Preface

In this post-genomic era, emphasis continues to be placed on the derivation of genetic information from the simplest to the most complex of organisms. Recent advancements in DNA sequencing technology, specifically with regard to speed, cost, and overall availability, promise a future that is awash with novel genomic information—information that will not only expand our knowledge of the processes that govern life, but also fuel innovation that will undoubtedly conquer many of today's most salient health and environmental challenges. Though emphasis is often placed on the "gene," it is the encoded product—the "protein"—that actually serves any real biochemical function. Compared to genes, which themselves hold information and more so describe potential function, proteins are the actual tangible components of the cellular machinery, carrying out the multitude of functions that support life, whether biochemical or structural. Thus, to fully understand and appreciate any biological process, one must take a hard look at the proteins involved, taking note of the exquisite functional interplay between themselves and amongst other biomolecules.

Given the intrinsic DNA–protein link and coupled with the routine use of molecular cloning/recombinant DNA technology, today's researchers can easily alter the amino acid sequence of any given protein. This powerful technology allows for precise engineering of specific proteins and enables unprecedented inquiry into both their structures and function. A common technique utilized by many molecular biologists interested in probing protein function involves genetically grafting a protein or short peptide sequence onto one or both terminal ends of a given protein of interest. By virtue of the appended protein/peptide sequence, this new recombinant, "tagged" protein can be easily purified by one of several routine methods. Aptly named, protein affinity tagging employs the use of known protein binding interactions in order to "fish out" a protein of interest or, with more advanced tags, a protein complex.

The intent of this collection is to provide researchers with the necessary information, tools, and strategy needed to properly interrogate a given protein's function. The development and use of recombinant affinity tags has been paramount in this endeavor, providing an efficient means to purify a protein of interest for downstream functional assessment. This volume is not intended, however, to be an all-inclusive listing of affinity tags but rather attempts to highlight the general strategies employed by researchers who use this technology on a daily basis. In fact, more emphasis has been placed on advanced affinity tagging procedures that introduce novel purification techniques. As follows, purposefully missing are chapters that focus on the one-step purification of proteins employing commonly used affinity tags *without* additional novelty (e.g., visualization, solubility enhancements, or enhanced tag removal strategies). These simple affinity tags have been covered *ad nauseam* in the literature and most are optimized, detailed, and readily available through commercial means. Furthermore, single tag purification methodologies can be easily extrapolated from the included chapters as many include these often used affinity tags packaged into more advanced constructs.

With regard to the volume at hand, the included chapters are roughly divided into tag constructs that feature one purification event and those that feature two or more. Though

engineering proteins with a single affinity tag is routine and generally useful for purifying ample amounts of recombinant protein, issues with proper folding, solubility, and localization often arise. Many of the chapters included here provide means to circumvent these shortcomings. Likewise, single tag-based strategies are sometimes afflicted with purity issues. Often, this is of no consequence. When purity does matter (i.e., certain sensitive applications such as LC-MS/MS-based identification of interacting protein partners) tagging proteins with multiple affinity motifs/domains provides enhanced specificity but at the expense of reduced protein recovery. Finally, readers will notice that many affinity-tag constructs include other features that aid in the purification process. These features can be grouped into three major categories: tags that enhance protein solubility, tags that include motifs/proteins/domains that allow for protein visualization, and tags that can be removed from the final purified product, either via the targeting of proteases or those that are self-cleavable.

Whatever the needs of the researcher, there is most likely an affinity tag available to accomplish the task at hand—they come in virtually every flavor, differing in size, complexity, binding/bait capture affinities, and types of interaction (i.e., antibody–antigen/epitope, enzyme–substrate, receptor–ligand, coordination of amino acid residues and metals, etc.). It is our hope that the collection of methodologies listed here will be an invaluable resource to those seeking to utilize protein affinity tags to study their biological system of interest.

Oak Ridge, TN, USA — *Richard J. Giannone*
West Greenwich, RI, USA — *Andrew B. Dykstra*

http://www.springer.com/978-1-4939-1033-5

Protein Affinity Tags
Methods and Protocols
Giannone, R.J.; Dykstra, A.B. (Eds.)
2014, X, 213 p. 46 illus., 31 illus. in color., Hardcover
ISBN: 978-1-4939-1033-5
A product of Humana Press