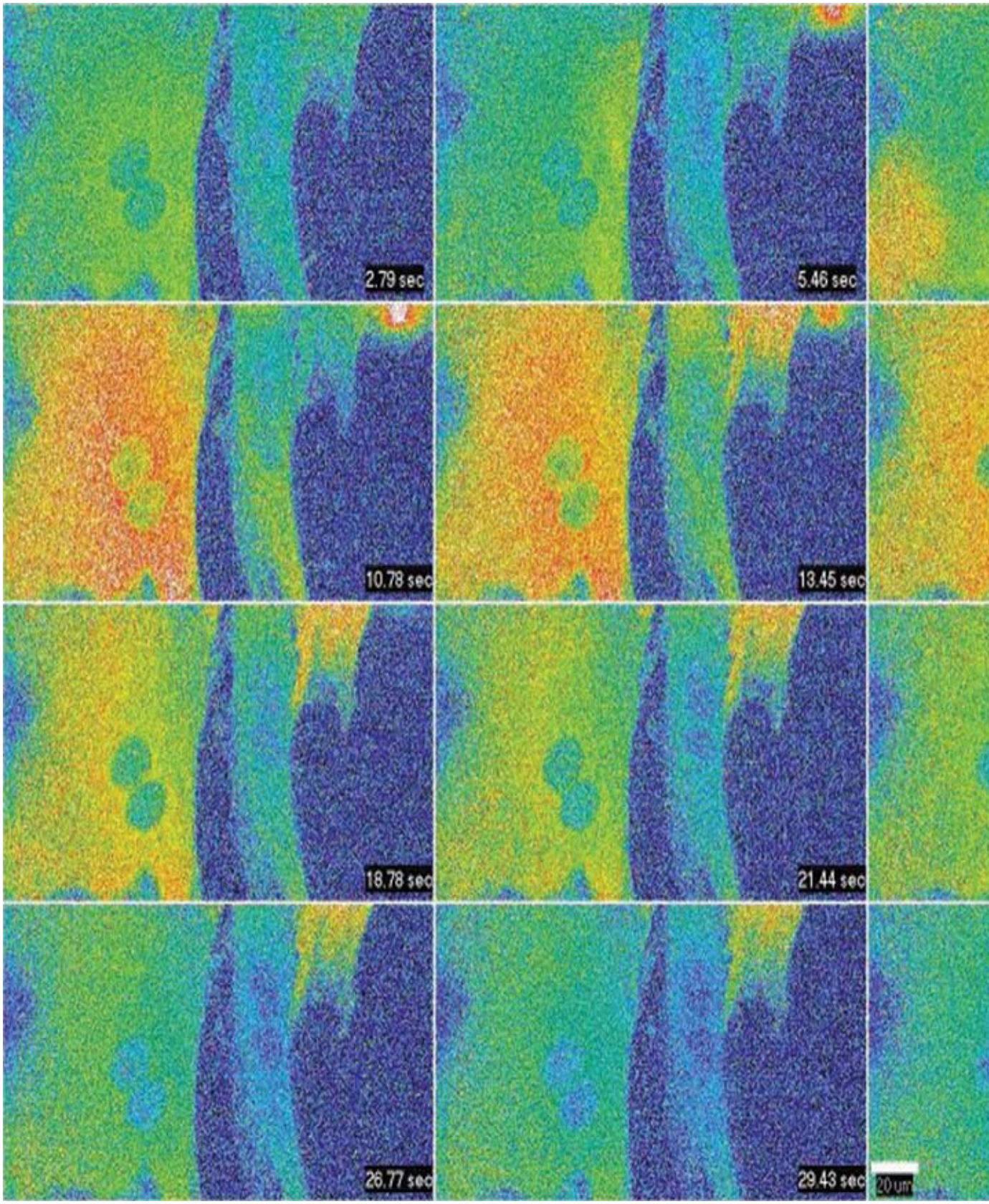

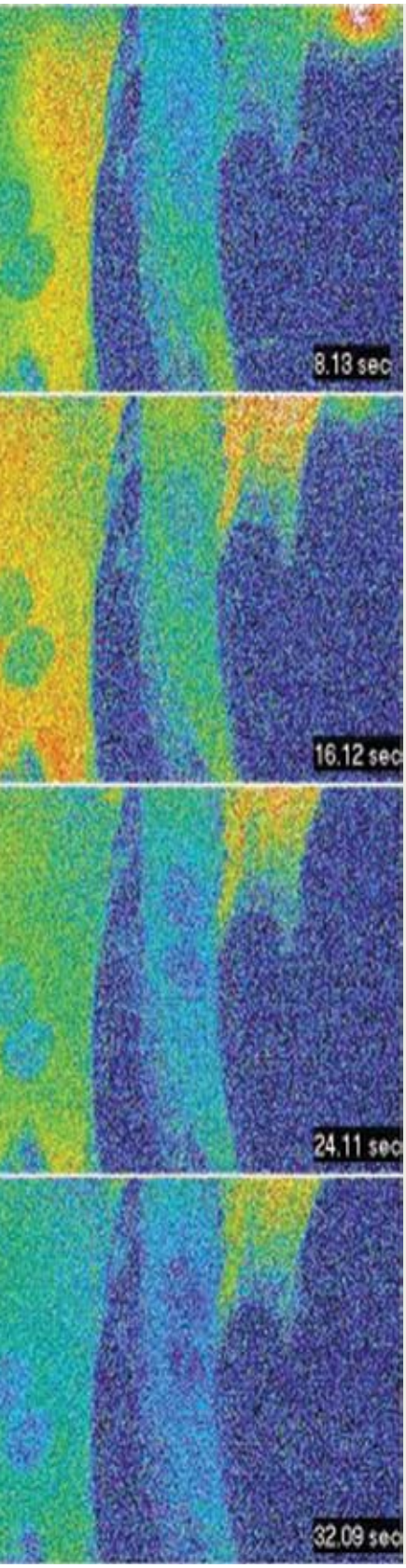

Listening in on cellular communication

PREMO™ CAMELEON CALCIUM SENSOR.

Cells are constantly talking. Extracellular signals such as hormones and neurotransmitters convey a barrage of information to the cell that is crucial for its survival and function. Within the cell, second messengers like Ca^{2+} and cAMP relay these extracellular signals to proteins that translate the signals into action. To be able to measure such dynamic signaling events within a cell in real time is to eavesdrop on the functional language of the cell in its native state. A variety of ion indicator molecules exist to allow certain insights into these important pathways and processes; however, there has not been a widely available genetically encoded tool to expand researchers' capabilities into targeted and iterative measurements. With the release of the Premo™ cameleon calcium sensor, a genetically encoded, fluorescence resonance energy transfer (FRET)–based tool for ratiometric measurement of Ca^{2+} is now available. →

Figure 1—Live-cell calcium imaging using the Premo™ cameleon calcium sensor. Porcine left atrial appendage (LAA) progenitor cells were transduced with the Premo™ cameleon calcium sensor, incubated overnight at 37°C, and imaged at 3 second intervals before and after stimulation with 10 µM ATP using a Zeiss® LSM 5 LIVE microscope. The time-lapse sequence of intensity profile images illustrates changes in YFP and CFP signal levels, which are pseudocolored to indicate Ca^{2+} flux. Images obtained in collaboration with Michael Rutten and Kenton Gregory, Oregon Medical Laser Center Bioimaging Suite, Providence St. Vincent Hospital, Portland, Oregon.

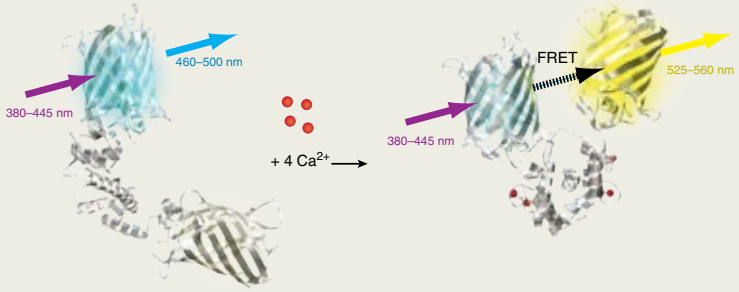

Figure 2—Schematic of the Premo™ cameleon calcium sensor mechanism.

The BacMam delivery system uses baculovirus to deliver the sensor into cells of interest and has been shown to be an efficient transducer of a wide range of mammalian cell types [4] (see **www.invitrogen.com/premo** for a full list), including stem cells and primary cells that have proven difficult to transfect or transduce by other methods. The delivery system is currently not effective in hematopoietic or macrophage cells. The Premo™ cameleon calcium sensor is provided as a ready-to-use solution of virus that is used in conjunction with the included Premo™ enhancer for increased expression of the cameleon sensor. The sensor provides an effective technique for measuring Ca^{2+} mobilization in transduced cells using microplate-based assays or fluorescence microscopy. Figure 5 illustrates the utility of the probe in agonist and antagonist modes for the measurement of Ca^{2+} in a microplate-based assay.

Interpret cell signaling in real time

The Premo™ cameleon calcium sensor combines a genetically encoded FRET-based calcium sensor with efficient BacMam (baculovirus) delivery to measure intracellular calcium in live-cell studies [1] (Figure 1). The sensor is based on the YC3.60 version of the cameleon family developed by Tsien, Miyawaki, and coworkers [2,3] (Figure 2). Binding of four Ca^{2+} ions to the calmodulin portion of the sensor induces a conformational change that brings Cyan Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP-cpVenus) closer to each other, allowing FRET to occur. This leads to an increase in YFP emission (535 nm) and a decrease in CFP emission (485 nm) indicative of Ca^{2+} release in the system under study (Figure 3). The ratiometric aspect of the readout (Figure 4) helps eliminate assay variations due to autofluorescence or differences in fluorescence between samples.

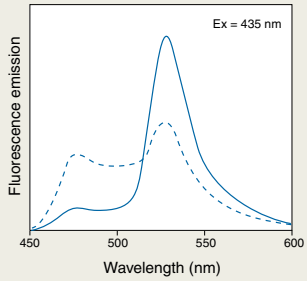

Figure 3—Fluorescence emission spectra of Premo™ cameleon calcium sensor. The dashed line indicates the spectra in the absence of Ca^{2+}; the solid line shows the fluorescence resonance energy transfer (FRET)-based change upon Ca^{2+} binding.

Real cells, real insight

Important advances in understanding cell signaling have led to new targeted therapies for cancer and other disease states, and continue to point toward interesting possibilities for new probes to map these pathways.[5,6] Tools for measuring Ca^{2+} are widely available and used routinely in research laboratories and for drug screening; however, these have been primarily limited to applications based on binding of the ion to a small chemical entity (e.g., fluo-3 and fluo-4) or to protein-based luminescent Ca^{2+} sensors (e.g., aequorin). Working with colleagues at the Oregon Medical Laser Center/Providence St. Vincent Hospital (Portland, Oregon), we have shown the capabilities of the Premo™ cameleon calcium sensor with BacMam delivery for visualizing and quantitating ATP-mediated Ca^{2+} flux in primary and progenitor cell types.

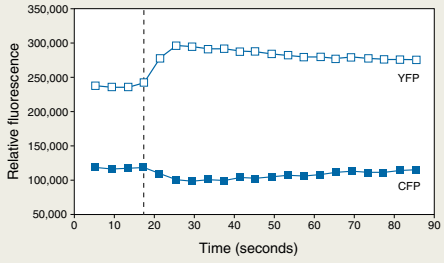

Figure 4—Example of single-well readings from a fluorescence-based microplate reader. HeLa cells were transduced with the Premo™ cameleon calcium sensor and plated in Opti-MEM® 1 medium + 1% FBS. The following day, a dose response assay for Ca^{2+} flux was performed using histamine. The agonist was injected at 16 seconds (dashed line) with readings acquired every 4–5 seconds.

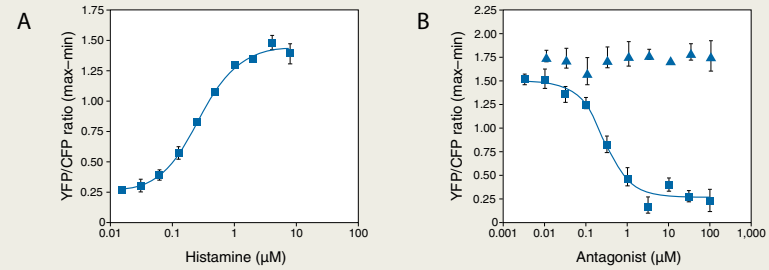

Figure 5—Agonist and antagonist dose response curves. HeLa cells were plated in a 96-well plate at a density of 15,000 cells/well, transduced with Premo™ cameleon calcium sensor, and incubated overnight at 37°C. The following day, a histamine dose response was performed (A). A separate plate was used to evaluate an antagonist dose response with pyrilamine (■) and thioperamide (▲) in the presence of an EC_{80} concentration of histamine (B). Pyrilamine is a known H1 receptor antagonist that couples through G_q proteins and the second messenger Ca^{2+}. Thioperamide is a known H3 receptor antagonist that couples through G_i proteins and the second messenger cAMP.

In this study, porcine primary cardiac and skeletal muscle cells and left atrial appendage (LAA) progenitor cells were transduced with the Premo™ cameleon calcium sensor. The cells were incubated overnight, followed by agonist induction for Ca^{2+} flux measurements. The LAA progenitor cells exhibited cytoplasmic expression as expected, and following administration of 20 µM ATP, we observed a receptor-mediated Ca^{2+} flux indicated by a change in color (Figure 1, page 2); quantitative measurements were also obtained (Figure 6). These results indicate a Ca^{2+}-dependent FRET response from the cameleon sensor. Other agonists (e.g., carbachol) and cell types were also analyzed successfully with the Premo™ cameleon calcium sensor (data not shown).

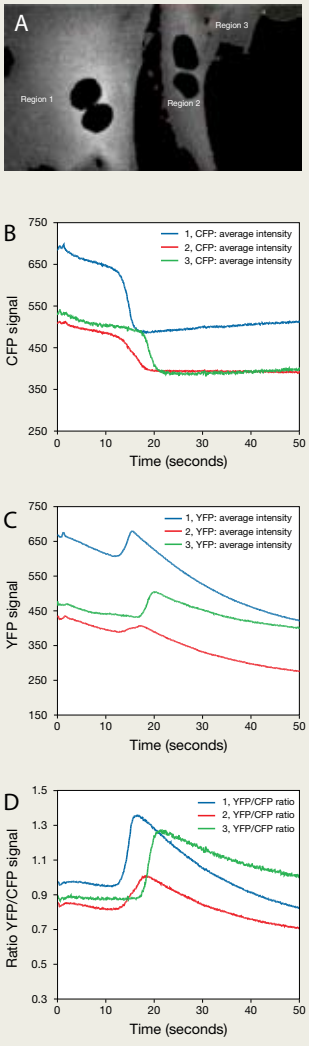

Figure 6—Analysis of YFP/CFP ratio to determine change in Ca^{2+} flux. Cells from Figure 1 were processed through a binary mask to remove the background noise and nuclear regions. The merged cell areas were divided manually, and a binary mask was used to define the regions (A). Panels B, C, and D show the change in CFP signal, YFP signal, and YFP/CFP ratio, respectively, as determined using Zeiss® LSM software.

Learn the language

The Premo™ cameleon calcium sensor pairs a fluorescent protein–based ion sensor with the very efficient, ready-to-use BacMam viral transduction delivery system to provide highly selective intracellular Ca^{2+} measurement. Building on this powerful new tool, Invitrogen plans a suite of second messenger indicators to support researchers in their quest to map and mine the dynamic events of cellular physiology. To learn more about the Premo™ cameleon calcium sensor, visit **www.invitrogen.com/premo**. ■

Michael Rutten, Ph.D., and Kenton Gregory, M.D., Oregon Medical Laser Center Bioimaging Suite, Providence St. Vincent Hospital (Portland, Oregon), contributed to this article.

References

1. Kost, T. et al. (2005) *Nat Biotechnol* 23:567–575.
2. Nagai, T. et al. (2004) *Proc Natl Acad Sci U S A* 101:10554–10559.
3. Miyawaki, A. et al. (1997) *Nature* 388:882–887.
4. Kost, T. and Condreay, J.P. (2002) *Trends Biotechnol* 20:173–180.
5. Cohen, P. (2002) *Nat Rev Drug Discov* 1:309–315.
6. Austin, C. et al. (2004) *Science* 306:1138–1139.

Product	Quantity	Cat. no.
Premo™ cameleon calcium sensor *for 10 microplates*	1 kit	P36207
Premo™ cameleon calcium sensor *for 100 microplates*	1 kit	P36208