



# Enhancing the baculovirus expression system with VANKYRIN technology

Kendra Steele, Ph.D.

# Insect cells

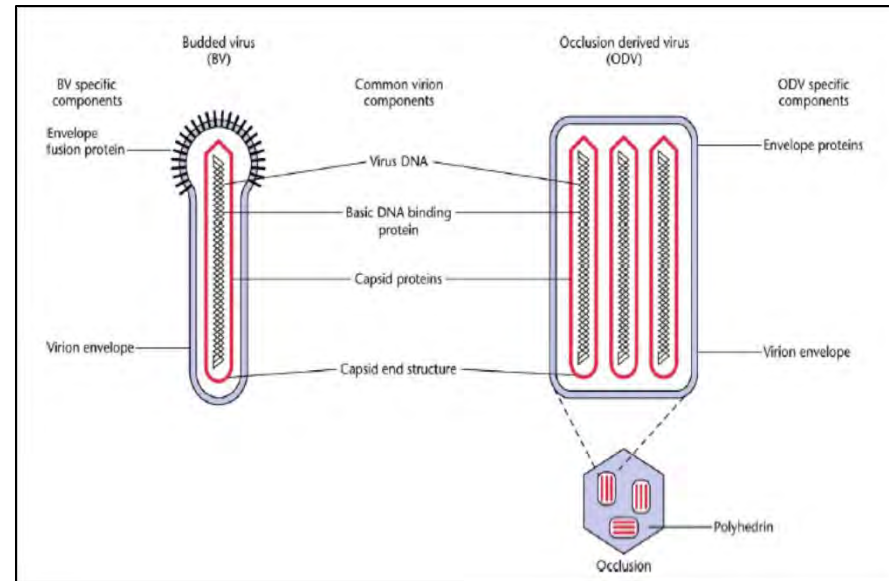
- Can express mammalian proteins
- Similarities with mammalian cells
  - ▣ Eukaryotic systems
    - How protein are produced
    - Protein folding
    - Protein trafficking
    - Simple post-translational modifications
  - ▣ Cell size
  - ▣ Growth rate

# Advantages of using insect cell systems

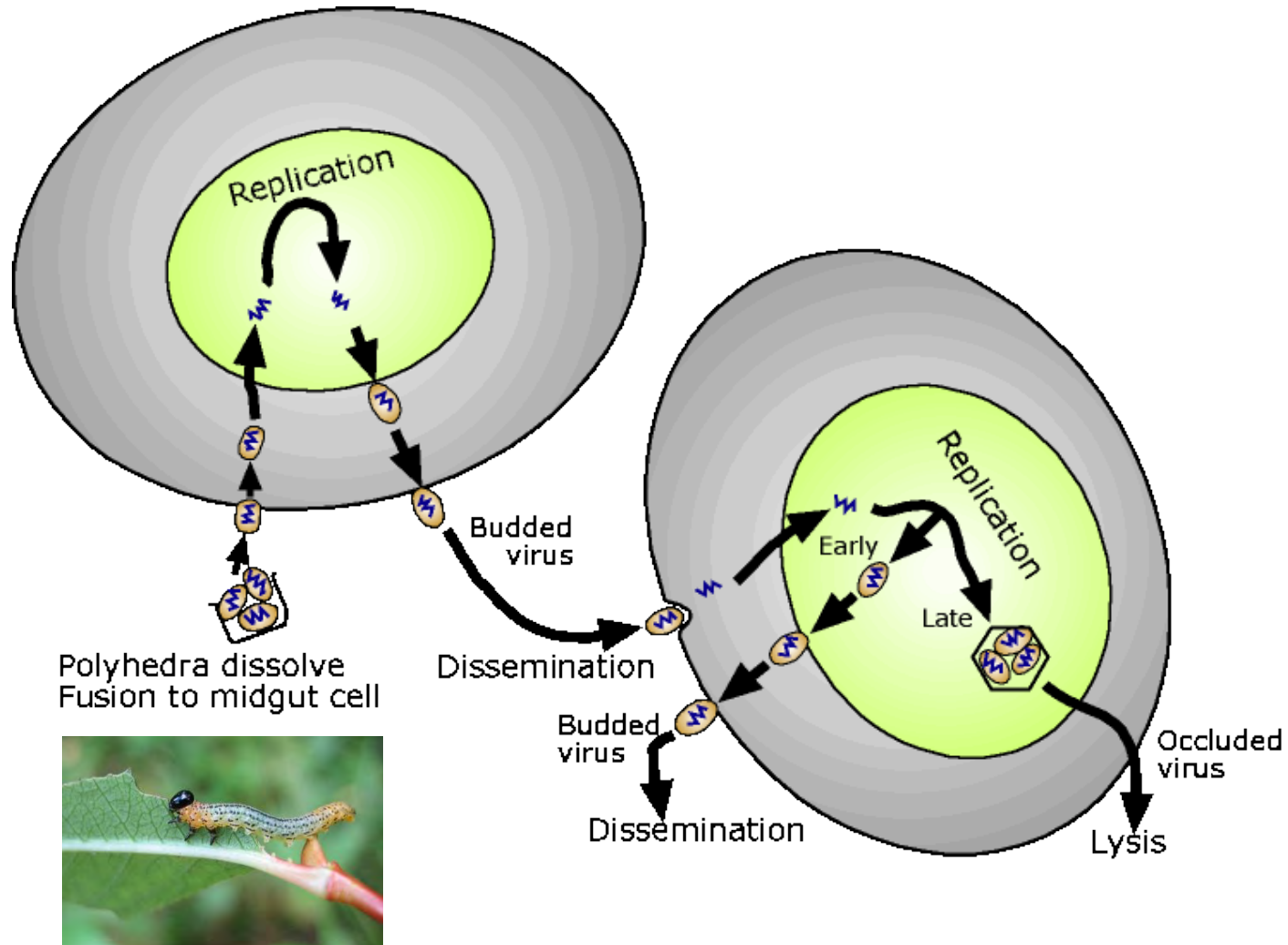
- Produce high levels of recombinant proteins
- Have cellular advantages
  - ▣ Can grow in monolayers and suspension
  - ▣ Capable of indefinite replication
  - ▣ Do not require CO<sub>2</sub>
  - ▣ Physical dislodgements of cells is easier
  - ▣ Preserved well by cryopreservation

# Insect-baculovirus expression system

- Baculovirus
  - ▣ Insect larva virus
  - ▣ Biphasic lifecycle
    - Occluded form-transmitted between insects hosts
      - Stable in environment
    - Budded form-transmitted between insect cells
      - Used in baculovirus expression system



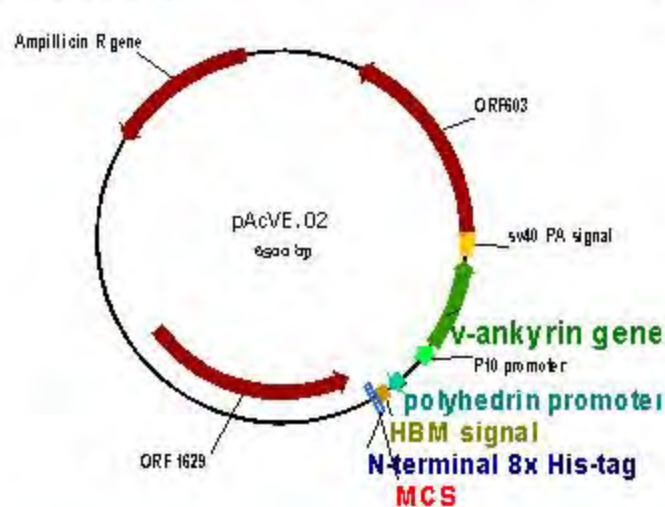
# Baculovirus infection cycle



# Baculovirus Expression System

method for producing high amounts of recombinant proteins

## 1. Cloning

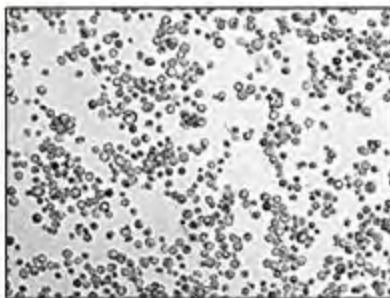


## 2. Construct recombinant baculovirus



- Homologous Recombination between transfer vector and baculovirus DNA
- Infect insect Sf9 cells with ligated baculovirus (P0 stock)
- Amplify virus (P1 stock)
- Titer P1 stock

## 3. Infection



- Infect insect Sf9 cells with a specific MOI of recombinant baculovirus for 3-5 days
- Collect supernatant or cell pellet (Analyze by Western blot)

## 4. Purify protein using a his-tag column



# Baculovirus Expression System

## Advantages

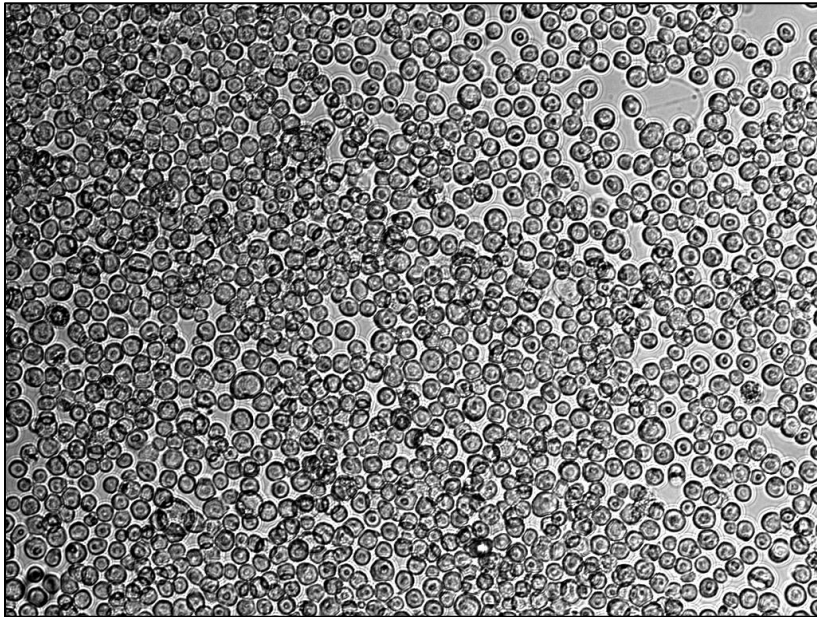
- Produces high levels of recombinant protein
- Efficiently cleaves signal peptides
- Simultaneous expresses multiple genes
- Can contain large DNA inserts

## Disadvantages

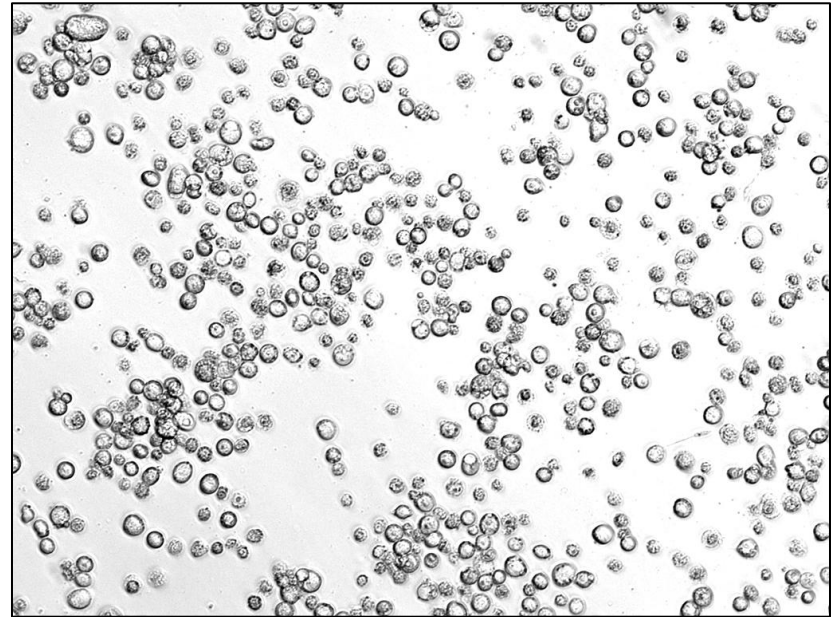
- **Lytic**
- Limits on post-translational processing

# Baculovirus infection stimulates host cell lysis

Day 2 post-infection



Day 5 post-infection





# Vankyrin protein inhibits apoptosis

Insect Molecular Biology (2009) 18(4), 497–506

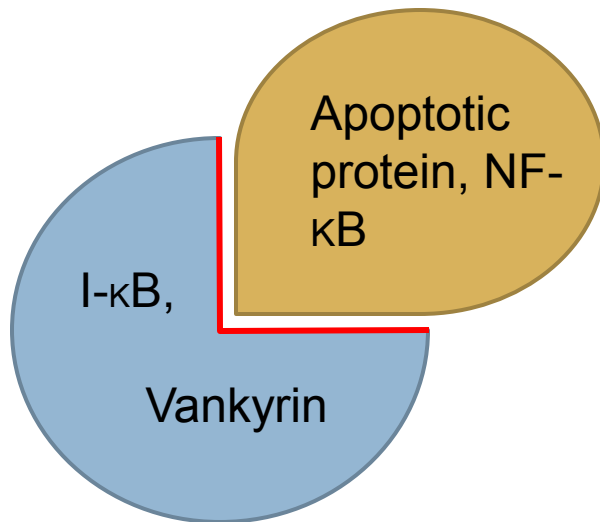
## The *Campoletis sonorensis* ichnovirus vankyrin protein P-vank-1 inhibits apoptosis in insect Sf9 cells

A. Fath-Goodin\*†, J. A. Kroemer† and B. A. Webb\*†

- *Campoletis sonorensis* ichnovirus produces 7 vankyrin proteins.
- **P-vank-1** disrupts the apoptotic pathway when expressed in Sf9 insect cells (**VE-CL cells**)
  - ▣ Nuclear fragmentation is inhibited
  - ▣ Internucleosomal degradation is inhibited
  - ▣ Apoptotic protein Caspase-3 activity was upregulated 22x in infected Sf9 but only 5x in infected VE-CL cells.

# Model of Vankyrin inhibition

-- ankyrin repeats

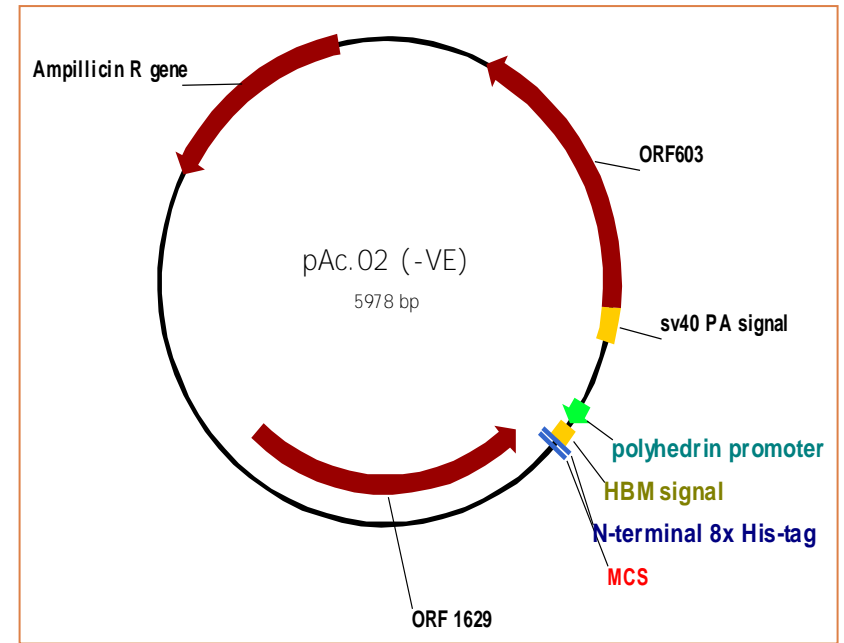
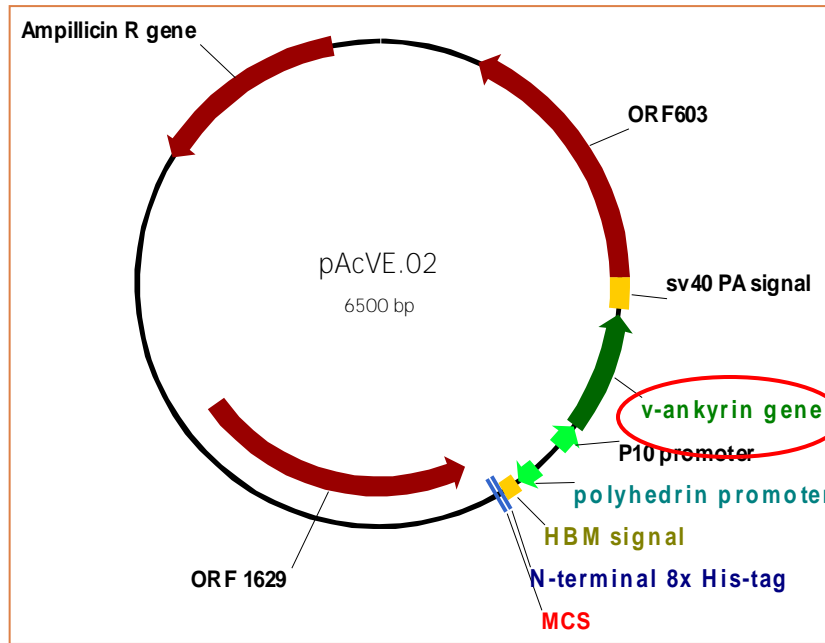


- Homology to I-κB, a NF-κB inhibitor
  - Both have ankyrin repeats
- Possibly, Vankyrin binds and inhibits an apoptotic protein such as NF-κB

# First Question

Can we produce more recombinant protein by co-expressing it with the **Vankyrin** protein?

# Created two new base transfer vectors



# Current therapeutic proteins

## Erythropoietin (EPO)

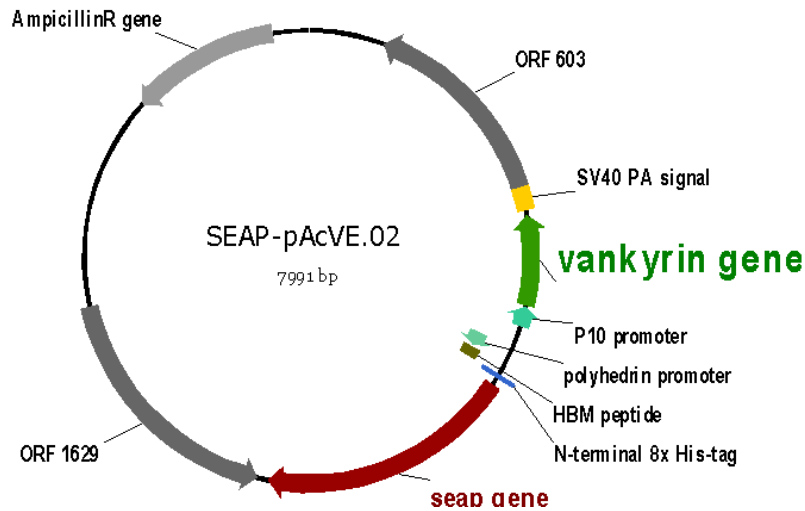
- A hormone that regulates red blood cell production
- Currently used to treat anemia from kidney failure
- Presently made in mammalian CHO cells

## Secreted alkaline phosphatase (SEAP)

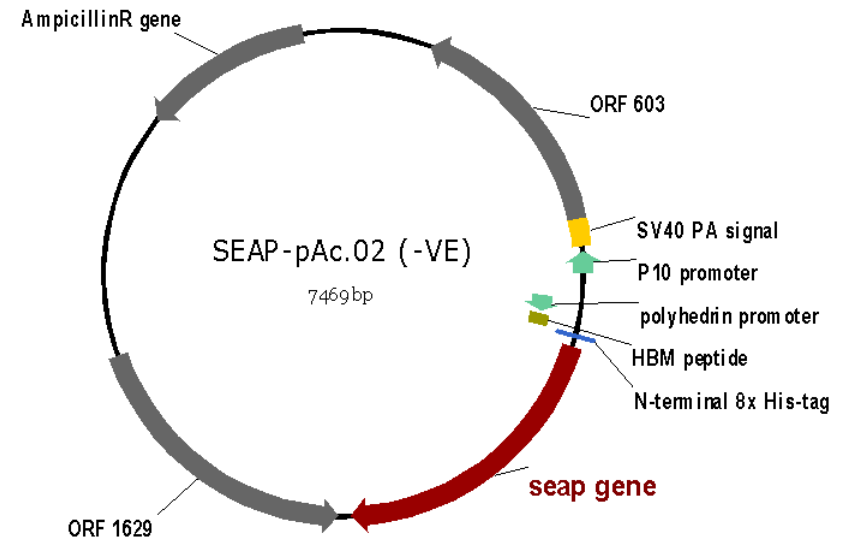
- Dephosphorylates and detoxifies LPS
- Currently used to treat
  - ▣ Endotoxin-induced systemic inflammation
  - ▣ Acute renal failure
  - ▣ Sepsis
  - ▣ Ulcerative colitis
- Enzymatic assay

# Inserted two recombinant genes into the base transfer vectors

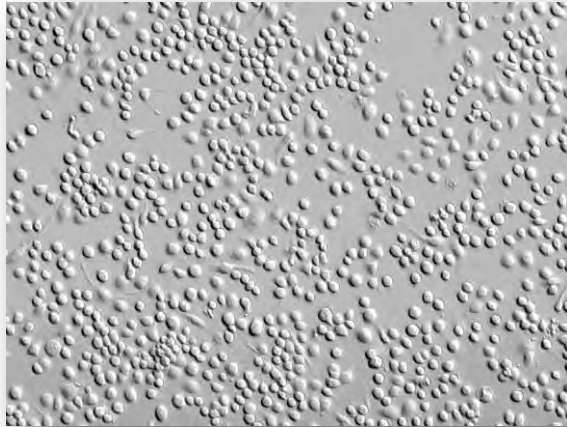
## SEAP-pAcVE.02



## SEAP-pAc.02 (-VE)

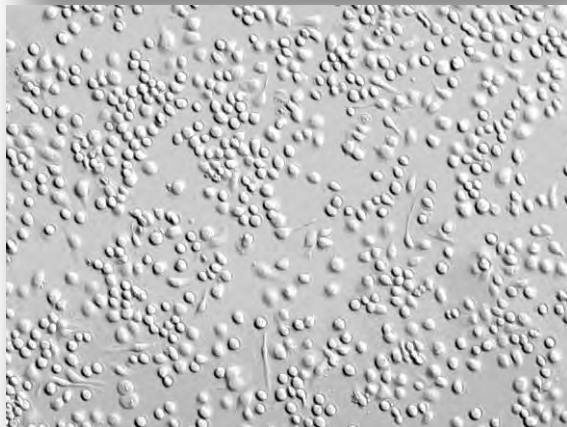
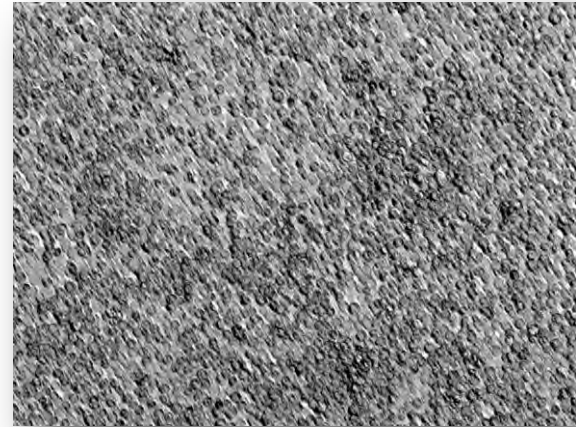


Day 1 post-infection

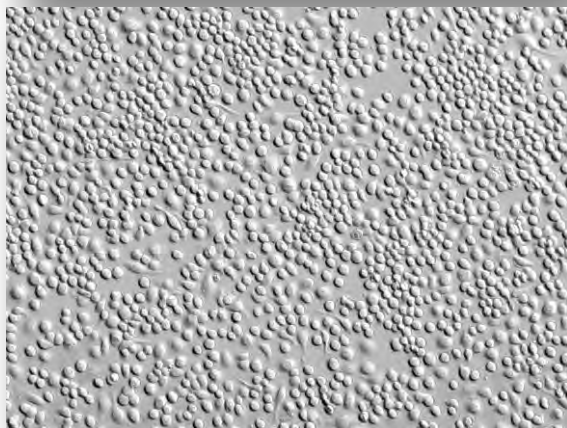
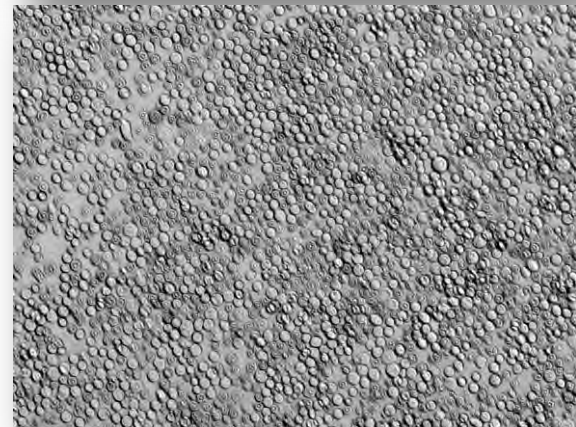


Uninfected

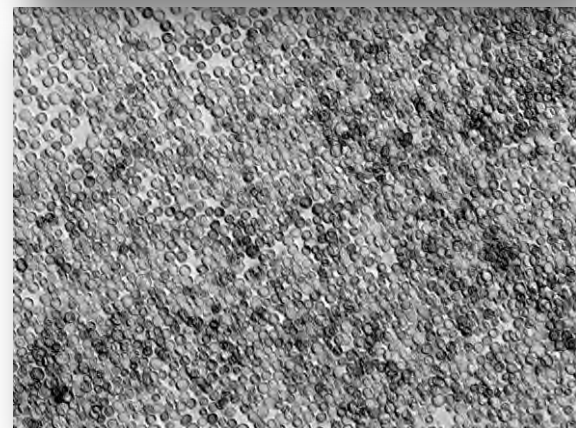
Day 5 post-infection



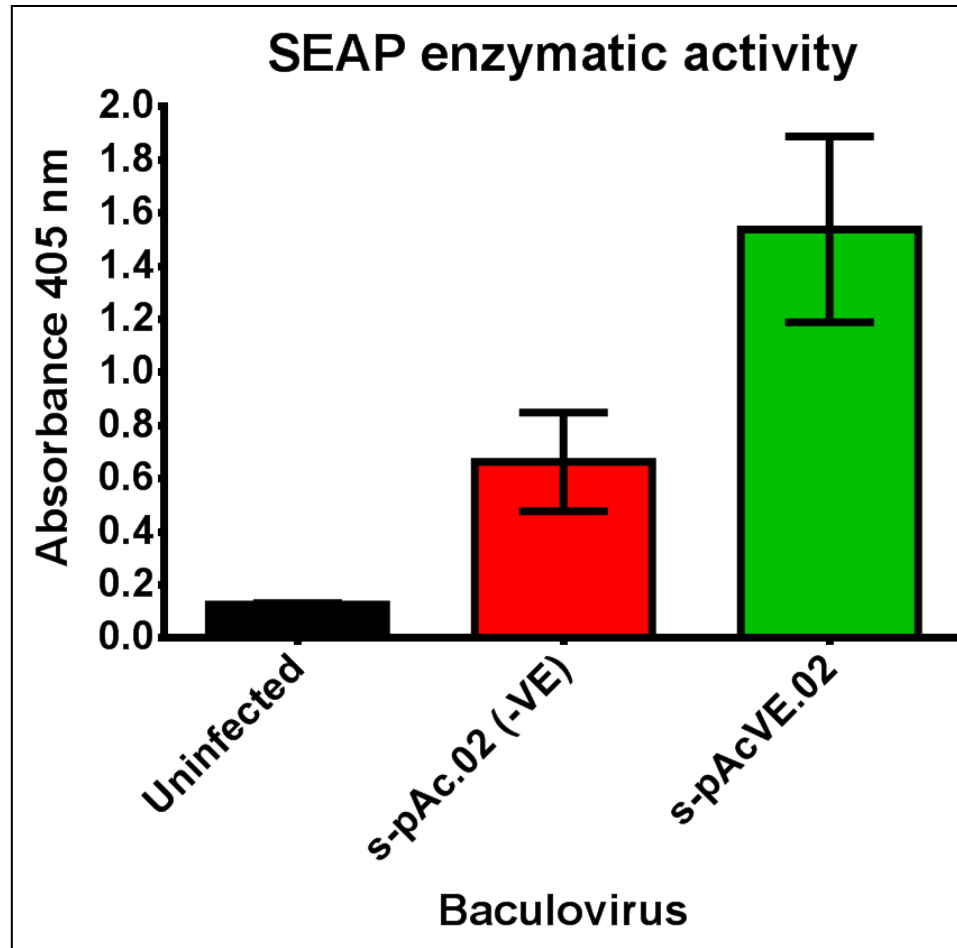
*BV*-VE



*BV*+VE

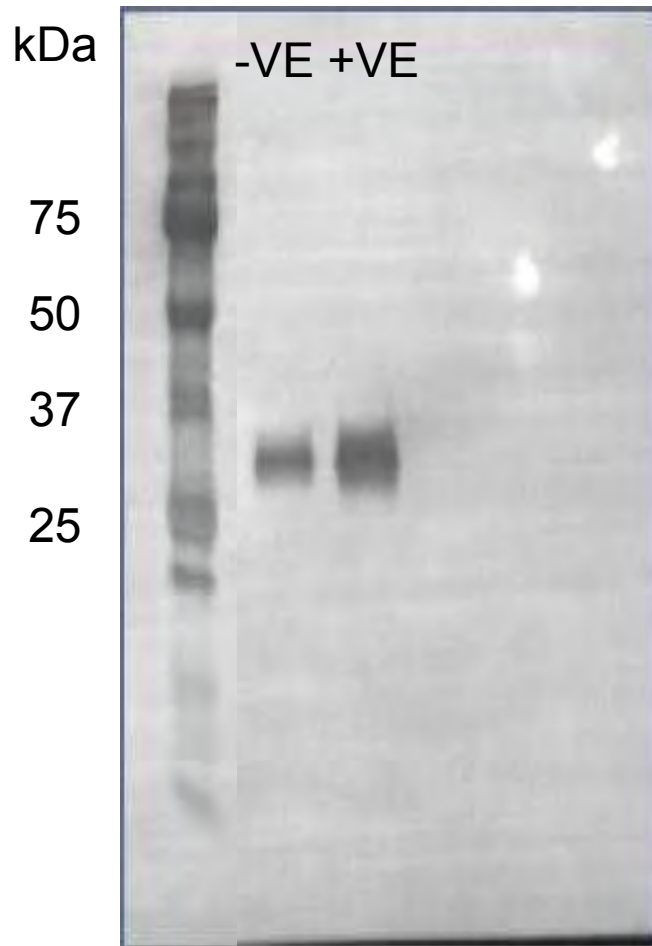


# Higher level of SEAP activity is detected when using a Vankyrin-enhanced baculovirus





# Higher levels of rEPO are produced using a Vankyrin-enhanced baculovirus



# Summary of question 1

- The Vankyrin protein delays apoptosis of infected Sf9 insect cells
- Increased levels of recombinant EPO and SEAP proteins are produced using a **vankyrin-enhanced baculovirus** compared to current baculovirus technology
- Current yields from a 50 ml culture are  $> 6$  mg

# Baculovirus Expression System

## Advantages

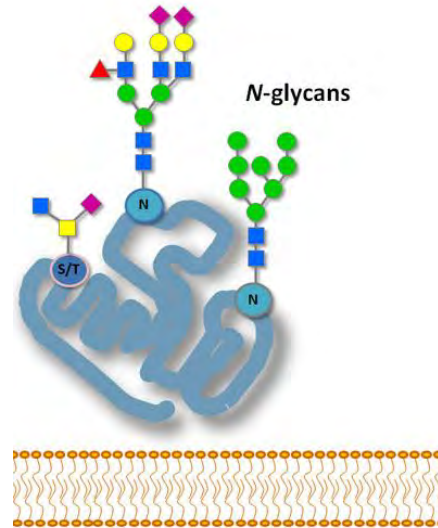
- Produces high levels of recombinant protein
- Efficiently cleaves signal peptides
- Simultaneous expresses multiple genes

## Disadvantages

- Lytic
- **Limits on post-translational processing**

# Glycosylation

- The addition of carbohydrates to a protein
- A post-translational modification
  
- Important for
  - Protein folding
  - Stability
  - Cell-to-cell adhesion
  - Enzyme activity
  - Receptor binding



Last accessed 4-3-14 . Modified from  
<https://www.neb.com/applications/glycobiology>.

# Importance of glycosylation

- Over 50% of all proteins are glycosylated
- Glycoprotein and carbohydrate therapeutics represent a \$20 billion market



# StSW14: a transgenic insect cell line capable of *N*-glycan processing

- Donald L. Jarvis' lab
- Expresses 6 mammalian glycosylation enzymes

## Second Question

Can we use the **vankyrin technology** to enhance production of **glycosylated mammalian proteins**?



# Created vankyrin-producing insect cells

Sf9 + *vankyrin* gene = VE-CL

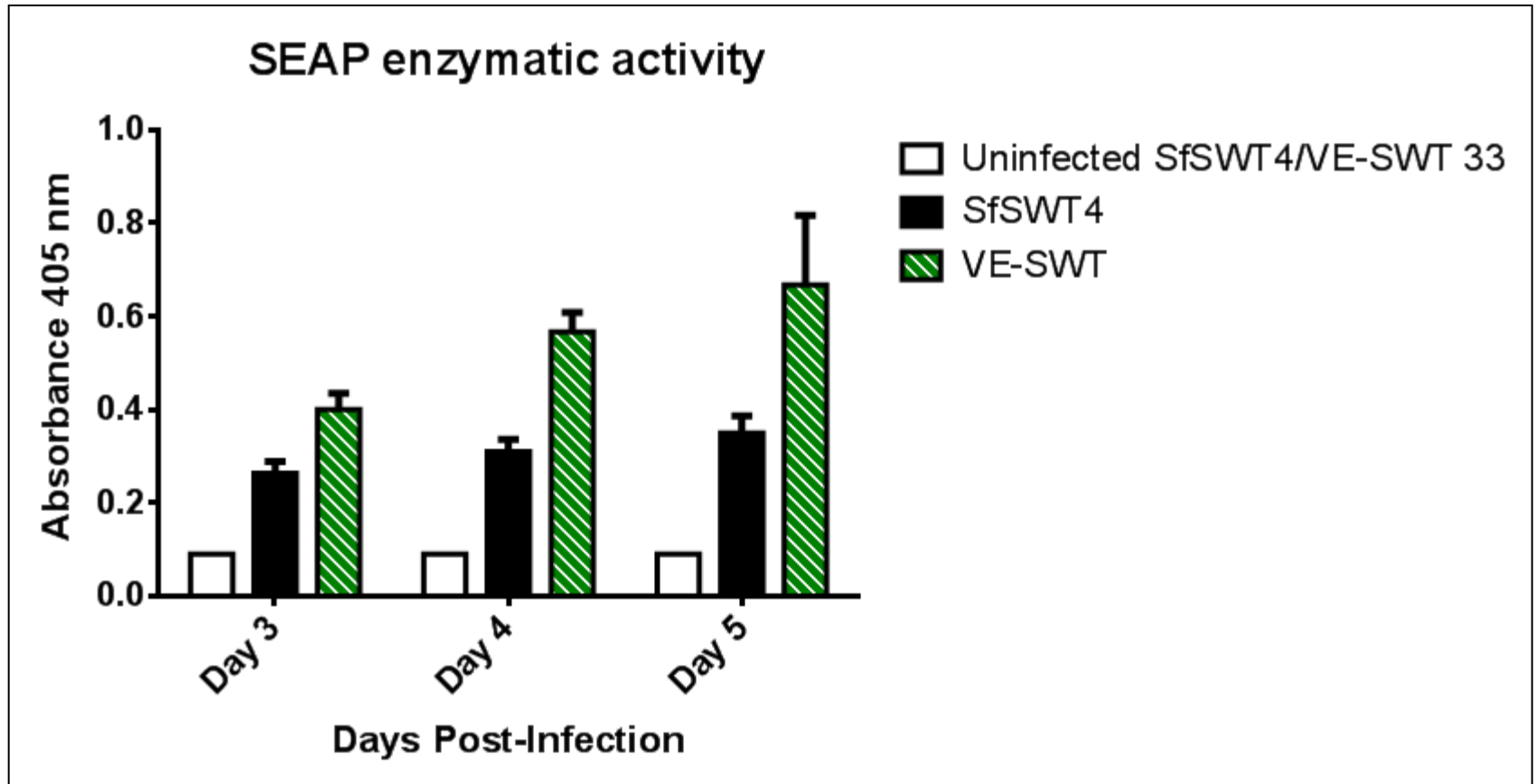
SfSWT4 + *vankyrin* gene = VE-SWT

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*seap*-BV → SfSWT4

*seap*-BV → VE-SWT

# Vankyrin-enhanced SfSWT4 cells also increase recombinant protein production



# Summary of question 2

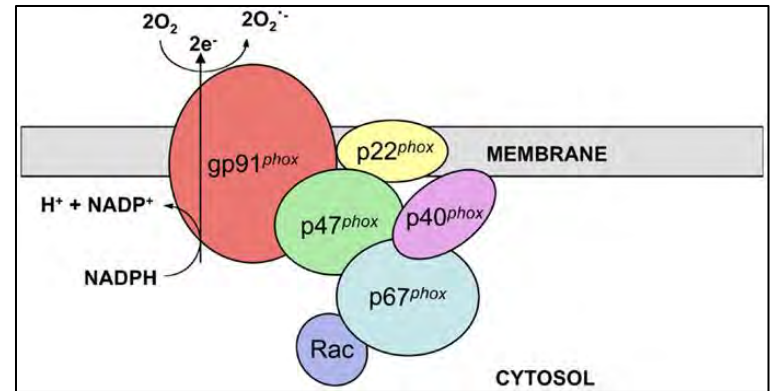
- The engineered insect cell line, SfSWT4, can properly glycosylate mammalian recombinant proteins
- SfSWT4 was further enhanced with the addition of the *vankryin* gene: VE-SWT
- Recombinant protein production is enhanced in VE-SWT insect cells

# Toxic proteins

The Vankyrin technology enhances production of hard to express or toxic proteins.

# NADPH oxidase

- NADPH oxidase
  - ▣ Generates superoxides in response to pathogen binding to phagocytic cells
  - ▣ Oxidative stress:  $O_2^-$  and  $H_2O_2$
  - ▣ Five subunits and a small GTPase Rac
    - Catalytic core comprises **flavocytochrome  $b_{558}$** :  $gp91^{phox}$  and  $p22^{phox}$



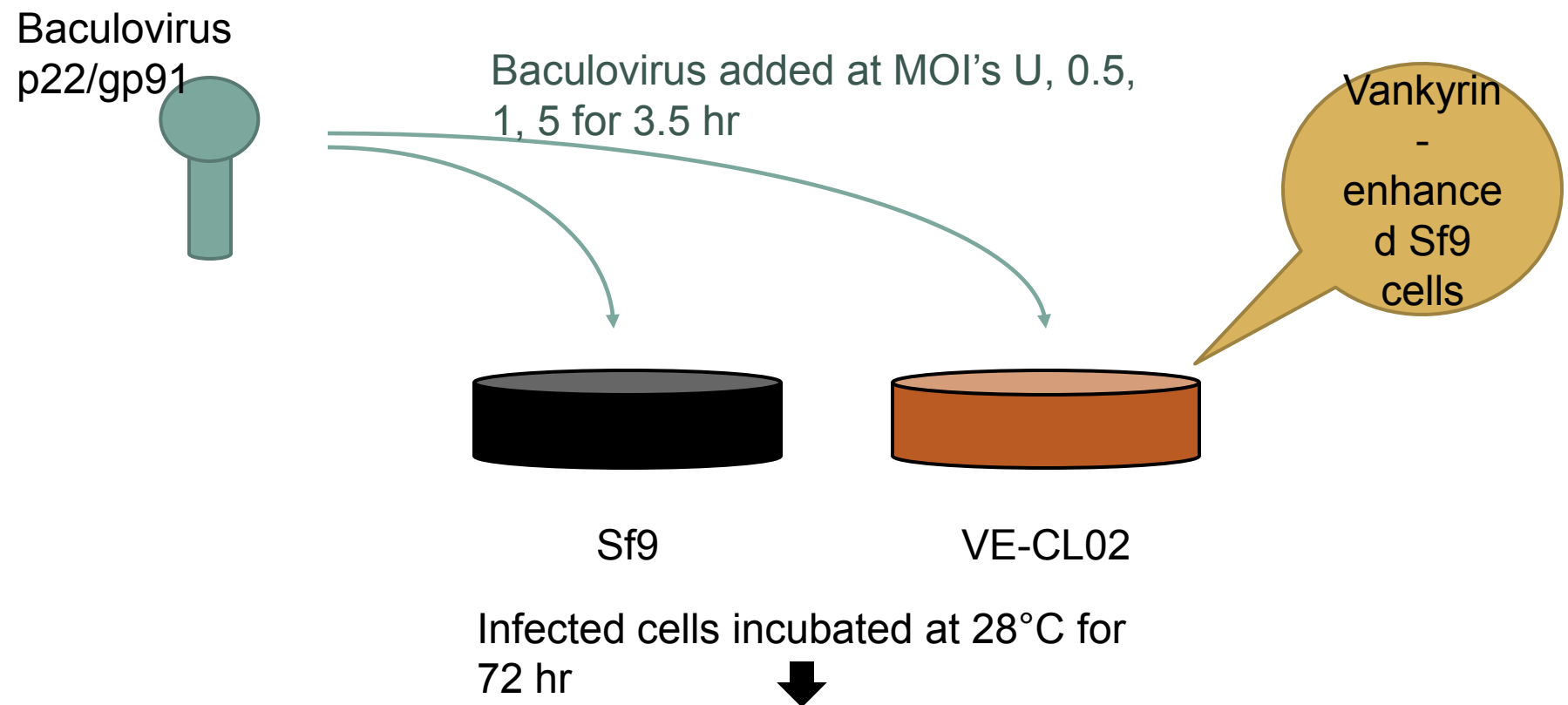
Gardiner *et al.* Front. Immunol. 2013. 4: Art. 295

# Problem

The production of  $gp91^{phox}$  and  $p22^{phox}$  were killing the insect cells.

Only **10%** of insect cells were viable at 40 hr post-infection

# Methods

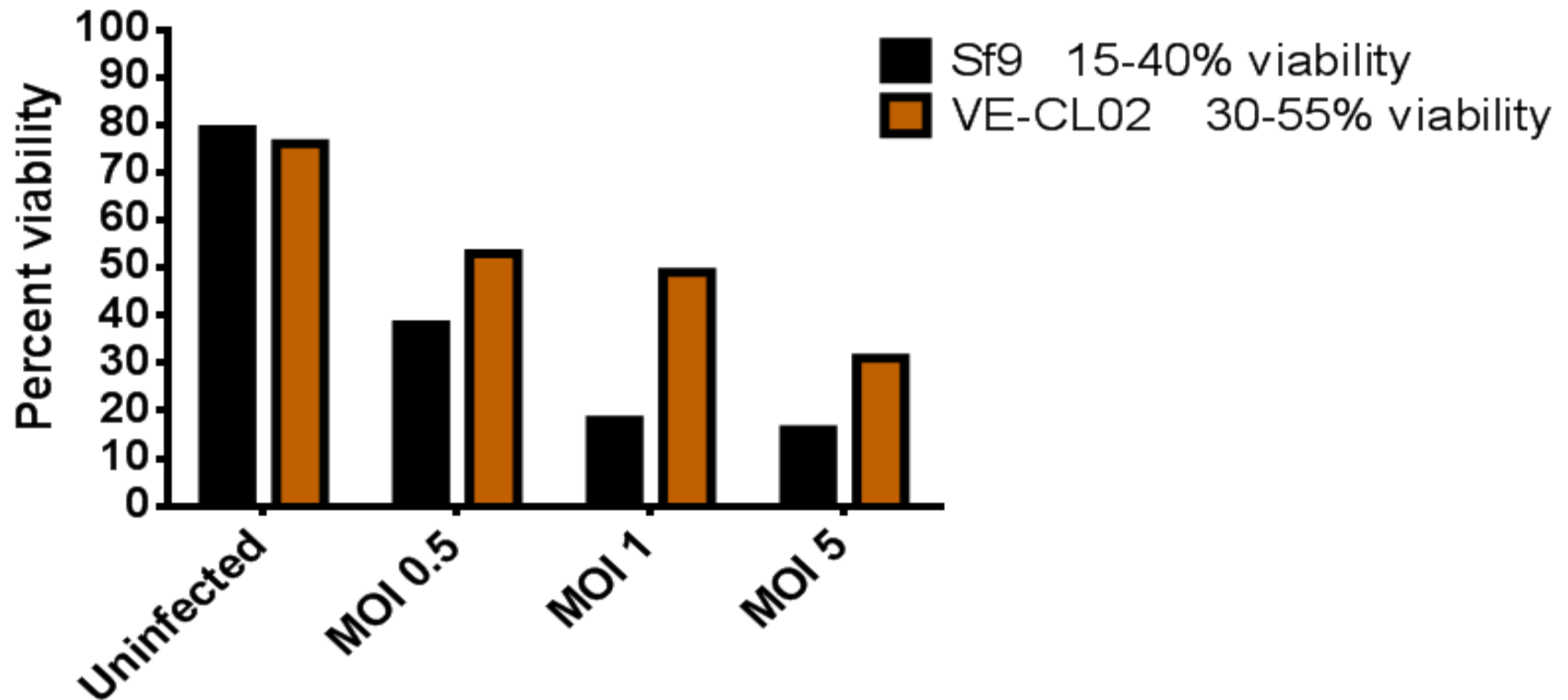


Cultures were stained with **trypan blue** to determine percent viability.

Remaining cells were collected and analyzed by Western blot.

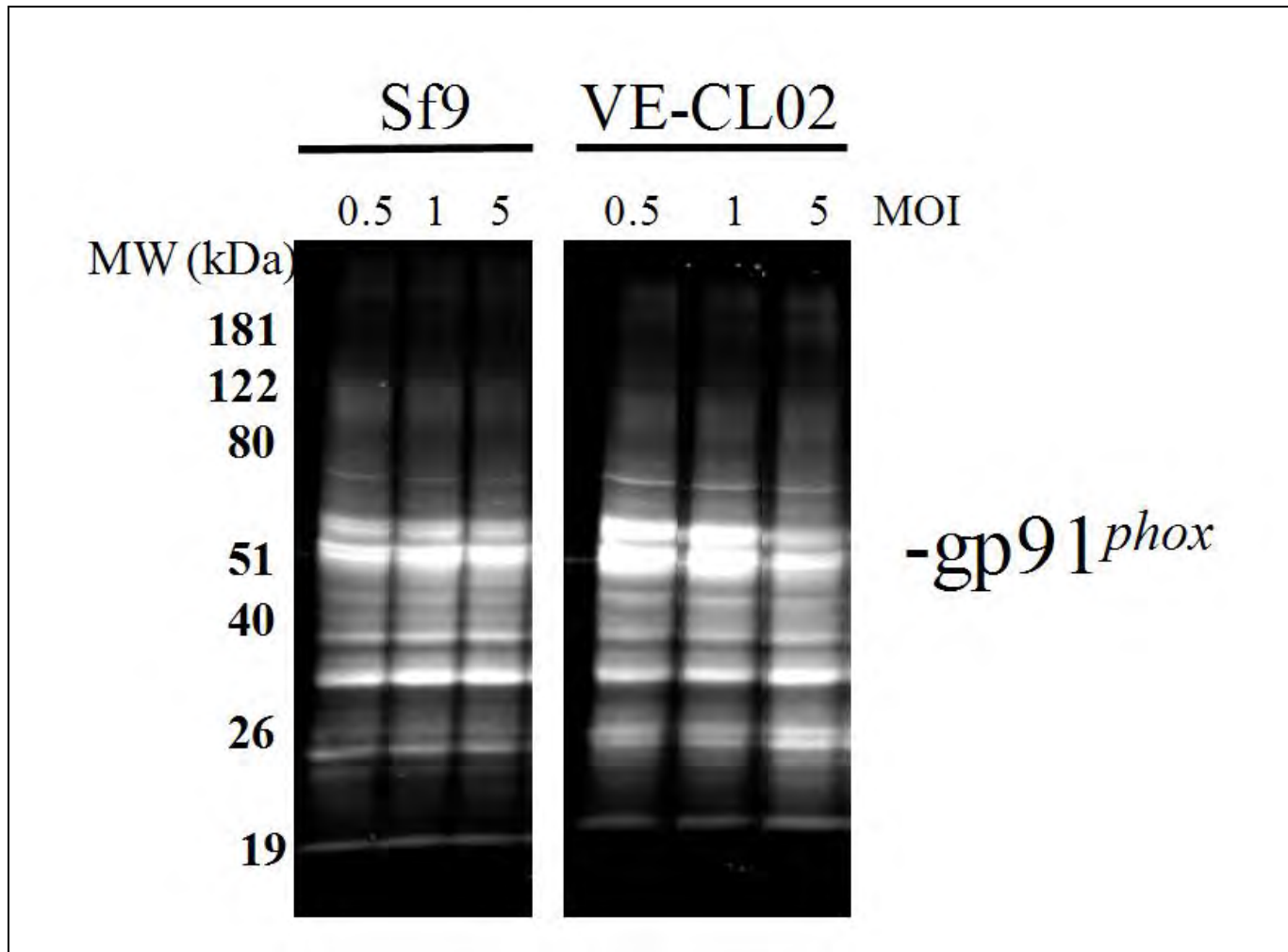
# Vankyrin-enhanced Sf9 cells have increased viability

72 hrs post-infection





# Vankyrin-enhanced Sf9 cells produce higher levels of gp91<sup>phox</sup>

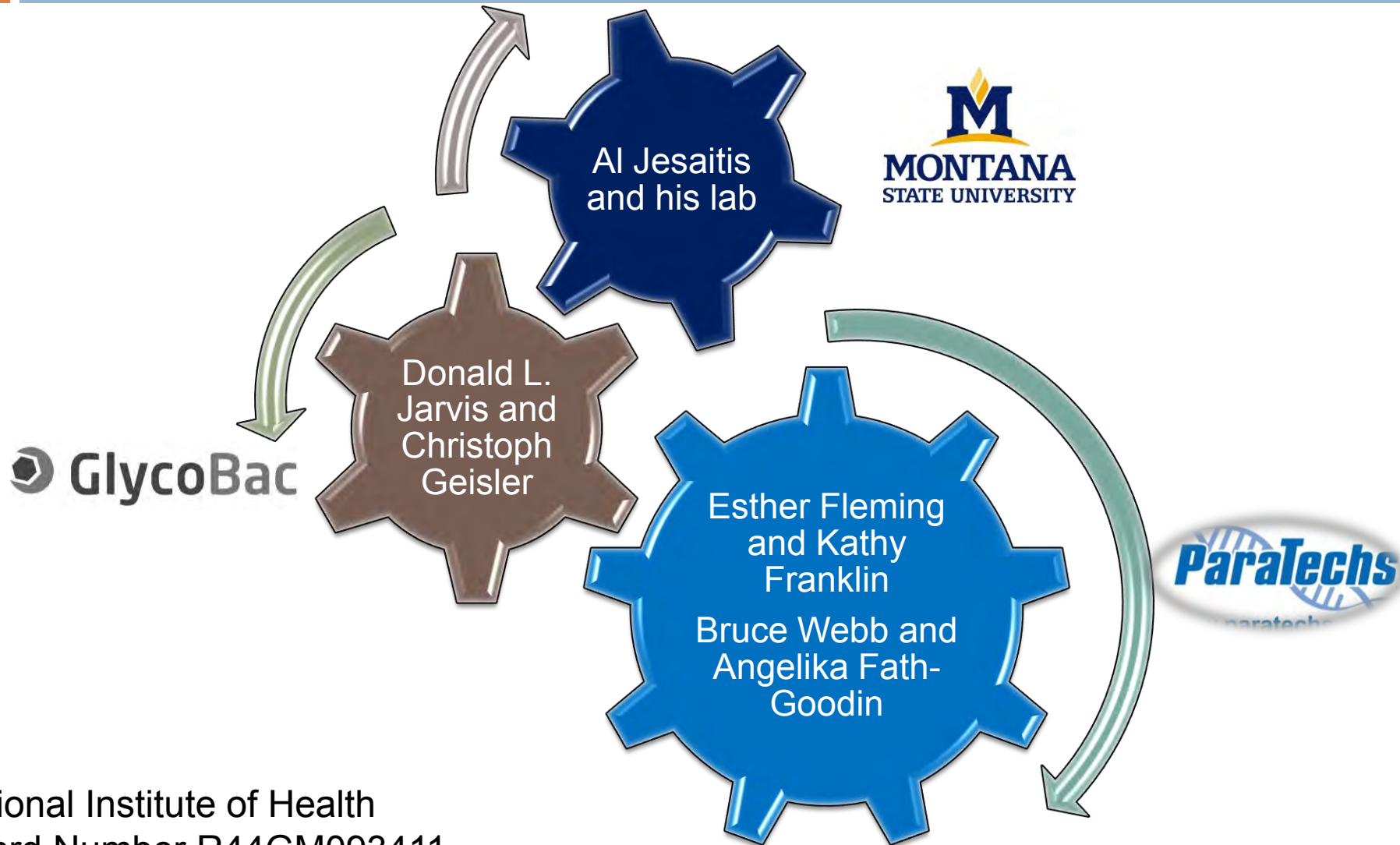


# Conclusions

- The Vankyrin protein delays apoptosis of infected Sf9 insect cells
- Many recombinant proteins are produced at higher levels using a...
  - Vankyrin-enhanced baculovirus
  - Vankyrin-enhanced insect cells
    - VE-CL02 (Sf9)
    - VE-SWT (SfSWT4)
- Includes for toxic proteins such as flavocytochrome  $b_{558}$

# Acknowledgements

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National Institute of Health  
Award Number R44GM093411.