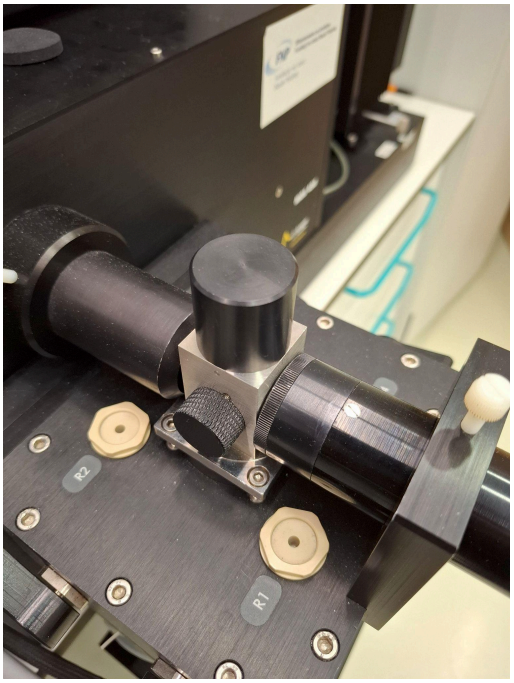
CD Measurements from technical point of view

for methyltransferase fusion protein

Maciej Sikora



Critical points:

– **Cuvette 0.1mm is fragile and cracked on one side! One needs to be extra careful with it. When mounting, the cracked side should be outside to avoid further damage**
– Remember to open Nitrogen valve and close it properly after!
– **Never open the cap when HV is "ON"!** One should only open it when it's turned off – especially after the finished measurement.

Requirements:

– Sample set for measurements
– Pipetor 10-100ul
– Tips box
– Antibacteria or ethanol
– White paper
– Plastic cup (for tips)
** Cuvette 0.1mm (near the machine)
** Cuvette mount (near the machine)

Turning on the machine order:

– Gently open the Nitrogen valve (Check the flow)
– Lamp (Best to be done by somebody from the lab)
—> Wait 30mins before continuing
– Computer
– Machine switch
– BioKine → Spectrometer → Wait 30sec

Turning off order:

– Lamp → Wait 5 mins
– Machine switch
– Computer
– Nitrogen valve closure

Software:

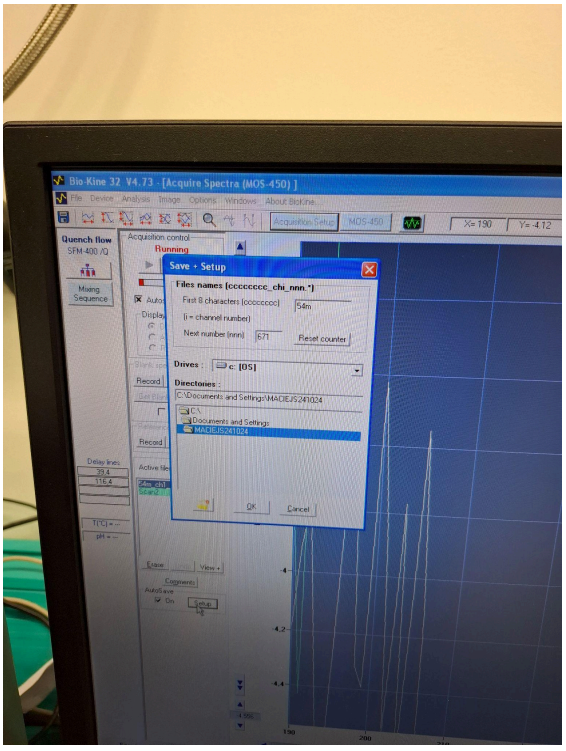
1: HV button
2: Setup with name
3: Acquisition setup
4. Start run
5. Erase old measurements

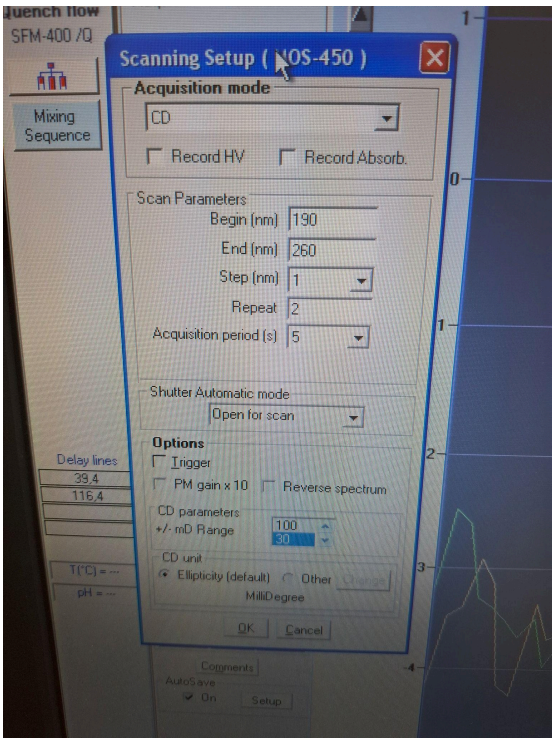
Acquisition setup
– Step: 1nm
– Acquisition period - 5s. (For test measurements 2s is enough)
– Repeat: 2
– +/- mD Range: 30

In loop:
– HV MUST BE TURNED OFF
– Insert the sample
– Change the name and folder
– Delete old measurements
– Start run
– After measurement TURN OFF HV!
– Take the sample out and clean the cuvette

Cleaning and setting up:

– Cuvette is cleaned with antibacteria and polished well
– Put 12ul of the sample into the well
– Smear the liquid along the sides of the triangle-shaped end
– Close the glass well
– Secure the glass with the mount
– Put the sample into the machine – glass towards the exterior





DSF Measurements from technical point of view

for methyltransferase fusion protein

Maciej Sikora

Sample preparation (For 1 measurement set):
Prepare 80ul of protein sample in 22uM concentration and make sure they are well mixed using vortex and mini-spin.
Separately prepare blank buffer (in 15ml Falcon) for dilutions and for the dye.

Plate loading:

The plate setup for a given row is as follows:

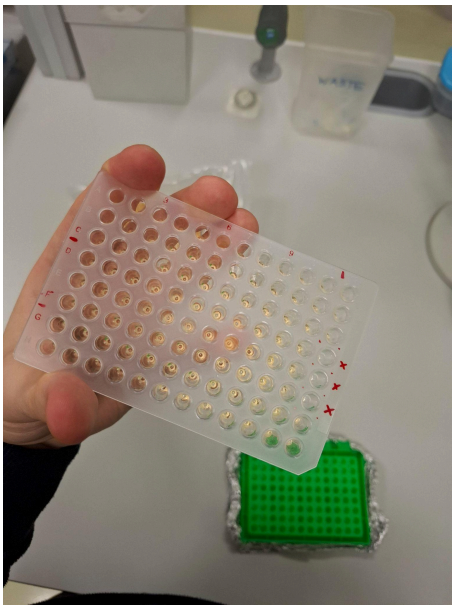
1	2	3	4	5	6	7	8	9	10	11	12
1uM	2uM	3uM	4uM	5uM	6uM	7uM	8uM	9uM	10uM	11uM	Blank

The total volume of the sample is 22ul and consists of:
– 11ul of buffer and protein mix:
- Column number equals to protein amount used (column 7: 7ul of protein)
- Dilute with buffer to 11ul (column 7: 11-7=4ul)

– 11ul of SYPRO Orange dye 10x (Diluted from stock with a buffer)

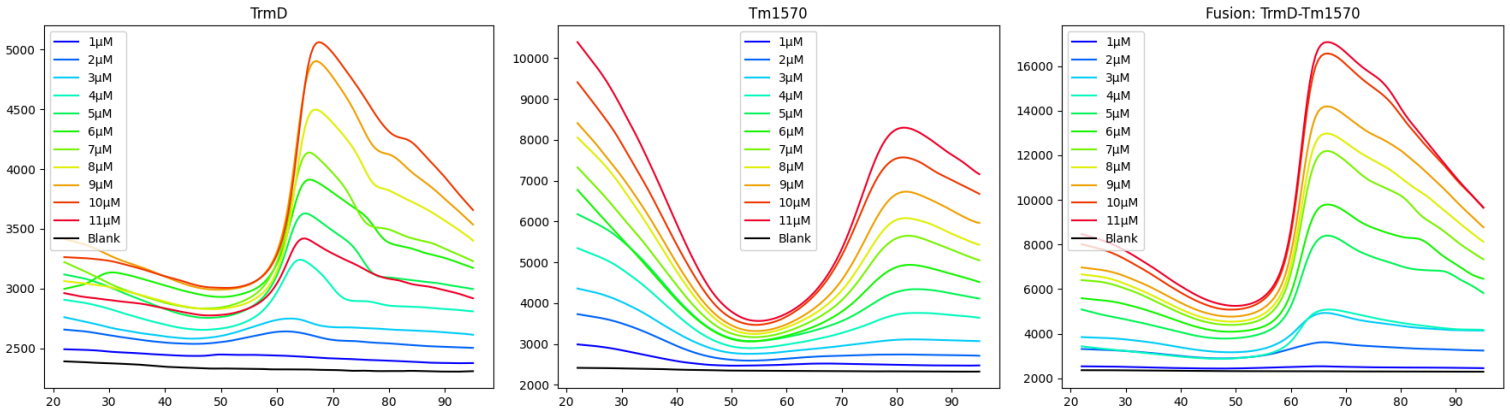
The order of adding components should follow:
- Buffer
- SYPRO Orange dye
- Protein

Pipette only to the first resistance and make sure you are adding to the very bottom of the well. Moreover, be extra careful pipetting 1ul, as the small drop will stick to the pipette tip.

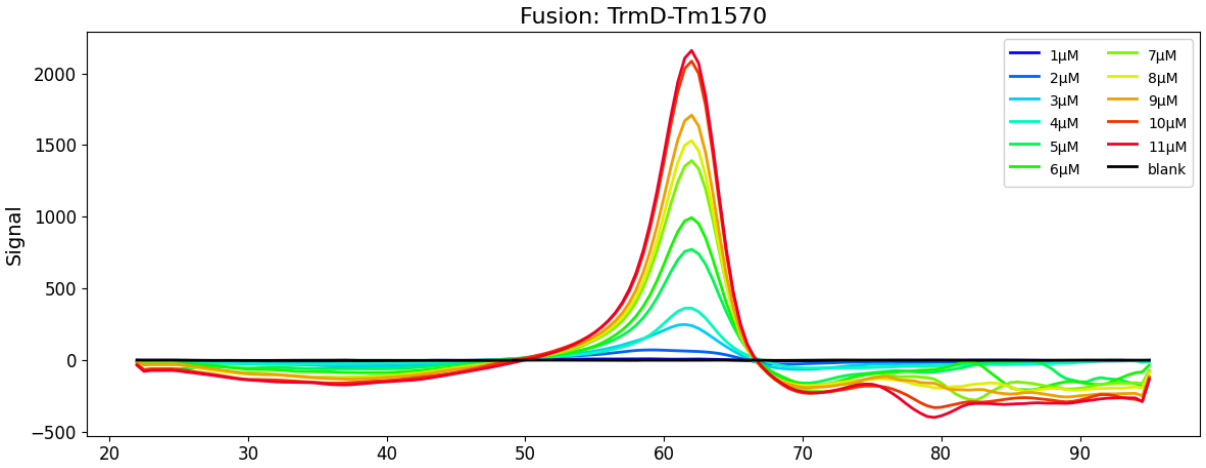


Results:

Scripts were prepared to plot and analyze the DSF data. The plots are presented below.



First derivative for Fusion protein



Results show Tma (Approximate melting temperature):

- 65°C (68°C peak) for TrmD protein
- 72°C (79°C peak) for Tm1570 protein
- 61°C (66°C peak) for fusion protein with secondary peak at 75°C

Those results agree for the TrmD protein with the current literature.