Human general transcription factor TFII D is a megadalton sized multiprotein complex containing the TATA-box binding protein (TBP) and 13 TBP Associated Factors (TAFs). Structural studies by cryo-electron microscopy (cryo-EM) have uncovered the overall architecture of TFII D, providing valuable insight into subunit assembly and promoter recognition at medium resolution. Despite considerable effort, high resolution structural information of TAFs to date have been largely limited to structures of individual domains and assemblies of TAF histone-fold pairs, which are prevalent in TFII D. We used our MultiBac baculovirus/insect cell technology to obtain recombinant TFII D complexes including holo-TFII D in unprecedented quality and quantity, setting the stage for high resolution structural analysis by X-ray crystallography. We identified stable subassemblies of the TFII D complexes by limited proteolysis, and utilized a rapid combinatorial co-expression approach to produce and purify specimens for high-throughput crystallization. We crystallized a thus defined complex formed by TAF5, TAF6 and TAF9 and determined the structure to a resolution of 2.7 Å, revealing intricate molecular interactions at the core of human TFII D.


Keywords: Human TFII D, TBP Associated Factor TAF, MultiBac