SPR experiments for the Atomwise (AW) library

Experimental conditions:
Sensor: NTA + amine coupling (including final wash with running buffer with 350 mM EDTA)

Samples immobilised:
- *Pa*MurE
- *Pa*MurC
- *Pa*MurD

Running buffer:
- 20 mM HEPES, pH 7.5
- 10 mM MgCl2
- 200 mM NaCl
- 5% DMSO

Temperature: 15 °C

I have carried out different SPR experiments: AMPPCP and AMPNP K_D determination, library screens in the absence and presence of AMPPCP, and dose response experiments for the hits from the screen in the absence of AMPPCP.

AMPPCP and AMPPNP K_D determination experiments
The experiments to determine the K_D values for AMPPCP showed 2 binding events for *Pa*MurD. The first event presents positive binding signal and a K_{D1} of 1.5 uM, while the second event showed a negative binding signal and K_{D2} of 290 uM. In the case of *Pa*MurE and *Pa*MurC, only one event was detected with K_D values of 295 and 124 uM respectively.

AW library Screening and dose response experiments
I performed a single concentration screening of the AW library. The compounds concentration was 500 uM. Some compounds binding signal would be due to non-specific binding (NSB) or multiple binding sites which could also be considered NSB. Negative binding signal usually corresponds to larger conformational changes.

The selection criteria for the compounds to perform dose response experiments are the following:
1. Compounds presenting a binding signal greater than 10 RU.
2. Compound presenting the largest negative signal.
3. All compounds that were a hit at the XChem experiments.

The dose response experiments were carried out using 500uM as the top concentration, and ten ½ serial dilutions were performed per compound.

*__Please note__, the K_D values for the AW compounds might not be accurate due to the compound solubility under these conditions and the potential presence of NSB. Some dose response graphs will show sigmoidal curves which could be an indication of both 2 binding sites or NSB interference.

Additionally, I performed a screen in the presence of AMPPCP (1mM). The experiments were carried out using a dual injection method:

- Injection 1: running buffer + 1 mM AMPPCP
- Injection 2: running buffer + 1 mM AMPPCP + 500 uM compound

The binding values cannot be compared to the first screen. I have inspected all binding curves and determined I am not fully confident about the presence of binding for anything with s binding signal lower than about 15 RU.

The main conclusion to take from this experiment in the presence of AMPPCP, XChem hit compounds Z224156390, Z225695112, Z1275565768, and Z1720524522 showed binding in the presence of AMPPCP. Follow up experiments to calculate K_D values would need a significant amount of AMPPCP and it was not feasible at the time the experiments took place.

I suggest the library should be tested on the enzymatic assay and compare the results to this SPR experiments. I would like to:

- determine if there is a correlation between the enzymatic assay results and the SPR,
- understand better how to interpret SPR data for future experiments,
- determine if negative binding means there exists binding at the ATP site,
- determine if the XChem hits present inhibit activity.