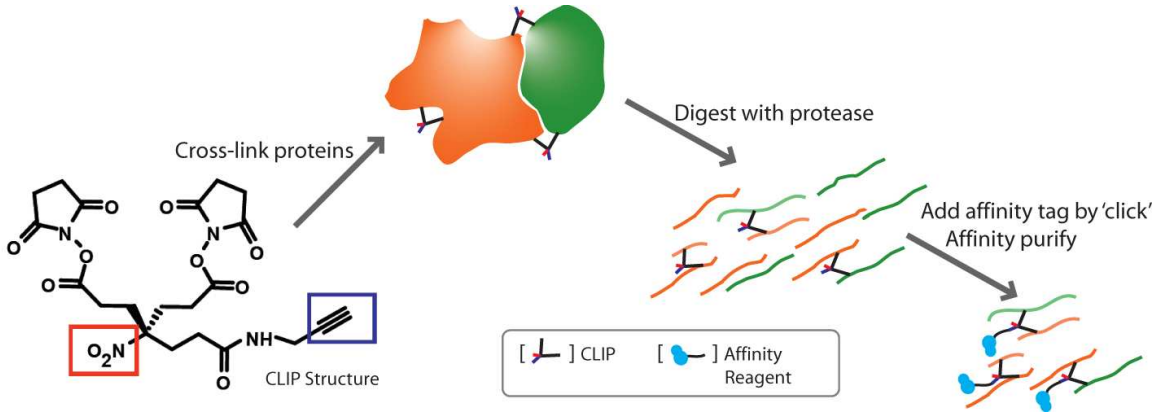
Identification of crosslinked peptides after click-based enrichment using sequential CID and ETD mass spectrometry

Saiful M. Chowdhury, Xiuxia Du, Nikola Tolić, Si Wu, Ronald J. Moore,
M. Uljana Mayer, Richard D. Smith, Joshua N. Adkins*
Biological Sciences Division and Environmental Molecular Sciences Laboratory,
Pacific Northwest National Laboratory,
Richland, WA 99352

Email: Joshua.Adkins@pnl.gov

Supporting Information



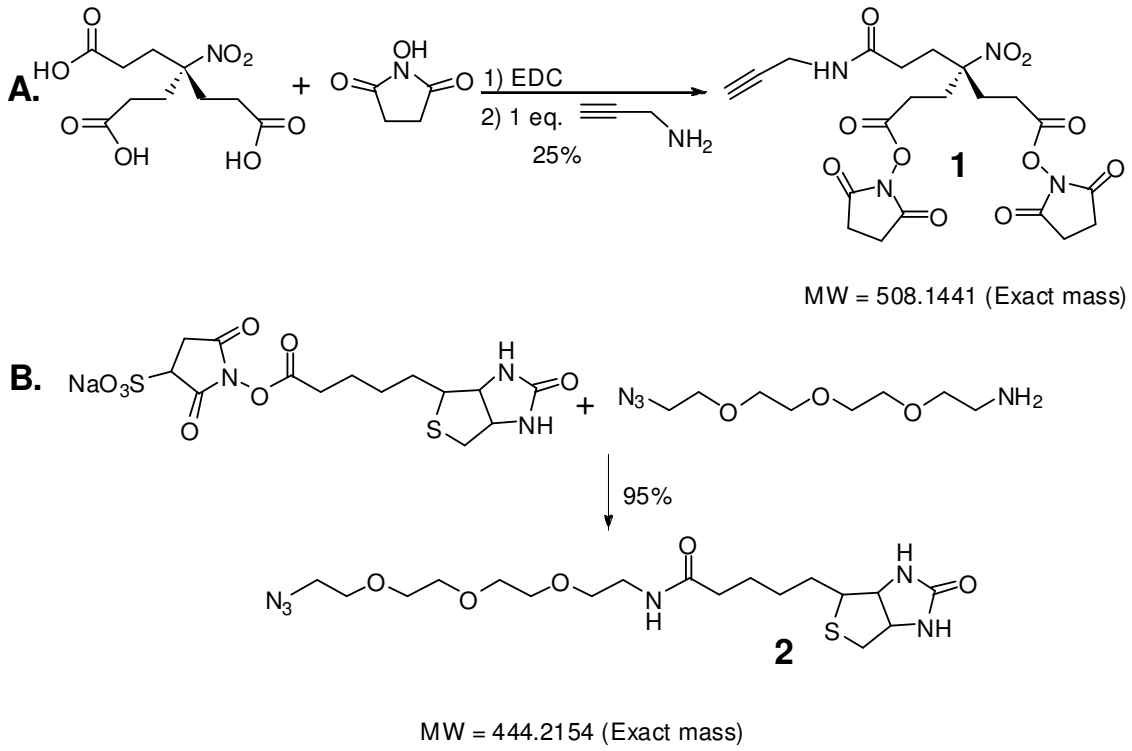


Figure S1: Synthesis scheme of crosslinker and enrichment reagent

Synthesis of Crosslinker (1):

Nitromethyltrispropionic acid (1.118 g, 1 eq. 4.04 mmol) and N-hydroxysuccinimide (1.44g, 3.1 eq., 12.1 mmol) were added to 15 mL dry acetonitrile in a dry round bottom flask equipped with stir bar under an argon atmosphere. After the reaction was determined to be complete by monitoring through thin-layer-chromatography (new spot at R_f 0.9 with 100% ethyl acetate, stained with potassium permanganate), a suspension of dry methylene chloride containing one equivalent of propargylamine hydrochloride (0.37g) and 0.16 mL triethylamine (1 eq.) was slowly added under vigorous stirring. The reaction was permitted to go to completion overnight. It had previously been found that the crosslinker hydrolyses with silica chromatography. Therefore purification consisted of evaporating the reaction mixture to dryness, taking it up in ethyl acetate and washing twice with 1 Molar phosphate buffer at pH7, and immediately drying over magnesium sulfate (yield 24.8%, compound contains 10% triple NHS ester). ^{1}H-NMR: (d-DMSO) 8.40(s , broad, 1H), 3.82 (s, broad, 2H), 3.1 (s, 1H), 2.85 (s, broad, 8H), 2.77 (q, J = 7.4 Hz , 4H), 2.04-2.40 (m, 8H). Theoretical mass = 508.1441, HRMS [M+H$^+$] = 509.1545, mass error 0.003 Da.

Synthesis of the enrichment reagent (2):

NHS-biotin (Pierce, 0.051g, 0.000149 mol, 1 eq) was combined with 11-azido-3,6,9-undecanamine (29.6 microliter, 0.000149 mol, 1 eq.) in dry chloroform (1mL) in a dry round bottom flask equipped with stir bar under Argon. Half an equivalent of triethylamine (10.4 microliter) was added twice, causing the material to go into solution. The reaction was allowed to stir for 14 hours at room temperature under argon, monitored by thin layer chromatography (25% MeOH/EtoAc). At completion, another 4 mL of chloroform was added and the solution extracted with 5 mL water. The water layer was washed with another 5 ml chloroform and the organic layers dried over magnesium sulfate and evaporated to dryness (yield: 23%). Theoretical Mass = 444.2154, HRMS [M+H$^+$] = 445.2187, mass error 0.0041 Da.

Calculation of precursor *m/z* of crosslinked peptides:

Note: the use of high mass accuracy instruments requires the use of high precision calculations of mass for analysis.

Inter-crosslinked peptide (two peptides connected with crosslinker)
= peptide mass + crosslinker mass – loss of two NHS group-2H

Intra-crosslinked peptide (one peptide connected with crosslinker)
= peptide mass + crosslinker mass – loss of two NHS group-2H

Dead-end crosslinked peptide
= peptide mass + hydrolyzed crosslinker mass – loss of one NHS group-H

After enrichment, crosslinked precursor peptide mass + mass of enrichment reagent

Crosslinker = 508.1441; hydrolyzed crosslinker mass = 411.1278; NHS leaving group = 114.0191; enrichment reagent = 444.2154

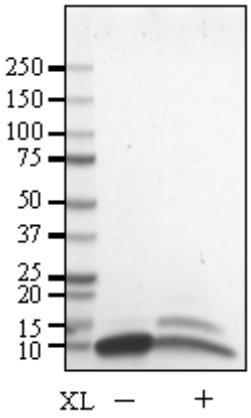


Figure S2: 1D-SDS-PAGE (Bio-Rad, 4-20% precast gel, Precision Plus Proteins standards) of the Ubiquitin crosslinking experiment.

Example of a mass spectrum of an intra-crosslinked peptide:

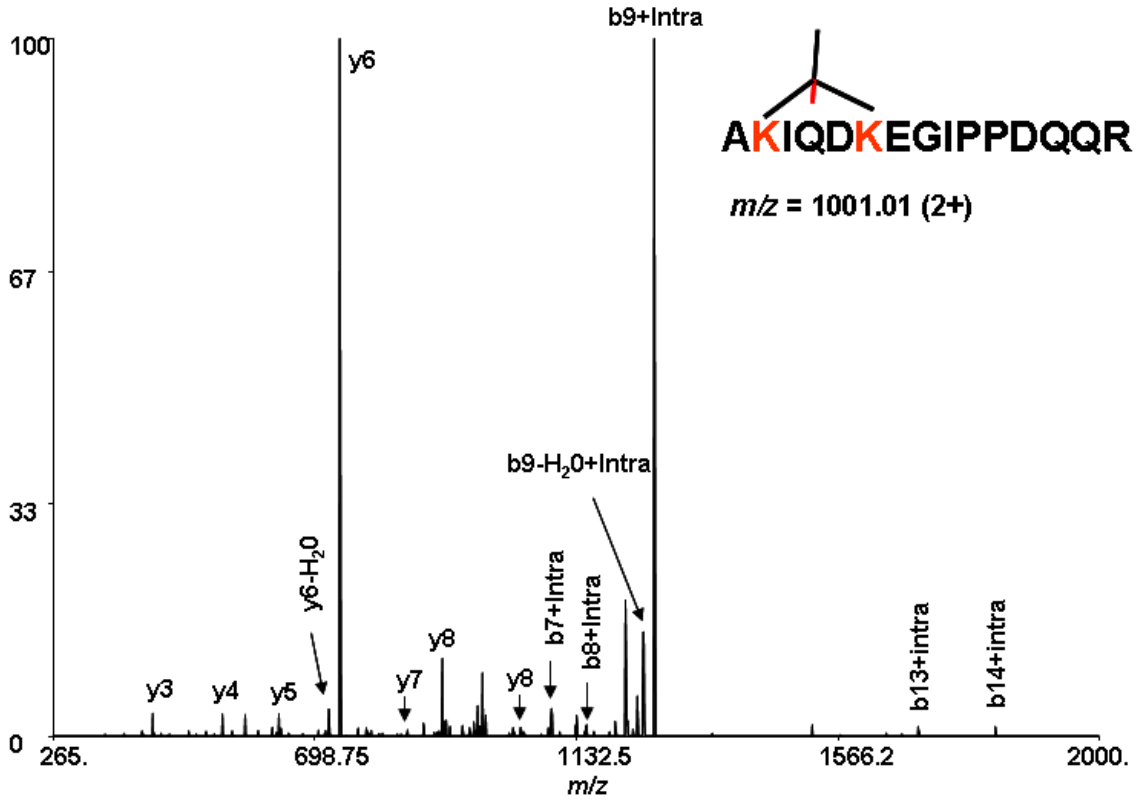


Figure S3: LTQ-mass spectra of an intra-crosslinked peptide of ubiquitin before click labeling.

Example spectra of the ubiquitin-ubiquitin crosslink:

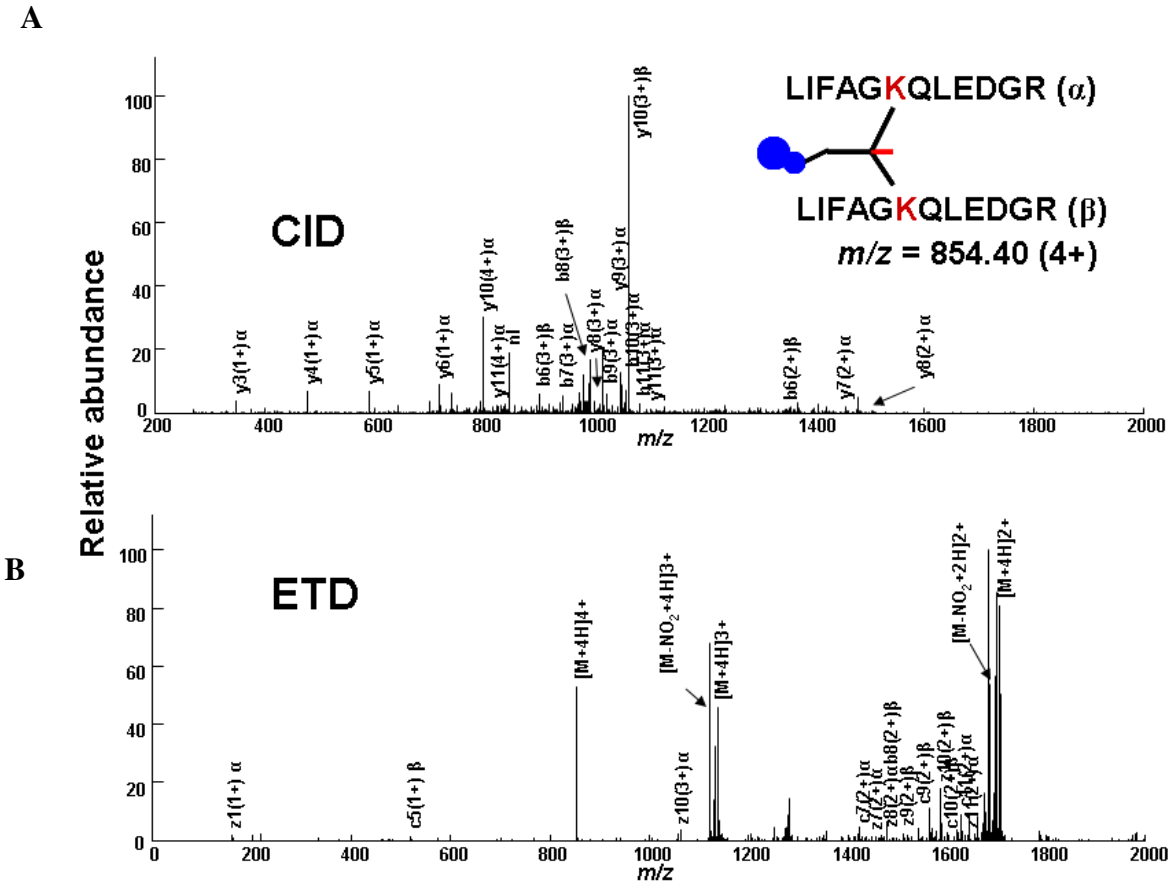


Figure S4: Example of sequential CID and ETD-MS/MS of an enriched inter-ubiquitin crosslinked peptides after in-solution digestion. CID and ETD-MS/MS unambiguously identify this crosslinked species. ETD shows the characteristic charge-neutralized peak from precursor *m/z*.

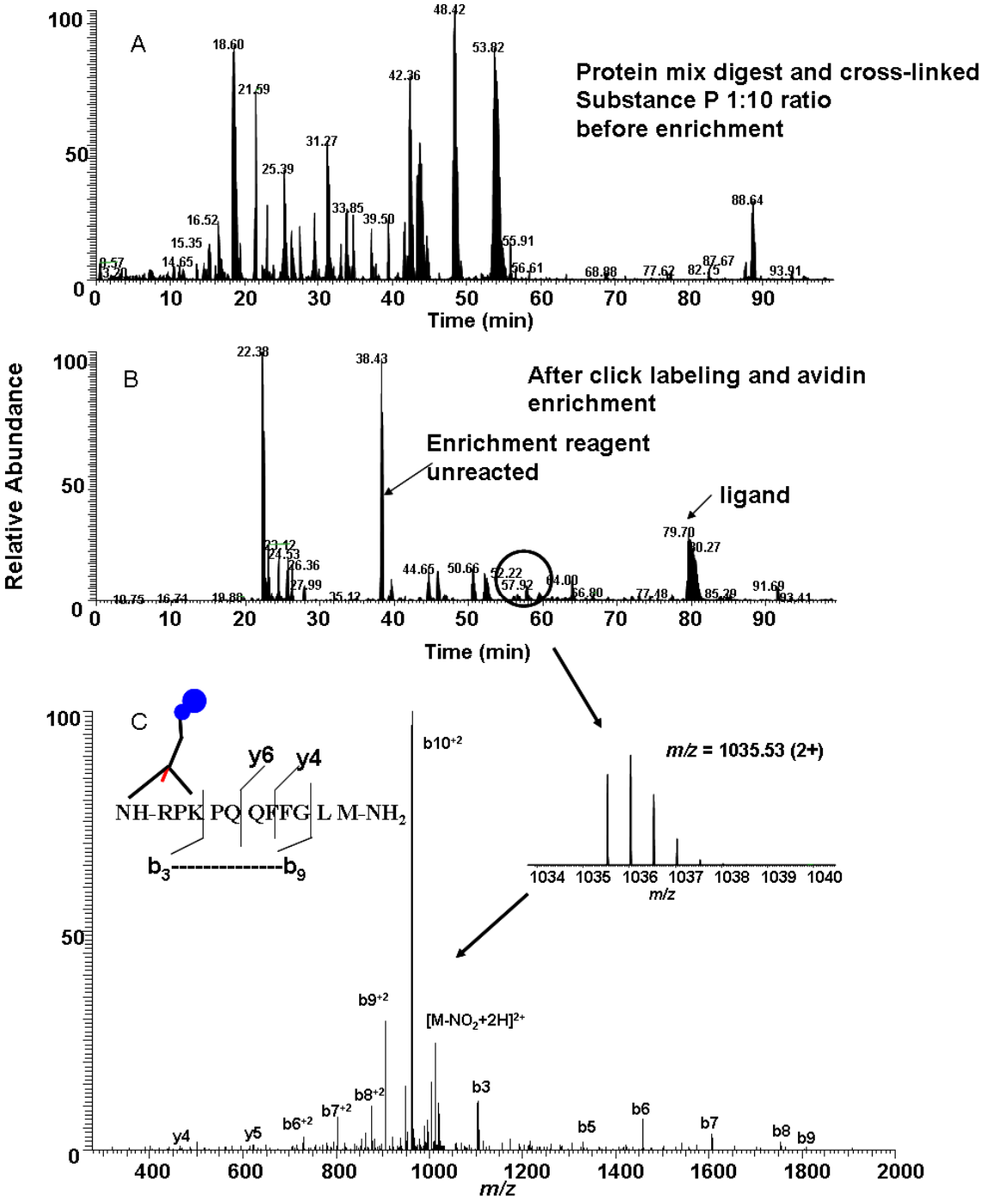


Figure S5. Demonstration of CLIP crosslinking and enrichment strategy in a moderately complex protein mixture. A) LC-MS/MS chromatogram of tryptic digest of three protein mixtures, BSA, RNase S and ubiquitin. A crosslinked Substance P was spiked in the mixture in 1:10 peptide to protein ratios. B) LC-MS/MS chromatogram after click labeling and avidin enrichment. C) MS/MS of click labeled intra crosslinked Substance P identified after enrichment. Raw data is available at http://omics.pnl.gov

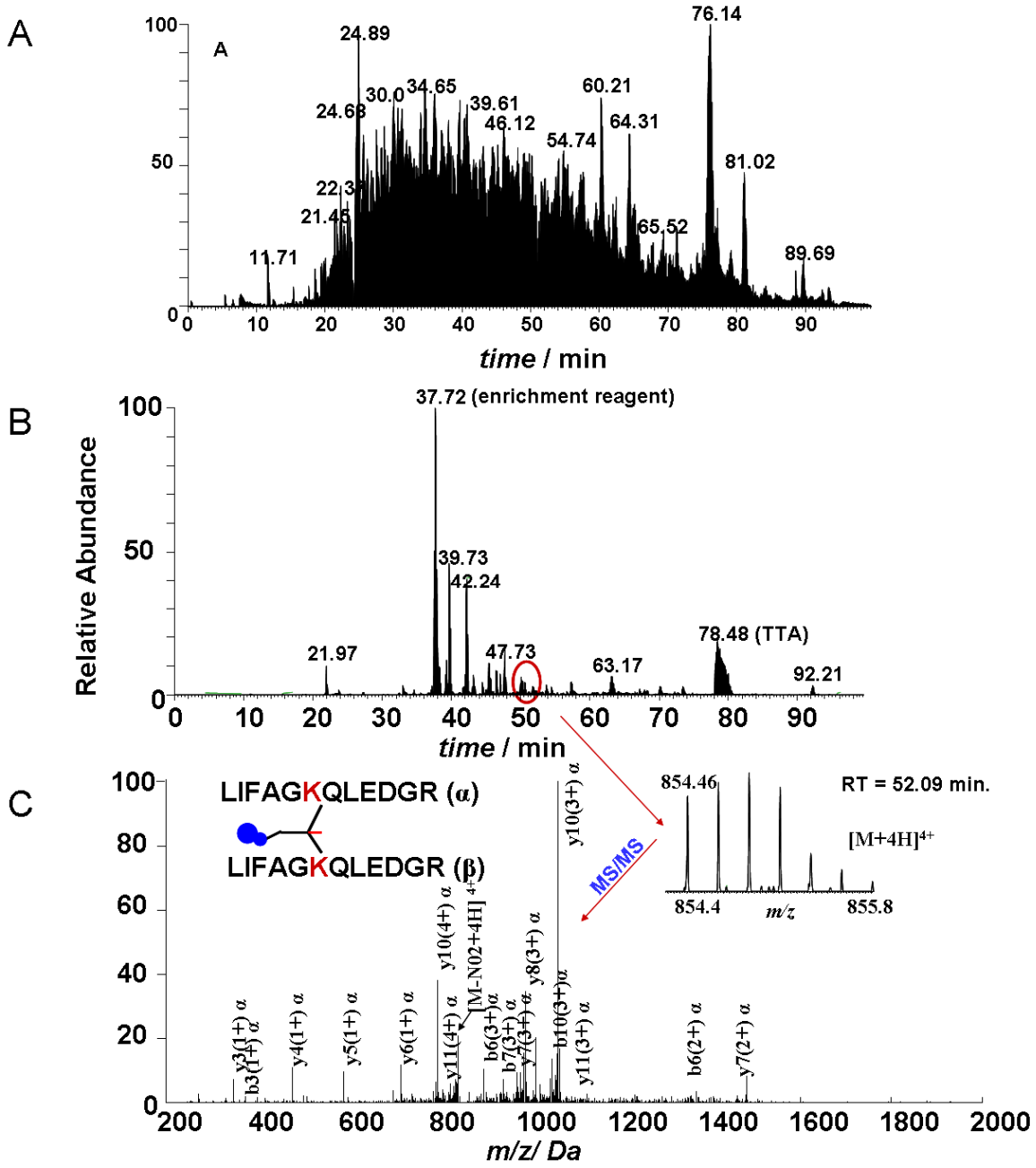


Figure S6. Demonstration of the "CLIP" enrichment strategy from complex biological samples A) LC-MS/MS chromatogram of a tryptic digest consisting of a 100:1 ratio of *E. coli* to crosslinked ubiquitin. B) LC-MS/MS chromatogram after click labeling and enrichment. C) MS/MS of one homodimeric inter–crosslinked peptide identification is shown. See supplementary figure S4 for sequential CID and ETD-MS/MS of this peptide