

Reading Guide: "Bio-inspired CO₂ Conversion by Iron Sulfide Catalysts . . ." ¹

What questions do you have that we have not covered here? Write them down and bring them to class.

Bring your copy of the paper with your notes to class! Bring your computer to class!

Please answer wherever possible with reference to specific figures, tables or sentences in the paper. Cite any outside source you use. Show your work where relevant.

Software

- Please install Chimera (or update your existing version) on your computer. Download Chimera
- Please install VESTA on your computer. Download VESTA
- I will send you some files with structures electronically.

Monday Discussion: Understanding the Fe–S Cluster Structures

Before class, please read the Wikipedia entry on structural motifs in iron–sulfur proteins.

Our main goal today will be to understand the various structures and structural terminology discussed in the paper that contain Fe–S bonds, and how this underpins the experiments the authors conducted.

Fe–S Clusters in Enzymes

1. Go to the PDB and look up 1SU8. This is carbonmonoxide dehydrogenase (CODH), an enzyme believed to represent one of the simplest Fe–S clusters that could be a model for LUCA and possibly some earlier form of proto-life.
 - (a) How many protein chains does it have?
 - (b) How many amino acid residues does CODH have?
 - (c) What is its EC number? It's general category/function?
2. Start up Chimera and do File → Fetch by ID . . . and fetch structure 1SU8 from the PDB.
3. Find the Fe–S clusters in this structure. You should see three clusters; focus on the two that are near the surface of the enzyme. Do Select → Residue → FES or SF4 to find each one. These are very typical Fe–S clusters.
 - (a) Draw out the structure of each Fe–S cluster, including the side chains of the amino acids that coordinate the cluster.
 - (b) Assuming the cysteine side chains are present as $-\text{CH}_2\text{S}^-$ and the non-cysteine sulfurs are present as S^{2-} what seems to be the oxidation state of the Fe in each cluster?

Standalone Fe–S Clusters

1. Launch VESTA and do File → Open . . . and navigate to the FAGREK.cif file which I sent you.² This is the structure of $[\text{Fe}_4\text{S}_4(\text{SH})_4]^{2-}$ which is pretty much the simplest Fe_4S_4 cluster one could make. The structure looks quite messy because the counter ion is huge: PPh_4^+ , making the overall formula $(\text{PPh}_4)_2[\text{Fe}_4\text{S}_4(\text{SH})_4]$.
 - (a) Take a good look at the structure; What does the black box represent? Hint: look through the information area at the bottom of the VESTA window for clues.
 - (b) In the panel on the left-side of the VESTA window, under the "Style" tab, change the style to "polyhedra". In the example of the CODH above, your eyes were probably drawn to the cube-like structure. But here, that's not what they have done. Describe what you see in terms of the polyhedra displayed.

The structure of greigite

1. Start VESTA and do File → Open . . . and navigate to the Greigite.cif file I sent you electronically.³

¹Roldan *et al.* *Chem. Commun.* vol. 51, pg 7501 (2015).

²Müller *et al.* *J. Chem. Soc. Chem. Commun.* pg 35 (1987).

³Retrieved from the database at rruff.geo.arizona.edu/AMS/amcsd.php

2. Turn the structure around to get a good look at it. Locate an Fe_4S_4 cluster analogous to the ones you saw above. How are the different Fe_4S_4 clusters in greigite connected to each other? Try looking at the structure parallel to each axis, then tip it slightly off center to see where other layers/atoms are located. Note the little coordinate system in corner of the main window.
3. Switch the style to "polyhedral". Each iron atom is at the center of a polyhedron. Name it.
4. Roldan *et al.* talk about the (001) and (111) "faces" or "edges" of greigite. These notations are called Miller Indices, which you can look up and in which case you will discover the definition is rather technical. Fortunately, VESTA will show you these faces visually which is vastly superior. Here's how:
 - (a) Display greigite with the polyhedra turned on (at least for now, feel free to experiment).
 - (b) On the menu, do Edit → Edit Data... → Crystal Shape... which opens a control panel.
 - (c) In the control panel, click the "New" button, then fill in the Miller indices, and click "Apply".
 - (d) The display will change to show the previous structure embedded in a hypothetical crystal whose faces are oriented along the specified Miller indices.
5. Display the (001) and (111) faces one at a time. Thinking about Roldan Fig. 2B, which shows various CO_2 -related species binding to one of these faces: Let's say you were HCO_3^- (bicarbonate ion), and you were making a 2-footed landing with your oxygen atoms on one of the faces as shown in the figure. What atoms on the face would you likely be interacting with? Keep in mind the geometry of HCO_3^- (Describe it).

Wednesday Discussion: The Rest of the Paper

1. Fe–S clusters are central to existing complex biochemical pathways and they have become a focal point for the metabolism-first viewpoint. Why? What role are Fe–S clusters playing in the proposed early metabolic steps (I hesitate to call them pathways)?
2. Many modern Fe–S cluster-containing proteins have several Fe–S clusters. Why?
3. In almost all cases the reaction intermediates coordinate with the greigite surfaces via their oxygen atoms. Why is this? What are the reaction intermediates coordinating to? Think about the atoms presented by a particular face of the crystal.
4. What is the purpose of the electrode in the experiments described in the paper? Be as specific as possible. What biological processes replace the electrode in real life?
5. The authors state that "... the transformation of HCO_3^- into formic acid is thermodynamically and kinetically favorable." How do they reach this conclusion?
6. The reaction rate falls off at both high and low pH values.
 - (a) What specific figure and portion of that figure show that the rate falls off?
 - (b) Why does the rate fall off at low pH?
 - (c) Why does the rate fall off at high pH?
7. What is the significance of the formation of acetic and pyruvic acid in these experiments?

Questions Due Friday Noon

1. Fe–S clusters take many forms, but the simplest form may be $\text{Fe}_2\text{S}_2(\text{H}_2\text{O})_4$.
 - (a) Draw the structure of this complex.
 - (b) Imagine one replaced the 4 H_2O with 4 cysteine side chains, giving $\text{Fe}_2\text{S}_2(\text{RCH}_2\text{S})_4$. What do you suppose would be the smallest peptide chain that would have a geometry that would allow 4 cysteines to coordinate iron appropriately? Draw this peptide and show it coordinating to the iron atoms.
2. Figure 2C represents the best pathway for the reduction of bicarbonate ion to formic acid. Write and balance each step shown in Figure 2C, including a sketch of the molecules interacting with a little piece of Fe–S (do this at acidic pH since the reaction works better in acid). Draw mechanistic arrows any place they would be appropriate. In the pathway, where does the reduction actually occur? In the experiment, what reactant sustains the overall reaction?