Post Translational Modifications

Oxidation and Carbonylation

- Oxidation of proteins by reactive oxygen species (ROS)
- H_2O_2 and OH^- radicals and O_2^-
- Oxidation can be irreversible

Oxidation of Amino Acids

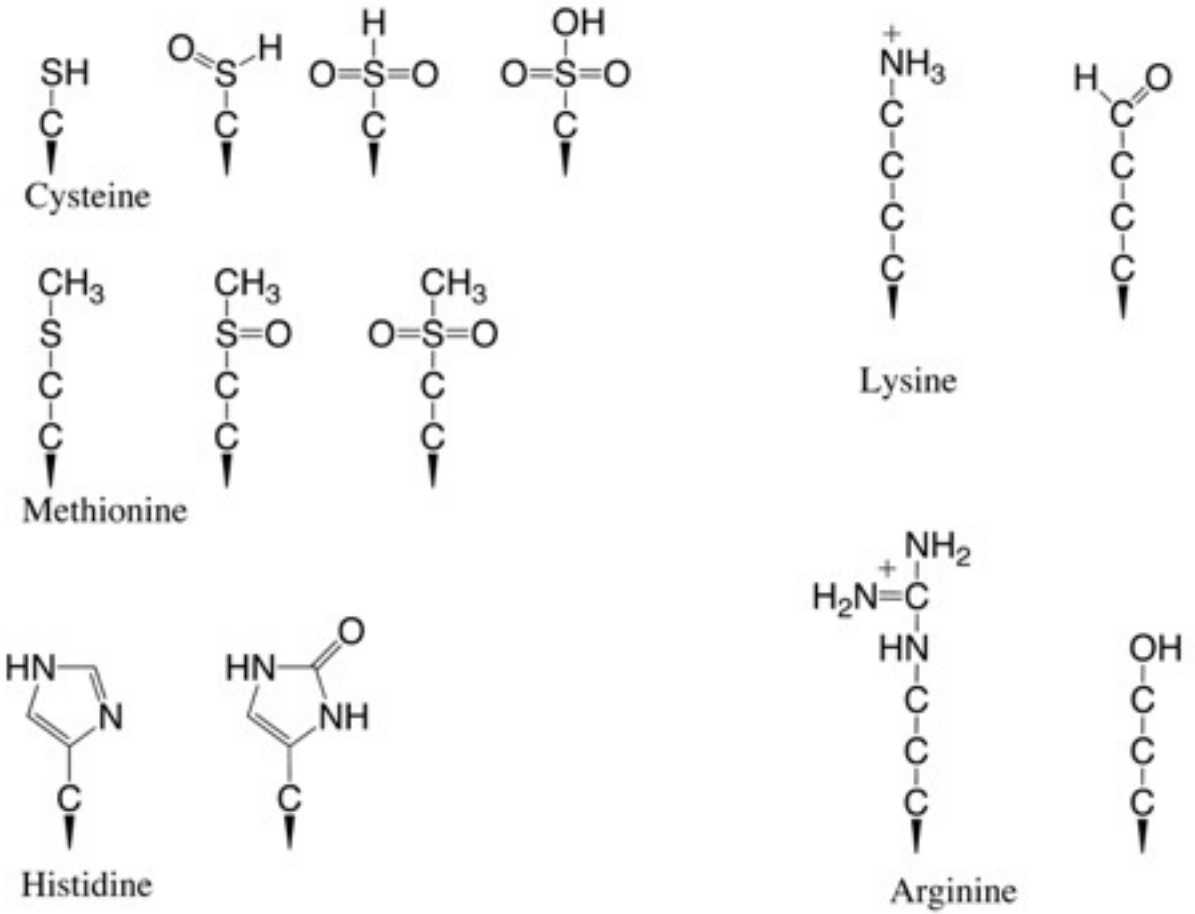


FIGURE E.1 ■ Examples of modification of some amino acid side chains by oxidation.

Deamidation of Asn and Gln Residues

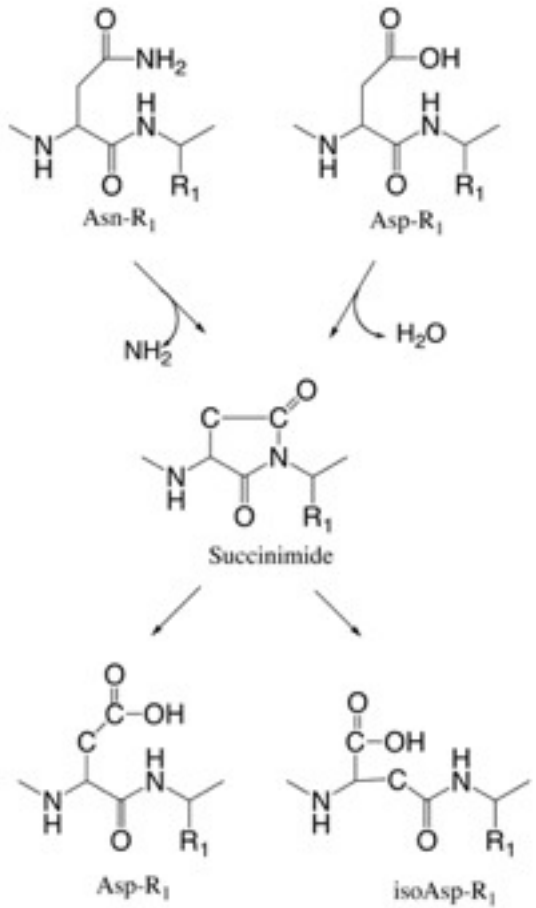


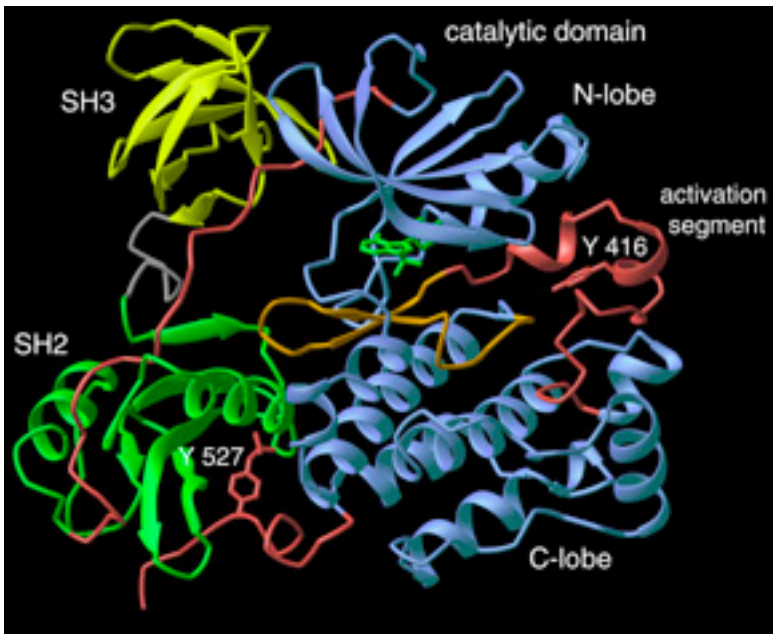
FIGURE E.3 ■ Pathways of deamidation of asparagine residues and cyclization of aspartate and asparagine residues. The succinimide may open to yield an isoAsp residue with both the α and β carbons of the main chain.

Phosphorylation

- Phophorylation is critical for intracellular signaling
- Kinases and phosphorylases
- Human genome has 700 kinases
- Eukaryotes - serine, threonine and tyrosine
- Bacteria - Histidine

Kinases are Highly Regulated

Kinases share a common fold of 250 residues



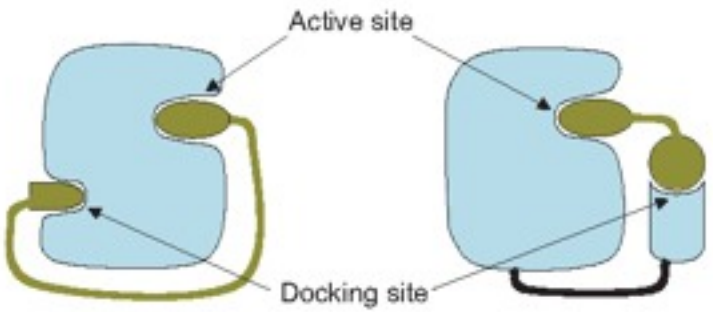


FIGURE E.4 ■ The regulatory role of kinases and phosphatases requires extensive control of the protein interactions. The enzymes (blue) partly need to be activated but also need to identify structures other than the part that will be phosphorylated or dephosphorylated. This is due to substrate (green) interactions with a docking site that can be part of the kinase domain or belong to different parts of the enzyme.

Methylation and Acetylation

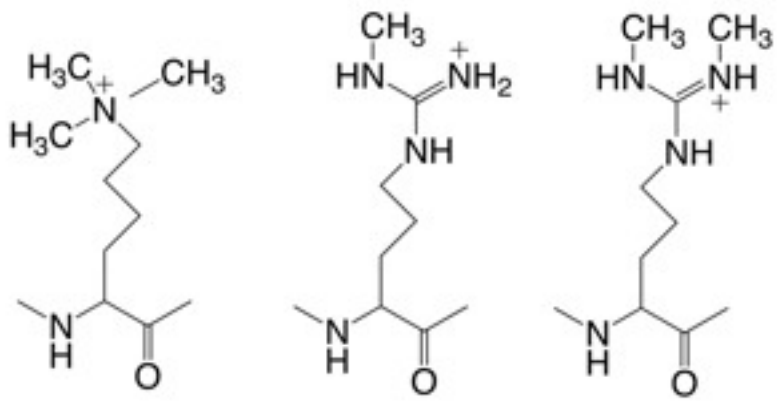


FIGURE E.5 ■ The methylation of lysines and arginines has important functional roles in systems interacting with nucleic acids. On the left a trimetyllysine is shown. The center and right-hand figures show a mono- and a symmetrically dimethylated arginine.

Disulfide Bond

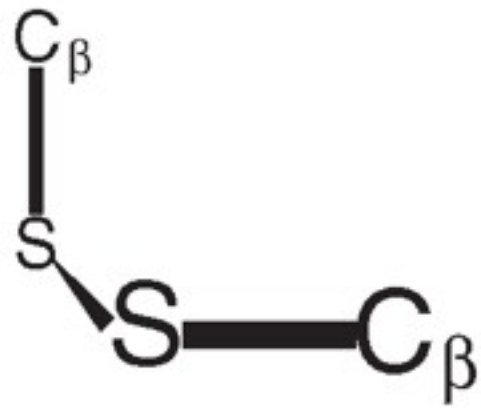
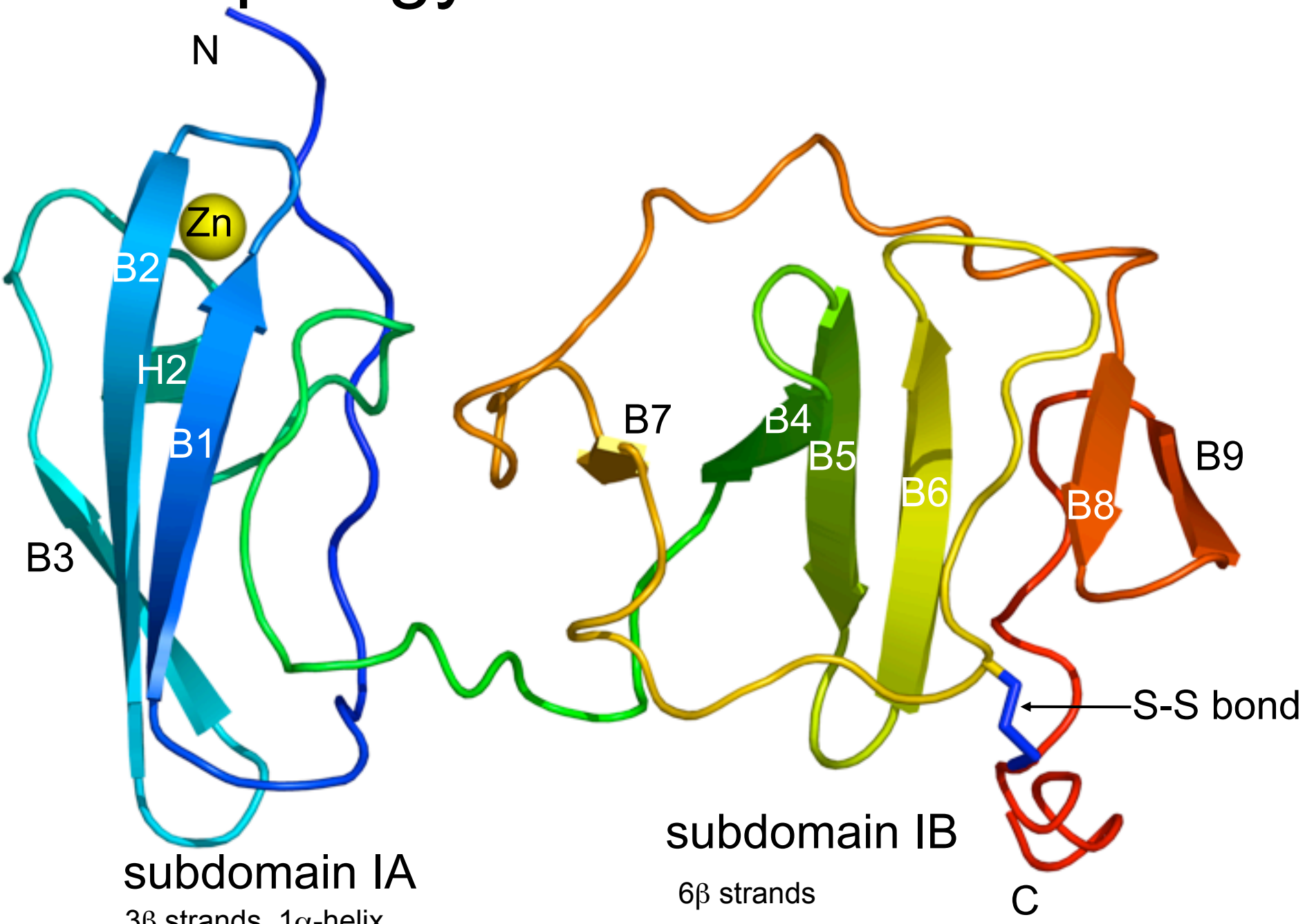
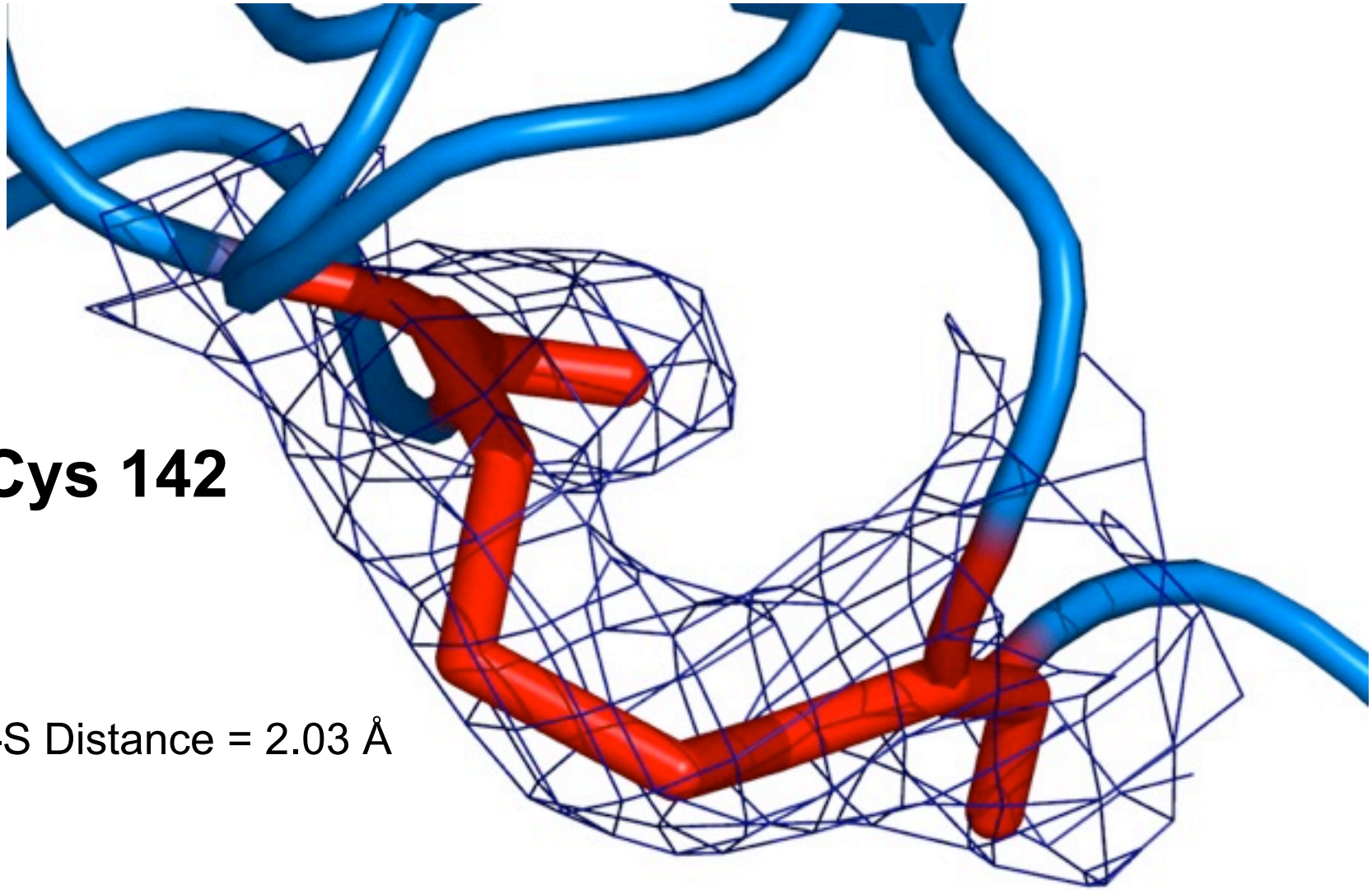


FIGURE A.1 ■ The preferred conformation of a disulfide bond with a 90° angle between the S–C_β bonds viewed down the S–S bond.

Topology of NS5A Domain I



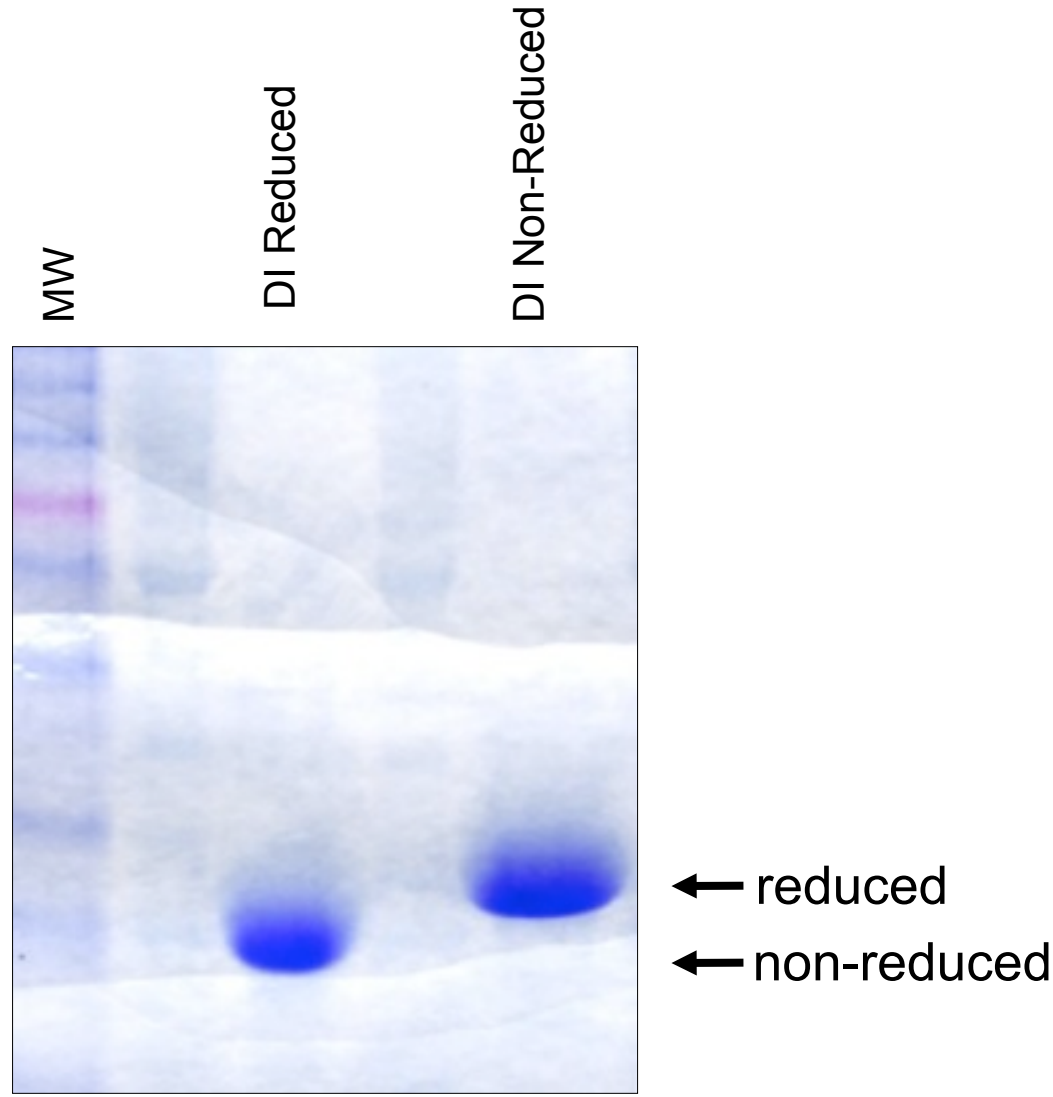
Domain I Disulfide Bond With Experimental Density



S-S Distance = 2.03 Å

MAD Experimental Electron Density at 1σ

Domain I Electrophoretic Mobility Shift



5 mM DTT treatment in reduced samples

Glycosylation

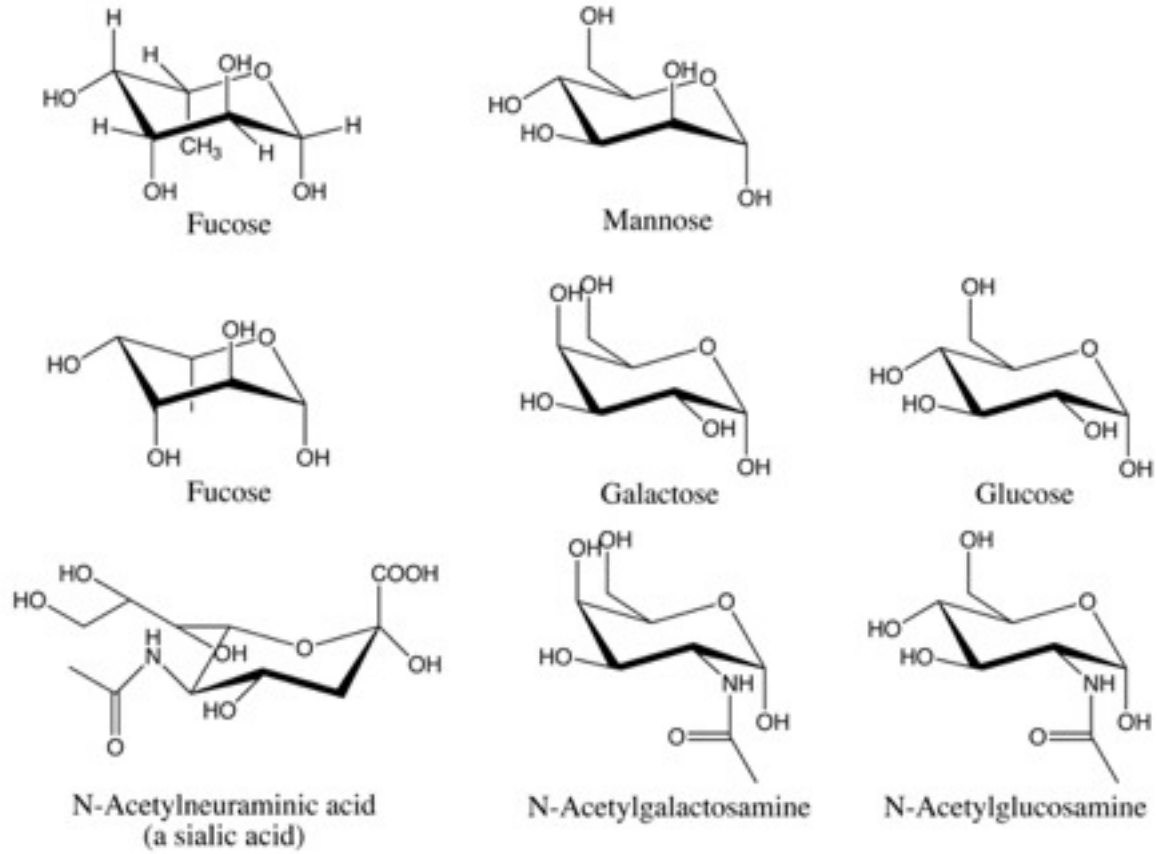
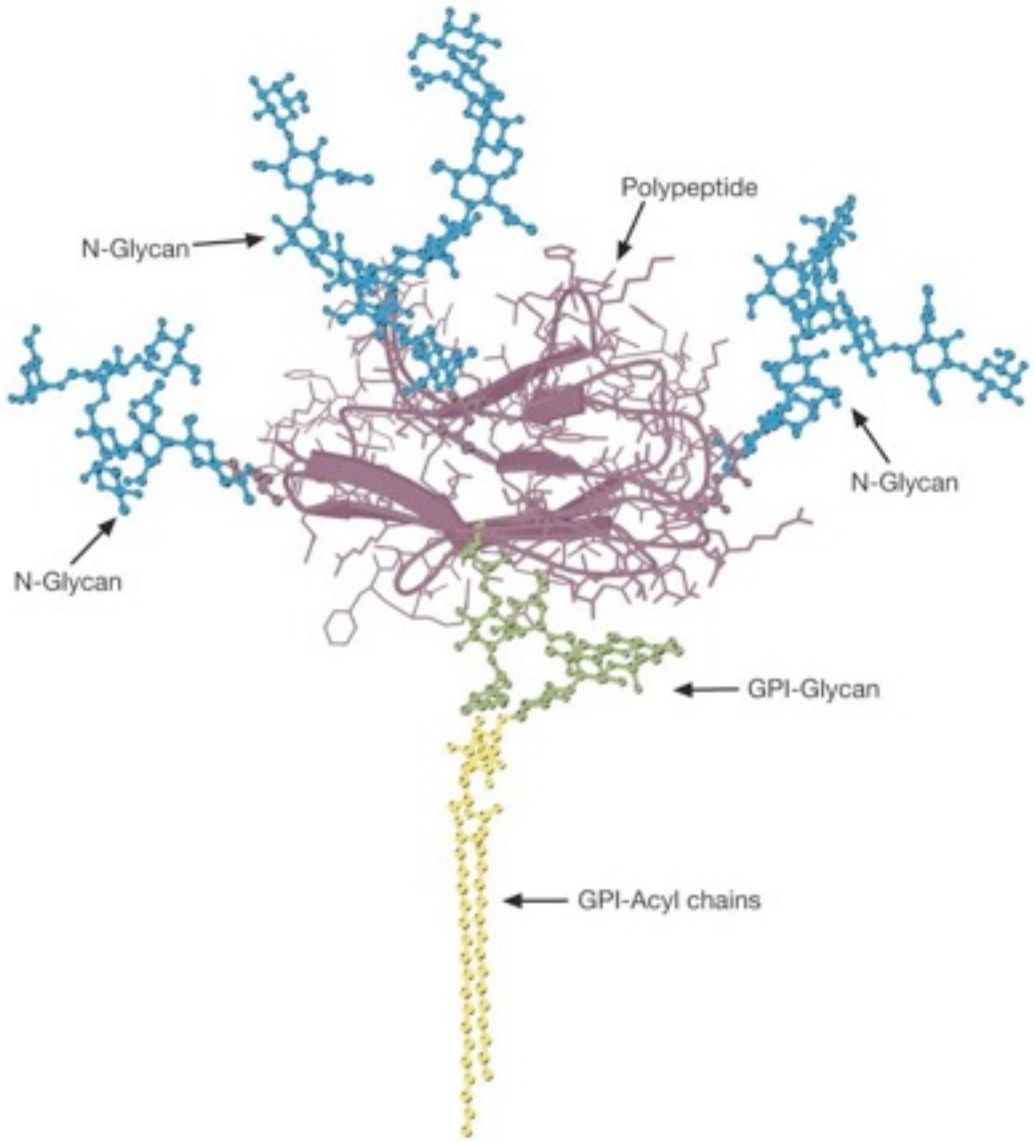


FIGURE E.7 ■ Some types of sugar residues that can be attached to proteins. The top *left* shows the more extensive formula for fucose where all hydrogens are included. *Below* is the same fucose without showing the hydrogens directly attached to the ring carbons.

http://www.ncbi.nlm.nih.gov/books/NBK1908/

http://www.ncbi.nlm.nih.gov/books/NBK21154/

Glycoproteins



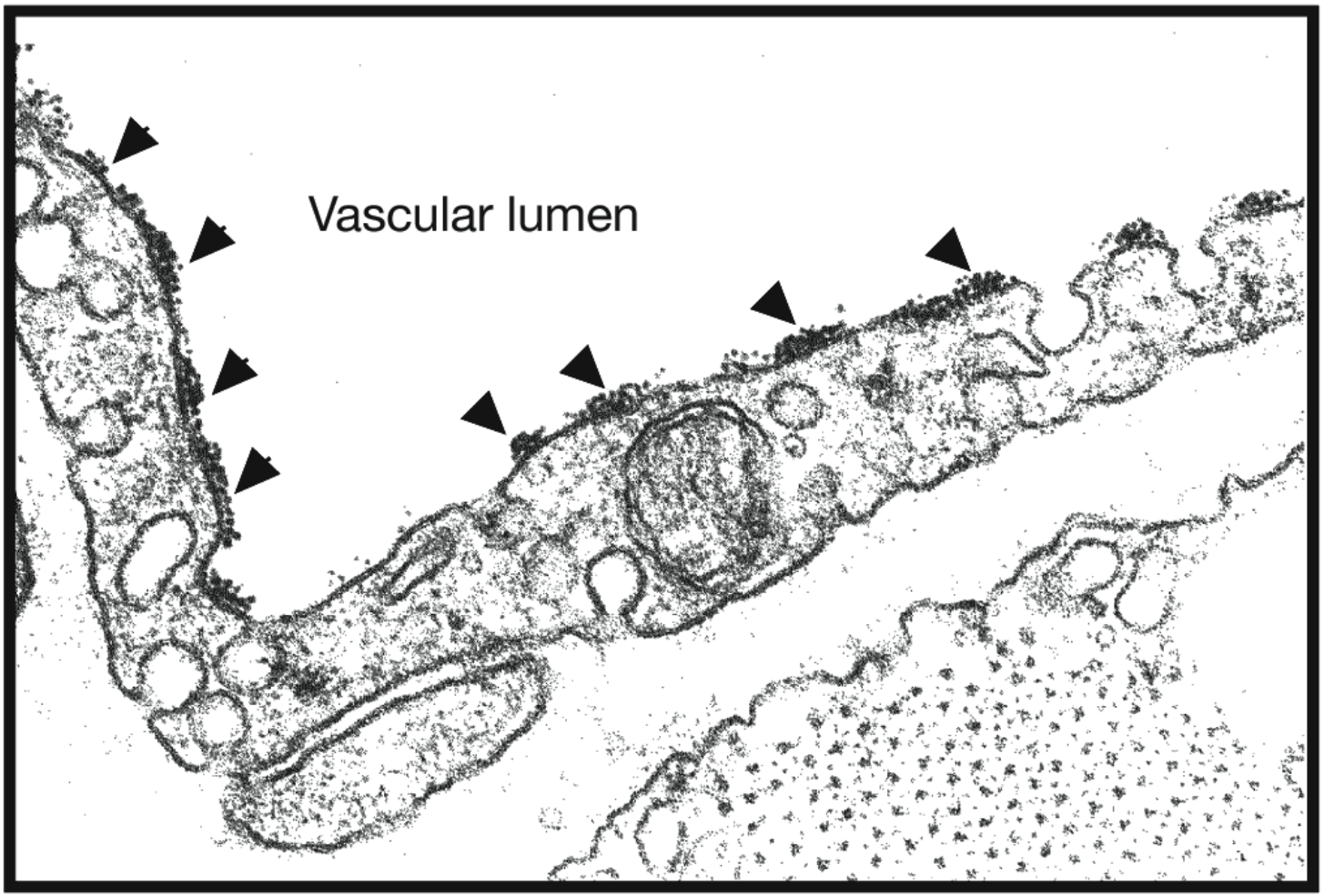
Schematic representation of the Thy-1 glycoprotein including the three N-glycans (*blue*) and a glycosylphosphatidylinositol (GPI-glycan, *green*) lipid anchor whose acyl chains (*yellow*) would normally be embedded in the membrane bilayer. Note that the polypeptide (*purple*) represents only a relatively small portion of the total mass of the protein.

Cell Surface

- Glycans make up a large portion of the mass of the protein.
  - In many cases it can consistute about 50% of the mass
  - one glycan is about 2-2.5kDa.

- The surfaces of all types of cells in nature are effectively covered with a dense array of sugars and glycoproteins giving rise to the so-called glycocalyx.

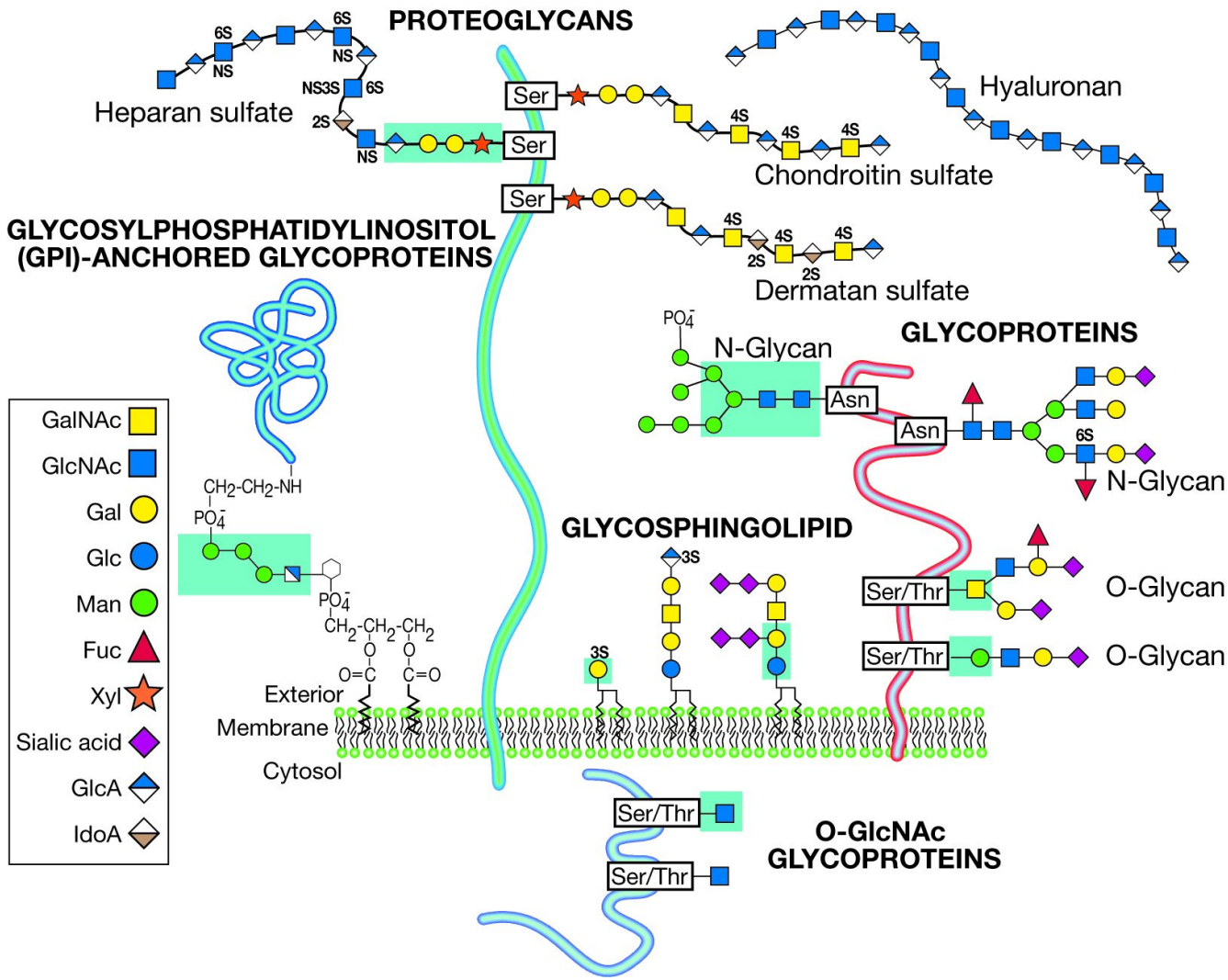
Electron Micrograph of Endothellal Cells



Types of Sugars

- Pentoses: Five-carbon neutral sugars, e.g., D-xylose (Xyl)
- Hexoses: Six-carbon neutral sugars, e.g., D-glucose (Glc), D-galactose (Gal), and D-mannose (Man).
- Hexosamines: Hexoses with an amino group at the 2-position, which can be either free or, more commonly, N-acetylated, e.g., N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-galactosamine (GalNAc).
- Deoxyhexoses: Six-carbon neutral sugars without the hydroxyl group at the 6-position (e.g., L-fucose [Fuc]).
- Uronic acids: Hexoses with a negatively charged carboxylate at the 6-position, e.g., D-glucuronic acid (GlcA) and L-iduronic acid (IdoA).
- Sialic acids: Family of nine-carbon acidic sugars(generic abbreviation is Sia), of which the most common is N-acetylneuraminic acid (Neu5Ac, also sometimes called NeuAc or historically, NANA)

Types of Glycans



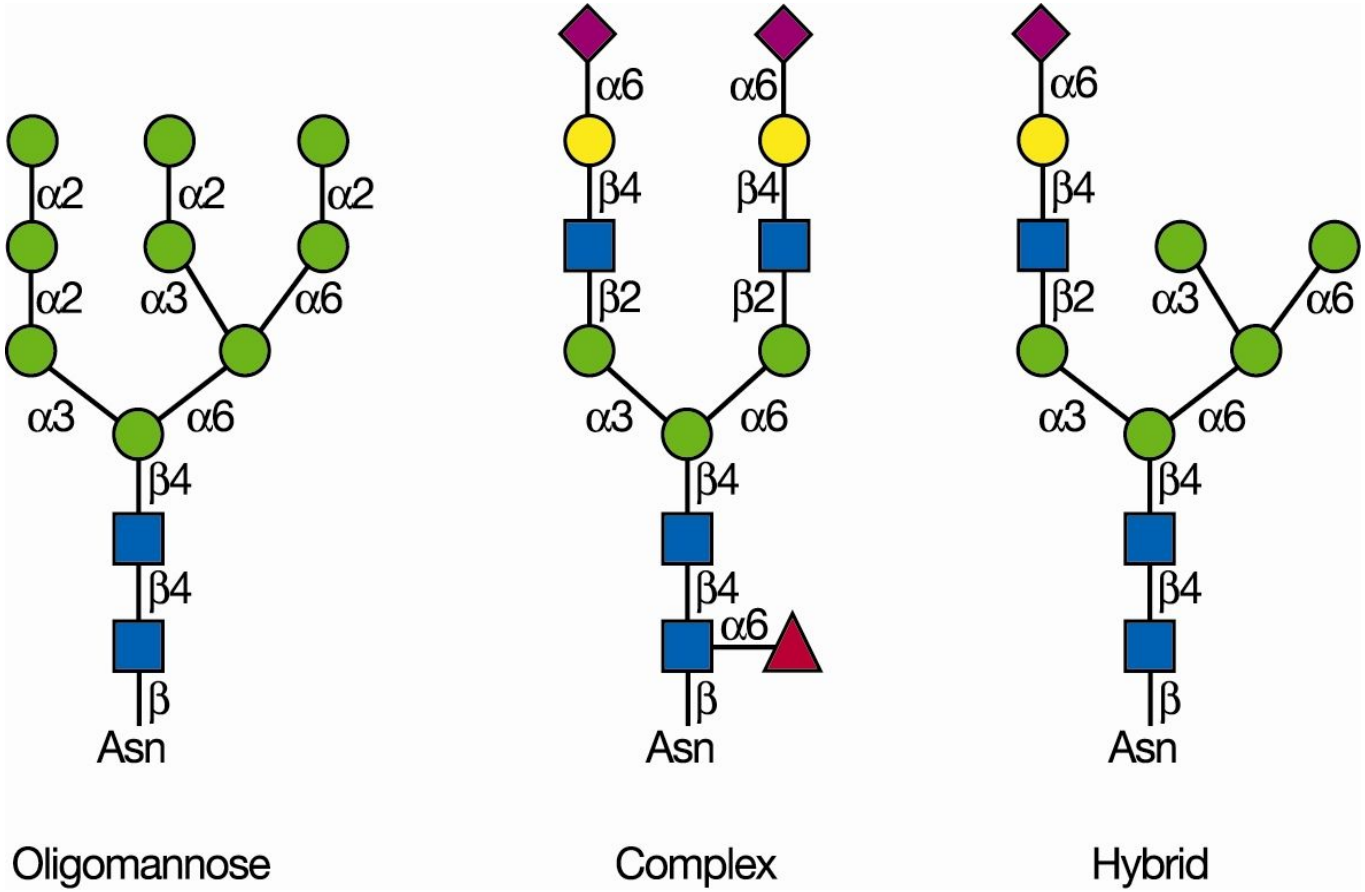
N-linked Glycans

- Glycan covalently modified to an Asn residue
- Five different N-glycan linkages have been reported, of which *N*-acetylglucosamine to asparagine (GlcNAcβ1-Asn) is the most common.
- The minimal amino acid sequence begins with asparagine followed by any amino acid except proline and it ends with serine or threonine (Asn-X-Ser/Thr) sequons.
- Glycans are initially synthesized on a lipid-like molecule termed dolichol phosphate (Dol-P), followed by “en bloc” transfer of the entire glycan of 14 sugars to protein.
- This synthetic pathway is conserved in all of the metazoa, in plants, and in yeast. Bacteria use a related pathway to synthesize their cell wall.

Glycan Diversity

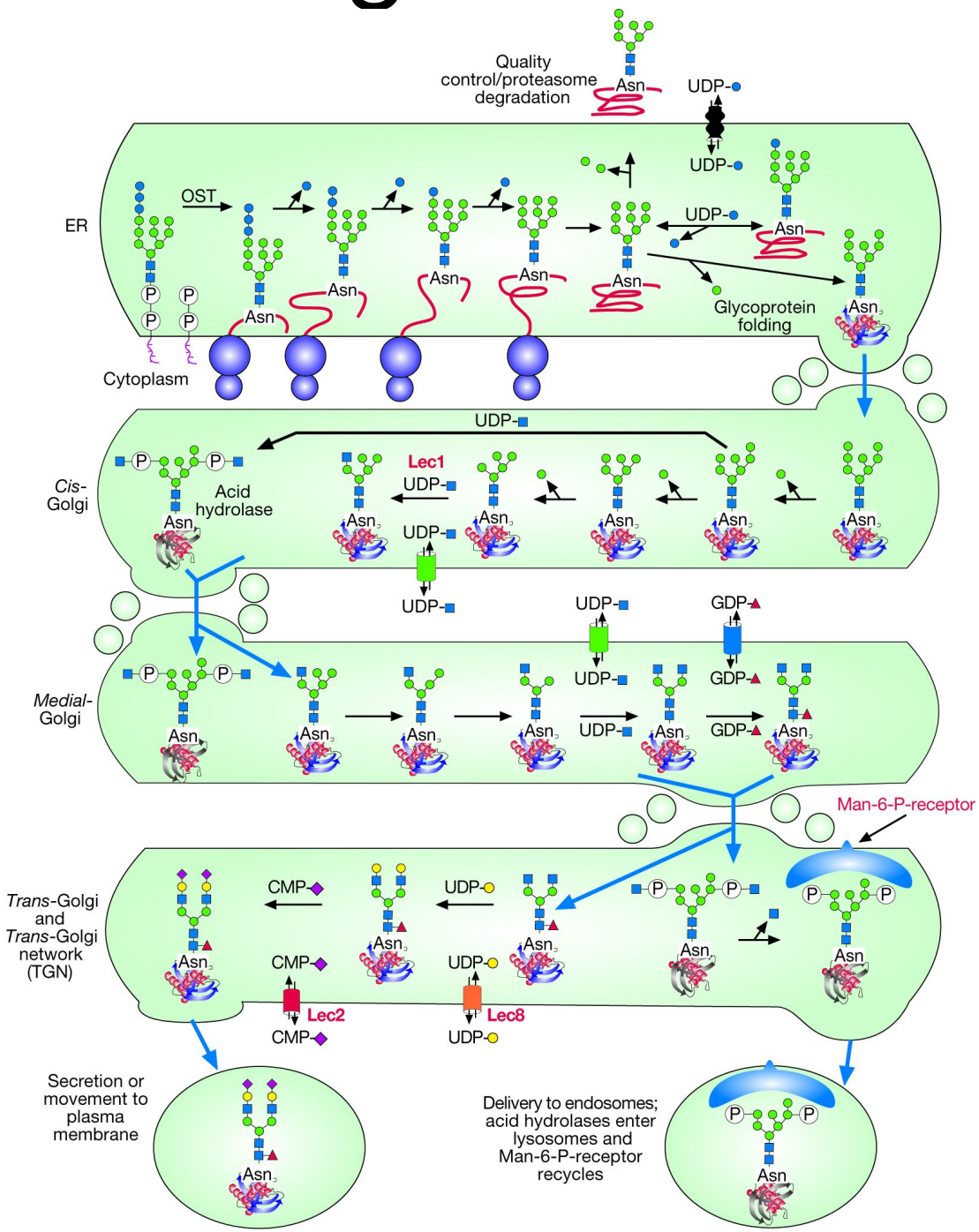
- Glycosylation is heterogenous
- At an attachment site on a given protein synthesized by a particular cell type, a range of variations can be found in the structures of the attached

Types of N-linked Glycans



- Oligosaccharyltransferase (OST) in the ER membrane catalyzes the transfer of $Glc_3Man_9GlcNAc_2$ from Dol-P-P to Asn-X-Ser/Thr
- Once linked further modification of the glycans are done by specific enzymes in the ER.

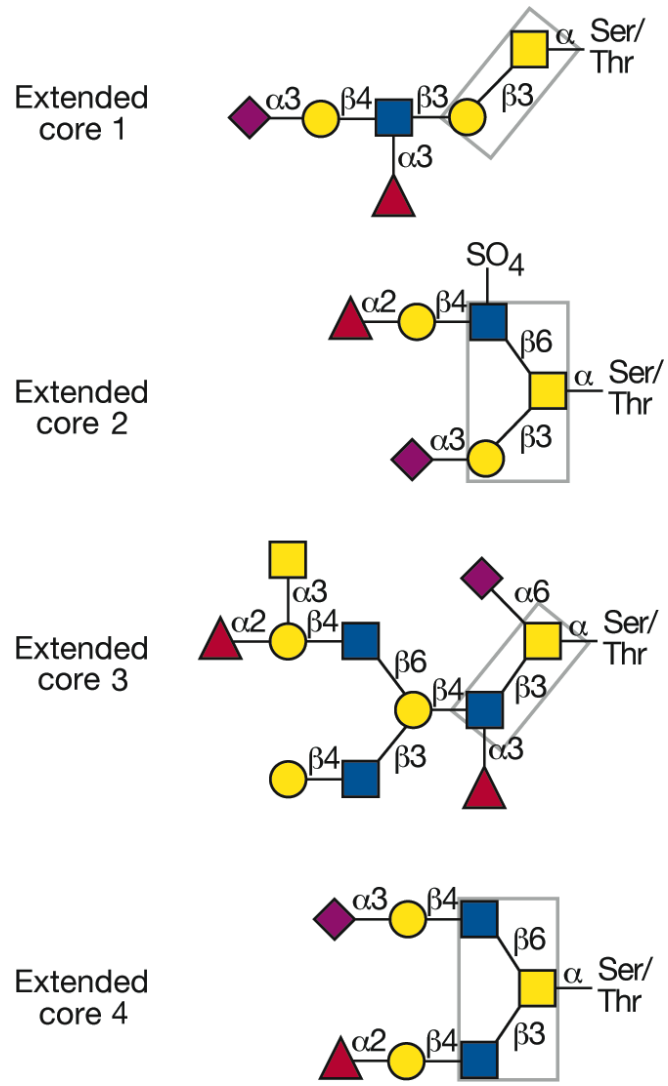
Processing of N-linked Glycans



O-Linked Glycosylation

- O- glycosylation is a common covalent modification of serine and threonine residues of mammalian glycoproteins (often referred to as mucins)
- α-linked via an N-acetylgalactosamine (GalNAc) moiety to the -OH of serine or threonine by an O-glycosidic bond.
- Other types
  - α-linked O-fucose, β-linked O-xylose, α-linked O-mannose, β-linked O-GlcNAc (N-acetylglucosamine), α- or β-linked O-galactose, and α- or β-linked O-glucose
- The simplest mucin O-glycan is a single N-acetylgalactosamine residue linked to serine or threonine. Named the Tn antigen, this glycan is often antigenic.
- The most common O-GalNAc glycan is Galβ1-3GalNAc-, and it is found in many glycoproteins and mucins
- transfer of N-acetylgalactosamine from UDP-GalNAc to serine or threonine residues, which is catalyzed by a polypeptide-N-acetyl-galactosaminyltransferase (ppGalNAcT)
- No defined amino acid sequons
  - Proline residues near the site of N-acetylgalactosamine addition are usually favorable, whereas charged amino acids may interfere with ppGalNAcT activity.

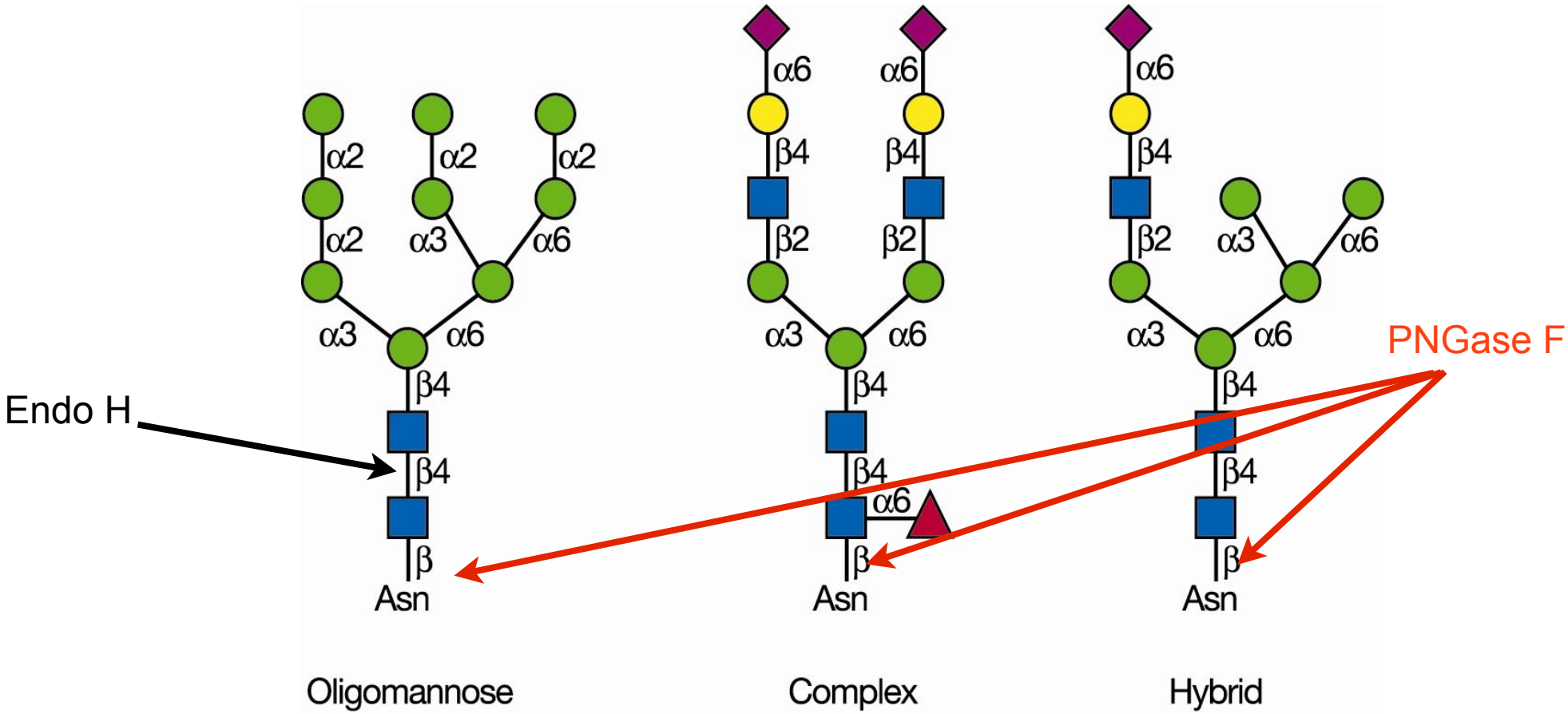
O-GalNAc Glycans with Different Core Structures



Glycan Analysis

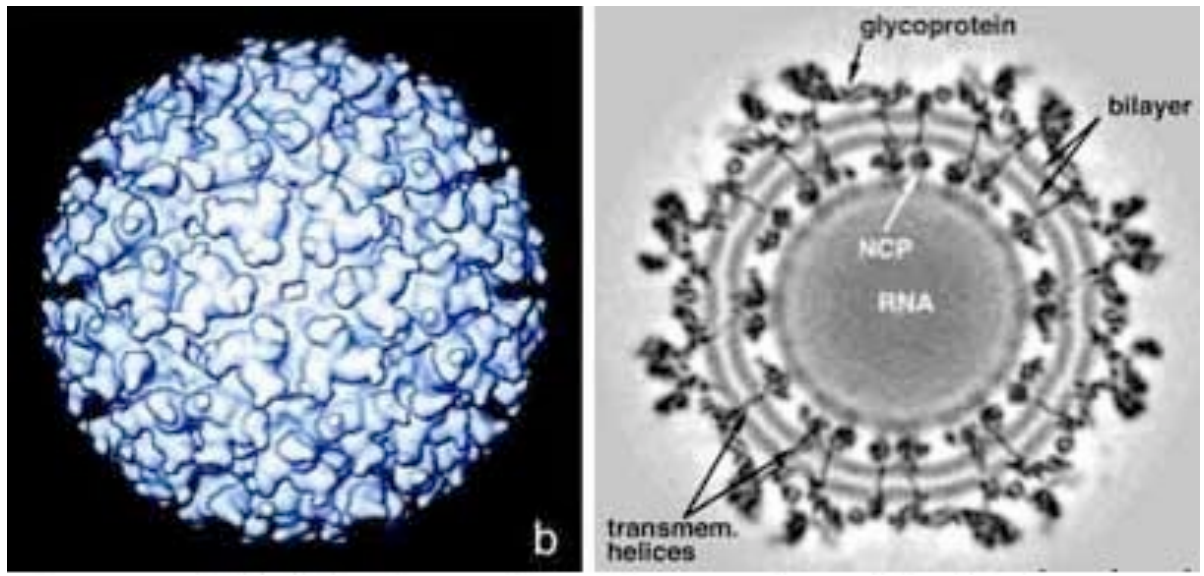
- Glycoproteins migrate as diffuse bands on SDS-PAGE due to the glycan heterogeneity
- Removal of N-linked glycans by treatment endoglycosidases: peptide-N-glycosidase F (PNGase F), endoglycosidase F2 (EndoF2) and endoglycosidase H (Endo H)
- Complete removal of N- and O- glycans can be achieved by chemical treatments (e.g., hydrazinolysis, or hydrogen fluoride treatment)
- O-Sialoglycoprotease can be used to remove clustered sialylated O-glycans

Types of N-linked Glycans



- PNGase F will hydrolyze all three types of N-linked glycans and results in a conversion of Asn to Asp
- Endo H will hydrolyze only oligomannose and some hybrids, leaving a single GlcNAc residue attached to the Asn

Viral Envelope Glycoproteins

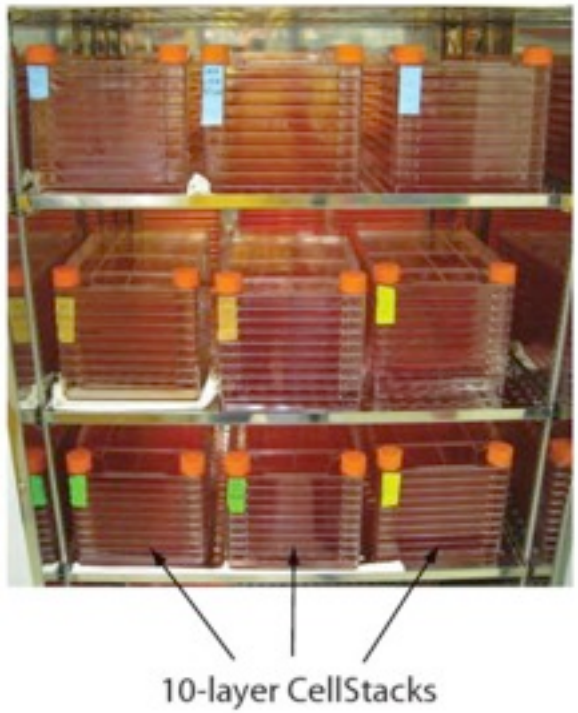


Zhang, Mukhopadhyay et al., 2002

Method to Grow Large-scale Mammalian Culture

roller bottles

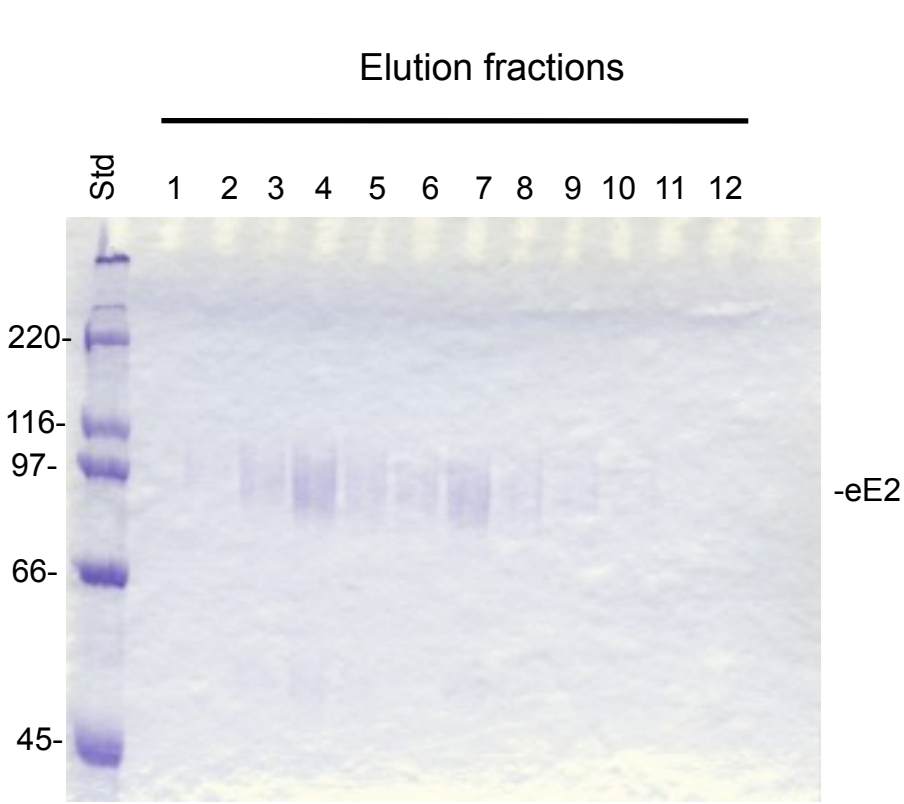
cell stacks



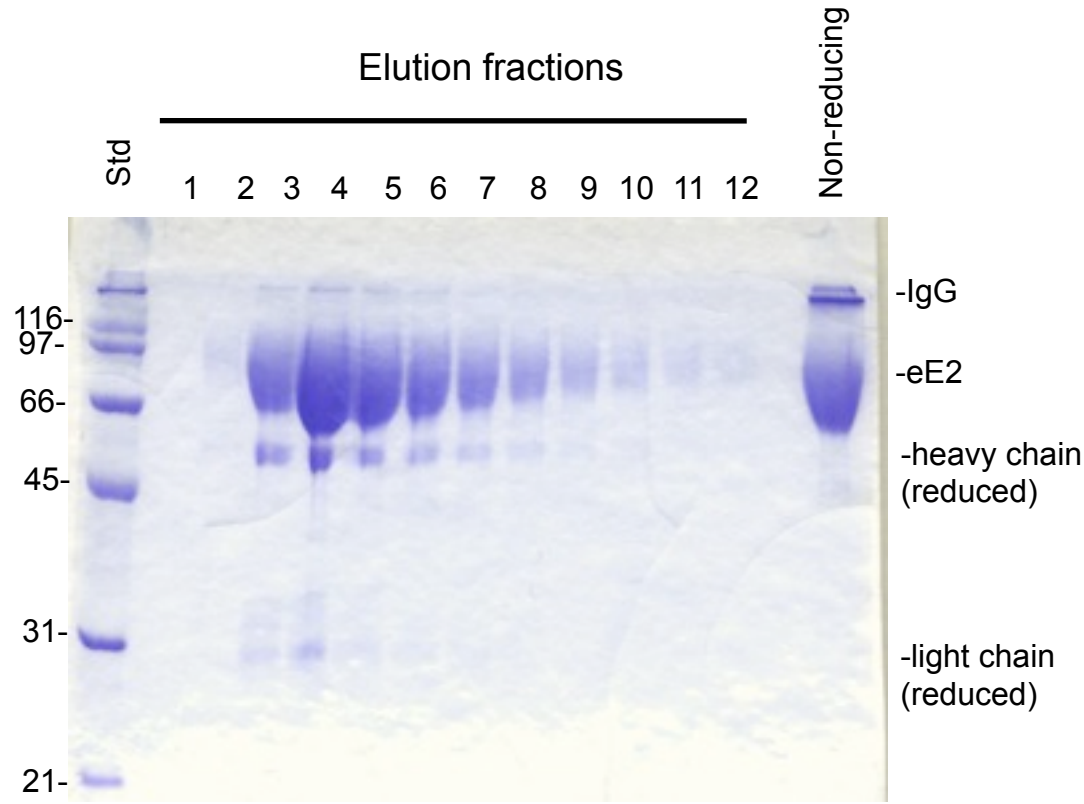
BelloCell

One BelloCell bottle has the surface area of 21 roller bottles or three cell stacks

Production and Purification of viral glycoprotein

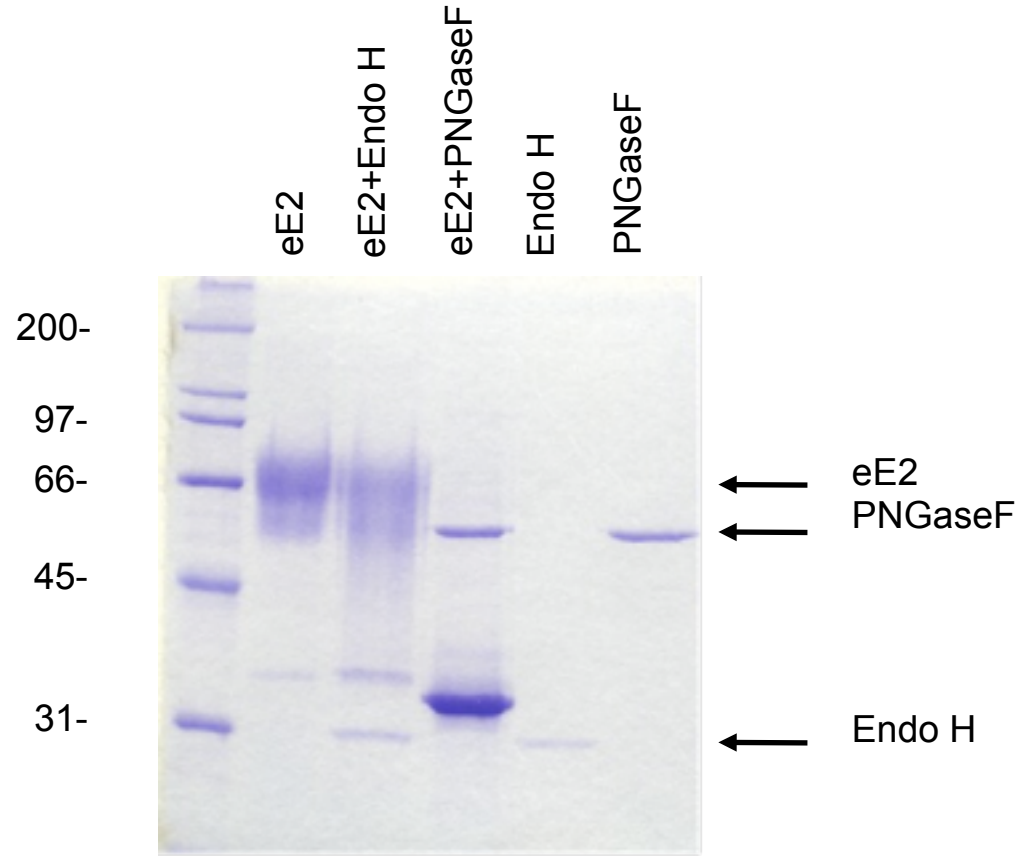


Yield = 0.1 mg/L



Yield = 20-30 mg/L

Deglycosylation



Denatured and reduced degylcosylation 37°C for 1hr