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4) streak out strains : DH10B(DE3)
DH10B pLysS
BL21 A1
BL21 (DE3) pLysS
DH10B? / TOP10?

5) prepare TUC01 template

6) order primers

20180619 | Cloning successful. - pAAG169 is pET9a GG-GFP vector
pAAG170 is pET9a-6xH-TEV-LacI1-331

Made frozen stock of sAAG170a (DH10B).

Transformed pAAG170 → 1) BL21 A1
2) TOP10
3) DH10B pLysS
4) DH10B (DE3)
5) BL21 (DE3) pLysS

Began O/N cultures of 20 ml each of sAAG1146, sAAG1666 for expression.
Began 2 ml O/N cultures of all above empty strains as well.

20180620 | Made frozen stocks of empty e. coli strains.
Transformation plates of pAAG170 in all strains ⇒
lots of colonies.
↳ ready for expression - stored plates at 4°C.

S9G10 MBPα Y197A, ARm3 protein expression cultures : 1L each.
- Subcultured 20 ml → 1L LB / Kan / Cm O/N culture
+ 5 ml 40% glucose for 1146
- Grew 2.5 hrs at 37°C / 225 rpm
- Induced with 500 μM IPTG and reduced temperature to 16°C.
- Grew cultures O/N.

Plan for crystallography : - dialyze into pH 8.3-8.5 buffer to set up Mosquito trays / 15-well S9G10 trays.

- optimize PEG3350 / Na Acetate conditions from previous Mosquito hits (after dialysis into pH 6.0 MES.)
⇒ JCSG1 / C8

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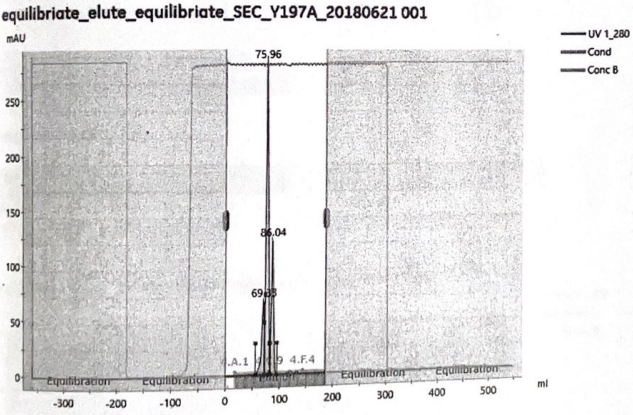
20180621 Spun down SAAG1146, SAAG1666 expression cultures at 6000 x g / 4°C / 20 min. Froze pellets.

Thawed 1146 pellet on ice (BL21(DE3) pLysS, pET28-MBP-Y197A). Added 225μL DNaseI, 225 μL $MgCl_2$, 45 μL $MnCl_2$, 4.5 μL $CaCl_2$, and protease inhibitor tablet to 40 ml 50 mM Tris, 150 mM NaCl, pH 8.0 buffer. Resuspended pellet in lysis buffer by vortexing for 15-20 min.

Lysed cells by 2x microfluidizer. Spun down insoluble fraction at 27000 x g, 30 min., 4°C. Decanted supe and stored on 4°C for 1 hour. Spun down at 3700 x g / 4°C / 10 min. and decanted again. Passed through freshly regenerated amylose column. Eluted in 14 ml Tris buffer + 30 mM maltose. Concentrated down to 1 mL in 10 kDa MWCO spin column, then applied to S200 for SEC overnight.

20180622 MBP Y197A SEC: 3 peaks



Peak Table - UV 1_280

Peak	Retention ml	Area mAU*ml	Area %	Ext coeff. mg ml⁻¹ cm⁻¹	Fraction(s)	Volume mL	Conductivity mS/cm
Peak A	69.330	322.5	15.5		4.B.8 - 4.C.3	16.023	15.41
Peak B	75.960	1238	59.51		4.C.5 - 4.C.8	7.999	15.41
Peak C	86.037	519.8	24.99		4.C.10 - 4.D.3	11.998	15.42

Thawed ARm3 pellet on ice and resolubilized in lysis buffer. Lysed by microfluidizer and spun down the insoluble fraction.

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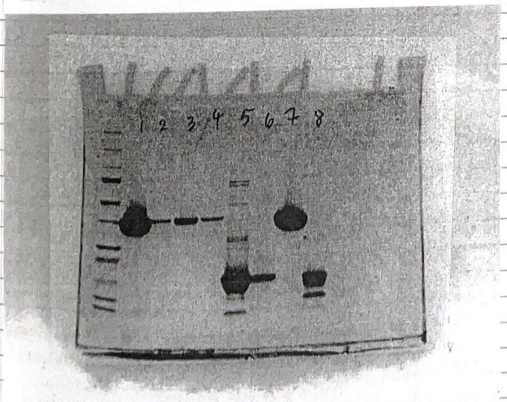
20180623 | ran gel on purified proteins. Destaining O/N.
Prepared dialysis buffer: 25 mM Tris, 150mM NaCl, pH 8.0.
Began O/N cultures: (1) BL21 AI / pAAG170
(2) BL21 (DE3) pLysS / pAAG170
(3) DH10B (DE3) pLysS / pAAG170
} 21 ml ea.

20180624 | Made frozen stocks: (1) sAAG170b : BL21 AI
(2) sAAG170c : BL(DE3) pLysS
(3) sAAG170d : DH10B (DE3) pLysS

Began protein expression cultures:
- subcultured O/N 20 ml → 1L LB + cm/kan for (2,3)
  + kan for (1)
  + 5 ml 40% glucose
- grew at 37°C / 225 rpm for 2-2.5 hours until OD600 ~ 0.4
- induced with 10 ml 20% arabinose (final conc. 0.2%) for (1) and with 1mM IPTG for (2,3)
- reduced temperature to 16°C at induction and grew O/N.

OD600 table —

	1 hour	1.5	2	2.5
(1)	0.13	0.29	0.49	
(2)	0.12	0.24	0.42	
(3)	0.08	0.13	0.20	0.45



SDS-PAGE

1 load MBP Y197A SEC
2 SEC Y197A peak 1
3 SEC Y197A peak 2
4 SEC Y197A peak 3

5 load ARm3 HTQ
6 SEC ARm3 peak

7 old sample Y197A
8 old sample ARm3.

Combined fractions and dialyzed at 4°C, spinning, O/N.

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20180625 | Spun down O/N cultures, 6000 x g / 4°C / 20 min. Froze pellets at -80°C. Took proteins ARm3, S9G10 MBP∝ Y197A out of dialysis and concentrated down to ~200 µL volumes.

Stocks: ARm3 : ~2.2 mM / 38.9 mg/mL

Y197A : 970 µM / 39.5 mg/mL

Prepared protein solution for Mosquito trays (pH 8.0) : 200 µL total

15.45 µL	ARm3	
35.0 µL	Y197A	
34.0 µL	FPP	(from 10 mM stock)
115.55 µL	buffer	(Tris / NaCl pH 8.0)

Buffer-exchanged into 25 mM MES, 200 mM NaCl, pH 6.0 for JCSG1 / C8 optimization trays. Conditions optimized around: 0.2 M Na Acet 20% w/v PEG3350

↳ set up one 15-well tray w/ 3 concentrations of protein / FPP

Also set up 6 x 15-well trays with 3 concentrations in each well of pH 8.0 ARm3 / Y197A in HIGH PEG S9G10 conditions — 3x no maltose, and 3x 170 µM maltose.

Protein final concentrations : 170 µM, 85 µM, 70 µM.

FPP at 10x protein concentration in each solution.

Stored all trays in crystallography room.

20180627 | OCTET experiments: large concentration range ARm3 / avi-Y197A BT + 200 µM FPP.

Fridge stocks : - non-BT avi-Y197A @ 0.9 mg/ml ~21 µM (large epi tube)

- Y197A-BT @ 1.9 mg/ml ~40 µM (2ml tube)

↳ diluted 12 µL → 2388 µL buffer for 200 nM experimental conc. (200 µL / well)

↳ buffer: 50 µM Tris, 150 mM NaCl, pH 8.0

- ARm3, 2.2 mM fresh conc. stock solution.

↳ diluted 5.5 µL (2.2 mM) → 49.5 µL buffer for 220 µM ARm3 stock

↳ diluted 1.1 µL (220 µM) → 9.9 µL buffer for 22 µM ARm3 stock.

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Rm experiment: Need 55 µL 220 µM ARm3, 11 mL 22 µM ARm3, 2.4 mL 200 nM avi-Y197A-BT, 36 µL 10 mM FPP

	sample conc.	sample ID	sample volume	buffer volume
A1	0 µM	22 µM ARm3	0 µL	196.0 µL
B1	0.1 µM	↓	0.9 µL	195.1 µL
C1	0.2 µM		1.8 µL	194.2 µL
D1	0.3 µM		2.7 µL	193.3 µL
E1	0.5 µM		4.5 µL	191.5 µL
F1	1.0 µM	220 µM ARm3	0.9 µL	195.1 µL
G1	1.5 µM	↓	1.4 µL	194.6 µL
H1	3.0 µM		2.7 µL	193.3 µL
A2	6.0 µM		5.5 µL	190.5 µL
B2	8.0 µM		7.3 µL	188.7 µL
C2	12.0 µM		10.9 µL	185.1 µL
D2	24.0 µM		21.8 µL	174.2 µL

★ +4 µL FPP/well from 10 mM stock

PROTOCOL:

1. RINSE / 60 s
2. LOAD / 300 s
3. RINSE / 60 s
4. BASELINE / 60 s
5. ASSOCIATION / 120 s
6. DISSOCIATION / 240 s.

Subtracted 0 µM AR well from each sample.
Local / partial fitting
Req. fit for steady-state analysis.

Ran the experiment 4x.

1:1 MODEL ~ the fit wasn't so great for any of the trials.

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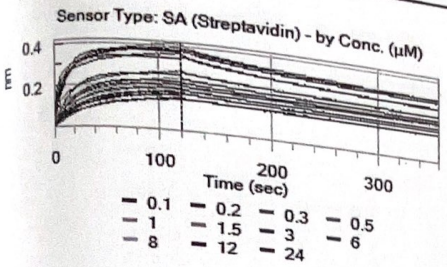

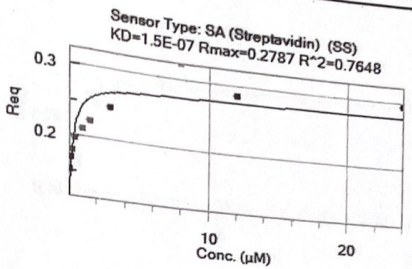

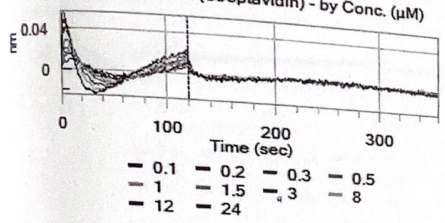

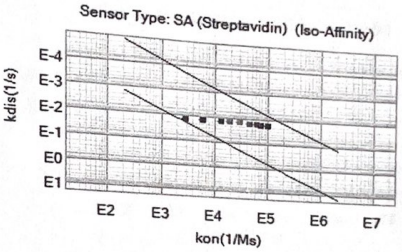

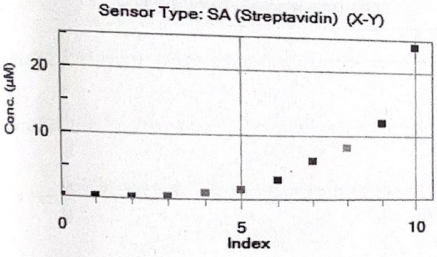

#1 - ALL DATA.

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