The aim of this research project was to understand the interactions between ribosome-nascent chain complexes (RNCs), specifically an FLN5 RNC with point mutation Y719E and a +47 nascent chain attached, and a Y719E nanobody, NB5. The project used 1H-15N HMQC NMR spectroscopy to look at the interaction between the nanobody and a 15N-labelled isolated nascent chain protein, and later between the nanobody and the +47 Y719E RNC. This was carried out to understand whether the nanobody had any effect on the folding of the nascent chain. The majority of my time at the lab was spent carrying out preparations of the RNCs, nanobodies and isolated Y719E proteins. This involved growing the proteins in cultures under different expression systems until the desired pellet could be extracted, and then purifying the resulting cell pellet in order to obtain the pure protein samples. Purification steps involved running metal affinity chromatography, size exclusion chromatography, butyl columns, sucrose cushions and physical cell lysis.

I spent the entirety of the seven weeks in the lab, doing practical work everyday. I began by recapping key molecular biology techniques such as PCR and gel electrophoresis. I was then introduced to my research project and began by focusing on purifying the Y719E FLN5 nanobody. This involved learning purification techniques including metal affinity chromatography on a nickel column and size exclusion chromatography on a Superdex 75 column. I was then supervised on my first growth prep, following a protocol for the +47 Y719E RNCs and isolated Y719E proteins. This provided lots of practice in essential microbiology techniques such as maintaining a sterile environment and preventing cross-contamination, and the process increased my confidence using different types of centrifuges independently. I then purified the RNCs and isolated proteins which allowed me to practice many techniques which I had been earlier introduced to, this time without direct supervision, and to learn some more purification techniques. These included lysis using a french press, centrifugation using sucrose cushions, nickel affinity chromatography and butyl columns, using an AKTA system, for the RNC cells and further practice with the nickel column affinity chromatography and S75 column size exclusion chromatography, also using the AKTA system, for the isolated protein purification. Once all of the required proteins had been purified, and the purification had been checked by running gels, such as SDS-PAGE and BisTris, and western blots, I analysed the interaction between the isolated Y719E protein samples and the nanobody using HMQC 2D NMR. I learnt how to prepare samples for NMR and then had the chance to observe and help with running the equipment. I was also shown how to use the TopSpin software to visualise the results. I had planned to also introduce the nanobody to the +47 Y719E RNCs but there was not enough of the RNC or of the nanobody from the purification in order to do so. Therefore, I began another round of RNC and nanobody growth and purification. However, this was a very useful opportunity to follow the protocol almost completely independently and allowed me to further practice using the aforementioned equipment and techniques.

After all of the samples had been purified, 3 samples were prepared for NMR, one with only Y719E isolated protein, one with a 2:1 ratio of isolated Y719E:nanobody and one with a 1:1 ratio of isolated Y719E:nanobody. The results from the NMR did not show any significant changes in protein folding with or without the nanobody, or between the two different nanobody concentrations. After analysing the samples with NMR, I ran SDS-PAGE gels and western blots with all the samples in order to confirm the presence and concentration of the nanobodies. These confirmed that there was no nanobody present in the isolated Y719E only sample, and that the 1:1 Y719E:Nb sample had double the amount of nanobody to the 2:1 Y719E:Nb sample. Therefore it is likely that the nanobody was present as expected but did not interact with the RNC to any significant effect.

While there were no statistically significant impacts on protein folding in this pilot study, in the future further research on the interactions between nanobodies and RNCs and their potential impact on protein folding could be carried out with other nanobodies which maybe target different parts of the RNC. Any significant impact on protein folding could then be manipulated to help prevent misfolding and aggregation and therefore could have therapeutic uses for disorders such as Huntingtons or Parkinsons. Therefore, this field of research has an important medical impact, whilst also improving the understanding of protein-folding processes, particularly whilst the nascent chain is still attached to the ribosome.

Throughout the project I was well supported by the whole group, particularly by my supervisor, and where possible I also learnt about the different research projects being carried by other members of the group. I even had the chance to observe and help with RNC growth for my supervisor's project looking at the HTT protein causing Huntington's disease. This interaction with different members of the team was an invaluable experience, allowing me to learn techniques in different ways. Being involved in such an important lab, with autonomy and independence over my own project, while still being supported and supervised throughout, allowed me to gain confidence and improve my awareness of current research being carried out and my understanding of how the research process works. This experience has improved my organisational skills, since I was often running multiple preps at the same time, and it has improved my analytical skills and time-management. This is an opportunity which I know has put me in a much better position going into the third year of my degree and especially for my Master's, and will improve my prospects when working towards a postgraduate degree and a career in research. Furthermore, it has given me a real-world perspective on the topics of protein-folding and ribosome biochemistry, deepening my appreciation and understanding for the topic and inspiring me to continue specialising in this field.