BIOSENSORS

Nature's pH meter

A new pH nanosensor changes color in acidic cell compartments by forming an unusual four-stranded DNA structure.

The pH is a measure of acidity or basicity in a solution and influences the activity of almost all enzymes and biochemical reactions. The cell is divided into compartments to allow specific biological processes to take place at optimal pH. Although researchers can easily measure and control pH in test tubes, monitoring the pH inside living cells is more elusive, partly because of their small scale.

Advances in nanotechnology have generated nanometer-sized devices that are beginning to find applications in biological systems. Yamuna Krishnan at the National Center for Biological Science in Bangalore, India is an expert at creating nanodevices based on DNA. As a chemist, Krishnan knows of many interesting DNA structures but she always wonders "what applications we can realize with these beautiful architectures to help other fields grow." "I like to work with biologists," says Krishnan, who enjoys helping solve challenges faced by her colleagues. One such challenge was finding an easy way to measure pH in living cells.

Krishnan and colleagues exploited a special DNA structure that forms when cytosine-rich DNA strands are exposed to low pH. The high proton concentration induces unusual cytosine-cytosine base-pairing and leads to the formation of an intercalating four-stranded DNA structure called the i-tetraplex. To create a pH sensor they placed cytosine-rich DNA sequences on both ends of a piece of B-form DNA. When the pH drops, the ends come together and form an i-tetraplex. This DNA nanomachine, which they call an I-switch, has two defined conformations: an open state at pH higher than 6.8 and a closed state in more acidic environments of pH less than 5.5. To provide a visual readout of pH, they added fluorescent tags to the ends of the I-switch. When the I-switch closes, a phenomenon known as fluorescence resonance energy transfer (FRET) occurs, resulting in a change of the relative fluorescence intensity from both tags. The ratio of fluorescence correlates linearly with pH values from 5.8 to 7.0 inside the cell. The change happens within a minute and is reversible, making the I-switch a useful tool for intracellular pH measurement.

Krishnan and her collaborators tested the I-switch in blood cells isolated from fly larvae. When added directly to the cells, the I-switch is taken up by endosomes, the specialized cellular structure formed by the bulged cell membrane. By tracing the ratio of the I-switch fluorescence over time, they precisely measured the pH in the endosomes at various maturation stages. The I-switch can also be conjugated to specific ligands, such as transferrin, to label and measure pH in certain subsets of endosomes.

"An advantage of the I-switch is that you can use any convenient FRET pair [of fluorescent tags], which could enable studies on compartment mixing," says Krishnan. This color flexibility was not available in existing pH sensors and it allows easier integration of the I-switch with other fluorescence-based methods. According to Krishnan, "another advantage is that the I-switch is very bright. Previous sensors were good at pH 7 but the intensity drops quickly at acidic pH. That's not a problem with ratiometric sensors like the I-switch."

Going forward, Krishnan is working on optimizing the DNA sequence to make faster versions of the I-switch, to target it to other organelles and to tune the dynamic range. She also continues working with biologists to study pH regulation and effects in animal models. "We are very keen on this technology to become a well-accepted method in biology because this is possibly one of the simplest ways to measure pH in living cells," says Krishnan.

As researchers begin to harness more and more biomolecules to make small biosensors, we are starting to gain tremendous insights into cellular processes. As Krishnan puts it, "we have to use nature's tools to study nature." We have just begun to learn how to use nature's pH meter.

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RESEARCH PAPERS
Modi, S. *et al.* A DNA nanomachine that maps spatial and temporal pH changes inside living cells. *Nat. Nanotechnol.* **4**, 325–330 (2009).