Supplemental Material

Mann et al. *Conformation-dependent recognition of HIV gp120 by Designed Ankyrin Repeat Proteins provides access to novel HIV entry inhibitors*
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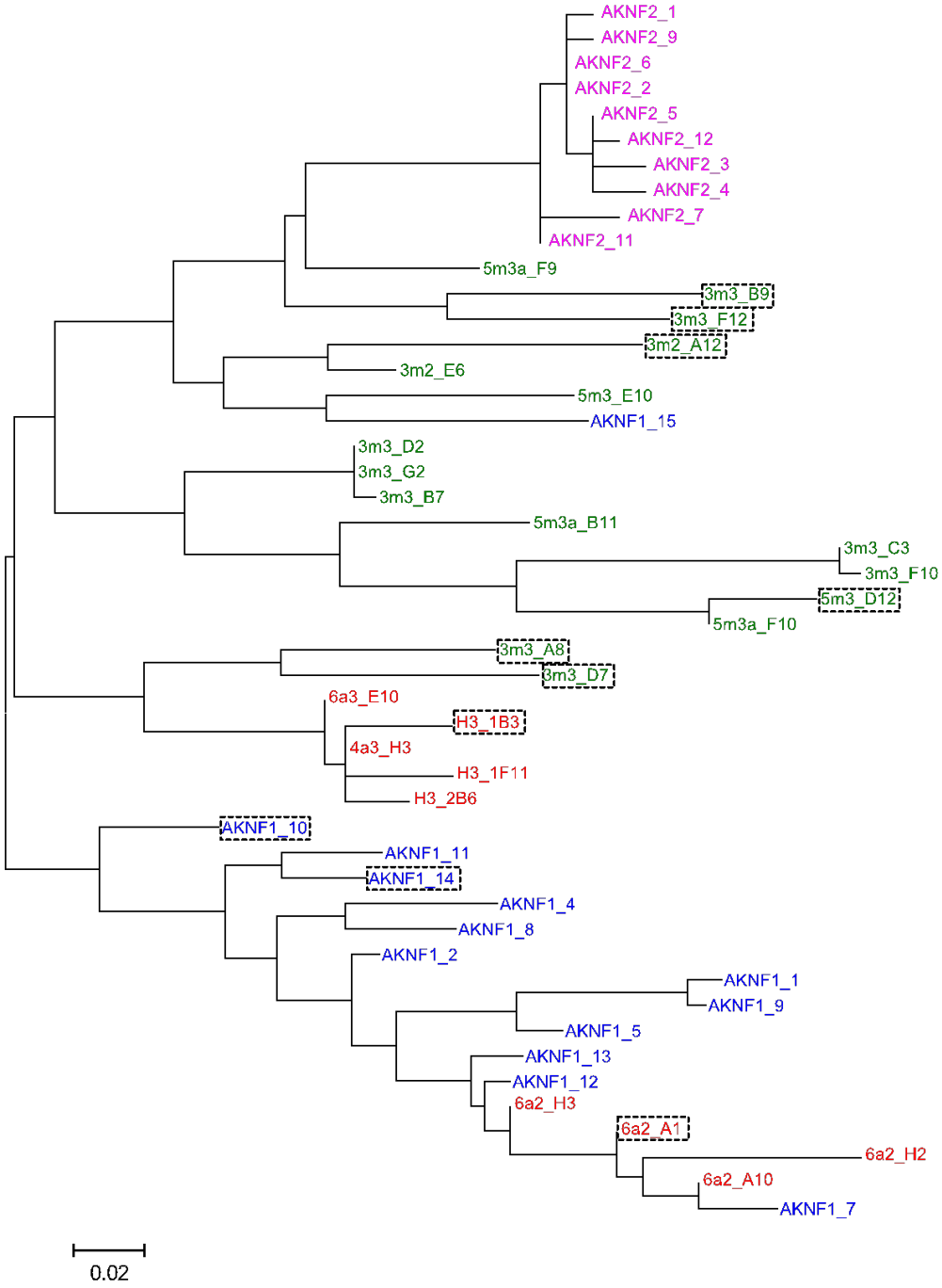


Figure S1. Phylogenetic analysis of DARPin sequences. A phylogenetic tree including amino acid sequences from all derived gp120 specific DARPin binders.

Binders from Selection I are shown in red, Selection II in green, Selection III in blue and Selection IV in pink. Binders that were followed up in detail are boxed with a dashed line. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (1). The tree with the highest log likelihood (-2689.0018) is shown. Initial tree(s) for the heuristic search were obtained automatically with the BIONJ method with MCL distance matrix. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 48 amino acid sequences. There were a total of 168 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (2).

NMR studies of cyclic peptides

^{1}H NMR measurements were performed in H_2O/D_2O (9:1) or pure D_2O, at pH 5.0, at 1-3 mM peptide. Spectra were acquired on a *Bruker* AV-600 spectrometer at 300 K. Data were processed using TOPSPIN 2.1 (*Bruker*) or XEASY (3). Water suppression was performed by presaturation. Spectral assignments were made using 2D DQF-COSY, TOCSY and NOESY spectra. *Cis* and *trans* peptide bond rotamers in slow exchange were observed at the Gly^{312}-Pro^{313} peptide bond, at the tip of the loop in each mimetic, in the ratios shown in the Figure below. A full assignment was only possible for the *trans* rotamers, due to signal overlap, and structure calculations were performed using NOE-derived restraints for only the *trans* rotamers. $^3J_{HNH\alpha}$ coupling constants were determined from 1D spectra or from 2D NOESY spectra by inverse Fourier transformation of in-phase multiplets (4).

Chemical shift assignments for the four cyclic peptides are given below.

Table S1 - Chemical shift assignments for HF.

Residue	NH	αH	βH	Others
Arg[1]	7.71	4.44	1.79, 1.79	γCH_2 1.49, 1.59; δCH_2 3.19, 3.19; εNH 7.23
Ile[2]	8.23	3.88	1.42	γCH_3 0.68; γCH_2 0.76, 0.81; δCH_3 0.64
His[3]	8.51	4.49	2.25, 2.26	δCH 6.91; εCH 8.44
Ile[4]	8.28	4.27	1.79	γCH_3 0.86; γCH_2 1.09, 1.39; δCH_3 0.79
Gly[5]	8.16	4.08, 4.32	-	-
Pro[6]	-	4.49	2.03, 2.28	γCH_2 2.04, 2.10; δCH_2 3.65, 3.65
Gly[7]	8.60	3.95, 4.04	-	-
Arg[8]	7.95	4.41	1.75, 1.83	γCH_2 1.59, 1.59; δCH_2 3.19, 3.19; εNH 7.15
Ala[9]	8.44	4.73	1.29	-
Phe[10]	8.14	4.92	3.10, 3.10	δCH 7.08; εCH 7.12
Tyr[11]	8.80	5.03	2.82, 3.23	εCH 6.74; δCH 7.04
Thr[12]	8.81	4.88	4.28	γCH_3 1.27
D-Pro[13]	-	4.81	1.92, 2.35	γCH_2 2.06, 2.16; δCH_2 3.60, 3.92
Pro[14]	-	4.57	2.13, 2.28	γCH_2 1.97, 2.11; δCH_2 3.75, 3.96

Table S2 - Chemical shift assignments for IY.

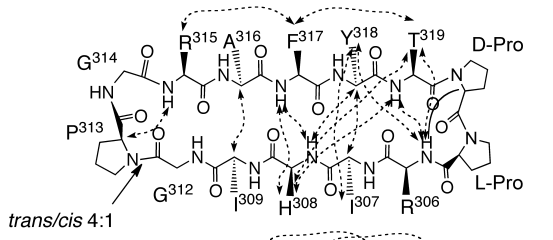
Residue	NH	αH	βH	Others
Lys[1]	7.89	4.40	1.80, 1.80	γCH_2 1.34, 1.43; δCH_2 1.66, 1.66; εCH_2 2.97, 2.97;
Arg[2]	8.44	4.85	1.46, 1.59	γCH_2 1.31, 1.46; δCH_2 2.57, 2.75; εNH 6.85
Ile[3]	9.01	4.35	1.40	γCH_3 0.84; γCH_2 1.10, 1.31; δCH_3 0.76
His[4]	9.00	4.53	3.00, 3.14	δCH 7.03; εCH 8.54
Ile[5]	8.07	4.29	1.77	γCH_3 0.95; γCH_2 1.04, 1.53; δCH_3 0.83
Gly[6]	7.88	3.75, 4.03	-	-
Pro[7]	-	4.63	2.16, 2.42	γCH_2 1.86, 1.96; δCH_2 3.53, 3.53
Gly[8]	8.88	4.01, 4.07	-	-
Arg[9]	8.86	4.42	1.79, 2.09	γCH_2 1.68, 1.71; δCH_2 3.25, 3.25; εNH 7.27
Ala[10]	7.64	4.39	1.31	-
Phe[11]	8.32	5.36	2.82, 2.82	δCH 7.08; εCH 7.26
Tyr[12]	9.00	4.98	2.99, 3.11	εCH 6.74; δCH 7.04
Thr[13]	8.56	5.13	4.11	γCH_3 1.18
Thr[14]	8.62	4.87	4.11	γCH_3 1.23
D-Pro[15]	-	4.76	1.91, 2.33	γCH_2 2.04, 2.15; δCH_2 3.68, 3.91
Pro[16]	-	4.55	2.11, 2.23	γCH_2 1.92, 2.08; δCH_2 3.71, 3.95

Table S3 - Chemical shift assignments for IF.

Residue	NH	αH	βH	Others
Lys[1]	7.74	4.43	1.81, 1.86	γCH_2 1.36, 1.43; δCH_2 1.65, 1.65; εCH_2 2.95, 2.95;
Ser[2]	8.32	4.49	3.39, 3.46	
Ile[3]	8.50	4.25	1.54	γCH_3 0.79; γCH_2 1.10, 1.28; δCH_3 0.77
His[4]	8.68	4.78	3.07, 3.17	δCH 7.14; εCH 8.46
Ile[5]	8.33	4.30	1.81	γCH_3 0.88; γCH_2 1.06, 1.36; δCH_3 0.79
Gly[6]	8.08	4.06, 4.15	-	-
Pro[7]	-	4.48	2.03, 2.28	γCH_2 2.03, 2.09; δCH_2 3.64, 3.64
Gly[8]	8.73	3.95, 4.04	-	-
Arg[9]	8.08	4.47	1.56, 1.70	γCH_2 1.55, 1.55; δCH_2 3.17, 3.17; εNH 7.15
Ala[10]	8.37	4.64	1.06	-
Phe[11]	8.37	4.79	2.98, 3.04	δCH 7.15; εCH 7.28
Tyr[12]	8.57	4.99	2.78, 3.10	εCH 6.75; δCH 7.06
Thr[13]	8.71	4.73	4.12	γCH_3 1.22
D-Pro[14]	-	4.92	2.01, 2.30	δCH_2 3.68, 3.91
Pro[15]	-	4.55	2.13, 2.27	γCH_2 1.98, 2.11; δCH_2 3.71, 4.01

Table S4 - Chemical shift assignments for HY.

Residue	NH	αH	βH	Others
Ser[1]	7.88	4.56	3.77, 3.77	
Ile[2]	8.28	4.44	1.63	γCH_3 0.75; γCH_2 1.02, 1.42; δCH_3 0.73
His[3]	8.76	4.75	2.38, 2.86	δCH 6.93; εCH 8.53
Ile[4]	8.42	4.33	1.72	γCH_3 0.85; γCH_2 0.98, 1.34; δCH_3 0.75
Gly[5]	8.57	3.94, 4.55	-	-
Pro[6]	-	4.44	1.93, 2.33	γCH_2 2.05, 2.08; δCH_2 3.61, 3.66
Gly[7]	8.84	3.86, 3.93	-	-
Arg[8]	8.31	3.95	1.88, 1.97	γCH_2 1.59, 1.59; δCH_2 3.19, 3.19; εNH 7.20
Ala[9]	7.44	4.39	1.31	-
Phe[10]	8.26	5.05	2.97, 2.97	δCH 7.15; εCH 7.30
Tyr[11]	8.63	4.86	2.94, 3.08	εCH 6.60; δCH 7.01
Thr[12]	8.58	4.95	4.19	γCH_3 1.17
Thr[13]	8.61	4.84	4.21	γCH_3 1.22
D-Pro[14]	-	4.84	1.95, 2.33	γCH_2 2.04, 2.17; δCH_2 3.66, 3.88
Pro[15]	-	4.53	2.11, 2.23	γCH_2 1.97, 2.05; δCH_2 3.71, 3.99

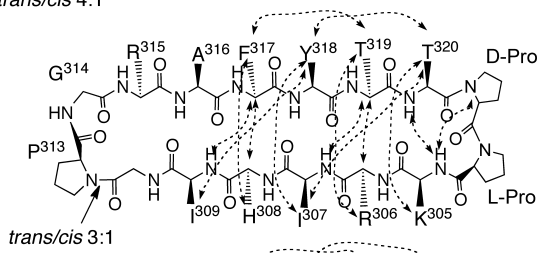


HF

10
R I H I G P G R A F Y T

$^3J_{HNH\alpha}$ 8.4 7.9 8.7 8.7 - - - 6.2 7.3 8.1 8.8 9.8

- Δδ/T (ppb/K) 2.4 11.1 3.0 8.4 7.1 - 6.8 13.3 9.9 0.6 6.1 4.5

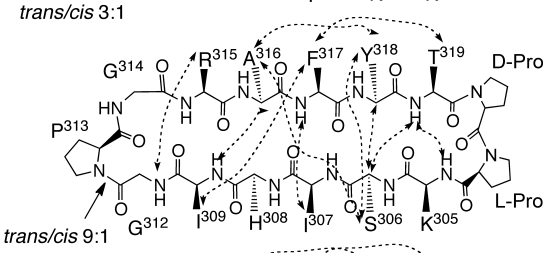


IY

10
K R I H I G P G R A F Y T T

$^3J_{HNH\alpha}$ 8.1 8.8 9.1 7.9 nd - - - nd nd 8.1 8.4 9.3 nd

- Δδ/T (ppb/K) 2.9 7.0 4.6 5.6 5.1 7.9 - 6.3 7.1 8.3 7.4 5.6 7.8 4.1

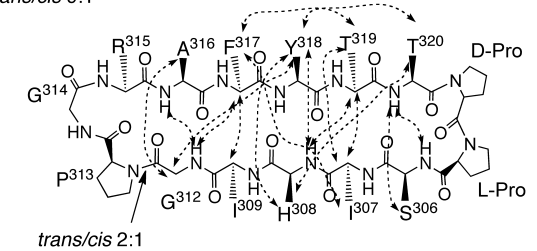


IF

10
K S I H I G P G R A F Y T

$^3J_{HNH\alpha}$ 8.3 8.6 9.2 8.3 9.9 - - - 7.7 5.8 nd 8.9 9.6

- Δδ/T (ppb/K 2.1 5.4 2.1 3.5 5.7 7.9 - 3.5 5.7 5.8 2.5 6.0 3.6



HY

10
S I H I G P G R A F Y T T

$^3J_{HNH\alpha}$ 8.4 9.1 9.3 8.9 - - - 7.2 6.3 8.0 8.4 9.4 9.4

- Δδ/T (ppb/K) 2.7 7.3 4.9 6.9 4.2 - 7.0 4.2 4.2 6.8 4.1 7.0 5.3

Figure S2. A summary of long range NOEs observed in ^{1}H NMR 2D-NOESY plots for each mimetic are shown, along with $^{3}J_{HNH\alpha}$ values (Hz) and the temperature-dependence of amide chemical shifts (-Δδ/T ppb/K).

Distance restraints were obtained from NOESY spectra with a mixing time of 250 ms. Spectra were typically collected with 1024 x 256 complex data points zero-filled prior to Fourier transformation to 2048 x 1024, and transformed with a cosine-bell weighting function. The structure calculations were performed by restrained molecular dynamics in torsion angle space using NOE-derived distance restraints and DYANA (5). Starting from 100 randomized conformations a bundle of 20 final structures were selected with the lowest DYANA target energy function. The program MOLMOL (6) was used for structure analysis and visualization of molecular models. DYANA structures were optimized by energy minimization using the program MOE (*Chemical Computing Group*, Canada).

Table S5 - Statistics from the DYANA structure calculations for each mimetic, having the HF, IY, IF and HY registers (see Figure 7)

Mimetic =	**HF**	**IY**	**IF**	**HY**
NOE upper-distance limits:	126	144	122	128
Intraresidue	43	42	31	32
Sequential	46	62	59	53
Medium-and long range	37	40	32	43
Residual target function value (Å^2)	1.04 ± 0.04	0.96 ± 0.05	0.89 ± 0.08	0.80 ± 0.05
Mean rmsd value (Å)				
All backbone atoms	0.93 ± 0.44	1.01 ± 0.50	0.53 ± 0.15	0.79 ± 0.25
All heavy atoms	1.88 ± 0.46	2.03 ± 0.63	1.58 ± 0.35	1.57 ± 0.32
Residual NOE violations				
Number > 0.2 Å	2	5	6	6
Maximum (Å)	0.36	0.28	0.27	0.25

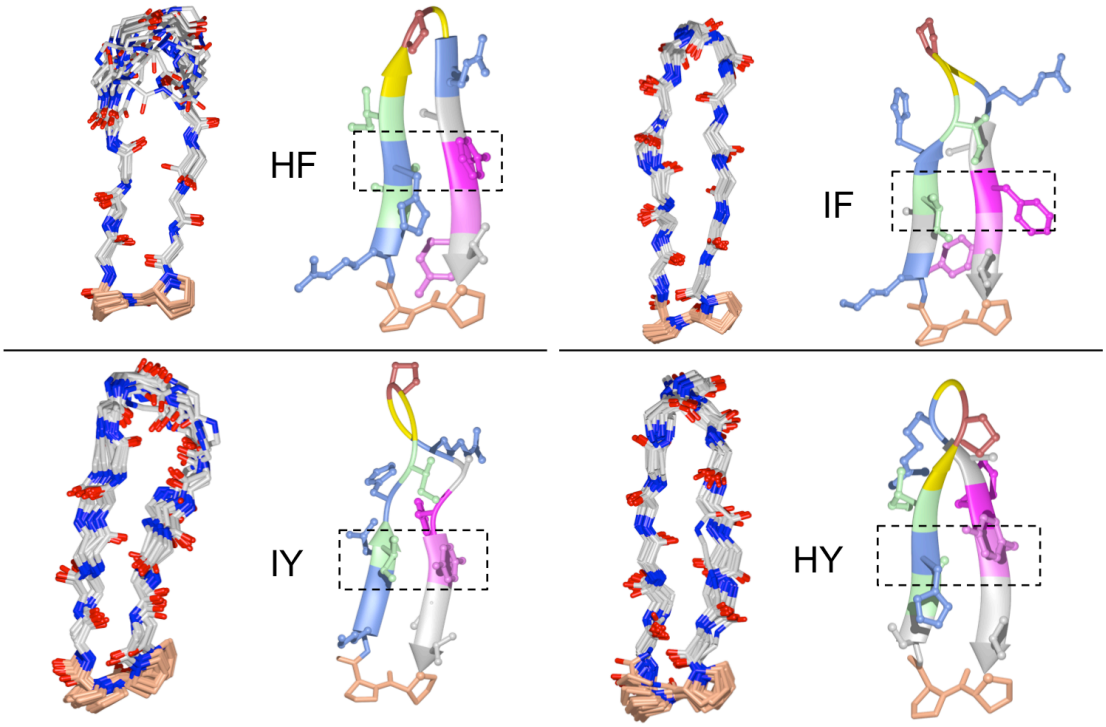


Figure S3. A backbone superimposition of the final 20 NMR structures having the lowest energy function for each of the cyclic peptides is shown (*left*) (no side chains included for clarity); one typical structure of each mimetic is shown (*right*), with same coloring of equivalent residues (Ile green, Phe dark pink, Tyr light pink, Pro orange, Gly yellow). The D-Pro-L-Pro template is at the bottom of each structure. Dotted boxes indicate for each mimetic residue pairs at a cross-strand hydrogen-bonding position that define the hairpin register.

Table S6. Alignment of V3 sequences of virus isolates probed in Figures 5 and 9. (Amino acid positions numbering according to HXB2).

Table A1		**V3 loop sequence**
		300 310 320 330
	HIV strain	....|....|....|....|....|....|....|
Sensitive to 5M3_D12	**JR-FL**	CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHC
	RHPA4259.7	...H.....N.....A..K.....
	NAB1pre-cl_39x	....S.....T.....A.....K...
	NAB2pre-cl_3	...L.....R..N.....W.....V.....K.N.
	NAB10pre-cl_2	.....R....S.....K...
	NAB12pre-cl_7	.....P.....A..D.....
Resistant to 5M3_D12	**6535.3**	.....NL.....A..D.....
	AC10.0.29	.I.....G.....D.....
	CAAN5342.A2	.....S...T.....A..R.....K...
	PVO.4	.....S.....A..D.....
	QH0692.42	....G.....A..D.....
	REJO4541.67	.....A.....A.....K.Y.
	SC422661	.....G.T.....V...-...V.....V..
	THRO4156.18	.....S....M...G..FA..R.....K.Y.
	TRO.11	.....R.....A..D.....
	WITO4160.33	....G....R..N.....A..A.....K...
	NAB3pre-cl_43	.....A..A...N.....
	NAB4pre-cl_1	.....R..P.....A.-D.....
	NAB5pre-cl_1	....S....R..T.....A..D.....K...
	ZA110_10.14	....S....R.....K....-.G.....

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