Supporting Online Material for

DNA-CNT Nanowire Networks for DNA Detection

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MATERIALS AND METHODS

Materials. Unless otherwise noted, all the starting materials were obtained from commercial suppliers and used without further purification. Purified HiPco Single-Wall Carbon Nanotubes were purchased from Unidym, Inc. Oligonucleotides were purchased from Integrated DNA Technologies. *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 98+%; Tween® 20; Triton X-100; polyethylene glycol (PEG) with average mol wt 10,000; octyltrichlorosilane (OTS), 97%; 6-mercapto-1-hexanol (MCH), 97%; hydrogen peroxide 30 wt % solution in water; sodium nitrate ≥99.0%; sodium phosphate monobasic (anhydrous); sodium phosphate dibasic; 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); peroxidase from horseradish, Type II (HRP); albumin from bovine serum (BSA); human serum from human male AB plasma and nonfat-dried bovine milk were purchased from Sigma-Aldrich. Hepes (free acid) was purchased from EMD

Biosciences, Inc. EnzMet™ kit was acquired from Nanoprobes, Inc. Microcon centrifugal filter devices and polycarbonate membrane filters (0.6 μm) were purchased from Millipore. NAP-5 columns were obtained from GE Healthcare. *N*-[γ-Maleimidobutyryloxy]sulfosuccinimide ester (Sulfo-GMBS) was obtained from Pierce Biotechnology, Inc. Phosphate-buffered saline (PBS×10) without calcium and magnesium was purchased from Mediatech, Inc. DL-Dithiothreitol (DTT) was acquired from J.T.Baker. Ultrapure water from a NANOpure Diamond (Barnstead) source was used throughout all of the experiments.

General Methods. Devices were fabricated with an ATC ORION 5 Sputtering system (AJA International, Inc.) using chrome and gold as sputtering substrates. All sonication procedures were conducted with an ultrasonic bath (Branson Ultrasonics Corporation, model 3510). Glass substrates and devices were cleaned with UV and Ozone using a UVO Cleaner® model No. 42 (Jellght Company, Inc.). Conductance measurements were recorded with a Fluke 287 True-RMS Electronic Logging Multimeter with TrendCapture (Fluke, Everett, WA). UV/Vis spectra were recorded on an Agilent 8453 diode-array spectrophotometer. Confocal Raman microscopy was performed using a Horiba Jobin Yvon Raman confocal microscope (model LabRAM-HR) with a 784.4 nm (1.58 eV) laser as the excitation light sources. A x50 objective was used for imaging with a pin hole size of 300 microns.

DNA sequences:

Probe DNA:

5'-HS-AAAAAAAAAATTGTTGATACTGTTC-3'

Linker DNA (capture strand):

5'-$H_2N(CH_2)_6$-AAGAATCCAACATTTACTCCAAAAA-NH_2-3'

Match and mismatch oligonucleotide sequences:

Match (target):

5'-GGAGTAAATGTTGGAGAACAGTATCAACAA-3'

1AA mismatch:

5'-GGAG**A**AAATGTTGGAGAACAGTATCAACAA-3'

1CC mismatch:

5'-GGA**C**TAAATGTTGGAGAACAGTATCAACAA-3'

2AA mismatch:

5'-GGAG**A**AAA**A**GTTGGAGAACAGTATCAACAA-3'

3AA mismatch:

5'-GGAG**A**AAA**A**G**A**TGGAGAACAGTATCAACAA-3'

Non-match (control):

5'-ATGATTAGGTTGCACTCACACTATTACATCTGGCT-3'

Buffers

Disulfide cleavage buffer: Phosphate buffer, 170 mM, pH 8.0.

Blocking buffer: 0.1 mM 6-mercapto-1-hexanol, 0.1% Tween 20 (v/v) aqueous solution.

Storage buffer: PBS, 1% BSA (wt/v), 1% Triton X-100 (v/v), pH 7.4.

Assay buffer: PBS, 0.1% nonfat milk, 0.025% Tween 20 (v/v), pH 7.4.

Slide washing buffer A: 0.5 M $NaNO_3$, 0.01% SDS (wt/v), 0.1% Tween 20 (v/v) in NANOpure water.

Slide washing buffer B: 0.5 M $NaNO_3$ in NANOpure water.

Slide washing buffer C: 0.1 M $NaNO_3$ (store at 4 °C) in NANOpure water.

Oxidation of SWCNT. Commercial HiPco SWNTs were oxidized according to our published procedure.[1]

Shielding SWCNT-COOH sidewalls. Sidewall shielding was performed according to our published procedure.[1]

Tethering linker DNAs to the shielded SWCNT-COOH terminus sites $(DNA\text{-}CNT)_n$. DNA conjugation was preformed according to our published procedure.[1]

HRP crystal structure analysis and rendering. The crystal structure of horseradish peroxidase (PDB 1hch) was analyzed, rendered, and solvent accessible surface area calculations were performed using the UCSF Chimera software package.[2]

HRP functionalization with DNA (HRP probe). 1.75 nmol of horseradish peroxidase (HRP) was reacted with 8.75 nmol of Sulfo-GMBS and 17.5 nmol thiolated DNA (Probe DNAs) in a ratio of 1:5:10, for 3 h at room temperature. The excess Sulfo-GMBS and DNA were removed using a Microcon centrifugal filter device unit (cut-off MW 30,000). Prior to modification, the disulfide bonds in all oligonucleotides were reduced by soaking in 0.1 M DTT in disulfide cleavage buffer for 2-3 h (10 OD of lyophilized DNA is typically reduced with 150 µL of freshly prepared solution of 0.1 M DTT). The deprotected DNA solutions were purified through desalting NAP-5 columns, and the amount of DNA from each column was determined by reading the absorbance of the solutions at 260 nm. The modified HRP was diluted to a final concentration of 4 μM with storage buffer and stored at -30 ºC.

Determination of number of DNA oligomers per HRP molecule. Concentrations of 5'-HRP-labeled oligonucleotides (probe DNA) were calculated either by the peak area at 260 nm (DNA concentration) and/or by incubating serial dilutions of the 5'-HRP oligonucleotides. Standard dilutions of native HRP were used for calibration at 402 nm. DNA-labeled HRP concentrations were interpolated from the calibration curve. Comparing the spectra of native HRP, free oligonucleotide, and oligonucleotide-modified HRP, the number of DNA strands conjugated per each HRP molecule was determined.

Enzymatic activity of the DNA–HRP conjugates compared to native HRP. The activity of the enzyme was determined colorimetrically using a UV–Vis spectrophotometer. A mixture of 8.7 mM ABTS, 0.01% hydrogen peroxide (wt/wt), 0.004% BSA (wt/v), 0.008% Triton X-100 (v/v) and 0.1 nM of the native HRP or the modified HRP, placed in PBS buffer, pH 7.4. The spectrophotometer was adjusted to 414 nm. Increase in absorbance was recorded for 5 min. The concentration of HRP was

diluted so that a reasonable reaction rate (usually ΔA/min = 0.02–0.04) could be obtained.

Device preparation. In a typical experiment, microelectrodes (50 nm Au on 10 nm Cr) with 250 μm gaps were prepared on glass substrates using a shadow mask. The exposed glass of the entire chip was treated with 0.2% OTS to block the surface Si–OH bonds as follows: the chips were cleaned with hot ethanol, dried with nitrogen, and cleaned for 30 seconds using a UV-Ozone cleaner. Next, the chips were soaked in a solution containing 0.2% OTS (v/v) in toluene for 15 minutes. The chips were washed with pure toluene to remove excess OTS, and dried using nitrogen followed by 30 minutes at 90° C in an oven. Finally, the chips were soaked in toluene for 5 minutes with sonication using an ultrasonic bath. SWCNT-DNA nanowires (0.5 μL, see section: Tethering linker DNAs to the shielded SWCNT-COOH terminus sites) were spin coated at 2,000 rpm between the two electrodes of each device, followed by a second 75 nm gold layer deposition on top of the nanowires. Chips were immersed in blocking buffer for 1 h to block the vacant gold surface with MCH and to block the vacant SWCNT surface with Tween 20. Finally, the chips were washed with NANOpure water to remove excess MCH and Tween 20, followed by drying with nitrogen.

DNA detection assay. The target DNA in assay buffer was reacted with the device surface followed by incubation at 25 °C for 2 h to allow hybridization between the captured DNA strands and the target DNA. Next, the devices were washed with the same assay buffer (5x1 mL). The HRP probe, 10 nM was added to the capture DNA-target complexes, and hybridization was allowed to proceed at 25 °C for 1 h. The devices were washed with assay buffer (5x1 mL), washing buffer A (5x1 mL), washing buffer B (5x1 mL) and washing buffer C (5x1 mL) to remove nonspecifically bound enzyme probe and chloride ions that can react with the silver ions in the next step. Finally, the devices were soaked briefly in NANOpure water and dried with nitrogen. The conductance was recorded with a Fluke 287 multimeter before and after silver deposition. Silver deposition was accomplished using an EnzMet^{TM} solution of reagents A, B and C in a 1:1:1 ratio for 2 minutes of incubation at room temperature.

Note: additional development time resulted more non-specific silver deposition and loss of sensitivity compared to controls.

Mismatch detection assay. The same procedure for the DNA detection assay was applied for mismatch detection. All DNA concentrations were fixed at 1 nM and in one set of experiments the temperature was changed to 32 °C.

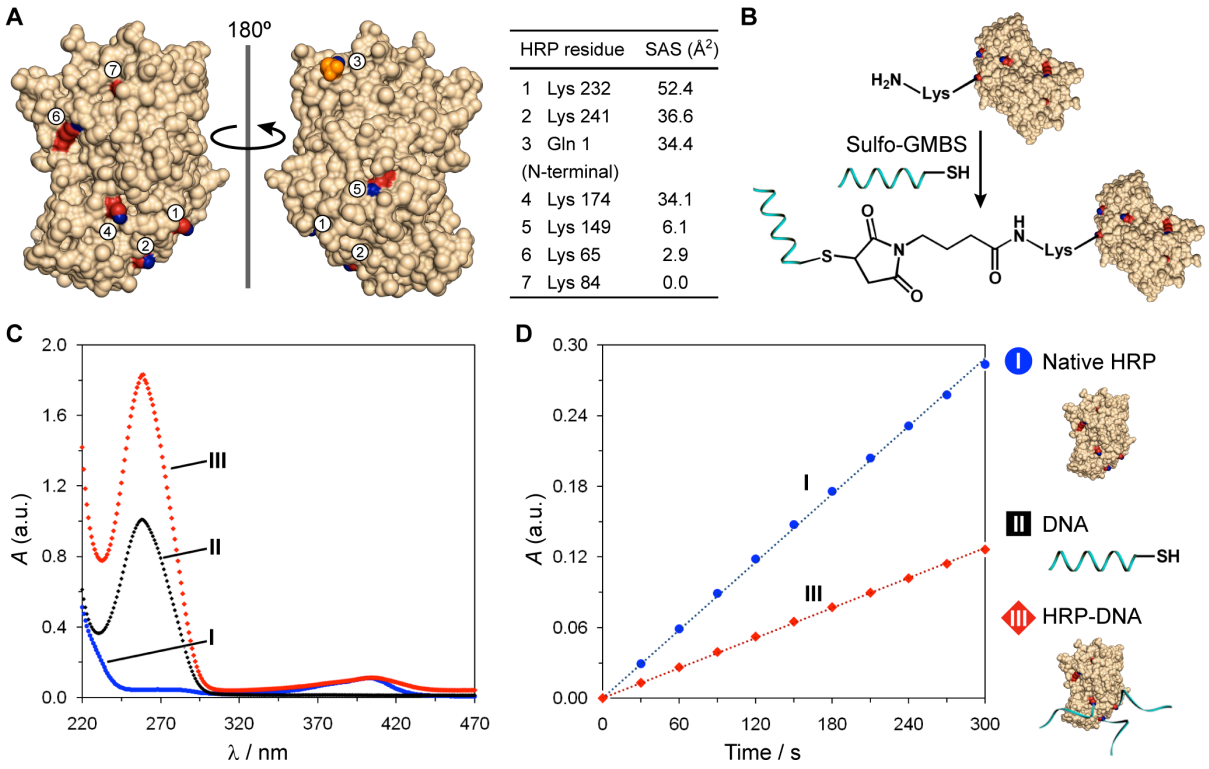
Analysis of the DNA target in serum. The same procedure for DNA detection assay applied for the serum detection assay. Except all DNAs were fixed at 1 nM concentration in 10 mM phosphate buffer, pH 7.4 that included 1 M NaCl. The serum was diluted to 30% with 10 mM phosphate buffer, pH 7.4 and the NaCl concentration was adjusted to 1 M.

References:

(1) Weizmann, Y; Chenoweth, D. M.; Swager, T. M. *J. Am. Chem. Soc.* **2010**, *132*, 14009-14011.

(2) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605-1612.

(3) Schütz, E.; von Ahsen N. *Biotechniques* **1999**, *27*, 1218-1224.



HRP residue	SAS (Å^2)
1 Lys 232	52.4
2 Lys 241	36.6
3 Gln 1 (N-terminal)	34.4
4 Lys 174	34.1
5 Lys 149	6.1
6 Lys 65	2.9
7 Lys 84	0.0

Figure S1. Peroxidase enzyme (HRP) modeling, conjugation, and activity data. (A) HRP crystal structure (PDB 1hch) with potential nucleophilic residues for DNA conjugation mapped on the surface, and corresponding table of calculated solvent accessible surface areas (SAS) for each residue. Front and back of HRP structure is shown with a 180º rotation. Nucleophilic primary amines are shown in dark blue, lysine side chains are in red, and glutamine side chain are in orange. (B) Conjugation strategy used for HRP labeling with DNA. (C) UV-Vis determination of DNA stoichiometry after conjugation to HRP, indicating that ~3-4 DNA strands are conjugated per HRP enzyme (native HRP, I; DNA, II; HRP-DNA, III). (D) Enzyme activity assay for native HRP (I) and HRP after DNA conjugation (III). HRP activity is reduced to ~44% of the native enzyme after DNA conjugation.

Match and Mismatch Oligonucleotide Sequences Thermodynamics				
			T_M (°C)	ΔT_M
1CC Mismatch	captured strand	5' - TCC AAC ATT TAC TCC - 3'		
	mismatch	3' - AGG TGG TAA ATC AGG - 5'	22.2	15.8
1AA Mismatch	captured strand	5' - TCC AAC ATT TAC TCC - 3'		
	mismatch	3' - AGG TGG TAA AAG AGG - 5'	30.2	7.8
2AA Mismatch	captured strand	5' - TCC AAC ATT TAC TCC - 3'		
	mismatch	3' - AGG TGG AAA AAG AGG - 5'	14.9	20.0
3AA Mismatch	captured strand	5' - TCC AAC ATT TAC TCC - 3'		
	mismatch	3' - AGG TAG AAA AAG AGG - 5'	0.4	34.5

1CC Mismatch		% bound at 38 °C	% bound at 25 °C	% bound at 30 °C	**% bound at 32 °C**	% bound at 35 °C
Exact Match T_M (°C):	38.0	50%	100%	99.1%	**97.1%**	85.1%
Mismatch T_M (°C):	22.2	0%	18%	1.6%	**0.6%**	0.1%
ΔT_M:	15.8					
1AA Mismatch		% bound at 38 °C	% bound at 25 °C	% bound at 30 °C	**% bound at 32 °C**	% bound at 35 °C
Exact Match T_M (°C):	38.0	50%	100%	99.1%	**97.1%**	85.1%
Mismatch T_M (°C):	30.2	2%	93.4%	52%	**28.4%**	8.2%
ΔT_M:	7.8					

Figure S2. Calculated melting temperature study using MeltCalc (Supporting Ref. 3).

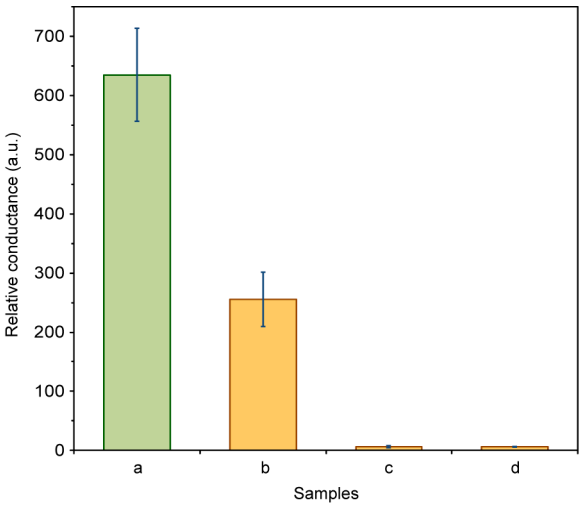


Figure S3. Serum samples. (a = target DNA in 10 mM phosphate buffer + 1M NaCl, pH 7.4, b = target DNA in 30% human serum dilute in 10mM phosphate buffer + 1M NaCl, pH 7.4, c = control DNA (our full mismatched DNA) in 30% human serum dilute in 10mM phosphate buffer + 1M NaCl, pH 7.4, d = No DNA (only probe) in 30% human serum dilute in 10mM phosphate buffer + 1M NaCl, pH 7.4) Note: Target DNA and control were at 1 nM concentration. The HRP probe concentration was 10 nM.

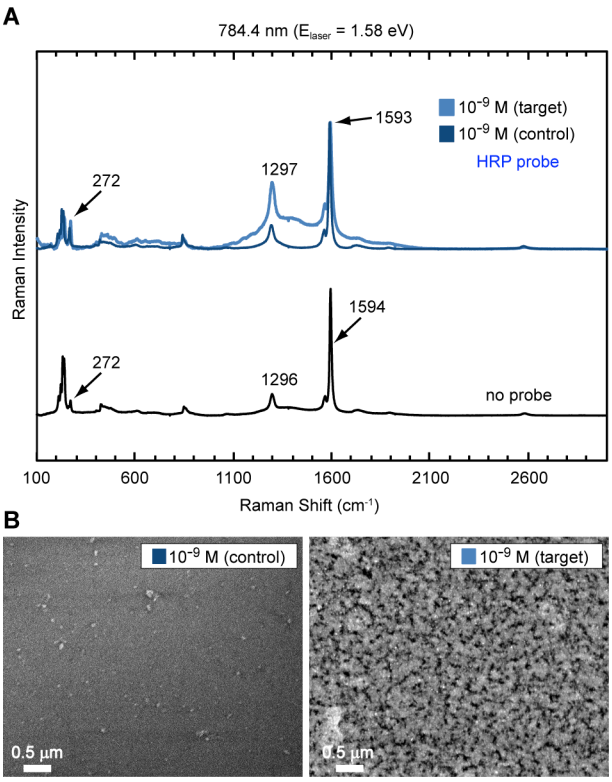


Figure S4. Confocal Raman and SEM characterization of device junctions. (A) Confocal Raman data for device junctions before (black, no probe) and after silver deposition (light blue top curve = target device, dark blue middle curve = mismatch control device). Data obtained using laser wavelength of 784.4 nm (Elaser = 1.58 eV). All DNA analyte and control concentrations are shown at 1 nM and a x50 microscope objective was used in all Raman experiments. (B) SEM images of DNA-CNT nanowire devices. (bottom left) SEM characterization of DNA-CNT nanowire device after mismatch DNA control experiment (1 nM conc.), showing a lack of silver deposition after development with HRP. (bottom right) SEM characterization of DNA-CNT nanowire device after target DNA detection experiment (1 nM conc.), showing silver deposition after development with HRP. Scale bar in all micrographs = 0.5 µm.