

Week of April 21-25: EC50s for Qc and Sec17 for stimulating Sec18 ATPase

I tested the EC50 for Qc for supporting Sec18/17/naked liposome ATPase, and it's over 400nM. So I went on to test it with Ypt7:GTP-VML liposomes +/-HOPS. HOPS made no difference, but I need to do 2 more repeats. Translocation also has R, Qa, and Qb. Do they lower the EC50 for Qc, or can I show in the same assays (with luminal fluorescent proteins which had been dialyzed to remove the phosphate buffer) that the EC50 of Qc for translocation is nM, while the EC50 for Qc for ATP hydrolysis by Sec18 is e.g. 400 nM? This would explain why there's not too much Sec17 release and/or SNARE disassembly, causing diminished fusion, in fusion reactions with e.g. 10 nM Qc.

I should repeat Sec18 ATPase with:

Sec17, a curve of Qc, and a) Naked, b) Ypt7-RPLs, and c) Ypt7/R, and Ypt7/R + sQa+sQa

I then looked at Sec18 ATPase with a) a curve of Sec17, b) Sec17 and naked, and c) Sec17, naked, and Qc.

a Without lipid, the curve is still slowly rising at 6.4uM Sec17.

b A lot higher ATPase with liposomes too, and a EC50 of around 0.6 uM

c With Qc as well, far higher ATPase but little or no shift in EC50.

This suggests that Qc isn't driving lower concentrations of Sec17 to engage with Sec18; rather, either Qc engages Sec18 directly or Sec17:Qc is a better activator of Sec18's ATPase than Sec17 is alone.

Sec17 is very cooperative for stimulating Sec18 ATPase when Qc is present, and without Qc (needs repeats!)

Sec18 ATPase shows little stimulation by Qc, liposomes, or even Sec17 alone.

April 22, 2025

① Concentration curve for Qc in support: Sec18 ATPase

2	Sec17	55.09 μ Rb, 6.12 μ Rb	Sec17	75 μ M	6 ATP	100.5 μ Rb; 13.6 μ M Qc, 12.6 μ M ATP	50mM	50mM
3	Qc	56.3 μ Rb, 9.7 μ Rb	Qc	32.4 μ M	7 EOTA	619.7 μ Rb, 9.44 μ M EOTA		
4	Lipos	0.11/1.7 sh, 24 vults			8 Mix	220 μ PColor Mix, 2.2 μ Accelerator		
5	Sec18	40.65 μ Rb, 14.33 μ Rb	Sec18	5.76 μ M	9 Stabilizer	100 μ		
6	ATP		ATP		10			
7	EOTA		EOTA		11			
8	Mix		Mix		12			
9	Stabilizer		Stabilizer		13			
10	Accelerator		Accelerator		14			
11	Qc		Qc		15			
12	Qc		Qc		16			
13	Qc		Qc		17			
14	Qc		Qc		18			
15	Qc		Qc		19			
16	Qc		Qc		20			
17	Qc		Qc		21			
18	Qc		Qc		22			
19	Qc		Qc		23			
20	Qc		Qc		24			
21	Qc		Qc		25			
22	Qc		Qc		26			
23	Qc		Qc		27			
24	Qc		Qc		28			
25	Qc		Qc		29			
26	Qc		Qc		30			
27	Qc		Qc		31			
28	Qc		Qc		32			
29	Qc		Qc		33			
30	Qc		Qc		34			
31	Qc		Qc		35			
32	Qc		Qc		36			
33	Qc		Qc		37			
34	Qc		Qc		38			
35	Qc		Qc		39			
36	Qc		Qc		40			
37	Qc		Qc		41			
38	Qc		Qc		42			
39	Qc		Qc		43			
40	Qc		Qc		44			
41	Qc		Qc		45			
42	Qc		Qc		46			
43	Qc		Qc		47			
44	Qc		Qc		48			
45	Qc		Qc		49			
46	Qc		Qc		50			
47	Qc		Qc		51			
48	Qc		Qc		52			
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61	Qc		Qc		65			
62	Qc		Qc		66			
63	Qc		Qc		67			
64	Qc		Qc		68			
65	Qc		Qc		69			
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83	Qc		Qc		87			
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86	Qc		Qc		90			
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96	Qc		Qc		100			
97	Qc		Qc		101			
98	Qc		Qc		102			
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100	Qc		Qc		104			
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214	Qc		Qc		218			
215	Qc		Qc		219			
216	Qc		Qc		220			
217	Qc		Qc		221			
218	Qc		Qc		222			

April 23, 2025

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(K) Do HOPS & Y₁₇ affect μ_{kin} for Qc?

2 RPLs: N 3/6 Not given, purple shape $\frac{V_{\text{HPL}}}{V_{\text{HPL}} + V_{\text{HPL}}}$
3 HOPS: 14.59 λ , 9.41 λ HOPS box 115.3 μM
4 Sec17: 62.29 λ Rb, 1.71 λ Sec17 box 257 μM
5 Sec18: 43.08 λ Rb, 22.92 λ Sec18 box 378 μM

6 Qc 59.06 λ Rb, 4.94 λ Qc box 46, 32.4 μM
7 ATP 84 λ Rb, 10.5 λ ATP, 10.5 λ ATP
8 EOTA 619.7 λ Rb, 9.44 λ 0.4M EOTA
9 Mix 270 λ P (Coloc Mix, 2.7 λ Accelerator)

Index	SL	Rb	Pi	RPL	HOPS	Sec17	Sec18	10 Stabilizer	110 λ	Qc
1	16								0	0
2	12	4 λ							.258	0
3	12			4 λ					.310	0
4	13				3 λ				-.005	0
5	13						3 λ		.083	0
6	10					3 λ			.143	0
7	6			4 λ					.507	0
8	3							3 λ 1:16	.538	0
9								1:8	.634	0
10								1:4	.649	0
11								1:2	.879	0
12								1:1	1.414	0
13					3 λ				.654	0
14	0							3 λ 1:16	.693	0
15								1:8	.683	0
16								1:4	.721	0
17								1:2	.811	0
18								1:1	1.234	0
19	3 λ								.491	0
20	0							3 λ 1:16	1.245	0
21								1:8	.460	0
22								1:4	.547	0
23								1:2	.565	0
24								1:1	.797	0

Finishing: HOPS
doesn't shift
the curve
ATP vs [Qc]
at all.

Repeat twice;
K', K''

* 80 λ + 3.2 λ 30mM EOTA
+ 3.2 λ 10mM ATP 1027°
12.8 λ 30mM Mpf2

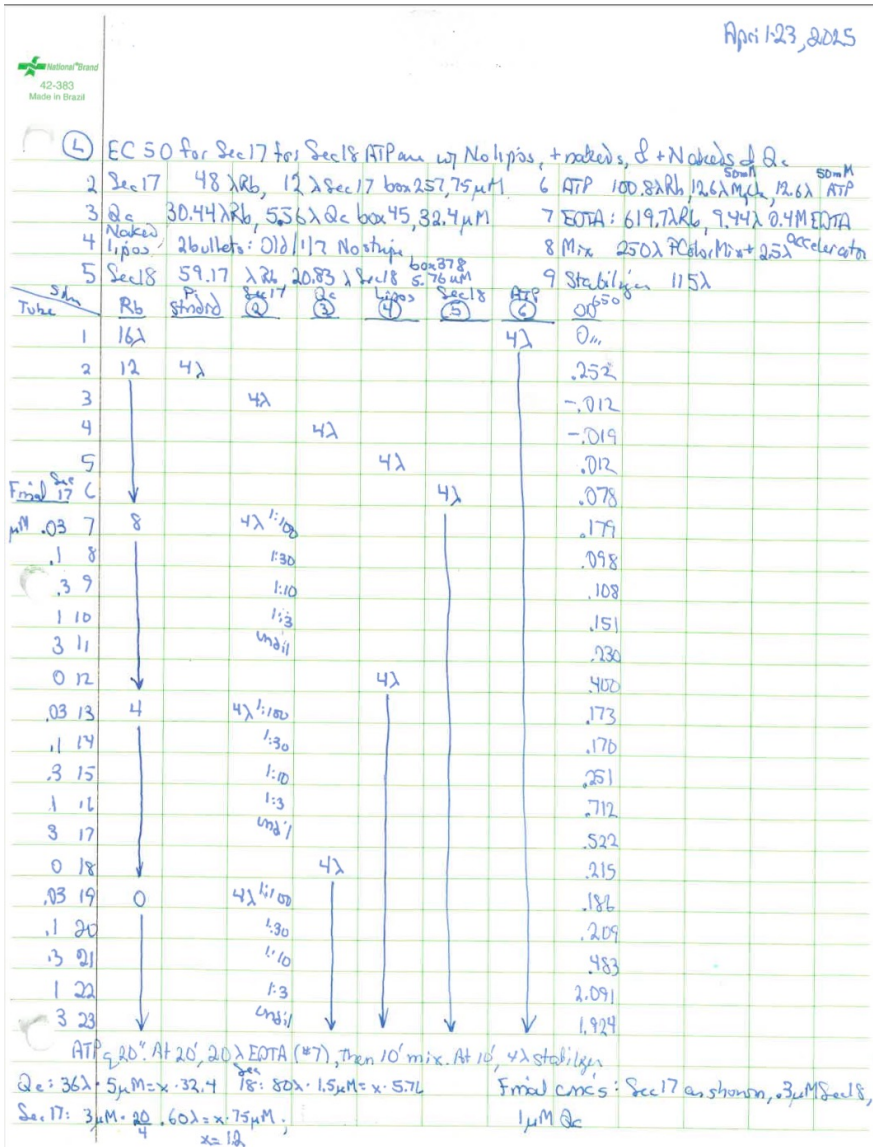
ATP: 105 λ 5mM =
x 50mM

HOPS: 24 λ = 28 λ 0.2 μM
= x 3.4

Final conc:
HOPS 3 μM
Sec17 3 μM
Sec18 3 μM
Qc 50-800nM

Sec17: 64 λ $\frac{20}{8}$ 0.3 = x 75
x = 1.71
Sec18: 66 λ $\frac{20}{8}$ 0.3 = x 57.6 μM
x = 22.92
Qc: 30 λ $\frac{20}{8}$ 0.8 μM = x 32.4 μM
x = 4.94

No effect of HOPS



By itself, Sec17 has little effect on Sec18 ATPase.

With liposomes, Sec18 has a big effect, maxing out at 1μM Sec17

With liposomes and Sec17, Qc has a huge effect, cooperative with respect to Sec17 concentration.

Apr 124, 2025

① A slight extension of L

6.4 μl 2. Sec 17: 100.8 μM, 20.64 λ, 15.36 λ, Sec 17

3 Qc: 30.48 λ, 6.12 λ, Qc box 45, 32.7 μM

4 Nucleoside 0.13111, 7 nucleoside

5 Sec 18: 79.88 λ, 28.12 λ, Sec 18: 5.76 μM

6 ATP 100.8 μM, 12.42 μM, 12.62 ATP

7 EDTA 619.7 λ, 9.44 λ, 0.4 μM EDTA

8 Mix 300 λ, PC Mix, 3 λ, 3 nucleoside

9 Stabilizer 125 λ

Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	16 λ					4 λ																				
2	12	4 λ																								
3			4 λ																							
4				4 λ																						
5					4 λ																					
6	↓					4 λ																				
7	8		4 λ	1:32																						
8				1:16																						
9				1:8																						
10				1:4																						
11				1:2																						
12				1:1																						
13	↓				4 λ																					
14	4		4 λ	1:64																						
15				1:32																						
16				1:16																						
17				1:8																						
18				1:4																						
19				1:2																						
20	↓				4 λ																					
21	0		4 λ	1:64																						
22				1:32																						
23		a	6	1:16																						
24		.1		1:8																						
25				1:4																						
26	↓			1:2																						

0.7

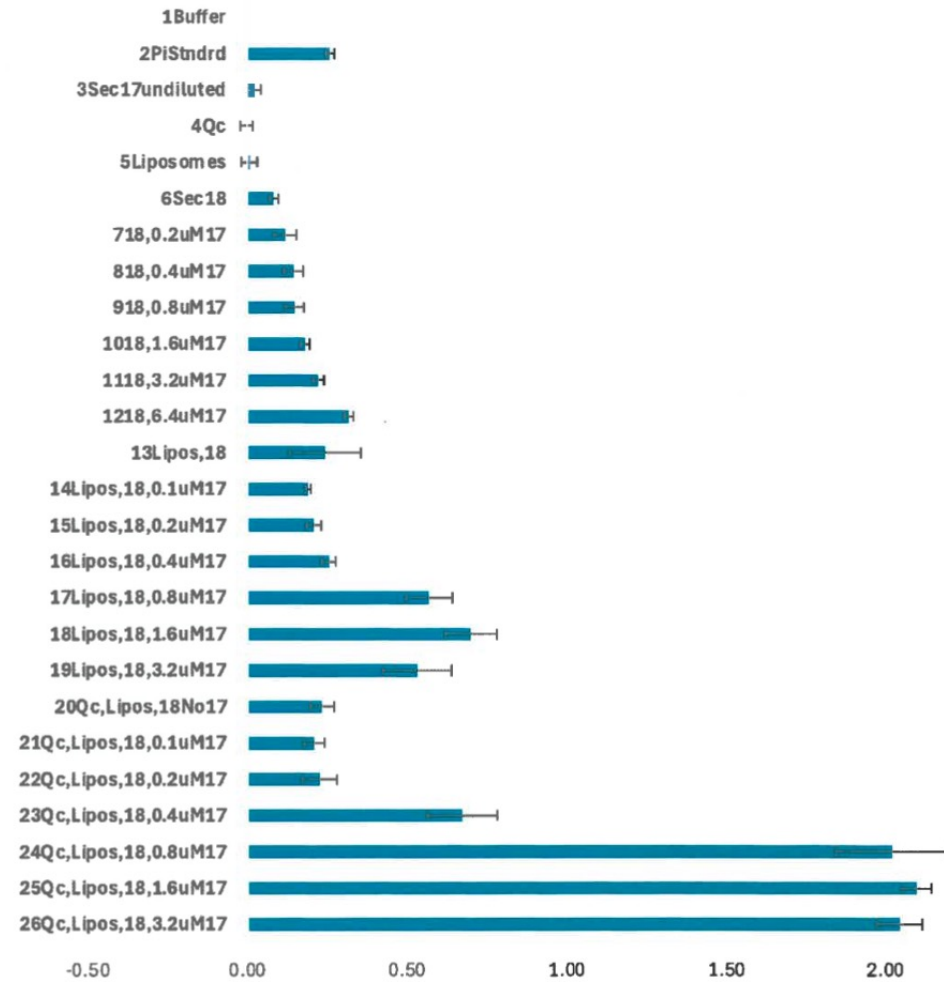
36 λ x 6.4 μM x 5 = 11.52

Sec17 gives a modest stimulation alone.

A much bigger, and perhaps cooperative, Sec17 stimulation in the presence of liposomes.

A yet huger, and definitely cooperative, Sec17 stimulation in the presence of liposomes and Qc.

Sec17EC50"L"Apr24,2025



4/25/25

(M) Is Sec17 cooperative for stimulating Sec18 ATPase?

Sec17	Rb	Sec17	Qc	Lipos	Sec18	ATP	Qc		
1	16λ					4λ	0%		
2	12	4λ					.293		
3			4λh				.2019		
4				4λ			.003		
5					4λ		.069		
6						4λ	.128		
7			4λh				.139		
8				4λ			.170		
9					4λ		.281		
10			4λh	4λ			.272		
11			4λh		4		.764		
12				4λ	4		.253		
13	0		4λa				.283		
14			b				.299		
15			c				1.081		
16			d				1.925		
17			e				2.179		
18			f				2.113		
19			g				2.064		
20			h				2.041		
	Rb λ	a	b	c	d	e	f	g	h
		9	8	7	6	5	4	3	2
		1	2	3	4	5	6	7	8
		1.0	1.5	2	2.5	3	4	5	6
		0.1 μM	0.2	0.3	0.4	0.5	0.6	0.8	1.0

70λ : 5 μM = x λ : 75 μM 18 : 66λ : 1.5 μM = x : 6.7λ

Sec18 ATP hydrolysis is highly cooperative with respect to Sec17; it takes a good number/concentration of Sec17 molecules working together to give a signal over background. If each Sec17 worked alone, then .1uM, .2uM, .3uM, .4uM, .5uM should have given signals over the 0uM background in the proportions 1, 2, 3, 4, 5.

ATP hydrolysis with Sec18 and Lipos, +/-Qc, but no Sec17

ATP hydrolysis is only stimulated a little by 0.1 or 0.2 uM Sec17

ATP hydrolysis goes up a LOT with 0.3 and 0.4 uM Sec17

We have physical evidence for 3Sec17:1Sec18Hexamer and the fusion assay shows Sec18 dependance at low Sec17. Now this is a 2nd catalytic assay for 3Sec17:1Sec18, especially if a Hill plot shows 3 Sec17's cooperating. Is Qc needed for cooperativity? Amazing that a single SNARE, not a 4-SNARE bundle, activates Sec18.

4/26/25

④ Is Sec17 cooperativity also seen without Qc?

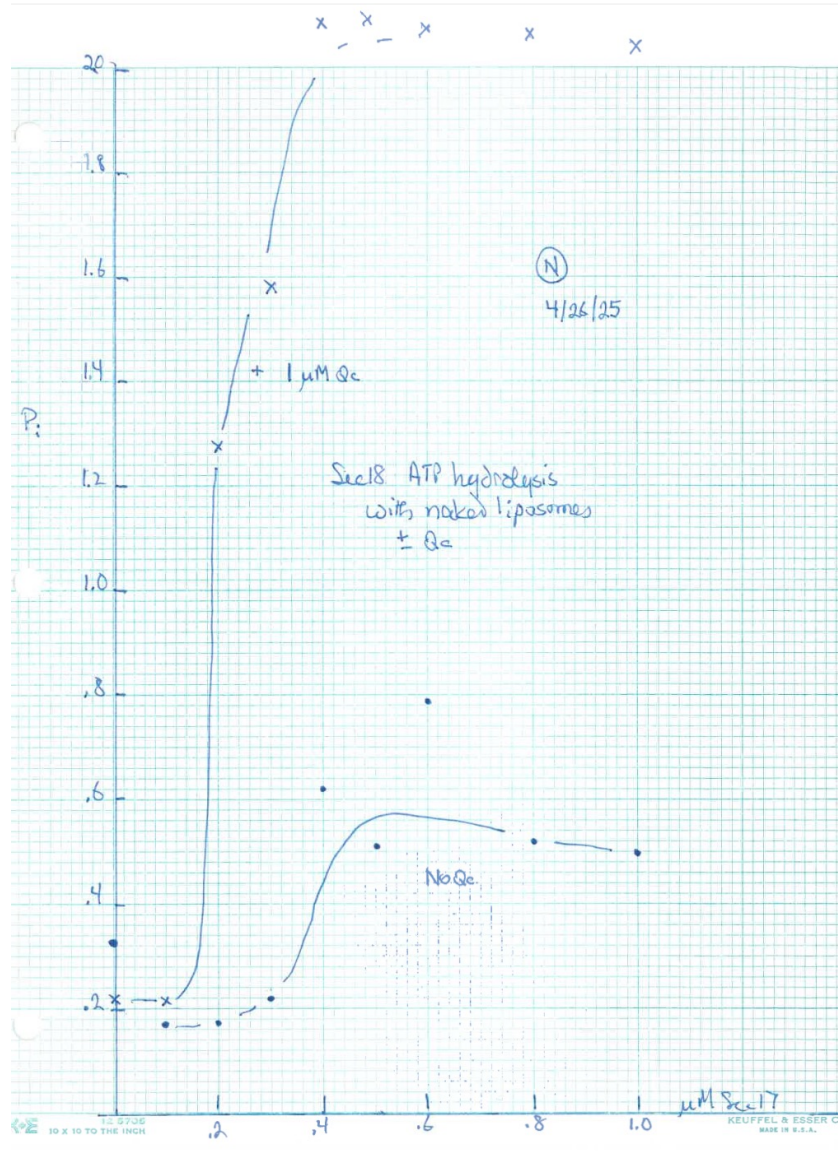
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
Notes		Sec17 (1.140M) 98.2λ Rb, 7λ Sec17 75μM	Qc 48.55λ Rb, 8.87λ Qc 32.7μM	Naked lipos 0.04λ Rb, 2.1λ Sec17 5.76μM	150nM Final Soln Rb: 16λ	150nM Final Soln Rb: 12λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ	150nM Final Soln Rb: 4λ		
ATP		6 ATP 100.8λ Rb, 12.6λ ATP	7 E/TA 619.7λ Rb, 9.44λ 0.4M E/TA	8 Mix 290λ PCol, 2.9λ Acceptor	9 Stabiligen 130λ																						
ATP		4λ 0.4	1.230	-0.15	-0.49	.004	.077	.847	.144	.323	.231	.218	.167	.172	.219	.621	.495	.789	.572	.496	.217	1.228	1.579	2.101	2.265	2.362	2.219
ATP																											

50nM 50nM
100.8λ Rb, 12.6λ ATP
619.7λ Rb, 9.44λ 0.4M E/TA
290λ PCol, 2.9λ Acceptor
Stabiligen 130λ

Assay: Add ATP 20%
At 20, add 20λ #7.
Add 10λ #8; at 10,
add 4λ #9

Sec18: 92λ · 0.75μM = x · 5.76

Apparent cooperativity even without Qc (see graph next) but needs repeats.



4/28/25

Curves of Sec18

	6 min 4 min soln	Time	Sec17	22.4λ Rb	1.6λ Sec17	box 258 75 μM	6 ATP	70.56λ Rb	8.92λ Rb	50 μM	50 μM
		2	Sec17	20.3	"	3.7λ Qc	32.4 μM	7 EDTA	619.7λ Rb	9.44λ	0.411 EDTA
		3	Qc	20.3	"	3.7λ Qc	32.4 μM	7 EDTA	619.7λ Rb	9.44λ	0.411 EDTA
		4	Naked Lipids	0.12 + 0.01 Wash	No shipping			P.Mix	190λ	PColonLook	+1.9λ Accelerator
		5	Sec18	14.4	APb	15.6λ	Sec18	6.76 μM	P.	9 Stabilizer	85λ
		6	Rb	2	Qc	3	Lipids	4	Sec18	5	ATP
		1	16λ				4λ				
		2	12	4λ P. Standard							
		3		4λ #2							
		4			4λ						
		5				4λ					
		6				4λ undil					
		7				1:2					
		8	✓			1:4					
		9	8λ		4λ	undil					
		10			"	1:2					
		11			"	1:4					
		12		4λ		undil					
		13		"		1:2					
		14		"		1:4					
		15		4λ		undil					
		16		"		1:2					
		17	↓	"		1:4	↓				

ATP q20. After 20', add 20λ EDTA. Add 10λ mix. After 10', add 4λ stabilizer. v. 00650

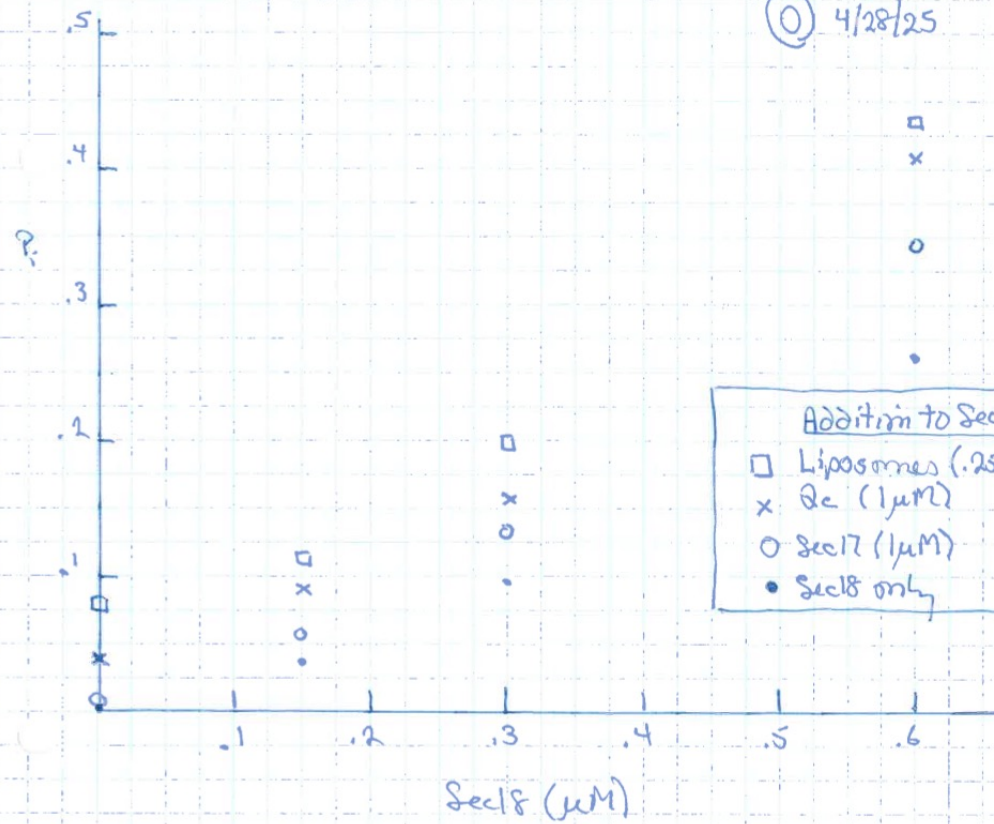
Curves:

Sec17 1 μM
Qc 1 μM

Sec17: 14λ · 5 μM = x · 75 Sec18: 30λ · 3 μM = x · 5.76

Neither liposomes, Qc, nor even Sec17 when added alone gives a particularly big stimulation of Sec18 ATPase.

① 4/28/25



Next:

A Is Sec17 stimulation of Sec18 ATPase with liposomes but no Qc also cooperative? I think so...

B Some repeats...

C Make 6 RPLs: naked, Qc-tm, Qc Δ N-tm, and Qc-SNAREdomainOnly-tm, RQaQb, and 3Q. Test them for Sec18 ATPase with a curve of added Sec17.

