**24/04/2018 - ACVR1A-c096 purification for co-crystallisation with LDN-193189 for fragment screening and co-crystallisation with other compounds**

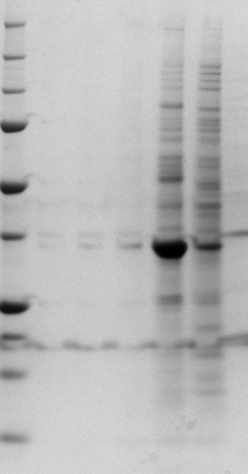
**Aim:** to purify ACVR1 (residues 208-499, constitutively active Q207D mutant) for crystallisation in complex with LDN-193189 to do fragment screening for allosteric compounds

The purification protocol has already been documented a few times in earlier posts. Suffice it to say, this was not a good purification for some reason, I only got 1 mg of protein, as opposed to the 10 mg I got last time, where I was able to set up 25 plates. This time I could only set up 3 plates.

**Ni purification**

One can already see from the Nickel purification that there is not much protein – normally the bands would be a lot fatter.

**E1 E2 E3 E4 E5 MW**



**250 kDa**

**150**

**100**

**75**

**50**

**37**

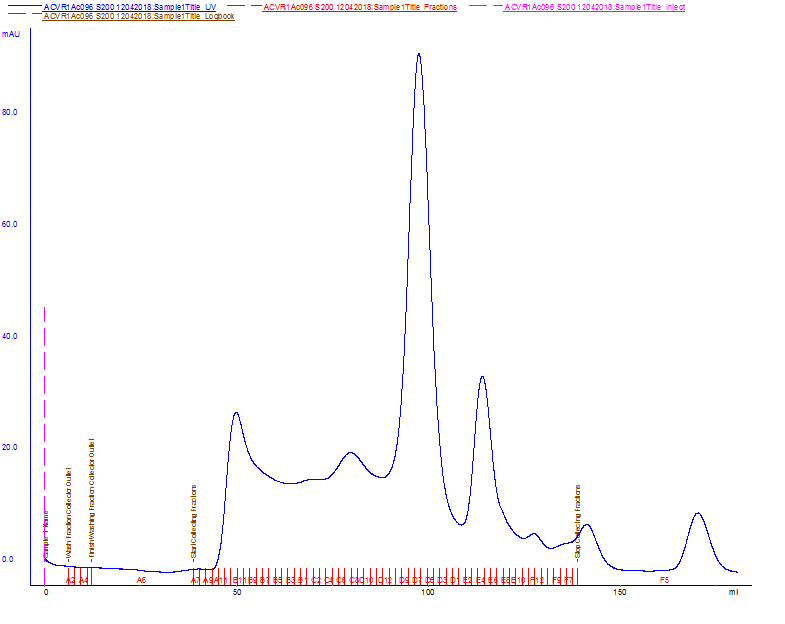
**25**

**20**

**15**

**10**

**ACVR1 (~34 kDa)**



**Superdex S200 gel filtration profile**

**Gel filtration gel**

Not much protein, and it’s contaminated.



**250 kDa**

**150**

**100**

**75**

**50**

**37**

**25**

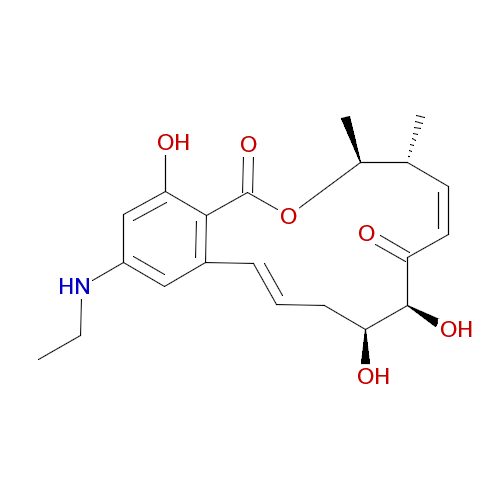
**20**

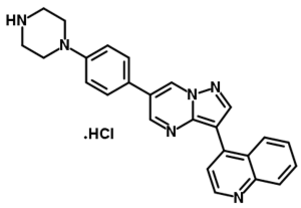
**15**

**10**

**ACVR1 (~34 kDa)**

Nevertheless, I concentrated this protein down to about 9.4 mg/ml, and set up one plate with 1 mM LDN-193189, and two plates with 1 mM E6201 (K63275a) ( structures below) that were follow-up fine screens from a couple of hits I got some time ago, where spindly little needles grew. These conditions were 20 % PEG 6000, 10 % ethylene glycol, 0.1 M tris pH 7.5, 0.2 M LiCl2. So I screened around the pH and PEG conditions.





**LDN-193189 K63275a**

Below are the original hits I got for ACVR1 with E6201. So far there’s nothing in the new follow-up plates, but I’ll keep an eye on them.

