

## **"Defining the Specificities of Broadly Neutralizing Sera"**

Challenges:

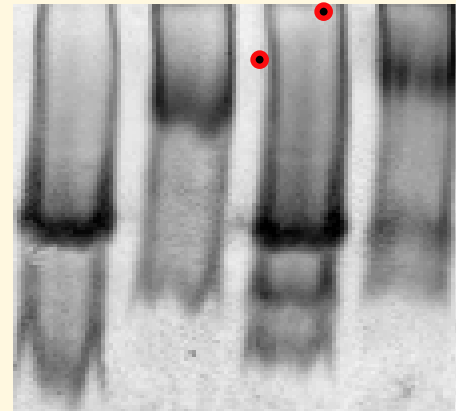
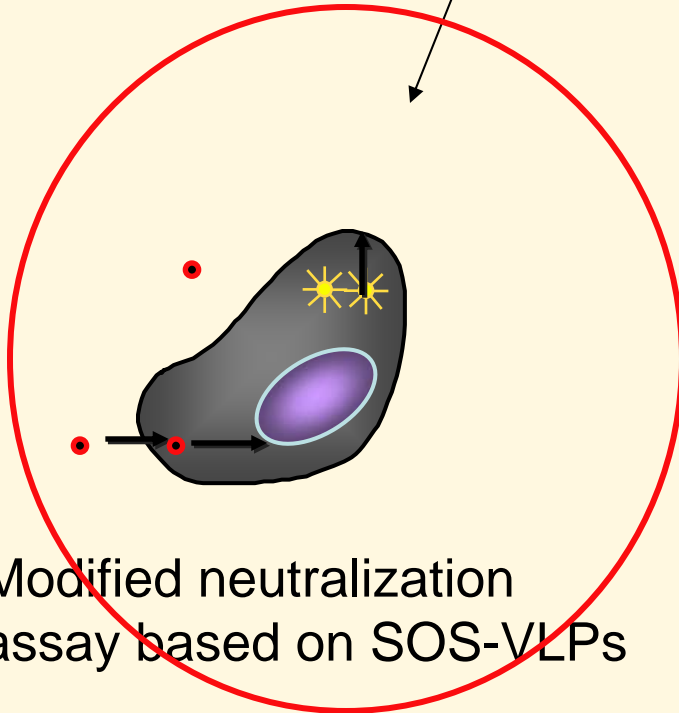
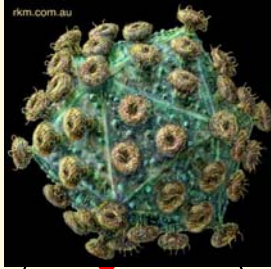
\*polyclonal

\*distinguishing neutralizing and  
non-neutralizing fractions

# Clade B, C, E cross-clade neutralization by mAbs and sera

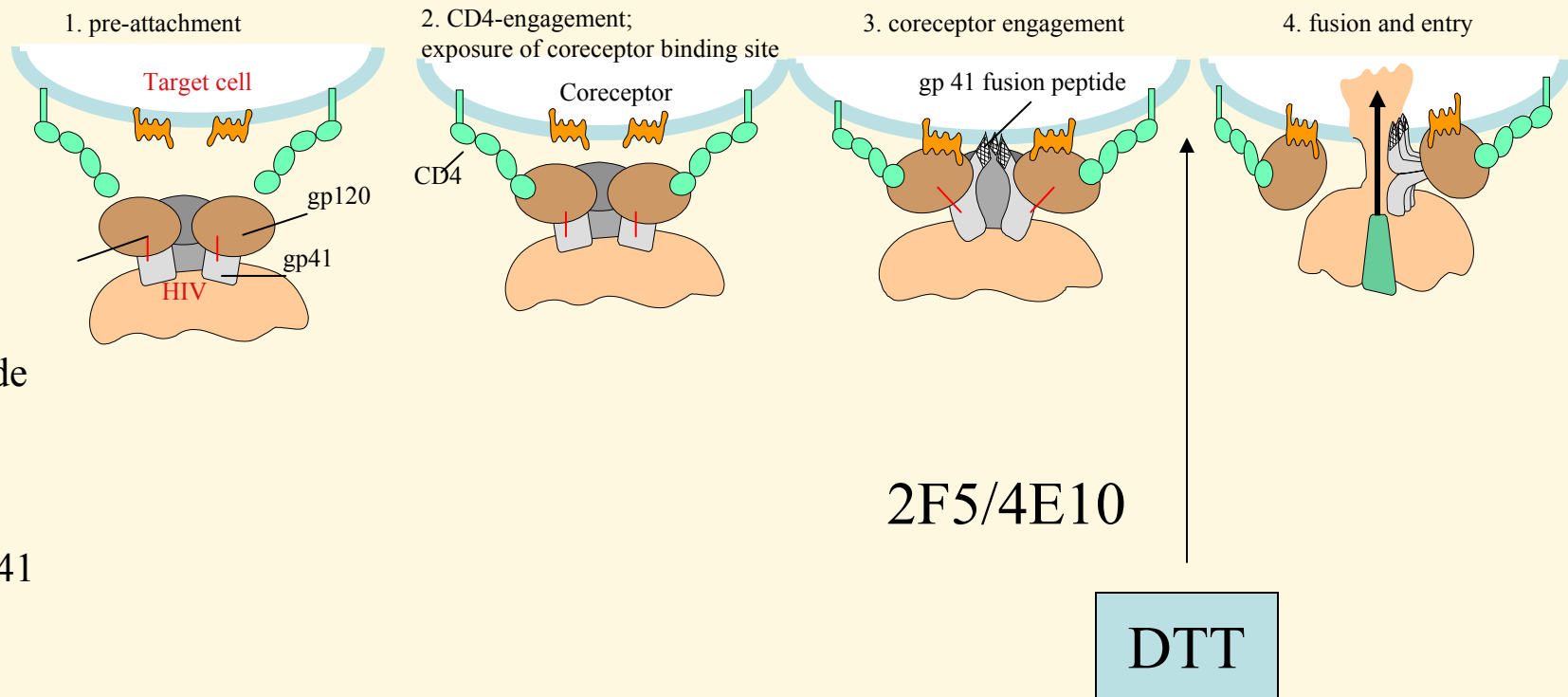
				IC50								1/dilution												
				MONOCLONAL ANTIBODIES								CLADE B PLASMAS				CLADE C PLASMAS				CLADE E PLASMAS				
Envelope	Origin	Cultured?¹	Clone?²	sCD4	b12	2G12	447D	F425	58.2	2F5	4E10	Z23	Z85	Z2	LTNP2	Z87	N16	C4	C10	C1	E17	E6	E3	
APV 10	USA	No	Q	>50	>50	>50	n.d.	>50	n.d.	>50	>50	158	94	46	136	87	44	<30	104	43	32	37	33	
APV 7	USA	No	Q	>50	>50	>50	n.d.	>50	n.d.	>50	26.58	166	104	27	33	25	26	<30	65	43	51	68	53	
APV 2	USA	No	Q	>50	2.97	1.62	n.d.	>50	n.d.	1.28	3.34	406	278	92	108	33	78	90	89	37	154	93	78	
TORNO	Italy	Yes	Q	>50	>50	0.26	>50	n.d.	>50	>50	1.15	304	348	88	60	162	79	178	61	129	260	130	53	
APV 4	USA	No	Q	>50	>50	>50	n.d.	>50	n.d.	2.80	5.77	193	<20	84	<20	<20	241	48	192	48	73	82	69	
1168	USA	Yes	Q	42.26	>50	>50	>50	n.d.	>50	3.53	7.18	129	94	31	67	104	29	36	61	<30	50	52	43	
APV 1	USA	No	Q	35.46	>50	23.94	n.d.	>50	n.d.	21.52	>50	234	158	54	107	86	46	<30	83	52	47	64	53	
1196	USA	Yes	Q	26.62	13.06	19.02	1.01	n.d.	0.52	42.31	5.36	183	169	84	196	89	73	31	138	154	<30	<30	31	
92UST12	USA	Yes	Q	25.29	9.22	>50	0.61	n.d.	1.36	5.80	7.68	173	183	79	177	148	39	84	42	33	114	36	41	
APV 9	USA	No	Q	24.81	>50	3.13	n.d.	>50	n.d.	>50	0.70	204	35	52	96	43	25	45	126	39	93	74	75	
JR-CSFc	USA	Yes	C	6.35	0.19	0.66	>50	5.03	32.80	3.36	6.00	270	449	149	57	176	184	222	59	57	126	58	50	
QH0515	Trinidad	Yes	Q	3.41	1.18	0.06	>50	n.d.	>50	3.38	5.22	217	306	62	128	57	31	53	62	41	46	57	72	
JR-FL	USA	Yes	C	2.69	0.07	1.42	32.61	n.d.	>50	5.70	12.80	732	364	192	3,629	1,957	76	649	96	120	530	66	62	
QH0692	Trinidad	Yes	Q	1.84	0.28	2.30	40.31	n.d.	39.31	2.40	4.13	185	77	60	41	165	51	73	81	70	75	52	61	
5768	USA	Yes	Q	1.19	1.52	>50	>50	n.d.	>50	9.24	21.89	463	414	211	140	104	71	97	137	78	64	49	58	
APV 5	USA	No	Q	0.99	>50	>50	n.d.	>50	n.d.	>50	9.19	581	319	112	156	202	147	117	65	31	118	73	41	
NL4-3	France	Yes	C	0.04	0.08	0.75	0.07	18.15	>50	2.41	4.95	4,942	3,750	2,149	2,702	1,474	1,558	2,110	3,137	3,077	213	140	450	
SF162	USA	Yes	C	n.d.	0.03	0.96	0.04	n.d.	0.07	1.00	3.95	24,296	35,691	12,520	5,653	5,744	6,988	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
average:												287	213	89	322	216	77	113	91	63	116	64	54	
												201				89				78				
MB C 12	unknown	No	Q	>50	9.13	>50	n.d.	>50	n.d.	>50	3.28	235	230	152	42	56	68	806	681	288	70	99	141	
MB C 1	unknown	No	Q	47.27	>50	>50	n.d.	>50	n.d.	>50	3.28	96	92	66	139	74	39	73	110	40	141	143	89	
21068	India	Yes	Q	35.87	2.24	>50	>50	n.d.	>50	>50	3.08	232	191	152	101	126	89	412	105	210	327	115	80	
MB C 4	unknown	No	Q	35.39	21.47	>50	n.d.	>50	n.d.	>50	7.25	231	27	43	45	88	102	98	187	52	49	95	41	
MB C 11	unknown	No	Q	31.98	>25	>50	n.d.	>50	n.d.	>50	20	199	94	41	58	51	61	75	78	59	51	99	71	
93IN101	India	Yes	Q	21.61	>50	>50	n.d.	>50	n.d.	>50	2.12	367	82	78	114	91	51	130	190	287	92	99	87	
MB C 5	unknown	No	Q	20.94	0.41	>50	n.d.	40.92	n.d.	3.49	4.00	80	166	24	21	21	31	35	44	32	45	60	34	
93IN959	Malawi	Yes	Q	9.68	>50	>50	n.d.	>50	n.d.	>50	4.23	456	556	64	125	33	83	188	118	177	110	88	70	
98BR004	Brazil	Yes	Q	9.02	>50	>50	>50	n.d.	>50	>50	6.13	615	140	210	73	36	74	124	46	65	55	52	54	
MB C 6	unknown	No	Q	8.98	>50	>50	n.d.	>50	n.d.	>50	>50	70	94	30	21	27	59	185	96	103	80	164	59	
98CN009	China	Yes	Q	8.46	1.25	>50	>50	n.d.	>50	>50	2.18	475	362	147	65	55	71	313	136	144	196	90	103	
97ZA012	S. Africa	Yes	Q	6.34	>50	>50	n.d.	>50	n.d.	>50	3.68	269	238	43	65	30	58	279	179	115	128	100	94	
MB C 3	unknown	No	Q	5.26	>50	>50	n.d.	>50	n.d.	>50	27.95	144	197	55	34	48	29	38	65	63	53	54	52	
98IN022	India	Yes	Q	0.90	0.80	>50	>50	n.d.	>50	>50	0.47	293	194	107	60	105	61	256	946	677	54	94	69	
MB C 7	unknown	No	Q	0.76	>50	>50	n.d.	>50	n.d.	>50	7.06	248	275	37	84	42	87	103	99	165	89	98	138	
93IN999	India	Yes	Q	0.59	10.14	>50	>50	n.d.	>50	>50	7.52	373	227	83	58	39	53	144	152	88	62	64	89	
MB C 8	unknown	No	Q	0.14	32.27	>50	n.d.	3.21	n.d.	>50	1.06	2,138	4,626	542	254	701	658	5,111	1,035	1,772	296	371	778	
average:												271	198	83	69	58	63	204	202	160	100	95	79	
												124				189				91				
92TH005	Thailand	Yes	Q	>50	>25	>50	>50	n.d.	n.d.	0.71	1.02	405	<20	118	21	40	111	58	93	65	1,209	234	263	
92TH024	Thailand	Yes	Q	>50	>25	>50	>50	n.d.	n.d.	0.49	0.39	274	62	109	<20	39	56	53	79	53	774	139	143	
MBAE12	unknown	No	Q	>50	0.70	>50	n.d.	>50	n.d.	1.34	1.08	294	<20	111	58	32	112	95	63	<50	284	159	219	
MB AE 2	unknown	No	Q	48.22	>50	>50	n.d.	>50	n.d.	2.74	2.96	158	174	132	23	21	33	59	35	<30	518	72	49	
92TH022	Thailand	Yes	Q	38.64	>50	>50	n.d.	>50	n.d.	0.48	0.92	116	63	55	<20	20	27	54	40	<30	2,286	176	302	
VLGC AE1	unknown	No	Q	28.30	>50	>50	>50	n.d.	>50	0.53	0.90	420	<20	64	<20	30	53	77	77	51	2,034	302	83	
MB AE11	unknown	No	Q	19.44	n.d.	n.d.	n.d.	>50	n.d.	n.d.	n.d.	254	<20	119	<20	29	71	93	65	84	111	109		
MB AE 4	unknown	No	Q	16.95	>50	>50	n.d.	>50	n.d.	1.39	1.94	86	<20	34	<20	<20	49	38	107	40	127	107	147	
MB AE 8	unknown	No	Q	14.25	>50	>50	n.d.	>50	n.d.	1.96	2.66	260	199	78	38	40	71	85	61	47	173	101	125	
MB AE 5	unknown	No	Q	13.51	>50	13.72	n.d.	>50	n.d.	9.65	19.76	216	129	173	45	29	38	616	204	45	1,012	70	48	
MBAE13	unknown	No	Q	13.11	15.58	>50	n.d.	>50	n.d.	1.28	1.03	185	100	157	<20	26	34	30	<50	<50	194	92	108	
MB AE 7	unknown	No	Q	12.53	31.83	20.71	>50	>50	n.d.	11.57	4.65	180	146	108	21	46	47	232	60	58	130	280	78	
CMU06	Thailand	Yes	Q	11.65	>50	>50	>50	n.d.	>50	1.21	1.05	315	195	91	95	31	59	53	165	41	253	47	77	
92TH001	Thailand	Yes	Q	6.60	>50	>50	>50	n.d.	>50	1.25	1.19	218	117	134	<20	30	45	70	76	52	844	184	140	
92TH019	Thailand	Yes	Q	4.89	>50	>50	>50	n.d.	>50	2.35	1.87	223	116	117	23	47	103	307	71	73	2,929	276	171	
MB AE10	unknown	No	Q	7.05	>25	n.d.	n.d.	>50	n.d.	n.d.	n.d.	205	192	88	25	28	37	48	99	34	172	99	114	
MB AE 1	unknown	No	Q	2.61	>50	>50	n.d.	>50	n.d.	2.09	2.25	330	305	100	32	38	49	430	284	168	255	41	46	
average:												244	112	105	31	32	56	140	97	56	781	146	131	
												97				98				353				
				aMLV	>50	>50	>50	>50	>50	>50	>50	>50	<40	<20	<20	<20	<20	<20	<30	64	<30	41	34	38

# Mapping methods

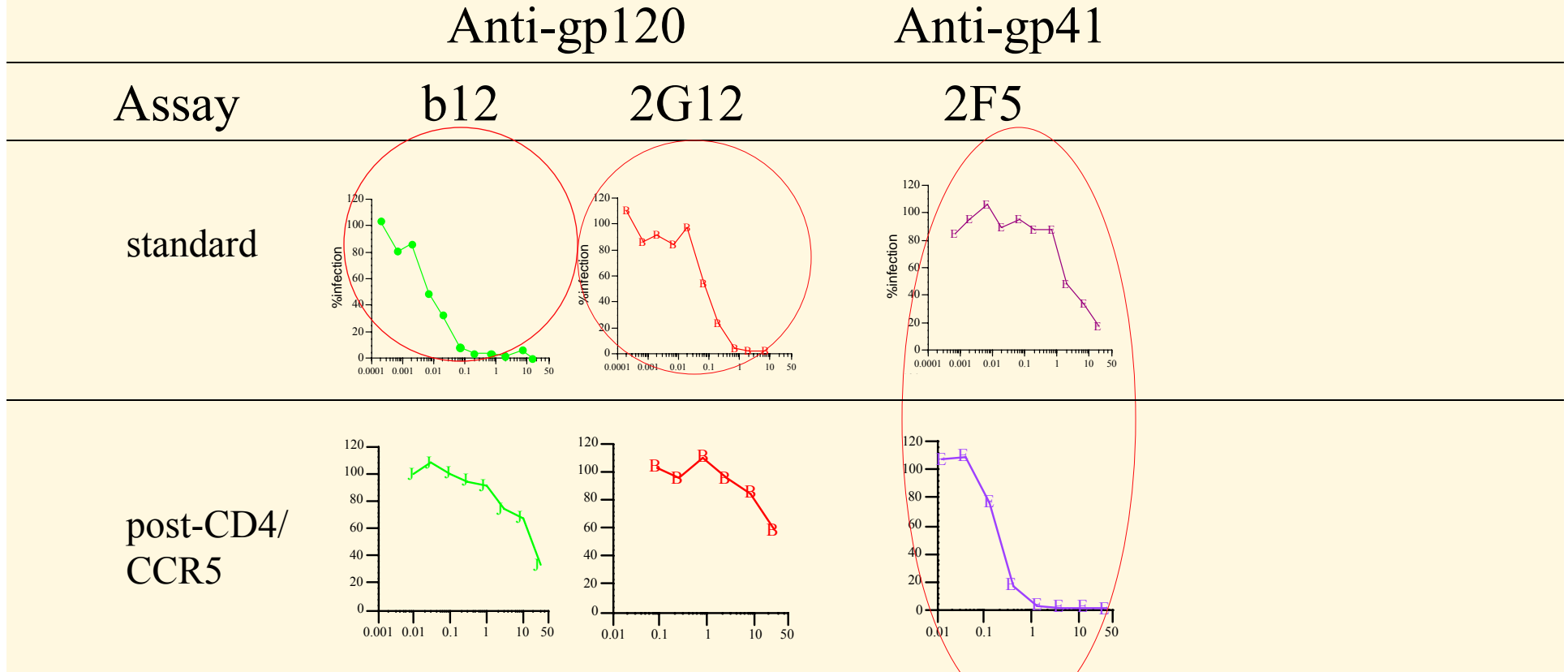


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# Infection and neutralization by mAbs



# Behavior of mAbs in different neutralization assay formats



Crooks *et al.* Human Antibodies 14:101 (2005)  
 Binley *et al.* J.Virol. 77:5678 (2003)

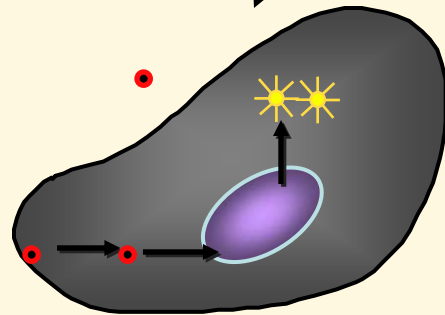
## Mapping HIV+ plasma neutralization

IC50 titer:

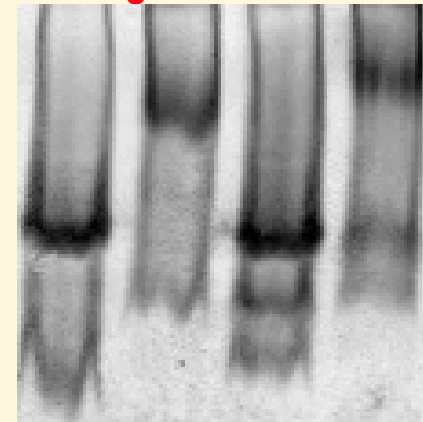
<50
50-300
300-1000
1000-3000
>3000

		format	
human plasma	gp120 titer	standard	post-CD4/CCR5
N308	250,000	9,000	<40
#11H	500,000	1,000	<40
L92	1,000,000	400	<40
L909	1,500,000	320	<40
R2	600,000	150	<40
739	80,000	100	<40
A62	100,000	100	<40
TN15	603,000	64	40
J864	248,000	50	<40
N160	500,000	<40	<40
K370	381,000	<40	<40
TN11	119,000	<40	<40
L503	612,000	<40	<40
HIV-	<300	<40	<40
spiked human plasma			
J864	248,000	50	<40
J864+b12	N.D.	950	<10
J864+2G12	N.D.	120	<10
J864+2F5	N.D.	110	71

\*Lack of post-CD4/CCR5 neutralization indicates mostly  $\alpha$ - gp120 neutralization (not  $\alpha$ - gp41)

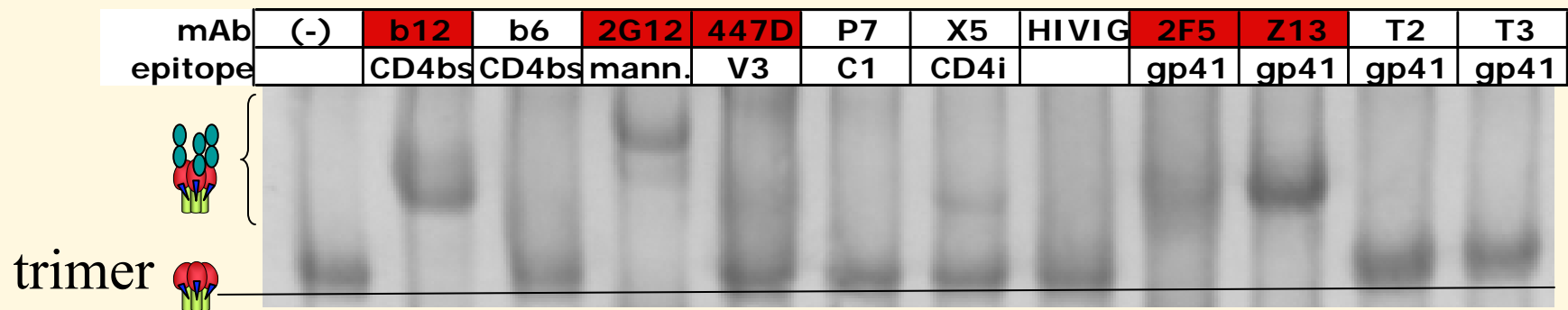


Neutralization  
mechanism



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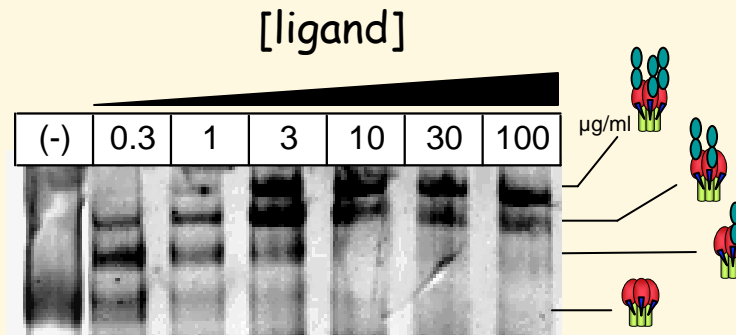
# Native PAGE trimer binding assay



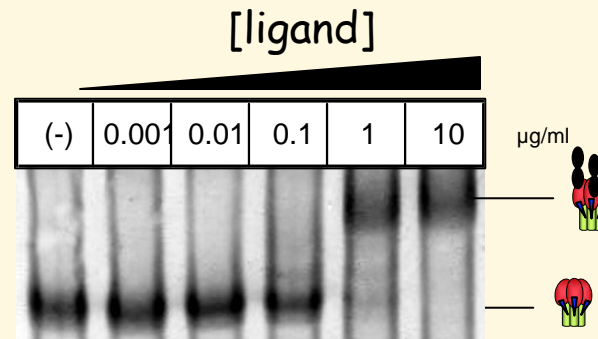
Infer: trimer binding directly correlates with neutralization

# 2G12 and soluble CD4

**2G12**  
(trimer  $IC_{50}=0.3\mu\text{g/ml}$   
neut  $IC_{50}=0.5\mu\text{g/ml}$ )

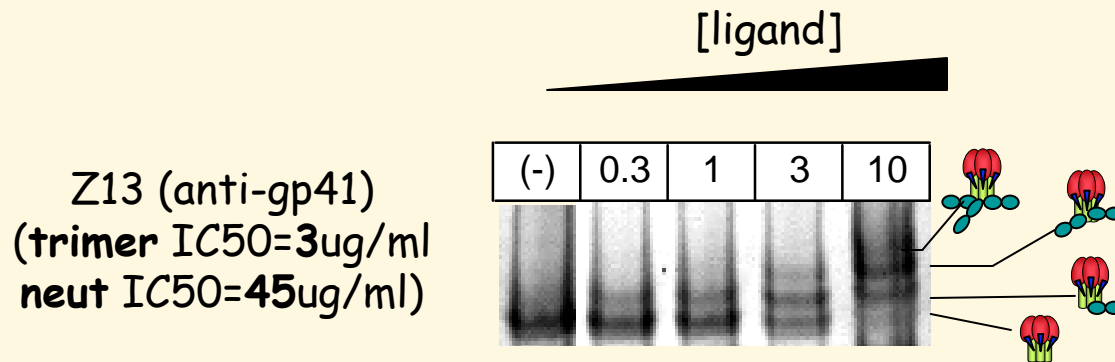
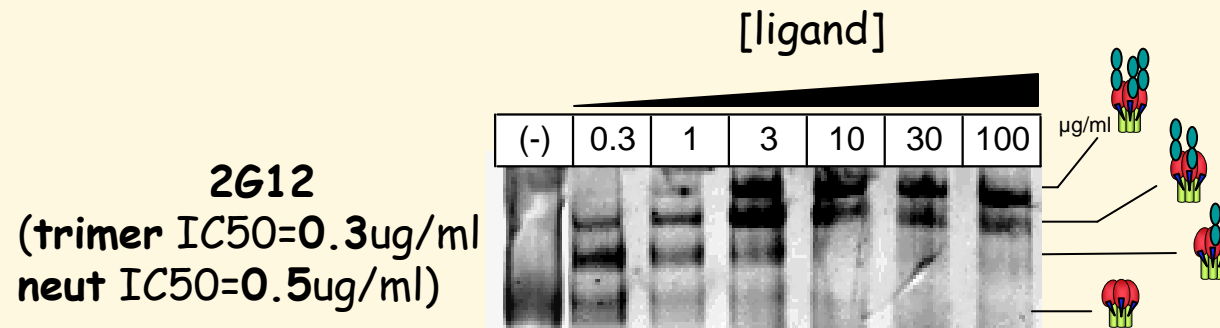


**soluble CD4**  
(trimer  $IC_{50}=0.3\mu\text{g/ml}$   
neut  $IC_{50}=0.8\mu\text{g/ml}$ )



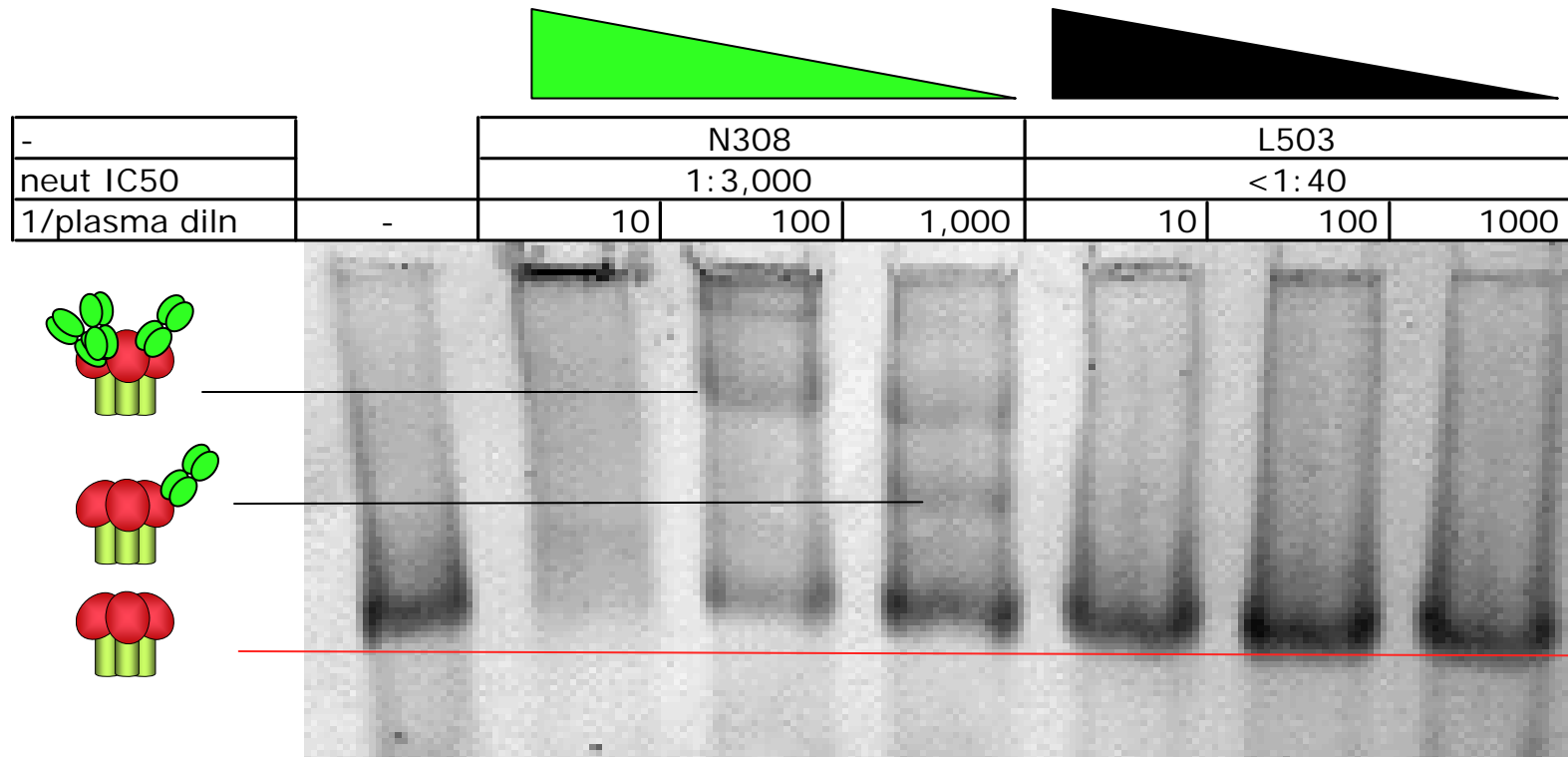
- \*3 x 2G12's bind per trimer
- \*direct correlation to trimer binding and neutralization  $IC_{50}$ s
- \*2G12 saturates over a large concentration range; sCD4 is "all or none" (co-operative?)

## Comparison of 2G12 and Z13



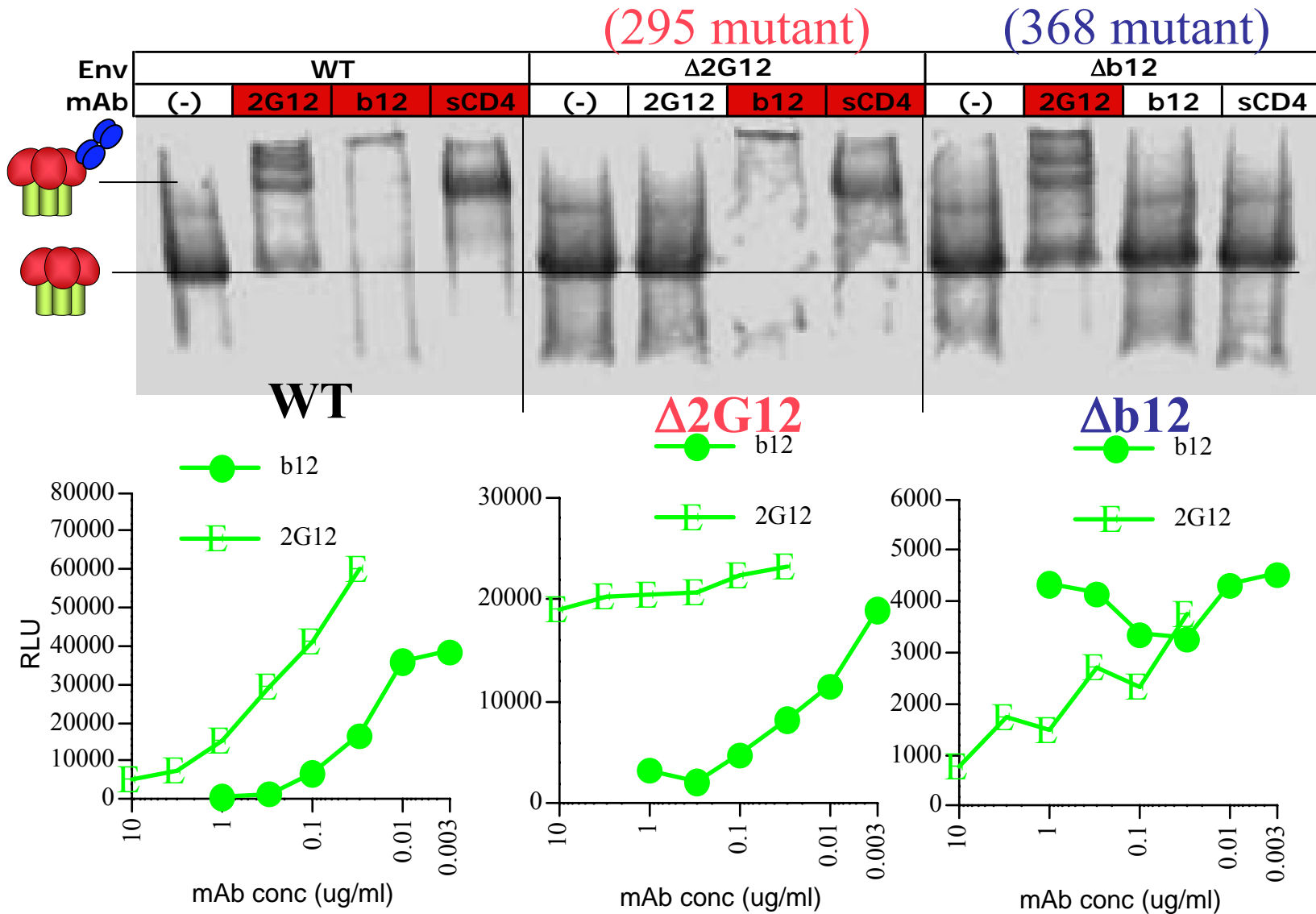
\*trimer binding is more sensitive than neutralization for detecting anti-gp41 Abs  
Perhaps more gp41 Ab required before neut effect (3x Abs)?

# Trimer shifts are mediated by **neutralizing** plasmas

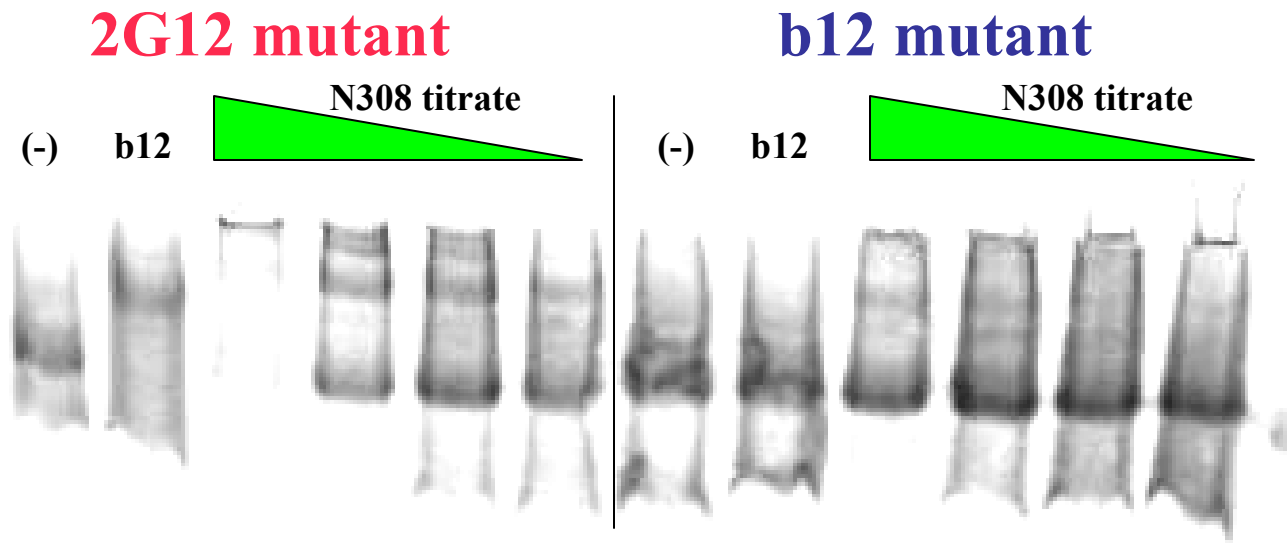




# Shifts of 2G12 and b12 knockout trimers

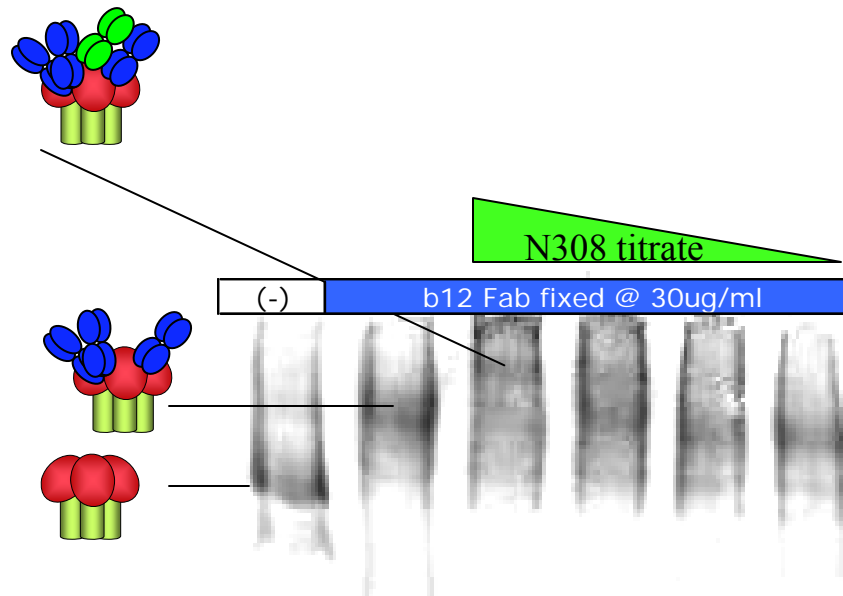


# N308 plasma binding to 2G12 and b12 “knockout” trimers



\*368 ( $\Delta$ b12) mutant knocks out most of HIV+ plasma (N308) neutralization.

...BUT: a fraction of trimer binding doesn't overlap the b12 epitope



...i.e. something completely different? Gp41 Abs?

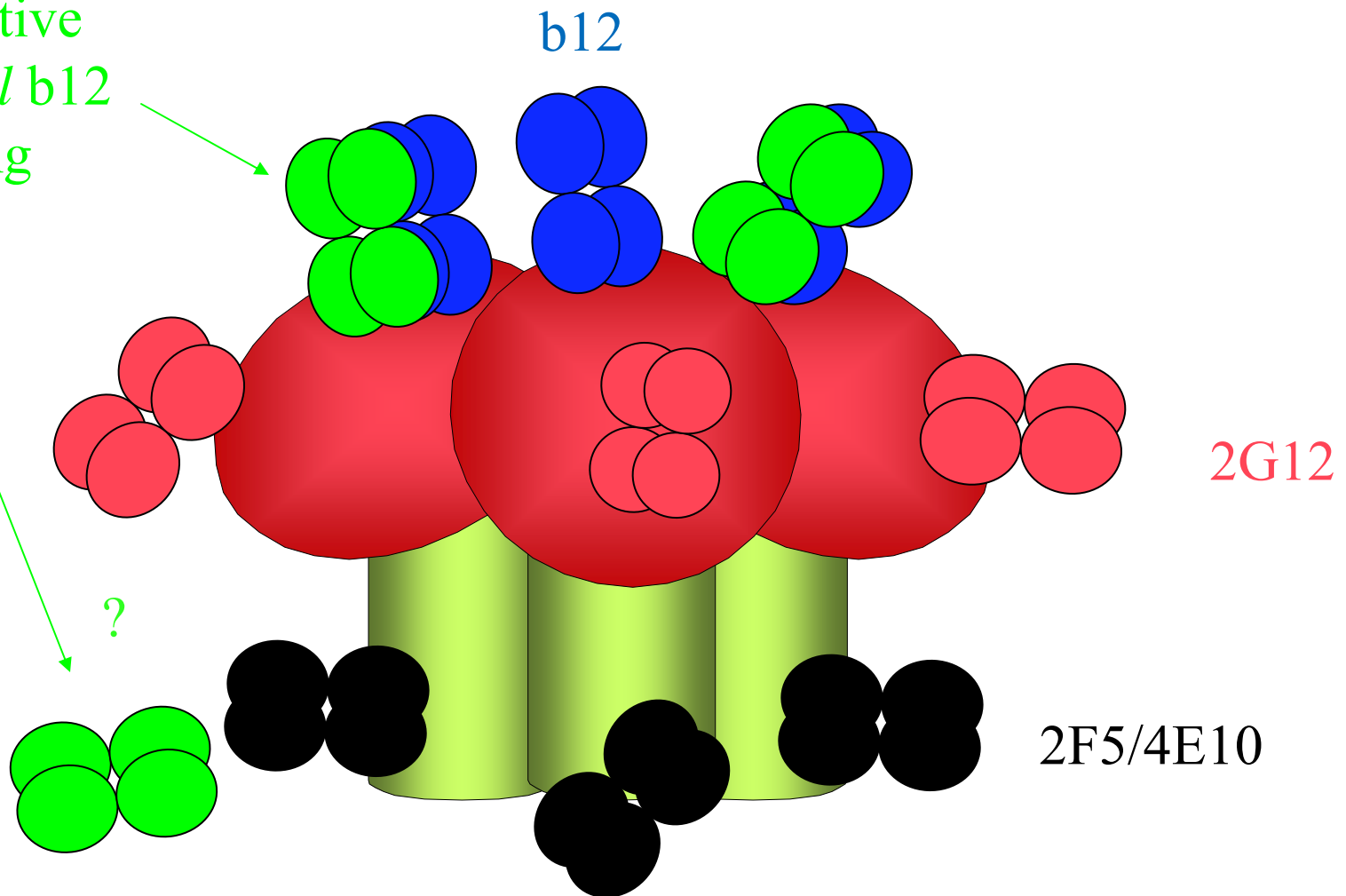
# Summary

Neutralizing HIV+ plasma:

-368-sensitive

-but not *all* b12

overlapping



# Thanks

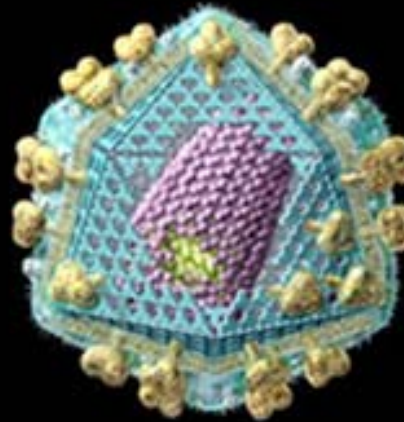


Torrey Pines Institute for Molecular Studies



Dennis Burton

Emma Crooks,  
Phenix Jiang



Doug Richman



National Institute of Allergy and Infectious Diseases  
National Institutes of Health



James Robinson

BILL & MELINDA  
GATES *foundation*



Promoting innovation and collaboration to  
speed the search for an HIV vaccine



the **collaboration**  
for AIDS vaccine discovery



NICD

Lynn Morris  
Penny Moore