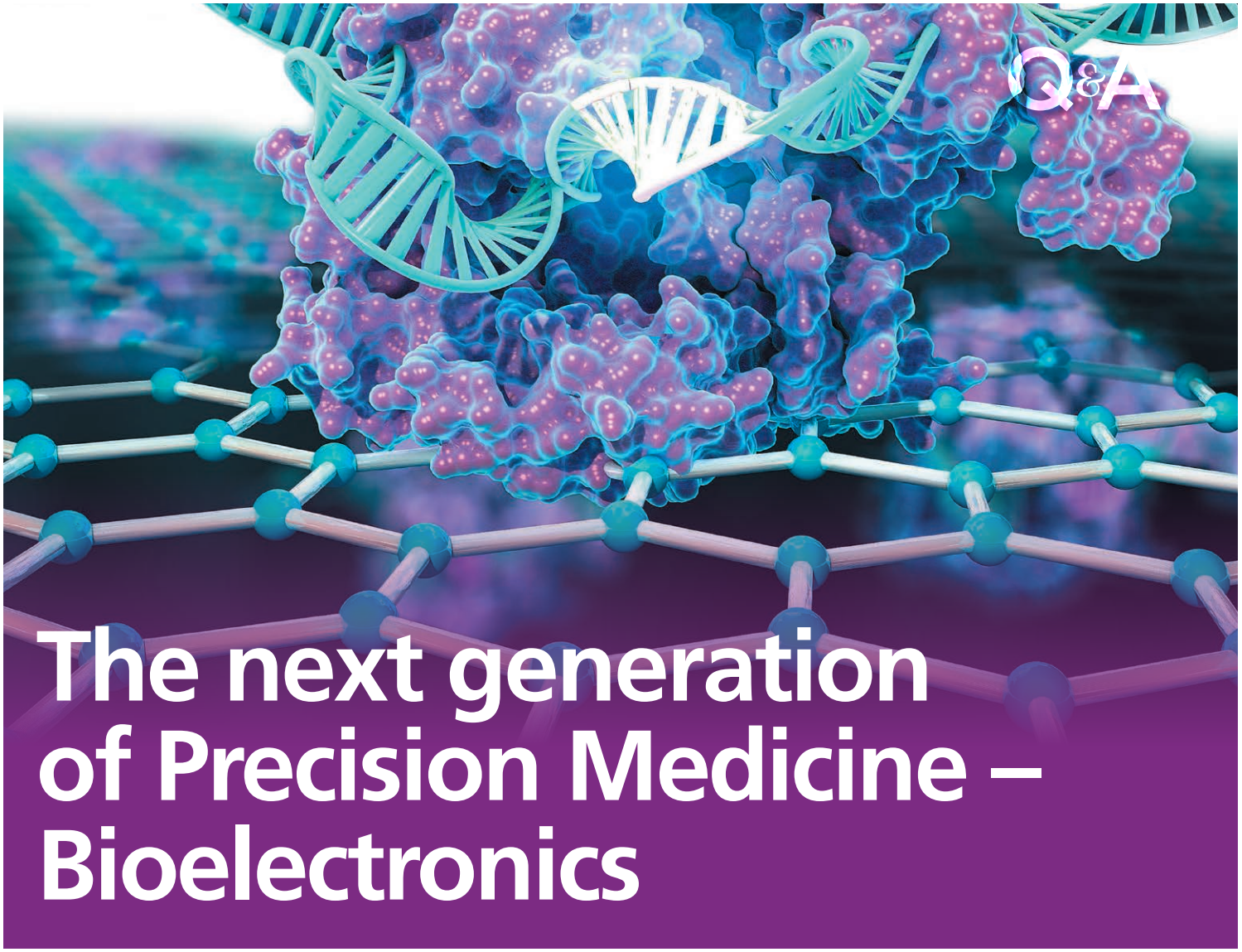

The next generation of Precision Medicine – Bioelectronics

An interview with **Michael Heltzen and Brett Goldsmith**

MANY EFFORTS IN the past have been made to link biology directly to various read-out technologies to make molecular control signals accessible for monitoring in near-real time. Cardea Bio has taken up this challenge with a new class of devices that transduce biological binding surface events to a signal generated in a substrate. The key to Cardea's approach is to stabilize receptor biomolecules on the gate of a graphene transistor that bridge biology binding events on the transistor's surface to an electrical signal in the transistor's substrate. Cardea Bio's mission is to provide a means to connect digital networks (the Internet, computers, and our cell phones) to the networks of biomolecular signals measure by the device.

We contacted Michael Heltzen and Brett Goldsmith of Cardea Bio to ask a few questions on the technology and applications of the device directed to how this platform could be used to advance precision medicine in drug development and in the clinic.

Q1. What are the benefits that Cardea Bio brings to the market compared to related measurement platforms in terms of e.g., sensitivity, selectivity, speed? In this regard, how does Cardea Bio differentiate its performance relative to competitive systems?

A. The Cardean Infrastructure has several distinct advantages over traditional measurement platforms (lateral flow, surface plasmon resonance (SPR), Bio-Layer Interferometry (BLI), next-generation sequencing (NGS), etc.), many of which contain outdated methods and technology (e.g. sample prep for optics) nearly a half-century old.

Optics-based technologies (SPR, BLI) contain an inherent limitation when it comes to measurement; the molecules they aim to detect are often smaller than the wavelengths of light needed to observe them optically. As a result, for example, technologies that measure nucleotides (e.g. NGS) require the mass-amplification of the target molecule to meet the minimum threshold for detection. Additionally, optics-based technologies cannot directly detect amino acids; rather, they rely on secondary tags bound to the target to produce a detectable signal, providing an indirect approximation at best. Where optics-based

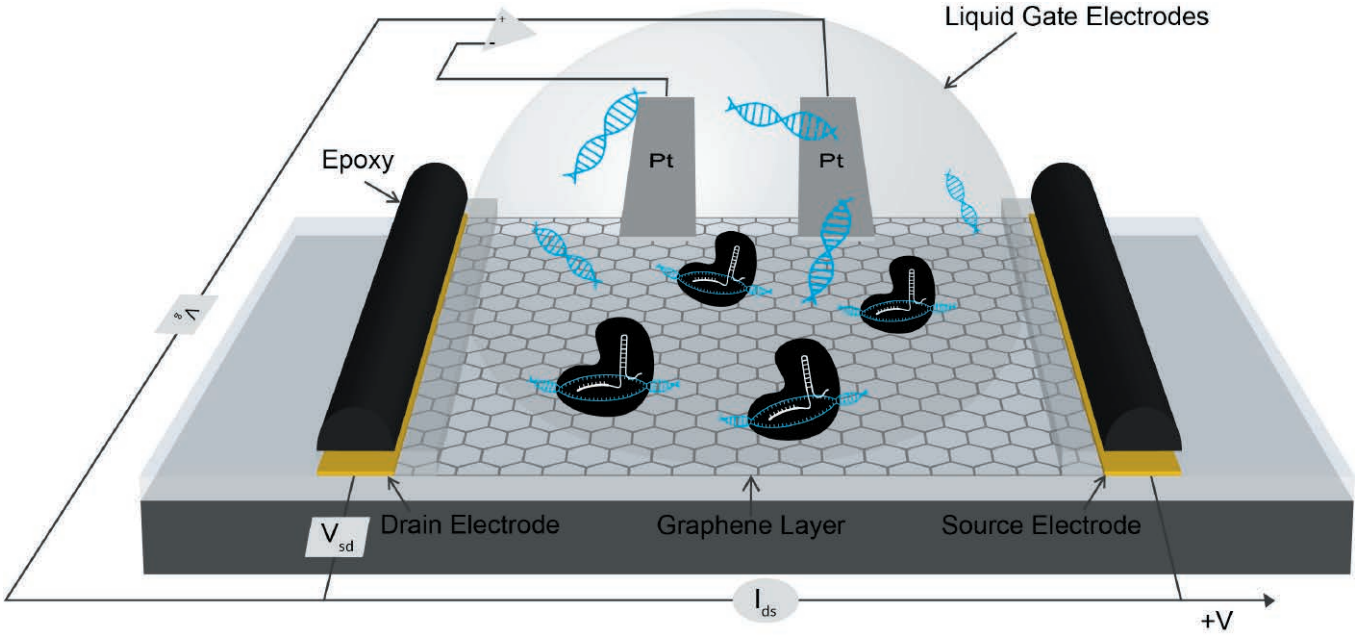

Figure 1: A diagram describing the action of the Cardean biology gated transistor. A capture chemistry such as an antibody is connected to the graphene surface. The graphene is constructed to be part of a liquid gated transistor. At the start, there is nothing bound to the capture chemistry.

technologies provide a snapshot of a biological interaction, electronic detection provide a near-real time monitoring capability over the course of the experiment. Finally, many traditional technologies require cumbersome equipment, complex protocols, and extensive training.

The Cardean Infrastructure overcomes limitations of optics-based technologies. Cardea's direct detection does not rely on any tags that can interfere with the biomolecules being studied, or cause non-specific interference that can influence results, as with optical technologies. Cardea is able to directly read and quantitate the biological signal in question in near real time in live data streams. Our system can detect molecules that are smaller than visible wavelengths of light – allowing us to directly detect biomarkers in minutes, as well as binding activity without amplification, including whole genes, single nucleotide polymorphisms (SNPs), and small molecules.

We further potentiate the capabilities of direct electrical detection by using graphene at the core of our transistor. Graphene is highly biocompatible, unlike gold and silicon because it can operate directly in salt-solutions, such as biological fluids. It is also very sensitive to changes in its charge environment such as those caused when two molecules bind near the surface. When our target binds to the capture mechanism immobilized to the surface of our transistor, the binding event changes the electrical properties of the graphene that can then be read as a robust signal (as diagrammed in **Figures 1 and 2**). These signals are fed into machine learning algorithms that improve accuracy and sample-to-answer times as well as letting us gain an understanding of the biology. The current generation of Cardea Infrastructure is already capable of unparalleled sensitivity, selectivity, and speed.

Q2. What portfolio of assays does Cardea Bio envision for precision medicine applications:

In drug development?

A. Cardean transistors are used by our partners to do drug discovery via primary and secondary screenings, especially with respect to "hit to lead" validation. This adds another fast, cost-effective, platform that has demonstrated comparable rank ordering to BLI.[1]

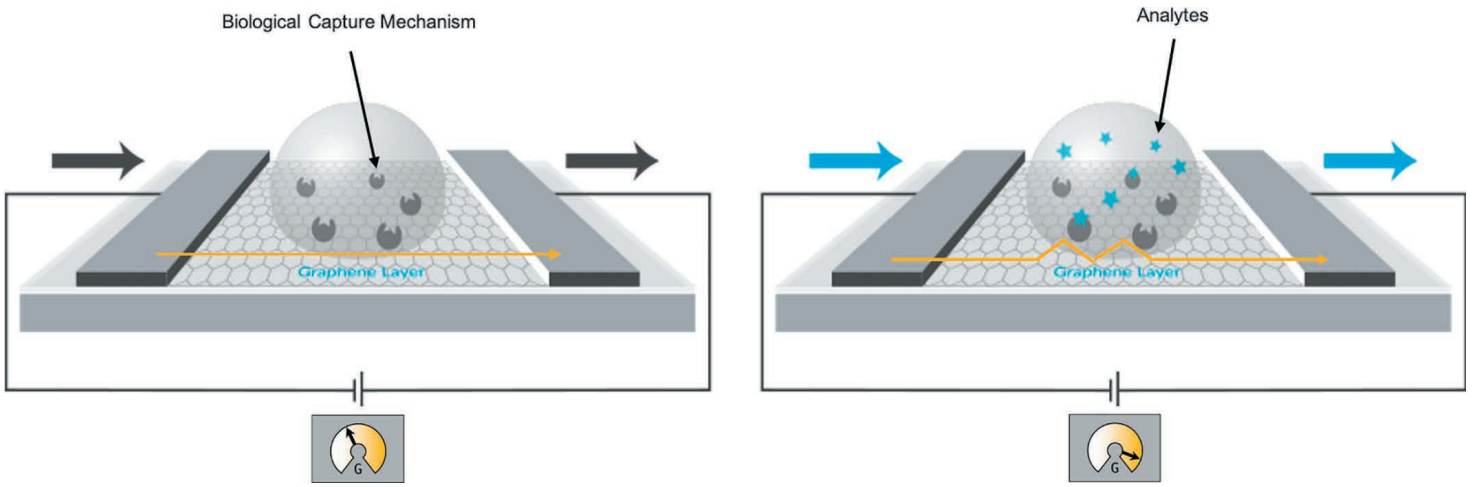

Figure 2: A diagram describing the action of the Cardean biology gated transistor after exposure to the analyte. Binding to the capture chemistry causes a change in the overall transistor properties.

In clinical trials?

A. Cardea's platform could be used for a wide range of applications during clinical trials. From real-time pharmaco-kinetics (PK), to biomarker assays, to genetic screening for inclusion/exclusion criteria, and many more; each of these presents an opportunity for our partners to provide a cutting-edge technology to support clinical trials.

In the clinic?

A. We see our technology as enabling in the field of rapid point of care (POC)/point of testing in ways not available previously. From enzymatic assays for newborn screening to infectious disease diagnostics to monitoring of immune responses after vaccination or immunotherapies, our partners have the ability to provide a transformational way to understand patient health in prognostic, diagnostic, or monitoring scenarios.

In the R&D lab?

A. R&D labs instruments and "quality control" applications like www.CRISPRqc.com are the fastest moving markets we see in regard to deploying our technology via product development. This path offers a combination of no or low regulatory hurdles and a strong market demand to solve a number of costly problems and bottlenecks identified and well understood by our "Powered by Cardea" partners.

Q3. Does Cardea Bio plan to develop companion diagnostics application for, say, an assessment of a patient in a doctor's office for a therapy?

A. Companion diagnostics represent a great place for our future partners to utilize our technology for rapid stratification of patients to the appropriate treatment. The opportunity with cancer treatments is a great example of this. The use of liquid biopsy techniques, such as with our InterCell-Chip (for intercellular communication) or CRISPR-Chip sets (for gene or SNP mutations), presents an opportunity not only to diagnose cancer early, but also to assess which treatment would be most effective in the context of identified genetic mutations or abnormalities, active cellular destress signals, or evidence of cancer cells hijacking cellular components like exosomes to metastasize. The beauty of our system is its imminent ability to combine several individual tests into one multi-omics chip. Instead of sending a sample to sequencing, another for an immunoassay, and a third to enzymatic assay, our partners will be able to build products that combine it all on a single disposable chip. Thus, a physician will get a more complete and near instant picture of a patient's condition and can use those actionable insights to improve patient outcome.

Figure 3: A standard Cardean chip containing multiple sensors inside the circular well.

'Linking up to Life'

Cardea is on a long-term mission, "Linking up to Life," to empower its "Powered by Cardea" partners with Tech+Bio solutions that will enable impact via innovative applications.
For more information about Cardea Bio Inc. visit www.cardeabio.com

Q4. In follow up to Q2 and Q3, what sample types does the Cardea platform support – blood, sputum, urine? What types of sample preparation are needed for in-office of in-laboratory applications?

A. Being an all-electric system that does not rely on optics, the platform can function in any biological fluid sample. The sample preparation would be dependent on the assay and end-use indication. For example, most POC tests require rapid results with minimal to no sample handling. For blood or urine, simply place a drop of the sample on a cartridge; the machine will process and read the sample. We are experts in integration of our biology-gated transistors into a wide range of off-the shelf microfluidic cartridges that would enable onboard sample processing designed specifically to meet individual assay requirements. At Cardea, we leverage what is already established in the marketplace, pulling the best "Lego" blocks together to create a product compatible with the workings of our Cardean transistors.

Q5. What are Cardea's plans with respect to FDA approval and marketing its products? Does Cardea Bio intend to market an FDA-approved in vitro diagnostic kit, or will it go the laboratory-developed test route?

A. Cardea works by enabling our Innovation Partners to utilize this technology to create disruptive products in their markets. This universe of opportunities for Cardea runs the breadth of markets, both regulated and unregulated. For our partners in regulated markets, we help their regulatory and commercial teams to devise an appropriate route, whether a cleared IVD kit or a CLIA-waived LDT depending on the market and the application. Cardea then relies on its partners to market and distribute their "Powered by Cardea" product.

Q6. What are Cardea Bio's plans for regulatory compliance in the US, the UK, the EU, and elsewhere? Does Cardea Bio have a roadmap and strategy along these lines?

A. As a manufacturer of biosensors that will be integrated into a wide variety of products, we intend to seek ISO 9001 and ISO 13485 certifications. We believe this will be a critical path for us to support our partners in regulated markets, and we are a firm believer in providing safe and effective products for our partners and their consumers. Each product we design with our Innovation Partners would require an individualized regulatory strategy determined by our Partner's commercial goals and intended use market. We work with our Partners to identify the most effect roadmap for each product to create a win-win partnership that delivers safe and effective products quickly to consumers.

Q7. In follow to Q6, does Cardea Bio have plans to apply for reimbursement with the CMS and other agencies worldwide?

A. The decision of which reimbursement strategy to use will be dependent on the

Linking computers

Cardea is linking computers to the LIVE molecular signals running biology. Its multi-omics technology consists of a Tech+Bio Infrastructure (hardware, software, wetware) and Cardean Chipsets manufactured with proprietary Graphene-based Biology-gated Transistors, or Cardean Transistors™.

partner's commercial and regulatory strategies. If our Innovation Partner desires to apply for CMS reimbursement, we highly recommend utilizing FDA's parallel review programs that enable communication with both organizations concurrently to effectively set up analytical performance and clinical trial studies to meet the unique demands of both CMS and FDA. Additionally, we are encouraged by the CMS decision to grant Medicare coverage to Breakthrough Devices that receive FDA authorization and lasting up to 4 years as part of the new Medicare Coverage of Innovative Technology reimbursement pathway. This will greatly assist companies like Cardea in providing highly disruptive technologies to our partners to create next-generation precision diagnostics.

CRISPR-Chip – Electronic DNA detection without the need of a DNA lab

The Cardea chipset called CRISPR-Chip has the capability to search through genomes without the need for amplification or other normal DNA workflow steps that require a DNA lab, as demonstrated in two Nature Biomedical Engineering (Nature BME) publications.[2,3] The CRISPR-chip can report if a genomics sample has a specific gene[2] or mutation[3] in it as a near real time measurement, and it can even be built as a handheld device. The CRISPR-chip works by combining the natural search power of CRISPR and the biology-gated Transistors from Cardea, that together allow for live signals in biology to be linked directly into electronics and computers.

Q8. Clinical laboratory tests take advantage of measuring standards and samples from many patients in a multi-well plates to reduce per-sample costs. How will Cardea position its device in light of the cost competition with data generated in clinical laboratories?

A. Our biology-gated transistors chips will sit in anything from a single sample handheld devices all the way up to large multi-well high-throughput screening instruments, and as our chips use semi-conductor technology manufactured in massively scaled semiconductor fabs, price falls with scale. Since the founding of our company, we have continued to beat Moore's law. When we first started in 2013, it cost tens of thousand dollars to produce a chip; now we are producing chips for under $50, a 100+ fold reduction in cost in 8 years (a photograph of a standard chip is shown in **Figure 3**). Future generations and new chip designs hold the potential to drive consumable cost below a dollar. On top of this, our readers are inexpensive and low-powered electronics that can range from a handheld POC reader where assays can be performed inside disposable microfluidic cartridges, to huge industry size and fully automated high-throughput lab-based liquid handlers and robots that do not require expert users to operate or analyze the data.

Additionally, our system is entirely reprogrammable to keep up with the need to update workflows to, for example, detect continually mutating pathogens and a wider breadth of biomarkers. The system is modular, and the hardware does not need to be reinvented for each new assay or class of biomolecule. This means not needing to buy a new machine for each new assay or a machine for each "-omic" parameter but instead having a truly modular and multiplexing testing or measurement solution that can be upgraded with new features or tests that are optimized for different markets.

Q9. What are Cardea Bio's plans for integrating its data with other data types ('omics, imaging, etc) used in precision medicine analyses?

A. The vision of our company is to be able to integrate all of life's signals to be able to understand it at a systems level. The capability of our technology allows for this new understanding – we provide a multiplex and multi-omic system that can detect and measure cytokines, pathogens, DNA, proteins, pH, and more all on the same system. This multi-omic data stream is available in near real-time provides a much clearer picture of the biology being studied than can be provided by a single data point from a single molecule. Our multi-omic data is electronic and digital so it can easily integrate with other data types and algorithms to draw meaningful conclusions and actionable insights. For example, we and our partners are already discussing the possibility of adding the Cardea technology to existing devices as either an additional feature or as a QC step.

Q10. Final thoughts?

A. In the 21st century we are continuing to use techniques that were invented 30-50 years ago to try to solve the complex problems of the modern age. Precision medicine requires a deep understanding of individuals' unique biology that cannot be provided by these 20th century techniques.

What we need is a revolutionary technology to deliver on the promise of precision medicine. Doctors need tools that provide modular, multiplex, and multi-omic streams of data at the bedside to power better informed decisions. Our technology provides the infrastructure needed to manufacture these tools. We are actively partnering with other diagnostic and life science companies through our Innovation Partnership Program to make the next generation of diagnostics and monitoring tools available at the bedside to enable the potential of precision medicine.

The pandemic painfully magnified the limitations of current diagnostic testing and life science technologies. It highlighted the global need for a significantly better and faster solution to understand our own biology and how it interacts with the world around us. We see our technology as the foundation for our many different partners' many different solutions. With unparalleled sensitivity, specificity, speed, modularity, scalability, and multiplexing capabilities, we are pushing forward to empower our partners with chipsets and a modern tech+bio infrastructure that is capable of providing a more complete understanding of people's health, and that someday may enable the global community to deliver on the promise of providing everybody with Precision Medicine. JoPM

Michael Heltzen

Michael spearheads Digital Biology leadership and strategy at Cardea. Michael has an extensive background in tech business development in Bioinformatics, Next-Gen Sequencing, Genomics, and Intercellular communication. He has held leadership positions at CLC bio, BGI, EXO Incubator, Nanosens and BlueSEQ before heading up the leadership team at Cardea Bio Inc.

Brett Goldsmith

Brett's passion is applying nanoelectronics technology to products that change people's lives. Brett was a post-doc at the University of Pennsylvania, from one of the world's leading graphene labs. Brett was an Intelligence Community Fellow and is the lead researcher on landmark Field Effect Biosensing papers in Science and Nature Nanotechnology.

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