

Hope College

## Hope College Digital Commons

---

22nd Annual Celebration of Undergraduate  
Research and Creative Activity (2023)

The A. Paul and Carol C. Schaap Celebration of  
Undergraduate Research and Creative Activity

---

4-14-2023

### Creation of an N-terminal xCT Mutant Lacking Lysines for Use in Protein Turnover Studies

Alexandria Switzer  
*Hope College*

Follow this and additional works at: [https://digitalcommons.hope.edu/curca\\_22](https://digitalcommons.hope.edu/curca_22)



Part of the [Biochemistry Commons](#), and the [Molecular Biology Commons](#)

---

#### Recommended Citation

Repository citation: Switzer, Alexandria, "Creation of an N-terminal xCT Mutant Lacking Lysines for Use in Protein Turnover Studies" (2023). *22nd Annual Celebration of Undergraduate Research and Creative Activity (2023)*. Paper 39.

[https://digitalcommons.hope.edu/curca\\_22/39](https://digitalcommons.hope.edu/curca_22/39)

April 14, 2023. Copyright © 2023 Hope College, Holland, Michigan.

This Poster is brought to you for free and open access by the The A. Paul and Carol C. Schaap Celebration of Undergraduate Research and Creative Activity at Hope College Digital Commons. It has been accepted for inclusion in 22nd Annual Celebration of Undergraduate Research and Creative Activity (2023) by an authorized administrator of Hope College Digital Commons. For more information, please contact [digitalcommons@hope.edu](mailto:digitalcommons@hope.edu), [barneycj@hope.edu](mailto:barneycj@hope.edu).



# Creation of an N-terminal xCT mutant lacking lysines for use in protein turnover studies

Alexandria Switzer<sup>a,b,c</sup>, and Leah Chase<sup>a,b,c</sup>

Departments of <sup>a</sup>Chemistry and <sup>b</sup>Biology and <sup>c</sup>Program of Neuroscience, Hope College, Holland, MI, 49423

## Background

### Function of xCT

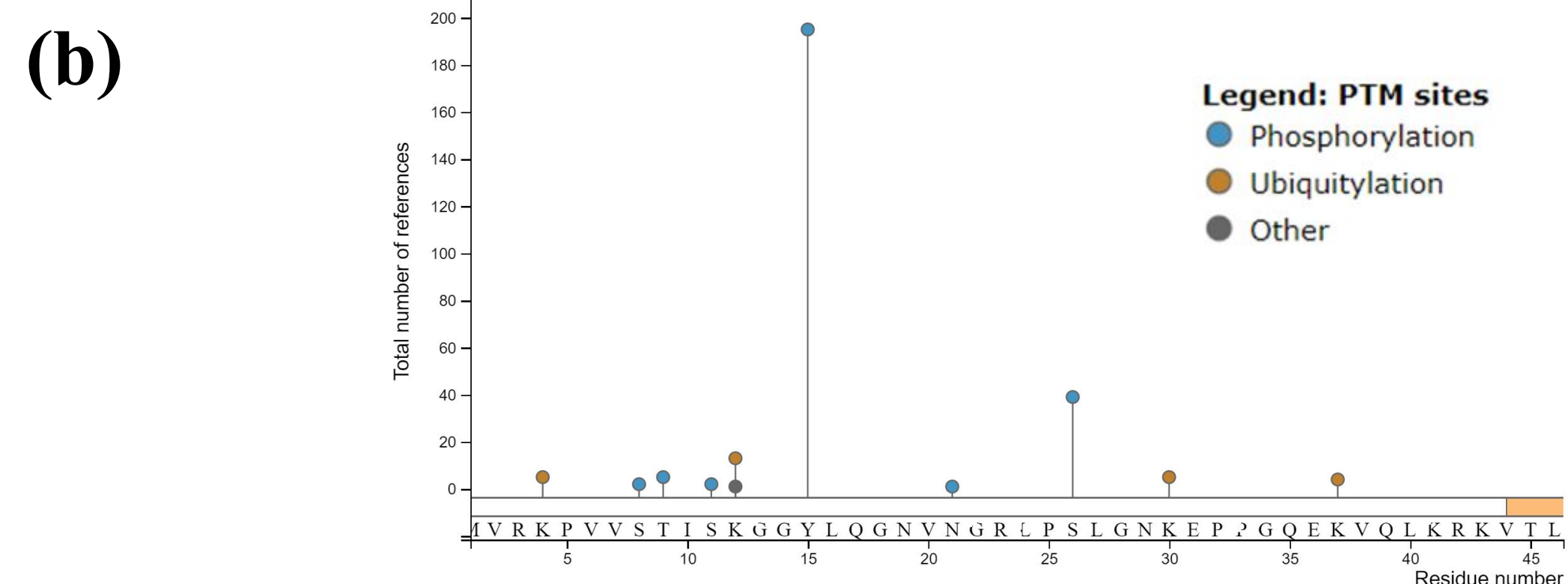
- System x<sub>c</sub><sup>-</sup> is an antiporter that exchanges intracellular glutamate for extracellular cystine
- Cystine is reduced to cysteine, which is necessary for the synthesis of glutathione (GSH)
- GSH is a reducing agent that mitigates oxidative stress in cells
- System x<sub>c</sub><sup>-</sup> consists of the light chain protein, xCT, that confers specificity, and the heavy chain protein, 4F2HC
- Trafficking of xCT is thought to modulate the oxidative stress response

### Effects of Ubiquitin

- Ubiquitin tags on proteins typically leads to their internalization or degradation
- Ubiquitin reversibly attaches to lysine (K) residues
  - 6 K's are in the N-terminus of xCT: 4, 12, 30, 37, 41, 43. Four K's are conserved across species (4, 37, 41, 43). (Figure 1)
- Other proteins, such as epithelial sodium channel, are regulated by ubiquitin, with increased ubiquitination resulting in decreased membrane expression

(a)

Human xCT	1	MVRKPVVSTI	SKGGYLQGNV	NGRLPSLGN	EPPGQEKVOL	KRKV	44
Mouse xCT	1	MVRKPVVATI	SKGGYLQGNM	SGRLPSMGD	EPPGQEKVVL	KRKI	44
Zebra Finch	1	MFRKAAPVT	SNGSYLQQA	NGKLSSTDSG	QFAREGRVVL	KRKV	44
Zebra Fish	1	MPRRTVSAPH	PNGDPTPSNF	GEKEPLKLN	EPPREKVEL	KRKV	44



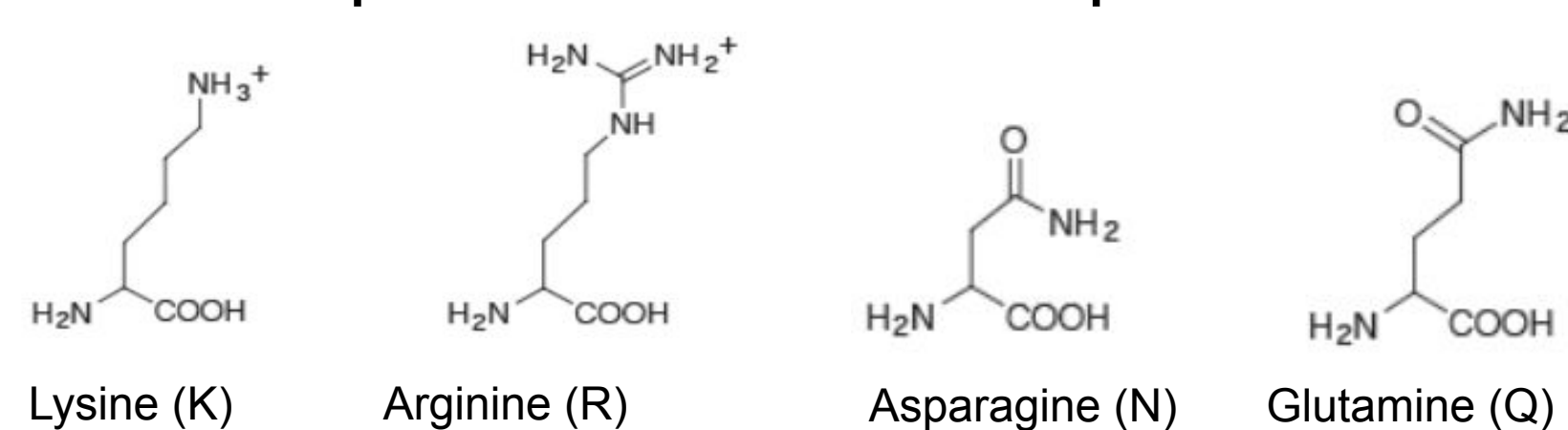
**Figure 1. N-terminus xCT DNA sequences for four species.** (a) A comparison of the DNA sequence of xCT for *Homo sapiens* compared to 3 other species. Most of these lysines are highly conserved across species. (b) Post-translational modifications of amino acids in the N-terminus of xCT identified through high-throughput screening (Phosphosite plus).

## Research Question

How does the lack of ubiquitination on the N-terminus of xCT affect the function of the protein?

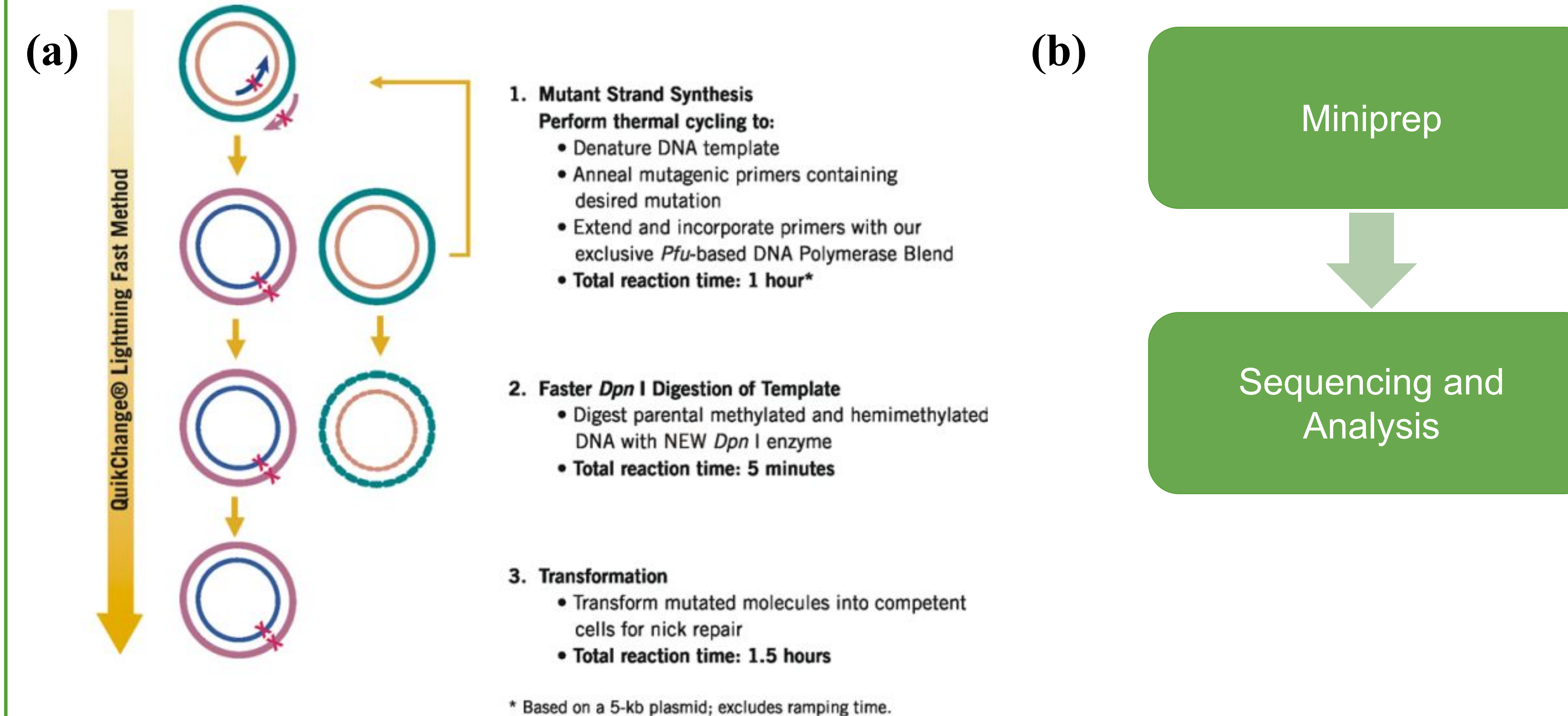
## Hypothesis

Replacing all of the lysines in the N-terminus of xCT with arginines will lead to a loss of ubiquitination and an overall increase in the activity and overall expression of the transporter.



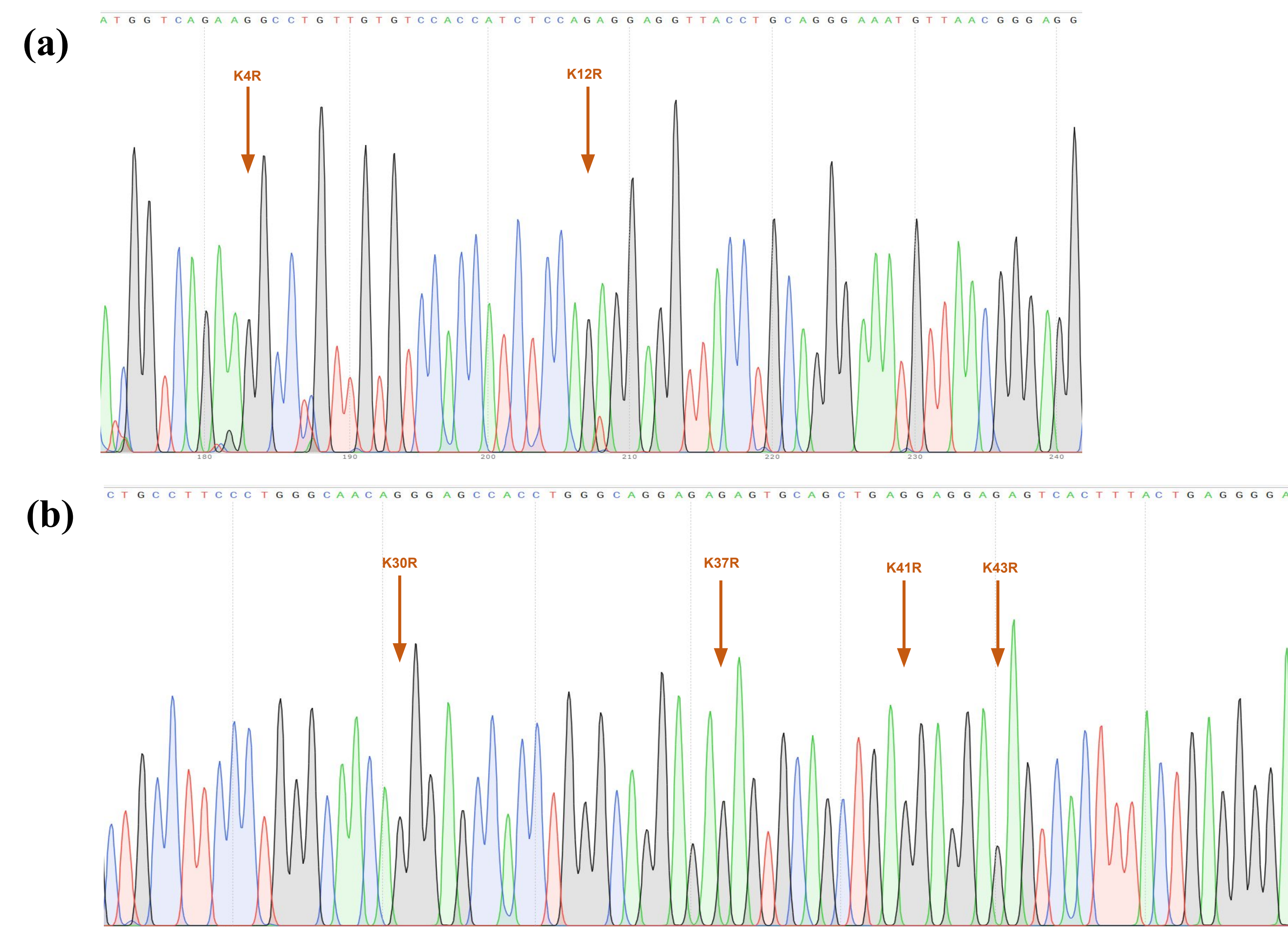
**Figure 2. Mutagenesis strategy.** Arginine and lysine are interchangeable because they are similar in size and charge, but arginine cannot be ubiquitinated, allowing for preservation of protein structure while allowing us to test the importance of ubiquitination. Asparagine and glutamine are amino acids that are found in other species (besides humans) at positions for K. These can serve as acetylated mimetics of lysines.

## Methods



**Figure 3. Schematic of overall experimental approach.** (a) Process for inserting successive mutations in the N-terminus of xCT (QuikChange Lightning Manual, 2015). We started with K37,41,43R and successively replaced K's at the other 3 positions. (b) Following transformation, the samples were mini-prepped and sent to Eurofins for DNA sequencing.

## Sanger Sequencing Trace for N-terminus of xCT



**Figure 4. DNA Sequencing results of final 6K mutant.** (a) Sanger sequencing trace of K4R and K12R on the N-terminus of xCT. A missense mutation occurred in both amino acids, changing Lys→Arg. (b) Sanger sequencing trace of K30R, K37R, K41R, and K43R on the N-terminus of xCT. In every case, the peak represented is guanine (G), which replaced adenine (A), leading to the change of the codon from AAA or AAG (lysine codons) to AGA or AGG (arginine codons), respectively.

## Comparison of 6K xCT mutant amino acid sequence to native *Homo sapiens*

Homo sapiens solute carrier family 7 member 11 (SLC7A11), mRNA  
Sequence ID: [NM\\_014331.4](#) Length: 9645 Number of Matches: 1

Range 1: 281 to 998 [GenBank](#) [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
1101 bits(596)	0.0	688/730(94%)	15/730(2%)	Plus/Plus

Query 166 ATGGTCAGAAAGCCTGTTGTGTCCACCATCTCCAGAGGAGTTACCTGCAGGGAATGTT 225  
Sbjct 281 ATGGTCAGAAAGCCTGTTGTGTCCACCATCTCAAAGGAGTTACCTGCAGGGAATGTT 340

Query 226 AACGGGAGGCTGCTCCCTGGGCAACAGGAGCACCTGGGAGGAGAGTGCAGCTG 285  
Sbjct 341 AACGGGAGGCTGCTCCCTGGGCAACAGGAGCACCTGGGAGGAGAAAGTGCAGCTG 400

Query 286 AGGAGGAGAGTCACTTTACTGAGGGAGTCTCATTATCATTGGACCATCATTGGAGCA 345  
Sbjct 401 AAGAGGAAAGTCACTTTACTGAGGGAGTCTCATTATCATTGGACCATCATTGGAGCA 460

**Figure 5. Nucleotide-blast for mutated xCT.** A nonsynonymous SNP from A→G at positions 291, 315, 369, 390, 402, and 408 causes a missense mutation of Lys→Arg in K4R, K12R, K30R, K37R, K41R, and K43R.

## Conclusions

- Previous work in the Chase lab has shown the replacement of individual lysines with arginines does not lead to obvious changes the ubiquitination of a transporter
- Currently, all 6 N-terminal lysines in xCT have been replaced by arginines.
- It is unknown if complete removal of lysines in the N-terminus will result in a lack of ubiquitination or change in transporter function or overall expression.

## Future Directions

- Transfect COS-7 cells (mammalian cells) with the 6K xCT mutant to study its level of expression and function in cells
- Immunoprecipitate the 6K xCT from the cells and perform Western blotting to examine the ubiquitination status of the 6K xCT mutant
- Examine the rate of turnover and cell surface expression of the 6K xCT mutant.
- If we find that xCT is still being ubiquitinated, investigate what other aspects of xCT may allow ubiquitin to bind to the protein.

## Acknowledgments

- Schaap Endowed Funds for Undergraduate Research
- Amanda Gibson for creating the initial 3KR xCT mutant

## References

- Lin, A., Hou, Q., Jarzylo, L., Amato, J., Gilbert, F., Shang, and H. Man. 2011. Nedd4-mediated AMPA receptor ubiquitination regulates receptor turnover and trafficking. *JNChem* 119:27-39.
- QuikChange lightning site-directed mutagenesis kit instruction manual. 2015.