Data processing for the chemical denaturation of GFP

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Loading of data for absorbance at 395nm.

```
abs_table <- read.csv("../data/exp_data.csv")
unfolded_abs <- mean(abs_table$Average[8:10]) # Assigning the average of 8x-10x as the absorb
ance of the unfolded state
native_abs <- abs_table$Average[1] # Assigning 1x as the absorbance of the native state
abs_table$fraction_unfolded <- 1 - (abs_table$Average - unfolded_abs)/(native_abs-unfolded_ab
s)

abs_table
```

```
##    Sample Final_urea_concentration Read_1 Read_2 Read_3 Average
## 1      1x                      0.0  0.589  0.581  0.589   0.586
## 2      2x                      0.8  0.565  0.566  0.556   0.562
## 3      3x                      1.6  0.570  0.589  0.557   0.572
## 4      4x                      2.4  0.551  0.551  0.551   0.551
## 5      5x                      3.2  0.561  0.567  0.567   0.565
## 6      6x                      4.0  0.552  0.557  0.557   0.555
## 7      7x                      4.8  0.525  0.538  0.539   0.532
## 8      8x                      5.6  0.539  0.545  0.545   0.543
## 9      9x                      6.4  0.545  0.552  0.552   0.550
## 10    10x                      7.2  0.546  0.547  0.546   0.546
##    fraction_unfolded
## 1          0.0000000
## 2          0.6050420
## 3          0.3529412
## 4          0.8823529
## 5          0.5294118
## 6          0.7815126
## 7          1.3613445
## 8          1.0840336
## 9          0.9075630
## 10         1.0084034
```

Sample 7x seems off, so I will filter it out for analysis.

```
processed_abs <- abs_table[c(1:6,8:10),c(1,2,6,7)]
processed_abs
```

```
##    Sample Final_urea_concentration Average fraction_unfolded
## 1      1x                      0.0   0.586         0.0000000
## 2      2x                      0.8   0.562         0.6050420
## 3      3x                      1.6   0.572         0.3529412
## 4      4x                      2.4   0.551         0.8823529
## 5      5x                      3.2   0.565         0.5294118
## 6      6x                      4.0   0.555         0.7815126
## 8      8x                      5.6   0.543         1.0840336
## 9      9x                      6.4   0.550         0.9075630
## 10    10x                      7.2   0.546         1.0084034
```

Performing a sigmoidal fit on our experimental data using a non-linear least squares model.

```
library(minpack.lm)
```

```
## Warning: package 'minpack.lm' was built under R version 4.4.2
```

```
sigmoidal_fit <- nlsLM(
  fraction_unfolded ~ 1 / (1 + exp(-m * (Final_urea_concentration - dfifty))),
  data = processed_abs,
  start = list(m = 0.1, dfifty = median(processed_abs$Final_urea_concentration))
)


summary(sigmoidal_fit)
```

```
## 
## Formula: fraction_unfolded ~ 1/(1 + exp(-m * (Final_urea_concentration - 
##     dfifty)))
## 
## Parameters:
##        Estimate Std. Error t value Pr(>|t|)  
## m        0.7288     0.3196   2.280   0.0566 .
## dfifty   1.6740     0.5482   3.053   0.0185 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 0.1994 on 7 degrees of freedom
## 
## Number of iterations to convergence: 8 
## Achieved convergence tolerance: 1.49e-08
```

Visualising our experimental data

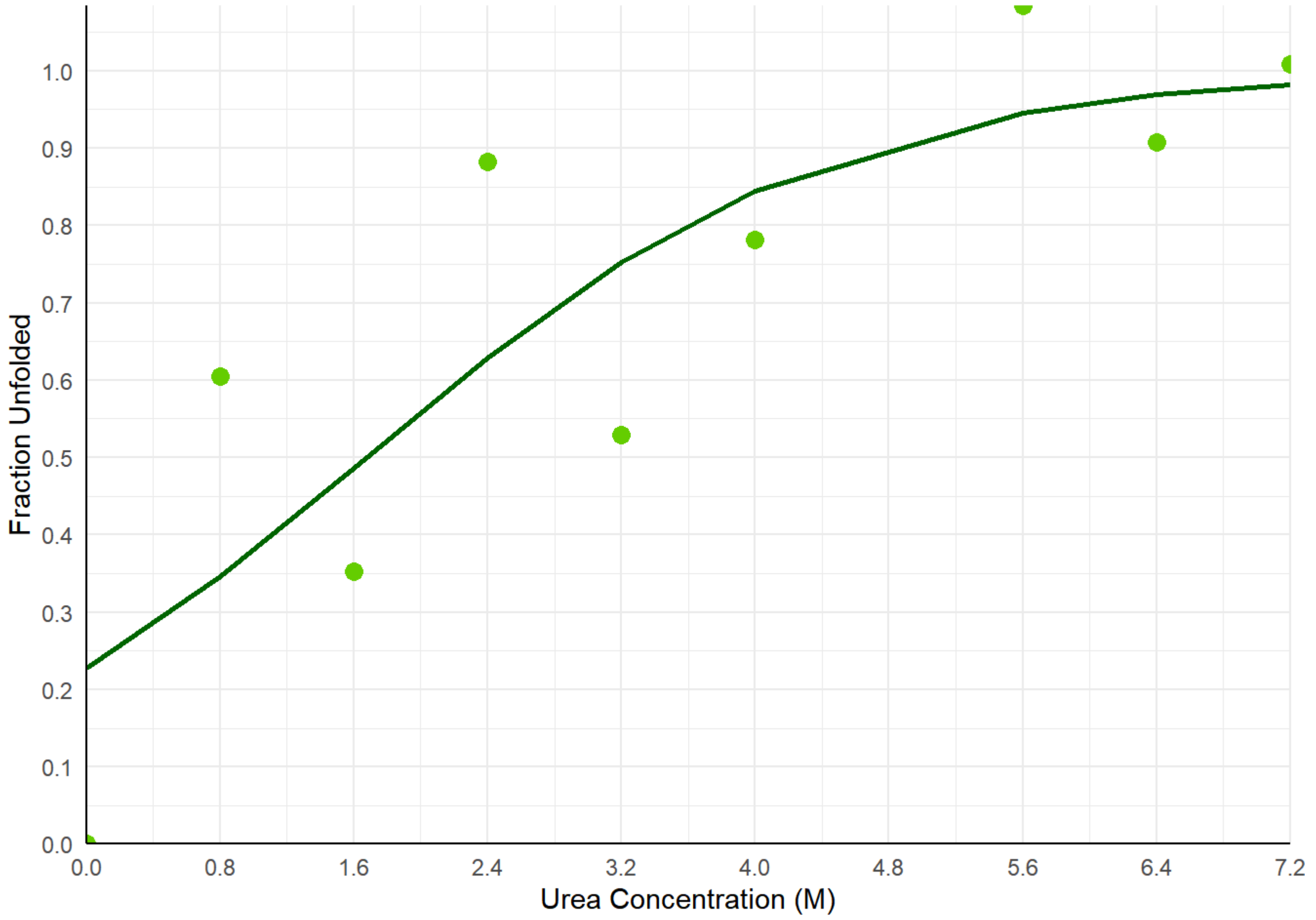
```
library(ggplot2)

ggplot(data = processed_abs, aes(x = Final_urea_concentration, y = fraction_unfolded)) +
  geom_point(color = "chartreuse3", size = 3) +  # Scatter plot of data points
  theme_minimal()+
  theme(axis.line = element_line(color = "black",
                                 linewidth = 0.5,
                                 linetype = 1),


      )+

  geom_line(aes(y = predict(sigmoidal_fit)), color = "dark green", size = 1) +  # Fitted curv
e
  labs(
    x = "Urea Concentration (M)",
    y = "Fraction Unfolded"
  )+
  scale_y_continuous(breaks = seq(0, 1, by = 0.1), expand = c(0,0))+
  scale_x_continuous(breaks = seq(0, 8, by = 0.8), expand = c(0,0))
```

```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## ℹ Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```



Data processing for equillibrium constants and Gibbs Free Energy of each data point

```
R <- 8.314  # J/(mol*K)
T <- 298.15 # Biochemical standard state (298.15 K)
abs_table$K <- abs_table$fraction_unfolded/(1-abs_table$fraction_unfolded)
abs_table$dGibbs <- -R*T*log(abs_table$K)
```

```
## Warning in log(abs_table$K): NaNs produced
```

```
gibbs_processed <- subset(abs_table, !is.na(abs_table$dGibbs) & !is.infinite(abs_table$dGibb
s)) #filtering NaN and infinite values
gibbs_processed
```

```
##   Sample Final_urea_concentration Read_1 Read_2 Read_3 Average
## 2     2x                      0.8  0.565  0.566  0.556   0.562
## 3     3x                      1.6  0.570  0.589  0.557   0.572
## 4     4x                      2.4  0.551  0.551  0.551   0.551
## 5     5x                      3.2  0.561  0.567  0.567   0.565
## 6     6x                      4.0  0.552  0.557  0.557   0.555
## 9     9x                      6.4  0.545  0.552  0.552   0.550
##   fraction_unfolded         K     dGibbs
## 2         0.6050420 1.5319149 -1057.2622
## 3         0.3529412 0.5454545  1502.5010
## 4         0.8823529 7.5000000 -4994.5801
## 5         0.5294118 1.1250000  -291.9628
## 6         0.7815126 3.5769231 -3159.2623
## 9         0.9075630 9.8181818 -5662.2077
```

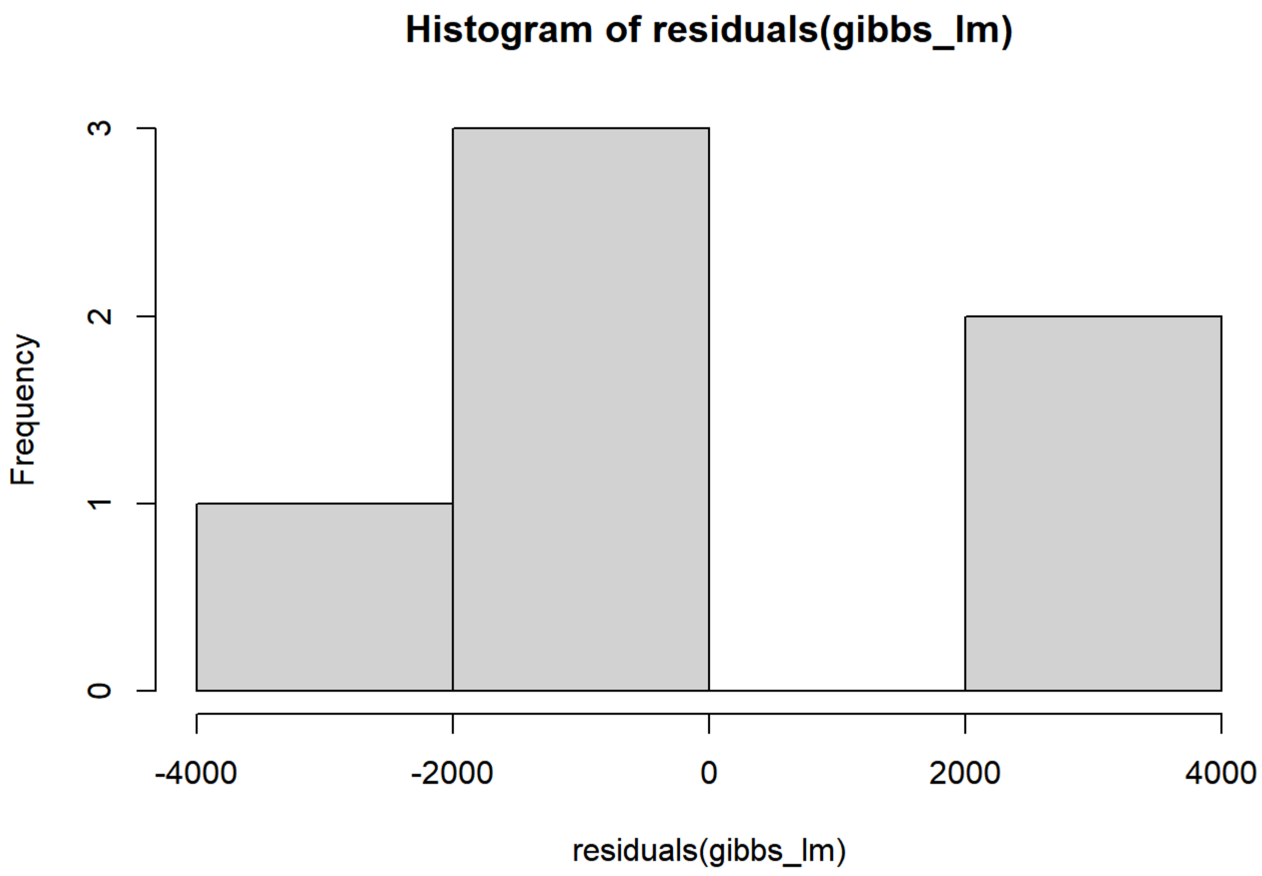
Linear regression of the Gibbs free energy change

```
gibbs_lm <- lm(dGibbs~Final_urea_concentration, data = gibbs_processed)
summary(gibbs_lm)
```

```
## 
## Call:
## lm(formula = dGibbs ~ Final_urea_concentration, data = gibbs_processed)
## 
## Residuals:
##        2        3        4        5        6        9 
##  -886.65  2416.59 -3337.01  2109.08   -14.74  -287.26 
## 
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 572.9     1893.4   0.303    0.777
## Final_urea_concentration   -929.3      531.4  -1.749    0.155
## 
## Residual standard error: 2361 on 4 degrees of freedom
## Multiple R-squared:  0.4333, Adjusted R-squared:  0.2916 
## F-statistic: 3.058 on 1 and 4 DF,  p-value: 0.1552
```

Testing the assumption of normal distribution of errors

```
hist(residuals(gibbs_lm))
```



Loading, wrangling and fitting reference data

```
reference_data <- read.csv("../data/ref_data.csv")

ref_unfolded_abs <- mean(reference_data$Absorbance395[8:10]) # Assigning the average of 8x-10
x as the absorbance of the unfolded state
ref_native_abs <- reference_data$Absorbance395[1] # Assigning 1x as the absorbance of the nat
ive state
reference_data$fraction_unfolded <- 1 - (reference_data$Absorbance395 - ref_unfolded_abs)/(re
f_native_abs-ref_unfolded_abs)


ref_sigmoidal_fit <- nlsLM(
  fraction_unfolded ~ 1 / (1 + exp(-m * (Final_urea_concentration - dfifty))),
  data = reference_data,
  start = list(m = 0.1, dfifty = median(reference_data$Final_urea_concentration))
)
summary(ref_sigmoidal_fit)
```

```
##
## Formula: fraction_unfolded ~ 1/(1 + exp(-m * (Final_urea_concentration -
##     dfifty)))
##
## Parameters:
##        Estimate Std. Error t value Pr(>|t|)
## m       1.65416    0.15063   10.98 4.20e-06 ***
## dfifty  3.25780    0.06243   52.19 2.01e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.03665 on 8 degrees of freedom
##
## Number of iterations to convergence: 9
## Achieved convergence tolerance: 1.49e-08
```

Estimation of protein unfolding at 2, 4 and 6 M of urea.

```
q2_concs <- c(2, 4, 6)

df_q2_concs <- data.frame(
  Final_urea_concentration = q2_concs
)

predict(ref_sigmoidal_fit, newdata = df_q2_concs) #Refernce dataset predictions
```

```
## [1] 0.1109964 0.7734184 0.9893978
```

```
predict(sigmoidal_fit, newdata = df_q2_concs) #Experimental dataset predictions
```

```
## [1] 0.5591197 0.8449121 0.9590225
```

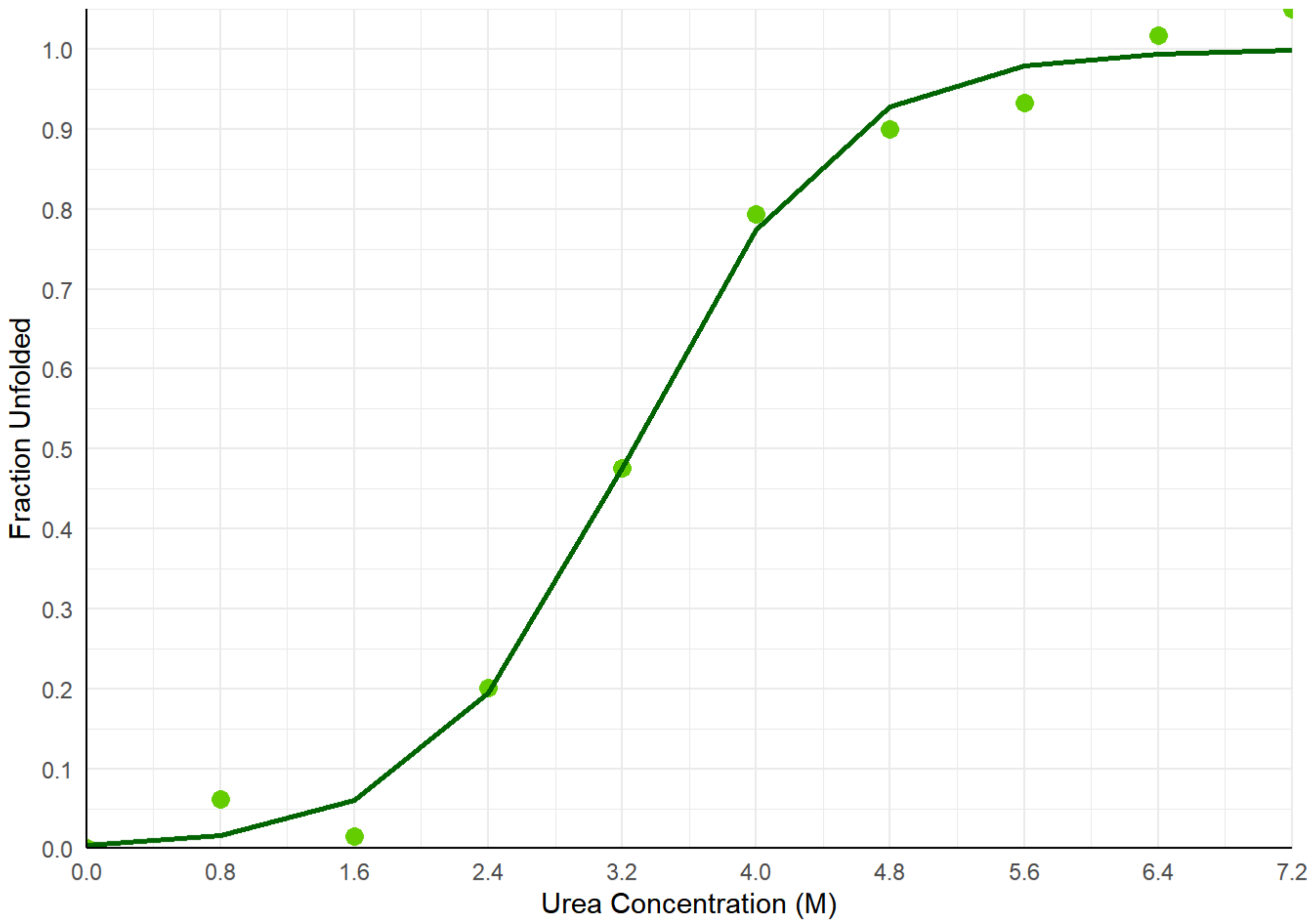
Visualising the reference data

```
library(ggplot2)

ggplot(data = reference_data, aes(x = Final_urea_concentration, y = fraction_unfolded)) +
  geom_point(color = "chartreuse3", size = 3) +  # Scatter plot of data points
  theme_minimal()+
  theme(axis.line = element_line(color = "black",
                                 linewidth = 0.5,
                                 linetype = 1),


      )+

  geom_line(aes(y = predict(ref_sigmoidal_fit)), color = "dark green", size = 1) +  # Fitted
curve
  labs(
    x = "Urea Concentration (M)",
    y = "Fraction Unfolded"
  )+
  scale_y_continuous(breaks = seq(0, 1, by = 0.1), expand = c(0,0))+
  scale_x_continuous(breaks = seq(0, 8, by = 0.8), expand = c(0,0))
```



```
ggsave("../results/ref_denaturation_curve.png", height = 7.47, width = 8.69, units = "cm")
```

Processing reference data for the Gibbs Free Energy

```
reference_data$K <- reference_data$fraction_unfolded/(1-reference_data$fraction_unfolded)
reference_data$dGibbs <- -R*T*log(reference_data$K)
```

```
## Warning in log(reference_data$K): NaNs produced
```

```
ref_gibbs_processed <- subset(reference_data, !is.na(reference_data$dGibbs) & !is.infinite(re
ference_data$dGibbs))
ref_gibbs_processed
```

```
##   Sample Final_urea_concentration Vol_urea Vol_buffer Vol_protein Absorbance395
## 2     2x                      0.8       40        365          95         0.394
## 3     3x                      1.6       80        325          95         0.407
## 4     4x                      2.4      120        285          95         0.356
## 5     5x                      3.2      160        245          95         0.281
## 6     6x                      4.0      200        205          95         0.194
## 7     7x                      4.8      240        165          95         0.165
## 8     8x                      5.6      280        125          95         0.156
##   fraction_unfolded           K     dGibbs
## 2        0.06219512  0.06631990  6725.6939
## 3        0.01463415  0.01485149 10434.9742
## 4        0.20121951  0.25190840  3417.5225
## 5        0.47560976  0.90697674   242.0281
## 6        0.79390244  3.85207101 -3342.9625
## 7        0.90000000  9.00000000 -5446.5222
## 8        0.93292683 13.90909091 -6525.5970
```

```
ref_gibbs_lm <- lm(dGibbs~Final_urea_concentration, data = ref_gibbs_processed)
summary(ref_gibbs_lm)
```

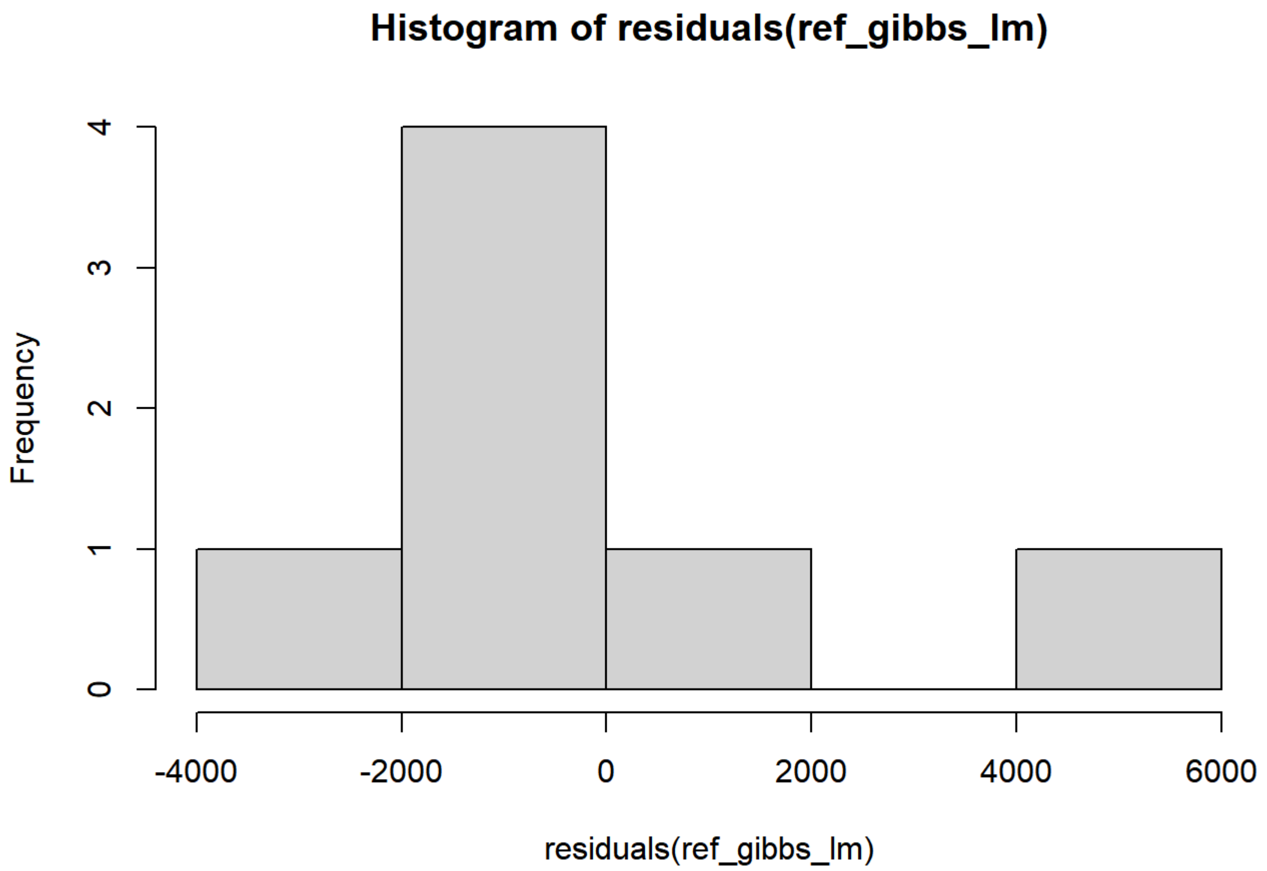
```
## 
## Call:
## lm(formula = dGibbs ~ Final_urea_concentration, data = ref_gibbs_processed)
## 
## Residuals:
##       2       3       4       5       6       7       8 
## -2447.6  4057.3  -164.5  -544.4 -1333.8  -641.7  1074.8 
## 
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)   
## (Intercept)               11968.9     1931.8   6.196  0.00160 **
## Final_urea_concentration  -3494.5      539.9  -6.472  0.00131 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 2286 on 5 degrees of freedom
## Multiple R-squared:  0.8934, Adjusted R-squared:  0.872 
## F-statistic: 41.89 on 1 and 5 DF,  p-value: 0.001312
```

```
confint(ref_gibbs_lm)
```

```
##                              2.5 %    97.5 %
## (Intercept)               7003.178 16934.676
## Final_urea_concentration -4882.494 -2106.556
```

Testing the assumption of normal distribution of errors

```
hist(residuals(ref_gibbs_lm))
```

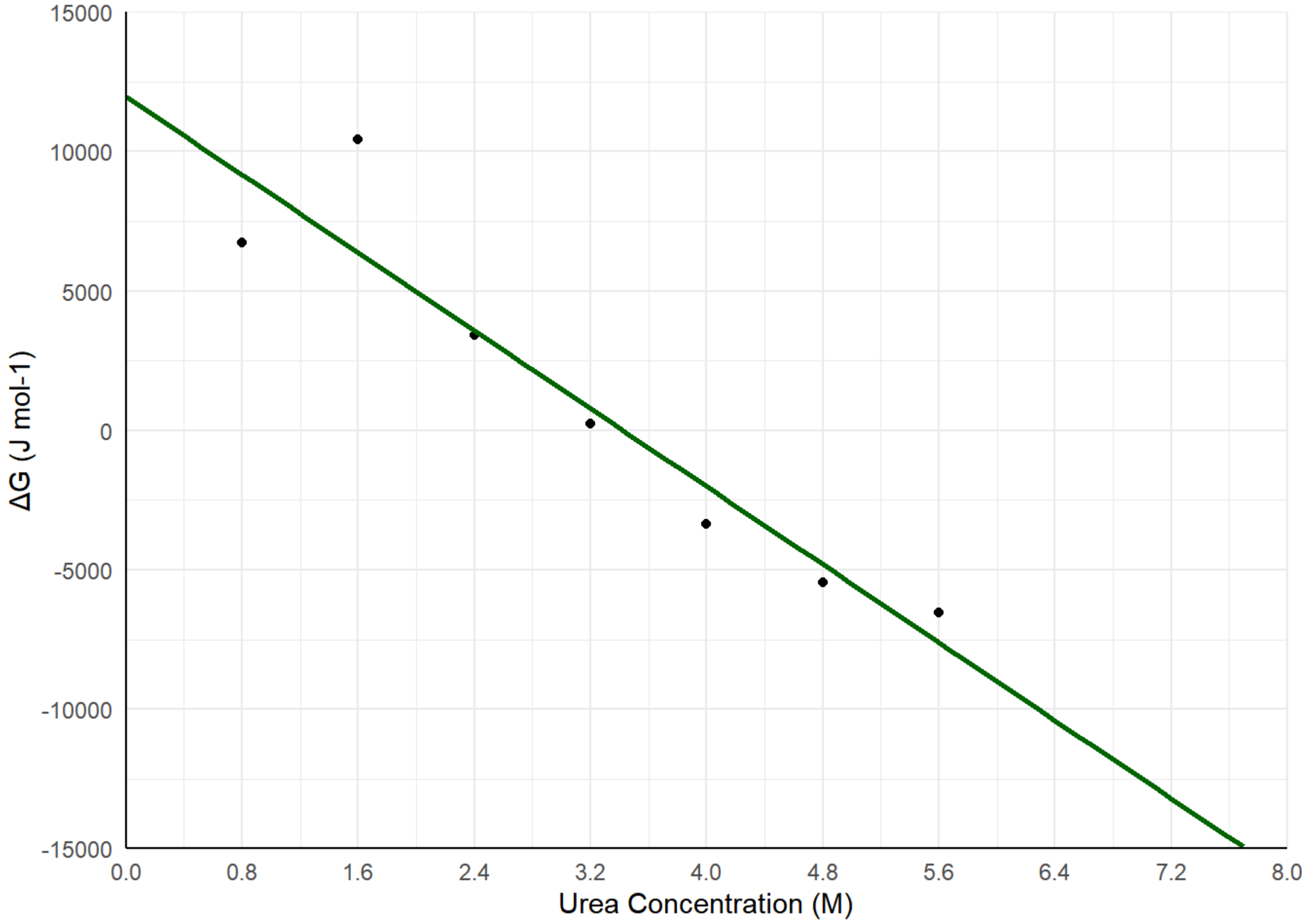


Visualising the Gibbs Free Energy distribution

```
ggplot(data = ref_gibbs_processed) + aes(x = Final_urea_concentration, y = dGibbs) + geom_poi
nt() + geom_smooth(method = "lm", se = FALSE, fullrange = TRUE, color = "dark green") +
  theme_minimal()+
  theme(axis.line = element_line(color = "black",
                                linewidth = 0.5,
                                linetype = 1))+
  labs(x = "Urea Concentration (M)",y = "ΔG (J mol-1)")+
  scale_x_continuous(limits=c(0, 8), breaks=seq(0,8,0.8))+
  scale_y_continuous(limits=c(-15000, 15000), breaks=seq(-15000, 15000, 5000))+
  coord_cartesian(expand = FALSE)
```

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

```
## Warning: Removed 3 rows containing missing values or values outside the scale range
## (`geom_smooth()`).
```



```
ggsave("../results/ref_gibbs.png", height = 7.47, width = 8.69, units = "cm")
```

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

```
## Warning: Removed 3 rows containing missing values or values outside the scale range
## (`geom_smooth()`).
```

The linear model calculates a highly significant ($p<0.001$) intercept of 11968 J mol-1, indicating a valid estimate of 12kJ mol-1 or 2.9 kcal mol-1 for the Gibbs Free Energy change associated with GFP folding.