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CRISPR quality control on a chip

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Combining the precision of CRISPR's DNA searching ability with the speed and scalability of electronics, we have developed an 'electronic DNA search engine', called a CRISPR–Chip, which not only enables DNA detection without amplification, but also showcases the untapped potential of merging molecular biology with nanomaterial electronics. Here, we discuss highlights and challenges on the journey from the initial idea to the commercialization of the CRISPR–Chip.

Nanoelectronics meets CRISPR

Biosignals typically arise from the interaction between two molecular elements as they bind to each other. In the context of CRISPR, the guide RNA (gRNA) binds to a matching target DNA sequence, an event that is essential in the CRISPR–Cas9 genome editing process, where the gRNA guides the Cas9 protein to the precise location on the DNA strand that needs modification. This process initiates sequence-specific cleavage and potentially DNA editing, making it a fundamental contributor to the precision of CRISPR technology. If we could monitor these binding events in real time with high sensitivity on an electronic platform, we could detect target sequences using 'programmable' biochemistry at high throughput.

A graphene biosensor is centred around a graphene field-effect transistor (gFET), using a liquid electrolyte gate to control electrical current. It offers high tunability, sensitivity and biocompatibility, making it valuable for interfacing with biological systems. However, this set of properties can also be a challenge. Any biomolecule in solution can interact with a graphene surface, leading to a sensing signal. Achieving a specific response thus requires either a robust and customized blocking chemistry or precise reagent control.

To exploit a gFET to monitor the binding events of CRISPR to detect DNA target sequences, we brought together a team of graduate students and postdoctoral researchers with diverse backgrounds and a unified sense of purpose focused on combining electronics and biology. We designed multiple versions of an assay with customized blocking chemistry suitable for nanoelectronics and we simplified our sample to purified DNA to minimize noise. We called our technology, CRISPR–Chip, which allowed CRISPR to function when chemically anchored to graphene, demonstrating the ability of a gFET to detect the binding of the Cas–gRNA complex to its DNA target (Fig. 1).

Scaling-up graphene transistors

Reproducibly manufacturing graphene transistors at a university nanofabrication facility posed a substantial challenge for us. In particular, developing a biological assay based on gFETs required hundreds of consistent devices for testing. In addition, although gFETs form the core of the biosensor, the entire system requires control of liquids, voltages and data processing, which can be difficult to engineer at the required scale and consistency in an academic setting. Thus, the Aran lab teamed up with B.R.G. and Ross Bundy, who had founded a startup dedicated to the scaled production and use of gFET biosensors, and who had already completed the lengthy journey of enhancing and scaling gFET production.

Applying the CRISPR–Chip to disease detection

With access to a commercial team to supply and quality check biosensors, we were able to show the capability of the CRISPR–Chip in detecting genetic diseases and infectious diseases without amplification in samples from people with Duchenne muscular dystrophy and sickle cell disorder[1–3]. On the basis of this initial success, we founded a startup to commercialize the CRISPR–Chip for amplification-free genotyping, together with Michael Heltzen and in close collaboration with B.R.G. and Ross Bundy. We merged our individual enterprises, giving rise to a new entity called Cardea. Cardea's primary product line was gFET biosensors with the CRISPR–Chip as its flagship product line. In 2023, Cardea was acquired by 2D electronics foundry, Paragraf.

Unveiling unexpected value

Within the process of technology development and commercialization, the actual value proposition of your technology is often not what you initially envisioned. This concept of product-market fit needs to be done right when moving work from basic science to commercialization. While we initially saw the CRISPR–Chip as a genotyping tool, its first notable commercial use was in monitoring the quality and performance of reagents throughout the CRISPR-based gene-editing process. The process of performing a CRISPR–Chip measurement involves several quality checks, including gRNA binding to Cas and the kinetics of the cleavage process. Importantly, the CRISPR–Chip is the only device capable of rapidly assessing the stability of the combined Cas and gRNA, that is, the CRISPR complex. Identifying the likelihood of low editing activity using a simple assay prior to attempted gene editing saves valuable reagents and time.

Therefore, we focused on developing assays that can identify the failure to form functional Cas9–gRNA complexes and the dissociation of gRNA from Cas proteins, and that can characterize effects of mutations in gRNA or Cas enzymes as well as competition between different gRNA strands to bind to Cas proteins.

Gene-editing quality control

In 2022, Cardea spun off CRISPR QC, which solely focuses on using the CRISPR–Chip to monitor the gene-editing workflow. CRISPR QC's analytical platform builds on the CRISPR–Chip technology, identifying the factors and reagents responsible for producing inconsistent outcomes in the CRISPR gene-editing process. This quality control assay improves the affordability, scalability and insurability of CRISPR-based gene-editing therapies, thereby expanding their accessibility to patients. Cost-effective reagent quality control may not be a critical need of basic research, but it is crucial for diagnostic and therapeutic companies.

However, to technically achieve such quality control with the CRISPR–Chip, we had to go beyond a platform-style biosensing technology and start considering how specific users interact with the technology and data. Therefore, the team had to develop an understanding of the specific needs and processes involved in gene editing to ensure that the technology was practical for this application.

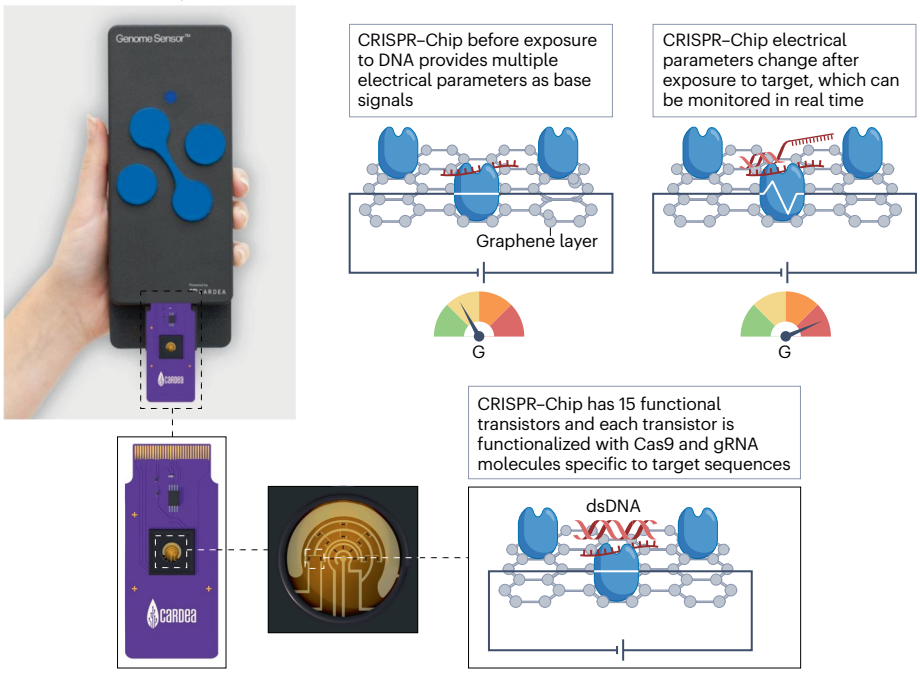

Fig. 1 | CRISPR–Chip. Each black rectangle represents one graphene field effect transistor (gFET) within the CRISPR–Chip construct, where each gFET is functionalized with thousands of CRISPR–Cas complexes. The gFET has a base electrical response, which is measured after immobilization of Cas–guide RNA (gRNA) atop the graphene surface of the gFET. Upon introduction of a genomic sample, the binding of Cas–gRNA to its target sequence creates a shift in electrical parameters of the gFET owing to proximity of the negatively charged DNA, which can be measured in real time. dsDNA, double-stranded DNA.

Getting the CRISPR–Chip on the market

Attracting commercial partners faced its own set of obstacles. Convincing pharmaceutical companies to adopt the CRISPR–Chip technology was challenging, especially because many companies were comfortable with existing cell-based methods for testing gene-editing formulations. The initial reluctance might have been due to sufficient budgets to cover the costs of cell-based assays. If something is expensive, but you have the budget for it, you are less inclined to change your process. However, in 2023, Vertex Pharmaceuticals received the first regulatory approval for a CRISPR-based treatment, and that came with an extraordinarily high price tag to patients and concerns that gene-editing treatments were commercially dead-on-arrival. This helped in convincing pharmaceutical companies to try a new technology that may ultimately expand the affordability and accessibility of gene-editing therapies.

Challenges

Every invention has its own unique challenges from initial idea to commercialization. For the CRISPR–Chip, finding the correct product-market fit was the most notable challenge, requiring patience, cooperation and determination to test different combinations of technical and business sales solutions. Prospective investors or customers have often expressed a positive view of our technology but would not follow through with the lifeblood of commercial science: cash. We also faced innumerable other challenges, from negotiating intellectual property agreements, qualifying new vendors, dealing with manufacturing variations, managing payment terms and cash flow and handling staff turnover, to working with board members who disagreed with our decisions. We applied the same patience, cooperation and determination to finding the proper product-market fit.

The road ahead is promising for the CRISPR–Chip. Paragraf continues to develop graphene chips at scale, new assays are being developed based on the CRISPR–Chip and new form factors and markets are being explored commercially. Each of these efforts will face challenges. However, the development and commercialization of the CRISPR–Chip demonstrates the power of true cooperation and the impact that can be made if academic and commercial teams work together.

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Author contributions

K.A. and B.R.G. prepared the manuscript.

Competing interests

K.A. is a co-founder of Cardea and CRISPR QC. B.R.G. is a co-founder of Cardea.

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