

cal to understanding device operation and improving OLED efficiency. For example, electrical and photoelectron spectroscopy measurements on OLEDs with different metal electrodes showed that charge injection improves when the energy barrier at the contact decreases (8). Modeling how these elementary steps affect parameters such as spatial distribution of electric field and charge inside the device has supported OLED optimization (9).

Translating this language to organic electrochemical devices is surprisingly straightforward and identifies which processes would be considered capacitive or Faradaic. For electrochemical oxidation of a conjugated polymer film, for example, there would be the following elementary steps: hole injection from the metal electrode into the HOMO of the polymer; hole transport within the delocalized HOMO of the polymer; anion injection from the electrolyte into the polymer; anion transport in the free volume between the polymer crystallites or chains; and electrostatic compensation of the two charges in the bulk of the film (see the figure, bottom right).

As in OLEDs, the injection and transport of the two charges are correlated through changes in local electric field and band structure. When these elementary steps work efficiently, the outcome will be volumetric charging of the film, which is a pure capacitive process akin to charging a stack of capacitor plates (4). There may still be CV peaks caused, for example, by the presence of an injection barrier or a voltage-dependent mobility (10); nonetheless, the overall process remains capacitive.

Faradaic processes result from the departure from this ideal picture because of poor efficiency of an elementary step or competing reactions that happen in parallel. For example, injection of electrons at energy levels of the polymer that lie above the LUMO of molecular oxygen leads to electron transfer from the polymer to oxygen and the formation of superoxide (11). Poor injection of holes or ions or slow transport of one of the charges will cause large interfacial fields that may trigger electrolysis and other Faradaic reactions. Electron transfer between the injected ion and the polymer chain (the equivalent of recombination in an OLED) would also be a Faradaic process. Thus, the electrochemical response of a particular material will depend on its properties and operating conditions.

PEDOT:PSS shows capacitive behavior because it has high mixed ionic and electronic

conductivity and is stable in common electrolytes under small doping changes. The response of other polymers may be different and requires detailed study. For example, so-called redox polymers such as poly(2-vinylanthraquinone) (12) could show Faradaic behavior given their much lower electronic carrier mobilities due to the highly localized states on the pendant redox groups.

Understanding of organic electrochemical devices requires isolating and probing each elementary step. Techniques such as “moving front” measurements to measure ion transport, chronoamperometry, and the recording of organic electrochemical transistor parameters can help to advance understanding of ion injection and transport and its coupling to electronic conductivity and energetics, at different doping densities. The impact of electrolytes on film morphology and dielectric environment must also be considered. An energy-level description of organic electrochemical devices will support the development of more sophisticated devices and will also help to explain the relationship between polymer energetics, charge transport, and reactivity.

With this understanding at hand, Faradaic reactions can be promoted or suppressed by tuning polymer energetics and the spatial distribution of electric field and charge. In neural electrodes, where capacitive response is desirable to avoid the generation of reactive species, care should be taken to avoid interfacial fields. Conversely, in electrocatalysis, engineering electron transfer to solutes throughout the volume of the polymer film will help to achieve a large steady-state current and maximize efficiency. ■

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GENOME EDITING

When genome editing goes off-target

Detecting unintended mutations could improve DNA-editing strategies

By Hannah R. Kempton¹ and Lei S. Qi^{1,2,3}

Editing DNA in eukaryotic cells with CRISPR-based systems has revolutionized the genome engineering field. Cas (CRISPR-associated) endonucleases are directed to a particular location in the genome by a short guide RNA, providing an easily programmable strategy to target any section of DNA. As of now, two CRISPR-based approaches can introduce targeted, permanent edits. DNA cleavage with the Cas endonuclease facilitates small insertions or deletions of nucleotides that can disable the targeted gene (1). A second modified “base editor” system can generate precise single-base mutations in the targeted DNA (2). For both approaches, it is imperative that DNA modifications are made in the intended region (“on-target”) and not elsewhere in the genome (“off-target”). On pages 286, 289, and 292 of this issue, Wienert *et al.* (3), Zuo *et al.* (4), and Jin *et al.* (5), respectively, describe methods that identify off-target activities, which will be invaluable in therapeutic contexts as well as for stringent evaluation of future iterations of gene-editing tools.

The specificity of gene editing tools is critical to their utility, which is why off-target potential is a major concern. For therapeutic applications, unintended mutations introduced in a patient’s DNA could permanently disrupt normal gene function and lead to unpredictable complications. CRISPR tools can also generate a variety of engineered cell lines and animal and plant models for research purposes. The data generated with these cellular and organismal model systems depend on the specificity of the DNA-editing tool because off-target mutations can confound experimental results. As a consequence, much research has gone into identifying and minimizing potential off-target sites of Cas activity.

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Traditional CRISPR-based genome editing introduces double-strand breaks in DNA using a catalytically active Cas enzyme. This break can be corrected by an error-prone nonhomologous end-joining process in which DNA bases are randomly inserted or deleted at the target site. For base editing, a nucleotide deaminase is fused to a catalytically impaired Cas enzyme. This tool does not generate double-strand breaks in DNA but instead uses the deaminase to convert one nucleotide to another in the targeted region. In both cases, it is critical to have an appropriate method to identify off-target activity for the given tool.

Early work profiling off-targets of Cas nucleases used computational methods to predict genomic sites likely to be cleaved by a particular guide RNA based on sequence similarity (6). However, such *in silico* investigations are limited because they only experimentally validate selected regions for unintended mutations. An ideal off-target detection platform should be unbiased and examine the entire genome. Newer *in vitro* approaches look for sites of DNA cleavage upon incubating Cas9, guide RNA, and purified genomic DNA (7, 8). Although highly sensitive, these methods do not account for cellular properties that present potential obstacles to accessing DNA such as chromatin and nuclear architecture. By contrast, *in vivo* experimental methods deliver Cas9 and guide RNA to living cells to identify resulting off-target events in a particular cellular context (9, 10). However, these approaches can have lower sensitivity and need additional components, which can be difficult to deliver, limiting application to many samples.

Wienert *et al.* developed a new approach to identify off-target cleavage by Cas endonucleases in cells and tissues. The technique, called DISCOVER-Seq (discovery of *in situ* Cas off-targets and verification by sequencing) relies on endogenous DNA repair machinery that is naturally recruited to sites of double-strand breaks in the genome (see the figure). The authors determined that the protein MRE11 (meiotic recombination 11), a subunit of a complex that repairs DNA, was recruited to sites of Cas9-induced genome breaks. By isolating MRE11 and sequencing the bound DNA, Wienert *et al.* identified the locations of cleavage events for an RNA guide of interest. The method worked in induced pluripotent stem

cells from a patient with Charcot-Marie-Tooth syndrome, as well as in mouse livers that were edited with virally delivered Cas9.

Previous *in vivo* methods required the introduction of additional components beyond the Cas enzyme and RNA guide, which can be a technical challenge for some cell types. Because DISCOVER-Seq utilizes endogenous DNA repair machinery to identify sites of double-strand breaks, no such additional factors are required. As a result, the approach of Wienert *et al.* can be applied to a variety of samples, including patient-derived primary cells. This opens a range of possibilities for stringently evaluating off-targets for therapeutic genome editing. By screening a panel of potential RNA guides in cultured

patient-derived cells, it may be possible to identify off-target sites that might have otherwise been missed because of differences between a particular patient's genome and a standardized reference genome. Additionally, RNA guides can be designed and tested for patient-specific mutations for rare genetic disorders. Such a personalized approach to prevalidate RNA guides for individual patients *ex vivo* before treatment could provide an additional level of safety for CRISPR-Cas endonuclease therapeutics.

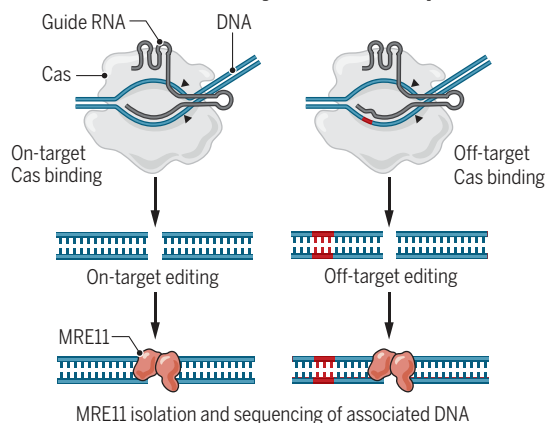
The detection of off-target activity from fusion Cas9 base editors poses additional technical difficulties. The single-nucleotide variants produced by base editors are difficult to detect, especially in heterogeneous samples in which rare off-target mutations may be masked within the population. There are also potential unexpected effects that could arise from introducing the nucleotide deaminase. Zuo *et al.* developed an approach called GOTI (genome-wide off-target analysis by two-cell embryo injection) to identify potential off-targets of cytosine and adenine base editors *in vivo* in mouse embryos. A Cas base editor and RNA guide were injected into a single blastomere of a two-cell embryo (along with a molecule to fluorescently mark the edited cell). Progeny cells of the edited or nonedited blastomeres were sorted on the basis of fluorescence and then sequenced, providing an internal control from the same embryo for accurate determination of editing-induced single-nucleotide variants. Zuo *et al.* found that the cytosine base editor (BE3) generated around 20 times more single-nucleotide variants than the adenine base editor, Cas9, or control. Most off-target mutations were independent of the RNA guide, implying that off-targets did not arise from Cas9 itself but rather from random off-target activity of the fused deaminase.

Jin *et al.* reported similar genome-wide off-target findings for cytosine and adenine base editors in rice. The authors introduced the base editors into single cells and then evaluated genetic changes in the resulting rice plant, using whole genome sequencing. They found substantially more off-target single nucleotide variants in plants treated with the cytosine base editor than in plants treated with the adenine base editor or control. The results of Zuo *et al.* and Jin *et al.* are an important supplement to other work examining deaminase

Detecting CRISPR off-targets

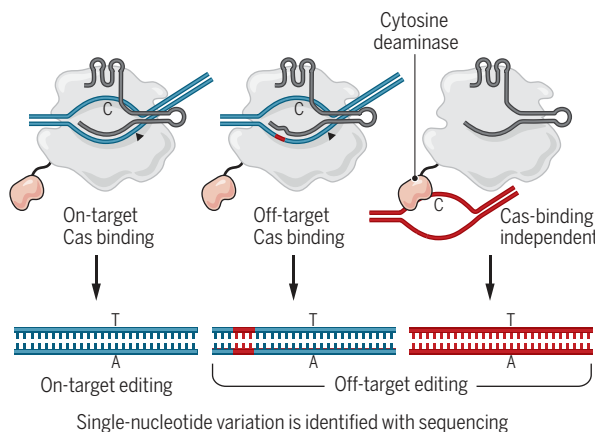
Two CRISPR-based approaches edit DNA, but neither system is perfect. Methods to detect the location of undesired edits provide information about editing accuracy.

Detection of Cas mistakes by DISCOVER-Seq



Cas endonucleases generate double-strand DNA breaks at targeted (on-target) or undesired (off-target) sites. A protein (MRE11) of the cell's DNA repair machinery binds to the breaks.

Detection of Cas mistakes by whole-genome sequencing



Cas-deaminase–base editors can generate single-nucleotide changes in DNA, such as changing a targeted cytosine (C) to thymine (T), but off-target base changes can occur. A, adenine.

off-targets in vitro (11, 12). By editing at the single-cell stage, both groups could sequence a homogeneous population of cells and observe off-target changes scattered throughout the genome that were previously undetected.

The observed off-targets are not entirely surprising given the properties of the deaminase effectors. The BE3 base-editor system uses rat APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like), a cytidine deaminase that can bind to single-stranded DNA independently from Cas9. This could explain why Zuo *et al.* and Jin *et al.* observed most of the random mutations in actively transcribed genes, where DNA is unwound by transcriptional machinery and single-stranded DNA is available for binding and editing by APOBEC. This raises additional concerns about the potential effect of these random mutations, because they could disrupt highly transcribed protein-coding genes. By contrast, the adenine base editor uses a modified TadA (tRNA-specific adenosine deaminase) protein from bacteria as a deaminase (13). Unlike APOBEC, TadA lacks the ability to bind DNA on its own, and thus its activity is more likely restricted to sites of RNA guide-specified Cas9 binding rather than acting independently on random DNA sequences it encounters.

Substantial work has already been done to minimize off-target effects of Cas9 itself, including RNA guide-design strategies, ribonucleoprotein delivery, and protein engineering (14). Similar efforts should be made to improve the specificity of base editors by limiting deaminase activity outside of Cas9 binding. This could be done by utilizing different deaminase effectors or rationally engineering the deaminase to decrease its DNA binding ability. Overall, improved identification of off-targets provides an opportunity to optimize guide development as well as improve gene-editing tools themselves, advancing the capabilities of genome editing. ■

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NEUROSCIENCE

Parsing signal and noise in the brain

Large-scale neuronal recordings reveal that brainwide activity is linked to behavior

By Alexander C. Huk and Eric Hart

Like engineers who characterize the fidelity of signals flowing through a circuit, neuroscientists focus on quantifying the degree to which neuronal signals are “noisy” (1, 2). Engineers have the benefit of designing the system and knowing the form of the signal, making identification of corrupting noise relatively straightforward. For neuroscientists, the task is harder, as it entails figuring out first what the signal is, and only then, what the noise is. On pages 254, 253, and 255 of this issue, Gründemann *et al.* (3), Allen *et al.* (4), and Stringer *et al.* (5), respectively, report findings from large-scale neural recordings in the brains of mice and find brainwide activity that correlates with behavior that might usually be ignored as noise. These studies prompt reconsideration of the origin and impacts of “noise” in the nervous system.

Although well-established tools and experimental approaches exist for addressing the signal-noise question (6), neuroscientists tend to rely on a seemingly necessary assumption: that neural noise can be defined simply as the variability in the neural response not accounted for by experimentally controlled inputs and measurable outputs. Such inputs and outputs might be sensory stimuli presented to an organism, or motor responses that the organism makes to those stimuli. Gründemann *et al.*, Allen *et al.*, and Stringer *et al.* each took different approaches to examine the cognitive and motivational processes that exist inside the brain. Gründemann *et al.* imaged large ensembles of neurons in the amygdala of freely moving mice. This brain region is thought to play a primary role in memory, decision-making, and emotional behaviors. The authors identified distinct populations that encode external stimuli (such as an auditory stimulus) and

internal behavioral states (such as hunger or thirst). Activity in these populations was correlated with switches between exploratory and nonexploratory behavioral states. Allen *et al.* recorded electrical activity from a large number of brain areas in the mouse by means of Neuropixels microelectrode arrays. This recording technology allows simultaneous monitoring of hundreds of neurons throughout an animal's brain. During thirst-motivated behavior, the authors observed that water-predicting sensory cues (an olfactory stimulus) gated brainwide neural dynamics and encoded a motivational state of behavior. Stringer *et al.* used both calcium imaging and Neuropixels arrays to record activity from neuronal populations in mouse visual cortex and

“To understand which sources of variability are actually noise, researchers will have to think more... about what the organism is doing, and what neural signals might be useful...for task performance.”

other brain areas. The spontaneous activity encoded a rich latent state related to the mouse's ongoing behavior (such as moving about and pupil dilation). Sensory input added to this signal in an orthogonal way, rather than disrupting it.

By leveraging large-scale recordings, Gründemann *et al.*, Allen *et al.*, and Stringer *et al.* all unpack a latent internal state of brainwide activity that corresponds to the ongoing behavior of the animal. In doing so, the studies collectively make a powerful case for breaking down the widely held reliance on associating neural activity solely with external variables. Although cognitive neuroscience focuses on neural signals related to mental processes, the subfield is often quite “zoomed in,” studying how a specific cognitive process might be implemented in a brain area of interest. The approach of Gründemann *et al.*, Allen *et al.*, and Stringer *et al.* is different, as they each focus less on hypotheses about par-

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