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LC-MS/MS for Proteomic Analysis of Post-translational Modifications on xCT

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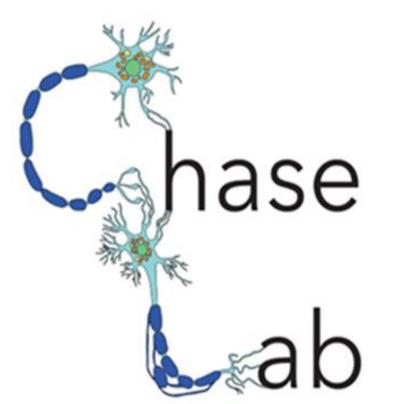
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LC-MS/MS for proteomic analysis of post-translational modifications on xCT

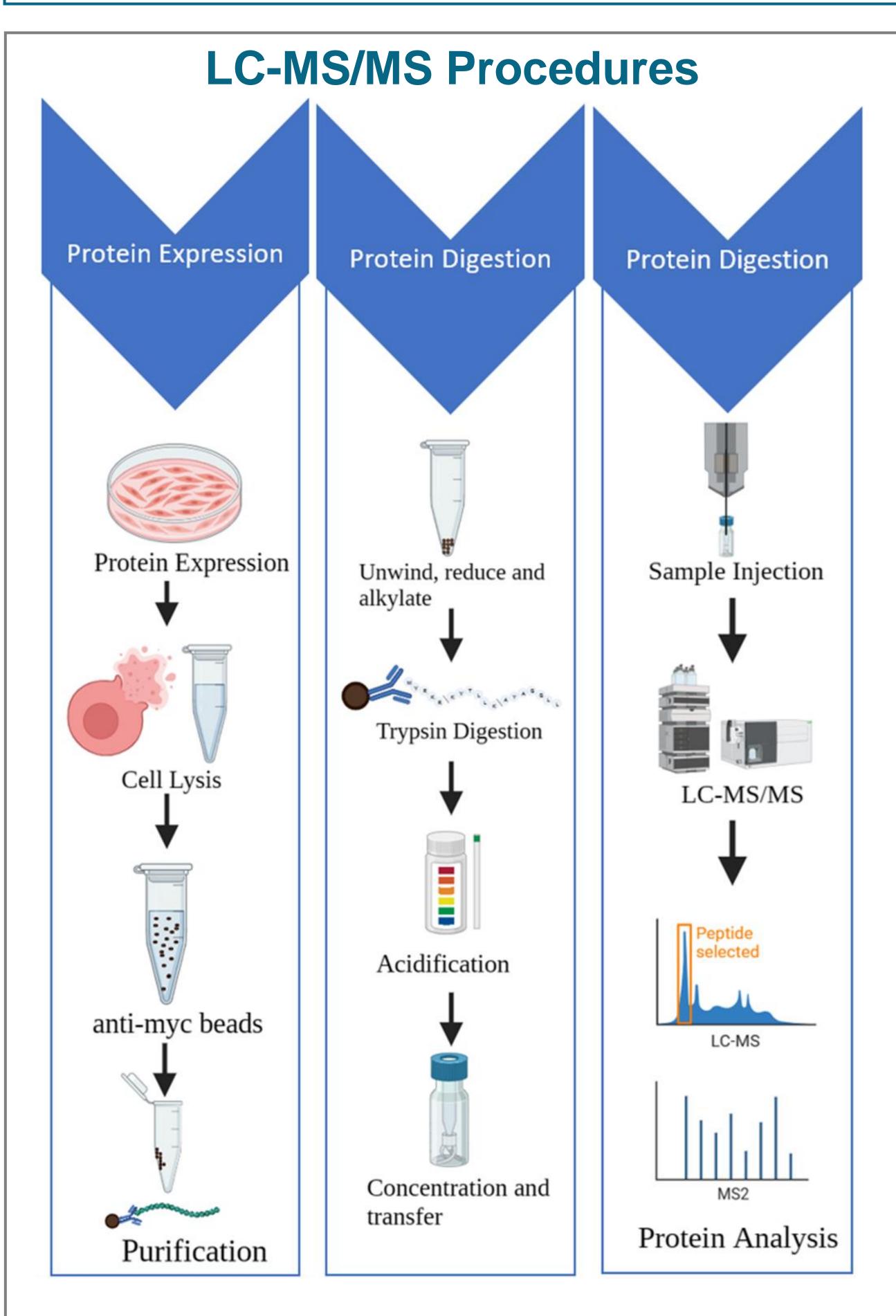


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BackF	urground	Protein Coverage		PTM Analysis Confident PTM Sites:				
xc- System					INI SITES:			
Function of System x _c ⁻ System x _c ⁻ is an antiporter that exchanges intracellular	Cys Glu	T under oxidative stress:		Modifications	Cell Conditions	# amino acids	matched ms/ms ions	Highest MS/MS score
glutamate for extracellular cystine. This cystine is used	Glutamate-cystine antiporter (X _c)	MVRKPVVSTISKGGYLQGNVNGRLPSLGNKEPPGQEKVQLKR <mark>KVTLL</mark>	RGVSIIIGTIIGAGIFISPK 67	Phospho S51, S56	Basal Stress		15	4 57.87
for glutathione production. Under basal conditions xCT resides in endosomes underneath the membrane,but when oxidative insult occurs we hypothesize that xCT is	Cys Glu Cys Glu Free Cys Glu Cys Glu GCL GSS + Gly	GVLQNTGSVGMSLTIWTVCGVLSLFGALSYAELGTTIKKSGGHYTYI	LEVFGPLPAFVRVWVELLII 134	Phospho T457, T459, Y464, Y465	Basal Stress		32	3 32.61
		RPAATAVISLAFGRYILEPFFIQCEIPELAIKLITAVGITVVMVLNS	MSVSWSARIQIFLTFCKLTA 201	Oxidation W249	Basal Stress		33	4 62.88
	Oxidative Stress	ILIIIVPGVMQLIKGQTQNFKDAFSGRDSSITRLPLAFYYGMY AYAG	WFYLNFVTEEVENPEKTIPL 268	No PTMs found 487- 501	Basal Stress		14	19 90.28
post-translationally modified (PTM) which functions to move it to the membrane	Fig.1 Shows system xc- as a transporter, then movement of xCT on and off the membrane.	AICISMAIVTIGYVLTNVAYFTTINAEELLLSNAVAVTFSERLLGNF	slavpifvalscfgsmnggv 335	Phospho S51, S56	Oxidative Stress		15	5 66.88
and increase cystine import. Project Goals		FAVSRLFYVASREGHLPEILSMIHVRKHTPLPAVIVLHPLTMIMLFS	GDLDSLLNFLSFARWLFIGL 402	Phospho T459, Y464, Y465	Oxidative Stress		59	3 31.97
Use LC-MS/MS to identify F endogenously occur on xCT	PTMS and associated proteins that T in the presence and absence of	AVAGLIYLRYKCPDMHRPFKVPLFIPALFSFTCLFMVALSLYSDPFS	TGIGFVITLTGVPAYYLFII 469	Oxidation W249	Oxidative Stress		65	3 32.19
oxidative stress. Significance		digestion and then resuspended in 5%ACN/0.1%FA and 70% isopropyl for injection. Spectra analyzed using		No PTMs found 487- 501	Oxidative Stress		14	17 87.93
By understanding how xCT is regulated in oxidative stress, we may be able to develop effective therapeutic strategies targeting System x_c^{-1}								

variable mounication reature on Diocommin 10.0. Sites with a wo/wo score above 50 and a clean chromatogram are considered authentic. Additionally, presence of PTM under both conditions and across samples increases reliability. C-terminus peptide of high confidence shows no oxidation or phosphorylation.



xCT under basal conditions:

- MVRKPVVSTISKGGYLQGNVNGRLPSLGNKEPPGQEKVQLKR**KVTLLRGVSIIGTIG**GAGIFISPK 67
- **GVLQNTGSVGMSLTIWTVCGVLSLFGALSYAELGTTIKKSGGHYTYIL**EVFGPLPAFVRVWVELLII 68
- RPAATAVISLAFGRYILEPFFIQCEIPELAIK**LITAVGITVVMVLNSMSVSWSARIQIFLTFCK**LTA 201 135
- 202 ILIIIVPGVMQLIKGQTQNFK**DAFSGR<u>DSSITRLPLAF</u>YYGMYAYAGWFYLNFVT**EEVENPEKTIPL
- 269 AICISMAIVTIGYVLTNVAYFTTINAEELLLSNAVAVTFSERLLGNFSLAVPIFVALSCFGSMNGGV 335
- 402 336 FAVSRLFYVASREGHLPEILSMIHVRKHTPLPAVIVLHPLTMIMLFSGDLDSLLNFLSFARWLFIGL
- 403 AVAGLIYLRYKCPDMHRPFKVPLFIPALFSFTCLFMVALSLYSDPFSTGIGFVITLTGVPAYYLFII

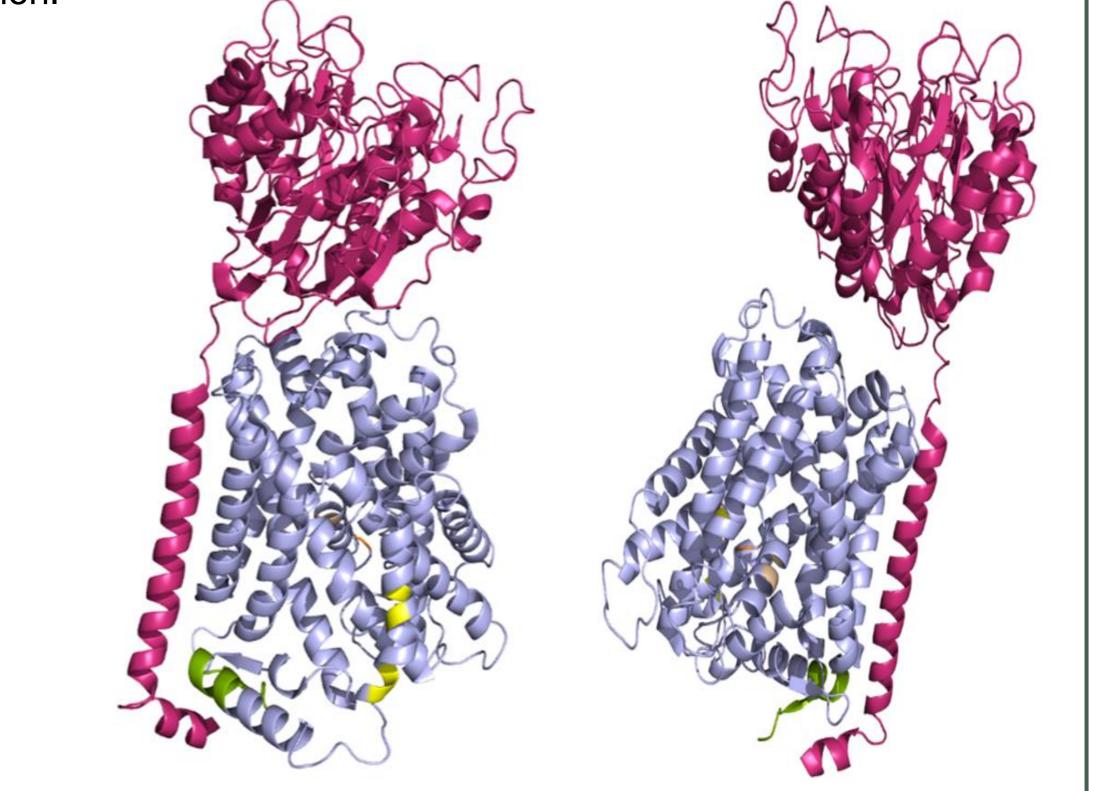


Figure.5 Shows two orientation of three dimensional xCT (blue) and 4FTHC (pink), with amino acids S51 and S56 marked in yellow; T459, Y464 and T465 marked in beige; and W249 marked in orange.

Conclusions

 Trypsin digestion is not sufficient in digesting xCT 501 • Nonspecific protein association with myc-beads could be reducing the identification of xCT peptides Amino Acids 487-501 in C-terminus appears as a low site of phosphorylation and oxidation • There are several potential sites of phosphorylation within N and C terminus and between transmembrane domains • There is a potential site of oxidation within the transmembrane domain

Fig.2 Expressed myc-tagged xCT and 4FTHC in COS7 cell cultures using standard transfection techniques. Cells lysed, and cell lysis incubated overnight with myc-tag beads. RapiGest, DTT and IAA used to prepare sample for overnight trypsin digest. Sample is acidified and resuspended with 5%ACN/0.1%FA and 70% isopropyl and concentrated before injection.

470 WDKKPRWFRIMSEKITRTLQIILEVVPEEDKL

Fig.4 xCT expressing COS-7 cells were lysed and prepared for trypsin digestion and then resuspended in 5%ACN/0.1%FA and 70% isopropyl for injection. Spectra analyzed using BioConfirm 10.0 and identified xCT peptides reported above. The total sequence coverage for combined xCT iterative samples was 36.5% of the total sequence.

Protein Association

Protein	Score	Matches (sig.)	Sequences (sig.)
Vimentin	3297	136	14
Actin beta	2840	170	11
Tubulin beta	2355	152	11
Keratin	1522	77	9

Table.1 Four highest scored proteins found in control sample identified using Mascot Daemon. A score over 300 is considered as an authentic call for a protein.

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Future Plans

- Use new enzyme combination to effectively digest xCT Reduced IP incubation time in order to decrease nonspecific protein binding
- Look for additional post-translational modifications (acetylation) or gly-gly modification of lysine residues)

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