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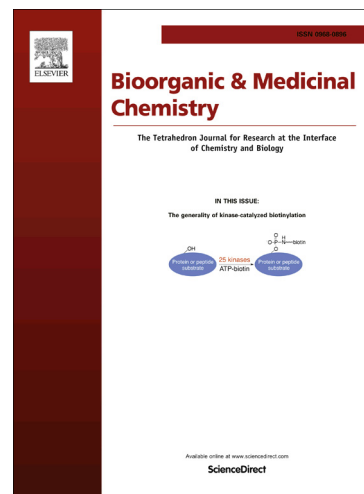
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Twelve ways to confirm targets of activity-based probes in plants

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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,

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