COMPUTATIONAL PREDICTION OF NANOBODY BINDING: TARGETING FRUCTOSE 1, 6-BISPHOSPHATE ALDOLASE

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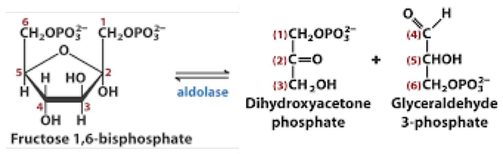
Aim

The aim is to develop and validate an *in silico* tool that can design novel nanobodies against target proteins with accurate and tuneable binding affinity. Fructose 1,6-bisphosphate aldolase (FBA), a glycolytic enzyme was chosen as the proof of concept protein for validating this computational tool.

Introduction

Fructose 1,6-bisphosphate aldolase (FBA) plays a role in several metabolic pathways in glycolysis, gluconeogenesis and the pentose phosphate pathway.



The structure of human FBA (hFBA) has been solved using x-ray crystallography. There are three human isoforms of FBA (A-Muscle, B-Liver and C-Neuronal) each with a distinct tissue expression profile. A Nanobody -FBA complex (PDB:5O0W, Nb474 and *Trypanosoma congoelense* FBA (*Tc*FBA)) is available and can be used as a template for designing novel nanobodies (Nb) against human FBA (hFBA) isoforms.

Protein Production

To carry out in vitro experiments, all hFBA isoforms and Nb474 were expressed, purified and characterized.

- His-Tag, Immobilised Metal Ion Chromatography (IMAC) using Nickle was used as the first capture step.
- Size Exclusion Chromatography (SEC) on a a 16-600 Superdex200pg column was carried out as the final polishing step.
- All protein produced to >95% purity

Comparison of *In silico* K_D & *In vitro* results

The results after each *in vitro* experiment was used to optimise the parameters of SMD. The *in silico* K_D predictions improved with each results. While no binding was measured, based on *in vitro* results a minimum K_D was estimated and compared to the optimised *in silico* K_D.

- *In vitro* K_D >800µM
- *in silico* K_D = ~700µM

In silico Modelling

1st Identify Nb474 binding epitope of *Tc*FBA and hFBA's using PYMOL
2nd Docked Nb474 onto the aligned epitope and analysed interactions using Discovery Studio Visualizer focusing on Hydrogen bonds and Electrostatic interactions and Van de Waals.
3rd Analyse the interactions between hFBA and Nb474 based on the *Tc*FBA binding epitope using Steered Molecular Dynamics (SMD). SMD pulls Nb474 from FBA and measures the force required to move a specified distance converting it into K_D.

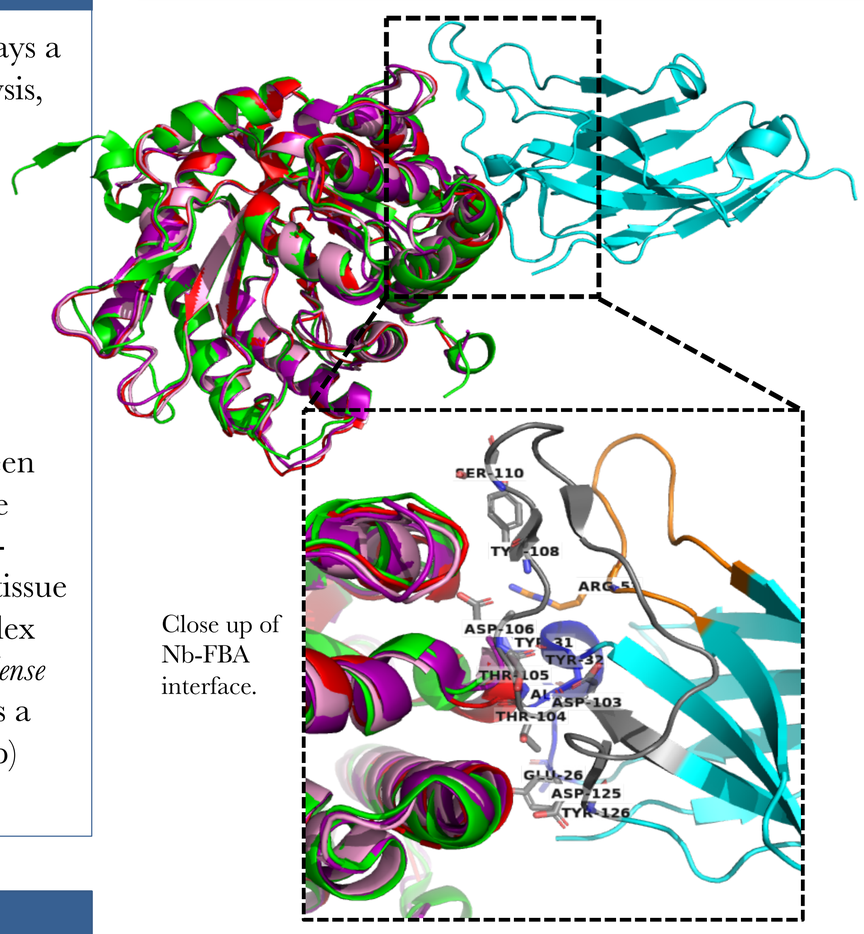


Figure 1. FBA-Nb474 *in silico* modeled interaction. Nb474 (teal) docked onto alignment of *Tc*FBA (green) with hFBA A (red) B (pink) and C (purple). CDR1 (blue) CDR2 (yellow) and CDR3 (grey). The interacting residues are labelled in the close up and highlighted in the Nb474 sequence shown above.

Conclusions

Nanobody-target protein binding affinity can be predicted using *in silico* methods. The optimization of the computational prediction for Nb474-hFBA binding using *in vitro* results is promising.

In Vitro Binding Studies

Native PAGE, Analytical SEC and Surface Plasmon Resonance were carried out to determine the *in vitro* hFBA-Nb474 K_D

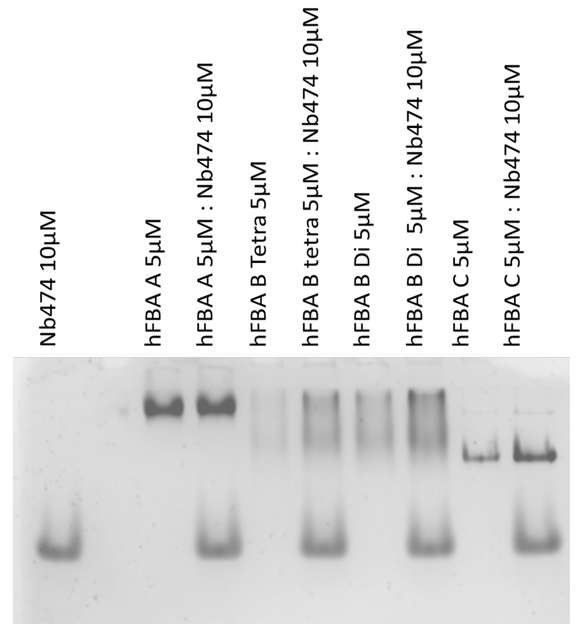


Figure 2: Native PAGE. 5µM of hFBA's incubated with 10µM of Nb474 on ice at 4°C for 30 mins.

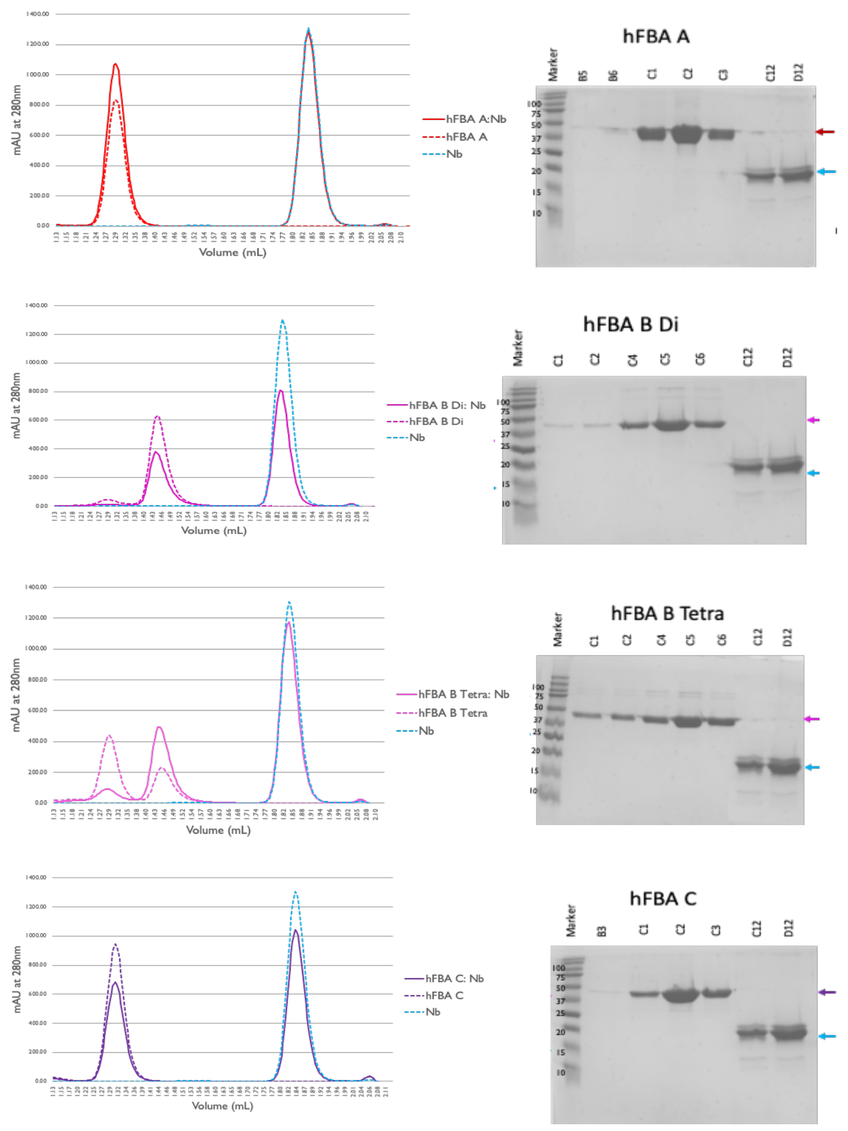


Figure 3 Analytical SEC. 2.4mL superdex200 PC column run at 50µL/ min using Nb474 at 100µM and hFBA at 50µM. Elution peaks were further analysed using SDS-PAGE

Future Work

- Design a tool that can generate novel nanobodies with tuneable binding affinity
- Examine the effect of site mutations on binding affinity.
- Generate a series of in silico site mutations with calculated K_D and validate them *in vitro*.
- Carry out and compare Phage Display Screening to investigate different Nb epitope sites on FBA
- Test the tool *in vivo* in cancer cell lines

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