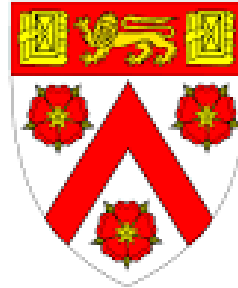
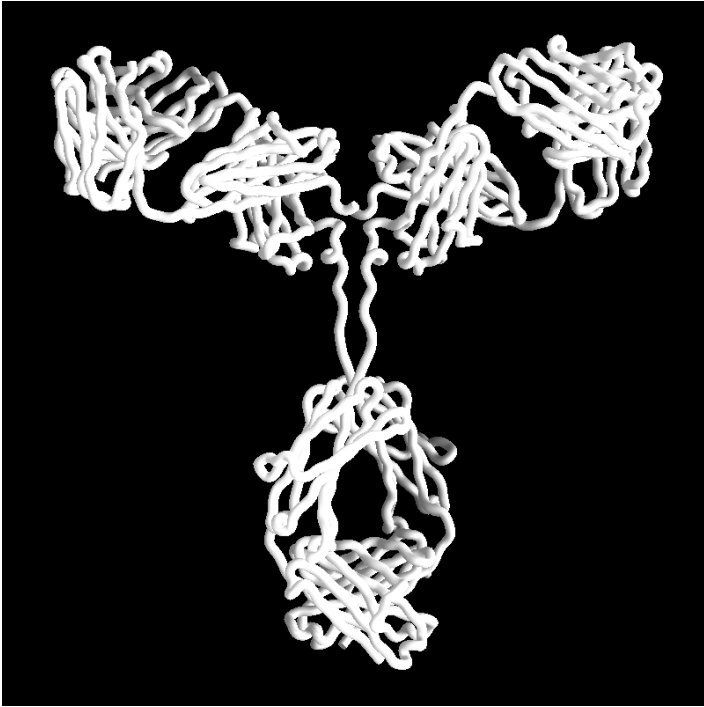


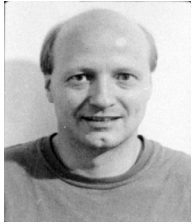
# Harnessing evolution to make medicines



MRC | Laboratory of  
Molecular Biology

MRC | Centre for  
Protein Engineering

C | a | T  
*Cambridge Antibody Technology*



**Peter  
Jones**



**Rosaria  
Orlandi**



**Detlef  
Gussow**



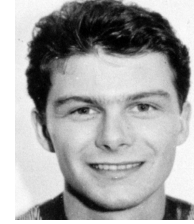
**Sally  
Ward**



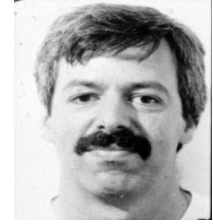
**Andrew  
Griffiths**



**John  
McCafferty**



**Tim  
Clackson**



**James  
Marks**



**Hendricus  
Hoogenboom**



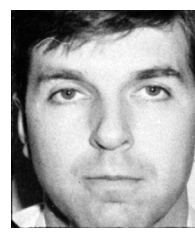
**Ian  
Tomlinson**



**Sam  
Williams**



**Gerald  
Walter**



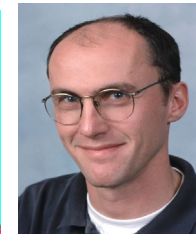
**Robert  
Hawkins**



**Steven  
Russell**



**Ahuva  
Nissim**

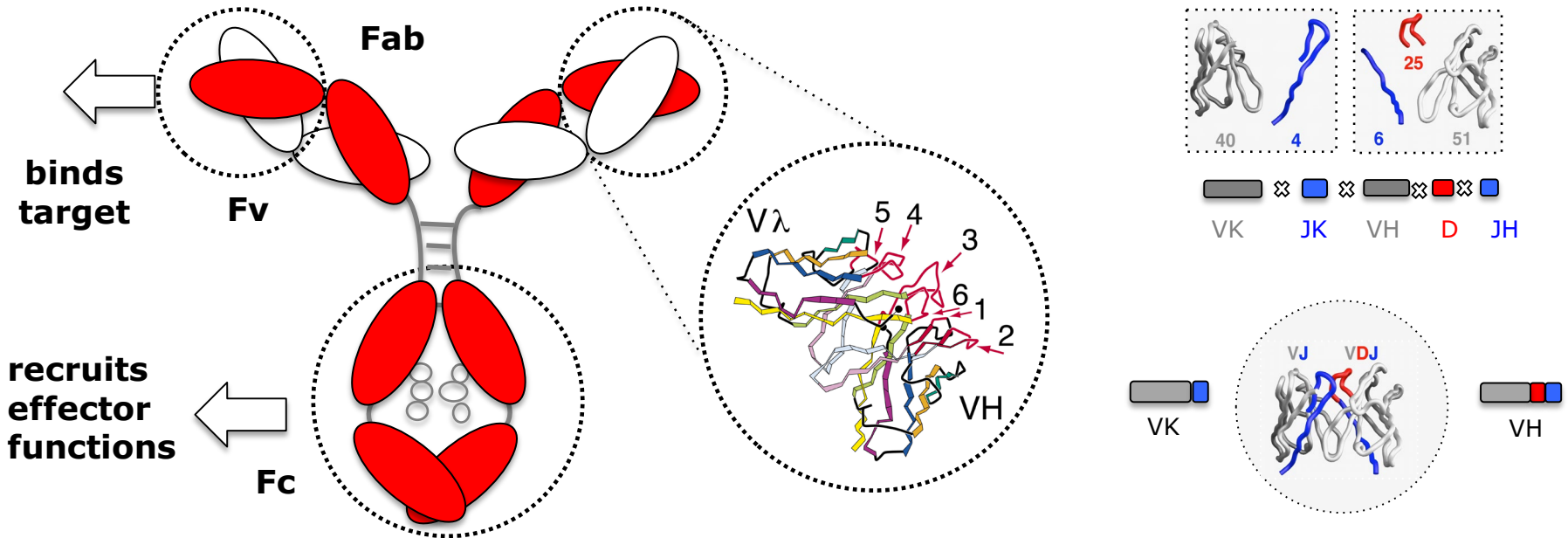


**Laurent  
Jaspers**



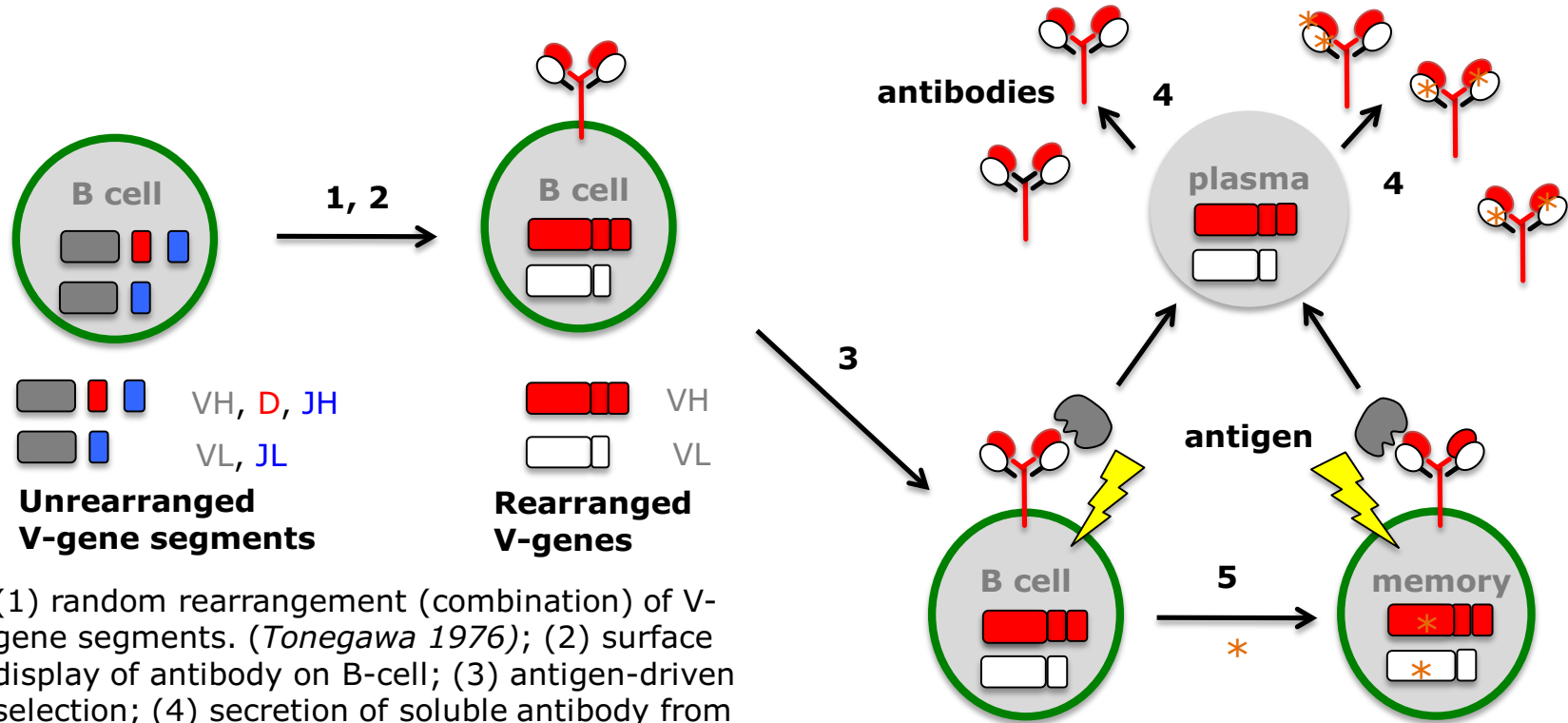
**Greg  
Winter**

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

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

# Mouse-human therapeutic antibodies



**Mouse monoclonal antibodies (mAbs) 1975**

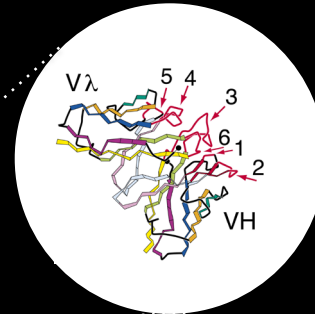
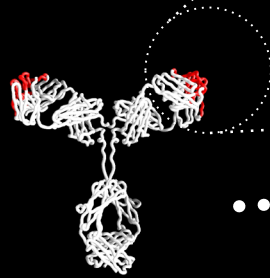
**Simple chimeric mAbs 1984**

CD20 Rituxan 1996  
EGFR Erbitux 2006

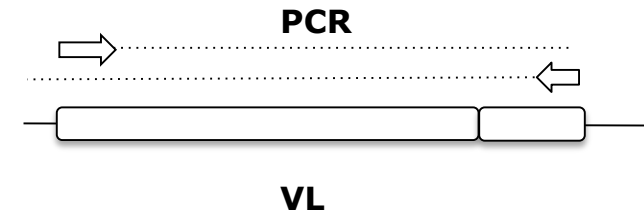
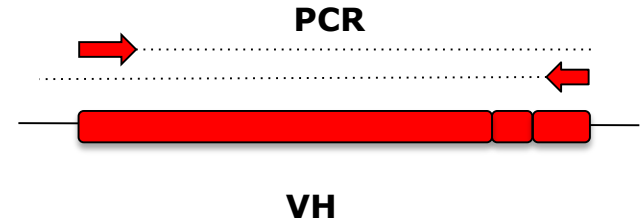
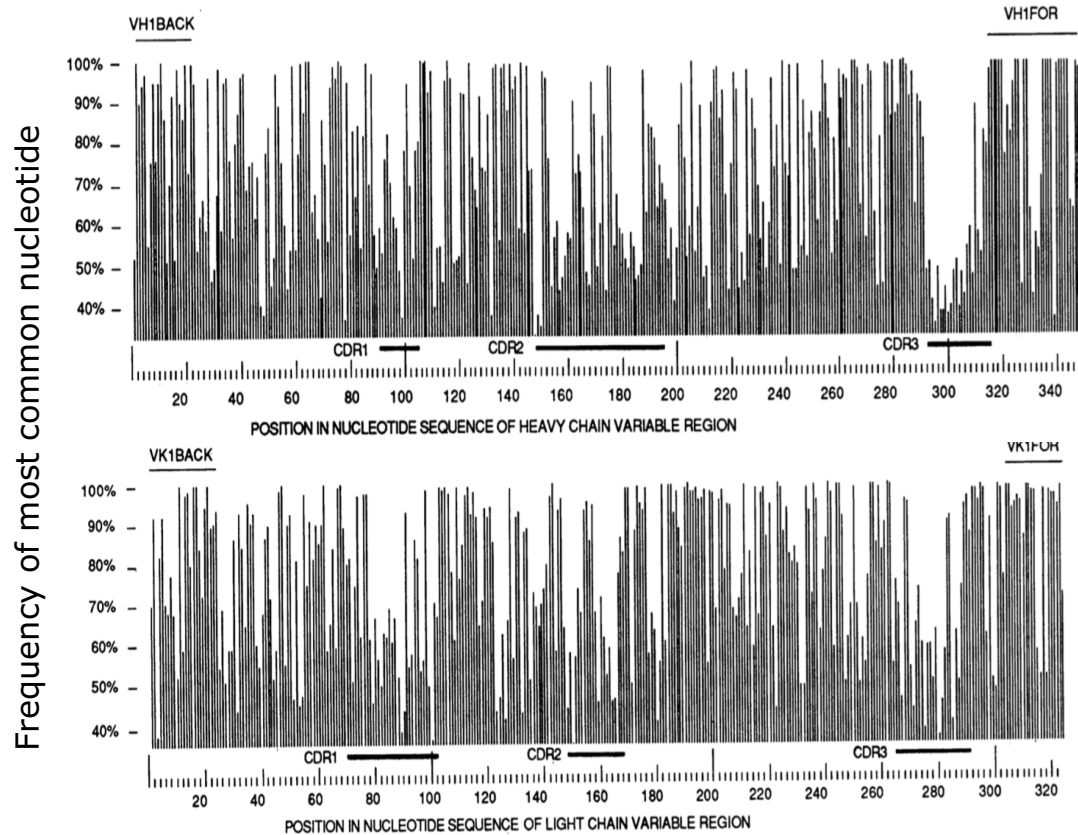


**Humanized mAbs 1986**

HER2 Herceptin 1998  
VEGF Avastin 2004  
PD-1 Keytruda 2014  
PD-L1 Tecentriq 2016

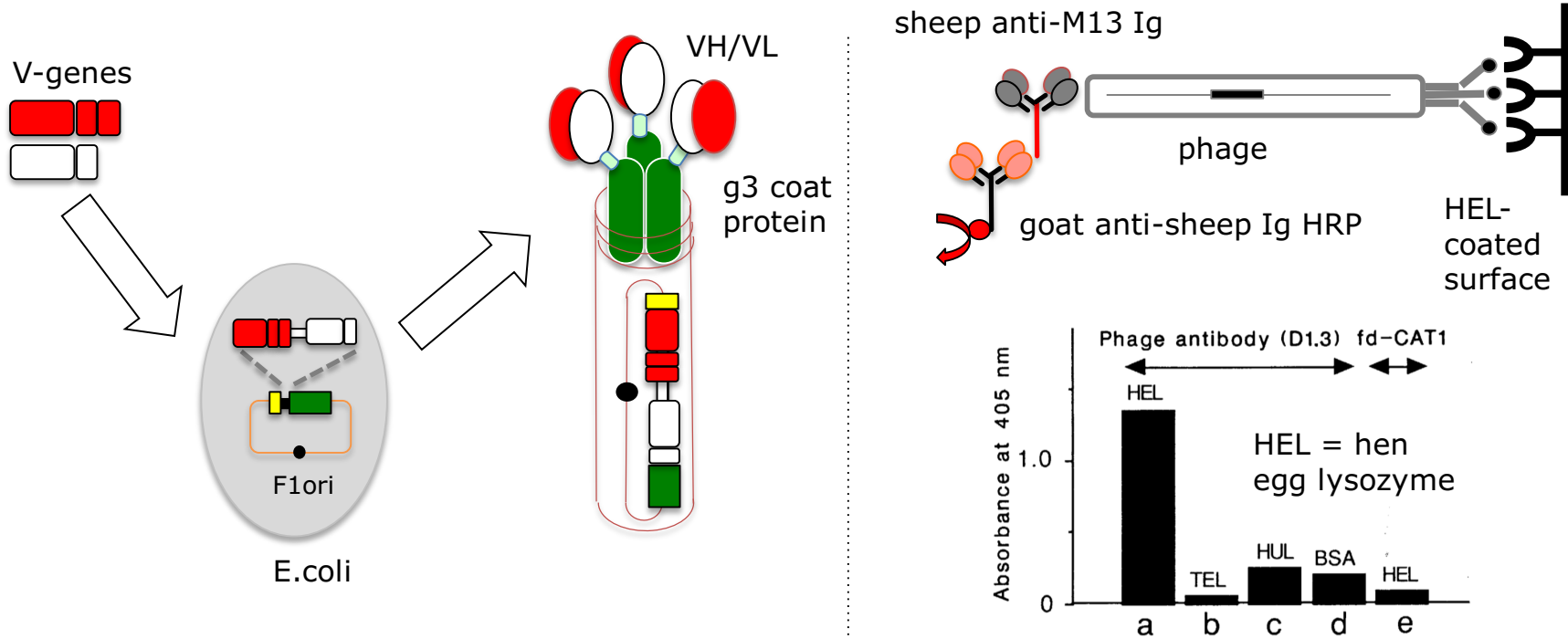


# Sequence conservation in V-genes



From hybridoma cDNA. (Orlandi 1989).

# Display of antibody fragment on phage

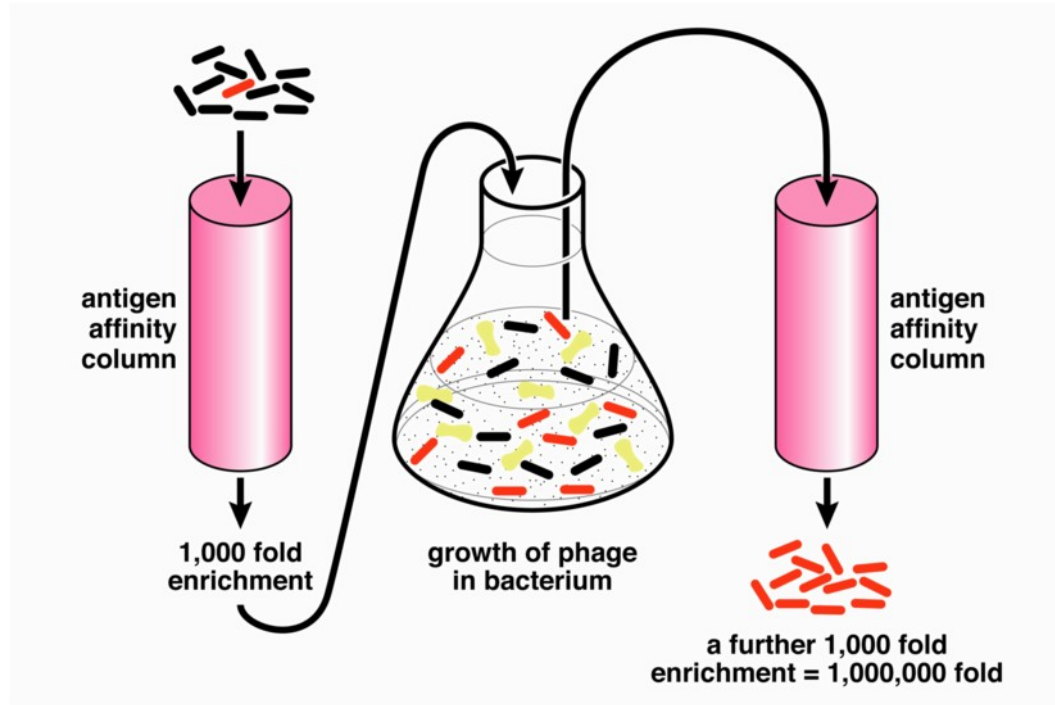


**Phage vector.** VH/VL from anti-HEL D1.3 mAb.  
(McCafferty 1990).

**Phage ELISA**

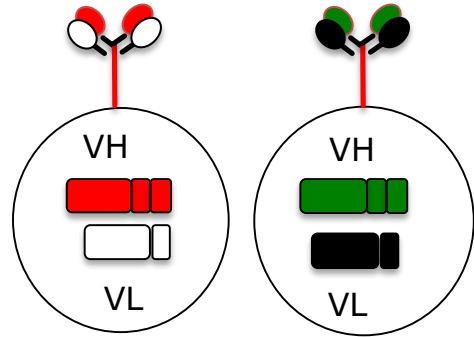


# Phage selection

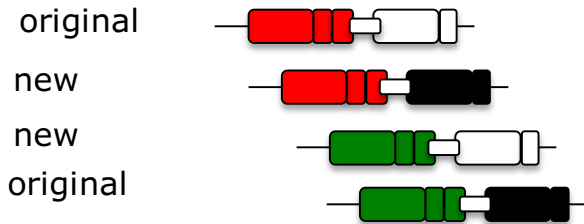


**Model selection:** rare binders (scFv D1.3 to target HEL) isolated by multiple rounds of affinity selection. (McCafferty 1990).

# Antibody libraries



**spleen B-cells**



**random combinatorial** [Huse 1989]

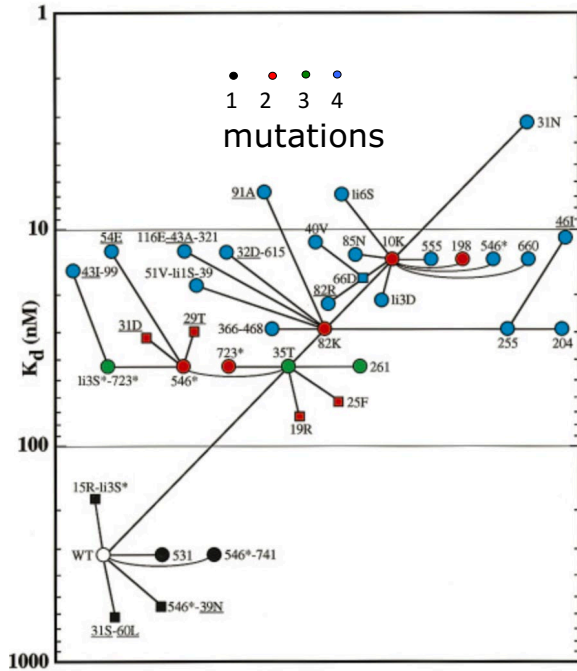
$10^6$  clones from mouse immunized with phOx.  $K_d = 10$  nM

**immune mouse library**  
(Clackson 1991)

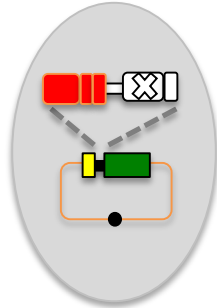
$10^7$  clones from human donors,  
 $K_d = 10$   $\mu$ M

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

# Variation

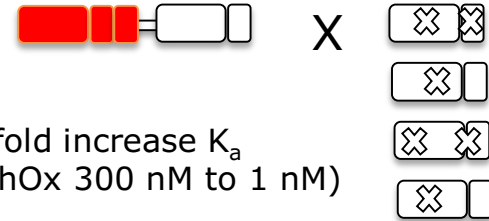


**Mutation *in vivo*.** (Low 1996)

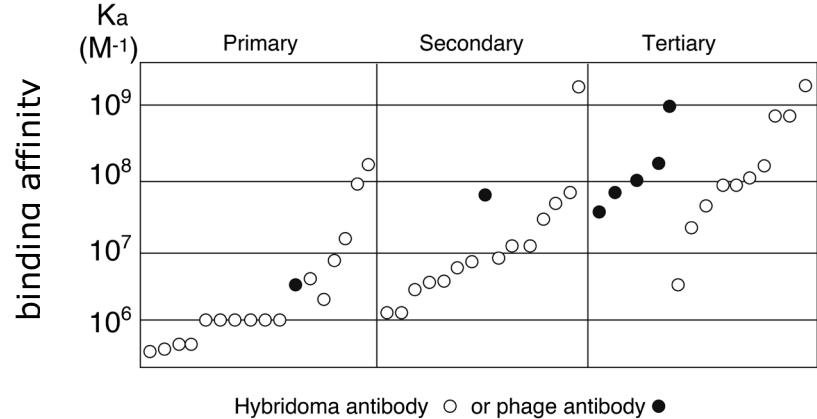


**Mutator host**

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

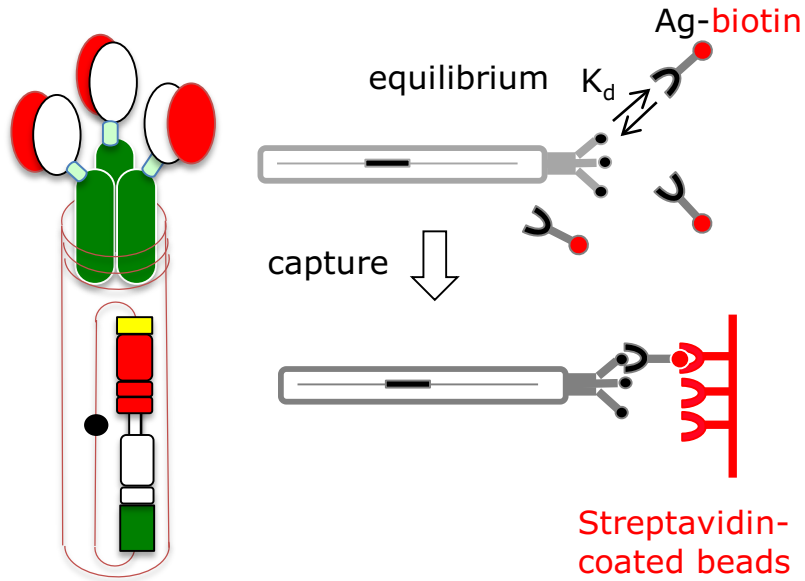


300-fold increase  $K_a$   
 ( $K_d$  phOx 300 nM to 1 nM)

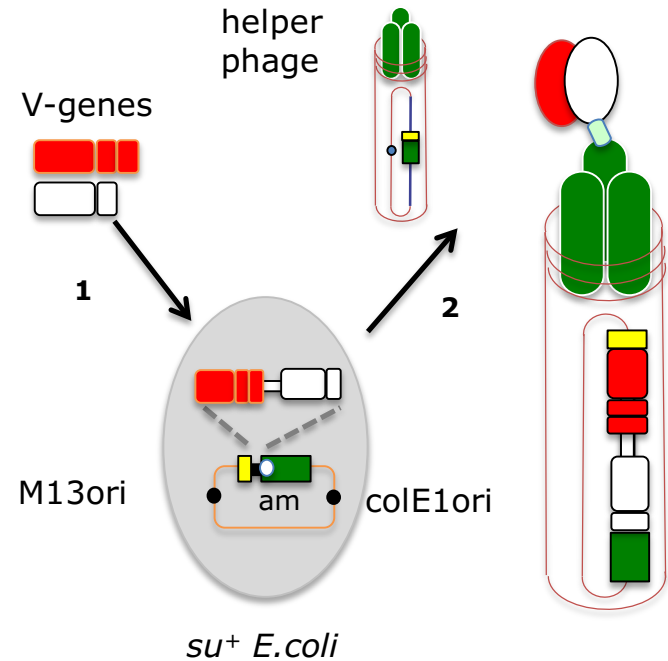


**Chain shuffling *in vitro*.** (Marks 1992)

# Selection stringency

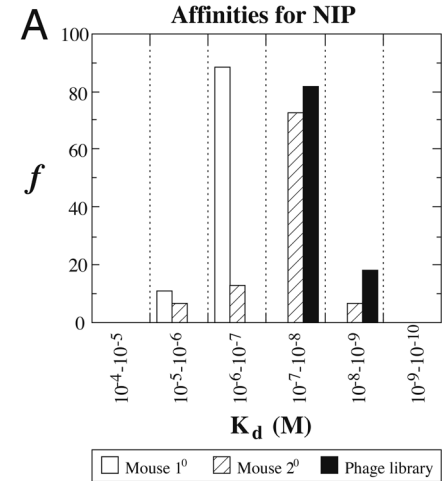
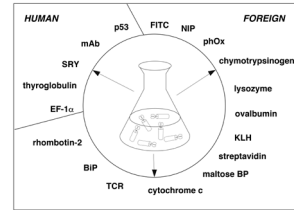
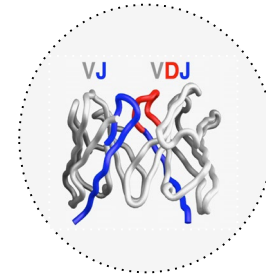
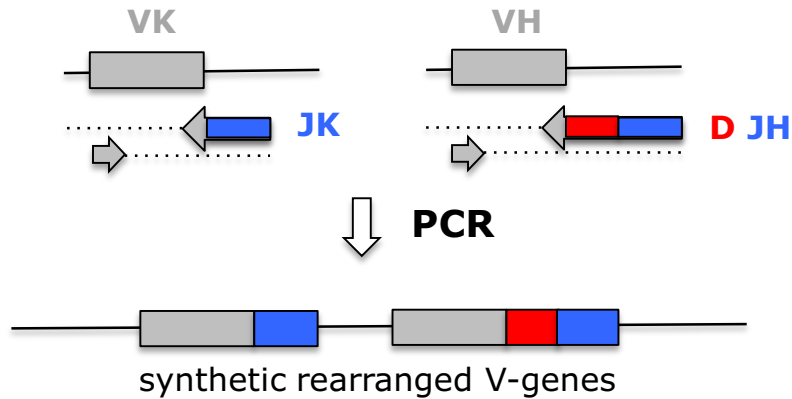


**Low [Ag] & capture** (*Hawkins 1992*)



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

# Large synthetic libraries



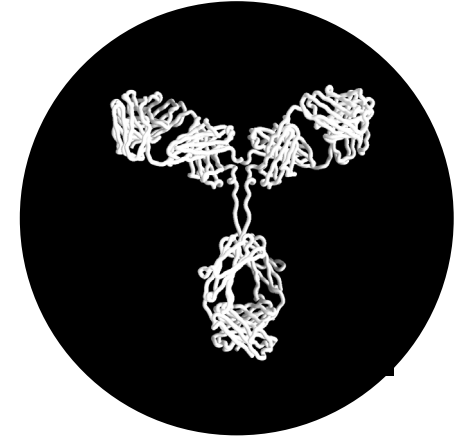
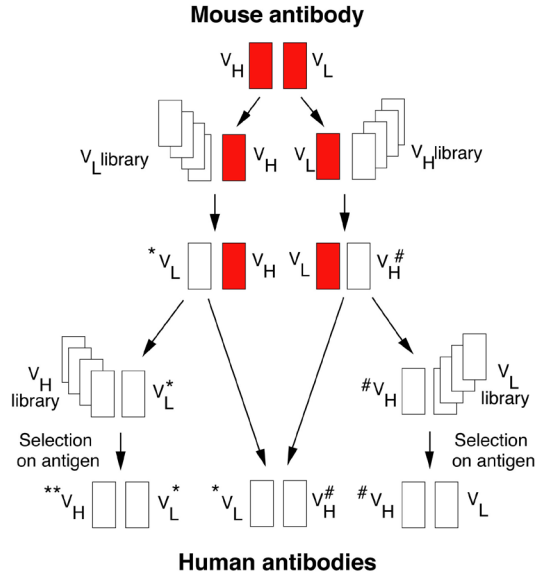
**Synthetic V-gene repertoires.** V-segment building blocks (Tomlinson, 1992; Williams 1994, Cox 1994): assembly into synthetic libraries (Hoogenboom 1992, Nissim 1994, Griffiths 1994)

**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

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**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

