

### Introduction

In biochemical, biophysical or pharmaceutical research, proteins are an important subject in the development of new drugs or treatments.

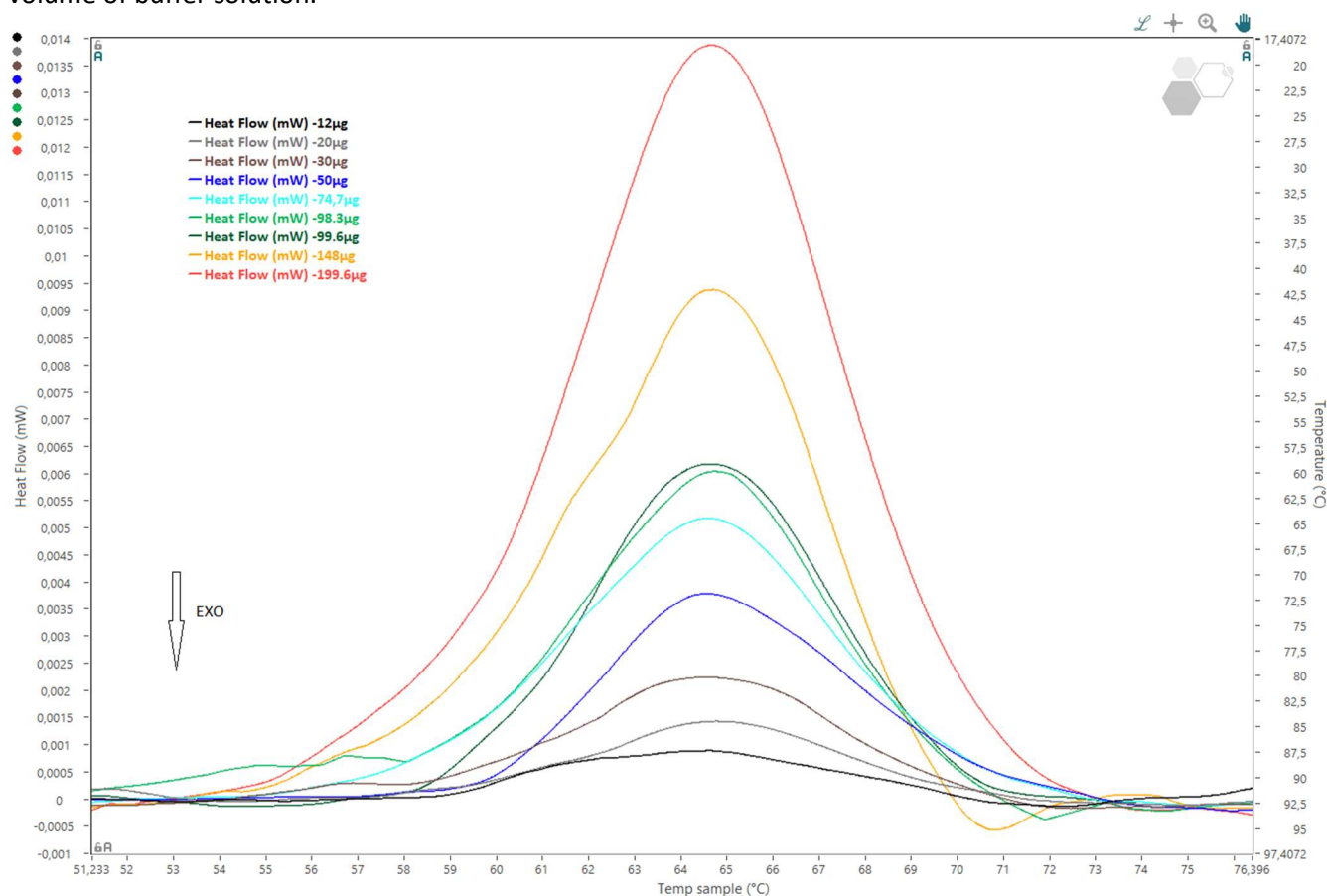
The stability parameters of these proteins are necessary for all these developments. It is therefore necessary to know, for example, the denaturation temperatures of the proteins studied, as well as the energy involved in these denaturations (denaturation enthalpy and temperature).

The technology most commonly used to access these thermodynamic parameters is differential scanning calorimetry (DSC). One of the major problems with this technology is the large quantity of protein required to obtain usable results: up to 1 ml per experiment, for proteins that can sometimes be very expensive to produce.

In this context, Calneos has developed the Ultimate DSC, which allows the use of less than 100  $\mu\text{L}$  of sample in extractable crucibles. One of the advantages is the drastic reduction in the amount of sample required to obtain usable thermograms. Another advantage is the elimination of tedious and sometimes unreliable cleaning procedures.

### Experimental protocol

Several buffered solutions of bovine RNase (50mM phosphate pH 7.3 150mM NaCl) were prepared at different concentrations. Between 50 and 80  $\mu\text{L}$  of these solutions were placed in crimped measuring crucibles. The corresponding RNase masses analysed ranged from 10 to 200  $\mu\text{g}$ . The reference crucible was filled with the same volume of buffer solution.



The Ultimate DSC was programmed to perform a temperature ramp from 20 to 95°C at 1°C/min. The thermograms obtained are shown above.

### Conclusion

The Ultimate DSC allows to measure the enthalpy and denaturation temperature of bovine RNase using quantities between 200 and 10 µg, for sample volumes between 50 and 80 µl. These sample volumes used are between 5 and 10 times smaller than those usually required with currently marketed instruments, which need between 350 and 1000 µl.

Each thermogram was obtained in less than 60 minutes, allowing many analyses to be carried out quickly. The Ultimate DSC saves large amounts of sample and allows faster operation.

An automated version of the Ultimate DSC is also available.

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