Metal-Directed Multiprotein Structures for Redox Catalysis by Copper

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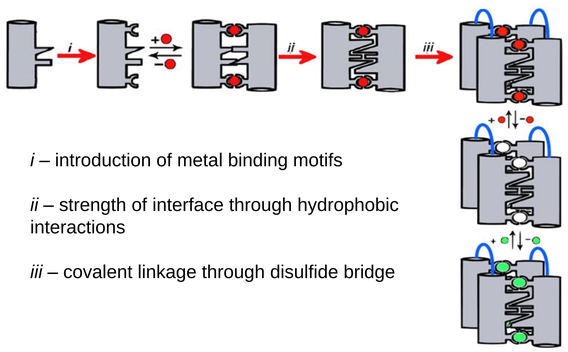
Abstract

We have demonstrated that we can control protein self-assembly by using metal coordination chemistry. Our goal is to design a copper-binding protein oligomer with tunable redox reactivity. The model protein cytochrome cb_{562} was modified by introducing metal binding residues, like histidine and aspartate, which allowed the protein to tetramerize in the presence of metal. Further modifications to this model, by using computational interface redesign, gave rise to a stable metal-free tetramer that we can further use as a template for our studies (known as RIDC-1 96C). We have been making mutations at the tetrameric interface including sulfur-containing amino acids like methionine because these have a high preference for Cu(I) binding. By using analytical ultracentrifugation and protein crystallography we are able to get a clear picture of the copper coordination environment. Once this is understood, we will study if these copper centers are capable of oxygen activation and catalysis.

Overview



i – introduction of metal binding motifs

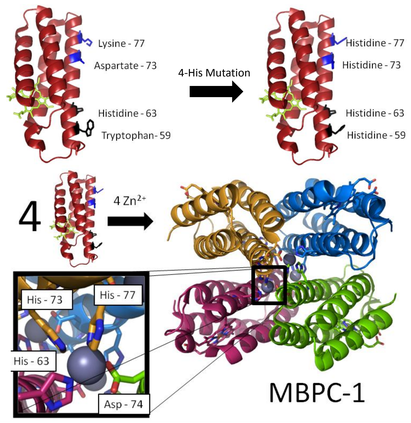
ii – strength of interface through hydrophobic interactions

iii – covalent linkage through disulfide bridge

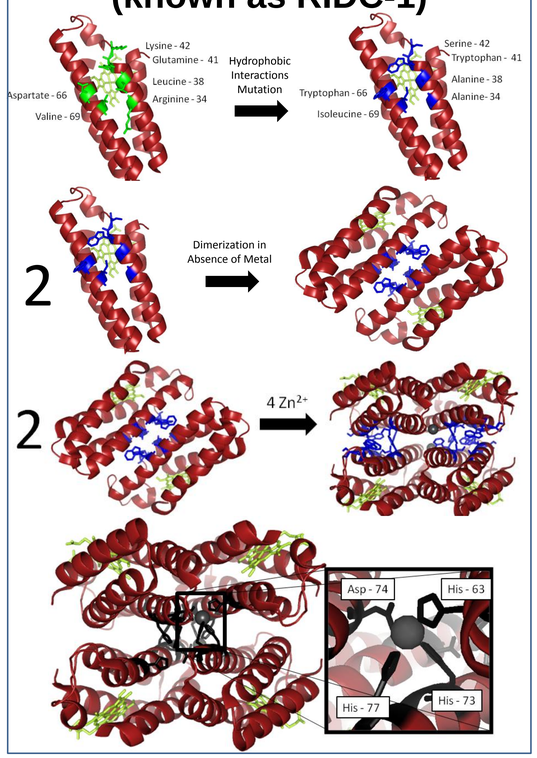
Cytochrome cb_{562}

- Four-helix bundle protein
- Stable
  - -Temperature
  - -pH
- Recombinantly expressed in large quantities
- Monomeric at millimolar concentrations
- Red protein, visible

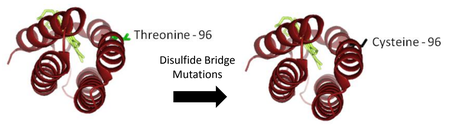
Tetramerization of 4-His Mutation (known as MBPC-1)



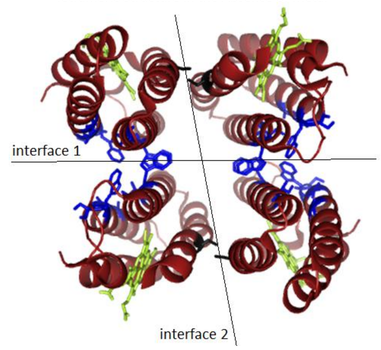
Interface Stabilization Based on $Zn_4(MBPC\text{-}1)_4$ Template (known as RIDC-1)



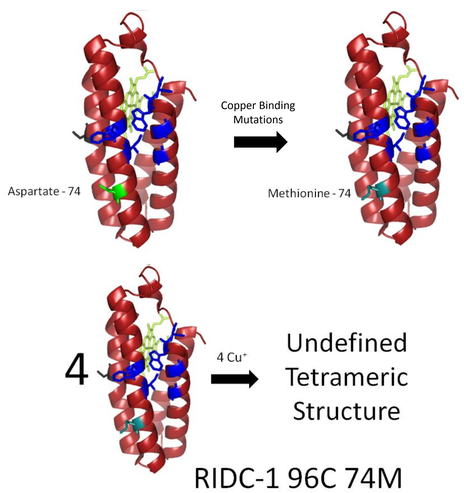
Covalent Linkage Between RIDC-1 Dimers (known as RIDC-1 96C)



Tetrameric Structure in the Absence of Metal

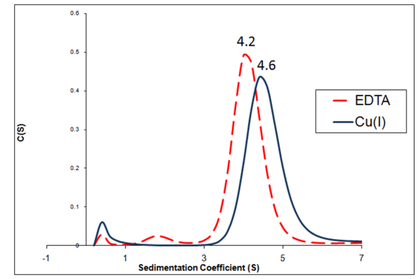


Introduction of Methionine for Cu(I) Binding (known as RIDC-1 96C 74M)

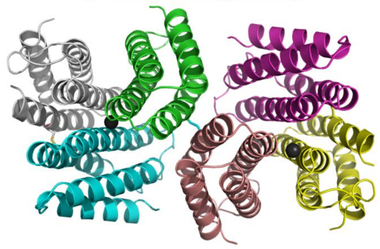


Experimentation Results on RIDC-1 96C 74M

Sedimentation Velocity Experiment of 40uM RIDC-1 96C 74M with 2mM EDTA or 1mM Cu(I)



Crystal Structure of RIDC-1 96C 74M in presence of Cu(I) (Determined with X-Ray Diffraction)



References

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