

## **Emission ratiometric imaging of ATP-evoked Ca<sup>2+</sup> transient in rat vagal sensory neuron**

### **Neurons**

The inferior vagal (nodose) ganglia from a Sprague-Dawley rat were dissociated enzymatically. The yield of nodose neurons was suspended in Leibovitz L-15 medium supplemented with 10% (v/v) fetal bovine serum and penicillin-streptomycin and plated onto No. 1 glass coverslips.

### **Loading Asante Calcium Red indicator into neurons**

Neurons were incubated for 50 min at room temperature with 3 μM Asante Calcium Red AM ester. Thereafter, the cells were washed and maintained in fresh L-15 medium for 40 min to permit intracellular enzymatic hydrolysis of the AM ester to proceed to completion.

### **Confocal fluorescence imaging**

Indicator-loaded neurons were positioned on the stage of an inverted microscope (Axiovert 100 M, Zeiss) and superfused with Locke solution (equilibrated with a mixture of 5% CO<sub>2</sub> and 95% O<sub>2</sub>). Confocal imaging microscopy was performed with the LSM 510 system (Zeiss) through a ×40 objective (N.A. 1.2; oil-immersion) at a frame rate of 0.5 Hz. Excitation was at 488-nm; a 545-nm dichroic mirror separated the fluorescence emission into two components: a short-wavelength component (designated  $F_{525}$ ) that passed through a 500-550-nm band-pass filter, and a long-wavelength component ( $F_{650}$ ) that passed through a 560-nm long-pass filter. Neurons were stimulated with a 10-sec pulse of 100 μM ATP delivered through the superfusate.