สรุปและวิจารณ์ผลการทดลอง และข้อเสนอแนะสำหรับงานวิจัยในอนาคต

The relaxed tRNA specificity of the non-discriminating Aspartyl-tRNA synthetase has been the center of attention for many years. Despite the fact that several mutations in the anticodon binding domain were studied, our work presented here is the first example of the complete introduction of foreign domain from different organism into the non-discriminating enzyme. The introduction of the N-terminal anticodon binding domains of the discriminating AspRS and AsnRS from *E. coli* revealed a contribution of the anticodon binding domain toward tRNA specificity. Although not quantitatively, this is the first example of domain swapping in any aminoacyl-tRNA synthetase that provides catalytically active enzyme (Chimera-D). Chimera-D and Chimera-N have similar secondary structure content to the wild-type enzyme as observed in CD spectra and the calculated secondary structure content. In theory, these two chimeras should preserve the structural architecture of class IIb of aminoacyl-tRNA synthetase and exist as homodimer in their catalytically active form. The native-PAGE in the presence of 0.01% denaturant confirmed the existence of both homodimer and monomer of the wild-type enzyme. The degree of dimerization is significantly lower in Chimera-D and the lowest in Chimera-N. This observation could be the result from the differences in amino acid content around the surface of the N-terminal anticodon binding domain and the catalytic domain. The ability to form dimmers for these chimeras also correlates well with their catalytic activity in which Chimera-N is nearly catalytically inactive. (Only slightly active with $tRNA^{Asp}$) Despite the fact that both chimeras are less active than the wild-type for aspartylation reaction, the Chimera-D prefers $tRNA^{Asp}$ over $tRNA^{Asn}$ as a substrate. In deed, the preference for $tRNA^{Asp}$ is significantly higher than those of wild-type enzyme, indicating that our hypothesis regarding the contribution of N-terminal domain of the ND-AspRS toward tRNA specificity is valid and verified. The catalytic activities of these chimeras also correlate nicely with the heterologous toxicity when expressed in *E. coli* host cells. Since the chimera-N is nearly catalytically inactive toward both tRNAs, its overexpression is slightly less toxic to the host compare to Chimera-D. Both chimeras are a lot less active than the wild-type enzyme, and therefore, show less toxic phenotype compare to the wild-type AspRS.

The future investigation will concern a closer look at the conformational change within the anticodon binding domain upon binding to substrates (ATP, Asp, $tRNA^{Asp}$, and $tRNA^{As}$). These experiments will complete the overall changes in the anticodon binding domain and will also provide more information regarding the interface between the anticodon binding domain and the catalytic domain. The study will employ solution

dynamic NMR experiments. To do this, the anticodon binding domain (approximately 104 amino acids) needs to be labeled, independently from the rest of the enzyme. This domain will be subjected to a series of ligand titration experiments. The independent overexpression of the anticodon binding domain is now completed. The next step for us is overexpression of N-15 and C-13 labeled protein. Once the experiments are completed, the information obtained will yield a complete mechanism of binding between the Non-discriminating AspRS to its tRNA substrates.