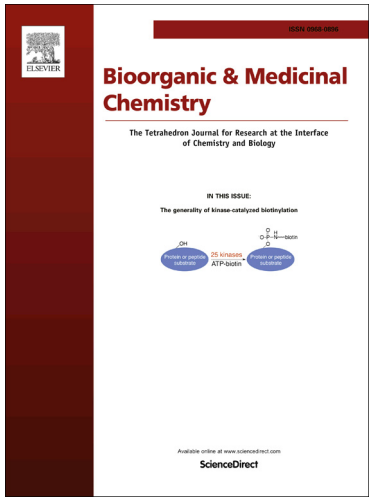

Twelve ways to confirm targets of activity-based probes in plants

Judit Kovács, Renier A.L. van der Hoorn

PII: S0968-0896(16)30360-1
DOI: http://dx.doi.org/10.1016/j.bmc.2016.05.036
Reference: BMC 13020

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 12 March 2016
Revised Date: 14 May 2016
Accepted Date: 19 May 2016

Please cite this article as: Kovács, J., van der Hoorn, R.A.L., Twelve ways to confirm targets of activity-based probes in plants, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.05.036

Twelve ways to confirm targets of activity-based probes in plants

Judit Kovács & Renier A. L. van der Hoorn
The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, South Parks Lane, OX1 3RB Oxford, United Kingdom.

For correspondence: Tel.: +44 1865 275077; Fax: +44 1865 275074
E-mail address: renier.vanderhoorn@plants.ox.ac.uk

Running title: Target confirmation for ABPP

Key words: Activity-based protein profiling; mass spectrometry; Arabidopsis; tomato; probe targets.

ABSTRACT

Activity-based probes are powerful tools to interrogate the functional proteome. Their covalent and often irreversible labeling of proteins facilitates the purification, identification and quantification of labeled proteins. However, the detection of labeled proteins often requires a confirmation, especially when unexpected proteins are identified, or to unravel fluorescent activity profiles. Here, we review twelve approaches towards target confirmation, grouped in approaches by direct target detection, target expression or target depletion. We discuss their proper use and limitations and illustrate these approaches with examples from plant science.

Introduction

Activity-based protein profiling (ABPP) is a powerful and robust functional proteomics tool that displays the active proteome in various biological systems (Cravatt et al. 2008; Serim et al., 2012; Willems et al., 2014; Nodwell & Sieber, 2012; Morimoto & Van der Hoorn, 2016). Chemical probes for ABPP react with active site of target proteins in a mechanism-dependent manner, resulting in a covalent and irreversible bond that facilitates the purification and detection of the labeled proteins.

There are four types of chemical probes (Cravatt et al., 2008; Morimoto & Van der Hoorn 2016). i) Mechanism-based probes are often inspired on a covalent,