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Using non-enzymatic chemistry to influence microbial metabolism

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The structural manipulation of small molecule metabolites occurs in all organisms and plays a fundamental role in essentially all biological processes. Despite an increasing interest in developing new, non-enzymatic chemical reactions capable of functioning in the presence of living organisms, the ability of such transformations to interface with cellular metabolism and influence biological function is a comparatively underexplored area of research. This review will discuss efforts to combine non-enzymatic chemistry with microbial metabolism. We will highlight recent and historical uses of non-biological reactions to study microbial growth and function, the use of non-enzymatic transformations to rescue auxotrophic microorganisms, and the combination of engineered microbial metabolism and biocompatible chemical reactions for organic synthesis.

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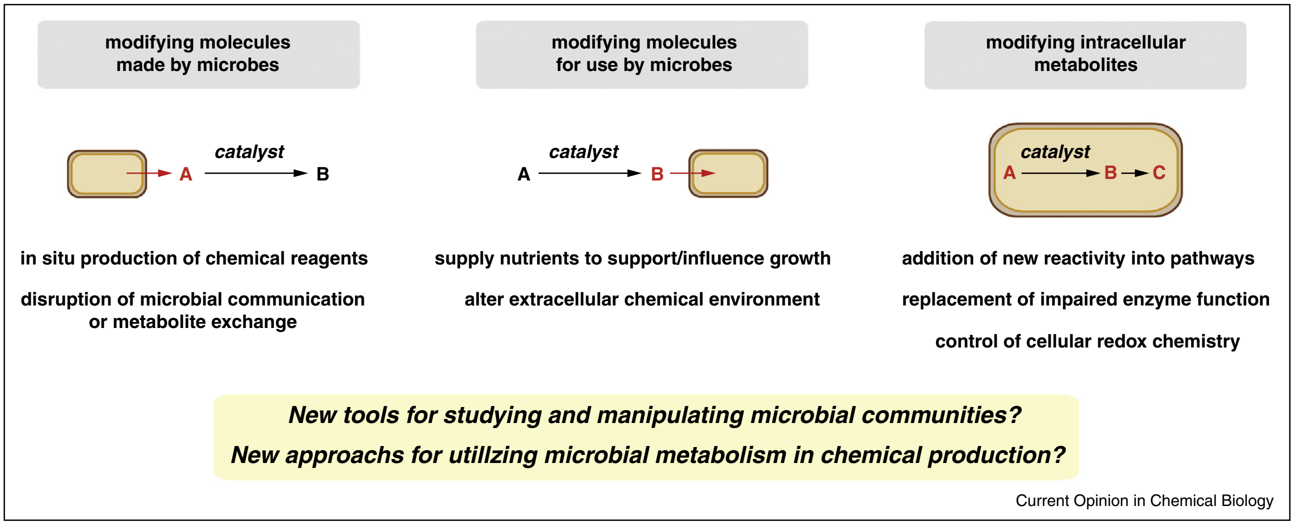
Introduction

Microorganisms are the most abundant and diverse organisms on Earth. Through billions of years of evolution they have acquired the ability to inhabit an astonishing array of environments [1]. These include the extreme temperatures found in deep-sea hydrothermal vents (hyperthermophiles) [2] and in subterranean ice sheets (psychrophiles) [3]; the high pressure of the Mariana Trench (piezophiles) [4]; and various sites in and on the human body (the human microbiota) [5]. To support growth in these habitats, many microbes have coevolved strategies to support their metabolism that involve chemistry not encoded by their genomes. In many cases survival is therefore dependent on a microbe's ability to interface its metabolism with the surrounding chemical environment. Conceptually this parallels the way synthetic chemists make molecules in a laboratory setting, using non-enzymatic reagents and catalysts to synthesize molecules essential to our everyday lives.

Over the past several decades there has been a steadily growing interest in developing non-biological chemical transformations for use in a cellular setting [6–12]. Although such reactions have been demonstrated to occur in both cells and in living organisms and are powerful tools for studying natural biological processes (bioorthogonal chemistry), the question of whether or not their reactivity can interface with cellular metabolism and alter biological function remains comparatively underexplored. One approach to achieving this goal is the development of biocompatible chemistry: non-enzymatic reactions that alter the structures of metabolites as they are produced by living organisms. There are multiple ways in which biocompatible transformations could be integrated with cellular metabolism (Figure 1). They could modify the end products of metabolic pathways, support metabolic functions by generating key substrates or nutrients *in vivo*, or be fully integrated into cellular metabolism.

One of the largest challenges encountered in merging non-enzymatic chemistry with metabolism is the apparent mismatch between the approaches used in synthetic organic chemistry (non-aqueous solvents, rare earth and transition metals, reactive intermediates, extreme temperatures and pH) and the growth conditions required to support a living organism (aqueous media, ambient temperature, neutral pH). The complexity of the cellular and extracellular environments and the typically low concentrations of cellular metabolites are also potential concerns. As has been the case with bioorthogonal reactions, we feel that these obstacles may be overcome through reaction screening optimization and that the potential benefits associated with the realization of this approach justify the challenges associated with developing such transformations. Biocompatible chemistry would provide scientists with a unique toolkit for manipulating and augmenting biological function *in vivo* that would not require genetic manipulation and could be applied to both cultured and uncultured organisms. This review will discuss recent achievements in combining non-enzymatic chemistry with metabolism, with a particular emphasis on studies involving microorganisms. We will focus on non-enzymatic reactions that have a direct influence on metabolic function. We will not include examples

Figure 1



Approaches to interfacing biocompatible chemistry with microbial metabolism and potential applications of this technology.

that use solely genetic approaches to manipulate metabolism or studies that use non-enzymatic de-caging strategies to control protein function *in vivo*. We will highlight key experiments that have seeded interest in this topic and provide our perspective on the future challenges and opportunities for this area of research.

The role of non-enzymatic chemistry in natural microbial habitats

Before discussing examples of non-biological transformations that have been designed by chemists and chemical biologists to function in the presence of living systems, we will briefly discuss several examples of how microbes have evolved to utilize non-enzymatic reactions to facilitate growth in natural settings. The influence of non-enzymatic chemistry on microbial metabolism is a phenomenon often observed in natural microbial populations. Non-enzymatic transformations have also frequently been proposed to play important roles in the origin of life and metabolic pathways. In 1924 biochemist Aleksandr Oparin first hypothesized that carbon-based metabolism could have originated from non-enzymatic chemistry enabled by the highly reducing geochemical environment of the early Earth [13–16]. In support of this hypothesis, ferrous iron has been shown to catalyze transformations that in modern organisms constitute glycolysis and pentose phosphate pathway chemistry, suggesting that the origins of cellular metabolism could date back to non-enzymatic chemistry operating in the iron-rich oceans of the prebiotic world [17•].

The chemistry of environmental iron impacts microbial survival in limiting habitats [18–22]. Under oxygen-limiting conditions many facultative anaerobic microbes use extracellular iron and manganese ions as terminal electron acceptors [23]. This use of redox-active environmental metals is especially remarkable due to the chemical challenges associated with their acquisition. For example, geological manganese and iron exist in metal oxides/oxyhydroxides that are inaccessible to reduction due to their insolubility. Microbes overcome this issue by excreting small molecules capable of solubilizing these metal ions (siderophores), as well as redox active small molecules that can directly reduce metal oxides (extracellular electron shuttles) [24]. An example of a microbe that uses redox-active small molecules to facilitate respiration of metals is the opportunistic pathogen *Pseudomonas aeruginosa*, which secretes redox-active pigments collectively known as phenazines [25]. Secreted, reduced phenazines support respiration under anaerobic conditions by transferring electrons to nearby extracellular oxidants (frequently $Fe^{2+/3+}$). This generates ATP through the Entner-Doudoroff pathway, which involves the non-enzymatic regeneration of NAD^+ from accumulating NADH (Figure 2A) [26]. Phenazines and Fe^{2+} have been detected in the sputum of cystic fibrosis patients at levels consistent with this process supporting the anaerobic survival of *P. aeruginosa* in this environment and contributing to chronic infection [27,28•]. This observation has led to research into iron-chelation therapy as a treatment for persistent *P. aeruginosa* infections [29–31].

Microbes in soil and aquatic ecosystems also use iron to support their metabolism by harnessing its reactivity to promote the non-enzymatic degradation of lignin [32]. Lignin is complex polymer containing aromatic functionality