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Accuracy-rate tradeoffs: how do enzymes meet demands of selectivity and catalytic efficiency?

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I discuss some physico-chemical and evolutionary aspects of enzyme accuracy (selectivity, specificity) and speed (turnover rate, processivity). Accuracy can be a beneficial side-product of active-sites being refined to proficiently convert a given substrate into one product. However, exclusion of undesirable, non-cognate substrates is also an explicitly evolved trait that may come with a cost. I define two schematic mechanisms. *Ground-state discrimination* applies to enzymes where selectivity is achieved primarily at the level of substrate binding. Exemplified by DNA methyltransferases and the ribosome, ground-state discrimination imposes strong accuracy-rate tradeoffs. Alternatively, *transition-state discrimination*, applies to relatively small substrates where substrate binding and chemistry are efficiently coupled, and evokes weaker tradeoffs. Overall, the mechanistic, structural and evolutionary basis of enzymatic accuracy-rate tradeoffs merits deeper understanding.

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Introduction

Living systems exhibit accuracy at all levels. We are, however, also aware of the limits of accuracy, through a diversity of phenomena ranging from genetic mutations and translational errors to enzyme promiscuity and metabolite damage [1]. Limited accuracy, noise, or messiness as defined by some, is also of crucial importance, as it provides the starting points for evolutionary innovations [2]. Take for example the existence of coincidental protein conformations and promiscuous enzymatic activities. Although never selected for, these latent, promiscuous conformers and activities serve as starting points if and when new enzymatic functions are needed [3]. The prevalence of inaccuracy, its evolutionary benefits but also its inevitable cost, prompt the question of why? Why is the accuracy of biological systems, and enzymes in particular, limited?

The evolutionary origins of inaccuracy

The evolutionary benefits of infidelity raise the tempting, albeit largely baseless, hypothesis that inaccuracy, and other phenomena related to life's complexity, are explicitly promoted by natural selection [4]. An alternative view is that accuracy comes with a significant cost, and is thus being maintained at an acceptable rather than maximal level [1,3]. Let us consider these two alternative hypotheses in more detail.

(i) *Inaccuracy is a selected trait*: Imagine an organism with a perfect replication and DNA repair machinery. Most mutations are deleterious, and indeed, the mutation rates in nearly all organisms are extremely low. In *Escherichia coli*, it takes, on average, about 10^6 generations for a gene to be hit by any one mutation. Even a mild increase in mutation rate is deleterious under optimal growth conditions, and following prolonged growth, also under challenging environments [5]. Mutations, however, are absolutely essential, as they provide the basis for adaptation. So are replication fidelity and repair explicitly kept from being too accurate? Namely, are life's molecular systems shaped by evolution with a dual purpose — performing their current function with maximal efficiency, and maintaining the capacity for innovation (evolvability [6])?

Despite its popularity, this hypothesis is rarely applicable, let alone unambiguously supported by data. As a rule, selection very rarely acts to limit the accuracy of replication, or of DNA repair enzymes, or in fact of any other enzyme. Most claims for noise or infidelity being explicitly shaped by evolution comprise Panglossian reasoning — if it is there, it must be for a good reason [1,2,4]. For example, the linkage between stress and higher mutational rates in bacteria is largely indirect [7]. One rare exception is RNA viruses where increasing replication fidelity impairs fitness [8]. The high error rate of Polio's RNA polymerase is under direct selection, and is tightly kept at approx. two mutations per genome per replication [8] (a rate that is *a priori* $>10^4$-fold higher than in non-viral organisms on a per base pair basis [9]).

(ii) *Inaccuracy — the lesser evil*: The alternative to (i) above, and in my view the default explanation for noise

and inaccuracy is that, at a population level, their deleterious effects are too weak to be eliminated by selection. Thus, as a default, biological molecules are as inaccurate as they can afford to be, and not as accurate as they could, or need to be, particularly so because accuracy comes with a cost. Proof-reading, or editing is an evident hallmark of cost: Hydrolysis of non-cognate aminoacyl-tRNAs by aminoacyl-tRNA synthases (AARSs), or the nuclease action of polymerases, impose a direct cost of ATP molecules for remaking these bonds (for a general model of speed-accuracy tradeoffs in systems with proof-editing see [10]).

Here, I discuss mechanisms of discrimination that occur within the primary active-site and do not involve undoing of enzymatic steps as in proof-editing. As discussed here, a potential cost of enzymatic accuracy is reduced rate, or lower processivity. Specifically, enzyme rates tradeoff with accuracy and with a range of other demands, thus resulting in a fine balance between the benefit and the cost of accuracy. Indeed, the catalytic efficiency (k_{cat}/K_M) of the ''average'' enzyme is $\sim 10^5\ M^{-1}\ s^{-1}$ [11], orders-of-magnitude lower than the diffusion rate limited 'perfect enzyme' [12]. Our understanding of the evolutionary constrains that shape enzymes is therefore relevant to our understanding of what is a 'perfect enzyme'.

The evolutionary origins of accuracy

Two alternatives can be noted — the first ascribes specificity to chance, and the second to a necessity:

(i) *Accuracy is an inherent side-product of catalytic proficiency*: Evolution shapes the active-sites of enzymes to maximize specific interactions between active-site residues and the reaction's transition-state (*positive selection*). Active-sites are unlikely to materialize the same interactions with substrates and transition-states that differ from the cognate one, certainly not to the same degree. Thus, selectivity can be an inherent side-product of catalytic efficiency. DNA polymerases may indeed represent such a case whereby catalytic efficiency for incorporation of then cognate base correlates with fidelity [13].

(ii) *Accuracy as an explicitly evolved trait*: In many enzymes, the inherent selectivity might be physiologically insufficient, especially when substrates similar to the cognate one are present at competing concentrations. In these cases, selectivity is likely to be the outcome of an explicit selection against certain substrates (*negative selection*).

How relevant is negative selection in explicitly shaping the specificity of natural enzymes? Laboratory evolution experiments suggest that positive selection on its own is insufficient to induce high selectivity [14]. As suggested by (i) above, selection toward one substrate and transition-state reduces the accommodation of other substrates. However, it seems that large losses in the original activity only come at the late stages of optimization toward a new activity [14]. Further, at this advanced stage, the improvement in the new activity per mutation is very small (diminishing returns). This means that inherent selectivity emerges slowly. High selectivity may inherently appear only when the newly evolving activity reached high levels [15], or may not occur at all if the selection pressure for high catalytic efficiency is weak [14]. Indeed, numerous natural enzymes show broad, or multi-specificity, and thus catalyze multiple transformations within the same cell [14,16]. Conversely, laboratory evolution experiments indicate that negative selection — selection against alternative substrates, induces selectivity much more effectively [17,18], and sometimes via mutations that abolish binding of the undesirable ligand with no effect on the evolving ligand [19]. Thus, in highly specific enzymes, selectivity is likely to be an explicitly evolved trait — that is, the combined outcome of *positive selection* for the cognate substrate and of *negative selection* against the nocognate ones.

Accuracy-rate tradeoffs

Accuracy-rate tradeoffs in enzymes relate to what degree increased accuracy leads to lower catalytic efficiency. Enzyme reactions being second-order, rates are usually expressed in the k_{cat}/K_M value for the cognate substrate. Accuracy is typically expressed as the *A value*, the ratio of k_{cat}/K_M values for the cognate substrate (the correct, physiological substrate) and alternative, non-cognate one(s) (Box 1). The magnitude of tradeoff cannot be derived from the kinetic parameters of a single enzyme variant or reaction. Rather, a comparison is needed, either of the wild-type enzyme to a mutant that exhibits higher or lower accuracy (as exemplified with DNA methyltransferaes below; see also Ref. [20]), or of the same enzyme under different reaction conditions, as discussed below with the ribosome's magnesium regulated rate-accuracy.

Measured as discussed above, the accuracy-rate tradeoff is a macroscopic property that does not relate to the molecular mechanism by which accuracy is achieved in a particular enzyme and to why it affects rate. Regarding the mechanism, two modes of discrimination can be schematically described: substrate, or *ground-state discrimination*, and catalytic or *transition-state discrimination* (Figure 1).

Theoretically, ground-state discrimination should be manifested in differences in K_M values, and transition-state discrimination in k_{cat}. However, enzymatic microscopic rate constants are coupled, and thus both K_M and k_{cat} may be affected.