hydrolysis. Asp–Y sequence has been reported to be 100 times more sensitive to hydrolysis than any other peptide bonds. At times, hydrolysis is the natural, subsequent reaction following deamidation of Asn residue, as is observed with insulin.

Furthermore, mAbs may contain certain residues that are sensitive to oxidation, such as Met, Tyr, Trp, His, and Cys. Although not as prevalent as deamidation and isomerization, oxidation has been reported to be the major pathway of degradation for certain proteins, e.g., OKT3 (IgG2), which demonstrated oxidation at several Met residues and free Cys under 5°C storage condition.

Several external factors induce oxidation, including exposure to light, contamination with a trace amount of transition metal ion, and presence of degradation product of an excipient (e.g., hydrogen peroxide from polysorbate degradation). The consequence of oxidation may include an increase in the aggregation. Oxidation is most commonly observed with the Met residue, and the major by-product of such reaction is the formation of sulfoxide and sulfone. Two factors that affect the rate of oxidation are the local structure around the oxidation-sensitive group (e.g., surface exposure and steric hindrance) and the solution pH. In some cases, an increase in solution pH has been found to increase the rate of oxidation, but the pH-dependent increase in oxidation is not a general phenomenon.

Protein oxidation is a covalent modification induced by reactive oxygen intermediates or other oxidants such as chemicals such as hydrogen peroxide (which often appears in formulations as a contaminant of polysorbates, leaching from disposable tubing, etc.), oxygen, metal ions, and other excipients. UV light is another major factor requiring proteins to be protected from light. Oxidation results in the modification of Met, Cys, Trp, His, and Tyr residues, which are primary formulation development relevant residues.

Photooxidation can change the primary, secondary, and tertiary structures of proteins and can lead to differences in long-term stability, bioactivity, or immunogenicity. Exposure to light can trigger a chain of biochemical events that continue to affect a protein even after the light source is turned off. These effects depend on the amount of energy imparted to a protein and the presence of environmental oxygen. Photooxidation is initiated when a compound absorbs a certain wavelength of light, which provides enough energy to raise the molecule to an excited state. The excited molecule can then transfer that energy to the molecular oxygen, converting it to reactive singlet oxygen atoms. This is how tryptophan, histidine, and tyrosine can be modified by light in the presence of oxygen. Tyrosine photooxidation can produce mono-, di-, tri-, and tetrahydroxy tyrosine as by-products. Aggregation is observed in some proteins due to cross-linking between oxidized tyrosine residues. Metal ion–catalyzed oxidation depends on the concentration of metal ions in the environment. The presence of 0.15 ppm chloride salts