Liskin Swint-Kruse, Associate Professor

Department of Biochemistry and Molecular Biology

The University of Kansas Medical Center

ABSTRACT:

To fully interpret differences between patient genomes, we must improve our understanding of the protein sequence/function relationship. Since experimental characterization of all protein polymorphisms is impossible, myriad bioinformatics algorithms are being developed to predict positions where a polymorphism might impact function. In currently-available analyses, we have noted untested assumptions and limitations in their implementation. We are experimentally testing a key assumption that underlies many analyses: All homologues in a family utilize the same nonconserved positions to create unique variation of the common function. To that end, we are (1) determining the functional contributions of nonconserved positions in several engineered homologues of the LacI/GalR family and (2) comparing results to existing bioinformatics predictions. Experiments are focused on the region of the LacI/GalR repressors that links the DNA-binding domain to the effector binding domain. This linker region does not directly contact ligand, and thus represents an oft-neglected protein region. Our results show that no single bioinformatics analysis predicts all functionally-important, nonconserved linker residues. We also find that the central assumption must be revised: Homologues *within a carefully defined subfamily* utilize the same positions to differentiate paralogue function. In addition to predicting the locations of important nonconserved positions, we must also understand their functional roles and whether the role is preserved among homologues. To that end, we have performed biophysical characterizations of repressor variants. Results for 4 linker positions show that these contribute variously to DNA-binding affinity, selectivity, and/or allosteric response. Future experiments will determine whether these roles are preserved among other LacI/GalR homologues.