Supporting Information

Terminal Protection of Small Molecule-Linked DNA for Sensitive Electrochemical Detection of Protein Binding via Selective Carbon Nanotude Assembly

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Estimation of DNA probe concentration in DNA-SWNT solution. The concentration of DNA probe in the DNA-SWNT solution was determined using the as-prepared complex of SWNT and the FITC-labeled probe **3**. Exo I (80 U) was added to 50 μL DNA-SWNT solution for complete digesting the probe **3**, allowing the release of the FITC tags from the SWNT surface with a concomitant precipitation of SWNTs from the aqueous solution. The resulting mixture was centrifuged at 6000 g for 5 min to remove the SWNT aggregates. A 40 μL aliquot of the supernatant was then collected and diluted to 100 μL followed by fluorescence measurements at an excitation wavelength 490 nm and an emission wavelength 520 nm on an F-7000 spectrofluorometer (Hitachi,. Japan). The concentration of the released FITC label was determined using a working curve of three calibration samples with known concentration of Exo I-digested probe **3** (100 pM, 5 nM, and 100 nM). The concentration of DNA probe in the DNA-SWNT solution was calculated as ~97 nM.

Fluorescence measurements of terminal protection assay. A 50 μl aliquot of folate-linked DNA-SWNT complex was added in 50 μl reaction buffer containing 40 mM tris-HCl (pH 7.9), 6 mM $MgCl_2$, 100 mM NaCl, and FR of a given concentration (0, 10, and 100 nM). The mixture was incubated at 37 ºC for 20 min to allow complete interaction between the protein and the folate-linked DNA-SWNT complex. Then, 80 U Exo I was added to the mixture and incubated at 37 ºC for 30 min followed by the addition of 20 mM EDTA to terminate the reaction. The resulting solution was immediately subjected to the fluorescence measurements. The fluorescence spectra were measured at

room temperature in a 100 μl quartz cuvette on an F-7000 spectrofluorometer (Hitachi, Japan). The excitation wavelength was 490 nm and the emission wavelength was in the range from 510 to 600 nm with both excitation and emission slits of 5 nm.

Scanning electron microscope (SEM) characterization of terminal protection assay. The gold disc substrate (99.99% polycrystalline, ~10 mm diameter, Eco Chemie Utrecht, Netherlands) was treated and modified with MHA SAM using the protocol previously described for the gold electrode**.** A 50 μl aliquot of folate-linked DNA-SWNT complex was added in 50 μl reaction buffer containing 40 mM tris-HCl (pH 7.9), 6 mM $MgCl_2$, 100 mM NaCl, and FR of a given concentration (0 and 100 nM). The mixture was incubated at 37 ºC for 20 min to allow complete interaction between the protein and the folate-linked DNA-SWNT complex. Then, 80 U Exo I was added to the mixture and incubated at 37 ºC for 30 min followed by the addition of 20 mM EDTA to terminate the reaction. The resultant solutions were deposited on the MHA-modified gold substrate for 30 min. Subsequently, the substrate was thoroughly rinsed with ethanol and ultrapure water to remove SWNTs weakly adsorbed on the electrode surface, and dried under mild nitrogen stream followed by the SEM imaging.

Oligonucleotides for further electrophesis assay of terminal protection. To further validate terminal protection for other pairs of small molecules and protein targets. Oligonucleotide probes, either with biotin tag or fluorescein isothiocyannate (FITC) label at the 3' terminus, were synthesized from Takara Biotechnology Co. Ltd. (Dalian, China). The sequences of the synthesized oligonucleotides are given in Table S1.

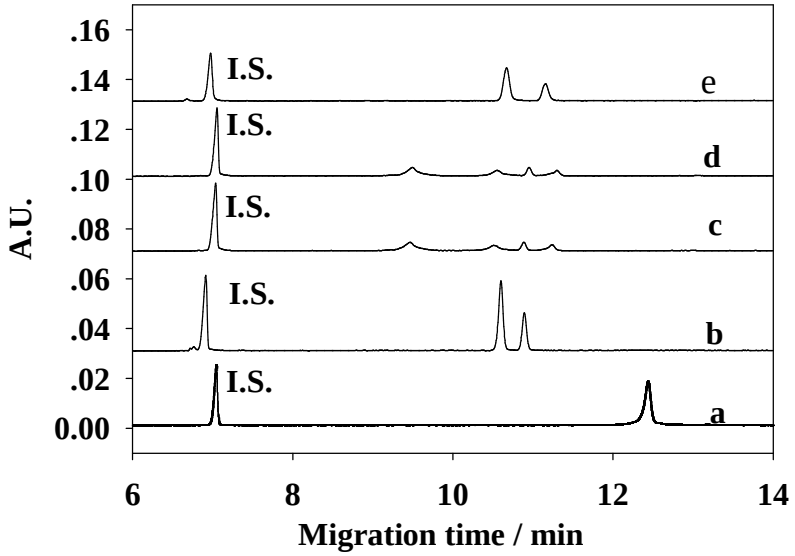
Table S1. Synthesized probes (5'→ 3') used in capillary and gel electrophesis

Probe **2**	GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT-NH2
Probe **4**	GT GT GT GT GT GT GT GT GT GT(-FITC) GT GT GT GT GT GT GT GT GT GT-NH_2
Probe **5**	GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT GT-Biotin
Probe **6**	GT GT GT GT GT GT GT GT GT GT (-FITC) GT GT GT GT GT GT GT GT GT GT-Biotin
Probe **7**	CTT TTA TAC TC-FITC
Probe **8**	CTT TTA TAC TC -NH_2
Probe **9**	Bodipy (493/503)-CTT TTA TAC TC -FITC
Probe **10**	Bodipy(493/503)-CTT TTA TAC TC -NH_2

Probes **2**, **4**, **5** and **6** were used in electrophoresis analysis for validating the terminal protection by the binding between biotin and streptavidin (SA). Probes **2** and **5** were used in capillary

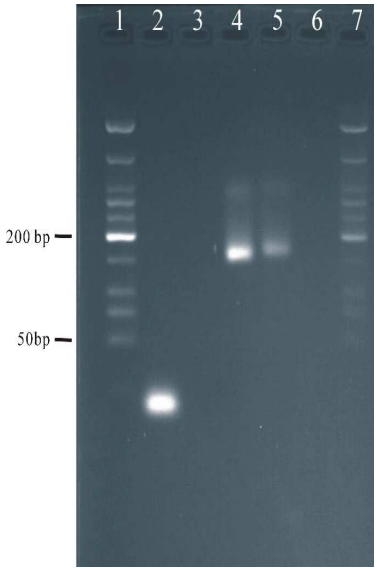
electrophoresis, and probes **4** and **6** were used in gel electrophoresis since the FITC labels could improve the detection sensitivity of the ssDNA in the gel. The FITC labels were conjugated to probes **4** and **6** via a modified nucleotide of T. Probes **7**, **8**, **9** and **10** were used in electrophoresis analysis for validating the terminal protection by the binding between FITC and its monoclonal antibody (Ab). Probes **7** and **8** were used in capillary electrophoresis, and probes **9** and **10** were used in gel electrophoresis since the Bodipy labels could improve the detection sensitivity of the ssDNA in the gel.

Figure S1. Electrophoretograms of 5 μM biotin-labelled probe **5** (a), 5 μM probe **5** digested by 40 U Exo I (b), 5 μM SA-bound probe **5** (c), 5 μM SA-bound probe **5** digested by 40 U Exo I (d), and 5 μM probe **2** plus 5 μM SA digested by 40 U Exo I (e). Internal standard is 80 μM adenosine.



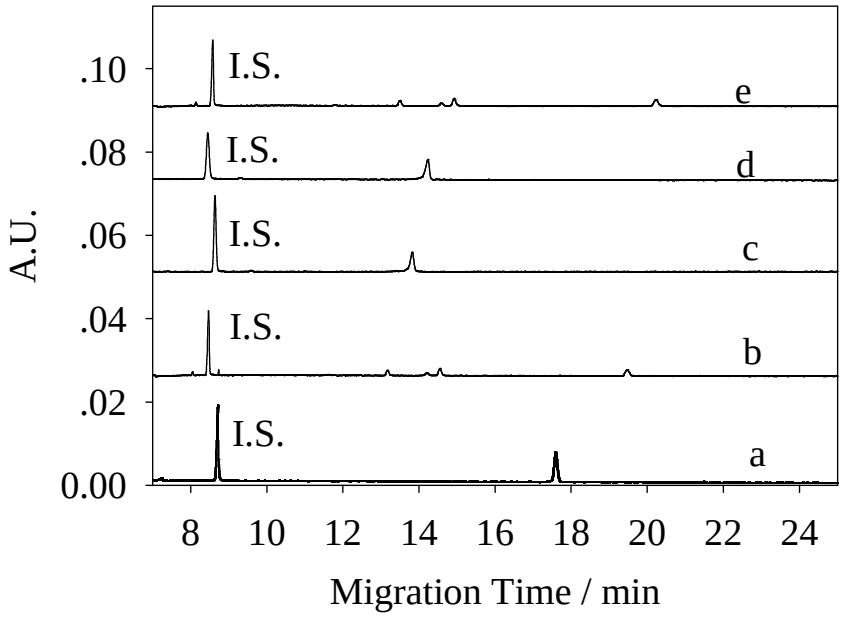
We can observe a sharp peak for biotin-labelled probe **5** and two separate peaks for Exo I-treated probe **5**, confirming a complete degradation of probe **5** into GMP and TMP by Exo I. After interacting with equimolar SA, four peaks with decreased migration times appeared, which are attributed to the complexes between SA and probe **5** with different complexation ratios (1 to 4). After extensive digestion of the complex using Exo I, no remarkable change in the electrophoretograms was observed for the complexes, implying terminal protection of biotin-labelled probe **5** by SA binding. A control experiment for Exo I digestion of the biotin-free probe **2** in the presence of SA also gave two completely-hydrolyzed peaks for GMP and TMP, evidencing that the protection of probe **5** was specific to the SA-biotin binding events rather than the binding of SA to the DNA sequence.

Figure S2. Agarose gel electrophoresis image for terminal protection assay. Lane 1, DNA size marker; lane 2, 5 μM biotin-labelled probe **6**; lane 3, 5 μM probe **6** digested by 40 U Exo I; lane 4, 5 μM SA-bound probe **6**; lane 5, 5 μM SA-bound probe **6** digested by 40 U Exo I; lane 6, 5 μM probe **4** plus 5 μM SA digested by 40 U Exo I; lane 7, DNA size marker.



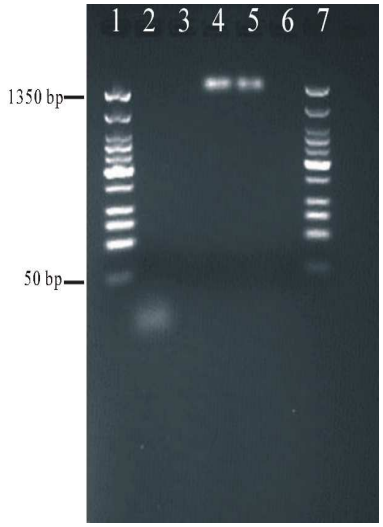
We observe a bright band for biotin-labelled probe **6** at lane 2 and no visible band for Exo I-treated probe **6** at lane 3, confirming a complete degradation of probe **6** by Exo I. After interacting with equimolar SA, a bright band with a long vertical span appeared at lane 4, which is attributed to the poorly-resolved complexes between SA and probe **6** with different complexation ratios (1 to 4). After extensive digestion of the complex using Exo I, the vertically-spanned bright band is still present at lane 5, visually confirming terminal protection of biotin-labelled probe **6** by SA binding. A control experiment for Exo I digestion of the biotin-free probe **4** in the presence of SA also gave no visible band at lane 6.

Figure S3. Electrophoretograms of 5 μM FITC-labelled probe **7** (a), 5 μM probe **7** digested by 40 U Exo I (b), 5 μM Ab-bound probe **7** (c), 5 μM Ab-bound probe **7** digested by 40 U Exo I (d), and 5 μM probe **8** plus 5 μM Ab digested by 40 U Exo I (e). Internal standard is 80 μM adenosine. Note that the capillary used in the experiment is different from that used for the other two CE experiments.



We notice a sharp peak for FITC-labelled probe **7** and four separate peaks for Exo I-treated probe **7** (the peak with longer migration time than probe **7** might be FITC-CMP, since FITC is also negatively charged in the running buffer), confirming a complete degradation of probe **7** into mononucleotides by Exo I. After interacting with equimolar FITC Ab, a peak with decreased migration time appeared (complex with 2:1 complexation ratio is not detected under the micromolar concentration). After extensive digestion of the complex using Exo I, no remarkable change in the electrophoretograms was observed for the complexes, implying terminal protection of FITC-labelled probe **7** by Ab binding. A control experiment for Exo I digestion of the FITC-free probe **8** in the presence of Ab also gave completely-hydrolyzed peaks for mononucleotides, evidencing that the protection of probe **7** was specific to the Ab-FITC binding events rather than the binding of Ab to the DNA sequence.

Figure S4. Agarose gel electrophoresis image for terminal protection assay. Lane 1, DNA size marker; lane 2, 5 μM biotin-labeled probe **9**; lane 3, 5 μM probe **9** digested by 40 U Exo I; lane 4, 5 μM Ab-bound probe **9**; lane 5, 5 μM Ab-bound probe **9** digested by 40 U Exo I; lane 6, 5 μM probe **10** plus 5 μM Ab digested by 40 U Exo I; lane 7, DNA size marker.



We observe a bright band for FITC-labelled probe **9** at lane 2 and no visible band for Exo I-treated probe **9** at lane 3. After interacting with equimolar FITC Ab, a bright band appeared at lane 4, which is attributed to the Ab-probe **9** complex with big molecule weight. After extensive digestion of the complex using Exo I, the bright band is still present at lane 5, visually confirming terminal protection of FITC-labelled probe **9** by FITC Ab binding. A control experiment for Exo I digestion of the FITC-free probe **10** in the presence of FITC Ab also gave no visible band at lane 6.