Developability Risk Assessment for dual variable domain immunoglobulin (DVD-Ig™)

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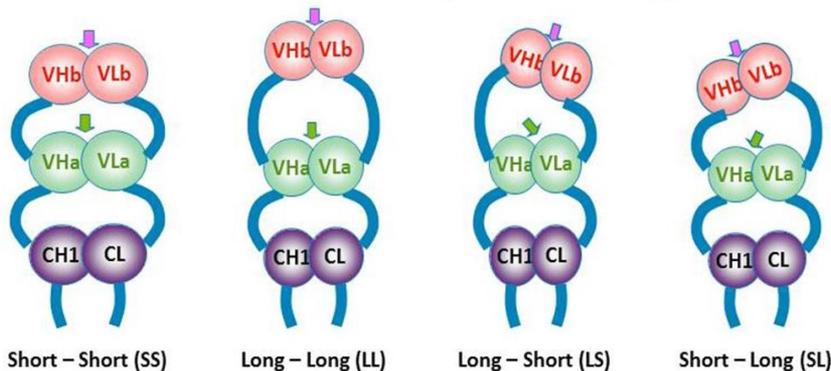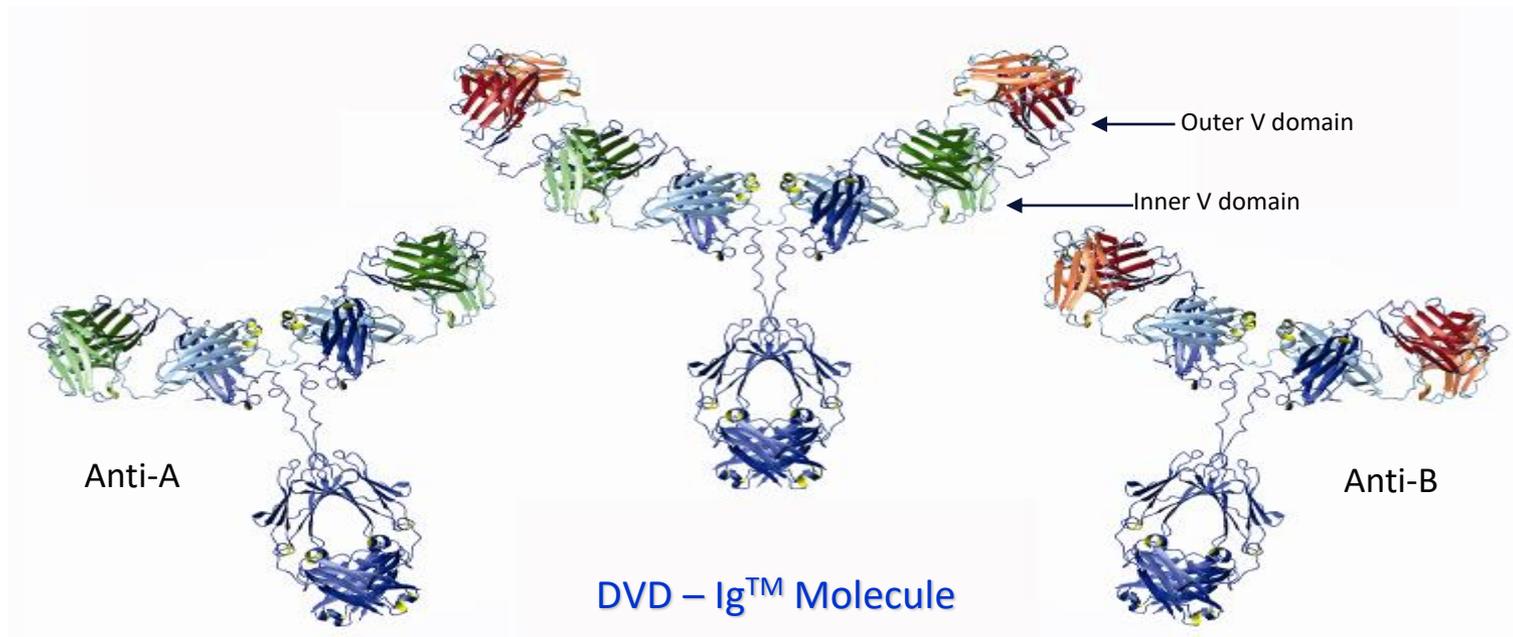
Virtual CASSS HOS

Disclosure

The author, <u>Dana I. Filoti</u> is a paid employee of AbbVie Inc.

All study design, research, analysis, data collection, and interpretation of data in this presentation, as well as the writing, reviewing, and approval of this presentation was conducted at AbbVie Inc. All materials and equipment used in the study described in this presentation, as well as all funding for the study and the presentation, were provided by AbbVie Inc.

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The dual variable domain immunoglobulin (DVD-Ig™) technology

Outer V domain

Inner V domain

Anti-A

Anti-B

DVD – Ig™ Molecule

Short – Short (SS)   Long – Long (LL)   Long – Short (LS)   Short – Long (SL)

Key Features of DVD-Ig Molecules

- Bi-specific tetravalent molecule
- Maintaining symmetry of the mAb
- Using naturally occurring sequences as linker
- Having modular structure of two binding domain on each arm

Jakob, C. et al mAbs, 5: 358-363, 2013
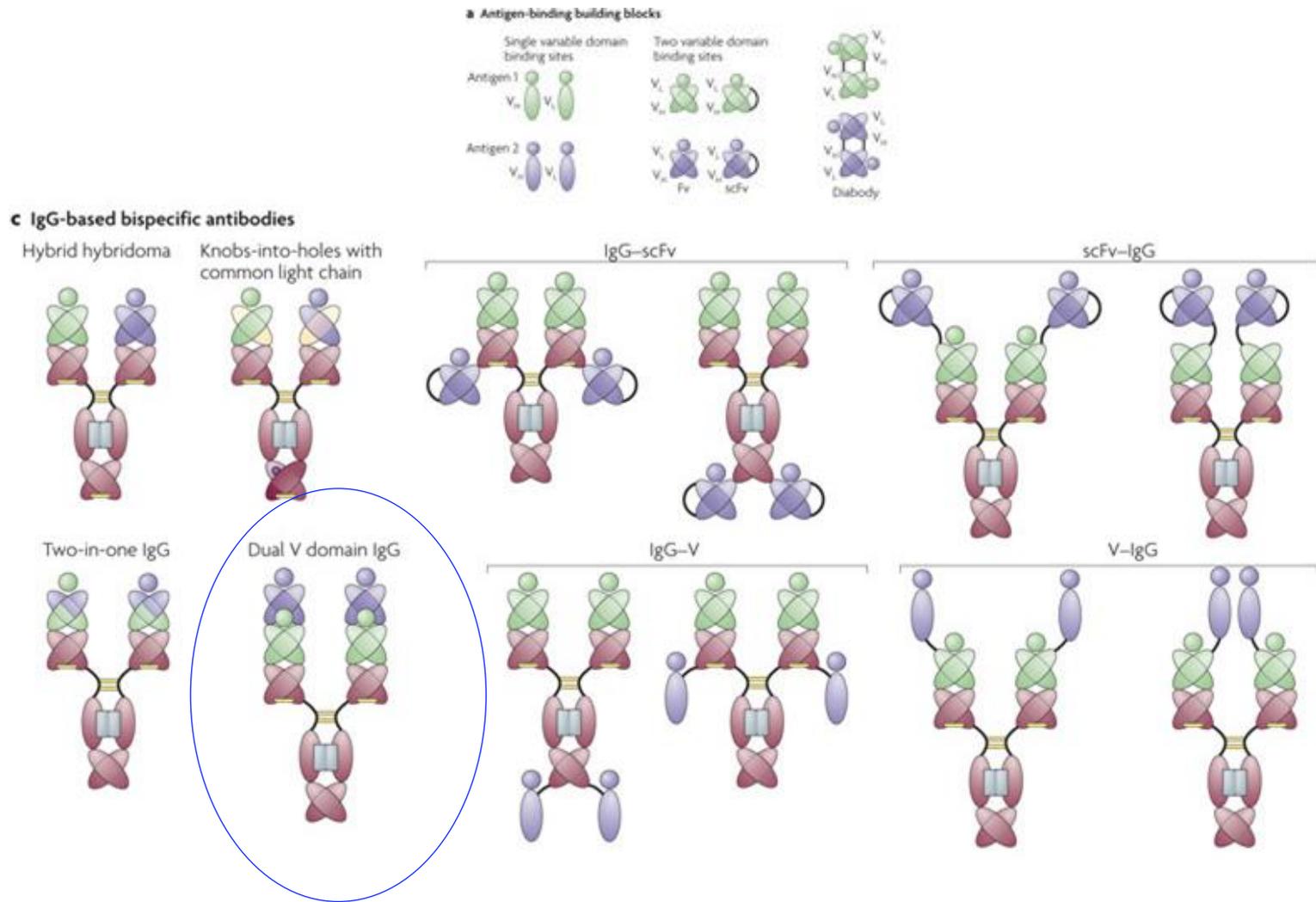
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Typical therapeutic monoclonal antibody
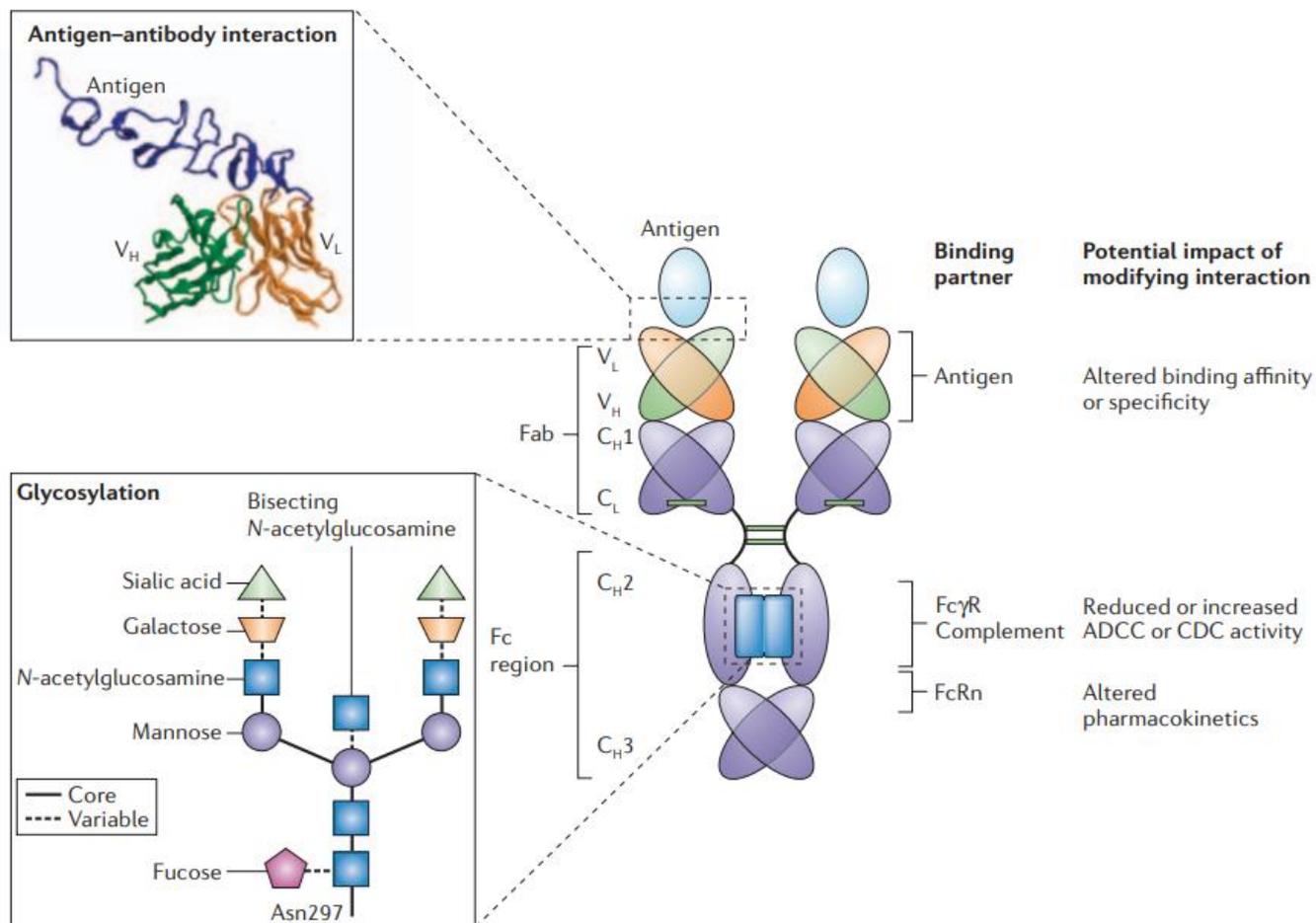
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Analytical characterization methods/tools available for analytical characterization

Analytical method	Properties Elucidated
Content	Protein concentration, oligosaccharide profile
General tests	pH, Osmolality, appearance, color, clarity
Purity	Charge isoforms, reduced LC-MS, bioburden, endotoxin, protein A, HCP, SEC and CE-SDS
Pharmaceutical Tests	Sub-visible particles
Amino acid Analysis	amino acid composition
Mass Spectrometry	Intact MW
	Reduced MW
	Deglycosylated MW
Non-reduced tryptic and Lys-C peptide mapping with MS detection	Disulfide bond pairing
Free sulfhydryl assay	
Circular Dichroism spectroscopy	2° structure
Differential scanning calorimetry	Melting temperature
Analytical ultracentrifugation (AUC)	Hydrodynamic shape
	% Monomer
Biacore	Antigen-antibody binding
Cell based bioassay	Inhibition of cell proliferation

primary — Amino acid Analysis, Mass Spectrometry

secondary — Non-reduced tryptic and Lys-C peptide mapping with MS detection, Free sulfhydryl assay, Circular Dichroism spectroscopy

tertiary — Differential scanning calorimetry, Analytical ultracentrifugation (AUC), Biacore, Cell based bioassay

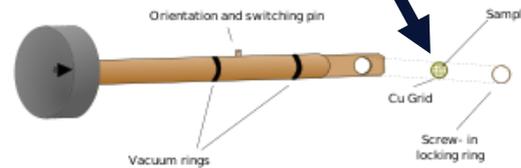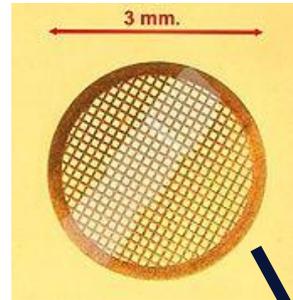abbvie

Alternative antibody formats

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Visualization and Animation of a DVD-IgG

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Electron Microscope (resolution = 0.2 nm) versus light microscope (resolution ~ 200 nm)

Electron source

Specimen

Objective lens

Ocular
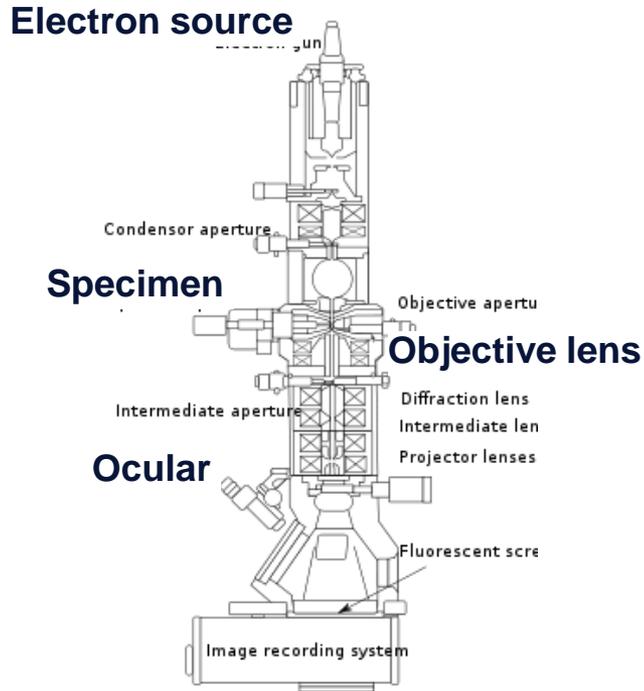
Grid: Protein + heavy metal

Negative stain
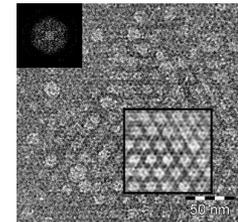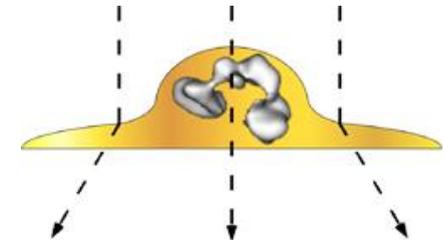
For better contrast specimens stained with heavy metals (U, Mo, W). Electron beam primarily interacts with the stain. When stain is added, the stain surrounds the sample but is excluded from the volume occupied by the sample

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2D Class Average Analysis

•**Particle selection:** Individual particles in the high magnification images were selected using automated picking protocols

•**Alignment:** A reference-free alignment strategy was used. Algorithms in this package align the selected particles

Classification: sort into similar classes. Twenty or thirty classes classified using XMIPP package

•The XMIPP package uses the Kernel Probability Density Estimator Self-Organizing Map classification method which maps a set of high dimensional input vectors into a regular two-dimensional grid, so that the proximity of the units in map reflects similarity of input data.

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Imaging of mAB using transmission electron microscopy + 2D class averaging

mAbs

Class average   Raw particles

- The left most column shows representative class averages from the samples and pictures on the right are examples of raw particles that went into each respective class average

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Imaging of DVD-Ig molecules using transmission electron microscopy + 2D class averaging

DVD

Inner   Outer

Fc

Hinge

Fab

Class average   Raw particles

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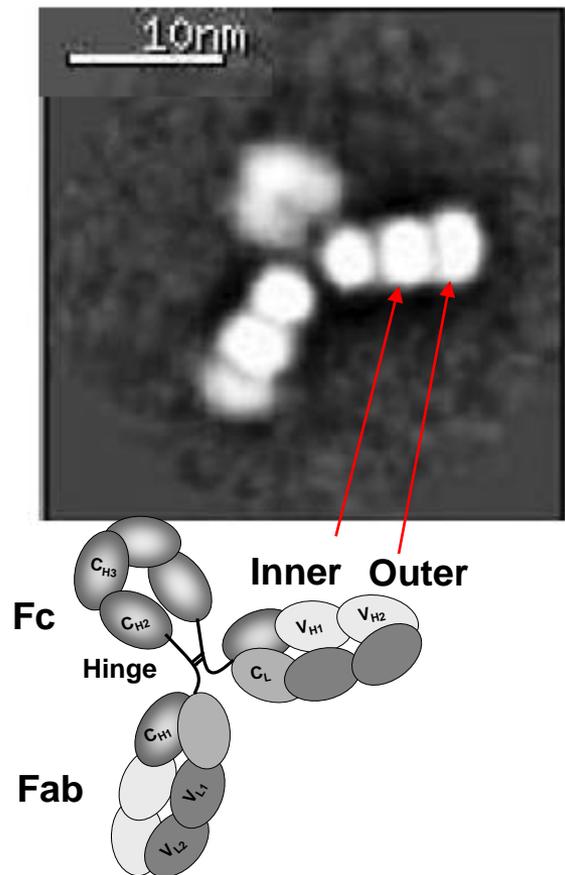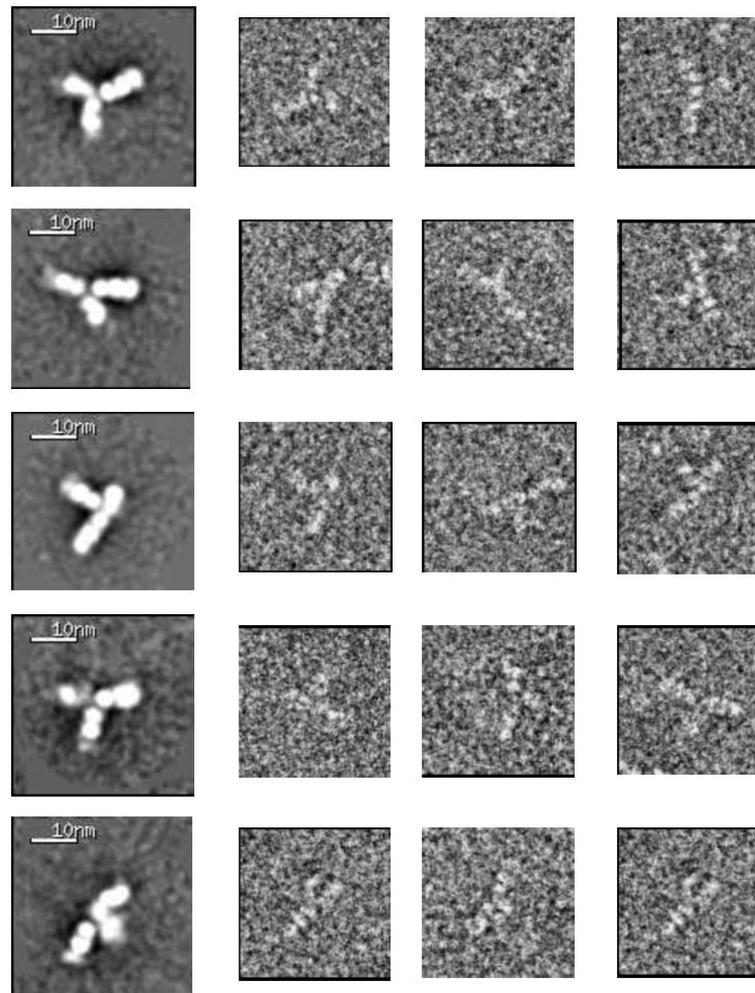
Dynamics of Fab arms allows binding of antigen of various size
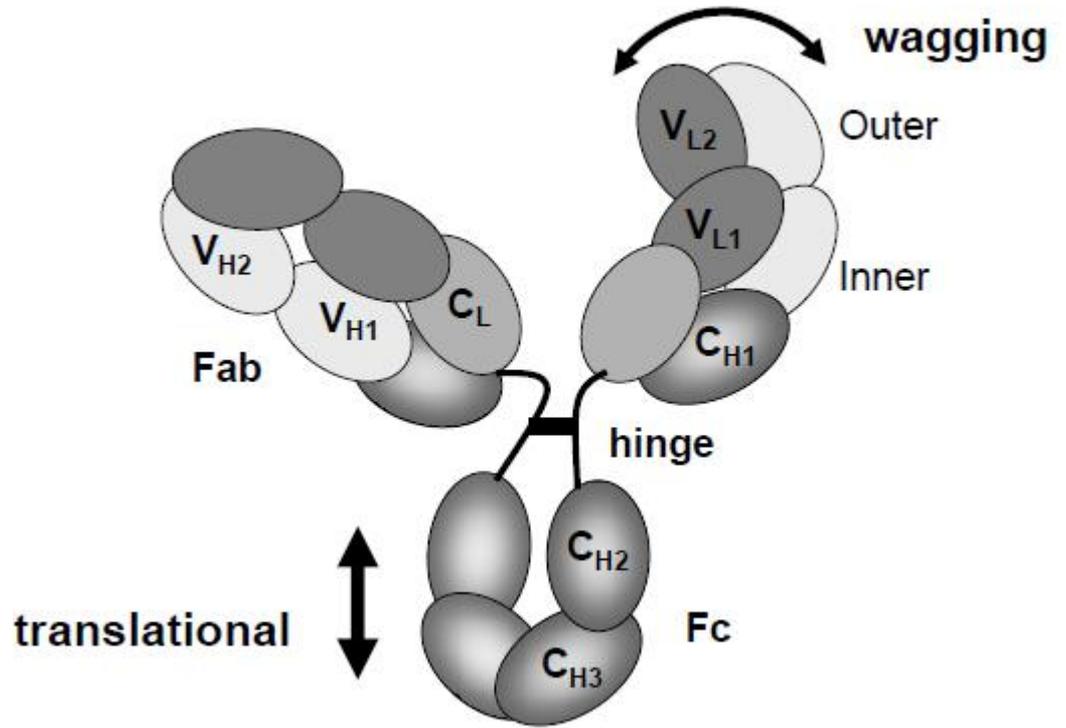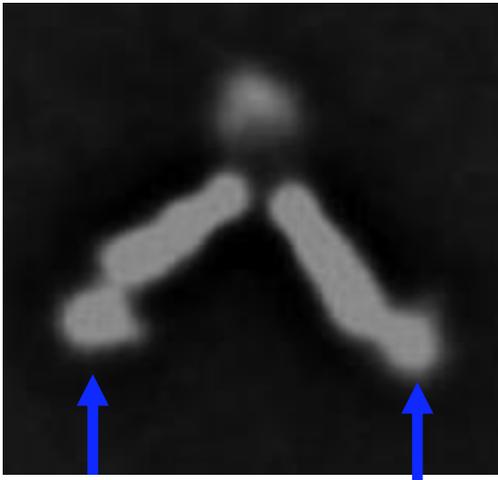
- We observe hinge region flexibility, rotation/wagging of the outer binding domain and translational movement and flexibility in the Fc region

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Developability risk Assessment for DVD-IgG

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Analytical Challenges with bispecific molecules
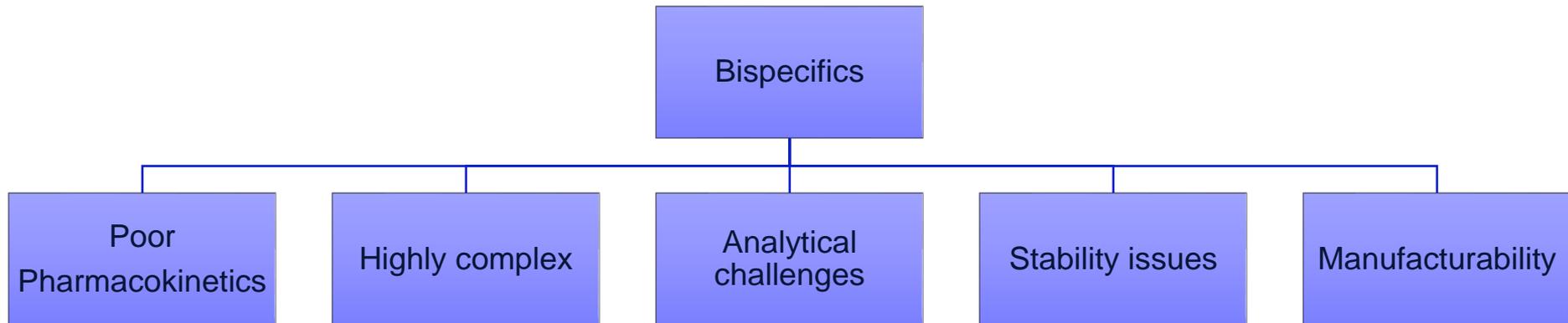
Screening for Continuous Improvement in Biologics Development

Developability Screening, Y

Formulation Screening, Z

Sequence Liability Screening, X

Novel Tools for Developability Screening, W

The n-dimensional Euclidian Space for Molecule Performance Optimizer = Wisdom

Screening Process for Biologic Candidate Selection

Total Antibody Diversity	Sequence Screening	Developability Tier 1	Developability Tier 2	Platform Suitability

Screening Libraries	HEK/CHO		CHO

Expression Host

Unique Sequences

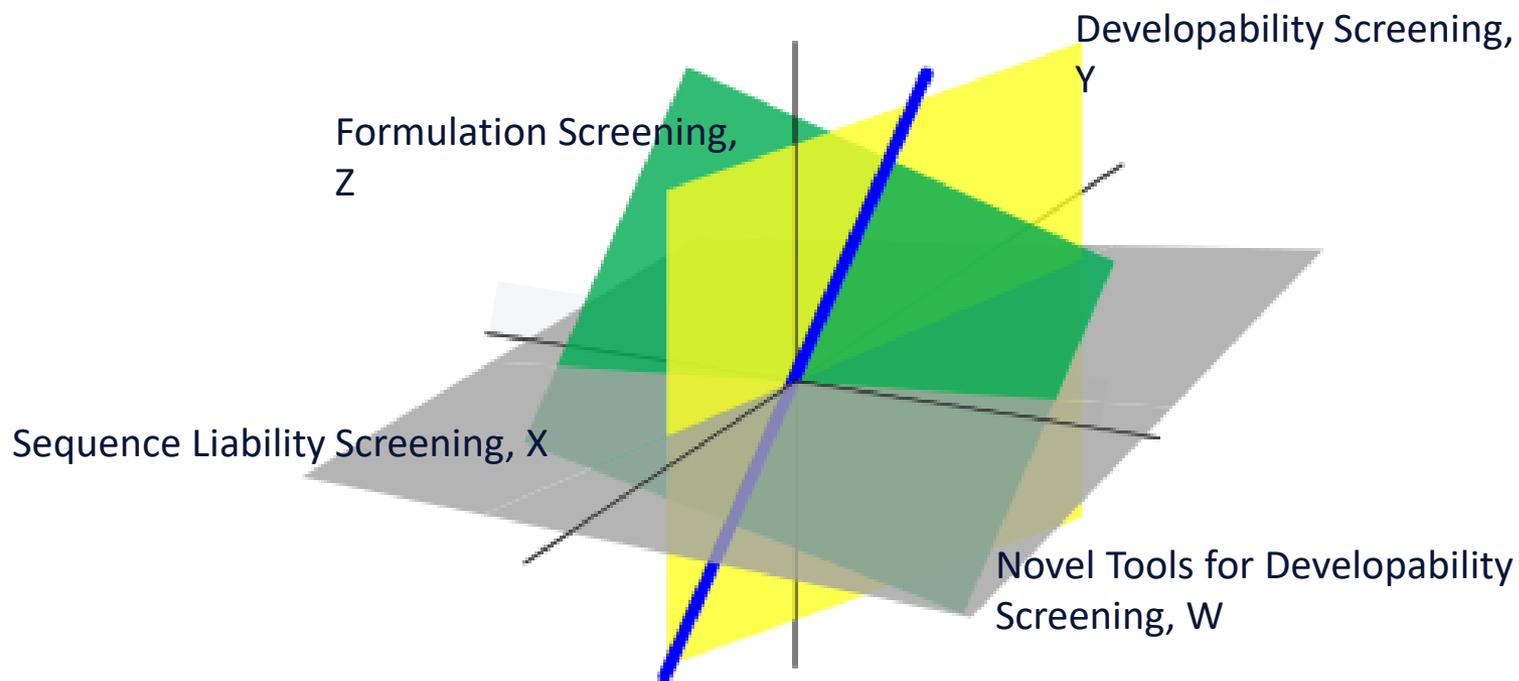| Millions	100's	10's	<10	1-2

Screen

➢ Antigen Binding

➢ In Silico Screen
➢ *In Vitro* Potency and Binding

➢ Intermediate concentration conformational and colloidal stability
➢ LC/MS Subunit analysis
➢ *In Vivo* Potency
➢ Affinity

➢ Forced degradation
➢ Comprehensive physicochemical screening
➢ High Concentration
➢ Serum Stability
➢ In vivo PK

➢ Additional physico-chemical investigations
➢ Targeted characterization as requested by project teams
➢ Process Sciences support
➢ Excipient screening
➢ Safety

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Sequence screening: *in silico* liability assessment and removal of motifs associated with post-translational modifications

Minimize heterogeneity to increase compatibility with platform manufacturing process of therapeutic candidates

Pyroglutamate formation: N-terminal Q

Glycosylation: N{P}S{P}, N{P}T{P}

Oxidation: M, C

Deamidation: NG, NS, QG

Isomerization: DG, DS

Cleavage: DP

High Risk

Deamidation: NH

Hydrolysis: NP

Cleavage: TS

Med. Risk

Deamidation: SN, TN, KN

Low Risk

L-Asparagine

deamidation

L-Aspartic acid

hydrolysis

L-Succinimide intermediate

hydrolysis

L- Isoaspartic acid
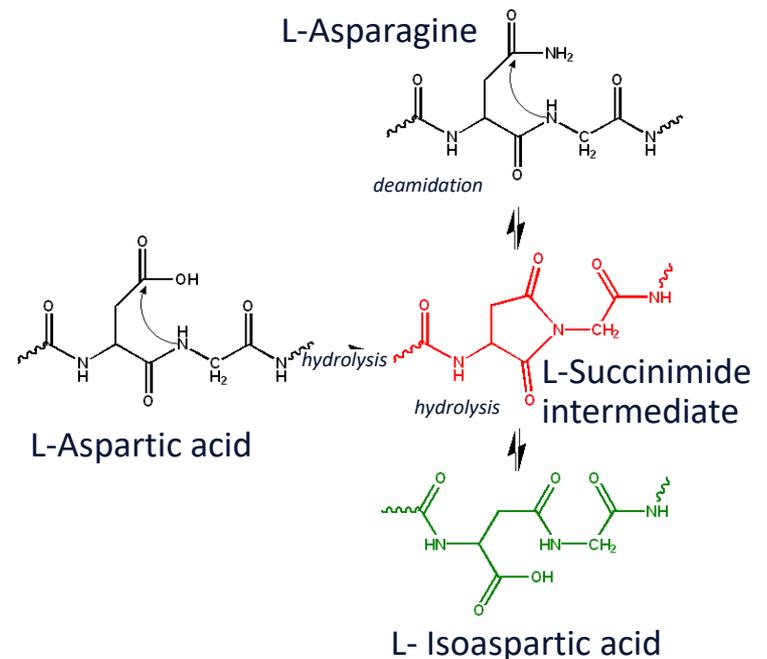
Chelius D., et. al. Analytical Chemistry 77:6004, 2005

Removing protein sequence liability motifs from the variable region without negatively impacting antibody function and project timeline

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Drug-Like Properties Screening for New DVD-Ig Candidates

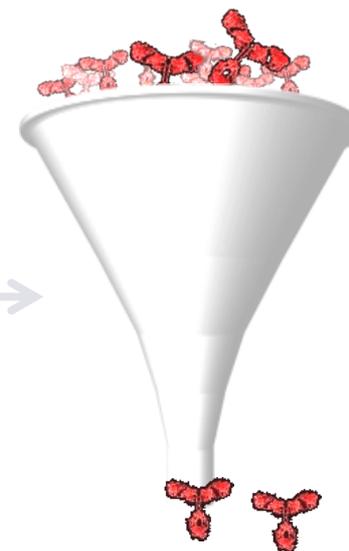| 19 **Tier 1a** DVD-Ig pre-liability engineered | 10 **Tier 1b** DVD-Ig Post-liability engineered | 5 **Tier 2** DVD-Ig Post-liability engineered |
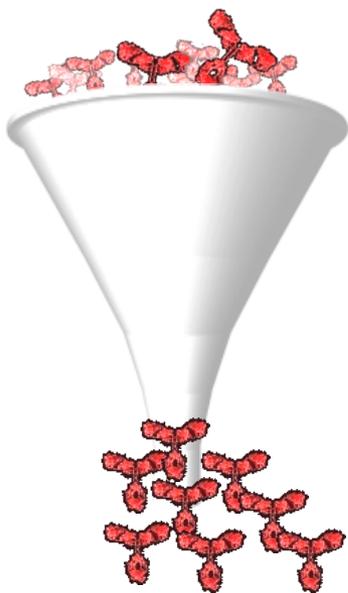
Liability engineering

Scale up

| 10 DVD-Ig post-liability engineered | 5 DVD-Ig Post-liability engineered | 2 LEAD DVD-Ig Post-liability engineered |

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Summary of Results from Tier 1b Screening of A/B and B/A DVD-Ig Candidates

Sample	Concentration		DSC	Cold Storage/High Concentration		Accelerated Storage		
	Achieved concentration	Appearance	Unfolding T_{onset} (°C)	%monomer loss	Appearance	Low concentration % monomer loss	High concentration % monomer loss	Appearance High concentration
S1		Clear			Clear		HMW	Clear
S2		Clear			Clear	LMW	HMW/LMW	Clear
S3		Clear			Clear		LMW	Clear
S4		Clear		HMW	Clear	LMW	HMW/LMW	Clear
S5		Clear			Clear			Clear
S6		Clear			Clear			Clear
S7		Clear		HMW/LMW	Clear	LMW	LMW	Clear
S8		Clear			Clear			Clear
S9		Clear		HMW	Clear	LMW	HMW/LMW	Clear
S10		Clear			Clear			Clear

This group of DVD-Ig candidates represents A/B and B/A formats as well as a variety of interdomain linker structures.

Summary of Results from Tier 2 Screening of A/B and B/A DVD-Ig Candidates

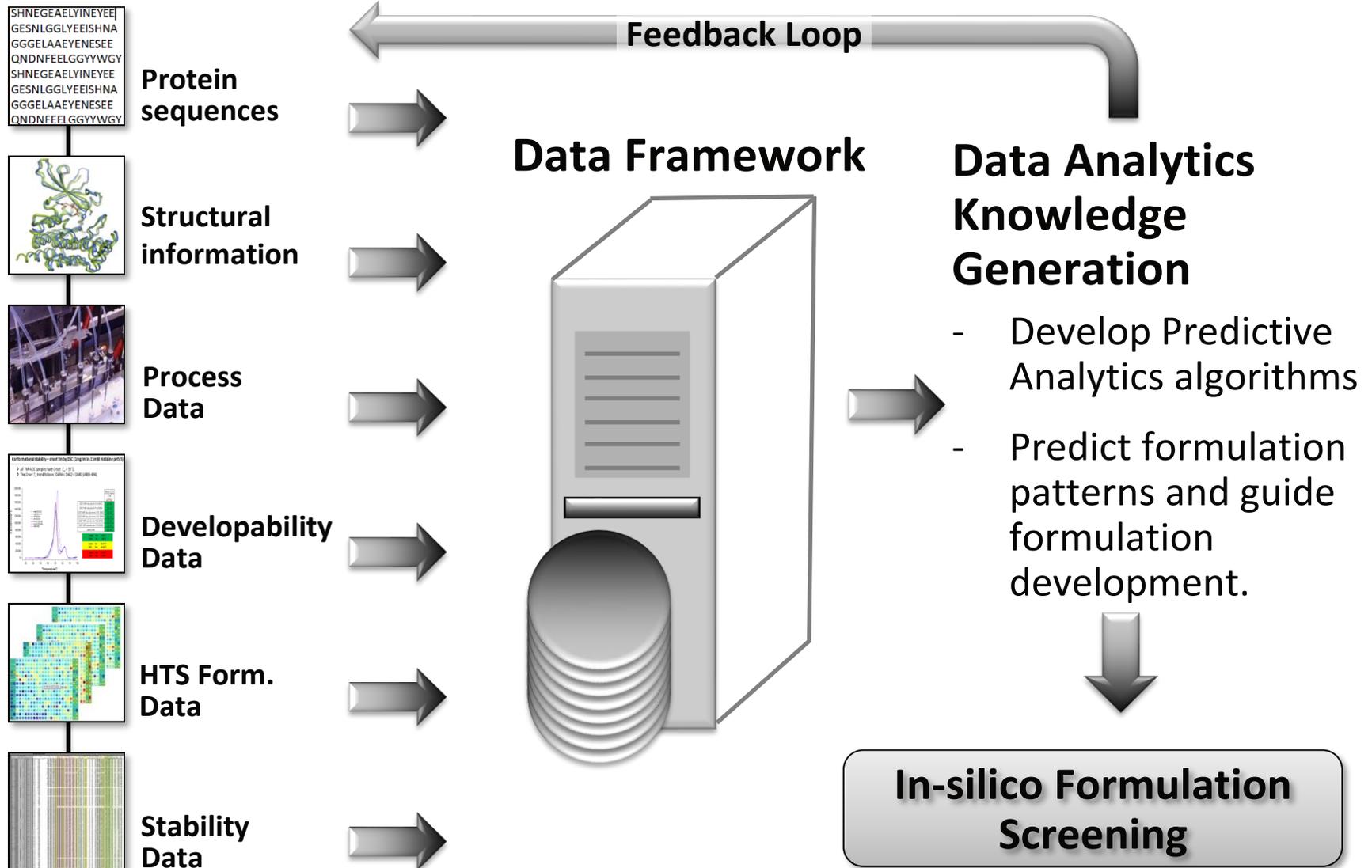| Sample Identifier	Measured pI (icIEF)	Maximum assessed Solubility (mg/mL)	Viscosity (cP)	Conformational stability — Unfolding via DSC, T_{onset} (°C)	Accelerated Condition — % High concentration monomer loss
S4	>7				HMW/LMW
S5	>7				HMW/LMW Gelled
S6	>7				HMW Gelled
S7	>7				HMW/LMW
S8	>7				HMW
DVD-Ig Negative control	>7				HMW
DVD-Ig Positive control	>7				HMW

- All samples in minimal buffer
- 100 mg/mL Concentration
- High aggregation and gelling of S6 resolved by changing buffer conditions.
- CHO cell line initiated for S6 and S8.
- Both lead candidates had different configurations: A/B and B/A as well as linkers.

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Overview of Formulation Screening Automation Line

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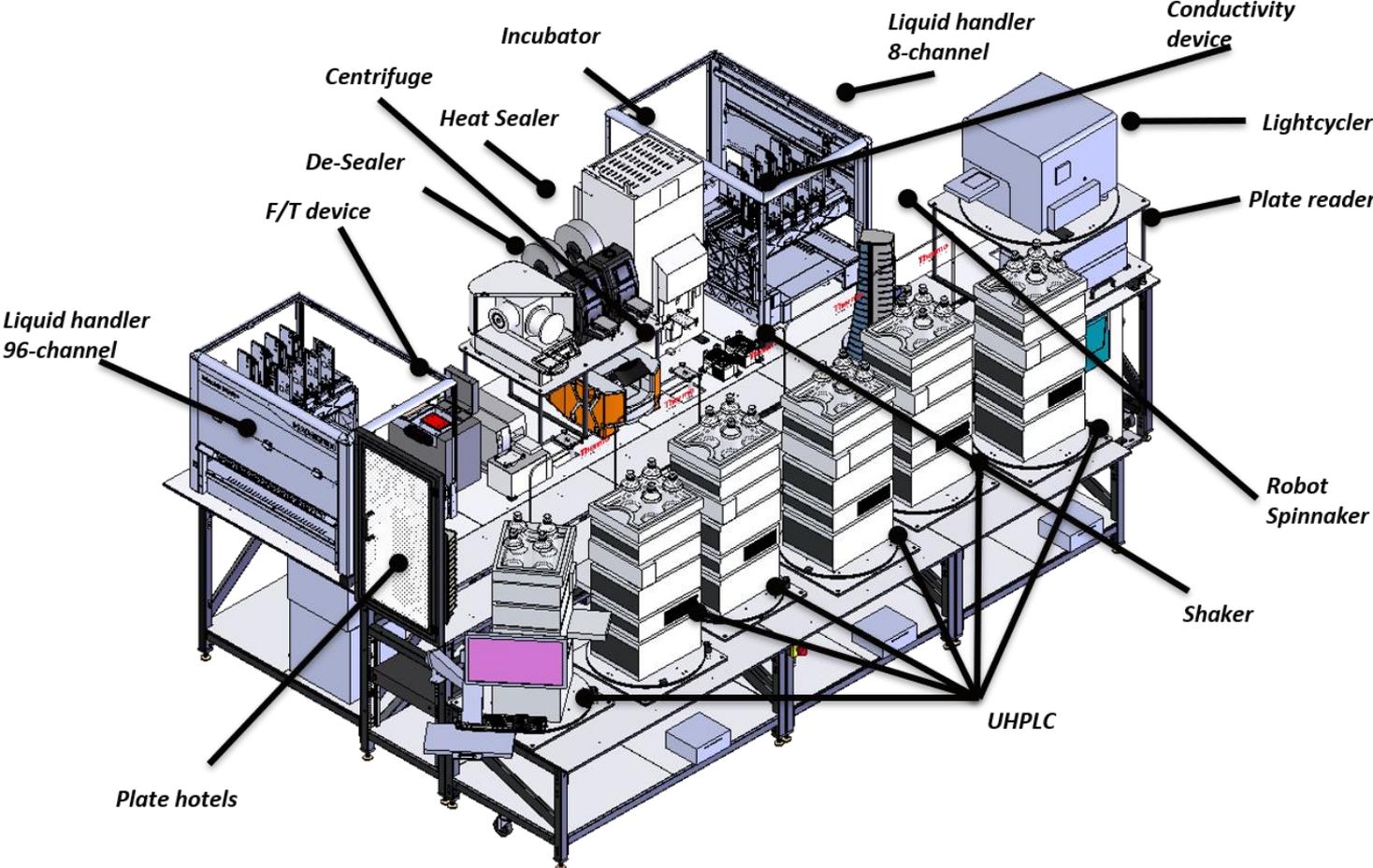
Outlook: Utilizing Predictive Analytics as a Tool for Continuous Improvement in Biologics Development

Protein sequences

Structural information

Process Data

Developability Data

HTS Form. Data

Stability Data

Feedback Loop

Data Framework

Data Analytics Knowledge Generation

- Develop Predictive Analytics algorithms

- Predict formulation patterns and guide formulation development.

In-silico Formulation Screening

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Relationship among data, information, knowledge and wisdom

| Aspects	Semantic load	
Data	Input of risk assessment	Developability and Process data
Information	Risk description	Critical Quality Attributes
Knowledge	Understanding the description and make decision	Standardization and Predictive analytics
Wisdom	(understand why) the ability to use results of analysis in the right way	Machine Learning

Why ? ····· Wisdom

How ? ····· Knowledge

What ····· Information

Who/ When/ Where ? ····· Data
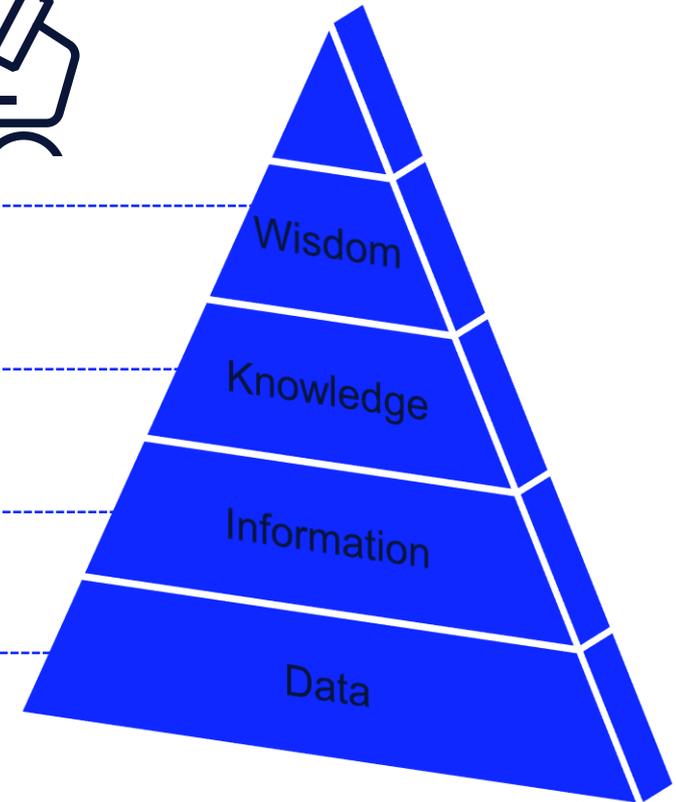
Duan Y, et.al., IEEE, DOI:10.1109/SERA.2017.7965747, 2017

How to use wisdom graph to predict unknown elements?

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Developability Risk Assessment for dual variable domain immunoglobulin (DVD-Ig™) Summary:

- Imaging studies suggest DVD-IgG molecules are more flexible than mAb's

  - The molecule retains the "Y" shaped structure of antibodies when not bound to antigen or bound to either antigen.

- New challenges are encountered with novel antibody formats and developmental stability information become imperative to address/mitigate some of these challenges

  - Depending on linker we observe different types of solution stability attributes and flexibility of the outer binding domain

  - Binding of antigen to inner domain resulted in a conformational change in outer binding domain. The outer domain folds out of the plane of inner binding and CH1/CL domains

- Utilizing Predictive Analytics as a Tool for Continuous Improvement in Biologics Development

  - Data analytics knowledge generation solutions are imperative for the development complexity of bispecific molecules

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Acknowledgments

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Research and Early Development

Global Protein Science

Biologics Generation

NBE Formulation

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