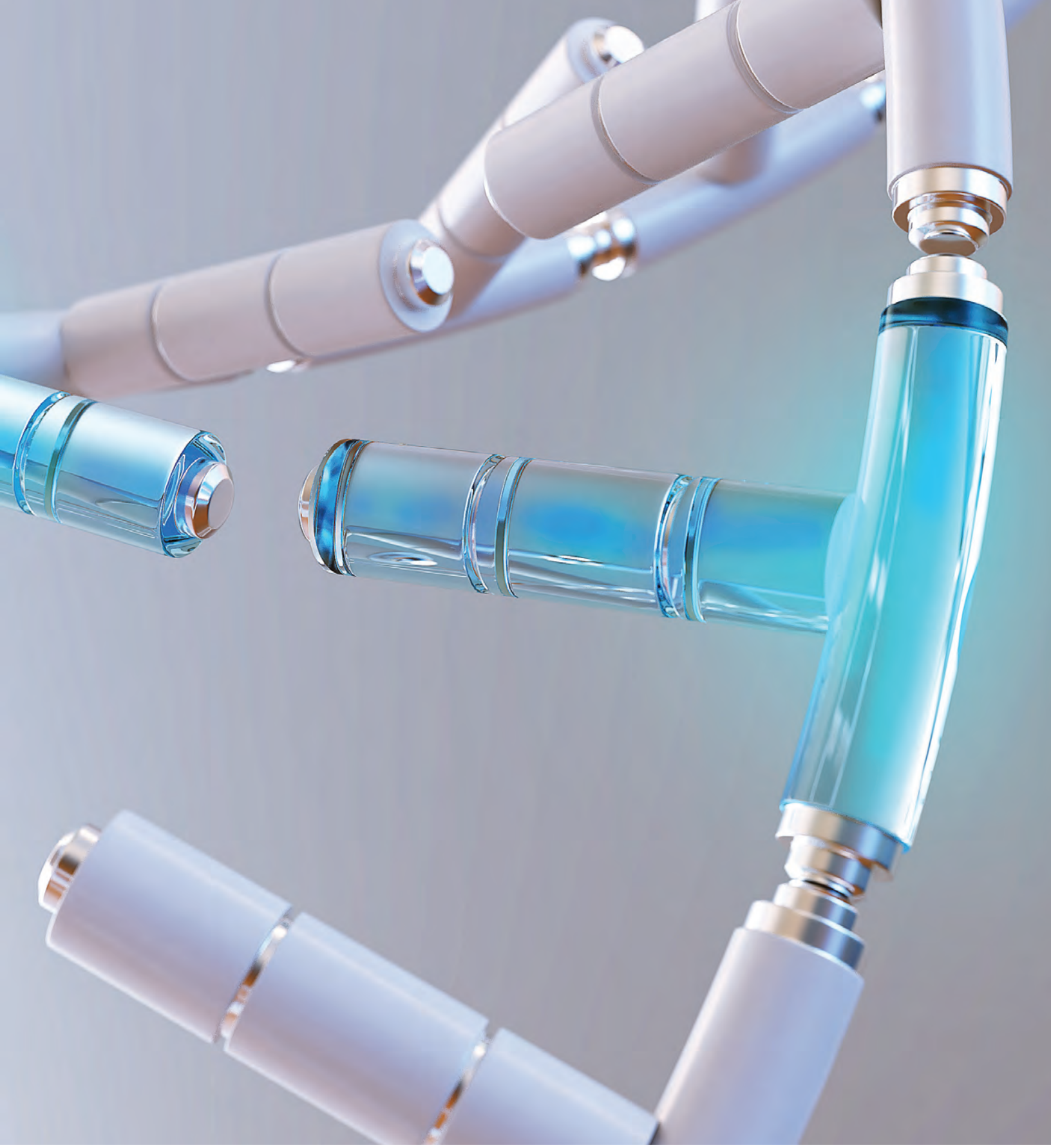

Electrochemical Probes

GLEN RESEARCH

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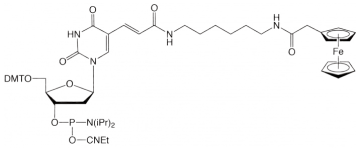
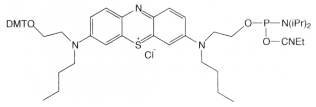
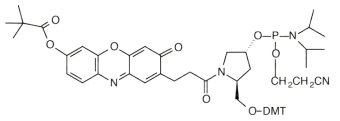
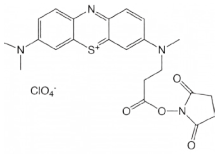
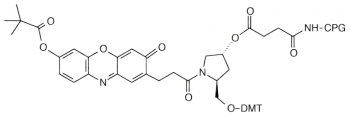
Glen Research provides numerous non-fluorescent labels for oligonucleotide incorporation. In this note, we will focus on electrochemically active labels, providing an introductory overview for interested readers to obtain some background information on the topic. We will then take a detailed look at an interesting recent research application of one label, methylene blue, with an aptamer.

Background

Electrochemically active labels attached to oligonucleotides have found many applications since the seminal discoveries and characterization of long-range electron transfer through double stranded nucleic acids. Electrochemical probe analogs that Glen Research provide include the stable iron (II) complex ferrocene and two molecules often thought of as fluorescent labels: methylene blue and Redmond Red®. These molecules also have excellent reversible redox properties that make them electrochemically useful.

Table 1. Electrochemically Active Probes

Ferrocene-dT	Methylene Blue	Redmond Red
 **Ferrocene-DT-CE-Phosphoramidite**	 **Methylene Blue II Phosphoramidite**	 **Redmond Red Phosphoramidite**
	 **Methylene Blue NHS Ester**	 **Redmond Red CPG**
Ferrocene features an excellent stability profile, attracting considerable interest for DNA labeling to generate probes for electrochemical detection.	Methylene blue has also been shown to be unmatched in performance as a redox-active reporter for electrochemical biosensors.	Redmond Red is a fluorescent dye tag that can be used as a probe to detect abasic sites electrochemically in double-stranded DNA.

Significant progress in developing electrochemical platforms for biomolecule detection has been motivated in part by the fact that these methods are low cost, portable, and require only modest instrumentation.[1] In addition to the electrochemical group, most of these platforms involve the use of oligonucleotides labeled with a thiol modifier. Thiols have a strong affinity for gold, and as such, the oligonucleotides will naturally form uniformly thick self-assembled monolayers on the surface of a gold electrode. When a voltage is applied to the electrode, the current will vary according to the proximity of the active label to the gold surface.[2] With this format, methylene blue, Redmond Red and ferrocene have been used in a wide variety of investigations that cover the detection of single-base mismatches, DNA methylation, important analytes, abasic sites, and DNA damage.[3]

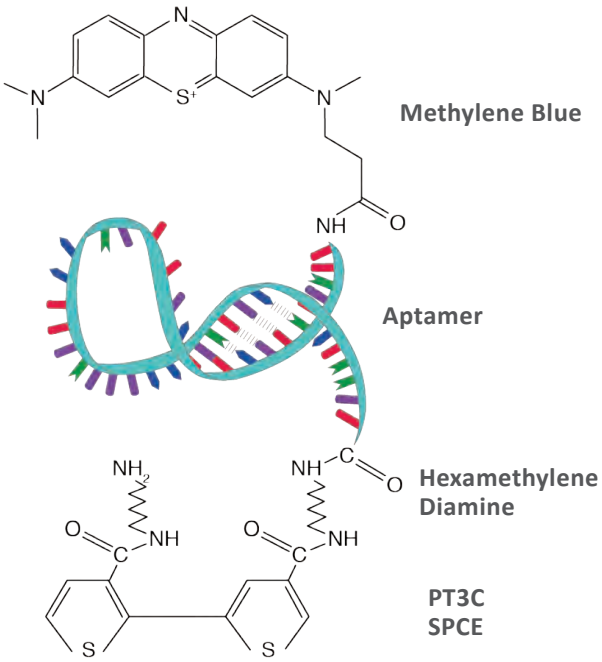
Biosensor Development

As noted earlier, the distance dependence of electrode current has been demonstrated and is now utilized for *in vitro* diagnostic applications using gold electrodes and well-defined oligonucleotide hybridization probes labeled with ferrocene.[4] An emerging method of sensor development is the coupling of analyte binding to an aptamer to an electrochemical signal. In this method, the aptamer will position the probe label at a certain average distance from the surface when analyte is not present, resulting in a low background current signal. When analyte binds to the aptamer, a conformational change occurs, bringing the attached label closer to the electrode surface and increasing the electrode current.

A recent example of this has been demonstrated in the Zelji lab, where researchers created an electrochemical sensor for aflatoxin B1 (AFB1) detection.[5] Aflatoxin B1 is a highly carcinogenic, well-known contaminant of nuts, cereals, beer, coffee beans, spices, and dried fruits. This and other mycotoxins present a serious health concern, so improved modes to detect them are of interest.

The researchers developed their electrochemical sensor by using a methylene blue redox probe labeled aptamer as a signaling fragment, and polythiophene-3-carboxylic acid (PT3C) as a signal-enhancer on the electrode surface. The aptamer was prepared with a carboxyl modification on the 5' end and a methylene blue modification on the 3' end.

Several aspects of this work were noteworthy. Instead of using a gold electrode and building a self-assembled monolayer surface, the researchers chose a carbon electrode with a polymer system (PT3C) that not only strongly adsorbed to the carbon surface, but could also later be modified to attach the aptamer after adsorption. The PT3C was first adsorbed onto the screen-printed carbon electrode (SPCE) interface, and the aptamer-methylene blue conjugate was then immobilized by attachment to the hexamethylenediamine (HMDA) spacer using carbodiimide coupling chemistry. Figure 2 depicts the chemical structures used, representing



the aptamer as a single stranded oligonucleotide with some secondary structure.

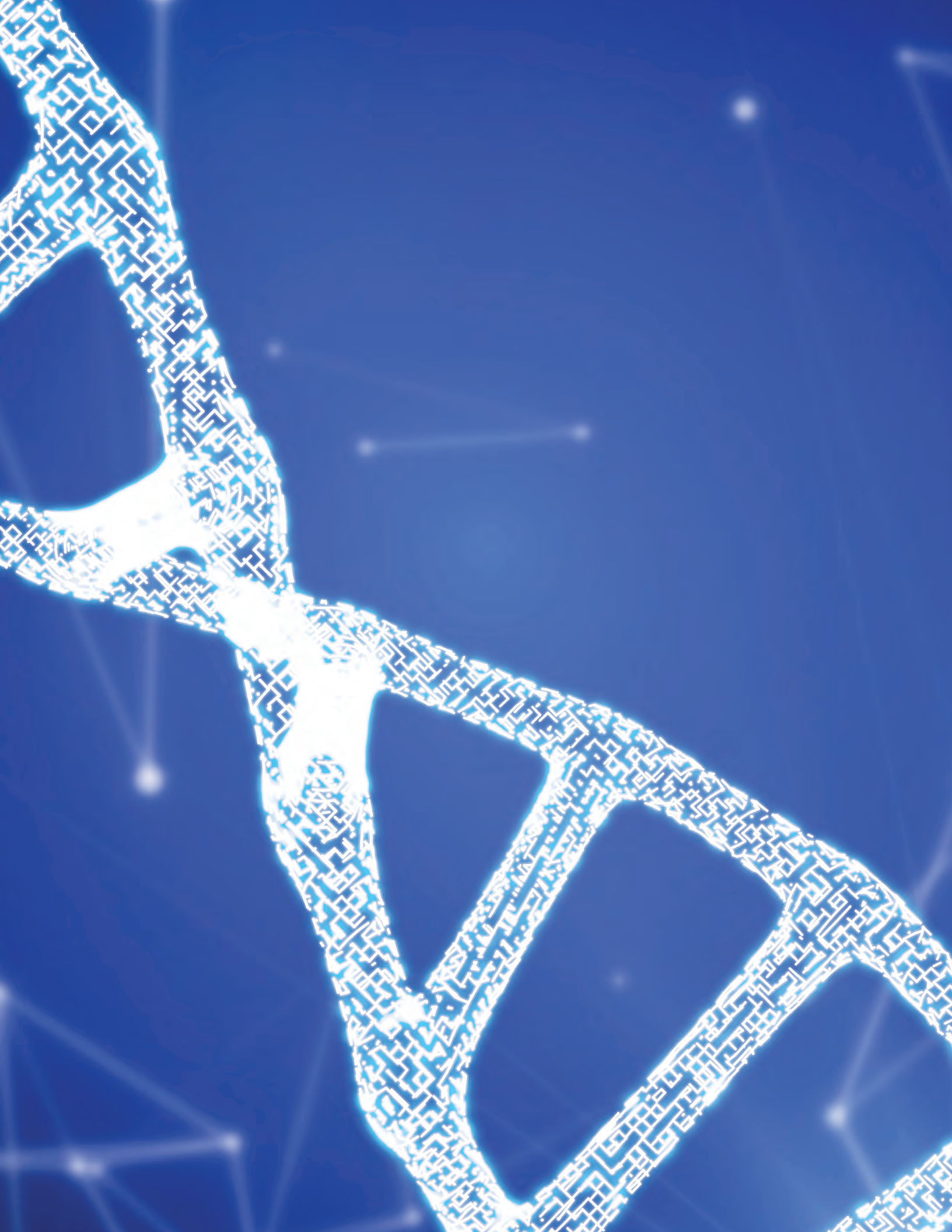


Figure 1. Methylene Blue Labeling of Aptasensor

The binding of AFB1 to the aptamer results in a conformational change that modifies the distance of the covalently attached methylene blue to the electrode surface, resulting in a significant change in current (Figure 3). The detection is specific to AFB1 versus the structurally related Ochratoxin, and the assay is linear, with a dynamic range from 8-100 pM and a limit of detection of 5 pM.

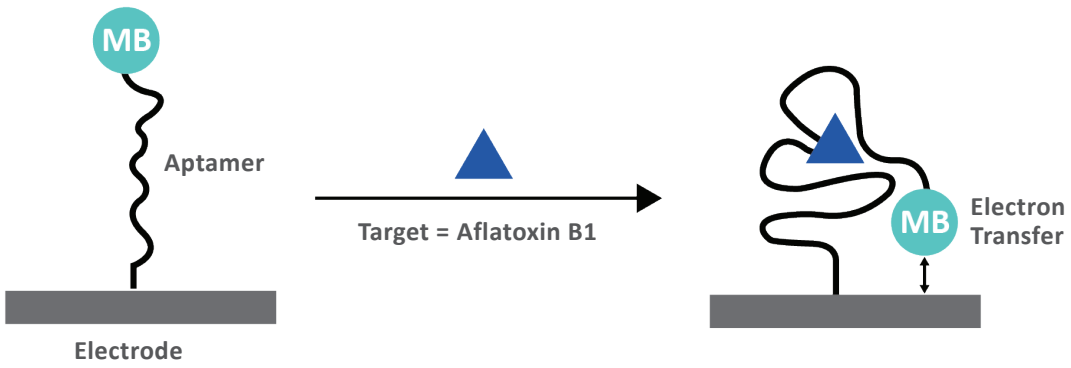


Figure 2. Aptasensor for Aflatoxin B1 Detection

Researchers continue to explore the best practices for implementation of optimal electrochemical biosensors.[6] In addition to the critically important aptamer itself, the chemistry and mode of attachment to the electrode must be considered in terms of a stable and reproducible support for the attached detection moieties. Although most published electrode designs have used gold electrodes and thiol-group attachment of self-assembled monolayers as the base for attachment of the detectable probes, the aforementioned Zelji report shows that a significantly different polymer adsorbed to carbon electrode preparation chemistry can be quite effective. The length of linker arms and the distances of the redox probe in the absence and presence of analyte must be carefully considered in order to effect the large signal change that benefits biosensor sensitivity. In addition, the composition of the solution, including pH and viscosity, may affect the disposition of the redox probes near the electrode surface during the detection assay and should therefore also be considered in optimal biosensor design.

The electrochemical options that Glen Research provides are listed in Table 2. The labels are available for 5', 3', or internal modification. Two modes for attachment of methylene blue to oligonucleotides are possible: it can be attached using the phosphoramidite at or near the 5'-end of oligonucleotides. We recommend mid- or near the 5'-end to avoid branching reactions that can occur on this molecule. The NHS ester is also available for post-synthetic modification of primary amino-modified oligonucleotides, providing further options for placement of the label and linker length. Redmond Red is available as both the phosphoramidite and the CPG support, and ferrocene is offered as a dT analog.

Table 2. Electrochemical Labeling Reagents

Item	Pack Size	Catalog No.
Ferrocene-dT-CE Phosphoramidite	0.25g	10-1576-02
	100µmol	10-1576-90
	50µmol	10-1576-95
	Custom Packaging	10-1576-SP
Methylene Blue NHS Ester	5.4mg	50-1960-23
Methylene Blue II Phosphoramidite	0.25g	10-5961-02
	100 µmol	10-5961-90
	50 µmol	10-5961-95
	Custom Packaging	10-5961-SP
Redmond Red® Phosphoramidite	0.25g	10-5920-02
	100µmol	10-5920-90
	50µmol	10-5920-95
	Custom Packaging	10-5920-SP
Redmond Red® CPG	0.1g	20-5920-01
	1.0g	20-5920-10
	1x10µmol	20-5920-13
	1x15µmol	20-5920-14
	4x1.0µmol	20-5920-41
	4x0.2µmol	20-5920-42
	Custom Packaging	20-5920-SP

References

1. A.O Sadik, A.O. Aluoch, A. Zhou, *Biosens. Bioelectron.,* 2009, **24(9)**,2749–2765
2. S. Creager, C. J. Yu, C. Bamdad, S. O'Connor, T. MacLean, E. Lam, Y. Chong, G.T. Olsen, J. Luo, M. Gozin, and J.F. Kayyem, *J. Am. Chem. Soc.,* 1999, **121**, 1059-1064
3. E. Palecek, M. Bartosik, *Chem. Rev.,* 2012, **112**, 3427-3481
4. R.M. Umek, S.W. Lin, J. Vielmetter, R.H. Terbrueggen, B. Irvine, C.J. Yu, J.F. Kayyem, H. Yowanto, G.F. Blackburn, D.H. Farkas and Y-P. Chen, *J. Mol. Diagn.,* 2001, **3(2)**, 74-84
5. H. Zejli, K.Y. Goud, J.L. Marty, *Sensing and Bio-Sensing Research,* 2019, **25**, 100290
6. S.M. Silva, R. Tavallaie, V.R. Goncales, R.H. Utama, M.B. Kashi, D.B. Hibbert, R.D. Tilley, J.J. Gooding, *Langmuir,* 2018, **34**, 1249–1255

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