Consequences of an NFU1 Mutation in the Fe-S Cluster Biosynthetic Pathway

Nathaniel Wesley[1], Christine Wachnowsky[2], Insiya Fidai[3], and James A Cowan[1,2,3]

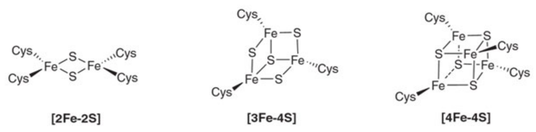
Department of Chemistry and Biochemistry[1], The Ohio State Biochemistry Program[2], The Biophysics Graduate Program[3], The Ohio State University

Abstract

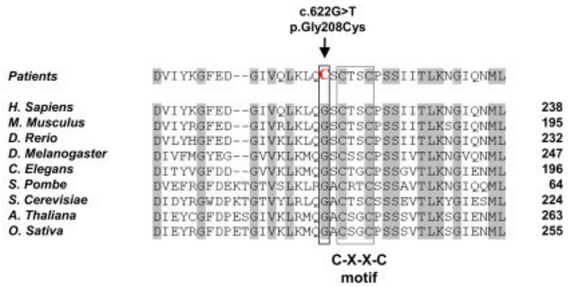
- Iron-sulfur (Fe-S) clusters are highly conserved molecules involved in various essential biochemical processes
- Multiple Mitochondrial Dysfunction Syndrome 1 (MMDS1) has been linked to a mutation near the active site of NFU1, an iron-sulfur scaffold protein
- The connection between the mutation in NFU1 and MMDS1 has recently been discovered, but little is known about the role of NFU1 and why the point mutation results in such drastic consequences
- An investigation into the structural and functional consequences of the mutation using *in vitro* analyses and kinetic assays has shown distinct differences between the wild-type and the mutant protein, which we hope will provide a better understanding of the mechanism of disease

Introduction

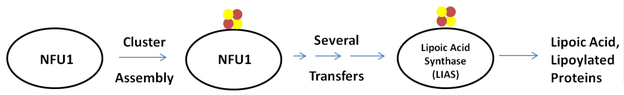
- Iron-sulfur clusters are highly conserved prosthetic groups found in several metalloproteins. They serve several essential and diverse roles in the mechanics of the cell, including electron transfer, regulation of gene expression, and disulfide reduction [1, figure 2]



- Mutations in Fe-S cluster-assembly and transfer proteins dramatically affect several crucial metabolic pathways [3]. Genetic mutations to a specific subset of mitochondrial cluster-delivery proteins are broadly categorized as Multiple Mitochondrial Dysfunction Syndrome (MMDS), and symptoms include impairment of neurological development, lactic acidosis, failure to thrive, and ultimately early death [4]
- MMDS1 arises as a result of the missense mutation in NFU1, which mutates a glycine near the Fe-S cluster binding pocket to a cysteine. The G208C mutation occurs near a highly-conserved CXXC motif in the C-terminal domain of NFU1 observed in species from yeast to humans [4]



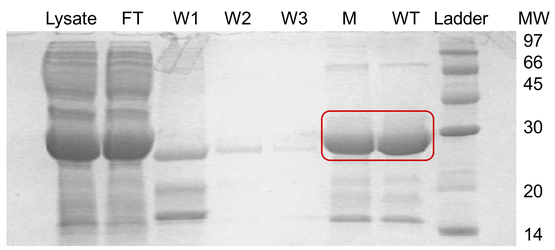
- MMDS 1 results in a phenotype of lipoic acid deficiency, but further examination into genetic data found no mutations in the gene for lipoic acid synthase (LIAS), but rather in NFU1, implicating NFU1 in the proper function of LIAS [5, 6]



Characterization of Apo Mutant Protein

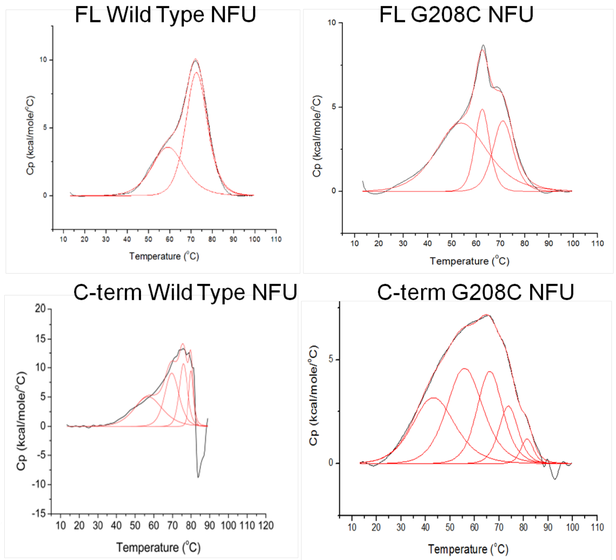
Growth and Purification of Mutant Protein

- Site-directed mutagenesis (SDM) was carried out using custom made primers in order to introduce the G208C mutation into a plasmid containing wild-type DNA for NFU
- The polymerase chain reaction (PCR) was used to amplify the mutated DNA and introduce the mutation into the NFU1 plasmid, which was transformed into *E. coli* expression cells
- The mutant was grown and purified over a Ni-NTA column. Protein purity was assessed using SDS-PAGE gel electrophoresis and shown to be as pure as the wild type



Assessing Structural Changes Using Differential Scanning Calorimetry

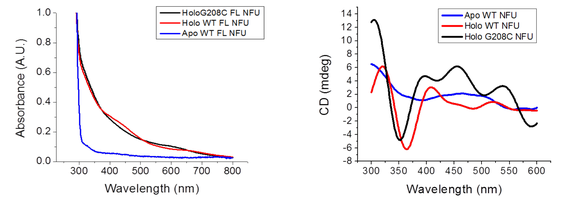
- Differential Scanning Calorimetry (DSC) provides key thermodynamic data such as the melting point (T_m), enthalpy (ΔH), and Gibbs free energy (ΔG) of a sample
- The melting profile that is generated can be contrasted with the melting profile of the wild type in order to detect any structural differences



Characterization of Holo Mutant Protein

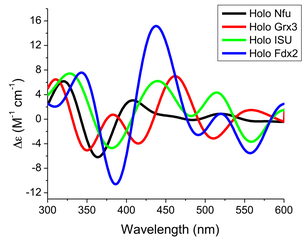
Chemical Reconstitution of Apo Protein

- An iron-sulfur cluster can be built on an apo protein *in vitro* under anaerobic conditions using $FeCl_3$ and Na_2S or L-cysteine with *Tm* NifS
- Features on UV-Vis at 330 nm and 415 nm and unique CD spectrum imply cluster binding

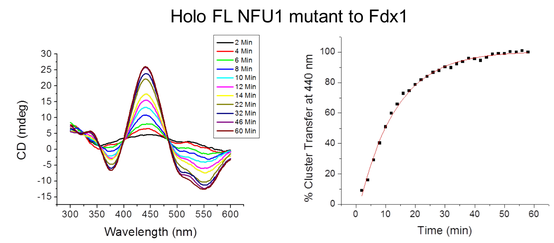


CD for Mapping the Kinetics of Cluster Transfer

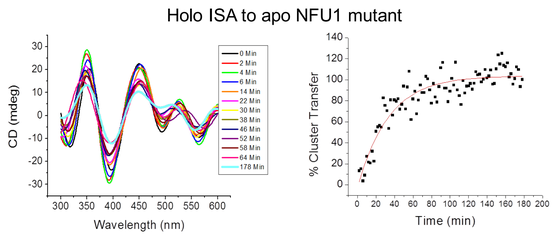
- Holo Fe-S proteins demonstrate unique CD spectra
- Spectra based on the specific Fe-S cluster binding site and the chirality of the cluster ligands
- The unbound cluster is achiral and demonstrates no CD spectra



Fe-S Cluster Transfer from NFU1



Fe-S Cluster Transfer to NFU1

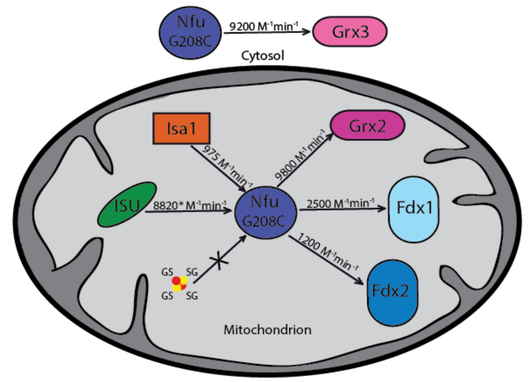


Summary of Rates Compared to the Wild Type

	WT ($M^{-1}min^{-1}$)	Mutant ($M^{-1}min^{-1}$)
Nfu to Fdx1	4700	2500
Nfu to Fdx2	3800	1200
Nfu to Grx2	3700	9800
Nfu to Grx3	36000	**9200**
ISU to Nfu	4750	8820
ISA to Nfu	6700	**975**

Conclusions

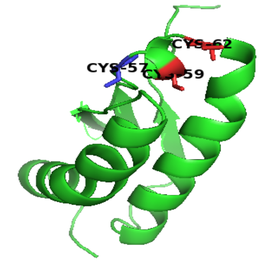
- Monitoring the protein transfers of the G208C NFU mutant using CD has shown a general trend of slower rates, with few exceptions compared to the wild type. This confirms that the G208C mutation significantly changes the chemical behavior of the protein. Most notably, the mutant transfers to Grx3 four times more slowly.



- Changes in the DSC protein melting profile indicate a structural modification as a result of the mutation

Future Work

- Evaluate the equivalency of each cysteine residue by analyzing behavior of twice-mutated NFU1 proteins
- Determine structure of C-terminus using NMR experiments and contrast to wild type
- Examine transfer to suggested final target LIAS



Citations

1. Johnson, D.C., et al., *Structure, Function, and Formation of Biological Iron-Sulfur Clusters*. Annual Review of Biochemistry, 2005. **74**(1): p. 247-281.
2. Fontecave, M. *Iron-sulfur clusters: ever-expanding roles*. Nature Chemical Biology, 2006. **2**: p. 171-174.
3. Ahting, U., et al., *Clinical, biochemical, and genetic spectrum of severn patients with NFU1 deficiency*. Frontiers in Genetics, 2015. **6**(123): p. 1-13.
4. Navarro-Sastre, A., et. al., *A fatal mitochondrial disease is associated with defective NFU1 function in the maturation of a subset of mitochondrial Fe-S proteins*. American Journal of Human Genetics, 2011. **89**: p. 656–667.
5. Invernizzi, F., et al., *Cavitating leukoencephalopathy with multiple mitochondrial dysfunction syndrome and NFU1 mutations*. Frontiers in Genetics, 2014. **5**: p. 1–6.
6. Nizon, M., et al., *Leukoencephalopathy with cysts and hyperglycemia may result from NFU1 deficiency*. Mitochondrion,2014. **15**: p. 59-64.

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