Contrastive Multiview Coding for Enzyme-Substrate Interaction Prediction

Apurva Kalia\textsuperscript{1}, Dilip Krishnan\textsuperscript{2}, Soha Hassoun\textsuperscript{1,3}
\textsuperscript{1}Department of Computer Science, Tufts University
\textsuperscript{2}Google Research
\textsuperscript{3}Department of Chemical and Biological Engineering, Tufts University
Apurva.Kalia@tufts.edu, dilipkay@google.com, Soha.Hassoun@tufts.edu

Introduction
Characterizing enzymes through sequencing, annotation, homology, and metabolic network analysis has provided insights into cancer, inborn errors of metabolism, human microbiome co-metabolism, and has facilitated transforming microorganisms into efficient cellular factories producing flavonoids and therapeutics products. Although traditionally assumed specific (transforming a single substrate), many enzymes are promiscuous and act on substrates other than those targeted by evolution alone \cite{1, 2}. Despite progress in assigning functional properties to enzyme sequences \cite{3-7} and extensively cataloging enzymatic activities through organism and enzyme databases, insufficient characterization of enzymes fundamentally limits our understanding of metabolism and hinders the development of many biological and biomedical applications.

This work explores the problem of predicting enzyme-substrate interactions. At some level, this problem is similar to the protein-ligand interaction prediction problem. While techniques based on deep learning \cite{8-10} for recent reviews) can be leveraged to solve the enzyme-substrate interaction problem, the two problems have different aims. Protein-ligand interaction analysis aims to identify side effects of drugs and/or repurpose existing drugs to inhibit/activate other proteins whereas in the enzyme-substrate interaction problem, we are interested in a large set of enzymes acting on a diverse set of molecules for a wide range of biological discovery and synthetic biology applications, and not only on drug-like molecules or a narrow set of health-related proteins. The drug-protein datasets (e.g., Davis \cite{11}, Metz \cite{12}, KIBA \cite{13}) are therefore not representative of enzymes, substrates, nor their interactions. Importantly, enzymatic datasets derived from biochemical reactions on natural (to living cells) and non-natural substrates provide rich auxiliary data that can enhance enzyme-substrate interaction prediction in ways not possible with the protein-drug datasets.

In this paper, we propose to leverage auxiliary data unique to enzymatic interactions to improve enzyme-substrate interaction prediction. Specifically, we apply Contrastive Multiview Coding (CMC) to the interaction prediction problem. CMC has been applied to image recognition tasks with great success \cite{14, 15}. CMC aims to learn view-invariant representations of the data. CMC therefore learns representations such that views of the same data (congruent views) map to nearby points in the embedding space when compared to views of different data (incongruent views). We identify congruent views of enzymatic-substrate interactions. Importantly, we show that data stratification gives rise to differing congruent views, a discovery that is uniquely applicable to enzymatic interaction data. We evaluate the impact of data stratification and the congruent views in achieving enhanced performance for the enzyme-substrate interaction prediction problem.

Methods

Multiviews of enzymatic interaction data. We investigate curated enzymatic interactions for natural substrates that are catalogued in the KEGG database for a large set of organisms \cite{16-18}. Each enzymatic reaction is associated with substrates and products, and enzyme sequences from different organisms that catalyze the reaction. The KEGG Reaction Classes (RCLASS) database catalogues a listing of biotransformations (e.g., methylation, decarboxylation, and others) between a substrate and product. Each RCLASS is associated with one or more substrate-product pairs, and a list of enzymatic reactions and therefore the sequences catalyzing the reactions. We therefore utilize the following three views of the enzyme-substrate interaction data:

- **View 1 (V\textsubscript{1}): Compound-compound pairs** representing a substrate-product pair for a given biochemical interaction. Such pairs share structural similarities to varying degrees depending on the type of enzymatic transformation.
- **View 2 (V\textsubscript{2}): Substrate-enzyme and product-enzyme pairs** representing enzymes acting on both substrate and product as reactions are reversible.
- **View 3 (V\textsubscript{3}): FASTA sequences** corresponding to enzyme sequences catalyzing a given reaction.
Importantly, the enzymatic data be stratified per reaction, per RCLASS, or per enzyme classification (EC) numbers, giving rise to different groupings on the data views. For example, when stratifying per RCLASS, the congruent views for compound-compound pairings are for all substrate-product pairings listed for a specific RCLASS. When stratifying per reaction, the congruent views of compound-compound pairings are across substrate-product pairings listed for each reaction. When stratifying per EC number, congruent views for compound-compound pairings are for all reactions catalyzed by the same EC number.

**Modeling using Contrastive Multiview Coding.**
Using CMC the model learns a representation where mutual information in congruent data is maximized. If $V_1$ and $V_2$ are two views of the data, and $\{v_i^1, v_i^2\}_{i=1}^N$ and $\{v_i^1, v_i^2\}_{i\neq j}^N$ are congruent and incongruent data pairs respectively, then the contrastive loss is defined as in [14] to optimize the log softmax of the cosine similarity between the congruent pair $\{v_i^1, v_i^2\}$ calculated over the sum of cosine similarities between all incongruent pairs $\{v_i^1, v_j^2\}$ of data – normalized using a hyperparameter over the range of the cosine similarity. For $N$ views, the total loss is calculated over all the pairwise losses and is given by $\sum_{1 \leq i < j \leq N} L(V_i, V_j)$ [14].

Similar to the original presentation of CMC, we train our model in two steps. In the contrastive training step, we train a Graph Neural Network based encoder for the molecules and a Convolutional Neural Network based encoder for the enzyme for all the views. In the interaction prediction step, the outputs of the encoders learnt in the contrastive training step are concatenated and passed through MLP layers for a binary prediction.

**Results**
From the KEGG database we curated 6,892 unique reactions. These reactions capture 119,350 unique enzyme-compound interaction pairs, 6,791 unique compound-compound pairs, and 20,074 unique FASTA sequences. For the contrastive learning step, this data is stratified either into 6,892 Reactions, 2,355 RCLASSes or 3,755 EC numbers. For the interaction prediction step, the 119,350 known enzyme-compound pairs were augmented with randomly generated pairs (negatives). This set was divided into 8:1:1 for training, validation, and test, keeping the test set at 1:1 positive to negative ratio. We also expanded the test set to create a harder test set with 6x more randomly generated negative pairs, and an “Unseen test set”, which is a subset of the test set with only unseen sequences and/or unseen molecules.

We highlight some of the results (Table 1). First, among the various stratification methods, reaction stratification achieves the best AP performance on the (1:1), (1:6), and unseen test sets. Second, the improvement over baseline (no contrastive learning) is maximum for the test set with 1:6 ratio, where CMC achieves an AP increase of 61% over baseline AP of 0.59. Third, our ablation studies for the reaction stratification shows that all three data views are useful, but that View 1 (compound-compound pairings) gives the highest performance contribution – as seen by the largest decrease in AP when $V_1$ is removed.

| Model | Test | Test(1:6) | Test Unseen |
|-------|------|-----------|-------------|
| a Baseline | 0.92 | 0.59 | 0.87 |
| b1 RCLASS Strata $V_1, V_2, V_3$ | 0.99 | 0.90 | 0.94 |
| b2 EC Strata $V_1, V_2, V_3$ | 0.99 | 0.93 | 0.98 |
| b3 Reaction strata $V_1, V_2, V_3$ | **0.99** | **0.95** | **0.98** |
| c1 Reaction strata $V_1, V_2$ | 0.98 | 0.85 | 0.93 |
| c2 Reaction strata $V_2, V_3$ | 0.96 | 0.73 | 0.88 |
| c3 Reaction strata $V_1, V_3$ | 0.98 | 0.88 | 0.94 |

**Table 1.** AP results for: (a) for GNN-CNN baseline interaction model, (b1-b3) three different stratification strategies, and (c1-c3) view-ablation studies for the reaction-based stratification.

**Conclusion**
This work applies CMC to the problem of enzyme-molecule interaction prediction. We explore how views and stratification of the enzyme-molecule interaction data affects performance. Our best model achieves a 61% improvement in Average Precision over a baseline with no contrastive learning. While we focused on the KEGG dataset, it is possible to apply CMC to other protein-ligand interaction prediction datasets [19-21] by judiciously identifying multiviews of the underlying data or by generating adversarial multiviews [22].

**Funding.** NIH R01GM132391 and NSF 1909536.
References

1. D’Ari, R. and J. Casadesús, *Underground metabolism*. Bioessays, 1998. **20**(2): p. 181-6.
2. Khersonsky, O. and D.S. Tawfik, *Enzyme promiscuity: a mechanistic and evolutionary perspective*. Annu Rev Biochem, 2010. **79**: p. 471-505.
3. Brown, S.D. and P.C. Babbitt, *New insights about enzyme evolution from large scale studies of sequence and structure relationships*. J Biol Chem, 2014. **289**(44): p. 30221-8.
4. Martínez Cuesta, S., et al., *The Classification and Evolution of Enzyme Function*. Biophys J, 2015. **109**(6): p. 1082-6.
5. Merkl, R. and R. Sterner, *Ancestral protein reconstruction: techniques and applications*. Biol Chem, 2016. **397**(1): p. 1-21.
6. Baier, F., J.N. Copp, and N. Tokuriki, *Evolution of Enzyme Superfamilies: Comprehensive Exploration of Sequence-Function Relationships*. Biochemistry, 2016. **55**(46): p. 6375-6388.
7. Finn, R.D., et al., *InterPro in 2017-beyond protein family and domain annotations*. Nucleic Acids Res, 2017. **45**(D1): p. D190-D199.
8. Bagherian, M., et al., *Machine learning approaches and databases for prediction of drug–target interaction: a survey paper*. Briefings in bioinformatics, 2021. **22**(1): p. 247-269.
9. Chen, X., et al., *Drug–target interaction prediction: databases, web servers and computational models*. Briefings in bioinformatics, 2016. **17**(4): p. 696-712.
10. Du, X., et al., *Insights into protein–ligand interactions: mechanisms, models, and methods*. International journal of molecular sciences, 2016. **17**(2): p. 144.
11. Davis, M.I., et al., *Comprehensive analysis of kinase inhibitor selectivity*. Nature biotechnology, 2011. **29**(11): p. 1046.
12. Metz, J.T., et al., *Navigating the kinome*. Nat Chem Biol, 2011. **7**(4): p. 200-2.
13. Tang, Z., C.C. Roberts, and A.C. Chia-en, *Understanding ligand-receptor non-covalent binding kinetics using molecular modeling*. Frontiers in bioscience (Landmark edition), 2017. **22**: p. 960.
14. Tian, Y., D. Krishnan, and P. Isola. *Contrastive multiview coding*. in *Computer Vision–ECCV 2020: 16th European Conference, Glasgow, UK, August 23–28, 2020, Proceedings, Part XI*. 2020. Springer.
15. Oord, A.v.d., Y. Li, and O. Vinyals, *Representation learning with contrastive predictive coding*. arXiv preprint arXiv:1807.03748, 2018.
16. Kanehisa, M. and S. Goto, *KEGG: kyoto encyclopedia of genes and genomes*. Nucleic Acids Res, 2000. **28**(1): p. 27-30.
17. Kanehisa, M., et al., *KEGG: integrating viruses and cellular organisms*. Nucleic Acids Res, 2020.
18. Kanehisa, M., *Toward understanding the origin and evolution of cellular organisms*. Protein Sci, 2019. **28**(11): p. 1947-1951.
19. Gilson, M.K., et al., *BindingDB in 2015: a public database for medicinal chemistry, computational chemistry and systems pharmacology*. Nucleic acids research, 2016. **44**(D1): p. D1045-D1053.
20. Placzek, S., et al., *BRENDA in 2017: new perspectives and new tools in BRENDA*. Nucleic acids research, 2016: p. gkw952.
21. Richard, A.M., et al., *ToxCast chemical landscape: paving the road to 21st century toxicology*. Chemical research in toxicology, 2016. **29**(8): p. 1225-1251.
22. Tian, Y., et al., *What makes for good views for contrastive learning?* arXiv preprint arXiv:2005.10243, 2020.