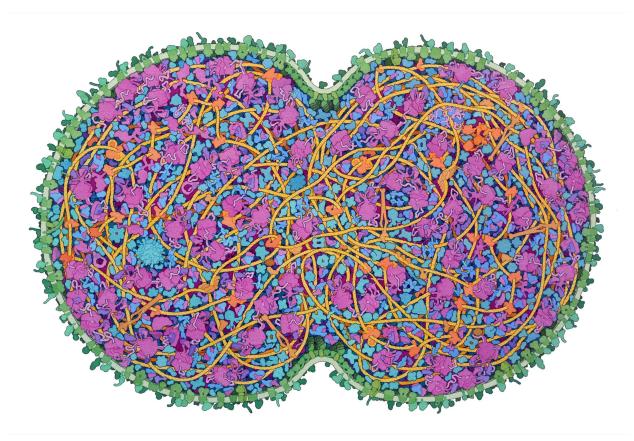
A Journey to the Center of Our Cells

Biologists are discovering the true nature of cells—and learning to build their own.

By James Somers



JCVI-syn3A, the "minimal cell," is a base model designed for expansion. Illustration by David S. Goodsell Listen to this story

It was by accident that Antoni van Leeuwenhoek, a Dutch cloth merchant, first saw a living cell. He'd begun making magnifying lenses at home, perhaps to better judge the quality of his cloth. One day, out of curiosity, he held one up to a drop of lake water. He saw that the drop was teeming with numberless tiny animals. These animalcules, as he called them, were everywhere he looked—in the stuff between his teeth,

in soil, in food gone bad. A decade earlier, in 1665, an Englishman named Robert Hooke had examined cork through a lens; he'd found structures that he called "cells," and the name had stuck. Van Leeuwenhoek seemed to see an even more striking view: his cells moved with apparent purpose. No one believed him when he told people what he'd discovered, and he had to ask local bigwigs—the town priest, a notary, a lawyer—to peer through his lenses and attest to what they saw.

Van Leeuwenhoek's best optics were capable of more than two hundred times magnification. That was enough to see an object a millionth the size of a grain of sand. Even so, the cells appeared minuscule. He surmised that they were "furnished with instruments for motion"—tiny limbs that must "consist, in part, of blood-vessels which convey nourishment into them, and of sinews which move them." But he doubted that science would ever advance enough to reveal the inner structure of anything that small.

Today, we take for granted that we are made of cells—liquidy sacs containing the Golgi apparatus, the endoplasmic reticulum, the nucleus. We accept that each of us was once a single cell, and that packed inside it was the means to build a whole body and maintain it throughout its life. "People ought to be walking around all day, all through their waking hours, calling to each other in endless wonderment, talking of nothing except that cell," the physician Lewis Thomas wrote, in his book "The Medusa and the Snail." But telescopes make more welcome gifts than microscopes. Somehow, most of us are not itching to explore the cellular cosmos.

Cell biologists know that the rewards for comprehension are substantial. The cell is the fundamental unit of life, shared by plants, animals, and bacteria. If we understood the cell in its entirety, biomedical progress would accelerate dramatically, the same way nuclear science did once physicists understood atoms. The trouble is that the interiors of cells are too small to easily see. Cells are hard to work with under controlled

conditions, and incredibly intricate. A poster hanging in many labs shows the Roche Biochemical Pathways diagram, a flowchart of cellular metabolism. It's oddly beautiful—like an engineering blueprint beamed down from an alien civilization.

Fifty years ago, we were less sure how to interpret the blueprint. The 1966 movie "Fantastic Voyage" imagined scientists who'd shrunk themselves in order to scuba dive inside a person's bloodstream; in one scene, antibodies attack a character in a wetsuit like a school of predatory fish. The film assumed that the cellular world would be a miniature version of our own. Today, although there's still no microscope capable of showing everything that's happening inside a living cell in real time, biologists grasp the strangeness of the zone, bigger than atoms but smaller than cells, in which the machinery of life exists. They've analyzed the tiny parts from which cells are made and learned how those parts interact. They've frozen cells, photographed them, and used computer simulations to revivify the pictures. They've studied the apparently empty spaces inside cells and discovered that they contain a world governed by unintuitive physical laws.

Several groups of "synthetic biologists" are now close to assembling living cells from nonliving parts. If we could design and control such cells with precision, we could use them to do what we want—generate clean energy, kill cancers, even reverse aging. The work depends on understanding a cell's inner workings to a degree that van Leeuwenhoek could not have imagined.

The first step is to reduce the problem to its essence. The human body contains brain cells and fingernail cells, blood cells and muscle cells, and dozens of species of single-celled bacteria. Each has been shaped to fit its niche by aeons of evolution. An alien trying to understand automobiles would be mystified by the differences between sedans and sports cars, and by the details of heated seats and infotainment systems. It would need to strip all that away, revealing the components common to all cars: engine, wheels, fuel tank, exhaust. A group of biologists

hoping to engineer cells have done something similar. They've modified a species of bacterium to create a "minimal" cell. It contains only what's necessary for life—it's the cellular equivalent of a stock car onto which new components can be bolted. John Glass, one of the project's leaders, described the minimal cell to me as "a platform for figuring out the first principles in biology." He said, "A way to get at big questions is to think small."

Glass, sixty-seven, leads the Synthetic Biology and Bioenergy Group, at the J. Craig Venter Institute, which occupies an artfully modern building set on a hill in San Diego. In the early two-thousands, when the minimal-cell project began, the field of genomics was only a few decades old. Biologists were sequencing DNA from every creature they could find—virus, bacterium, lab rat, human—and drowning in the data. J. Craig Venter, an instrumental player in efforts to sequence the human genome, felt a need to simplify. Why not create a cell with as few genes as possible, and use it as a model organism? If you wanted to understand a more complicated biological process, you could add the genes for it to your minimal cell. Their function would be easier to comprehend against a comparatively blank canvas.

Venter assembled a team of biologists that included Glass, who was one of the world's leading experts on a bacterium called *Mycoplasma*. "If you went to the zoo and lined up all the mammals and swabbed their urogenital tracts, you would find that each of them has some mycoplasma," Glass told me. Because the bacteria live in such a nutrient-rich environment, they rarely have to forage for food, or even do much to digest it; their lack of a sophisticated metabolism allows them to have the smallest known genome of any free-living organism. The researchers bombarded millions of these cells with special genes called transposons, which randomly splice themselves into a DNA strand, disrupting any gene they happen to land inside. Many of the bacteria died from this treatment, and the researchers sequenced the genomes of those which survived. It was like examining fighter planes that have returned from war: if you never saw bullet holes in the fuel

tank, you knew that damage there was always fatal. By 2016, after a few revisions, they had devised a minimal *Mycoplasma* genome half the size of the original. A researcher named Carole Lartigue spent years during her postdoc solving the daunting problem of implanting the genome in a cell. The bacterium that eventually resulted from the work was called JCVI-syn3.0. It was an engine bolted to some wheels.

One morning last fall, Glass greeted me at J.C.V.I. wearing a blue hoodie and black gym shorts. Upstairs, we met András Cook, a research associate, who led me to a bench on which some petri dishes were arranged. The dishes were a wan pink, with pinpricks in them; each pinprick was a colony of minimal cells—a version called JCVI-syn3A. Cook gestured to a nearby microscope. Through the lens, the colonies looked like fried eggs.

There was a higher-resolution microscope in another room. Glass took a seat on a stool nearby. The week before, he'd undergone a round of chemotherapy for colon cancer, and the treatment was slowing him down. "My hundred-year outlook is really bad," he said, smiling. "But my near-term outlook is quite good."

For contrast, Cook had prepared samples that contained both JCVI-syn3A and *E. coli*. The lab rat of biology, *E. coli* grows quickly and uniformly, and is genetically manipulable. It also hunts and eats, has a rudimentary kind of memory, and possesses around five thousand genes, compared with the minimal cell's roughly five hundred. After Cook loaded the syn3A slide, I peered through the eyepiece, but struggled to distinguish the minimal cells from the floaters in my eyes. Then I looked at the other slide. An *E. coli* swam by. It was about thirty-five times bigger than the minimal cell by volume, and crenellated with complexity—a destroyer rather than a dinghy.

In his office, Glass told me that the minimal cell was "a movement." He showed me a poster noting all of JCVI-syn3A's genes. About a third were labelled as having an unknown function. When the project began, there were a hundred and forty-nine mystery genes. Now about a

hundred were left. "In those hundred, there could be things going on that are essential to life," Glass said—not just syn3A's life, but all life on earth. Dozens of research groups from around the world are now using the minimal cell in their labs. Some are exploring its basic functions, while others are trying to add new capabilities, such as artificial photosynthesis, to the base model. The poster was really a scientific war plan—it outlined a mission. Decipher the labelled genes and you'd approach a comprehensive understanding of cellular life.

Generally, what a gene does depends on the protein it tells our cells to make. It's proteins that run the cellular world, by sparking chemical reactions, sending signals, and self-assembling into biological machines. To understand and control a cell, or to design a new one, biologists need to know exactly how a given protein behaves in the cellular environment. What shapes can it take? What does it interact with? What happens when a small molecule, like a drug, gets lodged in one of its crevices?

Until fairly recently, proteins have been too small to see except when they've been isolated outside a cell and crystallized. Our best pictures of the protein-rich cellular interior have come not from a microscope but from the brush of David S. Goodsell, a sixty-year-old biologist and watercolorist at the Scripps Research Institute. When I met Goodsell at Scripps, which is just down the road from J.C.V.I., he had long hair, a full beard, and a funky face mask. A painter since the age of ten, he illustrated his first E. coli during his postdoc, in 1991; the article that resulted, "Inside a Living Cell," became a sensation, and his cellular watercolors have since become ubiquitous in textbooks and databases and appeared on the covers of *Cell*, *Nature*, and other journals. Goodsell's work is partially funded by the Protein Data Bank—a project of the Research Collaboratory for Structural Bioinformatics—and while painting he frequently consults the P.D.B., which maps large biological molecules, including protein shapes, in atomic detail. He scours the literature for information about relative concentrations, metabolic rates, and the dynamics of protein interactions.

In his office, Goodsell was working on a new painting. A pencil sketch on an easel was to be a molecular-level depiction of milk. "We think of milk as just being this white, opaque, you know, nothing," he said. "This is going to help put some structure to it, showing all the bits and pieces that are inside." The sketch contained a few dots of color. Using a brush, he applied wash below a tangle of hourglass blobs representing casein proteins, which are abundant in milk. He started painting an antibody. In all, there were more than a thousand molecules to fill in.

Goodsell showed me some recent paintings: a particle of the coronavirus trapped in a respiratory droplet; a closeup of the flagellar motor of *E. coli*. One of his favorites was a portrait of JCVI-syn3A, the minimal cell. In order to capture it whole, he had made a painting nearly three feet across. A cleave was pinching the cell in half. Cells divide by splitting in two; it is believed that every cell in existence is a direct descendant of a single original—a split of a split of a split, through the generations. The membrane was light green, and the ribosomes—molecular machines that assemble proteins—were pink. Shaded coils and blobs of various sizes and orientations hung off one another, layered in a trippy cartoon.

The image communicated a sense of crowdedness. Diagrams often show a cell's "organelles," or specialized, factory-like structures, as islands in a sea of empty cytoplasm. But the cytoplasm is actually jammed with proteins, RNA, and other small molecules, all commingling at incredible speeds. It's sometimes tempting for biologists to think of proteins mainly in terms of their individual structures, or as nodes in an abstract biochemical flowchart. Goodsell's art makes vivid the messy reality in between.

As Goodsell painted, Arthur Olson, one of his colleagues, stopped by. Olson is a pioneer of 3-D computer modelling; among other things, his research group is working on CellPaint-VR, virtual-reality software that takes users into the cellscape. "It's a totally different world," he said.

Later, Olson showed me around the virtual cell. He put on a V.R. headset; I watched on a monitor, sharing his point of view. We began in

a void. Then, using a glove controller, he conjured some polio viruses—purple planetoids with bumpy, almost fuzzy surfaces. He added some antibodies—a host of pink, pockmarked shapes, which swarmed the invaders. "These are atomic representations that you can also interact with," Olson said, fiddling with a menu. He used his controller to select a ribosome, and attached it to a strand of RNA. It looked like a head of cauliflower.

Olson dragged the slider that controlled scale, so that the ribosome seemed to fill the world. There was nothing in view but individual atoms. He laughed, then reversed course, until the smoother contours on the ribosome's surface emerged. He tugged at the ribosome, trying to orient himself.

"That's the other thing," he said. "You can get lost."

Olson told me about an experience he'd had while building a virtual scene inside a red blood cell. The environment was so crowded that he had to make himself small. "I had this feeling that I was in a small plot of land in a huge valley that rose all around me," he said. "It gave me a totally different sense of the scale." He had been planting individual membrane proteins in the cell. "I mean, you can read in the literature that there are five hundred thousand of these in the red blood cell. But to actually experience it, in the sense of being in the landscape . . ." He trailed off. I thought of the ribosome extending all around us. It seemed like an environment you could get to know, like a park near your house.

The cellscapes created by Goodsell and Olson are best guesses—like an architect's 3-D renderings of an unbuilt house. The other side of the equation is microscope imaging, which, Goodsell told me, has made a "quantum leap" in recent years. A technology called cryo-electron microscopy, or cryo-EM, had developed to the point where it could help reveal the cellscape as it actually is, in startling detail. "They're getting really close to seeing cells at the level of the paintings I do," he said. "It's going to put me out of business."

Nearby, Elizabeth Villa, a physicist turned biologist, runs the cryo-EM lab at the University of California, San Diego. When I visited, Villa, who is originally from Mexico City, had whirlwind energy: in the past few months, she had become a U.S. citizen, received tenure, and been named a Howard Hughes Medical Investigator. The title comes with a grant that provides her lab with millions of dollars for at least the next seven years. "It's been a big summer," she told me. "I fell in love with cryo-EM. Now it's on the cover of every journal."

Light microscopes, like those you'd find on a high-school lab bench, have a fundamental limitation: light's wavelength is a quarter of a micron, about the size of three minimal cells laid end to end. Such microscopes have difficulty resolving anything smaller. In the nineteenthirties, scientists experimented with electrons, which can resolve individual atoms. But electron beams risk damaging the biological material at which they're fired. "Imagine if you took a picture with a camera and your subject melted," Villa said. By the eighties, a team led by a biophysicist named Jacques Dubochet discovered that samples could be better preserved by flash-freezing them: this was cryo-electron microscopy. The technique, which later won Dubochet and his collaborators a Nobel Prize, transforms water molecules into glasslike ice, in effect stopping life in medias res. By the twenty-tens, further advances, including better cameras and image-processing software, gave rise to the "resolution revolution": cryo-EM became powerful enough to image molecular structures inside living cells. Proteins could be captured in candid photos, not just in meticulously staged portraits.

Cryo-EM practitioners routinely produce highly detailed, panoramic views of cells. Some cells are easier to work with than others. *E. coli*, for instance, is often too thick to image at high resolution. "The minimal cells are very cute," Villa said. Inspecting one was like peering into a little glass house rather than into the Pentagon.

In Villa's lab, Lindsey Young, a postdoc, showed me a dish of what looked like tiny holes punched out of tinfoil. "Most of these are single

particle grids," she said—the cryo-EM equivalent of a glass microscope slide. Young handed me one of the grids. "That's like the size of the 'O' on your keyboard, right?" she said. "But, if you look at it under the microscope, it looks like a whole continent."

Villa demonstrated the cryo-EM process for JCVI-syn3A cells. The metallic grid is dipped in a solution containing cells, then flash-frozen in liquid ethane and stored in a cryo-chamber. We walked past a new microscope that was being installed. It was roughly the size of an Apollo moon lander, housed in a humidity-controlled, electrically shielded, acoustically dampened room designed to eliminate all vibrations.

"The higher energy an electron microscope has, the taller it is," Villa said. She pointed to a small metallic box within the machine, into which the cryo-chamber would be inserted like a VHS tape. "The microscope is, like, that thing and a couple of more lenses," she said. "Everything else is just electronics and stuff to keep it cool." This model cost around six million dollars, and would cost close to two thousand dollars a day to operate.

In her office, Villa pulled up an image of the inside of a human cell—an unprecedented view. To get a better look at cells that are larger and hardier than JCVI-syn3A, Villa's lab uses a technique called fib milling, in which a focussed ion beam is directed over the surface of a cell, carving little windows into it. The result, in this case, was hard to make out; the black-and-white image reminded me of television static. "The beauty and the horror of cryo-EM is that you see everything," Villa said. The data can be very hard to analyze. She pointed at the screen. "These are ribosomes, these big guys over here. Those are membranes. This is chromatin"—the complex structure into which our genetic material is coiled.

She clicked through a few slides, and soon everything was colored in.

"This is the picture from David Goodsell," she said. She'd overlaid his painting onto the raw image. It made the chaos more legible. "Look how

well it matches! It's nuts. And he did this without having these kinds of pictures."

With Wolfgang Baumeister, a German biophysicist, Villa helped develop an approach that combines fib milling with cryo-electron tomography—a technique in which a sample is rotated in place, allowing snapshots from different angles. Villa described it as "like a cat scan but a million times smaller." The physicist Richard P. Feynman once quipped that biology would be easy if you could "just look at the thing!" Villa supposed that we were nearly there. "All these questions that people have," she told me. "I think you're going to be able to say, 'Let's just do a tomogram.'"

Some biologists are now combining approaches. Their goal is to create an integrated view of life inside the cell, in the form of a computer simulation that puts the whole system into motion. In grad school, at the University of Illinois Urbana-Champaign, Villa studied under a biologist named Klaus Schulten, who, with his wife, Zan Luthey-Schulten, helped develop the field of whole-cell computational modelling. Klaus worked from the bottom up, favoring "all-atom" simulations, in which virtual atoms follow the laws of quantum mechanics, while Zan worked from the top down, with "kinetic" models that track the cell's larger traffic patterns. By the twenty-tens, the state of knowledge had advanced enough for them to try building a hybrid model. Klaus died in 2016. But, last month, Zan's group—which includes some of her current and former students—published a paper in Cell that outlined a computational model of JCVI-syn3A. The model drew on cryo-EM images from Villa's lab and on a genetic inventory supplied by John Glass's group at J.C.V.I. It included all four hundred and fifty-two of JCVI-syn3A's proteins, plus other cellular bits. In the simulation, these parts interact among themselves as they would in real life.

The software aims to simulate a world that's very different from ours. If a cell were blown up to the size of a high-school gym, you wouldn't be able to see across it. It would be filled with tens of thousands of proteins,

most about the size of a basketball. Other biomolecules no bigger than your hand, and water molecules the size of your thumb, would fill the spaces between. (To scale, your whole body would be about the size of a ribosome.) The mixture would have the consistency of hair gel. In such a world, gravity would be virtually meaningless—you would be weightless, as if suspended in a ball pit. And everything would be moving. The mixture would buzz constantly; spend just a few seconds inside it and every medium-sized object around you would have explored every square inch of your body. It would feel like pandemonium, but it wouldn't be.

In 2009, a bioengineer named Clifford Brangwynne and his colleagues made a discovery that filled in what could be the final piece of the new cellular picture. Brangwynne was studying a crucial early moment in the life of a small worm called *C. elegans*. Before it can build a body, the worm must figure out where to put its head and its tail; this process, called polarization, begins when it's a single cell. Small deposits form in the cytoplasm, creating what scientists call the P granule; the granule marks one side of the cell, and eventually the animal, as "left" and the other as "right." Biologists could spot the granule in their microscopes, but they couldn't say how it got to one side.

Brangwynne, who began his career in materials physics, was familiar with how liquids become solids and vice versa. Watching the P granule swirl into existence, he thought that it acted like an oily patch in liquid. If you poked it with a needle, it broke apart, then coalesced again. Through careful observation, he saw that it wasn't being built piece by piece by a molecular machine. Instead, it self-organized, like steam condensing into a droplet. Researchers soon found the same mechanism in other circumstances, and in other cells. In 2009, an article by a British cell biologist named Tony Hyman pinned down the phenomenon, sometimes called "liquid-liquid phase separation," and other articles began appearing; a trickle of papers became a flood. "There seems to be no end to the number of examples that are being discovered," James Rothman, a cell biologist and a Nobel laureate, told me recently. "Every

week, if you pick up your favorite journal in biology, you'll find another half a dozen."

The discovery requires a shift in our basic ideas about cellular life. For decades, biologists had assumed that activity in the cytoplasm was essentially random; the cellular world churned with such dramatic speed that the right proteins would eventually bump into one another. But it turned out that some molecules in the cytoplasm weren't randomly circulating. They were swirling in ways that brought related parties together. Suppose an important reaction involved five proteins out of ten thousand; the five tended to hang around one another, loosely attracted. (They sometimes had floppy regions that exerted a mutual pull, and which had been missed in images made of the proteins when they were in crystallized form.) Brangwynne and others found that, under the right conditions, groups of proteins could "phase separate," like bubbles of oil in a salad dressing, forming structures. For decades, researchers had known that complex biochemical reactions tended to happen faster in living cells than in test tubes. Now they knew why: the lava-lamp-like conditions inside a living cell allow chemicals to take advantage of subtle attractive forces more efficiently than is possible in the looser and more uniform environment of a tube or a dish. We've long imagined a spark of life—but it could be the physical structure of cytoplasm that's the key.

This new understanding has begun to open doors. In 2017, Glass helped found the Build-a-Cell consortium—a steering committee for hundreds of labs that are trying to build a working cell from scratch. Researchers in the consortium began combining nonliving parts—proteins, ribosomes, RNA, and other molecular constructions—into membranes that resembled cells, hoping that the mixture would come to life by expressing genes, doing metabolic work, and eventually dividing. Drew Endy, a professor of bioengineering at Stanford who is one of Glass's co-founders, described the group as trying to solve the Humpty Dumpty problem: could the parts add up to a whole? Such artificial cells could be used as living factories for the production of biofuels or drugs, or as

hyperefficient sites of artificial photosynthesis. But although the right parts are there, none have crossed the border from nonliving to living. Endy's group was experimenting with slightly different ingredients; if that failed, the problem might be in how they're physically arranged. He told me, "I think there's a milestone right in front of us. I don't think it's that far away."

Roseanna N. Zia, a physicist who studies cells, emphasized the importance of physicality in biology. She told me that there were other "colloidal" properties of the cytoplasm, besides liquid-liquid phase separation, that nature might be using to its advantage—for instance, the fact that a shove at one end of the cytoplasm propagates, nearly instantly, to the other. Her group models how individual molecules subtly interact. "This area of understanding how colloidal-scale physics is regulating and orchestrating cell function—this is the frontier," she said.

In Hooke and van Leeuwenhoek's time, it was easy to imagine that progress in biology was a matter of zooming in further—seeing what parts the parts were made of. But, having seen to the bottom, we've found that reductionism is a dead end. What's needed now is synthesis. Many of the scientists I spoke with work in different disciplines, at a cluster of separate institutions in San Diego; occasionally, they swirl together, and our understanding advances.

Before I left town, Glass gave me a memento. It was a strange-looking cube, a sort of clear plastic paperweight with a pink square suspended inside. Glass explained that the square was a plate of agar on which colonies of the minimal cell had been grown. The colonies were encased in a few inches of resin.

It's on my desk now. Holding it up to the light, I can make out perhaps a dozen pinpricks. I wonder what these colonies—some of the first examples of synthetic life—will come to be seen as initiating. In science, the consequences of understanding are often unpredictable. A year after neutrons were discovered, in 1932, a Hungarian American physicist

named Leo Szilard was waiting to cross the street in London. As the light turned green, he saw how one might use the new particle to create a chain reaction. He took a step, and his mind reeled. ◆

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