В качестве решения в данной задаче размещается ответ на задачу одного из участников (Е.Г.Евтушенко), поскольку оно лучше и подробнее стандартного решения, предоставленного автором, кроме того, это единственное решение, получившее максимальное количество баллов.

A) Explain the principle of work of this biosensor

This biosensor utilizes the quenching of quantum dot fluorescence by a proximal gold nanoparticle. Due to biologists are not experts in physics, I can't give a strict physical explanation of this phenomenon. Briefly, four models were suggested:

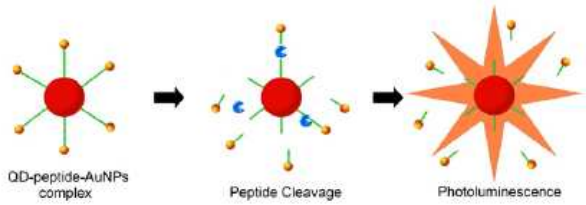
1) Förster dipole−dipole resonance energy transfer (FRET), with r^{-6} distance dependence;
2) Nanosurface energy transfer (NSET), which is similar to FRET, following a r^{-4} distance dependence;
3) Dipole−metal particle energy transfer;

Or even

4) Charge transfer, when Au NP donates an electron to valance band of excited QD with near exponential distance dependence.

Experiments show that such interactions extend significantly beyond the classical Förster range.

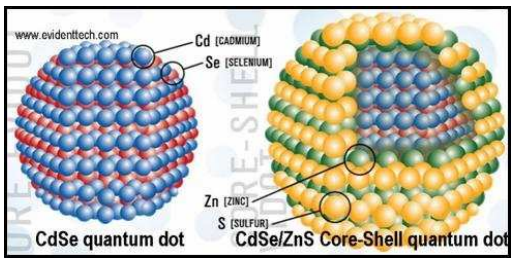
This phenomenon could find a practical application in many fields of nanotechnology, like nanoscale rulers, sensing of molecular interactions or enzymatic activity sensors. The latter should consist of QD and Au NP, joined with short cleavable linker. The enzyme (e.g. protease) with substrate specificity to linker sequence cleaves the linker, causing the restoration of fluorescence. If such protease is expressed by some cell types, this nanobiosensor could be used to visualize such cells.

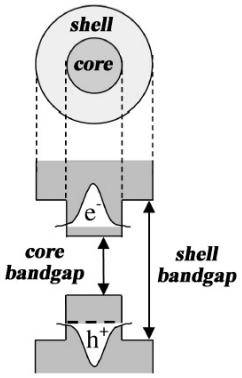


B) Why fluorescence is not observed in the case of normal cells?

As it was noted in the statement of a problem, normal cells don't express protease, specific to linker sequence and QD fluorescence remains quenched.

C) Explain why uncoated CdSe quantum dots cannot be used instead of core-shell CdSe/ZnS quantum dots.





Uncoated CdSe QDs contains structural defects, such as vacancies, local lattice mismatches, or dangling bonds at the surface. This defects act as effective traps for both holes and electrons in excited QD. The recombination of trapped charge carriers occurs in a nonradiative way. Thus, quantum yield of uncoated QDs is very low. If the CdSe core is coated with wide bandgap semiconductor, like ZnS, both electrons and holes remain localized in a core. At the same time structural defects are compensated. As a result quantum yield rises considerably. Even better quantum yield could be achieved with CdSe/$Zn_xCd_{1-x}S$ (x = 0.7, approx. 5 monolayers of $Zn_xCd_{1-x}S$ alloy) core-shell QDs.

D) What can you tell about amino acid sequence of the peptide used for the attachment of Au nanoparticles to core-shell CdSe/ZnS quantum dots?

First, it should contain a specific sequence, a substrate for cancer cell expressed protease. Second, to be specific, this sequence should not contain cleavage sites for other possible proteases. Furthermore, peptide sequence should not be a partner for any strict biomolecular interaction, because it could sterically block the cleavage by target protease. Third, peptide sequence must not exceed the energy transfer distance necessary for AuNPs to suppress QD fluorescence. Designing peptide sequence, we should take into account possible α-helical or any other secondary structure of the peptide, which shortens effective peptide length. We could use it, or we could disrupt it by introduction of several glycines. Glycine residue is very flexible and incompatible with α-helix.

Forth, if we use a dihydrolipoic acid-PEG capped QDs, we could utilize a self-assembly strategy for peptide attachment to QD. In this case peptide should have so called basic leucine zipper or polyhistidine tag (His_6) on its end.

E) Describe a detailed synthetic route to prepare the nanodevices described above. You can use any commercially available reagents (for uncommon reagent, please provide the name of the supplier and verifiable catalog number).

To synthesize the nanobiosensor we will use the following strategy:

1) Synthesize TOP/TOPO-capped CdSe/ZnS Core/Shell QDs;
2) Exchange native TOP/TOPO ligands with dihydrolipoic acid (DHLA)-PEG. Such coating results in water-soluble, highly fluorescent and highly stable QDs with reduced nonspecific binding to cells;
3) Buy an engineered peptide, containing His_6 tag on C-terminus and biotin on N-terminus;
4) Buy streptavidin-coated 2 nm Au NPs (typically 3-4 streptavidin molecules per NP);
5) Conjugate peptide with Au NPs using biotin-streptavidin interaction;
6) Fluorometrically titrate DHLA-PEG QDs with Au-NP-Peptide conjugate until total quenching of fluorescence. Peptide will attach to QD via His_6 tag.

Detailed protocol:

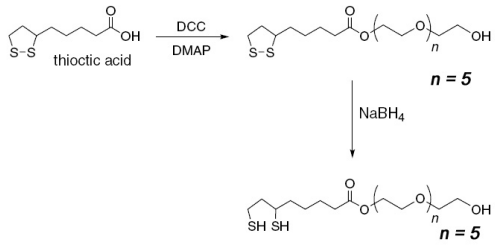
1) Synthesis of CdSe/ZnS Core/Shell QDs.

Se precursor, tributylphosphine selenide (TBP-Se) is prepared by dissolving 0.15 mmol of selenium shot in 100 mL of TBP under inert atmosphere and stirring vigorously overnight, forming a 1.5 M TBP-Se solution.

A Cd+Se precursor solution containing 0.5 mmol cadmium 2,4-pentanedionate ($Cd(acac)_2$), 0.25 mL dodecanal (DDA), and 2.8 mL tri-*n*-octylphosphine (TOP) should be degassed at 100 °C for 1 h, followed by the addition of 3.3 mL of 1.5 M TBP-Se after cooling to 24ºC. This mixture is loaded into a syringe under dry N_2 atmosphere. In a separate 3-neck round bottom flask, 6.25 g of 90% tri-*n*-octylphosphine oxide (TOPO) and 5.75 g of 90% hexadecylamine (HAD) are degassed at 135°C for 2 h and back-filled with N_2. The temperature is increased to 360 °C before rapidly injecting the precursor solution. The temperature of QD growth should be maintained at 280°C. During the growth stage the aliquots of reaction mixture are examined on fluorimeter to control the QDs size. Reaction stops when cores reached a first absorbance maximum at 565 nm. After cooling to 80°C, 4 mL of butanol is added to prevent solidification of the product. The sample is kept for 1 h at 24ºC and then centrifuged at 3000 g for 4 min. The pellet is discarded and acetone is added to the colored supernatant to precipitate the cores, followed by another round of centrifugation.

The pellet is re-dispersed in hexane, filtered through a 0.2 μm filter, and injected into a degassed solution of 10 g 99% TOPO and 0.4 g n-hexylphosphonic acid. After removing the hexane under reduced pressure at 80°C, the flask should be back-filled with dry N_2 and the temperature is increased to 130°C before adding 0.25 mL of decylamine and stirring for 30 min. Precursor solutions of $ZnEt_2$ and $(Me_3Si)_2S$ are prepared by dissolving the appropriate amounts of each in 4 mL of TOP and loading them into two separate syringes under inert atmosphere. The amount of $ZnEt_2$ is calculated by assuming a 3 monolayer overcoat according to the methods of Dabbousi. Typical mass is 56 mg. A two-fold molar excess of $(Me_3S)_2S$ is used. The precursor solutions are injected simultaneously into the 130°C bath at a rate of 4 mL/h. Aliquots should be taken in 10 min intervals to monitor the red-shift of the photoluminescence spectrum upon over-coating, and the injection is terminated after the desired wavelength is achieved, typically after the addition of three monolayers. The sample is annealed overnight at 80°C, and 4 mL butanol is added. The QDs are stored in growth solution under ambient conditions and centrifuged once more before use.

2) Synthesis of capping ligand dihydrolipoic acid (DHLA)-PEG.

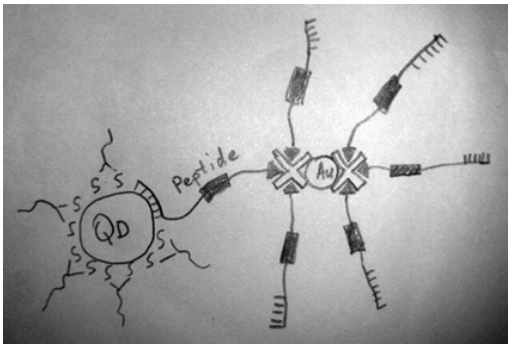


First stage: a round bottomed flask is filled with thioctic (lipoic) acid (5.16 g, 25 mmol), 4-dimethylamino-pyridine (915 mg, 7.5 mmol), poly(ethylene glycol) (125 mmol) and CH_2Cl_2 (300 mL). The mixture is stirred under nitrogen and cooled to 0°C while a solution of 1,3-dicyclohexylcarbodiimide (5.67 g, 27.5 mmol) in CH_2Cl_2 (20 mL) is added dropwise over 0.5 h. The reaction should be allowed to warm to room temperature and stirred for 20 h. The solution is filtered over a bed of Celite and rinsed with chloroform. The organics is washed repeatedly with water and brine to remove the unreacted poly(ethylene glycol). The combined extracts are dried over $MgSO_4$, filtered and evaporated to give a viscous yellow oil. The crude product is chromatographed on silica, using a solvent system consisting of $CHCl_3$, methanol and acetone.

Second stage: a round bottomed flask is filled with the pegylated lipoic acid derivative (1 mmol), ethanol (10 mL), and water (2 mL). $NaBH_4$ (150 mg, 4 mmol) is added and the reaction is heated gently until the yellow color vanished to give a cloudy colorless solution. The mixture is diluted with $CHCl_3$ (200 mL), dried over $MgSO_4$, filtered and evaporated to give a colorless oil.

3) The replacement of native TOP/TOPO ligands with DHLA-PEG.

0.2 mL QDs in growth solution are precipitated by adding 2 mL of methanol, centrifuged at 3000g. Clear supernatant is discarded; the precipitate is washed by 0.5 mL methanol and centrifuged again. QDs are dried using N_2.
50 μL of DHLA-PEG and 10 μL of methanol are added to 10 mg of dry QDs. The mixture is stirred gently under N_2 at 60°C for 2.5 h and precipitated by adding 0.3 mL of ethanol, 0.05 mL of $CHCl_3$, and 0.5 mL of hexane in succession. Centrifugation at 3000g for 2 min yielded a clear supernatant, which should be discarded. The precipitate is re-suspended in 0.5 mL of PBS (pH = 7.4) and filtered through a 0.2 μm filter. The sample should be purified using gel filtration chromatography in order to remove aggregated QDs, and the fractions are concentrated at 3500g using a Vivaspin-6 10 000 MW cutoff spin concentrator.
4) Engineered peptide
Peptide, containing His_6 tag on C-terminus and biotin on N-terminus could be purchased from Elim Biopharmaceuticals, Inc (http://www.elimbio.com).
5) Streptavidin-coated Au NPs
Highly spherical, non-aggregated Au NPs with narrow size distribution, with streptavidin corona could be purchased from BBI (http://www.britishbiocell.co.uk/products/lifescience/secondary_gold_probes.asp?navid=2).
Catalog numbers are EM.STP2, EM.STP5, EM.STP10 and EM.STP15 for 2, 5, 10 or 15 nm Au NPs. The optical density at 520 nm varies from 3.1 to 15 depending on NP's size.
6) Preparation of peptide-AuNP conjugate.
0.2 mL of 1.5 μM streptavidin-coated 2 nm Au NPs in PBS (pH = 7.4) is incubated with 0.2 mL of 14 μM biotinilated peptide for 1 h. Excess peptide is removed by ultrafiltration on Vivaspin-6 using 10 000 MW cutoff filter. Additionally, we have a possibility to recover the excess peptide using His_6 tag.
7) Titration of DHLA-PEG-capped QDs with peptide-AuNP conjugate.
10 μL of 8 μM DHLA-PEG-capped QDs in PBS (pH = 7.4) is placed in Corning Black 384-Well Polypropylene Assay Plate. 10 μL portions of 1.5 μM peptide-AuNP in PBS (pH = 7.4) are added with subsequent 30 minutes incubation and fluorescence measurements using FLUOstar Omega microplate reading fluorometer. Synthesis is finished when the fluorescence is totally quenched. Biosensor conjugate should be purified using gel filtration chromatography in order to remove aggregates (binding of His_6 to QDs is stochastic, so we have two multivalent partners and minor amounts of polymer could occur as a result) and free Au NPs.



Possible structure of the sensor is shown on the left picture. If we want to obtain the sensor with better sensitivity, we could use concurrent principle in stage 6, mixing biotinilated peptide with free biotin in defined ratio. Using this approach, we can decrease the average number of peptides down to 2-3 per Au NP. The number of biotin binding sites per Au NP could be determined using fluorometric titration with biotin-FITC.

F) Researchers decided to use the system described above to monitor the progression of metastasis for a squamous cell carcinoma in a mouse model. What modifications should be done to the sensor so that it could be used for this in vivo study?

Biological tissues are not transparent in visible region, but they have a “transparency window” in near infrared (NIR) region, 700-1000 nm. NIR radiation penetrates few centimeters deep into the tissue. Thus, we have to change visible fluorescent CdSe QDs to NIR fluorescent PbS, PbSe, PbTe or HgCdTe QDs, combining such core with appropriate wide bandgap semiconductor.

G) Would you recommend in vivo use of this biosensor in human patients? Provide your arguments.

This biosensor is a perfect tool for visualization of cancer cells in biopsy, but I’m afraid it will be not so efficient *in vivo*. Although NIR region is called a “transparency window”, the biological tissue is not so transparent to allow NIR radiation penetration trough the whole human body. So, deep tumors can’t be visualized. The second reason is the cytotoxicity of small Au NPs. It was shown, that certain cells could uptake 2 nm Au NPs. These NPs alter the expression of genes involved in processes of transcription, cell growth, and response to viruses. Also the products of QDs degradation, such as cadmium, lead, mercury, selenium and tellurium ions are highly toxic.