Molecular and structural insights into human pseudouridine synthase 3

Project goal. We aim to understand the fundamental role of a long-neglected RNA modification enzyme, the human pseudouridine synthase 3 (hPus3), on its potential mRNA substrates. We will employ structural biology and biochemistry to investigate hPus3 structure at atomic resolution as well as its catalytic reaction on different RNA substrate.

Description of research. RNA molecule as part of the central dogma plays an important role in deciphering DNA code into functional proteins. It only comprises of four basic nucleotides, including A, U, C, G. However, with the power of decoration by different chemical groups, there are over 170 different kinds of modified RNA nucleotides have been identified to date. We are interested in the pseudouridine (Ψ, 5-ribosyluracil), an isomer of U, which is one of the most abundant modified RNA nucleotides in transfer RNAs (tRNA) or ribosomal RNAs (rRNA) in all three domain of life and Ψ on these non-coding RNAs are permanent. By carrying the Ψ, it helps with proper RNA folding and the rigid structure greatly contribute to the molecular stability and function, such as tRNA in the protein translation regulation. Recently, our understanding on Ψ has expanded to gene regulation field. Several outstanding systemic studies have used a high throughput chemical-coupled next generation sequencing method to investigate more Ψ-modified targets. Surprisingly, many mRNAs are found to have Ψ. More importantly, these newly identified Ψ-modified sites are dynamic. In other word, this Ψ modification is inducible in response to external stimulation. This important finding strongly suggests that the induced Ψ modification involves in gene regulation for proper cellular function.

Reasons for attempting a particular research topic. Ten pseudouridine synthases are identified in eukaryotes, named Pus1-10 in human. They catalyze the pseudouridylation reaction on its own (without other associated molecules or proteins) and recognize the substrates with either specific sequences or secondary structures. Most knowledge on these enzyme activities, including structure and biochemical properties, are characterized in yeast system and little is known at human protein level. Some diseases are linked to the mutations in Pus enzymes, such as mitochondrial myopathy and sideroblastic anemia (MLASA) is due to the mutations in Pus1 whereas an autosomal recessive mental retardation (MRT55) is related to Pus3 mutations. We are particularly interested in characterizing Pus3 is because it might regulate certain gene expressions that involve in neuron development. How hPus3 recognizes these particular substrates and whether these Ψ-modified substrates affect neuron development are the two main questions we propose in this research.

Substantial expected results. To investigate the two main questions in this proposal, we propose to produce hPus3 for *in vitro* biochemical and biophysical studies and use protein crystallography and cryo-EM for structural characterizations. Altogether, we anticipate to present a comprehensive understanding on hPus3-meidated pseudouridylation on different RNA substrates and their roles in maintaining cellular function, especially the link to neuron development and the onset of related intellectual disorder.