are very complex due to the glycan structures that are added to the protein skeleton. The protein glycosylation step occurs in the endoplasmic reticulum and the Golgi apparatuses. Glycosylation consists of branching on the protein, on determined amino acids (for instance, for N-glycosylation, asparagine [Asn] which is in the Asn–X–Thr sequence), sugar groups such as mannose, fructose, or galactose following well-determined orders. These glycosylation chemical reactions will lead to the making of *sugar chains*, more or less complex and diversified, considering all the possible attaching combinations (number of antenna(e) on a glycosylation site, and the nature of sugars making up this antenna), even if some mandatory sequences are found in each structure.

Finally, the end of the sugar chain is most often capped by a sialic acid in the form of neuraminic N-acetyl acid (NANA) in human cells; as for many mammals, a part of the sialic acid is in the form of neuraminic N-glycolyl acid (NGNA) because the gene which codes for the enzyme that allows the NANA form to become NGNA is muted and inactive in humans. This species specificity is important when choosing systems involving carbohydrate expression/production of the recombinant protein of interest, to ensure that the sialylation is as close as possible to the human form. The mature protein, so glycolyzed and more or less sialylated, gets some characteristics that are more or less acidic with a changed isoelectric point (pI). Consequently, at the end of PTMs, the protein appears not as a single entity but as a mix, a molecular population with the same basic protein structure (primary sequence imposed by gene sequence) on which various types of sugar chains will have been attached, giving each protein molecule its own pI. These series of isoforms are studied qualitatively and quantitatively using appropriate analytical techniques that separate the various isoforms such as based on their charge.

Since the glycosylation profile of a protein is critical in determining its activity, proteins are characterized by their pI value and by a series of visible and quantifiable bandwidths, by separation methods of isoelectrofocusing.

There are four types of glycosylation links:

- N-linked glycosylation: N-linked glycosylation is the most common type of glycosidic bond and is necessary for the folding of some eukaryotic proteins and for cell–cell and cell–extracellular matrix attachments. The N-linked glycosylation process occurs in eukaryotes in the lumen of the endoplasmic reticulum and widely in archaea, but very rarely in bacteria.
- O-linked glycosylation: O-linked glycosylation is a form of glycosylation that not only occurs in eukaryotes in the Golgi apparatus, but also occurs in archaea and bacteria. Xylose, fucose, mannose, and N-acetyl glucosamine (GlcNAc) phosphoserine glycans have been reported in the literature.