Immune System

Branden & Tooze, Chapter 15

Protects complex multicellular organisms from pathogens, e.g. virus, bacteria, yeast, parasites, worms, etc

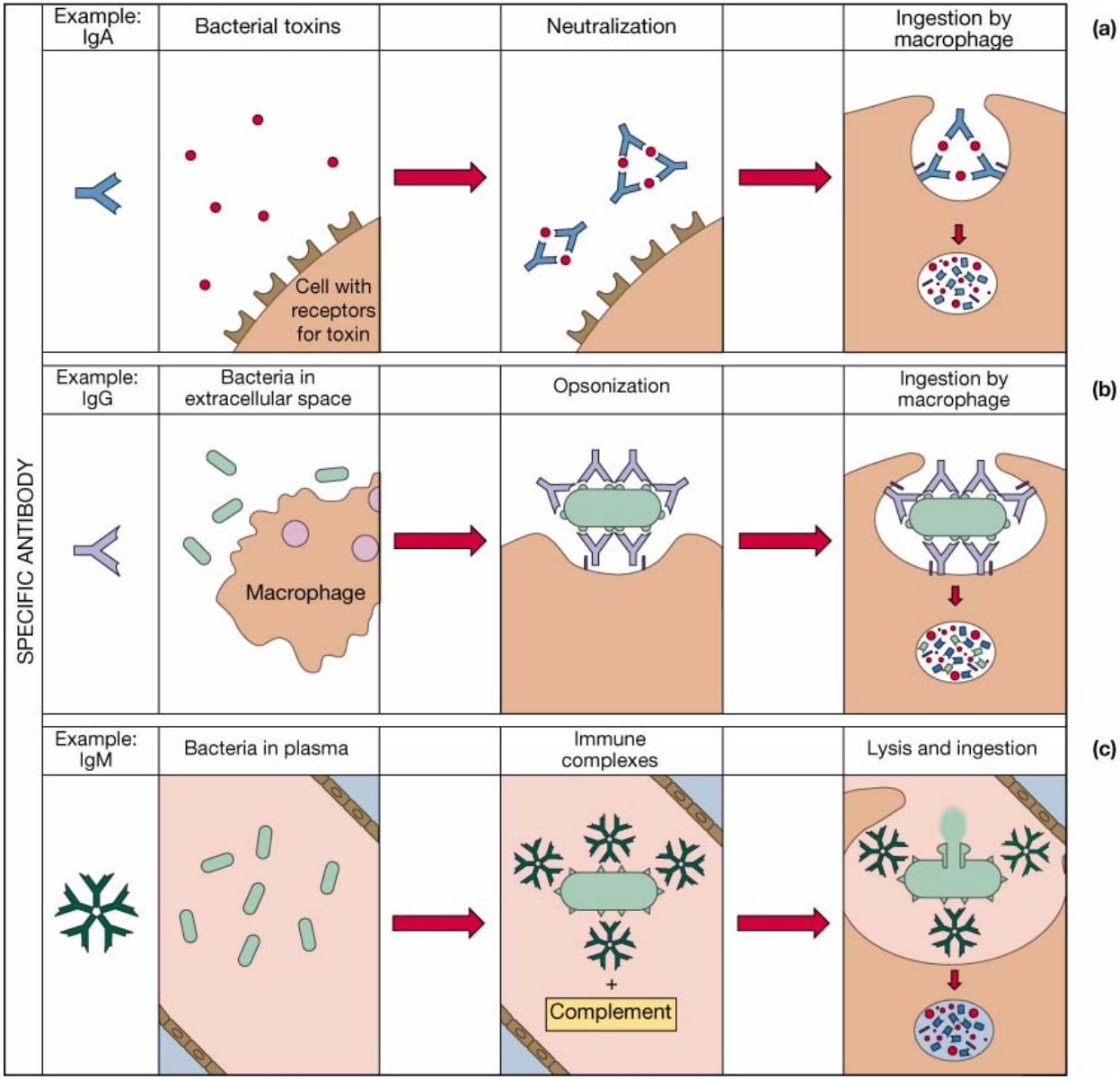
Innate immunity

- first line of defense past physical barriers, e.g. skin
- comprises molecules that recognize pathogen-associated molecular pattern (PAMP)
- carbohydrates, peptidoglycan, dsRNA, methylated CpG, bacterial flagella
- mannose binding protein, Toll-like receptors (TLR), complements

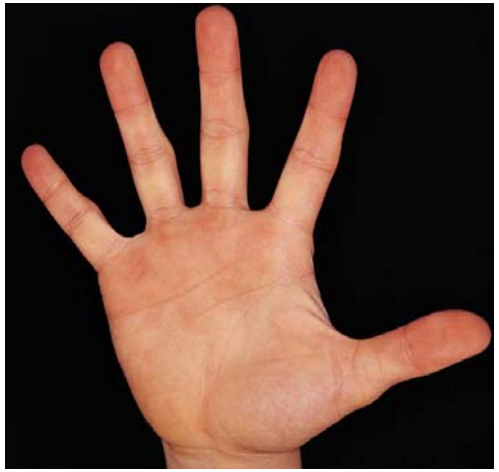
Acquired immunity

- remembers the molecular pattern unique to individual invading pathogens to launch a vigorous attack during subsequent exposure
- involves lymphocytes (B and T cells)
- antibodies, major histocompatibility complex (MHC), T-cell receptors

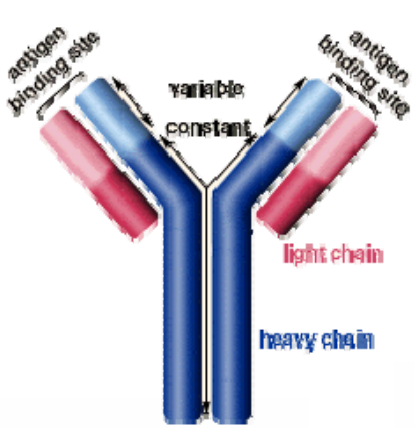
What antibodies do

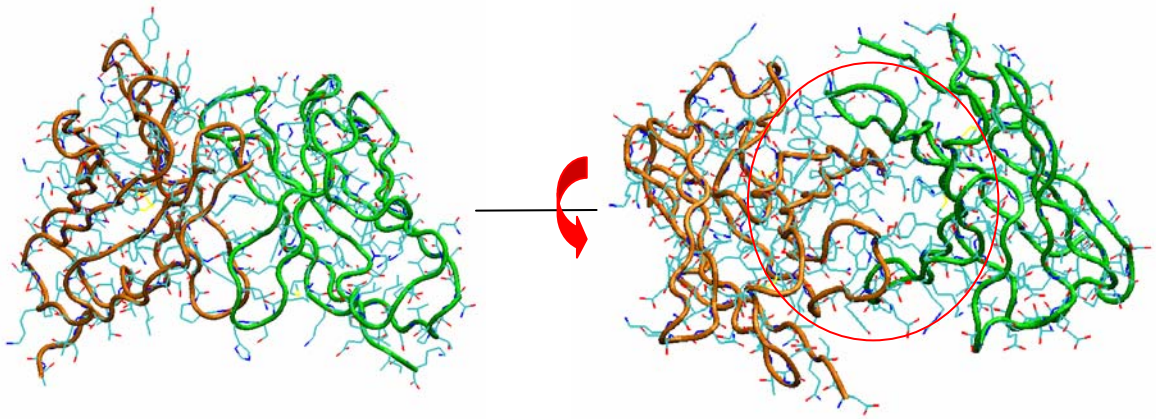


Antigen binding

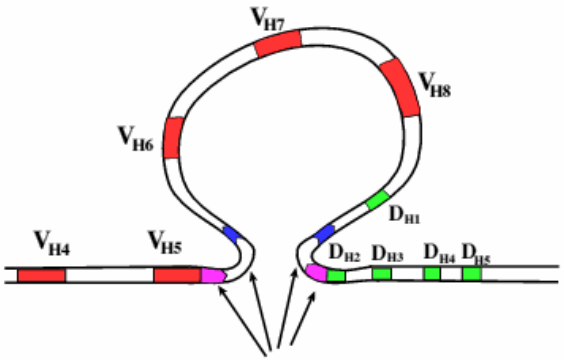


- Antibodies can learn to bind **anything**—but how?
- Somatic mutation generates diversity within the antibody binding loop

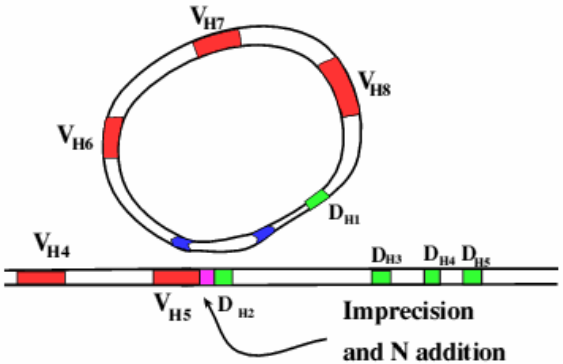




VDJ joining occurs by DNA recombination

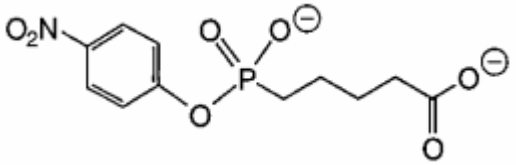


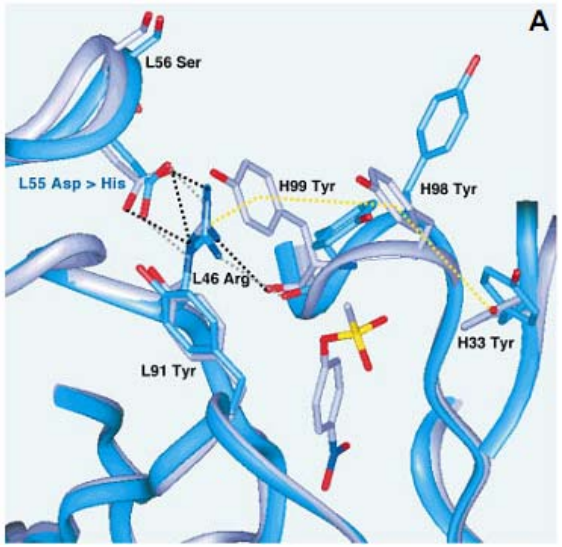
Heptamer and nonamer signal sequences



Evolution of antibody binding site

- Clonal selection and expansion based on affinity against foreign antigens underlies the evolution of antibody affinity
- What is the main structural difference between a germline antibody (Kd = 135 uM) and an affinity matured antibody (Kd = 4.5 nM)?
- Compare high resolution structures of hapten bound to a primary antibody or to an affinity optimized antibody
- Antigen binding site undergoes significant conformational changes upon hapten binding





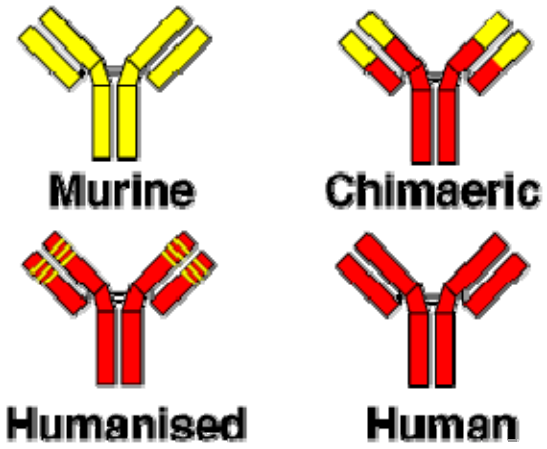
- Mutations (< 15 Å away) in the affinity-optimized antibody stabilize the bound conformation: the binding is closer to “lock and key” rather than “induced fit”

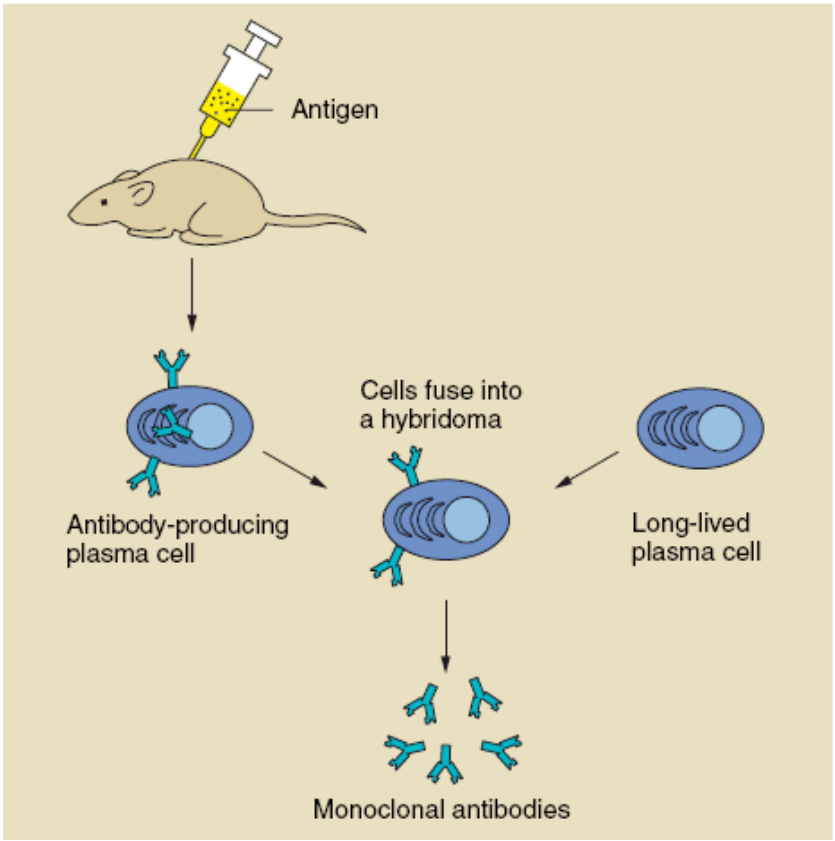
Wedemayer et al, Science 276, 1665 (1997)

Com-parison*	V_L		V_H		Both chains		V_LV_H rotation
	Cα	All	Cα	All	Cα	All	
g− to g+	0.38	0.66	0.51	0.99	0.60	0.94	4.60°
g− to m−	0.46	0.92	0.60	1.05	0.78	1.17	6.89°
g− to m+	0.41	0.78	0.30	0.65	0.77	1.11	6.94°
g+ to m−	0.41	0.84	0.66	1.15	0.60	1.05	3.63°
g+ to m+	0.40	0.79	0.65	1.02	0.59	0.95	3.51°
m− to m+	0.30	0.65	0.45	0.84	0.38	0.75	0.44°

Making antibodies in culture

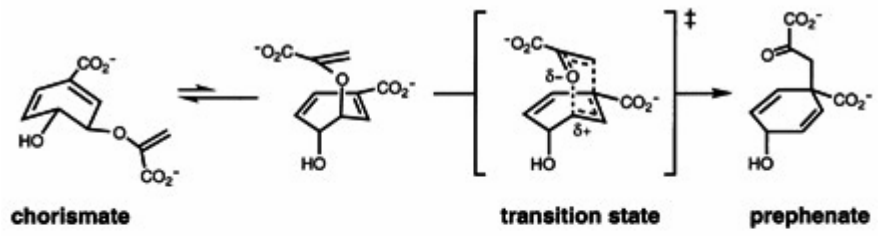
- Monoclonal antibodies are produced in model organisms, e.g. mouse, rat, rabbit, goat
- Antibody producing cells can be fused with immortal plasma cells to produce hybridoma cells that will continue to produce antibodies
- Functional residues from an antibody produced in mouse may be transplanted onto a human antibody to minimize immune reaction in human patients—important for therapeutic application

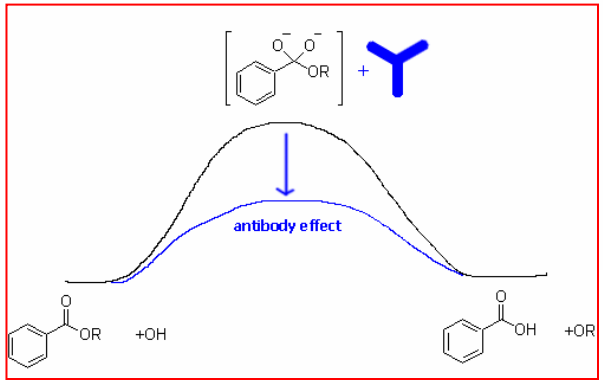


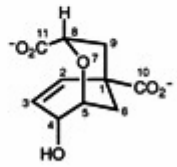


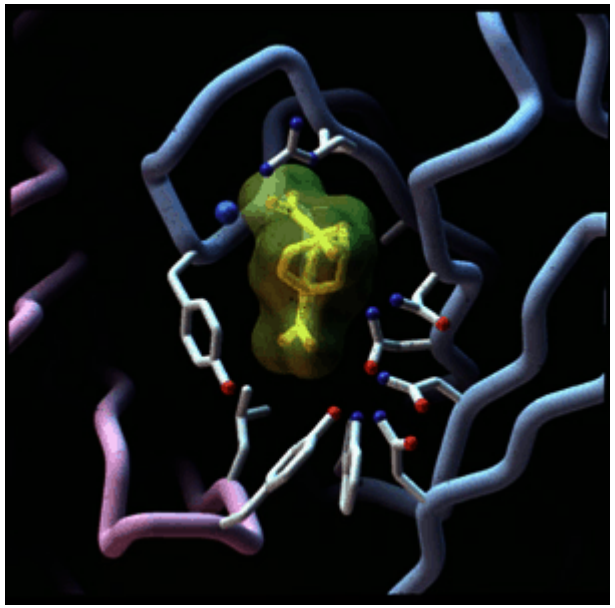
Catalytic antibody

- Antibodies can be “trained” to bind anything, including molecules that resemble the transition state of a chemical reaction
- Antibody binding would stabilize the transition state and thus lower the energy of the transition state—theoretically this should accelerate the rate of a reaction
- Design a transition state analog, i.e. a chemical that resembles the putative transition state—not always possible or easy





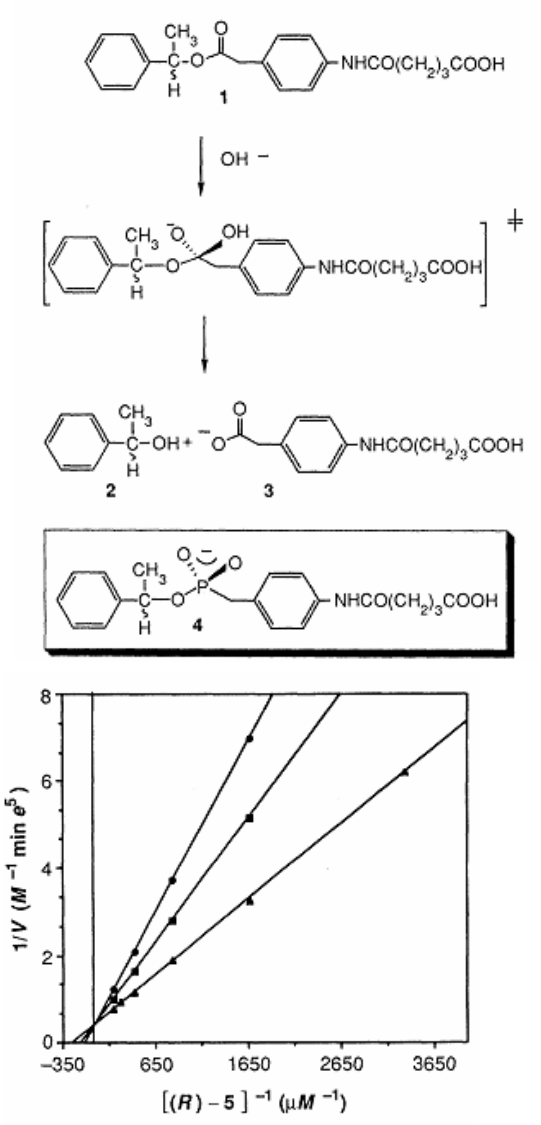




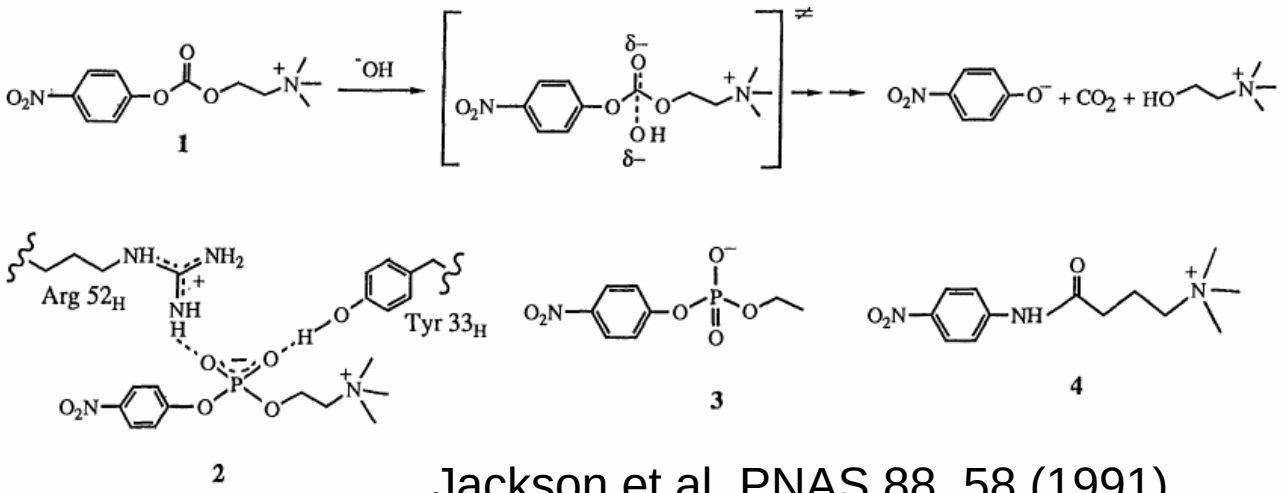
Antibodies to hydrolyze ester bonds

Antibodies can be engineered to mimic lipase activity

Janda et al, Science 244, 437 (1989)

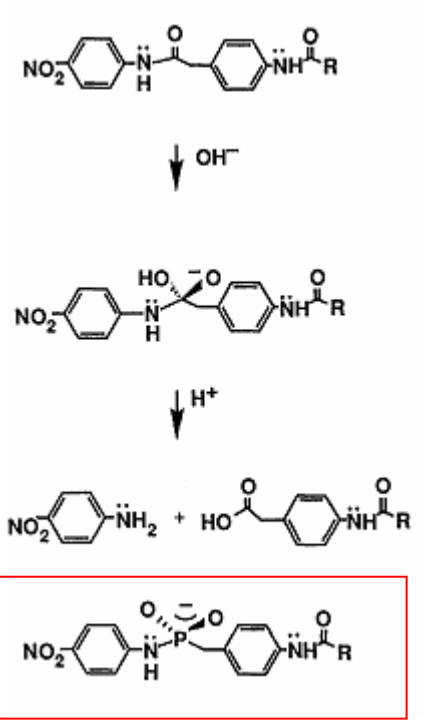


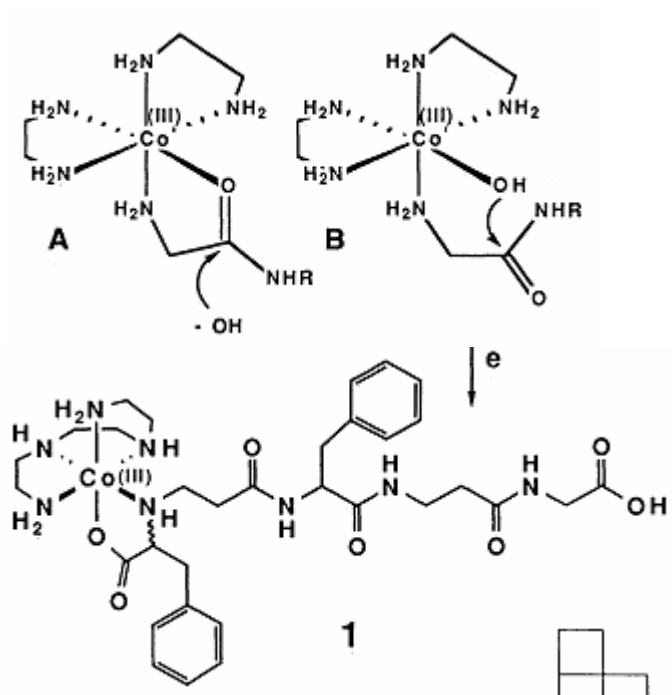
- Antibody S107 bind phosphorylcholine mono- and diesters
- Two variants, MOPC-167 and T15, can hydrolyze the carbonate esters, in which **R52** plays a key role in the catalysis
- Addition of a transition state analog inhibits the activity, presumably by binding to the antibody

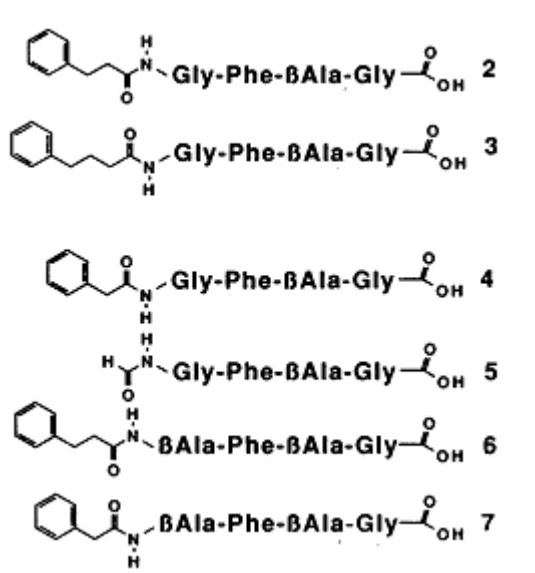


Jackson et al, PNAS 88, 58 (1991)

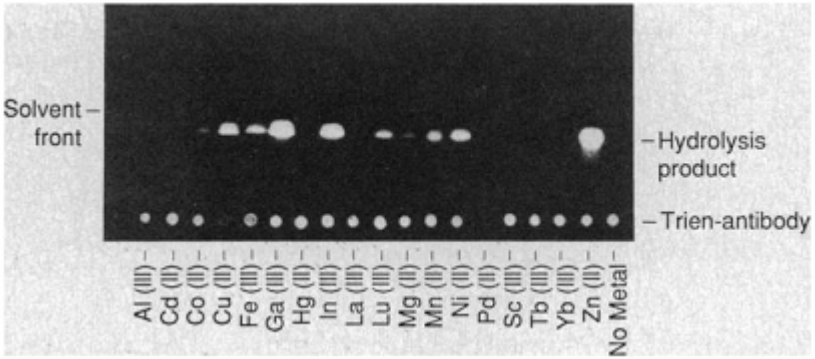
Antibody-catalyzed peptide bond hydrolysis

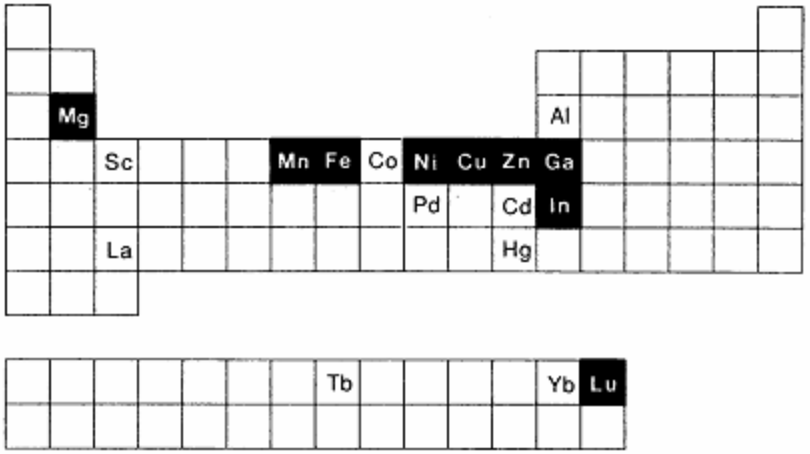




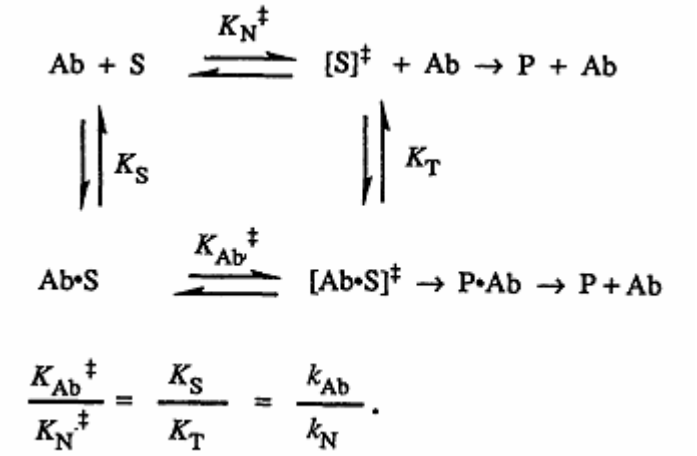


Janda et al, Science 241, 1188 (1988)

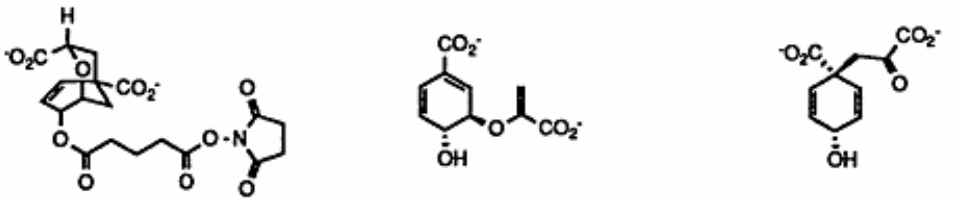




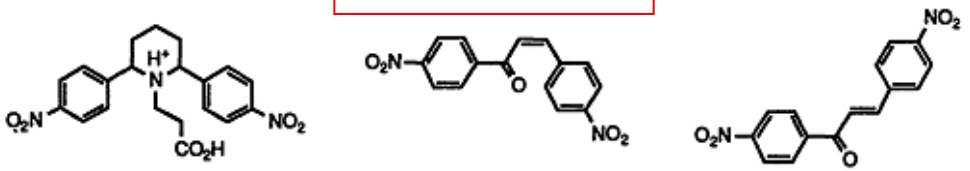
Iverson and Lerner, Science 243, 1184 (1989)



Claisen rearrangement

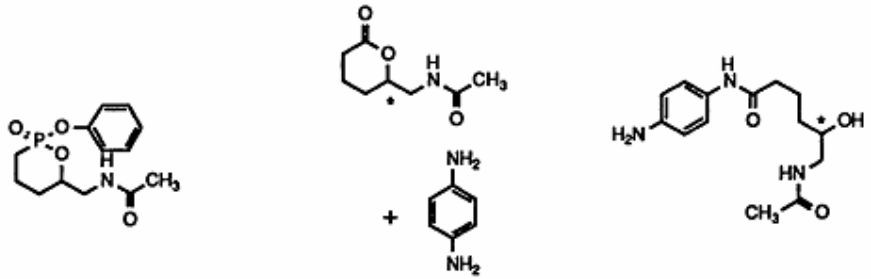


Cis-trans isomerization



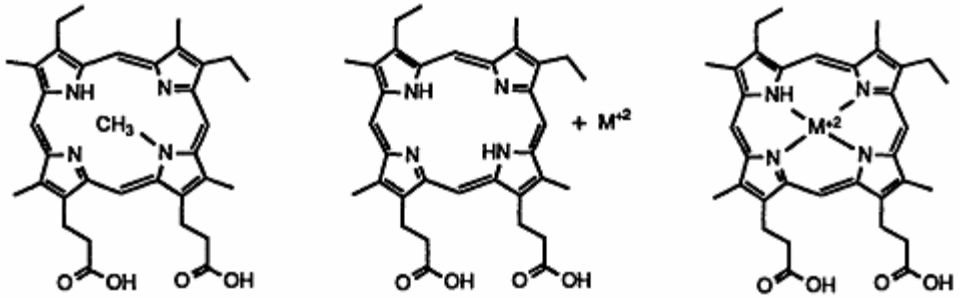
Reaction 34 (*74*)

Amide bond formation



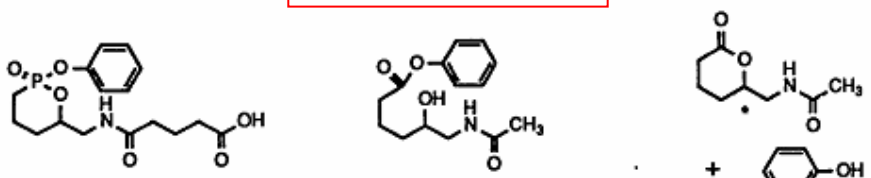
Reaction 23 (*46*)

Metal chelation



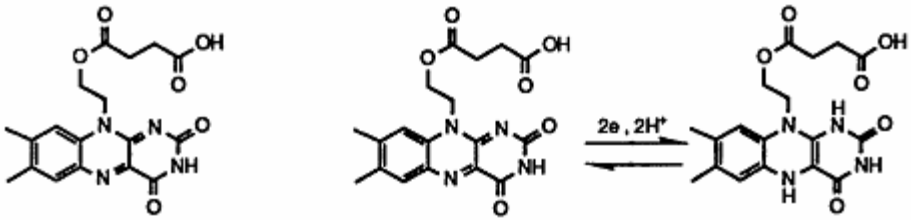
Reaction 35 (*55*)

Lactonization



Reaction 25 (*48*)

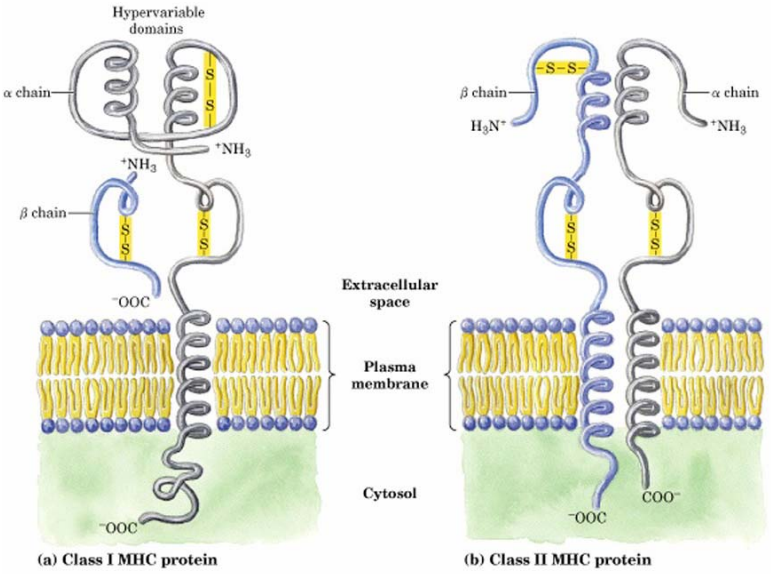
Redox

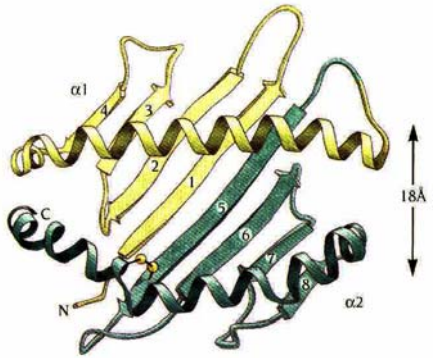


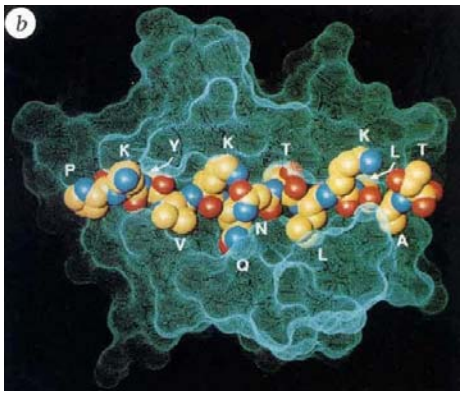
Reaction 30 (*76*)

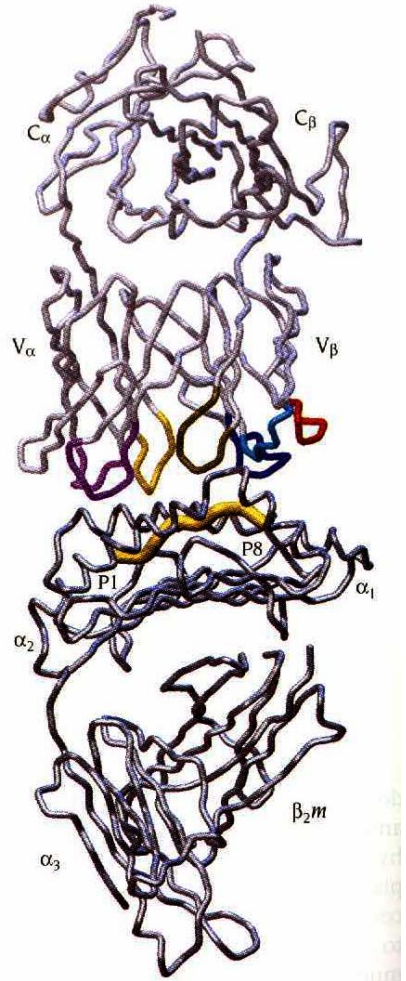
MHC

- Antigenic peptides are displayed in the context of the major histocompatibility complex (MHC) I and II
- MHCII is a heterodimer displayed on antigen presenting cells (APC), which alerts T cells of the presence of a foreign antigen
- MHC proteins have been linked to various diseases, including multiple sclerosis, type I diabetes, transplant rejection, rheumatoid arthritis





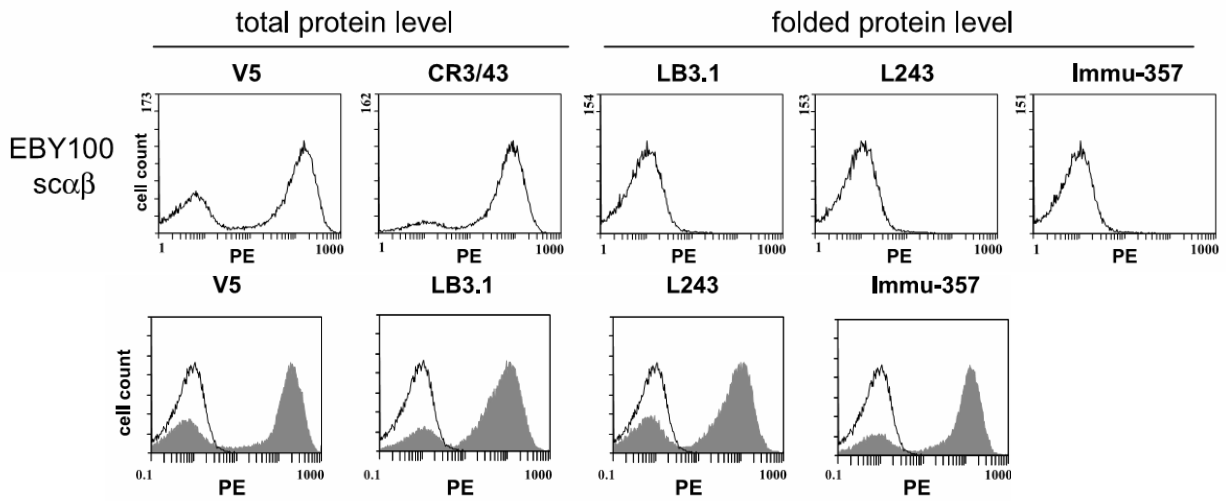


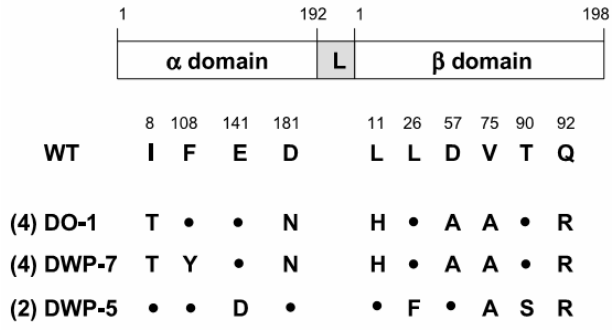


Stern et al, Nature 368, 215 (1994)

Single chain MHC II

- Peptides with high affinity for MHC are in general more immunogenic
- Developing MHC-based therapeutics requires expression of soluble and functional MHC molecules
- Yeast display utilizes the eukaryotic protein-processing machinery and is amenable to high throughput analysis based on directed evolution

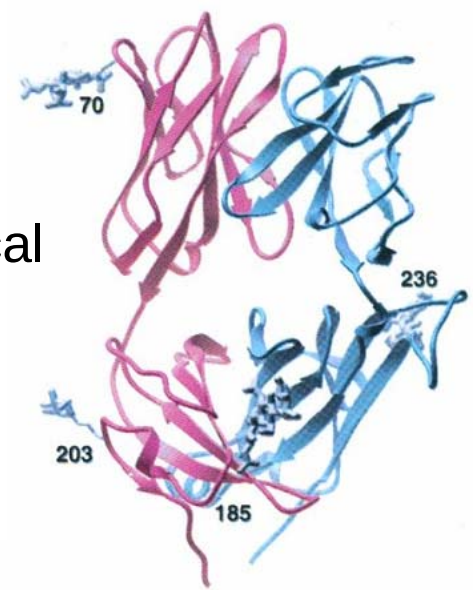


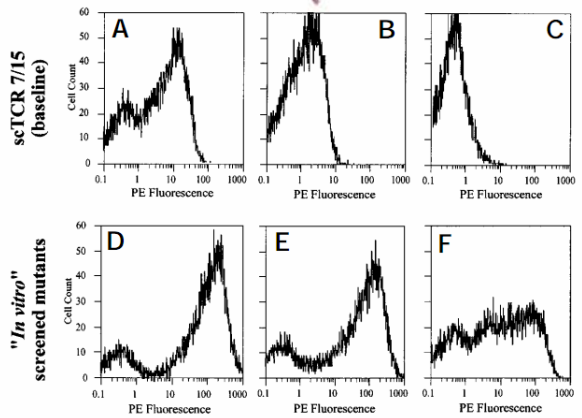


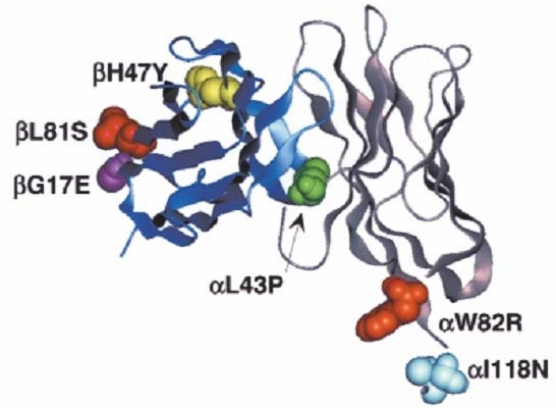
Esteban and Zhao, JMB 340, 81 (2004)

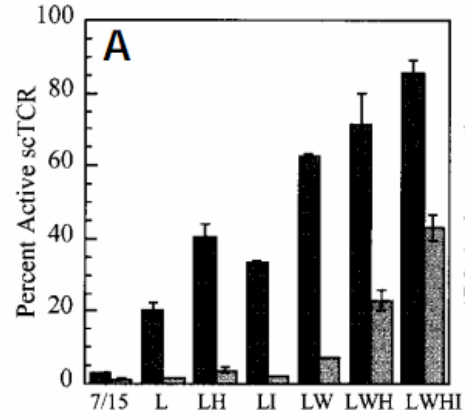
Single chain T cell receptor

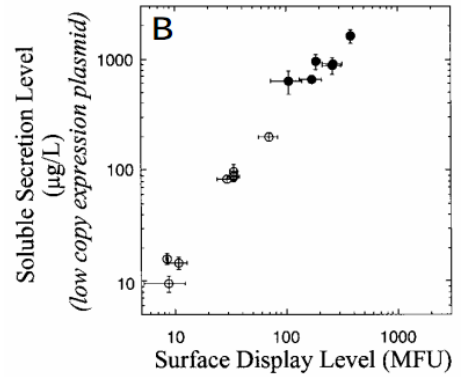
- Heterodimeric membrane bound protein with theoretical diversity exceeding that of antibody although binding affinity is weaker
- TCR recognizes antigenic MHC-bound peptides
- Binding of TCR to MHC-peptide triggers an immune
  response that may be important for clearance of virus and cancer cells
- Biophysical characterization of TCR requires a stable, soluble variant









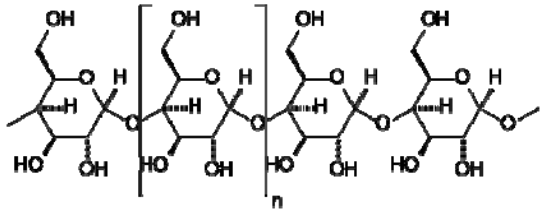


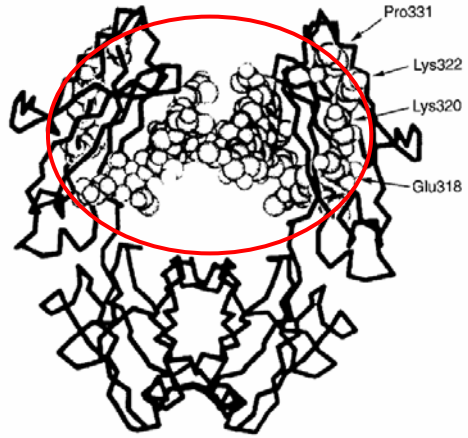
Shusta et al, Nat Biotech, 18, 754 (2000)

Innate immunity

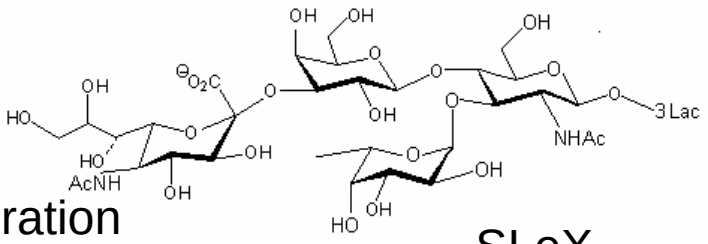
- Carbohydrates (saccharides, sugar) are ketones or aldehydes where most other carbons are hydroxylated
- Glycosylation (i.e. addition of a sugar) is a form of post-translational modification, and can change the biophysical properties of a protein
- Carbohydrate binding proteins (**lectins**) play important roles in development, immunity, cell-cell interactions
  - glycosylation of the Fc fragment of Ab
  - recognition of peptidoglycans (polymer of peptide and sugar found on the outside of bacteria) by the immune system
  - binding of complements to glycosyl groups on hypermannosylated proteins on yeast
  - sialyl Lewis x acid required for leukocyte migration to sites of injury
  - cancer cells express different levels and types of carbohydrates

amylose





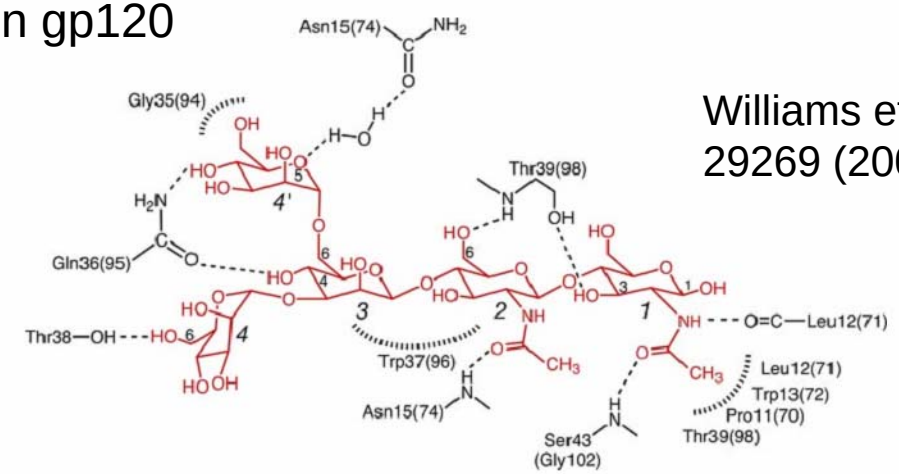
antibody Fc



SLeX

Protein-carbohydrate interactions are highly polar

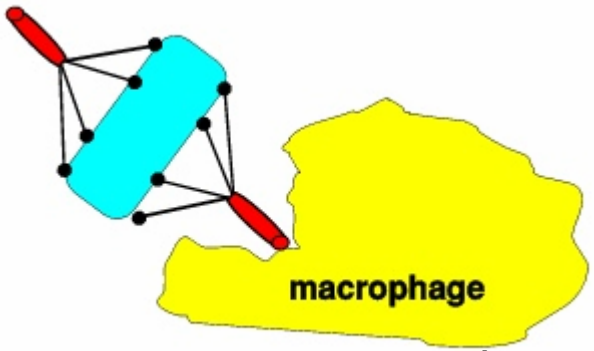
- accounts for their low affinity—typically in the μM to mM range
- e.g. HIV-1 inhibitory cyanobacterial protein MVL bound to $Man_3GlcNAc_2$ such as found on gp120

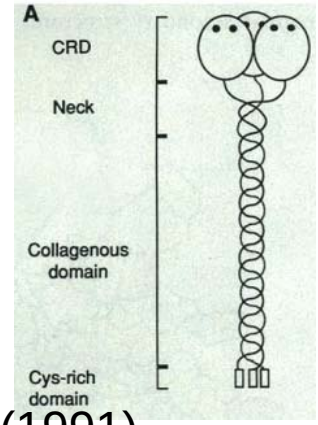


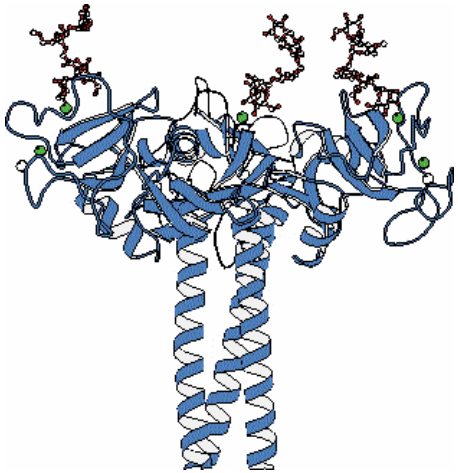
Williams et al, JBC 280, 29269 (2005)

Mannose binding proteins (MBP) are best characterized lectins

- microbes (e.g. bacteria and yeast) often express high levels of mannose
- involved in the lectin pathway of complement activation
- also found on macrophages





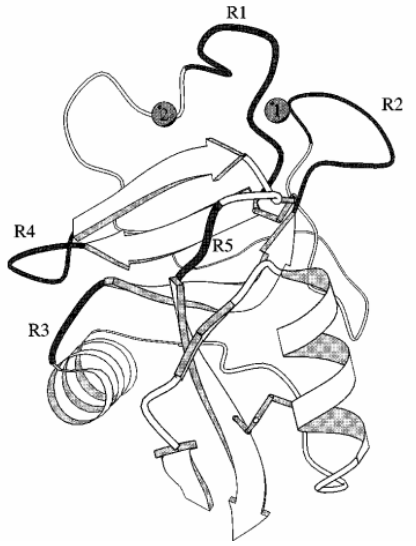


Weis et al, Science 254, 1608 (1991)

Changing the specificity of lectin

Selectins are lectins involved in cell adhesion

- E-selectins are found on endothelial cells
- share structural similarity with MBP



Introduce mutations in MBP where the two proteins differ in sequence

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                          160       170       180       190       200       210       220
MBP-A       AKT---SAFLGITDEVTEGQFMYV-TGGRLT--YSNWKKDEPNDHGSGEDCVTIV-----DNGLWNDISCQASHTAVCEFPA
Ca LIGANDS              1  1                          2 21   21              22

REGION 1              RK--VNNV
REGION 2                                        NRQKD

REGION 3    SYSPSY
REGION 4                                                  YIKREK
REGION 5                                                                  KKK

MUTANTS      ‡‡‡                                          -  -              ±  -
             AAF                                          F  K              A  A
E-SELECTIN  LSYSPSYYWIGIRK--VNNVWVWVGTQKPLTEEAKNWAPGEPNNRQKDEDCVEIYIKREKDVGMWNDERCSKKKLALCYTA-
                  50        60        70        80        90        100       110       120
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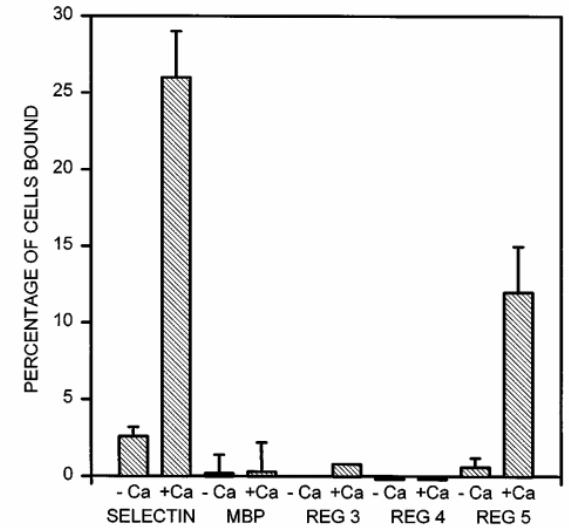

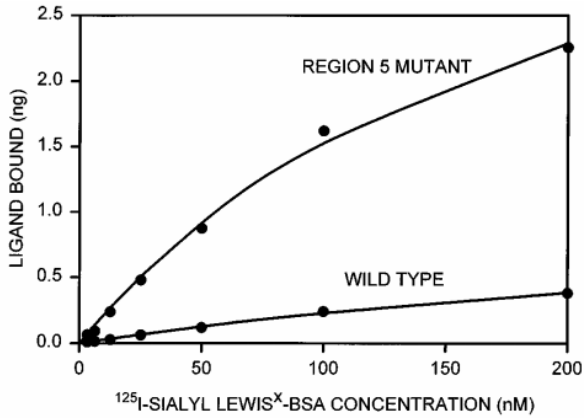

Blanck et al, JBC 271, 7289 (1996)