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Electronic Supplementary Material (ESM)

Fluorescent C-NanoDots for rapid detection of BRCA1, CFTR and MRP3 gene mutations.

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Synthesis of Carbon NanoDots

CNDs have been synthetized by a hydrothermal method using citric acid andethylenediamine as precursors. 1.26 g citric acid (99.0% (Sigma-Aldrich) and 1608 µL ethylenediamine (99.5% (Sigma-Aldrich) and 30 mL Milli-Q water were placed on a Teflon-lined autoclave at 200 ºC for 5 hours. After cooling down to room temperature, the obtained solution was filtered through a 0.22 µm membrane to remove bulk impurities. Subsequently, the solution was dialyzed against pure Milli-Q water through a dialysis membrane of 1 kD pore size (Spectra/Por® 6, Spectrum Laboratories Inc, http://spectrumlabs.com) for 3 days. This solution was kept in the fridge and used as stock solution of CNDs.

Procedures

For Elemental Analysis and Fourier Transform Infrared (FTIR) analysis, a dried sample of synthesized CNDs was directly used.

From the Elemental Analysis % C value and taking account the size of the CNDs obtained by TEM, a CNDs concentration of 20 µM was estimated for the stock solution.

For Atomic Force Microscopy (AFM) and XPS analysis, a dried sample of synthesized CNDs on a Si substrate was directly used.

UV-visible absorption and fluorescence emission spectra were performed using 0.1 M PB pH 7.0 in 1.0 cm quartz cells.

Fluorescence titrations were carried out at 2.0 μM of CNDs and varying the concentration of dsDNA or ssDNA from 0 to 30 μM. The binding constant (K_b) was calculated from a plot of I_o/I vs [DNA], where I_o and I are the fluorescence intensity of free and bound-to-DNA CNDs, respectively using a Stern-Volmer model.

DNA melting curves were acquired by monitoring DNA absorbance in 0.1 M PB pH 7.0 at 260 nm with temperature, over the range 30-100 ºC at a heating rate of 1 ºC min^{-1}. The melting temperature, T_m, was determined from the mid-point of the melting curve.

dsDNA-CNDs or ssDNA-CNDs nanohybrid samples for fluorescence microscopy, FTIR, TEM, DLS and Zeta potential experiments, were prepared by incubating 3.0 mL of a 20 μM CNDs solution with 2.0 mL of a 2.0 mM ds or ssDNA solution during 72 h at room temperature. The resulting solution was transferred to a filter unit (Amicon Ultracentrifugal 100K) and filled with 3.0 mL of water. Samples were centrifuged in a Hettich 320R centrifuge at 12000 rpm for 30 min. This centrifugation process was repeated 2 times to discard the CNDs which were not linked to the ds or ssDNA. Then, the supernatant was resuspended in 200 μL of water. 50 μL of this solution was drop casted and air-dried on a glass slide during 24 h. Finally, fluorescence images of these samples were obtained.

The fluorescence quantum yield of CNDs was calculated relative to a well-known standard, quinine sulfate in 0.1 M sulfuric acid, with excitation at 340 nm using the following equation:

$$\phi_{CNDs} = \phi_{quinine} \frac{I_{CNDs}}{I_{quinine}} \frac{A_{quinine}}{A_{CNDs}} \frac{\eta_{CNDs}^2}{\eta_{quinine}^2}$$

where Φ is the quantum yield, $\Phi_{quinine}$= 54%, I is the integrated emission intensity, A is the absorbance and η is the refractive index [22]. To calculate the quantum yield of CNDs after interaction with DNA, a 5 μM DNA concentration was used to keep the absorbance below 0.1 in order to avoid inner filter effects.

The interaction strength of CNDs and dsDNA was quantified by the binding constant (K_b), using the following equation, $F_0/F=1+K_b[DNA]$. From a plot of F_0/F versus [DNA] the binding constant was calculated.

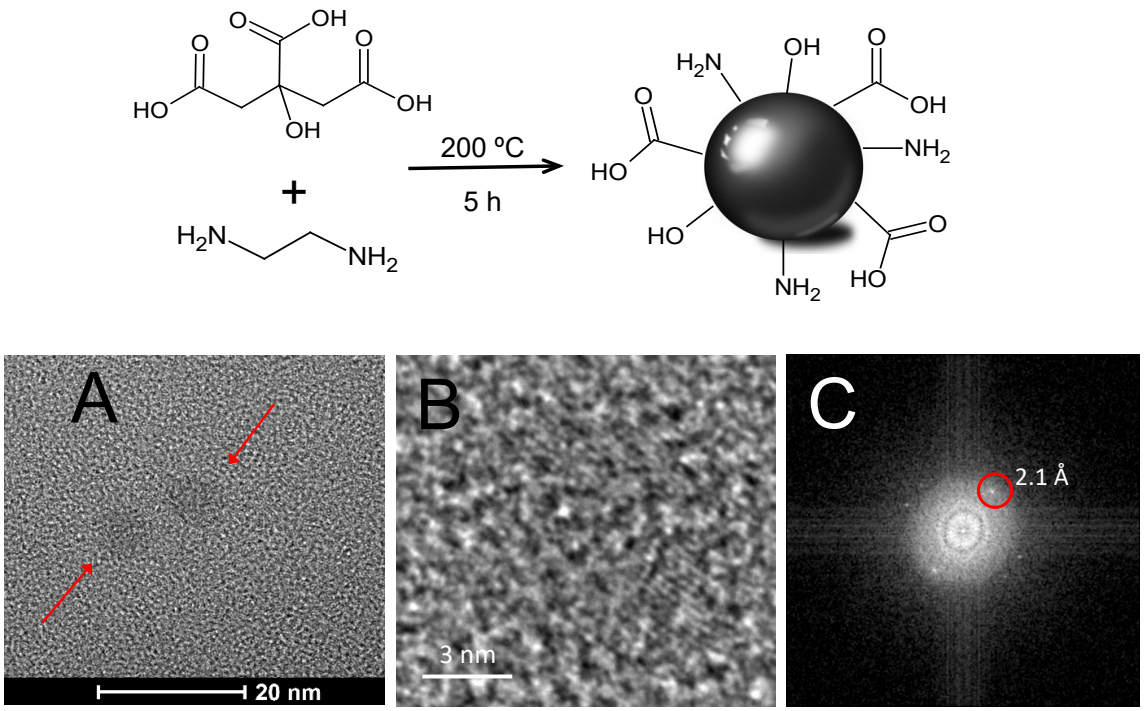

Figure S1. Reaction Scheme for CNDs synthesis. TEM images of the synthesized CNDs (A), magnification of a CND (B) and FFT analysis of the image (C).

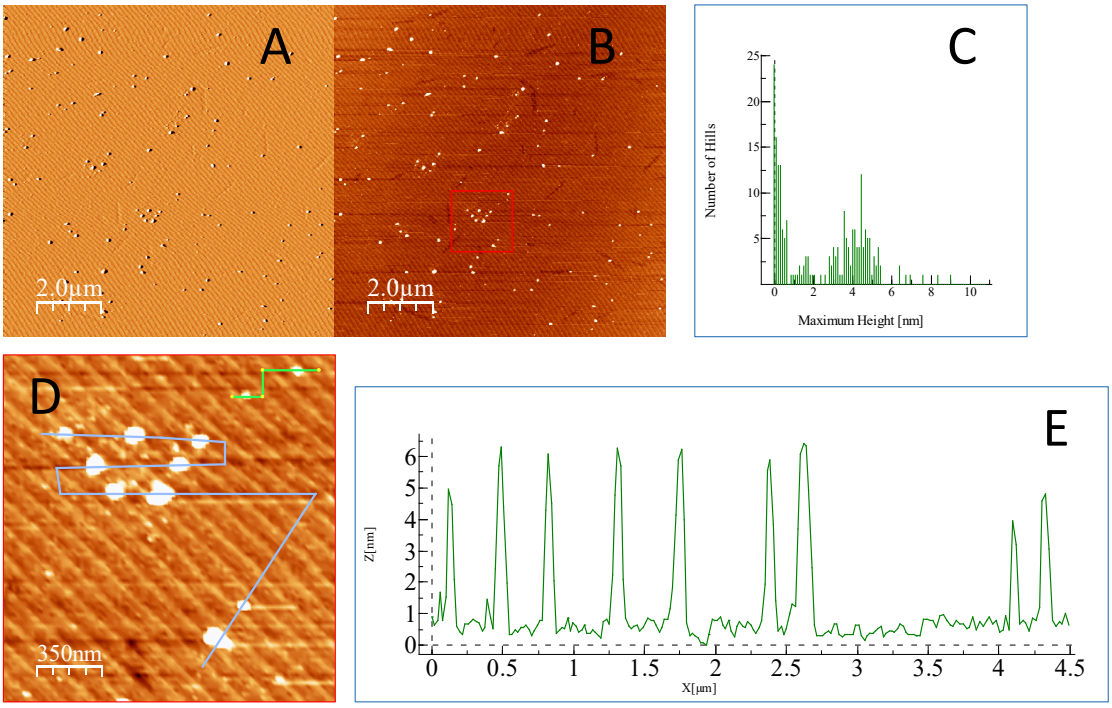

Figure S2. A,B) AFM image of a Si plate modified with the synthesized CNDs. C) Size distribution histogram of the CNDs in the red square of image B) . D) Magnification and scan of the red square of B image. E) High profile of the scan of D image.

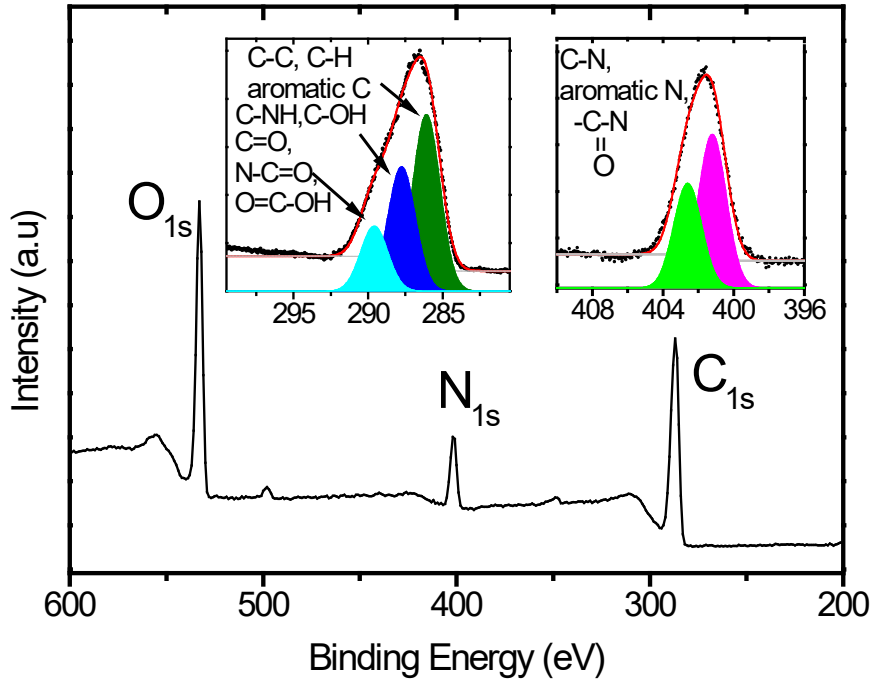

Figure S3. XPS spectra of the synthesized CNDs.

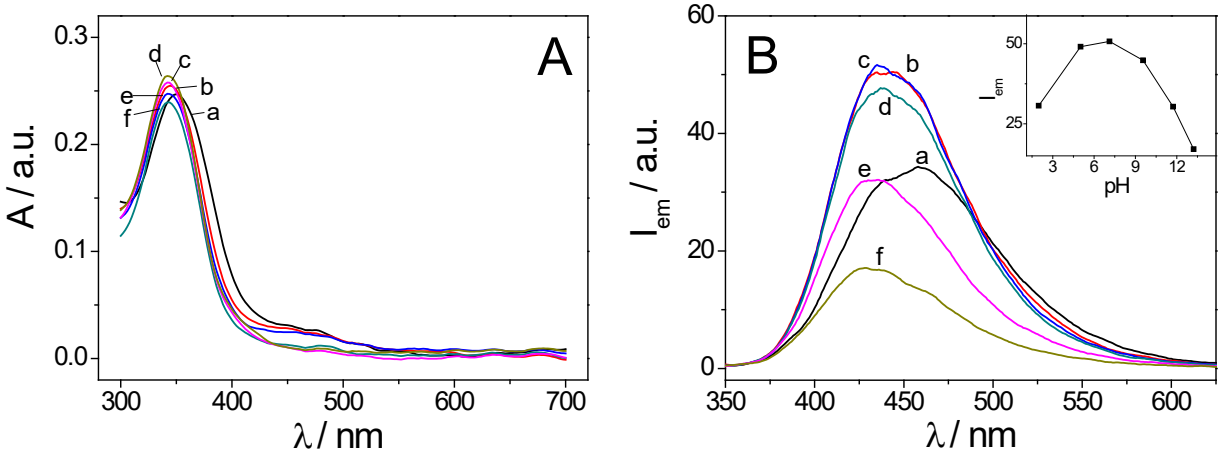

Figure S4. Absorbance (A) and Fluorescence emission (B) spectra of a 2.0 µM solution of the CNDs in 0.04 M Britton-Robinson buffer at different pHs: (a) 2.0, (b) 5.0, (c) 7.1, (d) 9.6, (e) 11.7 and (f) 13.2. Inset: emission intensity at 440 nm vs. pH.

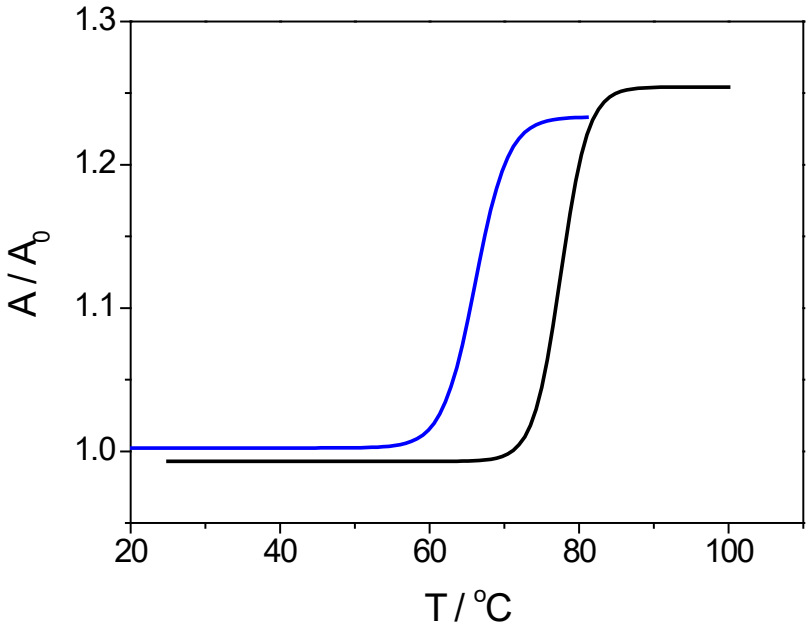

Figure S5. Melting curves of dsDNA (100 μM) in the absence (blue line) and in presence of 2.0 μM of CNDs (black line) in 0.1 M PB pH 7.0 solution.

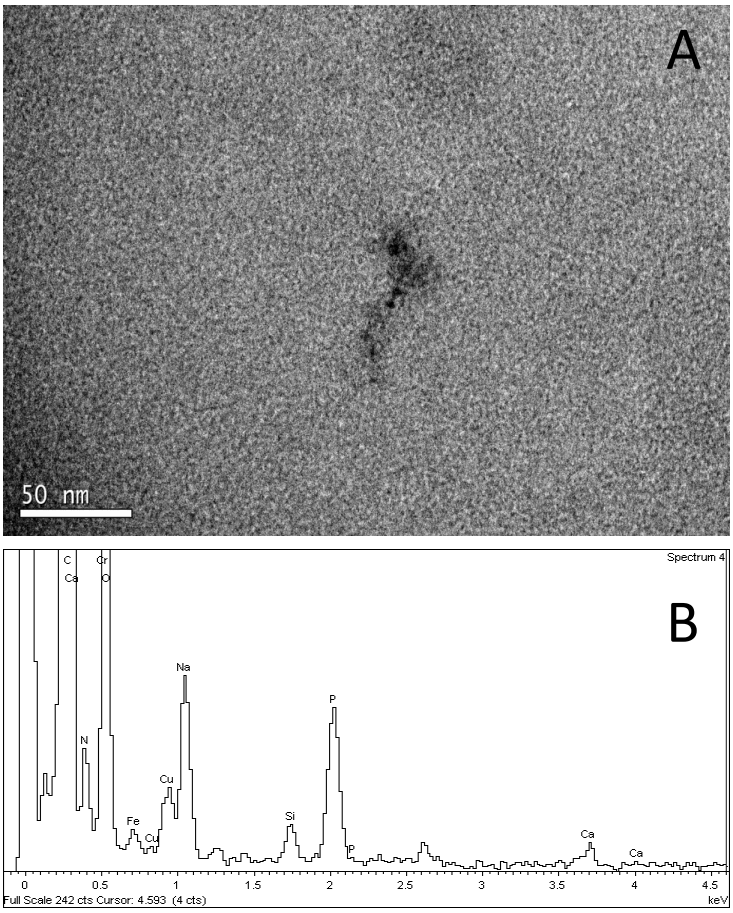

Figure S6. TEM image (A) and EDX (B) of the dsDNA-CNDs nanohybrid sample.

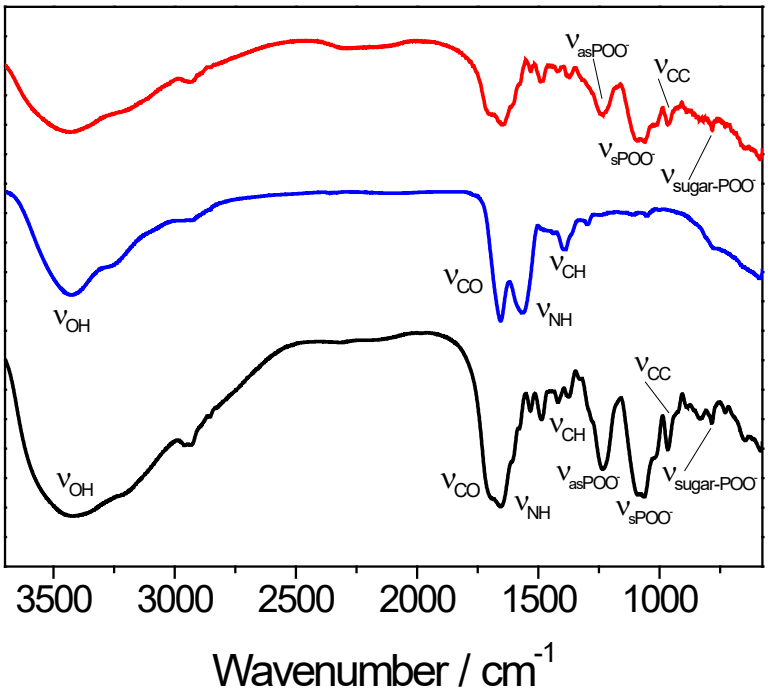

Figure S7. FTIR spectra of dried suspension of the synthesized CNDs (blue line), dsDNA (red line) and dsDNA-CNDs nanohybrid (black line).

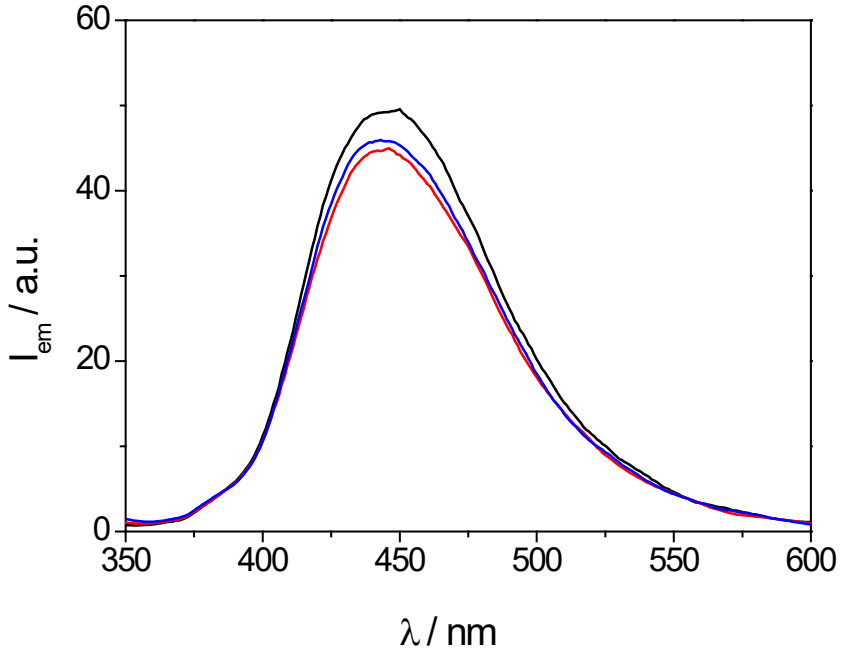

Figure S8. Fluorescence spectra of CNDs (black line) and of CNDs in the presence of 5 mM KI (red line) and in the presence of 300 μM DNA and 5 mM KI (blue line).

Table S1. DNA sequences used in this work

DNA SEQUENCES	
TCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCT TTGGTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAAGCAT GCC	PROBE $_{CFTR}$
GGCATGCTTTGATGACGCTTCTGTATCTATATTCATCATAGGAAACACC AAAGATGATATTTTCTTTAATGGTGCCAGGCATAATCCAGGAAAACTG AGA	WT $_{CFTR}$
GGCATGCTTTGATGACGCTTCTGTATCTATATTCATCATAGGAAACACC A___ATGATATTTTCTTTAATGGTGCCAGGCATAATCCAGGAAAACTGA GA	MUT $_{CFTR}$
ACAGAAGGGTGCGCGAGTTTCATTAACTCGAAAATTGTTAGAACTTTC TAAGCAGTGTGGTTCT AGTTCTGAGTGTTATGGTTAGTGCTTTTAGACT GCT	NC $_{CFTR}$
CTCAGATGGGGAGGGACAGGGTCGGCCTGTACCCCGGAGGCACCTGG GTCCATCAGAGAAGGTGCAGGTGACAGAGGCGAAGGCAGATGGGGCA CTGACC	WT $_{MRP3}$
CTCAGATGGGGAGGGACAGGGTCGGCCTGTACCCC**A**GAGGCACCTGG GTCCATCAGAGAAGGTGCAGGTGACAGAGGCGAAGGCAGATGGGGCA CTGACC	MUT $_{MRP3}$
ACAGTGCTTTTGTTTGAGTTTCATTAACTCGAAAATTGTTAGAACTTTC AAGCAGTGTGGTTCT AGTTCTGAGTGTTATGGTTAGTGCTTTTAGACT GAA	NC $_{MRP3}$
CTAAATAGGAAAATACCAGCTTCATAGACAAAGGTTCTCTTTGACTCA CCTGCAATAAGTTGCCTTATTAACGGTATCTTCAGAAGAATCAGATCCT AAA	PROBE $_{BRCA1}$
GGGGAAATTTTTTAGGATCTGATTCTTCTGAAGATACCGTTAATAAGGC AACTTATTGCAGGTGAGTCAAAGAGAACCTTTGTCTATGAAGCTGGTA TTT	WT $_{BRCA1}$
GGGGAAATTTTTTAGGATCTGATTCTTCTGAAGATACCGTTAATAAGGC AACTTATTG**T**AGGTGAGTCAAAGAGAACCTTTGTCTATGAAGCTGGTA TTT	MUT $_{BRCA1}$
CCCCTTTAAAAAATCCTAGACTAAGAAGACTTCTATGGCAATTATTCCG TTGAATAACATCCACTCAGTTTCTCTTGGAAACAGATACTTCGACCATA AA	NC $_{BRCA1}$

Table S2. Nanomaterial-based optical methods for determination of gene mutations.

Method used	Reagent used	Linear range	LOD	Gene	Reference
Luminescence	Cyclometallated Iridium (III) complex	0-0.5 μM	0.05 μM	BRCA1	[1]
Fluorescence	Perylene-labeled DNA probes	-	100 nM	BRCA1	[2]
Fluorescence	DNA silver nanoclusters	$1x10^{-4}$-2.4 μM	64 pM	BRCA1	[3]
Fluorescence	Gold nanoparticles	1-150 nM	1nM	CFTR	[4]
Luminescence	DNA silver nanoclusters	-	53 nM	LMP1 and CCR5	[5]
Fluorescence	Carbon nanodots	up to 200 nM	270 pM	BRCA1	Present work

References

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