**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 biosensor is developed, we would like to know: what is that biosensor good for? That is, what range of values of the cellular property of interest is that biosensor well-suited to measure accurately? Here, we present a theoretical framework to determine the suitability of biosensors with two states.

Two-state biosensors change conformation, and spectral properties, in response to a specific input. Existing two-state biosensors respond to various important biochemical properties, including pH, ATP, and glutathione redox potential (*EGSH*)[1-3](#_ENREF_1). The development of these biosensors has enabled real-time biochemistry in live animals. However, the potential of these biosensors has not been fully realized because fluorescence measurements do not provide absolute quantitative measurements of the biochemical property measured by the biosensor.

In our previous work with the roGFP1\_R12 biosensor 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[4](#_ENREF_4),[5](#_ENREF_5). This framework enables absolute quantitative measurements of redox potential but does not account for measurement limits set by imprecise ratio measurements. We extended this framework to predict the accuracy of biochemical measurements, given fluorescence ratio precision obtained from 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 biosensor 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 other biochemical properties; (iv) reclaim underused biosensors uniquely suited for certain conditions; and (v) identify what new biosensors 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.sensoroverlord.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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**Acknowledgements**

We thank \_\_\_\_\_ for critical reading and detailed comments on our manuscript. We benefitted from discussions with members of Erin Cram’s lab. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The research was supported by a National Science Foundation CAREER grant 1750065 to J.A.

**Competing interests**

The authors declare that no competing interests exist.

**Figure legends**

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