



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

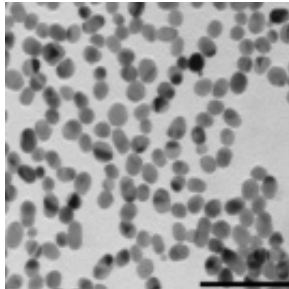
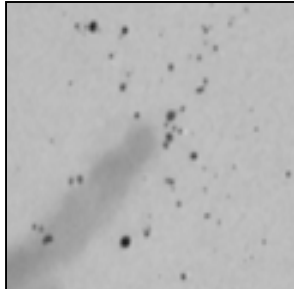
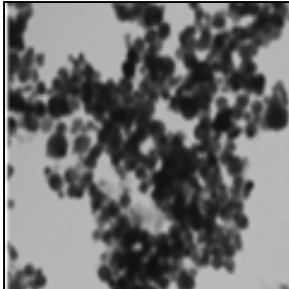

- Biosensors
- Antimicrobial Agents
- Munitions
- Propellants
- Coatings
- Smart Suits

Challenges

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



Silver Nanoparticles

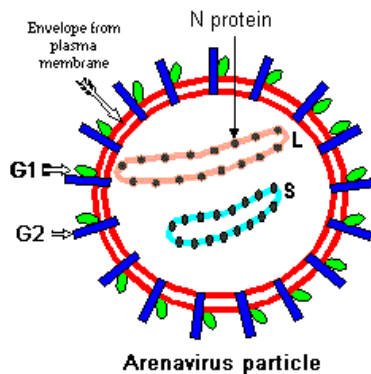
	UNCOATED	POLYSACCHARIDE COATED
10 nm	 <p>12.78 ± 0.13 Dr. Steve Oldenburg, NanoComposix</p>	 <p>9.48 ± 4.286 Dr. Dan Goia, Clarkston University</p>
25 nm	 <p>27.474 ± 9.062 Dr. Karl Martin, Novacentrix</p>	 <p>25.98 ± 8.38 Dr. Dan Goia, Clarkston University</p>



Hemorrhagic Fever Viruses

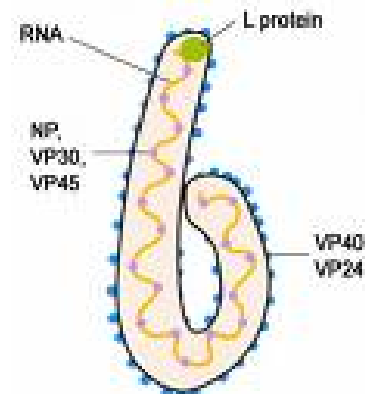
Arenaviridae

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



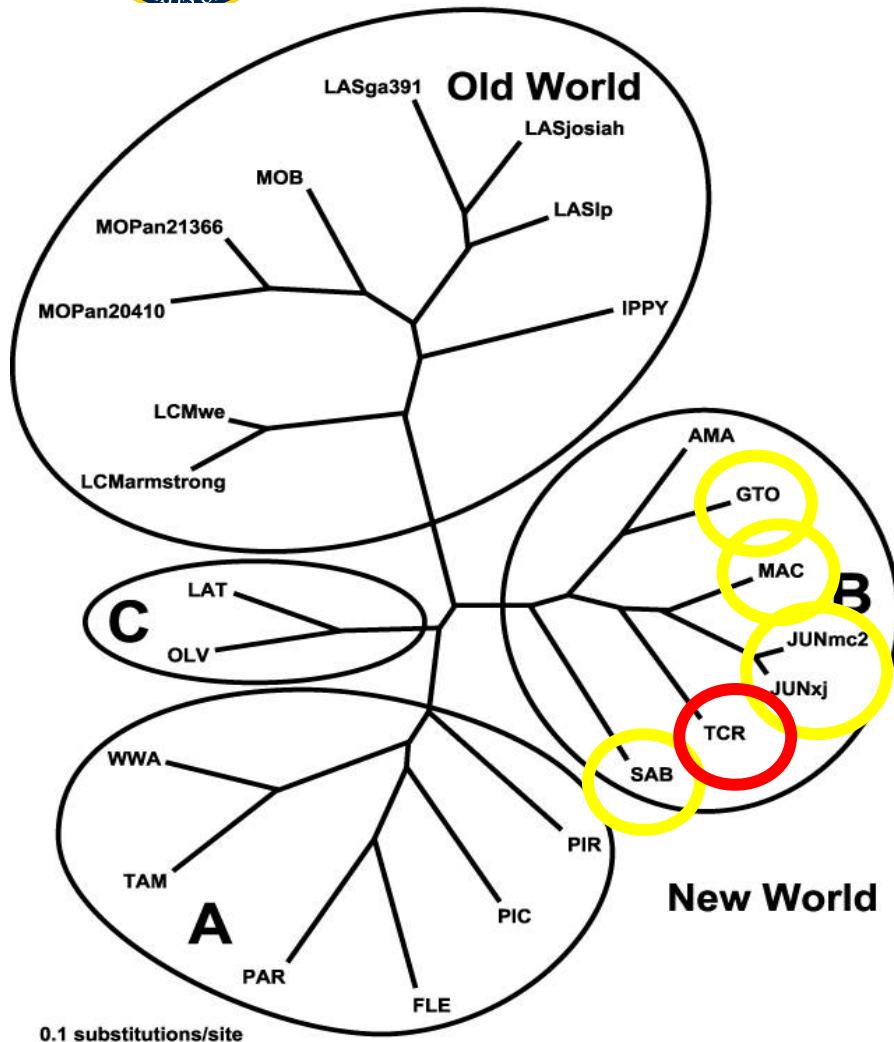
Filoviridae

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





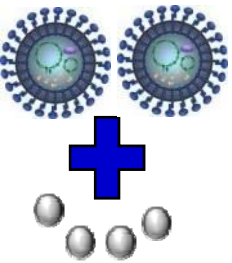
Tacaribe Virus



- 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
TCID₅₀ determination

0 hour

1 h.p.i



4 h.p.i
Confocal Microscopy
Virus internalization

4 d.p.i
qRT-PCR

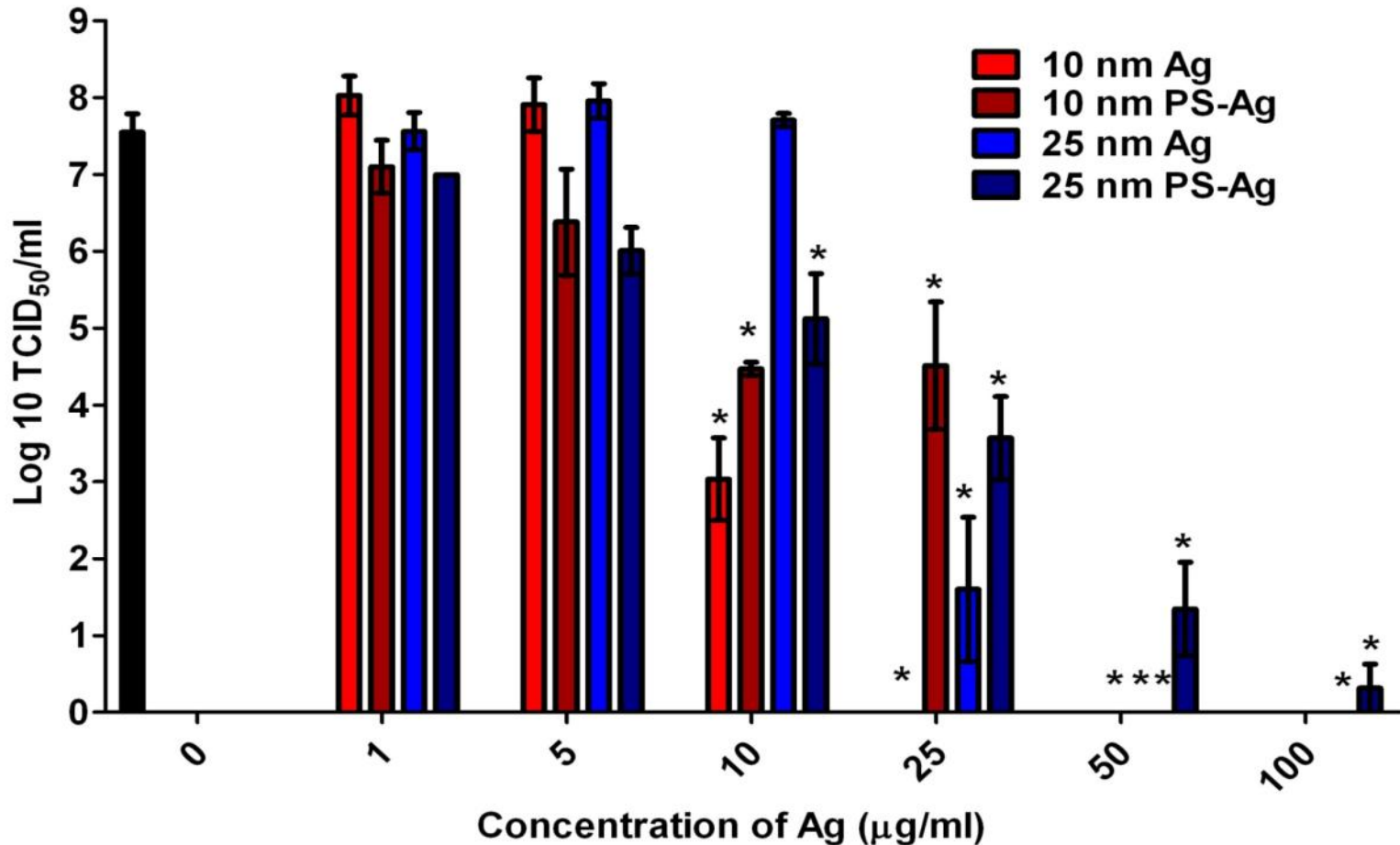


Vero cells



TCRV Progeny Virus Production

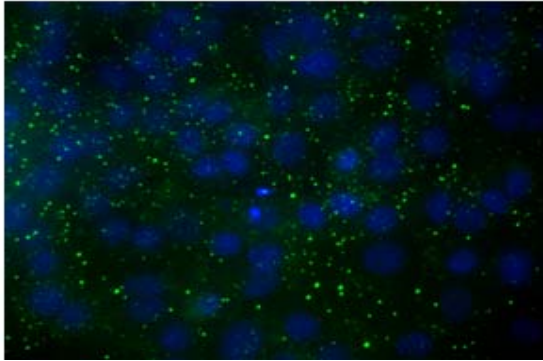
Tacaribe Virus Neutralization by Silver Nanoparticles



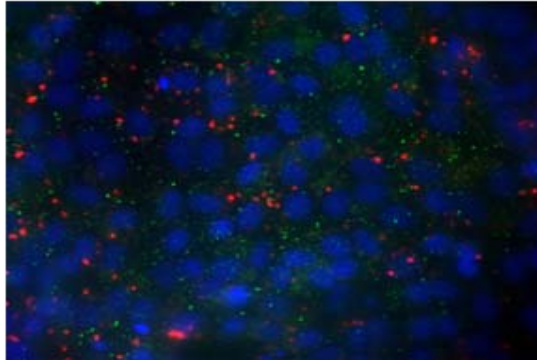


Cell Surface TCRV Expression

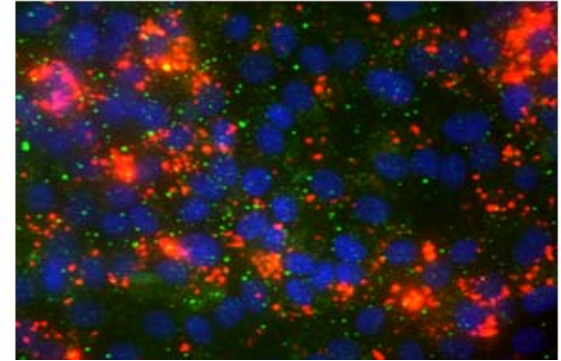
Negative Control



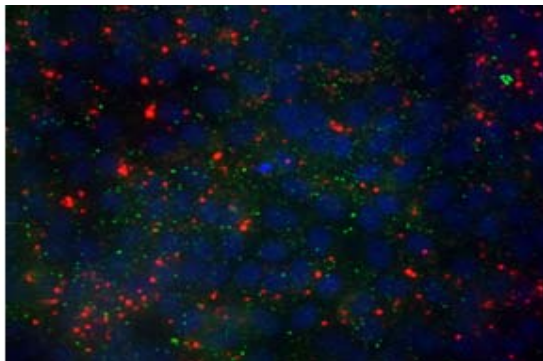
10 nm 50 $\mu\text{g/ml}$



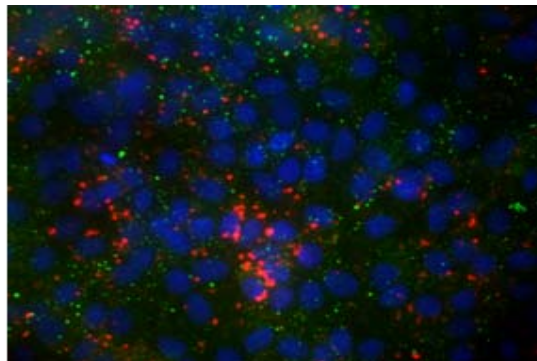
25 nm 50 $\mu\text{g/ml}$



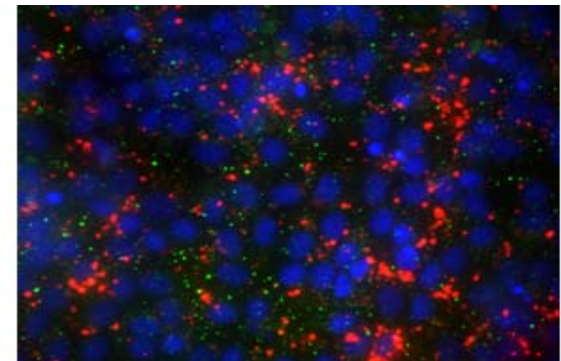
PositiveControl



10 nm 10 $\mu\text{g/ml}$

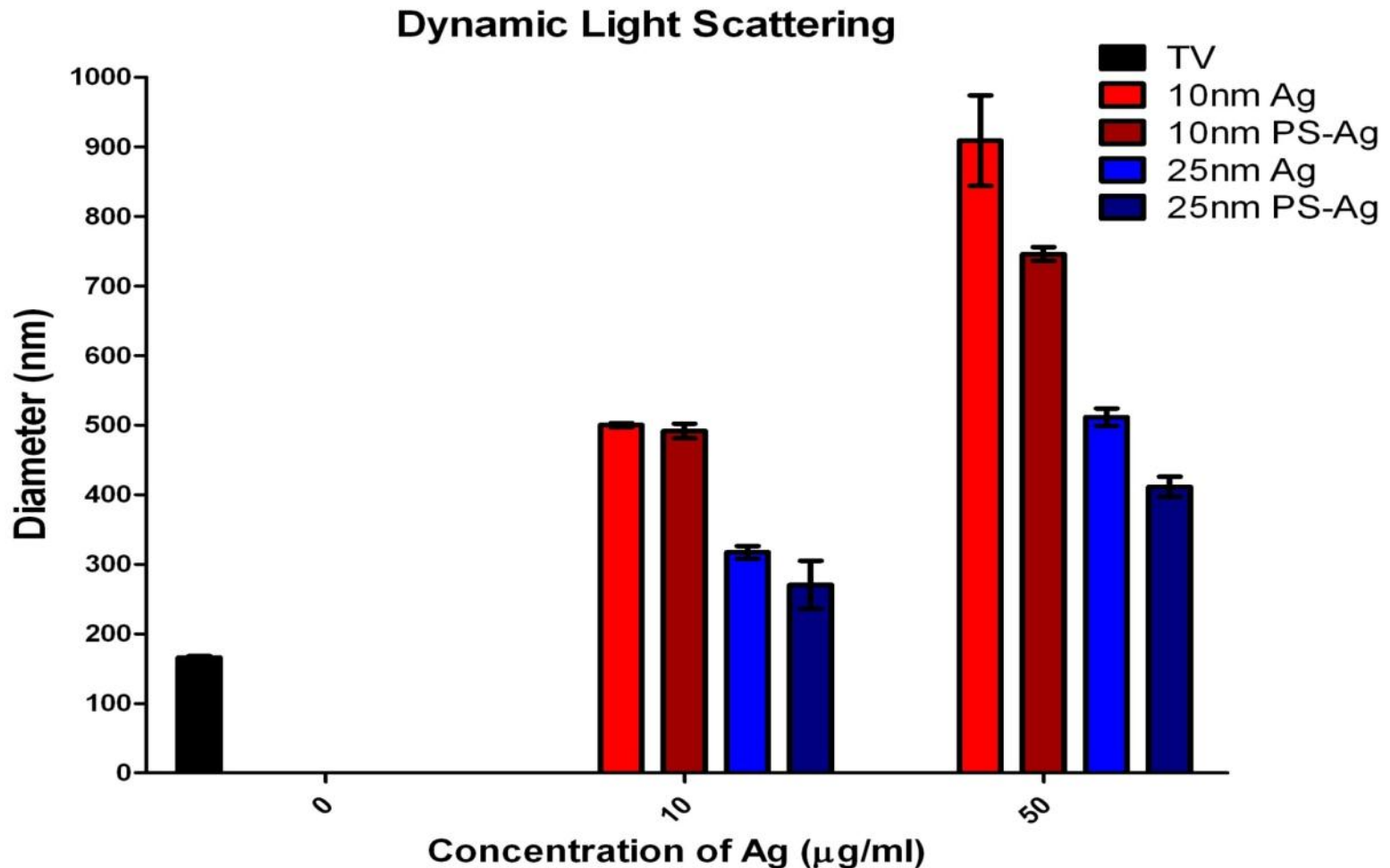


25 nm 10 $\mu\text{g/ml}$





Dynamic Light Scattering





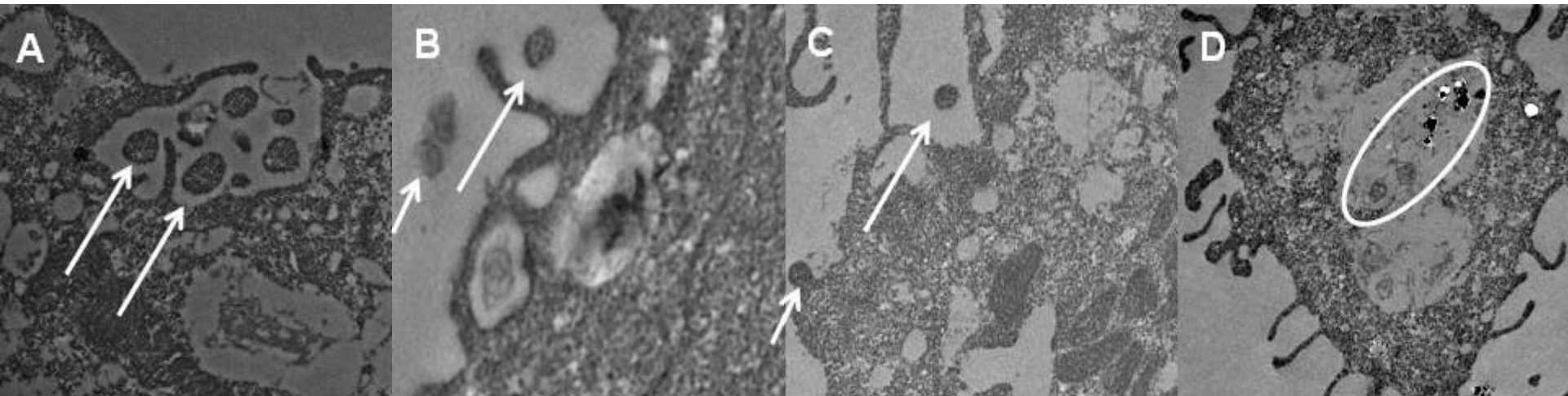
TCRV Internalization into Vero Cells

Untreated TCRV

TCRV + 10nm Ag

TCRV + 25nm Ag

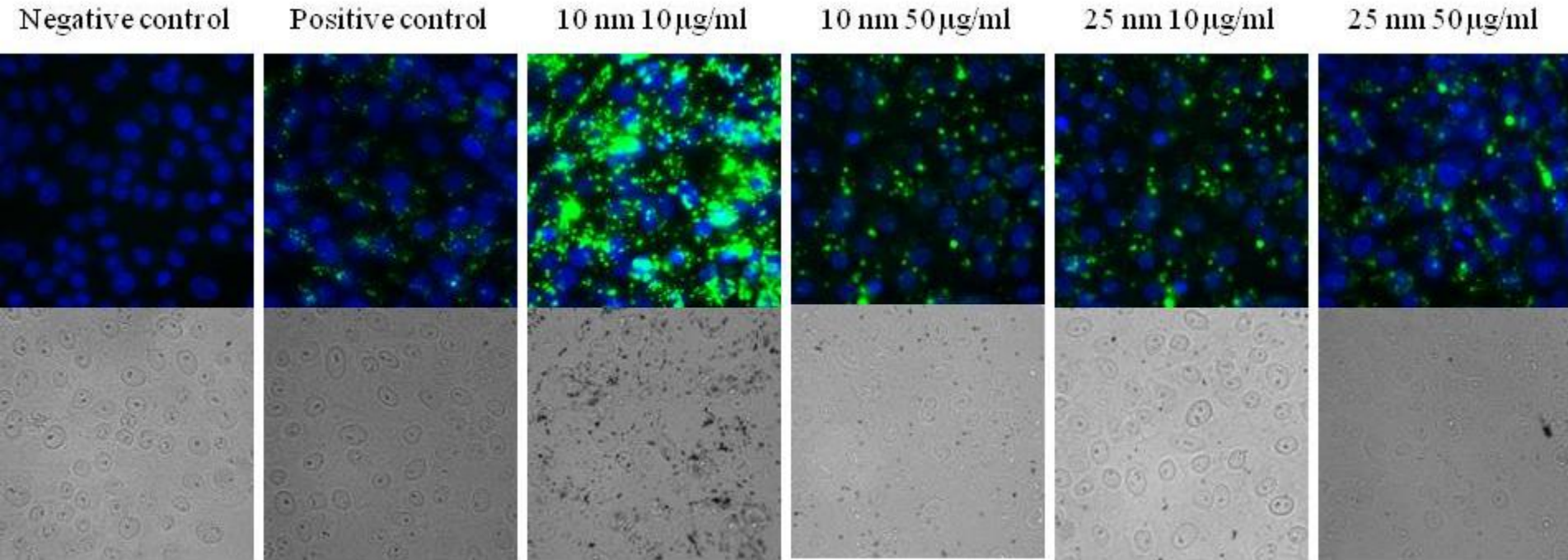
TCRV + 25nm Ag



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



TCRV Internalization into Vero Cells

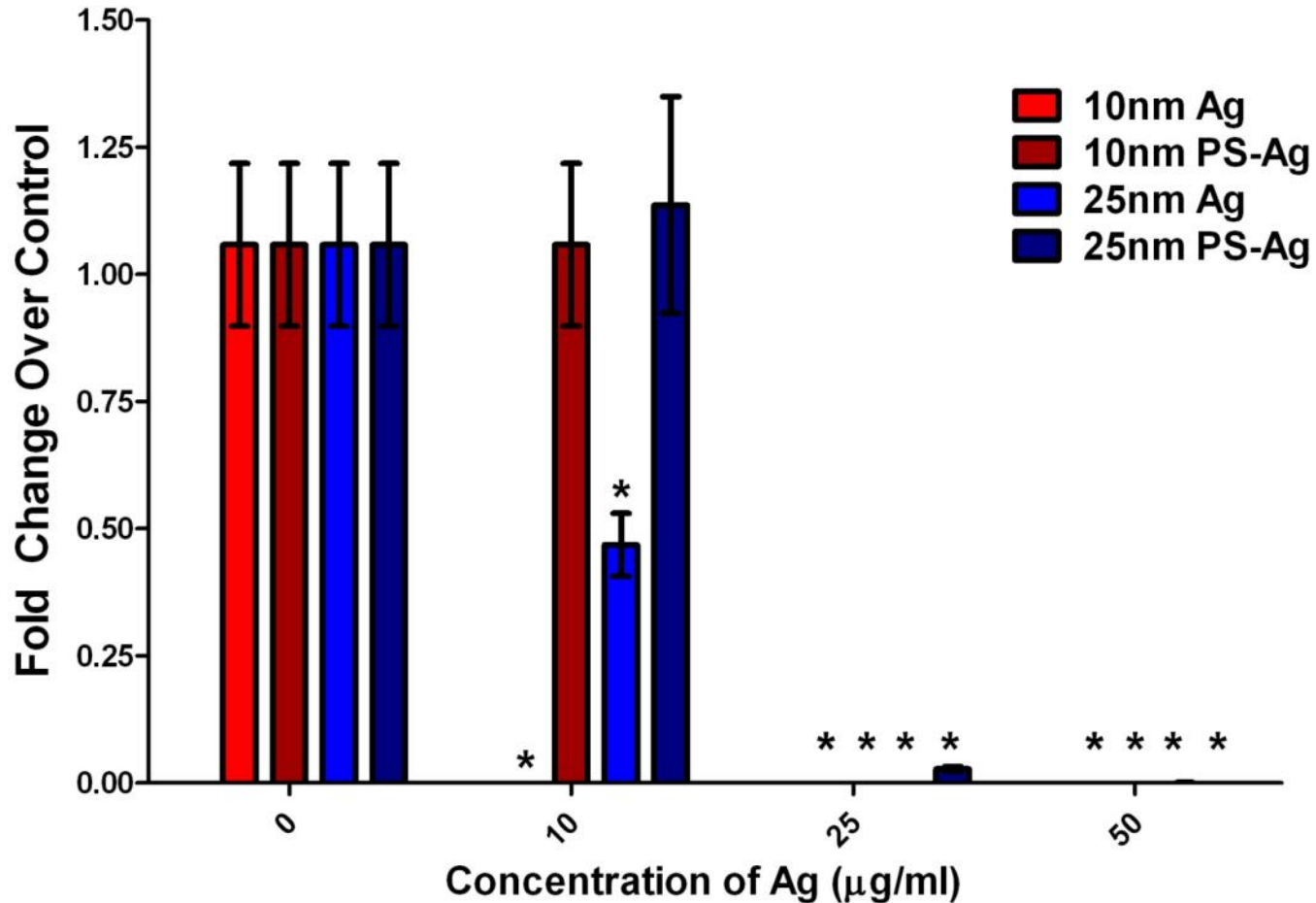


- Ag-NPs facilitate uptake of TCRV into Vero cells



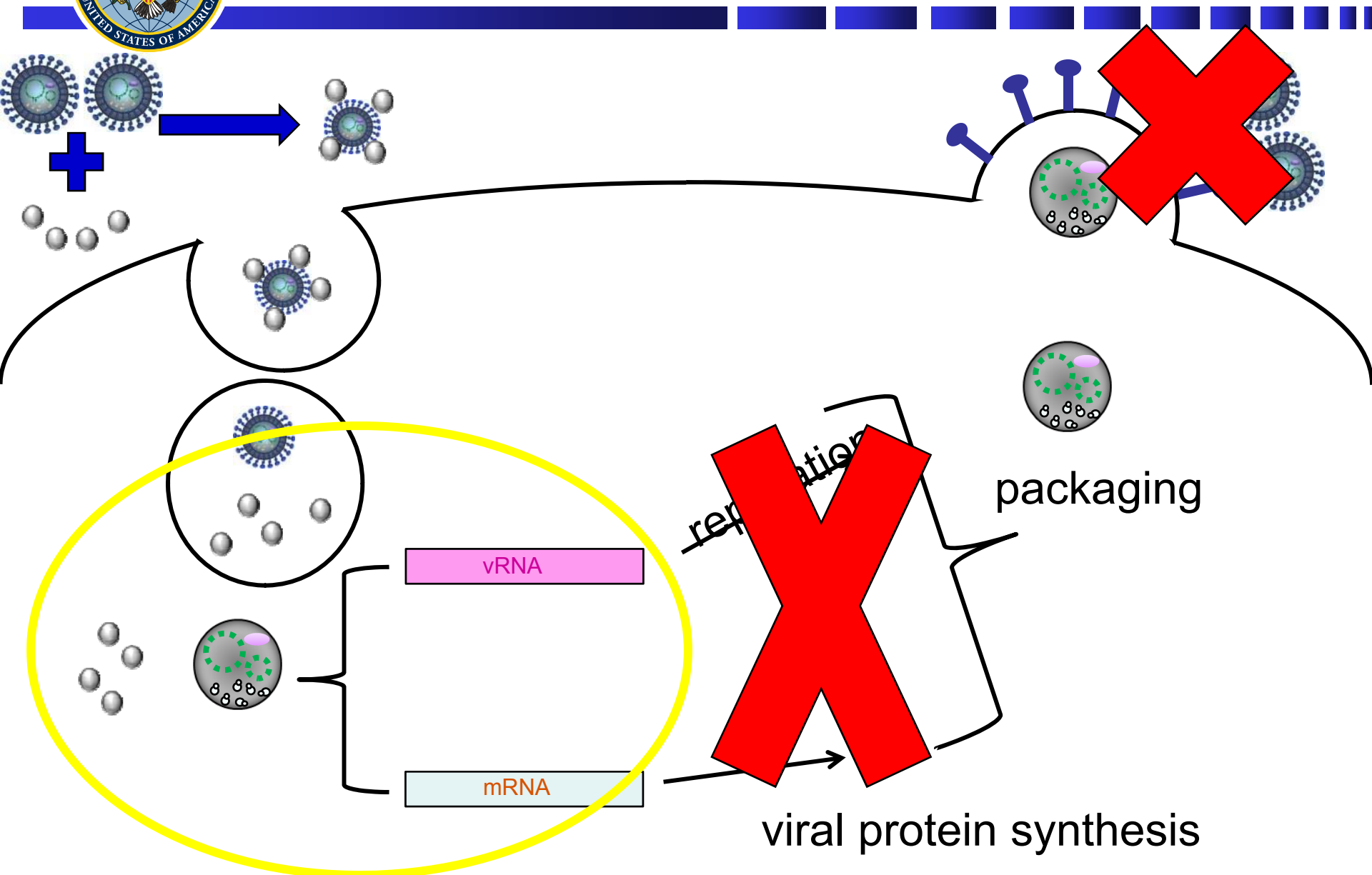
Nucleoprotein RNA Expression

N Protein Gene Expression





Mechanism of Ag-NP Inhibition





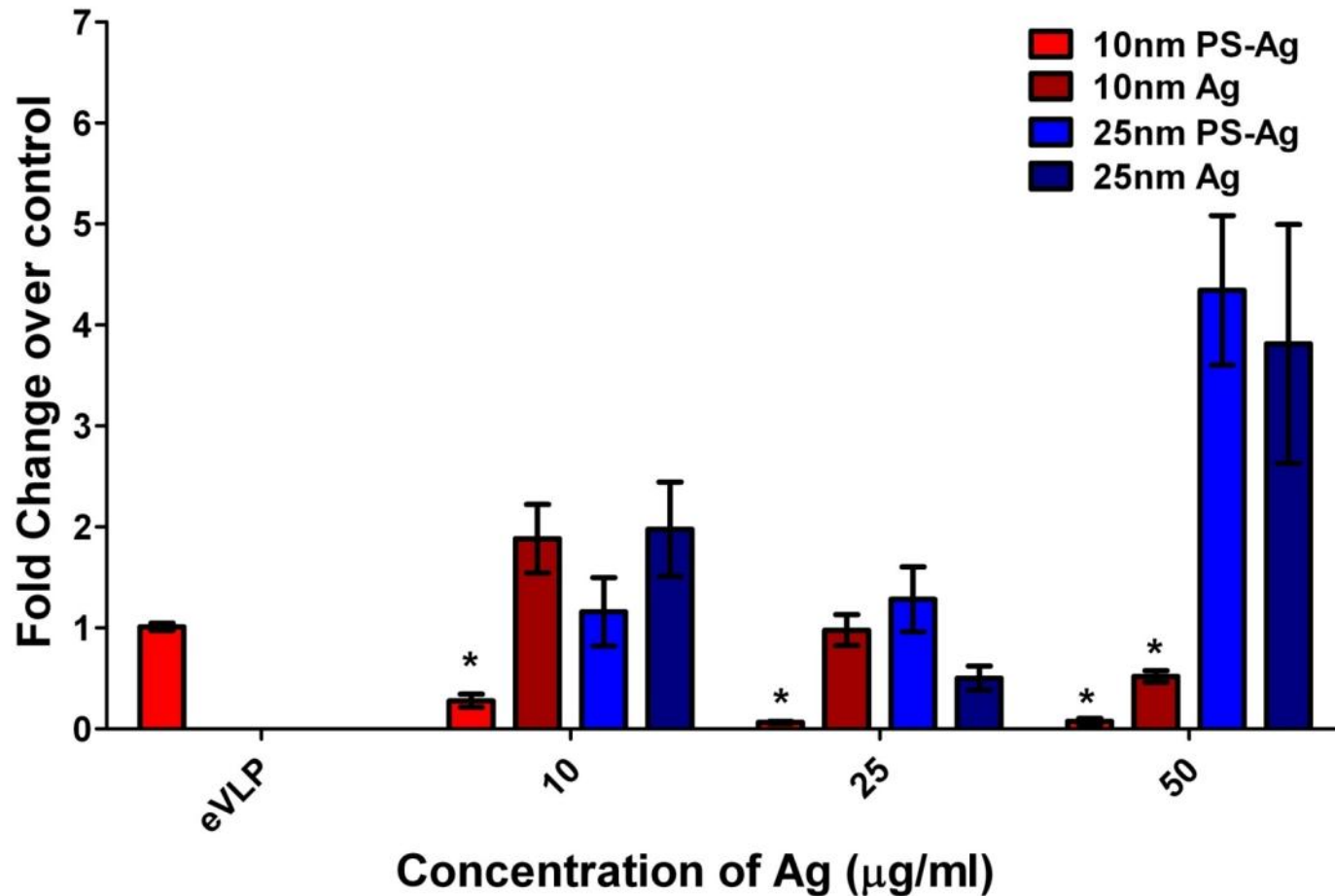
Filovirus

- 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

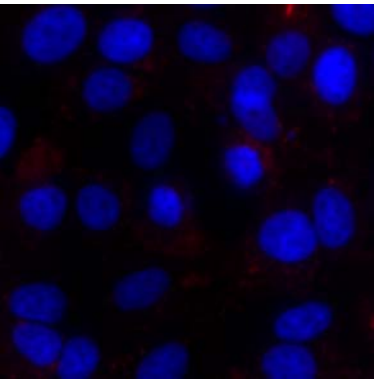
eVLP binding to Vero cells +/- Ag-NP



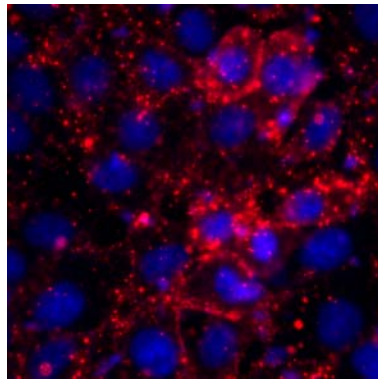


Cell Surface eVLP Expression

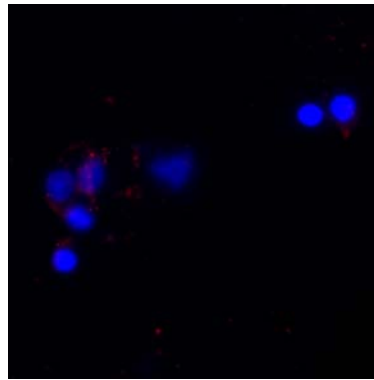
Vero cells
(negative control)



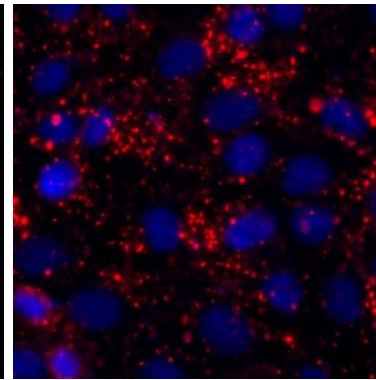
10nm uncoated
Ag 10 μ g/ml



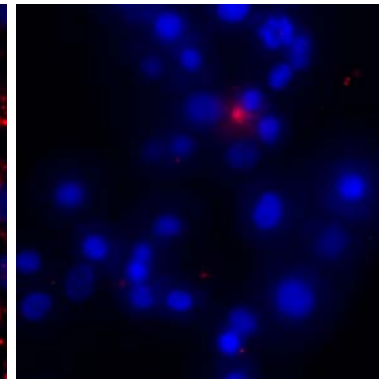
10nm uncoated
Ag 50 μ g/ml



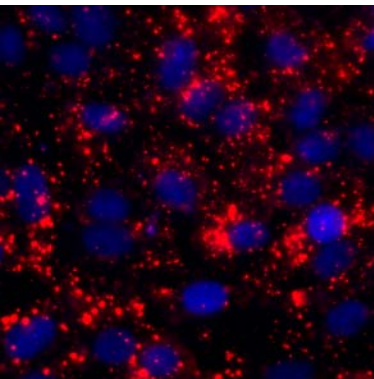
10nm PS-coated
Ag 10 μ g/ml



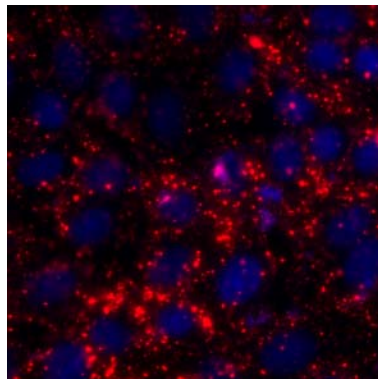
10nm PS-coated
Ag 50 μ g/ml



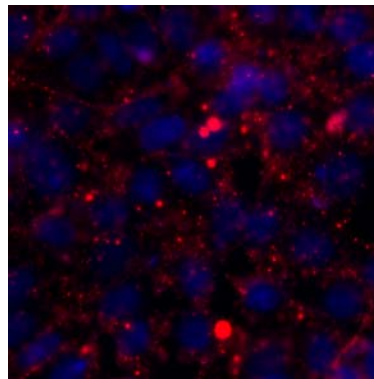
eVLPs
(positive control)



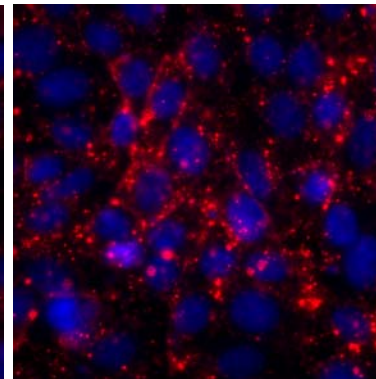
25nm uncoated
Ag 10 μ g/ml



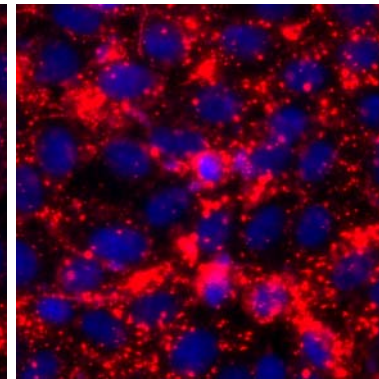
25nm uncoated
Ag 50 μ g/ml



25nm PS-coated
Ag 10 μ g/ml



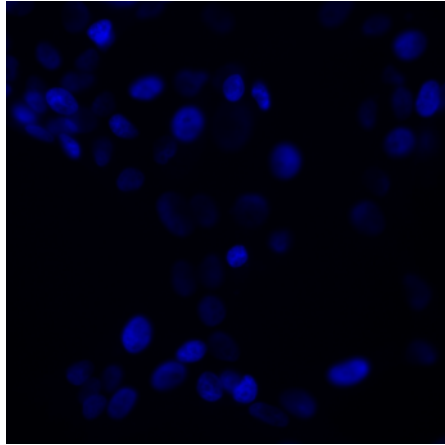
25nm PS-coated
Ag 50 μ g/ml



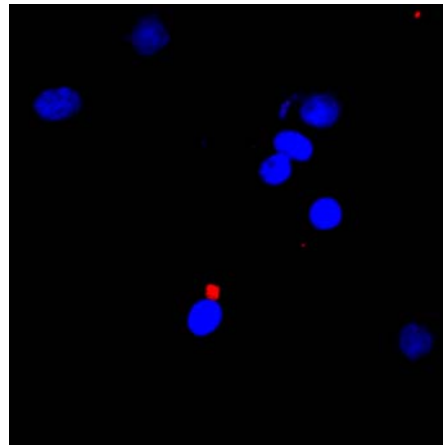


eVLP Internalization into Vero Cells

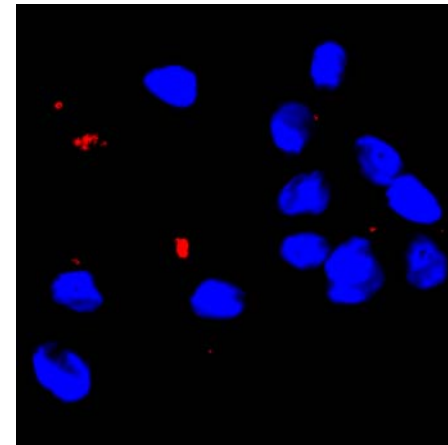
Negative Control



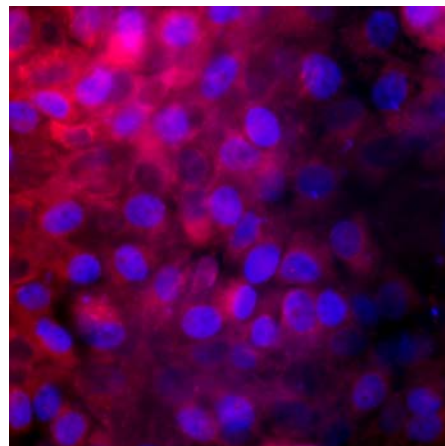
10nm 10 μ g



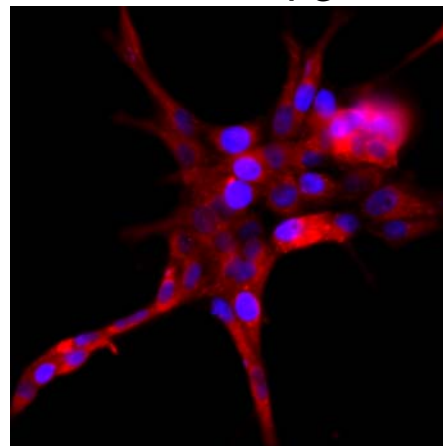
10nm 50 μ g



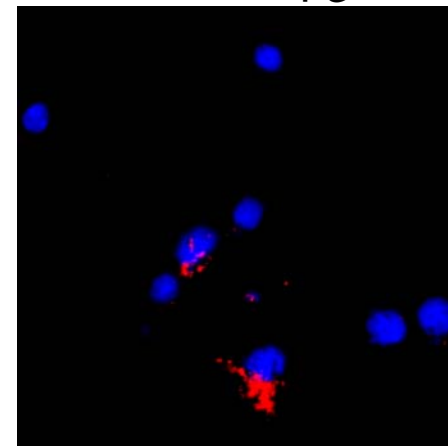
Positive Control



25nm 10 μ g



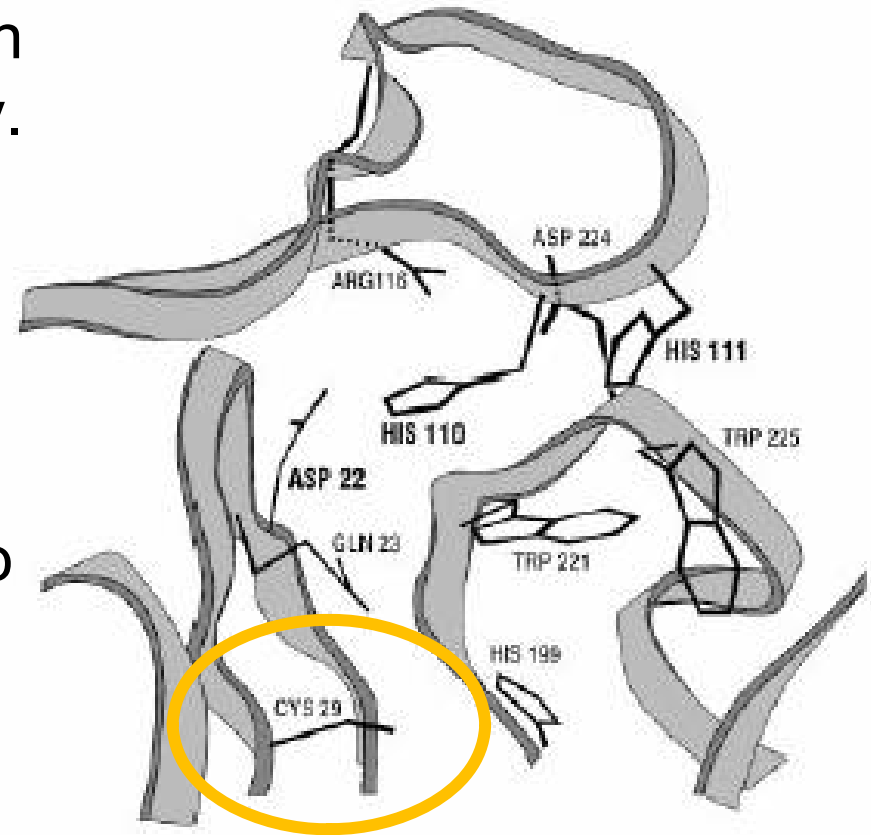
25nm 50 μ g





Cathepsin Activity

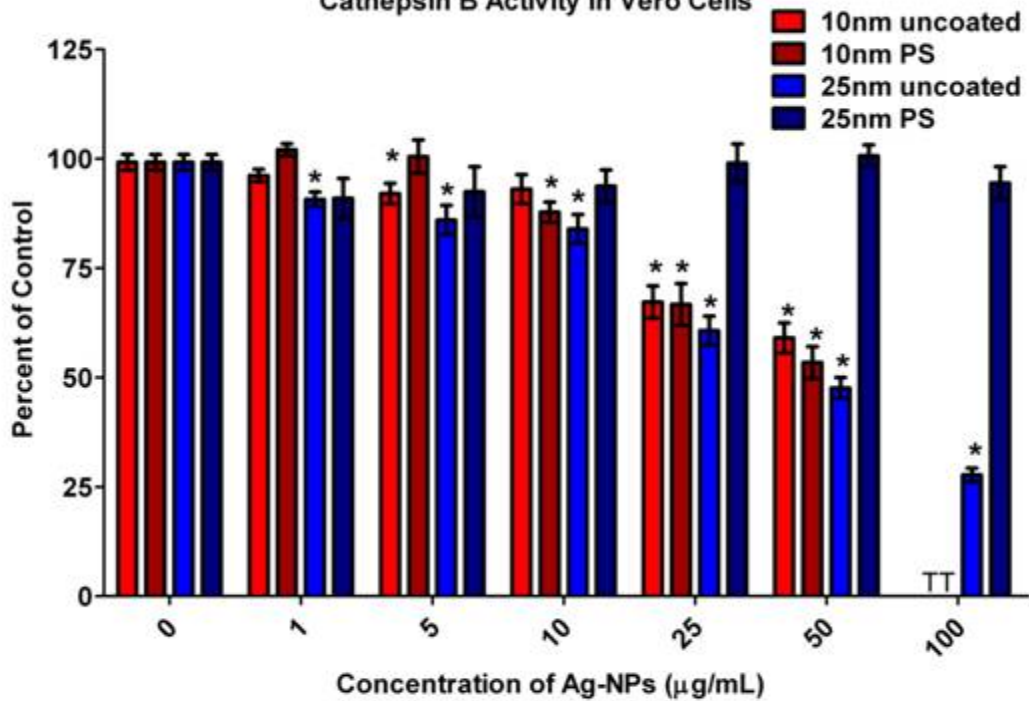
- Bulk and Nano Silver have been shown to inhibit enzyme activity.
- Silver binds readily to thiol groups.
- Cathepsin B has been shown to have an essential role in Ebola virus replication.
- Cathepsin L has an accessory role in Ebola virus replication.



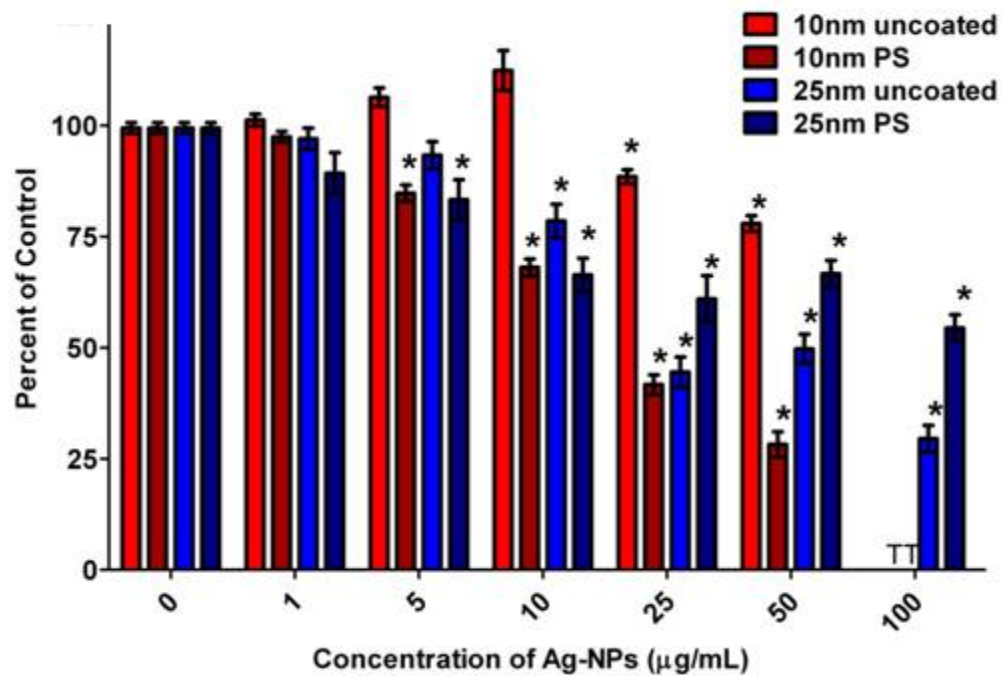
Cathepsin B

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