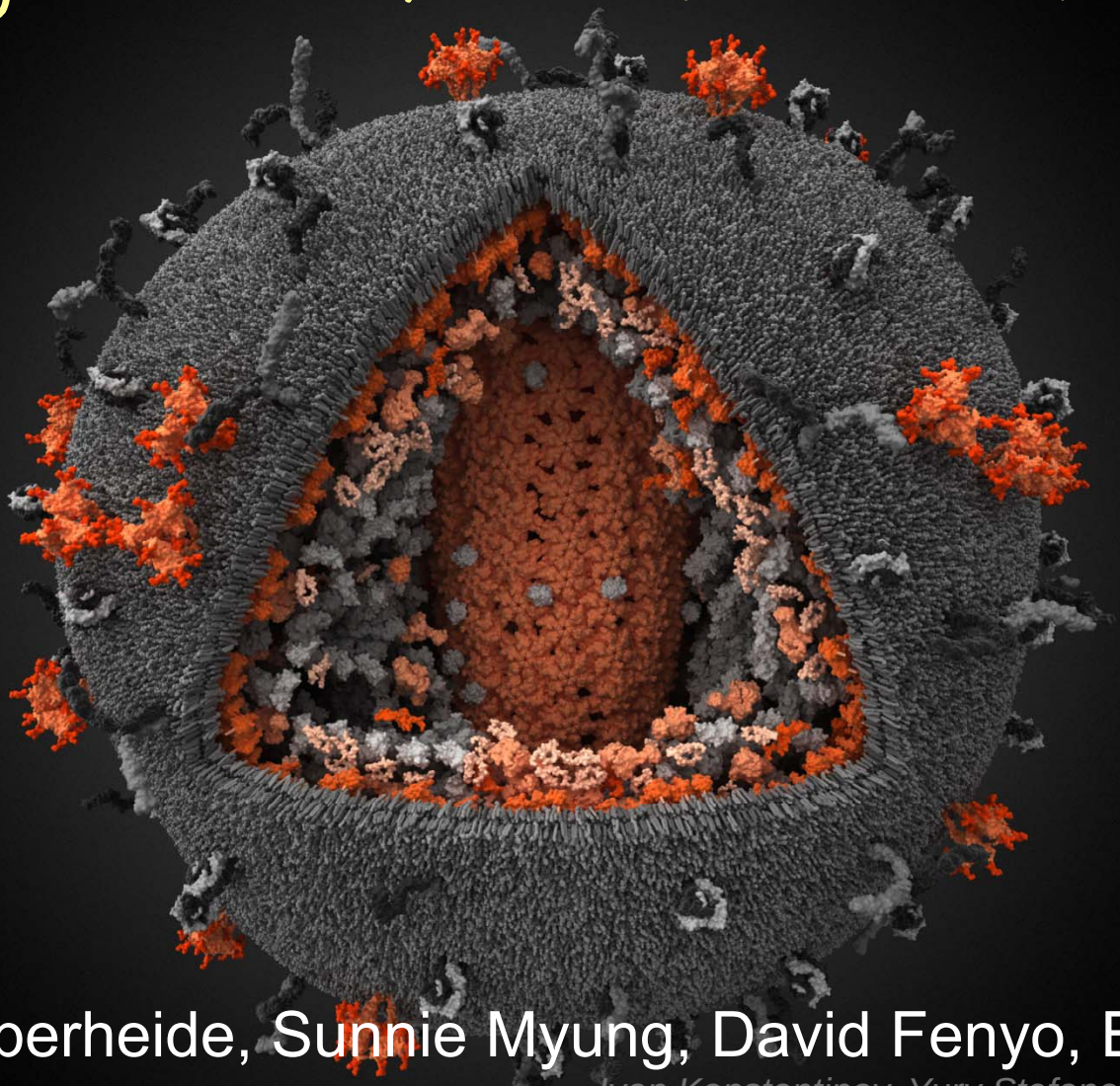
Finding Antibodies that Neutralize HIV

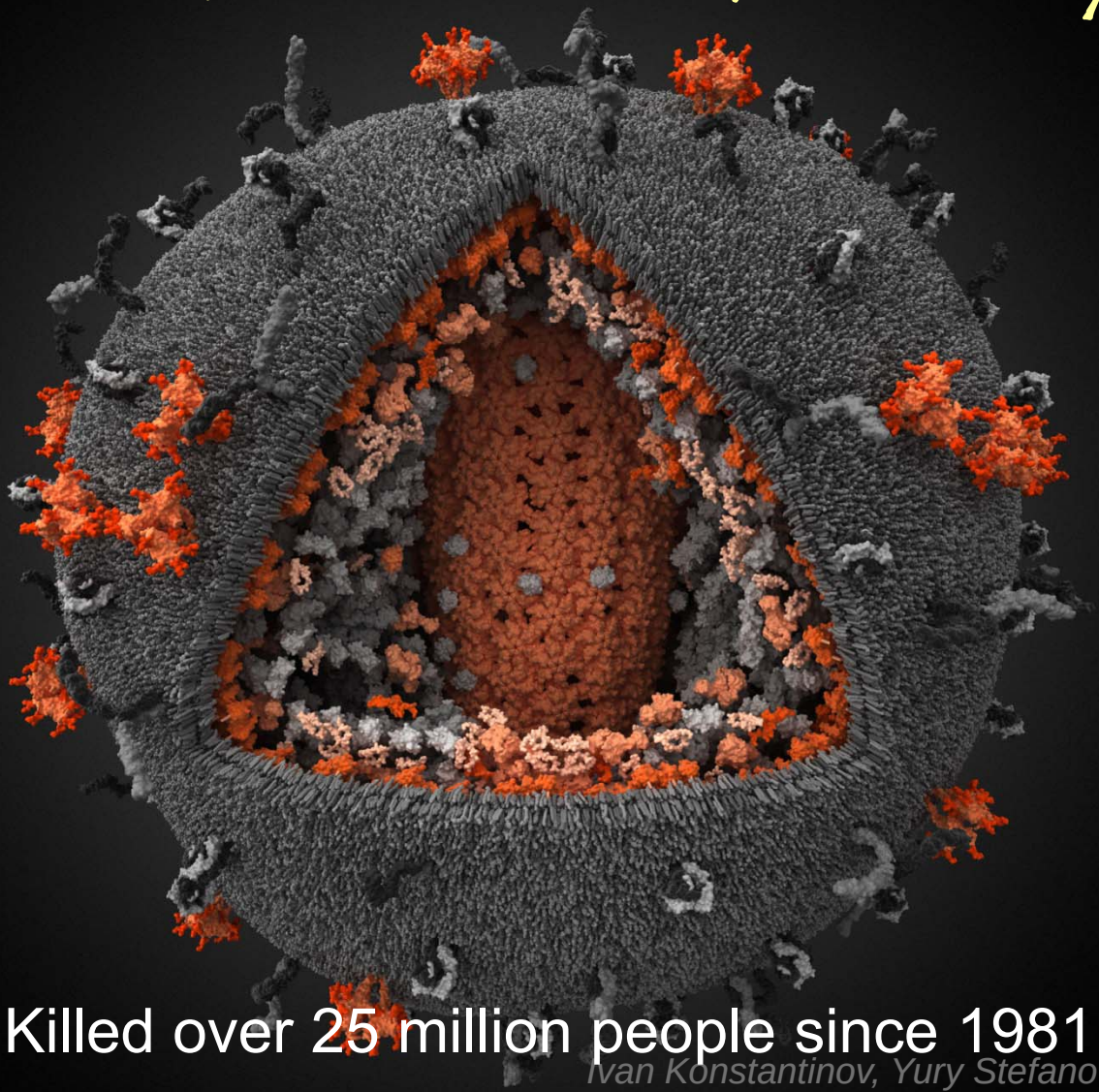


Beatrix Ueberheide, Sunnie Myung, David Fenyo, Brian Chait

Ivan Konstantinov, Yury Stefanov, Aleksander Kovalevskiy, Yegor Voronin (Visual Science Company)

Johannes Scheid, Michel Nussenzweig

HIV: Human Immunodeficiency Virus

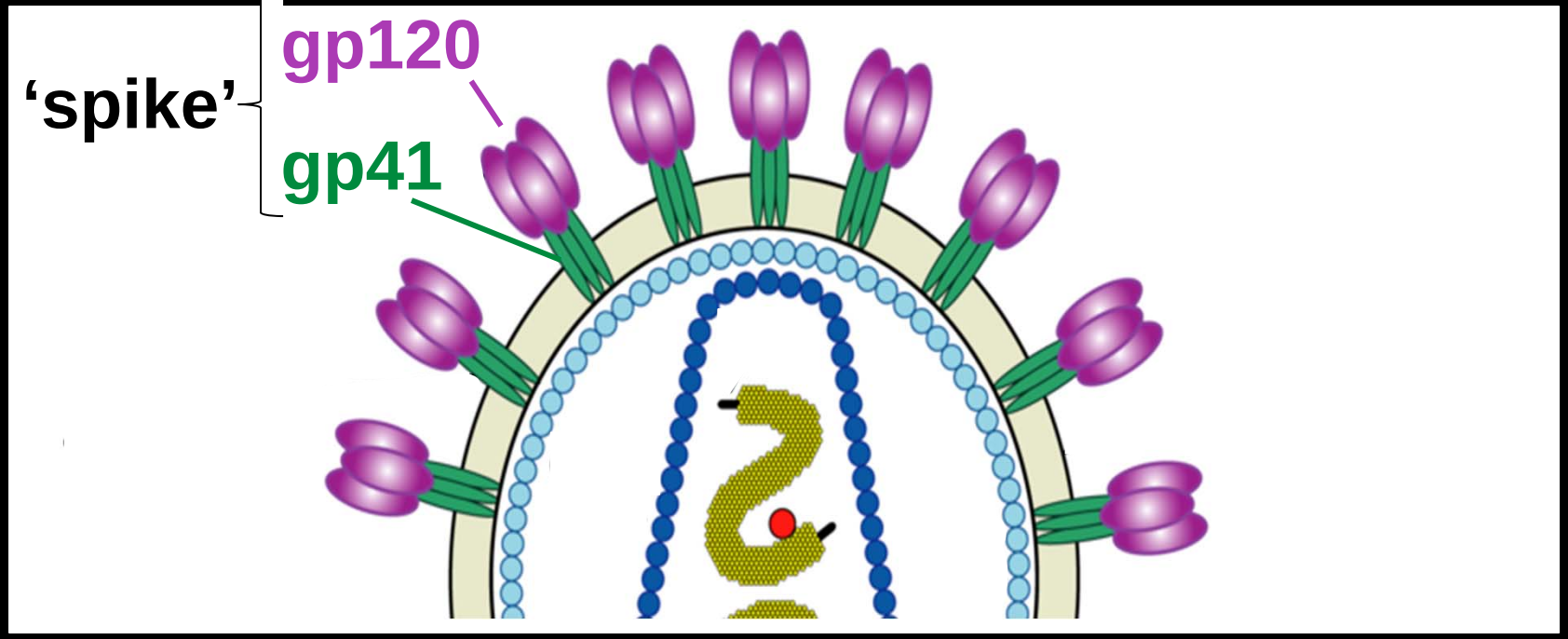


Killed over 25 million people since 1981

Currently 34 million people are infected

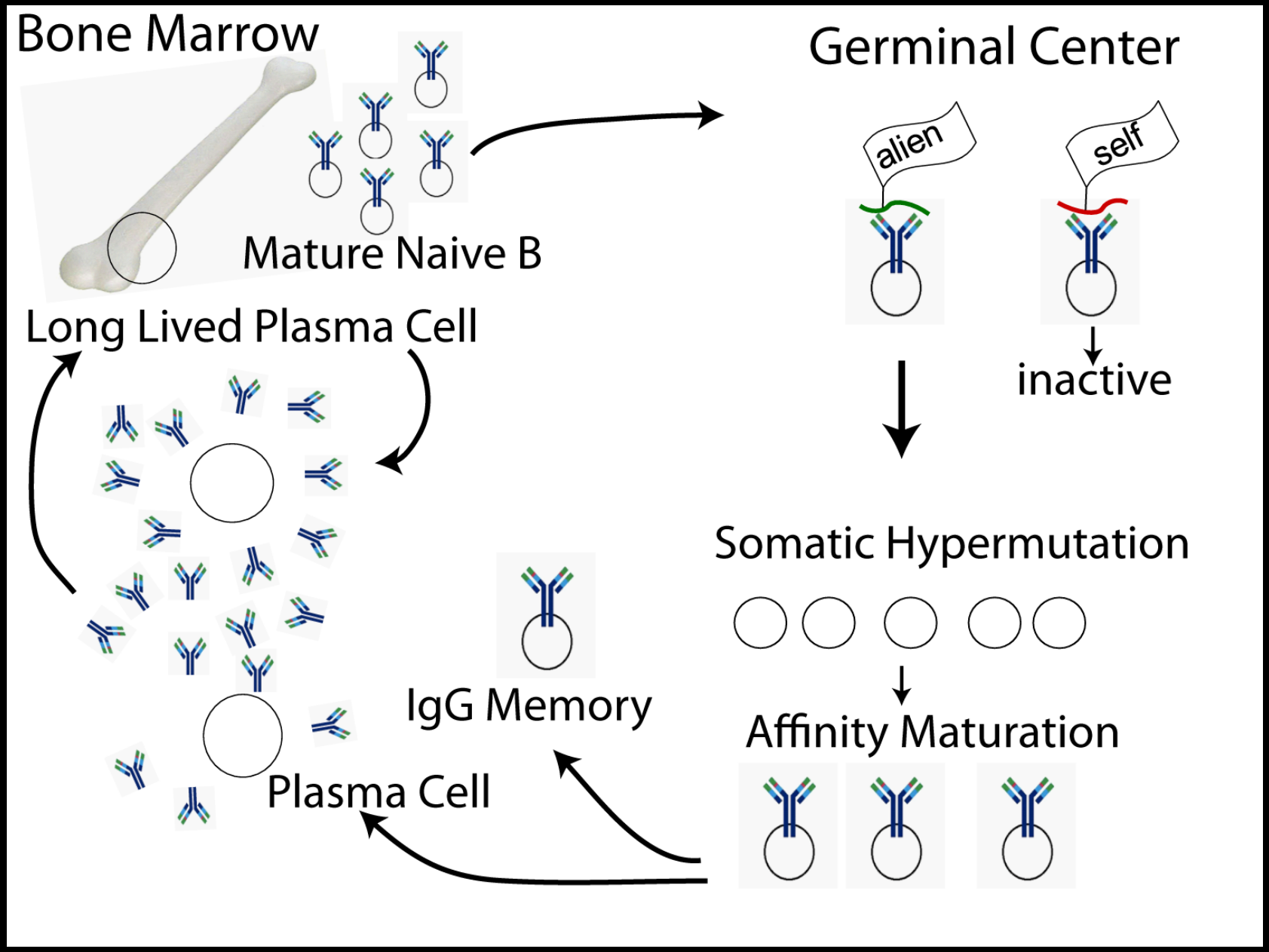
Ivan Konstantinov, Yury Stefanov, Aleksander Kovalevsky, Yegor Voronin/Visual Science Company)

Long-Term Non-Progressors & Elite Controllers of HIV

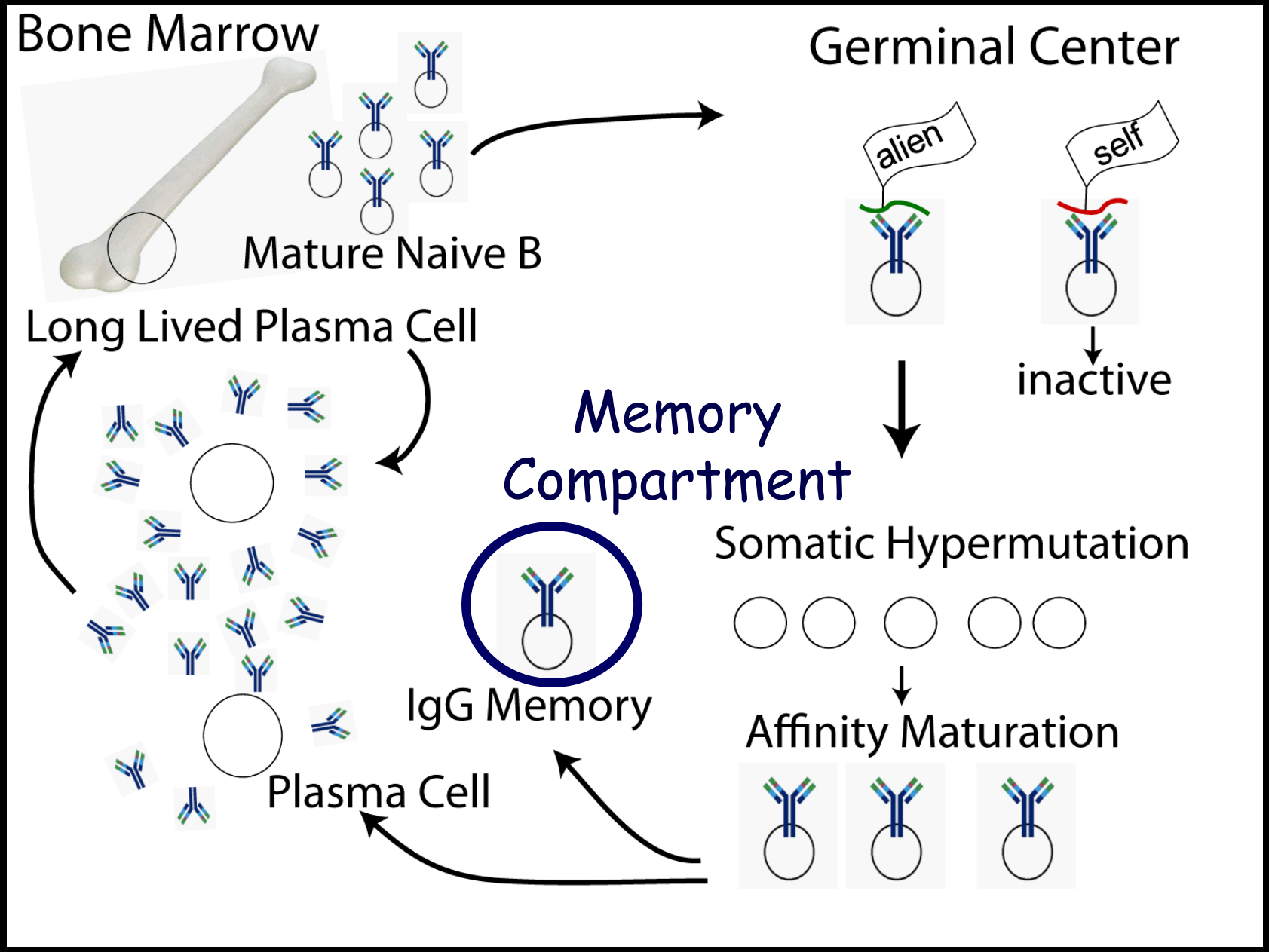


Individuals who control viral replication to low or undetectable levels without anti-retroviral therapy

Memory versus Plasma Compartments



Memory versus Plasma Compartments



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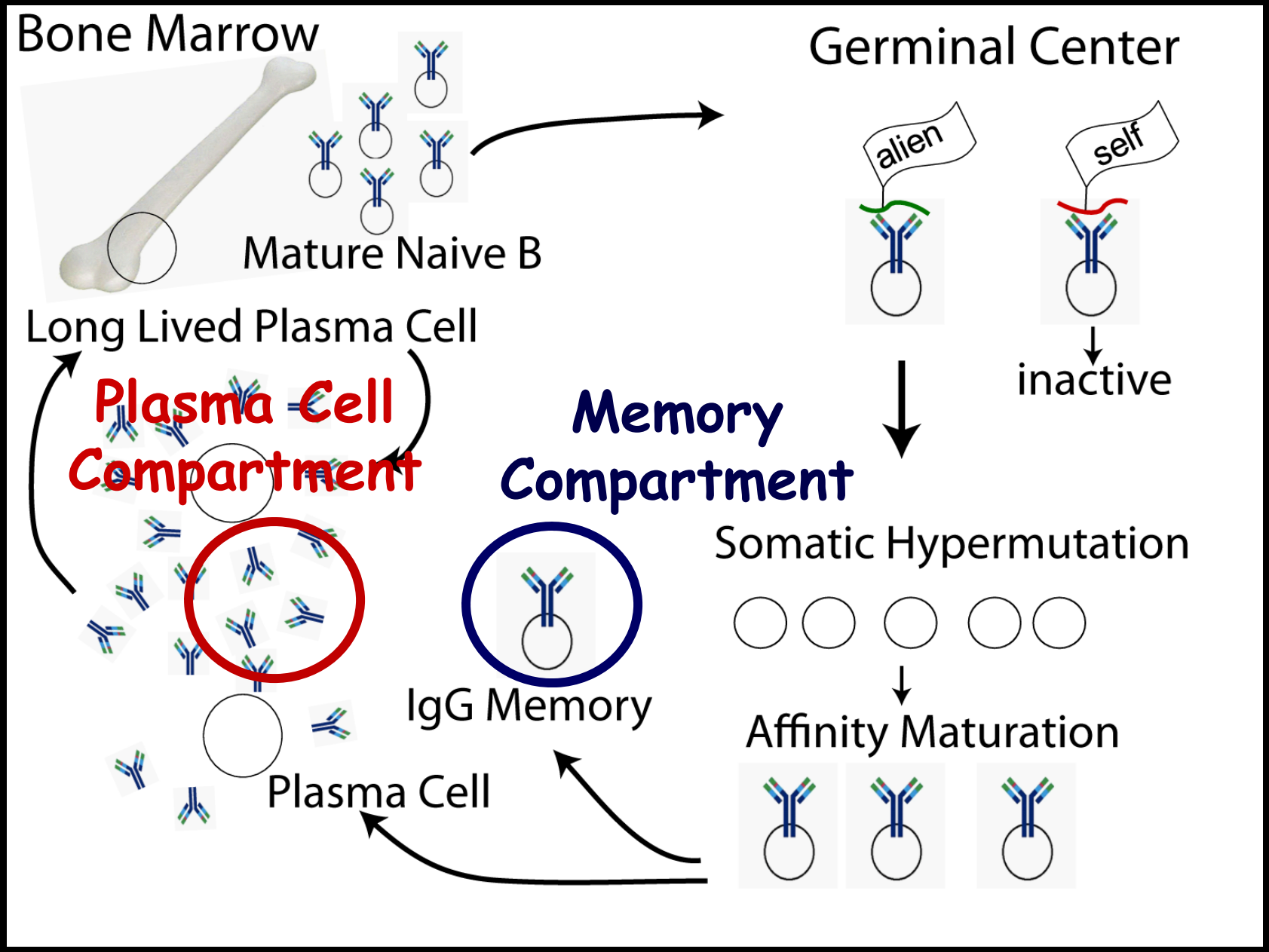
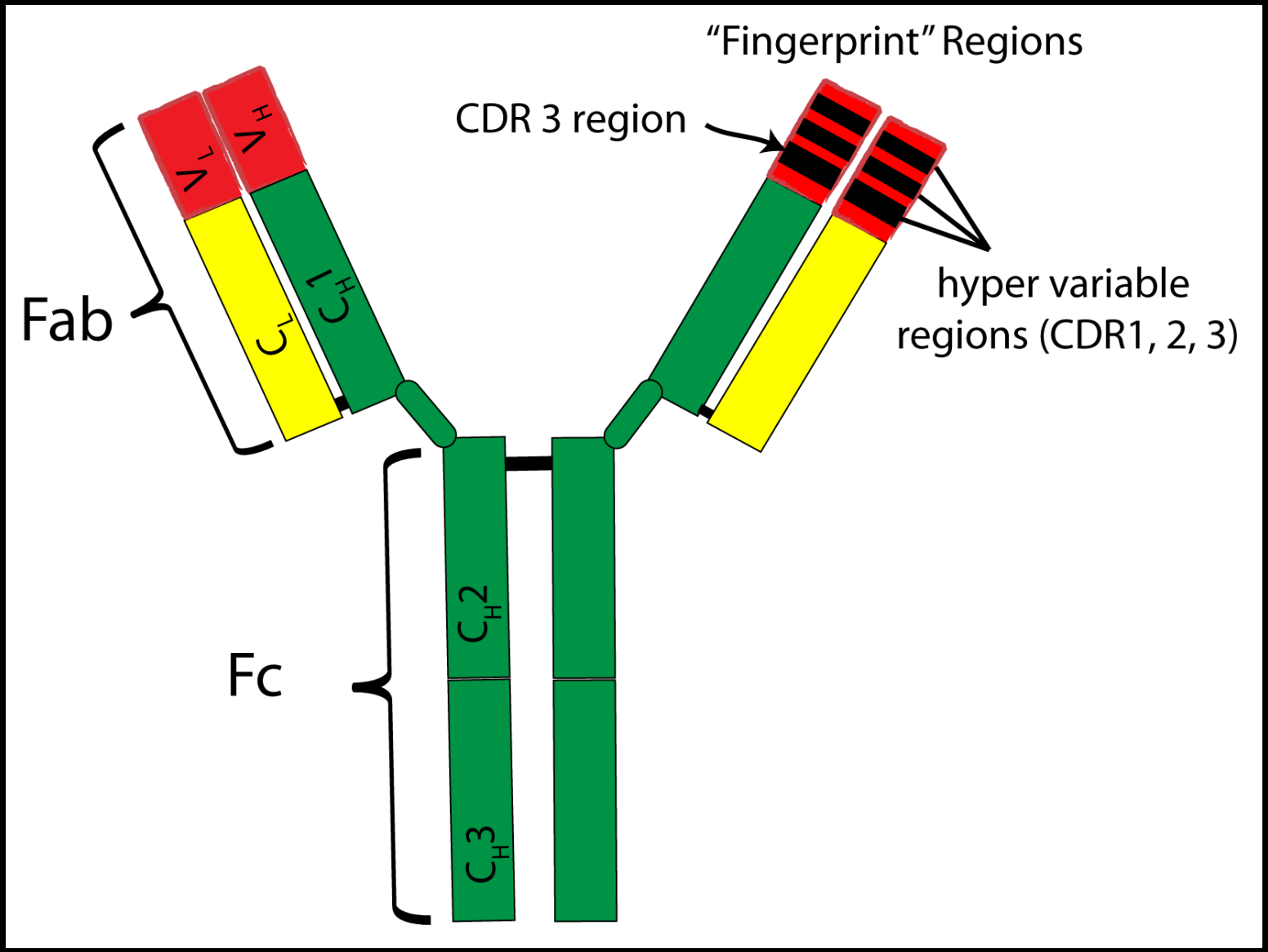
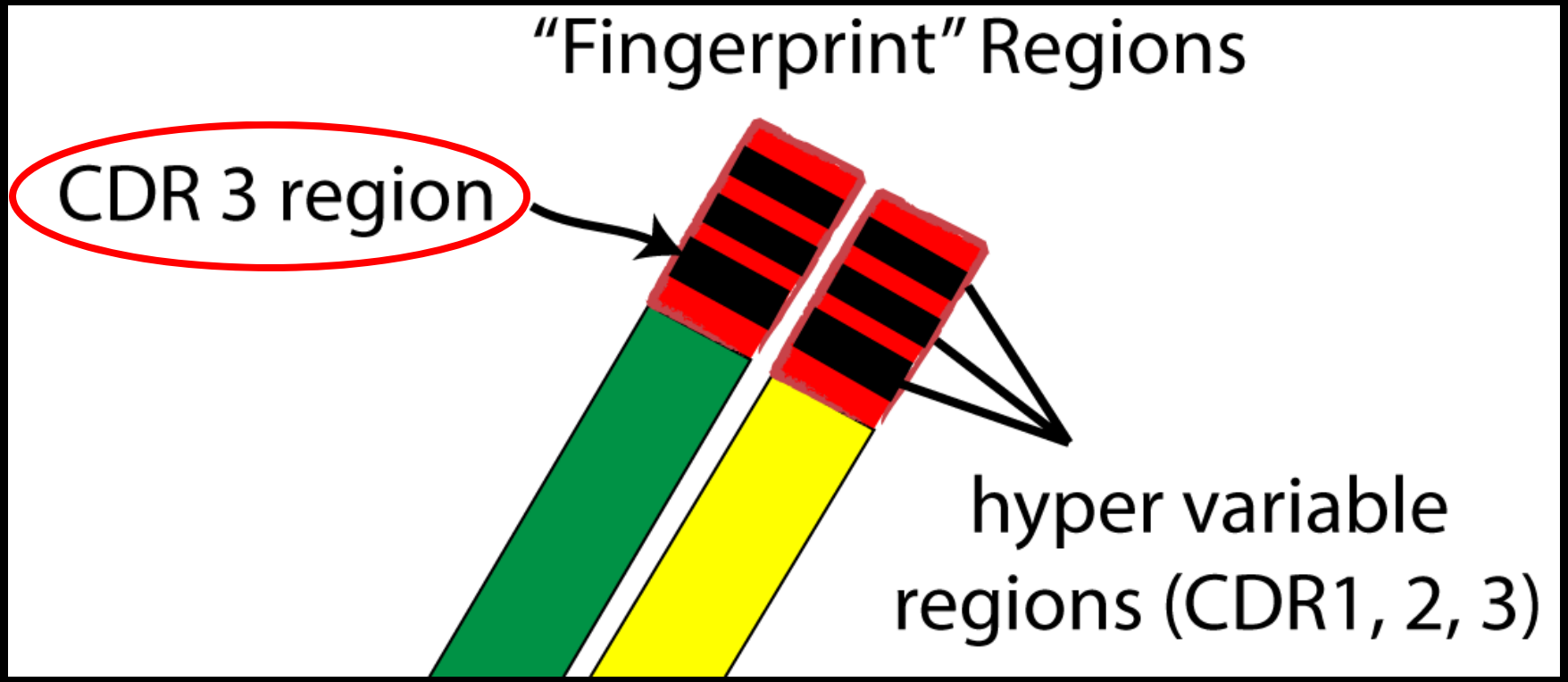


The IgG Molecule



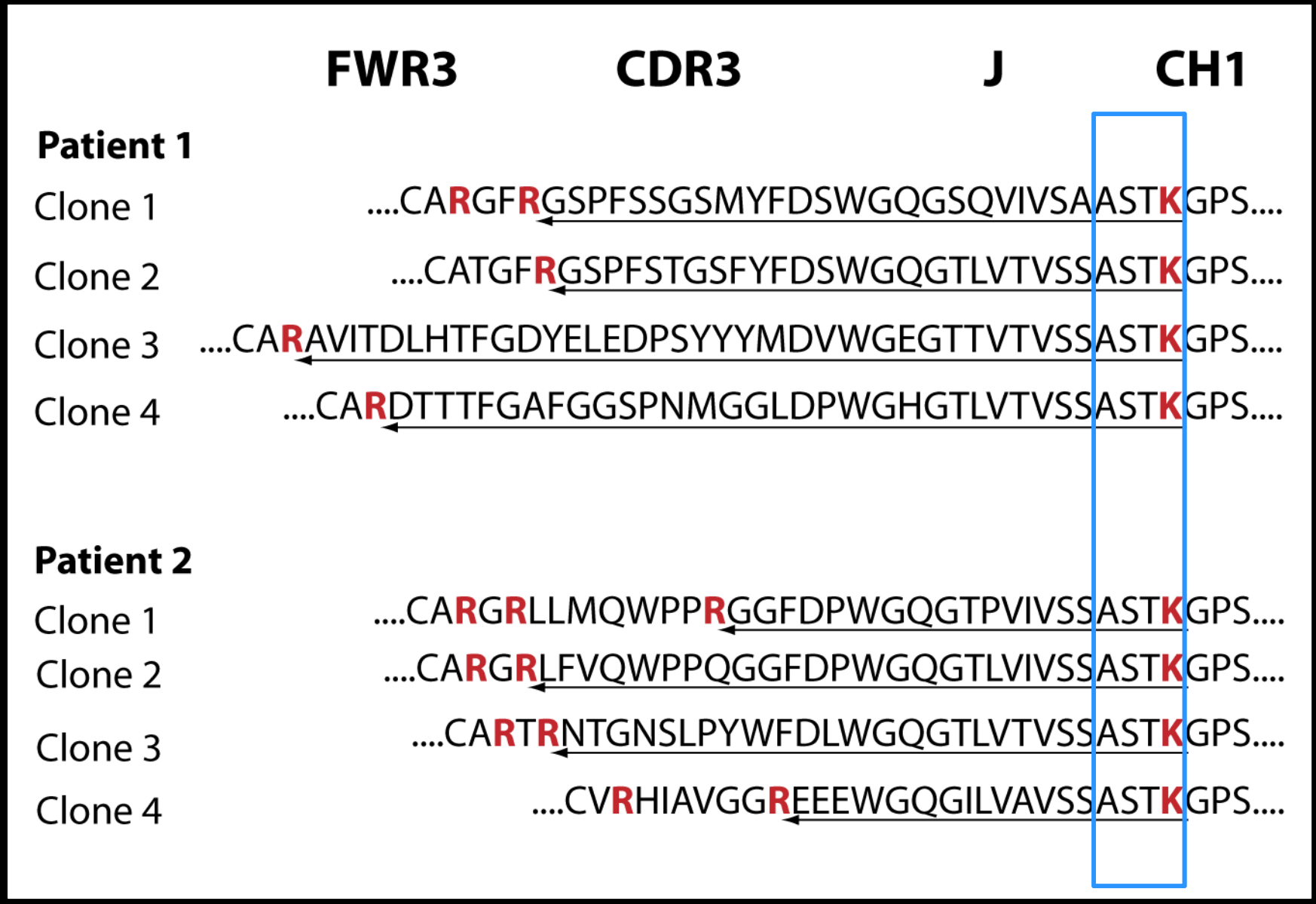
The IgG Molecule



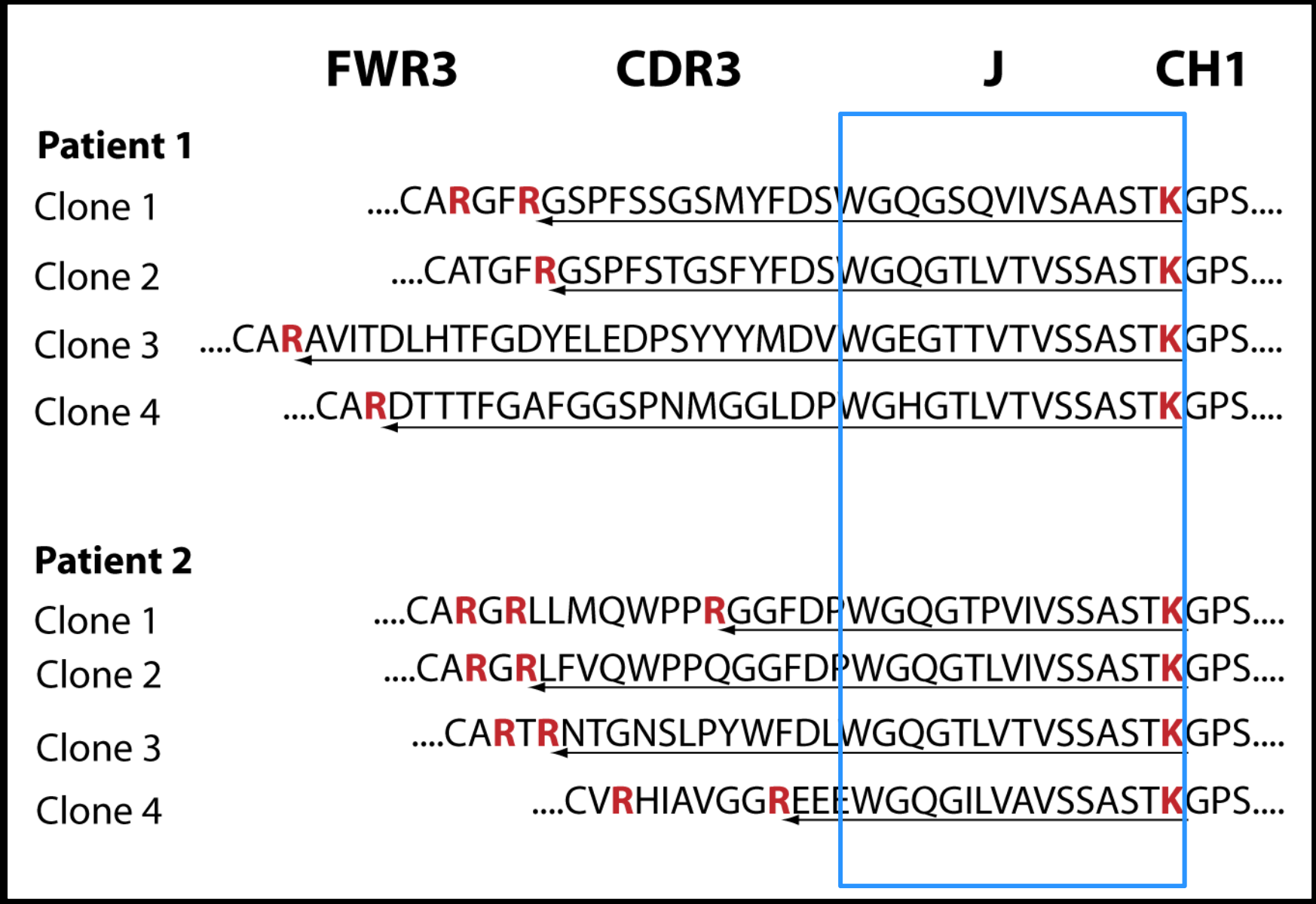
Representative Examples of CDR 3 regions

	FWR3	CDR3	J	CH1
Patient 1				
Clone 1	....CARGFRGSPFSSGSMYFDS		WGQGSQVIVSA	ASTKGPS....
Clone 2	....CATGFRGSPFSTGSFYFDS		WGQGTLVTVSS	ASTKGPS....
Clone 3	....CARAVITDLHTFGDYELEDPSYYYMDV		WGEGTTVTVSS	ASTKGPS....
Clone 4	....CARDTTTFGAFGGSPNMGGLDP		WGHGTLVTVSS	ASTKGPS....
Patient 2				
Clone 1	....CARGRLLMQWPPRGGFDP		WGQGTPVIVSS	ASTKGPS....
Clone 2	....CARGRLFVQWPPQGGFDP		WGQGTLVIVSS	ASTKGPS....
Clone 3	....CARTRNTGNSLPYWFDL		WGQGTLVTVSS	ASTKGPS....
Clone 4	....CVRHIAVGGREEE		WGQGILVAVSS	ASTKGPS....

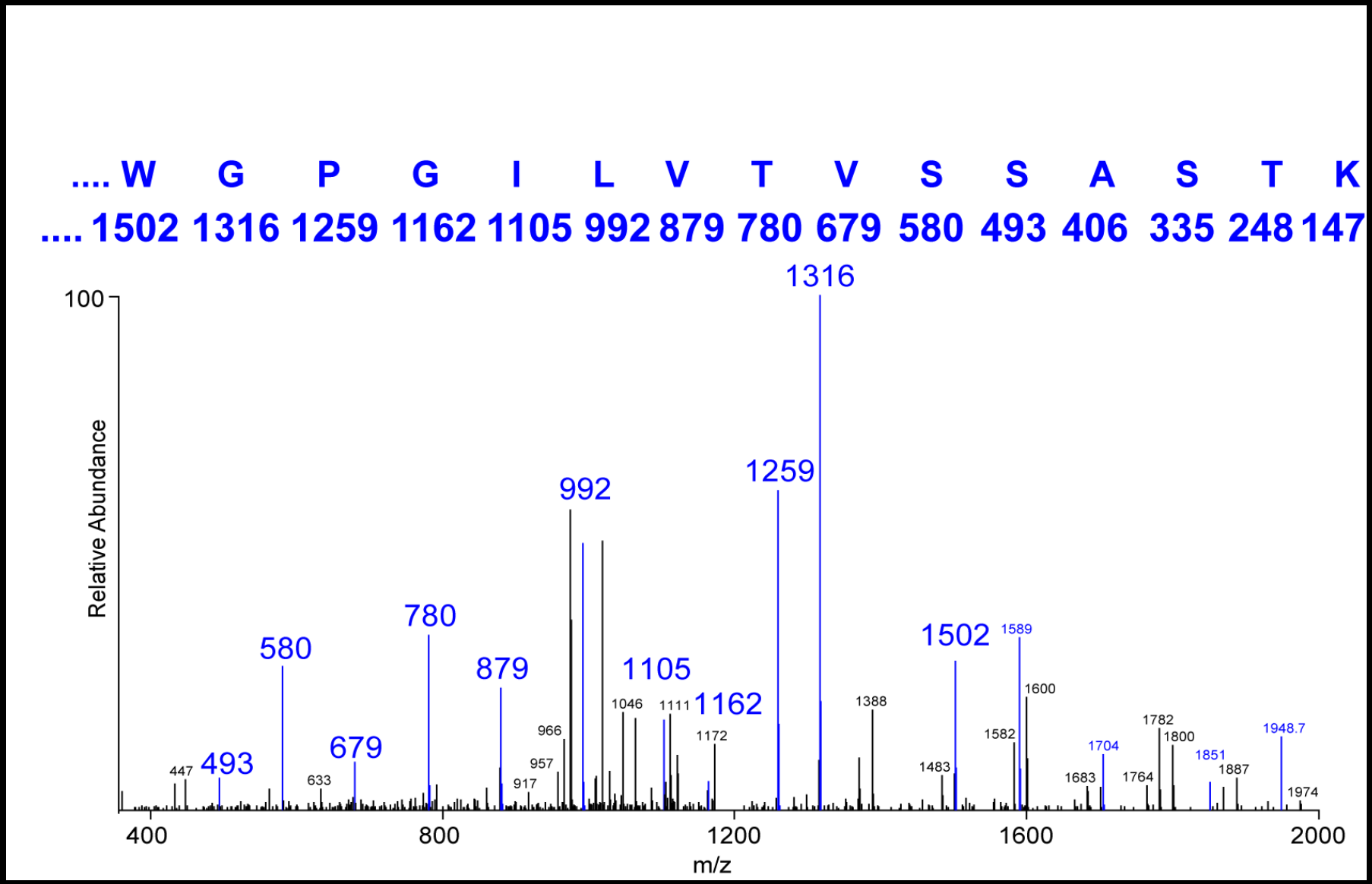
Representative Examples of CDR 3 regions Trypsin Cleavage



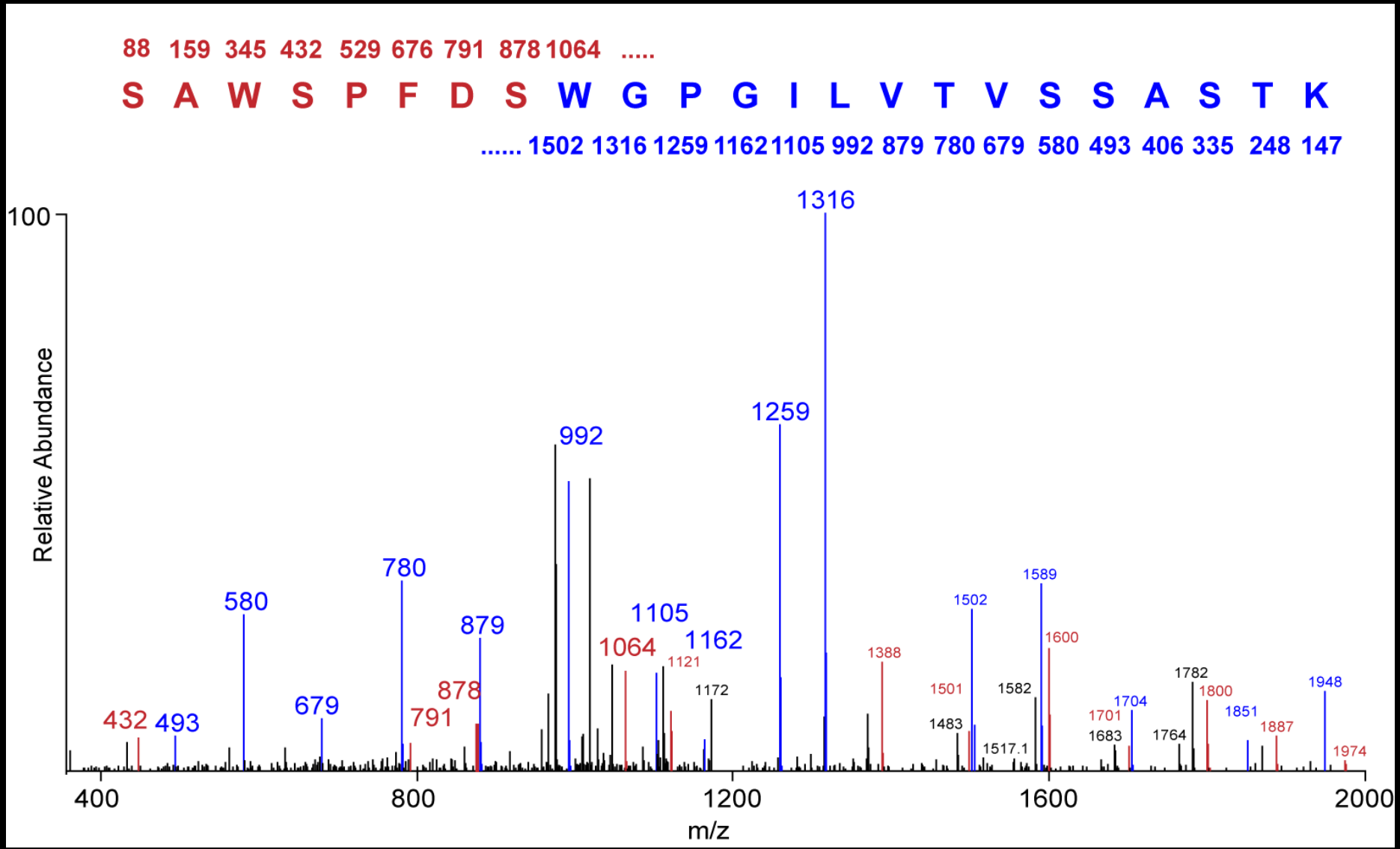
Representative Examples of CDR 3 regions Trypsin Cleavage



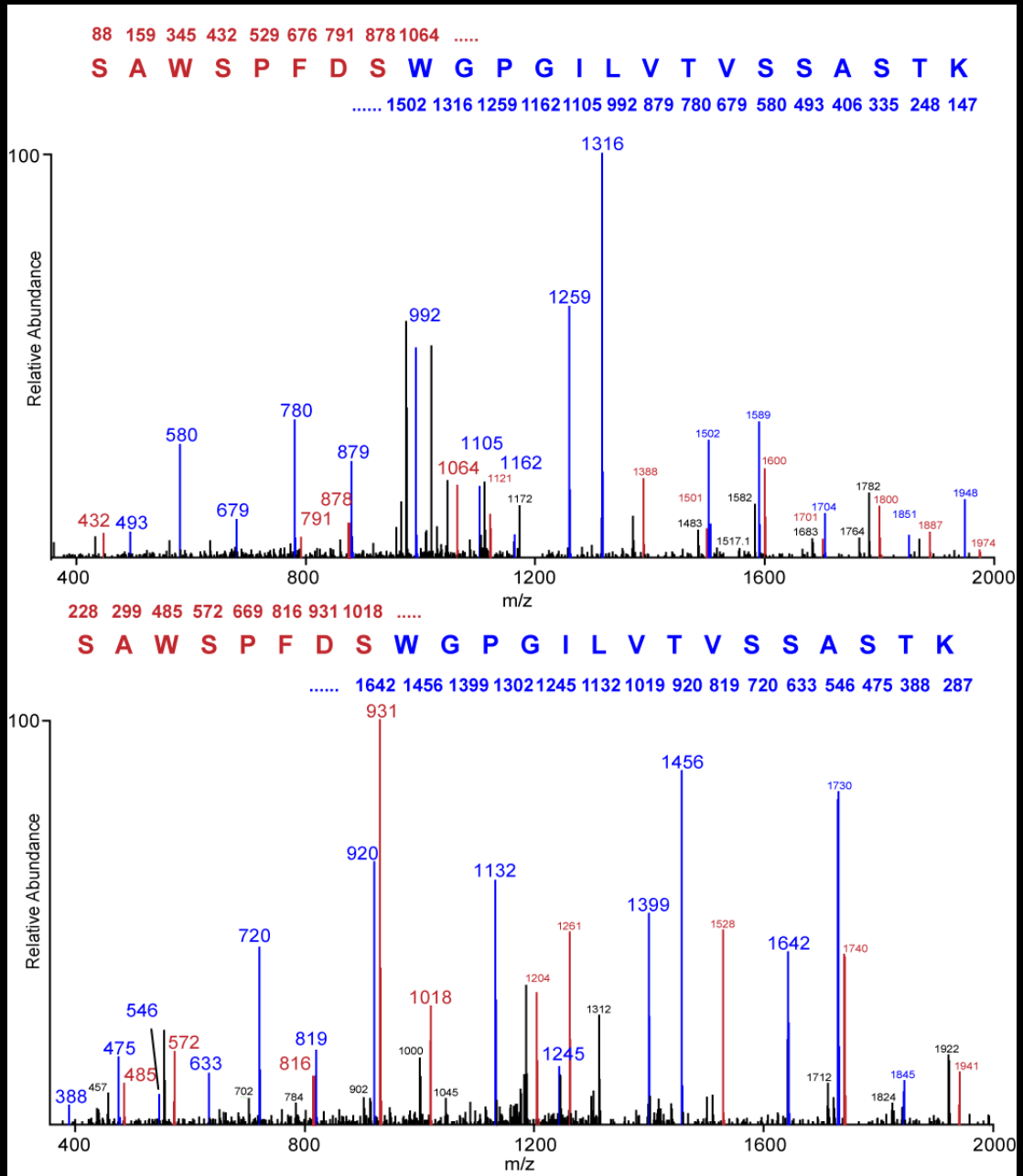
C-terminal Fragment Ions Serve as Markers for most CDR3 Peptides



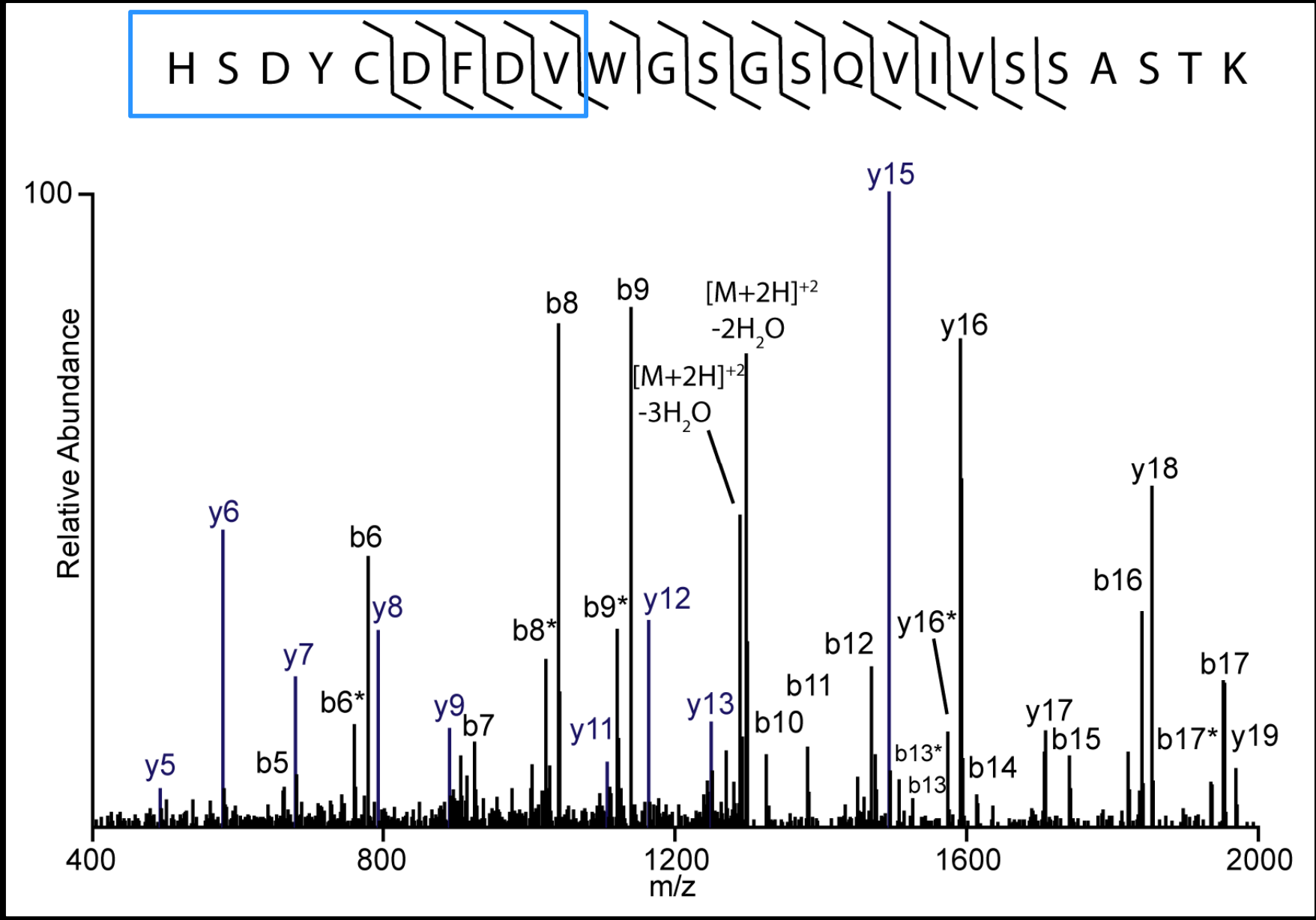
N-terminal Fragment Ions Identify the CDR3 Region



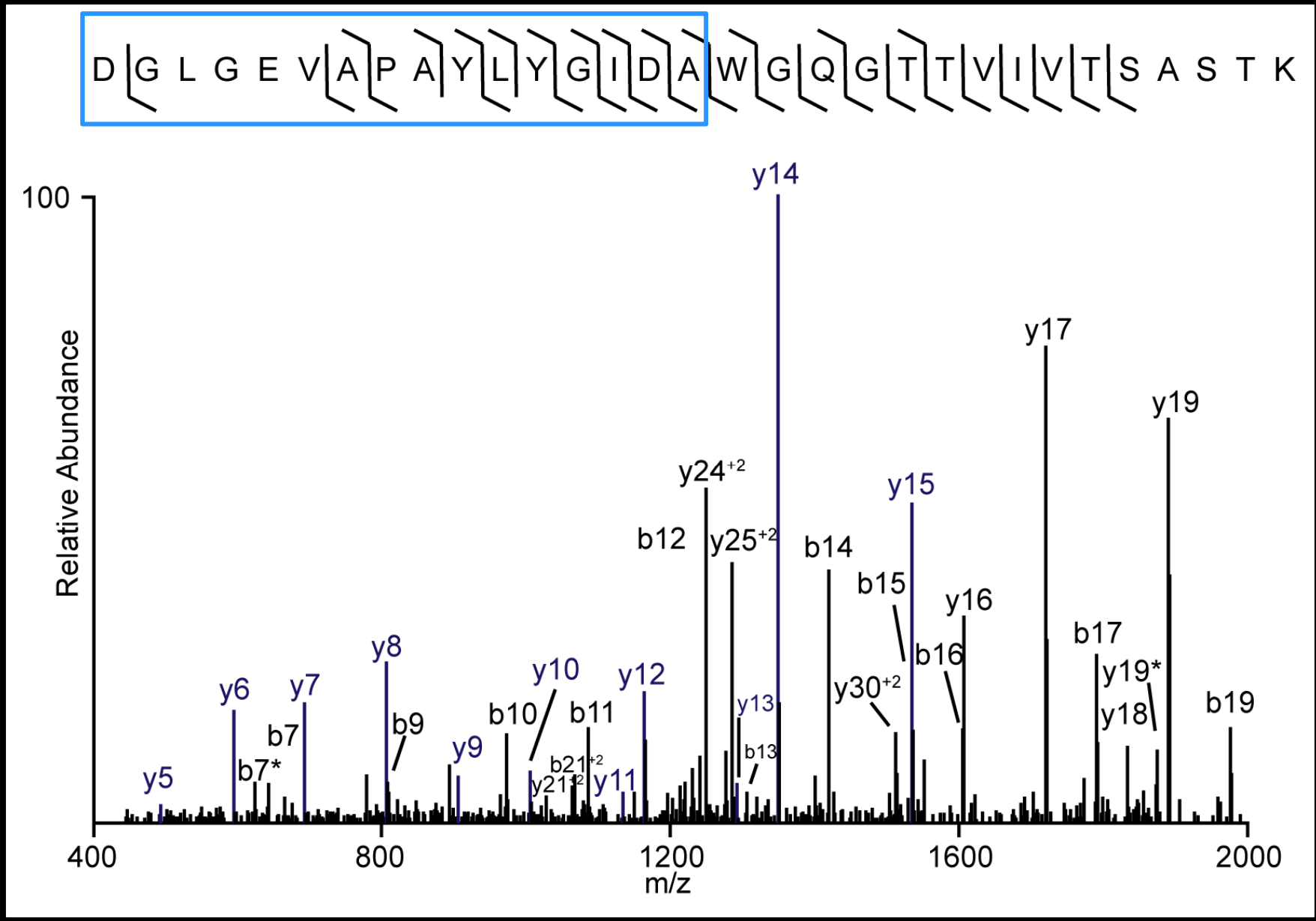
mTRAQ tag enhances N-terminal ion series



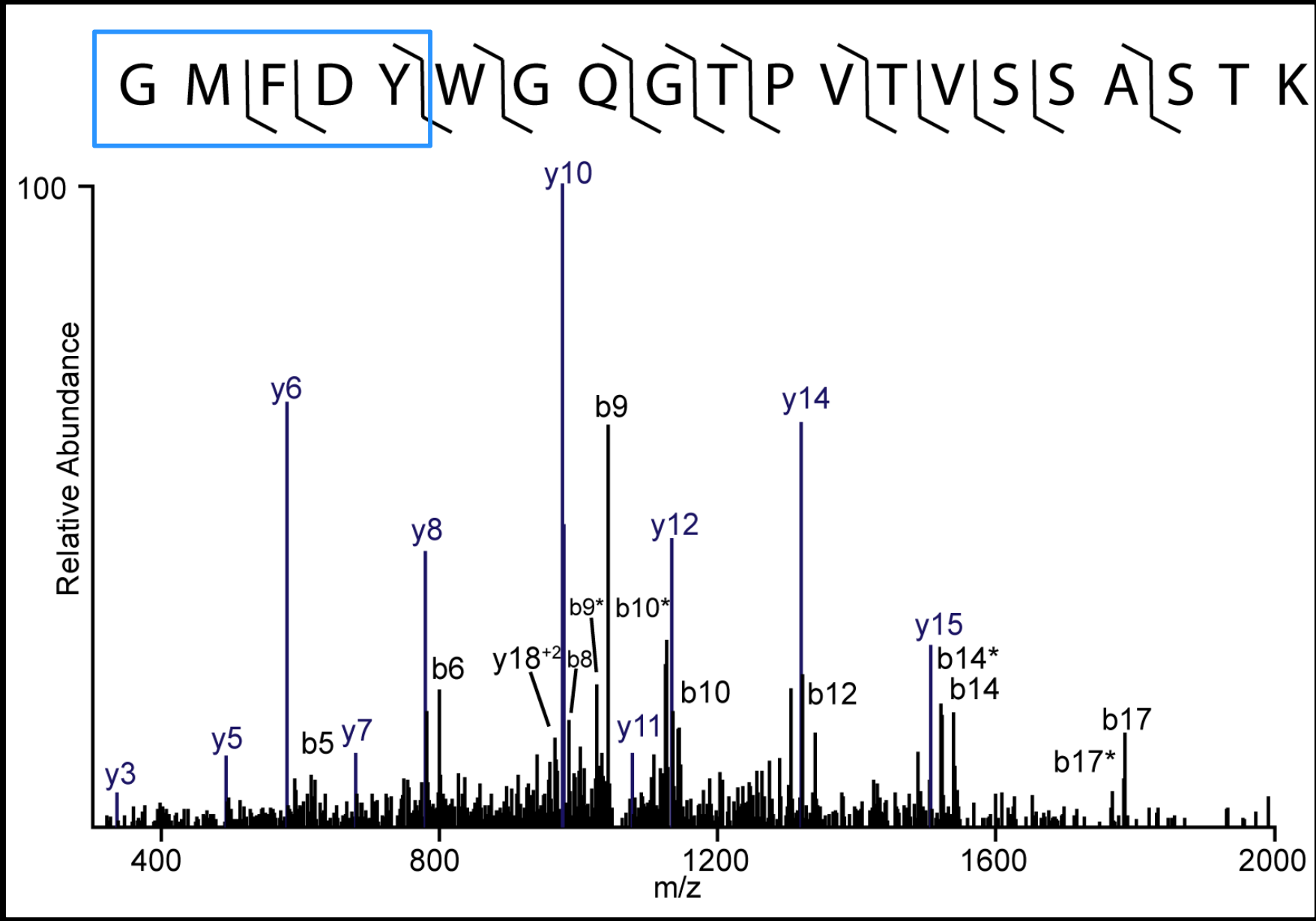
Individual 1



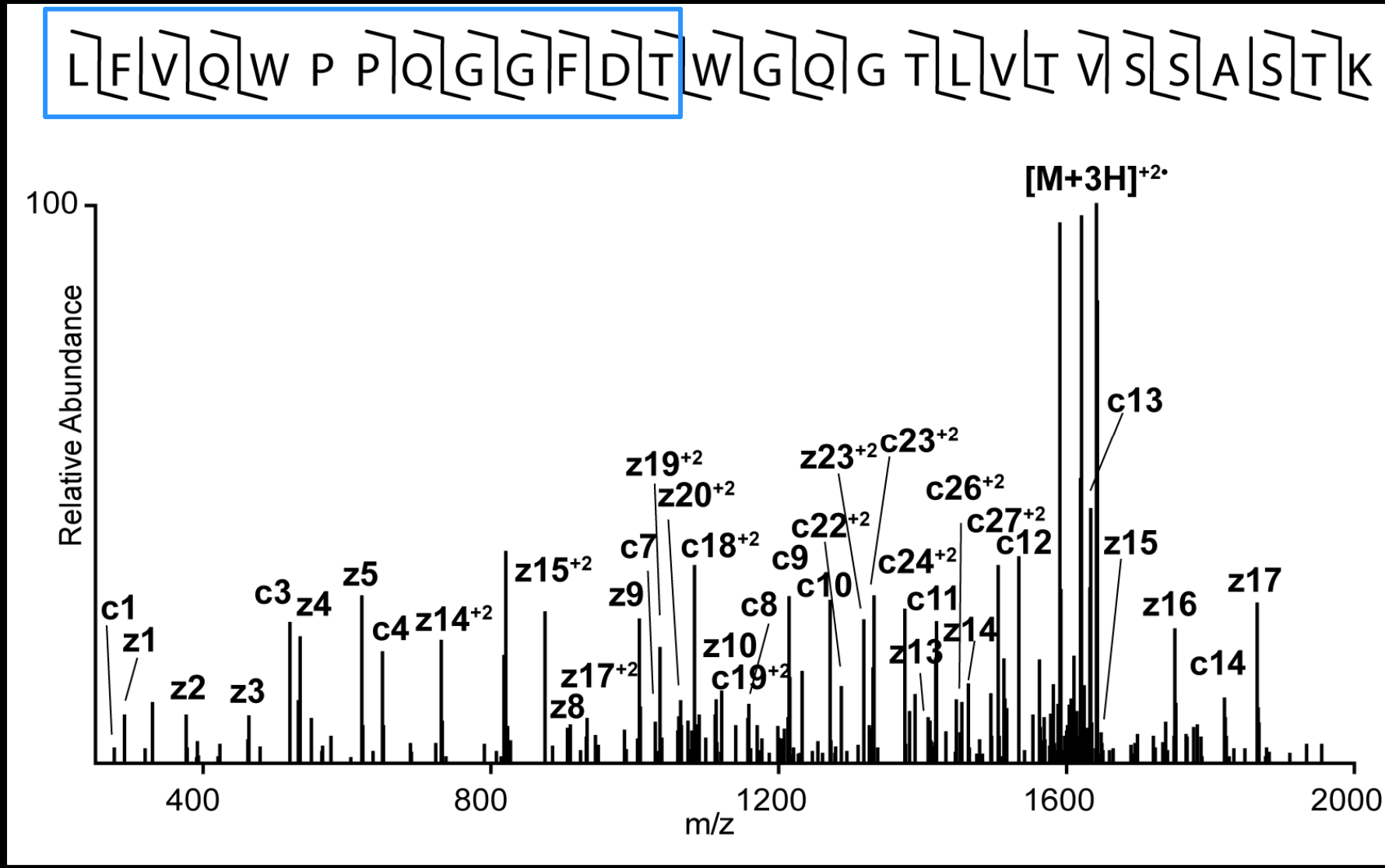
Individual 2



Individual 3



ETD after mTRAQ labeling



Protocol: Elucidating Memory B Cells

Enrich Patients B Cells

↓

Isolate HIV Spike Binding IgG^+ B Cells

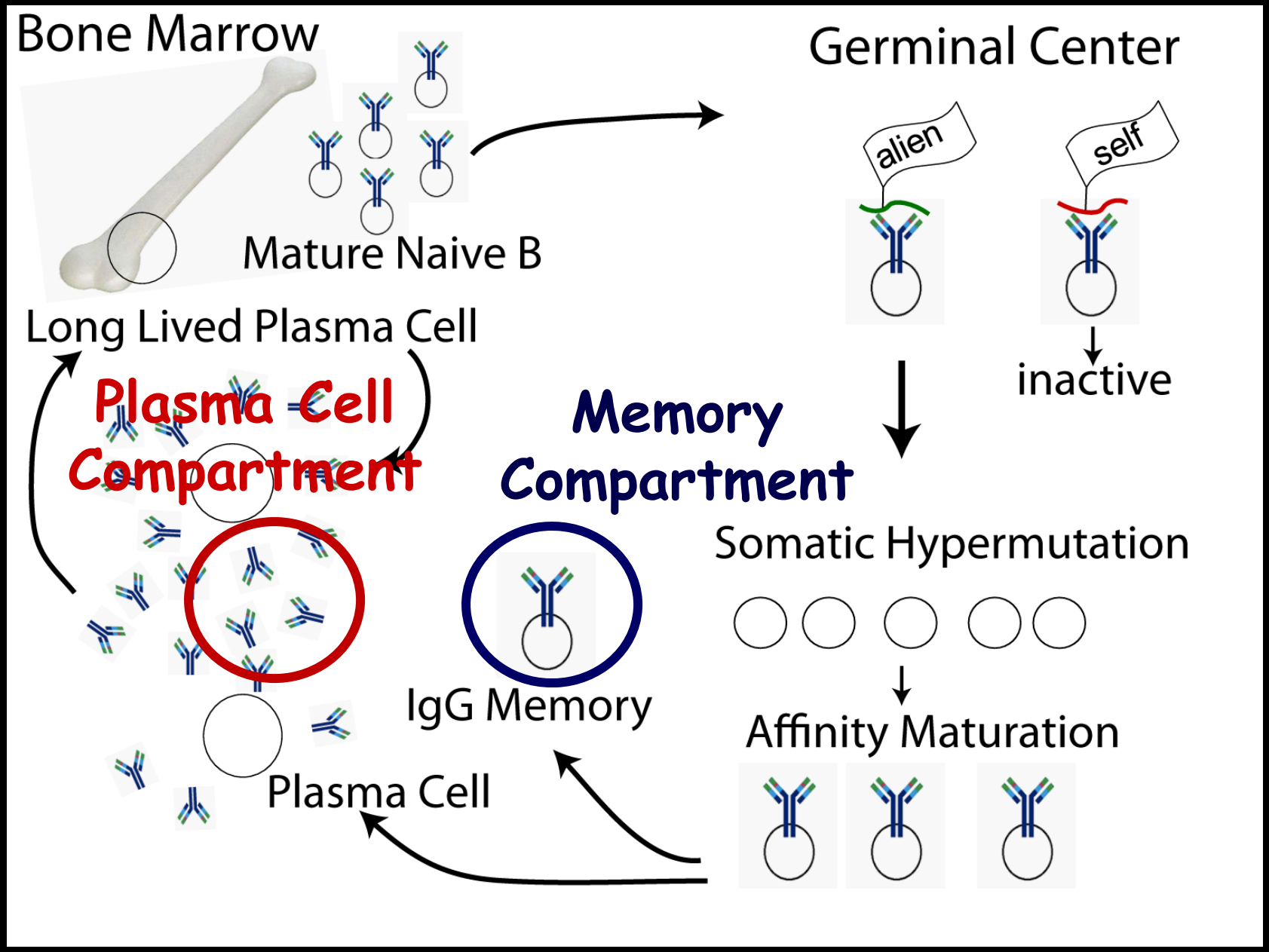
↓

Amplify & Sequence Heavy & Light Chains of IgG
from these HIV Spike Binding IgG^+ B Cells

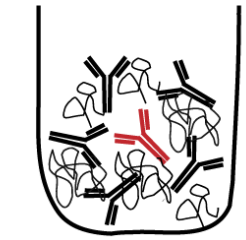
↓

Patient Specific HIV Spike Binding
Memory B Cell Antibody Database

Memory versus Plasma Compartments



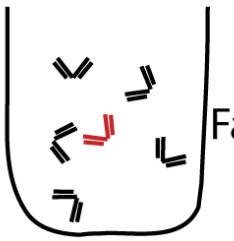
Protocol: Elucidating Circulating Antibodies



Patient Serum

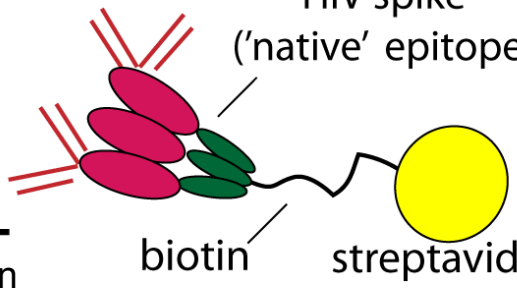
Protein G purification, Papain

Isolate IgG, remove Fc



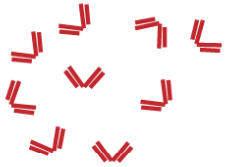
Fab fragments

Isolate HIV reactive Fabs



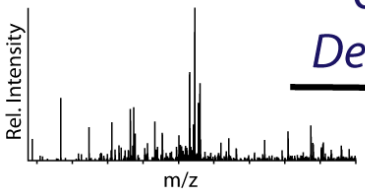
Elute Fab fragments

Digest with Trypsin



mTRAQ LC-MS

CAD/ETD/HCD



Patient-specific database search

De novo sequencing

Identify Antibody via "Fingerprint" peptides

First Findings

Unamigiously Identified Clones

AD27HC
LSS_167_LC
LSS_319_HC
LSS_479LC
LSS_491_HC
LSS_521_HC
LSS_527_HC
LSS_540_HC
LSS_684_KC
LSS_68HC
LSS_693_HC
LSS_705_HC
LSS_711_HC
LSS_774_KC
LSS_779_KC
LSS_79_HC
LSS_809_HC
LSS_81_LC
LSS_82_HC
LSS_880_HC
LSS_89_KC

'ASTK' peptides

GGPNMGGFDPWGQGTLVTVSSASTK
QIGDYWGQGTLVTVSSASTK
FTSPFDSWGQGTLVTVSSASTK
QIGDYLGQGTLVTVSSASTK
MGGLDPWGQGTLVTVSSASTK
FTSPFDSWGQGTLVTVSSASTK
GHSFTSPFDSWGQGTLVTVSSASTK
DAFDIWGPGTMVTVSSASTK
SPFDSWGPGILVTVSSASTK
NPLDIWGQGTMVTVSSASTK
LDVWGQGTTVTVSSASTK
STPLVWPPANGLDVWGQGTTVTVSSASTK
EGWGQGTLVTVSSASTK
LDHWGQGTLVTVSSASTK
DTTTFTTFGGGPNMGGFDPWGQGTLVTVSSASTK
YYHYGLDVWGQGTMVTVSSASTK

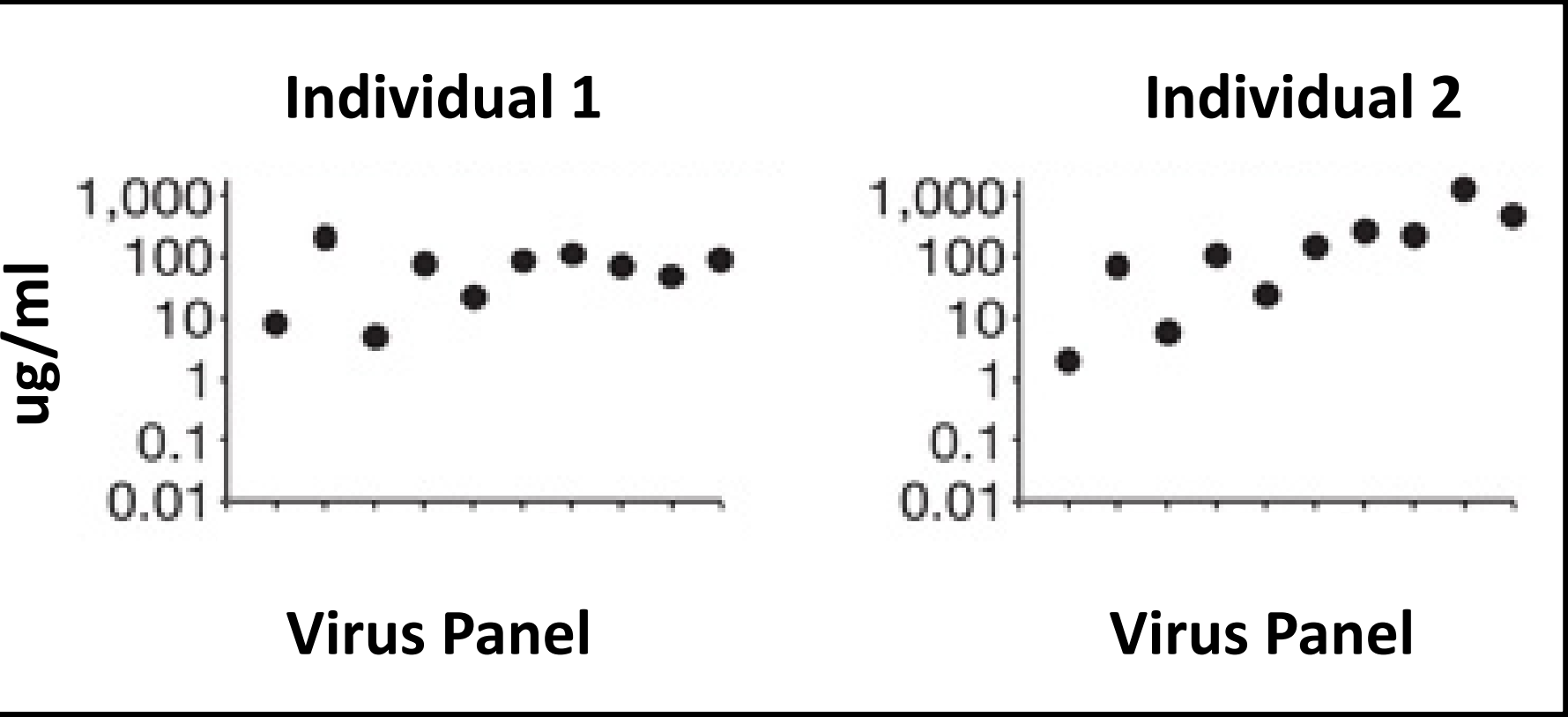
additional variants

Additional Clonal Variants (not in the database)

ASHPVSNGYLAWYQQKPGQAPR
ASQNIASSYLAWYQQKPGQAPR
ASQSVSSSSL(I/L)WYQQKPGQAPR
ATQTVSNNYLAWYQQKPGQAPR
GSPLSSGSLYFDSWGQGTPVTVSSASTK
QSELTQPPSVSGTPGQR
QSQRPSGVPDRFSGSK
LAWYQHKPGQGPR
LDPWGQGTLV(I/L)VSSASTK
NPLDIWGQGT(I/L)VTVSSASTK
SSASASLAISGLR
VNPTGEASSYAQKFQGR
VTISCSGSGSNVGGNLVSWYQHFPGAAPK
..... continued

about 25% of identified peptides carry further mutations

Neutralization (IC_{50}) of IgG Clones



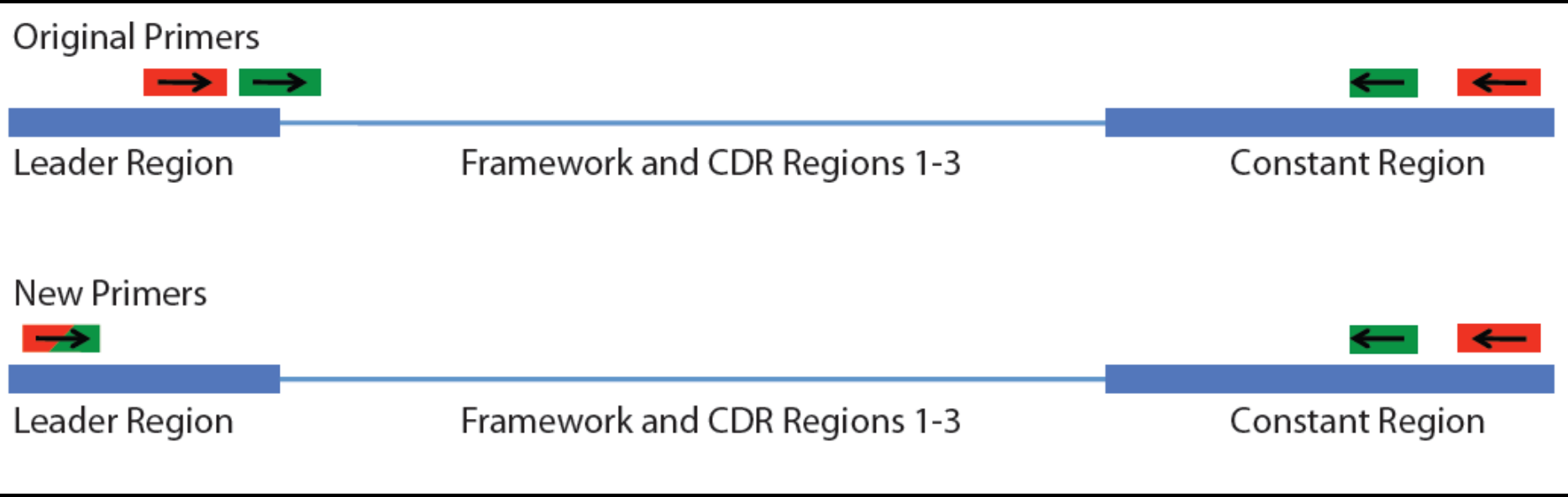
modified from Scheid et al., Nature 458 (2009)

Is there bias in our data?

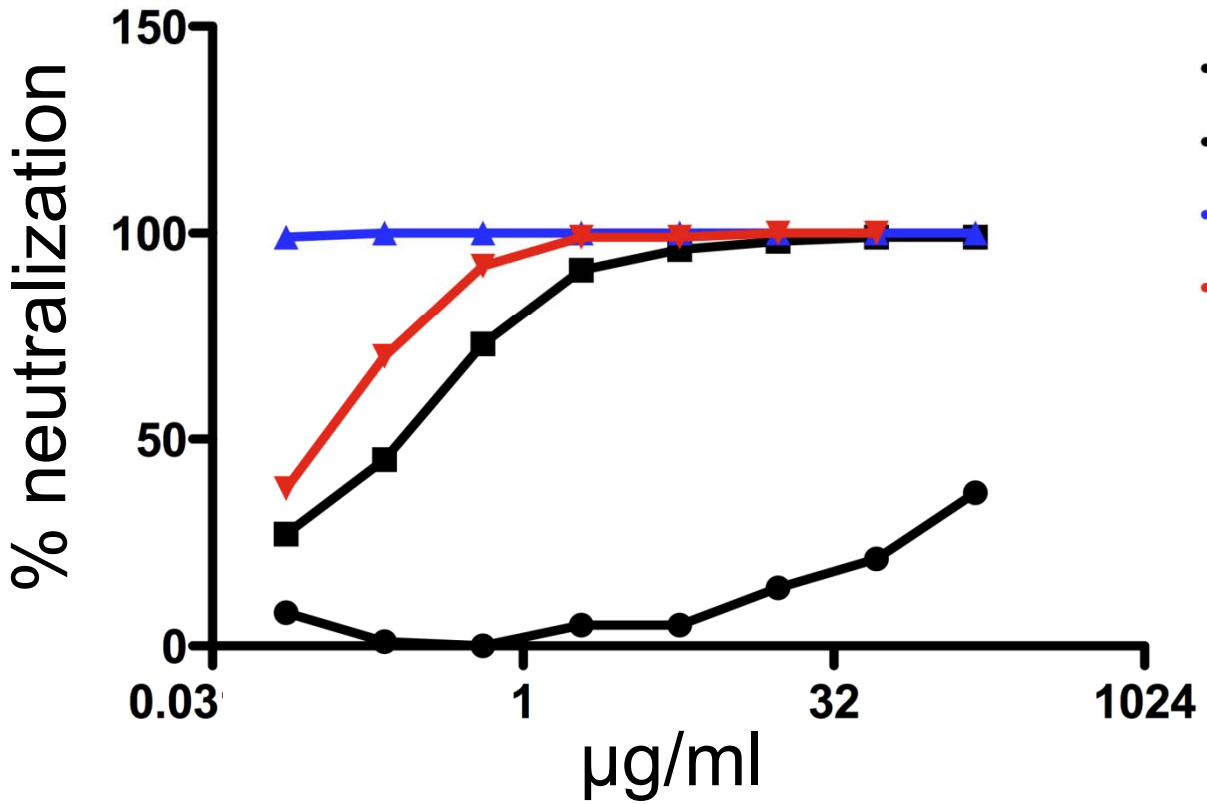
Is there bias in our data?

$\Rightarrow$ primers used for cloning the memory B cells

Highly mutated antibodies captured with new primers & amplification strategy



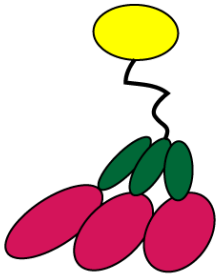
One Potent Broadly Neutralizing Clone per Elite Controller



* potent neutralizing antibodies

Are the potent neutralizers in the serum?

‘Native Spike’

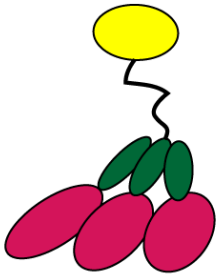


Identified Clones

3white_3A816HC	3white_3B8HC	3white_3A296KC
3white_3A816KC	3white_3B93HC	3white_3A334LC
3white_3A93HC	3white_3BNC149HC	3white_3A650HC
3white_3ANC3HC	3white_3BNC48HC	3white_3A67HC
3white_3ANC66HC	3white_3BNC58HC	3white_3A67KC
3white_3b129kc	3white_3BNC78HC	3white_3A381HC
3white_3B186HC	3white_3A125HC	3white_3A384HC
3white_3b27kc	3white_3A144HC	3white_3a426kc
3white_3B2HC	3white_3A160HC	3white_3A474KC
3white_3B44HC	3white_3A18KC	3white_3A518KC
3white_3B45HC	3white_3A204KC	3white_3A539HC
3white_3b45kc	3white_3A228HC	3white_3A539LC
3white_3b46HC	3white_3A244HC	3white_3A576HC
3white_3b46kc	3white_3A255HC	3white_3A64HC
3white_3B51HC	3white_3A296HC	3red_3bnc105kc
		3red_3B106HC
		3red_3BNC81HC

Are the potent neutralizers in the serum?

'Native Spike'



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3white_3ANC3HC	3white_3BNC48HC	3white_3A67HC
3white_3ANC66HC	3white_3BNC58HC	3white_3A67KC
3white_3b129kc	3white_3BNC78HC	3white_3A381HC
3white_3B186HC	3white_3A125HC	3white_3A384HC
3white_3b27kc	3white_3A144HC	3white_3a426kc
3white_3B2HC	3white_3A160HC	3white_3A474KC
3white_3B44HC	3white_3A18KC	3white_3A518KC
3white_3B45HC	3white_3A204KC	3white_3A539HC
3white_3b45kc	3white_3A228HC	3white_3A539LC
3white_3b46HC	3white_3A244HC	3white_3A576HC
3white_3b46kc	3white_3A255HC	3white_3A64HC
3white_3B51HC	3white_3A296HC	3red_3bnc105kc
		3red_3B106HC
		3red_3BNC81HC

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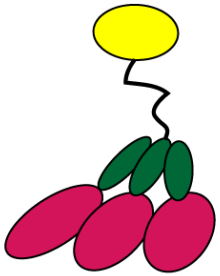

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3white_3b129kc	3white_3BNC78HC	3white_3A381HC
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3white_3b27kc	3white_3A144HC	3white_3a426kc
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3white_3B44HC	3white_3A18KC	3white_3A518KC
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3white_3b45kc	3white_3A228HC	3white_3A539LC
3white_3b46HC	3white_3A244HC	3white_3A576HC
3white_3b46kc	3white_3A255HC	3white_3A64HC
3white_3B51HC	3white_3A296HC	3red_3bnc105kc
		3red_3B106HC
		3red_3BNC81HC

Identified characteristic peptides from potent neutralizers

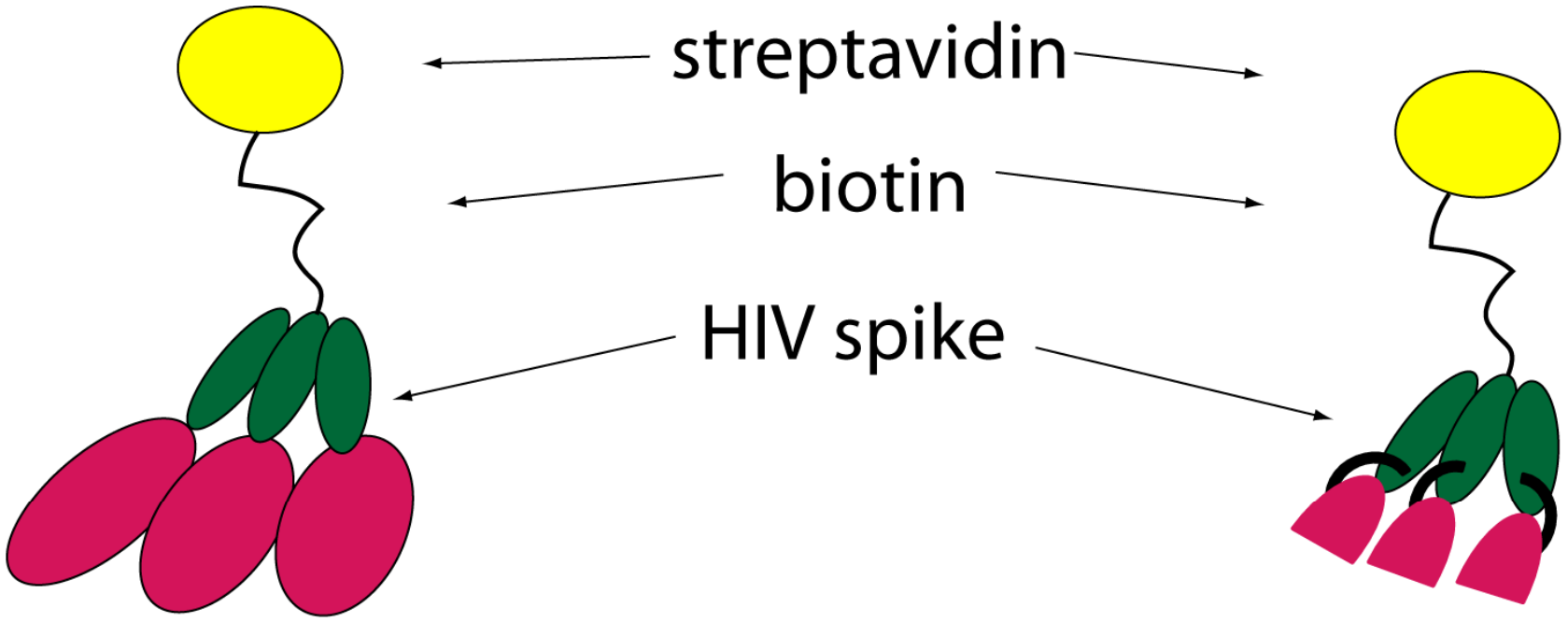
LLIYDGSR
TGQPNNPR
DTFQEILFMDLR

Is there any further bias in our data?

⇒ primers used for cloning the memory B cells

⇒ Bait used for enriching HIV reactive IgGs

Bait Construct



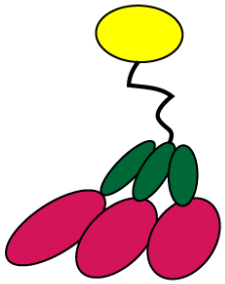
artificially stabilized
HIV spike

trimmed down gp120
artificially exposing
CD4 binding site

Chen et al Nature 433, 834 (2005)
Dey et al PLoS Pathog. 5, e1000445 (2009)

Are the potent neutralizers in the serum?

‘Native Spike’



3white_3A125HC
3white_3A144HC
3white_3A160HC
3white_3A18KC
3white_3A204KC
3white_3A228HC
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3white_3A255HC
3white_3A296HC
3white_3A296KC
3white_3A334LC
3white_3A381HC
3white_3A384HC
3white_3a426kc
3white_3A474KC
3white_3A518KC
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3white_3A539LC
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3white_3A67KC
3white_3A816HC
3white_3A816KC
3white_3A93HC
3white_3ANC3HC
3white_3ANC66HC
3white_3b129kc
3white_3B186HC
3white_3b27kc
3white_3B2HC
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3white_3B45HC
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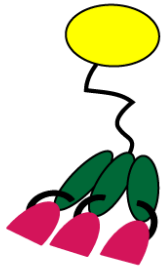
3white_3B8HC
3white_3B93HC
3white_3BNC149HC
3white_3BNC48HC
3white_3BNC58HC
3white_3BNC78HC
3red_3bnc105kc
3red_3B106HC
3red_3BNC81HC

‘exposed CD4 site’



LSSwhite_LSSNEC106HC
LSSwhite_LSSNEC77HC
LSSwhite_LSSNEC91HC
LSSwhite_154
LSSwhite_LSS_167_LC
LSSwhite_LSS_187_KC
LSSwhite_LSS_191_KC
LSSwhite_LSS_309_HC
LSSwhite_LSS_380_LC
LSSwhite_LSS_408_LC
LSSwhite_LSS_41_LC
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LSSwhite_LSS_505_KC
LSSwhite_LSS_531_KC
LSSwhite_LSS_612_LC
LSSwhite_LSS_687_LC
LSSwhite_LSS_693_HC
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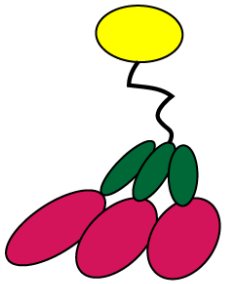
LSSwhite_LSS_73_LC
LSSwhite_LSS_74HC
LSSwhite_LSS_750_KC
LSSwhite_LSS_752_LC
LSSwhite_LSS_763_HC
LSSwhite_LSS_774_KC
LSSwhite_LSS_81_LC
LSSwhite_LSS_863_HC
LSSwhite_LSS_880_HC
LSSwhite_LSS_91_LC
LSSadd_LSSB2066HC
LSSgreen_LSSB2364LC
LSSgreen_LSSB2609HC
LSSgreen_LSSB2612HC
LSSgreen_LSSNEC108HC
LSSgreen_LSSNEC108LC
LSSgreen_LSSNEC109HC
LSSgreen_LSSNEC110HC
LSSgreen_LSSNEC110HC
LSSgreen_LSSNEC117HC
LSSgreen_LSSNEC122HC
LSSgreen_LSSNEC123HC
LSSgreen_LSSNEC18HC
LSSgreen_LSSNEC24HC
LSSgreen_LSSNEC24LC
LSSgreen_LSSNEC3HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC60HC
LSSgreen_LSSNEC70LC
LSSgreen_LSSNEC7HC
LSSgreen_LSSNEC89LC

Are the potent neutralizers in the serum?

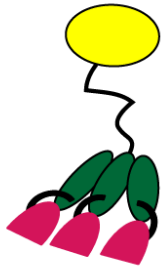
‘Native Spike’



3white_3A125HC
3white_3A144HC
3white_3A160HC
3white_3A18KC
3white_3A204KC
3white_3A228HC
3white_3A244HC
3white_3A255HC
3white_3A296HC
3white_3A296KC
3white_3A334LC
3white_3A381HC
3white_3A384HC
3white_3a426kc
3white_3A474KC
3white_3A518KC
3white_3A539HC
3white_3A539LC
3white_3A576HC
3white_3A64HC
3white_3A650HC
3white_3A67HC
3white_3A67KC

3white_3A816HC
3white_3A816KC
3white_3A93HC
3white_3ANC3HC
3white_3ANC66HC
3white_3b129kc
3white_3B186HC
3white_3b27kc
3white_3B2HC
3white_3B44HC
3white_3B45HC
3white_3b45kc
3white_3b46HC
3white_3b46kc
3white_3B51HC
3white_3B8HC
3white_3B93HC
3white_3BNC149HC
3white_3BNC48HC
3white_3BNC58HC
3white_3BNC78HC
3red_3bnc105kc
3red_3B106HC
3red_3BNC81HC

‘exposed CD4 site’



LSSwhite_LSSNEC106HC
LSSwhite_LSSNEC77HC
LSSwhite_LSSNEC91HC
LSSwhite_154
LSSwhite_LSS_167_LC
LSSwhite_LSS_187_KC
LSSwhite_LSS_191_KC
LSSwhite_LSS_309_HC
LSSwhite_LSS_380_LC
LSSwhite_LSS_408_LC
LSSwhite_LSS_41_LC
LSSwhite_LSS_479LC
LSSwhite_LSS_494_LC
LSSwhite_LSS_505_KC
LSSwhite_LSS_531_KC
LSSwhite_LSS_612_LC
LSSwhite_LSS_687_LC
LSSwhite_LSS_693_HC
LSSwhite_LSS_695_LC
LSSwhite_LSS_706_LC
LSSwhite_LSS_71_HC
LSSwhite_LSS_73_LC
LSSwhite_LSS_74HC
LSSwhite_LSS_750_KC
LSSwhite_LSS_752_LC
LSSwhite_LSS_763_HC
LSSwhite_LSS_774_KC
LSSwhite_LSS_81_LC

LSSwhite_LSS_863_HC
LSSwhite_LSS_880_HC
LSSwhite_LSS_91_LC
LSSadd_LSSB2066HC
LSSgreen_LSSB2364LC
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LSSgreen_LSSB2612HC
LSSgreen_LSSNEC108HC
LSSgreen_LSSNEC108LC
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LSSgreen_LSSNEC110HC
LSSgreen_LSSNEC117HC
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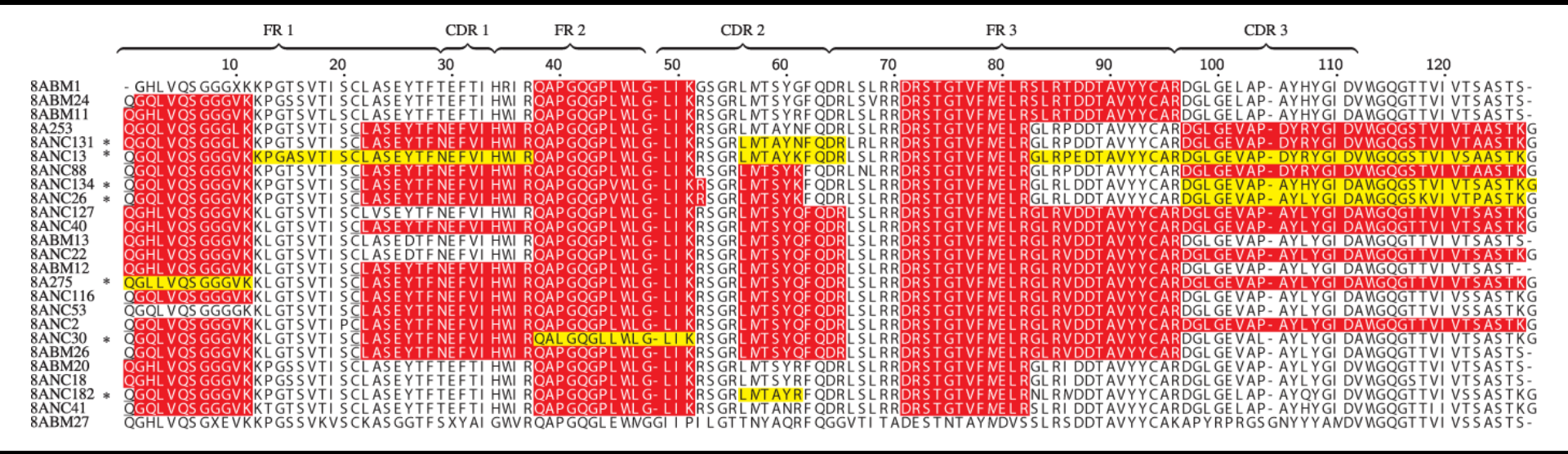
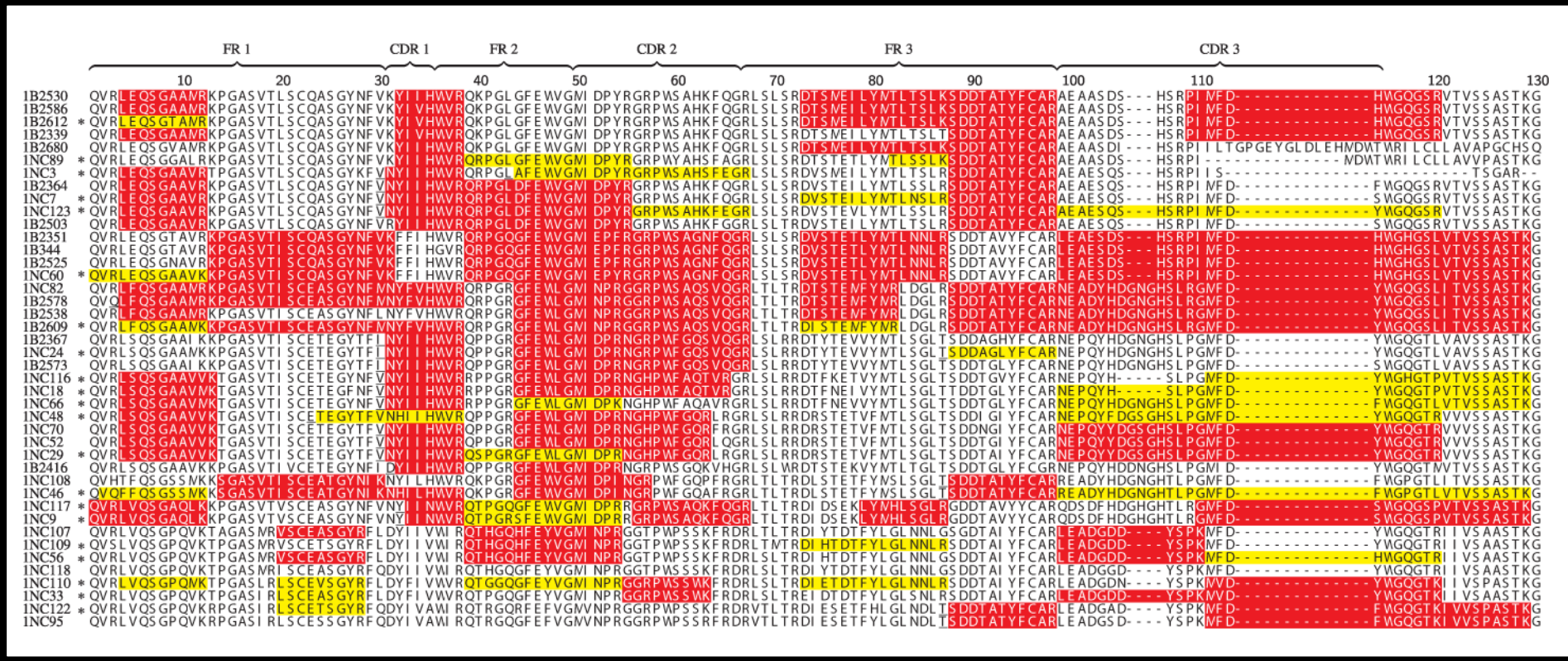
LSSgreen_LSSNEC24HC
LSSgreen_LSSNEC24LC
LSSgreen_LSSNEC3HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC46HC
LSSgreen_LSSNEC60HC
LSSgreen_LSSNEC70LC
LSSgreen_LSSNEC7HC
LSSgreen_LSSNEC89LC

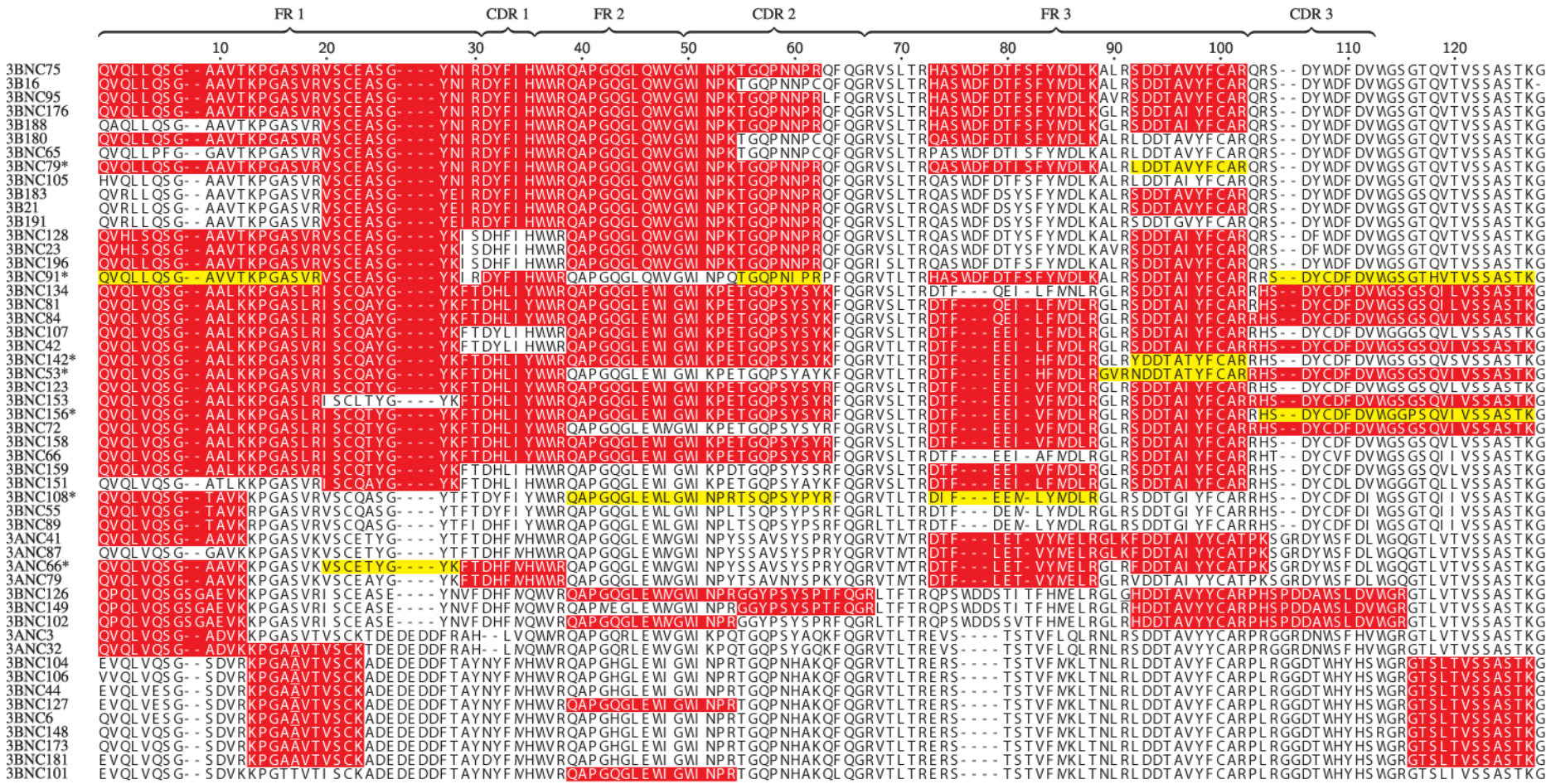
Elite Controller 1



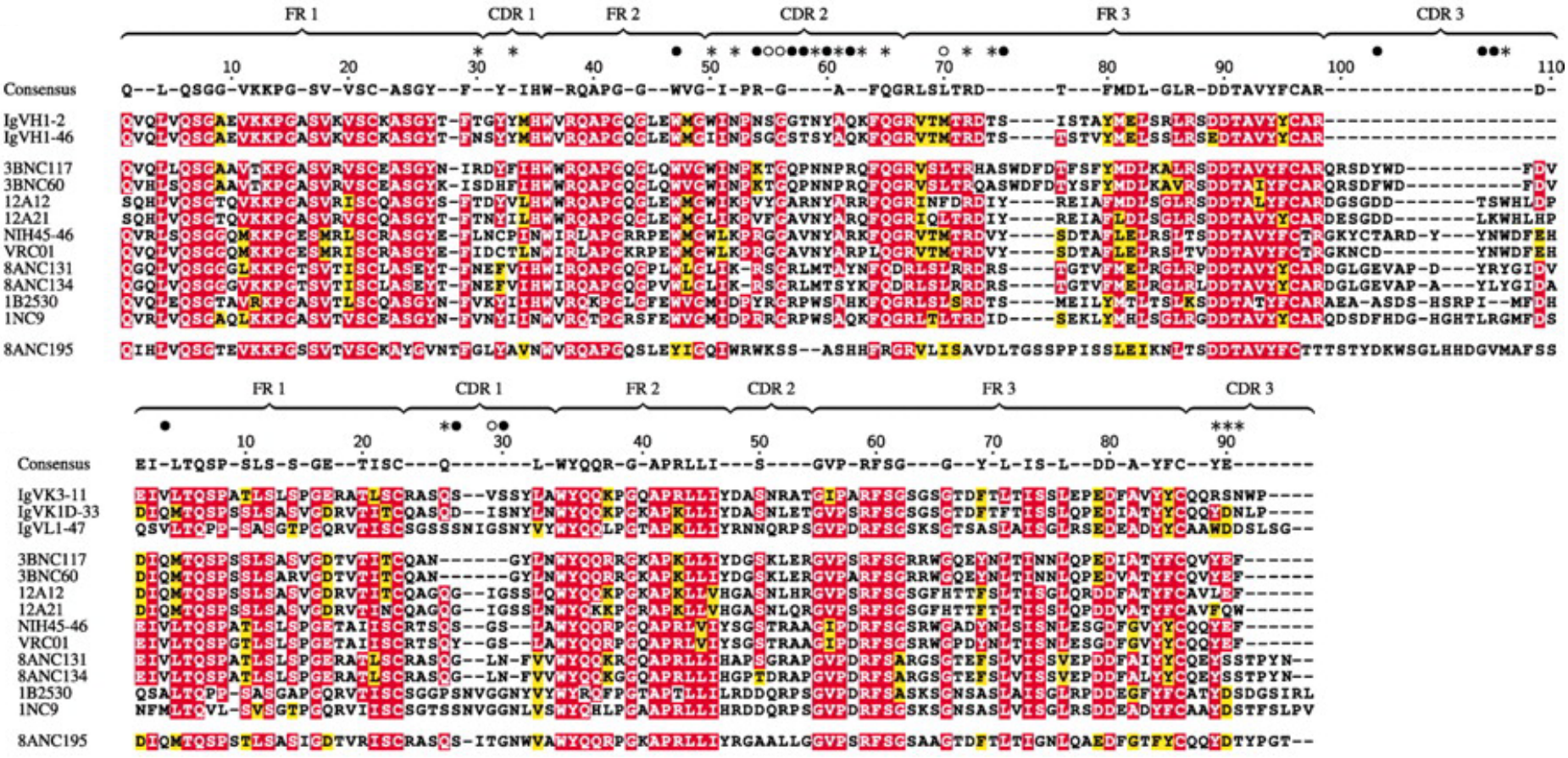
Elite Controller 2



Long-Term Nonprogressor 1



Sequence conservation of highly active agonistic CD4-binding site antibodies



J F Scheid et al. Science 2011;333:1633-1637

Conclusions

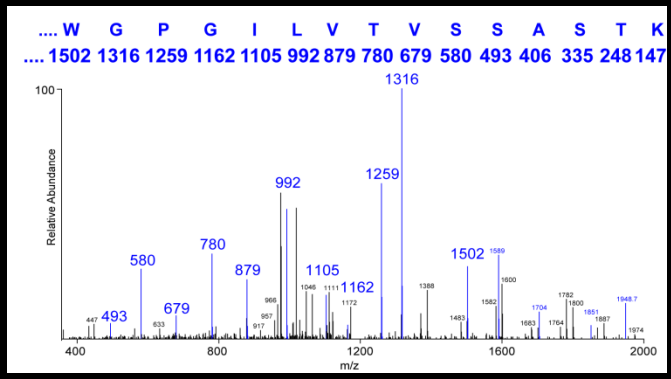
Cloned 576 new HIV Antibodies from 4 unrelated Individuals

All 4 individuals produced expanded clones of potent broadly neutralizing antibodies that mimic binding to CD4

Despite extensive hypermutation, the new antibodies share a consensus of 68 IgH chain amino acids

Conclusions

Our Marker Ion Strategy enables rapid estimation of the different clones present in patient sera with/without a database



For each individual under study we have provided the first direct proof that potent broadly neutralizing antibodies are circulating in the blood (<1% of all HIV reactive IgG)

These technologies are applicable to any disease in which antibodies are induced against a known antigen

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