Preparation of oleylethylene glycol-sreptavidin surfaces for SPR V.2

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Coronavirus Method Development Community P4SPR

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Surface plasmon resonance uses gold surfaces for sensing. Manufacturers provide a range of pre-functionalised surfaces, but these are often prone to non-specific binding problems. In other surface science sensing techniques a range of surface functionalisation approaches have been described. Here, the preparation of a self-assembled monolayer (SAM) of a thiolated oleyl ethylene glycol, incorporating a defined mole % of biotinylated ligands , on a gold surface is described. This allows the formation of a streptavidin layer on the SAM with control over the average surface coverage of streptavidin. Biotinylated ligands can then be immobilised on the streptavidin. Such surfaces have proved to be very resistant to non-specific binding and they are easily implemented on the sensor surfaces of commercial (surface plasmon resonance) SPR instruments.
This is adapted from a published method: Migliorini, E. et al. Well-defined biomimetic surfaces to characterize glycosaminoglycan-mediated interactions on the molecular, supramolecular and cellular levels. Biomaterials (2014) doi:10.1016/j.biomaterials.2014.07.017.

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Details relating to the stability the surfaces updated - even better than originally thought!

Surface plasmon resonance, SPR, gold, thiol, self-assembled monolayer, biotin, streptavidin, heparin, protein-glycosaminoglycan binding

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Glassware can be cleaned a few days in advance and oleyl ethylene glycol solutions prepared in cleaned glassware in advance.

MATERIALS

PEG

thiol **Polypure Catalog #10156-0795**

Biotin PEG

thiol **Polypure Catalog #41156-1095**

Glass vials: size dependent on sensor chip **Contributed by users**

Ethanol **Honeywell Catalog #34870-1L**

Gold sensor chip **Contributed by users**

Essential to use very high purity ethanol.

Glassware cleaning: this uses reagents that are corrosive and oxidising. A full local safety assessment must be performed, along with training of personnel. Full PPE and fume hood mandatory.

Plasma (or UV-ozone) cleaning: full training and local safety assessment must be performed.

Clean glass vials that will contain the oleyl ethylene glycol solutions and the sensor chips. Aqua regia, Piranha or chromic acid for glassware cleaning. After neutralisation of acid, wash glass vials with 10 volumes milliQ water and then ethanol. Put tops on and store.

Prepare stock solutions of the thiolated ligands 10m

1

10m

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Using standard plastic micropipettes, with tips that have been autoclaved (this prevents plasticiser on tips transferring into solutions) prepare 5 mL stock solution in cleaned glass vials:
100 mM thiol PEG in ethanol
1 mM biotin PEG in ethanol
These can be stored at -20°C in a spark-proof freezer. They should be warmed to room temperature before opening.

Prepare the thiolated ligand solutions 10m

2 10m

Prepare the thiolated ligand solutions in cleaned glass vials for the formation of a SAM on the sensor gold surface. Dilute the ligand stock solutions from (1) in ethanol mix thiol PEG and biotin-thiol PEG at the molar ratio necessary to obtain the desired surface overage of streptavidin. The final concentration of thiol-PEG ligands should be 1 mM.
1 % biotin-thiol PEG (mol/mol) will give ~100% streptavidin coverage.
0.3% biotin-thiol PEG (mol/mol) will give ~30% streptavidin coverage.
0.1 % biotin-thiol PEG (mol/mol) will give ~10% streptavidin coverage.
These can be stored at -20°C in a spark-proof freezer. They should be warmed to room temperature before opening.

Assembly of monolayer of thiolated ligands on gold surface 1d 13h

3 15m

Sensor chips should be plasma or UV/ozone cleaned. This removes trace organics that will interfere with PEG SAM formation and can also be used to clean a used chip.
If plasma cleaning, the chamber is sufficiently large that sensor chips may be placed in clean glass vials, gold surface up and cleaned for 10 min.
If UV-ozone clenaing, chambers are usually small and mayonly accommodate the sensor chip, 10 min.

3.1 30s

After plasma cleaning, vials are removed and immediately covered in the appropriate 1 mM thiol PEG/biotin-thiol PEG solution and the cap put on. It is critical to minimise the time the clean gold surface is in contact with the atmosphere, as absorption of organic molecules from the air will prevent the formation of a robust self-assembled monolayer.

3.2 1d

Incubate the gold sensor surface with the 1 mM thiol PEG/biotin-thiol PEG solution for 24 h to 36 h at room temperature, to allow the SAM to form. Avoid touching the gold surface from this point on, as this may damage the SAM. After assembly, wash with ethanol (5 volumes). The sensor surface may be

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stored in ethanol at 4 °C for at least 3 months.
Step 3.2 includes a Step case.

UV-ozone cleaning

Streptavidin and ligand functionalisation for a 1% mol/mol thiolated biotin PEG SAM 20m

step case

UV-ozone cleaning

UV/ozone cleaners tend to have small chambers, so the sensor surface will need to be removed with forceps and placed into a glass vial containing the appropriate 1 mM thiol PEG/thiol PEG biotin solution and the cap put on.

4 Insert the sensor surface chip (H_2O pre-washed) into the SPR instrument, lightly drying the chip (not include drying the SAM surface side) on surfaces where light falls and is collected from the sensor. 1m

4.1 Run H_2O, then PBS (or the experimental running buffer of choice) over the surface. The surface will withstand all the usual SPR running buffers. 5m

4.2 ⚠ 4m

Inject 20 mM HCl over the sensor surface, contact time 1 min, return to running buffer, then inject 2 M NaCl, contact time 1 min, return to running buffer.
These washes are essential to remove any PEG thiol associated with the SAM, but not bonded to the gold surface. This reduces non-specific binding substantially.

4.3 Inject 40 µg/mL streptavidin in running buffer over the surface. 500 µL/min flow rate, 1 mL injection (2 min cotacting time) to increase mixing. Slower flow rates will need longer contact times due to slow mixing under laminar flow. Return to running buffer for 1 min or longer. Repeat step 4.2 and return to running buffer again for 4 min to pull the (surface plasmon resonance) SPR response to be base. 3m

4.4 Surface is ready to capture biotinylated ligand. We use 160 µg/mL reducing end biotinylated heparin, 1 mL injection at 500 µL/min in the measurement channels, leaving the background channel as SAM + streptavidin. Return to running buffer for 1 min or longer, 1 mL injection of 2 M NaCl for 2 min and return to running buffer for 4 min or longer. 7m

4.5 ⏸ 2m

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Sensor surface may be removed at this point or any later stage and stored at 4 °C overnight in a clean glass vial in PBS containing 0.02% (w/v) sodium azide and then re-inserted into the instrument. Functionality is maintained for up to 10 days, including 5 days of use. How long functionality is maintained will depend in part on the stability of the immobilised biotinylated ligand, the intensity of experiments (cycles of binding & regeneration) and the harshness of the regeneration step.

4.6 Surface is ready for analysis of interactions of immobilised ligand and ligate.

Regeneration: we have tested repeated regeneration with 2 M NaCl, 20 m HCl and 0.2% (w/v) SDS with no loss of surface function.

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