SUPPORTING INFORMATION

Multiple functionalization of fluorescent nanoparticles for specific biolabeling and drug delivery of dopamine

Maria Ada Malvindi,[a,b] Riccardo Di Corato,[a,c] Annalisa Curcio,[d] Daniela Melisi,[d] Maria Grazia Rimoli,[d] Claudia Tortiglione,[e] Angela Tino,[e] Chandramohan George,[c] Virgilio Brunetti,[b] Roberto Cingolani,[a,c] Teresa Pellegrino,[a,c] Andrea Ragusa,*[a,c]

[a] *National Nanotechnology Laboratory of CNR-INFM, via per Arnesano km 5, 73100 Lecce, Italy,*
[b] *Italian Institute of Technology, Center for Biomolecular Nanotechnology (CBN), Via Barsanti 1, 73010 Arnesano, Lecce, Italy*
[c] *Italian Institute of Technology, via Morego 30, 16163 Genova, Italy*
[d] *Department of Pharmaceutical and Toxicological Chemistry, School of Pharmacy, "Federico II" University, via Domenico Montesano 49, 80131 Naples, Italy*
[e] *Istituto di Cibernetica ''E Caianiello'', Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy*

*To whom correspondence should be addressed. Andrea Ragusa, Ph.D., National Nanotechnology Laboratory of CNR-NANO, via per Arnesano km 5, 73100 Lecce; Italy and Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova, Italy.
Phone: +39-0832-298236; FAX: +39-0832-298230
e-mail: andrea.ragusa@unile.it

SUPPORTING DATA

Figure S1. TEM images of the starting QRs and of the bioconjugated nanocrystals in water.
Figure S2. Absorption and emission spectra of the bioconjugated nanocrystals in water.
Figure S3. ζ-Potential measurement graphics.
Figure S4. Cyclic voltammograms of QR-PEG and QR-PEG-DS nanocrystals.
Figure S5. Cyclic voltammograms of QR-Gal-DS nanocrystals before and after enzymatic cleavage.
Figure S6. DLS measurement on the aggregates formed by QR-Gal nanocrystals and the lectin.
Figure S7. *In vitro* hydrolysis assay. Gel electrophoresis of the bioconjugated QRs before and after incubation with porcine liver esterase.
Figure S8. Western Blot analysis of the dopamine transporter (DAT) in KB and A549 cells.
Figure S9. RT-PCR analysis on KB and A549 cells.

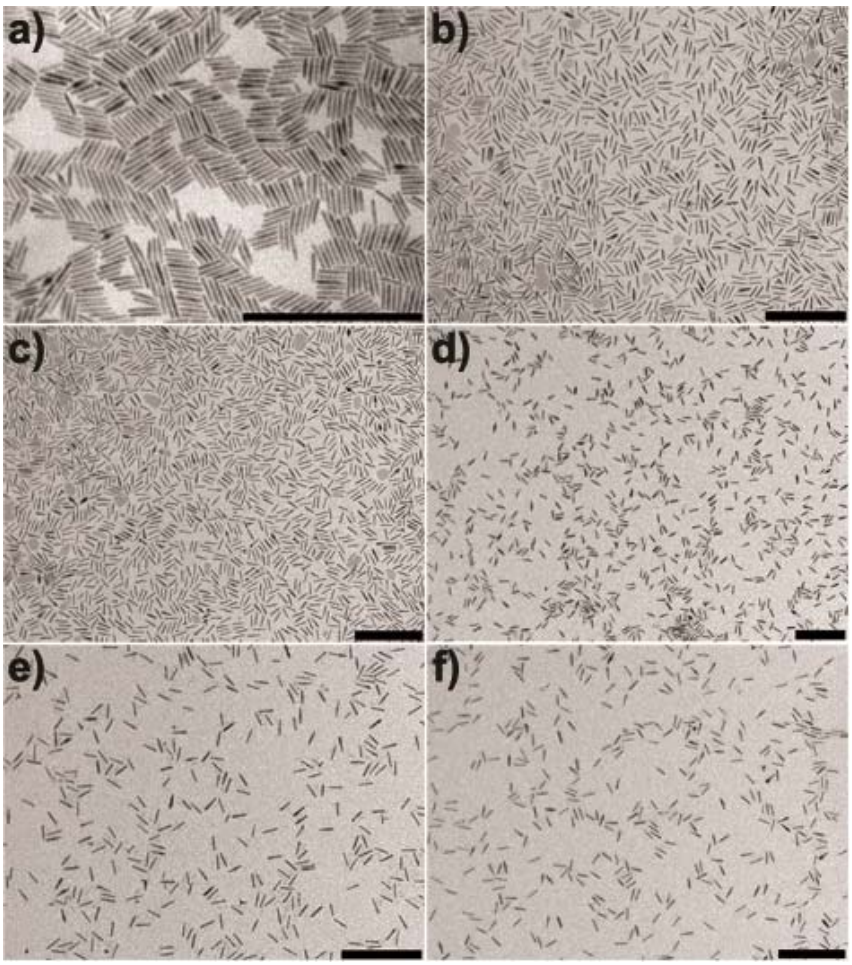


Figure S1. TEM images of a) the as-synthesized nanocrystals in toluene; and in water b) after polymer coating (QR-PC); c) after PEG conjugation (QR-PEG); d) after reductive amination (QR-Gal); e) after succinyl dopamine attachment (QR-DS); and f) after direct attachment of succinyl dopamine to QR-PEG nanocrystals (QR-PEG-DS). Scale bars are 200 nm.

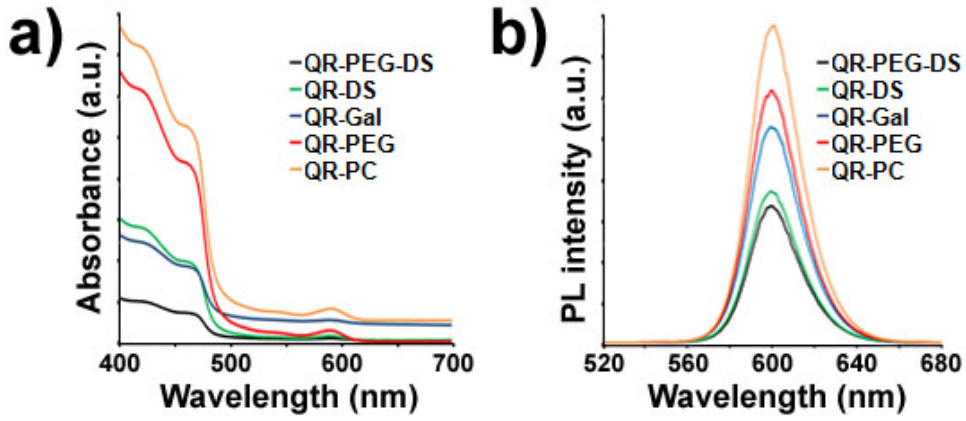


Figure S2. Absorption (a) and PL (b) spectra in water of the nanocrystals after each conjugation step. (QR-PEG-DS, black line; QR-DS, green line; QR-Gal, blue line; QR-PEG, red line; QR-PC, orange line). The excitation wavelength was 450 nm.

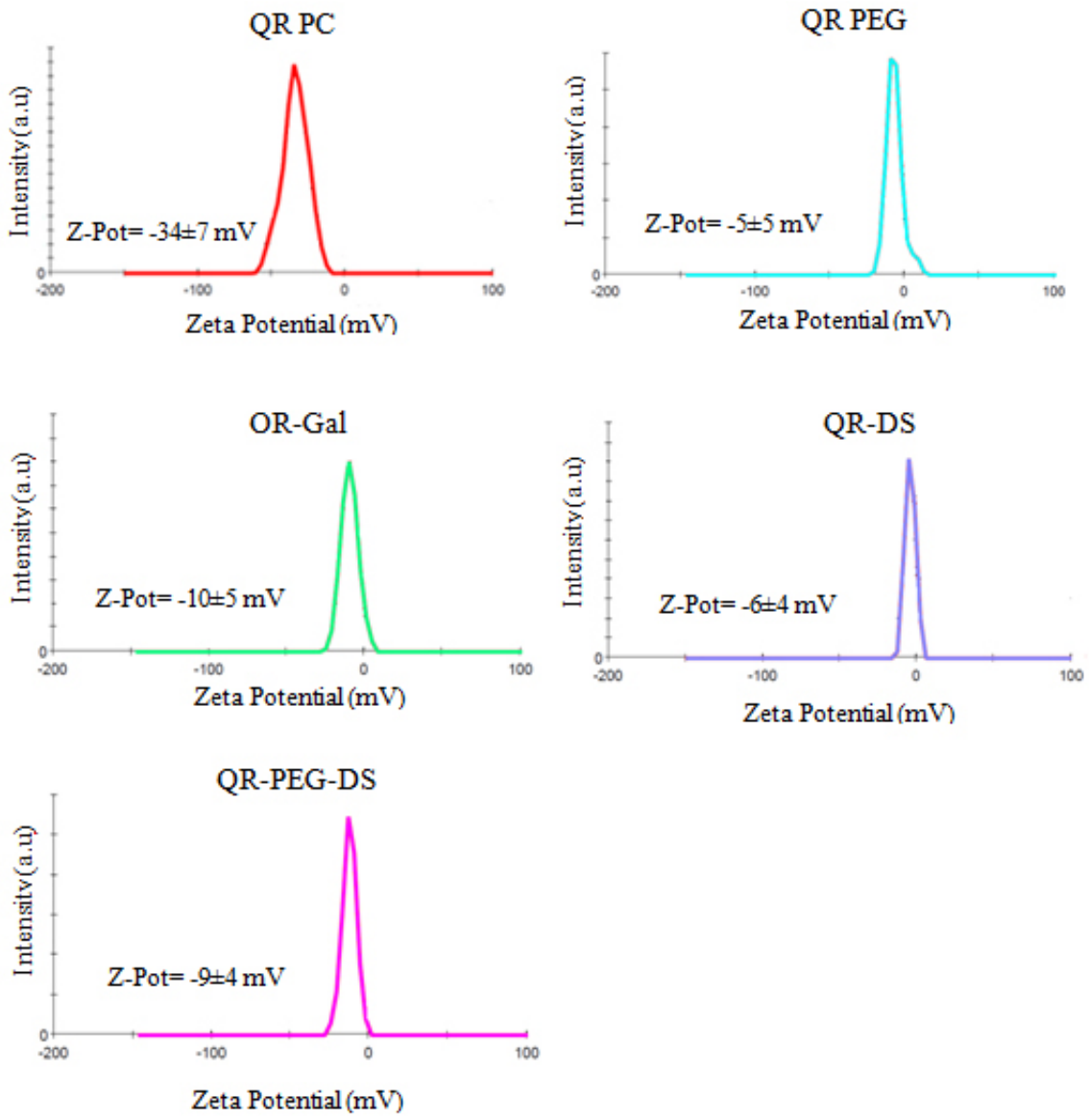


Figure S3. Graphics of the zeta-potential measurements after each conjugation step.

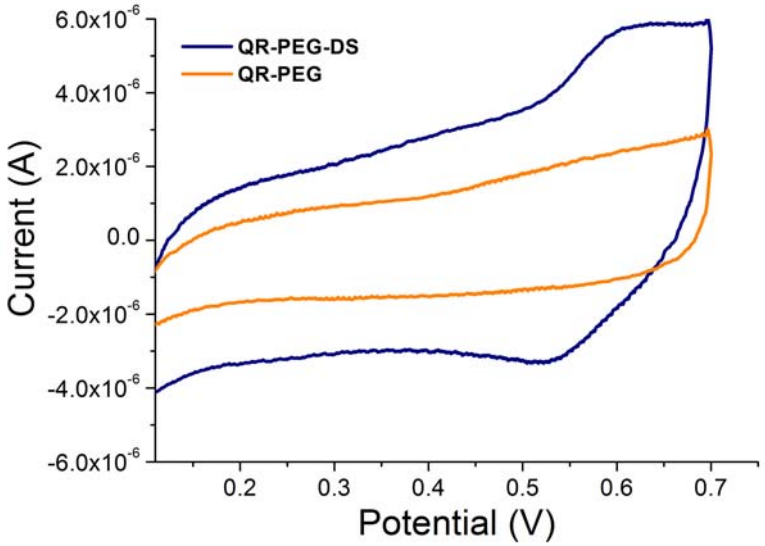


Figure S4. Cyclic voltammograms in PBS buffer solution (at 50 mV versus Ag/AgCl reference) showing the electrochemical response of 1 μM QR-PEG nanocrystals before (QR-PEG, orange line) and after (QR-PEG-DS, blue line) direct attachment of succinyl dopamine.

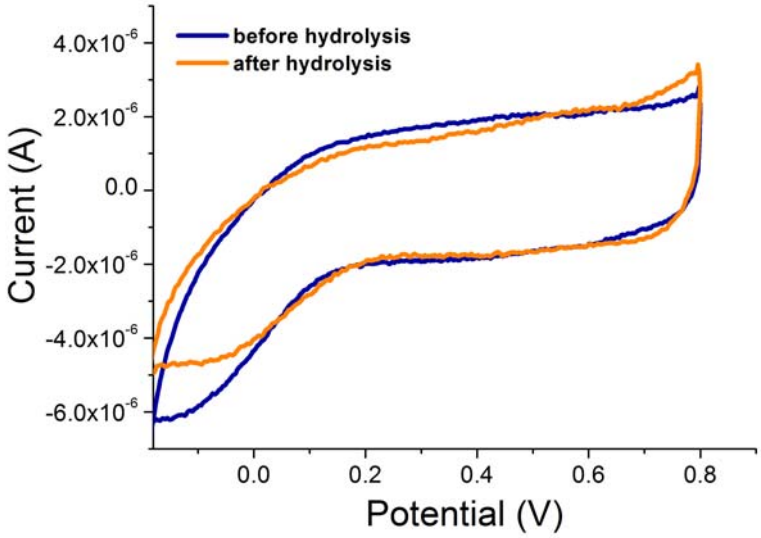


Figure S5. Cyclic voltammograms in PBS buffer solution (at 50 mV versus Ag/AgCl reference) showing the electrochemical response from 1 μM QR-DS nanocrystals before (blue line) and after (orange line) enzymatic cleavage.

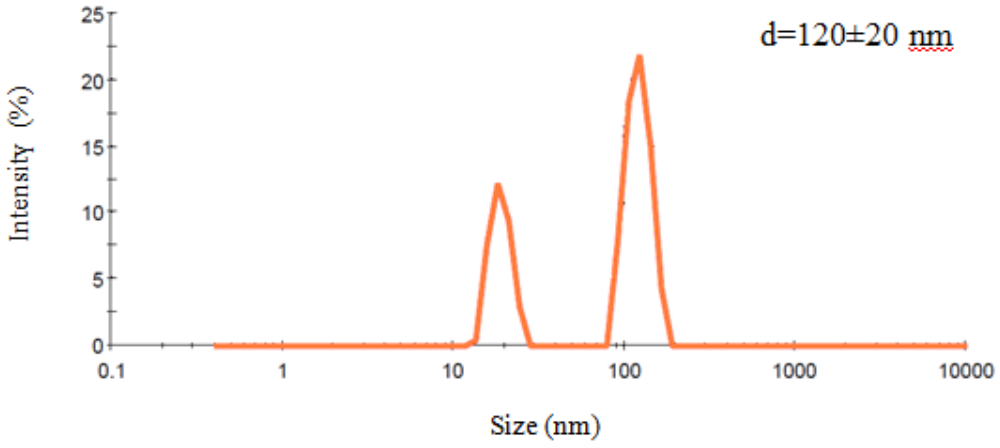


Figure S6. DLS measurement on QR-Gal nanocrystals after saturation with the lectin showing the formation of aggregates with sizes of about 120 nm and a new peak at about 14 nm corresponding to the free lectin in excess.

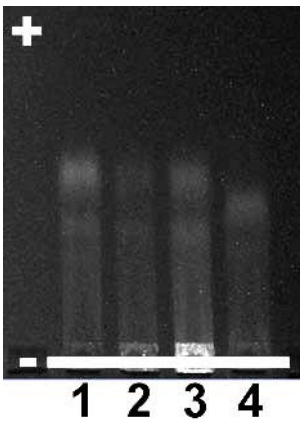
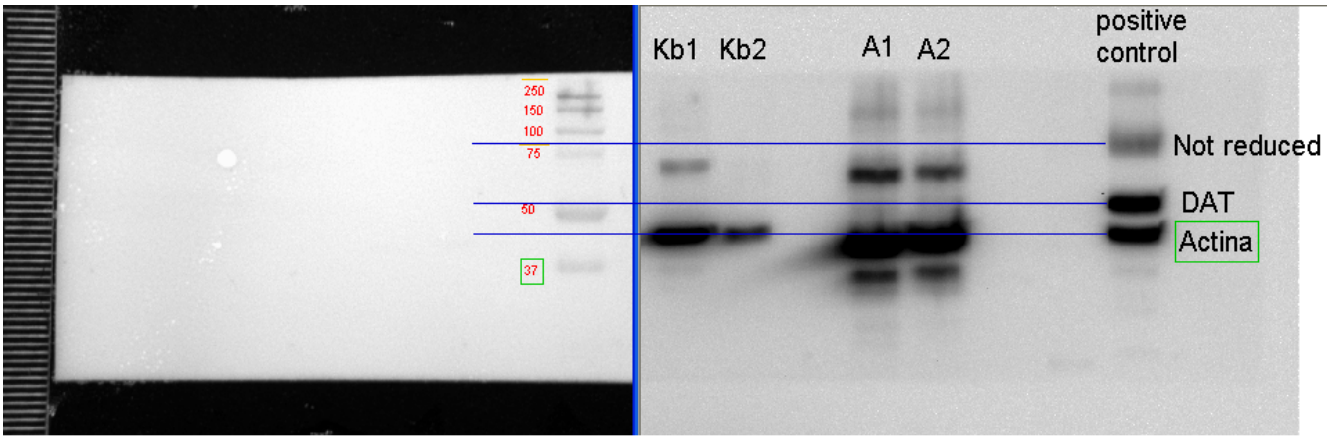


Figure S7. *In vitro* hydrolysis assay. Gel electrophoresis of QR-DS after incubation at 37 °C with porcine liver esterase for 24h. Lanes: 1) QR-Gal; 2) QR-DS + 5 mg/mL of esterase; 3) QR-DS + 2.5 mg/mL of esterase; 4) QR-DS. The starting QR-DS nanoparticles are more retained compared to QR-Gal nanocrystals (compare lanes 4 and 1). However, after incubation with the esterase, hydrolysis of the ester bond between the succinyl dopamine and the galactose occurs in the QR-DS nanocrystals, thus restoring the original QR-Gal nanoparticles (the nanocrystals present the same retention factor of the starting QR-Gal nanocrystals, compare lanes 2 and 3 with lane 1). No significant differences were observed between the addition of 2.5 or 5 mg/mL of esterase under these conditions (compare lanes 2 and 3).



- Actine MW: 43 kDa
- DAT MW: 80 kDa (not reduced), MW: 50 kDa (reduced)

Figure S8. Western Blot analysis of the dopamine transporter (DAT) in KB and A549 cell lines, in duplicate each. The proteic extracts obtained from Kb1, Kb2, A1, and A2 do not contain the DAT protein (positive control Nucleus Accumbens from NHE rat); not in the reduced form (MW: 50 kDa), neither in the not-reduced form (MW: 80 kDa). Reference protein: actine. The other bands at a different MW are aspecific bands.

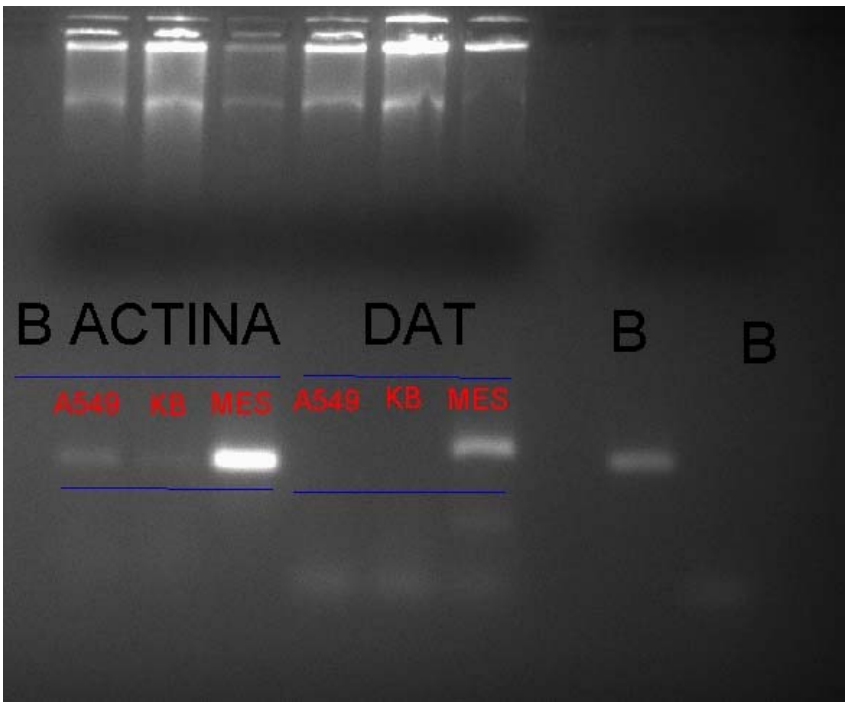


Figure S9. RT-PCR analysis on KB and A549 cells. 1 microgram of total RNA was retro-transcribed with reverse transcriptase (Invitrogen®), the sample has been diluted 1:200 and exposed to PCR (35 cycles) by utilizing specific DAT primers (positive control: beta actine). The DAT amplicon can be observed only in the positive control.