PREFACE

These two volumes of *Methods in Enzymology* cover engineering approaches to the development of protein biopharmaceuticals, which represent a significant and rapidly growing proportion of drug sales. Particular advantages of proteins as drugs relative to small organic molecules include high affinity and specificity afforded by a larger molecular recognition surface and much lower probability of off-target toxicities due to metabolic byproducts. The primary disadvantage to date has been the pharmacokinetic inaccessibility of intracellular drug targets to proteins and peptides, although vigorous efforts at overcoming this limitation are beginning to bear fruit.

The protein biopharmaceutical field was born with the advent of recombinant DNA expression systems for natural human protein agonists such as insulin, human growth hormone, erythropoietin, and granulocyte colony-stimulating factor. Humanization of mouse antibodies opened the playing field to novel molecules that specifically bound to and blocked receptors and ligands important in a variety of diseases. In vitro directed evolution technologies have enabled further exploration of nonnative structures and topologies for target antagonism or delivery of therapeutic payloads. It should be pointed out that to date protein engineering has made minimal progress in engineering the agonists which were the first protein biopharmaceuticals. Such engineered agonists could enable more subtle redirection of innate homeostatic regulatory pathways than the relatively crude tools of antibody antagonism or parenteral oversupply of naturally occurring protein and peptide agonists.

We provide here only an overview representing the wide spectrum of approaches in this field. The first section covers some aspects of antibodies, by far the dominant class of protein biopharmaceuticals at the present time. The second section provides examples where protein targeting is exploited to deliver a payload conjugated to the proteins. In the third section of the first volume, examples of engineered therapeutic enzymes are provided. In the first section of the second volume, peptides are considered, enabling chemical synthesis and more facile intracellular delivery, given their smaller size. The second section describes a number of leading efforts to engineer molecular recognition onto a scaffold other than that of an antibody. In the third and final section, pharmacokinetics of protein drugs is discussed with

respect to delivery to tumors, intracellular targets, and across the blood–brain barrier.

We particularly thank the authors of these contributions for their thorough and clear exposition of the state of the art in their respective specializations.

K. Dane Wittrup and Gregory L. Verdine