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Modeling the Effects of Microgravity On Oxidation in Mitochondria: A Protein Damage Assessment Across a Diverse Set of Life Forms

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December 2013

Motivation:

- Protein degradation (leading to muscular atrophy, for example) appears to be exacerbated by exposure to microgravity.

Study Objective:

- To determine some of the general trends of motifs which attract oxidative carbonylation across a wide set of organismal protein sequence data.

Conclusions:

- We show that there are less motifs attracting carbonylation in mitochondrial protein than in non-mitochondrial sequence data.

Houston, We have a Problem!

Rats in Space: Major Findings (corroborated by the literature)

- High degrees of oxidative protein stresses
- Evidence of damage: cell & mitochondrial (Mt) proteins
  - Rats acquired degraded and irregular-shaped Mt
  - Muscle protein: Reduced Mt function
  - Generalized myofibrillar edema (tissue *swelling*)
  - Onset of muscular atrophy
  - Cell death and on-set of heart failures

This is Houston: We Also Have the Same Problem!!

- Approximately 10% of the proteome is more prone to carbonylation during ageing, starvation or disease.
- Ageing causes oxidative stress to protein on Earth.
- Accumulation of oxygen radicals causes irreversible protein damage.

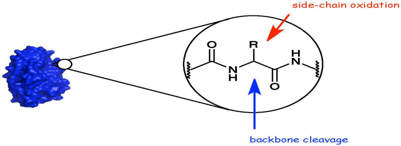
Damage By Carbonylation

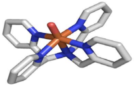
- Carbonylation refers to the oxidation of protein side chains.
- Oxidative Stress Condition: Irreversible, non-enzymatic protein modification
- Oxidative damage may lead to loss of protein function.
- Considered a widespread indicator of severe oxidative damage

Carbonylation: Some Causes

Protein Carbonyl Groups

- Protein degradation may come from free radicals generated in making energy.
- Reactive Oxygen Species (ROS)
  - ROS: peptide bond cleavage
  - Proteins are major targets for ROS and secondary by-products of oxidative stress.
- Direct oxidation of protein side chains: Lysine (K), Arginine (R), Proline (P), and Threonine (T)

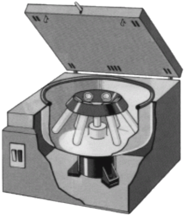




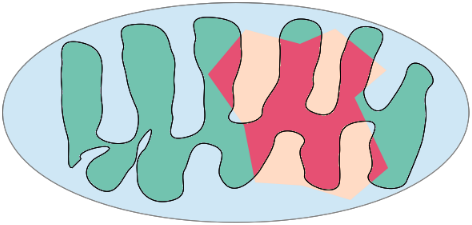
$[Fe^{IV}(O)(N4Py)]^{2+}$

Ekkati, A.R.; Kodanko, J.J. J. Am. Chem. Soc., **2007**, 129, 12390
Abouelatta, A.; Campanali, A.A.; Ekkati, A.R.; Shamoun, M.; Kalapugama, S.; Kodanko, J.J. Inorg. Chem., **2009**, 48, 7729

- Type of oxidative stresses could be explored and studied by simulation:
  - Prescribed immobility - Coma and bed rest patients
  - Suspension - Unused muscle tissue
- Common ailments:
  - Muscular atrophy; negative impact on heart function
  - Insulin resistance
  - Inhibited function of brain tissues



- The build-up of mutations and deletions in mtDNA may impair respiratory chain function (energy production).
- Mt function impairment and cell death
- May impact other Mt functions
    - Ageing: Protein degradation
    - Links to diseases: Parkinson's, Alzheimer's and Huntington's

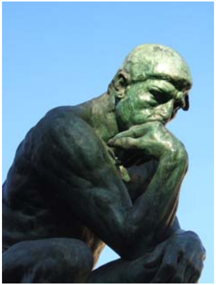


Recovery After Exposure to Carbonylation Source

- Healthy protein regrowth is not always certain
- Possible healing may be possible after a short-term exposure to microgravity
- Therapy is often necessary

Research Question

- Is it likely that Mt have fewer oxidative *accidents* due to protein composition?
- Since Mt perform oxidative processes to produce energy, does it appear that Mt protein has evolved some form of *protection* from the side effects of oxidation?
- Does is appear that non-Mt protein also have this protection?



Avoidance of Dangerous Words In Sequence Data

ISBRA 2012 – Dallas, TX

Distributions of Palindromic Proportional Content in Bacteria

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Abstract. DNA palindromes, the reversed and complemented genetic words, are read the same in the 3' to 5' as the 5' to 3' direction, and can form a unique restriction sites (RSs) where enzymes are able to cut DNA. Several studies have confirmed that short palindromes,

- There are dangerous words in biological sequence data.
- These words may be found in low abundance: Below expected rates.
- Words may be influenced evolutionary pressures.

Words Susceptible To Oxidative Damage

- Literature: Motifs (words) that may attract oxidation:
- **RKPT**: Contains a combination of Proline (P), Arginine (R), Lysine (K), Threonine (T)
  - RKPT-enriched motifs were often found at carbonylation sites in protein samples.
  - Mass spectrometry: Carbonylation sites may contain RKPT motifs (Maisonneuve *et al.*).
- **PEST**: A combination of Proline (P), Glutamic Acid (E), Serine (S) and Threonine (T)
  - Involved in proteolytic signaling for rapid protein degradation by cellular regulation
  - Dealing with stress: the up-regulation of genes for stress responses in plants

- Motifs: RKPT and PEST sets
- Protein Sequences:
  - One long sequence: All proteins from an organism are placed end to end with delimiters.
  - Obtained from Uniprot Protein Knowledgebase
  - Documented Protein Sequence Data:
    - *Mitochondrial* and *non-Mitochondrial*
    - *Enzymatic* and *non-Enzymatic*
  - Diverse organisms

Diverse Organismal Data

	Common Name	Scientific Name
1	African Clawed Frog	*Xenopus laevis*
2	Amoeba	*Acanthamoeba castellanii*
3	Mustard Plant	*Arabidopsis thaliana*
4	Aspergillus	*Aspergillus fumigata*
5	Bakers Yeast	*Saccharomyces cerevisiae*
6	Domestic Dog	*Canis familiaris*
7	Fruit Fly	*Sophophora melanogaster*
8	House Mouse	*Mus musculus*
9	Human	*Homo sapiens*
10	Maize	*Zea mays*
11	Norway rat	*Rattus norvegicus*
12	European Rabbit	*Oryctolagus cuniculus*
13	Nematode Worm	*Caenorhabditis elegans*
14	Zebrafish	*Danio rerio*

Number of Studied Proteins By Organism

	Organism	Mt	Non-Mt
1	African Clawed Frog	169	3202
2	Amoeba	32	17
3	Mustard Plant	707	11517
4	*Aspergillus fumigata*	87	794
5	Bakers Yeast	1056	6744
6	Dog	60	743
7	Fruit Fly	204	2994
8	House Mouse	973	15652
9	Human	1027	19240
10	Maize	38	680
11	Norway Rat	571	7287
12	European Rabbit	46	843
13	Nematode Worm	199	3232
14	Zebrafish	202	2696

Proportions

Find the Coverage of Each Motif in Each Protein Sequence

>Protein_length 20
MTKTTFLGRSLKPPRHVKPPR

>m1 RKPT	>m4 RRTR
>m2 TKTT	. . .
>m3 KPPR	>m256 RKTR

for each sequence (m_i) in the set

$$m_i \text{ in } S_L = \frac{count(m_i) * |m_i|}{|S_L|}$$

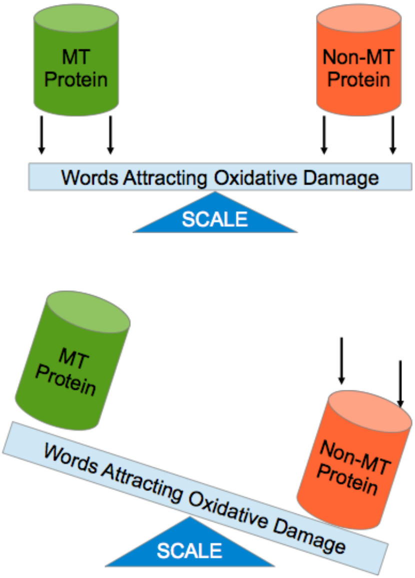
Percentages

>m1 0.0 %	>m4 0.0 %
>m2 19.05 %	. . .
>m3 38.1 %	>m256 0.0 %

Statistical Data for clustering

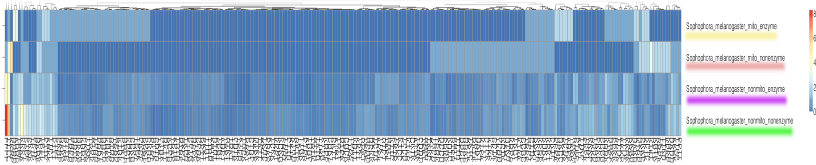
Where, *m_i* is a motif, *S_L* is a protein sequence, count(*m_i*) is the number of occurrences of *m_i* found in *S_L*, |*m_i*| and |*S_L*| are the lengths of the motif and sequence, respectively.

Generaly Less Oxidation-Attracting Motif Content in Mt Protein

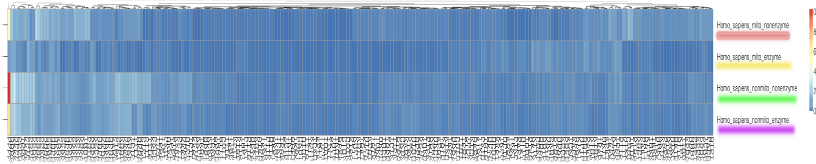


The absence of motif content in the large blank spaces in Mt proteins

Fruit Fly mitochondrial Proteins



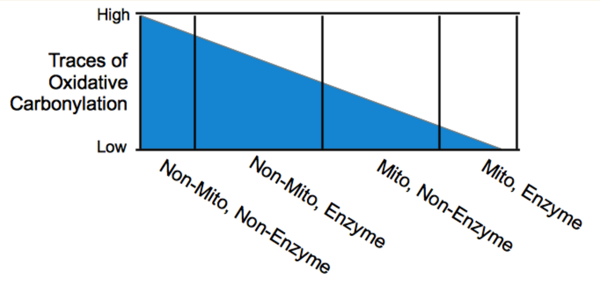
Human mitochondrial Proteins



Yellow = Mt,Enzyme; Red = MT,nonEnzyme;
Purple = nonMt, Enzyme; Green = nonMt, nonEnzyme

Conclusions

Mitochondrial Protein Data is Has Fewer Oxidation Motifs



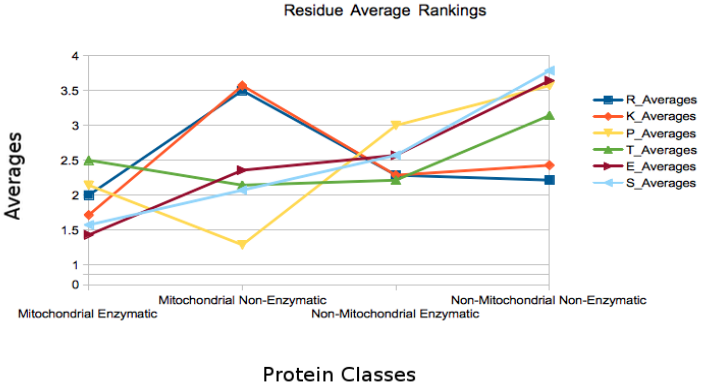
The average amount of RKPT and PEST motif content was least in mitochondrial proteins.

Average Proportions of RKPT and PEST across organismal proteins

Organism	ME PEST	ME RKPT	MN PEST	MN RKPT	NE PEST	NE RKPT	NN PEST	NN RKPT
African clawed frog	1.5	1.5	1.5	1.5	3	4	4	3
Amoeba	3	2	1.5	4	1.5	1	4	3
Aspergillus	2	1.5	1	1.5	3	3	4	4
Bakers yeast	1	1	2	2	3	3	4	4
Domestic dog	1.5	1.5	1.5	1.5	3	4	4	3
European rabbit	1.5	1.5	1.5	1.5	3	4	4	3
Fruit fly	1.5	1.5	1.5	1.5	3	3	4	4
House mouse	1	1	3	2	2	3	4	4
Human	1	1	3	3	2	2	4	4
Maize	1.5	1.5	1.5	1.5	4	3	3	4
Mustard plant	1	3	2	1	3	2	4	4
Nematode worm	1	1	2	2	3	3	4	4
Norway rat	1	1	3	3	2	2	4	4
Zebrafish	2	1.5	1	1.5	3	3	4	4
Averages	1.46	1.46	1.86	1.96	2.75	2.86	3.93	3.71

The average amount of RKPT and PEST motif content was least in mitochondrial proteins.
ME = Mt, Enzymatic, **MN** = Mt, Non-Enzymatic,
NE = Non-Mt, Enzymatic, **NN** = Non-Mt, Non-Enzymatic

Average Proportions of RKPT and PEST



Rankings of **R**, **K**, **T**, **P**, **E** and **S** residues across the protein classes of all organisms. Note how the enzymatic protein content had closer groupings of individual amino acid residues.

Future Works

- If there are motifs in Mt which attract oxidation:
  - How are these motifs distributed?
  - Do these motifs help form the same kinds of protein secondary structures (e.g., coils, sheets, helices?)
  - Do structures appear to be necessary (e.g., exist in small amounts to add some structure)?

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- Bonham-Carter, Oliver, Hesham Ali, and Dhundy Bastola. **A base composition analysis of natural patterns for the preprocessing of metagenome sequences.** *BMC Bioinformatics* 14. Suppl 11 (2013): S5.
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We would like to thank the support students, faculty and staff in the UNO-Bioinformatics Core Facility. This project has been funded by the grants from the National Center for Research Resources (5P20RR016469), the National Institute for General Medical Science (NIGMS) (8P20GM103427).

This study also funded by the NASA Nebraska Space Grant (2013).

Thank You! Questions?

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