



Introduction

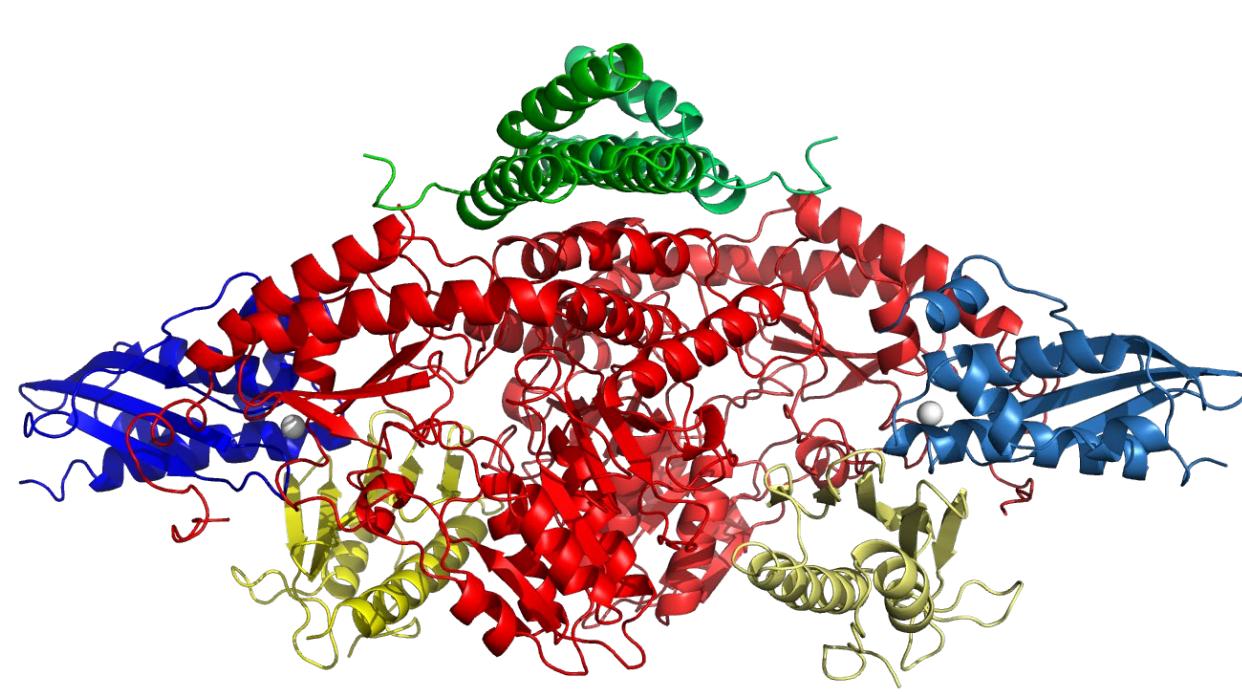
Friedreich's ataxia (FA) is the most common form of inherited ataxia (a group of disorders that affect coordination, balance and speech)

- FA is primarily caused by a mutation in the gene that encodes the frataxin protein (FXN), found in mitochondria
- FXN plays a role in iron storage, iron sensing, and acting as a chaperone for protein folding
- Deficiency can lead to the synthesis of iron-generated free radicals, which contributes to oxidative stress
- A mutation in the ISCU-encoding gene, ISCU M140I, has been hypothesized to bypass the frataxin ion facilitation, which could be a promising target for drug design to treat FA

Wild Type Iron-Bonded SDAUF Complex

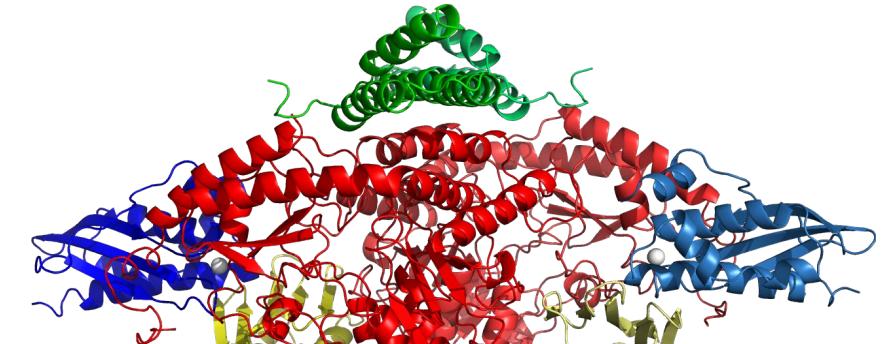
NFS1, ISD11, ACP, ISCU, FXN

Each of the represented proteins listed above has two chains in the SDAUF complex. All the generated mutants are on both chains of the respective protein. The white orbs in the protein structure are iron ions bound at the binding site, to give an indication of biological significance regarding the mutant analysis.

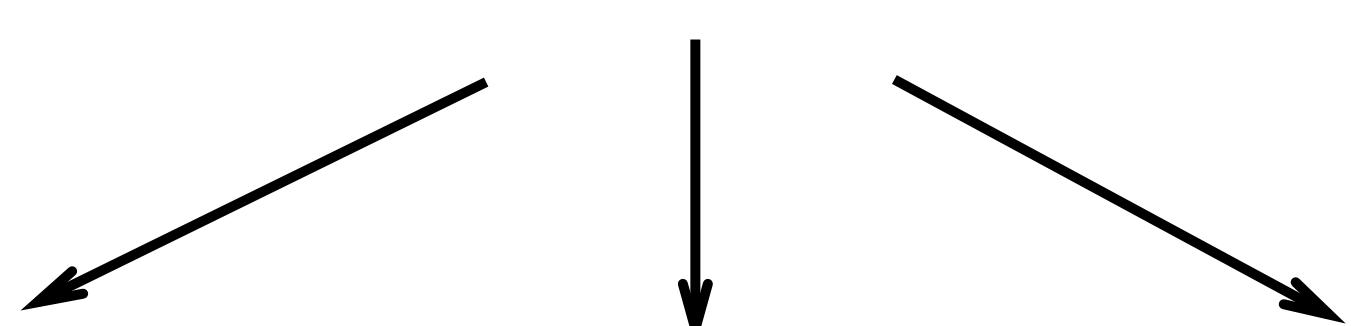


Methods

(1) Generate point mutations on the 6nzu protein complex in PyMol. 4 frataxin mutations and 1 ISCU mutation made. 10 molecular simulations run with Amber.



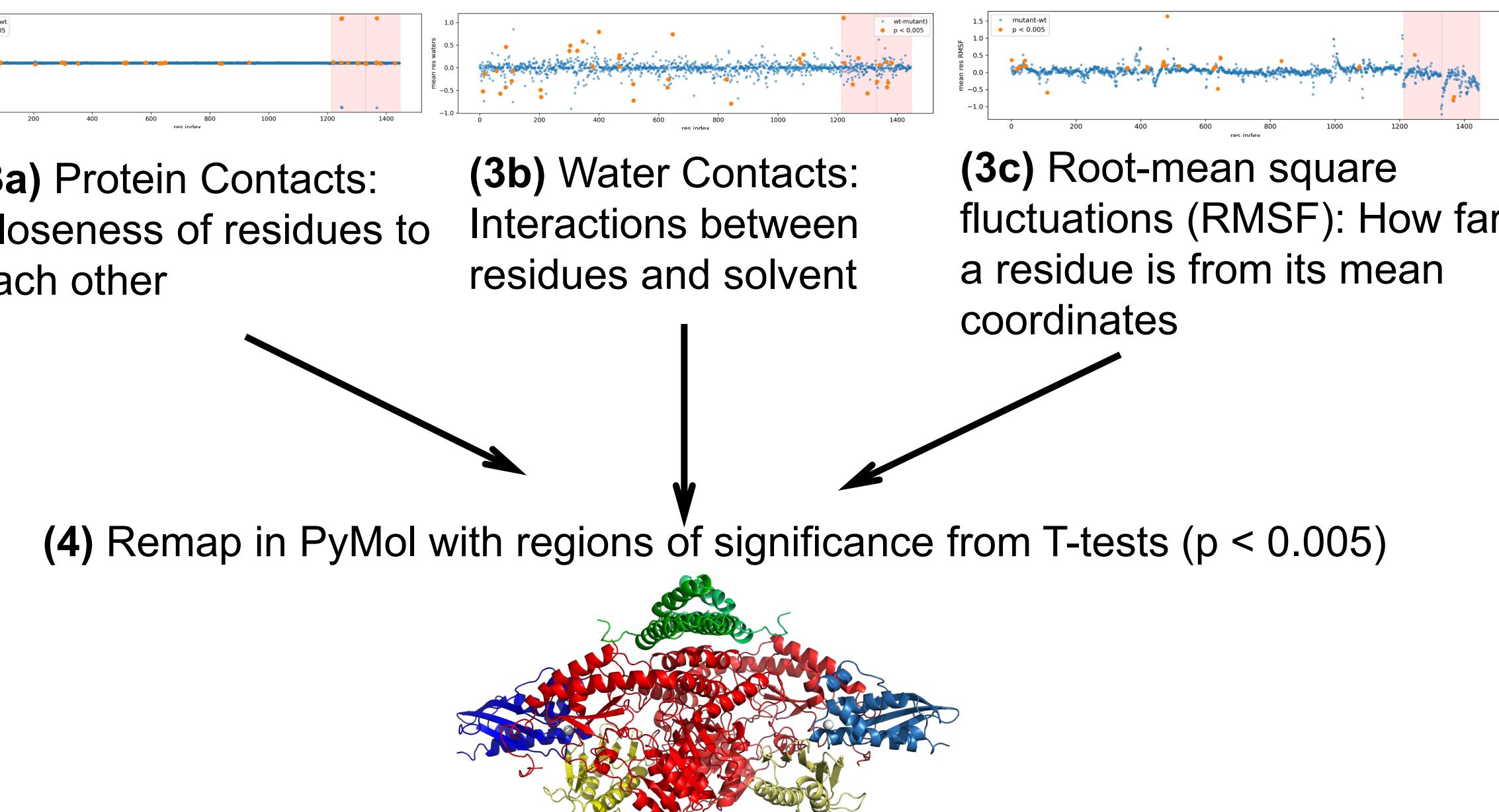
(2) Calculations were done with Python scripts and analyzed in Jupyter Notebook



(3a) Protein Contacts:
Closeness of residues to each other

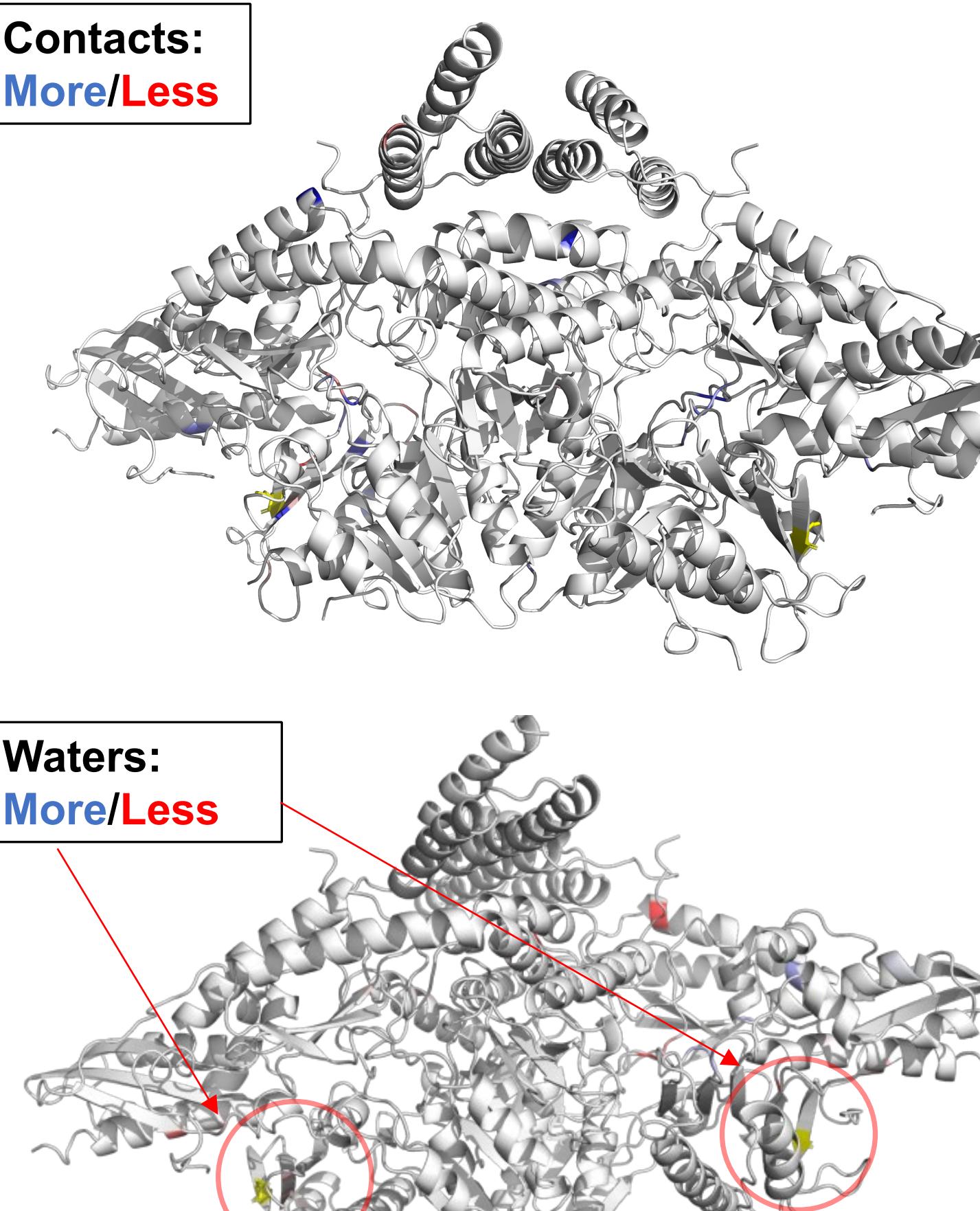
(3b) Water Contacts:
Interactions between residues and solvent

(4) Remap in PyMol with regions of significance from T-tests ($p < 0.005$)



FXN R165N

Contacts:
More/Less



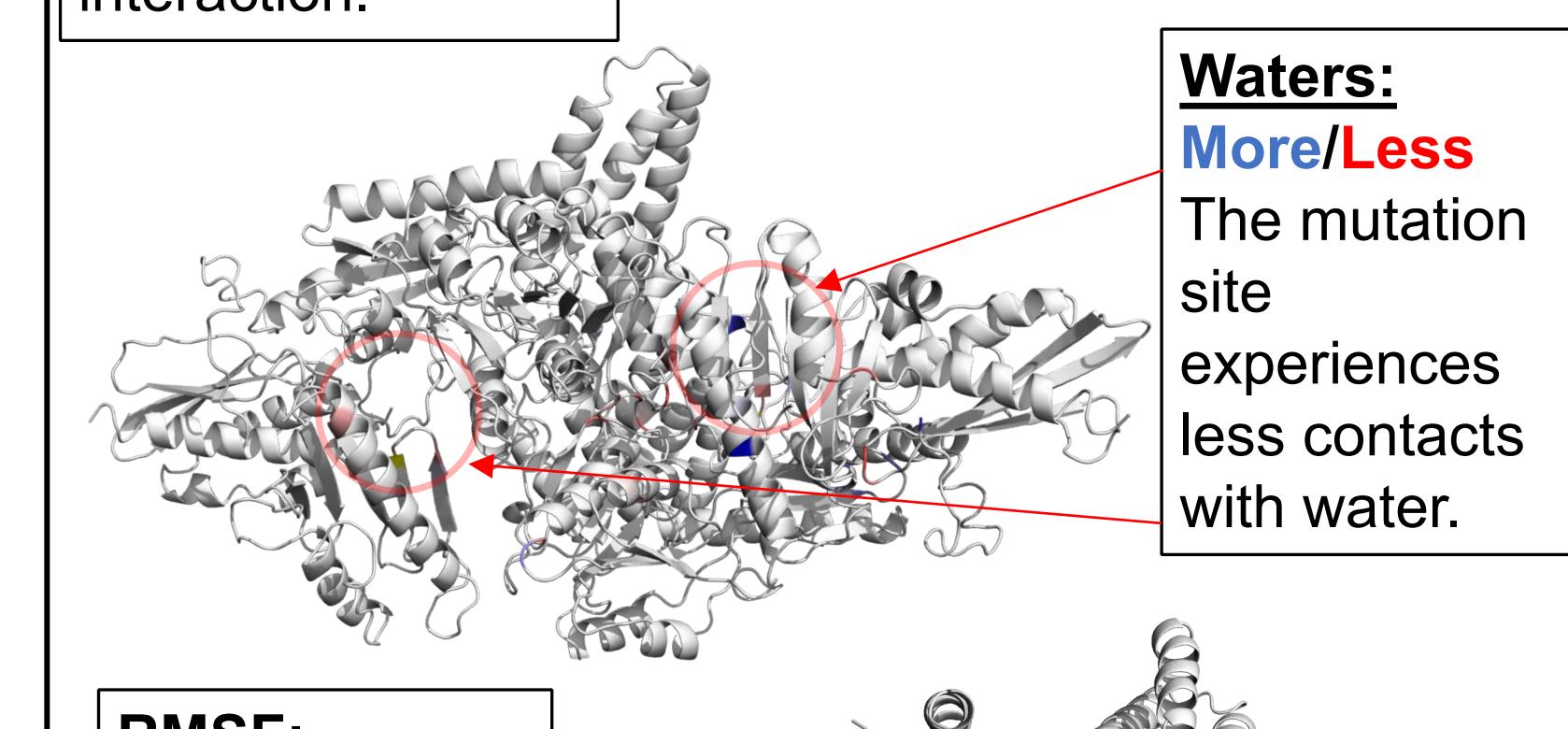
Waters:
More/Less

Hypothesis: The above images show the effect a mutation from Arginine to Asparagine at position 165 on the frataxin chains. The mutations are shown in yellow on the images, and other changes in color show affects throughout the molecule. Both images show a slight change in the amount of interaction around the site of the mutation, but there is nearly no change in the rest of the molecule. A mapping of changes in RMSF values showed absolutely no change. This lack of difference between mutant and wild type leads me to hypothesize that the mutation is not significant.

FXN G130V

Contacts:
More/Less

The mutation site typically sees less contacts, but adjacent residues may experience more and influence NFS1 and FXN interaction.



RMSF:
Flexible/Rigid

Several residues of NFS1 are more flexible, which may influence sulfur transfer.

Hypothesis: FXN G130V may decrease the stability of the complex and impact sulfur transfer due to increased flexibility around the mutation site and NFS1.

FXN W173G

Contacts:
More/Less

The area around the mutation has less contact between residues.

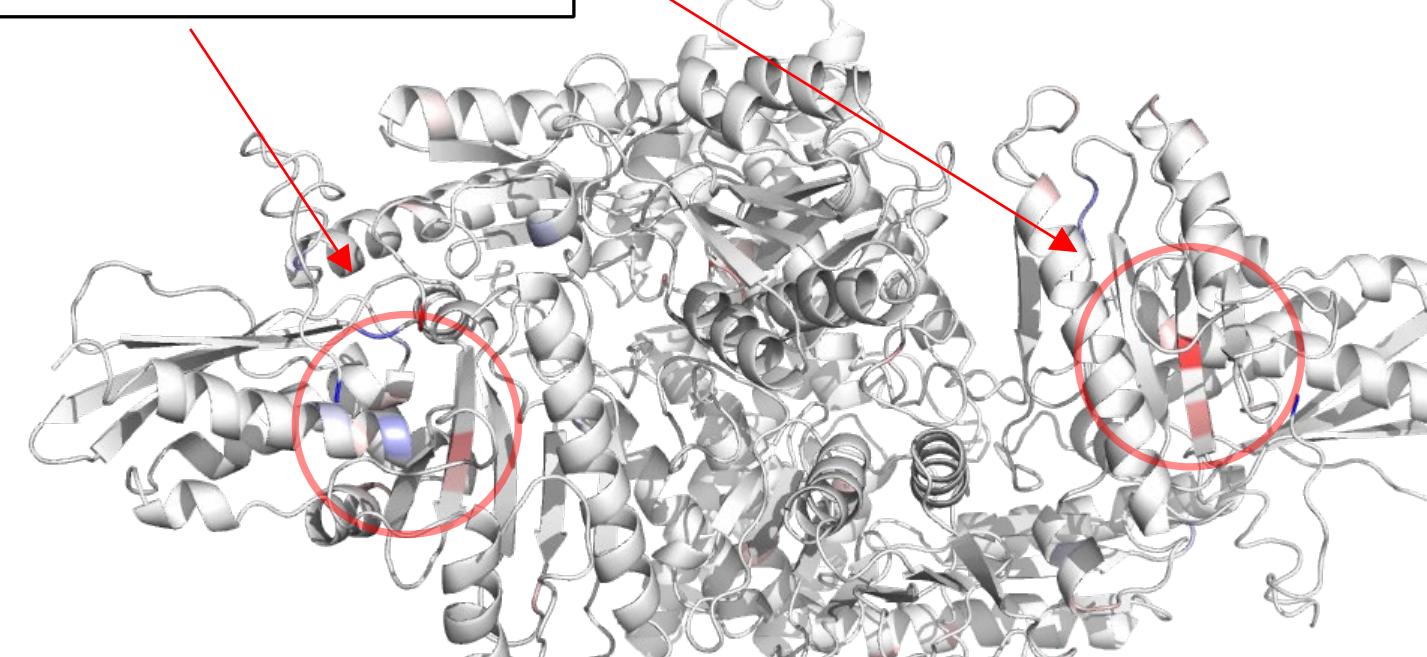
Waters:
More/Less

There are more contacts with water around the mutation.

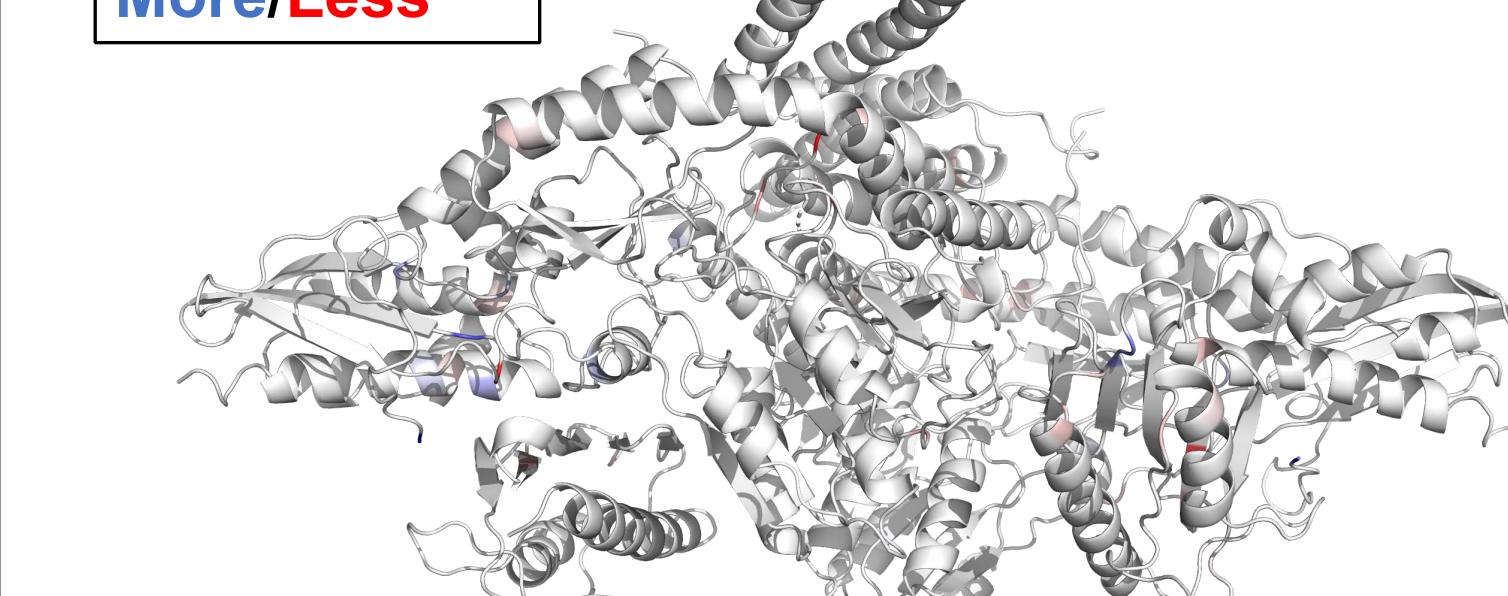
Hypothesis: FXN W173G likely decreases the stability and structure of the frataxin complex due to a decrease in size and decrease in reactivity through hydrogen bonding.

FXN W155R

Contacts:
More/Less



Waters:
More/Less



Hypothesis: W155R mutation decreases the structure of the complex due to the fewer physical contacts between residues. It has no significant change in the amount of interaction with water as the wild-type, and the RMSF of the complex showed little significant change compared to the wild-type. The primary structural differences were due to side-chain conformational changes near the mutation site and indicate lower affinity of iron-sulfur transfer.

Iron–sulfur cluster assembly scaffold protein (ISCU)

ISCU acts as a scaffold for cluster assembly, hence the name, and works with FXN and NFS1 to transport sulfur ions as well as to generate cluster intermediates. A team of researchers hypothesized that the mutation of the two ISCU chains in the SDAU complex, swapping Methionine at position 140 for Isoleucine, bypasses the need for FXN to act as an activator for certain enzymes related to complex formation. Experimentally, the mutant was shown to cause no structural change to the ISCU chain itself, and the Molecular Dynamics simulation revealed conditions conducive to increased binding affinity and a potential increase in function of the helper protein ISD11.

Step 1 – Cysteine desulfurase Step 2 – Sulfur Transfer
Step 3 – Cluster Synthesis Step 4 – Cluster Transfer

Source (text reference & image):
Das et al., *Journal of Biological Chemistry*, 2019
doi: <https://dx.doi.org/10.1074%2Fjbc.RA119.007716>

ISCU M140I

Contacts:
More/Less

Sharp decrease around the binding site/mutation points.

RMSF:
Flexible/Rigid

Increase in flexibility at the abutting point between ISD11 and NFS1 chains.

Hypothesis: ISCU M140I likely increases the binding affinity of iron at the two binding sites through a conformational change in the chains surrounding them, which points towards a higher affinity of iron-sulfur transfer and subsequent complex formation. The water analysis revealed no discernable trend, though some values were significant.