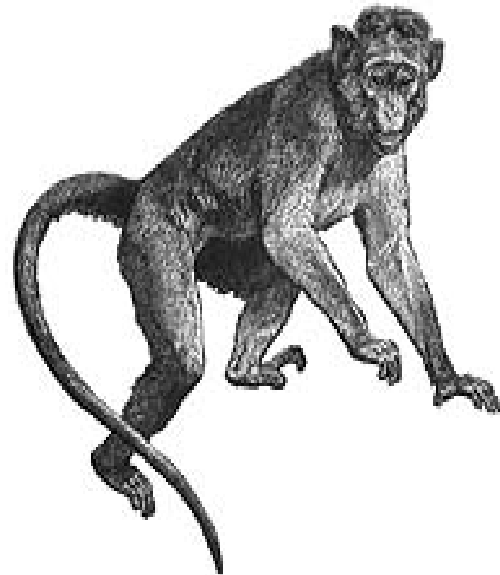
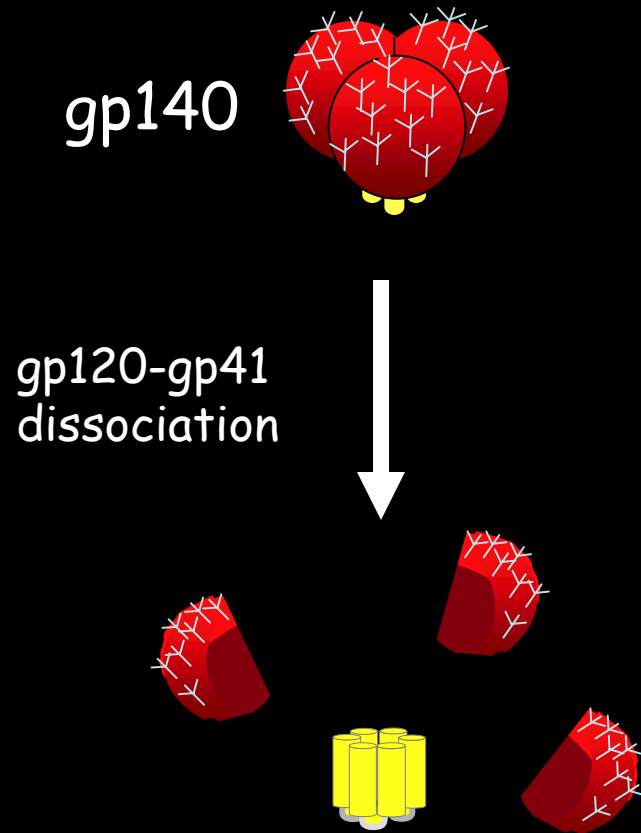


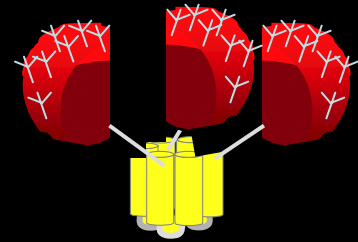
Immunogenicity of HIV VLPs
Bearing various forms of Env in
guinea pigs and macaques



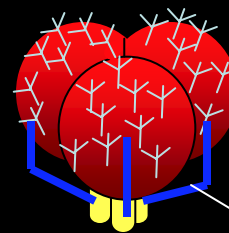
problem:
gp120 shedding



....possible solutions



solution 1: mutate so the gp120-gp41 precursor remains uncleaved



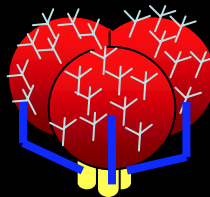
solution 2: stabilize assoc. of mature gp120-gp41

disulfide bridge (SOS mutant)

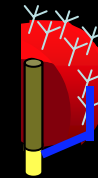
Stabilize Env trimers

problem:

cleaved
gp140_{sos}

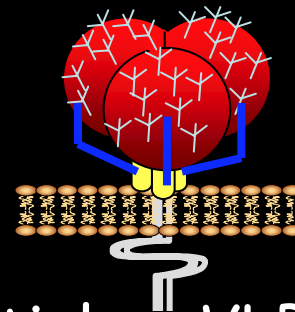


dissociation

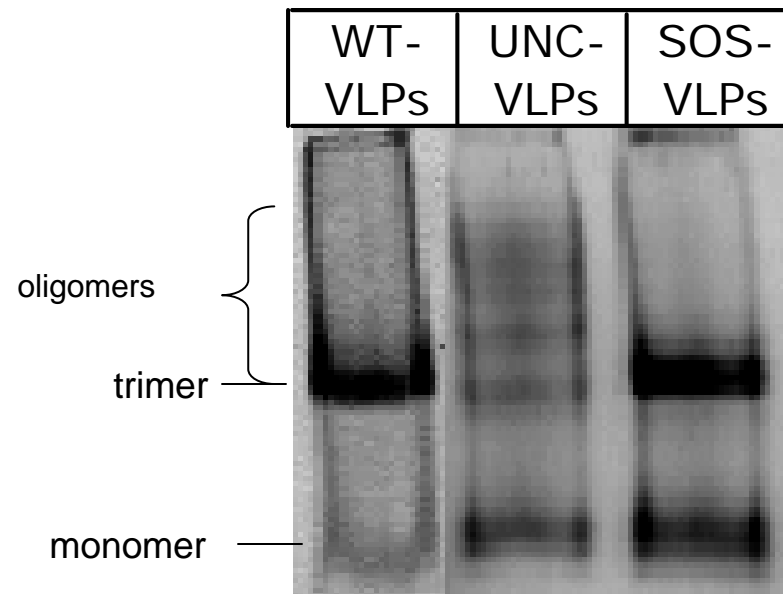


possible solution:

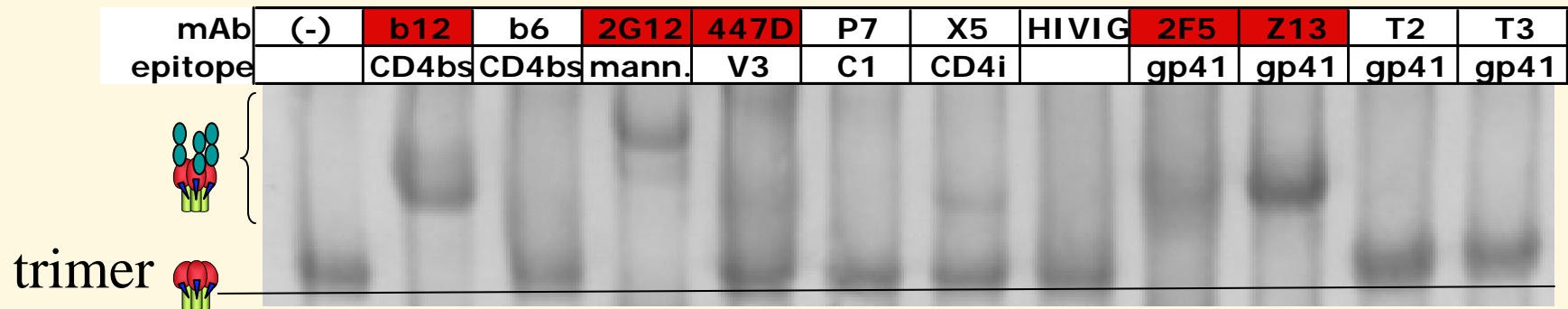
express
gp160_{sos}
in membranes



(on virus-like particles; VLPs)

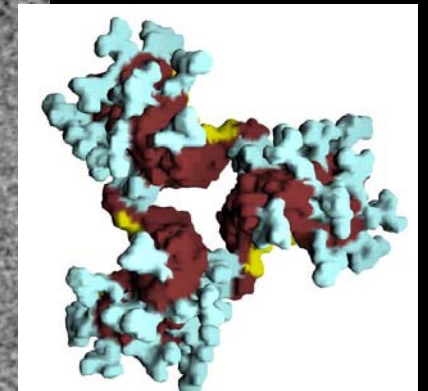
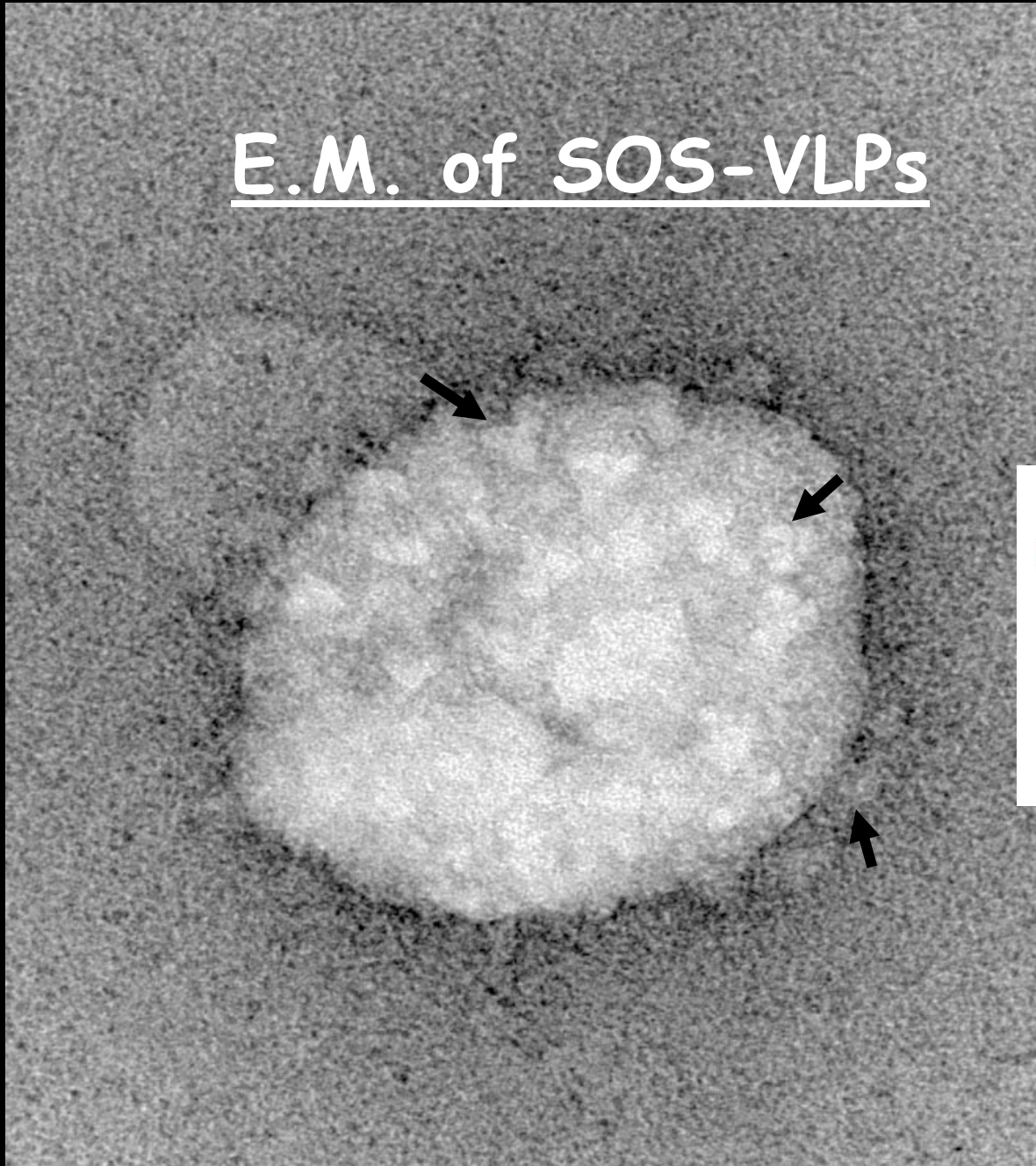


Native PAGE trimer binding assay



Infer: trimer binding directly correlates with neutralization

E.M. of SOS-VLPs



Immunogenicity of VLPs

Number: 20 guinea pigs; 4 macaques

Route:

intradermal/intramuscular inoculations monthly (g.pigs)

Intradermal (macaques)

Dose:

5ug Env equivalents -g.pigs

20ug Env equivalents - macaques

Types:

gp120

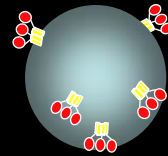


Adjuvants: CpG, QS-21

SOS-VLPs



UNC-VLPs

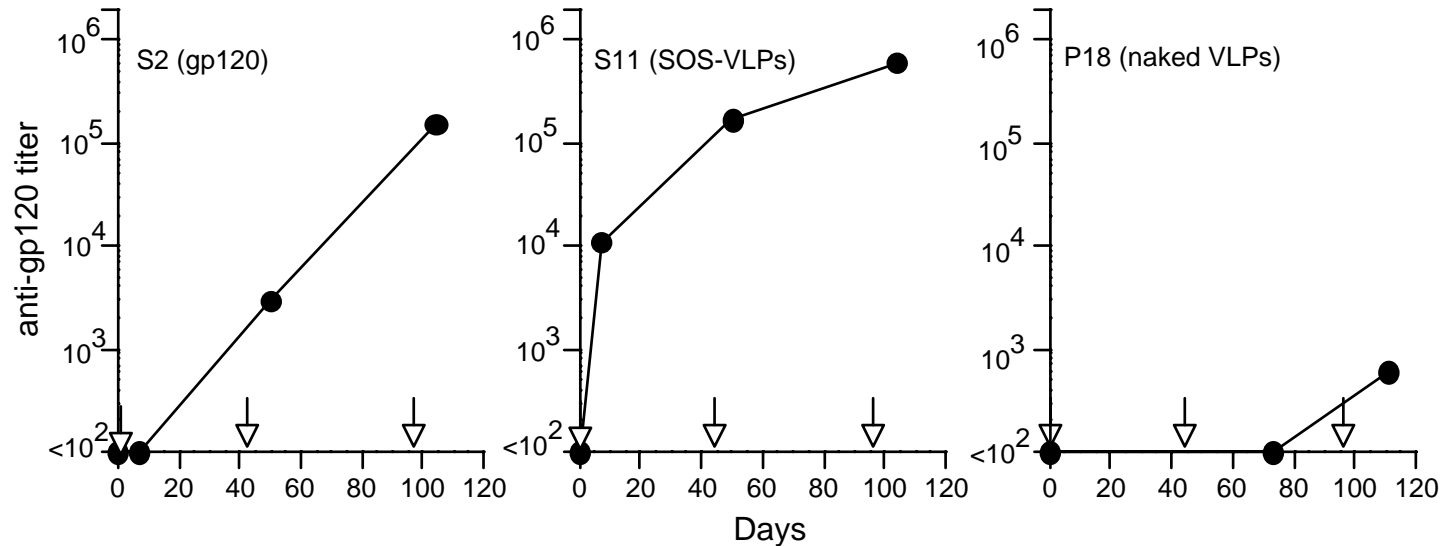


Naked-VLPs



Binding antibody responses in guinea pigs

Anima	Antigen/type	Adjuvant	ELISA titers			key:
			gp120	dgp120	gp41 AVERY	
S1	gp120	CpG	80,000	<300	<100	1,000-10,000
S2	gp120	CpG	150,000	5,000	<100	10,000-100,000
P1	gp120	CpG, QS21	600,000	300	<100	>100,000
S5	UNC-VLPs	CpG	100,000	n.d.	n.d.	
S6	UNC-VLPs	CpG	600,000	50,000	<100	
S7	UNC-VLPs	CpG	300,000	20,000	25,000	
S8	UNC-VLPs	CpG	150,000	60,000	25,000	
S9	SOS-VLPs	CpG	600,000	n.d.	n.d.	
S10	SOS-VLPs	CpG	160,000	10,000	<100	
S11	SOS-VLPs	CpG	180,000	45,000	<100	
S12	SOS-VLPs	CpG	150,000	4,000	<100	
P13	SOS-VLPs	CpG, QS21	190,000	10,000	1,300	
P14	SOS-VLPs	CpG, Ribi	260,000	9,000	<100	
P15	SOS-VLPs	CpG	650,000	18,000	700	
P16	naked VLPs	CpG	600	<100	<100	
P17	naked VLPs	CpG	6,000	n.d.	<100	
P18	naked VLPs	CpG	1,000	7,000	800	
A62	HIV+ plasma	N/A	100,000	50,000	70,000	



Inference: VLPs rapidly induce high titer antibody responses

Epitope Mapping of sera by virus capture and peptide ELISA

mapping by virus capture competition

Animal	Antigen/type	Adjuvant	LE311	39F	15e	b12	X5/sCD4	2G12	7B2	<40
			(V3 loop)		(CD4bs)		(CD4i)	(mann.)	gp41	40-100
S1	gp120	CpG	380	160	310	270	<40	<40	<40	100-1,000
S2	gp120	CpG	400	n.d.	n.d.	62	<40	n.d.	<40	>1,000
P1	gp120	CpG, QS21	850	260	3,300	2,400	430	<40	<40	
S6	UNC-VLPs	CpG	1,700	2,000	260	68	140	<40	<40	
S7	UNC-VLPs	CpG	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	760	
S8	UNC-VLPs	CpG	1,100	1,400	<40	<40	2,000	n.d.	80	
S11	SOS-VLPs	CpG	4,700	4,000	310	380	5,800	<40	<40	
S12	SOS-VLPs	CpG	1,600	1,400	<40	94	3,000	66	72	
P13	SOS-VLPs	CpG, QS21	1,300	4,000	40	<40	2,100	86	<40	
P14	SOS-VLPs	CpG, Ribi	4,400	4,100	50	47	150	<40	<40	
P15	SOS-VLPs	CpG	3,100	2,800	n.d.	40	1,200	<40	<40	
P16	naked VLPs	CpG	<40	<40	<40	<40	45	<40	n.d.	
P18	naked VLPs	CpG	<40	<40	<40	<40	<40	<40	<40	
A62	human HIV+	N/A	1,000	1,000	600	600	4,500	<40	2,000	
mAb self competition IC50 (µg/ml):			0.004	0.04	0.02	0.006	0.003	0.05	0.003	

mapping by peptide ELISA

Animal	Antigen/type	Adjuvant	V3 peptides							
			EINCFRPNNTKRSI	TRPNNNTRKSIHIGP	NNTRKSIHIGPGRAF	KSIHIGPGRAFYTT	IGPGRAFYTTGEIIG	RAFYTTGEIIGDIRQ	TTGEIIGDIRQAHCN	
S1	gp120	CpG	<100	710	1,300	280	<100	<100	<100	
S2	gp120	CpG	<100	6,000	60,000	400	<100	<100	100	
P1	gp120	CpG, QS21	<100	26,000	20,000	300	100	<100	100	
S6	UNC-VLPs	CpG	175	700,000	300,000	6,250	690	570	200	
S7	UNC-VLPs	CpG	394	56,000	320,000	600	600	<100	220	
S8	UNC-VLPs	CpG	337	5,000,000	2,000,000	6,200	450	700	190	
S10	SOS-VLPs	CpG	<100	4,600	20,600	150	160	<100	<100	<1,000
S11	SOS-VLPs	CpG	750	630,000	15,000	700	<100	<100	<100	1,000-10,000
S12	SOS-VLPs	CpG	<100	87,000	15,000	310	200	160	<100	10,000-100,000
P13	SOS-VLPs	CpG, QS21	<100	91,000	23,000	370	150	150	<100	>100,000
P14	SOS-VLPs	CpG, Ribi	210	2,000,000	500,000	4,400	370	490	300	
P15	SOS-VLPs	CpG	850	300,000	107,000	1,300	377	330	250	
P16	naked VLPs	CpG	120	130	100	1,400	<100	100	<100	
P17	naked VLPs	CpG	N.D.	410	400	<100	100	130	120	
P18	naked VLPs	CpG	155	110	<100	150	<100	<100	<100	
A62	human HIV+	N/A	<100	8,000	9,000	120	<100	<100	<100	

key:

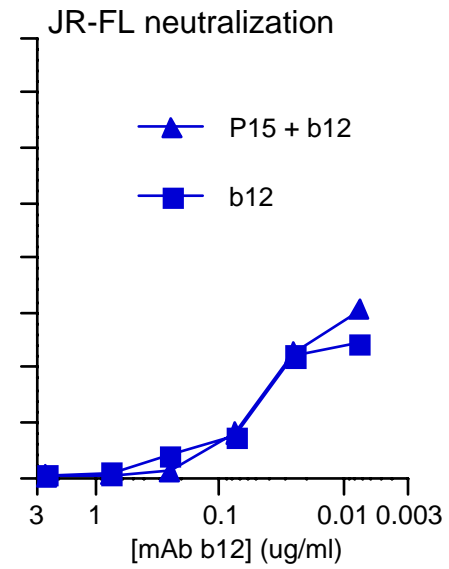
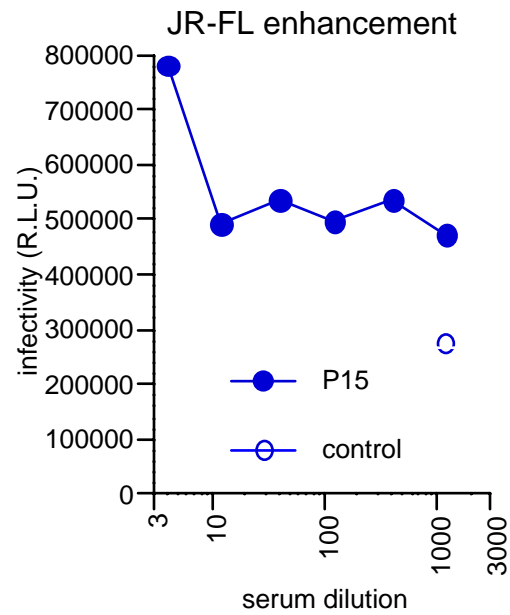
<1,000
1,000-10,000
10,000-100,000
>100,000

Neutralization activity in various assays

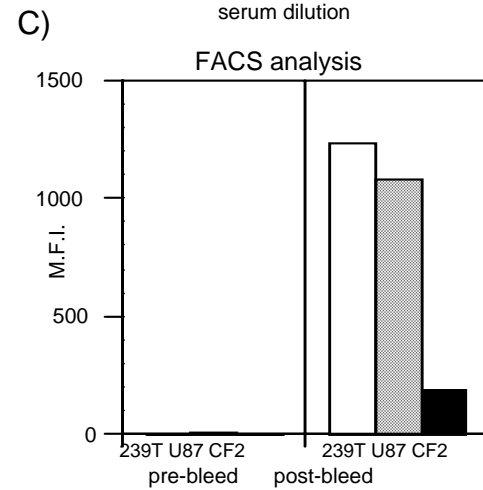
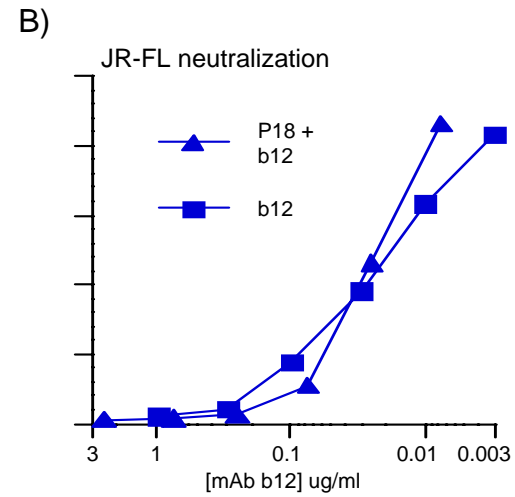
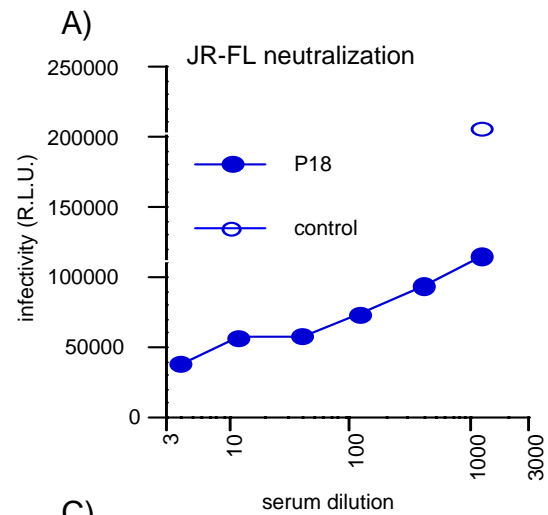
Column:		1	2	3	4	5	6	7	key:				
Assay type:		luciferase											
Virus grown:		293T cells											
Target cells:		CF2 cells											
Env isolate:		JR-FL	mac316		VSV-G		JR-FL	HXB2	1196	SF162 308T/H			
Animal	Antigen/type	(IC50)	(IC90)	(IC50)	(IC90)	(IC50)	(IC90)	post-CD4 (IC50)	(IC50)	(IC90)	(IC50)	(IC90)	(IC50)
S1	gp120	<4	<4	<4	<4	<4	<4	5,000	<4	<4	n.d.	n.d.	<4
S2	gp120	<4	<4	<4	<4	<4	<4	115,000	<4	<4	<4	<4	300
P1	gp120	<4	<4	<4	<4	8	<4	55,000	200	35	10	<4	110
S5	UNC-VLPs	<4	<4	n.d.	n.d.	<4	<4	n.d.	<4	<4	n.d.	n.d.	n.d.
S6	UNC-VLPs	<4*	<4*	<4*	<4*	<4	<4	270,000	4	<4	52	6	110
S7	UNC-VLPs	<4	<4	<4	<4	4	<4	80,000	<4	<4	79	<4	<10
S8	UNC-VLPs	<4	<4	<4	<4	4	<4	240,000	<4	<4	n.d.	n.d.	<10
S9	SOS-VLPs	<4	<4	n.d.	n.d.	<4	<4	n.d.	<4	<4	n.d.	n.d.	n.d.
S10	SOS-VLPs	<4	<4	<4	<4	<4	<4	30,000	<4	<4	4	<4	10
S11	SOS-VLPs	<4*	<4*	<4*	<4*	<4	<4	500,000	<4	<4	15	<4	250
S12	SOS-VLPs	<4*	<4*	<4*	<4*	<4	<4	52,000	<4	<4	130	<4	60
P13	SOS-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	200,000	<4	<4	110	15	300
P14	SOS-VLPs	<4*	<4*	<4*	<4*	<4	<4	200,000	<4	<4	30	5	150
P15	SOS-VLPs	<4*	<4*	<4*	<4*	<4	<4	86,000	<4	<4	n.d.	n.d.	70
P16	naked VLPs	500#	<4	540#	<4	30#	<4	<1,000	400#	40#	110#	10#	300#
P17	naked VLPs	550#	<4	800#	<4	n.d.	n.d.	<1,000	n.d.	n.d.	n.d.	n.d.	n.d.
P18	naked VLPs	300#	<4	800#	<4	30#	<4	<1,000	400#	40#	115#	13#	270#
A62	HIV+ plasma	100	12	<4	<4	<4	<4	89,600	n.d.	n.d.	n.d.	n.d.	n.d.
N308	HIV+ plasma	10,000	1,000	<4	<4	<4	<4	240,000	10,000	800	n.d.	n.d.	860

Column:		1	2	3	4	5	key:		6	7	key:	
Assay type:		luciferase							p24	p27		
Virus grown:		293T cells							hPBMC	hPBMC		
Target cells:		TZM-BL cells							hPBMC	hPBMC		
Env isolate:		SF162	JR-FL	1196	BaL	MLV			JR-FL	mac251		
Animal	Antigen/type	(IC50)	(IC50)	(IC50)	(IC50)	(IC50)			(@1:18)	(@1:18)		
S1	gp120	n.d.	n.d.	n.d.	n.d.	n.d.			-30%*	n.d.		
S2	gp120	<20	104	375	221	78			-29%*	n.d.		
P1	gp120	365	52	417	597	78			75%	n.d.		
S5	UNC-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			n.d.	n.d.		
S6	UNC-VLPs	83	27	235	298	<20			100%	46%		
S7	UNC-VLPs	<20	29	266	621	<20			100%	n.d.		
S8	UNC-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			100%	59%		
S9	SOS-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			100%	68%		
S10	SOS-VLPs	<20	25	48	49	<20			100%	61%		
S11	SOS-VLPs	25	24	795	390	<20			100%	53%		
S12	SOS-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			100%	50%		
P13	SOS-VLPs	135	47	1,585	1,474	<20			100%	n.d.		
P14	SOS-VLPs	67	<20	587	296	<20			100%	n.d.		
P15	SOS-VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			100%	n.d.		
P16	naked VLPs	23	46	<20	<20	31			n.d.	n.d.		
P17	naked VLPs	n.d.	n.d.	n.d.	n.d.	n.d.			n.d.	n.d.		
P18	naked VLPs	<20	23	<20	<20	<20			n.d.	n.d.		

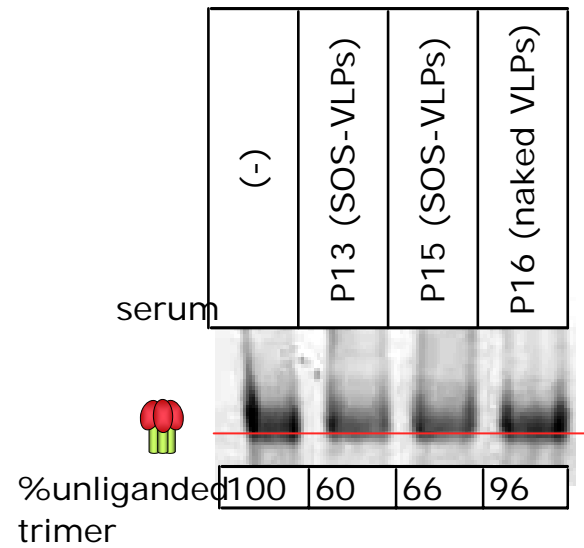
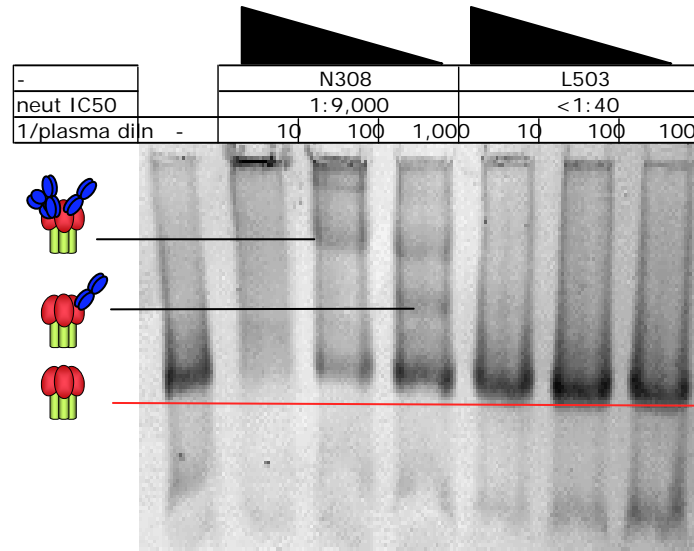
Enhancing Abs don't mask neutralization



Non-specific activity of Naked VLP sera



Trimer binding and neutralization

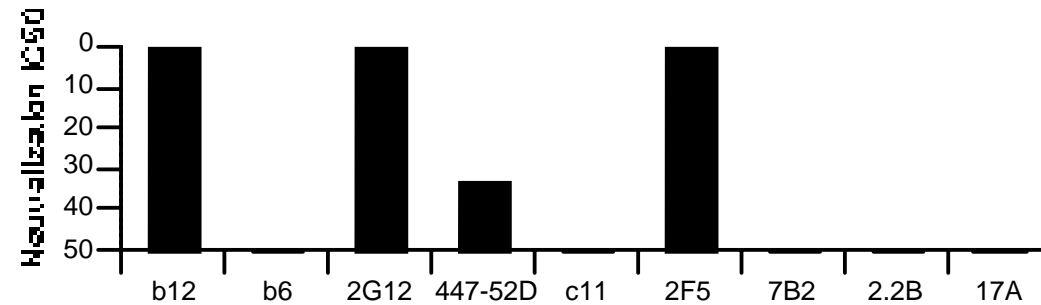


Summary of macaque VLP immunizations.

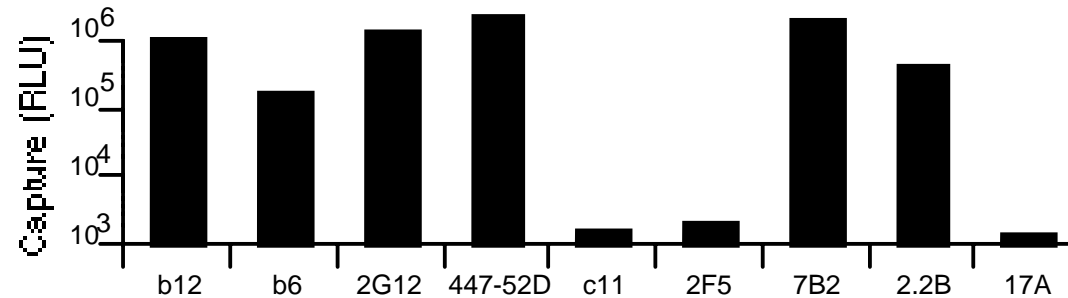
I.D.	Immunogen	ELISA EnFL gp120 Ps	Epitope mapping					Neutralization								
			39F (CD3 loc)	b12 (CD4bs)	X5/sCD4 (CD4i)	D2G12 (mann)	7B2 (gp41)	1196 bleed	SF162 308 3 bleed	JR+FL 3 bleed	SF162 bleed	SF162 2bleed	SIVmac316 bleed	92US715 bleed	US715 2bleed	
DV08	WT-VLP	3,930	<40	448	161	<40	550	<40*	<40*	<40*	<40*	<40*	<40*	<40*	<20	<40*
EK75	WT-VLP	28,275	40	350	800	<40	564	<40*	40	<40*	<40*	<40*	<40*	<40*	700	<40*
EN36	SOS-VLP	14,672	<40	150	300	<40	<40	<40*	<40*	<40*	48	<40*	<40*	<40*	35	<40*
EN37	SOS-VLP	45,318	40	489	300	<40	<40	<40*	50	<40*	54	<40*	<40*	<40*	50	<40*
							b12:	0.23	0.005	0.005	0.015	0.015	>50	1.03	1.03	

VLP capture and neutralization

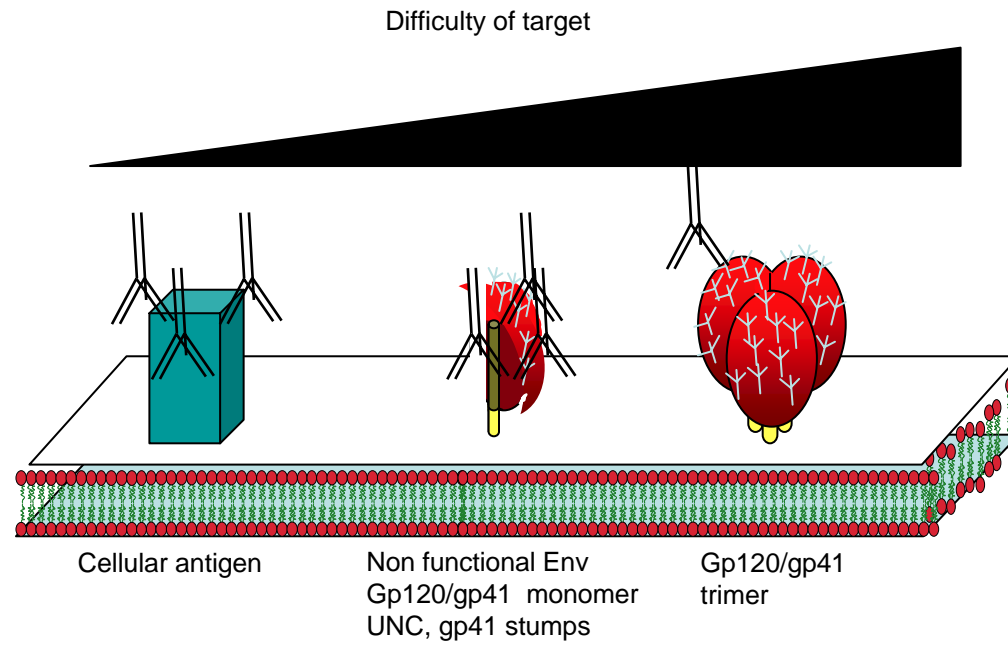
neutralization



WT-VLP
capture



Infer: mAb binding does not predict neutralization



P.Moore *et al.* J.Virol. 80:2515-2528 (2006)

Summary

- VLPs elicit high titer binding Abs
- macaques and guinea pig sera specific for different epitopes
- weak neutralizing activity, weak trimer binding
- need to refocus on trimer
- also anti-cell Abs are a problem for measuring neut
...the native trimer binding assay is a useful solution

Acknowledgments

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



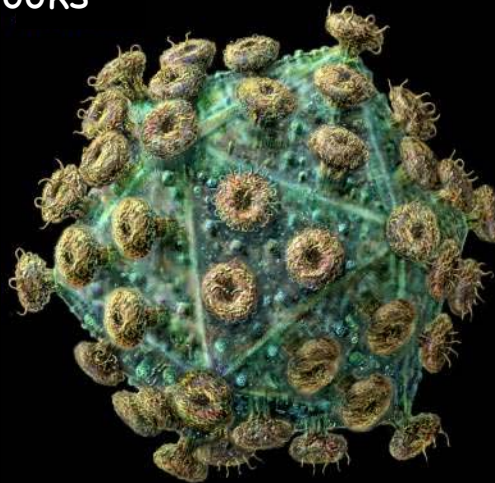
DUKE
UNIVERSITY

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Charmagne Cayanan
Cheryl Wiley
Irina Zharkikh
Dennis Burton

Pengfei Jiang
Emma Crooks



Paul Maddon
Norbert Schülke
Bill Olson



Cris Apetrei
Preseton Marx



Larry Arthur
Jeff Lifson
Julian Bess



Ping Zhu
Ken Roux

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