Antibodies

Structure of antibodies

General structure of antibodies

Structural differences between isotypes

Half-life

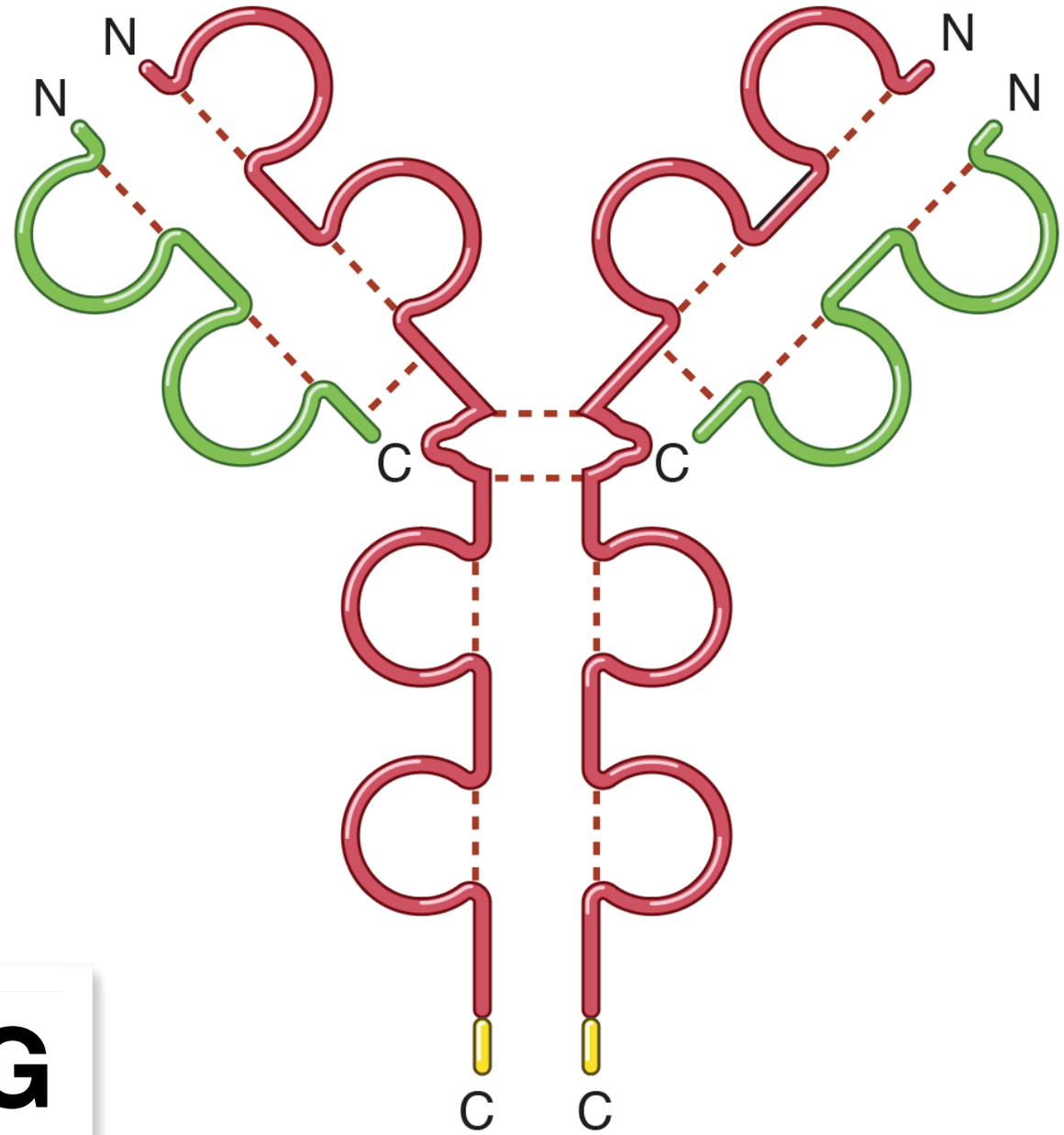
Monoclonal antibodies

Monoclonal vs polyclonal antibody

Development

Therapeutic applications

Structure of antibodies



Four polypeptide chains

Two identical heavy chains

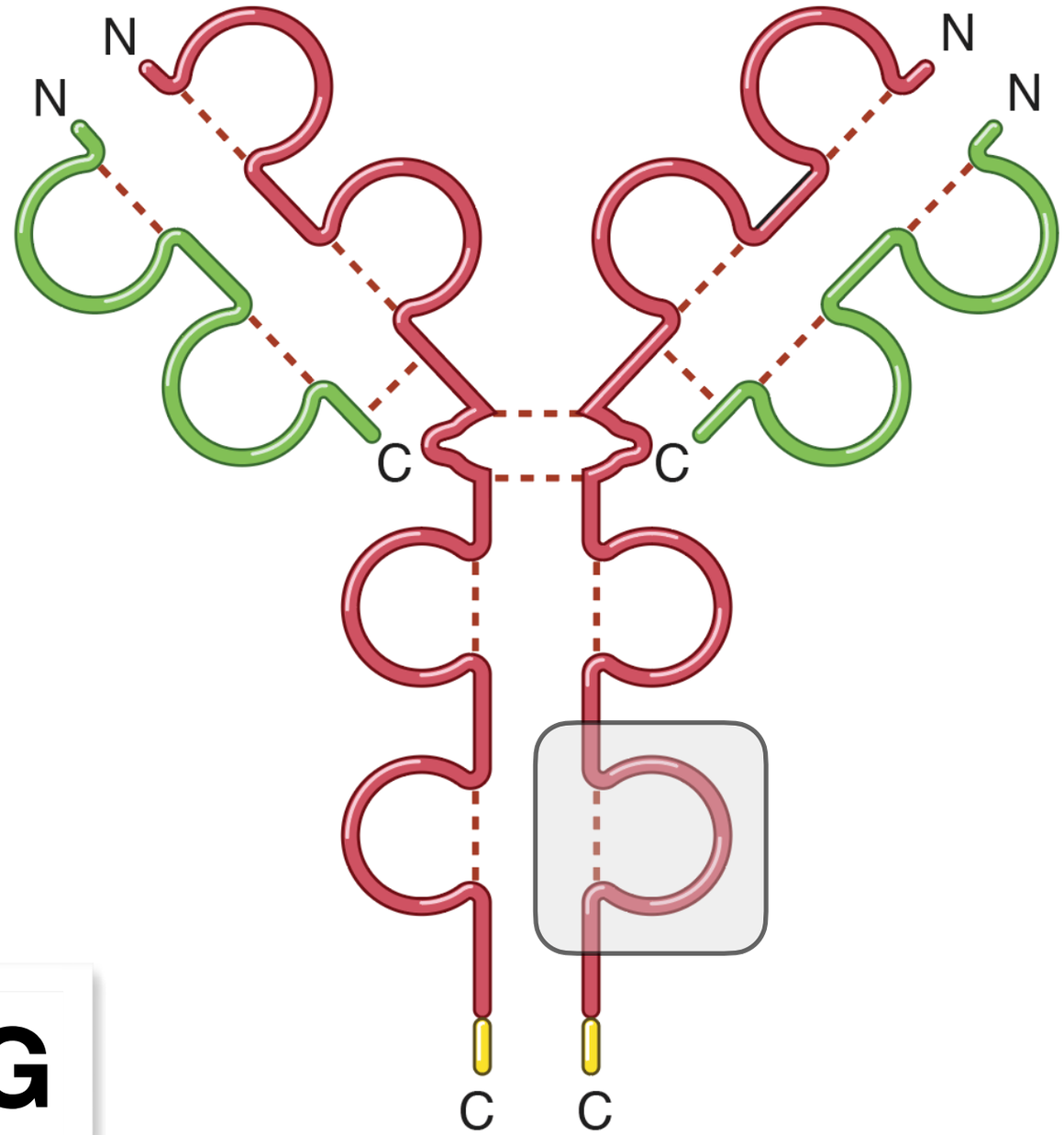
Two identical light chains

Four disulfide bonds

Disulfide bond -----

IgG

Structure of antibodies



IgG

Four polypeptide chains

Two identical heavy chains

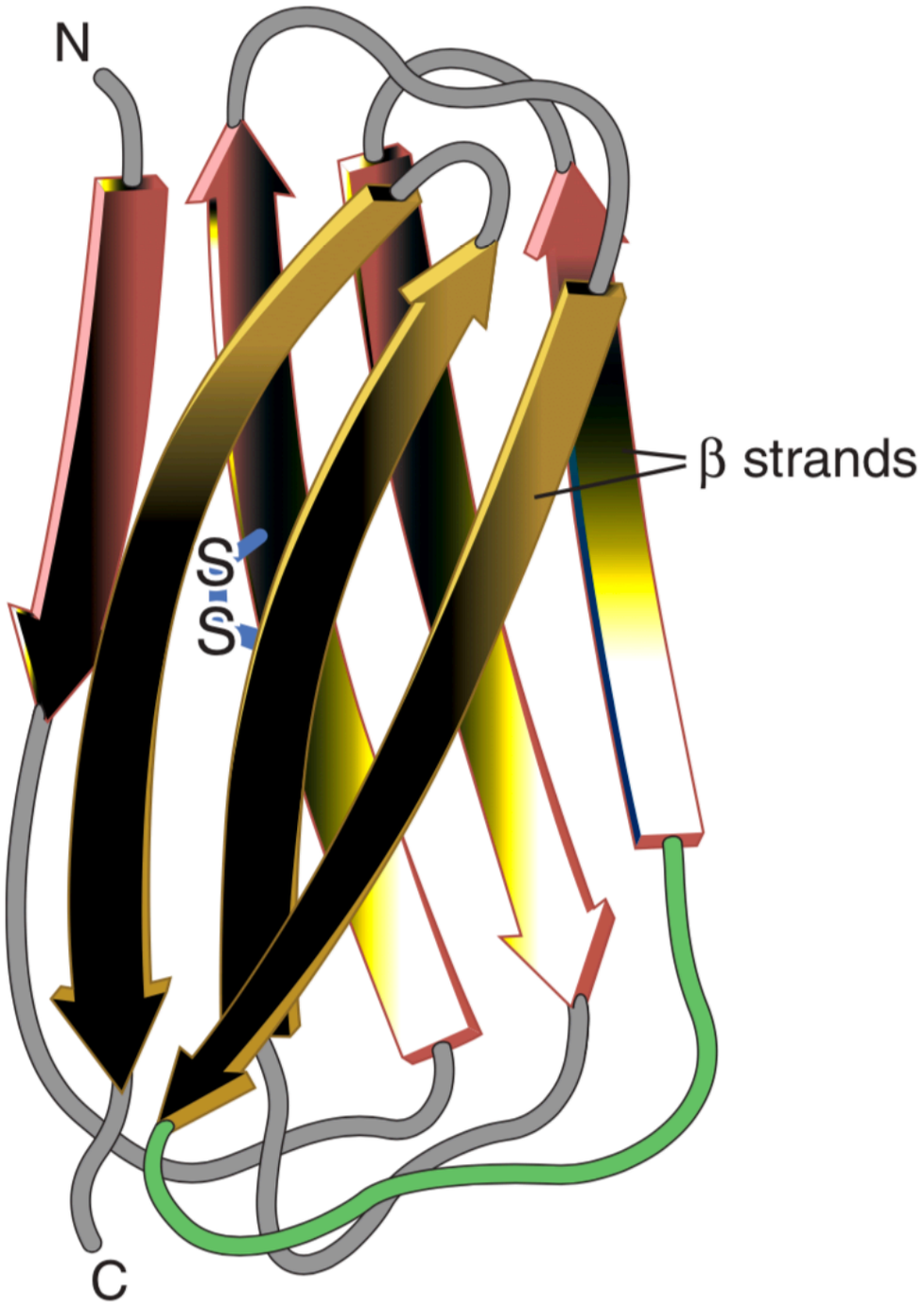
Two identical light chains

Both heavy and light chains contains globular domains called **Ig domain**

Disulfide bond -----

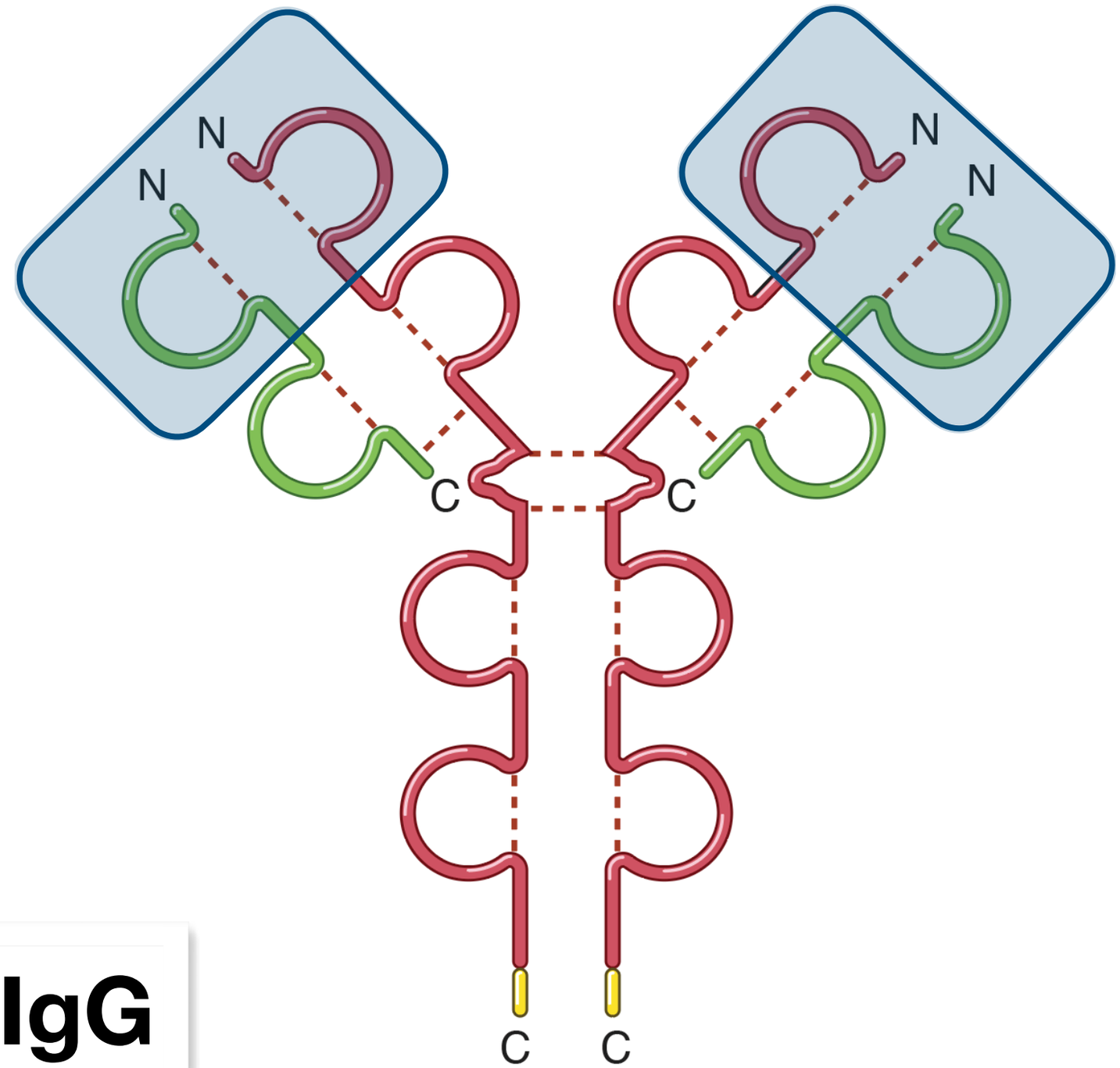
Ig domain 

Structure of IgG domain



Two β sheets held together by one disulphide bond

Structure of antibodies



IgG

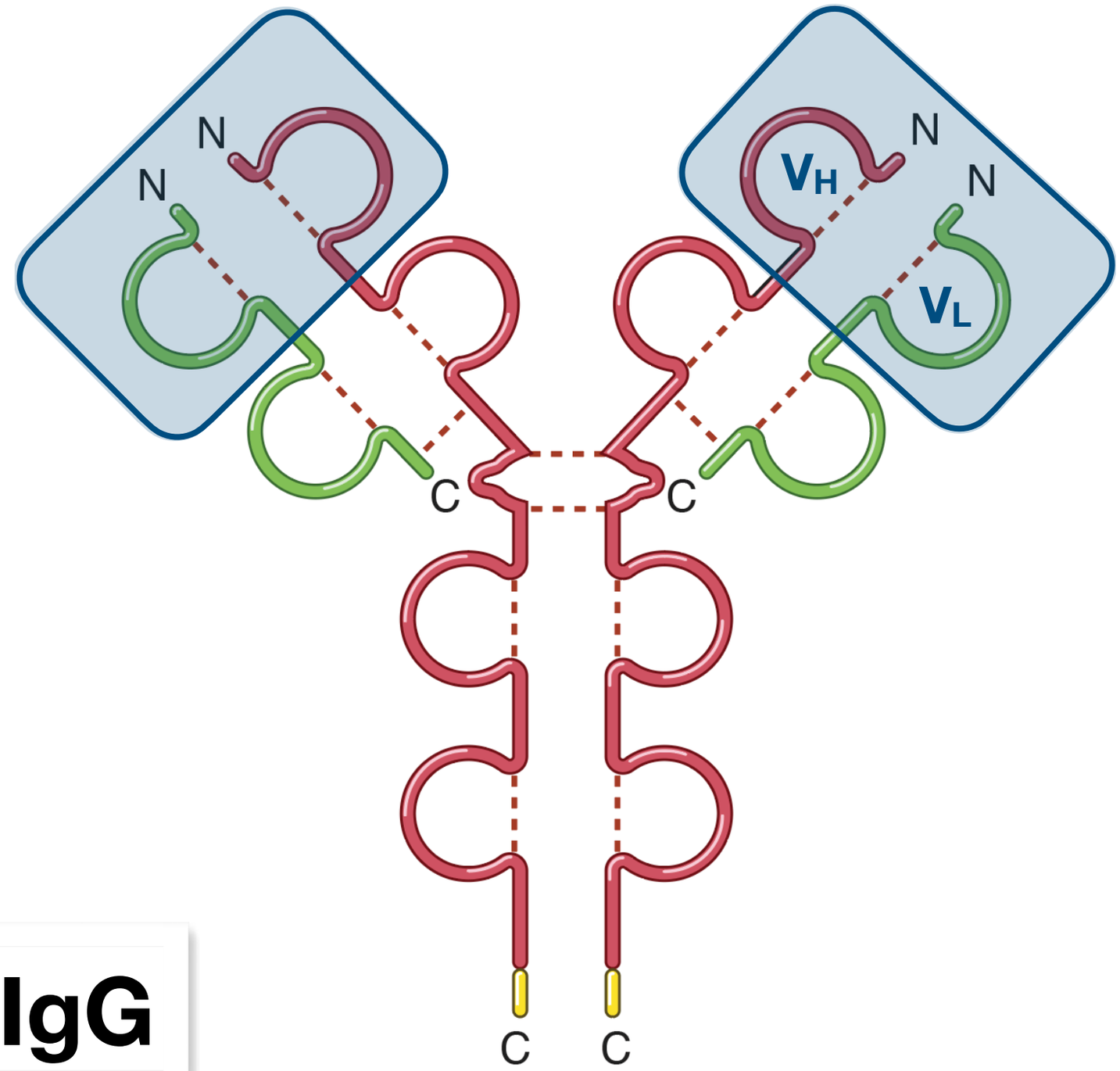
Variable (V) regions

Antigen binding

Constant (C) regions

Recognized by immune cells

Structure of antibodies



IgG

Variable (V) regions

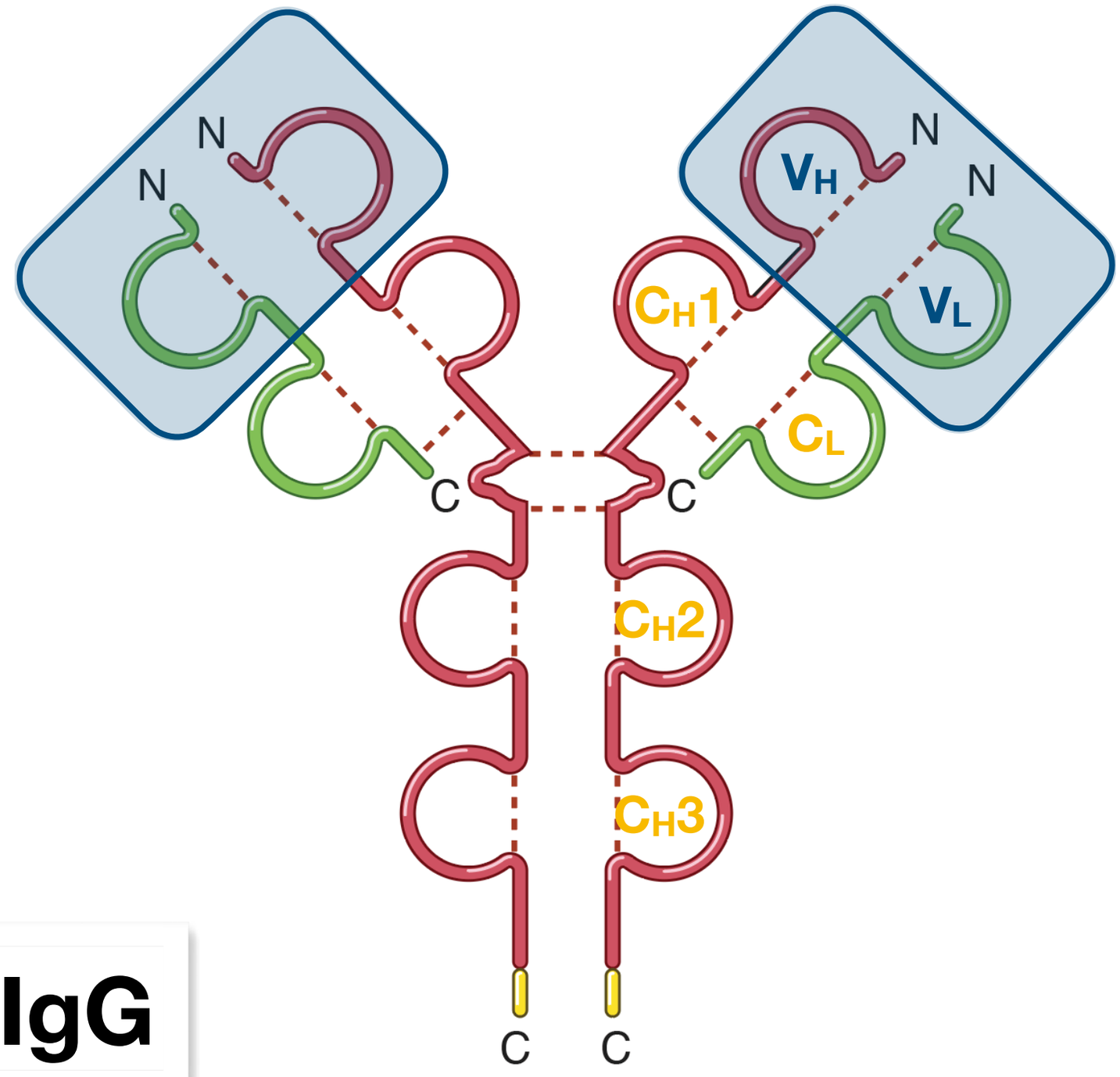
Antigen binding

Each variable region is composed by two Ig domains, one V_H and one V_L

Constant (C) regions

Recognized by immune cells

Structure of antibodies



IgG

Variable (V) regions

Antigen binding

Each variable region is composed by two Ig domains, one V_H and one V_L

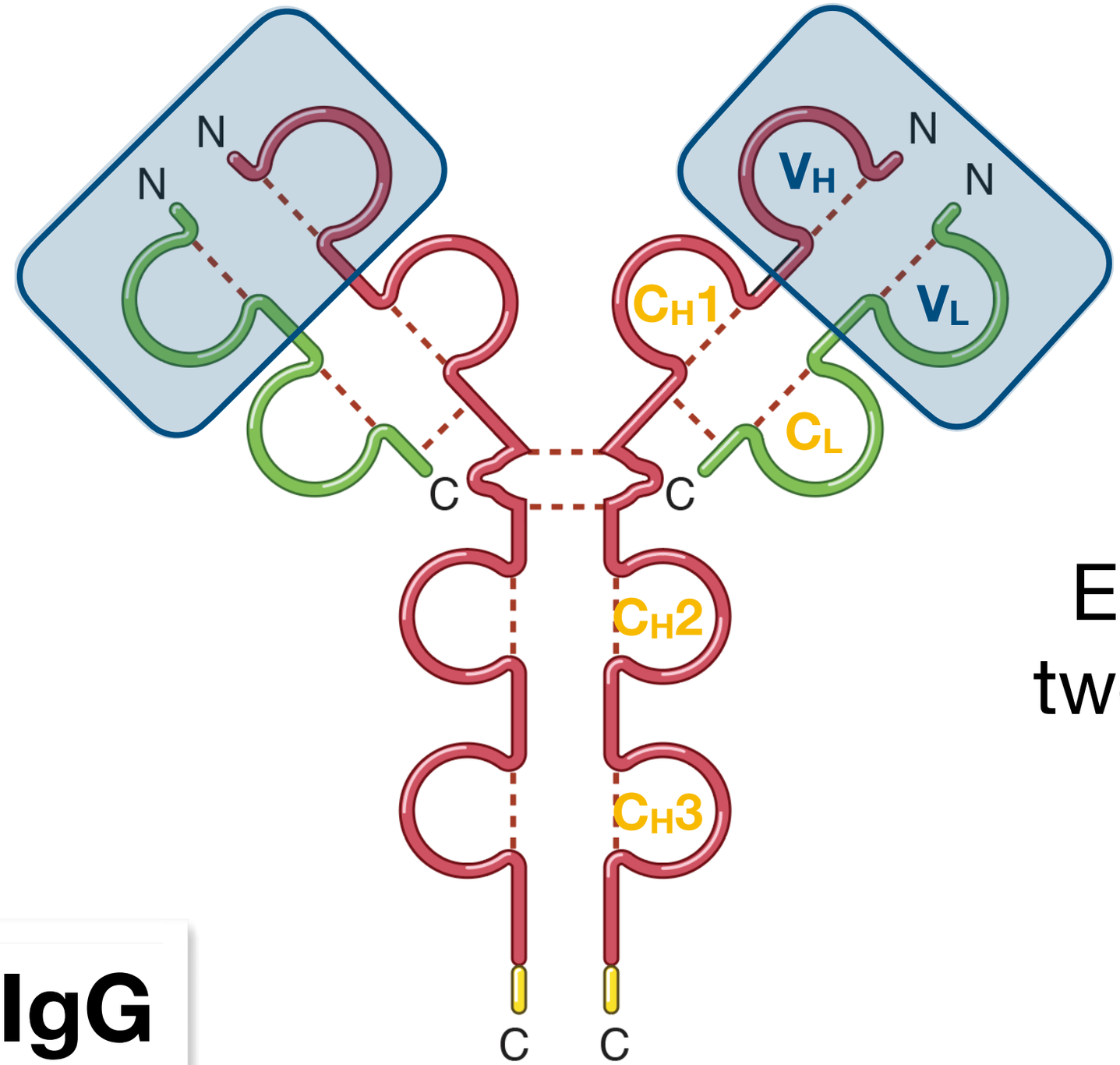
Constant (C) regions

Recognized by immune cells

Three Ig domains on the heavy chain, C_H1 C_H2 and C_H3

One Ig domain on the light chain, C_L

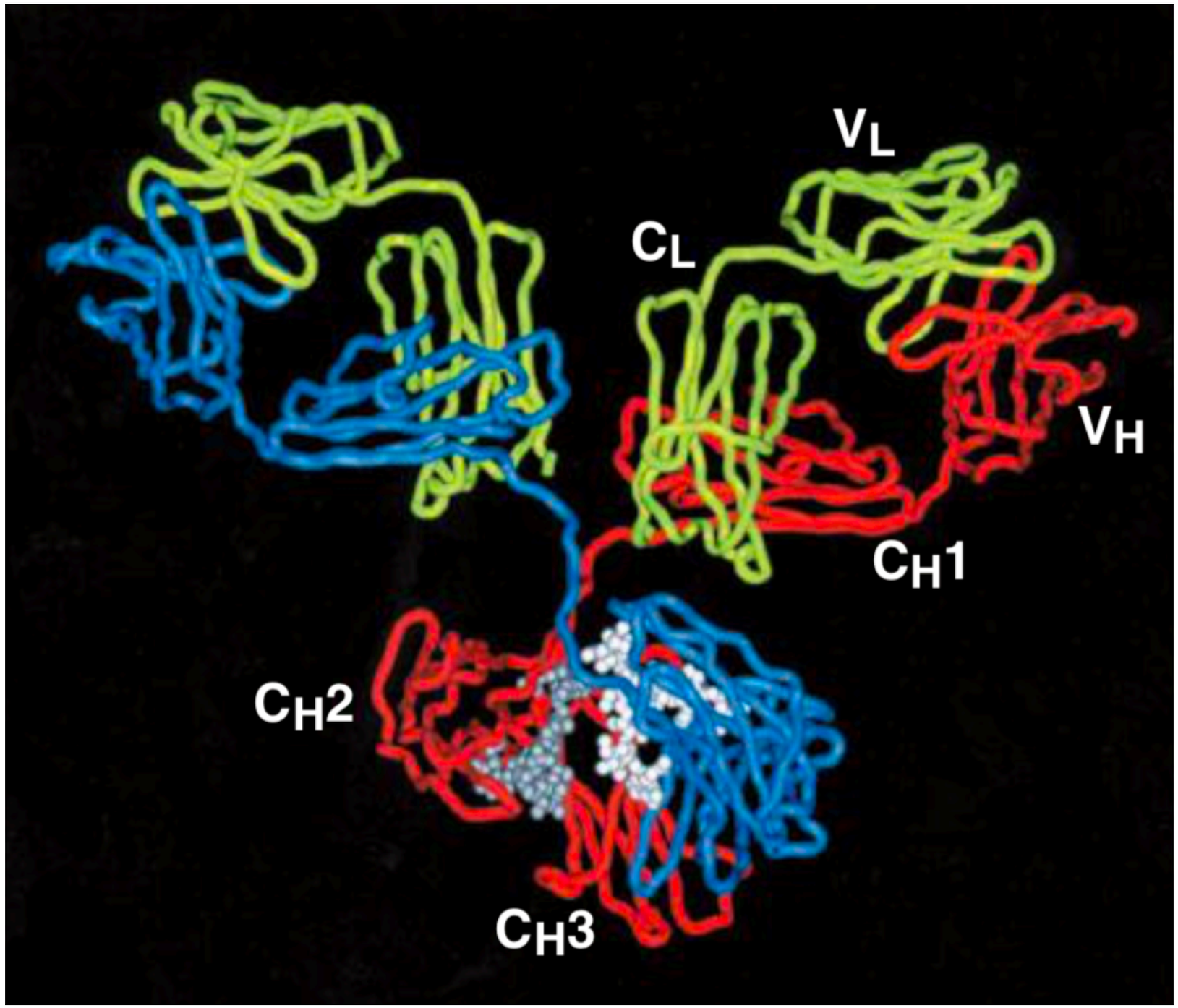
Structure of antibodies



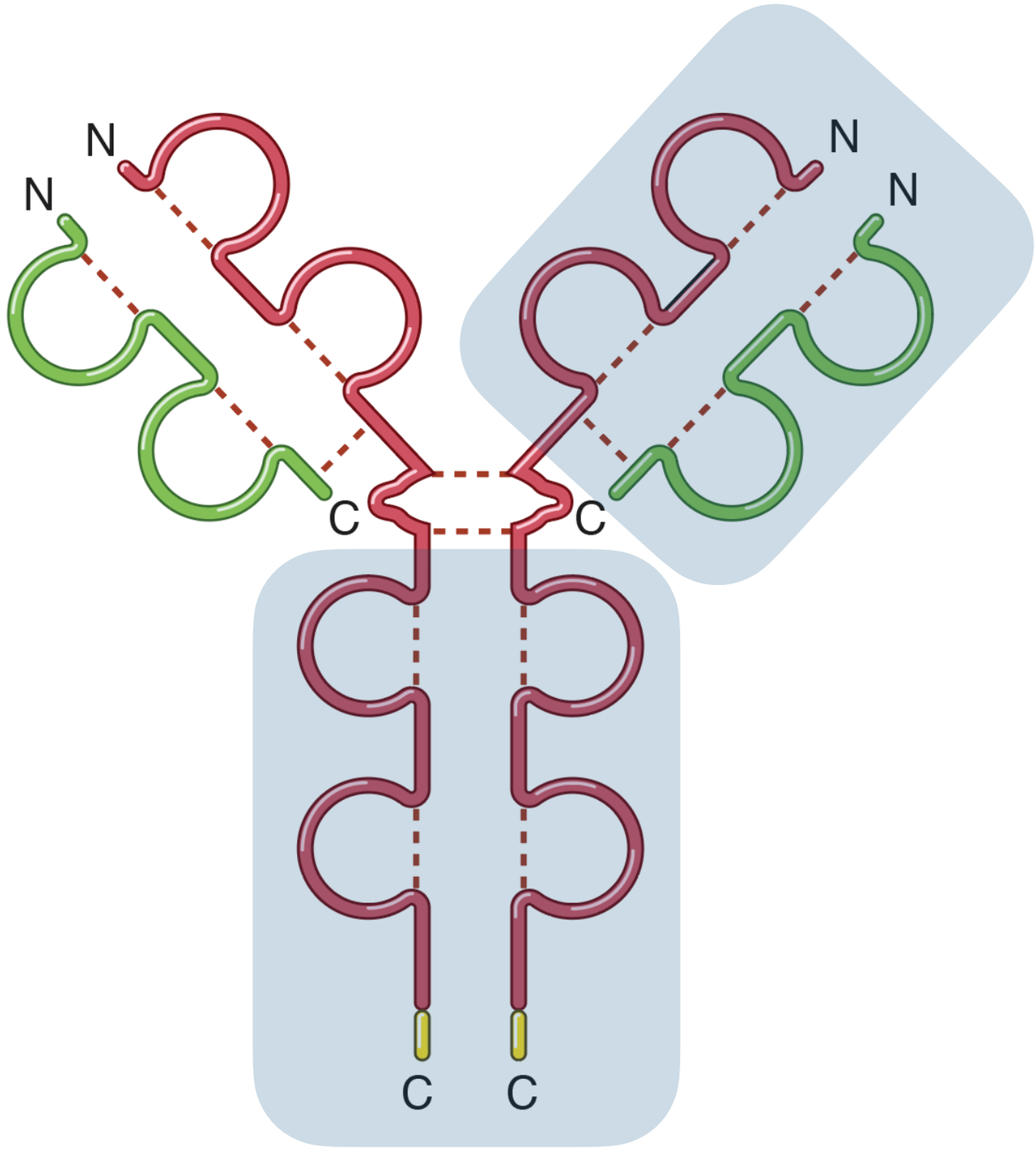
IgG

Each antibody has two identical antigen binding sites

Crystal structure of IgG



Fc and Fab



Fab region

Fragment **a**ntigen **b**inding

it contains the antigen binding portion

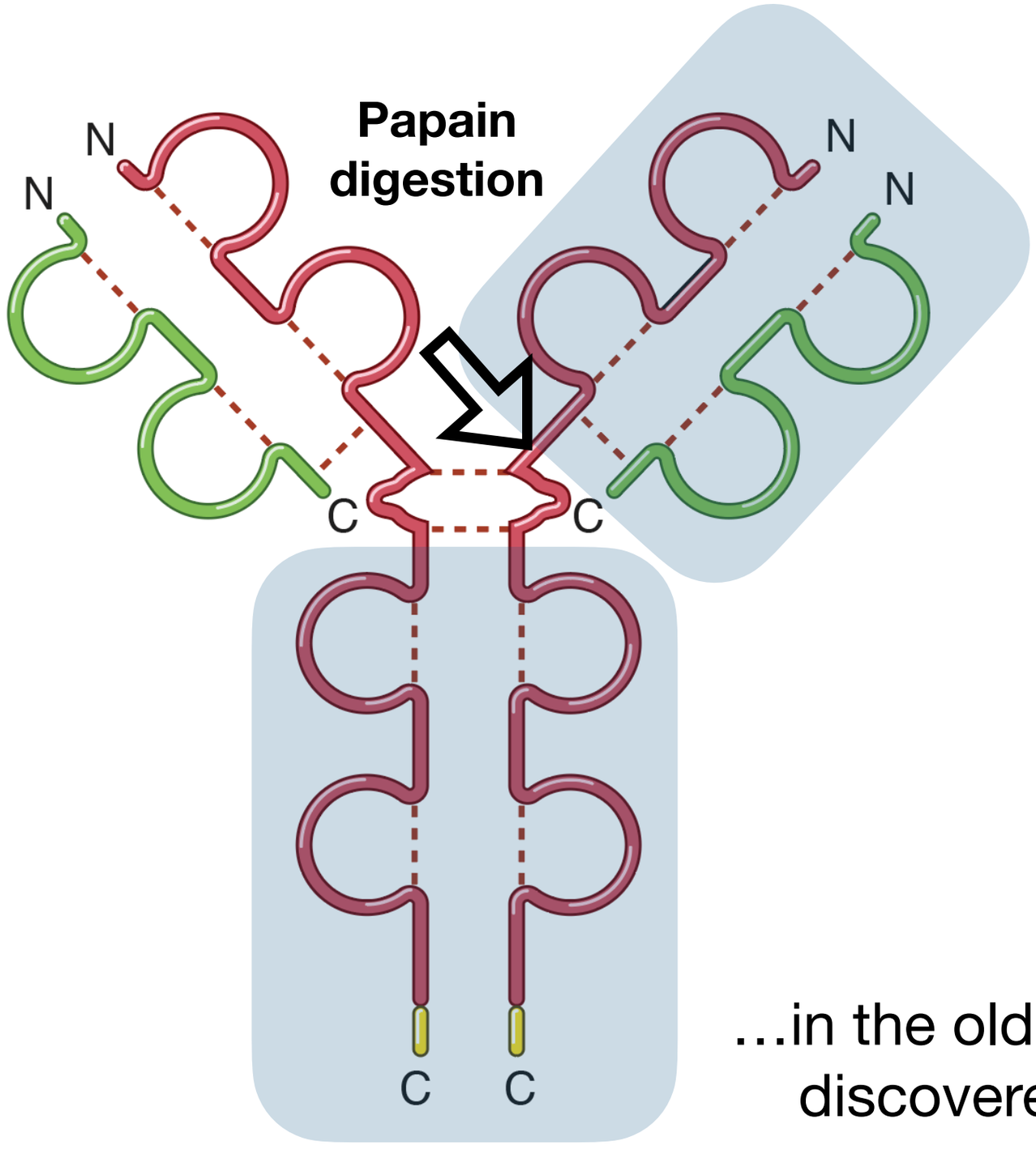
Fc region

Fragment **c**rystallizable

it’s involved in effector function

- **Fc receptor binding**
- **Complement binding**

Fc and Fab



Fab region

Fragment **a**ntigen **b**inding

it contains the antigen binding portion

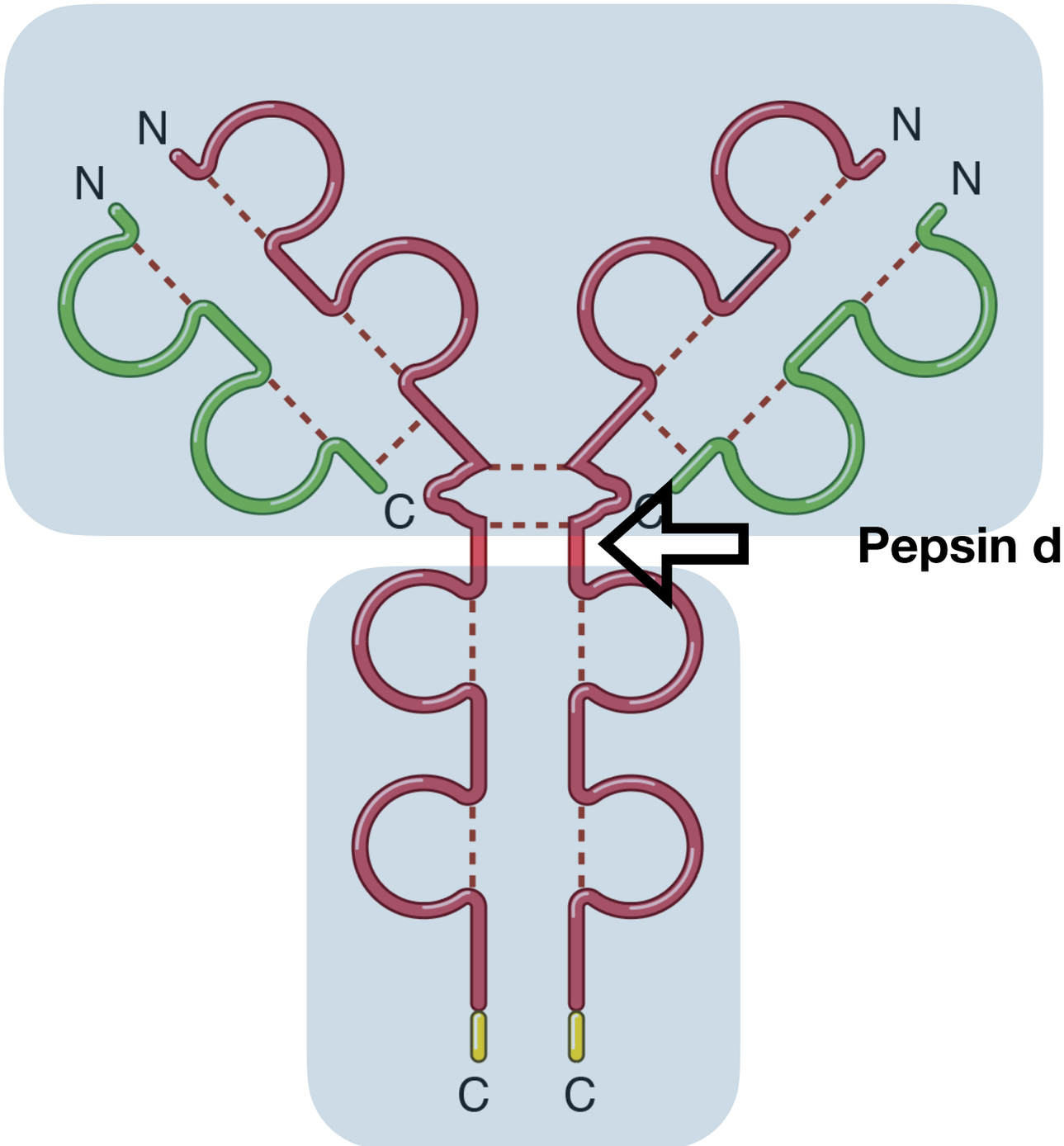
Fc region

Fragment **c**rystallizable

it’s involved in effector function

…in the old times, these features were discovered thanks to **proteolysis**

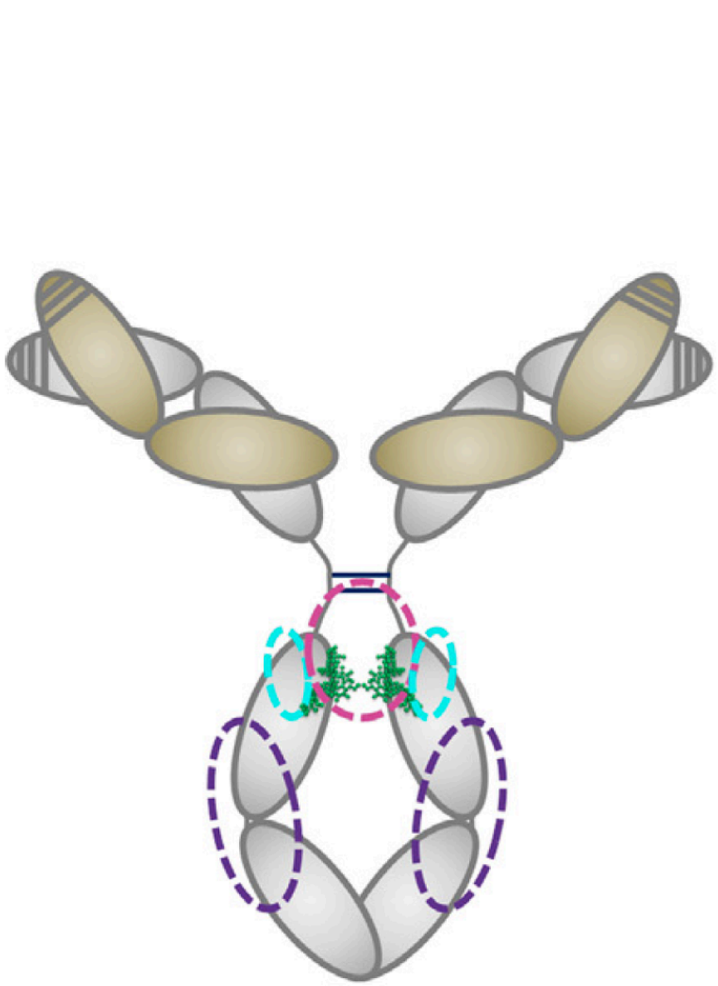
$F(ab')_2$

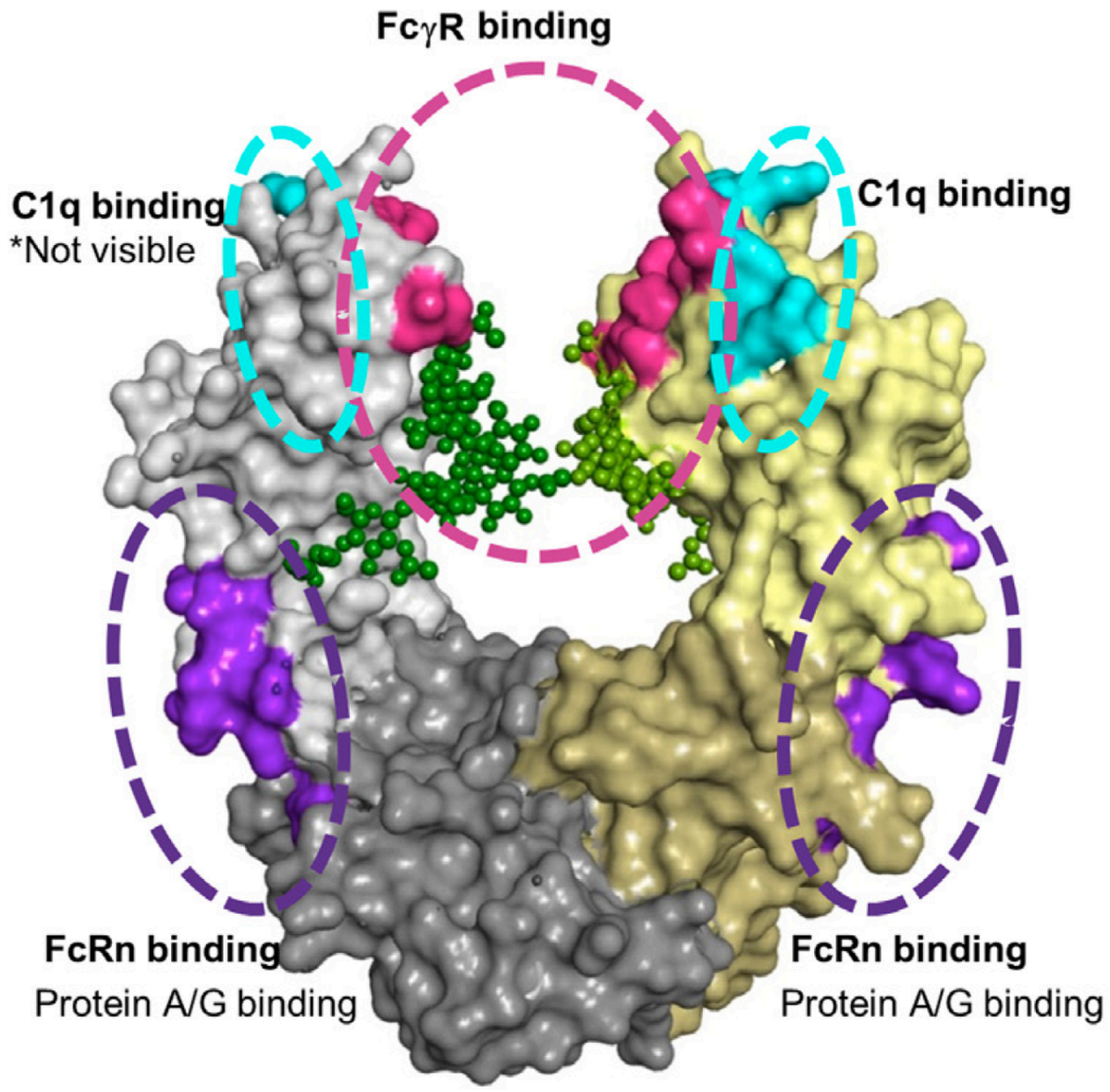


$F(ab')_2$ region

Pepsin digestion

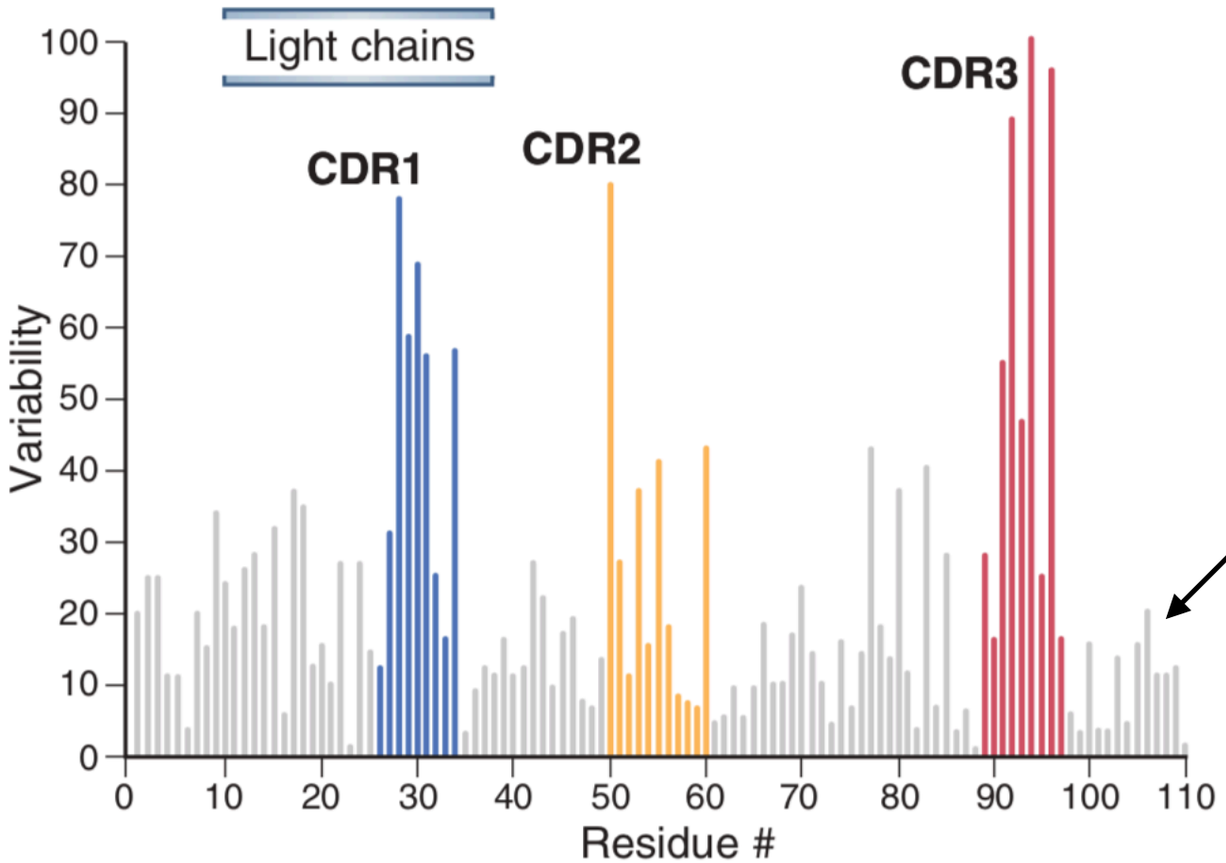
Interaction sites in the Fc region have now been fully characterised





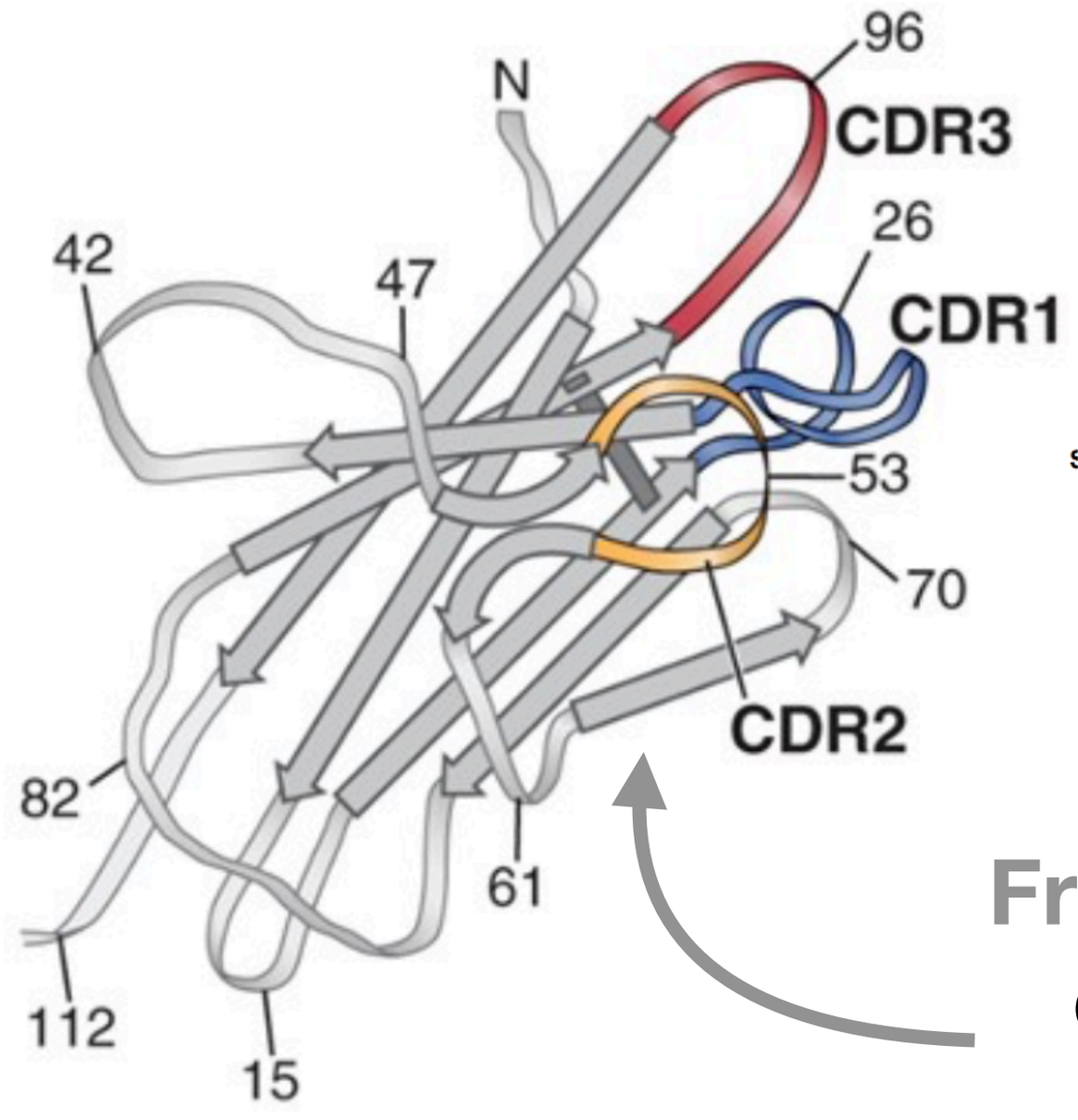
A closer look to the variable regions

In the V_L and in the V_H, sequence variability is higher in three stretches of residues, called complementarity-determining regions (CDR)



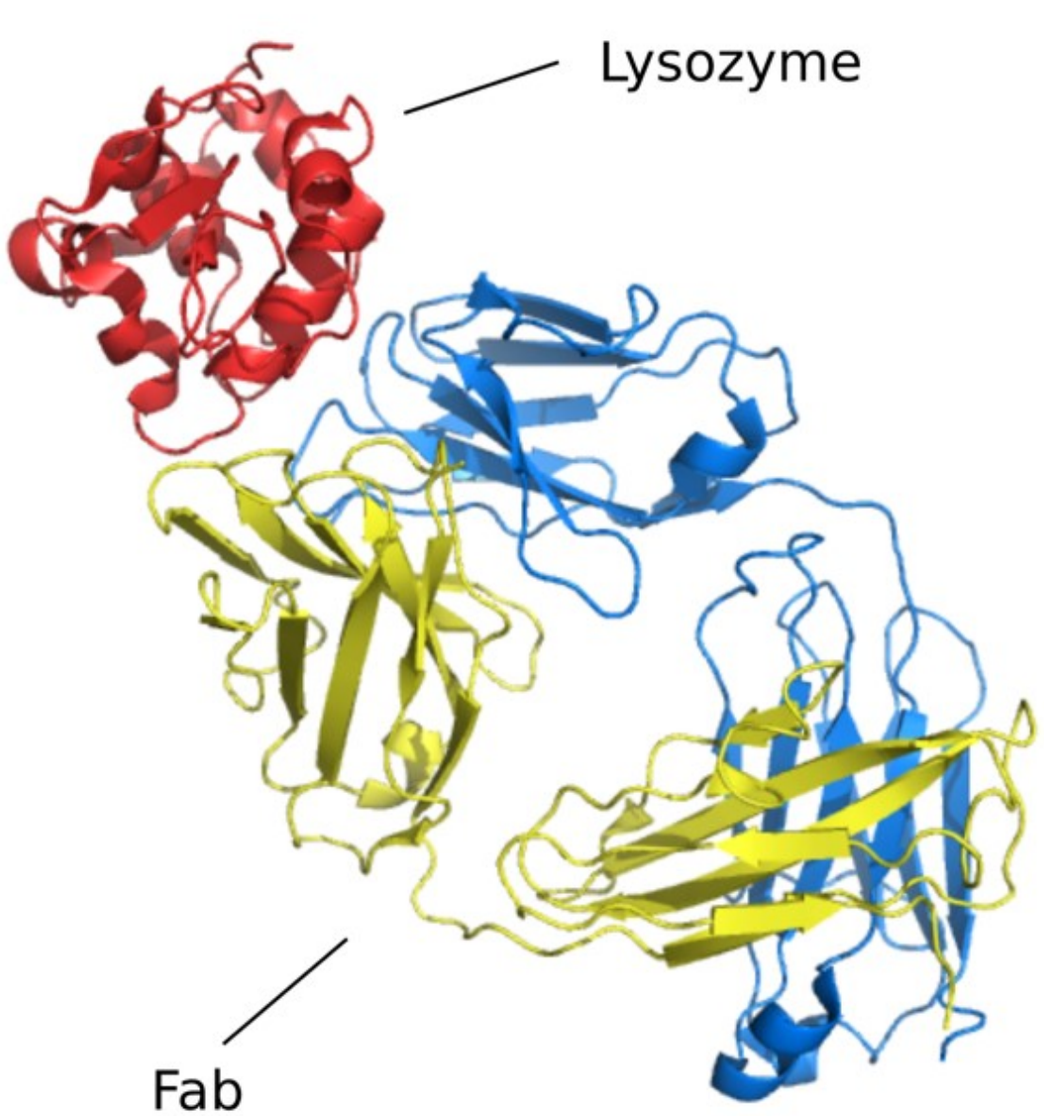
more conserved regions are called "framework"

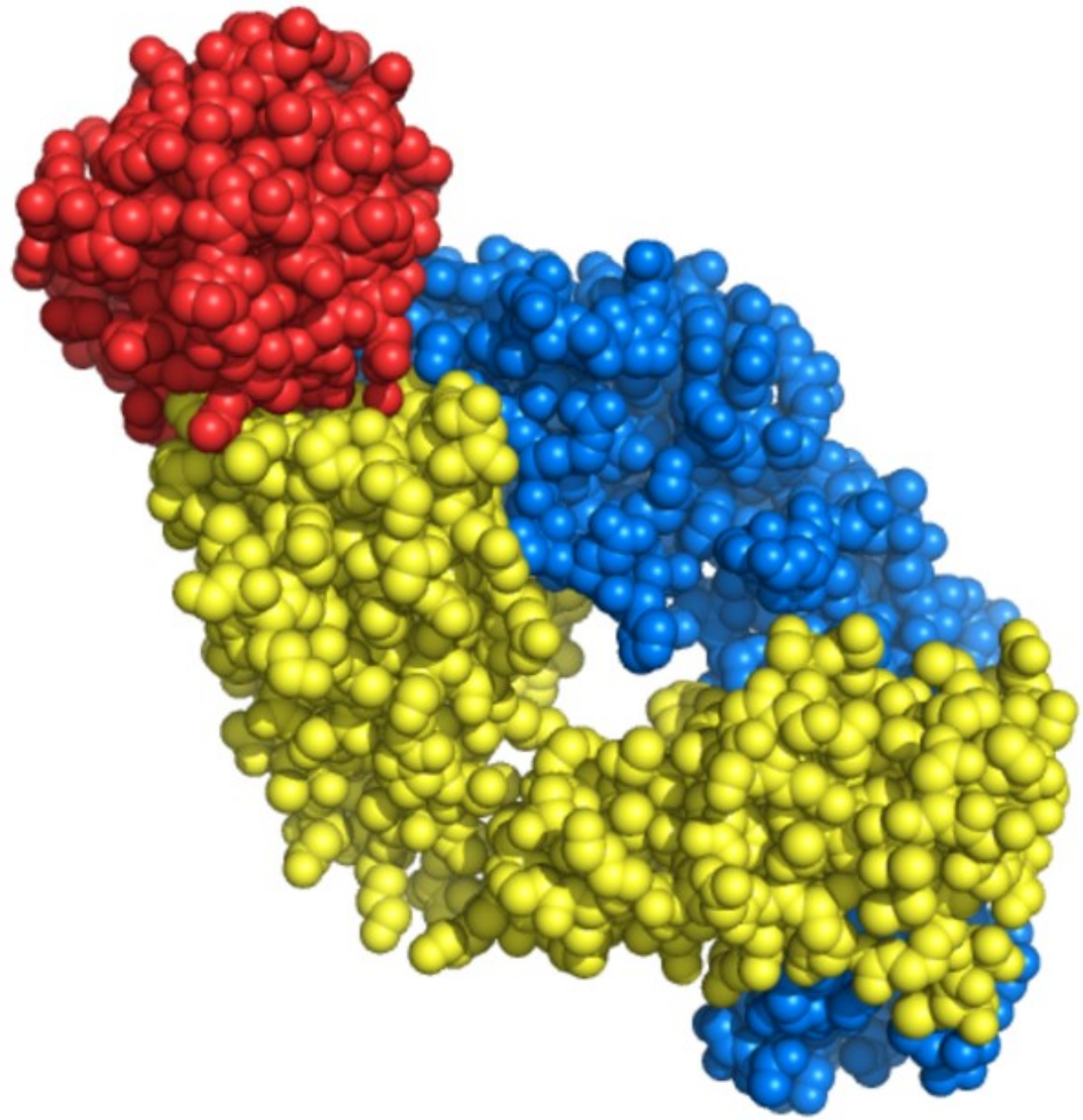
The three CDRs correspond to the loops



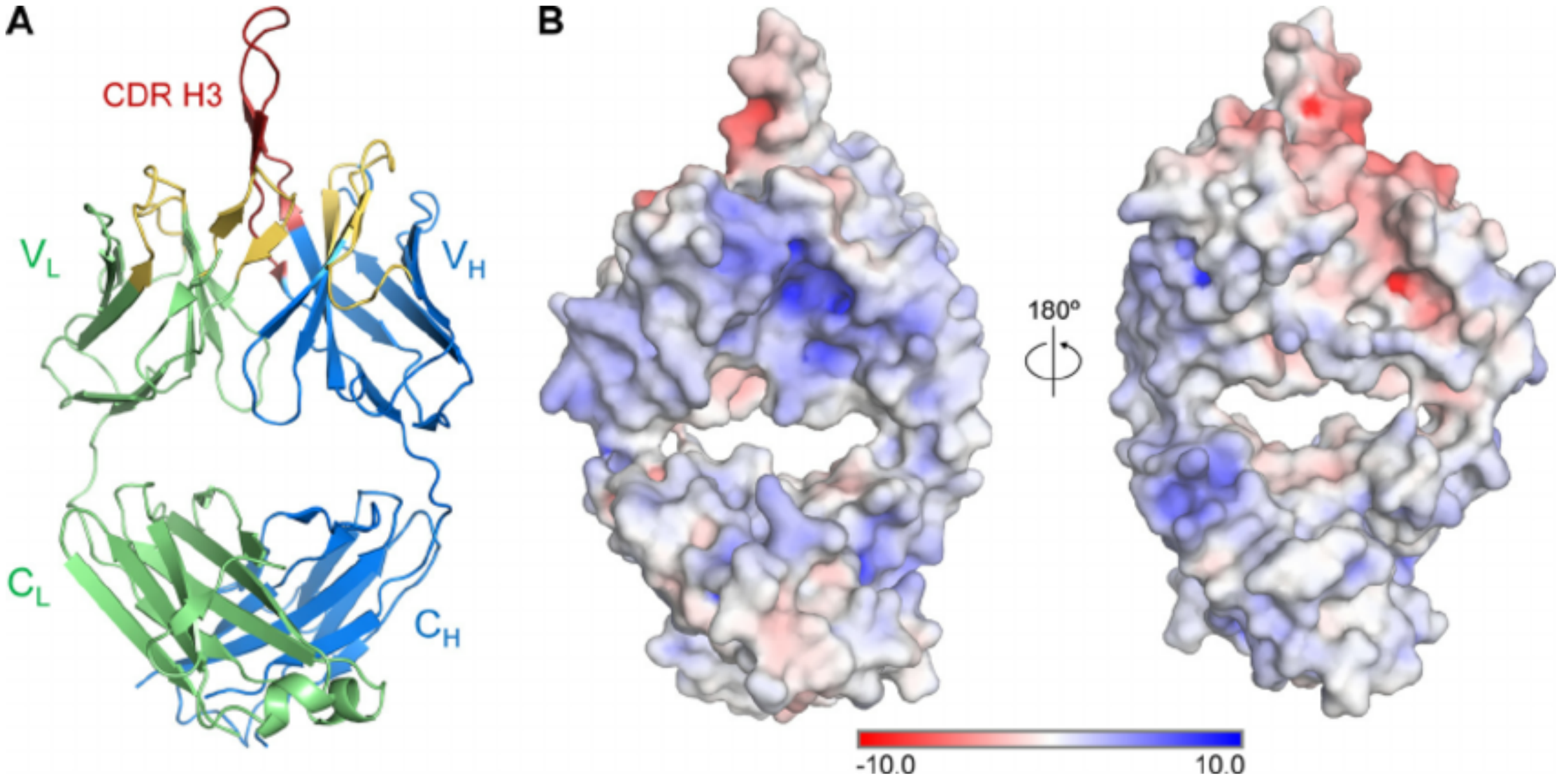
Framework regions correspond to β sheets

Heavy and light chain CDRs are typically engaged in Ag binding





Our immune system designed other creative solutions



4-5 The hinge region of the immunoglobulin molecule allows flexibility in binding to multiple antigens

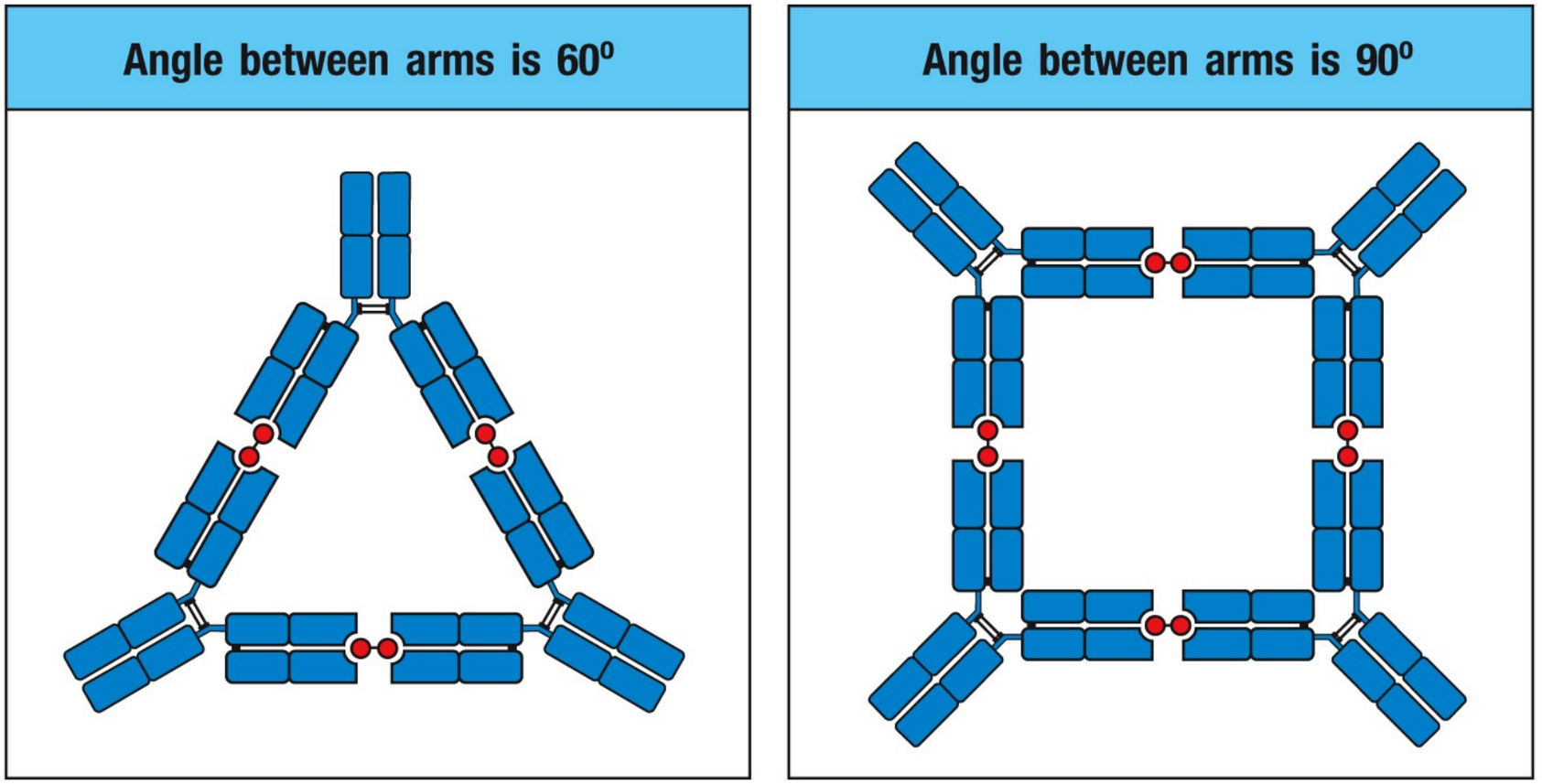
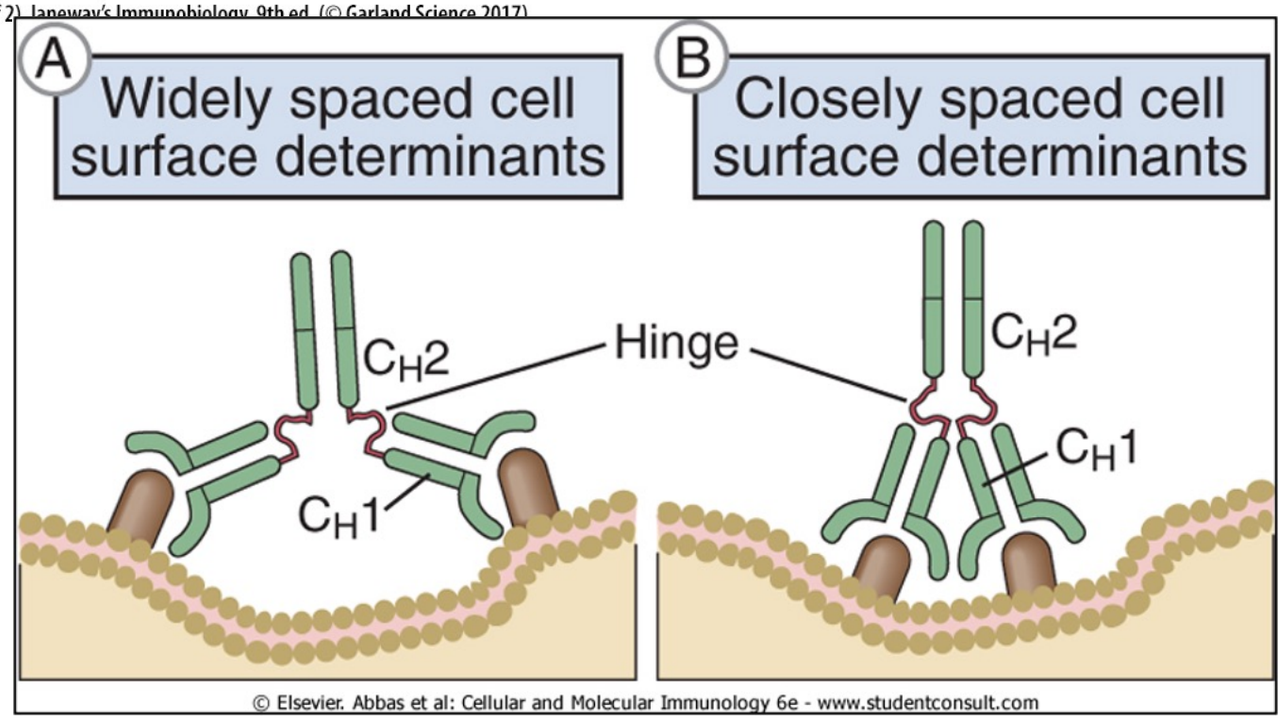
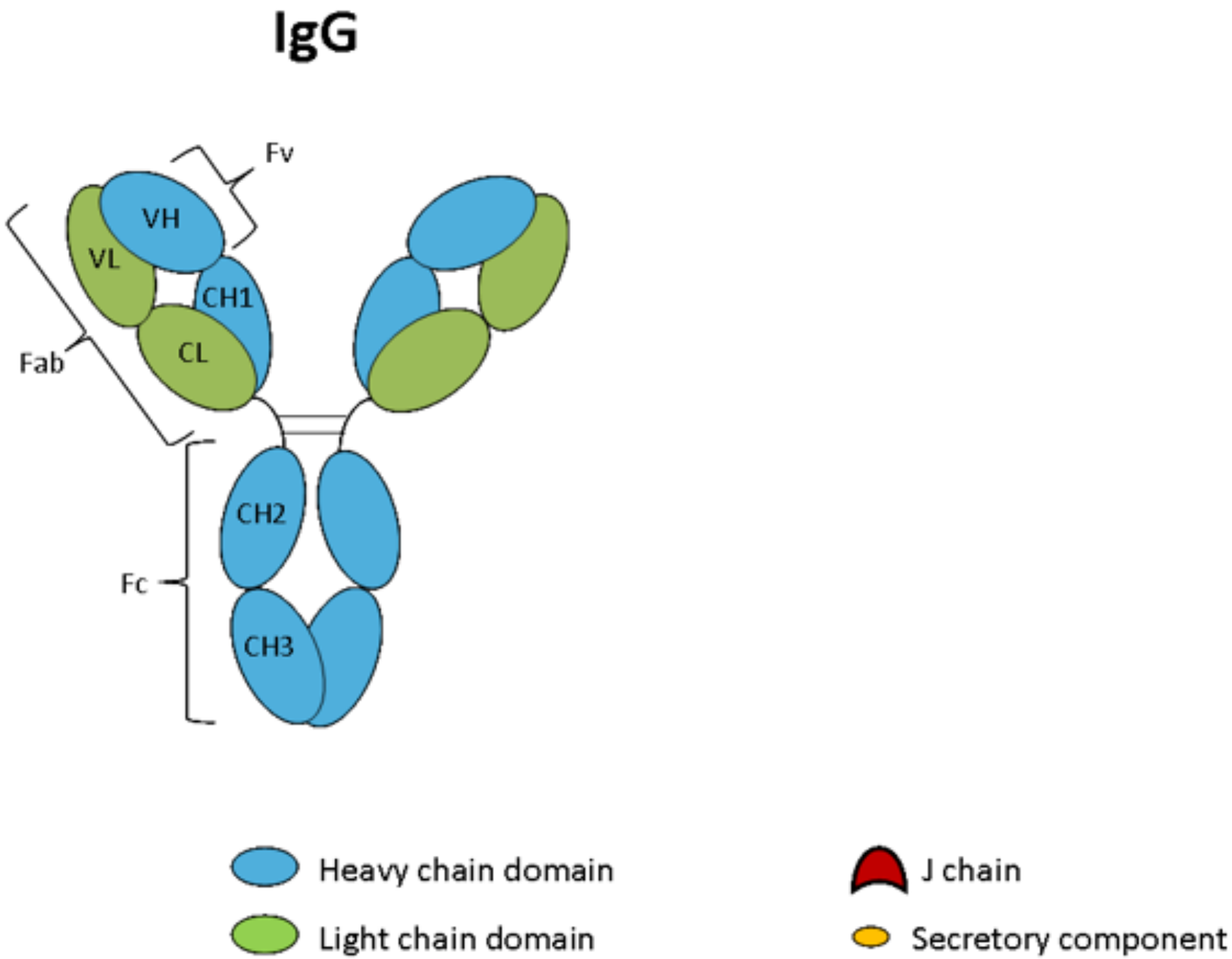


Figure 4.5 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)



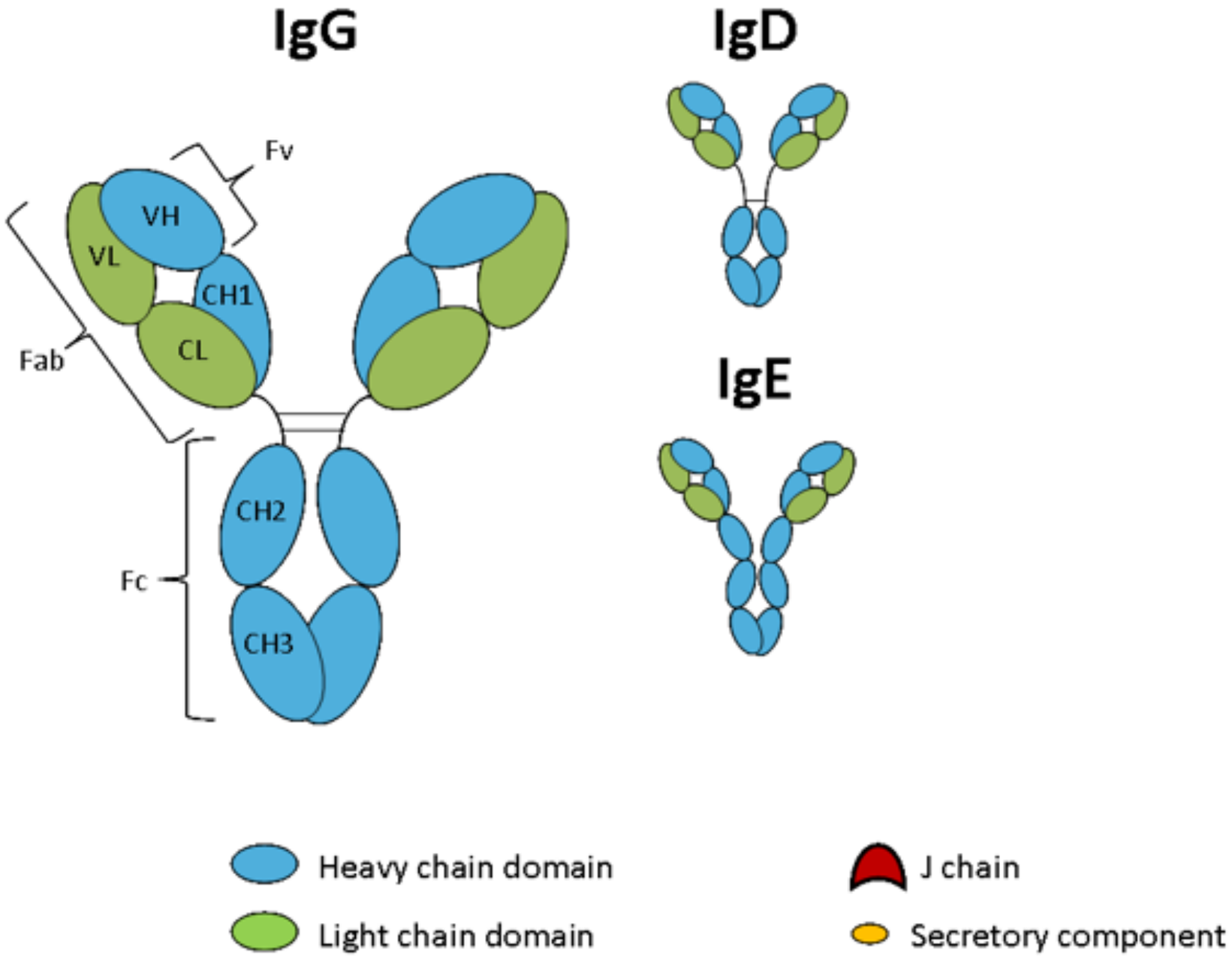
Isotypes

Five distinct classes based on the structure of the **heavy chain constant regions**



Isotypes

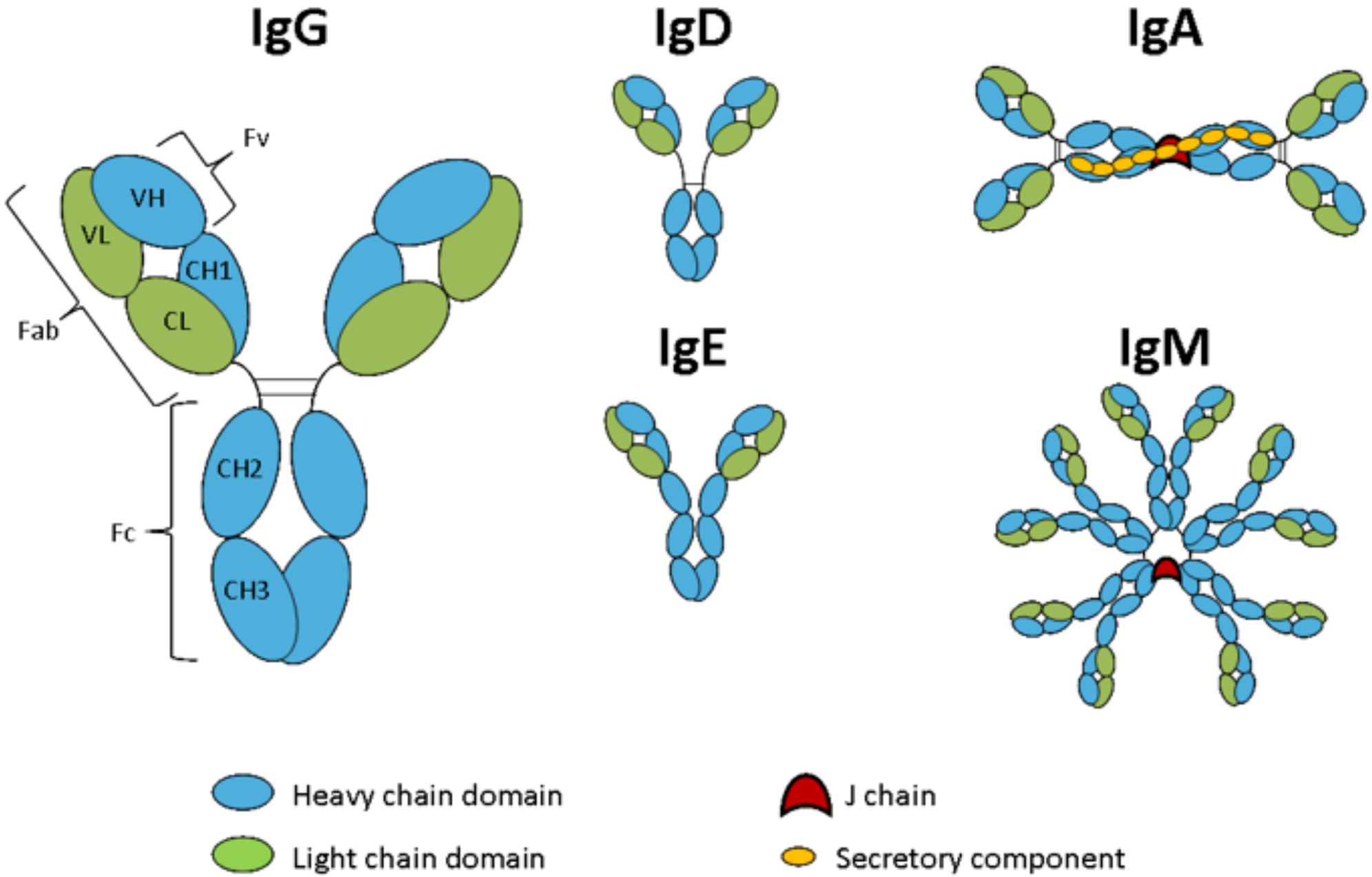
Five distinct classes based on the structure of the **heavy chain constant regions**



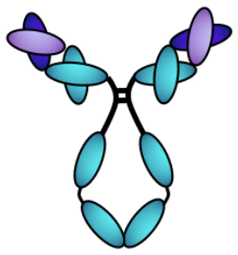
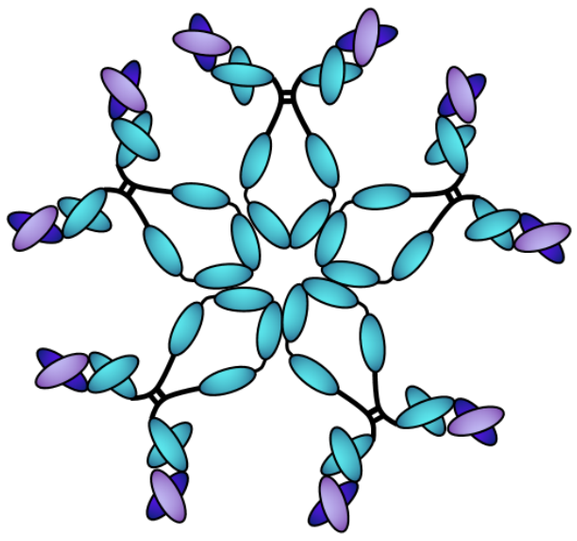
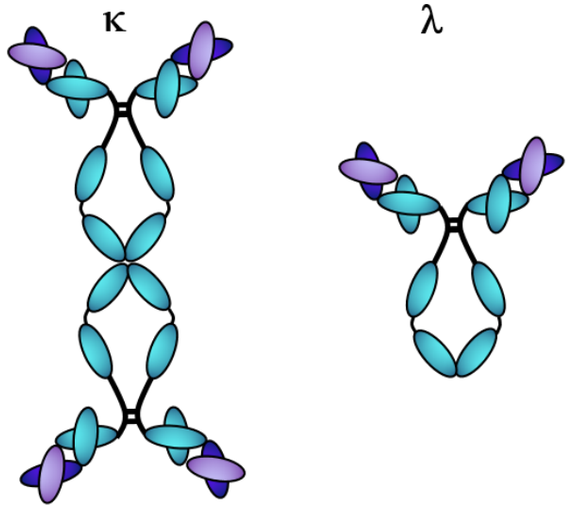
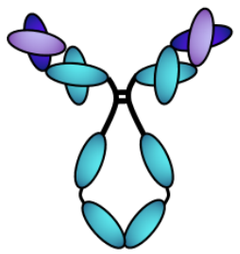
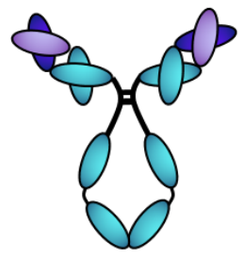
Isotypes

Five distinct classes based on the structure of the **heavy chain constant regions with same sequence**

C regions for
IgM and IgE contain 4 Ig domains
IgG, IgA and IgD contain 3 Ig domains

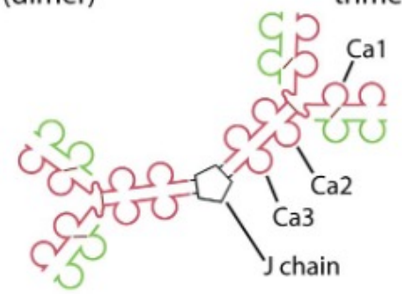
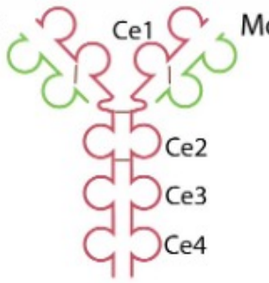
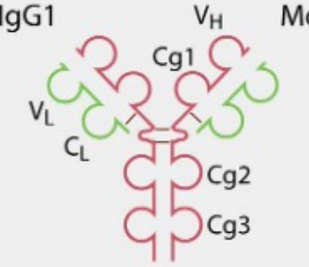
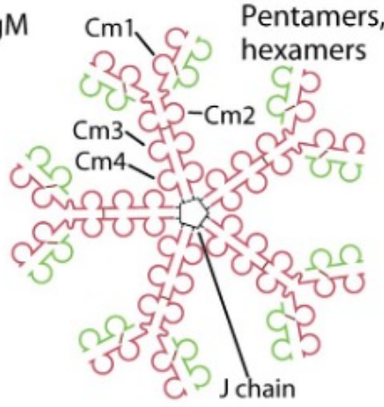


Isotypes - summary

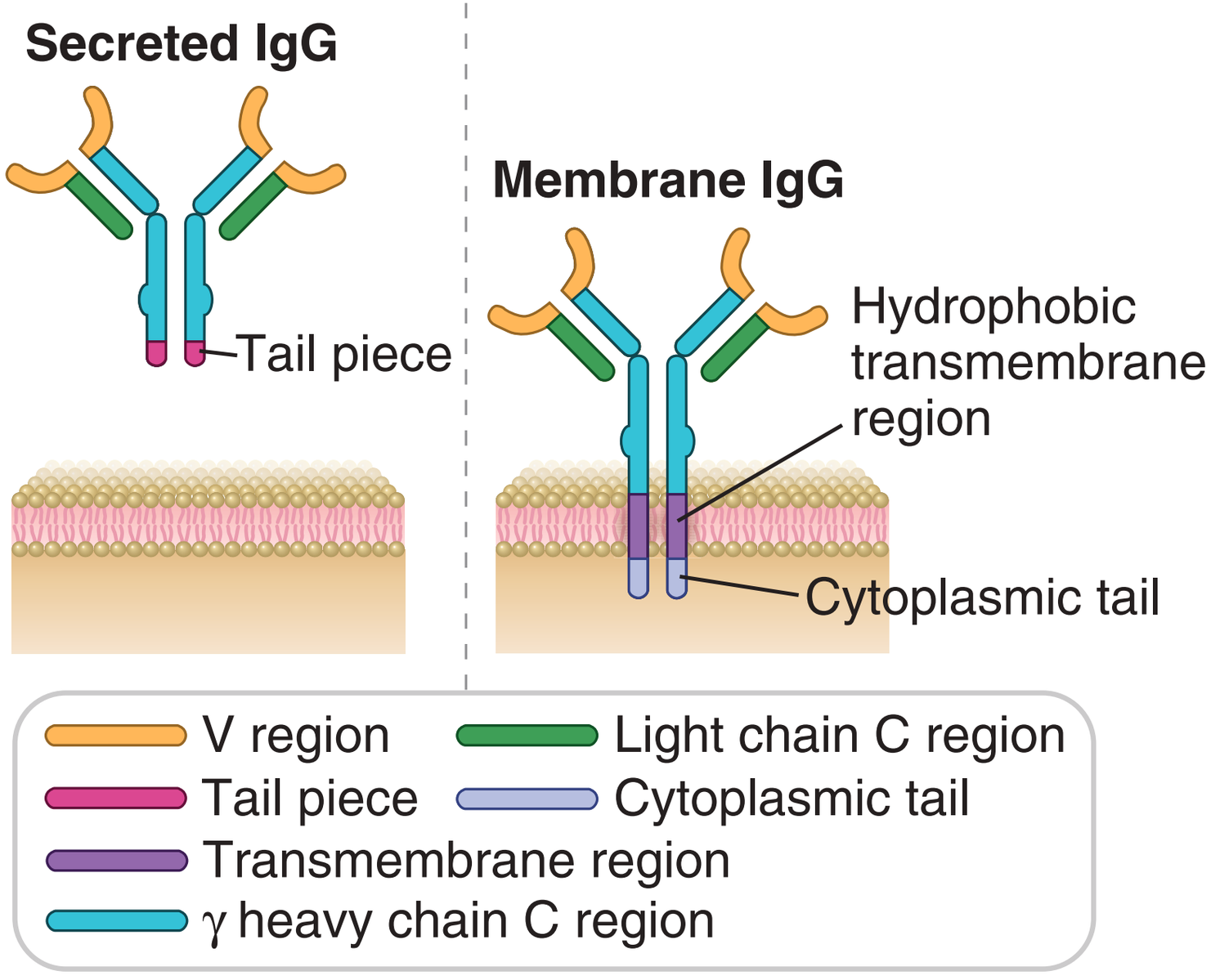
Antibody classes	IgG	IgM	IgA	IgE	IgD
Heavy chain	γ	μ	α	ε	δ
Light chain	κ or λ	κ or λ	κ λ	κ or λ	κ or λ
Structure					
Relative MW	146 ≤ X ≤ 170 kDa	~ 900 kDa	~ 320 kDa	~ 160 kDa	~ 184 kDa	~ 190 kDa

In humans and mice more frequent K chain. Ratio can be different in case of lymphomas

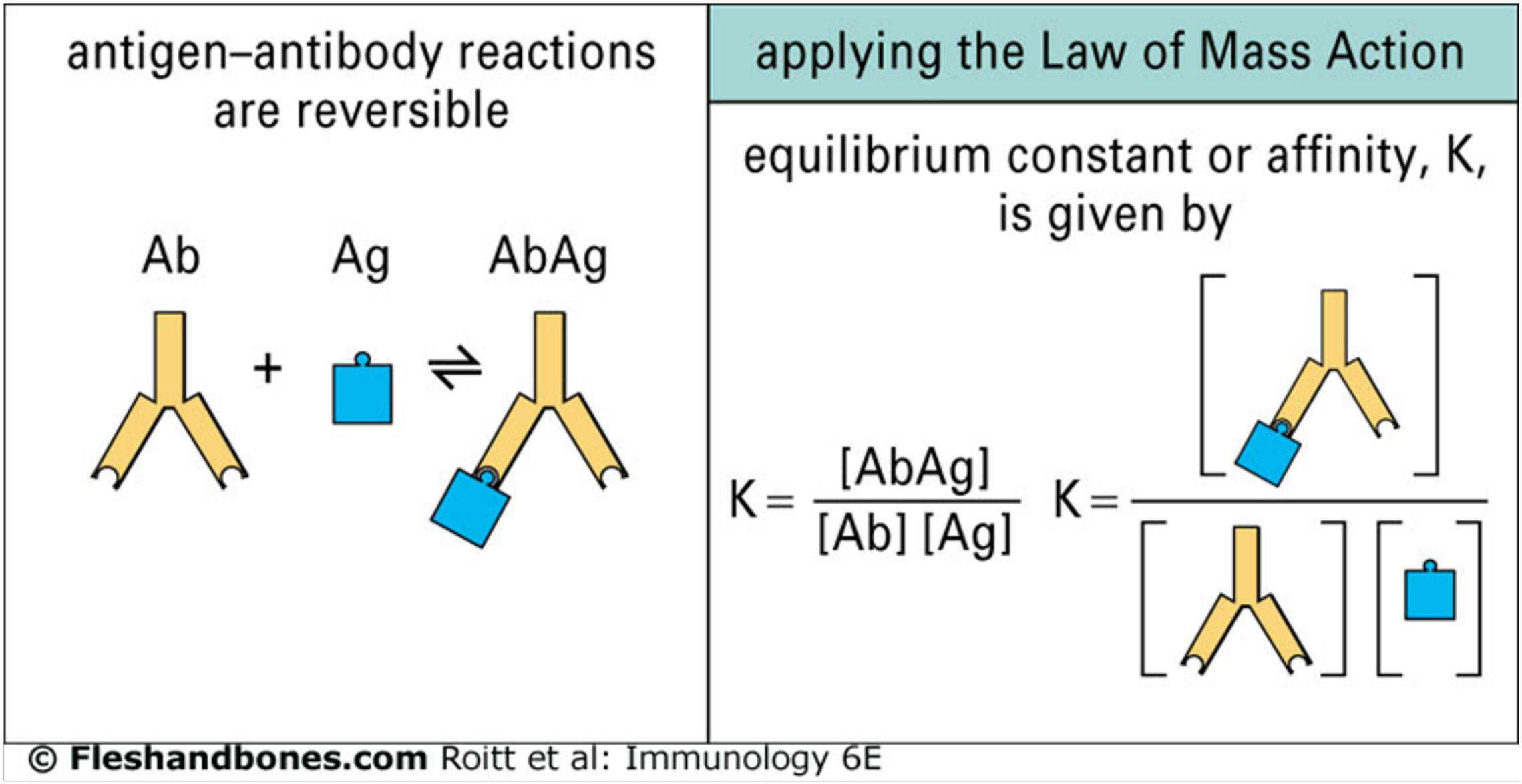
Functions of Isotypes

Isotype of antibody	Subtypes	H chain	Serum concentr. (mg/mL)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2	a(1 or 2)	3.5	6	IgA (dimer) Monomer, dimer, trimer 	Mucosal immunity
IgD	None	d	Trace	3	None	Naive B cell antigen receptor
IgE	None	e	0.05	2	IgE  Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4	g (1,2,3 or 4)	13.5	23	IgG1  Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None	m	1.5	5	IgM  Pentamers, hexamers	Naive B cell antigen receptor, complement activation

Secreted and membrane associated antibodies differ in the amino acid sequence of the carboxy-terminal end of the heavy chain C regions

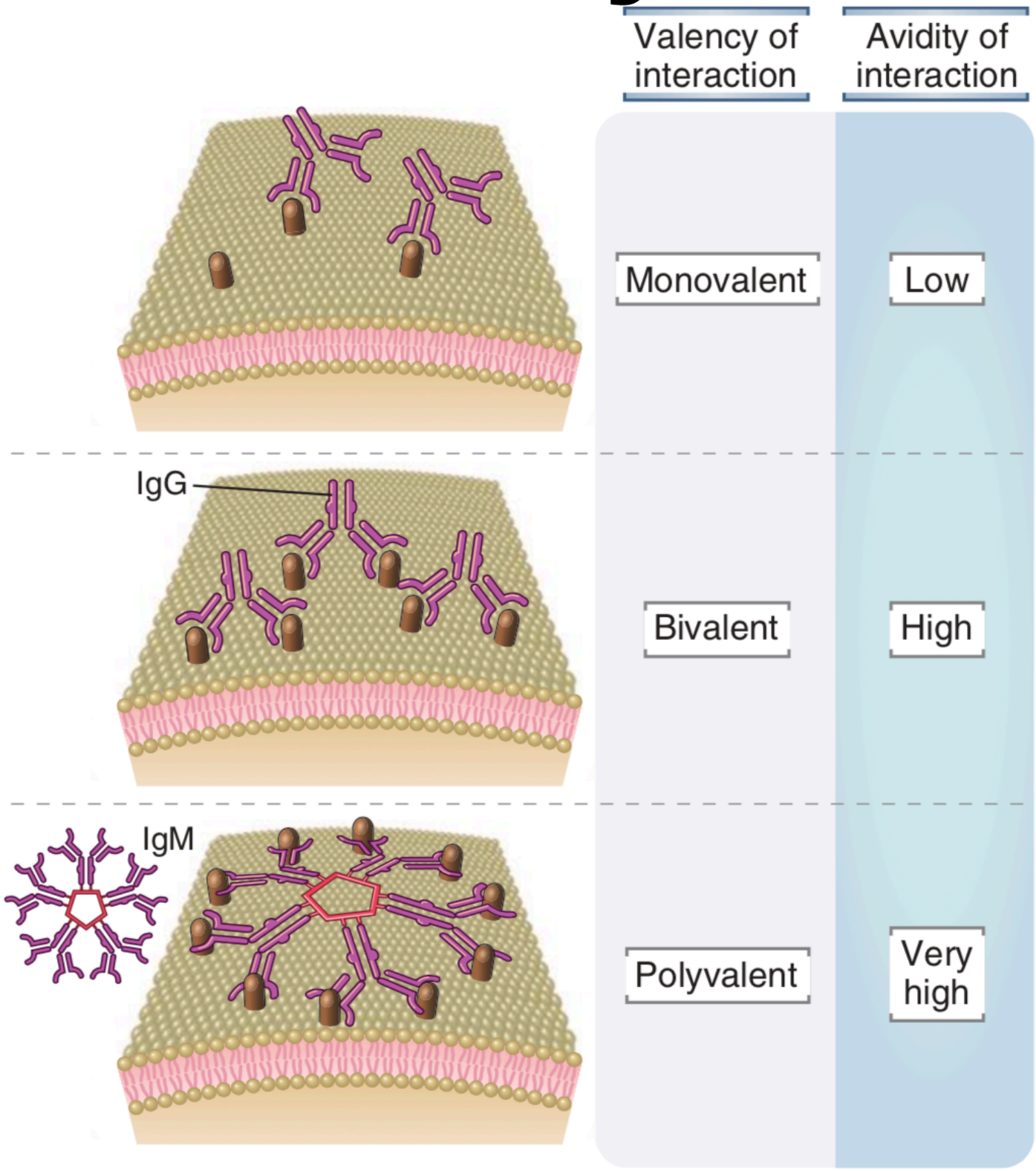


Antibody Affinity



The recognition of antigen by antibodies involves non covalent reversible binding

Affinity and avidity

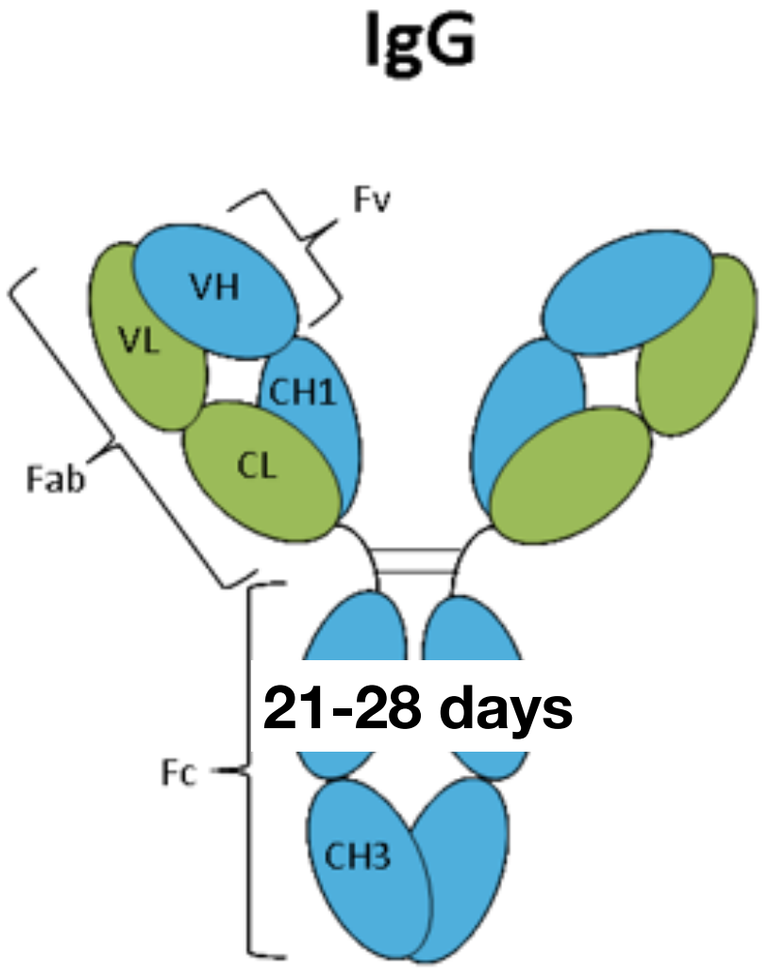


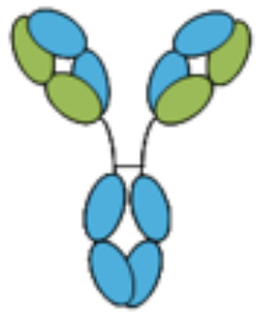
IgM are the first Ab to be made and are usually low affinity

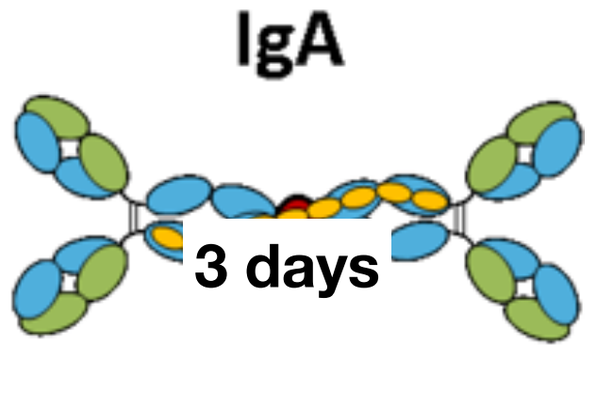
IgM have 10 antigen binding sites
IgM can "compensate" the low affinity with the high avidity

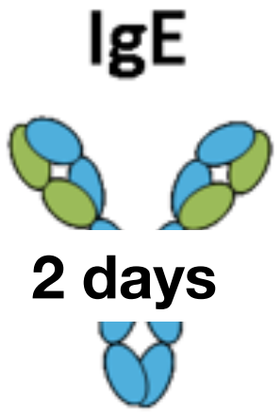
Antibody half-life

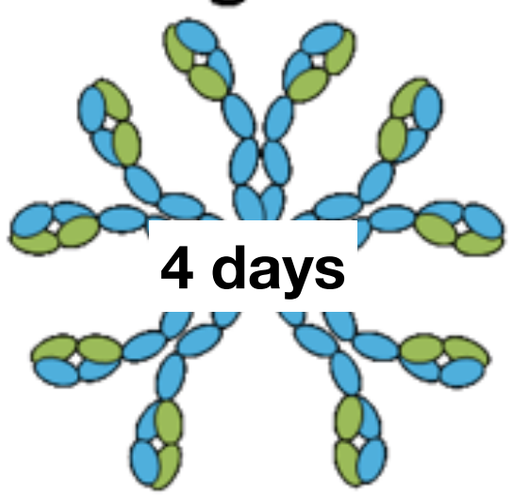
Te half-life is the mean time before the number of antibody molecules is reduced by half in the blood





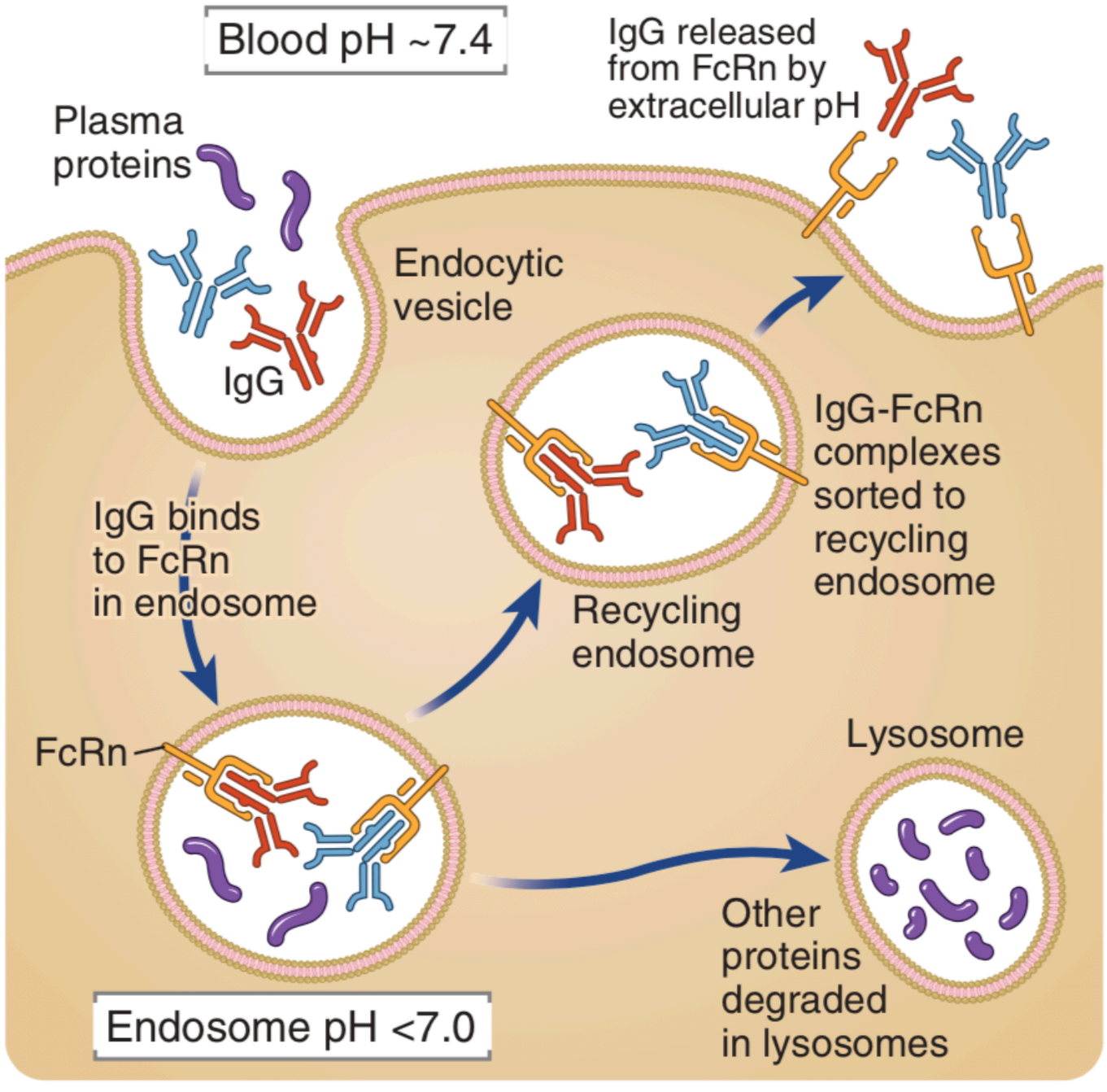






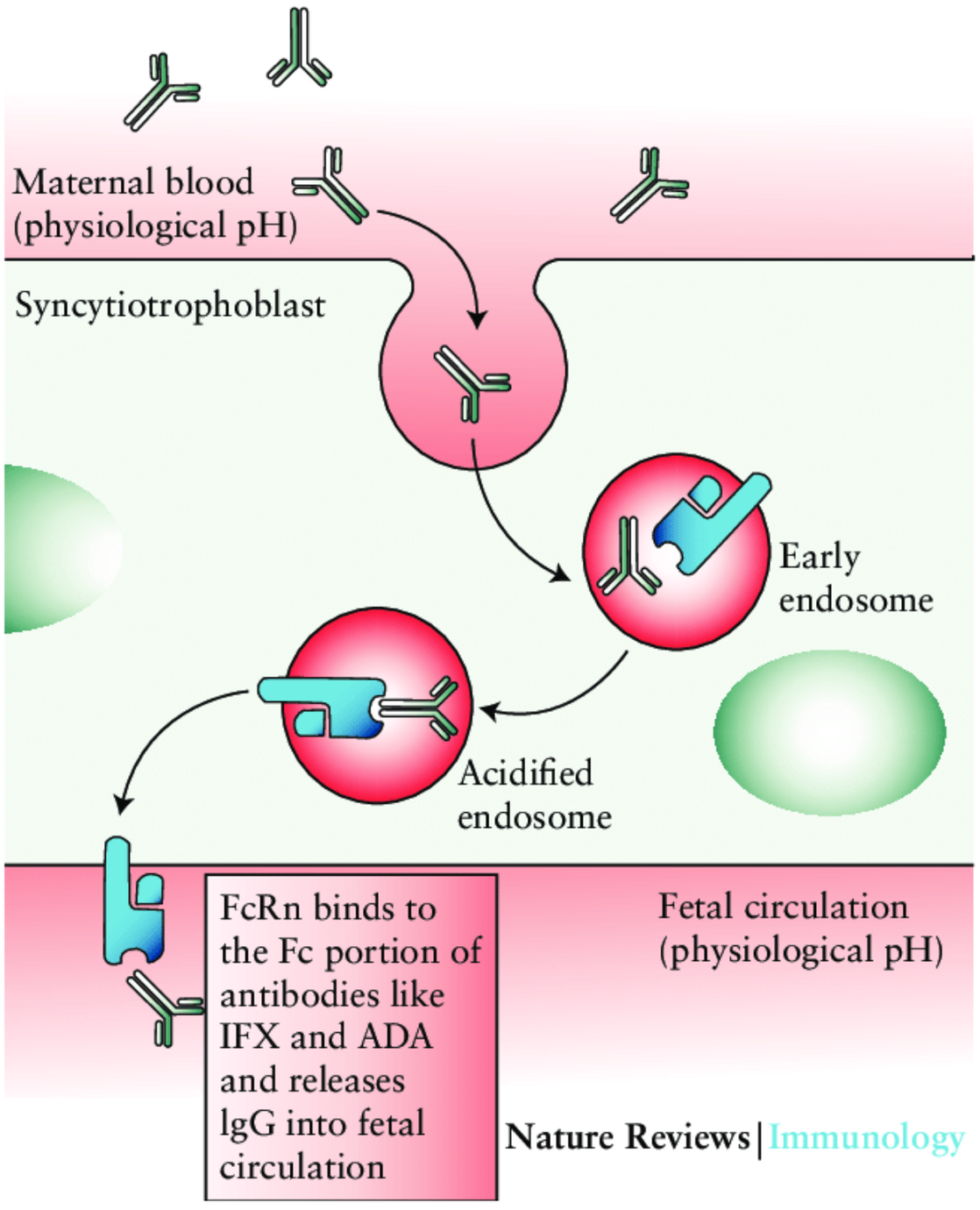
More high for IgG1 and IgG2 then IgG3

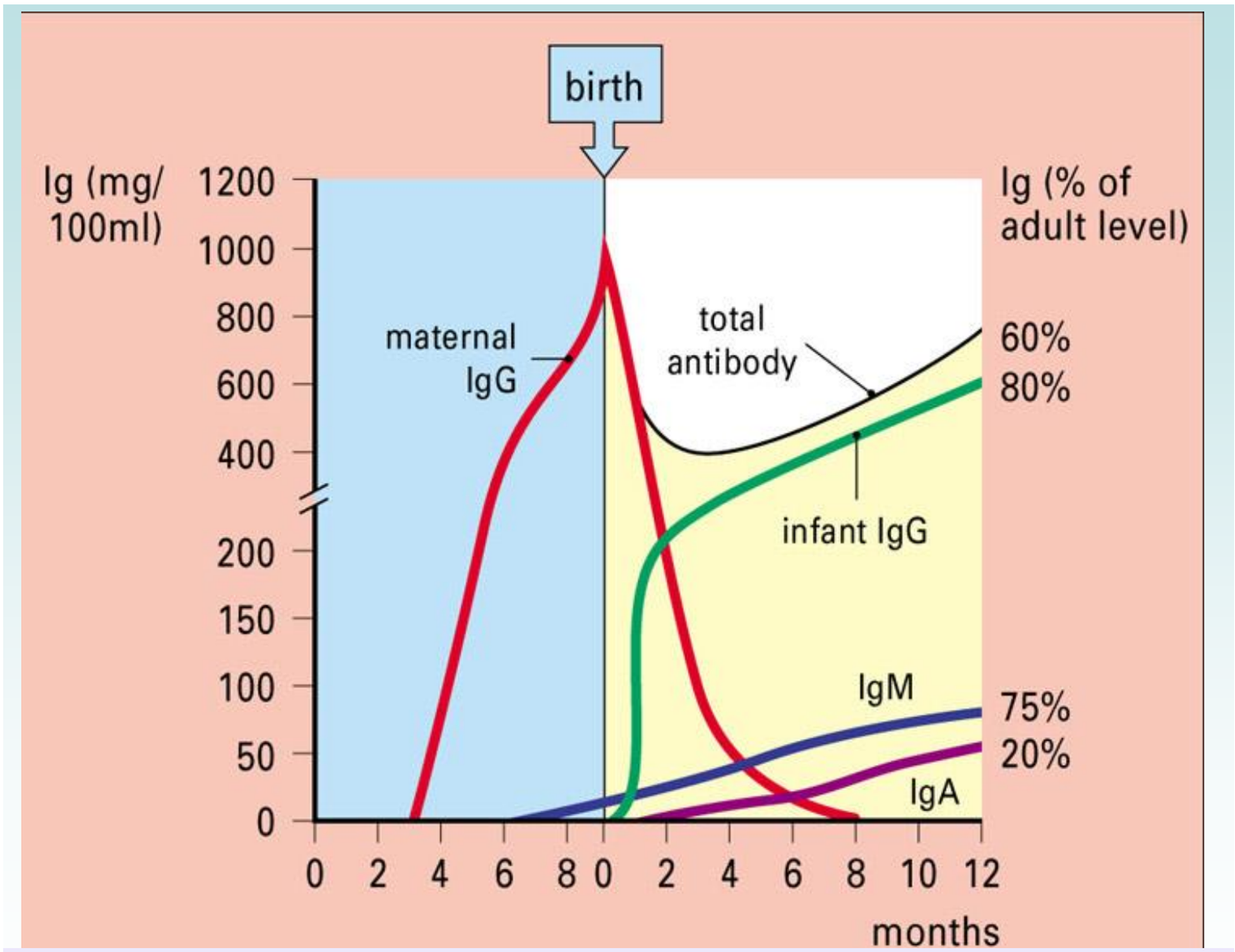
Why IgGs last longer?



FcRn (Neonatal Fc Receptor) recycles endocytosed IgGs back to the extracellular milieu, instead of targeting them to lysosomal degradation

The FcRn allow the transport of IgG through the placenta




birth
Ig (mg/ 100ml)
1200
1000
800
600
400
200
150
100
50
0
maternal IgG
total antibody
infant IgG
IgM
IgA
Ig (% of adult level)
60%
80%
75%
20%
0 2 4 6 8 0 2 4 6 8 10 12
months

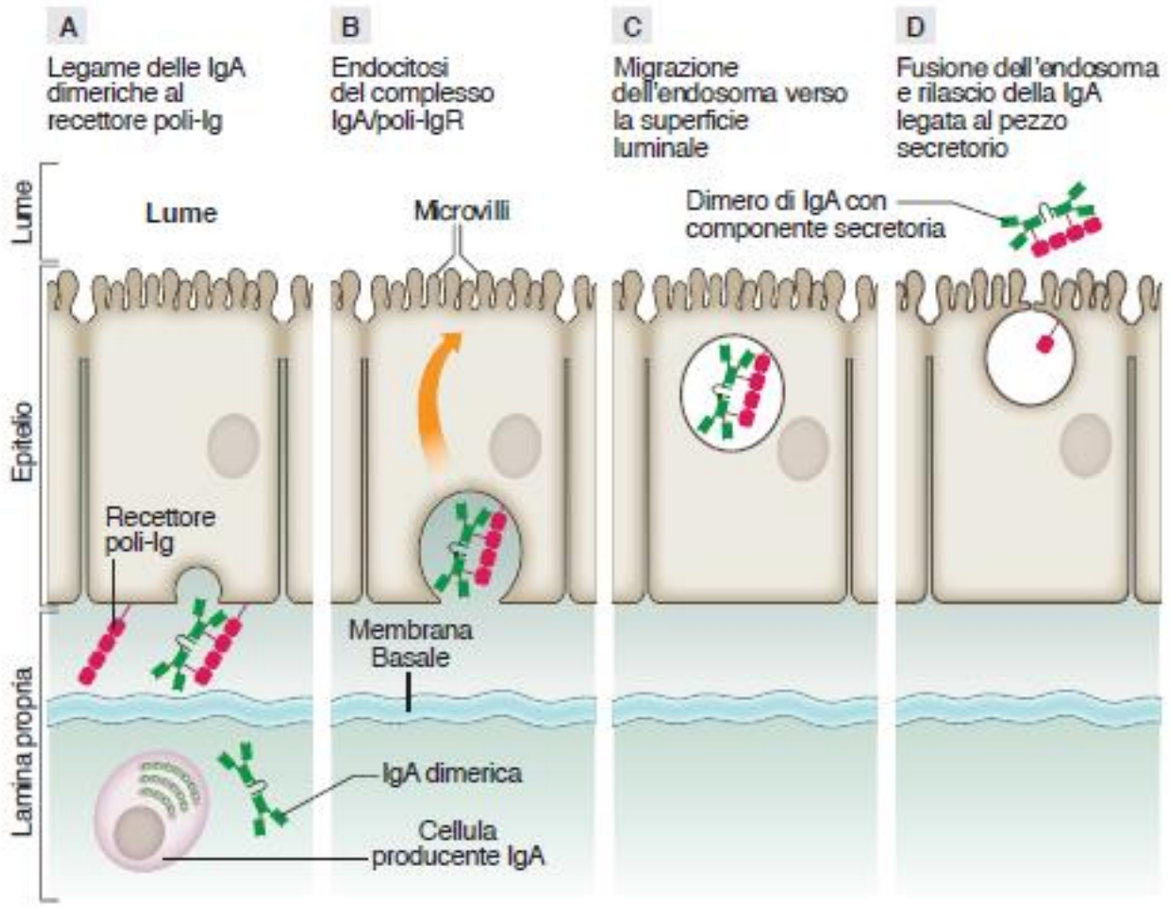


FIGURA 6.9.

La transcitosi delle IgA. La figura mostra le diverse tappe che portano all'esportazione delle IgA dimeriche dalla lamina propria intestinale o bronchiale al lume dell'organo, un fenomeno denominato transcitosi.