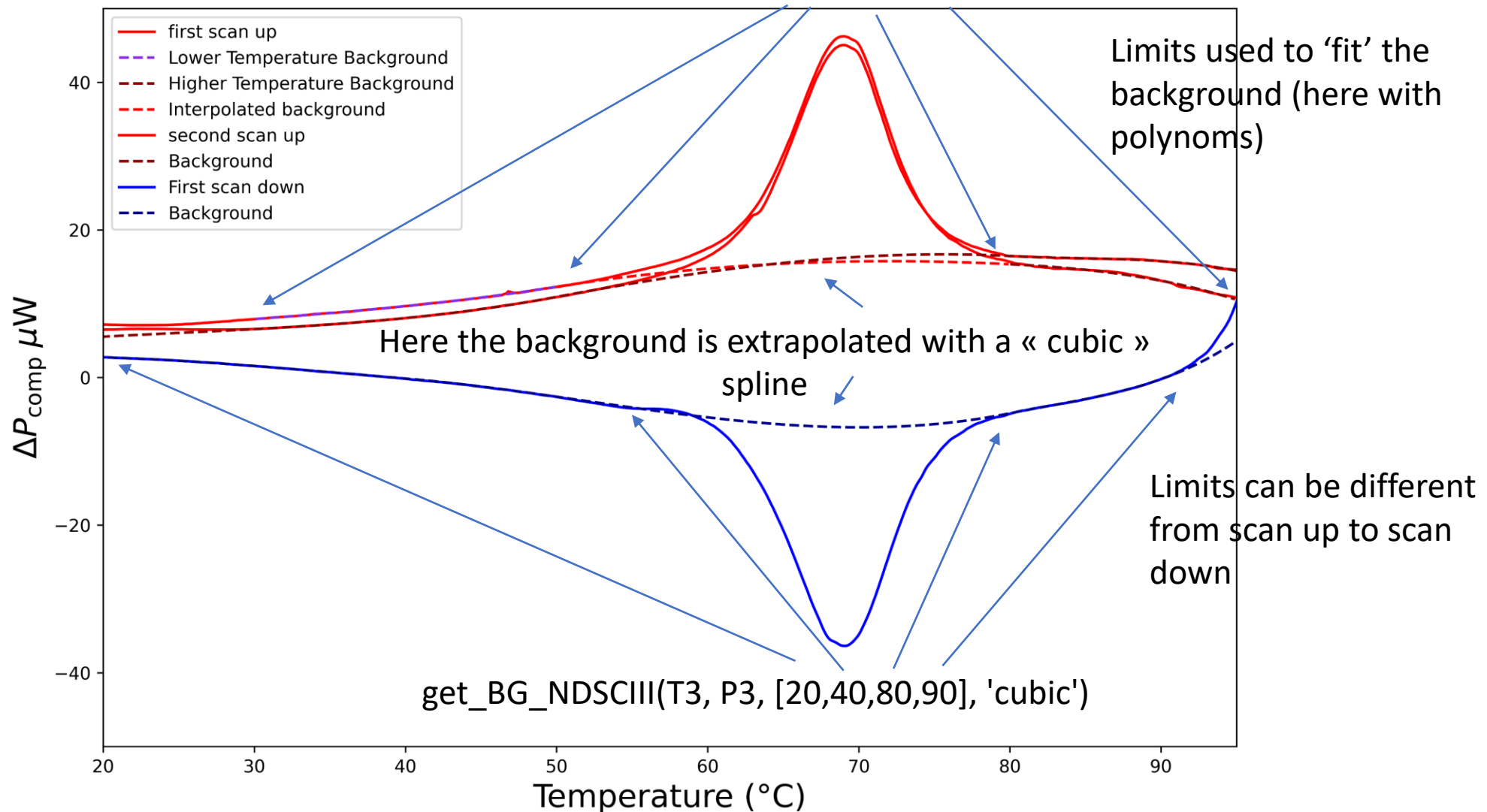Raw data for the 08/11/2022 1PTSPOOL 50uM STP2LOOP8 50uM experiment
At least one raw data should be plotted and discussed ….
With computed background (see python code)

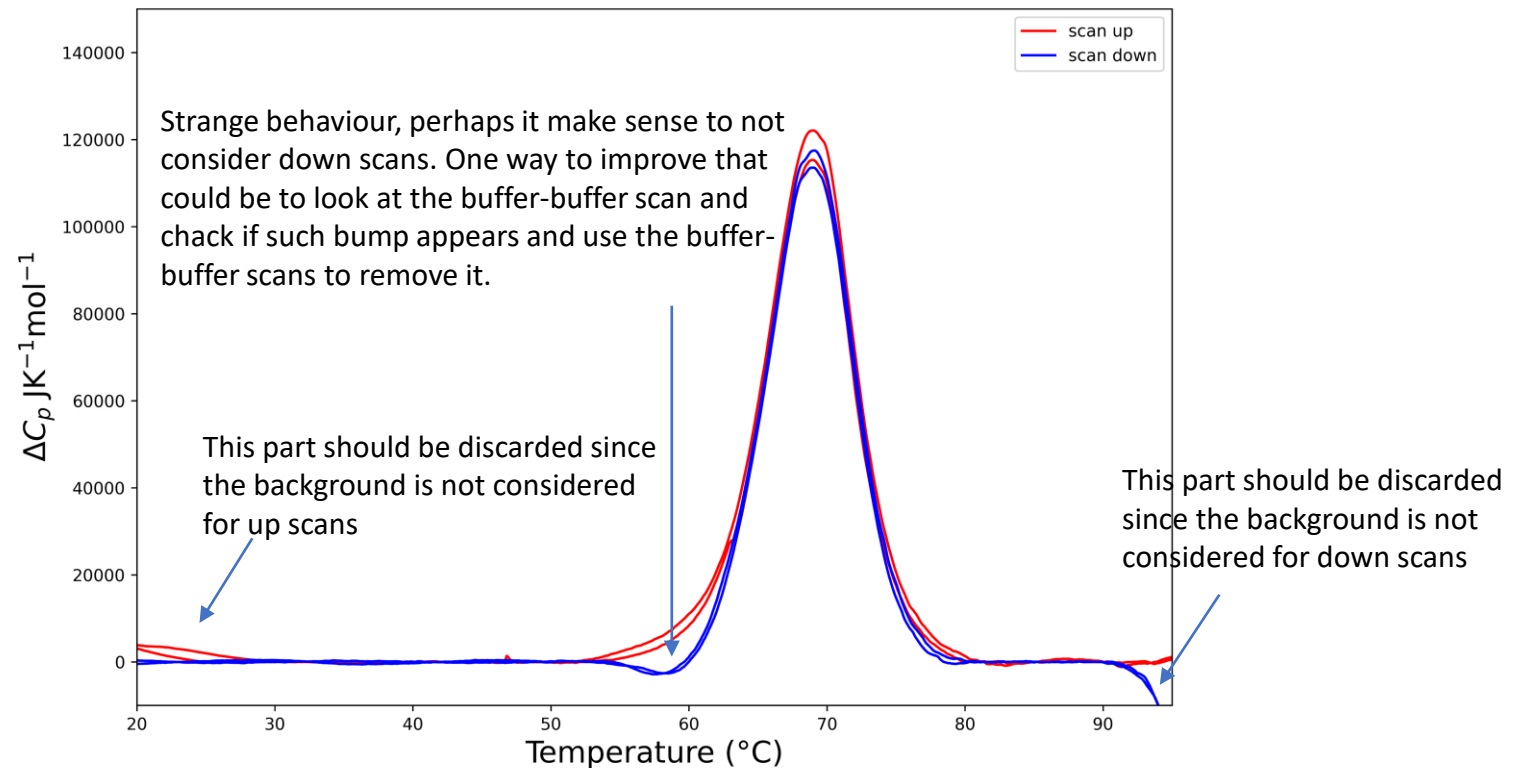get_BG_NDSCIII(T1, P1, [30,50,80,95], 'cubic')

Limits used to 'fit' the background (here with polynoms)

Here the background is extrapolated with a « cubic » spline

Limits can be different from scan up to scan down

get_BG_NDSCIII(T3, P3, [20,40,80,90], 'cubic')

The molar heat capacity is computed from the excess compensation power as:

$$\Delta C_p = \frac{P_{comp} - P_{background}}{\gamma \times V_0 \times C_0}$$

- P_{comp} is the measured raw data
- $P_{background}$ is the determined background
- γ is the scan rate (1/60 K/s)
- $V_0 = 300 \ \mu L$ is the sample volume
- C_0 is the experimental double strand concentration 50µM in our exemple

Strange behaviour, perhaps it make sense to not consider down scans. One way to improve that could be to look at the buffer-buffer scan and chack if such bump appears and use the buffer-buffer scans to remove it.

This part should be discarded since the background is not considered for up scans

This part should be discarded since the background is not considered for down scans

The molar enthalpy and molar entropy are computed from the molar heat capacity:

$$\Delta H = \int_{T_{min}}^{T_{max}} \Delta C_p \, dT \qquad \qquad \Delta S = \int_{T_{min}}^{T_{max}} \frac{\Delta C_p}{T} \, dT$$

Set the range of integration according to the range used for the background
In the exemples below it was typically between Tmin = 30°C and Tmax= 90°C.

Gives and idea of the experimental 'errors'
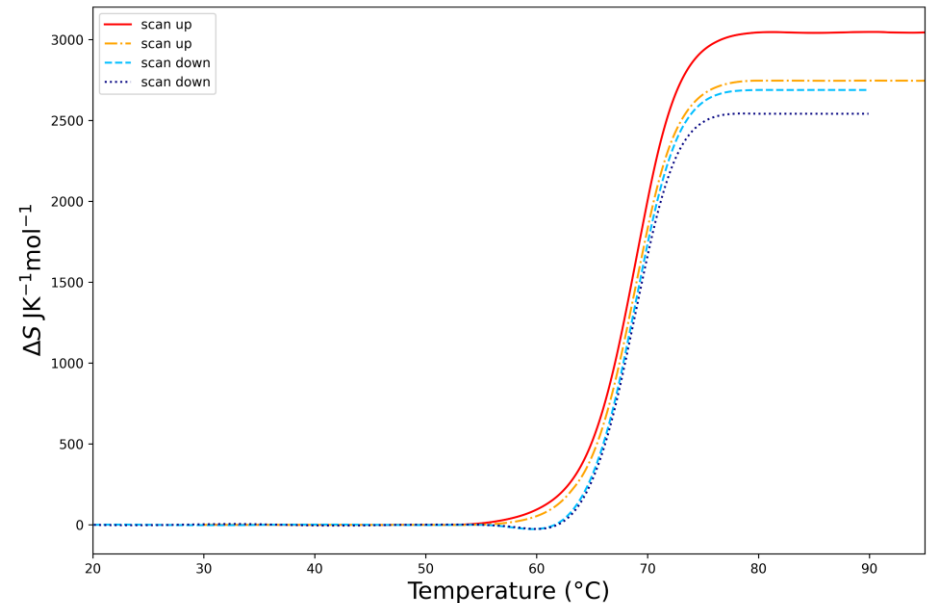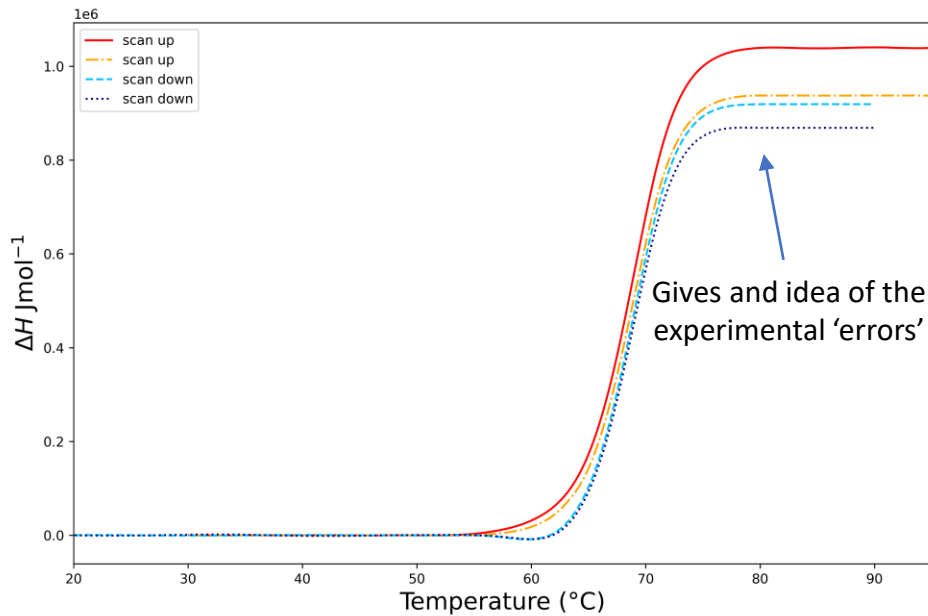
The graphs gives you a value

Theoretical analysis using standard thermodynamic tables or web servers

Nupack web server (http://www.nupack.org)

Analysis	Design	Utilities		Downloads
Input	Results		Demos	Help

Nucleic acid type: ○ RNA ⦿ DNA ❓

Temperature: [25] °C ❓   Compute melt: ☐ ❓

Number of strand species: [2 ▾] ❓   Maximum complex size: [2 ▾] strands ❓

Strand species

strand1 : CACCGTGCTTAGAGTGGCTTCTATTGACATGC

Concentration: [50] [µM ▾]

strand2 : CACTCTAAGCACGGTGTTTTTTTTGCATGTCAATAGAAGC

Concentration: [50] [µM ▾]

● A
● C
● G
● T
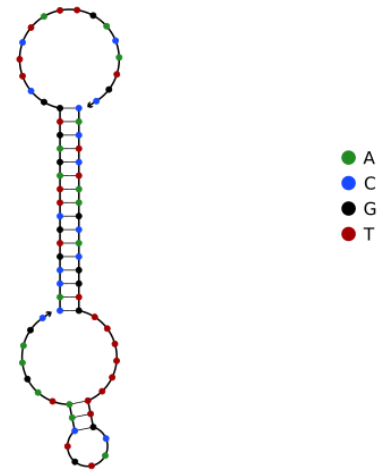
Free energy of secondary structure: -25.34 kcal/mol

Fails to find the structure

Mfold and Dinamelt web server:

http://www.mfold.org/Dinamelt/applications/hybridization-of-two-different-strands-of-dna-or-rna.php

The DSC measurements give a $\Delta H = 942 \pm 63 \ kJ/mol$ which its out of the 652 kJ/mol given by the simulations

A crude estimate of 8 kcal/bp gives 1042 kJ/mol which could be coherent with the measurements

Perfect complementary strands without loops

Hybridization of two Different Strands of DNA or RNA

Do you only have one sequence? Use this form.Use advanced form

Job Name:

Sequence 1: 5'- CACCGTGCTTAGAGTGGCTTCTATTGACATGC -3'

Rev. Comp.

Sequence 2: 5'- GCATGTCAATAGAAGCCACTCTAAGCACGGTG -3'

Temperature range: From 0 °C by 1 °C to 100 °C
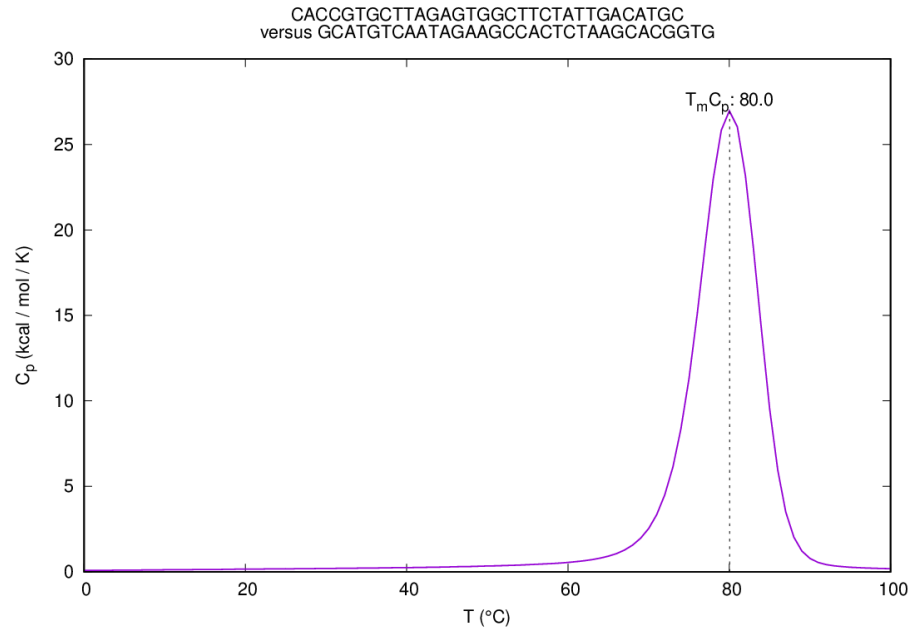
NA type: ⦿DNA ◯RNA ◯RNA2

Initial concentrations: [A₀]: 50 [B₀]: 50 Units: μM ⌄

Salt conditions: [Na⁺]: 0.3 [Mg⁺⁺]: 0 Units: M ⌄ ☐Polymer mo

Your email address (optional):

Submit Effacer

CACCGTGCTTAGAGTGGCTTCTATTGACATGC
versus GCATGTCAATAGAAGCCACTCTAAGCACGGTG
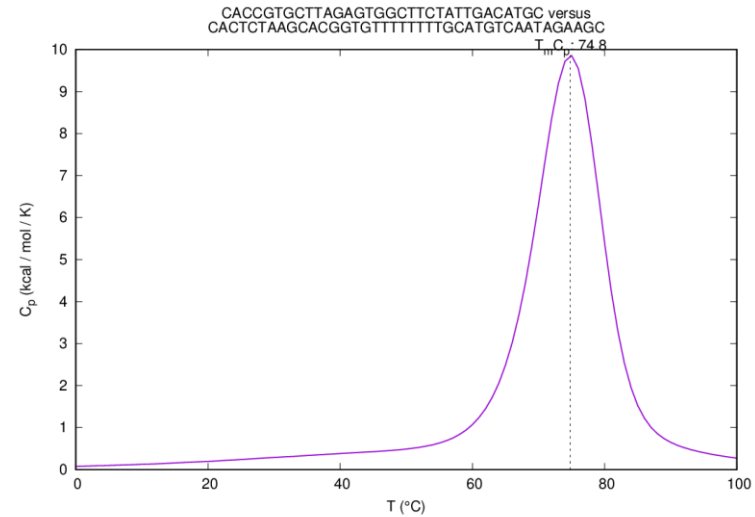
Important Numbers

T_m(Conc): 80.0°C

ΔG: 61.8 kcal/mol ΔH: 277.3 kcal/mol ΔS: 792.3 cal/mol/K $T_m(C_p)$: 80.0°C

T_m(Ext1): 78.8°C T_m(Ext2): 78.4°C

The melting temperature is much higher than what we observe by DSC and the enthalpy is also out of the bounds of the measurements.

So despite the simplicity of the system (2 strands) the models fail to describe the measurements

Interestingly Nupack is able to predict 3 strands structures: ITC stoechiometry will validate or not this possibility

What other structures could be assembled, how to check them ?