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 qualitatively and quantitatively studied using appropriate analytical techniques that separate the various isoforms, based on their charge, for example.

Since the glycosylation profile of a protein is important 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 important for the folding of some eukaryotic proteins and for cell–cell and cell–extracellular matrix attachment. 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 occurs in eukaryotes in the Golgi apparatus [6] but also occurs in archaea and bacteria. Xylose, fucose, mannose, and GlcNAc phosphoserine glycans have been reported in the literature.
- C-mannosylation: A mannose sugar is added to the first tryptophan residue in the sequence W-X-X-W (W indicates tryptophan and X is any amino acid). Thrombospondins are one of the most commonly C-modified proteins; however, this form of glycosylation appears elsewhere as well. C-mannosylation is unusual, because the sugar is linked to a carbon rather than a reactive atom such as nitrogen and oxygen. Recently, the first crystal structure of a protein containing this type of glycosylation has been determined—that of human complement component 8, PDB ID 3OJY.
- Formation of glycophosphatidylinositol (GPI) anchors (glypiation): A special form of glycosylation is the formation of a GPI anchor. In this kind of glycosylation, protein is attached to a lipid anchor, via a glycan chain.

Glycanic structures are obtained by combining the sugar group's nature Gal = Galactose, Man = Mannose, Fuc = Fucose, glcNAc = *N*-acetyl glucosamine and its organization in antennae (mono, bi, or even tri-antennae). Let us also note the presence of a "sialic acid" group that sometimes caps the antennae's ends. The sialic acid groups notably contribute to the protein molecule's half-life.

Posttranslational modifications, usually illustrated by the glycosylation profile, are intrinsic quality criteria of the protein, as well as critical parameters to consider during the assessment of the production process and its reproducibility, notably when changes are introduced in the production method, and a fortiori, when a new manufacturer offers a "biosimilar" version of a reference protein.

Indeed, for the new producer of a given glycosylated protein, one could fear an isoform distribution different from that of the original molecule. This different isoelectric profile, which is often difficult to distinguish by the only analytical methods offered by the manufacturer, will potentially have an impact on the pharmacokinetics or the biological activity of the therapeutic protein. Then, it will be the pharmacological and/or clinical data that will reveal the sometimes-subtle change in the isoform distribution when the quality control analytical data are detecting no noticeable difference.