roGFP Derivations & Sensitivity Analysis

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1 Converting 410/470 intensity ratio to the proportion of oxidized sensor molecules

The $roGFP_{12}$ sensor is genetically encoded into a tissue. The intensity values emitted from the $roGFP_{12}$-containing tissue are recorded after being stimulated 410nm and 470nm light. The ratios between these values describe relative levels of tissue oxidation. For example, a 410/470 ratio of 2.0 indicates that a tissue is more oxidized than a ratio of 1.0. In this brief summary, we algebraically convert the 410/470 intensity ratio into the more biologically-meaningful measure of redox potential (E) via the description of ratio of of $roGFP_{12}$ molecules in an oxidized (as opposed to reduced) state (OxD).

Assume a fully reduced state. Then, the intensities observed at a wavelength λ are equal to the product of N_T, the total number of roGFP molecules, and $I_{\lambda,R}$, the intensity of each roGFP molecule at a given wavelength in the reduced state.

$$I_{\lambda,R} = N_T * I_{\lambda,R} \tag{1}$$

The same is true for the fully oxidized state:

$$I_{\lambda,Ox} = N_T * I_{\lambda,Ox} \tag{2}$$

At a redox state between maximally reduced and maximally oxidized, the intensity at a given wavelength is a weighted sum of the molecules found at either discretely oxidized or reduced state. We therefore can rewrite any state in terms of equations (1) and (2)

$$I_\lambda = \frac{N_{Ox}}{N_T} * I_{\lambda,Ox} + \frac{N_{Red}}{N_T} * I_{\lambda,Red} \tag{3}$$

Because all sensor molecules must be in either an oxidized or reduced state, $N_{Red} = N_T - N_{Ox}$. So we can rewrite equation (3) :

$$I_\lambda = \frac{N_{Ox}}{N_T} * I_{\lambda,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{\lambda,Red} \tag{4}$$

Using equation (4), consider the intensity ratio of 410 nm / 470 nm:

$$\frac{I_{410}}{I_{470}} = \frac{\frac{N_{Ox}}{N_T} * I_{410,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{410,Red}}{\frac{N_{Ox}}{N_T} * I_{470,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{470,Red}} =$$

For brevity, let $OxD = \frac{N_{Ox}}{N_T}$. Then cross-multiply:

$$I_{410} * OxD * (I_{470,Ox} + (1 - OxD) * I_{470,Red}) =$$

$$I_{470} * OxD * (I_{410,Ox} + (1 - OxD) * I_{410,Red})$$

Simplify and express OxD in terms of known quantities:

$$OxD = \frac{I_{470} I_{410,R} - I_{410} I_{470,R}}{I_{410} I_{470,Ox} - I_{410} I_{470,R} - I_{470} I_{410,Ox} + I_{470} I_{410,R}} \quad (5)$$

To simplify, let:

$$R_{Red} = \frac{I_{410,R}}{I_{470,R}} \quad (6)$$

$$R_{Ox} = \frac{I_{410,Ox}}{I_{470,Ox}} \quad (7)$$

$$\frac{I_{410}}{I_{470}} = \frac{I_{410}}{I_{470}} \quad (8)$$

$$\delta_{470} = \frac{I_{470_{Ox}}}{I_{470_{Red}}} \quad (9)$$

We can now re-derive the definition of OxD in terms of ratio values.

Step: Re-arrange terms, multiply by $\frac{-1}{-1}$:

$$OxD = \frac{I_{410} I_{470,R} - I_{470} I_{410,R}}{I_{410} I_{470,R} - I_{470} I_{410,R} + I_{470} I_{410,Ox} - I_{470,Ox} I_{410}}$$

Step: Work to factor out $I_{470,R} i_{470}$ from the numerator and denominator write some in terms of ratio values:

$$OxD = \frac{I_{470,R} I_{470} (\frac{I_{410}}{I_{470}} - R_{Red})}{I_{470,R} I_{470} (\frac{I_{410}}{I_{470}} - R_{Red} + \delta_{470} (R_{Ox} - \frac{I_{410}}{I_{470}}))}$$

And simplify:

$$OxD = \frac{\frac{I_{410}}{I_{470}} - R_{Red}}{\frac{I_{410}}{I_{470}} - R_{Red} + \delta_{470} (R_{Ox} - \frac{I_{410}}{I_{470}})} \quad (10)$$

Where δ_{470} describes the sensor-specific dynamic range $470nm$.

2 Analyzing the conversion between intensity ratio and ratio of oxidized molecules

There are 4 parameters that fit into the determination of the fraction of oxidized molecules at any given point:

1. $R_{410/470}$ is the ratio of intensity at 410/470, which is usually the observed value of interest.
2. R_{Red} is the minimal intensity value at 410/470 (when the sensor is maximally reduced).
3. R_{Ox} is the maximal intensity value at 410/470 (when the sensor is maximally oxidized).
4. δ_{470} is the dynamic range of the sensor at 470 (ratio of 470 at maximally reduced and maximally oxidized, respectively).

Experimentally, our function's true input is $R_{410/470}$, so we can plot the fraction oxidized as a function of $R_{410/470}$.

Note that the $OxD \propto \frac{c}{d-\delta 470}$:

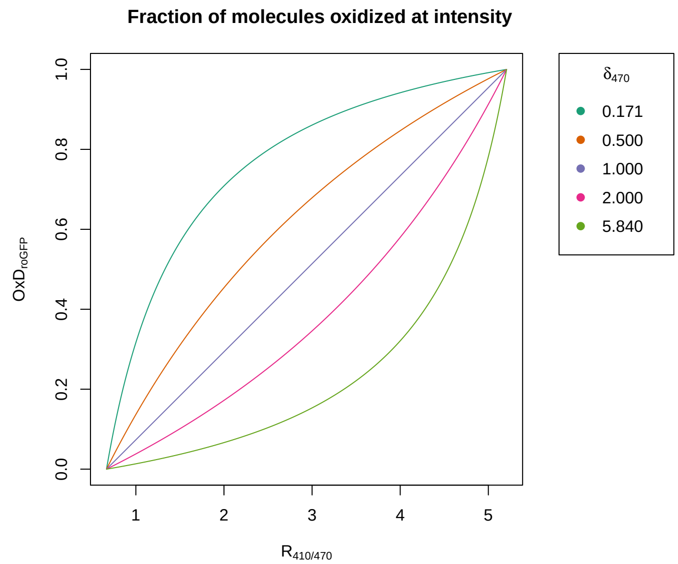

Figure 1: The relationship between observed intensity and OxD with different δ_{470} constants

3 Converting fraction of oxidized sensors into an electric potential

4 Reparameterizing the intensity ratio