

Projects ongoing:

1. Sec17/18 Studies

- Flootation assay with 2mM lipid liposomes+ all protein possible
- Production of UT-Sec17_D1-30 (to start)

2. Proteins Nyv1 and Vam3 with single Cys in the Juxta region

- Make 4-snare complex in detergent with ROC228 and Qa-3C262/ ROC and Qa0C
- Derivatization of ROC228 and Qa-3C262
- Cross-link assays
- Run samples in Mono-Q FPLC (ROC228 and Qa-3C262, regular and derivatized)

3. Nanodiscs reconstitution

4. Making Ypt7-tm and Nyv1 with split GFP tag using pDuet system

5. 4-Snare assembly studies with Cys-Vam7 (*Qc) and Cys-sdVti1 (*sQb)

Proteins 2024/2025:

- MBP-R_{JxCys} (Me)
- MBP-Qa_{JxCys} (Me)
- His-Sec17 (Basic-Ser) (Bill)
- MBP-ROC (Me)
- MBP-Qa0C (Me)
- MBP-QcY42A_tmQb (Bill)
- MBP-Tev-Qb3delta (Me)
- MBP-Qa_wt (Me)
- GST-Qc3delta uncleavable (Me)
- MBP-QcSnareDomain_tmQb (Bill)
- Strep Tags (Amy): MBP-Qb_Twin Strep, MBP-Qa_Twin Strep, MBP-Strep-Qb
- UT-Sec17_D1-30 (Bill)

Week (3/10-14)

- 1) Induction tests of MBP-Tev-Qb3delta and MBP-Qa_wt.
- 2) Worked on statistics and figures
- 3) Transformation of GST-Qc3delta uncleavable into stock and expression cells, digestion and induction test.
- 4) Made more His-Sec18 ATP free
- 5) Glycerol stocks and updated E. coli database
- 6) Ordered MBP-QcSnareDomain_tmQb vector

His-Sec18 ATP free

BSA		
0	0	0
1	0.02	0.057
2	0.04	0.107
4	0.08	0.196
6	0.12	0.232
8	0.16	0.294

The scatter plot displays the relationship between BSA (y-axis) and dil factor (x-axis). The y-axis ranges from 0 to 0.35, and the x-axis ranges from 0 to 0.2. A blue line with diamond markers represents the data points, and a black line represents the linear regression fit. The equation for the linear fit is $y = 1.7874x + 0.0226$.

dil factor	BSA
0	0
0.02	0.057
0.04	0.107
0.08	0.196
0.12	0.232
0.16	0.294

	forecast	dil factor	mg/ml	uM
His-Sec18 ATP free	0.04900397	10	0.490	5.76

MBP-Qa_wt and MBP-Qb3delta: transformations and confirmations

Working on MBP-Qa and MBP-Qb3Δ

- Vectors received on 03.04.2025

- Transformed into:

- A) DH5α (Amp)
- B) BL21(DE3) (Amp)
- C) BL21 STAR (Amp)
- D) Rosetta pLysS (Amp, chloro)

03.10

- Re-streak two colonies each in fresh plates

03.11

- Grow inoculums of c.1 of each transformation
- DH5α: make glycerol stocks and minipreps for digestion
- Expression cells: glycerol stocks and induction test

03.12

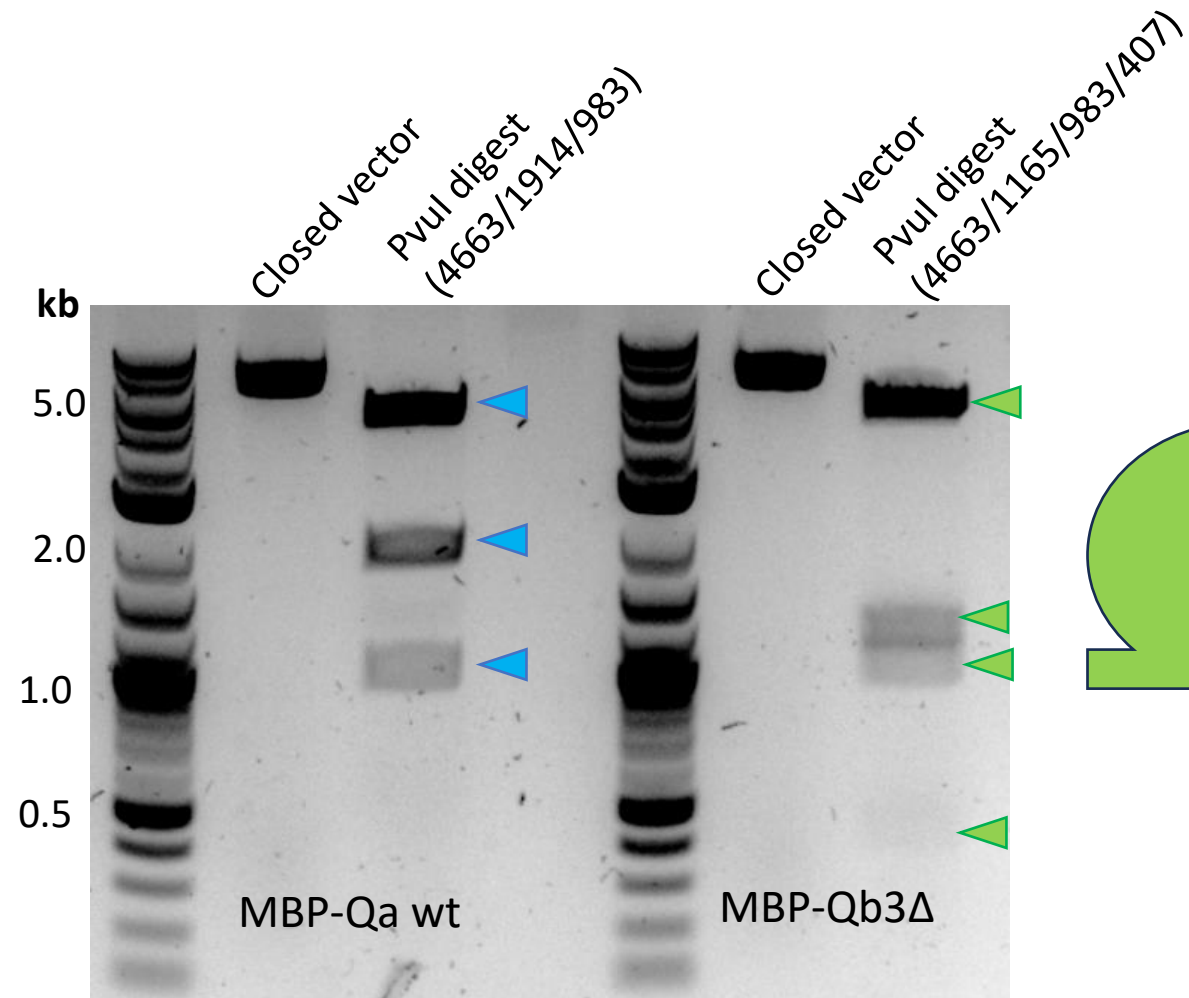
* Minipreps of DH5α

	nanodrop	for 500ng	Total (45μl)
1) MBP-Qa wt	94.6 μg/μl	5.3 μl	4.25 μg
2) MBP-Qb3Δ	126.5 "	4.0 μl	5.69 "

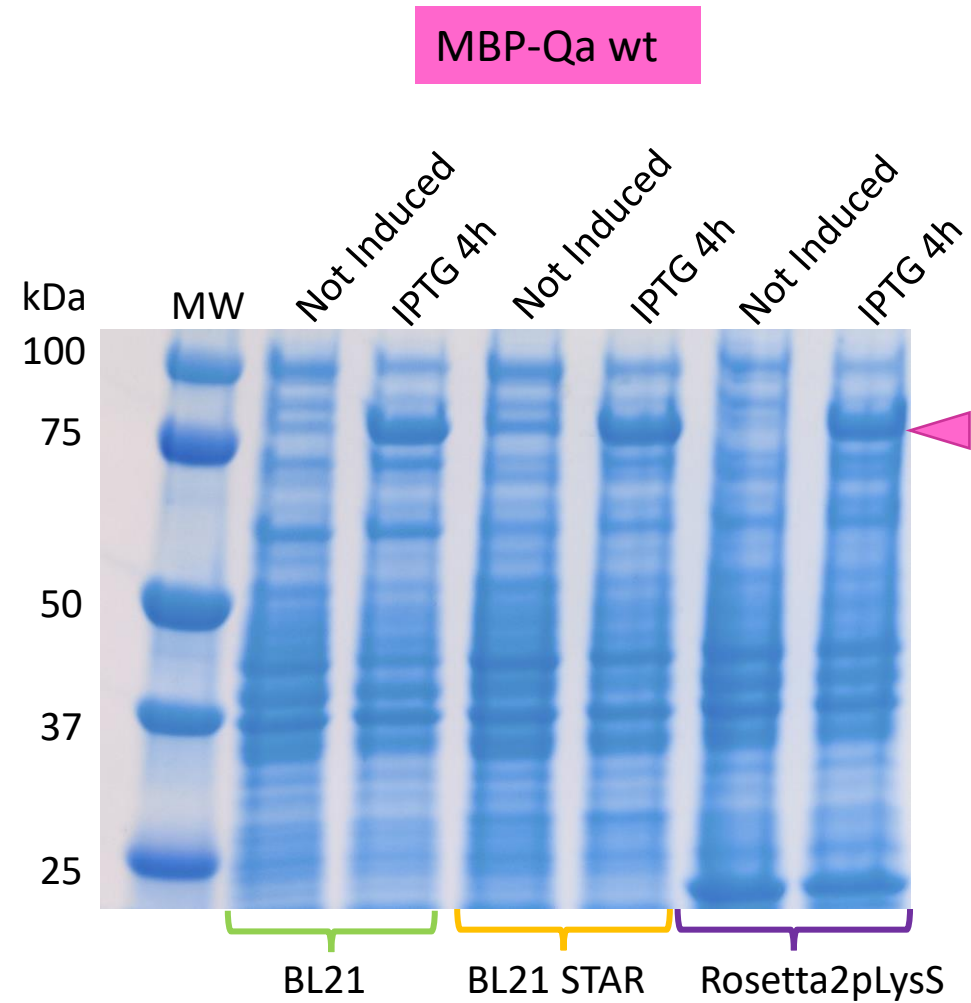
* Digestions

	MBP-Qa	MBP-Qb3Δ	} 25 μl rxn
500ng vector	5.3	4.0	
H ₂ O	16.2	17.5	
PvuI-HF	1.0	1.0	
Buf. CS	2.5	2.5	
Bands	4663/1914/983	4663/1665/983/407	

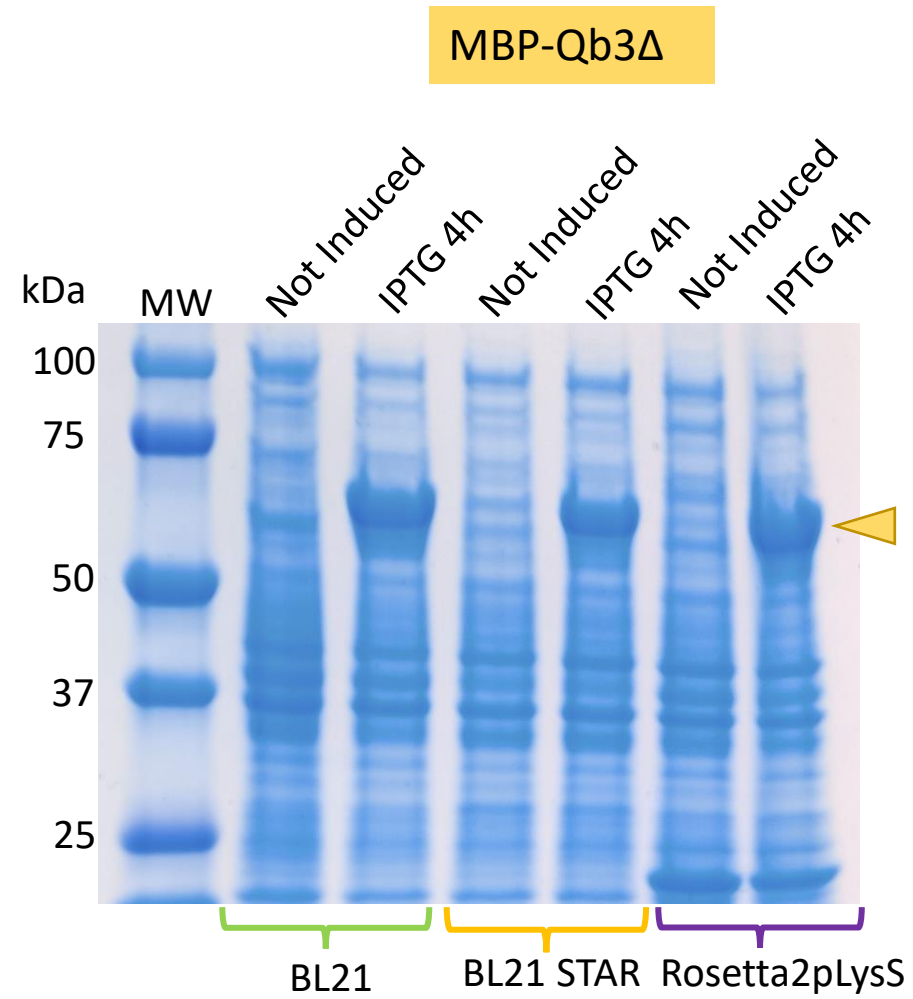
MBP-Qa_wt and MBP-Qb3delta: digestion



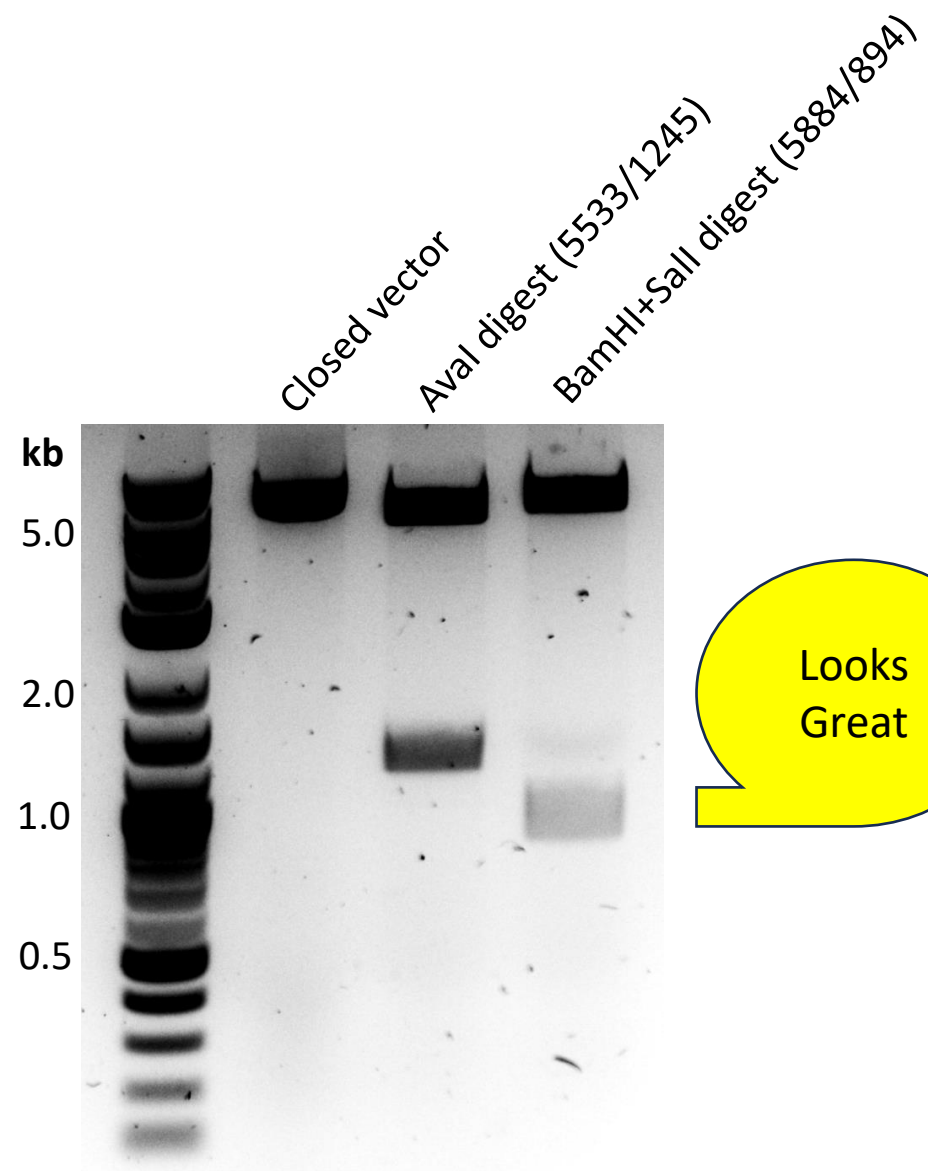
MBP-Qa_wt and MBP-Qb3delta: induction test



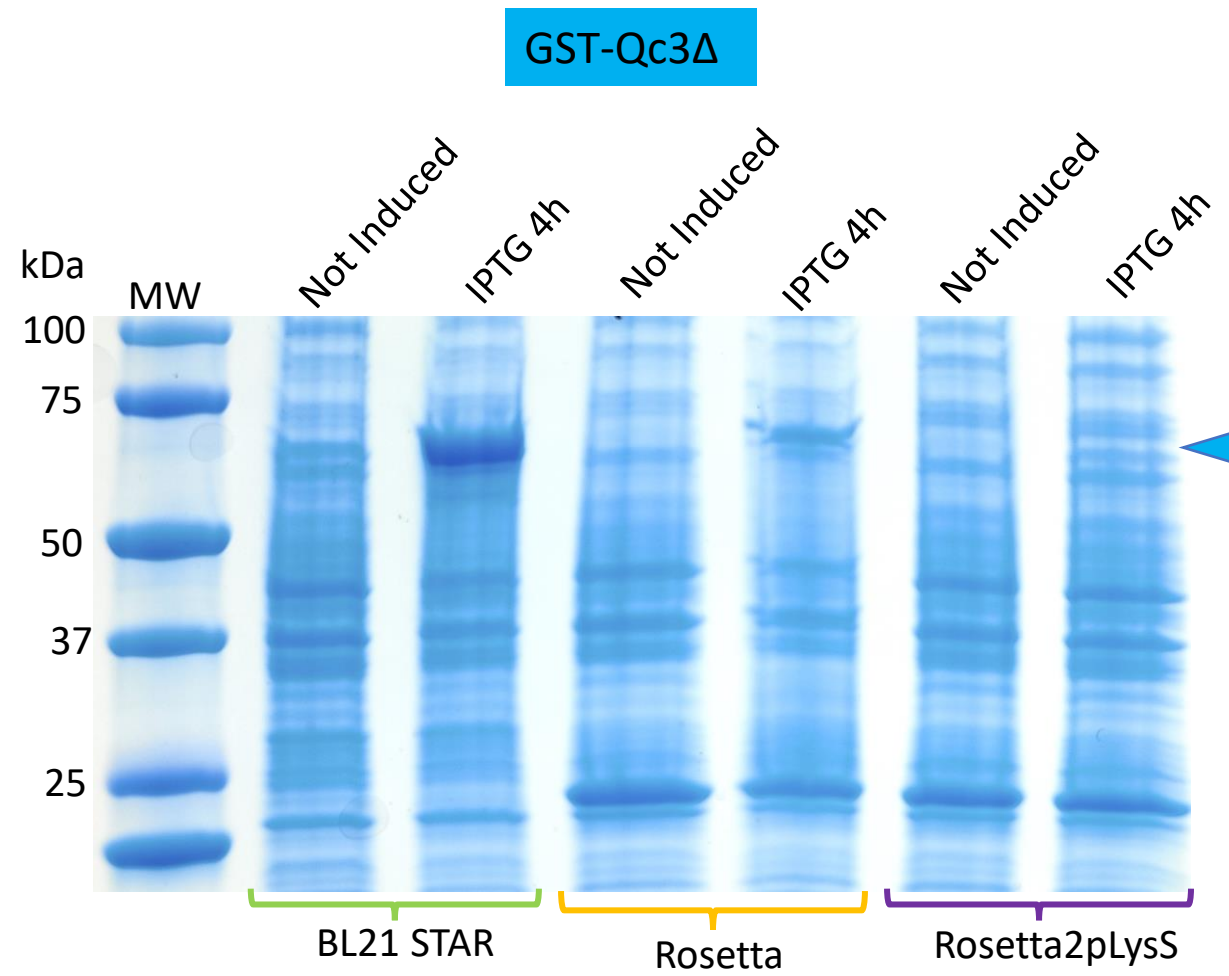
MBP-Qa_wt and MBP-Qb3delta: induction test



Uncleavable GST-Qc3Δ: digestion



Uncleavable GST-Qc3Δ: Induction test



Next steps:

- Production of MBP-Tev-Qb3delta, MBP-Qa_wt and GST-Qc3delta uncleavable.
- UT-Sec17_D1-30 production
- Try other combinations of cross-link with Qa+R(JxCys) and Qb3 Δ /Qc3 Δ , Qa0C/R0C...need proteins first!