Characterization of antigen-binding fragments to isolate native TOC complex

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Protein translocation across the chloroplast outer membrane is essential for photosynthesis in all green plants. This is because most chloroplast proteins (over 90%) are encoded in the nucleus, translated in the cytoplasm, and must be imported into the chloroplasts to perform their functions. The translocon on the outer chloroplast membrane (TOC) complex orchestrates this vital translocation process and consists of three components in terrestrial plants: Toc75, Toc33/34 and Toc159 with unknown stoichiometries. Our lab seeks to elucidate the structural architecture of the TOC complex to gain mechanistic insights into protein translocation in chloroplasts. However, the major bottleneck preventing structure determination of TOC has been the inability to produce or isolate the complex to sufficient yields and purity for structural studies. In this work, we demonstrate a novel method to isolate TOC directly from pea leaves. To that end, we have successfully purified the POTRA domains of Toc75 by in-vitro refolding methods. Subsequently, we generated antigen-binding fragments (sABs) that specifically recognize the POTRA domains from both *Arabidopsis thaliana* and *Pisum sativum*. Further, we characterized this interaction using size exclusion chromatography coupled with small angle X-ray scattering (SEC-SAXS), isothermal titration calorimetry (ITC), and X-ray crystallography. Finally, we show that we can use these sABs to pull down the native TOC core complex from pea leaves bought at a local grocery store. Currently, we are optimizing purification to make the sample amenable for structural studies using cryo-electron microscopy.