Efficient receptor-independent intracellular translocation of aptamers mediated by conjugation to carbon nanotubes


ELECTRONIC SUPPORTING INFORMATION
Materials and Instrumentation.

Pristine MWNTs (Short Thin MWCNT, 95+\% C purity) were purchased from Nanocyl S.A. (Belgium). The aptamer sequences were purchased from Integrated DNA Technologies, Inc. (Belgium). Transmission electron microscopy (TEM) analyses were performed on a TEM Philips CM 10. Atomic Force microscopy (AFM) analysis was performed using a Veeco Nanoscope IV Multimode AFM. The thermogravimetric analyses were performed with a TGA Q500 (TA Instruments). The samples were burned in N\(_2\) atmosphere at a heating rate of 10\(^{\circ}\)C/min. Confocal microscopy was performed using a Zeiss Axiovert LSM510 confocal with a 63\(\times\) oil immersion objective (Carl Zeiss Inc.). Fluorescence microscopy was done using a Zeiss Axio Observer A1 Inverted Microscope using a 40\(\times\) Neofluar objective (Carl Zeiss Inc.).

Synthesis of MWNT-Apt

To a suspension of ox-MWNT (5 mg) in water (5 mL) was added 10 mg EDC, 5 mg NHS and pyridine (100 \(\mu\)L) and the reaction was stirred at room temperature for 4 h. This mixture was filtered and resuspended in 5 mL water after which the aptamer solution (5 nmol in 50 \(\mu\)L) and 10 \(\mu\)L triethylamine were added. The resulting suspension was stirred for 3 days in dark and a black precipitate was obtained after filtration. This was subjected to repeated resuspension and filtration steps using 0.1 M K\(_2\)CO\(_3\), water and MeOH. Finally, the purified material was dried under vacuum. The filtrates from the washings were concentrated and analysed by fluorescence spectroscopy which indicated complete binding of the Cy3-aptamer.
Figure S1

Low resolution TEM imaging of ox-MWNT (A) and MWNT-Apt constructs (B). The aptamer molecules on the MWNT lead to a moderate degree of nanotube-nanotube binding, evidenced by the presence of more bundles (see arrows).
Figure S2

Low resolution (A) and high resolution (C) AFM images of the MWNT-Apt construct. Image B is shown for contrast and is identical to Figure 1C (bottom).
Figure S3

*Fluorescence microscopy images of MWNT-Apt in MUC-1 positive (MCF7) cells and MUC-1 negative (Calu-6) cells with pre-incubation of a MUC-1 monoclonal antibody (mAb).*  
A) MCF7 cells treated with Cy3-labelled aptamer alone (100 nM); B) MCF7 cells treated with Cy3-labelled aptamer alone, after MUC-1 mAb pre-incubation; C) MCF7 cells treated with MWNT-Apt; D) MCF7 cells treated with MWNT-Apt, after MUC-1 mAb pre-incubation.

Top panels show Cy3 (red) channel, bottom panels show merged phase contrast and Cy3 (white, red) channels. No nuclear stain was used.
**Figure S4**

*Cellular binding, internalisation and trafficking of MWNT-Apt(S) (scrambled sequence) in MUC-1 positive (MCF7) and negative (Calu6) cells.* A) MCF7 untreated cells; B) MCF7 cells treated with scrambled Cy3-labelled aptamer alone; C) MCF7 cells treated with MWNT-Apt(S); D) Calu-6 untreated cells; E) Calu6 cells treated with scrambled Cy3-labelled aptamer alone; F) Calu6 cells treated with MWNT-Apt(S). Panels show merged TOPRO-3 and Cy3 (blue and red) channels.

The scrambled sequence used was CTGCGG AGTTGTGAGCGGAGGGAGC.
Figure S5

Percentage of cell viability of MCF-7 cells after 4 hr incubation with aptamer (100 nM), ox-MWNT (10 μg/ml), non-covalent mixed control (mix) (100 nM Apt + 10 μg/ml ox-MWNTs) and MWNT-Apt (100 nM Apt/10 μg/ml). The data were collected 24 hr post-incubation. Untreated cells were used as a negative control. Incubation with 10 % DMSO was used as a positive control.