PROJECT ABSTRACT:
Expanded genetic alphabets such as the “hachimoji” or eight-letter base system include nucleotide pairs in addition to naturally-occurring A-T and C-G. These unnatural base-pairs (UBPs) have alternative hydrogen bond patterns but still obey ‘Watson and Crick’ rules and have potential in synthetic biology to develop microorganisms with artificially expanded genetic information systems. Key to unlocking the potential of UBPs, and a prerequisite for developing semi-synthetic microbes, is the identification of enzymes able to process them as substrates. DNA ligases are the enzymes that join breaks in the phosphodiester backbone of double-stranded DNA and are responsible for the penultimate step of DNA replication and many repair pathways. We have identified a large number of novel DNA ligases among bacteria, several of which have been structurally and functionally characterized, providing an outstanding starting point for investigating ligase on UBPs. In this project, the student will produce 1-2 of these DNA ligases (according to established protocols) and test their efficacy against UBP-containing substrates provided by our collaborators at University of Cardiff in Wales UK as well as mismatched natural substrates.

STUDENT SKILLS:
- Enthusiasm and interest in biochemistry/ enzymes
- Basic biochemistry laboratory skills (minimum 2nd year, preferred 3rd year)
- Ability to work with others

PROJECT TASKS:
- Purify two recombinant DNA ligase proteins from E. coli (established protocol)
- Test enzyme activity and binding with 3 UBP-containing substrates relative to all-natural DNA using urea-PAGE assay
- Test enzyme activity and binding with 4 natural mismatch-containing substrates relative to fully-matched DNA
- Examine the effect of crowding agents on ligase activity with substrates
- Analyze data to determine optimal conditions and enzymes for DNA ligase activity on UBP substrates

EXPECTED OUTCOMES:
- Student’s Research Poster (as per clause 6 of the Scholarship regulations)
- Expressed and purified two DNA ligases
- Tested 3 UBP substrates
- Tested all four mismatch substrates
- Tested effect of crowding agents on ligase activity
- Analyzed data and presented this as a poster