Supporting information

for

Bioactivation and Cell Targeting of Semiconductor CdSe/ZnS Nanocrystals with Phytochelatin-related Peptides

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Fig. S-1. Evaluation of the purification of peptide-coated nanocrystals by SE-HPLC and SDS-PAGE chromatography.

(a) For the same optical density of nanocrystals (NCs) injected, the 210 nm absorption chromatograms of unpurified (light blue) and dialysis purified (dark blue) peptide-coated NCs normalized at the NCs elution peaks, show a clear decrease in the amount of unbound peptides after purification. The luminescence signal of the NCs (red) is overlaid to confirm the identity of the peaks. Purification by dialysis was performed overnight with 100,000 Daltons MWCO membranes. (b) Silver stained 4-15% SDS-PAGE gel of two purification rounds of peptide-coated NCs using 100,000 Daltons MWCO centrifugal filters. Lane 1: Molecular weight standard (Biorad), Lane 3: Unpurified NCs, Lane 4: NCs after one purification round, Lane 5: NCs after two purification rounds.

Fig. S-2. Electrophoretic separation of three sizes of nanocrystals coated with positively charged peptide **10**.

Green (G), yellow (Y) and red (R) emitting CdSe/ZnS NCs coated with peptide **10** were separated on a 1% agarose gel in TBE buffer pH 8.3 for 1 hour at 120 Volts.

Fig. S-3. Agarose gel electrophoresis of CdSe core and CdSe/ZnS core/shell peptide-coated nanocrystals emitting at 565 nm.

NCs before and after ZnS shell deposition were coated with peptide **4** and run on a 2.5% agarose gel for 2.5 hours at 120 Volts. The difference in migration distance between the two samples relates to the larger size of the ZnS coated NCs. The core/shell NCs band appears wider than that of cores since the width of the size distribution after ZnS shell growth is increased.

Fig. S-4. Fourier transformed infrared spectroscopy of peptide-coated nanocrystals.

Methanol precipitated TOPO-coated NCs (a), peptides (b) and purified peptide-coated NCs in water (c) were dried under nitrogen, mixed with a KBr matrix and analyzed on a Brucker IPS66 spectrometer with a KBr beam splitter. The presence of peptides on the NCs is confirmed by the detection of the peptide bond amide I and II vibrational bands at 1650 cm^{-1} and 1535 cm^{-1} respectively.

Fig. S-5. Evaluation of the size of peptide-coated nanocrystals from SE-HPLC experiments.

The size of peptide-coated NCs was determined by comparing their elution time by SE-HPLC to a standard of globular proteins with known Stoke's radii: bovine thyroglobulin (85 Å), bovine gamma globulin (55 Å), chicken ovalbumin (27.3 Å), horse myoglobin (18.8 Å) and vitamin B-12 (7.5 Å). (a) The protein calibration curve was build by plotting the Stoke's radii *versus* $(-\log K_{av})^{1/2}$ where K_{av} is defined as $(V_e-V_0)/(V_t-V_0)$, V_e, V_t and V_0 being the sample, the column and the void volumes respectively[1]. (b) The Stoke's radius of red emitting CdSe/ZnS NCs coated with peptide **5** was evaluated at 82.4 Å for an elution at 17.368 min.

Fig. S-6. Avidin and NeutrAvidin gel retardation assays with biotinylated and non-biotinylated peptide-coated nanocrystals.

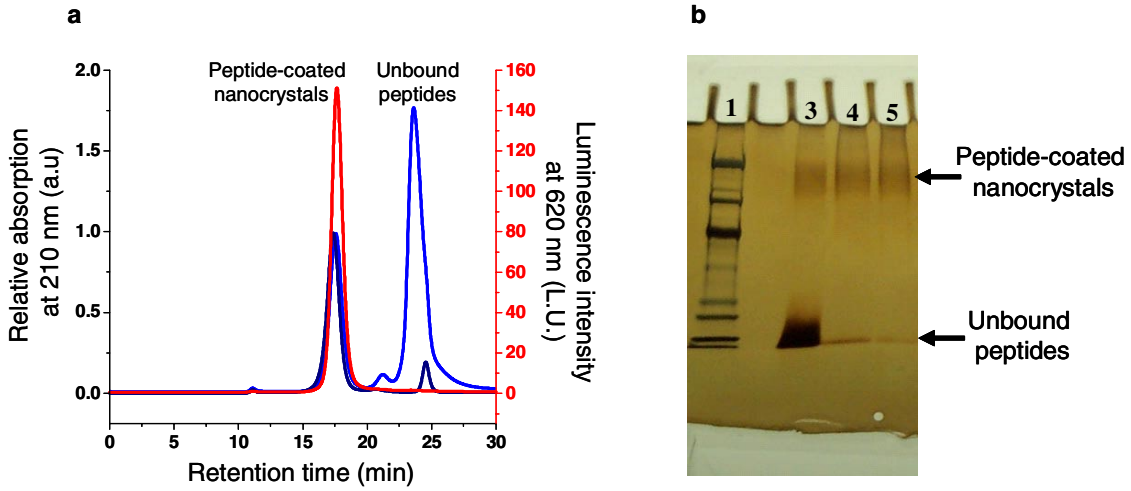
(a) 1% agarose gel electrophoresis of red emitting biotinylated nanocrystals coated with peptide **8** and non-biotinylated nanocrystals coated with peptide **7**. Both biotinylated and non-biotinylated samples aggregate for the same avidin concentration. The concentration of avidin tested were 1000, 500, 250, 1000, 50, 25, 10, 5 and 2.5 μg/ml. (b) 1% agarose gel electrophoresis of red emitting nanocrystals coated with 1:1 molar ratio of peptide **8** and peptide **4**. The concentrations of NeutrAvidin tested were 2000, 1000, 750, 500, 300, 250, 150, 100, 50, 40, 30, 25, 15, 10, 5, 2, and 1 μg/ml.

Fig. S-7. Dual labeling of live av-CD14 expressing HeLa cells with Hoechst nuclear fluorescent dye and biotinylated nanocrystals.

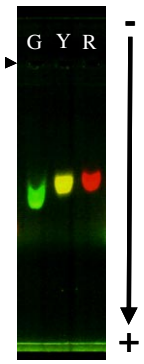
HeLa cells were treated as described in the experimental section with the exception that 3 μM of Hoechst (Molecular Probes) was added to the cells after 10 minutes of incubation with biotinylated CdSe/ZnS NCs emitting at 620 nm. The cells were imaged with a Hoechst filter set and the NCs filter set described in the experimental section. The internal compartments are located next to the nuclei (arrows).

Reference:

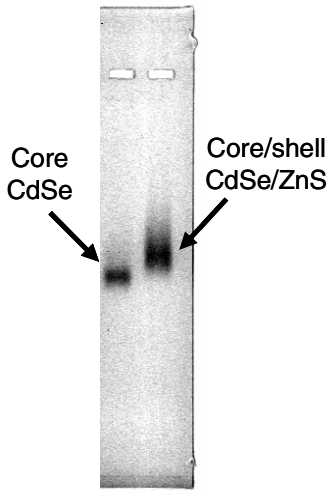
(1) Laurent, T.; Killander, J. *J. Chromatogr.* **1964,** *14*, 317.


a
Peptide-coated nanocrystals
Unbound peptides
Relative absorption at 210 nm (a.u)
Luminescence intensity at 620 nm (L.U.)
Retention time (min)
2.0
1.5
1.0
0.5
0.0
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
b
1
3
4
5
Peptide-coated nanocrystals
Unbound peptides

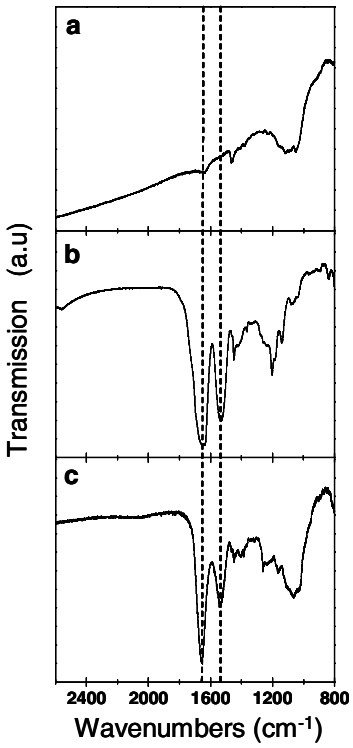
S-1.



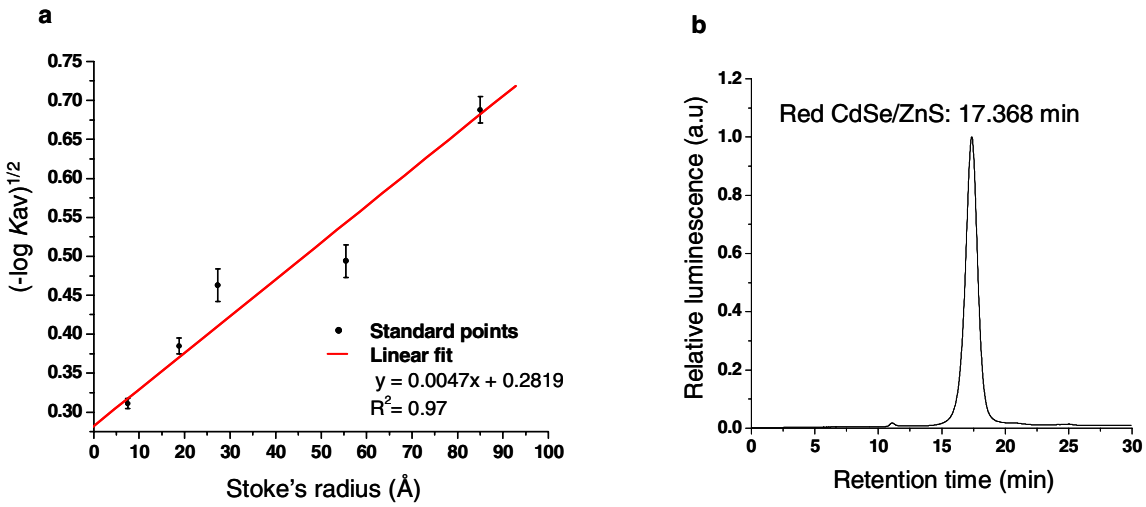
S-2.



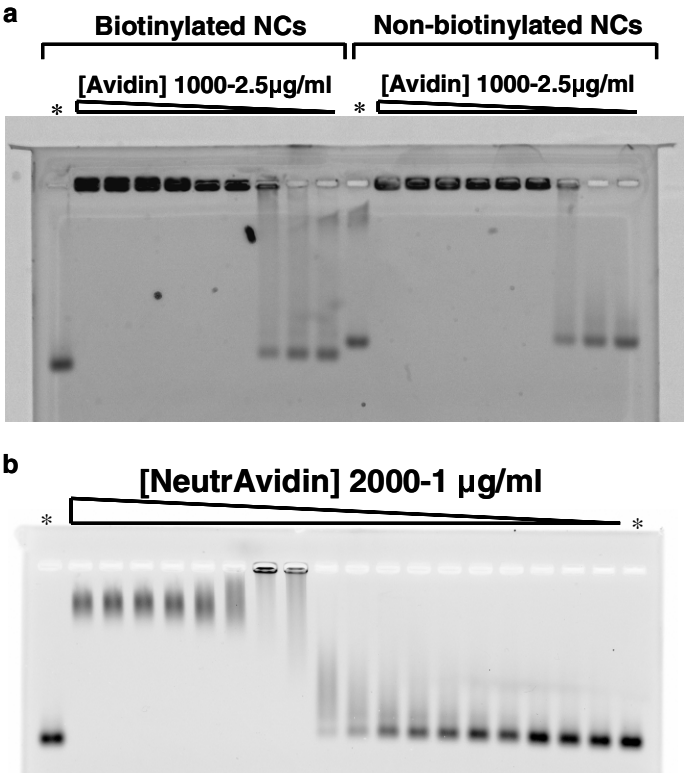
S-3.


a
b
c
Transmission (a.u)
2400
2000
1600
1200
800
Wavenumbers (cm-1)

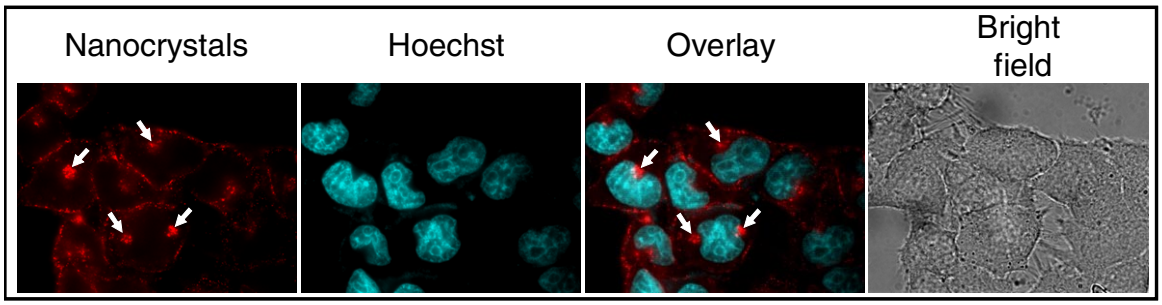
S-4.


a
(-log Kav)1/2
Stoke's radius (Å)
Standard points
Linear fit
y = 0.0047x + 0.2819
R2= 0.97
b
Relative luminescence (a.u)
Retention time (min)
Red CdSe/ZnS: 17.368 min

S-5.


a
Biotinylated NCs
Non-biotinylated NCs
[Avidin] 1000-2.5µg/ml
[Avidin] 1000-2.5µg/ml
*
*
b
[NeutrAvidin] 2000-1 µg/ml
*
*

S-6.



S-7.