Top Down Proteomics of Soluble and Integral Membrane Proteins from Human Mitochondria

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OVERVIEW

- Isolation of mitochondria followed by GELFrEE and nano LC-MS/MS allows for >100 identified intact mitochondrial proteins from human cell lines
- The developed Top Down proteomics platform is readily amenable to the analysis of membrane proteins on an LC timescale , capable of identifying post-translational modifications
- Examination of the fragmentation of integral membrane proteins has revealed extensive fragmentation on the transmembrane domain helices, leading to hyperconfident identification

INTRODUCTION

Mitochondria provide important cellular functions including oxidative phosphorylation, fatty acid biosynthesis, and acting as gatekeepers to apoptosis. These biological processes are highly regulated by post-translational modification and protein cleavage events, making them highly attractive to study at the intact protein level. With recent advances in protein separation technology and instrumentation it is now possible to detect, identify, and characterize hundreds to thousands of intact proteins in an isoform specific manner. Even with these advances, integral membrane proteins, which play vital roles in mitochondrial function, are often underrepresented in proteomic studies due to their hydrophobic character and lower abundance. In this work, we establish protocols for the enrichment, separation, identification, and characterization of mitochondrial proteins with particular emphasis on integral membrane proteins.

METHODS

- Intact mitochondrial were isolated from HeLa or H1299 cells by use of differential centrifugation followed by Percoll density gradient centrifugation
- Molecular weight fractions were prepared using the GELFREE 8100 Fractionation System (Expedeon). SDS was removed from the fractions using MeOH/$CHCl_3$/H_2O precipitation.
- Nano-LC separations were performed PLRPS columns utilizing gradients of 05% H_2O, 5% ACN and 95% ACN, 5% H_2O each with 0.2% formic acid.
- Mass spectra were collected on both 12 T LTQ Velos FT-ICR or Orbitrap Elite instruments.

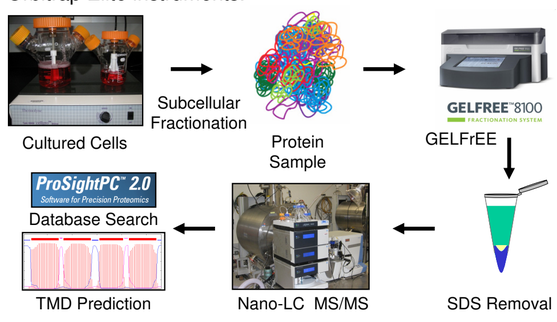


Figure 1: Platform for the Top Down analysis of mitochondrial proteins.

SAMPLE FRACTIONATION

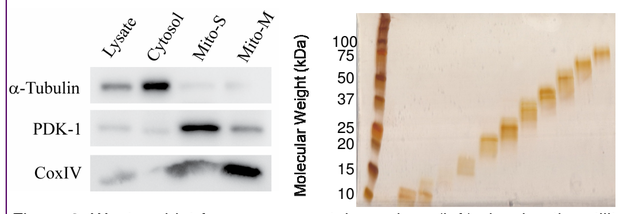
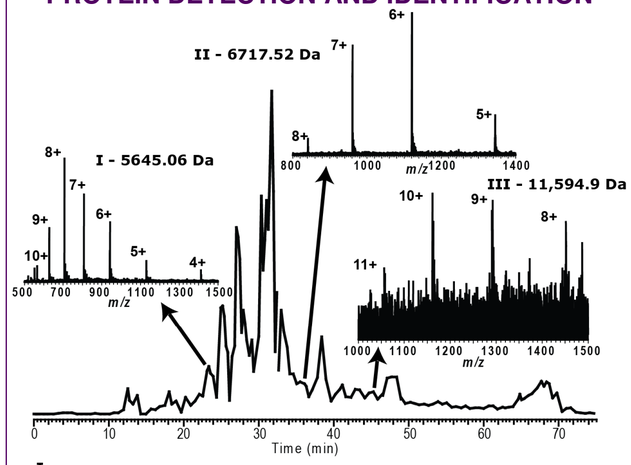


Figure 2: Western blot for common protein markers (left) showing the utility of the subcellular fractionation and a slab gel visualization of the GELFrEE separation (right) the mitochondrial membrane enriched fraction.

PROTEIN DETECTION AND IDENTIFICATION



I
-V[A]Y[W·R·Q·A]G·L[S]Y[I·R·Y·S·Q]I·C[A]K[A·V·R·D]A·L[K]T[E]F[K]
-A[N]A[E]K[T]S[G·S·N·V]K[I]V[K]VK[K·E·
ATP synthase subunit epsilon
q value: 1 x10^{-61}
Δ mass = -0.7 ppm

II
-F[K]N[K]V[P·E]K·Q·K·L·F[Q]E[D·D]E[I]P·L[Y·L]K[G·G]V[A]D[A·L·L]
-Y·R·A·T·M[I]L[T·V]G[G]T[A]Y[A]I[Y·E·L·A]V[A]S[F]P·K·K·Q]E·
Cytochrome *c* oxidase polypeptide 7A2
q value: 1 x 10^{-65}
Δ mass = -1.3 ppm

III
-M[Q·D·T]G·S·V[V]P·L·H·W·F[G·F]G·Y·A[A]L[V·A·S]G[G]I·I[G]Y·V[K·
-A[G·S·V]P·S·L[A]A[G]L[L]F[G]S[L]A[G]L[G]A[Y]Q·L[S·Q]D[P·R·N·V·
-W[V·F·L·A·T·S]G·T·L·A·G·I·M·G·M·R·F·Y·H·S·G·K·F·M·P·A·G·L·I[A·
-G·A·S·L]L·M·V·A·K·V·G·V·S·M·F·N·R·P·H·
Transmembrane protein 14C
q value: 3 x 10^{-53}
Δ mass = -4.4 ppm

Figure 3: Total ion chromatogram in which three protein mass spectra are shown. Fragmentation of each species resulted in confident identification of different modified forms Transmembrane helices are shown in red.

GLOBAL IDENTIFICATION

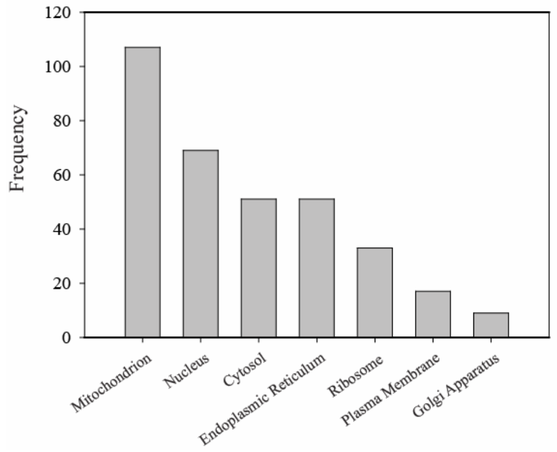


Figure 4: Subcellular localization of the 246 proteins identified from enriched mitochondrial membrane fraction derived from HeLa cells. Of the proteins identified within the mitochondrion, most were derived from the inner mitochondrial membrane.

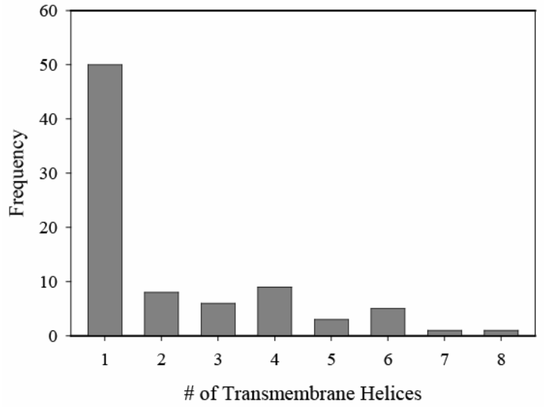
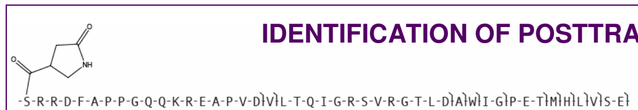


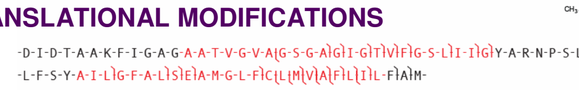
Figure 5: Distribution of the number of transmembrane helices for the 83 integral membrane proteins identified from HeLa. Thirty-three proteins contained two or more transmembrane helices.

IDENTIFICATION OF POSTTRANSLATIONAL MODIFICATIONS



-S-R-R-D-F-A-P-P-G-Q-Q-K-R-E-A-P-V-D[V]L-T-Q-I-G-R-S-V-R-G-T-L-D[A]W[I-G]P-E-T[M]H[L]L[V]S-E[
-S]S[S-Q]V[L]L[W]A[I]I[S]S-A[I]S[V-A]F-F-A[L-S]G-I-A-A-Q-L-L-N-A-L-G-L-A-G-D-Y-L-A-Q-G-L-K-L-S-
-P-G-Q-V-Q-T-F-L[L]L[W[G-A[G-A[L-V[V[Y[W-L-L[S-L[L[L[G-L-V[L[A-L-L-G-R-I-L-W-G-L-K-L[V[I[F-L-
-A-G-F-V-A-L-M-R-S-V-P-D[P-S-T-R-A-L-L-L-L-A-L[L-I-L-Y-A-L-L-S-R-L-T-G-S-R-A-S-G-A-Q-L-E-A-
-K-V-R-G-L-E-R-Q-V-E-E-L-R-W-R-Q-R-R-A-A-K-G-A-R-S-V-E-E-E-

Transmembrane protein 109 pyroglutamination



-D-I-D-T-A-A-K-F-I-G-A-G-A-A-T-V-G-V-A[G-S-G-A]G[I-G]T[V]F[G-S-L]I-I[G]Y-A-R-N-P-S-L-K-Q[Q]
-L-F-S-Y-A-I-L[G-F-A-L]S[E]A-M-G-L-F[C[L[M[V]A[F]L[I]L-F]A]M-

ATP synthase subunit c trimethylation

G-A-H-L-V-R-R-Y-L-G-D[A]S[V]E[P-D]P[L]Q[M-P-//-G-E-V-D[P-K-V-A-L-

NADH dehydrogenase 1 beta subcomplex subunit 7 myristoylation

ACKNOWLEDGEMENTS

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CID OF INTEGRAL MEMBRANE PROTEINS

-A-A-R-V-G-A-F[L]K-N-A-W-D[K-E]P-V-L[V]V[S]F[V]V[G]G[L]L[A]V[I]L[P[P-L-S-P-Y-F-K-Y-S-V-M-I-N-
-K-A-T-P-Y-N-Y-P-V-P-V-R-D-D-G-N-M[P-D]V[P-S-H[P-Q-D]P-Q-G-P-S-L-E-W-L-K-K-L-

NADH dehydrogenase 1 alpha subcomplex subunit 3

Average TMHMM score of protein: 0.25
Average TMHMM score of matching fragments: 0.69

-S-E-S-K-S-G-P-E-Y-A-S-F-F-A[V]M[G-A]S-A-A]M-V]F-S-A-L-G-A-A-Y-G-T-A-K-S-G-T-G-I-A-A-M-S-V-
-M-R-P-E-Q-I-M-K-S-I-I-P-V-V-M-A-G]I-I]A]I]Y]G-L-V-V-A-V]L]I-A[N-S-L-N-D-D-I-S-L-Y-K-S-F-L-
-Q-L]G-A]G]L]S-V]G]L-S]G[L-A]A-G[F-A-I-G-I-V-G-D-A-G-V-R-G-T-A-Q-Q-P-R-L-F-V-G-M-I-L]I-L[I]
[F-A]E-V]L[G[L]Y]G]L-I[V[A[L-I-L-S-T-K-

V-type proton ATPase 16 kDa proteolipid subunit

Average TMHMM score of protein: 0.59
Average TMHMM score of matching fragments: 0.96

Figure 6: Graphical fragments maps for two proteins which demonstrate the propensity for transmembrane helices to fragment relative to soluble regions.

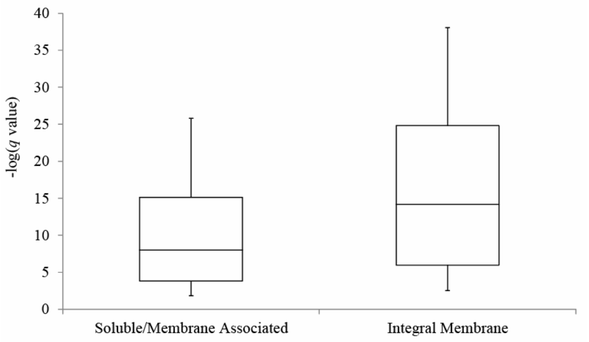

Figure 7: Box-plot demonstrating that the integral membrane proteins were on average identified with more confidence than the soluble and membrane associated proteins.

CONCLUSIONS

- Subcellular fractionation coupled to GELFrEE nano-LC MS/MS allows for effective Top Down study of the human mitochondrion
- Membrane proteins, with up to eight transmembrane domain helices, can be identified in a relatively high-throughput manner
- Top Down Proteomics is well suited for the identification of post-translational modifications on integral membrane proteins as well as lipid modifications on peripheral membrane proteins
- Collision-induced dissociation of integral membrane proteins leads to very confident identification through extensive fragmentation of the transmembrane domain helices

FUTURE WORK

- Future work will be focused on the comparison of untreated H1299 cells with those undergoing chemically-induced senescence
- Higher-energy collisional dissociation (HCD) and electron transfer dissociation (ETD) will be utilized fir fragmentation.
- Additional separation strategies will be applied to improve mitochondrial purity and proteome coverage