**Specific protein isotopic and fluorescence labeling (SPILL) for protein distribution and metabolism studies**

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Secondary ion mass spectrometry (SIMS) is extensively used for investigating the composition of materials at a lateral resolution of about 100 nm. In biology it is mostly used to visualize either the natural abundance of isotopic species in cells or to investigate metabolism using small molecules labeled with stable isotopes, such as 15N or 13C (1). However, these offer only a general labeling method and it is not possible to look at specific proteins or cell compartments without the aid of complementary approaches, such as optical microscopy.

In this study, we sought to establish a method to specifically label proteins for nanoscale SIMS and to render optional the correlation with fluorescence microscopy. The specific protein isotopic and fluorescence labeling (SPILL) technique is based on unnatural amino acid incorporation into the protein of interest and its coupling to an isotopic probe after fixation. For the unnatural amino acid incorporation procedure we co-transfected baby hamster fibroblast (BHK) cells with plasmids encoding for the protein of interest modified with an Amber stop codon at a specific position and an unnatural amino acyl-tRNA/synthetase pair (2). While expressing the proteins of interest, we provided the cells with the unnatural amino acid propargyl-l-lysine (PRK; Milles et al. 2012; Nguyen et al. 2009). Within transfected cells, the unnatural amino acid tRNA/synthetase pair directs the incorporation of PRK at Amber stop codons in the protein of interest. Upon fixation, the proteins containing PRK in their primary sequence are coupled to an azide-derivatized probe in a copper-catalyzed Huisgen azide-alkyne cycloaddition, generally known as a click reaction (5). We designed two different probes: **SK155** (6), enriched in 19F, and **TriazNF1**, enriched in 15N. To ease the analysis, both probes contain a fluorophore which helped us assess the specificity of the signal in both SIMS and optical microscopy. Using either of these probes we could visualize precisely the proteins of interest and to observe a good correlation of the isotopic and fluorescent markers. Both probes enabled the analysis of specific protein turnover in the context of general cellular metabolism, but **TriazNF1** presented the advantage that it is not sensitive to background or isotopic contamination.

In this proof-of-principle study we present two SPILL probes that can be employed in SIMS measurements for investigating any protein of interest without the constraint of correlating the results with fluorescence imaging.

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