fractionation of worm conditioned medium, they identified not only the previously identified daumone, but also two additional ascarosides that were more abundant in the medium extract than daumone. Each of these newly identified ascarosides was two orders of magnitude more potent than daumone in inducing dauer formation in either the natural or synthetic form. Combinations of these different ascarosides were slightly more active in inducing dauer formation than each compound alone. The authors noted that additional ascarosides were also present in the extracts at lower concentrations, which indicates that dauer pheromone may contain additional active components.

A number of interesting issues are raised by these findings. Do the individual components of dauer pheromone have distinct effects beyond triggering dauer entry? The effects of each of these ascarosides on other dauer pheromone–mediated effects have not yet been examined, leaving open the possibility that each of these components has specialized roles. This possibility is consistent with findings in other animals in which each component of an emitted chemical blend elicits a distinct response[2]. The precise ratio of each component in a mixture can also have a role in providing species-specific information[2]. Daumone was shown to induce dauer formation in the related species *C. briggsae*[9], which shares the same ecological niche as *C. elegans*. However, the effects of the ascarosides identified by Butcher *et al.*, or of mixtures of these compounds, on dauer formation in *C. briggsae* or other inhabitants of their ecological niche, have not yet been examined. It is also possible that the concentration of each component differs under different environmental conditions, or even at different developmental stages. In other animals, sexually mature individuals produce distinct pheromones from immature individuals; perhaps the chemical profile of pheromones produced by adult *C. elegans* is distinct from that of larval animals.

It has been argued that dauer pheromone is not a true pheromone—that is, a signal that provides selective advantages for both the sender and the receiver—but instead is merely a cue providing information only to the receiver[10]. The current study does not resolve this issue, but it is an important step toward dissecting the exact role of the dauer pheromone. The next important steps will be to dissect the functions of each individual component, investigate the metabolic pathways that result in their production, and explore the signal transduction pathways required for the responses. With specific compounds in hand, research on dauer formation has now graduated from medieval witches' brew to modern science.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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Calcium channels light up

Eric Green & Ricardo E Dolmetsch

The development of a technique for measuring calcium concentrations in nanodomains next to calcium channels provides new insights into calcium signaling.

Neurons and muscle cells express multiple types of voltage-gated calcium channels that control a wide range of cellular functions, including motility, secretion and synaptic plasticity. Activation of voltage-gated calcium channels produces an intracellular calcium elevation that is greatest next to the intracellular mouth of the channel and declines exponentially with distance[1]. The rise in the concentration of calcium in the region close to the mouth of calcium channels can be 10–100 times higher than the calcium rise in the rest of the cell. This nanodomain of elevated calcium around the intracellular mouth of calcium channels is thought to explain how voltage-gated calcium channels trigger the release of neurotransmitter-containing vesicles in milliseconds and how calcium influx through specific channels activates gene expression[2,3]. Despite the importance of calcium nanodomains for channel function, the magnitude and time course of the calcium elevation next to a calcium channel has never been measured directly. In this issue of *Nature Chemical Biology*, Tour *et al.*[4] report the development of a new method for measuring calcium signals generated at the pore of calcium channels; their study provides new insights into the mechanisms by which voltage-gated calcium channels regulate signaling pathways.

The main difficulty in measuring calcium in nanodomains is that nanodomains are smaller than the diffraction limit of most light microscopes. Even though small organic calcium dyes such as Fluo-4 and Fura-2 can measure calcium changes in the bulk of the cytoplasm, light microscopy limits the resolution of fast measurements to approximately 200 nM, which is too large to measure calcium that is nanometers away from the mouth of calcium channels. To improve the resolution of calcium measurements, an alternative approach is to fuse calcium channels to genetically encoded calcium indicators based on fluorescent proteins such as the cameleons, pericams or G-cams. Unfortunately these indicators are based on calmodulin binding to calcium, so they respond to calcium too slowly to measure calcium nanodomains, which are expected to last not more than 100 ms. In addition, fluorescent protein–based indicators are large and can disturb the function of calcium channels, which makes them non-ideal for measuring calcium next to channels.

Now, Tour *et al.*[4] have developed a strategy that combines the rapid response of small-molecule calcium indicators with the spatial discrimination of genetic targeting. Their technique is based on the FlAsH technology

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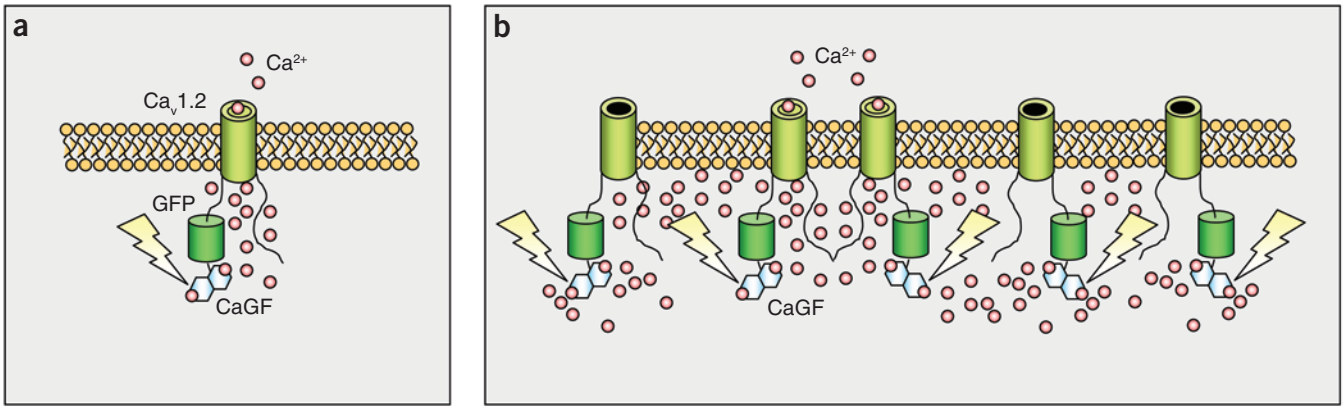

Figure 1 Targeting CaGF to ion channels to measure local calcium signals. (**a**) $Ca_v1.2$ channels fused to GFP and to a tetracysteine motif that is bound to CaGF. CaGF dramatically increases its fluorescence upon binding to calcium and thus reports calcium in nanodomains close to channels. (**b**) A substantial fraction of the $Ca_v1.2$ channels bound to CaGF did not conduct calcium but reported the calcium concentrations close to the cell membrane. Nonconducting channels are shown with black pores.

developed in Roger Tsien's laboratory, which takes advantage of the high-affinity binding between peptides that contain a tetracysteine motif and biarsenic-containing small molecules[5]. The authors synthesized Calcium Green FlAsH (CaGF), a small molecule containing a biarsenic domain and a low-affinity fluorescent calcium indicator. This calcium sensor binds to proteins containing tetracysteine motifs and dramatically increases its fluorescence in response to increases in calcium (**Fig. 1a**). By adding a tetracysteine motif to a version of $Ca_v1.2$, an L-type calcium channel, the authors could then target CaGF to the channel and measure calcium concentrations near the mouth of the channel.

The first iteration of this elegant approach for measuring the concentration of calcium near calcium channels revealed some unexpected limitations. When the authors added CaGF to cells containing the tetracysteine-tagged L-type channel, they could detect elevations of calcium upon depolarization of the membrane. They could also observe micron-scale hot spots of calcium influx that correlated with the location of $Ca_v1.2$ clusters (detected using green fluorescent protein (GFP)-tagged channels). They also inferred the existence of calcium nanodomains by showing that the hot spots persisted even in the presence of BAPTA, a high-affinity calcium buffer that should buffer all but the calcium closest to the channels. However, the authors were unable to quantify the calcium concentration next to the pore, and they could not measure the kinetics of calcium influx in detail either. This is because they made the unexpected discovery that only a small percentage of the channels expressed in 293T cells are activated by depolarizing pulses (**Fig. 1b**). Thus only a small percentage of the fluorescent signal was generated by CaGF bound to actively conducting calcium channels. The rest of the signal was produced by CaGF bound to nonconducting calcium channels. These CaGF molecules bound to inactive channels still changed their fluorescence in response to calcium, but they mostly reported calcium signals at the cell membrane, not those at the mouth of the channels. Therefore, the measured calcium signal lasted much longer than would be expected from models of calcium diffusion away from point sources, and it primarily reflected calcium elevations at the cell membrane, not at the mouth of $Ca_v1.2$ channels.

Despite these shortcomings, a couple of important conclusions can be drawn from the experiments of Tour *et al.*[4]. First, the rise in calcium concentration at the membrane of cells is significantly higher than the calcium rise in the cytoplasm of cells. This has important implications for the activation of calcium-regulated signaling cascades that are activated by membrane-associated proteins. Second, the operational unit that generates a calcium microdomain may be a cluster of calcium channels, not an individual calcium channel. Finally, many channels that seem to be at the membrane might not in fact be functional. This last conclusion has the caveat that these experiments were all conducted in non-excitable cells overexpressing a voltage-gated calcium channel; thus, whether this is physiologically important in neurons and other cells that express voltage-gated calcium channels remains to be established. Nevertheless, this paper introduces an important new tool for measuring calcium concentrations close to ion channels in cells, which will greatly expand our ability to study fast calcium signals in restricted domains next to proteins.

COMPETING INTERESTS STATEMENT
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