

Glass slide preparation

The glass slides are coated with an acrylamide brush which makes them hydrophilic and prevents proteins from sticking to the glass.

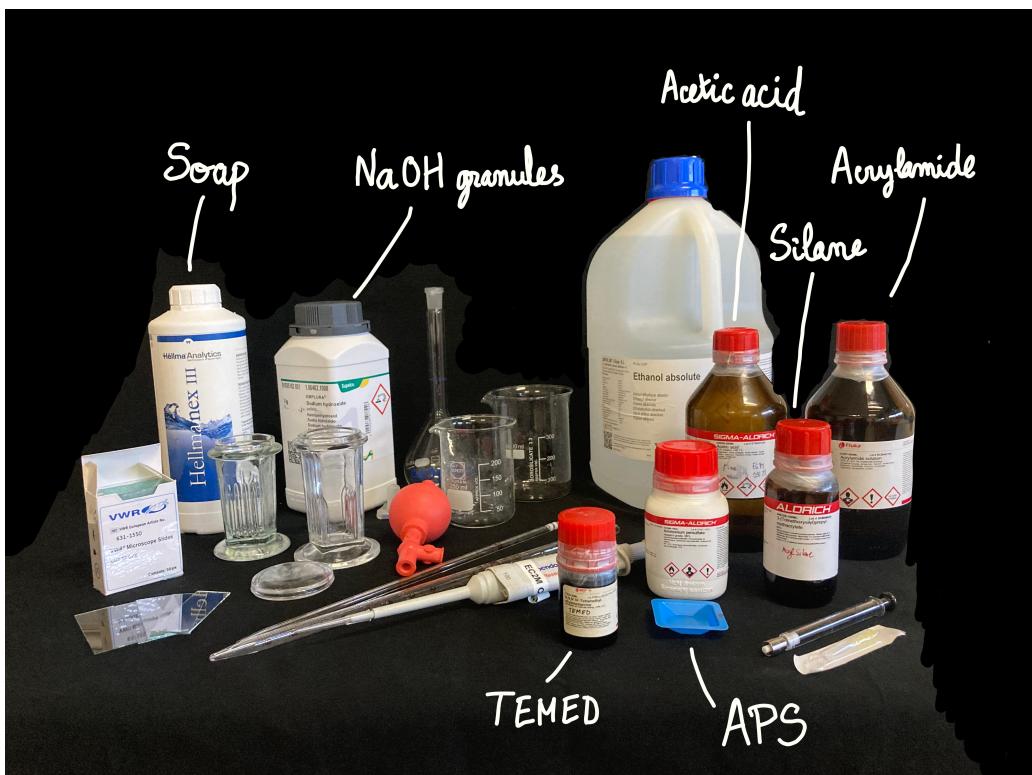
Wear gloves + blouse at all time

Hardware

- 2 « cuves de coloration de Coplin » + their glass lid (they have 5 slots, but you can add 4 more glass slides if you use the diagonals).
- 18 glass slides for microscope
- 1 Erlenmeyer 250 mL + cap
- 1 beaker of 500mL
- 1 beaker of 200mL
- 1 glass pipette of 10mL
- 1 glass pipette of 2 mL + red pipetting bulb
- 1 glass piston syringe of 2.5mL + needle
- 1 pipette for 100 μ L + tip
- precision balance+ blue plastic weighing cup
- ultrasound bath
- 1 source Nitrogen gas under a hood (in the clean room, there is a stick to hold the pistol)
- magnetic stirrer (+ magnetic stick)

Chemicals

- Ammonium persulfate solid (APS). It is a powder, moisture sensitive, bottle closed with parafilm.
- Hellmanex soap. (a white plastic bottle of 1L with a blue tag on it). This soap is strong, take some with a pipette pasteur.
- NaOH granules (3) SPM should have some if it is not inside chemistry room.
- ethanol 70%
- ethanol 99% (the purest you can find)
- TEMED (Tétraméthyléthylénediamine)
- acetic acid (98.5% purity)
- 3-(trimethoxysilyl)propyl methacrylate (let's call it silane)
- Acrylamid solution (40%), in the fridge in the chemistry room.
- distilled water



Protocol

Place 3 NaOH granules in the erlenmeyer, and fill with distilled water until 100µL.
Put the 18 glass slides in the 2 Coplin cuve

Cleaning

Step one : soap

-fill the cuves with water. With a pipette pasteur, add a few drops of Hellmanex soap in each cuve.
Put them in ultrasound bath for 10 min.

-Empty the cuves in the sink (with one finger accross on top so that the slides do not fall) and fill with distilled water. Manually shake them a little, and empty them. Rince this way 5 times in total.

Step two : ethanol

-fill the cuves with 70% ethanol. 10min in ultrasound bath

-Rince (like before)

Step three : NaOH

-fill the cuves with NaOH solution. 10min in ultrasound bath

-Rince (like before)

Acrylamide coating

Step One : prepare 2% acrylamide solution

In the 500mL beaker, place about 100mL of distilled water. Add :

-10mL of 40% acrylamide solution (glass pipette + red bulb)

-fill until 200mL with distilled water

-degas with N2 pistol under the hood (turned on) in the clean room for minimum 15min, up to 30 min

Step 2 : silane bath

While the acrylamide solution is being degassed, in 200mL beaker, place :

-about 100mL of pur ethanol

-2mL of acetic acid

-1mL of silane (use the glass syringe + needle)

-fill until 200mL with ethanol

-briefly stir with magnetic bead (1min)

-fill the 2 cuves with the glass slides with this solution and let them bath for 13 minutes (use a timer)

Step 3 : acrylamide bath

During the silane bath, bring back the acrylamide solution that has been degassed. Add :

-70µL of TEMED

-140 mg of APS

-briefly stir with magnetic bead

-Once the 13 min in silane solution are over, empty the cuves (holding the glass slides inside as usual), and immediately pour the acrylamide solution inside.

Close the cuves with their caps, label them and seal them with parafilm around. After 3 hours, the glass slides are ready to be used. Use within 2 months if possible.

Active solution preparation

It contains everything (motors, ATP, enzymes, PEP, crowding agent PEG, surfactant Pluronic, antioxydant...) but not the microtubules.

Take a polystyrene box, fill with 3 shovels of ice from the biology room. Place inside a red metallic tray. Grab hardware and chemicals :

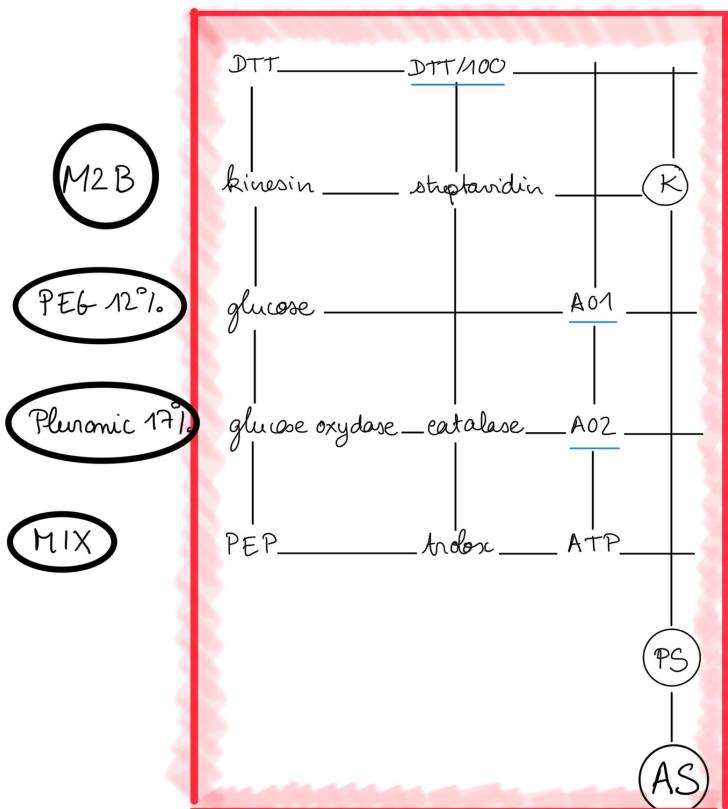
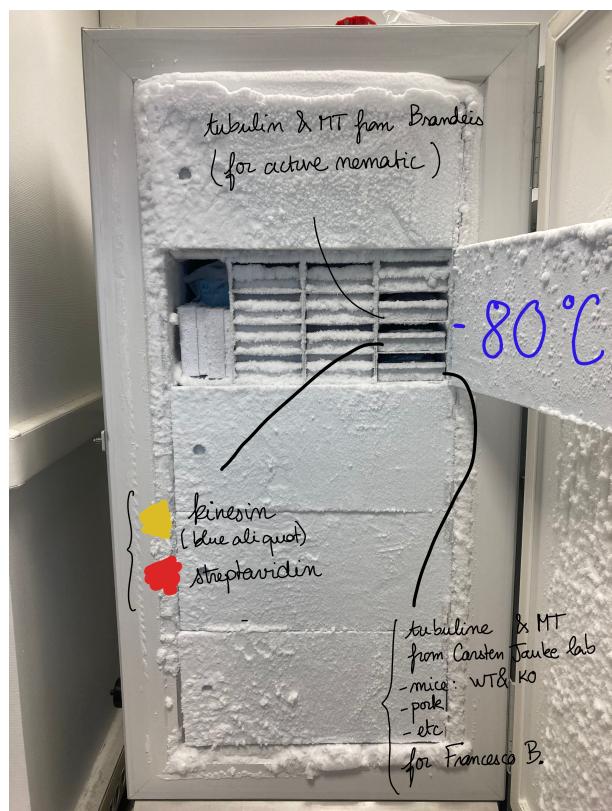
Hardware :

- pipette 2.5µL, 10µL and 100µL and associated pipette tips.
- 200µL aliquots, with flat cap

Chemicals

Fridge (4°C) chemistry room	Freezer -20°C chemistry room	Freezor -80°C biology room
<ul style="list-style-type: none"> -M2B (buffer) -Mix -PEG at 12% -Pluronic at 17% 	<ul style="list-style-type: none"> -PEP (ATP regenerator) -Trolox -DTT -ATP -Glucose oxydase (yellow) -Catalase (slightly green) -Glucose -PKLDH : enzymes to regenerate ATP, has to stay as less as possible out of the freezor) 	<ul style="list-style-type: none"> -kinesin (molecular motor) yellow sticker, in 10µL blue aliquots -streptavidin (to bind kinesins) red sticker -Microtubules (MT)

To avoid confusing the aliquots, place them according to this map representing the red metallic tray :



Protocol

- 1) Dilute x100 DTT : $49.5\mu\text{L M2B} + 0.5\mu\text{L DTT} = \text{DTT/100}$
- 2) Prepare motors K : add to the aliquot that contains the kinesin :
M2B ($10\mu\text{L}$) + streptavidin ($2\mu\text{L}$) + DTT/100 ($1\mu\text{L}$)
Vortex and let them sit on the metallic tray for 30min. The cold will help streptavidin-kinesin bindings. Meanwhile proceed to the next step :

3) AO1 and AO2

- $5\mu\text{L glucose} + 5\mu\text{L DTT} = \text{AO1}$
- $5\mu\text{L glucose oxydase} + 5\mu\text{L catalase} = \text{AO2}$

4) Prepare pre-solution PS by mixing :

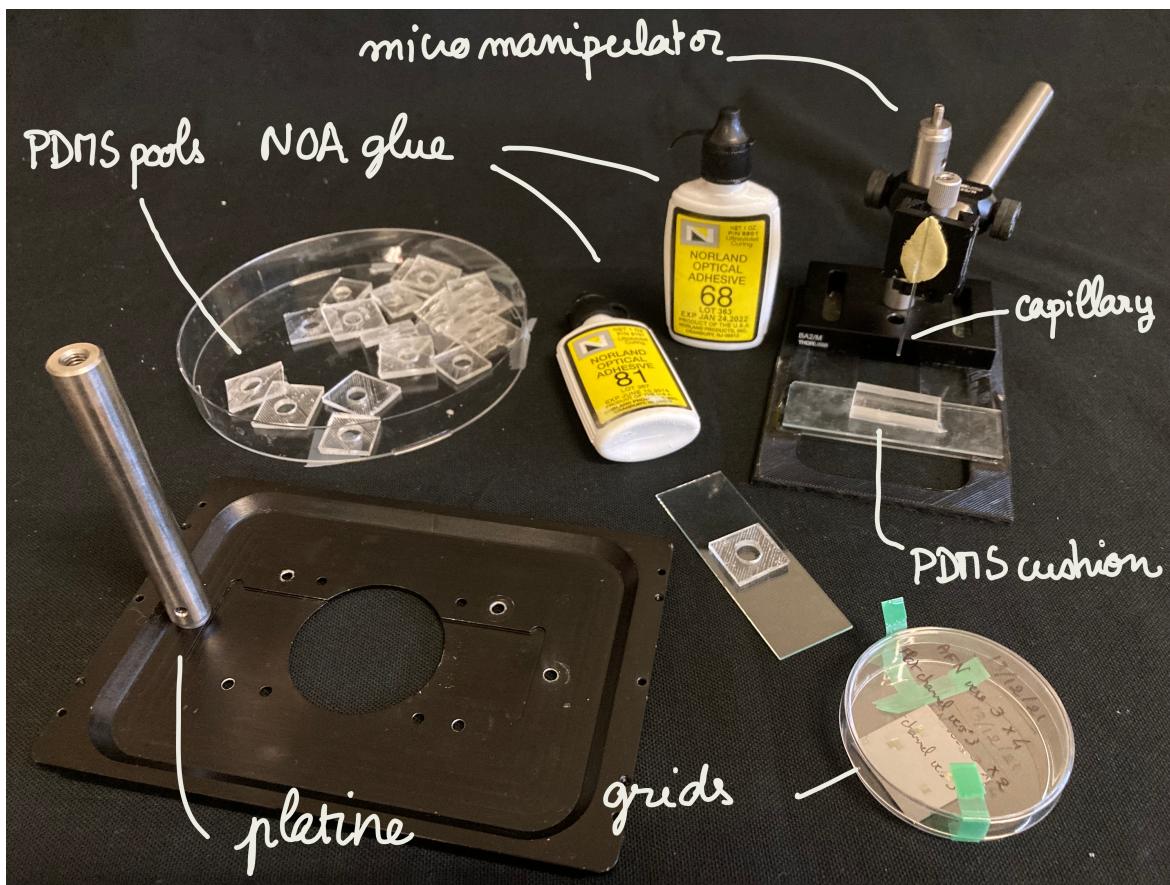
8 $\mu\text{L PEG}$
8 $\mu\text{L PEP}$
6 $\mu\text{L Trolox}$
2.9 $\mu\text{L Mix}$
1.7 $\mu\text{L ATP}$
1.5 $\mu\text{L Pluronic}$
1.33 $\mu\text{L AO1}$
1.33 $\mu\text{L AO2}$
1.7 $\mu\text{L PKLDH}$ (at the last minute)
Vortex PS

5) Active solution AS :

15 μL of K + 31.5 μL of PS = AS

Dispatch AS in aliquots of 2.5 μL each. Close well each of the aliquots. Place them inside an eppendorf falcon, label and store at -20°C in the freezor. Can be used without any issue for 1month.

Experimental set-up



Prepare the observation chamber :

With a glass slide tweezers, grab a glass slide from the acrylamide cuve, and rince it well with distilled water over the sink (both sides, water flow from the cleanest part to the dirtiest). Dry the slide with the air pistol.

Place a PDMS pool in the middle of the glass slide, the face with the trenches on the glass. Drop some glue Norland Optical Adhesive (NOA, yellow falcon inside the small fridge in the microscopy room) all around the pool. The glue seeps under the PDMS due to capillarity. As soon as the glue has reached the circular pool everywhere, expose to UV light for 2 good minutes. Use the hand-UV lamp, wear protective glasses, close the curtain around you.

Prepare the active gel :

In a polystyrene box filled with ice, place, on the red tray, 1 aliquot of AS + 1 aliquot of PEP. Get 1 aliquots of microtubules in the biology room, inside the -80°C freezer. **NEVER put microtubules on ice** (it will break them). When you take the MT aliquots out of the freezer, hold it between your fingers to warm it up quickly to 37°C. Then place it in the polystyrene. (Reversely, to freeze MT to -80°C, we flash-freeze them in liquid nitrogen.) One aliquot of MT contains 2µL at 8mg/mL in tubulin concentration. With one aliquot, you can thus do about 4 experiments. You do not need to get a new aliquot for each experiment you prepare during the day.

2.5µL of AS + 0.5µL of PEP + 0.5µL of MTs = AG (Active gel)

IMPORTANT : if you use the PEP from 21/01/2022, it is apparently not necessary to add 0.5µL of PEP.

Place 2.5 μ L of AG at the glass surface in the pool, then cover immediately with 100-200 μ L of silicon oil V20.

The active nematic layer will take about 20 to 45 minutes to assemble at the interface between water and oil. If the system is weird, it is often related to the microtubules. There is some variability in the aliquots, so it does not mean that you did something wrong ! Once the nematic layer has assembled, you can introduce the grid.

Grid preparation :

- 1) Remove the grid from the silicon wafer by covering with isopropanol for 30s, and pushing delicately with the tip of a scalpel. Transfer the grid from the wafer to the PDMS cushion (dedicated to this only). Dry gently with air pistol.
- 2) Cut one third of a 1mm capillary, fix it vertically to the « patafix » on the small support. Using the micro-screw, dip the unbroken end of the capillary in a drop of glue Norland Optical Adhesive (NOA). Lift up the grid once you see enough glue as climbed inside the capillary.
- 3) Place the grid on its PDMS cushion under the capillary. Bring the capillary in contact with the grid. Then reticulate the glue with the UV lamp (glasses + curtain).
- 4) Cover the grid with one drop of isopropanol to prevent it from sticking to the substrate, then lift the capillary with the micro screw. The grid should come with it. Dry gently with the air pistol, place in a petri box and label.

Observing and recording :

Remove the light condensator from the microscope. Remove the platine and replace it with the platine from my drawer (Claire). This one has a metallic pole screwed into it. We use it to fix the grid to it.

Switch on every device associated to the confocal microscope from left to right. Turn on the red button corresponding to the red laser on the big white box. Then open the software NIS. Press live, and turn on the laser by clicking on « CSU-CY5 ». You should see some red light.

Use the x10 objective, exposure time between 200 to 500ms. You can change the laser power, try to keep it below 60%. Changing the LUT will improve the contrast of the image on the screen. Make the focus on the active nematic layer (usually, z is about 2000 μ m to 5000 μ m).

Select a region of interest (ROI), so that you record only the field of view.

Typically, record movies of 2min or 5min, 2fps. (in the tab « ND acquisition »).

Setting the grid at the interface :

Once the AN layer has formed, Place the grid above the oil, approximately in the middle of the pool. Move in x y plane until you see a lot of red light diffracted by the grid. Increase the z until you see the grid in the field of view. Move to the part of the grid that is interesting (in the middle, it has channels/holes...). Then lower z to go back to the AN layer.

You can start lowering the grid using the microscrew. This process should be slow, especially when the grid start to appear in the background. When the grid reaches the interface, microtubules under the grid will pack into even brighter bundles, and « float away ». Wait 5 min, play with the focus and you should see the AN filling the grid channels.

After the experiment, remove the PDMS pool from the glass slide, clean it with isopropanol, dry and put it back in the box. They can be used multiple times.

