derivation_signal_vs_temp_fiting_model

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1 Use of the Integrated van't Hoff Equation to Treat Two-State Protein Denaturation

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Thermodynamics of Protein Denaturation Consider the equilibrium between the native (N) and random coil (C) states of a protein:

$$N \rightleftharpoons C$$

and recall that the equilibrium constant for this process is:

$$K = \frac{C}{N}$$

in which: C is the concentration of the random coil species, and N is the concentration of the native species. At low temperatures, the equilibrium constant will have values much smaller than 1, indicating that most of the protein molecules are found in the native state at those temeperatures. In contrast, at high temperatures, the equilibrium constant will have values much larger than 1, indicating that the population of the random coil state predominates over that of the native state. At intermediate temperatures, the equilibrium constant will have intermediate values, and at a particular temperature, the melting temperature T_M, the populations of native and random coil states will be precisely equal and the equilibrium constant will have a value of 1. The van't Hoff Equation The temperature dependence of the equilibrium constant is given by the linear form of the van't Hoff equation:[†]

$$ln(K) = -\frac{\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta S^\circ}{R}$$

If the value of the equilibrium constant is known at any one temperature, its value at another temperature, e.g. T_M, may be estimated using the integrated form of the equation:[‡]

$$ln(\frac{K}{K_{T_M}}) = -\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})$$

Because, in this case $K_{T_M} = 1$, this simplifies even further:

$$ln(K) = -\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})$$

To make use of this equation to estimate protein-specific parameters like melting temperature and enthalpy of denaturation, it is necessary to establish a relationship between the denaturation

equilibrium constant at an arbitrary temperature and an observable signal that varies as a function of temperature. Usually this signal is spectroscopic.

†: It is assumed that ΔH and ΔS are temperature-independent, which is often reasonable over small ΔT; in general ΔH and ΔS are temperature-dependent functions of the heat capacity. ‡: To prepare Brautigam's version, factor $\frac{1}{T}$ out of the parenthetical term in what follows.

Total and Fractional Protein Concentrations & Reformulation of the Equilibrium Constant

In a two-state protein denaturation equilibrium consisting of an N state and a C state, let: T be the total protein concentration, the sum of that in each of states N and C f_N be the fraction of total protein found in state N, and f_C be the fraction of total protein found in state C. Algebraically:

$$T = N + C$$

$$f_N = \frac{N}{T} = \frac{N}{N + C}$$

$$f_C = \frac{C}{T} = \frac{C}{N + C}$$

$$f_N + f_C = \frac{N}{N + C} + \frac{C}{N + C} = \frac{N + C}{N + C} = 1$$

If:

$$K = \frac{C}{N}$$

then, substituting $f_C * T$ and $f_N * T$ for C and N respectively, provides:

$$K = \frac{f_C * T}{f_N * T}$$

and T drops out to afford:

$$K = \frac{f_C}{f_N}$$

Substituting $1 - f_N$ for f_C eliminates an unnecessary parameter:

$$K = \frac{1 - f_N}{f_N}$$

Fractional Protein Concentrations &the Observed Signal Because the signal that is observed at any moment must derive from either the native conformation of the protein or its random coil conformation, it must be a linear combination of each of these signals weighted by the fraction of total protein molecules found in each of those states. Algebraically:

$$\sigma = f_N \sigma_N + f_C \sigma_C$$

Again f_C is eliminated as above:

$$\sigma = f_N \sigma_N + (1 - f_N) \sigma_C$$

from which an expression isolating f_N may be prepared:

$$f_N = \frac{\sigma - \sigma_C}{\sigma_N - \sigma_C}$$

Substitution of this result into that from the section above for the equilibrium constant provides:

$$K = \frac{1 - \frac{\sigma - \sigma_C}{\sigma_N - \sigma_C}}{\frac{\sigma - \sigma_C}{\sigma_N - \sigma_C}}$$

This rearranges to the tractable:

$$K = \frac{\sigma_N - \sigma}{\sigma - \sigma_C}$$

which may be substituted into the integrated form of the van't Hoff equation:

$$ln(K) = -\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})$$

to provide:

$$ln(\frac{\sigma_N - \sigma}{\sigma - \sigma_C}) = -\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})$$

Exponentiation yields:

$$\frac{\sigma_N - \sigma}{\sigma - \sigma_C} = e^{-\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})}$$

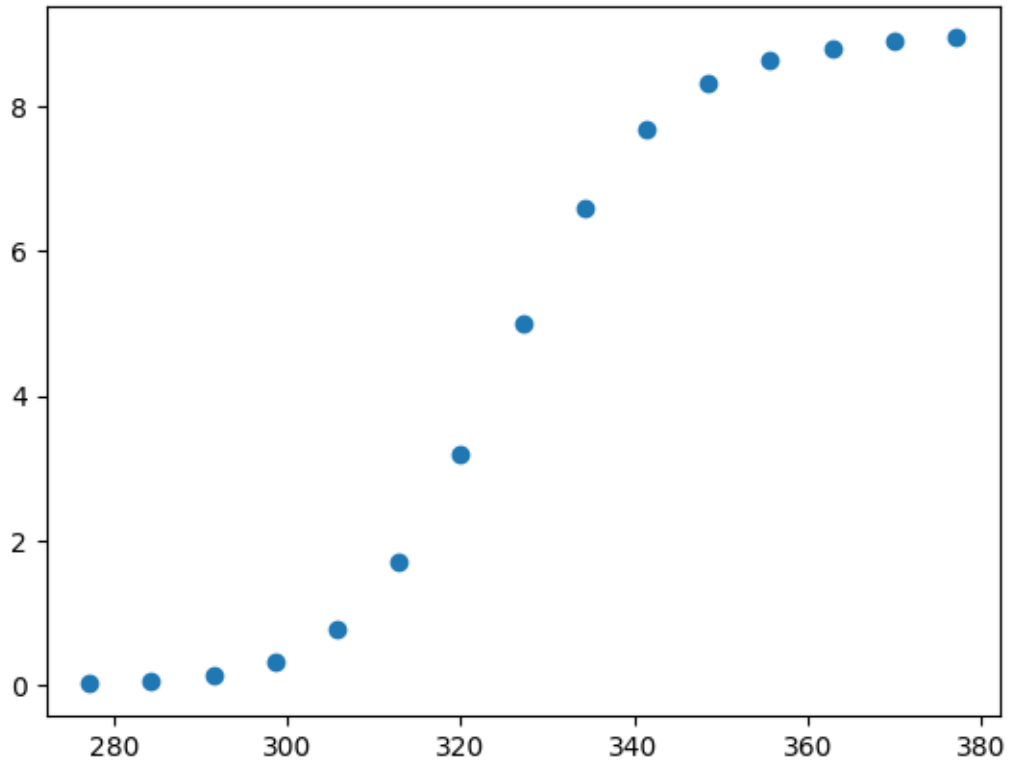
from which an expression for the observed signal in terms of temperature and fittable parameters ΔH° and T_M may be prepared:

$$\sigma = \frac{\sigma_N + \sigma_C e^{-\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})}}{1 + e^{-\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})}}$$

Here are computations for a plot with σ_N=0, σ_C=9, ΔH = 100 kJ/mol and T_M = 52°C.

[1]:
```
import os
import numpy as np
import matplotlib.pyplot as plt
from scipy.optimize import curve_fit
import scipy.stats as stats

def sigmafunc(T, sigmaN, sigmaC, deltaH, TM, R=8.3144):
    Q=np.exp(-deltaH/R*(1/T-1/TM))
    return ((sigmaN+sigmaC*Q)/(1+Q))

mintemp = 4 # degrees C
maxtemp = 104
mintemp += 273.15 # conversion to Kelvin
maxtemp += 273.15
T = np.linspace(mintemp, maxtemp, 15)

sigmaN = 0 # arbitrary
sigmaC = 9 # arbitrary, but != sigmaN
deltaH = 100000 # Joules per mol, because if undeclared, R=8.3144 Joules per␣
 ↪(Kelvin mol)
TM = 52 # degrees C
```

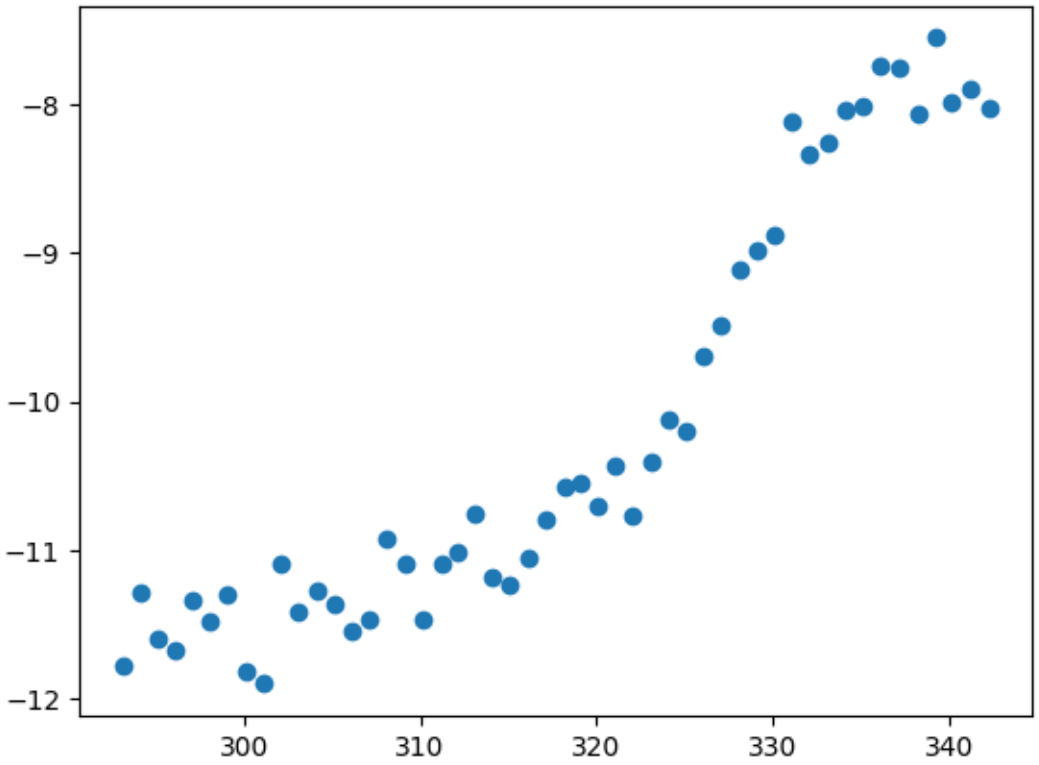
```
TM += 273.15 # conversion to Kelvin

sigma = sigmafunc(T, sigmaN, sigmaC, deltaH, TM)
plt.scatter(T,sigma)
plt.show()
```



Application to Experimental Date Here is a scatter plot of actual data CD data at 217 nm for the PupB NTSD L74A mutant in the temperature range ~290 K to ~350 K:

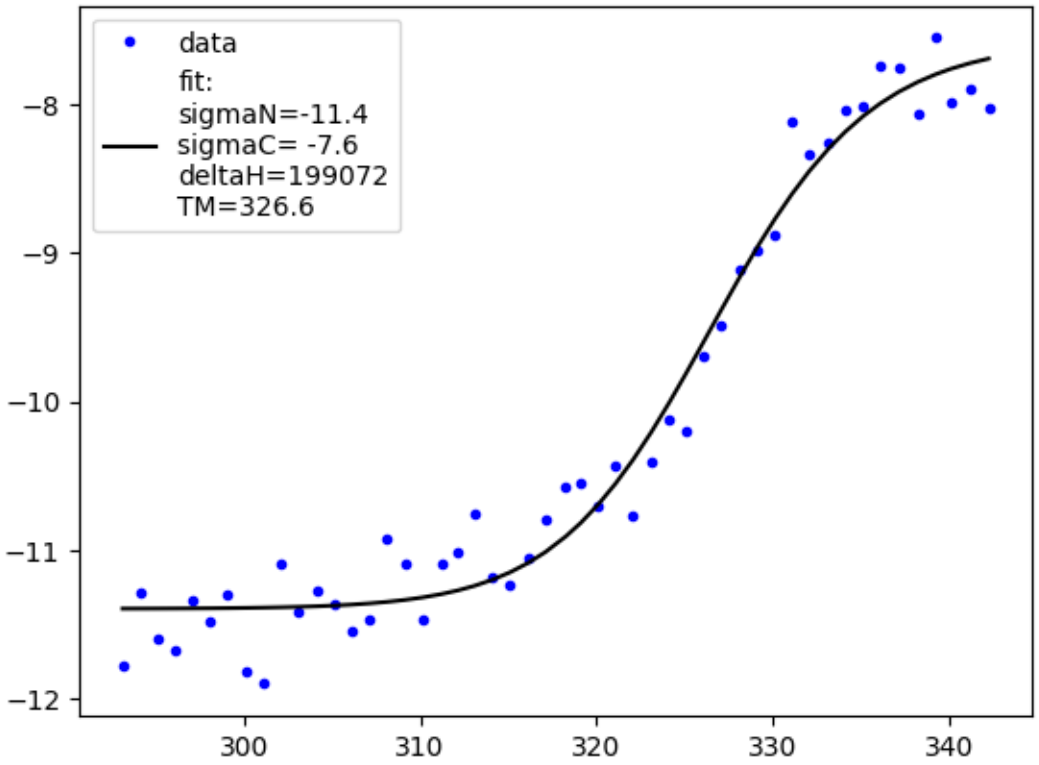
[2]:
```
basedir = '/home/david/gh/intro_curve_fitting_python'
datadir = basedir+'/thermal_denaturation_data'
fn = datadir+'/PupB NTSD L74A 25 uM 217 nm F.csv'
os.chdir(datadir)

# incidentally, here is another way to read csv data
my_data = np.genfromtxt(fn, delimiter=',')

# slicing syntax '[:,[i]]' = 'the i'th column';
# note the implied 'from the 0th row to the last row' in the
# absence of values before and after the colon
T = my_data[:,[0]].flatten()
y = my_data[:,[1]].flatten()
```

```
plt.scatter(T,y)
plt.show()
```



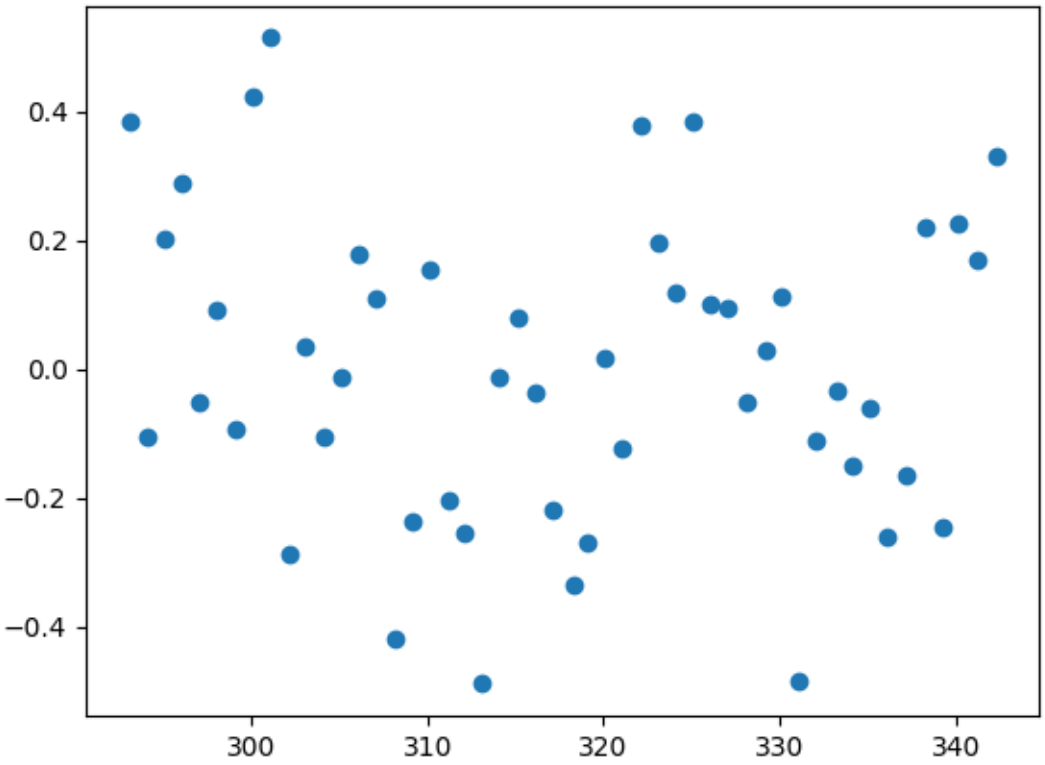
The lower temperature region shows a typical 'sloping' baseline. Although the fitting equation does not yet include appropriate corrections, I fit the data with it nonetheless to set up the exercise in F-statistics at the end of this document. Note that there are four adjustable (fittable) parameters in this equation: σ_N, σ_C, ΔH, and T_M.Carry out the fit to the four-parameter version:

[3]:
```
# sigmafunc(T, sigmaN, sigmaC, deltaH, TM, R=8.3144):
popt, pcov = curve_fit(sigmafunc, T, y, p0=[-11, -7.5, 28561, 329])

plt.plot(T, y, 'b.', label='data')
plt.plot(T, sigmafunc(T, *popt), 'k-',
        label='fit:\nsigmaN=%5.1f\nsigmaC=%5.1f\ndeltaH=%5.0f\nTM=%5.1f' %␣
 ↪tuple(popt))
plt.legend()
plt.show()
```



Compute residuals:

[4]:
```
# calculate residuals:
sigmafuncresiduals = sigmafunc(T, *popt) - y

# residuals plot
plt.scatter(T, sigmafuncresiduals)
plt.show()
```



Compute R-squared

[5]:
```
sigmafuncssres = np.sum(np.square(sigmafuncresiduals))
sigmafuncsstot = np.sum(np.square(y-np.mean(y)))
Rsquared = 1-sigmafuncssres/sigmafuncsstot
print('Rsquared: %1.3f' % Rsquared)
```

```
Rsquared: 0.972
```

Addition of Sloping Baseline Corrections to the Fitting Function Now, let's extend the model to encode the equations of lines in place of the constants σ_N and σ_C. These substitutions are simple:

$$\sigma_N \rightarrow m_1 T + b_1$$

and

$$\sigma_C \rightarrow m_2 T + b_2$$

and produce:

$$\sigma = \frac{m_1 T + b_1 + (m_2 T + b_2) e^{-\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})}}{1 + e^{-\frac{\Delta H^\circ}{R}(\frac{1}{T} - \frac{1}{T_M})}}$$

Note that there are six fittable parameters in this equation: m_1, b_1, m_2, b_2, ΔH, and T_M.

Define the revised function

[6]:
```
def sigmaslopingbaselines(T, m1, b1, m2, b2, deltaH, TM, R=8.3144):
    line1 = m1*T+b1
    line2 = m2*T+b2
    Q=np.exp(-deltaH/R*(1/T-1/TM))
    return ((line1+line2*Q)/(1+Q))
```

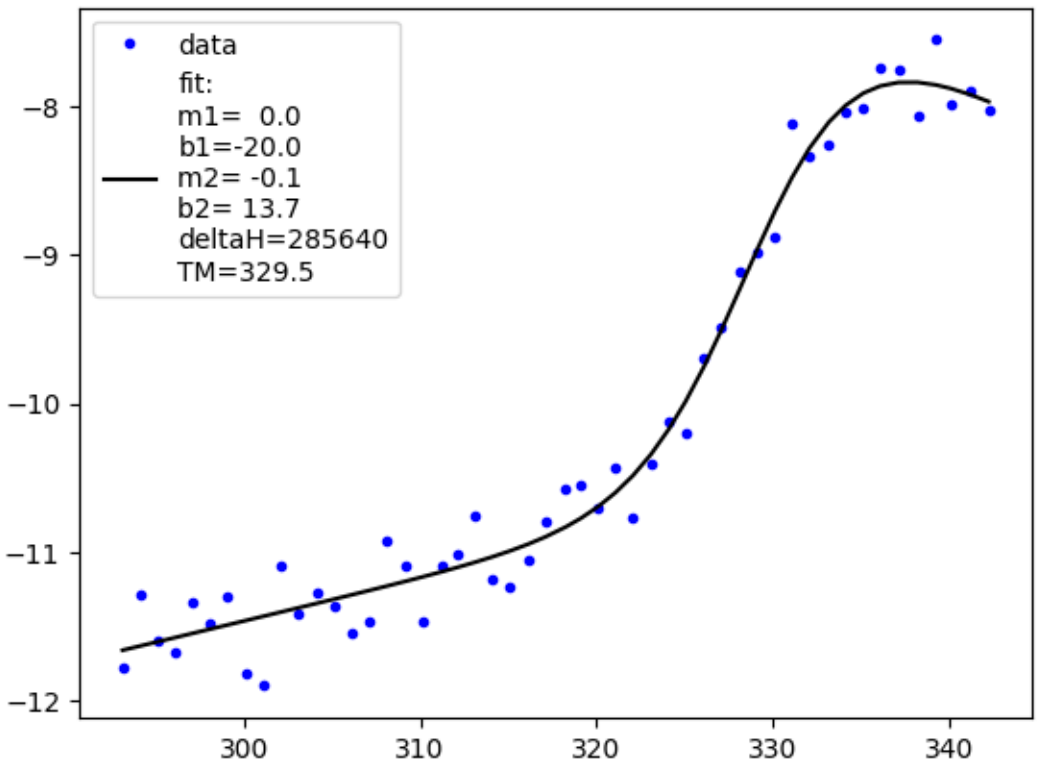
Estimate parameters for the low and high temperature lines:

[7]:
```
# get the first 20 points for a low temp line
eks = T[:20]
why = y[:20]
def line(x, m, b):
    return m*x+b

ltlineparam, pcov = curve_fit(line, eks, why)
print('low temperature line:\n\tslope: %1.4f\tintercept: %2.2f' %␣
 ↪tuple(ltlineparam))

# get the last 12 points for a high temp line
eks = T[-12:]
why = y[-12:]

htlineparam, pcov = curve_fit(line, eks, why)
print('high temperature line:\n\tslope: %1.4f\tintercept: %2.2f' %␣
 ↪tuple(htlineparam))
```

```
low temperature line:
        slope: 0.0255   intercept: -19.11
high temperature line:
        slope: 0.0321   intercept: -18.78
```

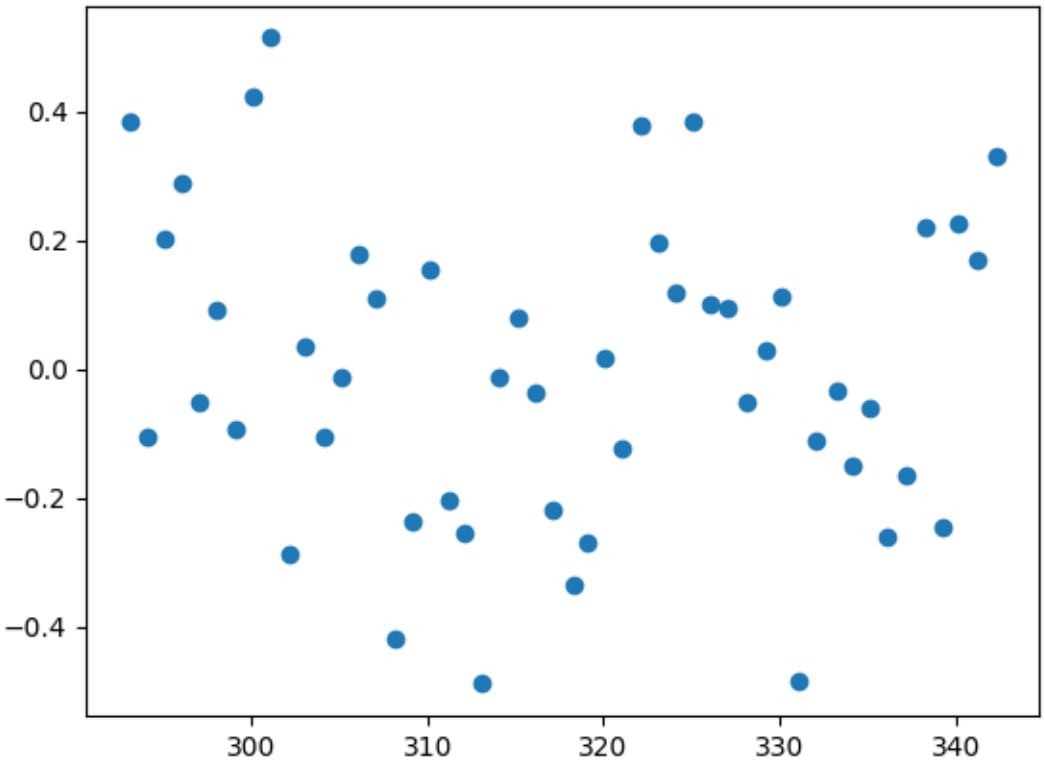
Carry out the fit to the six-parameter version:

[8]:
```
# def sigmaslopingbaselines(T, m1, b1, m2, b2, deltaH, TM, R=8.3144):
p=[ltlineparam[0], ltlineparam[1], htlineparam[0], htlineparam[1], 100000, 335]
popt, pcov = curve_fit(sigmaslopingbaselines, T, y, p0=p)

plt.plot(T, y, 'b.', label='data')
plt.plot(T, sigmaslopingbaselines(T, *popt), 'k-',
        label='fit:\nm1=%5.1f\nb1=%5.1f\nm2=%5.1f\nb2=%5.1f\ndeltaH=%5.
 ↪0f\nTM=%5.1f' % tuple(popt))
plt.legend()
plt.show()
```



Calculate residuals:

[9]:
```
sigmaslopingresiduals = sigmaslopingbaselines(T, *popt) - y

# residuals plot
plt.scatter(T, sigmafuncresiduals)
plt.show()
```



Compute R-squared:

[10]:
```
sigmaslopingssres = np.sum(np.square(sigmaslopingresiduals))
sigmafuncsstot = np.sum(np.square(y-np.mean(y)))
Rsquared = 1-sigmaslopingssres/sigmafuncsstot
print('Rsquared: %1.3f' % Rsquared)
```

```
Rsquared: 0.981
```

By comparison of R-squared values, there is a marginally better fit to the data using the six-parameter equation than using the four-parameter equation. But, as the number of parameters improve, so does R-squared. An F-test is a more sophisticated way to compare the two fits. A good introductory summary of F-statistics may be found hereCarry out F-test

[11]:
```
# borrows heavily from:
# https://www.geeksforgeeks.org/how-to-perform-an-f-test-in-python/

# populations to be tested
# sigmafuncresiduals
# sigmaslopingresiduals

# Calculate the sample variances
# variance1 = np.var(group1, ddof=1)
```

```
# variance2 = np.var(group2, ddof=1)
# rather, I wished to understand and implement the formula myself
var1 = 1/(-1+len(sigmafuncresiduals))*(np.sum((sigmafuncresiduals-np.
 ↪mean(sigmafuncresiduals))**2))
var2 = 1/(-1+len(sigmaslopingresiduals))*(np.sum((sigmaslopingresiduals-np.
 ↪mean(sigmaslopingresiduals))**2))

# Calculate the F-statistic
f_value = var1 / var2

# Calculate the degrees of freedom
df1 = len(sigmafuncresiduals) - 1 - 4 # 1 for the mean used in the computation␣
 ↪of the variance; 4 fittable parameters in sigmafunc
df2 = len(sigmaslopingresiduals) - 1 - 6 # 1 for the mean used in the␣
 ↪computation of the variance; 6 fittable parameters in sigmasloping

# Calculate the p-value
p_value = stats.f.cdf(f_value, df1, df2)

# Print the results
print('Degree of freedom 1:',df1)
print('Degree of freedom 2:',df2)
print('Variance 1:', var1)
print('Variance 2:', var2)
print("F-statistic:", f_value)
print("p-value:", p_value)
```

```
Degree of freedom 1: 45
Degree of freedom 2: 43
Variance 1: 0.0567750215081515
Variance 2: 0.03817603070863092
F-statistic: 1.4871902723851196
p-value: 0.9032124843017073
```

Interpretation

[ ]: