HHS Public Access

Author manuscript

J Proteome Res. Author manuscript; available in PMC 2021 June 05.

Published in final edited form as:

J Proteome Res. 2021 June 04; 20(6): 3017. doi:10.1021/acs.jproteome.1c00376.

Measuring Protein Shapes in Living Cells

Nikolai Slavov

Department of Bioengineering and Barnett Institute, Northeastern University, Boston, Massachusetts 02115, United States

Proteins fold into intricate shapes, known as conformations. The activation of many signal transduction proteins, kinases, and transcription factors requires a change in their conformations. Thus the conformation of a protein can indicate its biological activity. This importance of conformational changes has stimulated the development of numerous methods for analyzing protein conformations and interactions, such as native mass spectrometry[1] and cryoelectron microscopy.[2] These methods may achieve detailed characterizations of protein conformations, but they require highly purified proteins; they are challenged by the complexity of *in vivo* proteomes.

To enable the *in vivo* analysis of protein conformations, Bamberger et al.[3] report a covalent protein painting (CPP) of lysine residues, which directly determines the lysine residues that are accessible for covalent modification on protein surfaces. Specifically, the accessible amine groups on lysine residues are dimethylated in living cells, whereas the inaccessible amine groups are dimethylated with isotopically distinct methyl groups after protein extraction and digestion. Mass spectrometry analysis can distinguish and accurately quantify the peptides and their dimethyl labels, thus providing direct information for the fraction of lysine residues exposed on protein surfaces.[3] Therefore, CPP is a type of protein footprinting[4] approach that offers advantages over other footprinting methods. For example, CPP allows for the fast and highly specific covalent labeling of an amino acid that is frequently exposed on protein surfaces. A related footprinting method[5] uses tandem mass tags (TMT) to analyze the lysine surface accessibility, but it cannot be applied to living cells because cell membranes are not permeable to TMT. In contrast, the dimethyl labels used by CPP permeate cell membranes and allow labeling and analyzing the entire proteome of living cells without requiring protein purification. This ability to perform *in vivo* labeling offers the exciting potential to use CPP to quantify protein conformational changes in tissues of animal models.

The efficiency and specificity of dimethyl labeling can confer high-sensitivity to CPP and perhaps even enable single-cell sensitivity. One approach to this goal might include using isobaric carriers[6] to increase the sensitivity of CPP toward single-cell analysis. Then, the protein surface accessibility measured by CPP may provide information for protein activities and binding interactions, thus contributing a vitally needed dimension to single-cell analysis.

Corresponding Author: **Nikolai Slavov** – Department of Bioengineering and Barnett Institute, Northeastern University, Boston, Massachusetts 02115, United States; nslavov@alum.mit.edu, nslavov@northeastern.edu.

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jproteome.1c00376

[7] CPP will certainly find numerous applications, as hinted by the protein conformational changes in Alzheimer's disease already reported by Bamberger et al.[3]

REFERENCES

(1). Leney AC; Heck AJR Native Mass Spectrometry: What Is in the Name? J. Am. Soc. Mass Spectrom 2017, 28 (1), 5–13.

(2). Nakane T; Kotecha A; Sente A; McMullan G; Masiulis S; Brown PMGE; Grigoras IT; Malinauskaite L; Malinauskas T; Miehling J; Uchański T; Yu L; Karia D; Pechnikova EV; de Jong E; Keizer J; Bischoff M; McCormack J; Tiemeijer P; Hardwick SW; Chirgadze DY; Murshudov G; Aricescu AR; Scheres SHW Single-Particle Cryo-EM at Atomic Resolution. Nature 2020, 587 (7832), 152–156. [PubMed: 33087931]

(3). Bamberger C; Pankow S; Martínez-Bartolomé S; Ma M; Diedrich J; Rissman RA; Yates JR 3rd Protein Footprinting via Covalent Protein Painting Reveals Structural Changes of the Proteome in Alzheimer's Disease. J. Proteome Res 2021, 20, 2762. [PubMed: 33872013]

(4). Liu XR; Zhang MM; Gross ML Mass Spectrometry-Based Protein Footprinting for Higher-Order Structure Analysis: Fundamentals and Applications. Chem. Rev 2020, 120 (10), 4355–4454. [PubMed: 32319757]

(5). Yu K; Niu M; Wang H; Li Y; Wu Z; Zhang B; Haroutunian V; Peng J Global Profiling of Lysine Accessibility to Evaluate Protein Structure Changes in Alzheimer's Disease. J. Am. Soc. Mass Spectrom 2021, 32 (4), 936–945. [PubMed: 33683887]

(6). Specht H; Slavov N Optimizing Accuracy and Depth of Protein Quantification in Experiments Using Isobaric Carriers. J. Proteome Res 2021, 20, 880. [PubMed: 33190502]

(7). Slavov N Unpicking the Proteome in Single Cells. Science 2020, 367 (6477), 512–513. [PubMed: 32001644]