

# Source of Functional Annotations in UniProtKB and implications for Function Prediction

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## Abstract

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**Background:** Computational protein function prediction programs rely upon well-annotated databases for training their algorithms. These databases, in turn, rely upon the work of curators applying experimental findings from scientific literature to protein sequence data. However, with the advent of various high-throughput experimental assays, there are a few papers that dominate the protein annotation field. Here we investigate just how prevalent is the “few papers – many proteins” bias. We discuss how this bias affects our view of the protein function universe, and consequently our ability to predict protein function.

**Results:** We examine the annotation of UniProtKB by the Gene Ontology Annotation project (GOA), and show that the distribution of proteins per paper is a log-odd, with X papers dominating X% of the annotations. Since each of the dominant papers describes the use of an assay that can find only one function or a small group of functions, this leads to a substantial bias in what we know about the function of many proteins.

**Conclusions:** Given the experimental techniques available, the protein function annotation bias is unavoidable. Knowing that this bias exists and understanding its extent is important for database curators, developers of function annotation programs, and anyone who uses protein function annotation data to plan experiments.

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## Background

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Functional annotation of proteins is a cardinal challenge in molecular biology today. It has been said that while we may soon reach the \$1,000 genome (and for some prokaryotes, indeed we have), we will still need to pay \$20,000 analysis bill. There are and estimated XXX non-redundant open reading frame sequences, but less than 40% are functionally annotated. Moreover, the majority of functional annotations

## Results and Discussion

### Papers and proteins

As described in the Background section, with the advent of high-throughput experiments it has become possible to conduct large-scale interrogations of protein functions. Some papers therefore reveal one or more functional aspects of a large amount of proteins which respond to the particular type of interrogation conducted. To understand how prevalent this phenomenon is, we looked at the UniprotKB gene ontology annotation files, or UniProt GOA. UniProtKB is annotated by GO terms both manually and automatically using an exacting procedure described in [?]. Briefly, there is a six-step procedure which includes sequence curation, sequence motif analyses, literature-based curation, reciprocal BLAST [?] searches, attribution of all resources leading to the included findings, and a quality assurance phase. If the source is a research article, the attribution includes a PubMed ID. For each GO term associated with a protein, there is also an *evidence code* with which is used to explain how the association between the protein and the GO term was made. Experimental evidence codes include such terms as: *Inferred by Direct Assay* (IDA) which indicates that “a direct assay was carried out to determine the function, process, or component indicated by the GO term” or *Inferred from Physical Interaction* (IPI) which “Covers physical interactions between the gene product of interest and another molecule.” (Quotes are from the GO site, geneontology.org). The computational analysis evidence codes are generally considered less reliable than the experimental ones, and include terms such as *Inferred from Sequence or Structural Similarity* (ISS) and *Inferred from Sequence Orthology* (ISO). However, these are still assigned by a curator. There are also non-computational and non-experimental evidence codes, the most prevalent being *Inferred from Electronic Annotation* (IEA) “Used for annotations that depend directly on computation or automated transfer of annotations from a database”. IEA evidence means that the annotation was not made by a curator, and is not checked manually.

Different degrees of reliability are associated with the evidence codes, with experimental codes considered

to be of higher reliability than non-experimental codes. However, due to an ongoing increase in high-throughput experimental methods, we suspected that high-throughput experiments may dominate the protein annotation landscape.

To test our hypothesis, we first examined assignments with two degrees of reliability: experimental evidence codes (EXP, IDA, IPI, IMP, IGI, IEP), and all others. The reason for this partition into two groups is that we wanted to know which, if any, high throughput experiments dominate the protein function annotation landscape. This we compared with a baseline of annotations of all types of evidence, and with those which are non-experimental. The results are shown in Figure??.

As can be seen in Figure??, the distribution of proteins annotated per paper follows a log-odds ZZZcheckthis ratio. Many proteins are annotated by a few papers. (ZZZNeed to look at the percentage of proteins annotated by the top 10, top 20 papers.) We therefore conclude that there is indeed a bias in experimental annotations, in which there are few papers that annotate a large number of proteins.

Our next question was how much annotation bias these papers introduce. We decided to look who are the top contributing papers to protein annotation in UniProtKB-GOA, and what type of GO terms they contribute. The results to the first question are summarized in Table??. As can be seen, almost all of the papers are specific to a single species (typically a model organism) and assay that is used to annotate the proteins in that organism. Since a single assay was used, then typically only one ontology (MF, BP or CC) was annotated.

### **Annotation bias in model organisms**

To see how much a single species- and method- specific large-scale assay affects the entire annotation of a species, we examined the relative contribution of each paper to the entire corpus of experimentally annotated protein in that species. All the species we examined were model organisms, as all the top annotation-contributing papers dealt with model organisms. The results are summarized in Figure??.

### **Annotation quality**

One reflection on annotation coverage is the number of GO terms assigned to any given protein.

Ostensibly, the larger the number of GO terms that are assigned to a protein, the more comprehensive its annotation, provided that these terms are non-redundant, i.e. not direct parents of each other.

The median number of annotations per protein was 1.09, which means that most of the top-50 papers do not provide more than a single GO-term per annotation. Also, the mean number of annotations per

protein was 1.59. The difference between the mean and median reflects that most papers have an annotations-per-protein ration which is close to 1, whereas few have a higher annotation ratio. As shown in Figure ??, that is essentially the case. Seven papers had an annotations per proteins ratio which is above 2. Those were paper numbers 1, 4, 10, 23, 34 and 43 on the list. We decided to examine these papers to see whether these studies did indeed provide better coverage, and what distinguished them from the other high throughput studies.

## **Conclusions**

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## **Methods**

### **Databases used**

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## **Authors contributions**

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## **Acknowledgements**

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## **References**

### **Figures**

#### **Figure 1 - Sample figure title**

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#### **Figure 2 - Sample figure title**

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## Tables

### Table 1 - Sample table title

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A2	...	..
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### Table 2 - Sample table title

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## Additional Files

### Additional file 1 — Sample additional file title

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### Additional file 2 — Sample additional file title

Additional file descriptions text.