

Tiny Temperature Sensors

Alla Katsnelson

New nanothermometry techniques give cell biologists ways to measure temperature at the subcellular level.

Luis Carlos first realized he might be able to make a nanothermometer while developing light-emitting diodes more than 15 years ago. His team observed that lanthanide ions in the diodes reacted to changes in temperature by reliably shifting the color they emitted.

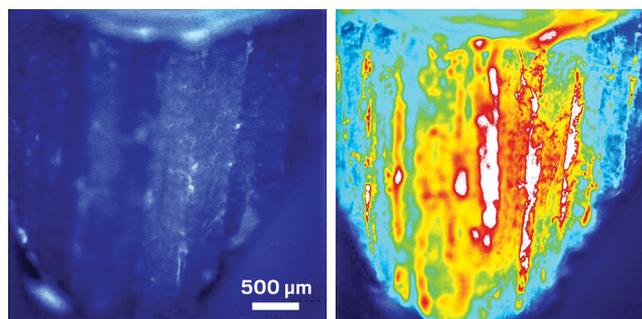
Carlos, a nanomaterials scientist based at the University of Aveiro, immediately realized that these ions might have a destiny beyond simply supplying the color for LED lights or displays. So he and his colleagues submitted a paper demonstrating that lanthanide ions could be used as temperature sensors.

But its publication was initially turned down. What's the point of using light emission to detect temperature, one reviewer asked, when easier and more robust techniques, like thermocouples or infrared imaging, are already commercially available?

"Of course this is true", Carlos says, "but [the reviewer] wasn't thinking on the nanoscale"—a size regime at which established thermometric techniques are too bulky and lack the sensitivity to work. It also wasn't clear what the value of temperature sensing on such a small scale could be, he adds.

Initially, Carlos developed lanthanide nanoscale thermometers to measure temperature gradients in electronic materials, but collaborators soon guided him toward another application: thermometric probes that could monitor tumor cells. At the time, scientists had begun testing an experimental cancer therapy that would kill tumor cells by heating them to above 40 °C. However, too much heat damages surrounding tissues, so the ability to monitor temperature change in targeted cells is key.

Carlos isn't alone in applying tiny temperature-sensing particles to biological systems. Nanoparticles are the right size and have properties that make them ideal for measuring the temperature of cells and even of the organelles within them. Conditions such as inflammation and diseases such as cancer are accompanied by hyperlocal temperature changes



A fluorescent nanosensor reveals temperature variation in the muscle of a beetle after it's stimulated to take flight (right). Without the nanosensor (left), the stimulated muscle displays its natural, background fluorescence. Blue is low-intensity fluorescence, and red is high. Reprinted with permission from *ACS Sens.* 2016, 1, 1222–1227. Copyright 2016 American Chemical Society.

in tissues, so nanothermometers could have broad applications for both health monitoring and treatment.

The tools are also poised to address basic questions about cell biology. Chemical reactions within a cell produce heat, but researchers know little about how such heat affects processes in the cell, or the organism in which it resides. An increase in temperature may serve as a cue to switch other reactions—or the transcription of certain genes—on or off. "Our hypothesis is that the living organism may use this ultra-locally-produced heat as another type of intracellular signaling", says Madoka Suzuki, a biophysicist at Japan Science & Technology Agency. "But nobody knows, because there has been no method" to detect it, he adds.

Scientists have known since the 1970s that temperature affects the light emission of certain chemical species, but not until the turn of the 21st century did they begin to harness this property for nanoscale sensing. A seminal article laying out the concept of using luminescent nanoparticles for thermometry was published in 2002 by researchers at Nomadics, Inc., an Oklahoma-based company that makes chemical and other sensors. It took another few years for efforts to blossom. Since about 2010, multiple groups have developed nanoscale thermometers that can detect temperature changes with a sensitivity of less than 1 °C. "The signal of the luminescence and the thermal resolution

Published: May 15, 2017

we can achieve are good enough now to study biological problems”, says Daniel Jaque, a biophysicist at the Autonomous University of Madrid.

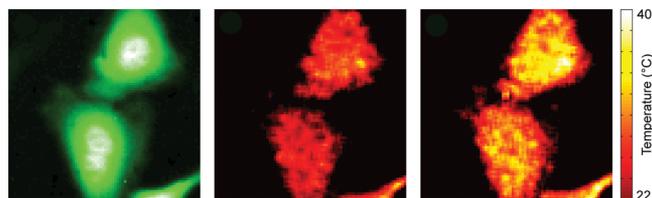
The most widely used probes for cell-based nanothermometry are light-emitting chemical species such as lanthanide ions, organic dyes, fluorescent proteins, and various nanoparticles such as quantum dots and nanocrystals. Many light-emitting species must be encapsulated in some sort of nanoparticle to prevent direct contact with the biological sample. This protects the probes from cellular conditions that could affect light emission and thereby skew the temperature reading.

In most cases, researchers excite these probes with an external light source and then analyze the resulting change in the intensity or other properties of the emitted light, translating it to a change in temperature by comparison to a standard measure such as a thermocouple. Different luminescent probes each have their own advantages and drawbacks. Fluorescent dyes are easy to use with other cell biology techniques, but currently available dyes are sensitive to non-temperature-related changes in the cell. They must also compete with the natural fluorescence generated by biomolecules in tissues and are prone to becoming altered and deactivated by the external light source, a process called photobleaching.

Quantum dots—nanosized particles of semiconductor material—emit an especially strong signal and are resistant to photobleaching, but most are made with cadmium or other toxic substances, limiting their use in living organisms. And like lanthanide ions, most quantum dots emit light in the visible spectrum, which doesn't penetrate tissue, thus making it hard to detect in living organisms. Researchers are working on finding the right combinations of lanthanide ions and developing quantum dots that emit in the infrared so that they can be used in living organisms. “At this point it is not yet established what is the best system”, says Carlos.

Carlos has continued developing thermosensing techniques for therapeutic ends. In 2015, he and colleagues created a nanoparticle that combined a magnetic bead with lanthanide complexes (europium and terbium ions) such that it both delivered heat and measured temperature, allowing researchers to monitor the amount of heat delivered to tumor cells. The group is now working to demonstrate the particles' double-duty power in living cells.

Jaque's lab, meanwhile, is developing a method in which a single nanoparticle, rather than many, would be deployed inside a cell. That's important, he says, because flooding a cell with nanoparticles may alter its function. Last year, Jaque's and Carlos's groups worked with Brazilian colleagues



A nanothermometry technique based on green fluorescent protein (left) maps temperature in human cells at room temperature (center) and when heated with an infrared laser (right). Reprinted with permission from *Nano Lett.* **2012**, *12*, 2107–2111. Copyright 2012 American Chemical Society.

to develop nanoparticles doped with neodymium and ytterbium ions that track temperature dispersal in the tissue of live mice, a feature that might be harnessed for diagnosing cancer or localizing oxygen shortages in tissue. Now that some basic technical issues have been worked out, says Carlos, a key goal is to “enlarge the borders of the field” by identifying new and innovative applications.

Scientists using nanothermometry to study basic properties of cells rely on essentially the same techniques, but such studies require higher spatial and temperature resolution, says Suzuki. In 2007, his lab published a study in which a thermometer consisting of a fluorescent dye enclosed in a glass micropipette measured temperature changes inside a cell as a drug triggered calcium ions to flow inside. Some of the energy it takes to power the ion pump regulating calcium concentration in the cell dissipates as heat, Suzuki explains, and his thermometer captured this local heating, plus perhaps heat that was triggered from other unknown sources.

Last year, Suzuki's group used fluorescent dyes embedded in polymeric nanoparticles to detect temperature changes generated in the muscle a beetle uses to take flight. The study achieved subcellular spatial resolution and a temperature resolution of 1.0 to 1.4 °C, Suzuki says, but notes that whether the technique would work as well in tissue that produces less heat is unclear. Earlier this month, Suzuki and an international team of colleagues reported using a fluorescent dye to visualize temperature increase in individual brown adipocytes, a type of fat cell known to produce heat.

Suzuki and colleagues have developed probes that they say can track temperature changes in specific organelles such as mitochondria and the endoplasmic reticulum. Using the probes, they have found differences of as much as 1 °C within the different parts of a cell. Some researchers, including nanoscientist Guillaume Baffou of the Fresnel Institute, have disputed the results, saying that current nanothermometers are not reliable enough to be sure these differences are valid and that such temperature increases are theoretically impossible under the laws of thermodynamics.

Suzuki agrees that his findings diverge from the theory, and explains that for precisely that reason, his team supported the findings with rigorous control experiments. Our understanding of equilibrium in thermodynamics was developed for large steam engines, not tiny cells, he notes, and therefore the challenge will be to bridge this gap. Carlos agrees that nature may hold some surprises. “At the microscale, and of course at the nanoscale, the laws that govern energy transfer and heat transfer could be different”, he says.

Still, Baffou speculates that factors other than temperature might be contributing to the fluorescent probes' signal. “The interior of a cell is very complex”, says Baffou. “There are many things in the cell that can affect the fluorescence.” Although he initially used [green fluorescent protein](#) to map temperature within a cell, Baffou has since moved to a technique that relies on a special camera to sense temperature by detecting the refractive index in a cell when it is heated.

Yet another approach that may be less prone to error, according to both Suzuki and Baffou, relies on [nanodiamonds](#)—nanosized bits of carbon—and was reported in 2013 by a team at Harvard. Nanodiamonds are nontoxic and don't interfere with cellular processes. When they are hit with a laser, they fluoresce at points of impurity in the diamond—spots where a nitrogen atom replaces a carbon atom. The variations in fluorescence intensity reveal temperature inside the cell at a [200 nm spatial resolution and 0.0018 °C temperature resolution](#). Because of their tiny size and stability, nanodiamonds could be ideal temperature sensors for detecting ultralocal heat production in the cell, Suzuki says, but so far, setting up and using the approach is complicated, so it has not been widely adopted.

For researchers working with nanothermometers, several priorities for improving these tools loom. Suzuki outlines a trio of qualities scientists are looking for in the perfect probe: It must be robust enough to remain unchanged by chemical factors inside the cell, it must consistently achieve a temperature resolution of 0.1–0.2 °C or better, and it must be targetable to specific subcellular locations. Another important goal, Suzuki says, is to gain a better understanding of the theoretical dimensions of thermodynamics at such a small scale in the cell. He adds: “People working in this field are still struggling to build the methodology.”

Alla Katsnelson is a freelance contributor to [Chemical & Engineering News](#), the weekly newsmagazine of the American Chemical Society.