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10. McDonald CB, El Hokayem J, Zafar N, Balke JE, Bhat V, **Mikles DC**, Deegan BJ, Seldeen KL, and Farooq A (2013). Allostery mediates ligand binding to Grb2 adaptor in a mutually exclusive manner. *J Mol Recognit 26*, 92-103.
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08. Bhat V, Kurouski D, Olenick MB, McDonald CB, **Mikles DC**, Deegan BJ, Seldeen KL, Lednev IK, and Farooq A (2012). Acidic pH promotes oligomerization and membrane insertion of the BclXL apoptotic repressor. *Arch Biochem Biophys 528*, 32-44.
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DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

MILLER SCHOOL OF MEDICINE UNIVERSITY OF MIAMI

Presents

# PhD DISSERTATION SEMINAR

## PHYSICOCHEMICAL INSIGHTS INTO THE EGR1-DNA INTERACTION

By

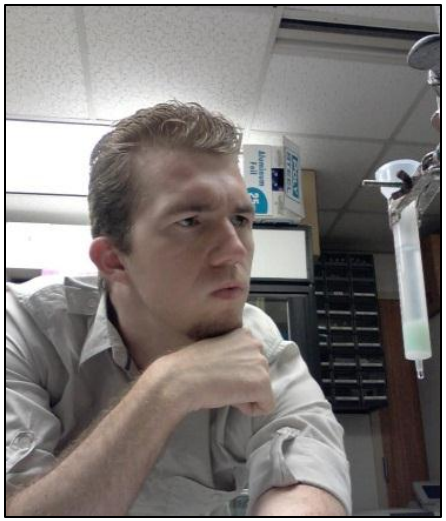

## David Mikles


**Tuesday, March 25, 2014**
**12:00pm-1:00pm**
**Gautier Rm. 118**
*(Defense to follow)*

### Thesis Committee

Thomas Harris PhD, *Committee Chair*
Yanbin Zhang PhD, *Committee Member*
Geoffrey Stone PhD, *External Examiner*
Amjad Farooq PhD DIC, *Research Mentor*

## BIOGRAPHY

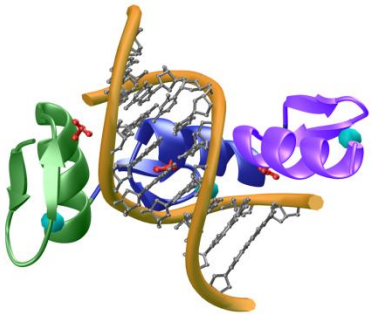


David was born and raised in the "Grand Canyon State" of Arizona. Early on, he developed a keen interest in the biological sciences. The urge to further study science led him to attend the University of Arizona for a Bachelor's degree in Molecular and Cellular Biology. As a part of his curriculum, he got the opportunity to work on several independent projects. These research projects helped him to understand the nuances of research and at the same time showed him the path to his true vocation in life. However, it was not until he took courses in biochemistry in college that David got captivated by protein biochemistry. Taken together, all his courses and the wet-lab work reinforced his decision to pursue higher studies in biological sciences.

After graduation from the University of Arizona, he was admitted into the Biochemistry and Molecular Biology PhD program at the Miller School of Medicine, University of Miami. Having parents who completely supported his goals, he embarked on a journey across the country and enrolled in the program. He had the vision of working at the interface of physics, chemistry and biology addressing problems in cancer. The department and the Farooq laboratory not only provided the ideal opportunity to fulfill these aspirations but also trained him extensively in the basic principles and approaches to protein biochemistry. Dr Amjad Farooq's guidance has been instrumental in developing his physicochemical approach and understanding of biology, particularly in the areas of molecular biophysics and structural biology.

Outside of science, David is passionate about college sports and is an avid Miami Hurricanes fan. In his time away from the lab, he enjoys basketball and listening to local bands. For all his gifts and successes in life so far, he gives all the credit to his parents and mentors.

## ABSTRACT

Early growth response 1 (EGR1) transcription factor orchestrates a plethora of signaling cascades in response to a wide variety of stimuli such as growth factors and hormones.

Herein, using a battery of biophysical tools, I explore the molecular basis of binding of EGR1 to its cognate gene promoters. My data show that the binding of EGR1 to DNA is tightly regulated by solution pH. The binding affinity undergoes an enhancement of more than an order of magnitude with increasing pH from 5 to 8, implying that the deprotonation of an ionizable residue accounts for such behavior. This ionizable residue is identified as H382 by virtue of the fact that its substitution to non-ionizable residues abolishes pH-dependence of the binding of EGR1 to DNA. Notably, H382 inserts into the major groove of DNA and stabilizes the EGR1-DNA interaction via both hydrogen bonding and van der Waals contacts.

I also provide evidence that the binding of EGR1 transcription factor to DNA displays virtually zero dependence on ionic strength under physiological salt concentrations and that such feat is accomplished via favorable enthalpic contributions. Importantly, I unearth the molecular origin of such favorable enthalpy and attribute it to the ability of H382 residue to stabilize the EGR1-DNA interaction via both intermolecular hydrogen bonding and van der Waals contacts against the backdrop of salt. Consistent with this notion, the substitution of H382 residue with other amino acids faithfully restores salt-dependent binding of EGR1 to DNA in a canonical fashion.

Finally, my biophysical analysis reveals that DNA sequence variations within the target gene promoters tightly modulate the energetics of binding of EGR1 and that nucleotide substitutions at certain positions are much more detrimental to EGR1-DNA interaction than others. In particular, the reduction in binding affinity poorly correlates with the loss of enthalpy and gain of entropy—a trend indicative of a complex interplay between underlying thermodynamic factors due to the differential role of water solvent upon nucleotide substitution.

In sum, my findings uncover an unexpected but a key protonation-deprotonation step in the molecular recognition of EGR1 and suggest that it may act as a sensor of pH within the intracellular environment. This study reports the first example of a eukaryotic protein-DNA interaction capable of overriding the polyelectrolye effect. The work presented herein also bears important implications on understanding the molecular determinants of a key protein-DNA interaction at the cross-roads of human health and disease.