**Title**

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**Abstract**

**Introduction**

Genetically-encoded biosensors have revolutionized our ability to measure a wide variety of cellular properties in live animals. As experimentalists, any time a new sensor is developed, we would like to know: what is that sensor good for? That is, what range of values of the cellular property of interest is that sensor well-suited to measure accurately? Here, we present a theoretical framework to determine the suitability of biosensors with two states.

Two-state biosensors are simple sensors that change conformation, and spectral properties, in response to a specific input. Existing two-state biosensors respond to a wide variety of important biochemical properties, including pH, ATP, and glutathione redox potential (*EGSH*). The development of these biosensors marks the start of the era of real-time biochemistry in live animals. However, the potential of these biosensors has not been fully-realized because fluorescence ratio measurements provide only qualitative information about the biochemical property measured by the sensor.

In our previous work with the roGFP1\_R12 sensor in *C. elegans*, we deployed a mathematical framework that enabled us to calculate glutathione redox potential from fluorescence ratio measurements given knowledge of the spectral and biochemical properties of the biosensor, and the properties of our microscope[1](#_ENREF_1). We have extended this framework to analyze how the precision of measured fluorescence ratios limits the accuracy of calculated biochemical properties for two-state biosensors with known spectral and biochemical properties.

This new framework enables to: (i) determine the range of values for which the roGFP1\_R12 sensor is well-suited to accurately measure *EGSH* in live *C. elegans*; (ii) optimize the precision of our imaging and image-analysis methods to improve that biosensor’s suitability; (iii) choose optimal biosensors for the measurement of *EGSH*, pH, and nucleotides; (iv) reclaim underused sensors uniquely suited for certain conditions; and (v) identify what new sensors are needed. To help the community find biosensors that are well-suited for their experimental needs, we developed web-based tool, the Sensor Overlord (<http://www.sensororverlord.com/>), that implements these analyses with a user-friendly interface.

**Results**

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**Discussion**

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**Materials and Methods**

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**Competing interests**

The authors declare that no competing interests exist.

**Figure legends**

**References**

1 Romero-Aristizabal, C., Marks, D. S., Fontana, W. & Apfeld, J. Regulated spatial organization and sensitivity of cytosolic protein oxidation in Caenorhabditis elegans. *Nature communications* **5**, 5020 (2014).