

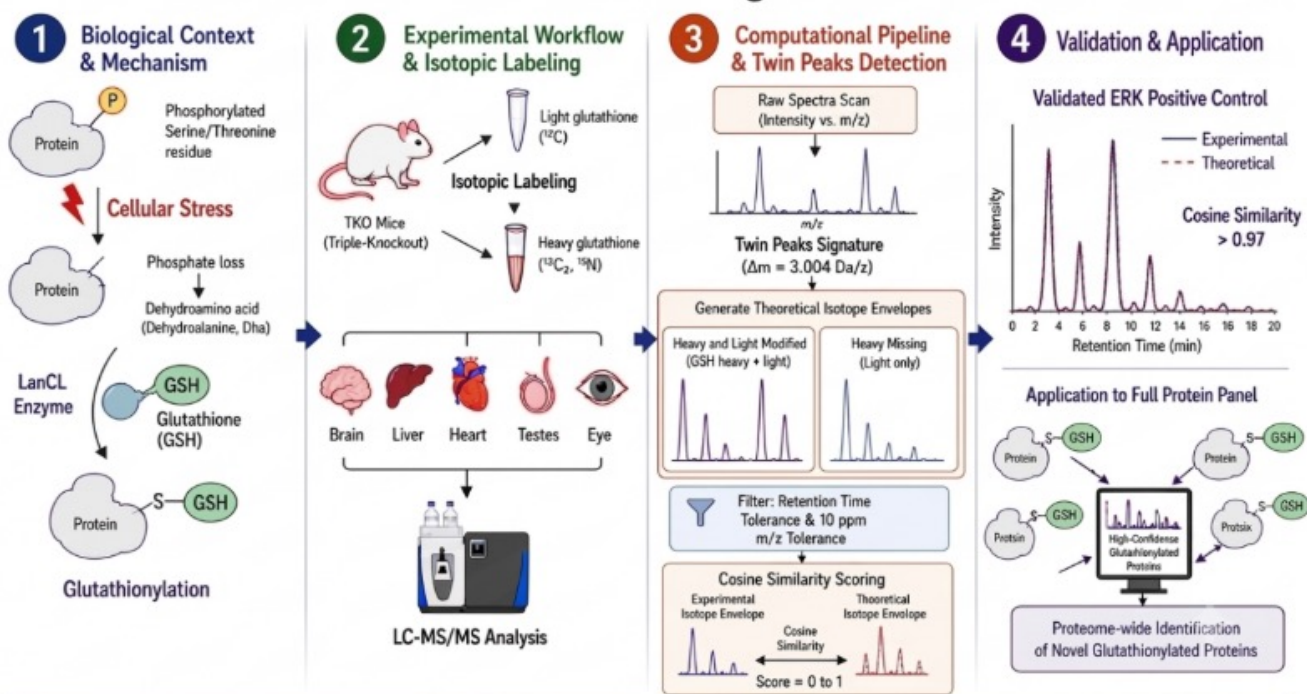
Identification of LanCL-Mediated Glutathionylation Targets in Mouse Tissues

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Glutathione (GSH), the cell's most abundant antioxidant, is known for neutralizing reactive species but can also be enzymatically attached to proteins as a post-translational modification. Under cellular stress, phosphorylated serine and threonine residues can lose their phosphate groups, forming the highly electrophilic dehydroamino acids dehydroalanine (Dha) and dehydrobutyrine (Dhb), which may dysregulate kinase signaling. LanC-like (LanCL) enzymes irreversibly add GSH to these damaged residues through glutathionylation, potentially preventing further signaling disruption. However, the full range of proteins LanCL modifies in living tissue remains unknown. This project aims to identify LanCL-mediated glutathionylation targets across male and female mouse brain, liver, heart, testes, and eye tissues. Triple-knockout tissues were isotopically labeled with light and heavy (+ ≈ 3.00374 Da) forms of GSH and analyzed by liquid chromatography/mass spectrometry. Authentic heavy and light modifications appear as characteristic "twin peaks" separated by ≈ 3.00374 Da per charge, distinguishing them from absent heavy modifications. To detect these events, we developed a computational pipeline that scans raw spectra for peptides carrying both the GSH modification and its isotopic partner. Candidate peptides are grouped by sequence and charge, theoretical light and heavy isotope envelopes are procedurally generated from composition, and experimental spectra are filtered by retention time and a 10-ppm m/z tolerance. Cosine similarity scoring against modified and unmodified theoretical spectra ranks candidates and exports the highest-confidence scans. Validated using a glutathionylated ERK positive control, the framework achieved a cosine similarity greater than 0.97 with its reference spectrum and is now being applied across the full tissue panel. Supported as a Grover Scholar by Dr. and Mrs. Gary Grover.

LanCL-Mediated Glutathionylation: Identification of Novel Protein Targets in Mouse Tissues



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