

# 利用基于肽段计数的无标记定量技术 揭示线粒体蛋白质组的功能特性

Quantitative Analysis of Mitochondrial Proteomes  
using Normalized Spectral Abundance Factor

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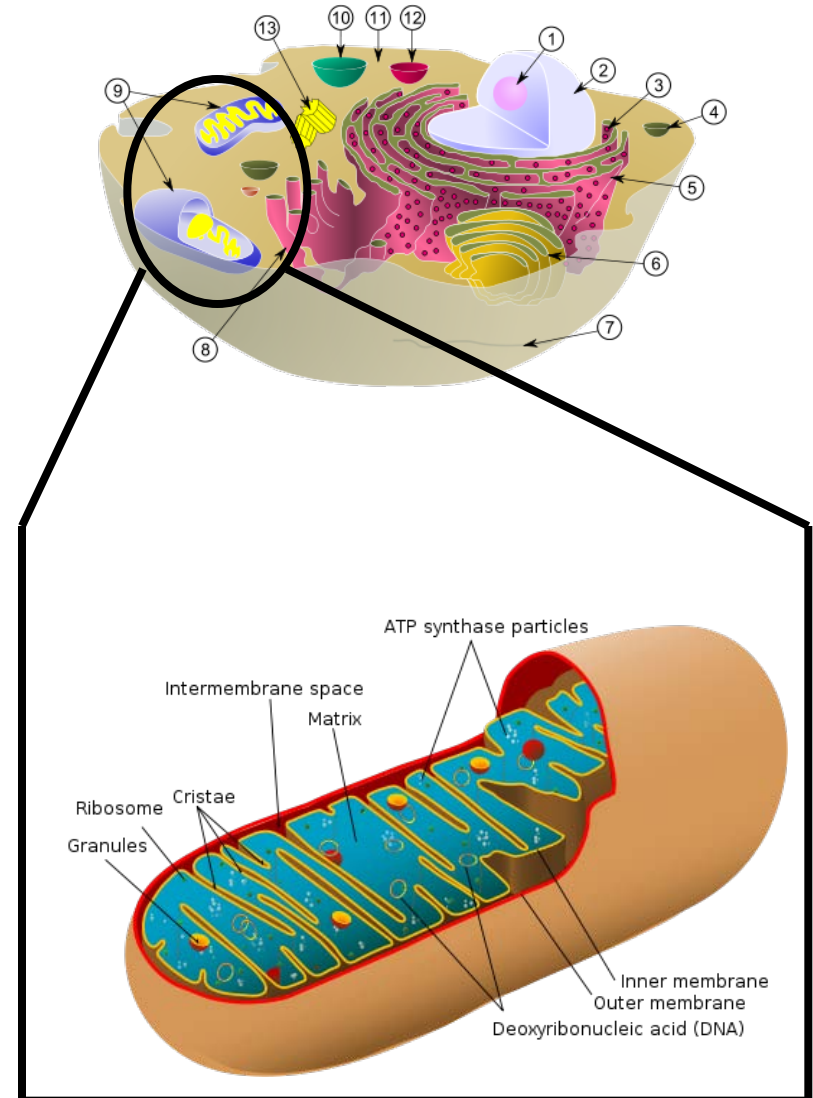
邓宁

浙江大学生物医学工程与仪器科学学院

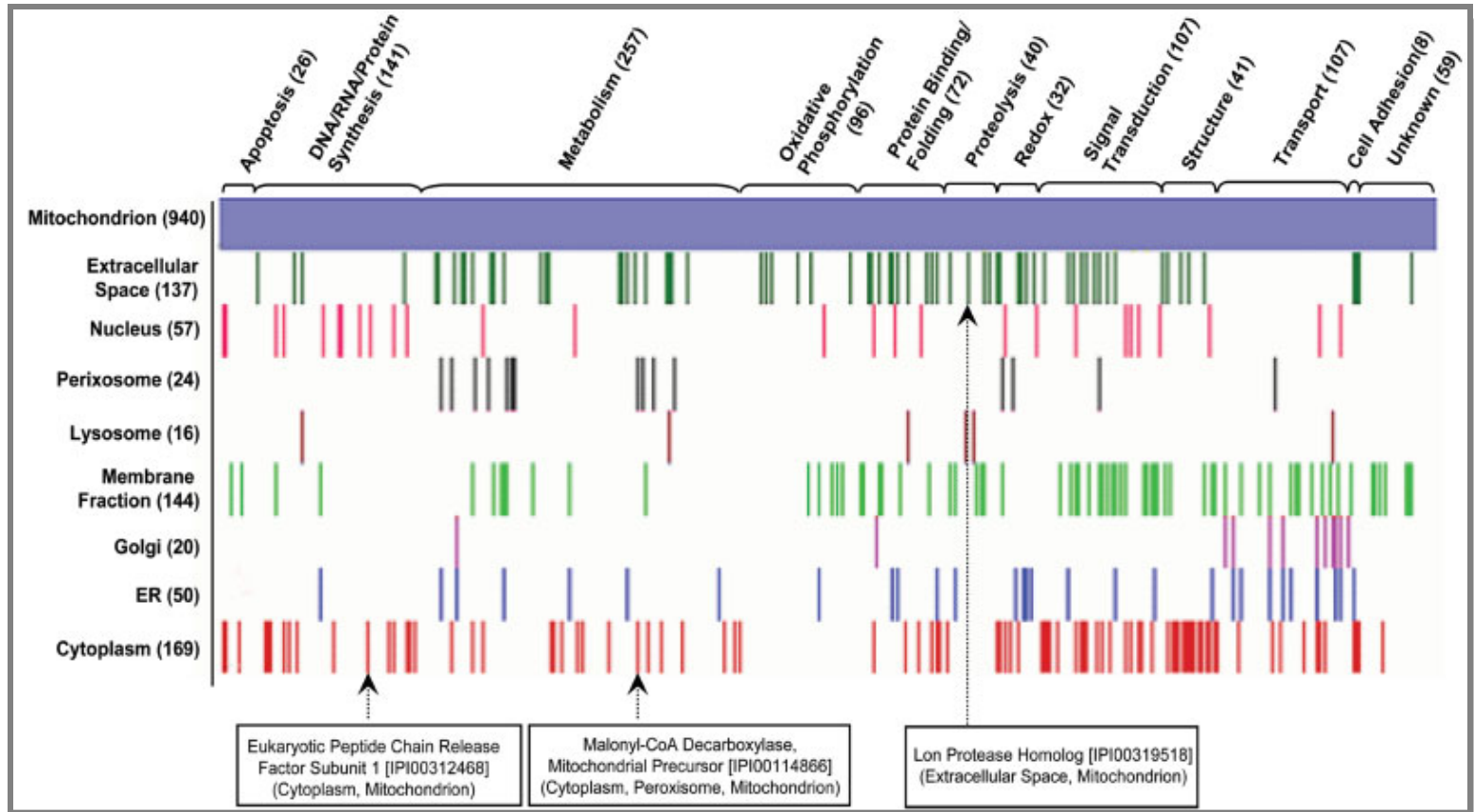
College of Biomedical Engineering and Instrument Science, Zhejiang University

# Overview of Mitochondria

- Subcellular organelle with four functionally distinct sub-compartments:
  - Outer membrane
  - Inner membrane
  - Matrix
  - Intermembrane space
  
- Produces adenosine triphosphate (ATP), the **energy** currency of the cell.
  
- Regulates programmed **cell death** (apoptosis).



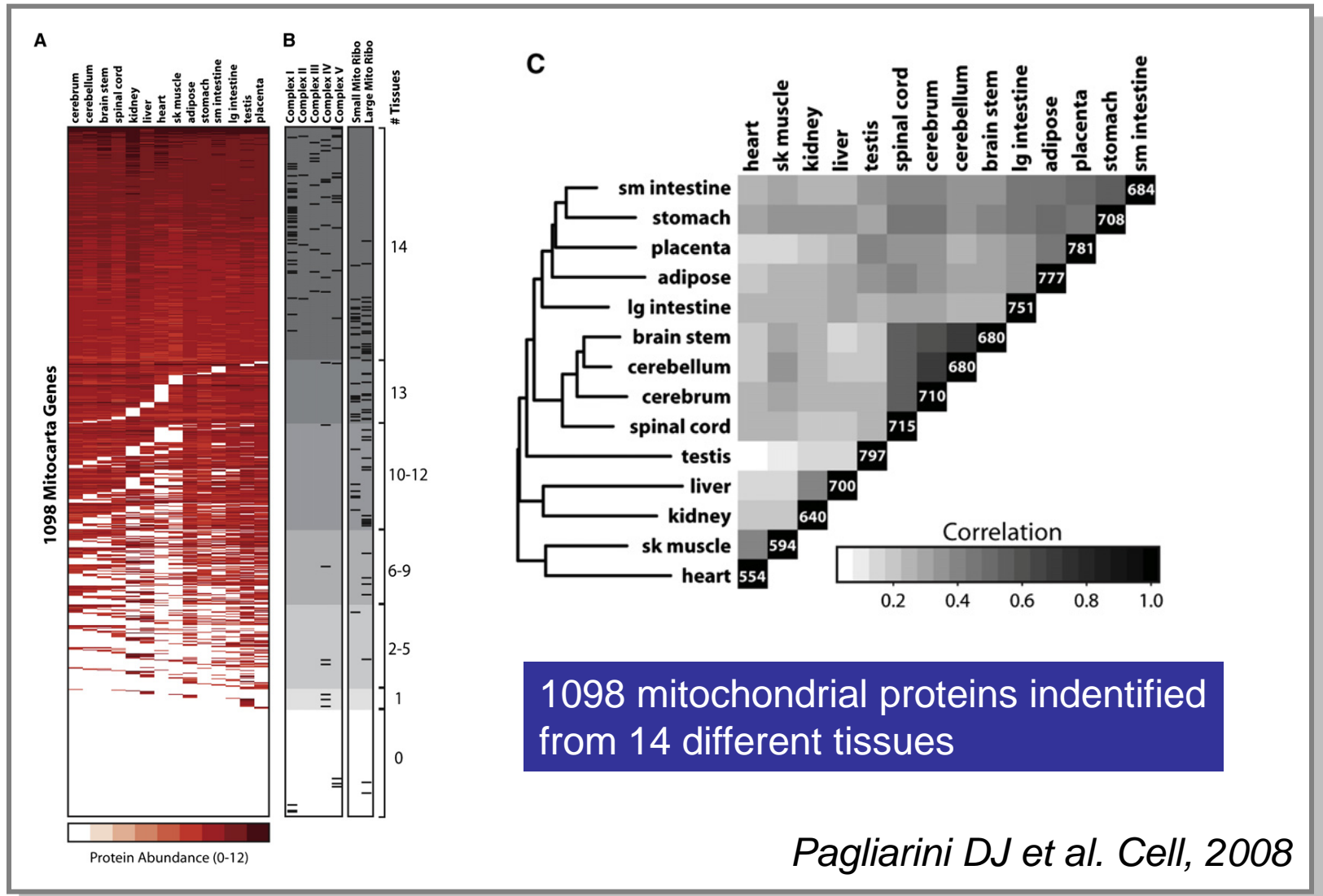
# Mitochondrion: A busy Society of Proteome



Zhang J et al. *Proteomics*, 2008

940 proteins identified from murine cardiac mitochondria

# Variety of Mitochondrial Proteome in Different Tissues



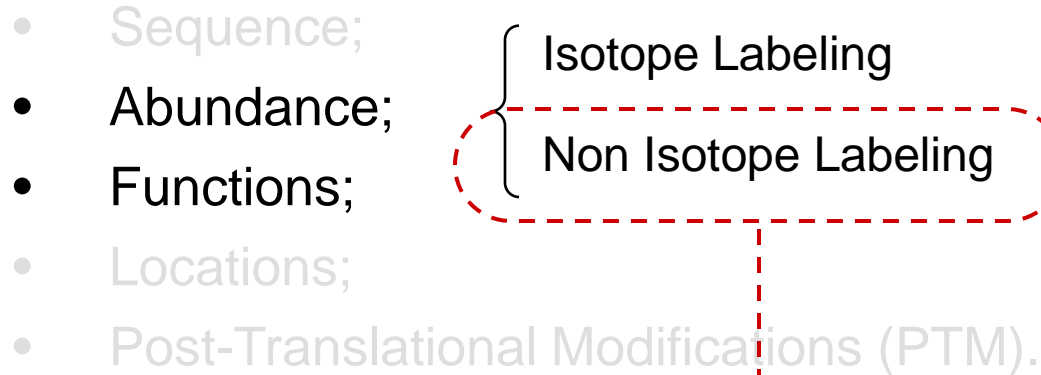
# Protein Properties of the Mitochondrial Proteomes

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- Sequence;
- Abundance;
- Functions;
- Locations;
- Post-Translational Modifications (PTM).

# Protein Properties of the Mitochondrial Proteomes

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- Sequence;
  - Abundance;
  - Functions;
  - Locations;
  - Post-Translational Modifications (PTM).
- Isotope Labeling
- Non Isotope Labeling
- 

Assessment of differential protein abundance from the observed properties of detected peptides is an essential part of protein profiling.

# Label-free Quantification of Mitochondrial Proteome using NSAF Values

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## Normalized Spectral Abundance Factor (NSAF)

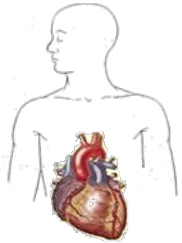
### Methods:

- a) All peptide spectra counts were summed for each identified protein, and then divided by protein sequence length, generating the values of **Spectral Abundance Factor (SAF)**.
- b) The SAF value of each identified subunit was then normalized against the sum of all SAFs within an individual biological sample, resulting in the **Normalized SAF (NSAF)** value.
- c) All NSAF values were then calculated separately for all biological samples. The average values of NSAF for each identified protein were used for further quantitative and biological analyses.

$$(\text{NSAF})_k = \frac{(\text{Spectra\_count}/\text{Protein\_length})_k}{\sum_{i=1}^N (\text{Spectra\_count}/\text{Protein\_length})_i}$$

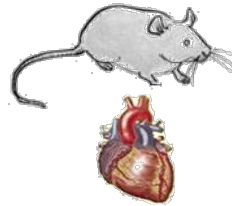
# Mitochondrial Proteome Data Sources

Human Heart  
Mito-datasets



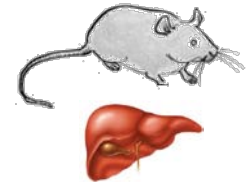
**6** Biological Samples  
from Heart Transplant Surgery

Mouse Heart  
Mito-datasets

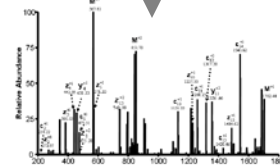


**10** Biological Samples  
from Normal Murine Heart

Mouse Liver  
Mito-datasets



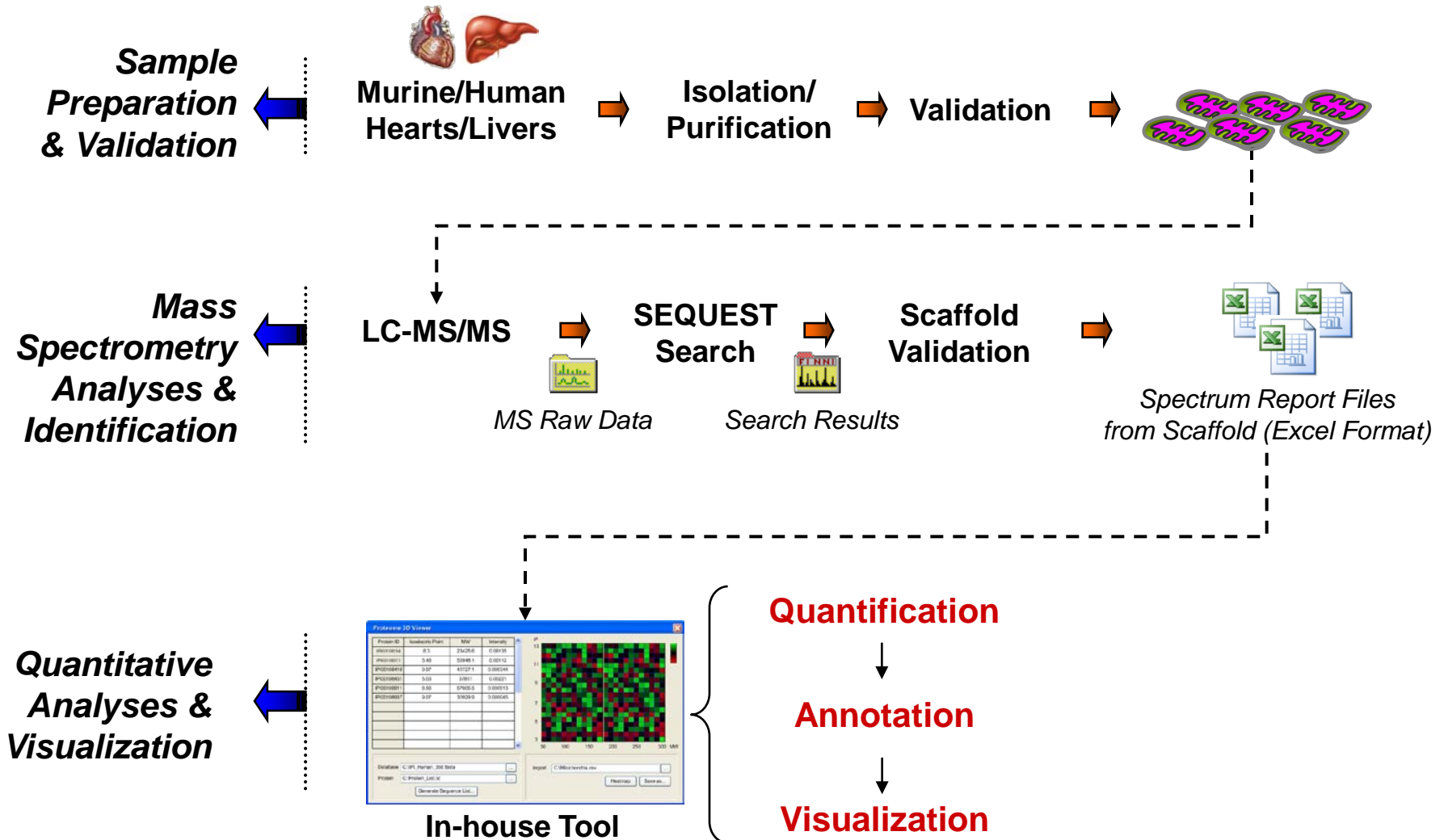
**8** Biological Samples  
from Normal Murine Liver



**3** technical runs by LC-MS/MS

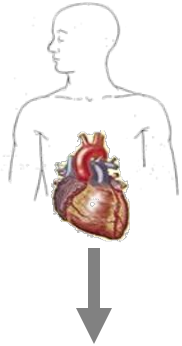


# Data Analysis Pipeline



# Shared Peptides

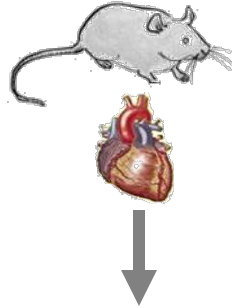
Human Heart  
Mito-datasets



**6.26%**

Shared Peptides

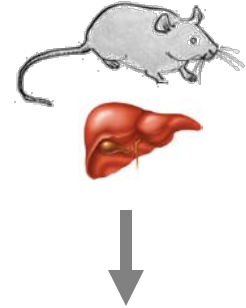
Mouse Heart  
Mito-datasets



**6.36%**

Shared Peptides

Mouse Liver  
Mito-datasets



**6.17%**

Shared Peptides

**Significant Impact on Protein Isoforms:**

Protein Isoform	Short Name	% of Shared Peptides
ADP/ATP Translocase 1	ANT1	59%
ADP/ATP Translocase 2	ANT2	58%
ADP/ATP Translocase 3	ANT3	34%
ADP/ATP Translocase 4	ANT4	14%

# Strategy to Deal with Shared Peptides Among Protein Isoforms

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- **Dismissive Strategy:** Only peptides unique to a particular protein are counted.

- **Distributive Strategy:** Shared spectral counts are divided among protein isoforms.



$$Spectrum\_Count_i = \sum_{band=1}^x [Nu_{band.i} + (\sum_{i=1}^n Ns_{band.i}) \times Percentage_{band.i}]$$

$$Percentage_{band.i} = \frac{Nu_i / Length_i}{\sum_{j=1}^n (Nu_j / Length_j)}$$

*Nu: Number of Unique Peptides*  
*Ns: Number of Shared Peptides*  
*Length: Number of Amino Acids*

# **Global Quantification of Mitochondrial Proteomes**

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*Human Heart, Mouse Heart and Mouse Liver*

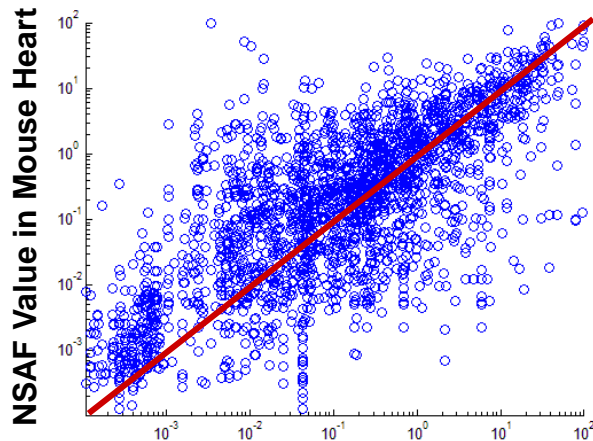
# Distribution of Protein Abundance

(Human Heart, Mouse Heart & Mouse Liver Mito-Datasets)

Function	Human Heart		Mouse Heart		Mouse Liver	
	Ave NSAF	% of Total	Ave NSAF	% of Total	Ave NSAF	% of Total
OXPHOS	7.12	32.10%	9.16	39.79%	7.16	23.39%
Metabolism	2.40	34.16%	2.49	32.68%	3.40	47.84%
Transport	2.26	13.90%	1.49	10.32%	1.36	8.22%
Apoptosis	3.30	7.06%	1.60	4.19%	2.38	3.26%
Redox	1.82	6.30%	1.17	3.19%	1.82	8.06%
Binding	0.58	6.30%	0.79	3.24%	0.90	4.37%
Signaling	0.61	2.05%	0.58	3.56%	0.64	2.21%
Biosynthesis	0.56	4.19%	0.67	4.53%	0.87	7.08%
Structure	0.27	1.34%	0.23	0.39%	0.18	0.39%
Proteolysis	0.13	0.18%	0.45	1.19%	0.50	1.04%
Cell Adhesion	0.11	0.07%	0.02	0.02%	0.0065	0.00%
Cell Cycle	0.00	0.00%	0.03	0.01%	0.032	0.00%
Unknown	0.40	1.47%	0.36	0.97%	0.45	1.55%

# Correlation of Mitochondrial Proteomes

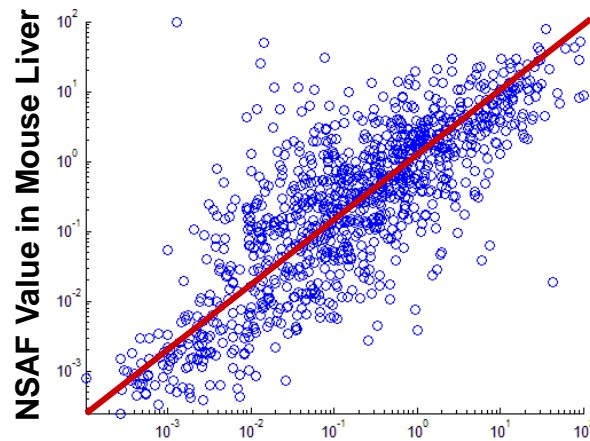
Human Heart vs. Mouse Heart



NSAF Value in Human Heart

**R = 0.5365**

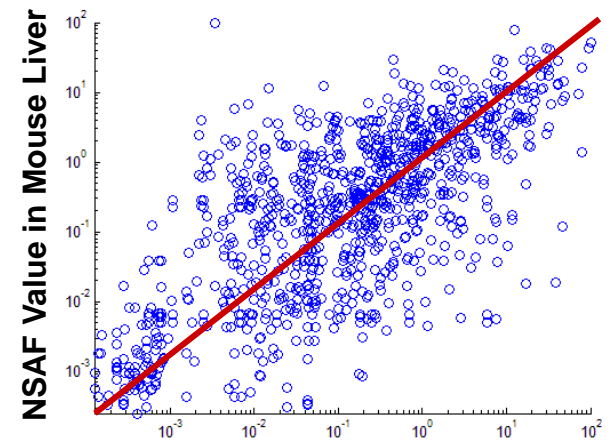
Mouse Heart vs. Mouse Liver



NSAF Value in Mouse Heart

**R = 0.6082**

Human Heart vs. Mouse Liver



NSAF Value in Human Heart

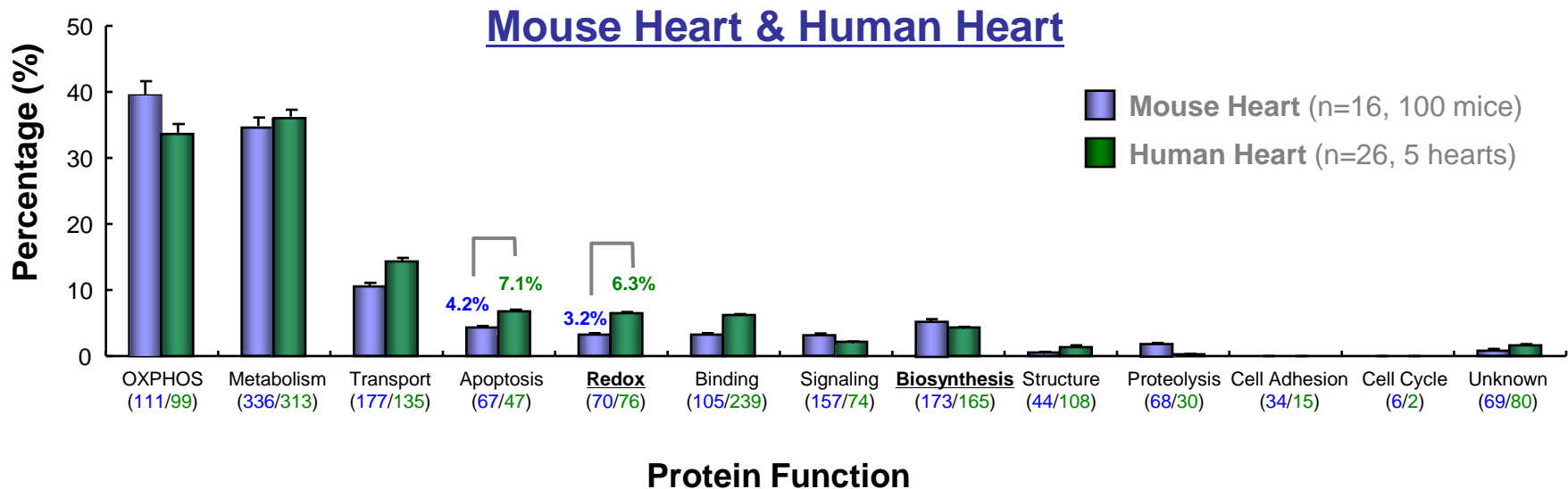
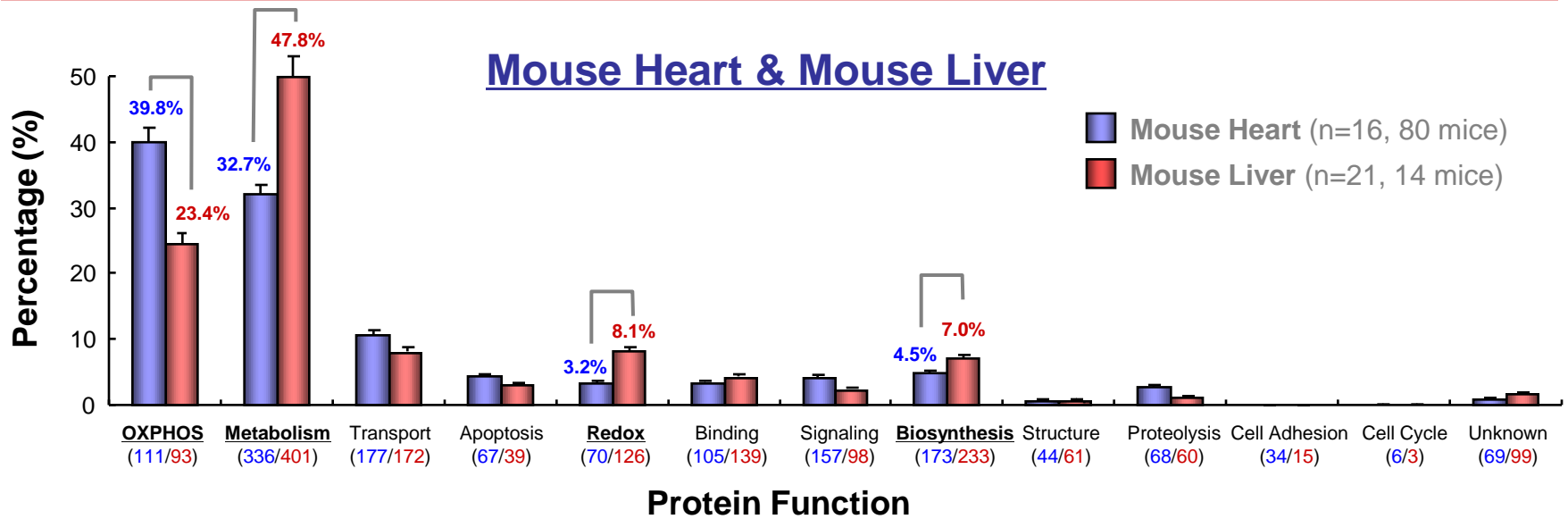
**R = 0.4770**

*Sample Correlation Coefficient:*

$$R = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n \sum x_i^2 - (\sum x_i)^2} \sqrt{n \sum y_i^2 - (\sum y_i)^2}}$$

# Distribution of Protein Abundance

## Distinct Functional Clusters in Organs and Species

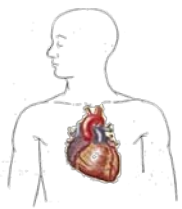


# **Top 10 Most Abundant Proteins**

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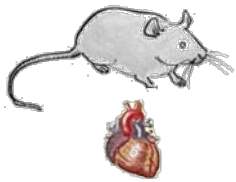
*Human Heart, Mouse Heart and Mouse Liver*





# Top 10 Most Abundant Proteins in Human Heart Mitochondrial Dataset

Rank	IPI	Gene	Name	Function	NSAF	Rank / NSAF Mouse Heart	Rank / NSAF Mouse Liver
1	IPI00022891	SLC25A4	ADP/ATP translocase 1	Transport	100	3 / 95.45	402 / 0.88
2	IPI00303476	ATP5B	ATP synthase subunit beta	ETC Complex	88.87	1 / 100	4 / 53.24
3	IPI00440493	ATP5A1	ATP synthase subunit alpha	ETC Complex	86.80	2 / 96.91	8 / 42.59
4	IPI00216308	VDAC1	Voltage-dependent anion-selective channel protein 1	Transport, Apoptosis	64.47	7 / 39.42	39 / 15.50
5	IPI00022314	SOD2	Superoxide dismutase [Mn]	Redox	57.60	22 / 23.18	29 / 19.43
6	IPI00003482	DECR1	2,4-dienoyl-CoA reductase	Fatty Acid Metabolism	42.00	45 / 15.69	35 / 17.74
7	IPI00015911	DLD	Dihydrolipoyl dehydrogenase	TCA cycle	41.14	93 / 8.09	109 / 6.13
8	IPI00291006	MDH2	Malate dehydrogenase	TCA cycle	41.13	4 / 91.42	17 / 29.33
9	IPI00294398	HADH	Isoform 1 of Hydroxyacyl-coenzyme A dehydrogenase	Fatty Acid Metabolism	37.28	70 / 10.41	36 / 17.13
10	IPI00015141	CKMT2	Creatine kinase	Metabolism	35.08	13 / 30.73	1136 / 0.015



# Top 10 Most Abundant Proteins in Mouse Heart Mitochondrial Dataset

Rank	IPI	Gene	Name	Function	NSAF	Rank / NSAF Human Heart	Rank / NSAF Mouse Liver
1	IPI00468481	Atp5b	ATP synthase subunit beta	ETC complex	100	2 / 88.87	4 / 53.24
2	IPI00130280	Atp5a1	ATP synthase subunit alpha	ETC complex	96.91	3 / 86.80	8 / 42.59
3	IPI00115564	Slc25a4	ADP/ATP translocase 1	Transport	95.49	1 / 100	402 / 0.88
4	IPI00323592	Mdh2	Malate dehydrogenase	TCA cycle	91.42	8 / 41.13	17 / 29.33
5	IPI00116753	EtfA	Electron transfer flavoprotein subunit alpha	ETC complex	50.35	14 / 30.29	3 / 53.44
6	IPI00223092	Hadha	Trifunctional enzyme subunit alpha	Fatty Acid Metabolism	43.72	17 / 28.40	25 / 20.62
7	IPI00122549	Vdac1	Voltage-dependent anion-selective channel protein 1	Transport, Apoptosis	39.42	4 / 64.47	39 / 15.50
8	IPI00133240	Uqcrcs1	Cytochrome b-c1 complex subunit Rieske	ETC complex	37.56	11 / 34.56	42 / 14.83
9	IPI00116074	Aco2	Aconitate hydratase	TCA cycle	37.41	12 / 31.51	88 / 8.24
10	IPI00230507	Atp5h	ATP synthase subunit d	ETC complex	36.22	13 / 30.53	5 / 50.34

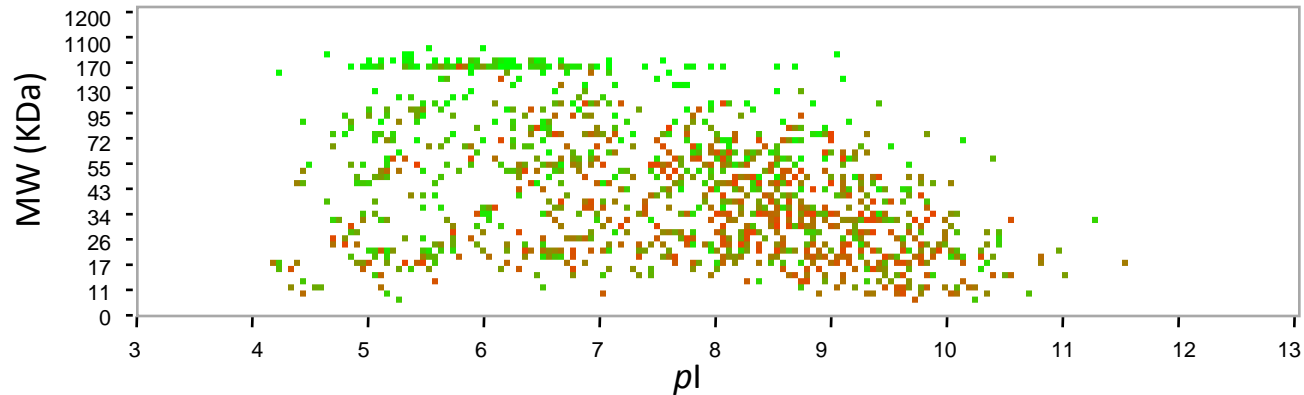
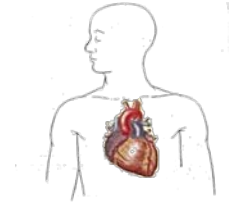


# Top 10 Most Abundant Proteins in Mouse Liver Mitochondrial Dataset

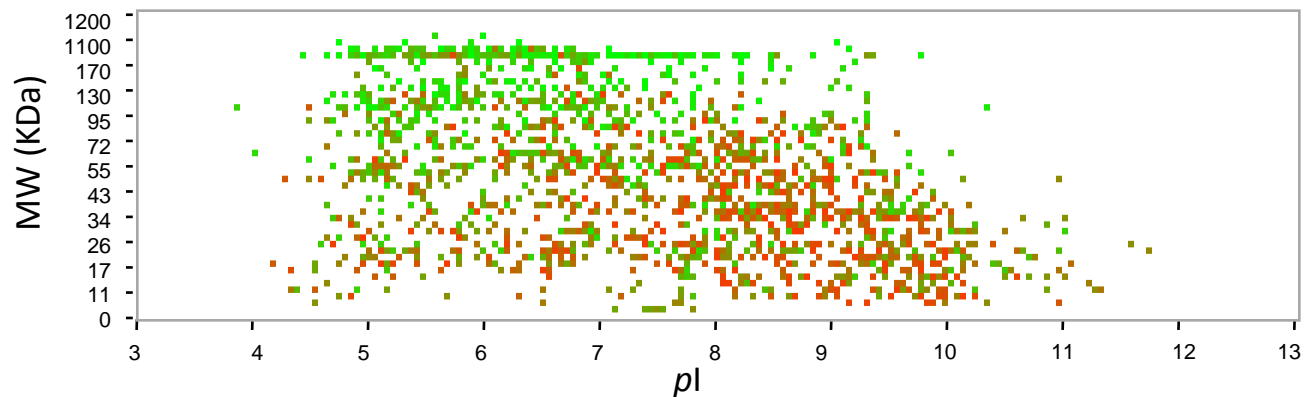
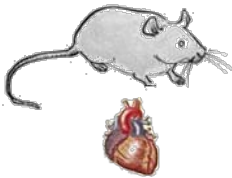
Rank	IPI	Gene	Name	Function	NSAF	Rank / NSAF Human Heart	Rank / NSAF Mouse Heart
1	IPI00111908	Cps1	Carbamoyl-phosphate synthase [ammonia]	Urea cycle	100	1004 / 0.003	1169 / 0.004
2	IPI00226430	Acaa2	3-ketoacyl-CoA thiolase	Fatty Acid Metabolism	73.61	51 / 11.02	11 / 33.42
3	IPI00116753	EtfA	Electron transfer flavoprotein subunit alpha	ETC complex	53.44	14 / 30.29	5 / 50.35
4	IPI00468481	Atp5b	ATP synthase subunit beta	ETC complex	53.24	2 / 88.87	1 / 100
5	IPI00230507	Atp5h	ATP synthase subunit d	ETC complex	50.34	13 / 30.53	10 / 36.22
6	IPI00420718	Hmgcs2	Hydroxymethylglutaryl-CoA synthase	Metabolism	48.98	N/D	868 / 0.028
7	IPI00116603	Otc	Ornithine carbamoyltransferase	Urea cycle	43.03	N/D	N/D
8	IPI00130280	Atp5a1	ATP synthase subunit alpha	ETC complex	42.59	3 / 86.80	2 / 96.91
9	IPI00626132	Hspd17b10	Hydroxysteroid (17-beta) dehydrogenase 10	Metabolism	42.00	N/D	N/D
10	IPI00466399	Mup2	major urinary protein 2	Urine-specific Transport	41.45	N/D	N/D

# Individual Heatmaps Show Protein Distribution

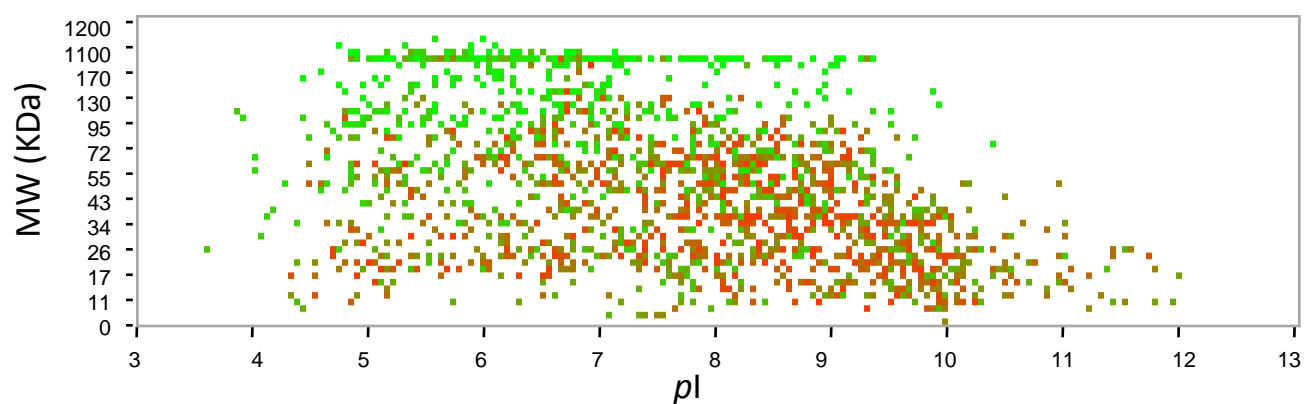
Human Heart  
Mito-dataset:



Mouse Heart  
Mito-dataset:



Mouse Liver  
Mito-dataset:



Color Metric  
of NSAF Value:



# **Quantitative Analysis of OXPHOS Proteins**

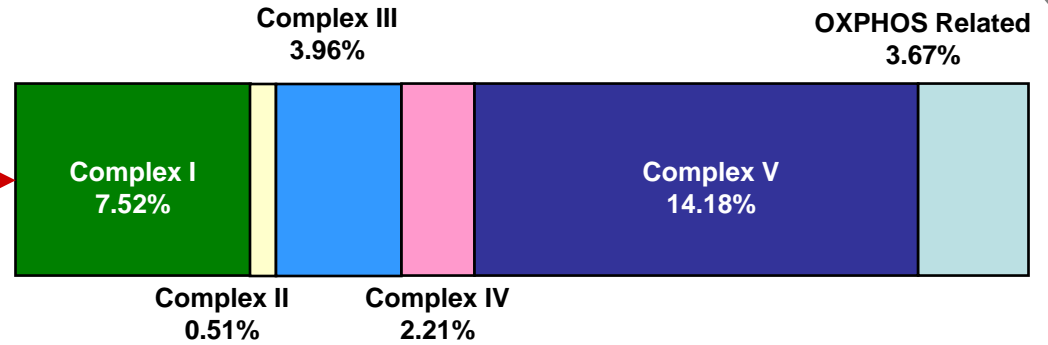
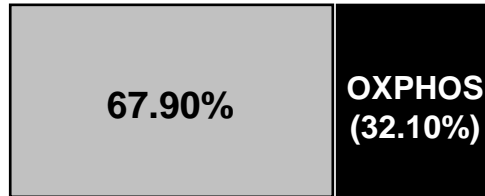
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*Human Heart, Mouse Heart and Mouse Liver*

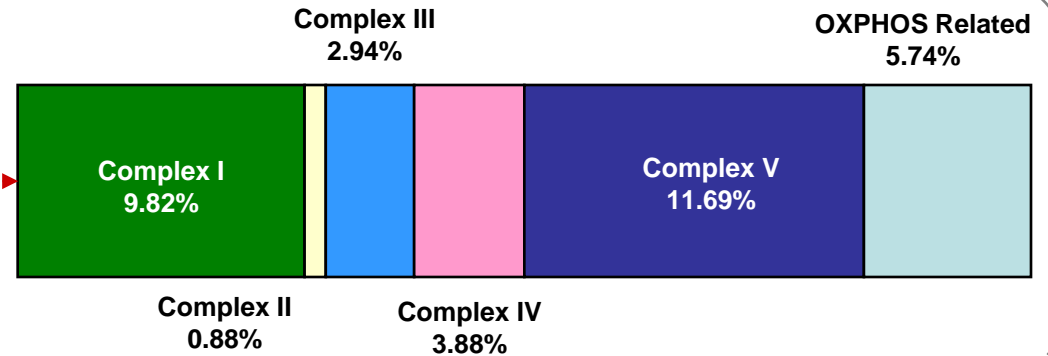
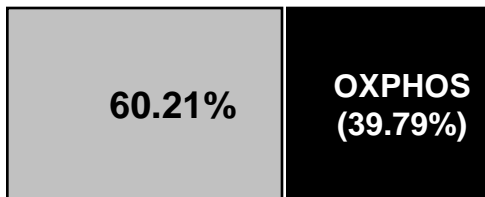
# Mito Proteomes

# OXPHOS Proteins

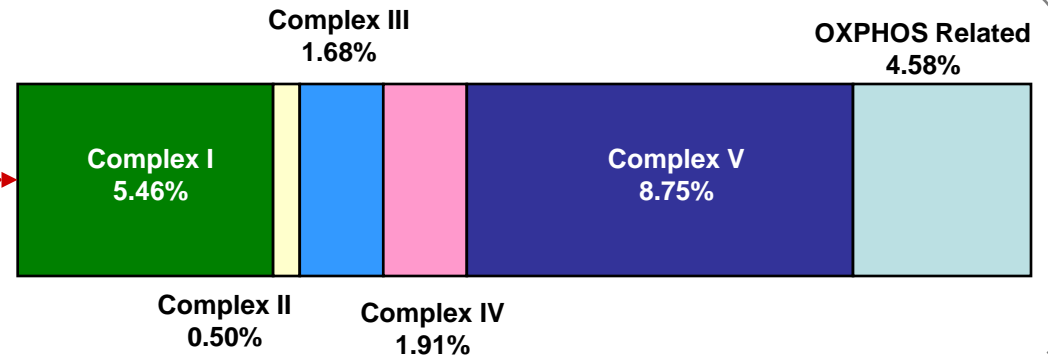
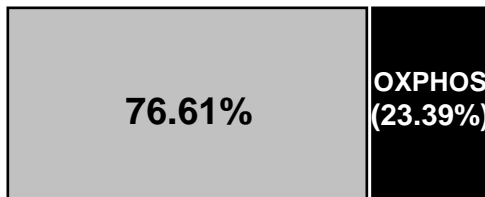
## Human Heart



## Mouse Heart

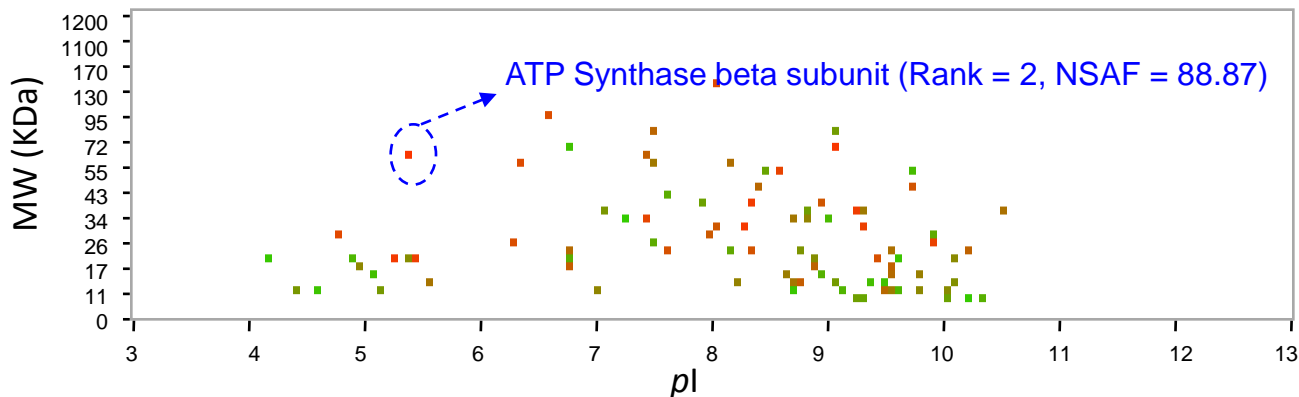
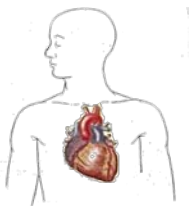


## Mouse Liver

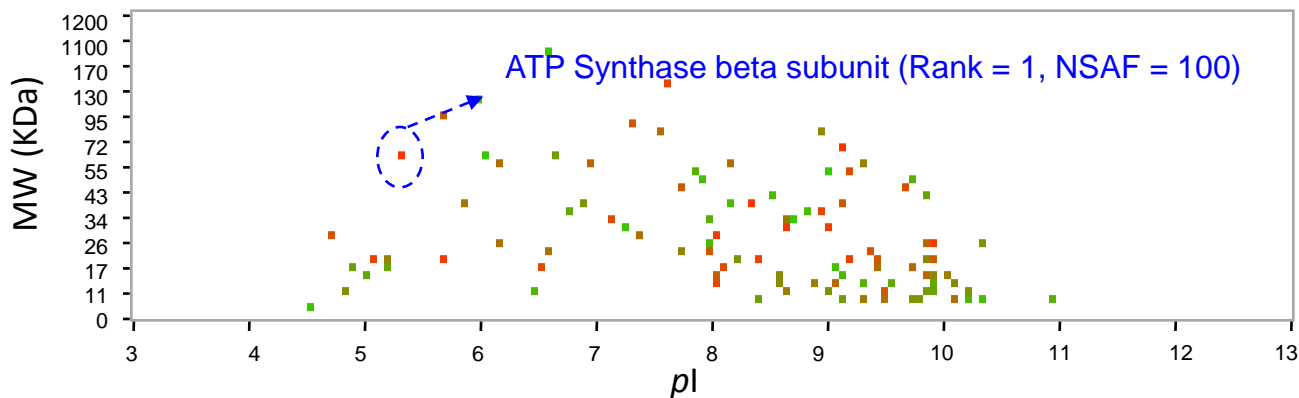


# Heatmaps of ETC Complexes

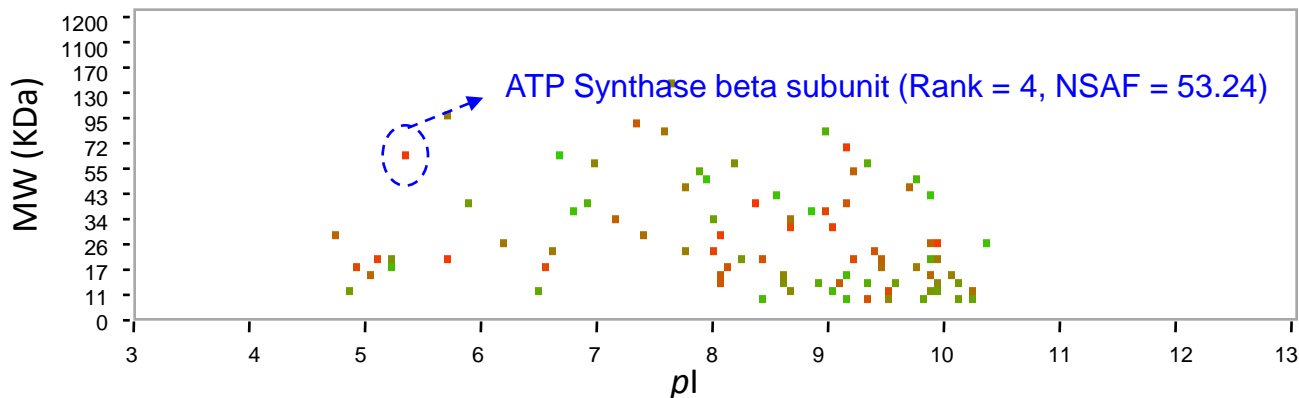
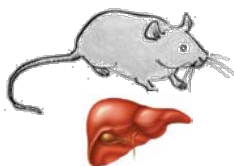
**Human Heart  
Mito-dataset:**



**Mouse Heart  
Mito-dataset:**

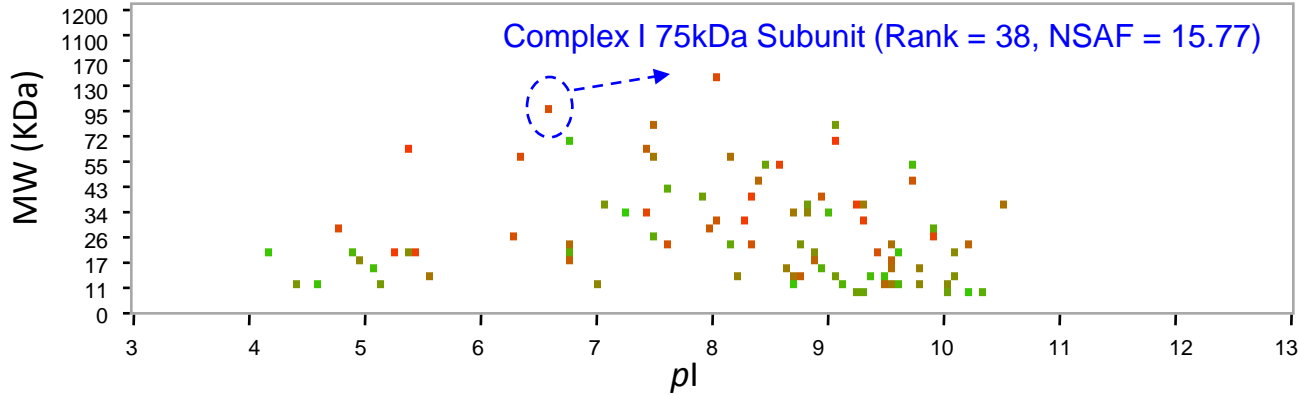
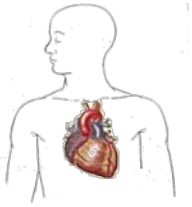


**Mouse Liver  
Mito-dataset:**

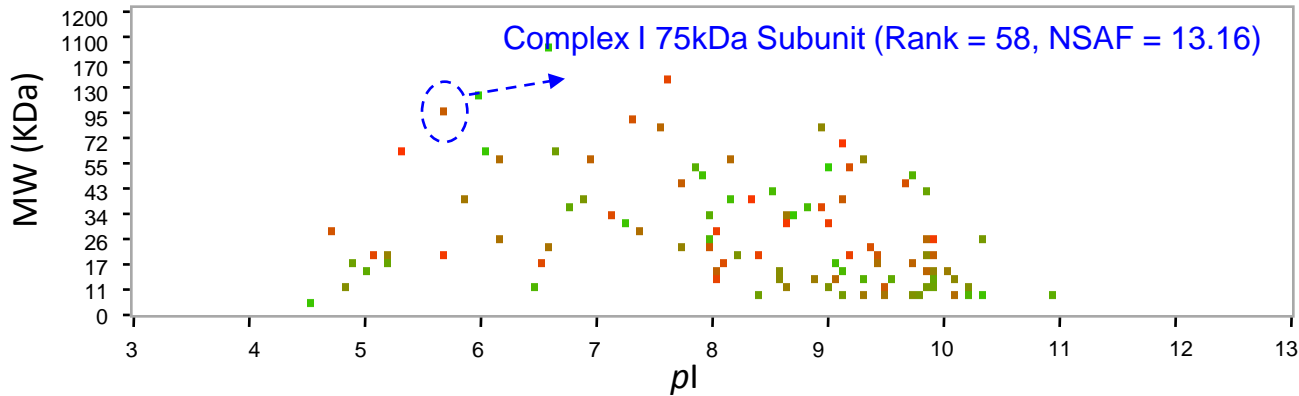
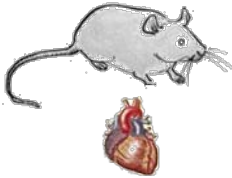


# Heatmaps of ETC Complexes

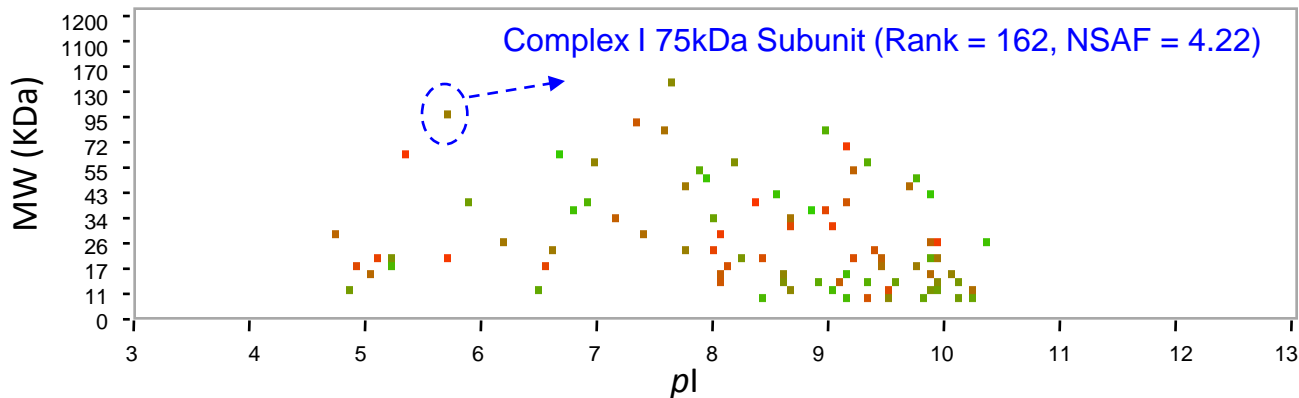
**Human Heart  
Mito-dataset:**



**Mouse Heart  
Mito-dataset:**



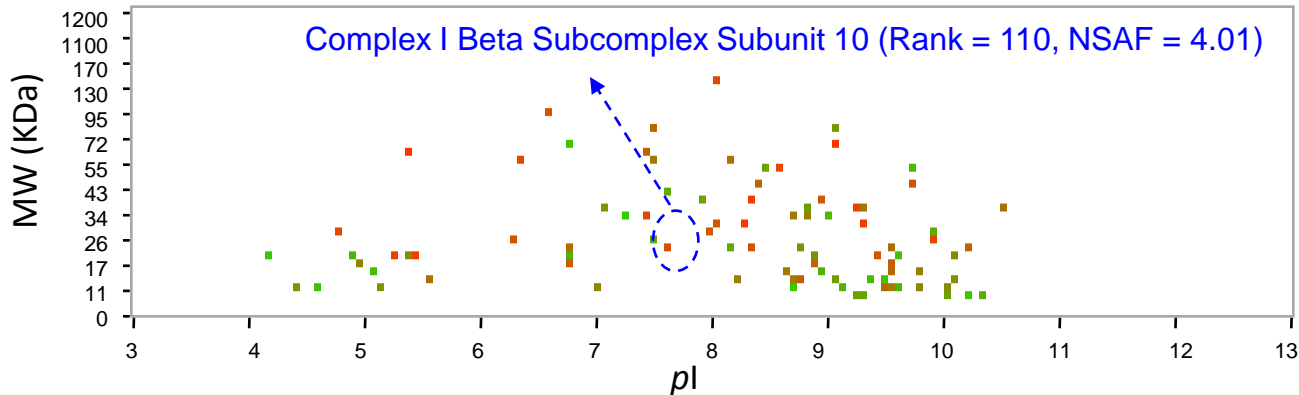
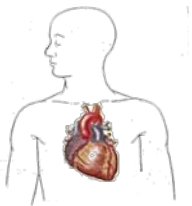
**Mouse Liver  
Mito-dataset:**



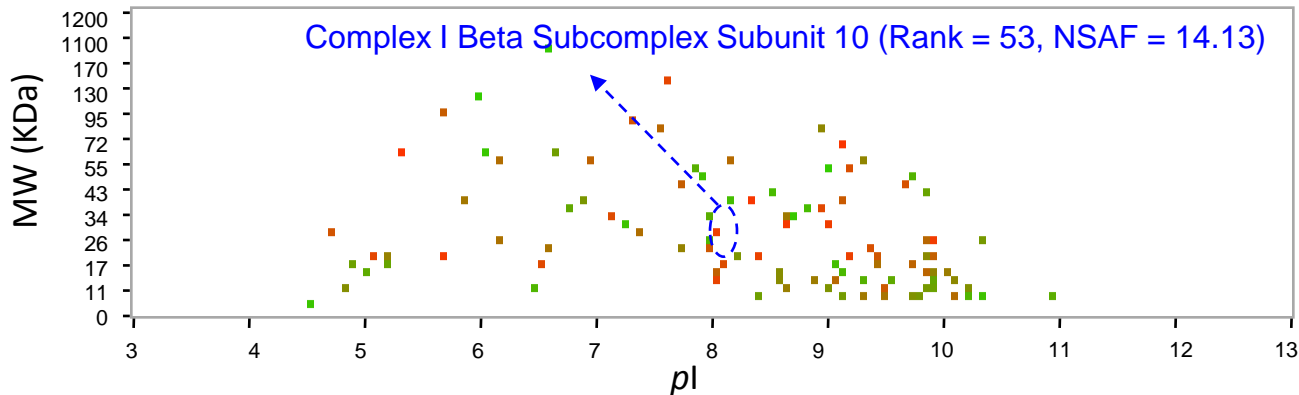


# Heatmaps of ETC Complexes

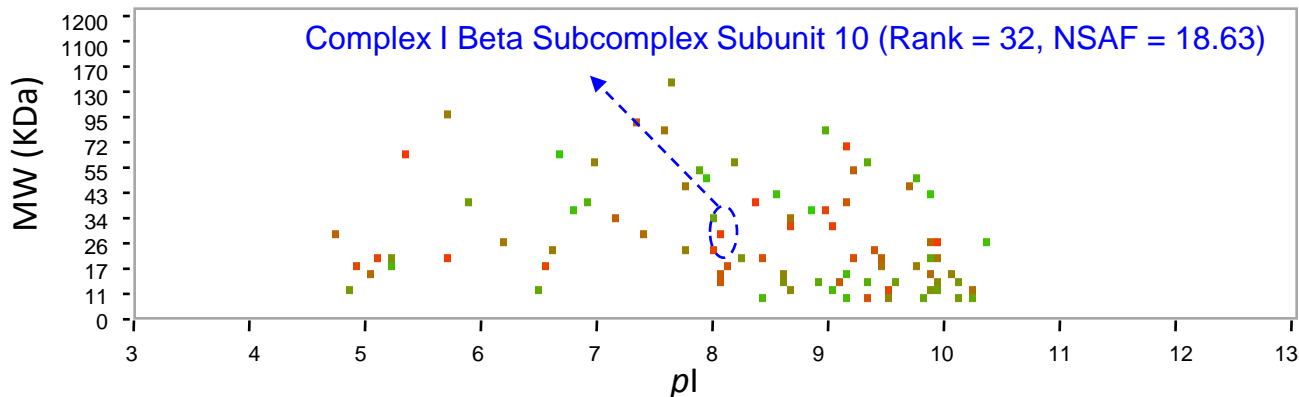
**Human Heart  
Mito-dataset:**



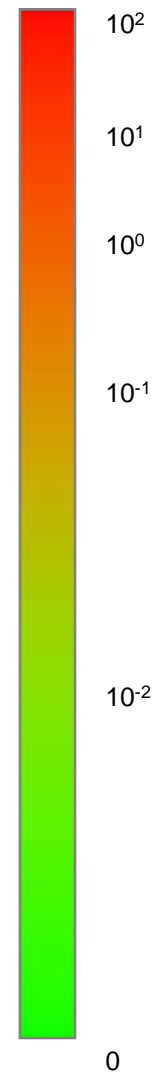
**Mouse Heart  
Mito-dataset:**



**Mouse Liver  
Mito-dataset:**



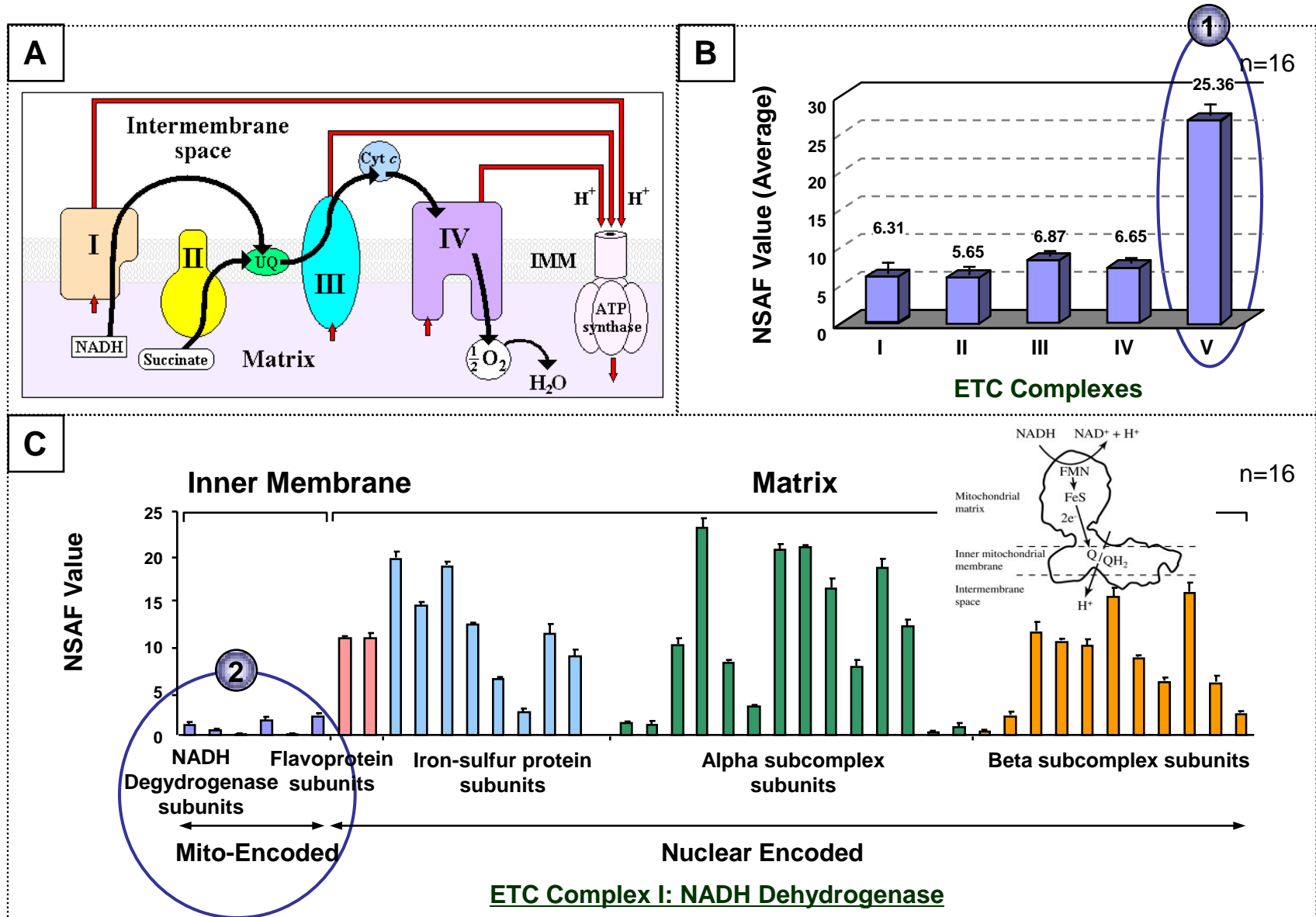
Color Metric  
NSAF Value



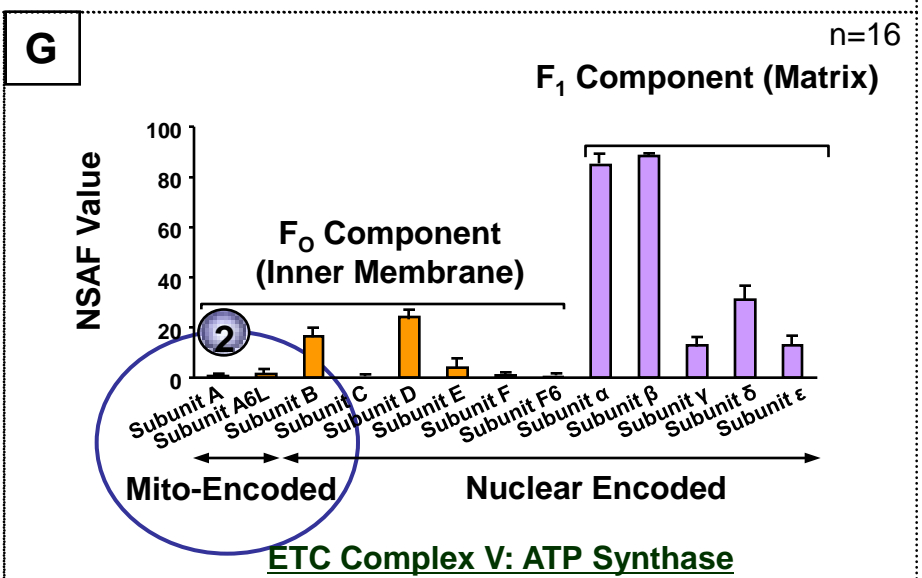
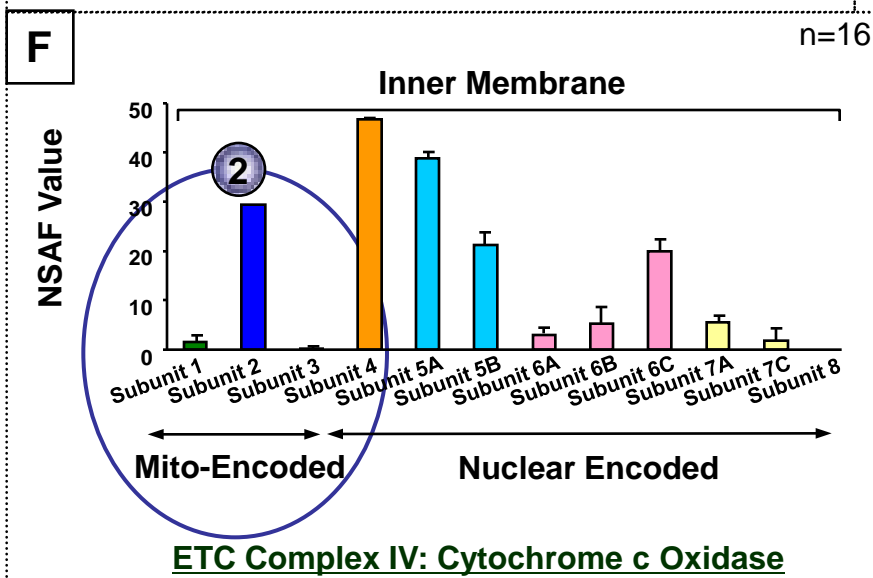
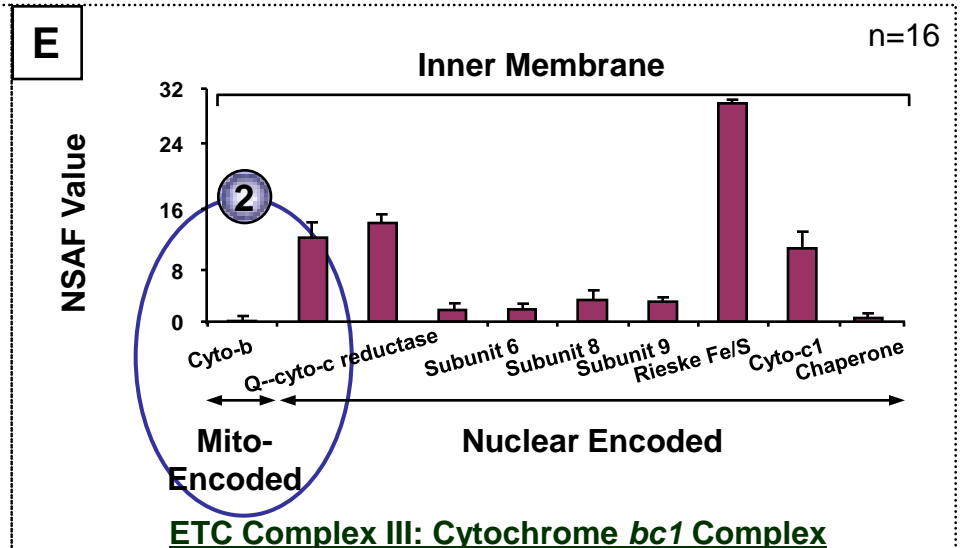
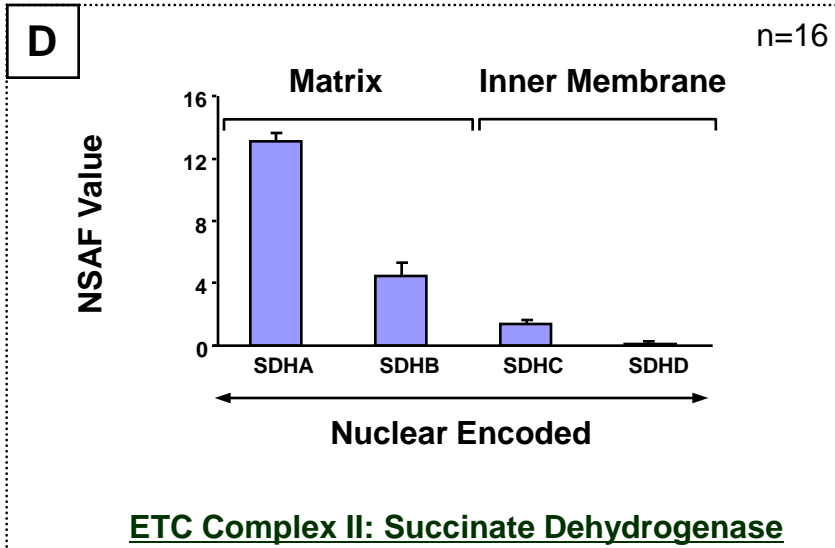
**Proteome Biology of Cardiac Mitochondria:  
One Protein Land Governed by Two Genomes**

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# Abundance Analyses of Murine Cardiac Mitochondrial ETC Subunits



# Relative Abundance of Murine Cardiac Mitochondrial ETC Components



# Assessment of Abundance on the Mitochondrial ETC Components

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## Main observations and insights:

- Stoichiometry.

Among all five ETC complexes, I to IV displayed very similar level of abundances. In comparison, complex V appeared to be in higher abundance (approximately 3-fold more than others).

- Mito Genome Coded Proteins.

The mitochondrial genome encodes 13 subunits of the ETC. Our study identified 12 out of the 13; these 12 subunits were in significantly low abundance when compared to the nuclear-encoded proteins, suggesting that these mitochondrial-encoded proteins maybe the limiting factors for effective assembly of the ETC I, III, IV, and V complexes.

# **Quantitative Analysis of Metabolism Proteins**

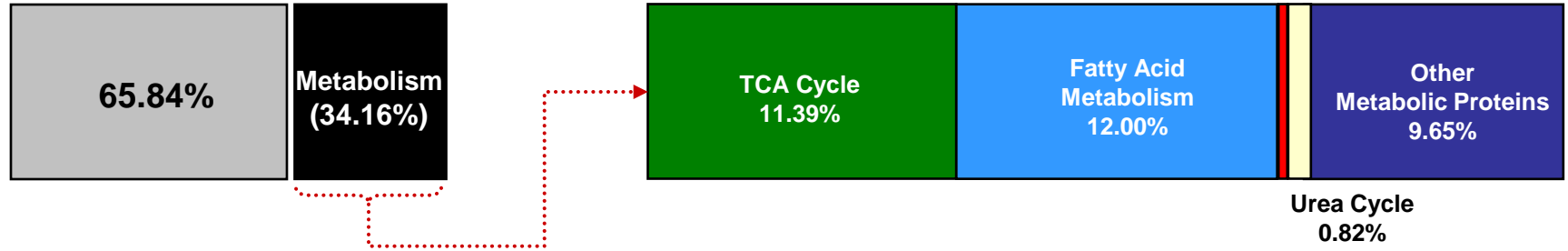
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*Human Heart, Mouse Heart and Mouse Liver*

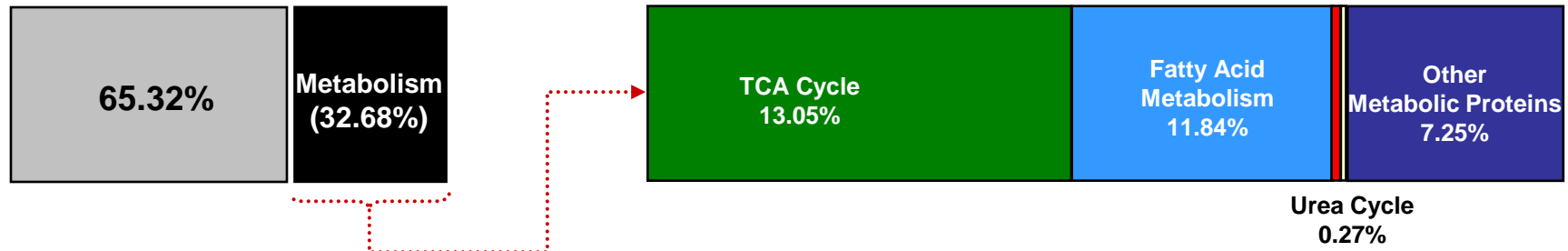
# Mito Proteomes

# Metabolism Proteins

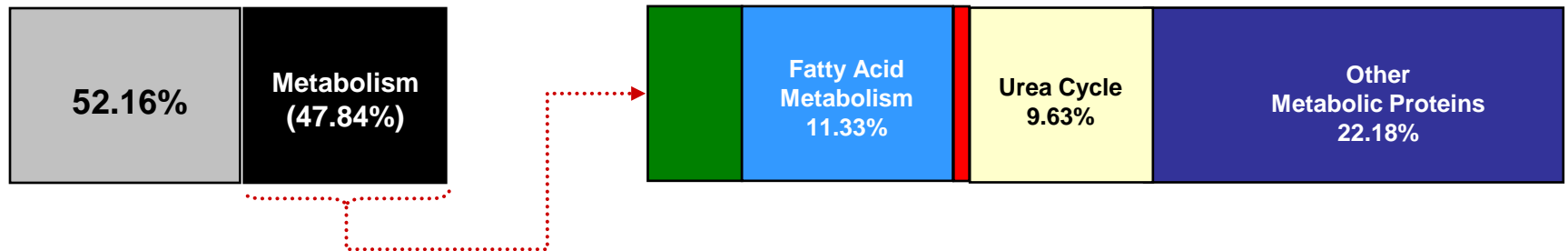
## Human Heart



## Mouse Heart

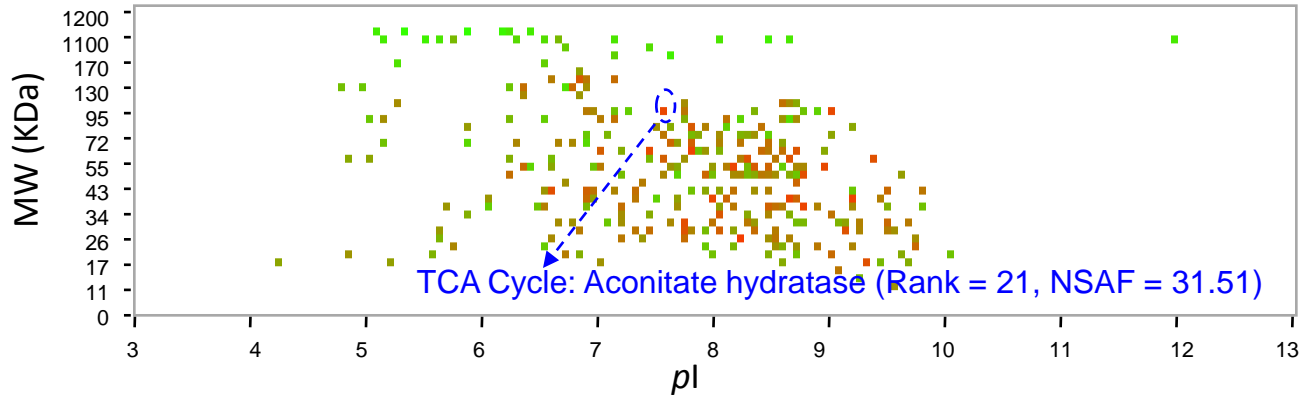
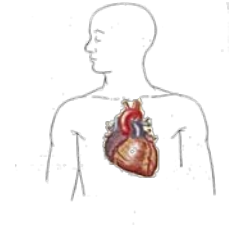


## Mouse Liver

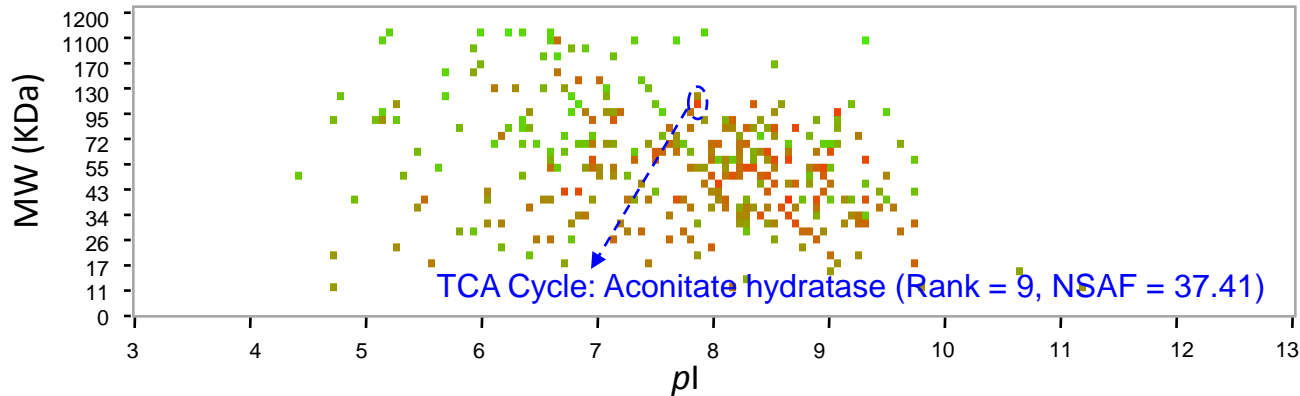
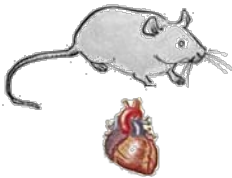


# Heatmaps for Metabolism Proteins

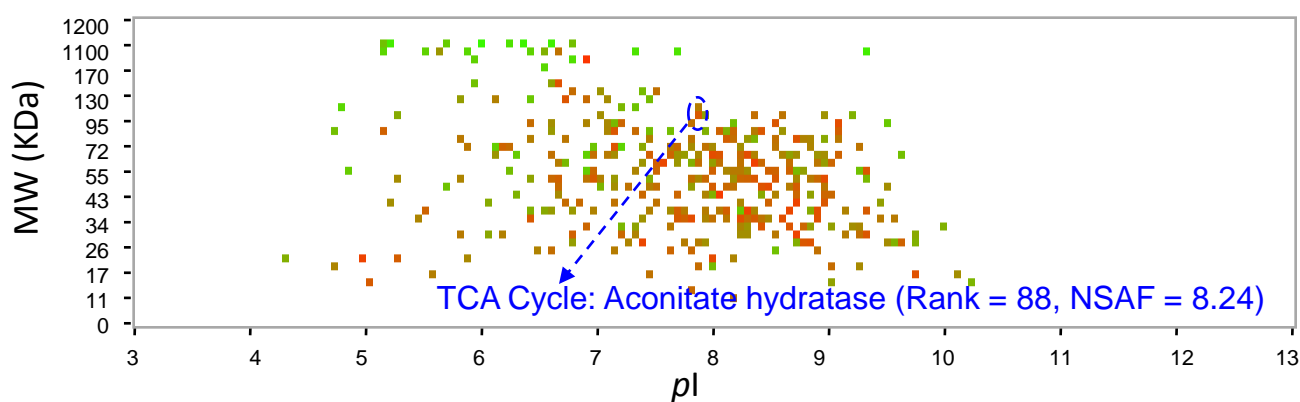
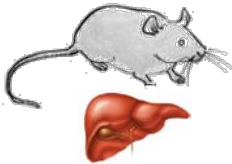
Human Heart  
Mito-dataset:



Mouse Heart  
Mito-dataset:



Mouse Liver  
Mito-dataset:



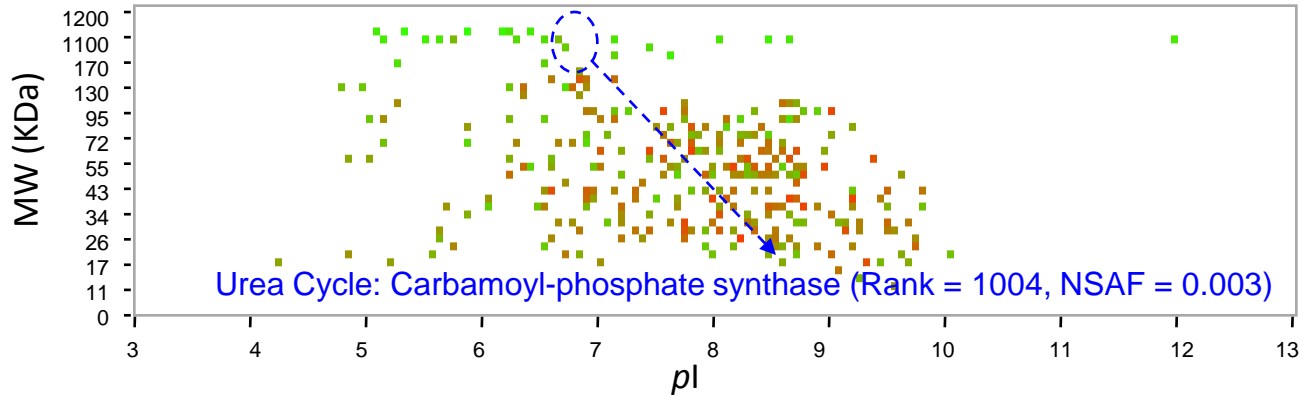
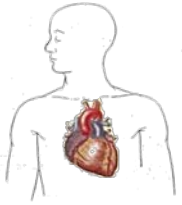
Color Metric  
of NSAF Value:



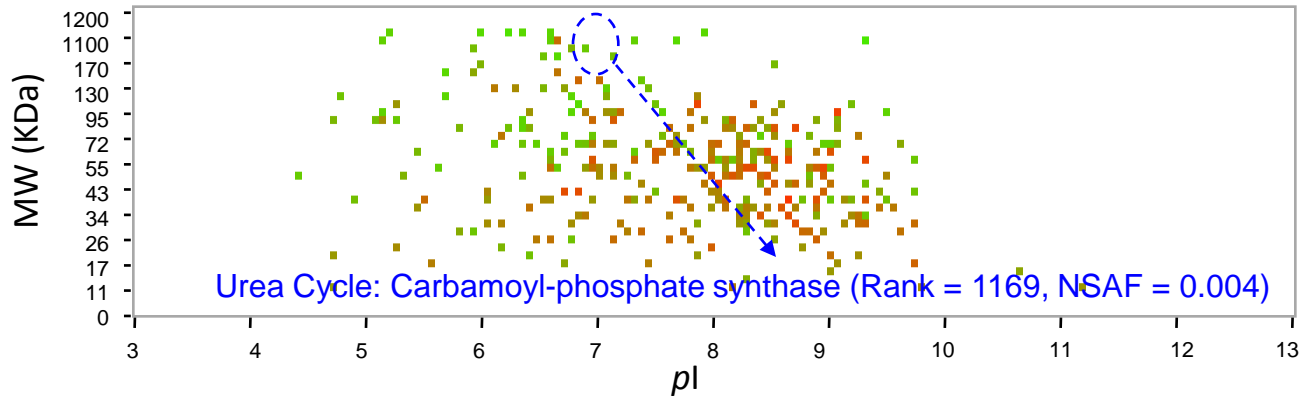
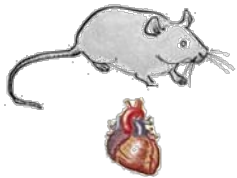


# Heatmaps for Metabolism Proteins

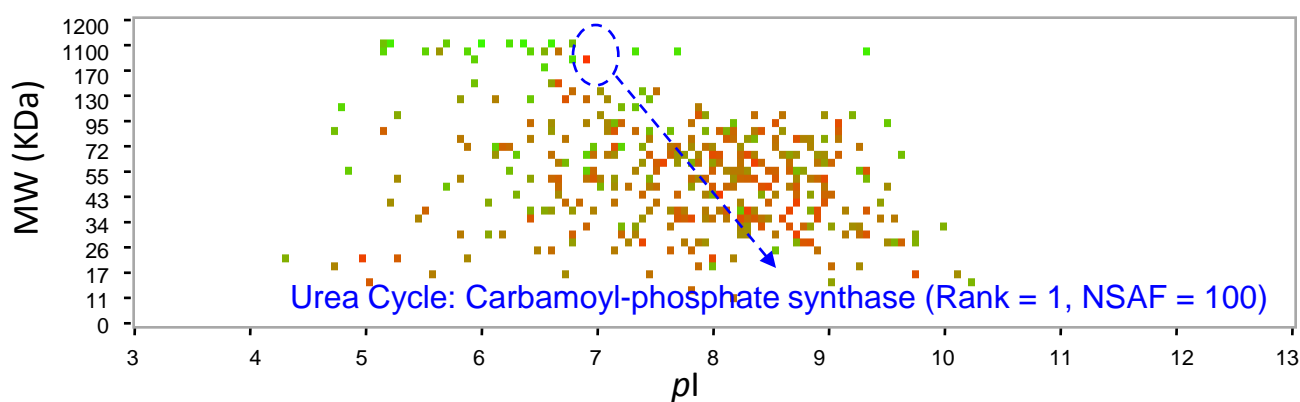
Human Heart  
Mito-dataset:



Mouse Heart  
Mito-dataset:



Mouse Liver  
Mito-dataset:



Color Metric  
of NSAFA Value:



# Urea Cycle

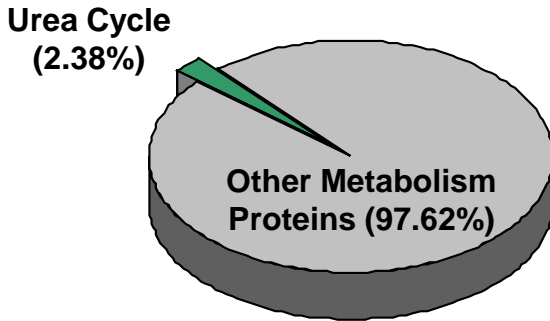
The **urea cycle** (also known as the **ornithine cycle**) is a cycle of biochemical reactions occurring in many animals that produces urea (NH<sub>2</sub>)<sub>2</sub>CO from ammonia (NH<sub>3</sub>). This cycle was the first metabolic cycle discovered (Hans Krebs and Kurt Henseleit, 1932). **In mammals, the urea cycle takes place only in the liver.**

---- *From Wikipedia*

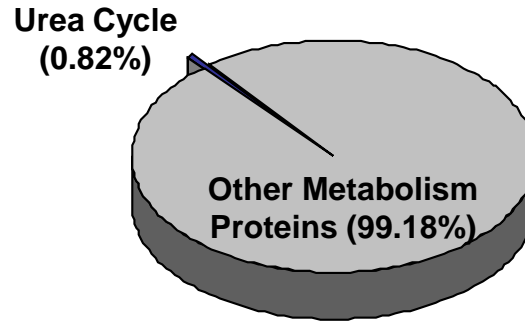
Gene	Protein	NSAF Human Heart	NSAF Mouse Heart	NSAF Mouse Liver
Cps1	Carbamoyl-phosphate synthase [ammonia]	0.0033	0.0004	100
Otc	Ornithine carbamoyltransferase	N/D	N/D	43.03
Slc25a15	Mitochondrial ornithine transporter 1	N/D	N/D	7.99
Aldh2	Aldehyde dehydrogenase	6.73	2.72	28.55
Aldh9a1	Aldehyde dehydrogenase 9, subfamily A1	0.33	N/D	0.94
Aldh1b1	Aldehyde dehydrogenase X	0.37	0.82	2.30
Aldh3a2	Fatty aldehyde dehydrogenase variant form	0.0068	N/D	1.37
Maoa	Amine oxidase [flavin-containing] A	5.55	0.0053	0.095
Maob	Amine oxidase [flavin-containing] B	4.85	1.43	2.99
Agmat	Agmatinase, mitochondrial	N/D	N/D	3.17
Ass1	Argininosuccinate synthase	N/D	N/D	0.21
Arg1	Arginase-1	N/D	N/D	0.12
Nags	Isoform 1 of N-acetylglutamate synthase	N/D	N/D	0.25
Mup2	Major urinary protein 2	N/D	0.079	41.45
Uox	Uricase	N/D	0.022	38.32
Oat	Ornithine aminotransferase	0.0030	1.81	3.45

# Urea Cycle Related Proteins

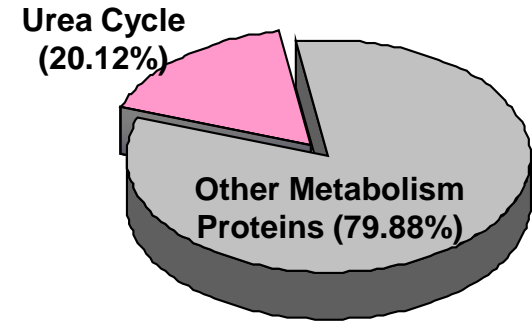
Human Heart



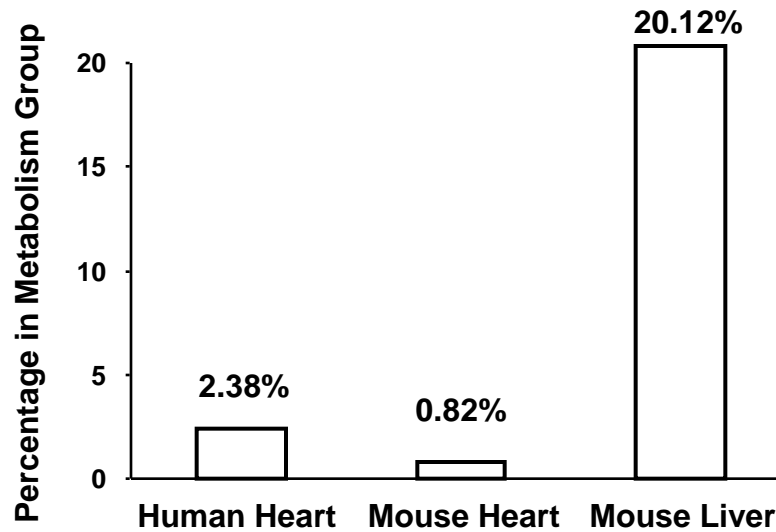
Mouse Heart



Mouse Liver



Urea Cycle Related Protein

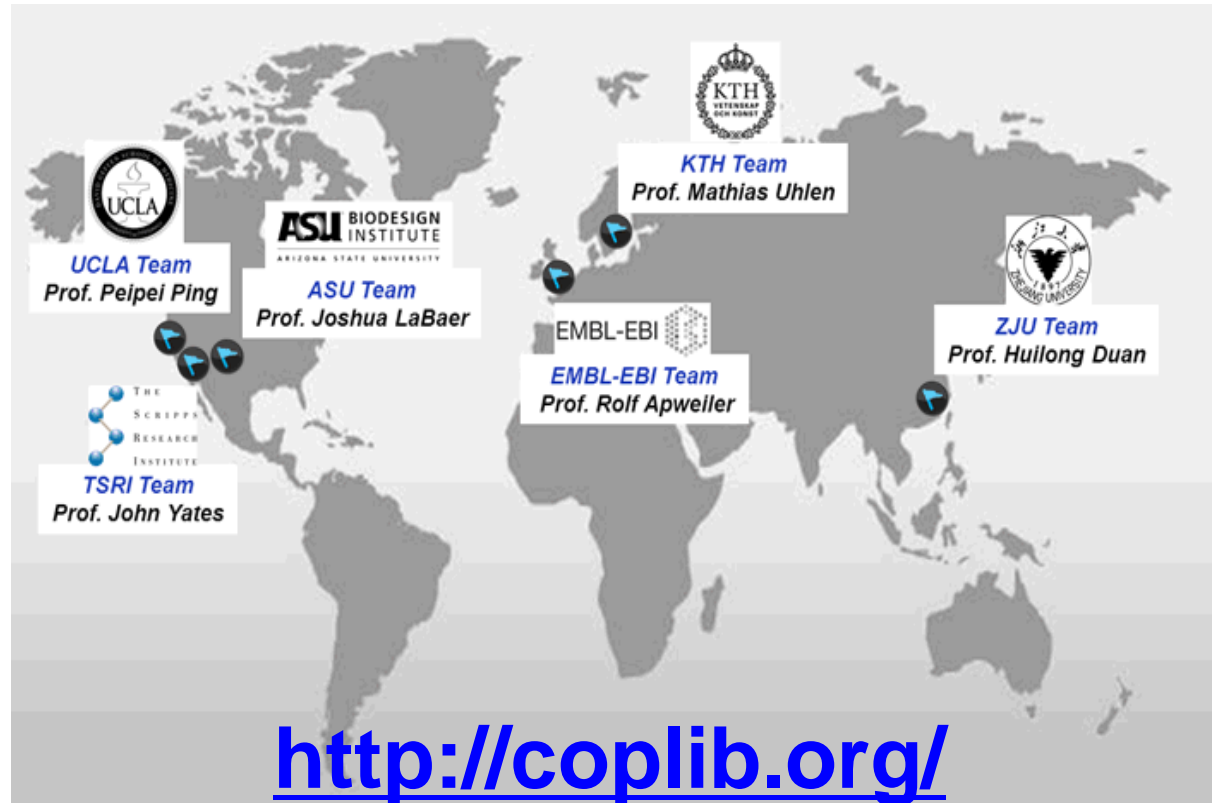


# 谢谢!

## The Cardiac Organellar Peptide Spectral Library

**COPa** Library

The Cardiac Organellar Peptide Spectral Library  
浙江大学/UCLA心脏蛋白质组谱图数据库计划



**COPa** Library

Cardiac Organellar Peptide Spectral Library  
Where proteomics meets cardiovascular biology and medicine

Protein ID



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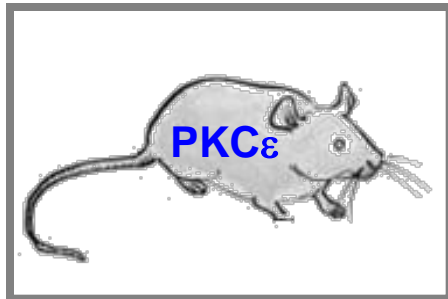
[COPa Wiki](#)

# Ongoing Work

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**Drosophila Mitochondrial Proteome**



**Protein Kinase C epsilon Transgenic Mice  
(Cardio-protection)**