# Research

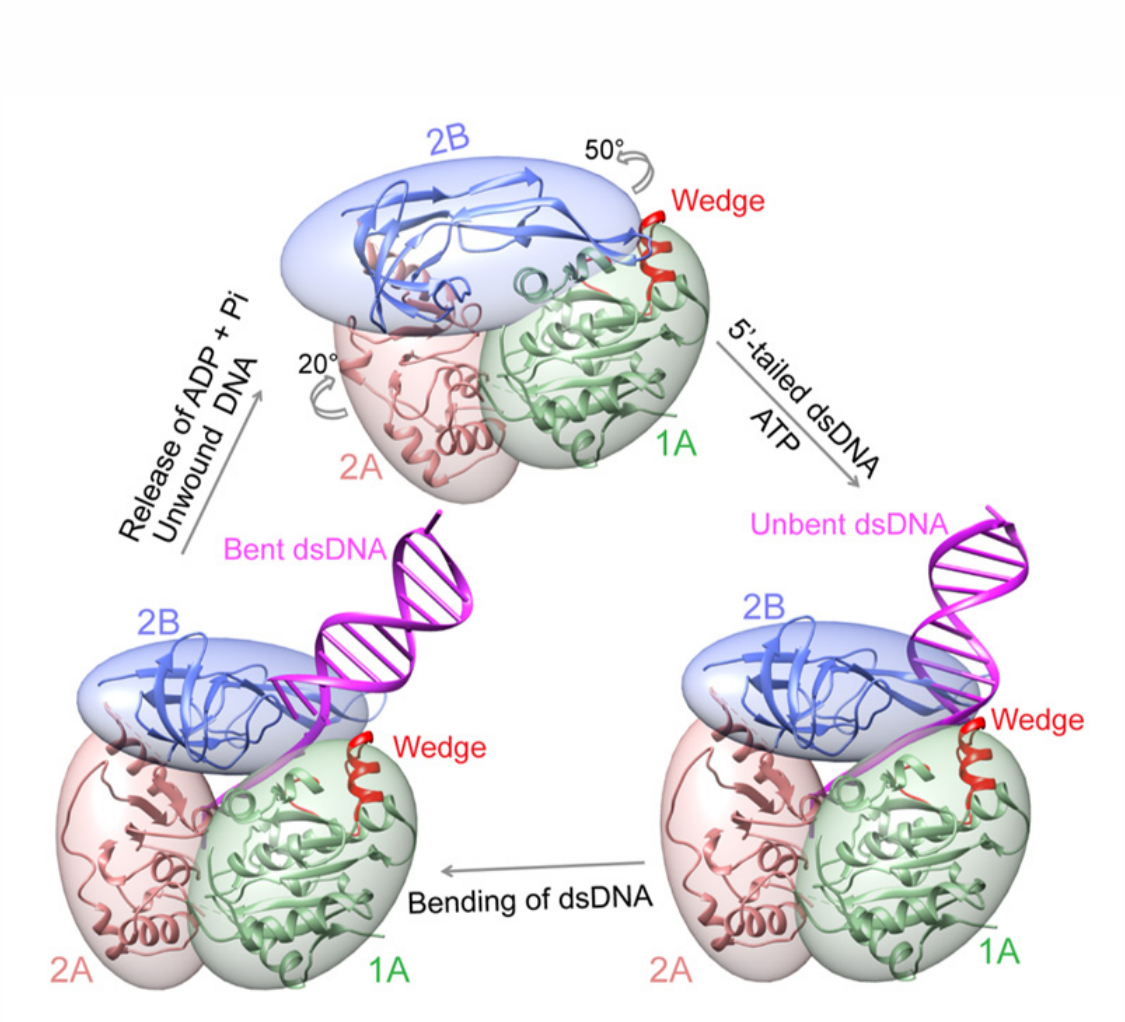
**Medical Structural biology**

Dr. Song's laboratory is interested in studying structure and function of proteins implicated in human diseases such as genetic and neurological disorders as well as cancers using a combinatorial approach of molecular biology, biochemical and biophysical methods, and structural biology (crystallography and cryo-EM). His current research aims to determine the structures of proteins involved in maitaining genome stability and the Hippo signaling pathway.

**Genome stability and human diseases**

The integrity of genomic DNA is constantly challenged by a variety of endogenous and exogenous DNA damaging agents such as replication fork collapse, oxidative stress and ionizing radiation (IR). Formation of G-quadruplex (also known as G4) structure also can lead to genomic instability. Failure to repair the damaged DNA and/or to resolve the G4 DNA strucures is associated with several human diseases including cancer.

Pif1 is a conserved SF1B DNA helicase involved in maintaining genome stability through unwinding dsDNAs, DNA/RNA hybrids and G-quadruplex (also known as G4) structure. We have solved the structures of the helicase domain of human Pif1 and Bacteroides sp Pif1 (BaPif1) in complex with ADP×$AlF_4^-$ and two different ssDNAs. The structural snapshots of BaPif1 combined with mutagenesis defined the functional role of the Pif1 signature motif and provided mechanistic insights into the unwinding activity of the Pif1 family helicases. We aim to solve a series of structures of Pif1 at different functional states including Pif1 in complex with DNA-RNA hybrid, Pif1 complexed with dsDNA and Pif1 with bound G4 DNA. These structures combined with subsequent mutational analyses would provide mechanistic view on how Pif1 resolves dsDNA, DNA-RNA hybrid and G4-DNA.



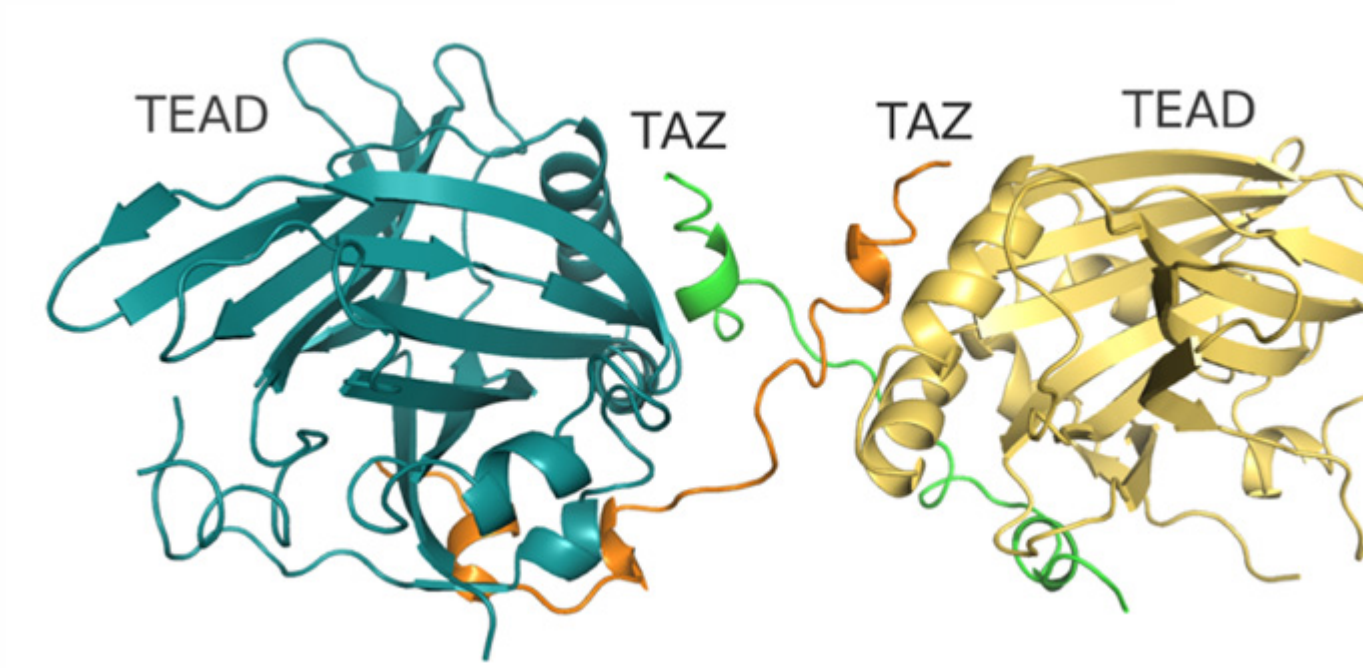

A model of dsDNA unwinding by Pif1

**RNA G-quadruplex and neurodegenerative diseases**

A hexanucleotide repeat expansion (HRE), $(GGGGCC)_n$ in the non-coding region of the gene C9orf72 is the most common genetic cause of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontoremporal dementia (FTD). The C9orf7HRE causes diseases by two possible pathomechanisms that involve formation of toxic RNA foci or production of toxic dipeptide repeat protein through repeat-associated non-ATG (RAN) translation. In the former case, the C9orf7HRE folds into stable G4 structures that disrupt RNA transcription and cause transcriptional pausing and abortion. The resulting abortive RNA transcripts with G4 structures, bind the essential nucleolar protein, nucleolin, in a conformation-dependent manner, which initiates molecular cascades leading to ALS/FTD pathologies. We aim to solve the structure of the nucleolin/HRE G4-RNA complex. This structure not only would elucidate the mechanism underlying nucleolin sequestration by the C9orf7HRE but also would aid structure-based design of small molecules that inhibit RNA foci formation or prevent RAN translation.

**The Hippo signaling pathway and tumerigenesis**

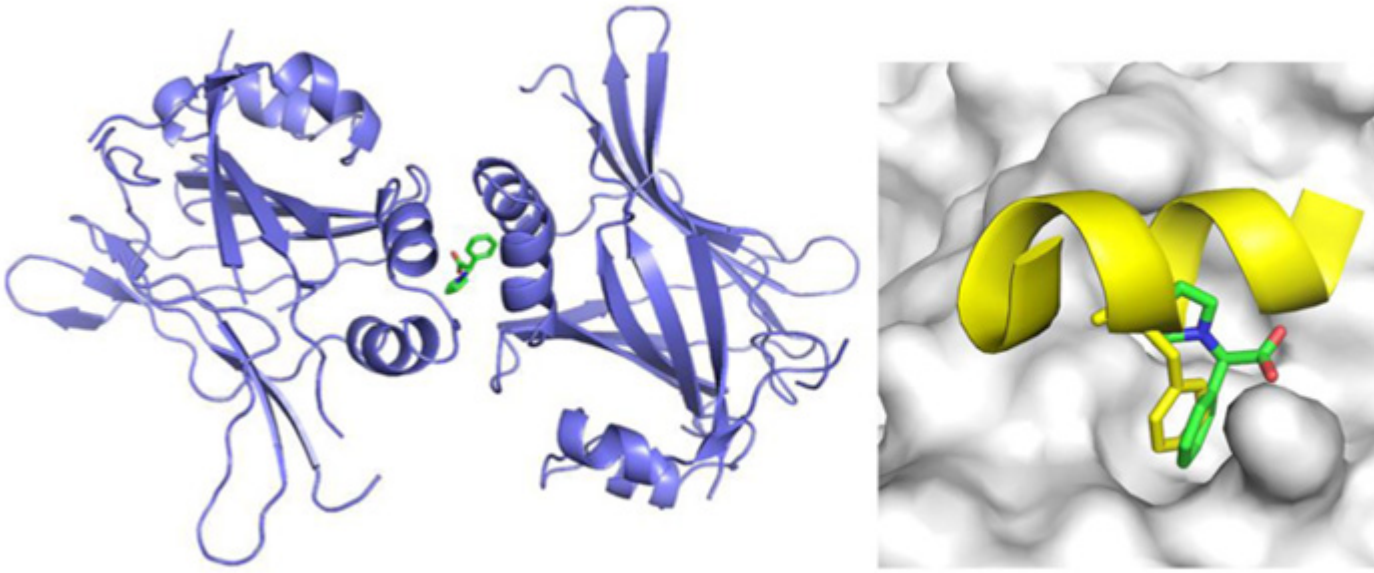
The Hippo signaling pathway controls cell growth, proliferation, and apoptosis by regulating the expression of target genes that execute these processes. While there are multiple proteins involved in the pathway, they can be grouped into three components: the upstream regulatory factors, the kinase core and the downstream transcriptional machinery wherein YAP functions as a transcriptional co-activator by interacting with the conserved TEAD family transcription factors. YAP mediates the output of Hippo pathway including growth control and cancer development. The abnormal activation of YAP has been associated with multiple types of cancer. We have solved the crystal structure of YAP in complex with TEAD4. Recently, we solved the crystal structure of TAZ-TEAD4 complex, which reveals two binding modes. The first is similar to the published YAP-TEAD structure. The second is a unique binding mode, whereby two molecules of TAZ bind to and bridge two molecules of TEAD4. These results provide mechanistic insights into the structural basis of YAP/TAZ-TEAD interaction and also offer a new strategy for cancer therapeutics by disrupting the YAP/TAZ-TEAD interaction. We will continue our efforts in elucidating the structures of proteins/complexes involved in this pathway including the key kinase complexes MST1/SAV1 and LATS1/Mob1.




Crystal structure of TAZ-TEAD showing a novel binding mode

Inhibiting the formation of the YAP/TAZ-TEAD complex has been shown to prevent cell proliferation. We have screened a total of 1000 fragments from the Maybridge Ro3 fragment library using the thermal stability shift assay and identified 20 fragments that increased the unfolding temperature of the protein by a range of 2 to 20°C. We have solved the crystal structure of TEAD in complex with one hit fragment. The structure shows that the hit fragment

occupies the same hydrophobic pocket as one of the important residues on YAP, suggesting that the fragment has the potential to disrupt interactions between YAP/TAZ and TEAD. In future, we will focus on growing this fragment into a more potent compound that can inhibit the YAP/TAZ-TEAD interaction.



Crystal structure of TEAD complexed with the hit fragment

**Prospective Graduate Students:**

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