Modular DSP approaches for complex non-mAB molecules

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SVP Process Science & Production
## Agenda

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<td>Virus inactivation</td>
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<td>Summary and conclusion</td>
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</table>
Expertise with a broad spectrum of proteins

- >100 therapeutic proteins produced 1997 - 2016
- 70% monoclonal antibodies and fusion proteins
- >40% biosimilars and biobetters
General considerations for DSP development

Basic requirements
- Protein concentration in intermediates ≥ 4 g L⁻¹
- Process steps at ambient temperature

General process related considerations/requirements
- Stability data on Capture (& Harvest and Intermediates at -20 °C, RT and 5 °C, +/- stirring)
- Aggregation/precipitation propensity (SEC, T₅₈₀nm)
- Contribution of steps for virus removal/inactivation (virus safety concept)

Definition of success criteria
- Overall process yield
- Removal factors of impurities:
  - process related (HCP, DNA, ProtA)
  - product related (fragments, aggregates, isoforms etc.)
- Final DS specification
Concept of modular toolbox

Major impurities?

Sequence of evaluation
- Capture
- pH-titration
- Stability assessment
- Polishing

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## Variety of DSP processes for complex proteins

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
<th>Step 4</th>
<th>Step 5</th>
<th>Step 6</th>
<th>Step 7</th>
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<th>Step 9</th>
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Getting order and structure

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### Modules of DSP unit operations and sequence of steps

<table>
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<tr>
<th>Conditioning</th>
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FP2: Potential folding pathways leading to variants

- Tetramer formation depends on linker length and pH
- Linker can be target for fragmentation or truncation
- Some intermediates occur in folding pathway
- Monomeric byproducts can be suppressed by additives in media
- Aggregate formation still possible

Closed monomer

Domain swapped tetramer

Kipriyanov et al. JMB, (1999), 293, 41-56
FP2: HCP removal & yield

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FP2: Effect of low pH and incubation

- At least 24 h are required for complete refolding and maximal restoration of activity
- Incubation at 20 °C leads to fragmentation (presence of acidic protease)
- In the process FP2 is incubated at 2-8 °C for 48 h
HCP removal by precipitation with caprylyc acid

- Precipitate can be removed by depth filtration
- Concentrations >3 mmol L\(^{-1}\) sufficient for virus inactivation
- At high concentration yield is reduced

- Optimization process:
  - Titration of pH and CA concentration
  - Select concentration that keeps API still soluble

- CA impacts subsequent viral removal steps!

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FcF6: HCP removal by precipitation with caprylyc acid

- HCP reduction already at low conc. of CA
- pH only has influence on aggregation
- Predictable model
Caprylic acid-induced impurity precipitation

HCP Clearance from a monoclonal antibody: Influence of pH and CA concentration

Robust HCP clearance at high mAb yield
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Example of product related impurities (mAb)

1. Full antibody
2. Incomplete antibody
3. Paired heavy chain
4. Half antibody
5. Free heavy chain
6. Free light chain

(non-red SDS-PAGE, silverstain)

Aggregates
FcF7: Peak cut in C20 eliminates incomplete molecules

Monomer Dimer Half-trimer Trimer
### Product related impurities

<table>
<thead>
<tr>
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<th>Aggregates</th>
<th>Truncations</th>
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- FcF4 seems to be low pH sensitive
- Aggregates can be successfully removed by CHT chromatography
- Non mammalian Vac1 contains a lot of LMW species (clipped isoforms) from the beginning
- LMW have lower net charge ➔ elimination in subsequent ion exchange chromatography
FP1: Glyco-isoform separation

Background:
- Molecule: highly glycosylated fusion protein
- Resin: DEAE Sepharose FF

Purpose of the step:
- Removal of HCP and DNA
- Removal of low glycosylated isoforms

Results:
- DNA reduction factor: 4 log
- Glycosylation: 100%
- Yield: 45%

Load \[\text{RP-HPLC}\]
Elute
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Virus inactivation alternatives and issues

- pH < 4 (key step for elution of mAbs/FcF from Protein A resin)
  - Can lead to denaturation and proteolysis
  - Might induce aggregation during high protein concentration at elution
- Detergents (see next slide)
- Organic solvents (see next slide)

Alternatives
- Arginine solutions >0.5 mol L\(^{-1}\)
- UV light (254 nm)
  - Might cause protein-DNA aggregates
- Gamma irradiation (>25 kGy)
  - Access to source and equipment
Virus inactivation with detergents (FP1)

- Detergent (e.g. 1 % Triton X-100, 0.3 % TNBP, 1 % Tween 80) for more than 4 h, at RT
  - Tend to oxidation and byproducts
  - permitted residual level 3.25 ppm ➔ removal in subsequent steps

Comparison of 2 experiments ➔ Reproducible results
Virus inactivation with organic solvent (Enz2)

- Variables: Conc, T, t
- Some effect on activity
- Low impact on oligomer
- Higher T more effective
- 180 min are sufficient

14.5% 2-propanol, 22°C
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Summary modular approach

- **General principles**
  - Identify major impurities in harvest ➔ select suitable analytic method
  - Assess molecular properties
  - Find specific capture with reasonable capacity

- **Frontload stability studies** (pH, mechanical, temperature, proteases)
  - Utilize that knowledge for definition of virus safety strategy

- **HCP issues**
  - Reduce HCP levels as early on ➔ otherwise reduction of column capacity
  - Think about alternative methods (e.g. caprylic acid precipitation)

- **Aggregation**
  - Identify underlying cause, eliminate according to properties (charge, hydrophobicity, size…)

- **Product related impurities**
  - Eliminate first mis-assembled variant, then aggregates and truncations
References

Kipriyanov et al., JMB, (1999), 293, 41-56
Data from our process development and production teams

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Director: BioProcess Science
Director: Process Development DSP
Director: Process Unit DSP Production

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Or contact me: Stefan.Schmidt@Rentschler.de