Re-Purification of ALK2 for crystallisation with different compounds from M4K.

Aim:

Re-purify the short construct of ALK2 (aka ACVR1A) for crystallisation with different M4K compounds. The protein had already been purified and flash frozen at -80C by standard methods by Ros Adamson and so any aggregates needed to be removed before use in crystallisation trials.

Protein Purification

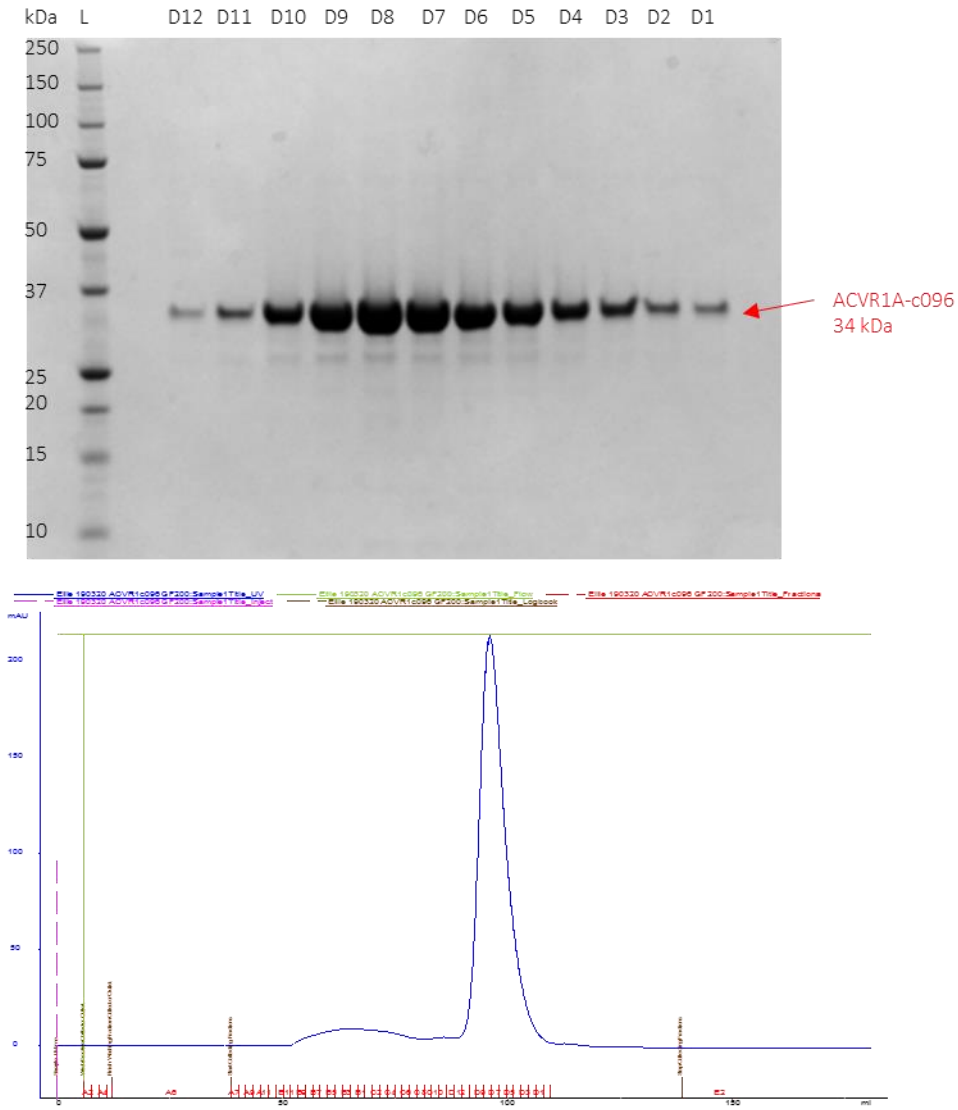
Proteins to be purified:

ACVR1A (no GS loop)
MGHHHHHHSSGVDLGTENLYFQ/*SMQRTVARDITLLECVGKGRYGEVWRGSWQGENVAVKIFSSRDEKSWFR
ETELYNTVMLRHENILGFIASDMTSRHSSTQLWLITHYHEMGSLYDYLQLTTLDTVSCLRIVLSIASGLAHLHIEIFGT
QGKPAIAHRDLKSKNILVKKNGQCCIADLGLAVMHSQSTNQLDVGNNPRVGTKRYMAPEVLDETIQVDCFDSYKR
VDIWAFGLVLWEVARRMVSNGIVEDYKPPFYDVVPNDPSFEDMRKVVCVDQQRPNIPNRWFSDPTLTSLAKLM
KECWYQNPSARLTALRIKKTLTKID

/* denotes Tev cleavage site

Clean up purification:

- Thaw protein sample from -80C storage
- Dilute from 0.5ml to 3ml with gel filtration buffer (50mM HEPES, 300mM NaCl, 0.5mM TCEP pH7.5)
- Run on a pre-equilibrated GF200 column at 1ml/min using standard gel filtration buffer. Run samples on an SDS PAGE gel
- Mix 5ul of loading dye with 15ul sample, boil for 3 minutes and load 10ul onto the gel. Run at 160V for 50 minutes.



Top: SDS-PAGE gel of re-purified ALK2 to remove aggregates from freeze/thaw process. Bottom: Gel filtration trace showing the UV absorbance of the fractions from the gel filtration process.

Protein crystallisation:

Coarse screen

- Sample concentrated down to 9.1mg/ml.
- Sample divided into 5 and 1mM compound added to each tube. (Compounds used M4K2163, M4K2143, M4K2192, M4K2207 and M4K2194)
- Sample spun at 13000rpm on a benchtop centrifuge for 10 minutes.
- Drops set up at 150nl volume using a mosquito dispenser at 1:2, 1:1 and 2:1 ratios. Two plates set up per compound for incubation at both 4C and 20C.
- Follow-up plates set up identically with 9.5mg/ml protein, 1mM compound (M4K2163 or M4K2143) with 150nl drops at ratios of 1:2 1:1 and 2:1.

FU screen design:

The follow-up screens designed from the hits identified from the initial coarse screen were made up as shown below based around the original coarse screen well condition.

Ros-composite-FU-C9-z001

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM	0.1M HEPES p 0.1M KPOD 0.4M NaPOM
B	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM	0.1M HEPES p 0.2M KPOD 0.4M NaPOM
C	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM
D	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM	0.1M HEPES p 0.6M KPOD 0.4M NaPOM
E	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM	0.1M HEPES p 0.4M KPOD 0.1M NaPOM
F	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM	0.1M HEPES p 0.4M KPOD 0.2M NaPOM
G	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM	0.1M HEPES p 0.4M KPOD 0.4M NaPOM
H	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM	0.1M HEPES p 0.4M KPOD 0.6M NaPOM

Ros-composite-FU-D9-z001

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX	0.1M MES pH 1M AmSO 5%(v/v) DIOX
B	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX	0.1M MES pH 1.2M AmSO 6%(v/v) DIOX
C	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX	0.1M MES pH 1.4M AmSO 7%(v/v) DIOX
D	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX	0.1M MES pH 1.6M AmSO 8%(v/v) DIOX
E	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX	0.1M MES pH 1.8M AmSO 9%(v/v) DIOX
F	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO	0.1M MES pH 2M AmSO 10%(v/v) DIO
G	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO	0.1M MES pH 2.2M AmSO 12%(v/v) DIO
H	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO	0.1M MES pH 2.3M AmSO 14%(v/v) DIO

FU screens based off the ALK2 refined coarse screen wells C9 and D9

Ros-composite-FU-G9-z001

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1M AmSO 0.05M NAAC 5% BPEGS	0.1M AmSO 0.1M NAAC 5% BPEGS	0.1M AmSO 0.2M NAAC 5% BPEGS	0.1M AmSO 0.3M NAAC 5% BPEGS	0.1M AmSO 0.4M NAAC 5% BPEGS	0.1M AmSO 0.5M NAAC 5% BPEGS	0.1M AmSO 0.6M NAAC 5% BPEGS	0.1M AmSO 0.7M NAAC 5% BPEGS	0.1M AmSO 0.8M NAAC 5% BPEGS	0.1M AmSO 0.9M NAAC 5% BPEGS	0.1M AmSO 1M NAAC 5% BPEGS	0.1M AmSO 1.1M NAAC 5% BPEGS
B	0.2M AmSO 0.05M NAAC 10% BPEGS	0.2M AmSO 0.1M NAAC 10% BPEGS	0.2M AmSO 0.2M NAAC 10% BPEGS	0.2M AmSO 0.3M NAAC 10% BPEGS	0.2M AmSO 0.4M NAAC 10% BPEGS	0.2M AmSO 0.5M NAAC 10% BPEGS	0.2M AmSO 0.6M NAAC 10% BPEGS	0.2M AmSO 0.7M NAAC 10% BPEGS	0.2M AmSO 0.8M NAAC 10% BPEGS	0.2M AmSO 0.9M NAAC 10% BPEGS	0.2M AmSO 1M NAAC 10% BPEGS	0.2M AmSO 1.1M NAAC 10% BPEGS
C	0.3M AmSO 0.05M NAAC 12% BPEGS	0.3M AmSO 0.1M NAAC 12% BPEGS	0.3M AmSO 0.2M NAAC 12% BPEGS	0.3M AmSO 0.3M NAAC 12% BPEGS	0.3M AmSO 0.4M NAAC 12% BPEGS	0.3M AmSO 0.5M NAAC 12% BPEGS	0.3M AmSO 0.6M NAAC 12% BPEGS	0.3M AmSO 0.7M NAAC 12% BPEGS	0.3M AmSO 0.8M NAAC 12% BPEGS	0.3M AmSO 0.9M NAAC 12% BPEGS	0.3M AmSO 1M NAAC 12% BPEGS	0.3M AmSO 1.1M NAAC 12% BPEGS
D	0.4M AmSO 0.05M NAAC 15% BPEGS	0.4M AmSO 0.1M NAAC 15% BPEGS	0.4M AmSO 0.2M NAAC 15% BPEGS	0.4M AmSO 0.3M NAAC 15% BPEGS	0.4M AmSO 0.4M NAAC 15% BPEGS	0.4M AmSO 0.5M NAAC 15% BPEGS	0.4M AmSO 0.6M NAAC 15% BPEGS	0.4M AmSO 0.7M NAAC 15% BPEGS	0.4M AmSO 0.8M NAAC 15% BPEGS	0.4M AmSO 0.9M NAAC 15% BPEGS	0.4M AmSO 1M NAAC 15% BPEGS	0.4M AmSO 1.1M NAAC 15% BPEGS
E	0.5M AmSO 0.05M NAAC 18% BPEGS	0.5M AmSO 0.1M NAAC 18% BPEGS	0.5M AmSO 0.2M NAAC 18% BPEGS	0.5M AmSO 0.3M NAAC 18% BPEGS	0.5M AmSO 0.4M NAAC 18% BPEGS	0.5M AmSO 0.5M NAAC 18% BPEGS	0.5M AmSO 0.6M NAAC 18% BPEGS	0.5M AmSO 0.7M NAAC 18% BPEGS	0.5M AmSO 0.8M NAAC 18% BPEGS	0.5M AmSO 0.9M NAAC 18% BPEGS	0.5M AmSO 1M NAAC 18% BPEGS	0.5M AmSO 1.1M NAAC 18% BPEGS
F	0.6M AmSO 0.05M NAAC 20% BPEGS	0.6M AmSO 0.1M NAAC 20% BPEGS	0.6M AmSO 0.2M NAAC 20% BPEGS	0.6M AmSO 0.3M NAAC 20% BPEGS	0.6M AmSO 0.4M NAAC 20% BPEGS	0.6M AmSO 0.5M NAAC 20% BPEGS	0.6M AmSO 0.6M NAAC 20% BPEGS	0.6M AmSO 0.7M NAAC 20% BPEGS	0.6M AmSO 0.8M NAAC 20% BPEGS	0.6M AmSO 0.9M NAAC 20% BPEGS	0.6M AmSO 1M NAAC 20% BPEGS	0.6M AmSO 1.1M NAAC 20% BPEGS
G	0.7M AmSO 0.05M NAAC 21% BPEGS	0.7M AmSO 0.1M NAAC 21% BPEGS	0.7M AmSO 0.2M NAAC 21% BPEGS	0.7M AmSO 0.3M NAAC 21% BPEGS	0.7M AmSO 0.4M NAAC 21% BPEGS	0.7M AmSO 0.5M NAAC 21% BPEGS	0.7M AmSO 0.6M NAAC 21% BPEGS	0.7M AmSO 0.7M NAAC 21% BPEGS	0.7M AmSO 0.8M NAAC 21% BPEGS	0.7M AmSO 0.9M NAAC 21% BPEGS	0.7M AmSO 1M NAAC 21% BPEGS	0.7M AmSO 1.1M NAAC 21% BPEGS
H	0.8M AmSO 0.05M NAAC 22% BPEGS	0.8M AmSO 0.1M NAAC 22% BPEGS	0.8M AmSO 0.2M NAAC 22% BPEGS	0.8M AmSO 0.3M NAAC 22% BPEGS	0.8M AmSO 0.4M NAAC 22% BPEGS	0.8M AmSO 0.5M NAAC 22% BPEGS	0.8M AmSO 0.6M NAAC 22% BPEGS	0.8M AmSO 0.7M NAAC 22% BPEGS	0.8M AmSO 0.8M NAAC 22% BPEGS	0.8M AmSO 0.9M NAAC 22% BPEGS	0.8M AmSO 1M NAAC 20% BPEGS	0.8M AmSO 1.1M NAAC 20% BPEGS

RoscompFUF4E12-z001

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.8M AmClTd	0.9M AmClTd	1M AmClTd	1.2M AmClTd	1.4M AmClTd	1.6M AmClTd	1.7M AmClTd	1.8M AmClTd	1.9M AmClTd	2M AmClTd	2.1M AmClTd	2.2M AmClTd
B	0.8M AmClTd	0.9M AmClTd	1M AmClTd	1.2M AmClTd	1.4M AmClTd	1.6M AmClTd	1.7M AmClTd	1.8M AmClTd	1.9M AmClTd	2M AmClTd	2.1M AmClTd	2.2M AmClTd
C	0.05M KPOD 0.5M NaPOM	0.05M KPOD 0.6M NaPOM	0.05M KPOD 0.7M NaPOM	0.05M KPOD 0.8M NaPOM	0.05M KPOD 0.9M NaPOM	0.05M KPOD 1M NaPOM	0.05M KPOD 1.1M NaPOM	0.05M KPOD 1.2M NaPOM	0.05M KPOD 1.3M NaPOM	0.05M KPOD 1.4M NaPOM	0.05M KPOD 1.5M NaPOM	0.05M KPOD 1.6M NaPOM
D	0.1M KPOD 0.5M NaPOM	0.1M KPOD 0.6M NaPOM	0.1M KPOD 0.7M NaPOM	0.1M KPOD 0.8M NaPOM	0.1M KPOD 0.9M NaPOM	0.1M KPOD 1M NaPOM	0.1M KPOD 1.1M NaPOM	0.1M KPOD 1.2M NaPOM	0.1M KPOD 1.3M NaPOM	0.1M KPOD 1.4M NaPOM	0.1M KPOD 1.5M NaPOM	0.1M KPOD 1.6M NaPOM
E	0.15M KPOD 0.5M NaPOM	0.15M KPOD 0.6M NaPOM	0.15M KPOD 0.7M NaPOM	0.15M KPOD 0.8M NaPOM	0.15M KPOD 0.9M NaPOM	0.15M KPOD 1M NaPOM	0.15M KPOD 1.1M NaPOM	0.15M KPOD 1.2M NaPOM	0.15M KPOD 1.3M NaPOM	0.15M KPOD 1.4M NaPOM	0.15M KPOD 1.5M NaPOM	0.15M KPOD 1.6M NaPOM
F	0.2M KPOD 0.5M NaPOM	0.2M KPOD 0.6M NaPOM	0.2M KPOD 0.7M NaPOM	0.2M KPOD 0.8M NaPOM	0.2M KPOD 0.9M NaPOM	0.2M KPOD 1M NaPOM	0.2M KPOD 1.1M NaPOM	0.2M KPOD 1.2M NaPOM	0.2M KPOD 1.3M NaPOM	0.2M KPOD 1.4M NaPOM	0.2M KPOD 1.5M NaPOM	0.2M KPOD 1.6M NaPOM
G	0.25M KPOD 0.5M NaPOM	0.25M KPOD 0.6M NaPOM	0.25M KPOD 0.7M NaPOM	0.25M KPOD 0.8M NaPOM	0.25M KPOD 0.9M NaPOM	0.25M KPOD 1M NaPOM	0.25M KPOD 1.1M NaPOM	0.25M KPOD 1.2M NaPOM	0.25M KPOD 1.3M NaPOM	0.25M KPOD 1.4M NaPOM	0.25M KPOD 1.5M NaPOM	0.25M KPOD 1.6M NaPOM
H	0.3M KPOD 0.5M NaPOM	0.3M KPOD 0.6M NaPOM	0.3M KPOD 0.7M NaPOM	0.3M KPOD 0.8M NaPOM	0.3M KPOD 0.9M NaPOM	0.3M KPOD 1M NaPOM	0.3M KPOD 1.1M NaPOM	0.3M KPOD 1.2M NaPOM	0.3M KPOD 1.3M NaPOM	0.3M KPOD 1.4M NaPOM	0.3M KPOD 1.5M NaPOM	0.3M KPOD 1.6M NaPOM

RoscompFUA2C10-z001

	1	2	3	4	5	6	7	8	9	10	11	12
A	15% PEG3.35 0.05M AmClT	15% PEG3.35 0.1M AmClTd	15% PEG3.35 0.15M AmClT	15% PEG3.35 0.2M AmClTd	15% PEG3.35 0.25M AmClT	15% PEG3.35 0.3M AmClTd	15% PEG3.35 0.35M AmClT	15% PEG3.35 0.4M AmClTd	15% PEG3.35 0.45M AmClT	15% PEG3.35 0.5M AmClTd	15% PEG3.35 0.55M AmClT	15% PEG3.35 0.6M AmClTd
B	18% PEG3.35 0.05M AmClT	18% PEG3.35 0.1M AmClTd	18% PEG3.35 0.15M AmClT	18% PEG3.35 0.2M AmClTd	18% PEG3.35 0.25M AmClT	18% PEG3.35 0.3M AmClTd	18% PEG3.35 0.35M AmClT	18% PEG3.35 0.4M AmClTd	18% PEG3.35 0.45M AmClT	18% PEG3.35 0.5M AmClTd	18% PEG3.35 0.55M AmClT	18% PEG3.35 0.6M AmClTd
C	22% PEG3.35 0.05M AmClT	22% PEG3.35 0.1M AmClTd	22% PEG3.35 0.15M AmClT	22% PEG3.35 0.2M AmClTd	22% PEG3.35 0.25M AmClT	22% PEG3.35 0.3M AmClTd	22% PEG3.35 0.35M AmClT	22% PEG3.35 0.4M AmClTd	22% PEG3.35 0.45M AmClT	22% PEG3.35 0.5M AmClTd	22% PEG3.35 0.55M AmClT	22% PEG3.35 0.6M AmClTd
D	25% PEG3.35 0.05M AmClT	25% PEG3.35 0.1M AmClTd	25% PEG3.35 0.15M AmClT	25% PEG3.35 0.2M AmClTd	25% PEG3.35 0.25M AmClT	25% PEG3.35 0.3M AmClTd	25% PEG3.35 0.35M AmClT	25% PEG3.35 0.4M AmClTd	25% PEG3.35 0.45M AmClT	25% PEG3.35 0.5M AmClTd	25% PEG3.35 0.55M AmClT	25% PEG3.35 0.6M AmClTd
E	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p
F	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p
G	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p
H	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p	1.3M NaClT 0.1M HEPES p	1.2M NaClT 0.1M HEPES p	1M NaClT 0.1M HEPES p	0.8M NaClT 0.1M HEPES p	0.6M NaClT 0.1M HEPES p	0.4M NaClT 0.1M HEPES p

FU screens based off the ALK2 refined coarse screen wells G9, F4/E12 and A2/C10

Crystal mounting:

- Crystals were mounted at the appropriate temperature using mitogen loops of an appropriate size.
- Ethylene glycol was added to mother liquor to make a 25% solution which was added to the drops.
- Crystals were flash frozen in liquid nitrogen before being transferred to a puck for long term storage.