants for boron (B) could not be acquired, but, given its prevalent presence in biology, it can be expected that bonding preferences fall within the top rank of nonbiological atoms amenable for incorporation in living cells. Abbreviations: Al, aluminium; As, arsenic; B, boron; Br, bromine; C, carbon; Cl, chlorine; F, fluorine; Ga, gallium; Ge, germanium; I, iodine; In, indium; N, nitrogen; O, oxygen; P, phosphorus; S, sulfur; Sb, antimony; Se, selenium; Si, silicon; Sn, tin; Te, tellurium.

landscape in which the two variables could overlap, thereby allowing for biologization. Although there appears to be no obvious trend recognizable on first glance, it is interesting that the Gibbs free energy values of already-biological chemical elements (and the bonds they can form) lie between the –280 kJ mol^{-1} and +210 kJ mol^{-1} range, which is only ~50% of the range covered when all elements are considered (from –350 kJ mol^{-1} to +550 kJ mol^{-1}). We speculate that these energy limits are relevant for biological systems and, subsequently, for the introduction of novel elements into biology via synthetic metabolism. For this reason, we counted the occurrence of nonbiological elements to assess whether some of them preferentially fall within these limits (Figure 5B, inset). Strikingly, 33 out of 58 such occurrences (57%) comprised only four elements: As (19%), Ge (14%), Se (12%), and Te (12%). The bonding types for these four elements included single and double bonds. Organic species derived from three out of these four elements have already been isolated from biological sources (See 'Organisms harbor a range

of small metabolites containing nonbiological atoms'). These observations suggest that the selection method via thermodynamic constraints could prove useful as a general methodology for identifying **new-to-biology** chemical elements to be incorporated with synthetic metabolism.

Concluding remarks

Bringing nonbiological elements to life represents a huge opportunity for sustainable, biotechnological production of new compounds and basic biochemical research, but also comes with many challenges and open questions (see Outstanding questions). To define which elements are in fact new to biology, we first researched publications and databases that reported the existence of molecules containing elements other than the canonical C, H, N, O, P, and S atoms. Here, we present evidence for the potential incorporation of many different elements, including B, Ge, Sn, As, Se, F, Cl, and I, into a variety of organic molecules, including: (i) methylated species; (ii) specialized, secondary metabolites (e.g., antibiotics, signaling molecules, and pigments); and also (iii) primary metabolites, including sugars, amino acids, and lipids. These findings highlight the incredible adaptability of organisms to their chemical milieu, and shows the enormous untapped potential that Nature continues to hold for synthetic biology and biotechnology. Certainly, these incorporated non-canonical elements are rather a (chemical) exception than the rule, mostly found in biotechnologically irrelevant or genetically intractable organisms, and are often not the most desired atoms or attached to useful metabolites. However, synthetic metabolism and genetic enzyme engineering offer remarkable tools for optimizing these 'off-target' activities to the creation of completely new bonds between an organic molecule and the atom of interest (i.e., the C–Si or C–B examples discussed herein [116,136]). Pathway engineering and expression in nontraditional production hosts will further aid the establishment of a bioeconomy around these compounds and will enable production of novel materials; the existing examples serve as a good indication that this is the way forward. Computational approaches could prove equally useful for the identification of reaction conditions that could be mimicked by biological systems.

To conclude, we used thermodynamic bonding preferences between biological and a set of nonbiological atoms to theoretically predict the most promising elements added to biological systems. As, Ge, Se, Te, and antimony were the resulting top five, based purely on thermodynamic properties (Figure 5). Naturally, the question remains whether these elements could be of substantial use for production purposes, but they represent 'low-hanging fruits' given the relative ease of bonding with canonical elements. Value-added organic compounds containing these atoms are already on the market. Halogens, especially F, but also B are, to date, relevant elements for industrial applications because they enhance the characteristics of many currently used materials, pharmaceuticals, or specialty chemicals. Biotechnology and synthetic biology have come a long way with the use of 'just' six chemical elements, and the addition of novel atoms to Biology's palette will certainly open doors to opportunities beyond our current expectations, satisfying needs in the future that we are only now starting to identify as relevant in our modern society.

Outstanding questions

What are the exact biochemical mechanisms/undiscovered routes that cells use to incorporate non-canonical trace elements into their metabolites?

Are these mechanisms conserved across (microbial) species?

What is the full scope of chemical elements that a cell could adopt?

Are there any genetic and biochemical prerequisites to blend nonbiological elements into microbial biochemistry?

Can organisms be made 'addicted' to nonbiological elements using synthetic biology approaches?

How will organisms adapt in an ever-changing environment with new compounds leaching from industrial activities?

Why do we not see all possible chemical-bonding preferences of canonical elements in biology?

Acknowledgments

The authors thank all members of the EU Synthetic biology-guided engineering of *Pseudomonas putida* for biofluorination (SinFonia) consortium for fruitful exchanges and discussions. We would also like to acknowledge the work of the many researchers who made authoritative contributions to the field of neo-biochemistry and synthetic metabolism, the work of whom could not always be cited in this article because of space constraints. Financial support from The Novo Nordisk Foundation through grants NNF20CC0035580, *LiFe* (NNF18OC0034818), and *TARGET* (NNF21OC0067996), and the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 814418 (*SinFonia*) to P.I.N. is gratefully acknowledged.

Declaration of interests

The authors declare that there are no competing interests associated with the contents of this article.

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