Supporting Information

Pseudouridine-modified RNA probe for label-free electrochemical detection of nucleic acids on 2D MoS_2

Prabhangshu Kumer Das,[1] Omair Adil,[1] Anthony P. Degregorio,[2] Minako Sumita,[2] and Mohtashim Hassan Shamsi[1]*

*[1]School of Chemical and Biomolecular Sciences, Southern Illinois University Carbondale, IL, 62901, USA. [2]Department of Chemistry, Southern Illinois University Edwardsville, Edwardsville, IL, 62026, USA. *Email: mshamsi@siu.edu.*

1.1. Synthesis and characterization of Ψ-RNA probe

The chemicals for the synthesis were obtained from Millipore Sigma unless mentioned otherwise. *Bis*-(tri-*n*-butylammonium)pyrophosphate was synthesized according to a previously reported method with some modifications mentioned below.[1] Tetrasodium diphosphate decahydrate (2.2 g, 5 mmol) was dissolved in 50 mL of water, followed by adding the solution to a DOWEX column and washing it with water. The eluent was directly dropped into a cooled and stirred solution of tri-*n*-butylamine (2.4 mM, 10 mmol) in 20 mL of ethanol. The column was washed until the pH of the eluent was lowered to 5. Then, the collected solution was evaporated under reduced pressure and re-evaporated twice with ethanol. Finally, the solution was dissolved in anhydrous DMF and dried using an oil pump. The residue (yellow oil) was dissolved in anhydrous DMF (the total volume was 10 mL), and the resulting pale yellow solution was stored over molecular sieves at −20 °C.

Pseudouridine 5'-triphosphate (ΨTP) was synthesized as previously described but with several modifications.[2] Semi-enzymatically synthesized Ψ (22.6 mg, 0.1 mmol) was suspended in 500 µL of trimethyl phosphate.[3] 1,8-bis(dimethylamino)naphthalene (proton sponge) (29.1 mg, 0.14

mmol) was added to the basic reaction mixture. The mixture was placed on ice and stirred for approximately 10 min. Phosphorus oxychloride (13 μL, 1.3 eq) was added to the mixture and stirred for 2 h. The reaction progress was monitored by thin-layered chromatography (2-propanol/NH_4OH/ddH_2O = 7:1:2). Tributylamine (119 μL, 5.0 eq) was added to the reaction mixture because a previous study demonstrated that the addition of an amine base improved the yields.[1] Then, 1.0 mL of *bis*-(tri-*n*-butylammonium)pyrophosphate (~5.0 eq) was added. After 30 min, the reaction was quenched with 0.5 M triethylammonium bicarbonate (pH 7.5, 500 μL). The crude product was purified using DEAE Sephadex A-25 chromatography (1.5 × 30 cm), employing an eluent ranging from 50 mM to 1 M in a linear gradient of triethylammonium bicarbonate. The obtained yield was 23.0 mg (white powder) and 52%.

The DNA templates of the RNA probes and T7 RNA polymerase universal promoter (18 nt) were obtained from Integrated DNA Technologies (Coralville, Iowa, USA). The sequences of the DNAs are as follows:

5′-CGCGCCAAACGCGCCTATAGTGAGTCGTATTA-3′ (template),

5′-TAATACGACTCACTATAG -3′ (promoter).

The RNA probes were obtained by *in vitro* transcription with T7 RNA polymerase and synthesized ΨTP using a HiScribe T7 High Yield RNA kit (New England Biolabs, Ipswich, Massachusetts, USA).[4] The transcription reaction with ΨTP was compared with the commercially available UTP transcription reaction. Before transcription, the DNA template and promoter were annealed in a 10-mM Tris-HCl buffer at pH 7.5. This was achieved by heating to 90 °C for 2 min, followed by a gradual cooling process to room temperature. Moreover, the transcribed RNAs were purified using 20% denaturing polyacrylamide gels (19:1 acrylamide: bisacrylamide, and 7M urea, typically at 350 V for 4 h). Before being loaded onto the gel, the RNA samples were dissolved in

urea loading buffer (7 M urea, 50% w/v glycerol, and 0.1% (w/v) each of xylene cyanol and bromophenol blue) and denatured at 90 °C for 2 min followed by rapid cooling on ice. The RNA bands were visualized on the gel by UV shadowing with a fluorescent TLC plate. Then, the bands corresponding to the 15-nt RNAs were excised from the gel, and the RNAs were extracted using a buffer (1 M NaOAc, pH 5.2, 1 mM EDTA) employing the crush and soak method. Finally, the extracted RNAs were desalted using a Sephadex G15 column and vacuum-dried.

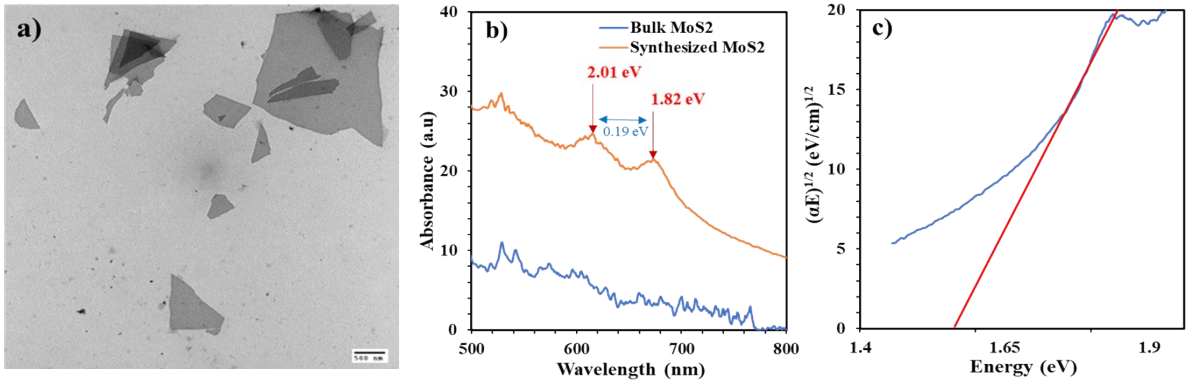

Figure S1. (a) TEM micrograph of the synthesized MoS_2 ink, **(b)** UV-vis spectrum of the MoS_2 solution dispersion, and **(c)** Tauc plot to determine Indirect band gap of the exfoliated MoS_2 a tangent to the line is drawn and the intercept to x-axis is found to be 1.56 eV.

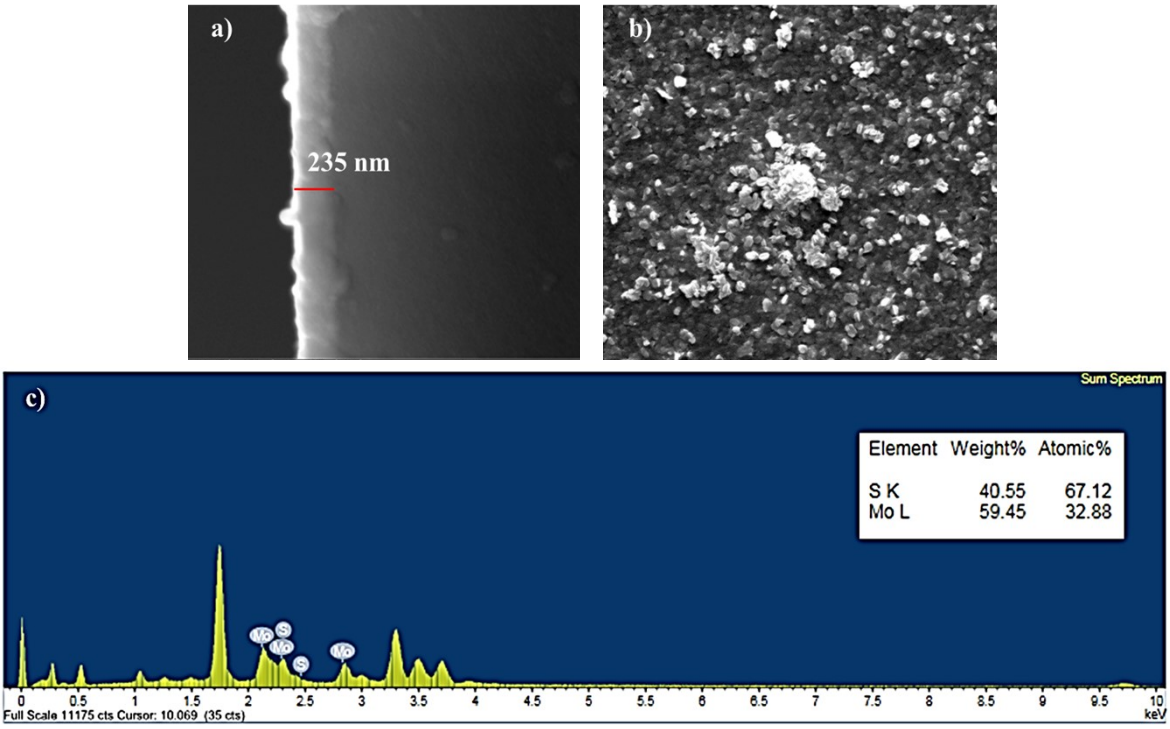

Figure S2. SEM micrographs of (**a**) cross-section and (**b**) surface of MoS_2 modified working electrode after annealing, and **(c)** EDS of **Figure S2b**. (**inset Figure S2c)** Atomic% and weight % of two main elements MoS_2 modified electrode, showing ratio of Mo:S around 1:2.

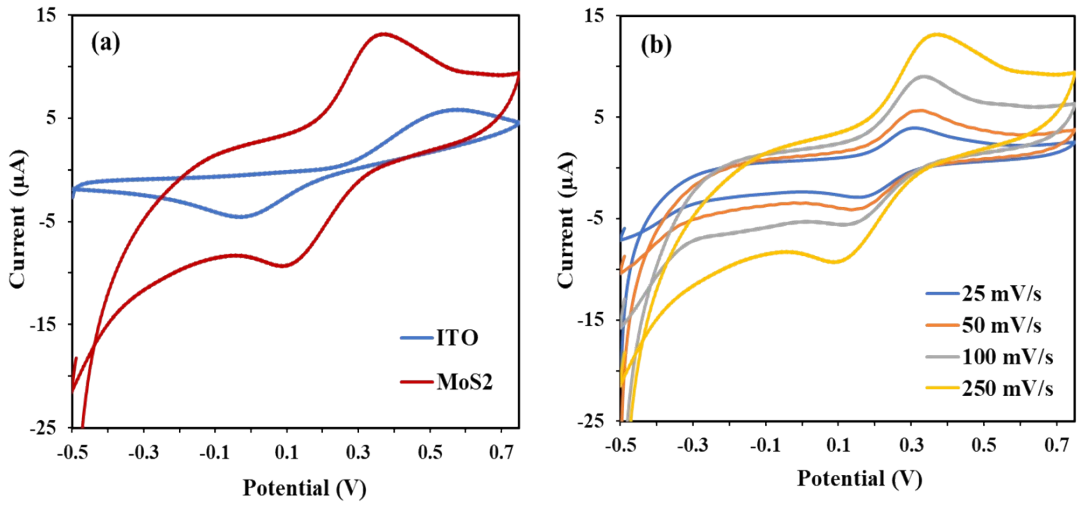

Figure S3. (a) Cyclic voltammograms of the bare ITO and MoS_2 modified ITO. (b) Scan rates effect on the MoS_2 modified working electrode. All the cyclic voltametric experiments were performed using $Fe(CN)_6^{3-/4-}$ redox probe in PBS solution at pH 7.2. Ag/AgCl and platinum wire were used as reference electrode and counter electrode respectively.

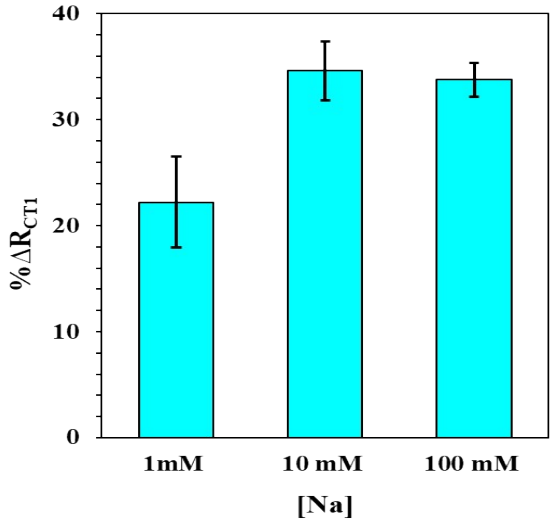

Figure S4. Charge transfer resistance change after ss$(GAA)_6$ adsorption on the MoS_2 surface at various NaCl concentrations. All the EIS experiments were done in the presence of $Fe(CN)_6^{3-/4-}$ redox probe at pH 7.2 with GAA-6 concentration 100 nM and incubated 30 minutes.

References

1. Ludwig, J.; Eckstein, F., Rapid and efficient synthesis of nucleoside 5'-*o*-(1-thiotriphosphates), 5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one. *J. Org. Chem.* **1989,** *54*, 631-635.
2. Mitsui, T.; Kitamura, A.; Kimoto, M.; To, T.; Sato, A.; Hirao, I.; Yokoyama, S., An unnatural hydrophobic base pair with shape complementarity between pyrrole-2-carbaldehyde and 9-methylimidazo[(4,5)-b]pyridine. *J Am Chem Soc* **2003,** *125* (18), 5298-5307.
3. Riley, A. T.; Sanford, T. C.; Woodard, A. M.; Clerc, E. P.; Sumita, M., Semi-enzymatic synthesis of pseudouridine. *Bioorg Med Chem Lett* **2021,** *44*, 128105.
4. Milligan, J. F.; Uhlenbeck, O. C., Synthesis of small RNAs using T7 RNA polymerase. *Methods Enzymol* **1989,** *180*, 51-62.