

# HIV Entry Inhibitors: Molecular Targets

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## Viral:

- gp120
- gp41

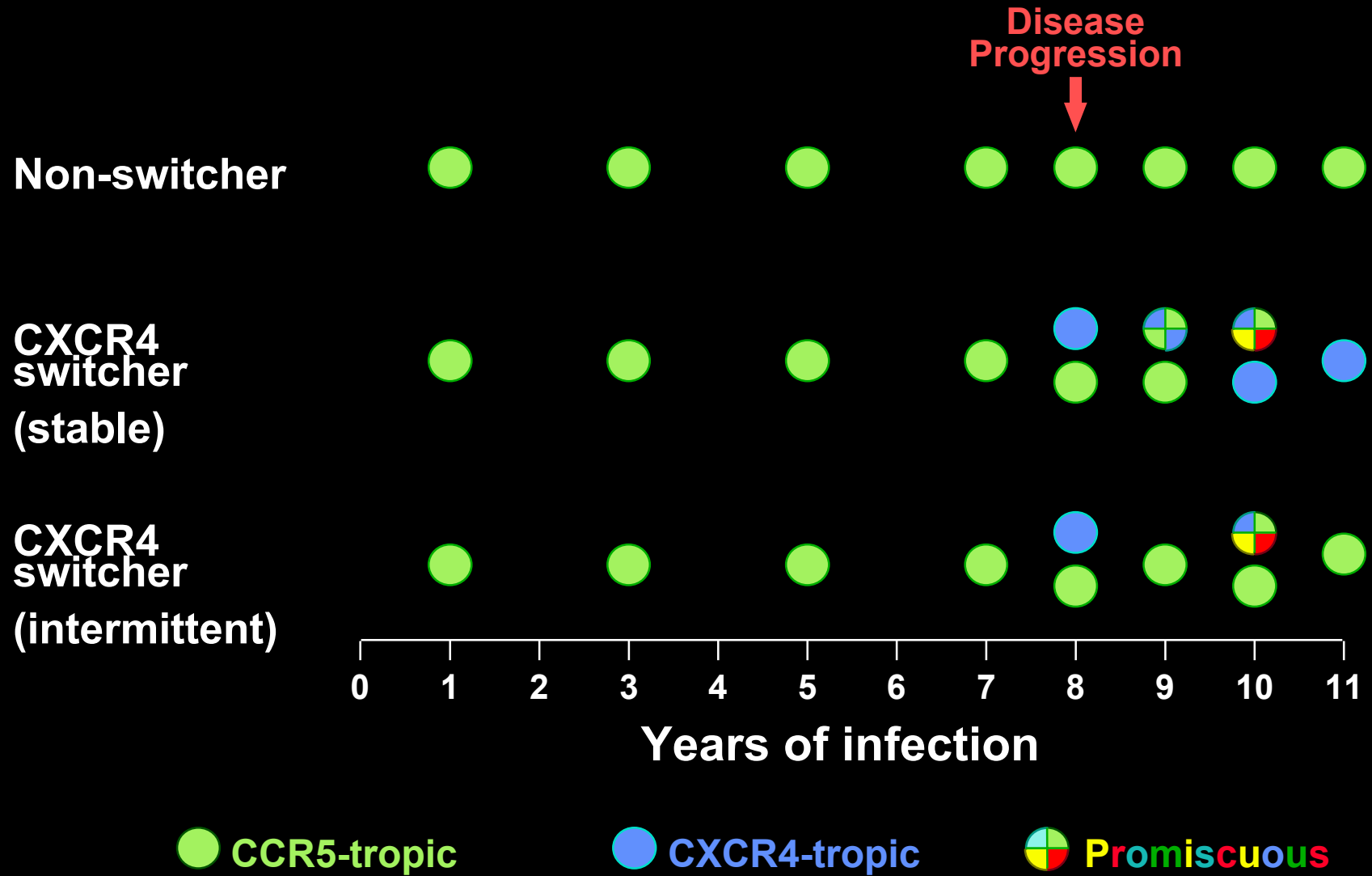
## Cellular:

- CD4
  - Coreceptors
-

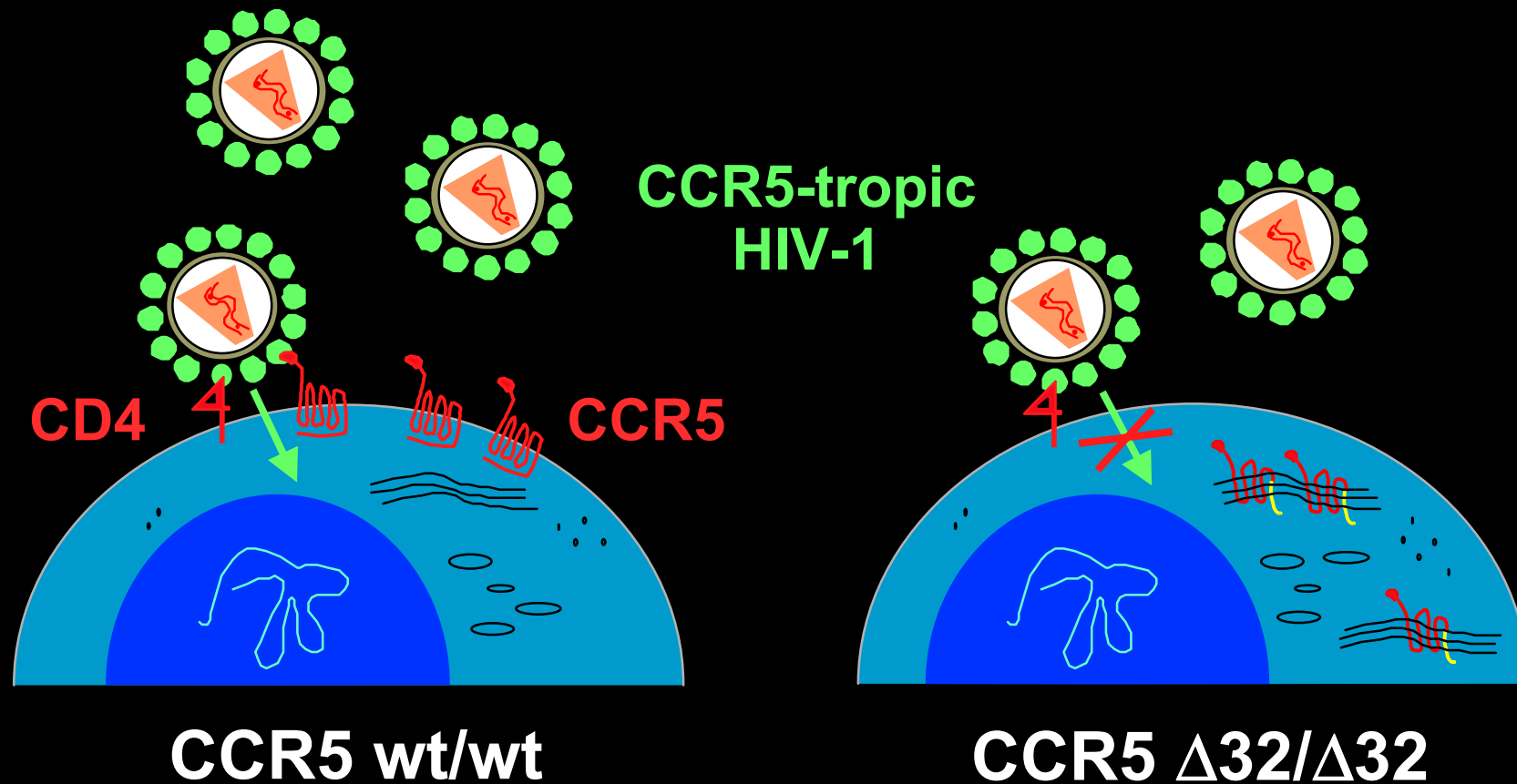


**Is CCR5 a prime target  
for microbicides?**

# Evolution of HIV-1 in Patients with Progressive Disease

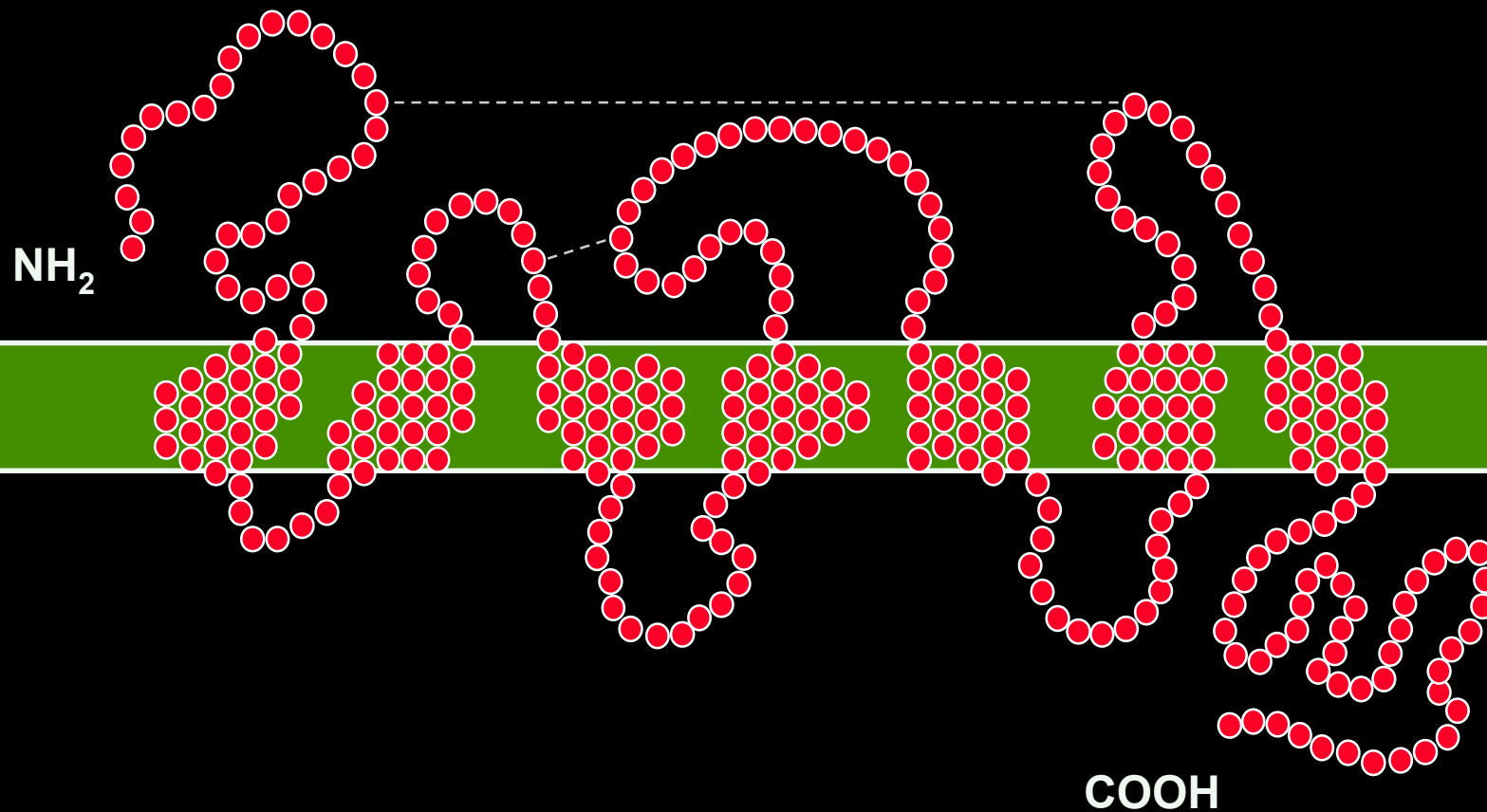


# Resistance to HIV-1 Infection in Cells from Homozygous CCR5- $\Delta$ 32 Subjects



**Can we rationally design  
effective CCR5 inhibitors?**

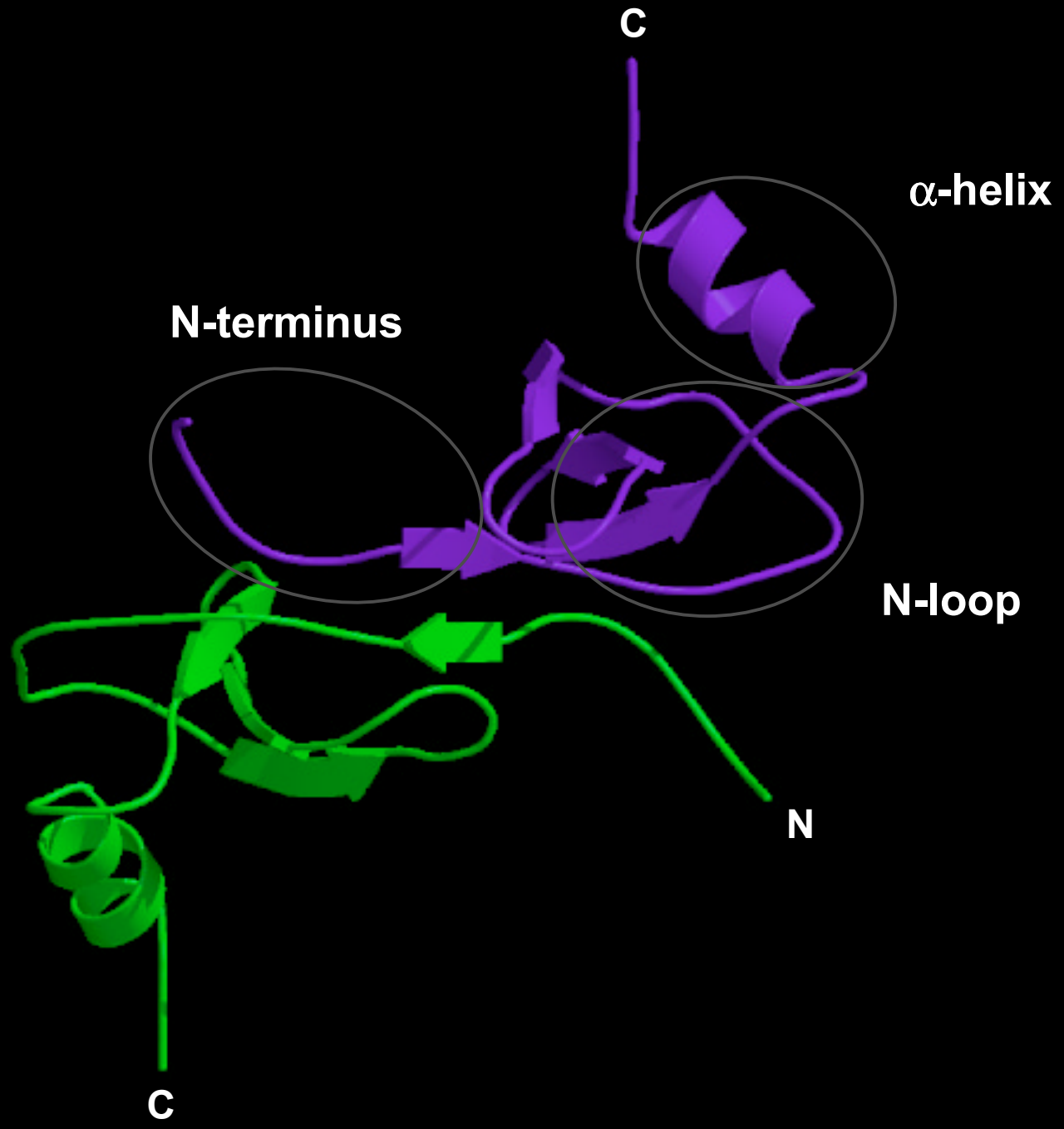
# 7-Transmembrane-Domain, G-Protein-Coupled Receptors



# Learning from Chemokines

# Solution Structure of RANTES





**N-terminus**



# N-Terminal Sequence of Two Mutated RANTES Analogues

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**RANTES**

**SPYSSDTPCCF-**

**L-RANTES**

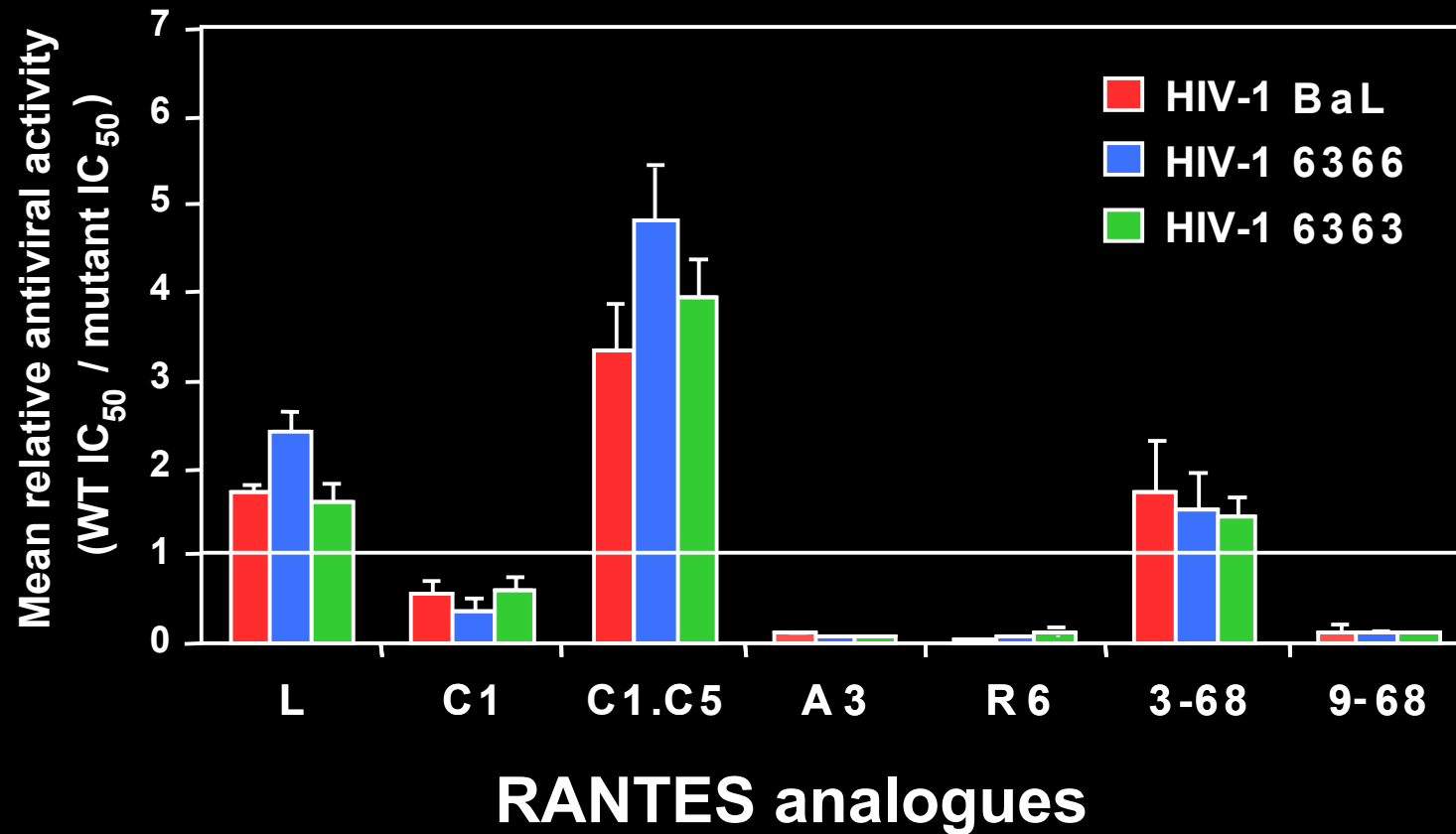
**L-SPYSSDTPCCF-**

**C1.C5-RANTES**

**CPYSCDTPCCF-**

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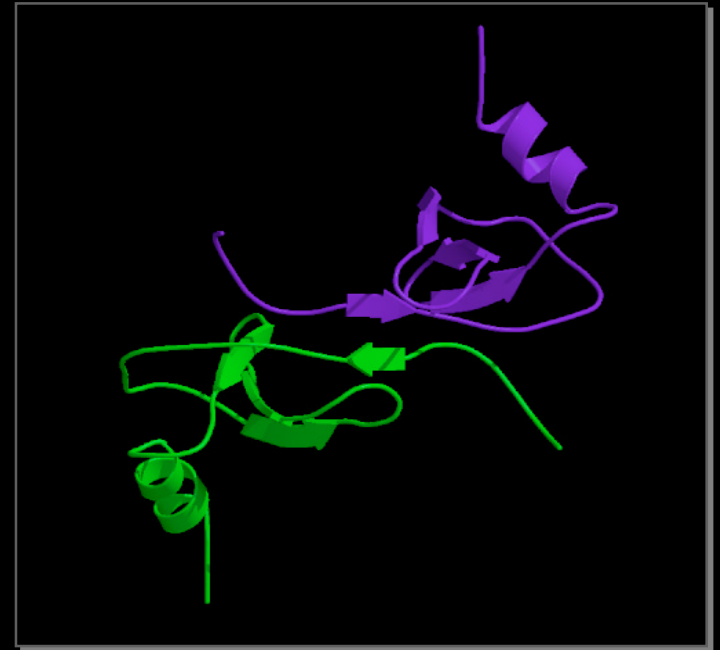
# Antiviral Activity of RANTES NH<sub>2</sub>-Terminal Mutants



**Identification of RANTES  
Functional Domains  
by Peptide Scanning**

# Peptide Scanning of RANTES

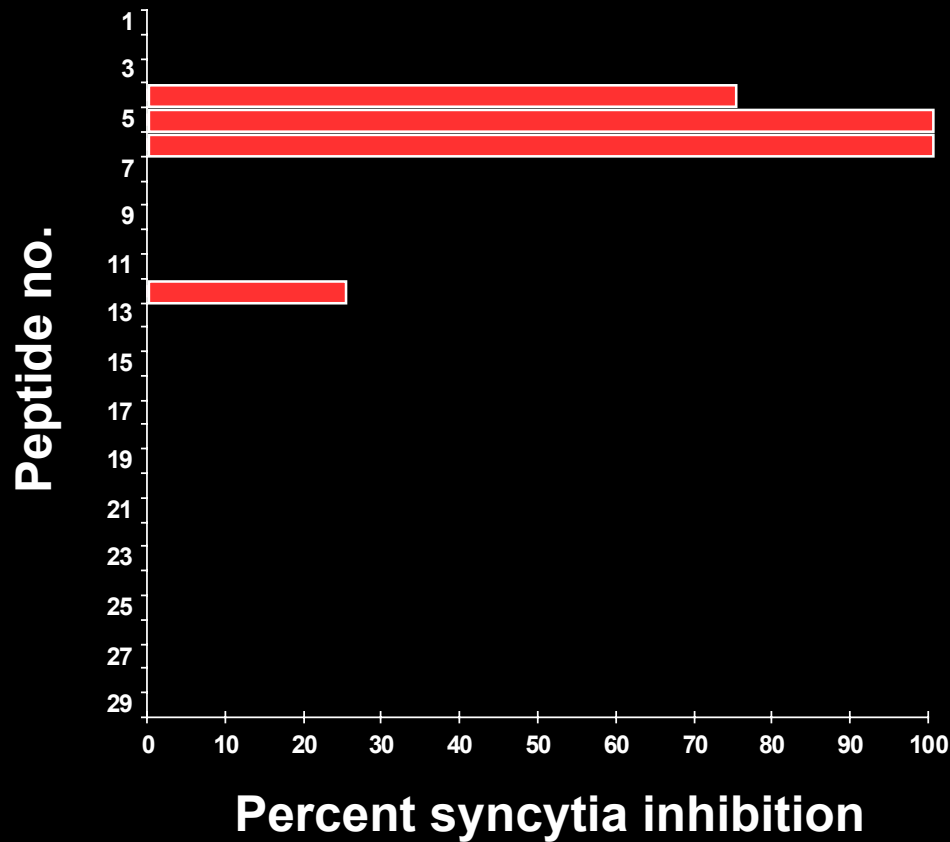
1	SPYSSD	TPCCF			
2	..YSSD	TPCCFAY			
3	....SD	TPCCFAYIA			
4	.....T	TPCCFAYIARP			
5	.....P	CCFAYIARLP			
6	.....C	FAYIARLPRA			
7	.....A	YIARLPRAHI			
8	.....I	ARLPRAHIKE			
9	.....R	PLPRAHIKEYF			
10	.....L	PRAHIKEYFYT			
11	.....R	AHIKEYFYTSG			
12	.....H	IKEYFYTSGKC			
13	.....K	EYFYTSGKCSN			
14	.....Y	FYTSGKCSNPA			
15	.....Y	TSGKCSNPAVV			
16	.....S	GKCSNPAVVFV			
17	.....K	CNPAVVFVTR			
18	.....S	NPAVVFVTRKN			
19	.....P	AVVFVTRKNRQ			
20	.....V	VVFVTRKNRQVC			
21	.....F	VTRKNRQVCAN			
22	.....T	RKNRQVCANPE			
23	.....K	NRQVCANPEKK			
24	.....R	QVCANPEKKWV			
25	.....V	CANPEKKWVRE			
26	.....A	NPEKKWVREYI			
27	.....P	EKKWVREYINS			
28	.....K	KWVREYINSLE			
29	.....W	VREYINSLEMS			



1            10            20            30            40            50            60  
 SPYSSDTPCCFAYIARLPRAHIKEYFYTSGKCSNPAVVFVTRKNRQVCANPEKKWVREYINSLEMS



# Inhibition of HIV-1<sub>BaL</sub>-Induced Syncytia Formation by RANTES Peptides



```

1 SPYSSDTPCCF
2 ..YSSDTPCCFAY
3 ...SDTPCCFAYIA
4 .....TPCCFAYIARP
5 .....PCCFAYIARLP
6 .....CFAYIARPLPRA
7 .....AYIARPLPRAHI
8 .....IARPLPRAHIKE
9 .....RPLPRAHIKEYF
10 .....LPRahiKEYFYT
11 .....RAHIKEYFYTSG
12 .....HIKEYFYTSgKc
13 .....KEYFYTSgKcSN
14 .....YFYTSgKcSNPA
15 .....YTSGKcSNPAVV
16 .....SGKcSNPAVVFV
17 .....KcSNPAVVFVTR
18 .....SNPAVVFVTRKN
19 .....PAVVFVTRKNRQ
20 .....VVFVTRKNRQVC
21 .....FVTRKNRQVCAN
22 .....TRKNRQVCANPE
23 .....KNRQVCANPEKK
24 .....RQVCANPEKKWV
25 .....VCANPEKKWVRE
26 .....ANPEKKWVREYI
27 .....PEKKWVREYINS
28 .....KKWVREYINSLE
29 .....WVREYINSLEMS
    
```

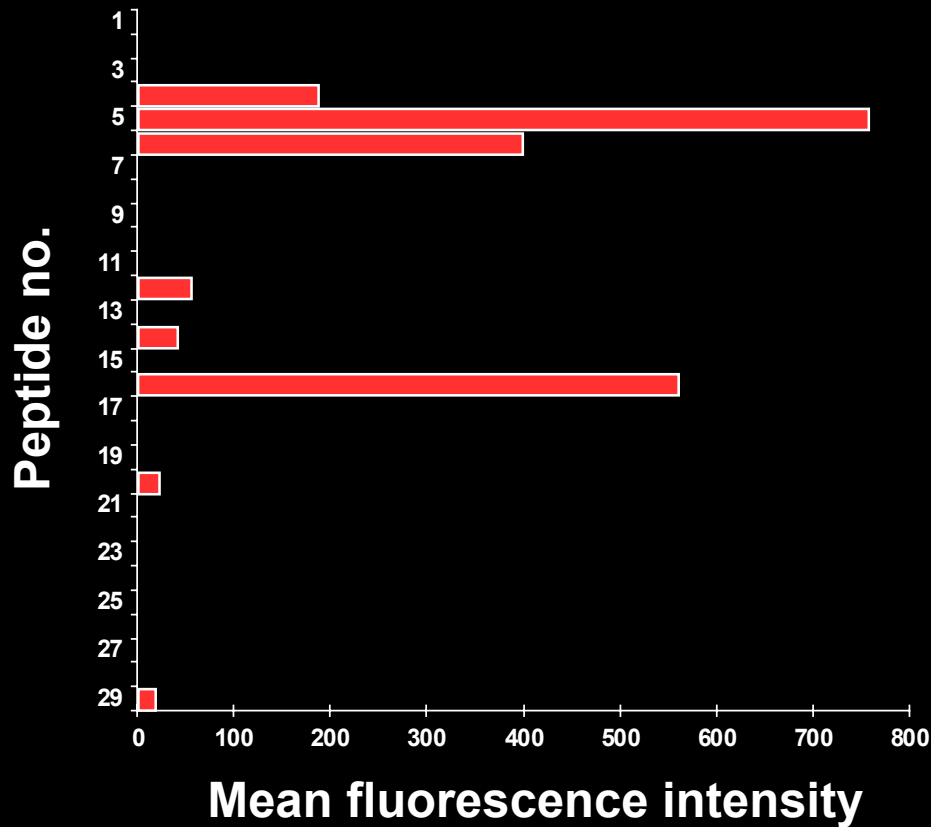
1 10 20 30 40 50 60  
 SPYSSDTPCCFAYIARPLPRAHIKEYFYTSGKcSNPAVVFVTRKNRQVCANPEKKWVREYINSLEMS





**N-loop**

# Binding of RANTES Peptides to CCR5-Expressing Cells



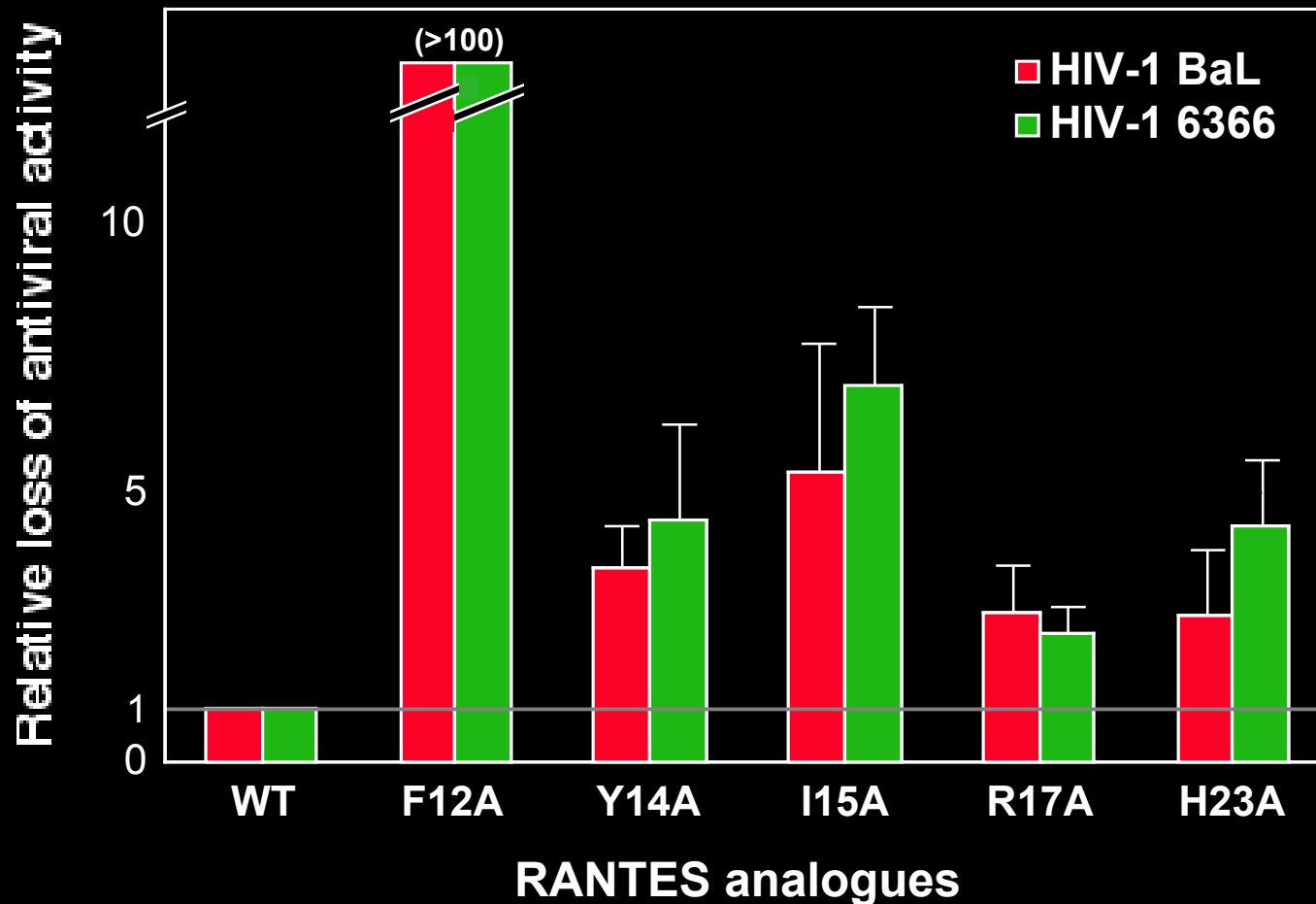
```

1 SPYSSDTPCCF
2 ..YSSDTPCCFAY
3 ...SDTPCCFAYIA
4 .....TTPCCFAYIARP
5 .....PCCFAYIARLP
6 .....CFAYIARLPRA
7 .....AYIARLPRAHI
8 .....IARLPRAHIKE
9 .....RPLPRAHIKEYF
10 .....LPRahiKEYFYT
11 .....RAHIKEYFYTSG
12 .....HIKEYFYTSGKC
13 .....KEYFYTSGKCSN
14 .....YFYTSGKCSNPA
15 .....YTSKCSNPAVV
16 .....SGKCSNPAVVFV
17 .....KCSNPAVVFVTR
18 .....SNPAVVFVTRKN
19 .....PAVVFVTRKNRQ
20 .....VVFVTRKNRQVC
21 .....FVTRKNRQVCAN
22 .....TRKNRQVCANPE
23 .....KNRQVCANPEKK
24 .....RQVCANPEKKWV
25 .....VCANPEKKWVRE
26 .....ANPEKKWVREYI
27 .....PEKKWVREYINS
28 .....KKWVREYINSLE
29 .....WVREYINSLEMS
    
```

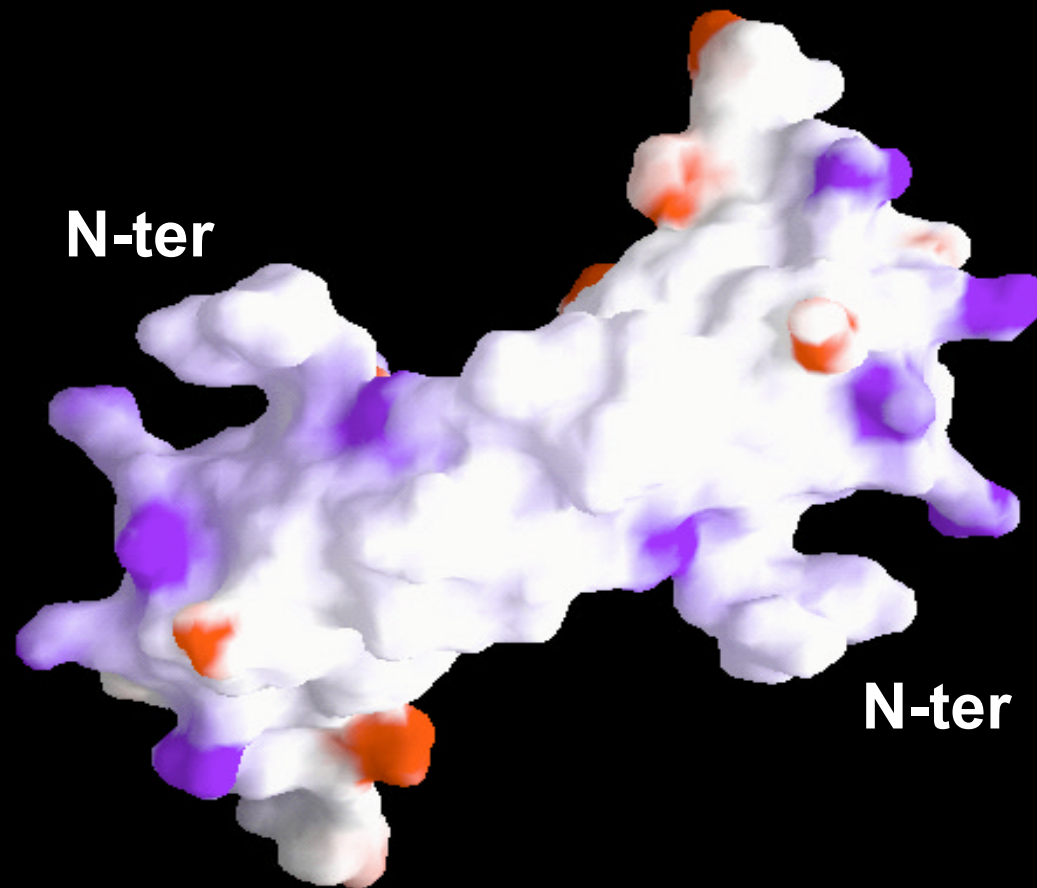
1 10 20 30 40 50 60  
 SPYSSDTPCCFAYIARLPRAHIKEYFYTSGKCSNPAVVFVTRKNRQVCANPEKKWVREYINSLEMS



# Effect of Single Alanine Substitutions within the N-loop Region on the Antiviral Activity of RANTES

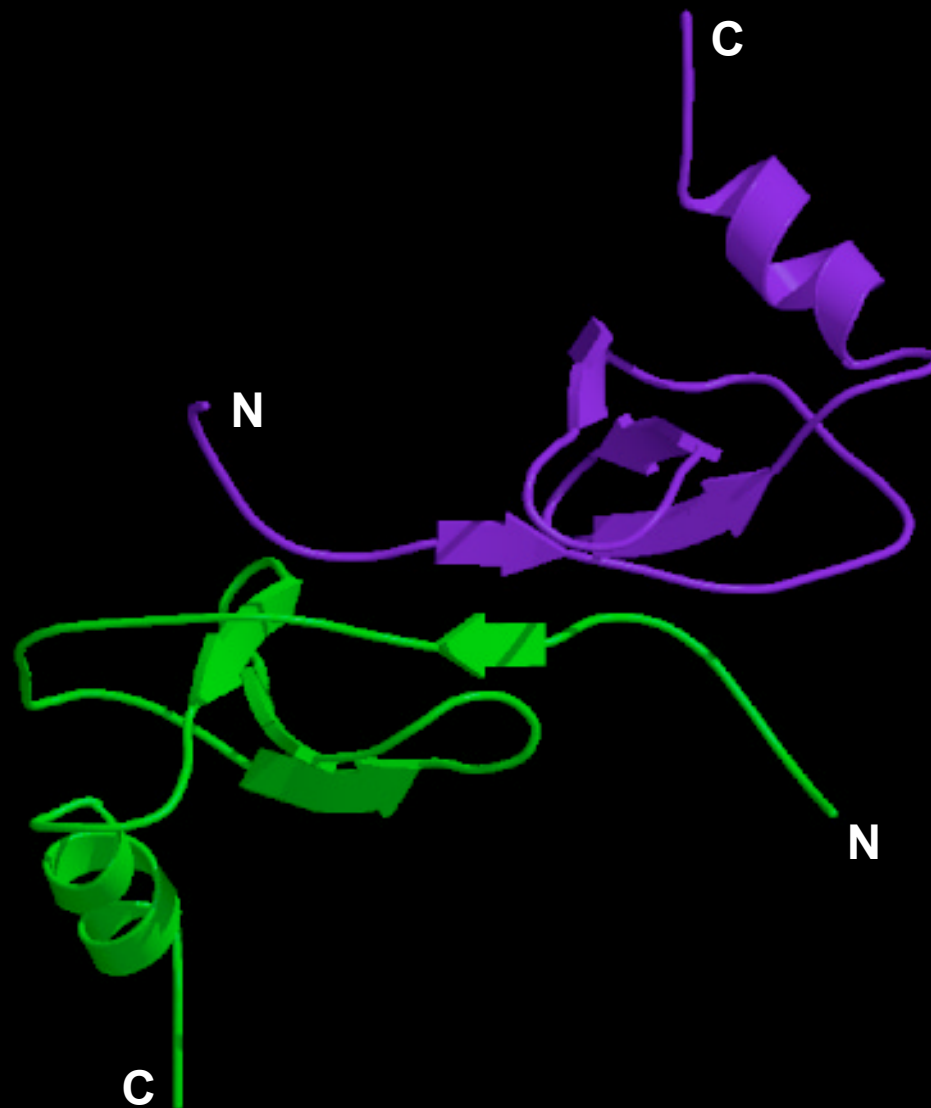


# Charge Distribution on the Surface of the RANTES Dimer

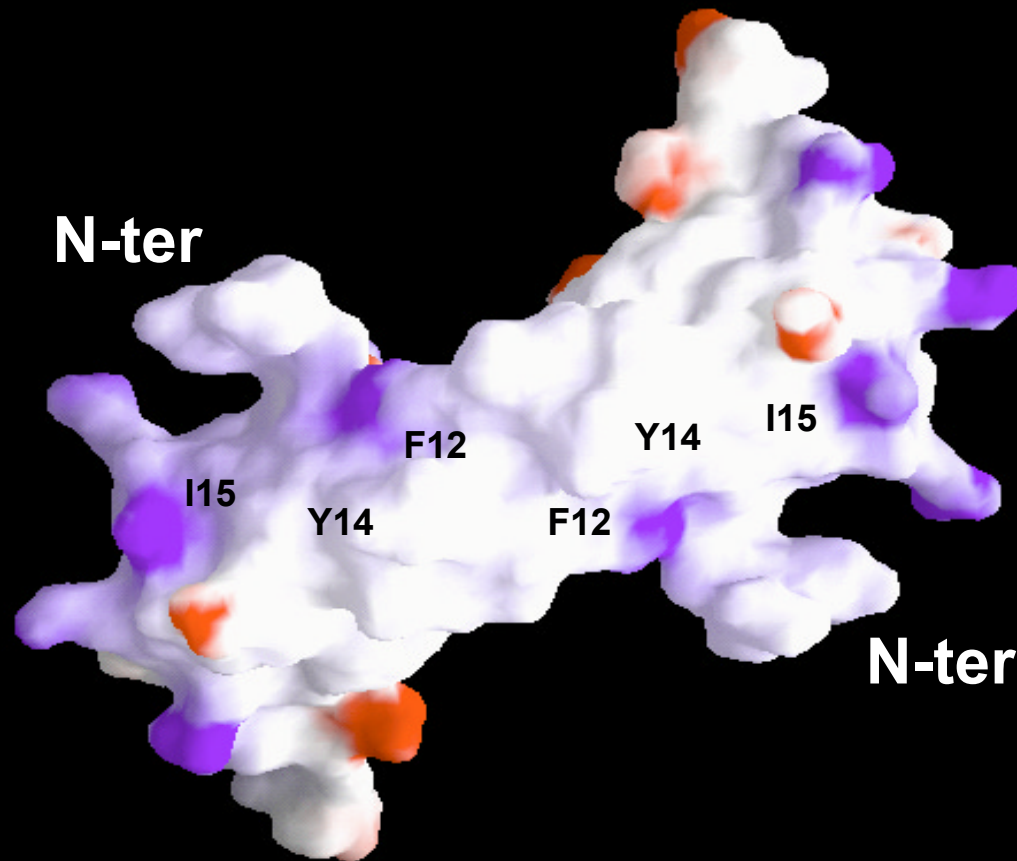


■ Positive charge  
■ Negative charge

# Solution Structure of the RANTES Dimer

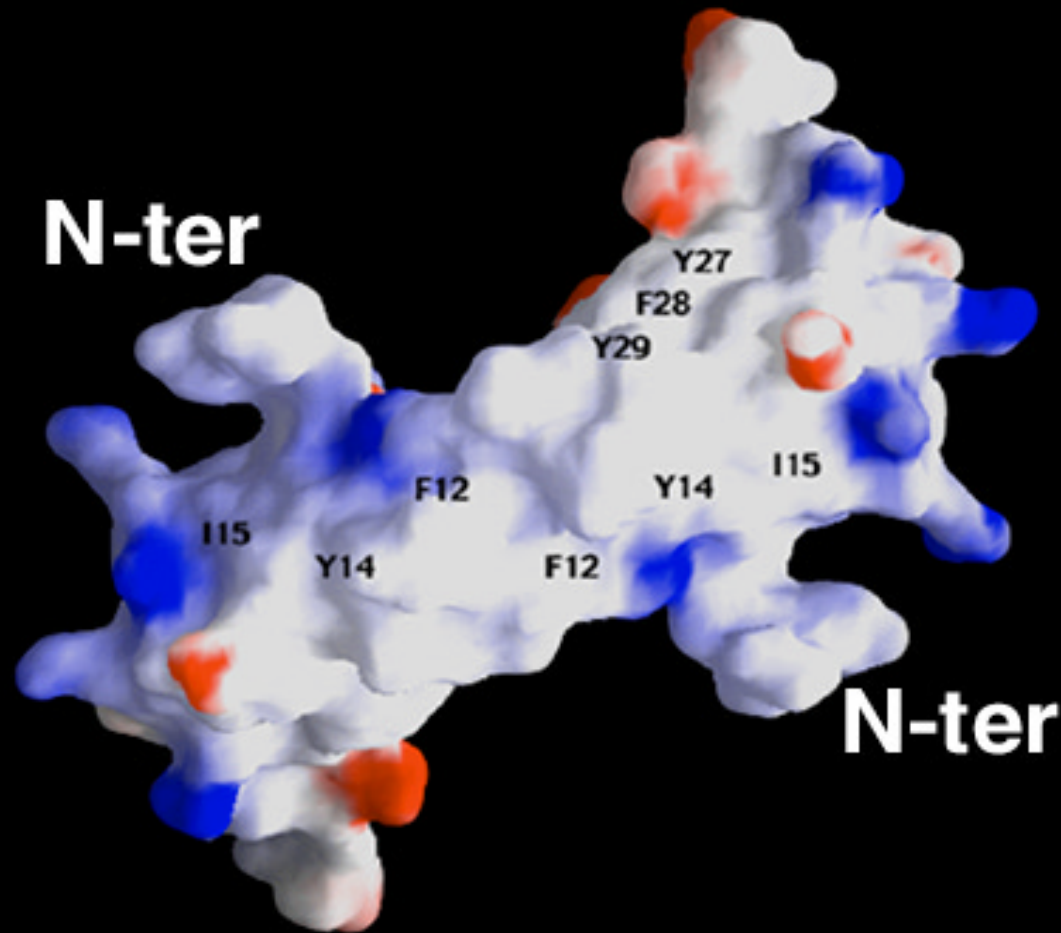


# Charge Distribution on the Surface of the RANTES Dimer



■ Positive charge  
■ Negative charge

# Charge Distribution on the Surface of the RANTES Dimer



- Positive charge
- Negative charge

# Synthetic Peptides Derived from the N-Loop Region of RANTES

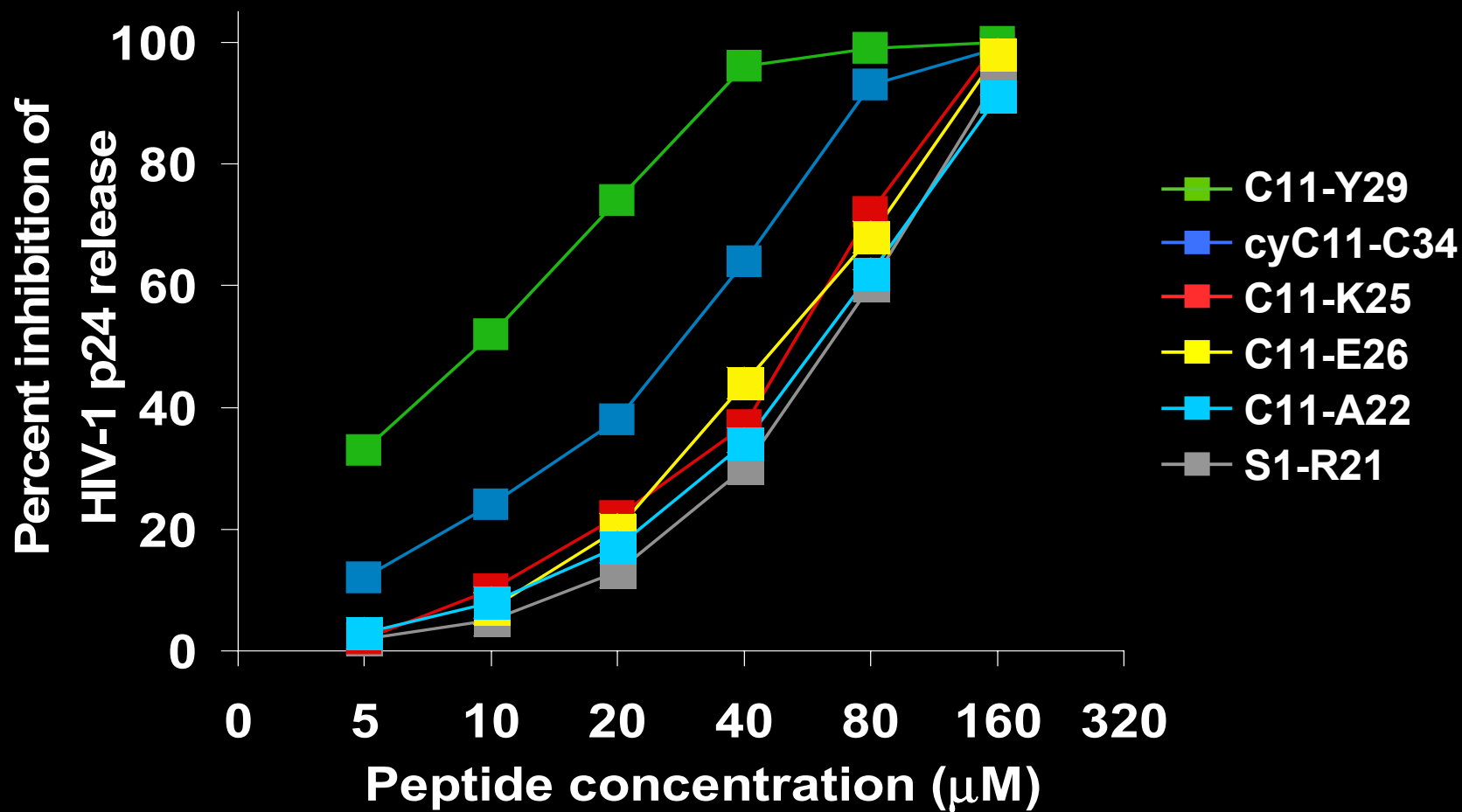
CFAYIARPLPRA  
SPYSSDTPCCCFAYIARPLPR  
CFAYIARPLPRAHIK  
CFAYIARPLPRAHIKE  
CFAYIARPLPRAHIKEYFY  
CFAYIARPLPRAHIKEYFYTSGKC

1 11 27  
SPYSSDTPCCCFAYIARPLPRAHIKEYFYTSGKC-

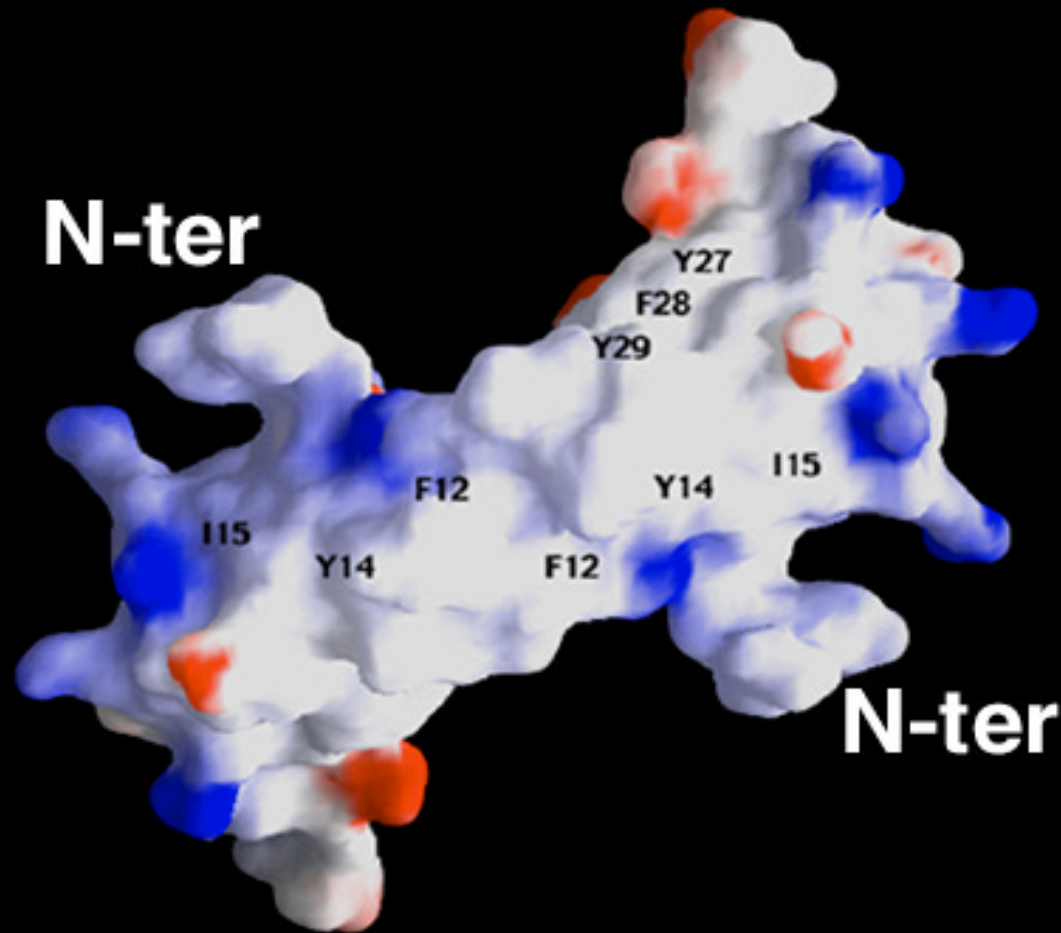
$\beta$ 0 N-Loop  $\beta$ 1

The diagram shows the full RANTES protein sequence: SPYSSDTPCCCFAYIARPLPRAHIKEYFYTSGKC-. The sequence is divided into three regions: a cyan arrow labeled  $\beta$ 0 points to the first 11 residues (SPYSSDTPCCCF); a white line labeled N-Loop spans residues 12 to 26 (AYIARPLPRAHIKE); and a cyan arrow labeled  $\beta$ 1 points to the last 10 residues (FYTSGKC-). Residue numbers 1, 11, and 27 are positioned above the corresponding residues in the sequence.

# Inhibition of HIV-1<sub>BaL</sub> Infection in Primary Human PBMC by RANTES Peptides



# Charge Distribution on the Surface of the RANTES Dimer



- Positive charge
- Negative charge

# Peptide RANTES 11-29

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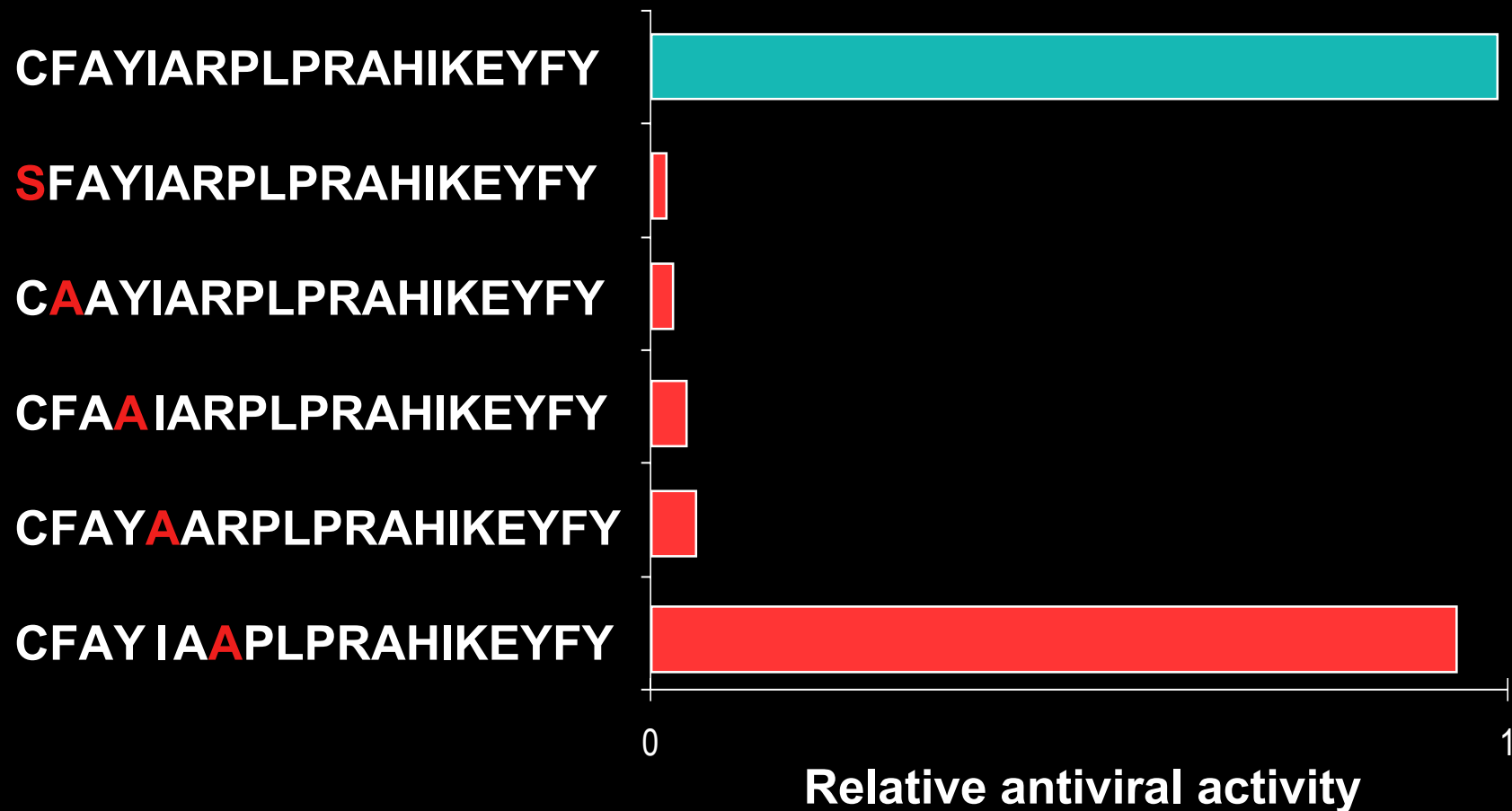
Linker

CFAYIARPLPRAHIKEYFY

Hydrophobic clusters

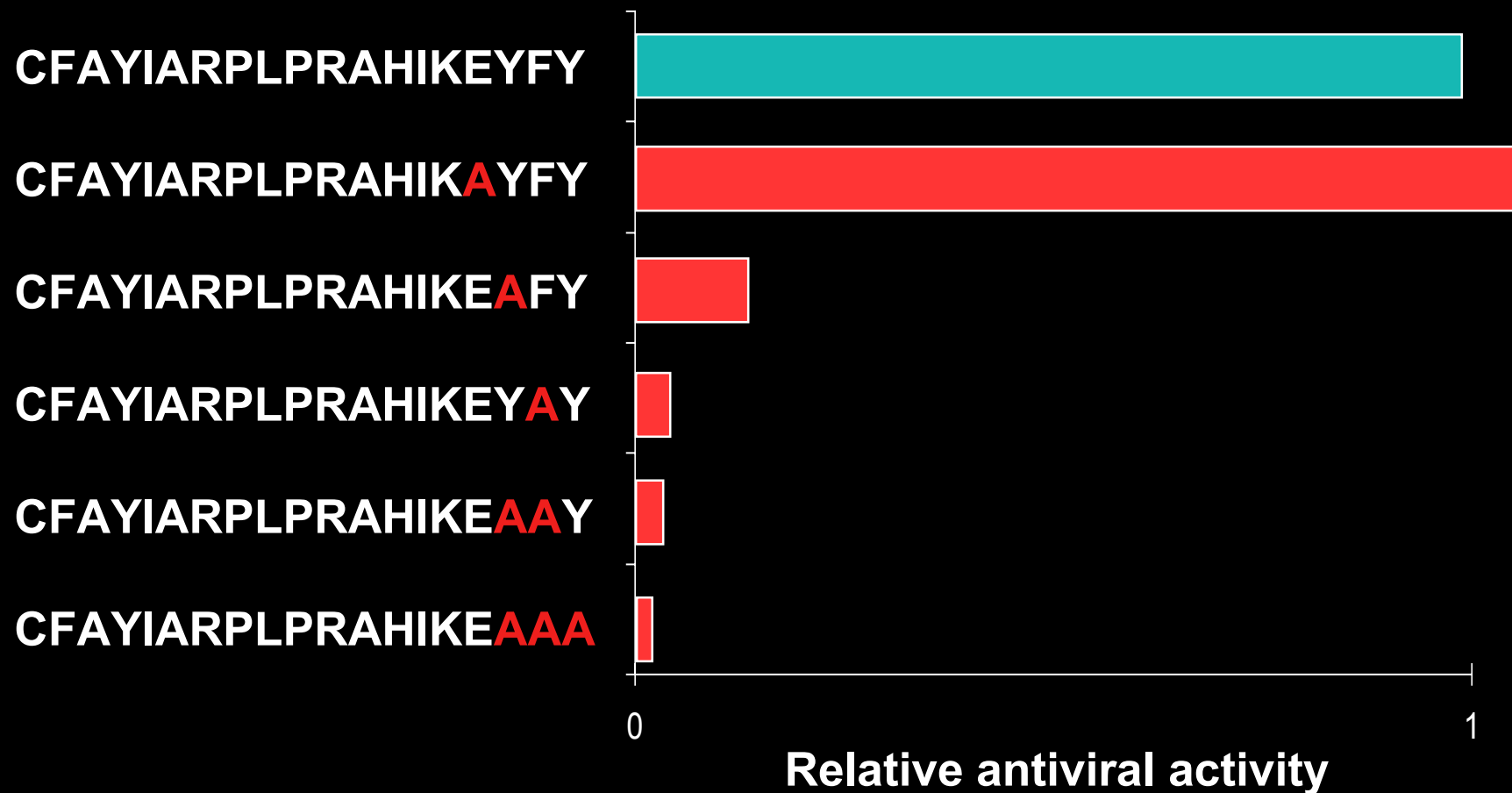
# The N-Terminal Hydrophobic Cluster of Peptide RANTES 11-29 Is Critical for HIV-1 Inhibition

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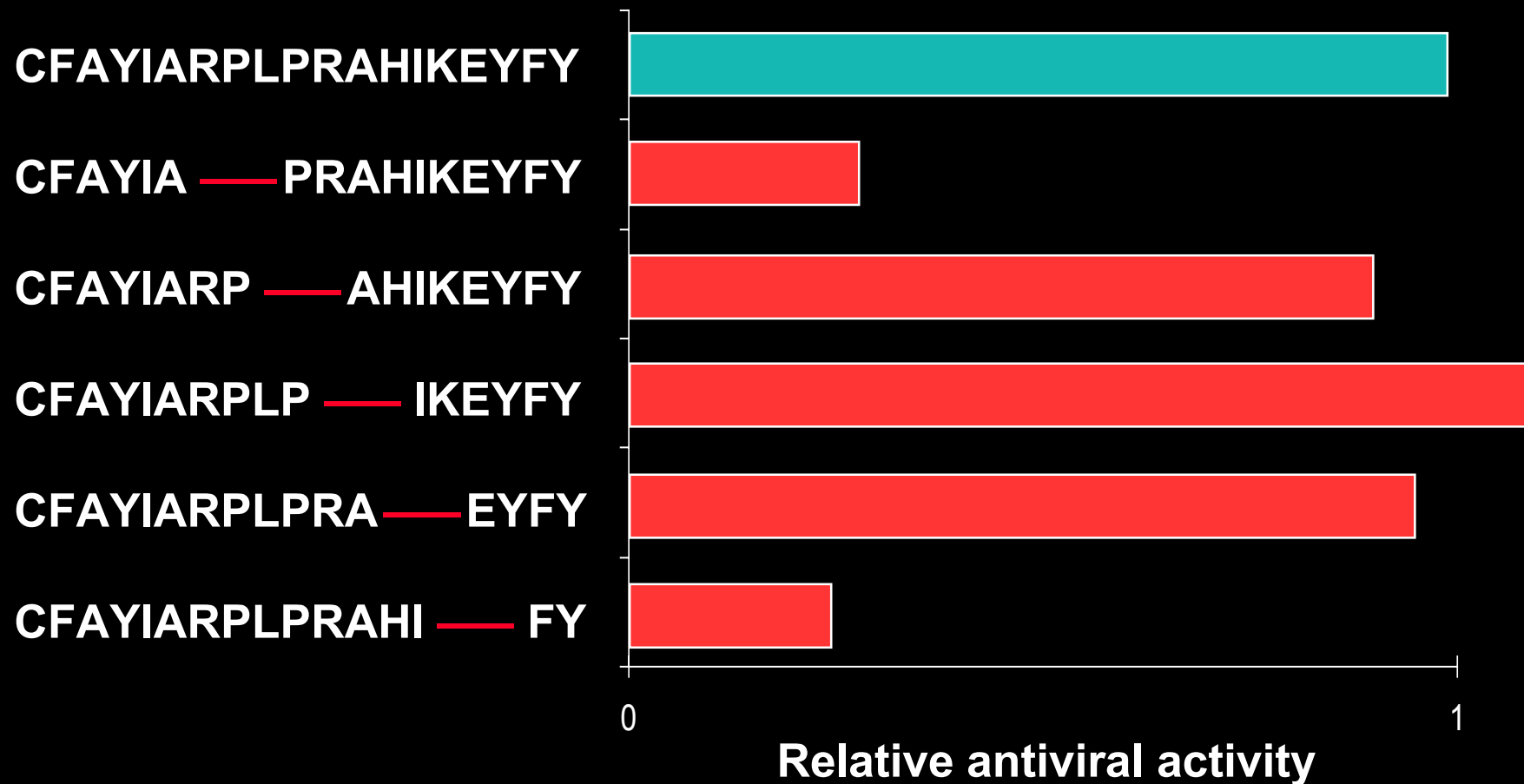
# The C-Terminal Aromatic Cluster of of Peptide RANTES 11-29 Is Critical for HIV-1 Inhibition

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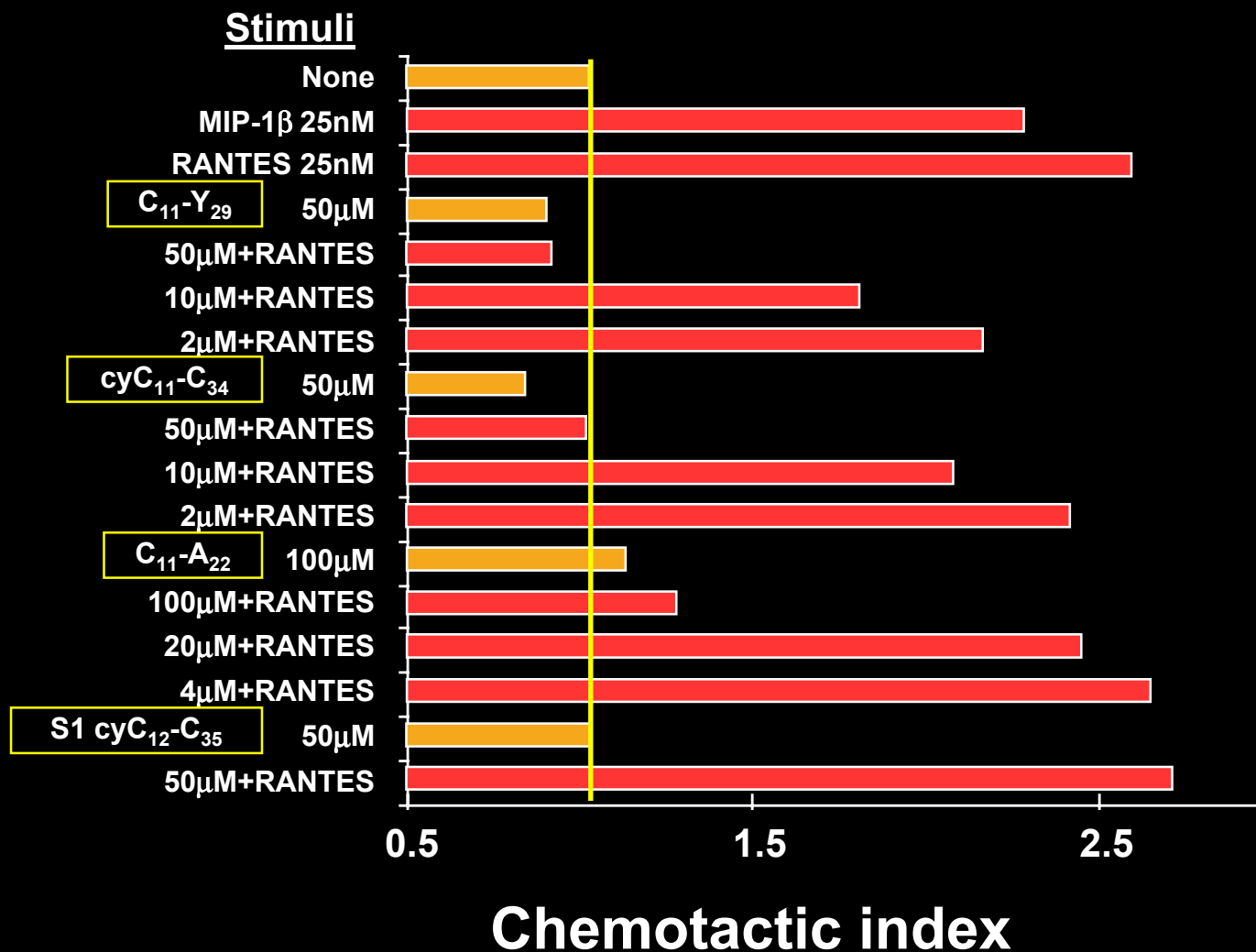
# The Central Region of Peptide RANTES 11-29 Is Dispensible for the HIV-Inhibitory Function

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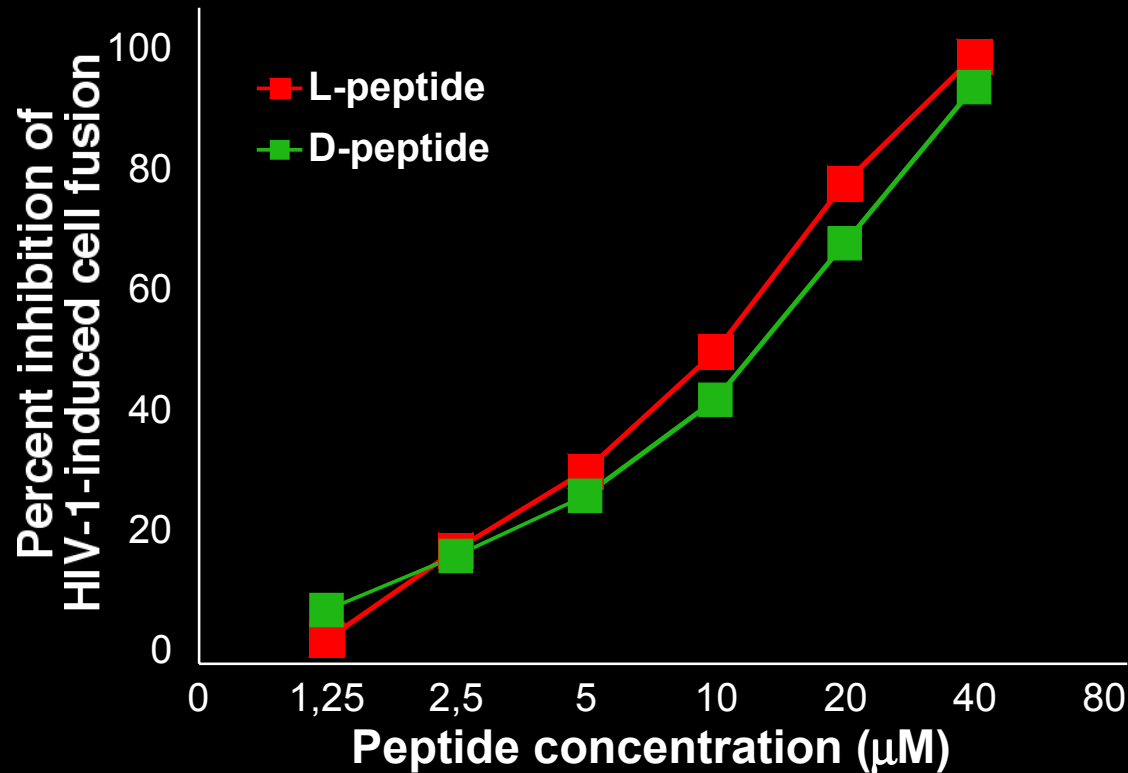
# **Uncoupling HIV-1 Blockade and Receptor Activation**

# Effect of RANTES Peptides on Primary Lymphocyte Chemotaxis



**Biological Activity of a  
Retro-Inverted Peptide  
Mimetic of RANTES 11-29**

# Inhibition of HIV-1-Induced Cell Fusion by Wild-Type and Retro-Inverted Peptide RANTES 11-29



L-peptide

**Ac-CFAYIARPLPRAHIKEYFY-nh<sub>2</sub>**

D-peptide

**nh<sub>2</sub>-CFAYIARPLPRAHIKEYFY-Ac**



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# Structural determinants of CCR5 recognition and HIV-1 blockade in RANTES

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Claudio DeSantis<sup>1</sup>, Paolo Sarmientos<sup>4</sup>, Menico Rizzi<sup>5</sup>,  
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