

# thermal\_denaturation\_fitting

September 29, 2024

Fitting thermal denaturation data

```
[11]: import numpy as np
import matplotlib.pyplot as plt
from matplotlib import gridspec
%matplotlib inline
from scipy.optimize import curve_fit
import os

basedir = '/home/david/gh/intro_curve_fitting_python'

try:
    os.chdir(basedir)
except:
    print('\n\nproblem changing to the directory you specified; does it exist?
↪\nthe kernel will now restart; rerun this program.\n\n')
    quit()
```

The Brautigam laboratory's equation for fitting protein thermal denaturation data is:

$$\frac{m_1 * T + b_1 + (m_2 * T + b_2) * e^{-\frac{\Delta H}{RT}(1 - \frac{T}{T_M})}}{1 + e^{-\frac{\Delta H}{RT}(1 - \frac{T}{T_M})}}$$

in which: | Symbol | Meaning | | —:| ———:| |  $T$  | absolute temperature, the independent variable | |  $\theta$  | arbitrary temperature-dependent signal reporting the denaturation  $K_{eq}$  | |  $m_1$  | slope of line in low-temperature region | |  $b_1$  | intercept of line in low-temperature region | |  $m_2$  | slope of line in high-temperature region | |  $b_2$  | intercept of line in high-temperature region | |  $\Delta H$  | denaturation enthalpy, units as per choice of  $R$  | |  $R$  | the gas constant, in whichever units are preferred | |  $T_M$  | melting temperature, Kelvin |

This equation is an adaptation of the integrated form of the [van't Hoff equation](#), and my own derivation of it may be found [here](#). As I derive both a four- and a six-parameter version of the equation, I have also included an exercise in F-statistics to evaluate if the more complex equation actually provides a better fit.

Encode the fitting function:

```
[12]: # if the value of a variable is specified in the definition of a function
# that is its default value, and the function may be called without specifying_
↪that parameter
```

```

# or its value. if, when called, the value of the variable is specified,
# that value overrides the default

# note then that the default units for deltaH are J/mol
# define the function
def tdmmodel(T, m1, b1, m2, b2, deltaH, TM, R=8.314):
    line1=m1*T+b1
    line2=m2*T+b2
    Q=np.exp(-deltaH/(R*T)*(1-T/TM))
    return (line1+line2*Q)/(1+Q)

```

Good initial guesses for fittable parameters Evidently this equation, which has 6 fittable parameters, is quite a bit more complicated than those necessary to fit linear and exponential decay data. With this number of parameters, the trick to getting the right fit, as opposed to a fit that is stuck in some pathological local minimum, is to make good initial guesses for the unknown parameters. In what follows I walk through one example of how I accomplished that for a PupB NTSD mutant using actual Colbert Lab data. In the ‘`../intro_curve_fitting_python/thermal_denaturation_data`’ directory, find the following files:

```

[13]: datadir = basedir+'/thermal_denaturation_data/'
      os.listdir(datadir)

```

```

[13]: ['PupB NTSD L74A 25 uM 217 nm F.csv',
      'PupB NTSD Q69K 25 uM 217nm F.csv',
      'PupB NTSD H72D 25 uM 217nm F.csv']

```

An alternative means of reading csv files: See [here](#) for more.

```

[14]: import csv

fn = datadir+'PupB NTSD L74A 25 uM 217 nm F.csv'
csvfile = open(fn)
csvreader = csv.reader(csvfile, delimiter=',')

# create empty x and y arrays
x = []
y = []

for row in csvreader:
    x.append(float(row[0]))
    y.append(float(row[1]))

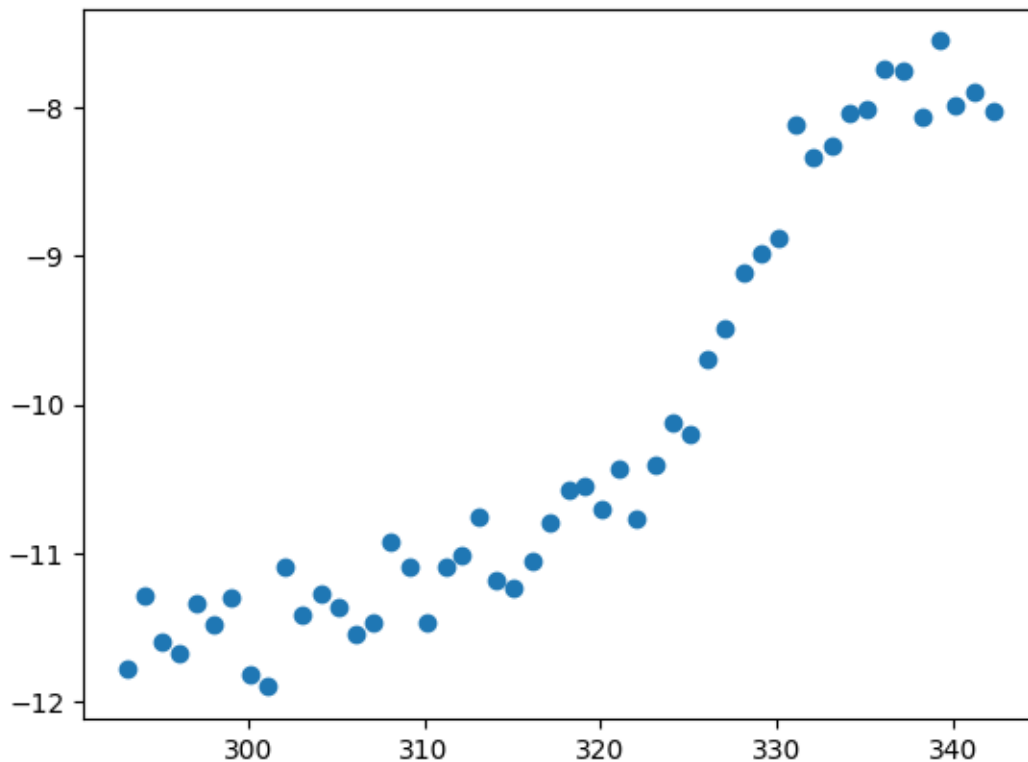
csvfile.close()

# make x and y proper np arrays
x = np.array(x)
y = np.array(y)

```

Scatter Plot Always look at your raw data:

```
[15]: # scatter plot:
plt.scatter(x,y)
plt.show()
```



Get the low- and high-temperature lines: As you've seen now, linear fits are insanely easy, so it is straightforward to get good initial estimates for  $m_1$ ,  $b_1$ ,  $m_2$ , and  $b_2$ . Let's do that:

```
[16]: # get the first 20 points for a low temp line

# the following 'slices' the array x beginning with the (implied)
# 0 prior to the colon, and ending with the 20 indicating the
# first array element _not_ to include in the 'slice'
eks = x[:20]
why = y[:20]

def func(x, m, b):
    return m*x+b

ltlineparam, pcov = curve_fit(func, eks, why)
m1 = ltlineparam[0]
b1 = ltlineparam[1]
```

```

# get the last 12 points for a high temp line

# more slicing syntax
# if the argument prior to the colon is negative, it means
# 'start counting from the end of the array'
# therefore, 'cut from the 12th from the last element of the
# array through to the end'
eks = x[-12:]
why = y[-12:]

htlineparam, pcov = curve_fit(func, eks, why)
m2 = htlineparam[0]
b2 = htlineparam[1]

```

Estimates for  $\Delta H$  and  $T_M$

For  $\Delta H$ , because an order of magnitude guess is sufficient, let's go with 100 kJ / mol.  $T_M$  may be eye-balled from the curve, I make it 335 K.

```

[17]: deltaH = 100000
      TM = 335
      print('initial guesses for fitting:')
      print('m1: %0.3f, b1: %0.3f, m2: %0.3f, b2: %0.3f, deltaH: %0.0f, TM: %0.0f' %_
            ↪(m1, b1, m2, b2, deltaH, TM))

```

initial guesses for fitting:

m1: 0.025, b1: -19.107, m2: 0.032, b2: -18.782, deltaH: 100000, TM: 335

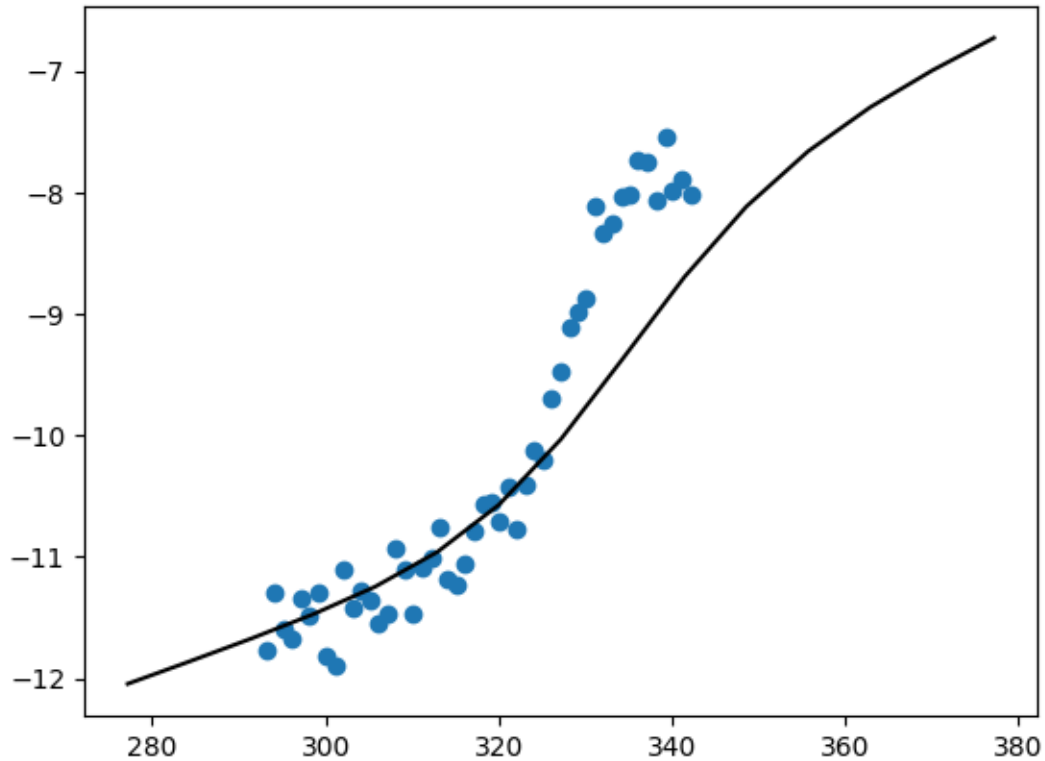
Compare Initial Paramters Against Data:

```

[21]: mintemp = 4 # degrees C
      maxtemp = 104
      mintemp += 273.15 # conversion to Kelvin
      maxtemp += 273.15
      eks = np.linspace(mintemp, maxtemp, 15)

      sigma = tdmodel(eks, m1, b1, m2, b2, deltaH, TM)
      plt.plot(T,sigma, 'k-')
      plt.scatter(x,y)
      plt.show()

```



Yes, that is close enough. Do the fitting using the values just estimated as initial guesses:

```
[22]: p = [m1, b1, m2, b2, deltaH, TM]

# do it
# argument 'p0=p' supplies initial guesses for fittable parameters
popt, pcov = curve_fit(tdmodel, x, y, p0=p)
popt

[22]: array([ 2.85739746e-02, -2.00378479e+01, -6.32331433e-02,  1.37202477e+01,
              2.85627011e+05,  3.29470759e+02])
```

Compute residuals and  $R^2$ :

```
[24]: residuals = y - tdmodel(x, *popt)
ssqresid = np.sum(np.square(residuals))
totsssq = np.sum(np.square(y - np.mean(y)))
rsq = 1 - ssqresid / totsssq
print('Rsquared: %0.3f' % rsq)
```

Rsquared: 0.981

Prepare a figure:

```

[38]: fn = basedir+'/thermal_denaturation_data/dmm_L74A_F_fit_figure.png'
eks = np.linspace(np.min(x), np.max(x), 100)
why = tdmodel(eks, *popt)

# create a figure
fig = plt.figure()
fig.set_figwidth(6)
fig.set_figheight(6)

# create grid for different subplots
spec = gridspec.GridSpec(ncols=1, nrows=2,
                           hspace=0.1, height_ratios=[4,
↳ 1])

ax0 = fig.add_subplot(spec[0])
ax0.scatter(x,y, marker='.', label='\ntheta, 217 mdeg\n')
ax0.plot(eks,why,'k-', label='$m_1$: %0.3f\n $b_1$: %0.1f\n $m_2$: %0.3f\n
↳ $b_2$: %0.1f\n deltaH: %0.0f J/mol\n $T_M$: %0.1f K' % tuple(popt))
ax0.legend()
ax0.set_ylabel('ellipticity at 217nm, mdeg')
plt.tick_params('x', labelbottom=False)

ax1 = fig.add_subplot(spec[1])
ax1.scatter(x, residuals, marker='.')
ax1.set_ylabel('residuals')
ax1.set_xlabel('Temperature, Kelvin')

plt.suptitle('Brautigam Equation Curve Fit to PupB NTSD L74A Forward Melting
↳ Data')
plt.savefig(fn)
plt.show()

```

## Brautigam Equation Curve Fit to PupB NTSD L74A Forward Melting Data

