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Editorial

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Making Intracellular Sensors Count

One of the first editorials of *ACS Sensors* was provocatively titled "Should *ACS Sensors* Publish Papers on Fluorescent Sensors for Metal Ions at All?" (**DOI:** 10.1021/acssensors.6b00213). The answer to that question was yes, but it depends on whether the sensor is fit-for-purpose, and whether there is a need for that sensor. In the case of metal ion sensors, the need to develop yet another metal ion sensor for environmental monitoring is often hard to justify, whereas fluorescent sensors that can be used intracellularly (*in situ*) can provide unique tools to answer key biological questions. The same editorial also identified some important requirements for a useful intracellular fluorescent sensor, such as water solubility, physiologically relevant affinity and specificity, and reversible binding.

The most important goal of any sensor, whether it is applied intracellularly or elsewhere, is to obtain quantitative information. We receive too many papers on intracellular sensors that only report the ability of the probe to respond to the analyte of interest when added exogenously. One step further is to show that the fluorescent signal responds to a biological trigger that is known to increase or decrease the concentration of the target analyte in the anticipated manner. For certain biological questions, being able to determine relative changes in concentration is sufficient—e.g., measuring the kinetics of signaling processes such as calcium signaling. However, relatively few studies try to determine the concentration of the analyte. Ultimately, it is concentrations that matter in order to understand and quantitatively describe the cell.

Intracellular quantification is not an easy task, in particular because calibration inside a cell can be challenging. A first prerequisite for quantitative measurements is control over the intracellular localization, and preferably also the concentration of a sensor. Control over intracellular localization is important not only because the concentrations of the analyte can vary widely in different compartments, but also because different compartments can represent a very different environment. Although functional groups that promote specific organelle targeting are known, control over intracellular localization remains particularly challenging for synthetic fluorescent sensors, requiring verification using an established intracellular marker.

Whether a fluorescent sensor is suitable for quantitative measurements is also determined by the sensor's mode of action. Intensiometric sensors are inherently difficult to use for quantification in cells, because the fluorescent signal depends on the concentration of both the analyte and the sensor. In contrast, ratiometric sensors are more suitable for quantitative measurements, as ratiometric signals are independent of sensor concentration and less dependent on background fluorescence. In fact, one of the early and most popular Ca^{2+} fluorescent sensors, Fura-2, was developed as a ratiometric sensor.[1] Another aspect that is important is whether the sensor's mode of action is reversible. Reversibility is relatively straightforward for metal ion and pH sensors, but many other fluorescent sensors undergo irreversible transformations—e.g., sensors that probe enzymatic activity or probes that target biological thiols. The fact that the signal of these sensors is time dependent makes the extraction of quantitative information using these sensors even more challenging. Developing intracellular sensors that respond reversibly is therefore an important goal in the area of intracellular sensing (see, for example, the recent work of Jin Wang and co-workers who developed a reversible reaction-based fluorescent sensor for glutathione (**DOI:** 10.1021/acssensors.7b00425)).

Even for ratiometric sensors, reliable intracellular quantification requires careful experimentation and proper control experiments. One of the key questions is whether the sensor behaves similarly in the test tube and in the crowded and complex environment of the cell. Ideally, this means that the sensor is calibrated *in situ*. If this is not possible the sensor should at least be characterized under conditions that mimic the intracellular milieu (pH, redox state) as closely as possible. An example is the effect of pH on metal ion sensors, which not only can affect fluorescent properties, but typically also have a large influence on metal affinity. Another caveat is that the sensor interacts with endogenous proteins, which has been documented for several protein-based sensors, and could be expected to be at least as big of an issue for small molecule fluorescent dyes. Some of these issues can be addressed in the design of a new sensor—for example, by mutating known protein interaction sites in protein-based sensors[2]—but non-anticipated effects are hard to address. A practical solution is to compare the performance of sensors with different affinities, or based on a different type of sensing mechanism. If different sensors yield the same numbers, this provides confidence that the numbers are probably correct, and the sensors work as anticipated. For example, using this approach, a consensus was reached in the field of zinc biology on the amount of free Zn^{2+} present in the cytosol, which was found to be between 0.10 and 1 nM in a wide variety of different cell types. However, comparing different sensors that target the endoplasmic reticulum reported very different concentrations, suggesting that one or more of the sensors were not functioning as anticipated.[3]

At *ACS Sensors,* we believe that these kinds of comparisons are very valuable, as they help the field to understand the validity of sensors and identify remaining issues. So, in addition to the development of new sensors that are designed to provide quantitative information, we also very much welcome studies that rigorously compare the performance of previously developed sensors, or provide new methods/strategies for intracellular calibration. Establishing rigorous sensor procedures for intracellular quantification at the single cell level is also important to allow quantitative intracellular measurements *in vivo*, which adds another level of complexity. We are fully aware that addressing all these issues in a single study may be

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unrealistic, but in making intracellular sensing more quantitative, every (small) step counts.

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Notes

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