Enhanced sample preparation for proteomic analysis utilizing reversible biotinylation and polymer based protein engineering

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Proteome sample preparation for gel based and mass spectrometry based studies has remained relatively unchanged for many years. The goal of these sample preparation schemes is to remove contaminants such as lipids, DNA, RNA and carbohydrates from cell or tissue homogenates. The vast majority of current methods rely on protein precipitation followed by centrifugation and re-solubilizing the proteins. Unfortunately none of the available techniques provide an unbiased sampling of the entire proteome. Regardless of the method (ammonium acetate precipitation, TCA/acetone precipitation, PEG precipitation, etc.), not all proteins will be precipitated and not all will be resolubilized. We have developed an unbiased method for proteome sample preparation based on protein capture, not precipitation. We recently showed that Biotin-CDM can be used as a tool to reversibly tag the entire proteome for purification. Biotin-CDM reacts with lysine residues present on the surface of proteins at elevated pH to covalently biotinylate proteins. Biotinylated proteins are bound to Avidin beads and then all non-protein contaminants are washed away. The biotin tag is then reversed by lowering the pH to release the proteins from the Avidin beads, resulting in PUR, unmodified and unbiased proteome sample. Because an excess of Biotin-CDM is required to tag all proteins, we utilized polymer based protein engineering to develop a novel method that separates free Biotin-CDM from Biotinylated proteins. Thus, we have developed a method to prepare proteome samples in an unbiased manner that could be used for any organism.

Biography

Amber Lucas has completed her Master’s degree at Texas State University in 2014 and she is currently studying in Graduate School at Carnegie Mellon University in the Department of Biological Sciences. She is also a Member of the NSF I-Corps site at Carnegie Mellon University.

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