Preliminary Results

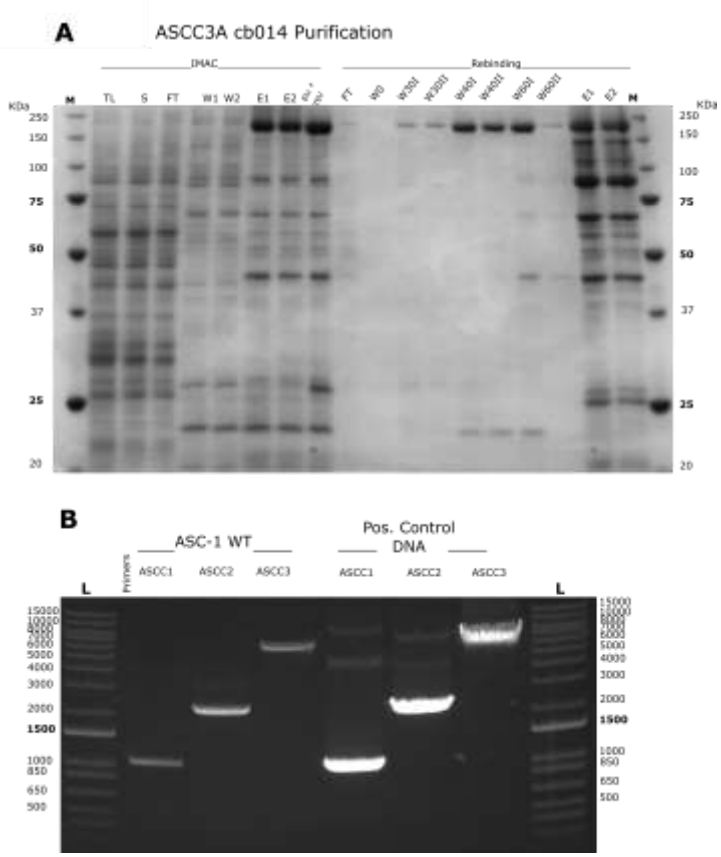

Figure 1. (A):SDS-PAGE analyses of ASCC3-cb014 purification fractions from IMAC, and rebinding after incubation with TEV protease. M: markers, TL: total lysate, S: supernatant, FT: flow-through, W: wash, E: elution. Rebinding: FT: flow-through, w0: wash with out imidazole, w30: wash 30mM imidazole, w40: wash 40 mM imidazole, w60: wash 60 mM imidazole, E1/E2: elutions. (B) PCR of the cre-recombined ASC1 genes, using multibac plasmids. Tree set of primers, one for each gene (ASCC1A, ASCC2A ASCC3A), was used confirming that the 3 sequences were recombined into the acceptor plasmid.

Large-Scale Expression and Purification of ASCC3-cb014: ASCC3 constructs were successfully cloned into vectors for expression in baculovirus (pFB-LIC-Bse and pFB-CT10HF). Test expressions were carried out to investigate which constructs would be soluble. For baculovirus expression, we are able to express and purify ASCC3 constructs in baculovirus in large scale to obtain high yields of purê protein for subsequent analyses, such as crystallography and cryo-EM. (Figure 1-A)

Cloning ASC1 complex proteins using multibac system: Currently, we have successfully cloned 3 proteins of the ASC-1 (RQT) complex (ASCC1A, ASCC2A and ASCC3A) into a cre-recombined plasmid for expression in Sf9 insect cells, using the multibac system (figure x.1-A). This system will enable co-expression of all member of the complex for structural and biochemistry experiments. This plasmid was transposed into DH10Bac cells for further bacmid DNA production fallowed by transfection and baculovirus generation. (Figure 1-B)