Week of April 28 to May 2

ATP hydrolysis

It's stimulated some by each of Sec17, Qc-SNARE, or liposomes alone, more by Sec17+liposomes, most by all 3.
Stimulation is cooperative with respect to Sec17 when liposomes and Qc are also present,
less clear for other combinations

Fusion inhibition by Sec18 hydrolysis of ATP is optimal when zippering is blocked and with Sec17 at 0.1 μM.
It can be seen with w.t.
It can be seen some with other Sec17 levels
It's seen as well with Qb3Δ as with Qc3Δ
It's not a matter of the ratio of Sec17 to Sec18, or of the sSNARE concentration.

4/29/25

National Brand 42-383 Made in Brazil

(N') Is Sec17 cooperativity seen without Qc?

2 Sec17 (140λ 5μM) 98λ Rb, 7λ Sec17 box 258 75μM
3 Qc 48.55λ Rb, 8.87λ Qc box 45 32.4μM
4 Naked Lipos Old tank Wand 1 box 7 Nostips 3 tubes
5 Sec18 68.04λ Rb, 23.96λ Sec18 box 378 5.76μM
No P: ATP
100 nM Final

6 ATP 100.8λ Rb, 12.6λ 50mM $MgCl_2$, 12.6λ 50mM ATP
7 EDTA 619.7λ Rb, 9.44λ 0.4M EDTA
8 Mix 270λ, 2.7λ accelerator
9 Stabilizer: 115λ

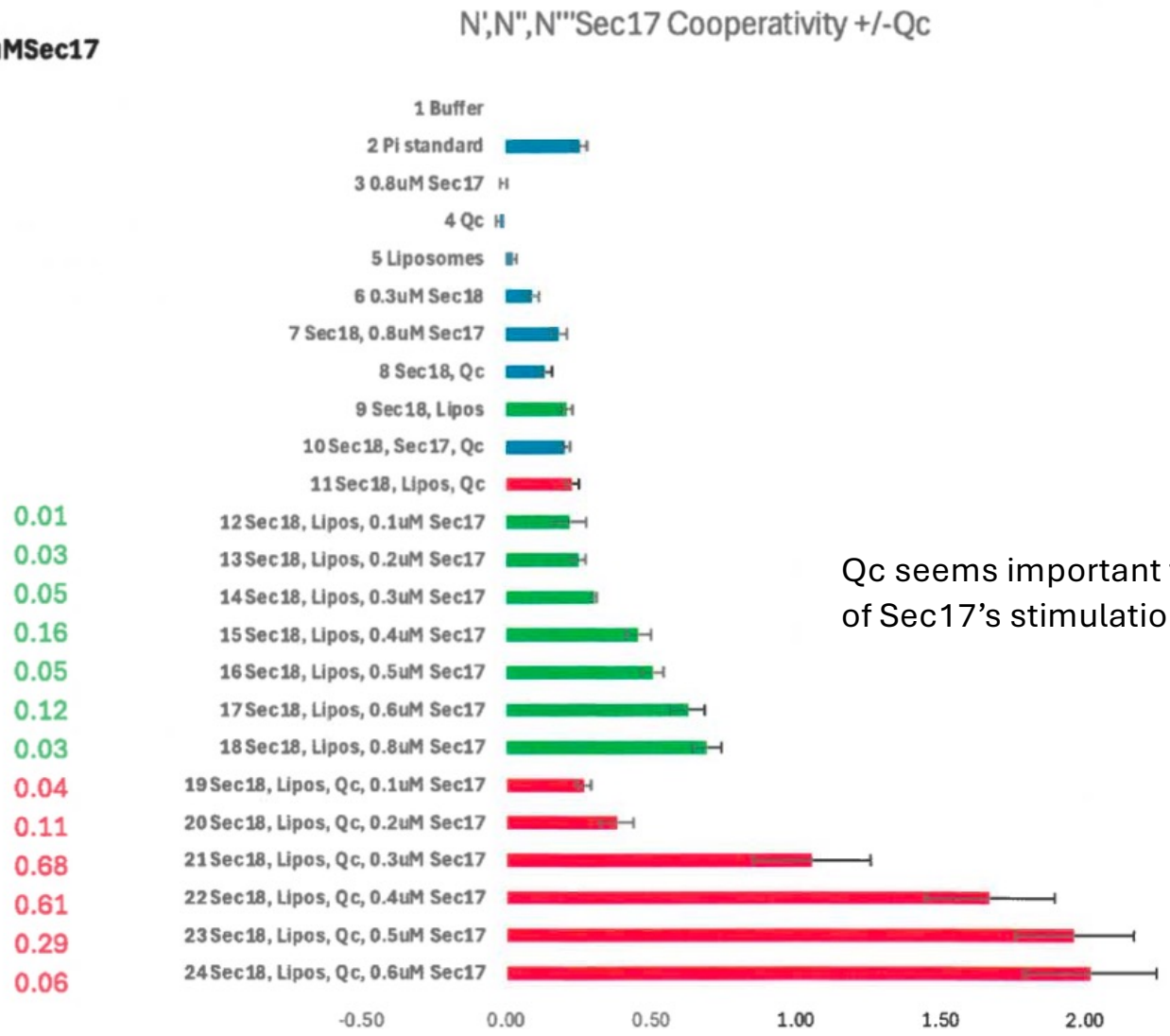
Tube \ Soln	Rb	Sec17 2	Qc 3	Lipos 4	Sec18 5	ATP 6	N'	N''	N'''	Final μM	Rb	#2
1	16λ					4λ	0m	0m	0m	a .1	18λ	2λ
2	12λ	4λ P: Stndrd					.250	.284	.242	b .2	16	4
3		4λ g					0	.004	-.020	c .3	14	6
4			4λ				-.007	-.014	-.032	d .4	12	8
5				4λ			.034	.032	.011	e .5	10	10
6	↓				4λ		.093	.117	.073	f .6	8	12
7	8λ	4λ g					.214	.178	.167	g .8	5	20
8			4λ				.145	.155	.114			
9	↓			4λ			.218	.229	.187			
10	4λ	4λ g	4	—			.218	.207	.186			
11			4	4			.239	.244	.204			
12		4λ a	—				.287	.191	.187			
13		b	—				.227	.275	.249			
14		c	—				.308	.294	.312			
15		d	—				.458	.503	.415			
16		e	—				.543	.466	.514			
17		f	—				.677	.645	.562			
18	↓	g	—				.692	.743	.642			
19	0	4λ a	4λ				.296	.249	.264			
20		b					.414	.315	.416			
21		c					1.131	1.210	.828			
22		d					1.848	1.418	1.748			
23		e					2.159	1.979	1.754			
24	↓	f	↓	↓	↓	↓	2.227	1.776	2.056			

#6 (ATP) q20'. 20', Then 20λ #7 (EDTA). Then 10λ #8 (Mix). 10', add 4λ #9. ✓ OD^{650}

Sec18: 92λ · 1.5μM = x · 5.76 x =

Assay	N'	N''	N'''	Average	Stndrd Dev
1 Buffer	0	0	0	0.00	0.00
2 Pi standard	0.25	0.284	0.242	0.26	0.02
3 0.8uM Sec17	0	0.004	-0.02	-0.01	0.01
4 Qc	-0.007	-0.014	-0.032	-0.02	0.01
5 Liposomes	0.034	0.032	0.011	0.03	0.01
6 0.3uM Sec18	0.093	0.117	0.073	0.09	0.02
7 Sec18, 0.8uM Sec17	0.214	0.172	0.167	0.18	0.03
8 Sec18, Qc	0.145	0.155	0.114	0.14	0.02
9 Sec18, Lipos	0.218	0.229	0.187	0.21	0.02
10 Sec18, Sec17, Qc	0.218	0.207	0.186	0.20	0.02
11 Sec18, Lipos, Qc	0.239	0.244	0.204	0.23	0.02
12 Sec18, Lipos, 0.1uM Sec17	0.287	0.191	0.187	0.22	0.06
13 Sec18, Lipos, 0.2uM Sec17	0.227	0.275	0.249	0.25	0.02
14 Sec18, Lipos, 0.3uM Sec17	0.308	0.294	0.312	0.30	0.01
15 Sec18, Lipos, 0.4uM Sec17	0.458	0.503	0.415	0.46	0.04
16 Sec18, Lipos, 0.5uM Sec17	0.543	0.466	0.514	0.51	0.04
17 Sec18, Lipos, 0.6uM Sec17	0.677	0.645	0.562	0.63	0.06
18 Sec18, Lipos, 0.8uM Sec17	0.692	0.743	0.642	0.69	0.05
19 Sec18, Lipos, Qc, 0.1uM Sec17	0.296	0.249	0.264	0.27	0.02
20 Sec18, Lipos, Qc, 0.2uM Sec17	0.414	0.315	0.416	0.38	0.06
21 Sec18, Lipos, Qc, 0.3uM Sec17	1.131	1.21	0.828	1.06	0.20
22 Sec18, Lipos, Qc, 0.4uM Sec17	1.848	1.418	1.748	1.67	0.23
23 Sec18, Lipos, Qc, 0.5uM Sec17	2.159	1.979	1.754	1.96	0.20
24 Sec18, Lipos, Qc, 0.6uM Sec17	2.227	1.776	2.056	2.02	0.23

Δ/0.1uMSec17



Qc seems important for the cooperativity of Sec17's stimulation of Sec18 ATPase

19.2
20.8

4/28/25

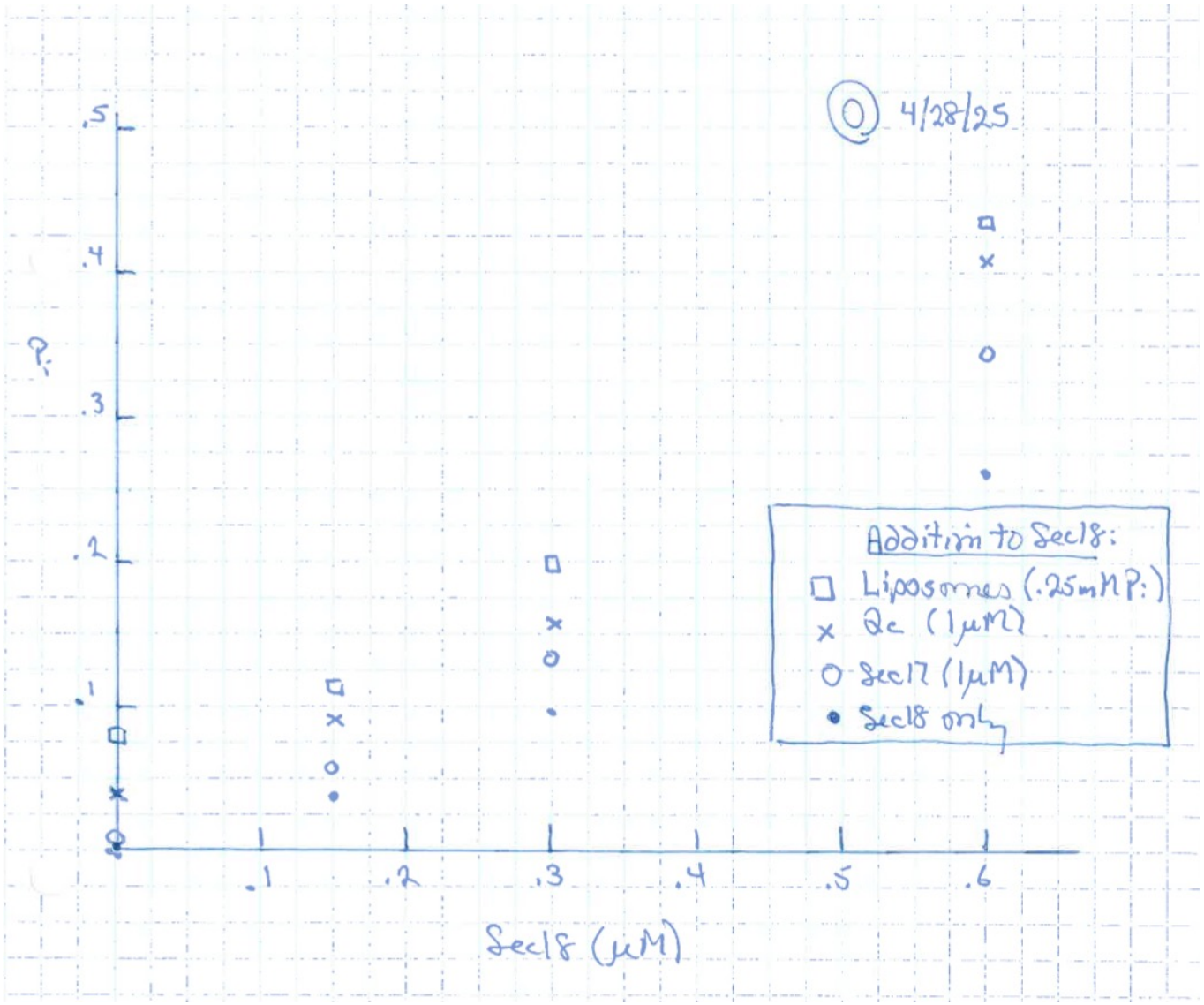
⓪ Curves of Sec18

2 Sec17 22.4λ Rb, 1.6λ Sec17 (box 258, 75 μM)
3 Qc: 20.3 " , 3.7λ Qc (box 45, 32.4 μM)
4 Naked Lipos: Old tenth Wand! Box 7, No shipm.
5 Sec18 19.2 λ Rb, 20.8 λ Sec18 (box 378, 6.76 μM) — .6 μM final
No P:
6 ATP 70.56λ Rb, 8.82λ $MgCl_2$ (50 mM), 8.82λ ATP (50 mM)
7 EDTA 619.7λ Rb, 9.44λ 0.4 M EDTA
8 Mix 190λ PColorLock + 1.9λ Accelerator
9 Stabilizer; 85λ

Soln / Tube	Rb	Sec17 2	Qc 3	Lipos 4	Sec18 5	ATP 6	OD_{650} "0"	0'	0''
1	16λ					4λ	0	0	0
2	12	4λ P: Stndrd				↓	.310	.285	.280
3	↓	4λ #2				↓	.042	.031	.014
4	↓		4λ			↓	.004	-.001	.015
5	↓			4λ		↓	.081	.051	.045
6	↓				4λ undil	↓	.266	.290	.262
7	↓				1:2	↓	.096	.130	.094
8	↓				1:4	↓	.039	.040	.051
9	8λ			4λ	undil	↓	.437	.440	.393
10	↓			"	1:2	↓	.200	.218	.178
11	↓			"	1:4	↓	.115	.102	.090
12	↓		4λ		undil	↓	.347	.351	.345
13	↓		"		1:2	↓	.136	.140	.186
14	↓		"		1:4	↓	.063	.034	.141
15	↓	4λ			undil	↓	.409	.412	.446?
16	↓	"			1:2	↓	.163	.183	(.431)?
17	↓	"			1:4	↓	.090	.075	.081

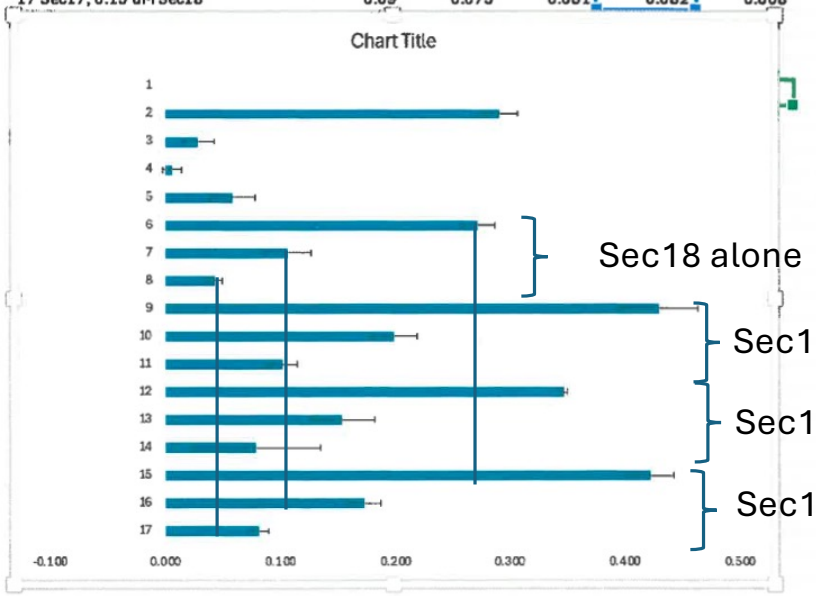
ATP q20". After 20', add 20λ EDTA. Add 10λ mix. After 10', add 4λ stabilizer. √ OD^{650}

Concs:
Sec17 1 μM
Qc 1 μM


4/28/25
Addition to Sec18:
Liposomes (.25mM P:)
Qc (1μM)
Sec17 (1μM)
Sec18 only
Pi
.5
.4
.3
.2
.1
.1
.2
.3
.4
.5
.6
Sec18 (μM)

O, O', O'' ATPase with 0, 0.15, 0.3, 0.6uM Sec18 with buffer, Liposomes, Qc, or Sec17

	O	O'	O''	Average	Std Deviation
1 Buffer	0	0	0	0.000	0.000
2 Pi Standard	0.31	0.285	0.28	0.292	0.016
3 Sec17	0.042	0.031	0.014	0.029	0.014
4 Qc	0.004	-0.001	0.015	0.006	0.008
5 Liposomes	0.081	0.051	0.045	0.059	0.019
6 0.6 uM Sec18	0.266	0.29	0.262	0.273	0.015
7 0.3 uM Sec18	0.096	0.13	0.094	0.107	0.020
8 0.15 uM Sec18	0.039	0.04	0.051	0.043	0.007
9 Liposomes, 0.6 uM Sec18	0.457	0.44	0.393	0.430	0.033
10 Liposomes, 0.3 uM Sec18	0.2	0.218	0.178	0.199	0.020
11 Liposomes, 0.15 uM Sec18	0.115	0.102	0.09	0.102	0.013
12 Qc, 0.6 uM Sec18	0.347	0.351	0.345	0.348	0.003
13 Qc, 0.3 uM Sec18	0.136	0.14	0.186	0.154	0.028
14 Qc, 0.15 uM Sec18	0.063	0.034	0.141	0.079	0.055
15 Sec17, 0.6 uM Sec18	0.409	0.412	0.446	0.422	0.021
16 Sec17, 0.3 uM Sec18	0.163	0.183		0.173	0.014
17 Sec17, 0.15 uM Sec18	0.09	0.075	0.081	0.082	0.008



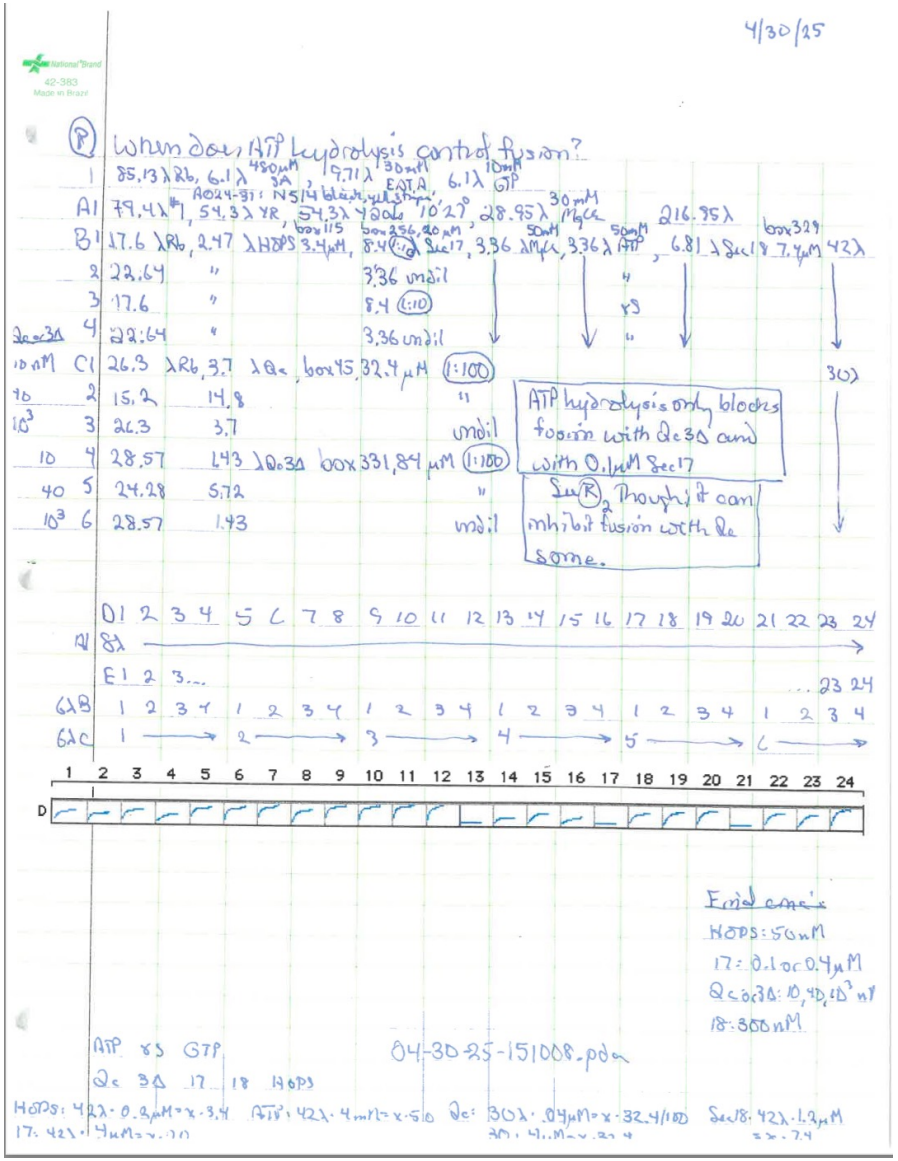
Sec18 alone

Sec18 + liposomes

Sec18 + Qc

Sec18 + Sec17

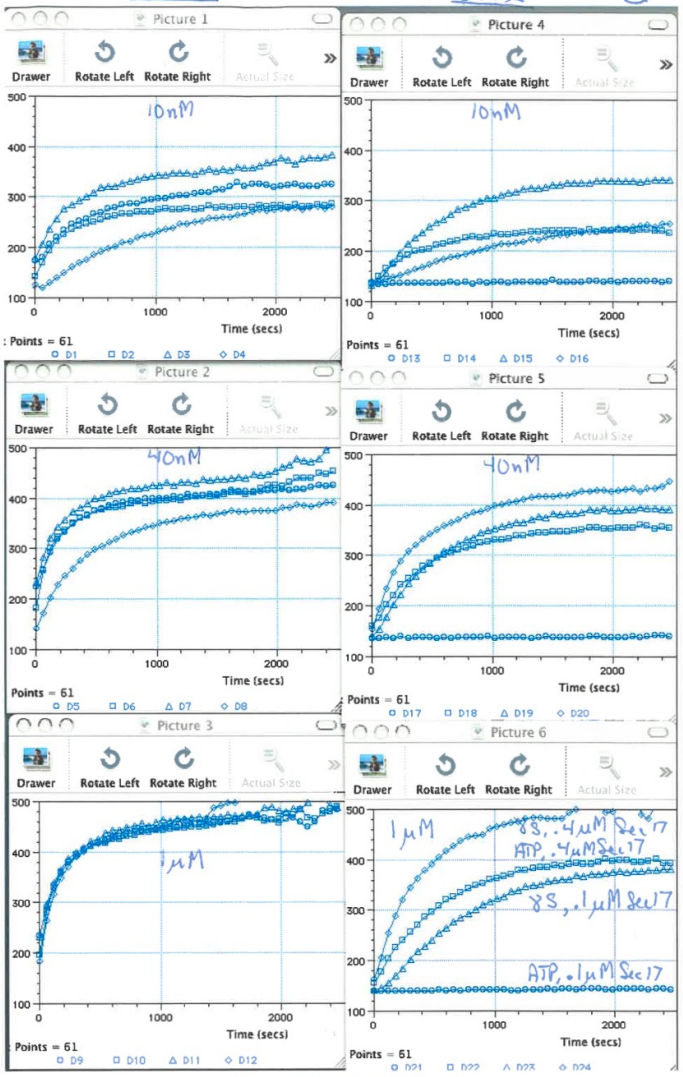
Qc, Liposomes, and Sec17 seem to each be comparable stimulants of Sec18 ATPase



Under what conditions is there less fusion with ATP than with ATPγS, i.e., when does ATP hydrolysis inhibit fusion?

It's seen consistently with Qc3Δ and 0.1 μM Sec17, though in "R" this is seen (though less) with w.t. Qc

Sc WT Sc3Δ R 4/30/25



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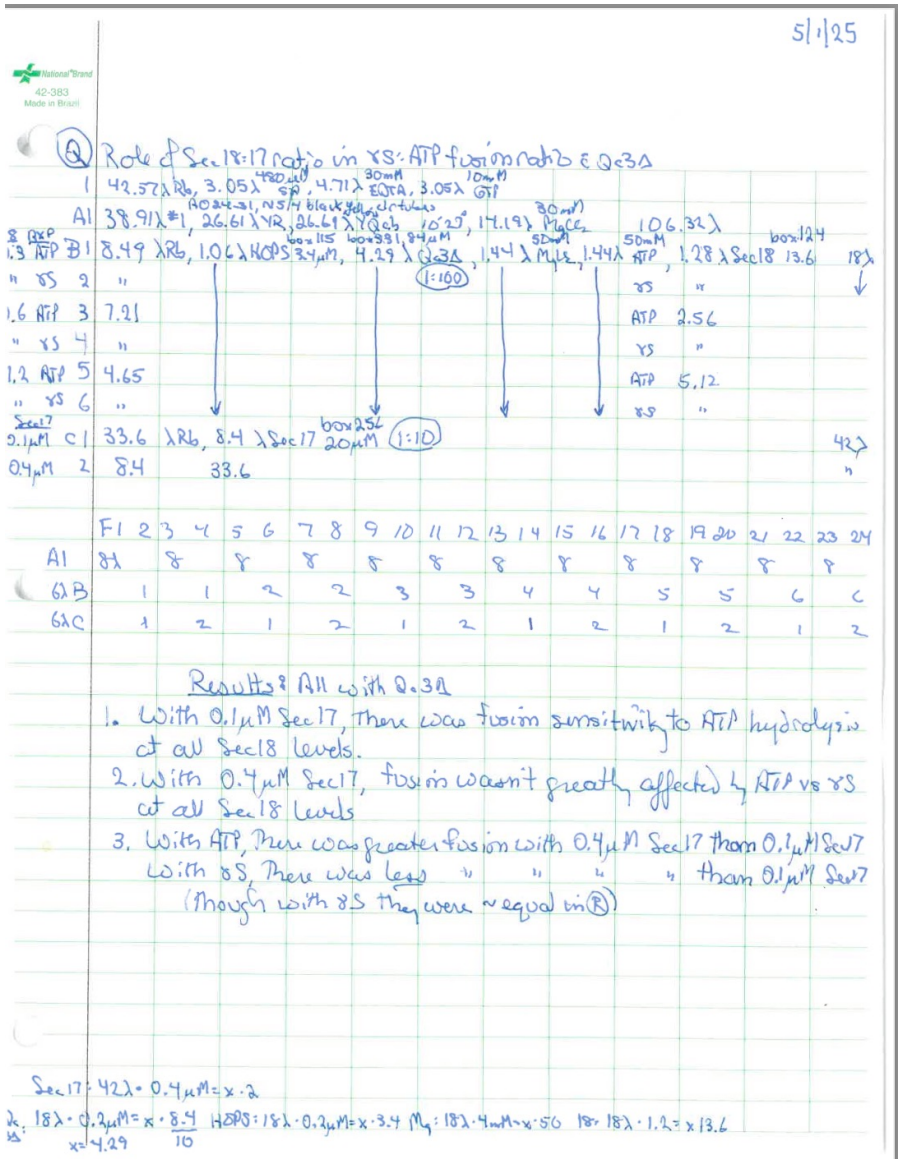
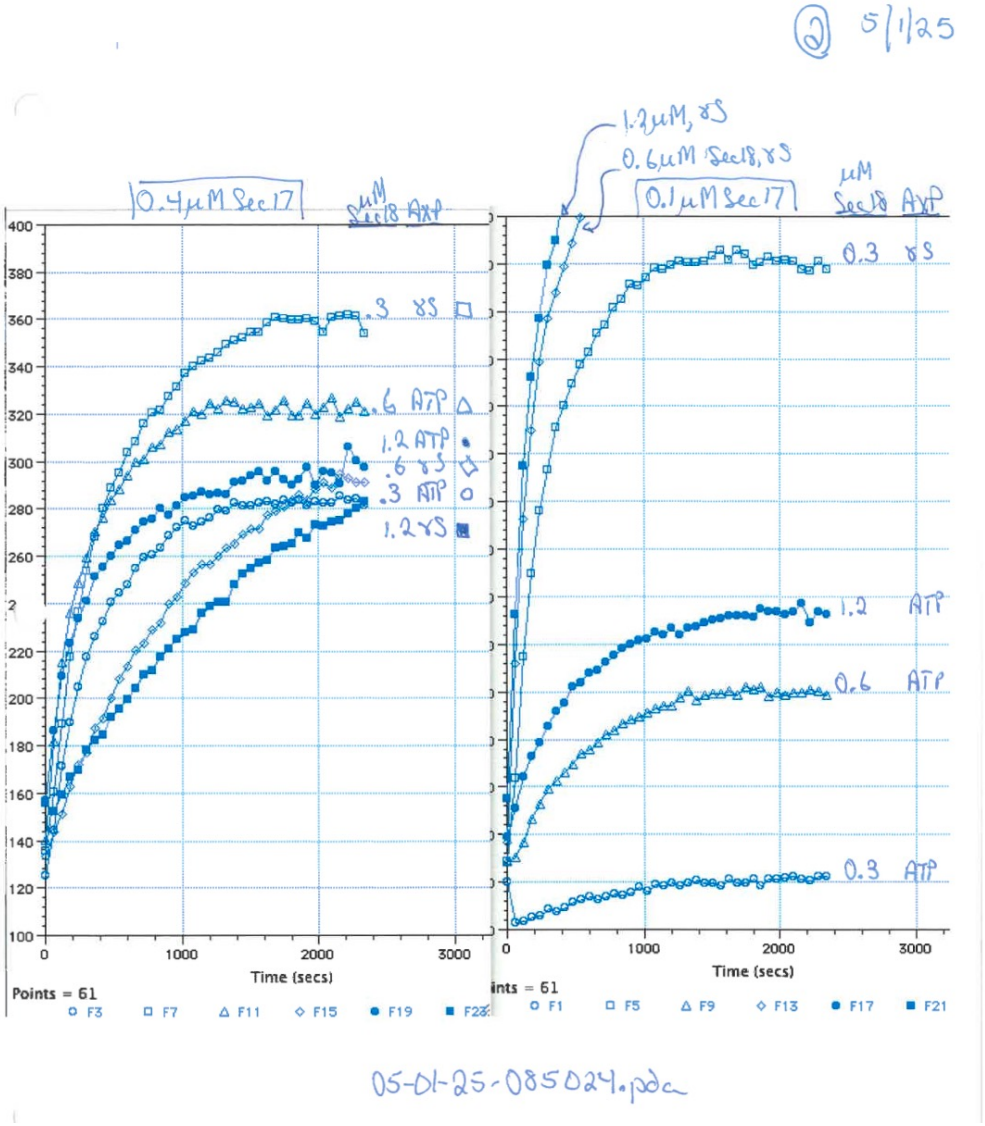


Is the relevant parameter the Sec18:Sec17 ratio?

Nope...



With Qc3Δ, varying levels of Sec18 show strong fusion suppression by ATP hydrolysis with 0.1 μM Sec17 but not with 0.4 μM Sec17

42-383
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Ⓡ A curve of [Sec17] with ATP vs γS and Qc vs Qc3Δ

1.7 | 1 | 72.37 λRb, 5.19λ SA (480μM), 8.0λ EDTA (30mM), 5.19λ GTP (10mM)
A1 | 77.82λ #1, 53.22λ YR (NS 14 / black dye), 53.22λ YQab, 10' 27°, 28.38λ $MgCl_2$ (30mM)
B1 | 23.37 λRb, 2.47 λHOPS (box 115, 3.4μM), 2.63λ Qc 32.4μM (box 45) (1:10), 3.36 λMgCl₂ (50mM), 3.36λ ATP (50mM), 6.81 λSec18 (box 329, 7.4μM), 42λ

	B1	23.37 λRb	2.47 λHOPS	2.63λ Qc 32.4μM (1:10)	3.36 λ$MgCl_2$	3.36λ ATP	6.81 λSec18	42λ
	2	"	↓	"	↓	γS	↓	↓
	3	24		2.0 Qc3Δ 84μM (box 331) (1:20)		ATP		
	4	"		"		γS		

Sec17					
0μM	C1	30 λRb,	0 λSec17 (box 256, 20μM)		30λ
.01	2	24	6	" (1:100)	
.03	3	12	18	"	
.1	4	24	6	(1:10)	
.4	5	6	24	"	
1.6	6	20.4	9.6	undil	↓

Final conc's:
HOPS 50nM
Sec18 300nM
Qc or 3Δ 50nM
Sec17 0→1.6μM

G 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A1 8λ →
H1 2 3 . . . . . .22 23 24
6λ B 1 → 2 → 3 → 4 →
6λ C 1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6

Findings: 1. Sec17 inhibits when > 0.4μM
2. Sec17 stimulates some with ATP, not at all with ATPγS, in the presence of Qc
3 With Qc3Δ, 0.4 - 1.6μM Sec17 is needed with ATP, 0.1 to 0.4 with ATPγS.
4. ATP hydrolysis inhibits with 0.4μM Sec17 ~2-fold but strongly inhibits at 0.1μM.
5. With Qc, ATP hydrolysis inhibits, even with Sec18 & no Sec17, Sec17 stimulates on top of Sec18.

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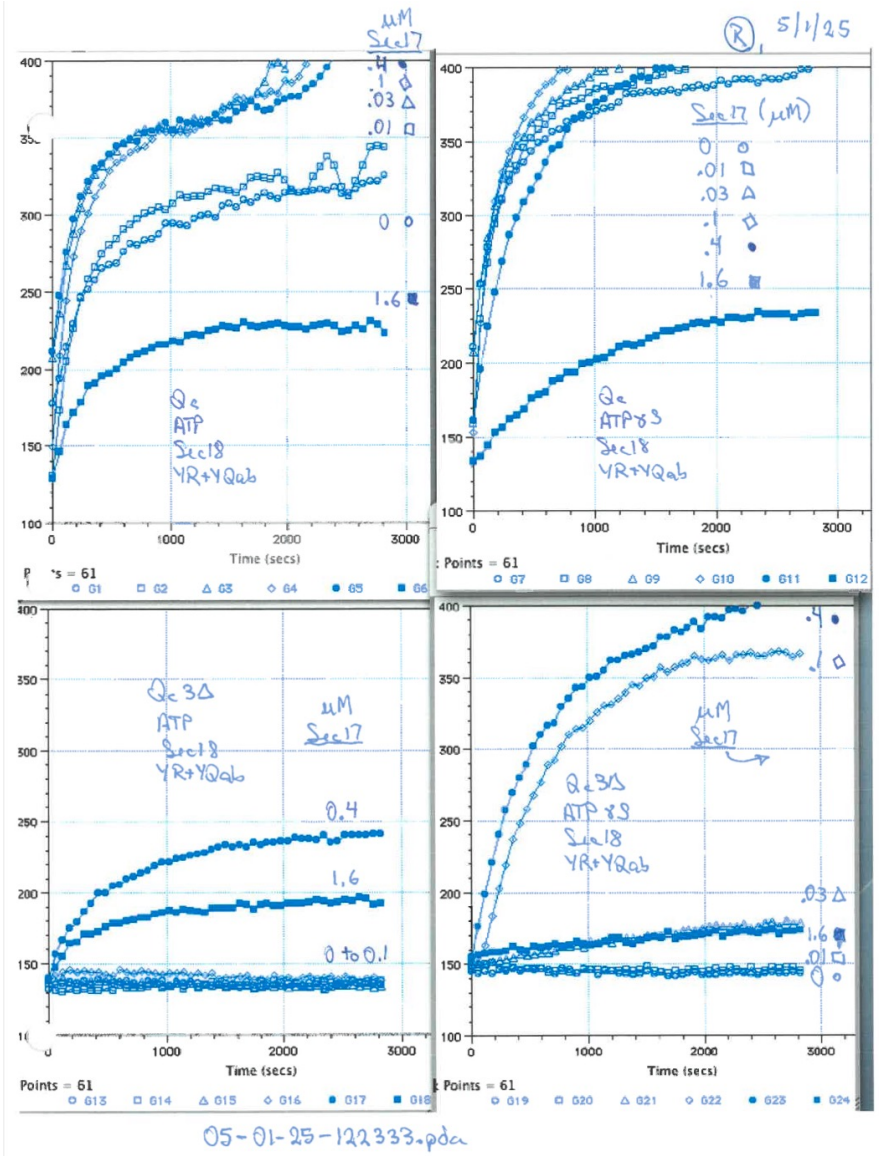
HOPS: 42λ · 0.2μM = x · 3.4 Qc: 42λ · .2μM = x · 32.4 Mg: 42λ · 4mM = x · 50 Sec18: 42λ · 1.2μM = x · 7.4
Sec17: 30λ · .04 = x · 20/100



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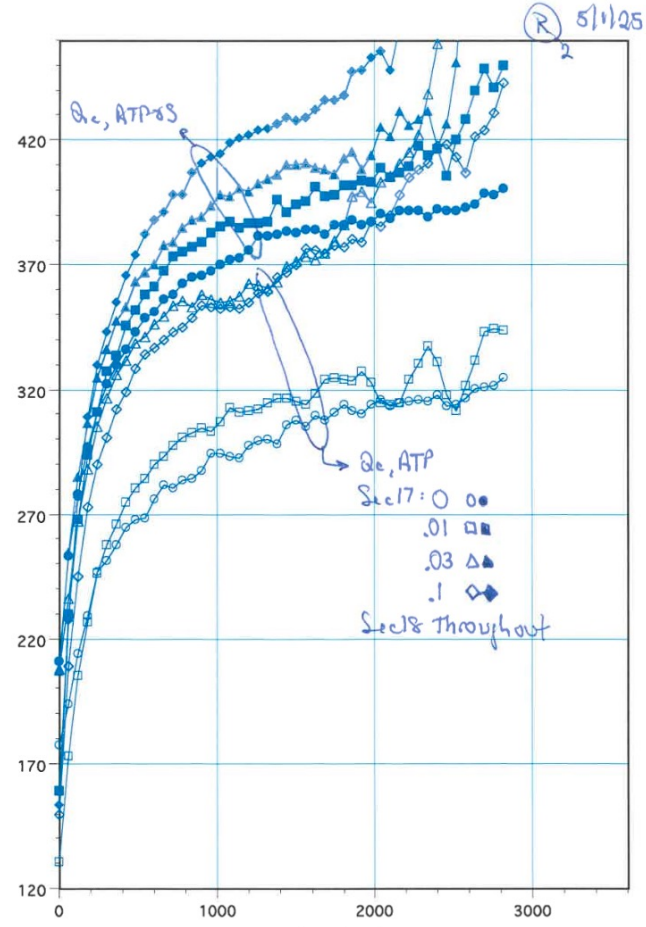

Points = 61

	G1	G2	G3	G4	G7	G8
Per Second	0.034	0.039	0.062	0.060	0.038	0.053
	0.740	0.607	0.827	0.792	0.657	0.691

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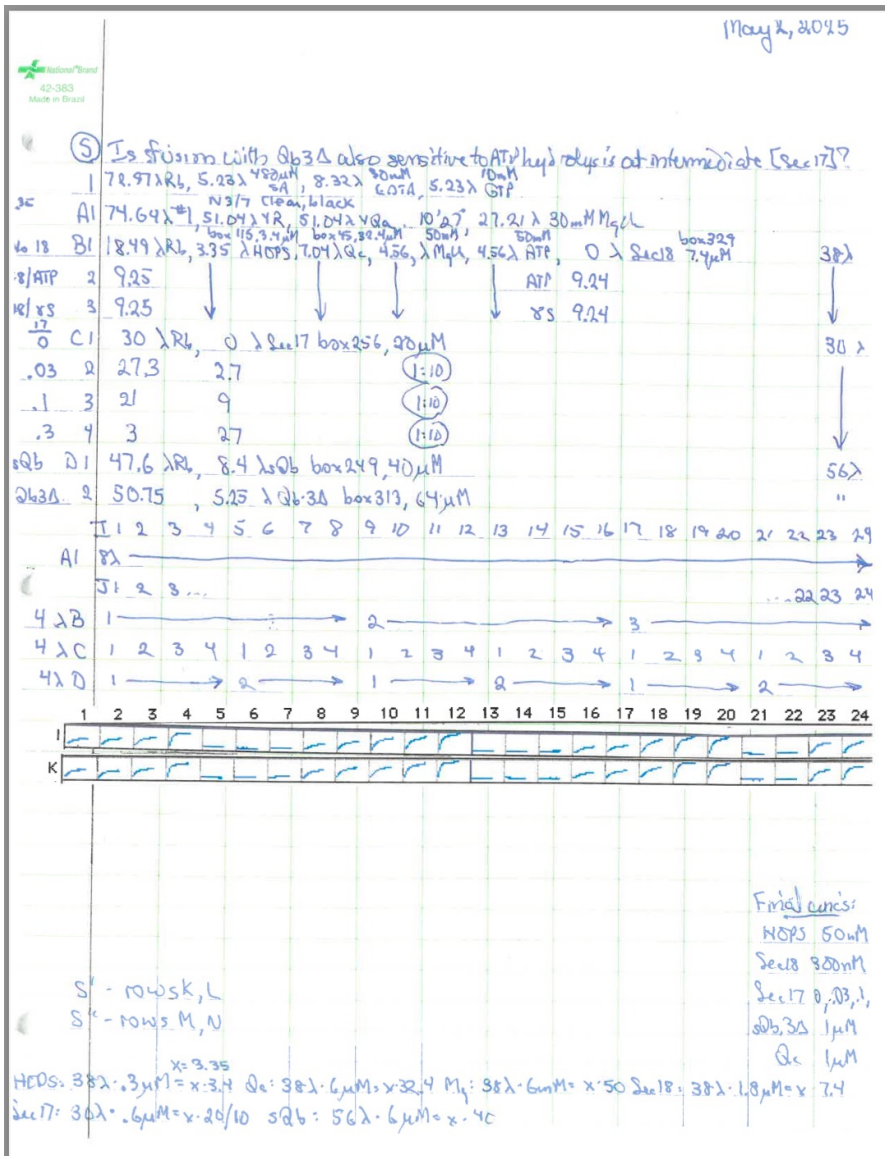
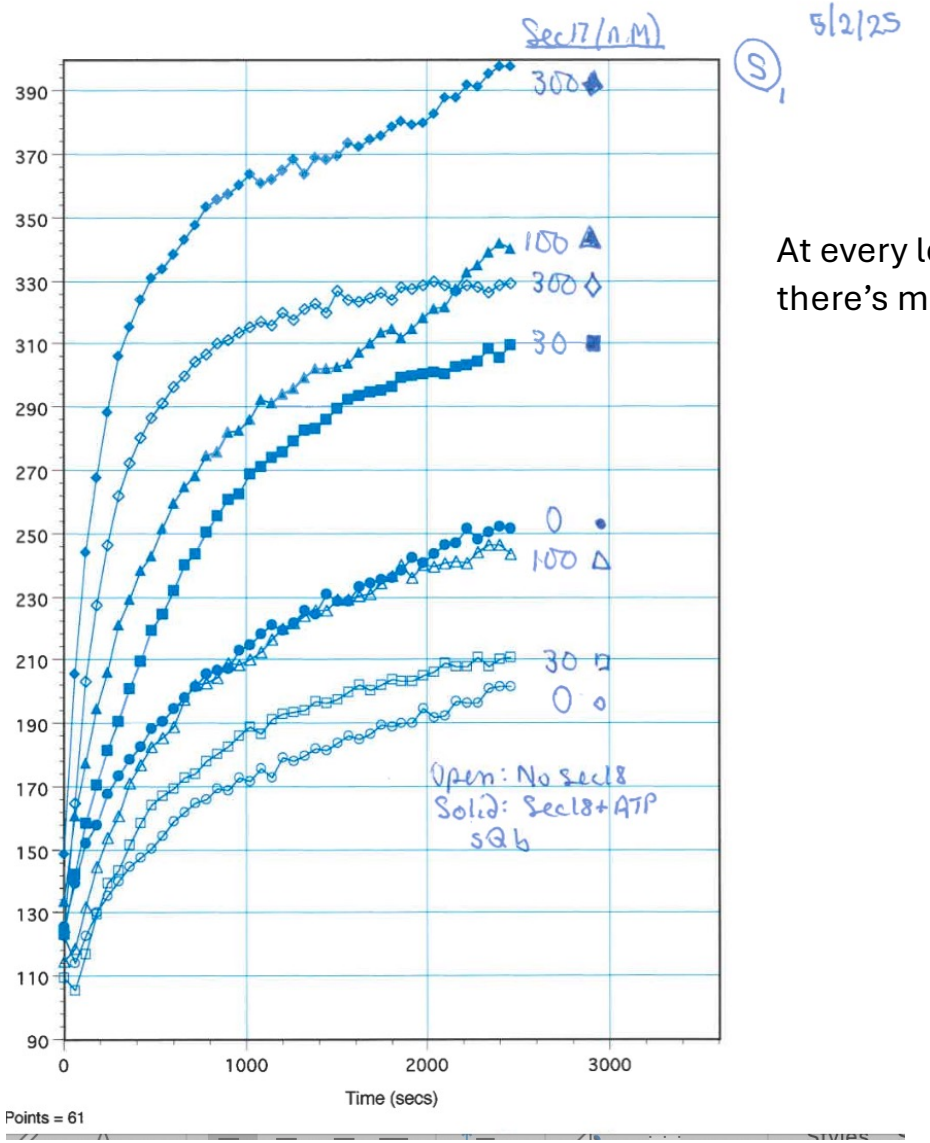


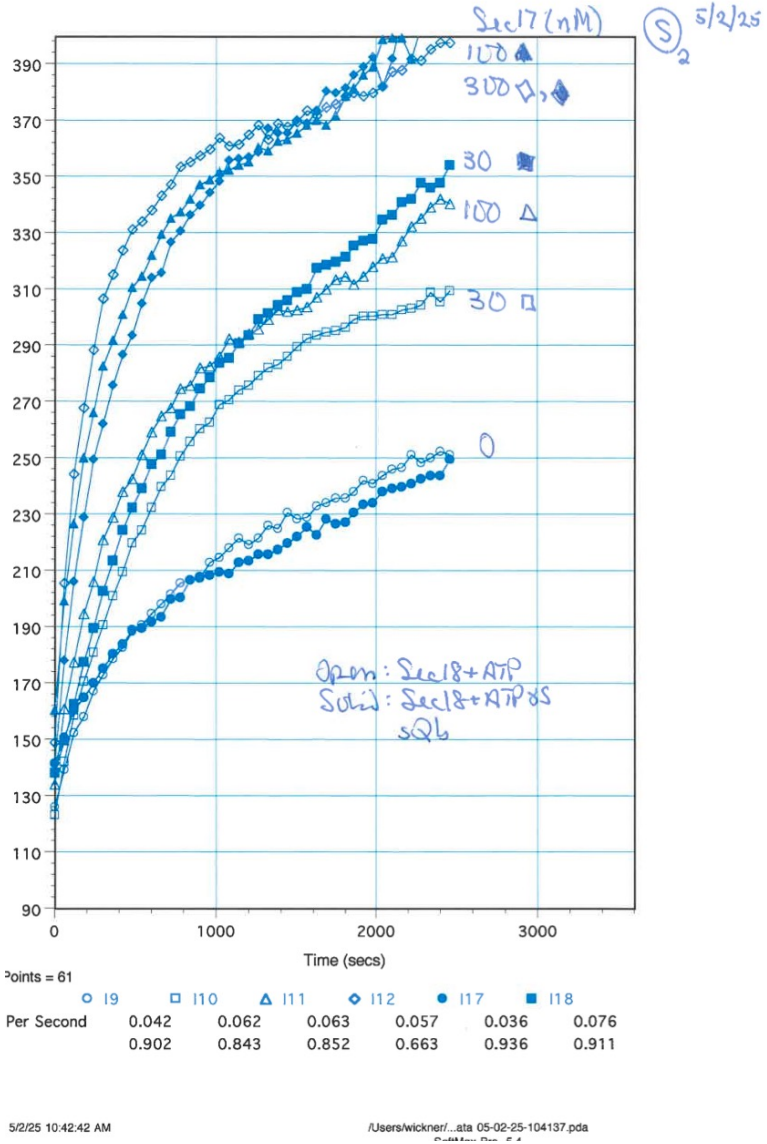
Earlier experiments used Qc vs Qc3Δ; here I try sQb vs Qb3Δ.

Result: ATP hydrolysis inhibits ~2-fold with sQb and 0.1μM Sec17, but totally with 0.1 μM Sec17 and Qb3Δ. So what's seen with Qc3Δ is also seen with Qb3Δ.

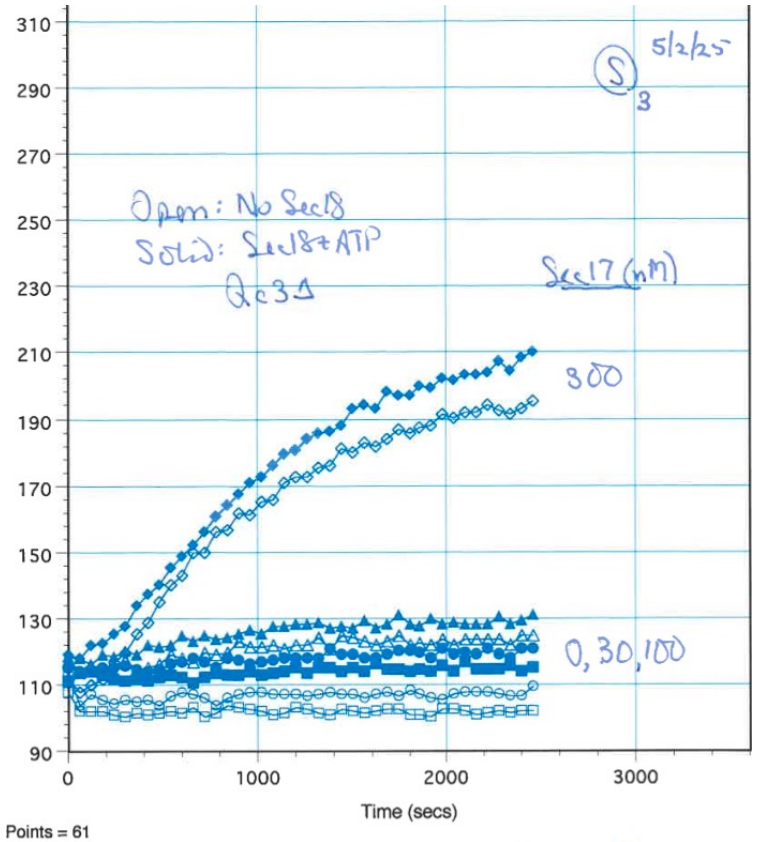
This experiment repeated well; I'll try for a 3rd time on Monday.



At every level of Sec17, including zero,
there's more fusion with Sec18/ATP than with only ATP.



Note that with with soluble Qb, allowing full zippering, and 30 or 100 nM Sec17, there is even more fusion with ATPγS than with ATP. ATP hydrolysis inhibits!



Points = 61

	I5	I6	I7	I8	I13	I14
Per Second	0.001	-0.000	0.004	0.037	0.002	0.001
	0.065	0.023	0.743	0.917	0.769	0.555

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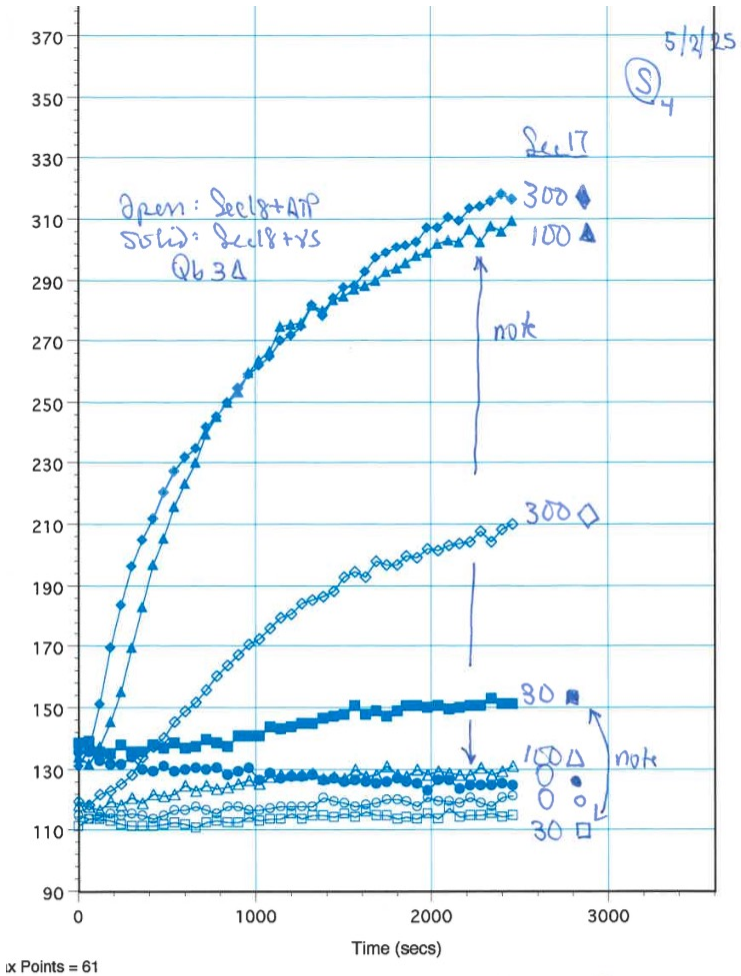

ix Points = 61						
I	○ I13	□ I14	△ I15	◇ I16	● I21	■ I22
ix Per Second	0.002	0.001	0.005	0.039	-0.004	0.008
	0.769	0.555	0.812	0.943	0.821	0.917

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