Identification of HIV-1 Env Mutations that Enhance Entry into Macaque Cells

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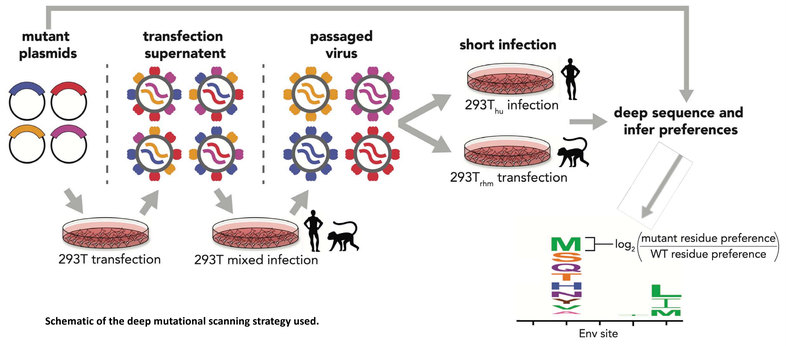
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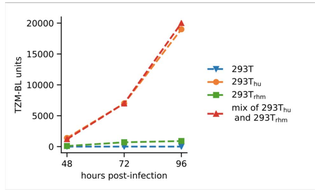
Motivation & Background

- Although rhesus macaques are a major animal model for HIV-1 vaccine development research, most transmitted/founder (T/F) HIV-1 strains, when introduced into SHIVs, replicate poorly in macaque cells.
- Research efforts employing either laboratory evolution or structure guided design strategies have discovered several *Env* mutations in gp120 that enhance binding of macaque CD4 by T/F HIV-1 viruses.
- In order to identify additional *Env* mutations that promote infection of macaque cells, we utilized deep mutational scanning to screen thousands of *Env* point mutants for those that enhance usage of macaque receptors.

DMS strategy to identify *Env* mutations that enhance usage of rhesus macaque receptors

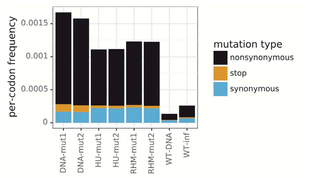


Schematic of the deep mutational scanning strategy used.

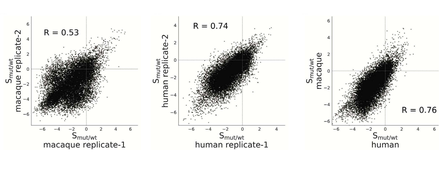


Wild-type BF520 infection of 293T cells engineered to express either macaque or human CD4/CCR5 receptors. TZM-bl units of supernatant harvested from cells infected with Q23 virus bearing wild-type BF520 Env (MOI of .01).

Overview of selection results

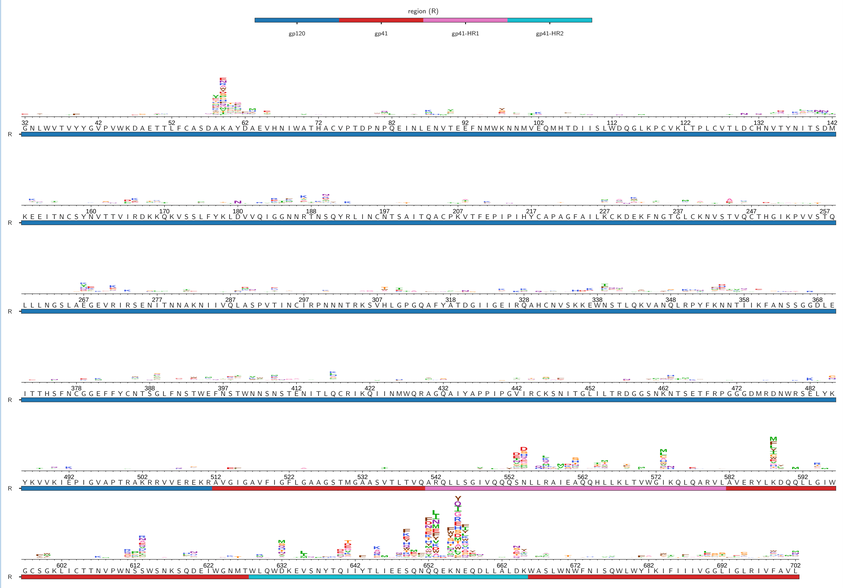


Frequencies of mutations in DMS of BF520 Env. Shown are nonsynonymous, stop, and synonymous mutations in the mutant plasmid DNA, the selected mutant viruses, and wild-type DNA and virus controls.



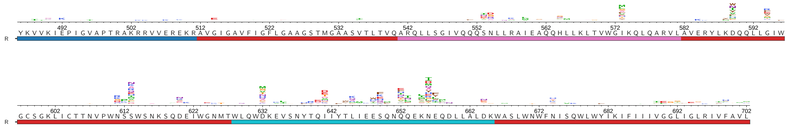
Pearson correlations among $S_{mut/wt}$ values. Correlation between replicate 293T$_{rhm}$ infections (left), 293T$_{hu}$ infections (middle) and average Smut/wt values from 293T$_{rhm}$ and 293T$_{hu}$ infections (right). $S_{mut/wt}$ is calculated as log$_2$ (mutant pref / wt pref)

Env mutations enriched through 293T$_{rhm}$ infection

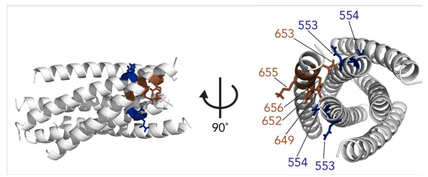


$S_{mut/wt}$ values of amino acids enriched during 293T$_{rhm}$ infection. BF520 Env wild-type amino acid identities are shown below the axes for residues 32-702. Logoplots above the axes indicate mutants that were enriched relative to wild-type residues ($S_{mut/wt}$ > 0) following infection of 293T$_{rhm}$ cells in our DMS. Site numbers correspond to HXB2 numbering.

Similar mutations improve usage of both macaque and human receptors

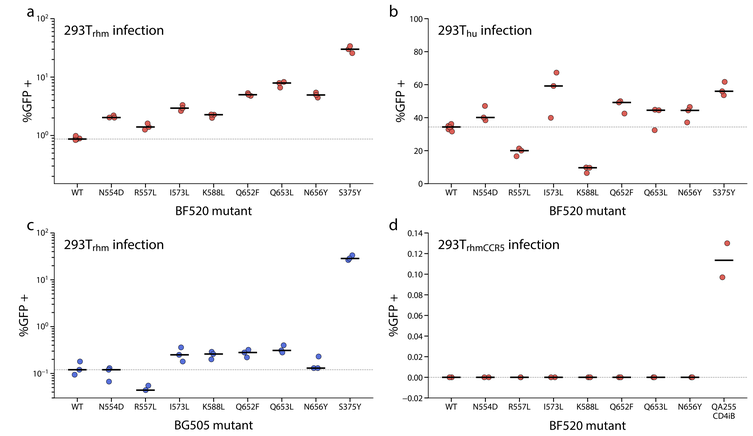


$S_{mut/wt}$ values of amino acids enriched during 293T$_{hu}$ infection. BF520 Env wild-type amino acid identities are shown below the axes for residues 486-702. Logoplots above the axes indicate mutants that were enriched relative to wild-type residues ($S_{mut/wt}$ > 0) following infection of 293T$_{hu}$ cells in our DMS. Site numbers correspond to HXB2 numbering.



Location of mutants highly enriched by 293T$_{rhm}$ infection within the six helical bundle. The 6HB conformation of NHR and CHR helices is shown in white. Sites and wt residues' side chains where mutations enhanced infection of 293T$_{rhm}$ cells greater than ten-fold over wild-type residues are shown in in blue (NHR) or red (CHR).

NHR/CHR mutations are CD4 dependent and enhance receptor usage by additional HIV strains



Infection of 293T$_{rhm}$, 293T$_{CCR5}$, and 293T$_{hu}$ cells by point mutant pseudoviruses. Each panel reports the percentage of the indicated cells that were GFP positive 48 hours post-infection with the indicated wild-type or mutant viruses. **a)** Infection of 293T$_{rhm}$ by point mutants in the BF520 Env background. **b)** Infection of 293T$_{hu}$ by point mutants in the BF520 Env background. **c)** Infection of 293T$_{rhm}$ by point mutants in the BG505 Env background. **d)** Infection of 293T cells bearing only rhesus CCR5 receptors by point mutants in the BF520 Env background.

NHR/CHR mutations expose MPER epitopes

		CD4bs			gp41 - heptad repeats		V3 loop	CD4i		V1/V2	MPER	
Env	Mutant	VRC01	b12	sCD4	T-20	NC-1	PGT121	17b	E51	PGT145	10E8	4E10
BF520	WT	0.18	>25	>25	0.36	>25	1.95	>25	>25	>25	3.15	>25
	Q653L	0.19	>25	>25	0.14	>25	3.05	>25	>25	>25	0.17	5.33
	N656Y	0.21	>25	>25	0.11	>25	1.28	>25	>25	>25	0.17	5.05
	I573L	0.20	>25	>25	0.20	>25	1.42	>25	>25	>25	1.13	10.59
BG505	WT	0.55	>25	>25	0.21	>25	0.25	>25	>25	0.22	2.44	>25
	Q653L	0.63	>25	>25	0.24	>25	0.32	>25	>25	0.28	0.29	7.73
	N656Y	0.54	>25	>25	0.13	>25	0.30	>25	>25	0.46	0.34	11.48
	I573L	0.49	>25	>25	0.27	>25	0.35	>25	>25	0.55	0.73	20.74

IC50: .1 — 2 — >25

Neutralization of BF520 and BG505 point mutants. Summary of IC50s for all mAbs and inhibitors assayed against mutant BF520 and BG505 viruses.

Conclusions

- N/CHR mutations enriched in our DMS following infection of 293T$_{rhm}$ cells enhance entry of cells bearing rhesus macaque receptors.
- N/CHR mutations improve usage of macaque receptors by a previously undescribed mechanism.
- Given that the N/CHR mutants we identified increase exposure of MPER epitopes, and in strain BF520, the NHR helix, we hypothesize that they may increase reactivity to macaque receptors by promoting sampling of a more open Env conformation.