NANOBODIES AS TOOLS IN BIOCHEMISTRY AND DRUG DISCOVERY IN CANCER

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Nanobodies (Nb) are single domain derivatives from the variable heavy chain region of Camelid antibodies. They can selectively bind to an antigen like antibodies (Ab) but have other distinct properties such as their significantly smaller size that make them an exciting new tool to be explored. However, like Ab, conventional Nb design has proven to be expensive, time consuming and lacks rational design seen in other areas of drug discovery research. The aim of my project is to design nanobodies against a cancer therapeutic target using *in silic*o and *in vitro* techniques. The unique computational tool developed will be experimentally validated and able to accurately predict Nb binding kinetics to a therapeutic target epitope. Fructose 1,6-bisphosphate aldolase (FBA), a glycolytic enzyme was chosen as the proof of concept protein for validating this combinational approach.

FBA plays a role across several metabolic pathways including glycolysis, gluconeogenesis and the pentose phosphate pathway. The structure of human FBA has been solved using x-ray crystallography. Several enzymatic (specific activity and substrate Km) and biophysical characteristics have also been defined. There are three human isoforms of FBA (A-Muscle, B-Liver and C-Neuronal) each with a distinct tissue expression profile. Unsurprisingly, the isoforms also have different expression profiles in different cancers; high FBA A expression linked with poor prognosis in Lung small squamous cell carcinoma; high FBA B expression linked with poor prognosis in colorectal cancer; and high FBA C expression linked with lower proliferation in glioblastoma. While the specific role of FBA in immune cells is not yet defined, the role of metabolism overall has been investigated with results showing its critical part in activation of T-cells. This suggests that FBA is an attractive target in both cancer and immune cells.

The first step in targeting FBA was to look for a binding epitope between a Nb and human FBA isoforms. This was done using *in silico* analysis. Briefly, a Nb-FBA (PDB:5O0W, Nb474 and *Trypanosoma congoelense* FBA (*Tc*FBA)) crystal structure was used as the template to model the interaction and predict binding using a range of computational techniques, programs and platforms. To validate the *in silico* analysis, all three isoforms were expressed, purified and characterised for use in binding studies with Nb. The next step is to produce Nb474 and *Tc*FBA provided by collaborators. Nb474 will be used in binding studies with the three human FBA's and *Tc*FBA will be used as a control.