$20200220_TRP_dN_nativeN_acrylamide_D1.R$

kelse

2022-06-01

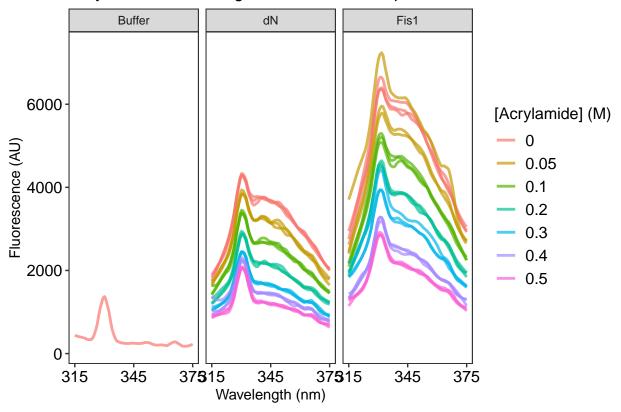
```
# Acrylamide quenching of Trp fluorescence of nativeN and dN-hFis1
# [nativeN] and [dN] = 10 uM
# [acrylamide] = 0-500 mM
# data collected on 20200219
# Buffer: 20 mM Hepes, 175 mM NaCl, 1 mM DTT, 0.02% NaAz
# Trp excitation: 295 nm
# Trp emission collected over: 300-400 nm
# slit widths set at 2.5 per Dash lab protocol
library(ggpmisc)
## Warning: package 'ggpmisc' was built under R version 4.0.5
## Loading required package: ggplot2
## Attaching package: 'ggpmisc'
## The following object is masked from 'package:ggplot2':
##
##
      annotate
library(tidyverse)
## -- Attaching packages ----- tidyverse 1.3.0 --
## v tibble 3.1.0
                  v dplyr 1.0.5
## v tidyr 1.1.3 v stringr 1.4.0
## v readr 1.4.0
                   v forcats 0.5.1
## v purrr 0.3.4
## Warning: package 'stringr' was built under R version 4.0.5
## -- Conflicts ----- tidyverse conflicts() --
## x ggpmisc::annotate() masks ggplot2::annotate()
## x dplyr::filter() masks stats::filter()
## x dplyr::lag() masks stats::lag()
```

```
library(broom)
library(readxl)
library(minpack.lm)
theme_set(theme_bw() +
            theme(axis.text = element_text(size = 12, color = "black"),
                  panel.grid.major = element_blank(),
                  panel.grid.minor = element_blank())
)
# Import data, tidy, and correct for buffer emission background signal
raw <- read_excel("20200219_TRP_dN_nativeN_acrylamide_d1.xlsx") %>%
  gather(., key = "tmp1", value = "fluor", 2:44 ) %>%
  separate(., "tmp1", into = c("prot", "conc", "tr"), sep = "_")
# Visualize data before buffer correction
raw %>%
  ggplot(., aes(x = Wavelength, y = fluor, color = conc, shape = tr)) +
  geom_line(stat = "smooth", method = "loess", span = 0.2,
            alpha = 0.7, size = 1) +
 facet_wrap(~ prot) +
  scale_x_continuous(limits = c(315, 375),
                     breaks = c(315, 345, 375)) +
 labs(color = "[Acrylamide] (M)",
       title = "Acrylamide Quenching of TRP Emission Spectra",
       x = \text{"Wavelength (nm)"},
       y = "Fluorescence (AU)") +
  guides(shape = guide_legend(override.aes = list(size = 2))) +
  theme_bw() +
  theme(axis.text = element_text(size = 12, color = "black"),
       panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.title = element_text(size = 12),
        legend.text = element_text(size = 12, color = "black")
 )
```

```
## 'geom_smooth()' using formula 'y ~ x'
```

Warning: Removed 1720 rows containing non-finite values (stat_smooth).

Acrylamide Quenching of TRP Emission Spectra

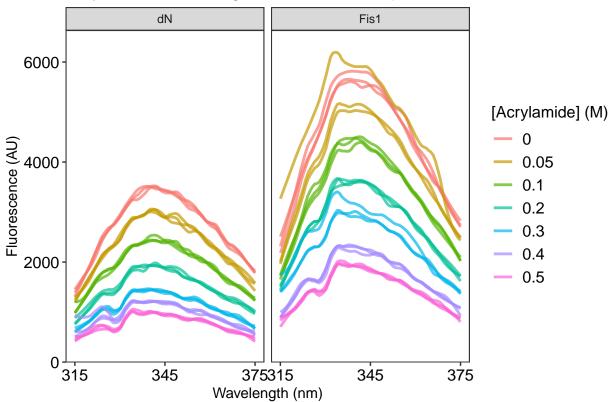


```
# Subtract buffer signal
corrected <- raw %>%
  filter(., Wavelength >= 315 & Wavelength <= 375) %>%
  mutate(., corrected_fluor = fluor - fluor[which(conc ==0 & prot == "Buffer")]) %>%
 filter(., prot != "Buffer")
# Visualize data
corrected %>%
  ggplot(., aes(x = Wavelength, y = corrected_fluor, color = conc, shape = tr)) +
         geom_line(stat = "smooth", method = "loess", span = 0.2,
                   alpha = 0.7, size = 1) +
  facet_wrap(~ prot) +
  scale_x_continuous(limits = c(315, 375),
                     breaks = c(315, 345, 375)) +
  labs(color = "[Acrylamide] (M)",
       title = "Acrylamide Quenching of TRP Emission Spectra",
       x = \text{"Wavelength (nm)"},
       y = "Fluorescence (AU)") +
  guides(shape = guide_legend(override.aes = list(size = 2))) +
  theme_bw() +
  theme(axis.text = element_text(size = 12, color = "black"),
        panel.grid.major = element_blank(),
       panel.grid.minor = element blank(),
        legend.title = element_text(size = 12),
```

```
legend.text = element_text(size = 12, color = "black")
)
```

'geom_smooth()' using formula 'y ~ x'

Acrylamide Quenching of TRP Emission Spectra



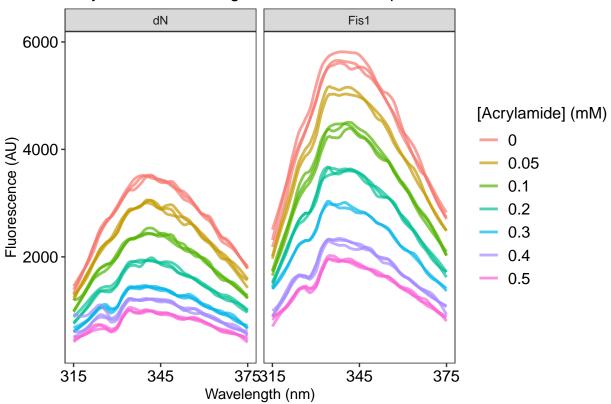
```
ggsave("nativeN_dN_hFis1_TRP_em_spectra_all.pdf",
    width = 20, height = 12, dpi = 300, units = "cm")
```

'geom_smooth()' using formula 'y ~ x'

```
labs(color = "[Acrylamide] (mM)",
    title = "Acrylamide Quenching of TRP Emission Spectra",
    x = "Wavelength (nm)",
    y = "Fluorescence (AU)") +
guides(shape = guide_legend(override.aes = list(size = 2))) +
theme_bw() +
theme(axis.text = element_text(size = 12, color = "black"),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    legend.title = element_text(size = 12),
    legend.text = element_text(size = 12, color = "black"),
)
```

'geom_smooth()' using formula 'y ~ x'

Acrylamide Quenching of TRP Emission Spectra



```
# Average corrected fluorescence intensities of corrected data

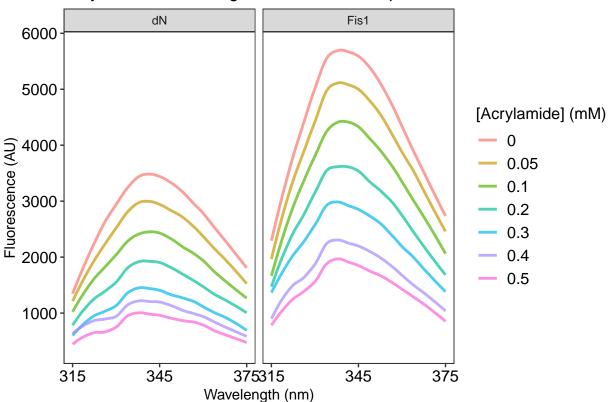
corrected_no_outlier_avg <- corrected_no_outlier %>%
  group_by(., prot, conc, Wavelength) %>%
  summarise(., avg_fluor = mean(corrected_fluor))
```

'summarise()' has grouped output by 'prot', 'conc'. You can override using the '.groups' argument.

```
# Visualize average fluorescence intensities of corrected data
corrected_no_outlier_avg %>%
  ggplot(., aes(x = Wavelength, y = avg_fluor, color = conc)) +
  geom_line(stat = "smooth", method = "loess", span = 0.35,
            alpha = 0.7, size = 1) +
  facet_wrap(~ prot) +
  scale_x_continuous(limits = c(315, 375),
                     breaks = c(315, 345, 375)) +
  labs(color = "[Acrylamide] (mM)",
       title = "Acrylamide Quenching of TRP Emission Spectra",
       x = \text{"Wavelength (nm)"},
       y = "Fluorescence (AU)") +
  guides(shape = guide_legend(override.aes = list(size = 2))) +
  theme_bw() +
  theme(axis.text = element_text(size = 12, color = "black"),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        legend.title = element_text(size = 12),
        legend.text = element_text(size = 12, color = "black")
```

'geom_smooth()' using formula 'y ~ x'

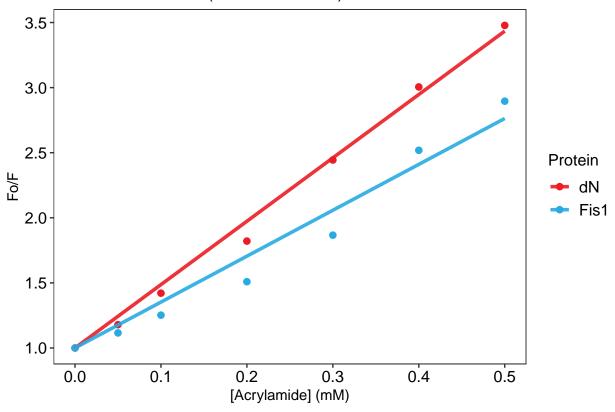
Acrylamide Quenching of TRP Emission Spectra



```
# Caclulate Fo/F for Stern-Volmer equation fitting
results <- corrected_no_outlier_avg %>%
  group_by(., prot, Wavelength) %>%
 mutate(., FoF = avg_fluor[which(conc == 0)] / avg_fluor)
write_csv(results, "nativeN_dN_hFis1_FoF_values.csv")
#Calculate average Fo/F for each protein/[acrylamide]
results_avg <- results %>%
  group_by(., prot, conc, Wavelength) %>%
  summarise(., avg_FoF = mean(FoF))
## 'summarise()' has grouped output by 'prot', 'conc'. You can override using the '.groups' argument.
# Determine lamda max at each concentration of NaI ----
max_wavelength <- results %>%
 group_by(., prot, conc) %>%
 summarise(., lamda_max = Wavelength[which.max(avg_fluor)])
## 'summarise()' has grouped output by 'prot'. You can override using the '.groups' argument.
write_csv(max_wavelength, "nativeN_dN_hFis1_iodide_quenching_lamda_max.csv")
avg_lamda_max <- max_wavelength %>%
  group_by(., prot) %>%
  summarise(., median_lamda_MAX = median(lamda_max),
            avg_lamda_MAX = mean(lamda_max),
            stdev = sd(lamda_max))
write_csv(avg_lamda_max, "nativeN_dN_hFis1_iodide_quenching_avg_lamda_max.csv")
# Visualize Stern-Volmer plots for nativeN and dN -----
results avg %>%
  filter(., Wavelength == 341) %>%
  ggplot(., aes(x = as.numeric(conc), y = avg_FoF, color = prot)) +
  geom_point(size = 2) +
  geom line(stat = "smooth", method = "nlsLM",
            formula = y \sim (1 + (Ksv * x)),
            method.args = list(start = c(Ksv = 0.5),
                               control = nls.control(maxiter = 100, tol = 1e-6)),
            se = FALSE,
            fullrange = TRUE,
            size = 1.25,
            alpha = 0.9) +
   scale_colour_manual(name = "Protein",
                      labels = c("dN", "Fis1"),
                      values = c("#EC1C24", "#29ABE2", "#EC1C24", "#29ABE2")) +
  scale_shape_manual(name = "Protein", labels = c("dN", "Fis1"),
                     values = c(17, 19)) +
   labs(color = "Protein",
```

```
title = "Stern-Volmer Plots (lamda = 341 nm)",
    x = "[Acrylamide] (mM)",
    y = "Fo/F") +
guides(shape = guide_legend(override.aes = list(size = 2))) +
theme_bw() +
theme(axis.text = element_text(size = 12, color = "black"),
    panel.grid.major = element_blank(),
    panel.grid.minor = element_blank(),
    legend.title = element_text(size = 12),
    legend.text = element_text(size = 12, color = "black")
)
```

Stern-Volmer Plots (lamda = 341 nm)



```
## It.
          0, RSS =
                       4.67509, Par. =
                                                0.5
## It.
          1, RSS =
                      0.269774, Par. =
                                            3.32373
          2, RSS =
                                            3.32373
## It.
                      0.269774, Par. =
                        4.9709, Par. =
          0, RSS =
                                                0.5
## It.
## It.
          1, RSS =
                      0.210258, Par. =
                                            3.43539
          2, RSS =
                      0.210258, Par. =
## It.
                                            3.43539
                       6.83763, Par. =
## It.
          0.RSS =
                                                0.5
          1, RSS =
                      0.141562, Par. =
## It.
                                            3.98132
## It.
          2, RSS =
                      0.141562, Par. =
                                            3.98132
## It.
          0, RSS =
                       5.57505, Par. =
                                                0.5
## It.
          1, RSS =
                      0.176175, Par. =
                                            3.62598
                      0.176175, Par. =
                                            3.62598
## It.
          2, RSS =
          0, RSS =
## It.
                       8.02953, Par. =
                                                0.5
                                            4.26287
## It.
          1, RSS =
                      0.206561, Par. =
                      0.206561, Par. =
          2, RSS =
## It.
                                            4.26287
## It.
          0, RSS =
                       5.01129, Par. =
                                                0.5
                                            3.45057
## It.
          1, RSS =
                        0.2013, Par. =
## It.
          2, RSS =
                        0.2013, Par. =
                                            3.45057
          0, RSS =
                       5.29711, Par. =
## It.
                                                0.5
## It.
          1, RSS =
                     0.0477185, Par. =
                                             3.5824
          2, RSS =
## It.
                     0.0477185, Par. =
                                             3.5824
          0, RSS =
                       4.68939, Par. =
## It.
                                                0.5
          1, RSS =
                     0.0896825, Par. =
## It.
                                            3.38535
                     0.0896825, Par. =
## It.
          2, RSS =
                                            3.38535
## It.
          0, RSS =
                       8.79808, Par. =
                                                0.5
## It.
          1, RSS =
                      0.216719, Par. =
                                            4.44105
          2, RSS =
                      0.216719, Par. =
                                            4.44105
## It.
## It.
          0, RSS =
                       6.14589, Par. =
                                                0.5
                      0.206431, Par. =
## It.
          1, RSS =
                                            3.77874
## It.
          2, RSS =
                      0.206431, Par. =
                                            3.77874
## It.
          0, RSS =
                       6.85983, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0307072, Par. =
                                            4.01574
## It.
          2, RSS =
                     0.0307072, Par. =
                                            4.01574
          0, RSS =
                       5.12494, Par. =
## It.
                                                0.5
## It.
          1, RSS =
                      0.127767, Par. =
                                            3.50743
## It.
                                           3.50743
          2, RSS =
                      0.127767, Par. =
## It.
          0, RSS =
                       7.76922, Par. =
                                                0.5
          1, RSS =
                     0.0774062, Par. =
                                             4.2312
## It.
## It.
          2, RSS =
                     0.0774062, Par. =
                                             4.2312
          0, RSS =
                       6.00151, Par. =
## It.
                                                0.5
          1, RSS =
## It.
                      0.188411, Par. =
                                            3.74368
          2, RSS =
                      0.188411, Par. =
                                            3.74368
## It.
## It.
          0, RSS =
                       8.54082, Par. =
                                                0.5
## It.
          1, RSS =
                      0.209881, Par. =
                                            4.38312
## It.
          2, RSS =
                      0.209881, Par. =
                                            4.38312
          0, RSS =
                       6.40369, Par. =
## It.
                                                0.5
## It.
          1, RSS =
                      0.250725, Par. =
                                            3.83715
          2, RSS =
## It.
                      0.250725, Par. =
                                            3.83715
## It.
          0, RSS =
                       6.06794, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0489372, Par. =
                                            3.80062
## It.
                     0.0489372, Par. =
                                            3.80062
          2, RSS =
## It.
          0, RSS =
                       5.48944, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0890861, Par. =
                                             3.6264
## It.
          2, RSS =
                     0.0890861, Par. =
                                             3.6264
```

```
## It.
          0, RSS =
                       8.75723, Par. =
                                                0.5
## It.
          1, RSS =
                      0.118556, Par. =
                                            4.45419
          2, RSS =
## It.
                      0.118556, Par. =
                                            4.45419
                       4.99107, Par. =
          0, RSS =
                                                0.5
## It.
## It.
          1, RSS =
                     0.0835525, Par. =
                                            3.48033
                     0.0835525, Par. =
## It.
          2, RSS =
                                            3.48033
                       10.8684, Par. =
## It.
          0.RSS =
                                                0.5
          1, RSS =
                      0.395152, Par. =
## It.
                                            4.85386
## It.
          2, RSS =
                      0.395152, Par. =
                                            4.85386
## It.
          0, RSS =
                       5.26247, Par. =
                                                0.5
## It.
          1, RSS =
                      0.146717, Par. =
                                            3.54291
                      0.146717, Par. =
                                            3.54291
## It.
          2, RSS =
          0, RSS =
## It.
                       13.0767, Par. =
                                                0.5
                      0.196493, Par. =
## It.
          1, RSS =
                                            5.32831
                      0.196493, Par. =
          2, RSS =
## It.
                                            5.32831
## It.
          0, RSS =
                       6.54644, Par. =
                                                0.5
          1, RSS =
## It.
                      0.164184, Par. =
                                            3.89876
## It.
          2, RSS =
                      0.164184, Par. =
                                            3.89876
          0, RSS =
## It.
                       13.9115, Par. =
                                                0.5
## It.
          1, RSS =
                      0.078568, Par. =
                                            5.50369
## It.
          2, RSS =
                      0.078568, Par. =
                                            5.50369
          0, RSS =
                       6.64263, Par. =
## It.
                                                0.5
          1, RSS =
                      0.180264, Par. =
## It.
                                            3.92003
          2, RSS =
                      0.180264, Par. =
## It.
                                            3.92003
## It.
          0, RSS =
                       14.5382, Par. =
                                                0.5
## It.
          1, RSS =
                      0.164337, Par. =
                                            5.60058
                      0.164337, Par. =
          2, RSS =
                                            5.60058
## It.
## It.
          0, RSS =
                       8.20498, Par. =
                                                0.5
                      0.184155, Par. =
## It.
          1, RSS =
                                            4.31016
                                            4.31016
## It.
          2, RSS =
                      0.184155, Par. =
## It.
          0, RSS =
                       25.3664, Par. =
                                                0.5
## It.
          1, RSS =
                      0.315431, Par. =
                                            7.23358
## It.
          2, RSS =
                      0.315431, Par. =
                                            7.23358
          0, RSS =
                       9.73464, Par. =
                                                0.5
## It.
## It.
          1, RSS =
                      0.219802, Par. =
                                            4.64987
          2, RSS =
                      0.219802, Par. =
## It.
                                            4.64987
## It.
          0, RSS =
                        18.018, Par. =
                                                0.5
          1, RSS =
                      0.424764, Par. =
                                            6.14296
## It.
## It.
          2, RSS =
                      0.424764, Par. =
                                            6.14296
          0, RSS =
                         8.6644, Par. =
## It.
                                                0.5
          1, RSS =
                      0.322446, Par. =
## It.
                                            4.38569
          2, RSS =
                      0.322446, Par. =
                                            4.38569
## It.
                       17.8068, Par. =
## It.
          0, RSS =
                                                0.5
## It.
          1, RSS =
                       0.17432, Par. =
                                            6.14925
## It.
          2, RSS =
                       0.17432, Par. =
                                            6.14925
                       8.03491, Par. =
          0, RSS =
## It.
                                                0.5
## It.
          1, RSS =
                      0.200149, Par. =
                                            4.26571
           2, RSS =
## It.
                      0.200149, Par. =
                                            4.26571
## It.
          0, RSS =
                       9.15115, Par. =
                                                0.5
                     0.0385037, Par. =
## It.
          1, RSS =
                                            4.56122
                     0.0385037, Par. =
## It.
          2, RSS =
                                            4.56122
## It.
          0, RSS =
                       5.43766, Par. =
                                                0.5
                      0.160535, Par. =
## It.
          1, RSS =
                                            3.59053
## It.
          2, RSS =
                      0.160535, Par. =
                                            3.59053
```

```
0, RSS =
## It.
                      6.46656, Par. =
                                              0.5
                     0.022931, Par. =
## It.
          1, RSS =
                                           3.91507
          2, RSS =
                                           3.91507
## It.
                      0.022931, Par. =
                        4.4744, Par. =
          0, RSS =
                                               0.5
## It.
## It.
          1, RSS =
                    0.0853123, Par. =
                                           3.31852
          2, RSS =
                    0.0853123, Par. =
## It.
                                           3.31852
                      7.33476, Par. =
## It.
          0.RSS =
                                               0.5
          1, RSS =
                    0.0234891, Par. =
## It.
                                           4.13773
## It.
          2, RSS =
                    0.0234891, Par. =
                                           4.13773
          0, RSS =
## It.
                       4.64689, Par. =
                                               0.5
## It.
          1, RSS = 0.0559316, Par. =
                                           3.38261
                    0.0559316, Par. =
          2, RSS =
                                           3.38261
## It.
## It.
          0, RSS =
                      8.77011, Par. =
                                               0.5
## It.
          1, RSS = 0.0536438, Par. =
                                           4.47195
          2, RSS =
                    0.0536438, Par. =
## It.
                                           4.47195
## It.
          0, RSS =
                        4.8711, Par. =
                                               0.5
          1, RSS = 0.0784329, Par. =
                                           3.44525
## It.
## It.
          2, RSS =
                    0.0784329, Par. =
                                           3.44525
## It.
          0, RSS =
                      10.7433, Par. =
                                               0.5
## It.
          1, RSS = 0.0441135, Par. =
                                           4.90056
## It.
          2, RSS = 0.0441135, Par. =
                                           4.90056
## It.
          0, RSS =
                      5.14926, Par. =
                                               0.5
          1, RSS =
                    0.0692001, Par. =
## It.
                                           3.53227
          2. RSS =
                    0.0692001, Par. =
## It.
                                           3.53227
## It.
          0, RSS =
                       10.0531, Par. =
                                               0.5
## It.
          1, RSS = 0.0644217, Par. =
                                           4.75194
          2, RSS = 0.0644217, Par. =
                                           4.75194
## It.
## It.
          0, RSS =
                      5.94609, Par. =
                                               0.5
## It.
          1, RSS = 0.0633265, Par. =
                                           3.76306
## It.
          2, RSS = 0.0633265, Par. =
                                           3.76306
## It.
          0, RSS =
                       9.43881, Par. =
                                               0.5
## It.
          1, RSS = 0.0576047, Par. =
                                           4.62063
## It.
          2, RSS =
                    0.0576047, Par. =
                                           4.62063
          0, RSS =
                        5.596, Par. =
## It.
                                               0.5
## It.
          1, RSS = 0.0764092, Par. =
                                           3.66073
## It.
          2, RSS =
                    0.0764092, Par. =
                                           3.66073
## It.
          0, RSS =
                       11.3331, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0953349, Par. =
                                           5.00996
## It.
          2, RSS =
                    0.0953349, Par. =
                                           5.00996
## It.
          0, RSS =
                      5.10229, Par. =
                                               0.5
          1, RSS = 0.0752465, Par. =
## It.
                                           3.51641
          2, RSS = 0.0752465, Par. =
                                           3.51641
## It.
                      9.85437, Par. =
## It.
          0.RSS =
                                               0.5
## It.
          1, RSS = 0.0324945, Par. =
                                            4.7163
## It.
          2, RSS =
                    0.0324945, Par. =
                                            4.7163
                      5.45885, Par. =
          0, RSS =
## It.
                                               0.5
## It.
          1, RSS =
                    0.0671645, Par. =
                                           3.62389
          2, RSS =
                                           3.62389
## It.
                    0.0671645, Par. =
## It.
          0, RSS =
                      10.5778, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0372883, Par. =
                                           4.86781
          2, RSS =
## It.
                    0.0372883, Par. =
                                           4.86781
## It.
          0, RSS =
                      5.17715, Par. =
                                               0.5
## It.
          1, RSS =
                     0.118116, Par. =
                                           3.52599
## It.
          2, RSS =
                     0.118116, Par. =
                                           3.52599
```

```
## It.
          0, RSS =
                       9.56145, Par. =
                                               0.5
                      0.144108, Par. =
## It.
          1, RSS =
                                           4.62855
          2, RSS =
                      0.144108, Par. =
                                           4.62855
## It.
                       5.96757, Par. =
          0, RSS =
                                                0.5
## It.
## It.
          1, RSS =
                     0.0958644, Par. =
                                           3.75999
                     0.0958644, Par. =
## It.
          2, RSS =
                                           3.75999
                       11.1117, Par. =
## It.
          0.RSS =
                                                0.5
          1, RSS =
                     0.0365413, Par. =
## It.
                                           4.97723
## It.
          2, RSS =
                     0.0365413, Par. =
                                           4.97723
## It.
          0, RSS =
                       5.18249, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0782035, Par. =
                                           3.53949
                     0.0782035, Par. =
                                           3.53949
## It.
          2, RSS =
## It.
          0, RSS =
                       10.5535, Par. =
                                                0.5
## It.
          1, RSS =
                      0.156276, Par. =
                                           4.83804
          2, RSS =
                      0.156276, Par. =
## It.
                                           4.83804
## It.
          0, RSS =
                       5.90269, Par. =
                                                0.5
## It.
          1, RSS =
                       0.07642, Par. =
                                           3.74735
## It.
          2, RSS =
                       0.07642, Par. =
                                           3.74735
## It.
          0, RSS =
                       10.9613, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0538666, Par. =
                                           4.94319
          2, RSS =
## It.
                     0.0538666, Par. =
                                           4.94319
          0, RSS =
                       6.25092, Par. =
## It.
                                                0.5
          1, RSS =
                     0.0771499, Par. =
## It.
                                           3.84279
          2. RSS =
                     0.0771499, Par. =
## It.
                                           3.84279
## It.
          0, RSS =
                       11.9084, Par. =
                                                0.5
## It.
          1, RSS =
                      0.117858, Par. =
                                           5.11956
          2, RSS =
                      0.117858, Par. =
                                           5.11956
## It.
## It.
          0, RSS =
                       6.57557, Par. =
                                                0.5
                      0.152749, Par. =
                                           3.90955
## It.
          1, RSS =
## It.
          2, RSS =
                      0.152749, Par. =
                                           3.90955
## It.
          0, RSS =
                       9.31356, Par. =
                                                0.5
## It.
          1, RSS =
                     0.0495342, Par. =
                                           4.59481
## It.
          2, RSS =
                     0.0495342, Par. =
                                           4.59481
          0, RSS =
                       6.21378, Par. =
## It.
                                                0.5
## It.
          1, RSS =
                      0.106969, Par. =
                                           3.82461
## It.
          2, RSS =
                      0.106969, Par. =
                                           3.82461
## It.
          0, RSS =
                       11.1517, Par. =
                                                0.5
          1, RSS =
                     0.0550836, Par. =
## It.
                                           4.98156
## It.
          2, RSS =
                     0.0550836, Par. =
                                           4.98156
          0, RSS =
                       5.38962, Par. =
## It.
                                                0.5
          1, RSS =
                       0.07391, Par. =
## It.
                                           3.60181
          2, RSS =
                       0.07391, Par. =
                                           3.60181
## It.
                       9.70898, Par. =
## It.
          0, RSS =
                                                0.5
## It.
          1, RSS =
                      0.083018, Par. =
                                           4.67403
## It.
          2, RSS =
                      0.083018, Par. =
                                           4.67403
                       6.00618, Par. =
          0, RSS =
## It.
                                                0.5
## It.
          1, RSS =
                      0.106298, Par. =
                                             3.7678
          2, RSS =
## It.
                      0.106298, Par. =
                                             3.7678
## It.
          0, RSS =
                       14.3918, Par. =
                                                0.5
## It.
          1, RSS =
                      0.139143, Par. =
                                           5.57904
## It.
                      0.139143, Par. =
                                           5.57904
          2, RSS =
## It.
          0, RSS =
                       6.61422, Par. =
                                                0.5
                     0.0776967, Par. =
## It.
          1, RSS =
                                           3.93959
                     0.0776967, Par. =
## It.
          2, RSS =
                                           3.93959
```

```
## It.
          0, RSS =
                       11.3666, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0645939, Par. =
                                           5.02284
## It.
          2, RSS =
                     0.0645939, Par. =
                                           5.02284
                       6.81657, Par. =
          0, RSS =
                                               0.5
## It.
## It.
          1, RSS =
                      0.087549, Par. =
                                           3.98987
                      0.087549, Par. =
## It.
          2, RSS =
                                           3.98987
                        13.183, Par. =
## It.
          0.RSS =
                                               0.5
          1, RSS =
                     0.0854527, Par. =
## It.
                                           5.36888
## It.
          2, RSS =
                     0.0854527, Par. =
                                           5.36888
## It.
          0, RSS =
                       5.82242, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0970729, Par. =
                                            3.7191
                     0.0970729, Par. =
          2, RSS =
                                            3.7191
## It.
## It.
          0, RSS =
                       11.0183, Par. =
                                               0.5
          1, RSS =
## It.
                    0.0252692, Par. =
                                           4.96058
          2, RSS =
                     0.0252692, Par. =
## It.
                                           4.96058
## It.
          0, RSS =
                       6.16476, Par. =
                                               0.5
          1, RSS =
                      0.132485, Par. =
## It.
                                           3.80426
## It.
          2, RSS =
                      0.132485, Par. =
                                           3.80426
                        11.199, Par. =
          0, RSS =
## It.
                                               0.5
                      0.049447, Par. =
## It.
          1, RSS =
                                           4.99223
## It.
          2, RSS =
                      0.049447, Par. =
                                           4.99223
## It.
          0, RSS =
                       6.38153, Par. =
                                               0.5
          1, RSS =
                      0.118497, Par. =
## It.
                                           3.86687
          2, RSS =
                      0.118497, Par. =
                                           3.86687
## It.
## It.
          0, RSS =
                       11.6702, Par. =
                                               0.5
## It.
          1, RSS =
                      0.101902, Par. =
                                           5.07582
          2, RSS =
                      0.101902, Par. =
                                           5.07582
## It.
## It.
          0, RSS =
                       5.38148, Par. =
                                               0.5
                    0.0730966, Par. =
## It.
          1, RSS =
                                           3.59967
## It.
          2, RSS =
                     0.0730966, Par. =
                                           3.59967
## It.
          0, RSS =
                       12.4265, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0355846, Par. =
                                           5.23572
## It.
          2, RSS =
                     0.0355846, Par. =
                                           5.23572
          0, RSS =
## It.
                       6.23941, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0914611, Par. =
                                           3.83579
## It.
          2, RSS =
                     0.0914611, Par. =
                                           3.83579
## It.
          0, RSS =
                       9.44982, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0233902, Par. =
                                           4.63055
## It.
          2, RSS =
                     0.0233902, Par. =
                                           4.63055
          0, RSS =
                       5.89397, Par. =
## It.
                                               0.5
          1, RSS = 0.0918637, Par. =
## It.
                                           3.74061
          2, RSS = 0.0918637, Par. =
                                           3.74061
## It.
## It.
          0.RSS =
                       8.83192, Par. =
                                               0.5
## It.
          1, RSS = 0.0279755, Par. =
                                           4.49184
## It.
          2, RSS =
                     0.0279755, Par. =
                                           4.49184
                       6.55273, Par. =
          0, RSS =
## It.
                                               0.5
## It.
          1, RSS =
                    0.0970309, Par. =
                                           3.91826
          2, RSS =
## It.
                     0.0970309, Par. =
                                           3.91826
## It.
          0, RSS =
                       11.1001, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0701701, Par. =
                                           4.96807
          2, RSS =
## It.
                                           4.96807
                     0.0701701, Par. =
## It.
          0, RSS =
                       7.30424, Par. =
                                               0.5
## It.
          1, RSS =
                      0.134583, Par. =
                                           4.10233
## It.
          2, RSS =
                      0.134583, Par. =
                                           4.10233
```

```
## It.
          0, RSS =
                       11.8424, Par. =
                                               0.5
## It.
          1, RSS = 0.0333966, Par. =
                                           5.12318
                                           5.12318
## It.
          2, RSS =
                     0.0333966, Par. =
          0, RSS =
                       5.71621, Par. =
                                               0.5
## It.
## It.
          1, RSS = 0.0933848, Par. =
                                           3.69015
          2, RSS =
                    0.0933848, Par. =
                                           3.69015
## It.
                       10.6949, Par. =
## It.
          0.RSS =
                                               0.5
          1, RSS =
                     0.0260347, Par. =
## It.
                                           4.89434
## It.
          2, RSS =
                     0.0260347, Par. =
                                           4.89434
## It.
          0, RSS =
                        5.7729, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0752248, Par. =
                                           3.71131
                     0.0752248, Par. =
## It.
          2, RSS =
                                           3.71131
## It.
          0, RSS =
                       13.2528, Par. =
                                               0.5
## It.
          1, RSS = 0.0284843, Par. =
                                           5.39239
          2, RSS =
                     0.0284843, Par. =
## It.
                                           5.39239
## It.
          0, RSS =
                       5.55202, Par. =
                                               0.5
          1, RSS =
## It.
                      0.118735, Par. =
                                           3.63592
## It.
          2, RSS =
                      0.118735, Par. =
                                           3.63592
                       12.7527, Par. =
## It.
          0, RSS =
                                               0.5
                      0.071967, Par. =
## It.
          1, RSS =
                                           5.29078
## It.
          2, RSS =
                      0.071967, Par. =
                                           5.29078
## It.
          0, RSS =
                        6.5424, Par. =
                                               0.5
          1, RSS =
                     0.0970149, Par. =
## It.
                                           3.91553
                     0.0970149, Par. =
## It.
          2. RSS =
                                           3.91553
## It.
          0, RSS =
                       10.5599, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0832647, Par. =
                                           4.85456
          2, RSS =
                     0.0832647, Par. =
                                           4.85456
## It.
## It.
          0, RSS =
                       5.87611, Par. =
                                               0.5
          1, RSS = 0.0930449, Par. =
## It.
                                           3.73529
## It.
          2, RSS =
                     0.0930449, Par. =
                                           3.73529
## It.
          0, RSS =
                       12.1553, Par. =
                                               0.5
## It.
          1, RSS =
                       0.01877, Par. =
                                           5.18686
## It.
          2, RSS =
                       0.01877, Par. =
                                           5.18686
          0, RSS =
                        6.3279, Par. =
## It.
                                               0.5
## It.
          1, RSS =
                      0.177442, Par. =
                                           3.83647
## It.
          2, RSS =
                      0.177442, Par. =
                                           3.83647
## It.
          0, RSS =
                       12.1516, Par. =
                                               0.5
## It.
          1, RSS =
                      0.164387, Par. =
                                           5.15793
## It.
          2, RSS =
                      0.164387, Par. =
                                           5.15793
## It.
          0, RSS =
                       6.07589, Par. =
                                               0.5
          1, RSS =
                      0.119516, Par. =
## It.
                                           3.78341
          2, RSS =
                      0.119516, Par. =
                                           3.78341
## It.
## It.
          0, RSS =
                       11.4173, Par. =
                                               0.5
## It.
          1, RSS =
                      0.126372, Par. =
                                           5.02063
## It.
          2, RSS =
                      0.126372, Par. =
                                           5.02063
          0, RSS =
                       5.91052, Par. =
## It.
                                               0.5
## It.
          1, RSS =
                     0.0890995, Par. =
                                             3.746
          2, RSS =
## It.
                     0.0890995, Par. =
                                             3.746
## It.
          0, RSS =
                       11.9555, Par. =
                                               0.5
## It.
          1, RSS =
                      0.048914, Par. =
                                           5.14224
          2, RSS =
## It.
                      0.048914, Par. =
                                           5.14224
## It.
          0, RSS =
                       6.74438, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0436165, Par. =
                                           3.98254
## It.
          2, RSS = 0.0436165, Par. =
                                           3.98254
```

```
## It.
          0, RSS =
                       14.8403, Par. =
                                               0.5
## It.
                       0.10404, Par. =
          1, RSS =
                                            5.6645
                       0.10404, Par. =
## It.
          2, RSS =
                                            5.6645
## It.
          0, RSS =
                        6.1725, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0977021, Par. =
                                           3.81589
## It.
                    0.0977021, Par. =
                                           3.81589
          2, RSS =
                       10.4828, Par. =
## It.
          0.RSS =
                                               0.5
          1, RSS =
                      0.101115, Par. =
## It.
                                           4.83479
## It.
          2, RSS =
                      0.101115, Par. =
                                           4.83479
## It.
          0, RSS =
                       5.69536, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0626388, Par. =
                                           3.69296
                     0.0626388, Par. =
          2, RSS =
                                           3.69296
## It.
## It.
          0, RSS =
                       12.7927, Par. =
                                               0.5
## It.
          1, RSS =
                      0.135476, Par. =
                                           5.28633
## It.
          2, RSS =
                      0.135476, Par. =
                                           5.28633
## It.
          0, RSS =
                        6.1908, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0557257, Par. =
                                            3.8323
## It.
          2, RSS =
                     0.0557257, Par. =
                                            3.8323
## It.
          0, RSS =
                        13.763, Par. =
                                               0.5
## It.
          1, RSS =
                      0.131954, Par. =
                                           5.46706
## It.
          2, RSS =
                      0.131954, Par. =
                                           5.46706
## It.
          0, RSS =
                       6.36817, Par. =
                                               0.5
                      0.167767, Par. =
## It.
          1, RSS =
                                           3.84999
          2, RSS =
## It.
                      0.167767, Par. =
                                           3.84999
## It.
          0, RSS =
                       10.0071, Par. =
                                               0.5
## It.
          1, RSS =
                     0.0901801, Par. =
                                           4.73664
## It.
          2, RSS =
                     0.0901801, Par. =
                                           4.73664
## It.
          0, RSS =
                       6.81497, Par. =
                                               0.5
## It.
                      0.212028, Par. =
          1, RSS =
                                           3.95702
## It.
          2, RSS =
                      0.212028, Par. =
                                           3.95702
## It.
          0, RSS =
                         12.51, Par. =
                                               0.5
## It.
          1, RSS =
                    0.0730136, Par. =
                                           5.24452
## It.
          2, RSS =
                     0.0730136, Par. =
                                           5.24452
                       6.50273, Par. =
          0, RSS =
## It.
                                               0.5
## It.
          1, RSS =
                      0.121557, Par. =
                                           3.89848
                                           3.89848
## It.
                      0.121557, Par. =
          2, RSS =
## It.
          0, RSS =
                       15.1764, Par. =
                                               0.5
## It.
          1, RSS =
                      0.208953, Par. =
                                           5.70484
## It.
          2, RSS =
                      0.208953, Par. =
                                           5.70484
## It.
          0, RSS =
                       7.18906, Par. =
                                               0.5
## It.
                    0.0997162, Par. =
          1, RSS =
                                           4.08209
## It.
          2, RSS = 0.0997162, Par. =
                                           4.08209
write_csv(Ksv_model, "nativeN_dN_Ksv_model_values.csv")
Ksv_model
## # A tibble: 122 x 6
  # Groups:
               Wavelength, prot [122]
##
      Wavelength prot std.error statistic
                                                             Ksv
                                                  p.value
##
           <dbl> <chr>
                            <dbl>
                                       <dbl>
                                                     <dbl> <dbl>
```

11.7 0.0000241

13.6 0.00000964

19.3 0.00000127

3.32

3.44

3.98

1

##

3

2

315 dN

316 dN

315 Fis1

0.285

0.252

0.207

##	4	316	Fis1	0.231	15.7	0.00000419	3.63
##	5	317	dN	0.250	17.1	0.00000258	4.26
##	6	317	Fis1	0.246	14.0	0.00000827	3.45
##	7	318	dN	0.120	29.9	0.000000936	3.58
##	8	318	Fis1	0.164	20.6	0.000000856	3.39
##	9	319	dN	0.256	17.4	0.00000233	4.44
##	10	319	Fis1	0.250	15.1	0.00000523	3.78

... with 112 more rows