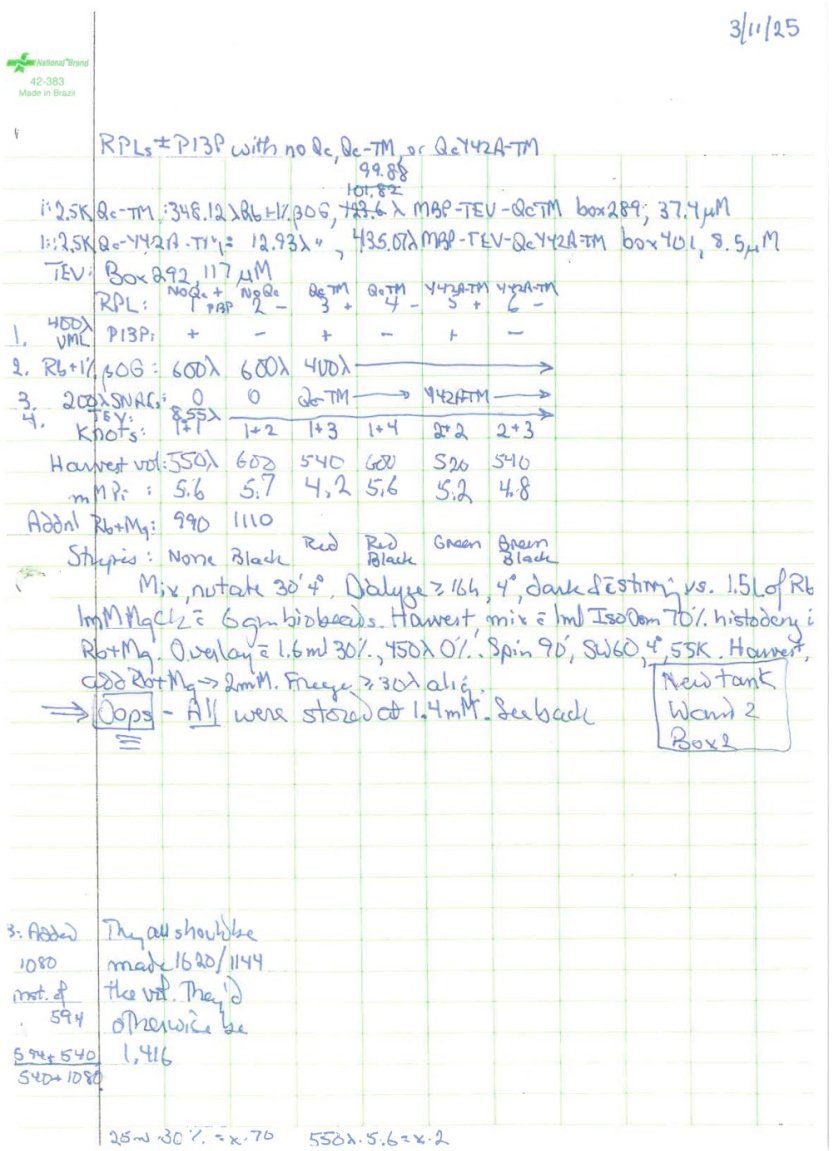
Week of March 10-14, 2025

I worked on Lopes et al (I'm Dr. Et) for resubmission

Qc-tm was shown earlier by Amy to bind Sec17 better than the naturally anchored SNAREs bind Sec17, and I found that Qc-tm works better than R or Qa to stimulate Sec18 ATPase in the presence of Sec17 + with PI3P in VLM.
This week: the PX:PI3P interaction of Qc is only needed for Qc membrane association for stimulating ATPase, as it can be mutated to Y42AQc-tm or even deleted entirely in QcΔN-tm and the Sec17-dep stimulation of Sec18 remains.

Remaining questions:

Is the Qc-SNARE domain the only part of Qc-tm needed to work with Sec17 to stimulate Sec18 ATPase?
Is anchored Qc SNARE domain better at this than anchored Qa SNARE domain?
Amy: compare, at 0.1 μM Sec17, the release of Sec17 by Sec18's ATP hydrolysis from:
a. "WT" 4-SNARE trans complex (as per Lopes et al)
b. *trans*-SNARE complex lacking Qc
An Amy floAtation experiment… Might Qc oligomerize and thus bind Sec17 better than e.g. sQa, etc?
Compare co-floatation of Qc and Sec17 to e.g. Sec17+ sQa and to 4sSNAREs or no sSNAREs



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RPLs ± PI3P with no Qc, Qc-TM, or QcY42A-TM

99.88
101.82

1: 2.5K Qc-TM : 348.12 λ Rb+1% βOG, 427.6 λ MBP-TEV-QcTM box 289; 37.4 μM

1: 2.5K Qc-Y42A-TM = 12.93 λ " , 435.07 λ MBP-TEV-QcY42A-TM box 401, 8.5 μM

TEV: Box 292 117 μM

RPL:	1 NoQc + MBP	2 − NoQc	3 + Qc TM	4 − Qc TM	5 + Y42A-TM	6 − Y42A-TM
1. 450λ VML PI3P:	+	−	+	−	+	−
2. Rb+1% βOG:	600λ	600λ	400λ →			
3. 200λ SNAPs:	0	0	Qc-TM →		Y42A-TM →	
4. TEV: 855λ Knots:	1+1	1+2	1+3	1+4	2+2	2+3
Harvest vol:	550λ	600	540	600	520	540
mM Pi :	5.6	5.7	4.2	5.6	5.2	4.8
Addnl Rb+Mg:	990	1110				
Stripes :	None	Black	Red	Red Black	Green	Green Black

Mix, nutate 30' 4°, Dialyze ≥ 16h, 4°, dark destim vs. 1.5L of Rb 1mM MgCl2 ± 6gm biobeads. Harvest, mix ± 1ml Isodom 70% histodenz in Rb+Mg. Overlay ± 1.6ml 30%, 450λ 0%. Spin 90', SW60, 4°, 55K. Harvest, add Rb+Mg → 2mM. Freeze ≥ 30λ aliq.

⇒ Oops - All were stored at 1.4mM. See back

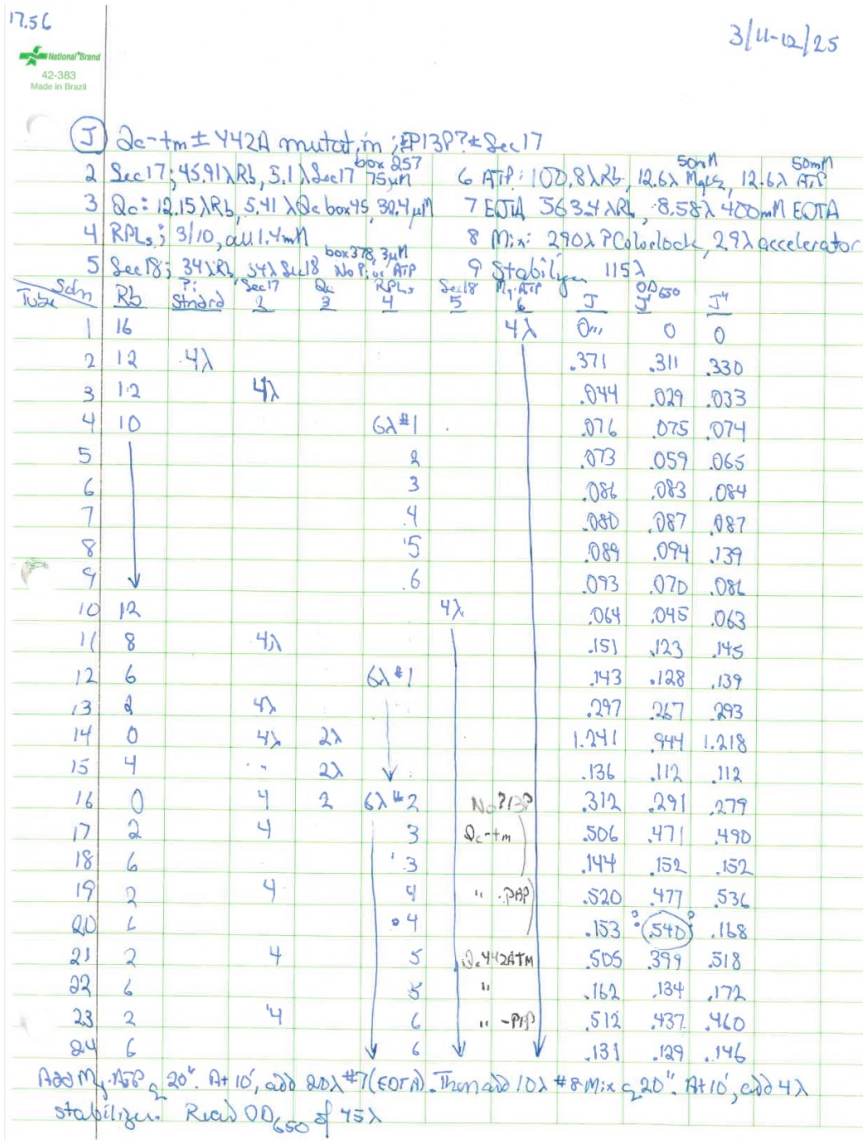
New tank
Wand 2
Box 2

3: Added 1080 mst. of 594 — 594+540 / 540+1080

They all should've made 1620/1144 the vol. They'd otherwise be 1,416

25 ml 30% = x .70 550λ · 5.6 = x · 2

VML Liposomes were made with no protein, Qc-tm, or QcY42A-tm, each +/-PI3P



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J) Qc-tm ± 442A mutant in EP13P? ± Sec17

2 Sec17: 45.91λ Rb, 5.1λ Sec17 box 257 75μM

3 Qc: 12.15λ Rb, 5.41λ Qc box 45, 30.4μM

4 RPLs: 3/10, all 1.4mM

5 Sec18: 34λ Rb, 34λ Sec18 box 378, 3μM No Pi or ATP

6 ATP: 100.8λ Rb, 12.6λ 50mM $MgCl_2$, 12.6λ 50mM ATP

7 EDTA 36.34λ Rb, 8.58λ 400mM EDTA

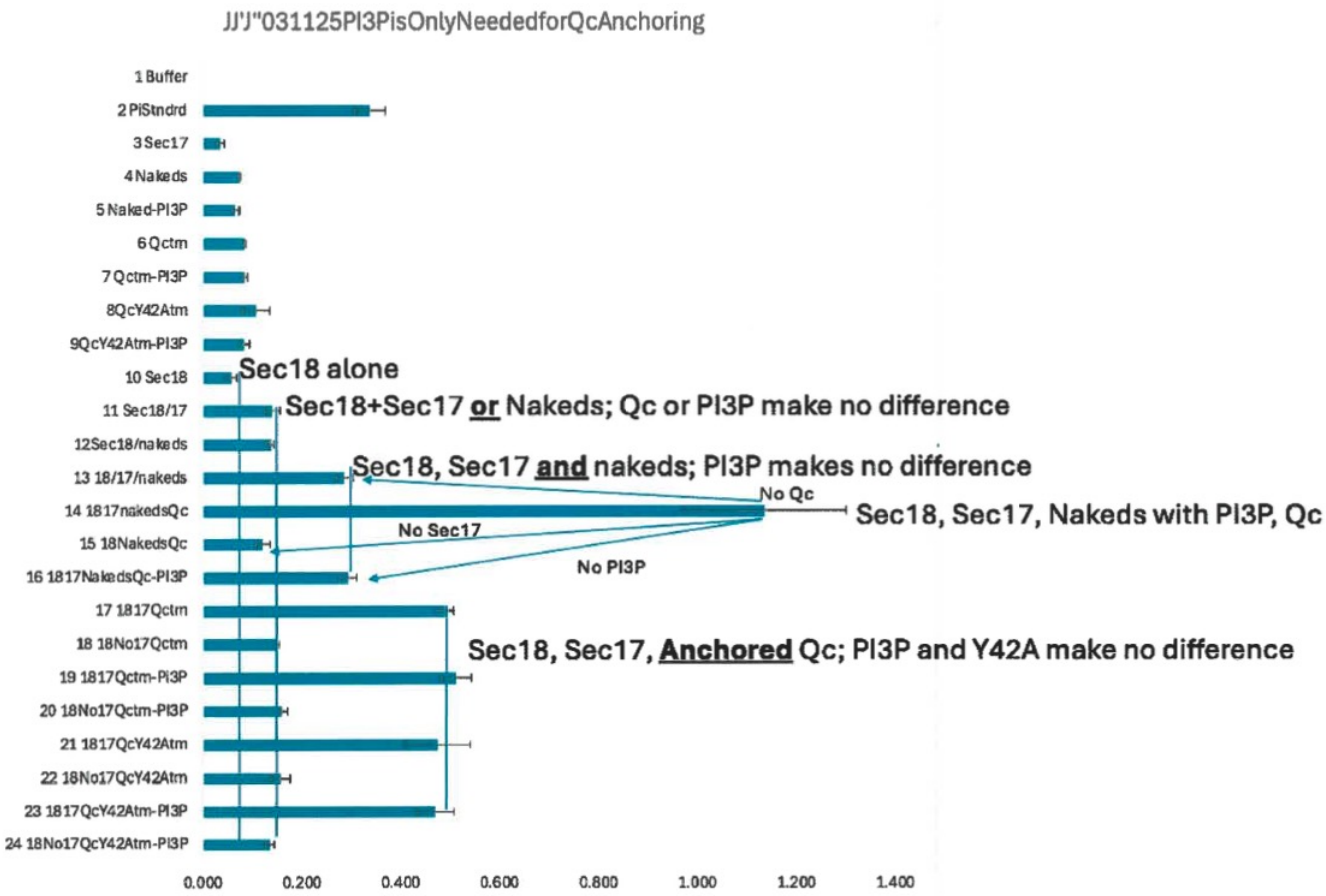
8 Mix: 290λ PCoolLock, 29λ accelerator

9 Stabilizer 115λ

Tube	Sdm Rb	Strand Pi	Sec17 2	Qc 2	RPLs 4	Sec18 5	Mix, ATP 6	J	OD_{650} J'	J''
1	16						4λ	0	0	0
2	12	4λ						.371	.311	.330
3	12		4λ					.044	.029	.033
4	10				6λ #1			.076	.075	.074
5					2			.073	.059	.065
6					3			.086	.083	.084
7					4			.080	.087	.087
8					5			.089	.094	.139
9					6			.093	.070	.086
10	12					4λ		.064	.045	.063
11	8		4λ					.151	.123	.145
12	6				6λ #1			.143	.128	.139
13	8		4λ					.297	.267	.283
14	0		4λ	2λ				1.241	.944	1.218
15	4			2λ				.136	.112	.112
16	0		4	2	6λ #2	No ?/ATP		.312	.291	.279
17	2		4		3	Qc-tm		.506	.471	.490
18	6				3			.144	.152	.152
19	2		4		4	" -PAP		.520	.477	.536
20	4				4			.153	(.540)	.168
21	2		4		5	3.4λ 2ATM		.505	.399	.518
22	6				5	"		.162	.134	.172
23	2		4		6	" -Pi?		.512	.437	.460
24	6				6			.131	.129	.146

Add Mix. Rxn @ 20°. At 10', add 20λ #7 (EDTA). Then add 10λ #8 Mix @ 20°. At 10', add 4λ stabilizer. Read OD_{650} of 45λ

Each was assayed for Sec18 ATPase, with or without Sec17, and with added Qc where it wasn't anchored.


JJ'J"031125PI3PisOnlyNeededforQcAnchoring
1 Buffer
2 PiStndrd
3 Sec17
4 Nakeds
5 Naked-PI3P
6 Qctm
7 Qctm-PI3P
8QcY42Atm
9QcY42Atm-PI3P
10 Sec18
11 Sec18/17
12Sec18/nakeds
13 18/17/nakeds
14 1817nakedsQc
15 18NakedsQc
16 1817NakedsQc-PI3P
17 1817Qctm
18 18No17Qctm
19 1817Qctm-PI3P
20 18No17Qctm-PI3P
21 1817QcY42Atm
22 18No17QcY42Atm
23 1817QcY42Atm-PI3P
24 18No17QcY42Atm-PI3P
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
Sec18 alone
Sec18+Sec17 or Nakeds; Qc or PI3P make no difference
Sec18, Sec17 and nakeds; PI3P makes no difference
No Qc
Sec18, Sec17, Nakeds with PI3P, Qc
No Sec17
No PI3P
Sec18, Sec17, Anchored Qc; PI3P and Y42A make no difference

JJ'J"

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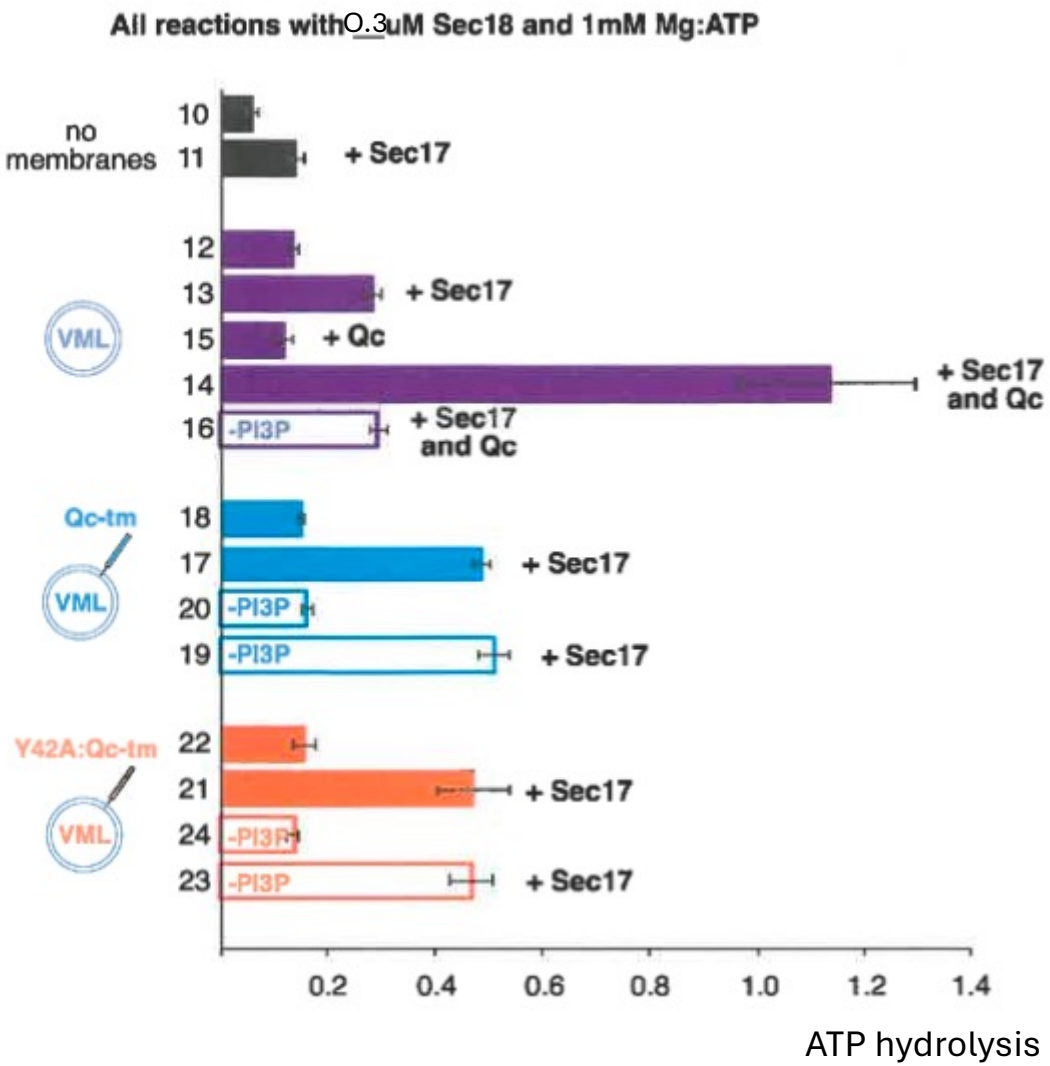
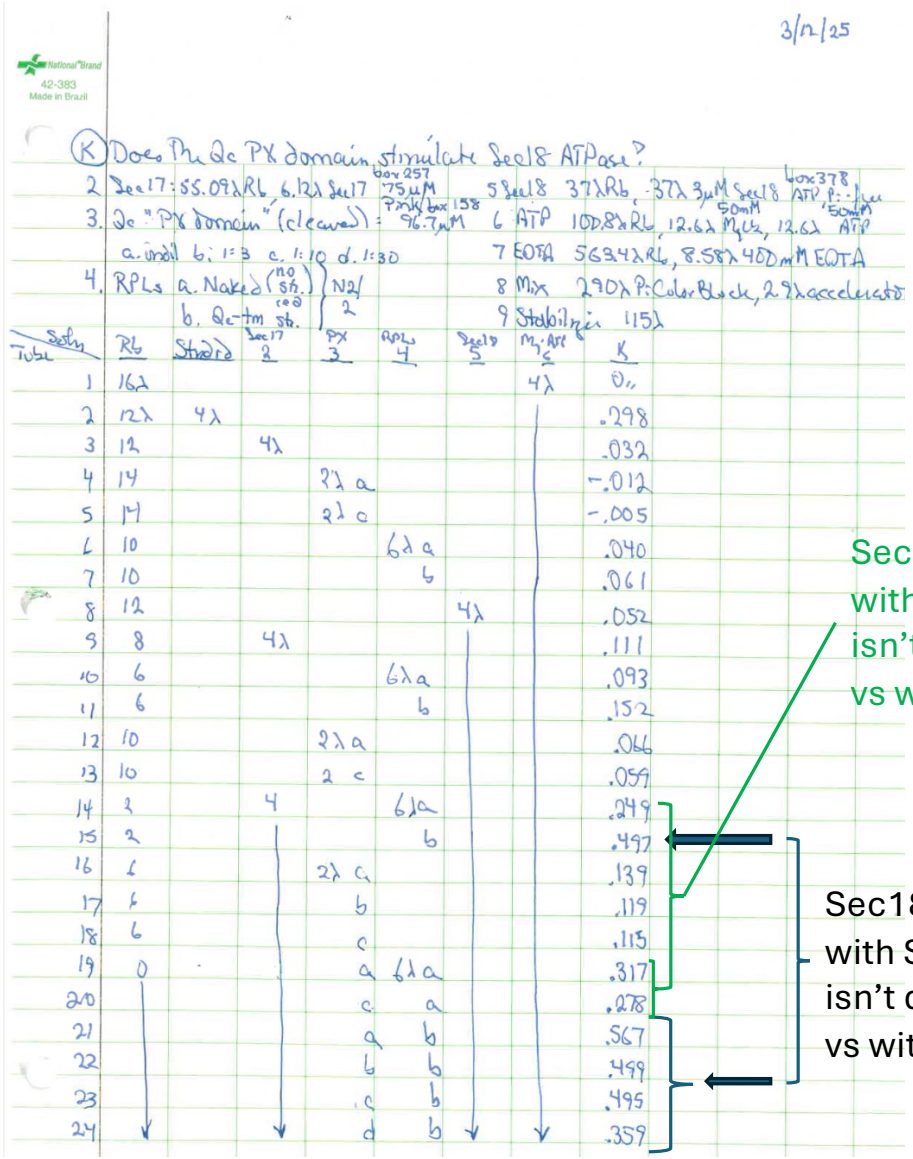


The AmyGram of this data!



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(K) Does Pn Qc PX domain stimulate Sec18 ATPase?

2 Sec17: 55.09λ Rb, 6.12λ Sec17 (box 257) 75μM
3. Qc "PX domain" (cleaved): (Pnk/box 158) 96.7μM
a. undil b. 1:3 c. 1:10 d. 1:30
4. RPLs a. Naked (no Sh.) N2/ b. Qc-tm Sh. (cod) 2

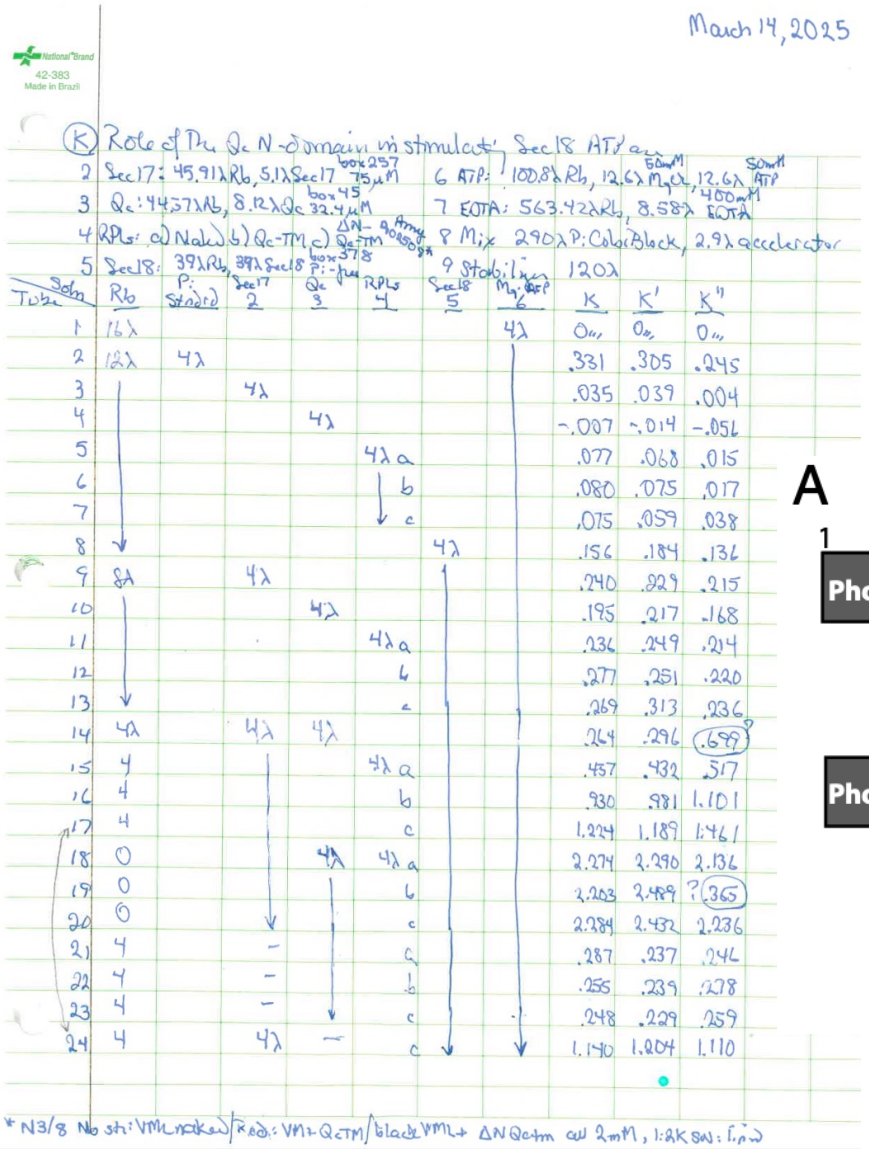
5 Sec18 37λ Rb, 37λ 3μM Sec18 (box 378) ATP P:: 50mM
6 ATP 100.8λ Rb, 12.6λ MgCl2, 50mM 12.6λ ATP
7 EGTA 563.4λ Rb, 8.58λ 400mM EGTA
8 Mix 270λ P: Color Block, 2.7λ accelerator
9 Stabilizer 115λ

Tube	Rb	Shake	Sec17 2	PX 3	RPLs 4	Sec18 5	MgATP 6	K
1	16λ						4λ	0
2	12λ	4λ						.298
3	12		4λ					.032
4	14			2λ a				-.012
5	14			2λ c				-.005
6	10				6λ a			.040
7	10				b			.061
8	12					4λ		.052
9	8		4λ					.111
10	6				6λ a			.093
11	6				b			.152
12	10			2λ a				.066
13	10			2 c				.059
14	3		4		6λ a			.249
15	2				b			.497
16	1			2λ a				.139
17	1			b				.119
18	6			c				.115
19	0			a	6λ a			.317
20				c	a			.278
21				a	b			.567
22				b	b			.499
23				c	b			.495
24				d	b			.359

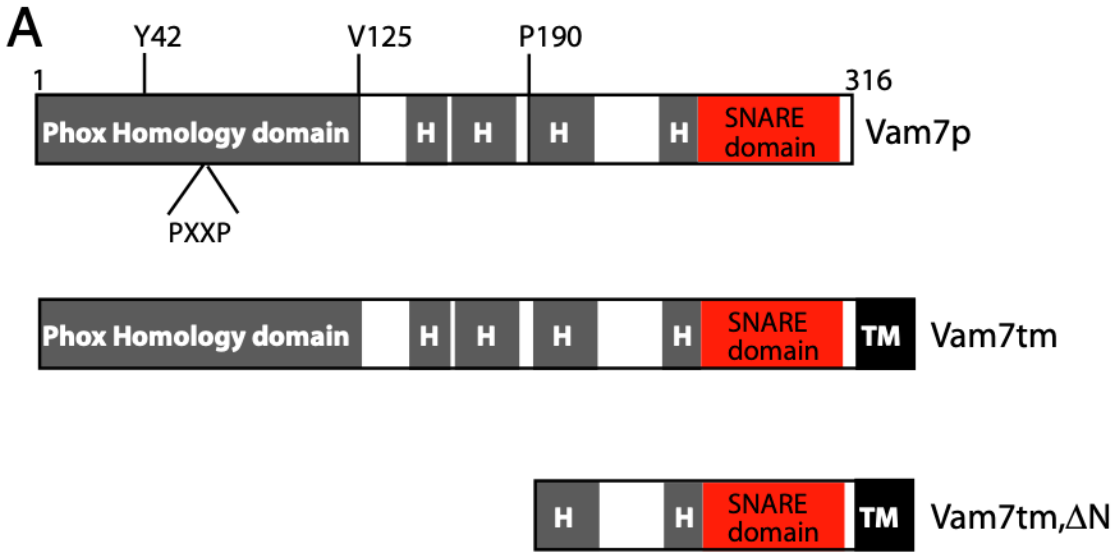
PX domain neither substitutes for Qc (green) nor inhibits when Qc is anchored

Sec18 ATP hydrolysis with Sec17 + Naked RPLs isn't different without PX domain (top) vs with a curve of PX (bottom)

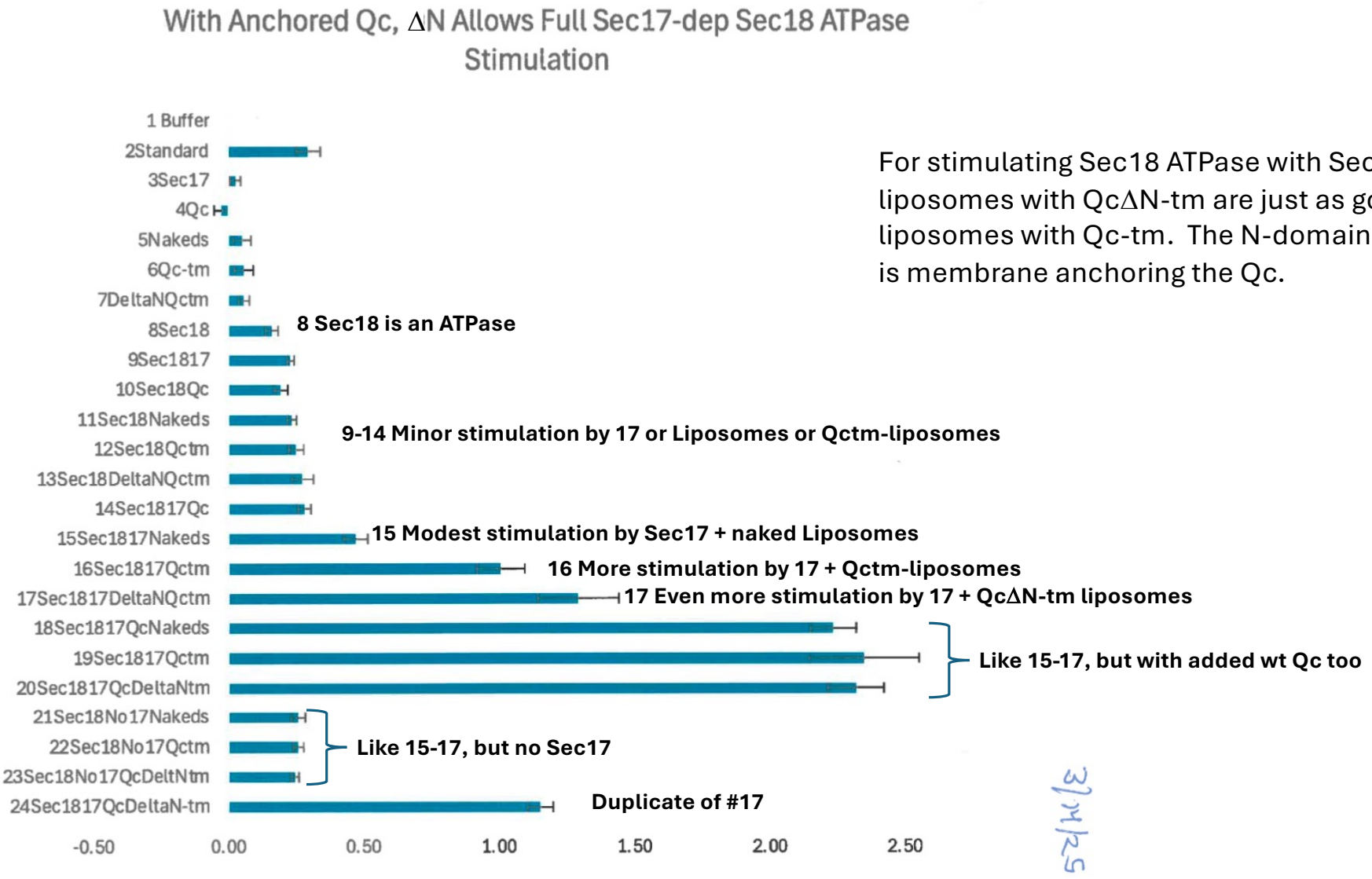
Sec18 ATP hydrolysis with Sec17 + Qc-tm RPLs isn't different without PX domain (top) vs with a curve of PX (bottom)



PX:PI3P recognition isn't needed if Qc is TM-anchored, BUT is the PX domain ALSO needed to interact with Sec17 or Sec18 to stimulate the Sec18 ATPase activity? To test this, Amy made RPLs that were naked, had Qc-tm, or QcΔN-tm



from Hao, 2012, PNAS



For stimulating Sec18 ATPase with Sec17 present, liposomes with QcΔN-tm are just as good as liposomes with Qc-tm. The N-domain's only function is membrane anchoring the Qc.

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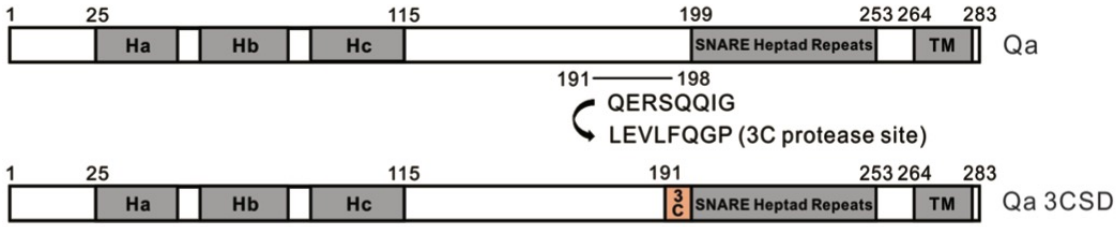
Next:

1 Karina's ordered a plasmid to make Qc-SNARE domain that's TM-anchored.
Is the stimulation only by Qc-SNARE domain:Sec17 interaction?

2 We have [anchored] Qa that has its entire N-domains deleted from Hongki.



How do RPLs bearing it compare to those with QcΔN-tm or (soon) QcSD-tm
for stimulation of Sec18 ATPase with Sec17 present?
Is the Qc SNARE domain special in this regard or is there an important upstream region?

"Sec17 binds the Qc SNARE domain to drive its activation of the Sec18 ATPase"

or

"Sec17 and a coiled coils region upstream of the Qc SNARE domain
work together to activate the Sec18 ATPase"

Remaining questions:

Is the Qc-SNARE domain the only part of Qc-tm needed to work with Sec17 to stimulate Sec18 ATPase?

Is it better at this than anchored Qa SNARE domain?

Compare, at 0.1 μM Sec17, the release of Sec17 by Sec18's ATP hydrolysis from:

a. "WT" 4-SNARE trans complex (as per Lopes et al)

b. *trans*-SNARE complex lacking Qc

Amy's experiment… Might Qc oligomerize and thus bind Sec17 better than e.g. sQa, etc?

Compare co-floatation of Qc and Sec17 to e.g. sQa or sR and to 4sSNAREs