AVIDD ASAP: MERS MPRO c900 (MVMPROA p001) crystallization and further concentration

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Experiment Started:
Projects: **Cloning**;**ASAP**
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According to Claire and Marco at DLS Original MERS MPRO foormulation something like

50 mM Tris, pH 8.0, 300 mM NaCl and stored at ~17 mg/mL. Crystallisation done at ~10 mg/mL and the salt dropped to 150 mM.

MVMPROA p001 in 10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP
17 mg/mL
Centrifuged 16000g 3 minutes
Plates setup at 20C
CI085080 HCS
CI085081 BCS
CI085082 MORPHEUS
CI085083 HIN
CI085084 JCSG
CI085085 LFS

Concentrating

100uL of protein stock added to 0.5 mL of

10 mM HEPES pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP
or
10 mM MES pH 6.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP
or
10 mM Bicine pH 8.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP

Concentrate 20 minutes 10,000g 10000 MWCO
A280 at pH 7.5 = 175
A280 at pH 6.5 = 133
A280 at pH 8.5 = 130

seems could probably go up to 1 mM or even 2 mM regardless of pH fairly simply.

Crystallization Summary

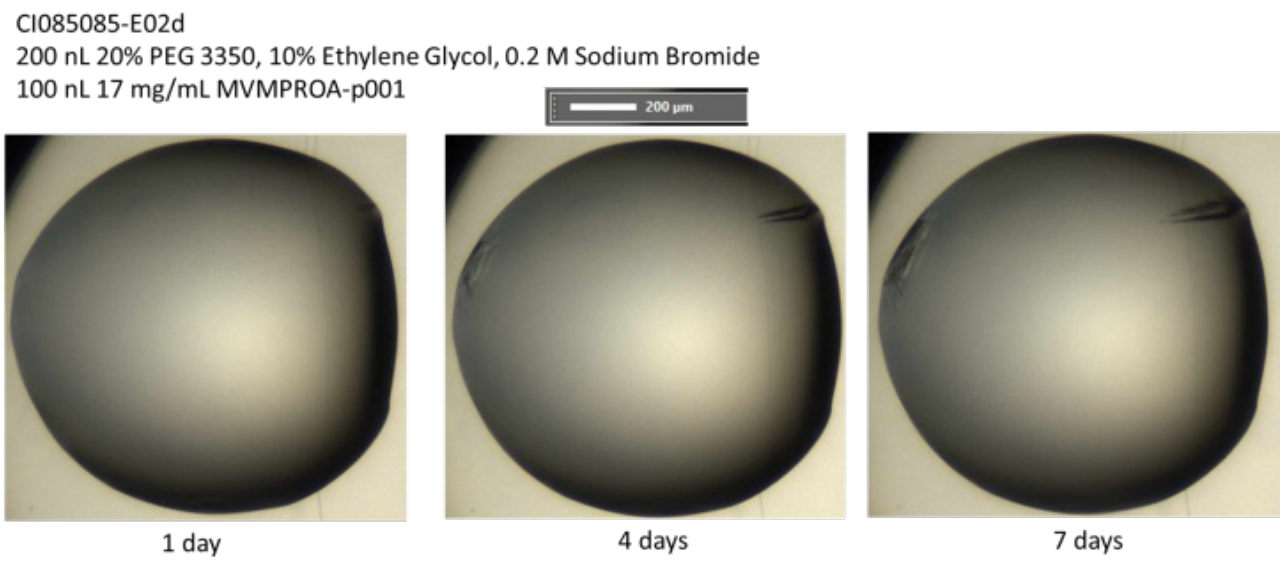
after 1 day general trend appears to be

pH 4.5-6 ppt
pH 7.5 cleat occasional needles
pH 8.5-9 needles some 3d

await 7 day inspection and see if can define specific trend and design follow up

Only real hit is in LFS (see below)
CI085085-E02d
200 nL 20% PEG 3350, 10% Ethylene Glycol, 0.2 M Sodium Bromide
100 nL 17 mg/mL MVMPROA-p001

CI085085-E02d



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crystal on the right (MVMPROA-x001) diffracted to 2.3ish A

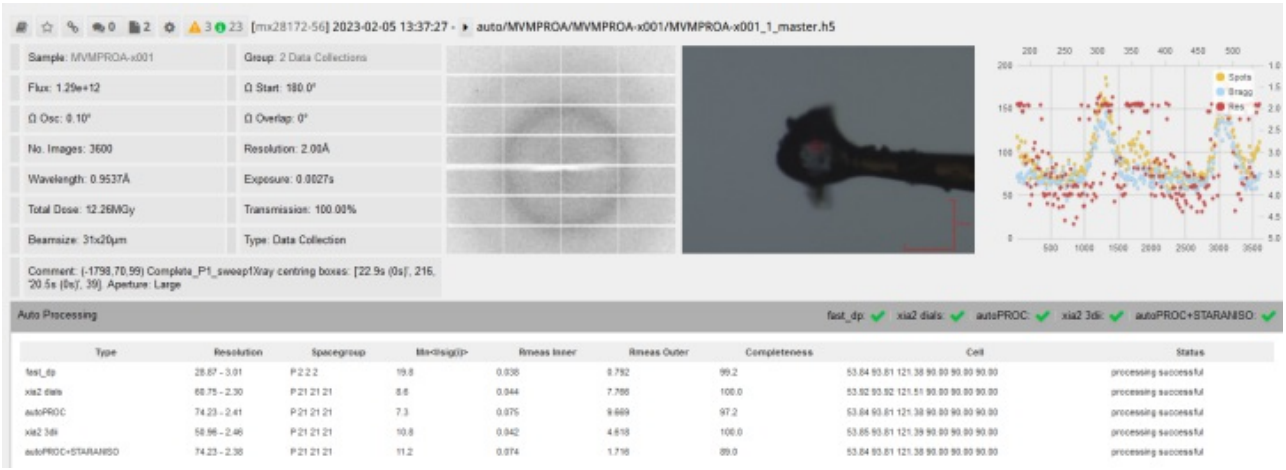
Space Group	A	B	C	α	β	γ
P 21 21 21	53.92	93.92	121.51	90.00	90.00	90.00

After phasing dimer in ASU but Rwork/Rfree remains pretty high after refining in Refmac (0.23/0.37) flags on data suggest anisotropic data

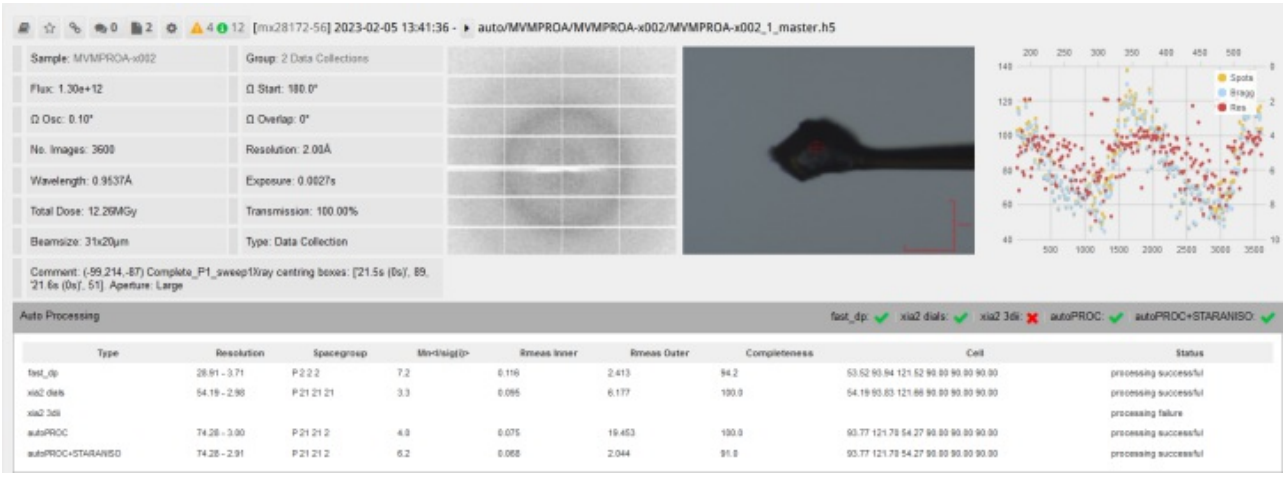
2nd crystal on the left (MVMPROA-x002) 3.0ish A

Space Group	A	B	C	α	β	γ
P 21 21 2	93.77	121.70	54.27	90.00	90.00	90.00

MVMPROA-x001



MVMPROA-x002



CI085085-E02 Follow up SCreen

CI085085-E02
20% PEG 3350, 10% Ethylene Glycol, 0.2 M Sodium Bromide

Follow up screen made based on this
MF-230207-MVMPROA-FU1-z001

1-12 = 5-35% PEG3350
A-H = 0.1M HEPES pH 6.8-8.2
All wells have 0.1 M NaBromide and 10% EG

CI085138, CI085139 300 nL drops

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Buffer Exchanged for BaseBuffer at different PHs, glycerol or salt concentrations

MVMPROA-p001	CI085571	LFS6	Base Buffer 10 % Glycerol
MVMPROA-p001	CI085579	LFS6	Base Buffer 20 % Glycerol
MVMPROA-p001	CI085520	LFS6	Base Buffer 30 % Glycerol
MVMPROA-p001	CI085521	LFS6	Base Buffer 50 mM NaCl
MVMPROA-p001	CI085522	LFS6	Base Buffer 100 mM NaCl
MVMPROA-p001	CI085523	LFS6	Base Buffer 250 mM NaCl
MVMPROA-p001	CI085524	LFS6	Base Buffer pH 8.5
MVMPROA-p001	CI085525	LFS6	Base Buffer pH 6.5