**Cobalt Schiff Base Zinc Finger Inhibitors**

These questions are intended to help you read and understand the paper “Spectroscopic Elucidation of the Inhibitory Mechanism of Cys2His2 Zinc Finger Transcription Factors by Cobalt(III) Schiff Base Complexes” by Heffern, Kurutz and Meade, Chem. Eur. J. 2013, 19, 17043-17053 (DOI: 10.1002/chem.201301659).

 **Background**

*Answer these questions before reading the article.*

1. Zinc Fingers.

Using the Ligand Explorer program at the Protein Data Bank (www.pdb.org), examine the structure of the zinc finger domain 2EPR with the Metal Interaction function turned on. Further help with Ligand Explorer is available at <http://www.pdb.org/pdb/staticHelp.do?p=help/viewers/ligandExplorer_viewer.html>

a. What is the bonding geometry around the Zn2+ ion?

b. Which specific amino acid residues are coordinated to the Zn2+ ion?

c. Which of these amino acids are located on the β-sheet portion of the peptide? Which are located on the α-helix portion?

2. Synthesis of the acacen Ligand.

a. What is a Schiff base condensation reaction?

b. Draw Lewis structures of ethylenediamine (en) and acetylacetone (acac).

c. Draw the product (acacen) of the reaction of one mole of ethylenediamine with two moles of acetylacetone.

d. Draw possible complexes formed between Co3+ and acacen.

e. What coordination number does Co3+ prefer? How do you reconcile this with your answer in part d?

3. Drug Development.

a. Find the structure of the drug Doxovir. How does this structure compare to the structure you drew in question 2?

b. What is Doxovir intended to be used for?

**Reading**

*Answer these questions while reading the article.*

1. Look at the structures of Complexes 1 and 2 in Figure 1. How do these structures compare to the Co(acacen) structures you drew earlier? What is the point group for complex 1? Which symmetry elements would be lost if the axial ligands are not identical?

2. In the zinc finger protein, the active site Zn2+ cation binds to both cysteine and histidine protein residues. To which amino acid residues does the [Co(acacen)]+ complex bind preferentially? How can this difference in binding affinity be explained?

3. Look at the ZF4 and CP1 peptides shown in Table 1. Why are these peptides being studied rather than the full zinc finger transcription factor?

4. For each peptide (ZF4 and CP1) which specific cysteine and histidine residues are involved in Zn2+ binding? According to Figure 3, which specific histidine residues are involved in Co3+ binding? How was this determined?

5. Why is Complex 2 in Figure 1, [Co(acacen)(4MeIm)2]+, used to model the interaction of [Co(acacen)]+ and the zinc finger protein? Based on the data given in Figure 6A, is it a good model? Explain your answer.

6. Using the spectrochemical series, would you expect Co3+ in the [Co(acacen)(4MeIm)2]+ complex to be high spin or low spin? What experimental results in the article support this conclusion?

7. Figure 2 presents the 2D 1H-1H TOCSY NMR spectra of the peptide residues from Table 1 in the absence (grey) and presence (red) of the [Co(acacen)]+ complex. According to these spectra, which specific residues are affected by the addition of the cobalt complex? How do you know?

8. How does this work help researchers study the mechanism of inhibition?

**Discussion**

*These questions should be considered and discussed by the class as a whole.*

1. What is the maximum number of histidine residues which could possibly bind to one [Co(acacen)]+ complex?

2. Figure 3 is described as a Δδ plot. Explain what Δδ means. How many histidines does this figure indicate are binding to cobalt? How can this observation be reconciled with the answer to discussion question 1?

3. Why does the paper focus on the NMR chemical shifts of the histidine H2 and H5 protons (see Figure 1C)? Why might the H5 proton be of interest to the researchers?

4. Compare and contrast the structures of Doxovir, Complex 1, and Complex 2 from this paper. How do their differences impact their ability to bind to histidine residues?

5. What synthetic avenues are available to improve the efficacy of the cobalt complexes as zinc finger inhibitors?