Visualizing the dynamics of enzyme annotations in Uniprot/SwissProt

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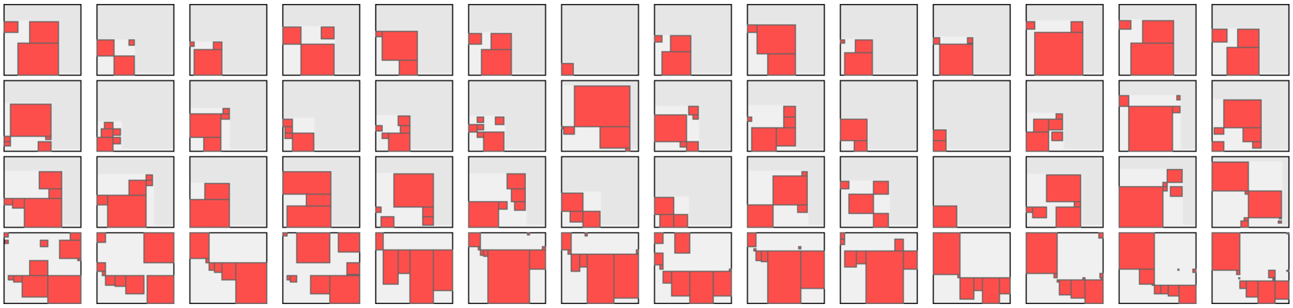

Abstract

In this paper, we tackle the problem of visualizing evolution in enzyme annotations across several versions of UniProt / SwissProt data base. More specifically, we visualize de dynamics of the EC numbers which are a numerical and hierarchical classification scheme for enzymes, based on the chemical reactions they catalyze. An EC number consists of four numbers separated by periods and they represent a progressively finer classification of the catalized reaction. The proposed interactive visualization gives macro panoramic view of the changes and presents further details on demand as, for instance, frequencies of types of changes segmented by levels of generalizations and spacializations as well as by the enzyme families.

Index Terms: Information visualization, Bioinformatics, Database dynamics, Enzymes, EC number, UniProt, SwissProt, Annotation, Processing.

1 Introduction

In recent decades there was a significant growth of biological data generated by experimental techniques such as the new generation DNA sequencing, protein sequencing and protein structure determination. Much of these data are organized and made publicly available to the scientific community in biological databases over the Internet. According to [11] these repositories not only store biological raw data but also relevant information related to them such as literature data, protein function, relationship between a protein and its encoding gene, among other metadata.

Given that these biological databases are growing at very high rates, most of these metadata are automatically assigned. In the majority of the cases, with no laboratory experiments at all, the roles of most genes in several organisms have been reported by homology propagation [4]. To ensure that these annotations remain reliable, studies about the confiability of the entries as well as measures of confidence should be developed. Many studies have called the attention to errors rates in the biological databases annotations [6, 8, 10, 12, 9]

In fact, the automatic identification of theses errors is still an open problem and several challenges have to be faced. Without laboratory experiments to verify automatically assigned annotations, it is impossible to know for certain. However, most of the studies present comparisons of diverse functional annotation methods and show they are widely incompatible what place a rough upper bound on their accuracy.

A major step toward automatic error detection is a description of how and to what extent biological databases entries annotations evolve. In other words, we have to be capable to understand why some entries seem to be more stable and and others more volatile and what are the factors that determines this different behaviours.

The research and development of models and algorithms as well as visualization ressources are very promissing toward understanding how biological databases evolve. Interactive visualizations can be specially powerful to represent in a macro/micro perspective this voluminous, high-dimensional and complex datasets and to help users to unveil trends and exceptions in those data sets.

1.1 Enzyme annotations

By the late 1950's it had become evident that the nomenclature of enzymology, in a period when the number of known enzymes was increasing rapidly, was getting out of hand. In many cases the same enzymes became known by several different names, while conversely the same name was sometimes given to different enzymes. Many of the names conveyed little or no idea of the nature of the reactions catalysed, and similar names were sometimes given to enzymes of quite different types. To meet this situation, the General Assembly of the International Union of Biochemistry (IUB) decided, in consultation with the International Union of Pure and Applied Chemistry (IUPAC), to set up an International Commission on Enzymes. Its objective was to consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assay, together with the symbols used

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in the description of enzyme kinetics. The Commission prepared a report,in 1961 and it was adopted and has been widely used in scientific journals, textbooks, etc. since then. The size of the Enzyme Commission number (EC number) list has increased steadily since the publication of the first report and also many corrections were done.

The EC number is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. Every enzyme code consists of four numbers separated by periods. Those numbers represent a hierarchical progressively finer classification of the catalized reaction. For example, the code: 3.4.21.4 is a:

3: hydrolase, which means the enzyme breaks a chemical bond using a water molecule.

3.4: peptidase, which means the broken bond is a peptide bond, i.e., a bond between amino acids in a protein chain.

3.4.21 : endopeptidase, because it breaks an intra-chain peptide bond.

3.4.21.4: trypsin, because enzyme has the specificity of cutting close the residues arginine and lisine.

When a new enzyme is annotated, one can add from one to four levels of the EC number, depending on the detail of existing knowledge. In the better case, we know all about the catalyzed reaction as well as the specific substrates and products involved. However, in many cases all we know is that the molecule is an enzyme. In this case, the annotation is left ”-.-.-.-”. An EC number ”3.4.21.-”, for instance, means we don’t know enzyme substrates specifically although we have information about the reaction catalyzed.

2 Problem modelling

Based on numerical and hierarchical nature of Enzyme Classification number, we proposed a model to characterize the EC changes observed over several versions of UniProt/Swiss-Prot. First of all, our focus was on visualizing what types of changes happens and with what frequency they occur. Considering it is important to know the hierarchical level in which a change occurs, since a move in higher levels (leftmost) are more severe than in lower ones, we decided to segment changes by common prefix length, number of generalizations and number of specializations a specific EC number has suffered.

An example of EC number change characterized by our model is provided below.

$$3.1.3.2 \rightarrow 3.1.3.5$$

It happened in 77 Hydrolases of releases 5 to 6. Observe that the common prefix length is 3 (the first three levels from left to right remains the same), there was 1 generalization (number 2 was deleted) and 1 specialization (number 5 was written). This change means that an Acid Phosphatase is now classified as a 5’-Nucleotidase.

More examples of EC moves characterized by our prefix / generalization / specialization model are provided in Table 1.

3 Data set

In this work we use the biological database UniProt [5], which aims to provide a centralized repository of protein sequences with comprehensive coverage and a systematic approach to protein annotation, incorporating, interpreting, integrating and standardizing data from a large number of disparate sources. It is the most comprehensive catalog of protein sequence and functional annotation and has four components optimized for different uses. As stated by [5] the UniProt Knowledgebase (UniProtKB) is an expertly curated database, a central access point for integrated protein information with cross-references to multiple sources.

In accordance with [1] UniProtKB consists of two sections, UniProtKB/SwissProt and UniProtKB/TrEMBL. SwissProt contains manually annotated records with information extracted from literature and curator-evaluated computational analysis. Annotation is done by biologists with specific expertise to achieve accuracy. TrEMBL contains computationally analyzed records enriched with automatic annotation and classification. As the Swiss-Prot is considered the gold standard for protein annotation, in this work we use its data to observe and analyze the changes in EC annotation.

The major releases available in the ftp of UniProt database when this study was started (March 2009) were downloaded. We analysed releases 1 (when SwissProt was integrated to UniProt) to 15 (the current release when this study was started).

In order to check if an EC move happened we need to look at a database entry EC annotation in two consecutive releases, therefore the mentioned releases were studied in pairs and the intersection of identifiers across two consecutive releases was taken.

The total number of entries as well as the number of entries annotated with EC number and its percentage for the fifteen used releases are provided in Table 2. Table 3 shows the number of entries in the set intersection of each release pair.

Table 3: Release pairs and number of entries in the intersection.

Release pair	Number of entries in $\cap$
1 and 2	141,249
2 and 3	151,318
3 and 4	162,812
4 and 5	166,933
5 and 6	181,005
6 and 7	193,382
7 and 8	207,069
8 and 9	222,181
9 and 10	241,189
10 and 11	260,065
11 and 12	269,152
12 and 13	276,011
13 and 14	356,036
14 and 15	392,597

4 Technique

The main objectives of the proposed visualization were:

1. to give a macro panoramic macro view of the evolution of EC number annotations

2. to allow users to explore the complete set of changes formulating and answering general questions about EC number changes

Concerning the first objective, we would like to present at once all the changes segmented by all the possible combinations of events considering the three parameters of the model (common prefix length and number of generalizations and specializations) across all the database releases.

4.1 Multivariate display

We have a multivariate problem where the fundamental activity is to compare multiple instances of several variables at once and to allow users to identify similarities and differences among them. Small multiples of Tufte [13] or trellis displays [2, 3], as proposed by Cleveland, are a straightforward approach to present our data. They consists of splitting the data into multiple graphs that are presented at the same time in close proximity in the screen and allows to examine data in any one grahh more easily, and comparison of values and patterns among graphs with relative ease. According to Few [7], individual graphs display a subset of a data set divided according to a categorical variable and the several graphs differ only in terms of data. Every graph is the same type, shape, size and shares the same categorical and quantitative scales. Scales in each graph must start and end with the same values (otherwise the accurate comparison is more difficult). Graphs can be arranged horizontally or vertically or as a matrix in a meaninful order.

Having this in mind, we proceed explaining the proposed visual representation. The basic graph of the proposed small multiple representation, which we call from now on *frame*, is presented in Figure 1. It is a 2D plot where we present in x-axis the number of generalizations and in the y-axis, the number of generalizations. Both x and y-axes varies in the interval [0,4]. Position (0,0) from frames represents entries with no changes in the corresponding pair of versions. It is important to point out that there are prohibited positions for some lengths of common prefixes. For instance, if a change keeps a common prefix of size 3, it is impossible to have 2 generalizations. They are presented in a darked shade of gray in Figure 1.

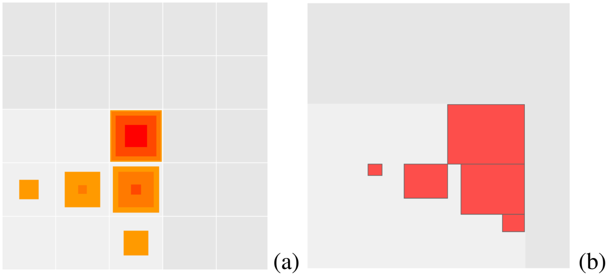

Figure 1: Basic frames for the proposed small multiple visualization. In (a), we present the heatmap version and in (b), the squaremap. Mudar para versão com heatmap de uma cor e com legendas

Several frames like this are then arranged in a small multiple fashion as in Figure 2. In x-axis, we represent the consecutive pairs of released versions. The y-axis presents the possible common prefixes in [0,4].

4.1.1 Heatmap

In a first version of the graph, we use a heatmap representation where color is a pre-attentive attribute that encodes the frequency of that configuration of change.

This representation aimed at giving an overview of the complete data evidencing trends and exceptions across the 15 releases. An interesting feature of this representation is that values in the lower right triangular matrix represents specialization and in the upper left triangular matrix, generalizations. Consequently, it is easy to recognize global trends towards generalization or specialization patterns in enzyme reaction annotations.

4.1.2 Squaremap

Heatmaps present important trends in terms of generalization and specialization occurences however we see two possible drawbacks in that approach.

Firstly, color is not a pre-attentive ??? which can precisely encode quantitative data. For sure, one can perceive that a intense color represent a higher value than a less intense one. Hoever, it is very difficult to estimate precisely the quantitative values from color intensities.

The second drawback is that our heatmap presents two much blank space. According to Tufte [13], the data density of a graph is the proportion of the total size of the graph that is dedicated displaying data. Tufte prefers high data density graphs as the human perceptual system is capable of detecting subtile patterns, trends and exceptions. On account of that, we decided to propose a second complementary view trying to reduce blank (non-data) space and also a representation which should use a more precise visual attribute to enconde the frequencies.

The Squaremap representation was inspired in 2D scatterplots where the points are squares whose area represent frequency. Even though area is not the most precise visual attribute to enconde quantity, one can estimate its area through square side length which users can precisely represent quantitative data. Notice, in Figure 1, that is easier to estimate quantities in the Squaremap (b) tnan in the Heatmap (a).

4.2 Analytical interaction and navigation

4.2.1 Filtering, scales and normalization options

The effective of the information visualization techniques hinge on the ability to clearly and accurately represent information and on the ability to interact with it to figure out what information means. Indeed, no matter how rich the display is, it will invite questions and the interaction is necessary to pursue an answer. Besides, different perspectives can lead to different insights. The proposed visualization allows pre-defined filters and different scalling and normalization options:

1. log scale on the frequencies
2. normalization of frequencies by frame or globally
3. filter by only changes (removing position (0,0)) or presentation of the complete data set

4.2.2 Micro/macro view

One particularly interesting way to create dense graphics is through what Tufte calls micro/macro readings [13]. These graphics convey one layer of information on a micro scale and another layer on a zoomed out, macro scale. One nice consequence of this technique is information is consumed hierarchically. The viewer may glance from a distance to observe an aggregate trend, and later peer in closely to examine individual pieces of that trend. Our multivariate view is a macro view of the whole changes data set. Users can click each frame and see it zoomed in a micro view.

4.2.3 Exploratory navigation

Besides having a micro/macro view of the possible changes in enzyme annotation, users can click the points in the micro view and see interactive histograms of each type of change. Through these histograms users can see the enzyme families which has suffered that change. These histograms are composed by small squares representing each change and by clicking individual points users can see details about the entry.

5 Discussions

In this section, we describe the insights we obtained through the proposed interactive visualization.

5.1 Trends

5.1.1 Stable enzyme annotations

The most common event over the entire data set is located at the bottom left corner of each frame and it represents pairs of observed EC numbers that remained constant in a certain pair of versions. It means that the two EC numbers involved were equal (i.e. 3.1.3.2 to 3.1.3.2) or that there was no EC number (-.-.-.- to -.-.-.-).

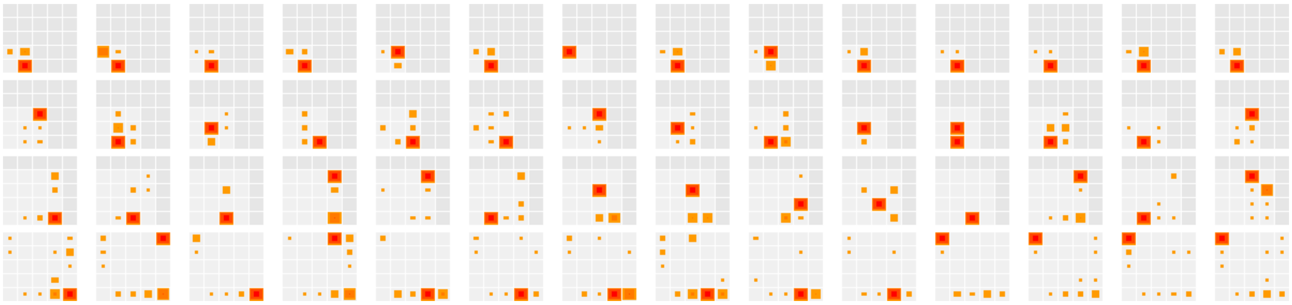

Figure 2: Multivariate view with heatmaps. Mudar para versão com heatmap de uma cor e com legendas

5.1.2 Generalization vs Especialization

Consider, for each frame, a diagonal that extends from the bottom left corner to the top right corner (marcar diagonal numa figura para dar exemplo). The matrix of points below this diagonal, called lower right triangular matrix, represents changes in which there are more specializations than generalizations. In a similar manner, the matrix of points above this diagonal, called upper left triangular matrix, represents changes in which there are more generalizations than specializations. In the figure as a whole, the lower triangular matrices have more points than the superior ones, and therefore in the entire data set there are more specializations than generalizations.

5.2 Exceptions

5.2.1 Annotation deletion

The four points, in the red rectangle of the last line of frames, whose parameters are $prefix = 0$, $generalization = 4$ and $specialization = 0$, represent a drastic change in which the four levels of involved EC numbers were deleted. The Table 4 shows the frequencies related to each point.

Table 4: Frequency of four-level EC number deletion from releases 11 to 15

Pair of releases	Frequencies
11 to 12	146
12 to 13	1,357
13 to 14	1,006
14 to 15	1,976

In UniProtKB/Swiss-Prot they try only to assign EC numbers to catalytic subunits. This means that in large protein complexes only one or a few of the subunits will be annotated with an EC number. When they discover cases where non-catalytic subunits are annotated with an EC number, they remove it completely since the subunits in question do not have any enzymatic activity on its own. Here we present three examples of UniProt/Swiss-Prot entries that experienced four-level EC number deletion from version 12 to 13.

- Identifier Q6FSJ2, which was annotated as 1.10.2.2 in version 12, is subunit 7 of cytochrome b-c1, but not the subunit with reductase activity

- Identifier Q8LX28, whose annotation was 3.6.3.14 in version 12, is subunit 8 of ATP synthase, which is part of the membrane proton channel

- Identifier Q6AY96, which was annotated as 2.7.11.1 in version 12, is a subunit of a transcription factorm, but not the subunit with serine/threonine kinase activity.

5.2.2 Deleted EC numbers

In the highlighted point with parameters $prefix = 2$, $generalization = 2$ and $specialization = 2$ in versions 7-8, a total of 1900 EC number changes are represented. The three most numerous changes depicted in this point are, respectively, 2.7.1.37 to 2.7.11.1 (frequency 918), 2.7.1.112 to 2.7.10.1 (frequency 215) and 2.7.1.112 to 2.7.10.2 (frequency 165). As stated by IUBMB [**?**], the EC number 2.7.1.37 was deleted and divided in 2005 into EC 2.7.11.1, EC 2.7.11.8, EC 2.7.11.9, EC 2.7.11.10, EC 2.7.11.11, EC 2.7.11.12, EC 2.7.11.13, EC 2.7.11.21, EC 2.7.11.22, EC 2.7.11.24, EC 2.7.11.25, EC 2.7.11.30 and EC 2.7.12.1. The same happened to the EC number 2.7.1.112, that was deleted and divided into EC 2.7.10.1 and EC 2.7.10.2. In such cases, transferase annotations, more specifically EC 2.7.*.* (transferring phosphorus-containing groups), underwent a revision caused by a change in the EC classification system, not by a change in enzyme function annotation.

6 Conclusion

Author contributions

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Table 1: Example of EC numbers across consecutive database releases and our prefix / generalization / specialization model

Previous EC number	Actual EC number	UniProt id	releases	Common prefix length	Number of Generalizations	Number of Specializations
-.-.-.-	-.-.-.-	Q9K5T1	1 to 2	0	0	0
3.1.4.14	1.7.-.-	P41407	7 to 8	0	4	2
1.1.1.-	1.-.-.-	P52895	5 to 6	1	2	0
5.3.-.-	5.3.1.27	P42404	14 to 15	2	0	2
2.5.1.64	2.5.1.-	P17109	13 to 14	3	1	0
4.1.1.22	4.1.1.22	P95477	1 to 2	4	0	0

Table 2: Releases 1 to 15 of UniProt/SwissProt.

Release	Release date (MM/DD/YYYY)	% of entries with EC	Number of entries with EC	Total of entries
1	12/15/2003	0.37	52,434	141,681
2	07/05/2004	0.38	57,931	153,871
3	10/25/2004	0.38	61,229	163,235
4	02/01/2005	0.38	63,221	168,297
5	05/10/2005	0.38	69,164	181,571
6	09/13/2005	0.38	74,468	194,317
7	02/07/2006	0.39	80,874	207,132
8	05/30/2006	0.40	89,245	222,289
9	10/31/2006	0.40	97,508	241,242
10	03/06/2007	0.40	105,225	260,175
11	05/29/2007	0.40	108,876	269,293
12	07/24/2007	0.40	111,230	276,256
13	02/26/2008	0.43	151,694	356,194
14	07/22/2008	0.43	168,849	392,667
15	03/24/2009	0.44	189,234	428,650