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

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## BackFurground

### Function of System $x_c^-$

System $x_c^-$ is an antiporter that exchanges intracellular glutamate for extracellular cystine. This cystine is used for glutathione production.

Under basal conditions xCT resides in endosomes underneath the membrane,but when oxidative insult occurs we hypothesize that xCT is post-translationally modified (PTM) which functions to move it to the membrane and increase cystine import.

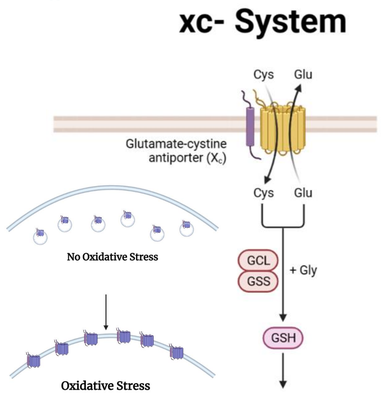


**Fig.1** Shows system xc- as a transporter, then movement of xCT on and off the membrane.

### Project Goals

Use LC-MS/MS to identify PTMS and associated proteins that endogenously occur on xCT in the presence and absence of oxidative stress.

### Significance

By understanding how xCT is regulated in oxidative stress, we may be able to develop effective therapeutic strategies targeting System $x_c^-$

## LC-MS/MS Procedures

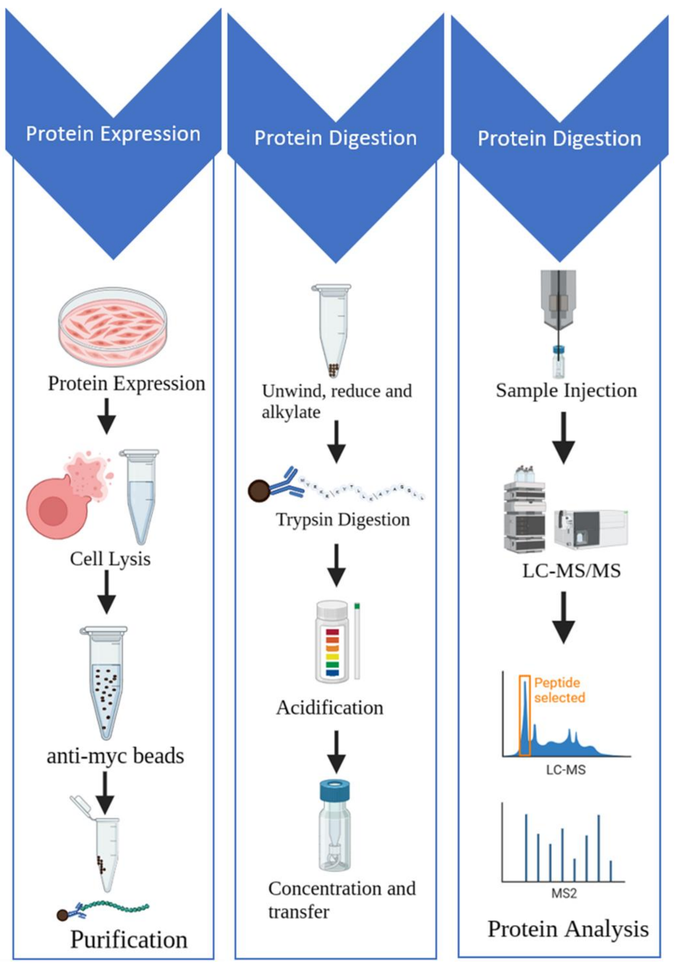


**Fig.2** Expressed myc-tagged xCT and 4FTHC in COS7 cell cultures using standard transfection techniques. Cells lysed, and cell lysis incubated overnight with myc-tag beads. RapiGest, DTT and IAA used to prepare sample for overnight trypsin digest. Sample is acidified and resuspended with 5%ACN/0.1%FA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

## Protein Coverage

### xCT under oxidative stress:


**Fig.3** xCT expressing COS-7 cells were exposed to 0.3% hydrogen peroxide then lysed, 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 38.9% of the total sequence.

### xCT under basal conditions:


**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.

## PTM Analysis

### Confident PTM Sites:

| Modifications | Cell Conditions | # amino acids | matched ms/ms ions | Highest MS/MS score |
|---|---|---|---|---|
| Phospho S51, S56 | Basal Stress | 15 | 4 | 57.87 |
| Phospho T457, T459, Y464, Y465 | Basal Stress | 32 | 3 | 32.61 |
| Oxidation W249 | Basal Stress | 33 | 4 | 62.88 |
| No PTMs found 487-501 | Basal Stress | 14 | 19 | 90.28 |
| Phospho S51, S56 | Oxidative Stress | 15 | 5 | 66.88 |
| Phospho T459, Y464, Y465 | Oxidative Stress | 59 | 3 | 31.97 |
| Oxidation W249 | Oxidative Stress | 65 | 3 | 32.19 |
| No PTMs found 487-501 | Oxidative Stress | 14 | 17 | 87.93 |

**Table.2** Sites of phosphorylation and oxidation identified using variable modification feature on BioConfirm 10.0. Sites with a MS/MS 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.

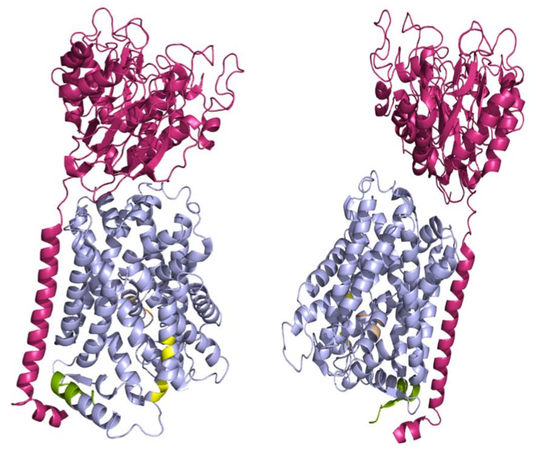

**Figure.5** Shows two orientation of three dimensional xCT (blue) and 4FTHC (pink), with amino acids S51 and S56 marked in yellow; T459, Y464 and T465 marked in beige; and W249 marked in orange.

## Conclusions

- Trypsin digestion is not sufficient in digesting xCT
- 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

## 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)

## Acknowledgments

- Schaap Undergraduate Research Fund
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