Quantifying tau fibril concentrations and characteristics

Fractionating fibrils by centrifugation

* Pre-cool ultracentrifuge chamber
* Transfer fibrils to **special eppendorfs** compatible with 100,000 g spins.
  + Usually do 1 ml per tube but can do as little as 100 ul and still see a pellet after spin
  + Make sure you have an **exact balance for each tube**
  + Spin for 1h at 100,000 xg at 4C
* Collect most of the supernatant and transfer to **non-binding tubes**
* Remove the rest of the supernatant without disturbing the pellet if possible. Discard this solution as it is likely contaminated with some pellet material
* Resuspend the pellet in ½ to 1/4 of the original volume. Use D-PBS + 2 mM MgCl. Use a disposable plastic pestle to break up the pellet. Transfer to **non-binding tubes** for storage.
* Optional:Supernatant can be concentrated with 3K protein spin filters.
  + Spin at 10,000 rpm for 2 minutes at a time
  + Check to see if desired concentration achieved
  + Pipette up and down to resuspend fibrils/tau and transfer **to non-binding tubes**

Fractionating fibrils by centrifugation

* Load up to 400 ul of fibril solution into 0.2 um spin filters.
* Centrifuge at 10,000 rpm for 2 min.
* Collect the flow-through and transfer to **non-binding tubes**
* Add up to 400 ul of D-PBS + 2 mM MgCl to the filter. Pipette up and down to resuspend fibrils and transfer to **non-binding tubes**
* Repeat with more of the same fibril solution on the same spin filter if desired. **Do not reuse a filter for a different tau fibril solution.**
* Optional:Supernatant can be concentrated with 3K protein spin filters.
  + Spin at 10,000 rpm for 2 minutes at a time
  + Check to see if desired concentration achieved
  + Pipette up and down to resuspend fibrils/tau and transfer to **non-binding tubes**

Quantify amount of tau in fractionated samples.

* Mix 5 or 10 uL of tau sample with 3X SB
  + 10 uM tau solution = 0.43 mg/ml
  + Depending on how you fractionated or concentrated your samples you can have anywhere between 0.01-2 mg/ml concentrations
    - E.g. 100,000g spin pellet resuspended in ¼ original volume ~2 mg/ml
    - E.g. 100,000 g spin supernatant, not concentrated~0.05 mg/ml
    - E.g. 0.2 micron spin pellet ~0.3 mg/ml
    - E.g. 0.2 micron spin supernatant, not concentrated~0.1 mg/ml
* Prepare aliquots of tau monomers to generate standard curve samples
  + E.g. 0N4R WT monomer stock=237 uM=10.2 mg/ml
  + 1:5 X dilution = 2 mg/ml
  + Made standards of 2, 1.5,1,0.5, 0.4,0.3,0.2,0.1 ug/ml in final of 1X SDS SB (5ul stand + 2.5 ul 3X SB)
    - You can pick your standards based on what samples you are running (see above estimates)
  + Load your samples and standards on the **same gel**
    - Use the gel imaging software to calculate your sample concentrations
    - Don’t forget to back-calculate if you loaded more sample than standard