Data-driven computer-aided synthesis planning utilizing organic or biocatalyzed reactions from large databases has gained increasing interest in the last decade, sparking the development of numerous tools to extract, apply and score general reaction templates. The generation of reaction rules for enzymatic reactions is especially challenging, since substrate promiscuity varies between enzymes, causing the optimal levels of rule specificity and optimal number of included atoms to differ between enzymes. This complicates an automated extraction from databases and has promoted the creation of manually curated reaction rule sets.

Here we present EHreact, a purely data-driven open-source software tool to extract and score reaction rules from sets of reactions known to be catalyzed by an enzyme at appropriate levels of specificity without expert knowledge. EHreact extracts and groups reaction rules into tree-like structures, Hasse diagrams, based on common substructures in the imaginary transition structures. Each diagram can be utilized to output a single or a set of reaction rules, as well as calculate the probability of a new substrate to be processed by the given enzyme by inferring information about the reactive site of the enzyme from the known reactions and their grouping in the template tree. EHreact heuristically predicts the activity of a given enzyme on a new substrate, outperforming current approaches in accuracy and functionality.
**EHreact: Extended Hasse diagrams for the extraction and scoring of enzymatic reaction templates**

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I. INTRODUCTION

Biocatalytic transformations nowadays comprise an ever expanding toolbox of chemo-, stereo- and regioselective reactions.1–7 The use of enzymes to catalyze reactions has several benefits, such as mild reaction conditions, aqueous media as solvents, compatibility of different reaction steps in multi-step syntheses, as well as a reduced need for protecting groups.3,4,8 Most enzymes are promiscuous to at least some extent, or can be engineered to accept a new substrate, so that the possible range of biocatalyzed transformations is large enough to be of interest to synthetic chemists, as testified by the large number of novel enzymatic cascades for the synthesis of diverse targets that were published in the last decade.2,3,6,7,9–15 Enzymatic transformations thus provide a promising and ecofriendly alternative to organic reactions in the synthesis of pharmaceutical intermediates or fine chemicals, amongst others.2

In practice, moderately promiscuous enzymes are often preferred when designing a pathway, where a small amount of activity can be increased via directed evolution.16 Enzymes can exhibit both substrate promiscuity and reaction promiscuity,17 but within bioretrosynthesis usually only the former is exploited.18 Substrate promiscuity refers to the ability to catalyze the native reaction on a non-native substrate, whereas reaction promiscuity describes the ability to catalyze a non-native reaction. In the following, we will only refer to substrate promiscuity.

To address the challenge of enzymatic synthesis pathway planning, a number of computational tools have been developed for general purpose bioretrosynthesis planning,18–22 enzyme selection,23,24 metabolic pathway exploration,18,25 and reaction rule extraction.26,27 in recent years. The tools usually extract the catalyzed transformation from a known reaction by identifying the reactive center, coding the changes of atoms and bonds into a reaction rule, and scoring the feasibility of a new substrate undergoing the same transformation on a set of criteria.

Here, a key challenge is to increase the accuracy of the employed scoring functions, and thus correctly rank reactions which are anticipated to be feasible higher than reactions which are most likely not catalyzed by a desired enzyme. Whereas some tools consider a reaction feasible if it suffices a reaction rule at a desired level of specificity,21,25 others score the feasibility of a transformation based on chemical similarity to known reactions or substrates via fingerprint vectors.20,22–24 However, methods relying on similarity or reaction rule specificity lack the distinction between generalist and selective specialist enzymes, i.e. they miss a description of enzyme promiscuity, as pointed out by Jeffries et al. recently.28 By treating each reaction in the database as a separate and independent data point, correlations between known substrates for the same enzymes are lost, and with them estimates for enzyme promiscuity and substrate ranges. On the other hand, describing enzymatic promiscuity on the basis of known substrates, as proposed by Nath and Atkins,29 can suffer from lack of data. In fact, a poorly studied enzyme with only a limited set of known substrates might be falsely viewed as highly specific.28 However, even an imperfect prediction of promiscuity

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adds to the accuracy of the predicted reaction feasibility. Furthermore, this limitation becomes less severe as enzymatic reaction databases such as BRENDA, RHEA, or KEGG grow. The ability to pool information across sets of substrates is especially true in the case of BRENDA, where enzymes are reported with a variety of activities on natural and non-natural substrates.

A missing description of enzyme promiscuity furthermore affects the quality of extracted reaction templates. Namely, the specificity of reaction rules extracted from databases of biocatalyzed reactions, i.e., the number of atoms included in the template, is usually set by a single user-defined value, treating specific and promiscuous enzymes the same. A few hand-curated sets of enzymatic reaction rules offer enzyme-specific levels of rule generality, but there is currently no method to automatically detect the promiscuity of an enzyme and extract reaction rules accordingly.

We therefore believe that a data-driven approach to extract enzymatic reaction templates at different levels of specificity, as well as to score new queries on criteria beyond fingerprint similarity, is needed, taking into account the estimated promiscuity of an enzyme and the diversity of chemical structures around the reactive center inferred from known substrates.

In this article we present a novel approach to compute enzymatic reaction templates and predict their applicability on non-natural substrates. We extract reaction templates at levels of specificities imposed by the set of known substrates, and arrange them in a tree-like structure (a Hasse diagram of molecule fragments) to allow for an estimation of enzyme promiscuity and substrate range. New substrates are scored on the basis of each template tree, by taking into account different measures of overall similarity and diversity, as well as a comparison of the structure of the query substrate to conserved substructures within the known substrates. Our open-source software allows a variety of different queries, including the scoring of a specific reaction, the proposal and ranking of possible reactions on a substrate including regioselectivity and choice of co-substrates, or scoring of substrates instead of full reactions if the products are unknown. We thus provide a valuable tool to describe and predict enzymatic reactions, which is freely available on Github.

The remainder of this article is organized as follows. The extraction algorithm, as well as details on the employed scoring functions and the preparation of literature datasets are explained in Section 2. We then analyze the number of known reactions per enzymes throughout different databases, showcase the template extraction routine on a small example, and compare the performance of the scoring routine regarding activity prediction, regioselectivity and co-substrate proposal against fingerprint based approaches on experimental screening data, as well as reactions from the enzyme database BRENDA in Section 3. Concluding remarks are given in Section 4.

II. METHODS

EHreact is implemented in Python and can be used either as a standalone command line application, or imported as a Python package. EHreact uses RDKit to process molecules and Graphviz to depict template trees.

A. Input format and transformation to imaginary transition structure

EHreact can operate in two different template tree generation modes: Taking reactions as input (default, recommended) or only the reactants (single substrates).

With standard settings, i.e., in reaction mode, EHreact takes a balanced, atom-mapped reaction SMILES as input, which must include explicit hydrogen atoms. If the atom-mapping is not known, it is automatically calculated via the Reaction Decoder Tool (RDT), a state of the art tool for atom-mapping enzymatic reactions. In this case the non-atom-mapped reaction SMILES can be given with or without hydrogens. Molecules not contributing atoms to the reaction, for example reagents, are omitted. Each reaction is then transformed into its imaginary transition structure (ITS), closely related to the condensed graph of reaction, by identifying every atom

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*a* EHreact code and documentation available at: https://github.com/hesther/ehreact

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and bond that changes during the reaction, where we take into account changes in the charge, hybridization, number of radical electrons, aromaticity or bond number and order into account, following the procedure outlined by Coley et al.\textsuperscript{38} The ITS of a reaction is a topologically superposition of the reactants and products, where bonds present in only the reactants, only the products, and both reactants and products can appear at the same time.\textsuperscript{39} It therefore describes the graph artificial transition state between reactants and products (but not a true transition state or mechanism). Fig. 1 shows an exemplary ITS for the oxidation of lactate via the enzyme lactate oxidase (EC 1.1.3.2). Such imaginary transitions structures, although known for decades, have recently attracted increased interest for parsing reaction databases, predicting structure-activity relationships and developing reaction descriptors.\textsuperscript{40–42}

We note that the extracted templates in EHreact do not take into account chirality, which is instead treated in the scoring algorithm. Handling stereochemistry on the scoring level instead of the template level has a number of advantages. First, reactions with stereocenters specified only in a part of the inputted reactions do not lead to different templates, and thus branching in the template tree, if stereoinformation is omitted. This is important because enzyme databases contain entries lacking stereocenters for select reactions, but the correct stereochemistry can usually be inferred from other reactions catalyzed by the same enzyme during scoring. At the template level, missing information on a stereocenter would cause different templates to be extracted for the set of reactions, making a comparison at the scoring stage difficult. Second, not every enzyme is perfectly stereoselective, making it favorable to consider all possible stereoisomers at the template level, and filter these stereoisomers later during scoring to account for the selectivity of the enzyme.

In single substrate mode, EHreact takes SMILES strings as input (e.g. ‘CC(O)C(=O)[O-]’ for lactate), which may be given with or without hydrogen atoms. Since no product is specified in this mode, one can additionally input a seed for the maximum common substructure search in SMILES format, to help the algorithm focus on the relevant part of the molecule. For the oxidation of lactate, Fig. 1, a meaningful seed would be ‘C([H])O[H]’, which is simply the secondary alcohol that lactate oxidase transforms to a ketone. If no seed is specified, the algorithm uses the maximum common substructure in all input substrates as seed.

In both reaction and single substrate mode, multiple seeds or reaction centers can be specified to describe enzymes that catalyze slightly different transformations as long as they are mutually exclusive.

\section*{B. Template tree generation}

After identification of the reactive center (or the seed atoms in substrate mode), the template is expanded in a step-wise manner, based on the structures of the known reactions or substrates. In reaction mode, the structures are ITS pseudo-molecules, and the initial atoms comprise the reaction center. In single substrate mode, the structures are real molecules, namely the input substrates, and the seed is either given manually, or automatically inferred from the maximum common substructure. Since EHreact is per default in reaction mode, we will use the ITS nomenclature in the following. An overview of the template tree generation is given in Fig. 2.

The algorithm iteratively adds more information to a template, creating a new, more specific template. To this aim, all atoms with unspecified neighbors are shortlisted in the current template. For example, in Fig. 1, the reaction center (the current template at the first iteration) can only be expanded at the atom C\textsubscript{3}, which is the only atom with unspecified neighbors. If only one atom is shortlisted, the new template is formed from the current template and all neighboring bonds and atoms of the shortlisted atom. If more than one atom is shortlisted, the algorithm searches for a combination of as many atoms as possible that lead to the exact same new, enlarged template. An example of this process is shown in Fig. 3 for a set of two known reactions, where from the shortlisted atoms 1, 7 and 8 only atoms 1 and 8 have the same neighbors in all pseudo-molecules. Thus, the new template is formed from the current template, and the neighboring bonds and atoms of only atoms 1 and 8. Multiple matches of the template to a pseudo-molecule may occur, in which case all options are explored, and the match leading to a maximum of nu-
The current template allows atoms 1, 7 and 8 to be extended (not all neighbors specified yet). The neighbors of each shortlisted atom in the template are compared by matching the template to each pseudo-molecule and identifying its neighbors. The algorithm chooses all atoms which have the same neighbors in all pseudo-molecules, here atom 1 and 8 (highlighted in gray), for extending the template, leading to a new, larger, more specific template.

The generated templates are saved in a template tree, that is, the template it emerged from. Each template can only have a single parent, but one or multiple children. A node in the tree without a child is simply one of the input pseudo-molecules, where all atoms are included in the template, leaving no atoms in the shortlist, and thus no more specific templates that could be attached as children. Mathematically, such a picture is called a Hasse diagram, which is simply a way of ordering and depicting a set of objects using partial orders. Hasse diagrams have been proposed in literature to be applicable for the ordering of substructures in molecules. Since we not only save the information of parent and child to the diagram, but also a number of additional features, we call the generated template trees “extended Hasse diagrams”.

In summary, if only a single reaction is known, templates are extracted at different diameters from the reaction center, creating a linear Hasse diagram without any branching, but if more than one reaction is known the algorithm makes use of the mutual structural information between them. In both cases, a number of properties of the template tree and its leaf nodes are then precomputed to speed up the subsequent scoring of a query reaction or substrate. The novelty of this approach is to add atoms and bonds to a reaction center making use of conserved substructures in all known reactions instead of some predefined radius around the reaction center. Implicitly we thus assume that conserved substructures indicate importance of the respective structures to the mechanism of the reaction, or to specific interactions with amino acids in the active pocket of the enzyme. Enzymes usually react only with certain types of substrates, whereas chemical reagents are typically only specific to a functional group, so that inferring information about important substructures in known substrates is especially relevant for biocatalytic transformations.
C. Queries on a template tree

There are three modes to score a reaction or substrate on a given template tree. First, if the Hasse diagram was produced in reaction mode, one can input a reaction SMILES (preferably atom-mapped, else automated mapping via RDT), i.e. one specific reaction, to obtain a score whether the given enzyme will catalyze the query reaction. If the reaction center of the query never occurs in the template tree, the score is zero, otherwise it is calculated as specified later in this article. Second, one can use a single substrate in SMILES format as a query for a Hasse diagram produced in reaction mode. In this case, the substrate is matched to the reactant fragments of the minimal template in the tree. If a match is found, EHreact identifies whether co-substrates are missing (for non-unimolecular reactions), as well as whether a transformation can happen in different parts of the molecule, and calculates all possible products of the transformation. For a unimolecular reaction with only one possible product, the substrate is transformed to the corresponding reaction ITS, and scored. For possibly regioselective reactions, i.e. different possible products, each possibility is translated to an ITS and scored individually. For each missing co-substrate in non-unimolecular reactions, the algorithm detects what type of co-substrate is necessary (for example, an amine donor in an amine transfer reaction, if the given substrate is an amine acceptor), and creates a reaction for each co-substrate that occurs in the tree, creating one or multiple possible ITSs, which are each scored individually. Third, one can specify one or multiple substrates in SMILES format and omit the co-substrate search, so that the score is zero if one or more co-substrates are missing. Multiple possible products due to regioselectivity are detected, and each reaction scored individually. This mode is beneficial for non-unimolecular reactions if all reactants are known, and thus no co-substrate search is necessary.

If the Hasse diagram was produced in single-substrate mode, it can only be queried by a single substrate. In this case, the product after transformation of the query substrate remains unknown. If the first substructure in the diagram (the seed) occurs in more than one location in the query, multiple scores are calculated, so that this mode still provides some measure of regioselectivity, but it cannot propose co-substrates or identify the product of a transformation. This functionality is only recommended for a quick scoring of related substrates if the products are not known; for all other cases we recommend training in reaction mode, so that the full capabilities of EHreact can be exploited during querying and scoring a new substrate or reaction.

D. Scoring function

EHreact scores are calculated as

\[ S_{\text{EHreact}} = S_S * (1 - S_P + S_M - 0.1 * S_L) \]  

where \( S_S \) is the maximum Tanimoto similarity of Morgan fingerprints (radius 2, no features) between the query substrate and the known substrates within the current branch. \( S_P \) is the average Tanimoto similarity between all pairs of substrates in the template tree, and is a measure of enzyme specificity. \( 1 - S_P \) is thus a measure of enzyme promiscuity. \( S_P \) was capped at 0.8 for practical reasons, i.e. not setting the promiscuity to zero for linear template trees with only a single substrate. \( S_M \) is the mean Tanimoto similarity between the query and all known substrates (within the whole tree, not only the current branch). A larger \( S_P \) (more specific enzyme) necessitates a larger \( S_M \) value to still yield a good overall score. Thus, the difference between \( S_P \) and \( S_M \) is either positive (increasing the overall score) if the query substrate is more similar to the known substrates than the specificity of the enzyme demands, or negative (decreasing the overall score) otherwise. \( S_S, S_P \) and \( S_M \) are calculated on the reactants in single substrate mode, or averaged over reactants and products in reaction mode. \( S_L \) scores the position of the highest applicable template within the tree by counting the minimum number of edges to the closest leaf node, where \( S_L \) is calculated as the minimum distance capped at 5, minus 1 so that it equals zero in the ideal case of only one edge to the closest leaf node. Since the range of \( S_L \) is thus much larger than the ranges of \( S_S, S_P \) and \( S_M \), its coefficient is smaller. The coefficients -1, 1 and -0.1 were determined empirically on part of the data. We note that there are several different ways to calculate a score from \( S_S, S_P, S_M \) and \( S_L \), as well as extract other metrics from the template tree. Various scoring schemes and metrics were evaluated during the course of this study, where Eq. 1 was found to have good performance and generalization qualities. The score is easily customizable in EHreact.

In this work we compare EHreact scores against simpler similarity scores, where only the maximum Tanimoto similarity between Morgan fingerprints (length 2048, radius 2, no features) to all known reactions is taken into account, similar to Ref. 23,24, as well as Ref. 22, which uses a different fingerprint, though. Different similarity metrics, fingerprint radii and fingerprints with/without features were tested for their ability to discern between active/inactive substrates (see Supporting Information), and the chosen metric and parameters performed best. We note that \( S_S \) is not always the same as such a simple similarity score, because \( S_S \) is the maximum similarity over the known substrates within the specific branch in the diagram, whereas the latter it usually the maximum similarity over all substrates.

Other scoring schemes in literature involve combining a similarity score with a further score, for example a
Table I. Summary of employed experimental data (reference, number of substrates, number of enzymes/reaction classes, and threshold into active/non-active (active if \( \geq \) threshold)).

| Ref. | # S | # E | Thresh. |
|------|-----|-----|---------|
| Nitrilases | [44] | 38 | 7 | 50% yield |
| Aminedehydrogenases | [45] | 18 | 12 | 50 mU/mg |
| Alcoholdehydrogenases | [46] | 65 | 2 | 100 min/mol mg |
| Carboxyl-methyltransferases | [47] | 17 | 3 | 50 min/mg |
| Transaminases | [48] | 10 | 12 | 95% yield |
| Tryptophan synthases | [49] | 9 | 42 | 50% yield |
| Aminotransferases | [50] | 42 | 1 | 30% yield |
| Dehalogenases | [51] | 46 | 1 | 30·\( \frac{nmol}{mg} \) |
| C(sp\(^2\))-C(sp\(^3\)) couplings | [52] | 52-117 | 7 | 70% yield |

"biological" score in Ref. 20, which incorporates a cluster analysis of enzymes, as well as the radius at which the rule was extracted. The novelty of our approach is that the difference between \( S_P \) and \( S_M \) characterizes the promiscuity of the enzyme in relation to the observed similarity, which is substantially different than the cluster analysis of Ref. 20 which characterizes sequence availability. Furthermore, counting the number of steps to the nearest leaf node, \( S_L \), instead of counting the number of steps from the most general rule to the current rule (radius of the rule) provides an advantage when differently sized substrates are known for an enzyme. Namely, scoring via the radius of a rule disadvantages small known substrates versus larger ones, since any change to a small substrate will substantially decrease the radius of the applicable rule, even if furthest away from the reaction center.

E. Data preparation

For validation of the scoring function, a set of experimental studies on the substrate ranges of various enzymes was extracted from literature manually, as well as a study on organic coupling reactions to test the performance of EHreact on organic, non-enzymatic reactions. Each study reported either the yield or activity of an enzyme/catalyst on a specified substrate under reaction conditions consistent throughout each study. Each reaction was classified as active or non-active to yield approximately 10-40% active reactions, thresholds listed in Table I, data available in the Github repository. For the organic coupling reactions, a larger threshold was chosen to limit the number of activate reactions due to the size of the dataset. Substrates with unknown products were omitted, as well as enzymes for which no substrate was labeled as active. The number of remaining substrates and enzymes is also listed in Table I. A full list of all employed enzymes/reaction classes, including identifiers as used in the respective references, is available in the Supporting Information.

To count the number of reported reactions for various EC classes and enzymes, we furthermore analyzed BRENDA, RetroRules and RHEA as described in the following.

To recover enzymatic reactions for various EC classes, the BRENDA database was parsed using a text download of the database as exported in December 2019. To resolve smiles strings from substrate and product names, BRENDA Ligands were also queried generically from the search portal to export Inchi values for various ligands included in the database. This does not cover all ligands. To supplement the ligands recovered from the search tool, all remaining compounds were queried against PubChem and the Opins name resolver. For downstream analysis, all reaction entries with unresolved compounds were removed and duplicates in each EC class were filtered. Code, including instructions for downloading necessary files, is available on Github.

Reactions from RetroRules (version rr02 based on MNXref version 3.0, compatible with RetroPath2) were determined for each 4-digit enzyme EC number in the forward direction at the lowest rule diameter after removing duplicate reaction entries where RetroRules splits multi-substrate reactions into multiple single-substrate rules.

For RHEA, reaction ids were crosslinked to their respective amino acid sequences in UniProt and SwissProt to determine the number of unique reaction annotations available per enzyme (not EC class). Since RHEA follows a hierarchical annotation technique, specific reactions are associated with a broader master class of reaction, if appropriate. If an enzyme was annotated both with its specific reaction class and master class, the master class was removed from the analysis, to avoid duplicate counting.

III. RESULTS AND DISCUSSION

A. Exemplary template tree construction

To illustrate the transformation of input reactions to their respective imaginary transition structures, as well as the iterative common substructure search around the reaction center we discuss the enzyme 4-hydroxy-2-oxoglutarate lyase (EC 4.1.3.42), for which BRENDA lists three known substrates, 4-hydroxy-2-oxoglutarate, 4-hydroxy-2-oxobutanoate and oxaloacetate. The substrates, together with the reaction catalyzed by 4-hydroxy-2-oxoglutarate lyase are depicted in Fig. 5a. The enzyme enables the splitting of a carbon-carbon bond adjacent to a hydroxyl group. The products are for all three cases pyruvate, as well as glyoxylate, formaldehyde or carbon dioxide respectively. Extracting reaction templates with literature methods, for example including

\[ b \text{ Code and instructions to parse BRENDA available at https://github.com/samgoldman97/brenda-parser} \]
all atoms up to one bond away from the reaction center (a common choice), would create three different templates, Fig. 5b, which all miss the mutual information inherent to the known reaction. The ITSs of the reactions show a large common substructure, Fig. 5c. One side of the substrate is highly conserved, namely the side which forms pyruvate (everything attached to C:10), while the other side (everything attached to C:4) is diverse in structure and size. This indicates that the pyruvate side is essential for a good fit into the active pocket of the enzyme and/or is involved in the mechanism. And indeed, a mechanistic study on 4-hydroxy-2-oxoglutarate lyase showed specific interactions of the amino acids in the active pocket with the pyruvate side of the substrate, as well as volume restrictions at the same side.\(^5\) EHreact exploits this mutual information between known reactions by iteratively adding atoms in conserved substructures to the minimum reaction template (first template in Fig. 5d). In each step, the algorithm adds only atoms and their corresponding bonds which are conserved in all reactions and are direct neighbours of current atoms in the template, eventually leading to the fourth template in Fig. 5d, which is the most specific template that applies to all input reactions. It identifies that 4-hydroxy-2-oxoglutarate lyase acts on substrates exhibiting the important pyruvate moiety next to the C-C bond to be split, and does not specify the other side of the molecule at all, thus corresponding perfectly to a template crafted with expert knowledge of the active pocket and mechanism in this system. Upon further addition of atoms to the template, the diagram splits up into three branches, where two branches lead to leaf nodes directly (the full reaction ITS), and one yields an additional template before ending in a leaf node as well. If the user is interested in a single template, extracting the most specific mutual template (the fourth template in Fig. 5d) is sufficient, and provides an advantage over traditional template extraction methods where the user decides on an appropriate level of specificity. However, saving the whole template tree and utilizing it in the scoring function was found to be highly beneficial, as demonstrated later in this article. Additional examples of Hasse diagrams (for the BRENDA entry EC 1.1.3.2 and the Uniprot entry P23525) are available in the Supplementary Information.

In general, the presented template tree extraction procedure can be useful in a number of scenarios. For example,
ample, EHreact reaction mode can be used to extract the single, most specific but mutually applicable template for a set of reactions. Furthermore, calculating a Hasse diagram in single substrate mode helps to quickly gain an overview over a set of molecules, and their common substructures and similarities. A further possible application of EHreact is the reduction of the number of extracted templates from reaction databases for enzymatic and organic reactions alike without losing generality, just as demonstrated in Fig. 5, where EHreact yields a single template for all reactions instead of the three different templates as extracted by other routines. It is well known that the number of extracted templates scales with the number of reactions in a database, and a large fraction of templates only occur once even in large datasets. One could thus reduce the number of templates by using EHreact to extract a template based on common substructures instead of a fixed number of bonds adjacent to the reaction center, or possibly even utilize the template-tree structure to speed up the application of a template to a molecule, where a missing match to the most general template in a Hasse diagram immediately disqualifies a reaction type, thus making computer-aided synthesis planning easier and faster.

### B. Composition of enzymatic reaction databases

The quality of EHreact templates and scoring directly depends on the number of reactions. The number of reactions determines the size and variety of each template tree and thus its ability to create meaningful templates and scores.

Fig. 6 depicts the number of known reactions per EC class or enzyme in different databases, namely RHEA (crosslinked with SwissProt and UniProt), BRENDA and RetroRules. For RHEA, reaction ids were associated with their respective amino acid sequences in UniProt and SwissProt, and unique reaction classes counted per enzyme. Nearly no differences arose between crosslinking with SwissProt or UniProt, and in the following we refer to the results from SwissProt only. For BRENDA, unique reactions that had valid entries for reactants and products could be parsed to SMILES strings were counted for each 4-digit enzyme EC number. If a reaction occurred in both forward and reverse direction in BRENDA, it was only counted once. For RetroRules, the number of reactions per 4-digit enzyme EC number was determined for transformations in the forward direction at the lowest rule diameter after removing duplicate reaction entries where RetroRules splits multi-substrate reactions into multiple single-substrate rules. 76% and 51% of all EC numbers and 17% of all enzymes are associated with more than one reaction for BRENDA, RetroRules and RHEA respectively, where EHreact can potentially exploit the mutual information between the reactions. Although there are certainly a number of cases where reported reactions are very dissimilar and thus limit the applicability of EHreact, we believe that template extraction based on sets of reactions is a possible and reasonable choice for enzymatic reactions, especially for databases such as BRENDA, and offers an advantage over the extraction of reaction templates from independent reaction precedents.

### C. Validation on experimental data

We compared the ability of EHreact scores to identify promising substrate/enzyme combinations observed in experimental screening studies against different similarity metrics. Nine recent datasets from literature were chosen toward this aim, for which both reactants and products were known (opposed to the more prevalent manner of only reporting reactants). Eight studies comprised enzymatic transformations, reporting the activity of different nitrilases, amidohydrolases, alcoholdehydrogenases, carboxyl-methyltransferases, transaminases, tryptophansynthases, amidotransferases and dehalogenases on diverse sets of substrates. An additional study on seven organic C(sp^3)-C(sp^3) couplings...
was utilized to showcase the performance of EHreact on non-enzymatic transformations.

Leave-one-out experiments were conducted on each dataset, where the feasibility of each reaction (substrate/product/enzyme combination) was evaluated by omitting it during the calculation of the template tree (one tree per enzyme), and subsequently calculating a score according to the above discussed scoring scheme. We calculated scores both using EHreact, as well as a traditional similarity metric (Tanimoto similarity on Morgan fingerprints of length 2048, radius 2, no features; see Supporting Information for other metrics and parameters). Data points labeled as active according to the thresholds in Table I were treated as known reactions (inputs) for both EHreact and similarity-based approaches. The area under the curve (AUC) of the receiver operating characteristics was then evaluated per assay (for all leave-one-out experiments), as well as the binary classification accuracy at a threshold of 0.5 (which is close to the mean optimal threshold averaged over all enzymatic systems for both EHreact and similarity-based approaches. The area under the curve (AUC) of the receiver operating characteristics was then evaluated per assay (for all leave-one-out experiments), as well as the binary classification accuracy at a threshold of 0.5 (which is close to the mean optimal threshold averaged over all enzymatic systems for both EHreact, 0.43, and similarity scores, 0.58; optimal thresholds for each system given in the Supporting Information). Other thresholds and F1-scores are available in the Supporting Information, as well as AUC and Acc. for running EHreact in single substrate mode (instead of reaction mode).

Table II lists the AUC and accuracy for the classification into active/inactive substrates in reaction mode. In general, EHreact leads to a similar AUC but higher accuracies, with the differences being especially prominent for carboxyl-methyltransferases, transaminases, tryptophansynthases and amidinotransferases. In these assays, substrates have high similarity scores between each other, but the enzymes only act on a very narrow range on substrates, i.e. are rather selective. In this case, a high similarity score does not necessarily ensure an enzyme being active toward a new substrate. Fig. 7 depicts the similarity scores of a new substrate, as well as the similarities between known substrates, over the observed classification accuracies for all eight assays. The classification accuracy of simple similarity metrics significantly decreases with increasing similarity scores (left panel), since the similarity between known substrates also rises, indicating very specific enzymes. Since the specificity/promiscuity of an enzyme is not taken into account, the high similarity scores cause a large number of false positives in the classification. In contrast, the accuracy of EHreact scores (center panel) does not show a dependence on the individual similarities and specificities, because they can both contribute to the score (higher specificities necessitate higher similarities to still observe a good overall score). The right panel shows the difference of accuracies via EHreact and similarity scores, which is largest for cases with high individual similarities and specificities. The shortcoming of similarity metrics to discern between specific and promiscuous enzymes was already identified in literature, but, to the best of our knowledge, EHreact offers the first systematic scoring scheme to correct for it. This observation is not tied to the threshold used (see Supporting Information for other thresholds), but a fundamental shortcoming in similarity metrics not discerning between generalist and specialist enzymes, and thus necessitating different thresholds for each enzyme. We thus find that the additional information in the shape, size and diversity of the template tree of known reactions of an enzyme is beneficial for the scoring of new substrates, and helps to find a universal scoring threshold across different datasets.

Table II furthermore lists classification metrics for a non-enzymatic assay, namely a set of organic C(sp²)-C(sp³) coupling reactions, where each name reaction (BF3K-Ni-photoredox, BF3K-Pd-Suzuki, CEC-Ni-Weix, CEC-Ni-photoredox, COOH-Ni-photoredox, MIDA-Pd-Suzuki, and Negishi-Pd) was used to group known reactions, similar to each individual enzyme in the enzymatic assays. Leave-one-out experiments were conducted to score each reaction within each name reaction group. EHreact scores provide an improvement regarding both AUC and accuracy compared to similarity scores, although EHreact scores were developed and tested solely on enzymatic reactions. We expect this improvement to hold for some other organic reactions, too, namely whenever the structure around the reaction center contributes to the reaction outcome or yield significantly. Although this is certainly not the case for organic reactions in general, it makes EHreact a useful tool for at least some reaction classes.

Next, we investigated whether EHreact still provided an improvement over similarity-based approaches if only a single substrate per enzyme was known. Thus, template trees and similarity comparisons were solely calculated for the most active substrate for each enzyme in each dataset, producing linear template trees without any branches. This analysis thus reflects the case of n = 1 in Fig. 6. In a linear template tree, the promiscuity scores do not naturally come into play, but the location score may still provide a means to penalize modifications close to the reactive center over modifications in other parts of the molecule compared to the reference structure. However, we found no significant trends in the AUC between scores based on similarity and EHreact.
Act outperforms similarity scores with an average.

For some systems a penalty based on the location score was beneficial but not for others, indicating that diameter based template scoring is not necessarily superior to overall similarity scoring.

D. Regioselectivity and cosubstrate proposal

To evaluate EHreact’s ability to propose meaningful cosubstrates for multi-substrate reactions, we selected EC classes from BRENDA which report on reactions with two substrates each, have more than ten known reactions, less than 70% occurrence of the most frequent substrate over all reactions, and molecular weights less than 200 g/mol per substrate. All reactions were then checked for balance, where unbalanced reactions where discarded, and then atom-mapped via RDT. Due to difficulties of RDT to map some of the reactions, mappings were checked manually and corrected if necessary. This yielded 555 reactions in 18 EC classes, namely 2.6.1.X with X=1, 12, 14, 15, 18, 2, 27, 28, 36, 39, 40, 42, 44, 45, 51, 57, 64, 70, 73 (transaminase reactions). For the reactions in each EC class the ability of EHreact and similarity scores to discern between combinations of amine-donors and acceptors as observed in BRENDA (positive data) and all other combinations (obtained by exhaustive combination of all donors and acceptors within a class, corresponding to negative data) was analyzed. We calculated the area-under-the-curve of the receiver-operator-characteristic to obtain a measure of how well the obtained scores can discern between true and artificial combinations of substrates, Fig. 8, left panel. EHreact outperforms similarity scores with an average AUC of 0.69 versus 0.59. We furthermore calculated the rank of the correct reaction partner for each substrate which occurred only once in the reported reactions but its partner occurred in multiple reactions by enumerating all possible reaction partners and calculating scores via EHreact and similarity on the basis of the other known reactions within an EC class. The average ranks are shown in Fig. 8, right panel, where EHreact ranks the correct partner on average at rank 2.5, and thus higher than a comparison via similarity, average rank 3.6. The fraction of reactions where the correct partner was identified at rank 1 (top-1-accuracy) is 64% for EHreact and 41% for similarity, respectively. Taking into account the first three suggestions (top-3-accuracy), EHreact correctly identifies the cosubstrate in 81% of cases, similarity for 62% of cases. EHreact thus outperforms similarity scores for both classifying whether a given combination of substrates is likely, as well as ranking suggestions for reaction partners.

Regarding regioselectivity, we selected 13 EC classes from BRENDA where some reactions had multiple possible sites of transformation, here alcohols for oxidoreductase enzymes catalyzing the oxidation of alcohols to ketons/aldehydes (EC 1.1.X with X = 1.145, 1.149, 1.209, 1.213, 1.239, 1.265, 1.283, 1.50, 1.6, 1.64, 1.72, 3.6, 3.9). We calculated scores for each reaction site using EHreact or similarity scores using the non-regioselective reactions within the same EC class as training reactions. Both EHreact and similarity scores showed 100% top-1-accuracy, thus identifying the correct site of transformation in all cases.

E. Limitations

In the following we briefly summarize current limitations of our tool, since we believe that a critical discussion helps to prevent unintentional misuse, as well as spark developments and solutions that overcome current shortcomings. Since the software is open-source, we furthermore invite interested users to contribute toward this effort.

An apparent limitation of the proposed method is its need for atom-mapped, balanced reactions, which can add additional burden to the preprocessing of databases, where reactions are often unbalanced, and not always atom-mapped, sometimes even incorrectly atom-mapped. Incorrect atom-mappings usually cause unique, non-meaningful ITSs, which branch off at the beginning of the Hasse diagram of templates, and are thus easy to detect, but can be tedious to remove or correct. On a similar note, input of inconsistent configurations, such as open and closed-loop sugars, leads to an undesired branching in the template diagram. Furthermore, full functionality of EHreact requires the knowledge of reactants and products for the training set, but especially substrate screening studies often only report on the reactants but not the products, measuring reaction success by consumption of the substrate or a cofactor.

We have shown in previous sections that EHreact functions best if more than one reaction per enzyme is known. If only a single reaction is known, the scoring scheme still profits from the multiple templates extracted at different specificities forming a linear template tree in some cases, but if the user wishes to only output a single reaction
template, then there is no advantage over other template extraction routines in literature. For a linear template tree, EHreact cannot determine which specificity or level of generality is best, and the specificity has to be determined by user input (for example, include all atoms up to one bond away from the reaction center, which is the second template in a linear template tree). This only comes into play where the primary use of EHreact is template extraction instead of scoring.

Lastly, there are some limitations to the scoring algorithm, too. Although EHreact uses a scoring scheme beyond simple chemical similarity metrics, it is still based on common structures and their similarities. Thus, for enzymes or systems where there is little structure-activity relation, we expect EHreact to not perform well, just as other similarity approaches fail for such cases as well. Also, an inherent limitation of all similarity and structure based methods is their inability to extrapolate to new substrates which are very different from known ones. Although the diversity of the EHreact scoring routine might help to perform better than a fingerprint similarity comparison for extrapolating to new substrates, we expect its extrapolation ability to be at best mediocre.

IV. CONCLUSION

We have introduced a novel method of extracting multiple reaction templates from a set of known reactions and utilizing the mutual information between them to obtain better predictions of the activity of non-natural substrates. The developed open-source software, EHreact, extracts, groups and saves templates as imaginary substructures in the imaginary transition structures to be an easy and promising alternative to conventional template extraction and scoring routines, especially where only a few reactions per enzyme are known. We acknowledge that different approaches, such as machine learning of structure-activity relationships of enzymes and substrates are a very promising alternative for large datasets, with a number of studies published recently. However, for regimes of little data, as presented in this study, we believe that simple heuristic scoring schemes are a more robust and interpretable route toward success, and estimate the performance of EHreact to be satisfactory for use in computer-aided pathway design. As outlook, we plan to utilize EHreact to design multi-step synthesis pathways and enzymatic cascades.

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Supporting information

EHreact: Extended Hasse diagrams for the extraction and scoring of enzymatic reaction templates

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I. COMPARISON OF FINGERPRINTS AND SIMILARITY METRICS

Fig. 1 depicts the AUC (for the classification into active/inactive substrates of leave-one-out experiments) for Morgan fingerprints of radius 2 (top) and radius 3 (bottom) with (right) or without (left) features. Twelve similarity metrics, all available in RDKit, were calculated and their performance compared. Fingerprints of radius 2 without features outperform other fingerprints throughout all similarity metrics. Tanimoto, Dice, Cosine, On-Bit, RogotGoldberg and Sokal similarity between the fingerprints of a query and the known substrates perform equally well in discerning between active and inactive substrates (and perform better than AllBit, Assymmetric, BraunBlanquet, Kulczynski, McConnaughey and Russel similarity). We chose Tanimoto similarity to be used throughout the remainder of this study, since it is routinely applied in several related studies.1–4

Figure 1: AUC (classification into active/inactive substrates for leave-one-out splits) for Morgan fingerprints at radii 2 and 3, with and without features, for different similarity metrics
II. ACCURACIES AT DIFFERENT_THRESHOLDS

Table I lists the classification accuracies at different threshold, Table II the corresponding F1 scores. Since the data is imbalanced, a very high threshold creating only negative predictions shows a high accuracy, but the F1 scores clearly label the corresponding classifier as performing badly. In general thresholds around 0.4 and 0.5 lead to good F1 scores and acceptable accuracies in all systems. Optimal thresholds for each system (which maximize the relation between the true positive and false positive rate) are given in Table III, and differ much between systems for similarity scores.

III. SINGLE SUBSTRATE MODE

Table IV lists the $AUC$ and Acc. for all systems similar to the main article, but with EHreact in single substrate mode (and thus the similarity scores also only taking the reactants into account). Again, similarity and EHreact scores perform equally well for the $AUC$ but no uniform threshold applies to all systems, so that the Acc. is higher for EHreact.

Table I: Classification accuracy Acc. (at different thresholds) for scores obtained via similarity or EHreact.

|                      | threshold = 0.3 | threshold = 0.4 | threshold = 0.5 | threshold = 0.6 | threshold = 0.7 |
|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                      | Sim. EHreact    | Sim. EHreact    | Sim. EHreact    | Sim. EHreact    | Sim. EHreact    |
| Nitrilases           | 0.62 0.84       | 0.83 0.89       | 0.87 0.88       | 0.87 0.88       | 0.87 0.87       |
| Aminedehydrogenases  | 0.72 0.86       | 0.90 0.89       | 0.89 0.90       | 0.90 0.90       | 0.90 0.90       |
| Alcoholdehydrogenases| 0.45 0.52       | 0.61 0.61       | 0.75 0.75       | 0.79 0.78       | 0.75 0.75       |
| Carboxyl-methyltransf. | 0.25 0.31     | 0.25 0.43       | 0.25 0.69       | 0.27 0.82       | 0.31 0.82       |
| Transaminases        | 0.36 0.55       | 0.47 0.69       | 0.53 0.79       | 0.51 0.62       | 0.61 0.63       |
| Tryptophansynthases  | 0.26 0.39       | 0.26 0.58       | 0.39 0.62       | 0.61 0.61       | 0.76 0.72       |
| Amidinotransferases  | 0.17 0.40       | 0.17 0.57       | 0.19 0.76       | 0.38 0.79       | 0.67 0.83       |
| Dehalogenases        | 0.48 0.65       | 0.61 0.74       | 0.70 0.78       | 0.74 0.78       | 0.78 0.78       |
| C(sp²)-C(sp³) couplings | 0.52 0.57     | 0.53 0.68       | 0.53 0.75       | 0.51 0.81       | 0.74 0.85       |
Table II: F1 scores (at different thresholds) for scores obtained via similarity or EHreact.

|                          | threshold = 0.3 | threshold = 0.4 | threshold = 0.5 | threshold = 0.6 | threshold = 0.7 |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                          | Sim.            | EHreact         | Sim.            | EHreact         | Sim.            | EHreact         | Sim.            | EHreact         | Sim.            | EHreact         |
| Nitrilases               | 0.40            | 0.59            | 0.57            | 0.66            | 0.51            | 0.43            | 0.00            | 0.11            | 0.00            | 0.00            |
| Aminatedehydrogenases    | 0.40            | 0.45            | 0.64            | 0.40            | 0.15            | 0.16            | 0.00            | 0.00            | 0.00            | 0.00            |
| Alcoholdehydrogenases    | 0.47            | 0.50            | 0.52            | 0.50            | 0.55            | 0.53            | 0.47            | 0.41            | 0.11            | 0.11            |
| Carboxyl-methyltransf.   | 0.41            | 0.43            | 0.41            | 0.47            | 0.41            | 0.62            | 0.41            | 0.64            | 0.43            | 0.64            |
| Transaminases            | 0.53            | 0.58            | 0.58            | 0.58            | 0.59            | 0.65            | 0.00            | 0.00            | 0.00            | 0.00            |
| Tryptophansynthases      | 0.41            | 0.35            | 0.41            | 0.43            | 0.35            | 0.43            | 0.45            | 0.41            | 0.15            | 0.25            |
| Amidinotransferases      | 0.29            | 0.36            | 0.29            | 0.40            | 0.29            | 0.55            | 0.35            | 0.40            | 0.50            | 0.46            |
| Dehalogenases            | 0.45            | 0.56            | 0.53            | 0.60            | 0.56            | 0.58            | 0.50            | 0.50            | 0.50            | 0.44            |
| C(sp²)-C(sp³) couplings  | 0.37            | 0.36            | 0.37            | 0.38            | 0.37            | 0.33            | 0.26            | 0.24            | 0.32            | 0.26            |

Table III: Thresholds which optimize the relation between true positive and false positive rate for scores obtained via similarity or EHreact.

|                          | threshold       |
|--------------------------|-----------------|
|                          | Sim.            | EHreact         |
| Nitrilases               | 0.48            | 0.28            |
| Aminatedehydrogenases    | 0.39            | 0.18            |
| Alcoholdehydrogenases    | 0.43            | 0.46            |
| Carboxyl-methyltransf.   | 0.84            | 0.57            |
| Transaminases            | 0.55            | 0.47            |
| Tryptophansynthases      | 0.68            | 0.46            |
| Amidinotransferases      | 0.71            | 0.54            |
| Dehalogenases            | 0.53            | 0.44            |
| Average                  | 0.58            | 0.43            |
Table IV: Area under the curve $AUC$ and classification accuracy $Acc.$ (at a threshold of 0.5) for scores obtained via similarity or EHreact in single substrate mode.

| Enzyme Class              | AUC  | Acc.  |
|---------------------------|------|-------|
|                           | Sim. | EHreact | Sim. | EHreact |
| Nitrilases                | 0.91 | 0.93   | 0.86 | 0.88   |
| Aminedehydrogenases       | 0.88 | 0.87   | 0.89 | 0.90   |
| Alcoholdehydrogenases     | 0.80 | 0.78   | 0.76 | 0.76   |
| Carboxyl-methyltransferases| 0.82 | 0.83   | 0.69 | 0.76   |
| Transaminases             | 0.44 | 0.70   | 0.60 | 0.62   |
| Tryptophansynthases       | 0.66 | 0.57   | 0.74 | 0.66   |
| Amidinotransferases       | 0.93 | 0.92   | 0.79 | 0.83   |
| Dehalogenases             | 0.74 | 0.72   | 0.74 | 0.78   |
IV. FURTHER TEMPLATE TREE DEMONSTRATION

A. BRENDA EC 1.1.3.2: L-lactate oxidase

L-lactate oxidase (EC 1.1.3.2) catalyzes the oxidation from L-lactate to pyruvate as depicted in Fig. 2. According to BRENDA\textsuperscript{5} other known substrates include modifications on the methyl carbon, such as linear or branched alkyl chains, or aromatic rings which are always substituted in para position, Fig. 3. The Hasse diagram of the known substrates (in transition mode) is depicted in Fig. 4. The structure of the tree depicts exactly the observations made previously, namely that modifications to the primary substrate lactate always occur on the methyl carbon, leaving the carboxyl-group unchanged. Further, the diagram splits into branched sidechains (first branch from the top), linear sidechains (second branch
Figure 4: Hasse diagram of the known reactions of enzyme EC 1.1.3.2.

from the top), no sidechains, which is simply the oxidation of lactate itself (third branch from the top) and aromatic sidechains (bottom branch). The branch with aromatic sidechains furthermore reveals that all known substrates are para-substituted, visible from the two first templates in the branch, the first characterizing that the sidechain is a 6-membered aromatic ring, the second that it is always para-substituted. If a query does not suffice this logic, it is penalized by $S_L$ since the first template (which fits ortho- and meta-substitutions) is at a distance of 2 edges to a leaf node.

The example of lactate oxidase furthermore reveals an advantage of $S_L$ as opposed to a traditional, radius based metric. Fig. 5 depicts two query substrates, one of which is known to be processed according to BRENDA. Both involve a change at a distance of two bonds from the reactive center, and thus only fit standard templates at radius 1. In a radius-based scoring scheme, both queries would be penalized exactly the same. Upon comparison of the templates with the Hasse diagram of the known reactions, however, the first query fits the first three templates, and the distance to the nearest leaf node is 1 ($S_L = 0$), whereas the second query only fits the first two templates, invoking a penalty ($S_L = 1$). In other words, the second query molecule does not feature an important conserved substructure in the known substrates, namely the carboxyl group, and should therefore be scored worse than the first query molecule. The Hasse diagram thus enables a more accurate scoring compared to a set of independent reaction rules extracted at different radii.
B. Uniprot P23525 - 2-methyl-6-phytyl-1,4-hydroquinone methyltransferase

The enzyme P23525 catalyzes an important methylation step in tocopherol and plastoquinone synthesis. Three reactions are reported, see Fig. 6, where three different substrates are methylated by S-adenosyl-L-methionine. The Hasse diagram of these reactions is very large due to the large molecule size, so we instead depict the first eight reaction templates in the diagram (before the diagram splits into branches) in Fig. 7 for the sake of readability. The reaction center is simply a carbon being transferred from sulfur to another carbon (first template), which is specified to be an aromatic carbon in the second template. As the templates grow in size, the common substructures, namely S-adenosyl-L-methionine being always the cosubstrate (methyl-donor), as well as the location of the methylation with large conserved substructures in the aromatic ring and its sidechains are identified by the iterative EHreact algorithm. Any change in these conserved structures in the methyl-acceptor, as well as the full methyl-donor molecule cause a penalty in the EHreact score via $S_L$. This is a major advantage against simple similarity metrics (equal importance of changes throughout the whole molecule) or radius-based similarity metrics (larger importance of changes close
Figure 6: Catalyzed reactions by 2-methyl-6-phytyl-1,4-hydroquinone methyltransferase

to the reaction center). For example, a change in the methyl-donor S-adenosyl-L-methionine
is likely to make the reaction unfeasible (according to the reported reactions), whereas vari-
ability in the substrate, depending on the location and preferably outside the conserved
substructures at the bottom of Fig. 7, may be permissible, although these changes might
be closer to the reaction center than changes in the methyl-donor (and thus predicted to be
less likely by radius-based metrics).
Figure 7: First eight reaction templates in the Hasse diagram of 2-methyl-6-phytyl-1,4-hydroquinone methyltransferase before diagram splits into branches.

For readability, atom-map numbers are only shown for the reaction center.
V. DATA DETAILS

Table V lists the name of each enzyme or system as used in this study and the system name and description as provided in the respective reference.

Table V: Description of experimental data. "Name" refers to the name of the system within this study (especially the CSV files available on Github), "Description" to the system details in the respective references.

| Class                  | Name                  | Description                                           |
|------------------------|-----------------------|-------------------------------------------------------|
| Nitrilases, Ref. 6     | E-1                   | Bradyrhizobium japonicum (Q89GE3) culture 1 (system 3a) |
|                        | E-2                   | Bradyrhizobium japonicum (Q89GE3) culture 2 (system 3b) |
|                        | E-3                   | Bradyrhizobium sp. BTAi1 (A5EKU8) (system 4)          |
|                        | E-4                   | Chaetomium globosum (Q2GR86) (system 5)               |
|                        | E-5                   | Rhodopseudomonas palustris (Q6N284) culture 2 (system 6b) |
|                        | E-6                   | PRO-NITR010 (system 19)                               |
|                        | E-7                   | PRO-NITR014 (system 22)                               |
| Aminedehydrogenases,   | E-1                   | ChatAmDH with NADH cofactor                           |
| Ref. 7                 | E-2                   | ChatAmDH with NADPH cofactor                          |
|                        | E-3                   | IGCAmDH1 with NADH cofactor                           |
|                        | E-4                   | SgorAmDH with NADH cofactor                           |
|                        | E-5                   | SgorAmDH with NADPH cofactor                          |
|                        | E-6                   | IGCAmDH5 with NADH cofactor                           |
|                        | E-7                   | IGCAmDH5 with NADPH cofactor                          |
|                        | E-8                   | MATOUAmDH1 with NADH cofactor                         |
|                        | E-9                   | MATOUAmDH1 with NADPH cofactor                         |
|                        | E-10                  | MATOUAmDH2 with NADH cofactor                         |
|                        | E-11                  | MATOUAmDH2 with NADPH cofactor                         |
|                        | E-12                  | AcolAmDH with NADH cofactor                           |
| Alcoholdehydrogenases, | E-1                   | R-ADH in Tris HCl buffer and 20% hexane               |
| Ref. 8                 | E-2                   | S-ADH in Tris HCl buffer and 20% hexane               |

Continued on next page
| Class, reference | Name | Description |
|------------------|------|-------------|
| Carboxyl-methyltransferases, E-1 | Hoya carnosa SAMT | |
| Ref. 9 | E-2 Datura wrightii SAMT | |
| E-3 M201H | |
| Transaminases, | Arthrobacter ArR-ωTA, AlaDH, no cosolvent | |
| Ref. 10 | Arthrobacter ArR-ωTA, AlaDH, DMSO 15% v/v | |
| E-3 | Arthrobacter ArR-ωTA, LDH, no cosolvent | |
| E-4 | Arthrobacter ArR-ωTA, LDH, DMSO 15% v/v | |
| E-5 | Aspergillus terreus AT-ωTA, AlaDH, no cosolvent | |
| E-6 | Aspergillus terreus AT-ωTA, AlaDH, DMSO 15% v/v | |
| E-7 | Aspergillus terreus AT-ωTA, LDH, no cosolvent | |
| E-8 | Aspergillus terreus AT-ωTA, LDH, DMSO 15% v/v | |
| E-9 | Hyphomonas neptunium HN-ωTA, AlaDH, no cosolvent | |
| E-10 | Hyphomonas neptunium HN-ωTA, AlaDH, DMSO 15% v/v | |
| E-11 | Hyphomonas neptunium HN-ωTA, LDH, no cosolvent | |
| E-12 | Hyphomonas neptunium HN-ωTA, LDH, DMSO 15% v/v | |
| Tryptophansynthases, | Pf2B9 | |
| Ref. 11 | TmAzul | |
| E-3 Tm9D8* | |
| E-4 TmTriple | |
| E-5 WT-100-1-B | |
| E-6 WT-100-1-C | |
| E-7 WT-100-2-B | |
| E-8 WT-100-2-C | |
| E-9 WT-100-2-D | |
| E-10 WT-100-2-E | |
| E-11 WT-003-1-B | |
| E-12 WT-003-1-C | |

Continued on next page
| Class, reference | Name | Description |
|------------------|------|-------------|
| E-13             | WT-003-1-A |
| E-14             | WT-003-1-D |
| E-15             | Q90*-003-1-C |
| E-16             | Q90*-003-1-D |
| E-17             | Q90*-003-1-E |
| E-18             | Q90*-003-1-A |
| E-19             | Tri-003-1-D |
| E-20             | Tri-003-1-E |
| E-21             | Tri-003-1-F |
| E-22             | Tri-003-2-B |
| E-23             | Tri-003-2-C |
| E-24             | Tri-003-2-D |
| E-25             | Tri-003-2-E |
| E-26             | Tri-100-1-B |
| E-27             | Tri-100-1-C |
| E-28             | Tri-100-1-D |
| E-29             | Tri-100-1-G |
| E-30             | Tri-100-2-C |
| E-31             | Tri-100-2-D |
| E-32             | Tri-100-2-E |
| E-33             | Tri-100-2-F |
| E-34             | Tri-100-2-A |
| E-35             | Tri-100-3-B |
| E-36             | Tri-100-3-C |
| E-37             | Tri-100-3-D |
| E-38             | Tri-100-3-E |
| E-39             | Tri-100-3-F |
| Class, reference | Name | Description |
|------------------|------|-------------|
| E-40             | Tri-100-4-D | |
| E-41             | Tri-100-4-E | |
| E-42             | Tri-100-4-F | |

Amidinotransferases, Ref. 12 SxtG

| Dehalogenases, Ref. 13 | LinB |
|------------------------|------|
| C(sp$^2$)-C(sp$^3$) couplings, Ref. 14 | E-1 BF3K-Ni-photoredox |
|                        | E-2 BF3K-Pd-Suzuki |
|                        | E-3 CEC-Ni-Weix |
|                        | E-4 CEC-Ni-photoredox |
|                        | E-5 COOH-Ni-photoredox |
|                        | E-6 MIDA-Pd-Suzuki |
|                        | E-7 Negishi-Pd |

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