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 IO3 however no diffraction was observed.

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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 1I 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