Supplementary Information (SI) for Lab on a Chip.

Supporting Information

WAFFLE - An automated platform for enhancing the performance of electrochemical biosensors

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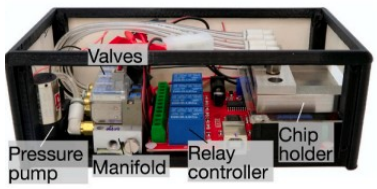
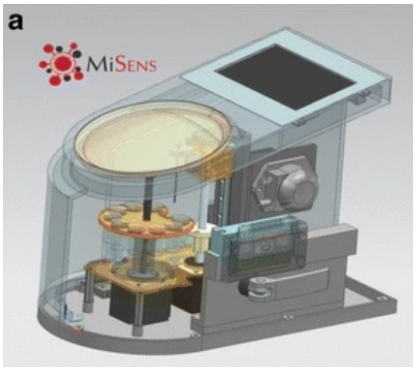
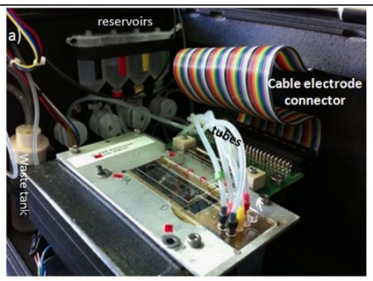
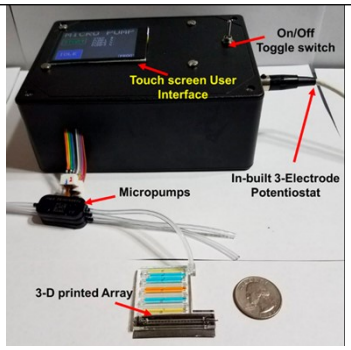
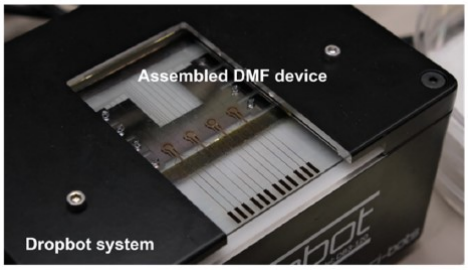
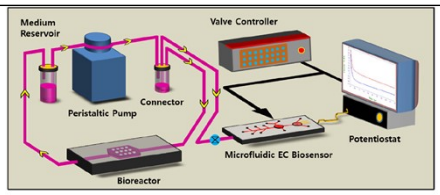
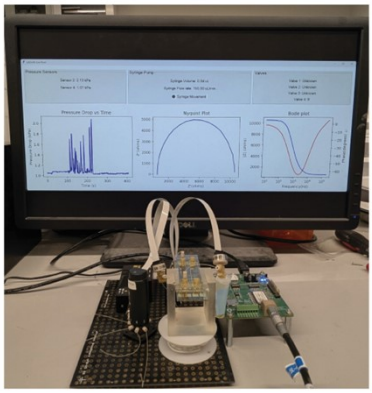
Table S1. Comparison of WAFFLE platform with prior art.

Platform	Image	Detection protocol	Steps automated	Ref.
eSIREN		1) Thiol modified oligonucleotides pipetted on electrodes 2) Flushing with PBST 3) Passivation with 5% BSA **4) <u>Fluidic device assembly</u>** 5) Sample/nanostructure/dNTP introduction by flow 6) Streptavidin-HRP 0.5% BSA introduced by flow 7) Wash buffer flushing 8) TMB substrate introduced by flow	5-8	1
MiSens		**For the detection of *E. coli* [2]:** 1) Immersing the sensor in 2 mM MUDA 2) Washing with ethanol/water **3) <u>Assembly into fluidic cassette</u>** 4) Injection of 0.4 M EDC and 0.1 M NHS 5) Injection of anti-*E. coli* antibody 6) Injection of 30 ug/ml BSA 7) Injection of 1 M ethanolamine 8) Sample addition 9) Secondary antibody-HRP injection 10) TMB injection	4-10	2–4
		For the detection of prostate-specific antigen (PSA)[3]: 1) Immersion in 2 mM MUDA and 2 mM mercaptoethanol 2) Rinsing with ethanol/water **3) <u>Fluidic device assembly</u>** 4) Injection of 400 mM EDC and 100 mM NHS 5) Injection of PSA antibodies 6) Passivation with 1 M ethanolamine 7) Antigen injection 8) Injection of PSA detection antibody/HRP Au nanoparticles 9) TMB substrate injection	4-9	
		For the detection of aflatoxin[4]: 1) Immersion in 2 mM MUDA and 2 mM mercaptoethanol 2) Rinsing with ethanol/water **3) <u>Assembly into fluidic device</u>** 4) Injection of 400 mM EDC and 100 mM NHS 5) Injection of Protein A 6) Passivation with 1 M ethanolamine 7) Aflatoxin/aflatoxin-HRP injection 8) TMB injection	4-8	
Dulay *et al.*		**For the detection of *Francisella tularensis*:** 1) Immersion in 1 µM DT2 (SAM assembly) 2) Rinsing with ethanol 3) Immersion in a 1:1 mixture of EDC (0.2 M) and NHS (0.05 M) 4) Incubation with monoclonal antibody 5) Blocking by immersion in ethanolamine **6) <u>Fluidic device assembly</u>** 7) Sample addition 8) Secondary antibody-HRP injection 9) TMB injection	7-9	5

Kadimisetty *et al.*		**For the detection of a multi-protein panel:** 1) Single-walled carbon nanotubes (SWCNT) attached to pyrolytic graphite sensor 2) SWCNTs activated using EDC−NHSS 3) Attachment of capture antibodies by amidization 4) Blocking with 2% BSA **5) <u>Assembly into fluidic device</u>** 6) Introduction of sample over sensor surface by flow 7) Wash buffer flown in 8) Introduction of functionalized silica nanoparticles by flow 9) Wash buffer 10) Introduction of tripropylamine coreactant	6-10	6
Dropbot system		**For the detection of PD-L1 checkpoint inhibitor**[7]: 1) 3D matrix comprised of rGO, BSA, and GA drop-casted onto electrodes 2) Activation with 400 mM EDC and 200 mM NHS 3) Incubation with primary antibodies 4) Surface blocked with Superblock **5) <u>Assembly within fluidic pathway/DMF device</u>** 6) Sample introduction to sensing area via Dropbox system 7) Wash droplets passed over the detection area 8) Secondary antibody droplet passed over the detection area 9) Poly-HRP-streptavidin droplet passed over the detection area 10) TMB droplet introduction	6-10	7, 8
		For the detection of LPS[8]**:** 1) Voltage assisted MUA and MCH SAM assembly 2) 100 mM EDC and 100 mM NHS immersion 3) Incubation with 10 µg/mL solution of TLR4/MD2 4) Washing with Tris buffer **5) <u>Assembly into DMF device</u>** 6) Sample droplets introduced to the sensing area 7) Washing with droplets of endotoxin free water supplemented with 90R4 8) Delivery of $[Fe(CN)_6]^{3-/4-}$ redox probe droplet to the sensing area	6-8	
Riahi *et al.*		**For measurement of cell-secreted biomarkers:** 1) Magnetic beads functionalized with antibodies off chip **2) <u>Fluidic device assembly</u>** 3) Introduction of beads in the detection chamber and immobilization *via* a magnet 4) Sample solution flown in 5) Secondary antibody-HRP loaded into the detection chamber 6) TMB substrate introduced into the chamber	3-6	9
Menon *et al.*		**For ssDNA sensing:** 1) Single-walled carbon nanotubes (SWCNTs) were functionalized with ssDNA probes using EDC/NHS coupling chemistry (off-chip) 2) SWCNT were drop cast into the sensing region of the fluidic chip **3) <u>Channel is enclosed</u>** 4) CNTs wetted using DI water 5) Device priming using an electrolyte buffer containing 10 mM potassium ferri/ferrocyanide 6) Sample introduction through flow 7) Measurement	4-7	10

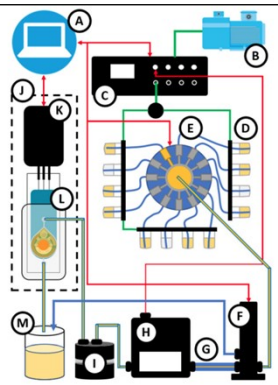
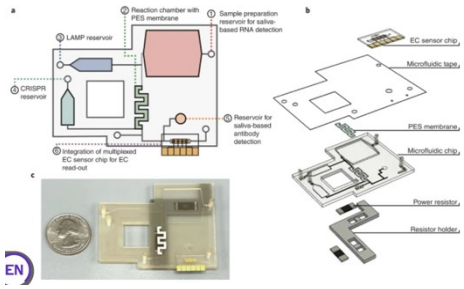
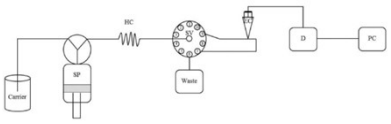
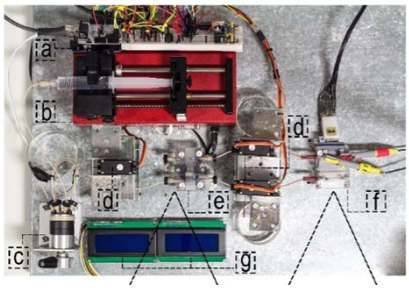
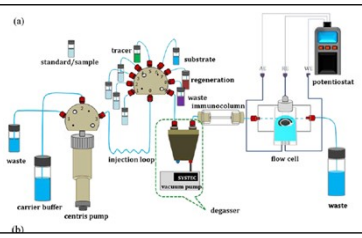
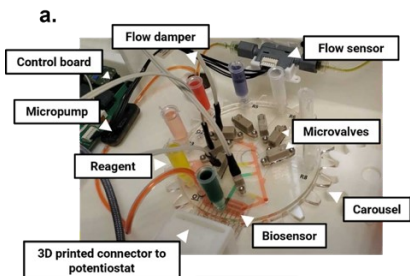
Ecke *et al.*		1) Magnetic bead immunoassay performed off-chip using previously reported protocol[11] 2) Final enzymatic products were collected into Eppendorf tubes **3) <u>Device assembly</u>** 4) iPrOH flushed through the system 5) Water and buffer flushed through the system 6) Reacted TMB substrate products were successively flushed through the system and measured	4-6	12
Najjar *et al.*		**For the preparation of a CRISPR-based RNA assay:** 1) The nanomaterial solution was crosslinked with 70% glutaraldehyde and deposited on the gold electrodes 2) Washing with PBS 3) Activation with EDC/NHS 4) Washing with ultrapure water 5) Capture molecule attachment 6) PNA spotted on the detection surface 7) Surface was quenched with 1 M ethanolamine 8) Surface was passivated using blocking agent **9) <u>Assembly within fluidic device</u>** 10) Sample processing by flow 11) Polystreptavidin-HRP addition 12) TMB addition and detection	10-12	13
Upan *et al.*		**For the detection of hepatitis B surface antigen:** 1) AuNPs-CNT are dropped onto the surface of the electrode and dried 2) AgNPs are dropped on the nanomaterial modified electrode 3) Surface was activated with 0.4 M EDC/0.1 M NHS 4) Electrode was dipped into antibody solution 5) Electrode is immersed into 1 M ethanolamine solution 6) Rinsing with PBS **7) <u>Assembly within fluidic device</u>** 8) Sample introduced by flow 9) Measurement using differential pulse voltammetry	8,9	14
Mercer *et al.*		**For the detection of various cancer biomarkers:** 1) Biotinylated antibodies and HRP-labels attached onto super- paramagnetic beads (MBs) off-chip 2) PDDA absorbed on the surface 3) Glutathione-decorated AuNPs were deposited on the detection area 4) Antibodies attached via EDC/NHSS coupling 5) Electrode washed 6) Backfilling with 2% BSA in PBS **7) <u>Assembly within fluidic device</u>** 8) Flooding of capture chambers with 0.05% PBS 9) Filling of capture chamber with Ab2-HRP-MB 10) Sample addition 11) Introduction of hydroquinone and H_2O_2 to generate amperometric signal	8-11	15
Lebogang *et al.*		**For the detection of Microcystin-LR:** 1) Antibodies covalently attached to CNBr-activated Sepharose beads **2) <u>Beads are assembled within an immunocolumn</u>** 3) Sample containing target and HRP-labelled target is flown through the column 4) Enzymatic substrate flow through and measured	3,4	16

TABLE S2
APTAMER SEQUENCES USED IN THIS STUDY

Component	5'-3' Sequence	No. of bases
Capture Aptamer (C)	(thiC6) - TTT TTT TTT TTT TTT TTT TTG TCG TCC CGA GAG	33
Cortisol Aptamer (A)	CTC TCG GGA CGA CGC CCG CAT GTT CCA TGG ATA GTC TTG ACT AGT CGT CCC	51

ss = single strand; thiC6 = thiol group

WAFFLE	a.   	**For the detection of IL-6/TnIC:** **1) <u>Sensors integrated within fluidic cartridge</u>** 2) MUA/MCH SAM assembled by flow 3) Flushing with deionized water 4) EDC/NHS injection 5) Primary antibody attachment 6) Backfilling the surface with BSA solution 7) Sample addition 8) Secondary antibody-biotin injection 9) HRP-streptavidin injection 10) TMB flow in and measurement	2-10 (All)	**This work**
		For the detection of cortisol: **<u>1) Sensors integrated within fluidic cartridge</u>** 2) Anchor aptamer attachment by flow 3) Backfilling through injection of MCH 4) Cortisol aptamer attachment 5) Sample injection 6) Redox probe injection and measurement	2-6 (All)	

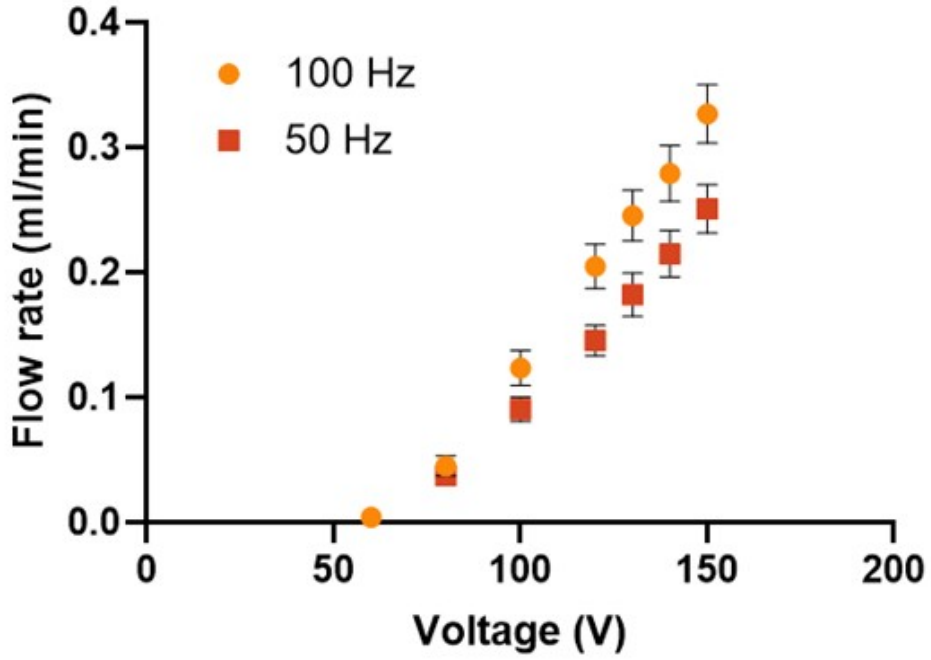


Figure S1. The effect of varying the pump frequency on the resultant flow rate (n=3 repeats for each condition). Each datapoint represents the average reading ±1 standard deviation

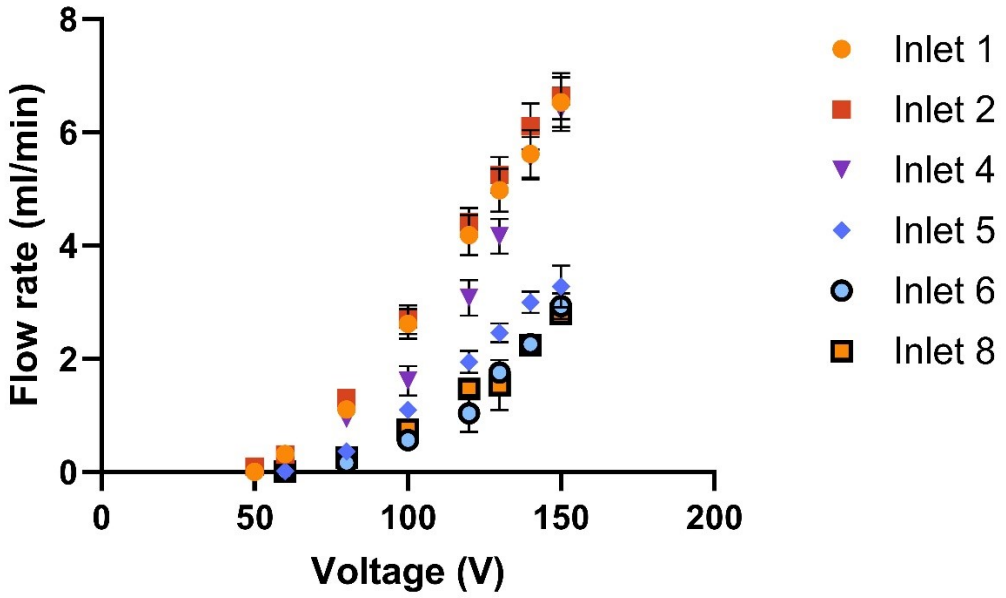


Figure S2. Differences in the resultant flow rates across the different inlets of the reagent cartridge as a function of the sine voltage amplitude applied to the Bartels micropump. Each datapoint represents the average reading ±1 standard deviation

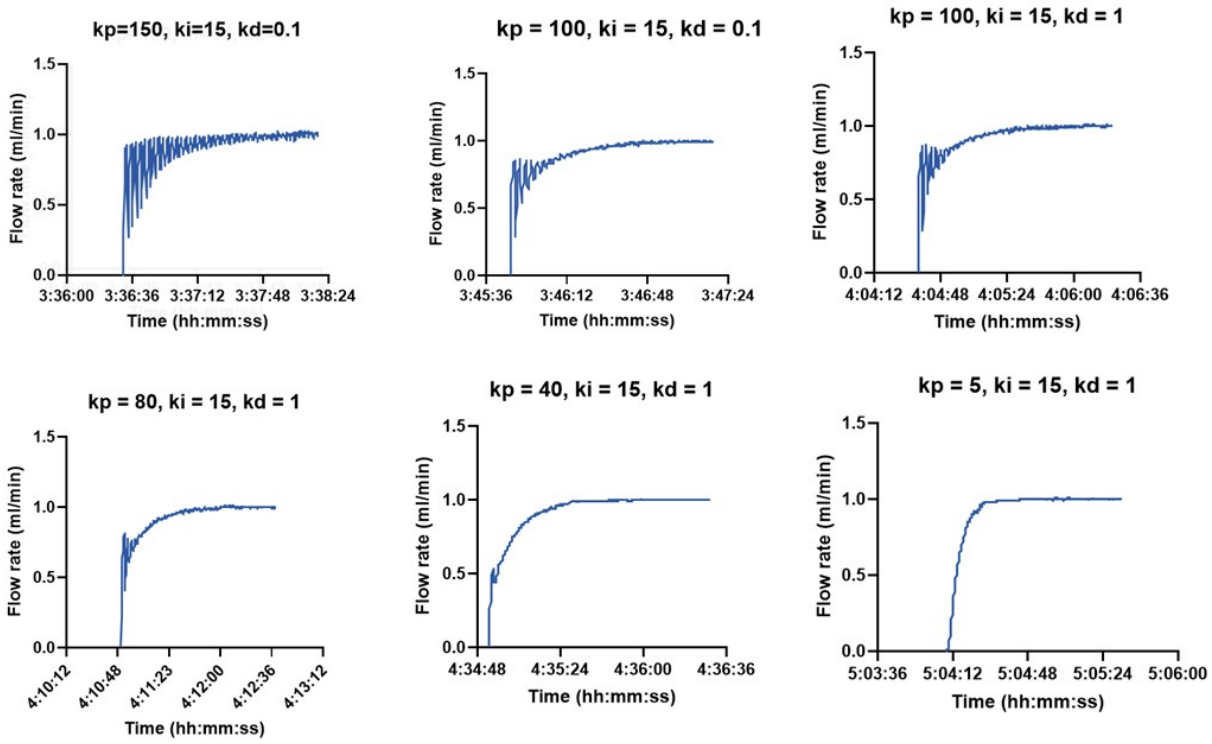


Figure S3. PID algorithm tuning. Flow rate setpoint = 1 ml/min

Table S3. Blank (0 pg/ml target) readings displayed in the main paper Figure 3

Condition/Sample tested	Figure	Blank current value ± 3 SD (nA)
Manual dose-response IL-6/PBS	3b – yellow (top) line	-4.0 ± 1.2
WAFFLE dose-response IL-6/PBS	3b – red (bottom) line	- 10.6 ± 2.5
Manual dose-response IL-6/neat serum	3c – yellow (top) line	-9.7 ± 1.8
WAFFLE dose-response IL-6/neat serum	3c – red (bottom) line	-32.5 ± 3.3
Manual dose-response TnI/neat serum	3e – yellow (top) line	-4.6± 1.5
WAFFLE dose-response TnI/neat serum	3e – red (bottom) line	-8.9 ± 1.2

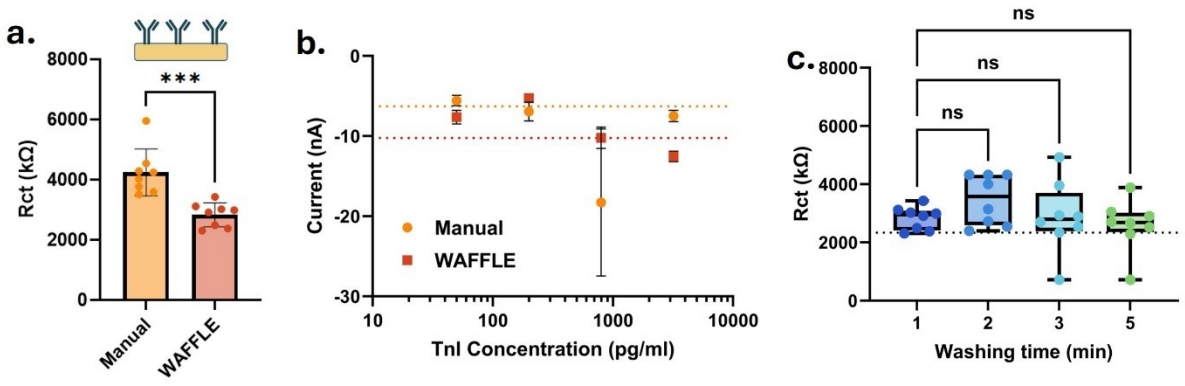


Figure S4. (a) The difference between a troponin antibody layer deposited on the electrode surface manually via pipetting or using the WAFFLE. (b) Dose responses of WAFFLE and manually made sensors to increasing concentrations of troponin (TnI) spiked in neat troponin-depleted human serum. Each marker represents the average over 8 electrodes +/− 1 SD. Dashed lines represent the blank reading -1SDs (n=8). (c) The effect of additional washing time on the troponin antibody layer (flow rate = 2 ml/min). Dashed line represents the impedance of the underlying EDC/NHS layer +1 SD (n=8), demonstrating effective attachment to the carbodiimide SAM.

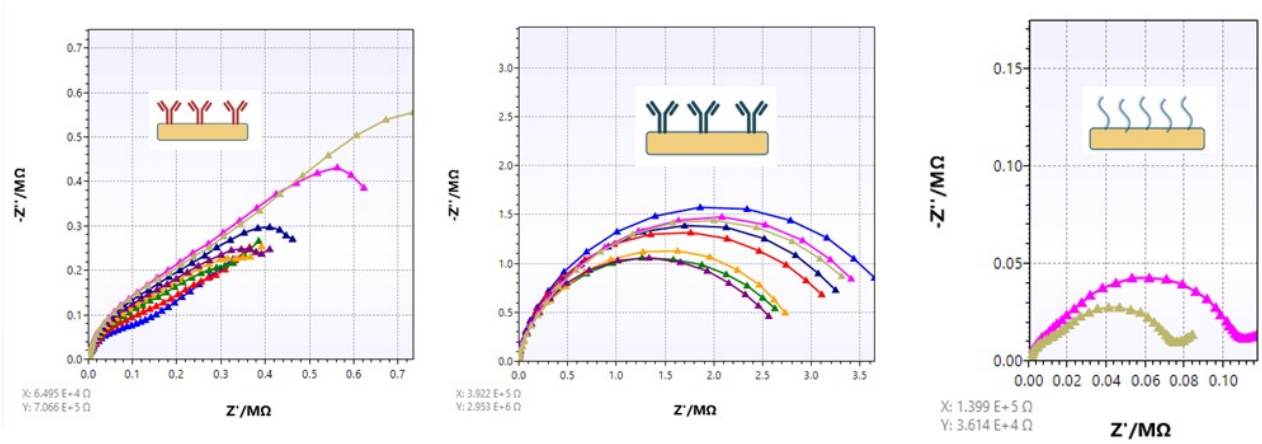


Figure S5. Different Nyquist plots acquired for electrodes (n=8 WEs per pannel) functionalised with (left) physisorbed IL-6 antibodies, (centre) TnI antibodies attached by EDC/NHS chemisorbtion on MUA SAM and (right) CSS.1 anchor aptamers attached to the surface via a thiol modification at the 5' end. Redox couple used for acquiring this data was 1 mM ferri-ferrocyanide in 1xPBS.

References

1. Zhao, H. *et al.* Accessible detection of SARS-CoV-2 through molecular nanostructures and automated microfluidics. *Biosens. Bioelectron.* **194**, 113629 (2021).

2. Altintas, Z., Akgun, M., Kokturk, G. & Uludag, Y. A fully automated microfluidic-based electrochemical sensor for real-time bacteria detection. *Biosens. Bioelectron.* **100**, 541–548 (2018).

3. Uludag, Y. *et al.* An integrated lab-on-a-chip-based electrochemical biosensor for rapid and sensitive detection of cancer biomarkers. *Anal. Bioanal. Chem.* **408**, 7775–7783 (2016).

4. Uludag, Y. *et al.* Lab-on-a-chip based biosensor for the real-time detection of aflatoxin. *Talanta* **160**, 381–388 (2016).

5. Dulay, S. B., Gransee, R., Julich, S., Tomaso, H. & O'Sullivan, C. K. Automated microfluidically controlled electrochemical biosensor for the rapid and highly sensitive detection of Francisella tularensis. *Biosens. Bioelectron.* **59**, 342–349 (2014).

6. Kadimisetty, K. *et al.* Automated 3D-Printed Microfluidic Array for Rapid Nanomaterial-Enhanced Detection of Multiple Proteins. *Anal. Chem.* **90**, 7569–7577 (2018).

7. Zhang, Y. *et al.* A digital microfluidic device integrated with electrochemical sensor and 3D matrix for detecting soluble PD-L1. *Biosens. Bioelectron. X* **19**, 100490 (2024).

8. Campos, R. P. S. De, Aggarwal, D., Chan, N. W. C. & Jemere, A. B. An integrated digital microfluidic electrochemical impedimetric lipopolysaccharide sensor based on toll-like receptor-4 protein. *Biosens. Bioelectron. X* **16**, 100433 (2024).

9. Riahi, R. *et al.* Automated microfluidic platform of bead-based electrochemical immunosensor integrated with bioreactor for continual monitoring of cell secreted biomarkers. *Sci. Rep.* **6**, 1–14 (2016).

10. Menon, N. H., Beshai, Y. & Basuray, S. Fully Automated Microfluidic Electrochemical Platform as a Versatile Biosensor. *IEEE Sensors Lett.* **2**, 1949–307 (2017).

11. Ecke, A., Westphalen, T., Hornung, J., Voetz, M. & Schneider, R. J. A rapid magnetic bead - based immunoassay for sensitive determination of diclofenac. *Anal. Bioanal. Chem.* 1563–1573 (2022) doi:10.1007/s00216-021-03778-7.

12. Ecke, A., Bell, J. & Schneider, R. J. A three-dimensional microfluidic flow cell and system integration for improved electrochemical substrate detection in HRP/TMB-based immunoassays. *Sensors & Diagnostics* 887–892 (2023) doi:10.1039/d3sd00095h.

13. Najjar, D. *et al.* A lab-on-a-chip for the concurrent electrochemical detection of SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies in saliva and plasma. *Nat. Biomed. Eng.* **6**, (2022).

14. Upan, J., Banet, P., Aubert, P. & Ounnunkad, K. Sequential injection-differential pulse voltammetric immunosensor for hepatitis B surface antigen using the modified screen-printed carbon electrode. *Electrochim. Acta* **349**, 136335 (2020).

15. Mercer, C., Jones, A. & Rusling, J. F. Multiplexed Electrochemical Cancer Diagnostics With Automated Microfluidics Short Communication. 208–211 (2019) doi:10.1002/elan.201800632.

16. Lebogang, L., Jantra, J. & Mattiasson, B. Electrochemical Flow-ELISA for Rapid and Sensitive Determination of Microcystin-LR Using Automated. (2017) doi:10.3390/s17071639.