GST/FLAG purification, mTEV cleavage, and NCL w/ test peptide

- A pellet from a 10 L fermentation was thawed, lysed and purified on an anti-GST, then an anti-FLAG column as described previously.

Biorad assay of fractions 4-12 of pure protein
(1 mL fractions were stored w/ ≈ 10% sterile glycerol at -20°C immediately after elution from the anti-FLAG column.)

fr. 4	46 µL	(← µL should say µg)
5Z	331 µL	
6Z	359 µL	1.05 mg of
7	206 µL	pure protein (uncut)
8	107 µL	
9	–	

Fraction 5 (330 µg of GST-ENLYFQ-Cys-Pax(38-557)-FLAG protein) was concentrated in two 50 KDa-cut off spin columns and redissolved in 616 µL of sterile water

mTEVp cleavage: ≈ 300 µg protein (in 616 µL water)
70 µL 10× mTEVp buffer
13.5 µL mTEVp (108 units)
The reactants were added to a sterile 1.5 mL eppendorf, flicked to mix, then incubated at 37°C for 1.5 hours.

The product mixture was exchanged into PBS buffer w/ 10 mM imidizole by concentrating in a 50-KDa spin column (this was cutting it close size-wise now that the GST tag has been removed - next time will use a 25 K-Da cut off) and then redissolving into 600 µL of the desired solvent

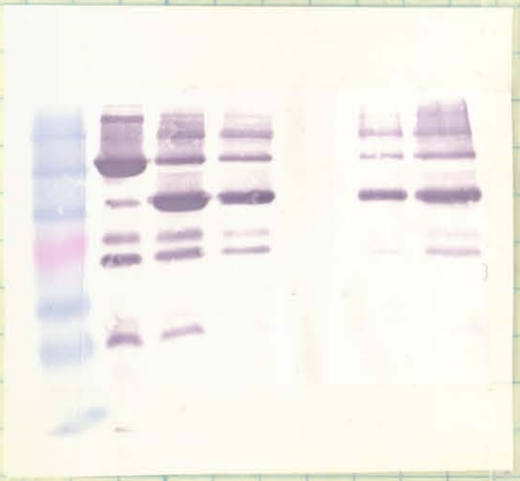
- Trapping of the mTEVp (w/ has a His_6 tag) w/ a Ni/NTA spin column

The column was equilibrate by adding 600 µL PBS buffer, then centrifuging with the cap open for two minutes at 2,000 rpm.

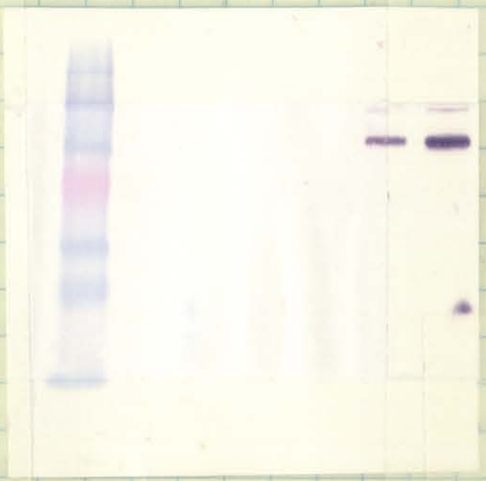
The Cys-Pax(38-557)-FLAG / mTEVp solution was added to the column, then centrifuged at 2 K rpm for 2 min. The flow through (which should contain the protein (Cys-Pax-FL) but not the His_6-tagged mTEVp) was collected in a sterile eppendorf.

Another 200 µL of PBS w/ 10 mM imidizole was added, centrifuged, and collected. The mTEVp remained on the column.

Native Chemical Ligation continued (from 111) Thursday, Jan. 22nd 2004



1 2 3 4 5 6 7
mouse anti-FLAG 1° ab
(7 µL in 15 mL TBST)



1 2 3 4 5 6 7
mouse anti-hexahistidine 1° ab
(5 µL in 15 mL TBST)

In 1 10 µL (plus 10 µL loading buffer) soln before mTEVp addition
2 10 µL (and 10 µL loading buffer) of the 700 µL mTEVp cleavage product mixture, t= 1.5 hours.
3 10 µL (plus 10 buffer) immediately after His column (flow through and wash) - before next concentration
4
5 10 µL (plus 10 buffer) of flow through from the last 50-KDa cut off concentration (to make sure the 59 KDa protein wasn't going through)
6. 3 µL (w/ 3 µL buffer) ligation product
7 10 µL (w/ 10 µL buffer) ligation product

← anti-hexahistidine 1° ab
30 25 µL of ligation product (plus 25 µL buffer)

← desired band

← unknown impurity - oh, silly, not unknown - that is some left over His6-tagged mTEV proteinase.