growth hormone have since demonstrated great therapeutic value. With the entrance of recombinant technology in the late 1970s it became possible to design analogs of natural proteins in expression systems like *E. coli*, yeast, or various mammalian cells. It was now possible to make mutations in a protein, or, native-like post-translational modifications such as glycosylation, giving rise to protein analogs with altered and/or improved properties. Rapid acting insulin analogs like insulin aspart or insulin lispro were the first protein analogs to enter the market. They were engineered so one or two mutations in the dimer interface destabilized the dimer formation leading to preferentially monomeric insulin which in turn led to a more rapid acting analog than native insulin.

In the early 1990s, high-throughput expression technologies such as the phage technology that could display billions of peptides and later even larger proteins such as antibodies were developed and this accelerated the discovery of protein analogs with improved or completely new pharmaceutical properties.

Protein–protein interactions (PPIs) are fundamental to any living organism and modulating these interactions can have profound importance. With the introduction of various humanized antibody screening technologies it quickly became feasible to identify antibodies that could interfere as PPI inhibitors and many blockbuster protein drugs today are based on this approach. However, although we recognize that antibodies and other domain scaffolds are some of the most important biopharmaceuticals on the market, they are beyond the scope of this Chapter. Rather, we will focus on ways to improve efficacy of naturally occurring peptides and proteins. The success of today's biopharmaceuticals is reflected by the fact that in 2013 among the top 20 top selling drugs 11 were proteins or peptide-based drugs.

9.1.2 Aspects of Peptide and Protein Drug Design

The design of a peptide or protein drug candidate needs to take a variety of different aspects into consideration in order to lead to a successful drug candidate. Thus, during the design process, a number of important factors that modulate the protein properties must be considered such as

1. Potency
2. Selectivity
3. Distribution
4. Elimination
5. Route of administration
6. Formulation
7. Toxicity and immunogenicity
8. Production process

In general, highly potent proteins and peptides that circulate in a low, picomolar range are much more specific than small molecules to the same target. This is of particular importance if homologous receptors are present, as in the case of the insulin and the insulin-like growth factor 1 (IGF-1) receptor, or, in the case of the neuropeptide Y (NPY) receptor family where four receptors Y1, Y2, Y4, and Y5 necessitate an even greater awareness concerning selectivity issues, since these are distributed across many organs and in the central nervous system (CNS) as well.

The half-life or rate of elimination is also an important parameter that affects efficacy. Elimination may be caused by a rapid receptor clearance, enzymatic instability, or renal clearance which rapidly removes peptides and proteins that are less than 60 kDa. Increasing half-life by modifying the size of the peptide or protein, or implementing other changes to make them less prone to proteolysis, is a major focus area in the engineering of therapeutic peptides and proteins.

The distribution and route of delivery of biopharmaceuticals versus small molecules is also very different. Intracellular targeting is generally reserved for small molecules since peptides and proteins do not readily pass the cell membrane, and most proteins likely do not cross the blood–brain