Bio-Layer Interferometry (BLI) using the ForteBio BLItz Protocol

This assay was used to detect binding between the AtzC and AtzA protein subunits. BLI works by detecting binding between a protein immobilized on the biosensor tip and an analyte with optical interferometry.

The biosensor is coated in streptavidin so our AtzC protein was biotinylated so that when exposed to the biosensor, the biotin-streptavidin interaction would immobilize our protein onto the biosensor. The proteins were biotinylated in 20-fold excess with 10mM Biotin in order to ensure complete biotinylation. After biotinylation, the proteins were dialyzed into 50mM HEPES, 100mM NaCl, 5% glycerol, pH 7.6 to remove any excess biotin.

Our AtzA proteins was phosphorylated and after AtzC was immobilized onto the biosensor, AtzA was flowed in. Then the ForteBio BLItz would measure any binding in nanometers.

The concentration of the AtzC protein was 4µM and the concentration for AtzA proteins was 6µM. All the dilutions were done with our buffer with the addition of .1% BSA and .2% Tween 20. This was to ensure there was no non-specific binding and acted as our negative control.

Protocol:

1. Biosensor is hydrated in 200µL of buffer for 10 minutes.
2. Biosensor is exposed to 250µL buffer in order to get an initial baseline reading.
3. Biosensor is exposed to 4µL of biotinylated protein (know as our load) for 120 seconds. This will give us our loading curve.
4. Biosensor is exposed to 200µL of buffer for 30 seconds for another baseline measurement.
5. Biosensor is exposed to 4µL of phosphorylated protein (known as our analyte/flow) for 120 seconds. This gives us our binding/association curve.
6. Biosensor is exposed to 250µL of buffer so that we can get a dissociation measurement. This gives us our dissociation cure.

Binding Curve Example: