Technology Available for License: Thiosulfonate Switch Technique – a three step protocol for detection of S-nitrosylated proteins

Background: Protein post-translational modification (PTM) increases the functional diversity of the proteome by the covalent addition of functional groups or proteins, proteolytic cleavage of regulatory subunits or degradation of entire proteins**.** PTM's influence almost all aspects of normal cell biology and pathogenesis. Therefore, identifying and understanding PTM's is critical in the study of cell biology, disease treatment and prevention.

S-Nitrosylation – the result of covalent bonding between a nitric oxide (NO) group into thiol groups to form S-nitrosothiol (SNO) - is a form of protein post-translational modification. S-Nitrosylation is a critical PTM used by cells to stabilize proteins and regulate gene expression. In many cases, SNO is believed to regulate protein function and activity with both physiological and pathological consequences.[1] To date, over 3000 peptides and proteins have been characterized and studied as SNO targets.

Developed in 2001(Jaffrey, et.al), the Biotin Switch Technique has become the most widely used protocol to study S-nitrosylation. However, this tool suffers from several drawbacks including false positives if all free thiols are not blocked before SNO reduction, disulfide scrambling, and thermal degradation. Approaches to address these limitations have produced second order problems. Regardless BST and related techniques have been described in the literature as "technically challenging and labor intensive". [2]

Finding: Thiosulfonate switch technique: a new three step protocol for SNO detection in aqueous medium that traps protein S-nitrosothiols as mixed disulfides bearing a fluorescent probe at pH 4.0.

Benefit: Faster process for detection of S-nitrosylation PTM's, efficiently blocks free thiols at pH 4.0. The process avoids denaturing agents, high temperatures and pH≥ 7.0. Protein purification steps are not required. A thiol modified rhodamine fluorescent probe provides the corresponding fluorescent mixed disulfides. This protocol is compatible with 1D non-reducing SDS PAGE and tryptic preolysis followed by peptide analysis.

Applications:

- Research in G-protein coupled receptor signaling, death receptor –mediated apoptosis , glutamate-dependent neurotransmission, vesicular trafficking, stimulation of prostaglandin synthesis, unfolded protein responses.
- Therapeutics to address areas such as tumor initiation and growth, neurodegeneration (Parkinson's, Alzheimer's and ALS), cancer, stroke.

This discovery was made at Montana State University by Dr.'s Ed Dratz, Paul Greico, David Singel and Benjamin Reeves, Dept. of Chemistry & Biochemistry. A patent is pending and a manuscript has been submitted for publication.

Contact for Inquiries: Gary Bloomer, Commercialization Specialist 406/994-7483 gary.bloomer@montana.edu	Montana State University, Technology Transfer Office 304 Montana Hall, Bozeman, MT 59717-2460 Phone: 406/994-7868; Fax: 406/994-4152 www.montana.edu/tto

1. http://www.piercenet.com/method/overview-post-translational-modification#introduction
2. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3120222/