# Engineering of Antibodies, a Knowledge Based Approach

*Nottingham: 21st March 2007*

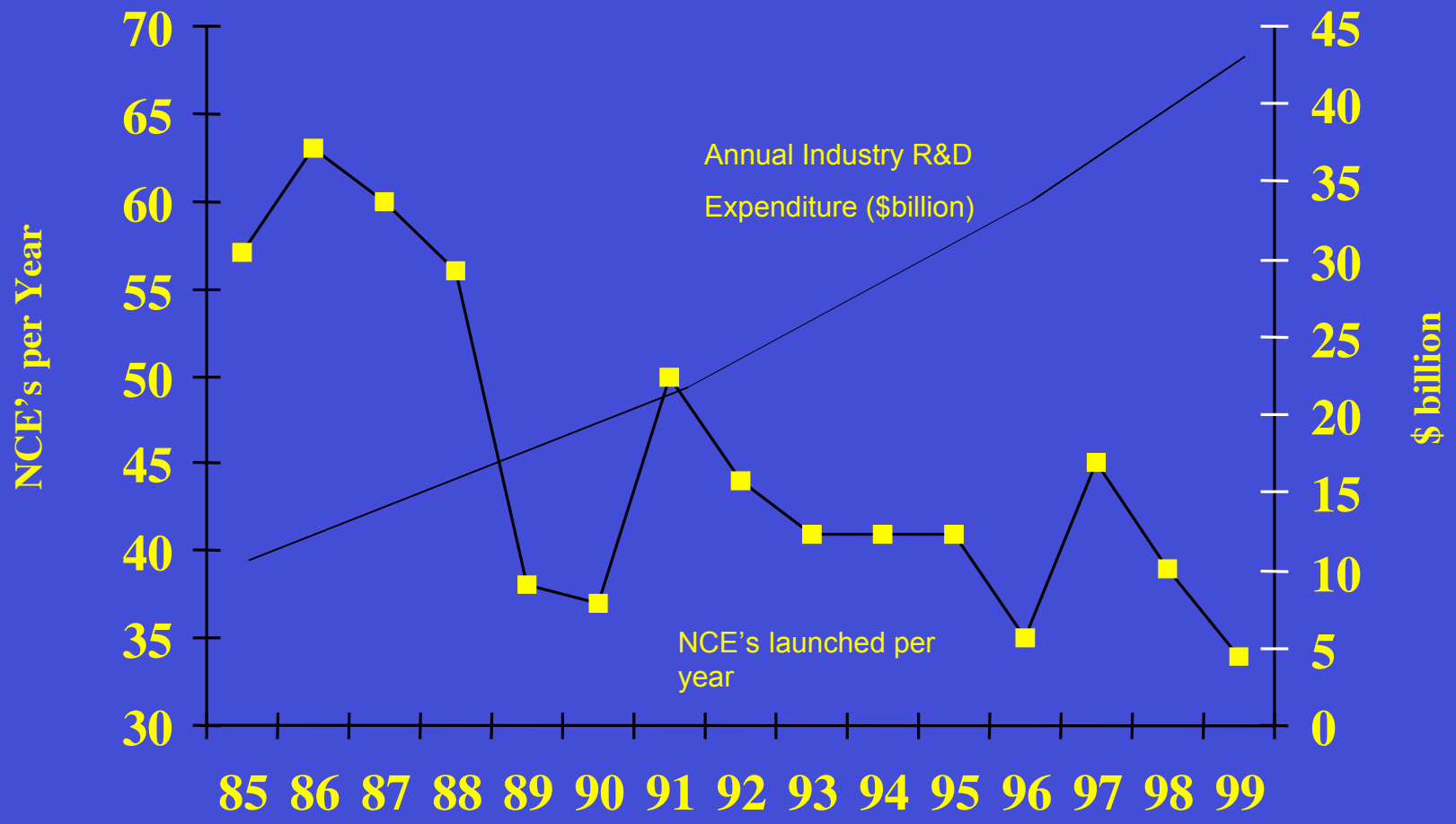
*dee@moksha8.com*

# Introduction

- Introduction
- Antibody structure
- Engineering Antibodies: Humanisation
- Expression
  - Mammalian
  - *E.coli*
- Clinical Utility
- Alternatives

# Commercial value of Antibodies

# Discovery Spending vs Output



# Licensed mAb Products

| Product | Indication | Year Licenced |
|---|---|---|
| **Reopro** | **Coronary artery disease** | **1994** |
| **Synagis** | **Prevent RSV infection** | **1998** |
| **Rituxan** | **Lymphoma therapy** | **1997** |
| **Herceptin** | **Breast Cancer** | **1998** |
| **Zenapax** | **Kidney Transplantation** | **1997** |
| **Remicade** | **Arthritis/Crohn's disease** | **1998** |
| **Orthoclone** | **Organ Transplantation** | **1986** |
| **Simulect** | **Kidney Transplantation** | **1988** |
| **Mylotarg** | **AML** | **2000** |

Total Global 1999 Sales ≈ $1.5 Billion

# Value of Antibody Technology

- Four marketed humanised antibodies had combined total sales in excess of $700M
  - Mylotarg
  - Herceptin
  - Synagis
  - Zenapax
- Total global sales in 1999 of therapeutic Mab products ≈ $1.5 Billion

**Avastin had approx sales of $1.1Billion in 2005**

# Value of Antibody Technology..contd

- one of the fastest growing and most lucrative sectors of the pharmaceutical industry, with exceptional 48.1% growth between 2003 and 2004.
- In 2005, the antibodies market was valued at an estimated US$14 billion, accounting for over 24 percent of the total protein therapeutics market.
- Potential to triple in value over the next few years and reach $30.3 billion in 2010
- A wave of fully human products are expected to launch from 2007 onwards, accounting for 12 of the 20 launches between 2007 and 2010.

# Immunogenicity of Therapeutic Antibodies versus other Biologics

- For naked, humanised antibodies or fragments, there is a high probability that the response will be directed towards the idiotype
- Important issues are
  - Hypersensitivity reactions (safety)
    - However it is unlikely that anti-idiotype antibodies will cross-react with native proteins
  - Blocking/ neutralising/ clearing of drug (efficacy)
    - Anti-idiotype antibodies will have the potential to neutralise if they are sufficiently high titre or affinity

# Immunogenicity of Therapeutic Antibodies – conjugates or chimeric Mabs

- Attachments have the potential to be immunogenic – even weak immunogens may provoke a response when attached to a large protein
- Linkers, neo-epitopes at joining regions, chimeric V-region framework sequences all have potential to be immunogenic

# Factors affecting Antibody Immunogenicity

- Molecule
  - Species homology (rat, monkey, man)
  - Expression of antigen
  - Location of antigen e.g. soluble vs surface of immune system cell; - Affinity of binding;
  - Attachments (e.g. cytotoxic drug, PEG)
- Manufacturing
  - Glycosylation
  - Aggregates
- Administration
  - Route of administration (SC vs IV)
  - Size of dose; - Number and frequency of doses
- Recipient
  - Patient population
  - Concomitant medication

# Technologies which have been developed to reduce Immunogenicity

- **Biovation:**For DeImmunisation™ of antibodies and proteins, helper T cell epitopes are identified within the primary sequence of the biopharmaceutical and these sequences are modified, principally by amino acid substitution, to avoid recognition by T cells.
- **AlgoNomicsl:**Epibase™ is the proprietary platform for T-cell epitope identification.
- **Genencor:** The i-mune Assay (Epitope Mapping Technology)
- **PEG:** Roche - PEGASYS® (IFN -α2a)

# Human/Humanised Antibodies

- 1st Generation - Chimeric Antibodies
- 2nd Generation - CDR Grafting
- Fully Human Antibodies:
  - Human Ab Phage Libraries
    - CAT
  - Hu Mice
    - Abgenix
    - Medarex
    - XTL
  - Hu Rabbits

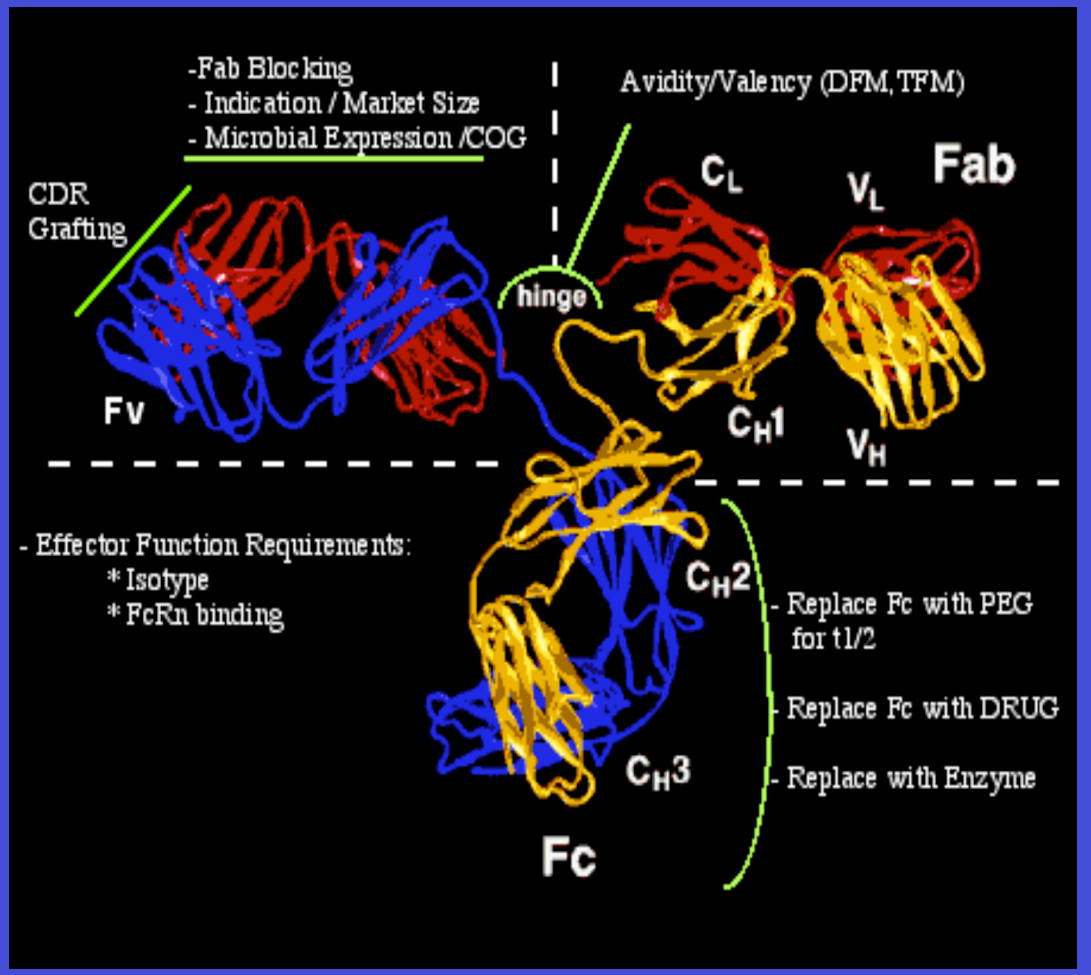
# HUMIRA™

- 1st approved "fully human" Mab (RA)
- Q - Is HUMIRA™ immunogenic ?
- A - Yes
  - Overall rate 5.5% in pivotal trials
  - Included 1gG2, IgG3, IgG4 & IgM (No igE)
  - Primarily anti Id's
  - Modest reduction in efficacy in patients on monotherapy
  - No effect on safety

Abbott Lab's presented above data in Sept 2003 (IBC)

# SAR of an Antibody

- Properties to be considered :
  - Half Life ($t_{1/2}$)
  - Number of binding sites (Avidity / Valency)
  - Immunogenicity
  - Blocking antibody ?
  - Internalising (TFM > DFM))
  - Deliver drug / effector functions / enzyme




CELLTECH therapeutics

# Antibodies

- Estimated that the humans can produce as many as 10 million different antibodies in the primary repertoire and this can be further expanded by several orders of magnitude by somatic mutation

- This diversity and specificity of antibodies can be understood by examination of antibody structure

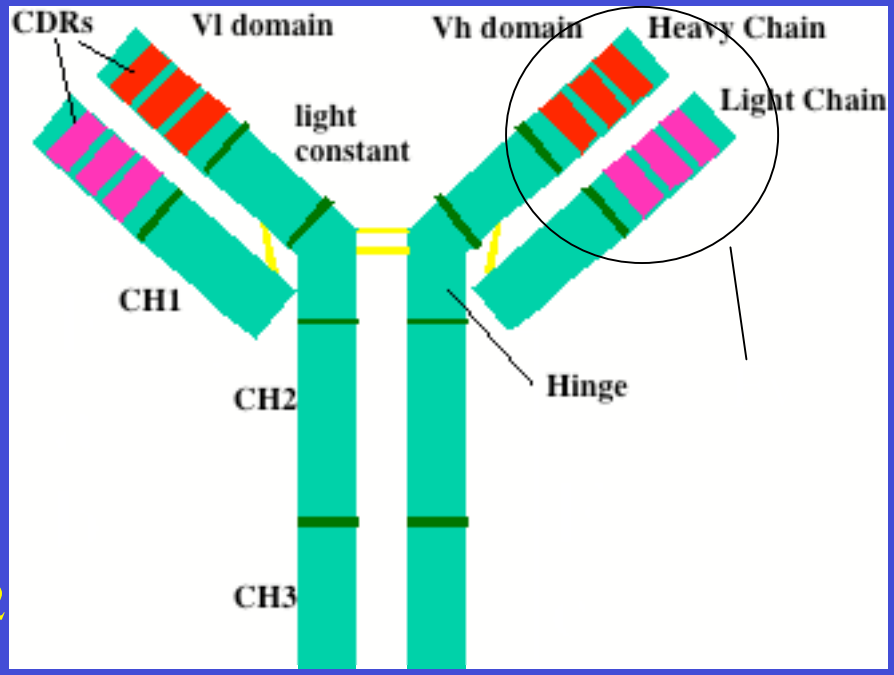
# IgG Structure

- Consists of 4 polypeptide chains
  - two heavy chains, approx. 50kDa
  - two light chains, approx. 25kDa

- Each is divided into domains of approx. 110aa stabilised by intra-disulphide bonds.

- Light chain composed of:
  - variable domain (VL)
  - constant domain (CL)

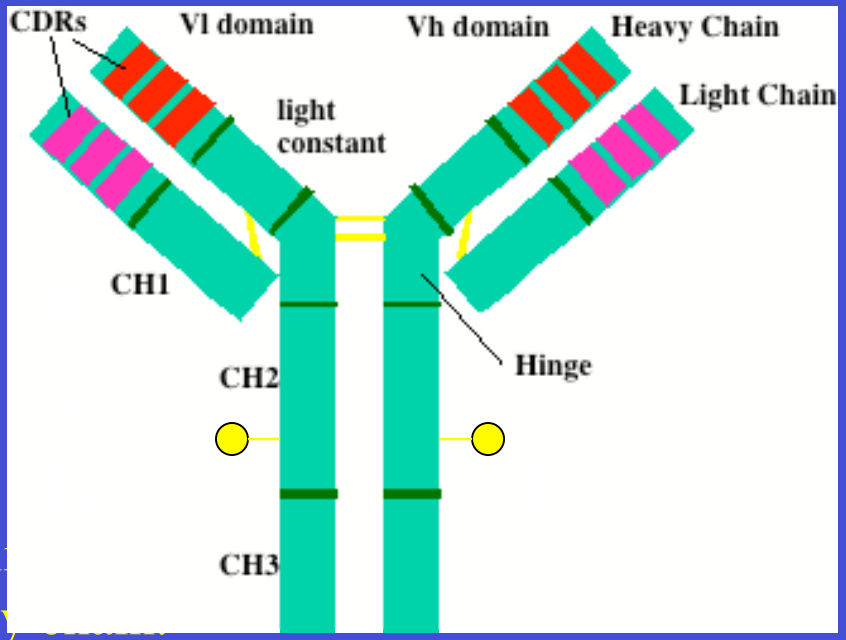
- Heavy chain composed of:
  - variable domain (Vh)
  - three constant domain (CH1,CH2




- Heavy and light chain variable domains associate to form the antigen binding site

# IgG Structure Contd.

- The IgG molecule has two antigen binding sites.
- The heavy and light chains are linked by a disulphide bond between the CL and CH1 domains.

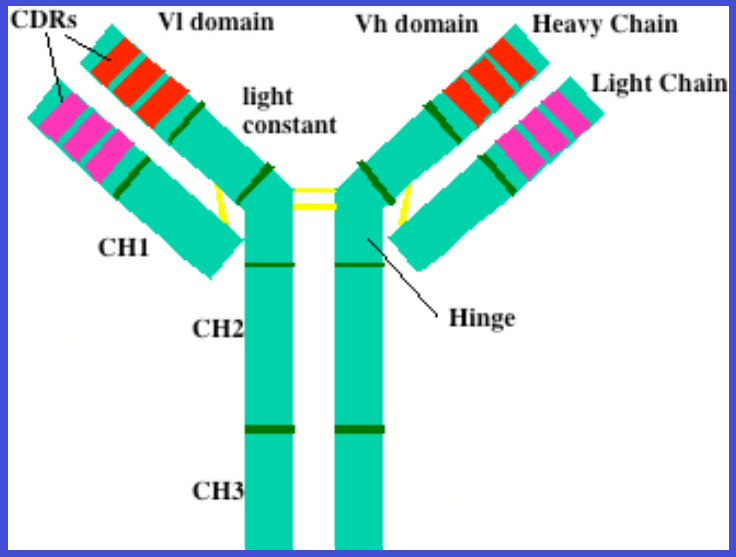


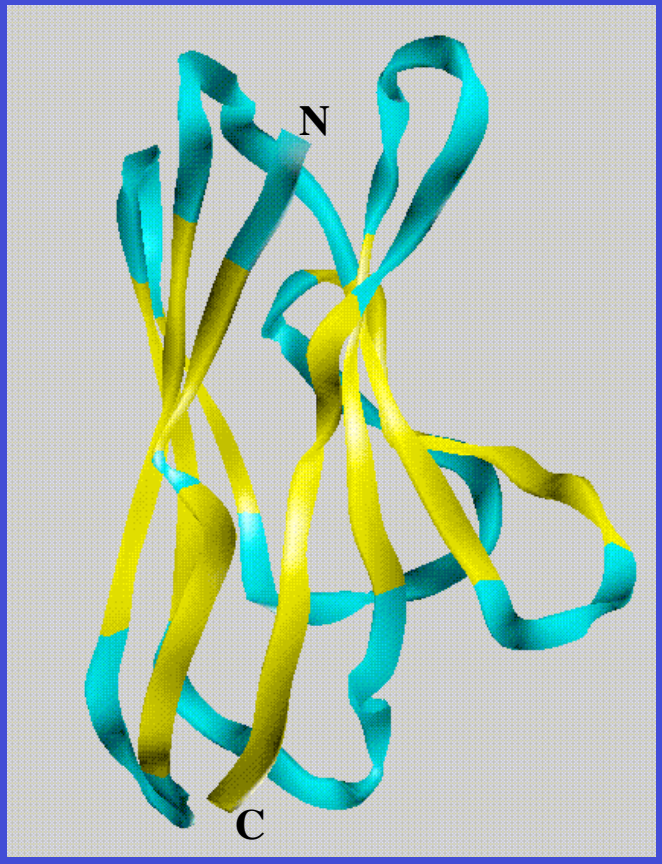

- A flexible region known as the "hinge" l
the CH1 and CH2 domains of each heavy
- The two heavy chains are linked together at the hinge by disulphide bonds.
- The CH2 domain is normally glycosylated.

# Antibody Architecture

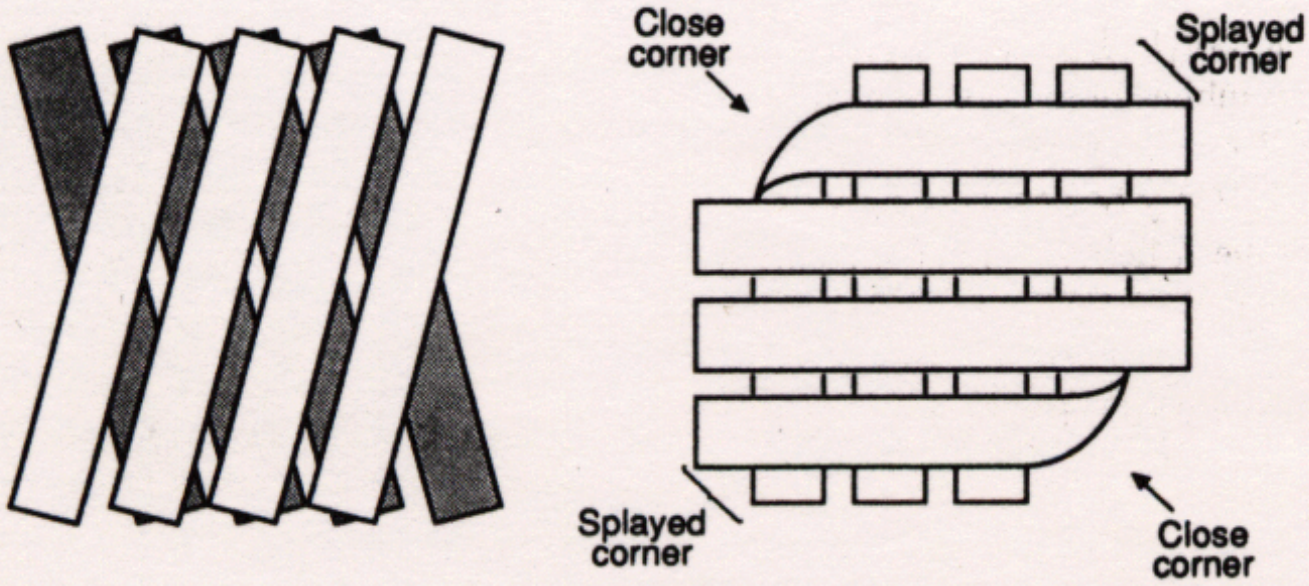
- Variety of immunoglobulin fragments from both human and mouse have been characterised by X-ray Crystallography

- The individual domains have similar conformation, "Immunoglobulin Fold"

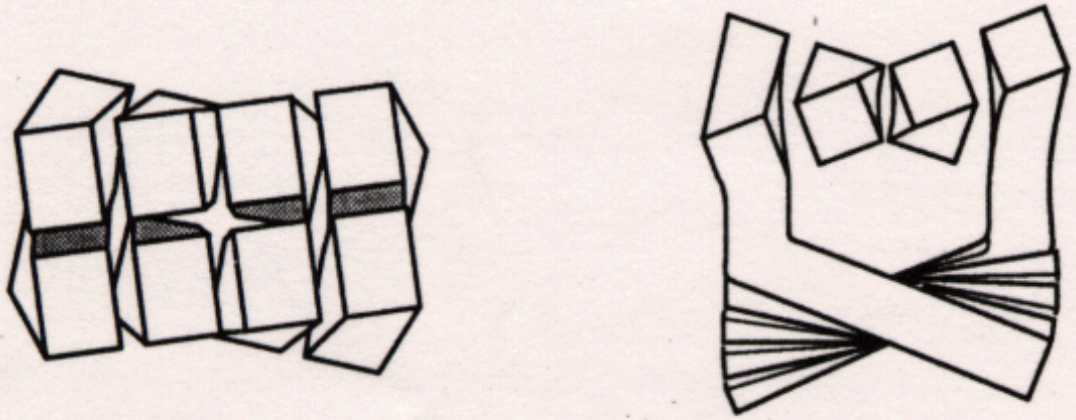



- The Ig Fold consists of 2 layers of anti parallel beta sheet




Front view



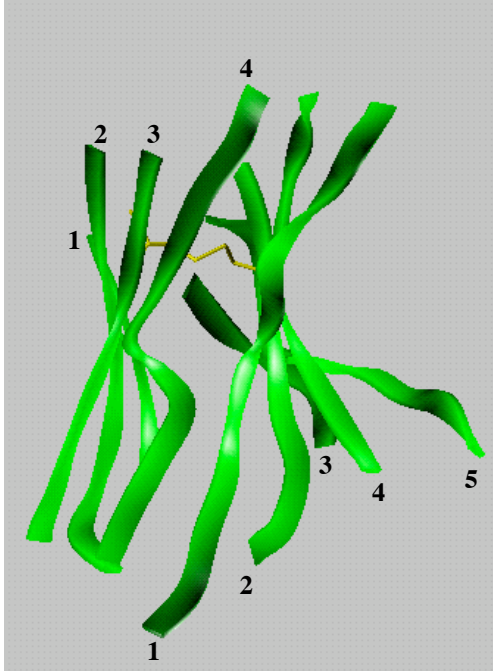
End view

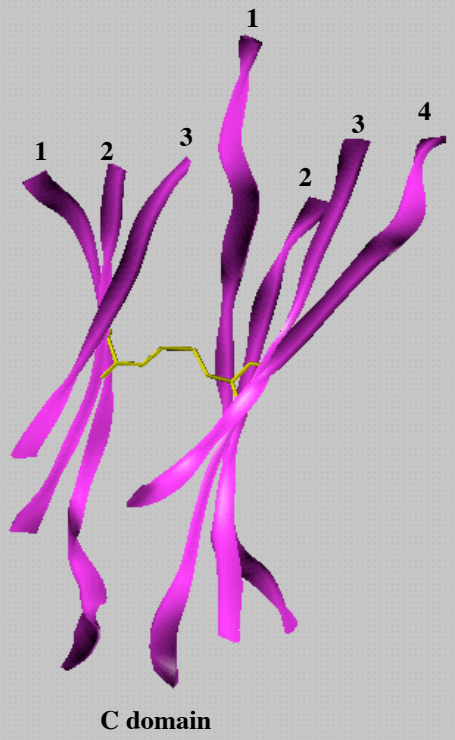
**(a)**
**Aligned packing**

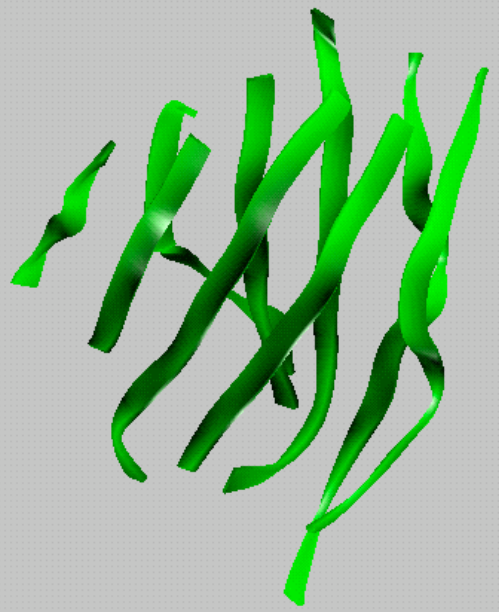
**(b)**
**Orthogonal packing**

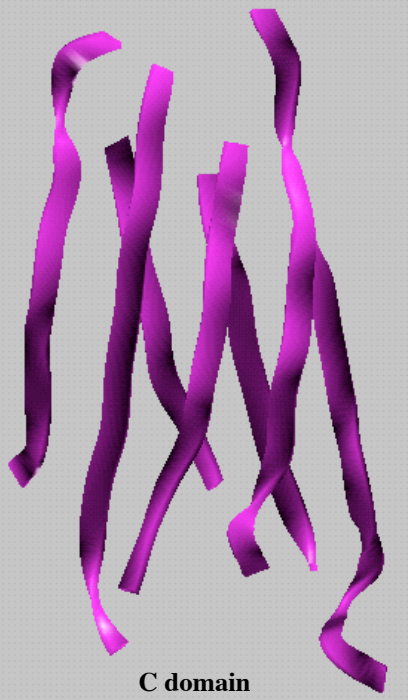
# Ig domain structure

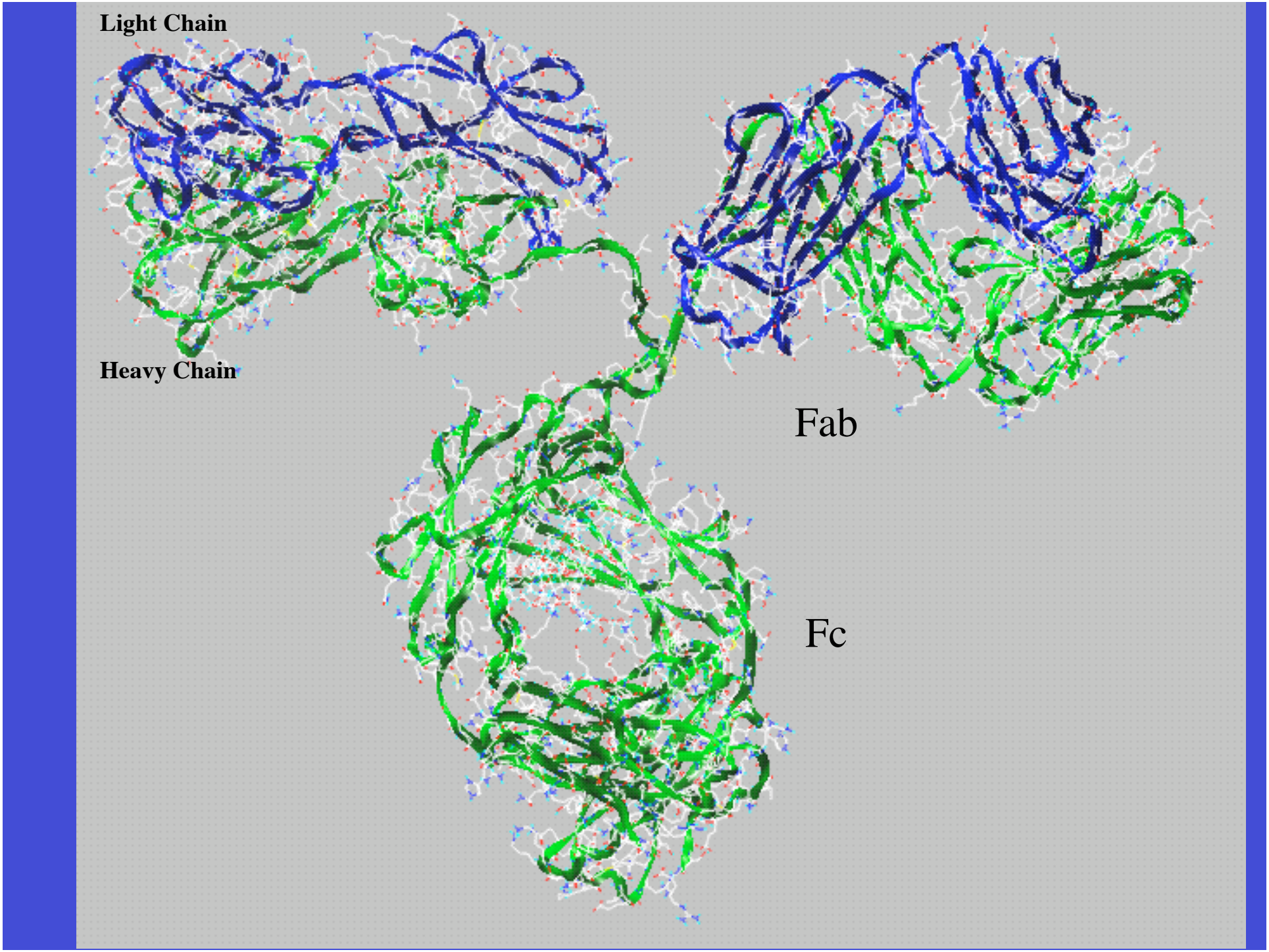
- The N-terminal variable domains differs from that of the C-proximal constant domains:
  - V domains β sheets consist of 5 & 4 strands
  - C domains β sheets consist of 4 & 3 strands




Light Chain
Heavy Chain
Fab
Fc

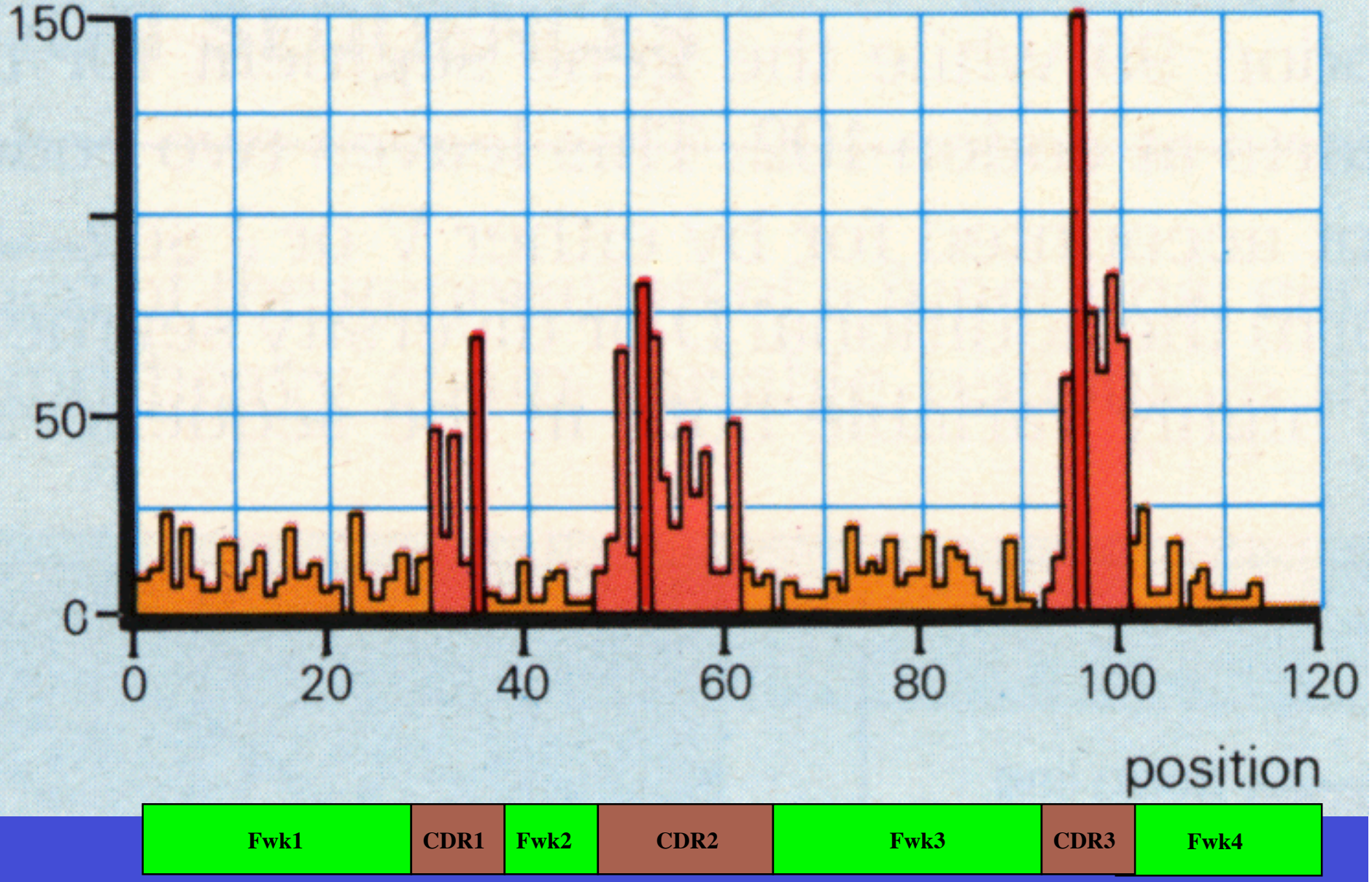
# Bio-informatics

- The primary sequence of several thousand antibody molecules are known (largest number of known sequences in one protein family).

- Analysis of these sequences revealed that the variability of the variable domains is largely restricted to three “hypervariable” regions, in each of the light and heavy variable domains.

- Comparatively little variation in the intervening “framework” regions”

# Kabat Plot


150
50
0
0
20
40
60
80
100
120
position
Fwk1
CDR1
Fwk2
CDR2
Fwk3
CDR3
Fwk4


39D10

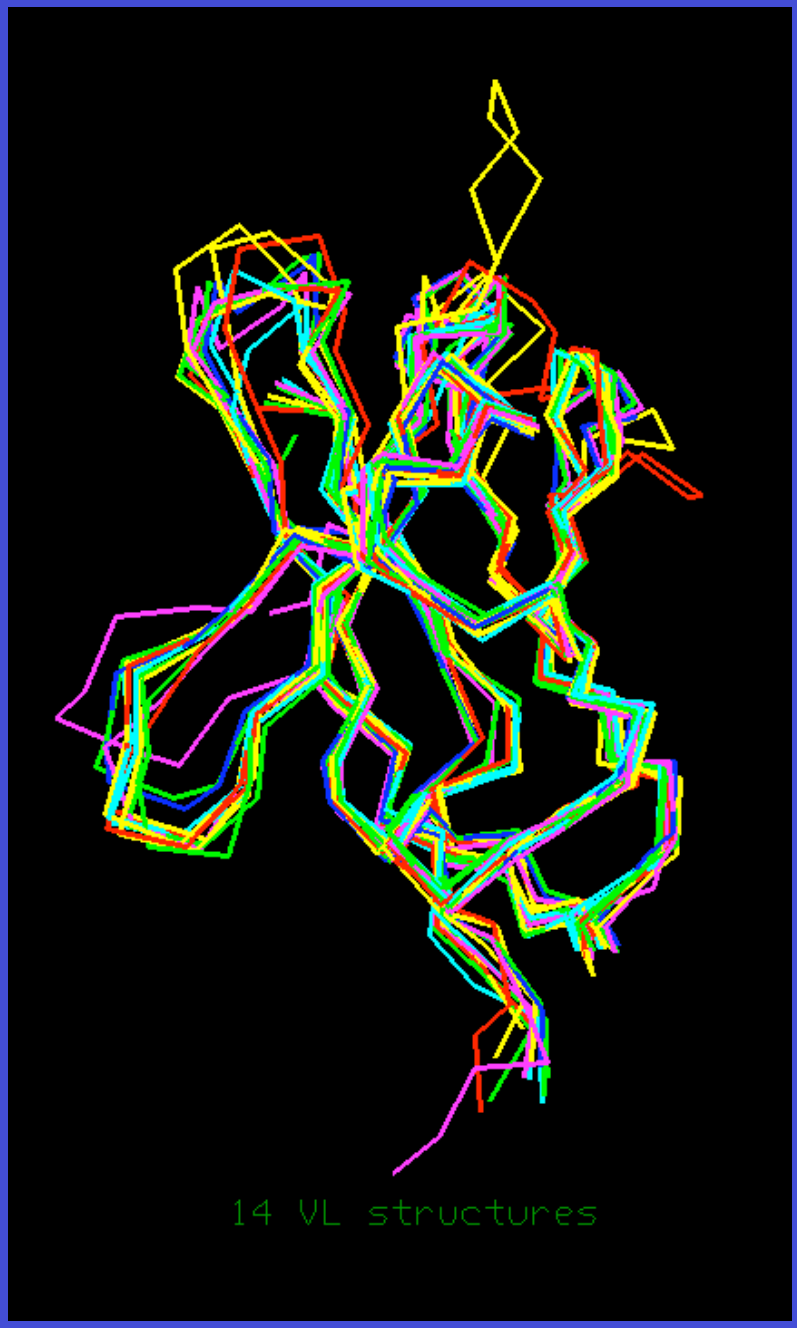
# CDRs

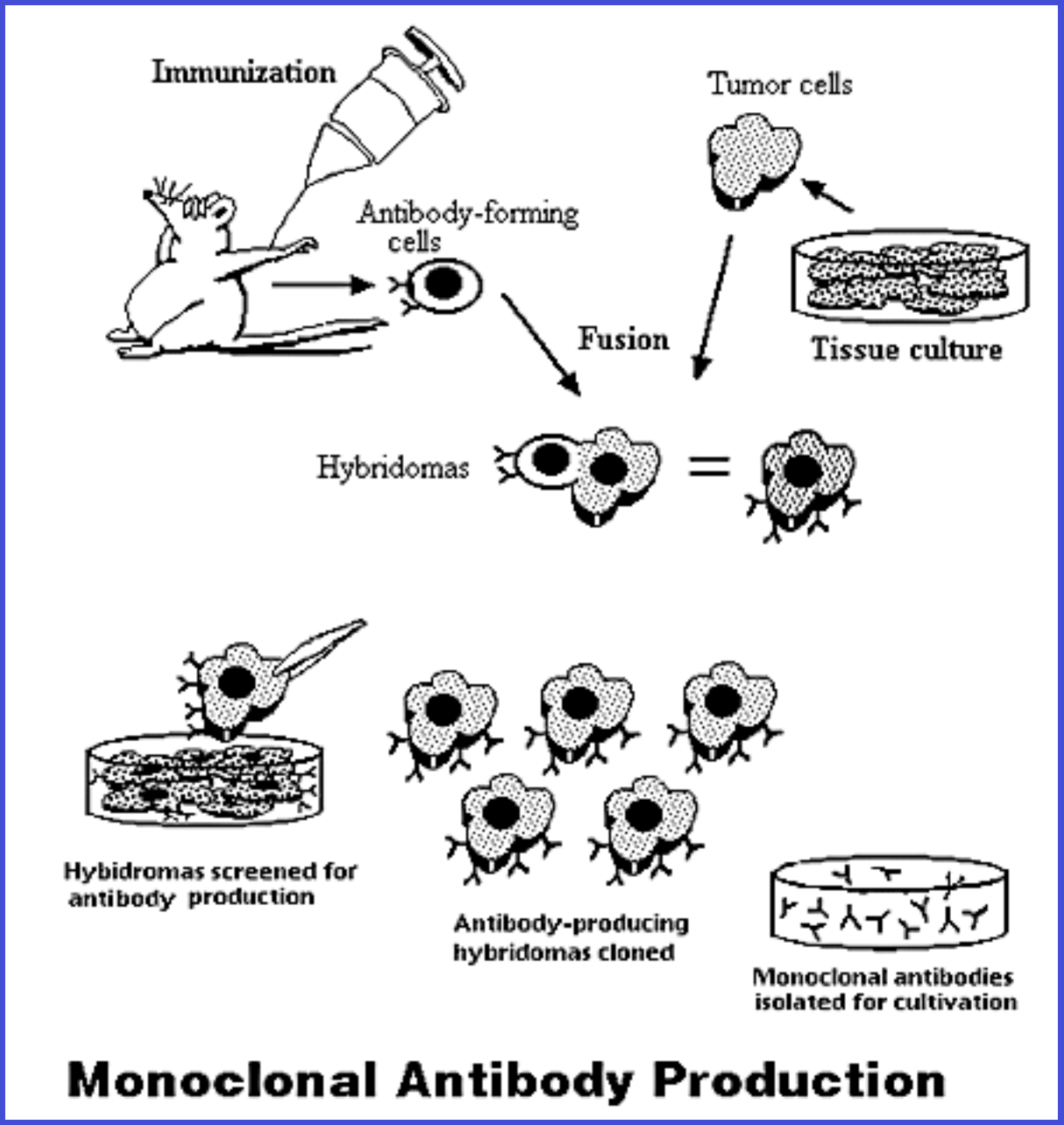
- The combining site of antibodies is almost entirely made up of 6 loops, 3 each from the light and heavy variable domains.

- Variability in these loops arises from:
  - sequence
  - Length

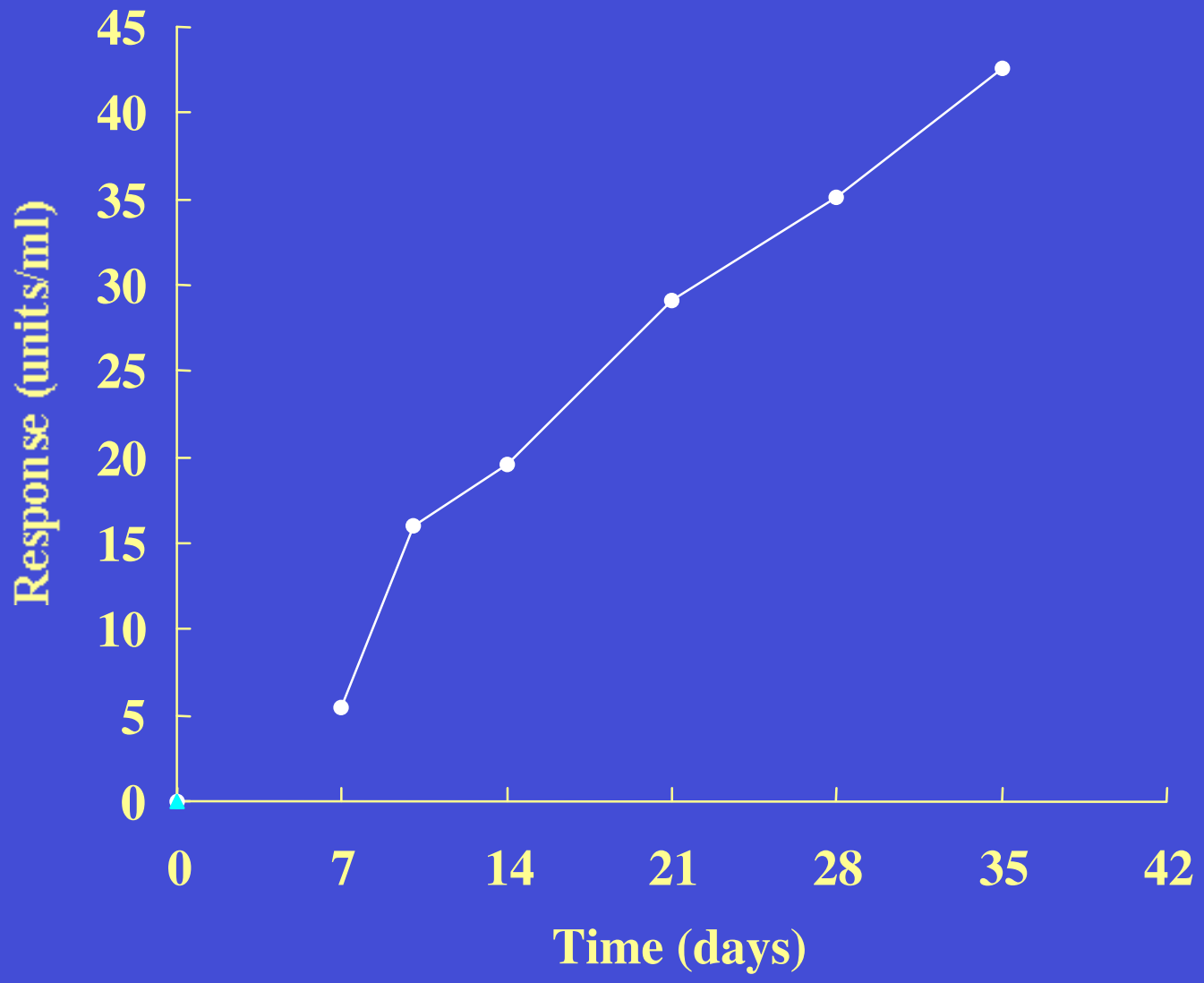
- Loops are commonly referred to as
  - Complementarity determining regions (CDRs)
  - Hypervariable loops


12 Vh structures


14 VL structures


Immunization
Antibody-forming cells
Tumor cells
Tissue culture
Fusion
Hybridomas
Hybidromas screened for antibody production
Antibody-producing hybridomas cloned
Monoclonal antibodies isolated for cultivation
Monoclonal Antibody Production

# Antibodies to Murine anti TNF-antibody in Cynomolgus Monkey



# Potential Disadvantages of a Human Anti -Mouse Antibody (HAMA) Response

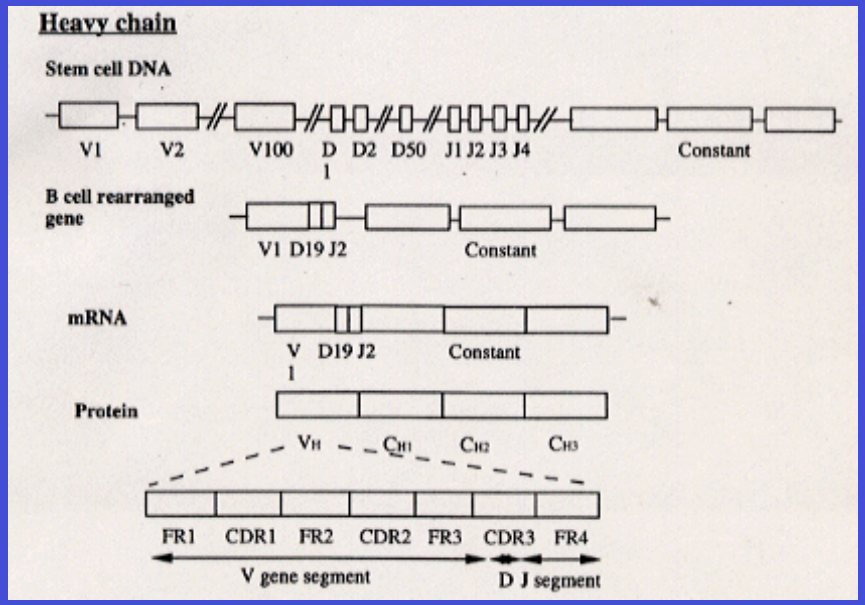
- Acute or Delayed Hypersensitivity Reactions
- Pharmacokinetic Changes
  - » High MW Aggregates
  - » Increased rates of clearance
- Reduced Targeting Efficiency / Efficacy

# Strategies to Avoid HAMA

- Co- therapy with immunosuppresent
- Sequential IgG type therapy regime
- Co- administration with anti-CD4 antibody
- Use of Antibody fragments
- Use of human or primate monoclonals
- Use of recombinant chimeric or humanised antibodies

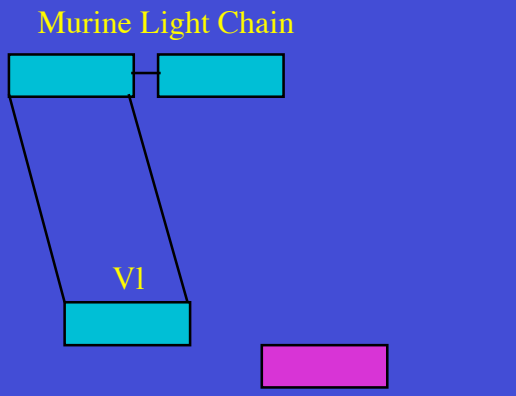
# Cloning V regions

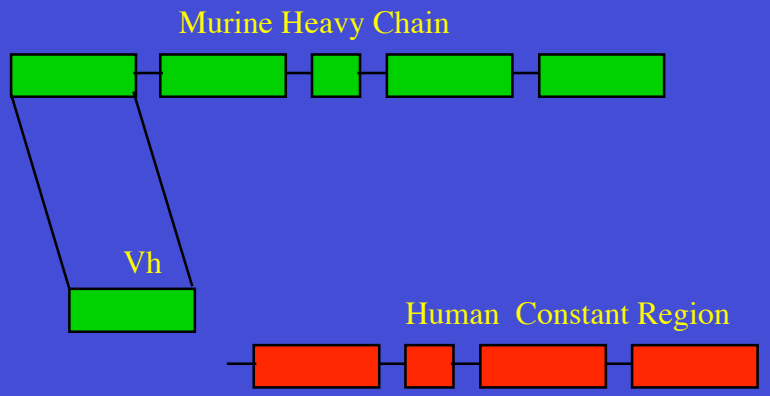
- Clone rodent variable domains using RT-PCR
- Primers designed based on leader sequence and constant regions




- Cloned directly into expression vectors containing human constant regions.

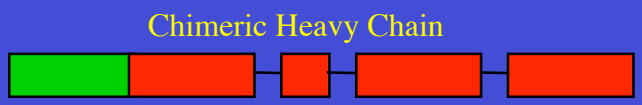
# Chimeric Construction



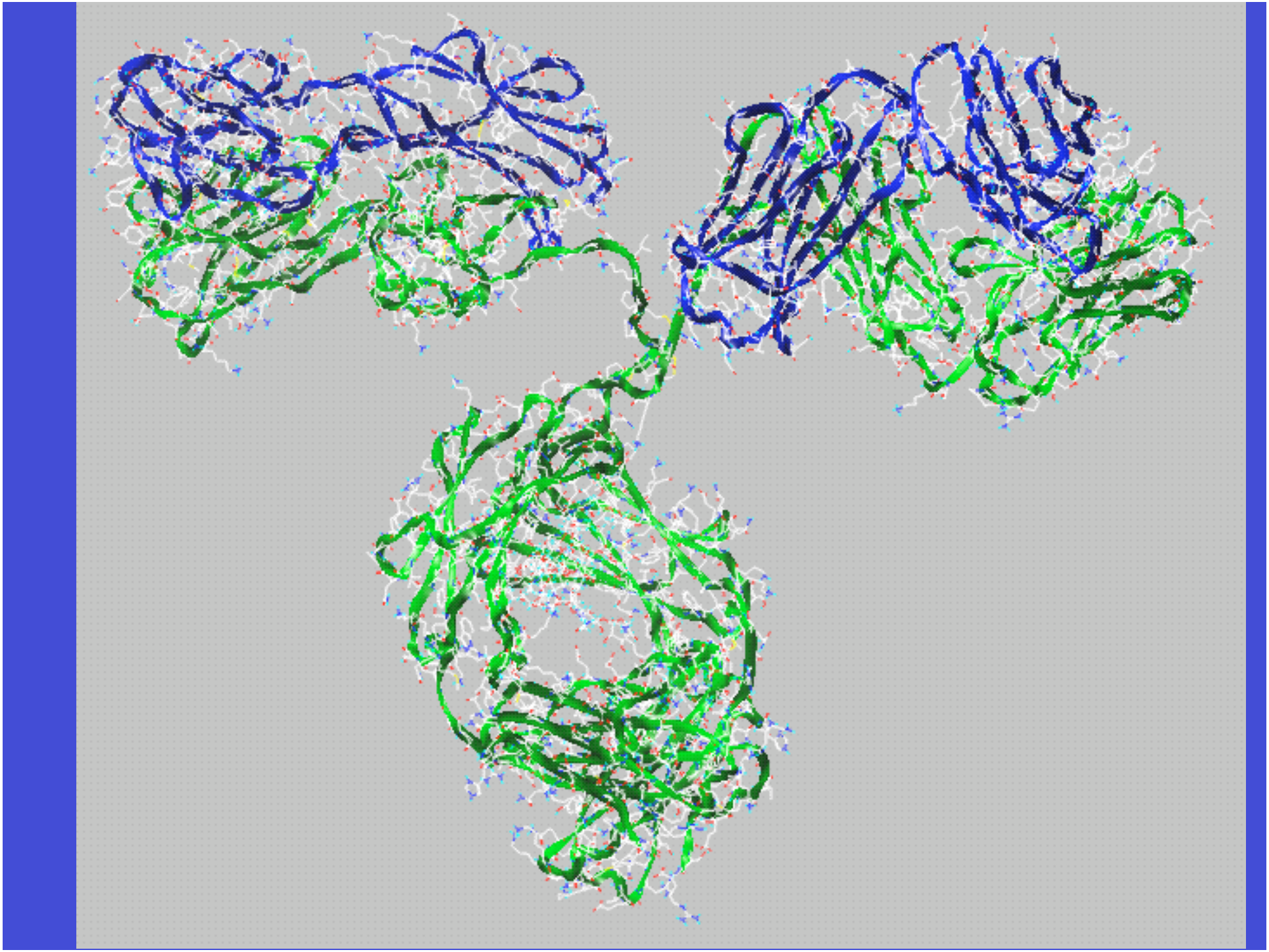



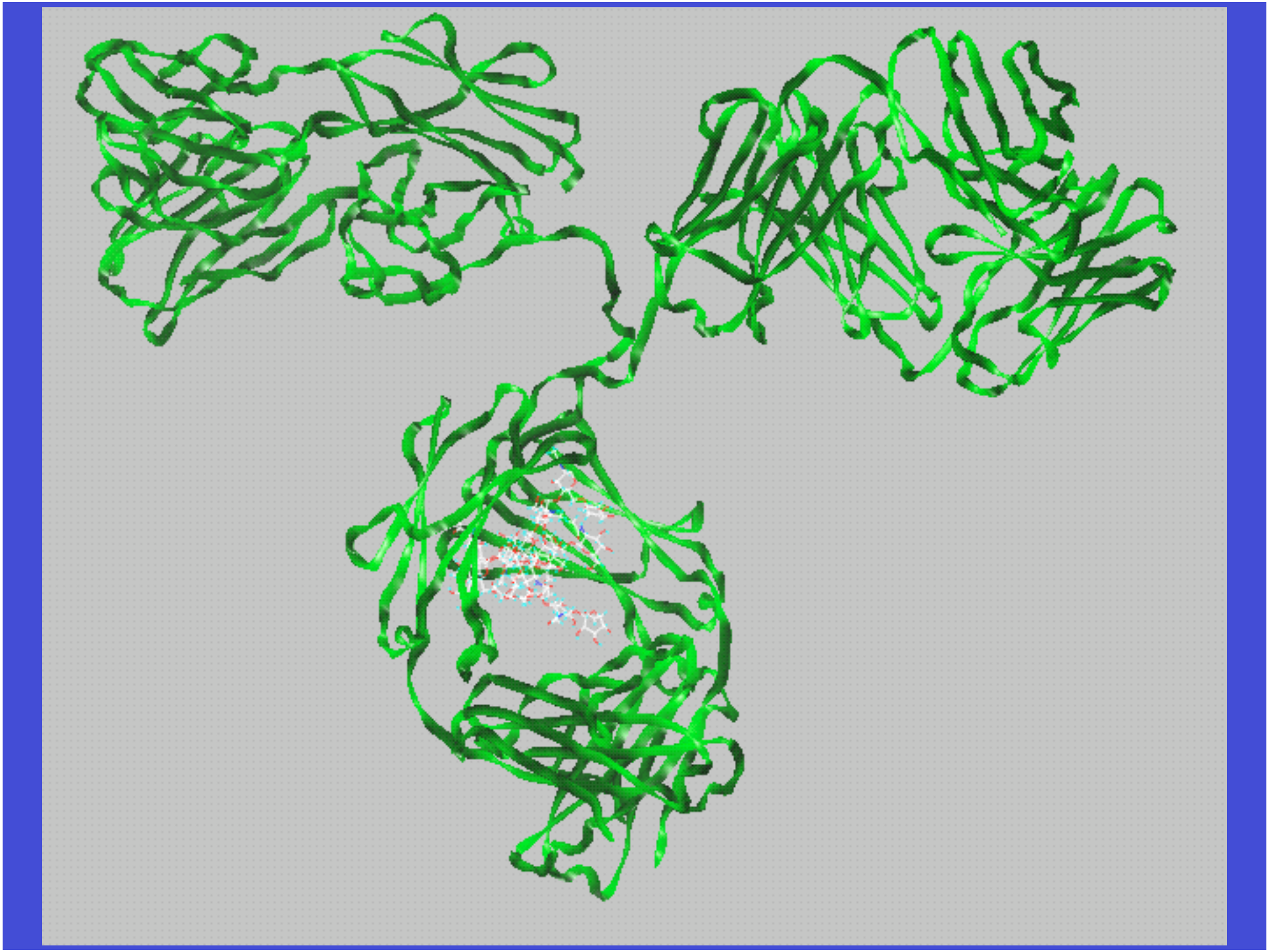


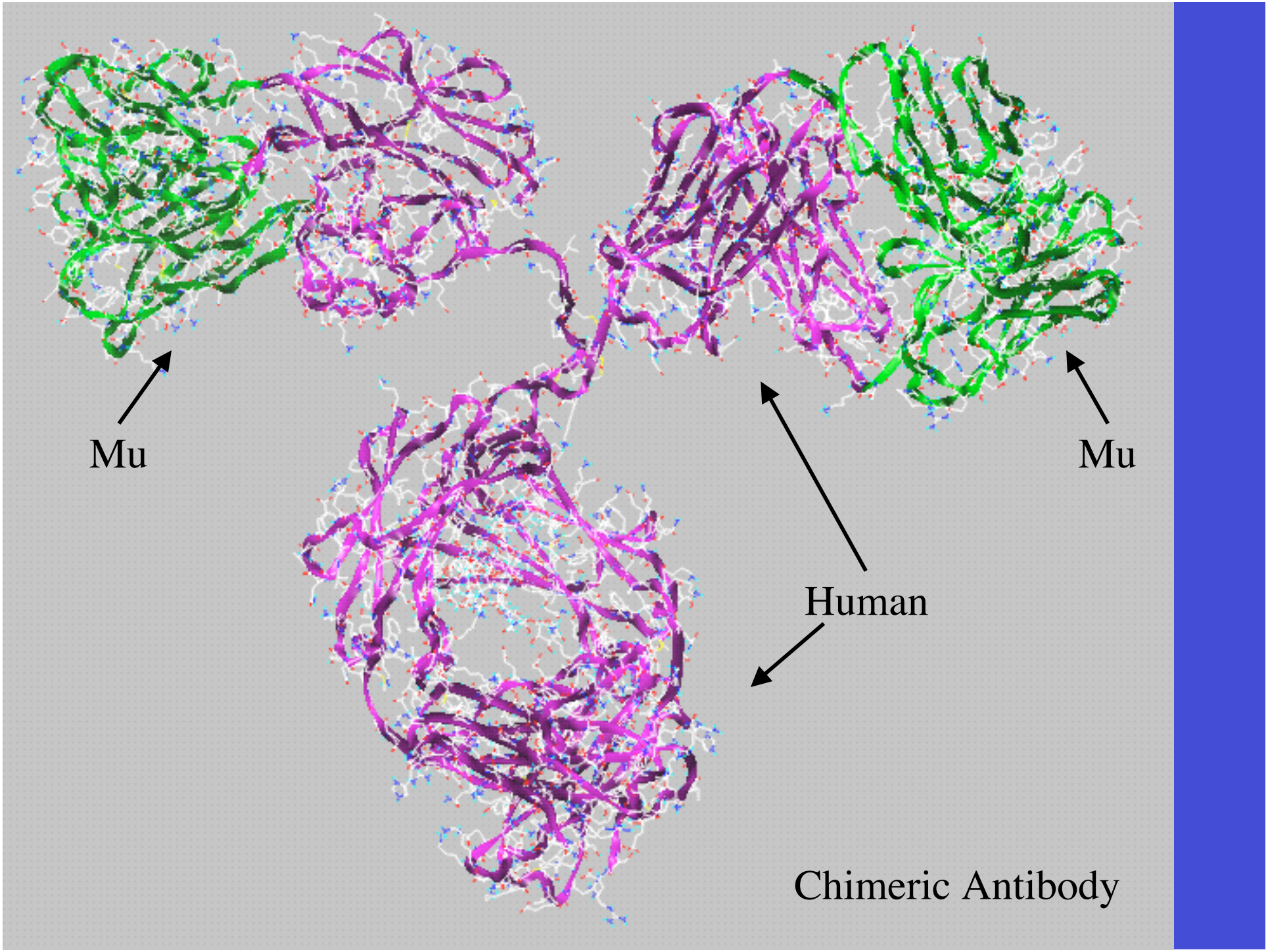
50% Human




75% Human





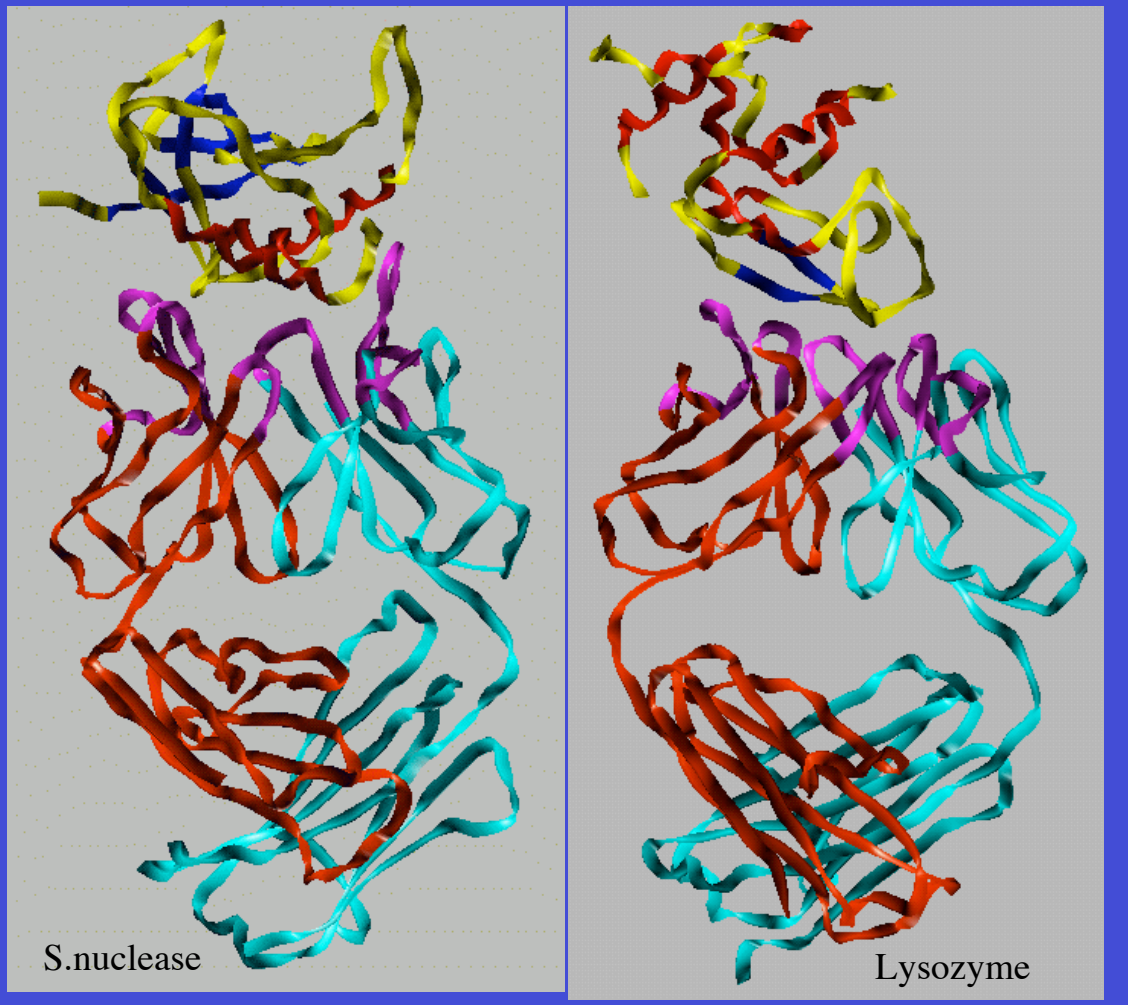

Mu
Mu
Human
Chimeric Antibody

# Antibody-antigen structures

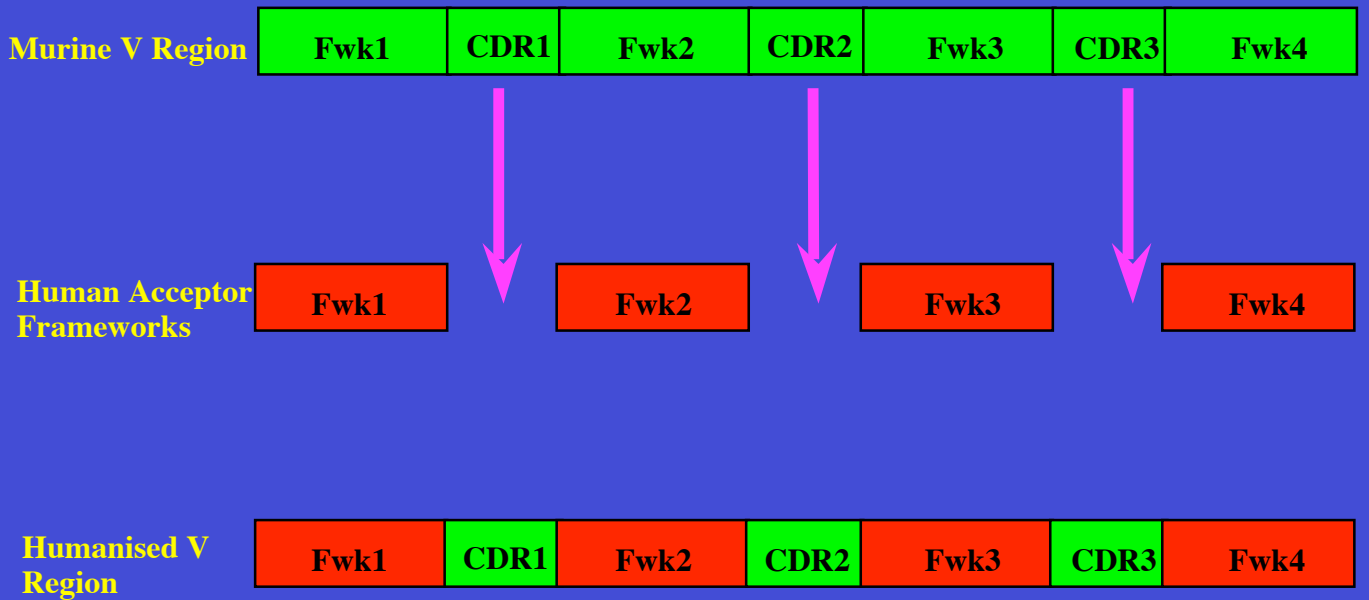
- Fab structures complexed with several antigens have been deposited in PDB:
  - Haptens
    - Phosphocholine
    - Vitamin $K_1OH$
    - Fluorescein
  - Proteins:
    - Lysozyme
    - Influenza virus neuraminidase
  - DNA
  - Carbohydrate

# Humanisation by CDR grafting

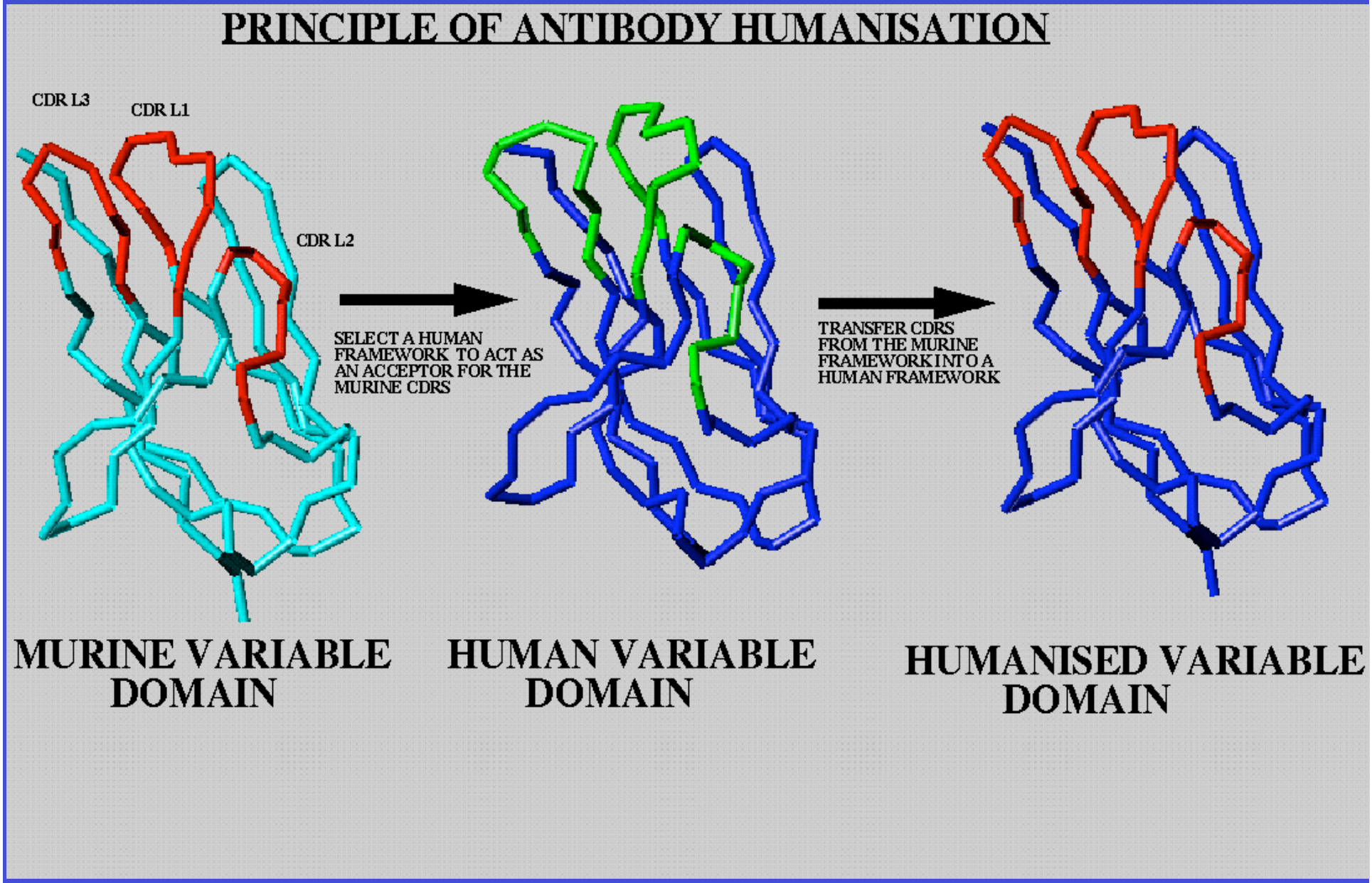
- Antibody - Antigen interactions occur at the tips of the Fab arms.
- CDR grafting transfers the binding specificity of the rodent antibody into a human antibody
- Transfer CDRs from mouse to human environment.



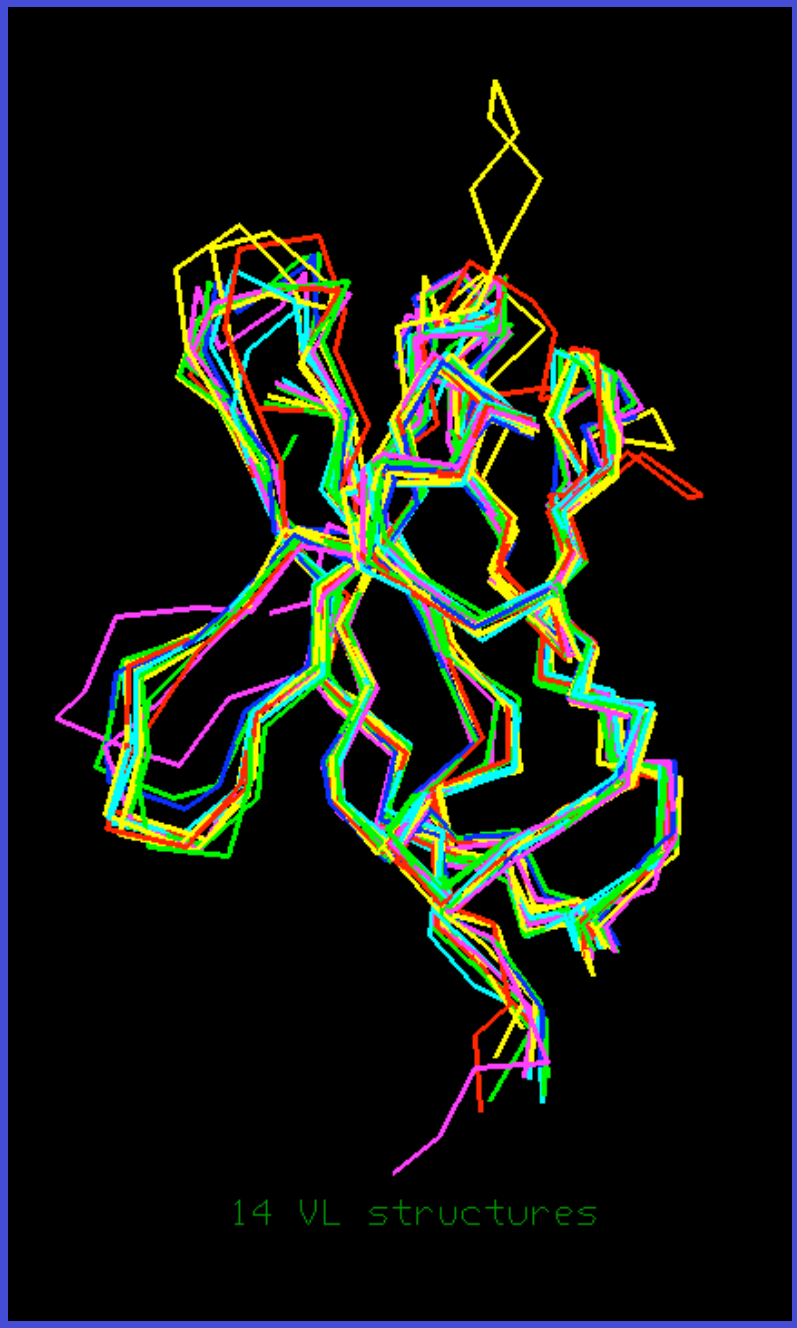
# Humanisation of V Region



# Kabat CDR grafting



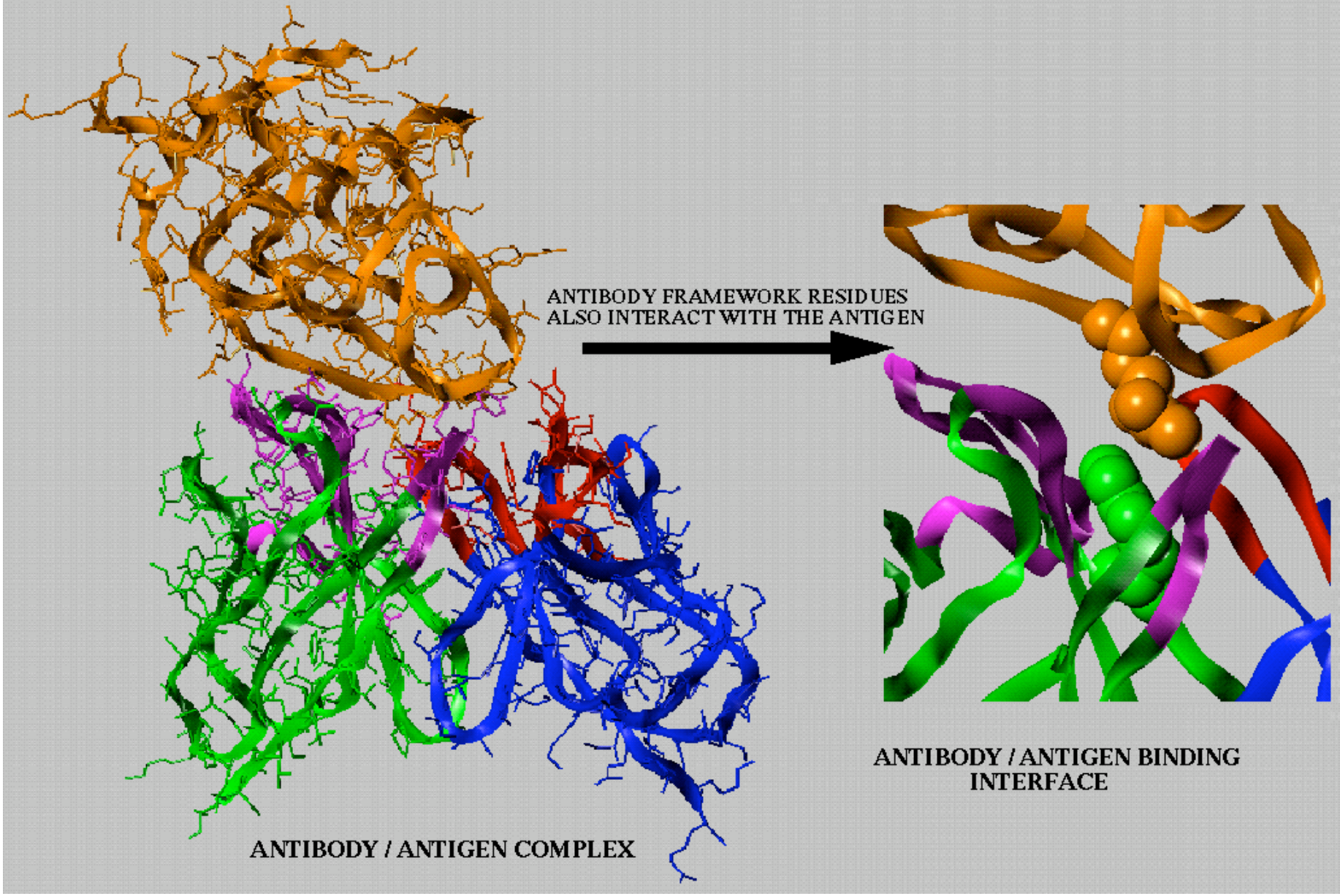

12 Vh structures


14 VL structures

# Initial Results of CDR grafting

- The affinities of Kabat grafts, transferring only the CDRs from rodent to human frameworks is generally poor.
- Most if not all of the original affinity is normally lost
- WHY?

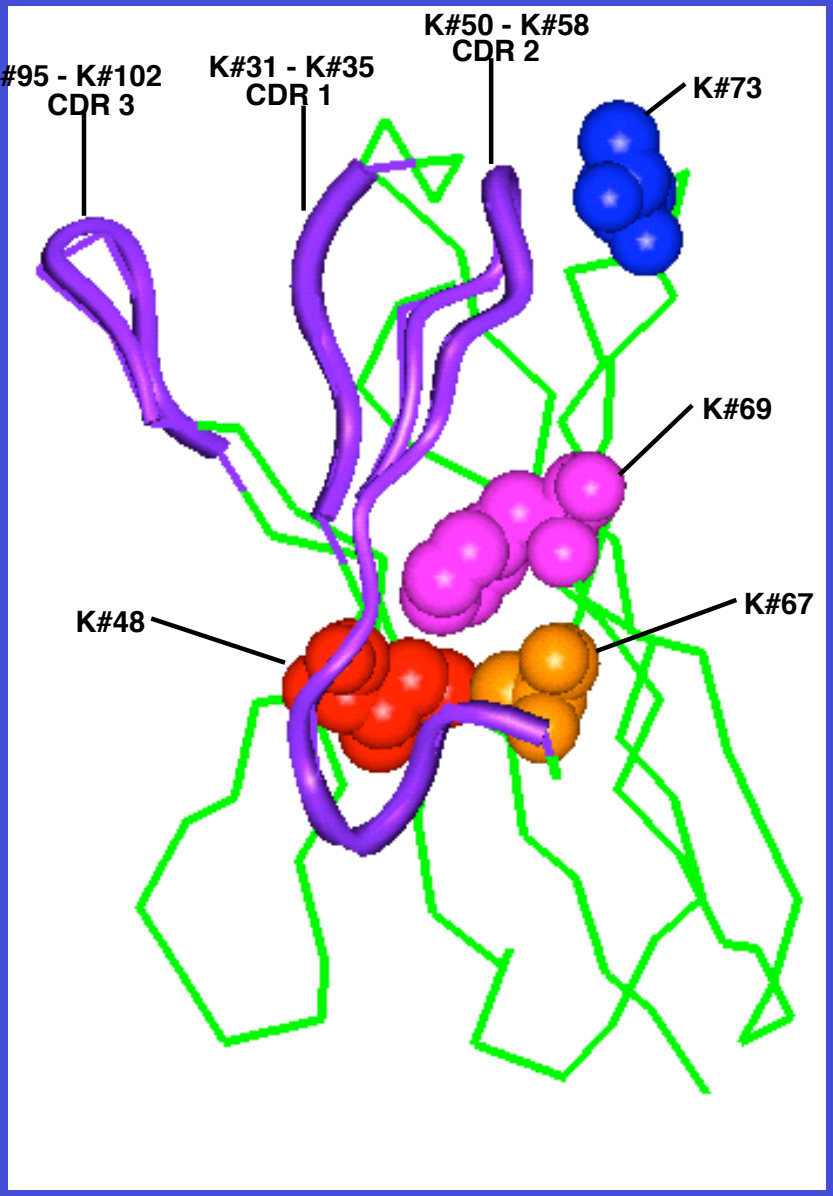
# PRINCIPLE OF ANTIBODY HUMANISATION



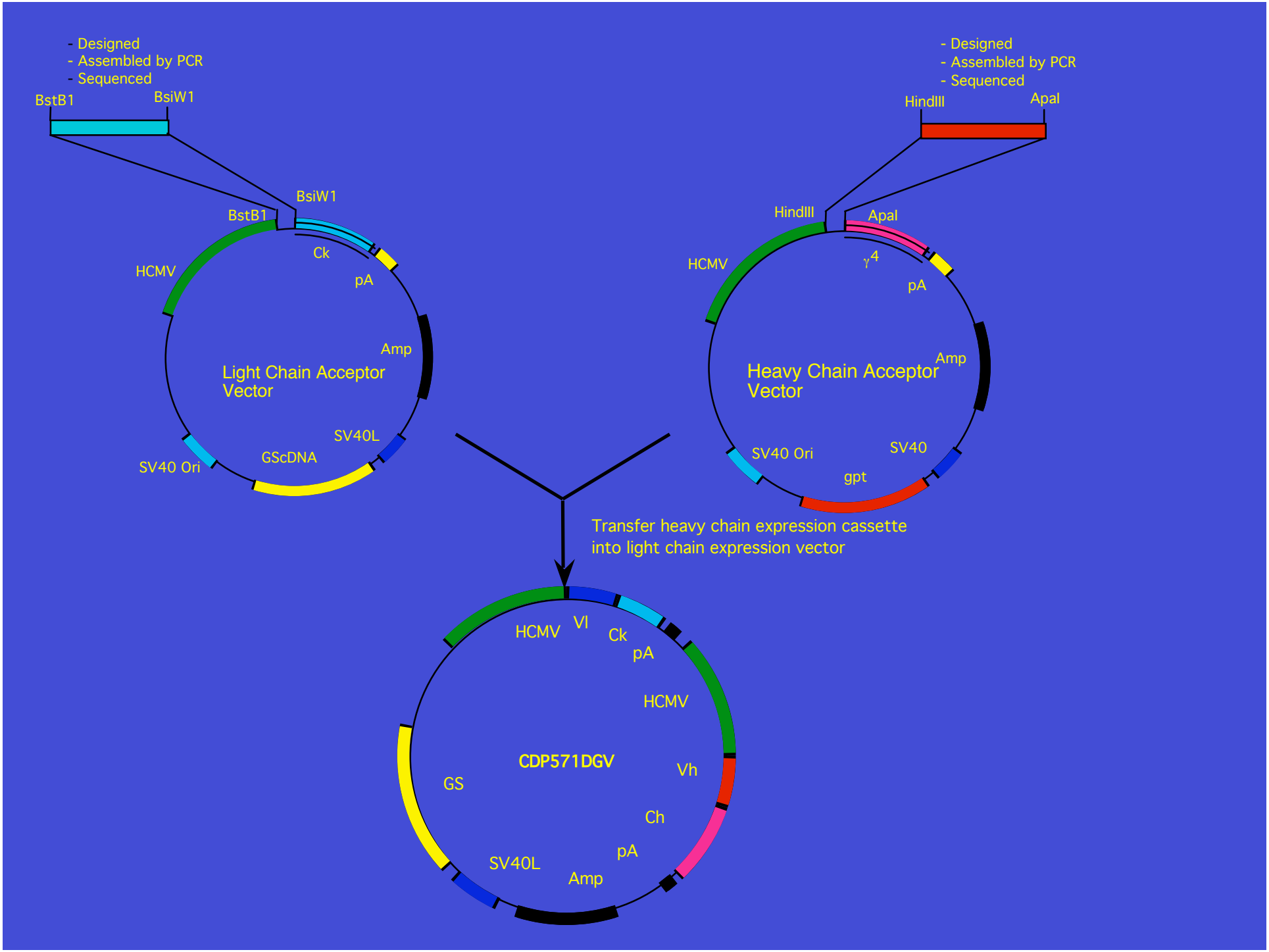
# Framework Residues

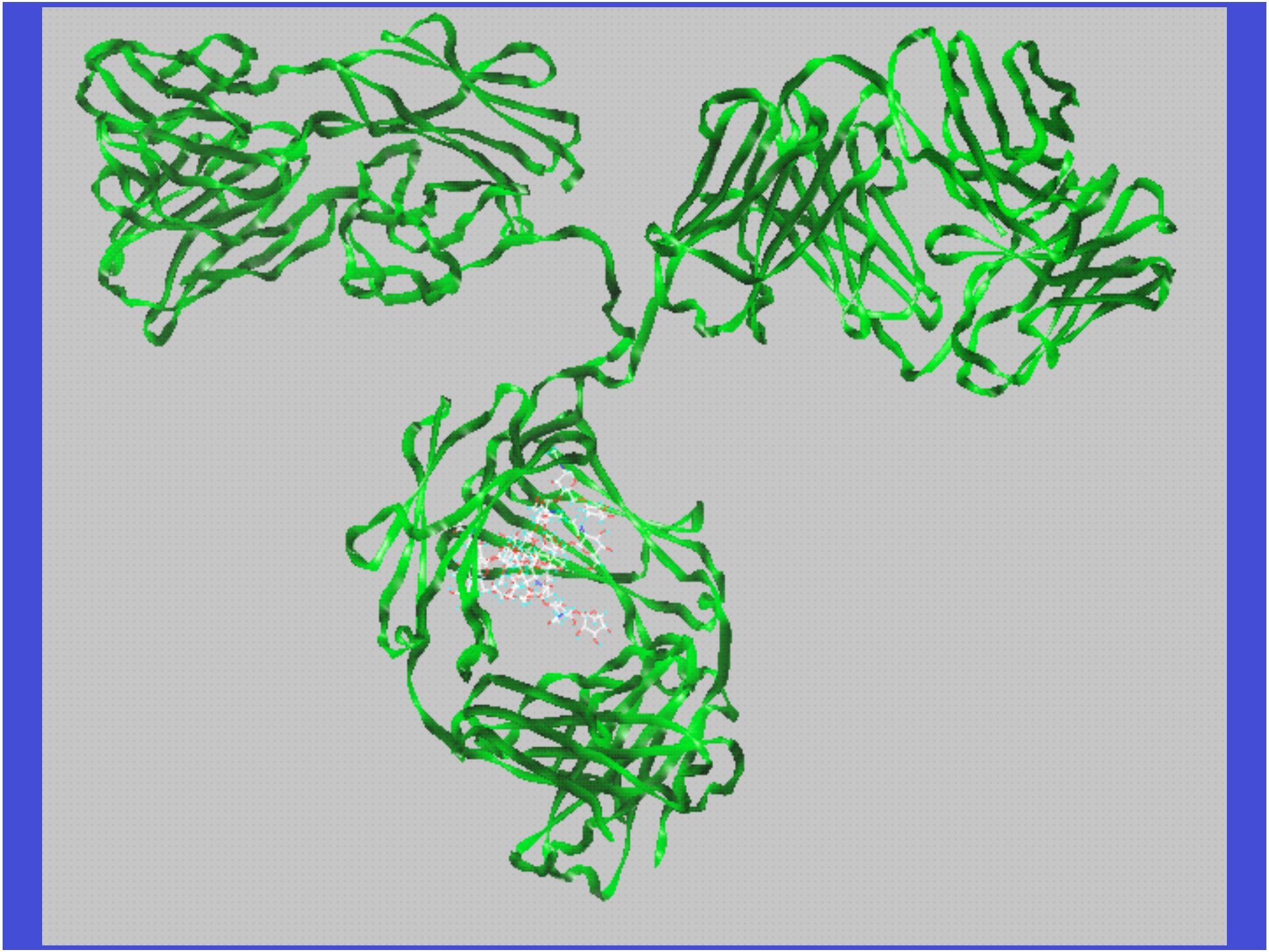
- Selection of framework residues is acheived by visual inspection of antibody models:
  - Exposed and spatially adjacent residues to CDRs
  - Buried residues which form Ig fold core
  - Buried residues which are capable of forming contacts with the CDRs

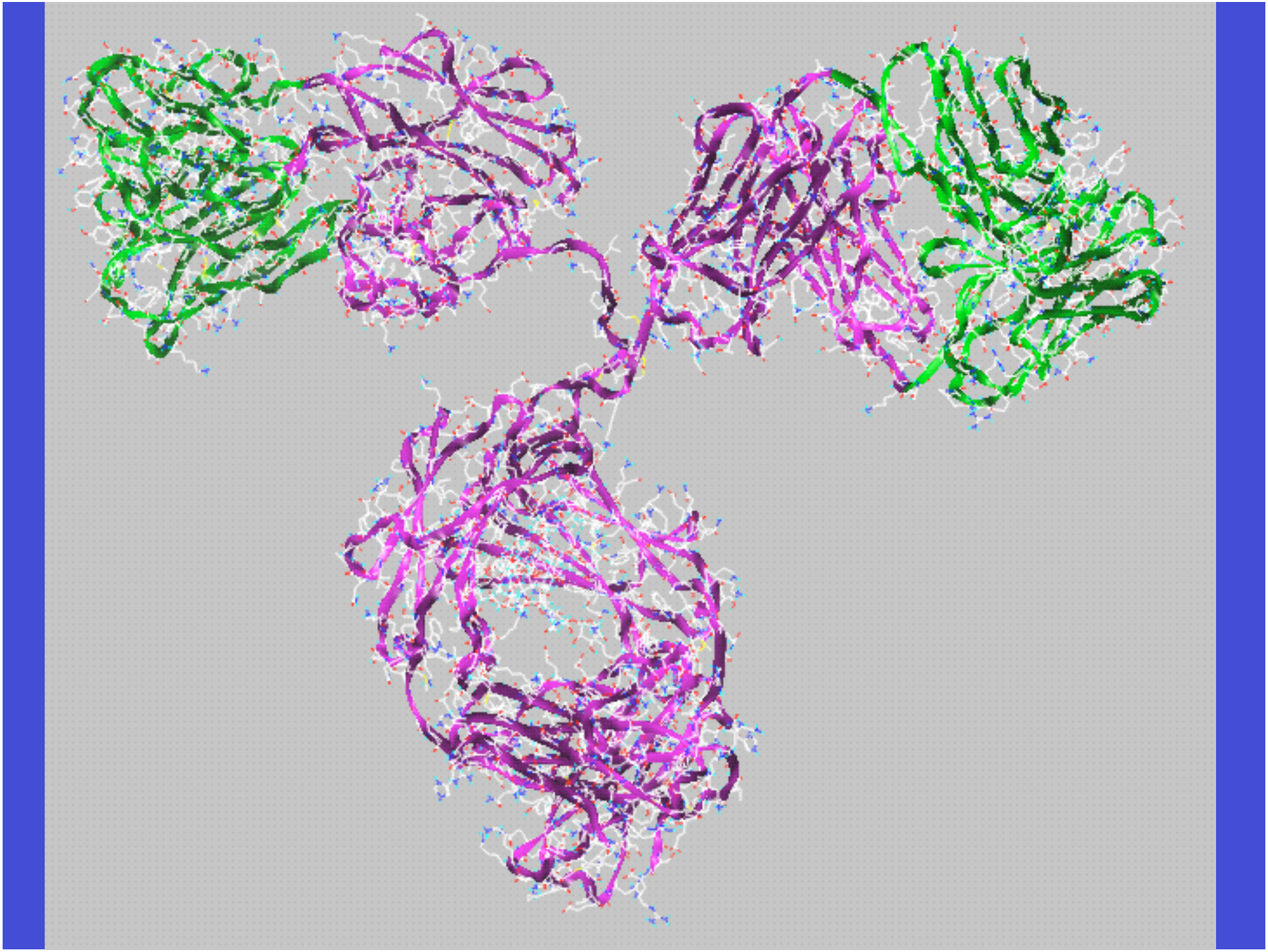
# Model of the variable heavy domain of CDP571

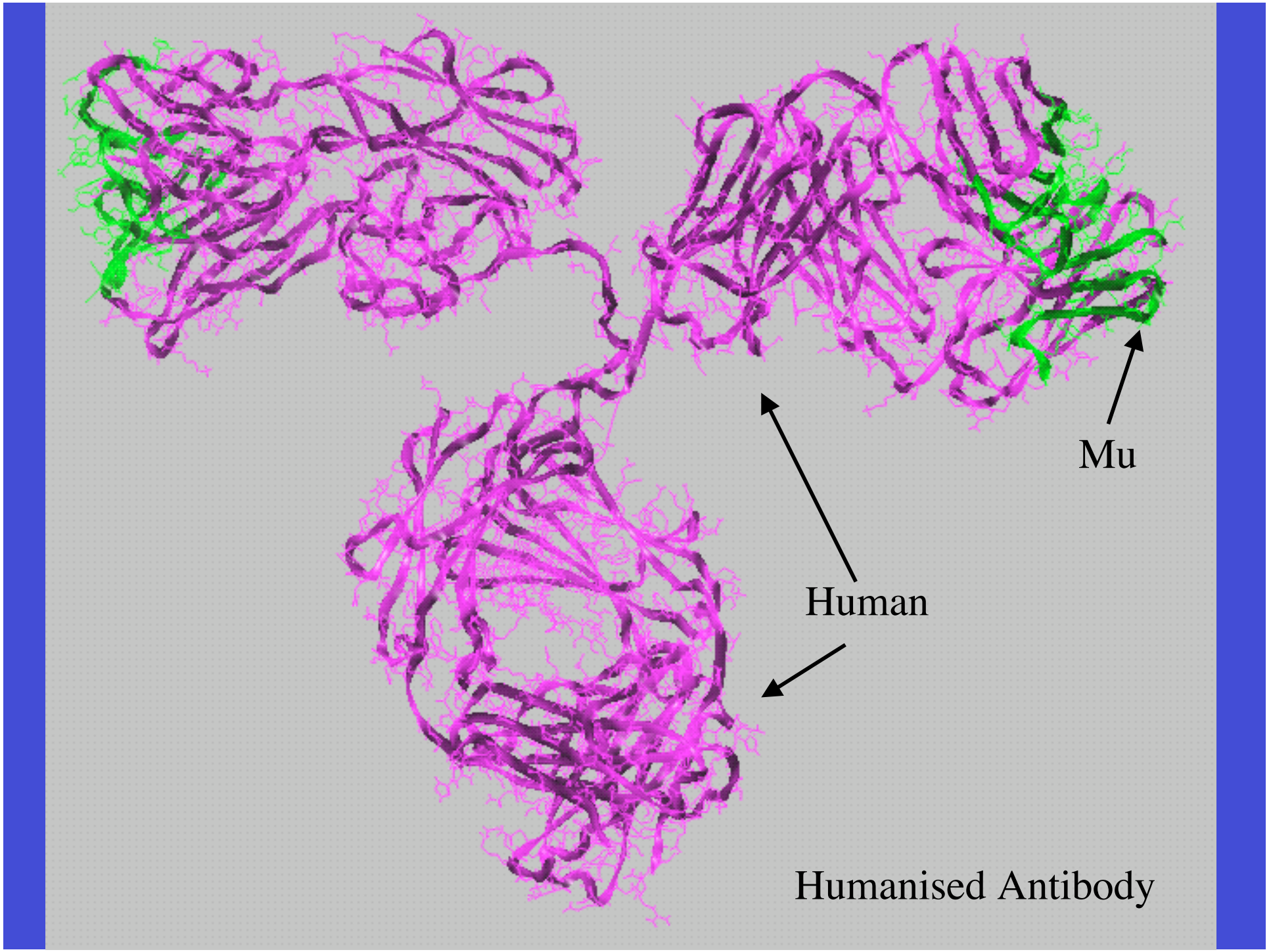



Model showing CDR's and 4 key amino acid substitution positions.


- Designed
- Assembled by PCR
- Sequenced
BstB1
BsiW1
BsiW1
BstB1
Ck
pA
HCMV
Amp
Light Chain Acceptor Vector
SV40L
GScDNA
SV40 Ori
- Designed
- Assembled by PCR
- Sequenced
HindIII
ApaI
HindIII
ApaI
HCMV
γ4
pA
Amp
Heavy Chain Acceptor Vector
SV40 Ori
SV40
gpt
Transfer heavy chain expression cassette into light chain expression vector
HCMV
Vl
Ck
pA
HCMV
CDP571DGV
Vh
GS
Ch
pA
SV40L
Amp






Mu
Human
Humanised Antibody

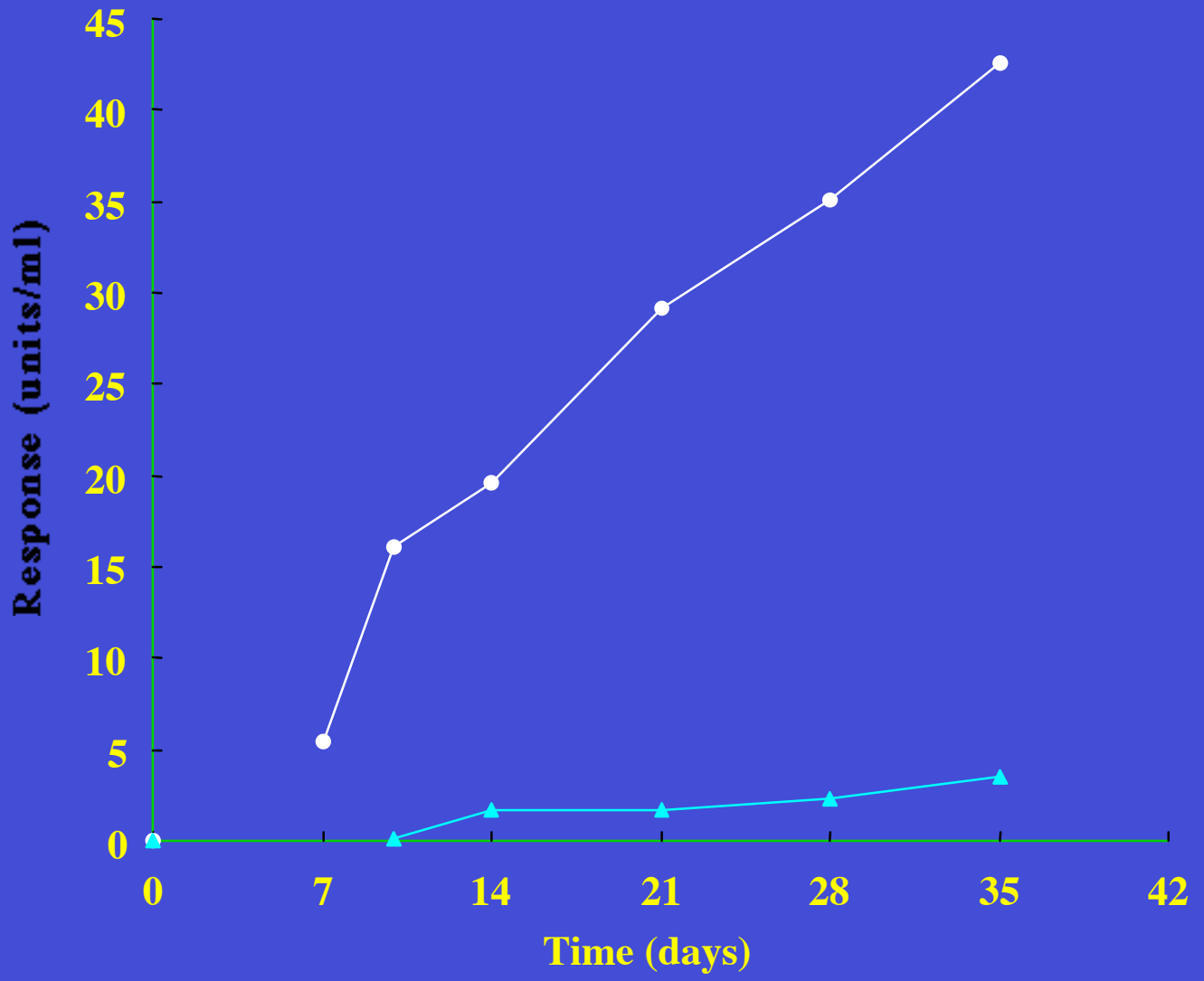
# Generation of High Affinity Antibodies

- Typical Affinities:

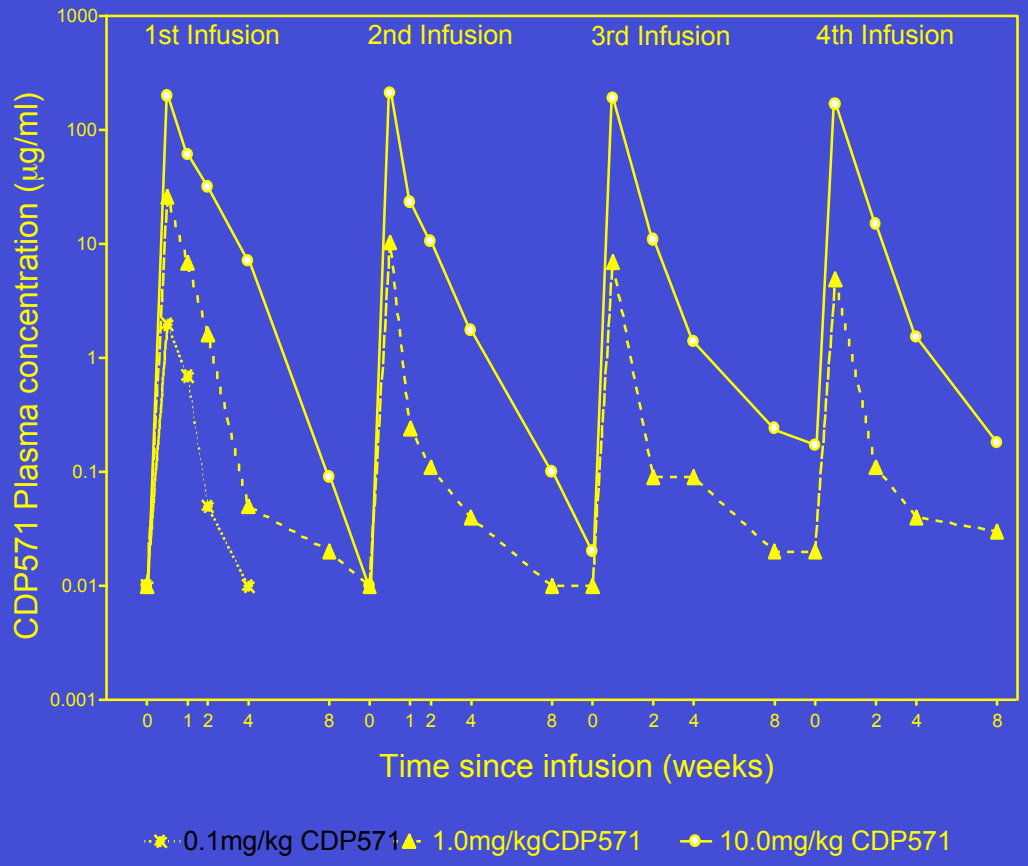
| Mab | Mu Affinity | Hu Affinity | Antigen |
|---|---|---|---|
| Ab1 | 3. 8 x10 $^{-10}$ | 3.2 x10 $^{-10}$ | TNF |
| Ab2 | 0.86 x10 $^{-10}$ | 0.5 x10 $^{-10}$ | Cytokine |
| Ab3 | 2.5 x10 $^{-10}$ | 4.5 x10 $^{-10}$ | Receptor |
| Ab4 | 4.2 x10 $^{-10}$ | 4.6 x10 $^{-10}$ | CD33 |
| Ab5 | 12.8 x10 $^{-10}$ | 12.7x10 $^{-10}$ | gp43 |

CELLTECH therapeutics

# Antibodies to Murine and Humanised TNF-antibody in Cynomolgus Monkey

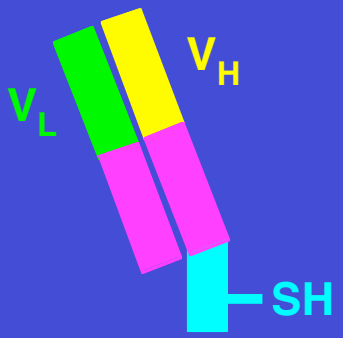


# *Repeat administration of CDP571 in Patients with Rheumatoid Arthritis*

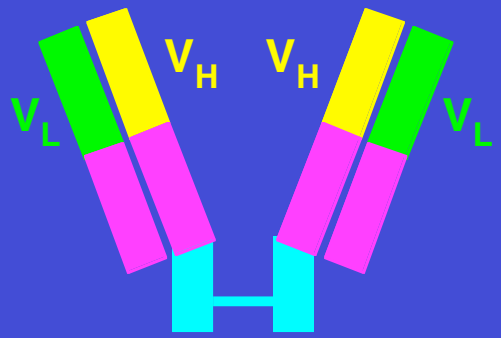



CELLTECH therapeutics

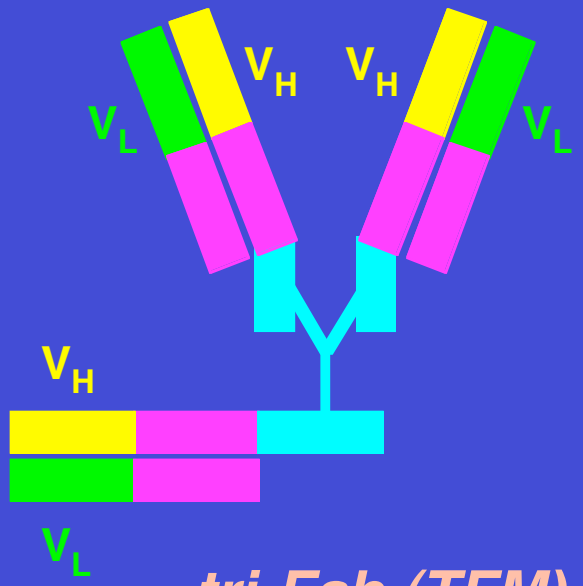
# Antibody Fragments Evaluated for Delivery of $^{90}Y$




Fab'

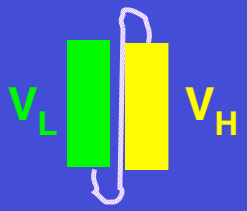



di-Fab (DFM)

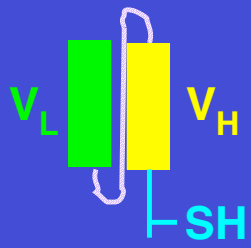



tri-Fab (TFM)

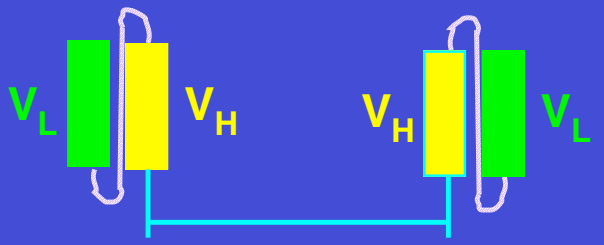

Fv




scFv

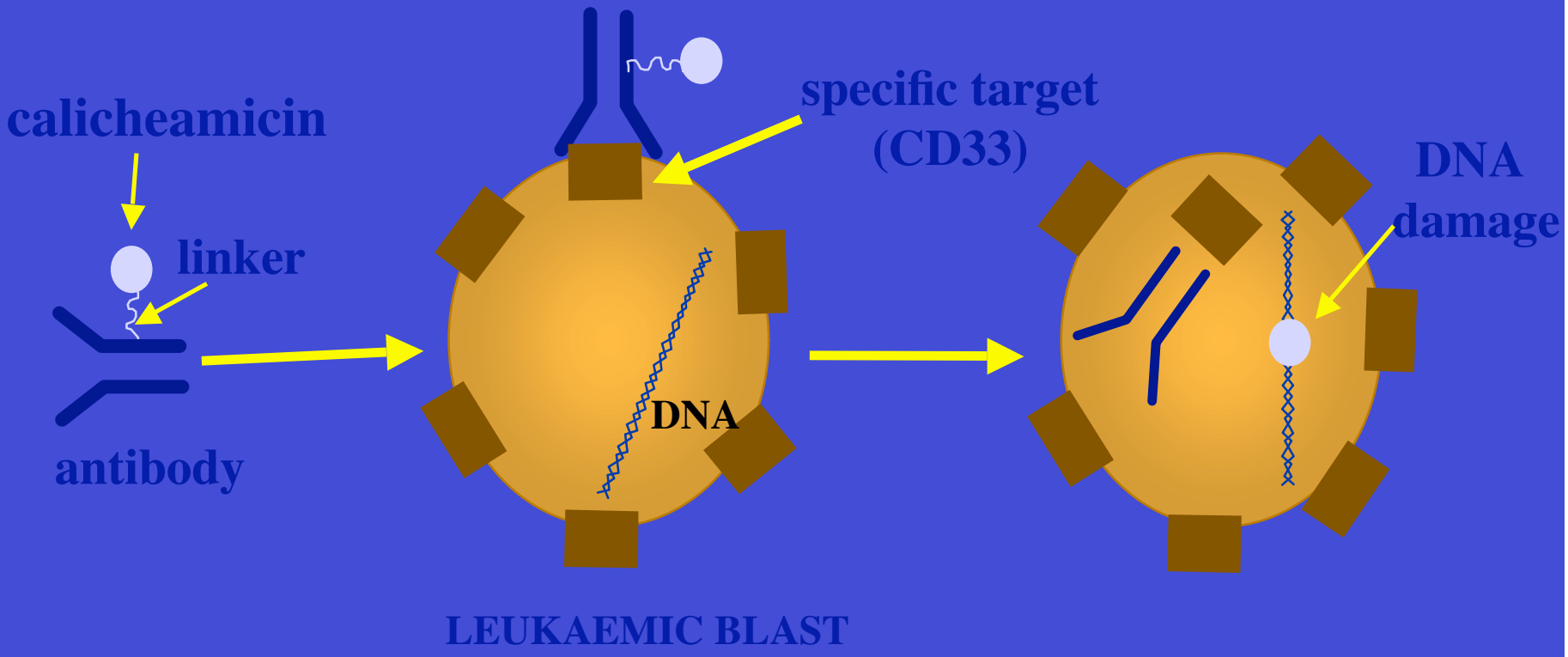


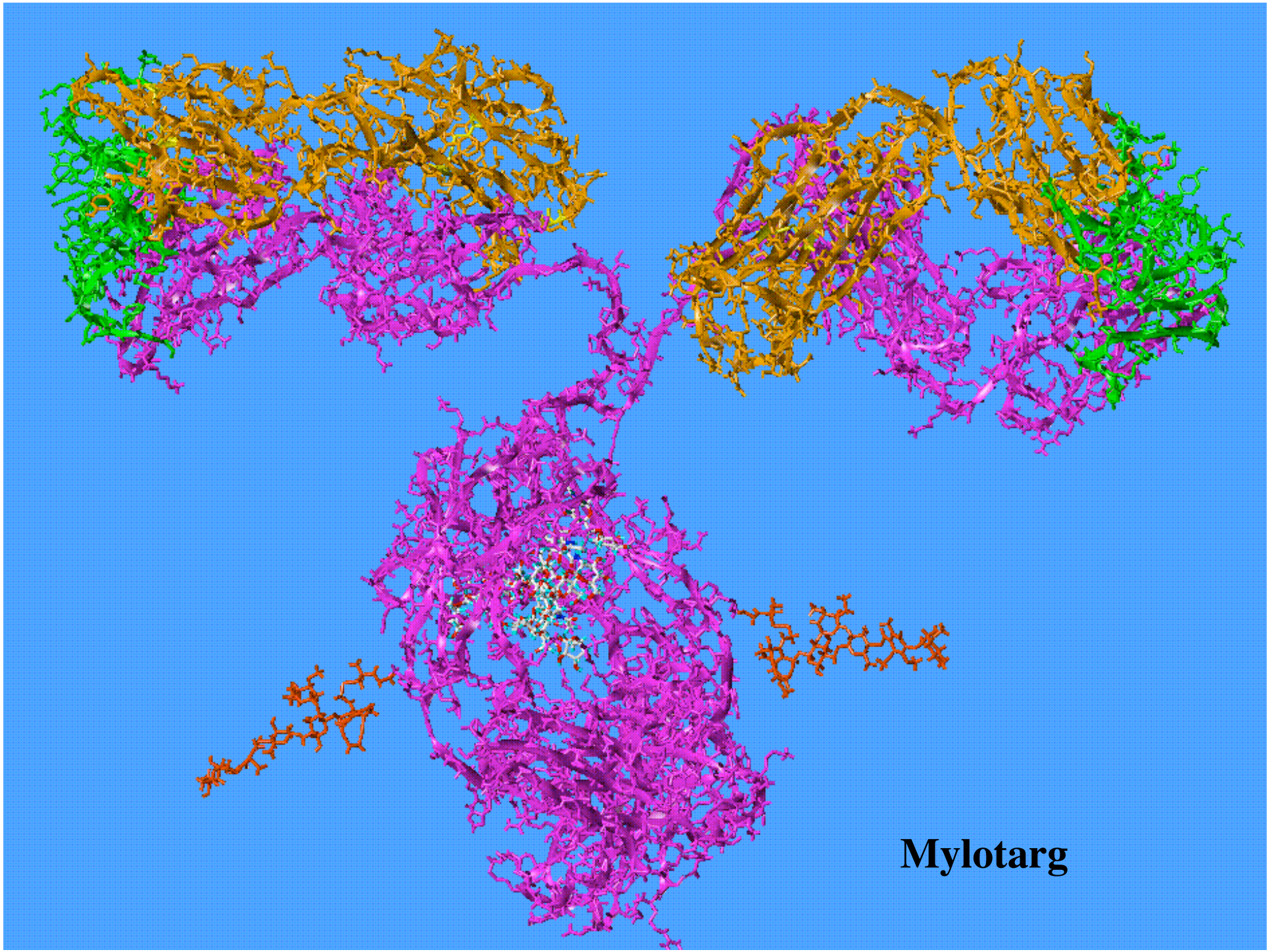

scFv-hinge

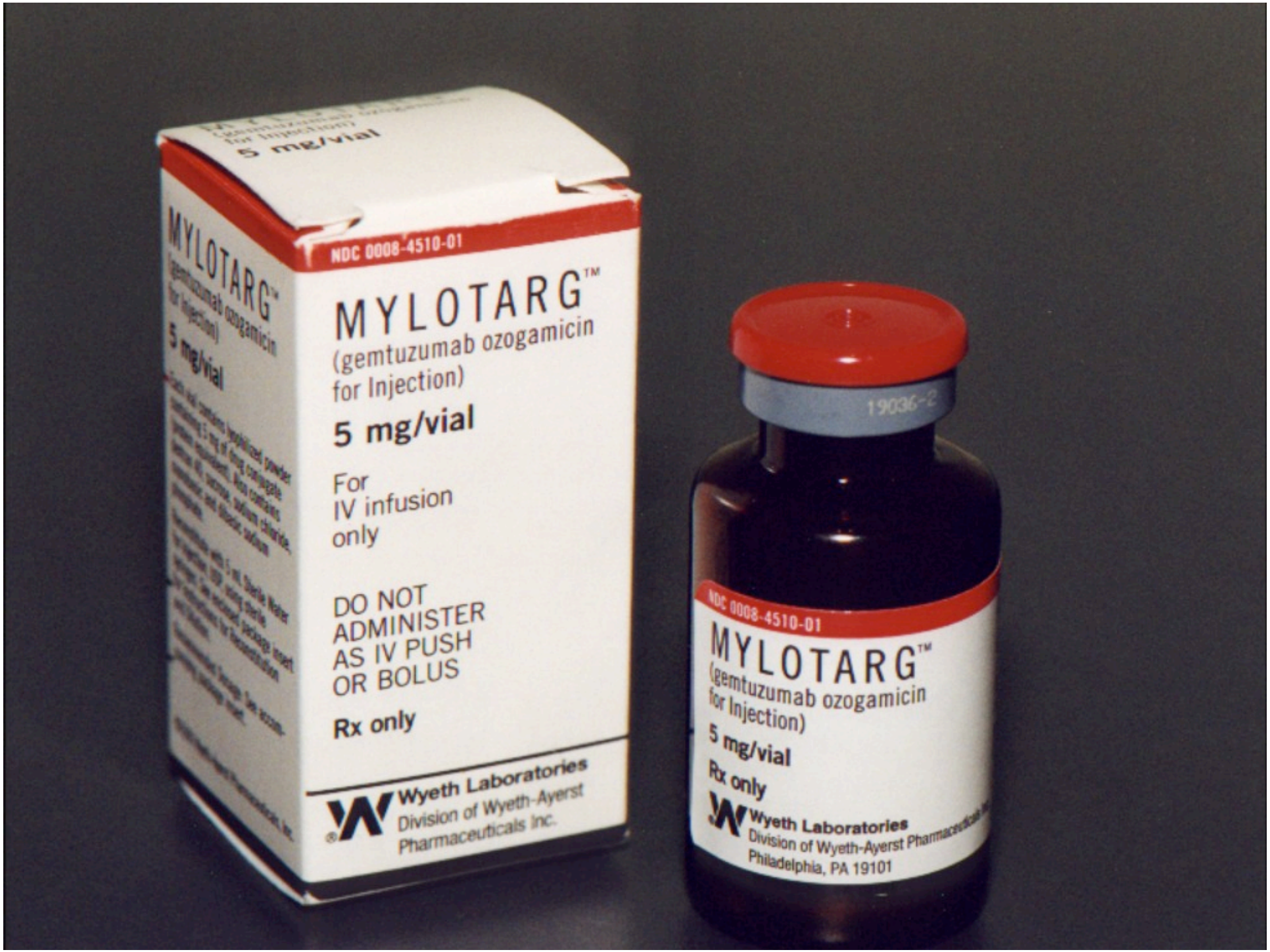



di-scFv

# Mylotarg™ - a pioneering treatment for acute myeloid leukaemia




Mylotarg


5 mg/vial
MYLOTARG™
(gemtuzumab ozogamicin
for Injection)
5 mg/vial
NDC 0008-4510-01
MYLOTARG™
(gemtuzumab ozogamicin
for Injection)
5 mg/vial
For
IV infusion
only
DO NOT
ADMINISTER
AS IV PUSH
OR BOLUS
Rx only
Wyeth Laboratories
Division of Wyeth-Ayerst
Pharmaceuticals Inc.
19036-2
NDC 0008-4510-01
MYLOTARG™
(gemtuzumab ozogamicin
for Injection)
5 mg/vial
Rx only
Wyeth Laboratories
Division of Wyeth-Ayerst Pharmaceuticals
Philadelphia, PA 19101