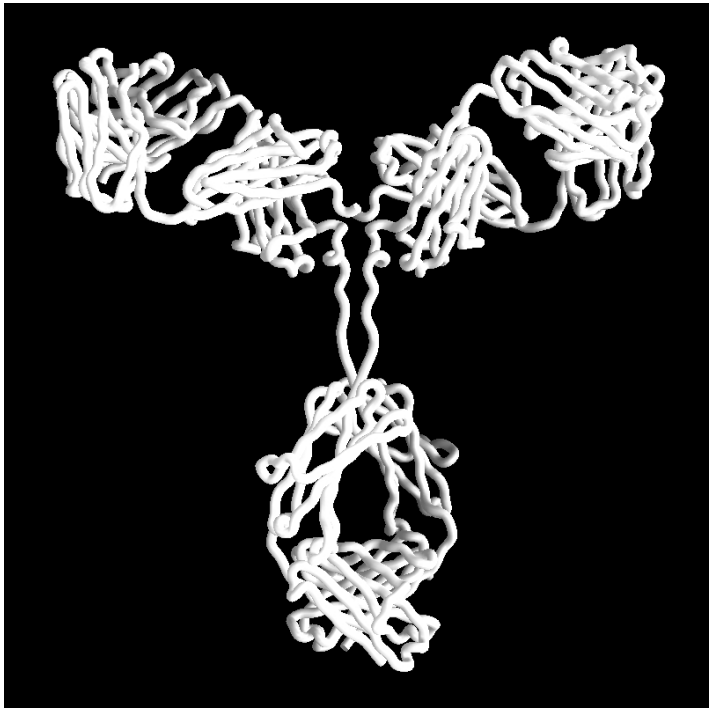
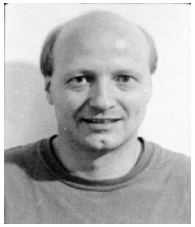
Harnessing evolution to make medicines



MRC Laboratory of Molecular Biology

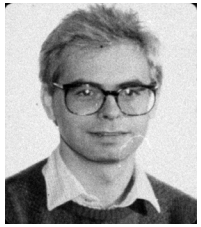
MRC Centre for Protein Engineering

CAT Cambridge Antibody Technology



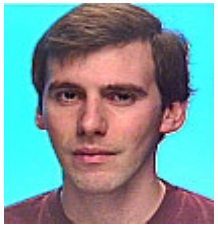
Peter Jones

Rosaria Orlandi



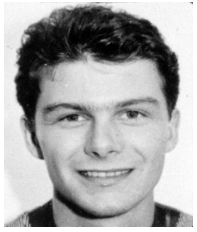
Detlef Gussow

Sally Ward

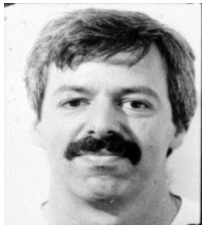


Andrew Griffiths

John McCafferty



Tim Clackson



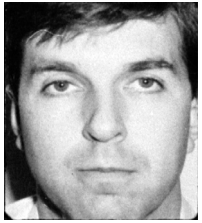
James Marks

Hendricus Hoogenboom

Ian TomInson

Sam Williams

Gerald Walter



Robert Hawkins

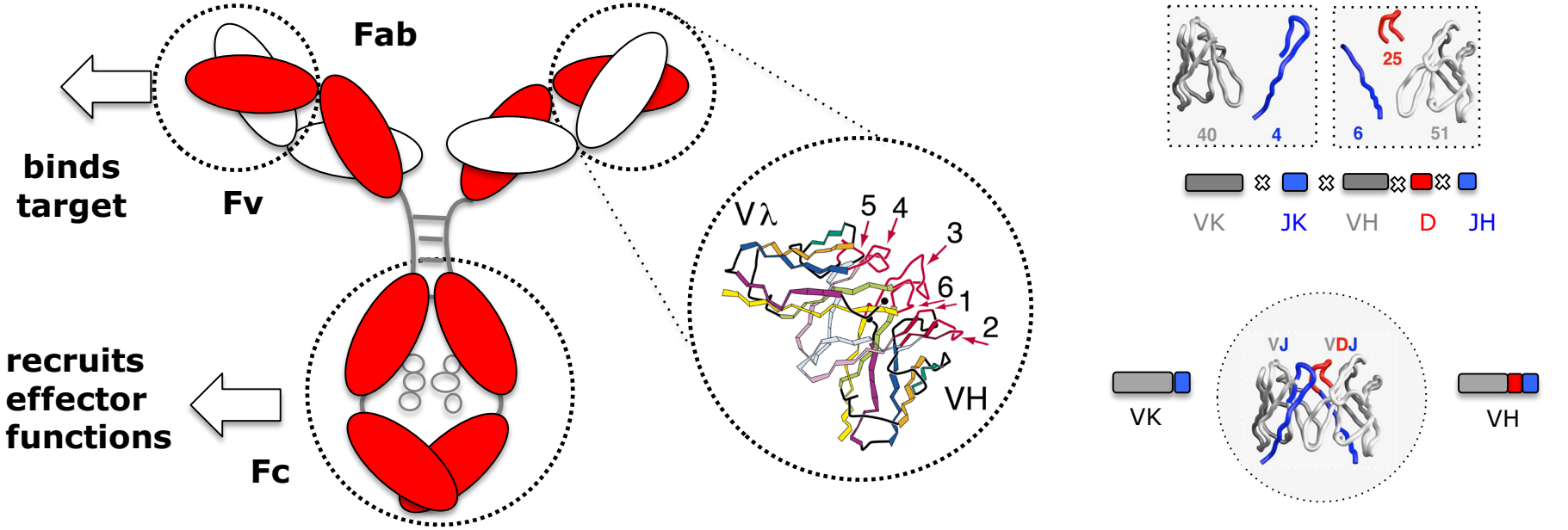
Steven Russell

Ahuva Nissim

Laurent Jespers

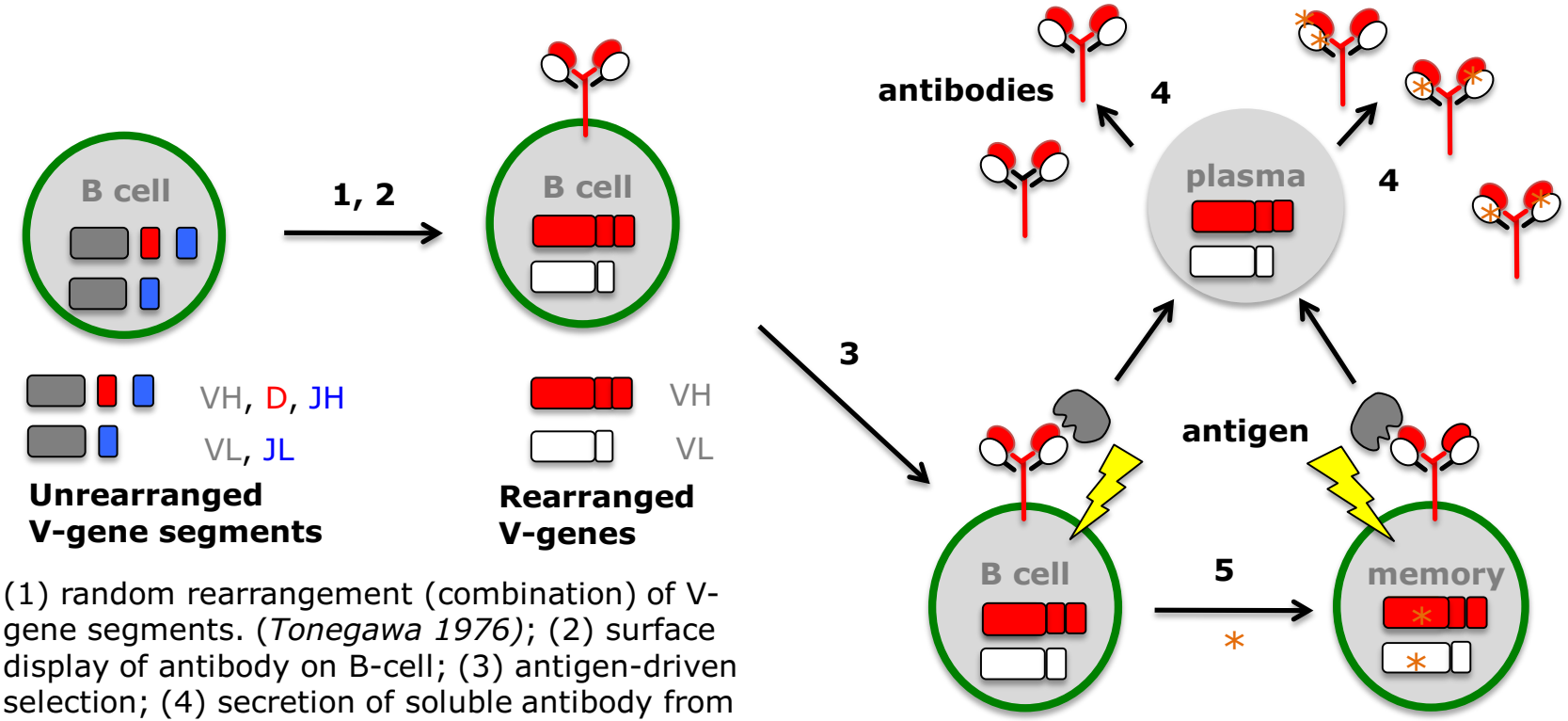
Greg Winter

Antibody structure and function



IgG mAbs are large (150,000 Da) Y-shaped protein molecules with two (H/L) chains. Associated VH/VL domains (=Fv at end of Fab arms) come together to form antigen binding site comprising a scaffold with six loops of variable sequence. Variability created by combinations of multiple genetic segments. Ab binds to infectious agent and can block infection, also can kill infectious agent by recruiting effector functions through Fc domains (stem).

Strategy of immune system



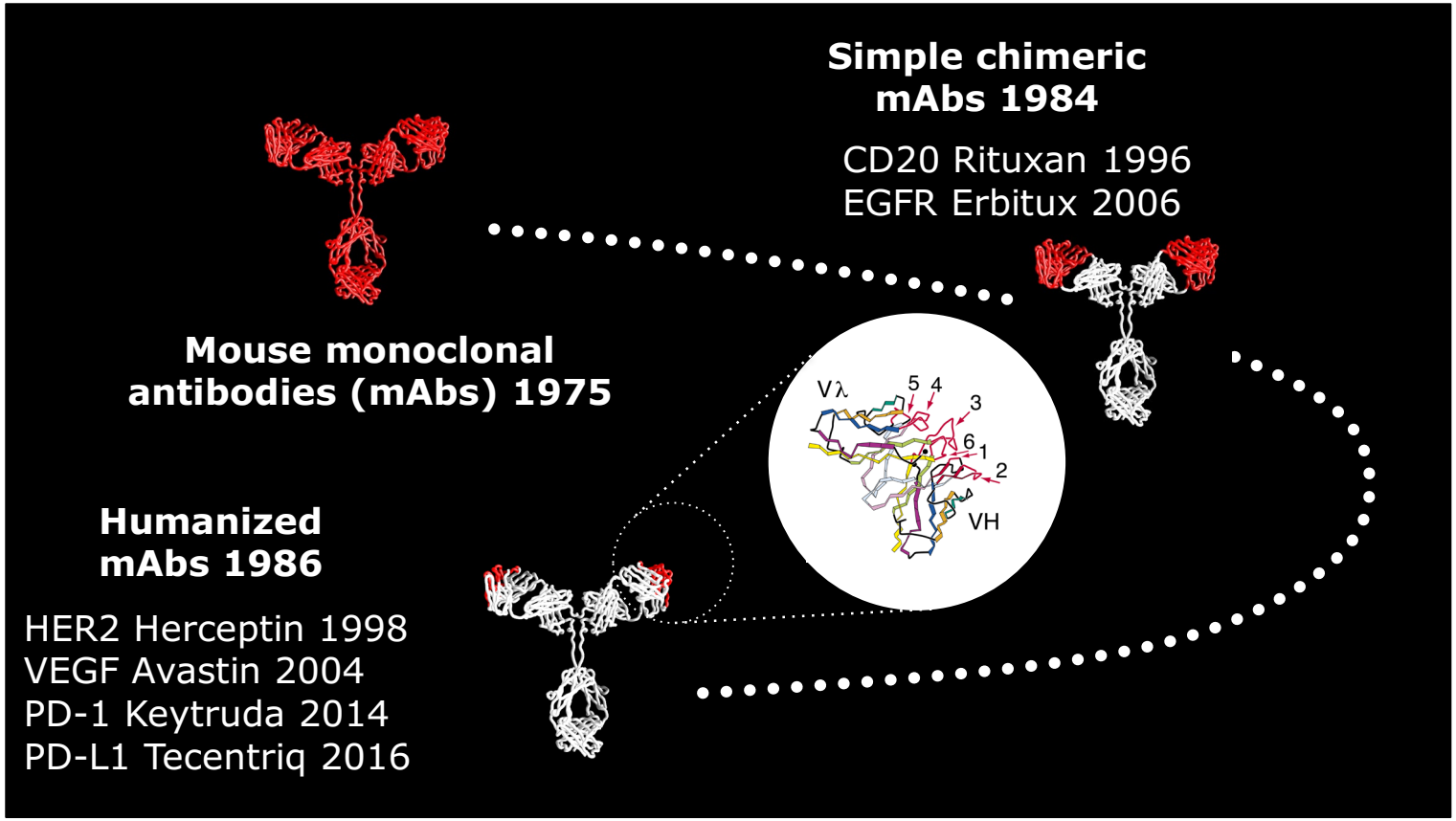
(1) random rearrangement (combination) of V-gene segments. (*Tonegawa 1976)*; (2) surface display of antibody on B-cell; (3) antigen-driven selection; (4) secretion of soluble antibody from plasma cell; (5) affinity maturation.

Best selling medicines

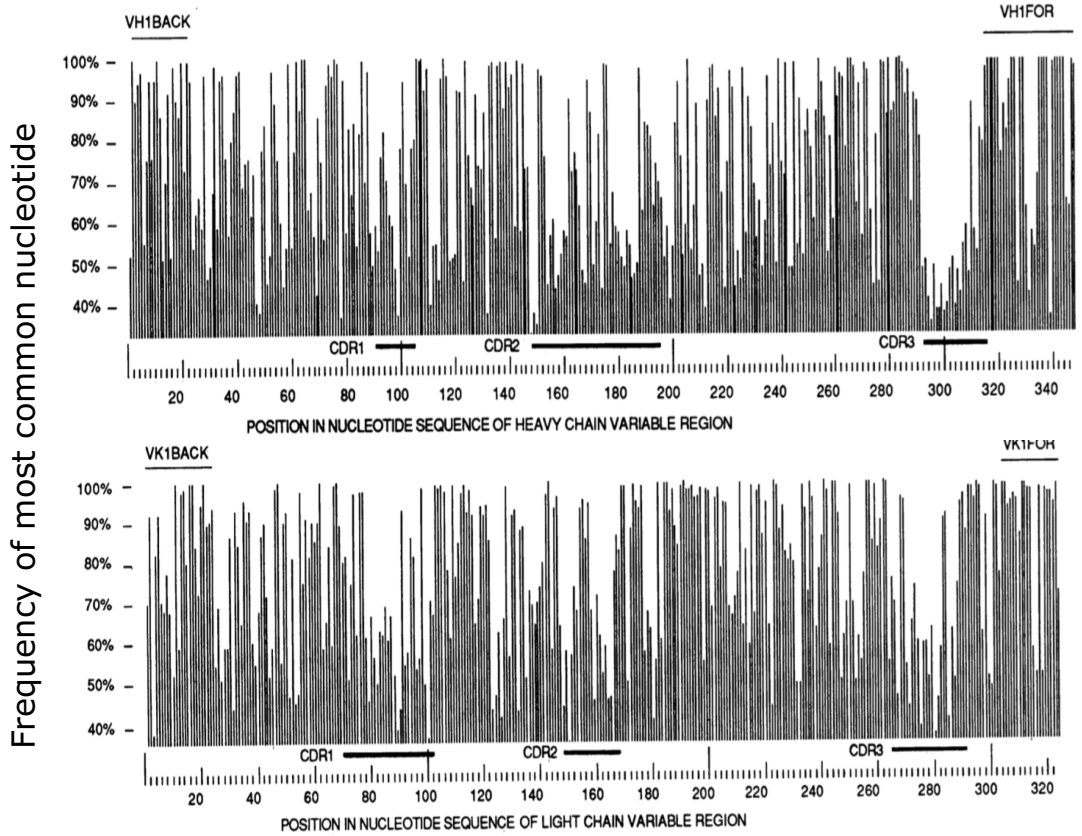
BRAND	DISEASE	COMPANY	SALES ($bn)
1. **Humira**	rheumatoid arthritis	AbbVie	**16.1**
2. **Harvoni**	hepatitis C	Gilead	**9.1**
3. **Enbrel**	rheumatoid arthritis	Amgen/Pfizer	**8.9**
4. **Rituxan**	NHL	Roche/Biogen	**8.6**
5. **Remicade**	rheumatoid arthritis	J&J/Merck	**7.8**
6. **Revlimid**	multiple myeloma	Celgene	**7.0**
7. **Avastin**	cancers	Roche	**6.7**
8. **Herceptin**	breast cancer	Roche	**6.7**
9. **Lantus**	diabetes (insulin)	Sanofi	**6.0**
10. **Prevnar**	pneumonia (vaccine)	Pfizer	**5.7**

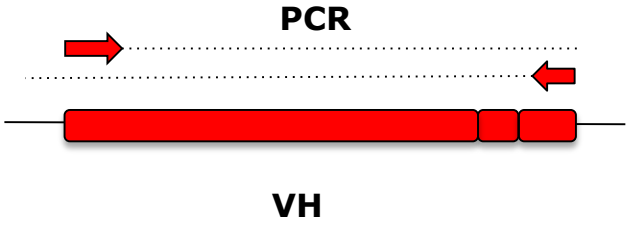
Year 2016. Source: from genengnews.com. **antibodies red, chemicals black, *others green***

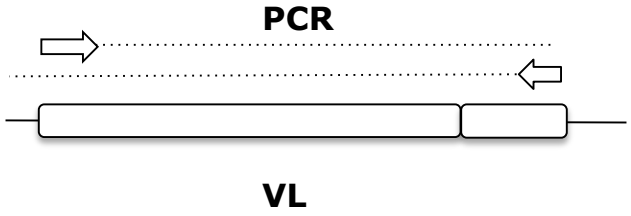
Mouse-human therapeutic antibodies



Sequence conservation in V-genes

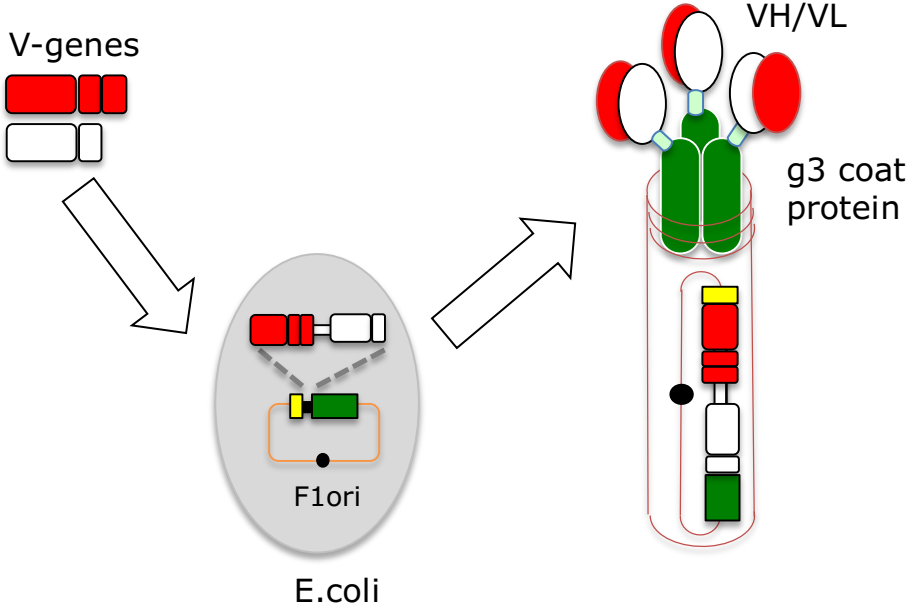




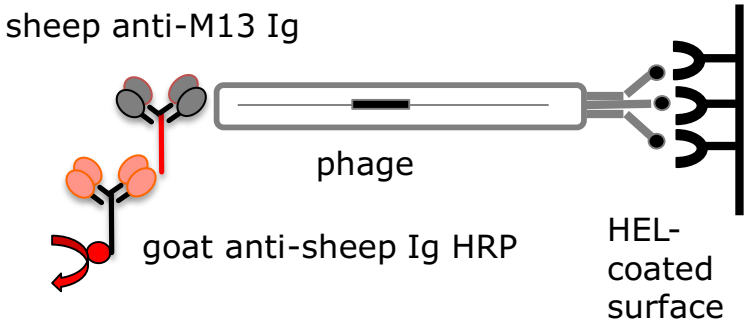


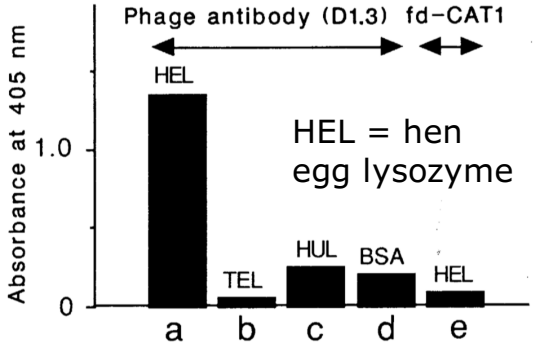
From hybridoma cDNA. (*Orlandi 1989)*.

Display of antibody fragment on phage



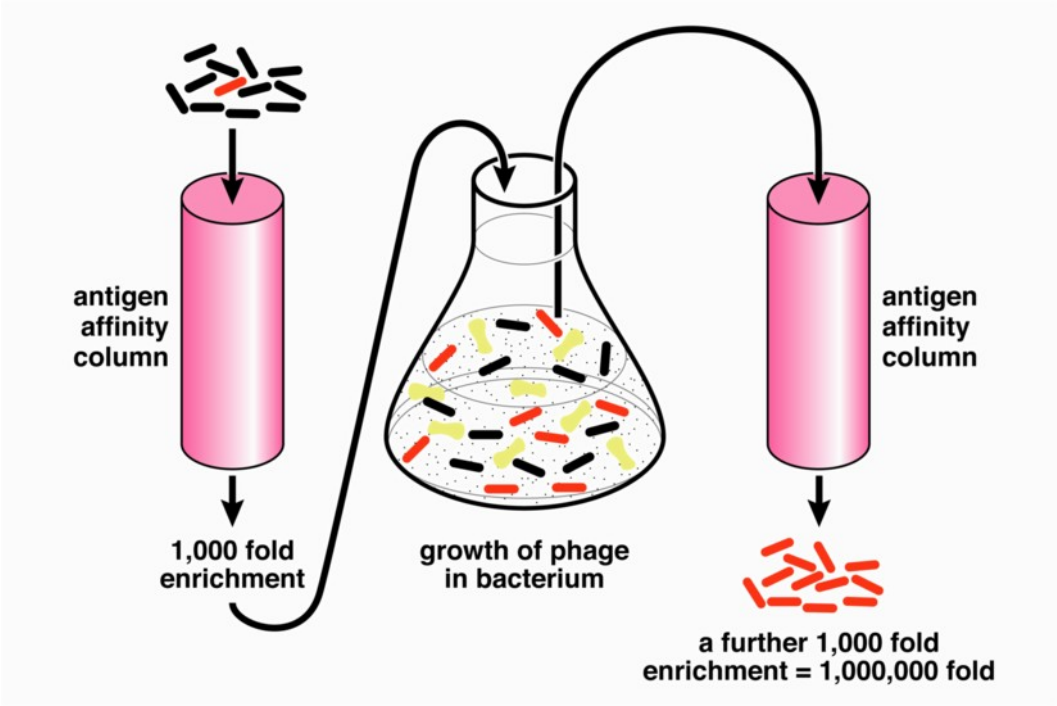
Phage vector. VH/VL from anti-HEL D1.3 mAb. (*McCafferty 1990)*.





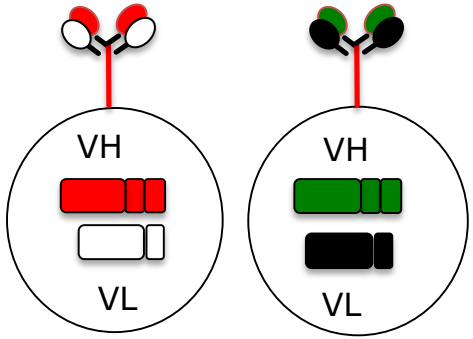
Phage ELISA

Phage selection

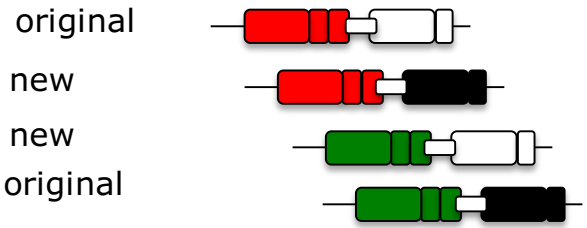


Model selection: rare binders (scFv D1.3 to target HEL) isolated by multiple rounds of affinity selection. (*McCafferty 1990)*.

Antibody libraries



spleen B-cells



random combinatorial [Huse 1989]

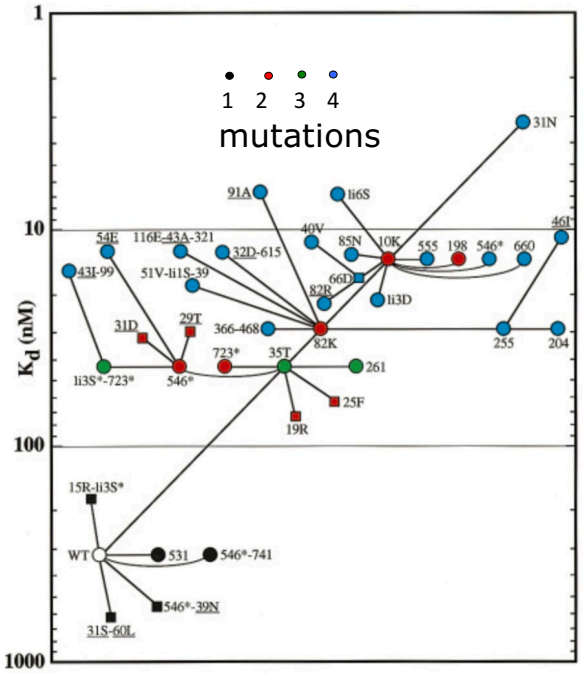
10^6 clones from mouse immunized with phOx. K_d = 10 nM

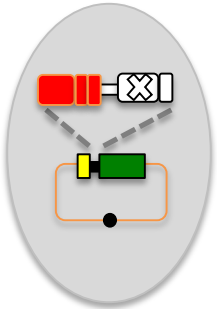
immune mouse library
(Clackson 1991)

10^7 clones from human donors,
K_d = 10 μM

non-immune human library
(Marks 1991, Griffiths 1993)

Variation

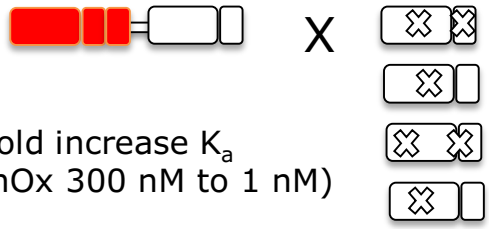




Mutator host

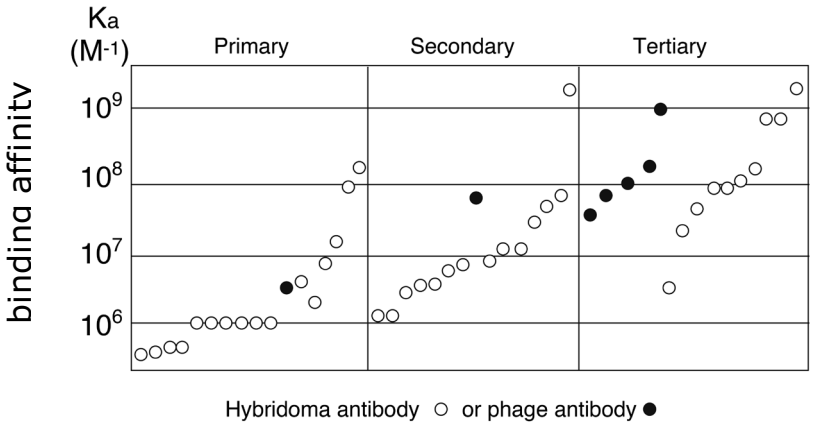
100-fold increase K_a (K_d phOx 300 nM to 3 nM)

Mutation *in vivo*. *(Low 1996)*



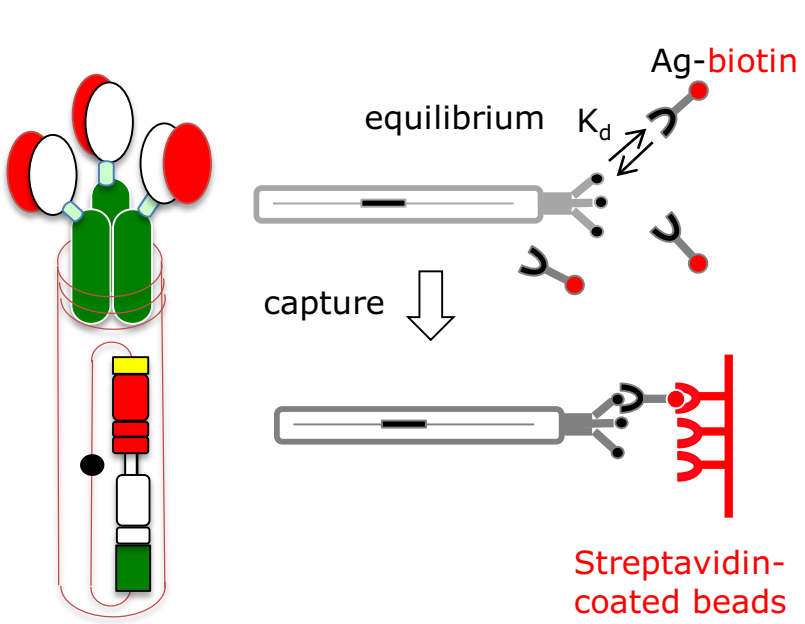
X

300-fold increase K_a (K_d phOx 300 nM to 1 nM)

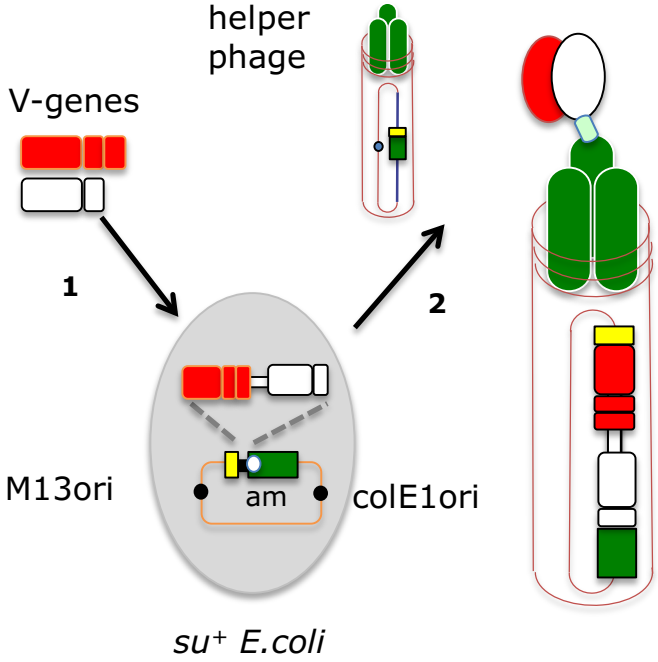


Chain shuffling in vitro. (Marks 1992)

Selection stringency

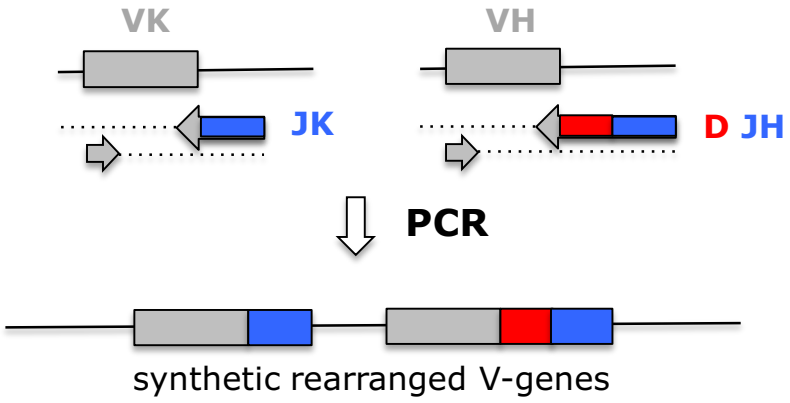


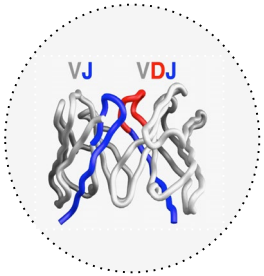
Low [Ag] & capture *(Hawkins 1992)*

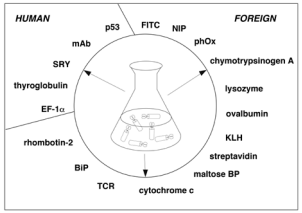


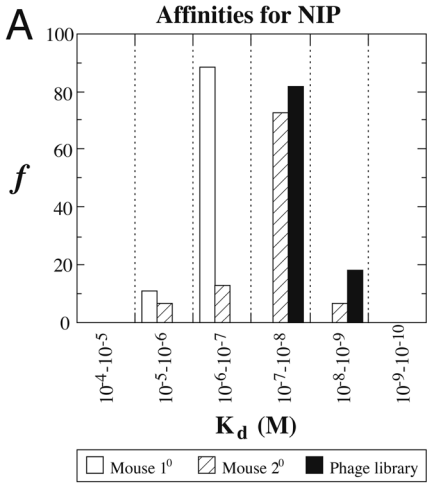
"Monomeric display"
[*Bass 1990*], (*Hoogenboom 1991*)

Large synthetic libraries







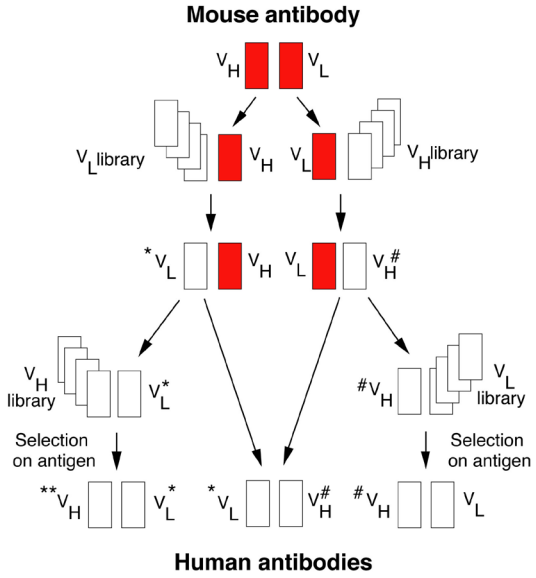


Synthetic V-gene repertoires. V-segment building blocks *(Tomlinson, 1992; Williams 1994, Cox 1994)*: assembly into synthetic libraries *(Hoogenboom 1992, Nissim 1994, Griffiths 1994)*

Binding specificities and affinities from large primary synthetic Fab library >10^{10} clones. *(Griffiths 1994)*

Human mAb templated by mouse mAb

mouse
(Knoll – Abbott)



human
MRC – CAT

Adalimumab (Humira). Developed through Cambridge Antibody Technology and Knoll (BASF Pharma), later sold to Abbott. First human therapeutic antibody approved by US FDA for rheumatoid arthritis. For strategy see (*Jespers 1994).*

Phage antibody pharmaceuticals

Growth factor: PIGF, VEGF-2, GDF-8
Chemokine: CXCL13
Ion Channel: P2X4
Receptor: IL-21R, PSGL-1, TRAIL-R1, GM-CSFa2

GPCR: GLP1R, GIPr
Cytokine: IL-6, Blys, APRIL
Protease inhibitor: PAI-1
Peptide: Ghrelin, NKB, gp41

Human pharma target classes

Adalimumab (TNF/Autoimmune)
Avelumab (PDL1/Cancer)
Belimumab (BAFF/Lupus)
Guselkumab (IL23/Psoriasis)

Necitumumab (EGFR/NSCLC)
Ramucirumab (VEGFR2/Cancer)
Raxibacumab (Anthrax)
Moxetumumab (CD22/HCL)

Phage antibodies on the market.
>60 antibodies from phage display have entered clinical trials; J. Osbourne, Medimmune