Substrate Prep for smFRET (or any single molecule immobilization experiment)

Tools needed:

- Sonicator with mesh basket accessory
- Glass Coplin jars
- Polypropyl Coplin jars
- Foodsaver + vacuum seal bags
- Pipette tip boxes
- Tweezers
- Nitrogen tank with blow gun accessory (D-20 from motor guard)

Reagents needed:

- Quartz slides
- 1M KOH
- MeOH
- Acetone
- MilliQ water
- Aminopropylsilane
- mPEG-SVA
- biotin-PEG-SVA
- acetic acid
- sodium bicarbonate

<u>Step 1:</u> Slide Cleaning (note: slides need more cleaning than coverslips bc quartz slides are reused while glass coverslips are fresh each time)

- Boil slides in a microwave for 5-10 minutes.
- Remove boiled slides. If reusing quartz slides from a previous experiment, use a razor blade to remove the epoxy and coverslip.
- Scrub slides with MeOH and tap water. Put them into a glass Coplin jar.
- Rinse Coplin jar with MilliQ water 3 times. Pour in 10% alconox and sonicate for 20 minutes
- Flush Coplin jar with MilliQ water.
- Pour in MilliQ Water and sonicate for 5 minutes.
- Rinse with MilliQ water
- Rinse with acetone. Fill with acetone and sonicate for 15 minutes.
- Pour out acetone. Rinse with MilliQ water.
- Transfer slides to polypropylene Coplin jars.
- Fill polypropyl Coplin jars with 1M KOH. Sonicate for 20 minutes.
- Dispose of KOH and rinse with MilliQ water
- Rinse slides with MilliQ water, dry with nitrogen and place in any empty containers.
- Burn slides thoroughly for a minute using propane torch. Place them into a clean, dry glass reaction container (glass Coplin jar).
- Allow the glass Coplin jar to cool for 30 minutes at room temperature.

<u>Step 2:</u> Coverslip Cleaning

- Place coverslips into polypropyl Coplin jars. Wash them with MilliQ water
- Pour in 1M KOH and sonicate for 20 minutes.
- Dispose of KOH and rinse with MilliQ water
- Rinse coverslips with MilliQ water, dry with nitrogen and place in any empty containers (empty pipette tip boxes).
- Burn coverslips with propane torch for ~1 second. Place them into a clean, dry glass reaction container (glass Coplin jar).

***Step 3:* Cleaning Reaction containers = glass Coplin jars**

- Sonicate reaction containers (glass Coplin jars) with 1M KOH inside for 20 minutes
- Dispose of KOH and rinse with MilliQ water
- Rinse with MeOH and fill with MeOH. Sonicate for 20 minutes.
- Dispose of MeOH. Rinse with MeOH and dry.

***Step 4:* Aminosilanization**

- Take aminosilane out of freezer. Leave at room temp for a few hours in dark.
- Pour 100 mL of MeOH into a reaction flask (glass flask). Using a glass pipette, add 5 mL of acetic acid.
- Using a glass pipette, add 1mL of aminopropylsilane. Mix well.
- Pour the mixture into the reaction containers from Step 3 (glass Coplin jars containing slides / coverslips) and incubate for 10 minutes.
- To prepare for the next use:
- Rinse the used flask with MeOH and MilliQ water. Fill with 1M KOH.
- Dehydrate aminopropylsilane in vacuo for >15 minutes.
- Seal aminopropylsilane bottle under nitrogen. Store in freezer.
- Sonicate the reaction containers (glass Coplin jars with aminopropylsilane-MeOH-acetic acid mixture) for 1 minute. Then incubate for another 10 minutes.
- Rinse coverslips with MeOH and water. Blow dry with nitrogen and place into clean pipette tip boxes.
- Rinse slides with MeOH and water. Blow dry with nitrogen and place into clean pipette tip boxes filled with water (See step 5-1 below).
- Rinse reaction containers with MeOH and MilliQ water. Fill with 1M KOH.

***Step 5:* PEGylation**

- Put water in the bottom of pipette tip boxes
- Take PEG bottles from freezer and place in dark at room temperature.
- Make fresh buffer for PEGylation = 10 mL MilliQ water + 84 mg sodium bicarbonate
- Place 1-2 mg biotin-PEG and 80 mg mPEG and place into a 1.5 mL eppendorf tube
- To prepare for next use
- Place PEG bottles in vacuum
- Seal under vacuum and store at -20C in a dessicant bottle.
- Add 320 uL of PEGylation buffer to the 1.5 mL eppendorf tube and mix gently by pipette. Spin for a minute at 10k rpm.
- Mix well (no bubbles) and drop 70 uL onto each slide (which is already in a pipette tip box).
- Place a coverslip gently onto the slide (no bubbles).
- Put the boxes in a dark and well-leveled place. Incubate for 2-3 hours.
- Check after 10 minutes and restore and coverslips that have become displaced.
- Disassemble slide-coverslips, rinse with plenty of MilliQ water and dry completely by nitrogen. Store them temporarily in clean pipette tip boxes, PEG side up.
- Assemble slides according to the application.
- Place the slides in a dark and dry place (Black tape + wrapped Corning tube, wrapped in Foodsaver)

Assembly of single molecule immobilization flow chambers

Supplies needed

Quartz slides
22 x 40 mm coverslips
5-minute epoxy
1 mm Tubing
Diamond drill bits
Crystallization dishes
Dremel and Dremel workstation
23G Syringe Needles
1 mL syringe
Flow solutions in 1.7 mL Eppendorf tubes
Pipette tips
Razor blades
Parafilm

- Set up Dremel workstation with 1 mm drill bits. Make sure the tool height is set such that it can go all the way through a glass slide
- Drill holes into quartz slide
- Mark off hole locations with black marker (according to design schematic)
- Peck drill all the way through the slide with a 1mm diameter diamond drill bit
- Clean / functionalize glass according to protocol
- Cut two 30-cm pieces of tubing. Discard the first ~2cm to avoid getting dust into the flow chamber. Cut the new ends at a ~30-45-degree angle (this helps insert the tubing into the drilled holes and helps avoid plugging of the flow device)
- Insert tubing into holes. Make sure the tubing can stand on its own.
- Apply epoxy to the tubing (make sure there are no gaps). Allow the epoxy to set for 5 minutes
- Cut away excess tubing using a razor blade.
- Flip over and apply double sided tape. Before applying, get rid of dusty tape. Also, get rid of bubbles by pushing down with a pipette tip.
- Put coverslip in place using tweezers. Push down using pipette tip to remove air bubbles.
- Cut away excess tape using a razor blade and epoxy the cover slip in place. Make sure there are no gaps.
- Slide a 23G syringe needle into the entry port tubing of the flow device. The needle may be sealed in using epoxy, but you don't have to.
- Seal both ends of tubing using Parafilm to avoid any dust getting into the device.
- Store the device in a Corning 50 mL tube. Wrap the tube with black construction paper and seal with Foodsaver.