Protocol to polymerize short microtubules

1. Make sure that liquid nitrogen is available

2. Turn on the water bath and set the temperature to 37°C.

3. Calculate the amount of M2B, GMPCPP, DTT, unlabeled and labeled tubulin you need to prepare the volume of microtubules you want. For this use the Matlab file "MT_preparation".

4. Take the GMPCPP, DTT and M2B out of the fridge and put them in a box with ice.

5. Take the unlabeled tubulin and the labeled tubulin out of the freezer, and remember to cover the labeled tubulin with aluminum before putting them in the box with ice.

6. Introduce all the ingredients in an Eppendorf tube (GMPCPP, DTT/25, M2B, unlabeled and labeled tubulin) and DO NOT VORTEX THEM. Instead mix them by gently sucking and realizing the solution in the tube approximatively 15 times.

7. Place and let the Eppendorf tube with the solution in the hot water bath for 30 minutes. You can seal the tube cap with parafilm to avoid losing the solution in the bath.

8. Take the tube out of the bath and cover it with aluminum. Let the microtubules solution stand for five hours at room temperature.

9. **PUT THE CRYOGENIC GLOVES AND GOGGLES** to fill an empty pipette tips box with liquid nitrogen.

10. Introduce 2 μL of microtubules in an Eppendorf tube and immediately throw the tube into the box with the liquid nitrogen. DO NOT INTRODUCE YOUR HAND IN THE BOX!! Repeat this process until the microtubules solution is over and that you filled as many tubes as possible.

11. Preserve all the microtubules aliquots in the freezer at -80°C.