Tau purification protocol
Written by Sue-Ann Mok
(Based on Baghorn et al. 2005, *Methods in Molecolar Bioiogy, vol 299, pg 35)*
Additional modifications by A. Gillies and Sue-Ann Mok
Buffers and solutions:

Betaine/NaCl	Prepare 5M betaine solution in ddH2O. Dilute 1:50 into 5M NaCl.	Need 100 ml per L of culture
1 M DTT	Need 25 mL for entire prep	
500 mM EGTA	Prepare in ddH2O and pH to 8 to dissolve	
100 mM PMSF	Prepare in 100% EtOH. Store at -20 to +4C	
5M NaCl	Need about 20 ml for prep in addition to volumes required for other buffers	
	stock solution	volume added
resuspension buffer (300 mL)		
20 mM MES, pH 6.8	1M	6 mL
1mM EGTA	500 mM	0.6 ml
0.2 mM MgCl2	1M	0.06 ml
	total volume	300 ml
Just prior to prep add:		
1 mM PMSF	100 mM	1 to 100
protease inhibitor tablets		3 tablets per prep
5 mM DTT	1M	1 to 200 dilution
cation exchange buffer A (need about 10 L)		
20 mM MES, pH 6.8	1 M	20 ml
50 mM NaCl	5M	10 ml
1mM EGTA	500 mM	2 ml
1 mM MgCl2	1 M	1 ml
	total volume	1000 ml
Just prior to prep add:		
2mM DTT		1 to 500 dilution
0.1 mM PMSF		1 to 1000

		dilution
cation exchange buffer B (need about 1L)		
20 mM MES, pH 6.8	1 M	20 ml
1M NaCl	5M	200 ml
1mM EGTA	500 mM	2 ml
1 mM MgCl2	1 M	1 ml
	total volume	1000 ml
Just prior to prep add:		
2mM DTT		1 to 500
0.1 mM PMSF		1 to 1000

1. 140 ml starter culture inoculated directly from BL21(DE3) glycerol stock. For most Tau constructs, resistance is Kan. Use TB media + 50 µg /ml Kan. Cultures grown overnight at 33C for 16-20 h.

2. Inoculate each litre of fresh TB + 50 µg /ml Kan with 20 ml of starter culture. I usually grow 6 L total which will yield between 100-200 mg or protein. Cultures grown at 37°C, 200 rpm. When cultures reach an OD600 of about 0.6 (usually 2h), add 100 ml of betaine/NaCl solution per litre of culture. Change temperature of incubator to room temperature and continue to grow culture with shaking for another 30 min. By this time the cultures should be cooled to 30C.

3. **Set temperature of incubator to 30C.** Inoculate cultures with 200 µM IPTG per litre and grown at 30C for 3.5 h. Pellet cells and pool into 2X50 mL falcon tubes. Store pellet at -80C.

4. Resuspend cell pellet in ice-cold resuspension buffer. Use 100 ml for 6 L culture. Homogenize the cell solution with a dounce homogenizer.

5. Lyse the cells with the microfluidizer at 17, 000 kPa under ice-cold conditions. Rinse system with 50 -100 ml extra resuspension buffer and collect most of elutant. Total volume after lysis should be around 175 ml. Place the lysed cells in a 500 mL erlynmeyer flask.

   Note: Cells can also be lysed with a sonicator but this will result in increased amounts of proteolytic fragments. Since these fragments are hard to remove the final prep will be less pure.

6. Immediately, Add 5M NaCl to a final concentration of 500 **mM (1:10 dilution)** and boil for 20 min. By this treatment, nearly all proteins are denatured apart from tau protein, which stays in solution and maintains its physiological function

Note: For this step, set up waterbath on hotplate in a fume hood (DTT fumes). Ensure water is boiling vigorously! Use clamp to hold flask in waterbath. There is no need to stir during boiling. Lysate will not boil but solution should turn cloudy and then curdle (within10 min).

7. Pellet the denatured proteins and cell debris by centrifugation at 35,000g for 45 **min** at 4'C.

8. Transfer supernatant into dialysis tubing (3.5-5 kDa molecular weight cut-off) and dialyze overnight in Buffer A with constant stirring. 4L of buffer for every 75-100 ml of cleared lysate is sufficient. Depending on the contrast the solution will become cloudy.

9. Clear the dialysate by centrifuging again at 35,000g for 30 min at 4'C.

10. Prepare cation exchange column. Column is about 30 ml of SP sepharose packed in a XK26 column. Column should be clean and stored in 20% EtOH. Use flow rate of 5ml/min. Wash with 3 volume ddH_2O then 4 volumes Buffer A. If you are worried about column cleanliness, run 4 volume of Buffer B before equilibrating with 4 volume buffer A.

11. Apply the cleared supernatant onto the cation-exchange column using the sample lines at a flow rate of 5ml/min.

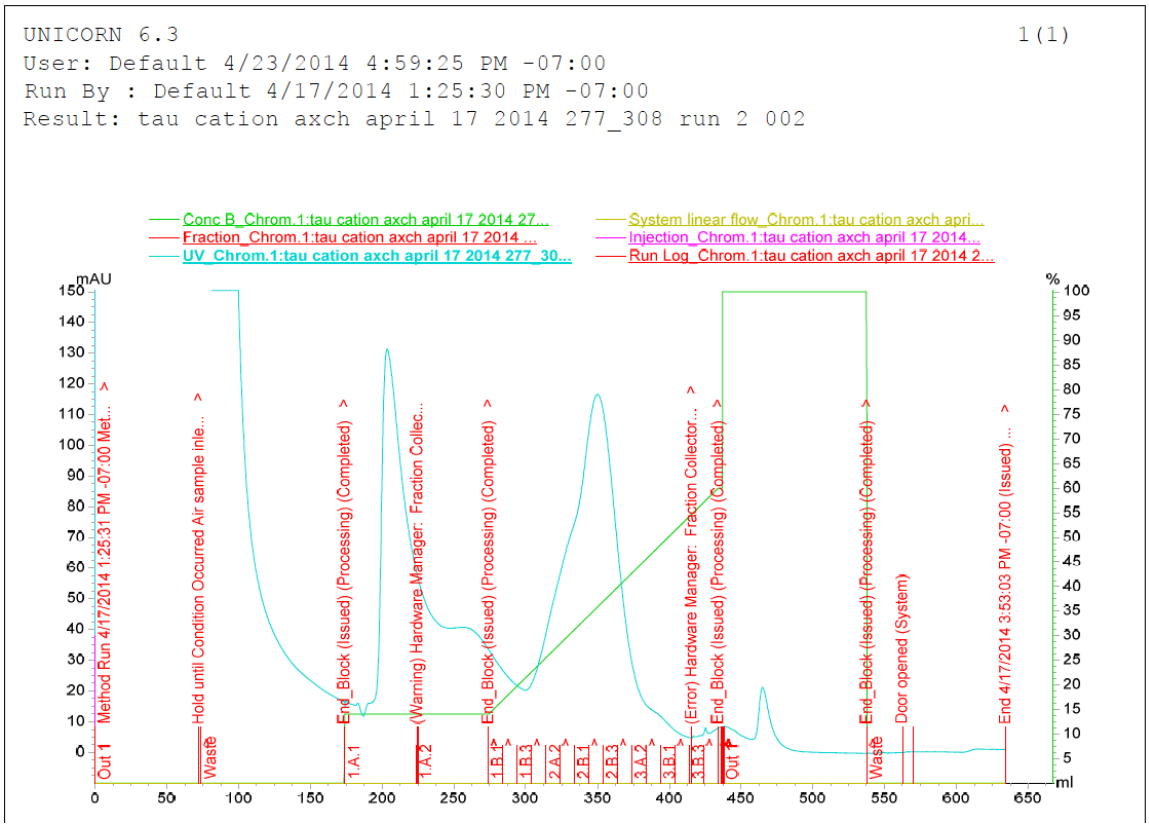
12. Wash out non-specific proteins with 3 to 5 column volumes buffer A until UV-absorption levels out. Flow rate 5ml/min.

13. Wash away more non-specific proteins with 100 ml of 14% B. Collect in 50 mL fractions (=fractions 1 and 2). Keep for analysis.

14. Elute protein with 14-50% B gradient over 124 ml. Collect in 7.5 ml fractions. Run 5 uL of each fraction on gel for analysis. Protein usually elutes in fractions 7-14 (se gel below). <u>Only pool fractions with highest ratios of full-length products.</u>

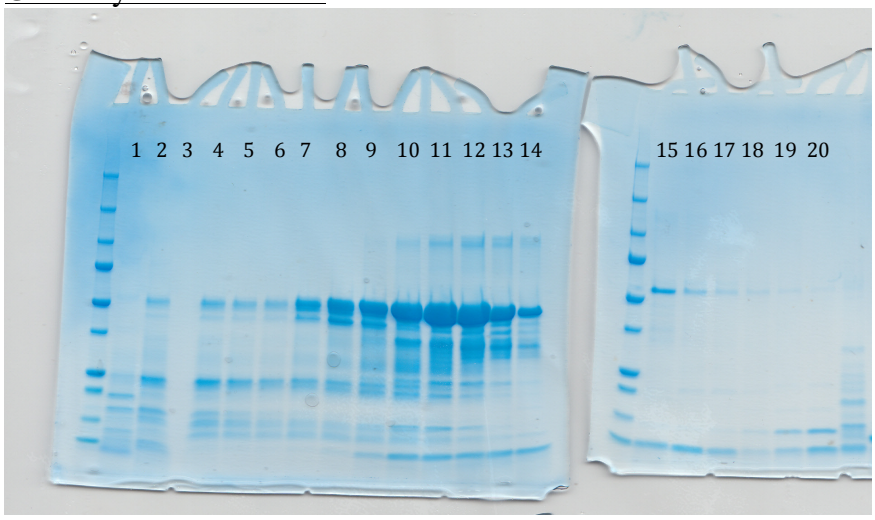
15. Purified tau can be concentrated with Amicon filters (10 kDa cutoff). Because tau is unstructured it may flow through larger pore filters. If required, dialyze protein O/N in the desired buffer (presence of 2 mM DTT or other reducing agent is necessary to minimize seed formation. Removal of some proteolytic fragments can be achieved using the SEC200 (PBS + 2mM DTT). Store protein in 0.5-1 ml aliquots at-80C.

<u>Representative chromatogram for cation exchange column:</u>



Note: Tau has no tryptophans leading to very low A280 readings. 100 mAu at peak of elution should represent about 100 mg of purified protein.

Gel analysis of fractions:



I usually pool only cleaner fractions. In this example, I would only keep fractions 10-14