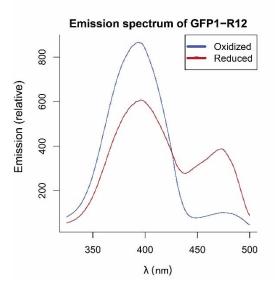
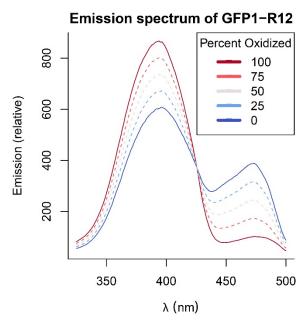
## Outline:

- Abstract 1: We use ratiometric measurements from genetically-encoded redox sensors to learn about how redox processes affect aging in C. elegans. If we know the precision of our ratiometric measurements, we can predict the range of glutathione redox potential values that our sensor is well-suited to measure within a certain accuracy.
  - a. Concept 1: If we have many redox-sensitive GFP proteins in a cell, we understand how to use the protein's excitation-emission pattern to estimate the cell's redox state.
    - i. Graph 1: Excitation-emission spectrum
    - ii. Graph 2: Spectrum: for a mixed population, spectrum is the weighted average of the two extremes.
    - iii. Graph 3: A ratio measurement *R* gives a concentration-independent map to the fraction of sensors that are oxidized and the redox potential.
    - iv. Graphs 4 and 5: A ratiometric *R* can be mapped into a fraction oxidized and a redox potential
    - v. Graphs 6 and 7: The map between R and the fraction oxidized, and redox potential is a function of a value called  $\delta$ .
    - vi. Graphs 8 and 9: The choice of the second wavelength in the ratiometric output has a predictable effect on the map between *R*, the fraction oxidized, and redox potential.
  - b. Concept 2: If we know the precision of our ratiometric measurements, we can estimate the accuracy of our predicted values of the redox potential.
  - c. Concept 3: If we know the precision of our ratiometric measurements, we can predict the range of redox potential values that our sensor is well-suited to measure within a certain accuracy.
- 2. Abstract 2: (TBD) If we know the precision of the measurements obtained from any ratiometric sensor, we can predict the range of values that the sensor is well-suited to measure within a certain accuracy.
- 3. Abstract 3: (TBD) We have created a publicly-available tool as a resource to identify the range of values a sensor is well-suited to measure within a certain accuracy.

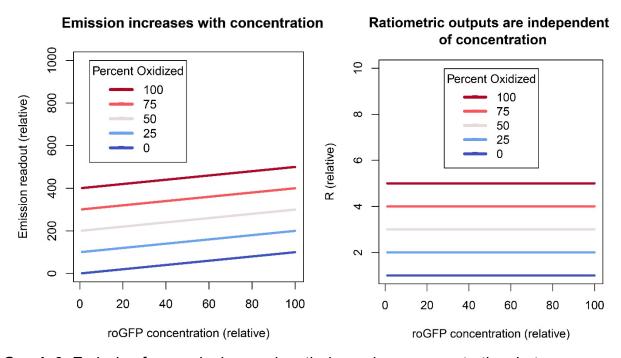
**Concept 1:** If we have many redox-sensitive GFP proteins in a cell, we understand how to use the protein's excitation-emission pattern to estimate the cell's redox state.



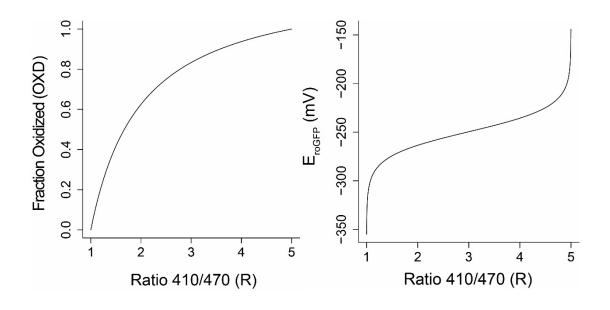
**Graph 1.** A redox sensor can be oxidized or reduced, and each sensor has a characteristic excitation-emission pattern.



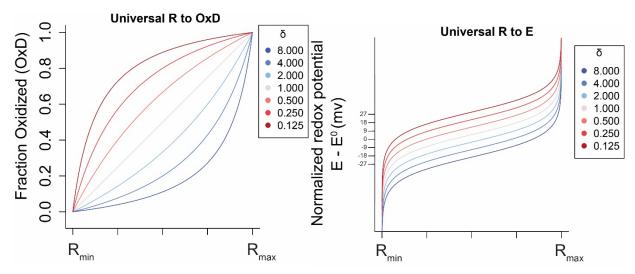
**Graph 2.** When you excite a population of sensors, the resulting spectrum is a weighted average of the emissions from the oxidized and reduced sensors.



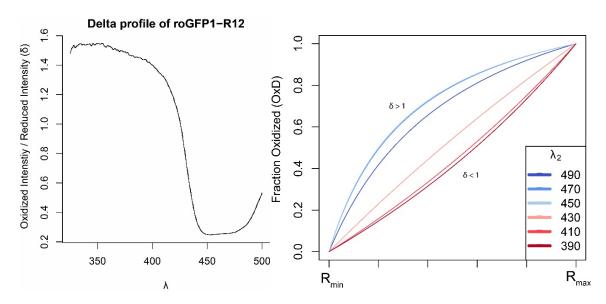
**Graph 3.** Emission from a single wavelength depends on concentration, but a ratiometric emission does not. Since, in an *in vivo* setting, we do not know the concentration of sensors, we take a ratiometric output.



**Graph 4 and 5.** A ratiometric output between two wavelengths can be mapped into two chemically meaningful values: the fraction of sensor molecules oxidized and the redox potential of the reaction between the redox sensor and the glutathione redox couple.



**Graphs 6 and 7.** The ratio between the emission value of an oxidized and reduced emission at the second of the two ratio wavelengths, which we call  $\delta$ , changes the way that the ratio emission maps to the fraction oxidized and redox potential.



**Graphs 8 and 9.** In graph 4, we chose 470~nm as our second wavelength but, had we chosen a different wavelength, our map between ratio and fraction oxidized would have been altered based on the ratio between the oxidized and reduced emission at that wavelength, or  $\delta$ .