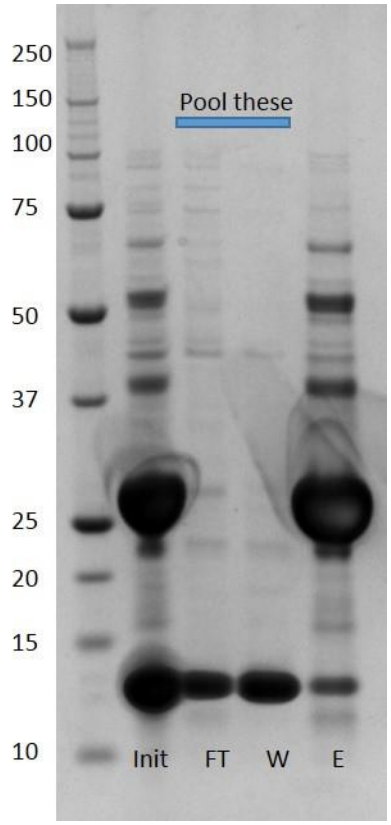
Complex formation of ACVR2 with XIAP:

XIAP-GST incubated with Tev overnight at 4C.

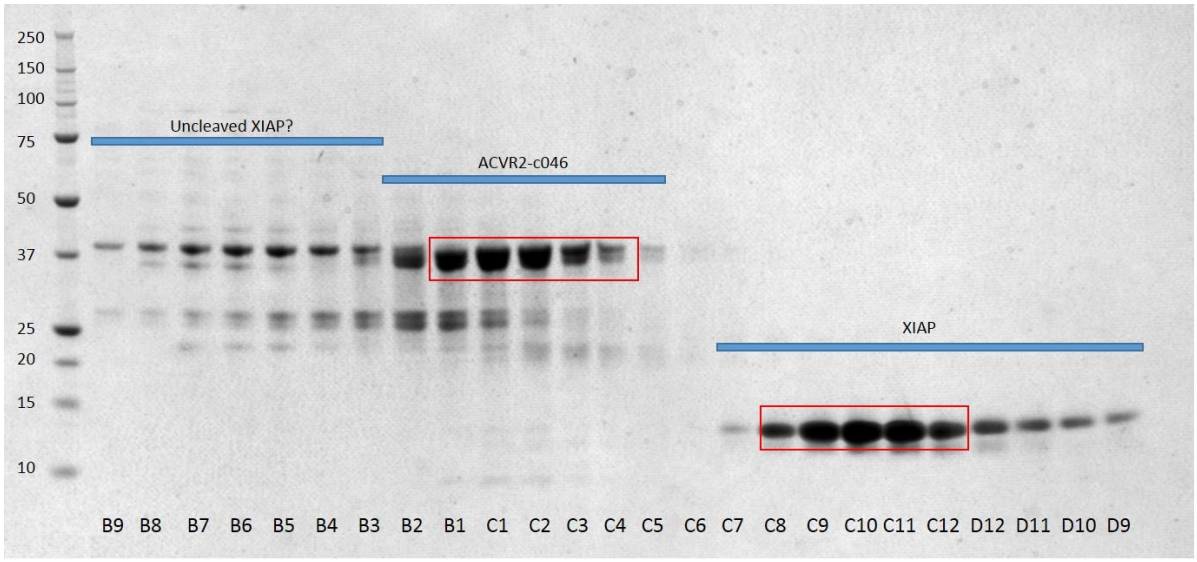
Sample loaded onto 0.5ml pre-equilibrated Ni NTA resin with binding buffer (500mM NaCl, 50mM HEPES, 5% glycerol, 5mM Imidazole, pH7.5) and flow through collected. Resin was then washed with 5ml wash buffer (500mM NaCl, 50mM HEPES, 5% glycerol, 30mM Imidazole, pH7.5) and eluted in 5ml Elution 4 buffer (500mM NaCl, 50mM HEPES, 250mM Imidazole, pH7.5).

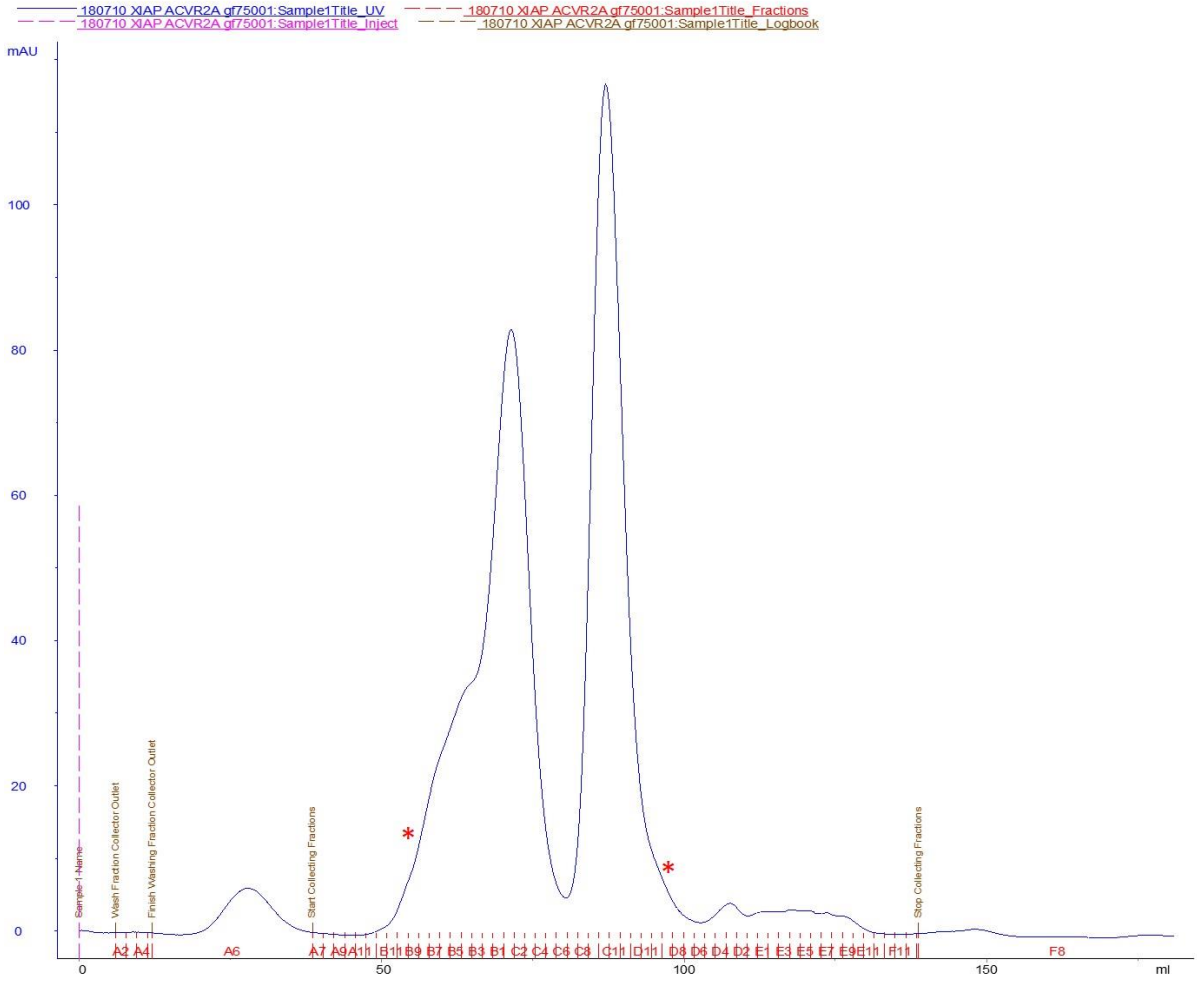
Cleaved XIAP found in flow through and wash fractions.



SDS-PAGE of cleavage and nickle rebind of XIAP-GST. Cleaved XIAP seen in FT and W fractions.

Cleaved XIAP was mixed at a 1:1 ratio with ACVR2 and purified using a GF75 size exclusion column equilibrated in 50mM HEPES, 300mM NaCl, pH7.5. Fractions were collected and run on an SDS-PAGE gel. This revealed no complex formation between proteins.



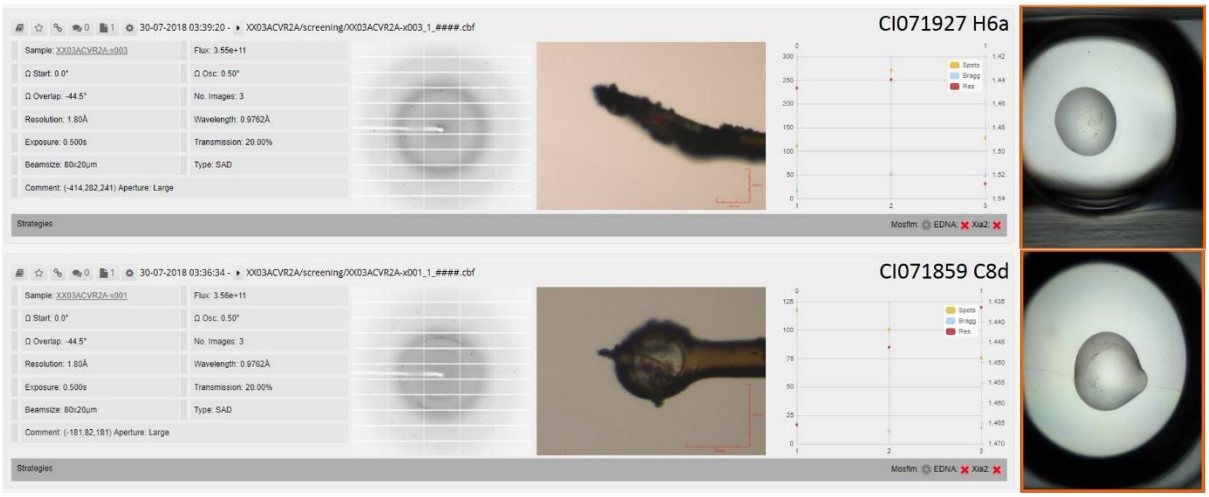


Top: SDS-PAGE of GF75 size exclusion run showing no complex formation between XIAP and ACVR2. Bottom: Corresponding UV trace from the AKTA

Individual proteins were concentrated down and mixed at a 1:1 ratio, LDN-193189 was added at 0.5mM concentration and set up in crystal trays. 150nl drops were used at 1:2, 1:1 and 2:1 ratios. Four coarse screens were used: JCSG7 & LFS6 at 20°C and HCS3 & HIN3 at 4°C.

Drops were monitored regularly and two crystals were mounted, one at 20°C and one at 4°C using 25% Ethylene glycol mixed with mother liquor.

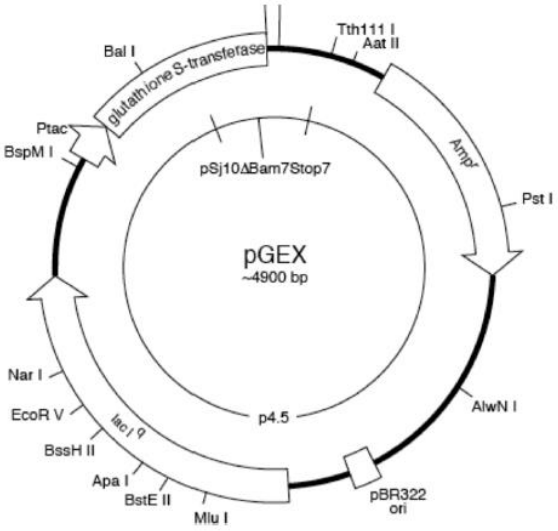
Crystals were sent to the diamond light source and screened on beamline I03 however no diffraction was observed.



Crystal drops, mounted crystals and diamond screening data for ACVR2/XIAP complex.

New XIAP-GST constructs:

Two constructs obtained from collaborator – full length XIAP and XIAP UBA-RING. Constructs were in pGEX-6P-1 vector.



Map of plasmid pGEX-6P-1

Plasmids were transformed into MACH1 and BL21 DE3 cells. (incubate 2ul of plasmid on ice with 50ul competent cells for 30 minutes. Heat shock at 42°C for 45 seconds before incubating on ice for a further 2 minutes. Add 300ul LB and incubate at 37°C while shaking for 2h. Plate 100ul onto LB +Amp and incubate at 37°C overnight)

Expression of full length XIAP was as follows:

Set up overnight from one colony of BL21 DE3 cells in Amp+ media. Grow while shaking at 37°C.

Inoculate 1l of LB with 10ml overnight and grow for 4h.

Induce with 0.4mM IPTG.

Reduce temperature to 18°C and grow overnight.

Harvest at 5000rpm for 15 minutes.

Freeze pellet at -20°C