Protocol for Coating QD-COOH on glass slides

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Cleaning glass slides prior to coupling and Amination with APTS (Aminopropyl triethoxysilane)

1. clean glass slides with 20% HCL or sulfuric acid in Ethanol for 20 min, blow dry (or use fresh glass-bottom dish)
2. ultrasonicate with acetone or toluene for 10 min, blow dry
   alternative cleaning with Piranha solution: "careful with Piranha solution, separate waste disposal!!
   immerse in hot 30:70 mixture of H2O2 and sulfuric acid, 60 C for 1 h
   rinse with distilled water and ethanol
   blow dry to store in sealed container (falcon tube)
3. add mixture of 1 mL EtOH, 200 uL APTS and 50 uL NH3 (usually conc.= 28%)
   and leave to react for 2 h
4. wash with EtOH x 3 and ddH2O x3, store aminated slides in distilled water for up to 5d

alternative amination procedure with Poly(ethylene Imine) solution

1. clean glass slides as above or use fresh glass bottom dish
2. prepare 1% solution of PEI in ddH2O
3. "add 200-300 uL of PEI solution to glass part of the dish, allow 20-30 min for adsorption, then wash with ddH2O 3-4 times"

Coupling of carboxylate Quantum Dots to the freshly aminated glass surface:

1. prepare solutions of EDC and NHS in PBS at pH 6 (with 0.1% SDS or CTAB) > "typically, prepare 20 mg/mL EDC solution and 20 mg/mL NHS solution, mix 1-to-1 and add 10 uL QD525 to 2 mL activation mix"
   > "always make these solutions fresh, let EDC defrost before opening and vortex mix for 15 min before adding to the aminated dish"
   > "0.1% SDS is added to increase colloidal stability of the QDs, shake for 15 min at room temperature"
2. "add 1 mL activated QDs to aminated glass surface in activation buffer (PBS pH6, SDS 0.1%), shake for 1 h"
   "add 1 mL borate bufer (50 mM at pH 9) to increase pH for optimum coupling conditions, shake another 1-2 h"
3. wash and block unreacted sites with ethanolamine if desired (not necessary usually) "(or add NTA-Ni if complexation of 6His-tagged proteins is the next
step, see below)" > "check surface density of QDs on glass, adjust starting concentration of QDs if required"
4 for imaging it helps to create a cross-pattern or scratch on the QD-coated glass surface by scratching with tweezers, this will help to locate a good spot for monitoring and also create an area for background reference

**Protocol for modification of immobilized QDs with 6His-tagged proteins**

A) Coordination with ZnS shell
1 "add 6His-tagged protein directly to QD-glass slide and leave to react for >2 h in fridge, overnight also ok" > "incubation in fridge only because the peptide is less likely to degrade, but it takes longer at lower temperature"
2 rinse with MMP buffer and analyze FRET

B) Coordination with NTA-Ni
1 activate QD-COOH surface with EDC/NHS (10 mg/mL in PBS pH 6) for 30 min > "alternatively, you can add the NTA-Ni or NTA-ZN mix directly after immobilizing the QDs or just after adding the borate buffer" (because the QD-COOH surface should still contain reactive NHS groups. If there is a long wait between QD immobilization and "NTA coupling, the QD-COOH groups may inactivate and need fresh activation with NHS/EDC (see above))"
2 premix 800 uL NTA solution of 2.62 mg/mL in pH 7-9 buffer with 200 uL NiCl2 of 6 mg/mL in water for< 6 h (usually 2 h sufficient) "(according to Bull Korean Soc 2010, 31, 6), alternatively ZnCl2 can be used"
3 add premixed Ni-NTA to activated QD-glass slide for 2 h, then wash with MMP buffer" 4 add 200 uL of 6His-tagged protein (1 uM) to Ni-NTA surface for 2 h (RT) - overnight (in fridge)
5 rinse with MMP buffer and analyze FRET

**Protocol for degradation of QD525-XRGD-Cy3 with soluble MT1-cat**
1 wash dish with MMP buffer and keep relatively dry except in centre (glass part), so when the protease is added it stays confined to the centre of the dish (~200 uL)
2 find a good spot (cross pattern) for imaging and collect baseline data (imaging usually every 60 second at 1/16 ND or lower to avoid bleaching) for 10-20 min or until baseline stable
3 "add 5-20 uL MT1-cat (of 0.7 uM stock) or at desired concentration to 100 uL MMP buffer, adjust image position if necessary"
4 monitor FRET ratio development over time at T=37 degrees
> after stabilization wash dish to remove physically adhering Cy3 (this may possibly reduce the FRET ratio further)

**buffer preparation**

<table>
<thead>
<tr>
<th>50 mL MMP buffer</th>
<th>weigh in</th>
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<tbody>
<tr>
<td>HEPES 50 mmol/L</td>
<td>238.3 g/mol</td>
</tr>
<tr>
<td>CaCl2 10 mmol/L</td>
<td>147.02 g/mol</td>
</tr>
<tr>
<td>MgCl2 0.5 mmol/L</td>
<td>203.31</td>
</tr>
<tr>
<td>ZnCl2 0.05 mmol/L</td>
<td>136.28</td>
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</tbody>
</table>

(make stock solution 100x and dilute)

| Brij35 | 0.01% | 2.5 mg in 50 mL |

> dissolve all ingredients in 50 mL water, adjust pH to 6.8 and filter with 0.2 um syringe filter to sterilize

**borate buffer pH 9** (need pH meter, room temperature)

| boric acid | 200 mM | 0.62 g |
| borax (sodium tetraborate) | 50 mM | 0.95 g |

> dissolve all ingredients in <50 mL (~40 mL) water (may take a while, shake at level 5, in a covered cup), adjust pH to 9 (by pH meter on shake at level 5), add more H2O to 50 mL, and filter with 0.2 um syringe filter to sterilize

**PEI 1 % solution**

> weigh in 1 g on balance (very viscous stock solution is 50 wt%) and dissolve in 50 mL ddH2O (or 0.5 M NaCl if thicker films preferred)

**PBS pH 6 with SDS 0.1%** (need PH meter, analytic balance)

> weigh in 50 mg of SDS powder and dissolve in <50 mL (~40 mL) sterile PBS pH 7.4, then adjust pH to 6 (by pH meter on shake at level 5, with HCl), add more PBS to 50 mL.

**Notes**

"QD imaging: when excited at 400 nm, fluorescence intensity will be 11x higher than for excitation at 633 (for QD655)"

"suitable filter sets recommended, intensity up to 5 fold higher"

"QD storage: do not store diluted, may decrease performance"
Chemical list

339350 ALDRICH
Nickel(II) chloride  98%  50 g

14580 ALDRICH
\(\text{Ni}^2+,\text{Ni}^2-\text{Bis(carboxymethyl)-L-lysine hydtrate}\)
≥97.0% (TLC)  1g

A3648 SIGMA-ALDRICH
(3-Aminopropyl)triethoxysilane
98%  100 mL

130672 ALDRICH
\(\text{N-Hydroxysuccinimide or sulfo-NHS}\)
98%  5g

03449 FLUKA
\(\text{N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride}\)
99.0% (AT)  1g

http://www.sigmaaldrich.com/catalog/product/sial/g6257?lang=en&region=SG
G6257 SIGMA-ALDRICH
Glutaraldehyde solution
Grade II, 25% in H2O 100 ml

P3143 FLUKA
Poly(ethyleneimine) solution
50% (w/v) in H2O 100 mL

**QD conjugation protocol using streptavidin-biotin interaction (from data sheet for ITK QD-COOH, Invitrogen)**

Conjugation Protocol Please read the entire protocol before starting.
1.1 In a small glass vial with a small stirbar, dilute 250 µL of 8 µM stock solution of Qdot® ITK™ carboxyl quantum dots to 2 mL using 10 mM borate buffer, pH 7.4. Mix well by stirring.

1.2 Add 0.48 mL of 10 mg/mL streptavidin to the Qdot® ITK™ carboxyl quantum dots reagent (step 1.1). Continue stirring.

1.3 Weigh ~5 mg of EDC in a 1.5 mL microcentrifuge tube and add 0.5 mL deionized water to obtain a 10 mg/mL EDC stock solution. Prepare EDC solution just before use.

1.4 Immediately, add 57 µL of 10 mg/mL EDC stock solution to the Qdot® solution (step 1.2).

1.5 Stir gently for 1–2 hours at room temperature for the conjugation.

1.6 Filter the conjugate solution through a 0.2 µm PES syringe filter to remove any large aggregates and transfer the solution to a clean centrifugal ultrafiltration unit (100 kDa cutoff).

1.7 Centrifuge at the recommended speed for the ultrafiltration unit for at least 5 buffer exchanges using 50 mM borate buffer, pH 8.3 to remove any excess unbound protein. Ensure that the volume of concentration is >10-fold (e.g., 4 mL to <400 µL) each time.

1.8 After ultracentrifugation is complete, filter the solution through a 0.2 µm syringe filter or a 0.8/0.2 µm combination syringe filter to remove any aggregates. Store the Qdot® conjugate solution at 4°C. Do not freeze the nanocrystal conjugate.