

Supporting Information

Selective Electrochemical Discrimination of 3' IsomiRs Differing by Two Nucleotides

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Experimental

Polyacrylamide electrophoresis analysis

Native polyacrylamide gel electrophoresis was used to examine the hybridization of the USL probe with the m and f strands, as well as in the presence and absence of the target. Individual oligonucleotides and their mixtures were prepared in hybridization buffer. Electrophoretic analysis of 4WJ complex formation was performed on an 8% polyacrylamide gel supplemented with 12.5 mM MgCl₂ under native conditions. Samples containing the indicated components were annealed in hybridization buffer at a final concentration of 2.5 μM for the USL probe, m and f strands, and the R+2 target. Each sample was mixed with 1× Blue/Green Loading Dye, and 10 μL was loaded onto the gel. Electrophoresis was carried out at 120 V for 75 minutes at 10 °C. Bands were visualized using SYBR Safe DNA Gel Stain.

Self-Assemble monolayer (SAM) evaluation

To evaluate the effect of the SAM in the electrochemical readout. The GDE-USL electrodes were drop-casted with 15 μL of either 2 mM MCE, MCH, MCO, HxT, or HxDiT in IB and allowed to incubate for 30 minutes. Then SWV was used to obtain signal measurements, as described before, and was recorded five times to evaluate the change of signal over time. Electrochemical impedance spectroscopy (EIS) measurements were performed in HB containing 1 mM K₄[Fe(CN)₆]·3H₂O/K₃[Fe(CN)₆]. This redox pair was selected because it is well established and thoroughly characterized. Measurements were carried out over a frequency range of 10 kHz to 10 Hz with an AC potential of 0.15 V. The experimental data are presented as Nyquist plots and were fitted to an equivalent circuit using the instrument software.

Table S1. Oligonucleotides sequences used in 5S-4WJ-based biosensor

Name	Sequence (5' to 3')
USL	HOCH ₂ (CH ₂) ₅ -S-S(CH ₂) ₆ - TTTTTTTTTCGCGTTAACATACAATAGATCGCG
UMeB	MeB-CGGTACATTGTTGAG
m_mm	ACAG <u>A</u> CTA/iSp9/TATGTTAACTTCTCAACAATGTACCG
m	ACAG <u>C</u> CTA/iSp9/TATGTTAACTTCTCAACAATGTACCG
f short	GATCTATTG/iSp9/TGGAATTCAGTT
f long	GATCTATTG/iSp9/TGGAATTCAGTTCT
miRNA 146b, isomiR (R+2)	TGAGAACTGAATTCCATAGGCTGT
miRNA 146b, canonical	TGAGAACTGAATTCCATAGGCT

(R)

MeB: Methylene Blue; iSp9: Triethylene glycol spacer. Fragments of the probes/targets complementary to each other are color coded.

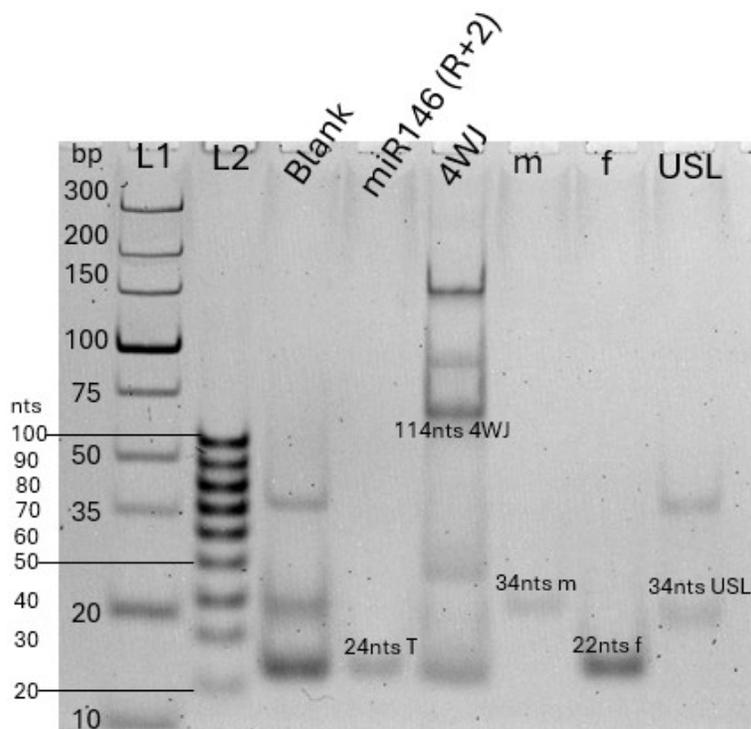


Figure S1. Electrophoretic analysis of the 4WJ complex formation in 8% polyacrylamide gel supplemented with 12.5mM MgCl₂ under native conditions. L1: Ultra Low range DNA ladder 10/300 bp, L2: 20/100 ssDNA ladder.

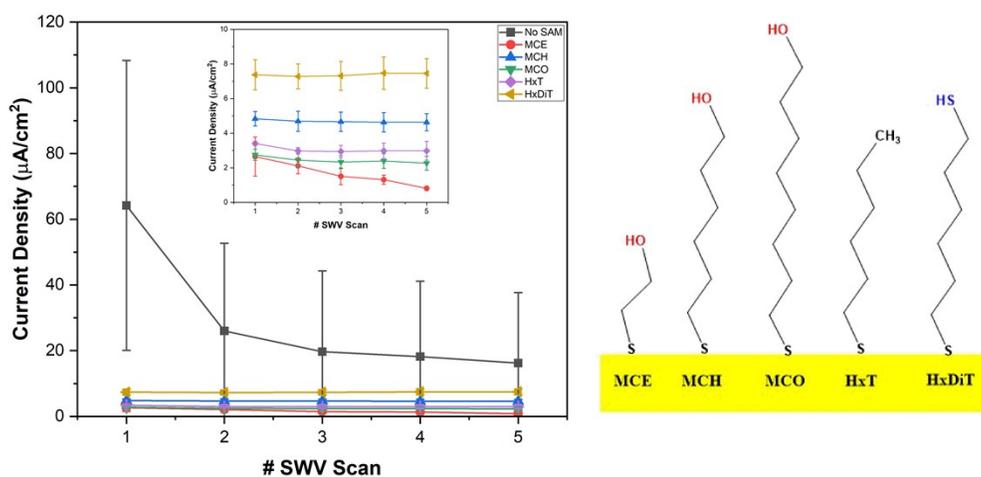


Figure S2. Left: SWV response of the E-Biosensor in the presence of 50nM of R+2 target under different SAM compositions. **Right:** Different SAM formed in the gold working electrode surface.

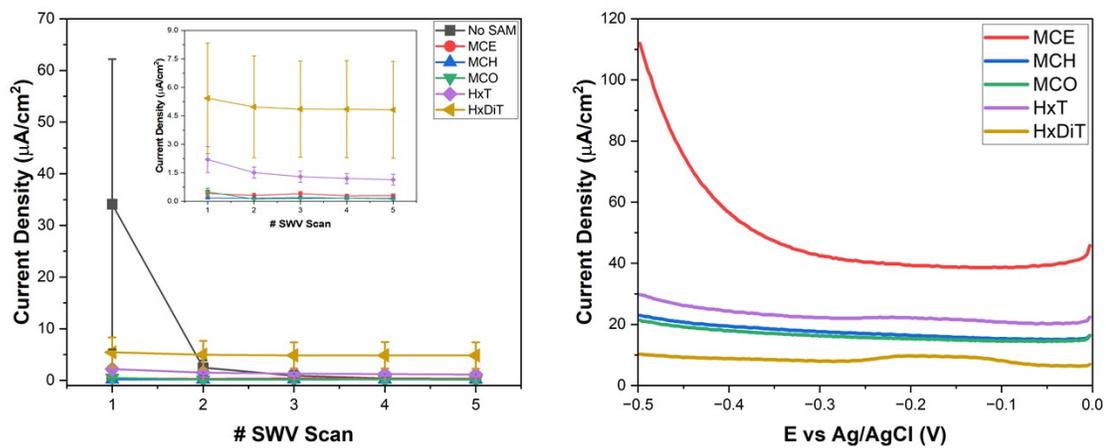


Figure S3. Left: SWV response of the E-Biosensor in the absence of target under different SAM compositions. **Right:** SWV voltammogram.

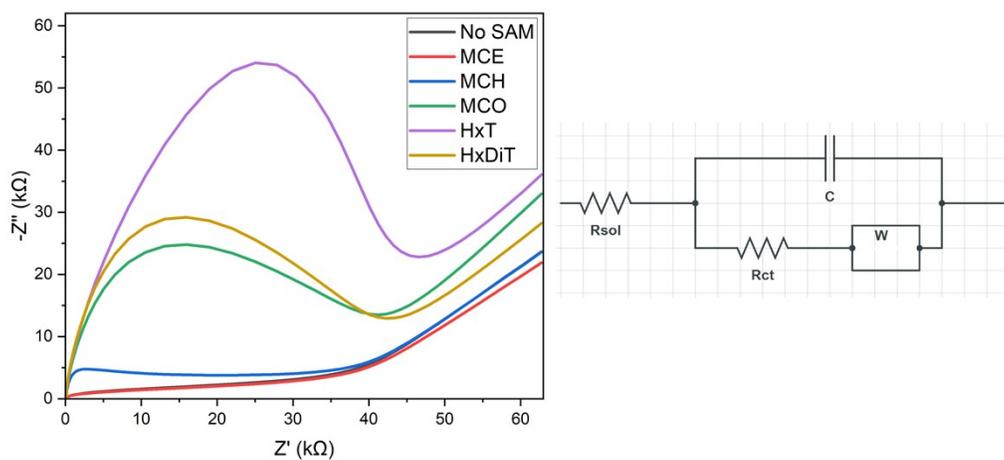


Figure S4. Left: Nyquist plots of the GDE/USL-SAM, and **Right:** Circuit model employed