**METABOLIC PATHWAY MEMBERSHIP INFERENCE USING AN
ONTOLOGY-BASED SIMILARITY APPROACH**

by

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ABSTRACT

Determining whether a protein belongs to a metabolic pathways is an important annotational task, can provide context to the basic functional annotation and aid reconstruction of incomplete pathways. In this work, we develop a method for pathway membership inference based gene ontology (GO) similarity between a query protein and proteins that are known to the members of a given pathway. We specifically use human metabolic pathway from KEGG and human gene annotation dataset from Gene Ontology in this experiment. By comparing with various existing GO term semantic similarity, we develop an effective and efficient way to take into both information content of individual GO terms and the whole GO hierarchy. We test the classifier using 10-fold cross validation for all metabolic pathways reported in KEGG database and demonstrate that our method either outperform with statistical significance or perform comparably with a suite of existing semantic similarity measures, as evaluated using ROC score. And our method outperforms other methods in running time by multiple orders of magnitude for long pathways.

Chapter 1

INTRODUCTION

A metabolic pathway is a chain of biochemical reactions in the cell. It consists of chemical compounds and protein enzymes where protein enzymes catalyze the conversion from substrate compounds into product compounds. The reaction in metabolic pathway then can be represented as a graph where each protein enzyme and its corresponding substrate and product compound are connected by edges (figure 1.1(a)). The enzymatic reaction is not isolated, but interrelated where the product of reaction A can be the substrate of reaction B and it can be represented as a graph of compound/substrate network (figure 1.1(b)). From protein perspective, a metabolic pathway can be viewed as a list of proteins in the pathway. These proteins can be represented as a graph where the protein enzymes are the vertices. Two proteins are connected by the edge if the product compound of first protein enzyme is the substrate compound of the second protein enzyme (figure 1.1(c)). As a result, we can model metabolic pathway from the proteins (or genes) as a graph by using this approach.

Figure 1.1: Schematic graph of metabolic pathway

(a)

(b)

(c)

Metabolic pathway network can be viewed as (a) the whole graph of chemical compound and protein enzyme , (b) enzymatic reaction which connect substrate and the product, or (c) a graph of protein (enzyme)

Identification of metabolic pathway is one of important task in biological science since understanding the metabolic profiles is needed to uncover the underlying cellular physiology. Any subtle changes in metabolic pathway may lead to the development of abnormal cell or disease. For example, changes in expression of metabolites can lead to colorectal cancer ([Brown et al. \(2016\)](#)) and lung cancer ([Cameron et al. \(2016\)](#)). Certain diseases such as phenylketonuria and Lesch-Nyhan syndrome are caused by the lack of enzymes that cause the disruption of metabolic pathways. The loss of phenylalanine hydroxylase in phenylketonuria patients can cause mental retardation, organ damage, unusual posture.

The Identification of metabolic mechanisms in laboratory involves many laborious experimental techniques mainly in characterization of protein enzymes in metabolic pathways. It requires advance technology, expensive equipment, and highly skills manpower to perform the experiments. Another approach is to use computational methods for modeling the pathway and to infer specific task by using the model. Pathway membership inference is to determine whether a protein is a member in the list of a given pathway. This is an important annotation task that can not only provide context to the basic function annotation of proteins but also more importantly aid reconstruction of incomplete metabolic pathways, which can subsequently help better understand metabolism and physiology of cells and provide complementary perspective to study evolutionary.

However, traditional sequence similarity based homology approaches to characterizing proteins for their enzymetic properties run into difficulties when sequence identity is lower than 60% ([Radivojac et al. \(2013\)](#)) due to multiple hits of homologous proteins. Facing this challenge, various efforts have been made to go beyond individual proteins and their homologs to leverage the large amount of annotations for proteins in their functional context.

This context may come from two perspectives. One perspective is based on gene ontology (GO) ([Duan et al. \(2006\)](#)), which provides a hierarchy of controlled terms define protein functions with varied levels of specificity for different cellular

functions/processes. The other perspective comes from metabolic pathways, or proteins that serve as enzymes catalyzing the reactions in a given pathway. While these proteins may not have the same function (thus with different GO terms), they function in a coordinated way to enable the pathway and hence possess certain functional relatedness (Chitale et al. (2011)). To expand on this concept, the counted co-occurrences of GO terms are used to score the functional relatedness or coherence and further to assign proteins to pathways (Chitale et al. (2011)).

For the GO term perspective, various similarity measures have been developed to quantify the semantic similarity of GO terms and applied it in quantitative comparison of functional similarity of gene products, although most of these methods are not developed for metabolic pathway membership inference. Essentially, those measures mainly involve two steps: 1) calculation of the term similarity, and 2) calculation of the gene product similarity. In the first step, the semantic similarity between two GO term is calculated to incorporate the GO hierarchy, via the following three approaches: edge-based, node-based, or a hybrid of the former two.

The edge-based approach relies on the structure of the GO graph (Wu et al. (2006), Chen et al. (2007), Al-Mubaid & Nagar (2008)). Basically, the semantic similarity between two GO terms is defined as the distance between two connected terms, which is the length of a path connecting the two terms in the GO graph. Since there may exist more than one path connect the two terms, this approach usually use the most recent common ancestor (MRCA), or the closest common ancestor to both terms, to quantify the semantic similarity between terms. The simplest method of this approach is by using directly the path length between two terms and convert it to similarity (Al-Mubaid & Nagar (2008)). However, this simple method suffers from shallow annotation problem (Guzzi et al. (2012)), in which the similarity of two term near the GO root term is very high. Another problem arises from the assumption that GO terms at the same level in the graph have the same specificity, which in biological ontology is not always true since different gene properties have different detailed information (Song et al. (2014)). Therefore, the same level of terms in the GO graph may have

different weight. Thus, more advanced techniques of edge-based approach such as RSS considers global structure of the DAG graph by assigning different weights of edges at different level.

The node-based approach focuses on information that is contained in the node. Methods in this approach such as Resnik’s ([Resnik \(1999\)](#)), Lin’s measure ([Lin \(1998\)](#)), and Jiang’s and Conrath’s measure ([Jiang & Conrath \(1997\)](#)) rely on information content (IC). IC is defined as the negative log of term frequency in a given annotation corpus, such as Gene Ontology Annotation (GOA). Given two GO terms, the information contained in the common ancestors is used alone or in combination with other terms to quantify the similarity between the two terms. Since it is likely that there are multiple common ancestors, most methods use the maximum informative common ancestor (MICA) which is the common ancestor that contain the maximum IC. Other methods, such as simGIC ([Pesquita et al. \(2007\)](#)) considers not only MICA in determining the gene similarity, but also all ancestor contributions to the similarity measurement of two terms. Unfortunately, similar to the edge-based approach, node-based approach also suffers from shallow annotation problem, especially when the MICA is located near the root term.

The third approach combine the node- and edge-based approaches. Essentially, this hybrid approach considers both the information contained in the nodes and the structure of the GO graph using path distance as weight ([Chen et al. \(2007\)](#), [Wu et al. \(2013\)](#), [Ehsani & Drablos \(2016\)](#)), in order to minimize the problem found in edge- and nod-based approach alone.

In this work, we proposed a method for metabolic pathway membership inference, bringing together both perspectives. From the pathway perspective, proteins from an incomplete pathway provide a ”model” that a query protein needs to fit in, and the similarity of GO terms for individual proteins provide some measure for functional relatedness. With respect to GO term similarity, our approach is a hybrid one, taking into account of both nodes information and GO graph topology, with a consideration of computational efficiency.

Chapter 2

RELATED WORKS

There are many methods that have been developed for calculating GO semantic similarity. One of the first method is Lord ([Lord et al. \(2003\)](#)) which applied Resnik similarity in GO term. The Resnik similarity itself relies on the information content(IC) that is contained by the terms. The IC is defined as negative log of term frequency in given corpus as:

$$IC(t) = -\log[p(t)] \quad (2.1)$$

where $p(t)$ is term frequency of t in a given annotation corpus, such as Gene Ontology Annotation (GOA). To find similarity between two terms, Resnik similarity measure finds the maximum IC of common ancestor, as:

$$sim(t_i, t_j) = \max_{t \in P(t_i, t_j)} IC(t) \quad (2.2)$$

where t is the common ancestor of t_i and t_j .

Wang is an edge-based approach that relies on the structure of GO term. Wang similarity measure ([Chen et al. \(2007\)](#)) considers contributions from all ancestor terms to a given term in determining the semantic value of term by using the distance between the two terms. Terms closer to term t_i contribute more to semantic value of term t_i . Contribution of term t_i to itself is 1, while contribution of terms t_{p_i} to semantic value

of term t_i is determined by semantic contribution of all t_{p_i} children term t' weight by semantic contribution factor w_e . Formally Wang semantic value S is defined as:

$$\begin{cases} S_{t_i}(t_i) = 1 \\ S_{t_i}(t_{p_i}) = \max\{w_e * S_{t'}\} | t' \in \text{childrenof}(t_{p_i}) | \text{if } t_{p_i} \neq t_i \end{cases} \quad (2.3)$$

where t_{p_i} is ancestor term of t_i , w_e is weight of edges, t' is children of t_p . Then the semantic contribution of all ancestor terms of t_{p_i} to t_i is defined as:

$$SV(t_i) = \sum_{t \in T_{p_i}} S_{t_i}(t_{p_i}) \quad (2.4)$$

where T_{p_i} is all ancestor term of t_i . The semantic similarity between $term_i$ and $term_j$ then is defined as:

$$S(t_i, t_j) = \frac{\sum_{t \in T_{p_i} \cap T_{p_j}} (S_{t_i}(t) + S_{t_j}(t))}{SV(t_i) + SV(t_j)} \quad (2.5)$$

RSS is also an edge-based similarity measure. Unlike other edge-based similarity measures, RSS considers both of the graph structure above (generality) and below (specificity) a given term. To quantify the similarity measure, RSS defines three definitions: α which defines the specificity, β defines generality, and γ defines local distance between two terms and the MRCA. The specificity is defined by the number of common parent terms between the two terms, and formally defined as:

$$\alpha = \max_{\substack{\text{path}_m \in \text{Paths}(term_i), \\ \text{path}_n \in \text{Paths}(term_j)}} \left\{ \frac{\text{thenumberofcommonterms}}{\text{betweenpath}_m \text{ and } \text{path}_n} \right\} - 1 \quad (2.6)$$

where $\text{Paths}(term_i)$ and $\text{Paths}(term_j)$ are the paths in the graph that are induced from $term_i$ and $term_j$ respectively. The generality β is defined as the minimum distance between the term, and its leaf terms (term without any descendant):

$$\beta = \max \left\{ \min_{u \in U} \{dist(term_i, u)\}, \min_{v \in V} \{dist(term_j, v)\} \right\} \quad (2.7)$$

where U and v are all leaf nodes descending from term t_i and term t_j respectively. γ is defined as:

$$\gamma = dist(MRCA, term_i) + dist(MRCA, term_j) \quad (2.8)$$

where MRCA is the most recent common ancestor of term t_i and t_j

Then similarity measure between two terms is define as:

$$RSS(term_i, term_j) = \frac{maxDepth^{GO}}{maxDepth^{GO} + \gamma} \frac{\alpha}{\alpha + \beta} \quad (2.9)$$

where $maxDepth^{GO}$ is maximum distance between the root term and leaf term.

TopoICSim (Ehsani & Drabløs (2016)) is a hybrid-based similiraty measure which uses both IC and topology of GO terms in measuring term similarity. First it calculates the weighted distance between two terms. Given two terms, there are multiple path that connect two terms. TopoICSim uses both the shortest path and longest path as define by:

$$wSP(t_i, t_j) = \underset{P \in A(t_i, t_j)}{\operatorname{argmin}} IIC(P) \times len(P) \quad (2.10)$$

$$wLP(t_i, t_j) = \underset{P \in A(t_i, t_j)}{\operatorname{argmax}} IIC(P) \times len(P) \quad (2.11)$$

where IIC is inverse IC, and P is all paths that connect t_i and t_j . To determine the distance between the two terms, TopoICSim uses a subset of common ancestors called disjunctive common ancestor as defined by:

$$DiscComAnc(t_i, t_j) = \{x \in ComAnc(t_i, t_j) | P(x, root) \cap C(x) = \emptyset\} \quad (2.12)$$

where $ComAnc(t_i, t_j)$ is common ancestors between t_i , t_j , $P(x, root)$ is the path between ancestor term x and root, and $C(x)$ is set of all immediate children of x . The distance of the two term through disjunctive common ancestor is defined as the ratio between the shortest weighted distance of two terms through the disjunctive common

ancestor x and the weighted longest distance between x and the root as defined by:

$$D(t_i, t_j) = \min_{x \in DisComAnc(t_i, t_j)} \frac{wSP(t_i, x) + wSP(t_j, x)}{wLP(x, root)} \quad (2.13)$$

The distance of two terms is then transformed into similarity by:

$$S(t_i, t_j) = 1 - \frac{Arctan(D(t_i, t_j))}{\pi/2} \quad (2.14)$$

There are other semantic similarity measures that do not use either the information content or the structure of the graph. CAS ([Chitale et al. \(2011\)](#)) is based on counting the term frequency, which is usually used in natural language problems. Specifically, CAS quantifies the GO term semantic similarity using PMI, the likelihood of finding two terms together in given corpus. Similarly, CAS calculates the probability of given two term presents together in given corpus as semantic similarity between two terms as :

$$CAS(i, j) = \frac{\frac{C(i, j)}{\sum_{i, j} C(i, j)}}{\left(\frac{C(i)}{\sum_k C(k)} \right) \left(\frac{C(j)}{\sum_k C(k)} \right)} \quad (2.15)$$

where $C(i, j)$ is the number of gene product that is annotated with both term i and term j , $C(i)$ and $C(j)$ are the number of gene product that is annotated with term i , term j respectively, and $C(k)$ is total number of gene product.

Table 2.1: Similarity measures and their mixing strategy found in literature

Similarity measures	Strategy	Ref
Lord	AVG	Lord et al. (2003)
Dice	set	Popescu et al. (2006)
CAS	RCMAX	Chitale et al. (2011)
Wang	BMA	Chen et al. (2007)
Topoicsim	RCMAX	Ehsani & Drabløs (2016)
RSS	MAX	Wu et al. (2006)
simGIC	groupwise	Pesquita et al. (2007)

Having obtained the term's semantic similarity, the next task is to combined the term similarity into gene similarity. In gene annotation, a gene can be annotated with multiple GO term. Calculating gene similarity based on the GO term thus need to consider multiple terms in each gene. There are several different strategies available for this purposes. Table 2.1 summarize some strategies that are used by different similarity measures to transform term similarity to gene similarity. The strategies are:

Average (AVG) strategy calculates the average similarity over all pair of terms of gene products being compared. The average strategy is used by Lord measure. It is defined as:

$$S(g_i, g_j) = \frac{1}{m * n} \sum_{i=1}^n \sum_{j=1}^m (S(t_i, t_j)) \quad (2.16)$$

where n and m are the number of terms in gene i and j respectively.

Maximum (MAX) strategy uses the maximum similarity of pair of terms from two gene products as gene product similarity measure. The maximum strategy is used by RSS measure. It is define as:

$$S(g_i, g_j) = \max_{1 \leq i \leq n, 1 \leq j \leq m} (S(t_i, t_j)) \quad (2.17)$$

rcmax (RCMAX) strategy uses matrix of gene terms. It uses the maximum score from the average of maximum value for each column and each row. This strategy is used by TopoICSIM and CAS measures. It is define as:

$$S(g_i, g_j) = \max \left(\frac{\sum_{i=1}^n \max_j S(t_i, t_j)}{n}, \frac{\sum_{j=1}^m \max_i S(t_i, t_j)}{m} \right) \quad (2.18)$$

Best match average (BMA) strategy also uses matrix of gene terms. It calculates the average of maximum term similarity value from each row and each columns.

TheBMA strategy is used by Wang measure. It is define as:

$$S(g_i, g_j) = \frac{(\sum_{i=1}^m \max S(t_i, t_j) + \sum_{j=1}^n \max S(t_i, t_j))}{m + n} \quad (2.19)$$

Unlike previously described pairwise approach, measures that uses groupwise approach do not need to perform two step calculations. For example, simGIC ([Pesquita et al. \(2007\)](#)) used set operation on calculating the semantic similarity of two genes. However, instead of counting the number of term occurrences in both genes, It sums the IC of term t . It is formally defined as:

$$S(g_i, g_j) = \frac{\sum_{t \in g_i \cap g_j} IC(t)}{\sum_{t \in g_i \cup g_j} IC(t)} \quad (2.20)$$

Other pairwise measures consider the term as a vector and calculate the semantic similarity of two genes by using vector operation such as cosine. Gan ([Gan \(2014\)](#)) represented gene as a vector of IC. The semantic similarity of two genes than is calculated by cosine similarity as:

$$S(g_i, g_j) = \frac{\sum_{1 \leq i \leq |V|} x_{g_i} x_{h_i}}{\sqrt{\sum_{1 \leq i \leq |V|} x_{h_i}^2}} \quad (2.21)$$

Chapter 3

DATA AND METHOD

3.1 The GO dataset and gene annotation

The GO term and the Gene Ontology Annotation (GOA) version 2018-10-01 were downloaded from the Gene Ontology Consortium, <http://archive.geneontology.org/>. This GO version contains 31024 BP, 11940 MF, and 4379 CC terms. We used human GOA to associate between the gene products and GO terms. There are 469,828 association of 19678 proteins were present in the human GOA dataset (390389 associations of 18084 proteins without IEA annotation).

3.2 UniProt dataset

We used the human UniProt identifier mapping (version 9606) to map the identifier used in KEGG dataset and BioGrid dataset.

3.3 The KEGG pathway database

We downloaded human KEGG pathway data set from Kyoto Encyclopedia of Genes and Genomes database ([Kanehisa & Goto \(2000\)](#)), <http://rest.kegg.jp>. The database consists of 320 pathways, and the number of gene in the pathways range from 1 to 448.

We evaluated our semantic similarity measure against other measures by predicting the genes membership in metabolic pathways. The assumption is that the genes that belong to the same pathways are more similar to each other since they share similar function ([Ehsani & Drabløs \(2016\)](#)).

To predict whether a gene belongs to certain metabolic pathways, functional relatedness. We generated positive and negative datasets for each pathway in KEGG pathway.

For a given pathway, we build a classifier and test its predictive power by 10-fold cross validation. Specifically, all genes that belong to the pathway make up a positive dataset, whereas a negative dataset is generated by choosing randomly from genes of all other pathways in KEGG database. We then randomly divided the positive dataset into ten subsets. Each subset is reserved as a positive test set, and the remaining nine subsets are merged into a positive train set. This process is repeated 10 times to ensure each subset is used once as test. For a classifier based on supervised learning, a negative train set and a negative test set are prepared in a similar manner as their positive counterparts. The performance of a classifier is evaluated on the test set and averaged over 10 fold cross-validation.

3.4 Gene representation

In order to calculate semantic similarity between two genes (or their corresponding proteins as we hereafter use gene and protein with nodistinction), we adopt the vector space model (VSM) approach, which is commonly used in document information retrieval. In this model, each document is represented by vector of words or keywords. Here, we represented the gene as vector of GO terms. We build the vector of genes by using GO terms as the gene’s features. While a simplistic approach is a binary vector – with 1 or 0 representing the presence or absence of GO terms in annotation of the given gene, our method considers both of the structure of the GO graph and the information content of the GO terms in building the vector of the gene and their corresponding annotations.

Specifically, before we build the gene vector, we first calculated the semantic value (SV) for each GO term in the annotation of a given gene. We calculated the SV as the information content of GO term.

We used a normalized information content by dividing the information content of the term t_i with the maximum IC in whole set of GO terms T as follows:

$$IC_n(t_i) = \frac{IC(t_i)}{\max_{t_j \in T} IC(t_j)} \quad (3.1)$$

Then we include the ancestor terms of original annotation in a given gene to build gene vector: for each annotation term t in a given gene, we assigned the weighted semantic value for all its ancestor terms t_p , defined as follows.

$$SV(t_p) = w^{d_p} IC_n(t_p), \quad (3.2)$$

where w is the weight, in this case we use a fix constant of 0.5, and d_p is the path length of the ancestor term p to the annotation term t . The path length is defined as the difference of the maximum depth between the two terms, i.e. between term t_i and its ancestor.

When there are multiple GO terms in the annotation of a given gene, it is possible that these GO terms may have ancestor terms in common. Therefore, during inclusion of the ancestor terms, for each annotation term in a given gene, a common ancestor term will have multiple semantic values, each for a descendant in the original annotation as the common ancestor term may receive a semantic value from all of its descendants differently. Hence, we calculated the average of these values as the new semantic values for a common ancestor term t_p .

After this procedure, a gene is represented as a vector of n -dimension, where $n = |T|$, each dimension corresponding to one GO term in the gene ontology hierarchy, with a semantic value being either a) the normalized information content if the GO term is present in the gene annotation, or b) a value assigned as above for a GO term whose descendant(s) is present, or c) a value of zero if a GO term is not of either of the two former cases.

The similarity score of two genes q and p in is then defined as dot product of

their corresponding vector as

$$s(q, p) = \sum_{t \in T} SV_q(t) * SV_p(t) \quad (3.3)$$

3.5 Pathway membership prediction

As mentioned in Introduction, our method brings together the two perspectives - incomplete metabolic pathway and GO annotation of individual proteins - to infer whether a query protein is a member of the incomplete pathway. While there exist some more sophisticated methods that construct a model for the pathway to integrate and leverage various information, our method simply use the set of proteins of the incomplete pathway P as a "model" and calculate a score $S(q, P)$ for a query protein q to determine whether q belong to the model, i.e., q as a member of the pathway P . For example, if $S(q, P)$ is higher than a threshold, q is predicted as a member, otherwise not a member. In this work, instead of using a specific threshold, a ROC score is used to evaluate the prediction performance.

The score $S(q, P)$ is the average similarity between the query protein and the proteins that are already known to be in the pathway P . Specifically,

$$S(q, P) = \sum_{p \in P} S(q, p) / |P| \quad (3.4)$$

where $s(q, p)$ is the similarity score between query protein q and a member protein p as calculated by 3.3 and $|P|$ is the number of known proteins for the incomplete pathway P .

3.6 Performance evaluation

We used receiving operating characteristic (ROC) curve analysis to evaluate the performance of our method. ROC curve measure the performance of classifier at

various threshold setting, and represents the trade off between true positive rate (TPR) and false positive rate (FPR). The TPR and FPR are defined as:

$$FPR = \frac{FP}{(FP + TN)} TPR = \frac{TP}{(TP + FN)} \quad (3.5)$$

where FP , TN , TP , and FN are the number of false positive, true negative, true positive and false positive respectively. The ROC curve of perfect classifier have the area under the ROC curve (AUC) of 1. The perfect curve rise steeply from bottom left to top left and move toward top right. The AUC is calculated using trapezoidal rule as:

$$AUC = \frac{1}{2} \sum_{k=1}^n (X_k - X_{k-1})(Y_k - Y_{k-1}) \quad (3.6)$$

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Random gene pair semantic similarity distribution

Biologically, functionally similar genes are far less common than those that are functionally dissimilar. Thus, a set of randomly pair of genes should have semantic similarity score close to zero ([Gan \(2014\)](#)). A good semantic similarity measures should reflect this, and should not overestimate the semantic similarity of set of random pairs, i.e. have semantic similarity score away from zero for set of random pairs. We follow the same approach as [Gan \(2014\)](#) to calculate the distribution of semantic similarity score of set of random pair of genes. We generated 100000 random pairs, and calculate the average score of the semantic similarity of the random pairs.

Table [4.1](#) shows the distribution of semantic similarity average score of random pairs for different methods. When comparing the average score across three ontologies, in most of the measures, BP ontology produces the minimum average score while CC ontology produces the maximum average score. For example, the average score of random pair combination of TopoICSim measure is lowest in BP (0.2), but for Lord Measures the lowest average score is in CC ontology (0.031). The highest scores in CC ontology for most measures might indicate that many of the random pairs produce high semantic similarity score. This is reasonable since the number of term in CC ontology is the lowest among three ontology. Thus the probability of two pairs of gene share common terms in CC are higher than in BP and MF. As a result the average similarity score is higher than BP and MF. The number of GO terms in BP, MF, and CC ontologies are 31024, 11940, and 4379 respectively.

Table 4.1: The average similarity score of 10000 random pair genes

Methods	BP	CC	MF
TopoICSim	0.200	0.432	0.386
Lord	0.043	0.031	0.047
Wang	0.329	0.594	0.565
RSS	0.463	0.612	0.556
CAS	0.005	0.001	0.002
simGIC	0.003	0.050	0.060
cos-bsc	0.007	0.107	0.155
GAN	0.005	0.075	0.075
our	0.009	0.092	0.074

4.2 Metabolic pathway membership prediction

We evaluate the performance of our method in predicting the membership of genes in KEGG metabolic pathway. The main idea behind this is that the genes in the same metabolic pathway are performing more similar biological process than the genes in different pathways ([Ehsani & Drabløs \(2016\)](#)).

We compare the performance of our method against other methods by using ROC analysis. Table 4.2 and figure 4.1 show ROC score and ROC curve for pathway membership prediction using our method, in comparison to using a suite of different ontology-based gene similarity methods mentioned in the Related Works. Because GO has three separate hierarchies: BP, CC, and MF, we evaluate prediction performance for each hierarchy. In general, the best result are obtained for all methods when BP ontology is used in calculating gene similarity, followed by either CC or MF depends on the methods used. This pattern is consistent with our intuition as metabolic pathways are better characterized as biological processes (BP). It is clear that our method achieves the best performance with a ROC score of 0.937, from using BP ontology. However, for CC and MF ontology, our method is not the best performance.

Since the prediction accuracy of our method is high, it is interesting to see whether our method is applicable in predicting the gene membership in the pathways for gene or gene product that has no annotations, or in other word for predicting the

membership of unknown gene or gene products. However, our method only applicable when the annotations are available for the gene. When calculating the semantic similarity between two genes, if the query gene vector is zero vector, then the cosine similarity for this pair will be zero. The query gene then will be categorized as not a member of metabolic pathway in question.

Table 4.2: The area under the curve ROC of semantic similarity measure for BP, MF and CC ontology

Methods	BP	CC	MF
TopoICSim	0.869	0.715	0.719
Lord	0.848	0.688	0.803
Wang	0.867	0.729	0.725
RSS	0.864	0.713	0.716
CAS	0.910	0.795	0.856
simGIC	0.922	0.795	0.746
cos-bsc	0.914	0.774	0.656
Gan	0.922	0.783	0.774
our	0.937	0.783	0.821

Figure 4.1: ROC curve plot for gene membership prediction

(a)

(b)

Figure 4.1: continued

(a)

ROC curve plot of KEGG genes membership prediction for (a) BP (b) MF, and (c) CC ontology

We then further evaluated the improvement of our measure against other methods. Table 4.3 shows that compared to other methods along with p-value from t-test on the 10-fold cross-validation results, our method can achieve better performance than most of measures in all ontologies. for example, our method outperformed other measures up to 11% when it is compared against Lord method. Similarly, in CC and MF ontologies our measure can outperform almost up to 14% (comparison against Lord in CC ontology) and 25% (comparison against cosine basic) respectively. However, our measures unable to outperform the CAS in both CC and MF ontology, as well as simGIC and GAN in CC ontology. The p-value score also shows that almost all differences are statistically significant (p value less than 0.05) with exception in CC ontology where there is no statistically significant improvement between our method and Gan.

Table 4.3: Improvement of our measure against other similarity measures in BP, CC and MF ontology (in percentage)

Methods	BP	p-value	CC	p-value	MF	p-value
TopoICSim	7.800	<2e-16	9.505	<2e-16	14.145	<2e-16
Lord	10.559	<2e-16	13.792	<2e-16	2.261	<2e-16
Wang	8.141	<2e-16	7.316	<2e-16	13.165	<2e-16
RSS	8.438	<2e-16	9.718	<2e-16	14.570	<2e-16
CAS	3.013	<2e-16	-1.575	<2e-16	-4.169	<2e-16
simGIC	1.636	<2e-16	-1.531	<2e-16	10.075	<2e-16
cos-bsc	2.563	<2e-16	1.189	4.27E-05	25.207	<2e-16
Gan	1.671	<2e-16	-0.030	0.915	5.992	<2e-16

While many ontology-based similarity methods evaluate their performance by predicting gene products membership in metabolic pathway, typically they only choose a subset of the metabolic pathways from KEGG database. For example, TopoICSim selected only 13 metabolic pathways with the number of genes in each pathway fewer than 40. In this project, we evaluated the performance of pathway membership prediction for all metabolic pathways in KEGG database, and we further evaluate the performance with different set of pathways based on the number genes in the pathways. Specifically, we divided the pathways into three groups, 1) pathways that contain

less than 100 genes, 2) pathways that contain between 101 to 200 genes, and 3) pathway that contain more than 200 genes.

Table 4.4 shows that in general, as the number of genes in the pathway increase, the performance of the methods decrease. Most methods perform best in pathways that contain less than 100 genes. For example in BP ontology, the ROC scores of methods in the less than 100 genes pathways ranges between .873 (Lord) to .947 (our method), while in pathways that contain 101 to 200 genes and more than 200 genes the ROC scores range between .820 (TopoICSim) to .919 (our method) and .782 (Lord) to .903 (our method) respectively. In comparison among three ontologies, most of the measures perform best when using BP ontology. For example, in the group of pathways that contain less than 100 genes, the ROC scores of BP, CC and MF are ranging between .873 (Lord) to .947 (our method), .720 (RSS) to .807 (our method) and .664 (cos-basic) to .830 (Lord and our method) respectively. In BP ontology, our measure outperformed other measures for all pathway groups. It is clearly seen in the table that our method is one of the top performances in all categories.

Table 4.4: Performance of measures in predicting genes membership of metabolic pathways that contains different number of genes.

Methods	BP			CC			MF		
	<100	101-200	>200	<100	101-200	>200	<100	101-200	>200
TopoICSim	0.893	0.820	0.816	0.738	0.666	0.653	0.731	0.687	0.728
Lord	0.873	0.794	0.782	0.723	0.617	0.583	0.830	0.741	0.760
Wang	0.886	0.823	0.840	0.744	0.696	0.712	0.750	0.665	0.701
RSS	0.883	0.823	0.833	0.720	0.699	0.701	0.731	0.679	0.712
CAS	0.922	0.888	0.867	0.801	0.783	0.776	0.846	0.879	0.881
simGIC	0.926	0.917	0.902	0.816	0.752	0.740	0.753	0.732	0.722
cos-bsc	0.920	0.904	0.884	0.794	0.729	0.725	0.664	0.637	0.636
GAN	0.926	0.916	0.900	0.806	0.736	0.719	0.776	0.772	0.764
our	0.947	0.919	0.903	0.807	0.734	0.715	0.830	0.801	0.799

Since genes in the same metabolic pathways are assumed to have similar biological process, we examined this by exploring the mean value of pairwise semantic similarity score between genes in the pathway (inside-inside genes) as well as the semantic similarity score between genes outside the pathway and genes inside the pathway (outside-inside genes). We reasoned that the inside-inside genes should have a higher similarity score than the similarity score of inside-outside genes since the inside-inside genes are more similar to each other than to the outside genes. Therefore, we can calculate the ratio of similarity scores of inside-inside genes against inside-outside genes to check whether the assumption holds. The ratio should be bigger than 1 if the inside-inside genes are more similar to each other than to outside genes.

Table 4.5 show the ratio of semantic similarity scores between inside-inside genes to inside-outside genes. All methods show the ratio bigger than 1, which indicates that the genes inside the pathway have higher similarity scores between them. Amongst the method being compared, CAS shows the highest ratio for all ontologies.

Table 4.5: The ratio of semantic similarity scores

Methods	BP	CC	MF
TopoICSim	1.666	1.201	1.362
Lord	1.746	1.341	1.942
Wang	1.329	1.138	1.101
RSS	1.254	1.140	1.207
CAS	3.672	3.892	7.828
simGIC	2.894	1.301	1.337
cos-bsc	2.636	1.230	1.203
GAN	2.636	1.230	1.203
our	2.271	1.222	1.459

Other semantic similarity based methods, such as those are mainly based on the structure of the graph, are limited to use only one of the three semantic similarities. The example of those methods are RSS and CAS. These methods calculate the similarity by including the structure of the graph. Since gene ontology consists a separate three ontologies, i.e. BP, MF and CC ontologies, each with its own tree structure and root node, combining all three ontologies to calculate the semantic similarity of two genes is

difficult. Our method on the other hand are based on vector. Each gene is represented as a vector of GO terms. If we want to use the three ontologies, the simplest approach is by combining the tree ontologies into a single vector to represent a gene. Table 4.6 show the ROC score for combine ontologies in 3 methods. The ROC scores of combine ontologies are lower than the score for BP ontology, however higher than the other two ontologies in three methods. For example, the ROC score of our method by using combined ontologies are .933 which is lower than ROC score for BP ontology (.937) but higher than ROC score for CC (.783) and MF (.821) ontologies. The difference of ROC score between BP ontology only and three ontologies, however, are very small, for cos-bsc, GAN and our methods, the differences are 0.074, 0.002, and 0.004 respectively. This pattern still consistent with our intuition that metabolic pathways are better characterized by BP ontology than other ontologies, although the application of all ontologies may be consider to represent the gene better as it includes all terms in three ontologies.

Table 4.6: ROC score for combine ontologies

Method	AUC
cos-bsc	0.840
GAN	0.920
our	0.933

We also compare the performance of our method by measuring the running time. For this purpose, we randomly chose 4 pathways, i.e hsa00072, hsa00532, hsa04979, hsa05169, of different sizes that contain 10, 20, 50, and 200 genes respectively. Table 4.7 shows that the running time of our method outperformed others. Methods that based on pairwise approach, i.e calculating pairwise semantic similarity of two terms between two pairs of genes, such as Lord, RSS or CAS are fast when the number of the genes are small. The time complexity of calculating the gene similarity in these methods are quadratic $O(nm)$, where n and m are the number of terms of the two genes. Calculating the similarity of genes in the pathways are not problem when the number of gene members in the pathway are small. However, it will need considerably more time

to calculate the semantic similarity when the number of gene members of the pathway are big. In addition, some methods, especially methods that used the structure of the graph such as RSS, calculating of pair of terms depends on the complexity of the tree. The time complexity of calculating the similarity of the terms is linear with the depth of the tree or the distance between the two terms is logarithmic $O(n \log n)$ where n is the depth of the graph or the length between the two terms depending what approach used in the methods.

On the contrary, vector based approach only need to build the gene vector once. Once the vector is build, the similarity between the two genes can be calculated directly by vector operation. Thus for this kind of methods, the time complexity is linear $O(n)$ where n is the number of the pair of genes.

Thus when comparing the running time for different methods, we can see clearly in table 4.7 that our method outperformed other methods. When compare to other vector based method, such as GAN, the running time is equal. Out method running time is linear as the number of pathway size, however other methods, especially the pairwise based method time requirement increase drastically when the pathways size getting bigger.

Table 4.7: Running time of similarity measures in seconds

Methods	hsa00072(10)	hsa00532(20)	hsa04979(50)	hsa05169(200)	total
TopoICSim	9.80	10.65	296.68	3734.78	4051.91
Resnik	0.06	0.27	7.89	2119.36	2127.57
Wang	0.05	0.25	8.64	1685.79	1694.73
RSS	119.69	547.79	9446.41	71 544.76	81 658.65
CAS	218.82	309.55	2313.55	11 126.51	13 968.43
simGIC	0.71	1.67	5.36	106.56	114.31
cos basic	0.79	0.85	1.45	3.12	6.21
cos	0.63	1.43	1.99	4.44	8.49
cos ic	0.57	0.71	1.54	3.67	6.49

We calculated the running time on Linux machine with Intel Xeon ES-2680 2.7GHz and 32GB of RAM.

4.3 Distribution of high semantic similarity score

Our method applies cosine similarity to determine the similarity between 2 genes which relies on semantic terms, i.e GO terms. If two genes have the same annotations, the similarity score of the two genes are 1. Although semantically the two genes are similar, the two genes may not always be member of the same pathway. Therefore, we explore the distribution of the similarity score of all possible gene pairs in the BP ontology in regard to the gene membership to the pathways. For each pair of genes, the possibilities of the genes in regard of the member of the pathways are: (1) both of the genes are member of the pathway(s) whether they are belong to the same pathway or different pathways (in-pathway), (2) or not member of any pathway (out-pathway), or (3) only one of the genes are belong to pathway(s) (in-out-pathway).

Table 4.8 shows the number of the pairs in those three categories. It shows that most of the pairs have both genes that are not member of any pathway, except where the similarity score are 1 or score between .1 and .2. This is as expected since the total number of the genes in the KEGG pathway are 7453 genes, which is only 12% of the total human genes listed in NCBI. However, for low semantic similarity score, especially score between 0.1 to 0.2, the number of pairs that both of the genes belong to pathways are bigger than any other type of pairs. In general, the number of both genes not belong to any pathway increases as the similarity score increases, while the number of pairs that the two genes or any one of the genes belong to pathway decrease as the similarity score increase.

For measuring the performance of our method, we used ROC analysis which based on ranking of the semantic similarity score. If the two genes being compared have high similarity score, the pairs will place itself in the higher ranking, and will be predicted to be member of the same pathway. For pairs that have semantic similarity score of 1, the in-pathway pairs are bigger than any other pairs categories. However, for most of the pairs in other range of semantic similarity, the number of the pairs that both of the genes are not the member of the pathway are the biggest.

For gene pairs that have identical annotation, it is likely that the two genes are

Table 4.8: Total number of pairs in regard of their gene's member status in the pathway

Similarity score	in-out	h		Total
		in	out	
sim = 1	1631 (5.6)	19337 (66.1)	8272 (28.3)	29240
.9 $\leq$ sim < 1	3024 (3.6)	2512 (3)	78664 (93.4)	84200
.8 $\leq$ sim < .9	3302 (6.8)	2637 (5.4)	42911 (87.8)	48850
.7 $\leq$ sim < 0.8	4372 (5.3)	30971 (37.7)	46916 (57)	82259
.6 $\leq$ sim < .7	17749 (16.8)	8708 (8.2)	79452 (75)	105909
.5 $\leq$ sim < .6	24047 (17.2)	16559 (11.8)	99314 (71)	139920
.4 $\leq$ sim < .5	54867 (22.4)	20114 (8.2)	170184 (69.4)	245165
.3 $\leq$ sim < .4	135260 (30.4)	30412 (6.8)	279715 (62.8)	445387
.2 $\leq$ sim < .3	361801 (38.5)	83528 (8.9)	495606 (52.7)	940935
.1 $\leq$ sim < .2	1211831 (46.3)	416528 (15.9)	988772 (37.8)	2617131

actually synonym genes, i.e. genes with multiple names, or belong to the same family member. Gene families are group of genes that are similar in nucleotide sequences or amino acid sequences and they also share similar cellular functions. These genes are commonly arise from genome duplication events ([Harris & Hofmann \(2015\)](#)). Where synonym genes refer to the same gene, genes in gene families may be different genes. Therefore, we try to map the genes in each pair to whether they are synonym or member of the same gene families. We found that the percentage of pair of genes belong to the same family member is quite high in all semantic similarity score, even for low semantic similarity score. The percentage for all score range are above 60% (figure 4.2). This indicates that the annotation for genes in the family genes can be very different, although it is widely accepted that family genes have similar cellular functions.

Figure 4.2: Distribution of gene family, interacting, and synonym genes in the pairs

(c)

Percentage of pairs that both genes in the pair are the same family, are interacting genes, or are synonym genes

Chapter 5

CONCLUSION

Metabolic pathway is important in understanding the underlying mechanisms in the biological process and cellular functions of cell. Predicting the membership of the gene to pathways in which the gene participate can greatly help to understand the gene function in the pathways. In this work, we developed a method for pathway membership inference based gene ontology (GO) similarity between a query protein and proteins that are known to the members of a given pathway. We use human metabolic pathway and human gene annotation dataset. We demonstrated that, by replacing binary vector of GO term annotation for a gene with the information content of individual GO terms and incorporating GO hierarchy with ancestor nodes that are directly present in gene annotation, we can create information rich vector representation for a gene, which enables accurate pathway membership prediction even with a simple classifier based just on positive training example and cosine similarity, outperforming some more sophisticated methods both by prediction accuracy and running time. In our test, the performance of our method outperformed other methods when using BP ontology. While other methods that rely on the structure of the graph use the three ontologies separately since the three ontologies are separate graph each with its own root node, our method can integrate the three ontologies easily. The performance of our method when using combine ontology smaller than when BP ontology use alone but higher than when using CC or MF ontologies. These result indicates that BP ontology is better characterize metabolic pathways than other ontology.

As our method only use a discriminative classifier that calculate the similarity between the set of proteins in incomplete pathway and query pathway, as future work use of a discriminative classifier that can take advantage of both positive and negative

training examples, such as support vector machines, will be explored. In addition, the use of dataset other than human may be interesting to be explored to see if the same result can be generalized. Another interesting future direction is to develop method to infer unannotated gene products membership in metabolic pathways since our method has limitation to only able to predict gene membership in the pathway only for genes that has GO annotation.

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Appendix

KEGG METABOLIC PATHWAY

Table A.1: List of KEGG Metabolic Pathway

KEGG pathway	Pathway's name	Number of genes
hsa00010	Glycolysis / Gluconeogenesis	68
hsa00020	Citrate cycle (TCA cycle)	30
hsa00030	Pentose phosphate pathway	30
hsa00040	Pentose and glucuronate interconversions	34
hsa00051	Fructose and mannose metabolism	33
hsa00052	Galactose metabolism	31
hsa00053	Ascorbate and aldarate metabolism	27
hsa00061	Fatty acid biosynthesis	13
hsa00062	Fatty acid elongation	30
hsa00071	Fatty acid degradation	44
hsa00072	Synthesis and degradation of ketone bodies	10
hsa00100	Steroid biosynthesis	19
hsa00120	Primary bile acid biosynthesis	17
hsa00130	Ubiquinone and other terpenoid-quinone biosynthesis	11
hsa00140	Steroid hormone biosynthesis	59
hsa00190	Oxidative phosphorylation	133
hsa00220	Arginine biosynthesis	21
hsa00230	Purine metabolism	174
hsa00232	Caffeine metabolism	5
hsa00240	Pyrimidine metabolism	101
hsa00250	Alanine, aspartate and glutamate metabolism	35
hsa00260	Glycine, serine and threonine metabolism	40
hsa00270	Cysteine and methionine metabolism	45
hsa00280	Valine, leucine and isoleucine degradation	48
hsa00290	Valine, leucine and isoleucine biosynthesis	4
hsa00310	Lysine degradation	59
hsa00330	Arginine and proline metabolism	50
hsa00340	Histidine metabolism	23

hsa00350	Tyrosine metabolism	36
hsa00360	Phenylalanine metabolism	17
hsa00380	Tryptophan metabolism	40
hsa00400	Phenylalanine, tyrosine and tryptophan biosynthesis	5
hsa00410	beta-Alanine metabolism	31
hsa00430	Taurine and hypotaurine metabolism	11
hsa00440	Phosphonate and phosphinate metabolism	6
hsa00450	Selenocompound metabolism	17
hsa00471	D-Glutamine and D-glutamate metabolism	5
hsa00472	D-Arginine and D-ornithine metabolism	1
hsa00480	Glutathione metabolism	56
hsa00500	Starch and sucrose metabolism	36
hsa00510	N-Glycan biosynthesis	49
hsa00511	Other glycan degradation	18
hsa00512	Mucin type O-glycan biosynthesis	31
hsa00514	Other types of O-glycan biosynthesis	22
hsa00515	Mannose type O-glycan biosynthesis	23
hsa00520	Amino sugar and nucleotide sugar metabolism	48
hsa00524	Neomycin, kanamycin and gentamicin biosynthesis	5
hsa00531	Glycosaminoglycan degradation	19
hsa00532	Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate	20
hsa00533	Glycosaminoglycan biosynthesis - keratan sulfate	14
hsa00534	Glycosaminoglycan biosynthesis - heparan sulfate / heparin	24
hsa00561	Glycerolipid metabolism	61
hsa00562	Inositol phosphate metabolism	74
hsa00563	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	25
hsa00564	Glycerophospholipid metabolism	97
hsa00565	Ether lipid metabolism	47
hsa00590	Arachidonic acid metabolism	63
hsa00591	Linoleic acid metabolism	29
hsa00592	alpha-Linolenic acid metabolism	25
hsa00600	Sphingolipid metabolism	47
hsa00601	Glycosphingolipid biosynthesis - lacto and neolacto series	27
hsa00603	Glycosphingolipid biosynthesis - globo and isoglobo series	15
hsa00604	Glycosphingolipid biosynthesis - ganglio series	15
hsa00620	Pyruvate metabolism	39
hsa00630	Glyoxylate and dicarboxylate metabolism	30
hsa00640	Propanoate metabolism	32
hsa00650	Butanoate metabolism	28
hsa00670	One carbon pool by folate	20
hsa00730	Thiamine metabolism	16
hsa00740	Riboflavin metabolism	8

hsa00750	Vitamin B6 metabolism	6
hsa00760	Nicotinate and nicotinamide metabolism	30
hsa00770	Pantothenate and CoA biosynthesis	19
hsa00780	Biotin metabolism	3
hsa00785	Lipoic acid metabolism	3
hsa00790	Folate biosynthesis	26
hsa00830	Retinol metabolism	67
hsa00860	Porphyrin and chlorophyll metabolism	42
hsa00900	Terpenoid backbone biosynthesis	22
hsa00910	Nitrogen metabolism	17
hsa00920	Sulfur metabolism	9
hsa00970	Aminoacyl-tRNA biosynthesis	66
hsa00980	Metabolism of xenobiotics by cytochrome P450	76
hsa00982	Drug metabolism - cytochrome P450	72
hsa00983	Drug metabolism - other enzymes	79
hsa01040	Biosynthesis of unsaturated fatty acids	23
hsa02010	ABC transporters	44
hsa03008	Ribosome biogenesis in eukaryotes	105
hsa03010	Ribosome	153
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