

enzyme-fit: automated nonlinear fitting of Michaelis-Menten and substrate-inhibition enzyme kinetics, with an explicit Lineweaver-Burk bias diagnostic

Karen Khachatryan^{*1}

¹Laboratory of Nanotechnology and Nanomaterials, Faculty of Food Technology, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Krakow, Poland

31 July 2026

Abstract

Determining the Michaelis constant (K_m) and maximum velocity (V_{max}) from initial-velocity vs. substrate-concentration data is one of the most common quantitative tasks in enzymology, food biochemistry, and related fields [Johnson and Goody, 2011]. Despite being known since at least the 1960s to give biased parameter estimates [Wilkinson, 1961], the double-reciprocal (Lineweaver-Burk) linearization is still routinely used because it can be fit by hand in a spreadsheet, whereas direct nonlinear regression requires dedicated software. **enzyme-fit** is a small, open-source Python command-line tool (with an optional desktop GUI) that fits both the classic Michaelis-Menten model and the Haldane substrate-inhibition model [Haldane, 1930] to initial-velocity data via nonlinear least squares, selects the best-describing model objectively by AIC, classifies the kinetic behavior (including the optimal substrate concentration under inhibition), and generates a report that displays the Lineweaver-Burk linearization alongside the nonlinear fit specifically to make their disagreement, and the recommended method, visually explicit.

Software: <https://github.com/karenkhachatryan-lab/enzyme-fit> **Software DOI:** [10.5281/zenodo.21724603](https://doi.org/10.5281/zenodo.21724603) **License:** MIT

1 Statement of need

Enzyme kinetics parameter estimation is commonly performed either by the Lineweaver-Burk double-reciprocal linearization in a spreadsheet, or in general-purpose statistical software without domain-specific conventions built in. The bias of the linearized approach is well documented: transforming v and $[S]$ to $1/v$ and $1/[S]$ and then applying ordinary least squares implicitly minimizes squared error in reciprocal space, which disproportionately weights the lowest-velocity (highest $1/v$, typically highest relative-noise) measurements [Wilkinson, 1961]. Direct nonlinear regression on the untransformed data does not have this problem, and has been the recommended method since the same era, yet the linearized method persists in routine use because no lightweight open tool made the comparison visible in a single figure. Substrate inhibition at high concentrations, common for many hydrolases and oxidases, is

^{*}ORCID: [0000-0001-7823-5406](https://orcid.org/0000-0001-7823-5406). Email: karen.khachatryan@gmail.com

a further complication: if undetected, a plain Michaelis-Menten fit systematically underestimates V_{max} and misrepresents the concentration range over which the enzyme is most active. To the author’s knowledge, no actively maintained, open-source tool exists that (1) fits both the Michaelis-Menten and Haldane substrate-inhibition models via nonlinear regression, (2) selects between them by an objective, AIC-based criterion (both models are fit on the same full dataset, so their AIC values are always directly comparable), (3) reports the optimal substrate concentration when inhibition is detected, and (4) displays the Lineweaver-Burk linearization next to the nonlinear fit as an explicit diagnostic of the bias described above, rather than as a fitting method in its own right. **enzyme-fit** addresses this gap with a compact, tested, MIT-licensed codebase intended for researchers who need consistent, reproducible enzyme kinetics analysis without adopting a full statistical modelling environment.

2 Functionality

Given a CSV file of (substrate, velocity) pairs (with optional per-point standard deviations), **enzyme-fit**:

- fits the Michaelis-Menten (2-parameter) and Haldane substrate-inhibition (3-parameter) models using `scipy.optimize.curve_fit` [Virtanen et al., 2020];
- reports R^2 , RMSE, AIC, and parameter standard errors for each model;
- selects the best-fitting model by AIC, both models being fit on the full dataset so the comparison is always valid;
- classifies the kinetic behavior: plain Michaelis-Menten, or substrate inhibition with the inhibition constant K_i and the optimal substrate concentration $[S]_{opt} = \sqrt{K_m K_i}$ at which velocity peaks;
- generates a report (PDF and/or PNG) showing the experimental points, all fitted model curves, a Lineweaver-Burk double-reciprocal panel comparing the linearized and non-linear parameter estimates on the same data, and a residuals plot for the best model;
- exports all fitted parameters, metrics, and the Lineweaver-Burk comparison to a JSON file for use in downstream analyses;
- provides an optional CustomTkinter desktop GUI (**enzyme-fit gui**) for interactive use without the command line.

The package is validated against synthetic datasets with known ground-truth parameters for both models, and against a literature-referenced Michaelis-Menten dataset for pectinase from *Aspergillus niger* LFP-1 hydrolyzing citrus pectin [Jalil and Ibrahim, 2021], for which **enzyme-fit**’s fit recovers the previously published parameters ($K_m = 3.89$ mg/mL, $V_{max} = 1701$ U/mg).

3 Availability

enzyme-fit is available on GitHub at <https://github.com/karenkhachatryan-lab/enzyme-fit>, under the MIT license. The archived software release is deposited on Zenodo at [10.5281/zenodo.21724603](https://doi.org/10.5281/zenodo.21724603); this document is a companion report and is not itself a substitute for the software citation.

References

- John Burdon Sanderson Haldane. *Enzymes*. Longmans, Green and Co., London, 1930.
- Mohd Termizi Mohd Jalil and Darah Ibrahim. Partial purification and characterisation of pectinase produced by *Aspergillus niger* LFP-1 grown on pomelo peels as a substrate. *Tropical Life Sciences Research*, 32(1):1–22, 2021. doi: 10.21315/tlsr2021.32.1.1.
- Kenneth A. Johnson and Roger S. Goody. The original Michaelis constant: Translation of the 1913 Michaelis–Menten paper. *Biochemistry*, 50(39):8264–8269, 2011. doi: 10.1021/bi201284u.
- Pauli Virtanen, Ralf Gommers, Travis E. Oliphant, Matt Haberland, Tyler Reddy, David Cournapeau, Evgeni Burovski, Pearu Peterson, Warren Weckesser, Jonathan Bright, Stéfan J. van der Walt, Matthew Brett, Joshua Wilson, K. Jarrod Millman, Nikolay Mayorov, Andrew R. J. Nelson, Eric Jones, Robert Kern, Eric Larson, C J Carey, İlhan Polat, Yu Feng, Eric W. Moore, Jake VanderPlas, Denis Laxalde, Josef Perktold, Robert Cimrman, Ian Henriksen, E. A. Quintero, Charles R. Harris, Anne M. Archibald, Antônio H. Ribeiro, Fabian Pedregosa, Paul van Mulbregt, and SciPy 1.0 Contributors. SciPy 1.0: Fundamental algorithms for scientific computing in Python. *Nature Methods*, 17:261–272, 2020. doi: 10.1038/s41592-019-0686-2.
- G. N. Wilkinson. Statistical estimations in enzyme kinetics. *Biochemical Journal*, 80(2): 324–332, 1961. doi: 10.1042/bj0800324.