
Virulence Factor-Based Drug Discovery

A Novel Approach for Identifying Drug Targets for
Autoimmune and Inflammatory Diseases

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Viral Logic Systems Technology

VLST

Office view:
July 8th, 2009, 3:43 PM



Office view:
The other 364 days of 2009



- Privately-held company founded in 2004
- ~35 Employees
- Focused on exploiting viral evolution to develop novel biotherapeutics
- Based in Seattle, WA

Virulence Factors as a Novel Route to Therapeutics

- Some viral proteins modulate/suppress host immune system
- Facilitate viral infection and influence severity of disease
- Can be homologous or unrelated to host genes
- Targets of viral proteins validated as treatment methods for autoimmune/inflammatory illness

Drug Development Strategy

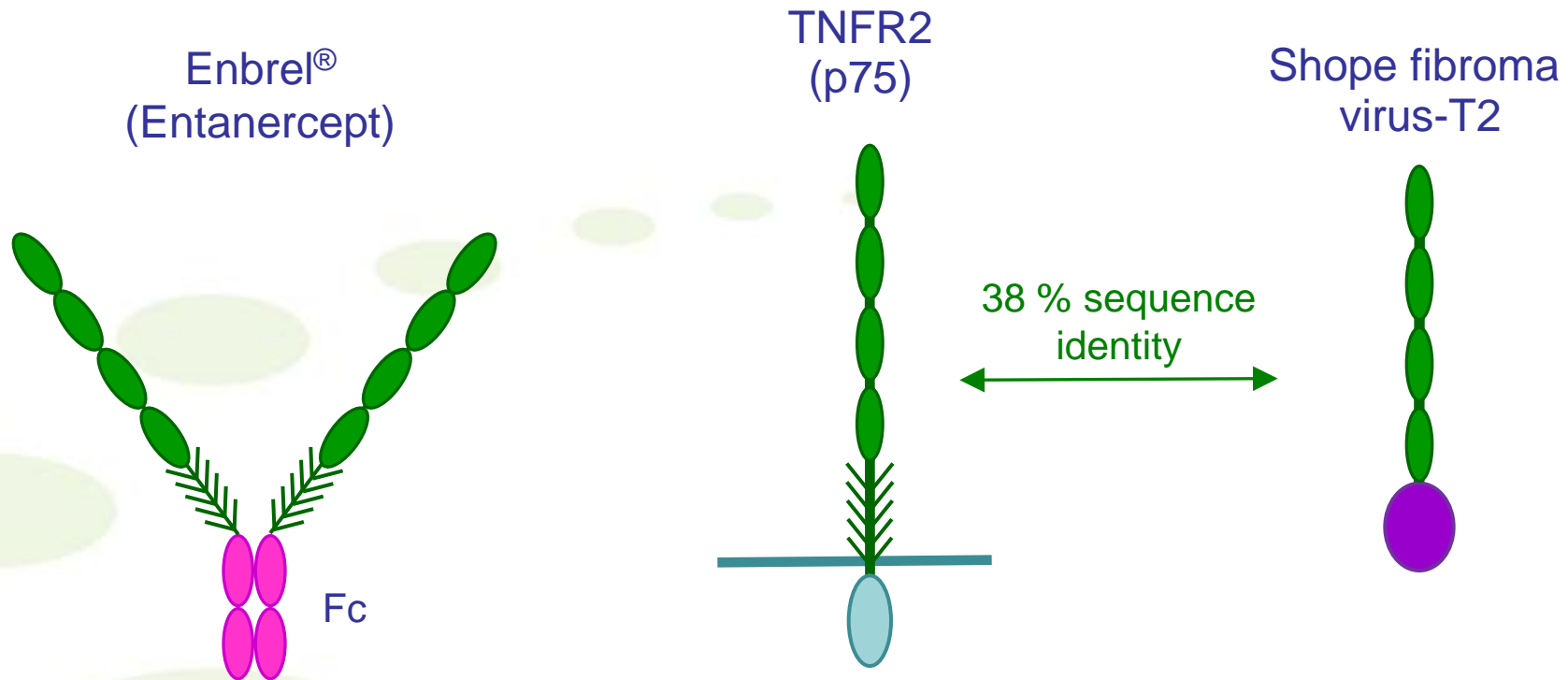
Identify
virulence
factors

Identify
cellular
targets

Define biologic
consequences
of interaction

Develop therapeutics
mimicking
virulence factors

Discovery of Soluble Viral TNF Receptor Key Step in Development of Enbrel[®]



Smith *et al* (1990) Science 248: 1019, Smith *et al* (1991) BBRC 176: 335

Cytokines, Chemokines and Their Receptors Encoded by Herpes Viruses

Alcami (2003)
 Nat Rev
 Immunol 3: 36

Soluble cytokine receptors or binding proteins



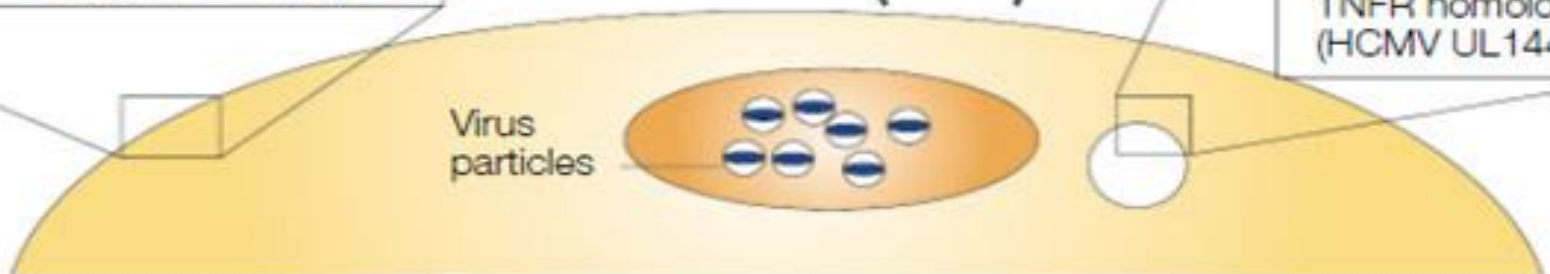
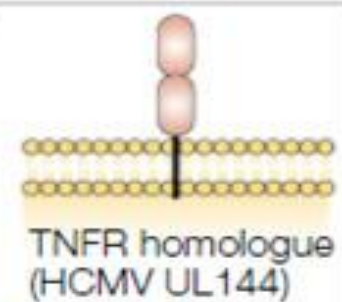
Cytokines and chemokines



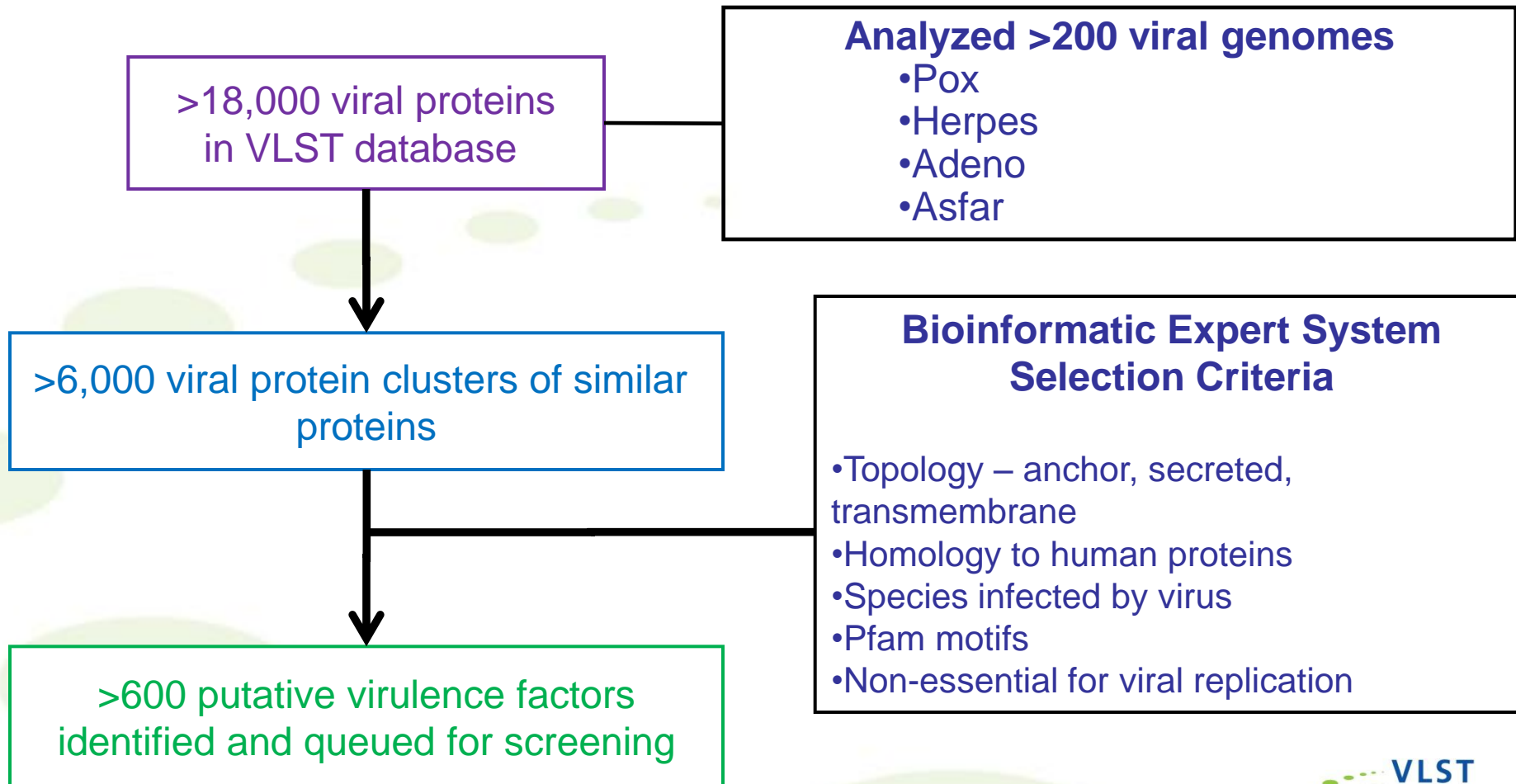
Membrane chemokine receptors



Cytokine receptors



Genomic Scale Search for Viral Virulence Factors

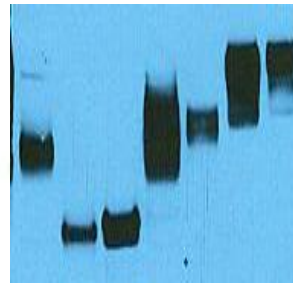


Identification of Host Targets of Virulence Factors

Bioinformatic mining for virulence factors



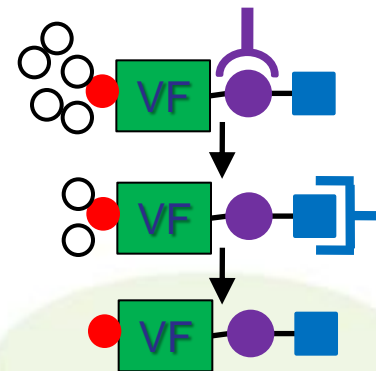
Transiently express affinity-tagged viral proteins



Target identification by LC-LTQ MS



Synthesize viral genes



Bind target(s) from supe and lysates from immune-related cell lines, using tandem affinity tag



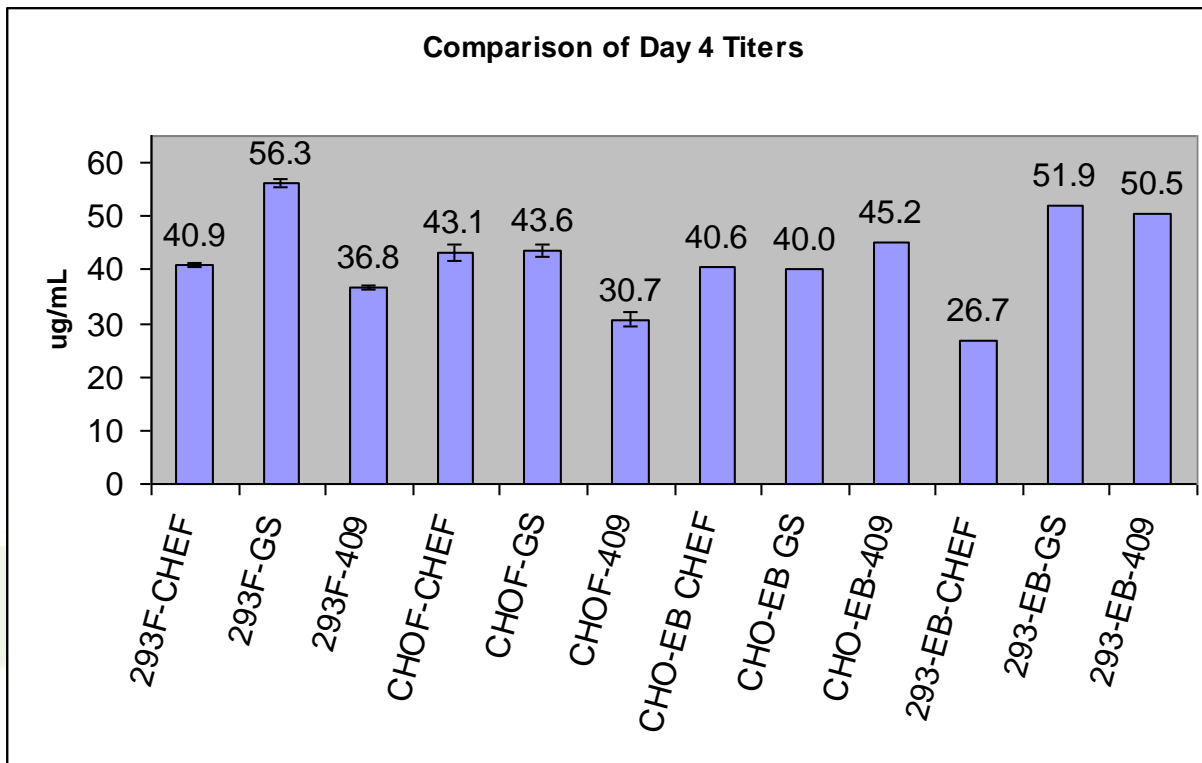
Viral Protein Expression

Target Discovery is a Numbers Game

Proteins expressed
Proteins screened
Targets identified

- Maximize number of viral factors going into screen
- Minimize effort, reagents spent on non-expressors
- Evaluate different vectors and cell lines with Freestyle transfection reagent
- **Scale up via technology, not FTE's!**

Effect of Cell line, Vector in Freestyle



Cells:

293-EBNA

293-F

CHO-F

CHO-EBNA

Vectors

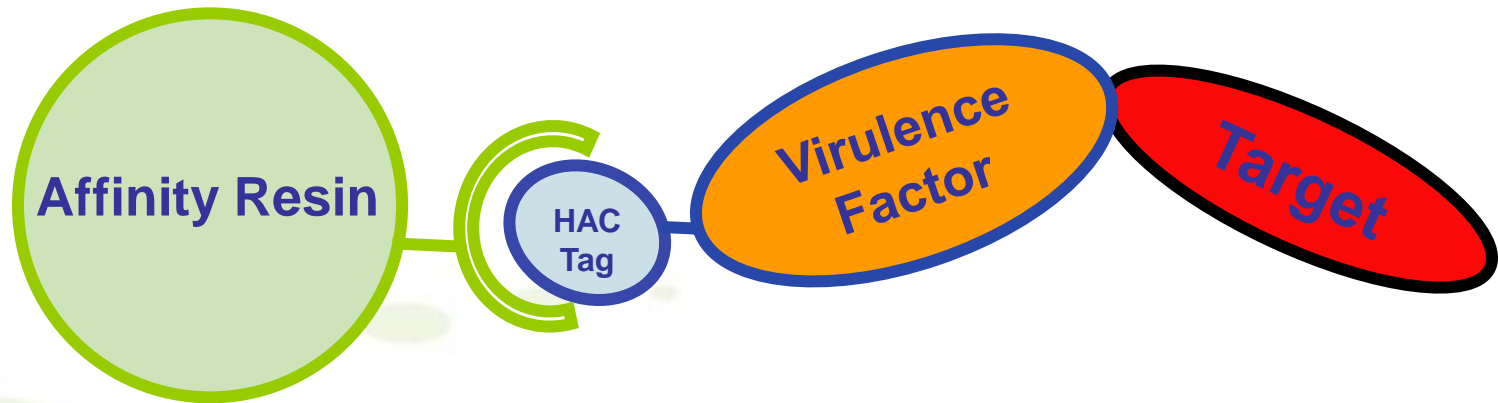
In-house

GS

CHEF

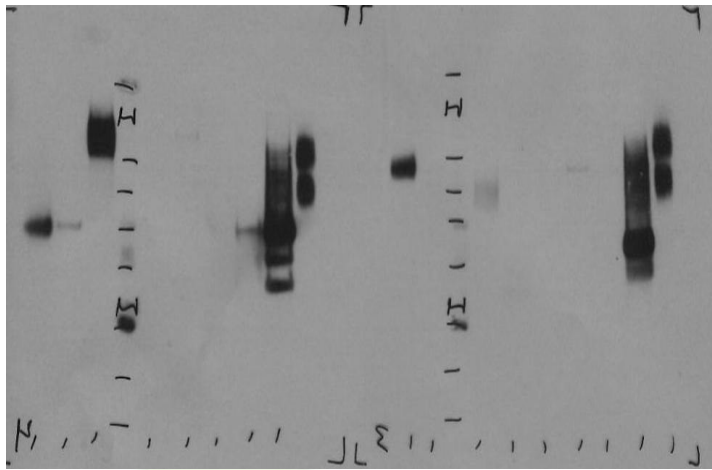
- Same gene in different vectors and cell lines
- Similar expression levels with all combinations tried

N- And C-Tagged Virulence Factors To Increase # Identified Targets



- “HAC”- tandem affinity tag on one end of virulence factor
- Position of HAC may block binding, affect expression
- Gene synthesize both N- and C-term. tagged vectors
- >90% express at least one version, ~20% increase over expressing C-tag alone
- Increases probability of identifying targets, in some cases, only one version binds target

24-well Shaker Plate Prescreen



CHO-EBNA transients Western blot

- Reagent, time, effort wasted on large-scale transfections if viral protein doesn't express
- Developed expression pre-screen of expression using 24-well shaker plates
- Can be used to make relative comparisons between different expression constructs, media, etc.

Platform Highlights

- Identified numerous immunologically relevant targets
 - **Validated targets of 4 approved, 11 investigational drugs**
- Partnership with Novo Nordisk in 2008 to provide research targets
- Therapeutic programs based on viral targets entering the clinic in 2010/2011

Therapeutic Proteins Based on Virulence Factor Platform

Possible approaches:

- Recombinant viral proteins to treat autoimmune diseases
 - Focus on acute indications to avoid immunogenicity from long-term dosing
 - Not currently being pursued
- If virus makes a homologue to a human protein (eg. soluble viral TNF α R), use recombinant human protein
- Develop antibodies to bind to/block the targets of viral proteins

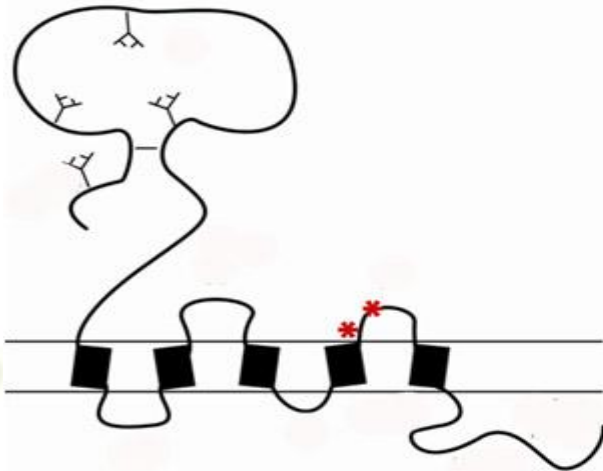
Challenges of Non-mAb Therapeutics

- mAb-like expression levels may not be achievable
- mAb-like purification process steps may not be feasible
- Platform processes may not be available
- More complex glycosylation patterns
- Greater stability issues with non-native structures/fusion proteins

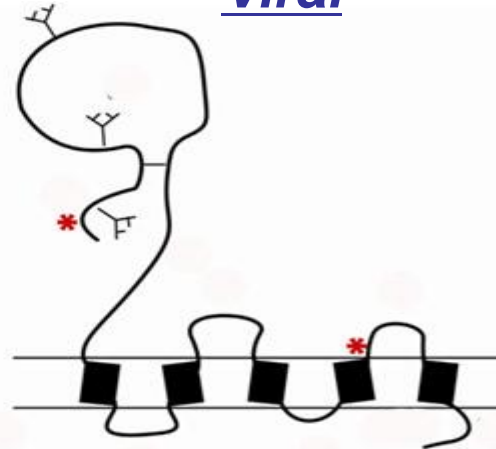
- Despite potential challenges, unique therapeutic approaches makes the effort worthwhile

Case study: VLST-007

Human



Viral



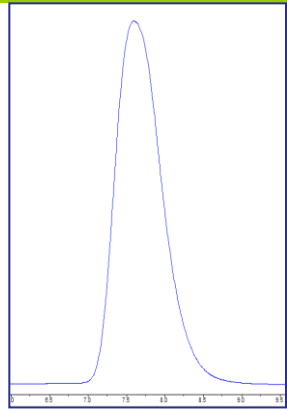
- Viral protein interrupts signaling by forming heterodimer with human transmembrane protein
- VLST's approach
- Make soluble Fc-fusion protein of bind to and block signaling through binding partner

Case study: VLST-007

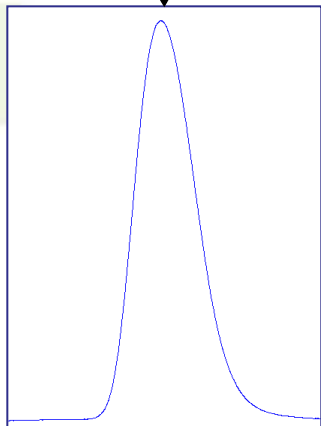
- Extracellular domain fused to IgG1 Fc
- 7 putative N-linked glycosylation sites (14 in dimer)
 - Process may impact glycoforms, potency
- Use “mAb-like” process, but customize as necessary
 - Anion exchange flow through step not possible
- Initial process development performed at VLST, tech transfer of process and reagents to CMO
- Collaborative effort to trouble shoot, assess impact of changes on product quality/activity

VLST-007 Development Challenges

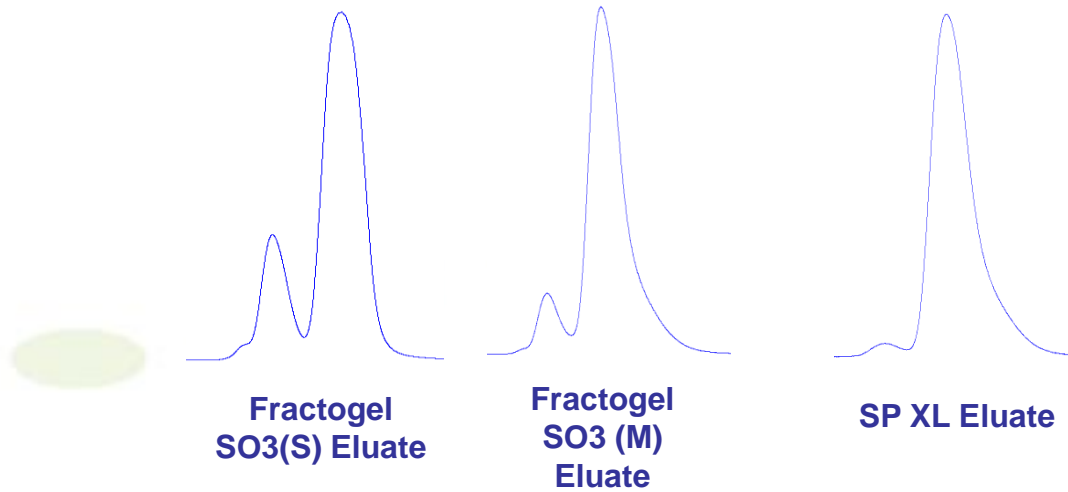
CEX Ligand-Dependent Aggregation



**Mabselect
Eluate**



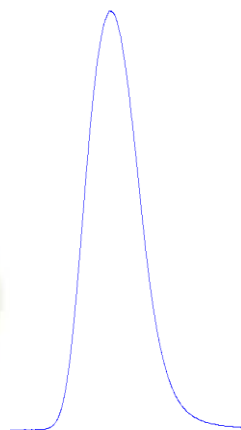
**GE CM FF
Eluate**



**Fractogel
SO3(S) Eluate**

**Fractogel
SO3 (M)
Eluate**

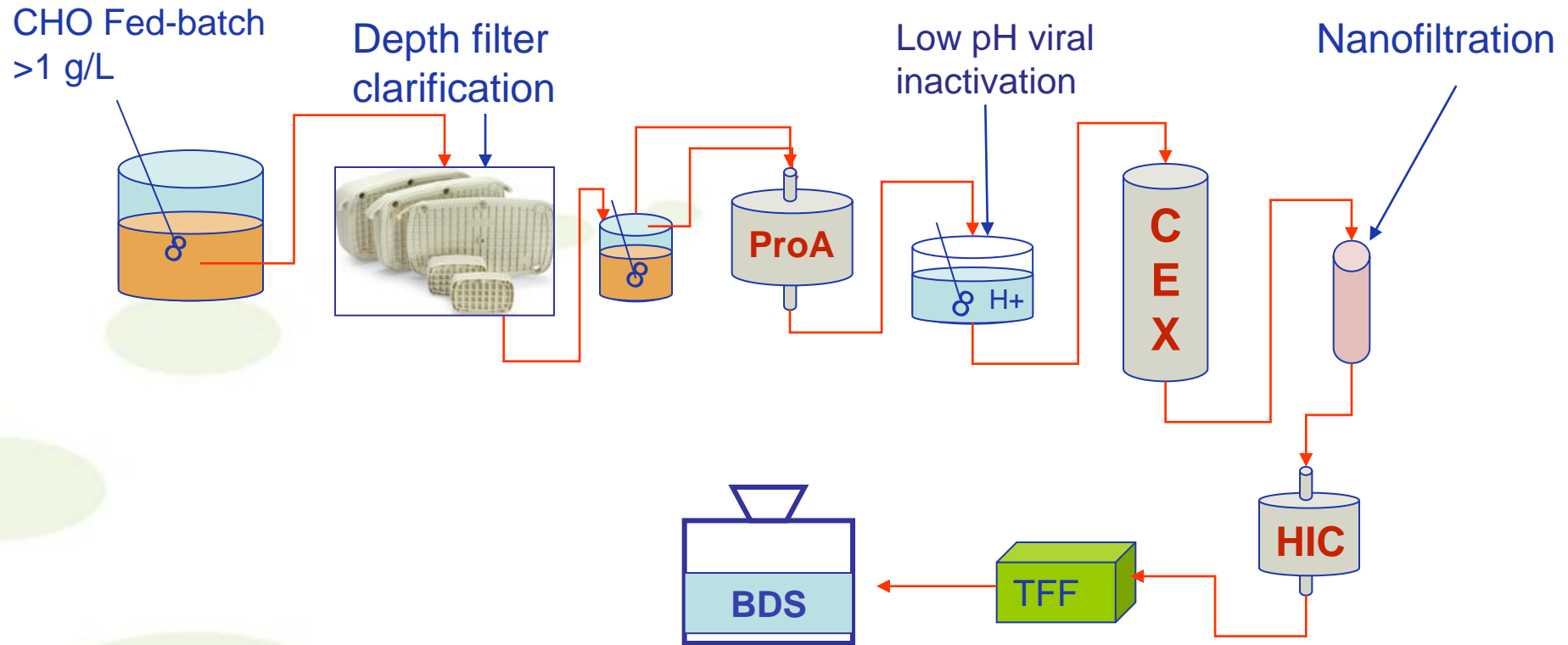
SP XL Eluate



**TOYO CM
650M**

- Strong CEX ligands induced product aggregation, as observed on SEC-HPLC
- Recommended that CMO switch resin (after tech transfer had already begun)
- CMO accommodated change with no impact to time-line

VLST and CMO Develop mAb-Like, Scalable cGMP Process

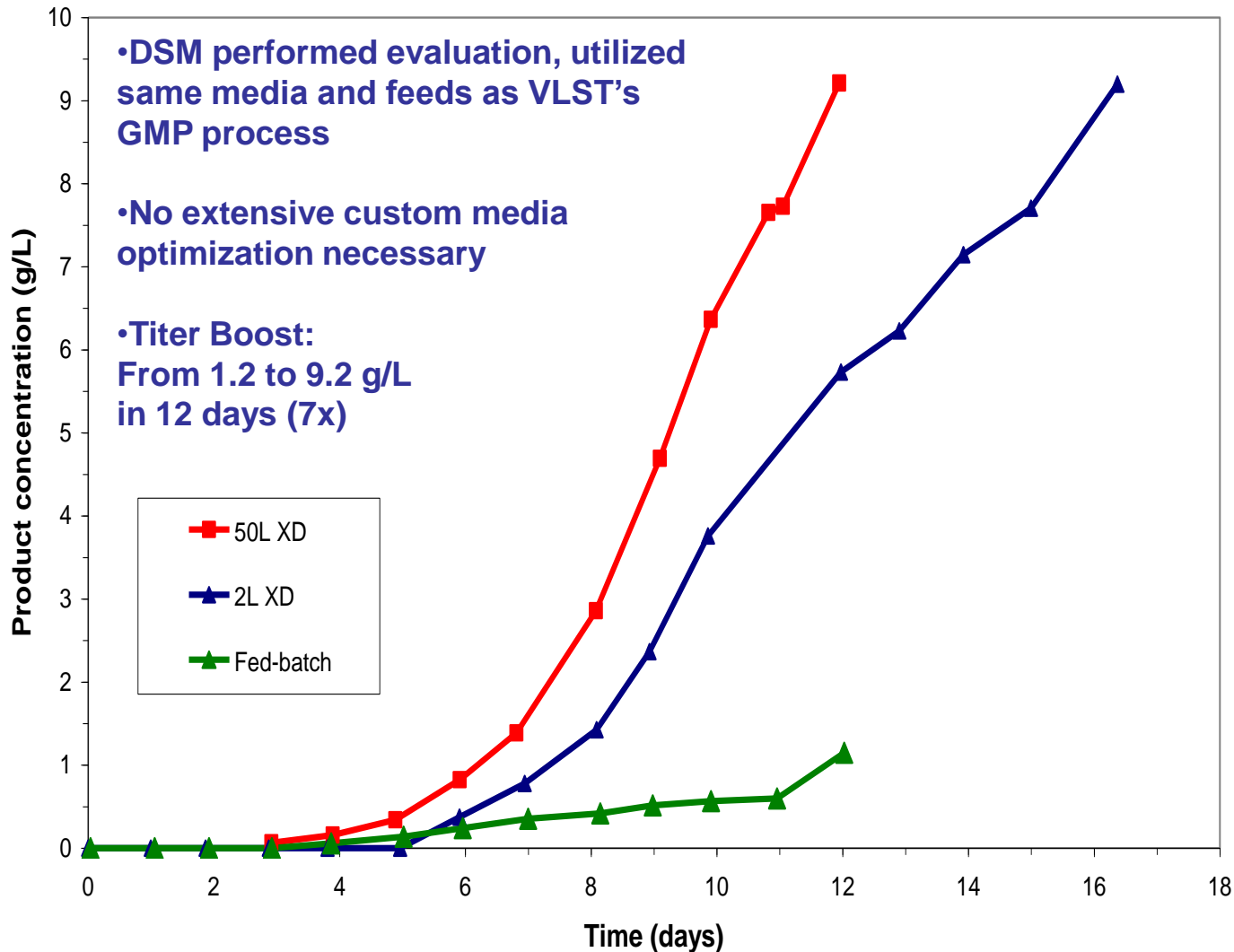


- 10L PD process yield: 75%
- 400L Engineering run process yield: 78%

Leveraging Technology to Boost Titer

CHO XD[®] Comparison

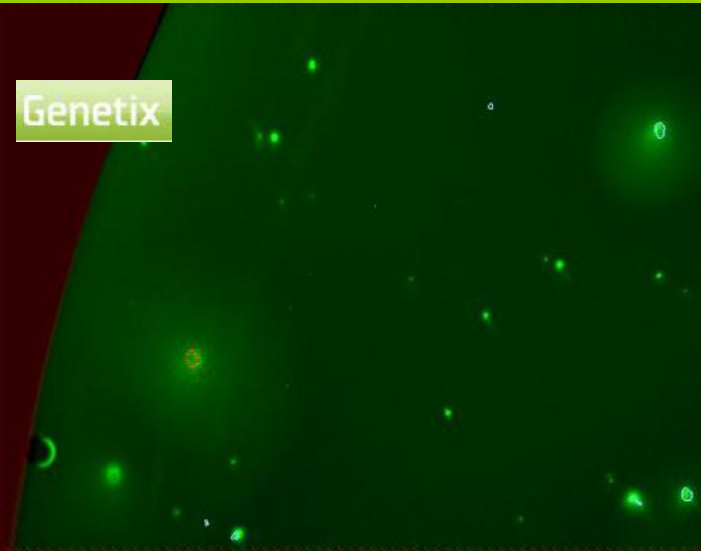
2L, 50L XD[®] and Fed-Batch



Case Study: VLST-018

- Mechanism validated by viral biology- multiple viral homologues mimic a membrane-bound human protein
- Generate soluble Fc-fusion of extracellular domain
- Multiple N-linked glycosylation sites
- Generate production cell lines, perform PD and tech transfer to CMO

Generation of Production CHO Cell Lines



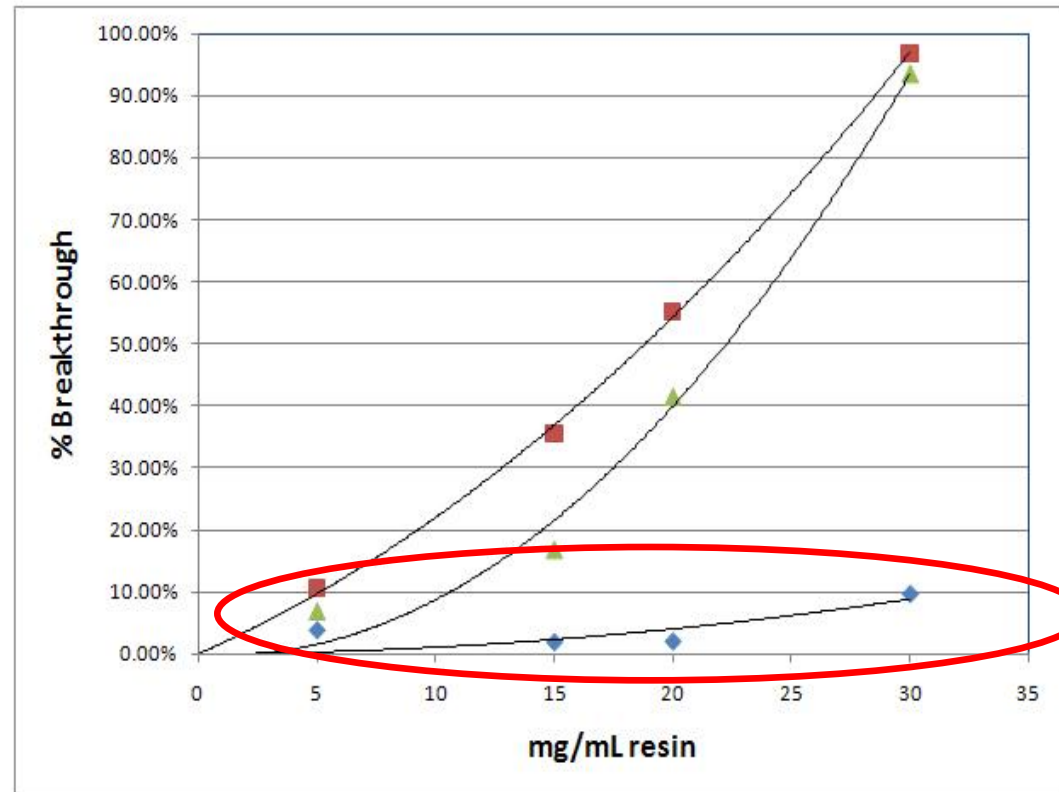
- Codon-optimize gene, and clone into two different proprietary expression vectors
- Screen transfectants via Clonepix
- Use to weed out low-expressing clones
- Permitted thousands of colonies to be pre-screened
- Titer results significantly better (40%) with top GS clones, will utilize for future cell line campaigns

Purification Development High-throughput Screening



96-well plate Chromatography

- Screen multiple conditions quickly and easily
- Facilitates use of DOE
- Rapidly identified lead CEX resin based on capacity, using little protein



Lessons Learned/Conclusions

- Leverage new technology, rather than adding FTE's, to do PD better, faster
 - 24-well prescreen
 - Alternatively-tagged constructs
 - Clonepix
 - Alternative cell culture methods, XD, perfusion to increase titer
 - Utilize vendor's expertise and help if possible
 - Plate-based chromatography development

Acknowledgements

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