conditions under which proteins are manufactured can significantly alter this profile.

Protein synthesis involves a complex array of cellular machinery, primarily ribosomes. Proteins are synthesized from the N-terminus to the C-terminus in a sequential manner at a rate of 50–300 amino acids/minute; the folding begins once the chain has acquired 50–60 amino acids—cotranslational protein folding that constraints and limits the pathways a protein can take into HOS, and this may explain why Levinthal's Calculations come short.

Chaperones are proteins that help other proteins fold correctly in addition to proteolytic apparatus available in the cells.

There are some proteins that have no well-defined HOS. These are disordered or unstructured random coils, like the synthetic polymer chains or denatured proteins. This state may be a transitory state during the binding process and may be responsible for a multitude of protein actions in the cell. This intrinsic disorder creates a challenge to the demonstrate structure–function relationship, and whereas these aspects are not yet recognized by the regulatory authorities, it is only a matter of time when the biosimilar product developers may be required to demonstrate the disordered state comparisons as well—that will significantly raise the bar on the development of biosimilar products.

There are two types of proteins that can be labeled as *unnatural* construction—the fusion proteins or the conjugate (e.g., pegylated) proteins and very a large assembly of virus particles or nanoparticle delivery systems. The fusion of an Fc part of an antibody (typically an immunoglobulin G1 [IgG1] antibody) with that of another pharmaceutically relevant protein through recombinant genetic technology results in fusion proteins. The Fc portion of an antibody increases the circulation time just as does the pegylation; examples include fusion of Fc to the blood-clotting factor VIII and factor IX. In June 2014, the FDA approved Eloctate, antihemophilic factor (recombinant), Fc fusion protein, for use in adults and children who have hemophilia A. Eloctate is the first hemophilia A treatment designed to require less frequent injections when used to prevent or reduce the frequency of bleeding. In March 2014, the FDA approved Alprolix, coagulation factor IX (recombinant), Fc fusion protein, which is a recombinant DNA–derived coagulation factor IX concentrate. It temporarily replaces the missing coagulation factor IX needed for effective hemostasis. Etanercept is a fusion protein produced by recombinant DNA technology. It fuses the TNF receptor to the constant end of the IgG1 antibody. First, the sponsors isolated the DNA sequence that codes the human gene for soluble TNF receptor 2, which is a receptor that binds to TNF-alpha. Second, they isolated the DNA sequence that codes the human gene for the Fc end of IgG1. Third, they linked the DNA for TNF receptor 2 to the DNA for IgG1 Fc. Finally, they expressed the