Structural modelling of the IFT-B complex: Combining AlphaFold, SAXS, XL-MS and MX

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Cilia, intricate microtubular structures that extend from the cell surface, are assembled through a process known as intraflagellar transport (IFT). This crucial procedure involves the polymerization of 24 unique IFT proteins into structures known as IFT trains. These trains, driven bidirectionally along the axoneme by kinesin and dynein motors, are composed of two types of complexes, IFT-A and IFT-B, which contain six and eighteen subunits respectively. High-resolution structures of IFT-A complexes have been recently achieved through single particle cryo-electron microscopy. However, understanding the IFT-B complex at a similar resolution level has been a formidable challenge. Up until now, we only had access to lower resolution cryo-electron tomography structures of larger complexes and X-ray crystallography structures of smaller 2-3 subunit IFT-B complexes.

In an attempt to bridge this resolution gap, we utilized AlphaFold, an advanced machine learning platform for protein structure prediction, to model the structure of the 16-subunit Chlamydomonas IFT-B complex. We combined this in silico approach with biochemical validation strategies, including chemical crosslinking coupled with mass spectrometry (XL-MS), small angle X-ray scattering (SAXS), and mutational analysis of in vitro reconstituted IFT-B complexes. Furthermore, we confirmed the structure of subcomplexes through X-ray crystallography, using AlphaFold-generated models for molecular replacement.

Our integrative approach has provided novel insights into the full-length structure and interactions of the IFT-B complex, contributing to a more comprehensive understanding of ciliary assembly and function. This synergistic combination of computational and experimental methodologies promises to be a powerful tool for resolving the architecture of other challenging protein complexes.