MONITORING THE TURNOVER OF ATP ANALOGS IN LIVING CELLS WITH FLIM-FRET MICROSCOPY

Anayat Bhat [1,3], Daniel Hammler [2,3], Andreas Marx [2,3], Andreas Zumbusch [1,3]

1. Division of Physical Chemistry, Department of Chemistry, University of Konstanz
2. Division of Organic Chemistry and Cellular Chemistry, Department of Chemistry, University of Konstanz
3. Konstanz Research School Chemical Biology, University of Konstanz

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Adenosine 5'-triphosphate (ATP) is the major energy currency of cells and is involved in various cellular functions like cell signaling and post-translational modifications of proteins. The high energy phosphoanhydride bonds of ATP are hydrolyzed to release ADP and a large amount of energy that is utilized for the functioning of various endergonic processes such as cellular transport and macromolecule synthesis [1].
Monitoring the hydrolysis activity of ATP in cells will be advantageous to understand ATP consuming cellular processes and can provide the basis for elucidating the mode of action and regulation of enzymes involved. Although a number of ATP analogs and other fluorescence sensors have been developed for this purpose, their applications in cells are limited either because they are not accepted by most of the cellular enzymes or they necessitate the overexpression of a fluorescently tagged protein. In this regard, some novel fluorogenic ATP probes were designed and synthesized recently. Upon enzymatic hydrolysis, these molecules display an increase in fluorescence intensity and fluorescence lifetime. The latter enables the readout of the hydrolysis of these probes and thus can be used for monitoring the enzymatic activity of ATP consuming enzymes [2].
We use these fluorogenic ATP analogs for the visualization of enzymatic activity in living cells by using confocal fluorescence microscopy and fluorescence lifetime imaging (FLIM) as the readout for measuring Förster resonance energy transfer (FRET) within probe molecules.
Like this, it is possible to monitor the hydrolysis of an ATP analog, adenosine tetraphosphate (Ap_4), in living cells with high spatial and temporal resolution. Our results demonstrate that the Ap_4 is hydrolyzed in lysosomes and autophagosomes. This provides a means to live-cell imaging of autophagy without the need to over-express fluorescently tagged proteins in cells. We show that this approach is suited to visualize lysosome distribution profiles and discuss how it can be employed to gather information regarding autophagic flux.

References:

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