In situ X-ray Protein Footprinting of Mitochondrial Proteins

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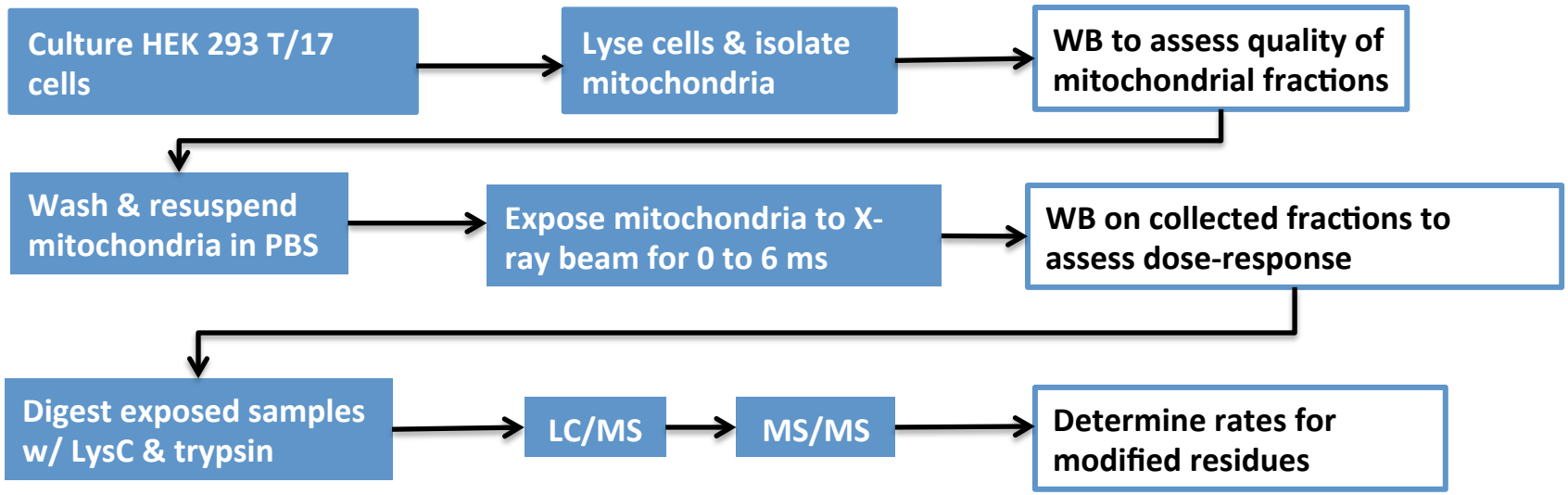
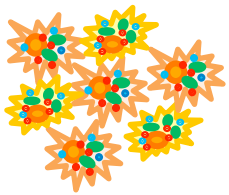
ALS User Meeting

Goal and Significance

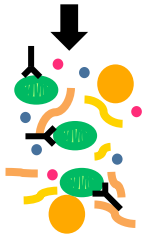
- **Goal**
  1. Develop a method for in situ X-ray exposure of intact, functional mitochondria
  2. Study the dynamics and function of respiratory chain proteins and complexes in their native environment under different metabolic states
- **Significance**
  1. Eliminates structural stability as a concern when studying membrane proteins and large macromolecular complexes
  2. Preserves native interactions potentially relevant to physiological function



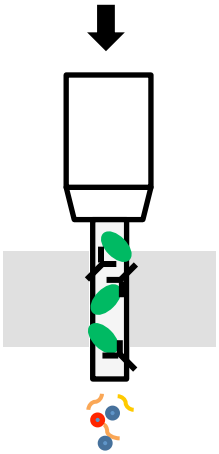
Mitochondrial Isolation



Cultured ~1.26 x 10^8 HEK 293 T/17 cells



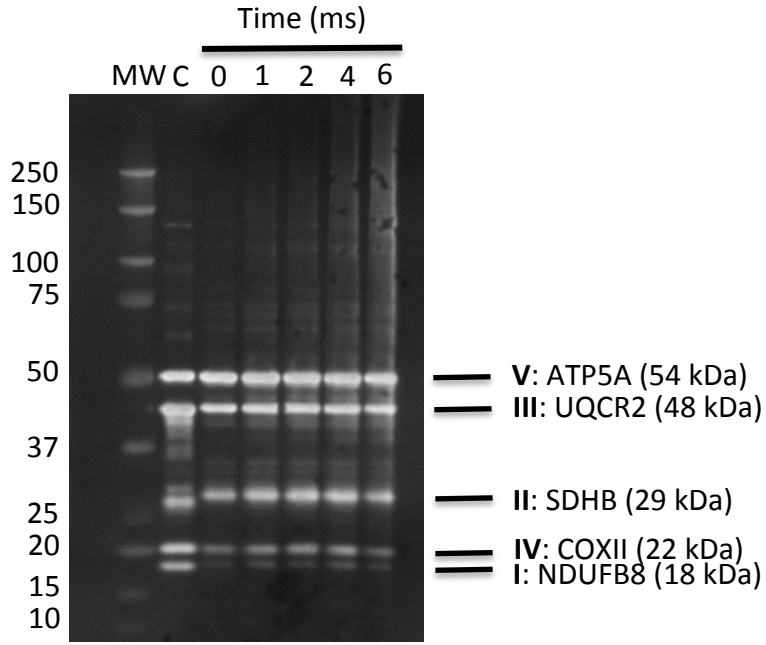
Lysed cells and labeled mitochondria w/ magnetic anti-TOM22 microbeads



Applied labeled cell lysate to magnetic column placed in MACS Separator.

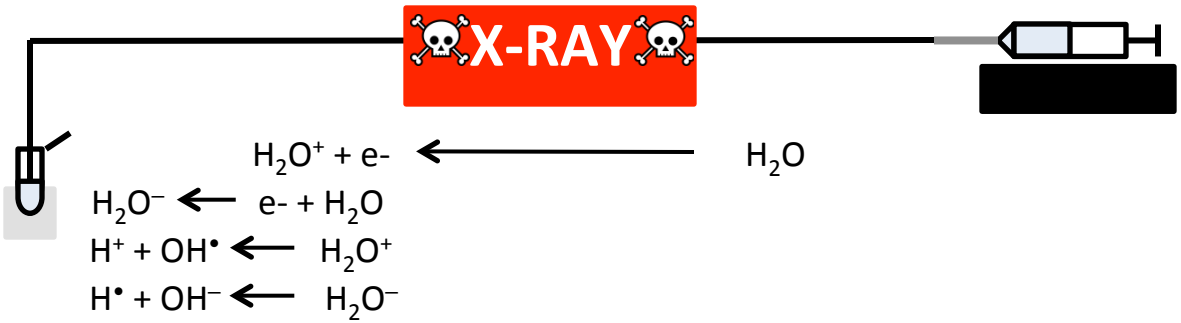
Removed column from MACS Separator in order to elute mitochondria.

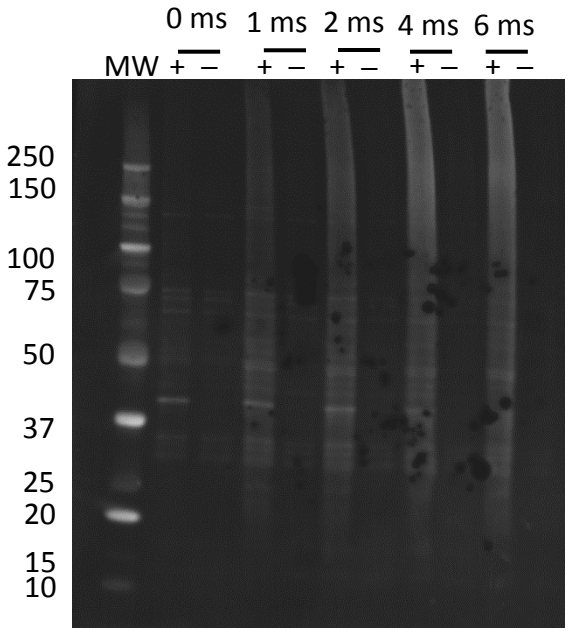
Isolated mitochondria was stored at -80°C in a buffer containing 300 mM trehalose

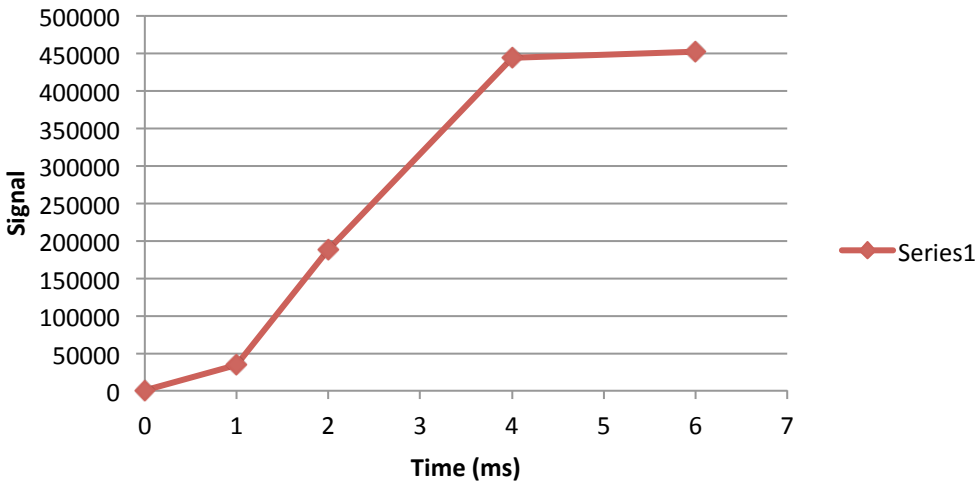


MACS separation isolated 383 ± 106 µg of protein from 1x10^8 cells.

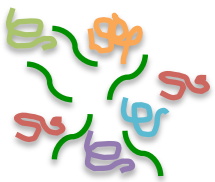
X-Ray Exposure of Mitochondria



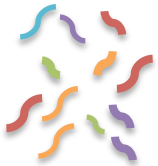




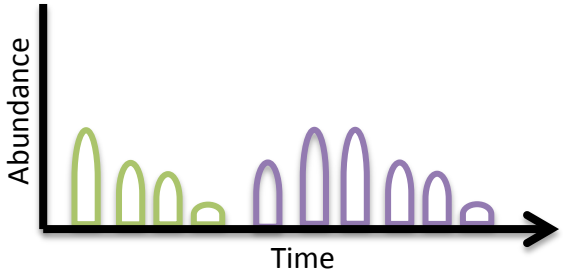
MS Analysis



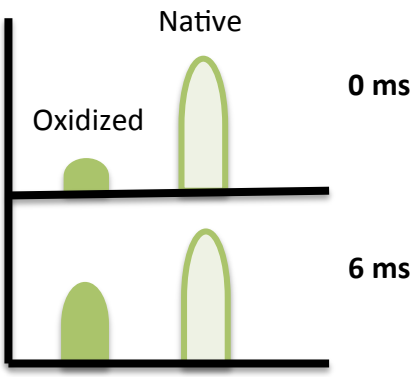
Lyse & solubilize mitochondrial membrane and proteins in SDS



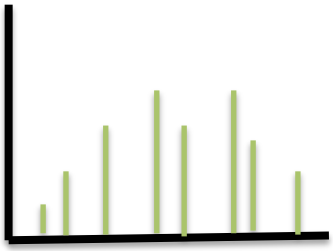
Cleave proteins into peptide fragments w/ Lys C (3hrs) & trypsin (O/N)



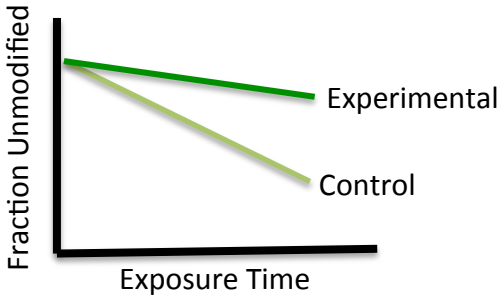
Separate peptides & determine m/z using reverse phase chromatography



Extract peptides and measure peak areas



Examine MS/MS of fragmented peptide to determine site of modified residue



Determine rate of modification for oxidized residue

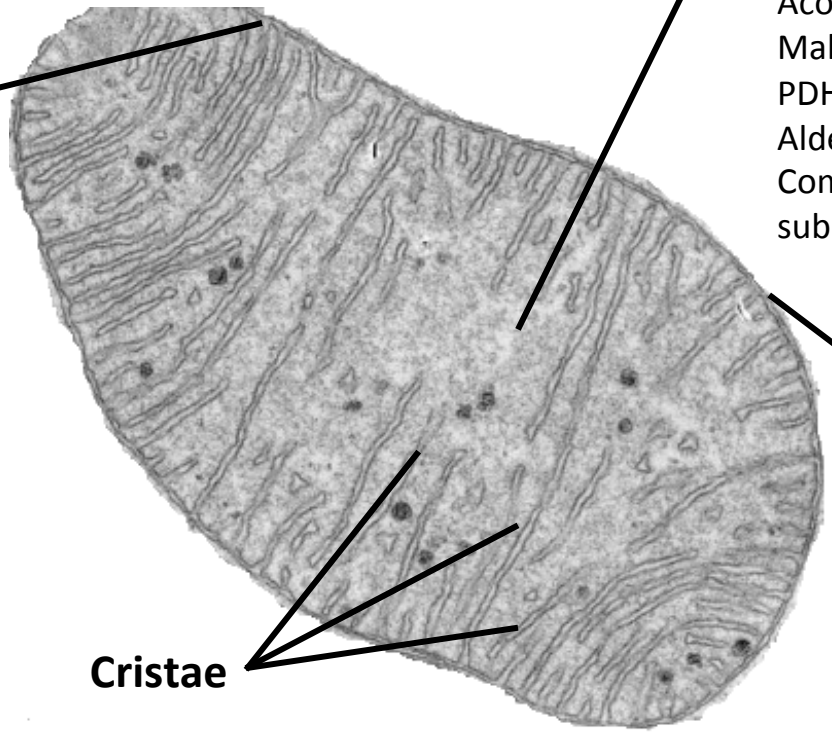
Top Proteins Detected via MS Analysis

Matrix

10, 60, & 75 kD heat shock proteins
Citrate synthase
Aconitase hydratase
Malate dehydrogenase
PDH E1 component subunits α & β
Aldehyde dehydrogenase
Complement component 1 Q subcomponent-binding protein

Inner membrane

Complex I:
NADH-ubiquinone oxidoreductase 75 kDa subunit
NADH dehydrogenase iron-sulfur protein 2
Complex II:
Succinate dehydrogenase, flavoprotein subunit
Complex III:
Cyt b-c1 subunit 1 & 2
Complex IV:
Cyt c oxidase 1,2
Complex V:
ATP synthase subunits α, β, γ, & d



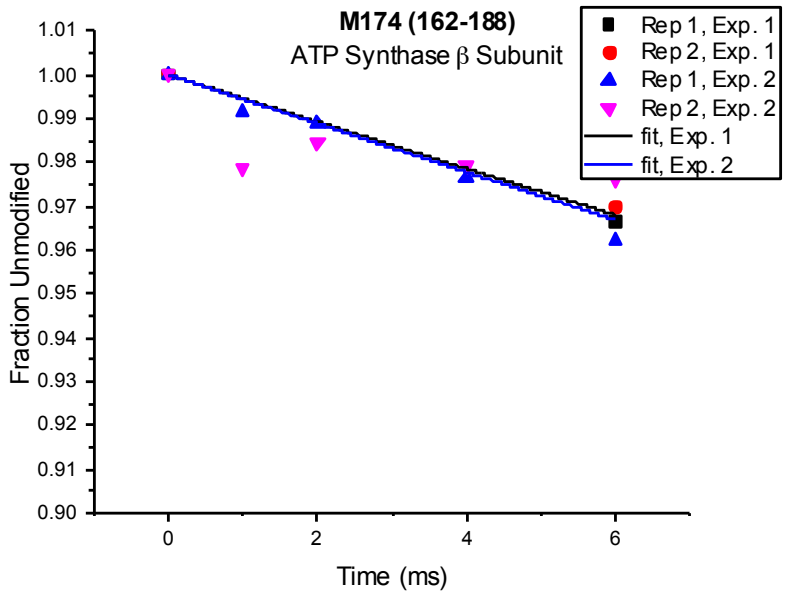
Cristae

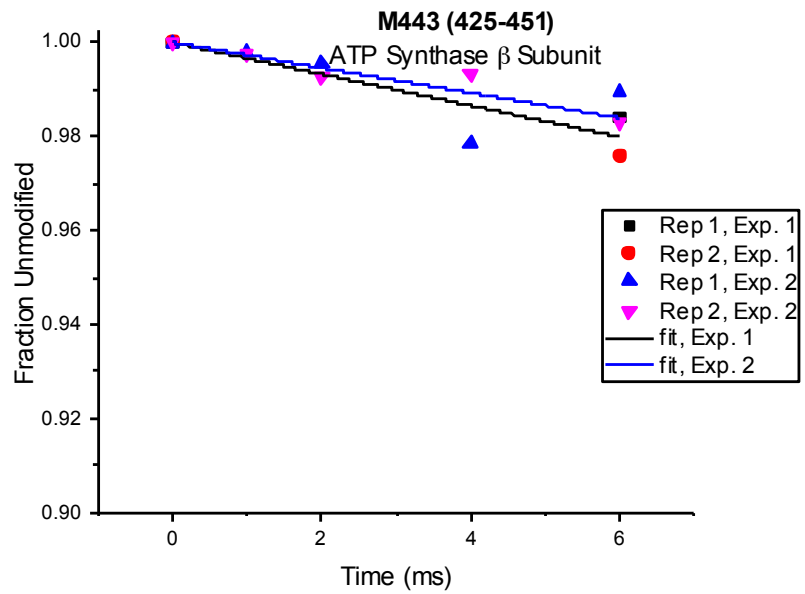
Outer membrane

Mitochondrial import receptor subunit TOM40
Voltage-dependent anion selective channel proteins 1-3

1504 Proteins Identified per sample → 233 Modified Proteins per Sample

ATP Synthase Beta Subunit (ATPB)

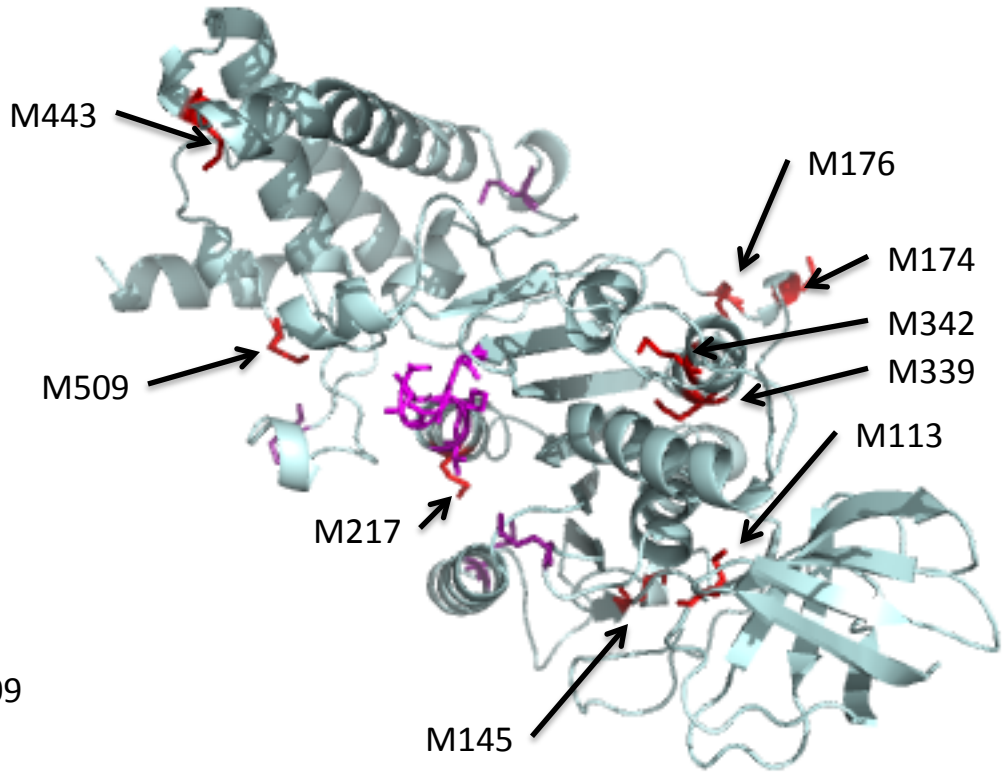




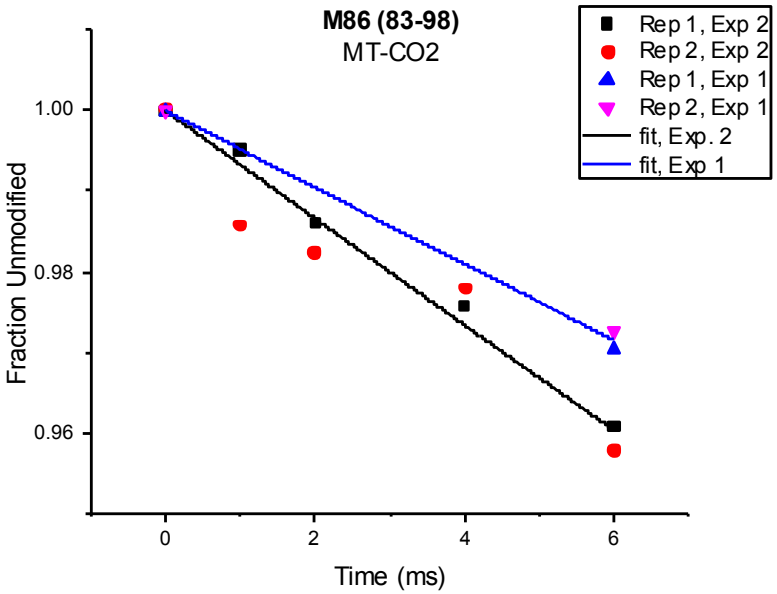
ATPB

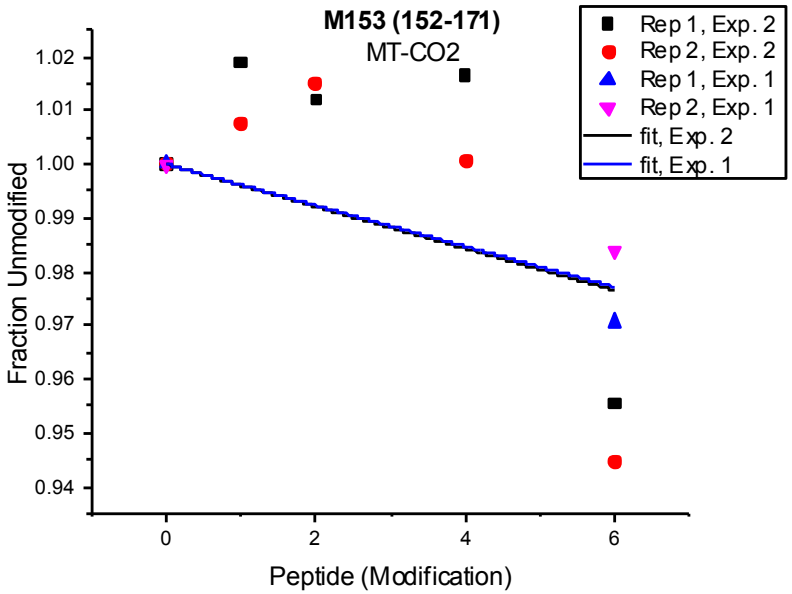
	Exp. 1	Exp. 2
Modification	**Rate (s^{-1})**	**Rate (s^{-1})**
M113	--	9.1
M145	--	6.3
M174	4.0	5.6
M176	1.0	6.8
M217	--	2.4
M339	--	1.0
M342	7.0	-0.6
M443	3.0	2.7
M509	1.0	1.2

Rate: M113 > M145 > M176 > M174 > M443 > M509



Cyt C Oxidase Subunit 2 (MT-CO2)





MT-CO2

	Exp. 1	Exp. 2
Residue	**Rate (s^{-1})**	**Rate (s^{-1})**
M86	6.75	4.82
M153	3.96	3.86

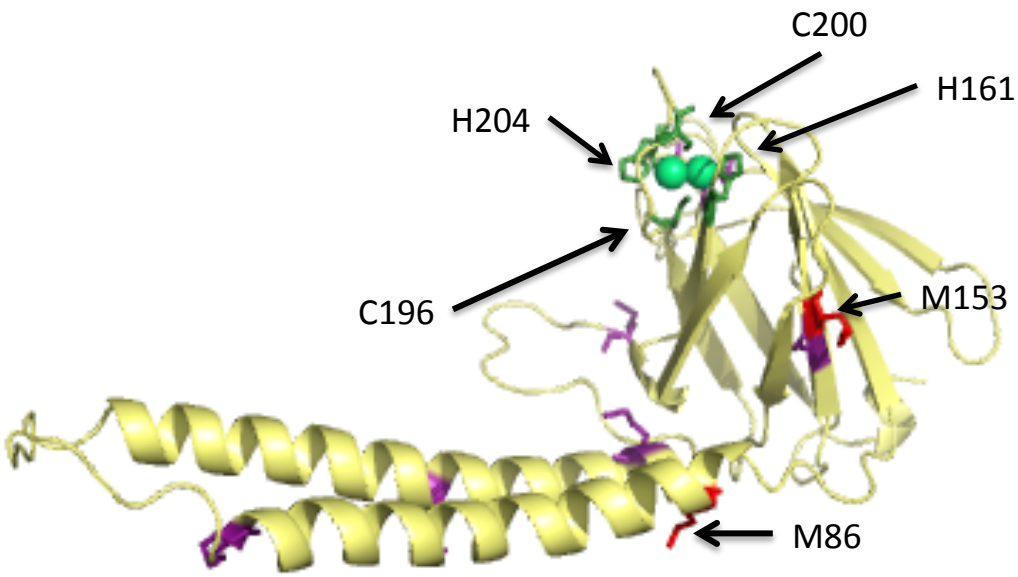
Rate: M86 > M153

Modified residue

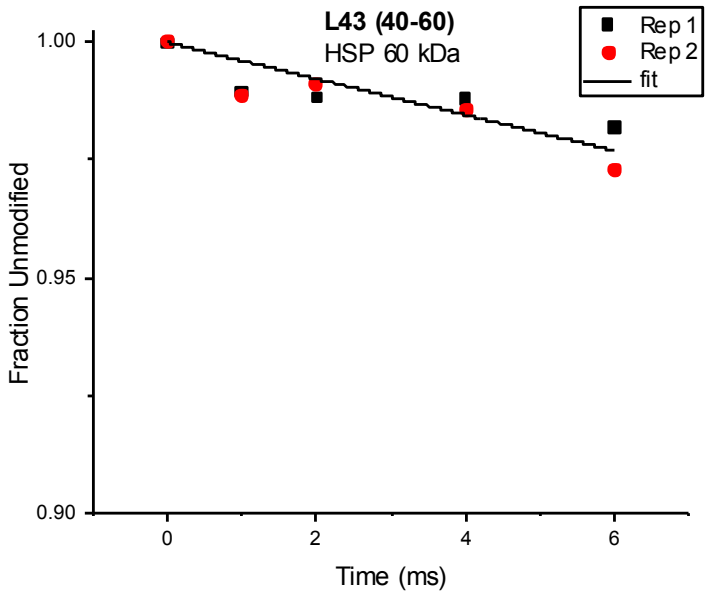
Unmodified M

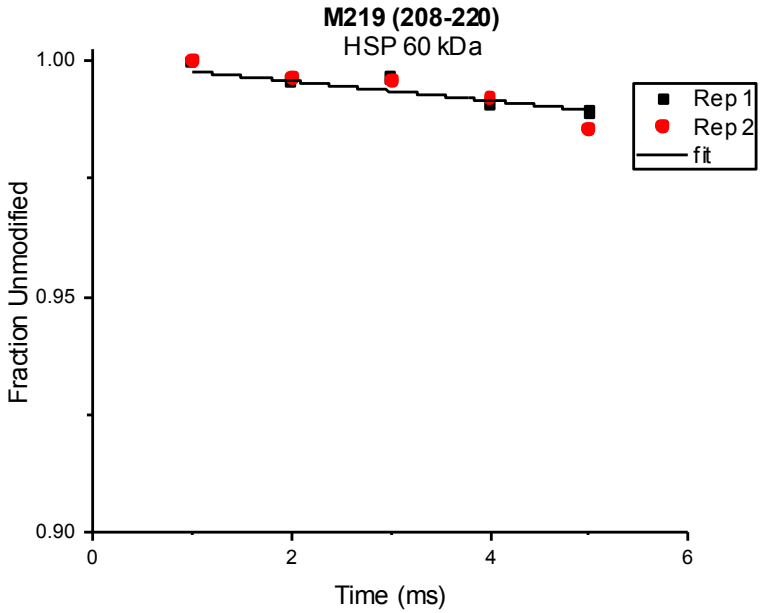
Copper-binding residues

Copper

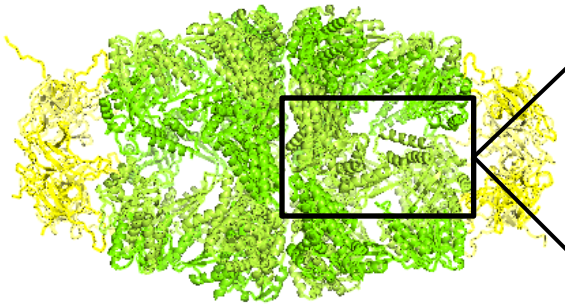


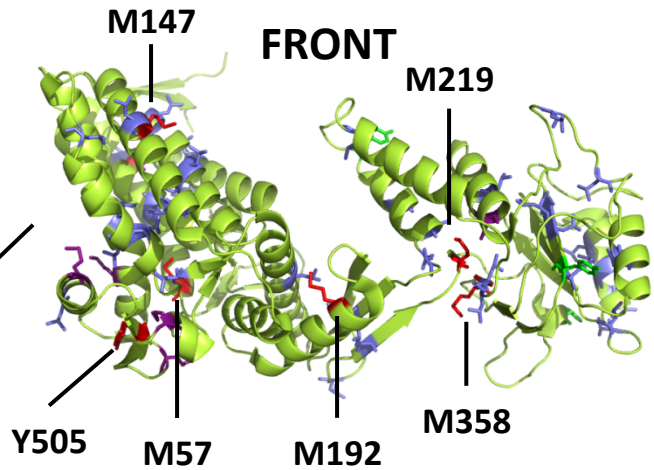
HSP60 D-R Curves

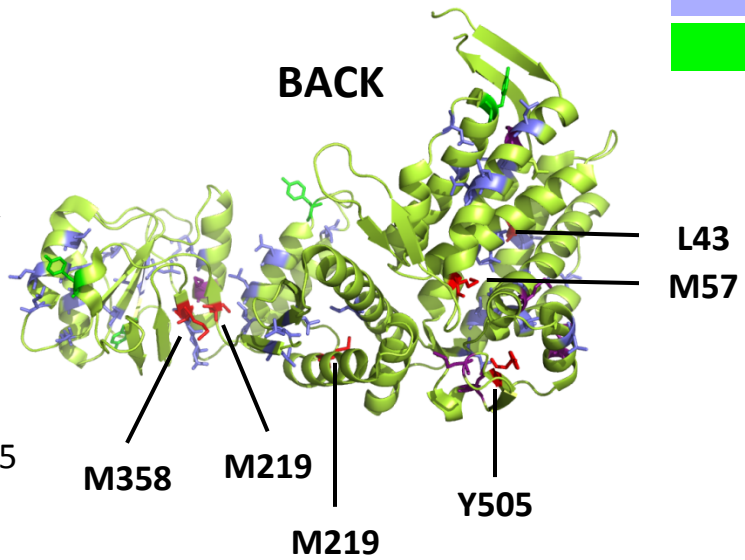




60 kDa Heat Shock Protein (HSP60)





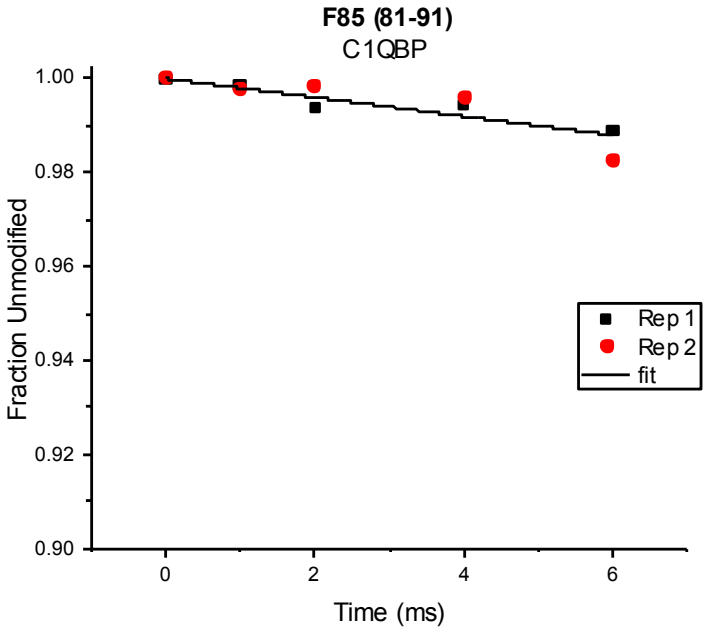


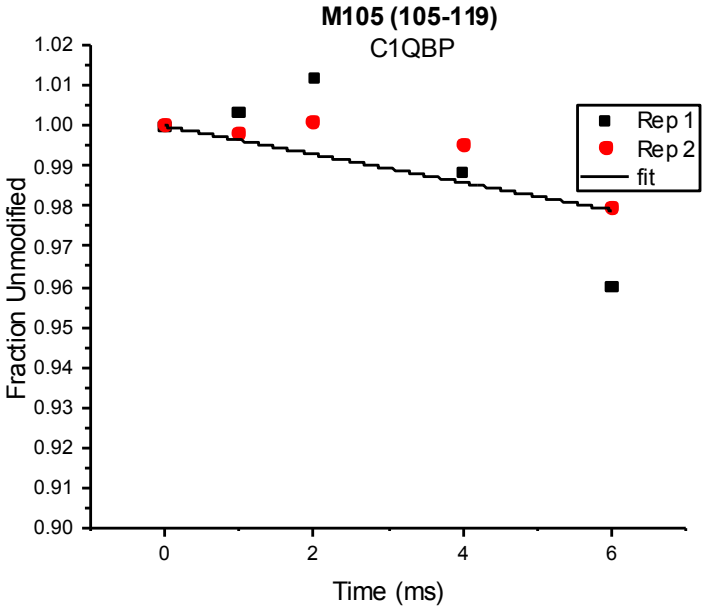
Modified residue
Unmodified M
Unmodified L
Unmodified Y

Residue	Rate (s^{-1})
L43	3.89
M57	4.53
M192	1.31
M219	2.09
Y505	-1.46

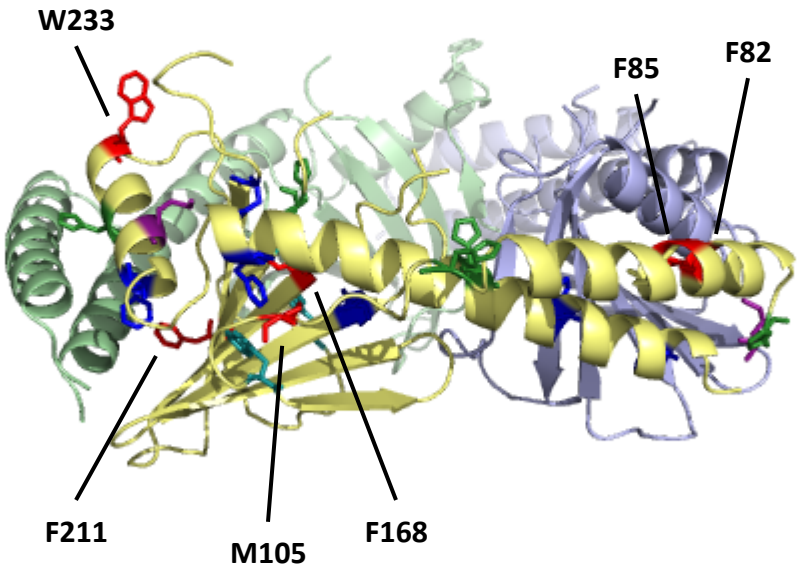
Rate: M57 > L43 > M219 > M192 > Y505

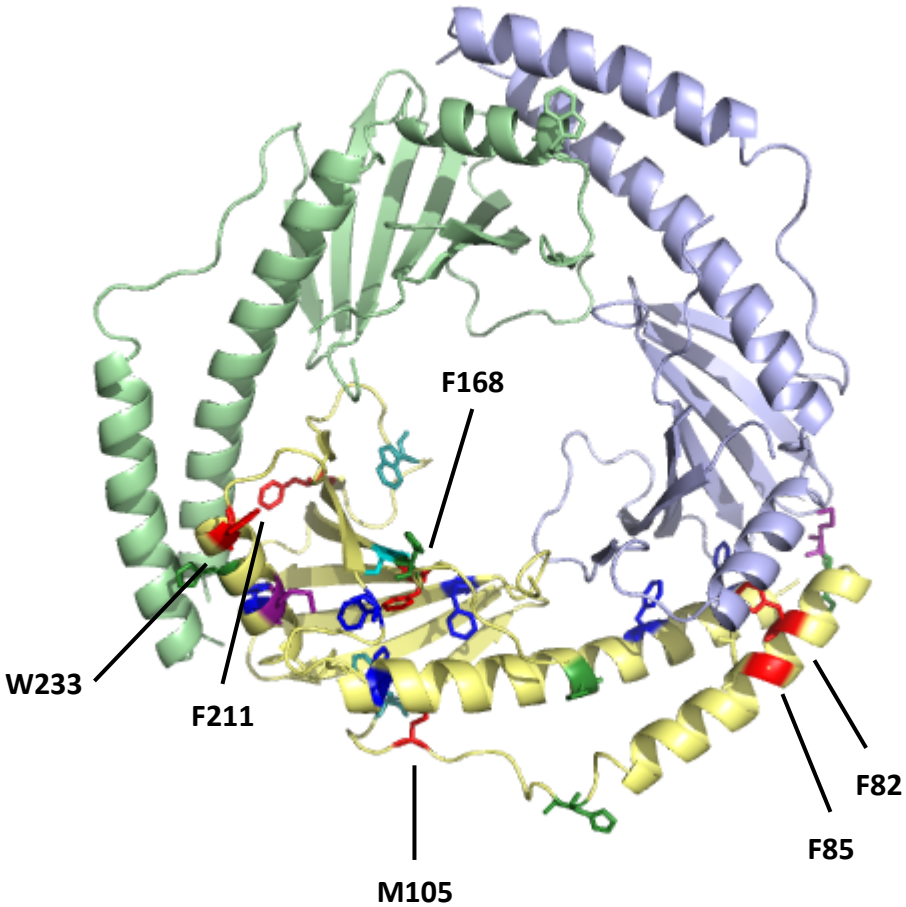
Complement Component 1 Q Subcomponent-binding Protein (C1QBP)





C1QBP





- Modified residue
- Unmodified M
- Unmodified F
- Unmodified W
- Unmodified H
- Unmodified C

Residue	Rate (s^{-1})
F82	0.76
F85	2.02
M105	3.53
F168	0.41
F211	1.25
W233	-0.08

Rate: M105 > F85 > F211 > F82 > F168 > W233

Conclusions & Future Work

- **Conclusions**
  1. Isolated highly pure mitochondria using MACS separation
  2. Oxidized mitochondrial proteins from various compartments in their native environment
  3. Demonstrated a dose-response with increased exposure time via WB and MS analysis
- **Future Work**
  1. Expose mitochondria in the presence of nitrous oxide
     - Nitrous oxide has been shown to increase the number of hydroxyl radicals generated during radiolysis of H_2O.
  2. Develop selected reaction monitoring (SRM) MS experiment to improve analytical sensitivity
     - SRM should increase instrument sensitivity by ten-fold.
  3. Perform oxidative phosphorylation assays to verify functionality of mitochondria prior to exposure
  4. Generate micrographs of mitochondria to monitor mitochondrial structural integrity under different conditions

Acknowledgements

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