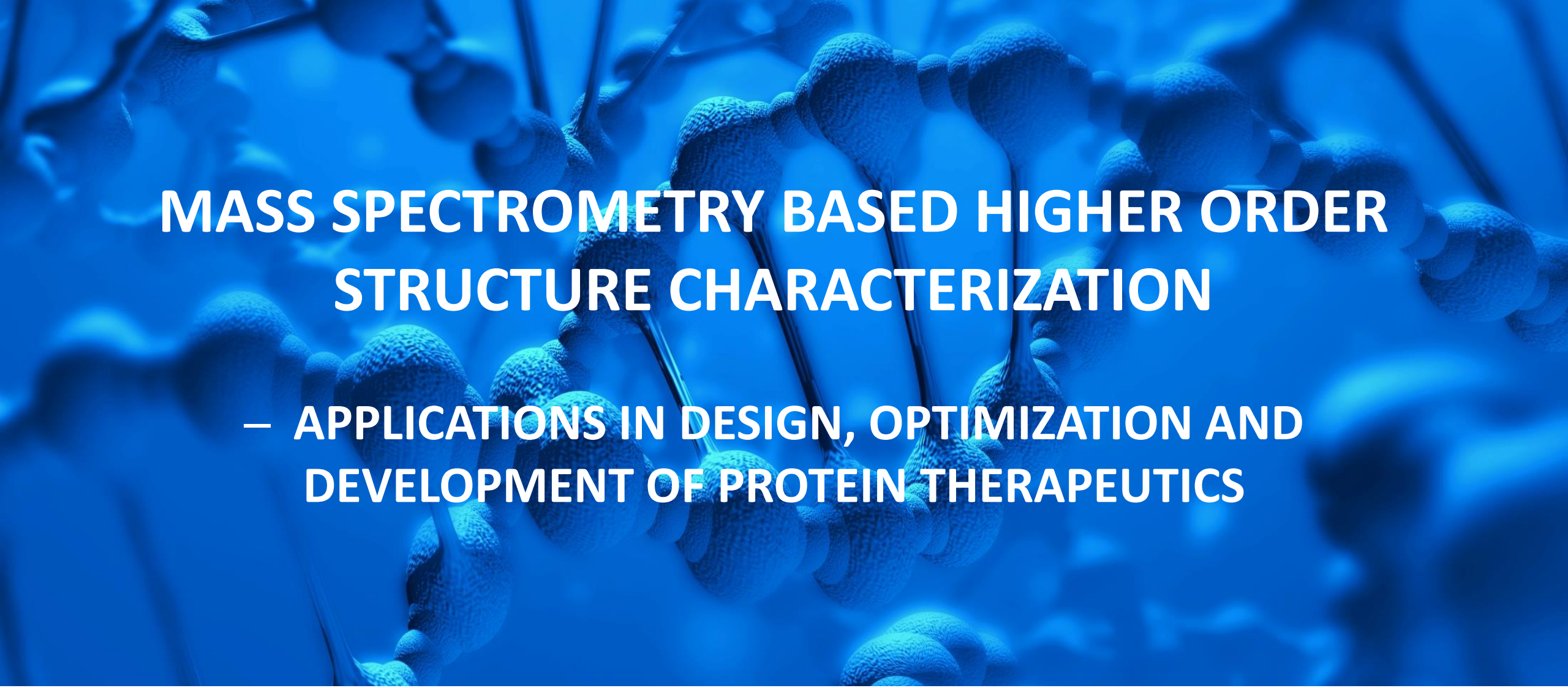


MASS SPECTROMETRY BASED HIGHER ORDER STRUCTURE CHARACTERIZATION

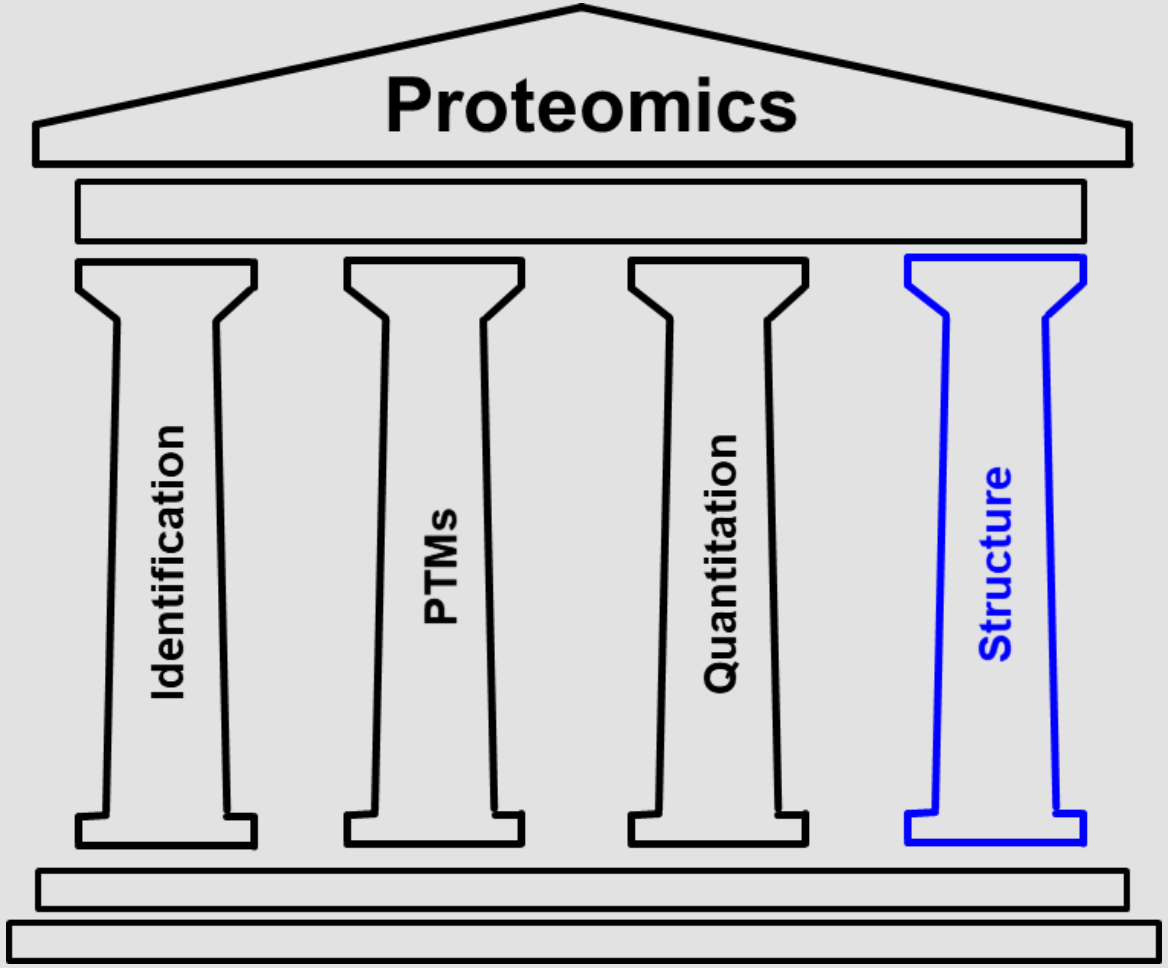
– APPLICATIONS IN DESIGN, OPTIMIZATION AND DEVELOPMENT OF PROTEIN THERAPEUTICS

JUN ZHANG

PROCESS DEVELOPMENT, AMGEN INC.

AMGEN® 40 YEARS

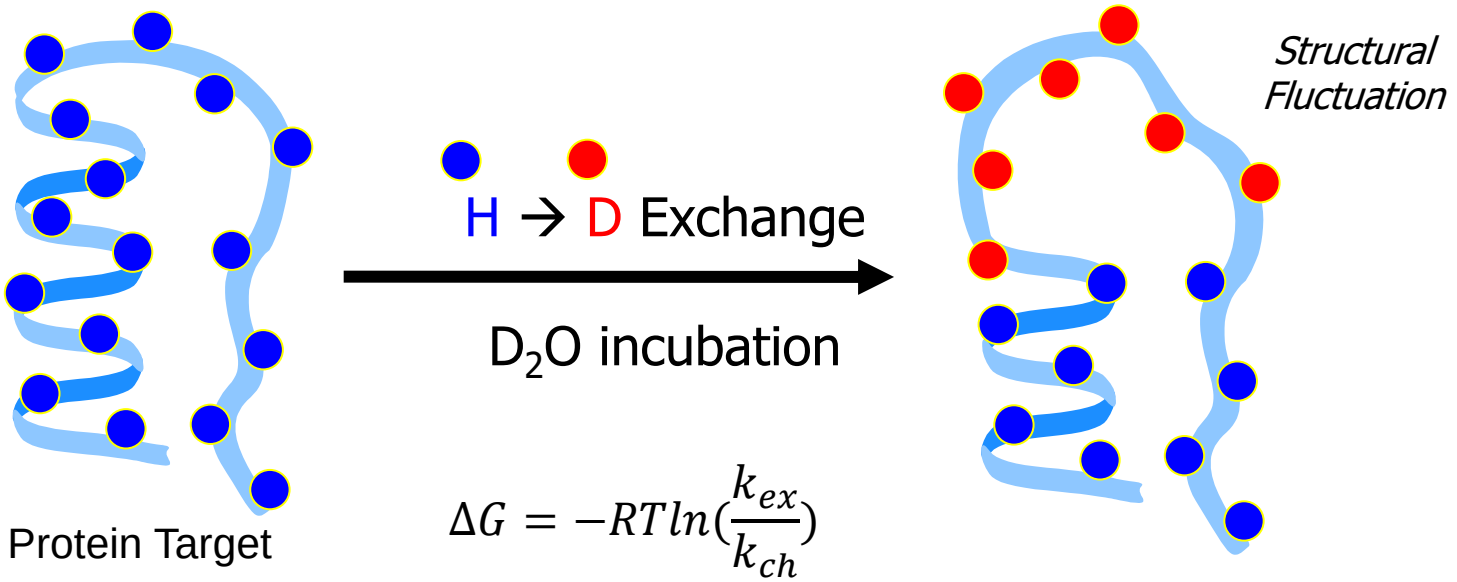
MS-BASED HOS CHARACTERIZATION – THE FOURTH PILLAR OF PROTEOMICS



STRUCTURAL PROTEOMICS

- Native Mass Spectrometry
- Limited Proteolysis
- Cross-linking
- Covalent Labeling
- Hydrogen Deuterium Exchange (HDX)

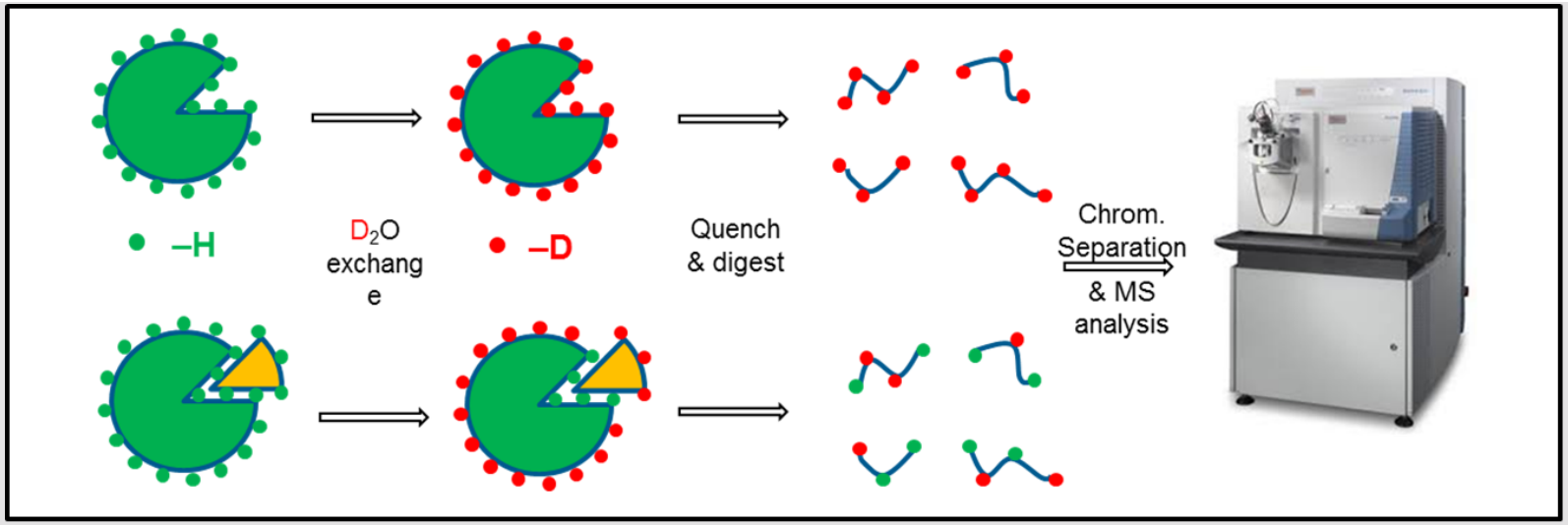
HDX-MS ANALYSIS OF PROTEINS



- **Protein dynamics influences rate of amide H atoms to exchange with solvent D atoms.**
- **The rate of HDX can be obtained by measuring the change in mass using mass spectrometry.**

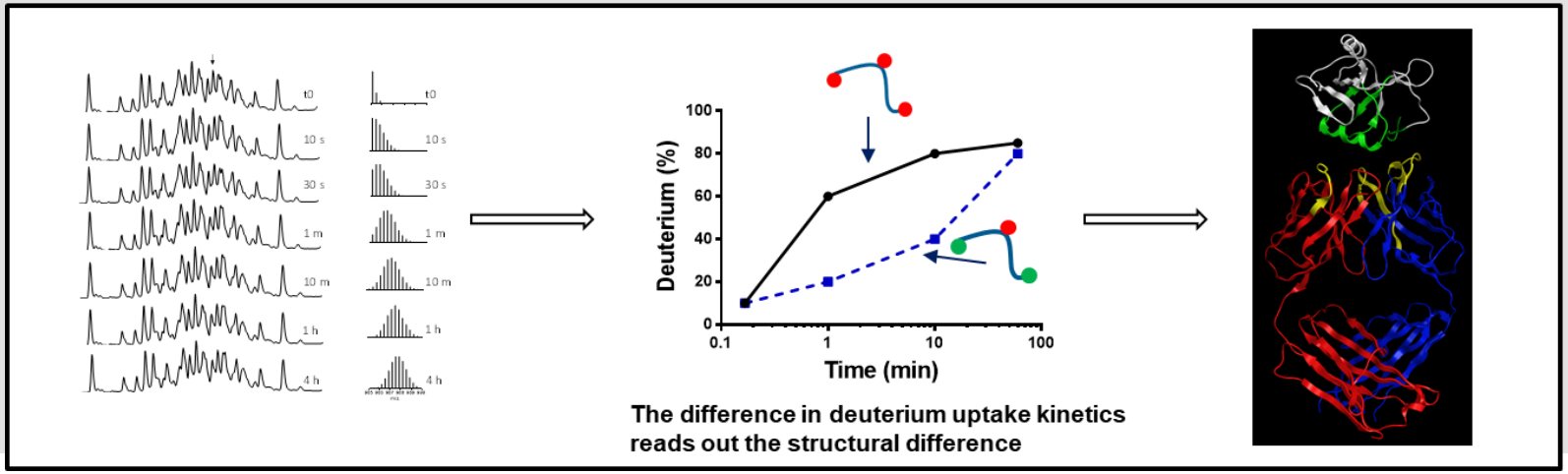
Englander *et al.* (1984) *Q. Rev. Biophys.*
Wales *et al.* (2006) *Mass Spectrom. Rev.*

STRUCTURAL INSIGHTS ENABLED BY HYDROGEN DEUTERIUM EXCHANGE MASS SPECTROMETRY (HDX-MS)



Sample Prep & Analysis:

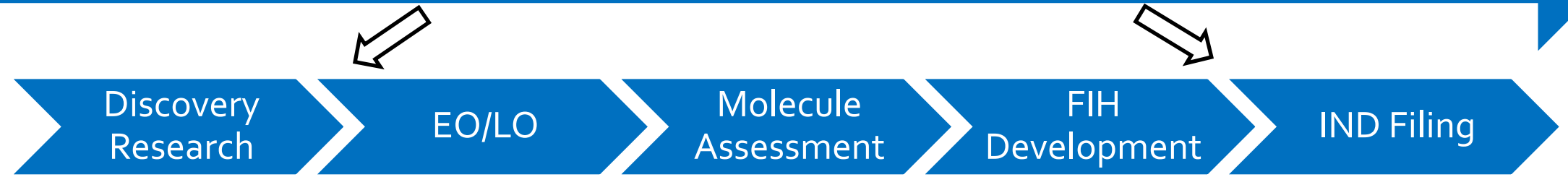
- Labeling, quenching , and digestion, and Detection
- Fully automated process controlled by HDX-PAL platform
- High resolution MS detection
- 1 day



Data Analysis:

- Structural information
- Data analysis by MassAnalyzer and HDX Workbench
- Map to sequence or structure
- Generate report
- 1-2 days

HDX-MS to Expedite Developing a Clinical Candidate



Identification

- Hit Generation
- Lead Selection
- Epitope and Paratope Mapping

Optimization

- Affinity Maturation
- Protein Engineering

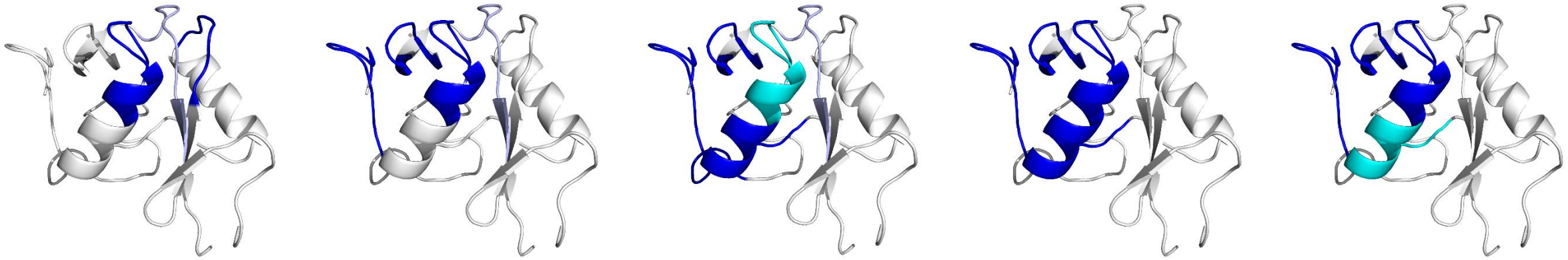
Selection

- Stability
- Activity
- Molecule Assessment

Formulation

- Protein-protein Interactions
- Protein-excipients Interactions

EXAMPLE 1: EPITOPES MAPPING BY HDX-MS

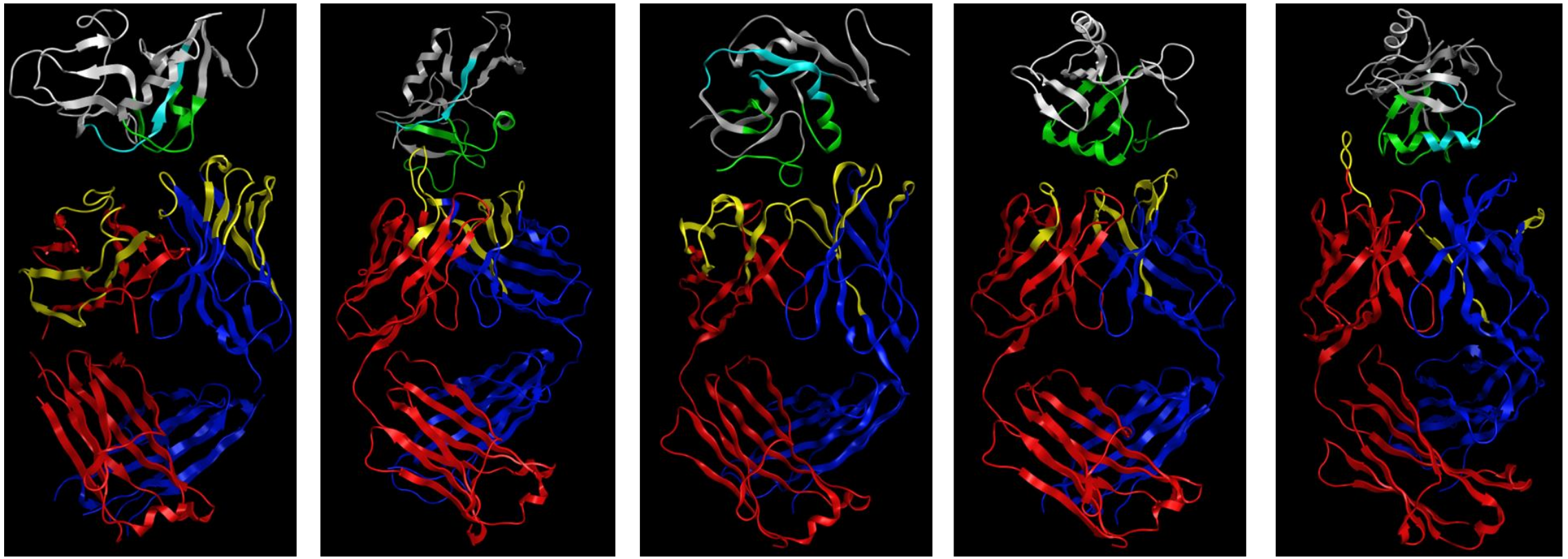


mAb-1 mAb-2 mAb-3 mAb-4 mAb-5

Blue: large protections from HDX
Cyan: relative smaller protections from HDX
Grey: no difference

Epitope mapping by HDX-MS enables fast lead mAb identification and mAb binning

EXAMPLE 2: PARATOPES MAPPING BY HDX-MS



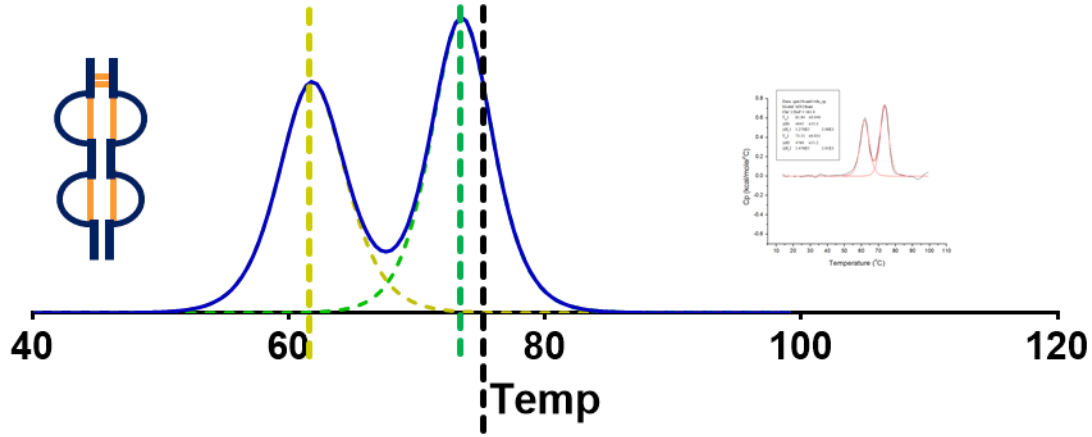
mAb-1 mAb-2 mAb-3 mAb-4 mAb-5

Paratope mapping by HDX-MS provides valuable information for affinity maturation

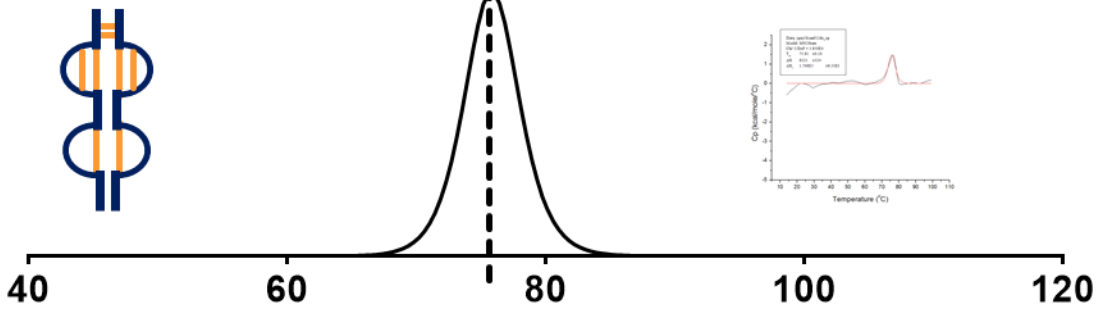
EXAMPLE 3: CANDIDATE SELECTION BY HDX-MS

- DSC measurements of SEFL-Fc

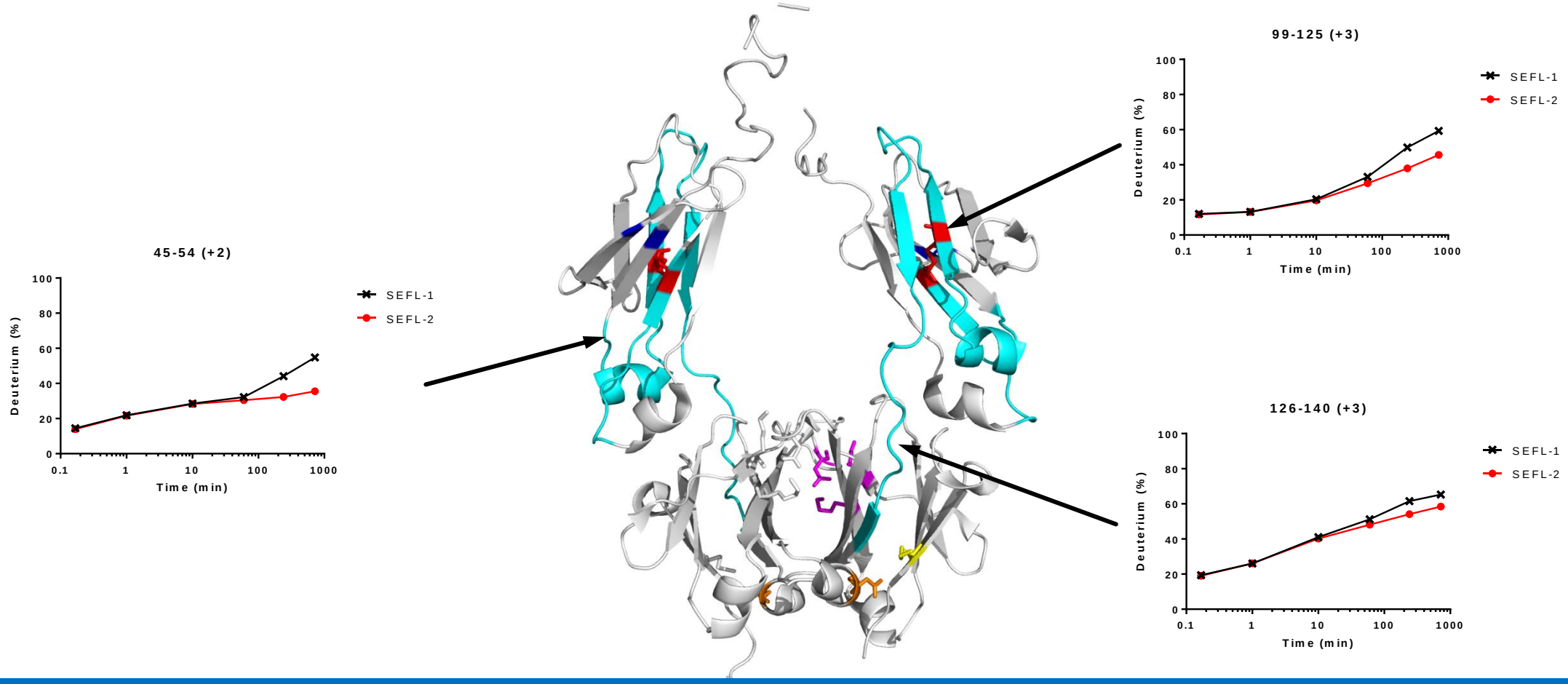
SEFL-1 T_{m1}: 61.8 °C
T_{m2}: 73.5 °C



SEFL-2 T_{m1}: 75.8 °C

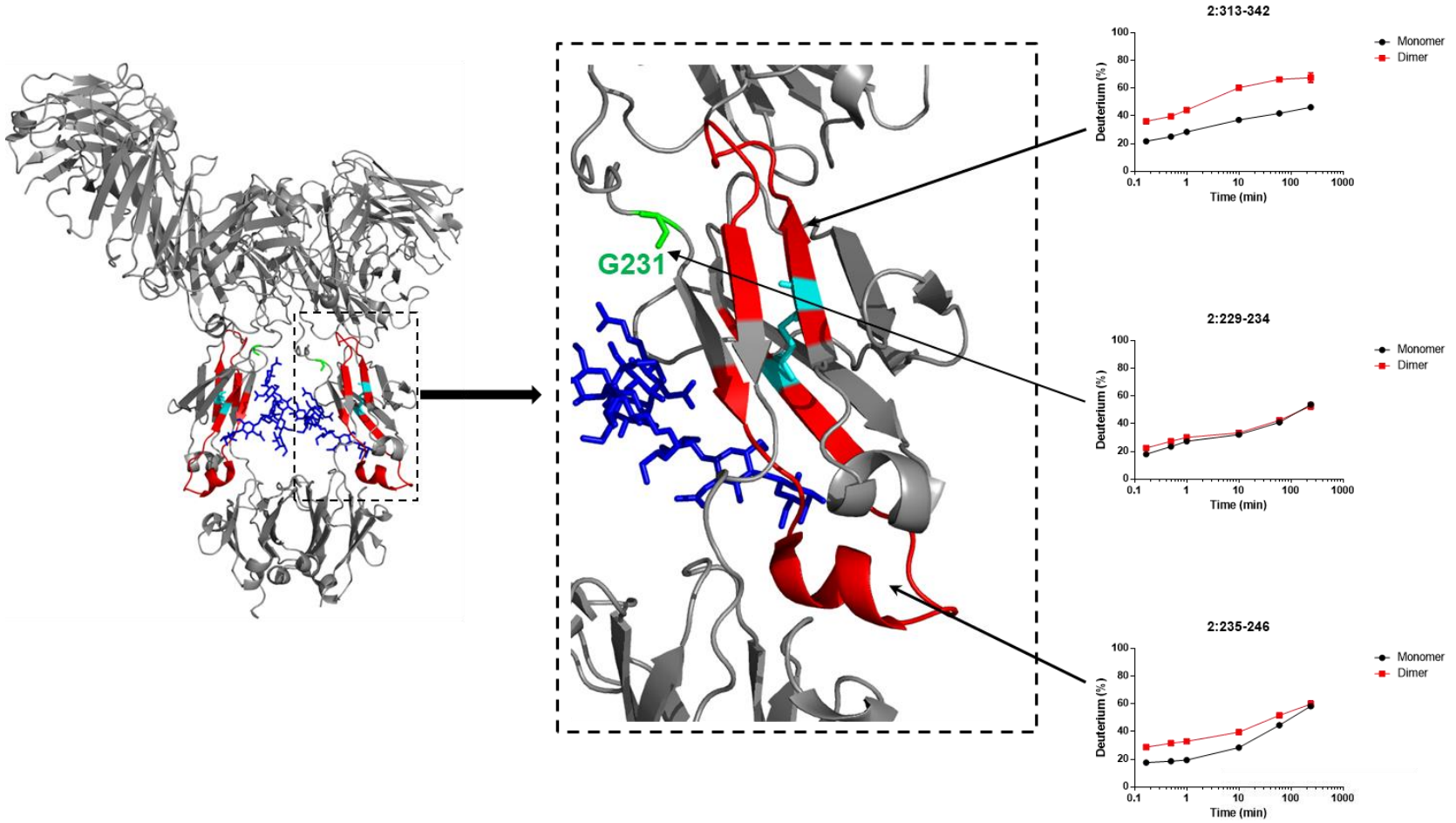


THE EFFECTS OF AN EXTRA DISULFIDE IN CH2 (SEFL2)



The extra disulfide significantly stabilizes Fc-CH2, and the effects extend to Fc-CH3 region

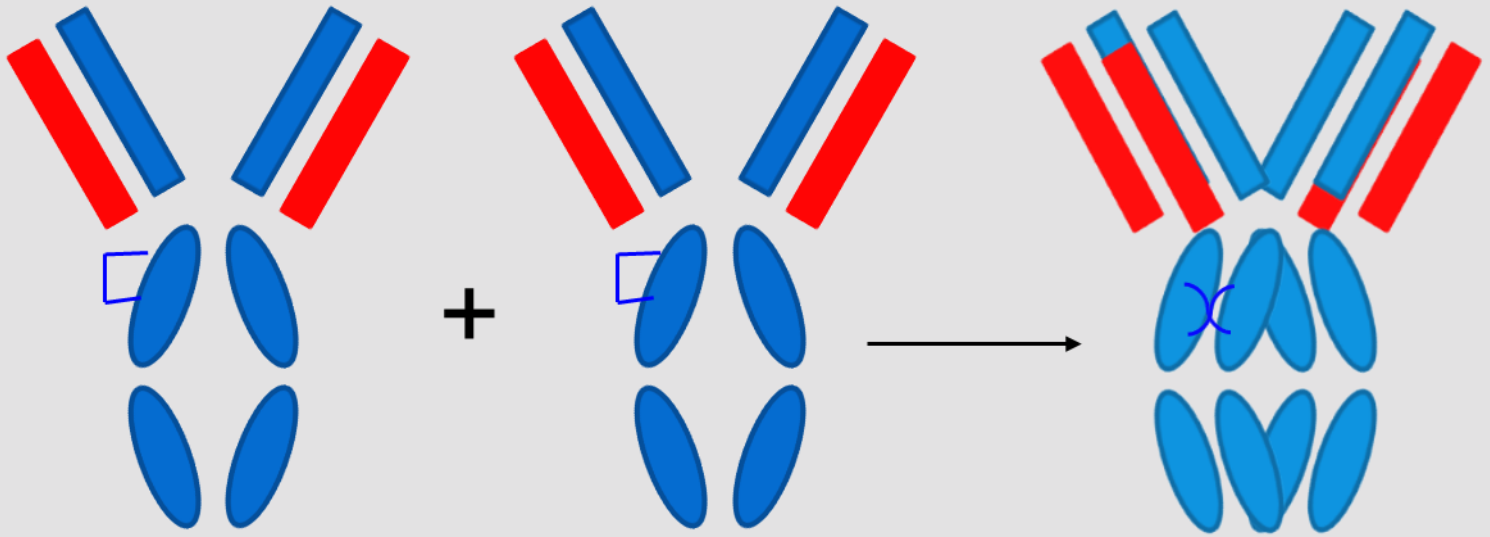
EXAMPLE 4: UNDERSTANDING THE AGGREGATION MECHANISM – A THERMAL DIMER VS. A NATIVE DIMER



Zhang et al. *Biochemistry* 2018

The thermal dimer showed significant destabilization effects in CH2 around the disulfide

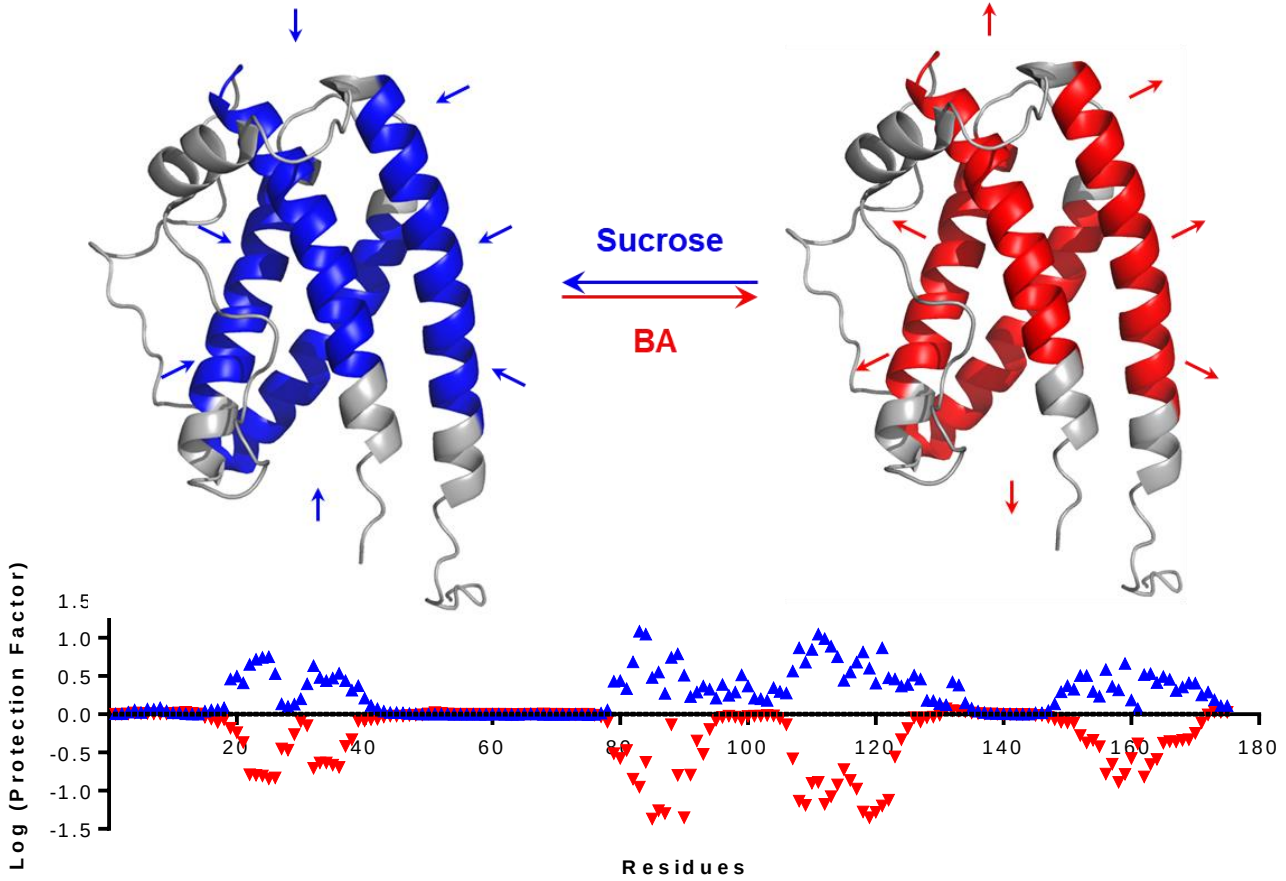
THE THERMAL DIMER FORMATION VIA DOMAIN SWAPPING



Zhang et al. *Biochemistry* 2018

Structural insights combined with other biophysical characterizations propose the thermal dimer formation via domain swapping

EXAMPLE 5: EFFECTS OF SUCROSE AND BENZYL ALCOHOL ON GCSF UNDER PHYSIOLOGICAL CONDITIONS



Sucrose
+
GCSF
+
BA

Zhang et al. *J. Pharm. Sci.* 2015

Sucrose partially counteracts benzyl alcohol induced high aggregation propensity by shifting the molecular population towards more compact conformations

SUMMARY AND FUTURE DIRECTIONS

- MS-based approaches are powerful analytical tools for drug discovery and development of therapeutic proteins
- HDX-MS continue playing a dominant role in higher order structure characterization
- Due to the unique capability, there will be more HDX-MS applications in structure-function/stability relationship analysis for therapeutic proteins
- Further improving automation and robustness will significantly increase applications of MS-based approaches for structural characterization in the biopharma industry

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