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Review

Biological messiness *vs.* biological genius: Mechanistic aspects and roles of protein promiscuity☆

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ABSTRACT

In contrast to the traditional biological paradigms focused on 'specificity', recent research and theoretical efforts have focused on functional 'promiscuity' exhibited by proteins and enzymes in many biological settings, including enzymatic detoxication, steroid biochemistry, signal transduction and immune responses. In addition, divergent evolutionary processes are apparently facilitated by random mutations that yield promiscuous enzyme intermediates. The intermediates, in turn, provide opportunities for further evolution to optimize new functions from existing protein scaffolds. In some cases, promiscuity may simply represent the inherent plasticity of proteins resulting from their polymeric nature with distributed conformational ensembles. Enzymes or proteins that bind or metabolize noncognate substrates create 'messiness' or noise in the systems they contribute to. With our increasing awareness of the frequency of these promiscuous behaviors it becomes interesting and important to understand the molecular bases for promiscuous behavior and to distinguish between evolutionarily selected promiscuity and evolutionarily tolerated messiness. This review provides an overview of current understanding of these aspects of protein biochemistry and enzymology.

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1. Introduction

The structural biology revolution that spanned the 1980s–1990s temporarily reinforced the long-held belief that enzymes and receptors were exquisitely specific in their substrate or ligand interactions. An explosion of published X-ray structures seemed to confirm the traditional perspective that receptors and enzymes were 'special' because of their specificity. It was easy to visualize

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directly, based on models derived from crystallography, that enzyme and protein active sites usually exploit all possible 'handles' for their interactions with their cognate ligands. Structurally similar ligands can be selectively recognized by different active sites because enzymes or proteins can exploit spatially optimized hydrogen bonds, ionic interactions and hydrophobic contacts, and they can also exclude non cognate ligands *via* steric clashes or charge repulsion [1–6]. The structural perspective revealed the mechanisms by which enzymes and proteins achieve the molecular recognition that had been heralded for decades.

However, the pendulum has swung. During the past 15 years that perspective has expanded to accommodate the growing realization that most enzymes or proteins are not as 'ligand specific' as the textbooks, or crystal structures, suggested, supporting the initial observations made by a few [7,8]. In fact, many enzymes are conspicuously promiscuous *in vitro* despite their critical roles in core metabolism *in vivo*. A search of the literature published in the past five years reveals a dramatic increase in the number of publications with 'promiscuity' in the title compared to 'specificity' (which remains high but more constant), as expected for the increased interest in the subject and the corresponding new insights that result.

More importantly, our growing awareness of promiscuity as a property of proteins has been accompanied by the realization that functional promiscuity or 'messiness' has clear roles in biology and biotechnology [9–17]. It is now apparent that promiscuity is as biologically important as specificity and a significant challenge lies in understanding how biological systems achieve controlled promiscuity and how they exploit it or tolerate it. Intuitively, it is a greater challenge to understand promiscuity than specificity, and definitive rules or concepts about promiscuity are still being developed.

My lab has devoted significant effort toward this aspect of protein structure and function. Our efforts with detoxication enzymes have revealed some useful lessons about the origins of molecular promiscuity and the behavior of promiscuous enzymes, which may be applicable to steroid enzymology. Many of the enzymes in steroid biosynthetic pathways are cytochrome P450s (CYPs) related to the highly promiscuous CYPs involved in detoxication, and we have considered the relative promiscuity of individual isoforms within this family and others. Many of our findings are relevant to this edition. This overview of promiscuity extends beyond enzymes to include other proteins and receptors.

2. Definitions

As with all new fields of study, it is critical to define terms. There have been many terms used to describe variations of promiscuous behavior, including terms defined in thoughtful and extensive reviews by Tawfik et al. [9,13]. I will limit the terms here to distinguish a few types of promiscuous behavior that are most well described, and those types of promiscuity most relevant to this edition. The definitions I find most useful are purely operational and less restrictive than those used by others [9,13] and are schematized in Fig. 1. If an enzyme or protein interacts with multiple structurally distinct ligands or substrates at a single binding site, this is promiscuous behavior. In contrast, others prefer to reserve the use of the term 'promiscuous' for cases where an enzyme or protein interacts with a ligand other than the ligand or set of ligands it is 'supposed to' interact with based on its biological role. With that definition, the term 'promiscuity' is applicable when an enzyme or receptor 'makes a mistake'. With this more restrictive definition, enzymes or proteins that interact with multiple substrates or ligands as part of their normal function would be called 'multispecific' rather than promiscuous. Arguably,

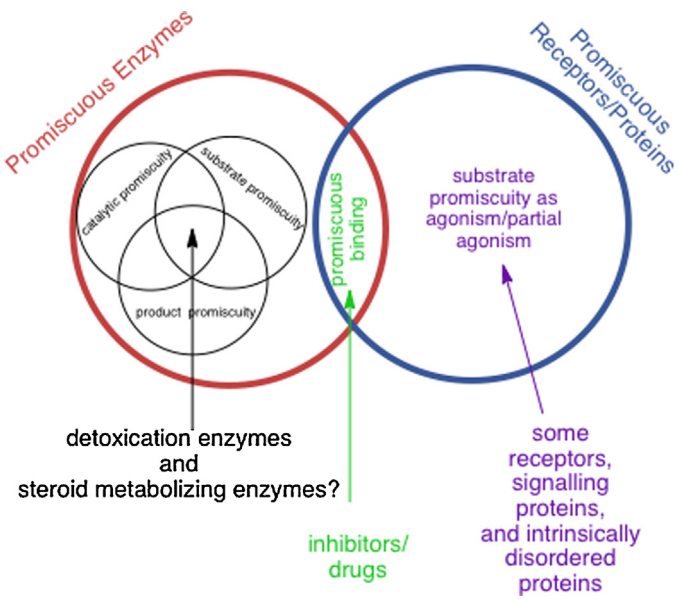

Fig. 1. Types of promiscuous behavior exhibited by enzymes, receptors and other proteins. The specific types of promiscuous behavior summarized are described in the text. The relative sizes of the compartments do not reflect their relative frequency or abundance. For example, promiscuous binding is more frequent than catalytic promiscuity, but this is not reflected in this figure.

'multispecific' would be a better term for enzymes that, in accord with their biological function, have clear specificity toward multiple substrates, as many do, rather than the proteins or enzymes that have no clear preference for any ligands. Therefore, to capture adequately the biological scope of the physico–chemical trait wherein enzymes and proteins are not as specific as once described, I apply 'promiscuity' to any case where multiple ligands can bind at a common site. Of course, then, essentially all proteins or enzymes are promiscuous to some degree and this demands consideration of how much promiscuity is tolerable *vs.* useful in different situations, as with steroid metabolism and signaling that are the focus of this edition. Even with these differences in the use of terms by different groups, several definitions are consistent with those depicted in Fig. 1 for both enzymes and receptors. Types of promiscuity that are relevant for enzymes are schematized in Fig. 2.

For enzymes, "catalytic promiscuity" is the ability of a single enzyme isoform to catalyze different types of chemical transformations, such as hydrolysis of esters or lactones *vs.* structurally distinct phosphotriesters. In this case a single enzyme has the ability to stabilize transition states of different reaction types. Also for enzymes, "substrate ambiguity", or "substrate promiscuity" refers to their ability to perform the same type of chemical transformation on different substrate structures. For example, some reductases metabolize fatty acyl CoAs of different acyl chain length [18] and some kinases recognize peptide motifs rather than specific peptide sequences [19]. In the case of substrate promiscuity the local transition states for the reaction are very similar or identical, but the structure of the substrate remote from the transition state varies. In addition to catalytic promiscuity and substrate promiscuity of enzymes, 'product promiscuity' should be acknowledged. Product promiscuity refers to the situation when a single enzyme converts a single substrate to multiple products in reactions that require different transition states. For example, proteases that cleave a peptide at a single peptide bond generate two product peptides from a single substrate peptide, but this requires a single transition state and does not represent product promiscuity. On the other hand a protease that hydrolyzes a single