Crystallization of the TOM Complex from *Neurospora Crassa* together with Monoclonal Antibodies

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The majority of mitochondrial proteins is nuclear-encoded and synthesized in the cytosol. Transport of these proteins into mitochondria is mediated by translocation machineries located in the outer and inner membrane. The multisubunit TOM complex (Translocase of the Outer membrane of Mitochondria) is responsible for protein sorting and translocation of proteins across the outer mitochondrial membrane. Our aim is crystallization and structure determination of the TOM complex in order to elucidate its architecture, functional mechanism and regulation. The low number of existing crystal structures of membrane proteins reflects the difficulties in obtaining good quality crystals of this class of proteins. The TOM complex can only be isolated in its native state from the outer membranes of mitochondria, which makes its purification a difficult and challenging task.

The filamentous fungi *Neurospora crassa* turned out to be an excellent model organism for studying the TOM complex due to its fast growth rate and simple manipulation procedures. We have been able to purify and crystallize the TOM complex from *Neurospora*, comprising Tom40, the major pore forming protein, Tom22, Tom5, Tom6 and Tom7. The obtained crystals already diffract to a resolution limit of 6 Å. Currently, we have been working on improvement of the reflection qualities of the TOM complex crystals. Crystallisation in complex with antibody fragments has been reported to facilitate the crystallization of membrane proteins and to improve the diffraction quality of such crystals. The binding of Fv or Fab antibody fragments to the epitops on the protein surface enlarges the hydrophilic part of integral membrane proteins, thereby providing additional surface for crystal contacts. We are working on the production of murine monoclonal antibodies against the TOM complex to improve the resolution of the TOM crystals.

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