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Towards mechanistic classification of enzyme functions

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Abstract

Classification of enzyme function should be quantitative, computationally accessible, and informed by sequences and structures to enable use of genomic information for functional inference and other applications. Large-scale studies have established that divergently evolved enzymes share conserved elements of structure and common mechanistic steps and that convergently evolved enzymes often converge to similar mechanisms too, suggesting that reaction mechanisms could be used to develop finer-grained functional descriptions than provided by the Enzyme Commission (EC) system currently in use. Here we describe how evolution informs these structure-function mappings and review the databases that store mechanisms of enzyme reactions along with recent developments to measure ligand and mechanistic similarities. Together, these provide a foundation for new classifications of enzyme function.

Introduction

The chemical reactions necessary to support life are catalyzed by enzymes representing a remarkable diversity of substrate and reaction specificities. The classification of their sequences and structures has been facilitated by underlying evolutionary and biophysical models, enabling quantitative assignment of sequence and structural similarity. In contrast, classification of enzyme functions currently relies on the Enzyme Commission (EC) system [1], which is based only on qualitative descriptions of the overall transformation catalyzed, a level of functional granularity too broad to allow direct correlation between enzyme functions and the structural features that are associated with them [2]. We discuss here some features of functionally diverse enzyme superfamilies and of convergently evolved enzymes and explain how the mechanistic steps in their catalytic mechanisms represent a more useful level of functional granularity than overall reactions for linking structure and function. As enzymes are discovered by genome sequencing, creation of resources to manage and enable investigation of their molecular diversity also becomes increasingly important. Thus, we also include here a review of the databases specifically developed to link active site structural features to their mechanistic capabilities in a computationally accessible manner.

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Finally, models for quantitative comparisons of ligands and mechanisms are described, along with a discussion about how these provide a foundation for a more robust and structurally contextual classification of enzyme functions.

Lessons learned from studying the evolution of enzyme functions

Early views of the enzyme universe assumed that a set of homologous enzymes catalyzed only one type of chemical reaction and that a particular chemical reaction was catalyzed by a single group of homologous enzymes. As more information has accumulated, larger-scale studies of the relationship between enzyme structure and function have shown both that divergent evolution from a common ancestor often generates superfamilies of enzymes catalyzing a diversity of reactions [3,4] and that convergent evolution independently generates unrelated enzymes that catalyze the same type of chemical reaction [5•]. To obtain a current estimate of the incidence of divergent and convergent evolution, we used PDBSprotEC [6] and SCOP [7] to map EC sub-subclasses (reaction types) to structural superfamilies. These counts show that over one third of the structurally characterized enzyme superfamilies are functionally diverse (Figure 1a) and that over two thirds of the currently defined EC sub-subclasses contain analogous enzymes (Figure 1b). Thus, many more enzymes now appear to belong to functionally diverse enzyme superfamilies or evolved via convergent evolution than has been previously appreciated. This underscores the importance of using knowledge of how new enzyme reactions evolve to develop new classifications of enzyme function.

Divergent Evolution of Enzyme Function

The type and extent of functional similarity shared by divergently evolved enzymes depends on which elements of the active site of the common ancestor were more amenable to change and which were more conserved. Building on earlier observations [8,9], Babbitt and Gerlt introduced the model for “chemistry-constrained” evolution of enzyme function [10], where reaction chemistry is conserved among homologs whilst substrate specificity changes. Subsequent large-scale analyses examining pairs of homologous enzymes within and across genomes suggest that this model represents a dominant natural strategy in the evolution of new enzyme functions [11,12]. Several recent reviews continue to enumerate cases where all members of a superfamily conserve at least one common mechanistic aspect linked to conserved features of their active sites [13–16]. Other evidence indicates that most superfamilies also share similarities among their substrates [17], with one article reporting that all but one of 42 such superfamilies indeed share a common substructure in all of their substrates [18]. Diversity of substrate and reaction specificities in divergently evolved superfamilies is thus conscribed by both catalytic and binding capabilities associated with the common active site features in all its members. The result is a specific structure-mechanism paradigm for each superfamily that provides valuable context for classification of their reaction specificities and to guide the prediction of function for new enzymes discovered in the genome projects [19].

Convergent Evolution of Enzyme Function

Several studies have suggested that convergent evolution is also widespread [5,20,21] and that some non-homologous enzymes share structurally and functionally equivalent active site residues and similar mechanisms [20,22]. Recently, a quantitative assessment of convergently evolved enzymes revealed that of those enzymes that have similar overall reactions one third also had similar catalytic mechanisms [23••], concluding that functional analogs, like homologs, often share common mechanistic aspects along their reaction pathways. This finding opens up the possibility of generating structure-mechanism links for

functionally analogous enzymes too, along with strategies for function annotation based on analogy rather than homology.

Databases storing mechanisms of enzyme reactions

As the genome projects continue to grow at a rapid pace and more enzymes are cataloged, resources for structure-function mapping in enzymes are needed to aid in leveraging this information for applications that range from functional inference to enzyme design. Four highly curated databases are described below that link information about enzyme sequences and structures to information about their catalytic mechanisms in a computationally accessible form, a necessary prerequisite for the development of functional classifications that are quantitative and can be applied on a large-scale. These include the Structure-Function Linkage Database (SFLD) [24], the Mechanism, Annotation and Classification in Enzymes (MACiE) database [25], the Enzyme Catalysis Database (EzCatDB) [26], and the Hierarchical Classification of Hydrolases Catalytic Sites (HCS) database [27].

The SFLD [24] focuses explicitly on functionally diverse enzyme superfamilies, linking structure-function relationships at three levels of granularity: *superfamilies, subgroups*, and *families* (see Figure 2). At each of these levels, a multiple sequence alignment and a corresponding hidden Markov model are generated, along with interactive protein similarity networks [28•] that provide mapping between sequence similarity clusters and many types of functional properties to facilitate classification and annotation of new sequences (see, for example [29•,30–32]). The SFLD currently contains information on nearly 200 reactions from a number of large, functionally diverse enzyme superfamilies, several of which were reviewed in [4]. An advantage of this approach is that it is structurally contextual, allowing enzymes to be classified according to mechanistic properties directly associated with the structural features it shares with other superfamily, subgroup, or family members. Thus, newly discovered structures and sequences are annotated only at the levels for which strong evidence of membership exists, thereby avoiding misannotation to a family, and thus to an overall reaction specificity, if evidence only exists to link it to the more general mechanistic attributes common to members of a subgroup or superfamily.

The MACiE database [25] focuses on enzymes with known structure and plausible reaction mechanisms published in the literature. It is based on a mostly non-homologous dataset, chosen to represent at least one reaction for each of the EC sub-subclasses. It currently contains nearly 300 fully annotated reactions, each accompanied by a complete stepwise description of catalytic mechanism, including the type of chemistry involved and the functional role of the catalytic amino acid residues participating in each step. New resources focusing exclusively on the role of metal ions [33] and of organic cofactors [34] now complement the mechanistic data presented in MACiE providing a broader view of the chemistry of protein catalysis [35,36,37•,38•]. Because of its design, MACiE does not sample well divergently evolved enzymes, however, it includes several examples of convergently evolved enzymes.

The EzCatDB [26] includes nearly 750 enzyme reactions, each linked with sets of homologous enzyme structures annotated with their catalytic and cofactor binding residues, their cognate ligands, and textual descriptions of their catalytic mechanism. EzCatDB includes examples of both divergently and convergently evolved enzymes, and it has been useful for comparing the catalytic mechanisms of hydrolases and transferases [22] and for comparing active sites and ligand biding residues in functionally diverse superfamilies [39]. Finally, the HCS database [27] classifies active sites based on their catalytic roles and also includes mechanistic information. Currently, the dataset contains over one thousand hydrolases classified into less than one hundred classes, most including a scheme describing

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their associated catalytic mechanism. The content of the HCS makes it best suited for studying convergently evolved enzymes.

Two additional resources deserve mention in the context of mapping reaction information to structural features in enzymes. The Catalytic Site Atlas (CSA) [40] stores hand-curated information about the catalytic residues of nearly one thousand enzyme structures that by homology can be transferred to nearly 30 thousand structures. The PROCOGNATE database [41], maps cognate ligands to enzyme domains, covering about ten thousand structures from the PDB. Both are suited for the study of divergent and convergent evolution.

Measuring similarity of ligands and mechanisms

Fueled by the increasing availability of enzyme ligand and mechanistic data on a computationally accessible form, several methods have emerged to compare ligands and mechanisms, adding to the toolset required for quantitative analysis of enzyme function.

Ligand similarity

Evaluation of ligand similarity is well developed in cheminformatics and drug design and various methods are available to describe the structures and properties of small molecules and compute their similarities (reviewed in [42]). Of interest here, several reports have clustered cognate ligands of enzymes within and across genomes [17,43–46]. In these works, similarity was usually calculated using Tanimoto coefficients [47] followed by assignment of the molecules to classes using machine learning algorithms, most notably by clustering. For describing the molecules themselves, 2D topological fingerprints and fingerprints containing information from physicochemical properties have been used. Graph-based descriptions have also been used to describe molecules, as these support calculation of the maximum common substructure (MCS) [48] between the ligands compared, providing a visually interpretable output. Among the implementations of the MCS algorithm that are available, we highlight the recent adaptation in the Small Molecule Subgraph Detector (SMDS) toolkit [49••] which incorporates chemical knowledge and was benchmarked on metabolomics data. The SMSD allows MCS searches between sets of molecules, which is valuable for determining common substructures among the ligands of groups of enzymes (Figure 3).

Mechanistic similarity

Several methods have been developed to identify similarities among overall reactions of enzymes [50–52,53••], including some that make use of physicochemical descriptors of the ligands involved in the reactions [54,55] as well as of the reacting bonds [56,57], thus indirectly capturing mechanistic information. Interestingly, all these (semi)-automated algorithms, some of which are also quantitative, identified issues with the EC classification and suggested improvements to make it more consistent. Currently, only one algorithm explicitly uses mechanistic information for quantifying similarity. This algorithm [58] encodes the transformation between successive reaction intermediates as either a set of bond changes or as a fingerprint that captures various chemical aspects of a mechanistic step. Similarities among each possible pair of steps between the reactions compared are computed using Tanimoto coefficients (for bond changes) or Euclidean distances (for fingerprints). A global alignment between steps is then generated and a Tanimoto coefficient computed using the similarity data as input. Recently, the algorithm was extended to allow local alignments of mechanistic steps and to handle comparisons of overall reactions [23] (Figure 4). One drawback for these studies has been their dependence on manually annotated bond change information available only from the MACiE database, thus limiting its application. A

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new algorithm is now available that automatically extracts information about bond changes from the chemical structure of substrates and products of reactions [53] enabling much easier and larger throughput calculations of mechanistic similarities.

Conclusions

The EC system is the *de facto* classification scheme for reactions in enzymes. It is based on the overall transformation catalyzed, establishing its worth for linking genes and gene products to reactions. However, because it was created when mechanistic and structural data were sparse and cheminformatics algorithms still in their infancy, classification of reactions using the EC is neither automated nor quantitative. Nor is it linked to sequence and structural information. Recent large-scale studies suggest that both divergently and convergently evolved enzymes share common mechanistic aspects along their reaction pathways, opening an opportunity for new classifications of function based on mechanistic information. In addition, because enzyme reactions are enabled by the structural elements in the enzymes that catalyze them, defining function in terms of catalytic mechanisms and bound ligands seems a more thorough solution. However, we still have a ways to go —while techniques for comparing similarities among ligands are already quite mature, algorithms for computing mechanistic similarity have only recently started to emerge. Still, it seems that the time has come for the widespread utilization of metrics for substrate and mechanistic similarity to complement functional classifications based on overall transformations. We envisage that these methods will help generate structure-function links for all known superfamilies as well as for those convergently evolved enzymes that are mechanistically similar. As information in the enzyme mechanism databases grows and the methods for comparison of mechanistic similarity continue to develop, we can perhaps soon expect to classify the diversity of enzyme functions using these more robust and quantitative measures.

Acknowledgments

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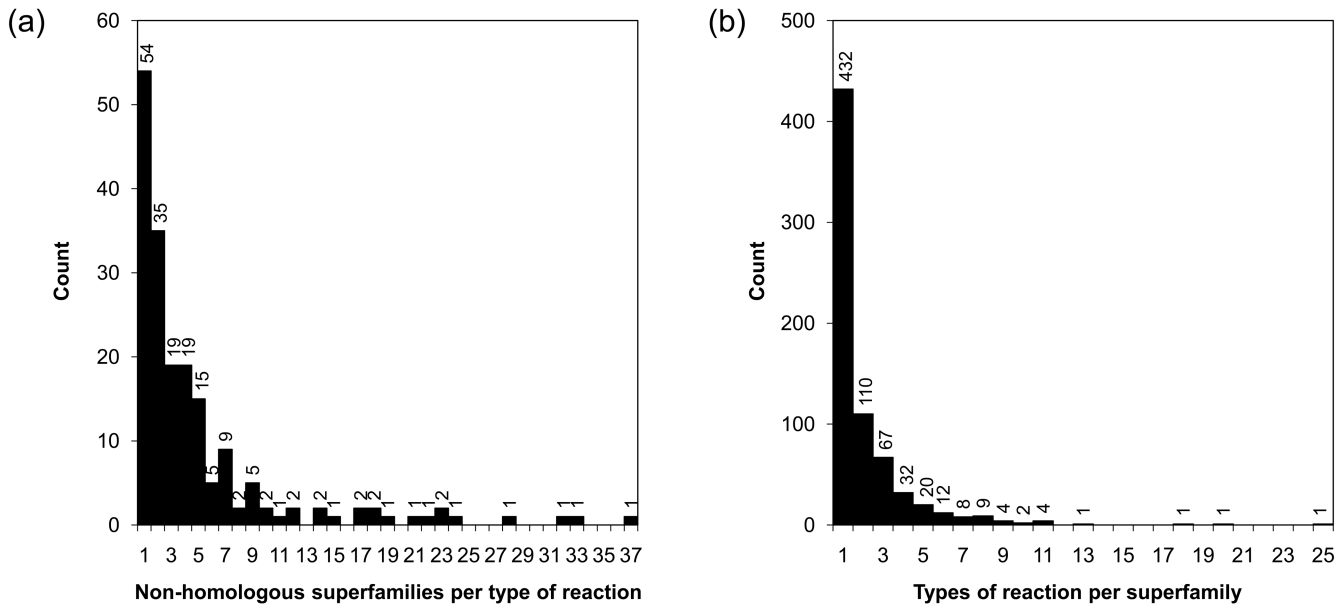

Figure 1.

Estimation of divergent and convergent function evolution in enzymes. In analogy to previous studies within and across genomes [59,60], we have used PDBSprotEC and SCOP to map EC sub-subclasses to structural superfamilies. EC sub-subclasses (first three numbers in the EC system) were used rather than EC serial numbers (all four numbers) to capture reaction specificities irrespective of substrate specificities. (**a**) The number of different EC sub-subclasses (types of chemical reactions) associated to members of structurally characterized enzyme superfamilies indicates that over one third of superfamilies (272 out of 704) are functionally diverse. (**b**) The minimum number of non-homologous superfamilies associated to each type of chemical reaction indicates that in over two thirds of the EC sub-subclasses (131 out of 185) Nature has convergently evolved independent enzymes to carry out the same function.

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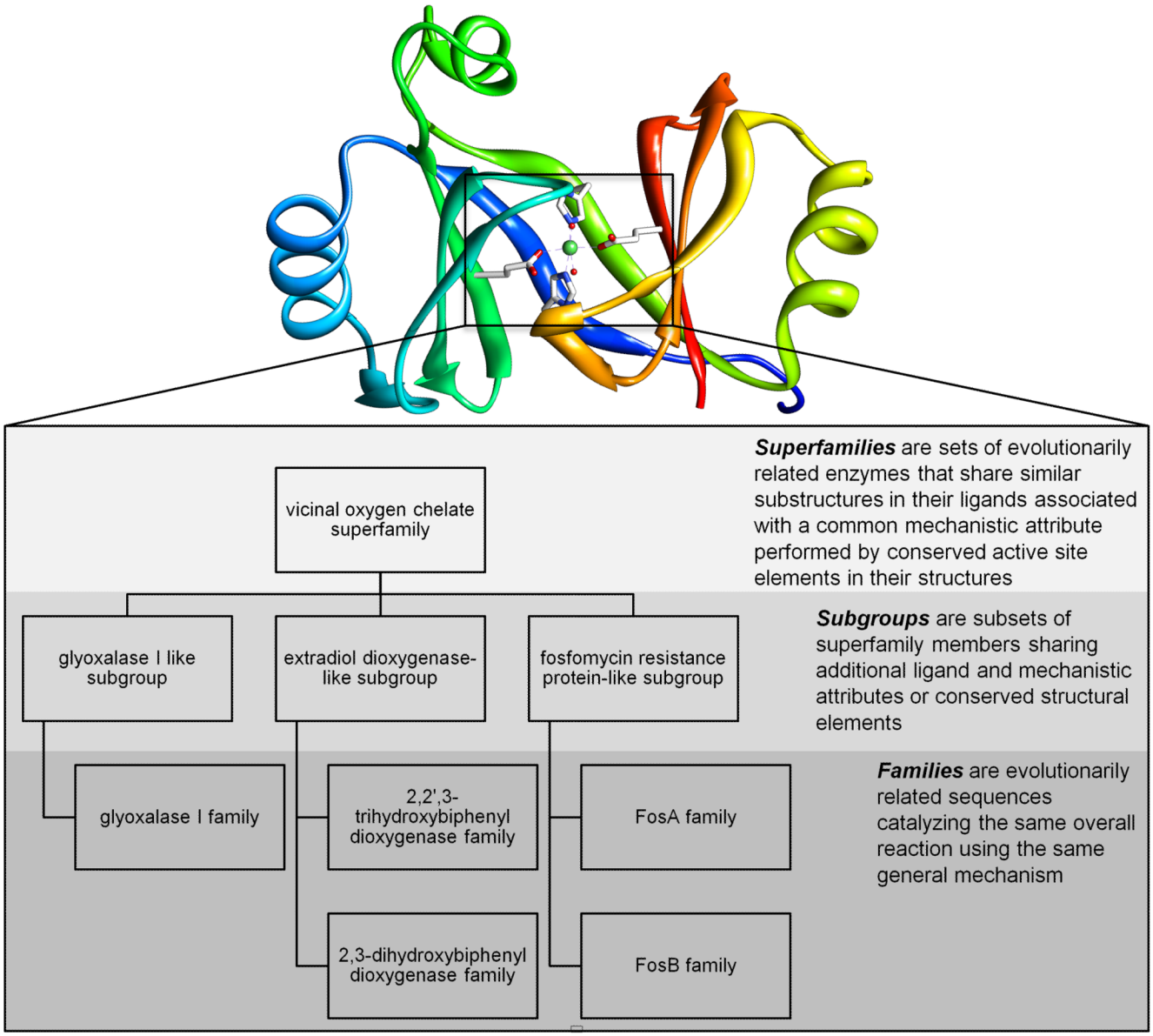

Figure 2.

Organization of a subset of subgroups and families of the vicinal oxygen chelate superfamily in the SFLD. The SFLD stores structure-function relationships for functionally diverse enzyme superfamilies at three levels of granularity: superfamilies, subgroups, and families. The structure corresponds to a member of the glyoxalase I family depicting the conserved positioning of the metal binding ligands in many of the members of the superfamily.

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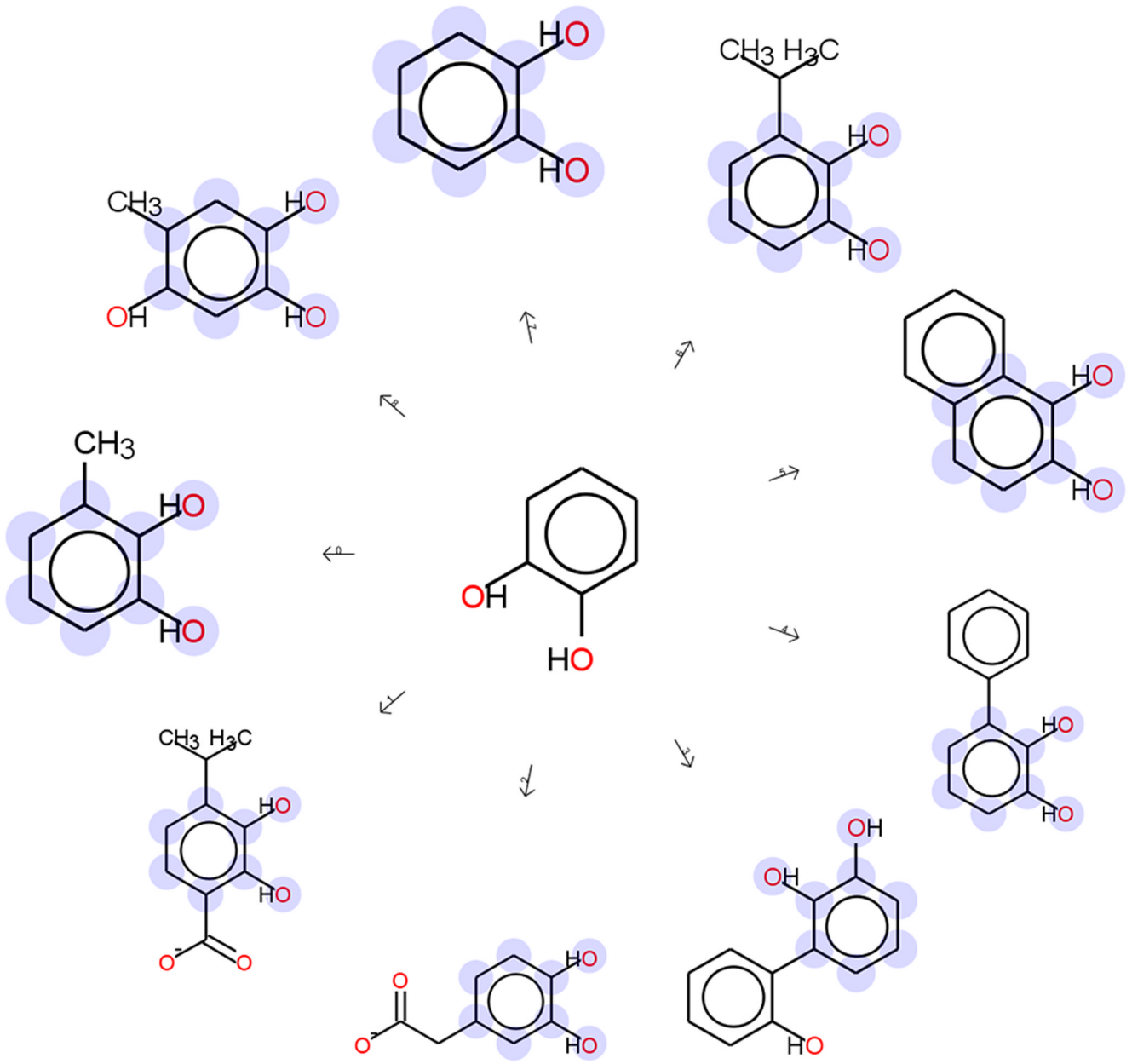
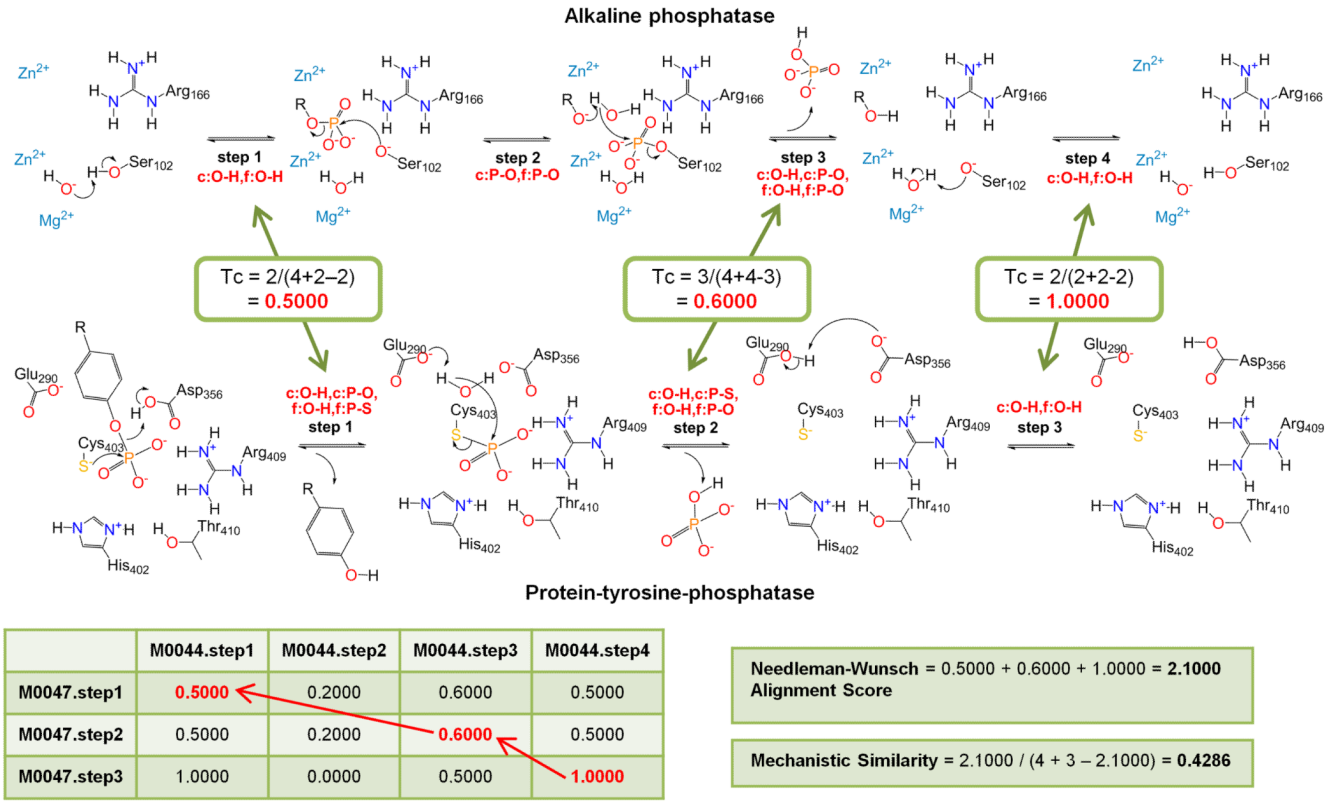


Figure 3.
Common substructure shared among some substrates of enzymes from the extradiol dioxygenase-like subgroup of the vicinal oxygen chelate superfamily curated by the SFLD. The maximum common substructure was computed using the Small Molecule Subgraph Detector (SMDS) toolkit [49]. Highlighting shows the substructures common in all of the substrates.



	M0044.step1	M0044.step2	M0044.step3	M0044.step4
M0047.step1	0.5000	0.2000	0.6000	0.5000
M0047.step2	0.5000	0.2000	0.6000	0.5000
M0047.step3	1.0000	0.0000	0.5000	1.0000

Needleman-Wunsch = 0.5000 + 0.6000 + 1.0000 = **2.1000**
Alignment Score

Mechanistic Similarity = 2.1000 / (4 + 3 – 2.1000) = **0.4286**

Figure 4.
Quantification of mechanistic similarity (adapted from [23]). The convergently evolved reactions catalyzed by alkaline phosphatase (MACiE M0044, EC 3.1.3.1, PDB ID: 1alk), and protein-tyrosine-phosphatase (MACiE M0047, EC 3.1.3.48, PDB ID: 1ytw) are used as examples. Mechanistic step are represented as the set of bond changes occurring in the transformation from substrates to products in that step, with c: bond cleaved, d: bond decreased in order, f: bond formed, and i: bond increased in order. Similarities between the sets of bond changes of the steps of the reactions compared are computed using Tanimoto coefficients (Tc) and stored in a similarity matrix. The maximum-match pathway is then obtained using the Needleman-Wunsch algorithm, and mechanistic similarity is computed as a new Tanimoto coefficient using the number of steps in each reaction and the Needleman-Wunsch alignment score as inputs.

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