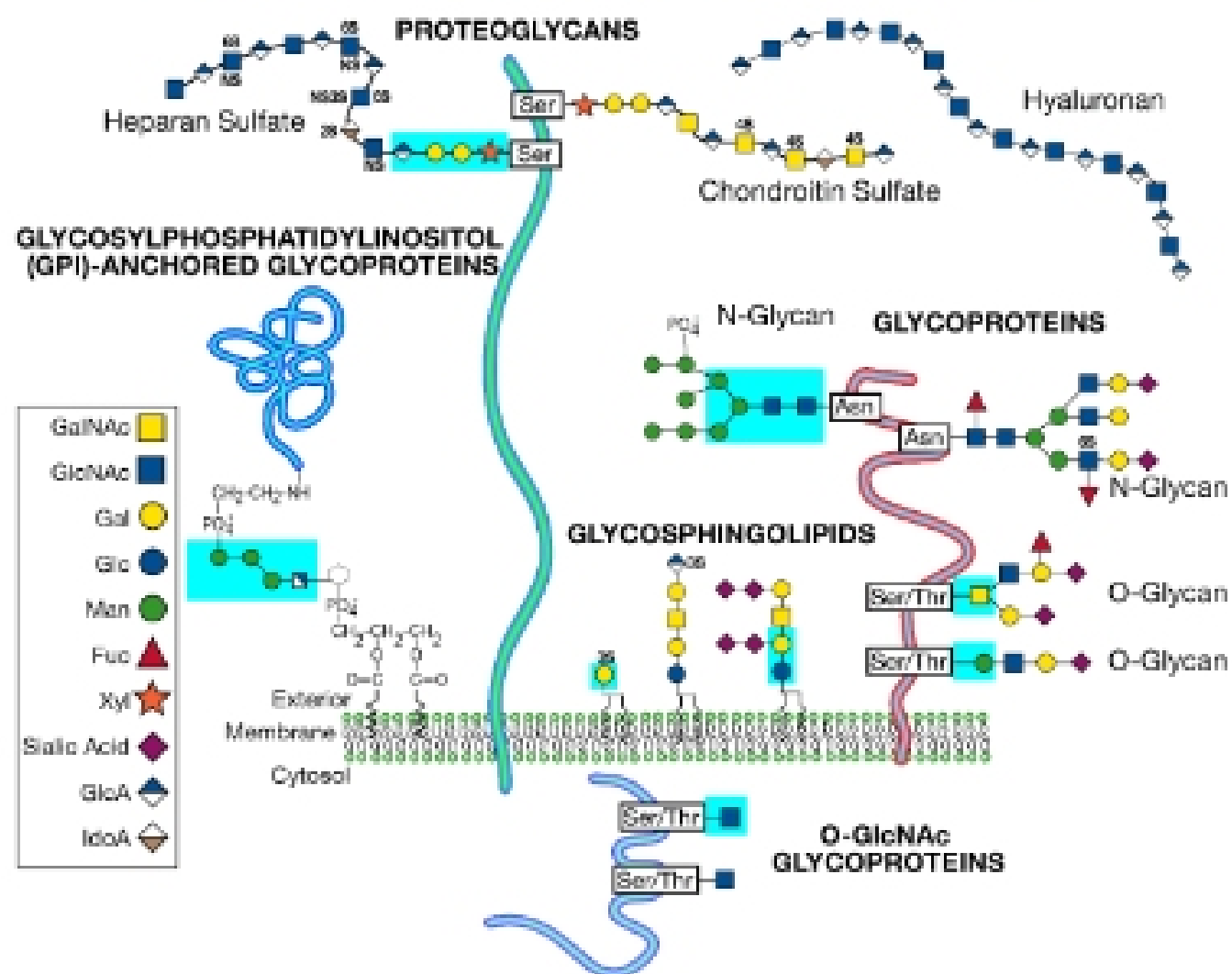


# N-Glycans

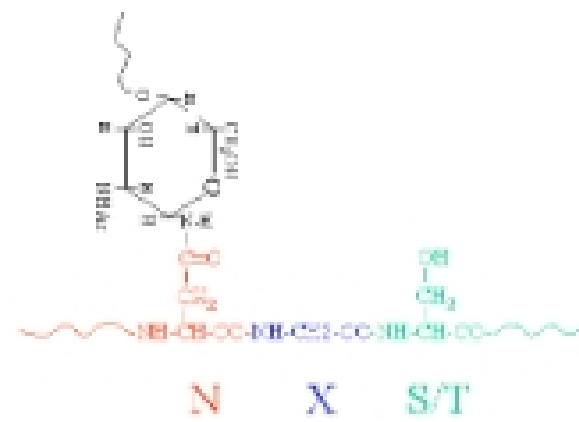
Dr. Lianchun Wang

## Common Classes of Animal Glycans

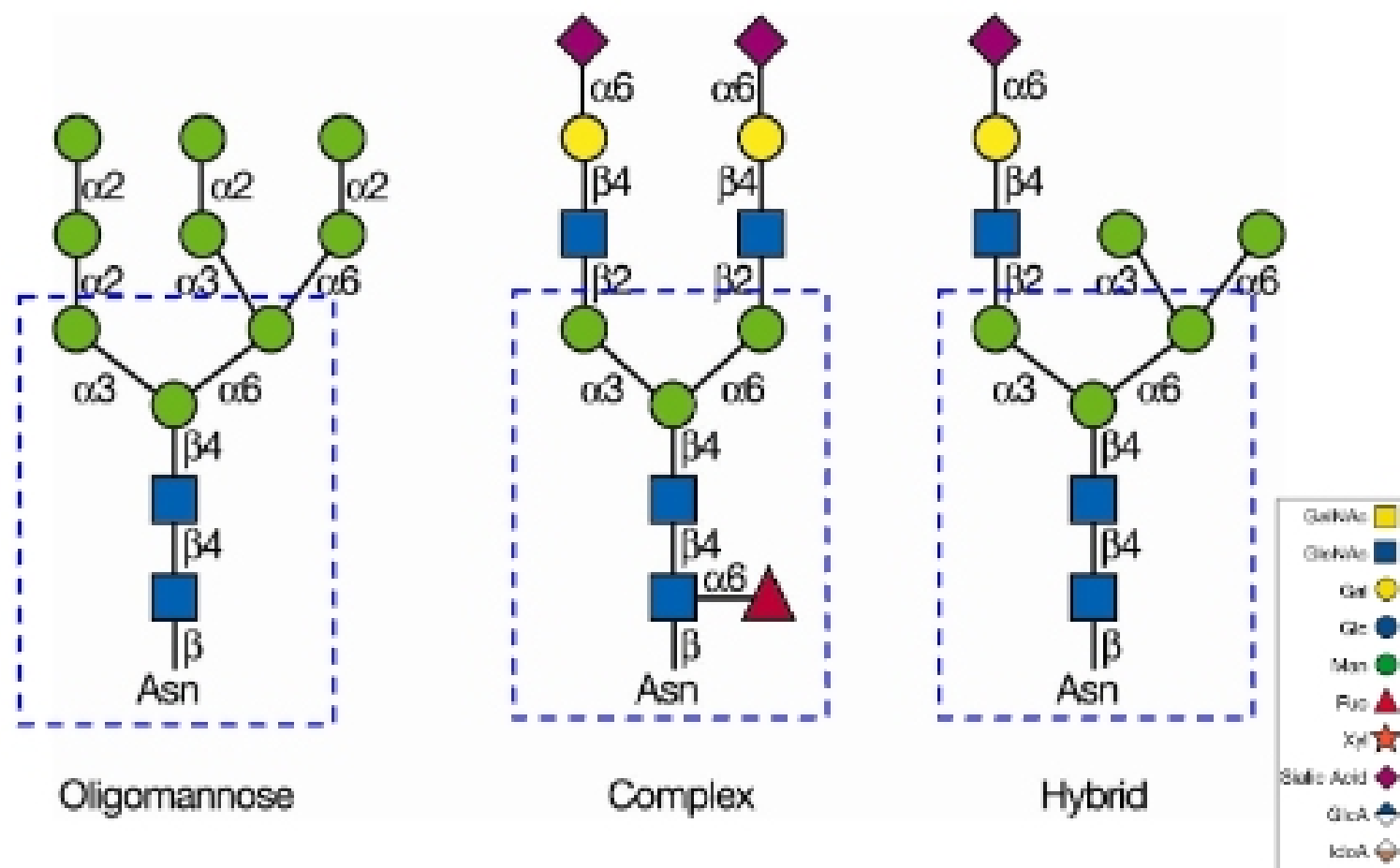


## N-glycans

- N-glycans are covalently attached to protein at asparagine (Asn) residues by an N-glycosidic bond
- Five different N-glycan linkages are known, of which N-acetylglucosamine to asparagine (GlcNAc! 1-Asn) is the most common
- Asn-X-Ser/Thr “sequons” in a protein are candidates for receiving an N-glycan
- Complicated biosynthesis
- Dolichol phosphate (Dol-P) as carrier for N-glycan biosynthesis
- N-glycan synthetic pathway is conserved in all of the metazoa, in plants, and in yeast
- Other linkages to Asn: glucose, N-acetylgalactosamine (GalNAc), rhamnose. And linkage to arginine: glucose
- N-glycans affect many properties of glycoproteins including their conformation, solubility, antigenicity, and recognition by glycan-binding proteins.
- Defects in N-glycan synthesis lead to a variety of human diseases



## Types of N-glycans



**Types of N-glycans.** N-glycans added to protein at Asn-X-Ser/Thr sequons are of three general types in a mature glycoprotein: oligomannose, complex, and hybrid. Each N-glycan contains the common core  $\text{Man}^{\text{II}} 1-6(\text{Man}^{\text{II}} 1-3)\text{Man}^{\text{I}} 1-4\text{GlcNAc}^{\text{I}} 1-4\text{GlcNAc}^{\text{I}} -\text{Asn}$  ( $\text{Man}_3\text{GlcNAc}_2\text{Asn}$ ).

## N-glycan Sites

- N-glycans occurs only on the Asn-X-Ser/Thr sequon
- About two thirds of protein contain the Asn-X-Ser/Thr consensus sequence. Among which more than two thirds of those sequons are likely to be N-glycosylated
- when Asn-X-Ser/Thr sequons are present in a deduced amino acid sequence encoded by a cDNA, they are not identified categorically as N-glycan sites, but are referred to as potential N-glycan sites. Proof that an N-glycan is actually present at a potential site requires experimental evidence
- Occasionally, N-glycans occurs at Asn-X-Cys
- The transfer of N-glycans to Asn-X-Ser/Thr sequons occurs on the luminal side of the endoplasmic reticulum (ER) membrane while the protein moiety is being synthesized on ER-bound ribosomes and is translocating through the translocon in the ER membrane

## N-glycan Isolation & Analysis

- Release
  - Peptide-N-glycosidase F (PNGase F): remove oligomannose, hybrid and complex N-glycan from ASN, but N-glycan core needs not to be modified.
  - PNGase A: remove all N-glycan from Asn
  - Endoglycosidase H: release oligomannose and hybrid N-glycans, but not complex N-glycans.
  - Endoglycosidase F: release simple biantennary N-glycans, but not oligomannose or hybrid N-glycans
  - Hydrazinolysis
  - Protease
- Purification and analysis
  - ion-exchange and size-exclusion chromatography, high-pressure liquid chromatography (HPLC) methods, and affinity chromatograph
  - composition, linkage and sequence