

## ABSTRACT

Author: Sanchez-Medina, Oscar, F. Ph.D.

Institution: Purdue University

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Title: Engineering Protein Probes for Monitoring Epigenetic Marks in Response to Environmental Chemicals

Major Professor: Chongli Yuan

Epigenetic modifications are inheritable changes of chromatin primarily consisting of post-translational modifications of histone protein (e.g., methylation and acetylation of histone proteins) and DNA methylation (e.g., addition of a methyl-group to a cytosine). Epigenetic modifications are dynamically regulated by epigenetic enzymes. Depending on the nature modifications, it can manifest itself either as a transient or stable modification that can be maintained throughout and across generations. The goal of my thesis is to elucidate the molecular traits of epigenetic changes induced by low-dose exposure of environmental chemicals like lead (Pb) and atrazine. The collective knowledge of this study will provide useful tools for assessing changes in epigenetic marks caused by the exposure environmental chemicals.

To do that, we designed protein-based probes that are capable of characterizing spatial distribution of epigenetic modification in live cells. The probes are based on epigenetic reader domains and are engineered for improved binding affinity and selectivity. The application of our engineered probes, i.e., H3K14ac and H3K9me3 probes, was demonstrated using the human embryonic kidney 293T (HEK293T) cell line as well as nuclear extracts from cell culture and animal tissues.

Pertinent to Pb-exposure, we found that Pb can act as non-competitive inhibitor of DNA methyltransferase and thus reduce the global DNA methylation level in exposed cells and a selected animal model (zebrafish). In addition, Pb causes reduction in H3K9me3. Pertinent to atrazine, it exhibits a similar behavior as Pb by inhibiting the DNA methyltransferase in a non-competitive way, and reducing the global DNA methylation as well H3K9me3 levels.

Our protein sensors can provide spatial resolution comparable to commercial antibodies but offer the flexibility of live cell tracking. They can be used to characterize epigenetic marks at a single-cell level. The probes can thus be used to advance our understanding of how environmental chemicals affect epigenetic changes at a mechanistic level.