
Expression and Purification of Virulence Factors

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

David Bienvenue, Ph.D.
VLST Corporation



Viral Logic Systems Technology

VLST

- Privately-held company founded in 2004
- ~35 Employees
- Focused on exploiting viral evolution to develop novel biotherapeutics
- Based in “sunny” 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

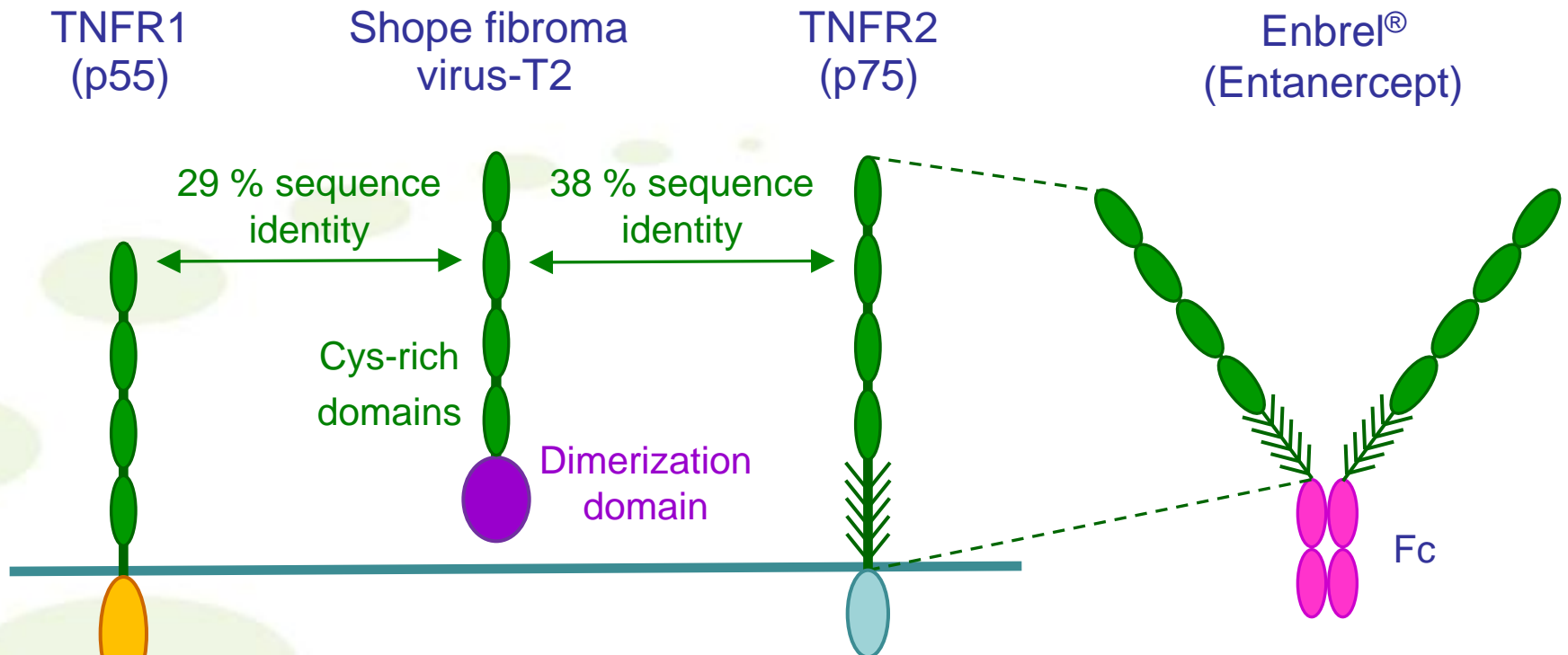
Identify
cellular
targets

Define biologic
consequences
of interaction

Develop therapeutics
mimicking
virulence factors

Discovery of Viral TNF Receptor

Key Step in Development of Enbrel®

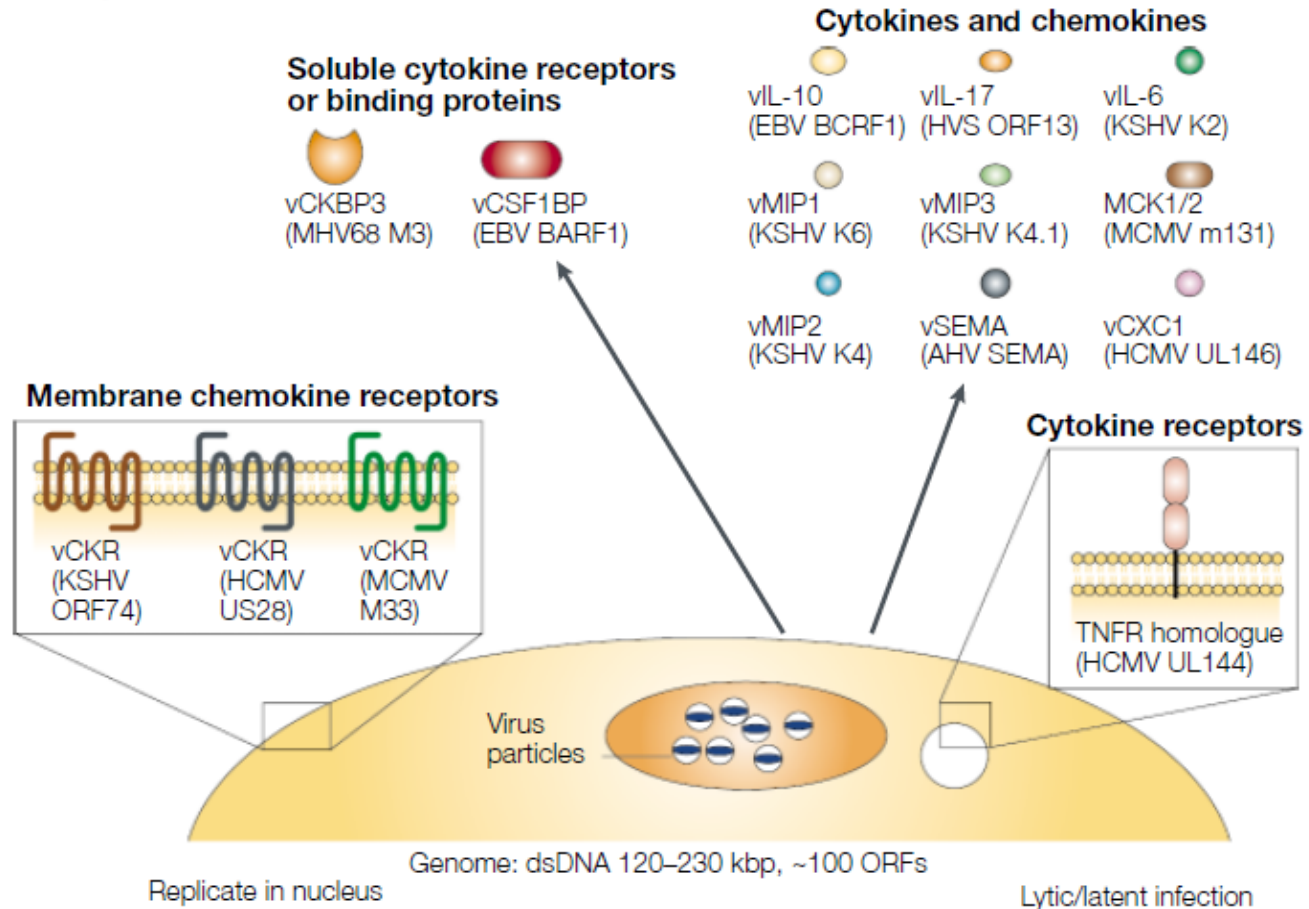


Smith *et al* (1990) Science 248: 1019
Smith *et al* (1991) BBRC 176: 335

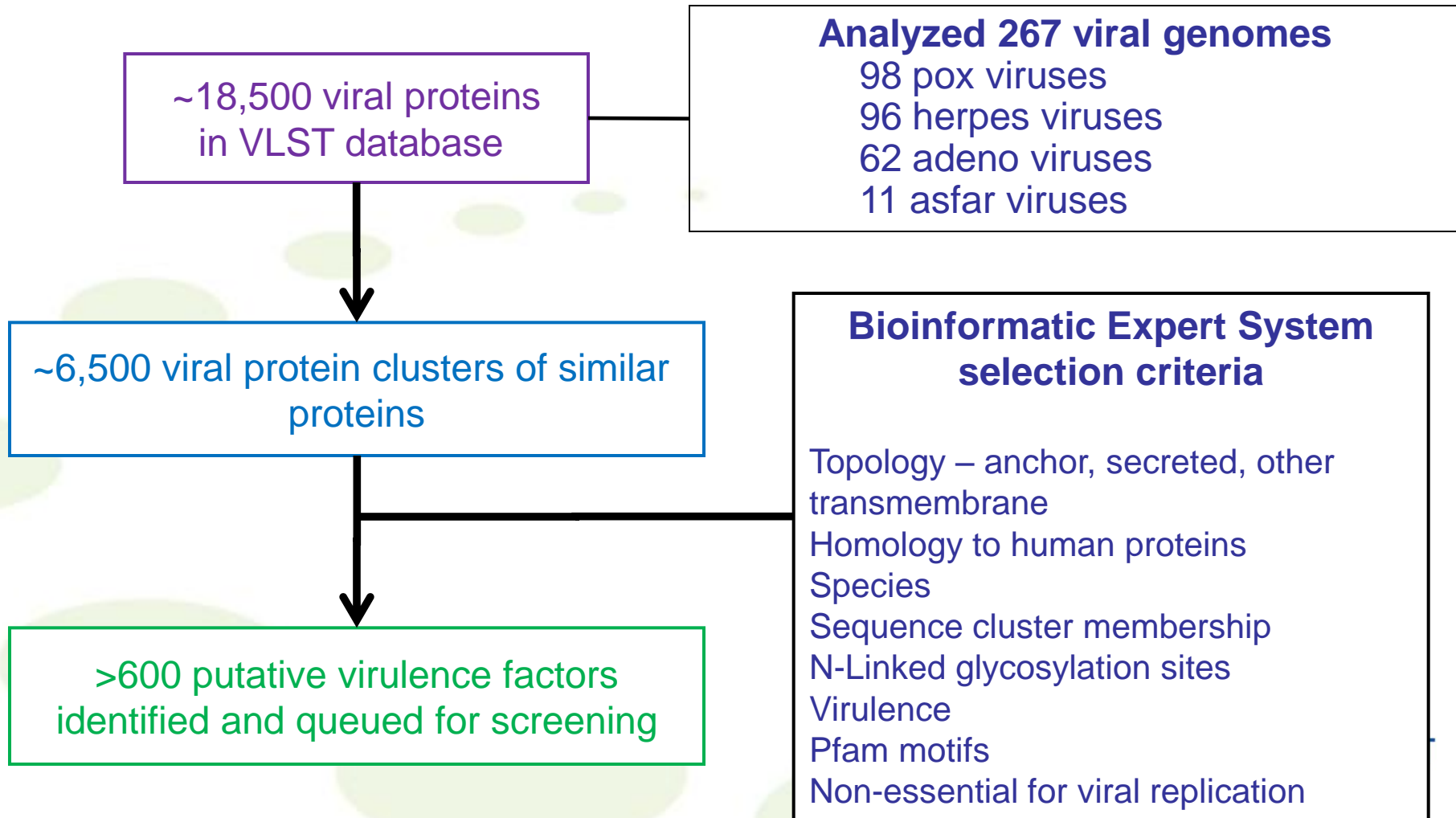
Cytokines, Chemokines and Their Receptors Encoded by Herpes Viruses

Alcami (2003)
Nat Rev
Immunol 3: 36

a Herpesviruses



Genomic Scale Search for Viral Virulence Factors

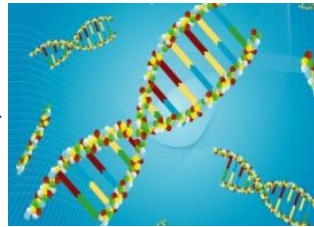


Identification of Host Targets of Virulence Factors

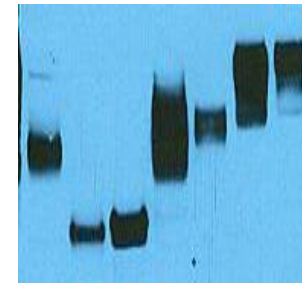
Bioinformatic mining for virulence factors



Synthesize viral genes



Transiently express tagged viral proteins

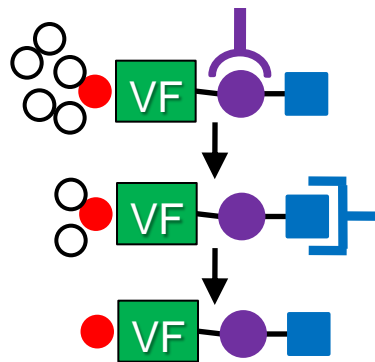


FACS screen and Bioassay panel

Target identification by LC-LTQ MS



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



Transient Expression of Viral Proteins

Challenge:

- Need high-throughput method to generate conditioned media for target discovery (goal ~20-30/wk)
- Adherent transfections not easily scaleable
- **Scale up via technology, not FTE's!**

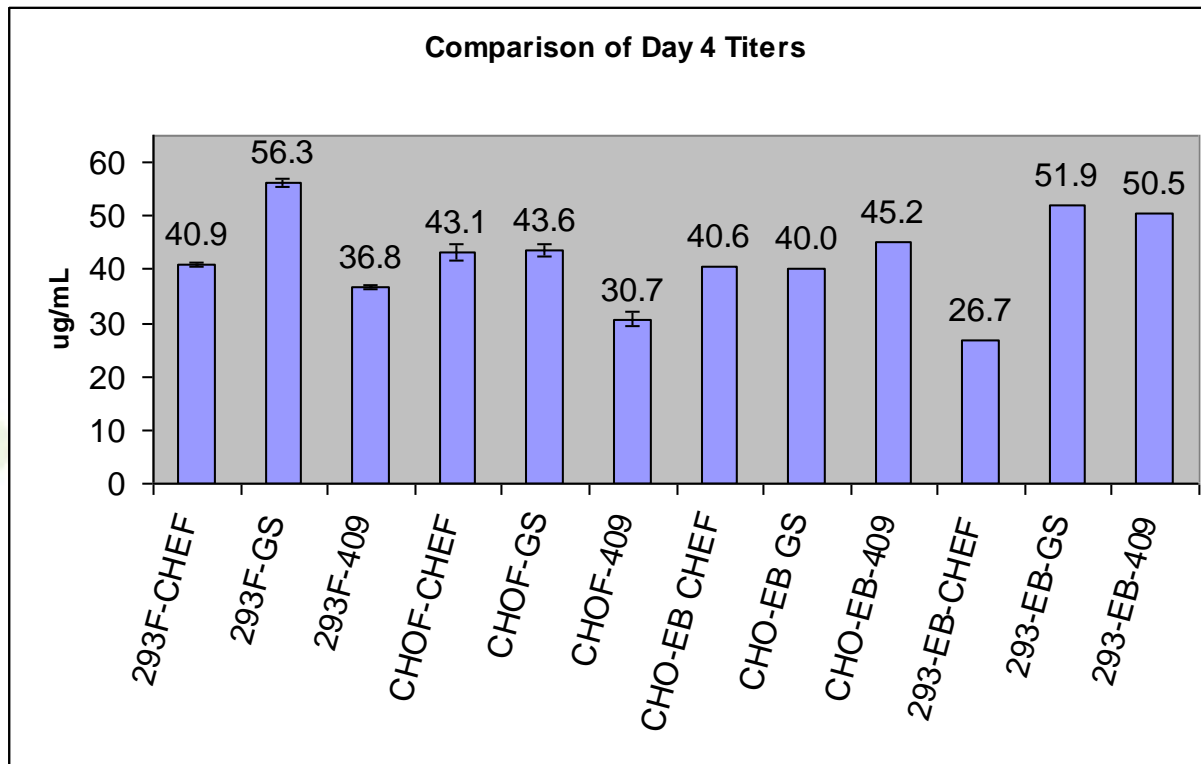
Solution:

- Transition away from adherent 293-EBNA's, to suspension transient transfections

Invitrogen's FreeStyle™ MAX Transfection System

- Optimized system for suspension CHO and HEK293 cells
 - Media and transfection reagent chemically defined, serum free
 - Cells adapted for suspension culture in FreeStyle™ media
- One shake flask vs. many T-flasks
 - Less labor intensive, higher throughput

Effect of Cell line, Vector in Freestyle




- Same gene in different vectors and cell lines
- Similar expression levels, GS vector has slight advantage

Viral Protein Expression

Target Discovery is a Numbers Game

Proteins expressed
Proteins screened
Targets identified



- Majority of putative virulence factors express in 293's
- Some 293 non-expressors can be expressed in CHO
- Maximize number of viral factors going into screen
- Minimize effort, reagents spent on non-expressors

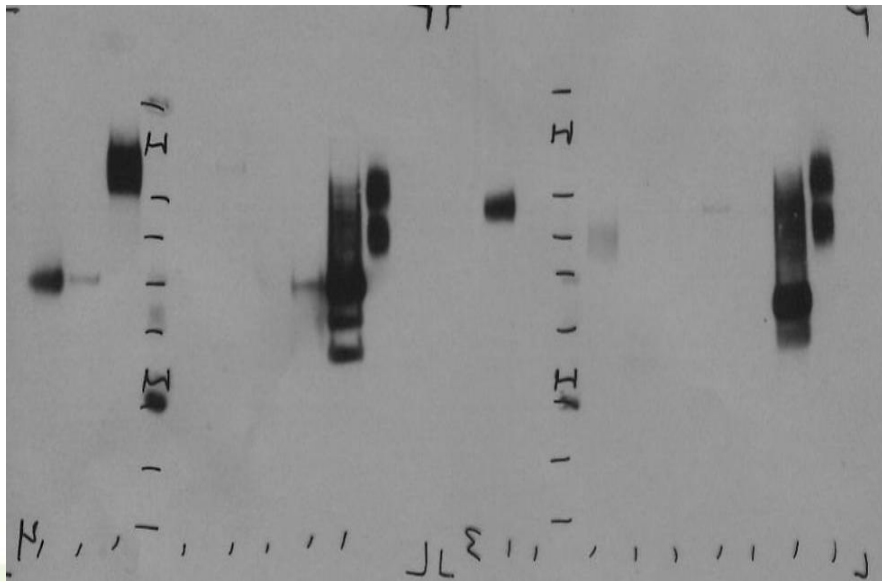
24-well Shake Plate Prescreen

- Reagent, time and effort wasted on large-scale transients, if protein doesn't express
- Typically if vectors failed in 293, re-try in CHO

Goal

- Minimize effort re-transfecting viral proteins in CHO
- Attempt prescreen expression in 24-well shaker plates
 - Mimic conditions in 100 mL shake flasks
 - Try ~70 constructs that didn't previously express in 293's

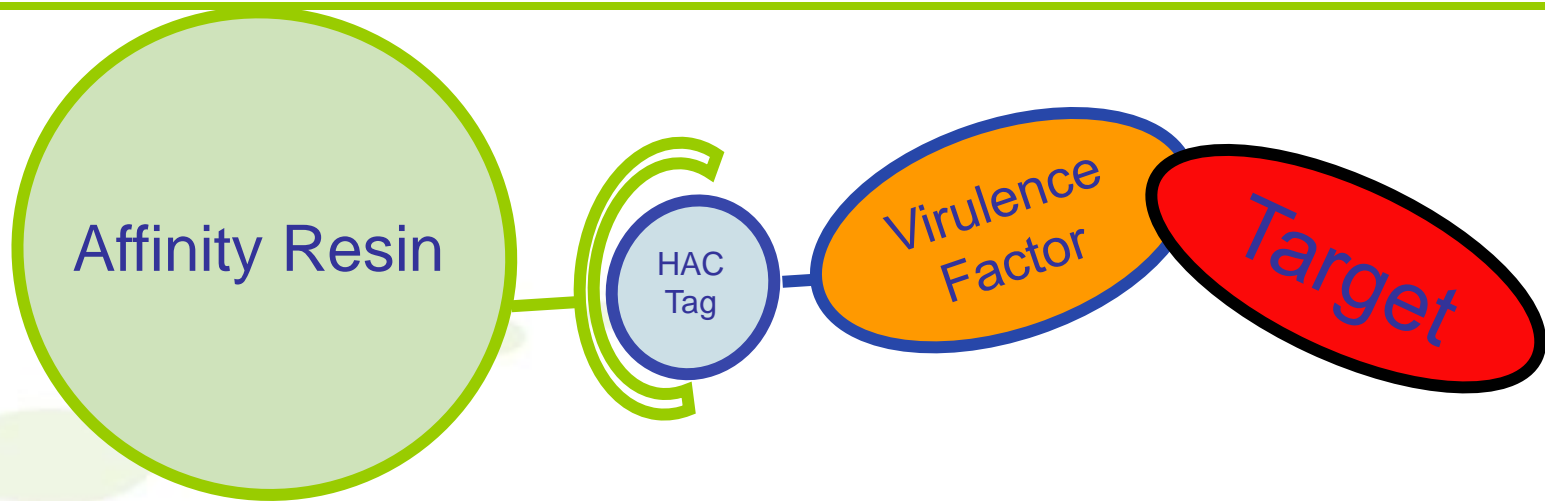
CHO Expression, 24-well Plate High-throughput Prescreen



CHO-EBNA transients, Anti HA blot

- ~30% (21 of 70) expressed in CHO
- Pre-screen all viral ORFs in 293 and CHO
- Only perform larger transients on vectors positive for expression

N- And C-Tagged Virulence Factors



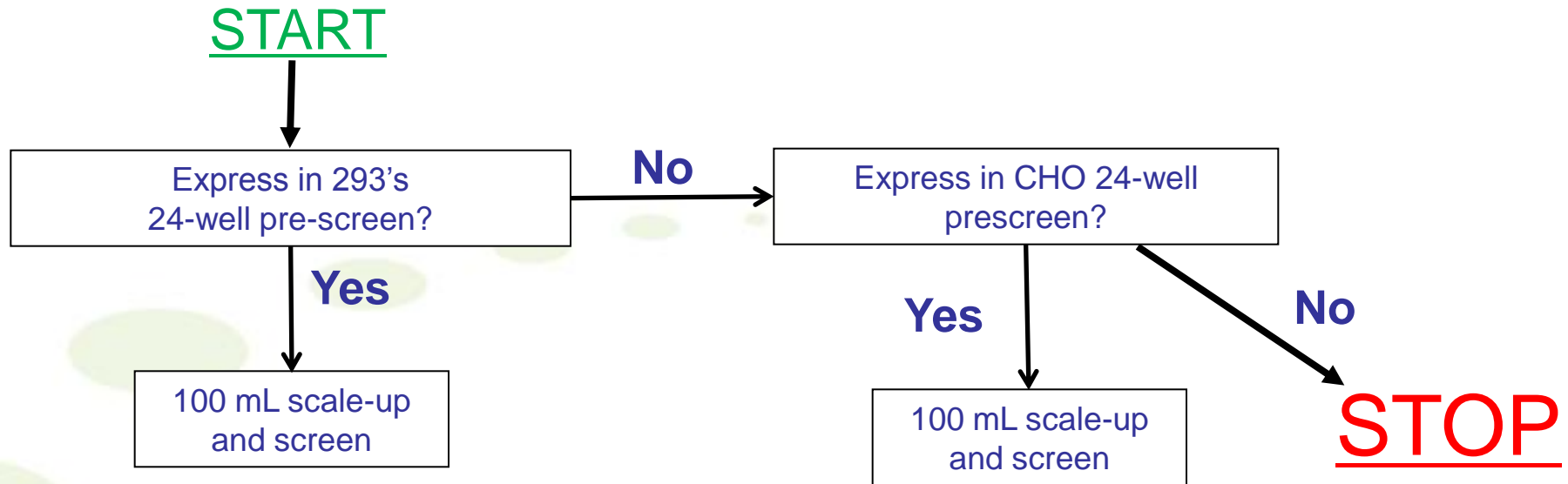
- “HAC”- tandem affinity tag expressed on one end of virulence factor
- Multi-epitope tag, used for purification and target discovery
- Possibility that HAC may block binding to target
- May also impact protein expression/secretion

Expression of N- and C-tagged Viral Proteins



- Gene synthesize both N- and C-term. tagged versions
- >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

Viral Protein Expression Salvage Strategy



- Use DNA from CRO to perform prescreen
- Minimize resources for DNA prep and large scale transients

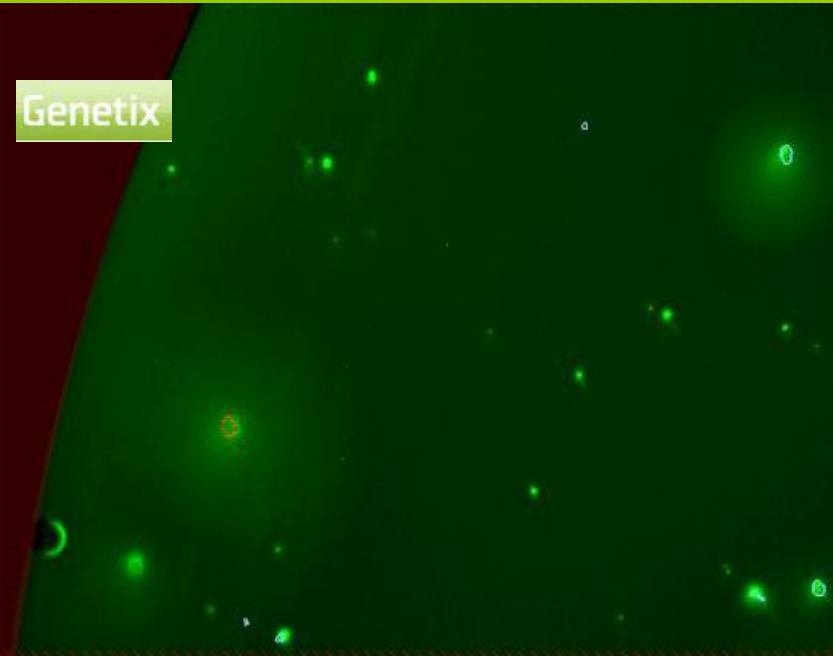
“No viral protein left behind”

Generation of Stable CHO Cell Lines

Large-scale Viral Protein Production

- Plan on evaluating some viral factors *in vivo*, mouse inflammation models
- Need quantity of protein that can't easily be obtained via transient transfection
- Codon-optimize gene expression in mammalian cells
- Add restriction sites to subclone into CHEF (CMC- ICOS) or GS (Lonza) expression vectors
- Utilize Genetix “Clonepix” colony picker to facilitate clone picking process

Clonepix-Based Colony Picking



- Secreted product detected via fluor. labeled probe
- Evaluate fluorescence compared to colony size
- Useful method for weeding out low/non-expressing clones
- Permits screening thousands, vs. hundreds of colonies
- Results in increased titer of final production clone (relative to limiting dilution cloning and picking)

Purification Development

High-throughput Screening



Atoll Media scout kit

- 100 μ L / 200 μ L formats
- 8 diff. resins per class

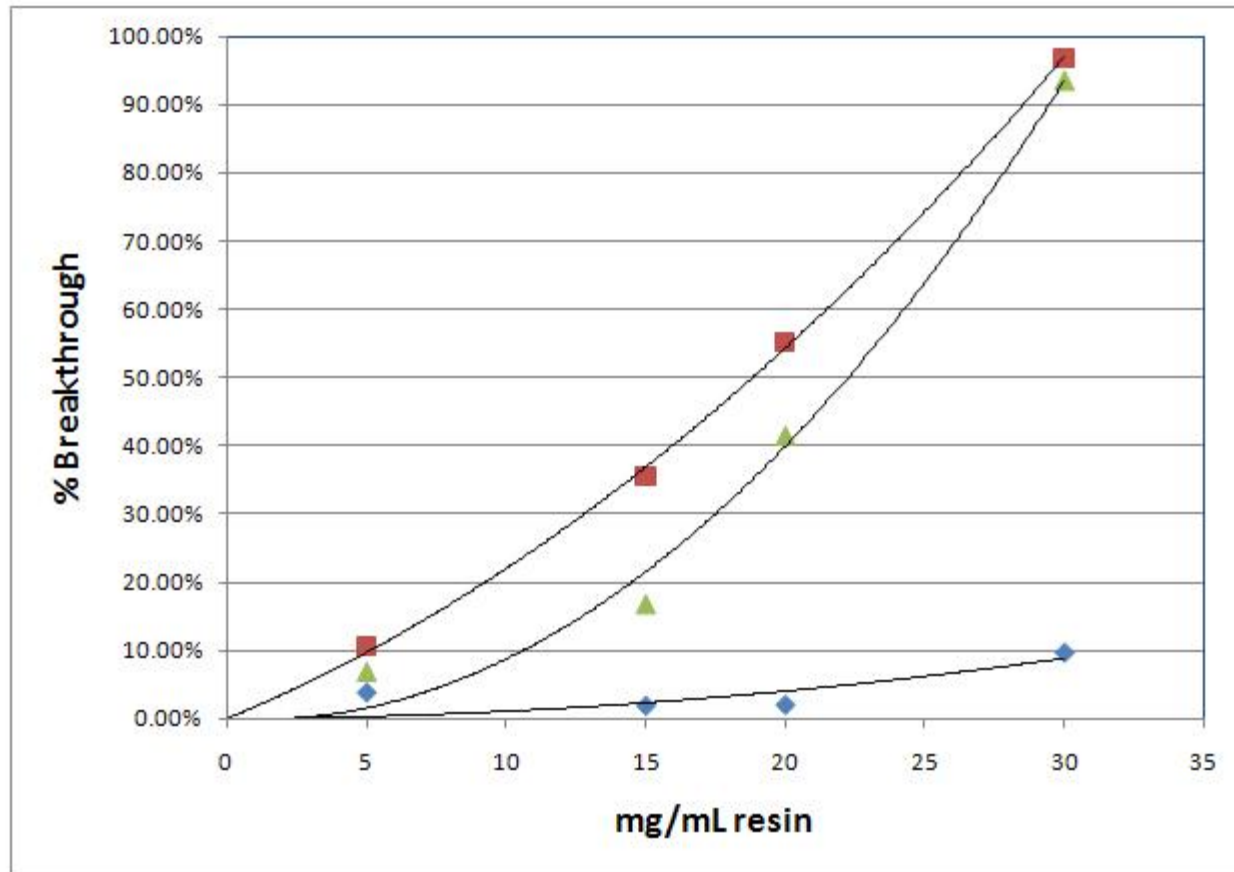
ProA
Anion
Cation
CHA
HIC



Millipore Vacuum Manifold

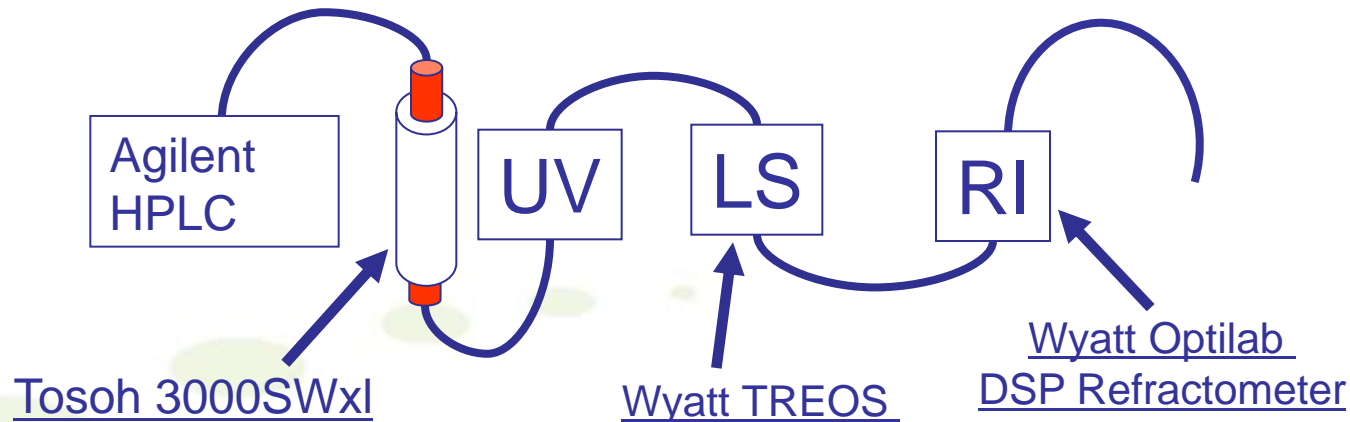
- Screen multiple conditions quickly and easily
- Facilitates use of “Design of Experiments”
- Consumes little protein

HTS-Capacity Determination



- Plate format quickly identified high capacity CEX resin

Multi-Angle Light Scattering To Characterize Viral Proteins

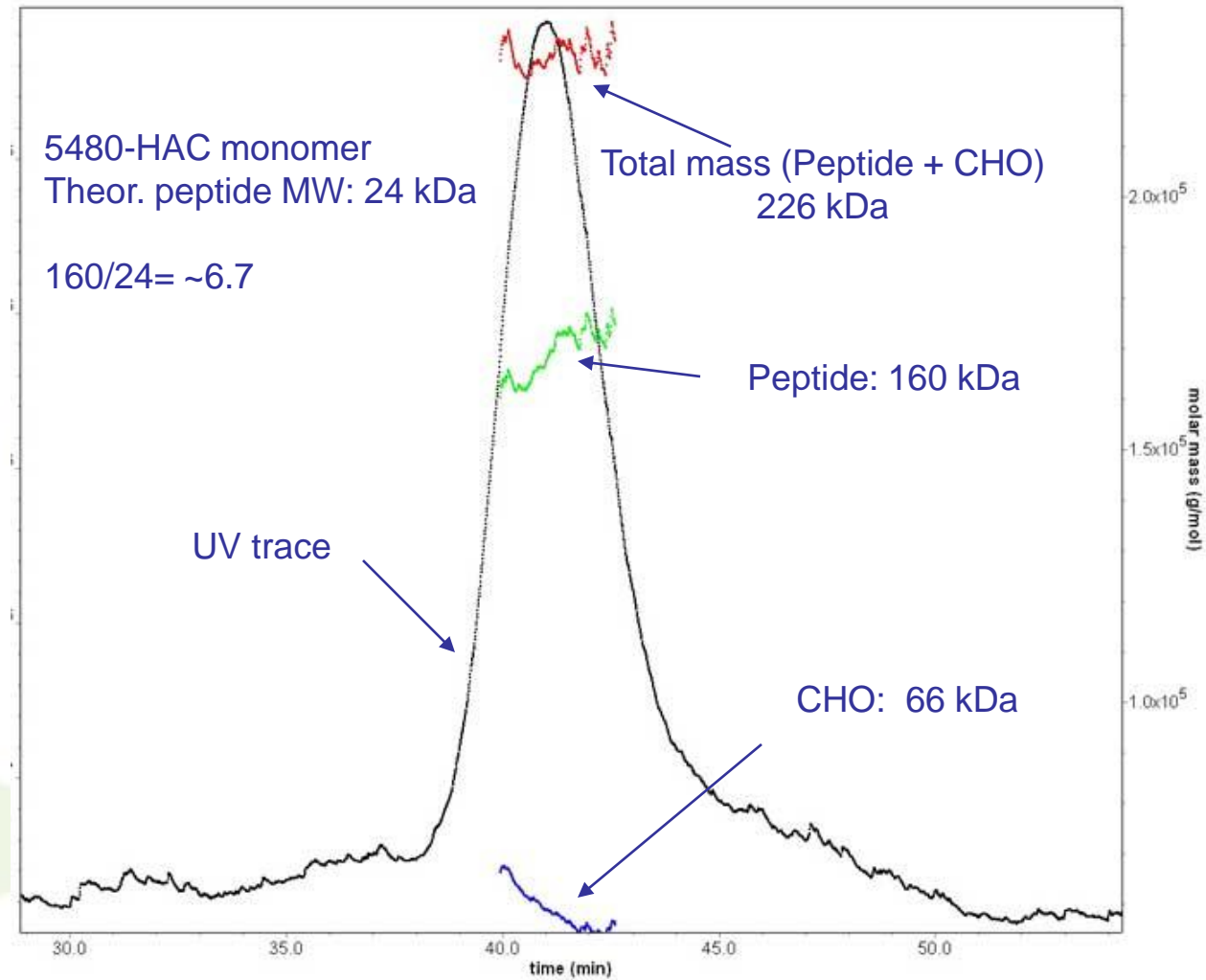


- Determine multimer state of novel viral proteins
- Often have several glycosylation sites
- Shape-dependent effects increase error of MW derived from std. curve of other proteins

Solution: Use SEC with Multi-Angle Light Scattering

5480-FLAG MALS

(Multi-Angle Light Scattering)



VLST 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

Acknowledgements

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