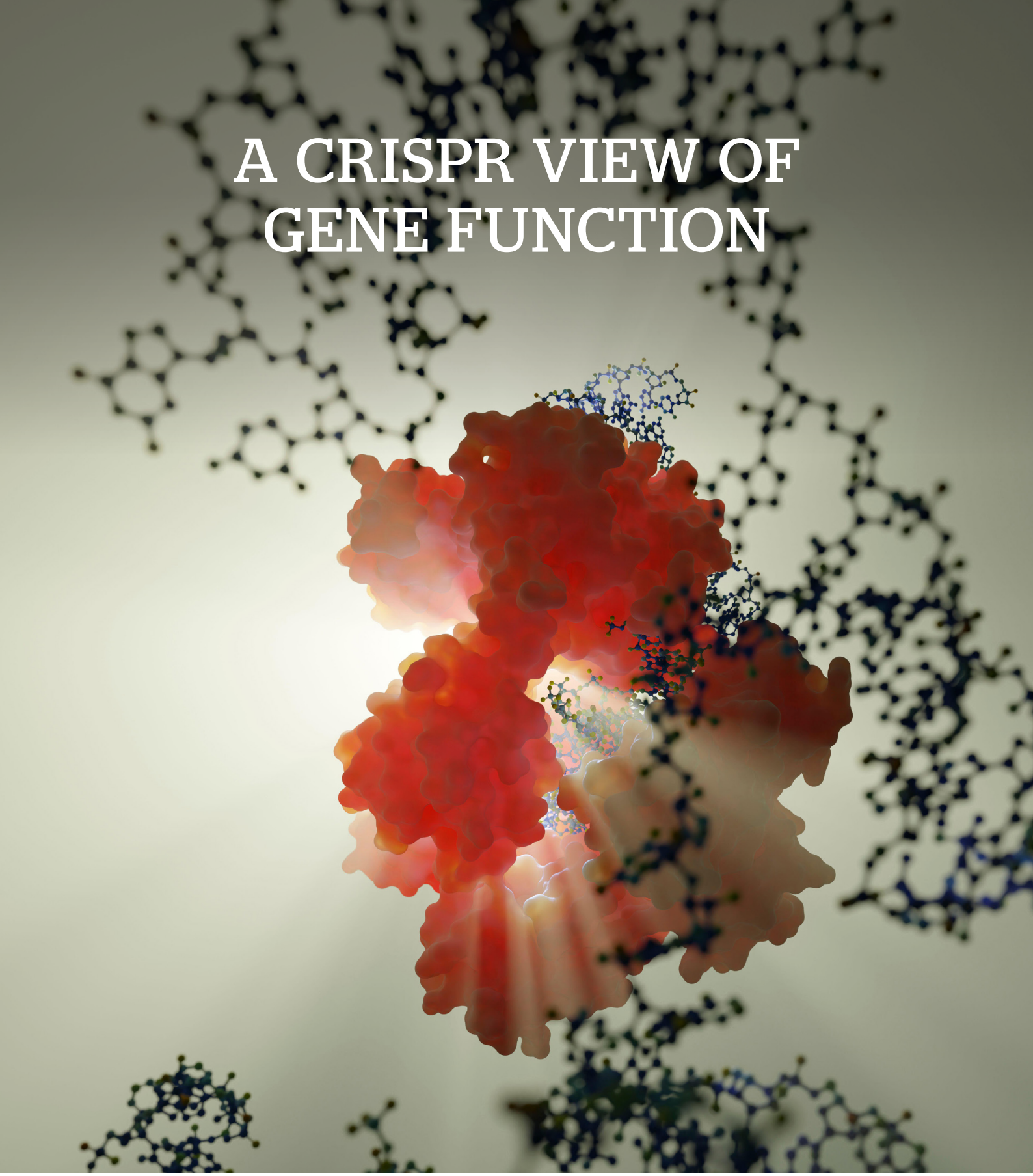
A CRISPR VIEW OF GENE FUNCTION



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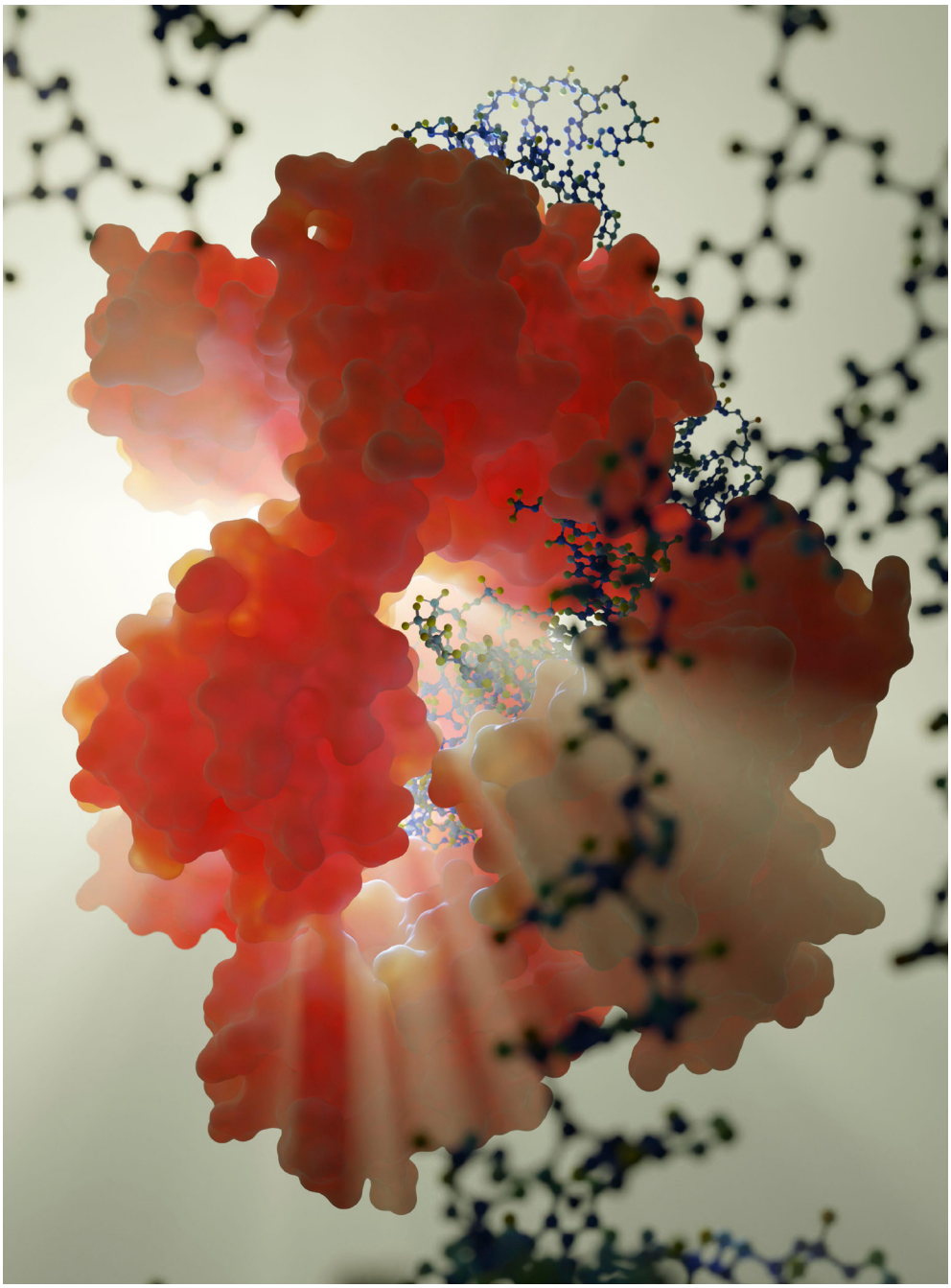

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▲ **RNA-seq reads mRNA, revealing which genes are active and transcribing proteins.**

A CRISPR VIEW OF GENE FUNCTION

NEXT-GENERATION SEQUENCING methods that provide multi-omic read-outs of CRISPR-modified cells reveal the relevance of genetic variants to disease states.

Benjamin Izar is trying to work out what happens when immune cells encounter cancer cells. He starts with large molecular profiling studies, such as whole-exome sequencing and RNA-seq. "They give dozens of putative targets or mechanisms that may play a role in disease or drug response, but it is impossible to functionally validate each of them individually," he laments.

To help, Izar, a physician-scientist at Columbia University's Herbert Irving Comprehensive Cancer Center in New York, turned to CRISPR screens. CRISPR allows researchers to precisely alter cells' DNA sequences, and modify gene function. With high-throughput screens, the effects of thousands of perturbations can be assessed in a single experiment. These tools aid research and drug discovery efforts by helping scientists identify the genetic variations, in both coding and non-coding regions, that contribute to disease.

However, until now, typical read-outs of CRISPR screens under different conditions, such as drug treatment or viral infection, have been quite simple cell growth and survival assays. These read-outs reveal genes that, when disturbed, either sensitize or confer a selective advantage to the challenged cells — but with no indication of how they do so.

Newly developed techniques provide single-cell, multi-omic readouts of CRISPR-modified cells. "Any large-scale profiling or screening effort may benefit from such methods as they help drill down to what might be functionally relevant," says Izar. With these 'high-content' CRISPR screens, researchers can start to evaluate the myriad nominated mechanisms and targets.

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High-content CRISPR screens combine genetic perturbations and stimuli with data-rich single-cell sequencing and imaging read-outs. Armed with these techniques, researchers can characterize screened cells with unprecedented detail and gain new biological insights into the function of genetic variants at scale (see 'CRISPR screening for large-scale variant-to-function analysis').

FROM GENETIC VARIANTS TO DISEASE PHENOTYPES

For genomic medicine to really transform patient health care, understanding the clinical relevance of genetic variation is key. Most efforts so far have concentrated on studying variants commonly found

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across many people with cancer, which proved less helpful than anticipated. "When you look at the genetic data from individual patients' tumours, the majority of cancer-associated mutations are actually quite rare, which means we have few insights into what these mutations do," says Jesse Boehm, principal investigator at MIT's Koch Institute for Integrative Cancer Research, and Chief Science Officer of the Break Through Cancer foundation, which aims to stimulate collaboration among cancer centres.

What researchers need is unbiased interrogation of gene function, which they can do with Perturb-seq. This technique pairs CRISPR perturbations with transcriptome responses. "There are tremendous opportunities at the intersection of these technologies," says Boehm.

Boehm and colleagues at the Broad Institute used Perturb-seq to measure the impact of 200 variants in two very common cancer genes, TP53 and KRAS, on the transcriptome of more than 300,000 single lung cancer cells[1]. They found that individual variants can have a wide range of effects on gene expression, downstream molecular pathways and cellular state, but that variants could be grouped into different categories based on their impact on cellular processes.

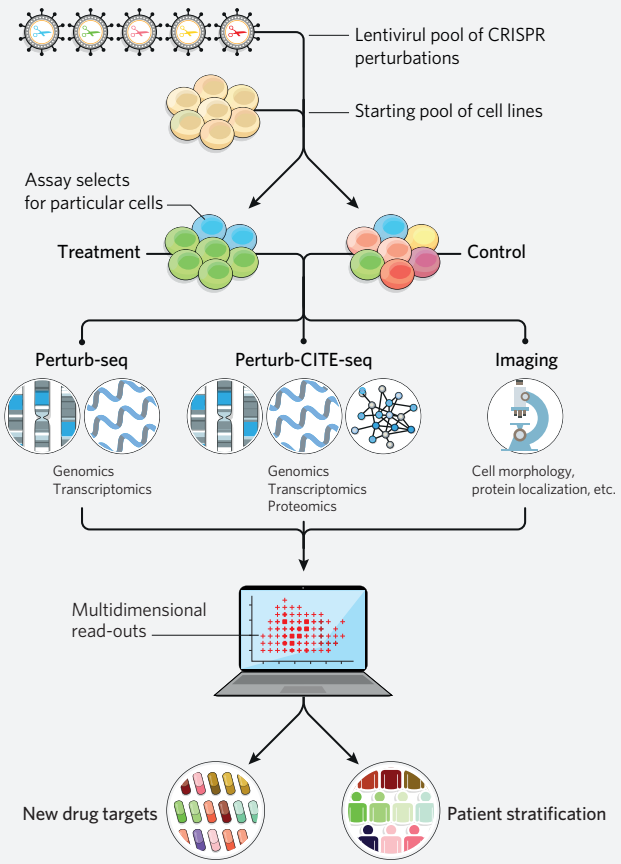
"We showed that there is a really systematic and cost-effective way to assess the impact of a very large number of common and rare cancer variants in the laboratory," Boehm says. "Methods such as Perturb-seq will enable the creation of a look-up table of information that tells us what each of the cancer variants is doing to drive tumour growth."

Boehm is the former scientific director of the Cancer Dependency Map project, which aims to systematically identify genetic and pharmacologic dependencies, and the biomarkers that predict them, in every type of cancer cell. Targeting these newly discovered cancer cell vulnerabilities could lead to the next generation of cancer therapeutics.

This work is closely aligned with that of researchers in the Atlas of Variant Effects Alliance, an international consortium that aims to develop, disseminate and democratize the use of technologies for mapping the effects of variants, and to coordinate the generation of high-quality variant-effect maps. "Because these types of methods are beginning to allow us to study genetic variants in any part of the genome and in any disease setting, they offer an opportunity to really advance genomic medicine," Boehm adds.

CRISPR SCREENING FOR LARGE-SCALE VARIANT-TO-FUNCTION ANALYSIS

Multiomic profiling of CRISPR-edited cells with approaches such as Perturb-seq and Perturb-CITE-seq are contributing to the generation of functional genomics maps that will speed up drug discovery and advance genomic medicine.



HIGH-CONTENT CRISPR SCREENS TO IMPROVE THERAPY

CRISPR screens with multidimensional read-outs can also contribute to investigations of drug sensitivity and resistance. Perturb-CITE-seq is an extension of Perturb-seq that provides information about both the RNA and protein content of CRISPR-modified cells. Izar and colleagues used Perturb-CITE-seq to investigate the mechanisms underlying cancer cell resistance to immunotherapy.

They analysed more than 200,000 patient-derived cancer cells, using CRISPR to disrupt hundreds of genes associated with resistance to immunotherapies[2]. The cells were co-cultured with patient-derived tumour-infiltrating lymphocytes before being screened. They profiled the single-cell transcriptomes and

20 surface proteins, and were able to identify most known mechanisms of drug resistance, such as those associated with defective IFN-κ signalling[3]. "The accuracy of the gene-gene and protein-protein interactions predicted by Perturb-CITE-seq validated the approach and gave us confidence that this is a robust method for informing important biology," Izar says.

Using this technique, Izar's team also found new cancer–immune cell interactions that could explain why immune checkpoint inhibitors fail or stop working in patients with metastatic melanoma. They have preliminary results that suggest that disruption of the T cell adhesion protein CD58, which has no known mouse homologue, could be an important new mechanism of resistance to T cell mediated killing[4]. This type of knowledge could help predict which patients are less likely to respond to immune checkpoint inhibitors and inform novel approaches to help overcome drug resistance in some patients.

Another application of these technologies is to improve cellular therapies. Perturbing cells and producing high-content phenotypic information at scale gives researchers the ability to engineer cells for specific fates. Screening cells in which certain transcription factors are overexpressed will help researchers learn more about key differentiation steps. If they can better direct stem cells to become specific cell types, then more-tailored cell therapies are possible.

A BESPOKE READ OUT

As with many cutting-edge techniques, cost is a major consideration. Daniel Schraivogel, a research staff scientist at the European Molecular Biology Laboratory (EMBL) working in Lars Steinmetz's laboratory in Heidelberg, Germany, has been working on a modified version of Perturb-seq, named targeted Perturb-seq (TAP-seq), which focuses on reading only a subset of genes of interest[5]. By doing so, it is possible to lower sequencing requirements up to 50-fold, and reduce experimental costs.

TAP-seq also increases sensitivity, so that lowly expressed genes and small effects can be detected more efficiently. Whole transcriptome read outs are very powerful if the outcome of perturbation is unknown, Schraivogel explains. "But in many cases, you know which particular genes or processes could be affected, so it makes sense to tailor your read-outs to those."

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Schraivogel and colleagues used TAP-seq to study the function of enhancers — DNA regions that cooperate with often distal promoters to control target gene transcription. They disturbed enhancers in two large genomic regions (2.5% of the human genome) and queried the effects on expressed genes in the same regions in single cells from five different cell types. To date, the effects of enhancers on target genes have been predicted from interactions in 3D space using techniques such as Hi-C. However, with TAP-seq the authors were able to obtain direct functional evidence of around 80 new enhancer–target gene pairs[6].

Only 4% of the enhancers they tested had a target gene, yet more than one third of their targeted protein-coding genes responded to the enhancer regulation. "Our findings are in line with our understanding that enhancers are very cell-type specific and can exert widespread effects," says Schraivogel. Thanks to TAP-seq, and machine-learning models, the authors are furthering understanding of the relevance of enhancer–target gene interactions to disease states.

EXPLORING NEW DIMENSIONS

The effects of genetic variants in living cells can also be imaged. Schraivogel used ultra-fast multi-channel fluorescent microscopy, combined with a flow cytometric cell sorting, to image and sort cells at speeds of up to 15,000 events per second. By rapidly isolating cells with complex cellular phenotypes, including cells with differently localized proteins and cells in different stages of mitosis, this image-enabled cell sorting (ICS) method adds a spatial dimension to CRISPR screens.

The researchers applied ICS to their CRISPR screens to identify regulators of nuclear factor κB (NF-κB), a fast-acting and crucial transcription factor that shuttles between the nucleus and the cytoplasm. "We are now able to complete a genome-wide image-based screen in less than a day — a big step forward compared with previous methods that took weeks or months," says Schraivogel. "We identified both known and novel NF-κB regulators, such as genes involved in chromatin remodelling not previously implicated in NF-κB signalling." Given that aberrant activation of NF-κB can cause chronic inflammation, oncogenesis and autoimmune disease, these findings hold promise for developing new drugs for a range of diseases.

"IT IS REALLY EXCITING TO SEE THE CONFLUENCE OF ALL OF THESE TECHNOLOGIES TO STUDY THE FUNCTION OF GENETIC VARIANTS IN A SYSTEMATIC WAY."

The next frontier for CRISPR screening is to move from using cell lines to primary cells and patient-derived cells. This will allow researchers to validate the hypotheses emerging from functional genomics maps and determine which targets are the most clinically relevant. "It is really exciting to see the confluence of all of these technologies to study the function of genetic variants in a systematic way," says Izar. "I am looking forward to seeing all the creative ways in which researchers will use them in the coming years." ■

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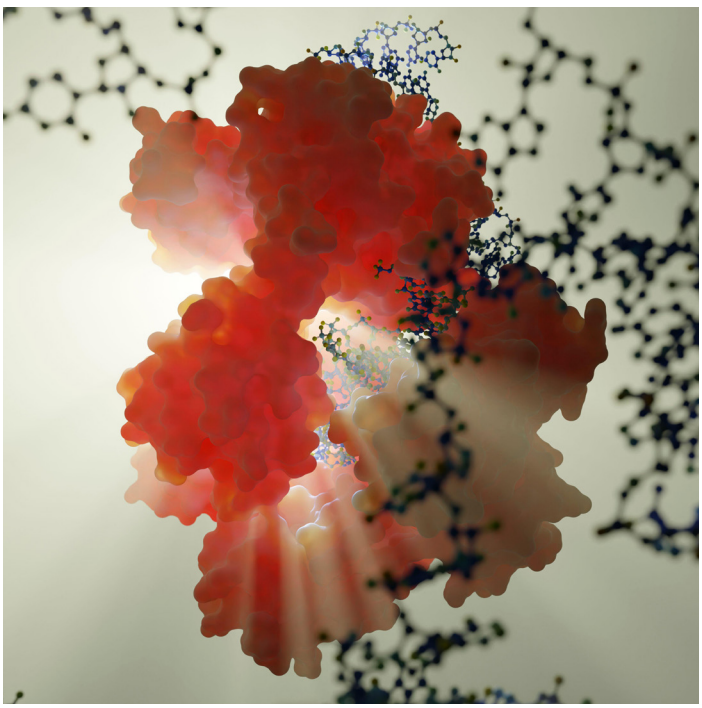

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