Supplementary Material (ESI) for Chemical Communications

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

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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°C/min. Confocal microscopy was performed using a Zeiss Axiovert LSM510 confocal with a 63× oil immersion objective (Carl Zeiss Inc.). Fluorescence microscopy was done using a Zeiss Axio Observer A1 Inverted Microscope using a 40 x 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 µL) 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 µl) and 10 µL 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_2CO_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).

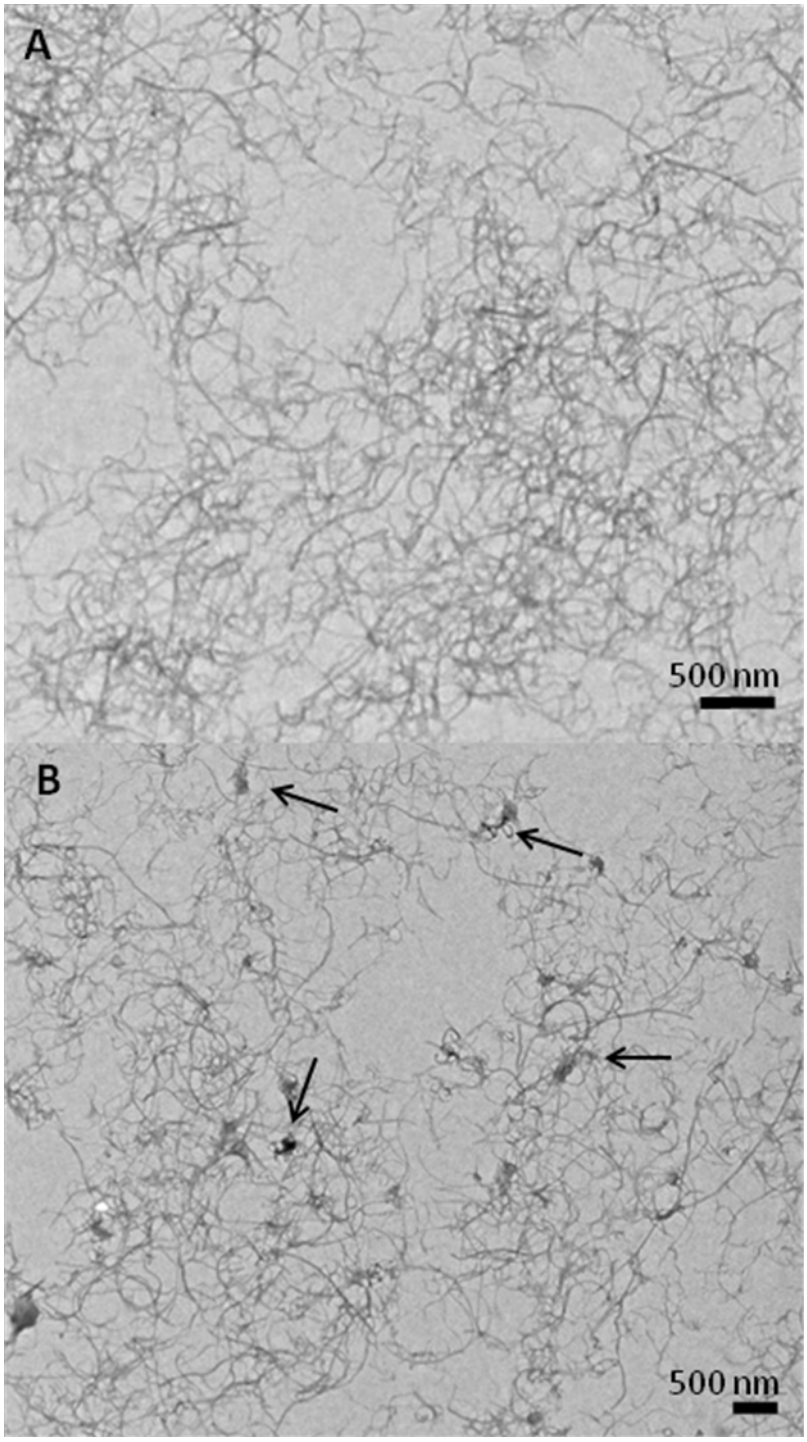
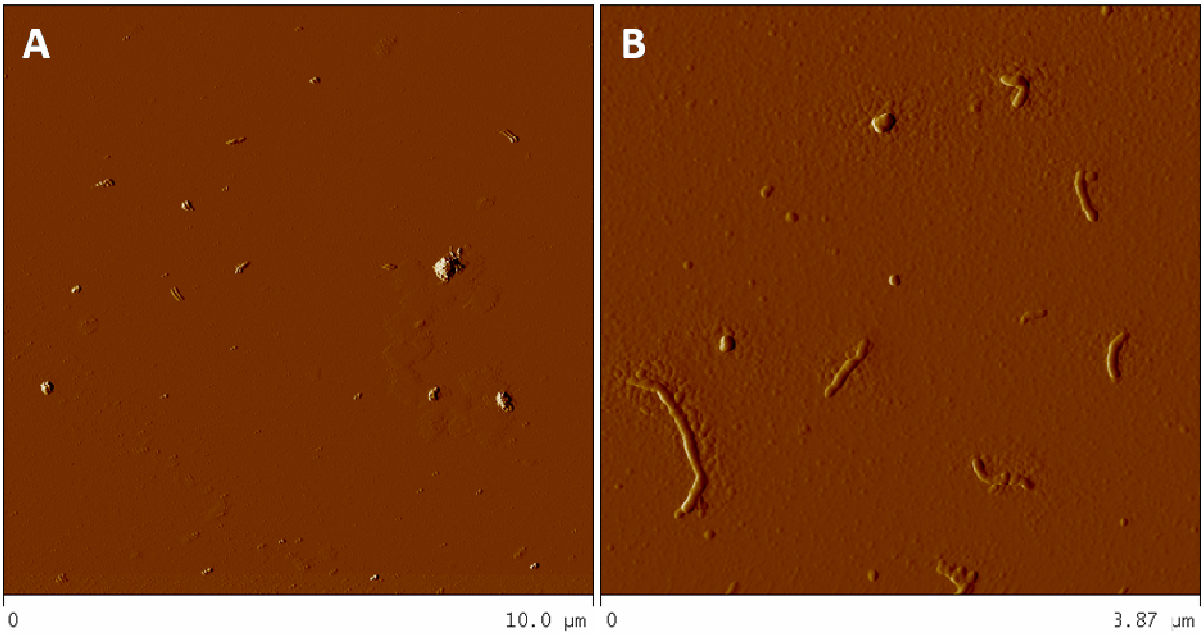


Figure S2

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).



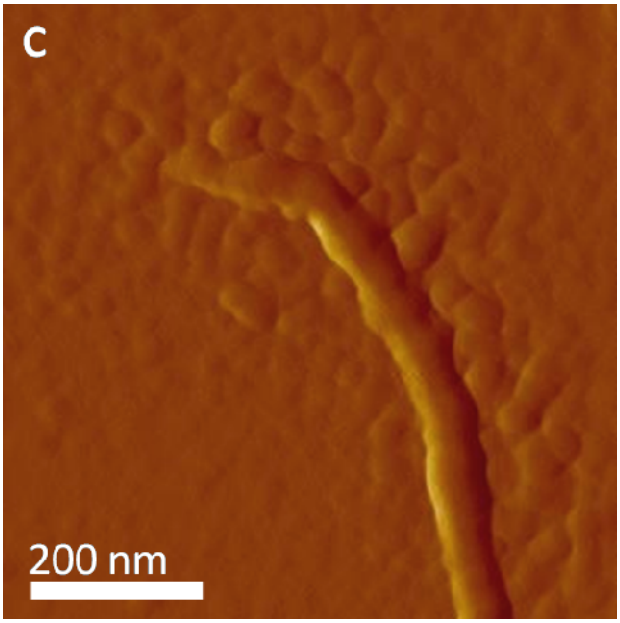


Figure S3

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.

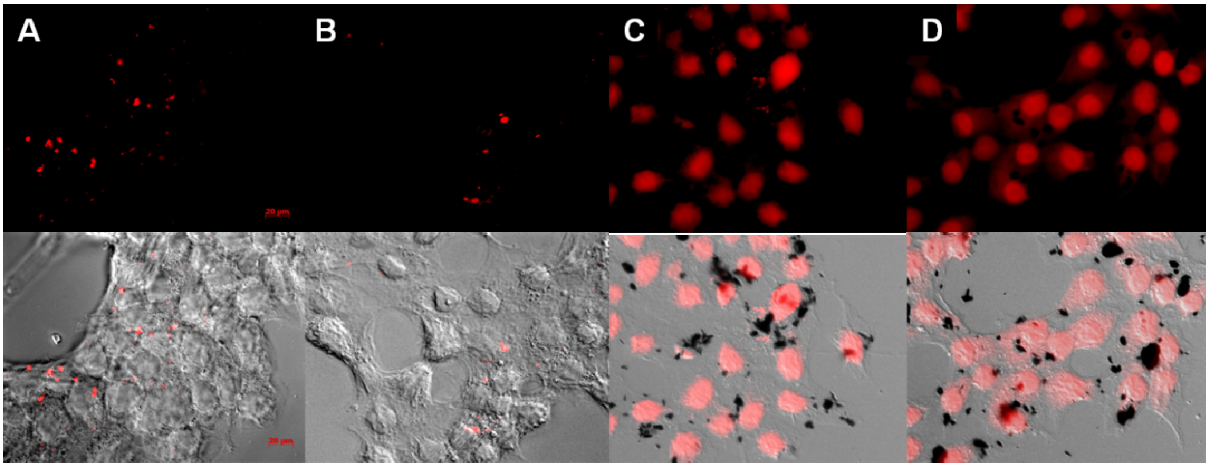


Figure S4

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.

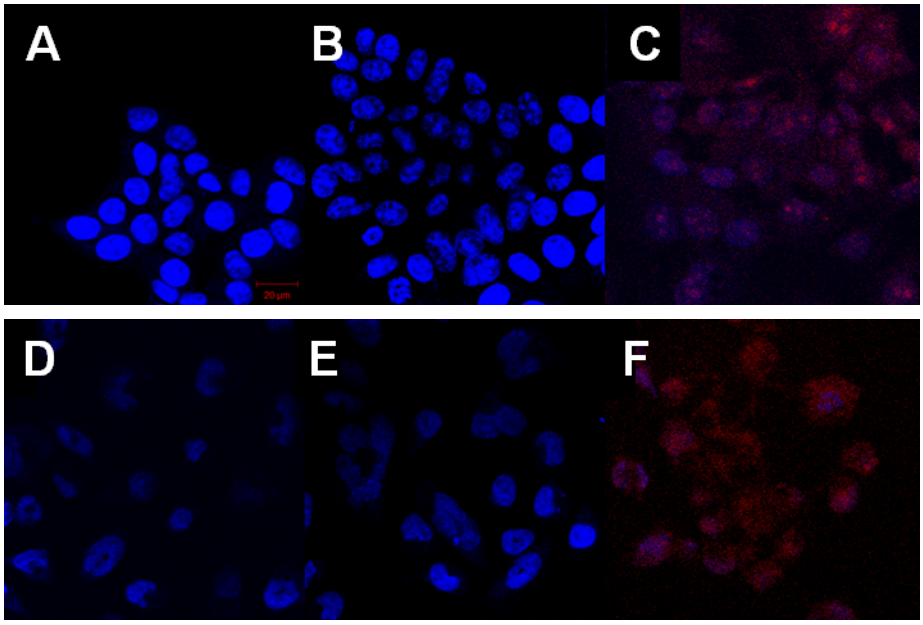


Figure S5

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.