



In vitro turnover numbers do not reflect in vivo activities of yeast enzymes

Downloaded from: <https://research.chalmers.se>, 2025-12-08 23:25 UTC

Citation for the original published paper (version of record):

Chen, Y., Nielsen, J. (2021). In vitro turnover numbers do not reflect in vivo activities of yeast enzymes. *Proceedings of the National Academy of Sciences of the United States of America*, 118(32). <http://dx.doi.org/10.1073/pnas.2108391118>

N.B. When citing this work, cite the original published paper.

In vitro turnover numbers do not reflect in vivo activities of yeast enzymes

Yu Chen^a and Jens Nielsen^{a,b,c,1}

^aDepartment of Biology and Biological Engineering, Chalmers University of Technology, SE412 96 Gothenburg, Sweden; ^bNovo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK2800 Kgs. Lyngby, Denmark; and ^cBiInnovation Institute, DK2200 Copenhagen N, Denmark

Edited by James J. Collins, Massachusetts Institute of Technology, Cambridge, MA, and approved July 9, 2021 (received for review May 5, 2021)

Turnover numbers (k_{cat} values) quantitatively represent the activity of enzymes, which are mostly measured in vitro. While a few studies have reported in vivo catalytic rates (k_{app} values) in bacteria, a large-scale estimation of k_{app} in eukaryotes is lacking. Here, we estimated k_{app} of the yeast *Saccharomyces cerevisiae* under diverse conditions. By comparing the maximum k_{app} across conditions with in vitro k_{cat} we found a weak correlation in log scale of $R^2 = 0.28$, which is lower than for *Escherichia coli* ($R^2 = 0.62$). The weak correlation is caused by the fact that many in vitro k_{cat} values were measured for enzymes obtained through heterologous expression. Removal of these enzymes improved the correlation to $R^2 = 0.41$ but still not as good as for *E. coli*, suggesting considerable deviations between in vitro and in vivo enzyme activities in yeast. By parameterizing an enzyme-constrained metabolic model with our k_{app} dataset we observed better performance than the default model with in vitro k_{cat} in predicting proteomics data, demonstrating the strength of using the dataset generated here.

Saccharomyces cerevisiae | turnover number | k_{cat} | proteomics | metabolism

Enzyme turnover numbers, also termed k_{cat} values, are fundamental parameters that specify the maximum rates of enzymatic reactions and hence determine the rates of biological processes such as metabolism. Determining k_{cat} is therefore essential for quantitatively understanding, modeling, and engineering cells. Traditionally, k_{cat} values are measured in vitro, which might differ from the in vivo situation. In addition, the coverage of measured k_{cat} is poor even for well-studied organisms (1). To address these issues, an approach for estimating in vivo enzyme catalytic rates, also termed k_{app} values, is to use the equation

$$v = k_{\text{app}} \cdot E, \quad [1]$$

where v is the metabolic flux through the enzyme and E the enzyme abundance (2). This approach was used to estimate k_{app} for *Escherichia coli* using absolute proteomics and flux data from various sources (2, 3), and it was found that the maximum k_{app} values across conditions, defined as k_{max} values, correlate well with in vitro k_{cat} values in log scale (2).

Here, we generate a k_{app} dataset for *Saccharomyces cerevisiae* under diverse conditions. We analyze our dataset and correlate in vivo with in vitro enzyme activities in yeast. Finally, we compare the predictive power of an enzyme-constrained metabolic model using in vivo and in vitro kinetic data.

Results and Discussion

To generate the yeast k_{app} dataset we collected absolute proteomics data of *S. cerevisiae* under diverse conditions (4–7) (Dataset S1). The absolute protein abundance can be directly adopted as enzyme abundance in Eq. 1. Note that we did not consider enzyme complexes composed of multiple distinct subunits due to the difficulty in calculating the abundance of catalytic sites (2). To determine the metabolic flux in Eq. 1, we performed flux balance analysis (FBA), as done previously (2), using the latest genome-scale metabolic model (GEM) of *S. cerevisiae* Yeast8 (8) (SI Appendix).

Using the absolute proteomics and flux data, we calculated k_{app} for 358 metabolic reactions under 26 conditions (Dataset S2). By correlating the estimated k_{app} in log10 scale, we found that yeast k_{app} varied between conditions with the lowest R^2 being around 0.4 (Dataset S3). This is different from the findings for *E. coli*, where log-transformed k_{app} values correlate strongly across conditions with the lowest R^2 being above 0.9 (9).

By comparing k_{max} (Dataset S4), i.e., maximum k_{app} across all the studied conditions, with the corresponding in vitro k_{cat} (Dataset S5) we obtained a fairly weak correlation in log scale with $R^2 = 0.28$ for *S. cerevisiae* (Fig. 1A), which is much lower than that of *E. coli* ($R^2 = 0.62$) (2). A weak correlation was also reported for the plant *Arabidopsis thaliana* (10). By examining in vitro k_{cat} , we found that some were estimated using purified enzymes obtained through heterologous expression in *E. coli*, with the others being estimated from yeast extracts. We therefore divided the in vitro k_{cat} dataset into two groups, i.e., heterologous and homologous expression. We found that there was no correlation for the heterologous expression group (Fig. 1B), suggesting that in vitro k_{cat} values obtained through heterologous expression poorly represent in vivo catalytic rates of yeast enzymes. This might be due to the lack of natural posttranslational modifications (PTMs) in the expression organism, which could regulate enzyme activity. Indeed, we found that 27 out of the 29 reactions have reported PTMs on the enzymes (Dataset S6), indicating that these PTMs could functionally affect enzyme activity (11). In the homologous expression group we observed an improved correlation of $R^2 = 0.41$ in log scale (Fig. 1C) and thus identified the data obtained through heterologous expression as the main source of deviations.

To evaluate how uncertainties in the FBA-based flux may impact our dataset we first investigated the effect of flux variability on the estimated k_{max} (SI Appendix). We found that less than 8% of k_{max} values could differ, due to flux variability, by more than one order of magnitude (Dataset S4), and after removing these data we found the correlation between in vivo and in vitro values in log scale to be almost unchanged, i.e., R^2 (all data) = 0.27, R^2 (heterologous data) = 0.12, and R^2 (homologous data) = 0.39. Second, we provided another set of k_{max} values (Dataset S7) estimated using unbiased flux random sampling (SI Appendix), which correlate strongly ($R^2 = 0.97$) with the FBA-based k_{max} values in log scale. To evaluate the effect of a single high outlier value due to protein measurement we also correlated in log scale the second largest k_{app} across all conditions with in vitro k_{cat} but found similar R^2 values, i.e., R^2 (all data) = 0.25, R^2 (heterologous data) = 0.1, and R^2 (homologous data) = 0.39. Moreover, by correlating k_{app} of

Author contributions: Y.C. and J.N. designed research; Y.C. performed research; Y.C. and J.N. analyzed data; and Y.C. and J.N. wrote the paper.

The authors declare no competing interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

¹To whom correspondence may be addressed. Email: jni@bii.dk.

This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2108391118/-DCSupplemental>.

Published August 2, 2021.

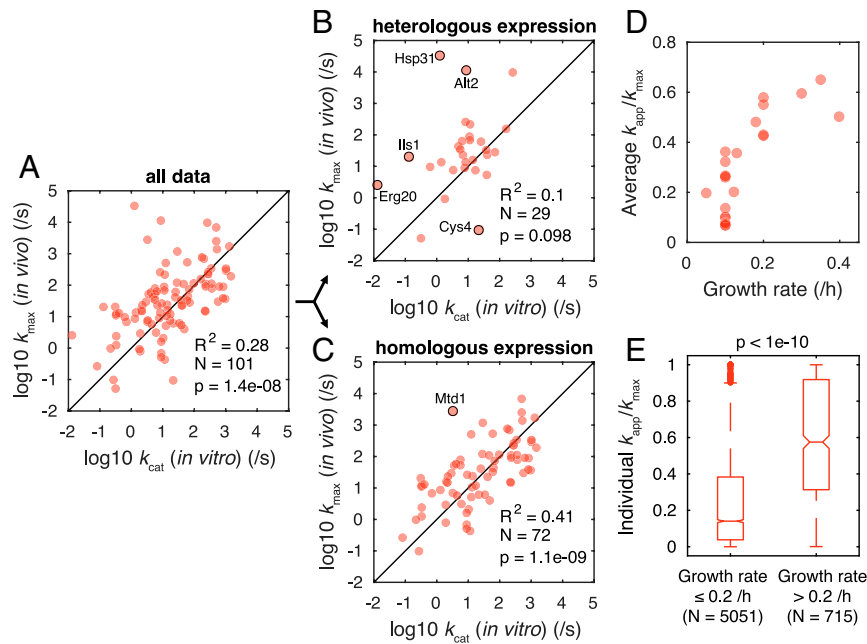


Fig. 1. Analysis of k_{app} and k_{max} of *S. cerevisiae*. Correlation in log scale between k_{max} and in vitro k_{cat} for all data points (A) and for the data points in which in vitro k_{cat} were measured using enzymes obtained through heterologous expression (B) and through homologous expression (C). The data points with deviations more than two orders of magnitude are labeled by the enzyme names. Student's t test was used to calculate P value for Pearson's correlation. (D) Change in average k_{app}/k_{max} of each condition with growth rate. (E) Comparison between k_{app}/k_{max} of individual reactions in two groups divided by a growth rate of 0.2/h. A two-sided Wilcoxon rank sum test was used to calculate P value.

each condition with in vitro k_{cat} we found most correlations to be poor in log scale (Dataset S8). A recent study, using another proteomic dataset and a different GEM, also showed a poor correlation in log scale of $R^2 = 0.27$ between yeast k_{app} and in vitro k_{cat} at one condition (12).

We analyzed the yeast k_{app} under various conditions based on the ratio of condition-specific k_{app} over k_{max} for each reaction. By plotting the average ratio for each condition versus the corresponding growth rate (μ), we observed an increasing trend (Fig. 1D), which is in line with findings for *E. coli* (13). Furthermore, we compared the ratio of individual reactions between slow ($\mu \leq 0.2/h$) and fast ($\mu > 0.2/h$) growth and found that k_{app} is significantly higher in faster-growing cells (Fig. 1E). We therefore conclude that k_{app} of yeast enzymes increase with growth rate. This suggests that proteome is more efficiently used at faster growth (13) and also indicates that growth could be controlled by efficiency of specific enzymes independent of conditions.

As turnover numbers are essential parameters in enzyme-constrained GEMs (ecGEMs) (9), we tested the use of k_{app} and in vitro k_{cat} in a yeast ecGEM ecYeast8 (8). We parameterized the model with 1) an assumed same k_{cat} for all enzymes, 2)

default in vitro k_{cat} in the original ecYeast8, 3) general k_{max} , and 4) μ -dependent k_{max} . Note that μ -dependent k_{max} is defined as the maximum k_{app} across the conditions under which growth rate is not greater than the given μ . To compare model performance, we used the model to predict proteomics data for growth on various carbon sources (14, 15), and we compared with the data not used to estimate our k_{app} dataset. We found that k_{max} outperforms the assumed k_{cat} and default in vitro k_{cat} (Fig. 2), confirming our estimation of k_{max} to be reliable. Notably, μ -dependent k_{max} can further improve the predictions (Fig. 2), meaning that it is more effective to use μ -dependent k_{max} values than condition-independent maximum values, which are adopted in most published ecGEMs (9). In addition, we found that default in vitro k_{cat} in the original ecYeast8 outperforms the assumed same k_{cat} for all enzymes (Fig. 2), meaning that it is still acceptable to use in vitro k_{cat} when k_{app} values are unavailable.

Overall, we present a k_{app} dataset of *S. cerevisiae* under various conditions, which can be used by ecGEMs for simulating the corresponding conditions. As k_{app} depends generally on growth rates rather than conditions (Fig. 1D), we believe that our μ -dependent

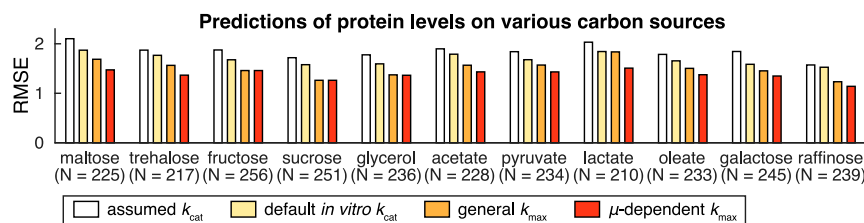


Fig. 2. Predictions of proteomics data on various carbon sources by ecYeast8 parameterized with an assumed same k_{cat} , default in vitro k_{cat} , general k_{max} , and μ -dependent k_{max} . Model performance is evaluated by root-mean-square error (RMSE) between predicted and measured protein levels on a log10 scale. N is the number of proteins with predicted nonzero concentrations by four parameterization strategies.

k_{\max} can be used to parameterize ecGEMs for predicting other conditions that are not involved in our dataset.

Materials and Methods

The metabolic fluxes were simulated using Yeast8 constrained by measurements. The absolute proteomics data were processed, i.e., the units of protein abundances were converted to millimoles per gram cell dry weight (gCDW). Details of all the materials and methods are provided in [SI Appendix](#).

Data Availability. The data and codes are available at https://github.com/SysBioChalmers/Yeast_kapp. All other study data are included in the article and/or supporting information.

ACKNOWLEDGMENTS. We acknowledge funding from the European Union's Horizon 2020 research and innovation program under grant agreement 686070. We also acknowledge funding from the Novo Nordisk Foundation (grant NNF10CC1016517).

1. D. Davidi, R. Milo, Lessons on enzyme kinetics from quantitative proteomics. *Curr. Opin. Biotechnol.* **46**, 81–89 (2017).
2. D. Davidi *et al.*, Global characterization of in vivo enzyme catalytic rates and their correspondence to in vitro k_{cat} measurements. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 3401–3406 (2016).
3. D. Heckmann *et al.*, Kinetic profiling of metabolic specialists demonstrates stability and consistency of in vivo enzyme turnover numbers. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 23182–23190 (2020).
4. P.-J. Lahtvee *et al.*, Absolute quantification of protein and mRNA abundances demonstrate variability in gene-specific translation efficiency in yeast. *Cell Syst.* **4**, 495–504.e5 (2017).
5. F. Di Bartolomeo *et al.*, Absolute yeast mitochondrial proteome quantification reveals trade-off between biosynthesis and energy generation during diauxic shift. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 7524–7535 (2020).
6. R. Yu *et al.*, Nitrogen limitation reveals large reserves in metabolic and translational capacities of yeast. *Nat. Commun.* **11**, 1881 (2020).
7. R. Yu, E. Vorontsov, C. Sihlbom, J. Nielsen, Quantifying absolute gene expression profiles reveals distinct regulation of central carbon metabolism genes in yeast. *eLife* **10**, e65722 (2021).
8. H. Lu *et al.*, A consensus *S. cerevisiae* metabolic model Yeast8 and its ecosystem for comprehensively probing cellular metabolism. *Nat. Commun.* **10**, 3586 (2019).
9. Y. Chen, J. Nielsen, Mathematical modelling of proteome constraints within metabolism. *Curr. Opin. Syst. Biol.* **25**, 50–56 (2021).
10. A. Küken, K. Gennermann, Z. Nikoloski, Characterization of maximal enzyme catalytic rates in central metabolism of *Arabidopsis thaliana*. *Plant J.* **103**, 2168–2177 (2020).
11. Y. Chen, J. Nielsen, Flux control through protein phosphorylation in yeast. *FEMS Yeast Res.* **16**, fow096 (2016).
12. A. D. Hanson *et al.*, The number of catalytic cycles in an enzyme's lifetime and why it matters to metabolic engineering. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2023348118 (2021).
13. E. J. O'Brien, J. Utrilla, B. O. Palsson, Quantification and classification of *E. coli* proteome utilization and unused protein costs across environments. *PLOS Comput. Biol.* **12**, e1004998 (2016).
14. J. A. Paulo, J. D. O'Connell, A. Gaun, S. P. Gygi, Proteome-wide quantitative multiplexed profiling of protein expression: Carbon-source dependency in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **26**, 4063–4074 (2015).
15. J. A. Paulo *et al.*, Quantitative mass spectrometry-based multiplexing compares the abundance of 5000 *S. cerevisiae* proteins across 10 carbon sources. *J. Proteomics* **148**, 85–93 (2016).