Electronic Supplementary Material (ESI) for Nanoscale.

From sensing the interaction to controlling the interaction: a novel approach to biological transistors for specific and label-free immunosensing

Soumadri Samanta[1], Vinay S. Tiwari[2], Sumesh Sadhujan[3], Sherina Harilal[3,] Avital Eisenberg-Lerner[5], Ziv Rotfogel[5,6], Evgeny Pikhay[4], Ruth Shima-Edelstein[4], Doron Greental[4], Muhammad Y. Bashouti[3,9], Barak Akabayov[2], Izhar Ron[1], Yakov Roizin[4], Offer Erez[7,8], Gil Shalev[1,9]*

[1] School of Electrical Engineering, Ben-Gurion University of the Negev, Israel

[2]Department of Chemistry and Data Science Research Center, Ben-Gurion University of the Negev, 8410501, Beer-Sheva, Israel

[3]Department of Solar Energy and Environmental Physics, Swiss Institute for Dryland Environmental and Energy Research, J. Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion 8499000, Israel

[4] Tower Semiconductor, PO Box 619, Migdal Haemek, Israel

[5] Ophthalmology Research Laboratory, Kaplan Medical Center, Rehovot, Israel

[6] Faculty of Medicine, Hadassah Medical School, The Hebrew University of Jerusalem, Jerusalem, Israel.

[7]Department of obstetrics and Gynecology Soroka University Medical Center, School of Medicine, Ben Gurion University of the Negev, Beer Sheva, Israel.

[8]Department of Obstetrics and Gynecology, School; of Medicine, Wayne State University, Detroit, MI, USA

[9] The Ilse-Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, POB 653, Beer-Sheva 8410501, Israel

* Corresponding author.

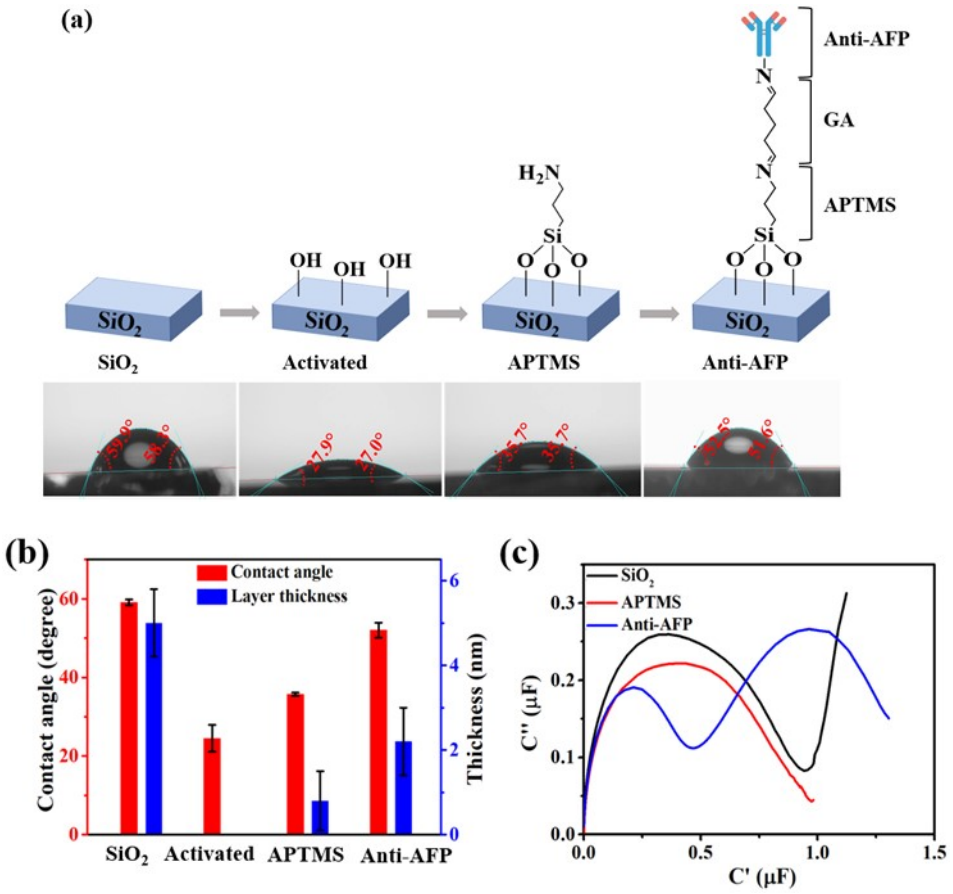
E-mail address: glshalev@bgu.ac.il

Supplementary information

SI1: biofunctionalization and characterization of Si/SiO_2 samples and MNC devices

Figure SI1a illustrates the main steps towards sensing area biofunctionalization with anti-AFP antibodies [1]. In short, the Si/SiO_2 sensing area is activated with piranha followed by surface chemical modification with APTMS linker molecules, and final surface-tethering of the anti-AFP antibodies (see Methods).[2] Figure SI1a also presents the corresponding contact angle measurements of Si/SiO_2 samples. The surface modifications were developed and characterized using 1 cm x 1 cm samples of silicon substrates decorated with 5 nm SiO_2 (Figure SI1b-c). Figure SI1b presents contact angle measurements for Si/SiO_2 samples post piranha activation, post APTMS modification and surface biofunctionalization with anti-AFP antibodies. The measurements are performed at three locations on the samples with two measurements performed at each location, such that the data points and the error bars reflect the averages and standard deviations, respectively, of six measurements. The contact angle dependency on the various surface modifications follows behavior well documented previously[34]. Figure SI1b also shows ellipsometry measurements performed on the same Si/SiO_2 samples. First, the SiO_2 sample is measured and the 5 nm SiO_2 thickness is validated. Afterwards, the sample is measured post APTMS modification where the SiO_2 is fixed and the APTMS thickness is fitted. Finally, the sample is measured post surface biofunctionalization with anti-AFP antibodies where both the SiO_2 and the APTMS thicknesses are fixed to the measured values and the antibody layer is fitted. The mean square error (MSE) for all measurements is smaller than 2. The thicknesses presented in Figure SI1b reflect the expected values of APTMS and the antibody layer[1,5,6]. Figure SI1c presents the EIS measurements of the imaginary capacitance (C") vs. the real capacitance (C') for unmodified Si/SiO_2, post APTMS modification and post biofunctionalization with anti-AFP antibodies. Three distinct curves are shown,

which reflect the effect of the modifications on the sensing area. The capacitance post APTMS modification is not significantly different from the unmodified sample, but the presence of an antibody layer is well reflected in the formation of an additional semi-circle characteristic of the generation of an additional layer. Finally, the biofunctionalization is applied to an MNC biosensor and Figure SI1d shows I_{DS} vs. V_{GL} for different V_{GF} values performed for unmodified, and MNC modified with anti-AFP antibodies. All the measurements are performed for 0.5 µL drops of 0.1 mM 7.4 pH PBS solution. Three drops are measured for the unmodified MNC biosensor, and three drops are measured for the modified MNC biosensor, and each drop is measured four times. Figure SI1d presents an excellent robustness and repeatability of a modified MNC device in 0.1 mM 7.4 pH PBS solution, as well as asserts the stability of the quasi-reference electrode under possible drop-to-drop variations[7]. Finally, the repeatability addresses the concern of possible drop-to-drop pH fluctuations[8].



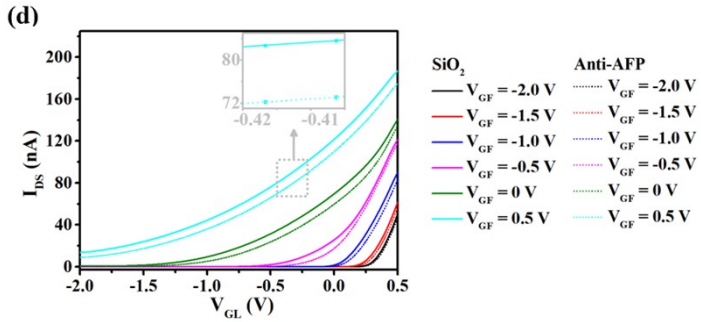


Figure SI1. (a) An illustration showing the process of Si/SiO_2 surface biofunctionalization. The corresponding contact angle measurements are also shown. **(b)** Ellipsometry measurements and contact angle measurements of Si/SiO_2 samples post various modification steps. **(c)** EIS measurements showing the real and imaginary capacitances for the various modifications steps. **(d)** I_{DS}-V_{GL} for various V_{GF} values for unmodified MNC device and biofunctionalized MNC biosensor. The data points and the error bars (see inset) reflect the averages and standard deviations of 12 measurements (3 drops each measured 4 times).

References

1 I. M. Bhattacharyya, I. Ron, A. Chauhan, E. Pikhay, D. Greental, N. Mizrahi, Y. Roizin and G. Shalev, *Nanoscale*, 2022, **14**, 2837–2847.

2 S. Samanta, I. M. Bhattacharyya, A. Prajapati, I. Ron, R. Shima-Edelstein, E. Pikhay, D. Greental, A. Eisenberg-Lerner, Z. Rotfogel, Y. Roizin, B. Akabayov and G. Shalev, *Adv Mater Technol*, 2023, **n/a**, 2202200.

3 R. G. Frieser, *J Electrochem Soc*, 1974, **121**, 669.

4 Z.-Z. Liu, Q. Wang, X. Liu and J.-Q. Bao, *Thin Solid Films*, 2008, **517**, 635–640.

5 K. Bierbaum, M. Kinzler, Ch. Woell, M. Grunze, G. Haehner, S. Heid and F. Effenberger, *Langmuir*, 1995, **11**, 512–518.

6 I. M. Bhattacharyya, I. Ron, R. Shima-Edelstein, E. Pikhay, Y. Roizin and G. Shalev, *Adv Electron Mater*, 2022, 2200399.

7 L. R. F. Allen J. Bard, *Electrochemical Methods: Fundamentals and Applications, 2nd Edition*, John Wiley and Sons Inc., 2008.

8 R. E. G. Van Hal, J. C. T. Eijkel and P. Bergveld, *Sensors and Actuators B*, 1995, **24–25**, 201–205.