



the
collaboration
for AIDS vaccine discovery

**Robin A Weiss on behalf of the
UCL Vaccine Discovery Consortium:**

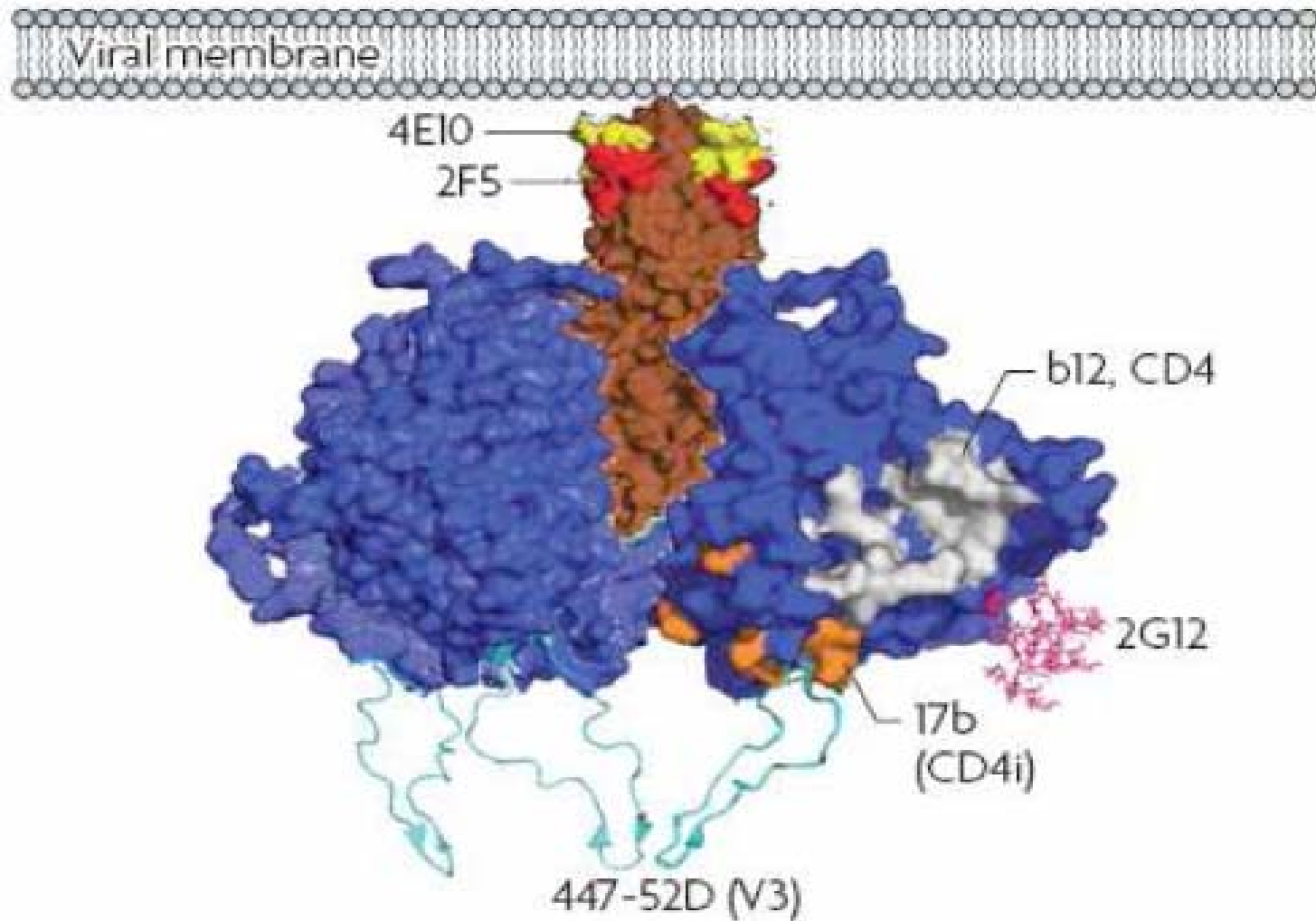
**“Vaccine-Induced Protective
Cross-Neutralization of HIV-1”**



BILL & MELINDA
GATES *foundation*

 Global HIV Vaccine
Enterprise

Neutralising epitopes on the HIV-1 envelope glycoproteins



‘Reverse vaccinology’ approach

- **Activity 1** Develop novel cross-neutralising monoclonal antibodies (NMABs)
(Chair: Quentin Sattentau, Oxford)

↓

- **Activity 2** Use NMABs to identify novel antigens
(Chair: Ralf Wagner, Regensburg)

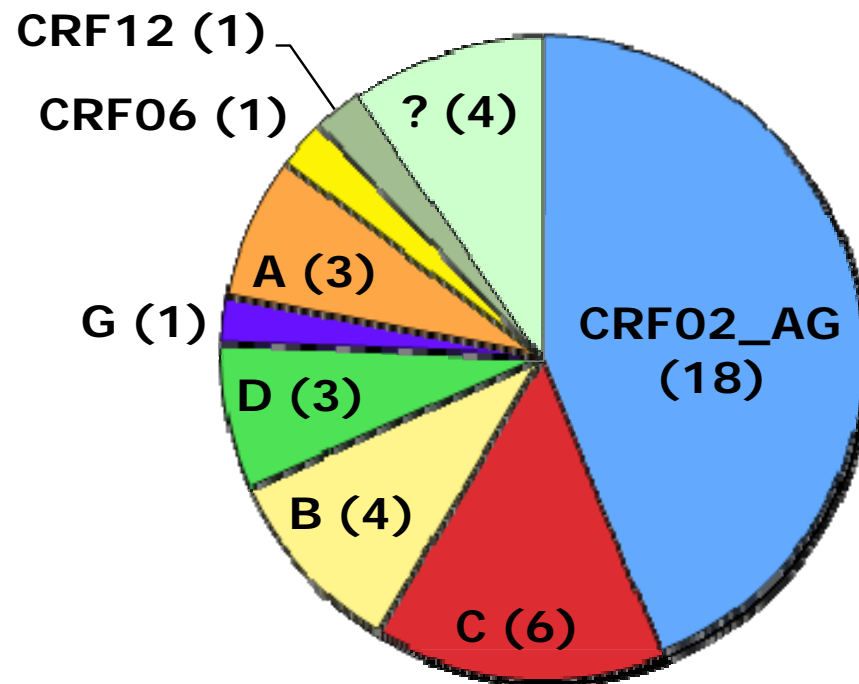
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- **Activity 3** Test novel antigens as immunogens for eliciting protective immunity
(Chair: Jonathan Heeney, Cambridge)

Methods for isolating cross-neutralising HIV-1 monoclonal antibodies (NMABs)

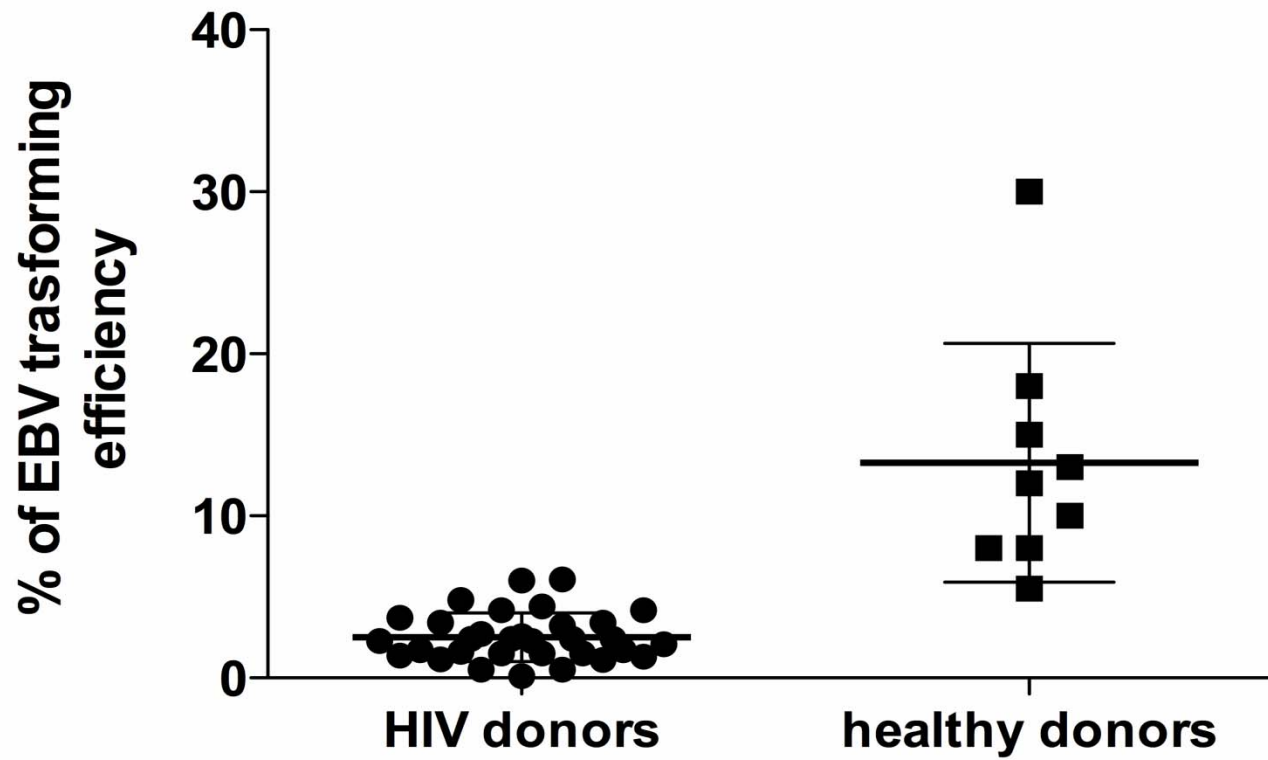
- 1. Immortalization of memory B cells taken from peripheral blood of infected human patients:**
 - **Patients from ITM Antwerp (Sunita Balla)
and QMUL London (Aine McKnight)**
 - **Isolation of NMABs at IRB Bellinzona
(Davide Corti & Antonio Lanzavecchia)**
- 2. Myeloma fusions of spleen cells from Hulg transgenic mice immunized with recombinant gp140 (ZM96 & UG37)
(Michael Neuberger & Quentin Sattentau, Cam/Ox)**
- 3. VHH phage libraries from llamas immunized with recombinant gp120 and gp140 (CN54, ZM96 & UG37)
(Theo Verrips & Robin Weiss, U Utrecht & UCL)**

Human NMAbs: HIV-1 clade (subtype) distribution of 42 donors of memory B cells



21 patients from ITM, Antwerp
21 patients from QMUL, London

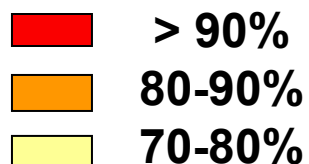
Relative efficiency of memory B-cell frequency and immortalisation



Neutralization breadth of human Mabs

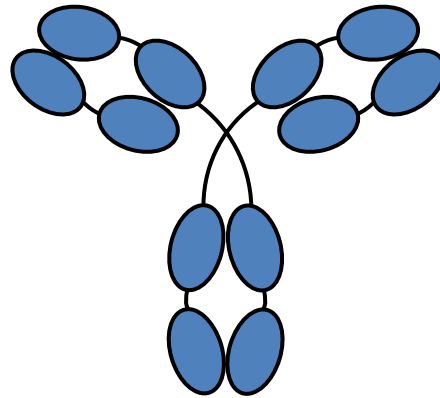
Daide Corti & Antonio Lanzavecchia

mAbs	A		B									C							AD	AG		BC			AB					
	Q769.d22	Q461.e2	H022.7	BaL.26	QH0692.42	SC42261.8	SF162	JRFL	TRO.11	CAAN5342.A2	RHPA4259.7	THRO4153.67	ZM249M.PL1	ZM53.PB12	96ZM651.2	DU151.2	CAP210.2.00.E8	93MW965	92BR025.9	DU422.1	ZM109F.PB4	Q168.a2	263-8	T257	CH110.2	CH064.20	CH181.12	AN527084	VSV-G	Donor's clade
HGA49	69	33	0	91	64	89	97	55	54	0	31	12	67	18	95	32	75	97	99	73	29	59	0	40	51	34	26	10	12	?
HGA9	0	0	0	92	28	0	82	23	0	0	0	0	0	0	91	0	69	98	91	0	60	0	32	0	26	0	0	0	22	
HGD129	79	0	0	99	76	62	95	59	0	0	50	0	37	1	35	30	0	72	0	0	0	29	51	31	29	0	0	0	33	
HGD14	44	0	0	84	0	0	81	76	0	27	0	20	0	0	40	0	0	0	0	0	0	0	0	42	13	0	0	0	33	B
HGD161	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	24	0	0	0	0	0	0	19	0	0	0	0	
HGD65	35	26	36	99	83	0	98	83	73	87	79	30	0	0	68	0	89	98	95	76	0	46	0	73	57	0	0	0	25	
HJ16	69	99	27	20	98	41	33	19	96	97	99	0	51	12	90	88	19	76	39	96	98	99	0	97	41	42	96	34	27	C
HK20	96	93	81	86	71	83	90	52	77	95	65	26	88	95	93	90	94	96	99	85	96	86	85	93	97	98	91	41	18	
HP12	19	0	0	0	0	0	0	0	71	71	44	0	0	0	0	0	0	54	69	13	0	0	13	0	0	0	0	0	0	
HR10	87	49	51	94	44	0	64	30	0	0	0	0	93	49	68	17	55	98	99	0	99	0	87	59	52	39	35	0	0	AG
HR12	71	0	0	0	0	52	0	0	0	66	0	26	0	0	41	0	0	20	0	67	51	42	41	74	46	0	0	0	18	
HZ74	68	31	0	99	64	65	95	72	0	0	0	0	40	0	23	16	0	96	48	0	86	20	61	48	17	47	21	0	25	
HX44	45	0	0	0	0	0	0	7	0	17	0	0	0	0	78	0	0	98	0	0	0	0	58	46	34	0	0	0	24	
HGF12	0	0	0	0	0	0	0	0	0	0	60	5	0	0	0	0	0	0	0	0	0	0	48	34	0	0	0	0	0	
HGF24	38	0	77	0	0	0	0	0	0	74	0	0	0	0	0	0	0	98	0	0	29	71	0	53	47	0	0	0	0	D
HGF9	0	38	24	10	14	27	57	28	44	69	0	15	62	50	96	41	90	97	99	0	0	64	74	54	60	0	35	0	9	
HGB33	83	0	0	0	0	0	0	77	0	0	0	0	0	0	72	0	0	50	59	92	55	0	0	91	67	0	0	0	13	G

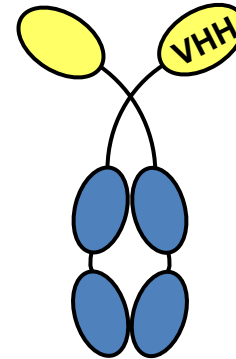


- 45 MAbs bind well to trimeric gp140
- 30 neutralize at least one primary HIV-1 isolate
- 14 are 'cross-neutralize' two or more clades
- Some recognize novel epitopes

***Camelidae* (llamas, dromedaries, camels)
have classical and non-classical IgG**



**Conventional
antibody**



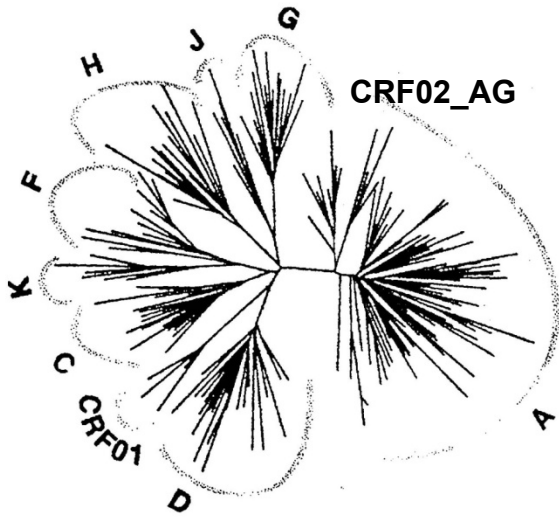
**Heavy-chain
antibody**



VHH

VHH recognising HIV may be useful as tools in vaccine development and also as vaginal microbicides (see Poster 18-04)

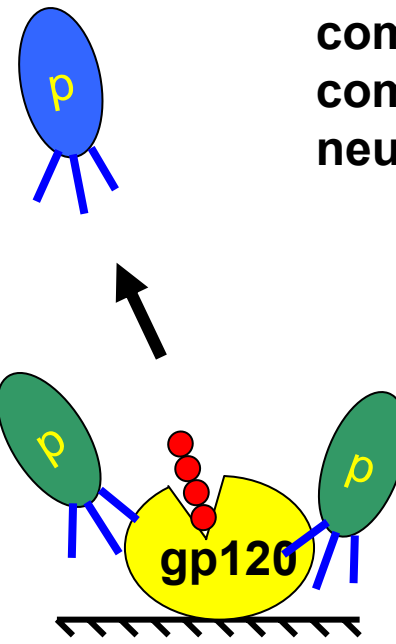
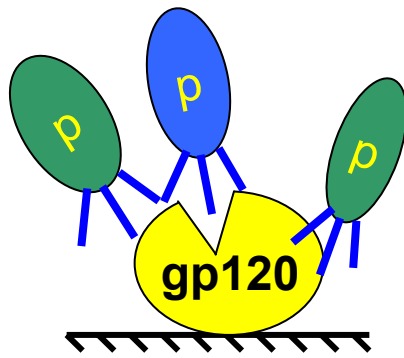
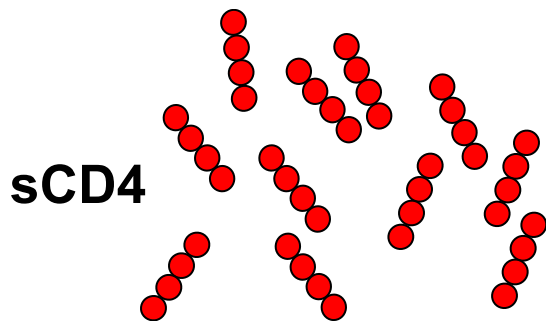
- **rapid screening of libraries**
- **long CDR3 loops**
- **small and stable 12-15kd proteins**
- **easy to mutate to improve affinity, stability, etc**



Screening for VHH targeting the CD4 binding site of gp120

**Selection strategy:
elution with soluble CD4**

Express eluted VHH



**Test: binding to gp120
competition with sCD4
competition with b12
neutralization of HIV-1**

(Forsman *et al*, J Virol, in press)

Neutralization of HIV-1 by llama VHH to the CD4-bs

IC₅₀ in TZM-bl cells (µg/ml)

Llama	VHH:	Clade: A					Clade: B					Clade: C								Clade: B'/C			Virus												
		Tier:	nd	2	2		1	1	2	2	2	2	nd	2	2	2	2	2	2	2	2	2		2	2										
		92UG037.A9	T257-71	T33-7		MN	IIIB	6535.3	THRO4156.18	TRJ04551.58																									
ZM96+UG37 gp140	2E7	16	45	38		34	10	41	•	•	18	3	8	22	38	22	6	•	17	30	•	20	36												
	1C2	16	•	•		>6	•	•	•	•	•	•	33	37	9	6	0.2	2	3	7	2	4	14												
	1F10	17	•	38		•	•	30	21	40	2	•	11	24	6	5	0.2	6	2	50	0.8	3	5												
	1B5	•	•	•		•	6	21	24	33	11	<2	5	•	16	32	48	•	20	7	6	15	8												
	1H9	•	•	•		•	6	•	•	•	9	<2	37	•	9	•	•	•	13	4	8	22	38												
CN54 gp120	A12	•	•	•		0.004	0.02	0.1	6	16	0.1	0.02	•	•	6	•	7	0.8	•	•	•	•	•												
	D7	•	•	•		0.06	0.03	0.2	7	•	•	0.03	•	•	22	•	34	6.6	•	•	•	•	•												
	C8	•	•	•		1	0.3	28	18	•	4	0.7	•	•	24	•	38	38	•	•	•	•	•												
mAb	b12	•	•	•		0.02	0.04	3	0.5	•	•	0.02	<2	<2	7	<2	•	•	•	<2	<2	•	•												

IC₅₀:
■ = <1 µg/ml
■ = 1-10
■ = 10-50
• = >50

VHH 2E7 neutralises 38/65 (58%)

VHH A12 neutralises 22/65 (32%)

MAb b12 neutralises 28/65 (39%)

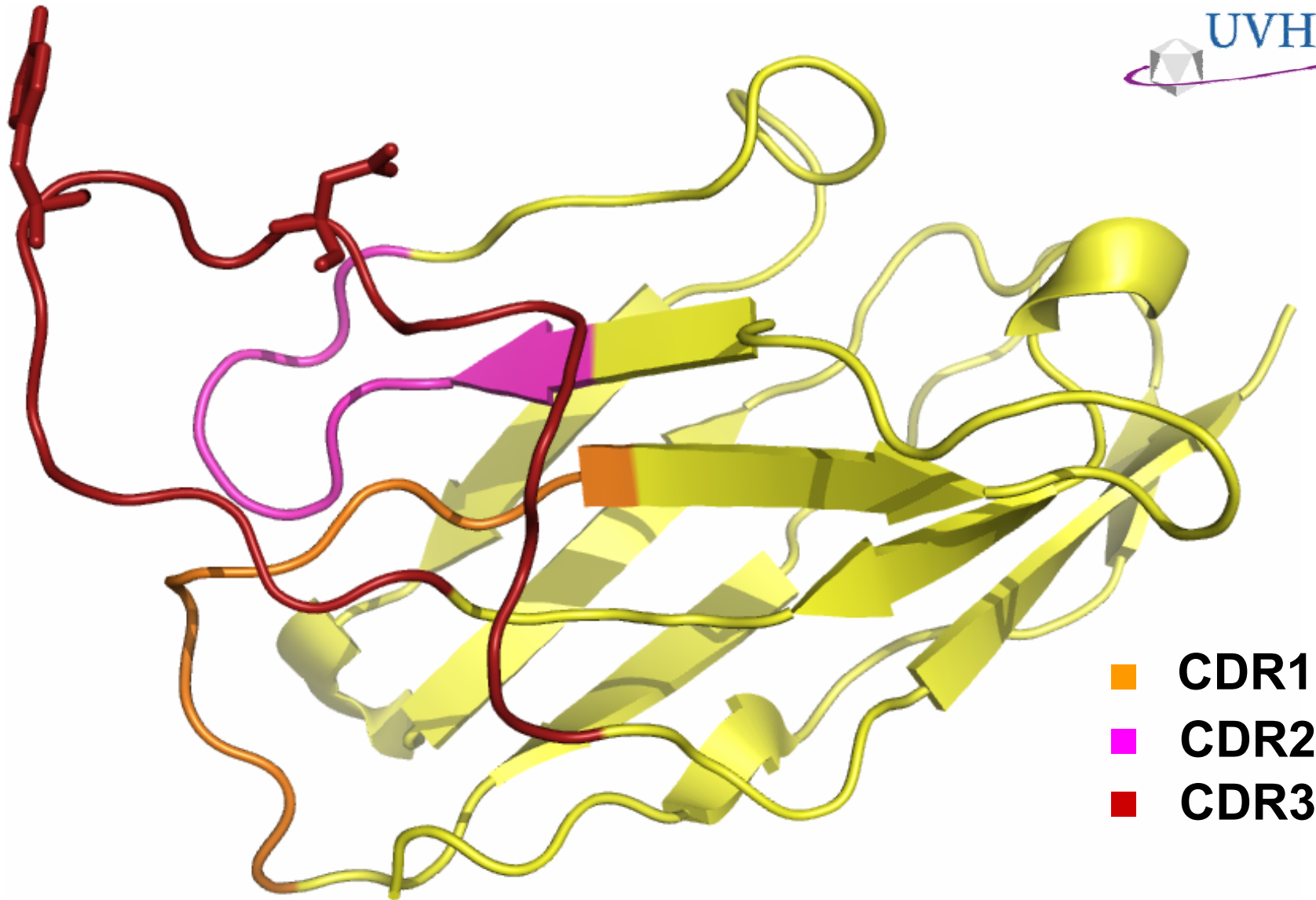
(Marlen Aasa-Chapman & Anna Forsman at UCL, Agnieszka Szynol, Utrecht)

Structure of VHH D7

(Poster 19-09)

Theo Verrips
Utrecht

W Weissenhorn
Andreas Hinz
Grenoble

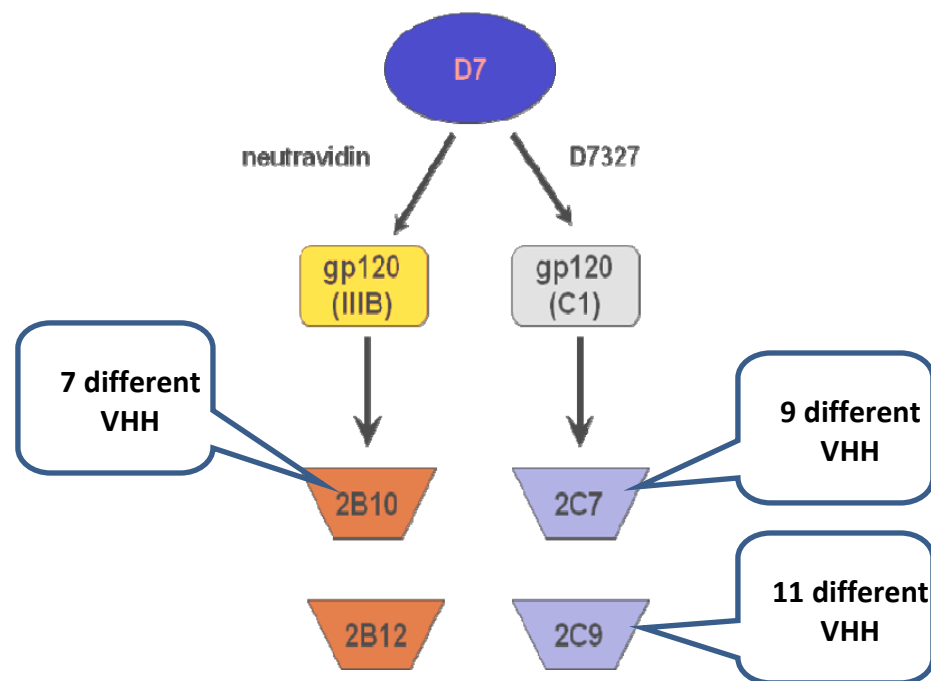


- CDR1
- CDR2
- CDR3

Selection of family-specific VHH using CDR3-degenerate reverse primers

(Willie Koh, UCL, and Hans de Haard & Theo Verrips, U Utrecht)

A sub-library related to D7 was created



49 unique clones from 2C7 & 2C9
27 VHH under study at present

Plate	1 st Round	2 nd Round
2B10 (D/E)	0.1nM gp120-IIIB	(D) 10pM gp120-IIIB (E) 1pM gp120-IIIB
2B12 (D/E)	0.01nM gp120-IIIB	(D) 10pM gp120-IIIB (E) 1pM gp120-IIIB
2C7 (C/D)	10nM gp120-C1	(C) 100pM gp120-C1 (D) 10pM gp120-C1
2C9 (C/D)	1nM gp120-C1	(C) 100pM gp120-C1 (D) 10pM gp120-C1

IC50 (ug/ml) titres against HIV-1 in Tzm-bl

VHH	IIIB	SVPB6	SVPB11	C222	92Br025	C27d	SVPC6	SVPC10	ZM214M
D7	0.30	•	16.49	•	•	0.03	9.48	18.59	•
A12	0.09	13.00	16.10	0.07	0.23	0.02	7.81	14.91	•
b12	0.21	0.70	16.82	•	•	0.02	6.73	5.39	12.29
2B10A2	0.07	•	15.78	0.13	2.01	0.02	5.43	9.97	
2B10C2	0.16	•	12.46	0.12	1.57	0.03	5.19	9.84	
2B10D7	0.12	•		0.20	1.42	0.03			
2B10D11	0.18	23.47	15.46	0.36	4.47	0.03	6.78	8.37	
2B10G5	0.40	9.52	13.02	0.04	0.28	0.05	5.19	7.77	
2B10H1	0.29	17.21	14.13	0.08	0.74	0.04	4.45	6.89	
2B10H3	0.29	19.14	10.72	0.06	0.39	0.04	2.23	8.05	
2C7B8	0.15			0.27	0.78				•
2C7D1	0.24			•	•				•
2C7D2	0.17			0.28	0.60				•
2C7D5	0.13			•	•				•
2C7E3	0.07			0.13	0.48				•
2C7E11	0.21			•	•				•
2C7F7	0.34			4.53	•				•
2C7G11	0.16			0.24	1.76				•
2C7H5	0.12			10.20	•				•
2C9A8	0.30			0.41	2.40				•
2C9B11	0.24			0.30	3.41				•
2C9C5	0.12			0.16	1.23				•
2C9D9	0.37			0.61	3.64				•
2C9E2	0.20			4.08	•				•
2C9E4	0.35			0.50	1.76				•
2C9E7	0.30			0.34	0.90				•
2C9F1	0.13			0.48	0.95				•
2C9F6	0.18			•	•				•
2C9F10	0.13			0.16	0.61				•
2C9H5	0.17			0.19	0.73				•

Key:	<0.10
	0.1<x<1
	1<x<10
	10<x<25
	• >25

Good (like A12)

2C7B8_1_	EVQLVESGGGLVQAGGSLRLSCTASG-RISSSSYDMGWFRQAPGKEREFVAAISWGGATD	59
2C9F1_18_	EVQLVESGGGLVQAGGSLRLSCTASG-RISSSSYDMGWFRQAPGKEREFVAAISWGGTKD	59
2C7G11_9_	EVQLVESGGGLVQAGGSLRLSCTASG-RISSSSYDMGWFRQAPGKEREFVAAISWGGTTI	59
2C9H5_21_	EVQLVESGGGLV-AGGSLRLSCTASG-RISSSSYDMGWFRQAPGKREFVAAISWSSGTTI	58
2C7D2_3_	EVQLVESGGGLVQAGGSLRLSCTASG-RISSSSDMGWFRQAPGKEREFVAAISWGGTTI	59
2C9E7_17_	EVQLVESGGGLVQAGGSLRLSCTAFG-RISSSSDMGWFRQAPGKEREFVAAISWGGTTI	59
2C7E3_5_	EVQLVESGGGLVQAGGSLRLSCTASG-RISSSSDMGWFRQAPGKEREFVAAISWGGTTN	59
2C9F10_20_	EVQLVESGGGLVQAGGSLRLSCTAPG-RISSSSDMGWFRQAPGKEREFVAAISWGGTTN	59
9A12	AVQLVESGGGLAQAGGSLRLSCTASG-RISSSSYDMGWFRQAPGKEREFVAAISWGGTTI	59
9D7	AVQLVESGGGLAQAGGSLRLSCTVSG-RISSSSDMGWFRQAPGKEREFVAAISWGGTTN	59
	*****.***** * * ***** : : *	
2C7B8_1_	YADSVKGRFAISKDNARNNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C9F1_18_	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C7G11_9_	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C9H5_21_	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	118
2C7D2_3_	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C9E7_17_	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C7E3_5_	YAESVKGRFVISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
2C9F10_20_	YAESVKGRFVISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
9A12	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
9D7	YADSVKGRFAISKDNAKNAVSLQMSLKPEDTAVYYCAAQWRPLRYSDDPSNSDYYDWGQ	119
	** : ***** . ***** : ***** ***** ***** *	

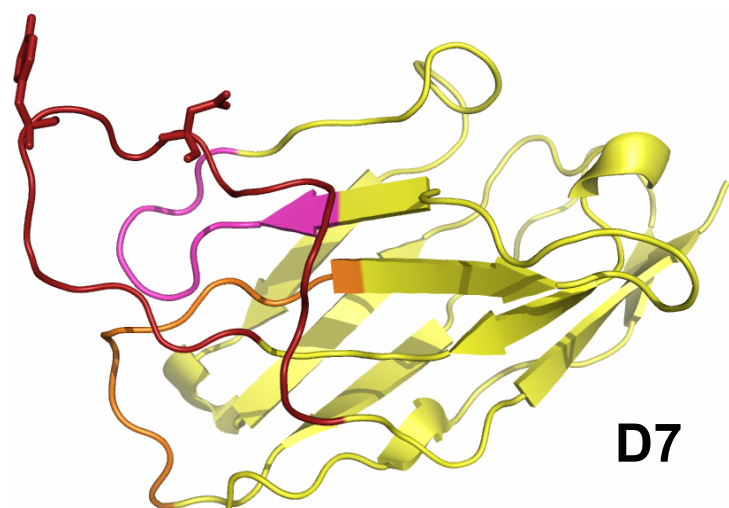
CDR1

CDR2

CDR3

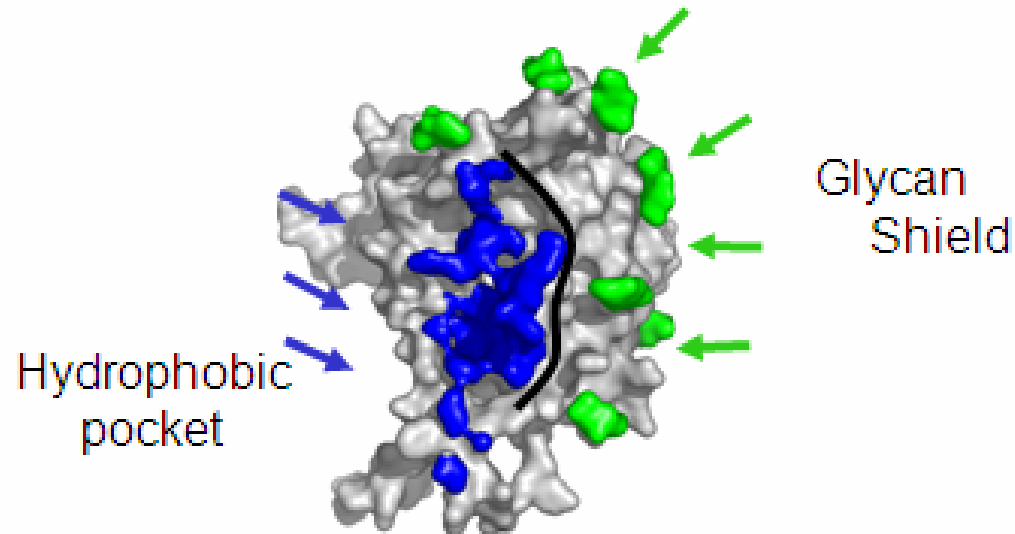
YYD

- Good neutralisers have a YYD (115) motif.
- Others have YYY, YNY (D7), YND or YYD
- Variations at the following residues did not affect neutralisation:
 - 108 – Y, D, S, N
 - 56-58 – D, N, T ↑
 - 32 – Y, F, H
 - 28 – I, T, V, L



Targeting the CD4 binding site

(Young Do Kwon & Peter Kwong, VRC NIH)



Neutralizing antibodies face two challenges:

1. Glycan shield
2. Hydrophobic pocket

12 kD VHH may overcome these challenges

Summary of new X-neutralising HIV-1 MAbs

- ❖ ~ 14 novel MAbs developed from memory B cells of human patients; more in pipeline
- ❖ ~ 8 novel MAb families to CD4-bs obtained from llama VHH phage libraries
- ❖ Llama VHH to gp41 and to CD4i being screened
- ❖ Structural studies initiated (Poster 19-09)
- ❖ Novel neutralization domains identified

