Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Capillary Electrophoretic Separation of Functionalized Nanoparticles

Sławomir Oszwałdowski, Katarzyna Zawistowska-Gibuła, Kenneth P. Roberts
Scheme S1

1. Exchange ligands

\[
\begin{align*}
\text{DHLA} & \quad \text{dihydrioloic acid} \\
\text{(PhSO}_3\text{)}_2 (\text{NN}) & \quad \text{bathophenanthroline disulfonic acid, disodium salt}
\end{align*}
\]

2. Surfactants used for nanoparticles coating

\[
\begin{align*}
\text{Triton X-100 (TX-100) (n = 9-10)} & \quad \text{DOSS} \\
& \quad \text{Polyethylene glycol tert-octylphenyl ether} \\
& \quad \text{Dioctyl sulfosuccinate sodium salt}
\end{align*}
\]

\[
\begin{align*}
\text{Sodium dodecylsulfate (SDS)} & \\
\text{Lauric acid sodium salt (LA)}
\end{align*}
\]

\[
\begin{align*}
\text{Poly(ethylene oxide) (PEO)} & \\
& \text{average } M_v \sim 100,000, \\
& \text{average } M_v \sim 600,000, \\
& \text{average } M_v \sim 8,000,000,
\end{align*}
\]
Preparation and characterization of CdSe/TOP NCs and NCs modification by amphiphilic molecules

Preparation of TOP-coated CdSe nanocrystals

The hydrophobic TOP-coated CdSe nanocrystals were prepared from cadmium oxide and elementary selenium using referenced procedures [Asokan S, Krueger KM, Alkhawaldeh A, Carreon AR, Mu Z, Colvin VL, Mantzaris NV, Wong MS (2005) The use of heat transfer fluids in the synthesis of high-quality CdSe quantum dots, core/shell quantum dots, and quantum rods. Nanotechnology 16:2000-2011]. Briefly, the selenium precursor was prepared by combining elementary selenium and TOP at room temperature. The cadmium precursor was prepared from cadmium oxide, ODE, and oleic acid at 250°C. Prepared solutions were mixed at different temperatures for the appropriate time period to obtain the desired size of CdSe nanocrystals. The resulting CdSe/TOP nanocrystals were purified by adding a large volume of acetone followed by adding methanol until a slightly turbid solution was obtained. The mixture was left to stand overnight. Larger NCs precipitated after the mixture stood overnight, but smaller NCs required centrifugation (3,000 rpm, 15 min). The pure nanocrystals were washed with methanol and then dissolved in either toluene or hexane for extended storage or in chloroform for the preparation of amphiphile-coated NCs.

Preparation of modified CdSe NCs

Samples of CdSe NCs surface passivated with trioctylphosphine (TOP) were dissolved in chloroform (3.0 μM) and 200 μL of the solution was added to 0.5 ml 0.2 M solution of sodium laurate in order to coat NCs to obtain CdSe/TOP//LA NCs. Alternatively, for preliminary experiments using a micellar plug, CdSe/TOP NC were dispersed in micellar solution containing TX-100 (10% w/w) and sodium laurate (0.2 M). In order to coat CdSe NCs with DOSS or SDS surfactant the CdSe NCs solution in chloroform was added to a solution of mixed surfactants containing 10% TX-100 and 0.6% anionic surfactant (SDS, DOSS) (wt/wt) (0.5 g 10% w:w water solution of TX-100 and 0.025-0.035 g of second surfactant). In all cases the obtained mixture was stirred
overnight to allow chloroform to evaporate, to form a bilayer between TOP and the amphiphilic surfactants.

The preparation of CdSe/ZnS NCs with \((\text{PhSO}_3)_2\text{(NN)}\) surface ligands was performed according to a general procedure previously published for ligands exchange at CdSe surface with the use of NC–pyridine as intermediate product [Schmelz O, Mews A, Basche T, Herrmann A, Müllen K (2001) Supramolecular complexes from CdSe nanocrystals and organic fluorophors. Langmuir 17:2861-2865]. The exchange of TOPO from the NC surface was facilitated through a pyridine intermediate to maximize surface coverage with \((\text{PhSO}_3)_2\text{(NN)}\). Both TOPO and pyridine derivatives of CdSe/ZnS did not dissolve in water and the formation of water soluble CdSe/ZnS/\((\text{PhSO}_3)_2\text{(NN)}\) indicated that the exchange process was successful. The obtained NCs were purified using centrifugation (Amicon Ultra-15, centrifugal filter (3000 rpm/15 min)) and stored in water.

**Dispersion of CdSe/TOP NCs to form CdSe/TOP//amphiphile NCs**

Amphiphilic molecules have already been applied to disperse nanostructures (nanocrystals, carbon nanotubes) in aqueous media. In the light of recently published results [Bulavchenko AI, Popovetsky PS (2010) Electrokinetic potential of nanoparticles in reverse AOT micelles: photometric determination and role in the processes of heterocoagulation, separation, and concentration. Langmuir 26:736–742], based on DLVO theory, the dispersion of particles and stability of dispersed particles is regulated by attraction/repulsion forces, between particles, imposed by hydrophobic/electrostatic interactions. Among several examples discussed in the work, the example (Ag nanoparticles dispersed in reversed DOSS micelles) seems to adequately explain nature of dispersion of surface modified nanocrystals applied in the present work.

In the present work, it was established that coating of CdSe NCs using amphiphilic molecules, according to Experimental Section, to form CdSe/TOP//amphiphile NCs, leads to formation of a lipid-like bilayer on nanocrystal surface for optimal aqueous solubility. Among anionic, cationic or non-ionic surfactants applied, the coating with carboxylate derivatives (R-COO\(^{-}\)Na\(^{+}\); LA, OA) ensures the
highest stability of dispersed NCs, without NCs aggregation, up to 1 month. Apart from this, coating procedure using LA assures efficient phase transfer of NCs from organic to aqueous phase, since absorbance of native CdSe/TOP NCs dissolved in chloroform and these dispersed in LA solution (Fig. S1) proved to be similar (inset, Fig. S1 allows for visual inspection of both NCs). In addition, it was seen that for both NCs, the wavelength position of the first exciton band in the UV-Vis range (Fig. S1) was the same, which means, that the core dimension was left unchanged during the coating process; this in agreement with ref. [Yu WW, Qu L, Guo W, Peng X (2003) Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. Chem Mater 15:2854-2860]. On the other hand, comparing emission efficiencies (PL; photoluminescence; Fig. S1), lower PL for CdSe/TOP//LA NCs than that for CdSe/TOP NCs was seen, as result of exchange of surface ligands attached to CdSe semiconductor nanocrystal.

*Fig. S1.* Comparison of UV-Vis (frame a) and photoluminescence (PL) (frame b) spectra for CdSe/TOP NCs; unmodified and coated with sodium laurate (LA). A, CdSe/TOP NCs dissolved in chloroform; B, CdSe/TOP NCs dispersed in 200 mM sodium laurate. PL spectra were obtained for both NCs samples with the same absorbance. Both NCs are shown to inspect them visually (inset).
**Fig. S2.** Ways to modify the conditions of migration of CdSe/TOP//LA NCs injected into capillary along with a micellar plug. Middle (a), the CdSe/TOP//LA NCs visualized as focused peak; Upper (b), modification a micellar plug by adding acetonitrile (ACN) to the plug; Below (c, d), electrophoresis using an electrolyte buffer modified of by ACN. Samples: (a, c, d) CdSe/TOP//LA NCs dispersed in mixture of TX-100 (10%, w/w) and 0.2 M LA; (b) the same sample mixed with ACN (1:1). Focused NCs (A), NCs outside the plug (B). Electrolyte buffers: (a, b) 10 mM sodium tetraborate; (c) 10 mM sodium tetraborate containing 20% ACN and (d) 10 mM sodium tetraborate containing 30% ACN. Applied voltage +20kV for all examples, detection $\lambda = 330$ nm (inset shows solutes spectra; vertical arrow shows the detector wavelength applied, selective for NCs).
Conclusions: Two ways of modification were examined in order to release NCs from a micellar plug. For sample (NCs/micellar plug) only modification of an electrolyte buffer allows for this.

Details:

1. Modification of a micellar plug with organic solvents (MeOH, ACN, up to 50% v/v) or by increasing ionic strength (up to 200 mM NH₄Cl, 50 mM LiCl) did not allow NCs to be released from a plug and only focused peak for NCs was seen. (using NaCl, KCl salts, even at low concentration, a precipitate, due to complexation of Na⁺ or K⁺ cations by a non-ionic surfactant was seen).

2. Modification of the electrolyte buffer allows for quantitative NCs release from micellar plug. This was observed, using 30% ACN in the BGE.

Both experiments above; experiment 1 vs. experiment 2 confirm, that NCs included to micellar plug are migrated at the micelle/electrolyte buffer boundary, which is accessible to electrolyte buffer (thus its modification). The release, thus transport of NCs from micellar plug to micelle-free zone leads to change in NCs peaks parameters (height, N) as result of transfer NCs through the micelle/electrolyte buffer boundary. Each situation (b-d) is graphically illustrated, where yellow layer denotes the micellar plug and red one the NCs zone, respectively. This issue is discussed more detailed in Electronic Support Section, text below with Fig. S4.
**Fig. S3.** Effect of changes in applied voltage on the shape of peaks for CdSe/TOP//LA NCs migrated within or outside a micellar plug, respectively. Peaks: (A) NCs being in the micellar plug (focused NCs), (B) NCs being outside the micellar plug. Both peaks (A, B) were obtained at 30 kV (red color) or at 10 kV (blue color).

Conclusion: Due to voltage decrease, an increase in the separation efficiency for focused NCs (peak A), and reverse effect (peak broadening) for NCs outside the micellar plug (peak B), was observed. Detection UV-Vis, $\lambda = 310$ nm.
In the previous work [Oswałdowski S, Zawistowska K, Grisby L, Roberts KP (2010) Capillary Electrophoretic Separation and Characterizations of CdSe Quantum Dots. Cent Eur J Chem 8:806–819] it was pointed out that CdSe NCs coated with (non-ionic, ionic) amphiphiles form a stable phase with regular micelles. This statement is supported by two observations. Firstly, applying the MEKC mode of separation of a sample containing NC/amphiphile and a surfactant, the relation between peak broadening for NCs and concentration of a surfactant in the sample leads to the conclusion about quantitative dispersion of NCs throughout the zone of micellar aggregates. Secondly, for CdSe NCs coated with a non-ionic surfactant the position of NCs peak close to EOF using CZE mode was opposite to the position of NCs peak due to MEKC mode, as NCs were migrated along with micellar aggregates, marked by Sudan III marker. The same can be concluded from Fig. 1 of the present work, where the position of CdSe/TOP//LA peak was shifted from expected position, within MEKC migration window, to the position of micellar aggregates.

Taking these features into account, it can be stated that CdSe/TOP NCs coated with amphiphilic molecules (CdSe/TOP//amphiphile NCs) forms a new system, which resembles, in some extend, regular micelle (similar radii, the presence of surface polar or charged groups). In this situation, the CdSe/TOP//amphiphile NCs can be regarded as pseudomicelles able to form a stable mixed pseudomicellar system containing pseudomicelles (CdSe/TOP//amphiphile) and regular micelles.

Migration phenomena for NCs, observed in the present work (NCs focusing, NCs release from micellar plug), in the context of the mixed pseudomicellar system can be discussed more detailed with the support of literature references.

Figure S4 (frame a) shows the initial conditions, where the micellar plug of non-ionic surfactant containing CdSe/TOP/amphiphile NCs injected into a capillary, is placed in between electrolyte buffer. When the voltage is applied, a decrease in conductivity of a micellar plug is induced by difference in ionic transfer between a micellar zone and electrolyte buffer solution [I]. This causes a strong electric field localized in a micellar plug. The velocity of the total flow ($v_{EOF}$) on any point in the separation channel must be
identical since the buffer solution is incompressible. Consequently, the hydrodynamic flow is generated in the micellar plug to maintain the uniformity of $v_{EOF}$. According to ref. [II], migration velocities for micellar plug ($v_M$) and front of buffer/micelles boundary ($v_{B/M}$) are different, as the micelles migrates under the $E_M$ electric field, whereas buffer/micelles boundary migrated under average electric field $E_{av}$, being mean of an electric field in a micellar and a buffer zone (Fig. S4b). This effect causes the micellar plug broadening, graphically illustrated in Fig. S4b and seen experimentally (eg. Fig. 3, frame b and c; main text).

On the other hand, the migration phenomena observed in the present work (mixed pseudomicellar system) may be a special case of mixed micellar systems (SDS/TX-100, SDS/C$_{12}$E$_8$), already analyzed using capillary zone electrophoresis [III, IV]. It was reported, that peaks of various fractions of mixed micelles with mobility between 0 and $-3.5 \times 10^{-4}$ cm$^2$V$^{-1}$s$^{-1}$ were observed, depending on polydispersity in terms of size and composition of mixed micelles. Typically, the mixed micelles with the most negative mobility, which give the sharp peaks at micelle/buffer boundary under CZE conditions, are almost monodisperse solutes (high and narrow mole fraction of one component, eq. SDS) (e.g. peak P2, frame d, e Fig. 2, ref. IV along with interpretation; Fig. 4, ref. III). Due to presumable resemblance between both systems (mixed micelles vs. mixed pseudomicelles) the observed a sharp peak for NCs on micelle/electrolyte buffer (M/B) boundary (Fig. 3, main text) can be explained in terms of the formation of thermodynamically stable mixed pseudomicellar phase with NCs, as one of the possible fraction, trapped on M/B border.

Due to these remarks above, two main effects are responsible for the focusing of amphiphile coated nanocrystals on the micelle/buffer (M/B) boundary.

Firstly, comparing electrophoretic mobility for the electrolyte buffer (sodium tetraborate), detected experimentally in the present work ($\mu_{EOF} = 5 \times 10^{-4}$ cm$^2$V$^{-1}$s$^{-1}$) with this for monodisperse pseudomicelle ($\mu_{pm} = -3.5 \times 10^{-4}$ cm$^2$V$^{-1}$s$^{-1}$), the relation ($v_{pm} \ll v_M$) can be concluded, which means that the pseudomicelles are moved towards M/B boundary (denoted by blue line, Fig. S4b). Secondly, further exit of pseudomicelle from narrow M/B boundary is hindered, due to the formation of a stable mixed pseudomicellar system, caused by a strong interaction between pseudomicelles and regular micelles, as
stated upper. These two features provide highly effective on-line sample preconcentration mechanism for surface modified nanocrystals. It should be noted that, the focusing of micelles on the micelle/electrolyte buffer boundary was predicted in work [ref: II supporting information, page 2].

The release of pseudomicelles (CdSe/TOP//amphiphile NCs) from a zone of mixed micelles (plug) to electrolyte buffer zone would be possible if velocity of pseudomicelle ($v_{pm}$) fulfills the requirement: $v_{pm}$ (micellar zone) > $v_{pm}$ (buffer zone) (for $v_M >> v_{pm}$ case). Taking into account the difference in an electric field ($E_M > E_{buffer}$) it would be possible only when $|\mu_{pm} \text{ (in buffer zone)}| < |\mu_{pm} \text{ (in micellar zone)}|$, which is unlikely probable taking into account high (maximal) $\mu_{pm}$ in micellar zone, which allows NCs to gather at the B/M boundary. This is supported by literature data for negatively charged nanoparticles, where mobility in aqueous electrolyte buffer for silica-encapsulated nanocrystals equal to $\mu \sim 4 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ was estimated, independent on electric field strength [V] and value of $\mu = 3.8 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ was calculated based on literature data for CdTe/CdS passivated by sodium mercaptoproprionate ligands [VI].

Due to this the another process should be considered (Fig. S4, frame c), which allows trapped NCs to be released from M/B boundary. Based on literature, where the micellar plug was applied as the separation/preconcentration tool in the capillary electrophoresis, it can be stated that in this system, migration of a solute, inside [II] or outside the micellar zone [VII-XI] is due to the release of a trapped solute from M/B boundary into micellar or into buffer zone. This step relies on a decrease in concentration of micelles at M/B boundary to the critical level, which causes a micelle decomposition with the subsequent release of solutes being trapped by a micelle on the M/B boundary. Thus, the transfer of micelles containing strongly bound analyte, from micellar zone to electrolyte buffer zone, with subsequent micelle decomposition (micelle $\rightarrow$ monomer) was found to release a solute to the buffer zone (new literature term: AFMC – analyte focusing by micelle collapse, [VII-XI]). It can be stated that the similar effect was observed in the present work, as a result of modification of an electrolyte buffer (Fig. S2 and Figs. 4 - 6). In this case, the modified electrolyte buffer was able to affect the pseudomicelle/micelle interaction for a fraction of nanoparticles (pseudomicelles) trapped on micelle/buffer boundary. This decreases integrity of the mixed pseudomicellar system.
at M/B boundary with consequence of releasing a pseudomicelle into an electrolyte buffer zone. However, in this case, the pseudomicelle decomposition (CdSe/TOP//amphiphile → CdSe/TOP + amphiphiles), due to sharp decrease in pseudomicellar concentration ($C_{pm}$, Fig. S4c) is unlikely (highly hydrophobic CdSe/TOP NCs would be generated, immediately expelled from aqueous solution), thus another cause should be considered. According to ref. [XII], the degree of dynamic coating of metal particles with surfactant molecules, under MEKC run, was proportional to the concentration of an ionic surfactant in an electrolyte buffer. By analogy, it can be stated that pseudomicelles (CdSe/TOP//amphiphile NCs) can be dynamically modified by surfactants of micellar plug and this feature is considered to be conditional and reversible, depending on concentration of ionic surfactants around the pseudomicelle. Note that the highest concentration of an anionic surfactant in the micellar plug of mixed micelles, is expected at M/B boundary, according to refs. [III]. In this case, a collapse of the dynamically modified pseudomicelles in a micelle-free zone (aqueous electrolyte buffer) leads to a release pseudomicelles (CdSe/TOP//amphiphile) and an excess of monomer surfactants (Fig. S4c), with subsequent appearance of the peak of NC/amphiphile NCs in a electrolyte buffer zone.

It should be noted that, the transport of pseudomicelles trapped on a micellar plug to an electrolyte buffer zone is energy consumption process. The former state can be characterized by ($E_{low}$) and the latest by ($E_{high}$) (Fig. S4c). The process is the demixing of a stable mixed pseudomicellar phase (release of a pseudomicelle from a phase of mixed micelles; demixing process), followed by a decomposition in buffer zone of dynamically coated by a surfactant pseudomicelles (collapse). The latest is related to the change in environment around pseudomicelles from micellar to aqueous. In the present work, this was available through the modification of a electrolyte buffer, in agreement with ref. [XI].
Fig. S4. Graphical interpretation of focusing of CdSe/TOP/amphiphile NCs dispersed in non-ionic/ionic surfactants, as well as releasing these NCs from micelle/buffer boundary (B/M) to the buffer zone. Frames: a, the starting point, a micellar plug of non-ionic/ionic surfactants containing NCs was injected into capillary in between electrolyte buffer; b, after applying voltage, the micellar plug was moved according to electroosmotic flow, generated by electrolyte buffer. Inside the plug the concentration gradient of micelles is
formed. CdSe/TOP/amphiphile NCs (pseudomicelles) were trapped into micelle/buffer boundary due to their lowest velocity. Because of a strong interaction between a pseudomicelle and a regular micelle (mixed pseudomicellar system), the release of NCs from micellar plug was suspended; c, modifying the electrolyte buffer (organic solvent, ionic strength), the NCs being trapped on micelle/buffer boundary were released into a buffer zone.

References:
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The effect of micellar plug for separating test solute and test NC
In the main text it was demonstrated that an introduction of a micellar plug, as well as modification of electrophoretic conditions allow for manipulating migration of surface modified NCs. In the present Section, the effect was examined using test molecules (phenol, DNA) and test NCs (amine-EviTag). These NCs (amine-EviTag) are known to be widely used to bioconjugation and were applied throughout the present work. The manipulation of separation selectivity is one of the crucial task in separation science and two examples below show that an introduction of a micellar plug leads to two important separation effects: (i) a shift in migration time for NCs, and (ii) an enhancement in a peak height for NCs, as result of NCs focusing (preconcentration). The first effect is illustrated (Fig. S5), where a sample containing the test molecule (phenol) and the test NCs (amine-EviTag) was separated using either the traditional CZE mode or the method with the use of a micellar plug. It was observed (Fig. S5a), that in the absence of a micellar plug both peaks (test molecule, amine-EviTag) co-migrate, close to the dead migration time. The DAD spectrum of the peak confirms this as the spectrum was found to be superposition of both spectra (phenol, NCs). An introduction of a micellar plug resulted to complete separation of both solutes (phenol vs. amine-EviTag) (Fig. S5c), due to the shift in migration of the amine-EviTags NCs. Similar result was observed in the separation of DNA oligomer and amine-EviTag NCs (Fig. S6). In this case, it was observed that although both were separated using the traditional CZE mode, the migration of amine-EviTag NCs was too close to the dead migration time, possibly greatly interfering the peak shape of NCs (Fig. S6a). An implementation of the micellar plug (Fig. S6b) changes migration time of both solutes (first effect). One–fold increase in peak height for amine-EviTag NCs, due to NCs focusing within micellar plug, is the second effect. To conclude this Section it can be stated that a micellar plug can be useful tool for manipulating electrophoretic migration of solutes (molecules, particles) and both examples above show that due to this tool double mechanism on the same run is available in the form of preconcentration (NCs focusing within a plug) and CZE mode for test molecules.
Absorbance vs. Time/ min.

Wavelength vs. Absorbance
**Fig. S5.** Application of the micellar plug to the CE separation of test molecule and amine-EviTag NCs. a, the mixture of phenol and amine functionalized NCs (T2-MP EviTag) in absence of a micellar plug in the sample. The peak being a mixture of both components is confirmed by spectrum being superposition of two spectra; b, only phenol in the presence of a micellar plug (mixture injected: 20 µl of phenol and 20 µl of surfactant solutions); c, the same as b with addition of 2 µl of amine-EviTag NCs. Peaks: A, phenol; asterisk *, peak from surfactant solution; B, amine-EviTag. Spectra from peaks: phenol (A) and amine-EviTag (B), respectively. Conditions: electrolyte buffer 10 mM sodium tetraborate with 10% MeOH. Voltage 5 kV. Phenol solution 10 mg ml⁻¹. Surfactants solution: 0.5 g TX-100 (10% w/w, water solution) was mixed with 0.07g DOSS.
**Peak A** (amine-EviTag)
Area: 230660
Height: 7454

Height ratio for both peaks A = 20 ($\text{SEF}_{\text{height}}$)
**Fig. S6.** Manipulation of a peak for NCs due to focusing effect: a, the mixture of amine functionalized, amine-EviTag NCs, and DNA oligomer was separated by means of CZE mode; b, the same mixture with the implemented micellar plug (TX-100/DOSS). Conditions: electrolyte buffer 10 mM sodium tetraborate. Voltage: a, 15 kV (CZE); b, 7 kV (focusing). Samples: a, 3 µl amine functionalized, amine-EviTag NCs, and 13 µl of DNA; b, to the previous sample (a) 10 µl of surfactant solution was added (composition of surfactant solution, see Fig. S5). To illustrate the separation effects, both samples were presented in the same frame (axis) allowing for visual inspection of the advantage of focusing mode. The preconcentration efficiency, in the terms of enhancement factor for peak height (SEF$_{height}$):

$$SEF_{height} = \frac{\text{peak height}_{\text{preconcentration method}}}{\text{peak height}_{\text{conventional method}}} \times \text{dilution factor}$$

shows one–fold NCs peak enhancement. The same level of SEF$_{height}$ was reported in the work [Quirino JP (2009) Analyte focusing by micelle collapse in CZE: Nanopreparation of neutrals. Electrophoresis 30:875–882].
**Fig. S7.** (a) The plain gel electrophoresis of amine-functionalized T2-MP Evi Tag (A) and the bioconjugated form aptamer/amine-EviTag (B). (b) CE Separation of amine-functionalized T2-MP Evi Tag (Evident) (A) and its aptamer derivative (B) with the use of the CE mode with micellar plug. Peaks: A, amine-EviTag; B, its bioconjugated form, aptamer/amine-EviTag. Spectra of appropriate peaks are included as inset. The sample of amine-EviTag NCs after bioconjugation (sample UV-VIS spectral data: \( \lambda_{abs} \) 590 nm, absorbance = 0.54) 10 µl was mixed with 5 µl of (TX-100/DOSS solution). Surfactants solution: 0.5 g TX-100 (10% w/w, water solution) was mixed with 0.07 g DOSS. Electrolyte buffer: 10 mM sodium tetraborate/15% ACN, voltage + 20kV, detection wavelength: 291 nm.
The silica encapsulation of CdSe/ZnS NCs and the plain gel electrophoresis of: CdSe/ZnS/silica NPs and NPs aptamer derivative: A, silica encapsulated CdSe/ZnS NPs; B, the same NPs after bioconjugation (CdSe/ZnS/silica/aptamer) (sequence of spots is due to fractions after SEC purification).

**Fig. S8.** The silica encapsulation of CdSe/ZnS NCs and the plain gel electrophoresis of: CdSe/ZnS/silica NPs and NPs aptamer derivative: A, silica encapsulated CdSe/ZnS NPs; B, the same NPs after bioconjugation (CdSe/ZnS/silica/aptamer) (sequence of spots is due to fractions after SEC purification).
Fig. S9. CZE conditions according to ref. [Vicente G, Colo´n LA (2008) Separation of bioconjugated quantum dots using capillary electrophoresis. Anal Chem 80:1988-1994] applied to the electrophoretic separation of silica coated CdSe/ZnS NPs and its bioconjugated product, due to reaction with aptamer. a, b, CdSe/ZnS silica encapsulated NPs; c, d, the same NPs after bioconjugation and SEC purification. Conditions: electrolyte buffer: 10 mM sodium tetraborate containing 0.05% PEO (600,000 MW), voltage: a, c, 25 kV; b, d, 3 kV. Detection wavelength 203 nm. The peaks due to NPs were verified by the characteristic DAD spectra of nanoparticles. Only one cumulative peak for NPs was obtained, despite various PEO applied to modify electrolyte buffer (Mv 100,000, 600,000 and 8,000,000), and varying POE content in electrolyte buffer.