Magnetoresistive Biosensors for Quantitative Proteomics

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ABSTRACT

Protein detection and quantification is routinely performed in laboratories around the world for in-vitro diagnostics. Many different sensing platforms, such as mass spectrometry, optical biosensors, electrochemical biosensors, magnetic biosensors, etc., have been developed for quantitatively detection. The sandwich immunoassay is widely used as a labeled detection method due to its high specificity and flexibility allowing multiple different labels. While optical sensors use enzyme and fluorophore labels to detect proteins with high sensitivity, they often suffer from high background signal and present challenges in miniaturization. Magnetic biosensors, including nuclear magnetic resonance sensors, oscillator-based sensors, Hall-effect sensors, and magnetoresistive sensors, use the specific binding events between magnetic nanoparticles (MNPs) and target proteins to measure the analyte concentration. Compared with other biosensing techniques, magnetic sensors take advantage of the intrinsic lack of magnetic background in biological samples to achieve high sensitivity and high specificity, and are compatible with semiconductor-based fabrication processes to enable low-cost and small-size devices for point-of-care (POC) applications. Although still in development, magnetic biosensing is a promising technique for in-home testing and portable disease monitoring.

Keywords: quantitative proteomics, sandwich immunoassay, magnetic biosensor, point-of-care

1. INTRODUCTION

The advent of the post-genomic era has revealed that, while incredibly powerful and informative, sequencing alone is not sufficient to elucidate the full physiological state of an organism. Where genomics stops at measuring mRNA gene expression, proteomics measures further downstream after translation and post-translational modifications enabling large-scale investigation of protein expression, function, pathways, and protein-protein interaction. Proteomics provides accurate and timely information about biological systems that directly reflect the current physiological state. Quantitative proteomics[1–7] is the study of steady-state protein expression and perturbation-induced changes caused by diseases, such as cancer, which have significant time-dependent protein expression changes[8–12]. As such, quantitative proteomics is the cornerstone of modern diagnostics and plays an increasingly important role in identifying new disease-specific biomarkers and therapeutic targets.

Historically, mass spectrometry (MS)[13] and tandem mass spectrometry (MS/MS)[14] have been the workhorses for large-scale proteomic studies. Detection strategies have been developed for both top-down proteomics that measure intact proteins (<50 kDa) or large peptide fragments and bottom-up proteomics that is used for peptides from proteolytic digestion. Both techniques require significant sample pretreatment (e.g., depletion of highly abundant proteins, enrichment of relevant complexes, and protein fractionation), demand extensive data post-processing to interpret the data, and are not highly quantitative. Despite tremendous interest and effort, MS remains a laboratory-based technique with significant barriers to miniaturization.

Biosensors, based today primarily on optical sensors[15–26] and electrochemical sensors[27–42], have been developed as a compact, low-cost, and ease-of-use platform for quantitative proteomics[43–45]. Unlike mass spectrometry, these sensors are miniaturizable and thus innately portable. These biosensors can be broadly categorized depending on if a label is used in the detection process (i.e., label-free and labeled). Although label-free biosensing techniques (e.g., ChemFET[46–49], microcantilever[50–52]) can be very sensitive via direct measurement of an intrinsic property of the analyte (i.e., charge, mass)[53], labeled detection is more specific and often preferred given the large heterogeneity of analytes in clinical samples[43,54]. Furthermore, external forces (e.g., magnetic force) can be used to manipulate the tagged analytes for precise positioning[17,55] or separation[56]. Most labeled detection schemes use a variant of the sandwich immunoassay, as shown in Figure 1, where the analyte is flanked on both sides by a recognition molecule, the second of which is attached to the label. A sandwich assay first immobilizes analyte specific receptors (e.g., antibodies, aptamers, etc.) on the surface of the sensor that selectively bind to the target analytes (antigens). This is followed with a second binding event using analyte specific

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receptors conjugated to a label (e.g., an enzyme, fluorophore, or magnetic nanoparticle) that is detected by a corresponding sensor. As such, these affinity biosensors indirectly measure the analyte concentration via the number of labeled complexes tethered to the surface.

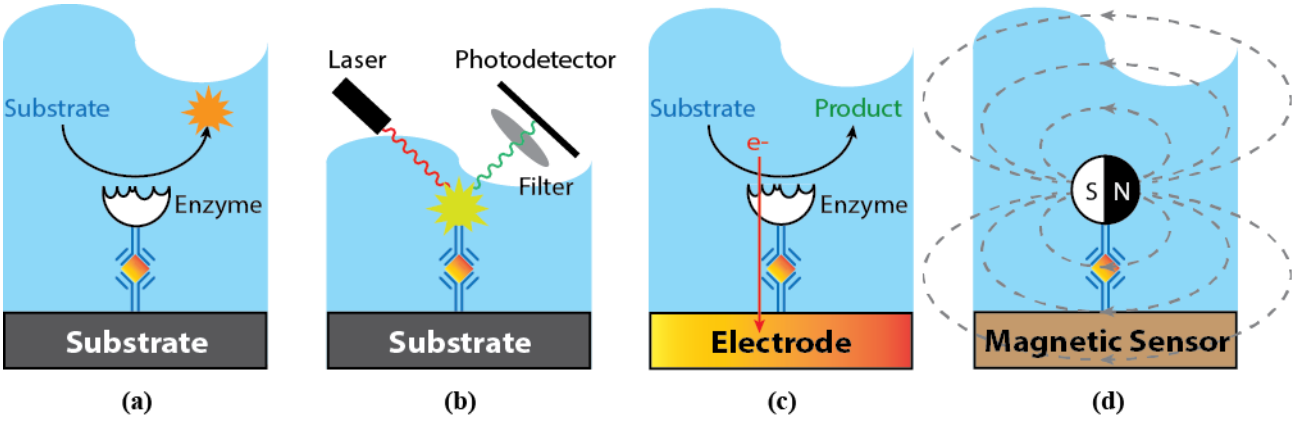


Figure 1. Illustration of sandwich immunoassay using (a) enzyme-labeled optical sensor, (b) fluorophore-labeled optical sensor, (c) enzyme-labeled electrochemical sensor, and (d) MNP-labeled magnetic sensor for quantitative proteomics.

Optical biosensors are highly sensitive and specific, easy to parallelize for multiplex detection, and low-cost[20]. While some optical biosensors, such as surface plasmon resonance (SPR), are label-free[15,16], most are enzyme-based[18,19], fluorescence-based[22,23], or chemiluminescence-based[24–26] labeled immunoassays. The enzyme-linked immunosorbent assay (ELISA), which uses an enzyme label that reacts with a substrate solution to generate a colorimetric signal (Figure 1a), is currently the gold standard in immunology. Whereas many other biosensing techniques have yet to make it out of research laboratories, this technique is currently one of the most widely used techniques[21]. Alternative formats using fluorophores[22] and quantum dots[57,58], where a laser excites the label and the fluorescent signature is measured (Figure 1b), have shown better sensitivity and quantification as there is a one-to-one relationship between the analyte and the label compared to the enzymatic assay where a single enzyme can repeatedly convert substrate. As such, traditional ELISAs require tight control over timing and routine calibration, often with every assay. These are not issues with fluorescent assays; however, fluorescent readout requires much more complex optical setups with narrow-band optical filters tuned to the excitation and emission frequencies. Chemiluminescence-based biosensors detect light emission due to a chemical reaction and therefore forego the excitation source, reducing the instrumentation complexity. Chemiluminescence-based biosensors also have very large dynamic range, up to 6 decades, and high sensitivity due to the low background signal[24,25]. However, tradeoffs between different types of chemiluminescence-based sensors result in none of them achieving high sensitivity, low cost, rapid assay time, and high quantum yield simultenously[25,26].

Electrochemical biosensors have been by far the most successful commercial biosensor to date, largely due to the glucometer, a critical device in managing healthcare for millions of diabetics worldwide[59]. Figure 1c illustrates an assay with an enzyme that catalyzes the substrate, resulting in an oxidation-reduction reaction[27–29]. Common electrochemistry techniques include potentiometry[30–33], amperometry[34–37], and impedance spectroscopy[38–41]. Glucometers are an example of an amperometry-based biosensor where the concentration of a byproduct (e.g., hydrogen peroxide) generated by an enzyme (e.g., glucose oxidase) reacting with glucose is detected and quantified. Although electrochemical biosensors have advantages of being very low-cost, easy to operate and miniaturize, they often suffer from high background, low specificity, dependence on pH and ionic strength of the solution, and may require highly specific enzymes[27,60].

Despite the success of optical and electrochemical biosensors, there remains an unmet need for quantitative proteomics platforms that are low-cost, highly sensitive with wide dynamic range, miniaturizable, require little to no sample pretreatment, and are scalable for point-of-care (POC) diagnostics. The remaining sections describe and compare different types of magnetic biosensors that address this need.

2. MAGNETIC BIOSENSING

Compared with its optical counterpart, magnetic biosensors do not require optical lasers, filters, or detectors. Thus, they can be more compact while maintaining the benefit of low-cost, high volume production due to the semiconductor-based fabrication process. This fabrication process also allows tight integration between the sensors and readout circuits

eliminating the need for external connections while simultaneously making large arrays of sensors possible. Another benefit of magnetic sensing is that biological samples are intrinsically non-magnetic, so the detection environment has very low background and does not require any sample pretreatment (i.e., the measurement is matrix-insensitive)[61]. As a result, magnetic sensors have very high sensitivity (down to femtomolar concentrations)[61–63] with wide dynamic range (6 decades)[61,62] and compact size, making them ideal for point-of-care applications[43,64,65]. Recently, several types of magnetic detectors have been demonstrated, including nuclear magnetic resonance (NMR)[66–68], oscillator-based sensors[69–72], Hall-effect sensors[73–79], and magnetoresistive sensors[61–63,80–89]. Although these sensors all use superparamagnetic nanoparticles to quantitatively detect analytes, their operation mechanisms are quite different.

2.1 Nuclear Magnetic Resonance

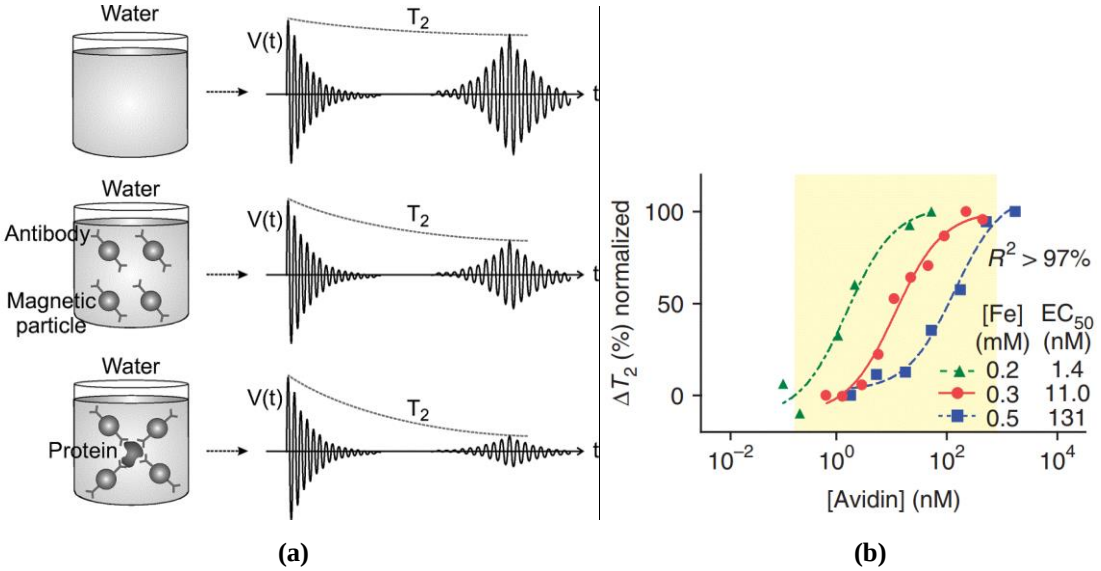


Figure 2. (a) Spin-spin relaxation time T_2 for pure water, antibody-coated magnetic particles, and antibody-protein complexes[66] and (b) measured NMR calibration curve[67].

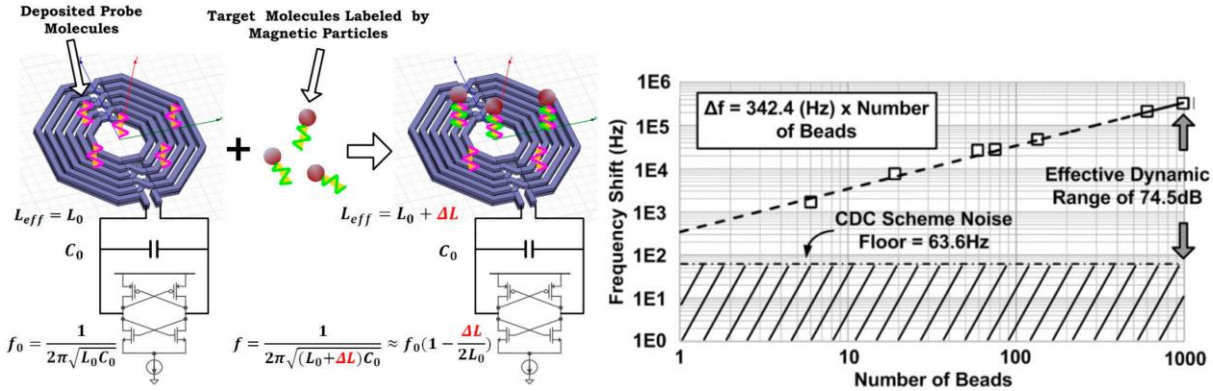
NMR is an indirect method to detect proteins by measuring the spin-spin relaxation time (T_2) of water molecules[90]. The setup requires at least one permanent magnet to provide a DC bias field B_0 and one electromagnet to provide an RF excitation field B_1[66]. By setting the frequency of B_1 to the Larmor frequency of the protons in water, which is proportional to B_0, the protons periodically absorb the energy from B_1 perturbing their alignment from B_0. Due to the proton-proton interaction, some protons will be out-of-phase and the total magnetic moment decays over time as the protons precess after B_1 is removed. The rate of the decay is characterized by the spin-spin relaxation time, T_2. By adding antibody-coated magnetic nanoparticles (MNPs) to the sample, the T_2 signal decreases as antibody-antigen complexes are formed, as shown in Figure 2a. These binding events produce large aggregates, further intensifying the perturbation and shortening the T_2 signal thus allowing the concentration to be quantified. Biological experiments have shown avidin detection with a dynamic range of 80 dB[67].

Miniaturization is the key challenge for this technique. Since it requires a permanent magnet and an electromagnet, the system is usually bulky[91]. A miniaturized NMR system, which can be held in the palm of a hand, was recently demonstrated[68]. However, since the signal amplitude is quadratically proportional to the DC bias field B_0, reducing the magnet size for portable applications significantly degrades the sensitivity. As a result, this NMR system only achieved a sensitivity of 3 nM[67] (Figure 2b) compared to benchtop equivalents which have a sensitivity of 140 fM[92]. Further improving the sensitivity and system size is difficult due to the fundamental tradeoff between the signal amplitude and the magnet size.

2.2 Oscillator-based Biosensor

Oscillator-based biosensors are usually tuned LC resonators where the resonant frequency is dependent on the number of tethered MNPs. Figure 3a shows one such example where a sandwich immunoassay labeled with MNPs is assembled on the surface of an inductor. The magnetic field generated by current passing through the inductor magnetizes the MNPs, which alters the inductance, and thus changes the resonant frequency[69,70]. These sensors are very attractive as they do not

need an external magnetic field and are fully CMOS compatible allowing them to be compact and low-cost. Techniques such as correlated double counting (CDC) have been employed to reduce correlated noise and environmental conditions such as temperature drift allowing detection of a single 1 μm MNP (Figure 3b)[70]. However, these sensors often have reproducibility issues due to the non-uniform magnetic field resulting in spatial dependency and non-linearities. This issue can be remedied using a bowl-shaped inductor, but this requires more exotic fabrication and is not always CMOS compatible[72]. Although this technique was not used for detecting proteins, it has successfully detected DNA[69] and cells[71].

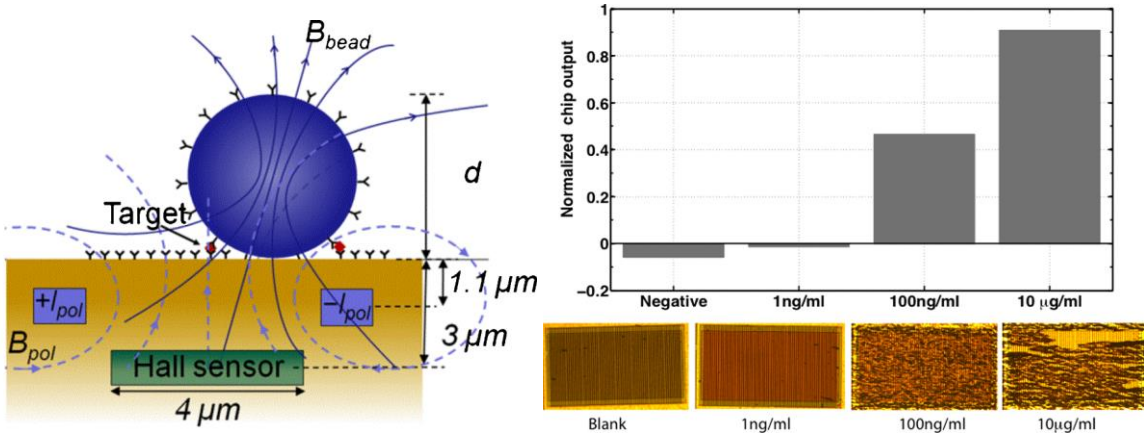


(a) **(b)**

Figure 3. (a) Illustration showing how tethered MNPs cause a resonance frequency shift in an LC oscillator[69] and (b) measured calibration curve[70].

2.3 Hall-effect Sensor

Hall-effect sensors measure an induced voltage caused by the force a perpendicular magnetic field exerts on a charge carrying ion. These sensors can be realized using the diffusion layer (n-well) of a transistor and thus are compatible with standard semiconductor fabrication processes. However, the diffusion layer is the bottom layer in a CMOS process, so post-processing is required to remove all (or most) metal and interlayer dielectric material above the sensor to minimize the distance between the sensors and MNPs[73]. Figure 4a shows a sandwich immunoassay on top of a Hall-effect sensor with an integrated electromagnet to magnetize the MNPs. The presence of the MNPs induces a voltage on the underlying sensor that is readout by the nearby circuitry.



(a) **(b)**

Figure 4. (a) Illustration of a Hall-effect sensor detecting a single captured MNP[78] and (b) measured concentrations with optical images for various concentrations of HSA at concentrations as low as 1ng/mL (15 pM)[79].

Researchers have demonstrated both magnetometry-based biosensing[73–75] and relaxation-based biosensing[76,77], where instead of leaving the magnetic field on constantly and measuring the perturbation, the magnetic field is pulsed and the temporal dynamic response is measured as the MNPs relax back to equilibrium. The detail of these mechanisms will be

discussed further in Section 3, but it should be noted that the relaxation of the MNPs is different from NMR sensors, which are based on the proton-proton interaction of water molecules. To maximize the signal in this work, the sensor was sized comparable to a single 4 μm MNP and thus each sensor can detect only one MNP[78]. To have a reasonable dynamic range, a large array containing 10k pixels was built with single MNP sensitivity and a dynamic range of 80 dB by combining all of the small sensors into one effective sensor[79]. This sensor array was used to detect Human Serum Albumin (HSA) with a sensitivity of 15 pM (Figure 4b). By limiting the design to one MNP per sensor and using relaxometry-based biosensing technique, Hall-effect sensors resolve the MNP location dependency and field non-uniformity issue that impedes oscillator-based sensors. However, one MNP per sensor requires a very large array to achieve sufficient dynamic range, which becomes a bottleneck for Hall-effect sensors.

2.4 Magnetoresistive Sensor

Oscillator-based sensors and Hall-effect sensors can both detect a single 1 μm MNP; however, micrometer sized MNPs diffuse very slowly in solution and are much, much larger than the target proteins, thus they require washing steps to remove unbound MNPs and have longer assay time[43]. Magnetoresistive sensors are used extensively in commercial applications as the read-head in a hard disk drive. These sensors are elaborately engineered stacks of magnetic and non-magnetic thin films (Figure 5a) and have much higher transduction efficiency, allowing them to detect nanometer sized MNPs. The operation of these sensors is deeply rooted in quantum mechanics and beyond the scope of this paper. At a high level, the tethered MNPs alter the local magnetic field and thus the sensor resistance (Figure 5b), which is measured by the readout circuitry to quantify the analyte concentration. Giant magnetoresistive (GMR) sensors and magnetic tunnel junction (MTJ) sensors are the two major types of magnetoresistive sensor used today.

GMR sensors have shown exceptional sensitivity compared to the other magnetic sensors. As few as 20 MNPs with a diameter of 16 nm have been detected[80,81]. A 256-pixel GMR biochip was reported that achieved a sensitivity of 10 fM while detecting a cancer biomarker, secretory leukocyte peptidase inhibitor (SLPI)[63]. In multiplexed protein assays (10 analytes), high sensitivity (5 fM limit of detection) and broad dynamic range (120 dB) were demonstrated, detecting another cancer biomarker, carcinoembryonic antigen (CEA) (Figure 5c)[61,62]. To achieve this level of performance, several calibration routines are required including a temperature correction scheme[82]. Notably, this work also performed a head-to-head comparison using the same antibodies in a traditional ELISA assay format and found that the magnetic immunoassay was over 1,000× more sensitive and had a larger dynamic range[61,62]. Although a more complicated fabrication process is required, GMR sensors have been successfully fabricated on CMOS chips[83,84].

MTJ sensors have even higher magnetoresistance than GMR sensors, potentially making them even more sensitive; however, they also have issues of higher noise[93] and pinhole defects[94], diminishing their utility. Despite these issues, they have found applications in hard disk drives and random-access memory. Researchers have used MTJ sensors to detect DNA and proteins, but with limited sensitivity (nanomolar concentrations)[88,89]. Nevertheless, given the high transduction efficiency, small size, and compatibility with semiconductor technology, MTJ biosensors remain extremely attractive.

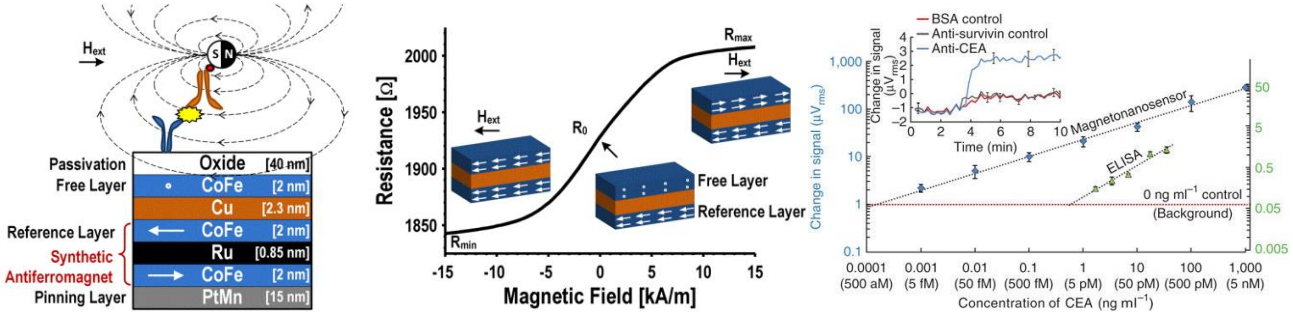


Figure 5. (a) GMR filmstack containing multiple magnetic and non-magnetic layers, (b) Plot of sensor resistance as a function of the external magnetic field[63], and (c) Measured calibration curve demonstrating extremely high sensitivity (5 fM)[61].

3. MAGNETIC BIOSENSING TECHNIQUES

While NMR uses a solution phase assay to measure the analyte concentration, all the other magnetic sensors share the same sandwich immunoassay with a magnetic nanoparticle label tethered to the surface of the sensor. There are two

common detection techniques that can be used with the aforementioned surface-based affinity assays: magnetometry and relaxometry, both of which are illustrated in Figure 6. In the absence of a magnetic field, the magnetic moment of the MNPs is randomly distributed resulting in zero net field for the underlying magnetic sensors. When an external magnetic field (DC or AC) is applied, the magnetic moments all align with the field generating a stray magnetic field that opposes the applied field at the sensor. Magnetometry measures the field difference with and without MNPs to quantify the number of tethered MNPs. For relaxometry, the applied magnetic field is rapidly removed and the sensors temporally monitor the change in magnetic field as the MNPs slowly randomize, capturing the dynamics.

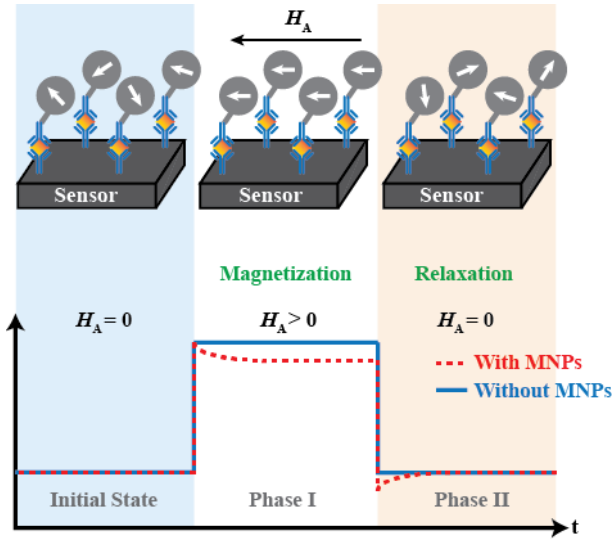


Figure 6. Illustration depicting magnetometry- and relaxometry-based biosensing techniques[86].

3.1 Magnetometry-based Biosensing

As the most straightforward method to detect MNPs, magnetometry-based biosensing was widely used by oscillator-based sensors[69–72], Hall-effect sensors[73–75], and GMR sensors[61–63,80–84]. However, this technique suffers from several drawbacks. First, the miniscule signal from the MNPs is superimposed on a large baseline signal from the applied field demanding very high dynamic range from the readout circuitry. This baseline can be rejected by a reference sensor, if one is able to achieve good matching between the sensors, which unfortunately is not always possible. Second, this technique requires a uniform external field to remove positional dependency, especially in low-concentration measurements. This is often accomplished using an off-chip electromagnet[63] or a special structure for an on-chip coil[72]. Lastly, magnetometry is sensitive to temperature drift, process variation, and environmental perturbation that requires precise calibration and signal processing to overcome[82].

3.2 Relaxometry-based Biosensing

Relaxometry-based biosensing technique resolves most of these issues. While there is no literature for oscillator-based sensors using relaxometry, Hall-effect sensors[76–79] and GMR sensors[85–87] have been demonstrated. By detecting the relaxation of the MNPs temporally after turning off the applied field, this technique pushes the difficulty from accuracy in amplitude to accuracy in time. It accordingly overcomes the low signal-to-baseline ratio and eases the field uniformity requirement. Furthermore, techniques such as magnetic correlated double sampling (MCDS) that repeat the relaxation and use the same sensor for correlated sampling remove the need for reference sensors and are immune to slowly changing environmental perturbations[79,86]. Since different sized MNPs have different relaxation times and dynamics, relaxometry introduces another degree of freedom that enables multiplexed bioassays[76,87].

However, relaxometry-based biosensing has some drawbacks that still need to be investigated. First, the applied magnetic field cannot collapse instantaneously resulting in a deadzone that makes detection of MNPs with very fast relaxation times impossible. Off-chip electromagnets have been reported to have a deadzone as low as 1.4 μs, which is still not fast enough for detecting some MNPs[86,87]. On-chip striplines can reduce the deadzone to just 16 ns[77], but suffer from area and device heating constraints[78]. Moreover, the relaxation signal decreases over time, thus high-speed readout circuitry is required to

capture the signal. As a result, low noise and high speed are both required for the readout circuitry, but they are often at odds with each other.

4. CONCLUSION

This paper reviewed the most commonly used magnetic biosensors for quantitative proteomics. Like an ELISA, magnetic sensors use MNPs instead of enzymes or fluorophores as labels to detect captured analytes in magnetic immunoassay. Magnetic biosensors take advantage of the low background signal since biological samples are non-magnetic and the compatibility with the semiconductor technology for low cost production. Table 1 summarizes the magnetic sensors and their respective performance. Each type of sensor and each sensing technique (e.g. magnetometry and relaxometry) has advantages and drawbacks, which make them suitable in different applications. Specifically, high sensitivity and wide dynamic range make magnetoresistive sensors a good candidate for measuring extremely low concentration analytes, which is usually the case in early stage disease detection. Due to low background and compact size, magnetic sensors have great potential for POC applications, such as in-home testing and continuous disease monitoring.

Table 1. Comparison of the magnetic sensors.

Sensor Type	**NMR**[67]	**Oscillator-based**[70]	**Hall-effect**[79]	**GMR**[61,62]
MNP Diameter	38 nm	1 μm	1 μm	50 nm
Minimum Detectable # of MNPs	N/A	1	1	70
Sensitivity	3 nM	N/A	15 pM	5 fM
Dynamic Range	80 dB	74.5 dB	80 dB	120 dB
Pixel Size	N/A	0.0144 mm^2	0.6 mm^2*	0.01 mm^2
# of Pixels	1	48	1*	64
Readout Time/Pixel	N/A	10 ms	8 s	62.5 ms
Technique	N/A	Magnetometry	Relaxometry	Magnetometry

* Hall-effect biosensors use the whole sensor array as an effective single sensor for biological experiment.

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