Thermodynamics of Antibodies

TA Instruments

Abstract

The development of monoclonal antibody (Mab) based drugs has undergone considerable growth since the release of the first Mab in the mid-eighties. Intuitive biophysical tools that examine and quantify interactions and conformational dynamics are important in demonstrating and understanding local and global conformational changes. The structure of antibodies as well as other macromolecules is related to its function and efficacy therefore control over these parameters is essential. The association of antigens to antibodies or of antibodies to delivery assemblies such as liposomes or gold nanoparticles can be addressed by isothermal titration calorimetry (ITC) while the folding of antibodies can be assayed using differential scanning calorimetry (DSC). Both techniques offer the advantage of using native solutions and both instruments are offered by TA Instruments.

Antibody Background

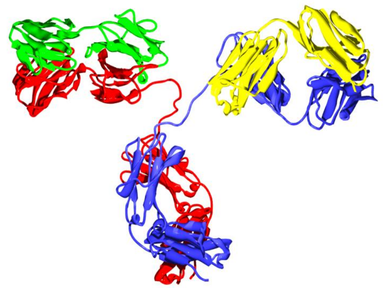

Figure 1. Structure of an IgG antibody. The red and the blue ribbons represent the heavy chains, which define the class of the antibody, and the green and yellow, the light chain portion (2Image taken from ; Wikipedia. *Antibody*. http://en.wikipedia.org/wiki/Antibody (accessed August 11, 2011).

Antibodies or immunoglobulins are a specific type of glycoprotein. There are billions of forms, each with a different primary sequence and antigen binding site.

The interaction between the antigen binding site and an antigen is extremely specific, which has made antibodies very highly desirable reagents when developing a wide variety of antigen detection assays.

Antibodies demonstrate a strong structure-function relationship. Because of this relationship, the unfolding or denaturation temperature measured by DSC is associated with functional differences. In a DSC scan, an unfolding event appears as an endothermic peak that can be approximated as a Gaussian or normal distribution curve. The area under the peak is proportional to the enthalpy change (ΔH) or unfolding and the temperature of the peak maximum (T_m) is related to the Gibbs energy change (ΔG) for unfolding.

The TA Advantage: Superior Sensitivity, Reproducibility, and Flexibility

Isothermal Titration Calorimeter (ITC)

Figure 2. The Low Volume ITC from TA Instruments

Isothermal titration calorimetry (ITC) is a straightforward method to determine basic chemical details of a binding interaction, affinity, thermodynamics and stoichiometry in a single experiment and under native conditions.

	Nano ITC Low Volume
Min detectable heat (μJ)[a]	0.05
Max detectable heat (μJ)[b]	3,000
Baseline stability (μW/hr)[a]	±0.02
Short term noise (μW)[c]	±0.0014
Active Cell volume (mL)	0.190
Sample Fill Volume (mL)	0.300
User- selectable stirring speeds	150-400 rpm

The twisted stir paddle and cylindrical cell shape enable slower stirring speeds during the experiment. These slower stir speed will prevent shearing of proteins or nucleic acids.

Figure 3. LV Nano ITC cell and paddle

Differential Scanning Calorimeter (DSC)

Figure 4 The Nano DSC and 96-well plate autosampler from TA INstruments

When the temperature is raised, as in a DSC scan, a protein unfolds and the sum of all of the enthalpy changes are measured and referred to as the $\Delta H_{calorimeter}$ value (ΔH_{cal}). Overall, the protein unfolding event is endothermic as the contribution from breaking hydrogen bonds out-weighs the contribution of the exothermic events such as the disruption of hydrophobic interactions.

	Nano DSC with Capillary Cell
Temperature Range	-10 to 130 ˚C
Baseline repeatability (μW)	±0.028
Short term noise (μW)[c]	±0.015
Active Cell volume (mL)	0.300

The capillary cell design attenuated protein precipitation after unfolding.

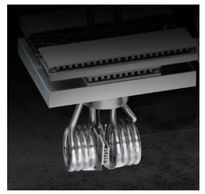

Figure 5. Nano DSC Capillary cell

ITC Examples

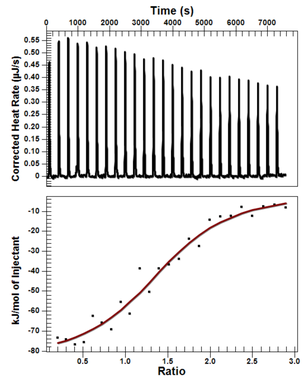

Figure 6. 30 um Antigen titrated into 3.6 uM antibody

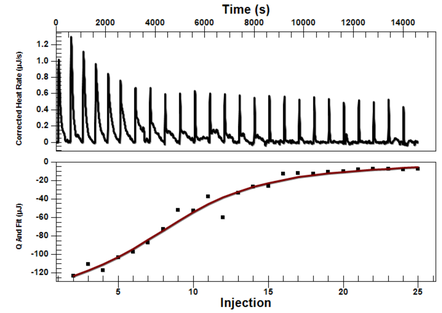

Figure 7. Micromolar (uM) Antibody titrated into Nanoomolar (nM) AuNps

DSC Examples

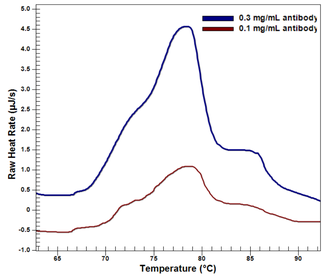

Figure 8. DSC Thermogram of lw concentrations of antibody., .2 um (blue) and 0.67 uM (red)

The shape of the peak in the thermogram provides further insight into the properties of the sample. If denaturation occurs within a narrow temperature range, then the transition is considered highly cooperative

When considering antibodies, there is no typical thermogram. Some exhibit a single peak in the thermogram, others show several distinct peaks, and others show overlapping peaks that appear as shoulders on a larger unfolding peak.

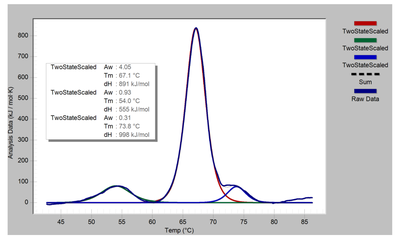

Figure 9. Thermograms are easily deconvoluted with NanoAnalyze™, the analysis software package provided by TA Instruments.

Conclusion

Antibodies are complex proteins and when analyzing binding and structural thermodynamics, require such an instrument and data analysis software capabilities that will allow one to fully differentiate between individual and interacting domains and report accurate T_m values. The combination of the Nano ITC, Nano DSC and NanoAnalyze™ can facilitate these needs.