Generation and validation of recombinant antibodies to study autoimmune diseases
M.Sc. project 30 hp for students in Biotechnology or similar

We are looking for enthusiastic, motivated students, who enjoy working as part of a team as well as independently. Ideally, candidates have some previous practical experience or interested in learning methods used in molecular biology/biochemistry. Please send a short description of your relevant work experience, your CV, and your motivation to Helena Persson Lotsholm (helena.persson@scilifelab.se).

Background
An important function of the immune system is being able to distinguish between foreign molecules invading the body, and the body's own molecules. If this process fails, a consequence can be the production of autoantibodies and development of an autoimmune disease. Idiopathic inflammatory myopathies (IIM, myositis) are rare autoimmune diseases, associated with high mortality and morbidity. One subgroup of IIM is called anti-synthetase syndrome and is characterized by the presence of autoantibodies targeting a protein family called aminoacyl tRNA synthetases.

Aminoacyl tRNA synthetases (aaRSs) have long been viewed as mere housekeeping proteins and have therefore often been overlooked in drug discovery. However, recent findings have revealed that many aaRSs have non-canonical functions and several of the aaRSs have been linked to autoimmune diseases, cancer and neurological disorders. Deciphering these roles has been challenging due to a lack of tools to enable their study. To help solve this problem, we have generated recombinant high-affinity antibodies for a collection of thirteen cytoplasmic and one mitochondrial aaRSs. Through this master thesis project we aim to generate recombinant antibodies for the remaining aaRSs in order to complete the set. By studying aaRSs and their connection to disease with validated research tools we hope to learn more about the pathogenesis of the disease and potentially understand how to better treat it.

Aim of project
To generate and validate recombinant antibodies using phage display technology, to targets associated with IIM and anti-synthetase syndrome, particularly aminoacyl tRNA synthetases.

Planned experimental activities
The activities have been divided into two work packages (WP I-II). Realistically, all activities of WPI should be completed before the end of the project.

WPI. Phage display selection and initial characterization of obtained antibody fragments
1. Phage display selections on 8-10 antigens.
2. Re-cloning of phage selected populations into vector for expression of soluble antibody fragments.
3. Small-scale bacterial expression and ELISA screening to check for binding.
4. Sequence analysis of generated hits.
5. Verification of binding of sequence unique binders by HTRF
6. Small-scale expression and purification of a sub-set of the selected binders
7. Surface plasmon resonance (SPR) measurements to determine binding kinetics on purified binders
WPII. More thorough characterization of obtained antibody fragments
   1. IP-MS on a subset of binders to confirm binding to endogenous target protein
   2. Establishing a method for epitope binning using ELISA/HTRF and applying the method on a sub-set of the obtained binders.

This translational project is a collaboration between researchers at KTH (Drug Discovery and Development Platform, SciLifeLab) and KI (Structural Genomics Consortium, Department of Medicine). For more information, see:

https://www.scilifelab.se/facilities/human-antibody-therapeutics
https://www.thesgc.org/profile/karolinska/sgraslund