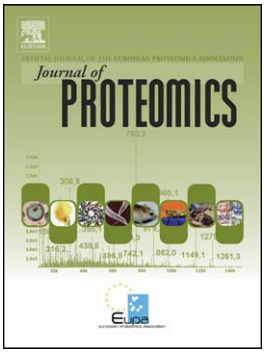

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Application of MeCAT-Click labelling for protein abundance characterization of *E. coli* after heat shock experiments

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Abstract

In a proof of concept study, metal-coded affinity tags based on click chemistry (MeCAT-Click) were used to analyze the proteome of *Escherichia coli* (*E.coli*) in response to heat stress. This allows high labelling efficiency, high detection sensitivity, and multiplex capabilities, which are pivotal for its application to protein quantification.

Two approaches are presented for relative quantification of differentially lanthanide-labelled proteins. The first approach uses isotope-labelling, where ESI-MS was utilized to quantify the differentially labelled proteins from different states of *E. coli*. With this approach, 14 proteins were found with changed abundance, among them five proteins upregulated.

In the second approach, differentially labelled samples were separated by two dimensional gel electrophoresis (2-DE) and scanned by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Comparison of the signal intensities of the different lanthanides was used to quantify different sample states. Based on this information, ESI-MS was used to identify the proteins with different abundance. The sensitivity of LA-ICP-MS allowed to find one upregulated protein that was nearly invisible by silver staining ("*Probable replication endonuclease from retron EC67*"). The advantage of this approach is to locate low abundant proteins with differential expression using LA-ICP-MS, which may be overlooked otherwise.

Keywords: heat shock response; Ln-MeCAT-Click labeling; *nano*LC-ESI-MS/MS; 2-D electrophoresis; LA-ICP-MS; protein quantification