PHRM 836 August 27, 2015

Protein structure-function relationship: Recognition – example of immunoglobulins

Devlin, section 9.2

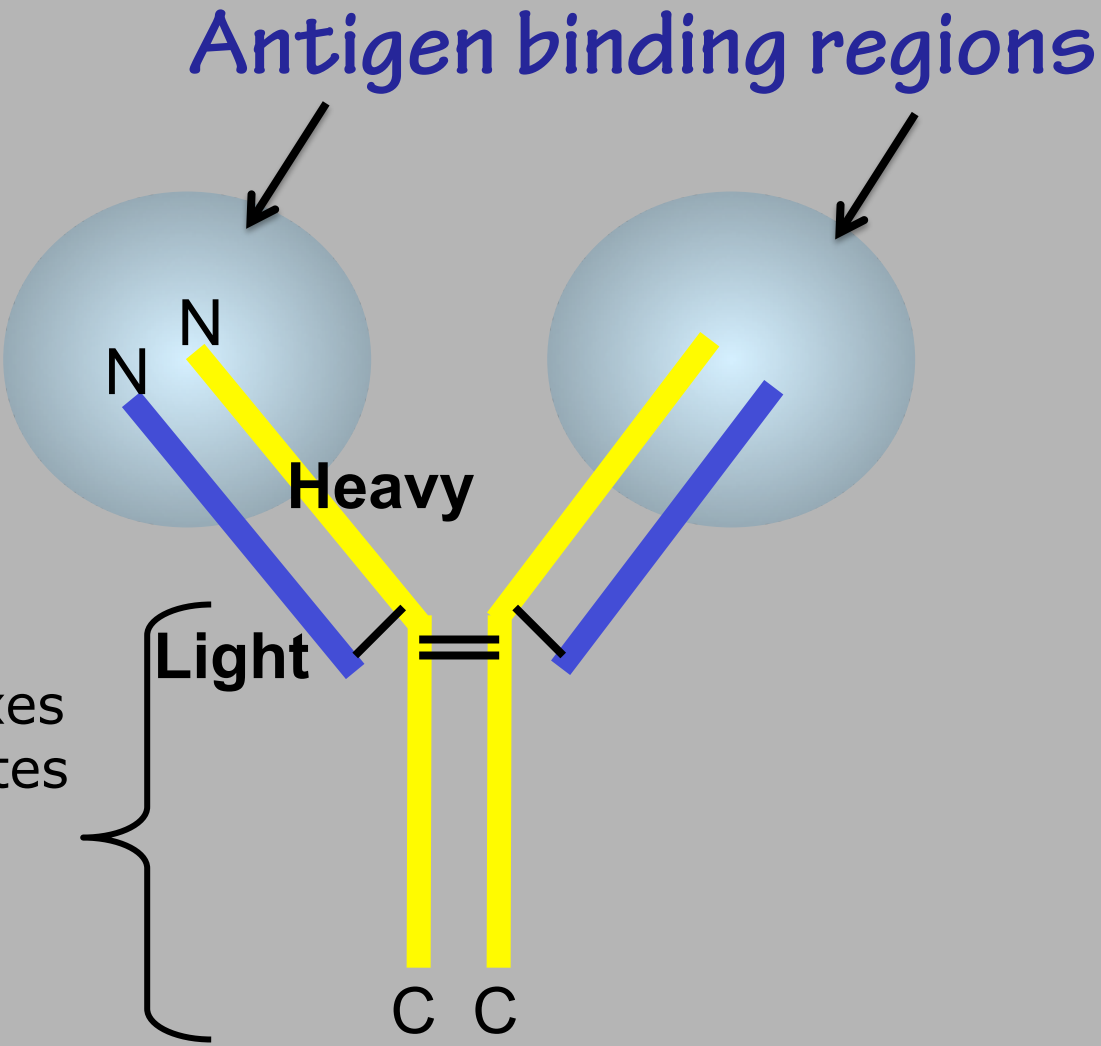
1. Overall structure of an antibody protein: quarternary and tertiary structure
2. Antibodies recognize antigen with high specificity
    1. Antibodies are therapeutic agents
3. Many proteins have an immunoglobulin fold

Protein Structure/Function: review

1. Amino acids
    a) Names of 20 common amino acids (3-letter and 1-letter abbreviations)
    b) Chemical structure and physical properties of each (polar, nonpolar, charged, pKa)
    c) Special structural features
        i. Gly
        ii. Pro
        iii. Disulfide bond
2. Polypeptide chain
    a) peptide bond
    b) Phi, psi conformation (Ramachandran plot)
3. Protein structure
    a) 4 levels of structure (primary, secondary, tertiary, quarternary)
    b) Stabilizing forces of the folded state

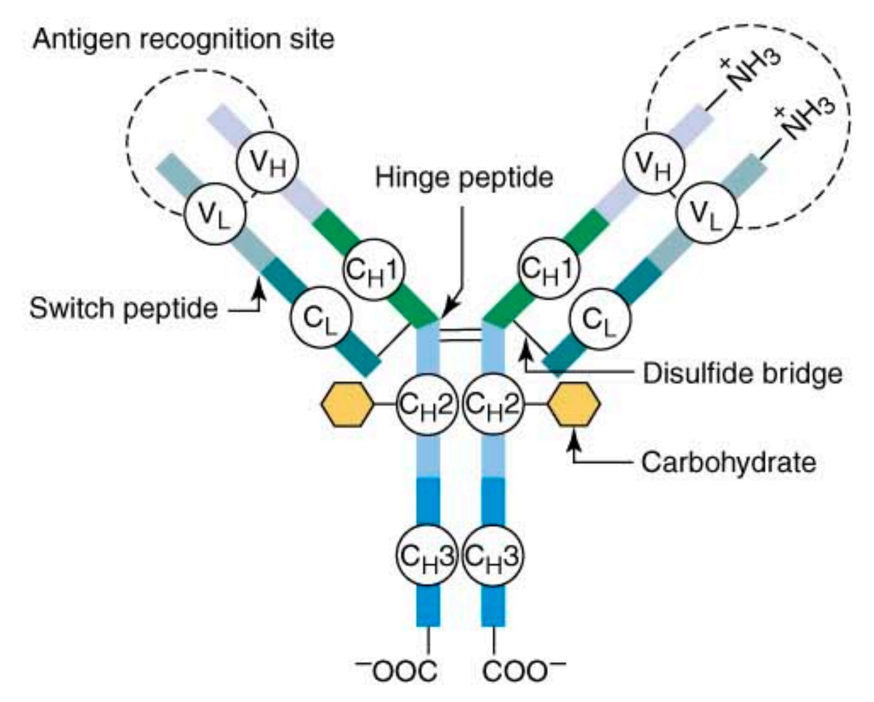
Immunoglobulins: IgG antibody chains

$(LH)_2$ =two copies of a heavy & light chain
H: ~440 amino acids
L: ~220 amino acids



F_c region: binds and fixes complement and activates complement-mediated cytotoxicity

Immunoglobulin Domains



Devlin
Figure 9.8

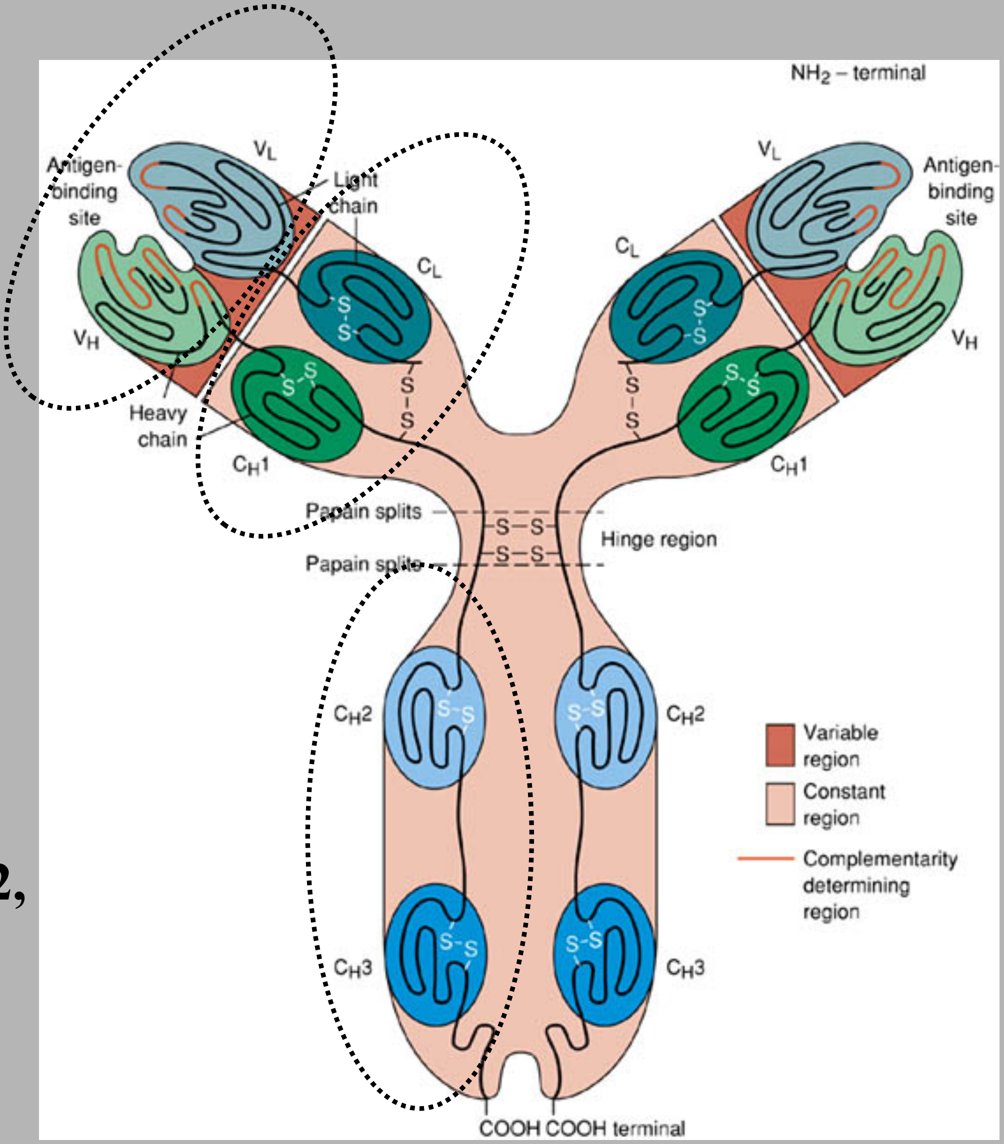
Chains:
Heavy vs Light

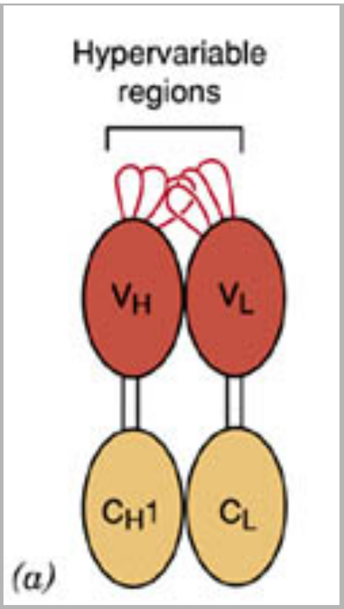
Sequence conservation:
Constant vs Variable

Ig domain (~110 aa):
2 on L and 4 on H

V_L C_L V_H $3C_H$

Immunoglobulin Domains





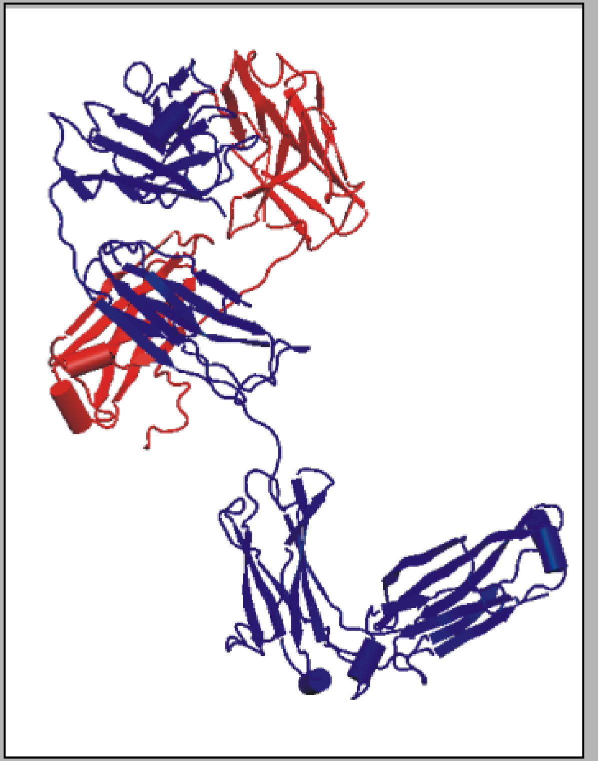


Figure 9.2, Devlin

Domains = Immunoglobulin Fold

The 110-amino acid domain

a "sandwich" of 2 antiparallel sheets

Disulfide bond links 2 sheets

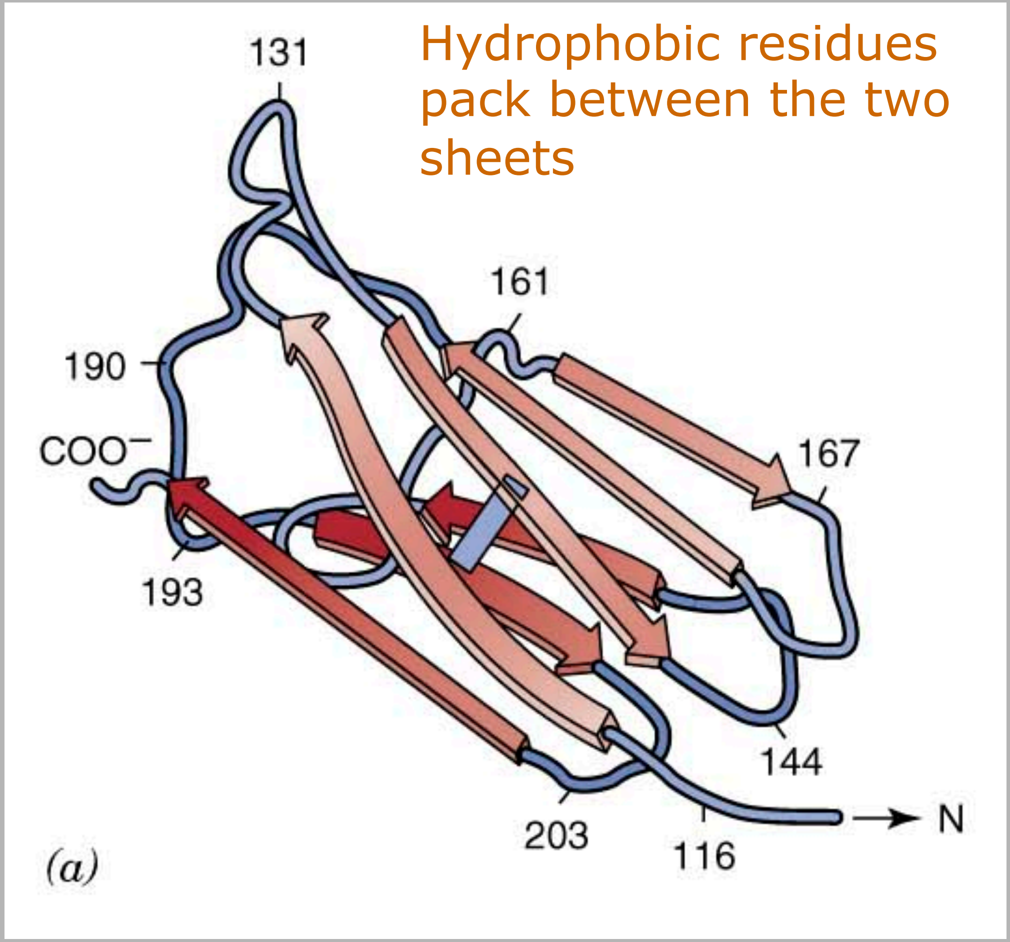


Figure 9.4, Devlin

Immunoglobulin Fragments

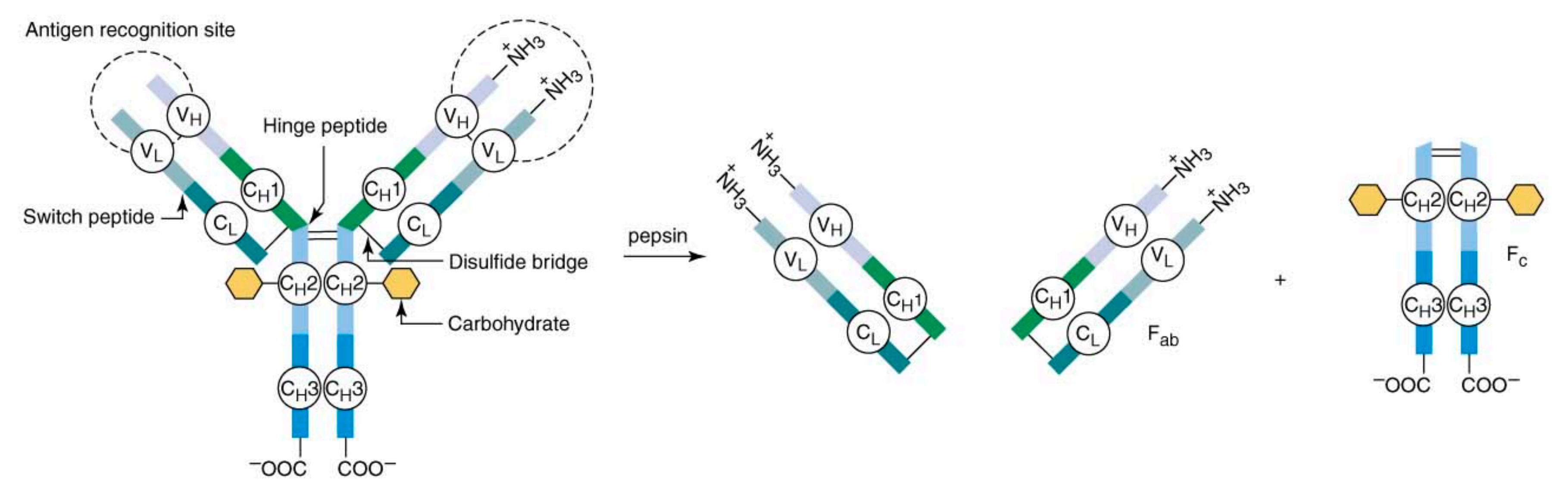
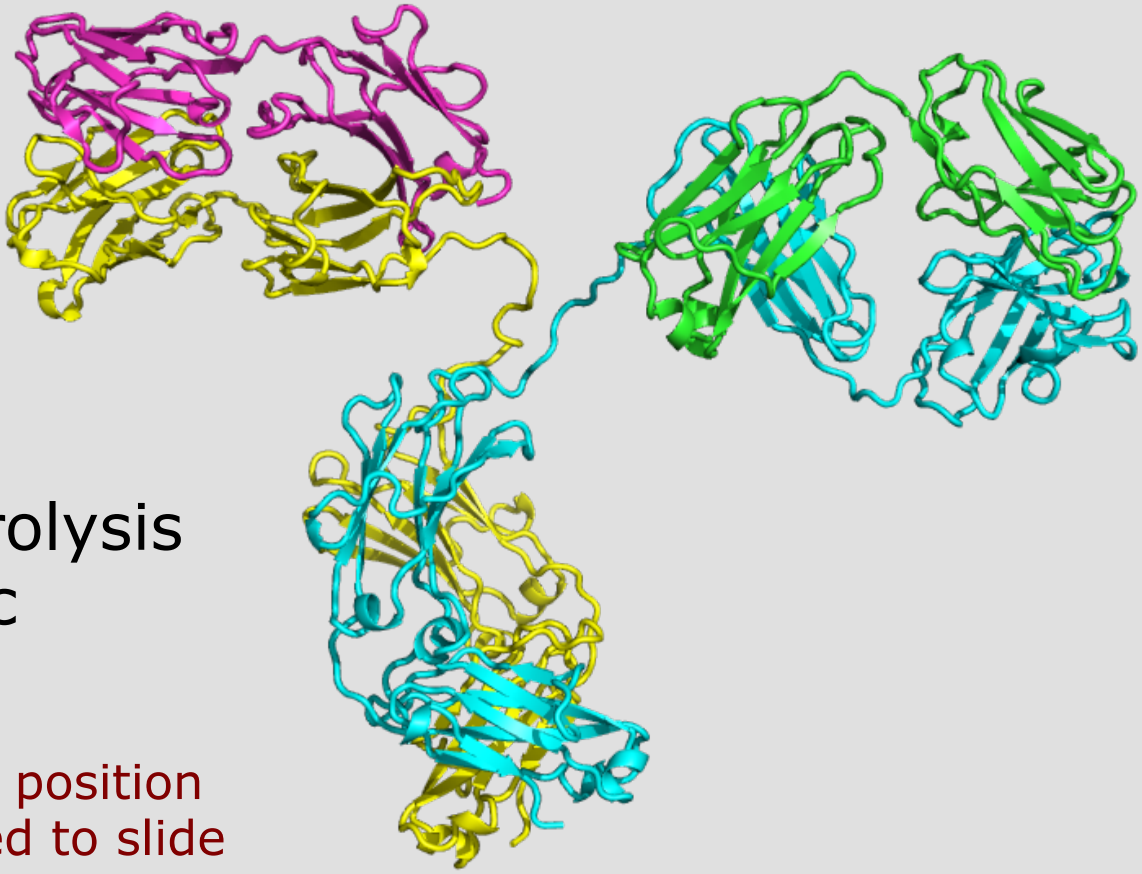


Figure 9.8

Full Immunoglobulin G Antibody



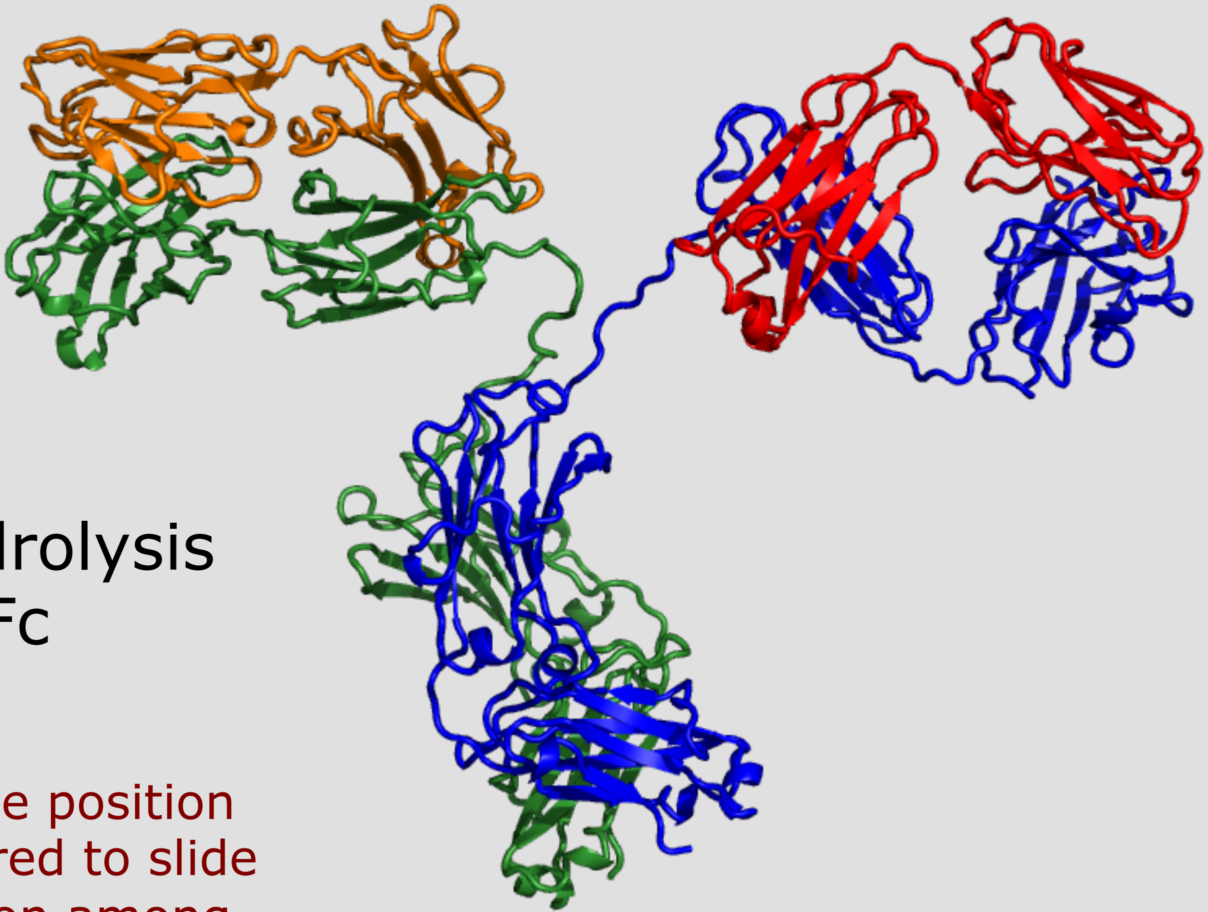
Two light chains with
V_L & C_L

Two heavy chains with
V_H & three C_H's

Where does the hydrolysis producing Fab and Fc occur?

NOTE: the different relative position of Fab and Fc here compared to slide 5. This variation is common among antibody molecules.

Full Immunoglobulin G Antibody



Two light chains with
V_L & C_L

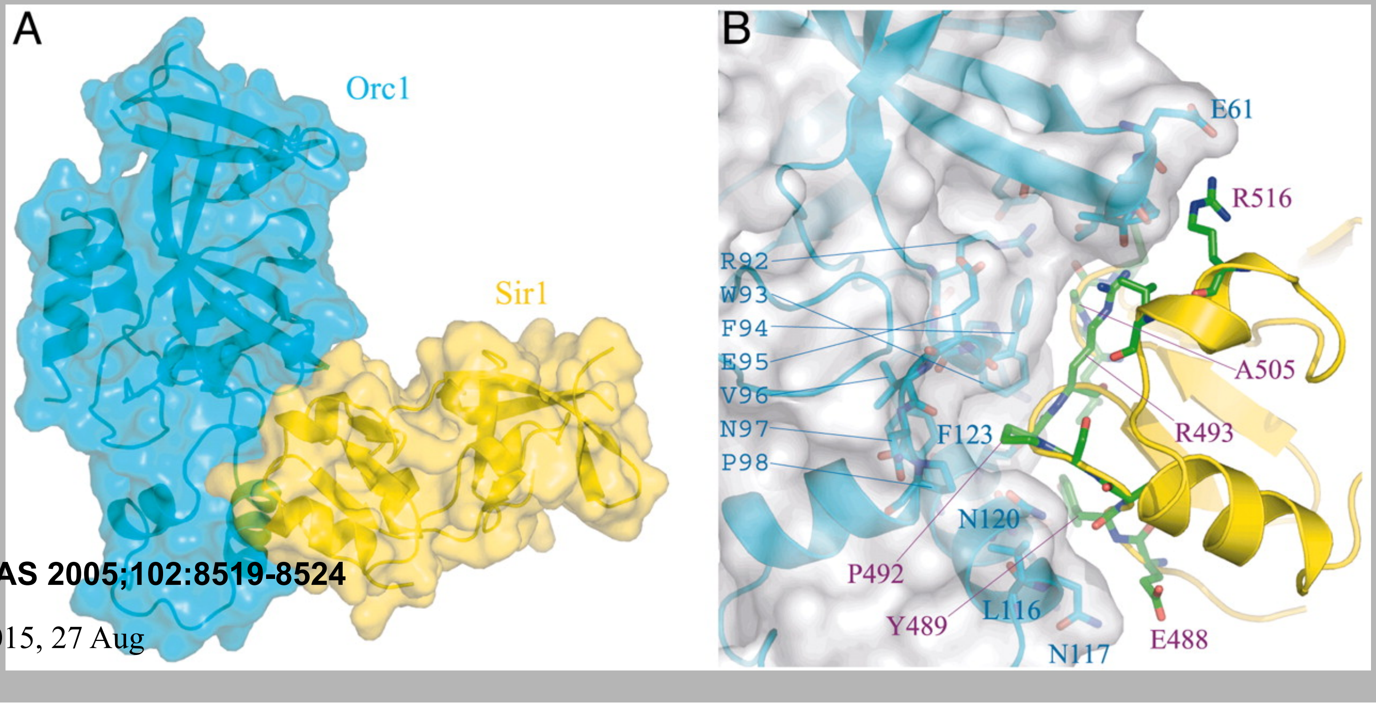
Two heavy chains with
V_H & three C_H's

Where does the hydrolysis producing Fab and Fc occur?

NOTE: the different relative position of Fab and Fc here compared to slide 5. This variation is common among antibody molecules.

Protein function: binding & molecular recognition

- The function of nearly all proteins, including antibodies, depends on binding other molecules, either small molecules or macromolecules.
- Recognition is highly specific.
  - complementarity
- Involves the formation of noncovalent interactions.
  - Such as what?
- Induced-fit structural changes occur to enhance specificity and complementarity



Hsu H et al. PNAS 2005;102:8519-8524

Protein function: binding & molecular recognition

- variable domains: comprise 3 hypervariable loops
- loops vary in length and sequence for diff IgG's
- both the H and L chains compose the site
- "Exact" complementary match between antigen and loops.
  - high affinity (up to 10^{10} M^{-1})
  - induced fit
  - remarkable specificity (exploited in diagnostics and research)

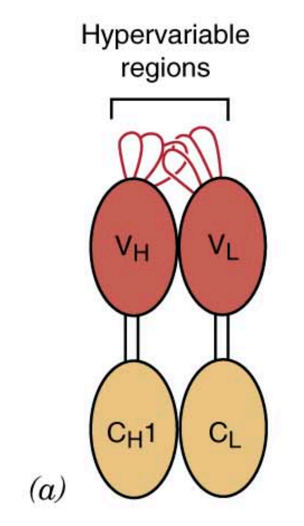
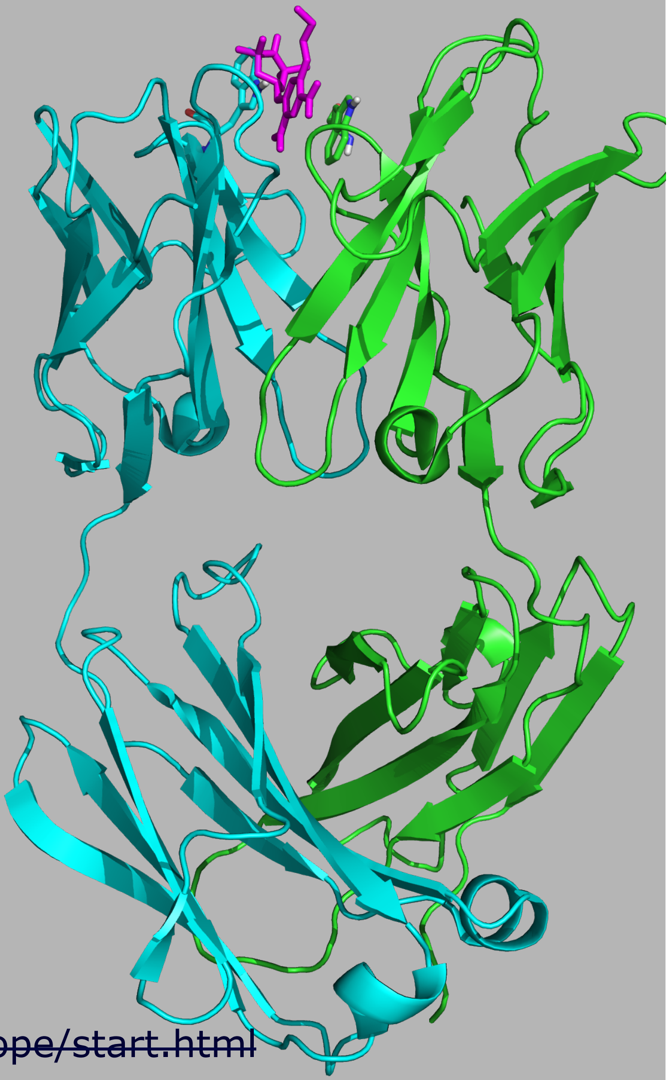


Figure 9.7, Devlin



~~http://bcs.whfreeman.com/immunology6e/content/cat_030/epitope/start.html (text for part II and IV)~~

Protein function: binding & molecular recognition

- variable domains: comprise 3 <u>hypervariable loops</u>
- loops vary in length and sequence for diff IgG's
- both the H and L chains compose the site
- "Exact" complementary match between antigen and loops.
  - high affinity (up to 10^{10} M^{-1})
  - induced fit
  - remarkable specificity (exploited in diagnostics and research)

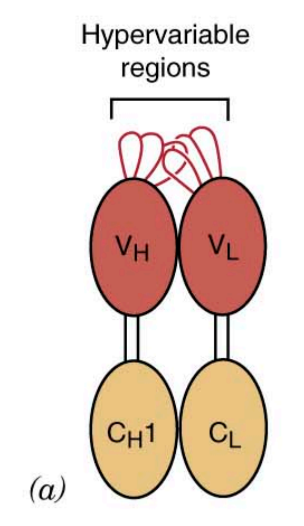
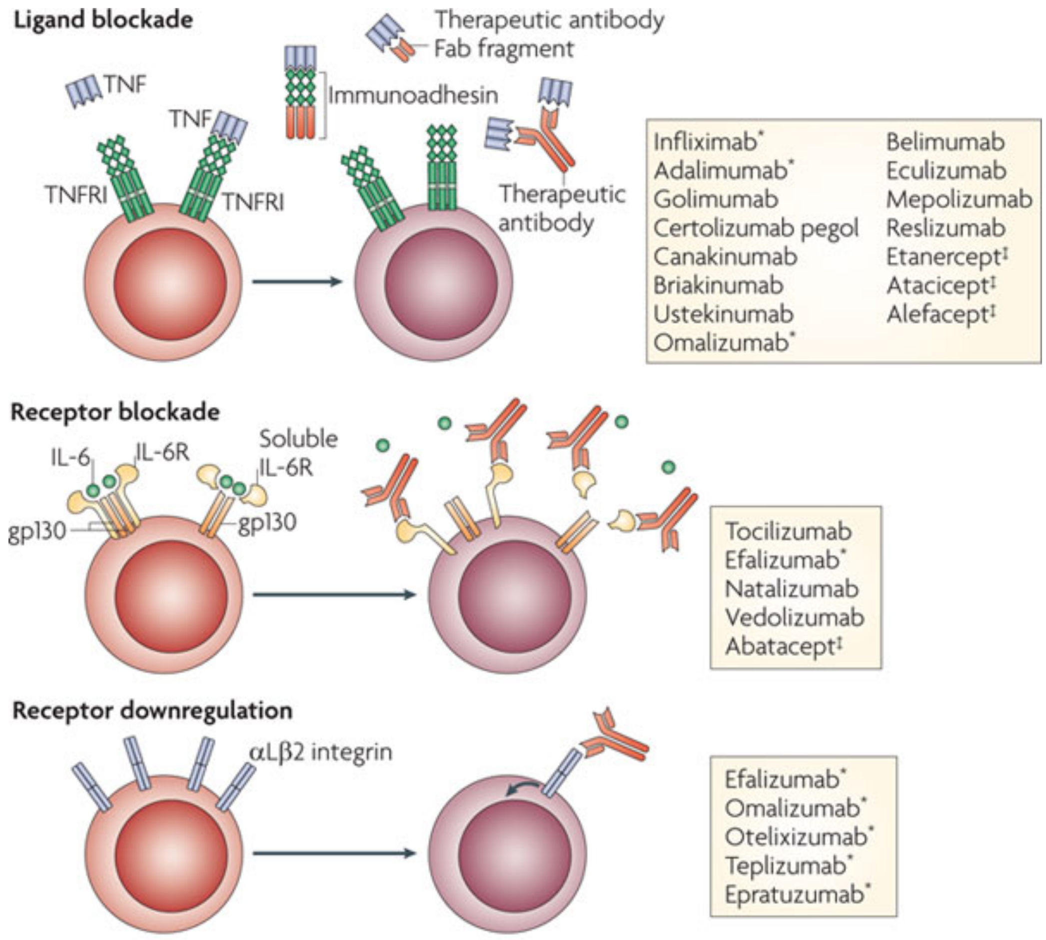


Figure 9.7, Devlin

Monoclonal antibodies in Pharmaceuticals

- Monoclonal: produced from a single B-lymphocyte clone and bind the same epitope
- Fastest growing group of pharmaceutical molecules
- ~30 are FDA-approved for clinical use to treat cancer, inflammatory disease, infectious disease and cardiovascular disease (Liu, Dec 2014, Annals of Medicine and Surgery)

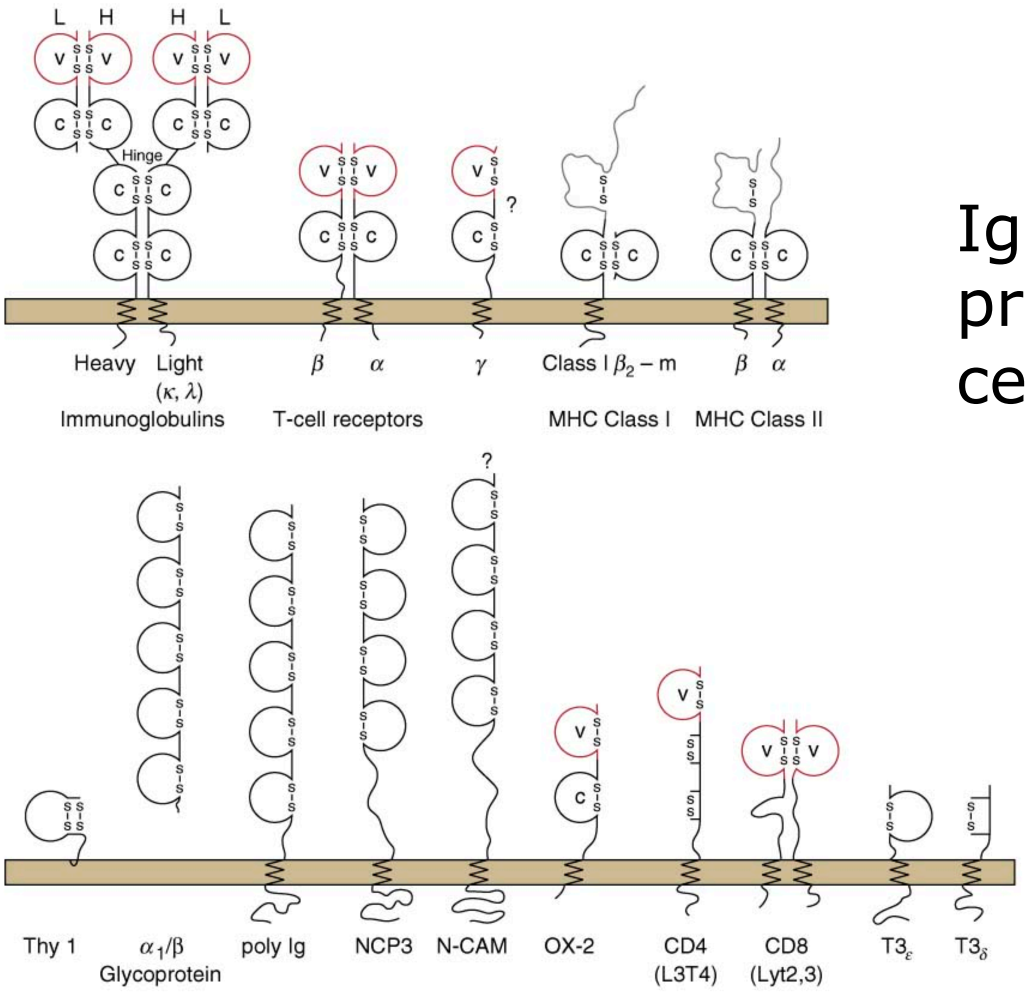
Therapeutic Antibody Mechanisms



- Targeting extracellular ligand-receptor interactions
  - Bind to ligand
  - Or, bind to receptor
  - Can lead to downregulation of receptor on cell surface
- Antigen binding affinity is key

Chan, Carter Nat Rev Immun 2010

Members of the Immunoglobulin Superfamily Have the Immunoglobulin Fold



Ig domains are present in many cellular receptors

Figure 9.10

Summary of Antibodies and Recognition

1. Two polypeptide chains fold into multiple domains, each domain being an immunoglobulin fold structure.

2. The basic immunoglobulin structure comprises two copies of each of the two polypeptide chains.
    1. Two immunoglobulin domains from each chain together form an FAB fragment
    2. The remaining two immunoglobulin domains from the heavy chains form an F_C fragment

3. One antibody molecule has two identical antigen binding sites and can bind two antigens

4. The antigen-binding site of the V_L-V_H domain, generated by hypervariable loops, forms a continuous surface complementary to and specific for the antigenic determinant.

5. The strong interactions between antigen and antibody hypervariable loops are noncovalent and include van der Waals, hydrogen bonding, and hydrophobic interactions.