Bioinformatics Approaches to Protein Interaction and Complexes: Application to Fe-S Cluster Biogenesis Model

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- **Friedreich’s Ataxia (FRDA)** is caused by **Frataxin** deficits.

- FRDA is a human, **neurodegenerative and hereditary disease** that affects the equilibrium and movement coordination, causes muscle weakness and heart hypertrophy among many other symptoms.

- This disease is the **most common autosomal recessive ataxia in Caucasians**.
• Frataxin is encoded by the gene \textit{fxn}.

• In FRDA a \textbf{GAA expansion} in the first intron of the gene generates an aberrant structure of DNA that reduces its transcription and Frataxin expression.

• In normal population, the \textbf{GAA motif is polymorphic} but in FRDA patients the repetitions are increased up to more than 1600 correlating with the severity of the disease.
• Frataxin is a **compact and globular** protein composed by two α-helices and five anti-parallel β-sheets that form an **α/β sandwich**.

• Frataxin is very **evolutionary conserved** and the most conserved domains correspond to these five β-sheets and one of the α-helices.

• Frataxin is a **mitochondrial** protein although encoded in the nucleus. It is an **ubiquitous** protein and its expression is higher in tissues that require huge amounts of energy.

Yeast Frataxin (Yfh1), 174 aa, PDB code: 2GA5
• Frataxin proposed **functions** have been always associated with **iron accumulation** inside the mitochondria and increased sensitivity to **oxidative stress**.

• Frataxin has been suggested to play an important role in the **ISC biogenesis** process donating the required iron.

Martinelli et al., 2012
• Iron-Sulfur Clusters (ISC) are **prosthetic groups formed by iron and sulfur** that are present in many proteins and are very flexible and ingenious.

• These structures are **ligated to proteins by cysteine** residues and perform many different functions such as mitochondrial respiration.

• The principal forms of ISCs typically present in proteins are \([2\text{Fe-2S}]\) and \([4\text{Fe-4S}]\).

**Structure of a \([4\text{Fe-4S}]\) ISC.** Iron atoms are shown in green and sulfur in yellow (Frazzon 2001).
• The biogenesis of ISC is carried out by complex protein machinery that, in eukaryotes, is placed in the mitochondria.

• In the human initial ISC assembly step, a protein complex composed by an iron donor (Frataxin), a sulfur donor (Nfs1), an accessory protein (Isd11) of unknown function but essential in the process, and a scaffold protein (Iscu) is formed.
ISC biogenesis key proteins. Schematic representation of the proteins involved in the initial ISC assembly process inside the human mitochondria.
Details of Iron and Sulfur transfer for ISC assembly.
• The **biogenesis of ISC** is carried out by **complex protein machinery** that, in eukaryotes, is placed in the mitochondria.

• In the **human initial ISC assembly step**, a protein complex composed by an iron donor (**Frataxin**), a sulfur donor (**Nfs1**), an accessory protein (**Isd11**) of unknown function but essential in the process. and a scaffold protein (**Iscu**) is formed.

• There are **high similarities between human and yeast** ISC molecular mechanisms and this makes yeast ideally suited to better understand the ISC biogenesis system.

• The yeast initial steps of yeast ISC assembly machinery are basically the same as shown before for humans where a protein complex composed by an iron donor (**Yfh1/Frataxin**), a sulfur donor (**Nfs1**), an accessory protein (**Isd11**) of unknown function but essential in the process. and a scaffold protein (**Isu/Iscu**) is formed.
ISC biogenesis key proteins. Schematic representation of the proteins involved in the initial ISC assembly process inside the yeast mitochondria.
• Characterize the proteins Frataxin (Yfh1), Nfs1, Isu and Isd11 from the sequence, structure, function and interaction point of view.

• Improve the current model of ISC biogenesis protein complex and study the dynamic behavior of its components to propose a new dynamic model of the ISC assembly process in yeast.

• Have a better knowledge about the molecular pathology of the ISC deficits occurring in FRDA.
The sequences of Frataxin, Isu and Nfs1 of different organisms were used to perform sequence multi-alignment analyses and see the most evolutionary conserved and important regions.
MATERIALS AND METHODS

Electronegative residues

Evolutionary conserved regions

Interaction regions prediction

Electronegative residues
• The sequences of Frataxin, Isu and Nfs1 of different organisms were used to perform sequence multi-alignment analyses and see the most evolutionary conserved and important regions.

• Several classical bioinformatics studies with protein sequence analysis servers were made to set up some characteristics for each of the proteins.

• Structure modeling of Isu and Nfs1 was done using applications specifically designed for this purpose like PsiPred, ESyPred3D. 3D-PSSM or Phyre.

• Almost nothing is known about the structure of Isd11, and for this reason we applied the “de novo” modeling tool Robetta.
MATERIALS AND METHODS

Model 1
Confidence 20.16
Score -9.81

Model 2
Confidence 15.58
Score -7.48

Model 3
Confidence 15.63
Score -8.29

Model 4
Confidence 19.02
Score -10.6

Structural fitting of the models

Models of the protein Isd11 obtained with Robetta.
• The sequences of **Frataxin**, **Isu** and **Nfs1** of different organisms were used to perform sequence **multi-alignment** analyses and see the most evolutionary conserved and important regions.

• Several **classical bioinformatics** studies with protein sequence analysis servers were made to set up some characteristics for each of the proteins.

• **Structure modeling of Isu and Nfs1** was done using applications specifically designed for this purpose like **PsiPred**, **ESyPred3D. 3D-PSSM** or **Phyre**.

• Almost nothing is known about **the structure of Isd11**, and for this reason we applied the “**de novo**” modeling tool **Robetta**.

• The 3D structure of **Frataxin** is available, **PDB code: 2GA5**.
• Protein interaction regions of all the proteins → **ProMate, meta-PPISP, and PPI-Pred**.

• ISC biogenesis protein interaction network → **APID, BIND, BOND, BioGRID, MINT, DIP, GRID, Mpact-MIPS, HPRD or IntAct**.

• Iron atoms were situated in Frataxin using **VEGA ZZ 2.3.2 Molecular Modeling Toolkit** and **ArgusLab 4.0.1**.

• All the previously mentioned studies were always complemented with the existing **literature** information to contrast the obtained data.
• The docking assays were performed with the programs **Escher NG, BiGGER, Hex** and **HADDOCK**.

• The most representative solutions of **Escher NG** and **Hex** were selected with **DockAnalyse**.

• These representative solutions were loaded in modeling tools like **RasMol**, **PyMOL** or **UCSF Chimera**, in order to monitor the surface and rotation displacements between the docked proteins.

• **HADDOCK** docking tool employs biochemical and/or biophysical interaction data such as bioinformatics predictions. Therefore, the previous data obtained from all the previous bioinformatics analyses and knowledge on the proteins could be used to refine the dockings.

• **EasyModeller** and **DeepView-Swiss-PdbViewer** programs were also used for modeling purposes and to finally propose a coherent model for the initial steps of the ISC biogenesis machinery.
The modeling process of the yeast iron-sulfur cluster assembly protein complex combining Modeller and DeepView-Swiss-PdbViewer.
• The protein **Isd11** does not seem to directly participate in ISC biogenesis, but it is essential in eukaryotes where it has a fundamental role **avoiding Nfs1 aggregation.**
• We propose that **Isd11 might be blocking the Nfs1 aggregation** region allowing or a proper functionality of the complex.

All solutions for Nfs1-Isd11 interaction

Solution with highest energy for Nfs1-Nfs1 interaction

**SAME REGION!**
• The Nfs1 structure indicates conformational plasticity both of the protein and of a long loop containing a cysteine essential for its function. These putative conformational changes were examined with several hinge prediction algorithms, and the expected movements were obtained both for the whole protein and the loop.
RESULTS AND DISCUSSION

A) Entire protein movements

B) Loop conformational change

C) Dynamic open-close model of the Nfs1 functional dimer

Nfs1 and its open-close conformational changes
• Our proposed open-close Nfs1 conformational changes allowed us for the improvement of the current ISC biogenesis model by putting the iron atoms, sulfur atoms, Nfs1 cysteine loop and Isu ISC assembly pocket close enough to allow for the formation of Iron-Sulfur covalent bonds required for ISC biogenesis.
RESULTS AND DISCUSSION

Structural details of iron and sulfur donation

A) Close loop conformation and iron donation
- Zoom and rotate to properly show the required details

B) Open loop conformation and sulfur donation
- Zoom and rotate to properly show the required details
• Taking all the previous analyses, models and studies into account, a structure of the initial ISC biogenesis protein complex and its dynamics could be postulated.
RESULTS AND DISCUSSION

Proposed model for the dynamics of the ISC assembly process.

A) TO monomer $\rightarrow$ Nfs1 opening, ISC assembling on Isu and Iron reloading of Frataxin

TC monomer $\rightarrow$ Nfs1 closing, Frataxin iron donation and Nfs1 loop persulfuration.

B) TO monomer $\rightarrow$ Contact of a free cysteine with Nfs1 PLP, Isu returning to its initial position and new assembled ISC donation

Close Nfs1 monomer – Open Nfs1 loop $\rightarrow$ Nfs1 loop opening, Isu approach, sulfur donation and Frataxin expelling

D) Close Nfs1 monomer – Open Nfs1 loop $\rightarrow$ Nfs1 loop opening, Isu approach, sulfur donation and Frataxin expelling

TO monomer $\rightarrow$ Contact of a free cysteine with Nfs1 PLP, Isu returning to its initial position and new assembled ISC donation
CONCLUSIONS

• The sequence, structure, function and interaction of Frataxin, Nfs1 and Isu have been deeply studied. A specific structure and function for the eukaryotic protein Isd11 has been proposed.

• A new dynamic model of the ISC assembly protein complex in yeast as well as the details concerning the iron and sulfur donation to the process have been suggested.

• This approach should help not only in the understanding of the function and molecular properties of the FRDA causing protein (Frataxin) and its protein partners, but also in increasing the knowledge about FRDA being helpful for a possible future treatments of FRDA.

GRACIAS POR VUESTRA ATENCIÓN