

# UV melting experiments

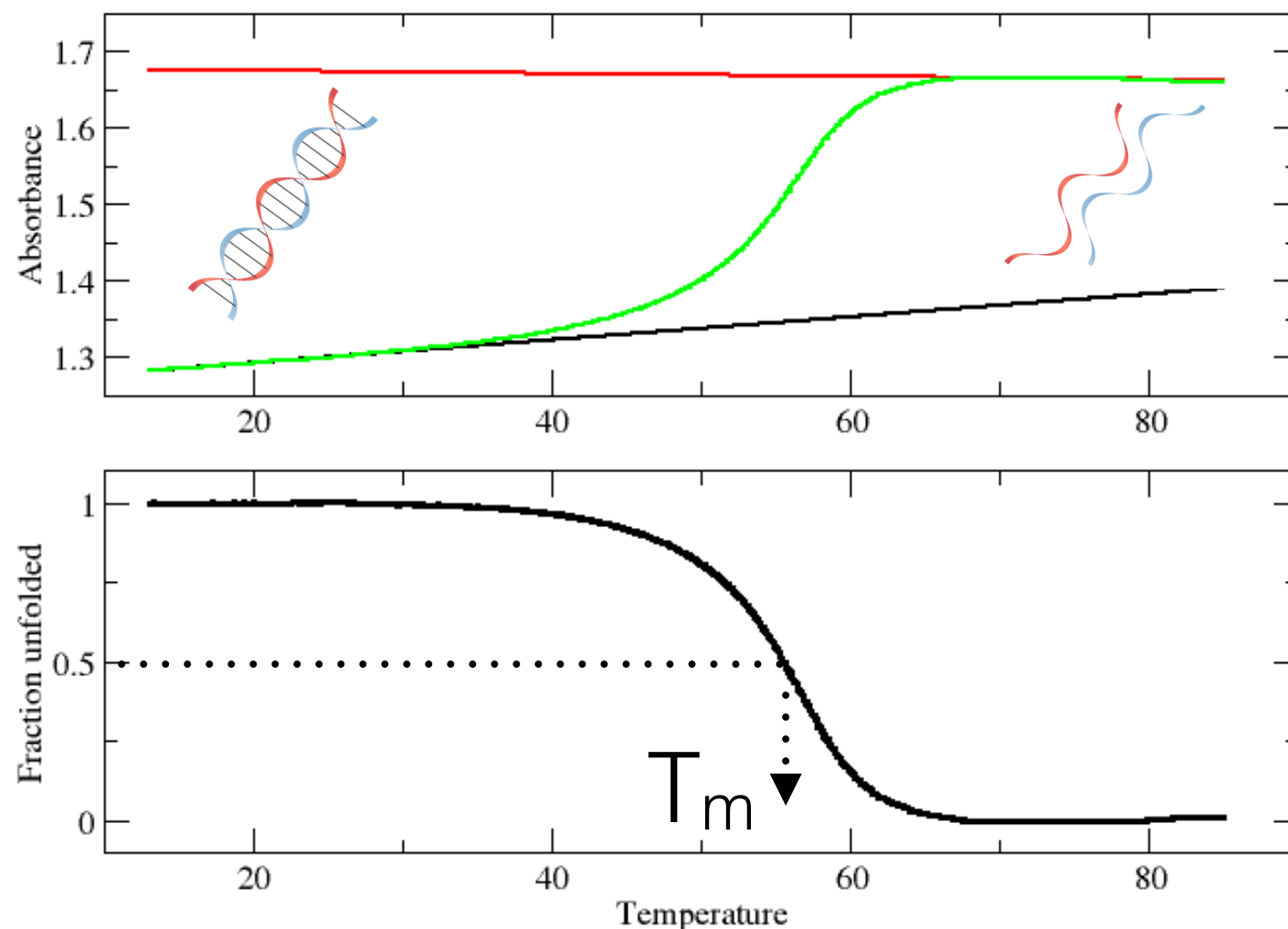
Simple setup - (many) useful informations



Biomolecular  
Machines  
Laboratory

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# UV melting experiments



Experimental curve

Linear fit of:

low T plateau ( $A_D$ )

high T plateau ( $A_S$ )

$$f_D = \frac{A - A_S}{A_D - A_S}$$

melting temperature  
definition:  $f_D(T_m) = 0.5$

# Why you are not using this method?

- You don't believe me, that it's useful
- You didn't know how to do it
- There wasn't a tool available that would do it automatically
- Most of us use functions from the software that came with the spectrophotometer (mainly differentiate).

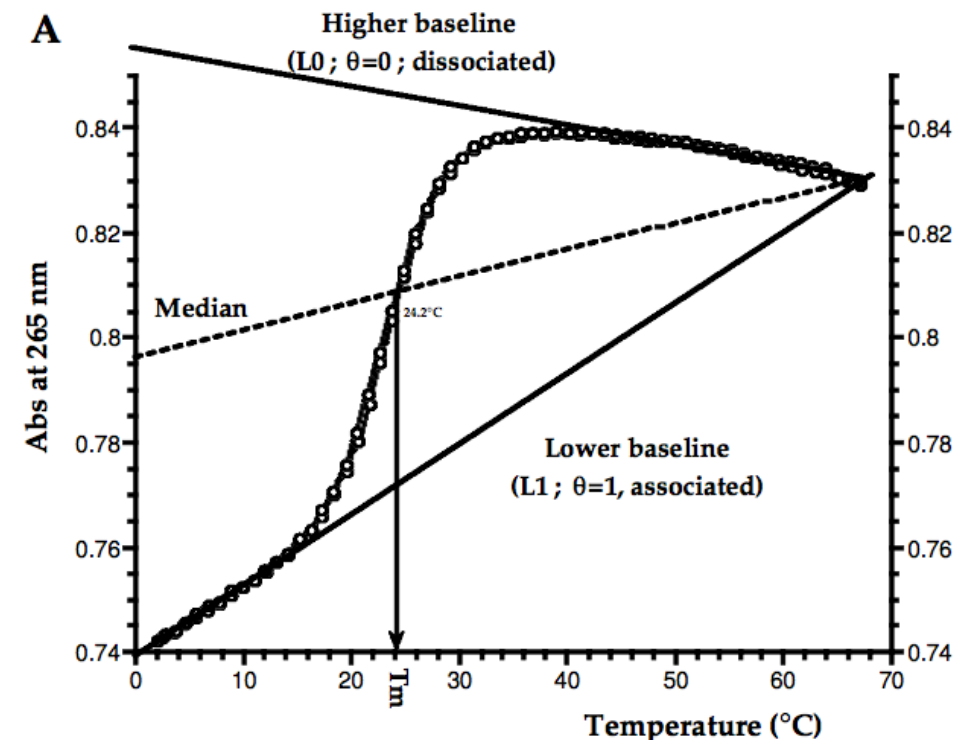
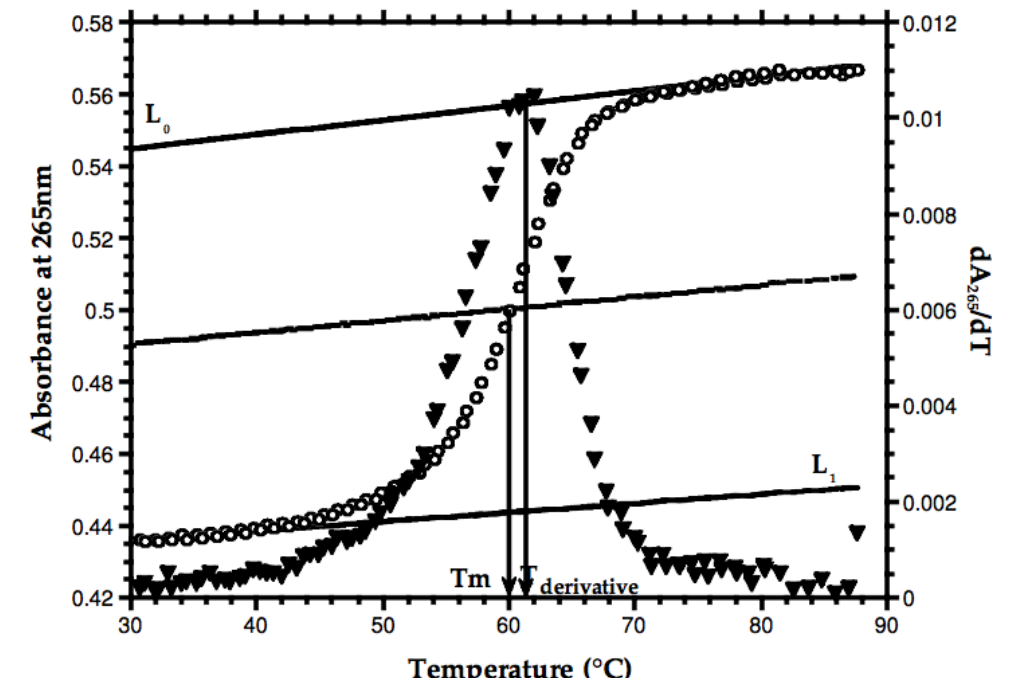
# Why it should be used?

- Because other methods do not work

TABLE 1. DOES THE FIRST DERIVATIVE MAXIMUM CORRESPOND TO THE  $T_M$ ?<sup>a</sup>

Derivative	Equilibrium	
	Intramolecular	Bimolecular
$dA/dT$	No <sup>b</sup>	No <sup>b</sup>
$dA/d(1/T)$	No <sup>b</sup>	No <sup>b</sup>
$d\theta/dT$	Almost <sup>c</sup>	No <sup>d</sup>
$d\theta/d(1/T)$	Yes	No <sup>d</sup>

Mergny, JL, Lacroix, L (2003). Analysis of thermal melting curves. Oligonucleotides, 13, 6:515-37.

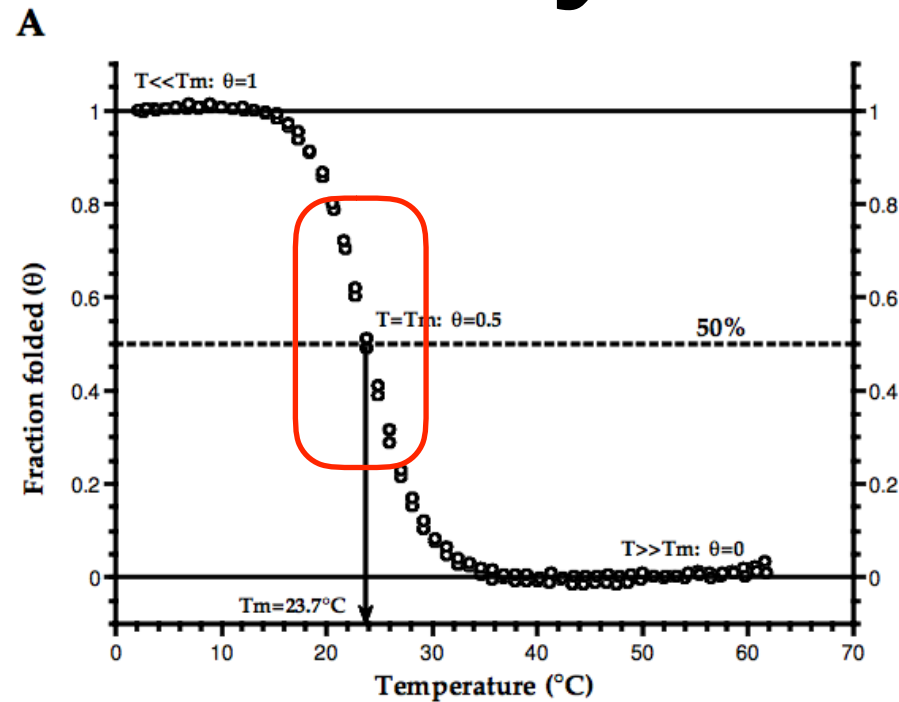


# From f, K vs T can be derived

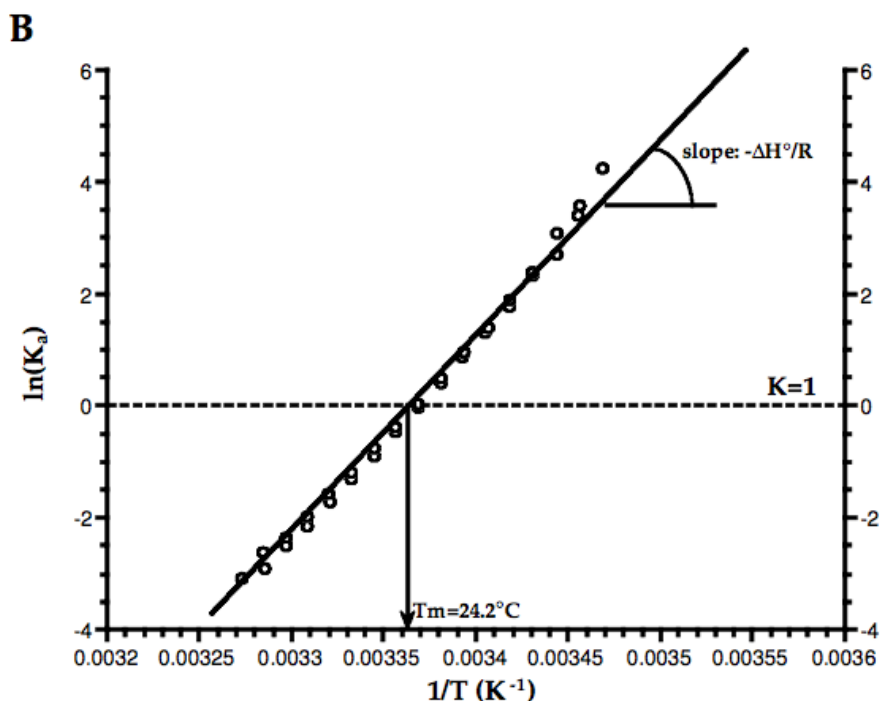
TABLE II  
TWO-STATE ANALYSIS OF NUCLEIC ACID TRANSITIONS

Reaction type	Equilibrium constants	$\Delta H^\circ$ from slope of $f$ versus $T$	Concentration dependence of $T_m$
Monomolecular $S = H$	$K = \frac{[H]}{[S]} = \frac{f}{(1-f)}$	$\Delta H^\circ = 4RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	
Bimolecular (self-complementary) $2S = D$	$K = \frac{[D]}{[S]^2} = \frac{f}{2(1-f)^2 c_i}$	$\Delta H^\circ = 6RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln c_i + \frac{\Delta S^\circ}{\Delta H^\circ}$
Bimolecular (non-self-complementary) $S_A + S_B = D$	$K = \frac{[D]}{[S_A][S_B]} = \frac{2f}{(1-f)^2 c_i}$	$\Delta H^\circ = 6RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - R \ln 4)}{\Delta H^\circ}$
Trimolecular (identical strands) $3S = T$	$K = \frac{[T]}{[S]^3} = \frac{f}{3c_i^2(1-f)^3}$	$\Delta H^\circ = 8RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{2R}{\Delta H^\circ} \ln c_i + \frac{[\Delta S^\circ - R \ln 4/3]}{\Delta H^\circ}$
Trimolecular (nonidentical strands) $S_A + S_B + S_C = T$	$K = \frac{[T]}{[S_A][S_B][S_C]} = \frac{9f}{c_i^2(1-f)^3}$	$\Delta H^\circ = 8RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{2R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - 2R \ln 6)}{\Delta H^\circ}$
Tetramolecular (identical strands) $4S = Q$	$K = \frac{[Q]}{[S]^4} = \frac{f}{4c_i^3(1-f)^4}$	$\Delta H^\circ = 10RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{3R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - R \ln 2)}{\Delta H^\circ}$
Tetramolecular (nonidentical strands) $S_A + S_B + S_C + S_D = Q$	$K = \frac{[Q]}{[S_A][S_B][S_C][S_D]} = \frac{64f}{c_i^3(1-f)^4}$	$\Delta H^\circ = 10RT_m^2 \left( \frac{df}{dT} \right)_{T=T_m}$	$\frac{1}{T_m} = \frac{3R}{\Delta H^\circ} \ln c_i + \frac{(\Delta S^\circ - 3R \ln 8)}{\Delta H^\circ}$

# Why is K important?



Perform fitting in the T range  
where f is linear:  $0.2 < f < 0.8$



It has an energy inside

$$\Delta G = -RT \ln K$$

$$\Delta G = \Delta H - T\Delta S$$

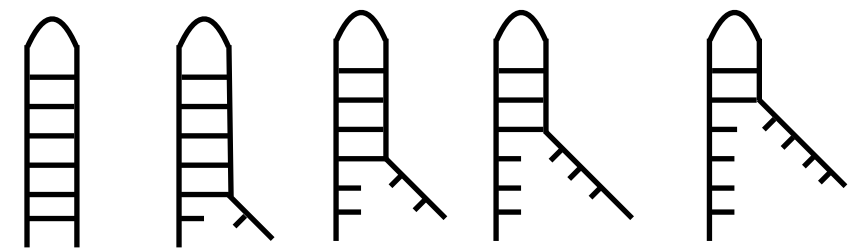
By fitting a linear function to the  
 $\ln(K)$  vs  $1/T$  one can derive  
both enthalpy and entropy

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

# Hill's coefficient

$$f(T) = \frac{T^b}{T_m^b + T^b}$$

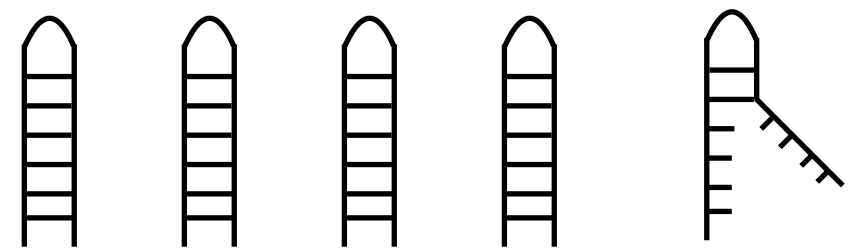
- $b$  = Hill's coefficient
- Describes cooperativity of binding



Hill's coefficient small

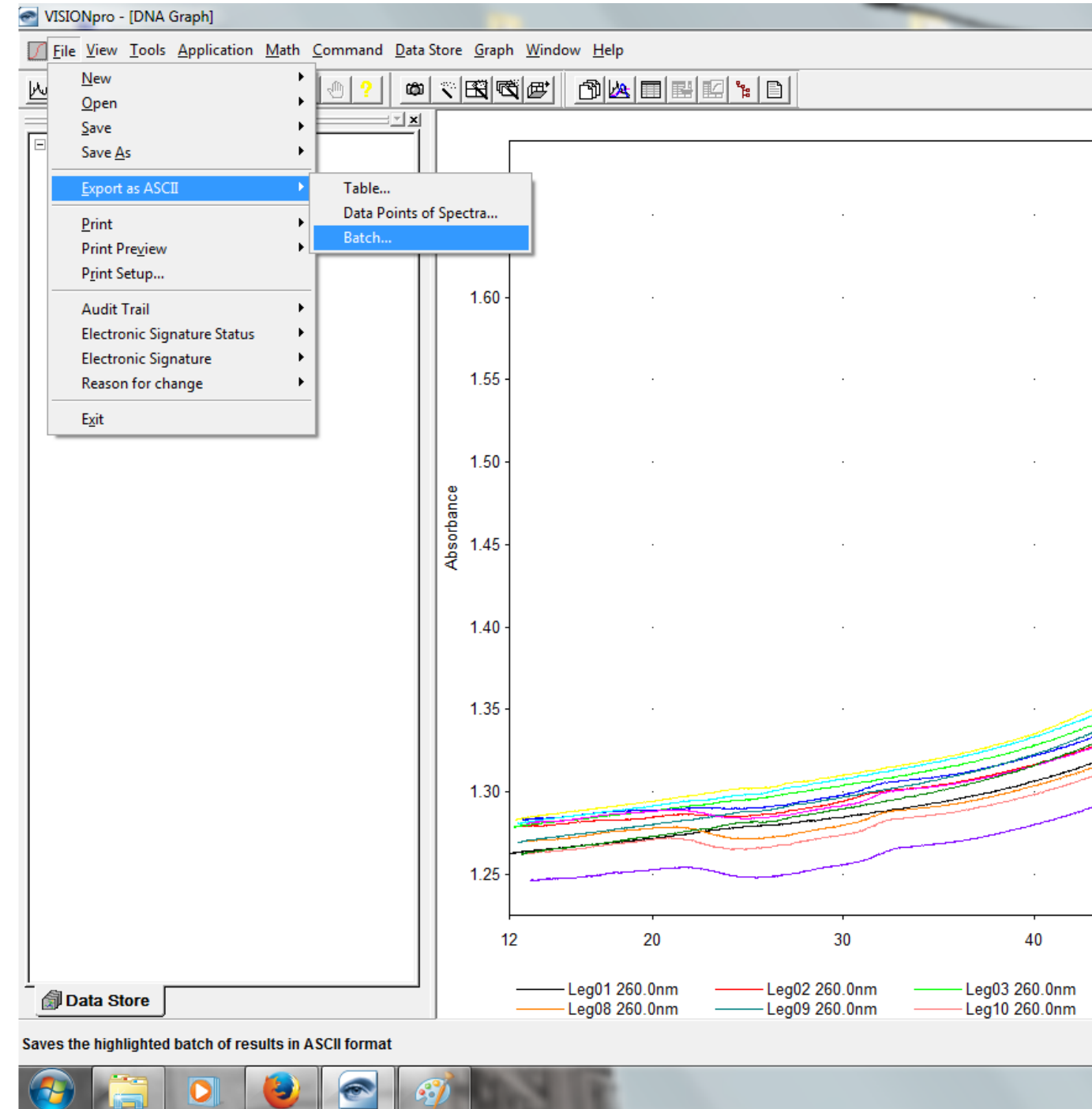


Hill's coefficient large



# I've made perfect tool

- Works on the ASCII (.txt) files from UV-Vis Nicolet Evolution 300 Spectrophotometer (Thermo Scientific)
- Analyses:
  - $T_m$
  - energy: dH, dS and dG
  - Hill's coefficient
- Need a Linux





# How to use it?

- Download from the <https://github.com/maciejj/UV-Tm>
- Adjust editable parameters in the Tm.py
- Run:  
`python Tm.py <filename>.txt`
- Let the magic happen

# Parameters

#####

#Adjustable parameters:

#Temperature in C for energy analysis:

Tenergy = 20

#Reaction type

#monomolecular=1; bimolecular(selfcomplementary)=2; bimolecular(non-self-complementary)=3

reactionType=1

#concentration in M; needed only when reactionType is not equal to 1

concentration=0.000002

#Temperature range in C for analyze. Zero values mean, it analyses whole curves

Tlow=0

Tmax=0

#Range for the lnK fitting. Usually 0.2 - 0.8 is OK

#If not try for example 0.3 to 0.7

lnKlow=0.2

lnKhigh=0.8

#####

# Outputs

- Files with calculated values of:
  - melting temperatures <filename>.Tms
  - thermodynamic parameters <filename>.Energy
  - Hill's coefficients <filename>.Hills
- Multiple xmgrace ready files
  - <filename>\_f.out
  - <filename>\_fit.out
  - <filename>\_lnK\_fit.out

# Magic happens

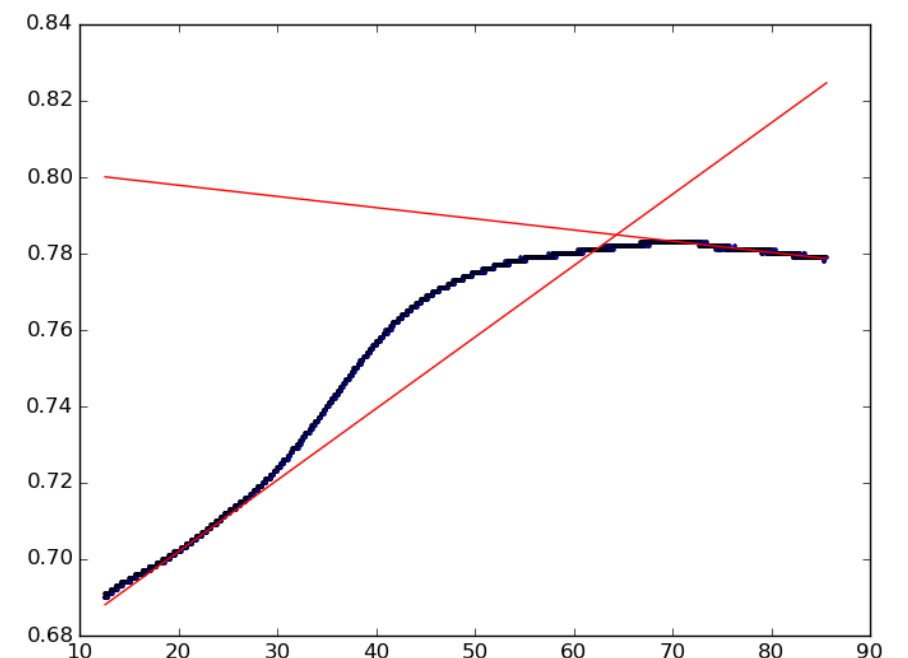
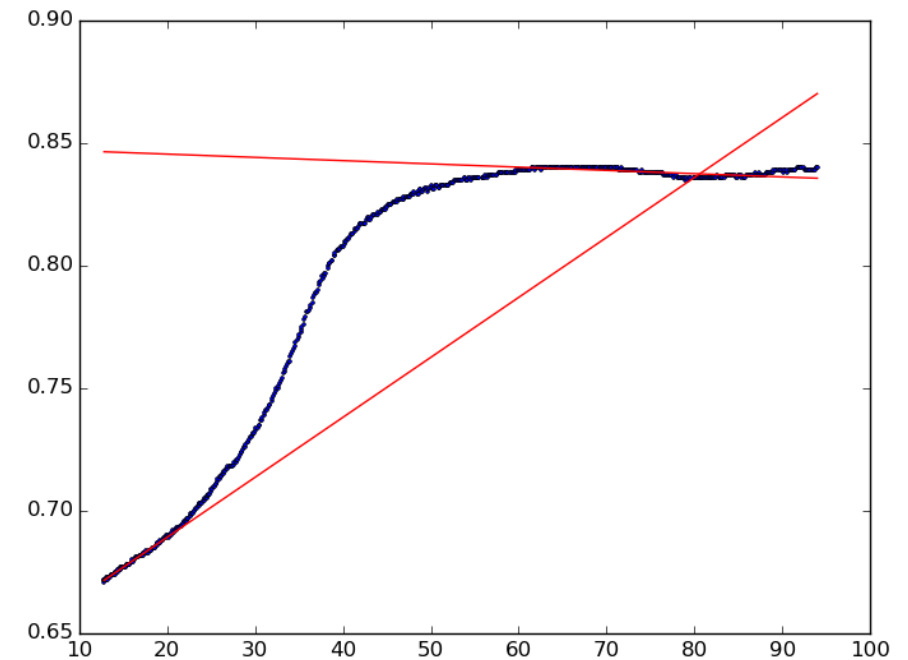


# What can go wrong?

- Lower and upper plateau should not intersect

$$f_D = \frac{A - A_S}{A_D - A_S}$$

- Set different temperature range for analysis e.g.  
Tlow=15  
Tmax=70



# What can go wrong

- The  $\ln(K)$  vs  $1/T$  is not linear
- Investigate the  $f$  vs  $T$  curve
- Set different  $f$  range for energy analysis e.g.  
 $\ln K_{\text{low}}=0.3$   
 $\ln K_{\text{high}}=0.7$

