FLIM-FRET MICROSCOPY FOR IN CELL IMAGING OF PROTEIN POST-TRANSLATIONAL MODIFICATIONS

Franziska Doll, Annette Buntz, Andreas Zumbusch
University of Konstanz
Universitätsstraße 10, 78464 Konstanz, Germany
E-Mail: Andreas.Zumbusch@uni-konstanz.de

KEY WORDS: FLIM, FRET, wide field, frequency domain, live cell imaging, protein post-translational modifications, glycosylation, acetylation.

Fluorescence lifetime imaging (FLIM) microscopy is the most robust and direct method to spatially and temporally resolve Förster resonance energy transfer (FRET). FLIM-FRET microscopy can be applied to investigate post-translational modifications of specific proteins inside cells.[1] Post-translational modifications are attachments of chemical groups onto amino acid side chains of proteins and until now, more than 400 of them are known. They have fundamental impacts on e.g. the function, localization, stability, and interaction of proteins and thereby modulate many biological processes. We recently used FLIM-FRET microscopy to visualize the modification of specific proteins with sugars (glycosylation) and nucleic acid polymers (PARylation) inside living cells.[2,3] In these studies, metabolic engineering is used to label the post-translational modification proteome-wide with chemical reporter analogs incorporated via the cells biosynthetic machinery. These analogs can be labeled with the acceptor fluorophore. Protein specificity is gained by expressing the target proteins with the donor fluorophore GFP. FRET between the donor and the modification-anchored acceptor is detected via the fluorescence lifetime of GFP to assess the modification state of the target protein. As an outlook, ongoing work towards the visualization of protein-specific acetylation inside single cells using a novel chemical reporter will be presented.

[1] W. Lin, L. Gao, and X. Chen, "Protein-Specific Imaging of Posttranslational Modifications", *Curr. Opin. Chem. Biol.*, **28**, 156-163 (2015).
[2] F. Doll, A. Buntz, A.-K.-Späte, V.F. Schart, A. Timper, W. Schrimpf, C.R. Hauck, A. Zumbusch, and V. Wittmann, "Visualization of Protein-Specific Glycosylation inside Living Cells", *Angew. Chem. Int. Ed.*, **55**, 2262-2266 (2016).
[3] A. Buntz, S. Wallrodt, E. Gwosch, M. Schmalz, S. Beneke, E. Ferrando-May, A. Marx, and A. Zumbusch, "Real-time Cellular Imaging of Protein Poly(ADP-ribos)ylation", *Angew. Chem. Int. Ed.*, **55**, 11256–11260 (2016).