**Design of a mega massive composite screen to streamline ACVR1 crystal screening**

**Aim:** To design a composite 96-well screen from the 5 coarse screens we have at the SGC: JCSG7, LFS6, HIN3, HCS3, BCS6, based on the PDB conditions ACVR1 is most frequently found to crystallise in. Analysis suggests most of these contain sulphate, phosphate or citrate. Currently it takes too much protein to do 5 screens at two temperatures for each compound, so if there are conditions we know the protein is unlikely to crystallise in, it doesn’t make sense to waste protein by screening those conditions.

**Experiment:**

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| **PDB code** | **Structure** | **Residues** | **Condition** |
| 4BGG | ACVR1 kinase in complex with LDN-213844 | 203-498 | 0.2M ammonium citrate, 20%(W/V) PEG 3350 |
| 3MTF | 2-aminopyridine inhibitor | 203-498 | 1.6M Na/KPO4, 0.1M HEPES pH 7.5, vapor diffusion, sitting drop, temperature 293.15K |
| 3Q4U | LDN-193189 | 202-499 | 20% PEG 3350, 0.2M ammonium citrate dibasic pH 5.0, vapor diffusion, sitting drop, temperature 293.15K |
| 4C02 | FKBP12.6 and dorsomorphin | 178-499 | 1.8M ammonium citrate, pH 7.2 |
| 5OXG | LDN-212854 | 203-498 | 18% PEG8000, 0.2M calcium acetate, 0.1M cacodylate pH 6.5 |
| 5OY6 | cyclical inhibitor OD36 | 204-498 | 0.3M magnesium formate, 0.1M bis-tris pH 5.5 |
| 6EIX | Q207E mutant in complex with a 2-aminopyridine inhibitor K02288 | 192-503 | 0.1M MES pH 6.5, 12%(w/v) PEG 20000 |
| 3H9R | FKBP12 and dorsomorphin | 172-499 | 30% PEG 3350; 0.25M Ammonium sulphate; 0.1M Bis-Tris, pH 6.0, vapor diffusion, sitting drop, temperature 277.15K |
| 3OOM | imidazo[1,2-b]pyridazine inhibitor K00507 | 203-499 | 20% PEG 3350, 0.2M Na/K PO4, 10% ethylene glycol, pH 7.5, vapor diffusion, sitting drop, temperature 293.15K |
| 4DYM | imidazo[1,2-b]pyridazine inhibitor K00135 | 207-499 | 1.60M MgSO4; 0.1M MES pH 6.5, vapor diffusion, sitting drop, temperature 293.15K |
| 6GI6 | Quinazolinone based ALK2 inhibitor with a 5-methyl core. | 202-499 | 1.5M ammonium sulfate, 0.1M sodium chloride, 0.1M bis-tris pH 6.5 |
| 6GIN | Quinazolinone based ALK2 inhibitor with a 4-morpholinophenyl solvent accessible group | 204-499 | 1.6M ammonium sulfate,12% glycerol, 0.1M tris pH 8.5 |
| 6GIP | Quinazolinone based ALK2 inhibitor with a 2, 5-dimethyl core. | 204-499 | 1.5M ammonium sulfate, 0.1M tris pH 8.5, 4% glycerol |

I analysed the crystal conditions for ACVR1 in 13 PDB structures:

I then designed a 96-well composite coarse screen in Screen Designer, our in-house screen design software, pulling out any conditions I could find in the coarse screens that contained sulphate, phosphate or citrate. This was not as trivial as it sounds, as Screen Designer can be buggy and crashes at the drop of a hat. I finally got the screen designed with the conditions shown below, in 800 μl blocks. I then needed to make the screens with our MPII liquid handler (which I have now renamed ‘mare). This required finding, setting out and pipetting 64 different reagents, many of which have short shelf lives, and so were not in stock. Thus I made up the following reagents:

Sodium citrate pH 2.2 (1 M)

Sodium citrate tribasic (2 M)

Sodium/potassium phosphate pH 6.2 (1 M)

Sodium phosphate dibasic (0.5 M)

Sodium phosphate pH 4.6 (1 M)

Rubidium chloride (0.5 M)

Potassium phosphate dibasic pH 9

Sodium citrate tribasic (2 M) did not want to dissolve, so I heated it to about 170 degrees C, and eventually it all went into solution. Of course, when I came in the next day, huge crystals had appeared in the bottom of the bottle, but I had already diluted what I needed in my screen, so it should be okay. The liquid handler crashed multiple times and had to keep being aborted all afternoon, but I got Nathan (in the protein crystallography group) to help me fix it, and I finally got the screens made. Have a look at my ACVR1 purification (uploaded at the same time as this) to see all the cool hits I got from this screen.

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| **Xtalln Screen Row** | **Xtalln Screen Column** | **Condition** | **Source** |
| A | 01 | 50% PEG400 -- 0.2M lithium sulfate -- 0.1M acetate pH 4.5 | **JCSG7 A1** |
| A | 02 | 20% PEG3350 -- 0.2M ammonium citrate dibasic | A3 |
| A | 03 | 20% PEG1000 -- 0.2M lithium sulfate -- 0.1M citrate pH 4.2 | A6 |
| A | 04 | 50% MPD -- 0.2M ammonium phosphate dibasic -- 0.1M tris pH 8.5 | A11 |
| A | 05 | 0.8M ammonium sulfate -- 0.1M citrate pH 4.2 | B1 |
| A | 06 | 5% PEG1000 -- 40% ethanol -- 0.1M citrate pH 4.2 | B6 |
| A | 07 | 20% PEG6000 -- 0.1M citrate pH 5.0 | B9 |
| A | 08 | 0.8M sodium phosphate monobasic -- 0.8M potassium phosphate dibasic -- 0.1M HEPES pH 7.5 | C5 |
| A | 09 | 2M ammonium sulfate -- 0.1M acetate pH 4.5 | C11 |
| A | 10 | 30% PEG8000 -- 0.2M lithium sulfate -- 0.1M acetate pH 4.5 | D4 |
| A | 11 | 40% PEG400 -- 0.2M lithium sulfate -- 0.1M tris pH 8.5 | D7 |
| A | 12 | 0.15M ammonium sulfate -- 25% PEG4000 -- 15% glycerol | D9 |
| B | 01 | 16% PEG8000 -- 0.04M potassium phosphate dibasic -- 20% glycerol | D12 |
| B | 02 | 2M ammonium sulfate -- 0.2M sodium chloride -- 0.1M cacodylate pH 6.5 | E2 |
| B | 03 | 1.26M ammonium sulfate -- 0.2M lithium sulfate -- 0.1M tris pH 8.5 | E4 |
| B | 04 | 0.8M ammonium phosphate dibasic -- 0.1M acetate pH 4.5 | E8 |
| B | 05 | 3M ammonium sulfate -- 0.1M citrate pH 5.0 | F2 |
| B | 06 | 2M ammonium sulfate -- 0.1M bis-tris pH 5.5 | G11 |
| B | 07 | 1% PEG3350 -- 1M ammonium sulfate -- 0.1M bis-tris pH 5.5 | H2 |
| B | 08 | 25% PEG3350 -- 0.2M ammonium sulfate -- 0.1M bis-tris pH 5.5 | H7 |
| B | 09 | 25% PEG3350 -- 0.2M lithium sulfate -- 0.1M bis-tris pH 5.5 | H9 |
| B | 10 | 0.4M ammonium phosphate monobasic | **HCS3 A3** |
| B | 11 | 2M ammonium sulfate -- 0.1M tris pH 8.5 | A4 |
| B | 12 | 30% MPD -- 0.2M sodium citrate tribasic -- 0.1M HEPES pH 7.5 | A5 |
| C | 01 | 0.8M ammonium phosphate monobasic -- 0.8M ammonium phosphate dibasic | A11 |
| C | 02 | 0.2M ammonium sulfate -- 30% PEG8000 -- 0.1M cacodylate pH 6.5 | B3 |
| C | 03 | 1.5M lithium sulfate -- 0.1M HEPES pH 7.5 | B4 |
| C | 04 | 30% PEG4000 -- 0.2M lithium sulfate -- 0.1M tris pH 8.5 | B5 |
| C | 05 | 0.2M ammonium sulfate -- 25% PEG4000 -- 0.1M acetate pH 4.5 | B8 |
| C | 06 | 0.2M ammonium sulfate -- 30% PEG8000 | C6 |
| C | 07 | 0.2M ammonium sulfate -- 30% PEG4000 | C7 |
| C | 08 | 2M ammonium sulfate | C8 |
| C | 09 | 0.4M sodium phosphate monobasic -- 0.4M potassium phosphate monobasic -- 0.1M HEPES pH 7.5 | C11 |
| C | 10 | 1.4M sodium citrate tribasic -- 0.1M HEPES pH 7.5 | D2 |
| C | 11 | 2M ammonium sulfate -- 2% PEG400 -- 0.1M HEPES pH 7.5 | D3 |
| C | 12 | 20% PEG8000 -- 0.04M potassium phosphate monobasic | D6 |
| D | 01 | 2M ammonium sulfate -- 0.1M acetate pH 4.5 | D11 |
| D | 02 | 0.8M ammonium phosphate monobasic -- 0.1M tris pH 8.5 | D12 |
| D | 03 | 2M ammonium sulfate -- 5% 2-propanol | E5 |
| D | 04 | 0.2M ammonium sulfate -- 30% PEG2000MME -- 0.1M acetate pH 4.5 | F1 |
| D | 05 | 0.2M sodium/potassium tartrate -- 2M ammonium sulfate -- 0.1M citrate pH 5.5 | F2 |
| D | 06 | 0.5M ammonium sulfate -- 1M lithium sulfate -- 0.1M citrate pH 5.5 | F3 |
| D | 07 | 1.6M magnesium sulfate -- 0.1M MES pH 6.5 | F8 |
| D | 08 | 0.1M sodium phosphate monobasic -- 0.1M potassium phosphate dibasic -- 2M sodium chloride -- 0.1M MES pH 6.5 | F9 |
| D | 09 | 1.6M ammonium sulfate -- 10%(v/v) dioxane -- 0.1M MES pH 6.5 | F11 |
| D | 10 | 1.8M ammonium sulfate -- 0.01M cobalt chloride -- 0.1M MES pH 6.5 | G1 |
| D | 11 | 0.2M ammonium sulfate -- 30% PEG5000MME -- 0.1M MES pH 6.5 | G2 |
| D | 12 | 30% MPD -- 0.5M ammonium sulfate -- 0.1M HEPES pH 7.5 | G5 |
| E | 01 | 5% MPD -- 10% PEG6000 -- 0.1M HEPES pH 7.5 | G6 |
| E | 02 | 1.6M ammonium sulfate -- 0.1M sodium chloride -- 0.1M HEPES pH 7.5 | G8 |
| E | 03 | 1M lithium sulfate -- 0.01M nickel chloride -- 0.1M tris pH 8.5 | H5 |
| E | 04 | 1.6M ammonium sulfate -- 12% glycerol -- 0.1M tris pH 8.5 | H6 |
| E | 05 | 50% MPD -- 0.2M ammonium phosphate dibasic -- 0.1M tris pH 8.5 | H7 |
| E | 06 | 2M ammonium sulfate -- 0.1M citrate pH 3.5 | **HIN3 A1** |
| E | 07 | 2M ammonium sulfate -- 0.1M acetate pH 4.5 | A2 |
| E | 08 | 2M ammonium sulfate -- 0.1M bis-tris pH 5.5 | A3 |
| E | 09 | 2M ammonium sulfate -- 0.1M bis-tris pH 6.5 | A4 |
| E | 10 | 2M ammonium sulfate -- 0.1M HEPES pH 7.5 | A5 |
| E | 11 | 2M ammonium sulfate -- 0.1M tris pH 8.5 | A6 |
| E | 12 | 1.26M sodium phosphate monobasic -- 0.14M potassium phosphate dibasic | B5 |
| F | 01 | 0.49M sodium phosphate monobasic -- 0.91M potassium phosphate dibasic | B6 |
| F | 02 | 0.056M sodium phosphate monobasic -- 1.344M potassium phosphate dibasic | B7 |
| F | 03 | 1.4M sodium citrate tribasic -- 0.1M HEPES pH 7.5 | B8 |
| F | 04 | 1.8M ammonium citrate | B9 |
| F | 05 | 1.5M ammonium sulfate -- 0.1M sodium chloride -- 0.1M bis-tris pH 6.5 | C6 |
| F | 06 | 1M ammonium sulfate -- 1% PEG3350 -- 0.1M bis-tris pH 5.5 | C8 |
| F | 07 | 1M ammonium sulfate -- 0.5% PEG8000 -- 0.1M HEPES pH 7.0 | C11 |
| F | 08 | 0.05M ammonium sulfate -- 30% pentaerythritol ethoxylate 15/4 -- 0.1M bis-tris pH 6.5 | E9 |
| F | 09 | 0.2M lithium sulfate -- 25% PEG3350 -- 0.1M bis-tris pH 5.5 | G2 |
| F | 10 | 0.2M lithium sulfate -- 25% PEG3350 -- 0.1M bis-tris pH 6.5 | G3 |
| F | 11 | 0.2M lithium sulfate -- 25% PEG3350 -- 0.1M HEPES pH 7.5 | G4 |
| F | 12 | 0.2M lithium sulfate -- 25% PEG3350 -- 0.1M tris pH 8.5 | G5 |
| G | 01 | 30% PEG Smear Low -- 0.1M citrate/phosphate pH 5.5 | **BCS6 A2** |
| G | 02 | 25% PEG Smear Medium -- 0.1M citrate/phosphate pH 5.5 | A5 |
| G | 03 | 20% PEG Smear High -- 0.1M citrate/phosphate pH 5.5 | A8 |
| G | 04 | 22% PEG Smear Broad -- 0.1M citrate/phosphate pH 5.5 | A11 |
| G | 05 | 25% PEG Smear Medium -- 0.1M cacodylate pH 5.5 -- 0.2M ammonium sulfate | C4 |
| G | 06 | 25% PEG Smear Medium -- 0.1M citrate pH 5.5 -- 0.1M potassium/sodium phosphate -- 0.1M rubidium chloride | C5 |
| G | 07 | 15% PEG Smear High -- 0.1M citrate pH 5.0 -- 0.15M ammonium acetate | C7 |
| G | 08 | 20% PEG Smear Broad -- 0.1M citrate pH 5.6 -- 0.15M magnesium acetate | C9 |
| G | 09 | 25% PEG Smear Broad -- 0.1M acetate pH 4.6 -- 0.2M ammonium sulfate | C10 |
| G | 10 | 20% PEG Smear Medium -- 0.1M phosphate pH 6.2 -- 0.2M sodium formate -- 10%(v/v) glycerol | D4 |
| G | 11 | 30% PEG Smear Medium -- 0.1M ADA pH 6.5 -- 0.2M lithium sulfate | D5 |
| G | 12 | 18% PEG Smear High -- 0.1M ADA pH 6.5 -- 0.2M ammonium sulfate | D7 |
| H | 01 | 28% PEG Smear Broad -- 0.1M phosphate pH 6.2 -- 0.2M sodium chloride | D10 |
| H | 02 | 22.5% PEG Smear Medium -- 0.1M citrate pH 5.5 -- 0.1M ammonium sulfate -- 0.05M magnesium sulfate | D11 |
| H | 03 | 15% PEG Smear High -- 0.1M HEPES pH 7.5 -- 0.1M potassium/sodium phosphate -- 10%(v/v) ethylene glycol | E6 |
| H | 04 | 25% PEG Smear Broad -- 0.1M HEPES pH 7.2 -- 0.2M lithium sulfate | E8 |
| H | 05 | 20% PEG Smear Medium -- 0.1M bicine pH 9.0 -- 0.2M ammonium sulfate -- 0.05M magnesium sulfate | F5 |
| H | 06 | 20% PEG Smear Broad -- 0.1M tris pH 8.0 -- 0.2M ammonium sulfate | F10 |
| H | 07 | 22.5% PEG Smear Broad -- 0.1M bicine pH 8.8 -- 0.02M magnesium sulfate -- 0.2M potassium chloride | F11 |
| H | 08 | 25% PEG Smear Low -- 0.1M bis-tris pH 6.8 -- 0.15M lithium sulfate -- 0.05M magnesium chloride | G2 |
| H | 09 | 25% PEG Smear Medium -- 0.1M HEPES pH 7.5 -- 0.2M ammonium sulfate -- 0.01M cadmium chloride | G3 |
| H | 10 | 25% PEG Smear Broad -- 0.1M HEPES pH 7.0 -- 0.1M ammonium sulfate -- 0.1M sodium formate | G10 |
| H | 11 | 22.5% PEG Smear Medium -- 0.1M HEPES pH 7.5 -- 0.2M potassium/sodium phosphate -- 10%(v/v) glycerol | G11 |
| H | 12 | 28% PEG Smear Broad -- 0.1M bis-tris-propane pH 8.5 -- 0.05M ammonium sulfate -- 0.05M lithium sulfate | H9 |