

# Diploma thesis

## Searching for microtubule inner proteins

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### Abstract

Microtubules (MTs) – cylindrical polymers of  $\alpha$ - and  $\beta$ -tubulin – maintain numerous irreplaceable functions in all eukaryotic cells. For this complex involvement of MTs in many cellular processes, precise tuning of their post-translational modifications, polymerization state, and interactome is crucial. Recently, a new mode of interactions with MTs was discovered – several microtubule inner proteins (MIPs) can enter the lumen of MTs.

Little is known about MIPs in dynamic MTs in the cytoplasmic network. Only two proteins have been shown to bind to the inside of dynamic MTs so far:  $\alpha$ TAT1 and MAP6; other proteins have been suggested to. Stabilised MTs, like the axoneme of the flagellum, contain dozens of orderly bound MIPs in the lumen and new ones are being added. MIPs are believed to play a role during axonemal assembly and to increase the stiffness required for flagellar beating.

This diploma thesis investigated MIPs in both dynamic and axonemal MTs. In the first part of the thesis, the goal was to identify candidates for new MIPs in the dynamic MTs by two independent approaches – proximity-labelling by promiscuous biotin ligase using  $\alpha$ TAT1 and MAP6 as baits, and direct isolation of MTs from cells and washing away outer proteins. Isolated proteins were then identified using mass spectrometry.

In the second part, the work aims to identify candidates for members of a newly discovered helical complex in the lumen of vertebrate sperm cell axoneme, termed TAILS (Tail Axoneme Intra-Lumenal Spiral). Candidates were identified by mass spectrometry analysis performed on isolated flagellum fragments, separated via cytometry sorting.

### Keywords

microtubules, microtubule inner proteins,  $\alpha$ -tubulin acetyltransferase 1 ( $\alpha$ TAT1), microtubule-associated protein 6 (MAP6), tail axoneme intra-lumenal spiral (TAILS)