IMAGING

# A new sensor with a sense for glucose

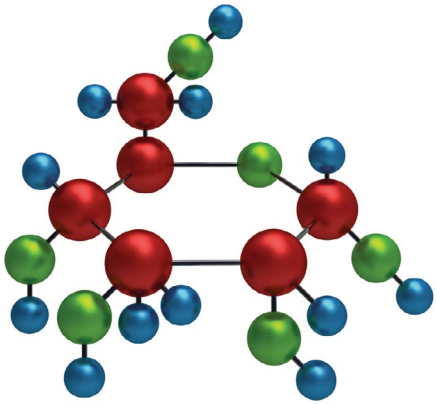
Keller, J.P. et al. *Cell Rep.* **35**, 109284 (2021)

The brain is a demanding organ, consuming a disproportionate amount of energy relative to the rest of the body. There has however been some debate in recent years about how it gets charged. Glucose is the primary energy source for the brain, but some studies suggest that neurons don't actually metabolize the sugar directly, as was long thought. Rather, the proposed astrocyte-neuron lactate shuttle hypothesis suggests that astrocytes in the brain take up glucose, convert it to another form – lactose – and then release that sugar to the extracellular space for other cells in the brain to consume.

Working with co-cultures of rat neurons and astrocytes and a novel, genetically encoded green fluorescent protein (GFP) sensor for glucose, new research from Loren Looger's lab at the HHMI Janelia Research Campus saw some potential evidence for the shuttle. They used an additional red marker to distinguish between the cell types, and then perfused glucose solutions into their petri dishes and measured the rate of glucose changes. The initial results indicate that astrocytes might transport glucose at a higher rate than the neurons.

Case closed? Hardly, says Looger. His lab didn't set out to settle the glucose-lactate debate – their goal is to build the tools needed to help *others* figure those details out. In the current work, published in *Cell Reports*, Looger and his colleagues describe iGlucoSnFR, a new sensor for sniffing out which cells are taking up glucose.

In addition to the rat neuron & astrocyte co-cultures, the team also demonstrates iGlucoSnFR's potential in larval *Drosophila* and zebrafish. For flies, they created a transgenic line that expressed the sensor throughout their neurons; they then excised larval brains and perfused those with glucose, observing unique spatiotemporal patterns: rather than simply diffusing throughout the tissue, glucose was taken up first in interior areas of the central nervous system. For zebrafish, they created a double transgenic line that expressed the green fluorescing glucose sensor throughout the fish as well as a red calcium indicator, jRGEC01a, in its muscles. Following the addition of insulin and epinephrine to the water, they were able to observe increases in glucose uptake in muscles, the liver, and brains of larval zebrafish that paralleled the activity captured by the calcium sensor.



Get that glucose! Credit: Borislav Marinic / Alamy Stock Photo

iGlucoSnFR consists of two elements: a binding protein – here, a periplasmic glucose binding protein found in the bacterium *Thermus thermophilus* – that serves as a scaffold for a fluorescent indicator. The binding protein is shaped a bit like a Venus flytrap, says co-author Jonathan Marvin. When it finds its ligand – in this work, glucose – it closes a little around it; that causes a conformational change in the hinge, and the attached fluorophore – GFP – undergoes local changes in its environment. That yields two different fluorescence states – dim and bright.

It's a pretty modular system, once you figure out how and where to attach your desired indicator to a protein with an affinity for your molecule of interest. "It's gotten to the point where making a sensor that works in a test tube is remarkably straight forward," says Marvin. "The hard part is getting it to function as we would like it to within the parameters that we need for someone who wants to study something in say, the brain."

Getting long-term expression in organs and other tissues does get a little more complicated that screening proteins in *E. coli*, notes Looger – you need to cope with 'gunk' in animal cells and test whether the sensor has the right affinity and can fold correctly and in the appropriate place, without becoming toxic. Fluorescence is also relative and can vary, so calibrating the sensor in a particular model system is also important, says co-author Jacob Keller.

Glucose was nevertheless a relatively easy analyte to work with, says Looger – there tends to be a lot of it around – millimolar amounts – so the sensor didn't need super high affinity, and its signals tend to be slow, on the order of seconds to minutes, which is easier to capture than say nanomolar calcium fluxes that occur in fractions of seconds. An improved version of iGlucoSnFR that localizes to the membrane and can report extracellular glucose is soon to be available, says Marvin. Plus, there are endless novel combinations of promotors, fluorophores, and ligand affinities to consider too.

"If you want to talk about all these other ligands, then our approach really is generalizable. This paper is about glucose, but we've got glutamate, GABA, dopamine, serotonin, arginine, aspartate, ATP, acetylcholine - all these million other things," says Looger, encouraging anyone interested in a particular sensor to get in touch. "We really like these scaffolds that generalize. We can hammer a lot of nails with one specific type of hammer."

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