

Investigation of the effects of storage and freezing on metabolites and amino acids

Rachel Culp-Hill, MSc

Department of Biochemistry and Molecular Genetics

University of Colorado - Anschutz Medical Campus

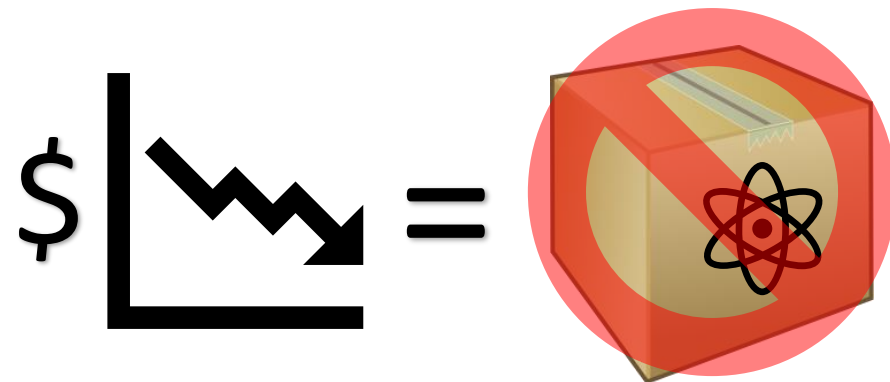
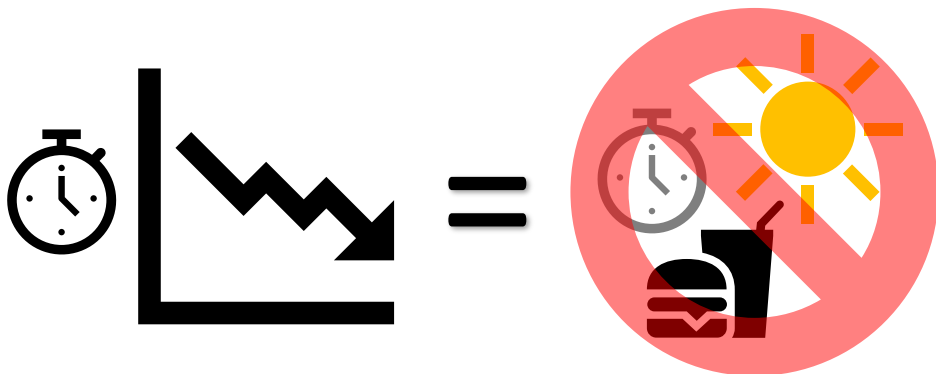
How much are we really spending on extractions?

15 min per standard, weigh/dilute
Avg. 2 standards per project
10+ projects per week

Avg. 2 standards per project
10+ projects per week
Avg. cost per standard per extraction = \$9.09

245 hours/year

\$1500 – \$16,000/year



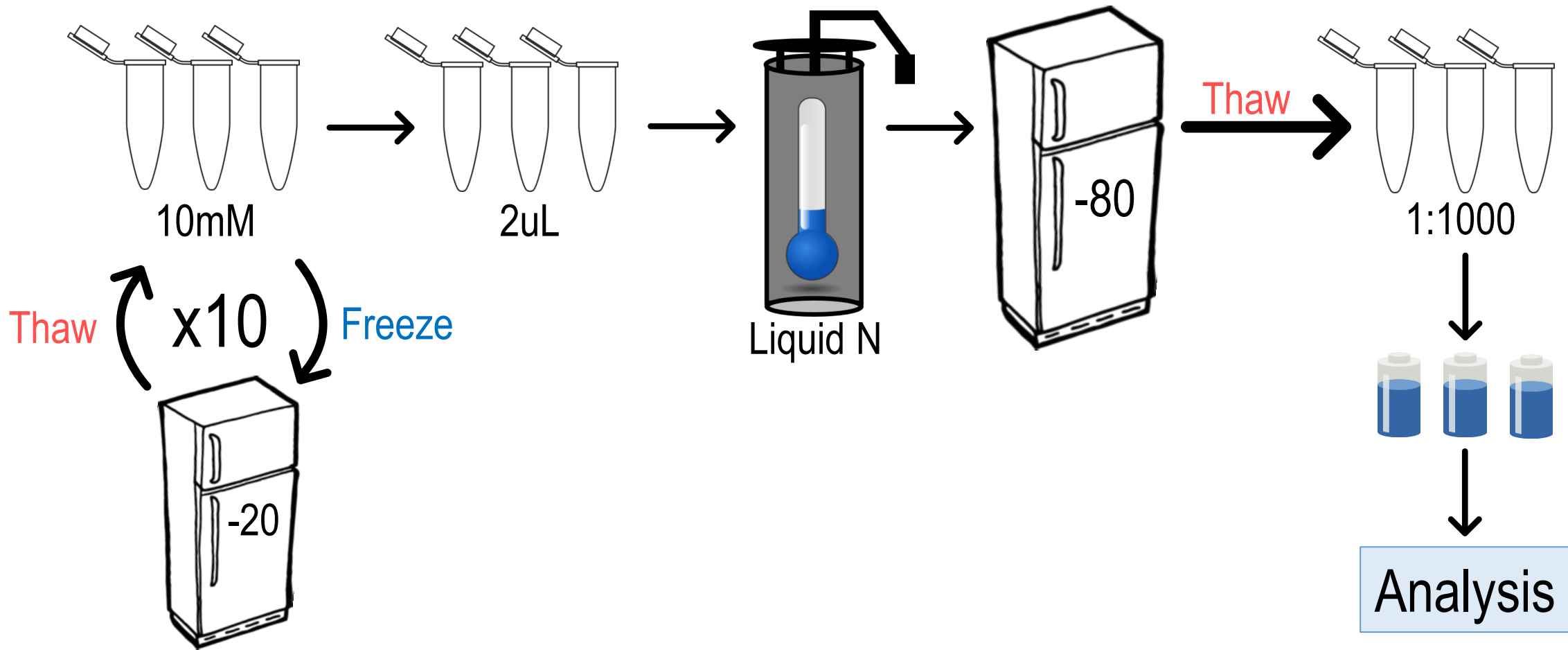
Questions to answer:

1. If we pre-dissolve individual standards in their appropriate solutions, store at -20C, and thaw for each use, will this affect the concentration over 10 freeze-thaw cycles?
2. If we pre-make a standard mix in lysis buffer and store at -20C, will this affect the concentration over 2 working weeks?
3. If we open a new ampule of amino acid mix and store at +4C, will this affect the concentration over 2 working weeks?

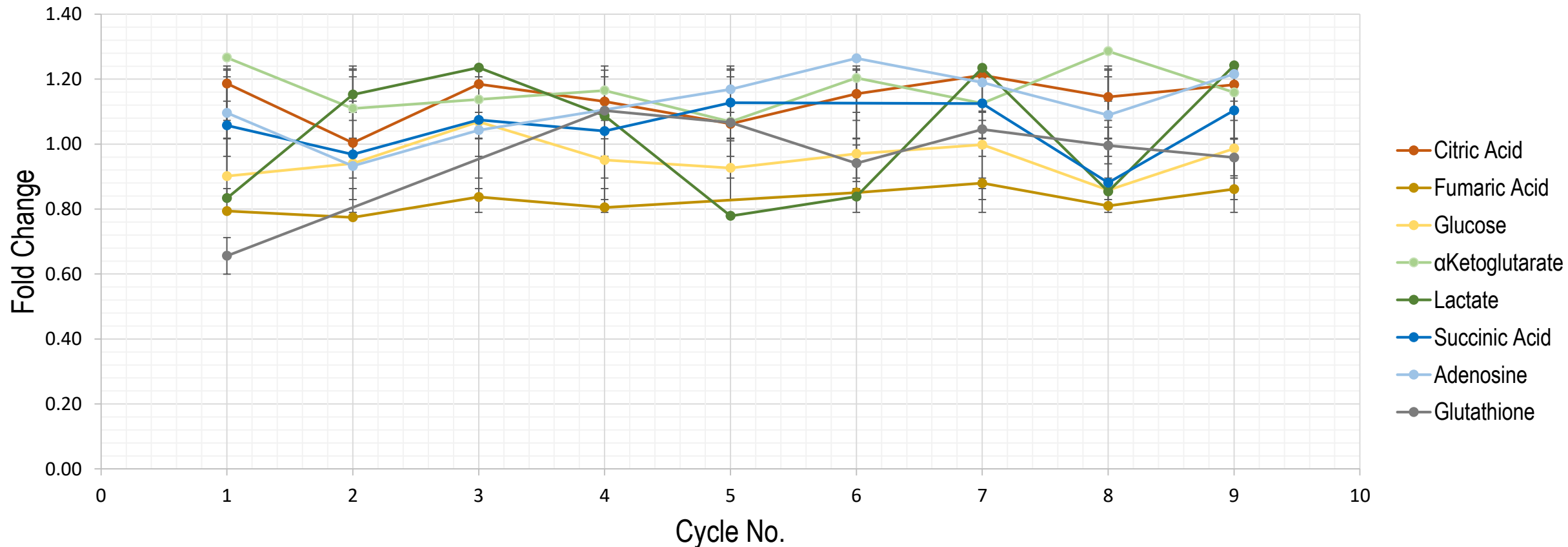
<u>Metabolite</u>	<u>Solvent</u>
Adenosine	water, heat
Citric acid	water
Fumaric acid	water, 1uL NH4OH
Glucose	water
Glutathione (GSH)	0.1% formic acid
a-Ketoglutarate	water
Palmitic acid	MeOH
Sodium lactate, 20% (w/w) in water	water
Succinic acid	water, 1uL NH4OH
Uric acid	water
Sphingosine 1-phosphate	10:3:2 MeOH: MeCN:H2O
Choline	water
N,N-dimethylglycine HCl	water
betaine (N,N,N-trimethylglycine) HCl	water
Trimethylamine N-oxide	water

Experiment 1

Metabolite Freeze-Thaw

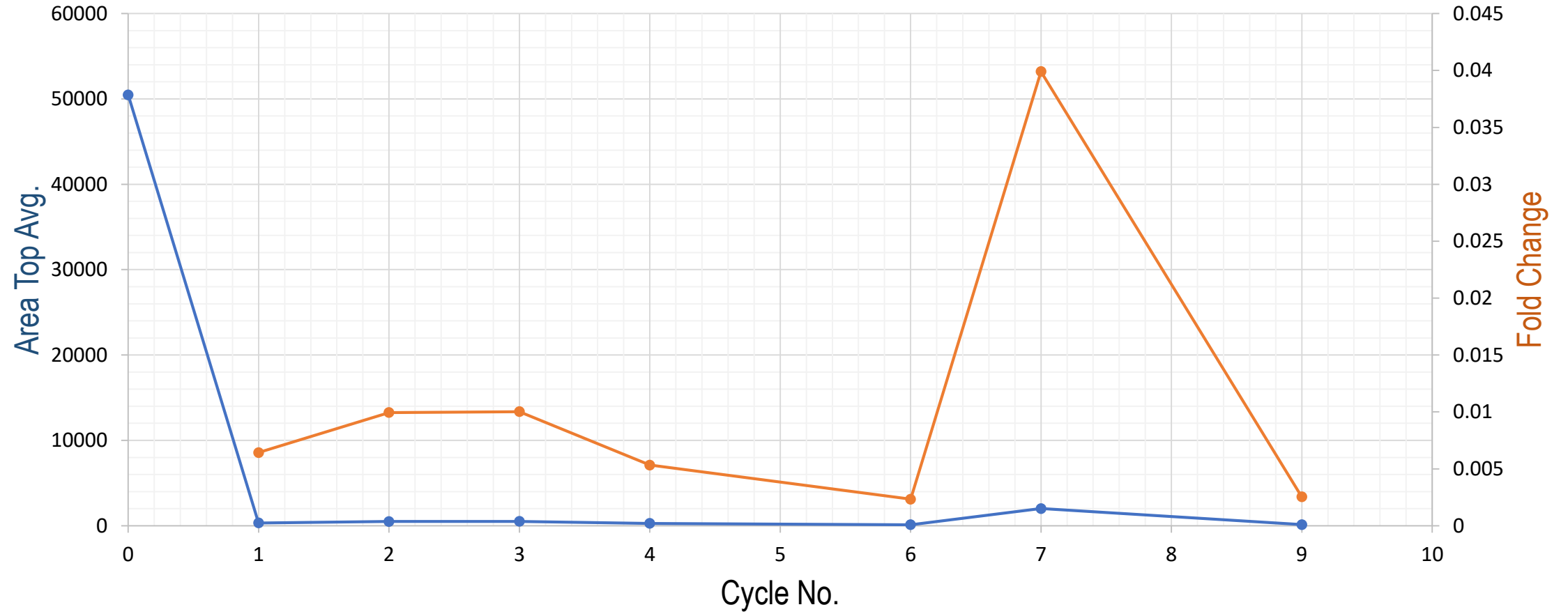


Freeze-Thaw Metabolites in Lysis Buffer



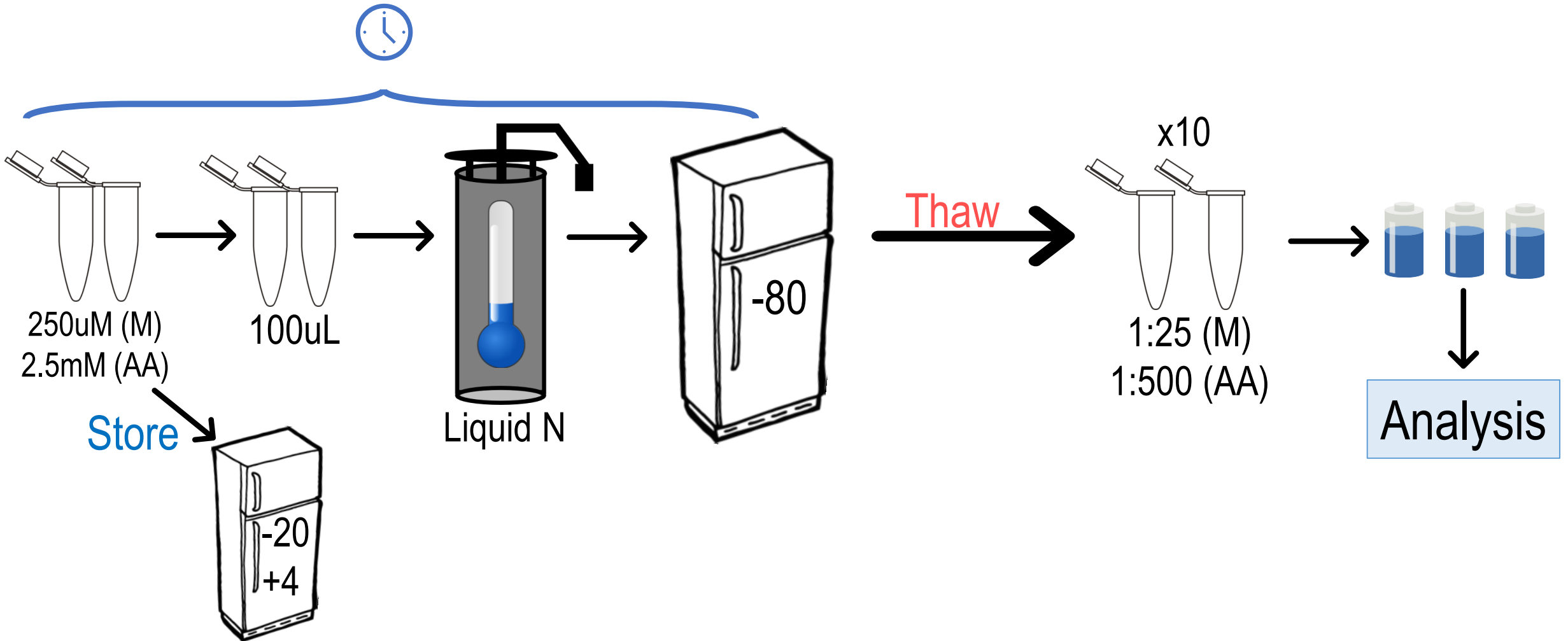
Notable Exception

Palmitic Acid

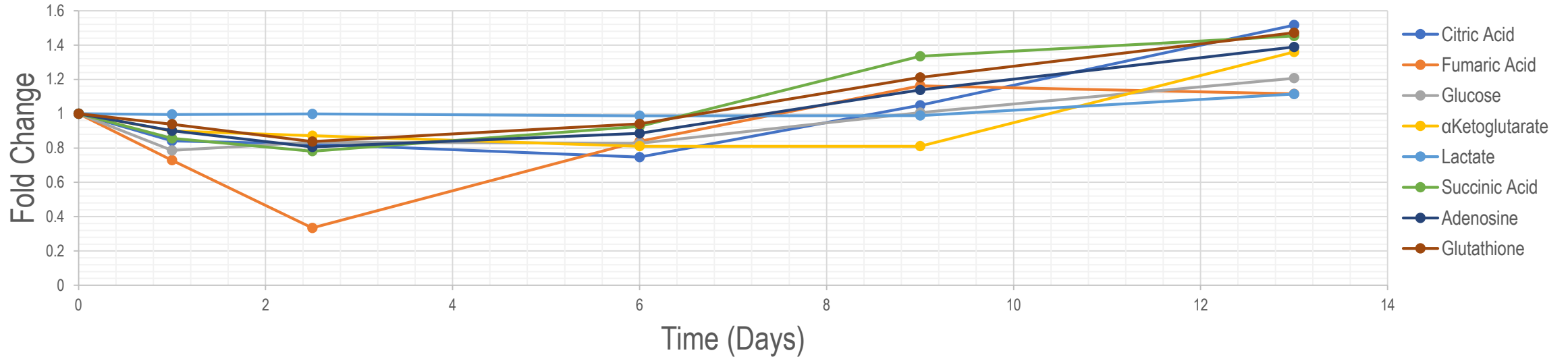


Experiment 2

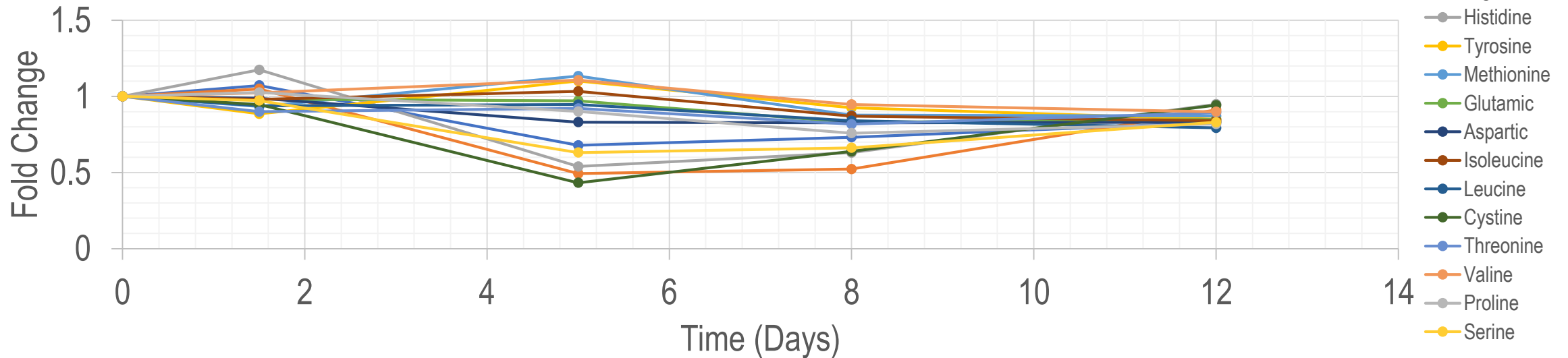
Metabolite & Amino Acid Mix Over Time



Metabolite Mix



Amino Acid Mix



Conclusions

1. Pre-dissolving individual standards in solution, storing at -20C and thawing for each use will not affect the concentration of the metabolite over 10 freeze-thaw cycles.
2. Pre-making a standard mix in lysis buffer and storing at -20C will not affect the concentration of the metabolites over the course of 13 days.
3. Opening a new ampule of amino acid mix and storing at +4C will not affect the concentration of the amino acids over the course of 12 days.

THEN:

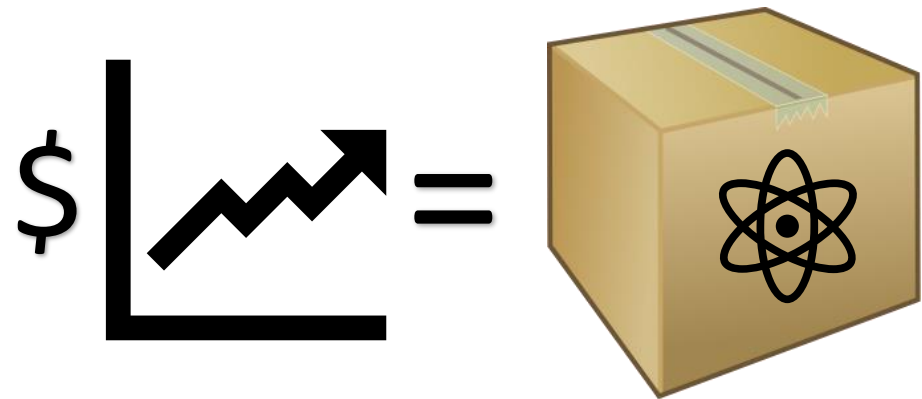
245 hours/year

\$1500 - \$16,000/year

NOW: Up to 10 freeze-thaw cycles kept at -20C/+4C before replacing

24.5 hours/year

\$150 - \$1600/year



Following up...

- Palmitic Acid
- Lipids
- Bile Acids
- Carnitine Mix
- ATP
- Longer study for amino acids (months)
- Abs. quant of metabolites in different biological matrices – create a “go-to guide”

Acknowledgements

