



# ***Novel Nanotechnology-Based Antiviral Agents:*** ***Silver nanoparticle neutralization of hemorrhagic fever viruses***

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# Nanomaterials

## Unique Properties

- Size (< 100nm)
- Optical (metal & Semiconductors)
- Magnetic (metal)
- Surface reactivity
- Catalytic activity (high surface area)
- Bioaffinity
- Surface modification

## DOD Applications

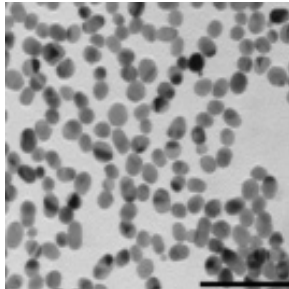
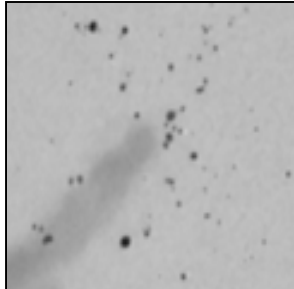
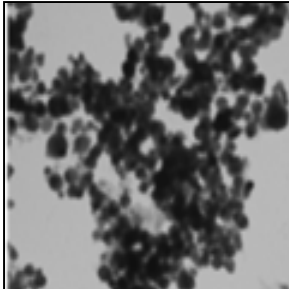

- Biosensors
- Antimicrobial Agents
- Munitions
- Propellants
- Coatings
- Smart Suits

## Challenges

- Toxicity
- Reproducibility
- Stability of coatings/functional groups
- bioaffinity
- Effects on protein activity
- Effects on gene expression



# Silver Nanoparticles

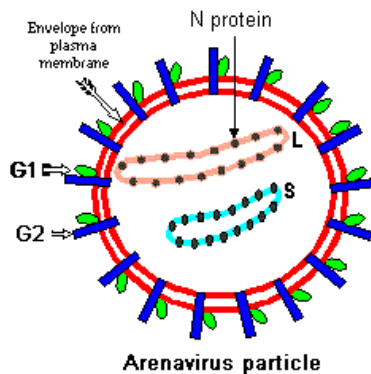
	UNCOATED	POLYSACCHARIDE COATED
<b>10 nm</b>	 <p><math>12.78 \pm 0.13</math> Dr. Steve Oldenburg, NanoComposix</p>	 <p><math>9.48 \pm 4.286</math> Dr. Dan Goia, Clarkston University</p>
<b>25 nm</b>	 <p><math>27.474 \pm 9.062</math> Dr. Karl Martin, Novacentrix</p>	 <p><math>25.98 \pm 8.38</math> Dr. Dan Goia, Clarkston University</p>



# Hemorrhagic Fever Viruses

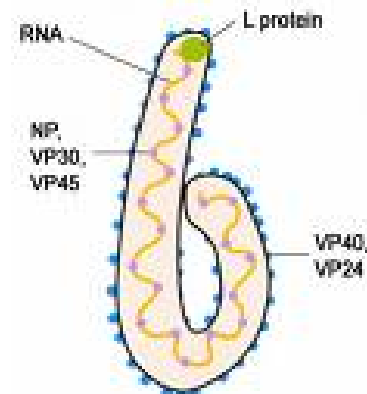
## Arenaviridae

- South american HFV, Lassa Fever, LCMV
- Enveloped, RNA viruses
- No effective therapies
- Candid#1 vaccine
- 5-35% fatality rate



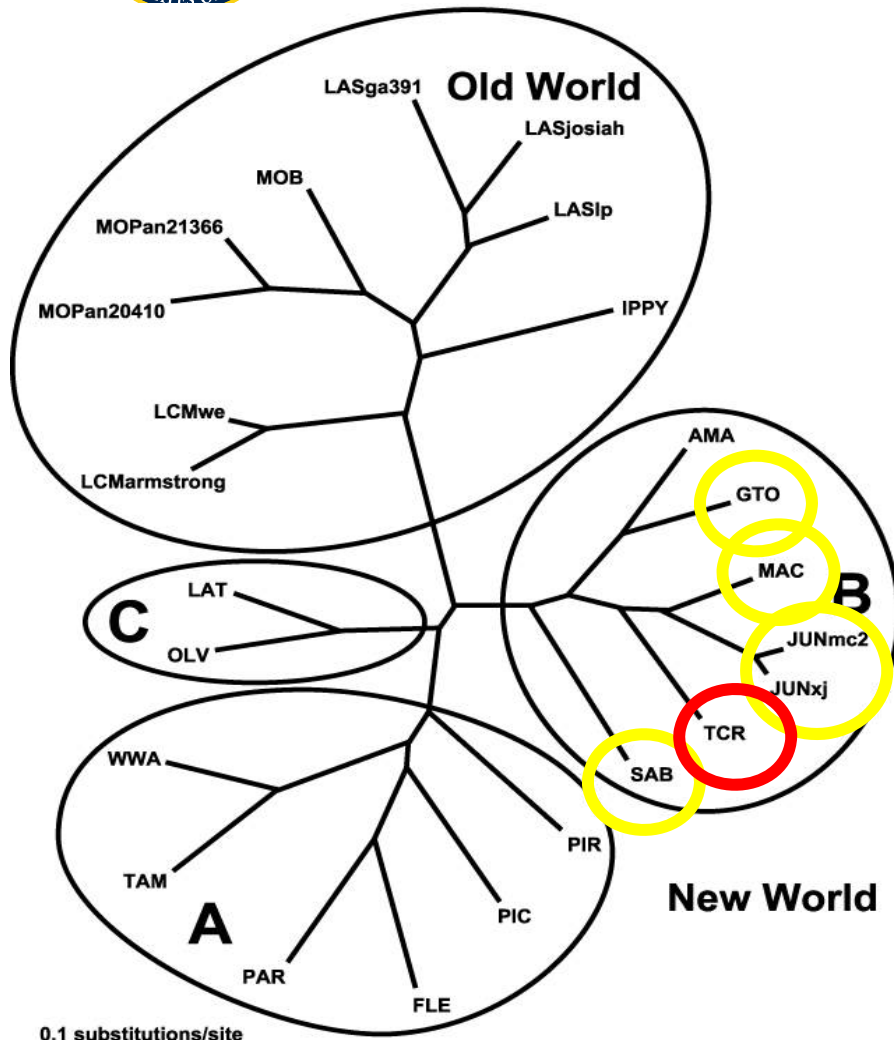
## Filoviridae

- Ebola and Marburg
- Enveloped, RNA viruses
- No effective therapies
- Vaccine in Phase I trials
- Up to 90% fatality rate





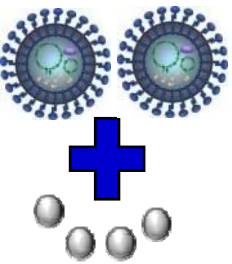
# Tacaribe Virus



- New World (Tacaribe) Complex
  - Junin, Machupo, Guanarito, and Sabia
- Tacaribe virus is a biochemically and serologically close relative of the CDC category A arenaviruses but has a low pathogenic potential for humans
- Experimentally:
  - Cytopathic effect in vero cells
  - lethal meningoencephalitis in mice



# Arenavirus Experimental Setup



2 h.p.i  
Confocal Microscopy  
Cell surface expression

12 h.p.i  
TEM  
Virus internalization

8 d.p.i  
Harvest Progeny Virus  
TCID<sub>50</sub> determination

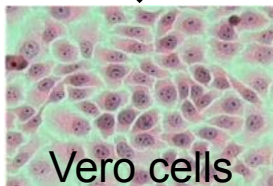
0 hour

1 h.p.i



4 h.p.i  
Confocal Microscopy  
Virus internalization

4 d.p.i  
qRT-PCR

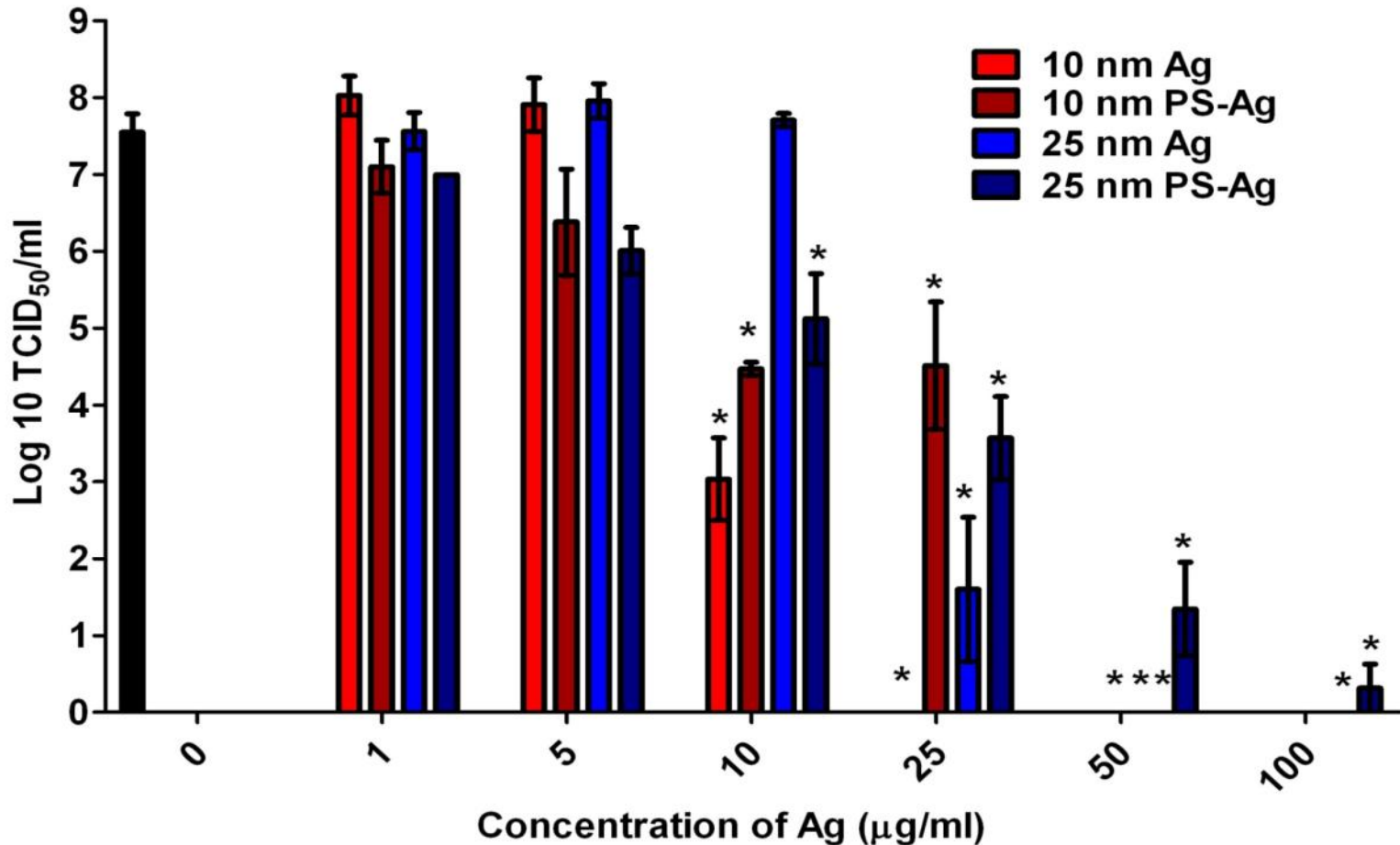


Vero cells



# TCRV Progeny Virus Production

## Tacaribe Virus Neutralization by Silver Nanoparticles

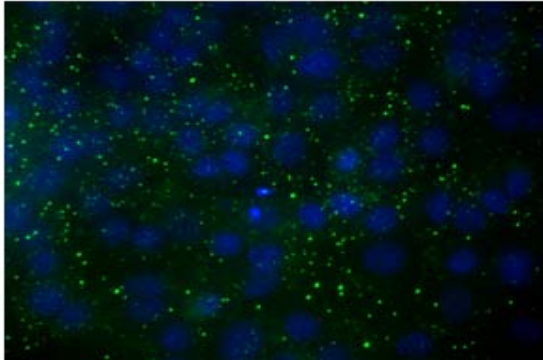




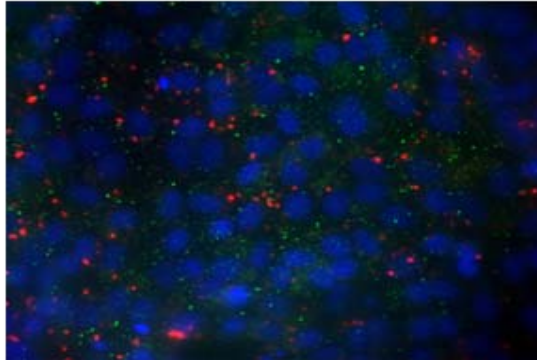


# Cell Surface TCRV Expression

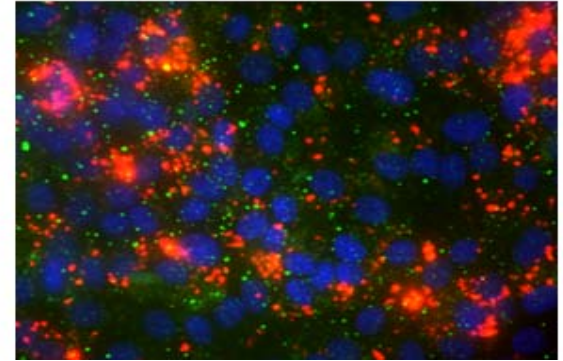
**Negative Control**



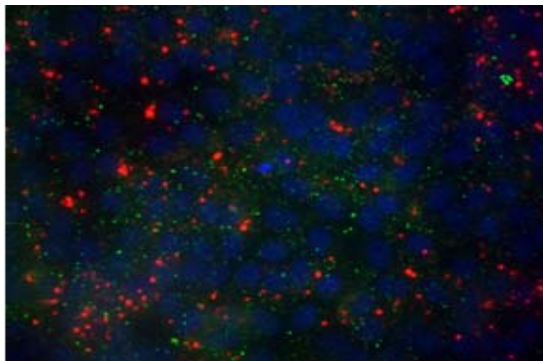
**10 nm 50 µg/ml**



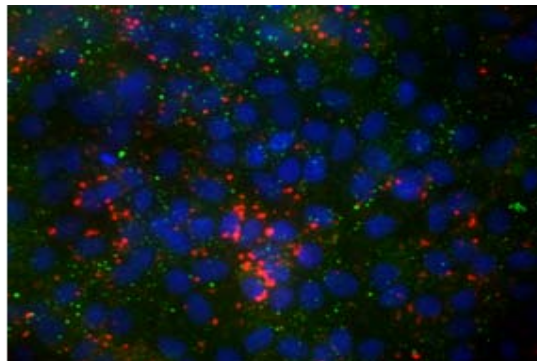
**25 nm 50 µg/ml**



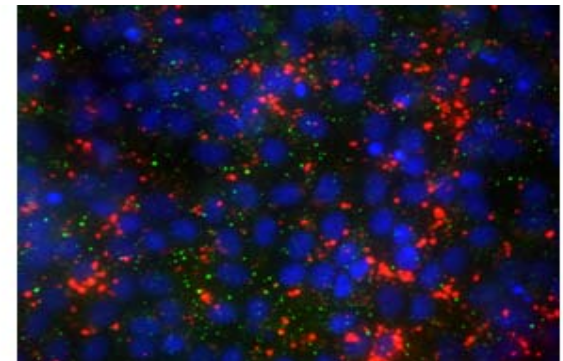
**Positive Control**



**10 nm 10 µg/ml**



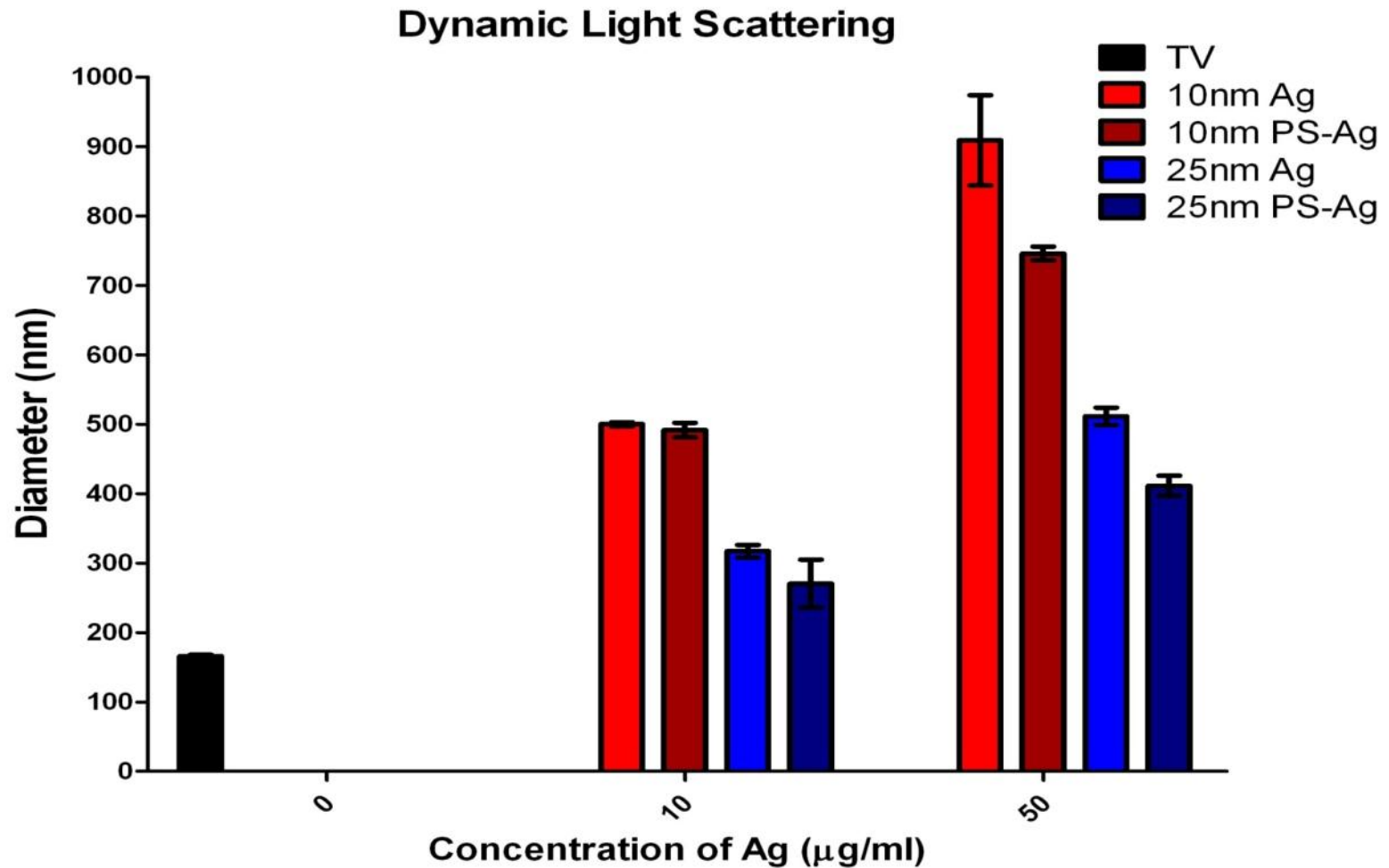
**25 nm 10 µg/ml**







# Dynamic Light Scattering





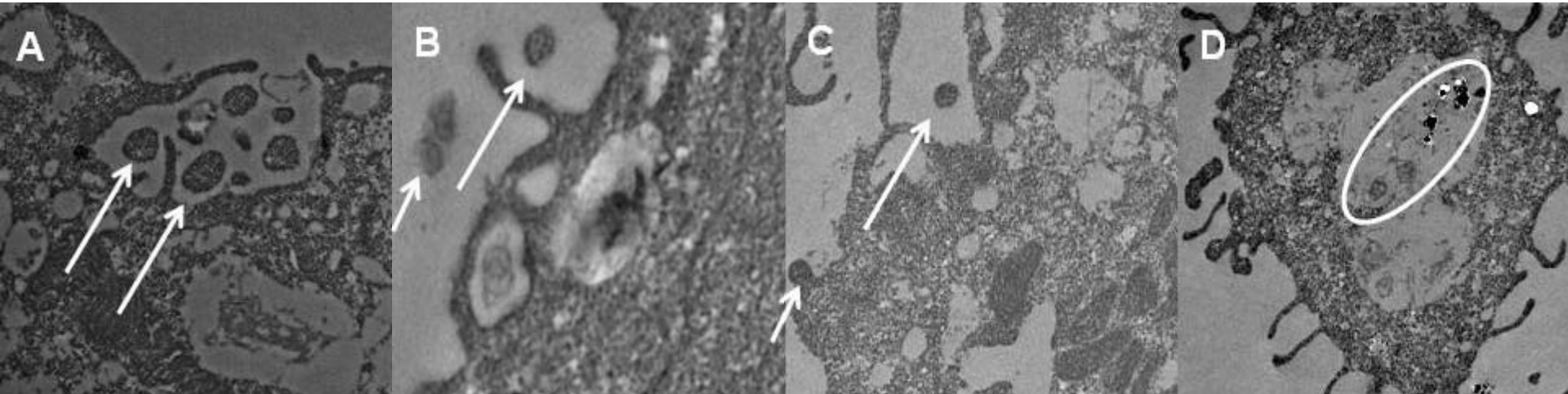
# TCRV Internalization into Vero Cells

Untreated TCRV

TCRV + 10nm Ag

TCRV + 25nm Ag

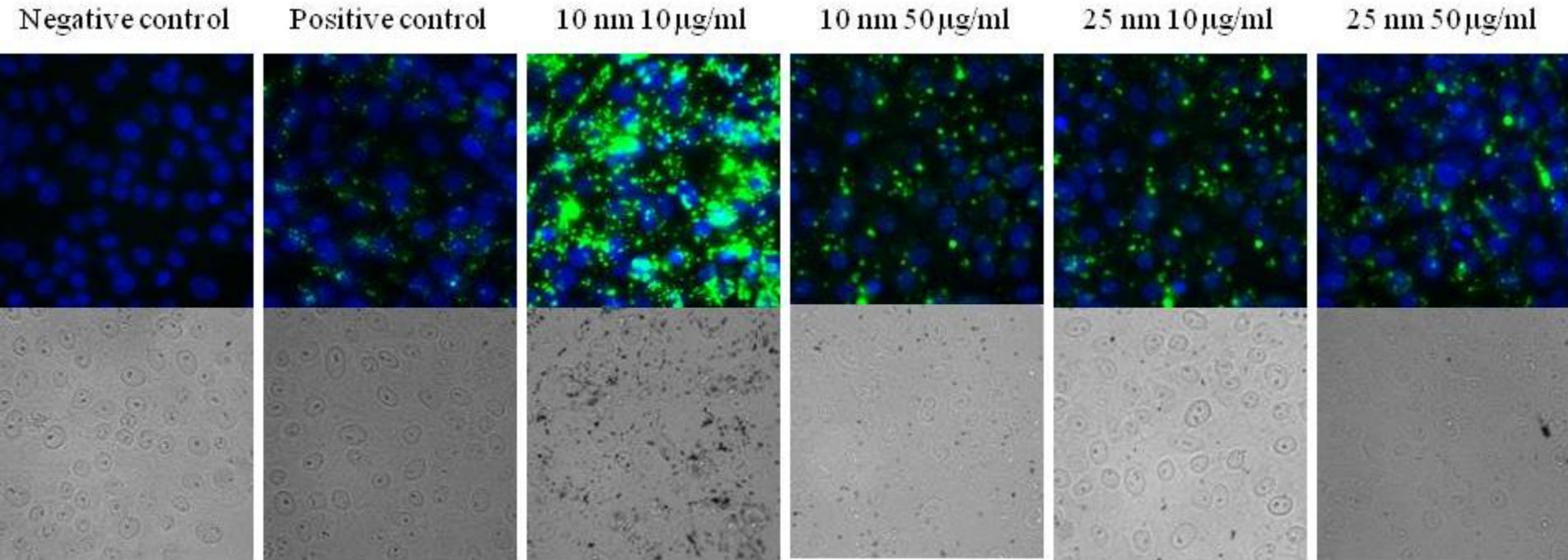
TCRV + 25nm Ag



- Ag-NP-treated TCRV is internalized into infected Vero cells
- Ag-NPs and TCRV interact inside the cell lysosomes



# TCRV Internalization into Vero Cells

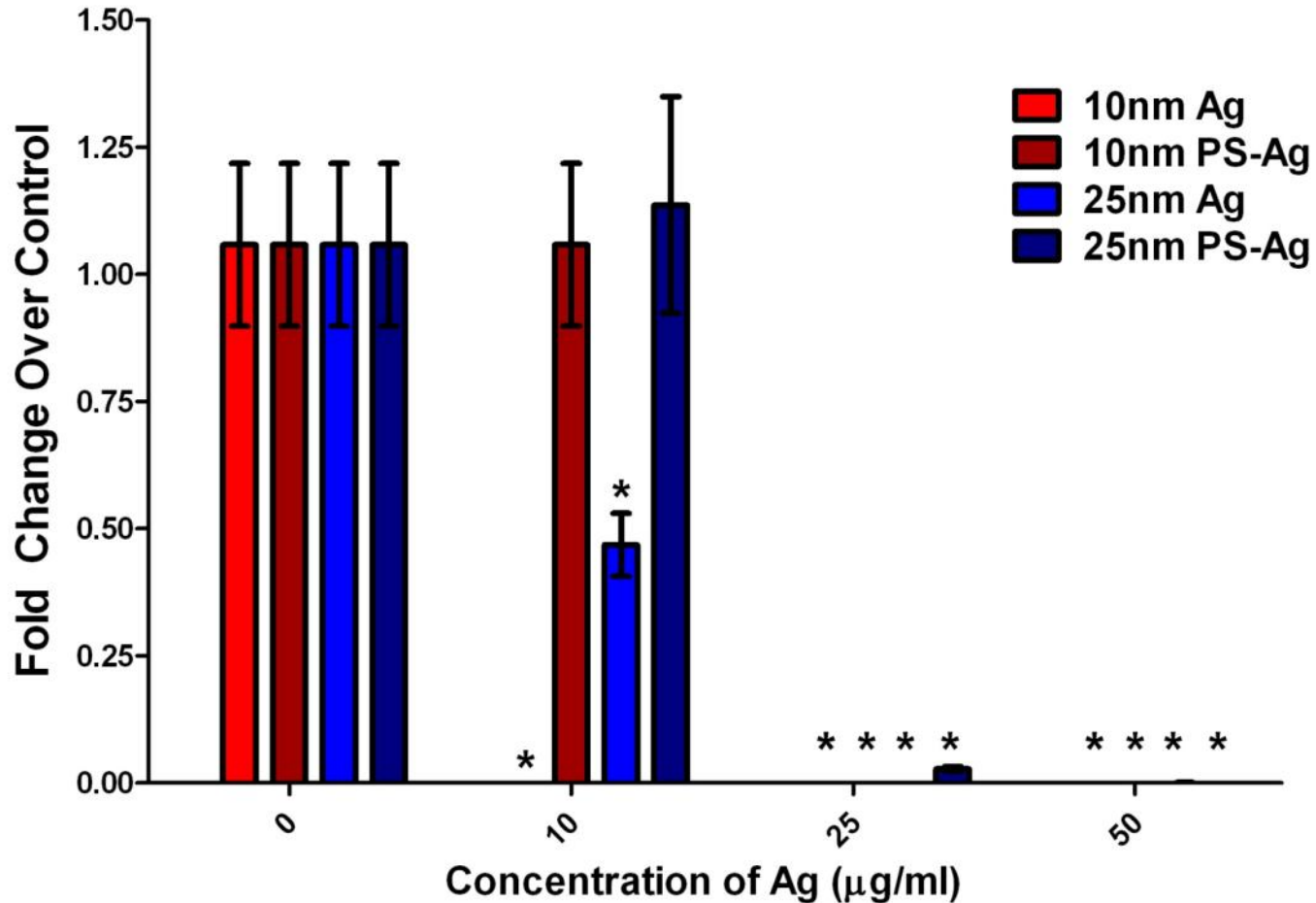


- Ag-NPs facilitate uptake of TCRV into Vero cells



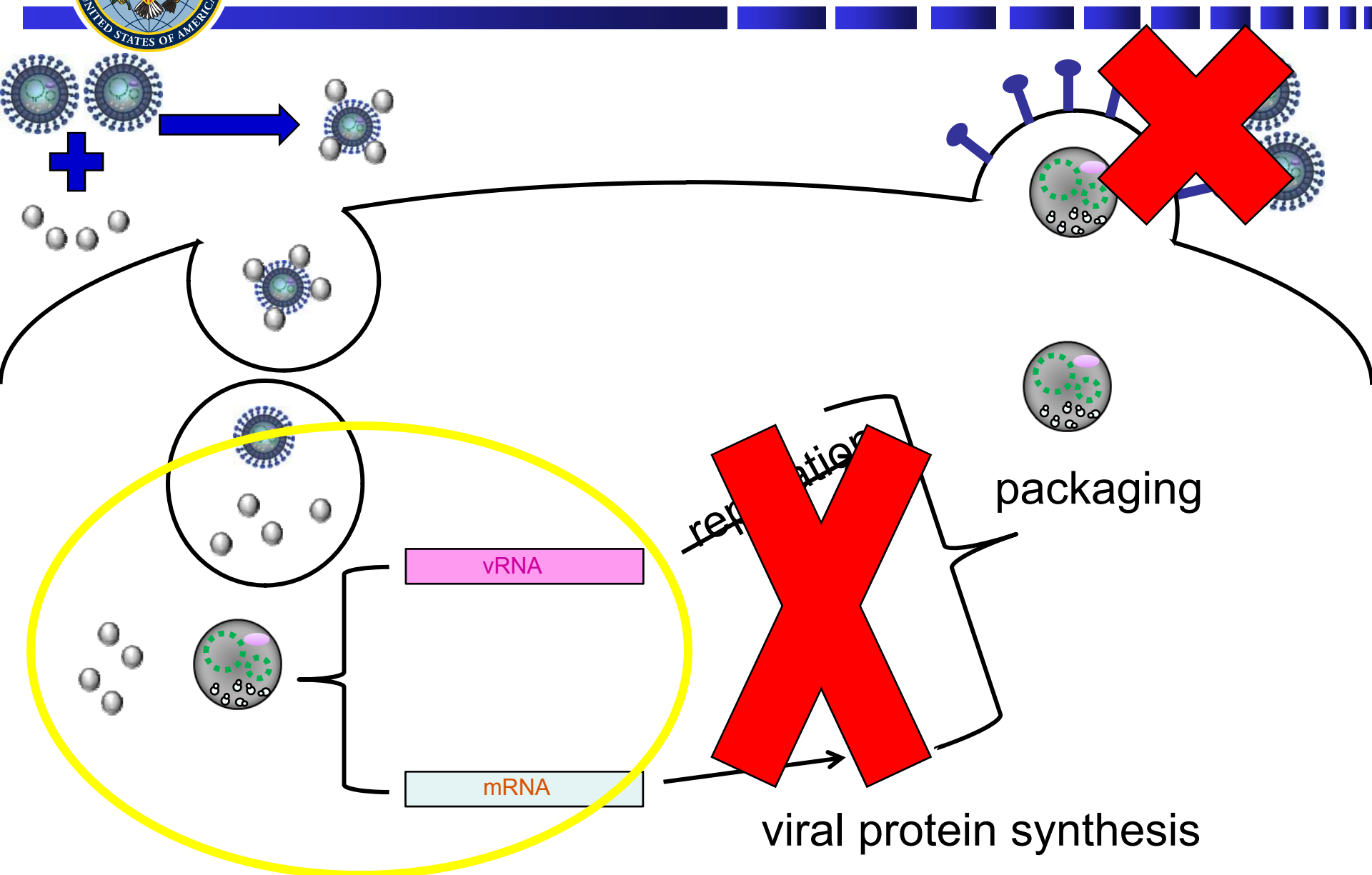
# Nucleoprotein RNA Expression

## N Protein Gene Expression





# Mechanism of Ag-NP Inhibition





# Filovirus

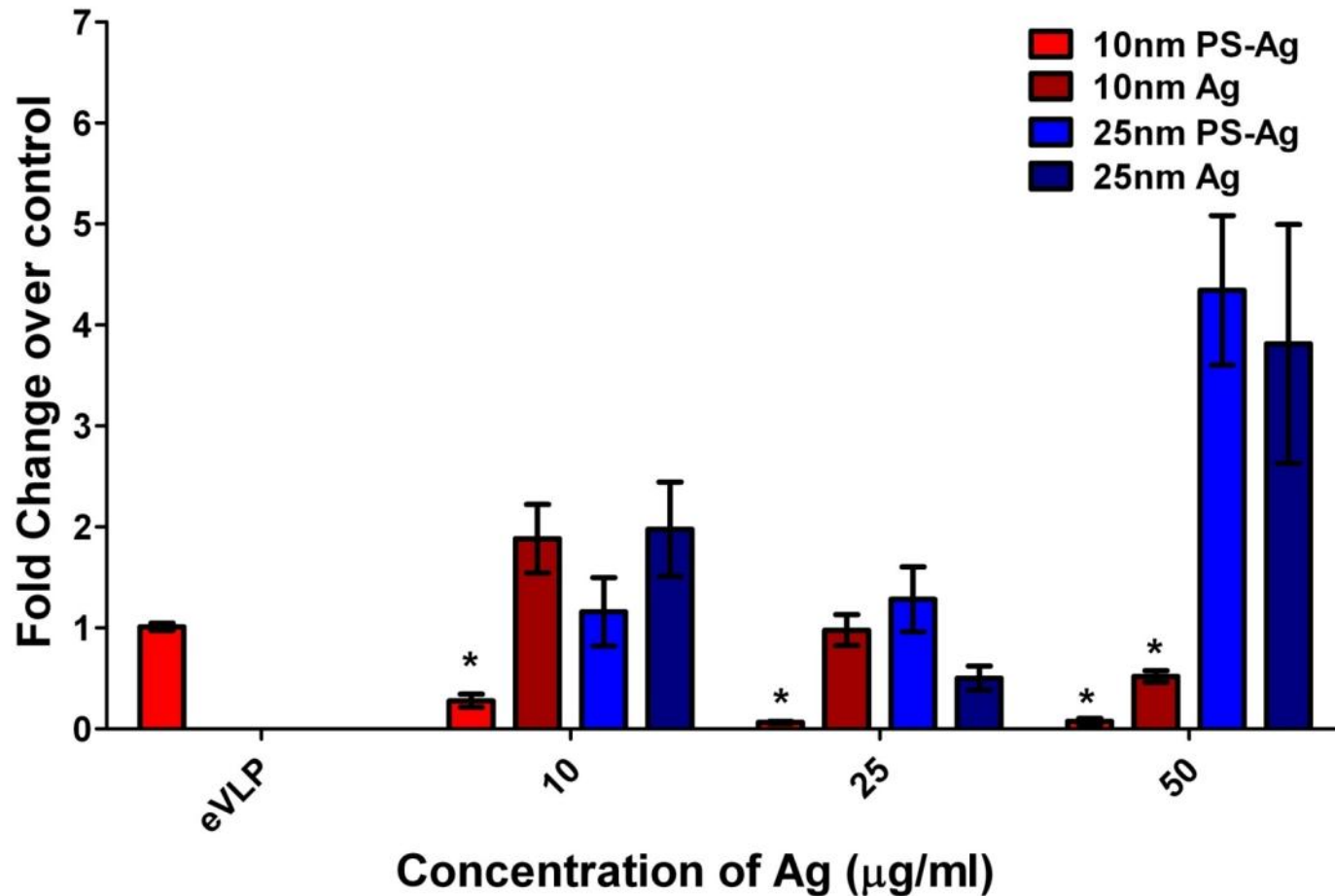
- qRT-PCR detection of internalized eVLPs using Gp as a marker
- Confocal Microscopy of eVLP cell surface binding
- Confocal Microscopy of eVLP internalization
- Cathepsin B and L activity in Vero cells.





# Ebola Virus-Like Particles

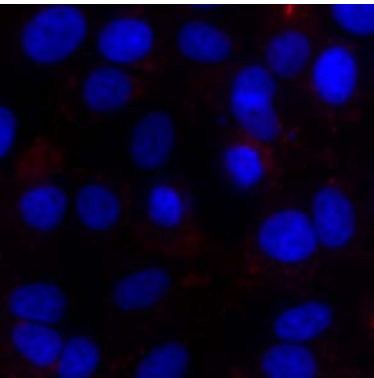
## eVLP binding to Vero cells +/- Ag-NP



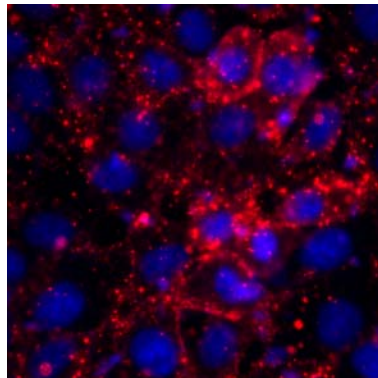


# Cell Surface eVLP Expression

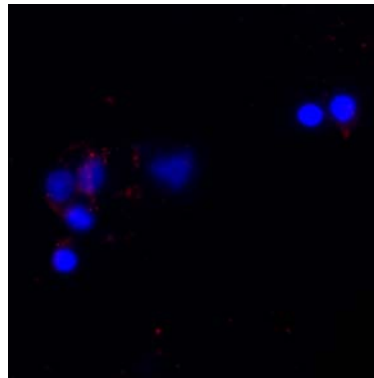
Vero cells  
(negative control)



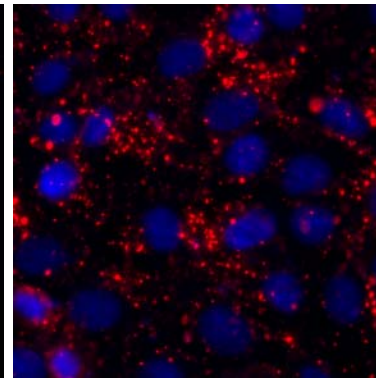
10nm uncoated  
Ag 10 $\mu$ g/ml



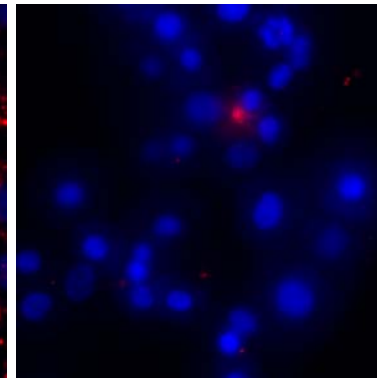
10nm uncoated  
Ag 50 $\mu$ g/ml



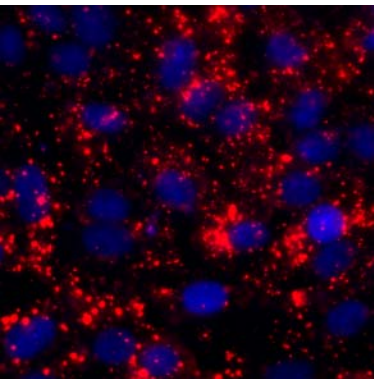
10nm PS-coated  
Ag 10 $\mu$ g/ml



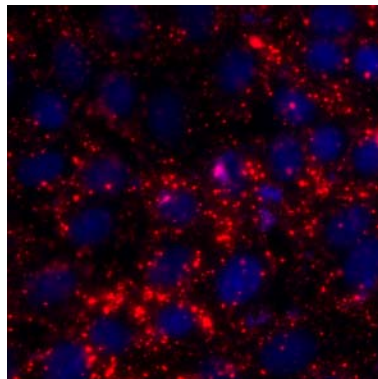
10nm PS-coated  
Ag 50 $\mu$ g/ml



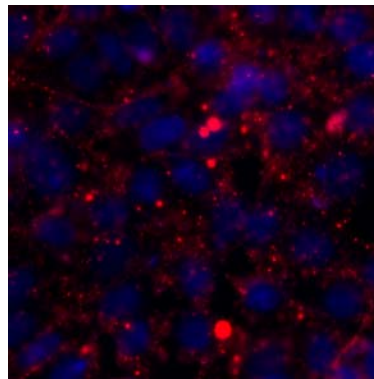
eVLPs  
(positive control)



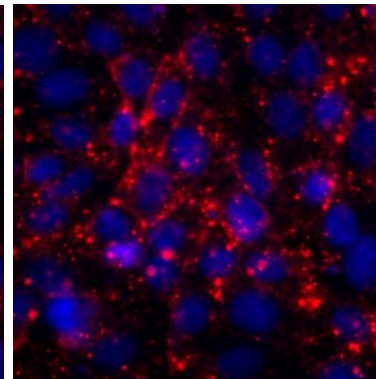
25nm uncoated  
Ag 10 $\mu$ g/ml



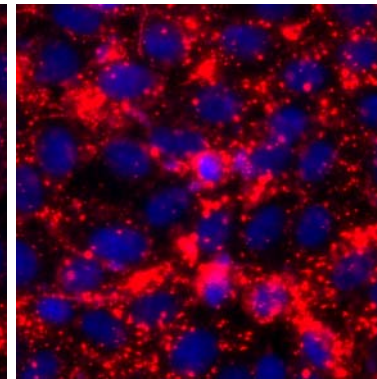
25nm uncoated  
Ag 50 $\mu$ g/ml



25nm PS-coated  
Ag 10 $\mu$ g/ml



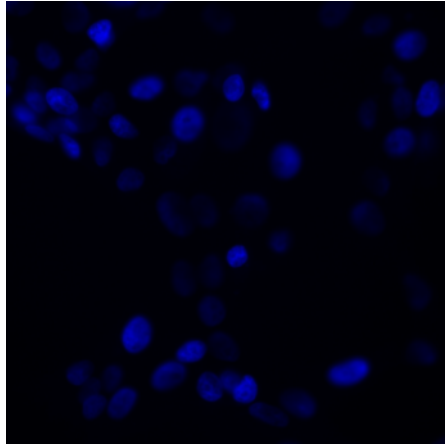
25nm PS-coated  
Ag 50 $\mu$ g/ml



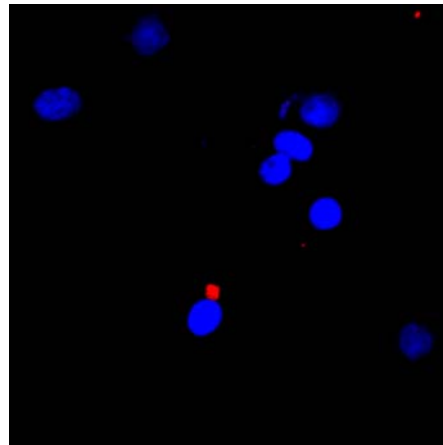


# eVLP Internalization into Vero Cells

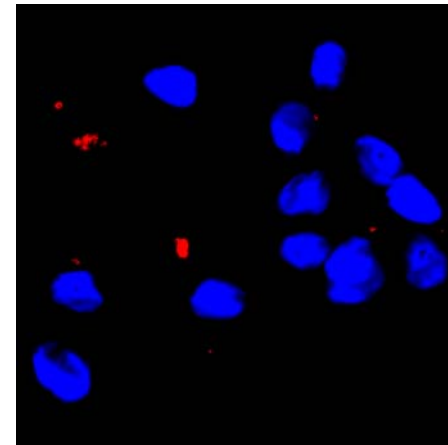
Negative Control



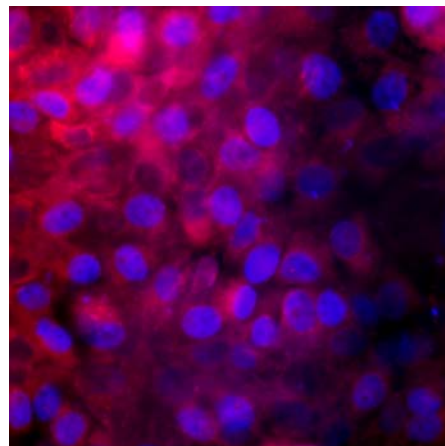
10nm 10 $\mu$ g



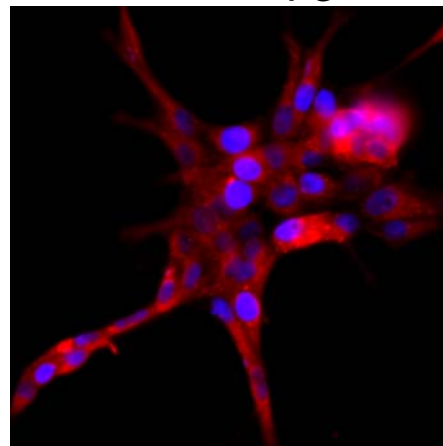
10nm 50 $\mu$ g



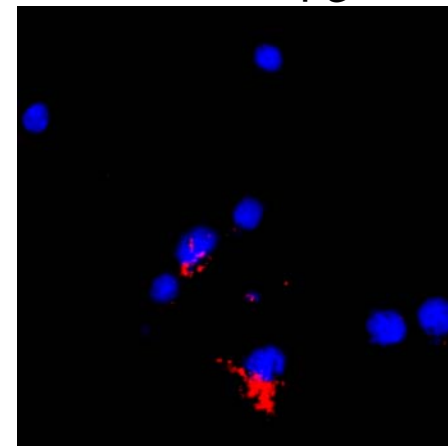
Positive Control



25nm 10 $\mu$ g



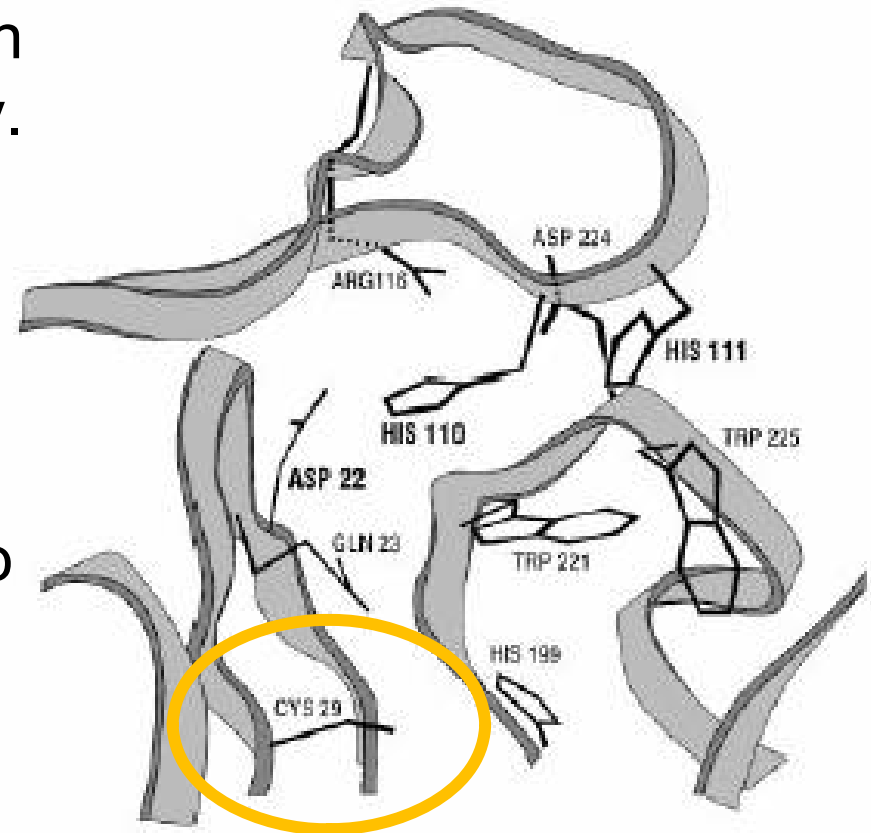
25nm 50 $\mu$ g





# Cathepsin Activity

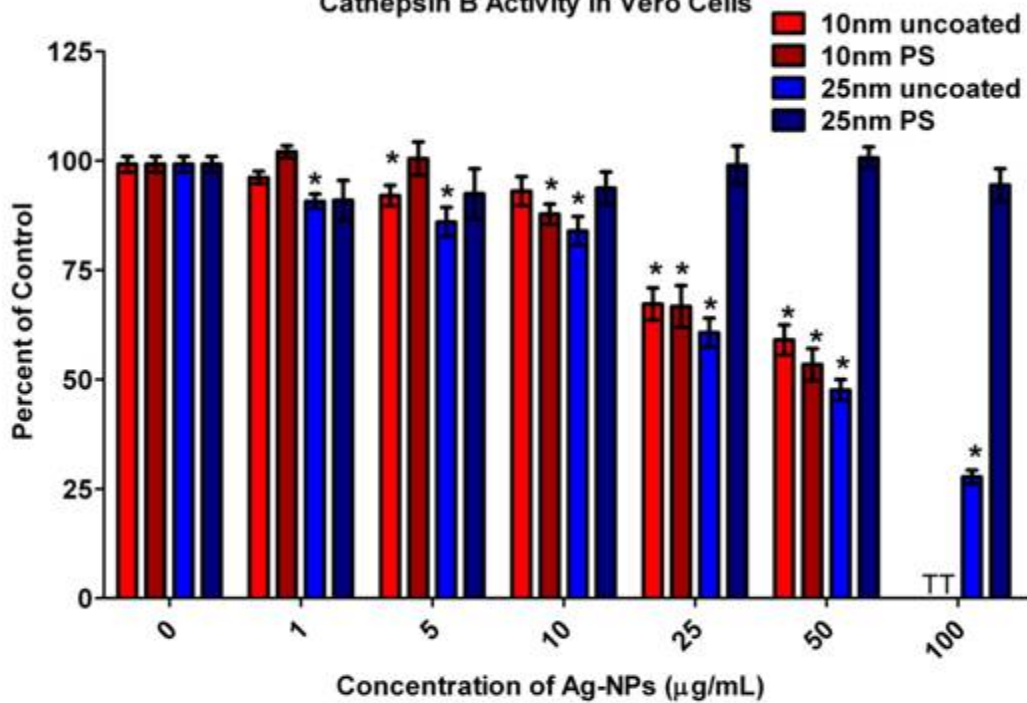
- Bulk and Nano Silver have been shown to inhibit enzyme activity.
- Silver binds readily to thiol groups.
- Cathepsin B has been shown to have an essential role in Ebola virus replication.
- Cathepsin L has an accessory role in Ebola virus replication.



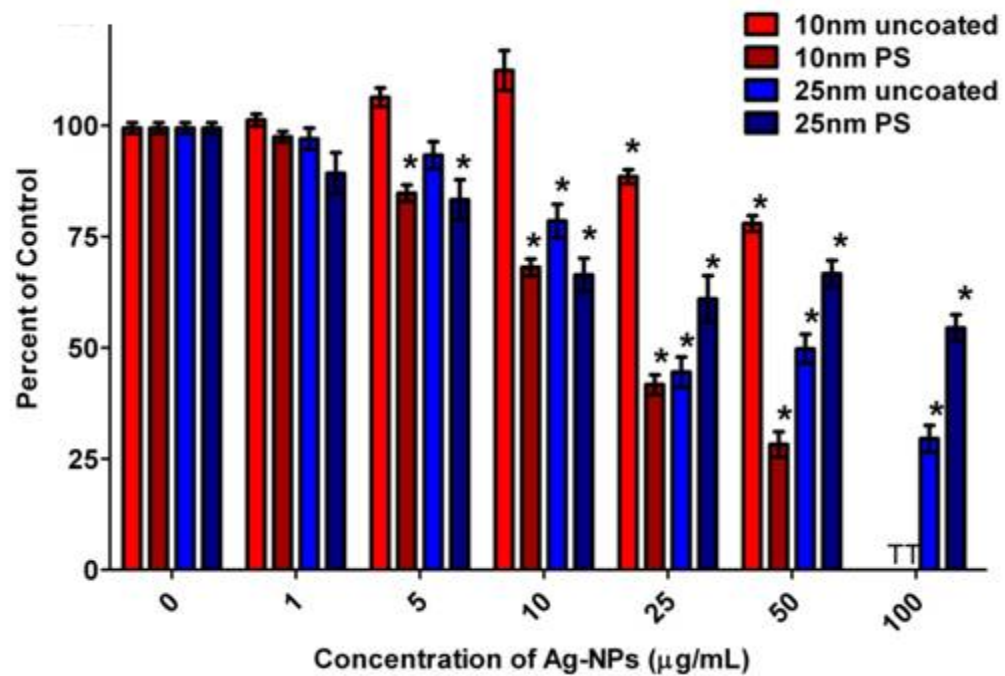
Cathepsin B

*L. Jayashankar, Acharya Nagarjuna University, Guntur*

Cathepsin B Activity in Vero Cells



Cathepsin L Activity in Vero Cells





# Conclusions

- Ag-NPs neutralize TCRV infection
  - Decrease in S segment gene expression
  - Decrease in progeny virus production
- Ag-NPs do not prevent the internalization of TCRV
  - Ag-NPs and TCRV interact inside the cell
  - Mechanism of inhibition occurs between endocytosis and vRNA gene production
- Ag-NPs have a similar effect on eVLPs
- Ag-NPs decrease cathepsin activity





# Acknowledgements



Dr. Laura Braydich-Stolle, Craig Murdock, Eric Szymanski  
Dr. Amanda Schrand – not pictured

## AFRL/RHPB BIN Group

### Nanoparticles

- **Dr. Karl Martin** (Novacentrix, Austin, TX)
- **Dr. Steven Oldenburg** (NanoComposix, San Diego, CA)
- **Dr. Dan Goia** (Clarkson University, Center for Advanced Materials Processing, Potsdam, NY)

### Ebola virus-like particles

- **Dr. Kelly Warfield** (USAMRIID)

- Dr. Schlager (AFRL/RHPB) and Col. Reilly (AFRL/RH)
- Funding: DTRA (proposal #4.10036\_07\_AHB\_B) & JSTO/DTRA – NRC Postdoctoral Fellowship Program (contract #F49620-02-C-0015)



# *Comments/Questions*