What is your biosensor good for?

A model to predict the accuracy of biochemical measurements made with two-state sensors

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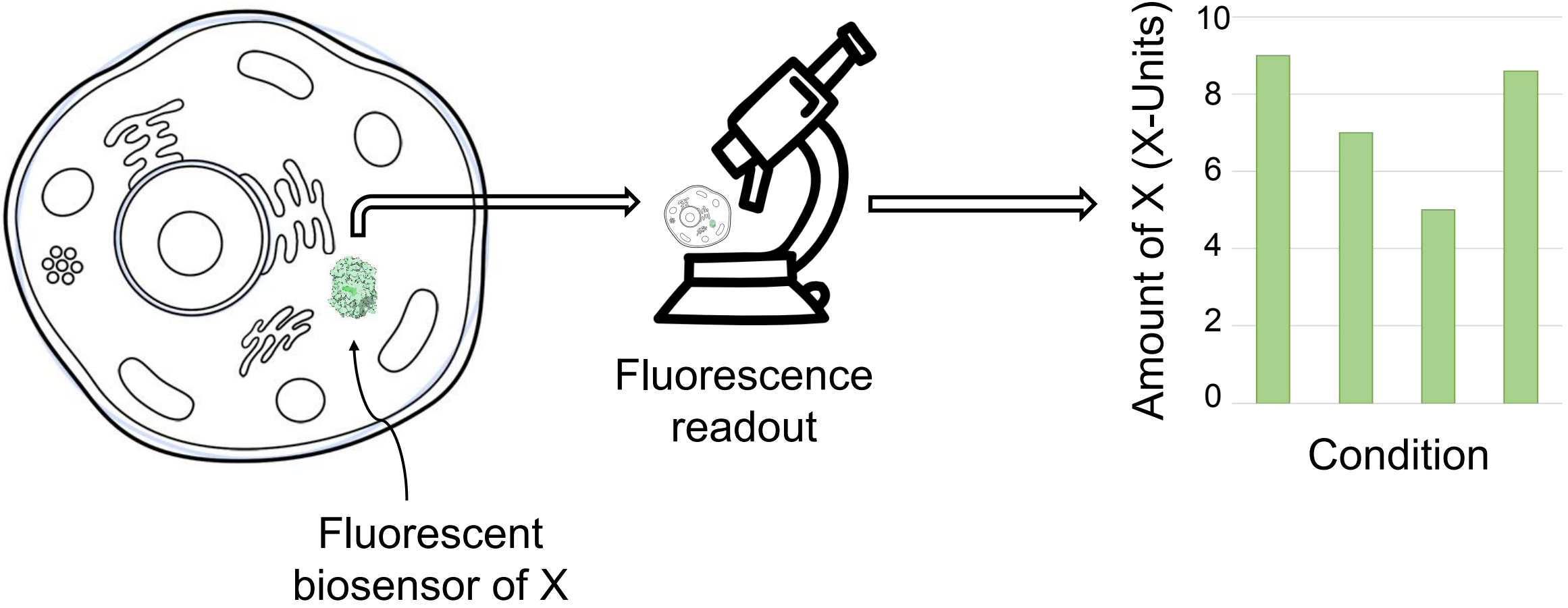
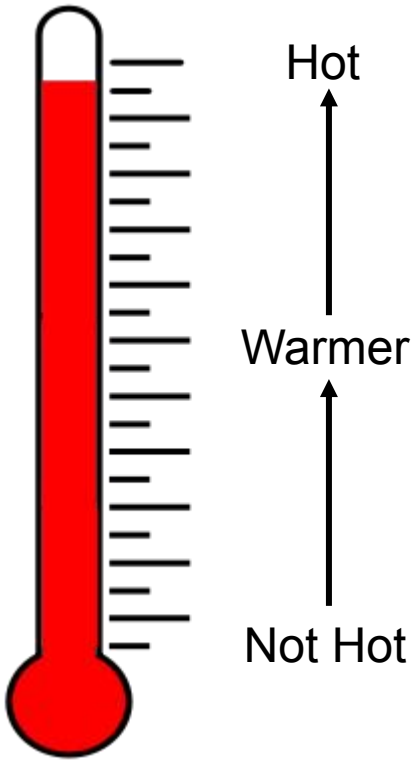
We use fluorescent biosensor proteins to make absolute measurements in cells

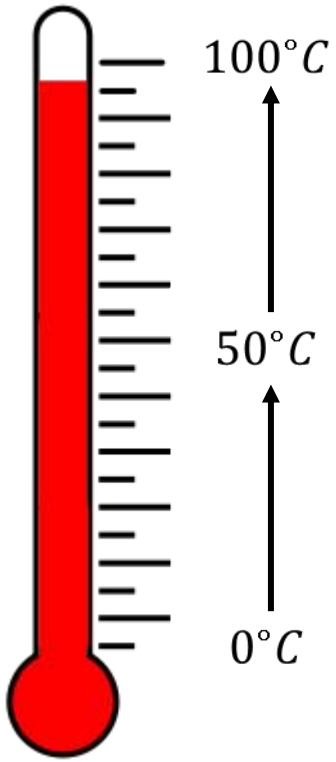


Absolute measurements allow us to make predictions that relative measurements do not

Relative measurements

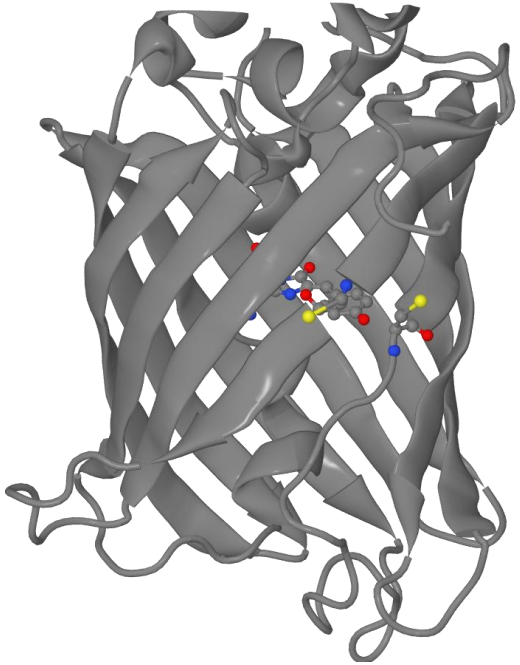


Absolute measurements



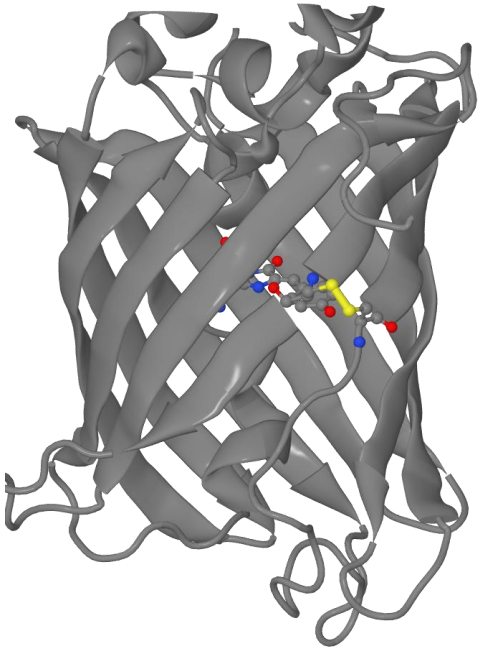
We use biosensors with two states

State A

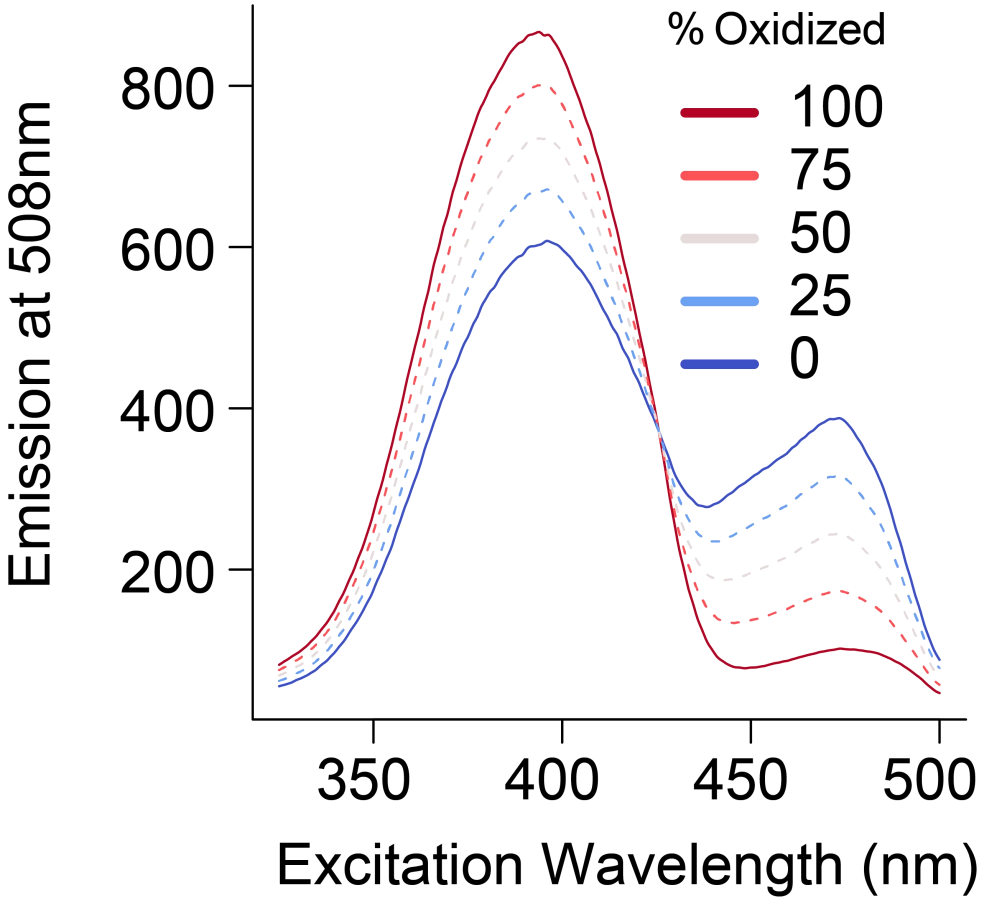


Thiol (Reduced)

State B



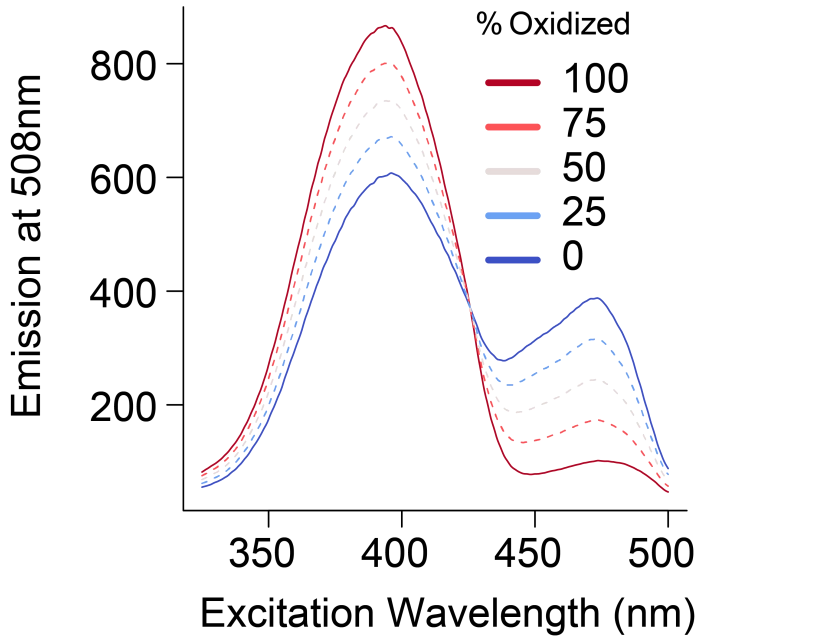
Disulfide (Oxidized)

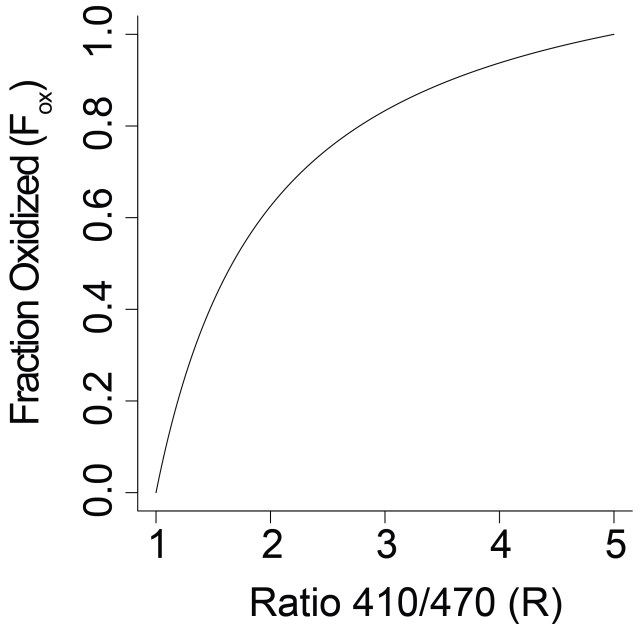


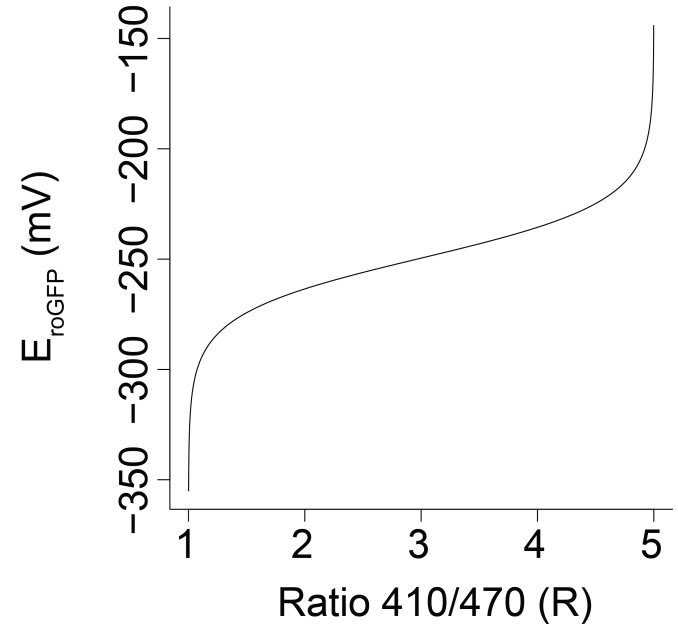
Hanson 2004, Cannon 2006

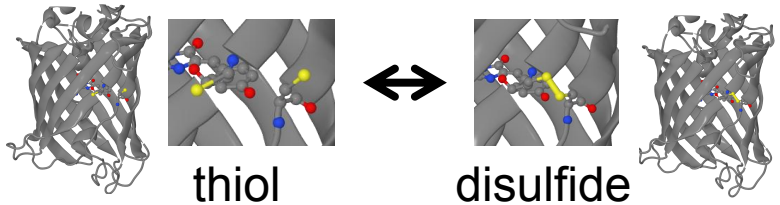
We use a mathematical map to make absolute measurements in cells

Fluorescence Ratio → **State Probability** → **Biochemical Property**



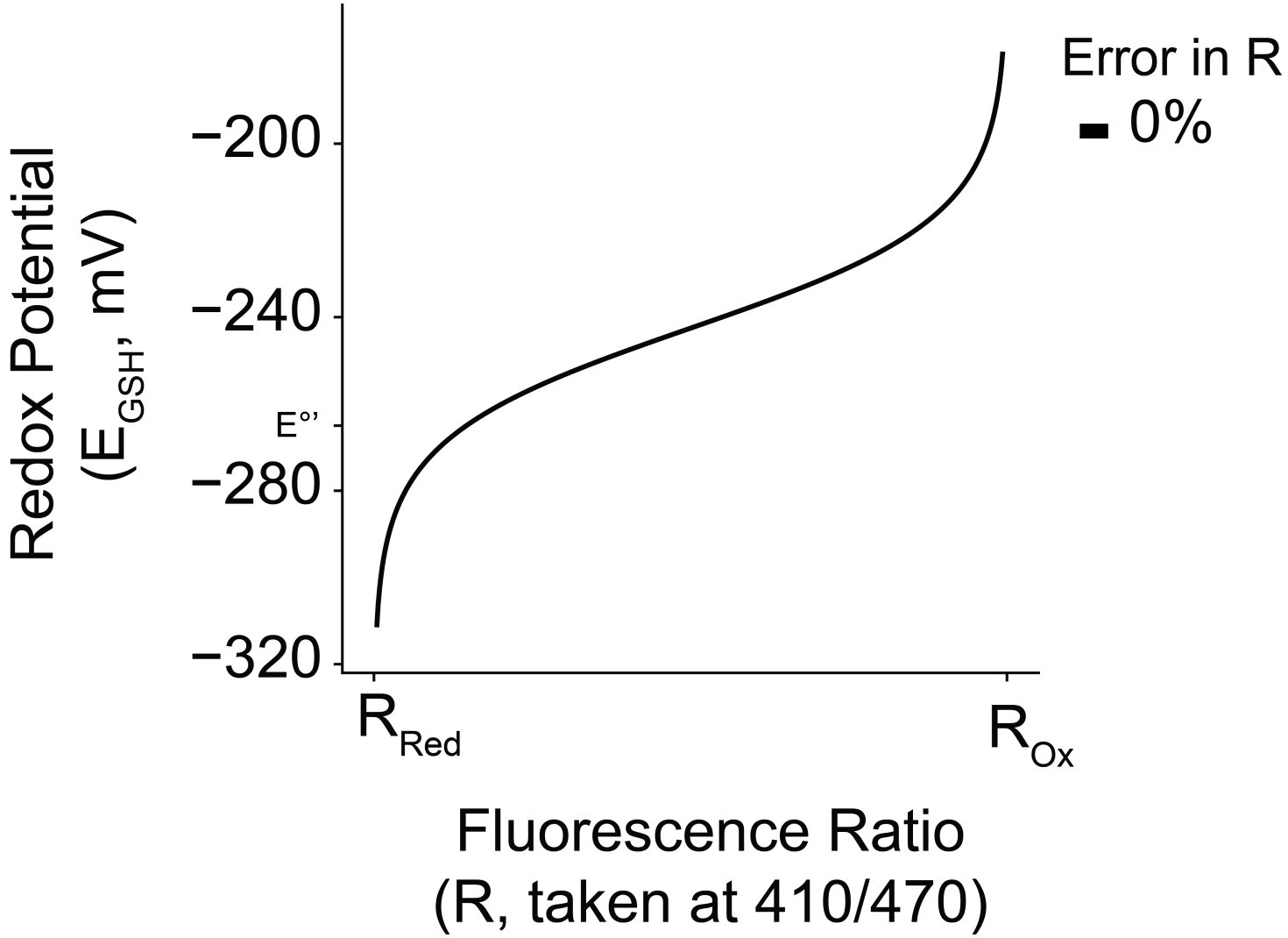






Romero-Aristizabal 2014, Meyer and Dick 2010

Errors in R influence redox potential measurements



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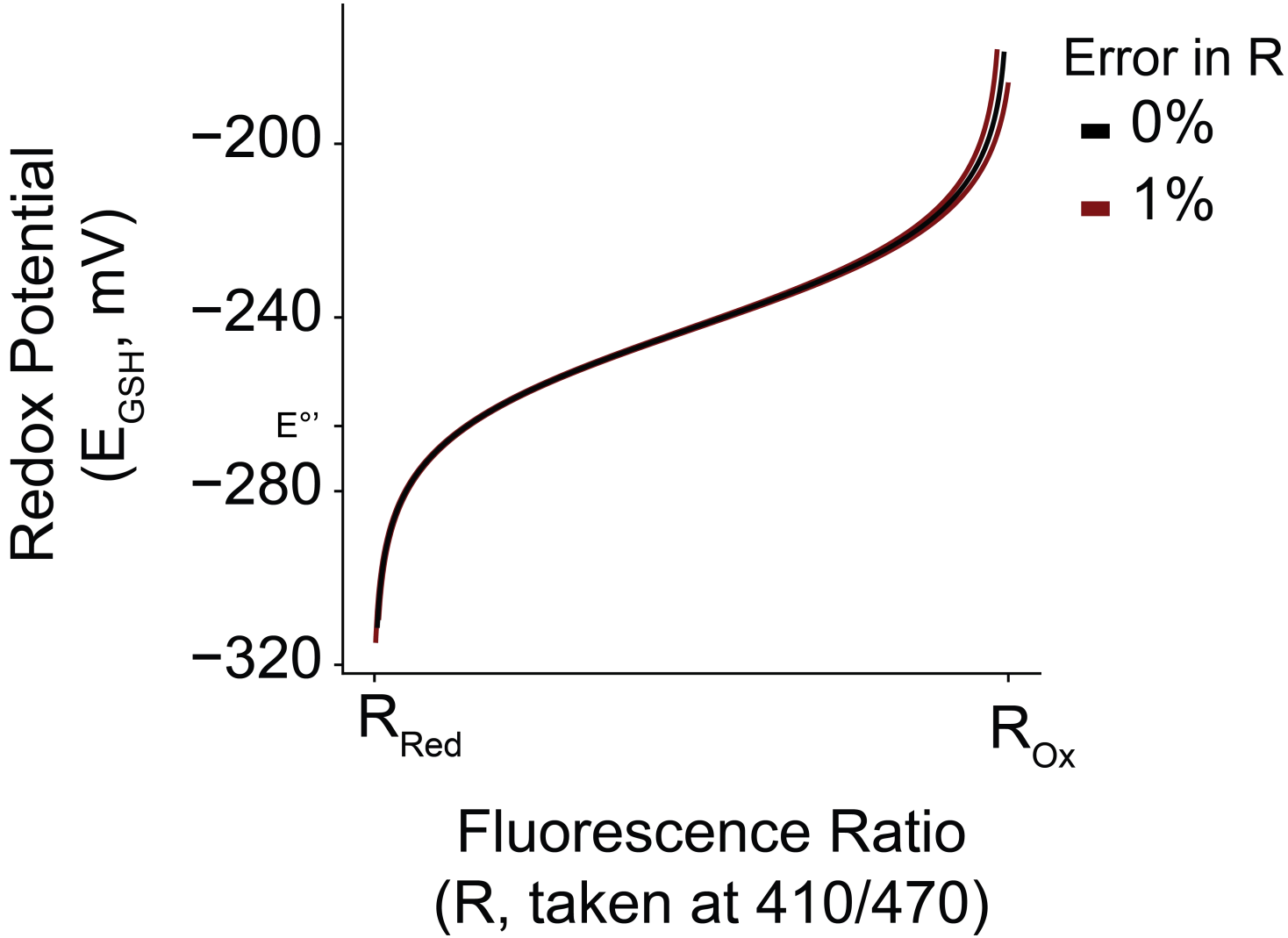

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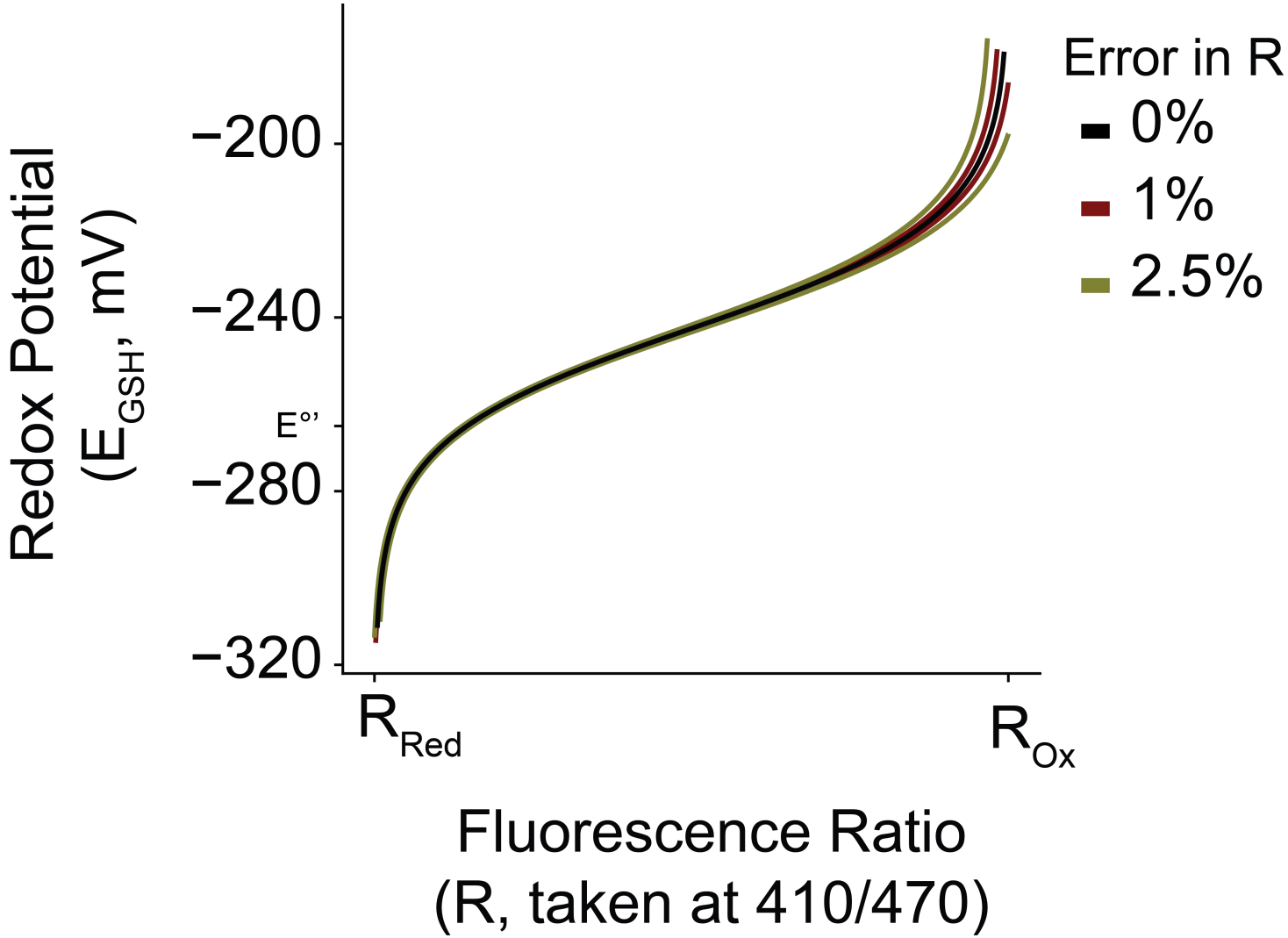

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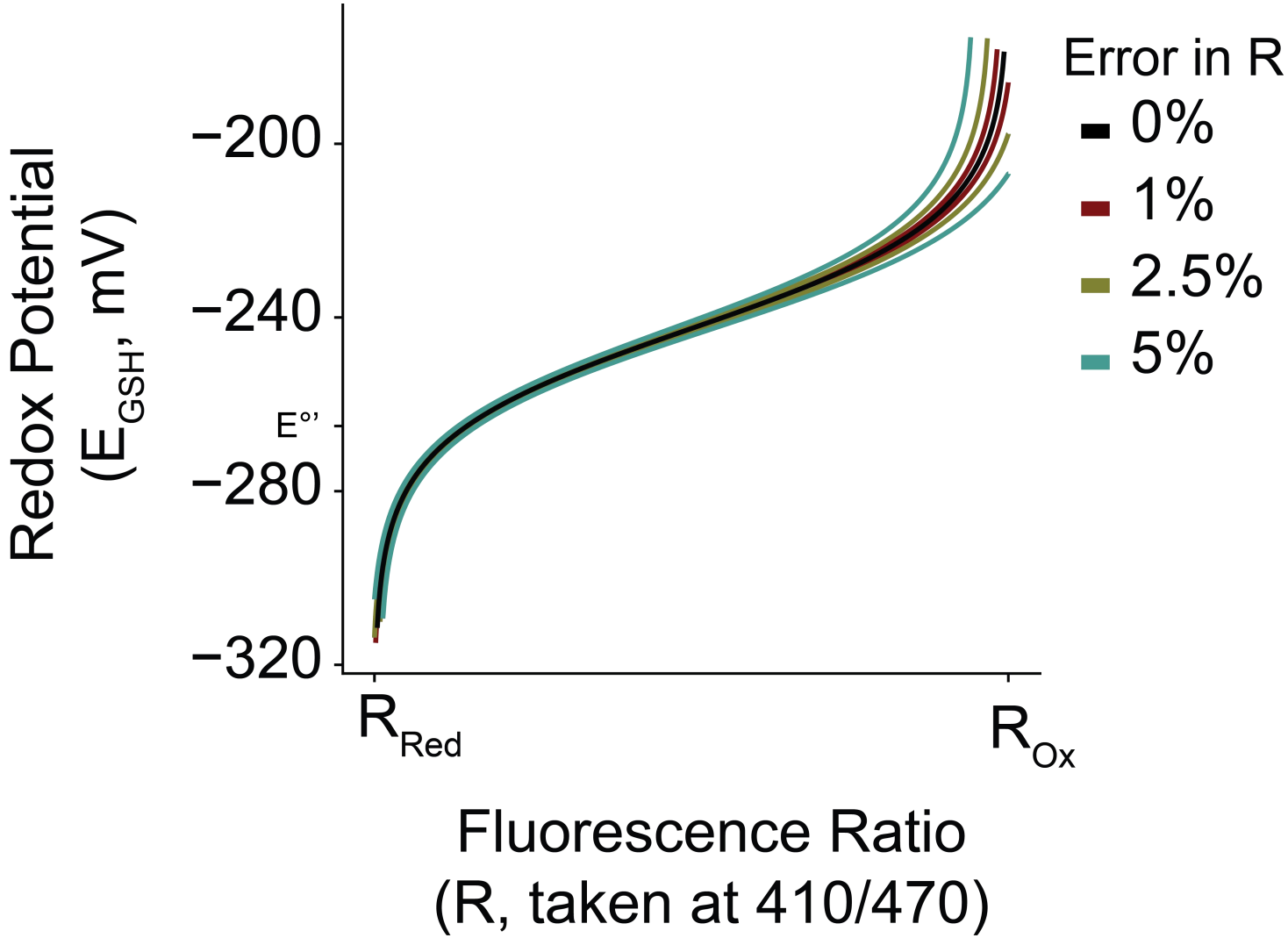

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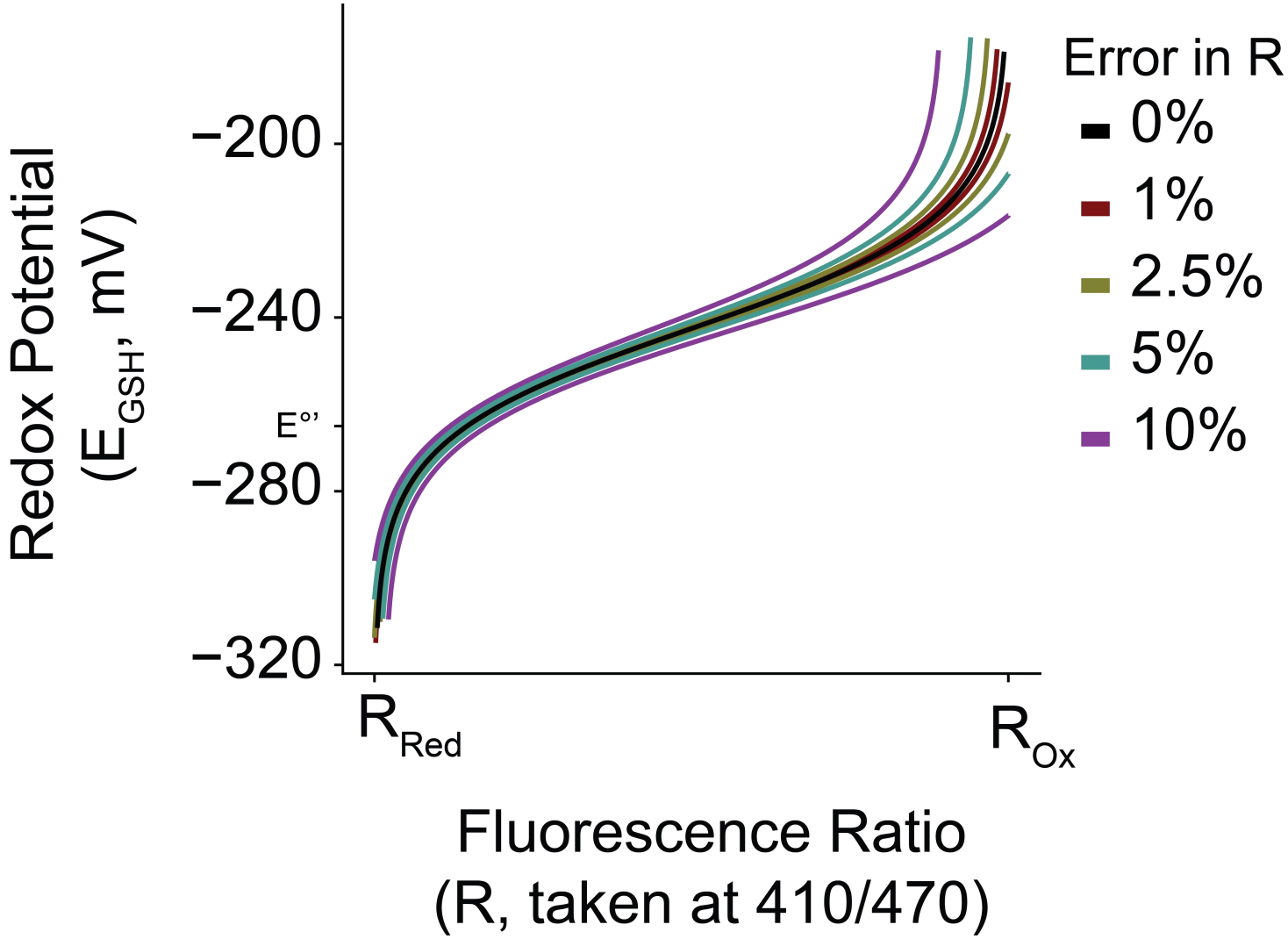

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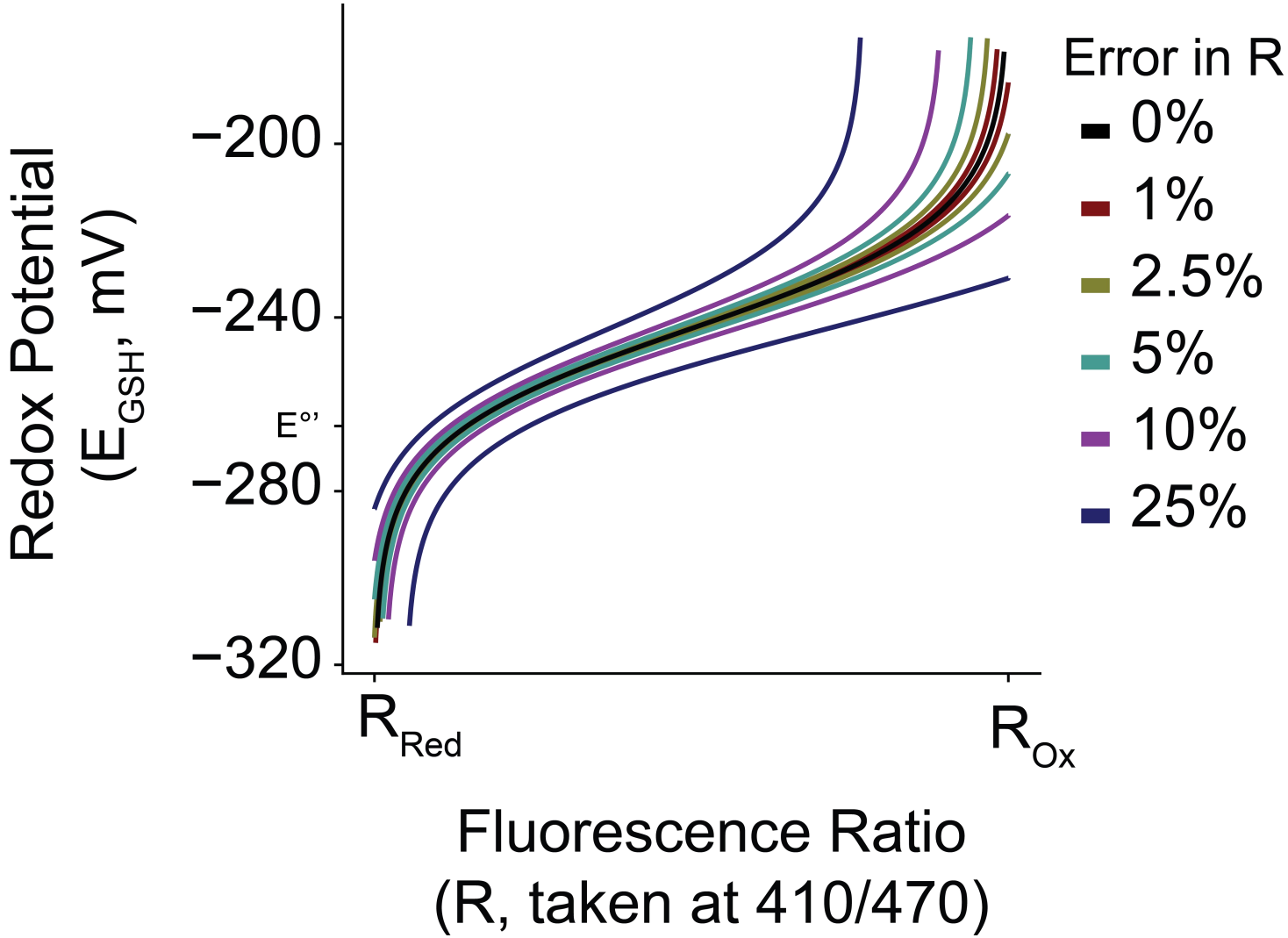
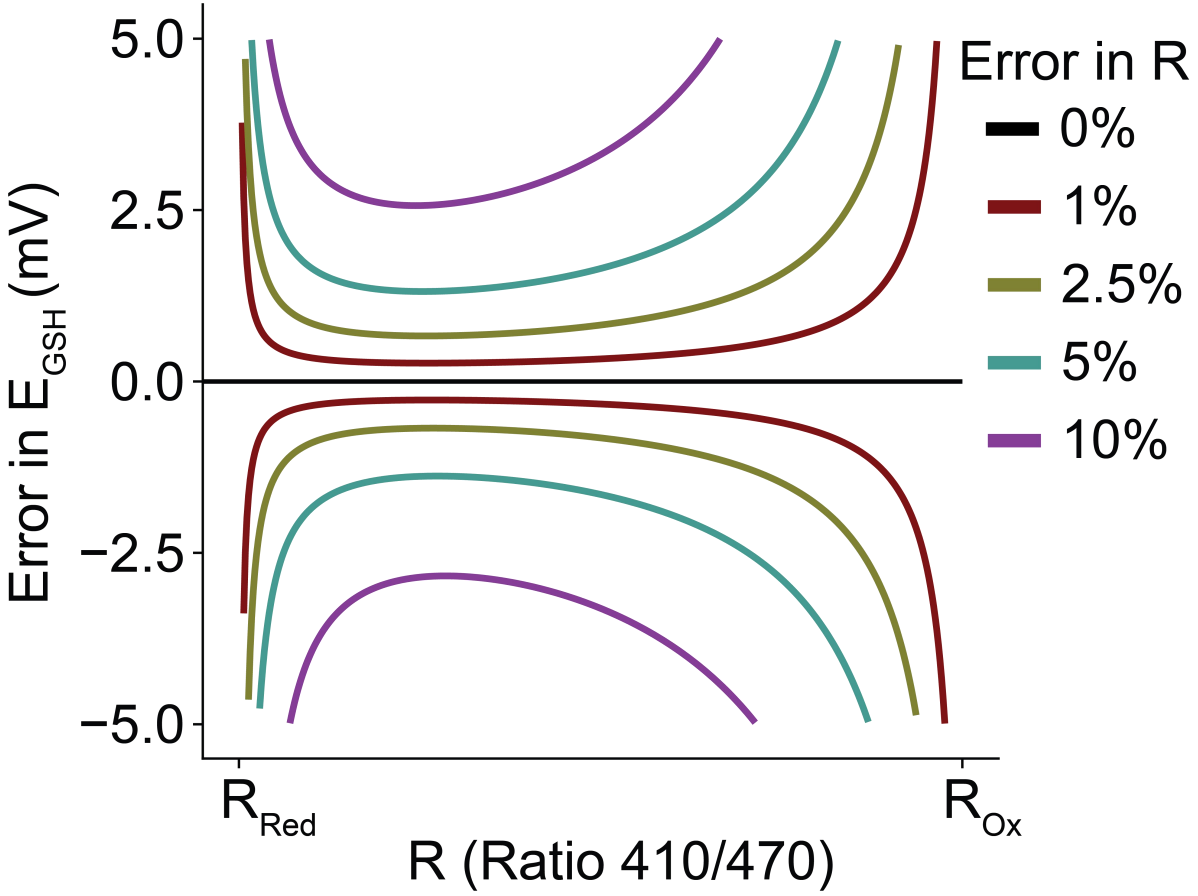
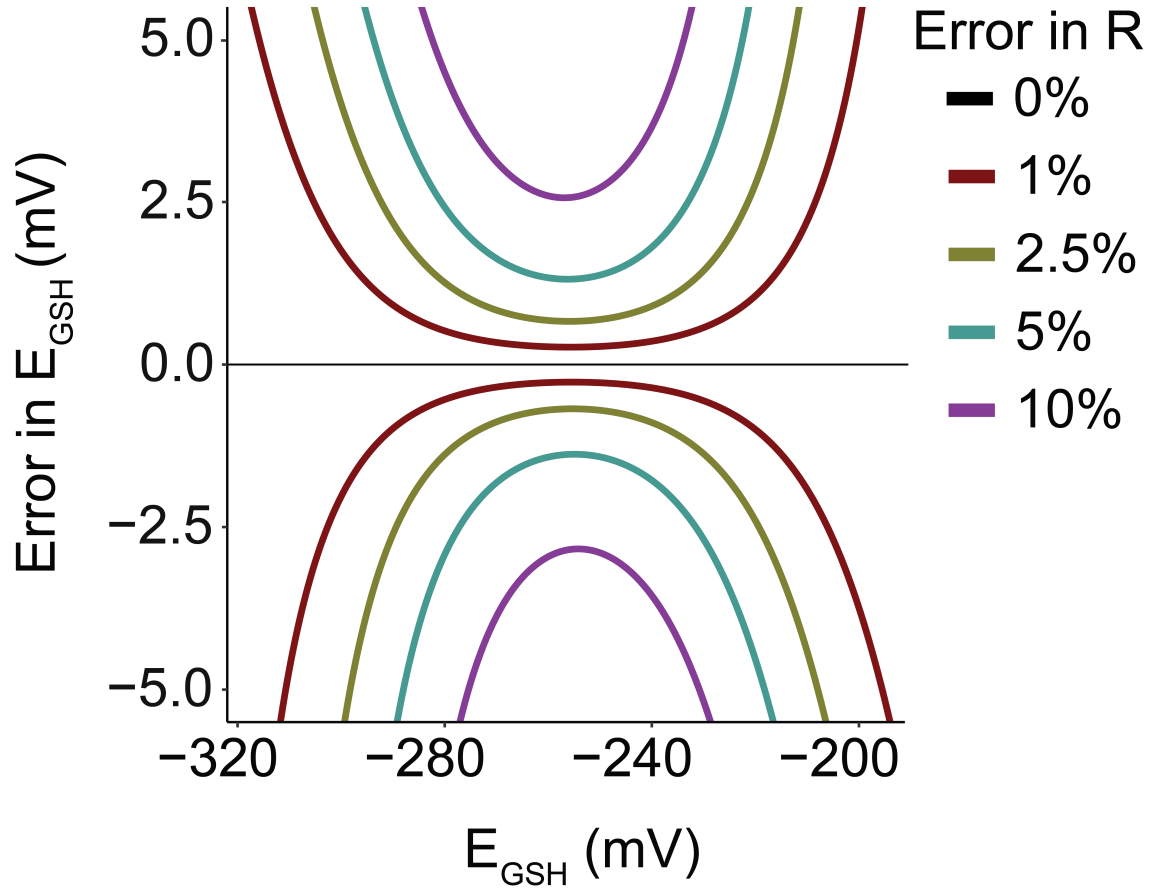
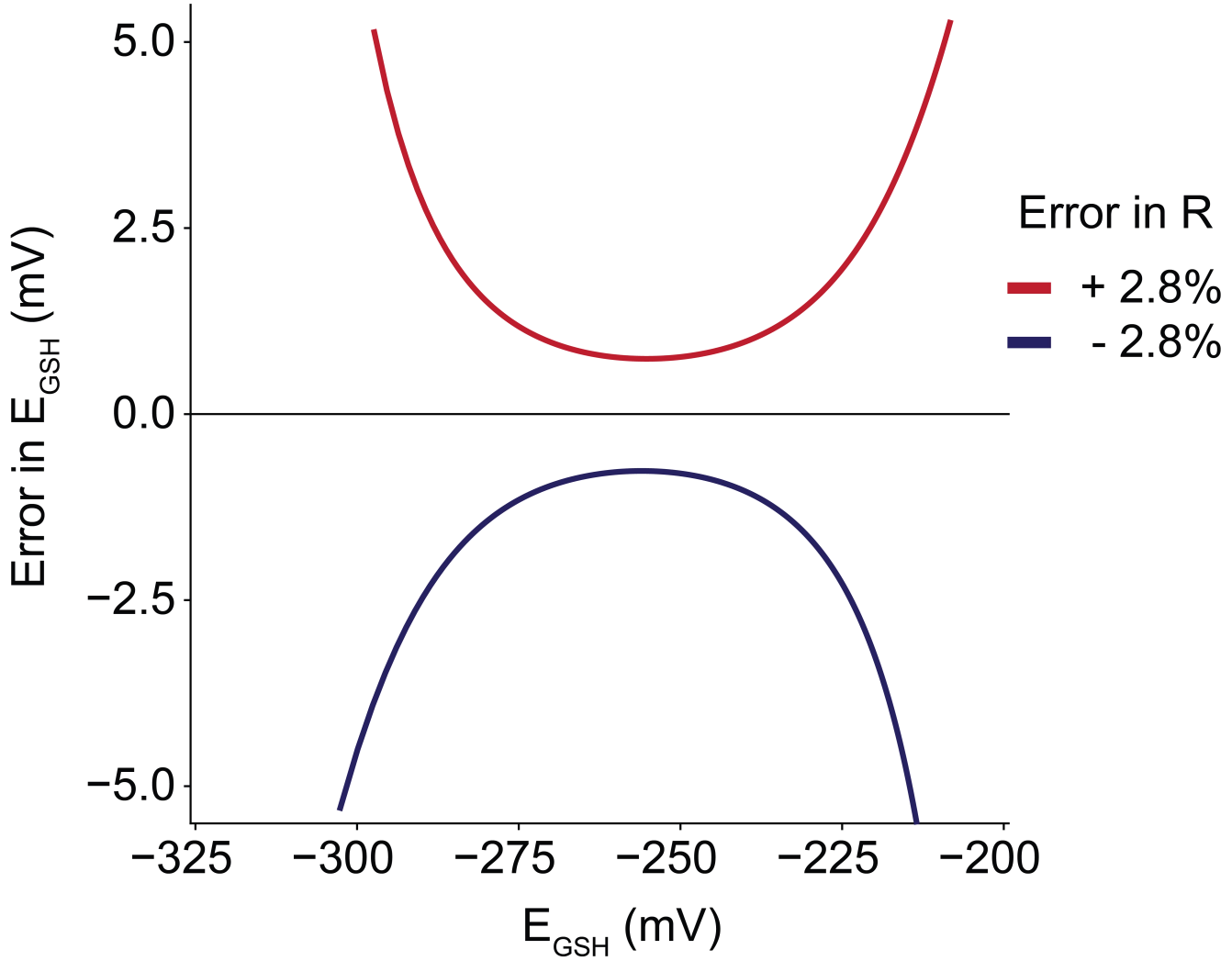


We can estimate the error associated with a true redox potential





How accurately can we can measure E_{GSH}?



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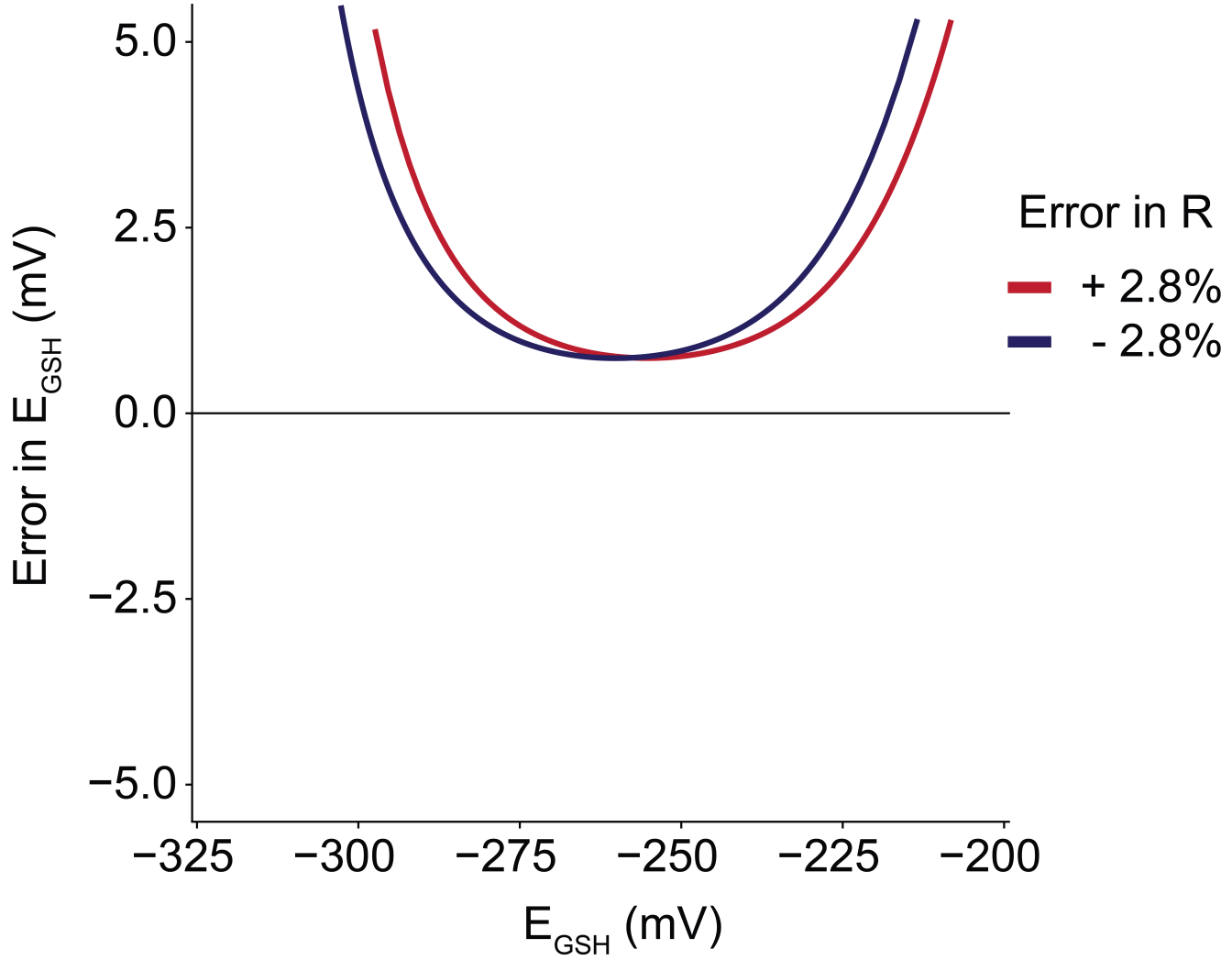
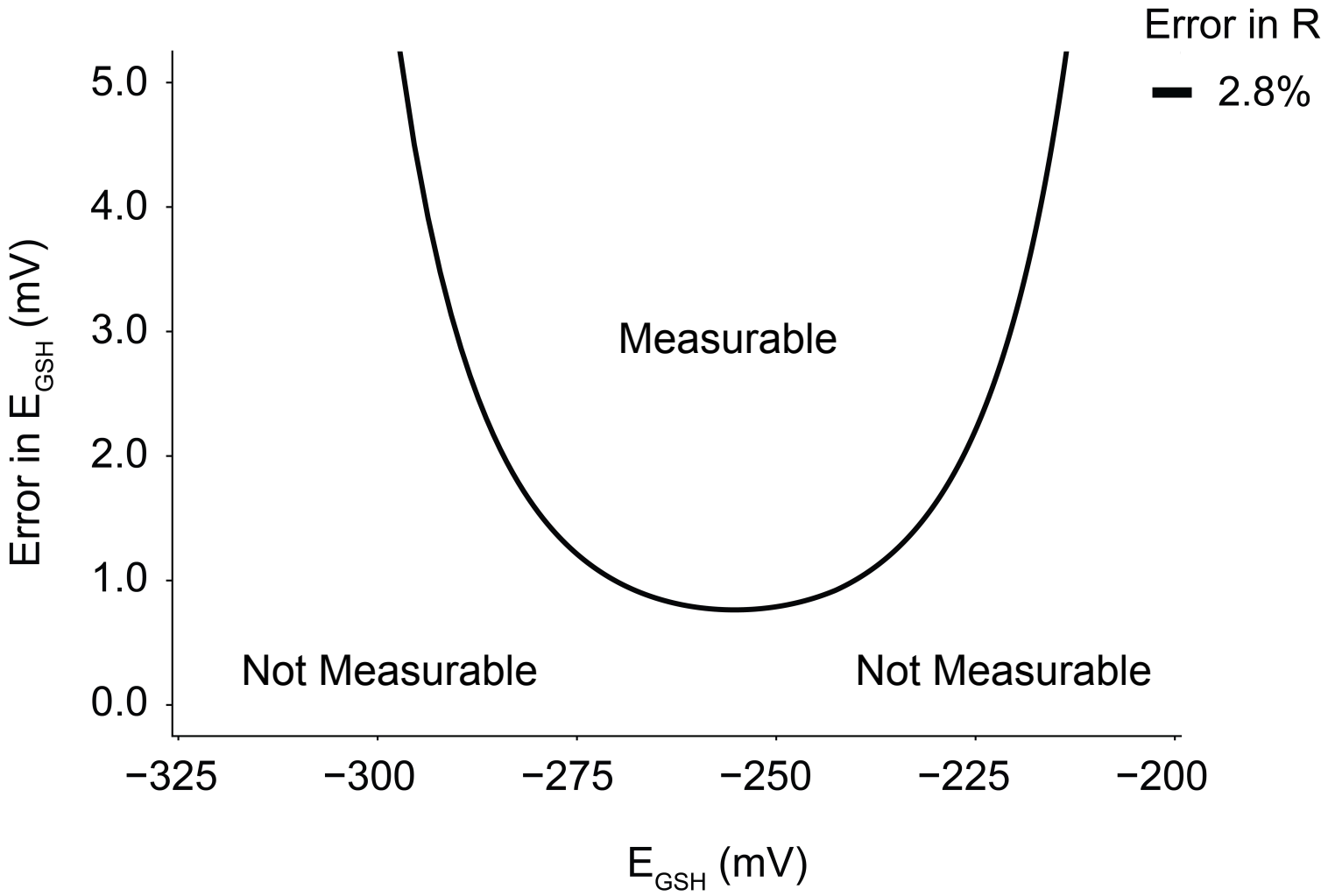
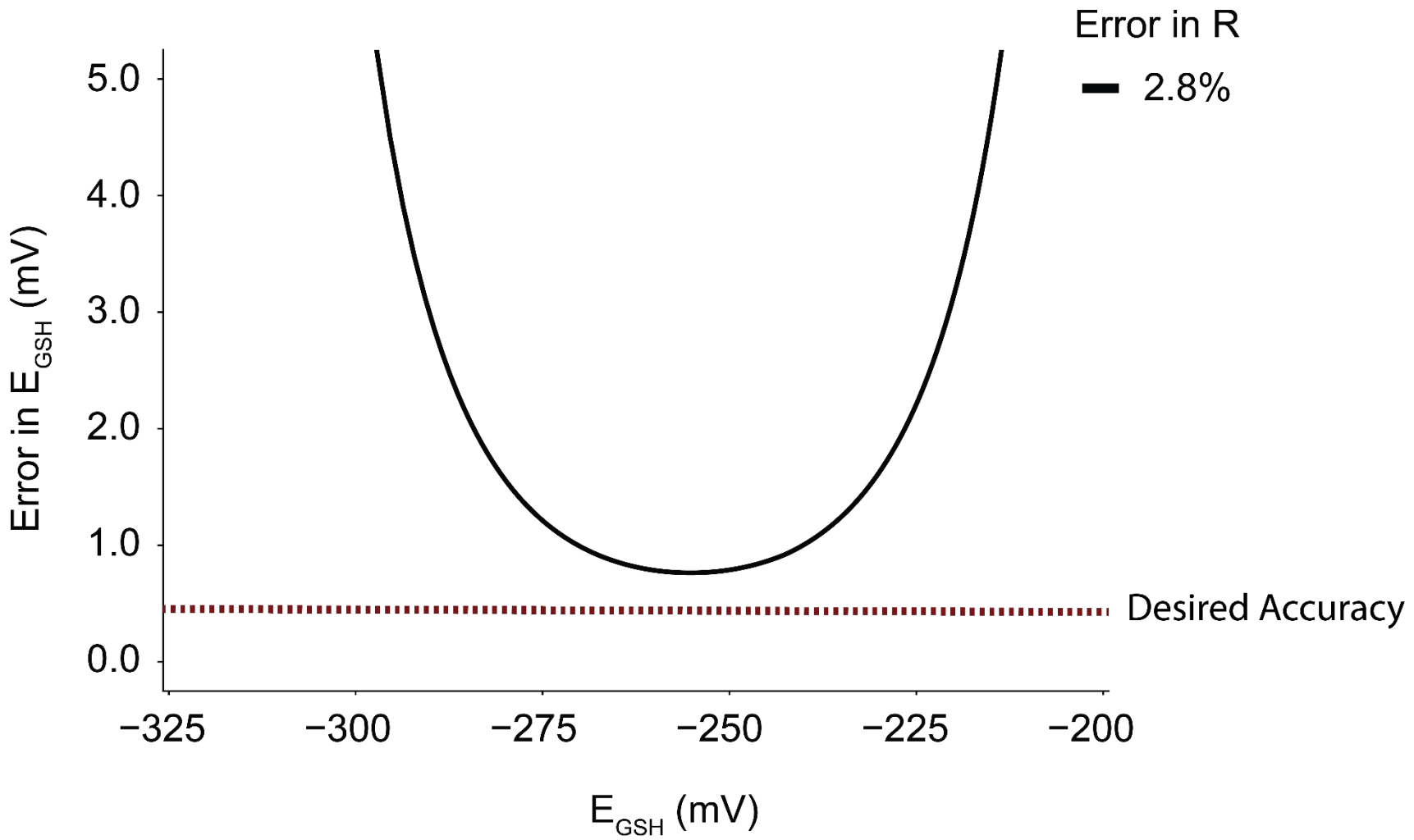


A phase plot shows how accurately we can measure E_{GSH}



A phase plot tells us what E_{GSH} values our sensor is well-suited to measure



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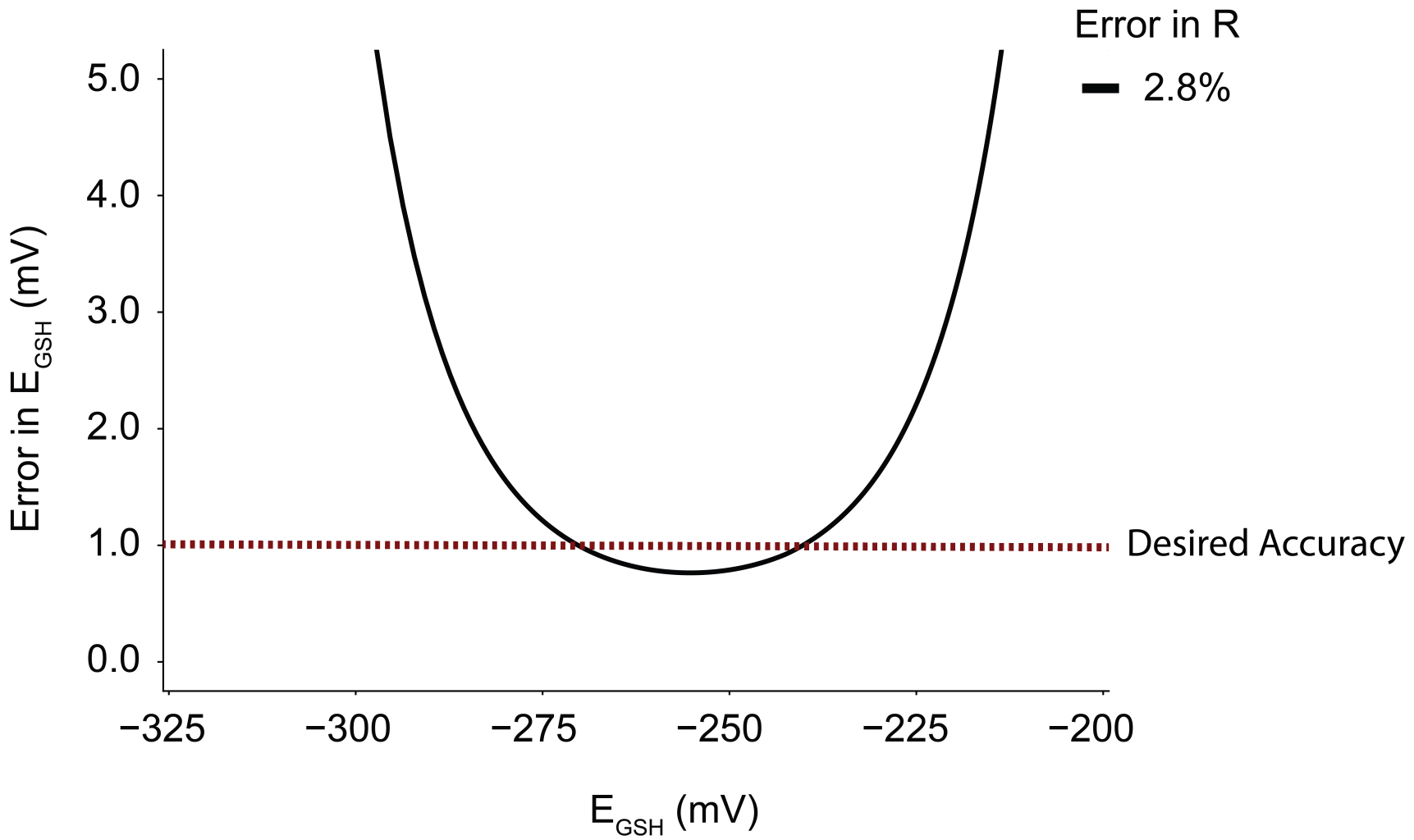

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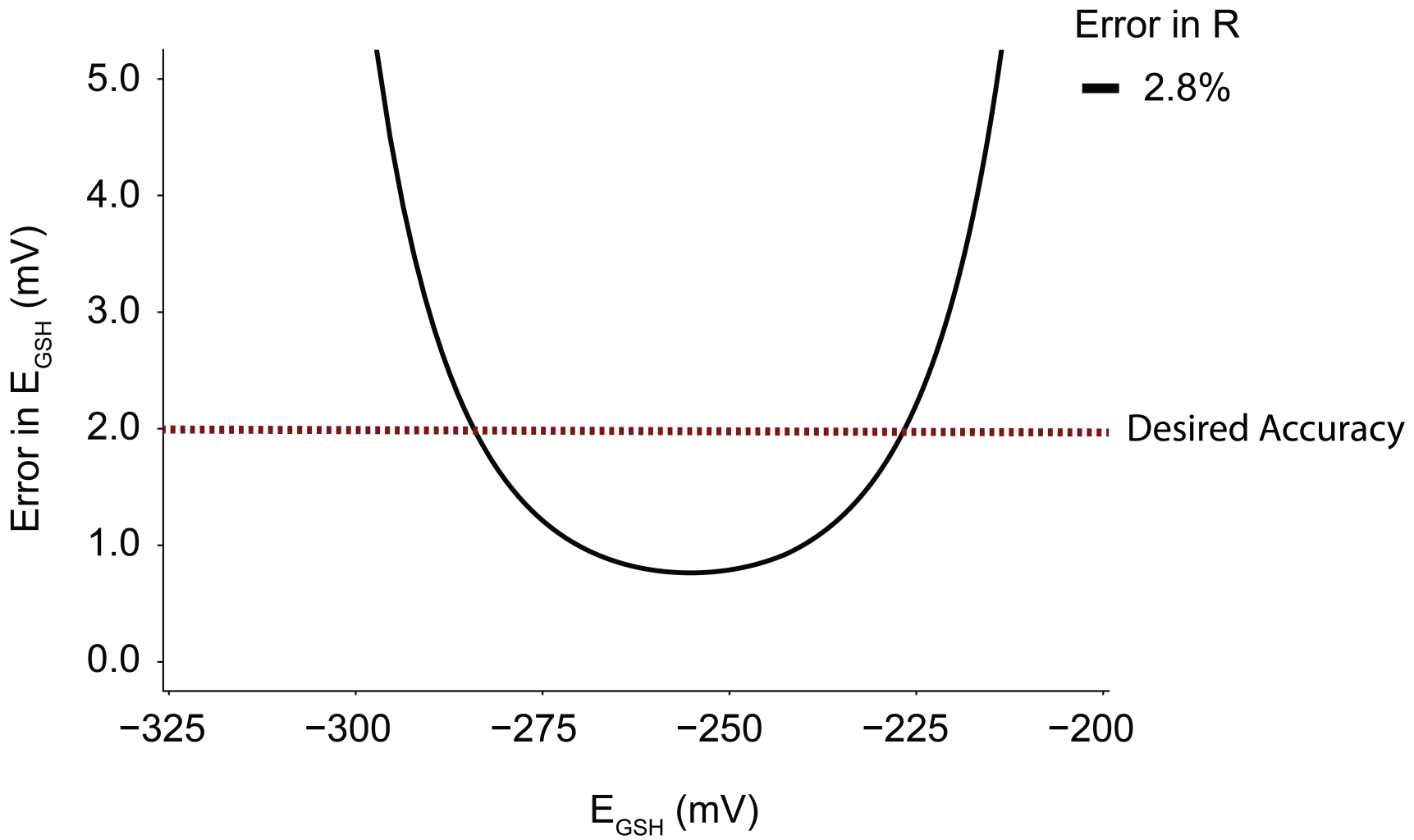

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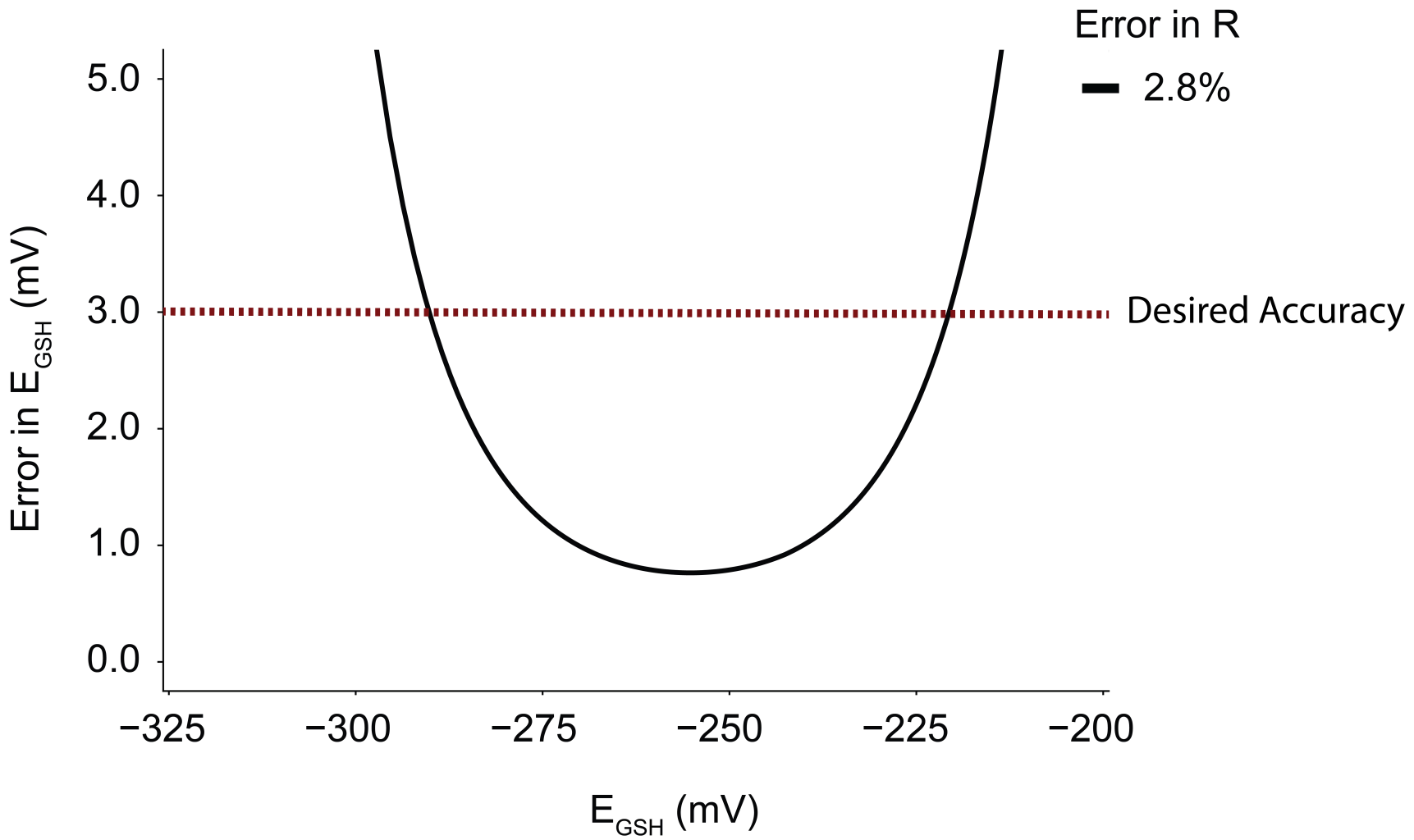
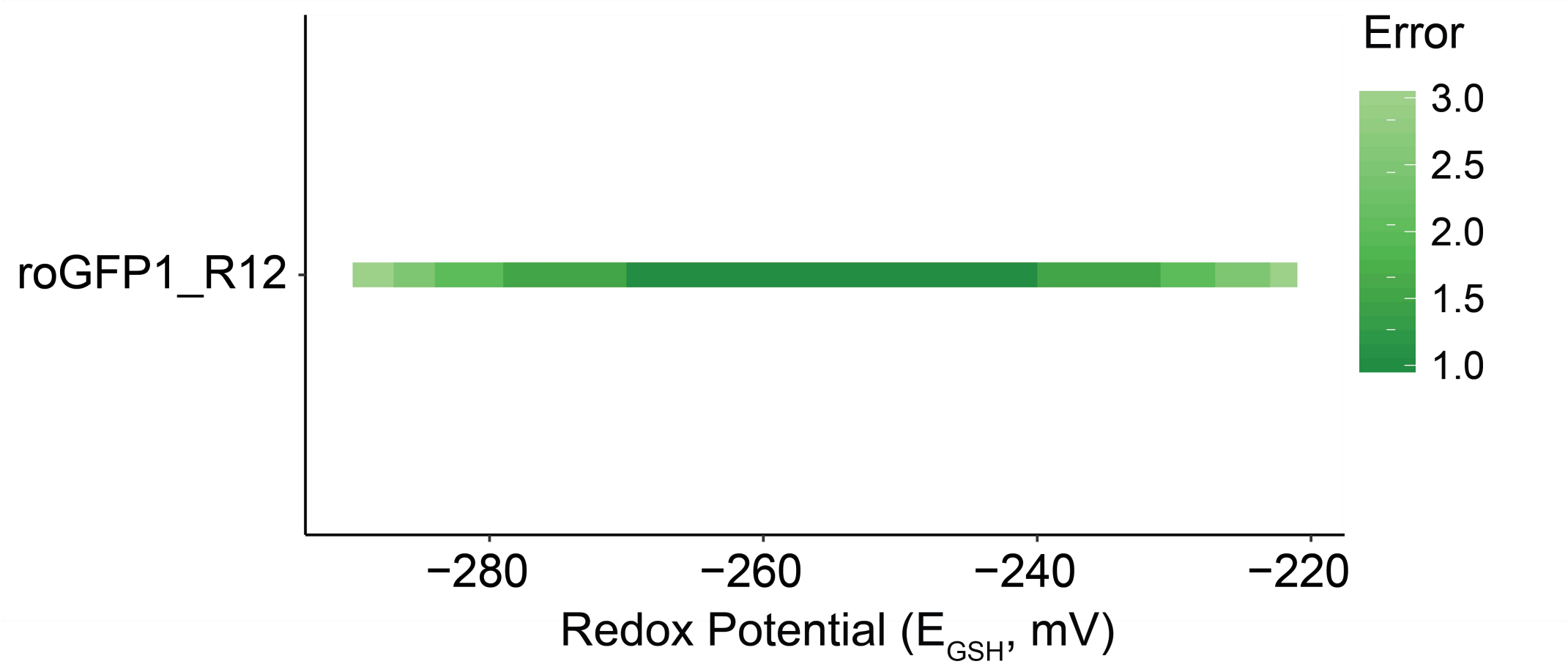
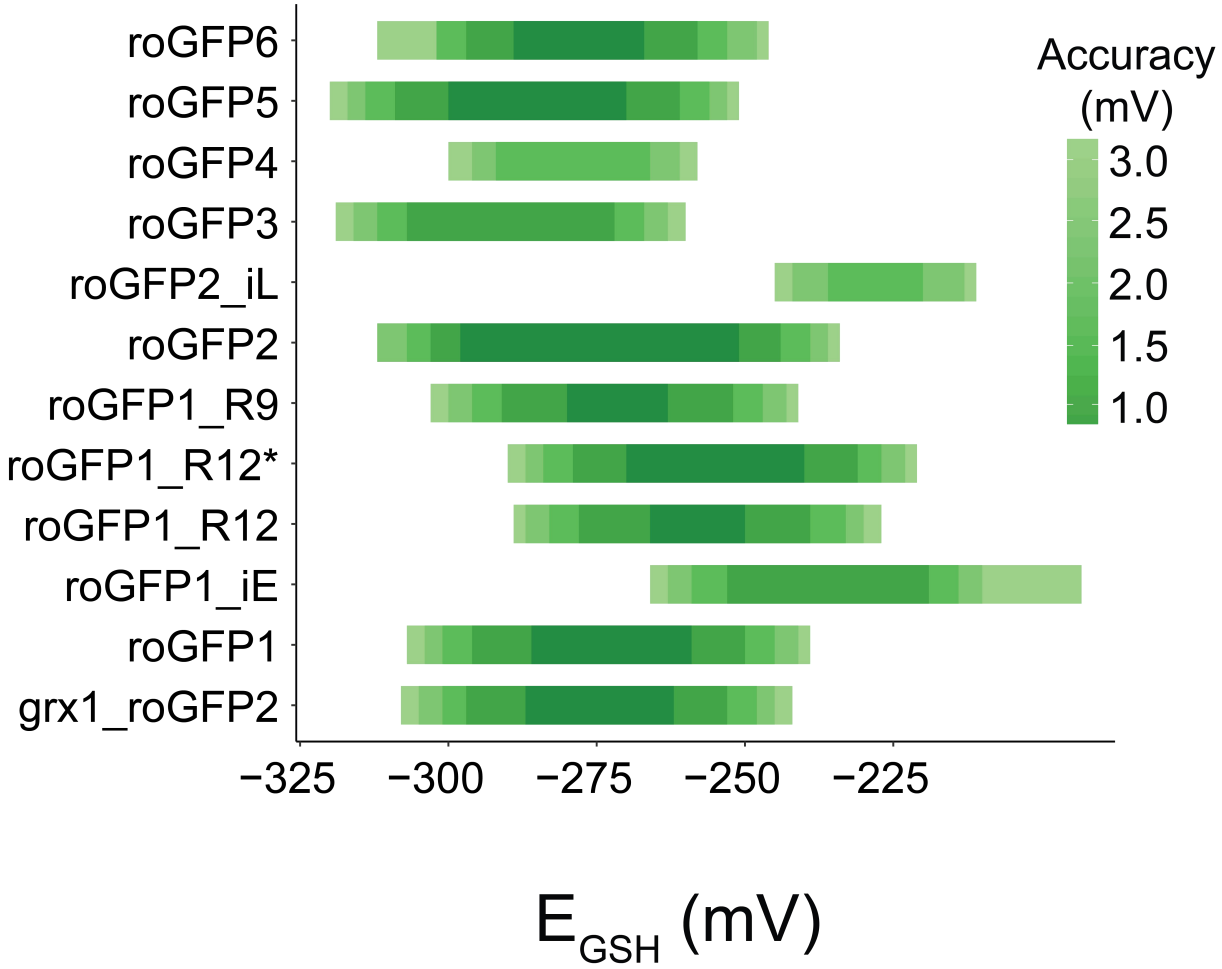
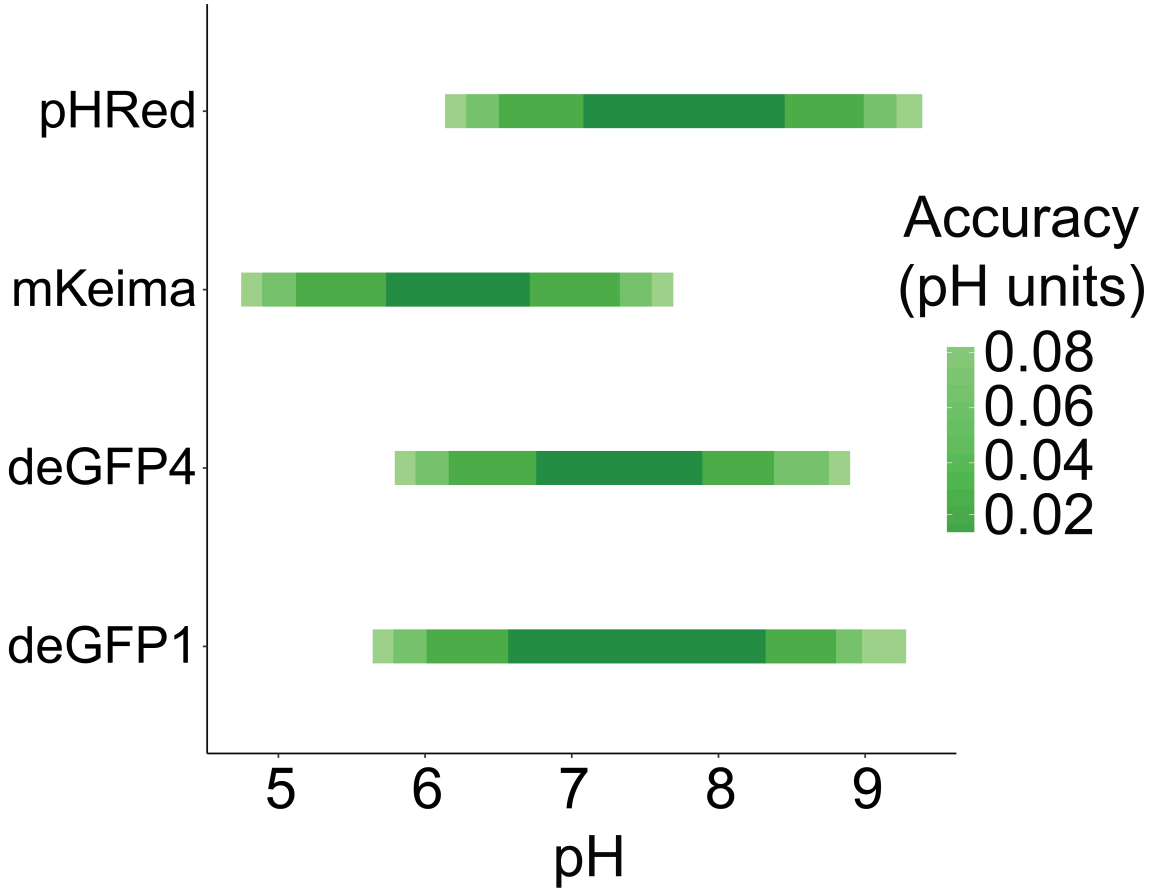


E_{GSH} values our sensor is well-suited to measure



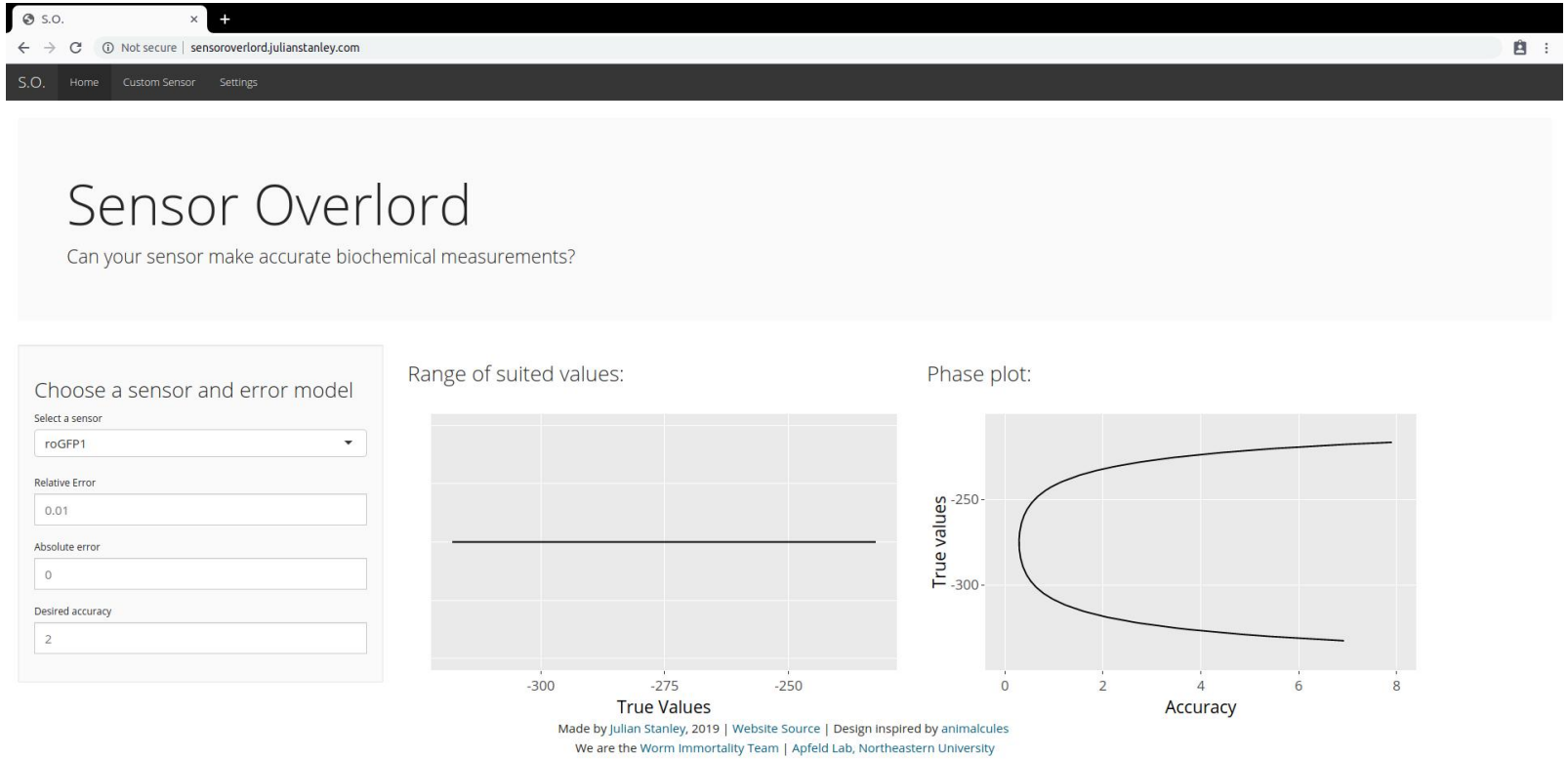
Suitable ranges can be compared within sensor families

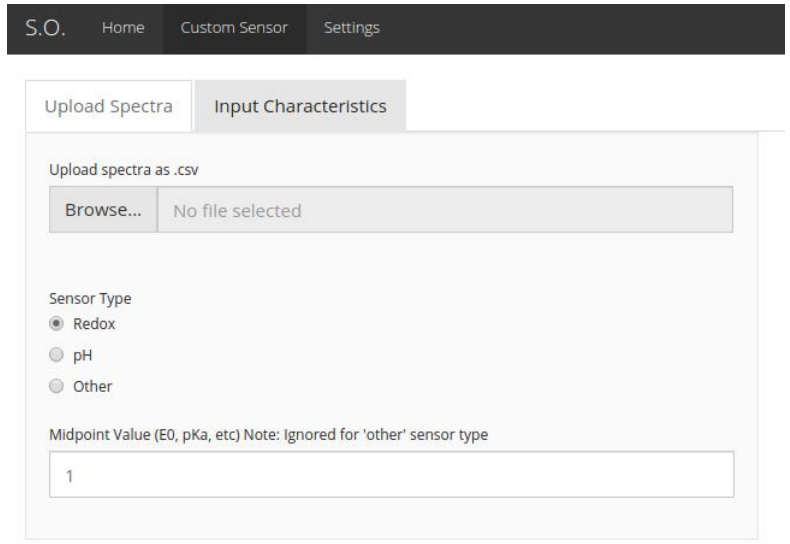




* Based on empirically-derived spectra characteristics

The Sensor Overlord can tell you what your favorite sensor can measure accurately (https://sensoroverlord.org)





github.com/julianstanley/sensoroverlord

Discussion

We can now can:

- Know which values of E_{GSH} we can measure accurately in the pharynx
- Quantify accuracy improvements from better imaging techniques
- Compare the accuracy of two-state sensors
- Identify of what new sensors are needed
- Reclaim underused sensors

Thank You!

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Jodie Norris (1014B)

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Sean Johnsen (300B)

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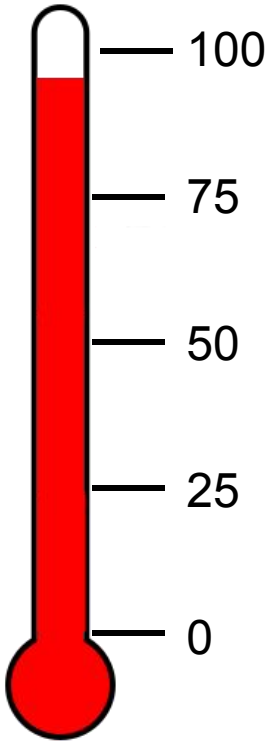
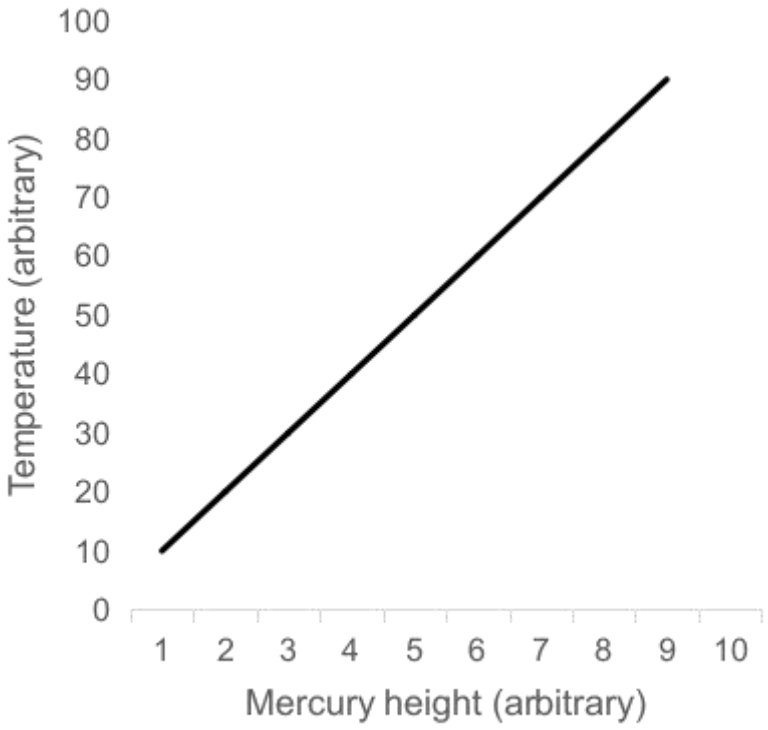
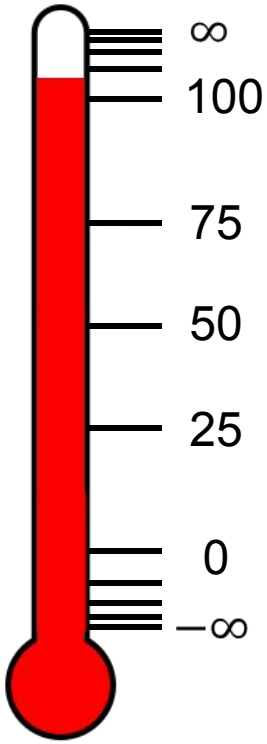
Questions?

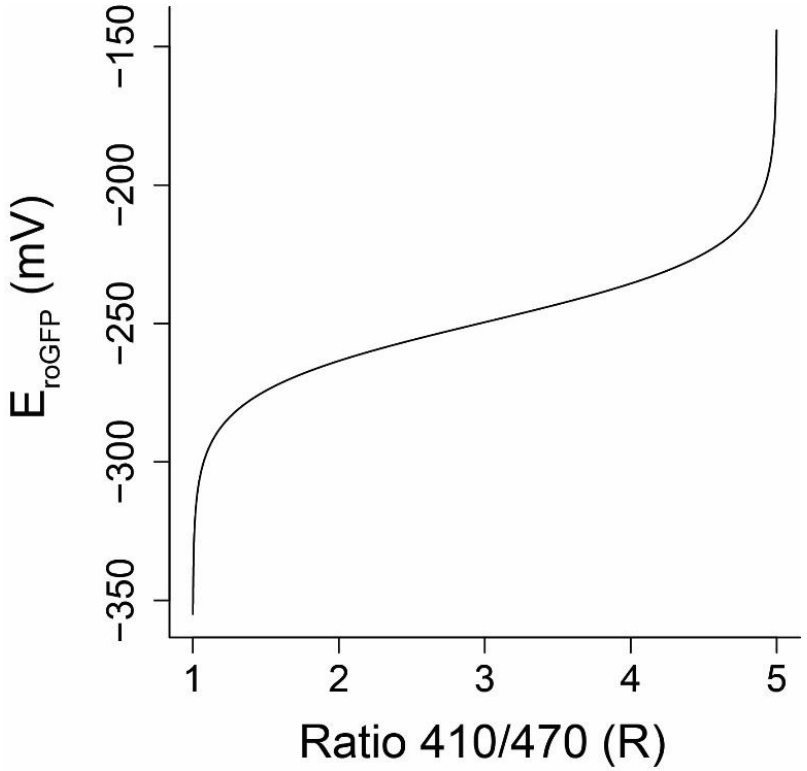
Appendix

How accurate is a biosensor?







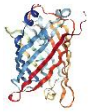


How do we tell the difference between quantitative sensors?

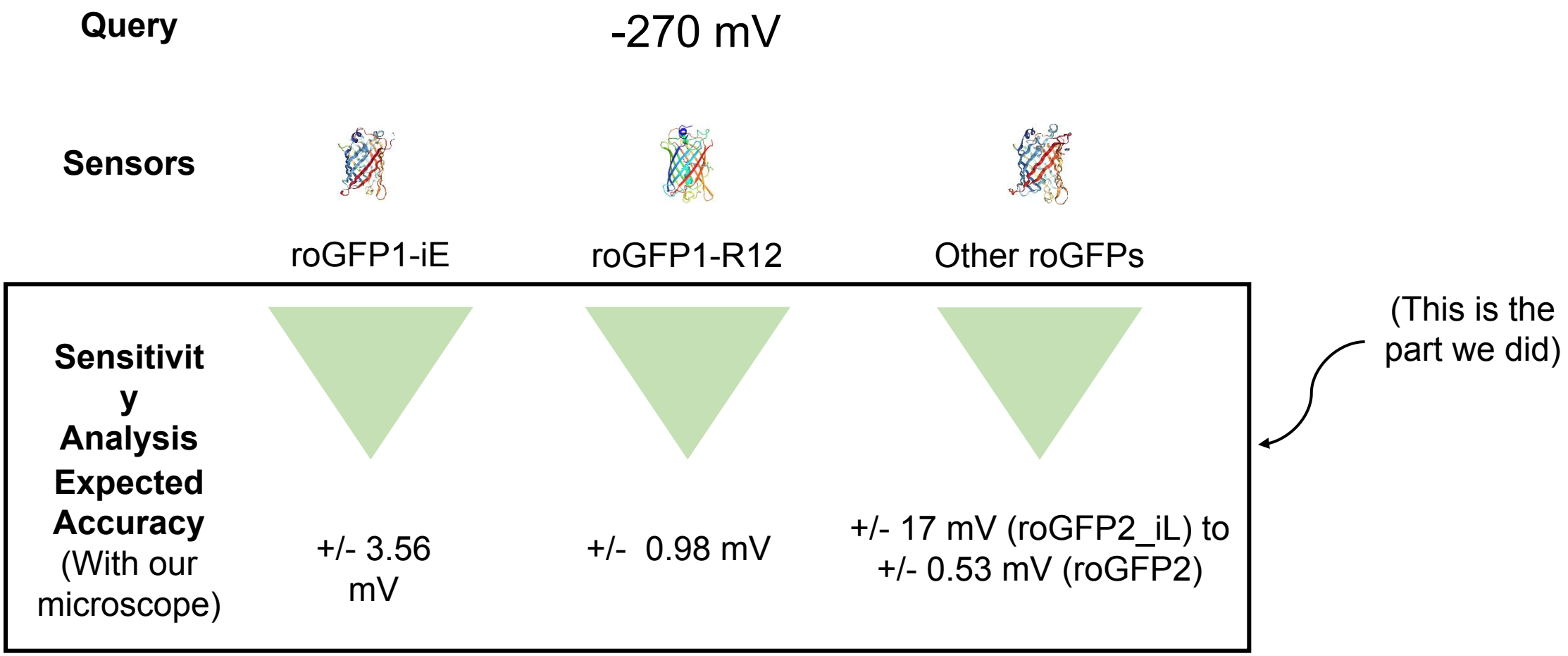
- Kinetics – well-established

- Suitable ranges—based loosely on midpoint potential, pKa, affinity, etc. Not well characterized. Here we characterize it for most biosensors with two-states, if you're taking ratiometric measurements.

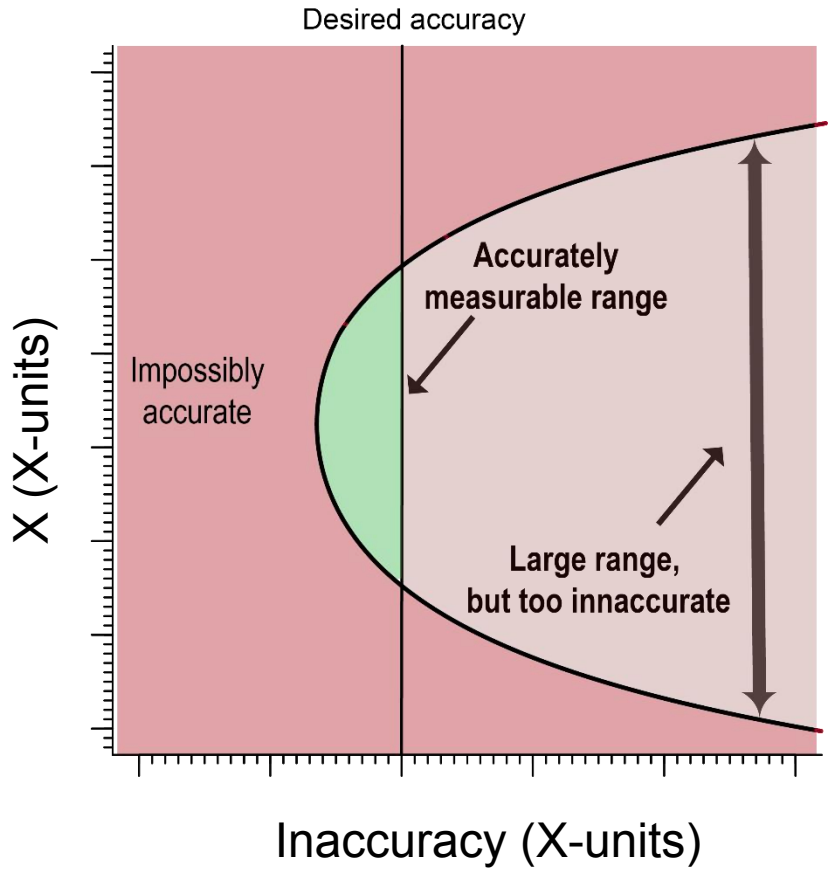
Other groups have developed redox biosensors to make measurements in different environments

Sensor	Design	Midpoint
 roGFP1-iE	Designed for “more oxidizing” environments	-236 mV
 roGFP1-R12	Designed for best kinetics in moderate environments	-265 mV
 Other roGFPs	Designed for a wide variety of environments	-287 mV to -229 mV

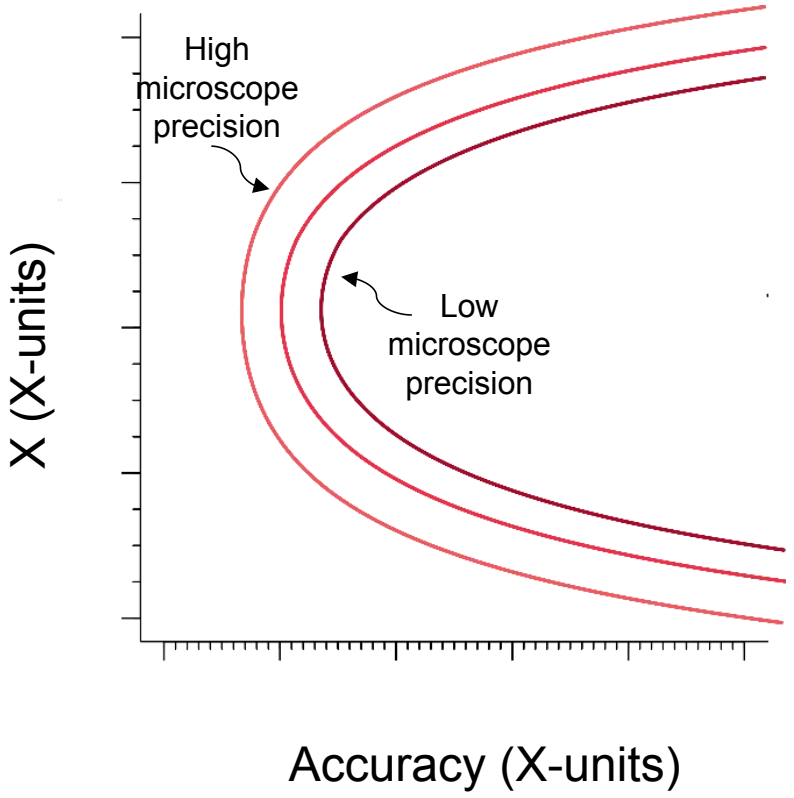
Challenge: how accurate is each biosensor?



We can present the analysis results as a phase plot



The phase plot depends on microscope precision



By predicting accuracy, we created a framework to compare similar biosensors

Sensor of X

Properties

(Spectra, Midpoints)

Experimental Parameters

(Microscope precision, desired accuracy)

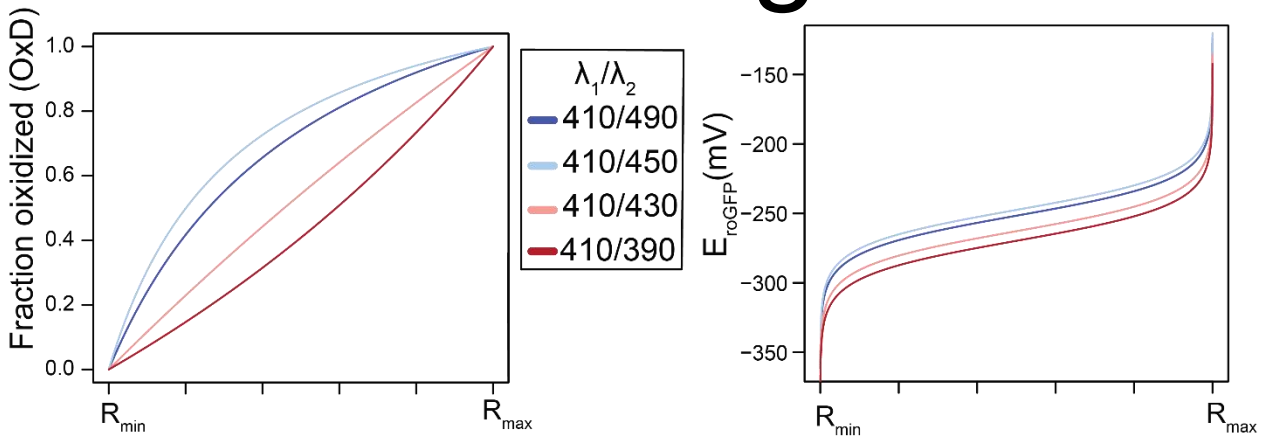
Sensor 1
Sensor 2
Etc.

The range of X's that this sensor is well-suited to measure at the desired accuracy

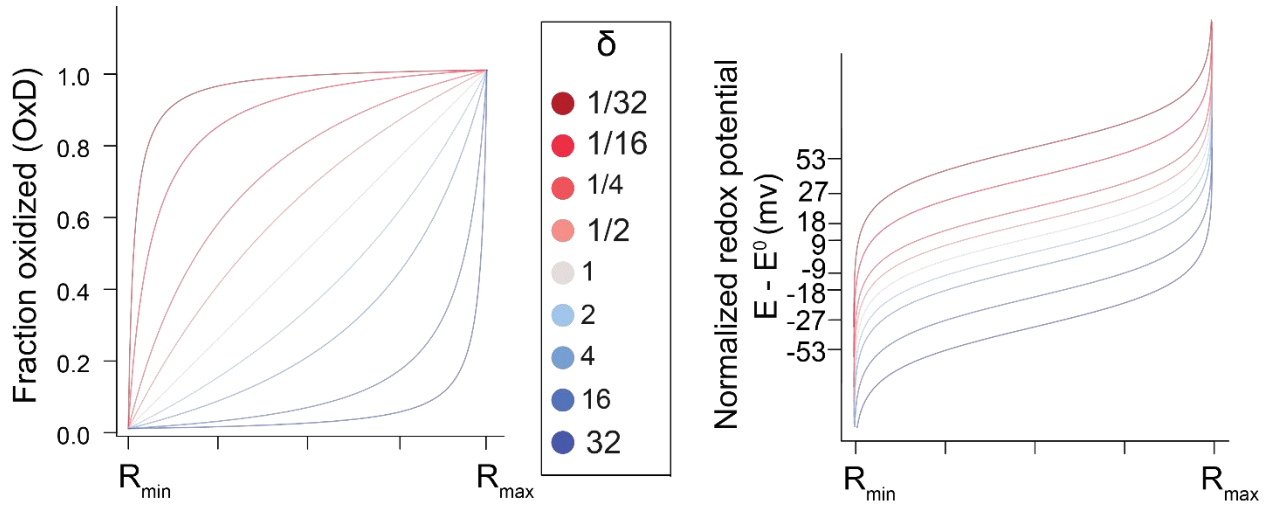
X (X-units)

Map between spectra and biochemistry depends on the sensor and ratio wavelengths

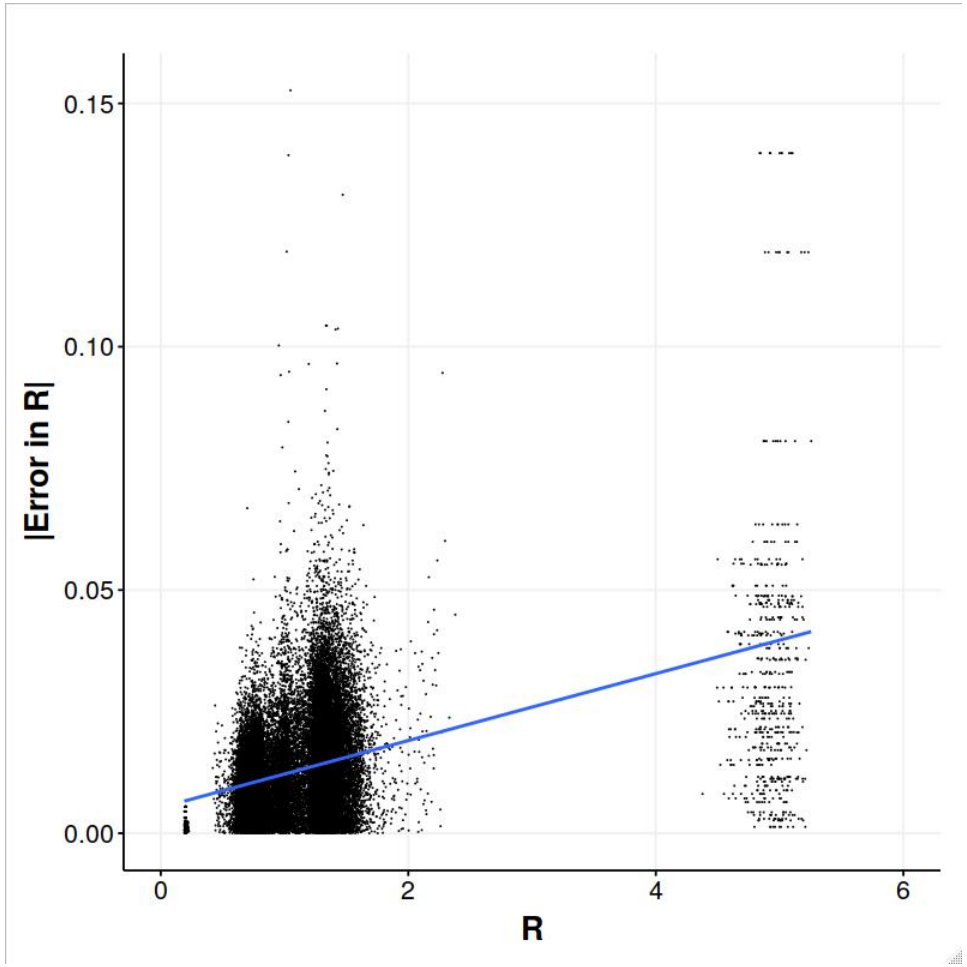
Specific examples

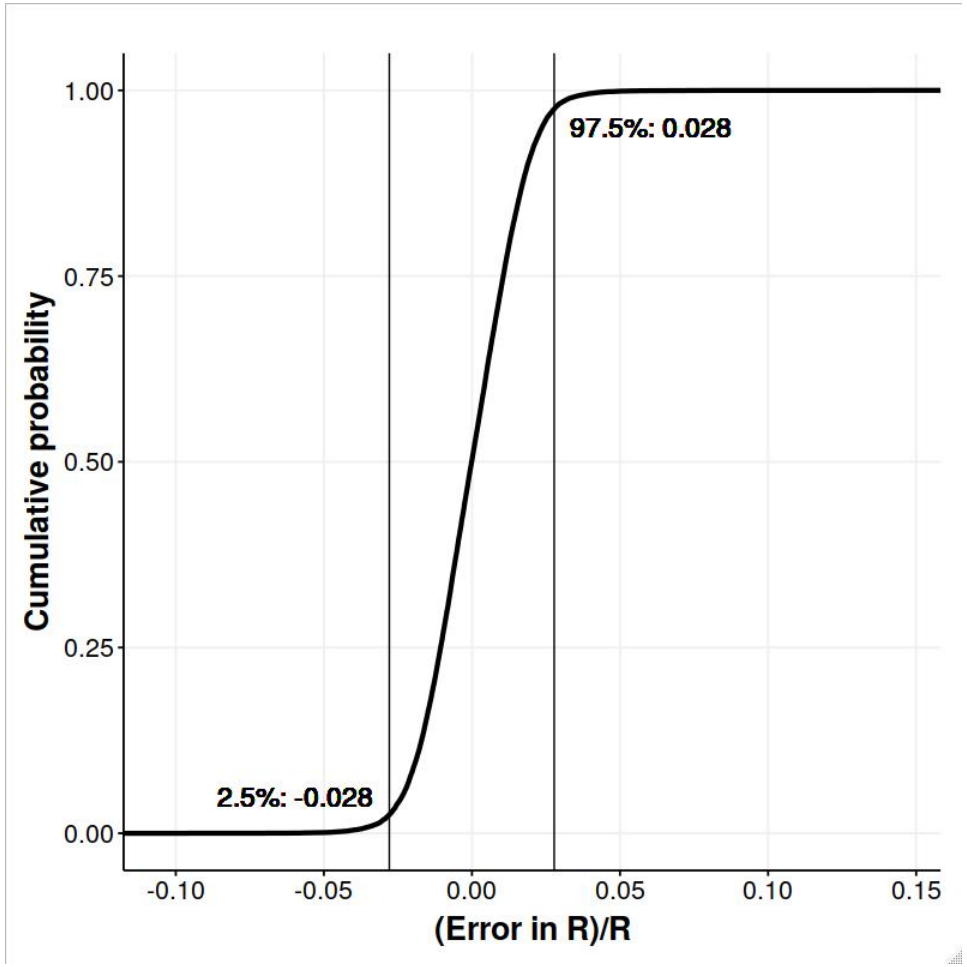


General case



We observe a ~2.8% relative error in microscopy





The framework generalizes to most two-state biosensors

Biochemical Property

$$E_{GSH} = E'^{\circ} - \frac{RT}{2F}\ln\left(\frac{Reduced}{Oxidized}\right)$$

$$pH = pK_a - \log\left(\frac{[HA]}{[A^-]}\right)$$

Generally:

$$Biochemical\ Property = C - \mathrm{D}\log_b\left(\frac{State_2}{State_1}\right)$$

State Probability

$$\frac{[Oxidized]}{[Oxidized] + [Reduced]} = \frac{R - R_{Red}}{R - R_{Red} + \delta_{\lambda 2}\,(R_{Ox} - R)}$$

$$\frac{[A^-]}{[A^-] + [HA]} = \frac{R - R_{[HA]}}{R - R_{[HA]} + \delta_{\lambda 2}\,(R_{[A^-]} - R)}$$

Generally:

$$\frac{[State_1]}{[State_1] + [State_2]} = \frac{R - R_{State_2}}{R - R_{State_2} + \delta_{\lambda 2}\,(R_{State_1} - R)}$$