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Electronic Supplementary Information for

Photoinduced production of NAD(P)H from an activated fluorescein-DNA monolayer

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Materials and Preparation. The DNA was synthesized and purified by standard DNA synthesis methods at the Nation Research Council (Saskatoon, SK, Canada) and the verification of purity and identity are included below. Gold electrodes were prepared by melting a 50 μm Au wire fixed into soft glass that was then polished with 0.05 μm alumina slurry then cleaned by soaking in hot Piranha etching solution (H_2SO_4:H_2O_2=3:1) for 10 min. (***Caution!*** *Piranha solution should be handled with extreme care and should never be stored in a closed container. It is a very strong oxidant and reacts violently with most organic material*s.) and finally sonicated in Millipore H_2O. Each electrode was inspected by light microscopy to ensure that the Au electrode surface was smooth and an effective seal was made between the glass and the Au. The electrodes were than electrochemically treated by cyclic scanning form potential –0.1 to +1.25 V vs. Ag/AgCl in 0.5 M H_2SO_4 solution until obtaining a stable gold oxidation peak at 1.1 V.

The sequences used for the photocurrent experiments are list in Table S1. The base sequence was chosen to minimize alternative secondary or tertiary structures and incorporate equal numbers of each base. DNA melting studies were done to confirm the presence/lack of double strand formation and to ensure the Fluorescein has no effect on duplex stability. DNA melting curves of **1**:**2** duplex show no

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change in T_m values versus a duplex of **2**:**3** (56.8 °C vs. 56.4 °C), indicating that the Fluorescein moiety does not interfere with duplex formation.

Table S1. DNA sequences used for photocurrent study. Fl = Fluorescein	
1	HO-$(CH2)_6$-S-S-$(CH2)_6$-5'-GTCACGATGGCCCAGTAGTT-3'-Fl
2	5'-AACTACTGGGCCATCGTGAC-3'
3	HO-$(CH2)_6$-S-S-$(CH2)_6$-5'-GTCACGATGGCCCAGTAGTT-3'

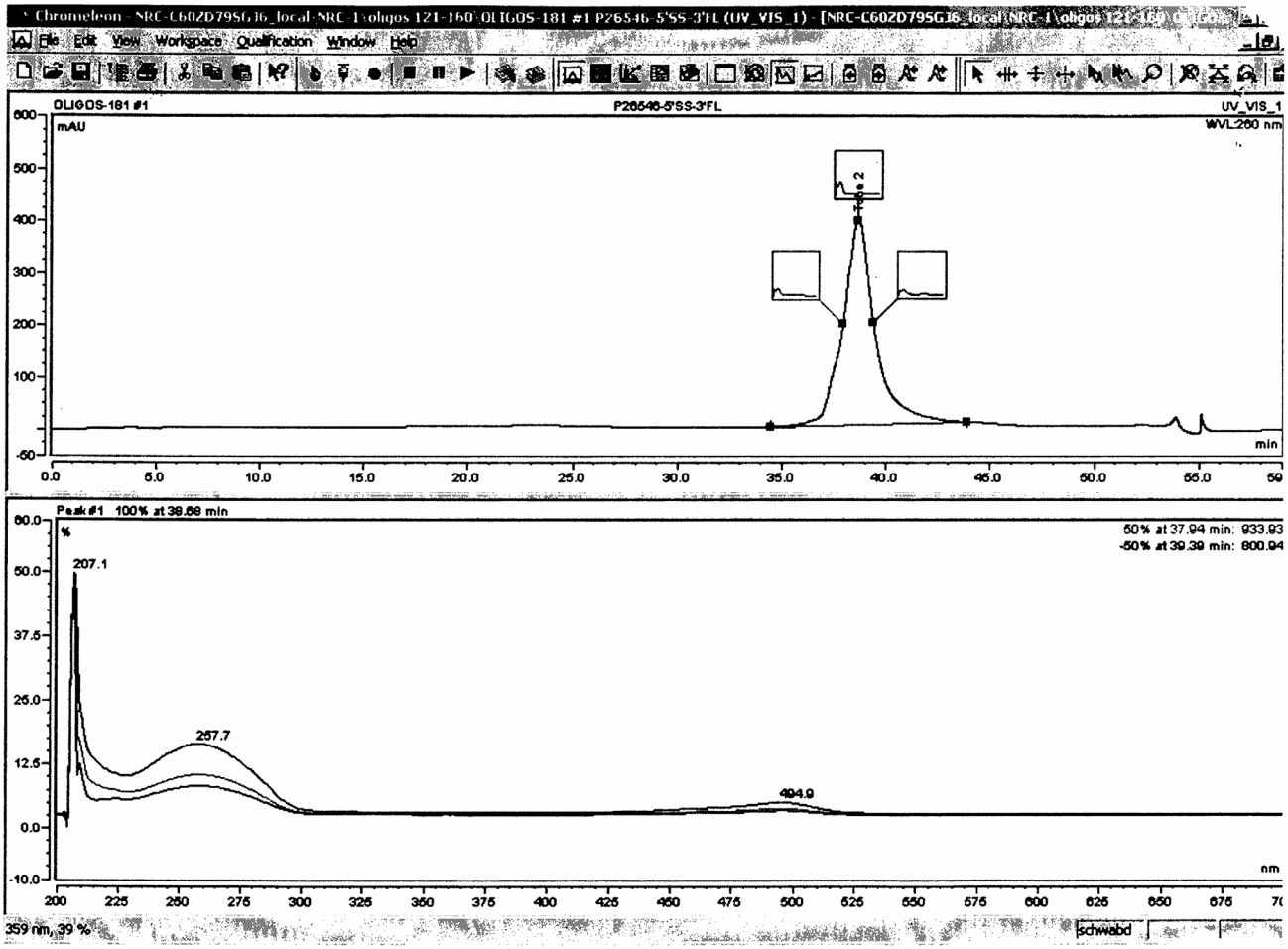

Figure S1. HPLC and UV-vis spectra of **1**.

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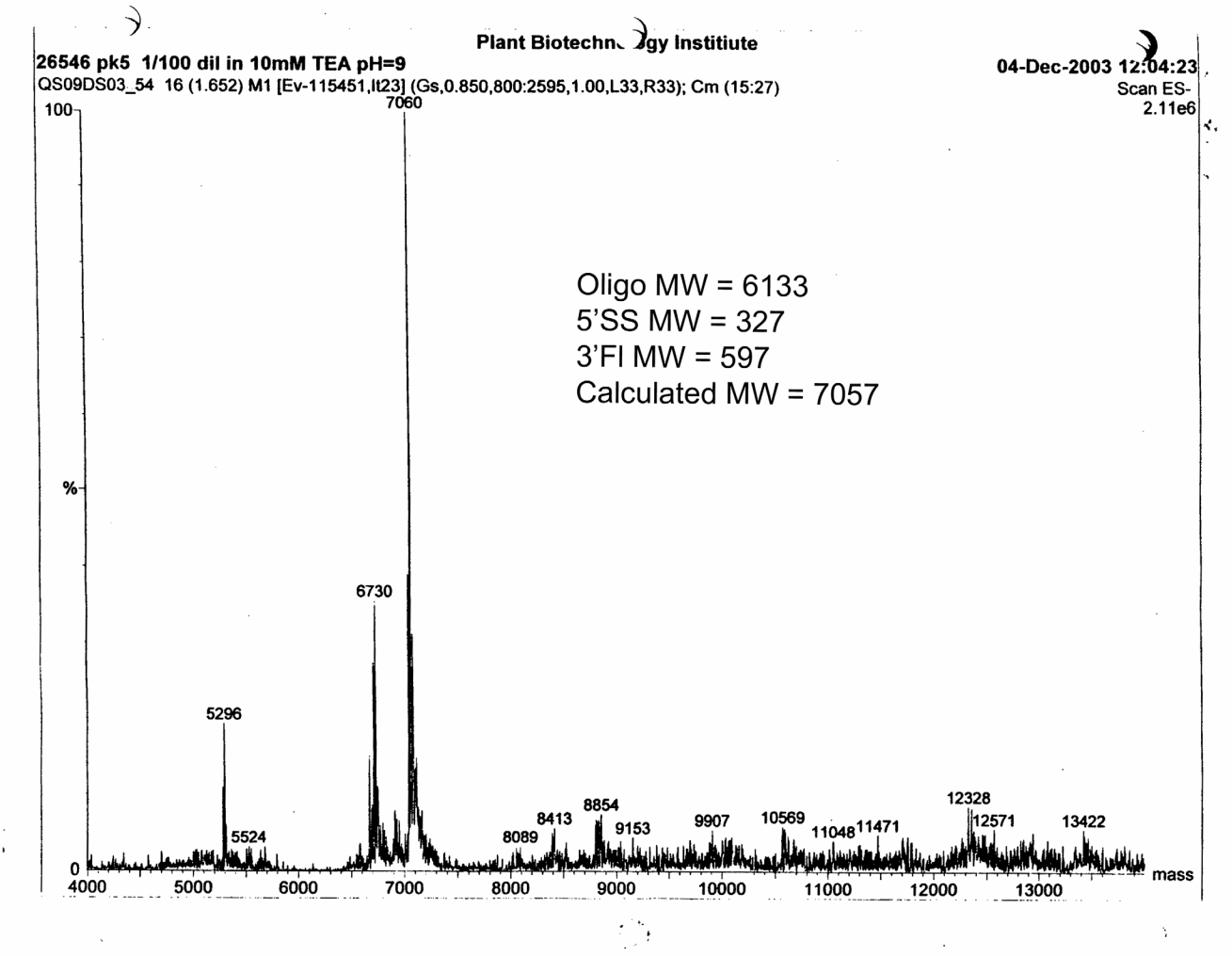

Figure S2. ESI-MS of Oligo **1**.

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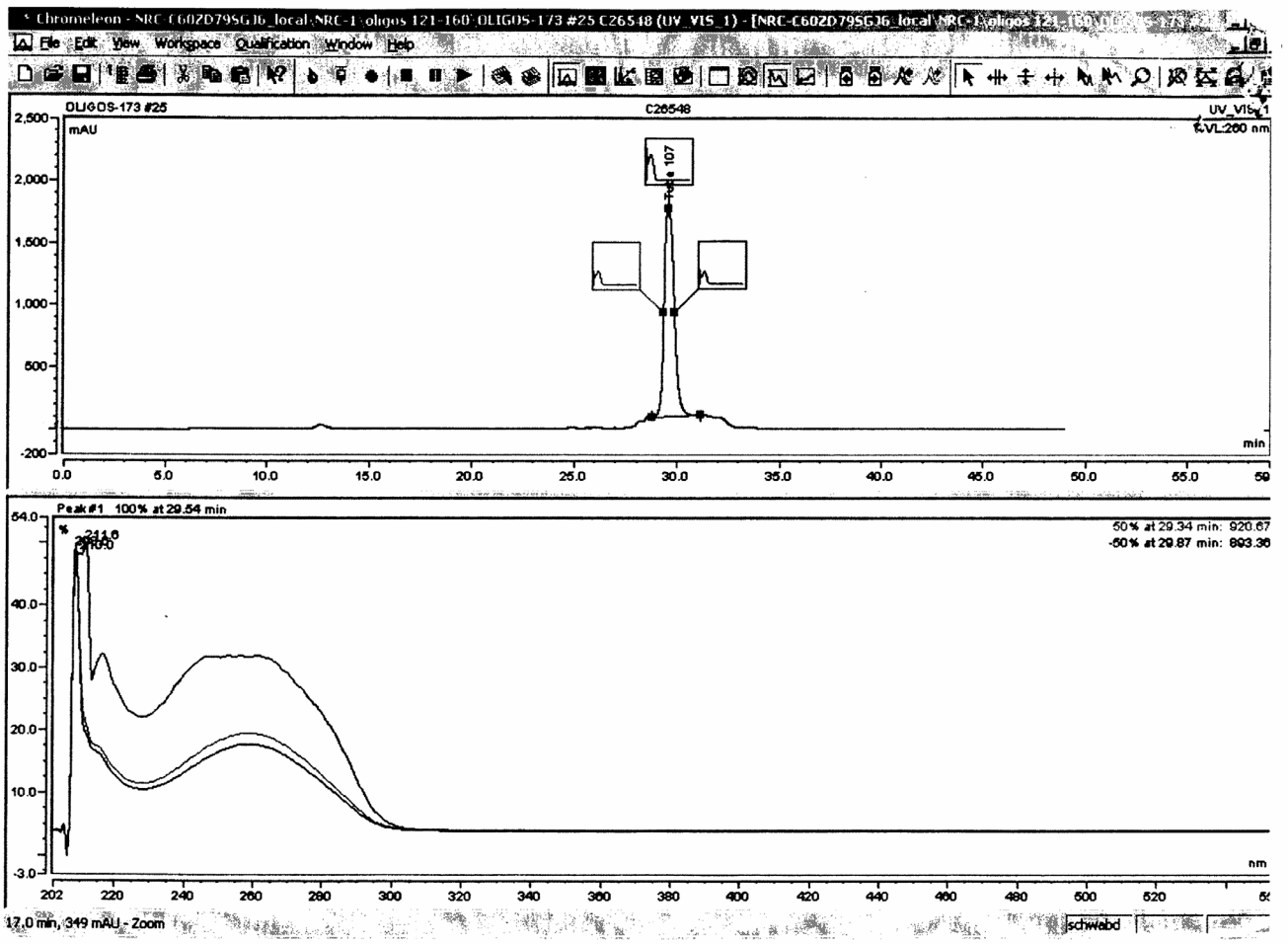

Figure S3. HPLC and UV-vis spectra of **2**.

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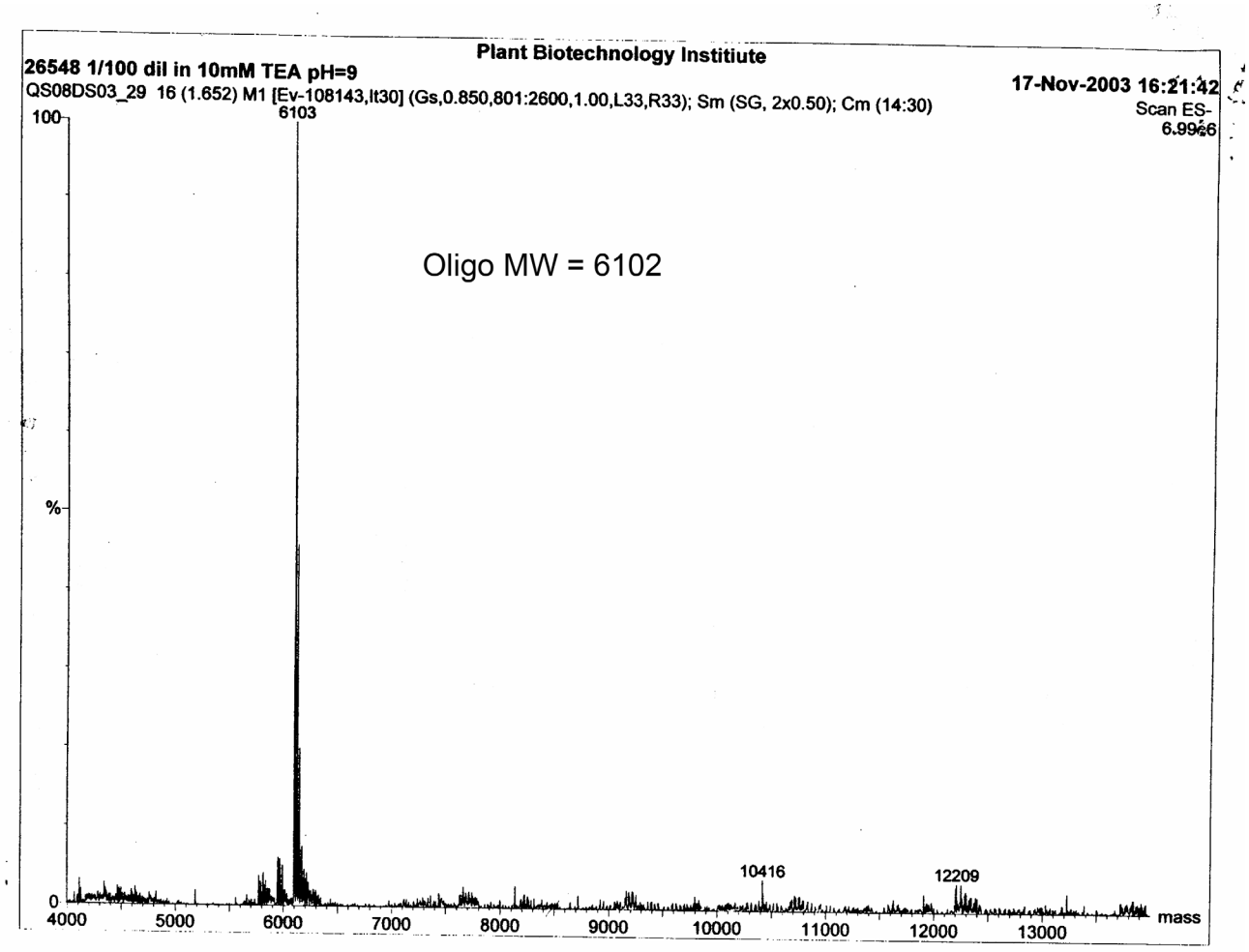

Figure S4. ESI-MS of Oligo **2**.

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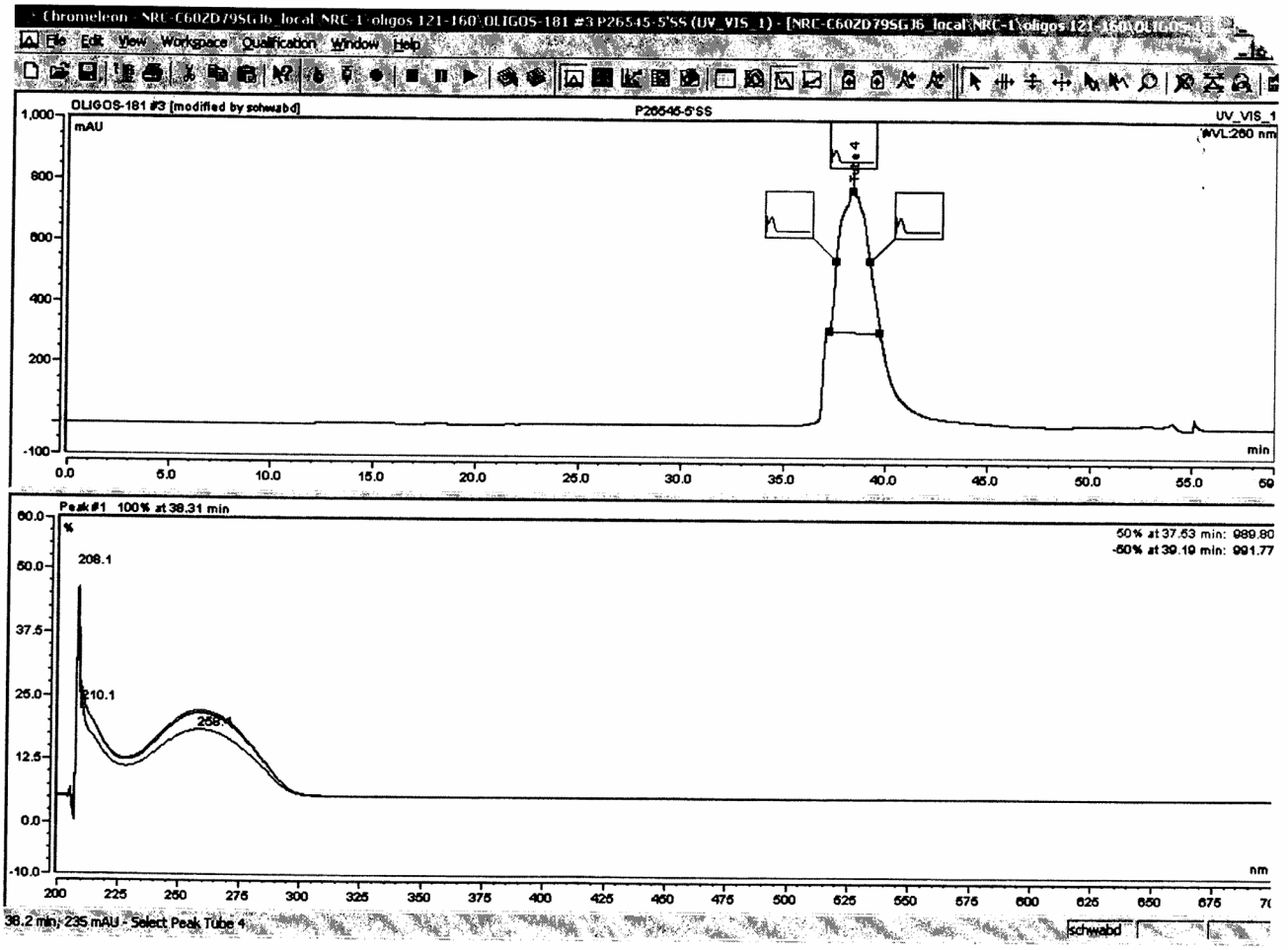

Figure S5. HPLC and UV-vis spectra of **3**.

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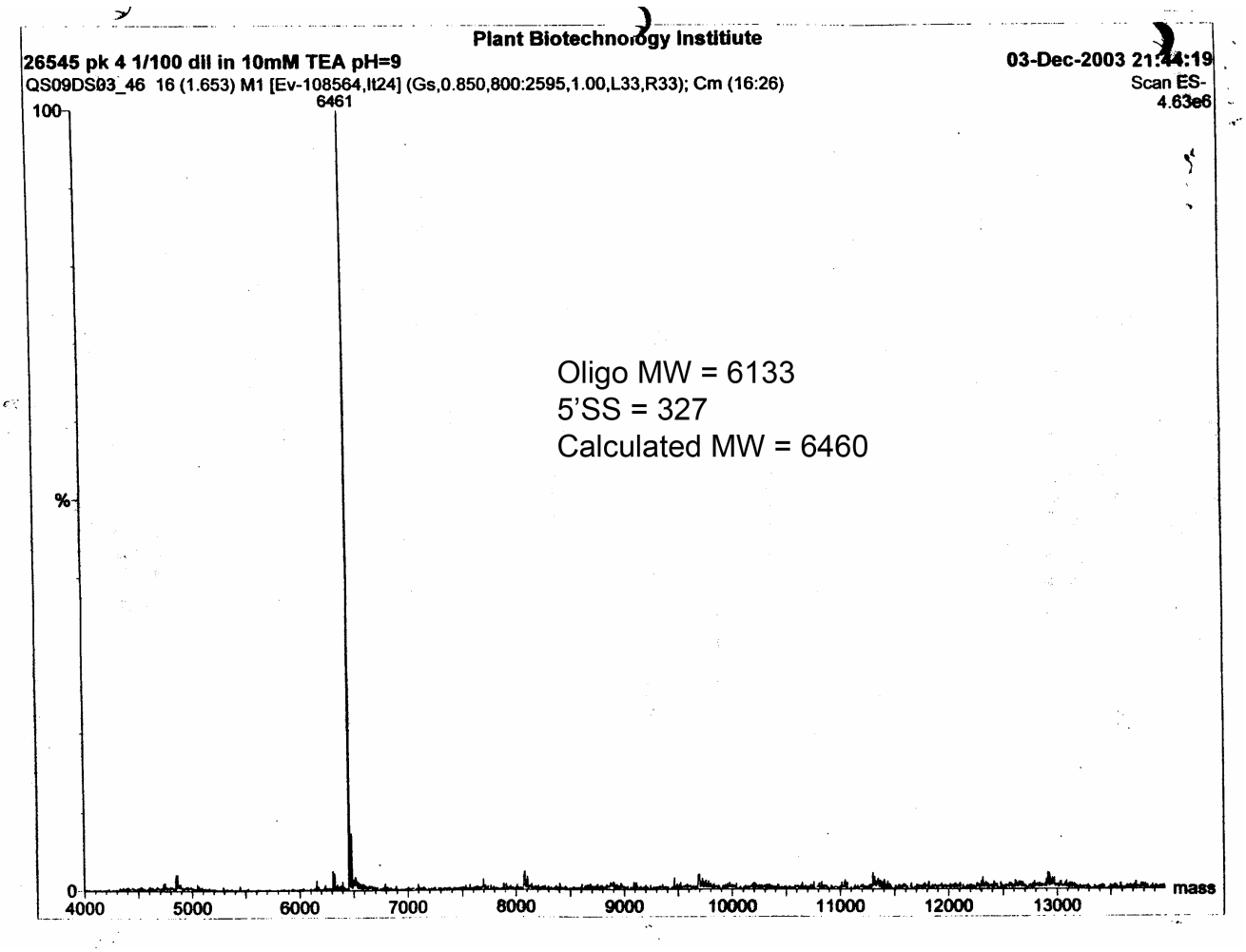

Figure S6. ESI-MS of Oligo **3**.

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Preparation of DNA modified gold electrodes. The microelectrodes were incubated in 0.05 mM double stranded DNA in 50 mM Tris-ClO_4 buffer solution (pH 8.6) for 5 days. The electrodes were then rinsed with the same Tris-ClO_4 buffer and mounted into a photo-electrochemical cell, illustrated in Figure S7. The isolation of the counter electrode was necessary to rule out counter electrode reactions that could contaminate the chronoamperometry.

Photocurrent conditions. A BM73-4V laser module (Intelite Inc., Genoa, NV, USA) laser power 4 $mW \cdot cm^{-2}$, wavelength 473 ± 5 nm and beam diameter less than 0.8 mm was used as the excitation source. Photocurrent experiments were run under voltage-clamp conditions using an Axopatch 200B amplifier (Axon Instruments) connected to a CV 203BU headstage. A two-electrode setup was used for voltage clamp conditions with the reference electrode as a Ag/AgCl wire in a 1 M KCl solution and working electrode as the modified Au microelectrode. The spectroelectrochemical cell was enclosed in a grounded Faraday cage (Warner Instruments) and resided on an active air anti-vibration (Kinetic Systems) table. Currents were low pass Bessel filtered at 1 kHz and were digitized at 5 kHz by DigiData 1322A (Axon Instruments) and recorded by a PC running PClamp 9.0 (Axon Instruments).

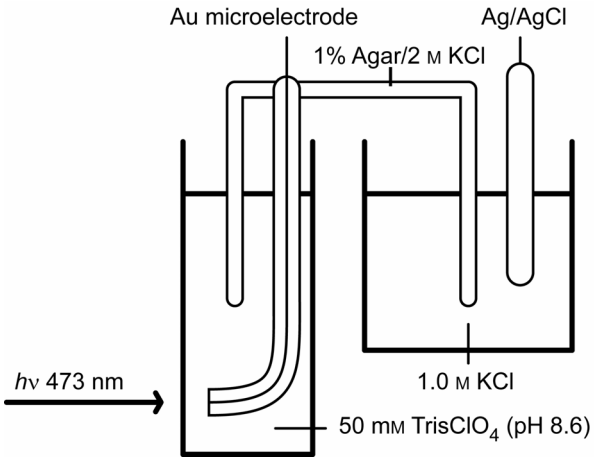

Figure S7. Experimental setup for photocurrent generation.

Further filtering was required and achieved by software methods using low-pass filter at 20 Hz.

Analysis of all data was performed by Origin 7.0 (OriginLab Corporation). Other electrochemical measurements were performed using CHInstruments 660B voltammetry analyzer using the standard 3-electrode setup. The gold microelectrode (50 μm diameter) serves as a working electrode. The reference/counter electrode was always isolated

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from the cell by a Luggin capillary containing the electrolyte. All experiments were conducted at room temperature.

Fl-labeled alkyl thiol monolayers. 11-Mercapto-undecylamine was purchased from Dojindo Molecular Technologies, Inc. (Maryland, USA) and used as received. Monolayers were formed in the same manner as described from DNA except the deposition solvent was EtOH. Fl-succimidyl ester was purchased from Molecular Probes, Inc. and used as received. The Fl labeling of the amino-surface was achieved using the procedure of Corn.[1] The resulting Fl-alkyl thiol gave a photocurrent, which was at least an order of magnitude lower than for the Fl-labeled DNA. A typical result is shown in Figure S8 under ideal conditions for a Fl-DNA photocurrent.

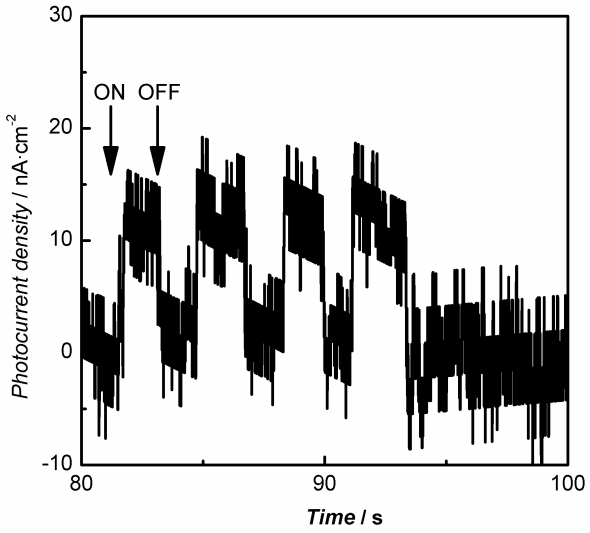

Figure S8. Photoinitiated current from a Fl-labeled undecylthiol monolayer at -750 mV (vs. Ag/AgCl), 4 mW·cm^{-2}, 473 nm.

Electron Paramagnetic Resonance (EPR). The EPR spectra were recorded using a Bruker ESP300 X-band field-swept spectrometer (resonant frequency ca. 9.4 GHz) equipped with a high-sensitivity cylindrical cavity (Model 4107WZ, Bruker Spectrospin). Modulation amplitude was 0.315 G, microwave power was 20 mW, conversion time of 41 ms, time constant of 20.5 ms and 32 scans were recorded. SimFonia software was used for simulation of EPR spectra.

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NAD(P)H production. A quartz cell containing a monolayer-protected Au mesh electrode (Alfa Aesar, MA, USA, 99.9%, 52 mesh, open area = 62.7%, wire diameter = 0.102 mm), a Pt counter wire and a Ag/AgCl quasi reference electrode. 120 μL of a 2.4 mM solution of $NAD(P)^+$ in 50 mM $NaClO_4$ and 20 mM Tris-ClO_4 buffer (pH 8.5) was added to the thin layer quartz cell (path length 0.3 mm). The mesh electrode was aligned such that the UV-vis beam (Cary 500, Varian) passed through the centre of the working electrode. Potentials were controlled by a home-built potentiostat. Incident laser light was positioned in such a manner that the laser-electrode junction was the same as the UV-vis beam-electrode junction. This setup allowed for the fastest NAD(P)H production rate.

NAD(P)H enzymatic assay. The general procedure followed Gorton and Dominguez.[2] To the above quartz cell, 1 μL of 100 $U \cdot mL^{-1}$ alcohol dehydrogenase (Sigma, Baker's Yeast, EC1.1.1.1,) and 5 μL of acetaldehyde (Aldrich) were added to the top of the cuvette very slowly to minimize mixing. As the enzyme and acetaldehyde diffused into the beam path the conversion of acetaldehyde to ethanol was assessed by the decrease in the NAD(P)H at 340 nm.

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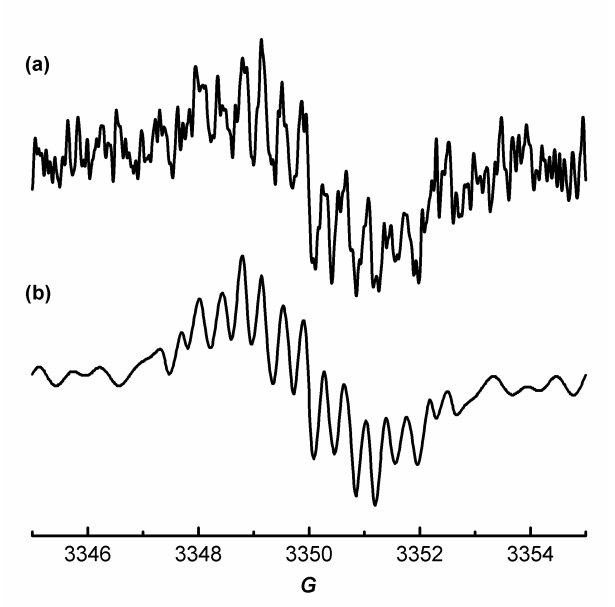
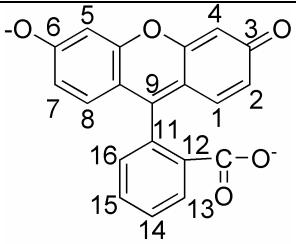


Figure S9. (a) EPR spectrum of **1**:**2** after bulk electrolysis at -750 mV (vs. Ag/AgCl) for 1 hour. (b) Simulated EPR spectrum of **1**:**2**.

Table S2. EPR Coupling constants and unpaired spin densities for reduced Fl and reduced Fl-DNA.

Position of Proton	*EPR for Fl-DNA* / G	*EPR for Fl* / G	*Literature Fl EPR* / G
	$\alpha_{H1,8} = 3.32$ $\alpha_{H2,7} = 1.38$ $\alpha_{H4,5} = 0.74$ $\alpha_{H13} = 0.47$ $\alpha_{H14} = 0.33$ $\alpha_{H15} = 0.22$ $\alpha_{H16} = 0.14$	$\alpha_{H1,8} = 3.35$ $\alpha_{H2,7} = 1.42$ $\alpha_{H4,5} = 0.71$ $\alpha_{H13} = 0.61$ $\alpha_{H14} = 0.30$ $\alpha_{H15} = 0.20$ $\alpha_{H16} = 0.17$	$\alpha_{H1,8} = 3.29^{\dagger}$ $\alpha_{H2,7} = 1.51^{\dagger}$ $\alpha_{H4,5} = 0.90^{\dagger}$ $\alpha_{H13} = 0.22^{\dagger}$ $\alpha_{H14} = 0.19^{\dagger}$ $\alpha_{H15} = 0.17^{\dagger}$ $\alpha_{H16} = 0.09^{\dagger}$
unknown structure of DNA radical.	(a) $\alpha_H = 1.52$, $\alpha_H = 1.15$, $\alpha_N = 1.11$, $\alpha_N = 0.36$, $\alpha_H = 0.69$ (b) $\alpha_H = 0.89$, $\alpha_H = 1.04$, $\alpha_N = 1.69$, $\alpha_N = 0.23$, $\alpha_H = 0.01$		‡(a) $\alpha_H = 1.61$, $\alpha_H = 1.00$, $\alpha_N = 0.52$, $\alpha_N = 0.24$, $\alpha_H = 0.04$ ‡(b) $\alpha_H = 1.71$, $\alpha_H = 1.16$, $\alpha_N = 1.16$, $\alpha_N = 0.52$, $\alpha_H = 0.01$

†(a) Compton, R. G., Coles, B. A., Pilkington, M. B. G., *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 4347-57. (b) Compton, R. G., Coles, B. A., Pilkington, M. B. G., *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 4347-57. (c) Compton, R. G., Daly, P. J., Unwin, P. R., Waller, A. M., *J. Electroanal. Chem.* **1985**, *191*, 15-29. (d) Compton, R. G., Harland, R. G., Pilkington, M. B. G., Stearn, G. M., Unwin, P. R., Waller, A. M., *Portugaliae Electrokimica Acta* **1987**, *5*, 271-9. (e) Compton, R. G., Harland, R. G., Unwin, P. R., Waller, A. M., *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1261-8. (f) Compton, R. G.,

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Mason, D., Unwin, P. R., *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 2057-68. (g) Compton, R. G., Mason, D., Unwin, P. R., *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 483-9. (h) Compton, R. G., Pilkington, M. B. G., *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 2255-71. (i) Nizuma, S., Sato, Y., Konishi, S., Kokubun, H., *Bull. Chem. Soc. Jpn.* **1974**, *47*, 2121-5.
[‡]Geimer, J., Hildenbrand, K., Naumov, S., Beckert, D., *Phys. Chem. Chem. Phys.* **2000**, *2*, 4199-206.

Electrochemical EPR studies of the **1**:**2** duplex and Fl have unambiguously identified the reduced Fl as Fl^- radical at potentials greater than -750 mV. The EPR spectra of **1**:**2** and their corresponding simulated spectra are shown in Figure S4. The simulated spectra values used are included in Table S2, and correlated well with Compton's work on Fl radical anions in the 80's. The **1**:**2** duplex EPR spectrum is slightly more complicated than the Fl spectrum because the simulated data required an additional radical species. The structural identity of this radical is unknown but has been observed before by Geimer in DNA. The simulated Fl-DNA EPR spectrum had the best fit when the $Fl^{-\bullet}$ was in 98% abundance with only 2% of the radical on the DNA.

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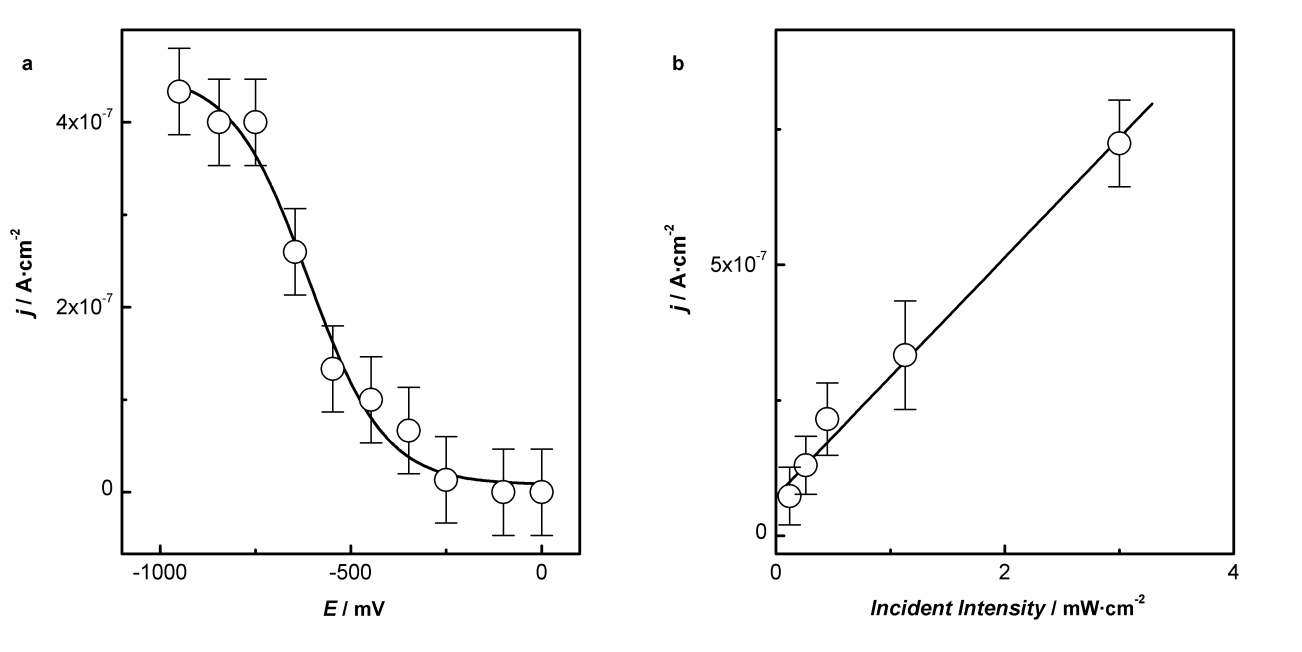

Figure S10. (a) Photocurrent response as a function of applied reductive potential (vs. Ag/AgCl). (b) Photocurrent response as a function of light intensity in the presence of $NADP^+$ or NAD^+.

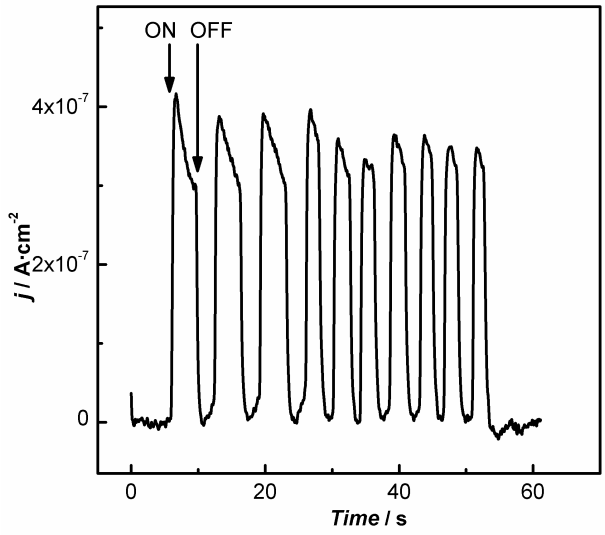

Figure S11. Multiple excitation responses showing a small decrease in the photocurrent as a function of repeat number.

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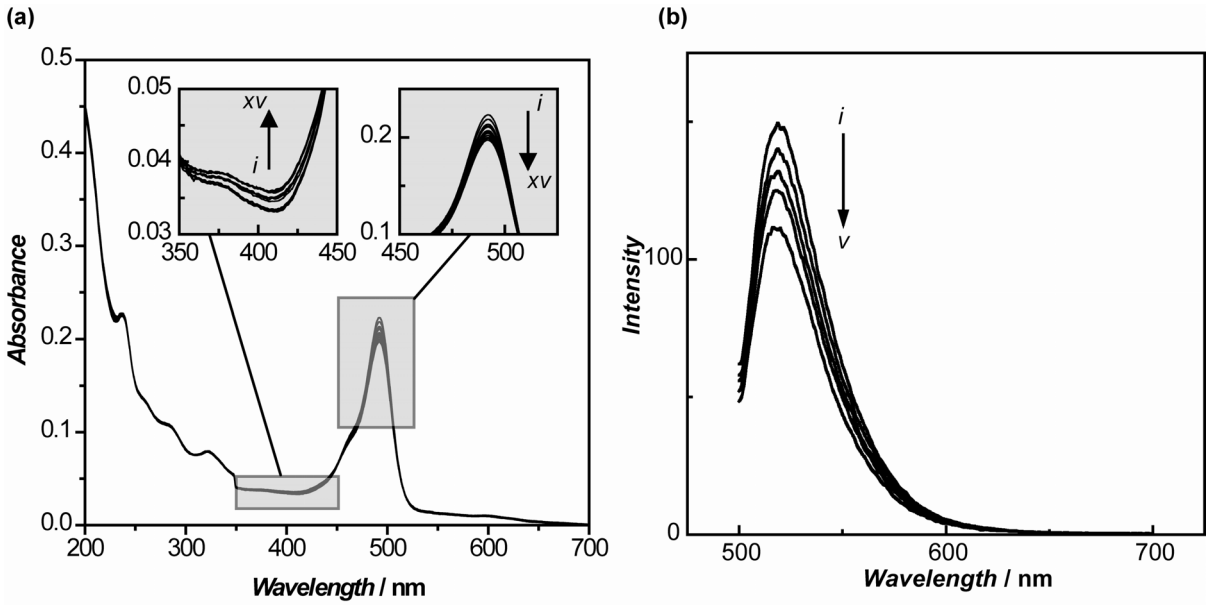

Figure S12. a) UV-visible absorbance spectra of Fl at -750 mV (vs. Ag/AgCl). Measurements, *i* to *xv*, were recorded at 3 min intervals. After 45 min (spectrum *xv*) there was no observable change. b) Emission spectra of Fl at various applied potential durations (vs. Ag/AgCl). Excitation = 494 nm (i) 0 mV, (ii) -750 mV for 1 min, (iii) -750 mV for 2 min, (iv) -750 mV for 3 min (v) -750 mV for 5 min. All spectra were recorded under the same conditions as described in the photocurrent section.

Quantum yield[3] was calculated with the following parameters.

1. Laser power = 4 $mW \cdot cm^{-2}$.
2. Wavelength = 473 nm
3. Surface coverage[4] = 8.3×10^{-12} $mol \cdot cm^{-2}$.
4. Molar absorptivity = 6.8×10^{4} $M^{-1} \cdot cm^{-1}$
5. Surface Area = 1.9×10^{-5} cm^{2}

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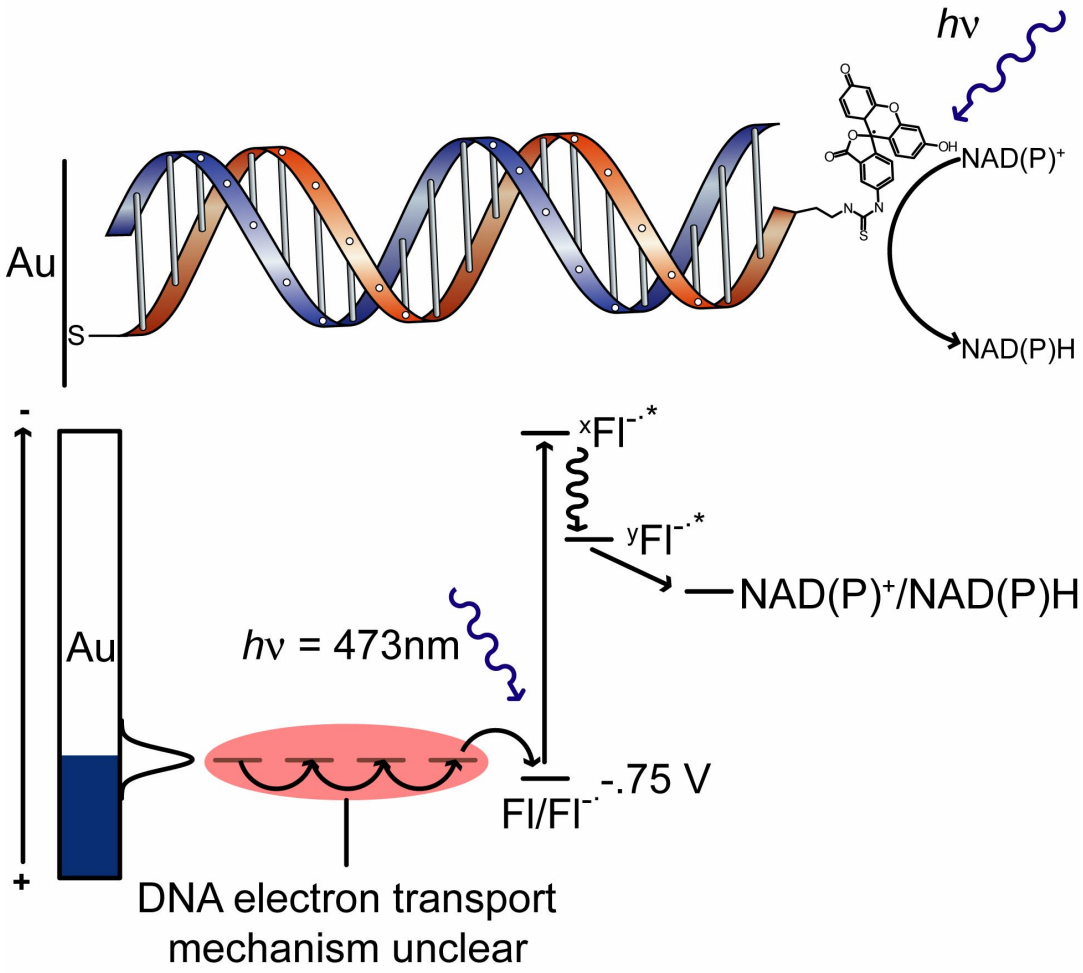

Figure S13. Energy level diagram of a Fl-labeled DNA monolayer at applied potential of -0.75 V. All potentials referenced to Ag/AgCl. Independent of DNA electron transfer mechanism, the Fl dye is reduced to its radical anion at sufficiently negative potentials. Upon irradiation at 473 nm, where the radical anion may only absorb to a small degree and the neutral Fl absorbs strongly, the monolayer is able to reduce $NAD(P)^+$ to NAD(P)H. The two electrons and one proton required to reduce $NAD(P)^+$ needs both the negative potential on the electrode and the incident laser at 473 nm. Control experiments that only supply either the negative potential or the incident laser (473 nm) did not produce any NAD(P)H.

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