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Circular Dichroism Spectroscopy of Clusterin

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We use this protocol and it's working

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Abstract

This protocol details how to record circular dichroism (CD) spectra and CD melting curves of the extracellular chaperone Clusterin.

Materials

Buffers and reagents:

- CD buffer: [M] 50 millimolar (mM) K-phosphate pH 7.0 .

Note

- Prepare 1 M K-phosphate pH 7.0 stock solution by titrating 1 M K_2HPO_4 with 1 M KH_2PO_4. Dilute stock solution 1:20 with deionized water, sterile-filter and de-gas in vacuum.
- Buffers for CD experiments should not absorb strongly in the far UV range. Chloride salts should be avoided.

- Clusterin variant (see protocol “Clusterin purification from HEK293E cells”, dx.doi.org/10.17504/protocols.io.bvvkn64w).
- Amersham NAP-5 columns (Cytiva, 17085301)

Note

Columns can be re-used when stored at 4 °C.

- De-ionized water
- Methanol

Safety information

Methanol is toxic. During handling, wear gloves and safety goggles.

Instruments

- Nanodrop microvolume spectrophotometer
- Jasco J-715 CD spectrometer equipped with a Peltier-thermostat
- 0.1 mm light path UV quartz cuvette

Preparation

1 Equilibrate NAP-5 column at Room temperature with 10 mL volume of CD buffer according to the manufacturer's protocol using gravity flow.

2 Prepare six 1.5 ml plastic vials in a microtube rack for fraction collection.

3 Thaw Clusterin variant stock solution aliquot.

4 Apply 25 µL Clusterin stock (approximately [M] 200 micromolar (µM)) to NAP-5 column. Allow liquid to completely enter gel bed.

5 Apply 475 µL CD buffer. Allow liquid to completely enter gel bed.

6 Place first fraction collection vial under the column outlet.

7 Add 100 µL CD buffer. Allow liquid to completely enter gel bed. Collect fraction. Move to next vial. Repeat five times.

8 Pool fractions 2-4. Determine absorbance at 280 nm (A280) of pool using Nanodrop, in triplicate. From the A280 average value, calculate Clusterin variant concentration using absorbance coefficients calculated with ProtParam (https://web.expasy.org/protparam/) and the respective sequence of the processed (starting at residue 23) Clusterin variant.

9 Prepare 300 µL solution of [M] 5 micromolar (µM) Clusterin variant for CD melting curve measurement. Dilute with CD buffer. Store On ice.

10 Prepare 300 µL solution of 0.1 µL Clusterin variant (~ [M] 2 micromolar (µM)) for CD spectrum measurement. Dilute with CD buffer. Store On ice.

CD Spectrometer Start-up

11 Open nitrogen valve to flush Jasco J-715 CD spectrometer with gaseous nitrogen.

12 Start cooling water flow at low rate.

13 Switch on detector power source (Power 1).

14 Switch on Xenon lamp (Power 2).

15 Start control computer and open Spectra Manager program.

16 Start external Peltier thermostat (Peltier accessory).

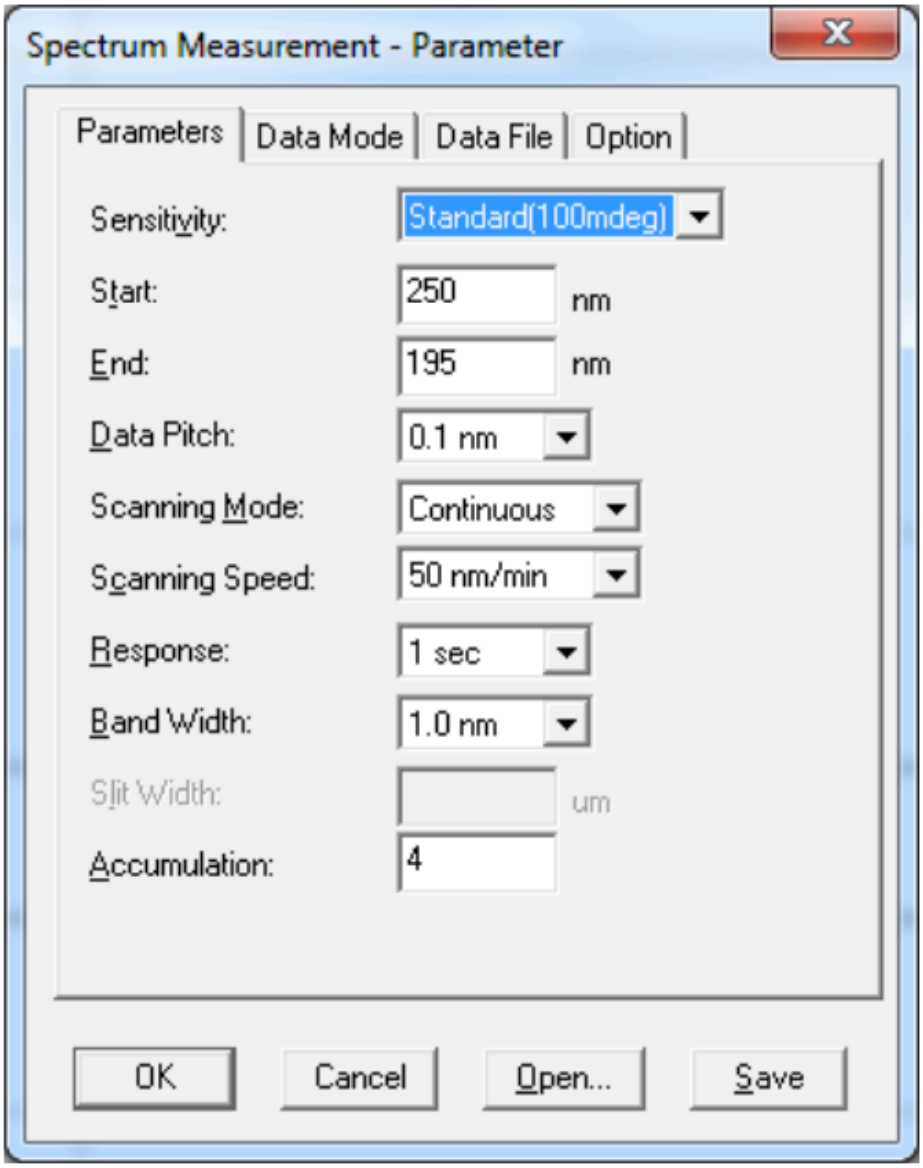
Note

NOTE: For CD spectrometer shut-down, stop the items in steps 11-16 in reverse sequence.

CD Spectrum Measurement

17 Start “Spectrum measurement”. Select Peltier thermostat under “Accessories” and set the temperature to 20 °C in “Control” under “Accessory”.

18 Set the “Parameters” like in the following:



19 Wash the UV cuvette with 3 times water and 3 times methanol. Dry with pressurized nitrogen.

20 Pipette ~ 300 µL CD buffer into the cuvette. Do not trap bubbles. Insert into spectrometer cuvette holder. Let cuvette equilibrate for 00:05:00 . 5m

21 Record a spectrum of CD buffer alone with above parameters.

22 Wash the cuvette as above (step 19).

23 Pipette ~ 300 µL Clusterin variant (0.1 µL) CD buffer into the cuvette. Do not trap bubbles. Insert into spectrometer cuvette holder. Let cuvette equilibrate for 00:05:00 . 5m

24 Record a CD spectrum of the Clusterin variant with above parameters.

Note

NOTE: The additional High Tension (HT) signal is a measure for sample absorbance at the wavelength. Ideally it should be below 500; above 800 the CD signal gets too noisy for quantitative measurement.

25 Subtract the CD buffer spectrum from the CD spectrum of the Clusterin variant in Spectra Manager.

26 In Spectra Manager I, under “CD Options” and “Optical Constants”, convert the units into molar ellipticities using the molar concentration and the residue number.

27 In Spectra Manager II, under “CD Options” and “CDPro”, analyze the secondary structure composition using the SMP56 dataset as a reference and the CONTIN algorithm.

Note

The CONTIN algorithm gives the best fit for Clusterin.

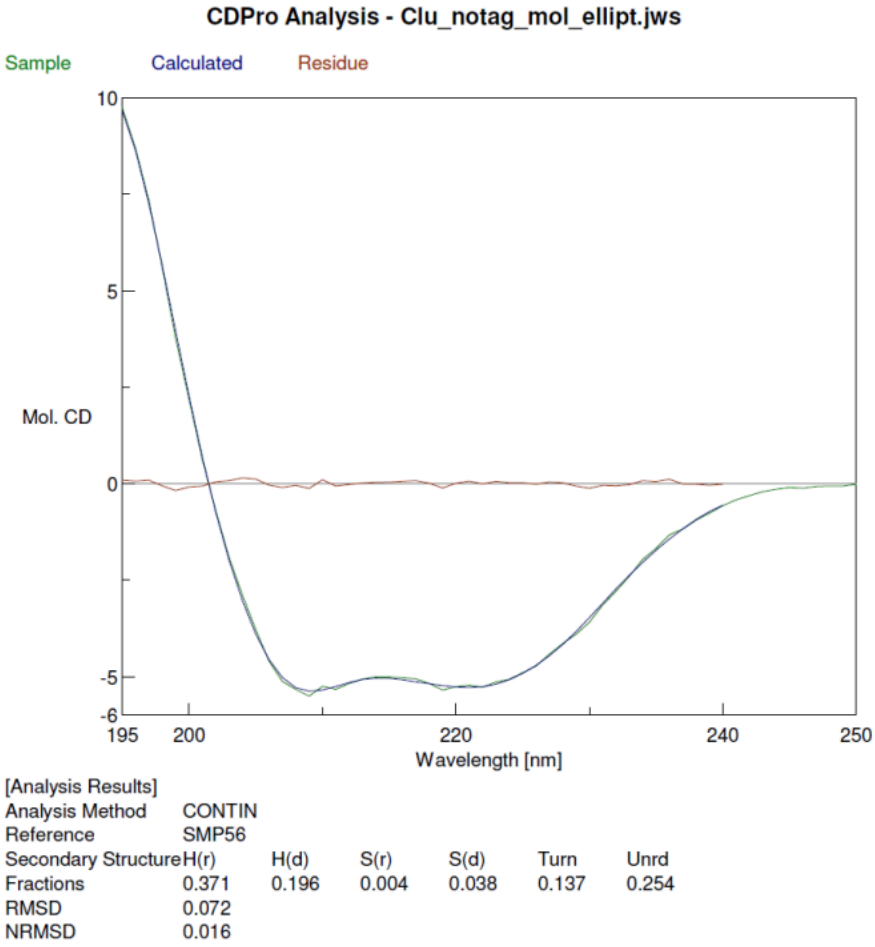
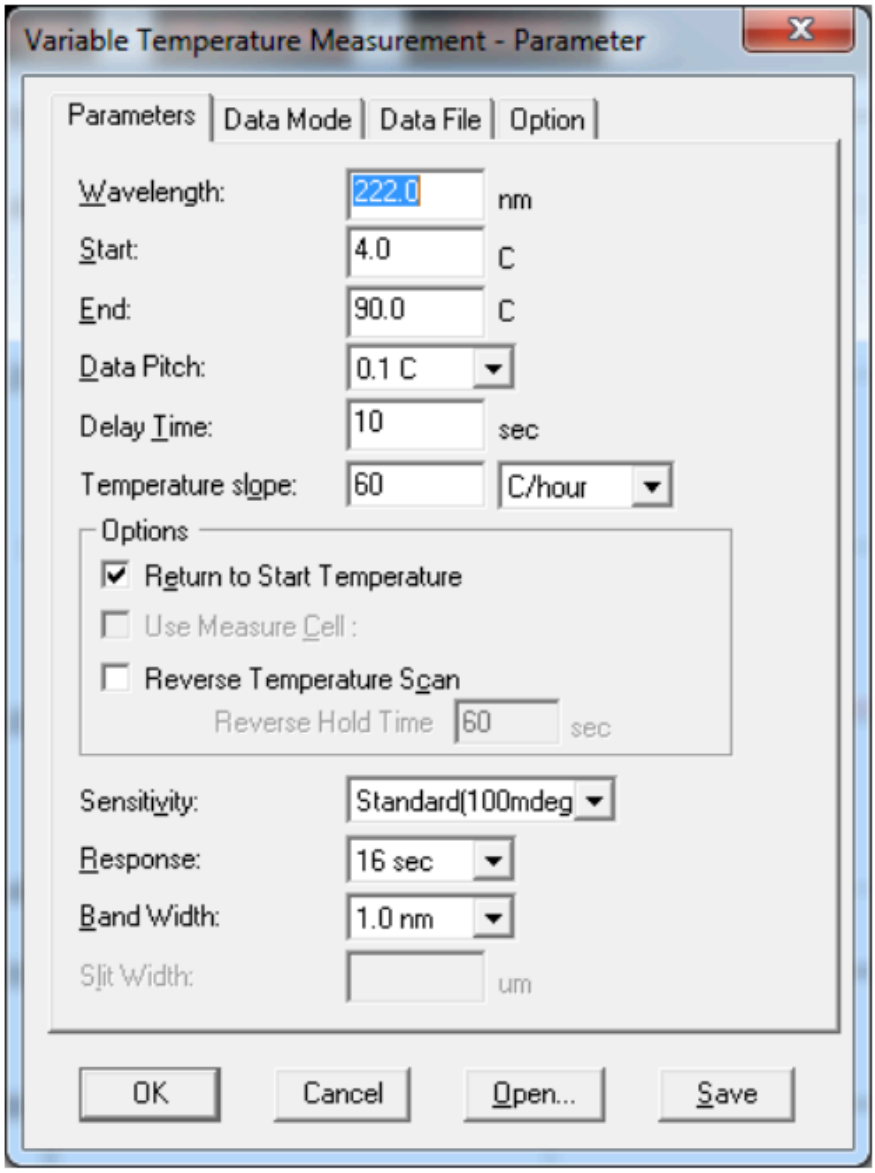


Fig. 1 Comparison of the CD spectrum of wildtype Clusterin (green) with the CONTIN algorithm fitted curve (blue) from CDPro analysis. Residuals are shown in brown.

CD Melting Curve Measurement

28 Start “Variable temperature measurement” in Spectra Manager. Select Peltier thermostat under “Accessories” and set the temperature to 4 °C in “Control” under “Accessory”.

29 Set the “Parameters” like in the following:



30 Wash the UV cuvette with 3 times water and 3 times methanol. Dry with pressurized nitrogen.

31 Pipette ~ 300 µL Clusterin variant ([M] 5 micromolar (µM)) CD buffer into the cuvette. Do not trap bubbles. Insert into spectrometer cuvette holder. Let cuvette equilibrate for 00:05:00 . 5m

Note

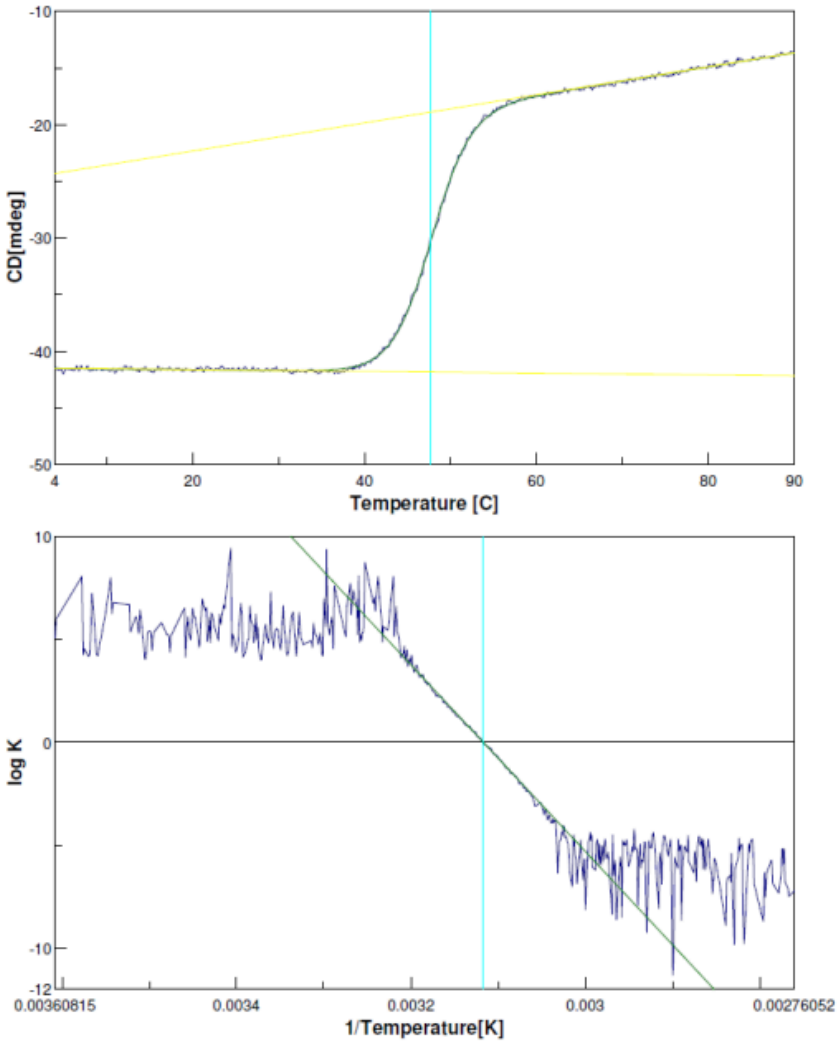
The higher Clusterin concentration compared to the CD spectrum measurement increases the sensitivity. The absorbance (HT signal) increase at 222 nm wavelength is negligible.

32 Record a CD melting curve of the Clusterin variant with above parameters.

Note

A steeper temperature slope will keep the sample further away from equilibrium, resulting in more pronounced overshoot. Heat denaturation of wildtype Clusterin at 5 µM concentration is not reversible. Therefore, the reverse temperature scan was omitted.

33 Under “Processing”, “Common Options” and “Denatured Protein”, analyze the melting curve.



(1) Range of denaturation
25.50 - 68.50 [C]

(2) Fitting line of stable range
A: Y = (0.124064)X + (-24.8292)
B: Y = (-0.00770209)X + (-41.4927)

(3) Residual of fitting curve
SIGMA: 0.151351

(4) Tm = 47.67 +- 0.007338 [C]

Fig. 2 Comparison of the CD melting curve of wildtype Clusterin (blue) with the fitted curve
(green). The fitting range suggested by the program was used.