Determination of optix regulators via Mass Spectrometry Analysis

Gene regulation is vital in maintaining normal cellular functions in all living organisms; transcription factors are proteomic molecules which are the key to understand this regulation. They bind with high specificity to regions in the chromatin called cis-regulatory elements. Many studies focus on elucidating where these proteins bind and their preferred DNA binding motif; this is a protein-centric approach. However, the methodology presented in this proposal aims to determine what transcription factors bind in a given chromatin region. This will be accomplished via -pulldowns to study DNA-protein interactions, as well as protein-protein interactions. Firstly, the DNA-pulldown will be performed with human K562 nuclear extract and targeted DNA probes that contain validated AP1 binding region. Afterwards, protein identification will be performed with mass spectrometry analysis. Once this procedure has been validated, it will be applied to isolated nuclear extract to elucidate the transcription factors that bind to the cis-regulatory elements of the optix gene in *Heliconius sp.* Results from this protocol could additionally help elucidate what other proteomic biomolecules were bound to the target factor at the moment of pulldown.