Characterizing Weak Binding Events with Two Advanced Biophysical Techniques: Hydrogen Deuterium Exchange (HDX-MS) and Differential Scanning Calorimetry (DSC)

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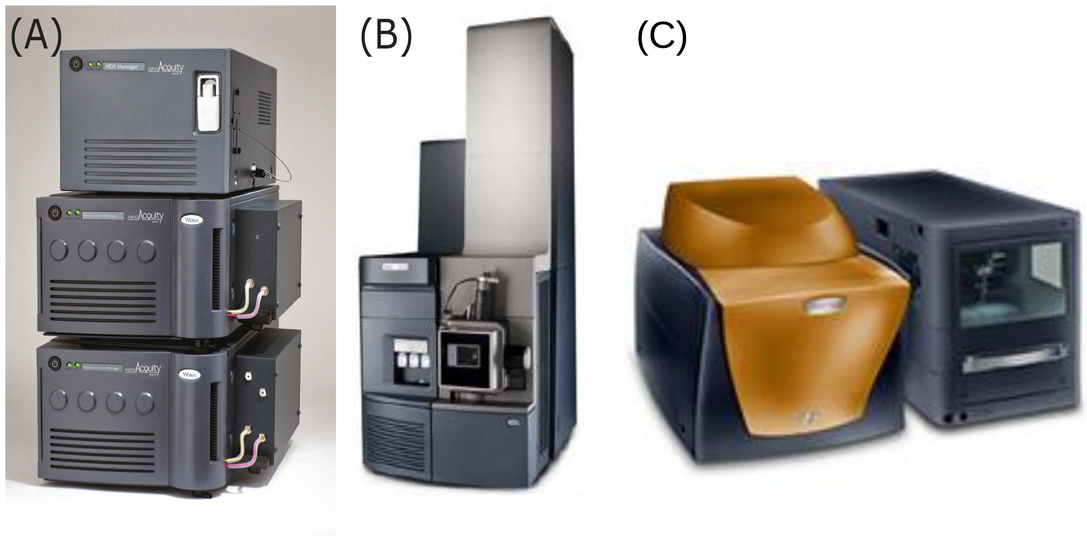
Background:

The objective of this study was to probe the conformational change caused by a site specific mutation in a human immunoglobin G (IgG).

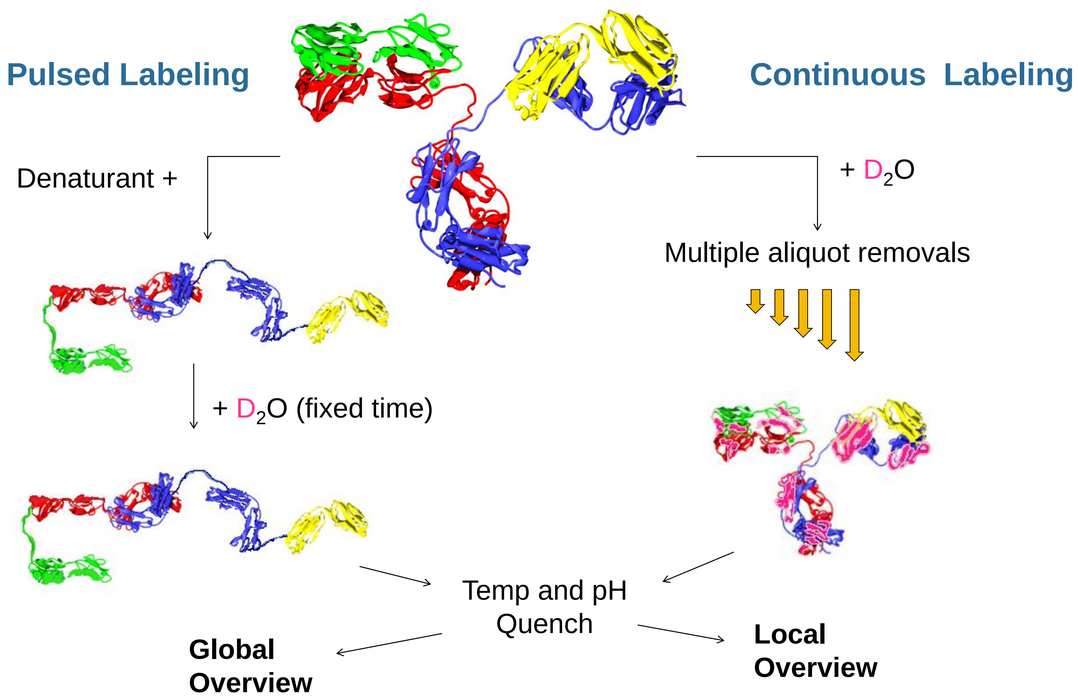
Hydrogen-Deuterium Exchange Mass Spectrometry (HDX -MS) and Differential Scanning Calorimetry (DSC) complement one another and have the laboratory-proven sensitivity and reproducibility to successfully characterize the structure and function of binding molecules.

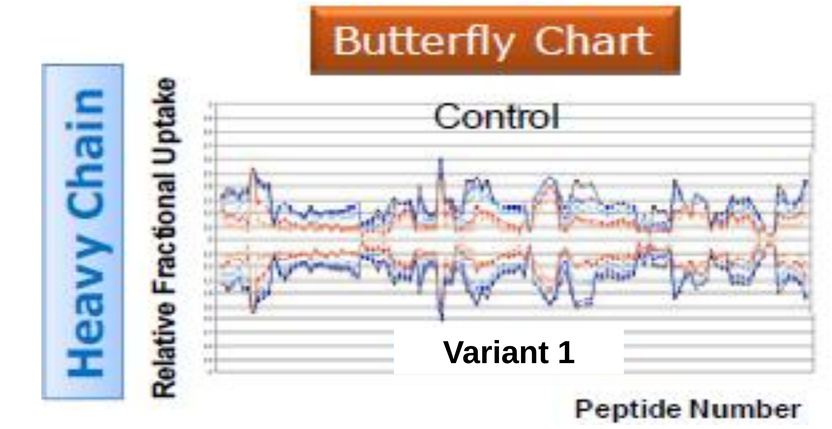
The HDX-MS/DSC data sets enabled a clear, comprehensive characterization of peptide maps and thermodynamic stability changes that help define weak binding events.

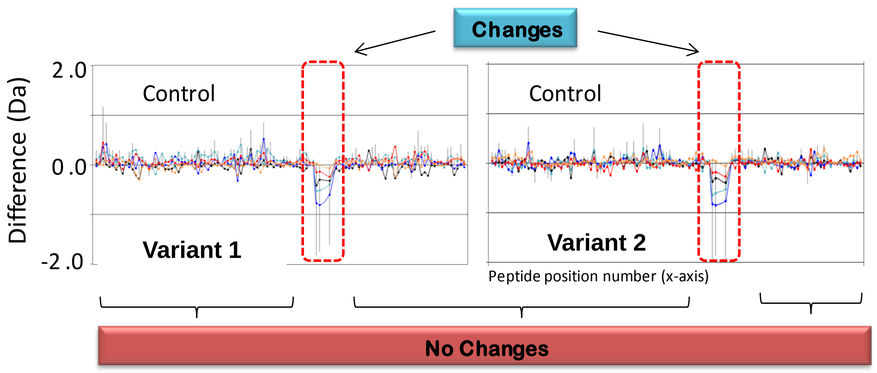


(A) nanoACQUITY UPLC HDX - Waters
(B) Xevo G2 Q-TOF MS - Waters
(C) Nano DSC Autosampler System - TA Instruments

HDX- MS Experiments / Data

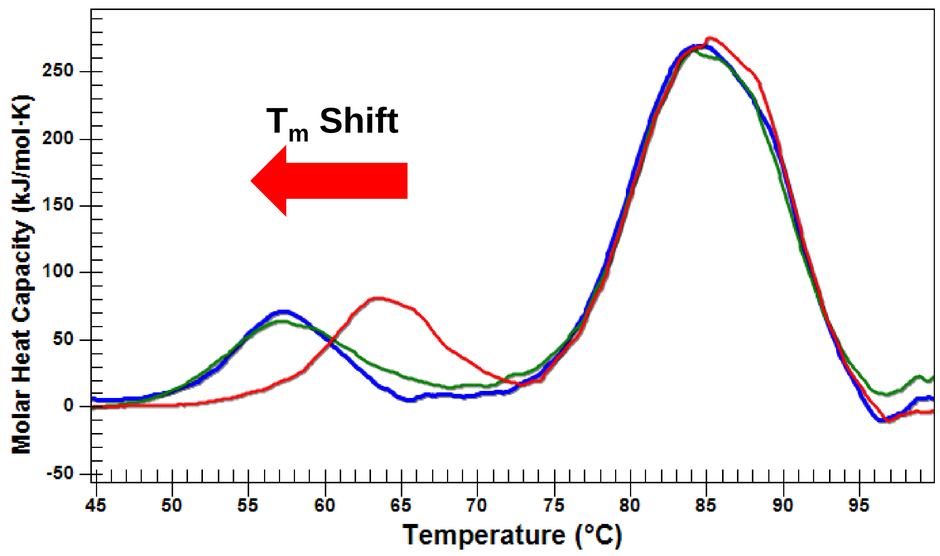






- HDX-MS data for Variant 1 & 2 indicates 3 AA substitution influences molecular structure
- Effective mapping of engineered changes in weak binding sites

DSC Experiments / Data



	Tm_1 (˚C)	ΔH_{vH1} (kJ/mol)	Aw_1	Tm_2 (˚C)	ΔH_{vH2} (kJ/mol)	Aw_2	Tm_3 (˚C)	ΔH_{vH3} (kJ/mol)	Aw_3
Var 1	57.6	376	1.4	83.4	444	4.7	88.4	640	1.4
Var 2	57.8	406	1.6	83.1	420	5.6	89.0	557	2.1
control	65	477	1.1	83.6	410	5.3	89.1	619	1.8

- Tm shift in Variant 1 & 2 indicates molecular structure changes in weak binding sites
- Data from DSC thermogram consistent with HDX-MS data

Summary / Conclusion:

A combination of HDX –MS and DSC is capable of revealing important structural details for biopharmaceuticals that cannot be obtained using other techniques

- ❖ HDX can map changes in binding regions and define structure of weak binding sites
- ❖ DSC characterization ensures highest quality structural data for differentiating weak binding molecular variants
- ❖ HDX-MS & DSC provide powerful, flexible analysis tools for DD, R&D and QC