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Shining a light on enzyme promiscuity

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Most, if not all, enzymes are capable of catalyzing physiologically irrelevant secondary reactions — termed 'promiscuous' reactions — in addition to the reactions that they have evolved to catalyze. Promiscuous activities can provide the starting point for evolution of new enzymes, both in nature and in the hands of protein engineers. Recent work suggests that the universe of promiscuous activities available in nature is enormous. New high-throughput approaches have significantly advanced our ability to identify promiscuous activities, setting the stage for synthetic biology efforts to construct novel pathways using catalysts derived from promiscuous enzymes via directed evolution.

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Introduction

Enzymes involved in primary metabolism are typically prodigious catalysts, capable of accelerating chemical reactions by up to 26 orders of magnitude [1] and of exquisite discrimination against other metabolites. However, even the most impressive enzymes are not perfectly able to control access to their active sites; natural selection only provides pressure for evolution of specificity to the point at which 'unauthorized' access does not impair the fitness of the organism.

Accidental binding of non-canonical substrates in a reactive active site environment sometimes results in a chemical reaction; such reactions are referred to as 'promiscuous'. 'Substrate promiscuity' occurs when enzymes carry out their typical catalytic functions using non-canonical substrates. By contrast, 'catalytic promiscuity' occurs when the catalytic abilities of the active site are used to catalyze a distinctly different type of reaction. Figure 1 illustrates how different the primary and catalytically promiscuous reactions can be.

Promiscuous activities are important because they provide the starting point for evolution of new enzymes. Even though they are usually less efficient than primary activities, promiscuous activities can accelerate reactions by factors up to 10^{26} [1]. When an environmental change makes a promiscuous activity important for fitness, gene duplication followed by divergence can allow evolution of a new enzyme while the original activity is maintained [2,3]. Promiscuous activities also provide useful starting points for novel enzymes in engineered pathways for synthesis of valuable chemicals and pharmaceuticals [4]. In some cases, even a single mutation can improve a promiscuous activity by more than 100-fold [5,6]. In a particularly notable case, changing Glu323 to Gly in muconate lactonizing enzyme II improved a promiscuous *o*-succinylbenzoate synthase activity by over 10^6-fold [7]. The evolutionary potential of promiscuous enzymes goes beyond just providing the starting point for individual new enzymes, however. Multiple promiscuous activities can be patched together, either in nature or in the laboratory, to generate new metabolic pathways. Examples include novel pathways for synthesis of pyridoxal 5′-phosphate [8], vanillin [9], and (*R*)-hydroxybutyrate and (*S*)-3-hydroxybutyrate [10], as well as for degradation of anthropogenic pollutants [11].

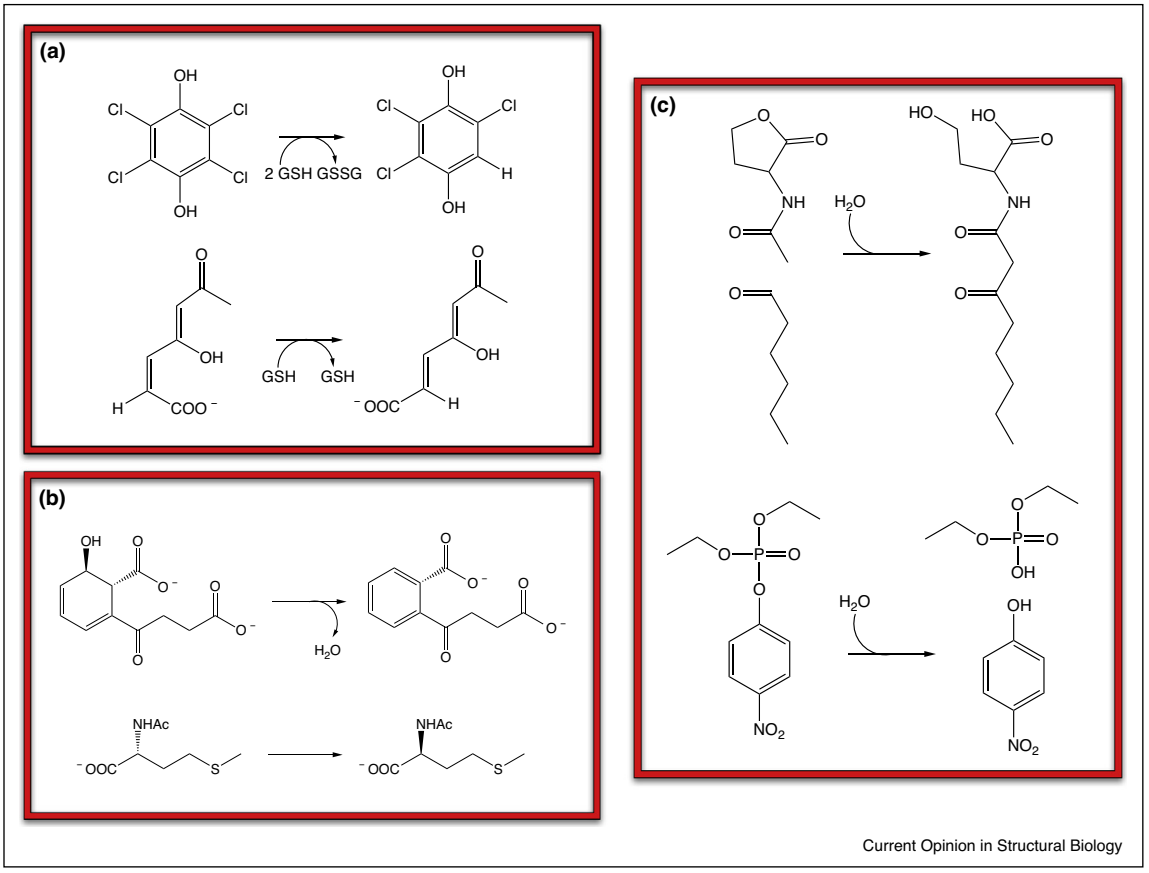
This review covers recent contributions to our understanding of the extent of enzyme promiscuity, as well as new ways of identifying promiscuous activities and insights into why they are often inefficient, with an emphasis on the years 2014–2016.

But first: when is a reaction promiscuous?

The term promiscuity is used in two different ways in the literature. Some authors refer to enzymes with broad substrate specificity as promiscuous. This is a reasonable use of the word, which can be defined as 'indiscriminate'. However, others [12] and I [13] prefer to restrict the term to secondary reactions that are *physiologically irrelevant*. The distinction is important. In some cases, such as the glutathione *S*-transferases that detoxify electrophilic compounds in the mammalian liver, broad specificity is clearly an evolutionarily beneficial trait. This is clearly a different situation from the existence of many very inefficient secondary activities in an enzyme, none of which makes a contribution to the fitness of the organism.

The situation is further complicated because broad specificity likely renders an enzyme more promiscuous.

Figure 1



Examples of catalytic promiscuity. **(a)** Tetrachlorohydroquinone dehalogenase catalyzes isomerization of maleylacetate [55]. (GSH, glutathione; GSSG, glutathione disulfide). **(b)** *o*-Succinylbenzoate synthase catalyzes racemization of *N*-acyl amino acids [56]. **(c)** PON1 (a homoserine lactone hydrolase) catalyzes hydrolysis of paraoxon [43].

Broad-specificity enzymes often have large active sites that can accommodate substrates of varying sizes and shapes [14,15]. Hydrophobic active sites that foster binding by hydrophobic effects rather than specific electrostatic and hydrogen bonding interactions also likely enable binding of physiologically irrelevant substrates.

The definition given above begs the question of how we can know whether a secondary activity is physiologically relevant or irrelevant. The distinction is obvious when a much more efficient enzyme exists within the organism. For example, the ability of *Escherichia coli* γ-glutamyl phosphate reductase (ProA) to catalyze reduction of *N*-acetylglutamyl phosphate is clearly promiscuous, as *E. coli* has an enzyme (ArgC) that is specialized for the latter function. Further, ProA cannot compensate for the loss of ArgC [16]. Secondary activities are also clearly promiscuous when the enzyme never encounters the substrate. A recent screen of two ω-transaminases with 32 amino donors showed that one or both had detectable activities with 31 substrates [17]. Many of these secondary activities are certainly promiscuous because the substrates are non-physiological.

Some circumstances, however, are less clear. The *E. coli* phosphatase NagD has high activity with UMP and CMP ($k_{cat}/K_M > 10^4\ M^{-1}\ s^{-1}$), but also respectable activities ($k_{cat}/K_M > 10^3\ M^{-1}\ s^{-1}$) with GMP, AMP, ribose 5-phosphate, pyridoxal 5′-phosphate, glucose 6-phosphate, glucosamine 6-phosphate, glycerol 3-phosphate and dTMP [18]. It is difficult to know whether the less efficient activities or the activities with substrates that are present at low concentrations *in vivo* (e.g. pyridoxal 5′-phosphate) are physiologically irrelevant or not. Ambiguous cases such as this one force us to confront the likelihood that multi-specific enzymes that do not fit neatly into linear metabolic pathways may in fact be beneficial in the context of an inter-connected metabolic network. In such cases, many, although perhaps not all, of the activities may be more properly described as due to multi-specificity rather than to promiscuity.

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