Non-covalent anchoring of oligonucleotides on single-walled carbon nanotubes via short bioreducible linker

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SWCNT characterization

SWCNT-COOH were characterized by means of IR and Raman spectroscopy. IR-spectrum of the SWCNT-COOH was recorded using a Scimitar FTS 2000 Fourier transform IR spectrometer (Digilab, US). Attribution of the IR spectrum was done according to Refs.\textsuperscript{1,2} The bands observed in the IR spectrum correspond to $\nu$(C-H), $\nu$(C=O), $\nu$(C=C), (C-O) vibrations. Raman spectrum of the SWCNT-COOH was recorded using a T64000 triple Raman spectrometer (Horiba Jobin Yvon, Italy) at room temperature, the Ar$^+$-laser (514.5 nm) was used, and the power of laser beam was 2-3 mW. D (1230 cm$^{-1}$) and G (1500–1550 cm$^{-1}$) bands and the radial breathing mode bands (155 and 170 cm$^{-1}$) are observed in the Raman spectrum of SWCNT-COOH. The radial breathing mode values correspond to the mean individual SWCNT diameters of 1.3 and 1.4 nm, according to the Ref.\textsuperscript{3} Calculated $I_D/I_G$ ratio value of 0.04 suggests that the number of defects of the SWCNT-COOH surface is negligibly low.

Fig. S1. IR (a) and Raman (b) spectra of SWCNT-COOH.
AFM studies of carbon nanotubes

SWCNT-containing samples were prepared as described in the Experimental section. An aliquot of sample solution containing SWCNTs (25 mg/L) was deposited onto mica slide for 1-2 minutes. The slide was then washed three times with deionized water and dried on air. Scanning was performed in tapping mode using Multimode 8 atomic force microscope (Bruker, US) with HA_NC cantilevers (NT-MDT, Russia) at scanning rate of 3 Hz. Images were processed using Gwyddion 2.36 software. SWCNTs are visualized as aggregates of bundles up to 1 µm length and up to 25 nm thick. The adsorption of oligonucleotide induces partial destruction of the aggregates.

Fig. S2. AFM images of SWCNT-COOH (left) and SWCNT-COOH+dON-L1 (right). Scale bar represents 500 nm.

Pyrene conjugates of oligonucleotides

The optical density of the solutions of the pyrene conjugates of oligonucleotides was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, US). The absorption ratio at 260 and 345 nm in the spectra of the pyrene conjugates are complied with the calculated ratio of the oligonucleotide and pyrene parts with consideration of their molar absorption coefficients. The molar absorption coefficients of the pyrene conjugates were calculated as a sum of the molar absorption coefficients of the corresponding oligonucleotides and one residue of pyrene attached to the oligomer (24000 M$^{-1}$cm$^{-1}$ at 260 nm).
The mass spectra of the oligonucleotide conjugates were recorded on a MALDI-TOF Autoflex Speed mass-spectrometer (Bruker Daltonics, Germany).

### Table S1. Structure of pyrene conjugates

<table>
<thead>
<tr>
<th>Oligo</th>
<th>Sequence*</th>
<th>MW calculated</th>
<th>MW found</th>
</tr>
</thead>
<tbody>
<tr>
<td>rON-L1</td>
<td>5′-L1-r(GACCUCGCGCUCCUUGGA)₃₋₅dT</td>
<td>6476.2</td>
<td>6476.9</td>
</tr>
<tr>
<td>rON-L2</td>
<td>5′-L2-r(GACCUCGCGCUCCUUGGA)₃₋₅dT</td>
<td>6436.7</td>
<td>6436.2</td>
</tr>
<tr>
<td>dON-L1</td>
<td>5′-L1-d(GACCTCGCGCTCCTTGGA)₃₋₅dT</td>
<td>6190.1</td>
<td>6189.6</td>
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<tr>
<td>dON-L2</td>
<td>5′-L2-d(GACCTCGCGCTCCTTGGA)₃₋₅dT</td>
<td>6152.2</td>
<td>6152.8</td>
</tr>
<tr>
<td>mON-L1</td>
<td>5′-L1-m(GACCUCGCGCUCCUUGGA)₃₋₅dT</td>
<td>6723.3</td>
<td>6746.7**</td>
</tr>
<tr>
<td>mON-L2</td>
<td>5′-L2-m(GACCUCGCGCUCCUUGGA)₃₋₅dT</td>
<td>6692.2</td>
<td>6692.2</td>
</tr>
</tbody>
</table>

*₃₋₅dT - ₃′-“inverted” thymidine; r – oligoribonucleotide, d – oligodeoxyribonucleotide, m – oligo(2′-O-methylribonucleotide); L1 – pyrene introduced by bioreducible linker (cystamine); L2 – pyrene introduced by stable linker (1,6-diaminohexane).

** Contains one Na⁺ counter-ion

Fig. S3. Electrophoregrams of the pyrene conjugates of oligonucleotides rON, dON, mON. Conditions: 15% acrylamide:N,N'-methylene-bis-acrylamide (19:1), 89 mM Tris-borate, pH 8.3, 10 mM Na₂EDTA, 12 V/cm. Staining: 0.05% Stains-All in 50% formamide (aq.). BP – bromophenol blue (marker dye).
Fig. S4. UV (a-c) and fluorescence (d-f) spectra of the pyrene conjugates of oligonucleotides rON, dON, mON (10 µM). Optical path length for UV spectra is 1 cm. Excitation wavelength for fluorescence spectra is 345±4 nm.

**Disulfide bond cleavage**

![Disulfide bond cleavage](image)

Fig. S5. Typical radioautographs used for calculation of degree of disulfide linkage cleavage: a) hybrid dON-L1+SWCNT-COOH; b) free oligonucleotide dON-L2. Glutathione concentration 10mM. For other conditions, refer to the Experimental section. GSH – glutathione
Fig. S6. Kinetic profiles of the disulfide bond cleavage in the pyrene conjugates of oligonucleotides rON-L1 (a), dON-L1 (b), and mON-L1 (c) and their hybrids with SWCNT-COOH. Conditions: refer to the Experimental section. Data represent mean ± S.D. (n = 3). GSH – glutathione.
References


