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Nispyrtase: A thermostable, N-terminal arginine and lysine specific protease for ≤ 1 hr digestion, simplified peptide fragmentation and increased MS/MS sensitivity

John P Wilson

Cold Spring Harbor Laboratory, USA

We present Nispyrtase, a novel Arg/Lys specific N-terminal peptidase. By placing all strongly basic centers (the amino terminus, Arg and Lys) at N-termini of peptides, most ion current is driven to N-terminal b-ions, thus simplifying MS/MS spectra. Because most protons remain on the N-terminal side of the peptide, ion current is not diluted between γ - and b-ions during fragmentation consequentially improving detection sensitivity. In limit of detection (LOD) MRM experiments, detection sensitivity is increased ~ 2 -4.5x (median) relative to trypsin. Nispyrtase is a thermophillic protease with a T_{opt} of ~ 65 °C. Digestion time can be very rapid, even less than 1 hr. The protease is highly stable and active in a wide variety of conditions including multiple buffers, pH of 5-12, detergents and salts. It withstands repeated freeze-thaw cycles and exhibits a level of specificity highly similar to trypsin ($> 95\%$ K/R). In comparison with trypsin, the number of identified peptides and proteins in shotgun experiments are highly similar. The protease is a metalloprotease allowing partial digestion to be precisely controlled by addition of EDTA. In conclusion, Nispyrtase increases detection sensitivity, increases spectral clarity (specificity), significantly decreases digestion time and allows for precise control of digestion.

Biography

John P Wilson researches at Cold Spring Harbor Laboratory in the Darryl J.C. Pappin Laboratory of Protein Analysis and Mass Spectrometry. The Pappin Lab focuses on technology development and has been the source of many technologies spun out into Protifi, LLC, where Dr. Wilson is CEO. As a graduate student at The Rockefeller University, Dr. Wilson discovered a new class of modifications on histones, fatty acylation including palmitoylation. Dr. Wilson is the founder of NYC Bio, a 501(c)3 forming a biotech cluster in the Greater New York City Region, and lives with his family on Long Island.

wilsonjp@gmail.com