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VABILO NA PREDAVANJE V OKVIRU DOKTORSKEGA ŠTUDIJA KEMIJSKE ZNANOSTI

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z naslovom:

Protein nitrosylation - from mass spectrometry to cardioprotection

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Vljudno vabljeni!

Povzetek

Mass spectrometry based proteomics can effectively identify and quantify proteins and their post-translational modifications (PTM). Most of the current approaches use electrospray ionization to ionize peptides and proteins, followed by measuring their masses in an analyzer (such as an Orbitrap). Protein post-translational modifications (phosphorylation, acetylation, nitrosylation, ubiquitination, etc.) are usually labile and sub-stoichiometric, thus, some form of enrichment is necessary before they can be analyzed by LC-MS.

In this presentation, I am going to give an overview of protein PTMs and how they are detected and analyzed. Our main interests are in protein and PTM changes in mitochondria isolated from heart tissues before and after ischemia and ischemic preconditioning.

One of the most important protein modifications in the heart is protein S-nitrosylation (SNO) which is a reversible modification that has been shown to modify the activity of target proteins and protect against thiol oxidation. The physiological production of NO leads to the formation of low levels of SNO under normal conditions in the cardiac myocyte. SNO is also greatly increased with myocardial preconditioning and following treatment with the S-nitrosylating agent S-nitrosoglutathione (GSNO), both of which have been shown to be cardioprotective. Numerous methodologies have been developed in order to detect S-nitrosylated proteins, but few methods have been able to effectively examine cysteine occupancy. We use isobaric cys-TMT mass tags which are normally used to label reduced cysteine residues for quantitative measurements of proteins. Using cys-TMT tags we are able to measure the percentage of S-nitrosylated cysteine sites (i.e. occupancy) in preconditioned mouse cardiac tissue.