An ELISA for studying Ebola Virus / VLP release

George Kallstrom, M. Javad Aman, Sina Bavari
Sina Bavari Lab, USAMRIID
Ft. Detrick, MD

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Introduction/Agenda

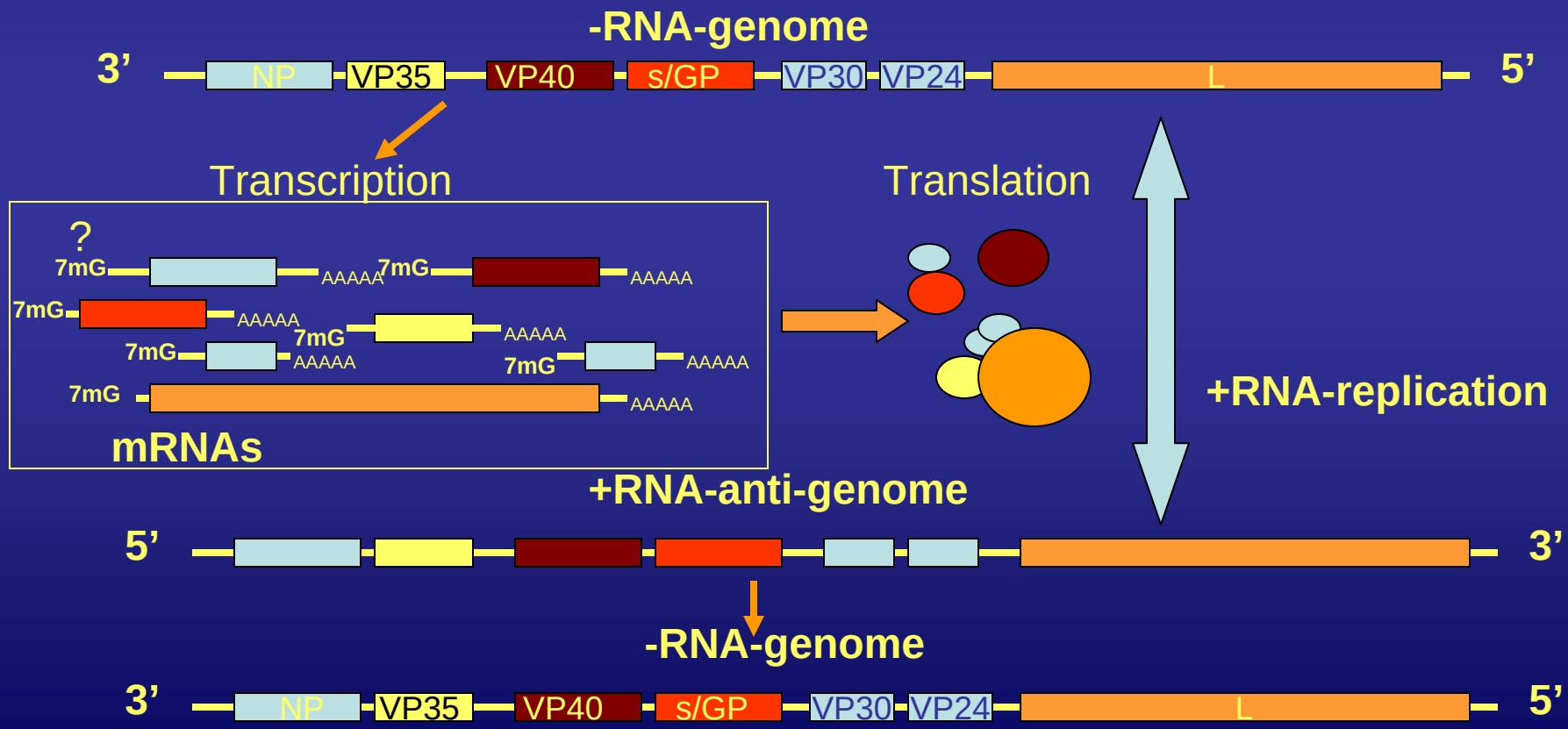
- Filovirus Background
  - Replication
  - Structure
  - VP40
- ELISA setup and optimization
- Effects of Ebola proteins on VLP release
- Effects of VP40 mutants on VLP release
- Therapeutic compound screening

Filoviruses

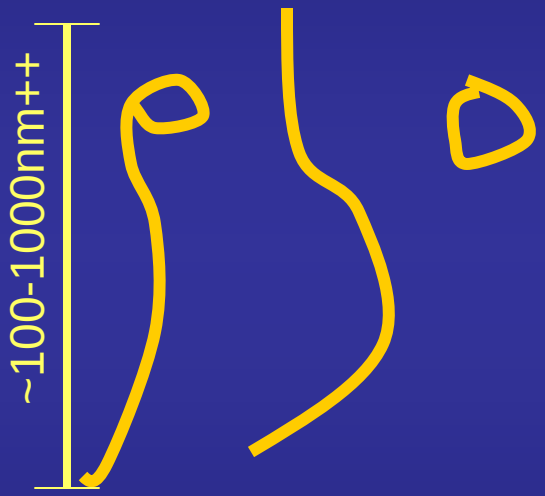
- *Mononegavirales, Filoviridae-2* species in family (Marburg and Ebola)
- Both clinically manifest as a rapidly progressive hemorrhagic fever with high mortality rates
- Marburg was weaponized and stockpiled by the USSR
- Sporadic outbreaks have been on the rise in Africa over the past several years
- Unknown Reservoir

Viral Replication

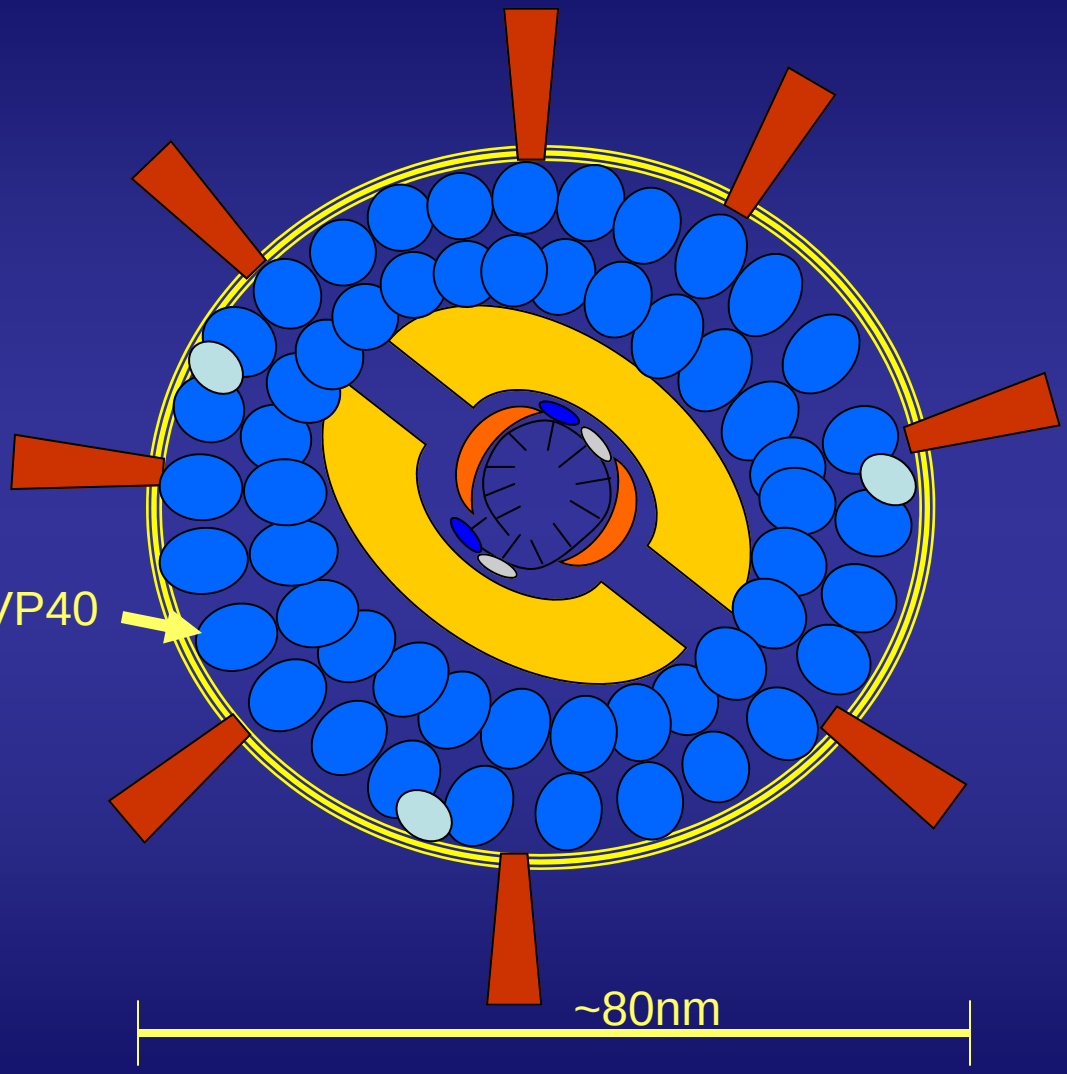
- Single strand negative sense RNA genome
- 7 genes, single segment ~19,000bp



Filovirus Structure

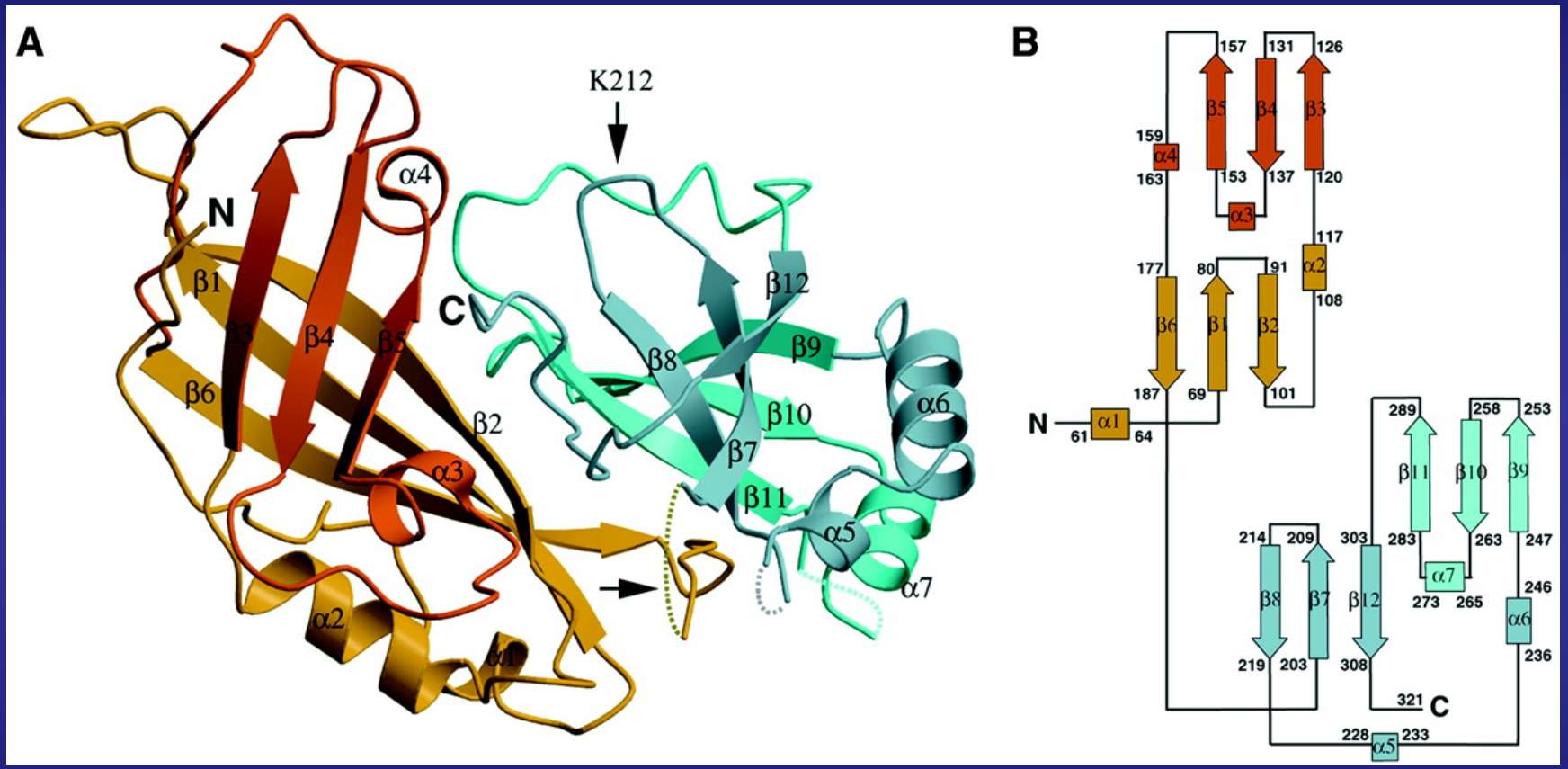


Longitudinal 2D Filamentous Particles



Transverse 2D section

VP40-Matrix Protein



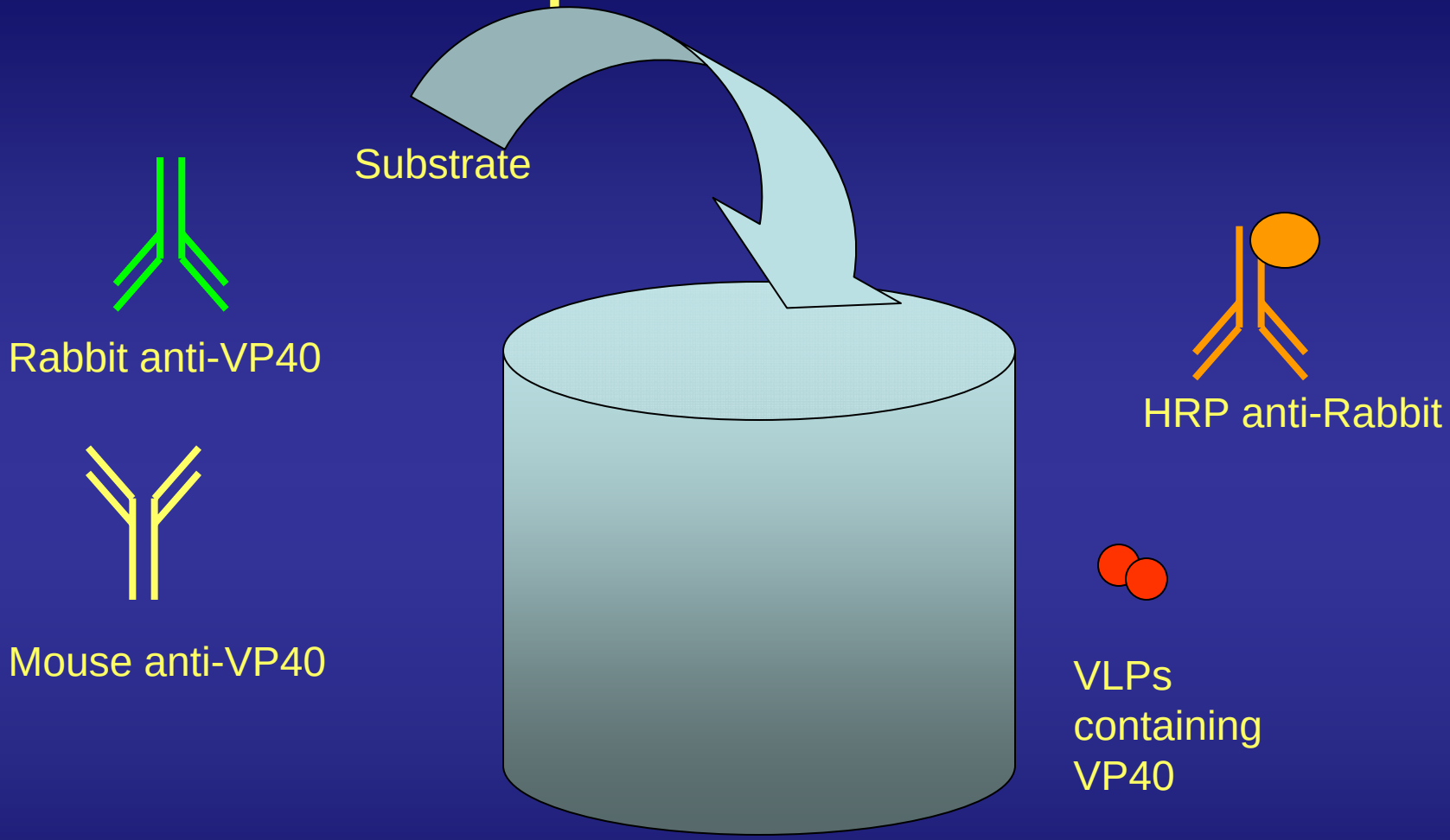
*Exists in both monomeric and oligomeric conformations

Dessen et al, 2000 EMBO Journal

Objectives

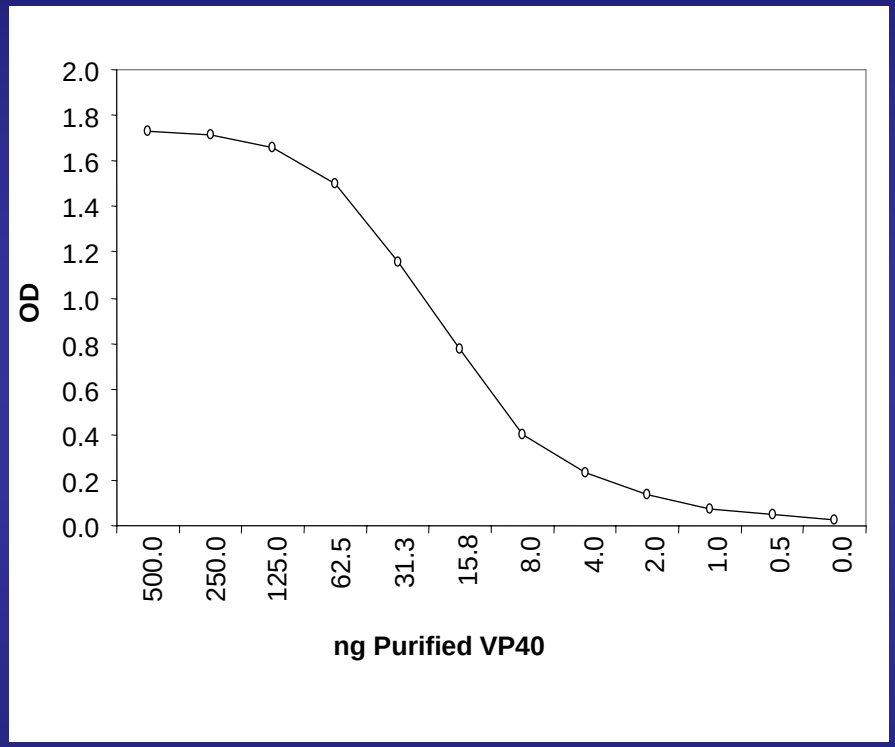
- Develop an assay that measures Ebola virus / VLP release
- Study the effects of Ebola proteins on VLP production
- Determine the regions of VP40 that are important for viral release
- Screen for potential anti-viral therapeutic compounds able to inhibit viral release

Capture ELISA

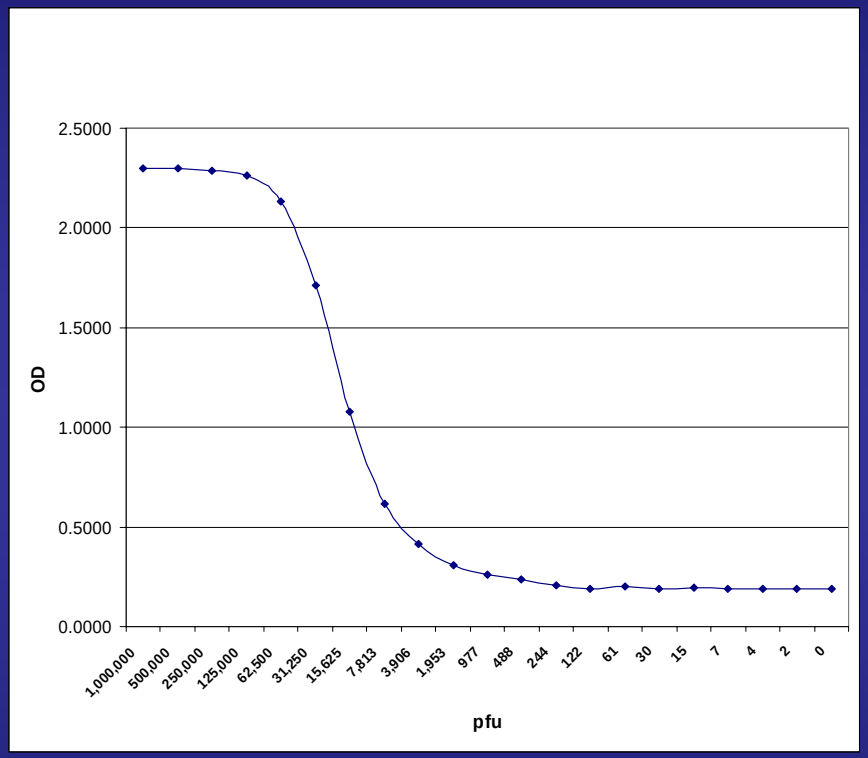


Standardizing the assay

Purified VP40 protein



Inactivated Ebola virus

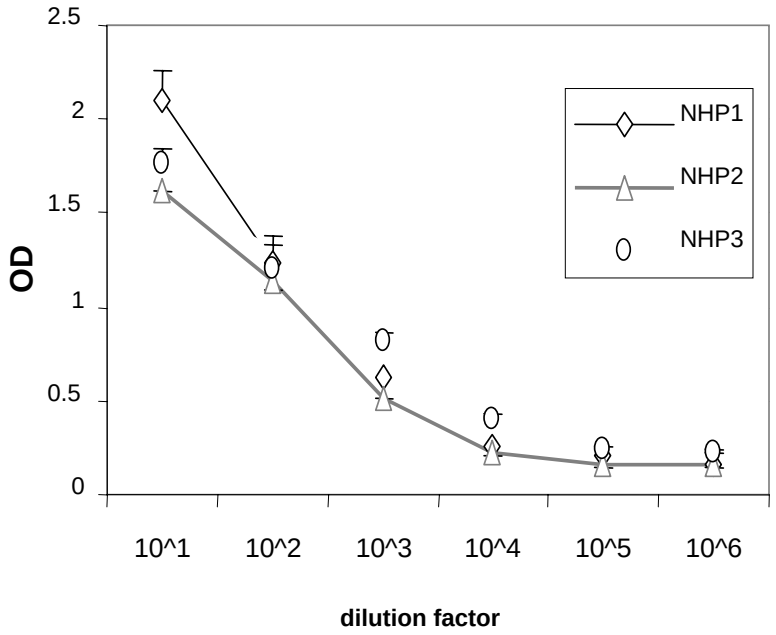


-Assay detects ~2ng of VP40 protein
-Linear from 10-125ng

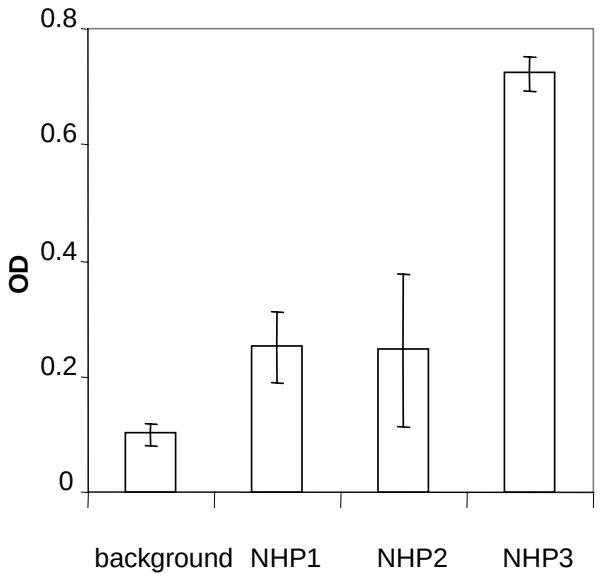
-Assay detects ~500pfu iEbola.
-Linear from 7000-100,000pfu

Detecting Ebola virus in non-human primate samples

Dilutions of Liver Homogenate



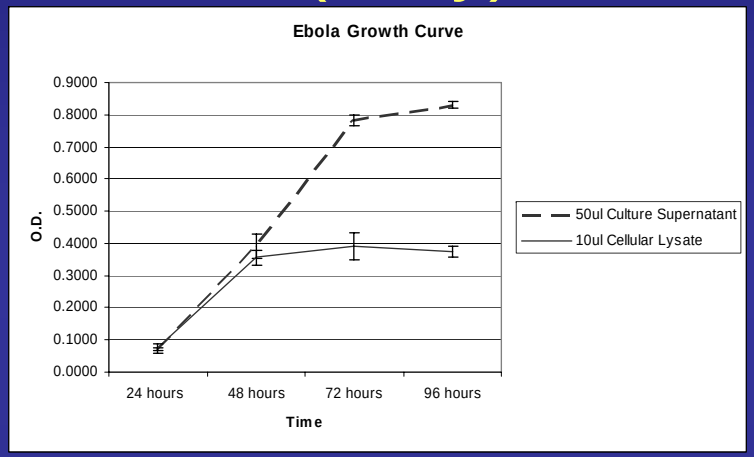
Plasma samples



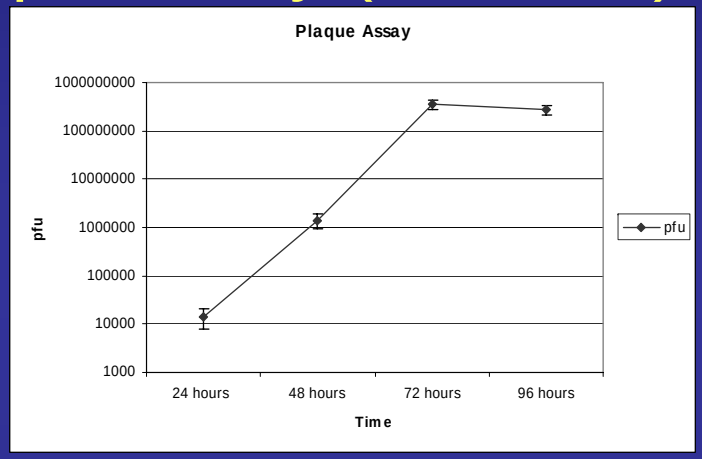
BL4 work, K. Warfield

Ebola virus growth curve
ELISA / plaque assay

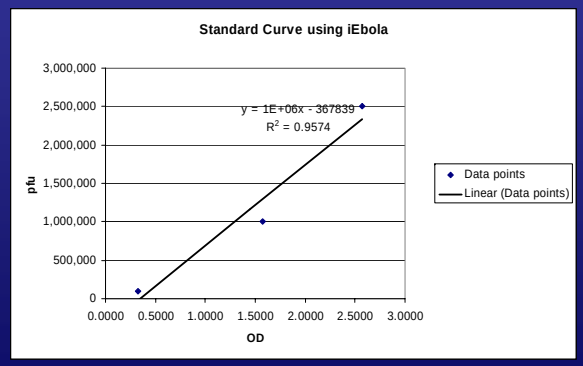
ELISA (1day)-BL2



Plaque assay (>1 week)-BL4



Standard Curve



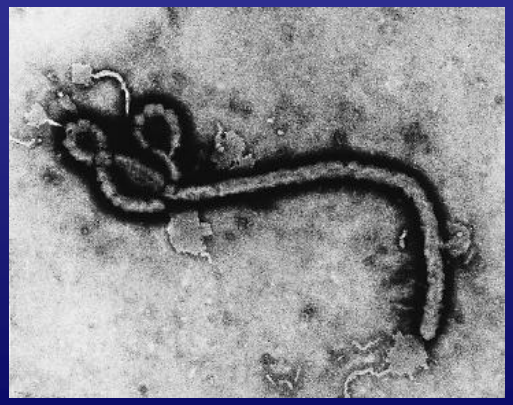
Time Point	Plaque Assay	ELISA
48h	1.2×10^7 pfu/ml	1.0×10^7 pfu/ml
72h	3.4×10^8 pfu/ml	1.7×10^8 pfu/ml
96h	2.7×10^8 pfu/ml	2.4×10^8 pfu/ml

BL4 work, D. Swenson

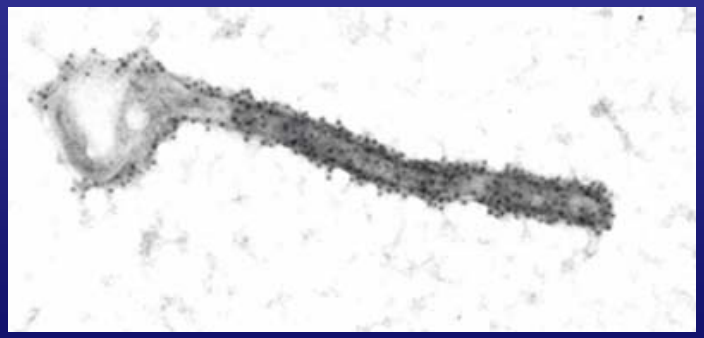
VLP Structure, GP+VP40

- VP40 alone makes filamentous structures that resemble native virus.
- Adding GP increases the production of VLPs and makes the VLPs immunogenic.

Ebola virus (F. Murphy, CDC 1976)

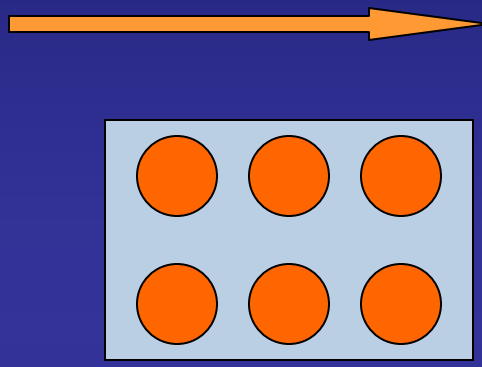


GP+VP40 Virus Like Particle



Standard VLP assay conditions

Transfect
VLP plasmids
into 6 well plate
@80% confluence

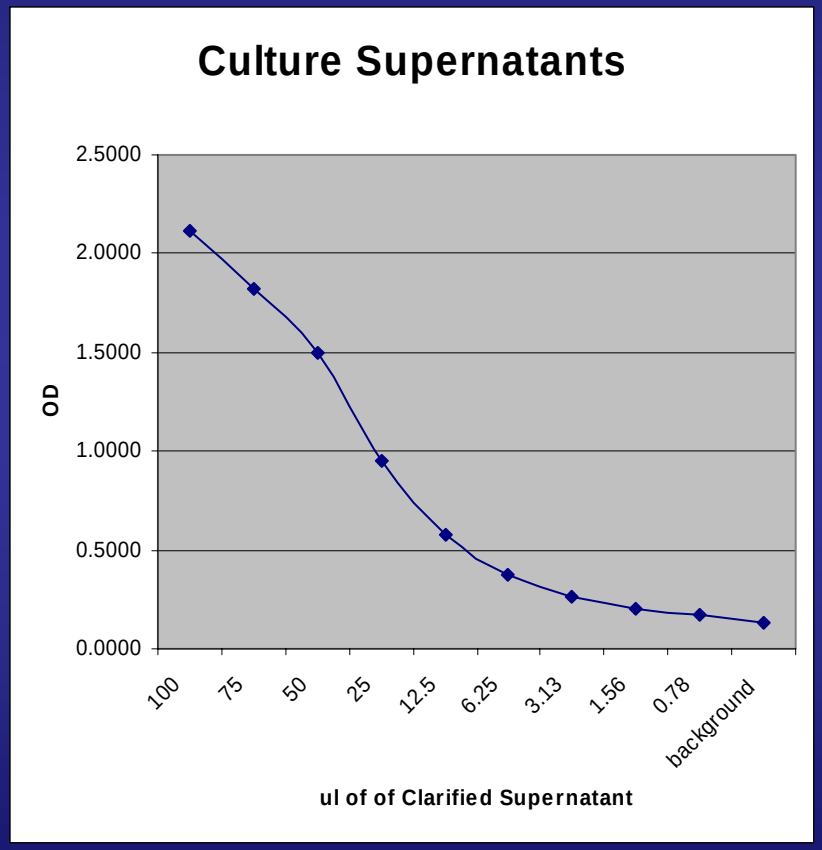


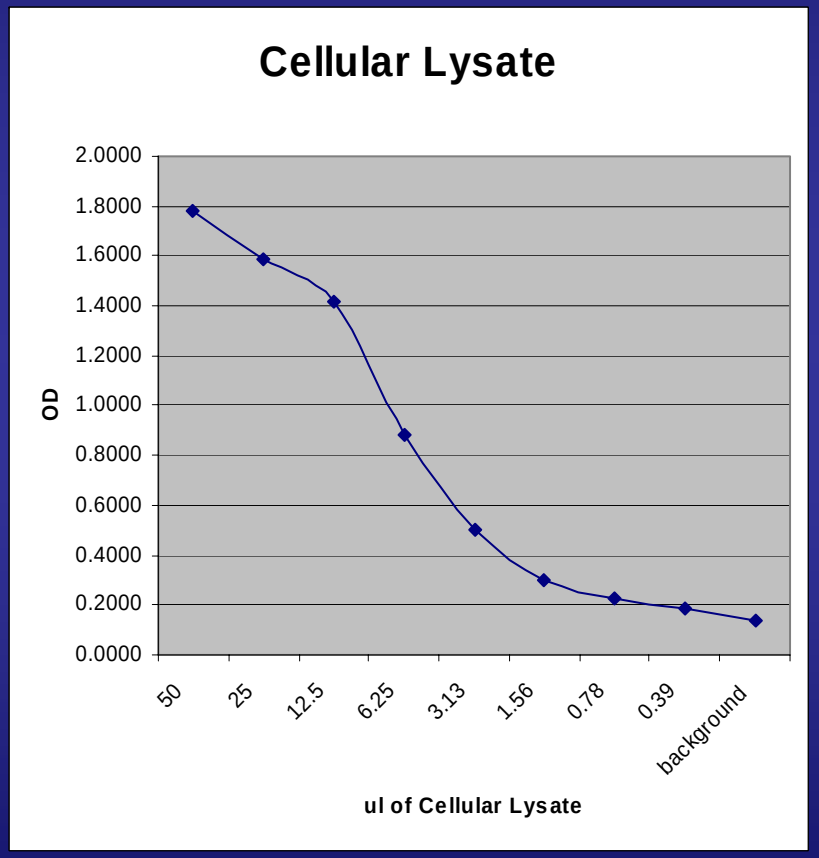
Incubate 48h

Harvest cell culture supernatant
Wash the cells with PBS, harvest wash
Harvest cell pellet and disrupt cells for cellular lysates

Perform ELISA

Testing VLP Supernatants / Lysates

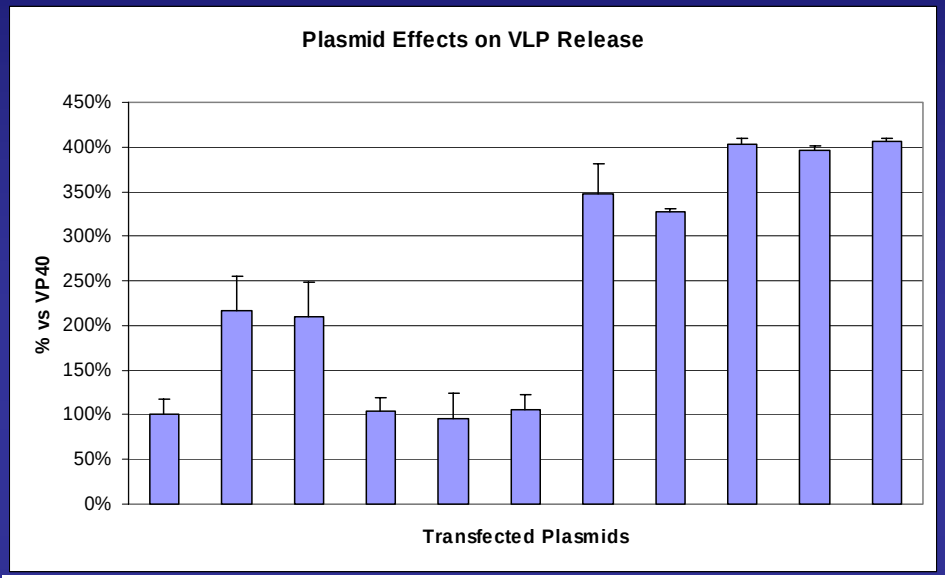




Can detect protein in 2ul of supernatant, linear range 12.5-75ul

Can detect protein in <1ul of lysate, linear range 3-25ul

Effect of other plasmids on VLP formation



VP40	+	+	+	+	+	+	+	+	+	+	+
GP	-	+	-	-	-	-	+	+	+	+	+
NP	-	-	+	-	-	-	+	+	+	+	+
VP35	-	-	-	-	+	-	-	-	+	+	+
VP30	-	-	-	+	-	-	-	+	-	+	+
VP24	-	-	-	-	-	+	-	-	-	-	+

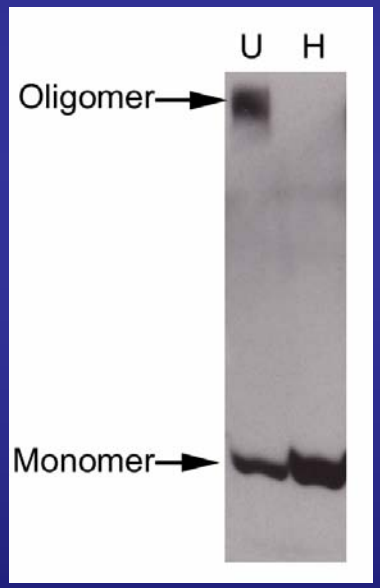
-GP and NP increase VP40 VLP release

-VP30 slightly inhibits release in the presence of VP40 and NP

-VP35 promotes VLP release in the presence of VP40 and NP

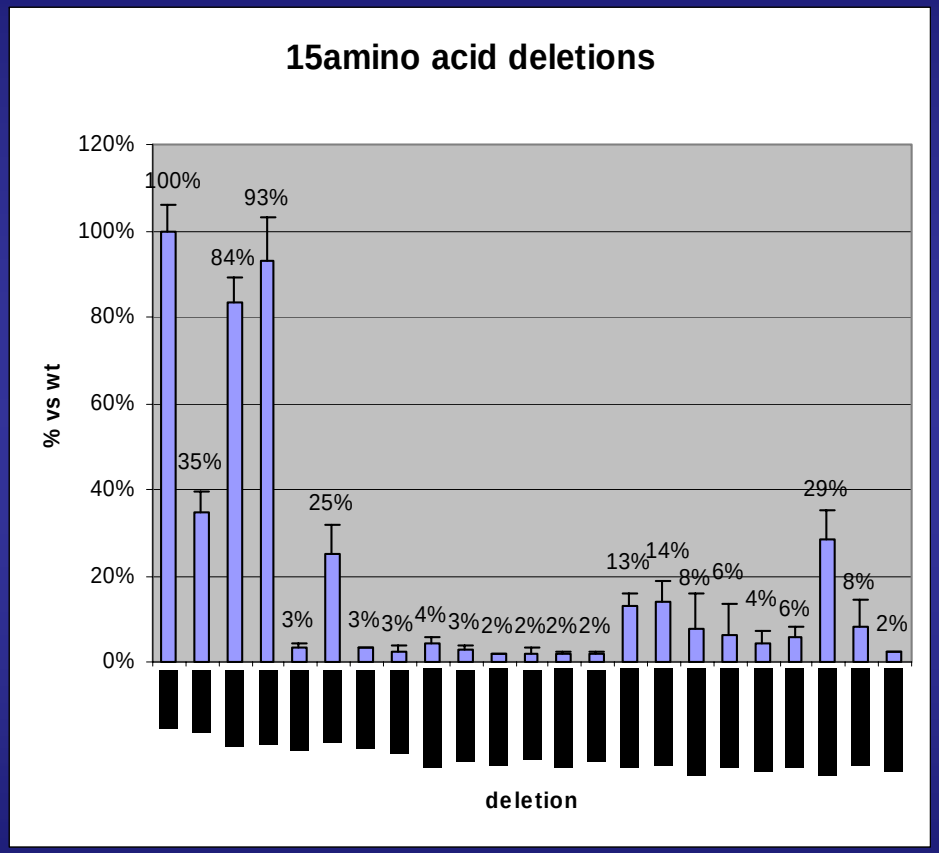
VP40 Oligomerization

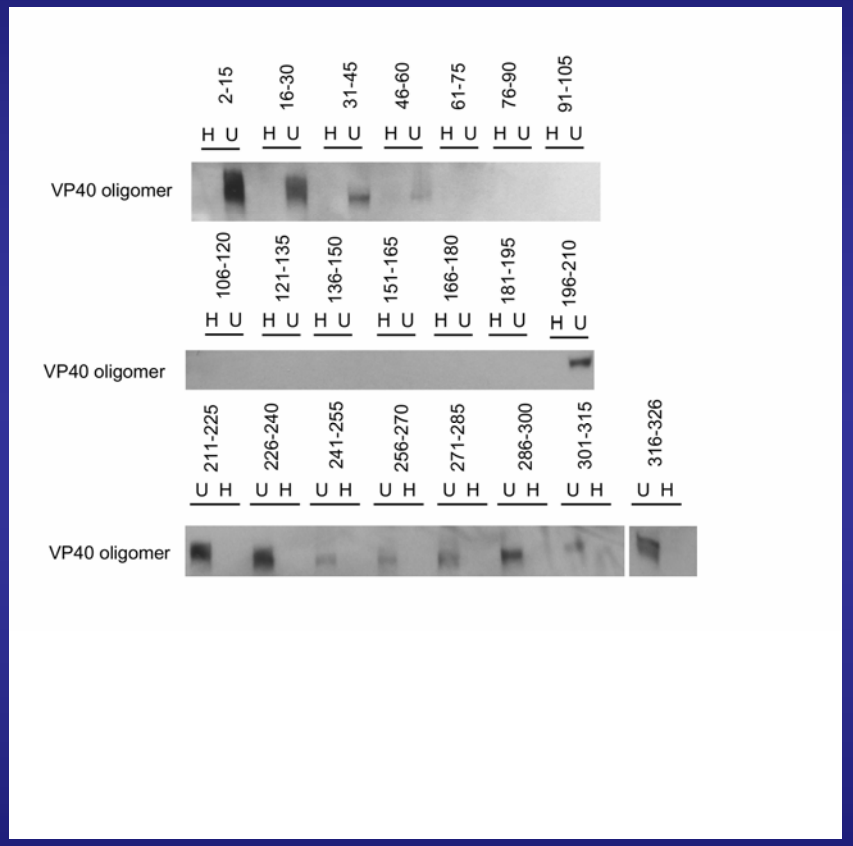
Anti-VP40 Western



- VP40 exists in both monomeric and oligomeric forms.
- VP40 oligomers are SDS resistant, but heat sensitive.
- Oligomerization of VP40 is important for formation of VLPs.

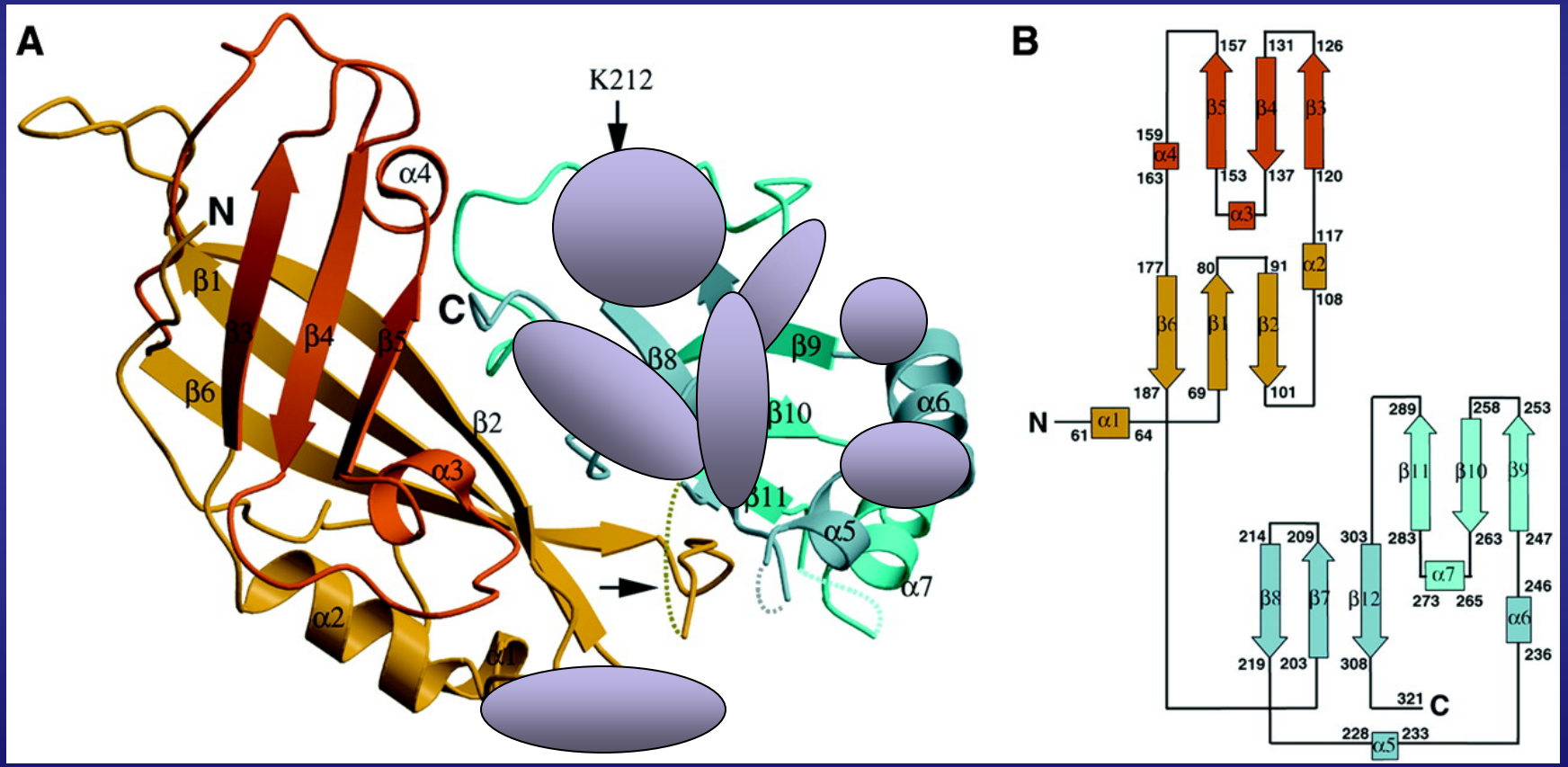
VLP/oligomer formation





T. Nelle constructed plasmids

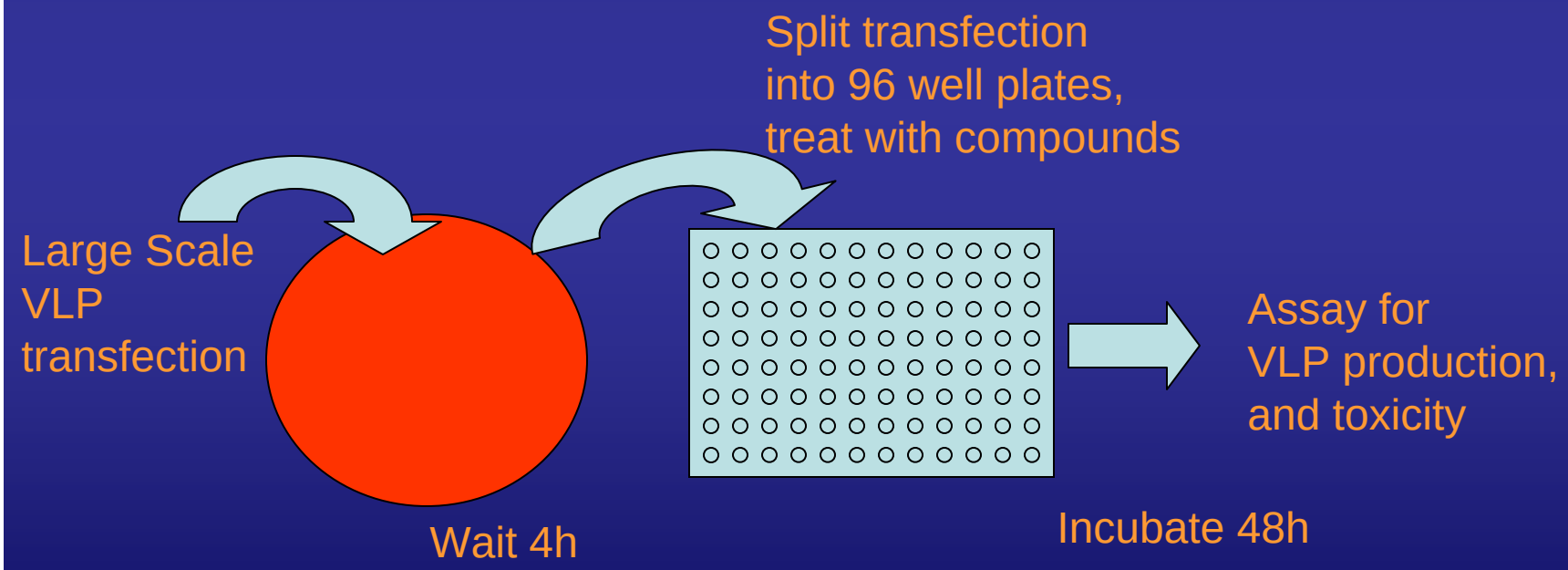
Shaded deleted regions made oligomer / released VLPs



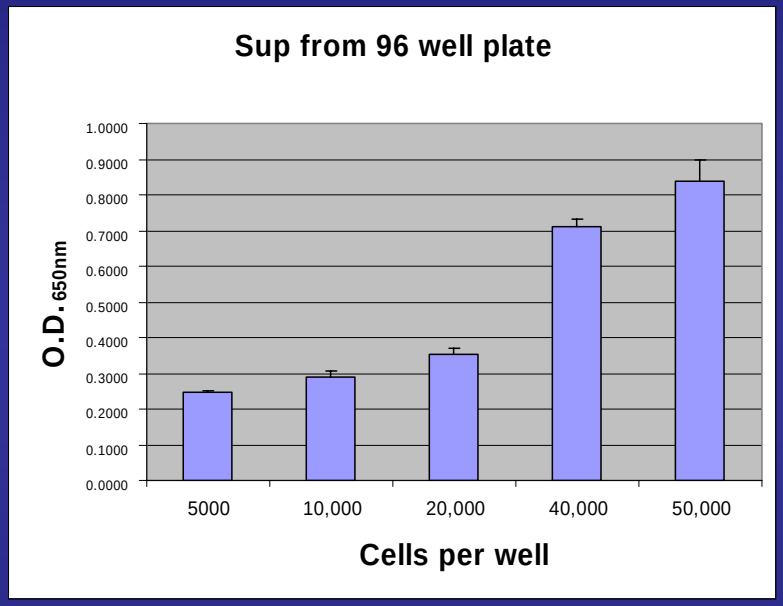
Dessen et al, 2000 EMBO Journal

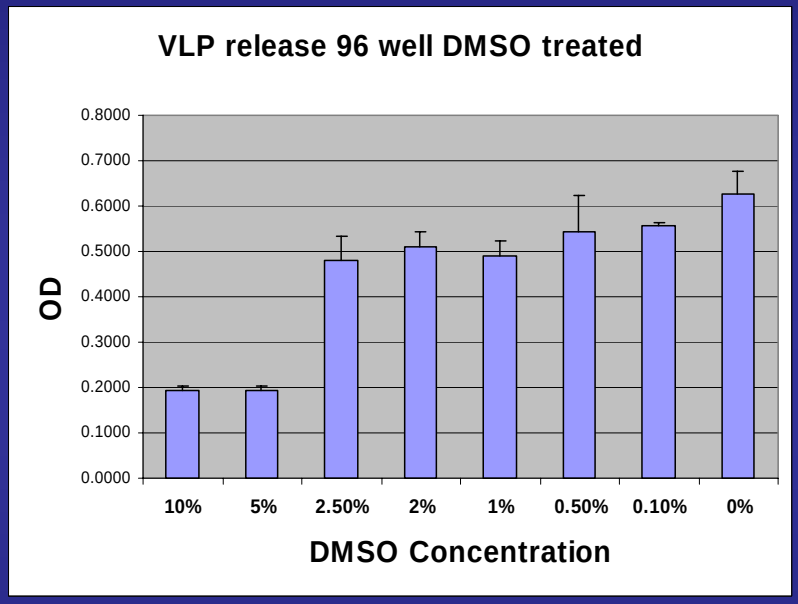
ELISA can be utilized for therapeutic drug screening

- Identification of compounds that inhibit viral egress.



Potential for therapeutic screening.





40,000 cells / well is optimal for splitting/signal, at 50,000 wells are overgrown

<2.5%DMSO did not affect VLP production or cell viability

Accomplishments

- Developed and optimized ELISA that measures Ebola virus / VLP release
- Quantified the contribution of filoviral proteins on VLP formation
- Identified regions of VP40 that are critical for VLP formation release
- Prepared for antiviral therapeutic compound screening

Summary

- We showed a sensitive ELISA for Ebola VLP / viral release
- Although not shown, the ELISA is useful for studying viral-host cell interactions
- Potentially useful as an alternative to plaque assays

Acknowledgements

Sina Bavari Lab

- Sina Bavari-leader of research group
- Javad Aman-research colleague
- Kelly Warfield & Dana Swenson-BSL4 experiments
- Gordon Ruthel-microscopy

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- Sally Charles-Undergraduate Student
- Shirin Badie-Graduate Student
- Tim Nelle-constructed 15aaΔ-VP40 plasmids
- Other lab members and USAMRIID staff who support our research