Electronic Supplementary Information (ESI)

Rapid sub-micromolar amperometric enzyme biosensing with free substrate access but without nanomaterial signalling support: Oxidase-based glucose detection as proof-of-principle case

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Table S1: Summary of analytical figures of merit for published biosensors that use nanomaterials of various types as components of the immobilization matrix to establish µM limits of detection. (Source of the reference collection: Web of Science).

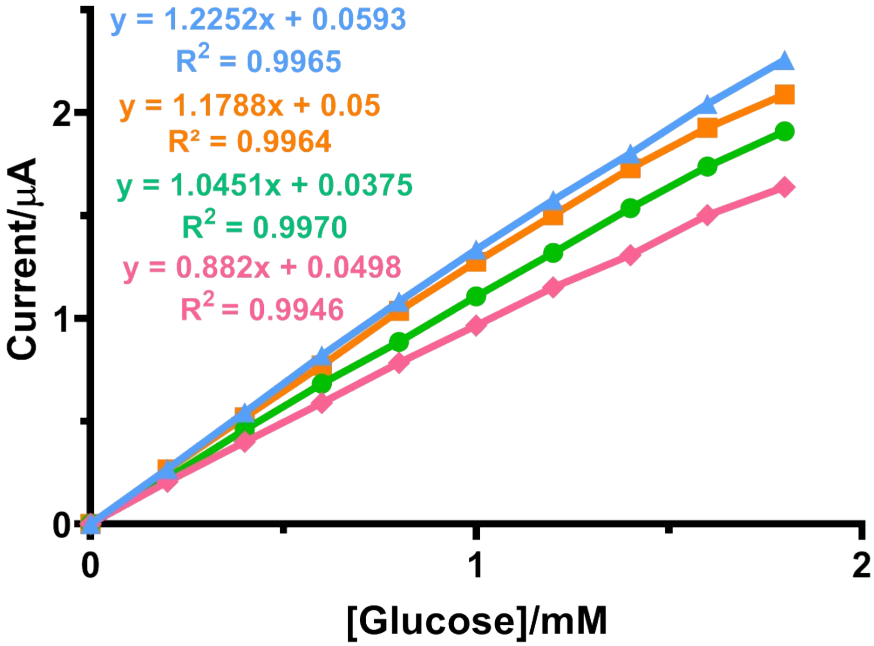
Nanomaterial in enzyme immobilization matrix	Study	Year	Linear Range	Sensitivity	LOD (µM)	Response time (s)
Carbon Nanotubes	Ref. 1	2013	0.42µM - 8.0mM	52.0 µAmM^{-1} cm^{-2}	-	-
	Ref. 2	2016	0.01mM - 4.7mM	2860.3 µAmM^{-1}cm^{-2}	5	3
	Ref. 3	2017	6µM – 2mM 10µM – 1mM	-	2.12 3.8	-
	Ref. 4	2018	Up to 1.0mM	0.44 ± 0.01 mA M^{-1}	3	28 ± 2
	Ref. 5	2019	50 – 500µM	4.83 µAmM^{-1}	1.2	-
Graphene	Ref. 6	2015	10µM – 12.55mM	6.36 µAmM^{-1}	1	-
	Ref. 7	2015	0.14 – 4.0µM	15.04 mAmM^{-1}	0.06	-
	Ref. 8	2015	-	221.0 mAmM^{-1}cm^{-2}	-	-
	Ref. 9	2019	0.1mM – 11mM	15.85 µAmM^{-1}cm^{-2}	20	-
	Ref. 10	2020	0.001 – 0.1mM	-	0.018	-
	Ref. 11	2020	0 – 1.2mg/mL	0.77 nm/(mg/mL)	-	2.16
	Ref. 12	2020	0.006 – 6mM	64.51 µAmM^{-1}cm^{-2}	1.8	-
Mesoporous carbon and carbon nanodots	Ref. 13	2015	48.6 – 12500µM	6.9 µAmM^{-1}cm^{-2}	2.7	-
	Ref. 14	2015	1 – 16mM	0.31 µAmM^{-1}	88	-
	Ref. 15	2015	0 – 0.64mM	6.1 µAmM^{-1}	1.07	-
	Ref. 16	2019	0.0002 – 0.1mM	123.28 µAmM^{-1}cm^{-2}	0.023	3.7
	Ref. 17	2019	250µM – 3mM	56.12 µAmM^{-1}cm^{-2}	4.1	-
Metal Organic Framework	Ref. 18	2017	25µM – 17mM	25.1 µAmM^{-1}	8	-
	Ref. 19	2018	0.51µM – 5.62mM	28.67 µAmM^{-1}cm^{-2}	0.48	-
	Ref. 20	2018	1µM – 2mM	-	0.333	
Prussian blue nanoparticles	Ref. 21	2000	-	3 µAcm^{-2}	-	
	Ref. 22	2016	5µM – 1.2mM	32 µAmM^{-1}cm^{-2}	0.5	3-4
	Ref. 23	2017	2 – 68mM	0.19 – 3.87 µAmM^{-1}cm^{-2}	-	-
Redox polymer and polymer nanoparticles	Ref. 24	2015	0.3 – 17mM	-	2.7	1
	Ref. 25	2015	-	120.87 mAM^{-1}cm^{-2}	37.6	-
	Ref. 26	2015	200µM – 3.4mM	1.59 nA µM^{-1}	46.2	15
	Ref. 27	2016	1 – 10mM	16.04 µAmM^{-1}cm^{-2}	820	10
	Ref. 28	2016	Up to 1.5mM	176 mAmM^{-1}cm^{-2}U^{-1}	9	<4
	Ref. 29	2016	9.0µM – 8.26mM	11.98 mAM^{-1}cm^{-2}	10	
	Ref. 30	2018	0.1 – 0.8mM 0.1 – 10mM 0.1 – 10mM	- - -	2.9 3.2 3.4	- - -
	Ref. 31	2018	5 – 30mM	-	550	< 3
	Ref. 32	2018	0.01 – 0.8mM	74.22 µAmM^{-1}cm^{-2}	2.9	2-3
	Ref. 33	2019	500nM – 10nM	-	0.01	-
	Ref. 34	2020	10µM – 1.94mM	35.56 µAmM^{-1}cm^{-2}	0.1	-
	Ref. 35	2015	0.005 – 10mM	175.49 µAmM^{-1}cm^{-2}	50	<2
	Ref. 36	2015	0.1 – 10.0mM	52.1 µAmM^{-1}cm^{-2}	24	-
	Ref. 37	2015	55.1µM – 16.53mM	390.5 µAmM^{-1}cm^{-2}	11	-
	Ref. 38	2015	0.05 – 2.25mM	9.62 µAmM^{-1}cm^{-2}	10	-

Metal, metal oxide and semiconductor nanoparticles	Ref. 39	2015	1 – 20mM	0.58 µAmM^{-1}	68	4
	Ref. 40	2015	0.02 – 6.0mM	6.31 µAmM^{-1}cm^{-2}	18	10
	Ref. 41	2015	50µM – 10mM	12.1 µAmM^{-1}cm^{-2}	1.02	-
	Ref. 42	2015	2.0 – 52 µM 0.052 – 2.3mM	68.58 mAM^{-1}cm^{-2}	0.5	-
	Ref. 43	2015	0.05 – 2.5mM	24.65 µAmM^{-1}cm^{-2}	20	-
	Ref. 44	2015	0.2 – 9.0mM	2.52 µAmM^{-1}cm^{-2}	100	-
	Ref. 45	2016	-	-	40	-
	Ref. 46	2016	5µM – 2mM	-	1.8	5
	Ref. 47	2016	0.1 – 2.7mM	6.37 mAM^{-1}cm^{-2}	14.1	5
	Ref. 48	2016	0.1 – 18mM	4.2 µAmM^{-1}cm^{-2}	5.9	-
	Ref. 49	2016	-	18.6 mAM^{-1}cm^{-2}	2.2	-
	Ref. 50	2017	1 – 6mM	6.82 µAmM^{-1}cm^{-2}	0.2	< 5
	Ref. 51	2017	0.025 – 2.20mM	3.19 µAmM^{-1}	0.2	-
	Ref. 52	2018	0.2 – 12mM	231.7 µAmM^{-1}cm^{-2}	6.3	3
Metal, metal oxide and semiconductor nanoparticles	Ref. 53	2018	5 - 100 µM	-	1.8	-
	Ref. 54	2019	0.02 – 2.5mM	42.8 µAmM^{-1}cm^{-2}	10	5
	Ref. 55	2019	0.008 – 1.13mM 1.13 – 5.53mM	18.9 mAM^{-1}cm^{-2}	1.8	< 8
	Ref. 56	2019	0.005 – 3.3mM	12.5 µAmM^{-1}cm^{-2}	0.9	< 3
	Ref. 57	2019	-	8.5 µAmM^{-1}cm^{-2}	3.2	< 10
	Ref. 58	2019	1 – 14mM	12.99 mAM^{-1}cm^{-2}	300	-
	Ref. 59	2019	0.003 – 3.43 mM	35.4 µAmM^{-1}cm^{-2}	1.34	< 3
	Ref. 60	2019	-	3.7277 mAmM^{-1}cm^{-2}	3	-
	Ref. 61	2019	0.1 – 2.6 mM	25.9 mAM^{-1}cm^{-2}	-	30
Nanomaterial-Free	This work	2020	Up to 1.8 mM	158 µAmM^{-1}cm^{-2}	0.5	1.4

Table References:

1. M. Ali Kamyabi, N. Hajari, A. P. F. Turner and A. Tiwari, *Talanta*, 2013, **116**, 801–808.
2. B. K. Shrestha, R. Ahmad, H. M. Mousa, I.-G. Kim, J. I. Kim, M. P. Neupane, C. H. Park and C. S. Kim, *J. Colloid Interface Sci.*, 2016, **482**, 39–47.
3. M. Baghayeri, H. Veisi and M. Ghanei-Motlagh, *Sensors Actuators B Chem.*, 2017, **249**, 321–330.
4. F. N. Comba, M. R. Romero, F. S. Garay and A. M. Baruzzi, *Anal. Biochem.*, 2018, **550**, 34–40.
5. P. A. Gallay, M. D. Rubianes, F. A. Gutierrez and G. A. Rivas, *Electroanalysis*, 2019, **31**, 1888–1894.
6. L. Meng, Y. Xia, W. Liu, L. Zhang, P. Zou and Y. Zhang, *Electrochim. Acta*, 2015, **152**, 330–337.
7. S. Sabury, S. H. Kazemi and F. Sharif, *Mater. Sci. Eng. C*, 2015, **49**, 297–304.
8. Z. Chu, Y. Liu, Y. Xu, L. Shi, J. Peng and W. Jin, *Electrochim. Acta*, 2015, **176**, 162–171.
9. B. Wang, *Int. J. Electrochem. Sci.*, 2019, **14**, 7495–7506.
10. S. H. Baek, J. Roh, C. Y. Park, M. W. Kim, R. Shi, S. K. Kailasa and T. J. Park, *Mater. Sci. Eng. C*, 2020, **107**, 110273.
11. B. Xu, J. Huang, L. Ding and J. Cai, *Mater. Sci. Eng. C*, 2020, **107**, 110329.
12. D. Uzak, A. Atiroğlu, V. Atiroğlu, B. Çakıroğlu and M. Özacar, *Electroanalysis*, 2020, **32**, 510–519.
13. D. Xiang, L. Yin, J. Ma, E. Guo, Q. Li, Z. Li and K. Liu, *Analyst*, 2015, **140**, 644–653.
14. E. Ghasemi, E. Shams and N. Farzin Nejad, *J. Electroanal. Chem.*, 2015, **752**, 60–67.
15. M. Zhao, Y. Gao, J. Sun and F. Gao, *Anal. Chem.*, 2015, **87**, 2615–2622.
16. T. Liang, L. Zou, X. Guo, X. Ma, C. Zhang, Z. Zou, Y. Zhang, F. Hu, Z. Lu, K. Tang and C. M. Li, *Adv. Funct. Mater.*, 2019, **29**, 1903026.
17. P. Barathi, B. Thirumalraj, S.-M. Chen and S. Angaiah, *Microchem. J.*, 2019, **147**, 848–856.
18. Y. Song, Y. Shen, C. Gong, J. Chen, M. Xu, L. Wang and L. Wang, *ChemElectroChem*, 2017, **4**, 1457–1462.
19. Y. Xie, Y. Song, Y. Zhang, L. Xu, L. Miao, C. Peng and L. Wang, *J. Alloys Compd.*, 2018, **757**, 105–111.
20. C. Hou, D. Zhao, Y. Wang, S. Zhang and S. Li, *J. Electroanal. Chem.*, 2018, **822**, 50–56.

21. R. Garjonyte and A. Malinauskas, *Biosens. Bioelectron.*, 2000, **15**, 445–451.
22. E. Jomma and S.-N. Ding, *Sensors*, 2016, **16**, 243.
23. A. Valiūnienė, P. Virbickas, A. Rekertaitė and A. Ramanavičius, *J. Electrochem. Soc.*, 2017, **164**, B781–B784.
24. M. Dervisevic, E. Çevik and M. Şenel, *Enzyme Microb. Technol.*, 2014, **68**, 69–76.
25. F. B. Emre, M. Kesik, F. E. Kanik, H. Z. Akpinar, E. Aslan-Gurel, R. M. Rossi and L. Toppare, *Synth. Met.*, 2015, **207**, 102–109.
26. Y. Lin, L. Hu, L. Yin and L. Guo, *Sensors Actuators B Chem.*, 2015, **210**, 513–518.
27. B. Hansen, M. A. Hocevar and C. A. Ferreira, *Synth. Met.*, 2016, **222**, 224–231.
28. V. I. Paz Zanini, M. Gavilán, B. A. López de Mishima, D. M. Martino and C. D. Borsarelli, *Talanta*, 2016, **150**, 646–654.
29. J. P. Hervás Pérez, B. López-Ruiz and E. López-Cabarcos, *Talanta*, 2016, **149**, 310–318.
30. S. Kurbanoglu, M. N. Zafar, F. Tasca, I. Aslam, O. Spadiut, D. Leech, D. Haltrich and L. Gorton, *Electroanalysis*, 2018, **30**, 1496–1504.
31. Onay, Ü. Dogan, H. Ciftci, D. Cetin, Z. Suludere and U. Tamer, *Ionics (Kiel).*, 2018, **24**, 4015–4022.
32. M. Z. Çetin and P. Camurlu, *RSC Adv.*, 2018, **8**, 19724–19731.
33. J. Yoon, S. N. Lee, M. K. Shin, H.-W. Kim, H. K. Choi, T. Lee and J.-W. Choi, *Biosens. Bioelectron.*, 2019, **140**, 111343.
34. H. Zheng, M. Liu, Z. Yan and J. Chen, *Microchem. J.*, 2020, **152**, 104266.
35. M. Ahmad, H. Sun, M. Hussain, S. Karim, A. Nisar and M. Khan, *Electroanalysis*, 2015, **27**, 1498–1506.
36. N. German, A. Kausaite-Minkstimiene, A. Ramanavicius, T. Semashko, R. Mikhailova and A. Ramanaviciene, *Electrochim. Acta*, 2015, **169**, 326–333.
37. C.-W. Hsu, W.-C. Feng, F.-C. Su and G.-J. Wang, *J. Electrochem. Soc.*, 2015, **162**, B264–B268.
38. Z. Miao, P. Wang, A. Zhong, M. Yang, Q. Xu, S. Hao and X. Hu, *J. Electroanal. Chem.*, 2015, **756**, 153–160.
39. M. Şenel, *Mater. Sci. Eng. C*, 2015, **48**, 287–293.
40. W. Tang, L. Li and X. Zeng, *Talanta*, 2015, **131**, 417–423.
41. C. Wu, H. Sun, Y. Li, X. Liu, X. Du, X. Wang and P. Xu, *Biosens. Bioelectron.*, 2015, **66**, 350–355.
42. Z. Yang, Y. Xu, J. Li, Z. Jian, S. Yu, Y. Zhang, X. Hu and D. D. Dionysiou, *Microchim. Acta*, 2015, **182**, 1841–1848.
43. X. Zhou, X. Dai, J. Li, Y. Long, W. Li and Y. Tu, *Mater. Sci. Eng. C*, 2015, **49**, 579–587.
44. A. Samphao, P. Butmee, J. Jitcharoen, Ľ. Švorc, G. Raber and K. Kalcher, *Talanta*, 2015, **142**, 35–42.
45. Y. Cai, Y. Ye, S. Wu, J. Liu and C. Liang, *RSC Adv.*, 2016, **6**, 78219–78224.
46. L. Han, C. Shao, B. Liang and A. Liu, *ACS Appl. Mater. Interfaces*, 2016, **8**, 13768–13776.
47. Q. Li, W. Luo, L. Su, J. Chen, K.-C. Chou and X. Hou, *RSC Adv.*, 2016, **6**, 92748–92753.
48. R. B. Rakhi, P. Nayak, C. Xia and H. N. Alshareef, *Sci. Rep.*, 2016, **6**, 36422.
49. J. Zhang, X. Yu, W. Guo, J. Qiu, X. Mou, A. Li and H. Liu, *Nanoscale*, 2016, **8**, 9382–9389.
50. S. K. Krishnan, E. Prokhorov, D. Bahena, R. Esparza and M. Meyyappan, *ACS Omega*, 2017, **2**, 1896–1904.
51. H. Yang, Q. Shi, Y. Song, X. Li, C. Zhu, D. Du and Y. Lin, *J. Electrochem. Soc.*, 2017, **164**, B230–B233.
52. N. Muthuchamy, R. Atchudan, T. N. J. I. Edison, S. Perumal and Y. R. Lee, *J. Electroanal. Chem.*, 2018, **816**, 195–204.
53. S. Alim, A. K. M. Kafi, J. Rajan and M. M. Yusoff, *Int. J. Biol. Macromol.*, 2018, **123**, 1028–1034.
54. Y. Zhou, S. D. Uzun, N. J. Watkins, S. Li, W. Li, A. L. Briseno, K. R. Carter and J. J. Watkins, *ACS Appl. Mater. Interfaces*, 2019, **11**, 1821–1828.
55. P. Yao, S. Yu, H. Shen, J. Yang, L. Min, Z. Yang and X. Zhu, *New J. Chem.*, 2019, **43**, 16748–16752.
56. Z. Hu, *Int. J. Electrochem. Sci.*, 2019, **14**, 10352–10362
57. Z. Hu, *Int. J. Electrochem. Sci.*, 2019, **14**, 9661–9670.
58. Z. Huang, A. Zhang, Q. Zhang, S. Pan and D. Cui, *J. Electrochem. Soc.*, 2019, **166**, B1138–B1145.
59. Y. Liu, X. Nan, W. Shi, X. Liu, Z. He, Y. Sun and D. Ge, *RSC Adv.*, 2019, **9**, 16439–16446.
60. L. Wang, W. Zhu, W. Lu, L. Shi, R. Wang, R. Pang, Y. Cao, F. Wang and X. Xu, *Biosens. Bioelectron.*, 2019, **142**, 111577.
61. P. Jakhar, M. Shukla and V. Singh, *J. Nanosci. Nanotechnol.*, 2019, **19**, 7605–7614.



Supplementary Figure S1: Response linearity of four gelatin/GOx biosensors, prepared identically on different days. The calibrations were carried out at day 1, after one-night storage in a refrigerator in 0.1 M Na-PB/0.1 M KCl. The electrolyte for all trials was 0.1 M Na-PB/0.1 M KCl, and amperometry was at 25° C, with a H_2O_2 detection potential of + 0.6 V vs. Ag/AgCl.

Experimental Section

Chemicals, materials, solutions. Anhydrous β-D (+) glucose, monobasic (NaH_2PO_4) and dibasic (Na_2HPO_4) sodium phosphate for buffer preparation and ascorbic acid, uric acid, dopamine and paracetamol for the interference tests were from Italmar (Thailand) Co., Ltd., Bangkok, Thailand. GOx (source: Aspergillus Niger, #G7141, 228,253 U/g) and glutaraldehyde (Grade I, 25 % in H_2O) were from S.M. Chemical Supplies Co., Ltd., Bangkok, Thailand. Food-grade 'McGarret' gelatin powder was from a local store. Ultrapure de-ionized water was used for solution preparation. The biosensor storage solution and supporting electrolyte for all biosensor tests was 0.1 M Na phosphate buffer pH 7.4 containing 0.1 M KCl.

Human serum samples. The serum glucose analysis with gelatin/GOx biosensors was conducted on aliquots of one particular whole-blood sample, which was an agreed donation of the principal investigator (PI) of the study (AS). Blood sampling from others, typical of large-scale clinical trials on many patients, was not part of this study, so institutional approval was not required. The blood sample from the PI was converted to serum by a standard procedure.[1] The glucose content of stockpiled serum samples was measured according to instructions with an Accu-Chek® Active blood glucose meter, a commercial hand-held device normally used by diabetic patients for blood tests. The mean of triplicate glucose meter assessments was used as the reference value for the data obtained with gelatin/GOx-based biosensors.

Biosensor fabrication. First, a 1-mm diameter platinum (Pt) disk electrode encased in a PEEK (polyether-ether-ketone) body of 3 mm outer diameter diameter from eDAQ Pty Ltd (Denistone East, Australia) was polished and cleaned, as usual for voltammetry, then 2 µL gelatin solution (5 mg mL^{-1} in H_2O) was placed on the Pt disk and an even gelatin film formed on the smooth metal surface by solvent evaporation for 10-15 min at room temperature, 25°C. Next, 2 µl of GOx solution (10 mg mL^{-1} in Na phosphate buffer) was dropped onto the gelatin-coated Pt disk and air-dried at room temperature for 10-15 min. Finally, the gelatin/GOx-covered electrode was pushed in an inverted position through a suitably-sized hole in the cap of an Eppendorf tube containing 200 µL of 10 % glutaraldehyde solution. Care was taken not to touch the central biolayer and a Parafilm® M wrap was then applied, to seal the tube cap to the electrode stem. The Pt disk coating was thus exposed to glutaraldehyde vapor in the tube, causing chemical crosslinking of NH_2-groups. The glutaraldehyde treatment was routinely continued for 6 h at 4°C to complete the desired gelatin/GOx-Pt sensors. Finished sensors were stored overnight in phosphate buffer, pH 7.4 at 4°C before performing calibrations or glucose measurements with model or real samples.

The 1-mm diameter copper (Cu) disk electrodes were home-made. In course of the procedure a 1-mm diameter electrical cable copper wire was placed in a tapered glass tube and the tube then filled with liquid superglue. After overnight superglue hardening the desired Cu disk was exposed by careful grinding of the tip of the assembly on emery paper. Polishing on progressively finer emery paper and finally on a soft polishing pad soaked with alumina paste of small grade created the circular and smooth Cu electrode, surrounded by a layer of insulating resin in the glass case. The gelatin modification of the Cu disk followed the procedure used for the Pt disks, but with 1.5 µL instead of the 2 µL of the gelatin and GOx solution used in the drop-and-dry coating procedure.

Biosensor amperometry. Electrochemical measurements with the developed gelatin/GOx-Pt and gelatin/GOx-Cu biosensors were performed with a PalmSens4 potentiostat (PalmSens BV, GA Houten, The Netherlands). The instrument was connected to a three-electrode electrochemical cell and controlled by the latest version of the PalmSens software. All amperometric trials took place at room temperature of 25°C in 5 mL of stirred Na phosphate buffer, pH 7.4. The biosensor of choice, an AgCl-coated silver wire and a Pt foil were the working-, pseudo-reference- and counter-electrodes, respectively. H_2O_2 currents on glucose addition to electrolyte were recorded at an anodic working electrode potential of + 0.6 V vs. Ag/AgCl for the Pt biosensor version and at a cathodic working electrode potential of – 0.25 V vs. Ag/AgCl for the Cu-based version.

References

1. M. K. Tuck, D. W. Chan, D. Chia, A. K. Godwin, W. E. Grizzle, K. E. Krueger, W. Rom, M. Sanda, L. Sorbara, S. Stass, W. Wang, D. E. Brenner, J. *Proteome Res.* 2009, **8**, 113-117.
2.