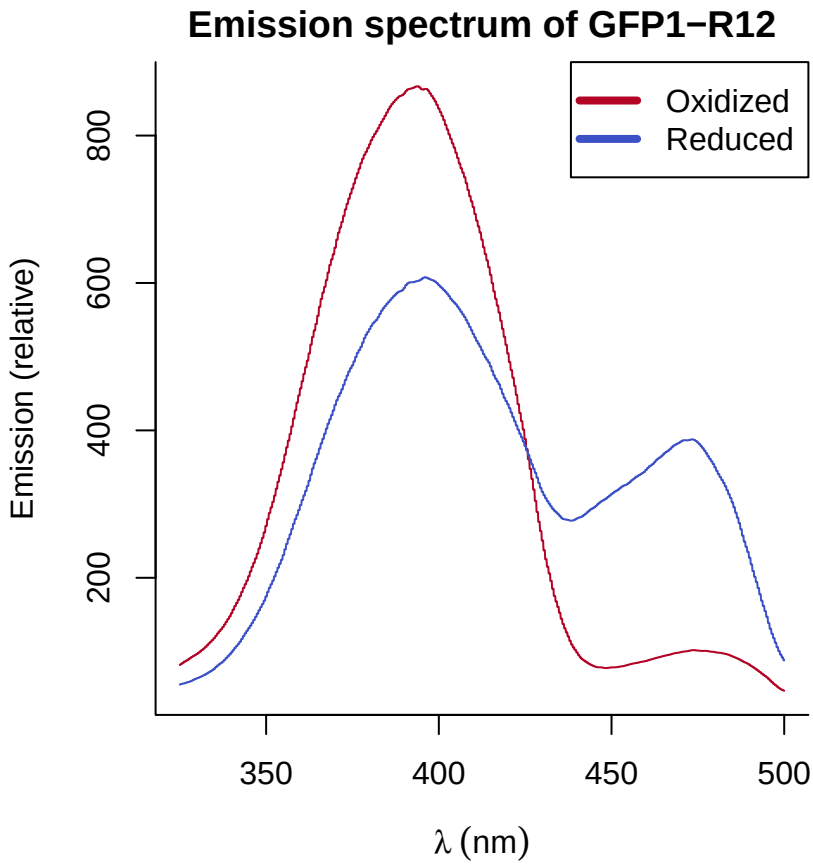
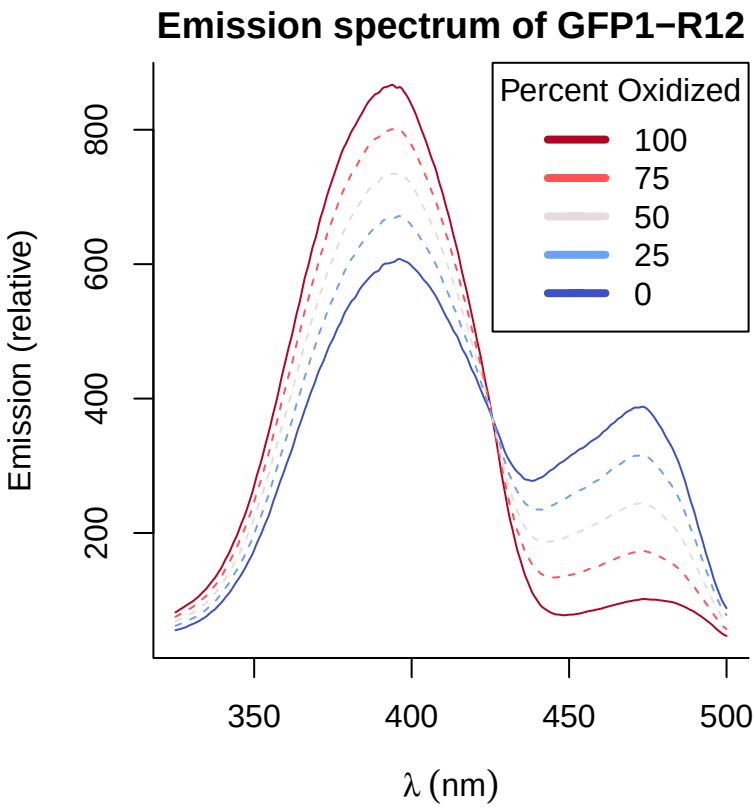
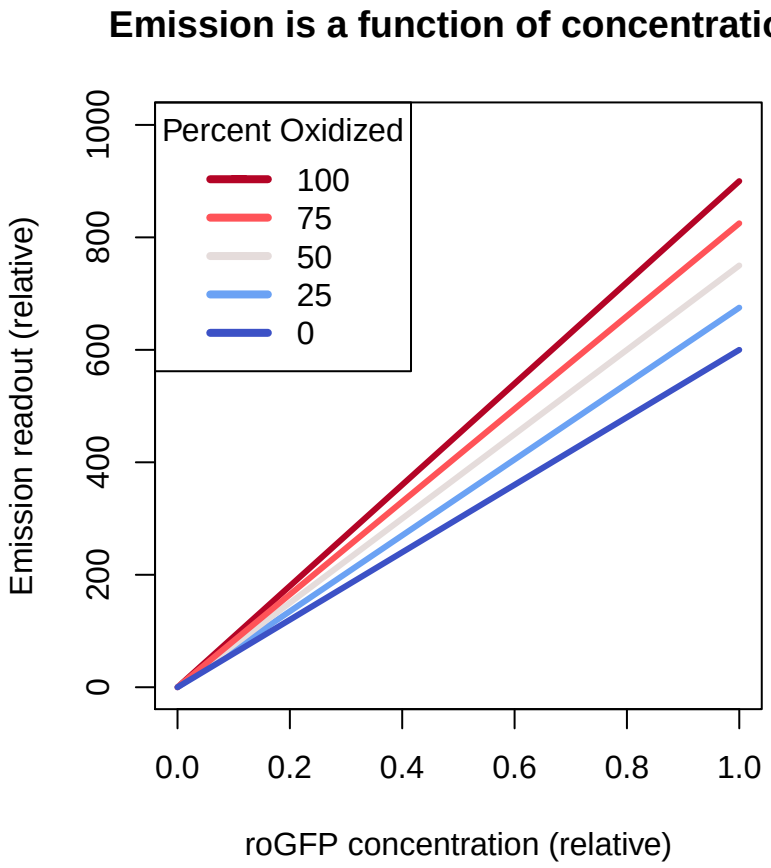
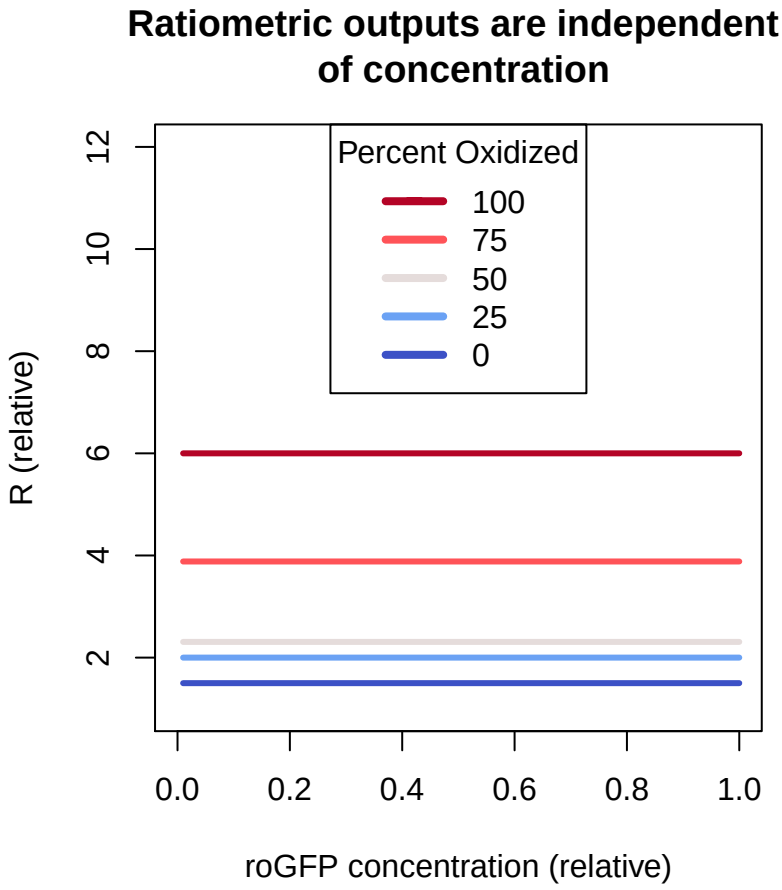
Excitation-emission profiles




Emission spectrum of GFP1−R12
Percent Oxidized
100
75
50
25
0
Emission (relative)
200
400
600
800
350
400
450
500
λ (nm)


Emission is a function of concentration
Percent Oxidized
100
75
50
25
0
Emission readout (relative)
0
200
400
600
800
1000
0.0
0.2
0.4
0.6
0.8
1.0
roGFP concentration (relative)


Ratiometric outputs are independent of concentration
Percent Oxidized
100
75
50
25
0
R (relative)
2
4
6
8
10
12
0.0
0.2
0.4
0.6
0.8
1.0
roGFP concentration (relative)

Delta profiles

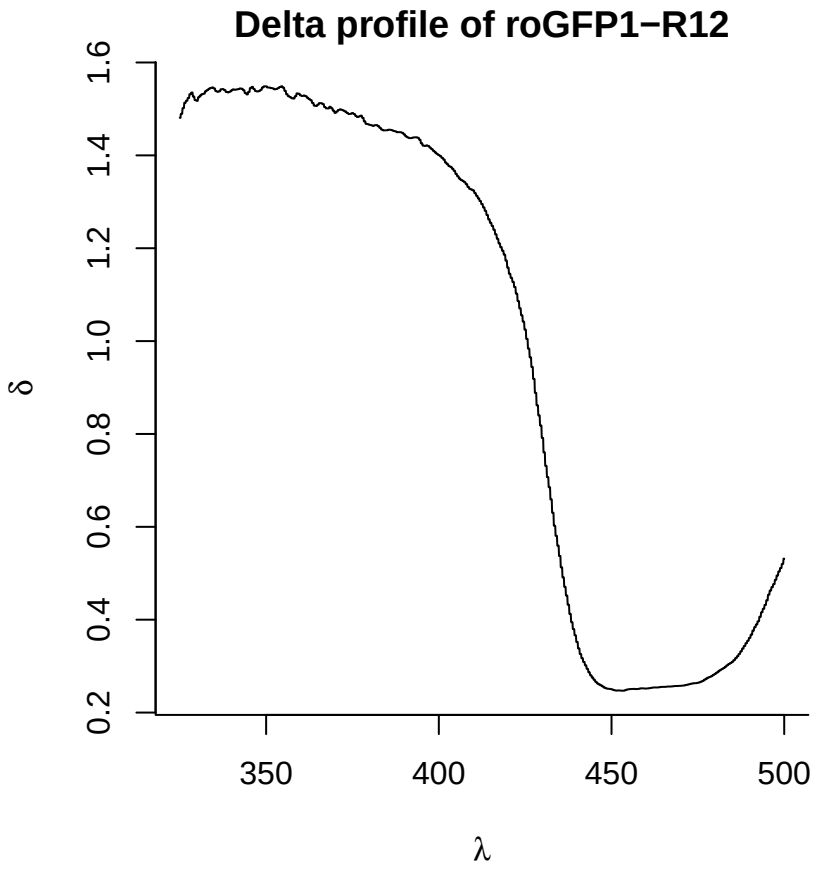


Table 1: Approximate delta-wavelength values for GFP1-R12

Characteristic	GFP1-R12
Delta ~ 1	425.3
Delta minimized	453.3
Delta maximized	354.3

Choose two sets of wavelengths for each sensor.

For GFP1-R12:

- Use $\frac{410+/-5nm}{425+/-5nm}$ for isobestic
- Use $\frac{410+/-5nm}{470+/-5nm}$ for maximum total dynamic range

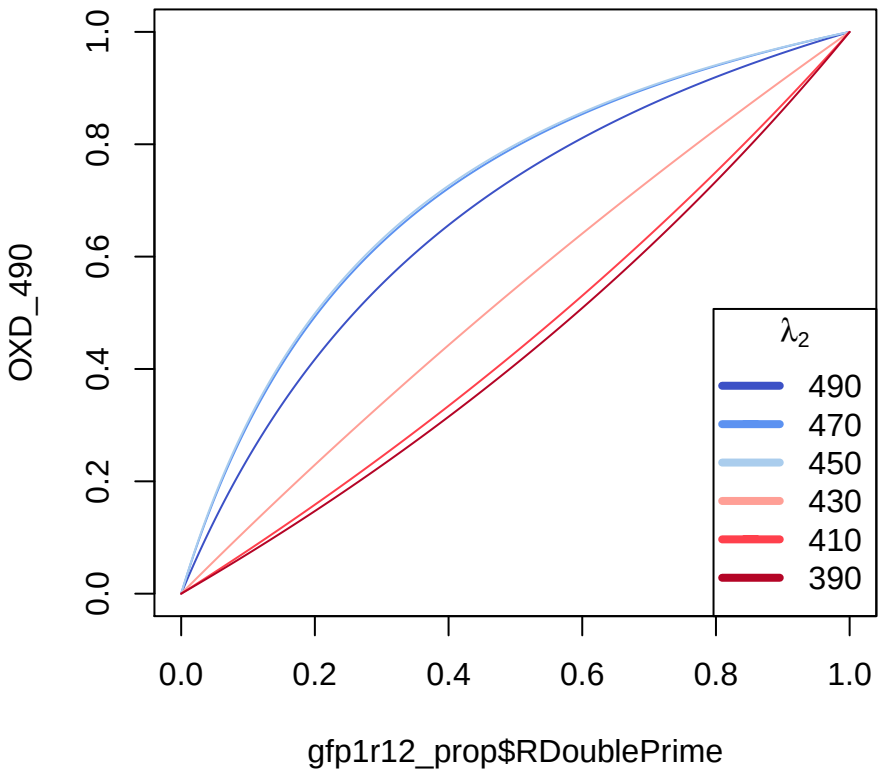
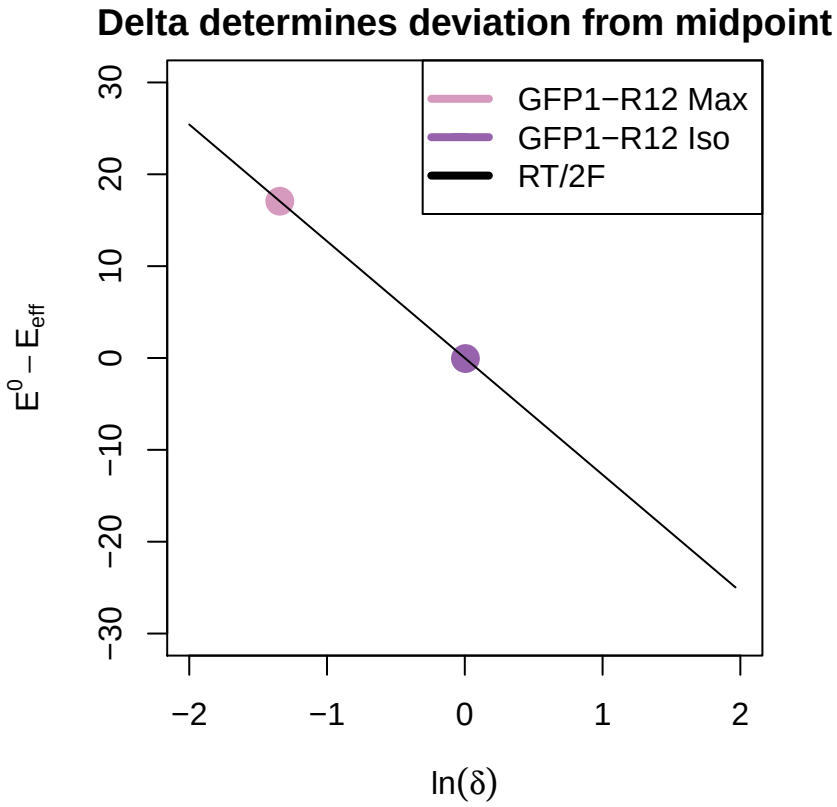
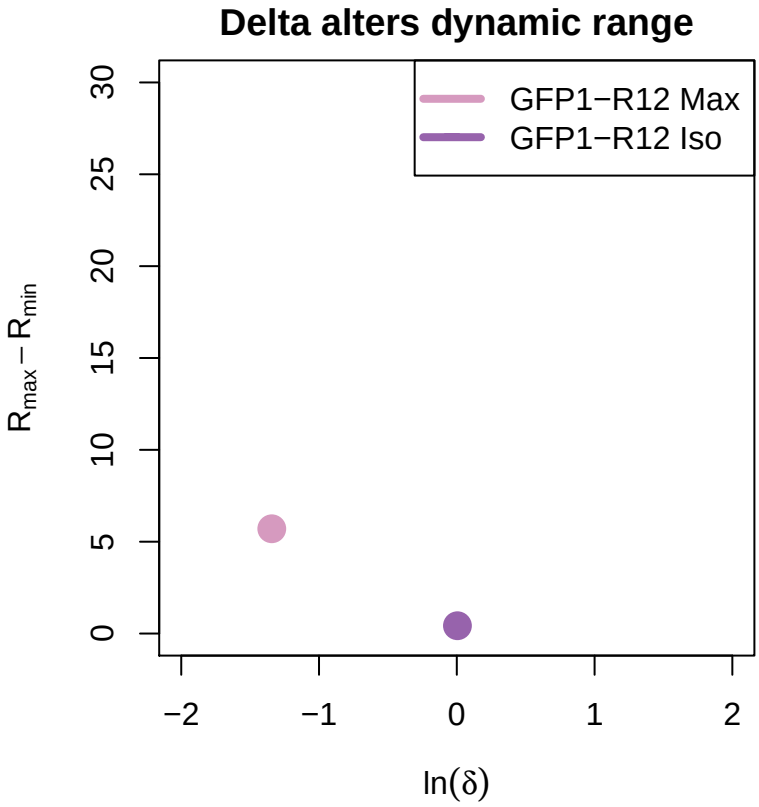


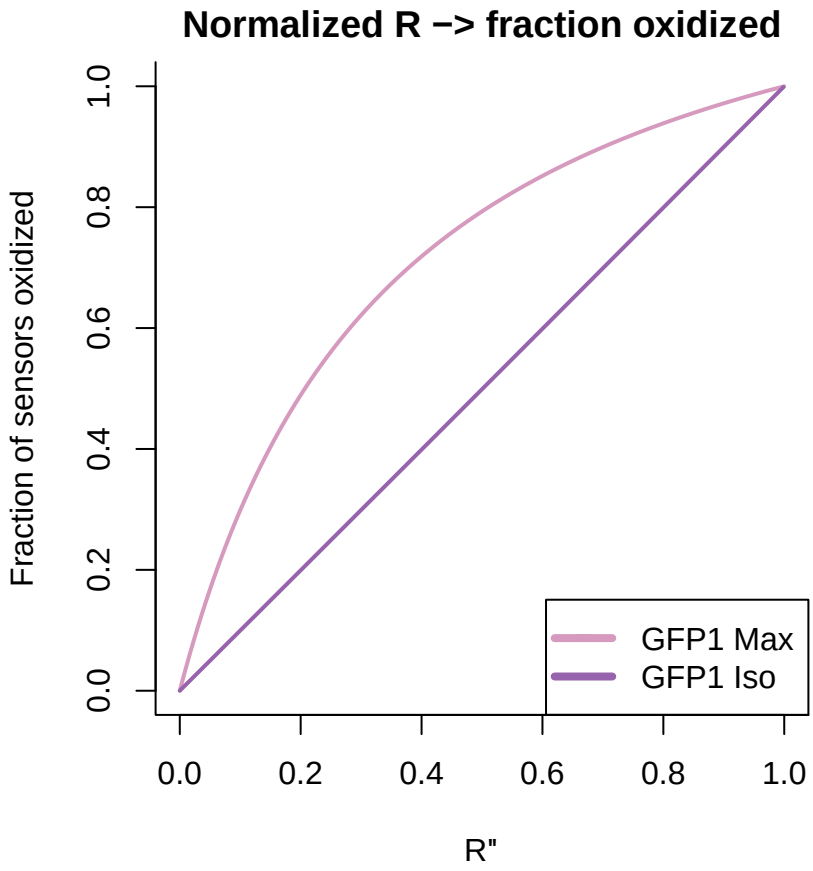
Table 2: Characteristics of GFP1-R12sensors

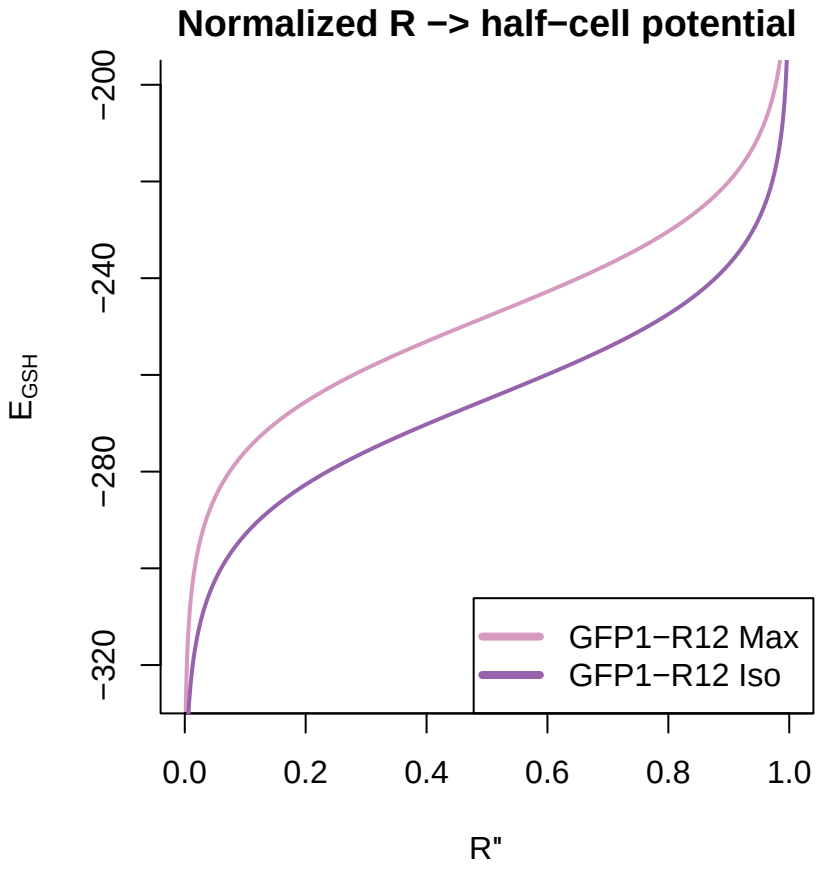
Characteristic	GFP1 Isobestic	GFP1 Max
Delta	1.0	0.3
Rmin	1.4	1.4
Rmax	1.8	7.1
E0	-265.0	-265.0
Adjusted E0	-265.1	-247.9
Rmax-Rmin	0.4	5.7
Rmax/Rmin	1.3	5.0


Delta determines deviation from midpoint
GFP1−R12 Max
GFP1−R12 Iso
RT/2F
$E^0 - E_{eff}$
30
20
10
0
−10
−20
−30
−2
−1
0
1
2
ln(δ)

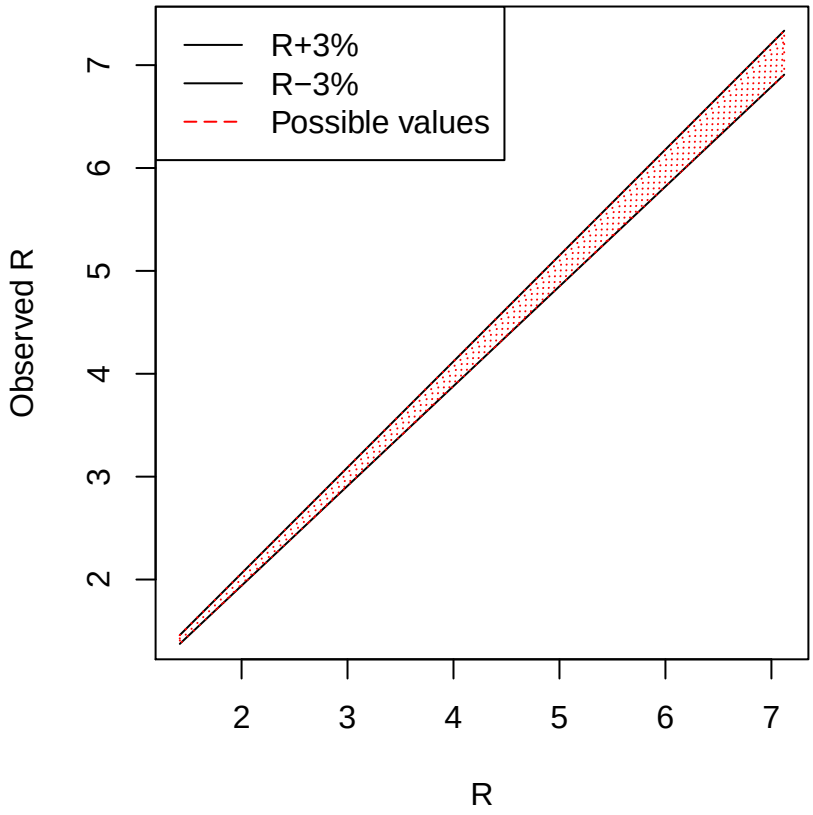

Delta alters dynamic range
GFP1−R12 Max
GFP1−R12 Iso
$R_{max} - R_{min}$
0
5
10
15
20
25
30
−2
−1
0
1
2
ln(δ)

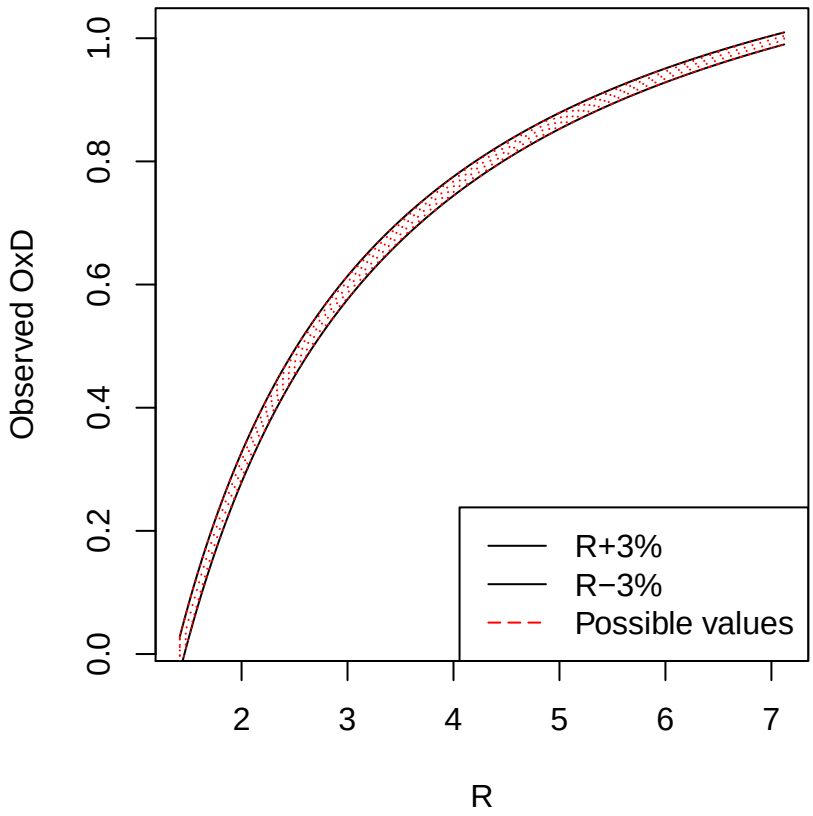
Fraction oxidized and redox potential



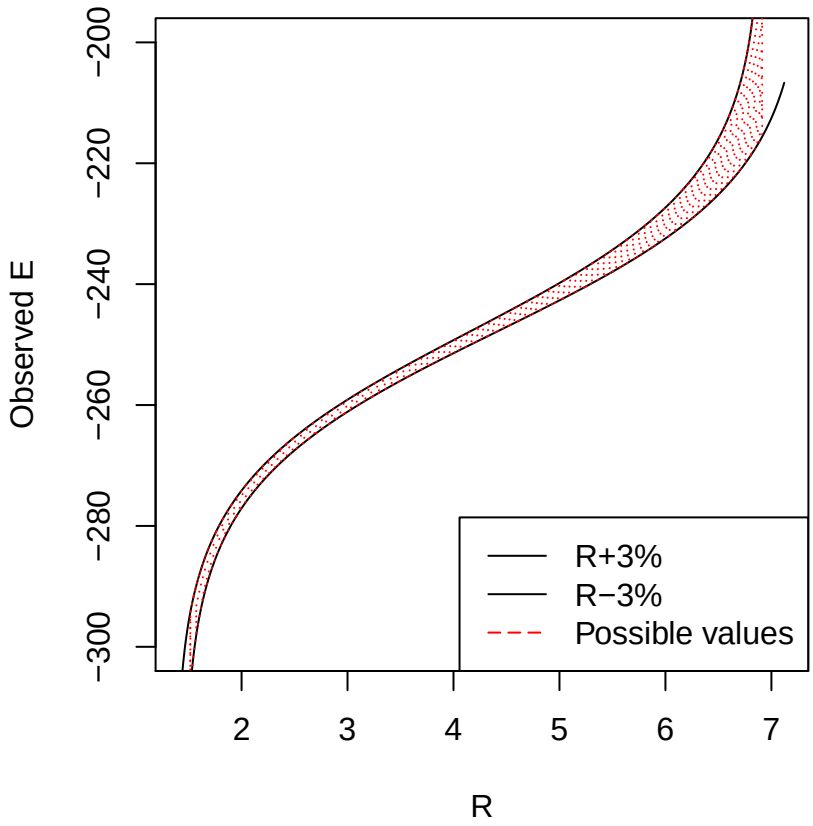

Normalized R -> half−cell potential
E_GSH
−200
−240
−280
−320
0.0
0.2
0.4
0.6
0.8
1.0
R"
GFP1−R12 Max
GFP1−R12 Iso

Error in R

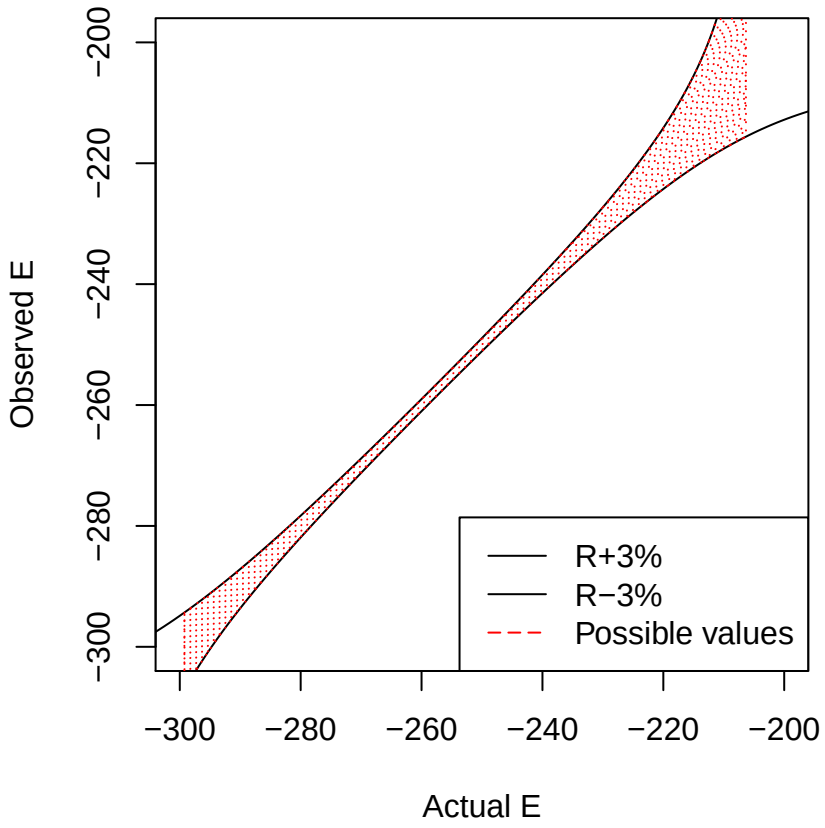




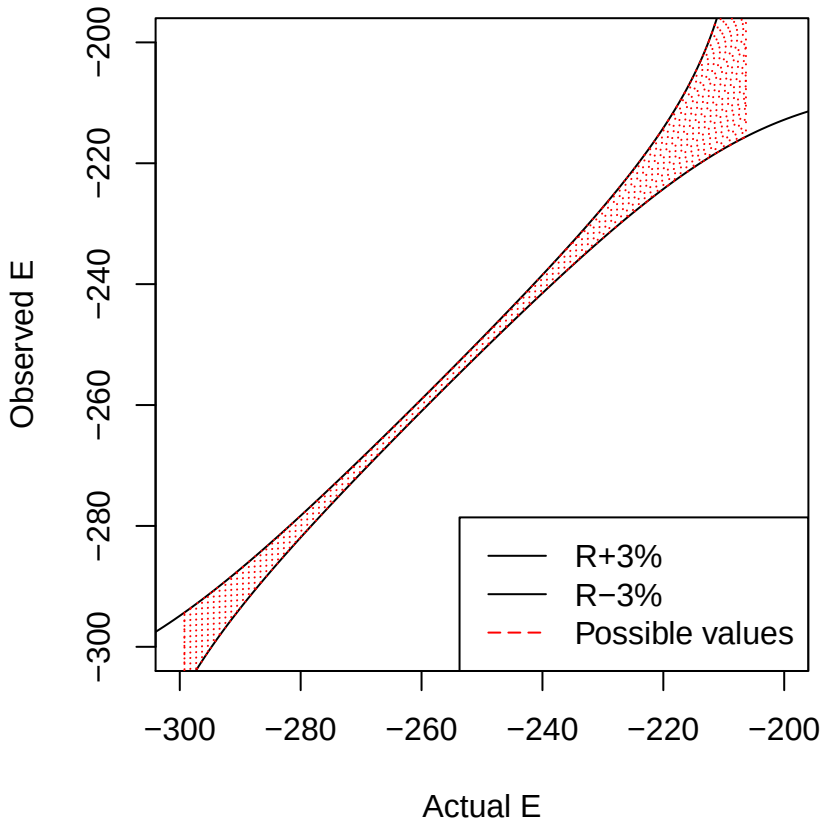

```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced

## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```




```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced

## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```



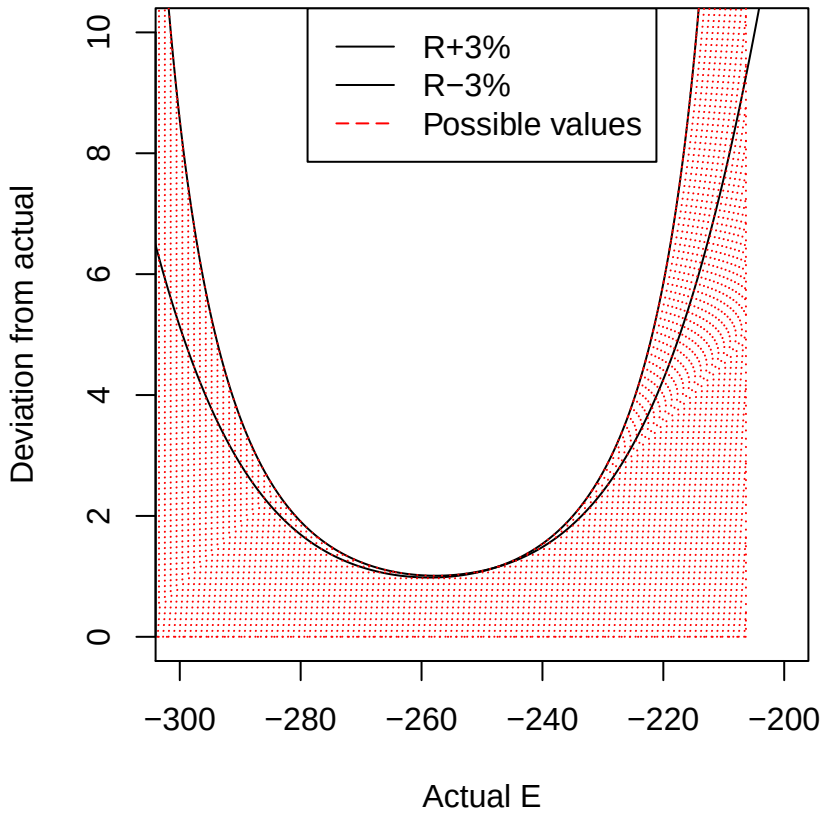

```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced

## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```



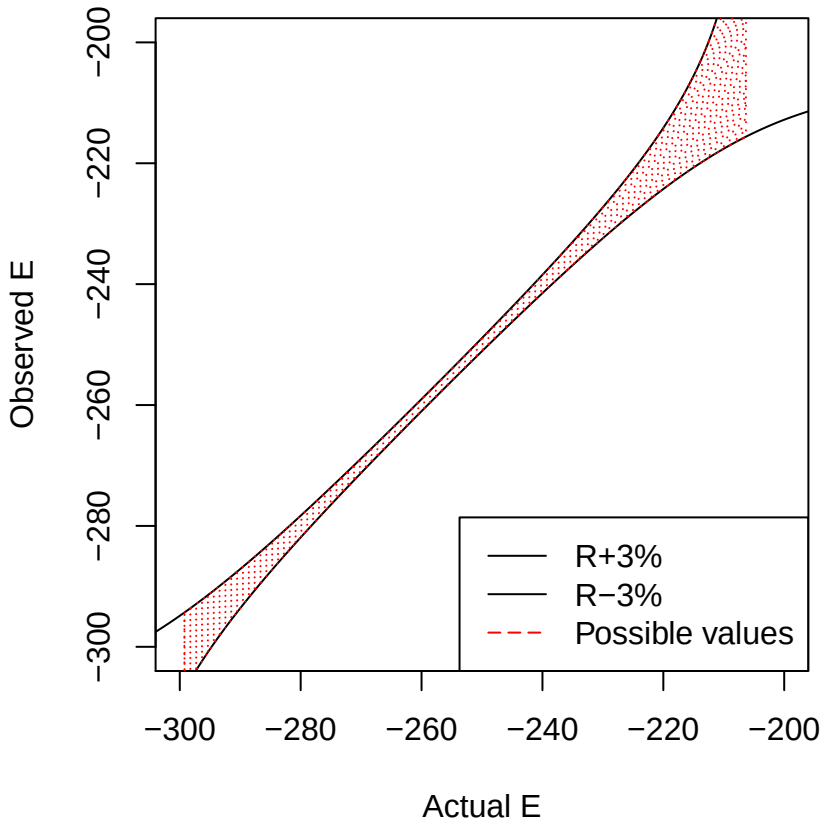
Here

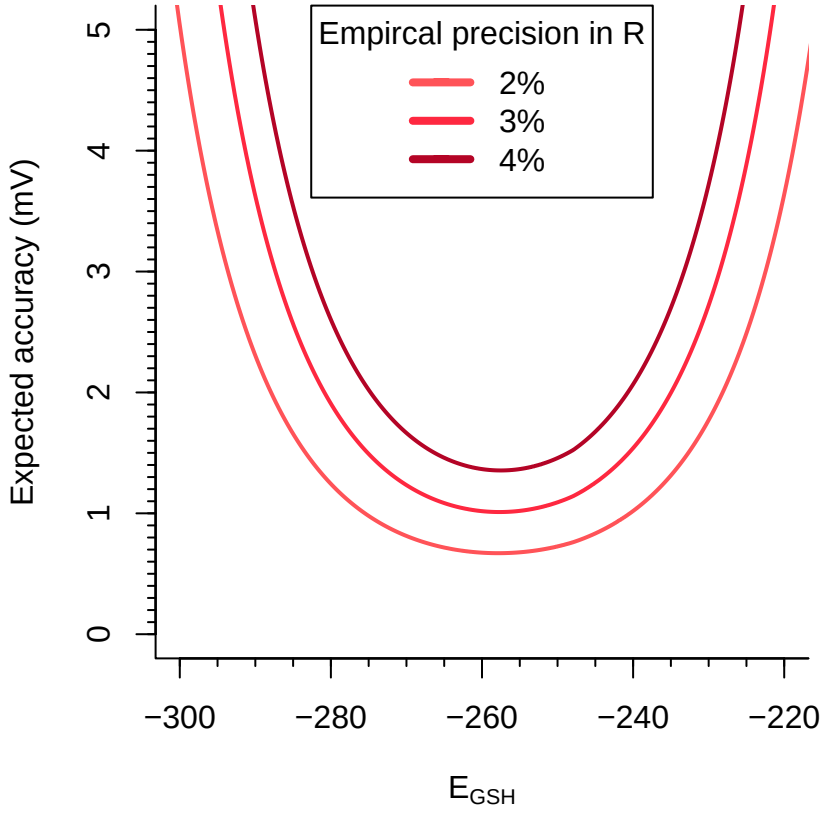
```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```

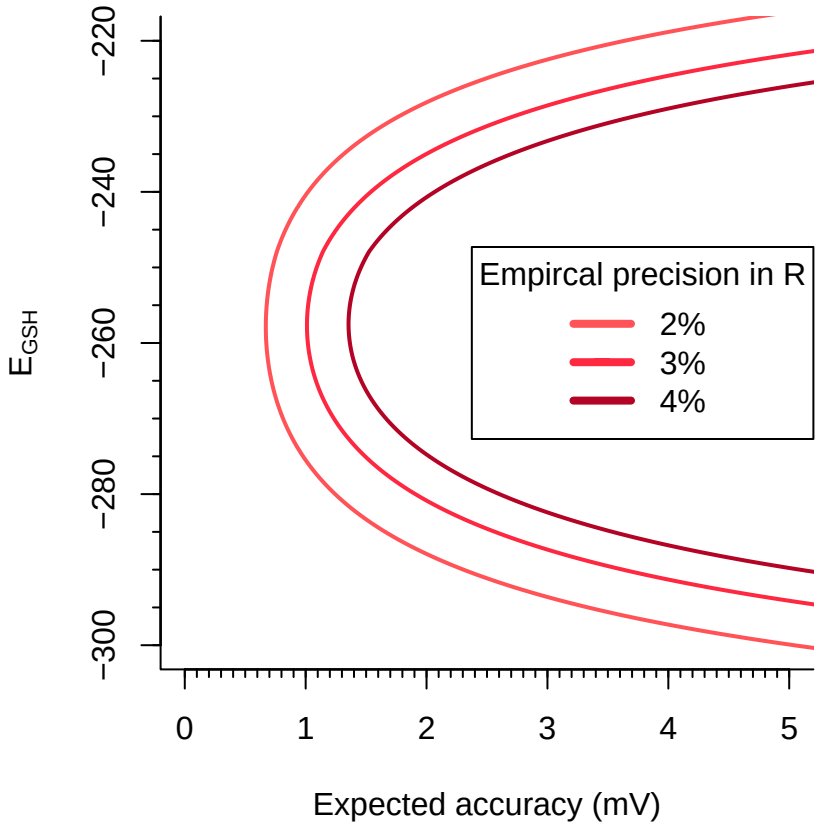
```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```

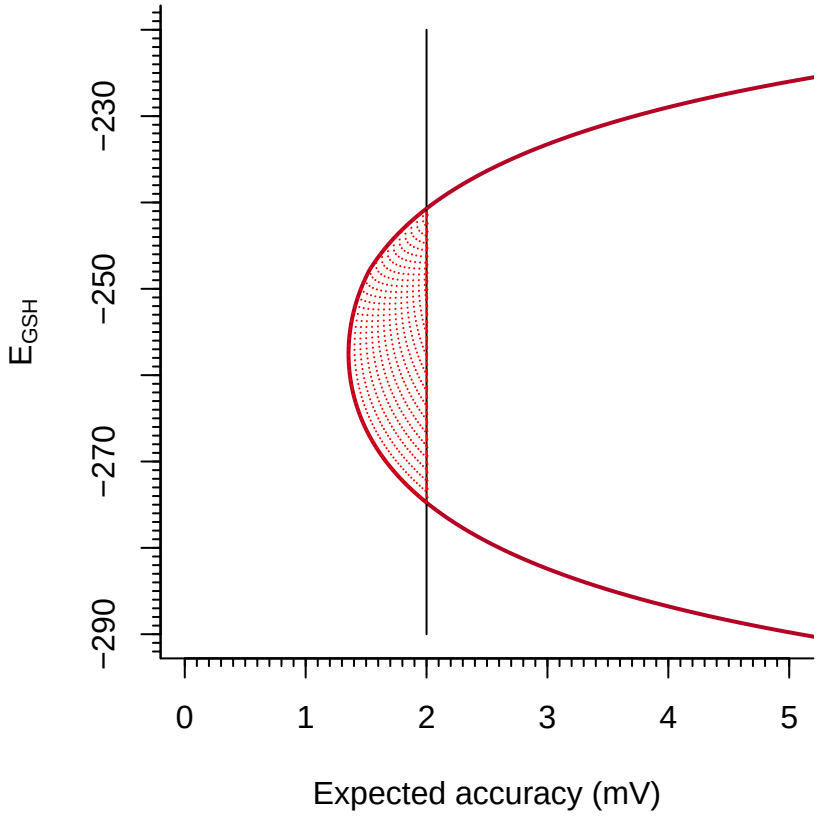


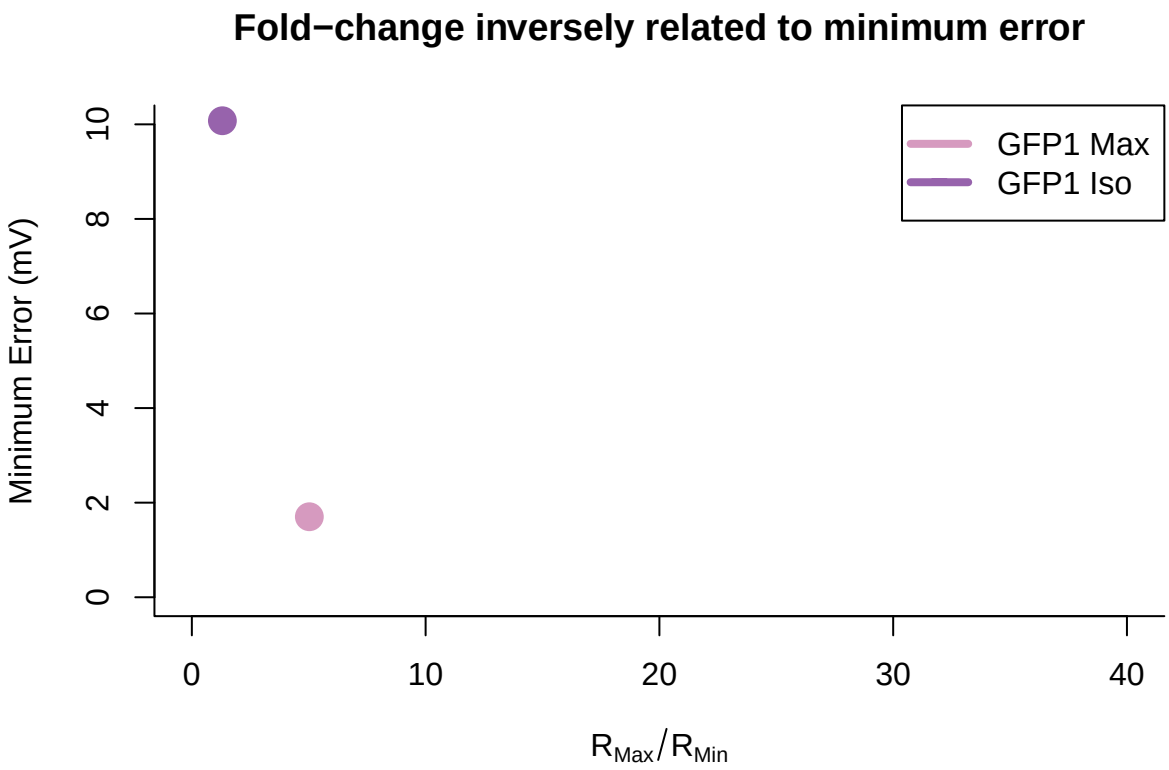

```
## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced

## Warning in log((delta * Rmax - delta * R)/(R - Rmin)): NaNs produced
```

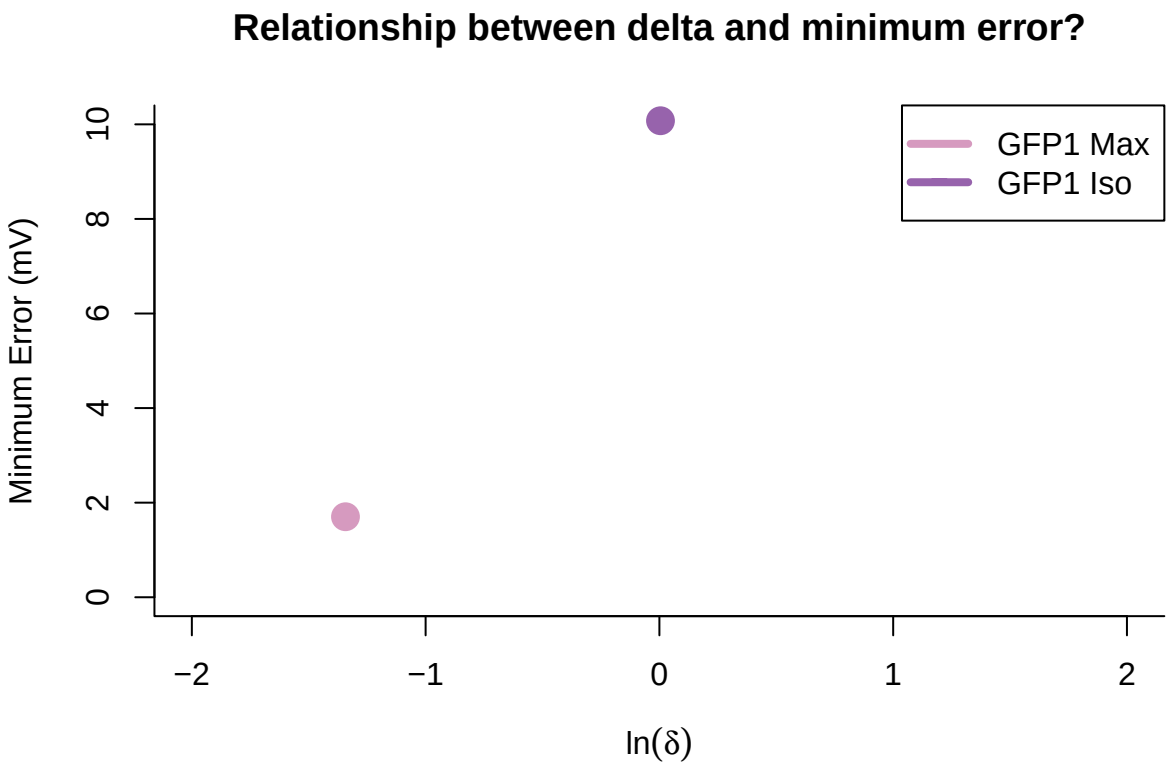

Observed E
−300
−280
−260
−240
−220
−200
Actual E
−300
−280
−260
−240
−220
−200
R+3%
R−3%
Possible values

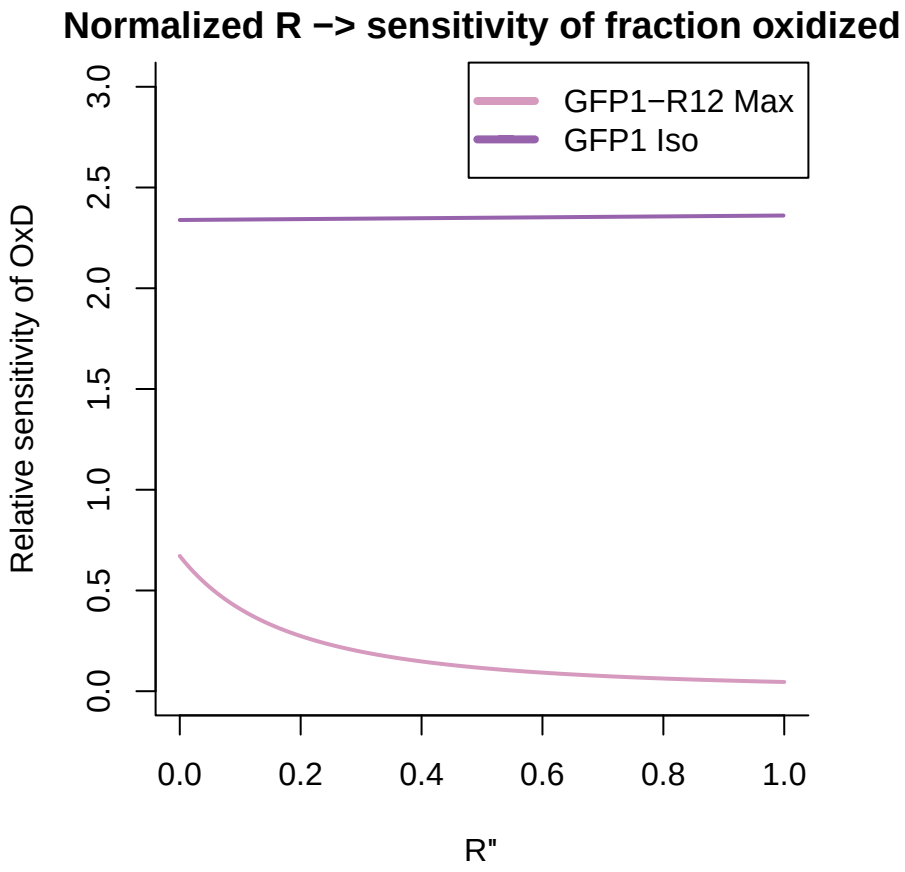

Empircal precision in R
2%
3%
4%
Expected accuracy (mV)
0
1
2
3
4
5
−300
−280
−260
−240
−220
E_GSH

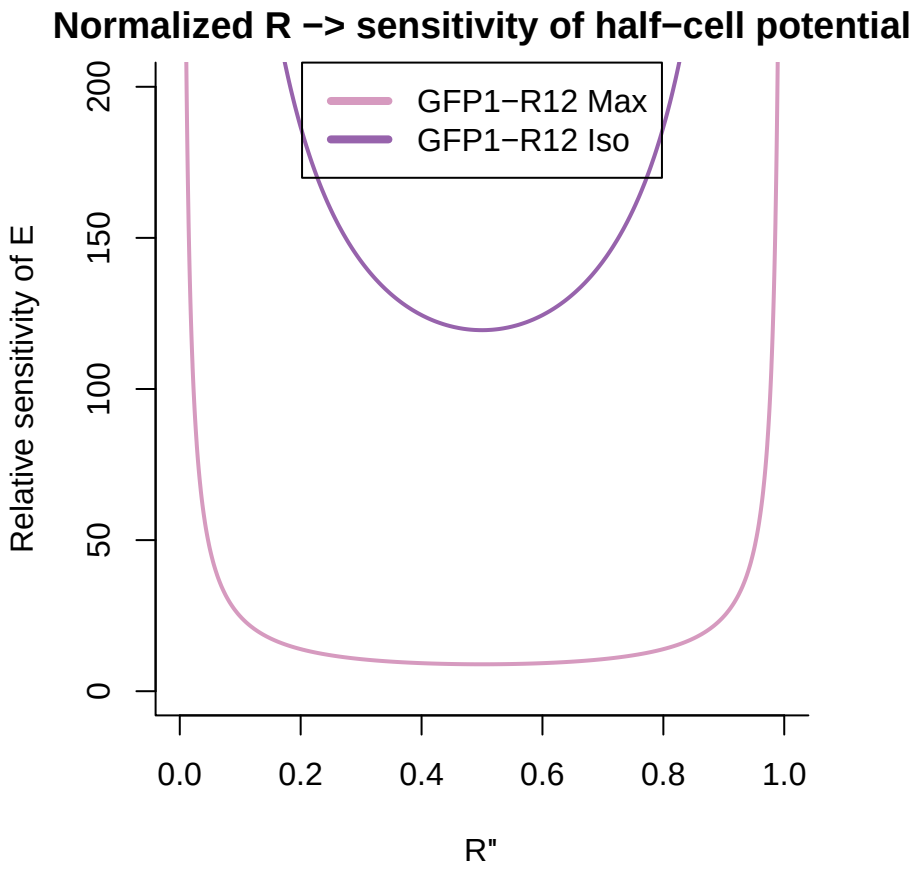

−220
−240
−260
−280
−300
E_{GSH}
0
1
2
3
4
5
Expected accuracy (mV)
Empircal precision in R
2%
3%
4%


E_GSH
−230
−250
−270
−290
0
1
2
3
4
5
Expected accuracy (mV)

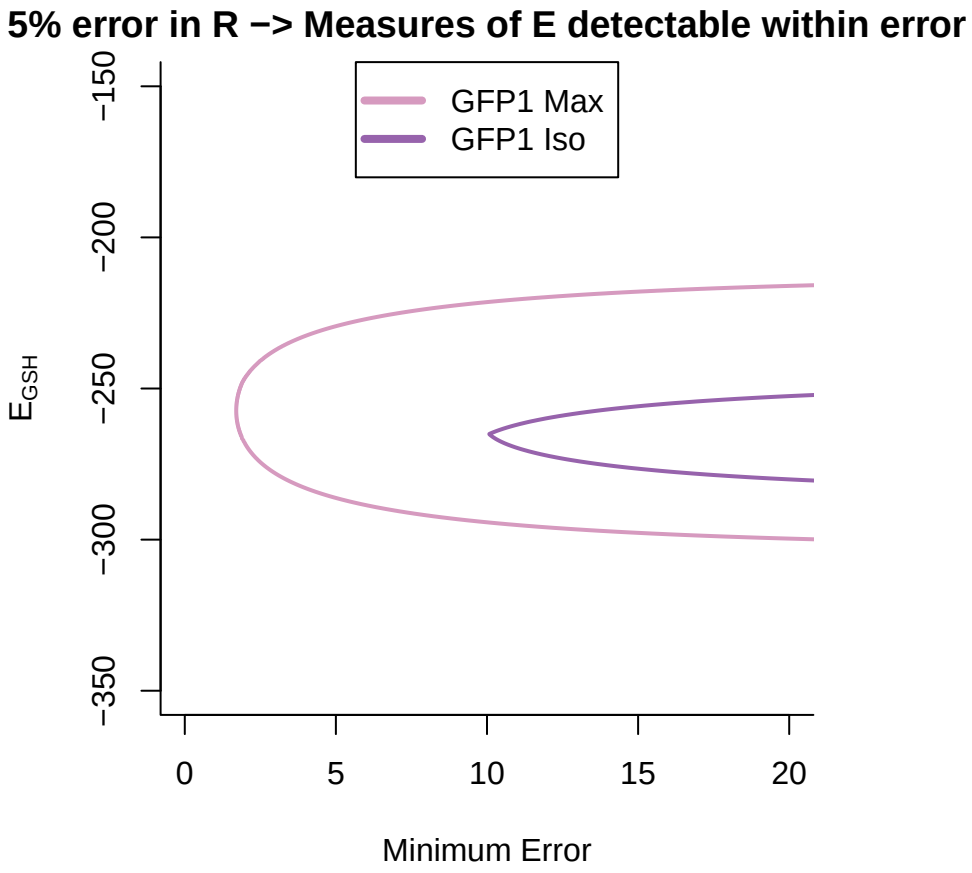

Fold−change inversely related to minimum error
GFP1 Max
GFP1 Iso
Minimum Error (mV)
0
2
4
6
8
10
0
10
20
30
40
R_Max/R_Min

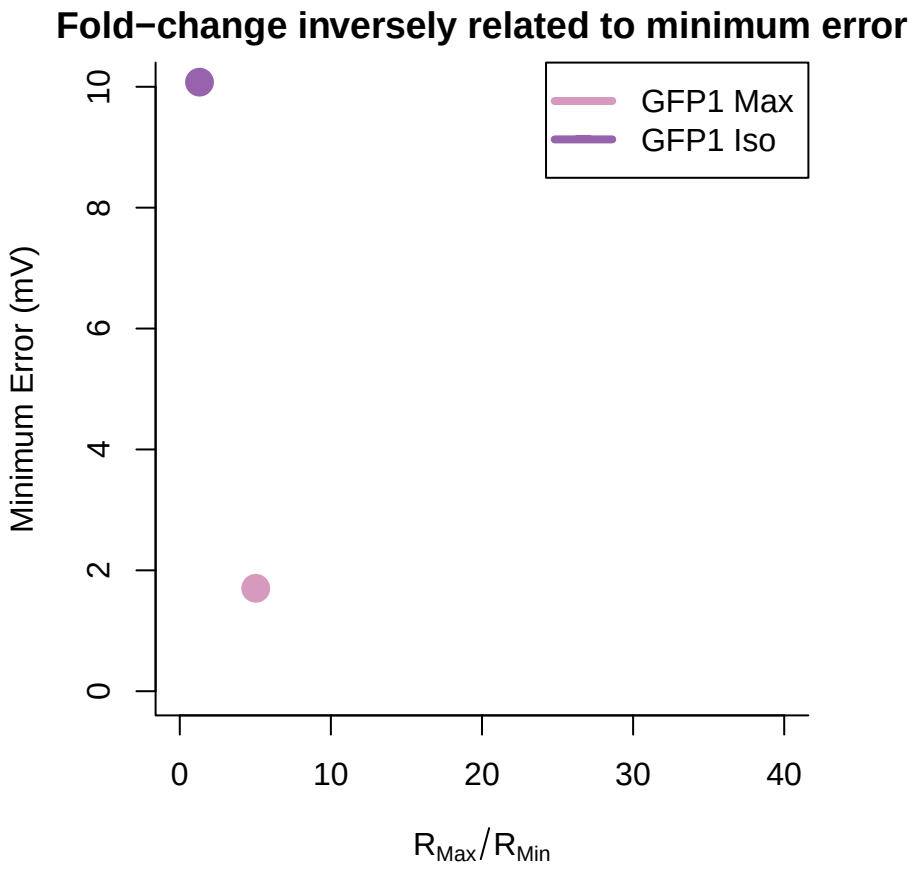

Relationship between delta and minimum error?
Minimum Error (mV)
0
2
4
6
8
10
−2
−1
0
1
2
ln(δ)
GFP1 Max
GFP1 Iso

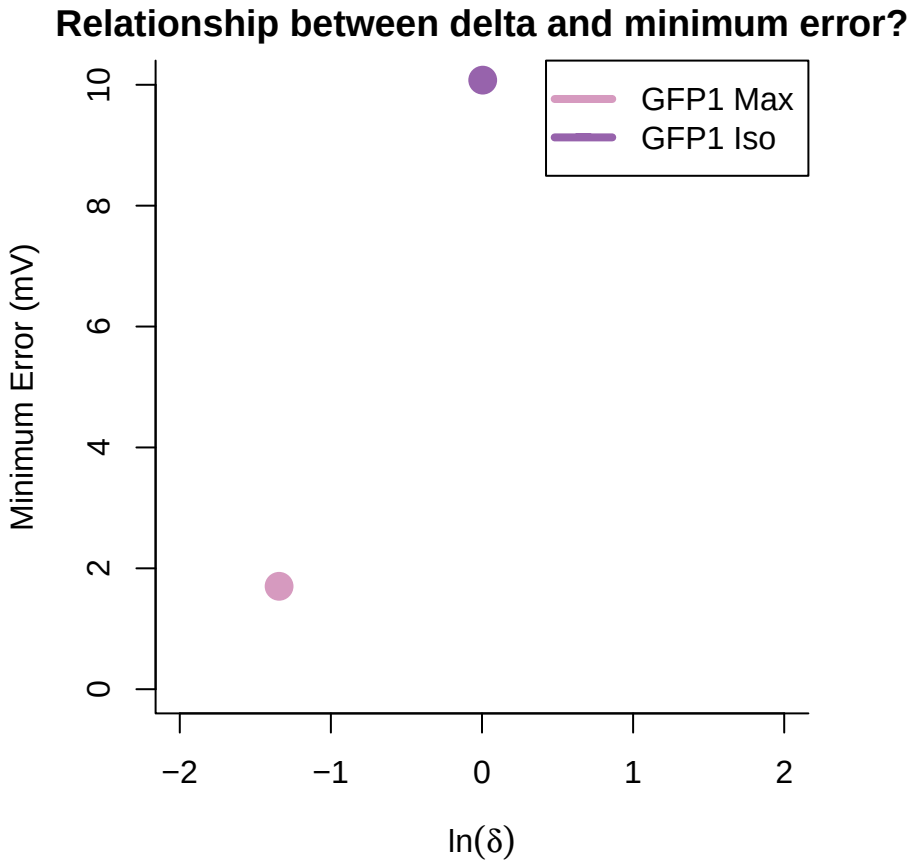

Normalized R −> sensitivity of fraction oxidized
GFP1−R12 Max
GFP1 Iso
Relative sensitivity of OxD
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.0
0.2
0.4
0.6
0.8
1.0
R"


Normalized R −> sensitivity of half−cell potential
GFP1−R12 Max
GFP1−R12 Iso
Relative sensitivity of E
0
50
100
150
200
0.0
0.2
0.4
0.6
0.8
1.0
R"

Error in half-cell potential readout given a 5% error in R




Fold−change inversely related to minimum error
GFP1 Max
GFP1 Iso
Minimum Error (mV)
0
2
4
6
8
10
0
10
20
30
40
R_{Max}/R_{Min}


Relationship between delta and minimum error?
GFP1 Max
GFP1 Iso
Minimum Error (mV)
0
2
4
6
8
10
−2
−1
0
1
2
ln(δ)

```
## pdf
##   2
```