Cilia/flagella are cell organelles conserved among eukaryotes and generate fluid flow. The bending motion of flagella is driven by axonemal dyneins and requires concerted activity of several hundreds of flagellar proteins.

To elucidate the mechanisms of dynein motor regulations in cilia/flagella, we have developed a new method to label specific proteins using biotin-streptavidin system. This labeling technique attaches the BCCP (Biotin Carboxyl Carrier Protein) tag to the target protein, which are subsequently enhanced by adding streptavidin and biotinylated cytochrome c. The 3D structure of labeled-flagellar were visualized by combining with cryo-electron tomography and sub-tomographic averaging. This enables us to locate flagellar proteins in 3D structure.

Using this system, we identified nonspecific intermolecular collision between central pair and radial spoke as one of the regulatory mechanisms for flagellar motility. By combining cryo-electron tomography and motility analyses of Chlamydomonas reinhardii flagella, we show that binding of streptavidin to radial spoke head paralyzed flagella. Moreover, the motility defect in a central pair projection mutant could be rescued by the addition of exogenous protein tags on radial spoke heads. Genetic experiments demonstrated that outer dynein arms are the major downstream effectors of central pair- and radial spoke-mediated regulation of flagellar motility. These results suggest that mechano-signaling between central pair and radial spoke regulates dynein activity in eukaryotic flagella.