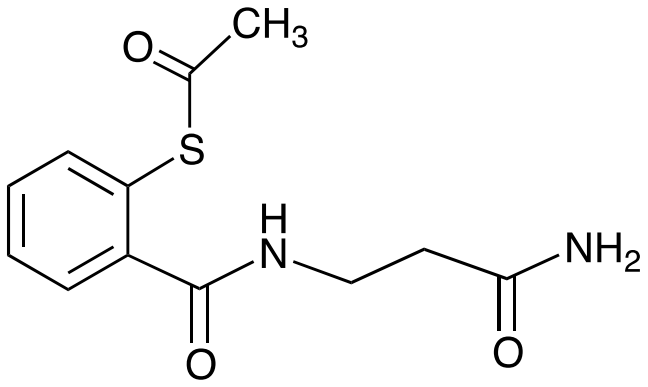
Disrupting HIV Maturation and Infectivity with Mercaptobenzamide Molecules

Daniel H. Appella

Mercaptobenzamide: 247



National Institute of Diabetes and Digestive and Kidney Diseases

Snapshot of HIV/AIDS today

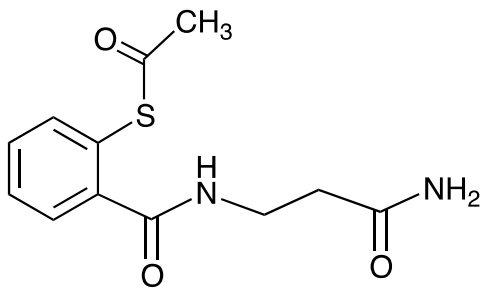
- 37 people million infected
  - 25.5 million in Sub-Saharan Africa
  - 5.1 million in India / Southeast Asia
  - 1.6 million Eastern Europe / Central Asia
  - 2.1 million Latin America and Caribbean
  - 1.1 million in U.S.A. and North America
  - 0.9 million Western / Central Europe

54% of people infected with HIV are aware of their infection.

- 20 Million people receiving antiretroviral therapy
- 30 drugs to treat HIV, used in combination, not a cure
- Cost: $20,000 to $36,000 per person per year for a lifetime (32 years)
- Countries where 10% or more new infections are resistant
  - Argentina, Guatemala, Namibia, Nicaragua, Uganda, Zimbabwe

Solomon, D. A. and Sax, P. E., *Curr Opin HIV AIDS*, **2015**, *10*, 219;
WHO Progress Report 2016, http://www.who.int/hiv/data/en
https://www.cdc.gov/hiv/programresources/guidance/costeffectiveness/index.html

Antiviral Activity



247

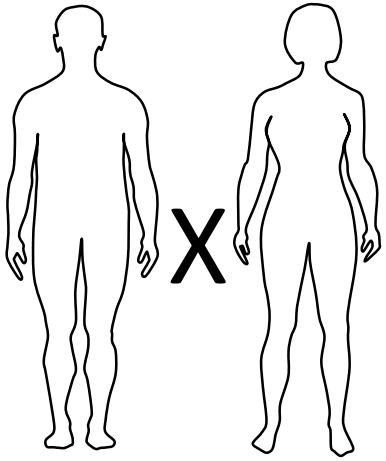
Cell Type	**247**	
	EC_{50} (μM)	TC_{50} (μM)
CEM-SS (HIV-1(IIIB))	0.6	>100
PBMC (HIV-1 Clade B)	5.74	>100
monocyte-macrophage	1.97	>100

- Remarkably non-toxic!
- Similar low micromolar activity against HIV Clades: A, B, C, D, E, F, G, O
- Antiviral activity is the same against HIV viruses with resistance to known drugs.
- No activity against HIV Reverse Transcriptase, Integrase, Protease, Viral Entry
- In combination with 23 FDA-approved HIV Drugs, synergistic or additive effects
  No synergistic toxicity
- After several attempts, not able to generate **247**-resistant HIV strains
- Not expensive to synthesize - $15 / gram
- **247** is a microbicide that can prevent SIV infection

Antiviral Res. **2016**, *134*, 216

Application as Microbicide

International Partnership for Microbicides
(impglobal.org)



HIV Positive *HIV Negative*

Protection for Uninfected Women and Men
who have sex with HIV-Infected Men

- Topical prevention
- Prevent HIV transmission during sexual intercourse
- CAPRISA Clinical Trial – July, 2010
- 1% tenofivir gel used by women in South Africa
- HIV transmission lowered 39%
- ASPIRE Clinical Trial – dapivirine in contraceptive ring
- February, 2016 – monthly usage reduces infections by 30%

Tenofovir

Nucleotide Reverse Transcriptase Inhibitor (NRTI)

Gel: Apply just before intercourse

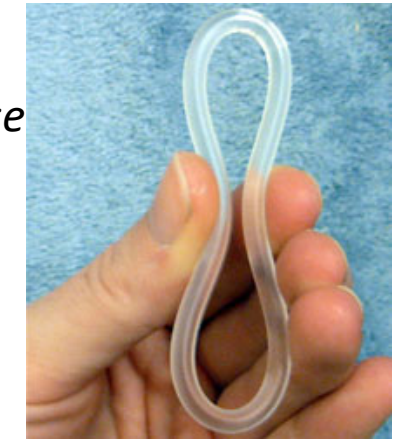
Dapivirine

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

Ring Formulation:
Slow release
over 1 month

(only for women)

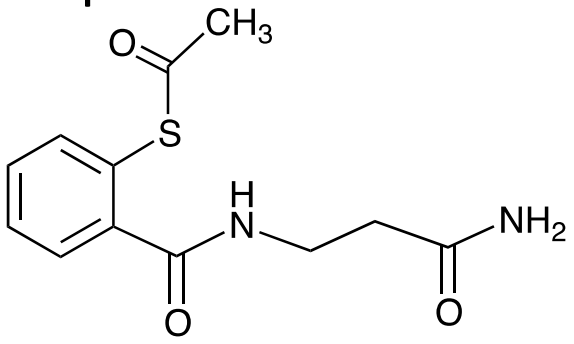
NuvaRing

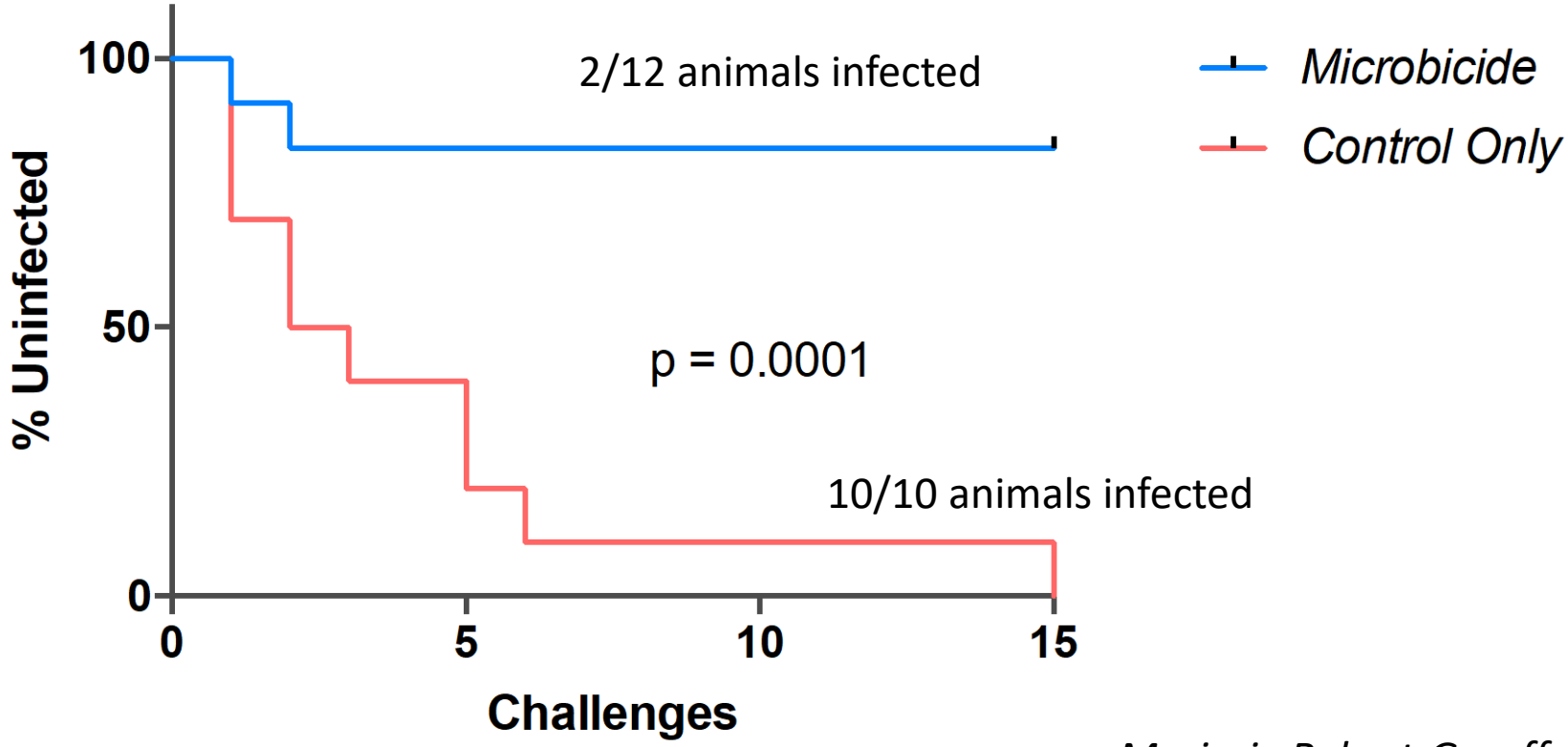


247 microbicide study in SIV-Infected Rhesus Macaques:

- Microbicide: 0.8% 247 in Hydroxyethylcellulose (HEC) Gel
- Animals challenged weekly, for total of 15 challenges

  (12 animals in microbicide group, 10 in control group)
- Intravaginal challenge 3 hours after application of microbicide
- Animals will be followed for 40 weeks after completion of challenges

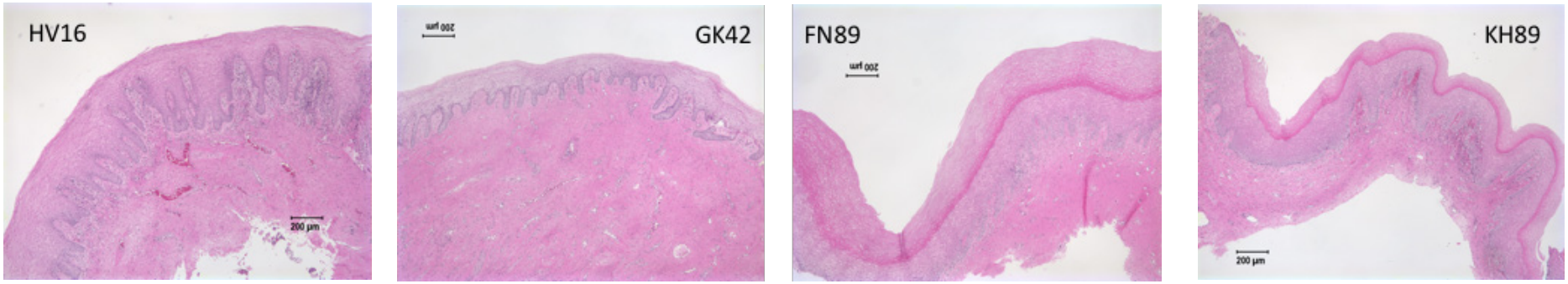




Marjorie Robert-Guroff, NCI

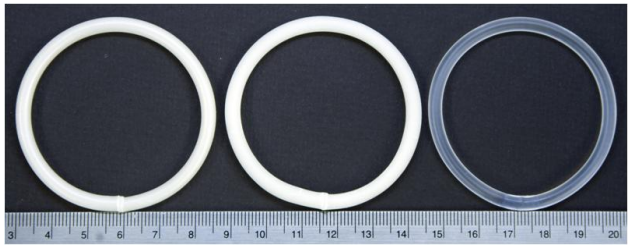
247 does not cause inflammation in SIV-Infected Rhesus Macaques:

Histology of vaginal biopsies collected day 7 post 1% SAMT vaginal gel administration



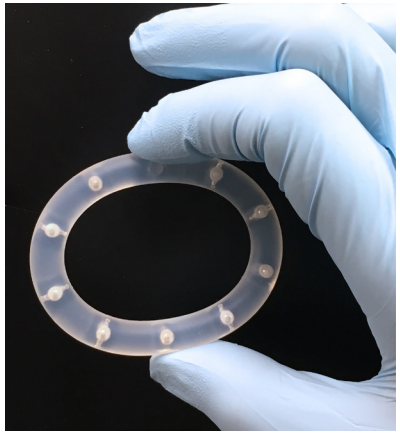
Ron Veazey, Tulane University School of Medicine

247 can be formulated into rings for slow release:



J. Pharm. Sci. **2015**, *104*, 3426

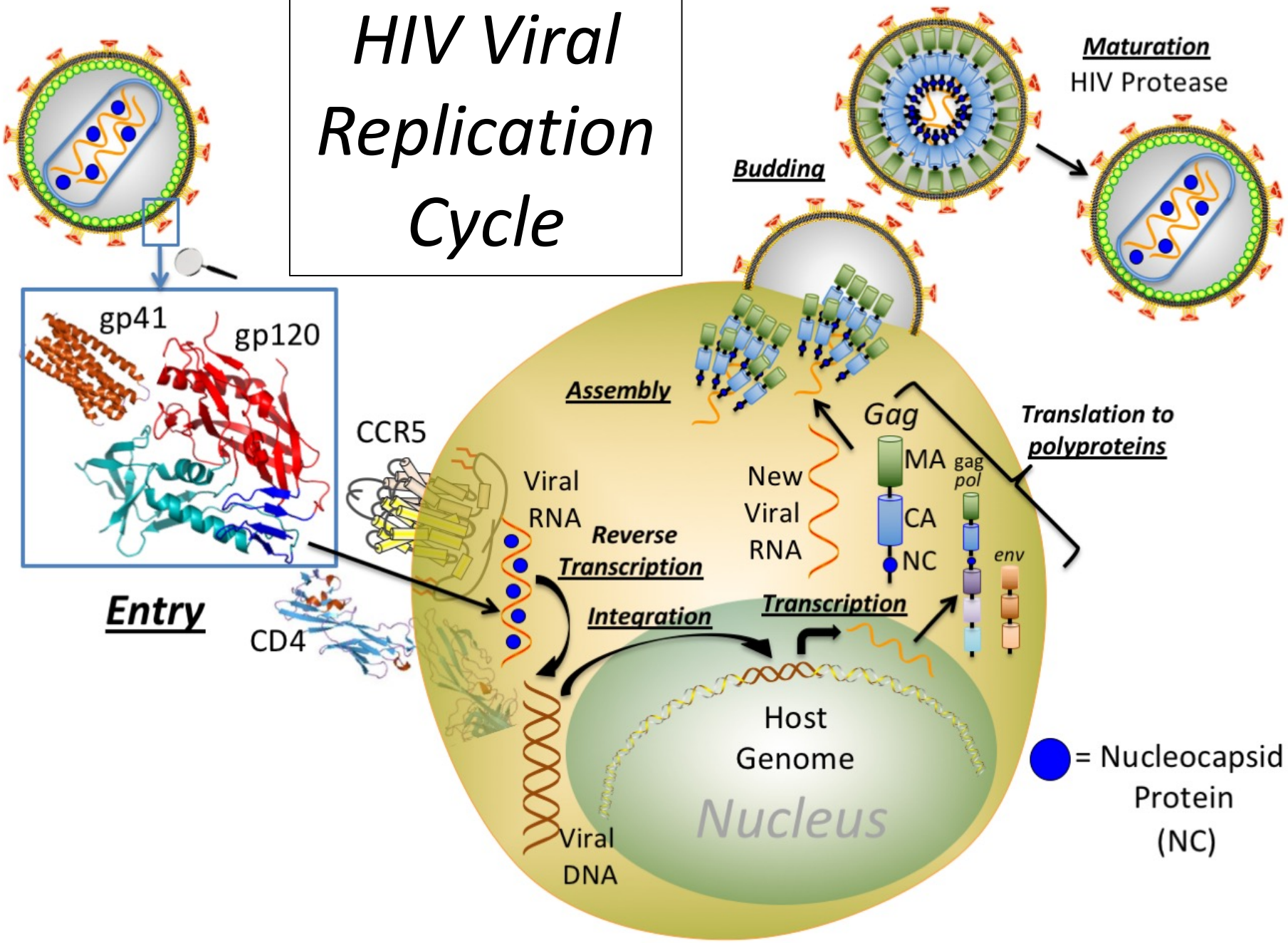
Patrick Kiser, Northwestern University



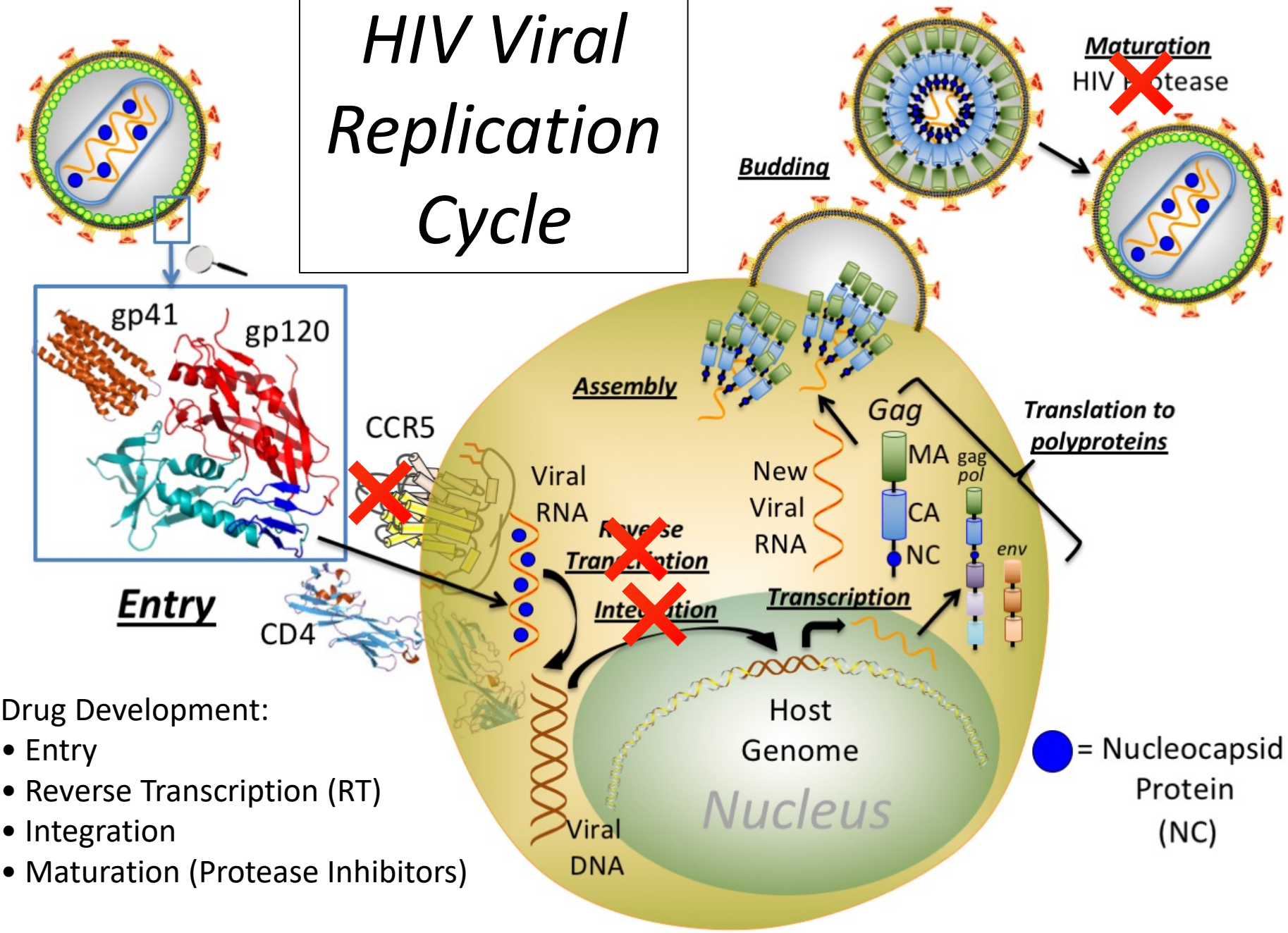
Slow release of **247** measured over 30 days

Marc Baum, John Moss
Oak Crest Institute of Science

HIV Viral Replication Cycle



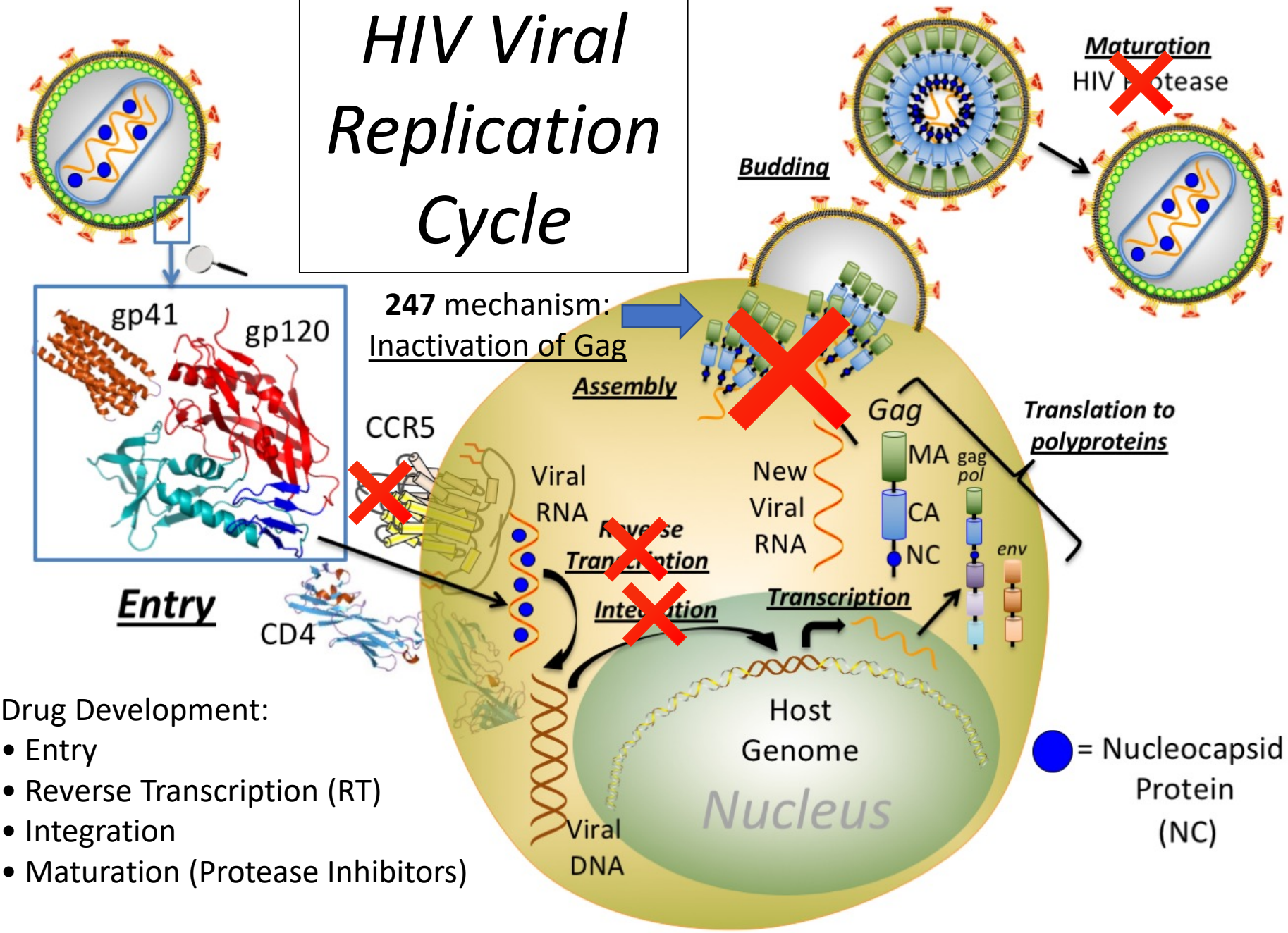
HIV Viral Replication Cycle



Drug Development:

- Entry
- Reverse Transcription (RT)
- Integration
- Maturation (Protease Inhibitors)

HIV Viral Replication Cycle



Drug Development:

- Entry
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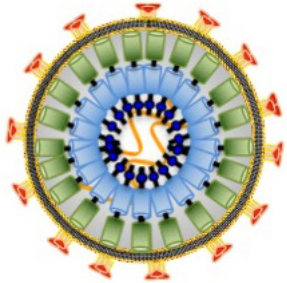
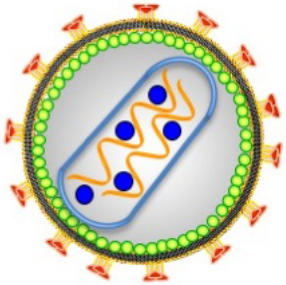
Immature HIV

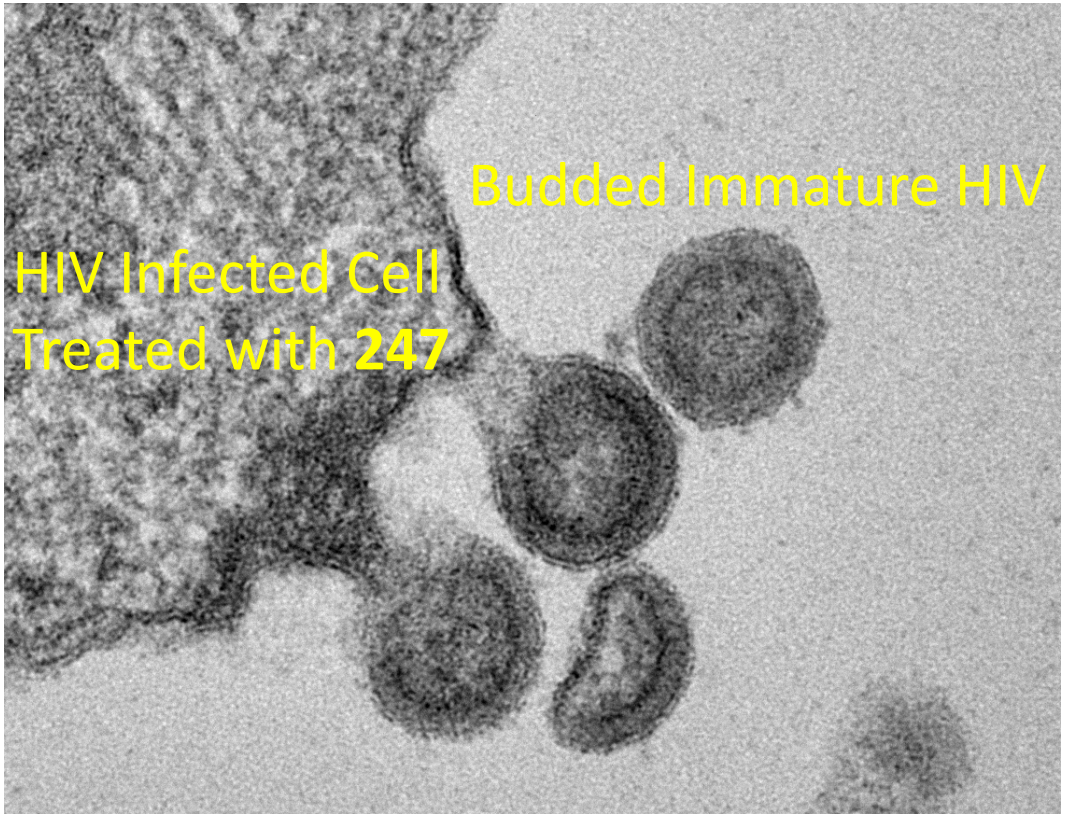


Electron Microscopy

Mature HIV



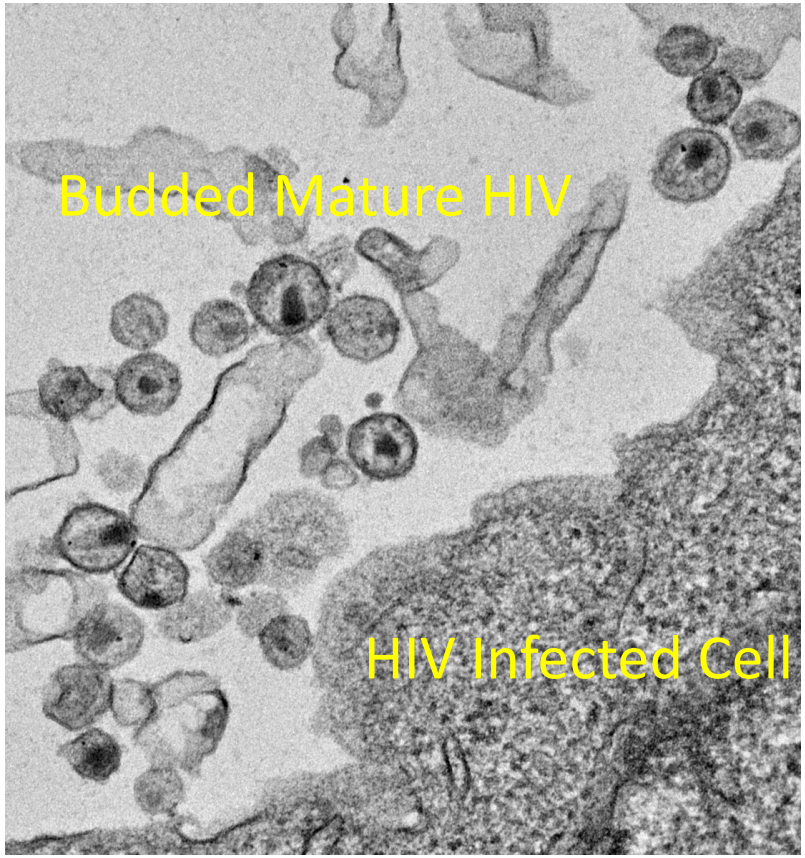
247 Treated Cells



David Ott (NCI)

100 nm

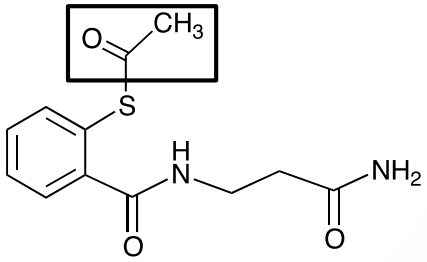
Untreated Cells



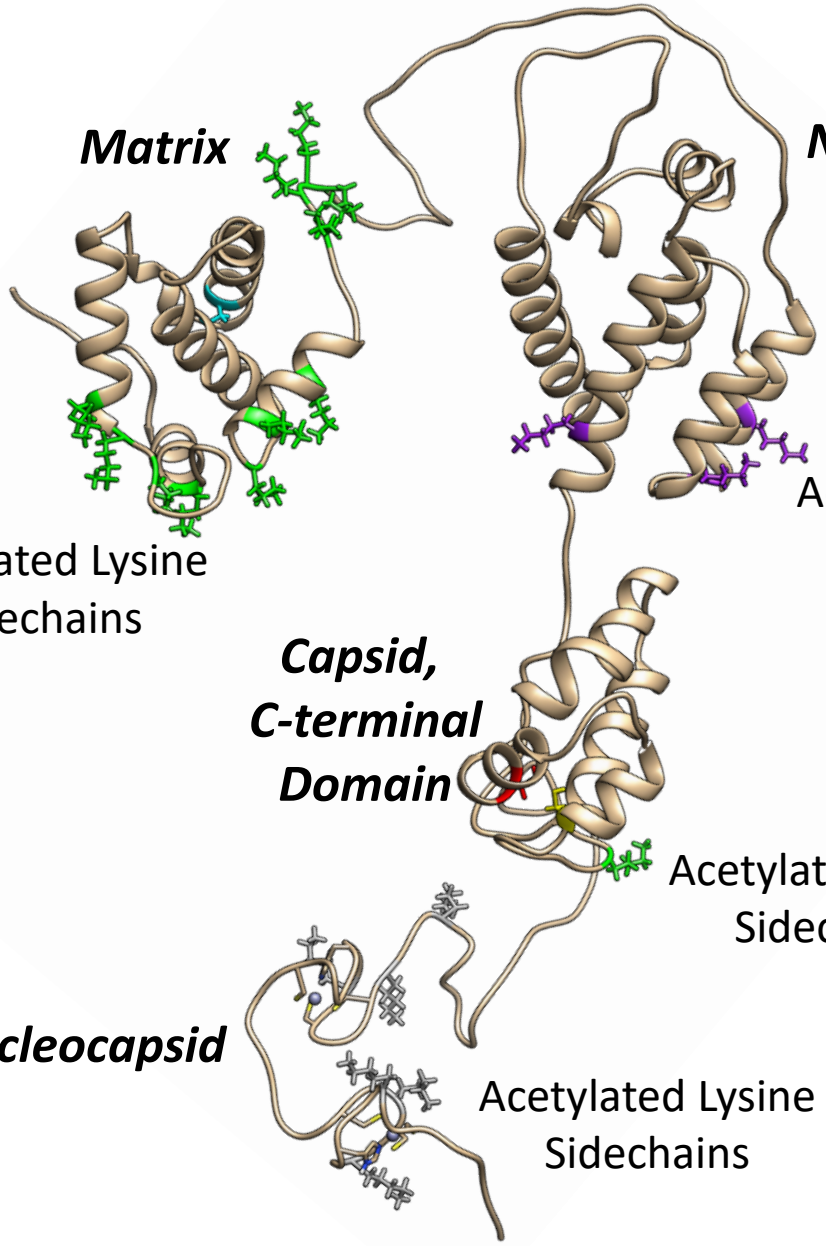
500 nm

247 specifically acetylates lysines and cysteines in HIV Gag

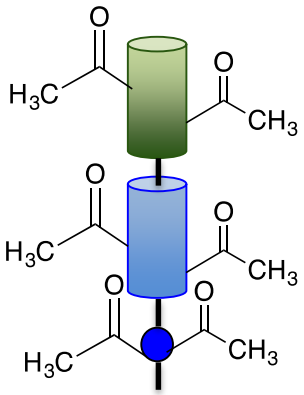
Acetyl Group of **247**



247

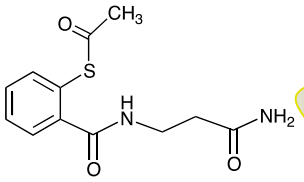


Acetylated HIV Gag

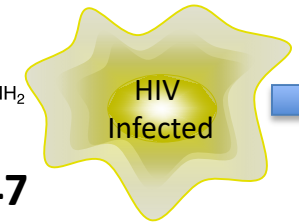


247 does NOT acetylate every lysine in HIV Gag.

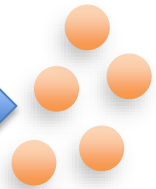
In HIV-infected H9 Cells:



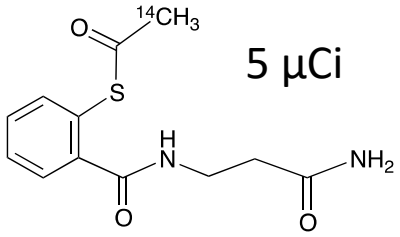
Treat with **247**



HIV Virions



Analysis



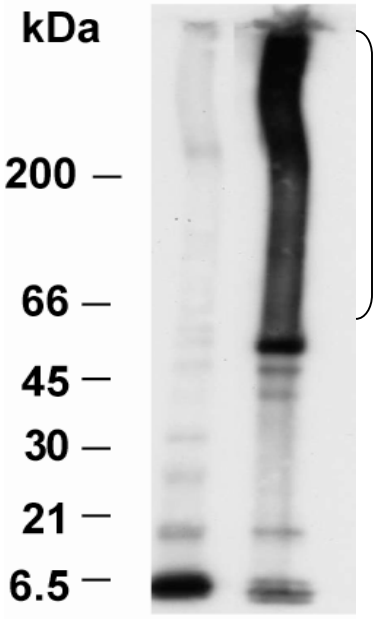
Not Infected

H9 Clone 4

HIV Infected

Examining isolated virions:
Gag aggregation and processing defects

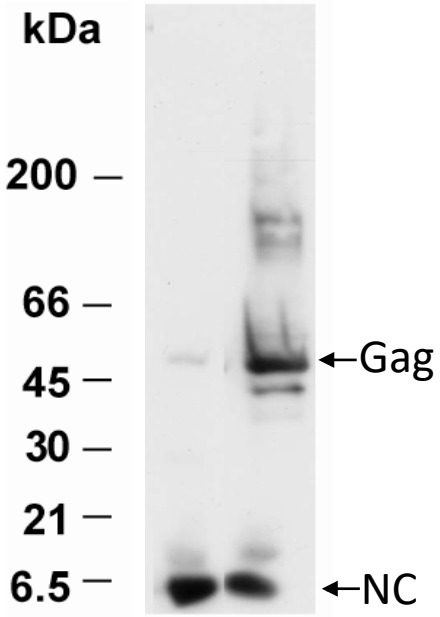
un. 247



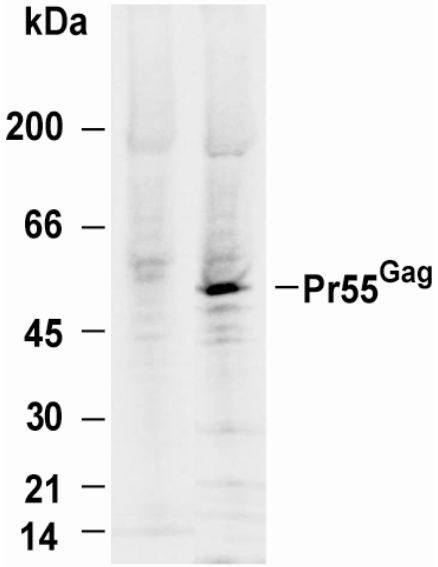
Gag_n

Non-Reducing

un. 247



Reducing

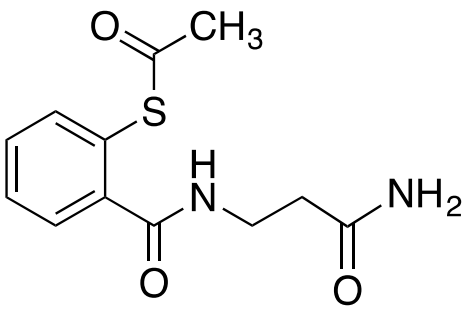


Radiolabel modifies Gag

Miller Jenkins, L.M. *et al.* *Nat. Chem. Biol.* **2010**, *6*, 887.

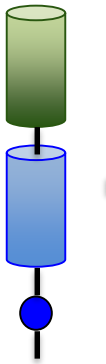
David Ott (NCI)
Lisa Jenkins (NCI)

Conclusions:

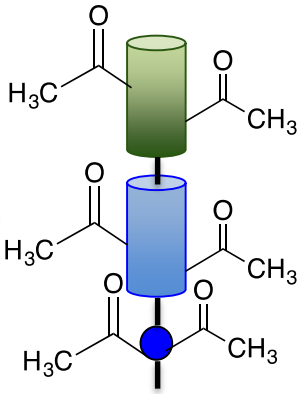


247

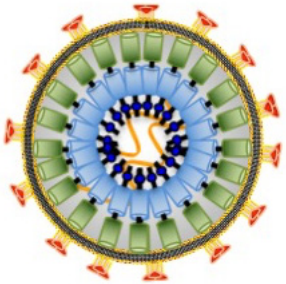
HIV Gag



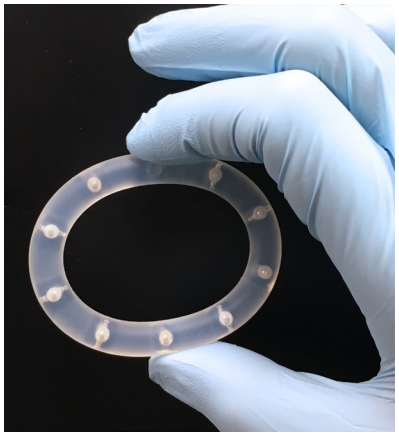
Acetylated HIV Gag



Block HIV Maturation



Translate to a microbicide



- **247** reacts with HIV Gag by acetylation of cysteines and lysines
- **247** acetylates Gag at several cysteines and lysines, both *in vitro* and in cells
- There is a unique pattern of HIV Gag acetylation that disrupts viral maturation
- Not able to generate HIV resistance to **247**
- **247** is an effective microbicide against SIV *in vivo*, rhesus macaques
- **247** does not cause irritation
- **247** can be formulated into different delivery devices

Acknowledgements

Collaborators:

Appella Lab:
Herman Nikolayevskiy
Marco Robello

NCI
Eric Freed
Marjorie Guroff
Lisa Jenkins
David Ott

NIDDK
Lalit Deshmukh
Marius Clore

ImQuest BioQual

Tulane University School of Medicine
Ron Veazey

Oak Crest Institute of Science
Marc Baum, John Moss

Northwestern University
Patrick Kiser

Funding:
Intramural Research Program of NIDDK, NIH
Office of AIDS Research (OAR)