

Identification of HIV-1 Fusion Inhibitors Derived from Synthetic Combinatorial Libraries

César Boggiano and Sylvie Blondelle

Poster # 394-T

César Boggiano and Sylvie Blondelle
Torrey Pines Institute for Molecular Studies
3550 General Atomics Ct
San Diego CA 92121, USA
Ph. (858) 455-3803
Fax (858) 455-3804
cboggiano@tpims.org
sblondelle@tpims.org

Scientific background

- There is **a need for new antiretroviral agents against HIV** since the currently used reverse transcriptase (RT) and viral protease inhibitors cannot eradicate the infection and produce long-term side effects [1].
- Clinical trials have shown that a 36 amino acid peptide, T20, an HIV **fusion inhibitor**, was **effective in reducing viral loads** in HIV infected people to undetectable levels [2].
- **Synthetic combinatorial libraries**, which have been successfully used to identify biologically active compounds such as antifungal and antimicrobial agents [3], represent a powerful approach for the development of new antiretroviral agents.

Strategy

- use **peptide synthetic combinatorial libraries** as a source of new antiretroviral agents
- **screen** libraries in **two fusion model systems** resembling either R5 or X4 HIV entry.
- **identify** peptides from these libraries having **antagonistic effect** to HIV mediated fusogenic activity
- **test** antiretroviral activity of individual peptides using **fusion and replication** assays

Possible inhibitory pathways

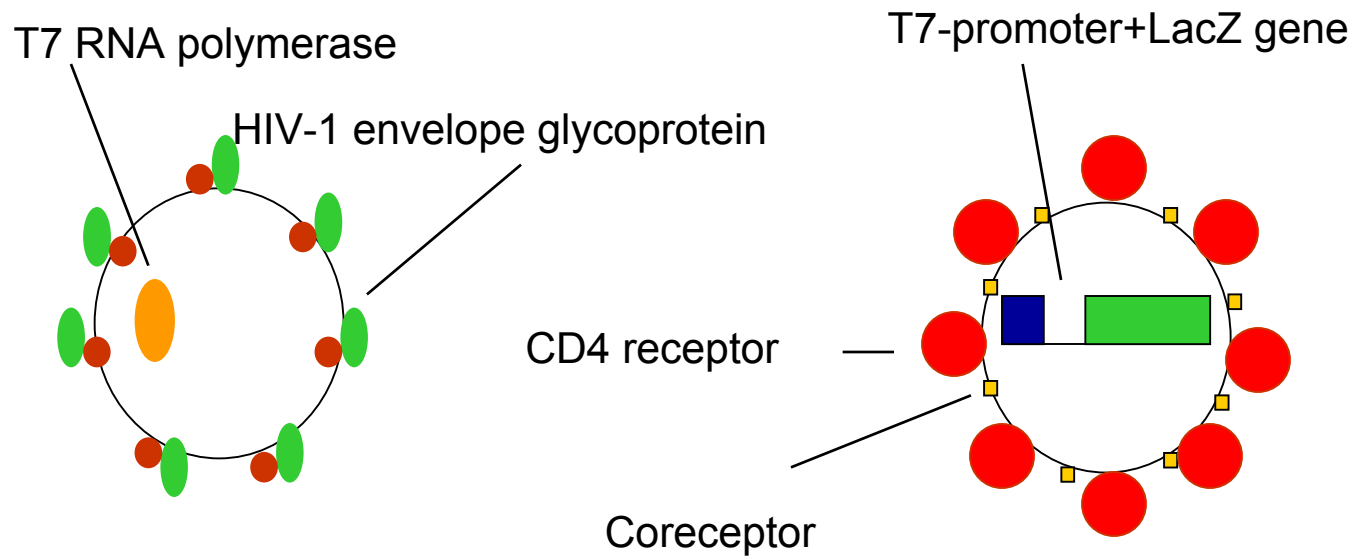
- inhibition of the **binding** between gp120 and the CD4/coreceptor complex
- inhibition of the **packing** of the C-terminal heptad repeat and **formation of the hairpin structure** of extended gp41 trans-membrane protein
- **interaction** with the gp41 N36 pocket

Model Systems

A recombinant vaccinia virus-based assay that measures the activation of a reporter gene (LacZ) upon fusion of two distinct cell populations [4].

Effector cells
[HIV surface mimic]

Target cells
[HIV host cell mimic]



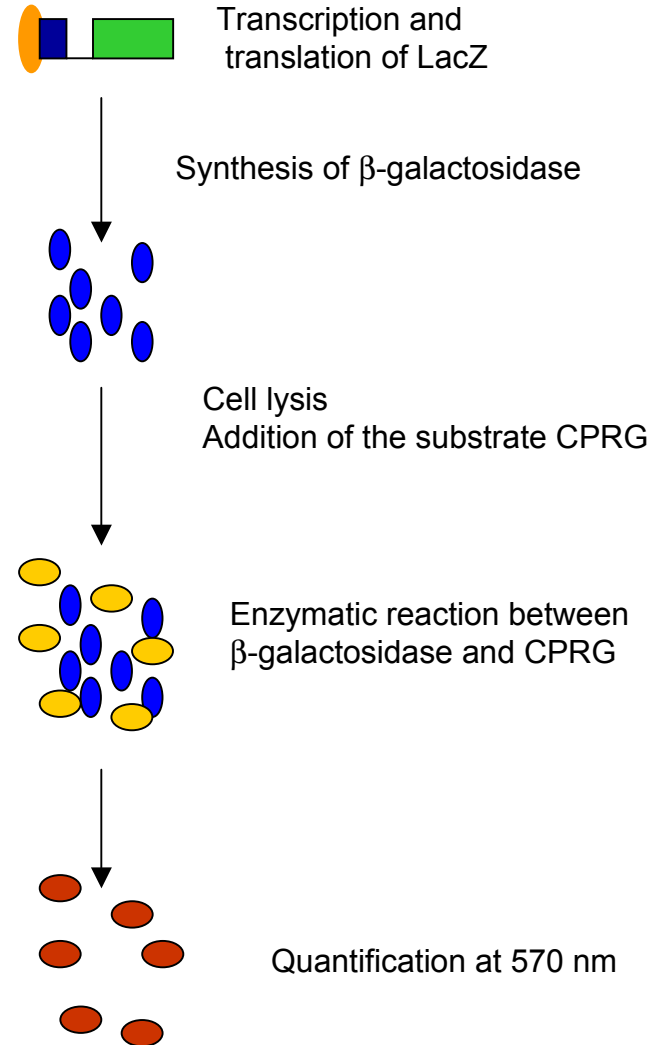
T-Tropic system: HIV envelope IIIb; coreceptor CXCR4

M-Tropic system: HIV envelope JR-FL; coreceptor CCR5

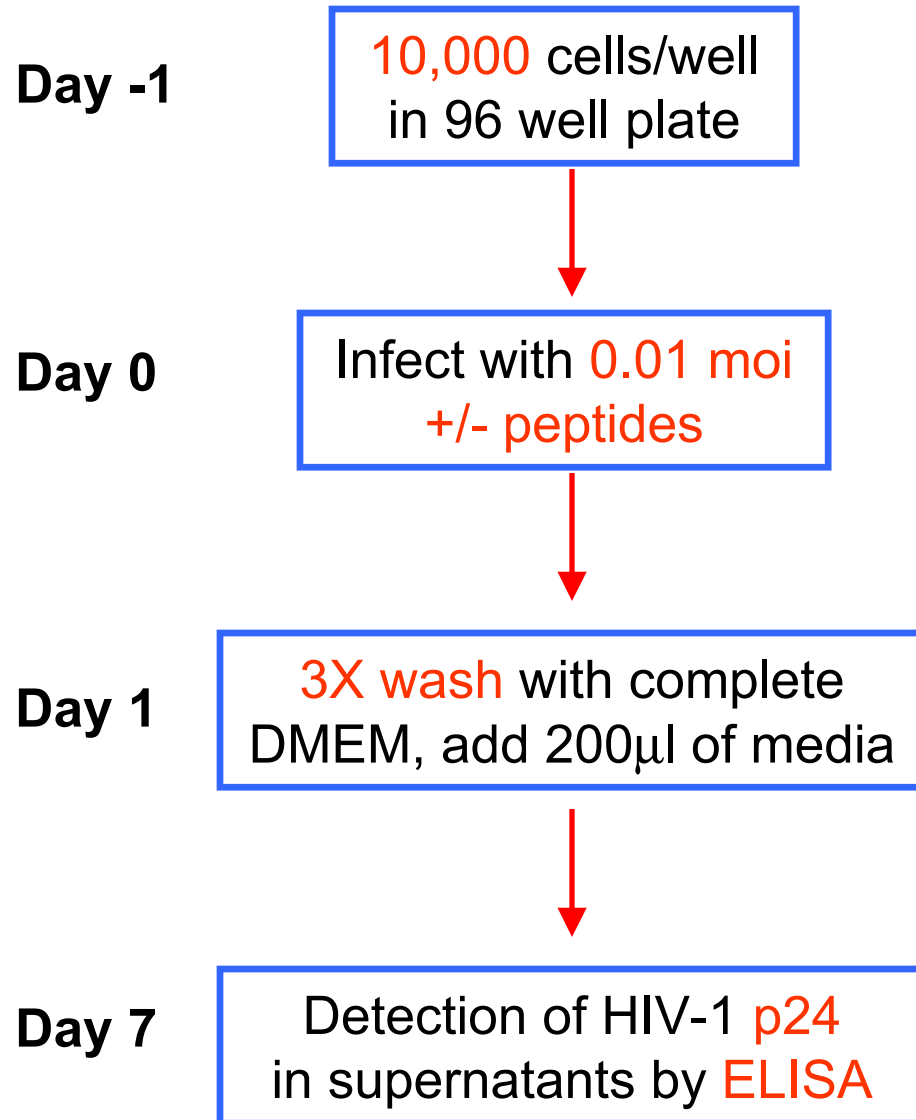
Fusion Assay

- Incubation of **effector** and **target** cells with or without peptides
- Fusion process:
 - 1) T7 RNA polymerase from **effector cells** binds to T7 promoter from **target cells**
 - 2) Transcription and translation of **LacZ gene** and synthesis of β -galactosidase
 - 3) Cell lysis
 - 4) Addition of chlorophenol red β -galactopyranoside (CPRG), substrate for β -galactosidase
 - 5) Quantification by spectrophotometry at 570 nm of the **reaction product** of β -galactosidase and CPRG

Complex T7 RNA polymerase +
T7 promoter-LacZ



Replication Assay



- Cells: U87 CCR5+
or CXCR4+
- Virus: IIIb (X4)
JRCSF(R5)
SF2(R5X4)
AB28*(R5)
*AZT resistant clinical isolate

Synthetic Combinatorial Libraries

- The libraries are composed of thousands to millions of compounds prepared in a **highly systematic manner**: all combinations of subunits of a class of compounds are synthesized simultaneously.
- They are **synthetically prepared** by solid phase method, cleaved from the resin support and **assayed free in solution**
- **Mixture-based** libraries: The compounds are pooled into mixtures of solubilized compounds and screened as mixtures
- The active individual active compounds are identified using a **positional scanning** (PS) deconvolution process [5] as explained below:
 - ✓ Sets of sublibraries to address each position in single screening assay
 - ✓ All sublibraries contain the **same total** individual compounds
 - ✓ The compounds are **grouped in a different manner** from one sublibrary to the others
- Successful applications in various assays of such libraries have been reported in the past 10 years [reviewed in 3]

Position Scanning Deconvolution: Illustration with Tripeptide PS-SCL

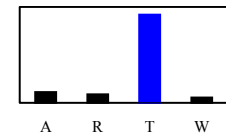
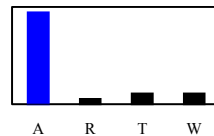
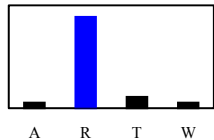
3 sublibraries containing 4 mixtures each

Amino acids at each position: A, R, T, W

$$4^3 = 64 \text{ peptides in the library}$$

$$4^2 = 16 \text{ peptides per mixture}$$

Sublibrary 1: O X X				Sublibrary 2: X O X				Sublibrary 3: X X O			
Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7	Mixture 8	Mixture 9	Mixture 10	Mixture 11	Mixture 12
A X X	R X X	T X X	W X X	X A X	X R X	X T X	X W X	X X A	X X R	X X T	X X W
A A A	R A A	T A A	W A A	A A A	A R A	A T A	A W A	A A A	A A R	A A T	A A W
A A R	R A R	T A R	W A R	A A R	A R R	A T R	A W R	A R A	A R R	A R T	A R W
A A T	R A T	T A T	W A T	A A T	A R T	A T T	A W T	A T A	A T R	A T T	A T W
A A W	R A W	T A W	W A W	A A W	A R W	A T W	A W W	A W A	A W R	A W T	A W W
A R A	R R A	T R A	W R A	R A A	R R A	R T A	R W A	R A A	R A R	R A T	R A W
A R R	R R R	T R R	W R R	R A R	R R R	R T R	R W R	R R A	R R R	R R T	R R W
A R T	R R T	T R T	W R T	R A T	R R T	R T T	R W T	R T A	R T R	R T T	R T W
A R W	R R W	T R W	W R W	R A W	R R W	R T W	R W W	R W A	R W R	R W T	R W W
A T A	R T A	T T A	W T A	T A A	T R A	T T A	T W A	T A A	T A R	T A T	T A W
A T R	R T R	T T R	W T R	T A R	T R R	T T R	T W R	T R A	T R R	T R T	T R W
A T T	R T T	T T T	W T T	T A T	T R T	T T T	T W T	T T A	T T R	T T T	T T W
A T W	R T W	T T W	W T W	T A W	T R W	T T W	T W W	T W A	T W R	T W T	T W W
A W A	R W A	T W A	W W A	W A A	W R A	W T A	W W A	W A A	W A R	W A T	W A W
A W R	R W R	T W R	W W R	W A R	W R R	W T R	W W R	W R A	W R R	W R T	W R W
A W T	R W T	T W T	W W T	W A T	W R T	W T T	W W T	W T A	W T R	W T T	W T W
A W W	R W W	T W W	W W W	W A W	W R W	W T W	W W W	W W A	W W R	W W T	W W W



Synthesis of individual peptides of residues defining the most active mixtures

RAT

Two separate libraries are used in these studies:

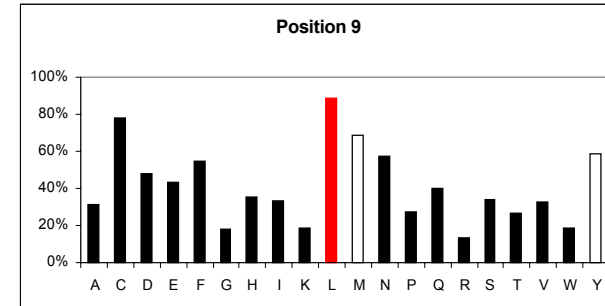
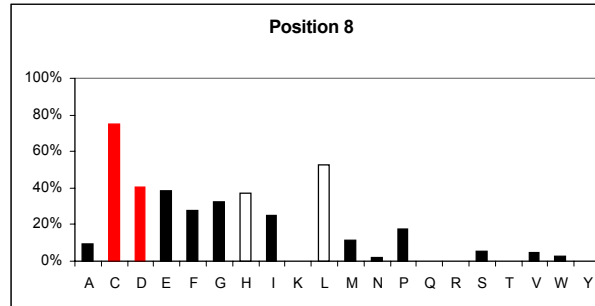
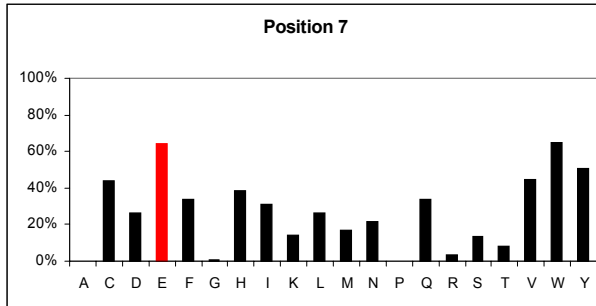
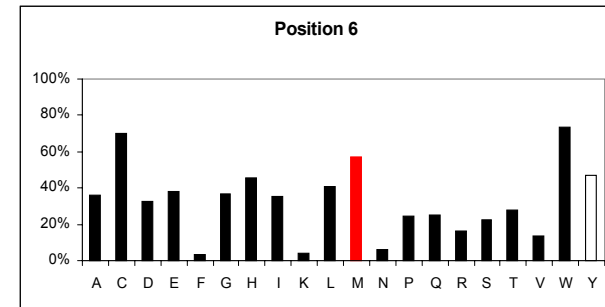
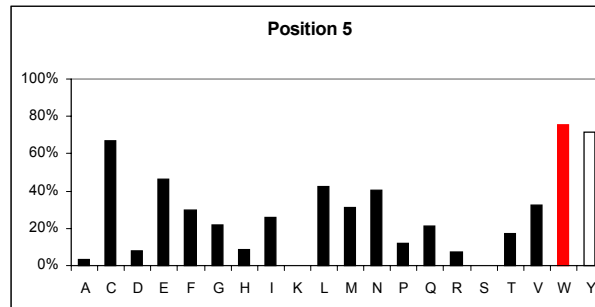
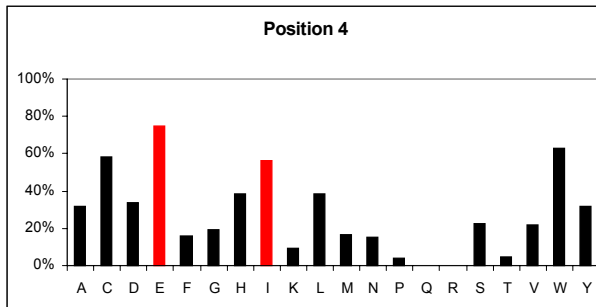
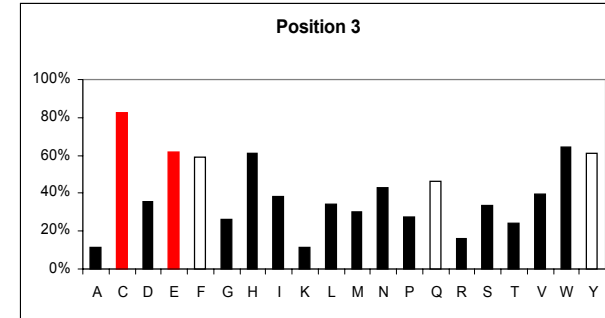
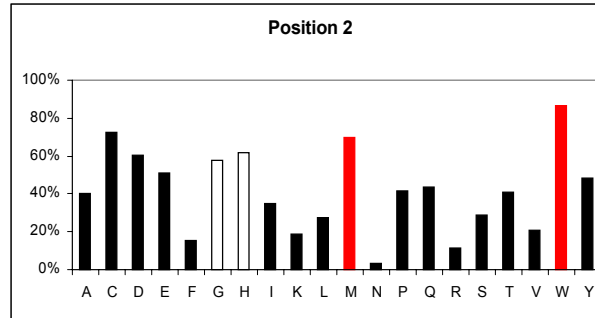
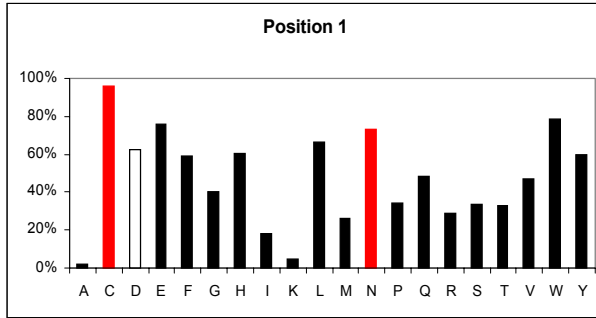
- a L-amino acid nonapeptide PS- SCL
- a D-amino acid decapeptide PS-SCL

Library nomenclature:

- **O**: All peptides present in a given mixture has the same amino acid “O” at a given position
- **X**: Equimolar mixture of all amino acids at a given position
- D-amino acids are represented by lower case letters

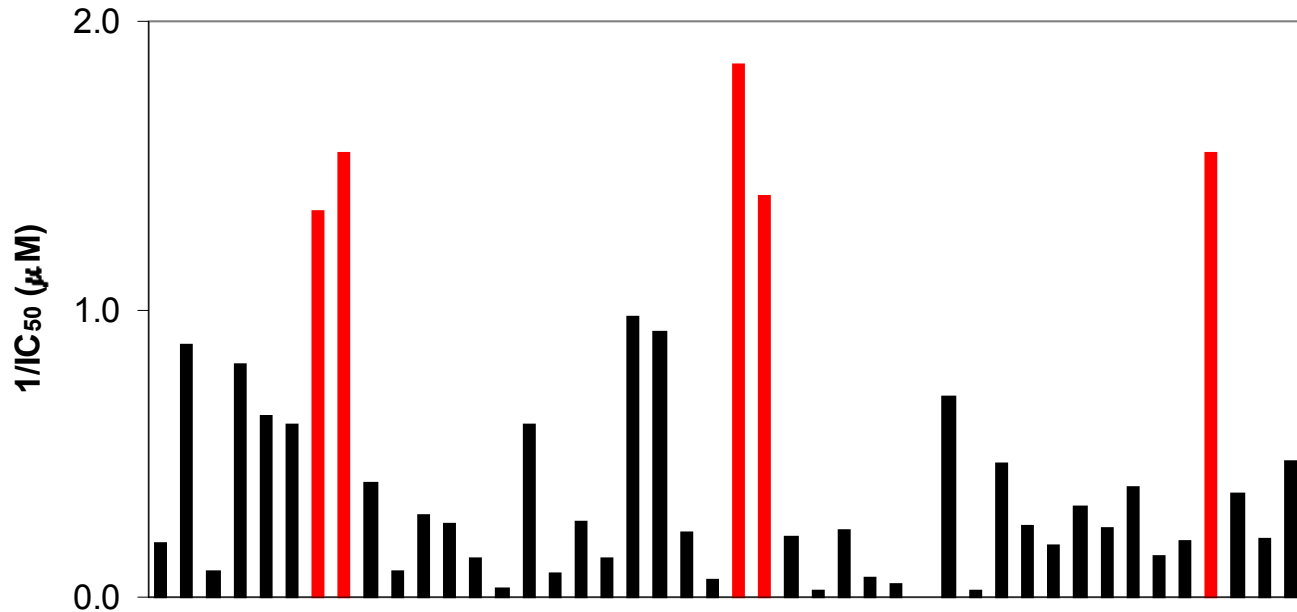
Inhibitory Activity in the X4 system

L-amino acid nonapeptide library



█ Mixtures selected based on X4 $\Rightarrow 2 \times 2 \times 2 \times 2 \times 1 \times 1 \times 1 \times 2 \times 1 = 32$ peptides
 Mixtures selected based on R5, low toxicity and different chemical character for additional synthesis of 12 selected peptides

Inhibitory activity in the X4 fusion system of individual L-amino acid nonapeptides identified from library



											<u>IC₅₀ (μ M)</u>	<u>IC₉₀ (μ M)</u>
C	W	E	I	W	M	E	C	L	-OH		0.7	1-3
C	W	E	I	W	M	E	D	L	-OH		0.6	3-5
N	W	E	I	W	M	E	C	L	-OH		0.5	1-3
N	W	E	I	W	M	E	D	L	-OH		0.7	3-5
C	W	C	E	W	M	E	L	L	-OH		0.6	5-10

IC₅₀ >40μM for all peptides in the R5 fusion system assay. No toxicity toward uninfected cells observed at 40μM.

Inhibition of fusion by a L-amino acid nonapeptide and its retro-inverso D-amino acid peptide

	X4 Fusion system		R5 Fusion system	
	IC ₅₀ (μM)	IC ₉₀ (μM)	IC ₅₀ (μM)	IC ₉₀ (μM)
N W E I W M E C L -OH	0.5	1-3	>40	>40
I c e m w i e w n -NH ₂	1.6	5-10	>40	>40

Decapeptide PS-SCL: 6.5×10^{12} different peptides

- 20 D-amino acids at the defined position o

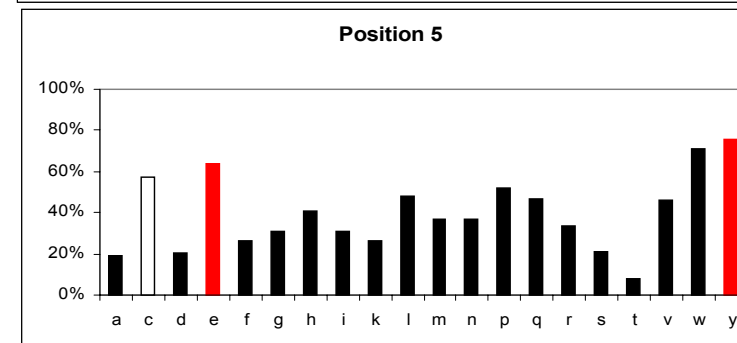
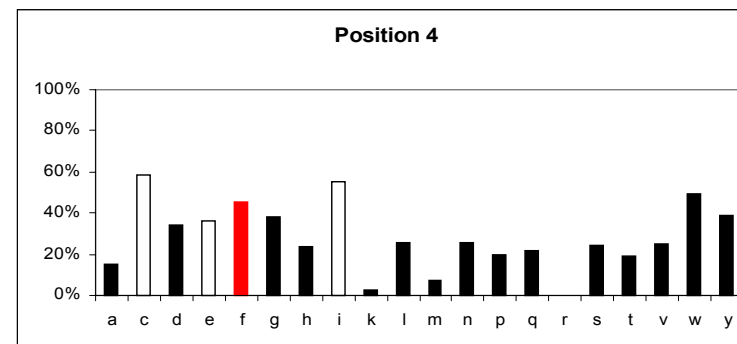
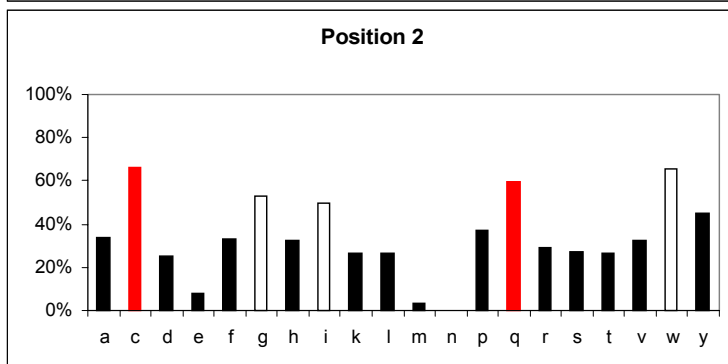
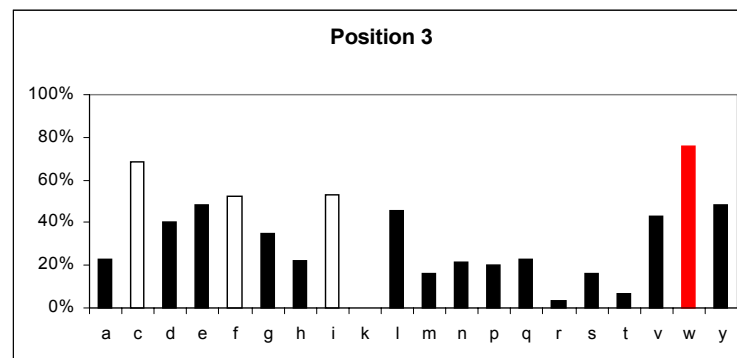
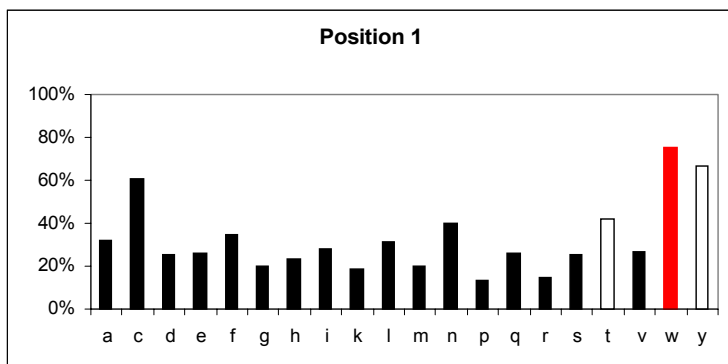
- 19 D-amino acids at the mixture position x

⇒ 200 mixtures with $19^9 = 3.2 \times 10^{11}$ peptides each

		1	2	3	4	5	6	7	8	9	10				
Sublibrary 10	Sublibrary 1	1 Ac-	a	x	x	x	x	x	x	x	x	x	-NH ₂		
		2 Ac-	c	x	x	x	x	x	x	x	x	x	-NH ₂		
		3 Ac-	d	x	x	x	x	x	x	x	x	x	-NH ₂		
		4 Ac-	e	x	x	x	x	x	x	x	x	x	-NH ₂		
		⋮													
		20 Ac-	y	x	x	x	x	x	x	x	x	x	x	-NH ₂	
		⋮													
		Sublibrary 10	X ₉ O	198 Ac-	x	x	x	x	x	x	x	x	x	v	-NH ₂
				199 Ac-	x	x	x	x	x	x	x	x	x	w	-NH ₂
				200 Ac-	x	x	x	x	x	x	x	x	x	y	-NH ₂

Inhibitory Activity in the X4 system

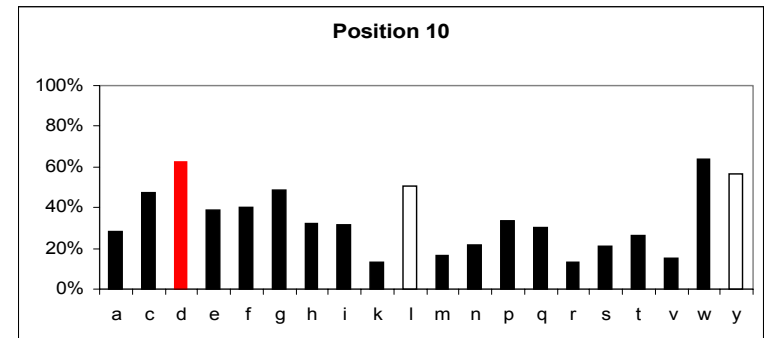
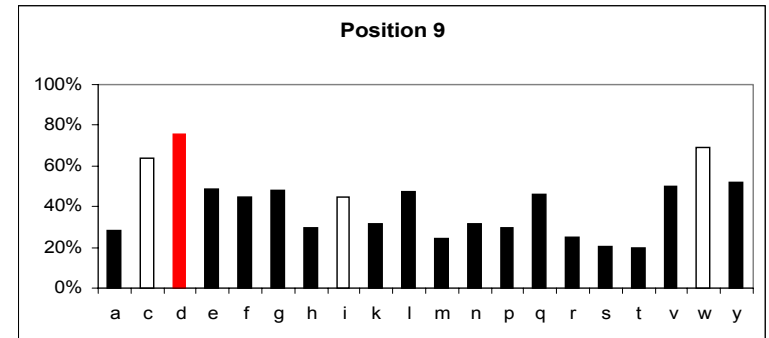
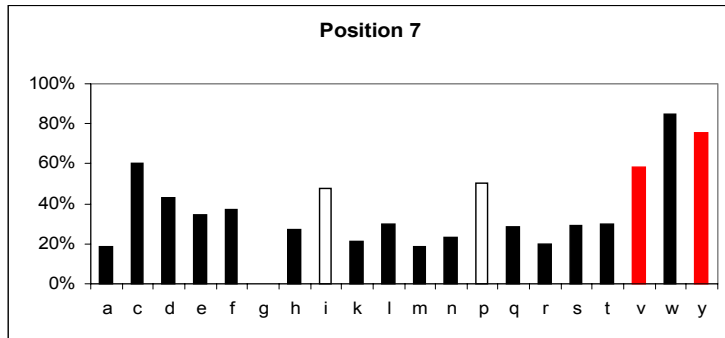
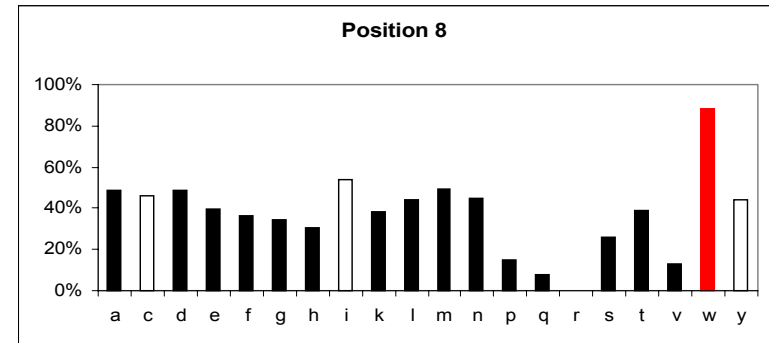
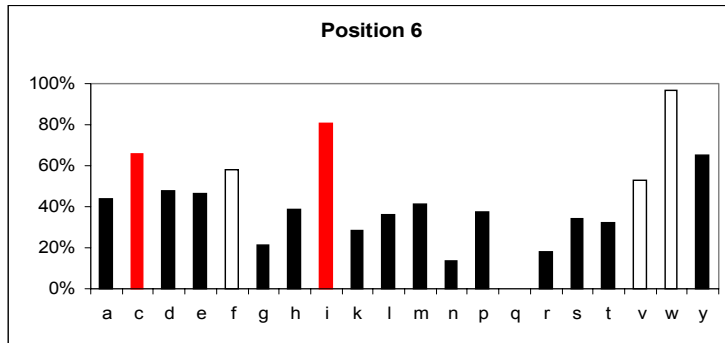
D-amino acid decapeptide library



█ Mixtures selected based on X4
 Mixtures selected based on low toxicity and different chemical character for additional synthesis

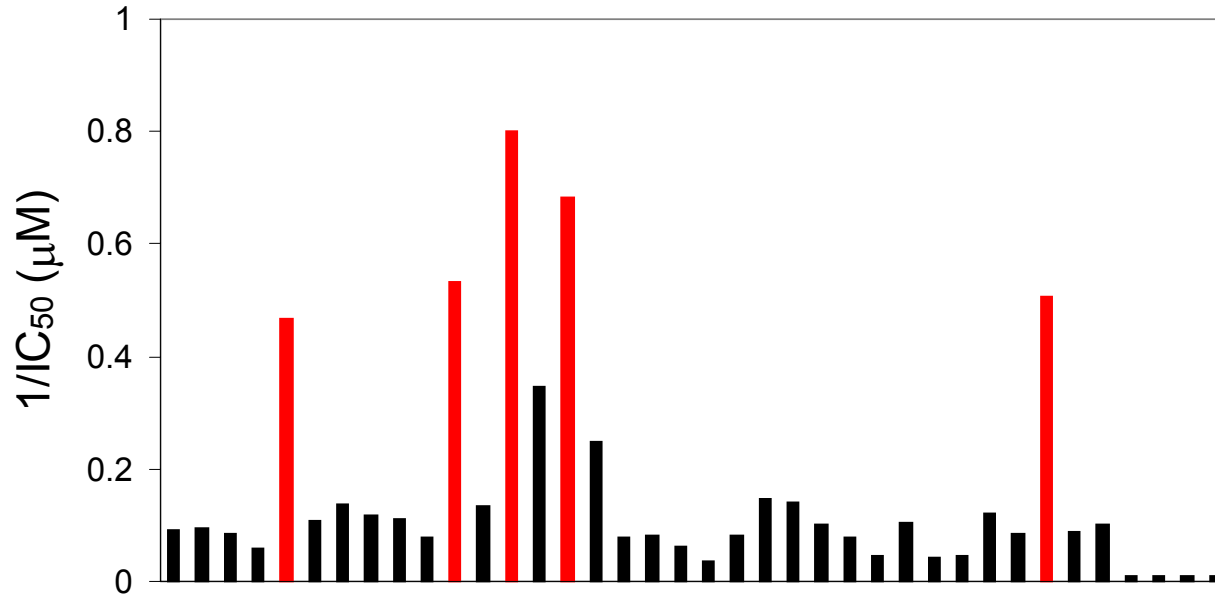
Inhibitory Activity in the X4 system

D-amino acid decapeptide library



- Mixtures selected based on X4 \Rightarrow
 $1 \times 2 \times 1 \times 1 \times 2 \times 2 \times 2 \times 1 \times 1 \times 1 = 16$ peptides
- Mixtures selected based on low toxicity and different chemical character for additional synthesis of 22 selected peptides

Inhibitory activity in the X4 fusion system of individual D-amino acid decapeptides identified from library



	<u>IC₅₀ (μM)</u>	<u>IC₉₀ (μM)</u>
Ac- w c w f e i y w d d -NH ₂	2.13	8-17
Ac- w q w f y c y w d d -NH ₂	1.87	4-8
Ac- w q w f e i y w d d -NH ₂	1.25	16-33
Ac- w q w f e c y w d d -NH ₂	1.46	4-8
Ac- w i c e y i y w d d -NH ₂	1.98	4-9

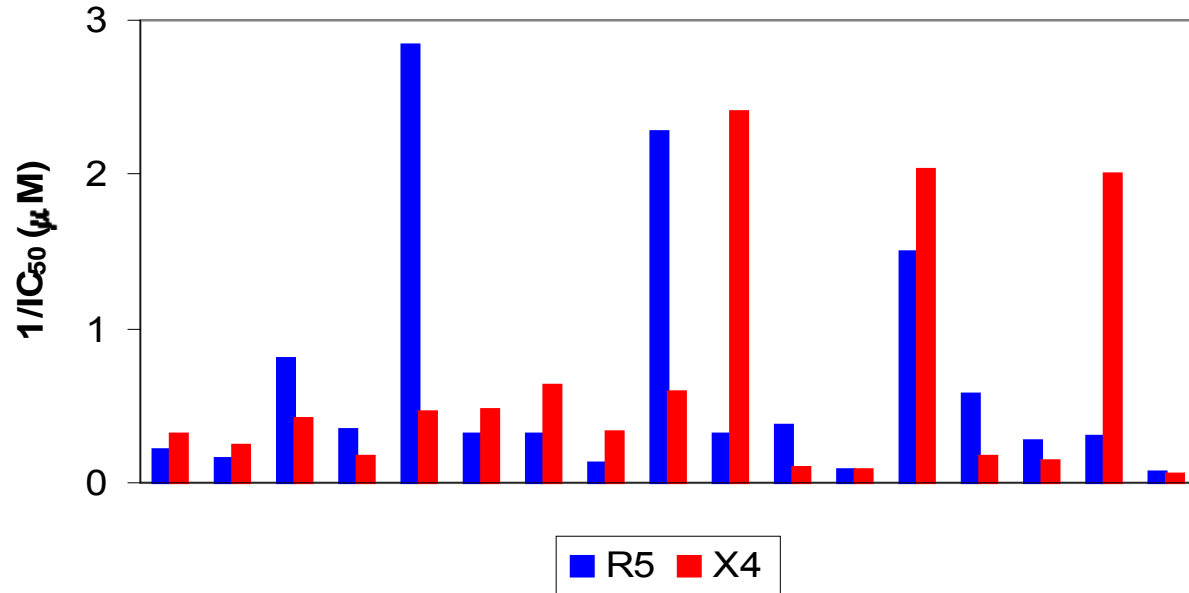
IC₅₀ >35μM for all peptides in the R5 fusion system assay. No toxicity toward uninfected cells observed at 35μM.

A second deconvolution process was performed based on the inhibitory activity of the D-amino acid decapeptide mixtures in the R5 fusion system



17 individual D-amino acid decapeptides were synthesized and assayed in the R5 and X4 fusion assay systems

Inhibitory activity in the fusion assay systems of individual D-amino acid decapeptides



	R5 Fusion system		X4 Fusion system	
	IC ₅₀ (μ M)	IC ₉₀ (μ M)	IC ₅₀ (μ M)	IC ₉₀ (μ M)
Ac- r r w r r r i y r r -NH ₂	0.35	1-2	2.18	4-8
Ac- r r w r r r i y y r -NH ₂	0.44	1-2	1.71	4-8
Ac- s f m y r r w r r r -NH ₂	3.22	4-8	0.42	1-2
Ac- r r m y r r i y r r -NH ₂	0.67	2-4	0.49	1-2
Ac- r r m y r y h r y i -NH ₂	3.32	8-16	0.50	1-2

No toxicity toward uninfected cells observed at 35μM.

Inhibitory activity of individual peptides in the replication assays

All nonapeptides and decapeptides exhibiting activity in the fusion assay systems were also tested for inhibitory of HIV replication.

L-amino acid nonapeptides

TPI-76 C W E I W M E D L -OH
TPI-91 N W E I W M E C L -OH

	IC₅₀ (μM)				
	IIIb (X4)	JRCSF(R5)	AB28 (R5)*	SF2 on R5	SF2 on X4
TPI-76	0.4	2.5	1.9	3.3	4.6
TPI-91	0.5	5.4	7.7	9.9	6.2

* AZT resistant clinical isolate

None of the peptides exhibits toxicity toward uninfected cells at 40μM

D-amino acid decapeptides

TPI-15 Ac- w q w f e c y w d d -NH₂

TPI-31 Ac- w g c f e c y w d d -NH₂

TPI-32 Ac- w i c e y i y w d d -NH₂

	IC ₅₀ (μM)				
	IIIb (X4)	JRCSF(R5)	AB28 (R5)*	SF2 on R5	SF2 on X4
TPI-15	6.5	1.2	2.6	6.3	7.4
TPI-31	35	3.7	7.4	>18	17
TPI-32	10	3	13.5	>18	18

* AZT resistant clinical isolate

None of the peptides exhibits toxicity toward uninfected cells at 35μM

Conclusions

- Fusion inhibitory peptides can be **rapidly identified** from positional scanning libraries made up of millions of peptides.
- The individual peptides identified from the libraries exhibit **significant inhibitory activity of HIV replication** including AZT resistant clinical isolates.
- The similar inhibitory activity observed in the fusion assay systems between a L-amino acid peptide and its retro-inverso counterpart indicates a potential **improvement in bioavailability** of the L-amino acid peptide candidates.

References

1. J.P. Moore, M. Stevenson (2000) *Nat. Rev. Mol. Cell Biology* **1**:40-49.
2. J.M. Kilby, S. Hopkins, T.M. Venetta, B. DiMassimo, G.A. Cloud, J.Y. Lee, L. Aldredge, E. Hunter, D. Lambert, D. Bolognesi, T. Matthews, M.R. Johnson, M.A. Nowak, G.M. Shaw, M.S. Saag (1998) *Nature Med.* **4**:1302-1307.
3. R.A. Houghten, C. Pinilla, J.R. Appel, S.E. Blondelle, C.T. Dooley, J. Eichler, A. Nefzi, J.M. Ostresh (1999) *J. Med. Chem.* **42**:3743-3778.
4. O. Nussbaum, C.C. Broder, E.A. Berger (1994) *J. Virol.* **68**:5411-5422.
5. C. Pinilla, J.R. Appel, P. Blanc, R.A. Houghten (1992) *Biotechniques* **13**:901-905.

Acknowledgment

The HIV-1 isolates and the vaccinia fusion systems were kindly provided by Dr. Mosier and Dr. Berger, respectively. We also thank Dr. Landau for his advices and comments.

This work was supported by NIH grant DE 12923