Chemical Biology, Chemical Probes, and Chemoproteomics

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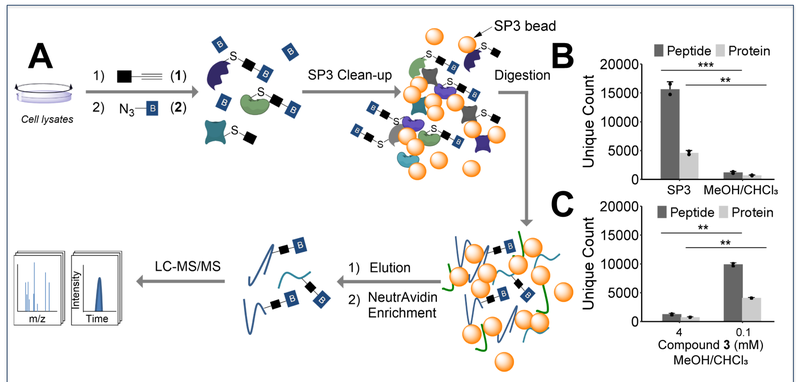
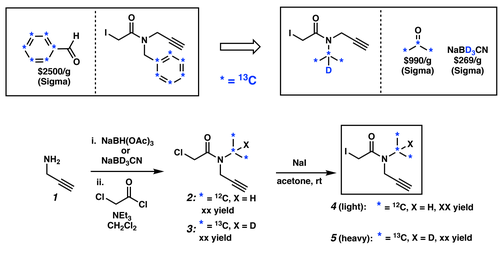
Research Overview

The Backus lab is a diverse, multidisciplinary group consisting of chemists, biochemists, and biologist with various backgrounds. Collaboration within the group is frequent and highly encouraged. Our research involves the use of a variety of chemical tools and probes to study protein function. This includes the use of applying Suzuki-Miyaura cross-coupling to label proteins, development of new chemoproteomic methods and tools for identification of ligandable cysteines, and new techniques to study protein-protein interactions (PPIs), as well as protein interactions with small molecules and other biomolecules.



We developed an optimized sample preparation workflow that combines enhanced peptide labeling with single-pot, solid phase-enhanced sample-preparation (SP3) to improve the recovery of biotinylated peptides, including from small sample sizes.

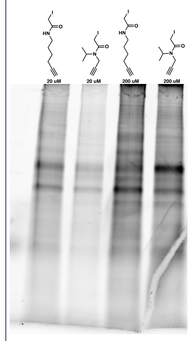


Cysteines, with high nucleophilicity and redox sensitivity, are involved in a variety of post-translational modifications, some of which are being investigated in the Backus lab.

We are using iodoacetamide alkyne probes to label biologically active cysteines. Recently, a 13C-labeled benzyl iodoacetamide probe was developed to enable quantitative profiling of cysteines in various environmental states of oxidation.

We synthesized a more inexpensive probe harboring a 13C-isopropyl group and are now using it in a variety of applications to label cell-surface and intracellular cysteines.

An Isobaric Iodoacetamide Alkyne Probe



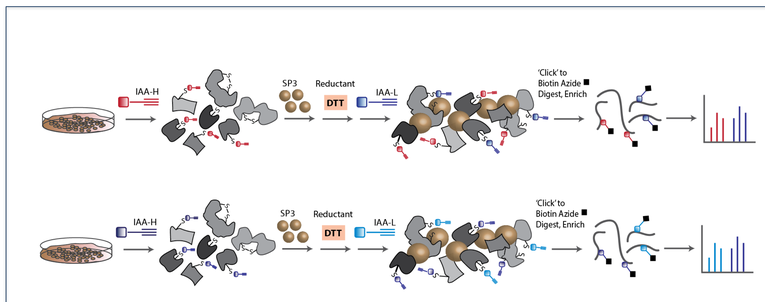
Distinct Labeled Proteins	495	827
Distinct Labeled Peptides	3865	6189
Total Proteins Labeled	4380	4729

*Data from average of 3 injections of a single sample

	Heavy IA 2mM	Light IA 2mM	1:1 ratio H:L 2 mM
Peptides ID'd	13519	14094	10745
Mods found	11680	11885	5541 L mods 7830 H mods

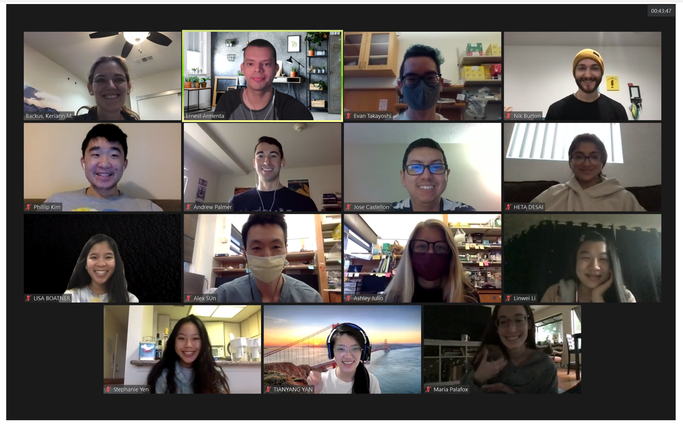
The click gel suggests our new IAA probe is comparable to existing an popular IAA probe.

We next performed mass spectrometry analysis of cell lysates labeled with our probe.



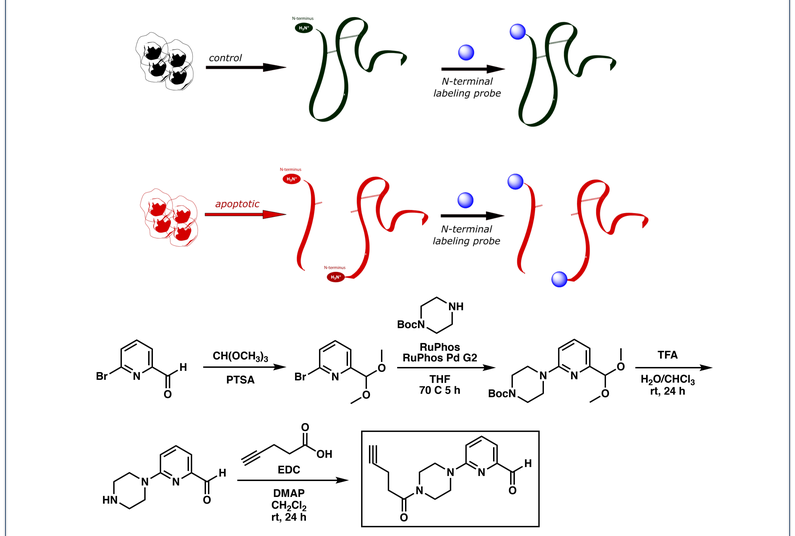
Currently, we are using these isobaric iodoacetamide probes to investigate redox states within various intracellular and cell-surface environments.

Backus Lab Members

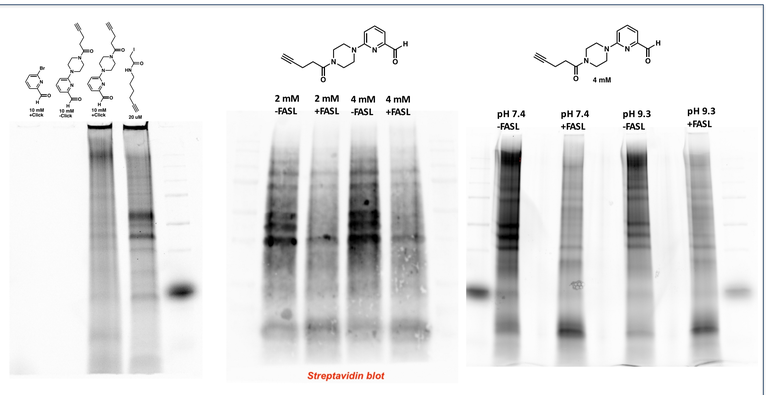


Numerous individuals to thank: Prof. Backus, and graduate students Ernest, Evan, Nik, Andrew, Jose, Heta, Lisa, Ashley, Sunny, Maria, Jian Cao (postdoc)

N-Terminal Labeling for Apoptosis



Caspases are proteases that are involved in diverse homeostatic functions including programmed cell death and inflammation. Their dysregulation leads to a host of diseases including cancers, autoimmune disease (eg, lupus), and auto-inflammation (eg, gout).



We have developed a small molecule probe that labels N-termini and is especially suited for identifying neo-N-termini formed from caspase cleavage. This probe will ideally be used to investigate caspase signaling biology.

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