BIOTRANS
LA ROCHELLE 2023

N°1184 / PC
TOPIC(s) : Enzyme discovery and engineering / Enzyme production, immobilization

Exploring mass photometry for monitoring hydrodynamic properties of enzymes

AUTHORS

Saniye Gul KAYA / MOLECULAR ENZYMOLOGY, GRONINGEN BIOMOLECULAR SCIENCES AND BIOTECHNOLOGY INSTITUTE, UNIVERSITY OF GRONINGEN, NIJENBORGH 4, GRONINGEN
Yulia D. YANCHEVA / SINGLE MOLECULE BIOPHYSICS, GRONINGEN BIOMOLECULAR SCIENCES AND BIOTECHNOLOGY INSTITUTE, UNIVERSITY OF GRONINGEN, NIJENBORGH 7, GRONINGEN
Marco W. FRAAIJE / MOLECULAR ENZYMOLOGY, GRONINGEN BIOMOLECULAR SCIENCES AND BIOTECHNOLOGY INSTITUTE, UNIVERSITY OF GRONINGEN, NIJENBORGH 4, GRONINGEN
Matthias HEINEMANN / MOLECULAR SYSTEMS BIOLOGY, GRONINGEN BIOMOLECULAR SCIENCES AND BIOTECHNOLOGY INSTITUTE, UNIVERSITY OF GRONINGEN, NIJENBORGH 4, GRONINGEN
Corresponding author : Katarzyna M. TYCH / k.m.tych@rug.nl

PURPOSE OF THE ABSTRACT

The growth and division of biological cells are crucial for life, yet the maximum rate of microbial cell growth is limited, and the reason for this is not fully understood [1]. Understanding this limit is important for biotechnological applications. Research suggests that cells, like machines, are unable to function beyond a critical rate of Gibbs energy dissipation, and this limit may be determined by the spatial displacement of certain enzymes during catalysis [2]. This displacement could lead to enhanced enzyme diffusion and cellular agitation, potentially disrupting biomolecular functions and limiting cell growth [3].

Enhanced enzyme diffusion (EED) is a debated phenomenon where some enzymes diffuse faster than expected from Brownian motion in the presence of their substrates and/or inhibitors [4]. Most experiments confirming EED have used fluorescence correlation spectroscopy (FCS), which requires fluorescent labelling, and is an ensemble averaging technique. Chen et al. [5] discuss an artefact in FCS caused by fluorescent labelling, which has been incorrectly interpreted as EED. Thus, there is a need for other suitable techniques to study the hydrodynamic properties of enzymes while being catalytically active.

We explored the use of a relatively new technique called mass photometry to monitor the hydrodynamic properties of several enzymes. The technique only requires small samples (µL range), is non-invasive, is a label-free approach, while enabling measuring protein masses. Results obtained using this technique with several prototype enzymes will be presented.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

enhanced enzyme diffusion | mass photometry | enzyme catalysis

BIBLIOGRAPHY

[1] Nielsen J, Keasling JD. Engineering Cellular Metabolism. Cell. 2016. doi:10.1016/j.cell.2016.02.004

[2] Niebel B, Leupold S, Heinemann M. An upper limit on Gibbs energy dissipation governs cellular metabolism. Nat Metab. 2019;1(1):125-132. doi:10.1038/s42255-018-0006-7

[3] Xu, M., Rogers, W. B., Ahmed, W. W., & Ross, J. L. (2020). Comparison of different approaches to single-molecule imaging of enhanced enzyme diffusion. arXiv preprint arXiv:2012.15424.

[4] Hari S. Muddana et al. Substrate catalysis enhances single-enzyme diffusion. In: Journal of the American Chemical Society 132.7 (2010). issn: 00027863. doi: 10.1021/ja908773a.

[5] Zhijie Chen et al. Single-molecule diffusometry reveals no catalysis-induced diffusion enhancement of alkaline phosphatase as proposed by FCS experiments. In: Proceedings of the National Academy of Sciences of the United States of America 117.35 (2020). issn: 10916490. doi: 10.1073/pnas.2006900117.