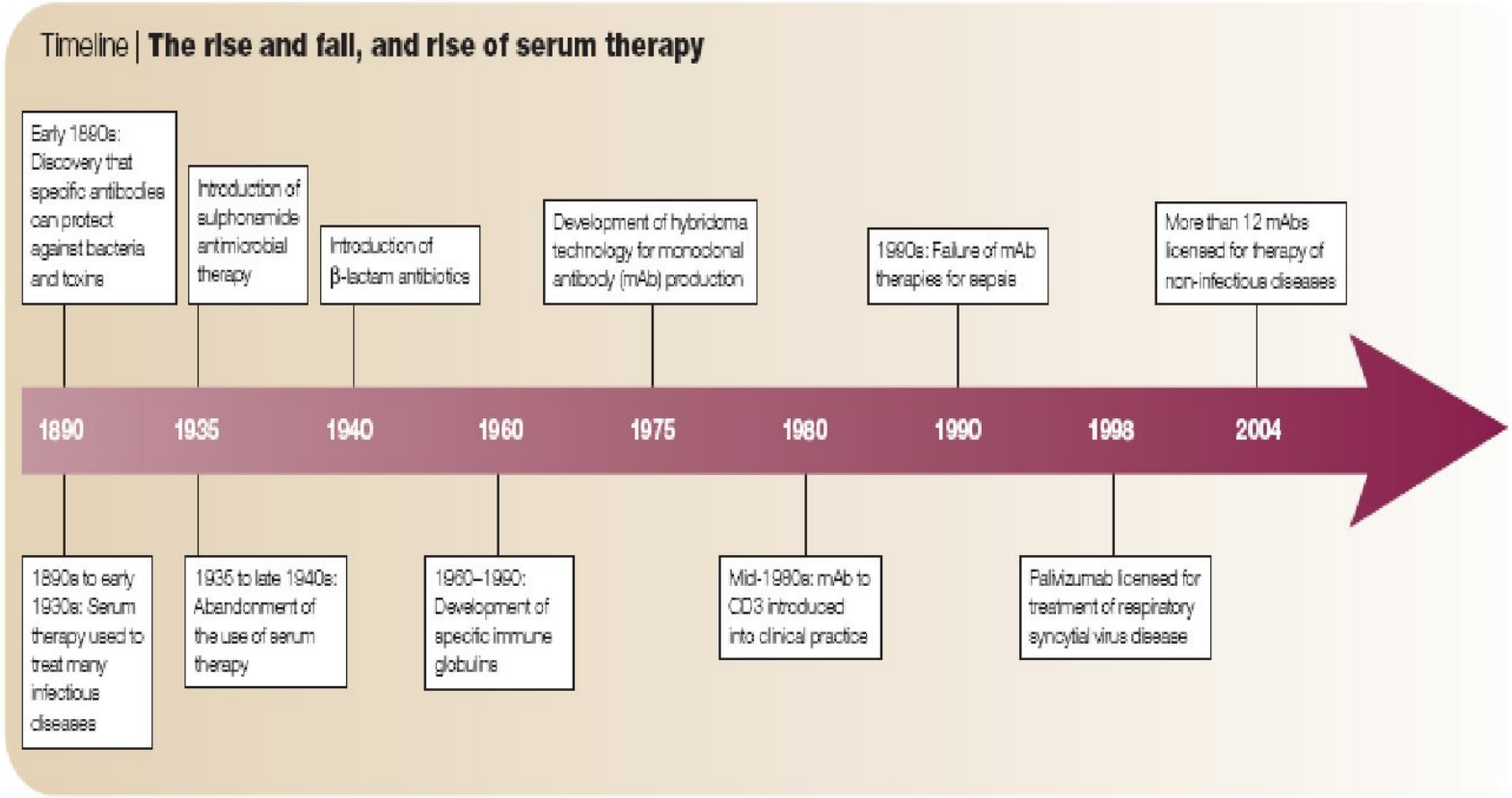
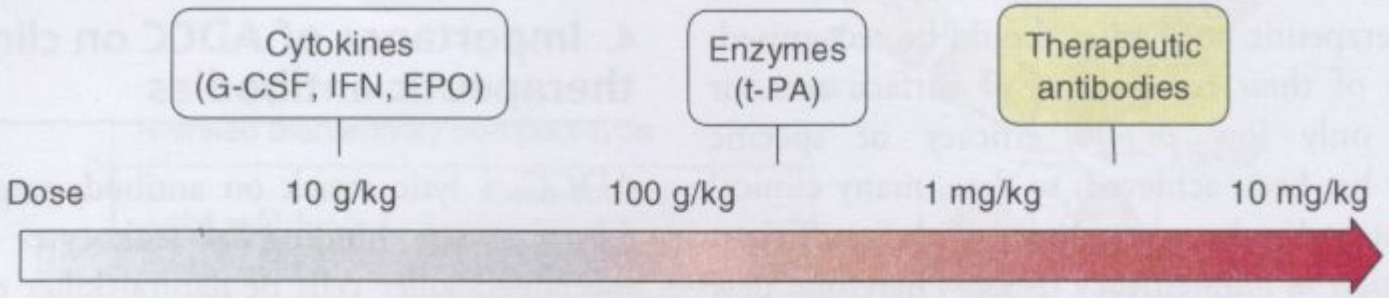
# Рекомбинантные антитела для диагностики и терапии


Timeline | The rise and fall, and rise of serum therapy
Early 1890s: Discovery that specific antibodies can protect against bacteria and toxins
Introduction of sulphonamide antimicrobial therapy
Introduction of β-lactam antibiotics
Development of hybridoma technology for monoclonal antibody (mAb) production
1990s: Failure of mAb therapies for sepsis
More than 12 mAbs licensed for therapy of non-infectious diseases
1890
1935
1940
1960
1975
1980
1990
1998
2004
1890s to early 1930s: Serum therapy used to treat many infectious diseases
1935 to late 1940s: Abandonment of the use of serum therapy
1960–1990: Development of specific immune globulins
Mid-1980s: mAb to CD3 introduced into clinical practice
Palivizumab licensed for treatment of respiratory syncytial virus disease

**Administrated dose/patient**




**Amount of drug products required for patients/year**

| Product | Company | Dose/year (g) | No. patients | Demand in 2002 (kg) |
|---|---|---|---|---|
| Erbitux® | ImClone/BMS/Merck | 6.0 | 5000 | 30 |
| Rituxan® | IDEC/Genentech/Roche | 4.3 | 60,000 | 255 |
| Herceptin® | Genentech/Roche | 3.5 | 26,000 | 91 |
| Enbrel® | Immunex/AHP | 2.5 | 100,000 | 250 |
| Remicade® | Centocor/J&J | 1.8 | 100,000 | 180 |
| Cerzyme® | Genzyme | 1.0 | 3100 | 3 |
| Epogen®/Procrit® | Amgen/J&J | 0.08 | 500,000 | 3 |

Table 2 | **Microorganisms against which antibody has been used to target human diseases***

| Microorganism | Disease in humans | References |
|---|---|---|
| *Bacillus anthracis* | Anthrax | 50 |
| *Bordetella pertussis* | Whooping cough | 51 |
| *Clostridium tetani* | Tetanus | 52 |
| *Clostridium botulinum* | Botulism | 53 |
| *Cryptococcus neoformans* | Cryptococcosis | 54 |
| *Cryptosporidium parvum* | Cryptosporidiosis | 55 |
| Enterovirus | Gastrointestinal-tract infections | 56 |
| Group A streptococci | Several illnesses including sore throats, necrotizing fasciitis | 57 |
| Hepatitis B virus | Hepatitis B | 58 |
| Measles virus | Measles | 59 |
| *Mycobacterium tuberculosis* | Tuberculosis | 60 |
| *Neisseria meningitidis* | Meningitis | 2,61 |
| Parvovirus | Aplastic anaemia | 62 |
| Rabies virus | Rabies | 63 |
| Respiratory syncytial virus (RSV) | RSV infection | 64 |
| *Streptococcus pneumoniae* | Pneumonia | 2 |
| Varicella–zoster virus | Shingles, chickenpox, pneumonia | 65 |
| Variola major | Smallpox | 66 |

*This is not a complete list.

**АНТИТЕЛО** - БЕЛОК СЫВОРОТКИ КРОВИ, ВЫРАБАТЫВАЮЩИЙСЯ В ОТВЕТ НА ВВЕДЕНИЕ АНТИГЕНА, КОМПЛЕМЕНТАРНЫЙ «СВОЕМУ» АНТИГЕНУ И СПОСОБНЫЙ СПЕЦИФИЧЕСКИ С НИМ СВЯЗЫВАТЬСЯ.

**АНТИГЕН** – ЛЮБАЯ СУБСТАНЦИЯ, КОТОРАЯ СПОСОБНА СВЯЗЫВАТЬСЯ С АНТИТЕЛАМИ.

**АНТИГЕННАЯ ДЕТЕРМИНАНТА (ЭПИТОП)** – ФРАГМЕНТ СТРУКТУРЫ АНТИГЕНА, С КОТОРЫМ СВЯЗЫВАЕТСЯ АНТИТЕЛО.

**ГАПТЕН** - НИЗКОМОЛЕКУЛЯРНОЕ СОЕДИНЕНИЕ, НЕ ОБЛАДАЮЩЕЕ АНТИГЕНЫИМИ СВОЙСТВАМИ, НО ВЫЗЫВАЮЩЕЕ ВЫРАБОТКУ АНТИТЕЛ ПРИ КОНЪЮГАЦИИ С БЕЛКАМИ .

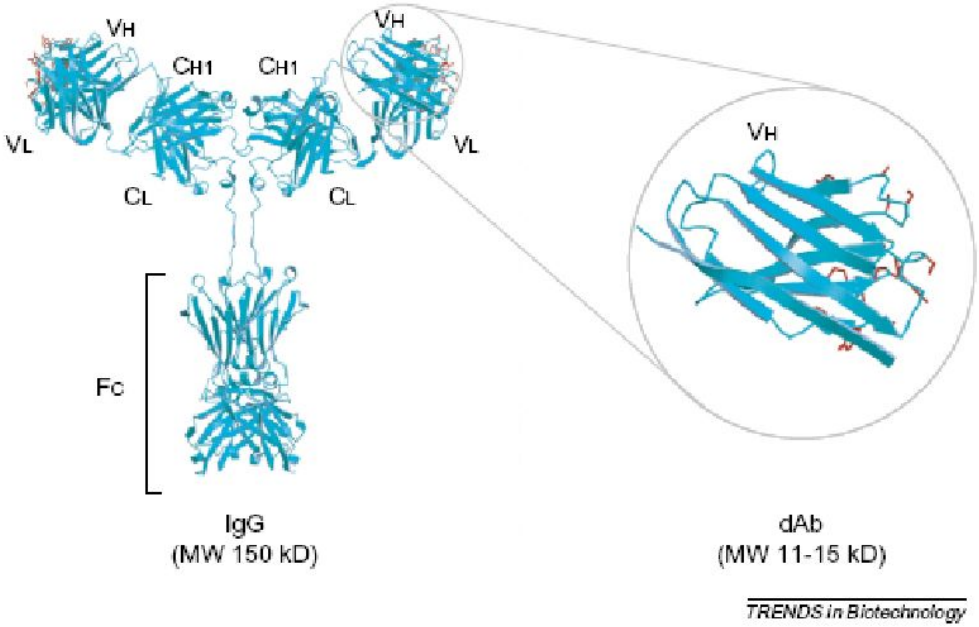
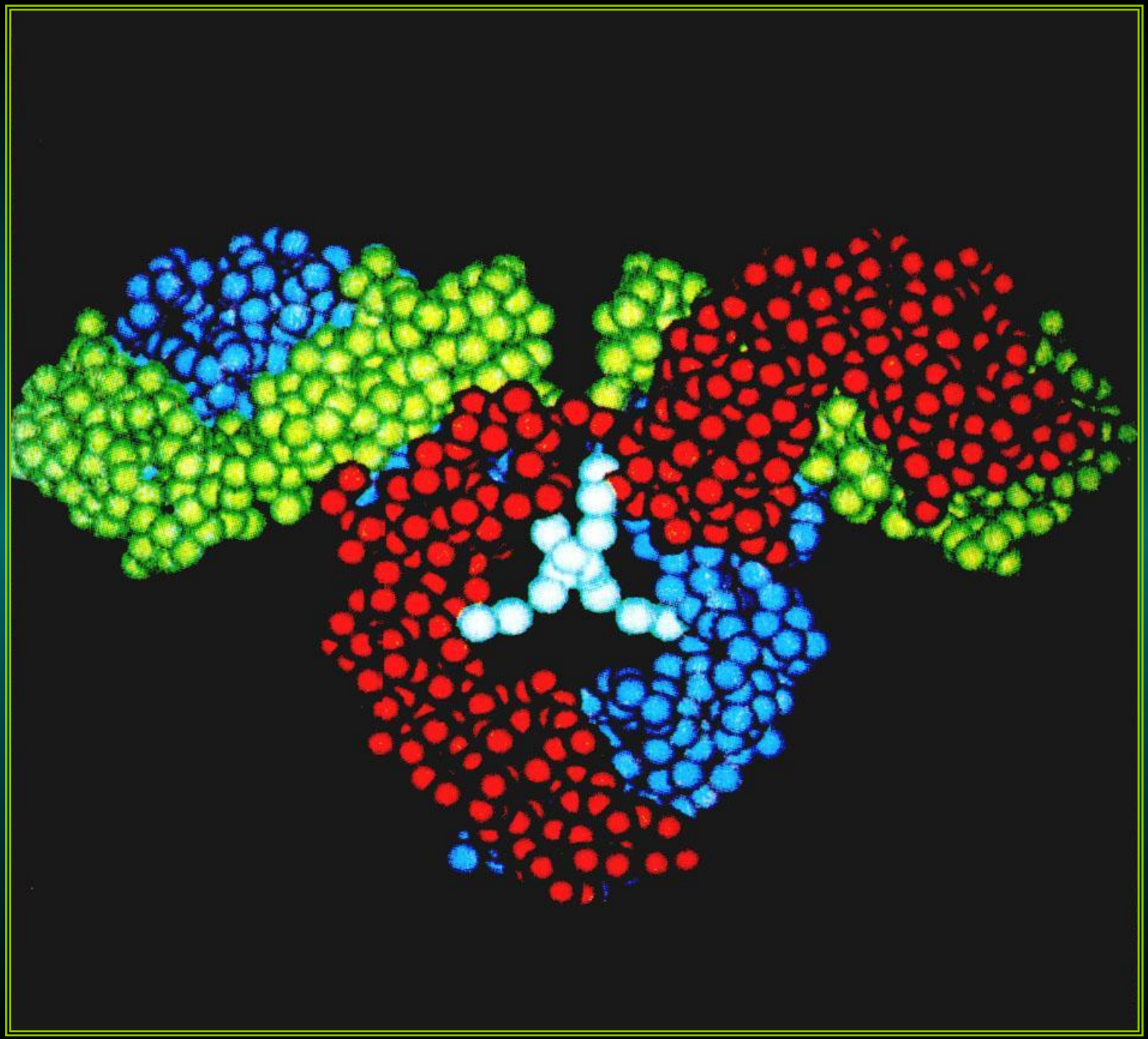
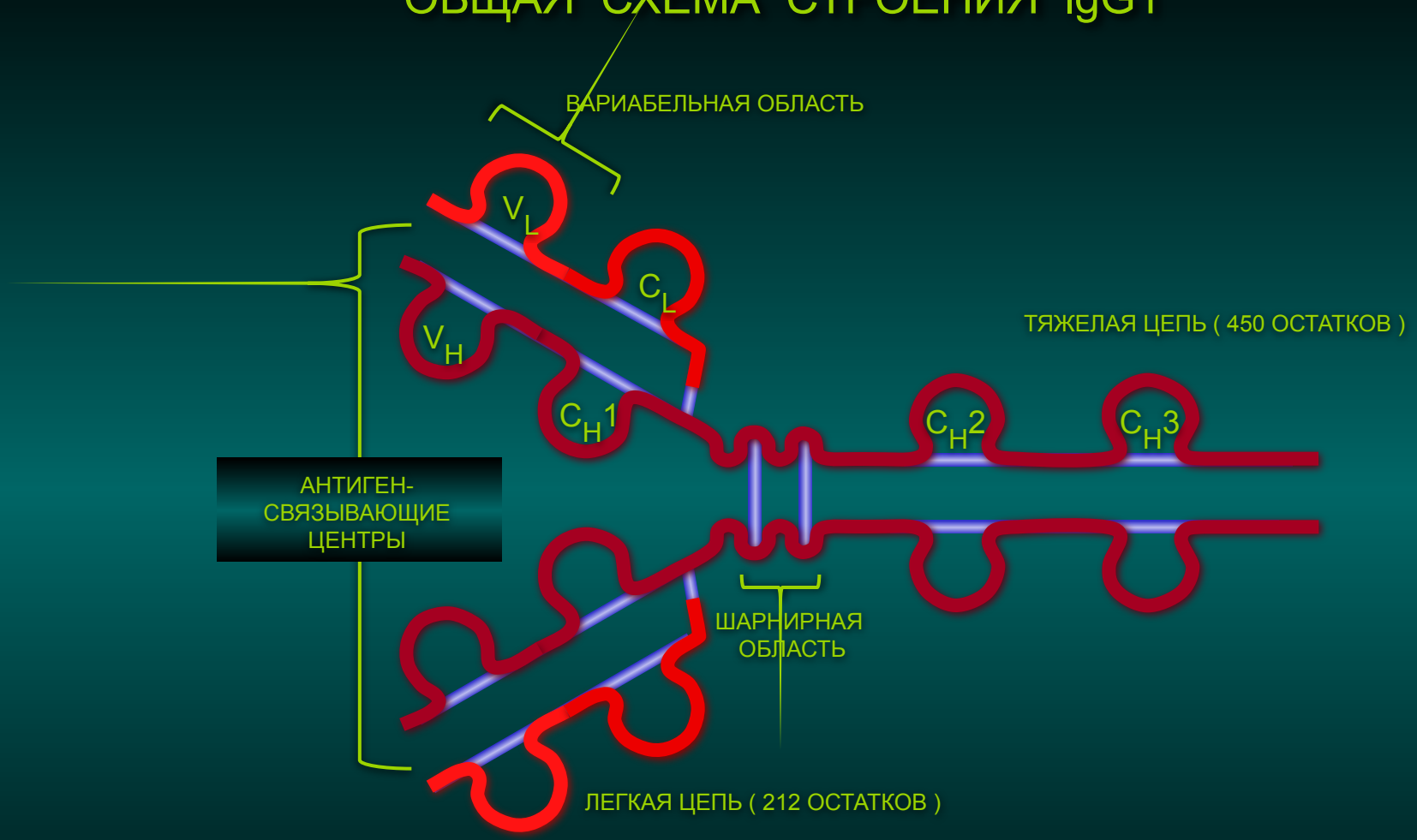


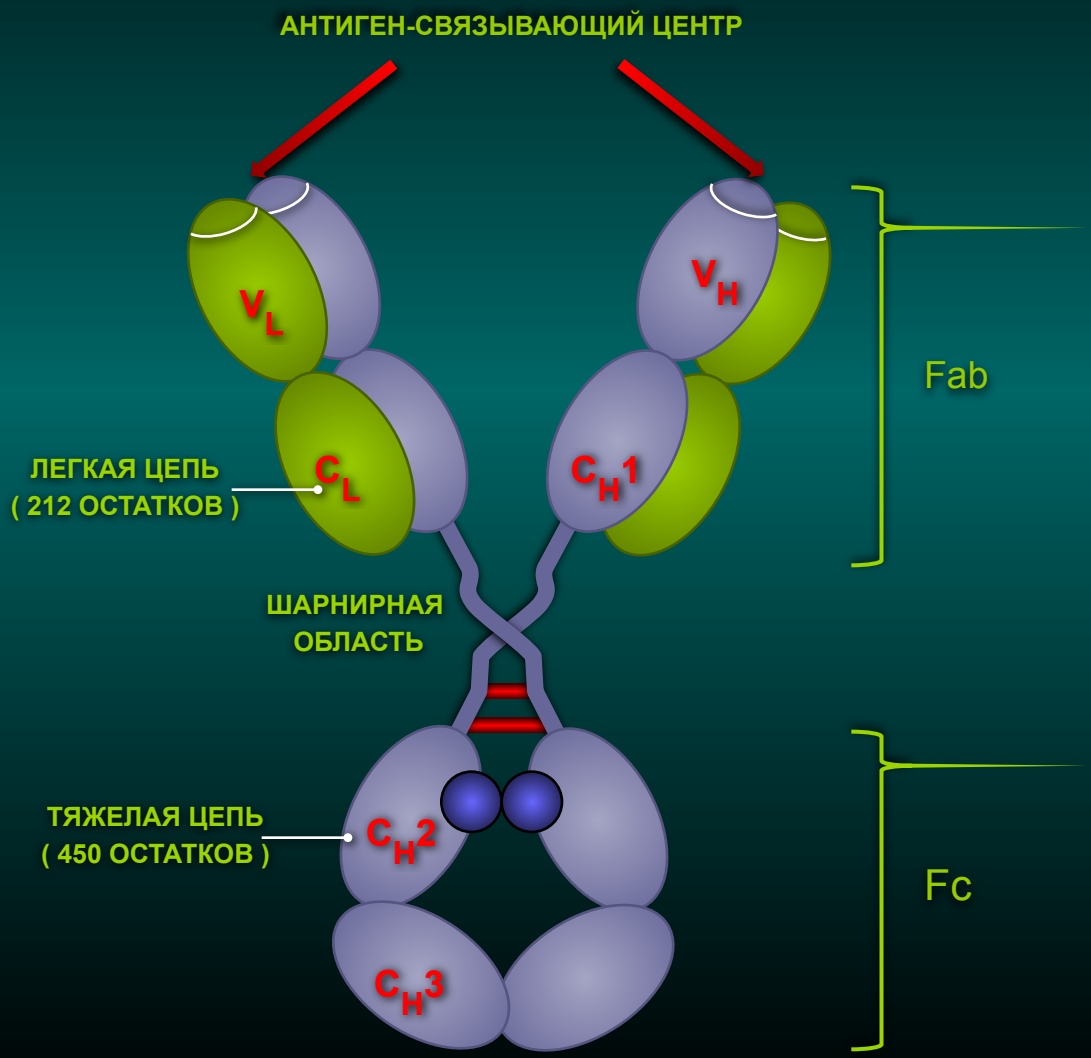

Figure 1. A human IgG molecule has both variable and constant regions. An IgG contains two variable regions (each composed of a $V_H$ and $V_L$ domain) that confer antigen-binding specificity on the antibody, and an Fc fragment in the constant region that recruits the effector functions of the immune system. Conventional recombinant antibody fragments contain one antigen-binding $V_H$–$V_L$ pairing. At ~57 kDa, a Fab fragment comprises a $V_H$–$C_H1$ polypeptide disulphide-bonded to a $V_L$–$C_L$ polypeptide. At ~27 kDa, a scFv fragment contains only the $V_H$ domain fused to the $V_L$ domain via a polypeptide linker. By contrast, the domain antibody, or dAb, of 11–15 kDa is either an isolated antibody $V_H$ domain [2], as shown here, or an isolated antibody $V_L$ domain [15]. Each dAb thus contains three of the six naturally occurring complementarity determining regions (CDRs) from a $V_H$–$V_L$ pairing. The side chains of the CDRs are highlighted in red.

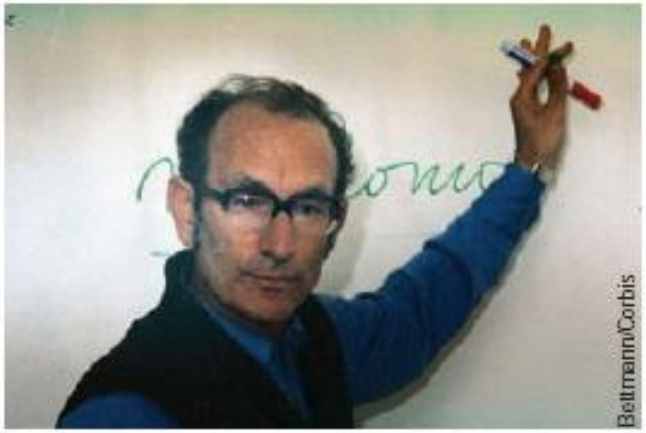


# ОБЩАЯ СХЕМА СТРОЕНИЯ IgG1

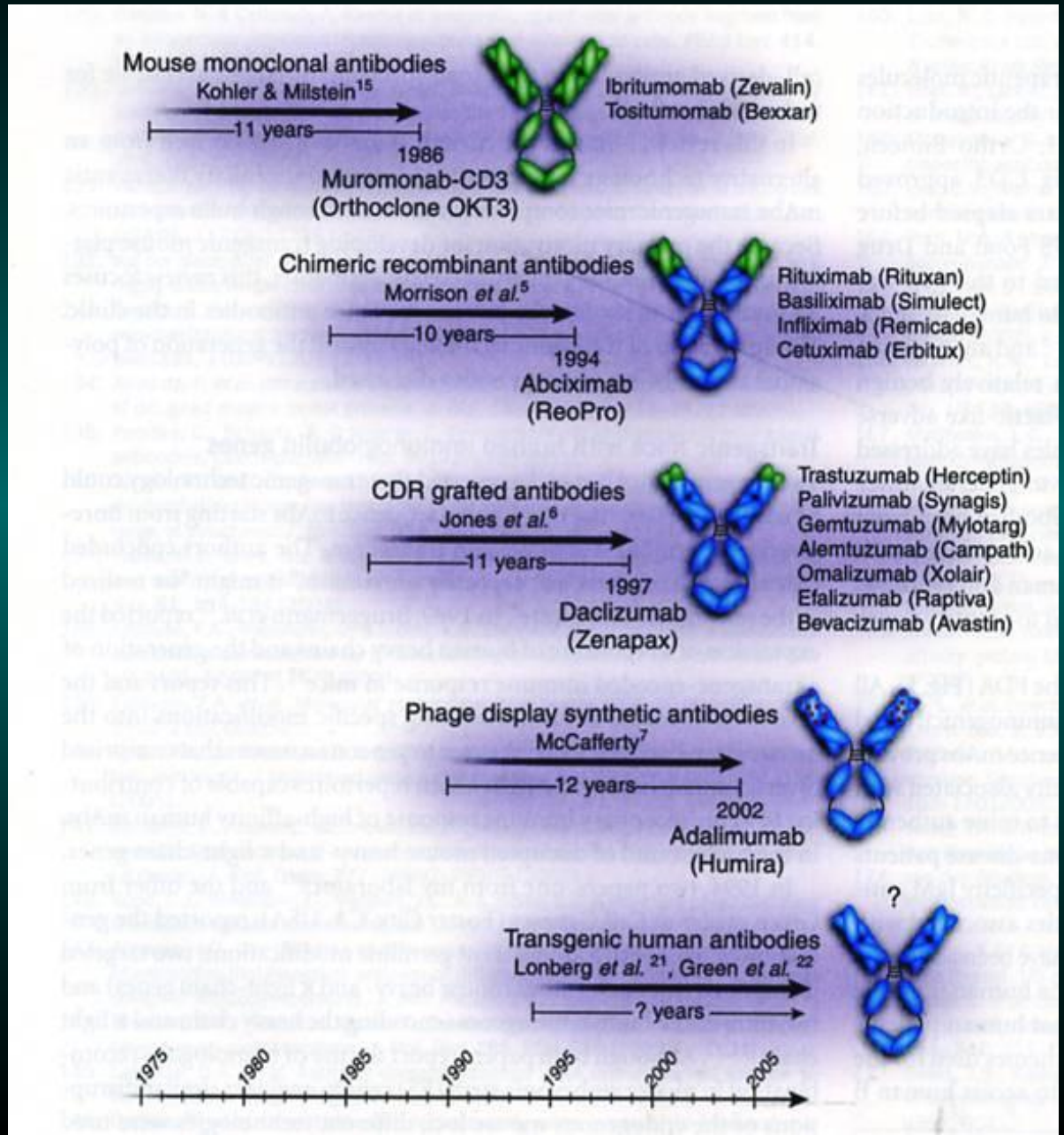


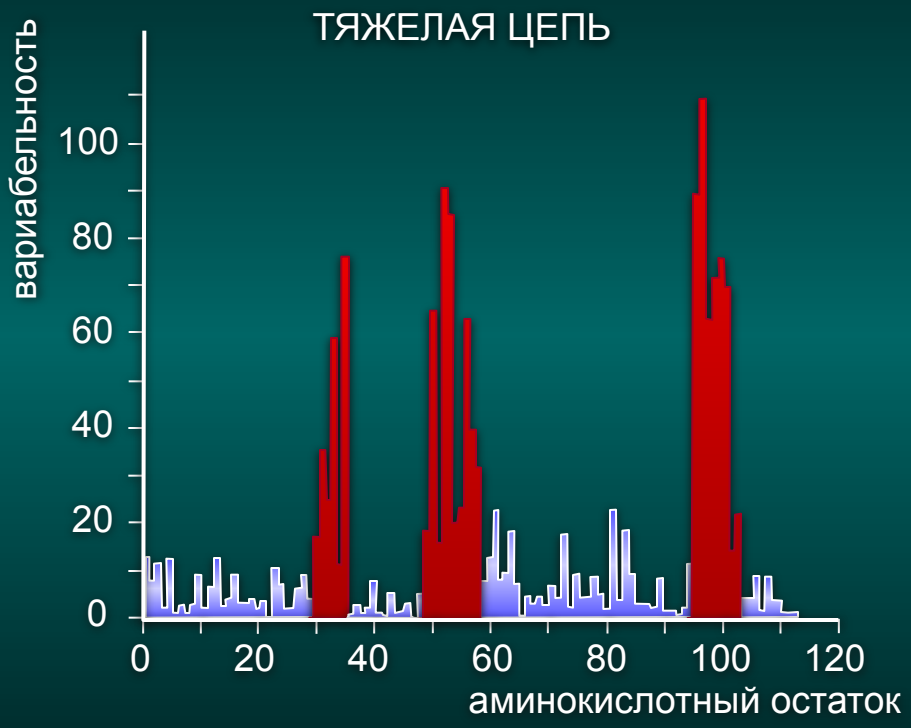
# СТРУКТУРА IgG1

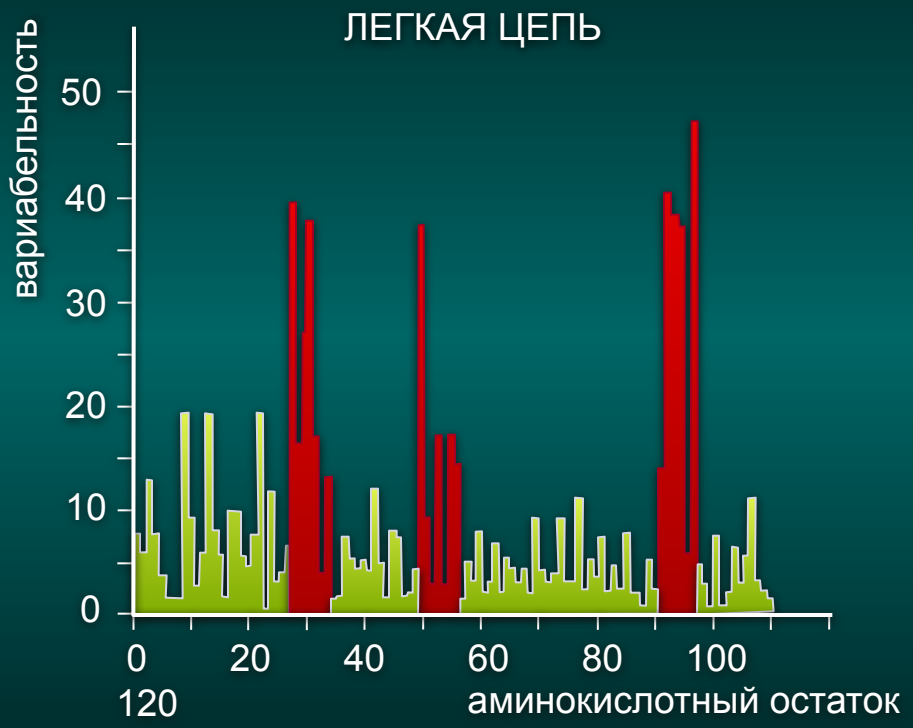





César Milstein, who together with Georges Kohler reported the creation of hybridomas to produce mAbs[1]. Milstein's decision not to patent the discovery, for which both Milstein and Kohler received the Nobel Prize in Physiology or Medicine in 1984, contributed to the rapid and wide dissemination of mAb technology.




ТЯЖЕЛАЯ ЦЕПЬ
вариабельность
0
20
40
60
80
100
0
20
40
60
80
100
120
аминокислотный остаток


ЛЕГКАЯ ЦЕПЬ
вариабельность
0
10
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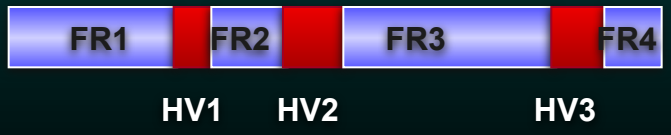
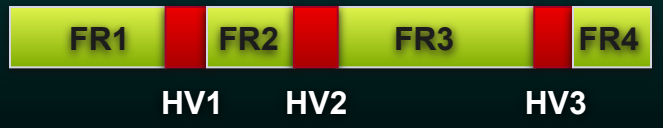
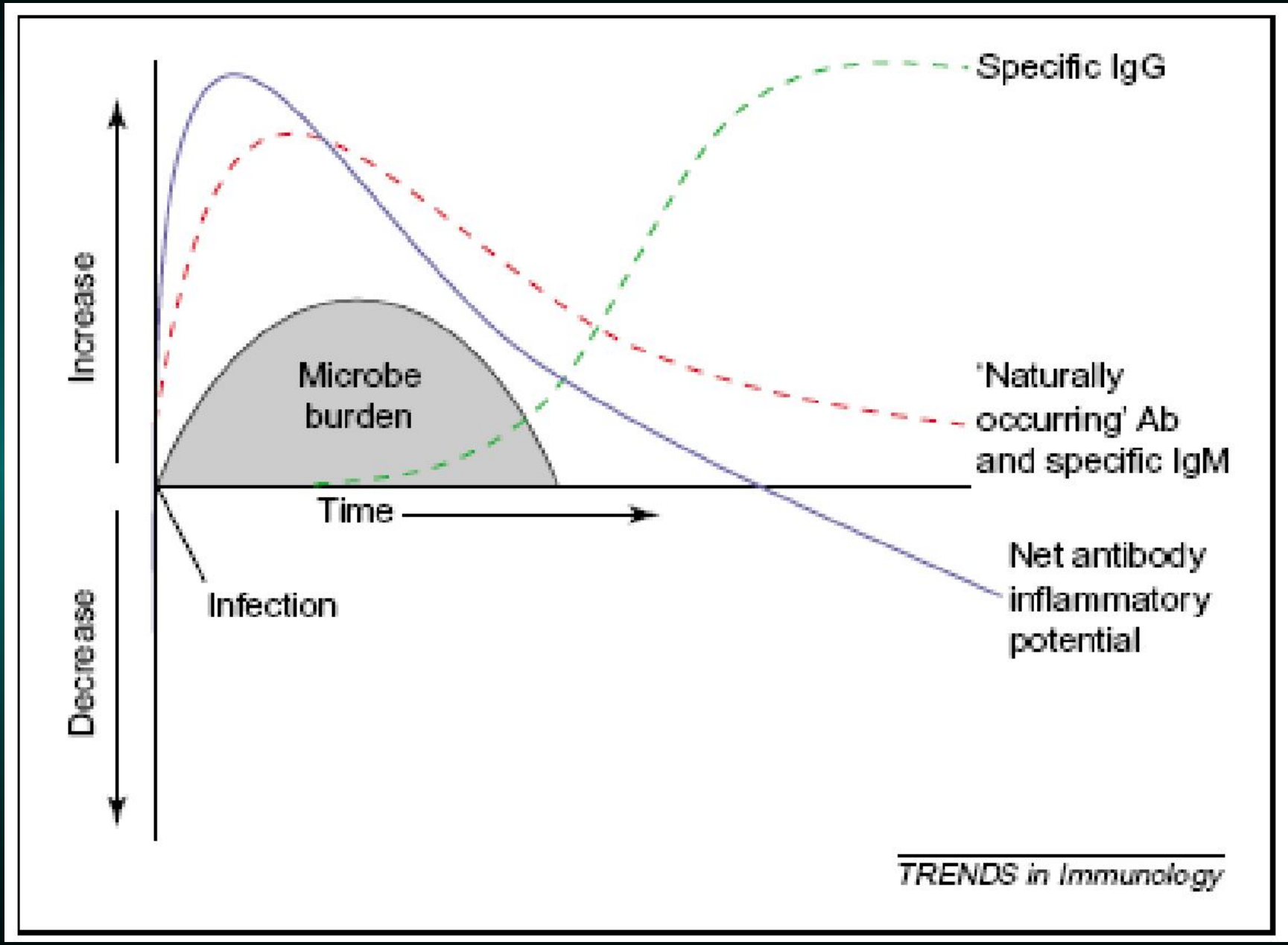
30
40
50
0
20
40
60
80
100
120
аминокислотный остаток


FR1
FR2
FR3
FR4
HV1
HV2
HV3


FR1
FR2
FR3
FR4
HV1
HV2
HV3

| | ИММУНОГЛОБУЛИН | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IgG1 | IgG2 | IgG3 | IgG4 | IgM | IgA1 | IgA2 | IgD | IgE |
| Тяжелая цепь | $\gamma_1$ $\varepsilon$ | $\gamma_2$ | $\gamma_3$ | $\gamma_4$ | $\mu$ | $\alpha_1$ | $\alpha_2$ | $\delta$ | |
| Молекулярная масса (кД) | 146 188 | 146 | 165 | 146 | 970 | 160 | 160 | 184 | |
| Содержание в сыворотке крови(мг/мл) | 9 $5x10^{-5}$ | 3 | 1 | 0.5 | 1.5 | 3.0 | 0.5 | 0.03 | |
| Время полужизни в кровотоке (дни) | 21 2 | 20 | 7 | 21 | 10 | 6 | 6 | 3 | |
| Активация классического пути комплемента | ++ | + | +++ | - | +++ | - | - | - | - |
| Активация альтернативного пути комплемента | - | - | - | - | - | + | - | - | - |
| Перенос через плаценту | +++ | + | ++ | - / + | - | - | - | - | - |
| Связывание с макрофагами | + | - | + | - | - | + | + | - | + |
| Связывание с тучными клетками и базофилами | + | - | + | - | - | + | + | - | +++ |


Specific IgG
Increase
Microbe burden
'Naturally occurring' Ab and specific IgM
Time
Net antibody inflammatory potential
Infection
Decrease
TRENDS in Immunology

# Эффекторные функции антител

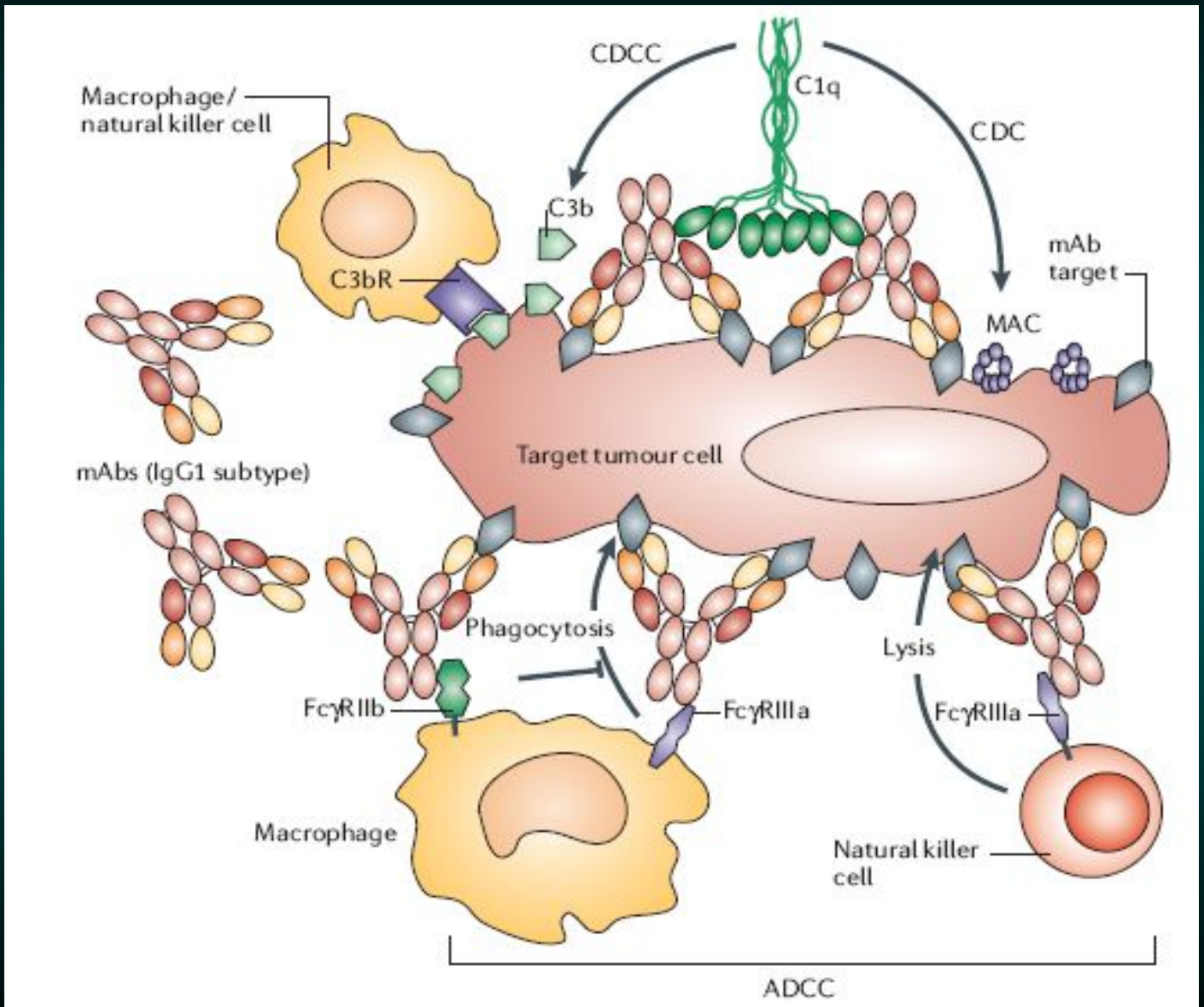
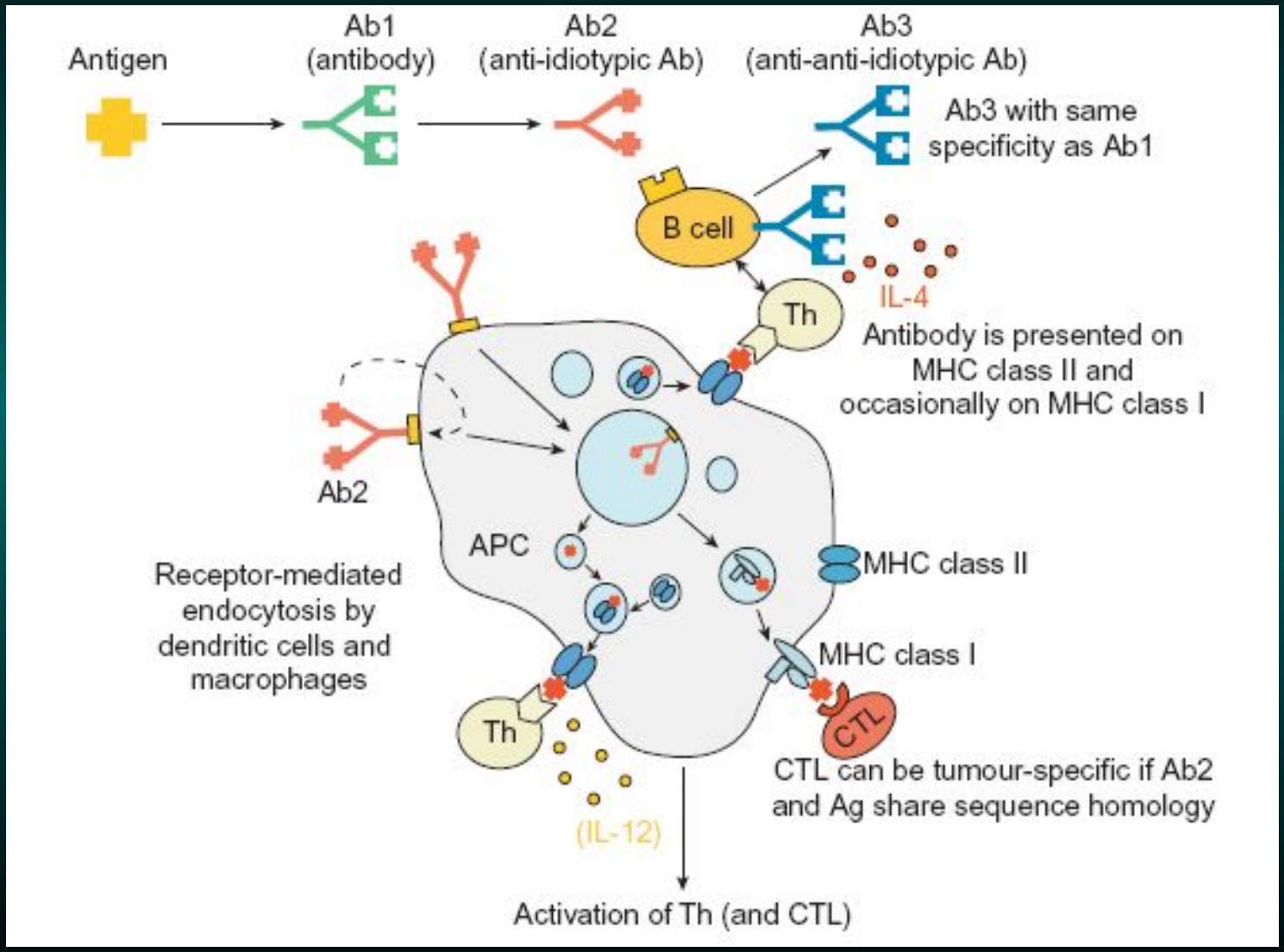



Figure 2 | **Schematic model of antibody action by immune mechanisms.**

# *Активация иммунного ответа через антиидиотипические антитела*



# Биологическая активность антител

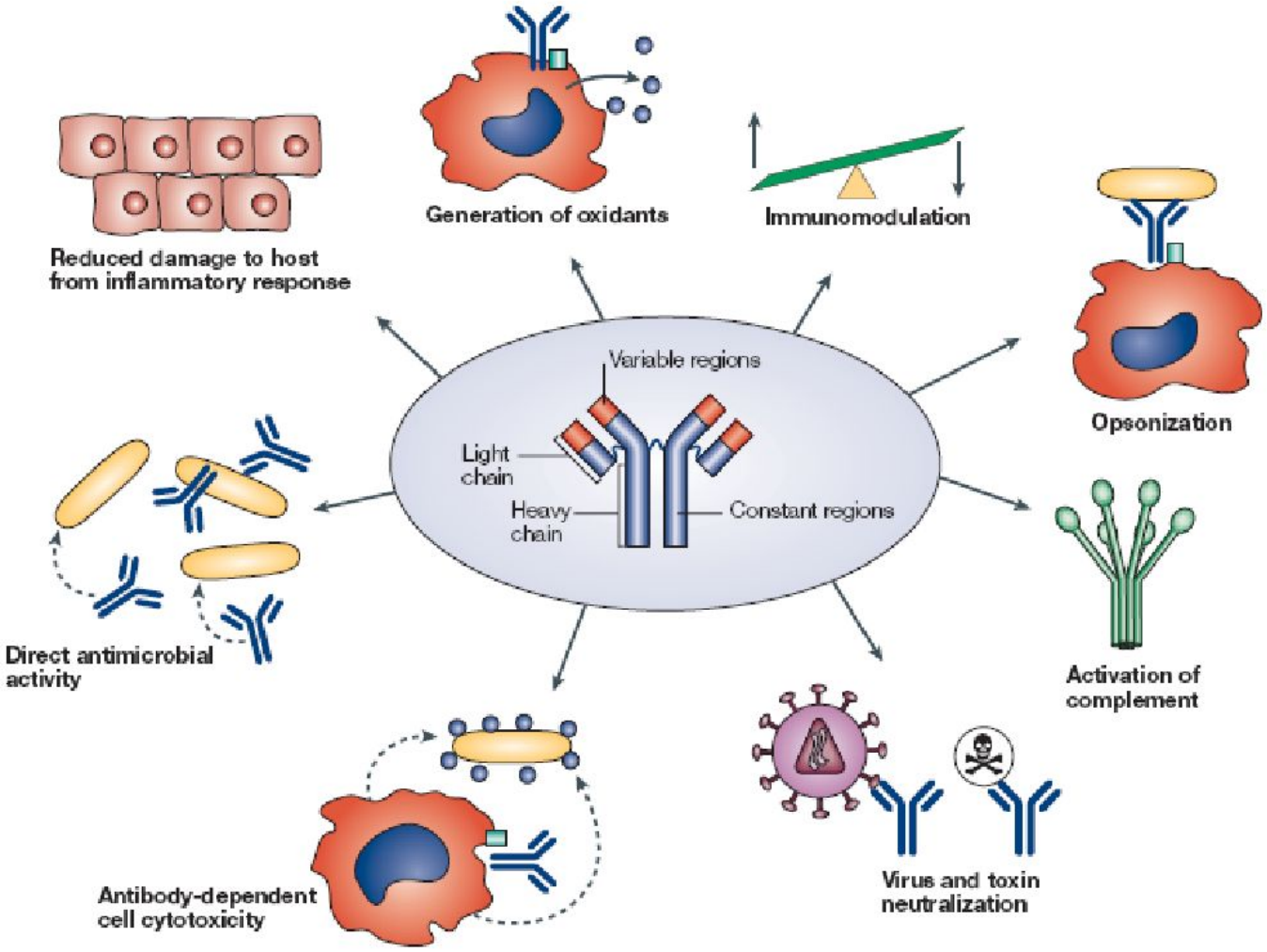



Figure 1 | **The different biological effects of antibodies.** Toxin and virus neutralization, complement activation and direct antimicrobial functions such as the generation of oxidants are independent of other components of the host immune system, whereas antibody-dependent cellular cytotoxicity and opsonization depend on other host cells and mediators.

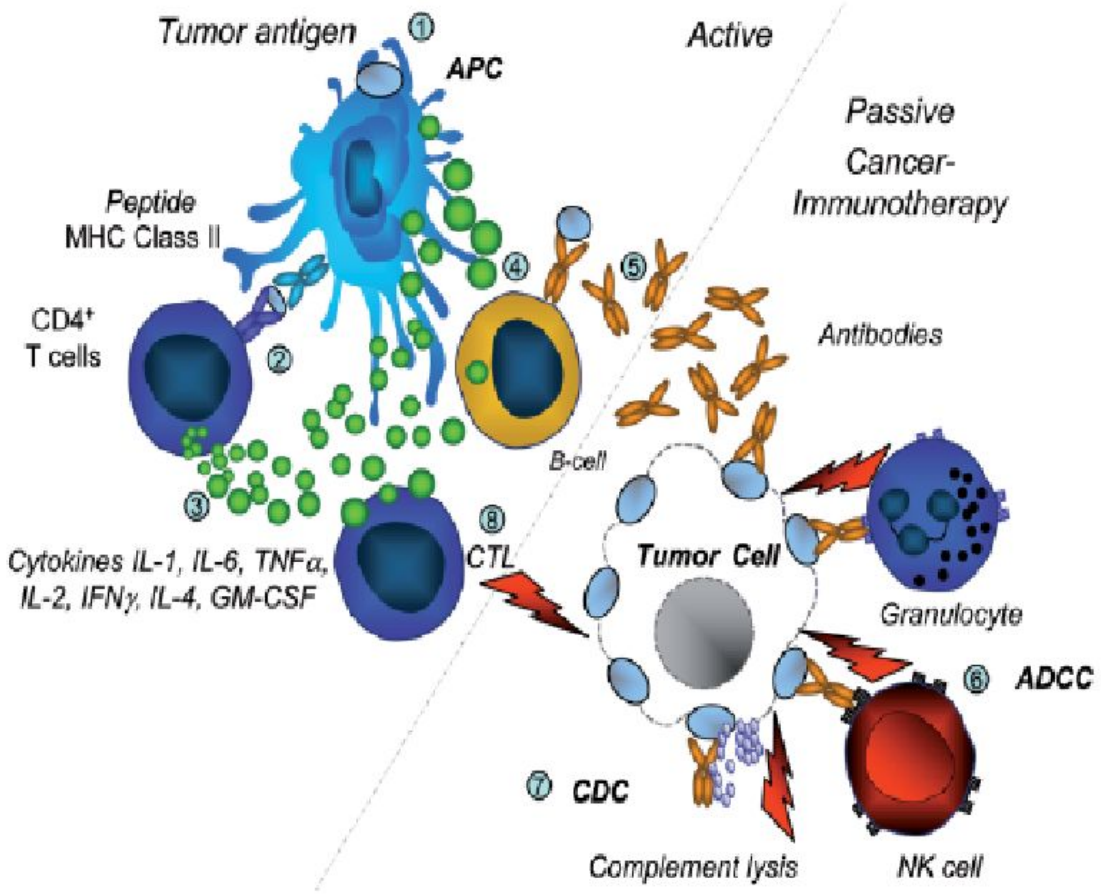



**Figure 1.** Cancer Immunotherapy. The whole spectrum of passive and active cancer immunotherapy is summarized. Active cancer immunotherapy comprises tumor antigen uptake by APCs (1), epitope (peptide) presentation to $CD4^+$ T cells (2), cytokine release (3), B cell activation (4), and antibody production (5), leading to lysis of tumor cells including different (passive) alternatives like ADCC (6), CDC (7) or unspecific attack by cytotoxic T lymphocytes (8).

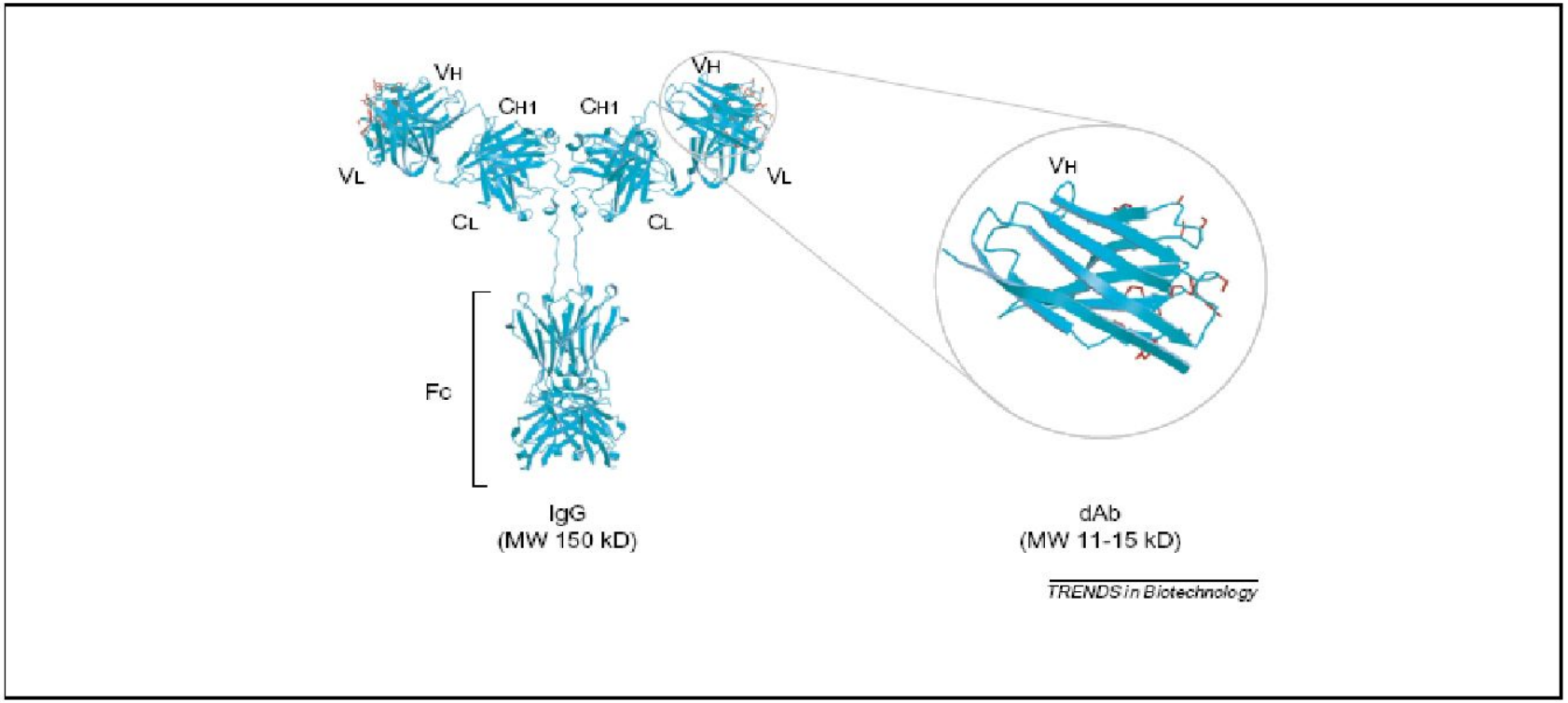



**Figure 1.** A human IgG molecule has both variable and constant regions. An IgG contains two variable regions (each composed of a $V_H$ and $V_L$ domain) that confer antigen-binding specificity on the antibody, and an Fc fragment in the constant region that recruits the effector functions of the immune system. Conventional recombinant antibody fragments contain one antigen-binding $V_H$–$V_L$ pairing. At ~57 kDa, a Fab fragment comprises a $V_H$–$C_H1$ polypeptide disulphide-bonded to a $V_L$–$C_L$ polypeptide. At ~27 kDa, a scFv fragment contains only the $V_H$ domain fused to the $V_L$ domain via a polypeptide linker. By contrast, the domain antibody, or dAb, of 11–15 kDa is either an isolated antibody $V_H$ domain [2], as shown here, or an isolated antibody $V_L$ domain [15]. Each dAb thus contains three of the six naturally occurring complementarity determining regions (CDRs) from a $V_H$–$V_L$ pairing. The side chains of the CDRs are highlighted in red.

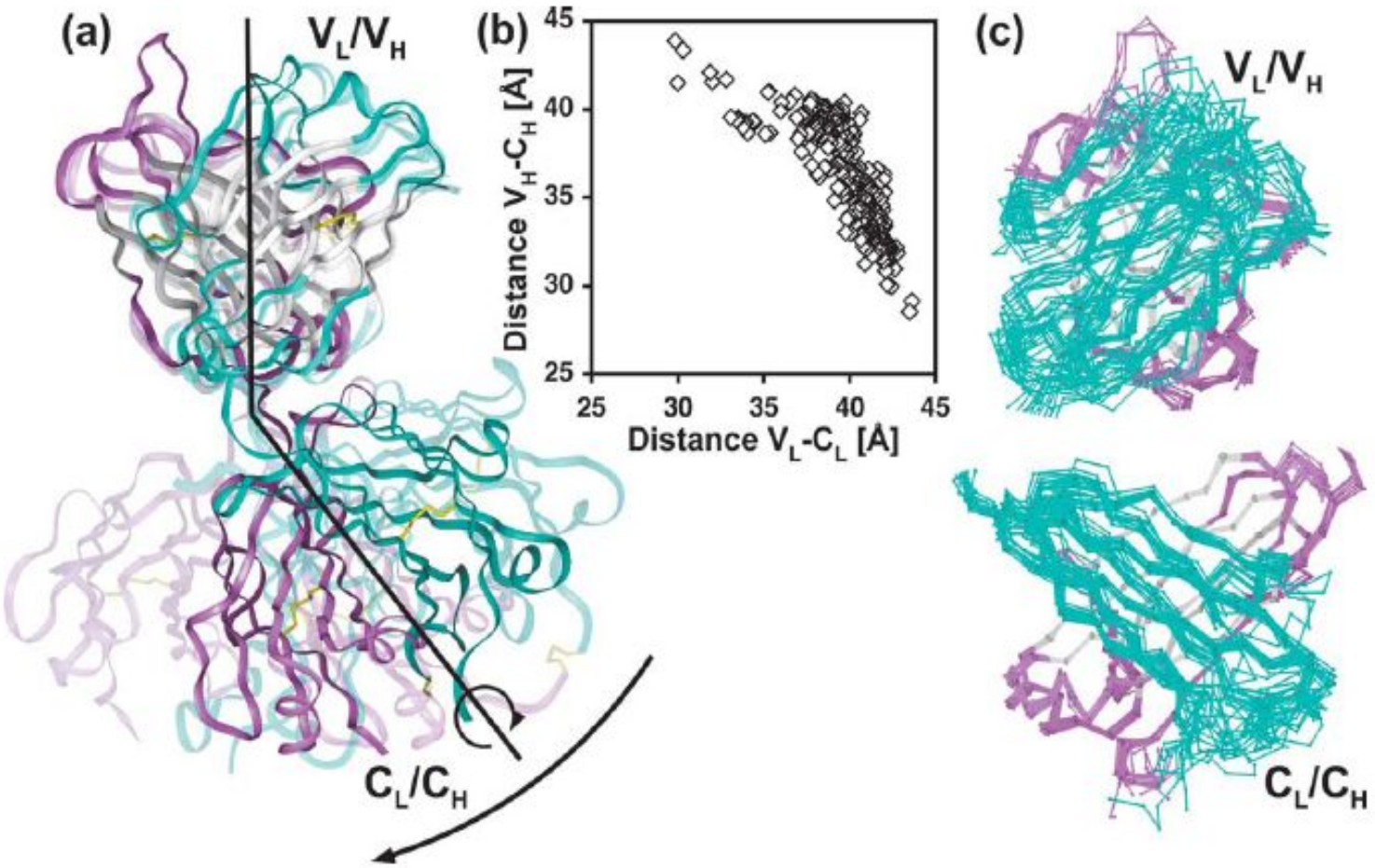
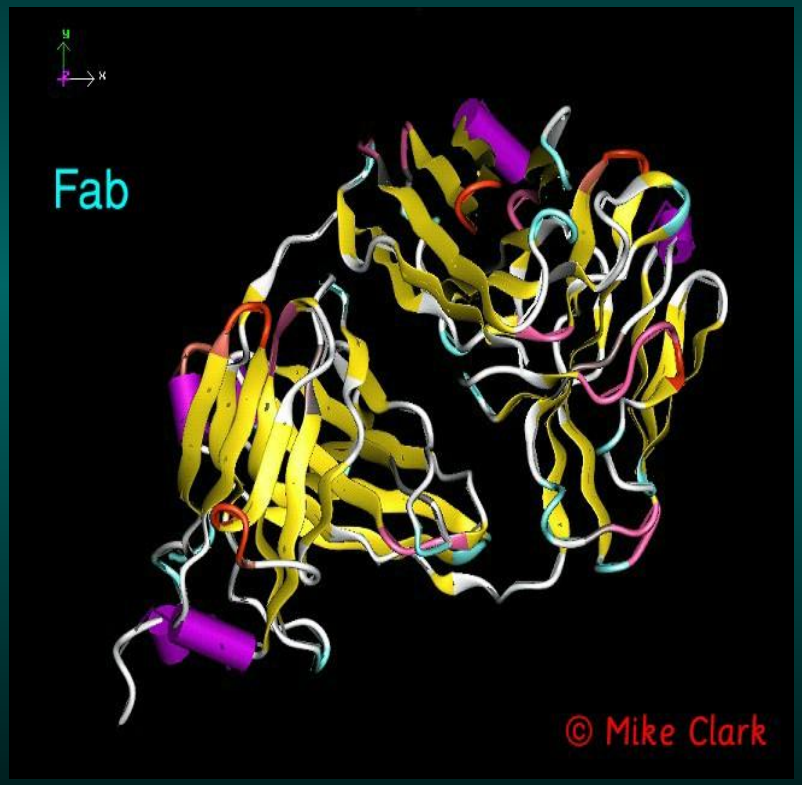
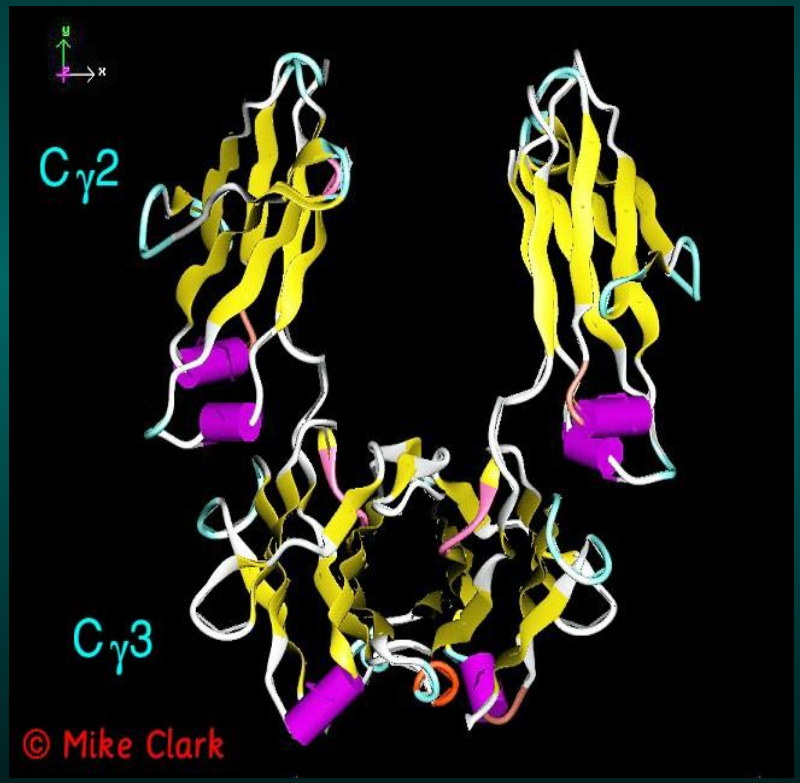
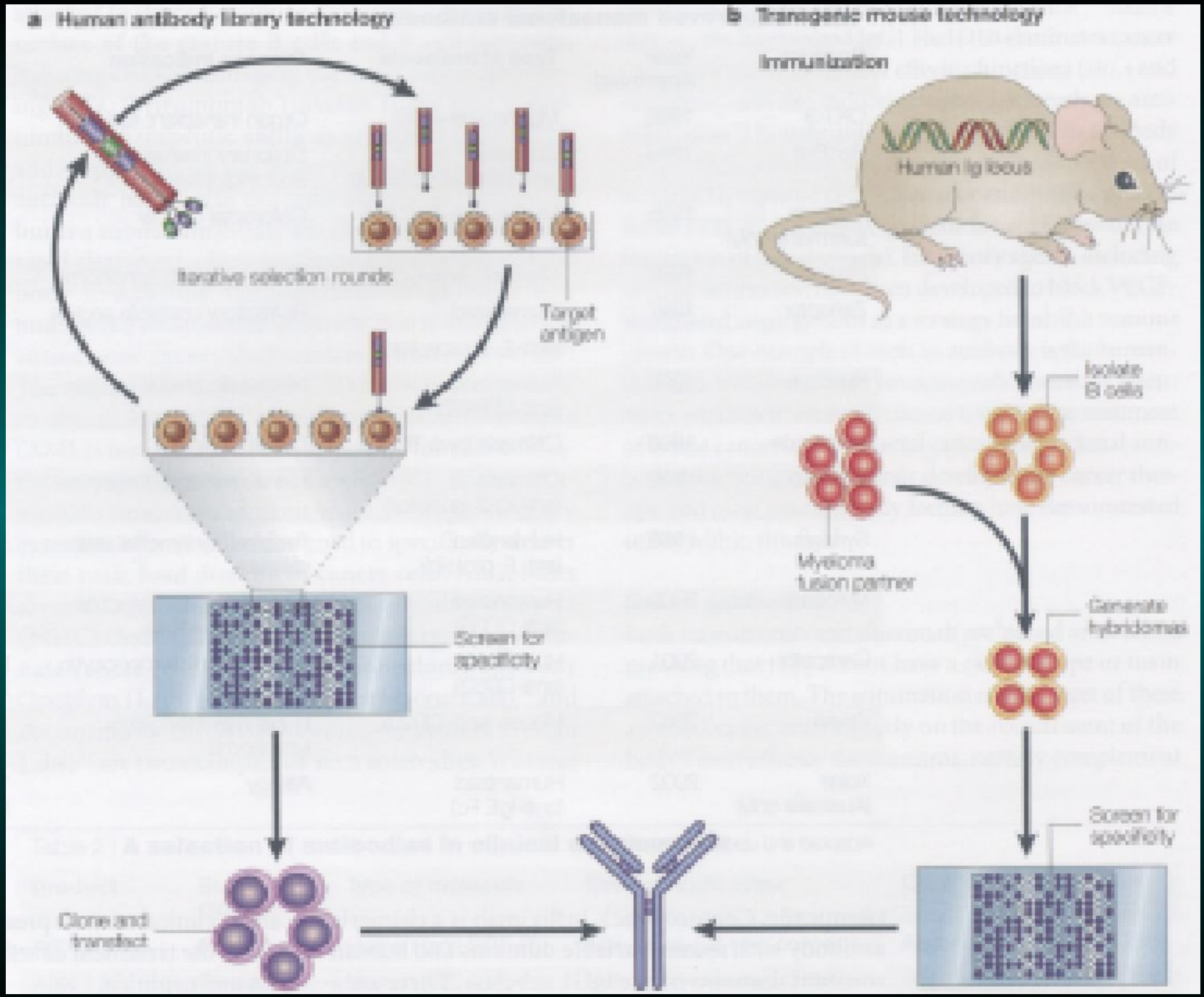


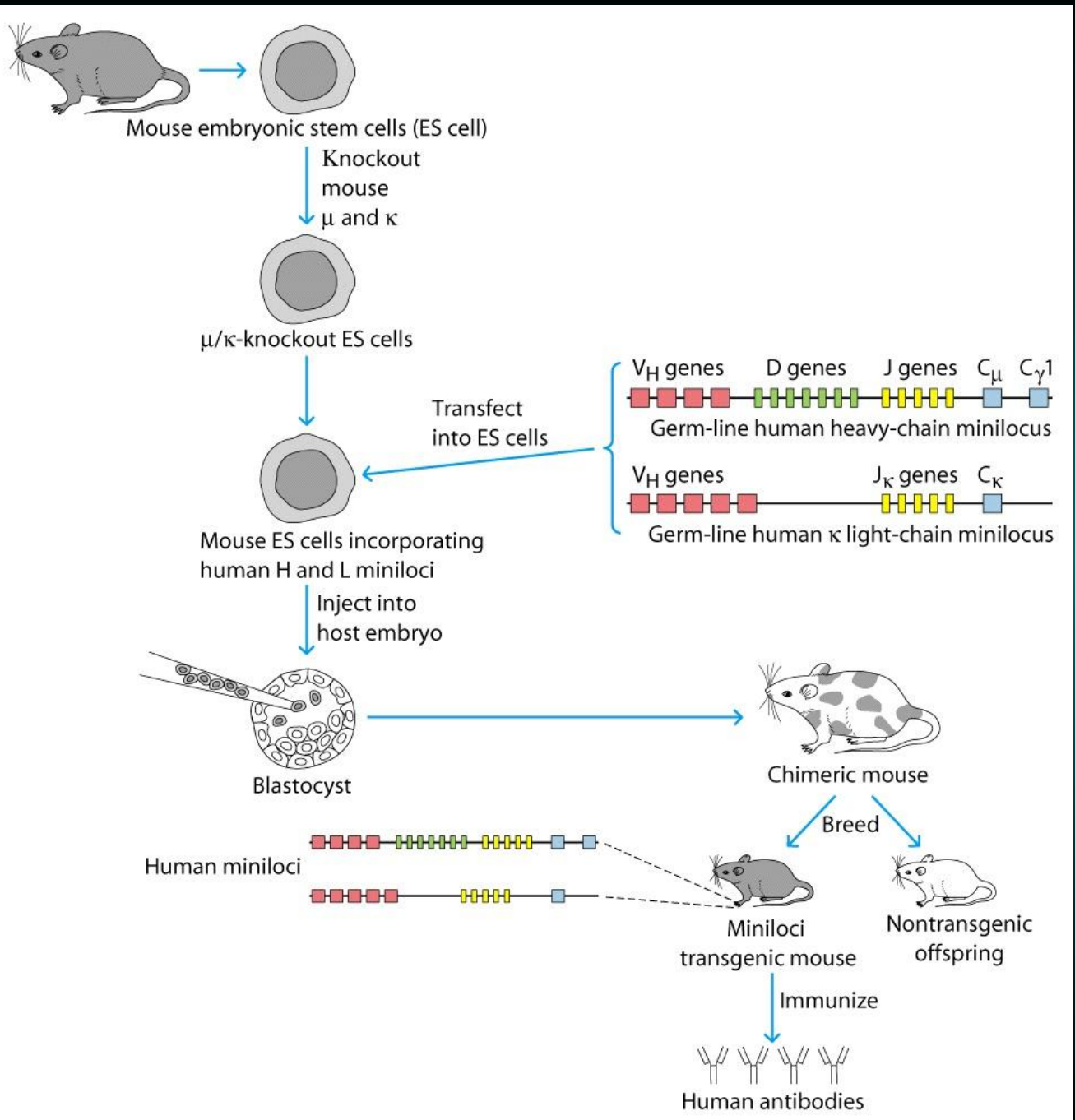

**Figure 7.** Flexibility of the relative orientations of the domains within the Fab fragment. (a) Three Fab fragments were superimposed by a least-squares fit of the structurally least variable $C^{\alpha}$ positions within the Fv part (indicated in white) to illustrate the flexibility of the variable/constant domain interface. (b) Plot of the distance between the centers of gravity of $V_H$ and $C_H1$ *versus* the distance between the centers of gravity of $V_L$ and $C_L$. Only the structurally conserved $C^{\alpha}$ positions (indicated by a gray background in Figure 6) were used for the calculation of the center of gravity. The observed anti-correlation is explained by a twist around the $C_H1/C_L$ pseudo 2-fold axis. (c) Limited flexibility of the $V_H/V_L$ and of the $C_H1/C_L$ interface, demonstrated by structurally aligning the $V_L$ and $C_L$ domains, respectively. From the comparison of (a) and (c), it becomes apparent that $V_H/V_L$ and $C_H1/C_L$ are each moving as a unit.

# Трехмерные модели Fab и Fc

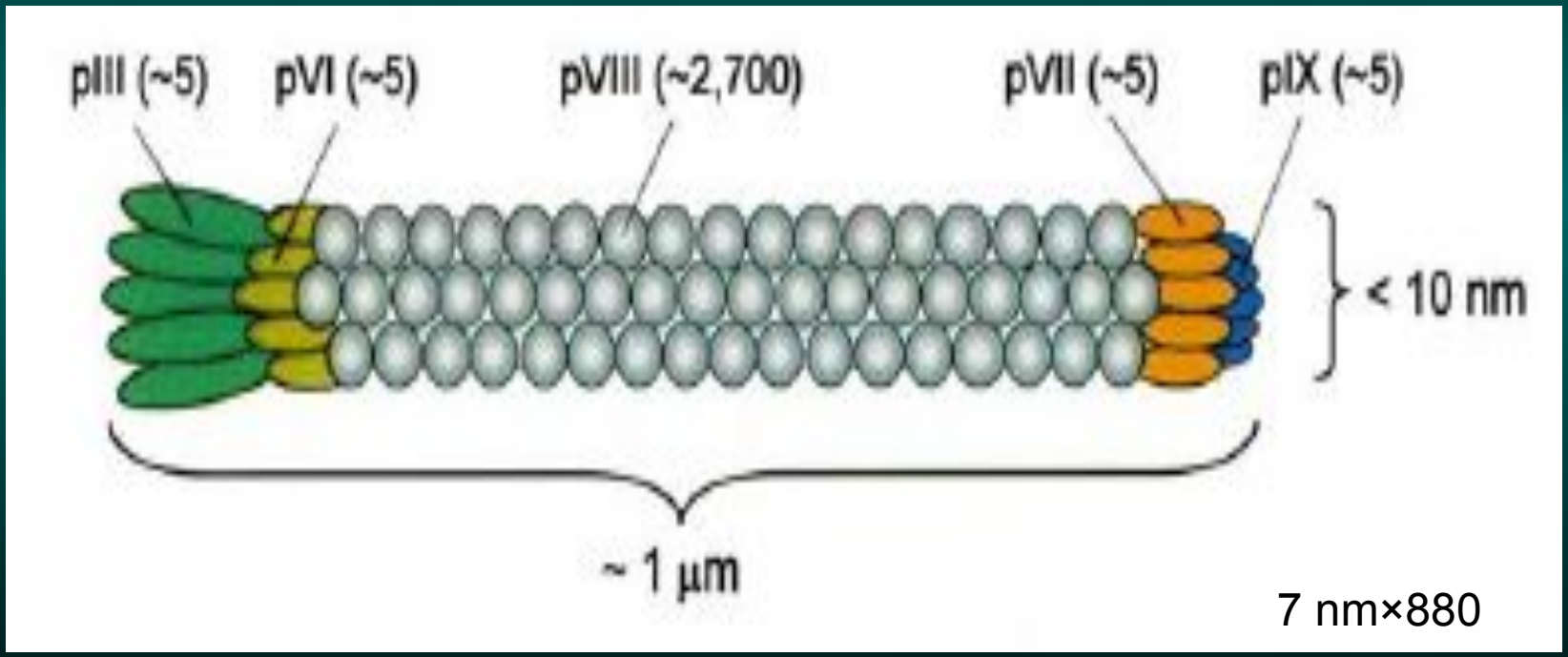


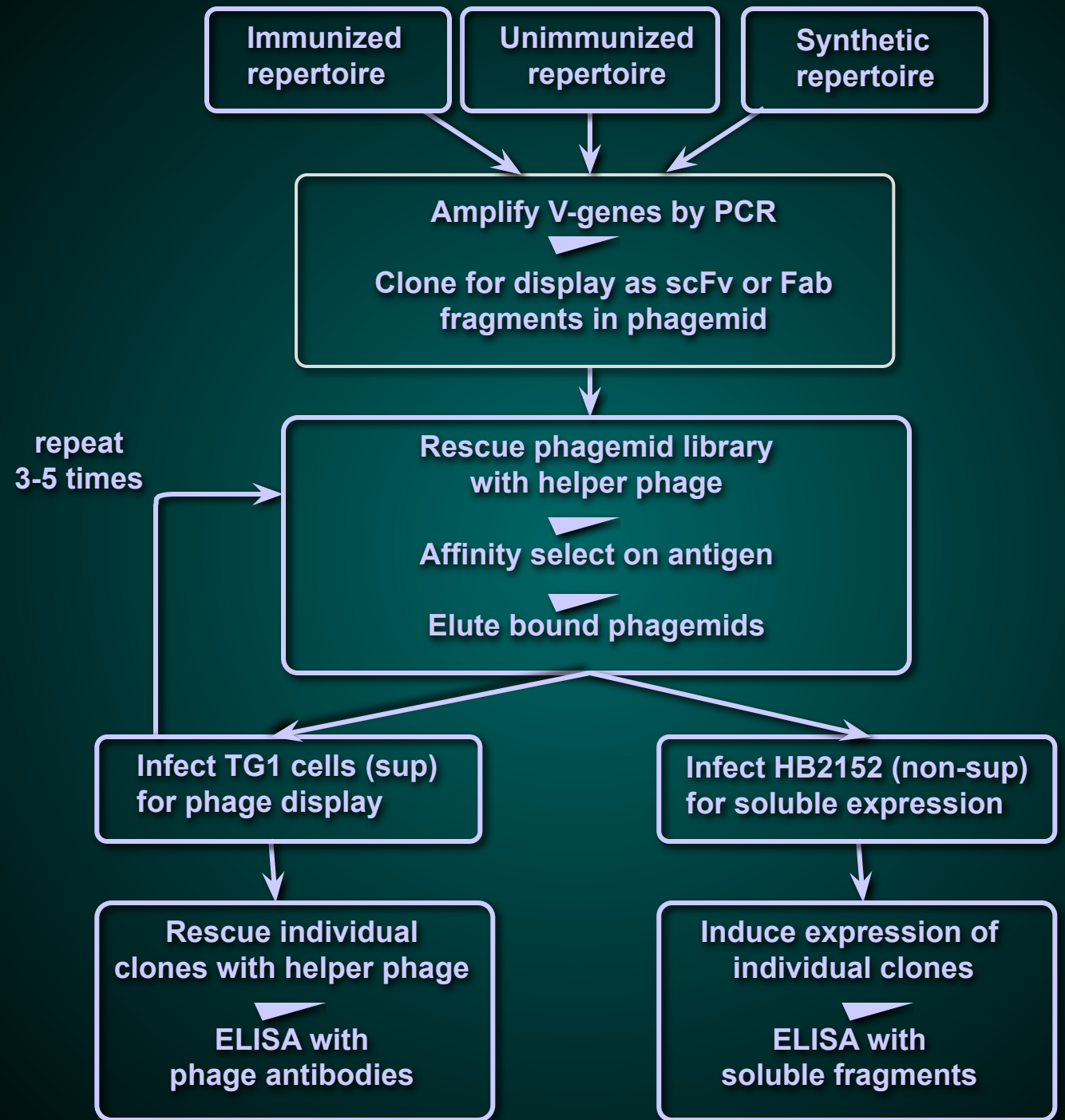




a Human antibody library technology
Iterative selection rounds
Target antigen
Screen for specificity
Clone and transfect
b Transgenic mouse technology
Immunization
Human Ig locus
Isolate B cells
Myeloma fusion partner
Generate hybridomas
Screen for specificity

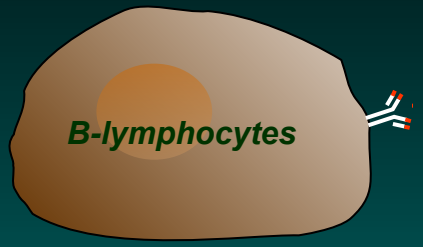

Mouse embryonic stem cells (ES cell)
Knockout mouse μ and κ
μ/κ-knockout ES cells
Transfect into ES cells
$V_H$ genes
D genes
J genes
$C_\mu$
$C_\gamma 1$
Germ-line human heavy-chain minilocus
$V_H$ genes
$J_\kappa$ genes
$C_\kappa$
Germ-line human κ light-chain minilocus
Mouse ES cells incorporating human H and L miniloci
Inject into host embryo
Blastocyst
Chimeric mouse
Breed
Human miniloci
Miniloci transgenic mouse
Nontransgenic offspring
Immunize
Human antibodies

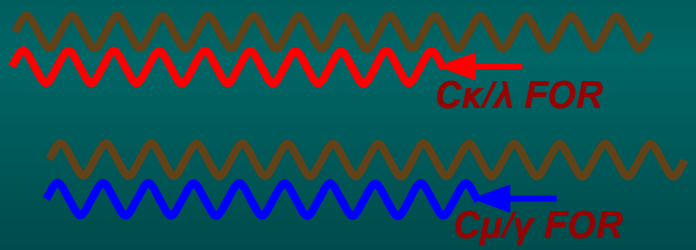
# *Строение бактериофага M13*




Immunized repertoire
Unimmunized repertoire
Synthetic repertoire
Amplify V-genes by PCR
Clone for display as scFv or Fab fragments in phagemid
repeat 3-5 times
Rescue phagemid library with helper phage
Affinity select on antigen
Elute bound phagemids
Infect TG1 cells (sup) for phage display
Infect HB2152 (non-sup) for soluble expression
Rescue individual clones with helper phage
ELISA with phage antibodies
Induce expression of individual clones
ELISA with soluble fragments

# *Construction of miniantibody library.*



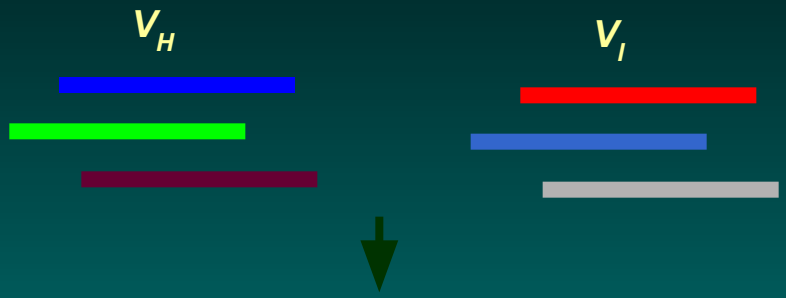

*mRNA isolation from immune volunteers*

*cDNA synthesis*

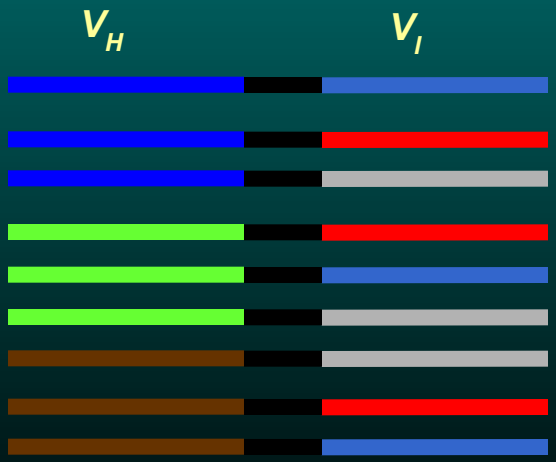
***Amplification of DNA fragment corresponding to variable part of light and heavy chain of Immunoglobulin***

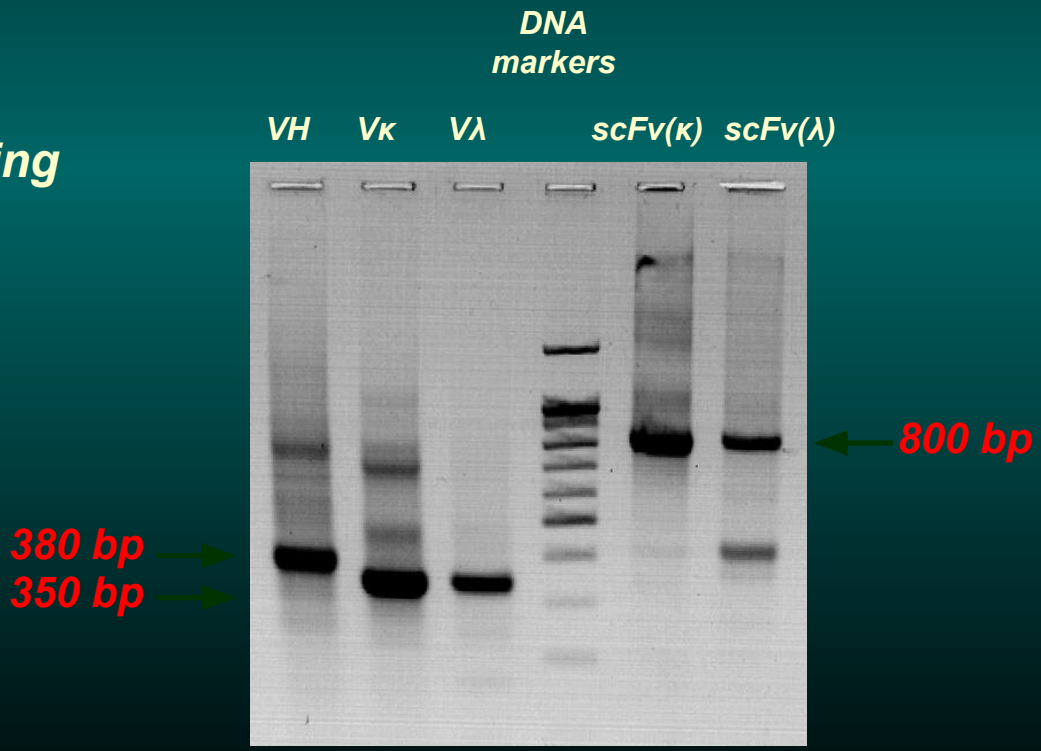
# Construction of miniantibody library.

## PCR amplification of DNA corresponding $V_H$ and $V_L$ Ig

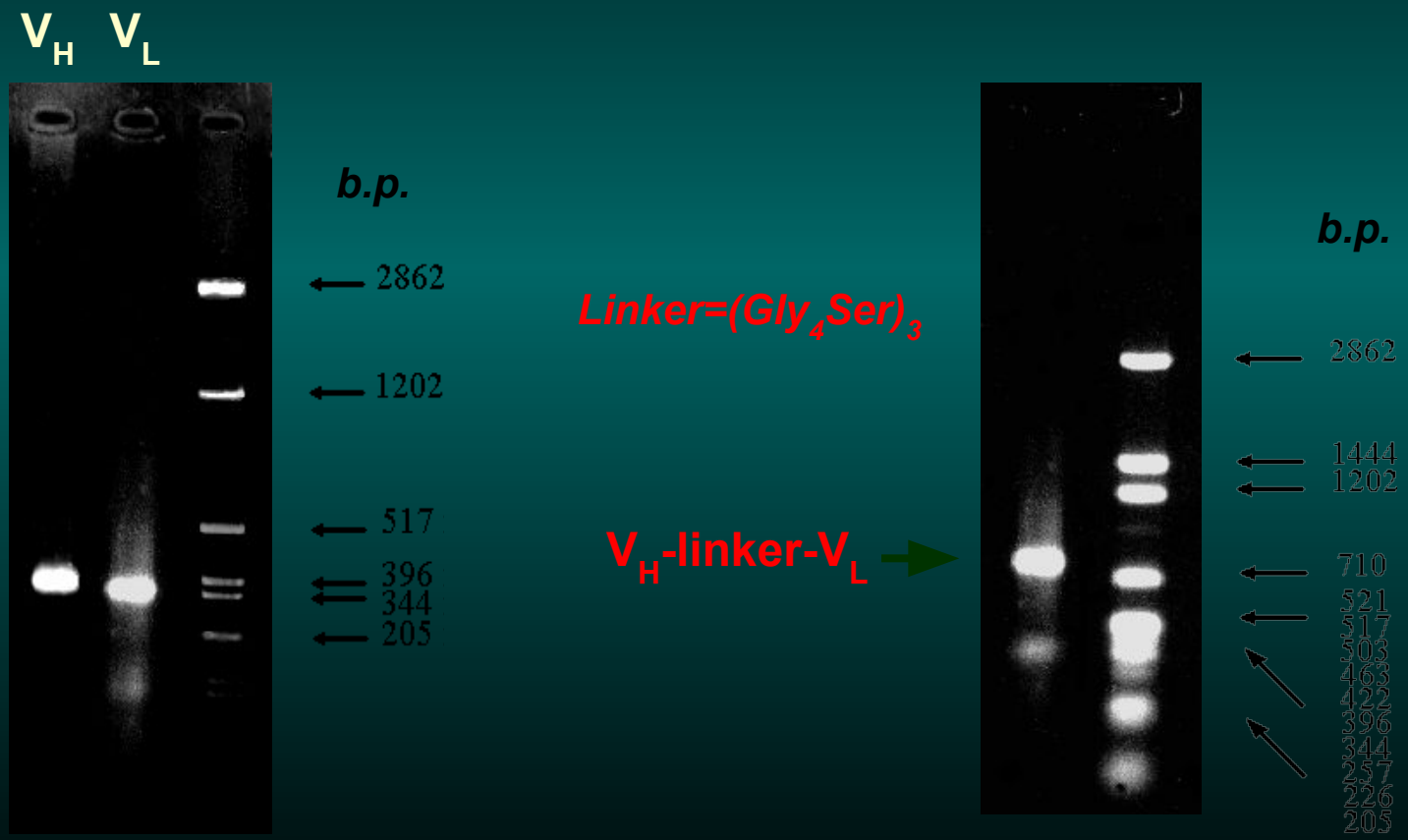



Gene assemble and stepwise cloning

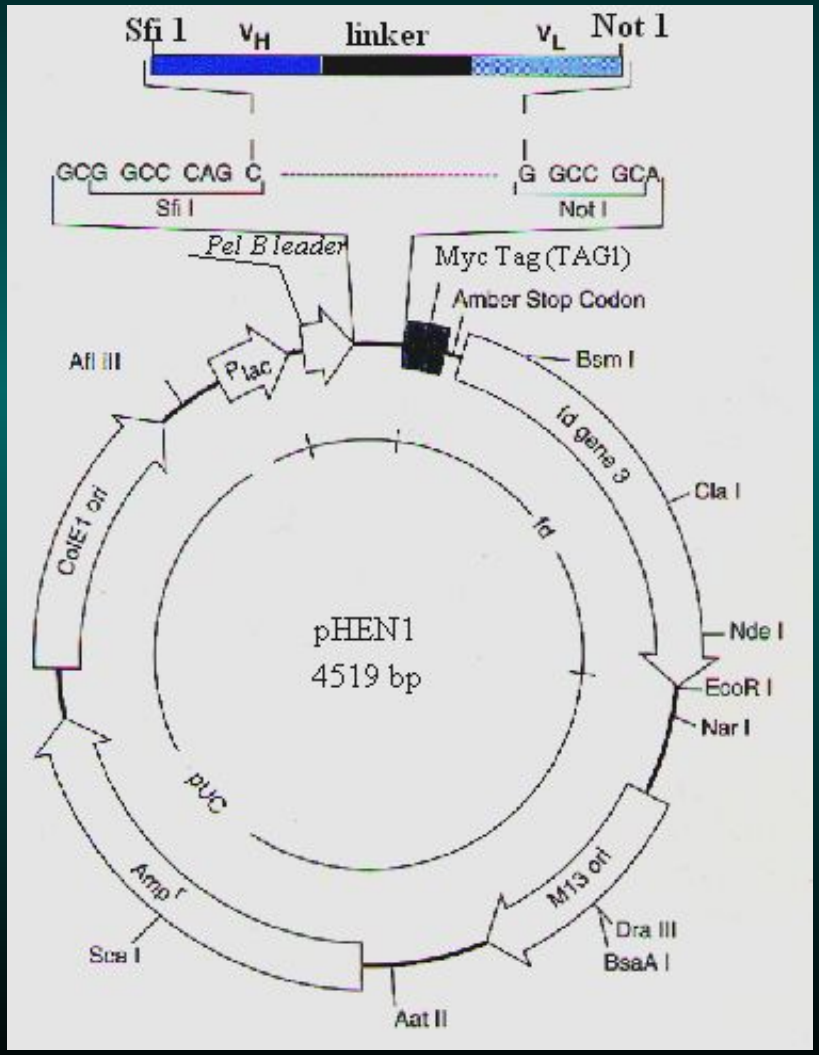


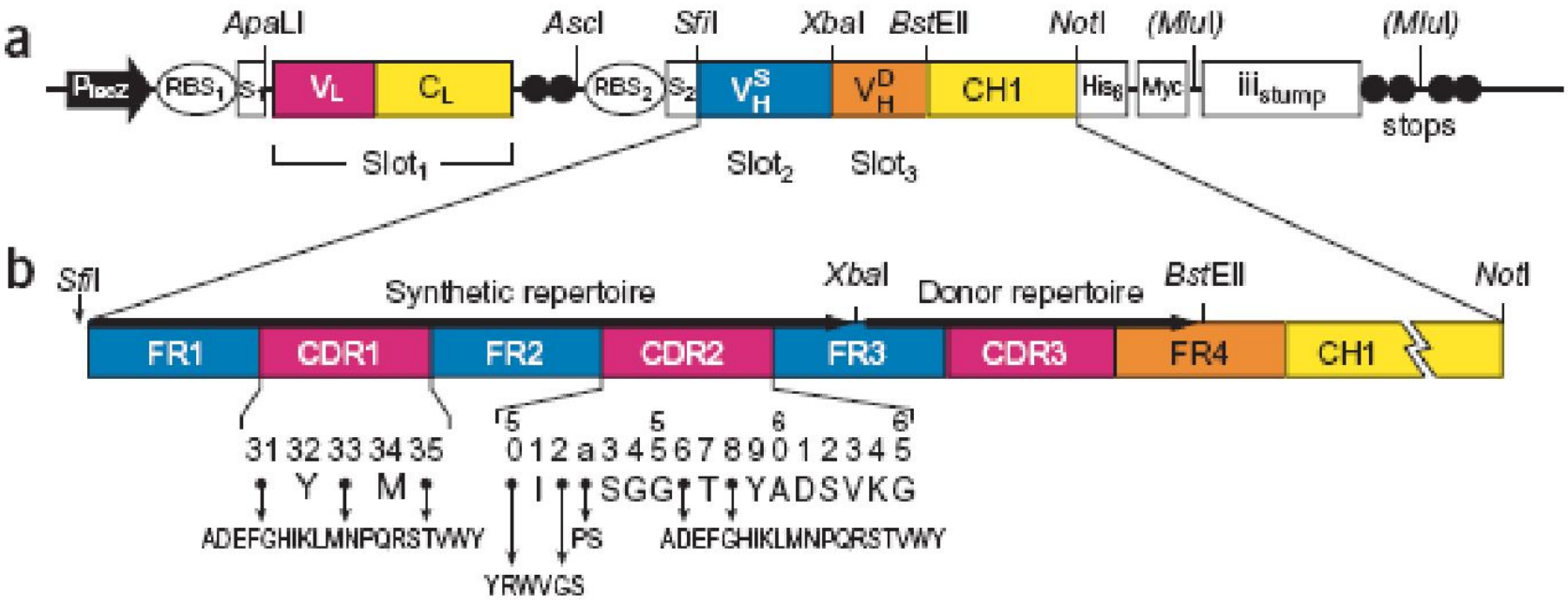



# *Electrophoresis of DNA fragments obtained after PCR.*

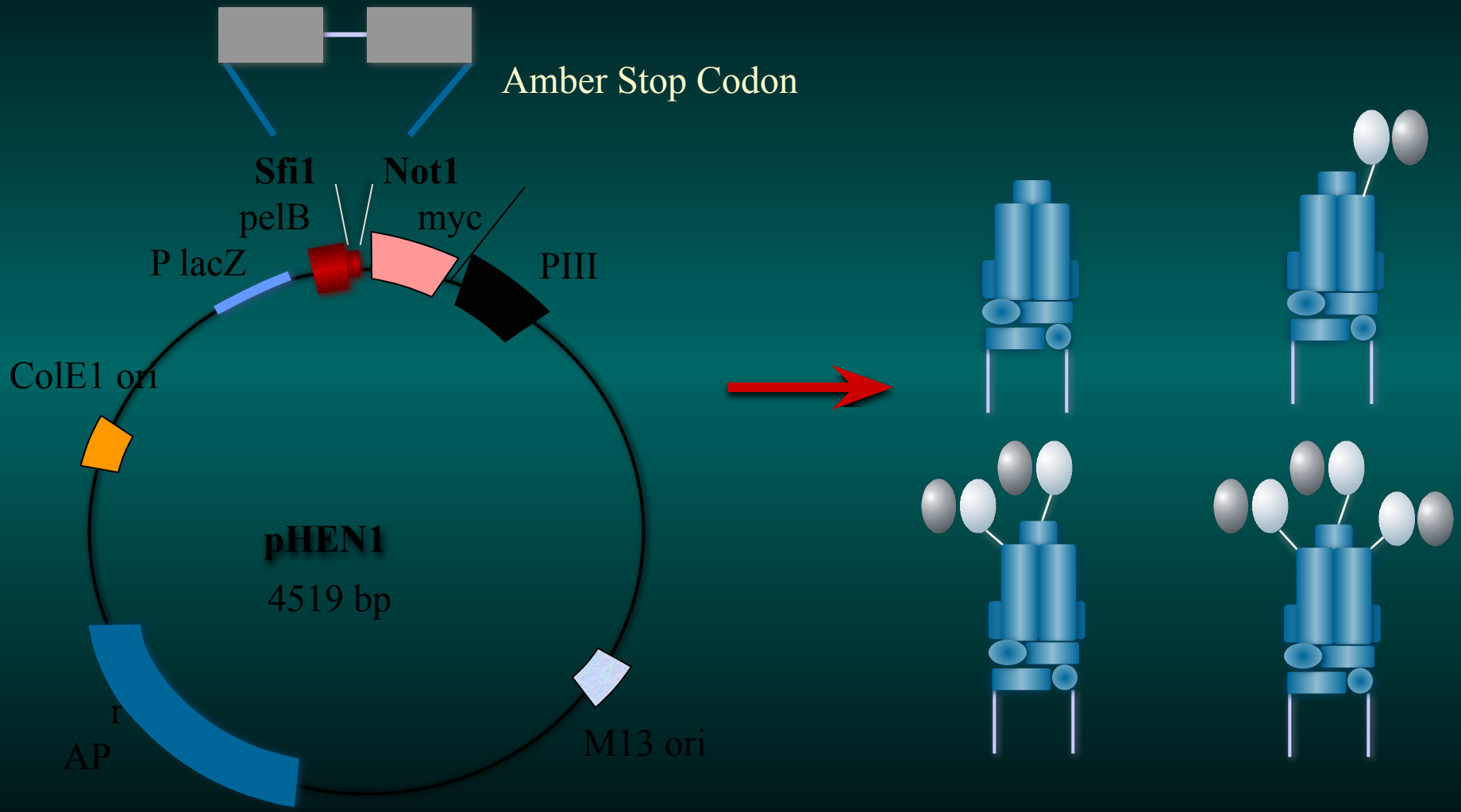


# *Cloning scFv DNA into a Phagemid Vector.*

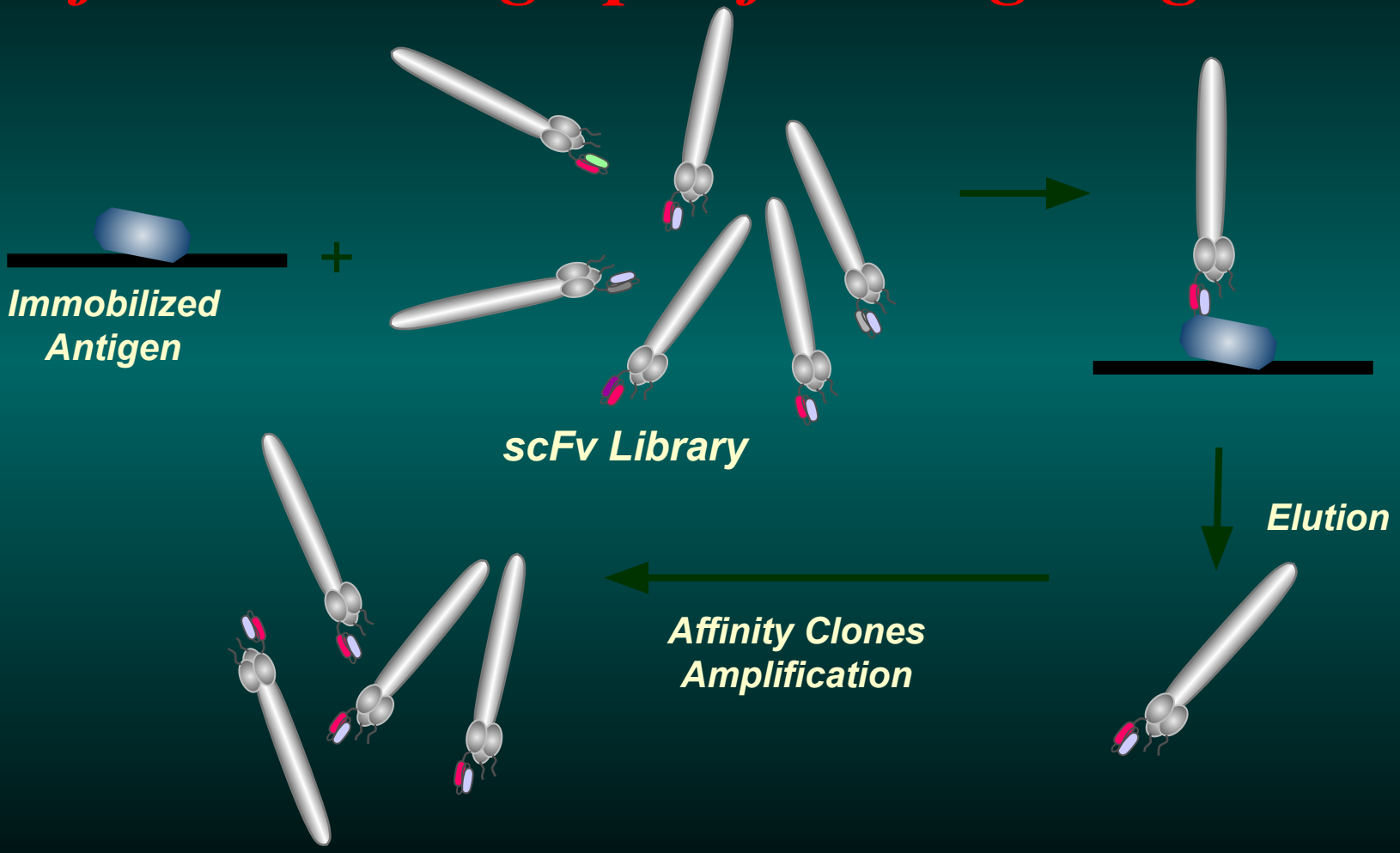


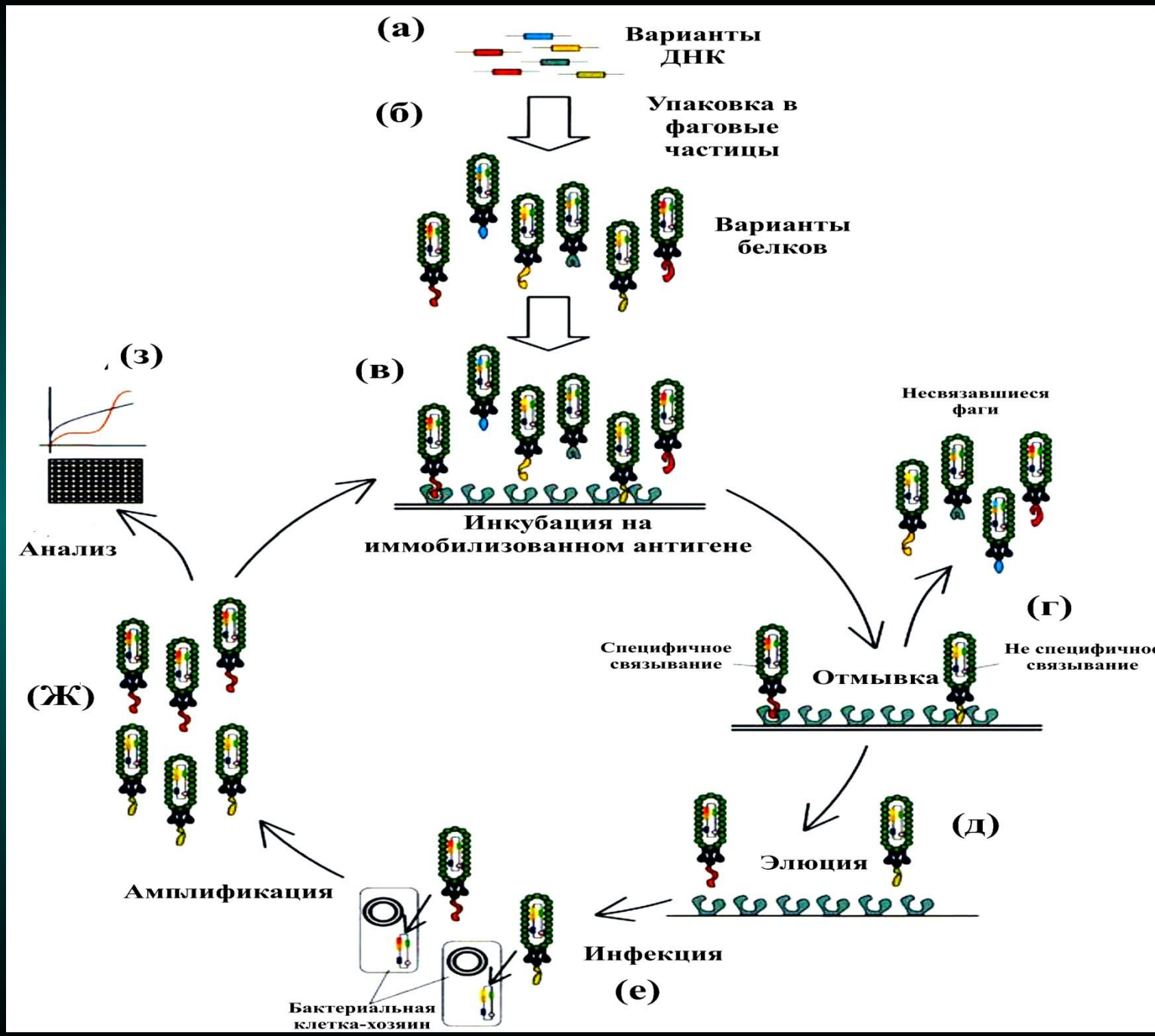

a
ApaLI
AscI
SfiI
XbaI
BstEII
NotI
(MluI)
(MluI)
Placz
RBS1
S1
VL
CL
RBS2
S2
VSH
VDH
CH1
His6
Myc
iii stump
stops
Slot1
Slot2
Slot3
b
SfiI
Synthetic repertoire
XbaI
Donor repertoire
BstEII
NotI
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
CH1
31 32 33 34 35
5 0 1 2 a 3 4 5 6 7 8 9 0 1 2 3 4 5
5
6
6
Y
M
I
SGGTYADSVKG
ADEFGHIKLMNPQRSTVWY
PS
ADEFGHIKLMNPQRSTVWY
YRWVGS

# Основы фагового дисплея

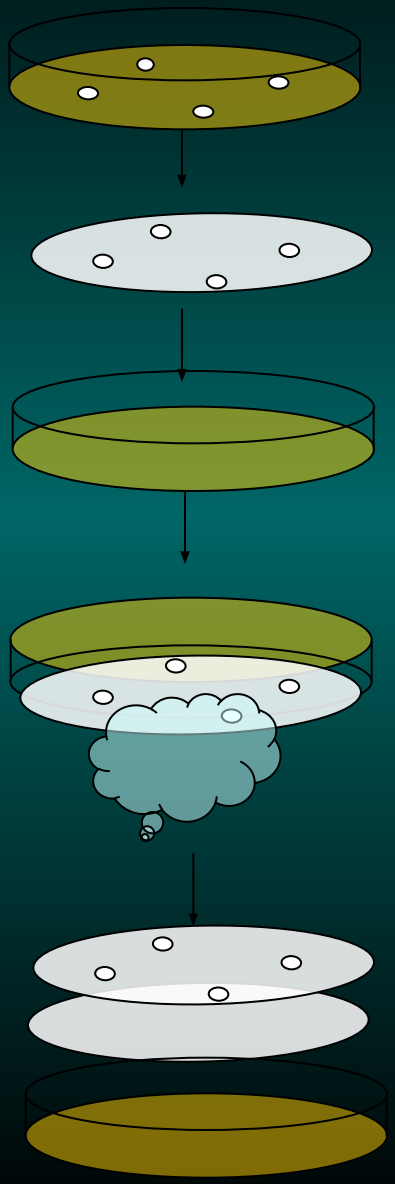


# *Selection Strategies for Obtaining Specific Phage Ligand.*




(а)
Варианты ДНК
(б)
Упаковка в фаговые частицы
Варианты белков
(в)
Инкубация на иммобилизованном антигене
Несвязавшиеся фаги
(г)
Специфичное связывание
Не специфичное связывание
Отмывка
(д)
Элюция
Инфекция
(е)
Бактериальная клетка-хозяин
Амплификация
(Ж)
Анализ
(з)

# *Скрининг клонов-продуцентов scFv E.coli к белковым антигенам*



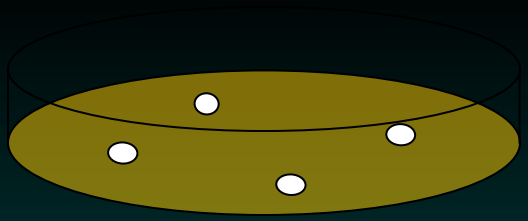
Чашка Петри с колониями E.coli .

Перенос колоний E.coli на нитроцеллюлозный фильтр

Инкубация фильтров с сорбированными колониями на питательной среде с IPTG(1 мМ) в течение 2-3 часов при 30 0С

Лизис колоний E.coli в парах хлороформа

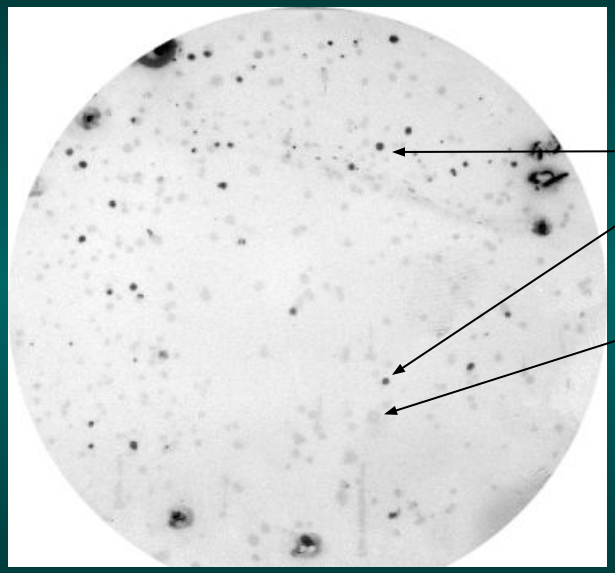
Совмещение фильтра с лизированными колониями E.coli с фильтром с сорбированным ботулиническим антигеном А (100 нг/мл)



trxA-scFv expressed E.coli

Analysis
~ $10^3$ clones

Analysis
~ $10^2$ clones



Positive signal

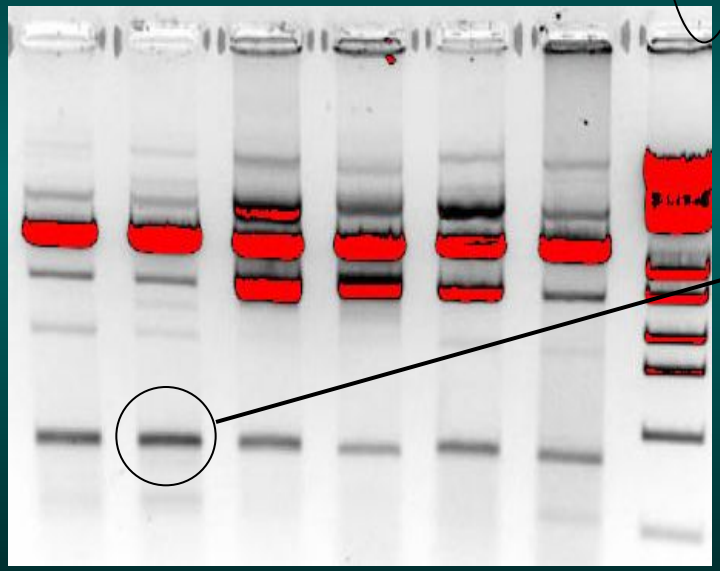
Negative signal

ELISA

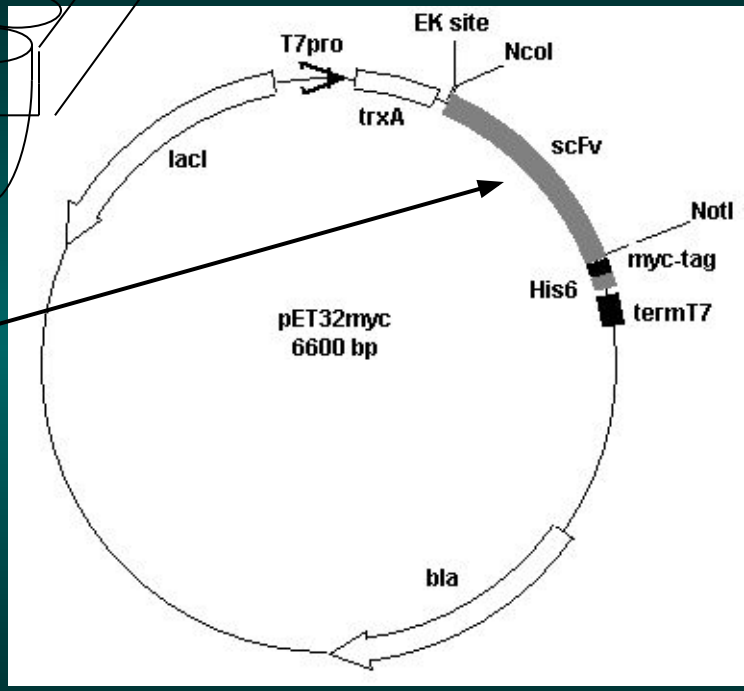
Colony lift screen

***Preparative protein expression, purification and analysis (sequence, Kd, specifity )***

# *Selection of antibody library*

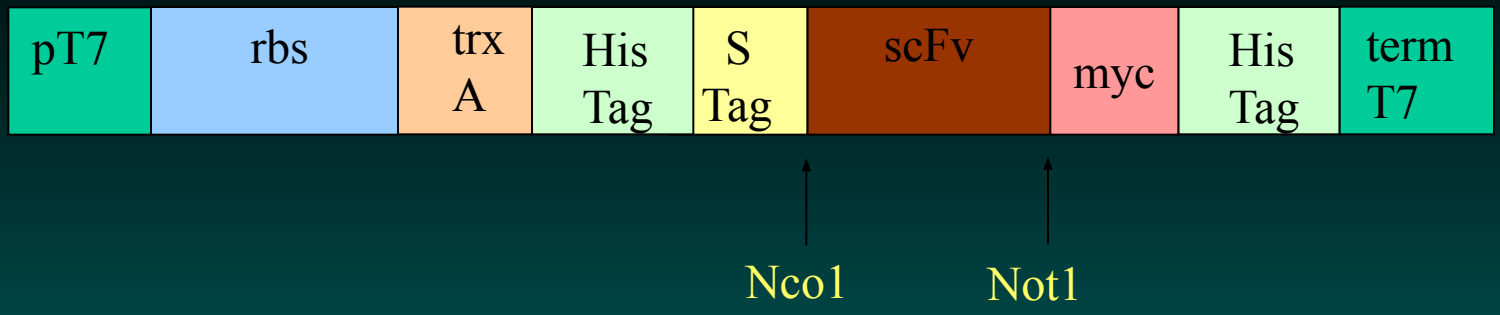


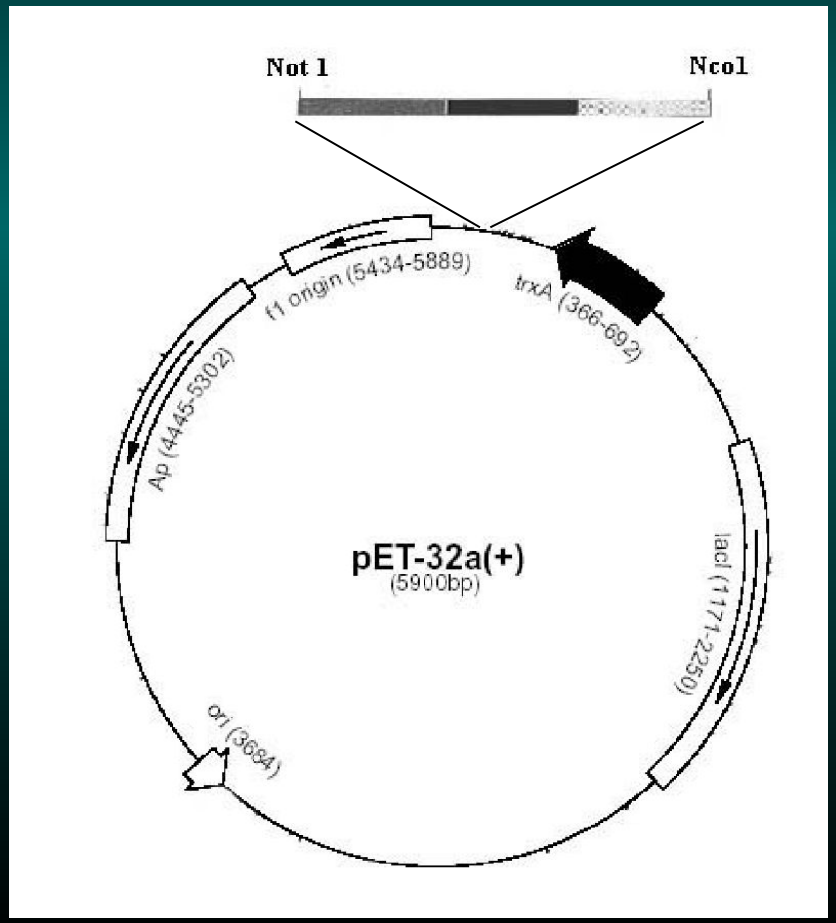
Restriction of total ScFv
gene from pHEN2



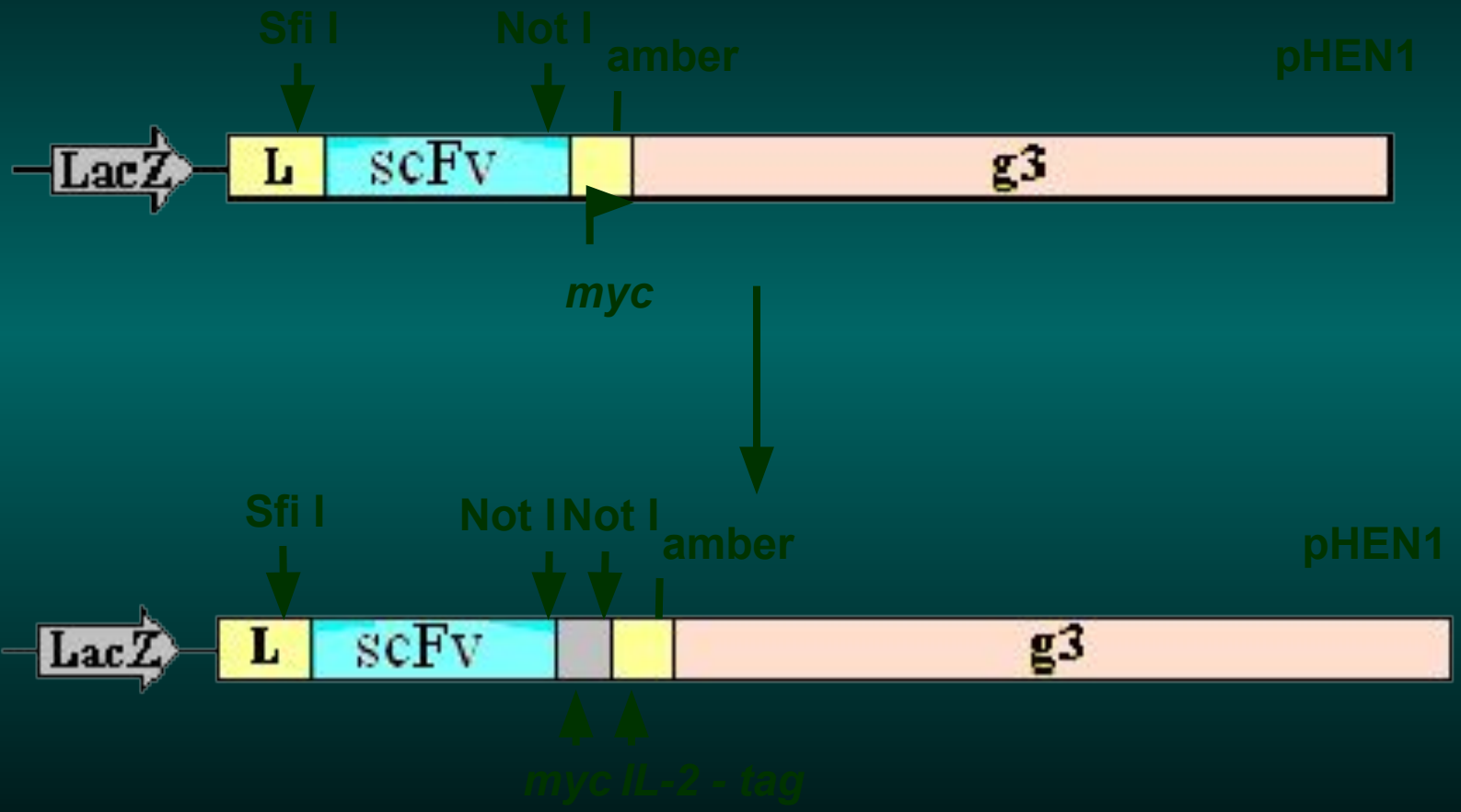

Recloning
in expression vector

# Клонирование генов scFv в экспрессионный вектор

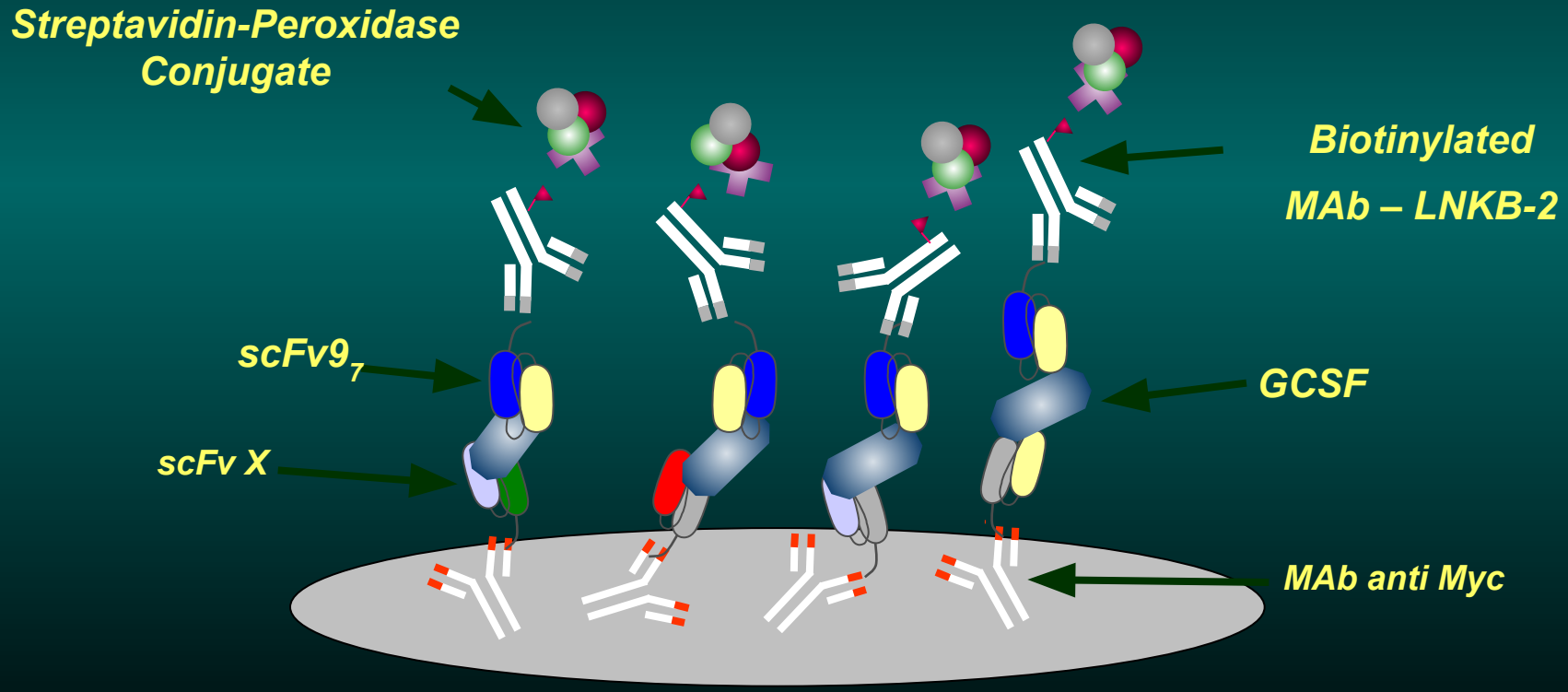


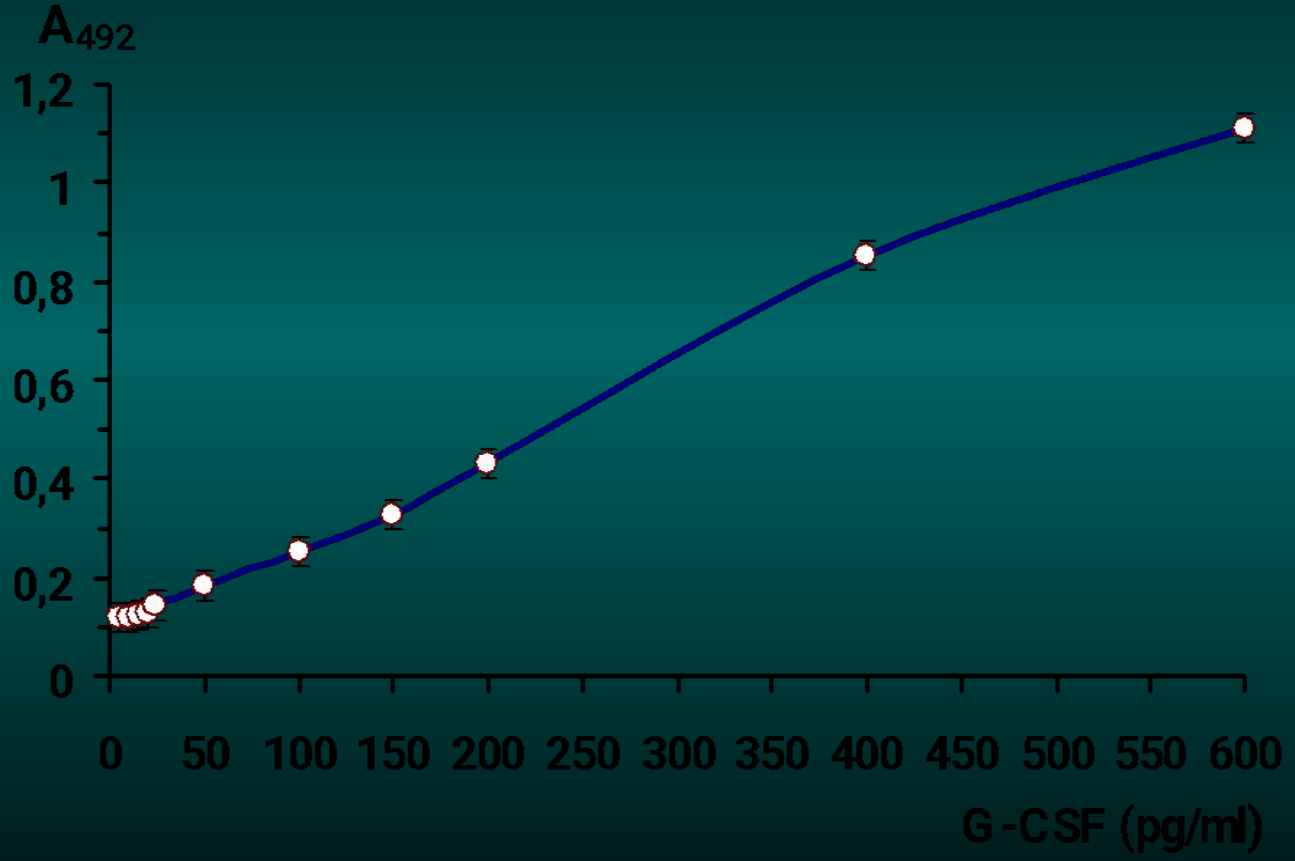
# *Insertion of c-myc and LNKB-2 epitop tags*



# *Selection of scFv clones for sandwich ELISA to analyze Granulocyte Colony Stimulation Factor.*



# *G-CSF titration with sandwich ELISA (F20-F16 scFv).*



# *Оптимизация условий очистки и ренатурации scFv*

Инкубация культуры E.coli Origami pRAREв условиях индукции при интенсивной аэрации при 30 0С

Экстракция внутреклеточного белка в денатурирующих условиях (8 М мочевина,pH8)

Очистка белка металл-хелатной хроматографией на Ni-NTA агарозе в денатурирующих условиях

Снятие целевого белка:
- понижением pH реакционной среды
- имидазолом
- EDTA

Рефолдинг scFv при понижении концентрации денатурирующего агента с использованием рефолдинг-буфера
Рефолдинг на колонке

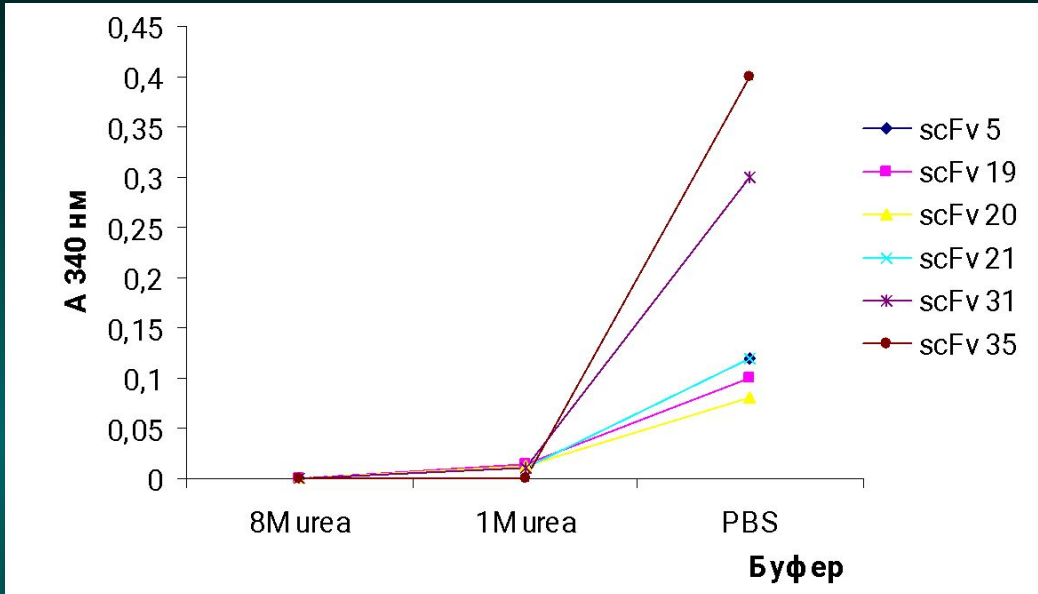
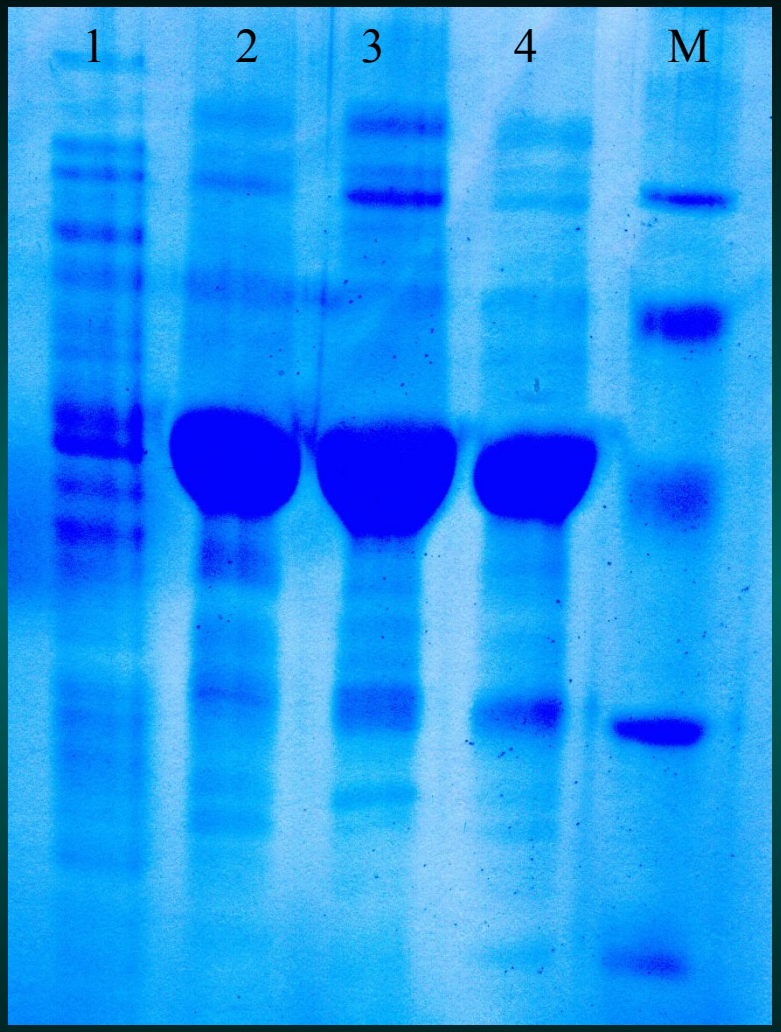



**Рис. 1 Рефолдинг scFv с постепенным понижением концентрации денатурирующего агента**

# Оценка чистоты препарата после ренатурации и концентрирования scFv




1- Тотальный клеточный экстракт E.coli (10 мкг) до очиски на Ni-NTA агарозе

2 - Очищенные, сконцентрированные scFv 21(15мкг)

3 - Очищенные, сконцентрированные scFv 31(15 мкг)

4 -Очищенные,

сконцентрированные scFv 5 (15мкг)

M - белковый маркер “Хеликон”

Рис. 4. Электрофоретический анализ белковых препаратов на разных стадиях очистки в денатурирующих условиях SDS PAGE(10%)

# *Оценка чистоты препарата после ренатурации и концентрирования scFv*

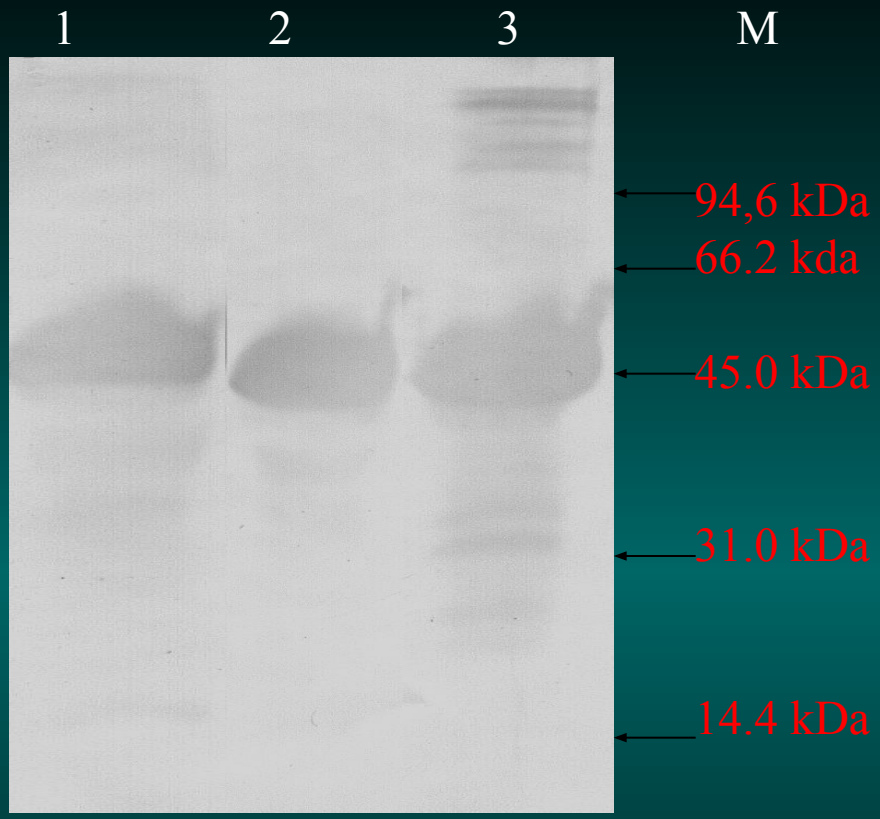



Рис.5. Иммуно-блот - анализ выделенных scFv.
Детекция белка с помощью мышиных моноклональных антител
MAb anti-myc и Streptavidin-Alkaline Phosphatase конъюгата

1 - Очищенные, сконцентрированные scFv 21(15мкг)

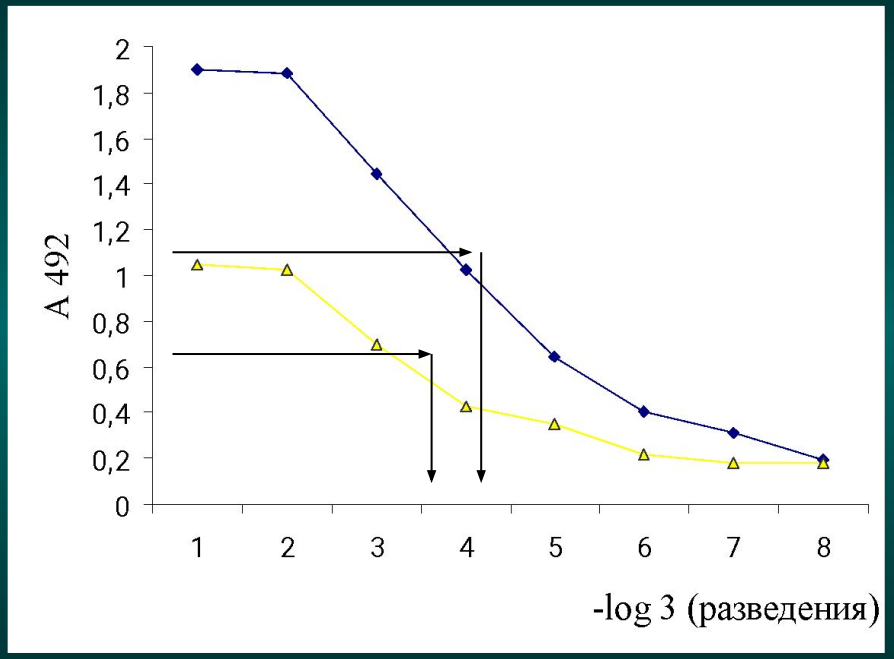
2 - Очищенные, сконцентрированные scFv 31(15 мкг)

3 -Очищенные,

сконцентрированные scFv 5 (15мкг)

M - белковый маркер “Хеликон”

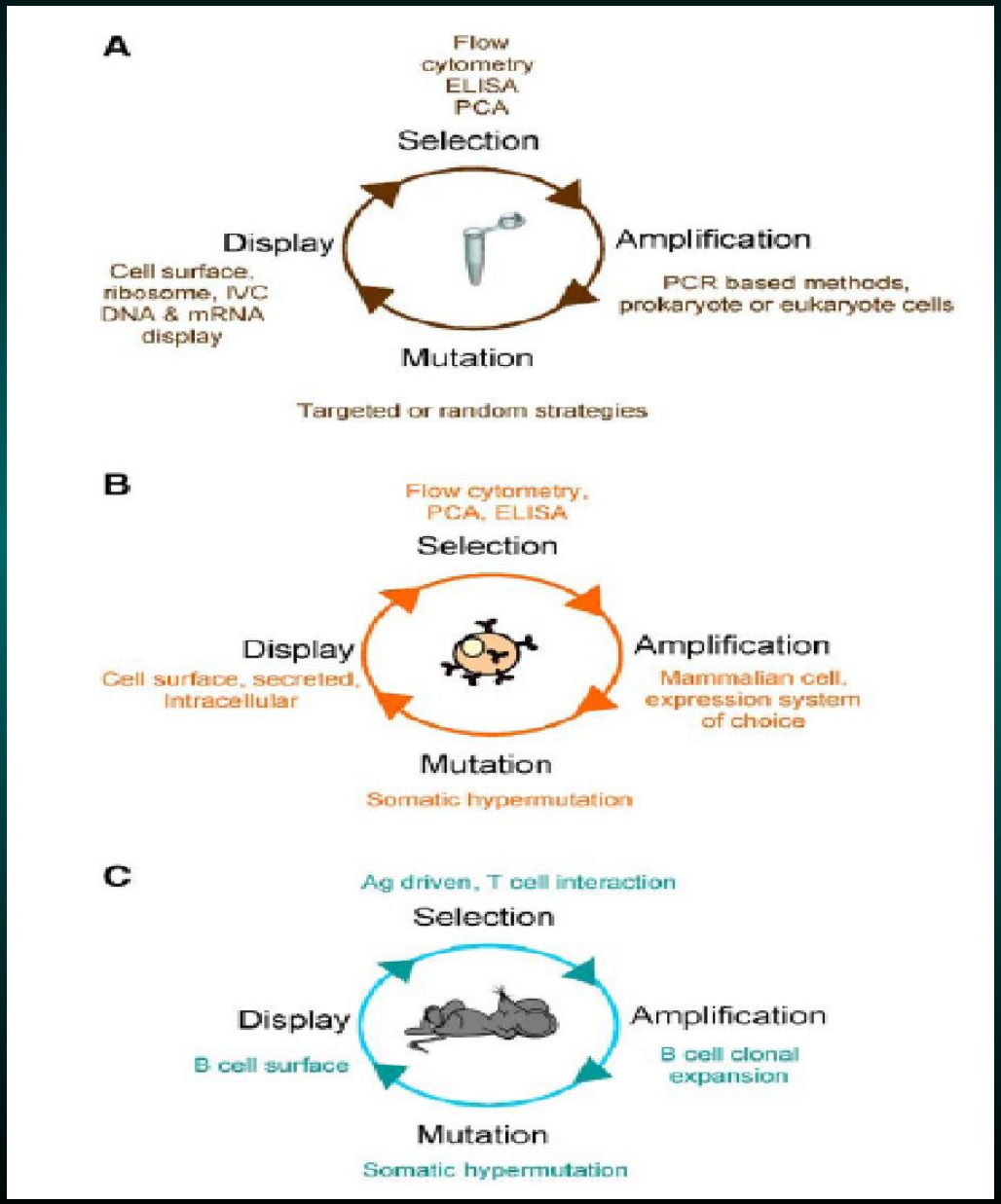
# *Определение констант аффинности миниантител*




| Миниантитело | $K_d$, M |
|---|---|
| scFv C 21 | $6*10^{-8}$ |
| scFv C 31 | $1,4*10^{-8}$ |
| scFv SE36 | $7*10^{-7}$ |
| scFv Tt 33 | $5.3*10^{-7}$ |
| scFv BA 5 | $5.3*10^{-7}$ |

Рис.6. Определение константы аффинности миниантител клона С31. с использованием непрямого ИФА.
$K_d$=1,4*10 $^{-8}$ М

# *Изменение аффинности рекомбинантных антител*



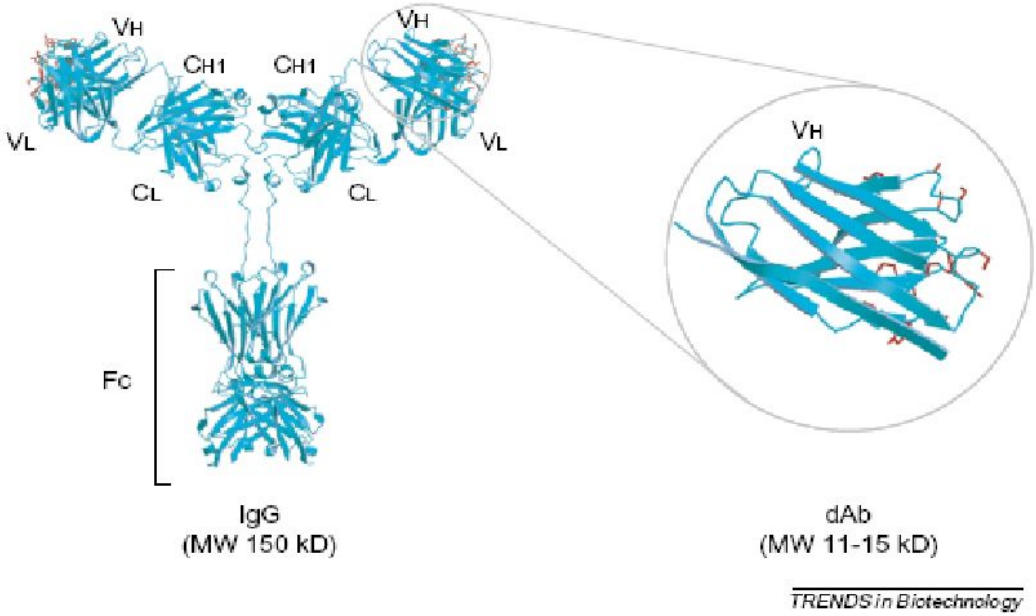


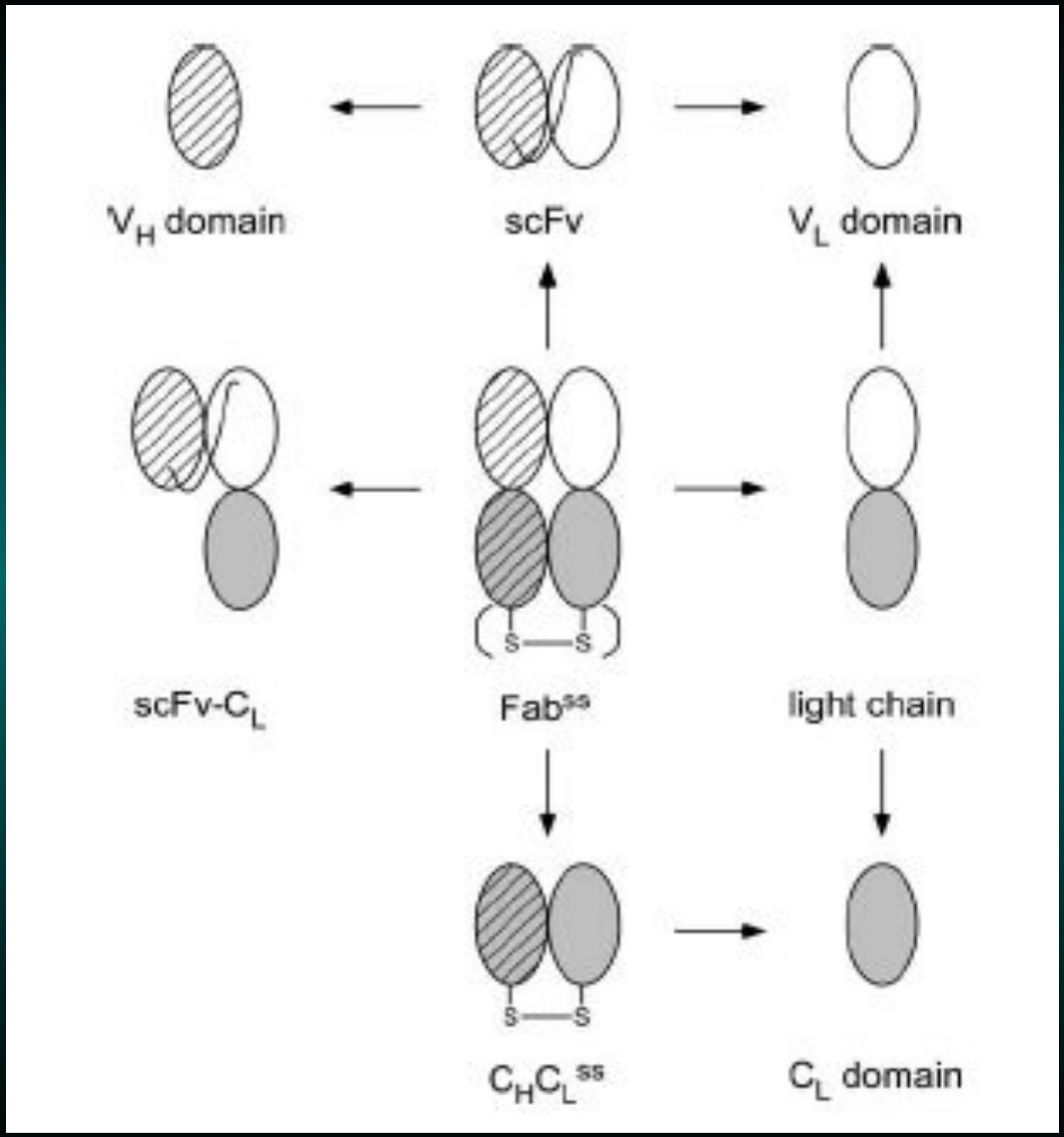

**Figure 1.** A human IgG molecule has both variable and constant regions. An IgG contains two variable regions (each composed of a $V_H$ and $V_L$ domain) that confer antigen-binding specificity on the antibody, and an Fc fragment in the constant region that recruits the effector functions of the immune system. Conventional recombinant antibody fragments contain one antigen-binding $V_H$–$V_L$ pairing. At ~57 kDa, a Fab fragment comprises a $V_H$–$C_H1$ polypeptide disulphide-bonded to a $V_L$–$C_L$ polypeptide. At ~27 kDa, a scFv fragment contains only the $V_H$ domain fused to the $V_L$ domain via a polypeptide linker. By contrast, the domain antibody, or dAb, of 11–15 kDa is either an isolated antibody $V_H$ domain [2], as shown here, or an isolated antibody $V_L$ domain [15]. Each dAb thus contains three of the six naturally occurring complementarity determining regions (CDRs) from a $V_H$–$V_L$ pairing. The side chains of the CDRs are highlighted in red.

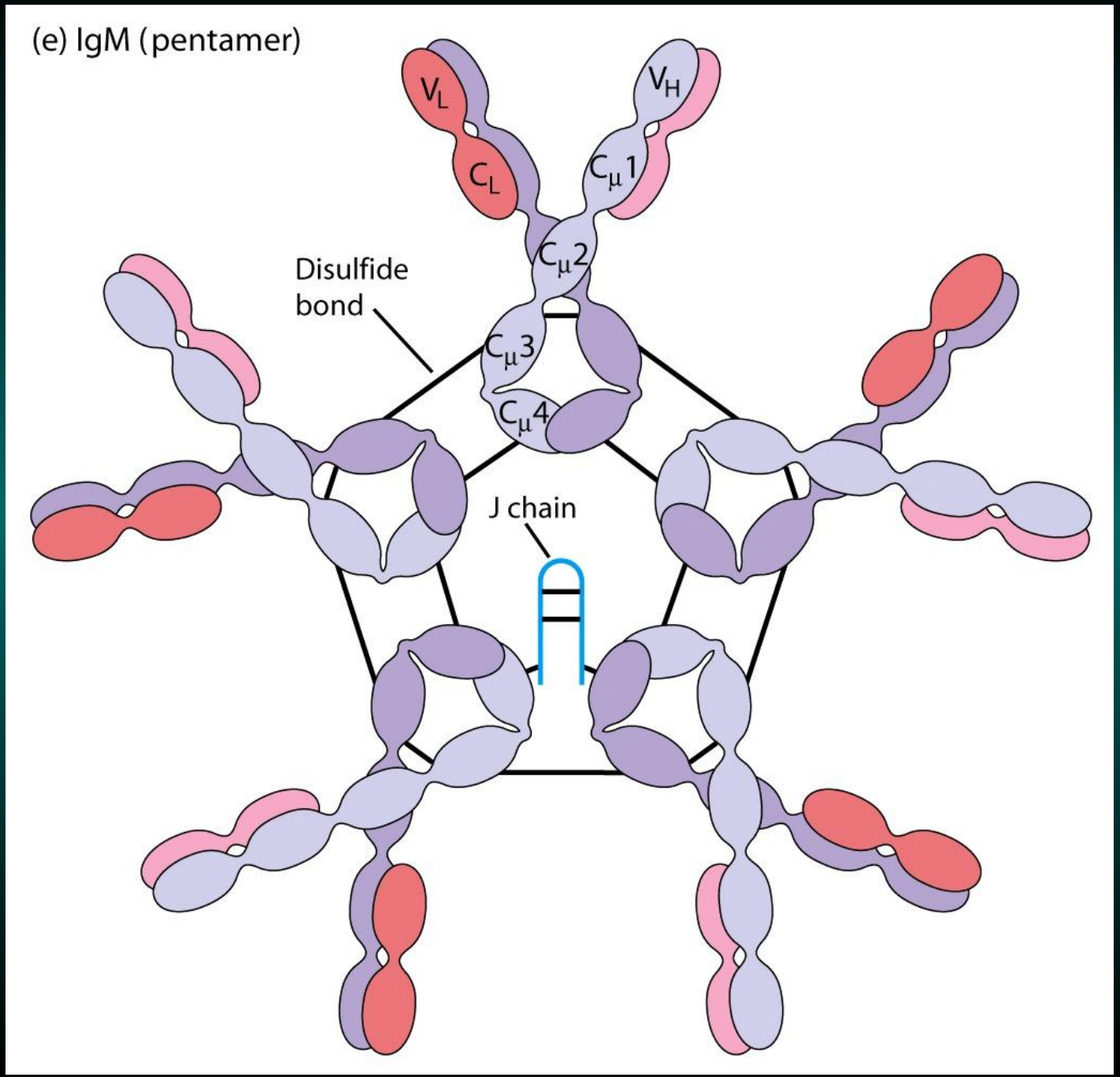
# *Стратегия улучшения антигенсвязывающих параметров антител*

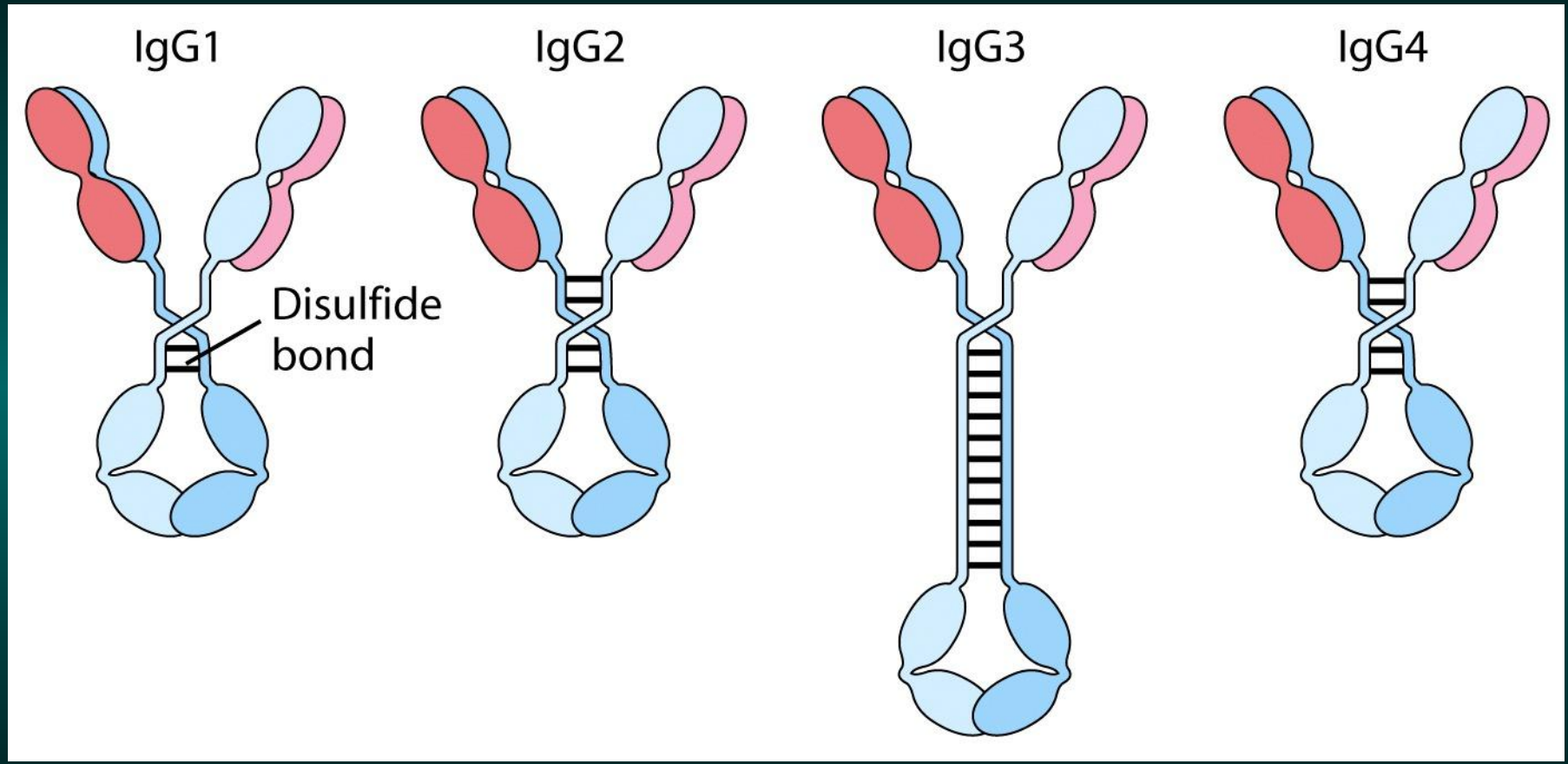
Table 2
Selected examples of mutation strategies to evolve antibodies

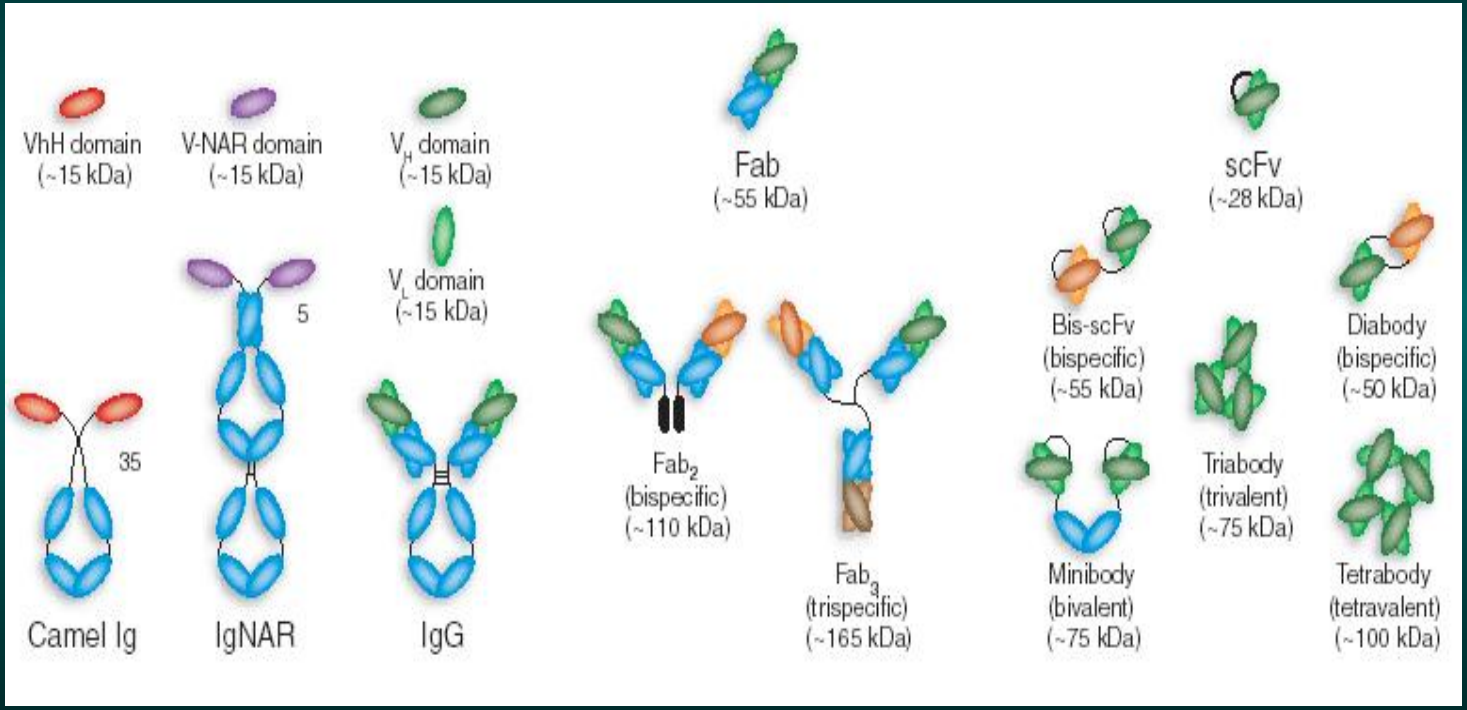
| | Target | Affinity gain/affinity ($K_D$) | Display |
|---|---|---|---|
| *In vitro mutagenesis* | | | |
| Targeted | | | |
| CDR walking | gp120 | 420 X to 15 pM | Phage |
| Site directed mutagenesis | Myelin axonal growth inhibitor | 8 X to 1 μM | Secreted |
| CDR target hotspot | CD22 | 7 X to 0.8 nM | Phage |
| | Parathyroid hormone | 30 X to 2 μM | Ribosome display |
| | CD22 | 14 X to 6 nM | Phage |
| Random | | | |
| Error prone PCR | Anthrax toxin | 200 X to 21 pM | Bacterial |
| | Streptavidin | 10.7 X to 3.2 nM | Yeast |
| Chain shuffling | HBV preS1 | 6.5 X to 520 nM[a] | Phage |
| | Hapten 2-phen-Yloxalazol-5-one | 320 X 1.1 nM | Phage |
| *Combined in vitro mutagenesis* | | | |
| Error prone PCR and DNA shuffling | GCN4 | 500 X to 5 pM | Ribosome display |
| Site directed and chain shuffling | αvβ3 | 90 X to 300 pM | Phage |
| Error prone PCR and site directed mutagenesis | Botulinum neurotoxin | 37 X to 23 pM | Yeast |
| Error prone PCR and site directed mutagenesis | Neural cell adhesion molecule | 85 X to 2.28 nM | Phage |
| Chain shuffling and DNA shuffling | Ep-Cam | 15 X 0.4 nM | Phage |
| *In vivo mutagenesis* | | | |
| Bacterial mutator cells (mutD5) | Hapten 2-phenyl-5-oxazolone | 100 X to 3.2 nM | Phage |
| Mammalian mutator | IgG mab | 5.8 nM[b] | Cell surface |
| Cells | Protein A | 0.32 nMb | |
| Transgenic mice (XenoMouse) | IL-8 | 610 fM | SLAM |
| | Undisclosed | 4 pM | SLAM |
| *Combined in vivo and in vitro mutagenesis* | | | |
| Yeast mating driven chain shuffle and error prone PCR and shuffle | Streptavidin | 6.8 X to 6 nM | Yeast display |

[a] $K_a$.
[b] Apparent affinity not based on a monomeric interaction.


$V_H$ domain
scFv
$V_L$ domain
scFv-$C_L$
$Fab^{ss}$
light chain
S—S
$C_HC_L{}^{ss}$
$C_L$ domain


(e) IgM (pentamer)
$V_L$
$V_H$
$C_L$
$C_\mu 1$
$C_\mu 2$
Disulfide bond
$C_\mu 3$
$C_\mu 4$
J chain


IgG1
IgG2
IgG3
IgG4
Disulfide
bond


VhH domain
(~15 kDa)
V-NAR domain
(~15 kDa)
$V_H$ domain
(~15 kDa)
$V_L$ domain
(~15 kDa)
35
5
Camel Ig
IgNAR
IgG
Fab
(~55 kDa)
scFv
(~28 kDa)
Bis-scFv
(bispecific)
(~55 kDa)
Diabody
(bispecific)
(~50 kDa)
$Fab_2$
(bispecific)
(~110 kDa)
$Fab_3$
(trispecific)
(~165 kDa)
Triabody
(trivalent)
(~75 kDa)
Minibody
(bivalent)
(~75 kDa)
Tetrabody
(tetravalent)
(~100 kDa)

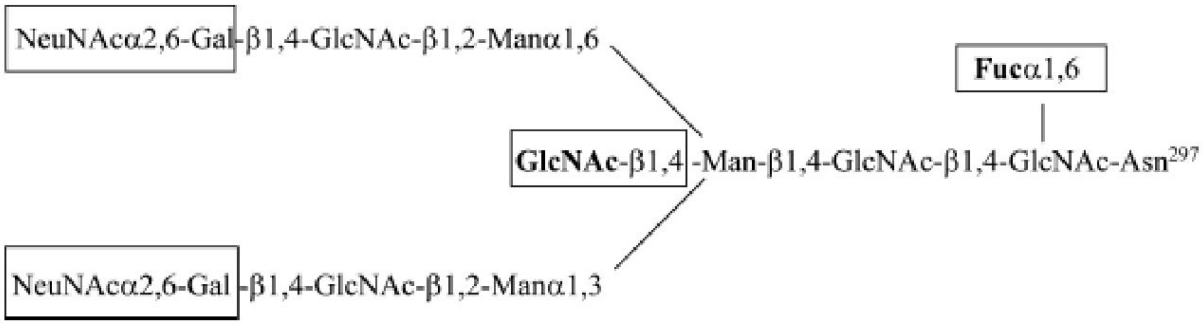



Fig. 2. Schematic representation of the glycan chain present in IgG Fc region. GlcNAc, *N*-acetyl glucosamine; Man, mannose; Fuc, fucose; Gal, galactose; and NeuNAc, *N*-acetyl neuraminic acid. Residues shown in box may be present or absent. The GlcNAc shown in bold represents the bisecting sugar. The Fuc shown in bold is known to be associated with reduction in ADCC activity.

# Влияние фукозилирования антител на антитело зависимую цитотоксичность

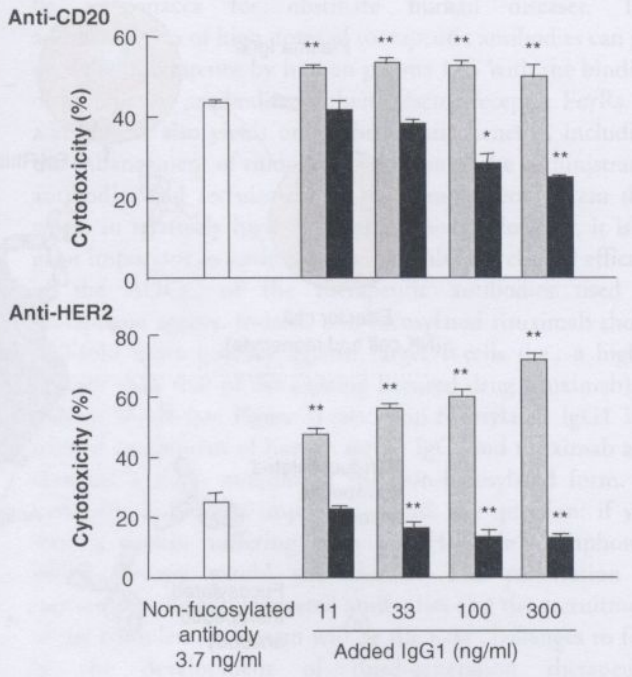



**Figure 4. Fucosylated therapeutic antibodies inhibit the *in vitro* ADCC of non-fucosylated therapeutic antibodies.** *In vitro* ADCC against CD20+ human B lymphoma WIL2-S cells (upper) and HER2+ human breast cancer MCF-7 cells (lower) was induced by the corresponding non-fucosylated anti-CD20 IgG1s or anti-HER2 IgG1s (3.7 ng/ml) (white columns). The corresponding non-fucosylated antibody (grey columns) or fucosylated antibody (black columns) was further added to the reaction at the indicated concentrations. Cytotoxicity was measured by a 4-h lactate dehydrogenase release assay using human PBMCs as effector cells at an effector:target ratio of 25:1. Columns: mean cytotoxicity (%) values of triplicates; bars: SD, statistically significant differences between non-fucosylated antibody alone- and antibody mixture-mediated ADCC, as determined by paired *t* test (see the details in [86]).

* $p < 0.05$; ** $p < 0.01$.

ADCC: Antibody-dependent cellular cytotoxicity; PBMC: Peripheral blood mononuclear cell; SD: Standard deviation.

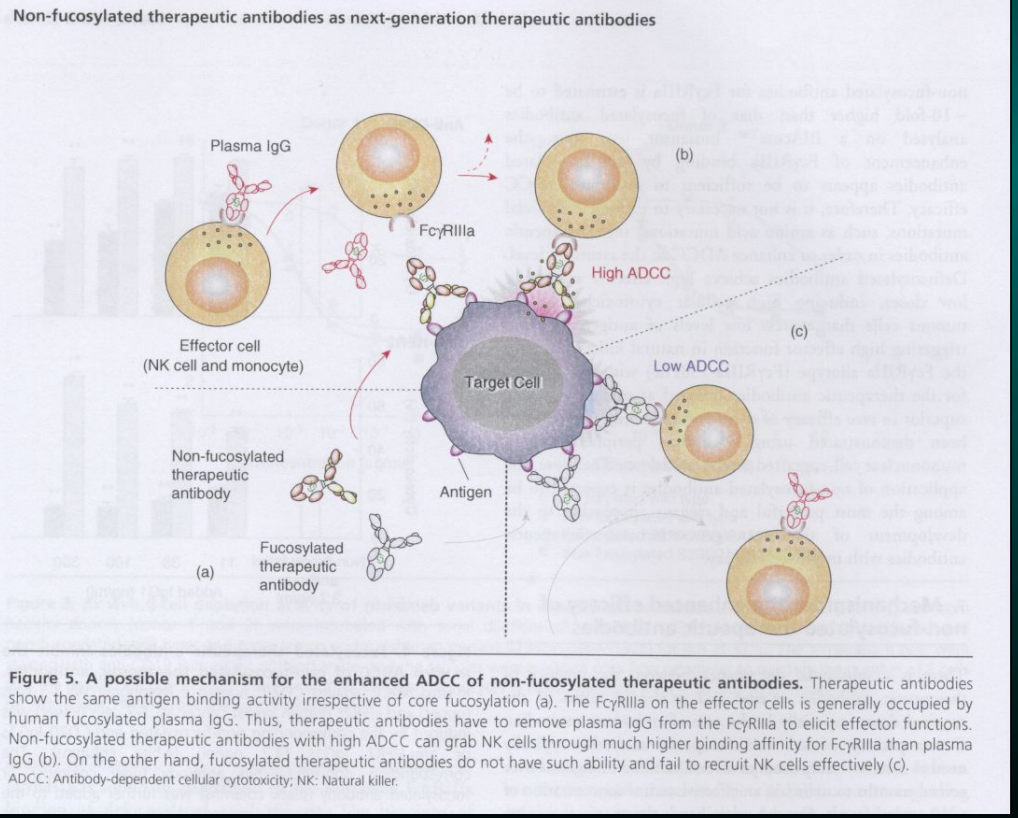
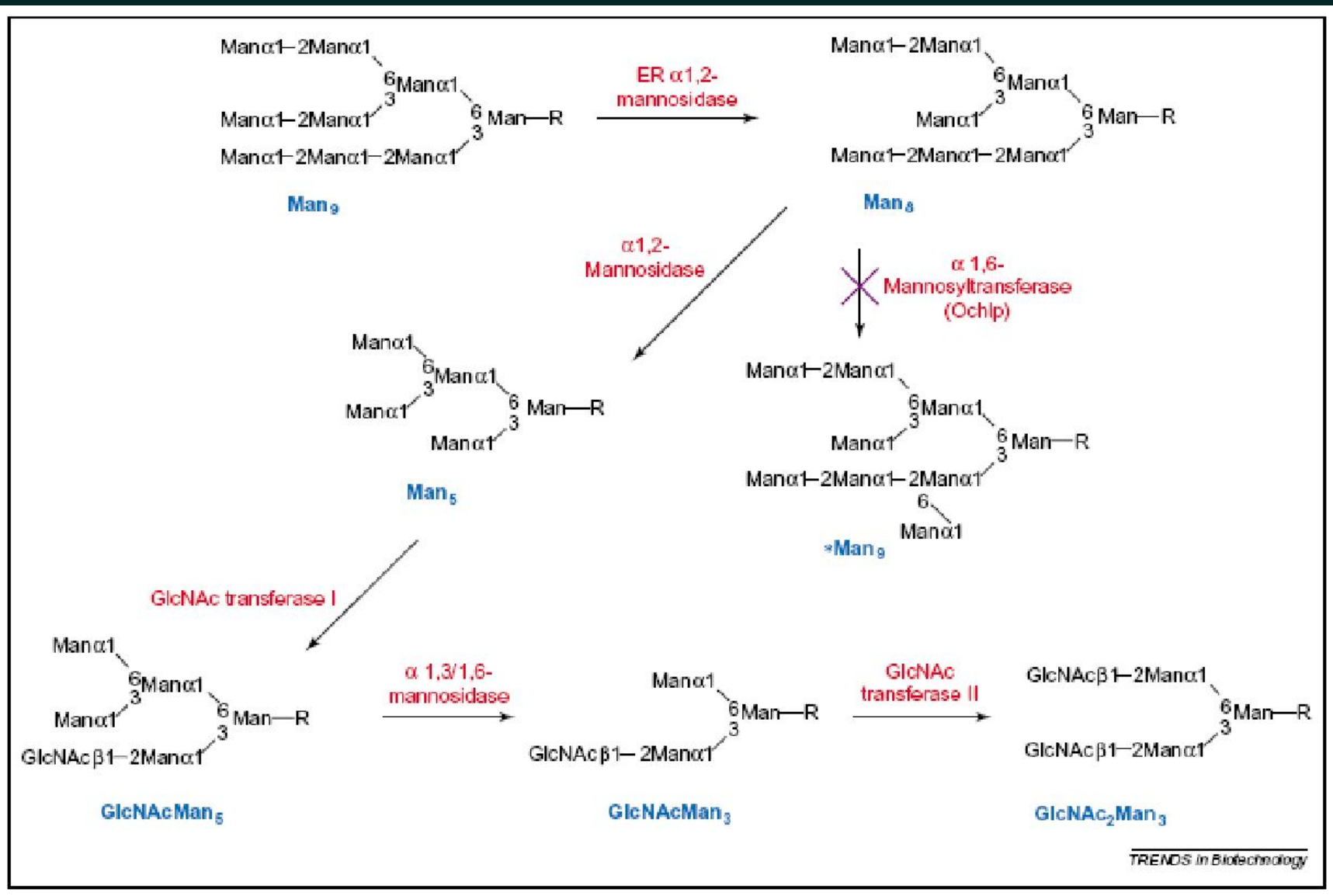



**Figure 5. A possible mechanism for the enhanced ADCC of non-fucosylated therapeutic antibodies.** Therapeutic antibodies show the same antigen binding activity irrespective of core fucosylation (a). The FcγRIIIa on the effector cells is generally occupied by human fucosylated plasma IgG. Thus, therapeutic antibodies have to remove plasma IgG from the FcγRIIIa to elicit effector functions. Non-fucosylated therapeutic antibodies with high ADCC can grab NK cells through much higher binding affinity for FcγRIIIa than plasma IgG (b). On the other hand, fucosylated therapeutic antibodies do not have such ability and fail to recruit NK cells effectively (c).

ADCC: Antibody-dependent cellular cytotoxicity; NK: Natural killer.

# Гликозилирование белков в диком и рекомбинантном штаммах Pichia Pastories



# Гликозилирование рекомбинантных белков

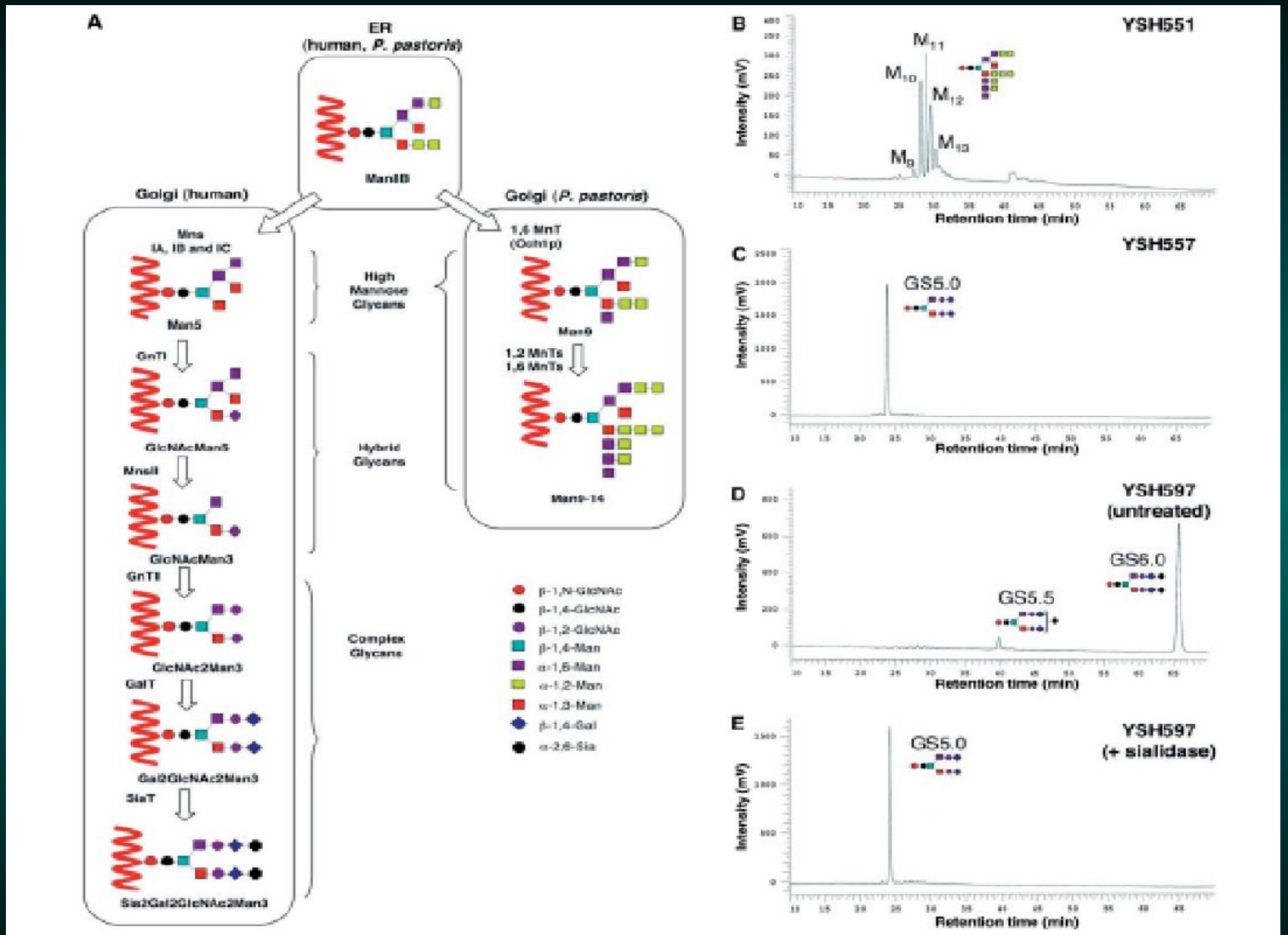



**Fig. 1.** N-linked glycosylation pathways and characterization of N-linked glycans released from recombinant rEPO. **(A)** Representative N-linked glycosylation pathways in humans and *P. pastoris*. Mns: α 1,2- mannosidase; MnsII: mannosidase II; GnTI: β 1,2-*N*-acetylglucosaminyltransferase I; GnTII: β 1,2-*N*-acetylglucosaminyltransferase II; GalT: β 1,4-galactosyltransferase; SiaT: α 2,6-sialyltransferase; MnT: mannosyltransferase. (**B** to **D**) rEPO secreted from *P. pastoris* strains YSH551 (B), YSH557 (C), and YSH597 (D) was purified from culture supernatants by Ni-affinity chromatography. Glycans were released by PNGase-F treatment and labeled with 2-AB before HPLC analysis. (**E**) Glycans secreted from YSH597 containing sialic acid were treated with α-2,3-/-2,6-/-2,8-sialidase. Elution times for commercial glycan standards corresponding to GS5.0, 5.5, and 6.0 were 24, 40, and 66 min, respectively.

# *Schematic presentation of whole IgG and different fragments*

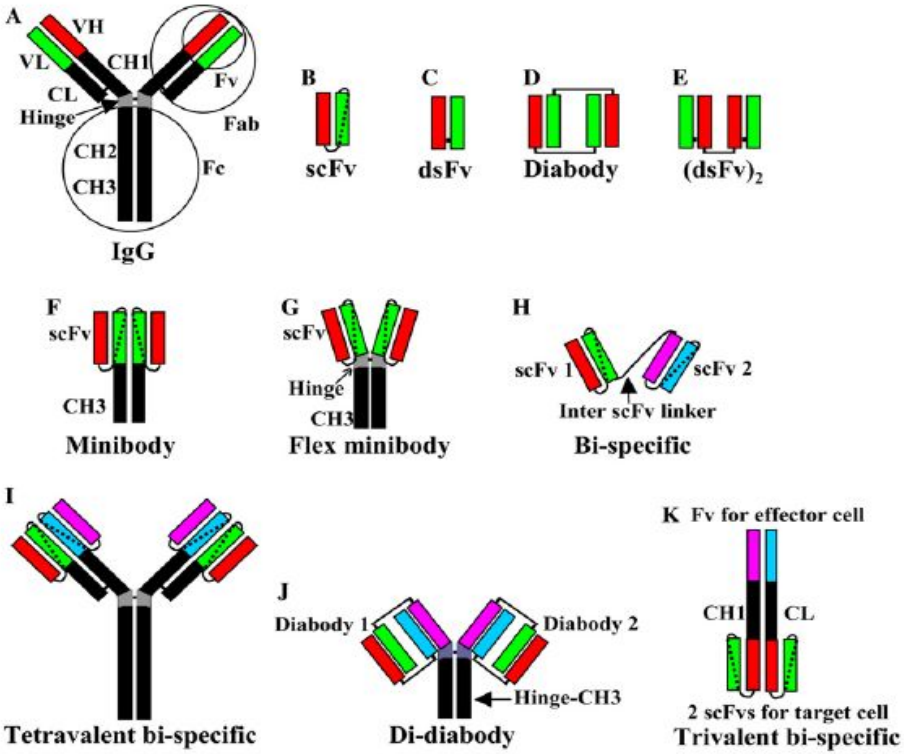
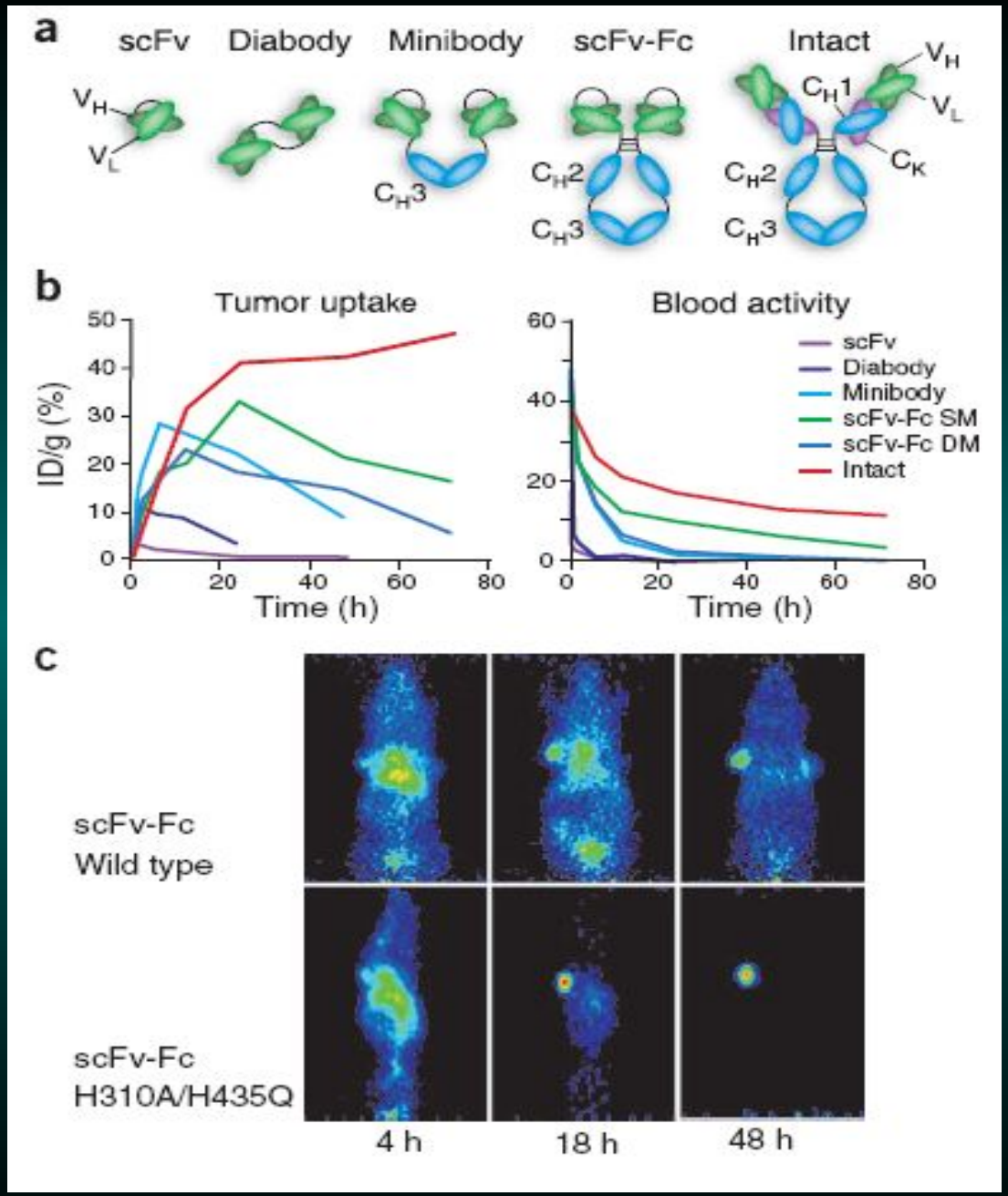



Fig. 1. Schematic representation of a whole IgG molecule (A) and different fragments that have been engineered (B–K). The diabody shown in (D) is a homodimer but can also be made in a heterodimer form when V-domains from two different antibodies are used.


a
scFv
Diabody
Minibody
scFv-Fc
Intact
$V_H$
$V_L$
$C_H3$
$C_H2$
$C_H1$
$C_K$
b
Tumor uptake
Blood activity
ID/g (%)
Time (h)
scFv
Diabody
Minibody
scFv-Fc SM
scFv-Fc DM
Intact
c
scFv-Fc
Wild type
scFv-Fc
H310A/H435Q
4 h
18 h
48 h

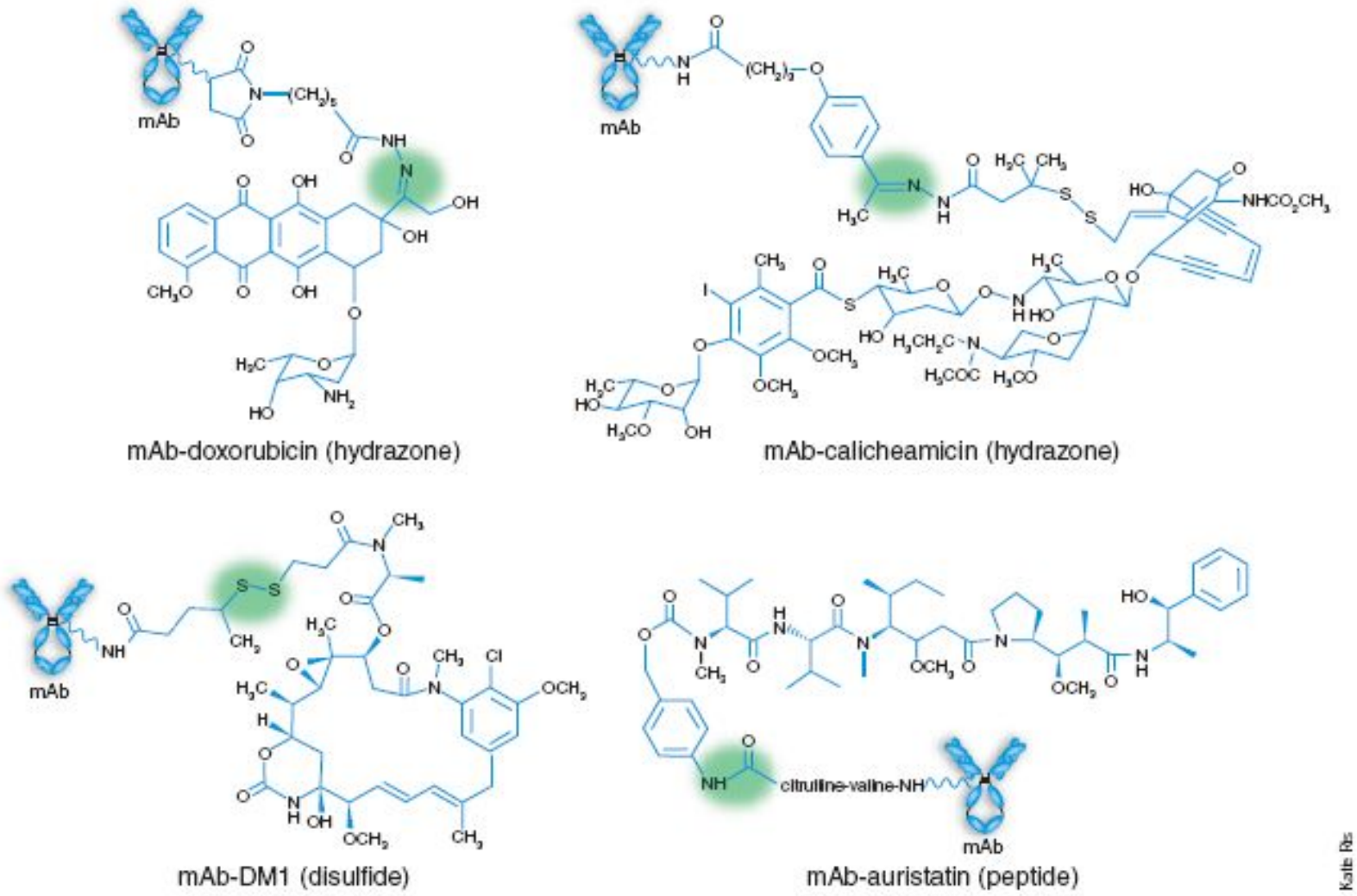
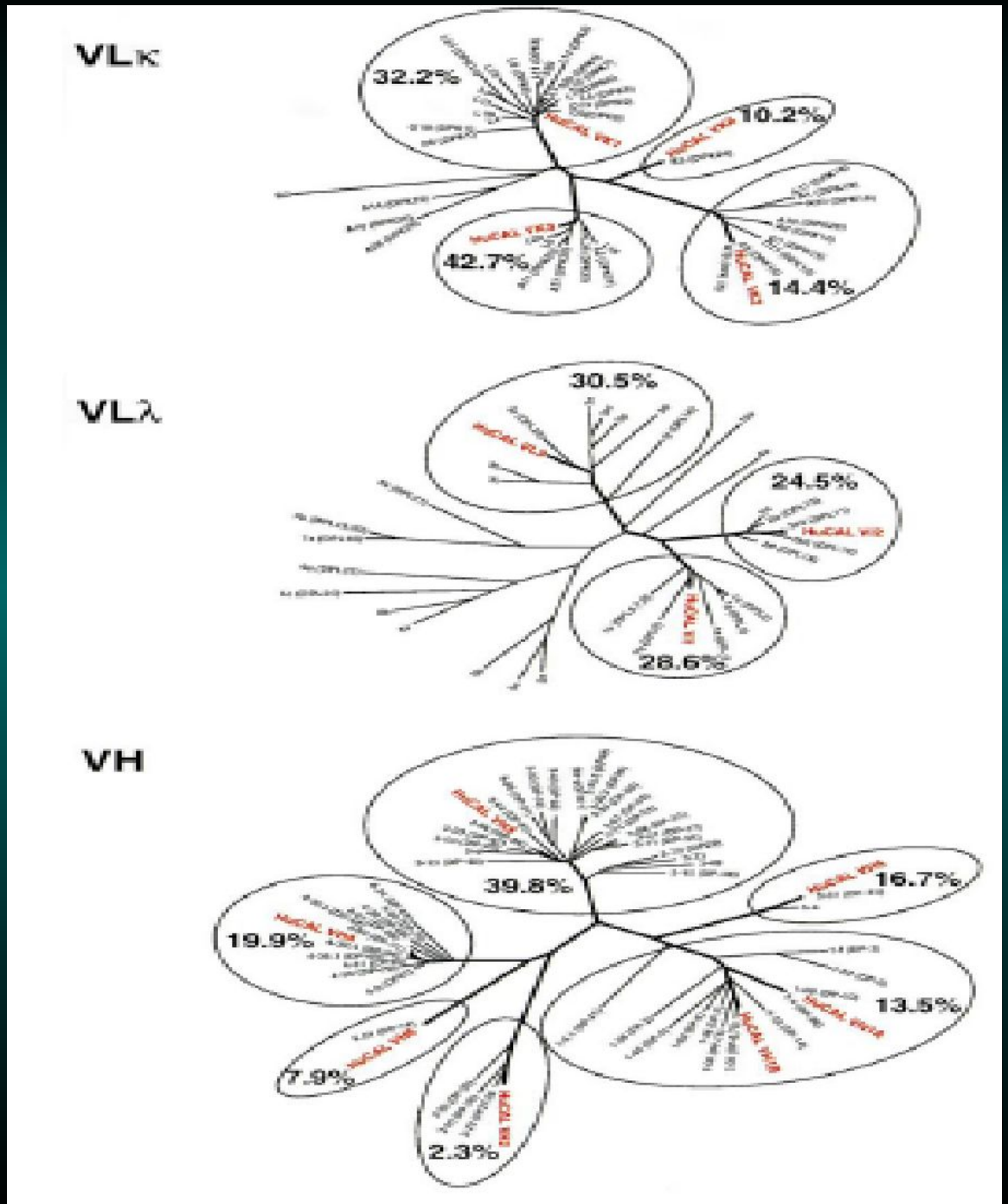



Figure 2 Chemical structures of some advanced mAb-drug conjugates. The linkers used for each drug are indicated in parentheses, and the labile bonds leading to drug release are shaded. For the examples shown, hydrazones release drug under acidic conditions within the lysosomes of target cells, disulfides undergo intracellular reduction and the peptides are enzymatically hydrolyzed by lysosomal proteases.

**Table 1.** Frequency of germline family usage and corresponding types of canonical structures

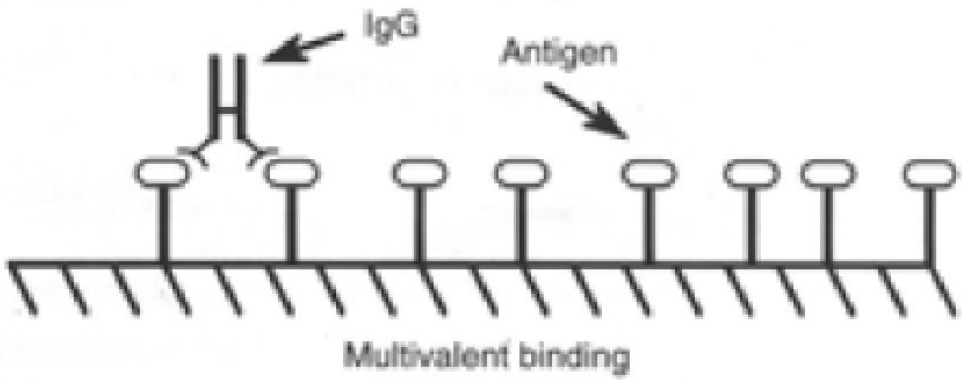
| | | Frequently used germline genes | | | Canonical structure prediction | | Chosen HuCAL canonical structures | |
|---|---|---|---|---|---|---|---|---|
| Subfamily | Family usage (%) | Locus | DP name | Usage (%) | CDR1 | CDR2 | CDR1 | CDR2 |
| | | 1-69 | DP-10 | 6 | | H2-2 | | H2-2 |
| VH1 | 19 | 1-18 | DP-14 | 4 | H1-1 | H2-3 | H1-1 | H2-3 |
| | | 1-02 | DP-8 | 4 | | | | |
| VH2 | 2 | - | - | - | H1-3 | H2-1 | H1-3 | H2-1 |
| | | 3-23 | DP-47 | 12 | | H2-1 | | |
| VH3 | 34 | 3-30.3 | DP-46 | 5 | H1-1 | H2-3 | H1-1 | H2-3 |
| | | 3-48 | DP-51 | 3 | | H2-4 | | |
| | | 4-34 | DP-63 | 5 | H1-1 | | | |
| VH4 | 12 | | | | H1-2 | H2-1 | H1-1 | H2-1 |
| | | 4-59 | DP-71 | 4 | H1-3 | | | |
| | | 5-51 | DP-73 | 16 | | | | |
| VH5 | 19 | | | | H1-1 | H2-2 | H1-1 | H2-2 |
| | | 5-a | - | 3 | | | | |
| VH6 | 14 | 6-01 | DP-74 | 14 | H1-3 | H2-5 | H1-3 | H2-5 |
| | | O12 | DPK9 | 9 | L1-2 | L2-1 | L1-2 | L2-1 |
| Vκ1 | 32 | | | | | | | |
| | | O8 | DPK1 | 7 | | | | |
| Vκ2 | 7 | A3 | DPK15 | 4 | L1-3 | L2-1 | L1-4 | L2-1 |
| | | | | | L1-4 | | | |
| | | A27 | DPK22 | 29 | L1-2 | | | |
| Vκ3 | 51 | L6 | - | 11 | L1-6 | L2-1 | L1-6 | L2-1 |
| | | L2 | DPK21 | 10 | | | | |
| Vκ4 | 10 | B3 | DPK24 | 10 | L1-3 | L2-1 | L1-3 | L2-1 |
| Vκ5-7 | 0 | - | - | - | L1-2 | L2-1 | - | - |
| | | 1b | DPL5 | 13 | 13 | | | |
| Vλ1 | 31 | | | | 14 | 7 | 13 | 7 |
| | | 1c | DPL2 | 11 | | | | |
| | | 2a2 | DPL11 | 18 | | | | |
| Vλ2 | 33 | | | | 14 | 7 | 14 | 7 |
| | | 2e | DPL12 | 11 | | | | |
| Vλ3 | 29 | 3r | DPL23 | 15 | 11 | 7 | 11 | 7 |
| | | | | | 12 | 7 | | |
| Vλ4-10 | 8 | - | - | - | 13 | 11 | - | - |
| | | | | | 14 | 12 | | |

The human immunoglobulin germline subfamilies are listed together with their percentage usage as calculated by comparison with rearranged sequences. The percentage usage is determined from using the initial database of rearranged sequences with 1006 entries. The percentage usage calculated from the updated database with 2460 entries is given in Figure 2. The most frequently used germline genes according to our analysis are also given (locus name as well as DP nomenclature, see Tomlinson *et al.* (1992) for $V_H$, Cox *et al.* (1994) for Vκ, and Williams *et al.* (1996) for Vλ) together with their corresponding usage (derived from analysis of the smaller database). For details of the calculation, see the text. The canonical conformations that are present in each subfamily are shown together with the canonical conformations that have been chosen for HuCAL design. The canonical structure nomenclature is according to Chothia *et al.* (1992) for $V_H$, Tomlinson *et al.* (1995) for Vκ, and Williams *et al.* (1996) for Vλ.


VLκ
32.2%
10.2%
42.7%
14.4%
VLλ
30.5%
24.5%
28.6%
VH
39.8%
16.7%
19.9%
13.5%
7.9%
2.3%

## Box 3. Affinity and avidity

As defined in Box 1, the affinity constant $K_a$ describes the 'strength' of the interaction between two binding partners in solution. Whenever one of the binding partners is present in multiple copies on a support that prohibits free diffusion (e.g. antigens on a cellular membrane or on a microtitre plate), the observed affinity constant $K_a^{obs}$ corresponds to the true $K_a$ constant only if the binder in solution is monovalent. Under the same conditions, a multivalent binder (e.g. an IgG) displays a higher apparent affinity (the 'functional affinity', or avidity) by virtue of rebinding effects and chelate binding. This apparent affinity constant is not a universal thermodynamic constant but is dependent on the antigen density on the solid support. While rebinding effects and multivalent binding are to be avoided to obtain 'true' affinity constants, avid interactions are the basis of several important biological and technological processes, for instance the stable binding of multivalent antibodies to cells exploited in flow cytometry. Avid 'apparent' affinity constants can be measured by a variety of methods, such as detecting cell binding by radio- or fluorescently-labeled antibodies with methodologies similar to those listed in Box 2.

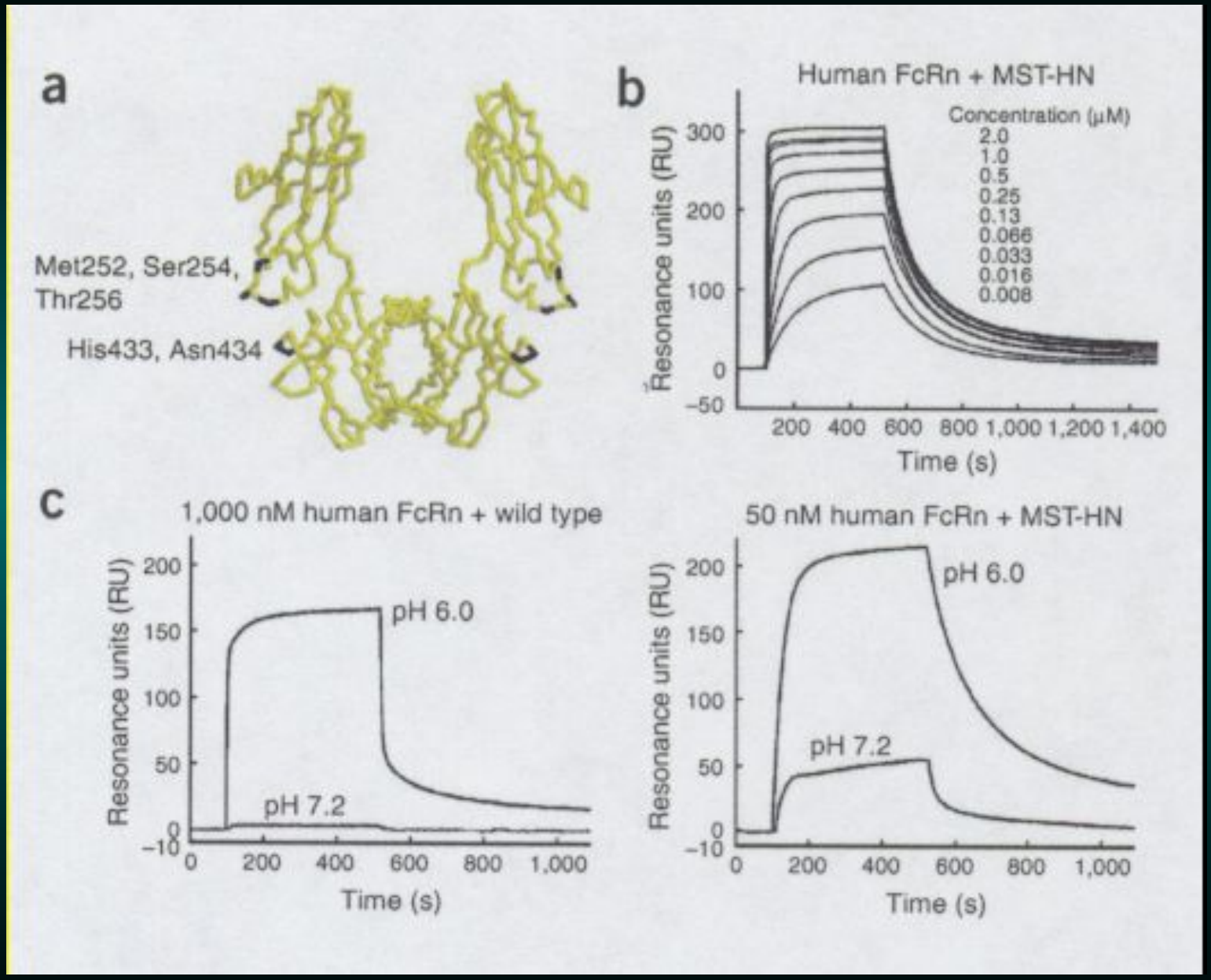


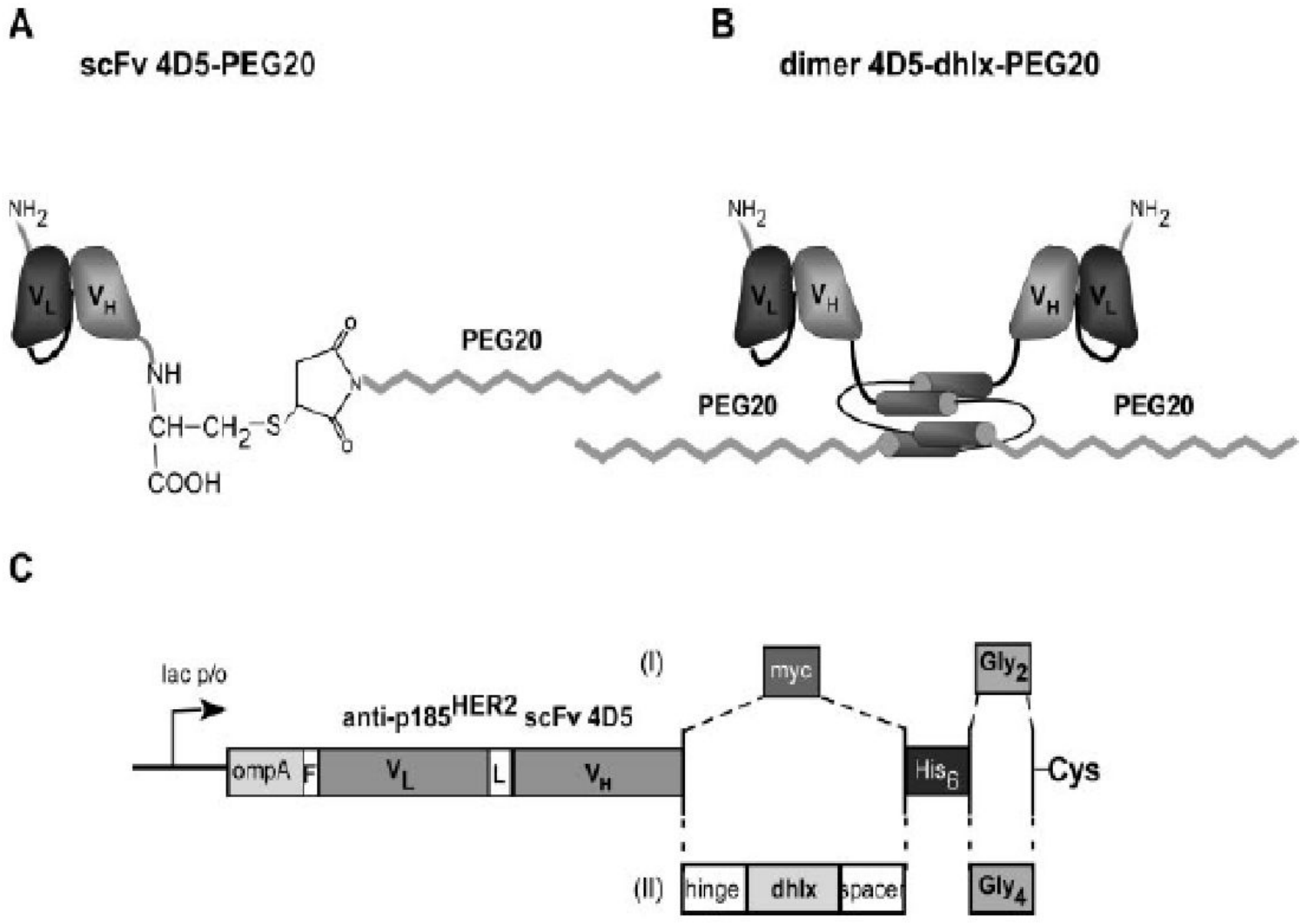

## Binding Site Barrier Hypothesis

*Anti –erbB2 scFv*
*Affinity from 10-7 to 10-9*
*Activity in cellular test not incriesed*

*Binding activity from 2 to 400 nM*
*Diabody and tumor targeting better for 2 nM*

*Anti mesothelin SS antibody (scFv) 0,8 nM 10 fold active that 11 nM,*
*But another mutant with 0,2 nM affinity have low activity that 0,8 nM*


a
Met252, Ser254, Thr256
His433, Asn434
b
Human FcRn + MST-HN
Concentration (μM)
2.0
1.0
0.5
0.25
0.13
0.066
0.033
0.016
0.008
Resonance units (RU)
300
200
100
0
−50
200 400 600 800 1,000 1,200 1,400
Time (s)
c
1,000 nM human FcRn + wild type
Resonance units (RU)
200
150
100
50
0
−10
pH 6.0
pH 7.2
0 200 400 600 800 1,000
Time (s)
50 nM human FcRn + MST-HN
Resonance units (RU)
200
150
100
50
0
−10
pH 6.0
pH 7.2
0 200 400 600 800 1,000
Time (s)


A
scFv 4D5-PEG20
B
dimer 4D5-dhlx-PEG20
NH2
VL
VH
NH
CH–CH2–S
COOH
PEG20
NH2
VL
VH
VH
VL
NH2
PEG20
PEG20
C
lac p/o
anti-p185HER2 scFv 4D5
ompA
F
VL
L
VH
(I)
myc
Gly2
His6
Cys
(II)
hinge
dhlx
spacer
Gly4

# *Антитела активаторы клеток иммунной системы как инструменты в онкоиммунологии*

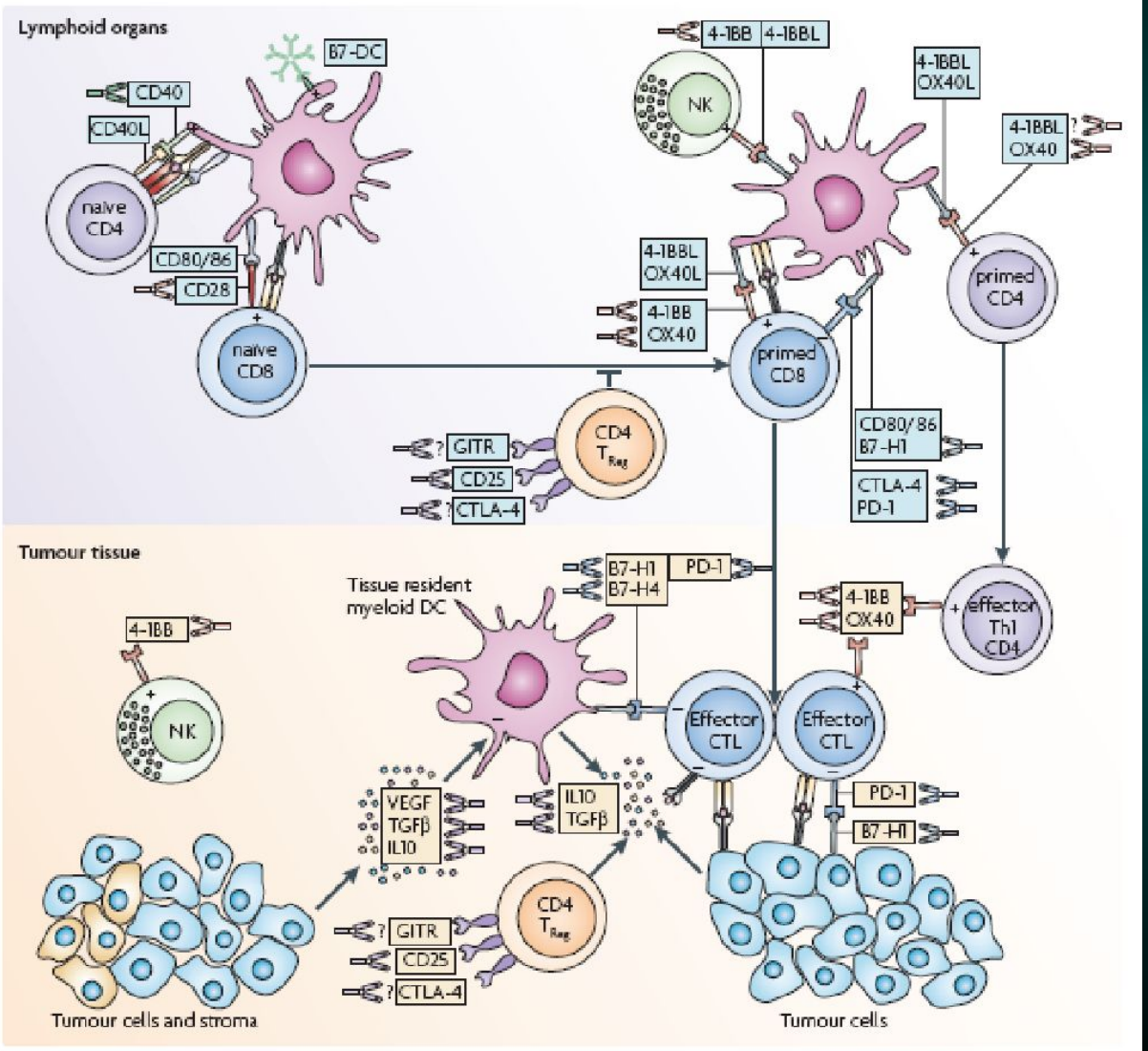



Figure 1 | **Schematic representation of points of intervention with immunostimulatory mAbs.** The rejection of

# Молекулярные мишени для активаторных – супрессорных антител

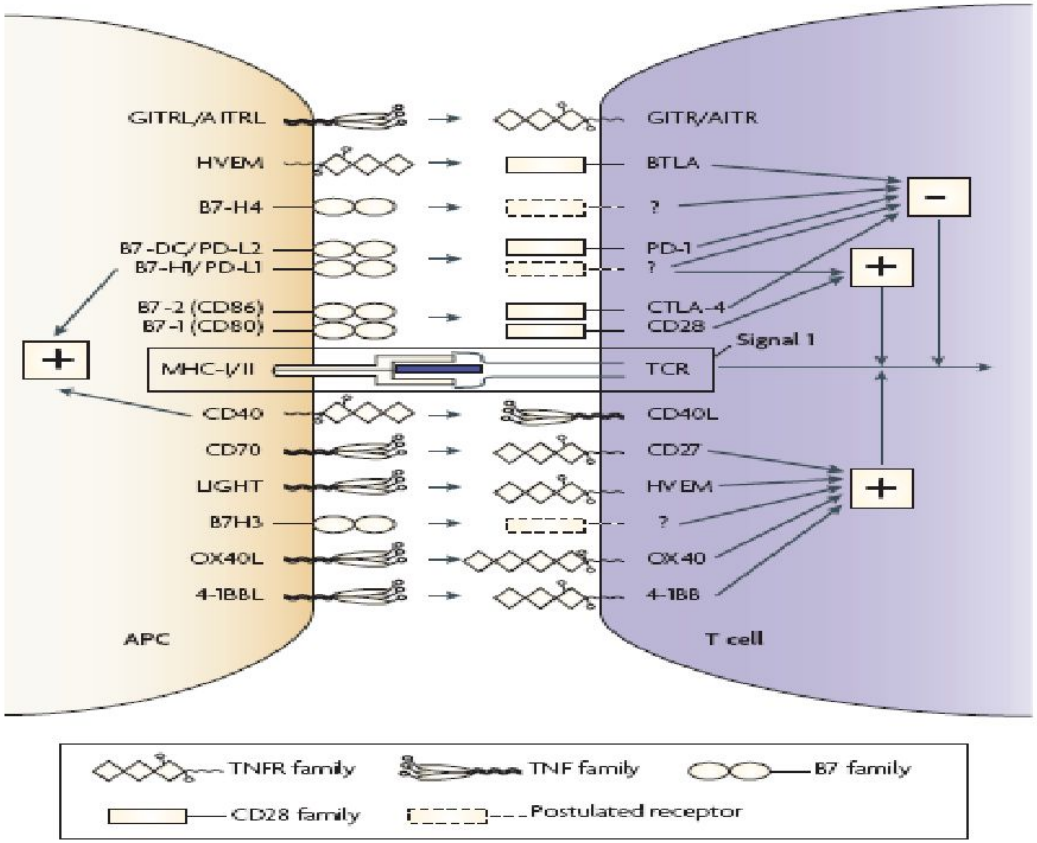



Figure 2 | **Co-stimulatory and co-inhibitory molecules targeted by immunostimulatory mAbs.** A schematic representation of surface co-signalling molecules on T cells relevant in cancer immunotherapy interacting with their ligands on an antigen-presenting cell (APC). These surface molecules belong to the tumour necrosis factor (TNF) receptor and immunoglobulin families, and their stimulation or inhibition can be exploited to increase the cellular immune response against cancer. The overactivation of co-stimulatory signals and inactivation of co-inhibitory functions can be artificially achieved by agonist and antagonist monoclonal antibodies (mAbs). Some of these lymphocyte surface molecules are constitutive (such as CD28), some are constitutively expressed on some cells and are inducible on others (such as CD27 and CD40), whereas others are induced only after antigen priming (such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), 4-1BB, OX40, CD40L, BTLA and GITR), and are therefore selectively expressed on responding lymphocytes. Inducible expression targets are conceivably more selective and less likely to cause systemic inflammatory syndromes.

# *Стратегия использования антител – иммуностимуляторов*

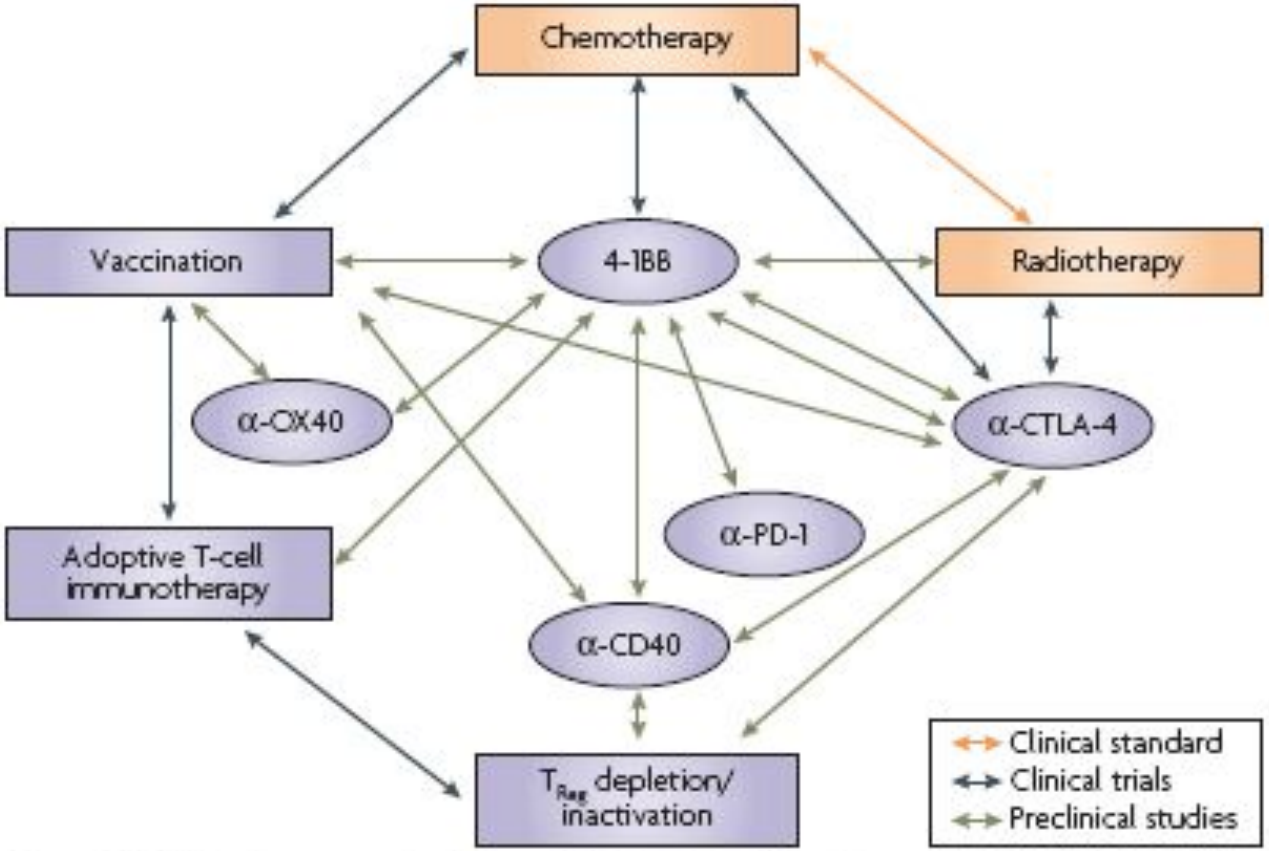



Figure 3 | **Efficacious combination strategies involving immunostimulatory mAbs.** A scheme of combinatorial therapeutic strategies with immunostimulatory monoclonal antibodies (mAbs) for cancer, in clinical and preclinical development. The status of each treatment or each combination (two-headed arrows) is referred to by the colour code. α, anti.

# *Молекулярные механизмы резистентности к терапии антителами*

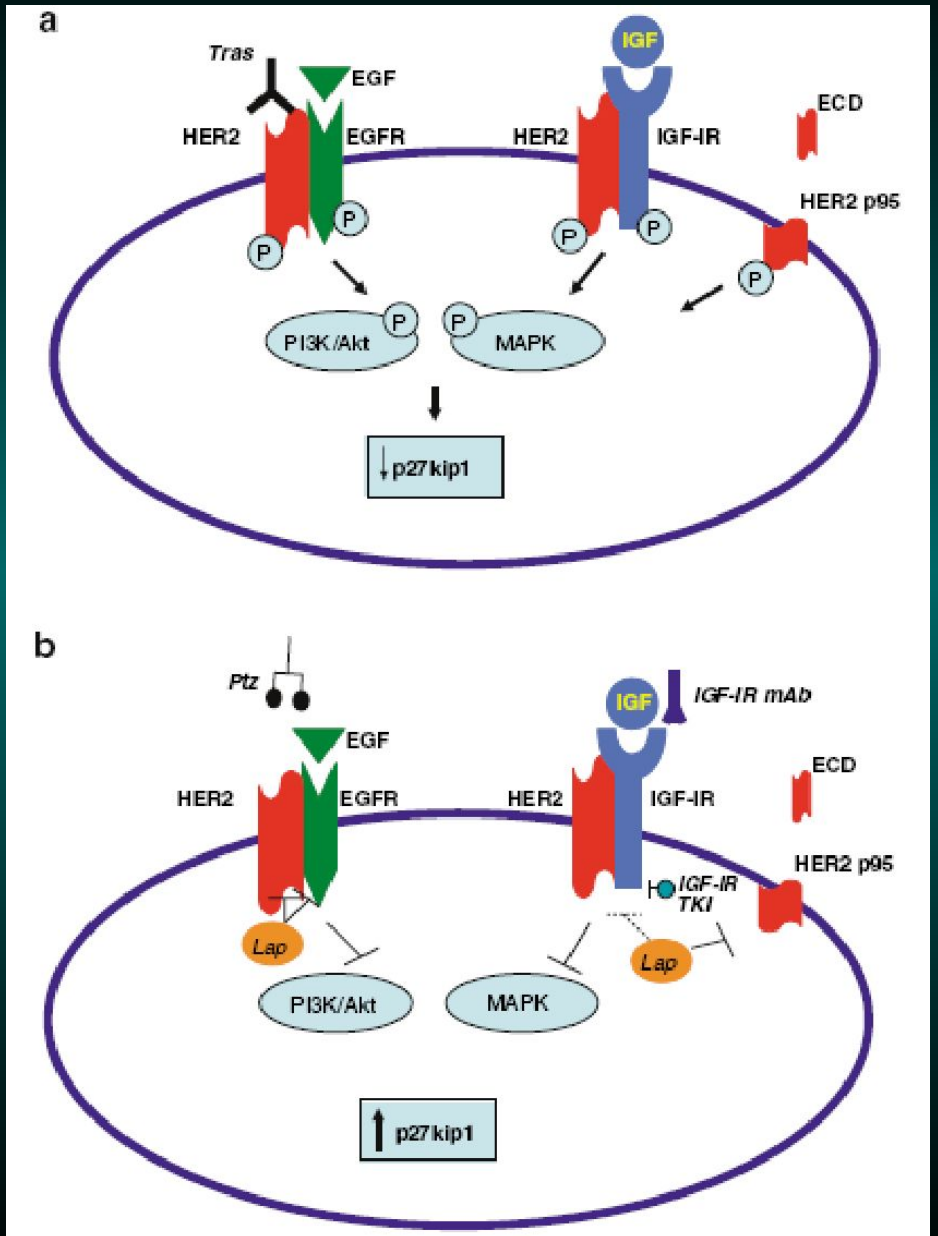



Figure 1 Trastuzumab resistance: mechanisms and novel agents.

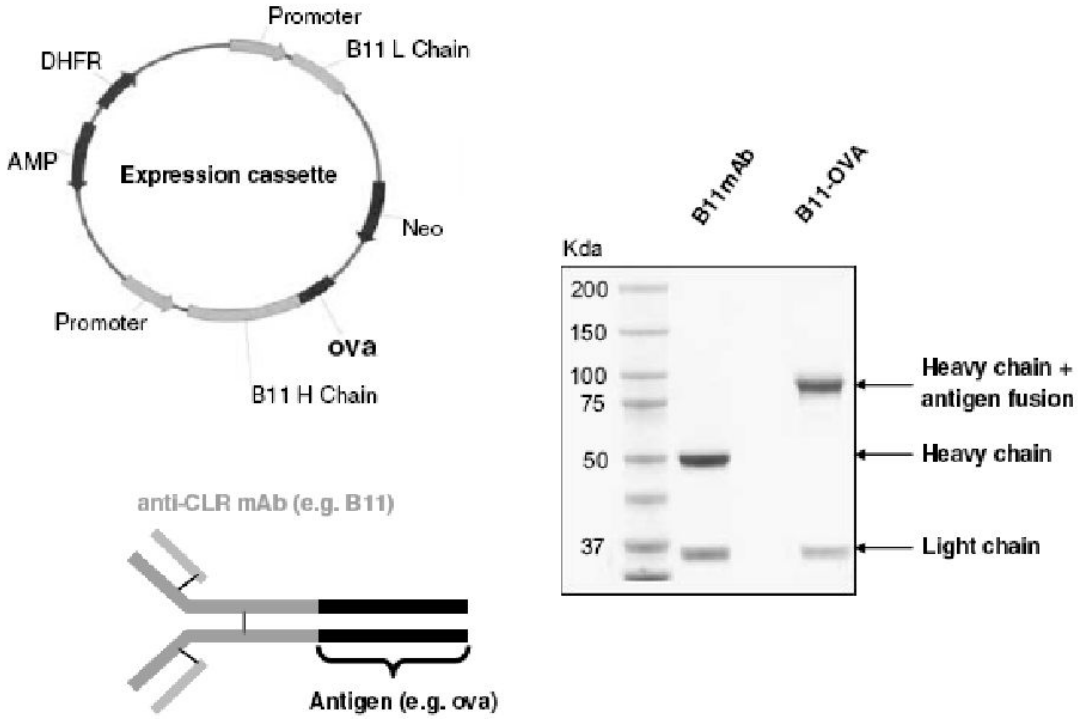



Figure 1 Illustration of a genetically engineered ATV. The illustration shows an example of an expression plasmid for generating an ATV using OVA as a model antigen. The plasmid was transfected into mammalian cells and the ATV purified by affinity chromatography. The ATV and the parental anti-MR mAb were separated by gel electrophoresis and stained with Coomasie Blue. DHFR, dihydrofolate reductase; AMP, ampicillin; NEO, neomycin; OVA, ovalbumin.

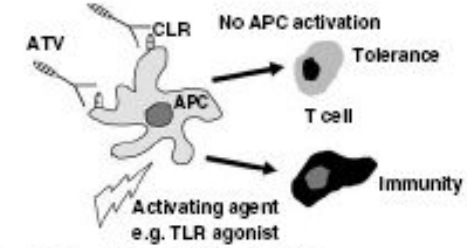
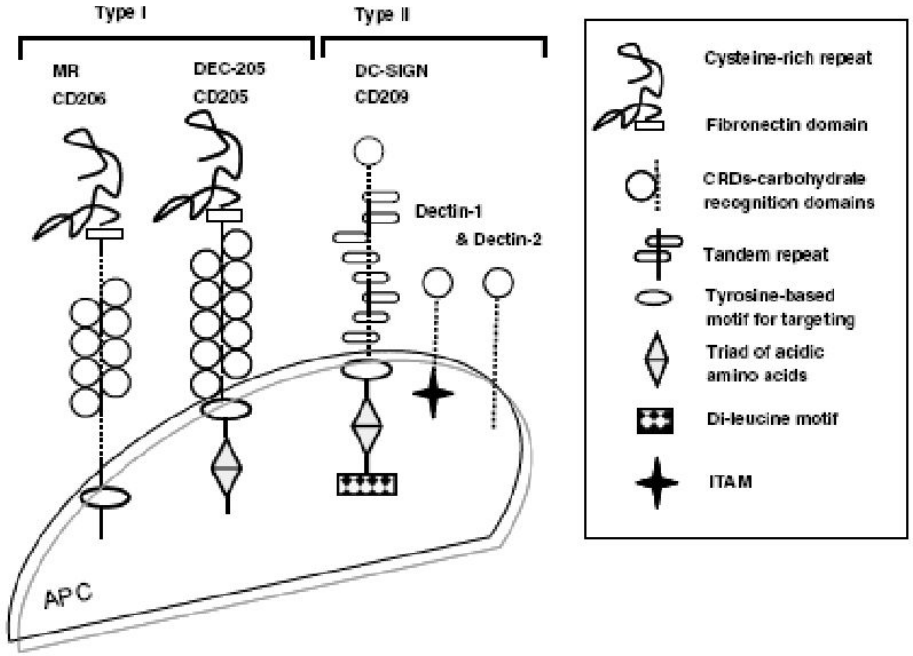



Figure 3 Illustration of ATV-mediated immune responses. ATV can elicit potent immunity when combined with appropriate APC activation, but in the absence of concomitant activation of the APC, targeted delivery of antigen may lead to tolerance. APC, antigen-presenting cell; TLR, toll-like receptor.




| CLR | Distribution | Ligand | Function |
|---|---|---|---|
| MR, CD206 | Macrophages, interstitial DCs, dermal DCs, Lymphatic endothelium | Mannose, fucose, N-acetyl glucosaimine | Antigen uptake, cell adhesion, serum glycoprotein homeostasis |
| DEC-205 CD205 | DCs, Langerhans Cells, thymic and gut epithelial cells, | Unknown | Antigen uptake (?) |
| DC-SIGN CD209 | DCs, Hofbauer cells, Decidual macrophages. Alveolar Macrophages. | Mannan, HIV gp120, ICAM-3 | Antigen uptake, DC-T cell interactions |
| Dectin -1 β-glucan receptor | Macrophages, Monocytes, Neutrophils, DCs | Beta-glucans | Binding and uptake of fungi |
| Dectin-2 | Macrophages | Fungal antigens | Binding and recognition of fungi |

ICAM- intercellular adhesion molecule; ITAM- immunoreceptor tyrosine-based activation motif.

Figure 2 CLRs targeted by ATVs.

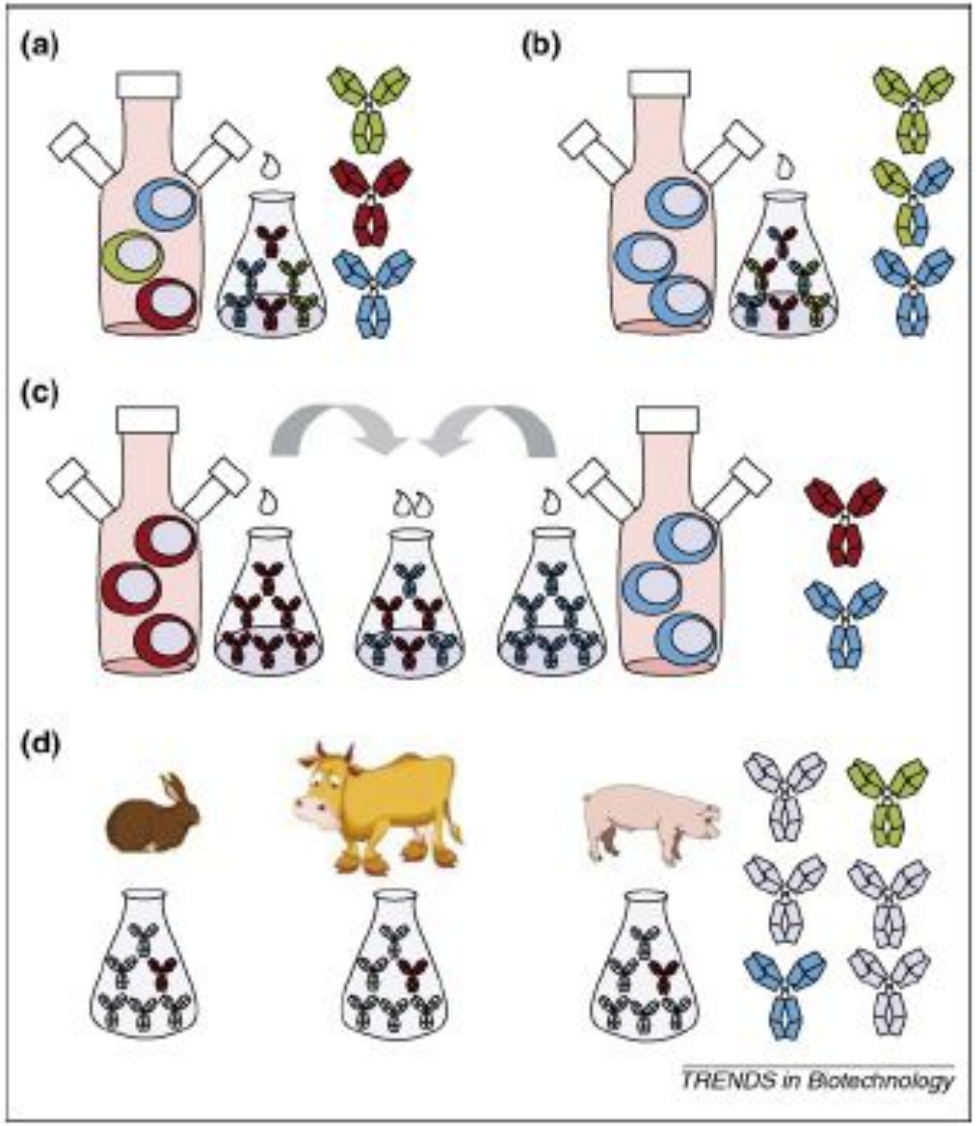



**Figure 2.** Manufacturing of cocktails of human mAbs. (a) Polyclonal cell bank producing up to 25 bivalent mAbs. (b) Clonal cell lines producing cocktails of 3–5 mAbs, consisting of bivalent and bispecific species. (c) Multiple clonal cell lines each producing single bivalent mAbs that are mixed to form a cocktail. (d) Transgenic animals producing human polyclonal antibodies in serum consisting of specific (colored) and non-specific (grey) IgG molecules.

Table I. Targets for which cocktails of mAbs have shown increased efficacy in *in vitro* and *in vivo* preclinical testing

| Target | Number of mAbs in mixture | Increased potency | | Clinical testing phase | Refs |
|---|---|---|---|---|---|
| | | *In vitro* | *In vivo* | | |
| **Viruses** | | | | | |
| HIV | 3 | ✓ | ✓ | | [14] |
| respiratory syncytial virus | 2–10 | ✗ | ✗ | | [19–21] |
| vesicular stomatitis virus | 2 | ✓ | ✗ | | [22] |
| Newcastle disease virus | 4 | ✓ | ✗ | | [23] |
| herpes simplex virus | 2 | ✓ | ✗ | | [37] |
| hepatitis B virus | 2 | ✓ | ✓ | II | [24] |
| hepatitis C virus | 2 | ✓ | ✓ | Ia | [25] |
| rubella virus | 2 | ✗ | ✗ | | [26] |
| La Crosse virus | 2 | ✓ | ✗ | | [27] |
| rabies virus | 2 | ✓ | ✓ | I | [28] |
| **Soluble molecules** | | | | | |
| botulinum toxin | 3 | ✓ | ✓ | | [29] |
| IL-6 | 3 | ✓ | ✓ | | [7] |
| IFN-2α | 3 | ✓ | ✓ | | [30] |
| tetanus toxin | 2 | ✓ | ✓ | | [31] |
| HGF | 3 | ✓ | ✓ | | [32] |
| Pneumolsyin | 3 | ✓ | ✓ | | [33] |
| **Cell-bound molecules** | | | | | |
| HER2/neu | 2–3 | ✓ | ✓ | II | [5,34] |
| CD20 × CD22 | 2 | ✓ | ✓ | II | [6] |
| EGF-R × VEGF-R | 2 | ✓ | ✓ | | [35] |
| CD4 × TNF-α (soluble) | 2 | ✓ | ✓ | | [36] |
| IL-1R × TNF-α (soluble | 2 | ✓ | ✓ | | [36] |

Antibody cocktails that are in early phases of clinical trials are indicated by roman numbers.

Abbreviations: EGF-R, epidermal-growth-factor receptor; HGF, hepatocyte growth factor; HIV, human immunodeficiency virus; IFN, interferon; IL-1, interleukin-1; IL-6, interleukin-6; TNF, tumor necrosis factor; VEGF-R, vascular endothelial-growth-factor receptor.

# *Антитела могут помогать патогенам*

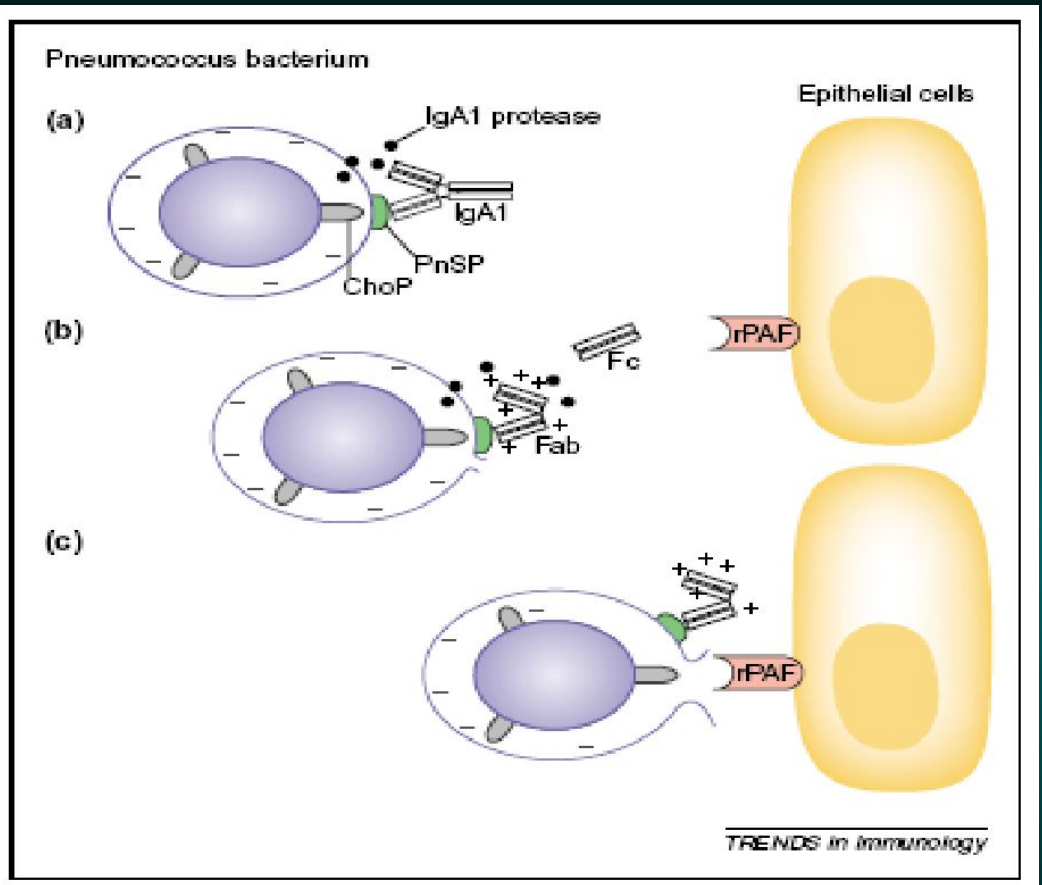



Fig. 1. IgA-Fab fragments 'unmask' ChoP (phosphorylcholine) from behind the polysaccharide capsule of *Streptococcus pneumoniae*, enabling enhanced bacterial binding to epithelial cells through the receptor for platelet-activating factor (rPAF). (a) Host IgA1 recognizes and binds to capsular polysaccharide (PnPS); *S. pneumoniae* releases IgA1 protease. (b) IgA1 protease cleaves IgA1 into Fab and Fc portions, leaving the Fab fragment attached. The cationic charge exposed on the Fab fragments by protease activity neutralises the negative charge of the capsule and results in disruption of the capsule at the site of antigen–antibody recognition. (c) The capsule is peeled away enabling the exposure of the bacterial surface ligand ChoP. Unmasking of ChoP surface molecules enables increased bacterial adherence to pharyngeal epithelial cells through the interaction with rPAF.

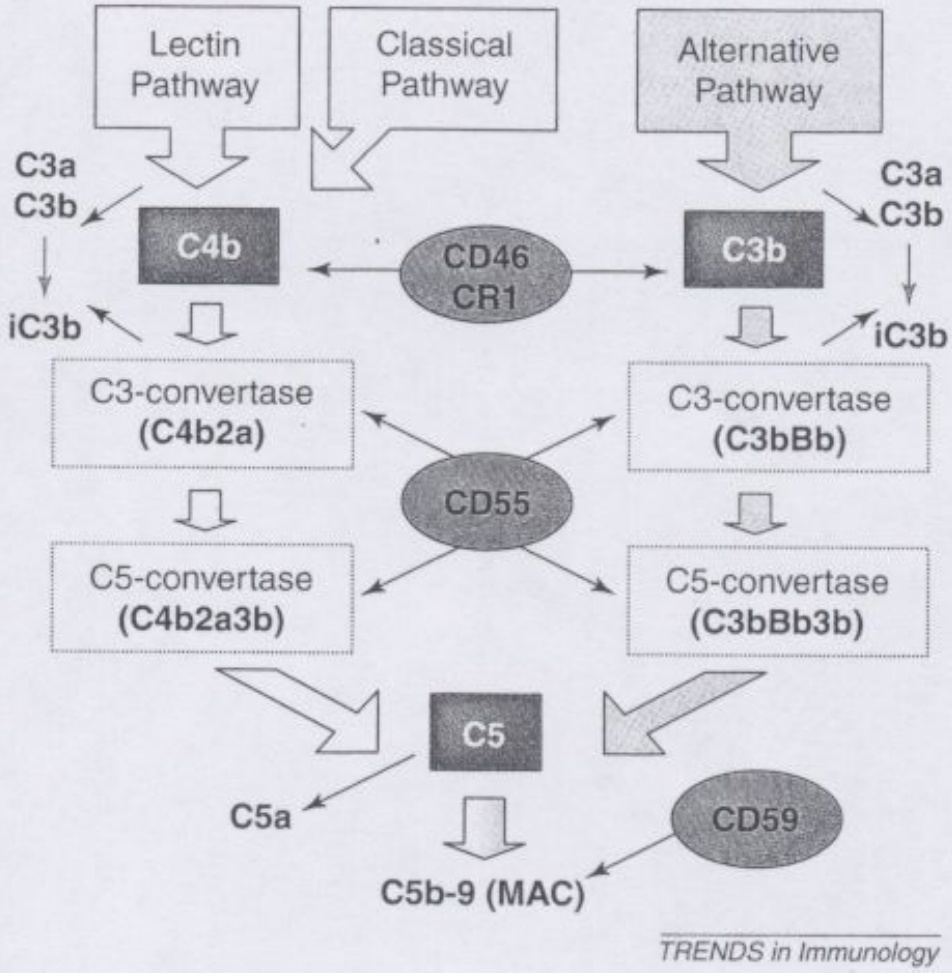



**Figure I.** The human complement system and complement regulatory proteins CR1, CD46, CD55 and CD59.

**Table 1. Currently approved mAbs for treating cancer and mAbs tested in Phase III clinical trials[a]**

| Isotype[b]/origin | Target | Mab names | Conjugated | Cancer type | Approved |
|---|---|---|---|---|---|
| hIgG1 | HER2/neu | Trastuzumab (Herceptin®) | No | Breast cancer | Yes (US) |
| hIgG1 | VEGF | Bevacizumab | No | Breast cancer, colorectal cancer, renal cell carcinoma, non-small cell lung cancer | Phase III |
| hIgG1 | CD33 | Zamyl™ | No | Acute myelogenous leukemia | Phase III |
| hIgG1 | CD52 | Alemtuzumab (Campath®) | No | Chronic lymphatic leukemia | Yes (US) |
| hIgG1 | CD22 | Epratuzumab | | Non-Hodgkin lymphoma | Phase III |
| hIgG4 | CD33 | Gemtuzumab (Mylotarg®) | Yes (cali-cheamicin) | Acute myelogenous leukemia | Yes (US) |
| cIgG1 | EGFR1 | Cetuximab | No | Colorectal cancer | Yes (Switzerland) |
| cIgG1 | CD20 | Rituximab (Rituxan®) | No | Non-Hodgkin lymphoma | Yes (US) |
| cIgG1 | DNA-associated antigens | Cotara™ | Yes ($^{131}$I) | Glioma | Phase III |
| mIgG2a | Ep-CAM | Edrecolomab | No | Colorectal cancer | Yes (Germany) |
| mIgG2a | GD3 | Mitomomab | No | Small cell lung cancer | Phase III |
| mIgG2a | CD20 | Tositumomab (Bexxar®) | Yes ($^{131}$I) | Non-Hodgkin lymphoma | Yes |
| mIgG1 | CEA | CeaVac™ | No | Colorectal cancer | Phase III |
| mIgG1 | CD20 | Ibritumomab (Zevalin™) | Yes ($^{90}$Y) | Non-Hodgkin lymphoma | Yes (US) |
| mIgG1 | CA125 | OvaRex® | No | Ovarian carcinoma | Phase III |
| mIgG1 | MUC1 | Pemtumomab | Yes ($^{90}$Y) | Ovarian carcinoma | Phase III |

[a]Abbreviations: CEA, carcino-embryonic antigen; cIg, chimerized Ig; EGFR, epithelial growth factor receptor; Ep-CAM, epithelial cell adhesion molecule; HER2/neu, EGFR2; hIg, humanized Ig; mAbs, monoclonal antibodies; mIg, mouse Ig; MUC1, human mucin 1 (episialin) VEGF, vascular endothelial growth factor.

[b]In general, the following isotypes efficiently fix human complement: mIgG2a, mIgG2b, mIgG3, mIgM, hIgG1, hIgG4, hIgM.

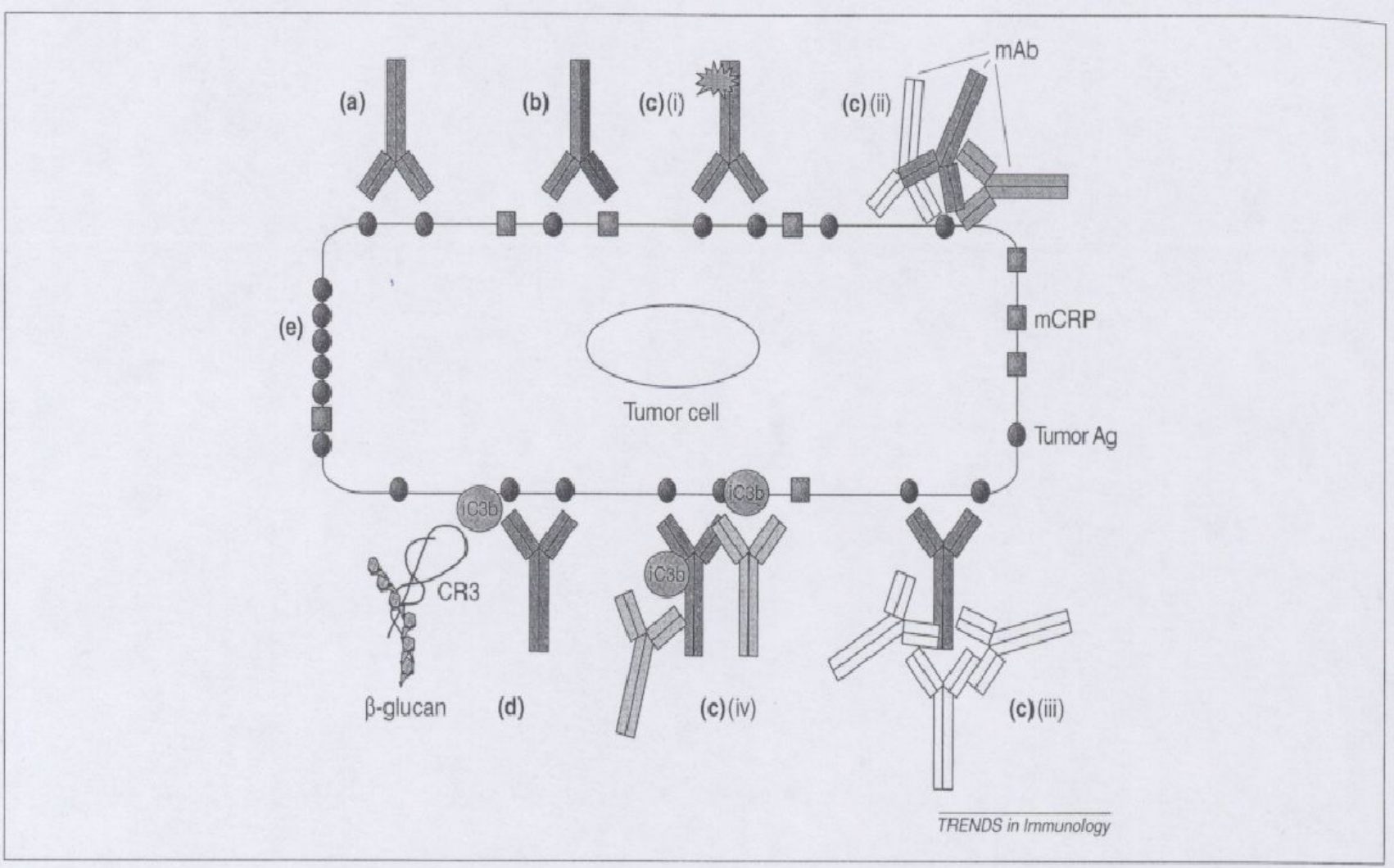



**Figure 2.** Strategies to improve monoclonal antibody (mAb)-mediated immunotherapy of cancer. **(a)** Engineering of a mAb to improve complement-activating properties. **(b)** Blocking the effect of membrane-bound complement regulatory proteins (mCRPs) with a bispecific mAb directed against a tumor antigen (Ag) and a mCRP. **(c)** Overwhelming the effect of mCRP, by: (i) a mAb conjugated to a complement-activating protein [e.g. cobra venom factor (CVF) or C3b]; (ii) a cocktail of mAbs directed against multiple epitopes of a tumor-associated antigen; (iii) secondary mAbs directed against the tumor-specific mAb; and (iv) mAbs directed against deposited complement fragments (iC3b). **(d)** Enhancing the CR3-dependent cellular cytotoxicity (CR3-DCC) of iC3b-opsonized tumor cells by priming leukocyte CR3 with soluble β-glucan. **(e)** Downregulating the expression of mCRP or increasing the expression of tumor-associated antigens with cytokines or gene therapy.

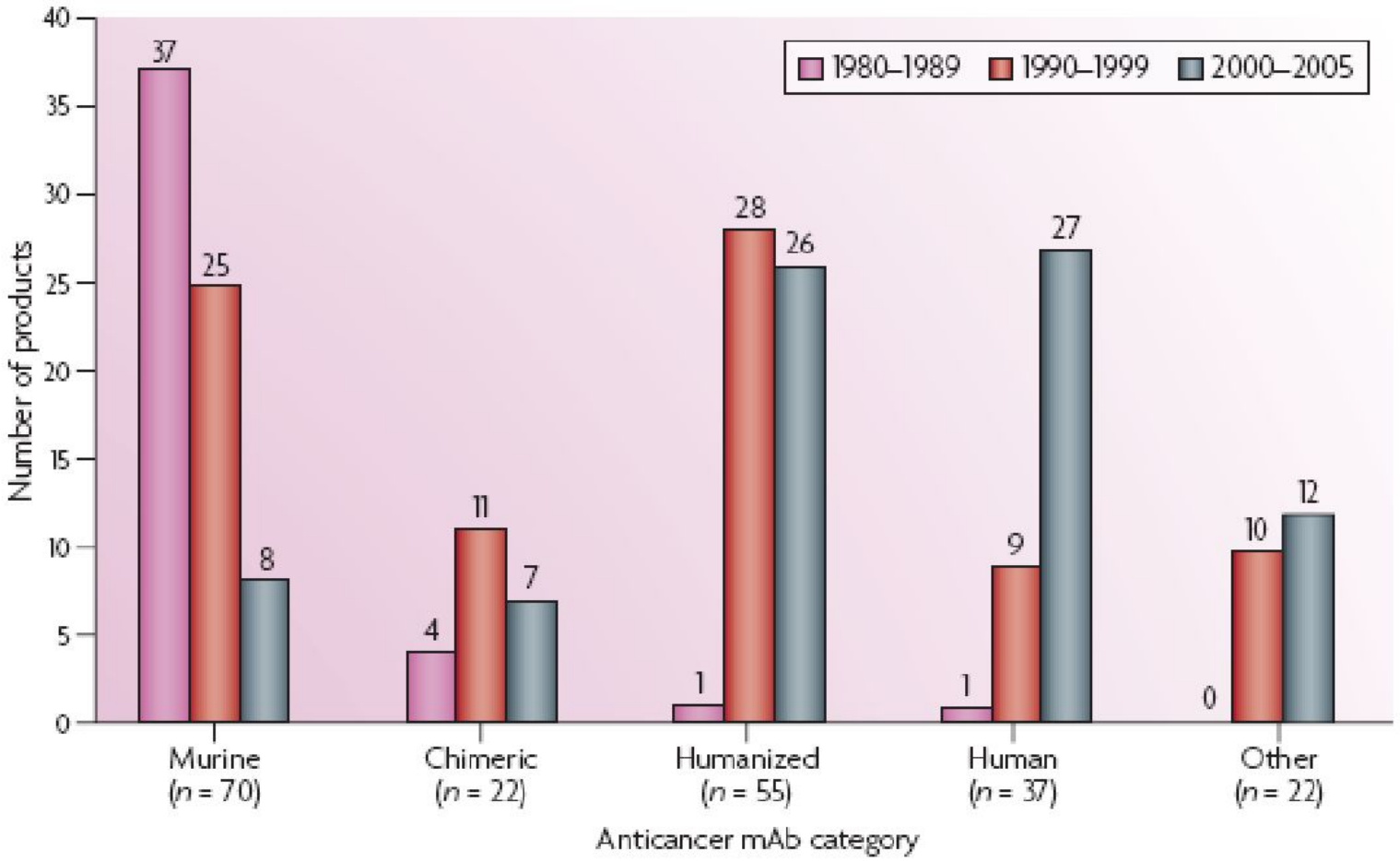



Figure 1 | **Categories of monoclonal antibody cancer therapeutics entering clinical study during 1980–1989, 1990–1999 and 2000–2005.** The 'Other' category includes bispecific monoclonal antibodies (mAbs) (13 products), primatized mAbs (2 products) and mAbs of unknown category (7 products).

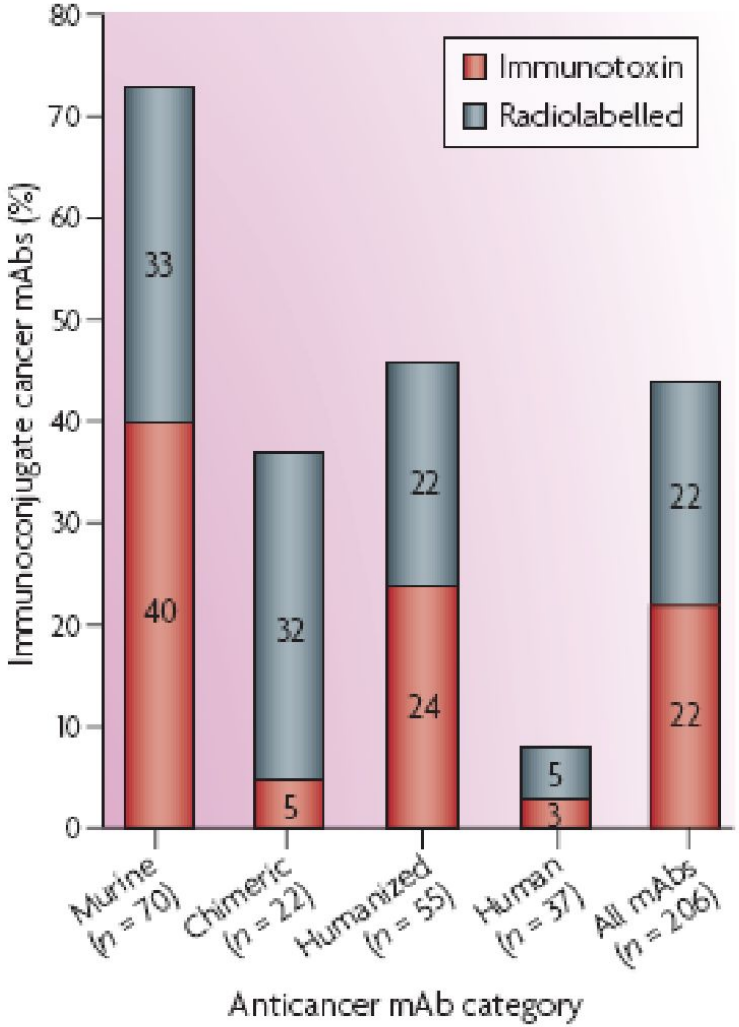


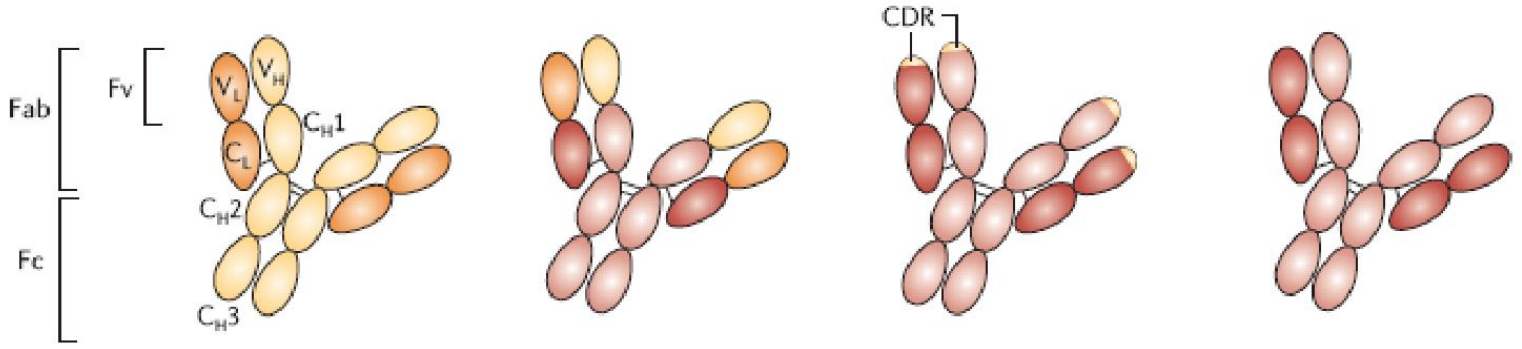

Figure 2 | **Modes of action for immunoconjugate cancer therapeutics in clinical study, 1980–2005.** mAb, monoclonal antibody.

Table 2 | **Targets of anticancer monoclonal antibodies in clinical study**

| Target | All mAbs* | Murine mAbs | Chimeric mAbs | Humanized mAbs | Human mAbs | Immuno-conjugated mAbs (any category) |
|---|---|---|---|---|---|---|
| 5T4 oncofetal antigen | 2 | 1 | 0 | 0 | 0 | 1 |
| Integrin αvβ3 | 2 | 0 | 0 | 2 | 0 | 0 |
| CA IX | 2 | 0 | 2 | 0 | 0 | 1 |
| CD5 | 2 | 2 | 0 | 0 | 0 | 1 |
| CD19 | 3 | 2 | 0 | 0 | 0 | 2 |
| CD20 | 10 | 4 | 2 | 2 | 1 | 4 |
| CD22 | 6 | 3 | 0 | 3 | 0 | 6 |
| CD30 | 4 | 0 | 1 | 0 | 1 | 0 |
| CD33 | 6 | 1 | 0 | 5 | 0 | 5 |
| CD40 | 2 | 0 | 0 | 1 | 1 | 0 |
| CD44V6 | 3 | 0 | 1 | 2 | 0 | 3 |
| CD55 | 2 | 1 | 0 | 0 | 1 | 1 |
| CD56 | 3 | 2 | 0 | 1 | 0 | 3 |
| CEA | 9 | 4 | 1 | 3 | 0 | 7 |
| CTA1 | 2 | 0 | 0 | 0 | 2 | 2 |
| CTLA-4 | 2 | 0 | 0 | 0 | 2 | 0 |
| EGFR | 12 | 3 | 2 | 3 | 3 | 3 |
| EpCAM | 17 | 8 | 2 | 5 | 1 | 10 |
| FAP | 2 | 1 | 0 | 1 | 0 | 1 |
| GD2 | 3 | 1 | 1 | 1 | 0 | 1 |
| GD3 | 3 | 2 | 1 | 0 | 0 | 0 |
| HER2 | 9 | 1 | 0 | 3 | 0 | 2 |
| HLA-DR10 (MHC II) | 3 | 1 | 0 | 1 | 1 | 1 |
| IGF1R | 3 | 0 | 0 | 0 | 3 | 0 |
| IL-6 | 2 | 1 | 1 | 0 | 0 | 0 |
| LEWIS Y | 6 | 3 | 1 | 1 | 0 | 4 |
| MUC1 | 10 | 4 | 0 | 6 | 0 | 9 |
| PSMA | 6 | 5 | 0 | 0 | 1 | 3 |
| TAG-72 | 5 | 3 | 0 | 1 | 0 | 4 |
| TAL6 | 3 | 1 | 2 | 0 | 0 | 1 |
| TRAILR2 | 3 | 0 | 0 | 0 | 3 | 0 |
| VEGF | 2 | 0 | 0 | 2 | 0 | 0 |
| VEGFR2 | 3 | 0 | 1 | 0 | 1 | 0 |
| Unknown target | 11 | 5 | 1 | 1 | 3 | 4 |
| **Total** | **163** | **59** | **19** | **44** | **24** | **79** |

*All monoclonal antibodies (mAbs) include murine, chimeric, humanized, human, bispecific and primatized mAbs, as well as mAbs of unknown category. Note that the table lists molecules that were the targets of a minimum of two mAbs in clinical study between 1980 and 2005. CA, carbonic anhydrase; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CTA1, cytokeratin tumour-associated antigen 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FAP, fibroblast activation protein; HER, human epidermal growth factor receptor; HLA-DR, human leukocyte antigen-DR; IGF1R, insulin-like growth factor 1 receptor; IL, interleukin; MUC, mucin; PSMA, prostate-specific membrane antigen; TAL6, tumour-associated antigen L6; TAG-72, tumour-associated glycoprotein 72; TRAILR2, tumour-necrosis factor-related apoptosis-inducing ligand receptor 2; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

# *Antibody Humanization*




| Type of mAb | Murine | Chimeric | Humanized | Human |
|---|---|---|---|---|
| | Ibritumomab tiuxetan (CD20); IgG1κ* | Cetuximab (EGFR); IgG1κ | Trastuzumab (ERBB2); IgG1κ | Panitumumab (EGFR); IgG2 |
| | Tositumomab-$I^{131}$ (CD20); IgG2aλ* | Rituximab (CD20); IgG1κ | Bevacizumab (VEGF); IgG1 | |
| | | | Alemtuzumab (CD52); IgG1κ | |
| | | | Gemtuzumab ozogamicin (CD33); IgG4κ* | |

# *Antibody Humanization*