Developability Evaluation During Early Pre-formulation: The Meeting Point Between Research and Development

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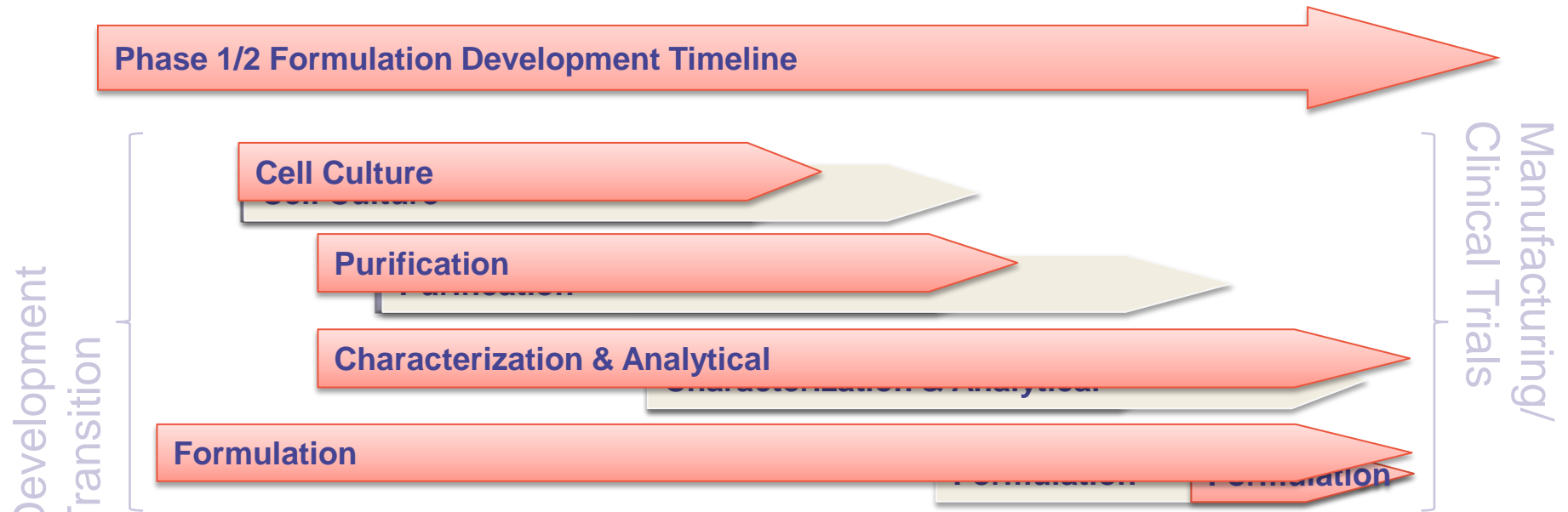One the Mountain Rd. Framingham MA 01701, USA

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Challenges Facing Protein Formulation and Product Development

- Time and budget allotted for developing and implementing formulations.

- Regulatory requirements.

- Requirements to work closely across sites and divisions as well as with external suppliers.

- Need for quality, innovation and rapid time to market.

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Product Development Timeline

Phase 1/2 Formulation Development Timeline

Development Transition

Manufacturing/ Clinical Trials

Cell Culture

Purification

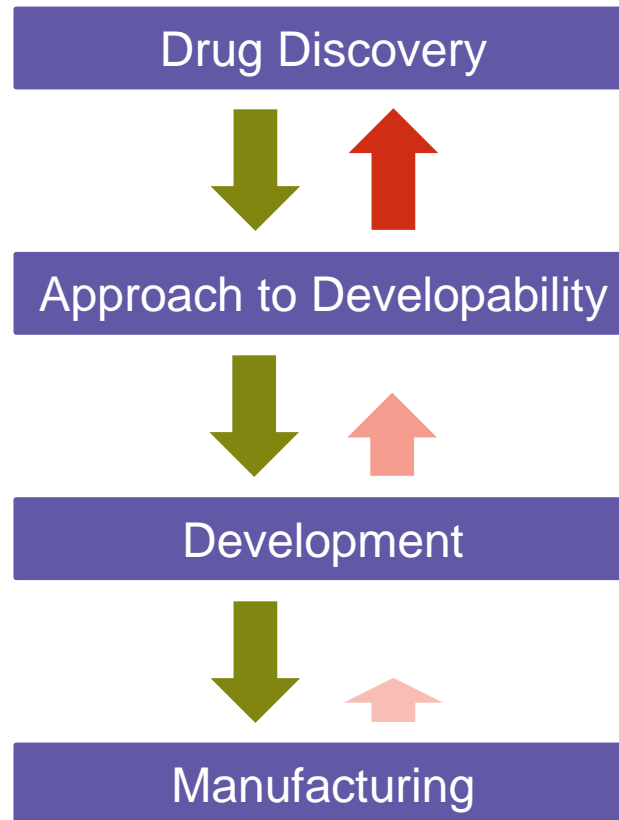Characterization & Analytical

Formulation

- Need time for robust formulation development
- Insufficient window for real-time data
- Start formulation development early (integration with research)
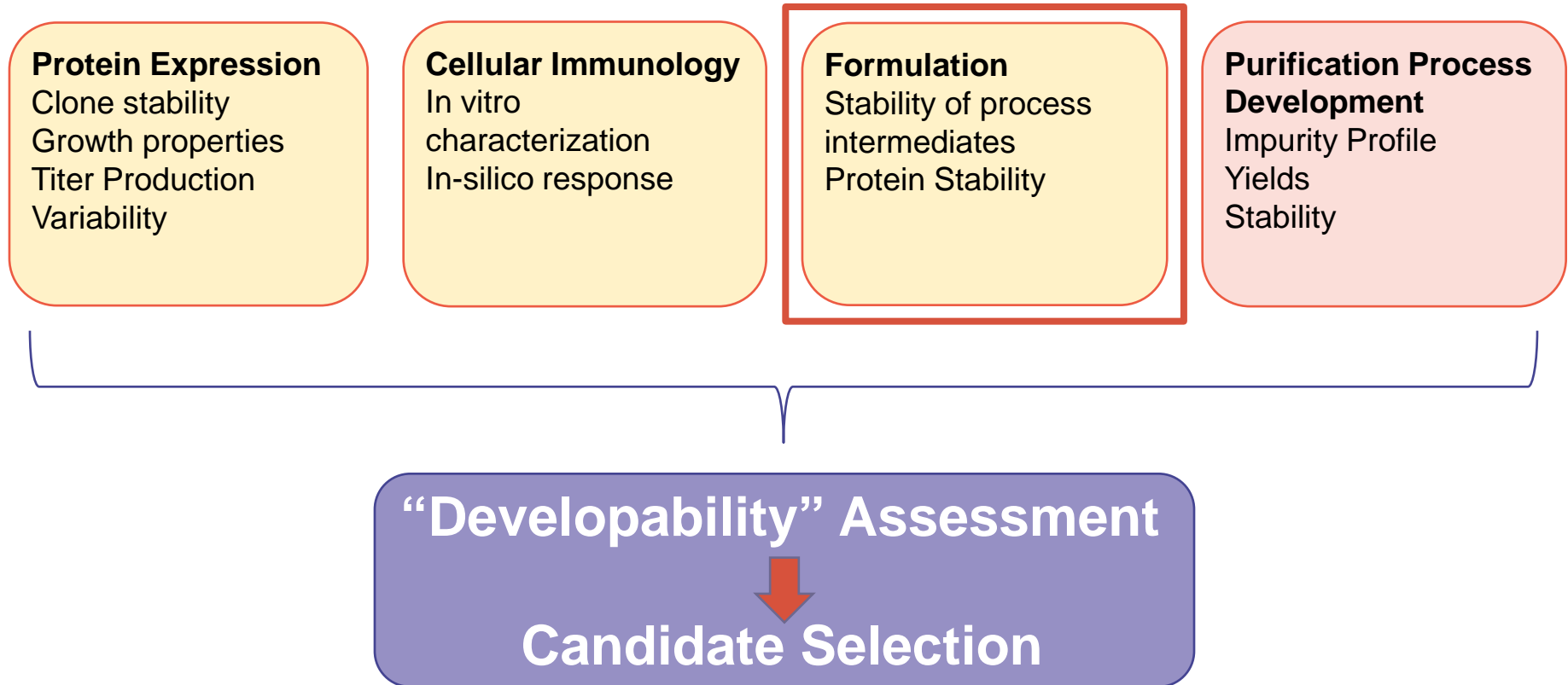
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Principles of the Formulation Developability Assessment: The Interface with Discovery Research

- **Intrinsic Quality of the Constructs**
  - Can it handle the stresses associated with development and manufacturing process?
  - What are the degradation "pathways" of the protein?
  - Rank candidates

- **Information on Proper Handling**
  - Reduce false negative results due to incorrect handling

- **Very Early Information on Protein Solution Behaviour**
  - Accelerates the formulation development process
  - Foundation for QbD

- **Great Return on Investment**
  - Cost ~20 mg and 4-8 weeks.

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Developability Evaluation During Early Pre-Formulation

Drug Discovery

Approach to Developability

Development

Manufacturing

Development Candidate Risk Assessment – Synergistic Collaboration

Protein Expression
Clone stability
Growth properties
Titer Production
Variability

Cellular Immunology
In vitro characterization
In-silico response

Formulation
Stability of process intermediates
Protein Stability

Purification Process Development
Impurity Profile
Yields
Stability

"Developability" Assessment

Candidate Selection

Formulations Role in Developability Assessment

Developability Evaluation

Relevant Process Stresses
Freeze/thaw
Shear/Mechanical stress
 -Viscosity, injectability, solubility
Mock viral inactivation

Forced Decomposition
Degradation (pH 5-8)
Temperature (45 °C)

- **Desired results: No change**

- **Desired results: Sufficient degradation to understand protein profile and differentiate candidate molecules**

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Stability and Solution Behavior Concerns

Physical Stability

- Soluble aggregation
- Self association
- Insoluble aggregation
- Particle formation
- Solubility
- Viscosity

Chemical Stability

- Oxidation
- Deamidation
- Isomerization
- Fragmentation
- Disulfide exchange

Function/binding

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Analytics

Physical Stability

A280 (concentration)
Turbidity
Circular dichroism (secondary and tertiary structure)
CE-SDS (purity)
SDS-PAGE (purity)
SEC (aggregation, fragmentation)
N-terminal sequencing (identity)
AUC (aggregates/oligomerization)

Chemical Stability

cIEF (charge variants)
Peptide mapping
Oxidation (methionines in CDR)
Isomerization (aspartic acid in CDR)
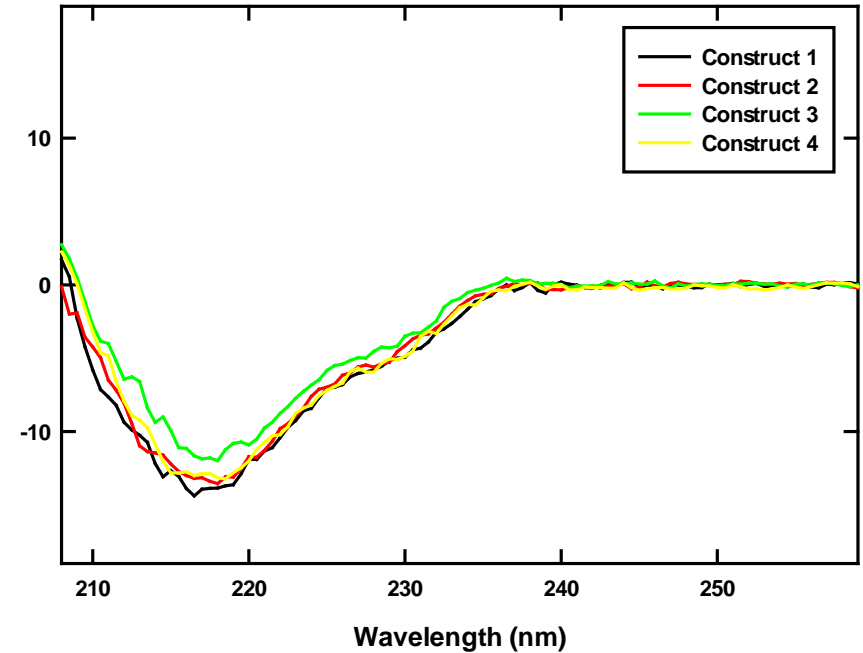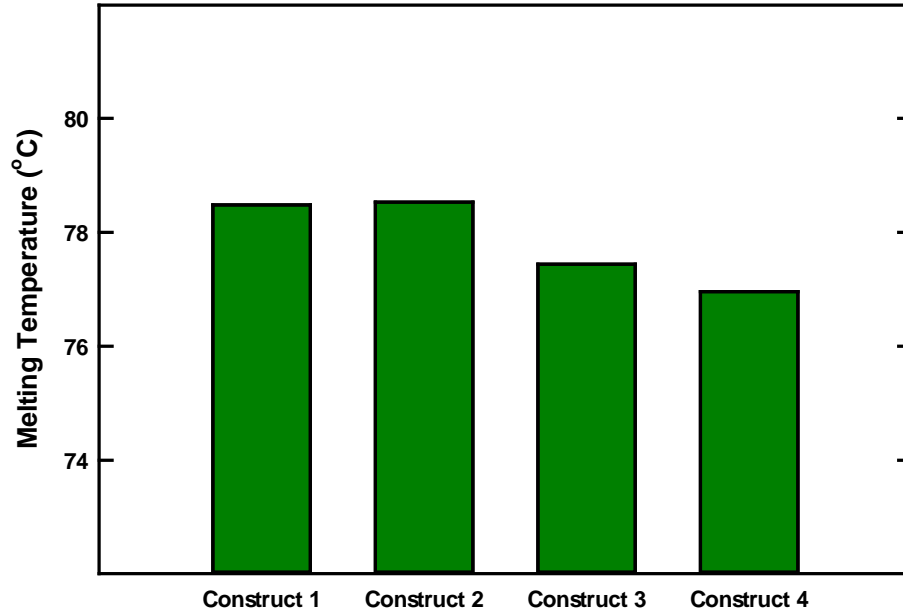Deamidation (PENNY peptide)

Functional Stability

Surface Plasma Resonance (Biacore)

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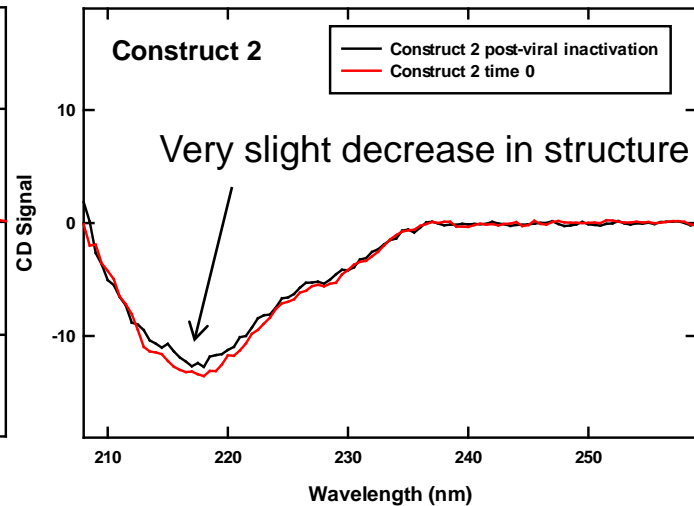# CASE STUDY: Which candidate has the best chance of making it through development and manufacturing…?
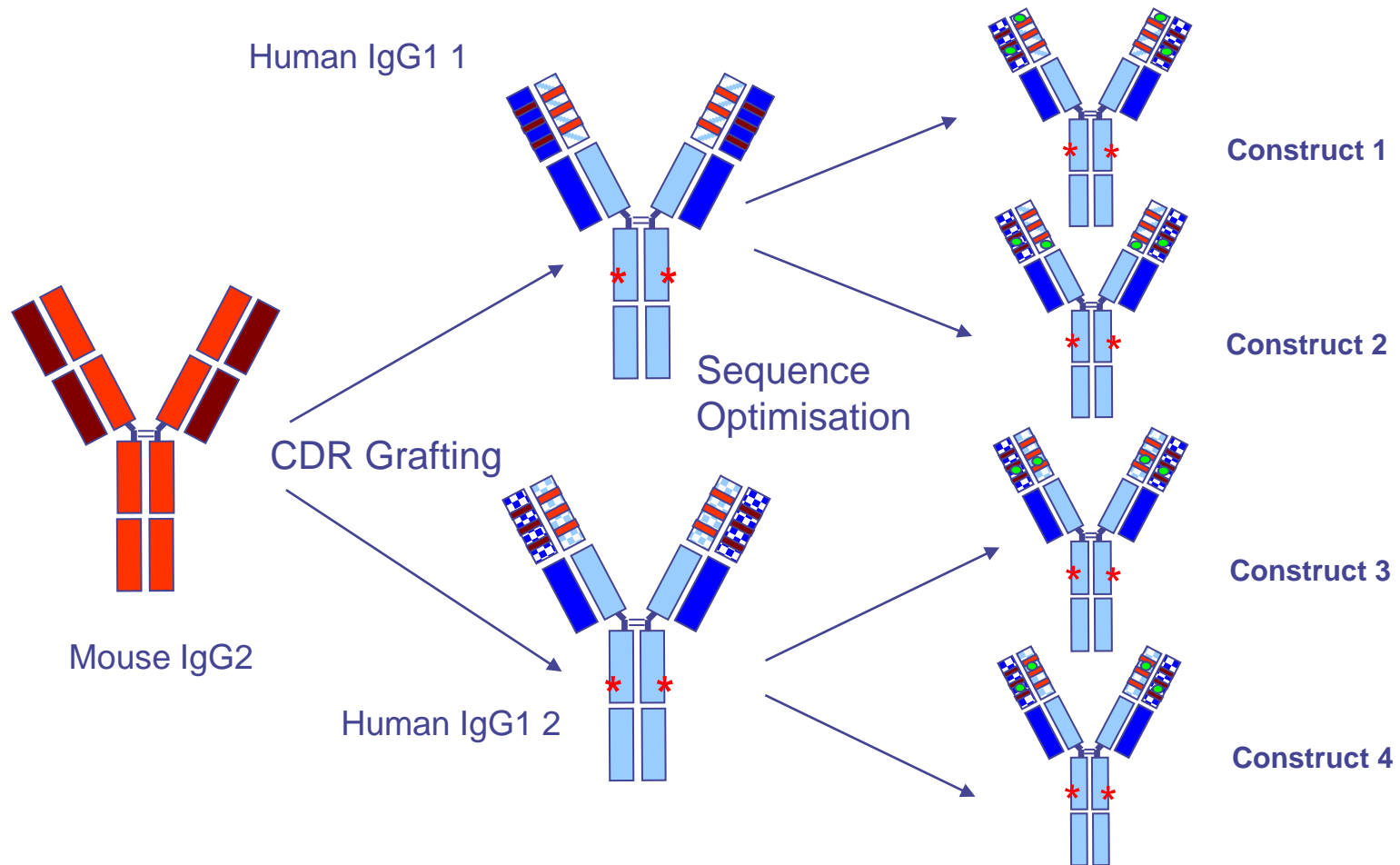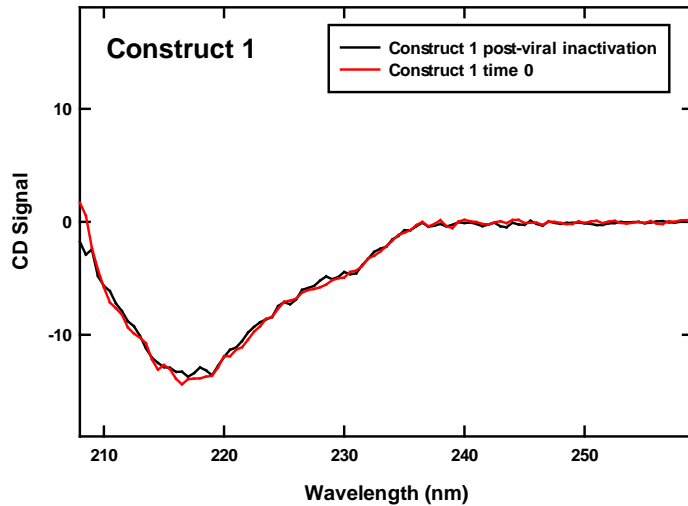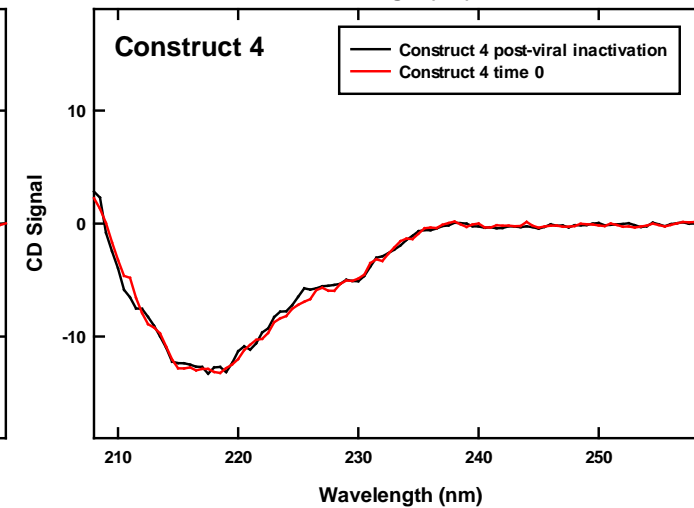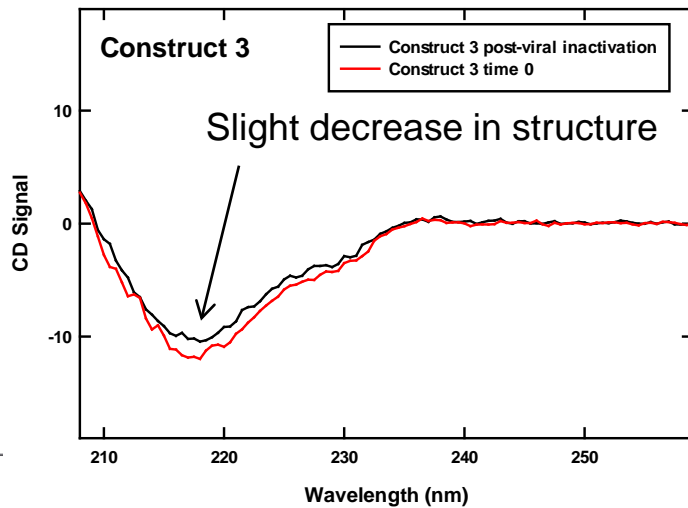
General Properties

More thermal stability in Constructs 1 and 2

Less secondary structure in Construct 3

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PROCESS STRESSES: Mock Viral Inactivation - Secondary Structure by CD

Red = time 0
Black = post vi

PROCESS STRESSES: Mock Viral Inactivation (VI)

Quality Attribute	C-1	C-2	C-4	Comments
Aggregation	🟩	🟩	🟩	
Purity	🟩	🟩	🟩	
Charge Heterogeneity	🟩	🟩	🟩	
Oxidation	🟩	🟩	🟩	
Secondary Structure	🟩	🟨	🟩	Possible ↓Construct 2
Turbidity	🟨	🟨	🟩	
Melt Point	🟩	🟩	🟩	

*Quality attributes ranked in order of criticality

🟩 No change 🟨 Minor change 🟧 Moderate change 🟥 Major change

C3 was not considered as a candidate due to its obvious weak performance on most analytics and lack of *in vitro* activity

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PROCESS STRESSES: Shear/Mechanical Stress

Quality Attribute	C-1	C-2	C-4	Comments
Aggregation	🟩	🟩	🟩	
Purity	🟩	🟩	🟩	
Charge Heterogeneity	🟩	🟩	🟩	
Secondary Structure	🟩	🟩	🟩	
Turbidity	🟩	🟩	🟩	
Melt Point	🟩	🟩	🟩	

*Quality attributes ranked in order of criticality

🟩 No change 🟨 Minor change 🟧 Moderate change 🟥 Major change

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PROCESS STRESSES: Freeze Thaw Study

Quality Attribute*	C-1	C-2	C-4	Comments
Aggregation	🟩	🟩	🟩	
Purity	🟩	🟩	🟩	
Charge Heterogeneity	🟩	🟩	🟩	
Secondary Structure	🟩	🟩	🟨	
Turbidity	🟩	🟩	🟩	
Melt Point	🟩	🟩	🟩	

*Quality attributes ranked in order of criticality

🟩 No change   🟨 Minor change   🟧 Moderate change   🟥 Major change

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FORCED DECOMPOSITION: pH Study - Deamidation of PENNY Peptide
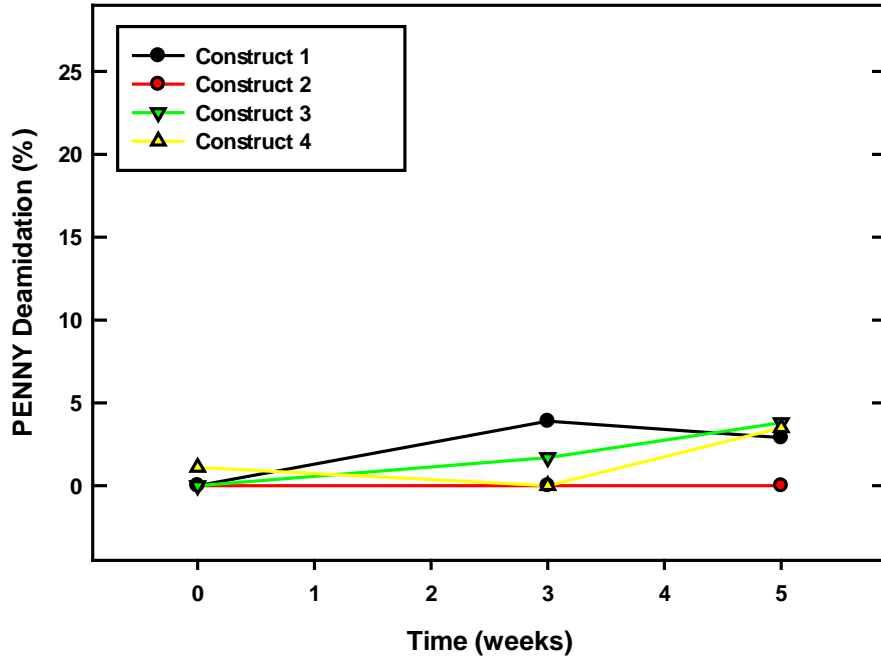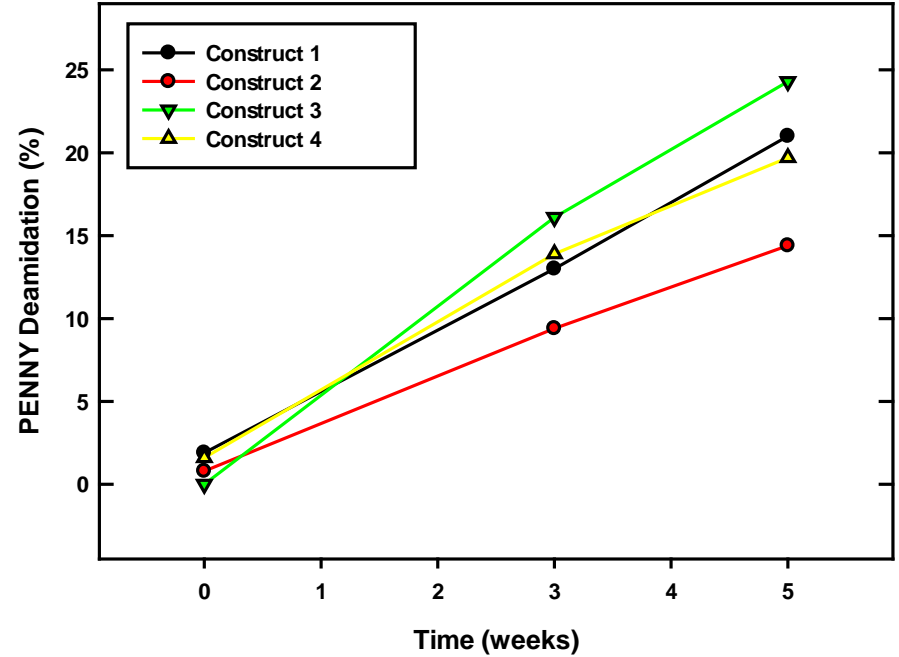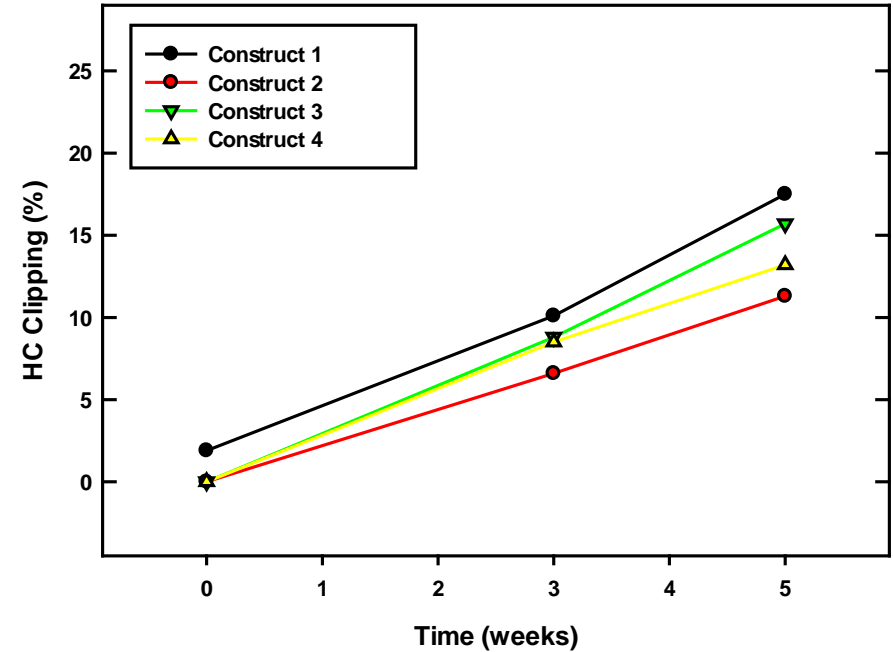
FORCED DECOMPOSITION: pH Study - Heavy Chain Clipping

pH 5.0
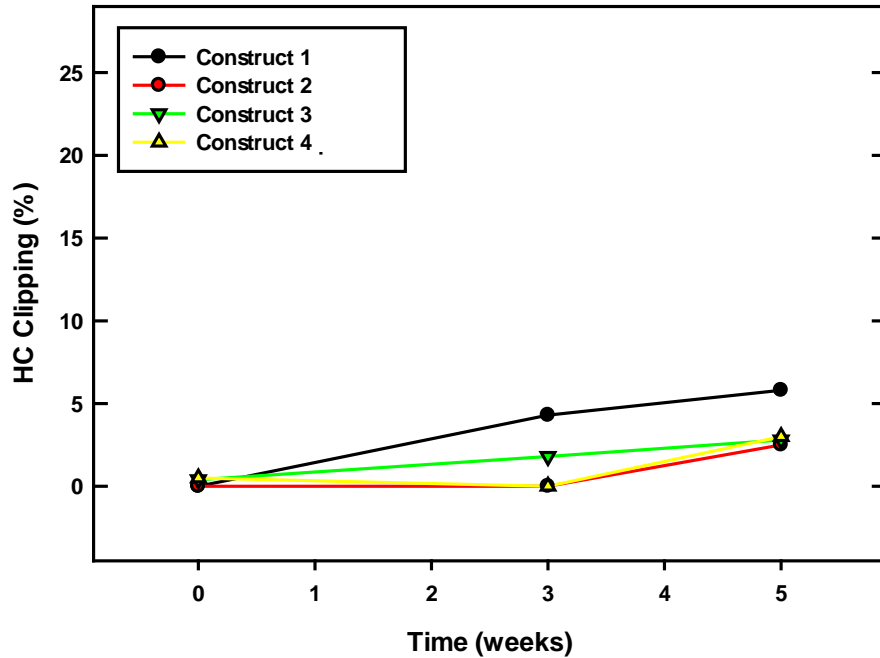
pH 8.0

More clipping in Construct 1

Significant clipping in all constructs.
Least in Construct 2, most Construct 1

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FORCED DECOMPOSITION: Low pH (5.0) and Elevated Temperature

Quality Attribute	C-1	C-2	C-4	Comments
Aggregation	🟩	🟩	🟩	
Purity	🟨	🟨	🟨	↑Fragmentation
HC Clip	🟧	🟨	🟨	
Isomerization	🟩	🟩	🟩	
Deamidation	🟨	🟩	🟨	
Charge Heterogeneity	🟨	🟨	🟨	
Oxidation	🟨	🟨	🟨	
Secondary Structure	🟩	🟩	🟩	
Turbidity	🟩	🟩	🟨	
Melt Point	🟩	🟩	🟩	

*Quality attributes ranked in order of criticality

🟩 No change   🟨 Minor change   🟧 Moderate change   🟥 Major change

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FORCED DECOMPOSITION: High pH and Elevated Temperature

Quality Attribute*	C-1	C-2	C-4	Comments
Aggregation	🟩	🟩	🟩	
Purity	🟥	🟥	🟥	↑Fragmentation
HC Clip	🟥	🟧	🟧	
Isomerization	🟩	🟩	🟨	
Deamidation	🟥	🟧	🟥	Significant ↑ in PENNY peptide
Charge Heterogeneity	🟥	🟧	🟧	Significant ↑acidic forms
Oxidation	🟨	🟨	🟨	
Secondary Structure	🟩	🟩	🟩	
Turbidity	🟨	🟨	🟧	Slight opalescent
Melt Point	🟩	🟩	🟩	

*Quality attributes ranked in order of criticality

🟩 No change   🟨 Minor change   🟧 Moderate change   🟥 Major change

Risk Analysis in Formulation
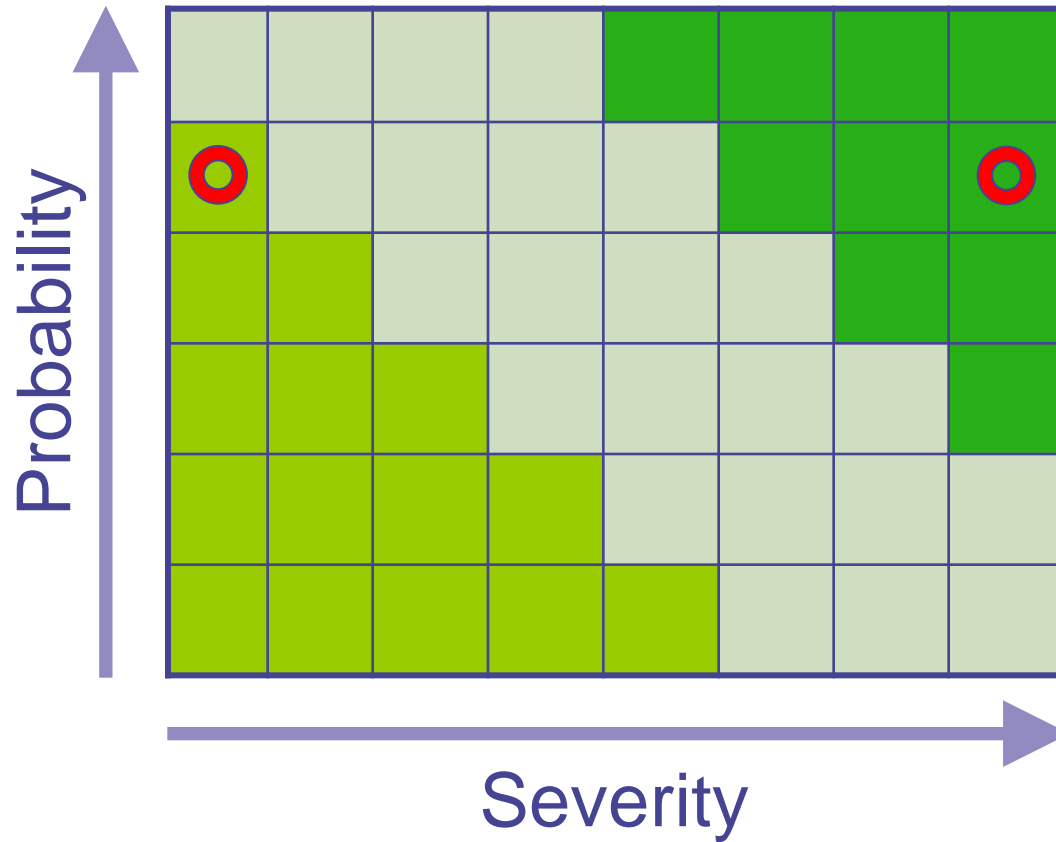
Developability Ranking

Stress	C-1	C-2	C-4
General	🟩	🟩	🟨
Freeze-thaw	🟩	🟩	🟩
Shear	🟩	🟩	🟩
Viral Inactivation	🟩	🟩	🟩
High temperature, pH 8.0	🟧	🟧	🟧
High temperature, pH 5.0	🟧	🟨	🟧

Formulation Developability Ranking
Construct 2>Construct 1>Construct 4>>Construct 3

🟩 Favorable   🟨 Neutral   🟧 Weak   🟥 Detrimental

SUMMARY: Improving formulation strategies and outcomes by strengthening the Interface with discovery research

- Identify problematic candidates

- Identify analytical challenges

- Accumulate solution behavior data which will help guide development and Quality by Design (QbD)

- Early information about the solution behavior of candidate proteins helps in development strategies for robust dosage form development in a timely fashion.

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