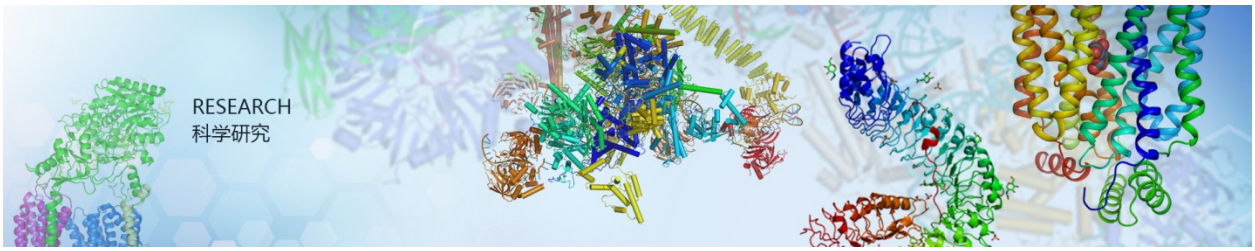

Tsinghua Press Release

A research team led by an American Postdoctoral Scientist, Lorenzo Finci, from the laboratory of Professor Yigong Shi from the Beijing Advanced Innovation Center of Structural Biology at Tsinghua University in an international collaboration with an American Pharmaceutical company in Cambridge, H3 Biomedicine, has published a research article in the journal Genes and Development. They reported the first human spliceosomal structure in complex with a spliceosomal modulator at near atomic resolution, and this represents the first example and sets precedence for using single particle cryo-EM for drug discovery.

Somatic mutations in spliceosomal proteins lead to dysregulated RNA splicing and are observed in a variety of cancers, like myelodysplastic syndromes, chronic lymphocytic leukemia, chronic myelomonocytic leukemia, uveal melanoma, skin melanoma, and breast and pancreatic cancers. These genetic aberrations may offer a potential intervention point for targeted therapeutics. The first splicing modulator to enter phase 1 clinical trials was E7107, and targets SF3b1, a component of the U2 snRNP. The first biochemical work providing insight into the mechanism of action of E7107 was published in Genes and Development in 2011 (Folco et al, Genes & Development 2011). However, the structural basis and precise mechanism of action of these modulators has remained elusive. Finci et al. present a single particle cryo-EM structure of the SF3b spliceosomal sub-complex bound to E7107 at 3.95 Å resolution (Figure 1).

Figure 1

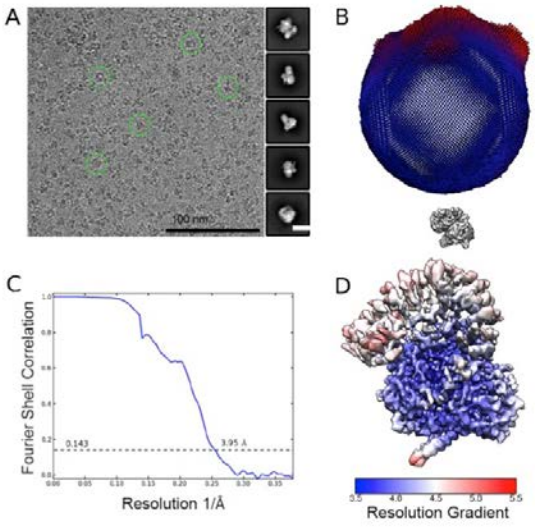

Figure 1. Cryo-EM analysis of the SF3b subcomplex A) (Left Panel) A representative electron micrograph of SF3b with some typical particles marked by green circles. (Right panel) Representative two-dimensional class averages of the particles. The scale bar is 100 nm. **B)** The angular distribution of the final reconstruction. Each column represents one view and the size of the column is proportional to the number of particles in that view. **C)** The Gold-standard Fourier shell correlation (FSC) curve for the 3D reconstruction of SF3b. **D)** Local resolution variations of the EM reconstruction. The resolution map is estimated with RELION 2.0.

The structure reveals how E7107 binds to the branch point adenosine-binding pocket via residues that also confer resistance upon mutation, and further suggest a substrate competitive model for how these natural products targeting SF3b modulate splicing (Figure 2). They further illustrate the structure activity relationship with chemical probes and use it to support their binding pose and mechanism of action. The work in this manuscript represents a significant advance in the field as it is the first human spliceosomal structure in complex with a modulator, and it demonstrates the potential for the utilization for single particle cryo-EM for drug discovery.

Figure 2.

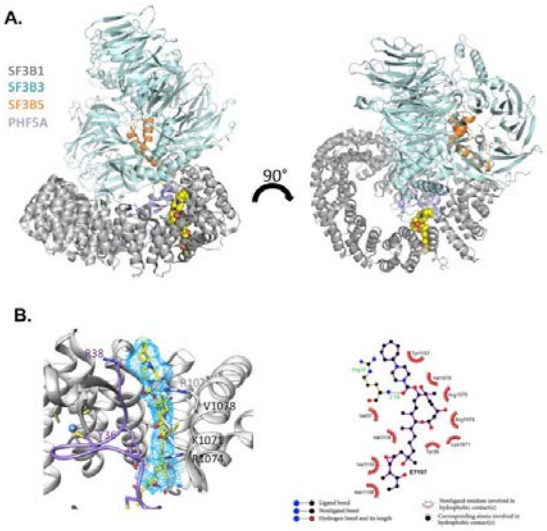

Figure 2. A) The overall structure of the four protein complex. HEAT repeats of SF3B1 shown in gray. PHF5a in light purple, SF3B3 in cyan, and SF3B5 in orange. E7107 is rendered as space filling spheres and colored by atom type. **B)** The cryo-EM map showing additional density in the branch point adenosine binding pocket. Map is contoured at 4 σ. Residue interactions are shown in 2-D schematic.

Lorenzo Finci was first and corresponding author from Tsinghua University. Xiaofeng Zhang, Xiuliang Huang, and Qiang Zhou also made significant contributions to the project. The team from H3 Biomedicine was led by Nicholas Larsen was corresponding author from H3 Biomedicine, and also included significant contributions from Jennifer Tsai, Teng Teng, Anant Agrawal, Betty Chan, Sean Irwin, Craig Karr, Andrew Cook, Ping Zhu, Dominic Reynolds, Peter G Smith, Peter Fekkes, Silvia Buonamici. This project was supported by the Tsinghua University Branch of China National Center for Protein Sciences (Beijing), the cryo-EM facility and the computational facility of the Bio-Computing Platform at Tsinghua University. This work was funded by the National Science Foundation of China (NSFC Grant no. 31650110470) Young Scientist Fellowship and the Beijing Advanced Innovation Center for Structural Biology Fellowship to Lorenzo Finci. Finally, Professor Yigong Shi provided guidance, support, critical discussion, and access to the Tsinghua electron microscope facility.