

# Retrocyclins: circular minidefensins active against HIV-1

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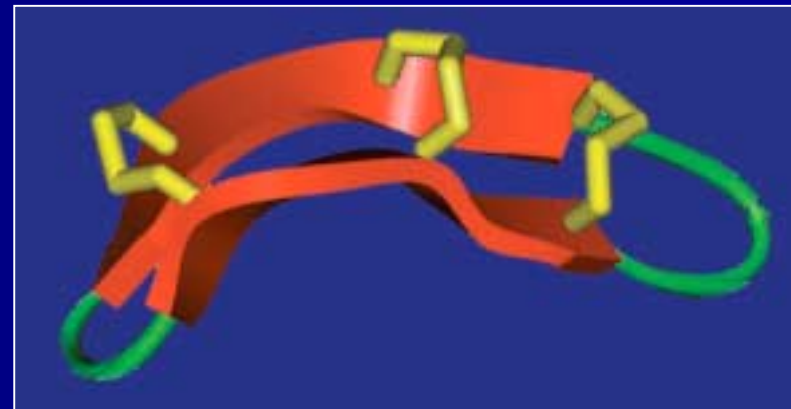
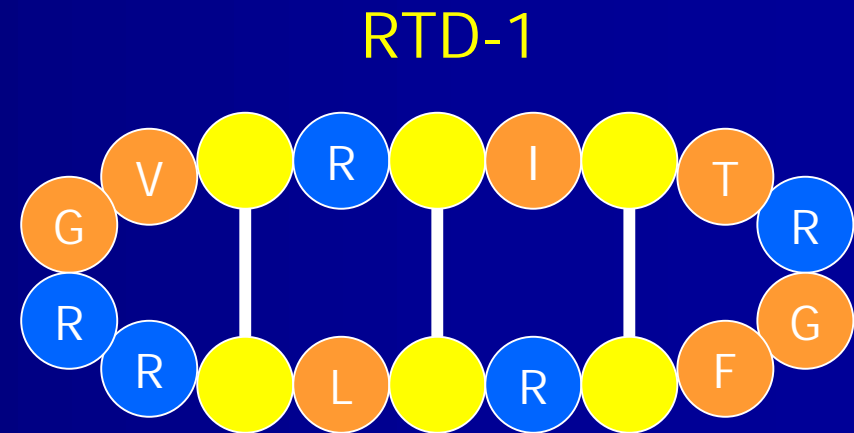
*Department of Medicine*

# Why study anti-HIV activity of antimicrobial peptides?

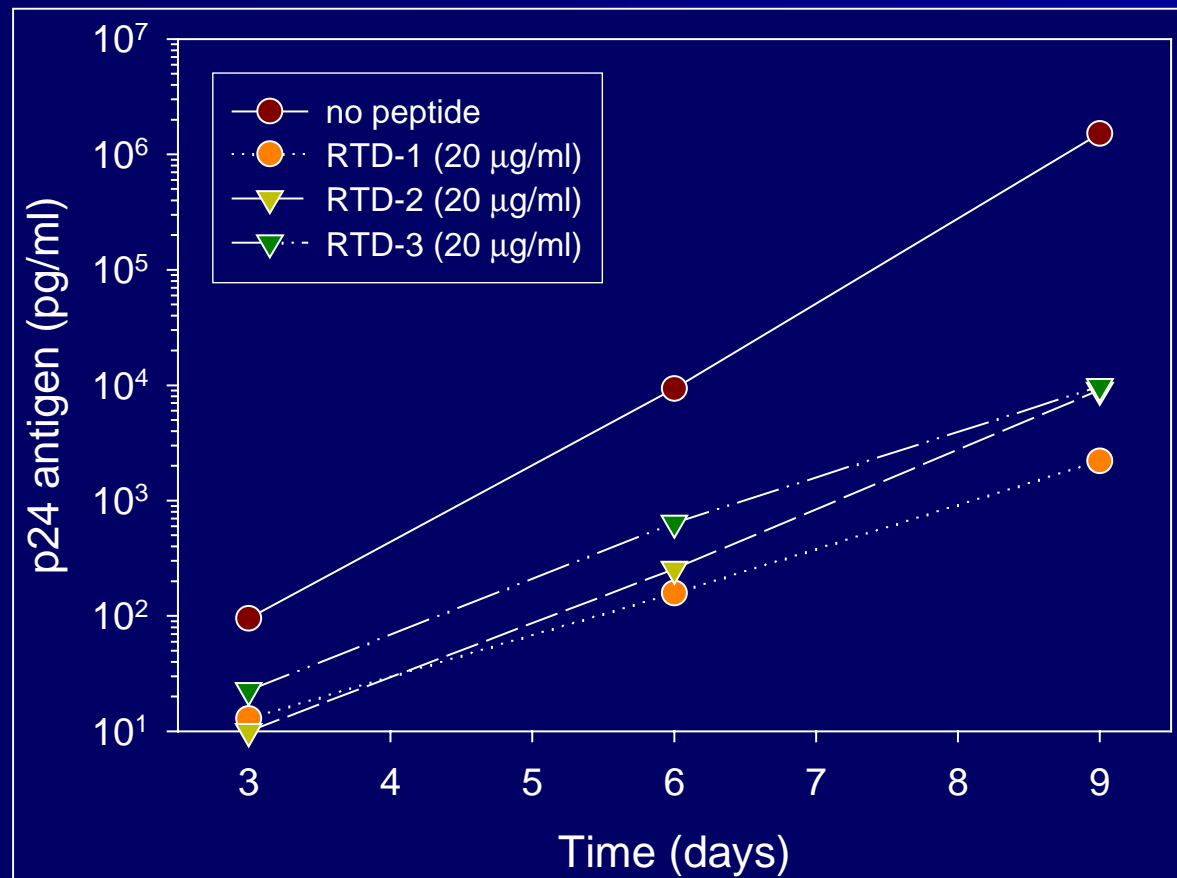
- Promising, yet understudied area:
  - Defensin (Nakashima et al. 1993)
  - Protegrin-related (Tamamura et al. 1995)
  - Mellitin and Cecropin (Wachinger et al. 1998)
  - Indolicidin (Robinson et al. 1998)
- T22, T140 - polyphemusin analogs
  - high activity, low cytotoxicity
  - shown to be effective only against X4 strains
- We discovered that circular minidefensins are remarkably active against both X4 and R5 strains

# Rhesus circular (theta) minidefensins

- Small (18 amino acids)
- Cationic
- 3 disulfide bonds
- Posttranslational generation of molecular diversity using *two* 9 aa precursors
  - **RTD-1** (precursor A + precursor B)
  - **RTD-2** (2x precursor A)
  - **RTD-3** (2x precursor B)



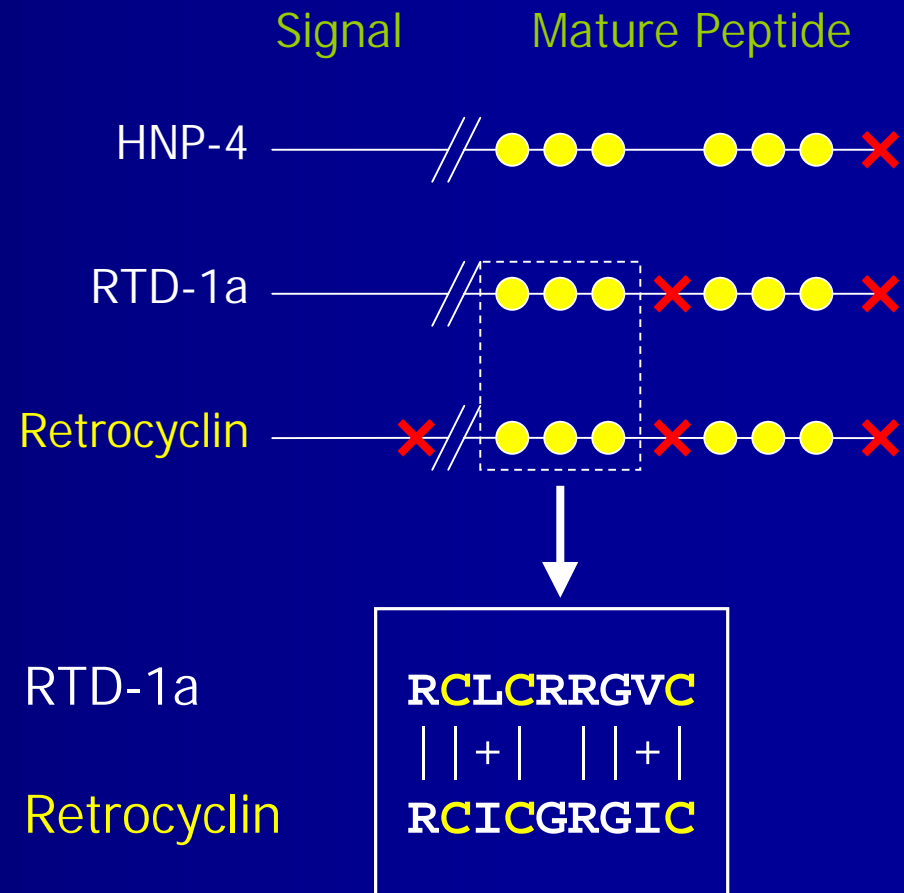
# Rhesus circular minidefensins inhibit infection by HIV-1



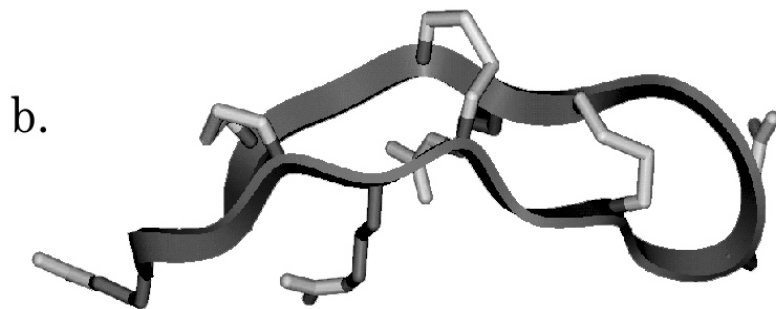
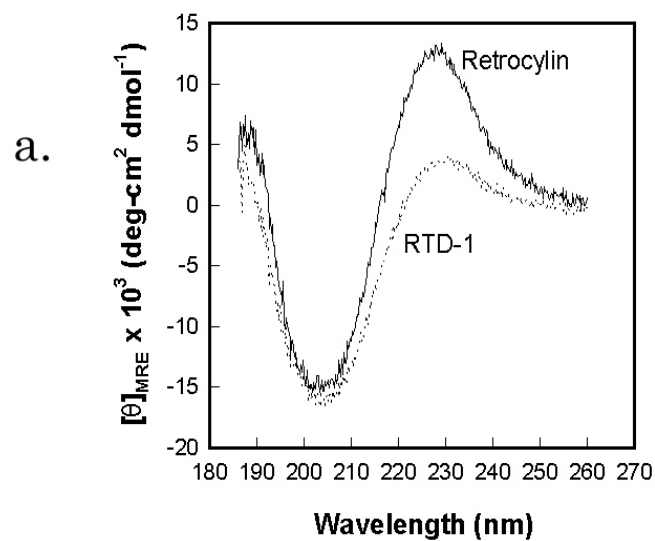
*H9 cells, HIV-1 IIB*

# Discovery of an ancestral human circular defensin

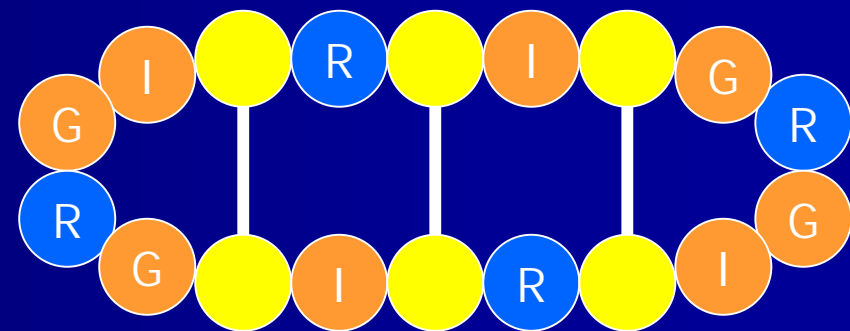
- Expressed pseudogene, *Stop codon* after putative signal region
- **Retrocyclin**
  - (retro = back or backward, cyclin [*L. cyclus*] = circle)
- Recreated this “dead” peptide:
  - synthesized, folded and cyclized similarly to RTD-1



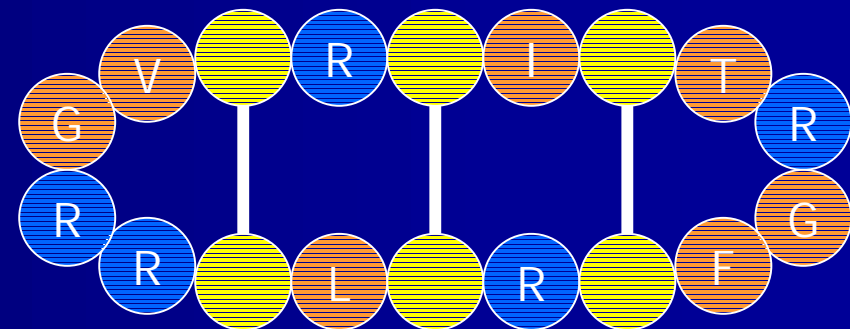
# Structural characterization of retrocyclin



Retrocyclin



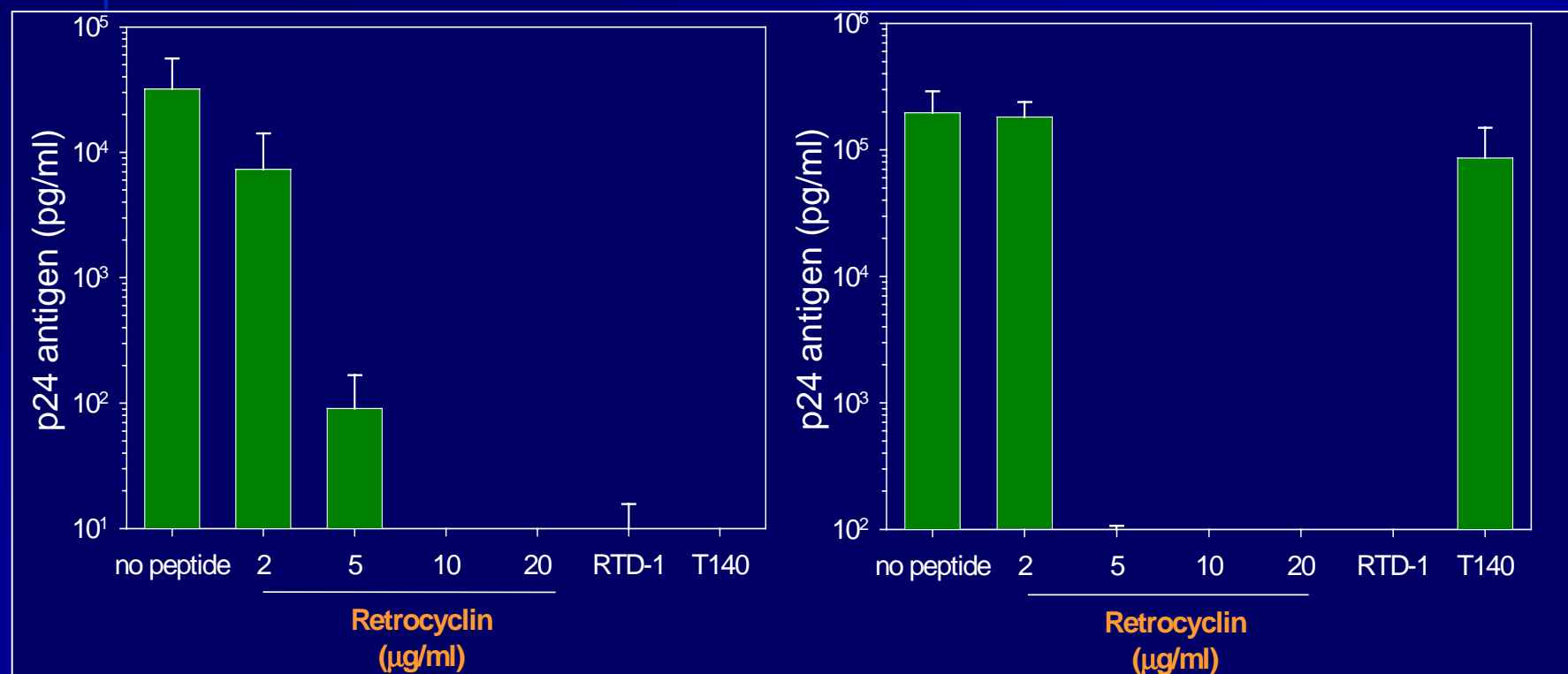
RTD-1



# Retrocyclin inhibits replication of X4 and R5 viruses

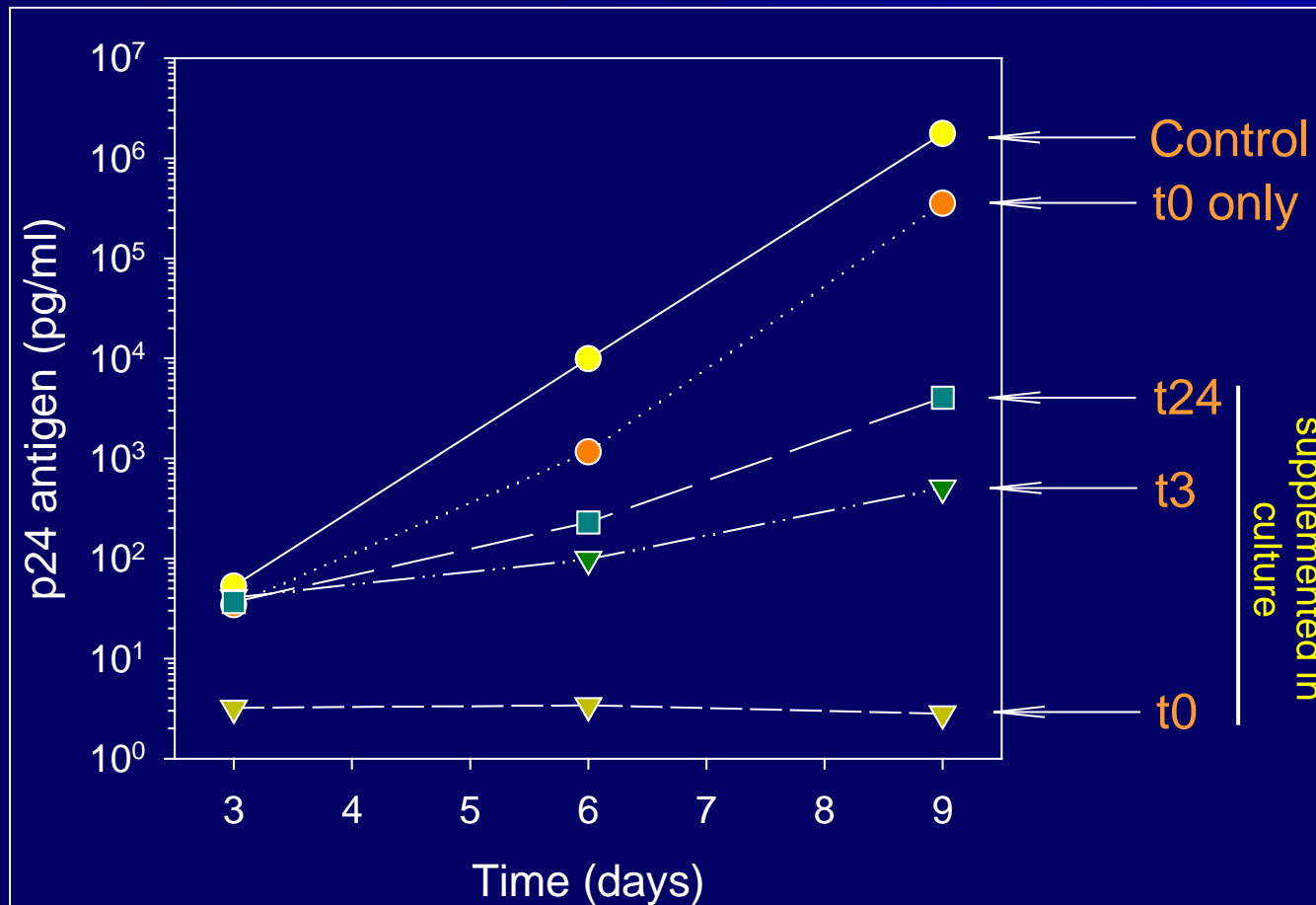
HIV-1 IIB (X4 strain)

HIV-1 JR-CSF (R5 strain)



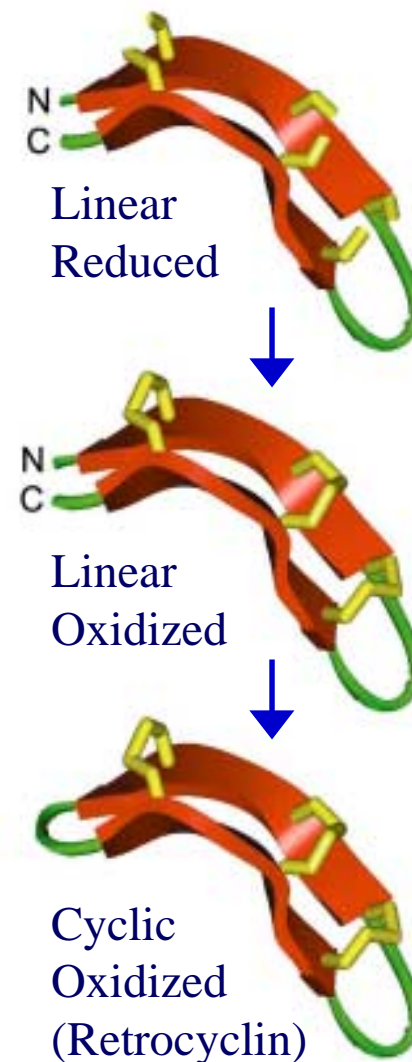
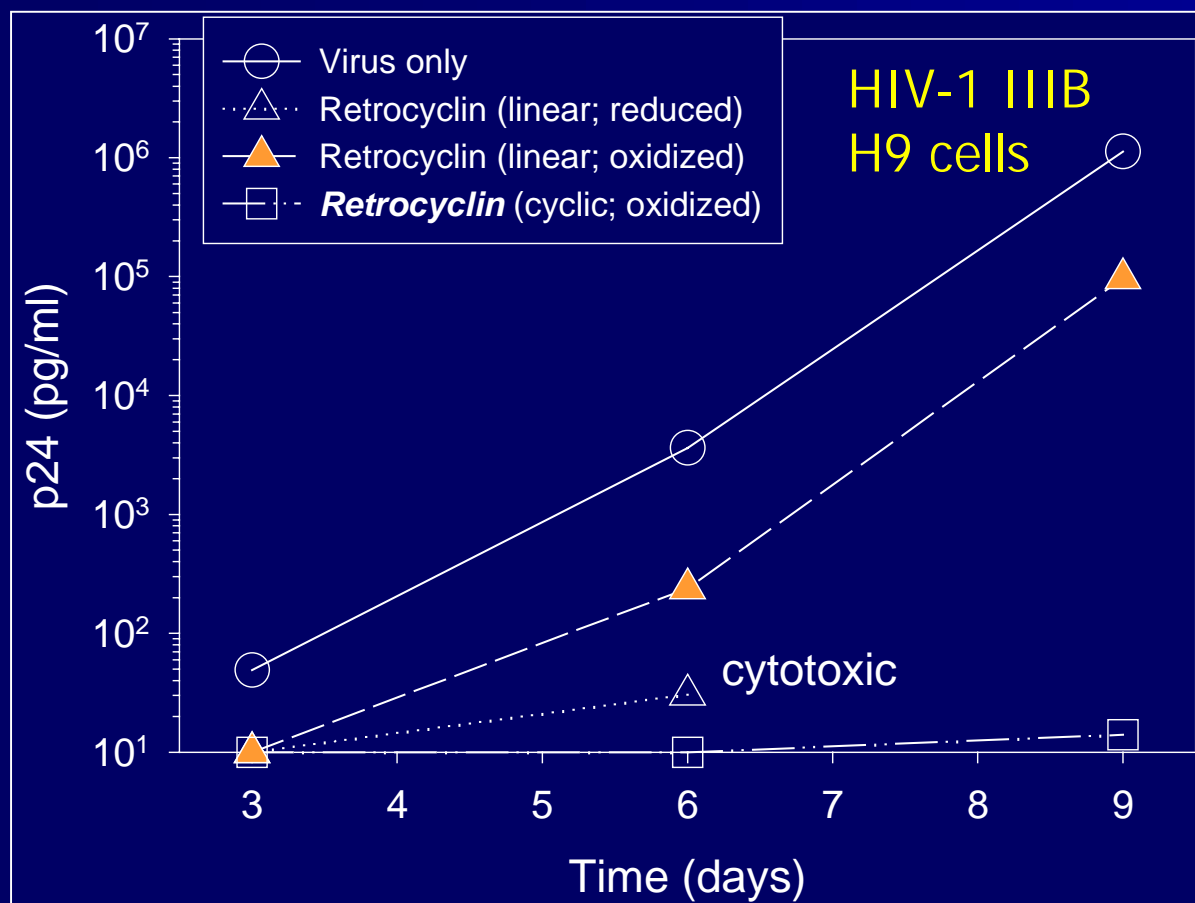
CD4<sup>+</sup>-selected PBMC; Day 9

# Retrocyclin is active when administered up to 24 hrs post-infection

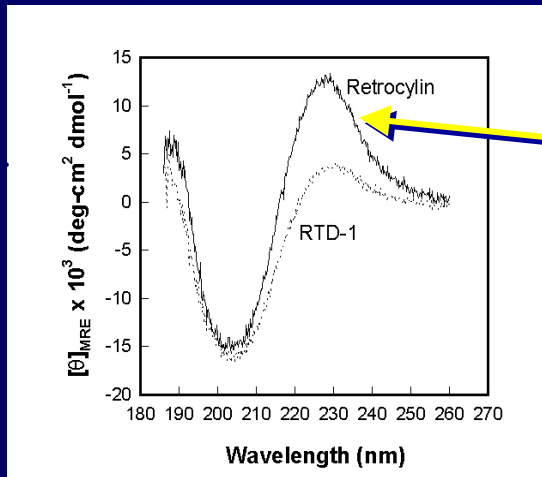


HIV-1 IIB  
H9 cells

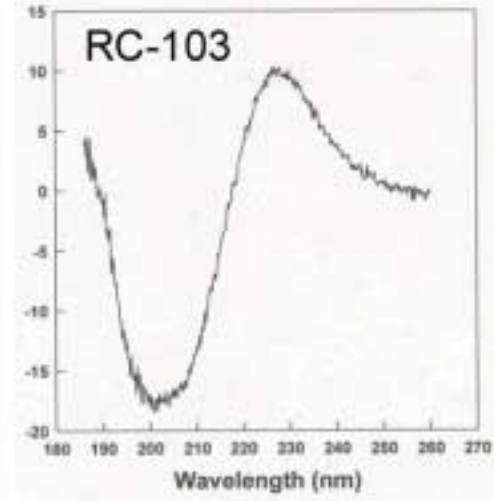
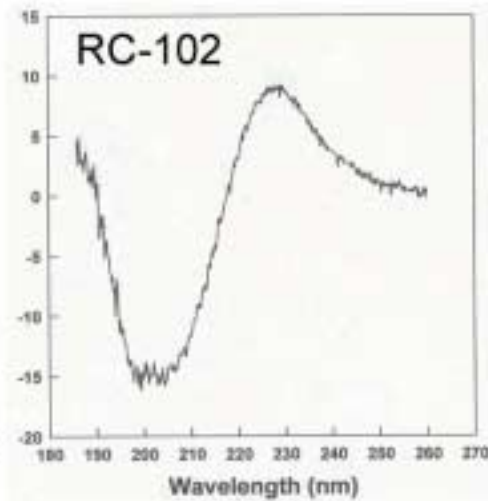
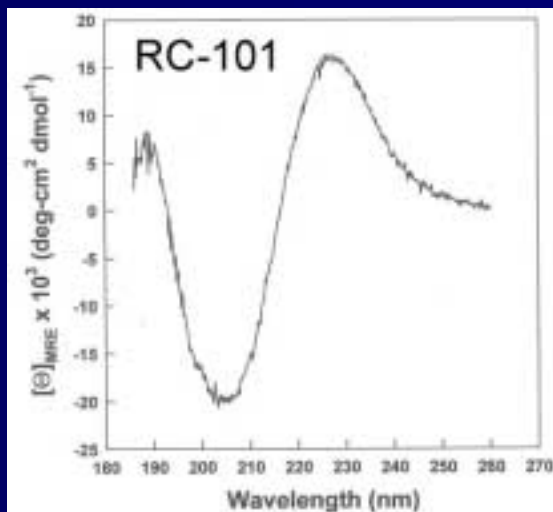
# Linear forms of retrocyclin do not inhibit HIV-1 replication



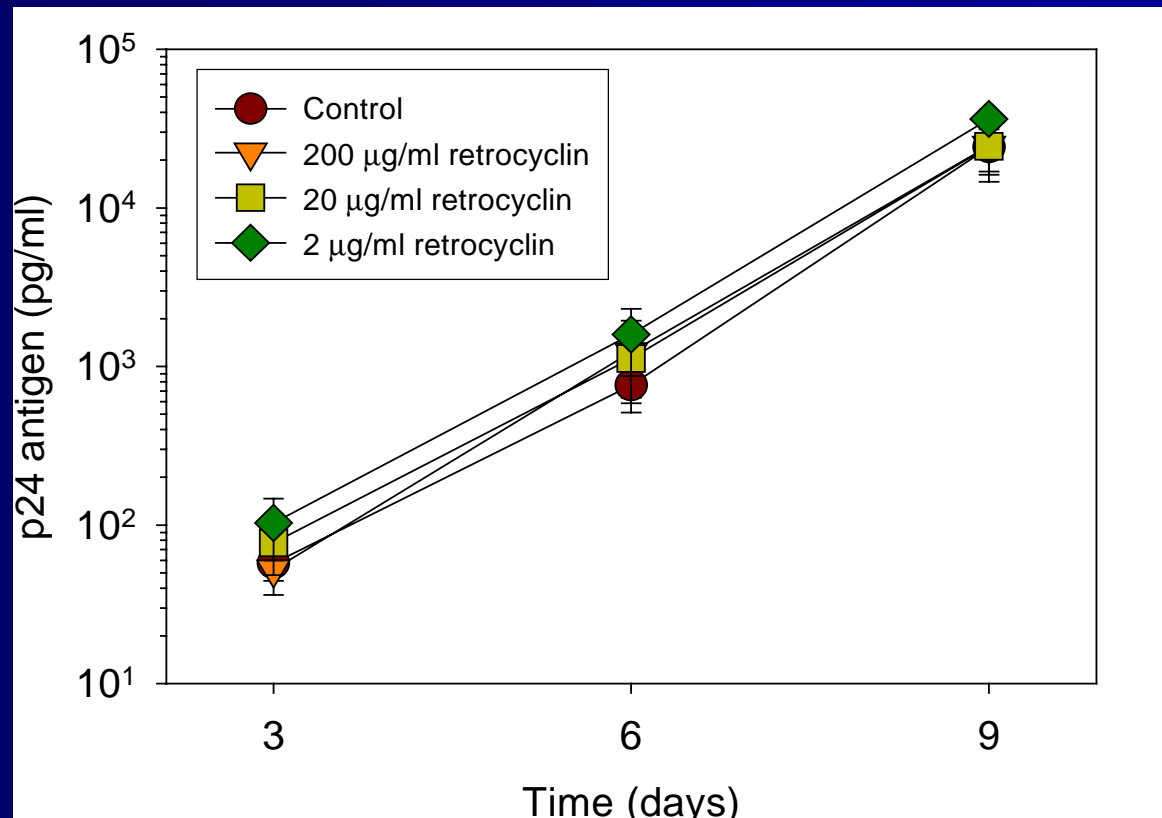
# RC-101, RC-102 and RC-103 share high structural identity with retrocyclin



Retrocyclin (RC-100)



# Adding retrocyclin directly to HIV-1 III B does not reduce infection of CD4<sup>+</sup> PBMC

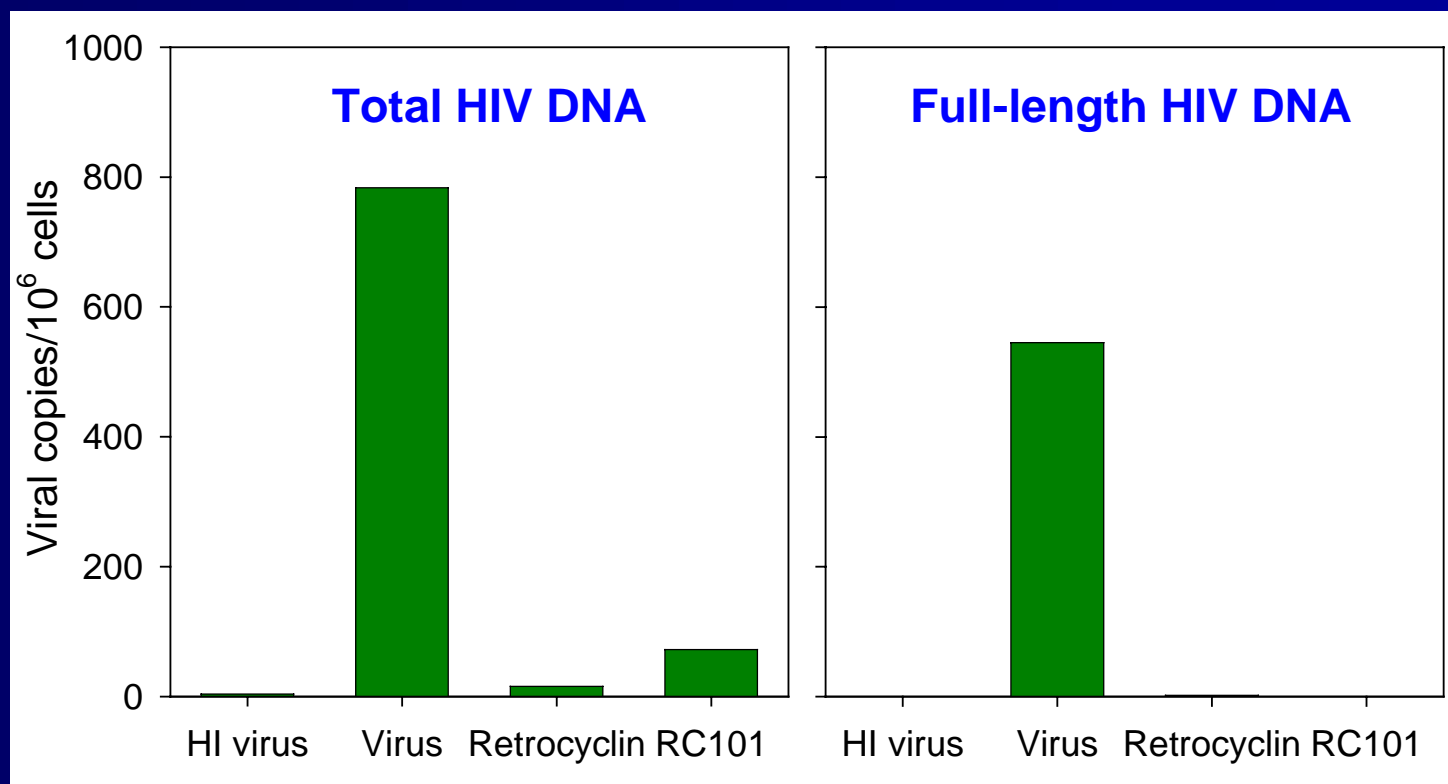


**Note:** Retrocyclin added to HIV-1 JR-CSF also did not reduce infection of CD4<sup>+</sup> PBMC (*not shown*).

# Retrocyclins inhibit the formation of HIV-1 proviral DNA

Quantitative real time PCR

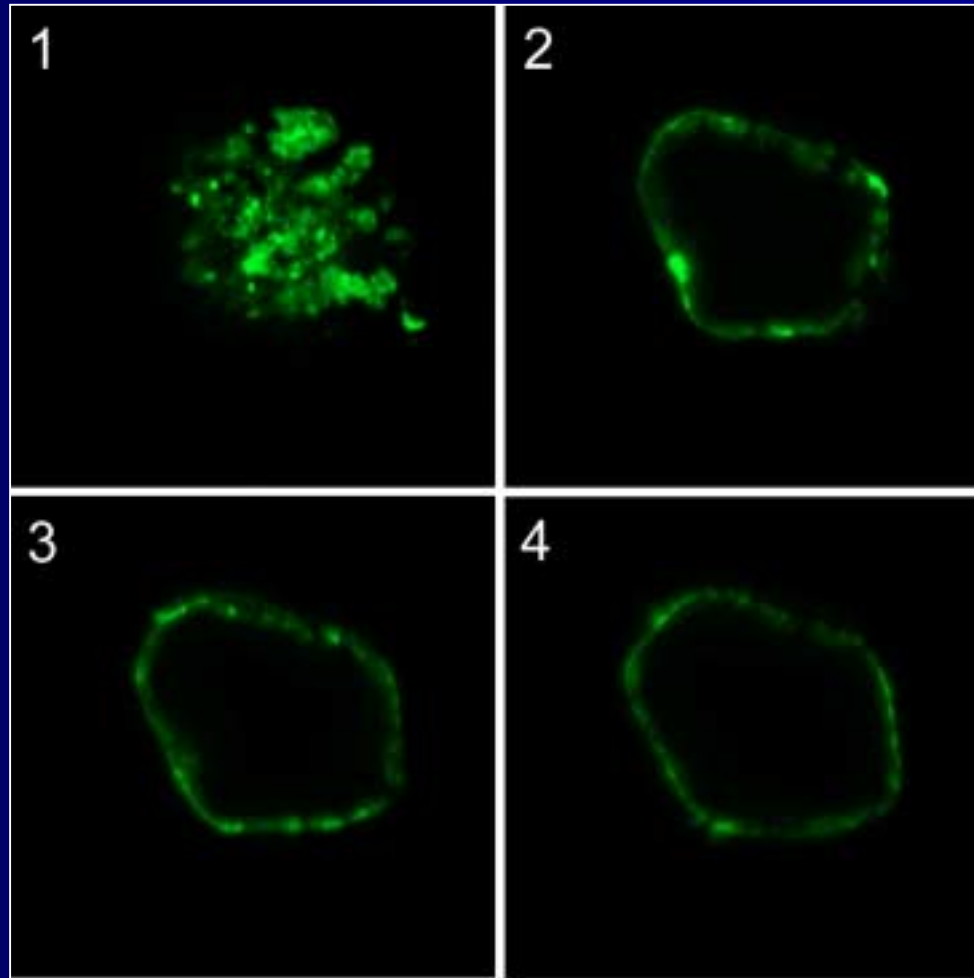
HIV-1 JR-CSF  
CD4<sup>+</sup> PBMC



(R/U5 junction)

(LTR/gag junction)

# Confocal microscopy localizes retrocyclin to membrane "rafts" on CD4<sup>+</sup> PBMC



*RC-101*<sub>BODIPY-FL</sub>

# Summary of select studies not shown ...

- Modest activity against other microbes:
  - Gram-positive bacteria
  - Gram-negative bacteria
  - HSV-1 and HSV-2 (Dr. Wagar)
  - *Suggests activity is more specific to HIV-1?*

# Acknowledgments

- *Robert I. Lehrer, M.D.*
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