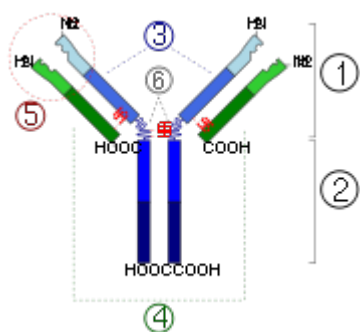


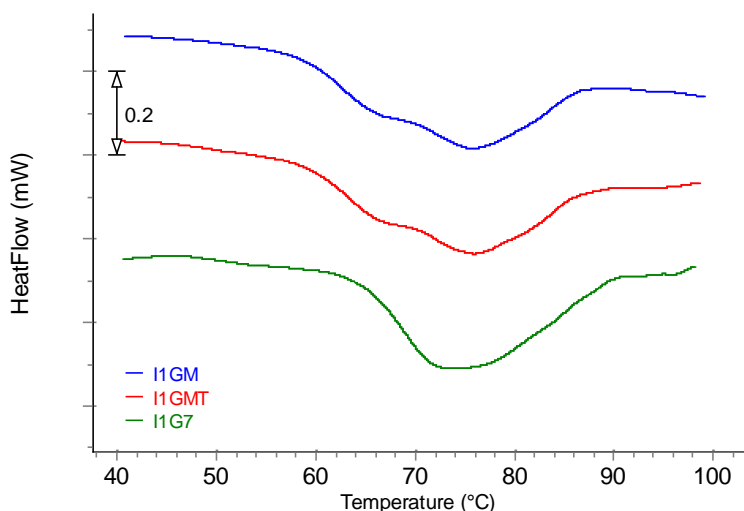
# Thermal stability of therapeutic proteins with MicroDSC3

## Introduction:

Antibodies are gamma globulin proteins that are found in blood or other bodily fluids of vertebrates, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. Antibodies can either neutralize targets directly by, for example, binding to an infection-leading part of a pathogen, or recognize an antigen and tag it for attack by other parts of the immune system. DSC allow to test and understand thermally induced unfolding of immunoglobulin.



**Figure 1 – Structure of an Antibody**  
 1. Fab region / 2. Fc region / 3. Heavy chain with variable (VH) and constant (CH) domains / 4. Light chain with variable (VL) and constant (CL) domains / 5. Antigen binding site – Source : [http://en.wikipedia.org/wiki/Immunoglobulin#Medical\\_applications](http://en.wikipedia.org/wiki/Immunoglobulin#Medical_applications)



## Experimental

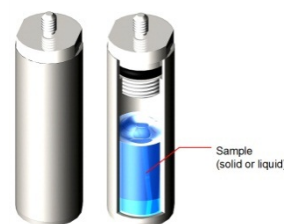
The analyzed samples are 10mg/mL aqueous solutions of different immunoglobulins. 800µL of sample were introduced in a µDSC3 Evo batch cell. For compensation, a buffer solution was introduced in a reference cell.

Sample	Amount of solution in sample cell (mg)	Concentration (mg/mL)
I1GM	806.3	10
I1GMT	783.65	10
I1G7	823.71	10

**Table 1 – Used amounts of sample**

The following thermal profile is programmed:  
 - Heating between 30°C and 100°C at 1 K.min<sup>-1</sup>

Closed « Batch » Cell



**Figure 1 – Scheme of standard batch cells**

µDSC3 evo  
 -20 °C to 120 °C



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## Results

Endothermic denaturation peaks are observed

Sample	T <sub>m</sub> (°C)		Heat (J/g sol – J/g protein)	
I1GM	67.63	75.89	0.194	19.55
I1GMT	68.43	75.93	0.222	22.30
I1G7	73.78	76.13	0.225	23.17

*Tab. 2 – results of peak integrations*

Literature reports that CH2 domain of an antibody unfolds at lower temperatures and CH3 domain unfolds at a higher temperature, while T<sub>m</sub> for the Fab fragment is difficult to predict. Thus, depending on the cooperativity of the unfolding process, there can be 1, 2 or 3 peaks.

It is observed that I1G7 unfolding process is more cooperative, as the T<sub>m</sub> of the first peak is higher. This first peak can be attributed either to CH2 or Fab domain, while the second peak may be attributed to CH3 domain.

μDSC3 evo  
-20 °C to 120 °C



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