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# Progress Towards Probing Conformational Changes in the Srs2 Helicase: A Proposed Intrinsically Unstructured Protein

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PROGRESS TOWARDS PROBING CONFORMATIONAL CHANGES IN THE SRS2  
HELICASE: A PROPOSED INTRINSICALLY UNSTRUCTURED PROTEIN

A Thesis

Submitted to the School of Graduate Studies and Research

in Partial Fulfillment of the

Requirements for the Degree

Master of Science

Kiel Charles Shaley

Indiana University of Pennsylvania

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We hereby approve the thesis of

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Title: Progress Towards Probing Conformational Changes in the Srs2 Helicase: A Proposed Intrinsically Unstructured Protein

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This thesis examines the beginning steps of the investigation into possible conformational changes in the C-terminus of the Srs2 helicase. This portion of the protein was predicted, using bioinformatics programs, like GlobPlot<sup>1</sup> and IUPred<sup>2</sup>, to possess characteristics related to the class of proteins known as intrinsically unstructured proteins. Initially, the full length Srs2 protein was expressed in BL21 (DE3) (Rosetta 2) cells and preliminary steps were taken to isolate it using an Ni-NTA column. Next, a set of bacterial transcription factors were expressed and subsequently isolated using an Ni-NTA column. To produce C-terminal portions of Srs2, two inserts were cloned separately into the pET 28a<sup>+</sup> plasmid. These plasmids were then transferred into a variety of cell lines. This includes the DH5<sub>α</sub> (plasmid isolation) and Rosetta 2 (protein expression) cells. After expression optimization, several protein purification trials were conducted.