Revolutionizing Chemical Design and Immobilization Techniques:

Chemistry at the Interface of Nanomaterials and Bioaffinity Binders for Point-of-Care Biosensors

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https://doi.org/10.51167/acm00071

The rapid strides achieved in nanomaterial synthesis and bioaffinity binder development have propelled the evolution of point-of-care biosensors, redefining healthcare and environmental monitoring. These biosensors have emerged as potent diagnostic tools, enabling real-time, precise detection of biomolecules and environmental analytes at critical junctures. The confluence of nanomaterials and bioaffinity binders, as alternatives to conventional antibodies, holds immense transformative potential for biosensing. This section delves into the forefront of advancements at the nanomaterial-bioaffinity binder interface, shedding light on cutting-edge innovations that catalyze the enhancement of point-of-care biosensor technology. With an active involvement in nanomaterial synthesis and portable biosensor development, research endeavors are firmly rooted in the fundamental principles of chemistry, forging pathways towards pioneering solutions that amplify biosensing capabilities.

1. Introduction

Point-of-care (POC) biosensors have garnered immense attention in recent years due to their potential to bring healthcare closer to the patient, obviating the need for centralized laboratory testing, and reducing the time required for diagnosis and treatment initiation. These portable devices offer a unique advantage in resource-limited settings, remote locations, and emergency situations, where prompt and accurate results can be life-saving. Additionally, they hold promise in environmental monitoring, enabling rapid detection of pollutants and facilitating timely interventions for pollution control and management.

The realm of POC biosensors has witnessed a profound transformation with the advent of nanomaterials. These materials, characterized by their nanoscale dimensions, have demonstrated remarkable physicochemical properties that enable unprecedented advancements in biosensing technologies. Nanoparticles, quantum dots, and polymeric nanomaterials have emerged as key players in this domain, revolutionizing sensor design and performance. Harnessing the unique properties of nanomaterials at the interface of bioaffinity binders has led to enhanced sensitivity, selectivity, and response time, making them instrumental in real-time diagnostics at the point of need.

Amidst the array of bioaffinity binders, traditional antibodies have long dominated biosensing applications, yet their limitations in stability, production cost, and size have spurred a quest for alternatives. The synthetic bioaffinity binders, such as abdurins, affitins, affibodies, affimers, anticalins, bicyclic peptides, DARPins, Fynomers, Kunitz domains, monobodies, and aptamers, have emerged as promising substitutes to antibodies. The distinct characteristics and advantages of these novel binders, including their efficient immobilization on nanomaterials, are expounded, underscoring their potential in revolutionizing POC biosensors. With an emphasis on chemical design, the rational design of bioaffinity binders for specific nanomaterials, optimizing their interactions at the interface and ensuring the efficient immobilization of binders on nanomaterial surfaces guarantee the performance of the resulted biosensor. State-of-the-art molecular simulations and computational tools augment these design strategies, providing invaluable insights into binder-nanomaterial interactions, which, in turn, influence sensor performance. Critical to the successful functioning of POC biosensors, the various immobilization techniques can be employed at the nanomaterial-bioaffinity binder interface. Physical immobilization methods, such as adsorption, encapsulation, and layer-by-layer assembly, are examined alongside chemical immobilization methods, including covalent bonding, click chemistry, and self-assembled monolayers. The inherent advantages and challenges of each method are meticulously evaluated, paving the way for future improvements in biosensor design and performance.

As we explore the interface of nanomaterials and bioaffinity binders, this article amalgamates knowledge from diverse disciplines, including chemistry, nanotechnology, materials science, and biotechnology. With a firm focus on the applications of these biosensors, the article highlights their potential in disease diagnosis, environmental monitoring, therapeutic drug monitoring, and personalized medicine. We shed light on recent advancements in miniaturization and portability, which enable field-deployable biosensors, poised to enhance healthcare accessibility across the globe. The prospective future outlook and challenges in this multidisciplinary domain mark the concluding sections of the article. From the emergence of wearable and implantable biosensors to regulatory and commercial hurdles, we underscore the need for continuous research and collaboration to fully harness the transformative potential of chemistry at the interface of nanomaterials and bioaffinity binders for POC biosensors.

2. Nanomaterials for POC Biosensors

2.1 Types of Nanomaterials Used in Biosensors

The versatility of nanomaterials stems from their diverse composition and properties, enabling their application in various biosensing scenarios[1]. Nanoparticles, encompassing metal nanoparticles, quantum dots, and metal oxide nanoparticles, offer exceptional optical and magnetic characteristics, making them ideal for signal amplification and label-free sensing strategies. Polymeric nanomaterials, including synthetic and biopolymers, have garnered attention for their facile functionalization and bioconjugation, facilitating targeted and specific biosensing applications. Quantum dots, with their tunable fluorescence emission, have emerged as valuable nanoprobes for bioimaging and multiplexed detection. The selection of nanomaterials in biosensor design is tailored to the specific needs of the target analyte and the intended application. Moreover, the strategic integration of nanomaterials in biosensor design bestows numerous advantages, propelling the field of POC diagnostics. The amplified surface area-to-volume ratio of nanomaterials facilitates efficient bioaffinity binder immobilization, maximizing the available binding sites for target recognition.

2.2 Synthesis Methods for Nanomaterials

The synthesis of nanomaterials is a critical aspect of biosensor development, as it dictates their size, shape, and surface properties. Researchers employ a range of synthesis methods to fine-tune nanomaterials for optimal biosensing performance[2]. Physical immobilization methods, such as adsorption and encapsulation, facilitate the integration of nanomaterials onto sensor surfaces, offering ease of implementation and versatility3-5. Meanwhile, chemical immobilization techniques, including covalent bonding, click chemistry, and self-assembled monolayers (SAMs), ensure robust and stable nanomaterial-bioaffinity binder interactions6. The choice of synthesis method significantly influences the sensor's sensitivity, stability, and ability to detect target analytes efficiently.

3. Bioaffinity Binders as Alternatives to Antibodies

Traditional antibodies have long been the cornerstone of biosensing applications, offering exceptional target specificity and affinity. However, their application in POC biosensors presents certain challenges that have spurred the exploration of alternative bioaffinity binders. The production of antibodies is a complex and time-consuming process, often involving the use of animals for immunization. This not only leads to batch-to-batch variability but also results in high production costs and ethical considerations. Additionally, the large size of antibodies may hinder their penetration into certain biological matrices, limiting their applications in certain diagnostic scenarios. The search for alternative bioaffinity binders with comparable or superior characteristics has been a focal point of research in the quest for innovative biosensing technologies.

In the quest for innovative bioaffinity binders, researchers have ventured beyond traditional antibodies, exploring a diverse array of engineered proteins. These alternative binders offer unique advantages, such as smaller size, enhanced stability, and reduced immunogenicity, making them promising candidates for nanomaterial-bioaffinity binder convergence in POC biosensors (Figure 1). In this section, we explore several examples of alternative bioaffinity binders, showcasing their diverse properties and applications in biosensing technologies.

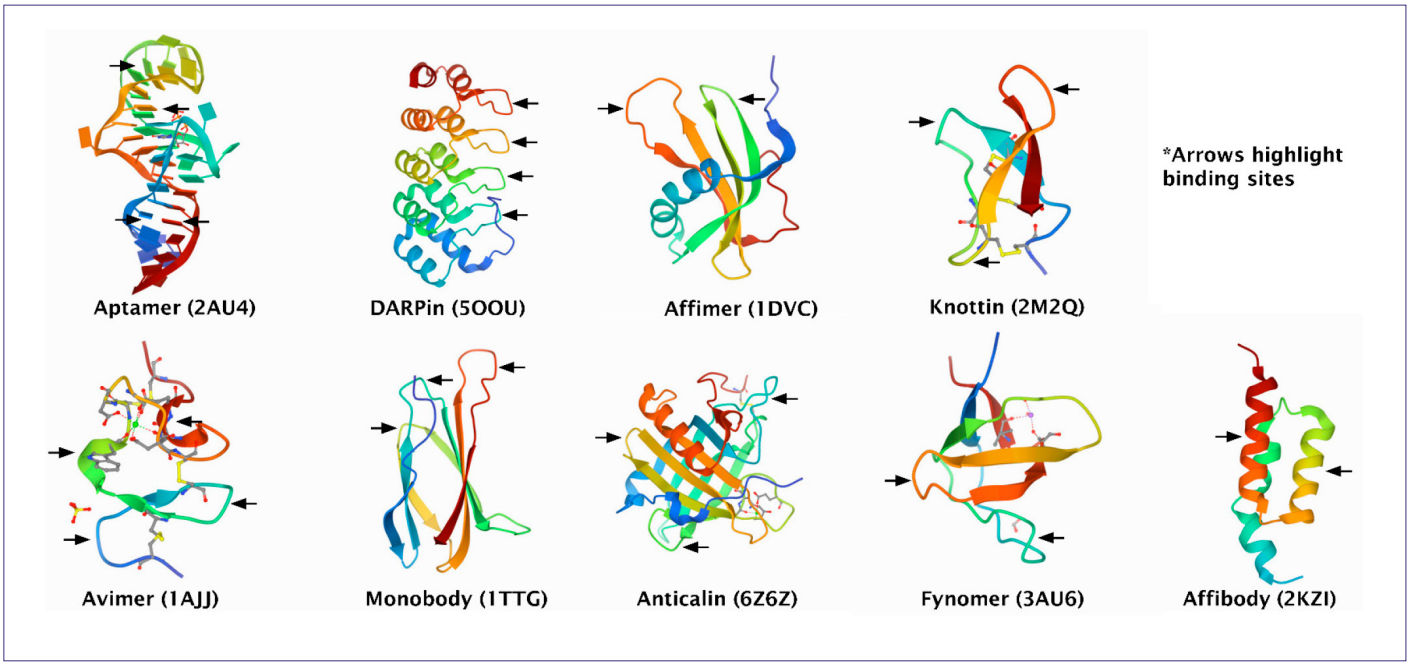

Figure 1. Crystal structures of non-antibody binders. Obtained from Protein Data Bank, codes enclosed in brackets (PDB, http://www.rcsb.org/ accessed on 11 August 2023)[7]. ©2021 The authors and published under open access license by MDPI.

3.1 Abdurins: A Fusion of Camelid-derived Nanobodies

Abdurins represent an exciting class of alternative bioaffinity binders that combine the unique properties of camelid-derived nanobodies with additional functionalities. Derived from the heavy-chain antibodies found in camelids, nanobodies are small, single-domain antibodies known for their remarkable stability and binding affinity to target antigens (Figure 2)[8, 9]. Abdurins take advantage of these inherent properties and fuse them with other functional domains, resulting in versatile and potent binding proteins.

The fusion of nanobodies with additional domains allows Abdurins to expand their applications beyond traditional antibodies. These engineered proteins exhibit enhanced characteristics, such as increased thermal stability, facile engineering for target specificity, and reduced immunogenicity, making them attractive candidates for various biosensing and therapeutic applications.

3.2 Affitins: Versatile and Stable Scaffold Proteins

Affitins are derived from the Sac7d protein scaffold, they are small, engineered binding proteins known for their exceptional thermal stability and robust performance under diverse environmental conditions [10, 11]. The small size and stability of Affitins allow for efficient production and customization for specific target recognition (Figure 3). Affitins can be engineered to bind with high affinity and specificity to a wide range of targets, making them valuable tools for diagnostics, therapeutics, and molecular imaging. Their compact structure also ensures minimal steric hindrance, promoting efficient target binding kinetics and enhancing biosensor sensitivity.

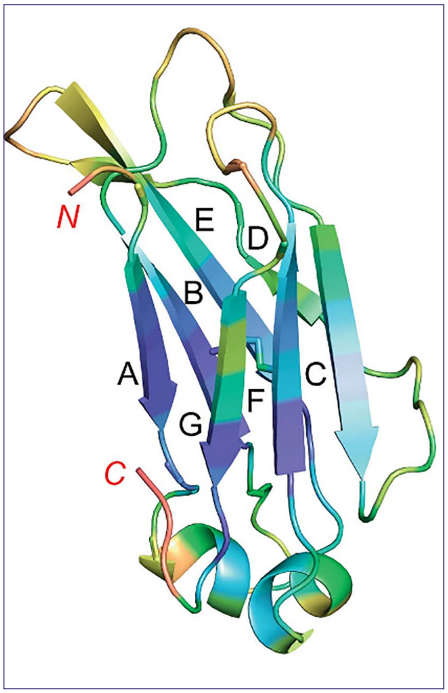

Figure 2. The configuration of the single-domain antibodies. Reproduced with permission[8]. ©2008 International Union of Crystallography.

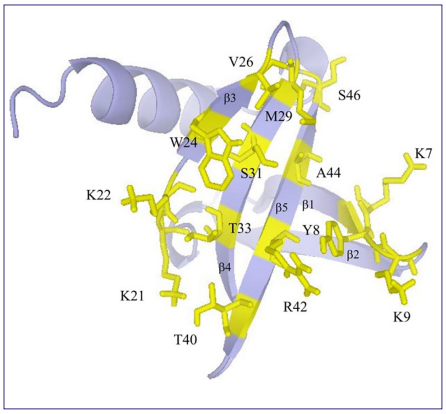

Figure 3. Illustrative depiction of wild-type Sac7d (PDB:1AZP). Notable amino acids for DNA binding and substitution are highlighted in yellow.

3.3 Affibodies: Engineered Protein Domains

Affibodies are a class of engineered protein domains derived from the Z domain of staphylococcal protein A (Figure 4)[12]. These small and stable protein binders offer a unique and versatile alternative to traditional antibodies for biosensing applications. Affibodies are engineered to bind with high affinity and specificity to a wide range of target molecules, making them valuable tools in POC biosensors. The compact structure of Affibodies allows for efficient synthesis and customization, enabling researchers to tailor their binding properties for specific analytes of interest. One of the key advantages of Affibodies lies in their reduced immunogenicity compared to traditional antibodies.

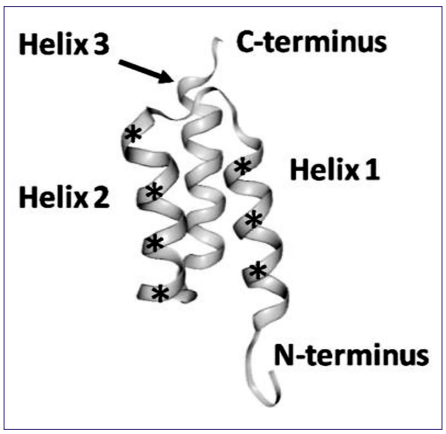

Figure 4. Structural presentation of an affibody scaffold. Asterisks indicate regions with randomized amino acids12. ©2020 The authors and published under open access license by MDPI.

Their non-immunoglobulin scaffold minimizes potential immune responses, making them ideal for repeated or long-term use in biosensing and therapeutic applications.

3.4 Affimers: Customizable Protein Binders

Affimers are a modular and customizable class of protein binders that have garnered significant attention in various biomedical fields[13]. The modular design of Affimers allows for the incorporation of various binding loops, enabling specific target recognition (Figure 5). Researchers can select and optimize these loops to achieve the desired binding affinity and specificity, making Affimers a versatile platform for diagnostic and therapeutic purposes. Their small size also ensures minimal steric hindrance, facilitating efficient interactions with target molecules and enhancing the sensitivity of biosensors.

Furthermore, Affimers' customizable nature offers great potential for multiplexing, enabling the simultaneous detection of multiple analytes in a single biosensor platform. This capability is particularly valuable in POC diagnostics, where the rapid and accurate detection of multiple disease biomarkers can provide comprehensive clinical information.

3.5 Anticalins: Lipocalin-derived Binders

Anticalins are a class of engineered protein binders that have emerged from the lipocalin protein family. Lipocalins are naturally occurring proteins that can bind to and transport small hydrophobic molecules (Figure 6). Anticalins with a specific and high-affinity binding capability to a wide range of target molecules and their potential as alternative binders with reduced immunogenicity, allowing for repeated and safe use in diagnostics and therapies[14]. The exceptional binding affinity and specificity of Anticalins make them well-suited for precision diagnostics in oncology and other diseases.

3.6 Bicyclic Peptides: Cyclized Scaffold Binders

Bicyclic peptides represent a promising class of bioaffinity binders that are characterized by their cyclized structure, containing two constrained loops which enhance their stability and target binding affinity[15, 16]. Bicyclic peptides provide a versatile platform for specific target recognition, while their cyclized scaffold confers exceptional resistance to proteolytic degradation, allowing for their use in demanding biological environments. This stability ensures prolonged binder activity and performance, making bicyclic peptides suitable for diagnostic applications requiring long-term biosensor stability. Furthermore, the design of bicyclic peptides allows for the incorporation of diverse amino acid residues within the constrained loops, enabling efficient customization for specific target recognition.

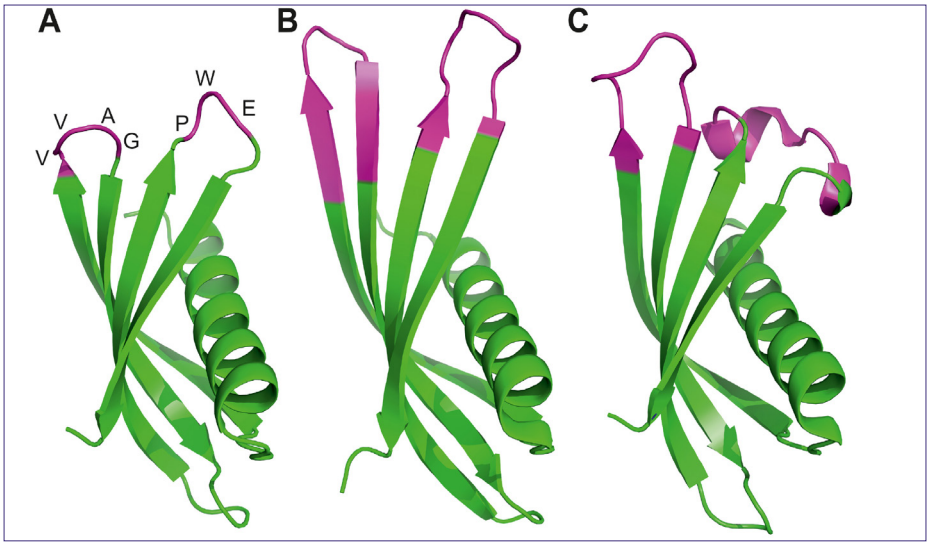

Figure 5. Ribbon diagrams portraying crystal structures of Affimer (Adhiron) reagents. Unaltered loops in pink (A), Exclusive to p300 (B), and for SUMO protein (C) with variable segments in pink13. ©2017 The authors and published under open access license by eLife Sciences Publications, Ltd.

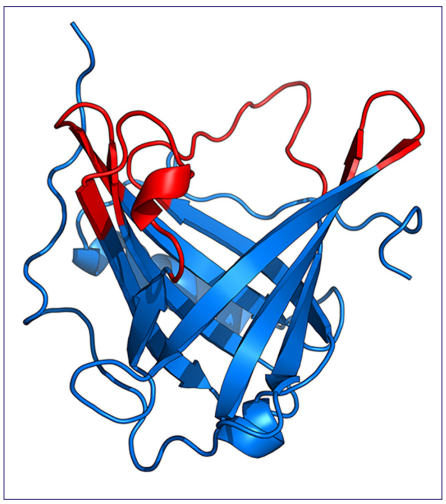

Figure 6. Adaptable nature of loop-centered binding sites in human tear Lipocalin (Tlc; Lcn1)14.©2018 The authors and published under open access license by Springer Nature.

3.7 DARPins: Engineered Ankyrin Repeat Proteins

DARPins (Designed Ankyrin Repeat Proteins) are a class of engineered protein binders derived from ankyrin repeat motifs. The unique structure of DARPins, composed of ankyrin repeat domains, allows for the efficient recognition and binding of target molecules (Figure 7)[17]. The repeat motifs contribute to the stable and robust interaction between DARPins and their targets, ensuring reliable performance in diverse biological environments18. One of the key advantages of DARPins is their small size, which minimizes steric hindrance and allows for efficient target binding kinetics. This characteristic is particularly beneficial in nanomaterial-based biosensors, where the binding efficiency and sensitivity are crucial for accurate and rapid detection.

3.8 Fynomers: Engineered Immunoglobulin Domains

Fynomers represent a class of engineered from the variable regions of immunoglobulin domains that offer great promise in the field of bioaffinity. Fynomers combine the advantageous properties of traditional antibodies with a simplified scaffold structure (Figure 8)[19]. The small size of Fynomers enables efficient production and customization for specific target recognition. Moreover, their immunoglobulin-like scaffold allows

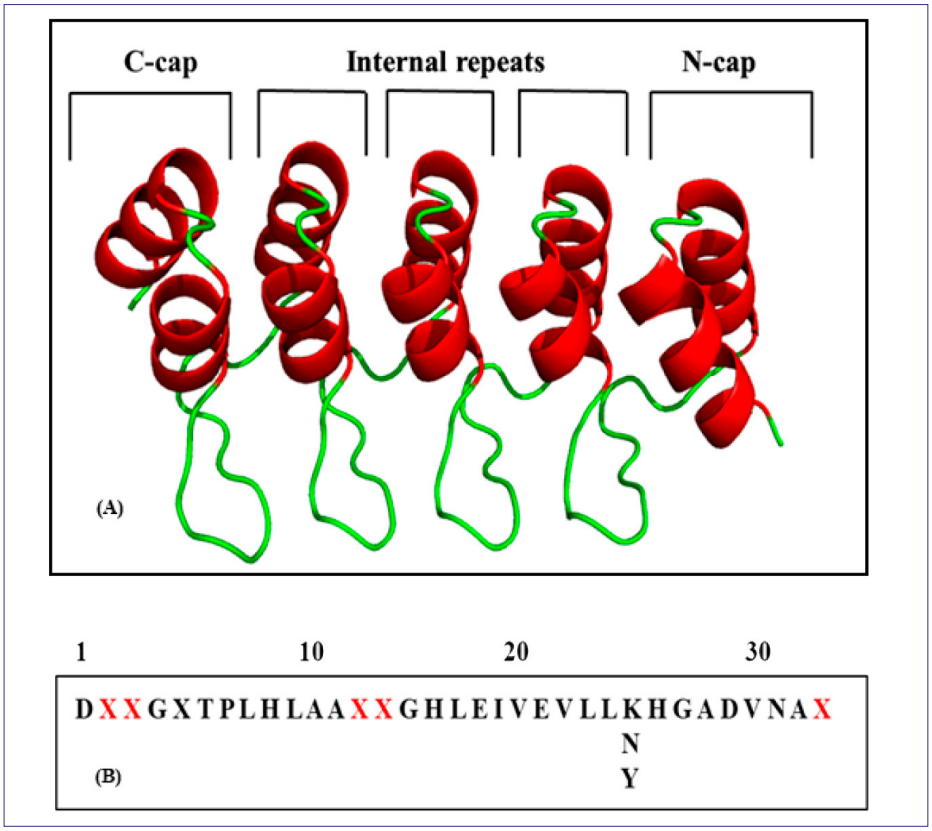

Figure 7. (A) DARPins (N3C) architecture featuring C-cap, N-cap, and internal iterations. (B) Consensus design of a DARPin repeat with 33 amino acids showcasing framework (black) and variable residues (red)17. ©2021 The authors and published under open access license by MDPI.

for the incorporation of various mutations and amino acid substitutions to fine-tune their affinity and selectivity, tailoring them for specific analyte detection.

3.9 Kunitz Domains: Proteinase Inhibitors with Binding Properties

Kunitz domains are a class of naturally occurring proteinase inhibitors which are known for their ability to inhibit serine proteinases, and their binding properties can be harnessed for specific target recognition through engineering[20]. The robust and stable nature of Kunitz domains ensures their functionality and performance in challenging biological environments. Furthermore, Kunitz domains can be engineered to optimize their binding properties and tailor them for specific biosensor applications. Their ability to be fine-tuned for target recognition enhances their versatility, allowing for the detection of a wide range of analytes with high sensitivity.

3.10 Monobodies: Protein Domains for Specific Interactions

Monobodies are a versatile class of bioaffinity binders derived from fibronectin type III domains[21]. These protein domains offer specific and high-affinity interactions with target molecules, and their small size and stable structure make them attractive candidates for integration with nanomaterials in biosensing applications. The fibronectin type III domain scaffold of Monobodies confers unique binding properties that enable specific interactions with target molecules (Figure 9). By engineering the loops of the domain, researchers can generate Monobodies with high affinity and selectivity for diverse targets, making them versatile binders for diagnostics and therapeutic purposes.

3.11 Aptamers: Nucleic Acid-Based Bioaffinity Binders

Aptamers, a burgeoning category of nucleic acid-based bioaffinity binders, have gained prominence in biosensing due to their unique attributes. These short single-stranded DNA or RNA molecules, derived via Systematic Evolution of Ligands by Exponential Enrichment (SELEX), exhibit exceptional specificity and affinity for target molecules. As potent alternatives to traditional antibodies, aptamers hold promise for reshaping POC biosensors, offering rapid and sensitive detection capabilities. SELEX

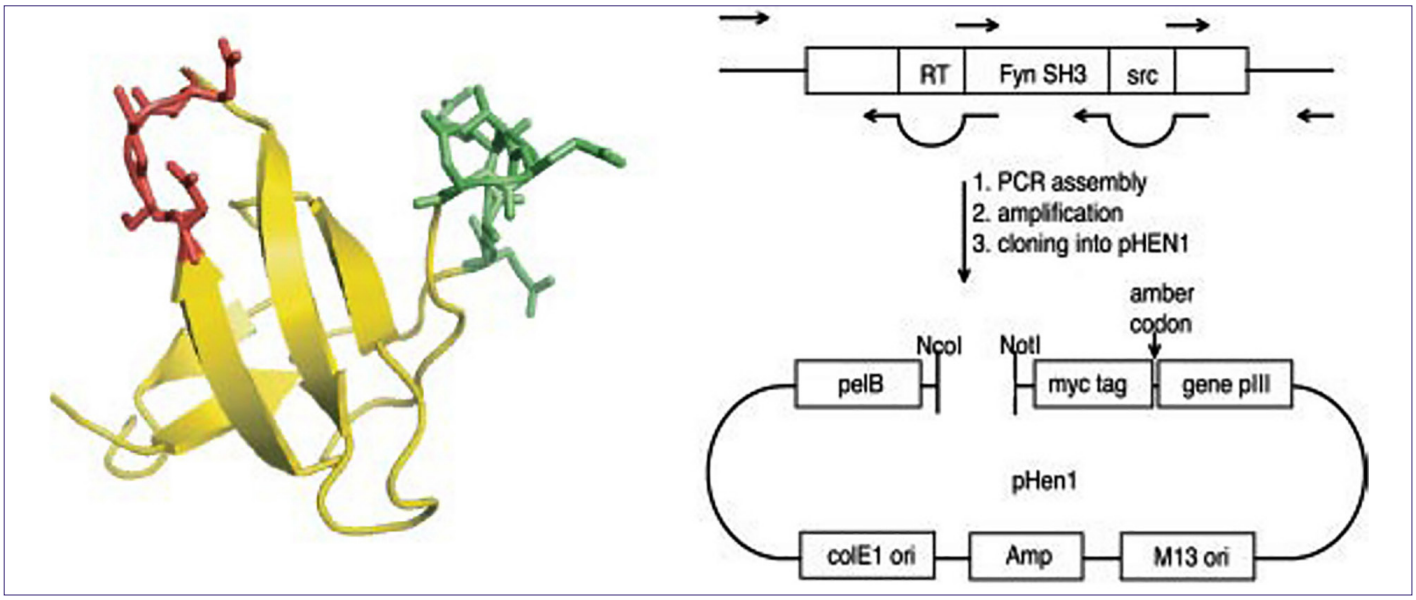

Figure 8. Fyn SH3 protein structure and approach to cloning libraries19. ©2007 The authors and published under open access license by Elsevier.

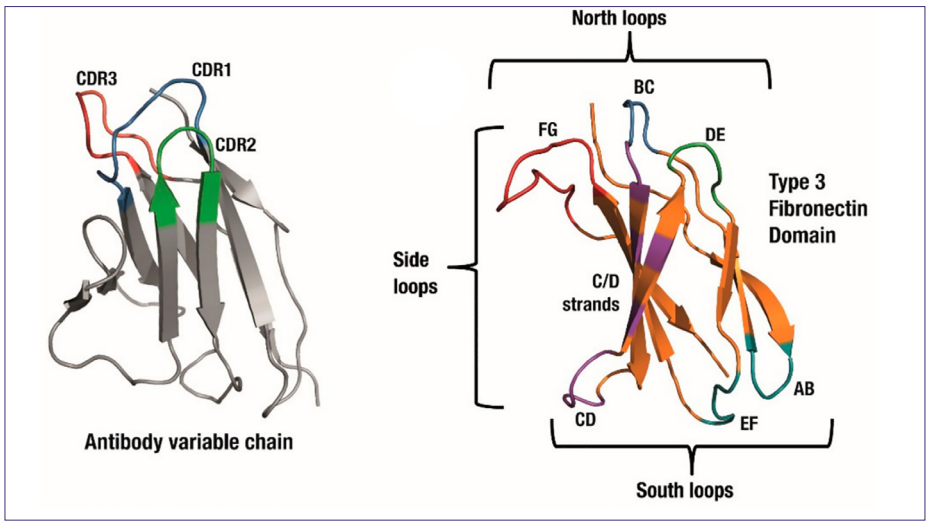

Figure 9. Antibody domains utilize a triad of highly variable binding loops (CDRs) forming a complementary region to target binding site (left). Fibronectin Type III (FN3) domains display similar loops for engineered binding function and expanded binding area in side and 'South' loops (right)22. ©2020 The authors and published under open access license by MDPI.

empowers researchers to tailor aptamers with specific affinities for diverse targets, including small molecules and proteins. This adaptability has fueled their use in diagnostics and therapeutics.

Aptamers' small size and strong binding affinity ensure rapid target recognition, crucial for POC biosensors. Their facile synthesis and modification allow seamless integration with reporter molecules or nanomaterials, amplifying signal generation[23].

Combining aptamers with nanomaterials presents intriguing biosensing prospects. Merging aptamer specificity with nanomaterial properties could yield highly sensitive biosensor platforms. Researchers are exploring aptamer-nanomaterial amalgamation, including nanoparticles and graphene, to enhance signal amplification. As aptamer exploration continues, they could revolutionize disease diagnostics, environmental monitoring, and personalized medicine. Aptamers' versatility, compact size, and potential for nanomaterial fusion make them essential in advancing biosensing solutions, underpinned by the guiding principles of chemistry[23].

The diversity and versatility of alternative bioaffinity binders showcased in this section exemplify the transformative potential of nanomaterial-bioaffinity binder convergence in POC biosensors. Each class of binders offers unique advantages in terms of stability, specificity, and functionality, expanding the horizon of biosensing technologies. Real studies have demonstrated their potential in diverse applications, ranging from cancer diagnostics and therapy to immunotherapy and inflammatory disease treatment. The following part briefly discusses the role of nanomaterial-bioaffinity binder chemistry community in rational design of POC diagnostics and personalized medicine.

4. Rational Design of Bioaffinity Binders for Specific Nanomaterials

The successful application of bioaffinity binders in POC biosensors heavily relies on their rational design and compatibility with specific nanomaterials. Understanding the interaction between bioaffinity binders and nanomaterial surfaces is essential for tailoring binding events that facilitate efficient analyte detection[24]. Researchers employ rational design strategies, combining computational modeling, structure-guided engineering, and molecular simulations to optimize the affinity and specificity of bioaffinity binders for targeted analytes. By engineering binders to precisely match the characteristics of nanomaterial surfaces, researchers can achieve enhanced sensor performance, leading to improved sensitivity and selectivity in POC biosensing applications.

4.1 Tailoring Surface Properties of Nanomaterials for Efficient Immobilization

In tandem with rational binder design, the optimization of nanomaterial surface properties is crucial for the efficient immobilization of bioaffinity binders. Nanoparticles, quantum dots, and nanocomposites possess diverse surface chemistries, including hydrophobic, hydrophilic, charged, or functional groups. These surface properties dictate the nature of interactions between bioaffinity binders and nanomaterials, influencing binder orientation, stability, and binding kinetics. Understanding the interplay between nanomaterial surfaces and bioaffinity binders is fundamental to tailor binding strategies for enhanced immobilization and biosensor performance. Researchers can modify the surface chemistry of nanomaterials to introduce functional groups that promote specific binding interactions with bioaffinity binders[25, 26]. Surface functionalization techniques, such as silanization, polymer coating, and ligand conjugation, offer precise control over the surface characteristics of nanomaterials, ensuring stable and specific binding interactions with bioaffinity binders. By tailoring the surface properties of nanomaterials, researchers can fine-tune the immobilization process, maximizing the availability of active binding sites and achieving enhanced biosensor sensitivity and stability.

4.2 Molecular Simulations and Computational Tools for Binder-Nanomaterial Interactions

Molecular simulations and computational tools have emerged as indispensable assets in elucidating the binding dynamics between bioaffinity binders and nanomaterial surfaces. Through molecular docking, molecular dynamics simulations, and quantum mechanical calculations, researchers gain valuable insights into the energetics and conformational changes that underpin the binding interactions. These computational approaches provide a deeper understanding of the binding kinetics, affinity, and specificity, guiding the rational design of bioaffinity binders and nanomaterial surfaces for optimal biosensor performance. By integrating experimental data with computational simulations, researchers can make informed decisions in the development of bioaffinity binder-nanomaterial interfaces, accelerating the progress of POC biosensor technology.

5. Immobilization Techniques at the Nanomaterial-Bioaffinity Binder Interface

Efficient immobilization of bioaffinity binders on nanomaterial surfaces ensures stable and specific binding interactions with target analytes, leading to enhanced sensor performance. Various immobilization techniques, both physical and chemical in nature, have been explored to achieve robust and reproducible binder-nanomaterial interfaces. This section delves into the intricacies of these techniques, highlighting their advantages, challenges, and applications in the construction of advanced POC biosensors.

5.1 Physical Immobilization Methods

Adsorption is a commonly employed physical immobilization method, wherein bioaffinity binders are adsorbed onto the surface of nanomaterials through non-covalent interactions. Van der Waals forces,

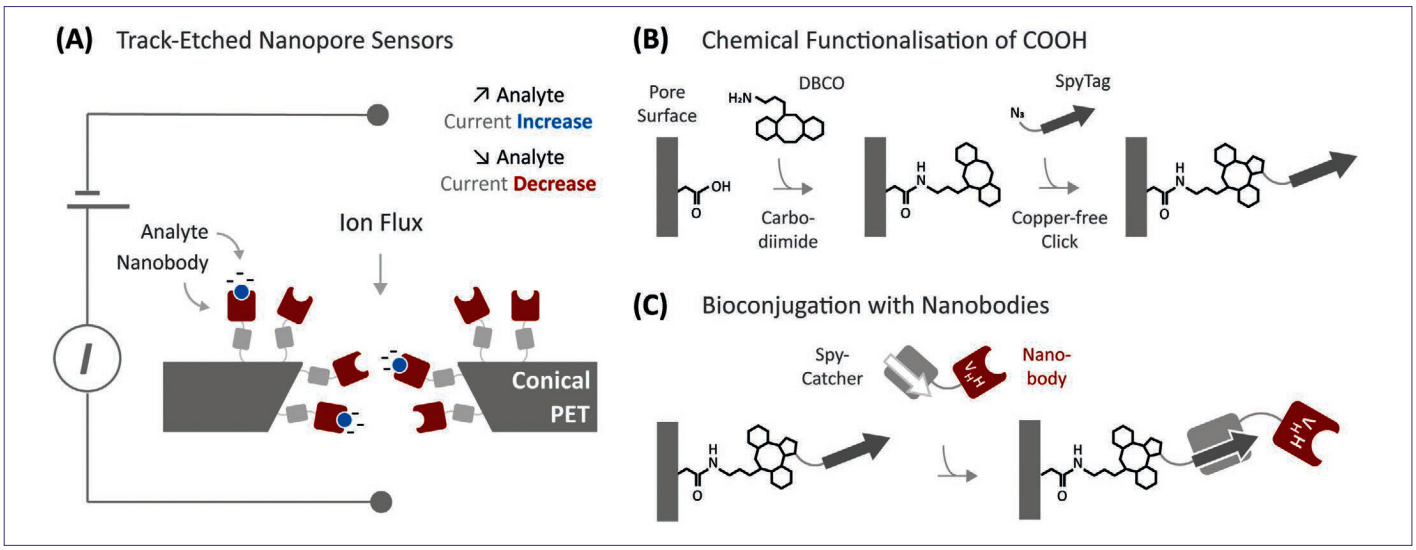

Figure 10. Systeatic approach in the oriented attachment of nanobodies over track-etched nanopores. (A) Interaction of protein analytes with corresponding nanobodies detected by altered current/voltage throughout the membrane where negative charges represented the analyte protein. (B) Diagram of DBCO attachment to COOH groups on surface; facilitating subsequent linkage of the azide-terminated SpyTag using Cu(II)-free Click chemistry. (C) SpyTag-SpyCatcher interaction facilitating covalent connection of nanobodies to the surface of nanopores and resulting in precise immobilization within nanopore with antigen binding site directed towards nanopore lumen32. ©2021 The authors and published under open access license by Wiley-VCH, GmbH.

hydrogen bonding, and hydrophobic interactions drive the adsorption process, facilitating the attachment of binders to nanomaterial surfaces[27, 28]. This method is facile and does not require complicated synthesis steps, making it suitable for rapid biosensor fabrication. However, adsorption may be reversible, leading to potential leaching of bioaffinity binders and affecting sensor stability. Efforts have been made to improve the stability of adsorbed binders by introducing stabilizing agents or using protective coatings, thus ensuring their retention on nanomaterial surfaces.

5.2 Chemical Immobilization Methods

5.2.1 Covalent Bonding

Chemical immobilization methods involve the formation of covalent bonds between bioaffinity binders and nanomaterial surfaces. Covalent bonding confers high stability and irreversibility to the binder-nanomaterial interface, ensuring robust sensor performance and minimized binder leaching (Figure 10). Various coupling chemistries, such as carbodiimide chemistry, maleimide-thiol chemistry, and NHS-ester chemistry, have been employed to facilitate covalent binding[29-31]. The versatility of covalent bonding allows for precise control over the binder orientation, ensuring optimal exposure of the binding sites for target analyte recognition. Although chemical immobilization techniques offer superior stability, they may require additional synthesis steps and, in some cases, surface functionalization of nanomaterials, adding complexity to the sensor fabrication process.

5.2.2 Click Chemistry

Click chemistry, a subset of chemical immobilization methods, has gained prominence for its efficiency and selectivity in bioconjugation reactions. Bioaffinity binders and nanomaterial surfaces can be functionalized with complementary click-reactive moieties, enabling rapid and specific covalent binding through click reactions (Figure 10B)[32-34]. Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) and strain-promoted azide-alkyne cycloaddition (SPAAC) are among the commonly used click reactions for biosensor construction. Click chemistry offers distinct advantages in terms of its mild reaction conditions, biocompatibility, and orthogonal selectivity, ensuring minimal interference with biomolecules and enabling versatile binder-nanomaterial conjugation strategies.

5.2.3 Self-Assembled Monolayers (SAMs)

Self-assembled monolayers involve the formation of a single layer of molecules on a nanomaterial surface through spontaneous adsorption (Figure 11). Thiol-based SAMs are frequently employed to facilitate covalent bonding between bioaffinity binders and nanomaterials, as thiol groups readily react with gold and other metal surfaces[35, 36]. SAMs provide a controlled and well-defined surface chemistry, offering precise control over the binder orientation and density. This method ensures minimal non-specific binding interactions and high binding capacity, leading to improved biosensor sensitivity and specificity. The versatility of SAMs in enabling the immobilization of diverse binders makes them attractive options in POC biosensor design.

6. Innovative POC Biosensors: Case Studies in Nanomaterial-Bioaffinity Binder Convergence

Infectious diseases remain a significant global health concern, necessitating the development of rapid and accurate diagnostic tools. Nanoparticle-based immunoassays have emerged as powerful diagnostic platforms for detecting viral antigens and antibodies with enhanced sensitivity and specificity. Gold nanoparticles, in particular, have been functionalized with alternative bioaffinity binders, such as nanobodies, affimers or affitins, targeting specific viral proteins[38-41]. This integration facilitates the rapid detection of pathogens, including influenza viruses and coronaviruses. The precise orientation of bioaffinity binders on gold nanoparticles ensures optimal exposure of their binding sites, leading to amplified signals in a label-free manner. These nanoparticle-based immunoassays offer the potential for POC diagnostics in resource-limited settings, where rapid and accurate detection of infectious agents is crucial for effective disease management.

The integration of nanomaterials and alternative bioaffinity binders has extended beyond diagnostics to the realm of personalized medicine. Nanoparticles functionalized with bioaffinity binders, such as affibodies or Kunitz domains, have been explored as targeted drug delivery vehicles42-44. These nanoconjugates can selectively recognize and bind to specific cellular receptors or

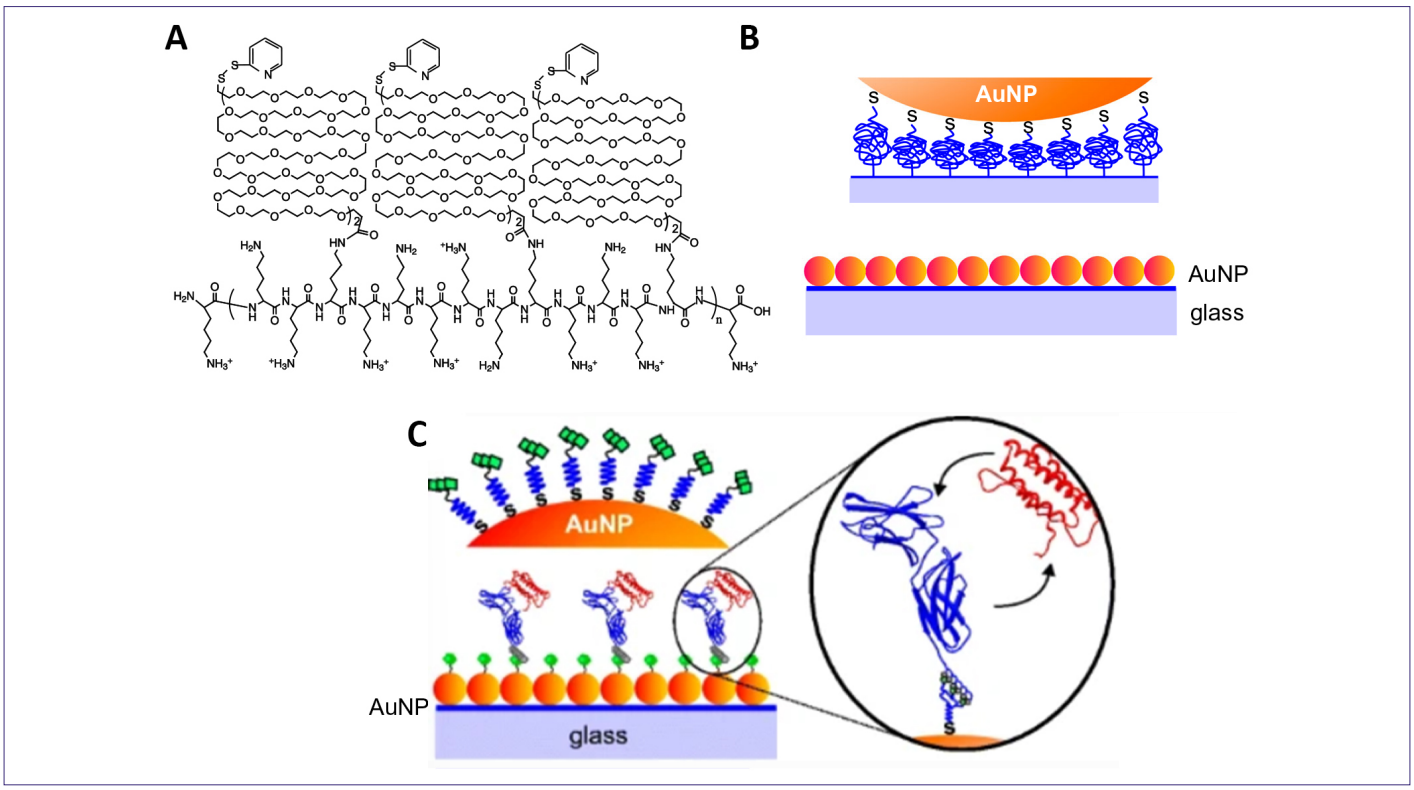

Figure 11. Self-assembly of tailored AuNP monolayers. (A) Molecular design of PLL-PEG-OPSS for coating substrate surface. (B) Conceptual presentation of AuNP monolayer on PLL-PEG-OPSS-coated glass slide. (C) Affinity binder-mediated surface functionalization of AuNP monolayers37. ©2020 The authors and published under open access license by Springer Nature.

disease biomarkers, facilitating targeted drug delivery and reducing off-target effects. The use of alternative bioaffinity binders with reduced immunogenicity enhances the biocompatibility of these drug carriers, ensuring safe and efficient drug delivery. By engineering bioaffinity binders to precisely match disease targets, researchers aim to revolutionize therapeutic interventions, leading to improved treatment efficacy and reduced side effects.

Our research team demonstrated their expertise by designing and implementing rapid, user-friendly electrochemical methamphetamine aptasensors, utilizing a variety of aptamer sequences. Employing the GO-SELEX method, distinct aptamer sequences (Apta-1, Apta-2, Apta-3, Apta-4) were developed and subsequently immobilized onto gold electrodes (GE) using gold-thiol affinity, employing appropriate surface chemistry45. In a parallel endeavor, we developed an original system for the selective analysis of Synthetic cannabinoids (SCs) through the application of tailor-made aptamers. By toggling amongst several SC analytes throughout successive selection sets, cross-reactive aptamers were generated. These amino-capped aptamers were then seamlessly attached to cysteamine-covered gold electrodes, as depicted in Figure 1246. Another notable achievement showcased the utilization of aptasensors as a potent tool for the sensitive and rapid recognition of Imidacloprid, a model analyte representative of pesticides. The team constructed a water analysis platform, highlighting the potential of aptasensors for efficient, cost-effective, and swift environmental monitoring[45].

Expanding our expertise, we ventured into peptide nucleic acids (PNAs), a unique aptamer class. In a recent study, an electrochemical biosensor utilizing PNAs as bio-recognition elements was crafted47. These PNAs, featuring a neutral "peptide-like" backbone, replaced the conventional charged sugar-phosphate structure in DNA. Leveraging this structure, PNAs exhibited high selectivity upon hybridization with target DNA and RNA chains. A distinctive innovation emerged by modifying the termini of "PNA capture probes," enabling targeted binding to DNA or RNA

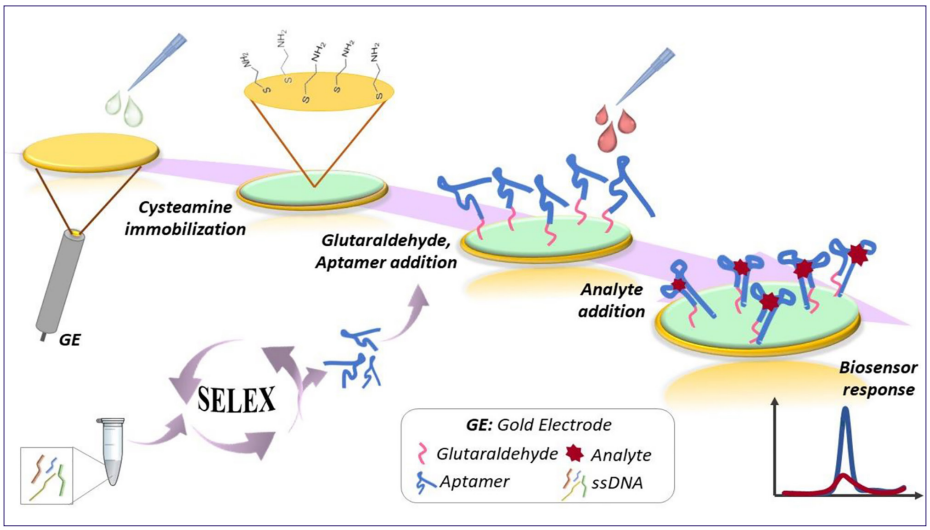

Figure 12. Sequence of modifications for tailor-made Aptasensor platform in SCs detection46. Reused with permission, © 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

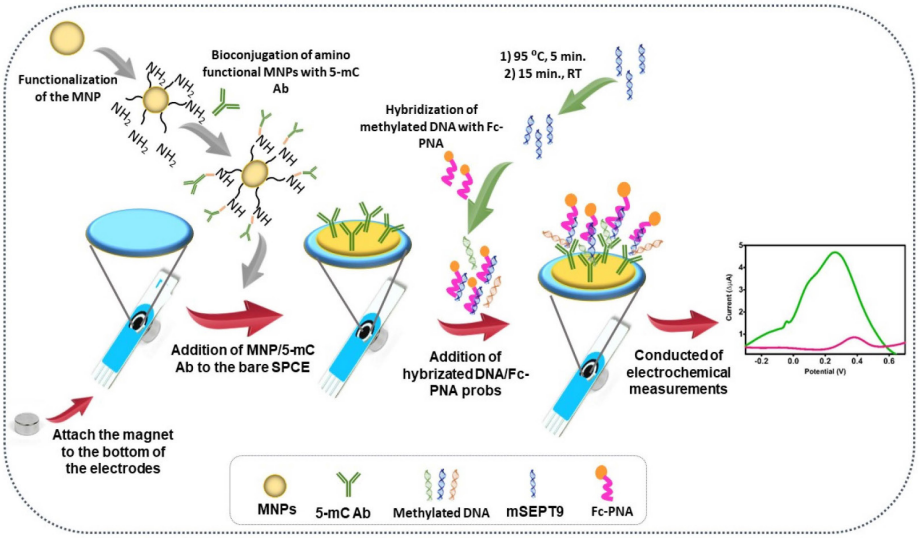

Figure 13. Surface design and sensing mechanism of the electrochemical sensor based on PNA for the early detection of colon cancer. Reproduced from47. ©2022 the Authors and published by MDPI.

fragments through a redox-active metal complex like ferrocene (Fc). This strategic integration of an electroactive label facilitated the development of an electrochemical sensing platform designed for the early diagnosis of colorectal cancer (CRC) (Figure 14). This platform, amalgamating magnetic nanoparticles (MNPs), 5-methylcytosine (5-mC) antibodies, and a hybridization system (Fc-PNA), gauged methylation levels of the CRC biomarker mSEPT9. Rigorous optimization and adaptation onto a POC device followed, showcasing the platform's clinical potential in early cancer diagnosis and treatment monitoring47.

7. Future Prospects and Challenges

The continuous exploration of advanced nanomaterials and innovative bioaffinity binders will be a driving force in shaping the future of biosensing technologies. Novel nanomaterials with unique physicochemical properties, such as 3D materials, metal-organic frameworks (MOFs), and peptide-based nanoparticles, hold immense potential in enhancing biosensor sensitivity, stability, and multiplexing capabilities. Likewise, the discovery and engineering of novel bioaffinity binders with exceptional target specificity and binding affinity will unlock new avenues for biosensor development. Moreover, multimodal sensing holds promise in complex diagnostic scenarios, where the detection of multiple disease biomarkers can provide comprehensive health assessments. Moreover, synergistic sensing platforms can enable rapid and accurate pathogen identification, environmental pollutant monitoring, and personalized therapeutic interventions. However, as nanomaterials become integral to biosensor design, addressing biocompatibility and nanotoxicity concerns is paramount. The safe deployment of nanomaterials for in vivo applications and environmental monitoring hinges on comprehensive biocompatibility assessments. Understanding the interaction of nanomaterials with biological systems will inform the design of biocompatible biosensors, minimizing adverse effects and ensuring their clinical relevance. Rigorous toxicity studies, using advanced in vitro and in vivo models, are essential to identify potential risks and mitigate environmental impacts.

The integration of data analytics and artificial intelligence (AI) algorithms with nanomaterial-based biosensors holds transformative potential in enhancing sensor performance. AI-driven data analysis enables real-time and automated interpretation of biosensor outputs, facilitating rapid and accurate diagnosis. Machine learning algorithms can identify complex patterns and correlations in large datasets, enabling early disease detection and precision medicine strategies. Additionally, AI-driven biosensors can adapt to dynamic environmental conditions and optimize sensor calibration, ensuring reliable performance in diverse settings.

The translation of nanomaterial-based biosensors into commercial products is a critical step in realizing their potential impact on global health and sustainability. To achieve widespread adoption, biosensor development must address scalability, cost-effectiveness, and regulatory compliance. The optimization of production processes and standardization of synthesis techniques are crucial to ensure consistent sensor performance. Cost-effective manufacturing methods and the use of sustainable nanomaterials will enhance accessibility to POC biosensors, particularly in resource-limited settings. Collaboration between academia, industry, and policymakers will be instrumental in navigating regulatory pathways and facilitating market entry. By addressing these challenges, nanomaterial-bioaffinity binder-based biosensors can reach healthcare facilities, clinics, and even remote regions, democratizing diagnostics and improving health outcomes for diverse populations.

8. Conclusion

In this comprehensive exploration of nanomaterial-bioaffinity binder convergence, a wide array of alternative bioaffinity binders has been discussed for their transformative potential in point-of-care biosensors. Among the innovative molecules discussed are Abdurins, Affitins, Affibodies, Affimers, Anticalins, Bicyclic peptides, DARPins, Fynomers, Kunitz domains, Monobodies, and Aptamers, each demonstrating unique attributes that make them valuable candidates for biosensing applications.

The seamless integration of these alternative bioaffinity binders with nanomaterials offers exciting possibilities for the development of advanced biosensor platforms. Their small size, stability, and specific binding capabilities enable efficient and robust target recognition, while the unique properties of nanomaterials enhance biosensor sensitivity and specificity. From cancer diagnostics to targeted therapy, real studies have showcased the exceptional capabilities of these binders in diverse biomedical applications, highlighting their potential to revolutionize precision medicine.

Moreover, the adaptability of these alternative binders through engineering allows for the fine-tuning of their binding properties, tailoring them to suit the demands of specific biosensor applications. As researchers continue to explore the potential of nanomaterial-bioaffinity binder convergence, they unlock new horizons in biosensor technologies, paving the way for the development of sensitive, specific, and multiplexed biosensors that hold promise for enhancing healthcare accessibility and personalized medicine. This journey of progress represents a critical milestone in the field of chemistry, with future research in this area set to shape the landscape of biosensing, ushering in a new era of scientific discovery and societal benefit.

References

1. Nagraik, R.; Sharma, A.; Kumar, D.; Mukherjee, S.; Sen, F.; Kumar, A. P., Amalgamation of biosensors and nanotechnology in disease diagnosis: Mini-review. ***Sensors International*** **2021,** *2*, 100089.
2. Malhotra, B. D.; Ali, M. A., Nanomaterials in Biosensors. In ***Nanomaterials for Biosensors***, 2018; pp 1-74.
3. Daneshvar Tarigh, G., Enantioseparation/Recognition based on nano techniques/materials. ***J Sep Sci*** **2023,** *46* (12), e2201065.
4. Joseph, T. M.; Kar Mahapatra, D.; Esmaeili, A.; Piszczyk, L.; Hasanin, M. S.; Kattali, M.; Haponiuk, J.; Thomas, S., Nanoparticles: Taking a Unique Position in Medicine. ***Nanomaterials (Basel)*** **2023,** *13* (3), 574.
5. Putzbach, W.; Ronkainen, N. J., Immobilization techniques in the fabrication of nanomaterial-based electrochemical biosensors: a review. ***Sensors (Basel)*** **2013,** *13* (4), 4811-40.
6. Ilhan, H.; Panhwar, S.; Boyaci, I. H.; Tamer, U., Optical Based Transducers for Biosensors. In ***Biosensors (Basel)***, CRC Press: 2022; pp 57-87.
7. Olaleye, O.; Govorukhina, N.; van de Merbel, N. C.; Bischoff, R., Non-Antibody-Based Binders for the Enrichment of Proteins for Analysis by Mass Spectrometry. ***Biomolecules*** **2021,** *11* (12), 1791.
8. Prabakaran, P.; Vu, B. K.; Gan, J.; Feng, Y.; Dimitrov, D. S.; Ji, X., Structure of an isolated unglycosylated antibody C(H)2 domain. ***Acta Crystallogr D Biol Crystallogr*** **2008,** *64* (Pt 10), 1062-7.
9. Ullman, C.; Mathonet, P.; Oleksy, A.; Diamandakis, A.; Tomei, L.; Demartis, A.; Nardi, C.; Sambucini, S.; Missineo, A.; Alt, K.; Hagemeyer, C. E.; Harris, M.; Hedt, A.; Weis, R.; Gehlsen, K. R., High Affinity Binders to EphA2 Isolated from Abdurin Scaffold Libraries; Characterization, Binding and Tumor Targeting. ***PLoS One*** **2015,** *10* (8), e0135278.
10. Behar, G.; Bellinzoni, M.; Maillasson, M.; Paillard-Laurance, L.; Alzari, P. M.; He, X.; Mouratou, B.; Pecorari, F., Tolerance of the archaeal Sac7d scaffold protein to alternative library designs: characterization of anti-immunoglobulin G Affitins. ***Protein Eng Des Sel*** **2013,** *26* (4), 267-75.
11. Mouratou, B.; Schaeffer, F.; Guilvout, I.; Tello-Manigne, D.; Pugsley, A. P.; Alzari, P. M.; Pecorari, F., Remodeling a DNA-binding protein as a specific in vivo inhibitor of bacterial secretin PulD. ***Proc Natl Acad Sci U S A*** **2007,** *104* (46), 17983-8.
12. Tolmachev, V.; Orlova, A., Affibody Molecules as Targeting Vectors for PET Imaging. ***Cancers (Basel)*** **2020,** *12* (3), 651.
13. Tiede, C.; Bedford, R.; Heseltine, S. J.; Smith, G.; Wijetunga, I.; Ross, R.; AlQallaf, D.; Roberts, A. P.; Balls, A.; Curd, A.; Hughes, R. E.; Martin, H.; Needham, S. R.; Zanetti-Domingues, L. C.; Sadigh, Y.; Peacock, T. P.; Tang, A. A.; Gibson, N.; Kyle, H.; Platt, G. W.; Ingram, N.; Taylor, T.; Coletta, L. P.; Manfield, I.; Knowles, M.; Bell, S.; Esteves, F.; Maqbool, A.; Prasad, R. K.; Drinkhill, M.; Bon, R. S.; Patel, V.; Goodchild, S. A.; Martin-Fernandez, M.; Owens, R. J.; Nettleship, J. E.; Webb, M. E.; Harrison, M.; Lippiat, J. D.; Ponnambalam, S.; Peckham, M.; Smith, A.; Ferrigno, P. K.; Johnson, M.; McPherson, M. J.; Tomlinson, D. C., Affimer proteins are versatile and renewable affinity reagents. ***Elife*** **2017,** *6*, e24903.
14. Rothe, C.; Skerra, A., Anticalin((R)) Proteins as Therapeutic Agents in Human Diseases. ***BioDrugs*** **2018,** *32* (3), 233-243.
15. Ahangarzadeh, S.; Kanafi, M. M.; Hosseinzadeh, S.; Mokhtarzadeh, A.; Barati, M.; Ranjbari, J.; Tayebi, L., Bicyclic peptides: types, synthesis and applications. ***Drug Discov Today*** **2019,** *24* (6), 1311-1319.
16. Gang, D.; Kim, D. W.; Park, H. S., Cyclic Peptides: Promising Scaffolds for Biopharmaceuticals. ***Genes (Basel)*** **2018,** *9* (11), 557.
17. Gautam, V.; Nimmanpipug, P.; Zain, S. M.; Rahman, N. A.; Lee, V. S., Molecular Dynamics Simulations in Designing DARPins as Phosphorylation-Specific Protein Binders of ERK2. ***Molecules*** **2021,** *26* (15), 4540.
18. Boersma, Y. L., Advances in the Application of Designed Ankyrin Repeat Proteins (DARPins) as Research Tools and Protein Therapeutics. In ***Methods in Molecular Biology***, Springer New York: 2018; pp 307-327.
19. Grabulovski, D.; Kaspar, M.; Neri, D., A novel, non-immunogenic Fyn SH3-derived binding protein with tumor vascular targeting properties. ***J Biol Chem*** **2007,** *282* (5), 3196-204.
20. Morais, K. L. P.; Ciccone, L.; Stura, E.; Alvarez-Flores, M. P.; Mourier, G.; Driessche, M. V.; Sciani, J. M.; Iqbal, A.; Kalil, S. P.; Pereira, G. J.; Marques-Porto, R.; Cunegundes, P.; Juliano, L.; Servent, D.; Chudzinski-Tavassi, A. M., Structural and functional properties of the Kunitz-type and C-terminal domains of Amblyomin-X supporting its antitumor activity. ***Front Mol Biosci*** **2023,** *10*, 1072751.
21. Hantschel, O.; Biancalana, M.; Koide, S., Monobodies as enabling tools for structural and mechanistic biology. ***Curr Opin Struct Biol*** **2020,** *60*, 167-174.
22. Chandler, P. G.; Buckle, A. M., Development and Differentiation in Monobodies Based on the Fibronectin Type 3 Domain. ***Cells*** **2020,** *9* (3), 610.
23. Yoo, H.; Jo, H.; Oh, S. S., Detection and beyond: challenges and advances in aptamer-based biosensors. ***Materials Advances*** **2020,** *1* (8), 2663-2687.
24. Gabriele, F.; Palerma, M.; Ippoliti, R.; Angelucci, F.; Pitari, G.; Ardini, M., Recent Advances on Affibody- and DARPin-Conjugated Nanomaterials in Cancer Therapy. ***Int J Mol Sci*** **2023,** *24* (10), 8680.
25. Chen, H.; Huang, J.; Palaniappan, A.; Wang, Y.; Liedberg, B.; Platt, M.; Tok, A. I., A review on electronic bio-sensing approaches based on non-antibody recognition elements. ***Analyst*** **2016,** *141* (8), 2335-46.
26. Huang, Z.; Hu, S.; Xiong, Y.; Wei, H.; Xu, H.; Duan, H.; Lai, W., Application and development of superparamagnetic nanoparticles in sample pretreatment and immunochromatographic assay. ***TrAC Trends in Analytical Chemistry*** **2019,** *114*, 151-170.
27. Goossens, J.; Sein, H.; Lu, S.; Radwanska, M.; Muyldermans, S.; Sterckx, Y. G. J.; Magez, S., Functionalization of gold nanoparticles with nanobodies through physical adsorption. ***Analytical Methods*** **2017,** *9* (23), 3430-3440.
28. Simoes, B.; Guedens, W. J.; Keene, C.; Kubiak-Ossowska, K.; Mulheran, P.; Kotowska, A. M.; Scurr, D. J.; Alexander, M. R.; Broisat, A.; Johnson, S.; Muyldermans, S.; Devoogdt, N.; Adriaensens, P.; Mendes, P. M., Direct Immobilization of Engineered Nanobodies on Gold Sensors. ***ACS Appl Mater Interfaces*** **2021,** *13* (15), 17353-17360.
29. Albert, C.; Bracaglia, L.; Koide, A.; DiRito, J.; Lysyy, T.; Harkins, L.; Edwards, C.; Richfield, O.; Grundler, J.; Zhou, K.; Denbaum, E.; Ketavarapu, G.; Hattori, T.; Perincheri, S.; Langford, J.; Feizi, A.; Haakinson, D.; Hosgood, S. A.; Nicholson, M. L.; Pober, J. S.; Saltzman, W. M.; Koide, S.; Tietjen, G. T., Monobody adapter for functional antibody display on nanoparticles for adaptable targeted delivery applications. ***Nat Commun*** **2022,** *13* (1), 5998.
30. Fan, R.; Du, J.; Park, K. W.; Chang, L. H.; Strieter, E. R.; Andrew, T. L., Immobilization of Nanobodies with Vapor-Deposited Polymer Encapsulation for Robust Biosensors. ***ACS Appl Polym Mater*** **2021,** *3* (5), 2561-2567.
31. Oberhaus, F. V.; Frense, D.; Beckmann, D., Immobilization Techniques for Aptamers on Gold Electrodes for the Electrochemical Detection of Proteins: A Review. ***Biosensors (Basel)*** **2020,** *10* (5), 45.
32. Duznovic, I.; Grawe, A.; Weber, W.; Muller, L. K.; Ali, M.; Ensinger, W.; Tietze, A.; Stein, V., Ultrasensitive and Selective Protein Recognition with Nanobody-Functionalized Synthetic Nanopores. ***Small*** **2021,** *17* (33), e2101066.
33. Hansen, R.; Adriaensens, P.; Guedens, W., Introducing SPAAC and SPIEDAC coupling chemistry into the VCAM-1 targeting nanobody for advanced medical biomaterials. In ***11th Biomedicasummit***, Eindhoven, The Netherlands, , 2017.
34. Hansen, R.; Smeets, S.; Adriaensens, P.; Guedens, W. In ***Introducing copper-free'Click'amino acids into Nanobodies by nonsense suppression for advanced biomaterials***, Annual Meeting of the Belgian Polymer Group, Hasselt, Belgium, 23-24/05/2016; Hasselt, Belgium, 2016.
35. Gao, S.; Guisan, J. M.; Rocha-Martin, J., Oriented immobilization of antibodies onto sensing platforms - A critical review. ***Anal Chim Acta*** **2022,** *1189*, 338907.
36. Oloketuyi, S.; Mazzega, E.; Zavasnik, J.; Pungjunun, K.; Kalcher, K.; de Marco, A.; Mehmeti, E., Electrochemical immunosensor functionalized with nanobodies for the detection of the toxic microalgae Alexandrium minutum using glassy carbon electrode modified with gold nanoparticles. ***Biosens Bioelectron*** **2020,** *154*, 112052.
37. Flesch, J.; Kappen, M.; Drees, C.; You, C.; Piehler, J., Self-assembly of robust gold nanoparticle monolayer architectures for quantitative protein interaction analysis by LSPR spectroscopy. ***Anal Bioanal Chem*** **2020,** *412* (14), 3413-3422.
38. Bao, G.; Tang, M.; Zhao, J.; Zhu, X., Nanobody: a promising toolkit for molecular imaging and disease therapy. ***EJNMMI Res*** **2021,** *11* (1), 6.
39. Chen, X.; Kang, S.; Ikbal, M. A.; Zhao, Z.; Pan, Y.; Zuo, J.; Gu, L.; Wang, C., Synthetic nanobody-functionalized nanoparticles for accelerated development of rapid, accessible detection of viral antigens. ***Biosens Bioelectron*** **2022,** *202*, 113971.
40. Pagneux, Q.; Roussel, A.; Saada, H.; Cambillau, C.; Amigues, B.; Delauzun, V.; Engelmann, I.; Alidjinou, E. K.; Ogiez, J.; Rolland, A. S.; Faure, E.; Poissy, J.; Duhamel, A.; Boukherroub, R.; Devos, D.; Szunerits, S., SARS-CoV-2 detection using a nanobody-functionalized voltammetric device. ***Commun Med (Lond)*** **2022,** *2*, 56.
41. Yu, X.; Yang, Y. P.; Dikici, E.; Deo, S. K.; Daunert, S., Beyond Antibodies as Binding Partners: The Role of Antibody Mimetics in Bioanalysis. ***Annu Rev Anal Chem (Palo Alto Calif)*** **2017,** *10* (1), 293-320.
42. Hu, Y.; Liu, C.; Muyldermans, S., Nanobody-Based Delivery Systems for Diagnosis and Targeted Tumor Therapy. ***Front Immunol*** **2017,** *8*, 1442.
43. Koenning, D.; Schaefer, J. V., Alternative Binding Scaffolds: Multipurpose Binders for Applications in Basic Research and Therapy. In ***Learning Materials in Biosciences***, Springer International Publishing: 2020; pp 215-249.
44. Vazquez-Lombardi, R.; Phan, T. G.; Zimmermann, C.; Lowe, D.; Jermutus, L.; Christ, D., Challenges and opportunities for non-antibody scaffold drugs. ***Drug Discov Today*** **2015,** *20* (10), 1271-83.
45. Bor, G.; Bulut, U.; Man, E.; Balaban Hanoglu, S.; Evran, S.; Timur, S., Synthetic antibodies for methamphetamine analysis: Design of high affinity aptamers and their use in electrochemical biosensors. ***Journal of Electroanalytical Chemistry*** **2022,** *921*, 116686.
46. Balaban, S.; Man, E.; Durmus, C.; Bor, G.; Ceylan, A. E.; Pinar Gumus, Z.; Evran, S.; Coskunol, H.; Timur, S., Sensor Platform with a Custom-tailored Aptamer for Diagnosis of Synthetic Cannabinoids. ***Electroanalysis*** **2020,** *32* (3), 656-665.
47. Hanoglu, S. B.; Man, E.; Harmanci, D.; Tozan Ruzgar, S.; Sanli, S.; Keles, N. A.; Ayden, A.; Tuna, B. G.; Duzgun, O.; Ozkan, O. F.; Dogan, S.; Ghorbanizamani, F.; Moulahoum, H.; Guler Celik, E.; Evran, S.; Timur, S., Magnetic Nanoparticle-Based Electrochemical Sensing Platform Using Ferrocene-Labelled Peptide Nucleic Acid for the Early Diagnosis of Colorectal Cancer. ***Biosensors (Basel)*** **2022,** *12* (9), 736.