Identification of specificity determining residues in enzymes using environment specific substitution tables

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Abstract

Environment specific substitution tables have been used effectively for distinguishing structural and functional constraints on proteins and thereby identify their active sites (Chelliah et al. (2004)). This work explores whether a similar approach can be used to identify specificity determining residues (SDRs) responsible for cofactor dependence, substrate specificity or subtle catalytic variations. We combine structure-sequence information and functional annotation from various data sources to create structural alignments for homologous enzymes and functional partitions therein. We develop a scoring procedure to predict SDRs and assess their accuracy using information from bound specific ligands and published literature.
1 Introduction

Enzymes are critical to cellular machinery. Enzymes are believed to have developed different specificities following gene duplication events that ease the evolutionary pressure on copies and allow exploration of novel avenues to greater organismal fitness. Each copy then develops its own niche, characterized by expression and localization, catalytic mechanism, substrate specificity, cofactor dependence and catalysis products. Such paralogous enzymes should have an evolutionary imprint corresponding to their specific niche, in addition to maintenance of structural fold. Thus evolutionary analysis of available structural and sequence data should enable identification of key residues responsible for specificity of various kinds. Enzyme specificity can be estimated with functional assays without structure determination, but identification of SDRs (specificity determining residues) remains difficult. While ENZYME [Bairoch (2000)] - a database of enzyme sequences with detailed functional annotation - exists, there is no such database of SDRs. Time, cost and technical limitations slow down structure determination and even when structure is known, it is not trivial to identify the residues important for binding cofactors and substrates. Hence it is important to be able to identify such residues computationally. Reliable detection of such residues will aid in deciding whether a SNP is deleterious or neutral and suggest mutation studies. Function assignment to sequence could be done at a finer level, e.g. by verifying that SDRs necessary for certain substrate are present. Computational SDR identification has received a lot of attention and several methods have been proposed. Evolutionary trace (ET) is one of the most important methods [Madabushi et al. (2002), Mihalek et al. (2004)]. It builds a phylogenetic tree based on sequence comparisons, such that branch lengths are indicative of evolutionary divergence. Functional subgroups consist of sequences in subtrees determined from this tree using a divergence cutoff. Residues common to a subtree are considered specificity-conferring rather than the ones common to entire tree. Spatial cluster identification can be used with ET to reduce the number of false positives. Inferring phylogeny correctly remains the main cause of concern in this approach, hence attempts have been made to use existing annotation with various statistical techniques. Another important direction is to use spatial proximity of residues.

Cornerstone of our approach is that structural environment influences residue substitution patterns, illustrated by Overington et al. (1990) and later used effectively for structure-sequence alignment and fold recognition [Shi et al. (2001)]. Structural environment of a residue is described in terms of secondary structure, solvent accessibility, sidechain-sidechain and sidechain-mainchain hydrogen bonding. Residue substitution tables derived from a set of high quality sequence-structure alignments represent the expected substitution rate in a structural environment. Unexpected conservation of a residue is indicative of functional restraint acting on it.
Advantage of using ESSTs is that the structurally conserved residues are masked, which is why active sites of homologous enzymes can be identified reliably with this approach. This approach has been extended in the present work by using functional annotation information.

A set of homologous enzymes is generally a union of smaller functionally specific subsets, e.g. substrate-specific subsets in serine proteinases (trypsin, chymotrypsin etc.), cofactor-specific subsets in ferrodoxin reductases (NAD and NADP specific) and so on. In multiple sequence alignment of a homologous protein family, SDRs generally appear as differentially conserved subcolumns. But all such appearances would not be SDRs. Our hypothesis is that SDRs would be identified by combining differential conservation with ESST-based detection of functional restraint.

2 Families, functional partitions and profiles

In order to test our hypothesis, we need to construct a dataset of homologous enzyme families with reliable functional partitions in them. While SCOP classification can be used in a straightforward way for making families, identifying functionally specific subsets is not a trivial task. Some automated approaches to detect functional shift, e.g. Abhiman and Sonnhammer (2005), exist to infer such partitions but manual annotation remains the most reliable. Additionally, protein function is not a precise and quantifiable entity. This restricted our study to enzymes which are the the most well studied and well annotated class of proteins. Enzyme function is fairly well defined and well classified according to hierarchical Enzyme Classification scheme (EC). We use the mapping between SCOP domains and EC numbers (George et al. 2004) to make EC-specific subgroups within a SCOP domain family. We generate profiles (multiple structure-sequence alignments) for SCOP families and functional partitions. Sequence homologs for structural families were found using PSIBLAST (Altschul et al. 1997) on nonredundant sequence database, whereas function-specific partitions were enriched using PSIBLAST searches on ENZYME database (Bairoch 2000). PSIBLAST hit on ENZYME database is retained only if the EC number of hit matches that of query. All PSIBLAST searches were with 5 rounds and e-value 0.01, hits smaller than 75% of query length were ignored. All structure-sequence alignments were carried out with fugueseq (Shi et al. 2001) which has been shown to improve alignment quality over PSIBLAST. This process is summarized in Fig.1.

Another constraint on the choice of dataset comes from the need for sufficient functional diversity in a SCOP domain family. In its absence, the contrast between the domain family and EC-specific subgroup within it might not be detectable. Hence we chose the SCOP families with at least two different EC annotations.

To be able to test the hypothesis quantitatively, a gold standard set of SDRs for every enzyme
Figure 1: Workflow

- SCOP
- SCOPEC
- Functional partition EC1
- Functional partition EC2
- Nonredundant sequences
- PSIBLAST
- Homologs
- fugueseq
- Multiple Structure-Sequence Alignment for whole SCOP family
- ENZYME
- PSIBLAST
- Homologs with same EC
- fugueseq
- Multiple Structure-Sequence Alignment for whole EC-specific partition
is needed. But SDRs are generally a topic of lively debate among researchers, partly due to the infeasibility of performing all necessary mutation studies. Thus there is no such dataset in our knowledge. Hence we use the information of bound ligands and close-by residues to assess the hypothesis. Due to this, the dataset gets restricted to only those cases where at least one EC-specific domain group has a relevant ligand bound. A relevant ligand is the one unique to the reaction carried out by that EC-group among all possible reactions in that domain family. For example, in SCOP family c.1.10.4 there are two functional subgroups:

3-deoxy-8-phosphooculonate synthase (EC 2.5.1.55): Phosphoenolpyruvate + D-arabinose 5-phosphate + H(2)O = 2-dehydro-3-deoxy-D-octonate 8-phosphate + phosphate

3-deoxy-7-phosphoheptulonate synthase (EC 2.5.1.54): Phosphoenolpyruvate + D-erythrose 4-phosphate + H(2)O = 3-deoxy-D-arabino-hept-2-ulosonate 7-phosphate + phosphate

Here D-arabinose 5-phosphate is unique to EC 2.5.1.55 and is present in domain 1fxqA as A5P. Hence it is taken as an indicator of SDR locations and not phosphoenolpyruvate which is common cofactor in both reactions. We sometimes use products also as such indicators. Ligand is considered relevant if its name from the PDB file (HETNAM, HETSYM records) matches its name in the reaction or PDBsum (Laskowski et al. (2005)) finds it sufficiently similar to ideal ligand molecule. Our final dataset consists of 97 examples drawn from 68 families. Very few SDR identification studies are carried out with these many examples.

3 Profiles and substitution patterns

Structural and sequence information in MSSA can be misleading if dominated by very close homologs, hence each MSSA was filtered with 90% sequence identity cutoff to avoid redundancy.

Observed substitution pattern for a column in profile MSSA (multiple structure-sequence alignment) was calculated after weighing down contributions from similar sequences (> 60% sequence identity). Gaps were ignored while calculating the observed substitution pattern but the ratio of gaps to amino acids in a column was computed. Columns with high gap content are generally not functional hence gap content was used as a filtering criterion as described later. Observed substitution patterns are normalized and sequence entropy was also calculated to get a measure of variability in the column as $\sum_{i=1}^{20} -f_i \log(f_i)$, where $f_i$ is the fraction of $i^{th}$ amino acid in the distribution.

Expected substitution patterns for a column were calculated using environment specific substitution probability tables derived from high quality multiple structure alignments from 371 families (Shi et al. (2001)). Substitution probabilities from every structure were averaged to get expected substitution probabilities for each column in MSSA. Again, sequence-based clustering was used to avoid expected substitution pattern getting dominated by very similar structures.
Functional restraint is calculated as the city-block distance between normalized observed and predicted substitution patterns \((\sum_{i=1}^{20} o_i - e_i, \ o_i \ \text{being observed fraction of} \ \text{ith amino acid and} \ e_i \ \text{being the fraction of times it is expected to occur})\). Thus, for both MSSAs (whole family and EC-specific) we have the following quantities: functional restraint \((famF, ecF)\), gap content \((famG, ecG)\) and sequence entropy \((famE, ecE)\). Moreover for each MSSA, number of sequences < 80% identical to each other was taken as an indicator of evolutionary information available in it.

4 Benchmarking

In order to assess the differences in residues important for whole family and EC partition, baseline predictions were made by choosing top-ranking residues according to whole family functional constraint from residues which are not highly gapped \((famG < 0.5)\). Number of baseline and SDR predictions is same whenever they are compared or an overlap between them is computed. This helps in assessing whether information in the EC-specific MSSA is distinct.

The likelihood of a residue to be an SDR is presumably proportional to its proximity to the specific ligand. Hence, to quantify the merit of a prediction, we defined mean proximity as the ratio of mean separation between predicted residues and ligand. Mean relative proximity is defined as the ratio of mean proximity to the mean separation between all residues in the domain and the ligand. Distance between a residue and ligand is taken to be the closest distance between residue sidechain (mainchain for glycine) and ligand atoms. Smaller the mean relative proximity, better the prediction. Prediction quality will also depend on the number of distinct homologous sequences available. In case of multiple ligands close to a domain, a residue’s proximity to the ligand is calculated with respect to the closest ligand. The basis for SDR prediction is that it be sufficiently distinct between whole family and EC-specific MSSAs. As Abhiman and Sonnhammer (2005) describe it, an SDR should be a rate-shifted or conservation-shifted site. Additionally, SDR should be sufficiently functionally constrained from ESSTs perspective \((ecF)\). For a residue with low entropy in EC MSSA, if change in entropy \(dE\) (family MSSA sequence entropy - EC MSSA sequence entropy) is high, it indicates that it could be SDR. Since each MSSA will be different in its variability, it is not advisable to use same functional constraint cutoff or entropy cutoff for all of them. This immediately suggests two 2-step approaches: choose top \(N1\) residues with high difference in sequence entropy between whole and EC MSSAs, then select top \(N2\) according to functional constraint in EC MSSA and vice versa. But there could be a third and more attractive approach that combines functional constraint from EC MSSA and sequence entropy difference. We pursue the third approach.

We assume that SDR score of a residue is a linear combination of its functional constraint,
Table 1: Optimal values of $a$ and $b$ for various levels of evolutionary information available.

| Criteria for choice of examples | Mean proximity $(0,0.8)$ | Mean proximity $(0.4,1.2)$ | #close ($<6\text{Å}$) residues $(0,0.8)$ | #close ($<6\text{Å}$) residues $(0.4,1.2)$ |
|---------------------------------|--------------------------|---------------------------|---------------------------------|---------------------------------|
| >5 homologs (67 examples)       | 10.84                    | 11.24                     | 3.35                            | 3.01                            |
| >10 homologs (55 examples)      | 10.41                    | 10.64                     | 3.45                            | 3.2                             |
| >10 homologs, >1 EC (23 examples) | 9.36                     | 9.24                      | 4.08                            | 3.6                             |

entropy and change in entropy, given that the residue passes certain quality checks ($ecF > 0.5$, $ecG < 0.5$, $ecE < 1$, $dE > 0.5$):

$$SDR_{score} = ecF + a \ast (famE - ecE) - b \ast ecE$$

In order to optimize the parameters $a, b$ and test the optimal ones, we created a high quality test set from our examples, consisting of 23 examples drawn from SCOP families with at least 2 EC groups, each with at > 10 distinct sequence homologs from ENZYME database. Parameters $a, b$ were varied from 0 to 5 in steps of 0.2 and 10 SDR predictions were made. For each value of $a$ and $b$, SDR and baseline predictions are made, each consisting of 10 residues. Note that baseline predictions are not affected by values of $a, b$. Optimization can be done with two objectives, either to minimize the mean proximity or to maximize the number of close ($<6\text{Å}$) residues. $a, b$ values of 0.4, 1.2 minimize the prior objective to 9.24Å and yield 3.6 close residues per prediction, whereas 0, 0.8 maximize the latter to 4.08 residues while yielding 9.36Å for the prior. Performance of these two $a, b$ values on different sets of examples is shown in Table 1.

This suggests that optimal $a, b$ parameters are 0, 0.8. It is surprising that there is no importance for the value of $dE = famE - genE$ in SDR score. Perhaps this is due to the quality checks applied prior to calculation of SDR scores, which demand $dE > 0.5$.

Fig.2 shows the distribution of mean proximity in various sets derived according to number of distinct homologs in ENZYME. This shows that quality of evolutionary information available has great impact on quality of predictions.

Mean relative proximity indicates how far from random is the prediction. Table 2 shows that mean relative proximity depends on quality of evolutionary information and is far from random for both SDR and baseline predictions.

The fraction of SDRs present in baseline predictions is 15% in all > 0, > 5, > 10 homologs classes, which suggests that SDR predictions are fairly different than baseline. This also suggests that baseline and SDR predictions are complementary to each other.
Figure 2: Frequency of observing a certain mean proximity of SDR predictions (binned in 1Å bins) for different qualities of evolutionary information available.

Table 2: Mean relative proximity in various datasets made according to number of available distinct homologs.

| Dataset       | Mean Rel. Prox. | Mean Rel. Prox. | Frequency of MRP(SDR) ≤ MRP(baseline) |
|---------------|-----------------|-----------------|--------------------------------------|
| >0 homologs   | 0.67            | 0.66            | 34% (33/97)                          |
| >5 homologs   | 0.57            | 0.66            | 60% (40/67)                          |
| >10 homologs  | 0.57            | 0.62            | 85% (47/55)                          |
5 Some examples

When quality sequence information is available, SDR predictions are closer to specific ligand than baseline predictions which in turn are closer than random. Here we compare our Top10 predictions with information from literature for some examples.

5.1 Aminotransferases

Aminotransferases or transaminases are important to amino acid biosynthesis and unique due to their specificity to two substrates: a glutamate and an amino-carrier. Our dataset contains two SCOP families (c.67.1.1 and c.67.1.4) that contain transaminases. Of those, we focus on SCOP family c.67.1.1 which contains the functional categories aspartate transaminase (AspAT, EC 2.6.1.1) and histidinol phosphate transaminase (HspAT, EC 2.6.1.9). Other non-transaminase members of this family include threonine adolases (EC 4.1.2.5) and alliin lyase (EC 4.4.1.4).

When Top10 predictions were analyzed in 1gex, an HspAT, we found that SDR predictions are very well clustered around the ligands PLP and HSP, but 5 of the 10 predictions were shared with Top10 baseline predictions. This overlap can be attributed to degrees of functional diversity in the SCOP family, i.e. large entropy reduction in HspAT residues could be due to their importance to general transaminase mechanism (as opposed to aldolase mechanism) or for substrate specificity to histidinol phosphate (as opposed to aspartate in AspATs). In order to increase the number of distinct predictions, Top20 baseline and SDR predictions were used. Fig.3 shows the predictions for 1gexA, an HspAT from E. coli - 7 predictions are common. Catalytically important residues (Haruyama et al. (2001)) Asn-157, Tyr-187, Lys-214 are identified as baseline, SDR and common respectively. Tyr-55, which interacts with substrate of the other subunit, is predicted as SDR\footnote{This is confirmed from a similar prediction in 1g4, an AspAT.} Tyr-20, believed to be important for specificity, is not predicted as such because it is conserved only 80% of times, whereas a similarly placed Tyr-55 from other subunit is much better conserved (98% times) and could be equally important for specificity. Ala-186, considered important for restricting rotation of PLP’s pyrimidine ring and thereby contributing to strain essential for enzyme function, is predicted as both SDR and baseline. Most other predicted SDRs lie close to the substrate. Their location and AspAT counterparts suggest their role in conferring specificity towards histidinol phosphate (see Table 3).

5.2 Phosphoric monoester hydrolases

SCOP family e.7.1.1 in our dataset contains 4 classes of phosphoric monoester hydrolases, 3’(2’),5’-bisphosphate nucleotidase (EC 3.1.3.7), Fructose-bisphosphatase (EC 3.1.3.11), Inosi-
Table 3: Residues from speculated roles [Haruyama et al. (2001)] for HspAT 1gex and how well they were predicted. The aligned residues in other subfamilies with transaminases are also shown.

| Residue | role | Predicted? | Asp | Arom | Tyr | Thr | Alin
|---------|------|------------|-----|------|-----|-----|------
| Tyr-20(20) | phosphate, Hsp | no, 80% conservation | Asp | Val | Asp | Asn | Thr
| Tyr-55(70) | Hsp, Glu | Top10 SDR | Tyr | Tyr | Tyr | Asp | Tyr
| Asp-85(109) | Hsp, Glu | no, 70% Asp, 30% Asn | Lys | Ser | Thr | Ser | Thr
| Asn-157(194) | phosphate, Glu, mechanism | Top10 baseline | Asn | Asn | Asn | Asn | Asn
| Asp-184(222) | mechanism | no, structurally conserved | Asp | Asp | Asp | Asp | Asp
| Ala-186(224) | mechanism | Top10 baseline and SDR | Ala | Ile | Ala | Ile | Ala | Val
| Tyr-187(225) | mechanism | Top20 SDR | Tyr | Tyr | Tyr | Tyr | Tyr
| Lys-214(258) | mechanism | Top10 baseline and SDR | Lys | Lys | Lys | Gly | Lys
| Tyr-243(296) | Hsp, Glu | no, 56% Phe, 41% Tyr | Thr | Ser | Cys | Gly | Glu
| Arg-322(382) | phosphate | Top20 SDR | Val | Leu | Met | Val | Ile | Leu | Asn | Gln
| Glu-38 | 6.6A | Top10 SDR | Glu | Val | Ala | Leu | Thr | Asp | Glu | Gly
| Thr-211 | 7.6 | Top10 SDR | Gly | Ser | Ser | Gly | Cys | Thr
| Ser-213 | 6.6 | Top10 SDR | Ala | Ser | Ser | Ala | Ser | Ser
| Ala-219 | 7.0 | Top10 SDR | Thr | Tyr | Thr | Pro | - | Ser
| Arg-222 | 4.5 | Top10 SDR | Arg | Arg | Arg | Val | Arg
| Asn-298 | 8.1 | Top10 SDR | Phe | Met | Met | Met | Asn | Ser
| Tyr-299 | 7.0 | Top10 SDR | Tyr | Tyr | Met | Tyr | Met | Tyr


Figure 3: SDR (green) and functional residue (red) predictions for 1gex, a HspAT. Residues predicted both as functional and specificity-conferring are colored blue. Top left panel shows Top5 predictions, top right panel shows Top10 predictions and bottom panel zooms in on the region around ligand in the Top10 case.
tolphosphate phosphatase (EC 3.1.3.25) and Inositol-1,4-bisphosphate 1-phosphatase (EC 3.1.3.57). Here we look at the SDR and baseline predictions for 1cnq, a member of FBPase category. FBPases are of key importance to regulation of gluconeogenic pathway and catalyze the hydrolysis of fructose 1,6-biphosphate to fructose 6-phosphate. They are metal dependent and are allosterically controlled by AMP which triggers a conformational change and masks the fructose active site. Fig.4 shows the Top10 baseline and general predictions, the overlap in this case of 2 residues. F6P molecule around which most predictions are clustered lies in the active site whereas the other F6P molecule is similarly located as AMP (from comparison with PDB 1yyz). Baseline predictions Tyr-279, Glu-280, Tyr-244, Met-244 and common prediction Tyr-264 are within interacting distance of F6P ligand in the active site. Most predicted SDRs form the active site walls and differ between FBPase and IMPase (1awb): Arg-276 to His, Ser-96 to Gly, Ser-123 to Thr, Ser-124 to Thr (see Table 4). It is surprising to see that the allosteric site is only mildly detected. Predictions Ala-161 (Top10 SDR), Lys-290 (Top10 baseline) and Val-178 (Top20 SDR) are close and suggestive of some role in AMP binding.

| Residue | role                  | Predicted? | ABPase(7) | 1D-MIPase(25) | MIPase(57) |
|---------|-----------------------|------------|-----------|---------------|------------|
| Asp-68  | Lys                   | metal      | no, high entropy |                | Val        | Lys Thr    |
| Glu-97  | metal                 |            | Asp       | Asp           | Asp        |
| Glu-98  | catalysis, metal      |            | Glu       | Glu           | Glu        |
| Asp-118 | metal                 |            | Asp       | Asp           | Asp        |
| Leu-120 | close to active site  |            | Ile       | Ile           | Ile Val    |
| Asp-121 | metal                 | Top20 SDR  | Asp       | Asp           | Asp        |
| Arg-276 | catalysis             | Top10 SDR  | Lys       | His Arg       | Phe Lys    |
| Glu-280 | Asp                   | Top10 baseline, Top20 SDR | Asp       | Asp           | Asp        |
| Ser-96  | 12.8                  | Top10 SDR  | Gly       | Gly Ser       | Gly        |
| Ser-123 | Thr                   | Top10 SDR  | Thr       | Ser Thr       | Thr        |
| Ser-124 | Phe                   | Top10 SDR  | Lys       | Ser Thr       | Thr        |
| Ala-161 | Phe                   | Top10 SDR  | Leu       | Phe Tyr       | Met Ala    |
| Arg-243 | 12.2                  | Top10 SDR  | Leu       | Arg           | Phe Leu    |
| Val-249 | Ala                   | Top10 SDR  | Gly       | Gly Ala       | Gly        |
| Tyr-264 | Asp                   | Top10 SDR  | Arg       | Asp Gly       | Phe        |

Table 4: Speculated roles of residues in FBPase for 1cnq from literature and how well they were predicted. Aligned residues in other subfamilies of hydrolases are also shown.
Figure 4: SDR and functional residue predictions for 1cnq, a FBPase. Residue-coloring scheme same as Fig.3. The bottom panel is a closer view of the region around ligand in the top panel.
5.3 Dehydrogenases

L-3-hydroxyacyl-CoA dehydrogenase (HAD, EC 1.1.1.35) is penultimate enzyme in -oxidation spiral and catalyzes conversion of hydroxy group to keto group while converting NAD+ to NADH. It consists of NAD-binding and C-terminal domains, which undergo relative movement between NAD binding and substrate binding events [Barycki et al. (2000)]. Its SCOP family is c.2.1.6, other members of which are other NAD/NADP-dependent dehydrogenases (ECs 1.1.1.8, 1.1.1.22, 1.1.1.44). HAD is represented in our dataset by NAD-binding domain of 1f0y (residues from A-12 to A-203). Fig.5 shows Top10 baseline and SDR predictions. Catalytically important pair of Glu-170 and His-158 is identified as SDRs. Ser-137, interesting due to its contact with substrate as well as NAD, is also identified as SDR. With the exceptions of Leu-122, Ala-35 (baseline) and Gly-29, Ala-107 (SDR), all other predictions are within interacting distance of either NAD or substrate. Ser-61 and Lys-68 are not detected due to their high entropy.

5.4 Tryptophan biosynthesis enzymes

Phosphoribosylanthranilate (PRA) isomerase (TrpF) is a ($\beta\alpha)_8$ barrel enzyme which is the most common fold adopted by enzymes and popular among non-enzymes. TrpF (EC 5.3.1.24) shares its SCOP family (c.1.2.4) with indole-3-glycerol-phosphate synthase (EC 4.1.1.48) and tryptophan synthase (EC 4.2.1.20), which are all involved in Trp biosynthesis. Top10 baseline and SDR predictions are shown in Fig.6. His-83 and Arg-36, considered important for catalysis, are predicted. Gln-81 (Glu in Trp synthase 1kfc), predicted as baseline and SDR, could be important for catalysis due to its location. A few baseline predictions are far from active site and their conservation suggests protein-protein binding interface. Predicted SDRs lie close to ligand and are either replaced by other residues in Trp synthase (Arg-36 to Asn) or deleted (Gln-184, Asp-178), which suggests that they could be specificity determining.

5.5 tRNA synthetases

Aminoacyl-tRNA synthetases catalyze the process of attaching an amino acid to its tRNA carrier so that it can be incorporated into a protein. SCOP family c.26.1.1 contains tyrosyl-tRNA synthetase (EC 6.1.1.1) along with other (Trp-, Glu-, Gln-) tRNA synthetases. Fig.7 shows baseline and SDR predictions for tyrosyl-tRNA synthetase 1h3e from a thermophilic bacterium T. thermophilus [Yaremchuk et al. (2002)]. Residues important for catalysis from 51-HIGH and 233-KMSKS regions are predicted as baseline (His-52, Gly-54, His-55, Lys-235). Predicted SDRs lie close to the substrate and cofactor. Residues specific for L-tyrosine binding, according to Kobayashi et al. (2003) (e.g. Thr-80, Tyr-175, Gln-179, Asp-182, Glu-197), are detected.
Figure 5: SDR and functional residue predictions for 1f0y, a HAD. Residue-coloring scheme same as Fig.3.
Figure 6: SDR and functional residue predictions for TrpF. Residue-coloring scheme same as Fig. 3.
Table 5: Residues in other tRNA synthetases aligned to predicted SDRs in tyrosyl tRNA synthetase.

| TyrTS | TrpTS | GluTS | GlnTS | Comment |
|-------|-------|-------|-------|---------|
| Thr-80 | His   | Asp   | Asn   | Tyr, Trp, Glu, Gln |
| Tyr-175 | Tyr | Tyr | Tyr | functionality other than AATS in the SCOP family? |
| Gln-179 | Met   | Asn   | His   | Tyr, Trp, Glu, Gln |
| Gly-193 | Val   | Arg   | Cys   | Tyr, Trp, Glu, Gln |
| Gly-194 | Gly   | Ala   | Thr   | Tyr/Trp, Glu, Gln |
| Asp-196 | Asp   | Glu   | Glu   | Tyr/Trp, Glu/Gln |
| Gln-197 | Gln   | Trp   | Phe   | Tyr/Trp, Glu, Gln |
| Pro-222 | Ala   | Pro   | Ser   | Tyr, Trp, Glu, Gln |
| Asp-182 | Asp   | Ser   |       | Tyr/Trp/Glu, Glu |
| Gly-43  | Gly   | Ala   | Pro   | Tyr/Trp, Glu, Gln |

Note that substrate similarity makes 2 broad divisions in this family corresponding to Trp/Tyr and Glu/Gln, each of which is subdivided into finer groups. Table 5 shows residues structurally aligned to SDRs in these tRNA synthetases.

Residues distinct for each substrate-group could be specific for it, e.g., Gln-179. Detection of residue Tyr-175 as SDR suggests that there could be more functions associated with this structural family than these four AATs. Detection of residues close to cofactor indicates different/no cofactors used by other functions of this structural family. Some residues speculated by Kobayashi et al. (2003) to be functional, stay undetected, e.g., Asn-128 which is not predicted due to high entropy (Ser dominates the MSSA column, not Asn).

6 Conclusion

We have combined structural and sequence information, functional annotation, residue entropy and environment specific substitution tables to predict specificity determining residues. We tested the predictions by using information of specific ligands and in some cases, published literature. We found that the predictions are far from random and functionally relevant, which suggests that our approach is effective. Predictions obtained with functional annotation (SDRs) and without it (baseline) are different, suggesting that available functional annotation is valuable. SDR and baseline predictions are complementary because they enlarge the set of functionally significant residues that can be computationally identified. We expected and found that our method cannot identify significant residues in absence of high quality evolutionary information, hence the importance of identifying chemically interesting patches remains undiminished. A major concern is how to obtain functional partitions in absence of annotation, which is similar
as establishing ortho/paralogy relationships. We plan to explore structure-sequence scoring schemes that would help establish functional partitions reliably. Alternatively, it would be useful to analyze the effects of constructing a functional partition based on sequence identity. We plan to use residue proximity information and residue contact conservation to detect clusters which may not be conserved in the obvious sense. We expect that cluster identification will alleviate the problem of not identifying structurally conserved residues. The most important purpose of SDR and catalytic residue identification is to help classify SNPs into normal/deleterious classes and this would be an important avenue to explore in near future.

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