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Electrifying biology in a bubble

The origin of life from Earth's primordial chemistry has long fascinated and perplexed us. Generations of scientists have endeavored to understand how complex biochemistry developed from organic compounds.

Researchers at UC Santa Barbara found that the conditions inside certain, naturally forming droplets promote reduction and oxidation (redox) reactions, which are crucial for life. The results support the idea that these droplets could have acted as proto-enzymes, enabling the formation of more complicated organic molecules. The findings, published in the Proceedings of the National Academy of Science, have important implications for understanding the origin of life and the development of biochemistry.

"We develop a way to see inside biologically important liquid droplets using electrochemistry to learn about how they create a suitable environment for chemical reactions," said co-lead author Nick Watkins, a former postdoctoral researcher in Professor Lior Sepunaru's lab.

This research project builds upon previous works by UCSB professor Herbert Waite, and along-standing collaboration with Professors Daniel Morse and Mike Gordon on protein assemblies. The study itself was made possible by a MIRA grant from the National Institutes of Health and an award from UCSB's Stanley and Leslie Parsons Fund in Biochemistry.

A chemistry crash course

A few theoretical principles govern which reactions occur in a system. The first is entropy: the amount of disorder. There's also enthalpy: the amount of heat absorbed or released during a chemical reaction at constant pressure. A thermodynamic potential called the Gibbs energy explains how entropy and enthalpy interact at a given temperature. It also reveals whether a reaction is likely to happen on its own, which chemists call "spontaneous," or whether it requires an energy input.

Chemists look at how these values change to predict and understand reactions. The environment — temperature, pressure, charge distribution, etc. — all affect whether a given reaction will occur on its own, like an iron nail rusting in a puddle.

A major question guiding this research concerns early organic chemistry on Earth. There is a hypothesis that, in pre-biotic Earth, the chemistry that led to the first lifeforms occurred within small droplets. "These droplets, called coacervates, can be thought of like droplets of oil in water," Watkins said. However, unlike oil droplets, coacervates form from macromolecules — like proteins, RNA or other polymers — that coalesce within a solution.

Sepunaru, Watkins and co-lead author Gala Rodriguez wanted to know whether coacervates create an environment that promotes biologically interesting reactions. In this paper, they looked at redox reactions. These involve transferring electrons between two substances: one substance loses electrons (is oxidized) and the other gains electrons (is reduced). These two processes always happen simultaneously, forming a single redox reaction. About a third of biochemistry involves redox reactions, which often underlie the pathways for making or transferring energy in a living system.

Peering inside micro-environments

The authors wanted a stable suspension that would mimic an environment that could've formed on prebiotic Earth. So they used an RNA molecule (polyuridylic acid) and a peptide (poly-L-lysine) to create their coacervate.

Rather than putting complicated biochemical reactants inside this droplet, the team chose to look at the reduction of ferricyanide to ferrocyanide. This only involves

adding or removing an electron to convert between $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$. While this redox pair is not itself a biological reaction, iron is a major player in biochemistry. What's more, the electrochemistry of this pair is well studied. "It is a textbook reaction you learn about in redox chemistry," Seunaru said.

With their system set up, Watkins and Rodriguez set out to measure the coacervates' Gibbs energy, which was a surprisingly straightforward matter: measure the voltage across the sample. "The voltage that you're measuring from a redox reaction is linearly proportional to Gibbs energy. In some way, we can think about electrochemistry as a Gibbs energy meter," said Sepunaru, an associate professor in the Department of Chemistry & Biochemistry.

The authors use a metal electrode coated with a thin film of coacervates suspended in water. The standard arrangement is that two electrodes connect the circuit, while a third electrode provides a reference ground to calibrate the voltage and obtain useful information. This provided a measurement across the whole sample, which the team used to calculate the voltage for an average droplet.

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A fundamentally different environment

The team found that the environment within the coacervate increased the probability of redox reactions occurring on their own. Sepunaru, Watkins and Rodriguez aren't the first, second, or even third team to demonstrate that these droplets facilitate redox reactions. But they are the first to describe how the micro-environment drives this.

What they achieved is unprecedented insight into the cause of the energetic changes. The team was able not only to measure how the droplet environment changes the energy of the reaction, but also to explain why these changes occur. “We showed that the water surrounding the iron behaves differently inside the droplet than in ordinary water,” Sepunaru said.

The team used Raman spectroscopy to link changes in the voltage to shifts in the droplet’s internal environment. In particular, they tracked changes in the vibrational modes of the iron-containing molecules as different concentrations of these were partitioned into the droplet. This provided direct molecular-level evidence for their electrochemical observations.

“Beyond finding that the droplets' internal environment makes redox reactions more likely, we also found that molecules have an easier time donating electrons within this unique environment,” said Rodriguez, a doctoral student in Sepunaru’s group.

Most researchers believed that the coacervates served as tiny reaction chambers that concentrate reactants. But the new study reveals that this is only part of the mechanism; there is actually a change in the Gibbs energy within this microenvironment. That means the environment is different within these coacervates in ways that alters the probability of these reactions occurring on their own. “Inside these droplets, the chemistry is very different from normal water,” Sepunaru explained. “So, you can make chemical and biochemical reactions that are otherwise impossible in water, which is very important for the origin of life.”

The authors think of coacervates as proto-enzymes because they actively catalyze certain reactions, much like an actual enzyme would. Both are made of polymers, and both alter their microenvironment in ways that promote specific reactions (although enzymes are much more selective, efficient and sophisticated). But instead of being complex, highly evolved proteins, coacervates are naturally occurring droplets with much simpler chemistry. By functioning as proto-enzymes, coacervates could have enabled more complicated bio-molecules to arise.

A need for speed

Sepunaru had several false starts with this project until Watkins and Rodriguez took it on. “It took a lot of innovation and creativity on their part to actually solve the

problems,” he said.

While Gibbs energy explains which reactions are likely to occur, it often doesn’t correlate with the reaction rate. Making a reaction more spontaneous does not necessarily mean it happens faster. However, Gibbs energy and rate are correlated in the special case of electron-transfer reactions, the class of redox reactions the team studies. They are using this to their advantage by investigating how coacervates influence reaction speed. They also plan to look at more complicated redox reactions.

A lot of teams have their instruments trained on coacervates. “But we’re the only lab with our eyes on the Gibbs energy,” Seupunaru said.

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