Four separate protein experiments were carried out throughout the course of eight weeks. Expression, purification and crystallisation were achieved with CDK2, PFK and HSD (proposed project protein) using defined constructs and conditions. Soaking experiments were performed on cyclophillin and CDK in the presence of various ligands, and a crystal structure was obtained for cyclophillin in the presence of benzamide.

**CDK2**

Cyclin-dependent kinase 2 is a Ser/Thr protein kinase, involved in cell cycle regulation. GST::CDK2 expression constructs were cloned into DH5α cells. The plasmids were purified using a QIagen miniprep kit, transformed into Rosetta, pLysS and BL21 DE3 host cells, which were grown on selective media plates. For each host, three starter cultures of selective media were each inoculated with one colony, and left to grow overnight at 37°C.

Each starter culture was then inoculated into 500ml selective media, and grown until mid log phase (OD$_{600} = 0.6$). The cultures were induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG), and for each vector, grown at three temperatures (25°C, 30°C and 37°C).

The Rosetta cultures were centrifuged, and the cell pellets collected and lysed. The supernatant from a further centrifugation was then filtered and purified using a GSTrap column. The purified CDK2 protein was cleaved using thrombin, however this was unsuccessful as most of the protein was completely degraded.

Crystal trays were prepared according to predefined conditions, and crystals were successfully grown at 17°C. Ligand soaks, including a time course experiment and soaking experiments at various concentrations of the ligand, were prepared, however, crystal structures were not obtained.

**tbPFK**

Phosphofructokinase is a kinase from *Trypanosoma brucei*, which acts upon Fructose-6-phosphate in the glycolytic pathway. It differs from mammalian PFK, and so is a potential drug target.

Expression cultures for *tbPFK*:His-tag constructs were provided, and inoculated into selective medium broths, and grown overnight at 37°C. As with CDK2, started cultures were transferred to selective media, and grown to mid log phase prior to induction with IPTG. The expression cultures were then grown at 30°C overnight.

The protein was collected as with CDK2, and the supernatant passed through an IMAC column, using a step gradient of Imidazole. Approximately 40mg of pure protein was obtained, and was passed through a Sephadex G-25 M desalting column, in addition to gel filtration. Analysis
using Mass Spectroscopy and Dynamic Light Scattering, produced ideal results, and co-crystallisation trials with Adenosine monophosphate (AMP) were prepared.

**HSD**

11β-hydroxysteroid dehydrogenase (Type 1) is an NADPH-dependent enzyme responsible for the activation of the stress hormone cortisone to cortisol. Unlike CDK2 and PFK, two variants of HSD were used – murine and human (Amgen), however expression of the protein followed a similar procedure. Murine HSD and Amgen HSD were transformed into Rosetta hosts, and colonies transferred to the appropriate medium. The cultures were grown to mid log phase, and induced with IPTG. In addition to IPTG, hydrocortisone (ethanol) was added to aid in folding of the protein.

This method resulted in low yield due to low solubility of hydrocortisone leading to a high concentration of ethanol being added. As an alternative, a double transformation of the chaperone GroEL and HSD into BL21 cells was performed, however with a low success rate.

A further attempt to produce a higher yield of protein was tried by adding the competitive inhibitor carbenoxolone to aid in folding, however, this too did not produce sufficient quantities. In addition to compounds that would aid in folding, different temperatures were investigated. When grown at 25°C, a noticeable amount of murine HSD was expressed with hydrocortisone, however purification using a HITrap column on an AKTA purifier was not successful, due to a system error (all eluted protein was not collected).

**Cyclophilin**

Soaking solutions of various ligands were prepared and soaking drops were placed next to existing crystal drops, and left to equilibrate. Crystals were then transferred to the ligand soaks, and left overnight to bind at 4°C. Most crystals degraded, however a benzamide soak was successful, and a crystal structure was obtained from the in-house x-ray machine.

**Discussion**

Although most of the experiments performed produced satisfactory results, focus on one project would have been ideal, as there was not enough time to successfully replicate successful expression and crystallization trials for all four proteins. As all proteins have been implicated in diseases affecting humans, it is of interest to perhaps explore more soluble ligands that can bind these proteins as potential scaffolds for drug design, and study the kinetics and mechanism through crystallography.

**Acknowledgement**

I am very grateful for this very valuable experience, which was made possible by the Biochemical Society. Not only did I learn new techniques, I was also able to put theory into practice, in addition to helping colleagues produce protein (PFK) that was required by researchers abroad.