Activity-based lipid esterase profiling of M. bovis BCG at different metabolic states using Tetrahydrolipstatin (THL) as bait

Book Item (Buchkapitel, Lexikonartikel, jur. Kommentierung, Beiträge in Sammelbänden)

ID 3677486
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Year 2016
Title Activity-based lipid esterase profiling of M. bovis BCG at different metabolic states using Tetrahydrolipstatin (THL) as bait
Editor(s) Overkleeft, H. S.; Bogdan, I. F.
Book title Activity-based proteomics: methods and protocols
Volume Chapter 6
Publisher Humana Press
Place of publication New York
Pages 75-85
ISSN/ISBN 978-1-4939-6437-6
Series title Methods in Molecular Biology
This chapter provides a step-by-step protocol using activity-based protein profiling (ABPP) as a chemical-proteomic tool to survey the antibiotic properties of a small molecule. Here, we investigate the molecular mechanism behind the bactericidal activity of tetrahydrolipstatin (THL). ABPP relies on small molecule probes that target the active site of specific enzymes in complex proteomes. These probes in turn are equipped with a reporter tag that allows capturing, visualization, enrichment, identification, and quantification of its targets either in vitro or in situ. THL possesses bactericidal activities, but its precise spectrum of molecular targets is poorly characterized. Here, we used THL analogs functionalized to enable Huisgen-base cycloaddition, commonly known as ”click chemistry,” to identify target proteins after enrichment from mycobacterial cell lysates obtained from different physiological conditions.

Digital Object Identifier DOI 10.1007/978-1-4939-6439-0