

Current Trends in Ubiquitous Biosensing

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Current Trends in Ubiquitous Biosensing

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Abstract

Biosensing technology is not currently capable of widespread use outside of a laboratory environment due to significant limitation in bioreceptor function and production, as well as in the overall size, weight, and cost of the sensing platform. However, as technology continues to advance, biosensors could truly become ubiquitous, employing social media and personal electronic devices for mundane yet powerful capabilities. In the near future, point-of-care diagnostics in third-world countries could save millions of lives and revolutionize the healthcare industry worldwide. Recent trends show many of the traditional barriers to realizing this vision will soon be overcome. In this paper we review exciting trends in the development of synthetic reagents, fluidic integration, and mobile platforms that are necessary for ubiquitous biosensing capabilities.

Keywords: Biosensing; Ubiquitous sensing; Synthetic molecular recognition; Synthetic reagents; Peptides; smart phone; Personal electronic devices; Bacterial display; Biomolecular recognition; Biosensor

Abbreviations: SWaP-C: Size, Weight, Power and Cost; PEDs: Personal Electronic Devices; POC: Point of Care; SERS: Surface Enhanced Raman Spectroscopy

Introduction

Biosensors of the future promise to be as ubiquitous as mobile phones, and if current sensing trends continue, the mobile phone itself could become an integrated platform for everyday use. For example, biosensors could bring about the next revolution in public health monitoring in third-world countries, providing low-cost point-of-care solutions with advanced diagnostic capabilities. Homeland security and the spread of disease could be monitored in real time, and the impact could be minimized through embedded sensors throughout the public transportation system. As technology continues to advance, biosensors could truly become a part of everyday life, employing social media and personal electronic devices to fuse biosensed data from the real world into virtual realities. There are several technological barriers that must be overcome before this scope and intensity is fully realized [1]. In this paper we review exciting trends in the development of synthetic reagents, fluidic integration, and personal electronic devices as platforms that are necessary for ubiquitous biosensing capabilities (Figure 1).

Biosensors comprise two primary components: the bioreceptor, or recognition reagent responsible for specific binding to the biological

analyte of interest, and the transducer, which converts this binding event to a measurable signal (Figure 2) [1,2]. A variety of bioreceptors (nucleic acid, antibody, etc.) can be used to bind to an analyte or target of interest, and similarly, a variety of transduction mechanisms have been used to translate this recognition event into an electronic signal (e.g., mass, electrochemical, optical, magnetic, label-free, etc.) [3,4]. Significant limitations in bioreceptor function and production, as well as in the overall size, weight, and cost (SWaP-C) of the transducer, have been the primary technological barriers to the vision of ubiquitous biosensing. Ideally, a bioreceptor reagent should have the characteristics outlined in table 1 to enable ubiquitous implementation across a variety of material systems and operational environments.

The bioreceptor (i.e., reagent) must be robust and resistant to degradation stressors encountered in real-world applications, including temperature extremes (cold and heat), pH variations, enzymes, etc. [5]. Furthermore, the ideal bioreceptor should be not only robust, but also

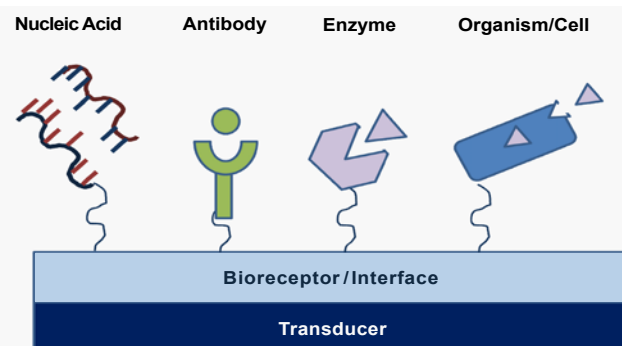


Figure 2: Conceptual biosensor concept employing traditional bioreceptor species.

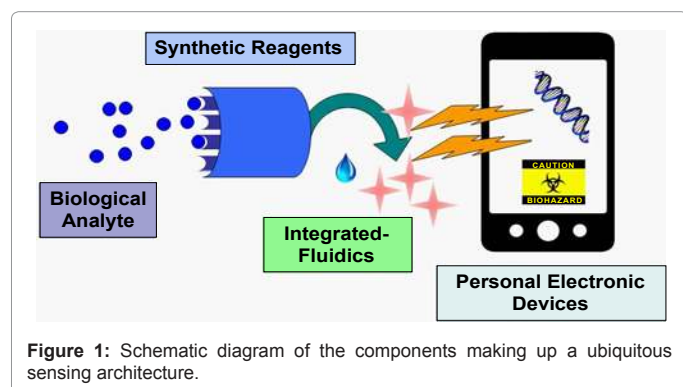


Figure 1: Schematic diagram of the components making up a ubiquitous sensing architecture.

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Desired Reagent Feature	Necessary for Ubiquitous Biosensing
Robust	Temperature, pH, enzyme degradation, and a long shelf life
Thermoplastic	Maintains full function under extreme temperature conditions
Universal	Encompasses all biosensing analytes irrespective of charge, size, etc.
Rapid Discovery	Rapid development without extensive knowledge of the target analyte, critical to adapt technology to new and emerging threats
Manufacturing Scale Production	Cost-effective production necessary for ubiquitous scale
On-Demand Production	Circumvent any shelf life or supply/demand issues
Low Cost	Critical for universal implementation
Adaptable	Readily incorporate into variety of platforms; drop-in replacement technology
High Affinity	Equivalent (or better) than antibody gold standard to meet sensing requirements
High Specificity	Critical for practical application in complex environments

Table 1: Table of affinity reagent features important to ubiquitous sensing.

Desired Platform Feature	Necessary for Ubiquitous Biosensing
Standardized	Universal interface capable of operation across platforms
Open and Programmable	Necessary for graphical user interface and software application to be rapidly modifiable
SWAP-C	For disruptive technologies, platforms must meet economic demands, including size, weight, power, and cost
Other Standard Capabilities to Leverage	Integrated archival data storage (GPS, time, date, readout, images, etc.) available for after-action processing
Networked	Data transmitted and recorded for further analysis and processing
Easy to Use	Operation and readout on platform must be simple for broad acceptance
Multiplexed Analysis	Extendable to include biothreat sensing, POC diagnostics, small molecule (cocaine, TNT, etc.), and nuclear materials

Table 2: Mobile platform reagent features important to ubiquitous sensing.

thermoplastic in nature (i.e., operational at elevated temperatures). The current state-of-the-art in reagent technology (i.e., antibody bioreceptors) capable of recognizing protein biomarkers and toxins (i.e., nongenetic materials) is fraught with difficulties including poor mass production, stability, and large overall production costs.

Many research groups have investigated and developed synthetic chemical and biological affinity reagent alternatives in an attempt to overcome limitations in natural protein antibody-based recognition. Most commonly these include nucleic acid aptamer, phage display, yeast display bacterial display, mRNA display, and one-bead-one-compound solid phase chemical libraries technologies [6-17].

Synthetic Reagents

Recent advances in synthetic affinity reagent technology and discovery have shown the potential to meet all of the desired features of ubiquitous biosensor bioreceptors outlined in table 1. In addition to the stability, a key advantage of many synthetic reagent technologies is the speed at which they can be discovered, a critical capability when considering rapidly changing detection needs, including newly engineered threat agents to which no current bioreceptor technologies exist. Antibodies usually require several weeks to months to isolate from living hosts, whereas recent advances have demonstrated that synthetic routes can be employed using various synthetic peptide recognition element technologies (e.g., bacterial display and phage display) to produce bioreceptors in as little as a few days to a couple of weeks [5,12,18,19].

Coupled with the speed at which a new synthetic bioreceptor can be manufactured is the scale and cost of the manufactured product. Currently, major issues in antibody technology include time to supply and shelf-life stability. To avoid these standard pitfalls, ideal bioreceptor materials should be stable and manufacturable on demand [17]. In the case of ubiquitous biosensing, a plethora of platforms and materials integration issues will be utilized, making adaptability a key feature of the synthetic bioreceptor technology [1,3]. Of course, any synthetic reagent needs to meet affinity and specificity requirements to a standard of equivalency to antibody technology for practical use in

any biosensor system and real-world operational environments full of interfering background species [20].

Not surprisingly, synthetic peptide receptors show some of the greatest potential as synthetic antibody alternatives, as the mechanisms for specific binding from peptides (protein building blocks) are similar to antibody-antigen interactions [21]. However, rapid development of stable synthetic antibody replacements can be accomplished through bioengineered combinatorial libraries and advanced screening methods followed by mass-production through standard synthesis techniques. Several variations of combinatorial peptide technologies, including yeast, phage, and bacterial display and other chemically synthetic techniques, are currently used to isolate synthetic reagents. In peptide display, a small section of protein from the surface of a biological system (bacterium, virus, etc.) is engineered to present a randomized segment of amino acids (the building blocks of proteins) [11,16,22,23].

However, with unconstrained bacterial peptide display technology, the extremely fast replication rate of bacteria is exploited, and biological components (e.g., modified proteins) on an *E. coli* cell surface are harnessed to produce the peptide library of binders (Figure 3) [11,12,19]. This creates billions of individual peptide display clones, together creating a combinatorial library of peptide binding elements (i.e., candidates of synthetic affinity reagents). The large diversity of sequences presented by this library is then exposed to a target of interest, and stringency controls, in an alternating fashion. A built-in expression tag allows normalization of the expression library and affinity screening-a key enabling feature for reproducible reagent discovery. Although discovery is rapid, the characterization, optimization, and integration into assays and devices can still be a bottleneck to successful biosensor development using synthetic alternatives.

More recently, advances in peptide *in-situ* click chemistry have allowed scientists to develop a new class of highly manufacturable synthetic antibody alternatives: protein-catalyzed capture (PCC) Agents [7,17]. In this technology, the target protein is used as a highly selective catalytic scaffold for assembling its own capture agent. PCC Agents are assembled stepwise from comprehensive, chemically

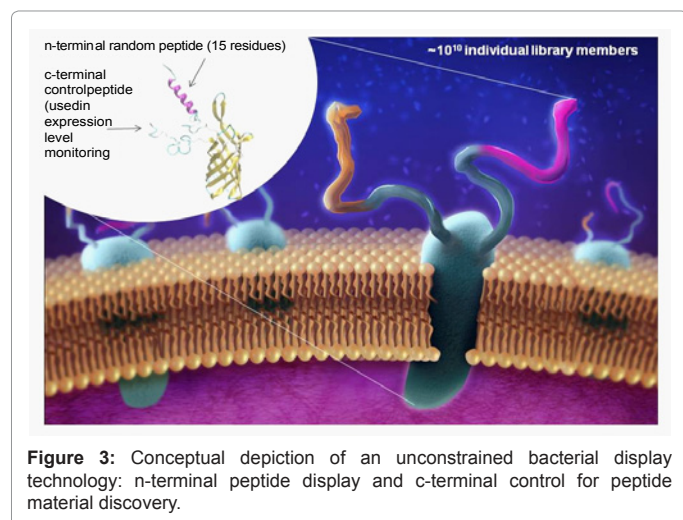


Figure 3: Conceptual depiction of an unconstrained bacterial display technology: n-terminal peptide display and c-terminal control for peptide material discovery.

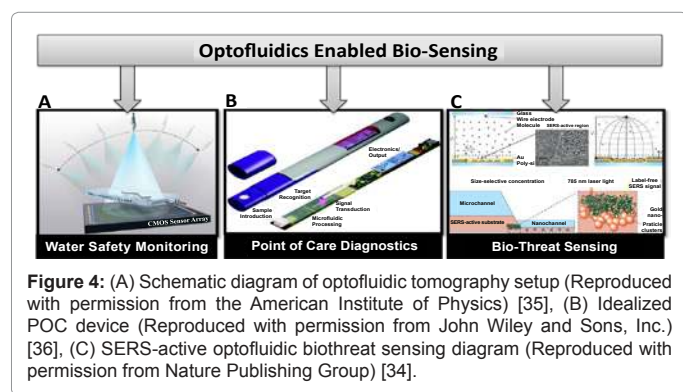


Figure 4: (A) Schematic diagram of optofluidic tomography setup (Reproduced with permission from the American Institute of Physics) [35], (B) Idealized POC device (Reproduced with permission from John Wiley and Sons, Inc.) [36], (C) SERS-active optofluidic biothreat sensing diagram (Reproduced with permission from Nature Publishing Group) [34].

synthesized peptide libraries that incorporate non-natural amino acids into the starting library to produce a final product that is highly stable, both on the shelf and *in vivo*. Additional functionality can be built into the initial design to produce a synthetic “drop-in replacement” that is highly adaptable and easily integrated into assays and sensor systems. The process is repeated to build biligand and triligand structures, each time refining the binding affinity and specificity through avidity with the target. The final synthetic peptide can be scaled up on-demand by automated chemical synthesis, avoiding the problem of batch-to-batch reproducibility.

Integrated Fluidics

The combined advances in microfabrication and micro-optic devices in optofluidics are key to enabling sensing of biological analytes, which are typically performed in aqueous systems. In the last decade, exciting and fundamental advances have been made in the synergistic combination of research in the fields of microfluidics and optics, coined “optofluidics” [24-26]. Optical techniques have long been used to analyze and characterize biological samples. Optical systems have traditionally served as a laboratory workhorse and include such instruments as flow cytometers, spectrophotometers, and microplate readers. More recently, these systems have been implemented in microdevices, such as on-chip waveguides and resonators. In parallel, we have seen the miniaturization of device architectures using microfabrication and clean-room techniques for the development of microfluidic devices [27]. Advances in the rapid fabrication of nano- and microfluidic devices bridge the SWAP-C

requirement gap and enable low-cost, small-volume sample handling and processing. Specifically for ubiquitous and mobile biosensing applications, optofluidics incorporates sample preparation and delivery with integrated transduction. The integrated optical transduction can take a variety of forms, including refractive index, fluorescence, Raman scattering, absorption, and polarization used individually or in concert to generate a robust signal output [24,26,28].

The versatility and broad application space of optofluidics is evidenced by a number of recent reviews and publications [24,26,28,29]. Figure 4 illustrates several examples of optofluidic technologies that enable biosensing applications. Technologies such as lens-free imaging and on-chip optofluidic tomography (Figure 4A) may prove to be invaluable where high-throughput, 3D lab-on-a-chip imaging of the specimen is necessary, such as water safety monitoring [30]. Optofluidics is also well-poised to revolutionize the field in point of care (POC) diagnostics. An idealized and ruggedized POC device is illustrated in Figure 4B, where the optofluidics is integrated into an all-encompassing device, which includes everything from sample introduction to electronic output [31]. Finally, the area of biothreat sensing has been covered by a number of reviews [20]. Figure 4C shows a sample surface enhanced raman spectroscopy (SERS) active, biothreat sensing modality [28]. This optofluidic sensor could be readily incorporated and modified and multiplexed into a variety of devices for detection of biological threats.

Personal Electronic Devices

Widespread sensing will likely be accomplished across a variety of platforms such as cell phones or other personal devices (PEDs), medical devices, autonomous vehicles, and a host of other data collection systems. Table 2 outlines the primary features needed for ubiquitous sensing including a universal interface, and an open and programmable architecture. Overarching engineering goals of low size, weight, power and cost (SWAP-C) have been the focus of point-of-care and persistent surveillance in particular, but are key to widespread use. Integration and fusion of onboard sensor suites and reporting through an agile and archival network will also be necessary. Recent trends in smartphone and other personal electronic device platforms show tremendous advances over the last several years, rapidly positioning smart-phone technology as a ubiquitous sensing platform [32]. Smartphones are open and programmable personal electronic devices that are equipped with a growing number of powerful embedded sensors, such as an accelerometer, digital compass, gyroscope, GPS, microphone, and camera. Combined these enable new sensing applications across a wide variety of domains such as mobile health, transportation, social networking, gaming, entertainment, and education.

Most recently, simple, cost-effective, compact, and lightweight imaging has been achieved on personal electronic platforms through the use of a smart-phone in conjunction with lens-free imaging. When lens-free imaging is used, high-resolution images are possible on a field-portable platform, which is ideal for affordable POC devices and is broadly extendable to ubiquitous sensing applications (e.g., food safety, environmental monitoring, homeland security). Researchers are exploring the use of personal electronic devices for biosensing applications ranging from food and water defense to explosive checkpoint analysis to point-of-care diagnostics [32-34]. Bridging the dimensional gap between single-channel analysis to microscopy provides a powerful approach to portable systems.

Conclusion

To conclude, biosensing is a powerful tool used in research and

clinical laboratories that if implemented on a global scale could transform our ability to monitor biological species in any location and environment. Specific advances in lab-on-a-chip technology to optofluidics as well as low-cost, highly networked mobile platforms open up the possibility, for the first time, to truly have a ubiquitous and archived biosensing network. Advances in the ability to produce highly manufacturable and robust synthetic bioreceptor alternatives now make biosensing in austere environments a reality. For example, smart skins used to be considered science fiction; several reports of patch electronic devices that stretch and conform to skin have been produced for physiological monitoring and drug delivery [35,36]. If trends continue, the fusion of these technological advances could revolutionize biosensing, with far-reaching impact, and make way for more commercial applications.

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