**Protocol for Coating QD-COOH on glass slides** cjochs@smart.mit.edu Chris Ochs 19/09/12

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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)

1. add mixture of 1 mL EtOH, 200 uL APTS and 50 uL NH3 (usually conc.= 28%) and leave to react for 2 h
2. 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** **50 mL MMP buffer** weigh in HEPES 50 mmol/L 238.3 g/mol 0.05 L 595.75 mg in 50 mL CaCl2 10 mmol/L 147.02 g/mol 0.05 L 73.51 mg in 50 mL MgCl2 0.5 mmol/L 203.31 0.05 L 5.1 mg in 50 mL ZnCl2 0.05 mmol/L 136.28 0.05 L 0.34 mg in 50 mL (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

http://www.sigmaaldrich.com/catalog/product/aldrich/339350?lang=en&region=SG

339350 ALDRICH

Nickel(II) chloride 98% 50 g

<http://www.sigmaaldrich.com/catalog/product/aldrich/14580?lang=en&region=SG>

14580 ALDRICH

NÎ±,NÎ±-Bis(carboxymethyl)-L-lysine hydrate

â‰¥97.0% (TLC) 1g

<http://www.sigmaaldrich.com/catalog/product/sial/a3648?lang=en&region=SG>

A3648 SIGMA-ALDRICH

(3-Aminopropyl)triethoxysilane

98% 100 mL

<http://www.sigmaaldrich.com/catalog/product/aldrich/130672?lang=en&region=SG>

130672 ALDRICH

N-Hydroxysuccinimide or sulfo-NHS

98% 5g

<http://www.sigmaaldrich.com/catalog/product/fluka/03449?lang=en&region=SG>

03449 FLUKA

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

<http://www.sigmaaldrich.com/catalog/product/fluka/p3143?lang=en&region=SG>

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.

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| --- |
| 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. |