Delft University of Technology

A mass-based aptasensor for real-time, continuous quantification of TNF-alpha with quartz crystal microbalance

Fasching, Lena; Zhu, Pancheng; Mulder, Atticus; Beltrao, Gabriel Spiller; Neeleman, Yannick; Rwei, Alina

DOI
10.1109/BioSensors58001.2023.10280820

Publication date
2023

Document Version
Final published version

Published in
2023 IEEE BioSensors Conference, BioSensors 2023 - Proceedings

Citation (APA)
Fasching, L., Zhu, P., Mulder, A., Beltrao, G. S., Neeleman, Y., & Rwei, A. (2023). A mass-based aptasensor for real-time, continuous quantification of TNF-alpha with quartz crystal microbalance. In *2023 IEEE BioSensors Conference, BioSensors 2023 - Proceedings* IEEE. https://doi.org/10.1109/BioSensors58001.2023.10280820

Important note
To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright
Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy
Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

This work is downloaded from Delft University of Technology.
For technical reasons the number of authors shown on this cover page is limited to a maximum of 10.

Green Open Access added to TU Delft Institutional Repository

'You share, we take care!' - Taverne project

https://www.openaccess.nl/en/you-share-we-take-care

Otherwise as indicated in the copyright section: the publisher is the copyright holder of this work and the author uses the Dutch legislation to make this work public.

A mass-based aptasensor for real-time, continuous quantification of TNF-alpha with quartz crystal microbalance

Lena Fasching*, Pancheng Zhu, Atticus Mulder, Gabriel Spiller Beltrao, Yannick Neeleman, and Alina Rwei†
Department of Product and Process Engineering, Delft University of Technology
Delft, The Netherlands
Email: *l.r.fasching@tudelft.nl, †a.rwei@tudelft.nl,

2023 IEEE BioSensors Conference (BioSensors) | 979-8-3503-4604-6/23/$31.00 ©2023 IEEE | DOI: 10.1109/BioSensors58001.2023.10280820

Abstract—**Accurate and real-time monitoring of biomarker proteins, such as Tumor Necrosis Factor (TNF) alpha, plays a vital role in early disease diagnosis, effective treatment design, and personalized health management strategies. However, existing detection methods, including enzyme-linked immunosorbent assay (ELISA), radioimmune assays (RIA), and polymerase chain reaction (PCR), have significant drawbacks regarding sensitivity, cost, time, and labor efficiency, emphasizing the urgent need for alternative biosensing techniques. Here, we present a mass-based biosensing approach utilizing aptamers for the real-time detection of proteins, using TNF-alpha as the model analyte. The recognition process is based on the selective binding of the target molecule to the aptamer's unique three-dimensional structure. By utilizing a quartz crystal microbalance (QCM) as the transducing element, real-time detection of target binding is translated into a linear decrease in resonant frequency due to the change in mass upon target binding. The developed aptasensor enabled real-time quantification of TNF-alpha with high reliability, sensitivity, and specificity. The sensitivity of the sensor ranged from 14.5 nM to 115.6 nM, in which a linear correlation between target concentration and frequency decrease rate was found. Successful sensor regeneration demonstrated potential for continuous measurements in solution. By directly monitoring the change in mass during sensor fabrication and upon analyte binding, this platform provides key mechanistic insights in the surface functionalization process during sensor fabrication and analyte binding kinetics during sensor operation. In the future, incorporation of alternative target receptors, by simply changing the aptamer sequence, can broaden the analyte spectrum, making this platform highly versatile. We hereby demonstrate a technology that can be utilized for various biosensing platforms upon minimal modifications, including electrochemical and optical systems, for a wide range of macromolecular analytes.**

Index Terms—**continuous, real-time cytokine biomarker monitoring; aptamer-based biosensor; mass-sensitive aptasensor**

I. Introduction

Cytokine biomarkers have gained increasing attention in recent years as potential diagnostic and therapeutic targets for diseases [1]. Cytokines are a group of small proteins with a molecular weight below 30 kD that act as inter-cellular messenger molecules between different cell types and play a crucial role in regulating immune responses [2]. Hence, their concentration in bodily fluids such as saliva [3], blood [4], and sweat [5] can provide an indication of the immune system's current status. Among pro-inflammatory cytokines, Tumor Necrosis Factor-alpha (TNF-α), which has been linked to various inflammatory diseases [6], including rheumatoid arthritis [7] and Crohn's disease [8], is particularly intriguing as a diagnostic biomarker, because of its abundance in the earliest stages of the disease [9]. Currently, cytokine measurements are typically performed using methods such as Enzyme-Linked Immunosorbent Assays (ELISA), lateral flow assays, and bead-based immunoassays [10], [11]. However, these well-established methods of measuring cytokine biomarkers are complex, time-consuming, invasive [12], and provide single-time-point measurements [13], highlighting the need for a novel sensing device that enables the continuous and real-time measurement of cytokine biomarker levels. Aptamer-based biosensors have been gaining significant attention for the detection of various biological molecules including proteins, DNA, metabolites, and small molecules [14]. As receptors these biosensors employ short, single-stranded DNA or RNA molecules called aptamers, which have unique three-dimensional conformations allowing for highly specific and affine target binding [15], [16]. Compared to traditional ligands like antibodies, aptamers have several advantages including higher specificity, stability, affinity, and lower manufacturing costs [17].

In this paper, we propose the development of a mass-based aptasensor interface for specific, reliable, and real-time quantification of the cytokine biomarker TNF-α (schematic depiction given in Fig. 1). The biosensor comprises a self-

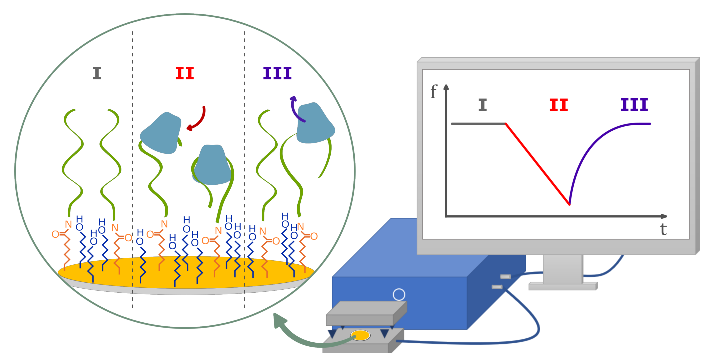

Fig. 1. Schematic depiction of the proposed QCM aptasensor measurement setup zooming in on the assembled sensing layer (I), TNF-α capturing (II), and aptamer regeneration (III).

Authorized licensed use limited to: TU Delft Library. Downloaded on November 29,2023 at 13:20:07 UTC from IEEE Xplore. Restrictions apply.

assembled monolayer (SAM) that governs the number of aptamers and the gap between them, and bound aptamers, in turn, are capable of selectively binding to the target analyte. Our biorecognition platform was immobilized on a quartz crystal microbalance (QCM), which detects changes in the resonance frequency of a thin quartz crystal due to alterations in the mass adhering to its surface [18]. The sensor assembly and target recognition were instantly detected by a decrease in frequency, which translates to mass by the Sauerbrey equation [19].

II. Materials and Methods

Characterization was conducted using a QE401 electronics unit with a QFM401 flow module as well as QCM QSX 301 gold chips from Biolin Scientific. The same preparation protocol was followed for all sensors performing the SAM formation outside the QCM flow module and all the subsequent steps inside. Acquired data were recorded via QSoft (Biolin Scientific) software and processed in Python. The sensor assembly protocol used in this study was adapted from a previously developed method by our group [16]. A SAM was created on a QCM chip gold surface by overnight incubation in a 5:95 mixture of $2\,\mathrm{mM}$ $HSC_{11}EG_6OCH_2COOH$ and $2\,\mathrm{mM}$ $HSC_{11}EG_5OH$ (ProChimia Surfaces) dissolved in ethanol (99.5 %; Sigma-Aldrich). After SAM formation, the QCM chip was introduced into the flow module, and a $100\,\mathrm{mM}$ 1:1 N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC): N-hydroxysuccinimide sodium salt (NHS) (Sigma-Aldrich) mixture in Phosphate Buffered Saline (PBS) was led over the chip to activate the carboxylic groups of the SAM forming an NHS ester. After a PBS washing step, the QCM flow module inlet tube was switched to a $1\,\mu\mathrm{M}$ FL11 aptamer (with the final sequence 3'-AATTAACCCTCACTAAAGGGTGGTGG ATGGCGCAGTCGGCGACAACTATAGTGTCACCTAAA TCGTA-5', containing an amine group modification at the 5' end and a thiol group modification at the 3' end, Merck). To ensure that no double-stranded aptamers were present, they were heated above their melting temperature of $85\,°\mathrm{C}$ for $15\,\mathrm{min}$ prior to use. After $30\,\mathrm{min}$ of flowing aptamer solution, the inlet tube is switched back to PBS concluding the sensor assembly.

For sensitivity measurements, the assembled sensor was exposed to TNF-α (Abcam) in PBS (concentration range $14.5\,\mathrm{nM}$ to $115.6\,\mathrm{nM}$). Specificity was tested by exposing the assembled sensor to $115\,\mathrm{nM}$ glucose in PBS (Sigma-Aldrich) and $115\,\mathrm{nM}$ Bovine Serum Albumin (BSA) in PBS (Sigma-Aldrich). Regeneration studies were conducted in terms of a $20\,\mathrm{min}$ washing step with MilliQ water at increased flow rates and subsequent comparison of TNF-α binding capability and PBS stabilization frequency before and after target exposure.

III. Results and Discussion

A. Validation of SAM formation and sensor assembly

The functionality of both the aptamers and the self-assembled monolayer (SAM) was examined using the Sauerbrey equation to correlate the frequency change observed in QCM resonance measurements to the amount of mass adsorbed onto the sensor surface [19]. Fig. 2 depicts the frequency shifts resulting from the different stages of the sensor preparation process and the subsequent incubation with TNF-α. The experimental procedure began with the formation of a self-assembled monolayer (SAM) containing 5 % -COOH and 95 % -OH end groups on a gold-coated QCM sensor. After its introduction to the flow chamber, the QCM chip with a SAM layer was immersed under a flow of PBS until a stable frequency was observed. The NHS:EDC mixture was led over the SAM layer (Fig. 2, orange). The EDC:NHS reacted with the -COOH groups of the SAM's linkers, resulting in the formation of an NHS ester and a drop in frequency (Fig. 2, orange). Following a short rinse step with PBS, the aptamer was incubated (Fig. 2, green), and the amine group at the 5' end reacted with the NHS ester, resulting in another frequency drop (approximately $12\,\mathrm{Hz}$). Based on the Sauerbrey equation, it is estimated that roughly 23 % of the -COOH groups coupled with the -NH_2 group of the FL11 aptamers (indicated by a calculated value of $2.50 \times 10^{12}\,\mathrm{molecules/cm^2}$ compared to the expected value of $1.09 \times 10^{13}\,\mathrm{molecules/cm^2}$ -COOH present on the surface). After a thorough rinse step with PBS, a solution of $58.6\,\mathrm{nM}$ TNF-α was introduced (Fig. 2, red). Due to diffusion-limited interaction of the target with the aptamer, a linear frequency decrease with a slope of $-0.1349\,\mathrm{Hz/min}$ (total frequency drop of $4\,\mathrm{Hz}$) was observed upon exposure to the sensor platform (Fig. 2, red). This suggests that 19 % of the aptamers have bound a target molecule ($8.36 \times 10^{11}\,\mathrm{molecules/cm^2}$). These results indicate the successful functionalization of the QCM chip with the FL11 aptamer and confirm the system's ability to bind TNF-α. To confirm the surface passivation by SAM and rule out non-specific binding of the aptamer's thiol functionality to the gold film, a SAM-modified QCM chip was exposed to aptamers without precedent EDC:NHS activation. In the absence of EDC:NHS activation, there was no discernible frequency decrease following aptamer exposure.

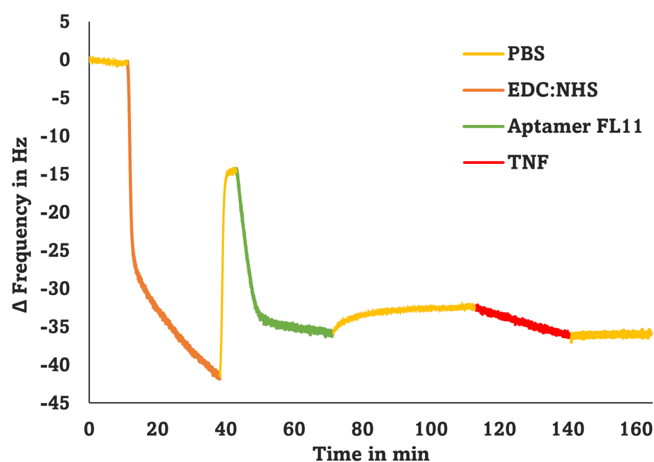

Fig. 2. Representative QCM frequency plot following the sensor assembly and the exposure of the assembled sensor to 58.6 nM TNF-α in PBS.

Authorized licensed use limited to: TU Delft Library. Downloaded on November 29,2023 at 13:20:07 UTC from IEEE Xplore. Restrictions apply.

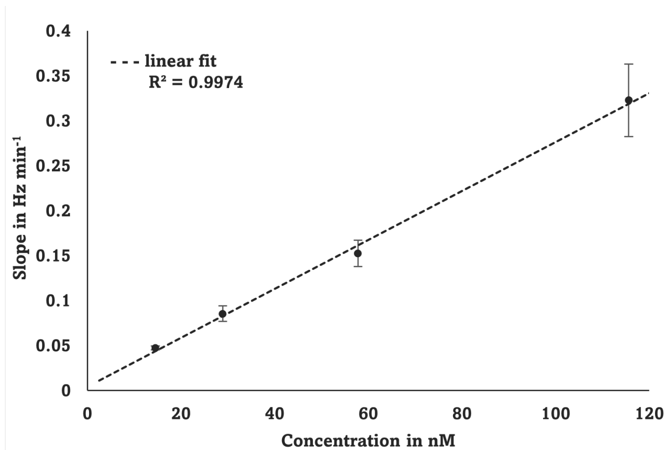

Fig. 3. Linear fit of averaged sensitivity measurement results: TNF-α concentration is plotted against the measured slope of frequency decrease

This observation implies that there was no substantial non-specific binding of the aptamer (-SH to the gold surface) when the activation step was omitted, indicating that the SAM provided complete coverage of the gold surface. Hence, it is likely that the aptamers only bind to the surface through amide binding on the active sites, as intended by our experimental design.

B. Analytical Sensitivity

To assess the reliability and potential of the developed aptasensor for accurate quantification of TNF-α in solution, five concentrations of TNF-α ranging from $14.5\,\mathrm{nM}$ and $115.6\,\mathrm{nM}$ were measured in triplicate. Fig. 3 represents the relation between the applied concentration of the target TNF-α and the measured slope of the frequency decrease as a fitted linear relation including SD. Assuming a constant distance between the surface and the bulk fluid, a linear relationship between the adsorption rate and the concentration of the target in the bulk can be postulated. Consequently, a doubling of the concentration of the target translates to a proportional doubling of its adsorption rate. The obtained results confirm the diffusion-limited model that was hypothesized and allow the linear correlation of the slope of frequency decrease and target concentration. The strong linearity demonstrates the high reliability of our sensor and hence shows the potential of the developed aptasensor for accurate quantification of TNF-α in solution (LOQ $14.5\,\mathrm{nM}$). With further optimization and fine-tuning of the aptamer concentration by controlling the SAM layer composition, the sensor platform can be expected to achieve even higher sensitivities towards TNF-α.

C. Specificity

The specificity of the proposed sensor was tested by exposing the sensing layer to two distinct competitor biomolecules, glucose ($115\,\mathrm{nM}$ in PBS) and BSA ($115\,\mathrm{nM}$ in PBS). Both competitor molecules were administered at the same concentration as the maximum tested TNF-α target concentration. Upon exposure, no significant decrease in frequency but slightly positive linear slopes coupled with high errors were observed for both competitor molecules. The acquired results indicate negligible adsorption of glucose or BSA on the sensor platform (glucose $0.001 \pm 0.059\,\mathrm{Hz/min}$; BSA $0.002 \pm 0.008\,\mathrm{Hz/min}$), thus validating the high specificity of the aptamer towards the target molecule.

D. Regeneration

The first regenerability test results of the sensing platform show partial washing off of TNF-α due to MilliQ water rinsing at a high flow rate, represented in the frequency increase of the PBS baseline before and after rinsing ($1.2 \pm 0.1\,\mathrm{Hz}$). Furthermore, the sensor's ability to rebind the target molecule was indicated by the linear frequency decrease upon repeated TNF-α ($28.9\,\mathrm{nM}$) incubation, albeit with decreased affinity ($-0.08\,\mathrm{Hz/min}$ before regeneration compared to $-0.05\,\mathrm{Hz/min}$ after regeneration). This simple, preliminary regeneration experiment indicates the potential for repeatability and reproducibility after regeneration. However, the observed frequency increase after regeneration with water was found to be only one-third of the expected, suggesting only partial removal of TNF-α. The optimization of the regeneration process will be investigated in the future, by following protocols with increased temperature, different buffer systems, or varied pH of the solvent as reported in other studies [20], [21].

IV. Conclusion and Outlook

In this study, we have demonstrated the quantification of TNF-α using a mass-sensitive, aptamer-based biosensor. The sensing platform exhibits a linear correlation between the frequency response and the concentration of TNF-α. Furthermore, upon exposure to competitor molecules, the sensor showed negligible responses confirming its high selectivity towards TNF-α. While first studies of the sensor's regenerability are promising, further research will be required to increase regeneration protocols' efficiency and enable real-time, continuous measurements. The development of simple and effective regeneration methods enables the acquisition of multiple measurements utilizing a single sensing device, a capability particularly advantageous for clinical settings. Future work will also focus on integrating the sensing platform in electrochemical or optical setups to enhance sensitivity. Further, integration in different read-out methods would also allow for investigating possible miniaturization of the system and integration in wearable devices to enable point-of-care sensors and their application in medical sensors. To summarize, the developed platform exhibits a high degree of specificity and reliability when detecting TNF-α, with the potential to increase sensitivity and regeneration capacity. The versatility of this platform lies in its ability to apply the sensing mechanism to other biomolecules by substituting the TNF-α aptamer with one that recognizes the target molecule, making it a promising and adaptable platform for various applications.

Authorized licensed use limited to: TU Delft Library. Downloaded on November 29,2023 at 13:20:07 UTC from IEEE Xplore. Restrictions apply.

REFERENCES

[1] J. Mondet, K. Hussein, and P. Mossuz, "Circulating cytokine levels as markers of inflammation in philadelphia negative myeloproliferative neoplasms: Diagnostic and prognostic interest," *Mediators of Inflammation*, vol. 2015, pp. 1–10, 10 2015.

[2] P. J. Barnes, "The cytokine network in chronic obstructive pulmonary disease," *American Journal of Respiratory Cell and Molecular Biology*, vol. 41, no. 6, pp. 631–638, 2009, pMID: 19717810. [Online]. Available: https://doi.org/10.1165/rcmb.2009-0220TR

[3] L. Barhoumi, F. Bellagambi, F. Vivaldi, A. Baraket, Y. Clément, N. Zine, M. Ben Ali, A. Elaissari, and A. Errachid, "Ultrasensitive immunosensor array for tnf-alpha detection in artificial saliva using polymer-coated magnetic microparticles onto screen-printed gold electrode," *Sensors*, vol. 19, no. 3, p. 692, Feb 2019. [Online]. Available: http://dx.doi.org/10.3390/s19030692

[4] E. Stern, A. Vacic, N. K. Rajan, J. M. Criscione, J. Park, B. R. Ilic, D. J. Mooney, M. A. Reed, and T. M. Fahmy, "Label-free biomarker detection from whole blood," *Nature Nanotechnology*, vol. 5, no. 2, pp. 138–142, 2010. [Online]. Available: https://doi.org/10.1038/nnano.2009.353

[5] W. Gao, S. Emaminejad, H. Y. Y. Nyein, S. Challa, K. Chen, A. Peck, H. M. Fahad, H. Ota, H. Shiraki, D. Kiriya, D.-H. Lien, G. A. Brooks, R. W. Davis, and A. Javey, "Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis," *Nature*, vol. 529, no. 7587, pp. 509–514, 2016. [Online]. Available: https://doi.org/10.1038/nature16521

[6] E. W. Orava, N. Jarvik, Y. L. Shek, S. S. Sidhu, and J. Gariépy, "A short dna aptamer that recognizes tnf alpha and blocks its activity in vitro," *ACS Chemical Biology*, vol. 8, no. 1, pp. 170–178, 2013, pMID: 23046187. [Online]. Available: https://doi.org/10.1021/cb3003557

[7] R. Caporali, F. B. Pallavicini, M. Filippini, R. Gorla, A. Marchesoni, E. G. Favalli, P. Sarzi-Puttini, F. Atzeni, and C. Montecucco, "Treatment of rheumatoid arthritis with anti-tnf-alpha agents: A reappraisal," *Autoimmunity Reviews*, vol. 8, no. 3, pp. 274–280, 2009, special Issue on Signal Processing by Immune System in SLE. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S1568997208002218

[8] S. O. Adegbola, K. Sahnan, J. Warusavitarne, A. Hart, and P. Tozer, "Anti-tnf therapy in crohn's disease," *International Journal of Molecular Sciences*, vol. 19, no. 8, p. 2244, Jul 2018. [Online]. Available: http://dx.doi.org/10.3390/ijms19082244

[9] B. Jagannath, K.-C. Lin, M. Pali, D. Sankhala, S. Muthukumar, and S. Prasad, "Temporal profiling of cytokines in passively expressed sweat for detection of infection using wearable device," *Bioengineering & Translational Medicine*, vol. 6, no. 3, p. e10220, 2021. [Online]. Available: https://aiche.onlinelibrary.wiley.com/doi/abs/10.1002/btm2.10220

[10] Z. Hao, Z. Wang, Y. Li, Y. Zhu, X. Wang, C. G. De Moraes, Y. Pan, X. Zhao, and Q. Lin, "Measurement of cytokine biomarkers using an aptamer-based affinity graphene nanosensor on a flexible substrate toward wearable applications," *Nanoscale*, vol. 10, pp. 21 681–21 688, 2018. [Online]. Available: http://dx.doi.org/10.1039/C8NR04315A

[11] C. Parolo, A. Idili, G. Ortega, A. Csordas, A. Hsu, N. Arroyo-Currás, Q. Yang, B. S. Ferguson, J. Wang, and K. W. Plaxco, "Real-time monitoring of a protein biomarker," *ACS Sensors*, vol. 5, no. 7, pp. 1877–1881, 2020, pMID: 32619092. [Online]. Available: https://doi.org/10.1021/acssensors.0c01085

[12] Y. Park, B. Ryu, B.-R. Oh, Y. Song, X. Liang, and K. Kurabayashi, "Biotunable nanoplasmonic filter on few-layer mos2 for rapid and highly sensitive cytokine optoelectronic immunosensing," *ACS Nano*, vol. 11, no. 6, pp. 5697–5705, 2017, pMID: 28489942. [Online]. Available: https://doi.org/10.1021/acsnano.7b01162

[13] J. Dong and H. Ueda, "Elisa-type assays of trace biomarkers using microfluidic methods," *WIREs Nanomedicine and Nanobiotechnology*, vol. 9, no. 5, p. e1457, 2017, e1457 NANOMED-461.R2. [Online]. Available: https://wires.onlinelibrary.wiley.com/doi/abs/10.1002/wnan.1457

[14] S. Song, L. Wang, J. Li, C. Fan, and J. Zhao, "Aptamer-based biosensors," *TrAC Trends in Analytical Chemistry*, vol. 27, no. 2, pp. 108–117, 2008. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0165993607002658

[15] M. Famulok, G. Mayer, and M. Blind, "Nucleic acid aptamersfrom selection in vitro to applications in vivo," *Accounts of Chemical Research*, vol. 33, no. 9, pp. 591–599, 2000, pMID: 10995196. [Online]. Available: https://doi.org/10.1021/ar960167q

[16] P. Zhu, V. A. Papadimitriou, J. E. van Dongen, J. Cordeiro, Y. Neeleman, A. Santoso, S. Chen, J. C. Eijkel, H. Peng, L. I. Segerink, and A. Y. Rwei, "An optical aptasensor for real-time quantification of endotoxin: From ensemble to single-molecule resolution," *Science Advances*, vol. 9, no. 6, p. eadf5509, 2023. [Online]. Available: https://www.science.org/doi/abs/10.1126/sciadv.adf5509

[17] H. Yoo, H. Jo, and S. S. Oh, "Detection and beyond: challenges and advances in aptamer-based biosensors," *Mater. Adv.*, vol. 1, pp. 2663–2687, 2020. [Online]. Available: http://dx.doi.org/10.1039/D0MA00639D

[18] S. Kurosawa, J.-W. Park, H. Aizawa, S.-I. Wakida, H. Tao, and K. Ishihara, "Quartz crystal microbalance immunosensors for environmental monitoring," *Biosensors and Bioelectronics*, vol. 22, no. 4, pp. 473–481, 2006, selected Papers from the 2nd International Meeting on Microsensors and Microsystems. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0956566306003010

[19] H. J. Lim, T. Saha, B. T. Tey, W. S. Tan, and C. W. Ooi, "Quartz crystal microbalance-based biosensors as rapid diagnostic devices for infectious diseases," *Biosensors and Bioelectronics*, vol. 168, p. 112513, 2020. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0956566320305054

[20] Y. Liu, J. Yan, M. C. Howland, T. Kwa, and A. Revzin, "Micropatterned aptasensors for continuous monitoring of cytokine release from human leukocytes," *Analytical Chemistry*, vol. 83, no. 21, pp. 8286–8292, 2011, pMID: 21942846.

[21] W. Su, M. Lin, H. Lee, M. Cho, W.-S. Choe, and Y. Lee, "Determination of endotoxin through an aptamer-based impedance biosensor," *Biosensors and Bioelectronics*, vol. 32, no. 1, pp. 32–36, 2012. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0956566311007536

Authorized licensed use limited to: TU Delft Library. Downloaded on November 29,2023 at 13:20:07 UTC from IEEE Xplore. Restrictions apply.