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Expression of regions of Apm1p in E coli. and purification of the expressed proteins

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An active area of cell biological research is the study of intracellular protein trafficking. Improper function of protein trafficking has pathological consequences, as the malfunction of certain transport systems can give rise to certain cancers. Our lab focuses on the dynamic mechanisms of transport between the trans-Golgi network (TGN) and endosomes using the model protein Ste13p. The goal of my project is to deepen our understanding of Ste13p retrieval from the early endosome to the TGN via binding of the adaptor complex (AP-1). Previous research suggests that the C-terminal domain of the AP-1 subunit Apm1p interacts with a sorting signal contained within amino acids 1-12 of the cytosolic domain of Ste13p. With this in mind, we developed plasmids that expressed different regions of the C-terminal domain of Apm1p in E coli. The hope was to find a construct that provided both high expression and ease of purification. As part of a collaboration, we intended to discover how the 1-12 region of Ste13p binds to Apm1p on an atomic level. Each construct was contained within the expression vector pET28a(+) which is under the control of a T7 promoter. The results led us to choose Apm1p 161-475 as the best construct. Using a nickel column, we purified this Apm1p construct due to the nickel's binding affinity for the engineered 6xHistidine tag. Soon, we will perform a pull down assay to confirm the binding of Apm1 161-475 to a peptide that represents amino acids 1-12 of Ste13- GST. After completion of the assay, we will send our construct to our collaborator who will co-crystallize the AP-1 derivative with a Ste13p peptide to structurally define the binding between these two proteins. Structural data will make it easier to understand how mutations could affect the binding of the signal with the adaptor protein, thus allowing more detailed manipulations of both proteins.