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"

<u>Coupling of carboxylate Quantum Dots to the freshly aminated glass surface:</u>

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

<u>Protocol for modification of immobilized QDs with 6His-tagged proteins</u>

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 $NiCl_2$ of 6 mg/mL in water for< 6 h (usually 2 h sufficient)

"(according to Bull Korean Soc 2010, 31, 6), alternatively $ZnCl_2$ 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

<u>Protocol for degradation of QD525-XRGD-Cy3 with soluble MT1-cat</u>

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®ion=S
G

339350 ALDRICH
Nickel(II) chloride 98% 50 g

http://www.sigmaaldrich.com/catalog/product/aldrich/14580?lang=en®ion=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®ion=SG
A3648 SIGMA-ALDRICH
(3-Aminopropyl)triethoxysilane
98% 100 mL

http://www.sigmaaldrich.com/catalog/product/aldrich/130672?lang=en®ion=SG
130672 ALDRICH
N-Hydroxysuccinimide or sulfo-NHS
98% 5g

http://www.sigmaaldrich.com/catalog/product/fluka/03449?lang=en®ion=SG
03449 FLUKA
N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride
99.0% (AT) 1g

http://www.sigmaaldrich.com/catalog/product/sial/g6257?lang=en®ion=SG
G6257 SIGMA-ALDRICH
Glutaraldehyde solution
Grade II, 25% in H_2O 100 ml

http://www.sigmaaldrich.com/catalog/product/fluka/p3143?lang=en®ion=SG
P3143 FLUKA
Poly(ethyleneimine) solution
50% (w/v) in H_2O 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.