

# Structural Elucidation of Interactions of Carcinogenic Metal Ions with Zinc Finger DNA Repair Proteins - Toward Molecular Mechanism in Carcinogenesis

## Faculty:

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## Project for Scholars:

Zinc fingers (ZnFs) are small protein motifs with one or more Zn(II) as a structural factor and conserved cysteine and/or histidine residues as ligands. The Zn(II) is tetrahedrally coordinated by the side chains of the ligands. ZnF-containing proteins are among the most abundant protein superfamilies in eukaryotic genomes and participate in a variety of cellular processes, such as replication and repair, transcription and translation, metabolism and signaling, and cell proliferation and apoptosis, where they typically mediate sequence-specific nucleic acid binding, protein-protein interactions, and protein-lipid interactions.

Zn(II) is crucial for the function of the ZnF-containing protein mainly through maintaining protein structure. ZnFs have been proposed to be cellular targets for the carcinogenic metals having high affinities for sulfhydryl groups that are increasingly available in the environment due to the industrial discharge of waste, e.g., Pb(II), Hg(II), and Cd(II). The interactions of the carcinogenic metal ions with ZnFs have been proposed to distort or damage the structure of ZnFs and thus may induce a number of adverse physiological effects on human health including carcinogenesis, reproductive and developmental defects, and neuropathies. For example, these interactions could perturb the regulation of gene expression in the ZnF-driven transcriptional events and inhibit DNA repair in the ZnF-containing enzyme involved DNA repair pathways.

The bacterial formamidopyrimidine-DNA glycosylase (Fpg) is a 30.2 kDa DNA repair protein involved in base excision repair (BER) of oxidatively damaged DNA primarily by removal of 8-oxoguanine in *Escherichia coli*. Fpg contains a CCCC-type ZnF located at its C-terminus (Fig. 1). The ZnF is essential for damaged DNA recognition in Fpg and the enzyme is inhibited in the presence of Cd(II), Cu(II)/Cu(I), or Hg(II). In this project, the interactions of toxic metal ions, Pb(II), Hg(II), and Cd(II), and Fpg will be studied using various spectroscopic methods including UV-Vis, Circular Dichroism, and NMR Spectroscopy. Our goal is to determine the detailed structure basis of the interactions of toxic metal ions with ZnF-containing DNA repair proteins. The atomic-level investigation of the toxic metal-bound sites are crucial to understand the relationship between the altered structure upon metal binding and metal carcinogenesis. A better understanding of molecular mechanisms of metal carcinogenesis will be obtained.

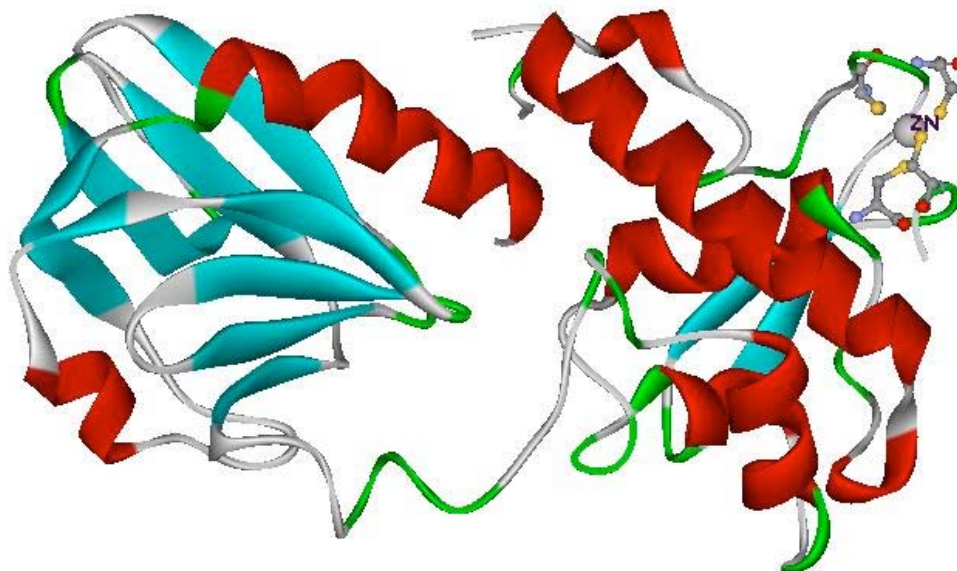


Figure 1. Tertiary structure of Fpg (PDB code 1K82). The ZnF is located at C-terminus of the protein. The zinc is shown as a ball in grey and the four Cys ligands shown as a ball and stick model.

We will use *E. coli* to express Fpg constructs. The stoichiometry of the metals and Fpg can be determined by a standard titration curve using a UV-Vis spectrophotometer. The  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -labeled Fpg and apoFpg before and after the addition of toxic metal ions will be collected and compared. Our hypothesis is that the residues with the most altered resonances will be those involved in toxic metal binding. However, the toxic metal ions may also bind to non zinc-binding residues. The resonance changes for the specific residues will also be studied.

Correct identification of the ligands will be confirmed by assessing the metal binding properties of Fpg mutants, in which all of the putative non toxic metal-binding Cys, His, and/or other residues are replaced by Ala, Phe, and/or relative amino acids, respectively. Similar experiments will be performed on the mutants and the results are expected similar to those of Fpg in the presence of the metal ions. The resonances of Fpg in the presence of toxic metal ions will then be assigned to localize the residues in the proximity of the toxic metal ions and to identify the toxic metal binding site in Fpg. The metal binding map will thus be defined.

The Student Scholar will be able to work in both places. Most of protein preparation and spectroscopic experiments will be performed at CSULA. The different activities of toxic metal substituted Fpg will be monitored using either  $^{32}\text{P}$  substrates or use near-infrared dyes at COH. Previous experience of protein purification is desired.

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