Poster # **219**

Session Title: Envelopes, Receptors, and Tropism

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HIV Co-receptor Tropism Switching Is Correlated With Binding Affinity to CXCR4

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BACKGROUND

HIV-1 co-receptor tropism switch from CCR5 (R5) to CXCR4 (X4) has been associated with an increase in net charge in the V3 loop. Despite the lack of experimental structures of the gp120-CD4-R5/X4 complexes, structure-based computational prediction of the thermodynamic effects of these charge fluctuations is important to understand the mechanism and implications of co-receptor switch.

METHODS

(1) Interaction Models: R5 (JRFL) and X4 (HBXc2) V3 loop sequences were modeled onto the backbone of V3, followed by Molecular Dynamic simulations and docking of simulated structures onto CCR5 and CXCR4.

(2) V3 Training sequence data-set: 165 R5 and 165 X4 sequences were mapped onto the simulated interaction models. Thermodynamic parameters were *computed* by well known free energy calculation methods **(a) Continuum-electrostatics poison-Boltzmann, (b) Generalized Born radii for electrostatic approximation, (c) Empirical Force field for protein-protein interaction energies.** Reported here as: V3 charge and Complex Binding Affinity by, **(a)** Electrostatic energy of V3 by, **(b)** and Association rate constant of complex formation by, **(c)** denoted by:

$$(e)_{computed} \qquad \Delta\Delta G_{BA}^{Complex} \qquad \Delta G^{V3} \qquad \Delta K_{on}$$

(3) Mathematical modeling: to derive a Binding Affinity Function in terms of the computed thermodynamic parameters. Structural validation: by implementation of the derived BA function into a Support Vector Machine algorithm, to assess the predictive power of the interaction models in terms of their ability to correctly classify a database of 1150 R5 and 230 X4 sequences.

(4) Dually infected sequence database with reported co-receptor switch: 38 macaques, 4 patients was modeled onto the the interaction models including the gp120 core, as well as CD4 followed by computation of BA.

CONCLUSION

- Charge fluctuations of the V3 loop suggests that electrostatic interactions modulate the choice of co-receptor use. An increase in charge on V3 strongly correlates to an increase in both predicted BA, and electrostatic rate of association to CXCR4 (R^2=.99, p<.001), with a concomitant decrease in V3 binding to CCR5 (R^2=.90, p<.001).
- Cooperative binding as a molecular mechanism for co-receptor switch along with the mathematical derivation of thermodynamic parameters as functions of charge, account for the observed increase of gp120 BA to CD4 (R^2=.94, p<.001) and CXCR4 (R^2=.99, p<.001) in macaques and patients with reported switch.

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RESULTS

(1) Interaction Models

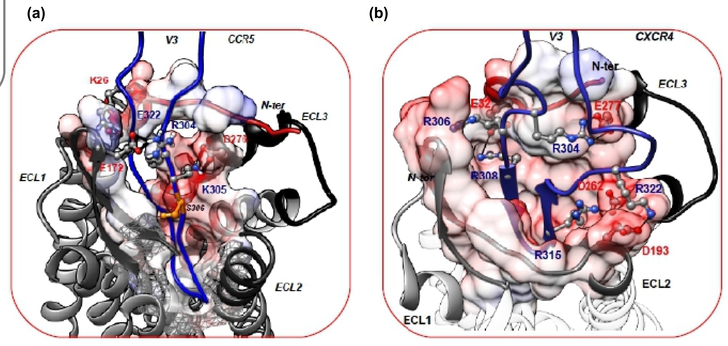
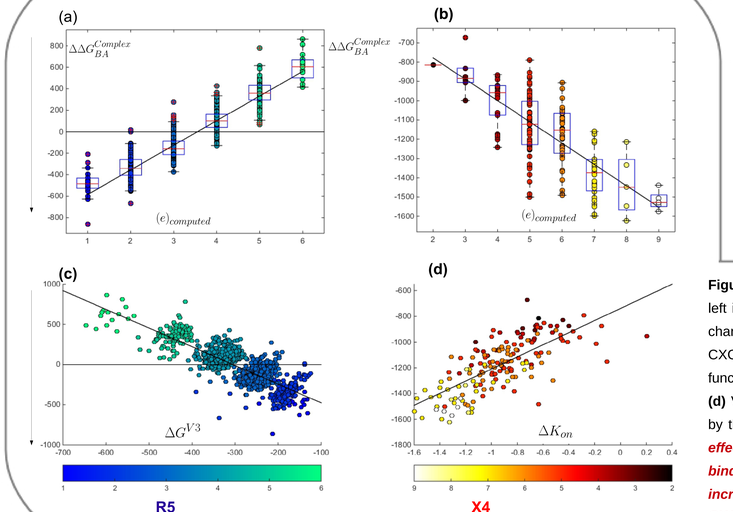


Figure1: Interaction models of V3 with **(a)** CCR5 and **(b)** CXCR4. Electrostatic interactions are labeled and highlighted as well as the charge density at the co-receptor binding site.

(2) V3 Training sequence data-set



(3) Mathematical Model of Cooperative Binding of X4 V3 to CXCR4

$$\varepsilon V3^{X4} + CXCR4 \underset{K_{on}}{\rightleftharpoons} CXCR4 : V3_{\varepsilon}^{X4}$$

$$K_d = [V3^{X4}]^{\varepsilon} \cdot [CXR4 : V3_{\varepsilon}^{X4}]^{-1} \quad (1)$$

$$En = log(\varepsilon) \cdot log(K_d)^{-1} \quad (2)$$

$$rate = \frac{Kd}{[V3]^{En}} \quad (3)$$

$$(e)_{predicted} = m \cdot En + b \quad (4)$$

$$\Delta\Delta G(V3) = \left(\frac{1}{\alpha} \cdot \frac{Kd}{\gamma \cdot exp^{\beta \cdot e}} - \beta\right)^{\frac{1}{En}} \cdot \int dV3 \quad (5)$$

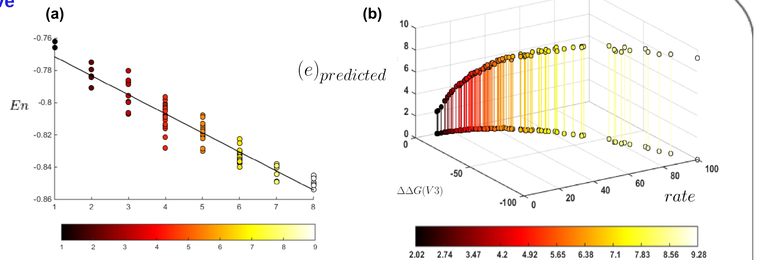


Figure 3: Predicted thermodynamic parameters by the Mathematical model plotted as a function of charge **(a)** Electrostatic rate enhancement En against the computed charge. Its linear regression equation equals to the predicted charge. **(b)** BA on the x-axis and rate on the y-axes against the predicted charge on the z-axis, the predicted curves for these functions follow an exponential rather than a linear increase, and the training data set is distributed evenly throughout the function.

	computed			*predicted*			
	$\Delta\Delta G_{BA}^{Complex}$	ΔG^{V3}	ΔK_{on}	K_d	En	$\Delta\Delta G(V3)$	$rate$
$(e)_{computed}$	**0.61**	**0.8**	**0.56**	**0.92**	**0.9**	**0.83**	**0.8**
$(e)_{predicted}$	**0.72**	**0.95**	**0.61**	**0.99**	**0.97**	**0.99**	**0.99**

Table 1. Coefficients of Determination (R^2) values from the linear association of net V3 charge to charge related thermodynamic parameters. "Computed" values were obtained as raw data (see methods), and "Predicted" values from the equations derived in the mathematical modeling of the interaction of an X4-V3 with CXCR4. *Kd* refers to the complex electrostatic dissociation constant, *En* is the electrostatic rate enhancement, *DDG(V3)* is the binding affinity as a function of the V3 loop, and *rate* is the Electrostatic Association rate. These predicted values all a function of the charge of X4 V3 loop.

(4) BA analysis of Env sequences of dually infected Macaques with reported co-receptor switch.

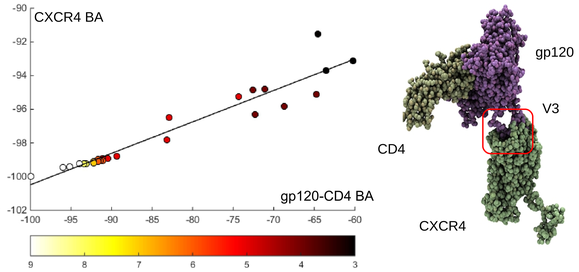


Figure 4. BA Calculations of gp120 Env sequences demonstrated that the binding of CD4 to gp120 also enhances cooperative binding of gp120-CD4 to CXCR4 as the charge on V3 increases. **Table 2** summarizes the sequences used for the scattered plot.

Monkey ID	Sequence ID	Tropism	Specific AA mutations	Env Region	R5 coreceptor BA kcal/mol	%	X4 coreceptor BA kcal/mol	%
DBJE	***Major Variant***	X4			***88.15***		***4.84***	
	D23	X4			2.22	97.5	1.11	77.1
	H9	X4	T303I*	V3	1.48	98.3	1.18	75.6
	J12	X4	R444K	V5	1.48	98.3	-0.14	102.9
		X4	N339K*	V3	3.70	95.8		
DBX9	***Major Variant***	X4			***0.74***		***-14.40***	
	O19	X4	R315K	V3	88.15	-99.2	-13.71	-4.8%
CE8J	***Major Variant***	X4			***86.67***		***7.82***	
	D9	X4	E187D, 188T, W395D	V2/V4	87.41	-0.8	3.67	53.1
	A15	X4	N135V*	V1	11.11	87.2	0.42	94.6
	J18	X4	N135V*	V1	8.89	89.7	0.69	91.2

2B.

SHIV ID	Sequence ID	Tropism	V3 *env* region	R5 coreceptor BA kcal/mol	%	X4 coreceptor BA kcal/mol	%
$SHIV_{ADE}$		R5	CT RPNNNTRKSI HIGPGRAFYT TGDIIGDIRQ AHC	+2.18		+5.59	
$SHIV_{SHT2}$		X4	-- --------G- T L----V--- --E-V----K ---	+1.36	37.6	+2.12	67.8
SHIVrecomb	197	X4	-- --------R- T L----/V/-- --E-V----K ---	+1.53	29.8	+3.22	51.1
	213	X4	-- --------R- T L----VL-- --E-V----K ---	+1.40	35.8	+3.18	51.7
	214	X4	-- --------R- T L----/V/-- --E-V----K ---	+1.48	32.1	+3.21	51.2
	217	X4	-- --------R- T L----/V/-- --E-V----K ---	+1.53	29.8	+3.21	51.2
	335	X4	-- --------R- T L----VL-- --E-V----K ---	+1.38	36.7	+3.18	51.7

Figure 2. y-axis on all panels is binding Affinity. Downward arrows on the left indicate the direction of increasing BA. Color bar indicates increase in charge from dark to light **(a)** V3 R5 BA to CCR5 and **(b)** V3 X4 BA to CXCR4, as a function of computed charge. **(c)** V3 R5 BA to CCR5 as a function of increase in Solvation energy of V3 noted by the negative sign **(d)** V3 X4 BA to CXCR4 increasing complex binding stability *DKon*, noted by the negative sign. ***An increase in charge on V3 R5 has a negative effect on charge related thermodynamic parameters that influence binding, an enhancing effect is seen in X4 V3. This may suggest increase in net charge leads to cooperative binding of X4 V3 to CXCR4.***