BCHM 421/422 – 2018/19

Project Outline: The development of B-cells, a process termed lymphopoeisis, is a tightly regulated process at that occurs primarily at the transcriptional level. Dysregulation of B-cell development can result in the development of several disease states, including childhood acute lymphoid leukemia (ALL). The underlying biochemical mechanism associated with normal lymphopoeisis, ALL, and other associated diseases involves transcription factor:ligand intearctions. EBF1 and E2A are two transcription factors that play particularly key roles in gene expression during lymphopoiesis, and whose modifications have been directly associated with ALL.

We are interested in characterizing the structural and functional properties of these transcription factors, and their interactions with each other and with other transcriptional regulators. We would also like to better understand how post-translational modifications might alter these interactions in health and disease.

Supervisor: Steven Smith

Project Title: Structural and functional analyses of transcriptional networks associated with B-cell development and cancer

Keywords (3-5):

1. **Transcription factors**
2. **Cancer**
3. **Protein-ligand interactions**
4. **Structure-function relationship**

Project Goals:

1. Assess the structural properties of full-length EBF1 and E2A and their interaction with the coactivator CBP/p300 and other components of the transcriptional machinery.

2. Identify the sites on full-length E2A that undergo post-translational modifications (i.e., acetylation, phosphorylation, and ubiquitination) and characterize their structural and functional impacts.

3. Assess the ability of small molecules to disrupt the interaction of transcription factor CBP/p300 interactions.

Experimental Approaches:

Towards pursuing our research goals, you will use expression various E2A, EBF1, and CBP/p300 protein constructs in *E. coli* and purify them using standard chromatographic methods. You will also receive training in the use of NMR spectroscopy, isothermal titration calorimetry, biochemical assays, and cell-based assays to assess transcription factor-ligand interactions.

References:

1. Denis, C.M., et al. (2014) Functional redundancy between the transcriptional activation domains of E2A is mediated by binding to the KIX domain of CBP/p300. *Nucleic Acids Res* 42: 7370-7382.
2. Denis, C.M., et al. (2012) Structural basis of CBP/p300 recruitment in leukemia induction by E2A-PBX1. *Blood*. 120: 3968-3977.
3. Belle, I. & Zhuang, Y. (2014) E proteins in lymphocyte development and lymphoid diseases. *Curr Top Dev Biol* 110: 153-187.
4. Boller, S. & Grosschedl R. (2014) The regulatory network of B-cell differentiation: a focused view of early B-cell factor 1 function. *Immuno Rev* 261: 102-115.
5. Dyson H.J. & Wright, P.E. (2016) Role of intrinsic protein disorder in the function and interactions of the transcriptional coactivators CREB-binding protein (CBP) and p300. *J. Biol Chem* 291: 6714-6722.