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Supplementary Information

Directed Evolution of the Optoelectronic Properties of Synthetic Nanomaterials

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Design of the mutant sequences

The mutant sequences were created computationally using Matlab (Matlab R2017b, Mathworks) according to the following approach: (1) the selection of a random nucleotide position number, (2) a random nucleotide substitution (25% probability for A, C, G, or T). The number of mutations per cycle was chosen to be three based on preliminary results where the fluorescence intensities of ssDNA-SWCNT complexes containing one, two, or three random mutations were compared. Whereas small variations in fluorescence intensity were observed between the sequences containing either one or two mutations, we found that sequences containing three mutations showed the greatest variability. In addition to the two evolution cycles described in the main text, a third round of mutation was performed on the M13-1-SWCNT with a library size of 10, though no additional improvements in fluorescence were observed for any of the mutants.

Fluorescence and absorbance characterization of ssDNA-SWCNT complexes

The ssDNA-SWCNT complexes were characterized using absorbance and fluorescence spectroscopy (Fig. S1). The samples were prepared using a wrapping exchange technique that involved the replacement of sodium cholate (SC) with ssDNA (see Materials and Methods).[1] Briefly, SC-suspended SWCNTs were incubated with ssDNA and methanol (which is known to increase the critical micelle concentration (CMC) of bile salts, such as SC[2]). Incubation with methanol further solubilizes the SC wrapping, allowing SWCNT surface exchange with the ssDNA. After the wrapping exchange, the ssDNA-SWCNT complexes are further purified in order to remove remaining traces of methanol and SC and finally solubilized in 100 mM NaCl.

The validation of ssDNA wrapping was done by monitoring the position of the absorbance and fluorescence peaks before and after the exchange (Fig. S1 and S2). Our observations follow those of Nakashima *et al.* regarding the replacement of SC on the surface of SWCNTs by ssDNA[3]. In this work, the replacement of SC by ssDNA was found to result in a change in the absorbance peak position towards higher wavelengths (red-shift). Following the replacement of SC by ssDNA, we observed a similar shift of the absorbance and fluorescence peaks towards higher wavelengths, confirming the ssDNA was successfully adsorbed onto the SWCNT surface.

We also independently confirmed DNA wrapping by comparing the behavior of the SC-SWCNT suspension in methanol in the presence and in the absence of ssDNA (Fig S6). Methanol addition increases the CMC of the solution. This increase in CMC would result in SWCNT aggregation for SC-SWCNTs. In agreement with this theory, we observe aggregation of SC-SWCNTs upon addition of 60% (v/v) methanol (Fig. S6). In contrast, no aggregation is observed upon addition of 60% (v/v) methanol to SC-SWCNTs in the presence of ssDNA (Fig. S6b). In this case, the ssDNA is believed to replace the SC, and thus, suspend the SWCNTs.

SWCNT concentration and chirality distribution for the selected mutants

Figures S1 and S2 show the absorbance (Fig. S1) and fluorescence (Fig. S2) spectra of the SWCNT complexes (a) before DNA wrapping, (b) after DNA wrapping, and (c) after the replacement of the DNA wrapping with a surfactant, sodium deoxycholate (SDC). Initially, the DNA-SWCNT complexes are all prepared by replacing SC–suspended SWCNTs (spectra shown in Fig. S1a) by DNA (spectra shown in Fig. S1b). The initial SC-SWCNT batch used is the same for all preparations, so the initial SWCNT chirality distribution is therefore considered to remain constant. In addition, after the DNA wrapping, the concentrations of all suspensions are adjusted to yield the same absorption value at 632 nm (ε_{632nm} = 0.036 L mg^{-1} cm^{-1} for HiPCO SWCNTs). Following wrapping with DNA, we observe heterogeneities in the absorbance and fluorescence spectra (Fig. S1b and S2b). In order to determine whether these differences were due to changes in SWCNT chirality distribution, the DNA wrappings were replaced with SDC. However, all complexes had overlapping absorbance and fluorescence spectra (Fig. S1c and S2c) following SDC replacement of the DNA, suggesting that there was no change in the chirality distribution after the DNA wrapping.

The replacement of DNA with SDC was previously reported by Jena *et al.*[4] in a study that showed SDC was capable of displacing, and eventually completely replacing, the DNA wrapping on the surface of SWCNTs. We confirmed that the surfactant wrapping replaced the DNA by comparing the positions of the absorbance and fluorescence peaks before and after the displacement. We can observe that the positions of the absorbance and fluorescence peaks (Fig. S1b and S2b) shifted towards shorter wavelengths (Fig. S1c and S2c), indicating a change in the SWCNT wrapping, in accordance with previous observations.[4] As discussed above, following the replacement of the DNA wrapping by SDC, the SWCNT complexes showed comparable absorbance and fluorescence spectra for all samples both in terms of peak intensity ratios and peak position. No variations were observed for the position of neither the absorbance nor fluorescence peaks after wrapping replacement, suggesting that the DNA wrapping was replaced to the same extent for all complexes. Hence, considering that all SDC-coated SWCNTs have the same optoelectronic properties, the lack of difference in peak intensity between the SDC-replaced complexes suggests that all complexes have the same SWCNT chirality distribution. We can therefore state that the ssDNA-SWCNT complexes did not present any differences in chirality distribution. As a result, the observed changes in fluorescence intensity must stem from variations in the quantum yield of the complexes.

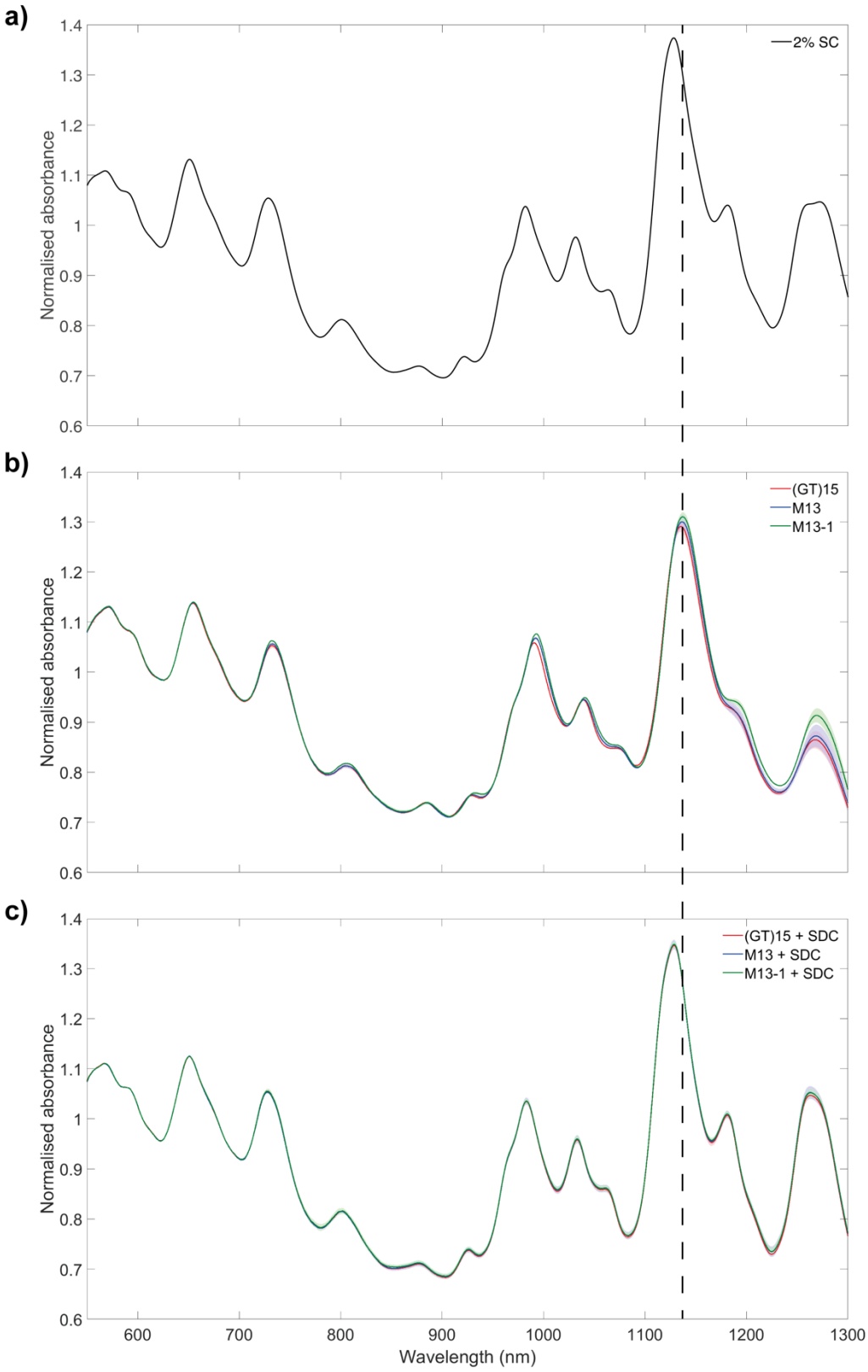


Figure S1 Absorbance spectra of (a) SC-suspended SWCNT and (b) the ssDNA-SWCNT complexes before and (c) after wrapping replacement with 0.1% SDC as a function of the wavelength. All absorbance spectra are standardized to the absorbance at 632 nm, which is used to determine the SWCNT concentration of HiPCO nanotubes. The dotted black line illustrates the change in peak position before and after wrapping replacement.

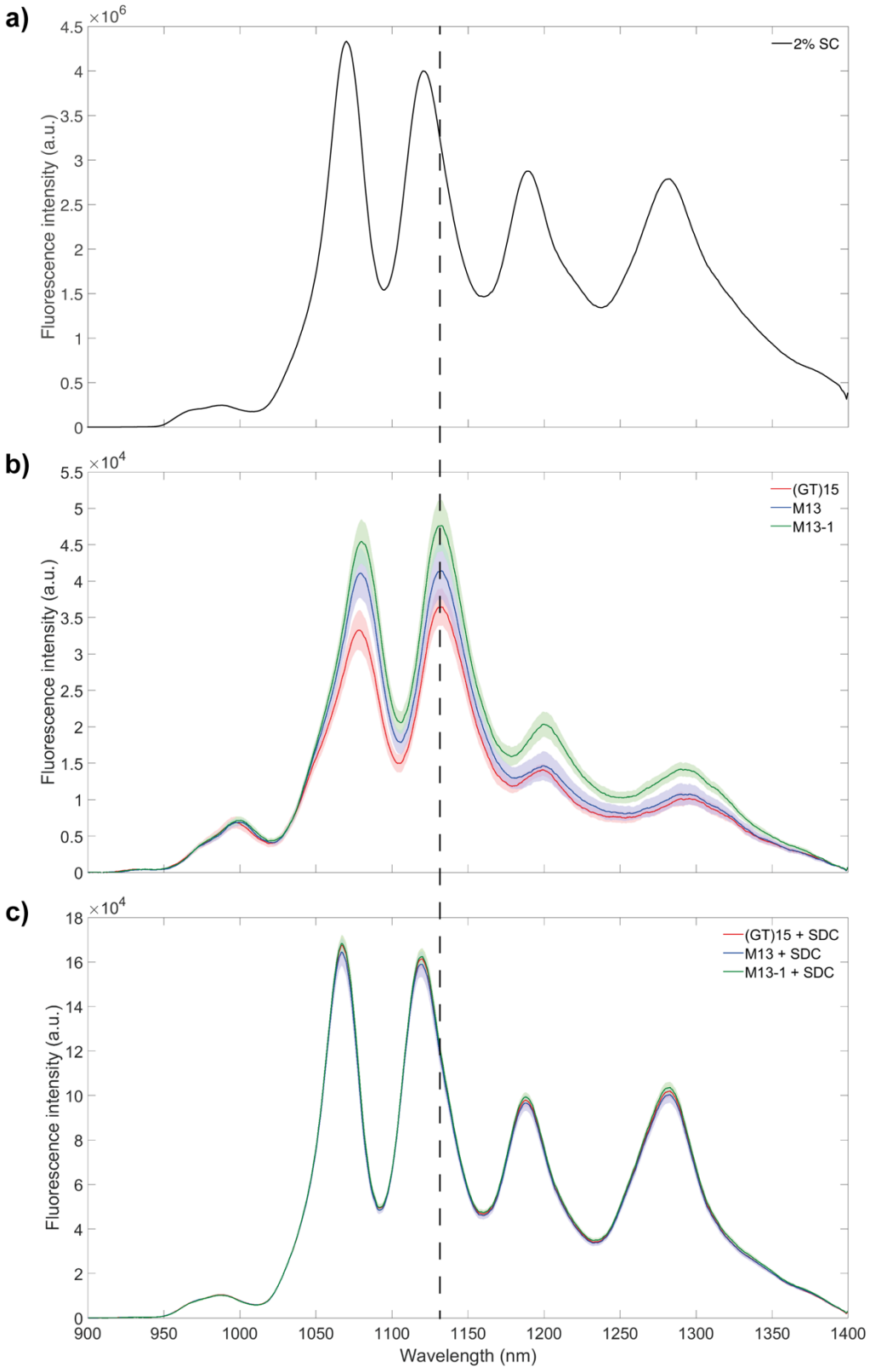


Figure S2 Fluorescence spectra of (a) SC-suspended SWCNT and (b) the ssDNA-SWCNT complexes before and (c) after wrapping replacement with 0.1% SDC as a function of the wavelength. The dotted black line illustrates the change in peak position before and after wrapping replacement.

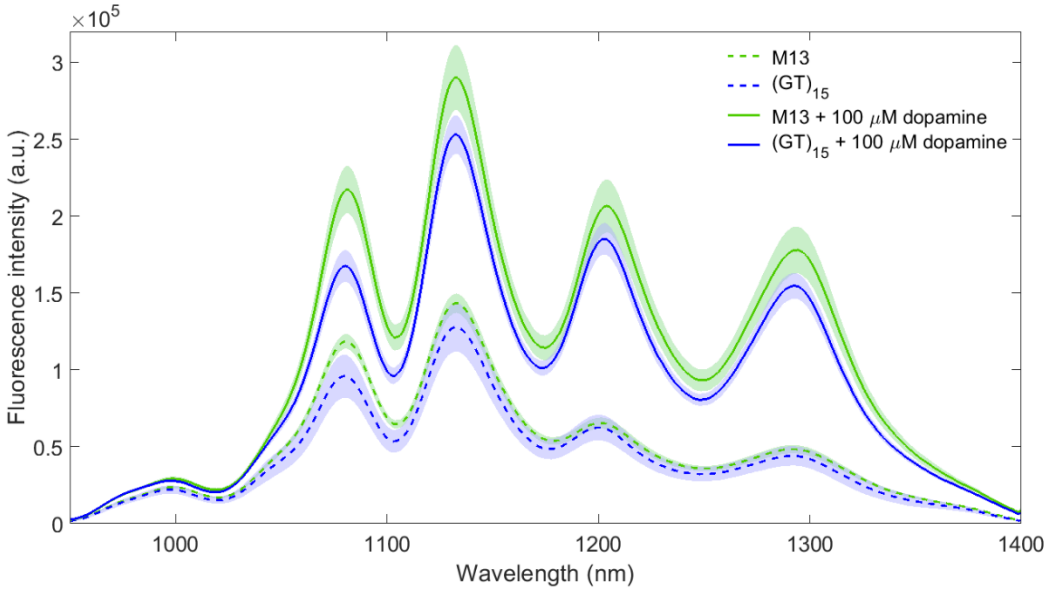
Table S1 Description of the ssDNA sequences used in the study. The mutations are indicated in red.

Label	DNA sequence (from 5' to 3')
$(GT)_{15}$	GTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
M13	GTGT**TCA**TGTGTGTGTGTGTGTGTGTGT
M47	GTGTGTGT**T**TGTGTGTGT**A**TGTGTGTGTG**C**
M71	**CC**GTGT**A**TGTGTGTGTGTGTGTGTGTGT
M13-1	GTGT**TCA**TGTGTGTGTGTGTGT**A**T**T**TGTG**C**
M13-6	GTGT**TCA**T**C**TG**C**GTGTGTGT**T**TGTGTGTGT

Table S2 Response of the ssDNA-SWCNT complexes towards dopamine and glutamic acid for the (9,4), (10,2), and (8,6) chiralities, as well as the integrated intensity under 745 nm excitation (labelled as "ALL"). The response (in %) corresponds to the ratio $(I-I_0)/I_0$ with I denoting the signal after analyte addition and I_0 denoting the signal before analyte addition. The response is shown with the specified standard deviation (n=5). Both values are rounded to the closest integer.

Label	Response to dopamine (%)				Reponse to glutamic acid (%)			
	(9,4)	(10,2)	(8,6)	ALL	(9,4)	(10,2)	(8,6)	ALL
$(GT)_{15}$	62 ± 8	46 ± 8	107 ± 12	72 ± 8	4 ± 5	2 ± 5	6 ± 5	4 ± 4
M13	73 ± 10	63 ± 9	125 ± 18	84 ± 11	4 ± 4	3 ± 4	4 ± 5	3 ± 4
M13-1	58 ± 5	58 ± 6	90 ± 8	70 ± 6	1 ± 4	-1 ± 4	3 ± 5	1 ± 4
M13-6	64 ± 10	65 ± 10	93 ± 12	74 ± 9	1 ± 5	0 ± 5	2 ± 6	1 ± 5

a)



b)

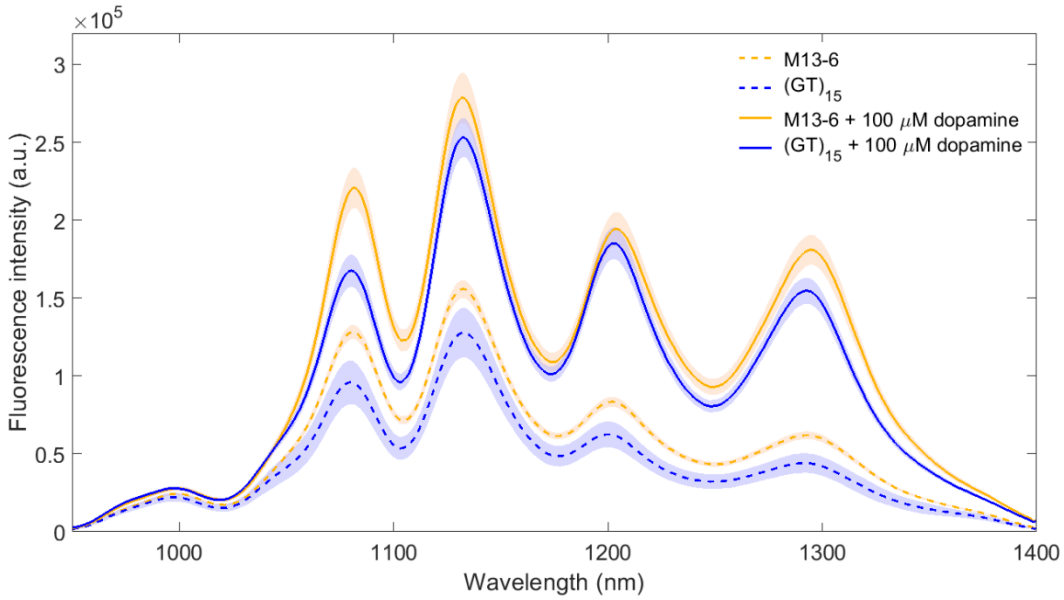
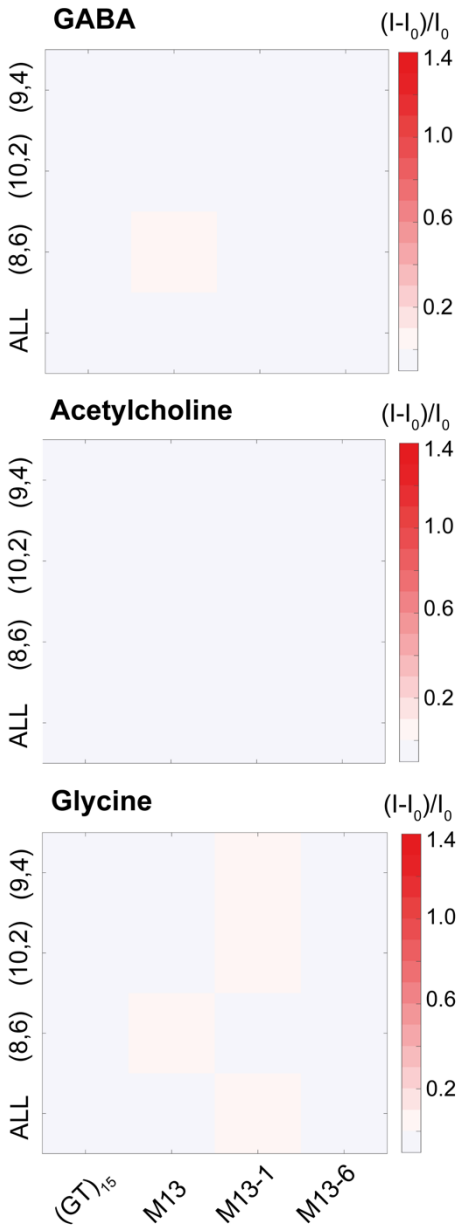


Figure S3 Fluorescence spectra of the reference and the mutant complexes, before (dotted lines) and after (solid lines) dopamine addition under 745 nm excitation. (a) Fluorescence spectra of the $(GT)_{15}$-SWCNT (blue curves) and the M13-SWCNT complex (green curves). (b) Fluorescence spectra of the $(GT)_{15}$-SWCNT (blue curves) and the M13-6 –SWCNT complex (orange curves). The shaded areas represent 95% confidence intervals.

a)



b)

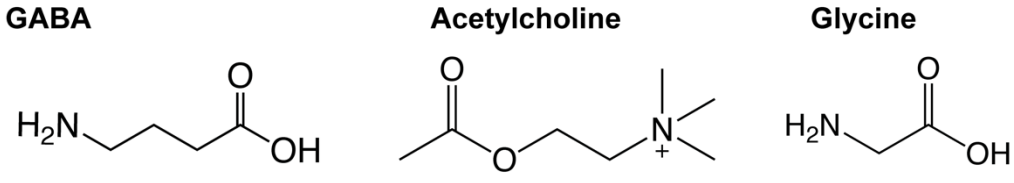


Figure S4 Response of the ssDNA-SWCNT complexes to γ-aminobutyric acid (GABA), acetylcholine, and glycine (final analyte concentrations of 100 µM). (a) Response of the ssDNA-SWCNT sensors towards GABA, acetylcholine, and glycine as a function of the intensity of the (9,4), (10,2), and (8,6) chiralities as well as the integrated intensity under 745 nm excitation (labelled ALL). The response (I) is normalised to the intensity before analyte addition (I_0). (b) Chemical structures of GABA, acetylcholine, and glycine.

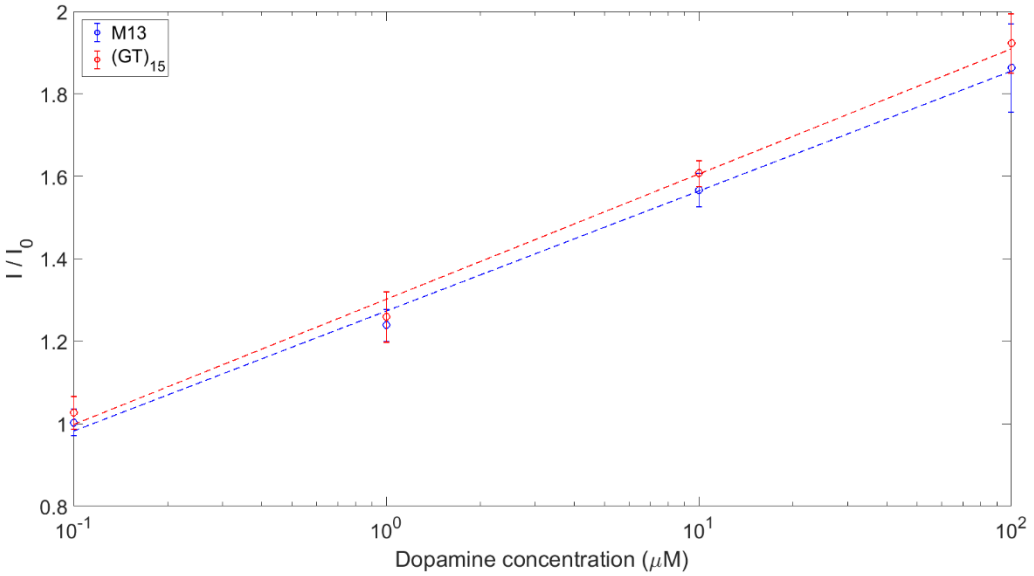


Figure S5 Dopamine calibration curves for the $(GT)_{15}$ - and M13-SWCNT complexes. The ratio I/I_0, with I denoting the integrated fluorescence intensity (under 745 nm excitation) after dopamine detection and I_0 denoting the integrated fluorescence intensity (under 745 nm excitation) before dopamine introduction, is represented as a function of the dopamine concentration in µM (logarithmic scale). The M13-SWCNT complex is shown in blue and the $(GT)_{15}$-SWCNT complex in red. The error bars represent 1 standard deviation.

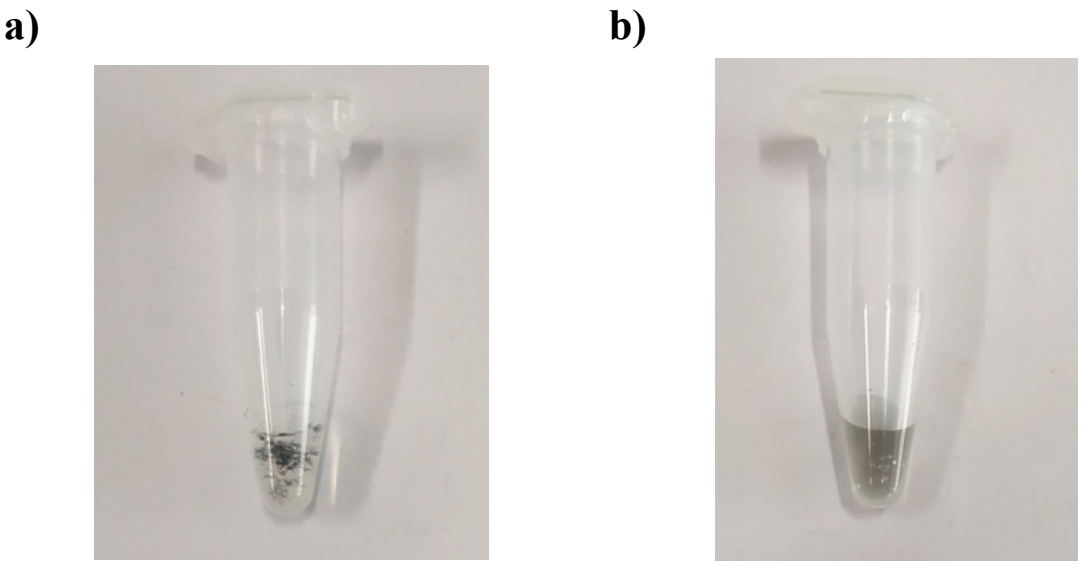


Figure S6 Picture of the SC-SWCNT suspensions with methanol (a) in the absence of ssDNA and (b) with the $(GT)_{15}$ DNA sequence. The samples consist of a mixture of 20 of µL SC-SWCNT solution with 60 µL of methanol in the presence of either 20 µL of water (a) or 20 µL aqueous ssDNA solution. The concentrations and conditions are equivalent to those described in the Materials and Methods section. In the absence of DNA, we observe the formation of SWCNT aggregates, while the suspension remains stable in presence of the $(GT)_{15}$ sequence.

Light penetration depth enhancement

The following calculations are based on the findings of Lee *et al.* for determining light penetration depth in biological tissue[4]. We apply these calculations to the $(GT)_{15}$ and the M13-1 –SWCNT complexes:

$$\log\left(\frac{F_0 1}{F1}\right) = \gamma_{em} d1$$

$$\log\left(\frac{F_0 2}{F2}\right) = \gamma_{em} d2$$

with γ_{em} representing the extinction coefficient of the tissue at the emission wavelength; $F_0 1$ and $F_0 2$ representing the initial fluorescence intensities before tissue penetration for the $(GT)_{15}$ and M13-1 –SWCNT complexes, respectively; *F1* and *F2* representing the fluorescence intensities after tissue penetration; and *d1* and *d2* representing the corresponding penetration depths in biological tissue. The initial fluorescence intensities are defined by:

$$F_0 2 = \alpha\, F_0 1$$

where α is a scaling factor relating the fluorescence intensity with that of the mutant. Since we want to compare the difference in light penetration depth between the $(GT)_{15}$ and M13-1 –SWCNT complexes under the same conditions, we set *F1 = F2* to indicate that the final recorded signal should be equal for both complexes. Hence, we have:

$$d2 = \mathrm{d1} + \log(\alpha)\frac{1}{\gamma_{em}}$$

We consider an extinction coefficient γ_{em} of approximately 10 cm^{-1} for biological tissues like the brain cortex. For the wavelengths considered in this study for SWCNT emission (1000 to 1300 nm), we can estimate the increase in penetration depth between the mutant and the reference complexes by calculating the difference *d2 – d1*:

	γ_{em} (cm^{-1})	α	d2 - d1 (μm)
Integrated I	10	1.41	149
(9,4)	10	1.43	155
(10,2)	10	1.54	188
(8,6)	10	1.56	193

Materials and Methods

Materials

Purified SWCNTs were ordered from NanoIntegris (HiPco, batch HP29-064). All DNA oligomers were purchased from Microsynth. All chemicals were purchased from Sigma-Aldrich, unless otherwise indicated.

ssDNA-SWCNT complex preparation

The DNA sequences were mutated using a custom-built code in Matlab (Matlab R2017b, Mathworks), as discussed above. We adapted a previous procedure used to suspend SWCNTs with ssDNA.[1] Briefly, wrapping exchange between surfactant-suspended SWCNTs and ssDNA was performed in the presence of methanol, which was added to increase the critical micelle concentration (CMC) of the surfactant. In this study, sodium cholate (SC) was used as the dispersant for SWCNTs instead of sodium dodecyl sulfate (SDS).

Preparation of SC-SWCNT complexes

30 mg of SWCNTs (HiPco, batch HP29-064, NanoIntegris) were suspended in 30 mL of 2% (w/w) SC in ddH_2O. The suspension was homogenized for 20 min at 5000 rpm (PT 1300D, Polytron) and sonicated for 1 h using a probe-tip ultrasonicator (1/4 in. tip, Q700 Sonicator, Qsonica) at 10% amplitude in an ice bath. The SWCNT suspension was centrifuged at 30 000 rpm (164 000 x g) for 4 h at 25°C (Optima XPN-80, Beckman Coulter), and the supernatant was collected. The SWCNT suspension was diluted to a final concentration of 108 mg/L in 2 wt% SC in ddH_2O.

SWCNT wrapping exchange

The DNA oligomers were dissolved in ddH_2O and diluted to a final concentration of 50 µM. The concentration of DNA was adjusted based on absorbance measurements (Nanodrop 2000, Thermo Scientific). To yield a final volume of 500 µL, 100 µL of DNA solution was mixed with 100 µL of SC-suspended SWCNT and 300 µL of methanol (VWR Chemicals). The suspension was incubated at room temperature for 2 h.

ssDNA-SWCNT complex purification

In order to remove the methanol and SC from the suspension, the complexes were purified using a protocol similar to the ethanol precipitation procedure commonly used in the purification of nucleic acids.[5] 76.9 µL 1.5 M sodium chloride (NaCl) solution was added to yield a final concentration of 200 mM NaCl. Following this addition, 1142 µL of ice-cold ethanol (VWR Chemicals) was added (2.5x total volume). The suspension was incubated at -20°C for 1 h and centrifuged at 15 000 rpm (21 130 x g) for 30 min (5424 R, Eppendorf). The supernatant was discarded and the pellet washed vigorously and vortexed with 1666 µL of 70% (v/v) ethanol (2.9x total volume). The suspension was centrifuged at 15 000 rpm (21 130 x g) for 1 h (5424 R, Eppendorf). The supernatant was discarded, and the pellet was air dried for 12 min and re-suspended in 500 µL of 100 mM NaCl in ddH_2O. The final suspension was centrifuged at 15 000 rpm (21 130 x g) for 30 min (5424 R, Eppendorf) and the supernatant collected to remove potential aggregates. The suspension was diluted to achieve an absorbance of 0.1 at 632 nm for a 100 µL aliquot placed in a 96-well plate (EIA/RIA plate, Corning) using a plate reader (Varioskan LUX, Thermo Scientific).

Near-infrared microscopy setup

A custom-built near-infrared microscope was used to monitor SWCNT fluorescence. The setup consists of a supercontinuum laser (SuperK Extreme EXR-15, NKT Photonics) coupled to a tunable band-pass filter unit (SuperK Varia, NKT Photonics) that operates between 400 and 830 nm at a 80 MHz pulse frequency. A short-pass filter (890 nm BrightLine, Semrock) was used to remove near-infrared

contributions from the laser optical fiber. The excitation light passes through a 20× objective (M Plan Apo NIR, NA 0.4 air, Mitutoyo Corporation) using silver-coated mirrors and a dichroic beam-splitter (LP 830nm, Semrock), resulting in an illumination spot of 350 × 350 μm^2. The emission from the SWCNT complexes is collected in the epi-direction and focused onto the entrance slit of a spectrometer (IsoPlane SCT-320, Princeton Instruments). The light is redirected into an InGaAs NIR camera (NIRvana 640 ST, Princeton Instruments) using a 70 lines mm^{-1} grating. Measurements were recorded with LightField (Princeton Instruments) in combination with a custom-built LabView (National Instruments) software for automation of the measurements.

Fluorescence spectroscopy measurements

The fluorescence measurements were performed using the setup described above. The measurements were done in 384-well plates (MaxiSorp, Nunc) using an exposure time of 5 s for all experiments except the surfactant replacement assay experiments, which were performed using an exposure time of 2 s. For all measurements, the laser excitation used a bandwidth of 10 nm and a relative power of 100%. The photoluminescence excitation (PLE) maps were acquired between 500 nm and 800 nm with a 5-nm step. The dopamine and glutamic acid experiments were recorded under an excitation of 745 nm. The excitation of 745 nm was chosen for the integrated fluorescence measurements in this study because it simultaneously excites the (10,2), (9,4), and (8,6) chiralities. All measurements were performed at room temperature and the plates were sealed (Empore, 3M) to avoid evaporation. The results were analyzed using a custom Matlab code (Matlab R2017b, Mathworks). The fluorescence results were standardized by the SWCNT concentration of each sample. The fluorescence of the individual chiralities was determined by performing a Lorentzian fitting of the peaks in the fluorescence spectrum.

Surfactant replacement assay

The ssDNA wrapping was replaced using sodium deoxycholate (SDC) surfactant. The SDC was dissolved to a concentration of 1% (w/w) in ddH_2O. 45 µL of ssDNA-SWCNT suspension was mixed with 5 µL of 1% (w/w) SDC in a 384-well plate (MaxiSorp, Nunc) and incubated at room temperature for 15 min. The near-infrared fluorescence of the suspension was recorded before and after SDC addition. The absorbance was recorded in quartz cuvettes (10mm Quartz SUPRASIL, Hellma Analytics) using a UV-VIS-NIR spectrophotometer (UV-3600 Plus, Shimadzu).

Analyte detection assay

10mM solutions of glutamic acid (L-glutamic acid monosodium salt monohydrate), γ-aminobutyric acid (GABA), acetylcholine (acetylcholine chloride), glycine, and dopamine (dopamine hydrochloride) were prepared in 100 mM aqueous NaCl solution. The fluorescence of 49.5 µL of ssDNA-SWCNT was measured initially in a 384-well plate (MaxiSorp, Nunc). After this measurement, 0.5 µL of analyte (final concentration of 100 µM) was introduced to the SWCNT suspension and mixed by pipetting up and down several times. The suspension was incubated for 20 min at room temperature prior to recording the second fluorescence spectrum. For the calibration curves, stock solutions of 1 mM, 0.1 mM, and 0.01 mM dopamine were prepared from the 10 mM stock solution and analysis was carried out using the same procedure described above.

Statistical analysis

Statistical analysis of the data was performed using Matlab (Matlab R2017b, Mathworks). Unless otherwise indicated, the p-values reported correspond to one-way ANOVA tests.

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