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CHEMICAL & ENGINEERING NEWS
NOVEMBER 30, 2020

CORONAVIRUS: THE LATEST

LOOKING FOR LIFE'S SIGNAL

Chemists search for molecular signatures beyond Earth

P.30

ACS
Chemistry for Life®

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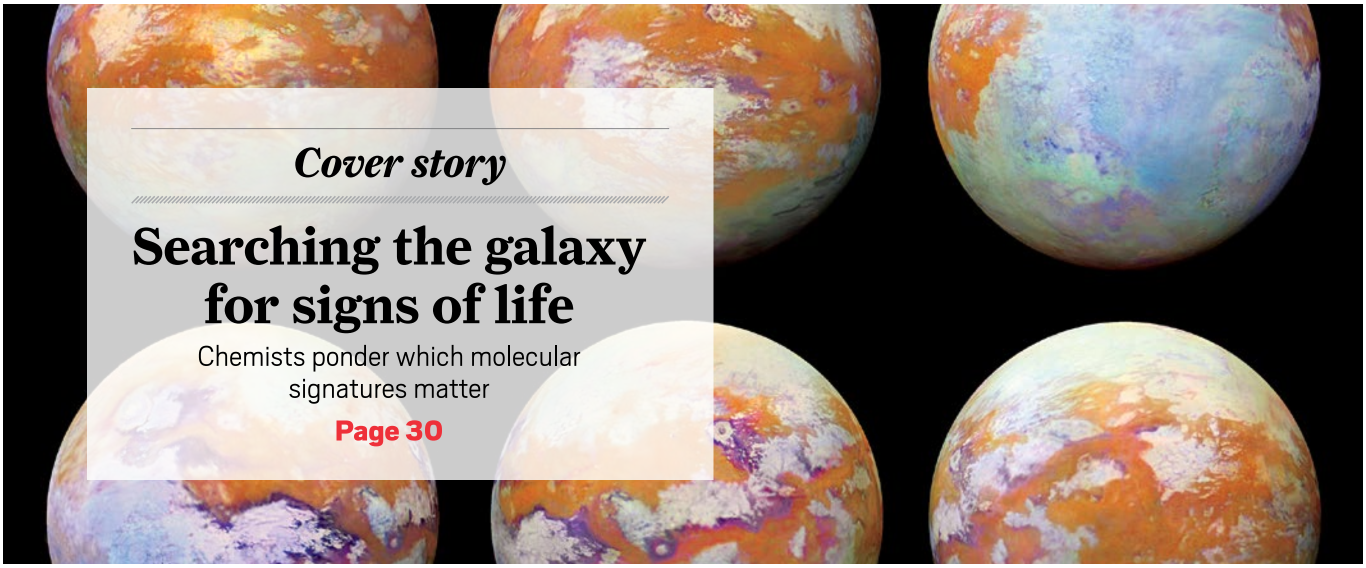
VOLUME 98, NUMBER 46
November 30, 2020



Quote of the week

"A 90% efficacy is impressive; a 62% efficacy is not."

—**Hildegund Ertl,** vaccine scientist, the Wistar Institute
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Illustration by Chad Hagen

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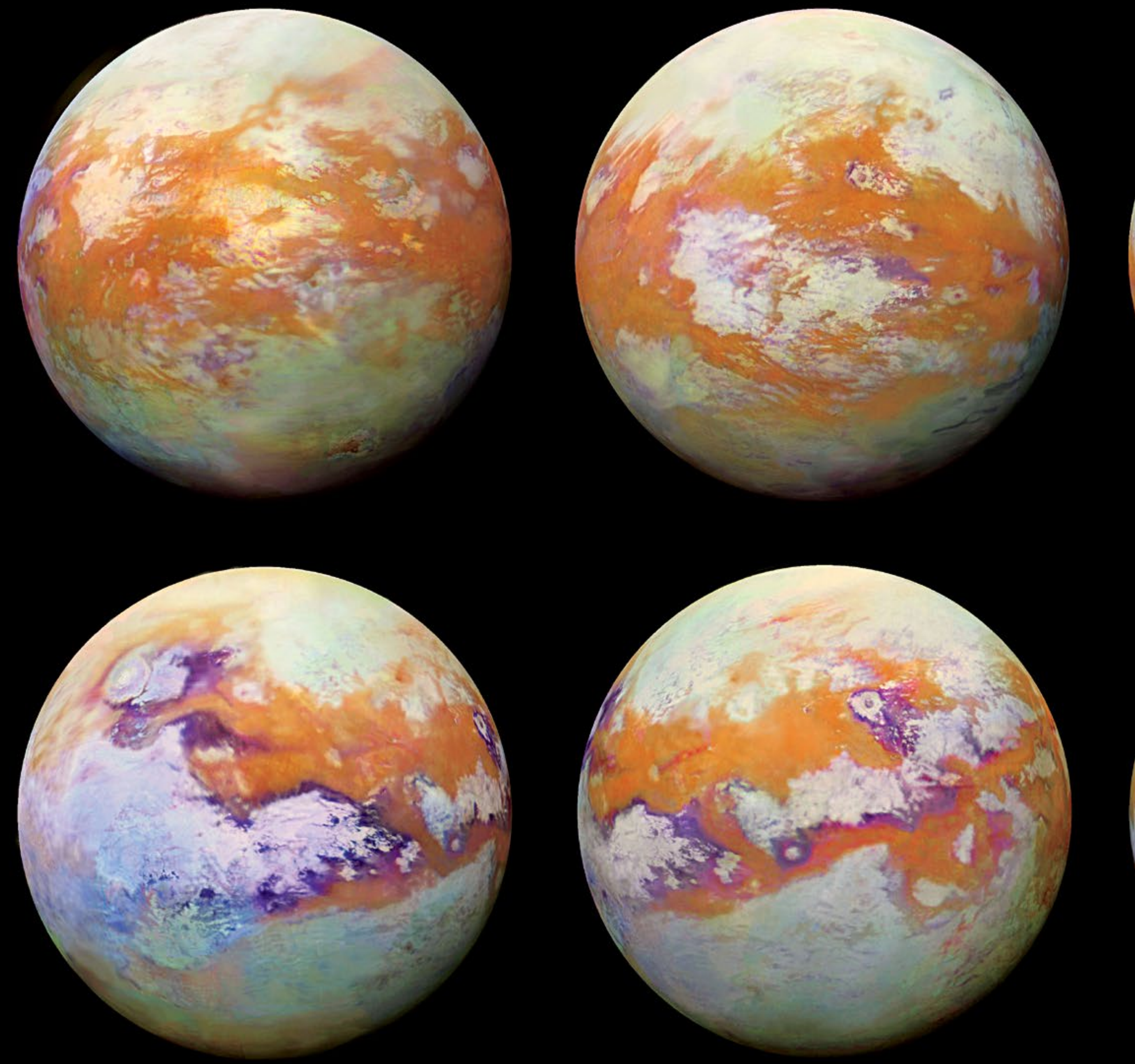
CENEAR 98 (46) 1–40 • ISSN 0009-2347



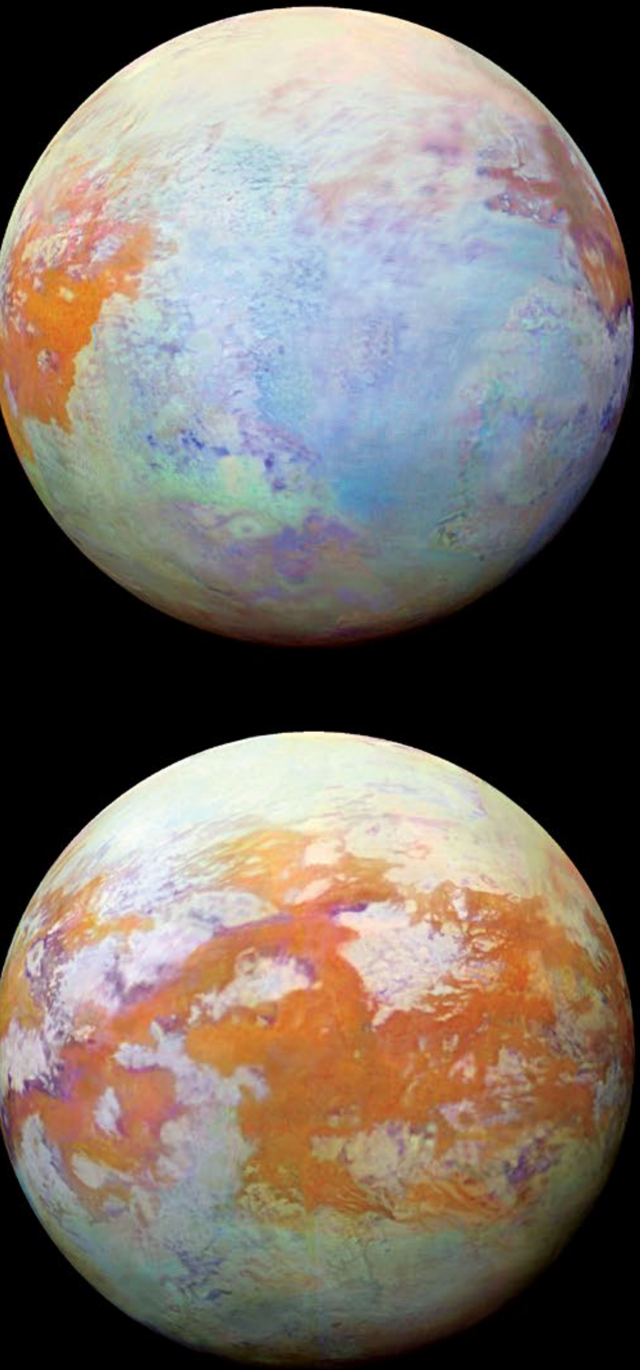
In brief

When astrobiologists look for physical evidence of past or present life beyond Earth, they search for biosignatures, like molecules with chemistry that doesn't make sense on the basis of nonliving processes. But determining if a molecule from another world is out of place enough to come from life means that scientists first have to understand the nonliving chemistry of the planetary body where it was found. While some scientists are developing tools like the Ladder of Life Detection to effectively evaluate biosignatures, others are trying to figure out how to differentiate biological chemistry from the rest. This conceptual work could help scientists who are analyzing data collected by missions searching for life in our solar system or beyond.

SEARCHING THE GALAXY FOR SIGNS OF LIFE

Chemists ponder which molecular signatures matter

ARIANA REMMEL, C&EN STAFF



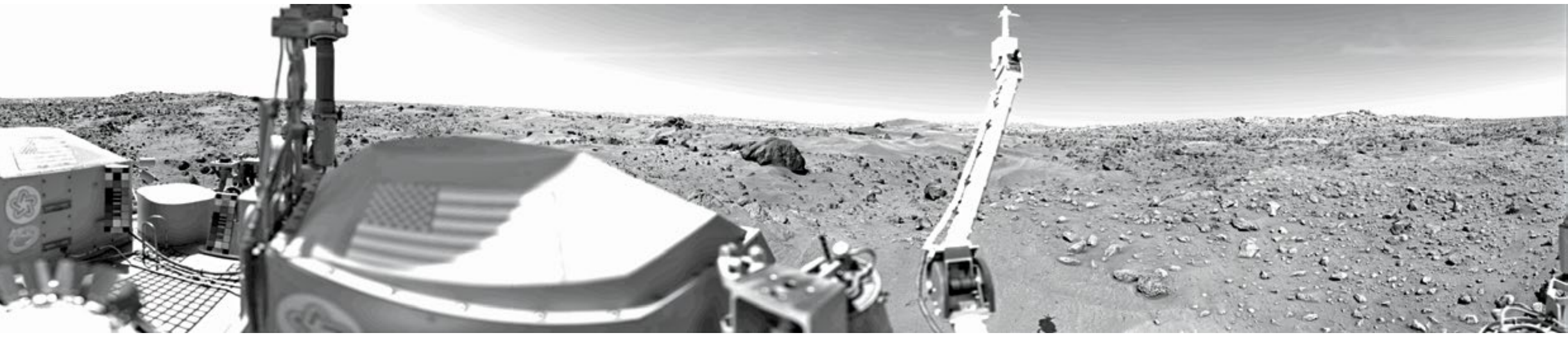
Titan's subsurface ocean is rich in organic molecules, making it a promising place to look for alien life in our solar system. These infrared images depict Titan from different angles.

CREDIT: NASA/JPL-CALTECH/UNIVERSITY OF NANTES/ UNIVERSITY OF ARIZONA

In 1976, two probes from NASA landed on Mars to conduct the first experiments in search of life beyond Earth.

The *Viking 1 and 2* landers were looking for evidence of living martian microbes. They treated soil samples with nutrients or other compounds that microbes could metabolize and then monitored for molecules that indicated active biochemistry.

Initial results had scientists excited: one experiment detected radiolabeled gases emitted from samples treated with carbon-14-labeled nutrients. "If information from other experiments on board the two *Viking* landers had not been available, this set of data would almost certainly have been interpreted as presumptive evidence for biology," writes Harold Klein, a NASA astrobiologist involved with the original *Viking* missions, in a paper published about the results (*Icarus* 1978, DOI: 10.1016/0019-1035(78)90053-2).



But other instruments on the *Viking* landers detected only trace amounts of organic molecules—like chloro- and dichloromethane. The lack of complex molcules, organic or otherwise, precluded a biological explanation for the radiolabeling results. Other experiments run by the landers were inconclusive at best. After many years of intense debate, the scientific community eventually concluded that nonliving, or abiotic, processes—like unknown oxidants in the soil—were a more likely explanation for the *Viking* results.

These experimental results demonstrated just how challenging it can be to identify physical signs of life, or biosignatures, much less make a definitive claim for having found life on another planet. The *Viking* missions led scientists to develop new techniques for evaluating biosignatures and instrumentation for detecting them. But these initial experiments also caused scientists to ask: How do we determine if something is alive in the first place?

"By and large, what we do in biosignature science is chemistry," says Heather Graham, an organic geochemist at the Catholic University of America and NASA's Goddard Space Flight Center. Biosignatures can be fossilized cells or active microbial communities. But they can also be molecules that are made only by living organisms. These biosignatures are molecules that would be out of place in a planet's geochemistry if it were not for some living organism churning them out.

"BY AND LARGE, WHAT WE DO IN BIOSIGNATURE SCIENCE IS CHEMISTRY."

—**Heather Graham,** organic geochemist, Catholic University of America and NASA's Goddard Space Flight Center

Yet without understanding the fundamental chemistry of our universe, scientists can't determine whether a physical indicator is weird enough to come from life. Now, scientists are trying to figure out what distinguishes biological chemistry from other types of chemistry and how we can quantifiably detect it. This work includes reevaluating what chemists have assumed about how biochemistry evolved on Earth. Astrobiologists hope this fundamental chemical research will help researchers collect and assess data from within our solar system and beyond.

LADDER OF LIFE

Before scientists can start to look for molecular signs of life, they need to define what life is. NASA's working definition is "a self-sustaining chemical system capable of Darwinian evolution." NASA scientists see life as a system of molecules that can reproduce, store information, and generate energy through metabolizing molecules in its environment.

NASA researchers have used that definition to establish a system for assessing whether a molecule or material from outer space—or even ancient Earth—is a biosignature. They call this framework the Ladder of Life Detection (*Astrobiology* 2018, DOI: 10.1089/ast.2017.1773). Developed by a research team led by Marc Neveu, an astrobiologist with the University of Maryland, College Park, and the Goddard Space Flight Center, the ladder consists of rungs corresponding to key features that scientists might look for in life, going from ones that are not strongly indicative of life to those that are.

"The key starting point here is that life has many features, but no single feature is a telltale sign of life in and of itself," Neveu says. He thinks the ladder can help scientists think about how to compile a chain of evidence in a "practical way."

This photo of the martian landscape was taken by the *Viking 1* lander on July 23, 1976.

For example, amino acids are the building blocks of proteins on Earth. If scientists found these molecules on another planet, that would correspond to the rung for potential biomolecule components. But that's only if amino acids can't be produced by any nonliving systems on that planet. A chemical hint of life can be deemed a biosignature only if the compound deviates from abiotic distributions, the authors write, meaning its presence or abundance doesn't make sense given the planet's general geochemistry.

"It really puts a lot of the burden of proof that you found life on understanding the context of what your environment looks like and what abiotic processes that don't involve life are at play," Neveu says. "The key here is to understand where the baseline is." Even if scientists can be reasonably sure that they've detected a potential biosignature, the ladder says that life has to be the hypothesis of last resort.

Frances Westall, a geologist with France's National Center for Scientific Research and a scientist with the European Space Agency (ESA), says the ladder's usefulness can be demonstrated by applying the framework to results from past experiments.

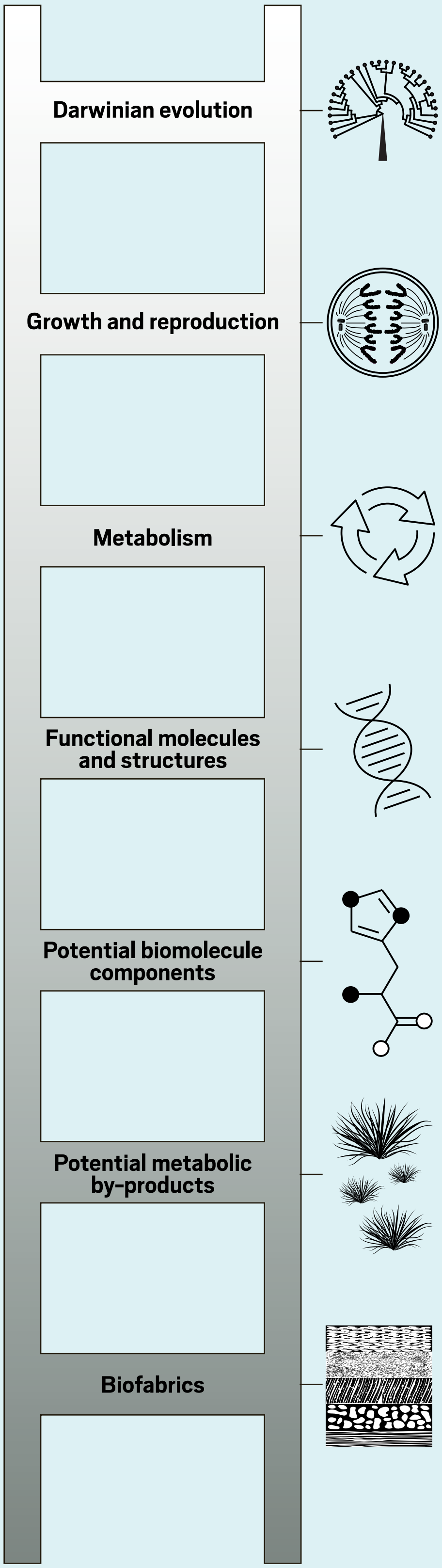
For example, when reevaluating the *Viking* experiments, scientists today would place the detection of those radiolabeled gases on the rung for metabolism because the gases suggested a response to the addition of possible metabolic fuels. But the *Viking* experiments produced no other data that could go on the ladder. Even after scientists confirmed that the signals detected by the *Viking* landers' instruments are real, the biosignatures fail to rule out enough abiotic processes to claim life as a last-resort hypothesis. Researchers can thus conclude that "there certainly is evidence for life, just not sufficient evidence to exclude abiotic processes," Neveu says.

It's not that an experiment should be ex-

CREDIT: NASA/JPL

Ladder of Life Detection

This framework helps scientists build a chain of evidence to confirm a potential observation of life. Features on the rungs ascend from weakly (bottom) to strongly (top) suggesting a living organism has been observed. Scientists would need to find features from multiple, but not all, rungs to claim that life has been found.



Darwinian evolution: Cultured microbes that show signs of adaptation to selective pressure would be a strong biosignature of life. These features are impractical to detect in current missions in our solar system.

Growth and reproduction: Observation of a suspected microbe at multiple stages of its life cycle would be needed to confirm growth and reproduction. Microbe motility could also indicate life on this rung.

Metabolism: Metabolic cycles can extract energy from molecules in the environment. These cycles often show a preference for certain isotopes or molecules, which change their abundance in biomass compared with the nonliving environment.

Functional molecules and structures: This class of molecules includes polymers with repeating charges or structures that might support information storage or other biological functions.

Potential biomolecule components: This category includes the smaller building blocks that could make up complex biomolecules—like the amino acids that build proteins on Earth. Some of these monomers are not produced abiotically on Earth.

Potential metabolic by-products: These complex molecules accumulate in a distinct way in the environment or contain features that follow a pattern, suggesting a living metabolic cycle is at play. These features—such as the carbon accumulated in a desert shrub—are more generic than those of the metabolism rung.

Biofabrics: Biofabrics are structures, like mats or layered morphologies, created by microbial colonies. They can be living or fossilized and can be observed with microscopy.

CREDIT: ADAPTED FROM *ASTROBIOLOGY*/C&EN/SHUTTERSTOCK

pected to find a feature on every rung of the ladder, Neveu says, but one feature is not enough to claim that you've found an alien life-form. Neveu hopes that the ladder will help scientists designing missions in search of life think about what kinds of evidence they would need to build a case for life.

The Ladder of Life Detection is still a work in progress and is meant to spur further discussion in the astrobiology community, Neveu says. One major limitation is that the ladder centers on NASA's working definition of life. "It all depends on what definition of life you're starting from," Neveu says, "and that's definitely an issue that has not been resolved." The order of rungs is also up for debate. Neveu expects that scientists will continue to add features and criteria to the ladder as our understanding of chemical traces of life evolves.

THE FUTURE OF MARS

Despite the disappointing results from the *Viking* missions, Mars remains a favorite destination for astrobiologists. Though the Red Planet's climate is harsh and its surface is bombarded with biology-zapping ultraviolet radiation, planetary scientists believe that Mars may have once looked a lot like Earth, coursing with rivers that could have been home to microbes.

The ESA and Russia's Roscosmos are jointly planning a mission called ExoMars 2022 that will explore Oxia Planum, a region of Mars rich in clay deposits that may have been left behind by an ancient river delta. The rover, named *Rosalind Franklin*, is specially equipped to look for signs of past and present life.

Because the martian surface is a harsh environment for preserving organic molecules, the *Rosalind Franklin* will drill down 2 m below the surface to collect samples that have been protected from the elements. A suite of onboard instrumentation, including the Mars Organic Molecule Analyzer (MOMA), will then interrogate the collected samples.

The samples can be processed one of two ways. In one, a sample is heated in an oven where volatile molecules are separated by gas chromatography before entering the ion-detection trap of MOMA's mass spectrometer. This process is not ideal for large organic molecules that might break apart with heat, so MOMA also has a laser to vaporize soil samples and directly inject the released molecules into the mass spectrometer.

Fred Goesmann, the principal investigator for MOMA and a scientist at the Max Planck Institute for Solar System Research, says the different sample preparation platforms allow MOMA to detect a broad array of organic molecules. So the researchers "can start with very few assumptions on what we might encounter," Goesmann says.

Unlike the *Viking* experiments, the MOMA instruments aren't trying to elicit a response from samples that could indicate ongoing biochemistry. Instead, the equipment is designed to look for inherent features of organic molecules that could suggest they came from living systems.

Goesmann says that when scientists look for such features, "the underlying assumption is that life creates order." He says that "life is choosy," meaning it prefers some molecules over others, so its presence can change the distribution of chemical species on a planet. For example, organisms on Earth prefer lighter isotopes in biomolecules, so the amount of carbon-13 and carbon-14 in organisms differs from their relative abundances on the planet in general. Such isotopic fractionation is a feature of metabolism on the Ladder of Life Detection and can easily be probed with a mass spectrometer.

Another feature of Earth's biochemistry, which is also found on the ladder's rungs, is a preference for chiral molecules. Most sugars and amino acids used in biology are exclusively one enantiomer, for example. Goesmann's MOMA instruments will be the first to directly analyze the chirality of organic molecules on another world. Because chiral molecules are difficult to characterize with gas chromatography/mass spectrometry, MOMA contains a tiny wet lab to modify the chiral molecules in a way that makes them distinguishable from one another and detectable in the mass spectrometer. Complex organic molecules featuring isotopic fractionation or an excess of one enantiomer could be important results for building a chain of evidence in favor of life on Mars.

In the meantime, the *Perseverance* rover, part of NASA's Mars 2020 mission, is equipped to prepare samples that may one day return to Earth for thorough analysis in traditional wet labs. The mission launched this summer and is scheduled to land in February 2021 at Jezero Crater, where the rover will also conduct experiments on the planet itself.

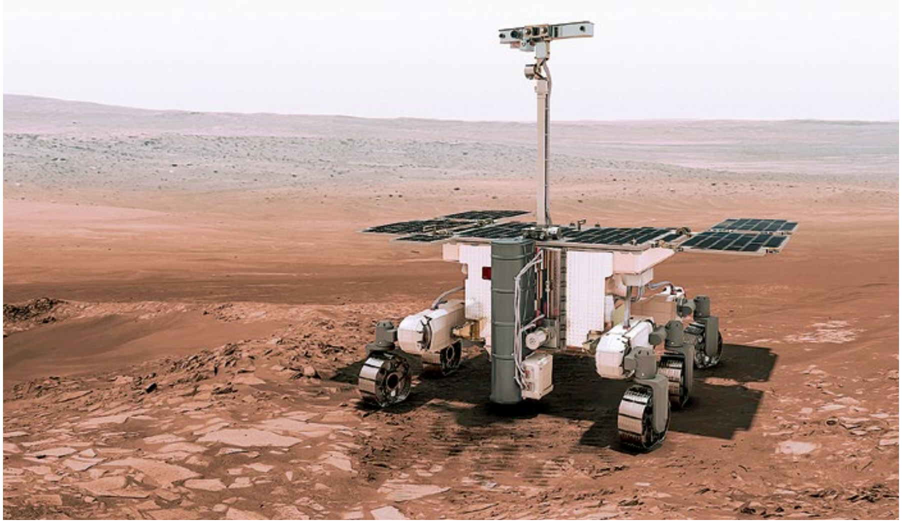
BACK TO THE DRAWING BOARD

But even as missions in search of life are planned for Mars and other bodies in our solar system, chemists on Earth continue to debate the basic molecular signs of life.

"I think the assumption within the prebiotic chemistry community and much of the biological community is that metabolism is a result of evolution," says Joseph Moran, an organic chemist at the University of Strasbourg. According to this prevailing view, molecules like enzymes evolved before the metabolic cycles they perform inside cells to produce energy and build cellular components. Moran takes the opposite view. His research with enzyme-free catalysis suggests that many biochemical reactions on Earth were possible under prebiotic conditions—before life was present.

For example, Moran has shown that iron can reduce carbon dioxide to form key metabolic intermediates of the reverse Krebs cycle and acetyl coenzyme A pathway, two ancient metabolic pathways that bacteria still use (*Nat. Ecol. Evol.* 2018, DOI: 10.1038/s41559-018-0542-2, and 2017, DOI: 10.1038/s41559-017-0311-7). His team has also found that pyruvate and glyoxylate can produce almost all components of the forward Krebs cycle in the presence of ferrous iron (*Nature* 2019, DOI: 10.1038/s41586-019-1151-1). "I guess I've made it a habit of trying to show that processes that we thought of as biotic can actually occur abiotically," he says.

And Moran is not the only one to argue that some biochemical reactions could have preceded life. A recent study from the Center for Chemical Evolution demonstrates how key analogs of the Krebs cycle can be produced under mild conditions without enzymes or metals (*Nat. Chem.* 2020, DOI: 10.1038/s41557-020-00560-7). Meanwhile, a team led by Bartosz Grzybowski, a physical organic chemist at South Korea's Institute for Basic Science, used computer algorithms to model how complex prebiotic chemical processes could have emerged from a handful of starting materials (*Science* 2020, DOI: 10.1126/science.aaw1955). Grzybowski previously developed software that uses chemical reaction rules to plan syntheses of complex organic molecules like pharmaceuticals. In this new study, his team taught a computer program rules based on possible prebiotic chemical reactions found in the literature and then watched what reactions it could plan starting with six simple molecules that probably existed on a prebiotic Earth. The researchers were excited when their software identified chemical cycles—synthetic routes that reproduce their starting materials—as you would expect from a rudimentary metabolism.



The *Rosalind Franklin* rover will search for life on Mars as part of the ExoMars 2022 mission.

CREDIT: ESA/ATG MEDIALAB

As chemists learn more about how chemical complexity can arise from simple mixtures of molecules, Moran and others say that astrobiologists will need to rethink what constitutes a biosignature or at least where metabolism fits on the Ladder of Life Detection.

The Laboratory for Agnostic Biosignatures (LAB) is a consortium of scientists funded by a grant from NASA to do just that. LAB is interested in looking at biosignatures that aren't biased by Earth's biochemistry.

Lee Cronin, a chemist with the University of Glasgow and a LAB researcher, thinks it's more than likely that the chemistry that led to the existing biology on Earth is no longer evident in the biochemistry we see. This means it may be impossible to reverse engineer what prebiotic chemistry on early Earth—or another planet—might have looked like solely from the life that's present today. As a result, a biosignature based on Earth's current biochemistry may not help us spot signs of developing life somewhere else.

LAB is looking for agnostic biosignatures—physical indicators that don't rely on an analogy to Earth's biochemistry—such as elemental accumulation. To understand the concept of elemental accumulation, for example, imagine an aerial view of a desert landscape peppered with sage brush, suggests NASA's Graham, LAB's deputy principal investigator. The amount of carbon that has accumulated in the sage plants is significantly different from that of the surrounding landscape, indicating that some biotic process—in this case, the plants' growth—is at work. This perspective even works down to the scale of microbes. "If you think about it, that's kind of a rudimentary way of describing a cell: it's a defined area where there's an accumulation and chemical abundance pattern that differs from its surrounding environment," she says. Looking for elemental accumulation patterns like these doesn't rely on an analogy to life on Earth, making it agnostic and possibly more broadly useful to astrobiologists.

BEYOND MARS

Mars isn't the only extraterrestrial body where life might exist or might have once existed. Recently, our nearest planetary neighbor, Venus, intrigued astronomers when a research team led by Jane Greaves at Cardiff University reported the first signs of phosphine in the planet's cloud decks (*Nat. Astron.* 2020, DOI: 10.1038/s41550-020-1174-4). This molecule is associated with anaerobic microbes on Earth, which had many astrobiologists excited for the possibility of alien life in the venusian atmosphere.

Phosphine could be a biosignature on Venus because it doesn't seem to belong. The planet's atmosphere is highly oxidizing—yet PH_3 is a highly reduced molecule. In the Ladder of Life Detection, this gas is a possible feature of metabolism. New evidence suggests that the phosphine signal could be an artifact of data processing (arXiv 2020, arXiv: 2010.09761). The new study was published on a preprint server, meaning it has not yet been peer-reviewed. Even so, some critics wonder if there might be an abiotic explanation for phosphine's presence on Venus.

"What it [the phosphine signal] shows is something weird is going on on Venus," says Matthew Pasek, a geochemist with the University of South Florida who specializes in phosphorus chemistry. He thinks that the authors of the first paper may have been too quick to dismiss abiotic avenues for phosphine production on Venus. For example, not knowing the composition of Venus's rocky surface makes it hard to rule out the possibility that acid rain from the cloud decks volatilized phosphorus in the planet's crust to produce phosphoric acid, which eventually formed phosphine. There's just too much we don't know about Venus's geochemistry without sending missions to probe it directly, Pasek says.

Farther out in our solar system, astronomers have identified other celestial bodies that may host life. In 2026, NASA will launch a mission to Titan, an icy moon orbiting Saturn. Titan is one of the few planetary bodies in our solar system with a dense atmosphere composed of nitrogen gas and methane. Scientists are particularly intrigued by the aqueous ocean hidden below Titan's icy crust. This carbon-rich sea occasionally explodes into the moon's atmosphere through ice-spewing volcanoes, a process called cryovolcanism. Michael Malaska, a planetary scientist studying Titan at NASA's Jet Propulsion Laboratory (JPL), believes that the moon's vast oceans and plentiful carbon make it "one of the most likely places in the solar system to find life." But on an alien moon chock full of organic molecules, it will be challenging to distinguish biosignatures from complex molecules made through background carbon chemistry.

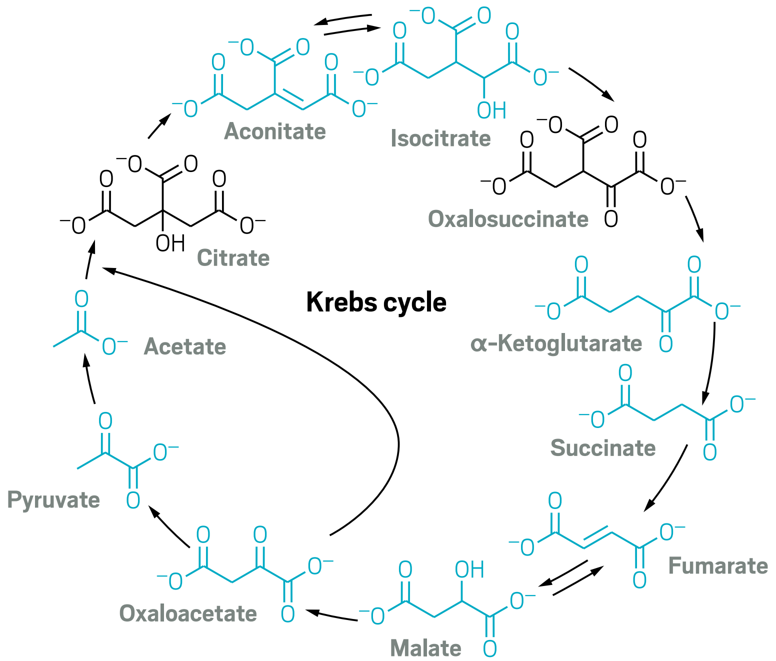
Malaska is part of a team at JPL led by planetary geologist Rosaly Lopes that is investigating how geochemical processes on the moon transport and alter carbon-based molecules. Lopes thinks the subsurface ocean is the most likely place for life to occur on Titan, so part of the team's mission is to understand what kinds of biosignatures might arise from the moon's carbon-rich waters. Because of Titan's complex geological processes, the researchers also have to consider how "these biosignatures might be modified as they go through the ice crust and come out as either gases or part of cryolava," she says.

The search for life beyond Earth also continues into the distant galaxy. Soon satellites like the James Webb Space Telescope will be able to study the habitability of exoplanets far outside our solar system.

What will scientists find inside or outside our solar system? "We're more likely to find traces of a prebiotic system than a biological system" on another planet, Westall says. She worries that we still don't know enough about the fundamentals of abiotic chemistry to suss out "the in-between bits" of a system with the potential to develop into biology.

Many scientists believe that given the right tools and enough time, we will find life beyond Earth. Others remain uncertain. "Do I think it's there? Yeah, probably," Graham says. "Do I think we'll find it? Maybe."

"The chase is half the battle," Malaska says. "If we did all of this and we found out that there are no other places in the solar system that has life, that would have very huge implications. We'd have to consider how absolutely lucky we are to have had this accident happen to us." ■



Joseph Moran's team found that pyruvate, glyoxylate, and ferrous iron can produce all but two (shown in black) molecules in the Krebs cycle.

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