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'Negative' and 'positive catalysis': complementary principles that shape the catalytic landscape of enzymes

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Our understanding of enzyme catalysis is dominated by transition state theory. According to this concept, an enzymatic reaction is guided along a desired reaction coordinate through the stabilization of favorable transition state. But how much is the outcome of an enzyme reaction controlled by the destabilization of unwanted transition states? Here, we revive and critically review the hypothesis that the active site of enzymes also features elements of 'negative catalysis'. We provide examples that show that enzyme catalysis can be achieved by the combined action of positive and negative constraints at the active site of an enzyme. This integrated view of enzyme catalysis has direct consequences for our studies on the catalytic landscape of enzymes, as well as current efforts in enzyme engineering and the *de novo*-design of enzymes.

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Enzymes are remarkable catalysts that can achieve rate accelerations of up to nineteen orders of magnitude at high regio specificity, stereo specificity and reaction specificity compared to the uncatalyzed reaction [1]. The concept of enzyme catalysis as formulated by Pauling states that enzymes accelerate reaction rates by binding transition states better than substrates, thereby lowering the activation energy of the reaction [2]. Pauling's original concept was expanded and further refined, by including additional factors that also contribute to the lowering of activation energies, such as ground state destabilization, conformational substrate stabilization and enzyme preorganization [3–6]. Besides catalyzing a given reaction enzymes are very efficient at preventing alternative reaction outcomes, which would be favored in solution or in the gas phase over the desired reaction. This enables enzymes to catalyze chemically 'difficult' or 'improbable' reactions involving highly reactive intermediates [7••]. The exquisite control of intermediates over the course of a reaction is a key feature of enzyme catalysis. Here we focus on the question how active sites confer reaction specificity, that is how they prevent alternative reactions during catalysis. We do not discuss how substrate specificity is achieved, which is reviewed elsewhere [8–10]. We would however be remiss not to mention that similar negative selection mechanisms may apply for substrate specificity [8].

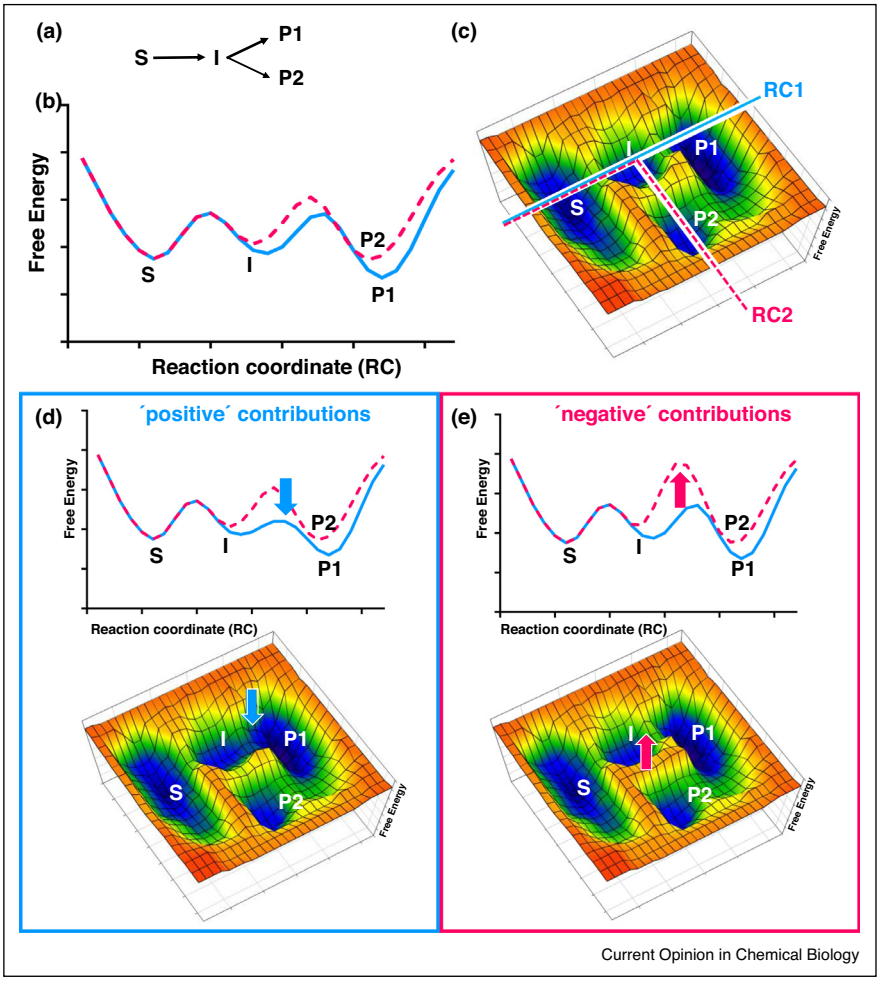
The catalytic landscape of enzymes is multi-dimensional and shaped by positive and negative contributions

The progress of enzyme reactions is typically visualized in two-dimensional plots, the free energy along the reaction coordinate (RC). In these graphs, transition states (TSs) are depicted as local energy maxima along the reaction path (Figure 1b). Although used very often to describe enzyme reactions, the two-dimensional representation of catalysis is rather misleading. On a three dimensional global energy landscape the above mentioned TS represents a energetic minimum compared to alternative TS of neighboring RC's (including RC's of side reactions). Conceptually it is thus more correct (and more helpful) to think of enzyme catalysis in a multi-dimensional landscape, where TSs represent saddle-points along different possible reaction outcomes (Figure 1c).

How do enzymes control their catalytic landscape to form the correct reaction product? In principle the outcome of a reaction can be determined through two different mechanisms: Enzymes could guide a reaction along a minimum energy path by lowering the energy of productive TSs that lead to formation of the wanted product, as formulated by Pauling (Figure 1d). Alternatively, enzymes could increase the energy of competing TSs that would lead to the formation of alternative reaction products (Figure 1e). Even though the final product of the reaction would be the same, it is a conceptual, as well as a mechanistic difference whether a preferred reaction is promoted, or whether competing side reactions are suppressed by an enzyme during catalysis.

In the past, most of the research focused on understanding the principles that are used by active site residues to promote catalysis. In contrast, much less is understood

Figure 1



Visualization of a chemical reaction. (a) Simple reaction scheme depicting the possible outcomes of a chemical reaction. **(b)** Two dimensional free energy plot along the reaction coordinate (RC). RC1 (light blue) shows the RC for the formation of the desired product P1. RC2 (pink) shows the RC for the formation of the side product P2. Transition states (TSs) are shown as local maxima. **(c)** Three dimensional free energy landscape showing the TSs as saddle points along the RCs. **(d)** 'Positive' contributions that stabilization of the productive TSs lead to increased formation of the desired product (P1). **(e)** 'Negative' contributions that destabilize the unwanted TSs also lead to the increased formation of the desired product (P1) compared to the side product (P2).

about the mechanisms that suppress the formation of competing TSs leading to unwanted side reactions, even though a concept of 'negative catalysis' was developed by Rétey almost 30 years ago [7••]. According to this concept, the suppression of unwanted side reactions ('negative catalysis') can be equally important as the promotion of the desired reaction ('positive catalysis').

A simple way to visualize positive and negative contributions of active site residues to catalysis

How can positive and negative contributions of active site amino acids to catalysis be assessed? In principle, the role of a given amino acid can be described in two dimensions. One dimension is the contribution of an active site residue to the apparent turnover frequency of an enzyme (k_{cat}), while the other is the contribution of an active site residue to reaction specificity, expressed as the relative ratio of side product (*P2*) to reaction product (*P1*), expressed as $-log[P1/P2]$.

In a first approximation residues conferring positive contributions would mainly affect the k_{cat} of an enzyme (measured as rate of substrate consumption or formation of all products). Residues conferring negative contributions on the other hand would mainly affect $-log[P1/P2]$. Thus, plotting the effect of an active site mutation onto k_{cat} versus $-log[P1/P2]$ in an activity/specificity-graph provides an intuitive way to visualize and understand the contributions of an individual residue to catalysis (Figure 2), although it