bonds involve the interaction of amino acid side chains and the polypeptide chain. Since the transition from a polypeptide chain to HOS requires a significant loss of entry (structuring), it must be compensated for by enthalpy released from the forming of a bond (energy is released when a bond is formed); as a result, the protein structure can remain a dynamic state of structuring that may affect its activity as well as its stability. In most instances, the changes are transitory, and the protein returns to its native structure. However, the possibility of dynamic changes to a protein structure makes it possible for a molecule to have a different activity if its physicochemical properties are altered; additionally, if there is aggregation, this may lead to a loss of activity and a likely increase in the immunogenicity of the protein. Protein aggregation is caused by two factors: colloidal and conformational stabilities. The attractions on the surface of proteins can make colloidal dispersions that can be dynamic and significantly affect the safety and the effectiveness of proteins under stress conditions; the conformational changes are brought about by the hydrophobic interactions of the buried functional groups. There is a likelihood of both types of aggregates and, in some instances, one leads to another. So far, the regulatory authorities have not focused on these differentiations but over time, it is likely that these would be included as part of the risk analysis of the manufacturing process.

There is also a likelihood of aggregation due to molecular crowding when the drug is exposed to a high concentration of other proteins in the plasma. Would it ever be a requirement to study the nature of a circulating protein drug? This remains to be seen. However, a recent trend in the reformulation of proteins like adalimumab and rituximab in high-concentration formulations is an alarming trend; motivated by intellectual property (IP) protection as these drugs come off patent, the originators are reformulating their products, without fully realizing that the molecular crowding at the site of administration, if not in the vial or the syringe, is likely to increase the aggregation potential. The regulatory agencies should require a demonstration of safety at this level when a formula change request is made. This aspect of safety consideration is a topic of a citizen's petition filed by the author with the FDA.

The HOS a protein takes is intrinsically dictated by its primary structure and posttranslational modifications (PTMs). In the 1960s, Cyrus Levinthal proposed an interesting observation regarding protein folding. In a 100-residue protein, allowing 5×10^{47} conformations possible and if each confirmation takes 1 ps, it will take 10^{18} times as long as the age of the universe. It is truly amazing how a protein HOS is repeatedly formed approximately the same way, even if there are a few defects left in the primary structure. It is this possibility of variability that makes the development of biosimilar products challenging. Misfolded proteins often reach a stage of energy level that may be difficult to overcome and return them to their native state—the