Noa Erlitzki
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Poster Submission

TITLE: Direct chemical control of PU.1, a hematopoietic regulator

AUTHOR: Noa Erlitzki

FACULTY ADVISOR: Gregory Poon, Ph.D., Associate Professor, Department of Chemistry

INTRODUCTION: Direct chemical control of transcription factors at their sites of action, i.e. at the protein-DNA level, is currently unavailable for most transcription factors, including the ETS-family transcription factor PU.1, an essential regulator of hematopoiesis. A major challenge in developing direct activators of PU.1 is the strong structural homology it shares with the ETS-family protein Ets-1 (and the ETS family in general). This project aims to overcome the challenge of achieving specificity in targeting PU.1 over its structural homologs. Recent studies by the Poon Lab on the mechanisms of DNA site recognition by PU.1 and Ets-1 show striking differences in molecular hydration accompanying DNA recognition, an exploitable feature that we use in this study to identify and characterize new compounds that directly activate the transcription factor PU.1.

METHOD: Compounds that bind to the PU.1/DNA complex and retain PU.1's osmotic sensitivity are candidates for acting as direct activators of PU.1. We employ osmotically-directed phage display screening of 7- and 12-residue peptide libraries against the PU.1/DNA complex in our search for these compounds. Successful enrichment is expected to yield 5 to 10 optimized sequence candidates. The thermodynamic, kinetic, and structural basis of PU.1 activation by these candidate peptides will be determined using biophysical and biochemical methods including surface plasmon resonance, hydrolytic probes, and mass spectrometric analyses.

RESULTS: Preliminary experiments to validate the phage display system were conducted initially. After validation of the experimental design, panning was carried out against a DNA-only target using magnetic streptavidin beads. Eight clones were identified as potential candidates for peptide binders of the high affinity *in vivo* "consensus" DNA sequence for PU.1.

CONCLUSION: Fluorescence anisotropy studies for characterization of the binding properties of the potential candidates are currently underway. In addition, N-hydroxysuccinimide (NHS) coupling is being explored as an option for solid-surface binding assays that preserve the dynamic nature of PU.1/DNA interactions with phage in solution. Panning using the NHS method of target immobilization will be done against the PU.1/DNA complex as a target.