

Advances in Protein Biosensors for Medical Diagnostics: Technologies, Challenges, and Applications

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Abstract: Protein assays play an important role in medical diagnostics and can be used for early disease screening. Traditional protein detection methods (e.g., Enzyme-linked immunosorbent assay (ELISA), Mass Spectrometry (MS), etc.) usually have limitations such as high cost, long detection time, and complex operation, which limit their application in prompt oncology testing (POCT) and mass screening. In recent years, biosensors have become an important development direction for protein detection technology due to their high sensitivity, rapid detection, portability, and real-time monitoring capabilities. This article reviews the research progress of biosensors in protein detection in recent years, focusing on the innovative applications of key technologies such as nanomaterials, highly selective biorecognition elements and intelligent and integrated biosensors. The article also analyses the advantages and challenges of current biosensors in medical diagnostics, including issues such as detection stability, anti-interference ability and compatibility of sensing platforms. Finally, the article looks into the future development trend of protein biosensors, such as the development of novel nanomaterials and biorecognition elements, multi-target and high-throughput detection and wearable smart biosensors, to promote the application in early disease screening.

Keywords: Biosensors, Protein Detection, Medical Diagnostics, Nanomaterials.

1. Introduction

The precise detection of proteins, as core targets for drug discovery, disease diagnosis and life science research, is critical to advancing precision medicine and health monitoring. For example, early cancer screening relies on the detection of very low abundance markers (e.g., CEA, AFP, PSA), and the detection of viral and infectious disease markers can provide critical data for disease management as well as the monitoring of viral and infectious diseases. However, traditional detection technologies (e.g. mass spectrometry, ELISA) are limited by high cost, complexity and static mode of detection, which make it difficult to meet the needs of prompt oncology testing (POCT), dynamic tracking and complex sample analysis. This technological barrier has spawned the rapid development of the biosensor field - through the integration of biology, nanotechnology and information science, it has the advantages of high sensitivity, high specificity, can be detected in real time and rapid response, etc., and has become the most transformative research direction in the field of protein detection.

2. Basic principles and classification of biosensors

2.1. Definition and basic components of biosensors

A biosensor is a device that converts a biometric event into a measurable signal, typically consisting of a biometric element, a transducer, and a signal processing system:

Biorecognition elements (e.g., antibodies, aptamers, molecularly imprinted polymers, etc.) are responsible for the specific binding of target analytes. For example, antibodies selectively capture cancer markers through antigen-antibody reactions [1].

Transducers (e.g. gold nanoparticles, surface plasmon resonance devices, optical fibers, etc.) [1,2] convert biometric events into optical, electrochemical or mechanical signals.

The signal processing system amplifies, analyses and displays the signal output from the transducer. For example, the LSPR peak displacement is detected by UV-Vis spectroscopy [1].

2.2. Classification of biosensors

Depending on the recognition element and signal conversion method, biosensors mainly include the following types:

2.2.1. Electrochemical biosensors

Electrochemical biosensors can be divided into three types. The first type is potentiometric sensors: Determine the target protein by detecting changes in potential in solution, such as ion-selective electrodes[3].

The second type is amperometric sensors: Detection by change in current based on the reaction of the target protein with an enzyme or other electroactive substance [3].

The third type is impedance-type sensors: analyses protein binding or conformational changes by measuring changes in the impedance of the electrode interface [3].

2.2.2. Optical biosensors

Optical biosensors can also be divided into three types. The first is surface plasmon resonance sensors: detect protein interactions by monitoring changes in light reflection on metal surfaces [3].

The second type is fluorescent sensors: detection of proteins using fluorescent labelling or fluorescence resonance energy transfer (FRET) [3].

The third type is colorimetric sensors: Protein detection using nanomaterials (e.g. gold nanoparticles) or enzymatic reactions to produce color changes [3].

2.2.3. Mechanical biosensors

Mechanical biosensors can be divided into two types. The first type is quartz crystal microbalance: detection of protein adsorption by measuring resonance frequency changes [4]. The second type is microcantilever beam sensors: when a protein binds to the surface of the sensor, it causes bending or vibrational changes in the microcantilever beam, thus enabling detection [5].

2.2.4. Thermal biosensors

Calorimetric sensors: analyzed by detecting changes in the amount of heat released or absorbed when a target protein binds to a recognition molecule (e.g. an antibody or a receptor) [6].

3. Key technological advances in biosensors for protein detection

3.1. Application of nanomaterials in biosensors

Nanomaterials significantly enhance the sensitivity and specificity of electrochemical sensors by increasing electrode surface area, catalytic activity and biocompatibility [7]. Carbon nanotubes (SWCNT) are ideal materials for biosensors due to their high electrical conductivity, large specific surface area, and environmental sensitivity, and can be used to achieve label-free detection through the electroosmotic effect (antigen-antibody binding disrupts the conductive network, leading to a rise in electrical resistance), for example, for the rapid and cost-effective detection of bovine serum proteins [8]. The other example is gold nanoparticles (AuNPs), quantum dots (QDs), graphene, etc., which can amplify the signal and thus enhance the sensitivity (e.g., thrombin detection limit up to 0.2 pM) [9].

3.2. Development of highly selective biometric elements

Aptamer sensors are characterized by high specificity, stability, ease of synthesis and low cost [3]. Molecularly imprinted polymers are also highly selective for the measurement of potential changes induced by target binding achieved by binding to rough gold nanofilms [7]. Antibodies, aptamers, molecularly imprinted polymers, and other highly specific recognition elements can be combined with CRISPR technology to amplify the signal and improve the sensitivity and specificity of detection [10-12].

3.3. Intelligent and integrated biosensors

Microfluidic chip integrates electrodes and fluid control for better control of fluid volume on a tiny scale and can also be combined with nanomaterials for simultaneous detection of multiple viruses [13]. The combination of microfluidic chip with Natural Language Processing (NLP) techniques can break through the limitations of traditional analyses based on structural or sequence similarity and achieve efficient mapping from sequence to function [14].

4. Application of biosensors in protein detection

Early detection of cancer-associated proteins (e.g. CEA, AFP, PSA) is crucial for diagnosis and prognosis [9] can be performed using AuPt NWs/THI/rGO sensor for CEA with low detection limit of 6 fg/ml [15]. Early detection and diagnosis of AFP is helpful for the treatment of hepatocellular carcinoma [16], which can be detected using Au@Pt dendritic nanorods/MoSe₂ composites with a low limit of detection of 3.3 fg/mL [17]. PSA, a biomarker of prostate cancer, can be detected using a wearable sensor based on a pleated gold membrane with a low limit of detection of 0.38 fg/mL and a linear range of 0.5 fg/mL [LU, W large]. with a low detection limit of 0.38 fg/mL and a linear range spanning six orders of magnitude [18].

Biosensors also have great application in detecting viral infectious diseases. For example, Au nanorods/g-C₃N₄ sensors can be used to detect NS1 protein, a biomarker for dengue fever [19]. Reduced graphene oxide (rGO) FETs can also be used to directly detect inactivated viruses for the detection of Ebola virus [20]. Quantum dot labelled sensors for COVID-19 detection can achieve a rapid detection in 30 seconds with an accuracy of up to 90% [21].

5. Discussion

In the field of nanomaterials and biorecognition components, new nanomaterials that are more biocompatible can be developed to improve the sensitivity and biosafety of biosensors [7] and to

develop more accurate sensors. For example, DNAzyme, which can be used in precision medicine due to its unique catalytic and programmable properties, but there is still a need to develop new DNAzyme in the direction of in vitro screening, such as targeting of non-metallic or biomolecules [22]. There is also a need to develop multi-channel biosensors for simultaneous detection of multiple protein markers to further improve efficiency. Developments in intelligence are also particularly important for biosensors, with the development of AI-driven data analysis to improve the automation of detection [14]. The development of wearable biosensors suitable for continuous monitoring, such as protein detection in sweat, tears and saliva and combined with smartphones, wireless sensing technology can achieve real-time telemedicine monitoring.

6. Conclusion

In recent years, the rapid development of biosensors, through the combination of nanomaterials to achieve a variety of viral markers detection and the use of nanomaterials electrodes with a large surface area, catalytic activity and biocompatibility and other characteristics of electrochemical sensors to improve the sensitivity and specificity. In terms of intelligence and integration, the microfluidic chip integrates electrodes and fluidic control, effectively reducing reagent consumption, lowering costs and shortening detection time. Accurate screening of proteins for specific functions by structure can be achieved using Natural Language Processing (NLP) technology.

However, in complex samples (e.g. saliva, blood) the mechanism of interference will reduce the specificity of the detection, and the stability and reproducibility of the biosensor still need to be further improved to adapt to extreme environments or multi-testing. In addition, the development of multi-target simultaneous detection and portable detection devices puts high demands on the compatibility of sensing platforms, and solutions to these problems may also be found in the development of multidisciplinary cross-disciplinary development, such as the use of biomimetic materials to enhance the ability of anti-jamming, through the combination of CRISPR technology to achieve multi-target simultaneous detection.

With the potential for high specificity, fast response and miniaturization, and through the integration of nanotechnology, artificial intelligence and bioengineering, the next generation of sensors will push the boundaries of performance and revolutionize precision medicine and health monitoring. The rise of biosensors in the field of protein detection marks a shift in bioanalytical technology from "static detection in vitro" to "dynamic sensing in vivo". However, technological breakthroughs need to be deeply integrated with clinical needs, and stability, accessibility and ethical issues need to be addressed through interdisciplinary collaboration. Future research should abandon the "sensitivity-only theory", and instead build scenario-adapted, user-friendly and socially acceptable solutions to truly realize the technological leap from the laboratory to the bedside.

References

- [1] Tai, J., Fan, S., Ding, S., & Ren, L. (2022). Gold Nanoparticles Based Optical Biosensors for Cancer Biomarker Proteins: A Review of the Current Practices. *Frontiers in bioengineering and biotechnology*, 10, 877193. <https://doi.org/10.3389/fbioe.2022.877193>
- [2] Peltomaa, R., Glahn-Martínez, B., Benito-Peña, E., & Moreno-Bondi, M. C. (2018). Optical Biosensors for Label-Free Detection of Small Molecules. *Sensors (Basel, Switzerland)*, 18(12), 4126. <https://doi.org/10.3390/s18124126>
- [3] Lou, B., Liu, Y., Shi, M., Chen, J., Li, K., Tan, Y., Chen, L., Wu, Y., Wang, T., Liu, X., Jiang, T., Peng, D., & Liu, Z. (2022). Aptamer-based biosensors for virus protein detection. *Trends in analytical chemistry : TRAC*, 157, 116738. <https://doi.org/10.1016/j.trac.2022.116738>
- [4] Lim, H. J., Saha, T., Tey, B. T., Tan, W. S., & Ooi, C. W. (2020). Quartz crystal microbalance-based biosensors as rapid diagnostic devices for infectious diseases. *Biosensors & bioelectronics*, 168, 112513. <https://doi.org/10.1016/j.bios.2020.112513>

- [5] Hansen, K. M., & Thundat, T. (2005). Microcantilever biosensors. *Methods* (San Diego, Calif.), 37(1), 57–64. <https://doi.org/10.1016/j.ymeth.2005.05.011>
- [6] Alodhayb A. (2019). Modeling of an Optically Heated MEMS-Based Micromechanical Bimaterial Sensor for Heat Capacitance Measurements of Single Biological Cells. *Sensors* (Basel, Switzerland), 20(1), 215. <https://doi.org/10.3390/s20010215>
- [7] Dong, T., Matos Pires, N. M., Yang, Z., & Jiang, Z. (2023). Advances in Electrochemical Biosensors Based on Nanomaterials for Protein Biomarker Detection in Saliva. *Advanced science* (Weinheim, Baden-Wurttemberg, Germany), 10(6), e2205429. <https://doi.org/10.1002/adv.202205429>
- [8] Janssen, J., Lambeta, M., White, P., & Byagowi, A. (2019). Carbon Nanotube-Based Electrochemical Biosensor for Label-Free Protein Detection. *Biosensors*, 9(4), 144. <https://doi.org/10.3390/bios9040144>
- [9] Meirinho, S. G., Dias, L. G., Peres, A. M., & Rodrigues, L. R. (2016). Voltammetric aptasensors for protein diseases biomarkers detection: A review. *Biotechnology advances*, 34(5), 941–953. <https://doi.org/10.1016/j.biotechadv.2016.05.006>
- [10] Joung, J., Ladha, A., Saito, M., Kim, N. G., Woolley, A. E., Segel, M., Barretto, R. P. J., Ranu, A., Macrae, R. K., Faure, G., Ioannidi, E. I., Krajewski, R. N., Bruneau, R., Huang, M. W., Yu, X. G., Li, J. Z., Walker, B. D., Hung, D. T., Greninger, A. L., Jerome, K. R., ... Zhang, F. (2020). Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *The New England journal of medicine*, 383(15), 1492–1494. <https://doi.org/10.1056/NEJMc2026172>
- [11] Zuckermann, M., Kawauchi, D., & Gronych, J. (2017). Applications of the CRISPR/Cas9 system in murine cancer modeling. *Briefings in functional genomics*, 16(1), 25–33. <https://doi.org/10.1093/bfpg/ew021>
- [12] Lee, M. H., Thomas, J. L., Lin, C. Y., Li, Y. E., & Lin, H. Y. (2023). Activation of Insulin Gene Expression via Transfection of a CRISPR/dCas9a System Using Magnetic Peptide-Imprinted Nanoparticles. *Pharmaceutics*, 15(4), 1311. <https://doi.org/10.3390/pharmaceutics15041311>
- [13] Kraiem, H., Zouari, F., Abderrazek, R. B., Manon, Y., Ayeb, M. E., Fillaudeau, L., Bedoui, J., & Bouhaouala-Zahar, B. (2017). Two-Dimensional Isoelectric Focusing OFFGEL, Micro-Fluidic Lab-on-Chip Electrophoresis and FTIR for Assessment of Long-Term Stability of rhG-CSF Formulation. *IEEE transactions on nanobioscience*, 16(8), 694–702. <https://doi.org/10.1109/TNB.2017.2763779>
- [14] Ofer, D., Brandes, N., & Linial, M. (2021). The language of proteins: NLP, machine learning & protein sequences. *Computational and structural biotechnology journal*, 19, 1750–1758. <https://doi.org/10.1016/j.csbj.2021.03.022>
- [15] Lu, W. et al. Large-scale synthesis of ultrathin Au-Pt nanowires assembled on thionine/graphene with high conductivity and sensitivity for electrochemical immunosensor. *Electrochim. Acta* 130, 335–343 (2014).
- [16] Jiang, L. et al. A sandwich-type electrochemical immunosensor based on multiple signal amplification for -fetoprotein labeled by platinum hybrid multiwalled carbon nanotubes adhered copper oxide. *Electrochim. Acta* 160, 714 (2015).
- [17] Zhang, S., Zhang, C., Jia, Y., Zhang, X., Dong, Y., Li, X., Liu, Q., Li, Y., & Zhao, Z. (2019). Sandwich-type electrochemical immunosensor based on Au@Pt DNRs/NH₂-MoSe₂ NSs nanocomposite as signal amplifiers for the sensitive detection of alpha-fetoprotein. *Bioelectrochemistry* (Amsterdam, Netherlands), 128, 140–147. <https://doi.org/10.1016/j.bioelechem.2019.03.012>
- [18] Guo, L., Zhao, Y., Huang, Q., Huang, J., Tao, Y., Chen, J., Li, H. Y., & Liu, H. (2024). Electrochemical protein biosensors for disease marker detection: progress and opportunities. *Microsystems & nanoengineering*, 10, 65. <https://doi.org/10.1038/s41378-024-00700-w>
- [19] Ojha, R. P., Singh, P., Azad, U. P., & Prakash, R. (2022). Impedimetric immunosensor for the NS1 dengue biomarker based on the gold nanorod decorated graphitic carbon nitride modified electrode. *Electrochimica Acta*, 411, 140069.
- [20] Jin, X., Zhang, H., Li, Y. T., Xiao, M. M., Zhang, Z. L., Pang, D. W., Wong, G., Zhang, Z. Y., & Zhang, G. J. (2019). A field effect transistor modified with reduced graphene oxide for immunodetection of Ebola virus. *Mikrochimica acta*, 186(4), 223. <https://doi.org/10.1007/s00604-019-3256-5>
- [21] Zhao, Y., Chen, J., Hu, Z., Chen, Y., Tao, Y., Wang, L., Li, L., Wang, P., Li, H. Y., Zhang, J., Tang, J., & Liu, H. (2022). All-solid-state SARS-CoV-2 protein biosensor employing colloidal quantum dots-modified electrode. *Biosensors & bioelectronics*, 202, 113974. <https://doi.org/10.1016/j.bios.2022.113974>
- [22] Gong, L., Zhao, Z., Lv, Y. F., Huan, S. Y., Fu, T., Zhang, X. B., Shen, G. L., & Yu, R. Q. (2015). DNzyme-based biosensors and nanodevices. *Chemical communications* (Cambridge, England), 51(6), 979–995. <https://doi.org/10.1039/c4cc06855f>