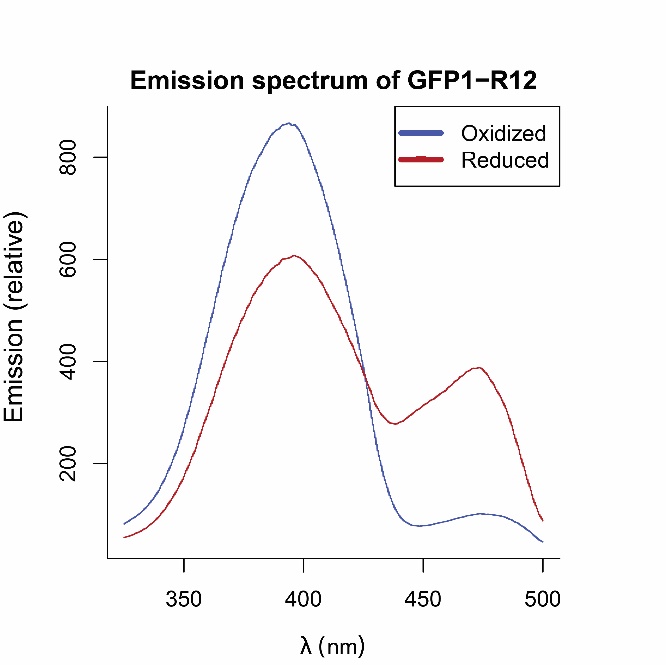
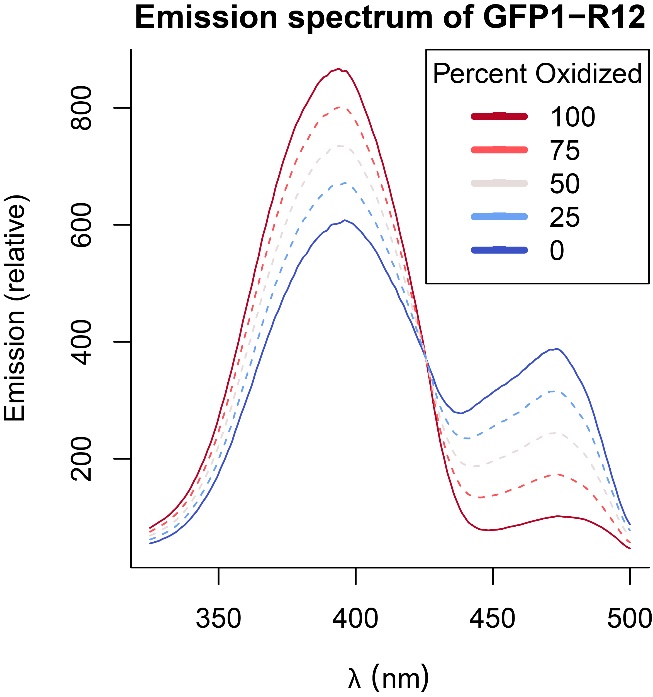
**Outline:**

1. 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.
   1. 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.
      1. Graph 1: Excitation-emission spectrum
      2. Graph 2: Spectrum: for a mixed population, spectrum is the weighted average of the two extremes.
      3. Graph 3: A ratio measurement gives a concentration-independent map to the fraction of sensors that are oxidized and the redox potential.
      4. Graphs 4 and 5: A ratiometric can be mapped into a fraction oxidized and a redox potential
      5. Graphs 6 and 7: The map between and the fraction oxidized, and redox potential is a function of a value called .
      6. Graphs 8 and 9: The choice of the second wavelength in the ratiometric output has a predictable effect on the map between , the fraction oxidized, and redox potential.
   2. Concept 2: If we know the precision of our ratiometric measurements, we can estimate the accuracy of our predicted values of the redox potential.
   3. 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.

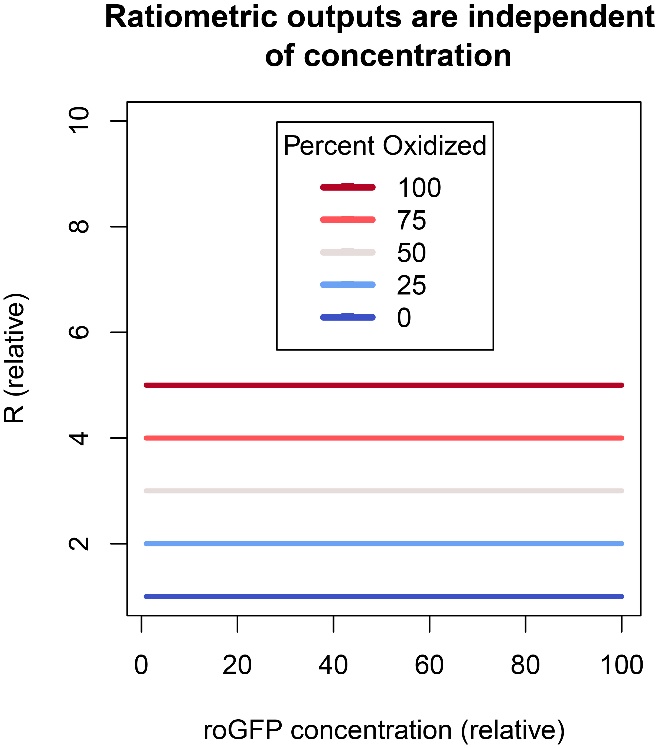
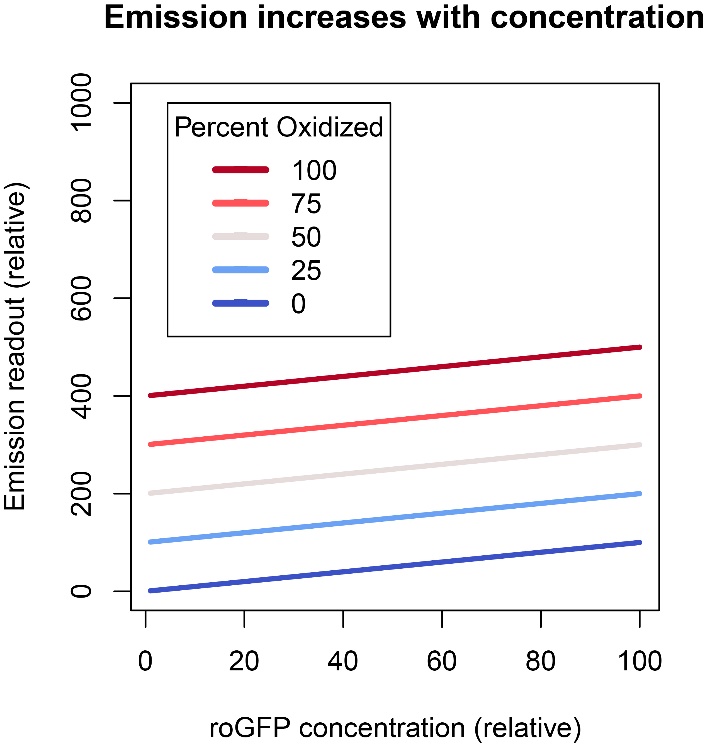
## **Concept 1:** If we have many redox-sensitive GFP proteins in a cell, we understand how to use the protein’s excitation-emission pattern to estimate the cell’s redox state.



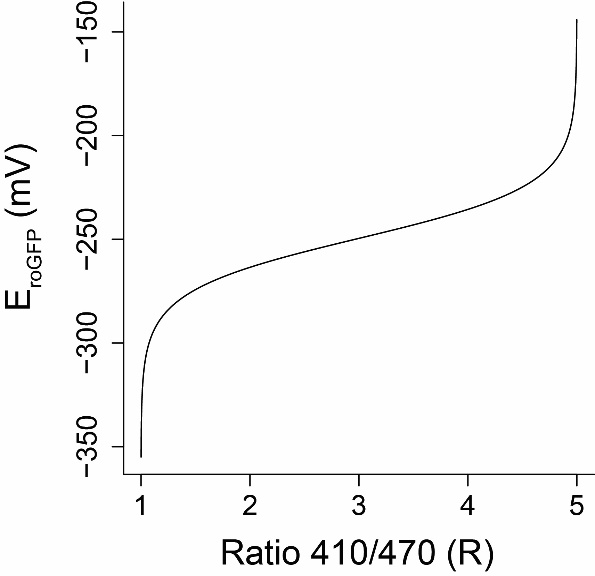
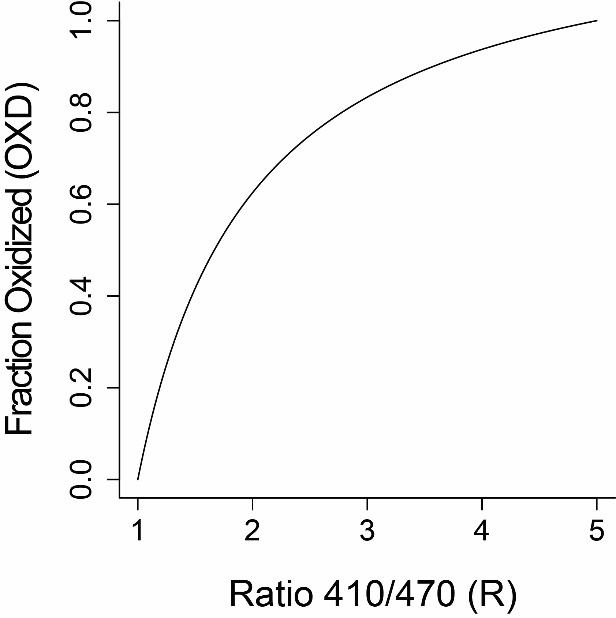
### **Graph 1.** A redox sensor can be oxidized or reduced, and each sensor has a characteristic excitation-emission pattern.

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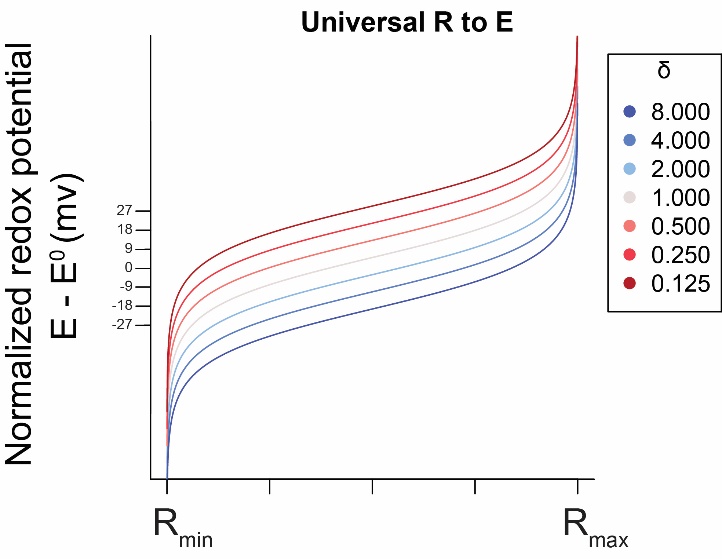
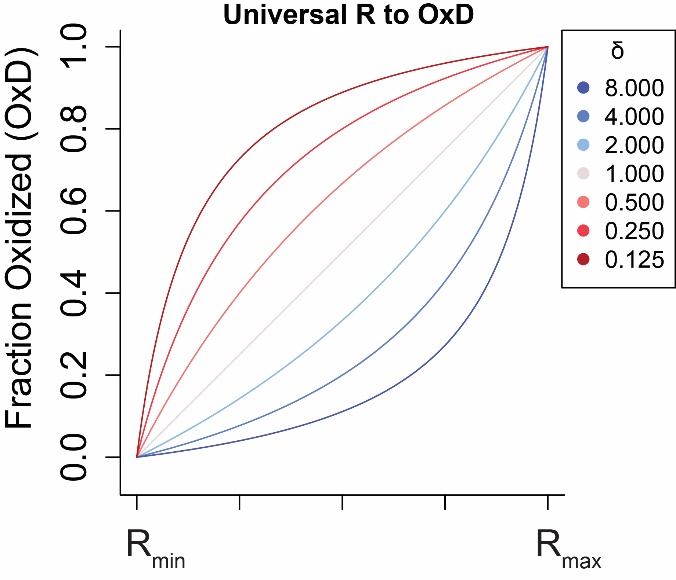
**Graph 2.** When you excite a population of sensors, the resulting spectrum is a weighted average of the emissions from the oxidized and reduced sensors.



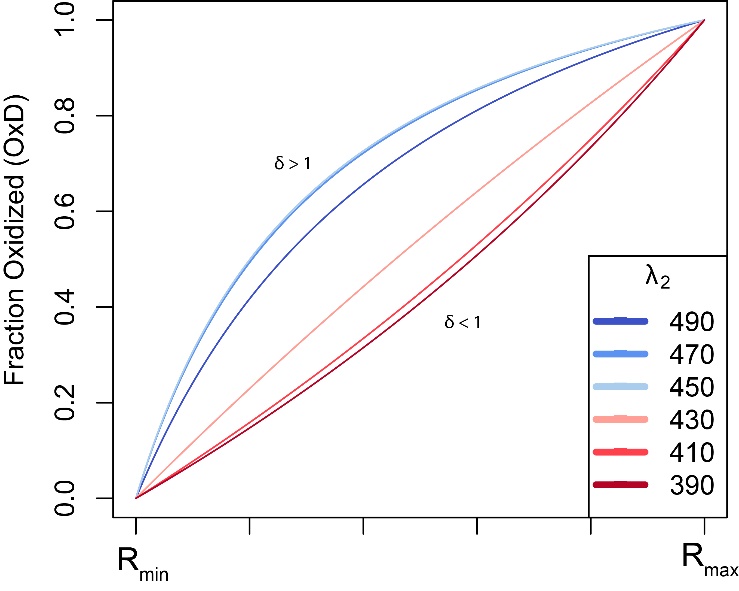
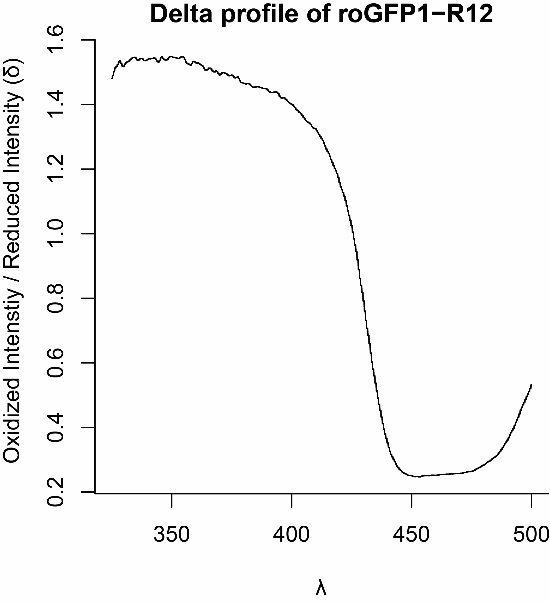
**Graph 3.** Emission from a single wavelength depends on concentration, but a ratiometric emission does not. Since, in an *in vivo* setting, we do not know the concentration of sensors, we take a ratiometric output.



**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.

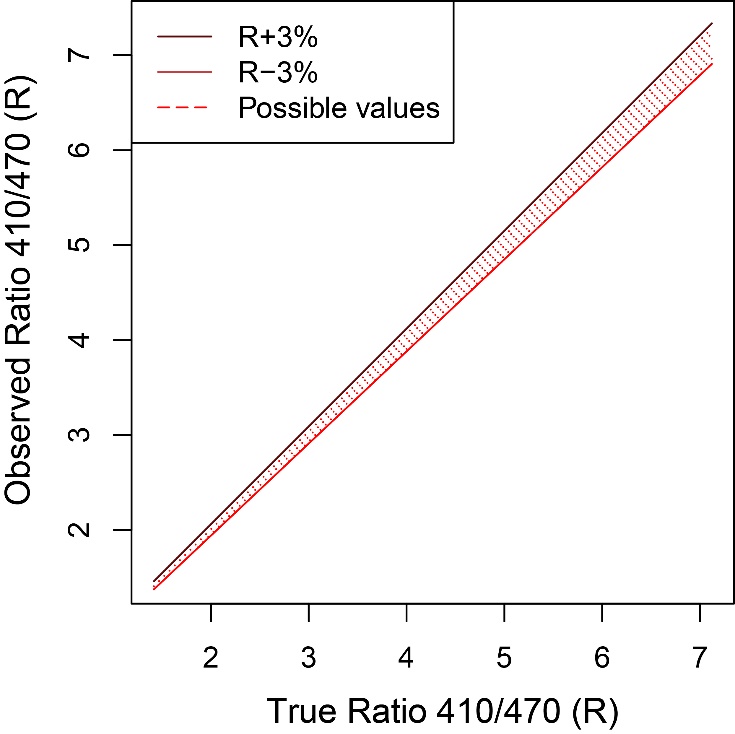


**Graphs 6 and 7.** The ratio between the emission value of an oxidized and reduced emission at the second of the two ratio wavelengths, which we call , changes the way that the ratio emission maps to the fraction oxidized and redox potential.

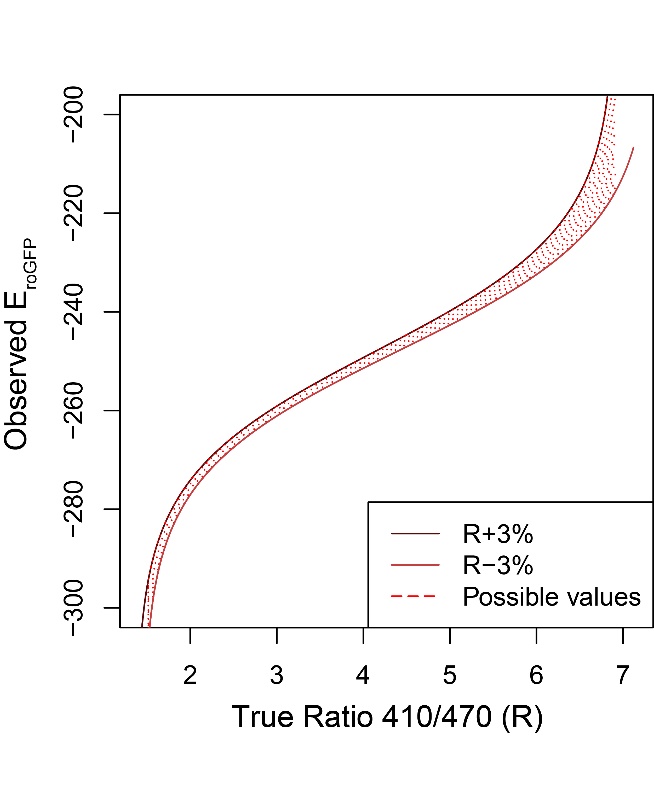
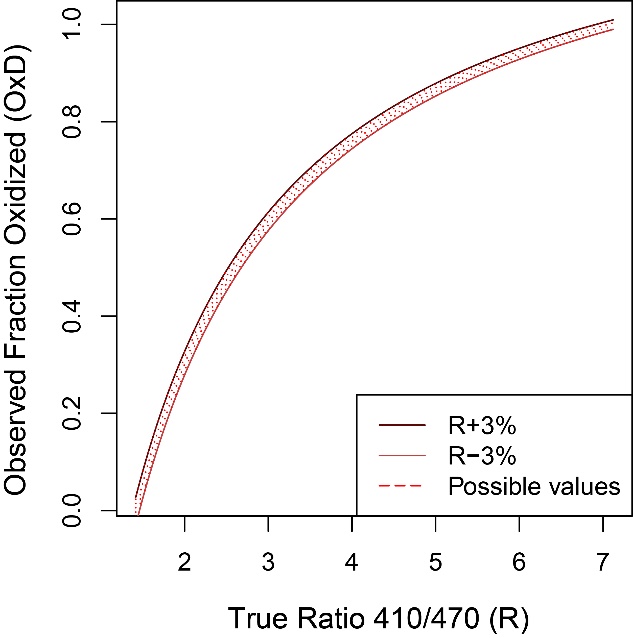


### **Graphs 8 and 9.** In graph 4, we chose as our second wavelength but, had we chosen a different wavelength, our map between ratio and fraction oxidized would have been altered based on the ratio between the oxidized and reduced emission at that wavelength, or .

## **Concept 2:** Our measurement of ratiometric emission has limited precision and we understand how that lack of precision alters the accuracy of interpretation of the redox state.

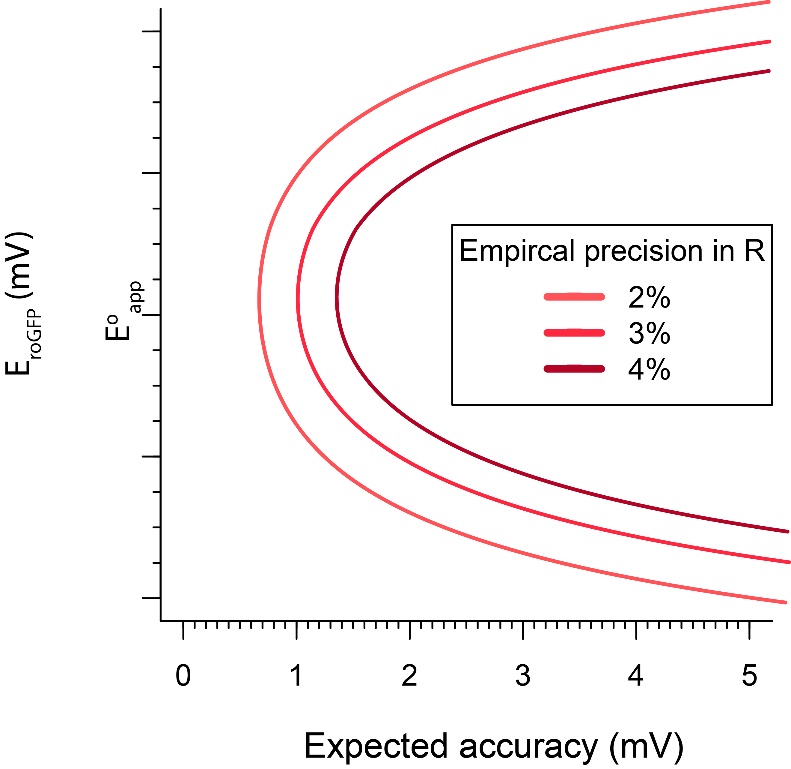


### **Graph 9.** Empirically, we observe a precision in the ratiometric emission of around 3%. Given that precision, there is a small range of emission values that we could observe for any true emission value.

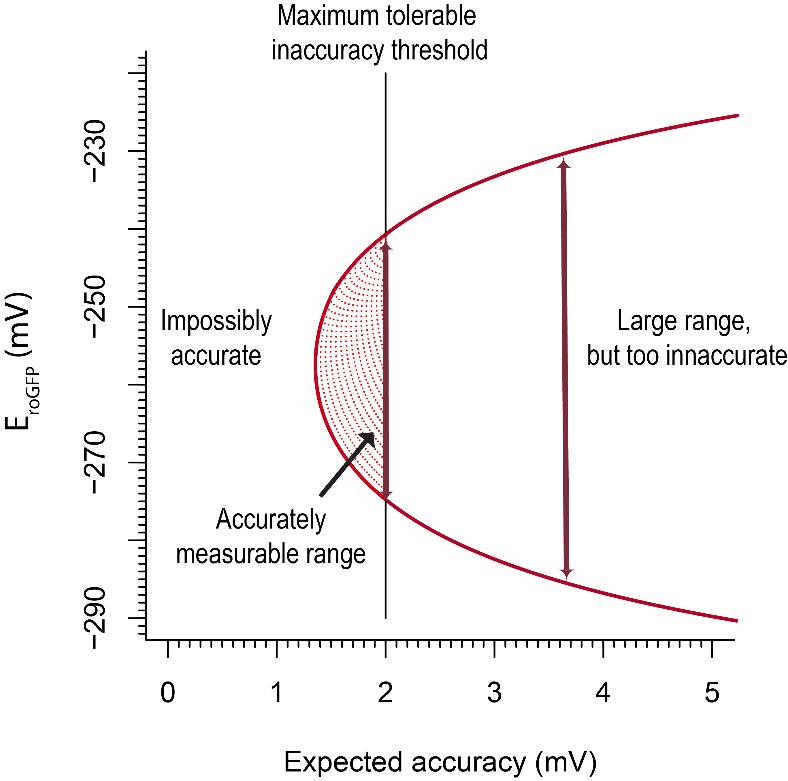


### **Graphs 10 and 11:** Since we know the range of ratiometric emission values we would expect to see with a precision of 3%, we can also determine the range of values we could predict for the fraction of sensors that are oxidized and the associated redox potential.

## **Concept 3:** For any set of sensor measurements with a known precision, we can predict the ranges of redox potentials that the sensor can accurately measure.

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### **Graph 12.** The more precisely we are able to measure the ratiometric emission, the more accurately we can measure a wider range of redox potentials.



### **Graph 13.** At some empirical level of precision in ratiometric emission, we can find the range of redox potentials that a sensor can measure accurately.