Engineering New Metalloprotein Scaffolds Through Disulfide Bond Formation

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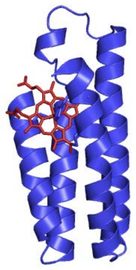
Abstract

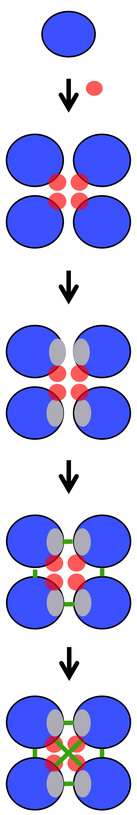
Proteins are the building blocks of nature. In order to understand how natural proteins function, and to extend their capabilities, we seek to engineer new metalloprotein scaffolds. To do this, we use metals to assemble proteins into larger structures, allowing us to engineer them further. In doing so, we ultimately seek to understand the natural behavior of proteins , and to engineer new artificial enzyme active sites for enzymatic activity. Using cytochrome cb_{562} as a model system we have engineered new tetrameric scaffolds called the RIDC1 family. Currently, we are engineering RIDC1 for increased stability through the use of disulfide bond formation between protein monomers.

Cytochrome cb_{562}

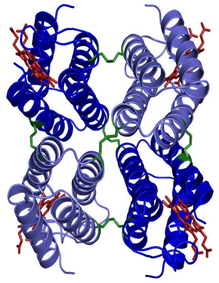


- Monomeric structure at high protein/ metal concentration
- Small and stable structure
- Can be crystallized and studied
- Soluble
- Color red (easy to track)

Protein Engineering Methods

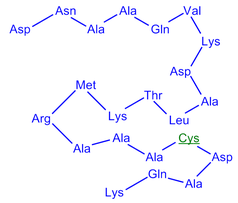


RIDC1 96/81/38C



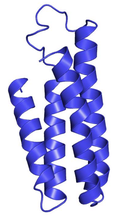
- Structure obtained by X-ray crystallography
- Tetramer is held together by six disulfide bonds

Levels of RIDC1 Structure

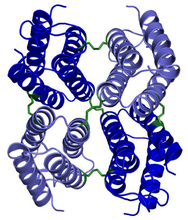


Primary Structure

Secondary Structure

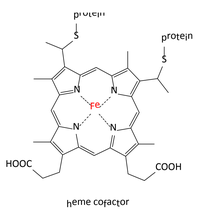


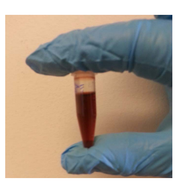
Tertiary Structure

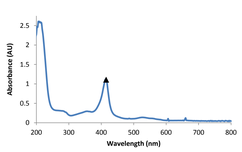


Quaternary Structure

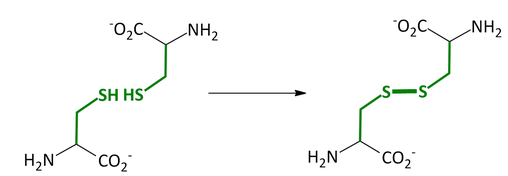
Heme Cofactor







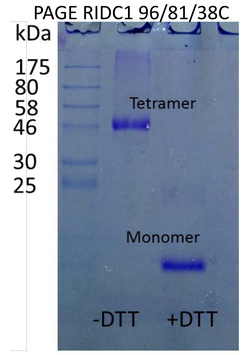
Cysteine and Disulfide Bonds



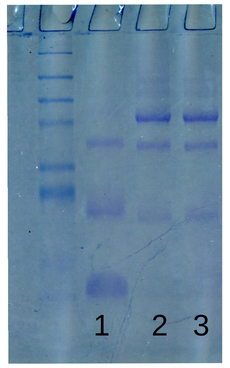
- Free thiol form
- Reduced state
- Disulfide bond form
- Oxidized state

Gel Electrophoresis Analysis

12% Non-reducing SDS-PAGE RIDC1 96/81/38C



12% Non-reducing SDS-PAGE RIDC1 96/38C Templating

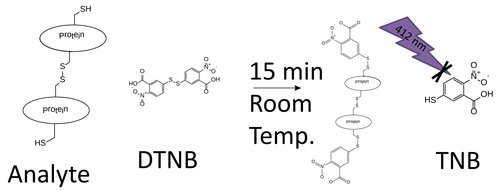


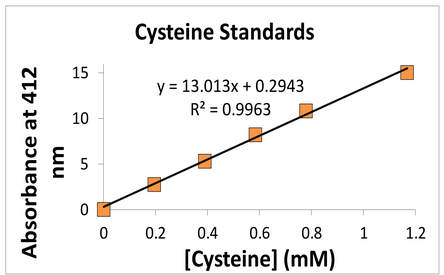
1: RIDC1 96/38C + IAA

2: RIDC1 96/38C + 1eq $ZnCl_2$ at room temperature

3: RIDC1 96/38C + 1eq $ZnCl_2$ at 37 ˚C

Counting Free Thiols with Ellman's Assay





Protein Sample Results

Protein Sample	# Cys/ Monomer	[thiol] (µM)	[protein] (µM)	Measured Thiols/ Monomer
RIDC3+59H	0	22.8	5.3	4.3
RIDC1 96/38C	2	32.0	4.3	7.5
RIDC1 96/81/38C	3	28.8	4.9	5.9
RIDC1 96/81/38C + DTT	3	40.9	4.9	8.3

Future Directions

- Conduct assay in oxygen-free conditions
- Count cysteines by mass spectrometry methods
- Characterize *in vivo* by a periplasmic extraction
- Introduce additional cysteines by site-directed mutagenesis
- Characterize metal binding characteristics

Conclusions

- RIDC1 96/81/38C tetramer is held together by disufide bonds
- Ellman's assay needs to be improved to count the number of free thiols

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