

Unit 9.9: Overview of Affinity Tags for Protein Purification

Michelle E. Kimple^{1*} and John Sondek^{1,2†}

¹Department of Biochemistry and Biophysics, Program in Molecular and Cellular Biophysics, ²Department of Pharmacology, and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

* Current address:

Department of Pharmacology and Cancer Biology
Box 3613
Duke University Medical Center
Durham, NC 27710
Phone: (919) 613-8615
Fax: (919) 613-8642
e-mail: pliskemi@duke.edu

† Corresponding Author

Department of Pharmacology
CB# 7365
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: (919) 966-7530
Fax: (919) 966-5640
e-mail: sondek@med.unc.edu

Keywords: protein purification, protein detection, affinity chromatography, affinity tag, epitope tag

Diskette Information: Current Protocols Affinity Tags.doc, prepared with IBM Microsoft Word XP

ABSTRACT:

Addition of an affinity tag is a ubiquitous method for differentiating recombinant proteins expressed in bacterial and eukaryotic expression systems from the background of total cellular proteins, and for detecting protein-protein interactions. The material in this chapter describes the historical basis for the development of affinity tags, affinity tags that are commonly used today, how to choose an appropriate affinity tag for a particular purpose, and several recently-developed affinity tag technologies that may prove useful in the near future.

SECTION 9.1: HISTORICAL OVERVIEW

The first affinity tags used were large proteins utilized almost exclusively for protein expression and purification in *E. coli*. Staphylococcal Protein A is 280 amino acids in length, and because of its size and proteolytic stability can increase the solubility and/or expression of heterologous proteins (Sambrook et al. 1989). Protein A fusions can be purified by affinity chromatography to IgG sepharose and eluted by a low pH buffer. The large size of the tag and/or the low pH elution can alter the functional activity of the fusion protein. In addition, the binding of fusion proteins to IgG complicates immunological analysis, and thus Protein A fusions are unsuitable for use as immunological reagents.

LacZ (a.k.a. β -galactosidase or β -gal), a proteolytically-stable 1024 amino acid protein, can also be used as an affinity tag to increase expression of fusion proteins in *E. coli* (Sambrook et al. 1989). LacZ fusions can be purified by substrate affinity chromatography to p-amino-phenyl- β -D-thiogalactosidase (APTG) and eluted with a high pH borate buffer, as well as detected using a colorimetric enzymatic assay. Because LacZ is relatively immunologically neutral, it is a useful fusion for the generation of antibodies to a protein of interest. The LacZ

affinity tag can alter the functional activity of the purified fusion protein because of its extremely large size and predominance to form homotetramers in solution. In addition, LacZ fusion proteins are often insoluble. This insolubility has advantages and disadvantages: Protein targeting to inclusion bodies can allow the expression of gene products that are toxic to *E. coli*, but because LacZ must be correctly folded to bind APTG, LacZ fusion proteins must be refolded prior to purification and/or detection.

SECTION 9.2: COMMONLY-USED AFFINITY TAGS

Although Protein A and LacZ are still used as affinity tags today (see Table 9.2.1 for commercially-available systems), many affinity tags have more recently been developed that improve upon these original protein fusion partners.

Polyhistidine

The polyhistidine affinity tag (a.k.a. His-tag or His₆) usually consists of six consecutive histidine residues, but can vary in length from 2 to 10 histidines. Polyhistidine was first used to purify recombinant galactose dehydrogenase by immobilized metal affinity chromatography (IMAC) in 1991 (Lilius et al. 1991). Previous to this, IMAC had been used to purify proteins that contained naturally-occurring histidine or tryptophan-containing sequences, but these sequences were not as generally applicable to protein expression and purification as would be desired for an affinity tagging system (Smith, Furman et al. 1988; Ljungquist et al. 1989).

Polyhistidine is such a ubiquitous affinity tag that most companies providing expression vectors or protein expression and purification reagents offer products related to the polyhistidine tag (see Table 9.2.1 for examples). Histidine readily coordinates with an immobilized transition metal

ion. Immobilized Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Ca^{2+} , and Fe^{3+} can all be used to purify polyhistidine fusion proteins, but Ni^{2+} is the most commonly used. Empirical determination of the most effective transition metal ion for purification of a specific polyhistidine fusion protein can be performed if purification by Ni^{2+} is unsatisfactory. There are several companies that offer IMAC resin. Iminodiacetic acid (chelating sepharose, Amersham Biosciences), nitrilotriacetic acid agarose (Ni-NTA resin, Qiagen), and carboxymethylaspartate agarose (Talon resin, Clontech), are all used for the immobilization of transition metal ions. Commercially-available resins are unaffected by protease or nuclease activity, and are appropriate for purification of fusion proteins from crude cell lysates. Most resins can be regenerated and reused indefinitely. Although polyhistidine does not usually cause a protein to be targeted to *E. coli* inclusion bodies, IMAC is amenable to denaturing agents (8 M urea, 6 M guanidine HCl, ionic and non-ionic detergents, and low concentrations of reducing agents) for the purification of insoluble or membrane-bound proteins. High concentrations of reducing agents such as dithiothreitol (DTT) can reduce the immobilized metal ion and should be avoided. The relatively small size and charge of the polyhistidine tag rarely affects protein function, and elution by imidazole gradient is relatively mild, preserving the immunogenicity of polyhistidine fusion proteins.

While purification of a highly-expressed polyhistidine fusion proteins can lead to relatively pure protein in one chromatographic step (> 80% pure), purification from insect and mammalian cells, which contain a higher percentage of His residues in their proteins than *E. coli* cells, can lead to significant background binding to immobilized metal ions. This may be circumvented with stringent wash conditions (5-10 mM imidazole), although a stringent wash may cause premature elution of the protein of interest. The location of tag (N-terminal, C-

terminal, or internal) can also have effect on IMAC. If a change in terminus does not increase the effectiveness of IMAC, a denaturing purification can be attempted.

Primary antibodies have been developed for the detection of polyhistidine fusion proteins *in vitro*. Again, because of the predominance of histidine residues in mammalian and insect systems, anti-polyhistidine antibodies are notoriously promiscuous. Ni²⁺ resin can also be used to pull-down a polyhistidine-tagged protein, for the detection of protein-protein interactions.

Glutathione S-transferase (GST)

The pGEX *E. coli* expression vectors, which encode for N-terminal GST molecules followed by protease cleavage sites, were first designed in 1988 and used to express and purify antigens of the parasite *Taenia ovis* (Smith and Johnson 1988; Smith 2000). Currently, pGEX vectors are available from Amersham Biosciences in all three reading frames and with three different protease cleavage sites—thrombin, factor Xa, and PreScissionTM. GST fusion proteins can be purified by affinity chromatography to commercially-available glutathione (γ -glutamylcysteinylglycine) sepharose, which is affected by γ -glutamyl transpeptidase activity in crude cell lysates. Therefore, glutathione resin has a finite lifetime and can only be regenerated and reused between 4 and 20 times. Glutathione affinity chromatography is amenable to low concentrations of denaturing agents (2-3 M urea or guanidine hydrochloride), reducing agents (<10 mM β -mercaptoethanol or dithiothreitol), and non-ionic detergents (2% Tween 20), depending on the nature of the fusion protein. Elution of GST fusion proteins with 10 mM glutathione is relatively mild, often preserving protein function and antigenicity. A 70-kilodalton *E. coli* heat-shock-induced chaperonin often co-purifies with eluted GST fusion proteins. This contaminant can be removed by treatment of cell lysates with 5 mM magnesium chloride and 5

mM ATP prior to purification. Furthermore, GST can be cleaved from its fusion protein while still bound to glutathione agarose, providing a convenient method for separating the 26 kilodalton GST from the protein-of-interest.

GST fusion proteins are often expressed at high levels in *E. coli* (typical yields ~10 mg/L), which may result in accumulation of aggregated protein in inclusion bodies. Purification from inclusion bodies has both advantages and disadvantages. Some advantages are that protein targeting to *E. coli* inclusion bodies allows the high-level expression of toxic genes, and the separation of inclusion bodies serves as a significant purification step from whole-cell lysate. Unfortunately, since glutathione affinity chromatography depends on the proper three-dimensional fold of GST, insoluble fusion proteins must be refolded and buffer exchanged before purification. Some insoluble proteins may not refold solubly and/or correctly, though. Another potential disadvantage of the GST tag is that the large size of tag and its dimerization in solution may affect the properties of the fusion protein.

GST fusion proteins can be detected by a colorimetric assay with the GST substrate 1-chloro-2,4-dinitrobenzene (CDNB) or with anti-GST antibodies. GST-pulldown assays with glutathione-coupled beads are commonly used for the detection of protein-protein or protein-DNA interactions.

Maltose binding protein (MBP)

The pMAL *E. coli* expression vectors were designed in 1988 (di Guan et al. 1988; Maina et al. 1988). MBP is often used to increase the expression level and/or solubility of its fusion partner, with typical yields of 10-40 mg fusion protein per liter of culture. pMAL vectors are available for cytoplasmic or periplasmic expression in all three reading frames, with factor Xa,

enterokinase, or gene I protease cleavage sequences (New England Biolabs). Other MBP fusion vectors include pIVEX (Roche), which can be used for coupled *in vitro* transcription/translation.

MBP fusion proteins can be purified by affinity chromatography to cross-linked amylose resin. Amylose resins are commercially-available, but are affected by amylase activity in crude cell lysates and can be regenerated and reused only 3 to 5 times. Adding glucose to bacterial growth media helps suppress amylase expression. Amylose affinity chromatography is not amenable to denaturing or reducing agents. Low concentrations of non-ionic detergents (0.2% Triton-X or Tween 20) may be used depending on the nature of fusion protein.

Like GST fusion proteins, high-level expression of MBP fusion proteins in *E. coli* may result in the accumulation of insoluble protein aggregates in inclusion bodies. The large size of the MBP tag (45 kD) may also affect protein function. Unlike glutathione affinity chromatography, proteolytic cleavage of the tag while bound to amylose resin is not effective, and the fusion protein must be eluted by free maltose before cleavage. The 10 mM maltose elution is mild, though, which often preserves protein function and antigenicity. If proteolytic removal of the MBP tag is performed, free maltose must be removed from MBP by an additional chromatographic step if MBP is to re-bind the amylose column. Anti-MBP antibodies are available for the detection of MBP fusion proteins.

Intein-chitin binding domain (CBD)

The intein-CBD tag is a combination of a protein self-splicing element (intein) with a chitin binding domain, allowing for the purification of a native recombinant protein without need for a protease. Intein-CBD *E. coli* expression vectors were designed in 1997 (Chong et al. 1997).

Commercially-available vectors provide for intein-CBD expression on the N-terminus, C-terminus, or both termini of a heterologous protein-of-interest (IMPACT system, New England Biolabs).

CBD fusion proteins are purified by affinity chromatography to chitin resin.

Commercially-available resins can be regenerated at least five times. Non-specific binding to chitin resin can be reduced by a stringent wash including high salt or detergent. Chitin affinity chromatography is not amenable to denaturing reagents such as urea or guanidine hydrochloride. Low concentrations of reducing agents (<1 mM dithiothreitol or <5 mM β -mercaptoethanol) may be used during purification, but higher concentrations will prematurely activate the intein self-cleavage reaction. Low concentrations of non-ionic detergents (0.2% Triton-X or Tween 20) may also be used depending on the nature of the fusion protein.

The intein self-cleavage reaction is induced by overnight incubation with 50 mM DTT at 4 degrees Celsius. β -mercaptoethanol, cysteine, or hydroxylamine may also be used, but are less effective (Chong et al. 1997). Cysteine and hydroxylamine form permanent complex with the cleaved protein. The production of proteins possessing an N-terminal cysteine and/or C-terminal thioester can be useful for protein labeling, ligation or cyclization (Chong et al. 1997). Typical yields from intein-CBD expression and purification are about 0.5-5 mg cleaved protein per liter of bacterial culture.

Biotin Carboxyl Carrier protein (BCCP)

In vivo biotinylation of heterologous proteins containing a biotinylation signal peptide (BCCP) was first used in 1990 (Cronan 1990). The PinPoint Xa vectors provide all three reading

frames and a factor Xa cleavage site (Promega). Typical yields from the PinPoint expression and purification system are in the range of 1-5 mg fusion protein per liter of bacterial culture.

Biotinylated proteins can be purified by affinity chromatography to avidin resin, although purification by traditional avidin resin requires a denaturing elution (e.g. heat, urea, or guanidine hydrochloride). Commercially-available resins (i.e. SoftLink™ avidin resin, Promega) can be regenerated at least 10 times and allows elution of biotinylated fusion proteins with a mild 10 mM biotin buffer. Denaturing reagents are not compatible with avidin affinity chromatography. Low concentrations of reducing agents or non-ionic detergents may be used depending on the nature of the fusion protein.

There is little non-specific binding to avidin in *E. coli* systems. Native BCCP does not bind to avidin. Mammalian systems have at least 4 biotinylated protein species which can co-elute with the biotinylated protein-of-interest, though. Biotinylated proteins can be detected by alkaline phosphatase (AP)- or horseradish peroxidase (HRP)-coupled streptavidin, primary antibodies against biotin, or labeling with fluorescent or radioactive biotin. Biotin-pulldown assays with streptavidin-coupled beads can be used for the detection of protein-protein or protein-DNA interactions. In addition, the high-affinity interaction of biotin and streptavidin makes the biotin tag useful for the immobilization of fusion proteins on streptavidin-coated surfaces, such as surface plasmon resonance chips (Sensor Chip SA, Biacore).

In addition to the *in vivo* biotinylated BCCP tag, other purification systems utilize the high-affinity interaction between biotin and streptavidin or avidin. Streptavidin-binding peptide (SBP) is 38 residues in length and can be used to immobilize fusion proteins on a streptavidin matrix. In addition, The Strep-tag and Strep-tag II (Sigma) are 8-9 residue peptides that bind a mutant form of streptavidin (Strep-Tactin®). Because the affinity of biotin for streptavidin is

higher than that of any of the streptavidin-binding peptides described above, streptavidin-bound SBP-tagged or Strep-tagged fusion proteins can be efficiently eluted by free biotin. Streptavidin itself can also be used as an affinity tag, for purification or immobilization of fusion proteins on a biotinylated matrix.

Epitope Tags

Relatively short epitope tags such as FLAG, hemagglutinin (HA), c-myc, T7, and Glu-Glu, among others (Table 9.2.1), are used for the detection of fusion proteins *in vitro* and in cell culture. Their short, linear recognition motifs rarely affect the properties of the heterologous protein-of-interest and are usually very specific for their respective primary antibodies. One exception is anti-myc antibody, which is somewhat promiscuous. Specificity can be increased by using an enzyme-linked secondary antibody to detect an unlinked anti-myc primary antibody, though.

Epitope-tagged proteins can be purified using immobilized primary antibodies to the epitope tag, although antibody affinity chromatography often involves a low or high pH elution which can irreversibly affect the properties of the fusion protein, and uses resin which has limited reusability. Epitope tags are therefore not the first choice when the main desires for a fusion protein are high-level expression and protein purification.

Reporter Tags

Enzymes such as LacZ, alkaline phosphatase (AP) and chloramphenicol acetyl transferase (CAT) serve as convenient reporters of protein expression and protein-protein interaction. These reporter tags are not normally used solely for protein purification, but a purified fusion protein containing a reporter tag can be a useful tool for protein detection.

LacZ interacts with its substrate X-Gal (5-bromo-4-chloro-3-indoyl- β -D-galactopyranoside) to yield a blue precipitate. The advantages and disadvantages of LacZ as a protein purification tag have been discussed in section 9.1.

AP interacts with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) to yield a purple precipitate. AP is commonly fused to primary antibodies for western blotting, but it can also be fused to any number of non-immune proteins for far-western blotting (i.e. detection of a membrane-transferred protein by interaction with another protein). Since AP is normally targeted to the *E. coli* periplasmic space, crude AP fusion protein lysates can be purified by periplasmic extraction. AP fusion proteins can be further purified by immunoaffinity chromatography.

The ease and sensitivity of the assay for CAT activity has made CAT one of the first reporter genes used for studies of mammalian gene expression (Podbielski et al. 1992). The CAT gene is not found in eukaryotes, and therefore eukaryotic cells contain no background of CAT activity, which can be detected using the radioisotope-labeled or fluorescently-labeled substrates acetyl-CoA or chloramphenicol. CAT fusion proteins can be purified by affinity chromatography to immobilized chloramphenicol, and eluted by free chloramphenicol.

SECTION 9.3: DEVELOPING AFFINITY TAG TECHNOLOGIES

Some newer affinity tags have not been described enough in the literature to justify their recommendation above the tried-and-true affinity tags described in section 9.2. Still, many of these novel affinity tags contain unique properties that warrant their mention, and may make them quite useful in cases of troublesome purifications.

His-Patch ThioFusion™

Thioredoxin has the ability to accumulate to about 40 percent of the total cellular protein in *E. coli*. In addition, the thioredoxin moiety can in some cases confer solubility to formerly insoluble heterologous proteins expressed in *E. coli*. Unfortunately, the phenylarsine oxide matrix used to purify thioredoxin fusion proteins does not give high-yield, high-purity product (Terpe 2003). To solve this problem, Invitrogen has developed a mutant thioredoxin molecule that has a cluster of histidines, such that when the thioredoxin molecule is properly folded the histidines will form a patch amenable to IMAC (His-patch ThioFusion™ expression system). In addition, the tag is cleavable by Factor Xa, allowing the fusion protein to be separated from the His-Patch ThioFusion molecule, which is over 100 residues in length and readily dimerizes in solution. While the cost of this expression system (> \$600.00) might discourage its use as a first-line affinity tag, the His-Patch ThioFusion tag might be useful when other attempts at producing large amounts of soluble protein have failed.

NorpA

The type II N-terminal PDZ domain (PDZ1) of InaD binds the C-terminus of no receptor potential A (NorpA), the relevant phospholipase C-β isozyme in the *Drosophila* phototransduction pathway. The crystal structure of PDZ1 in complex with a peptide corresponding to the NorpA C-terminus shows that only the last five residues of NorpA contact PDZ1 and that a disulfide bond is the major intermolecular interaction (Kimple et al. 2001). The short PDZ1 binding motif of NorpA, coupled with its covalent yet dissociable interaction, led to the hypothesis that the NorpA C-terminal residues (Thr-Glu-Phe-Cys-Ala) could be used as an affinity tag for protein detection and purification by appropriately modified PDZ1 (Kimple et al.

2002). In fact, PDZ1 specifically detects and purifies not only its physiological ligand NorpA, but also a heterologous protein expressing the NorpA C-terminal five residues. Because of its short, linear recognition sequence, and its specific interaction with PDZ1 that is both covalent and dissociable, the NorpA tag may improve upon most currently available affinity tags.

Optimization of the purification protocols, such as addition of a flexible linker to PDZ1 and different coupling chemistries, could yield a moderately reusable, high-affinity resin comparable to glutathione sepharose. This would make the NorpA C-terminus one of the most useful affinity tags, comparable with the His₆ tag in length, but potentially more useful for protein detection because of the specific, high-affinity interaction of NorpA with PDZ1. Specifically, the covalent interaction between PDZ1 and NorpA is ideal for protein-protein interaction techniques such as SPR, where a strong attachment of the protein of interest to the biosensor chip is required, but covalent attachment of proteins via primary amino groups can sometimes result in loss of biological activity. A PDZ1 surface would “catch” NorpA-tagged proteins, which would then be analyzed for their binding to various ligands. The entire surface could be regenerated by flowing over a reducing buffer, allowing the covalent attachment of a different NorpA-tagged protein for a new set of binding experiments.

SECTION 9.4: AFFINITY TAGS AND PROTEIN PURIFICATION

Sometimes it is necessary to determine prior to protein purification what the best affinity tag might be for the desired protein use. Figures 9.9.1, 9.9.2, and 9.9.3 give general schemes for which affinity tag to choose based on three different protein needs: A large quantity of highly pure, untagged protein, a medium quantity of highly pure tagged or untagged protein, and a small amount of moderate-to-highly pure tagged protein. These flow charts should be used as an initial

guide, but more difficult protein purification problems may require the use of a different system than those recommended.

Literature Cited

- Berlot, C. H. 1999. Expression and functional analysis of G-protein alpha subunits in mammalian cells. *In G-proteins: Techniques of Analysis*, D. R. Manning). pp. 37-57. CRC Press, New York.
- Bornhorst, J. A. and J. J. Falke 2000. Purification of proteins using polyhistidine affinity tags. *Methods Enzymol* 326: 245-54.
- Chong, S., F. B. Mersha, et al. 1997. Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element. *Gene* 192: 271-81.
- Crespo, P., K. E. Schuebel, et al. 1997. Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* 385: 169-72.
- Cronan, J. E., Jr. 1990. Biotination of proteins in vivo. A post-translational modification to label, purify, and study proteins. *J Biol Chem* 265: 10327-33.
- di Guan, C., P. Li, et al. 1988. Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. *Gene* 67: 21-30.
- Fritze, C. E. and T. R. Anderson 2000. Epitope tagging: general method for tracking recombinant proteins. *Methods Enzymol* 327: 3-16.
- Gerdes, H. H. and C. Kaether 1996. Green fluorescent protein: applications in cell biology. *FEBS Lett* 389: 44-7.
- Goldstein, D. J., R. Toyama, et al. 1992. The BPV-1 E5 oncoprotein expressed in *Schizosaccharomyces pombe* exhibits normal biochemical properties and binds to the endogenous 16-kDa component of the vacuolar proton-ATPase. *Virology* 190: 889-93.

- Jones, C., A. Patel, et al. 1995. Current trends in molecular recognition and bioseparation. *J Chromatogr A* 707: 3-22.
- Kaldalu, N., D. Lepik, et al. 2000. Monitoring and purification of proteins using bovine papillomavirus E2 epitope tags. *Biotechniques* 28: 456-60, 462.
- Karp, M. and C. Oker-Blom 1999. A streptavidin-luciferase fusion protein: comparisons and applications. *Biomol Eng* 16: 101-4.
- Kimple, M. E., D. P. Siderovski, et al. 2001. Functional relevance of the disulfide-linked complex of the N-terminal PDZ domain of InaD with NorpA. *EMBO J* 20: 4414-22.
- Kimple, M. E. and J. Sondek 2002. Affinity tag for protein purification and detection based on the disulfide-linked complex of InaD and NorpA. *Biotechniques* 33: 578, 580, 584-8 passim.
- Kolodziej, P. A. and R. A. Young 1991. Epitope tagging and protein surveillance. *Methods Enzymol* 194: 508-19.
- Kuliopulos, A. and C. T. Walsh 1994. Production, purification, and cleavage of tandem repeats of recombinant peptides. *J Am Chem Soc* 116: 4599-4607.
- Kwatra, M. M., J. Schreurs, et al. 1995. Immunoaffinity purification of epitope-tagged human beta 2-adrenergic receptor to homogeneity. *Protein Expr Purif* 6: 717-21.
- Lazzaroni, J. C., D. Atlan, et al. 1985. Excretion of alkaline phosphatase by Escherichia coli K-12 pho constitutive mutants transformed with plasmids carrying the alkaline phosphatase structural gene. *J Bacteriol* 164: 1376-80.
- Lilius, G., M. Persson, et al. 1991. Metal affinity precipitation of proteins carrying genetically attached polyhistidine affinity tails. *Eur J Biochem* 198: 499-504.

- Ljungquist, C., A. Breitholtz, et al. 1989. Immobilization and affinity purification of recombinant proteins using histidine peptide fusions. *Eur J Biochem* 186: 563-9.
- Maina, C. V., P. D. Riggs, et al. 1988. An Escherichia coli vector to express and purify foreign proteins by fusion to and separation from maltose-binding protein. *Gene* 74: 365-73.
- Makrides, S. C. 1996. Strategies for achieving high-level expression of genes in Escherichia coli. *Microbiol Rev* 60: 512-38.
- McLean, P. J., H. Kawamata, et al. 2001. Alpha-synuclein-enhanced green fluorescent protein fusion proteins form proteasome sensitive inclusions in primary neurons. *Neuroscience* 104: 901-12.
- Morandi, C., M. Perego, et al. 1984. Expression of human dihydrofolate reductase cDNA and its induction by chloramphenicol in Bacillus subtilis. *Gene* 30: 69-77.
- Nelson, R. W., J. W. Jarvik, et al. 1999. BIA/MS of epitope-tagged peptides directly from E. coli lysate: multiplex detection and protein identification at low-femtomole to subfemtomole levels. *Anal Chem* 71: 2858-65.
- Nilsson, J., M. Bosnes, et al. 1997. Heat-mediated activation of affinity-immobilized Taq DNA polymerase. *Biotechniques* 22: 744-51.
- Nilsson, J., S. Stahl, et al. 1997. Affinity fusion strategies for detection, purification, and immobilization of recombinant proteins. *Protein Expr Purif* 11: 1-16.
- Podbielski, A., J. A. Peterson, et al. 1992. Surface protein-CAT reporter fusions demonstrate differential gene expression in the vir regulon of Streptococcus pyogenes. *Mol Microbiol* 6: 2253-65.
- Rubinfeld, B., S. Munemitsu, et al. 1991. Molecular cloning of a GTPase activating protein specific for the Krev-1 protein p21rap1. *Cell* 65: 1033-42.

- Sambrook, J. and E. F. Fritsch 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press.
- Sano, T., S. Vajda, et al. 1998. Genetic engineering of streptavidin, a versatile affinity tag. *J Chromatogr B Biomed Sci Appl* 715: 85-91.
- Skerra, A. and T. G. Schmidt 2000. Use of the Strep-Tag and streptavidin for detection and purification of recombinant proteins. *Methods Enzymol* 326: 271-304.
- Smith, D. B. 2000. Generating fusions to glutathione S-transferase for protein studies. *Methods Enzymol* 326: 254-70.
- Smith, D. B. and K. S. Johnson 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* 67: 31-40.
- Smith, M. C., T. C. Furman, et al. 1988. Chelating peptide-immobilized metal ion affinity chromatography. A new concept in affinity chromatography for recombinant proteins. *J Biol Chem* 263: 7211-5.
- Stevens, R. C. 2000. Design of high-throughput methods of protein production for structural biology. *Structure Fold Des* 8: R177-85.
- Tai, T. N., W. A. Havelka, et al. 1988. A broad-host-range vector system for cloning and translational lacZ fusion analysis. *Plasmid* 19: 175-88.
- Terpe, K. 2003. Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. *Appl Microbiol Biotechnol* 60: 523-33.
- Wang, L. F., M. Yu, et al. 1996. BTag: a novel six-residue epitope tag for surveillance and purification of recombinant proteins. *Gene* 169: 53-8.

Figure legends:

Figure 9.9.1: Flow chart describing general scheme for choosing an affinity tag for protein purification if a large amount (> 1 mg) of highly pure, untagged protein is needed. Experimental methods requiring this high quantity and purity of native protein include, but are not limited to, protein crystallography, NMR spectroscopy, isothermal titration calorimetry, and surface plasmon resonance.

Figure 9.9.2: Flow chart describing general scheme for choosing an affinity tag for protein purification if a mid-range amount (100 μ g – 1 mg) of highly pure tagged and/or untagged protein is needed. An example of an experimental method requiring this quantity of tagged protein is surface plasmon resonance (SPR), where a biotin moiety could be used to attach a protein ligand to a streptavidin-coated SPR chip. Certain variants of the intein-CBD tag are useful for methods requiring a completely native protein, as it is possible to cleave off every intein residue from the fusion protein, leaving a native N- or C-terminus. Finally, the His-tag and GST-tag are useful because both tagged and untagged proteins can be easily prepared during the same protein preparation, and there are many secondary reagents, such as antibodies, resins, SPR chips, ELISA assays, etc., that are based on these fusion systems.

Figure 9.9.3: Flow chart describing general scheme for choosing an affinity tag for protein purification if a small amount of moderate-to-highly pure, tagged protein is desired. Since only small amounts of protein are required, it is usually not necessary to optimize protein expression, leaving the decision of affinity tag based mainly on the final use for the fusion tag: As a reporter or for general detection.

Table 9.2.1: Characteristics of Protein Affinity Tags

Tag	Length	Position	Matrix/Elution	Commercial Systems	Typical use(s)	Comments	References
Albumin-binding protein (ABP)	137	N-terminal (N-term) or C-terminal (C-term)	Albumin/Low pH or denaturation (e.g. heat, urea)	N/A	Purification, Increased expression, Increased solubility	May increase proteolytic stability of fusion proteins to increase expression; May improve fusion solubility; High background binding to albumin in eukaryotic systems; Matrix has limited reusability; Large tag or low pH elution conditions may affect fusion properties	(Nilsson, Bosnes et al. 1997)
Alkaline phosphatase (AP)	444	C-term	monoclonal antibody (mAb)/Low pH	PhoA* color system, Qbiogene; gWIZ secreted alkaline phosphatase expression vectors, Gene Therapy Systems	Detection	Colorimetric detection; Useful for far-western blotting, southern blotting, protein quantization, sandwich ELISA, and sub-cellular localization, in bacterial and mammalian expression systems; Convenient purification of crude periplasmic extract from bacteria; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability; AP dimerization and very large size may affect properties of fusion	(Lazzaroni et al. 1985)
AU1	6 DTYRYI	N-term or C-term	mAb/Low pH	N/A	Detection, Purification	Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Goldstein et al. 1992)
AU5	6 TDFYLK	N-term or C-term	mAb/Low pH	N/A	Detection, Purification	Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Crespo et al. 1997)
Bacteriophage T7 epitope (T7-tag)	11 MASMTGGQ QMG	N-term or internal	mAb/Low pH	T7-tag affinity purification kit, Novagen; pGEMEX vectors, Promega	Purification, Increased expression	N-term 11 amino acids of phage T7 gene 10; May increase expression of fusion proteins; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Makrides 1996)
Bacteriophage V5 epitope (V5-tag)	14 GKPIP NPL LGLDST	C-term	N/A	Selected pET directional TOPO, pBAD, and Gateway systems, Invitrogen	Detection	Short, linear recognition motif; Antibody specific in bacterial lysates, although some cross-reactivity in mammalian lysates; Often used in combination with His-tag tag for protein purification	(McLean et al. 2001)

Biotin Carboxyl Carrier Protein (BCCP)	100	N-term or C-term	Avidin or streptavidin/Biotin or denaturation (e.g. heat, urea)	PinPoint system, Promega	Detection, Purification, Immobilization	Tag is biotinylated in vivo; One-step purification of fusion protein; Protein may be secreted for convenient purification; Biotin tag can be used to immobilize fusion to streptavidin-coated surfaces (e.g. surface plasmon resonance (SPR) chips); High background binding to avidin in eukaryotic systems; Elution with biotin may be inefficient, and denaturing elution requires refolding of fusion protein; Promega SoftLink avidin allows elution under mild conditions	(Nilsson, Stahl et al. 1997)
B-tag	6 QYPALT	N-term or C-term	mAb/Low pH	N/A	Detection, Purification	VP7 region of bluetongue virus; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Wang et al. 1996)
Calmodulin binding peptide (CBP)	26	N-term or C-term	Calmodulin/EGTA or EGTA and high salt	pCAL system, Stratagene	Purification	Relatively short recognition motif; No endogenous E. coli proteins that bind calmodulin; PKA target sequence allows ³² P-labelling; High yield matrix is compatible with reducing agents and detergents, but of limited reusability; Not useful for purification from eukaryotic cells; Tag at N-term may reduce translation efficiency	(Terpe 2003)
Cellulose binding domain (CBD)	27-189	N-term, C-term, or internal (domain-dependent)	Cellulose/Denaturation (Family I CBDs) or ethylene glycol (Family II or III CBDs)	pET CBD Fusion System, Novagen	Purification, Immobilization	Irreversible binding of some CBDs to cellulose useful for immobilization; Reversible binding of CBD I, II, and III families useful for purification; Purification of Family I CBD fusion necessitates protein refolding; Purified Family II CBD fusions must be buffer exchanged	(Terpe 2003)
Chitin binding domain (CBD)	51	N-term or C-term	Chitin/When fused with intein: thiol-containing reducing agent [e.g. dithiothreitol (DTT) or beta-mercaptoethanol (beta-ME)]	IMPACT system, New England Biolabs	Purification	Typically used in conjunction with self-splicing intein tag. One-step purification of nearly 100 percent pure protein, with low milligram yields; Matrix compatible with high salt and non-ionic detergents. Purification must be done in absence of thiol-containing reducing agents until elution step; Fusion protein may have effect on intein cleavage efficiency	(Terpe 2003)
Chloramphenicol acetyl transferase (CAT)	218	N-term	Chloramphenicol/Chloramphenicol	gWIZ CAT mammalian expression vector, Gene Therapy Systems	Detection, Purification, Increased solubility	Enzymatic assay available for protein quantization; Chloramphenicol purification does not give high yields; Large tag may affect properties of fusion protein	(Podbielski et al. 1992)
Choline-binding domain (CBD)	145	N-term	Choline/Choline or CBD peptide	N/A	Purification, Immobilization	N-term CBD can immobilize fusion protein on choline-coated gold chip for SPR or microscopy studies; Large tag or C-term tag placement may affect fusion properties	(Jones et al. 1995)

dihydrofolate reductase (DHFR)	227	N-term or C-term	Methotrexate/Folate	pQE vector set, Qiagen	Increased expression	May increase proteolytic stability of fusion proteins to increase expression; Little immunogenicity in mouse and rat, therefore useful for generation of antibodies to fusion; Methotrexate purification does not give high yields; Usually coupled with a short affinity tag (e.g. His-tag, Strep-tag) for purification	(Morandi et al. 1984)
E2-tag	10 SSTSSDFR DR	N-term or C-term or internal	mAb/Low pH	E2-Tagging and Detection Kit, Abcam; E2 tagging technology, Quatromed	Detection, Purification	Derived from Bovine papilloma-virus type-1 transactivator protein E2; Primary antibodies available for colorimetric and chemiluminescent detection; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Kaldalu et al. 2000)
FLAG	8 DYKDDDK	N-term or C-term	mAb/Low pH, EDTA, or FLAG peptide	FLAG system, Sigma	Detection, Purification	Short, linear recognition motif; Moderately pure protein in one step; Enterokinase cleaves after C-terminal Lys to completely remove tag, depending on identity of first amino acid of fusion; M1 antibody can only bind tag at N-term; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Terpe 2003)
Galactose-binding protein (GBP)	509	N-term or C-term	Galactose/Galactose	N/A	Purification, Increased solubility	Fusion protein may be targeted to periplasm for convenient purification of crude periplasmic extracts; May increase solubility of fusion proteins; Large tag may affect properties of fusion protein	(Jones et al. 1995)
Glu-Glu (EE-tag)	6 EYMPME or EFMPME	N-term, C-term or internal	mAb/Low pH or 30 degrees C incubation	N/A	Detection, Purification	Short, linear recognition motif; Available antibody recognizes only EYMPME motif; Antibody purification does not give high yields, low pH or 30 degrees C elution may irreversibly affect protein properties, and matrix is of limited reusability	(Rubinfeld et al. 1991)
Glutathione S-transferase (GST)	211	N-term or C-term	Glutathione/Reduced glutathione	pGEX system, Amersham Biosciences; GST-Bind system, Novagen	Detection, Purification, Increased expression, Increased solubility; Immobilization	Very common purification tag; One-step purification of relatively pure protein; Detection antibodies specific; Kits for GST fusion proteins common (e.g. SPR, enzymatic assays); Matrix relatively reusable (4-20 times); GST is highly antigenic; Purification under native conditions only; Some GST fusions insoluble; GST dimerization and/or glutathione elution may affect fusion protein properties	(Smith 2000)

Green Fluorescent protein (GFP)	220	N-term or C-term	N/A	gWIZ GFP mammalian expression vector, Gene Therapy Systems; CT-GFP Fusion TOPO Cloning Kit, Invitrogen	Detection	Intrinsic fluorescence can permit native detection without antibody; Anti-GFP antibodies specific; Expression in prokaryotic and eukaryotic expression systems, as well as whole organisms (worms, plants, mice, etc); Useful to monitor gene expression, protein folding and targeting, and protein-protein interactions by fluorescence resonance energy transfer (FRET); Some GFP fusions non-specifically targeted to nucleus; Very large tag or GFP dimerization may affect properties of fusion	(Gerdes et al. 1996)
Hemagglutinin (HA)	31	N-term, C-term or internal	mAb/Low pH or HA peptide	<u>RTS pIVEX HA-tag vector set</u> , Roche	Detection, Purification	Anti-HA antibodies specific; Useful in mammalian expression systems; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Tai et al. 1988)
Herpes simplex virus peptide (HSV-tag)	11 QPELAPED PED	C-term	mAb/Low pH	N/A	Purification	C-term tag placement only; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Fritze et al. 2000)
Histidine-affinity tag (HAT)	19 KDHLIHNV HKEFHAH AHNK	N-term or C-term	Divalent metal (Ni ²⁺ , Co ²⁺ , Cu ²⁺ , or Zn ²⁺)/Imidazole or low pH	HAT protein expression and purification system, Clontech	Purification	Natural protein sequence that was found to chelate divalent metals; Short recognition motif; Contains six histidines interspersed among other amino acids; Semi-pure protein in one step; Matrix may be regenerated and reused almost infinitely; Does not bind metal affinity resin as tightly as His-tag tag; Tag or elution conditions may affect fusion properties	(Terpe 2003)
Ketosteroid isomerase (KSI)	125	N-term	N/A	pET-31b(+) vector, Novagen	Increased expression	Insoluble protein is targeted to inclusion bodies; Usually coupled with a short affinity tag (His-tag, Strep-tag) for purification; Convenient denaturing purification of toxic proteins, but refolding necessary	(Kuliopulos et al. 1994)
KT3	11 KPPTPPPE PET	N-term or C-term	mAb/Low pH	N/A	Detection, Purification	Short, linear recognition motif; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Kwatra et al. 1995)
LacZ	1024	N-term or C-term	p-amino-phenyl-beta-D-thiogalactosidase (APTG)/High pH borate buffer	pMC1871 fusion vector, Amersham Biosciences; gWIZ beta-galactosidase mammalian expression vector, Gene Therapy Systems	Detection, Purification, Increased expression	Also known as beta-galactosidase or beta-Gal; Enzymatic assay available for protein quantization; May increase proteolytic stability of fusion proteins to increase expression; Fusion proteins may be insoluble; Extremely large tag which tetramers in solution may affect properties of fusion protein	(Tai et al. 1988)

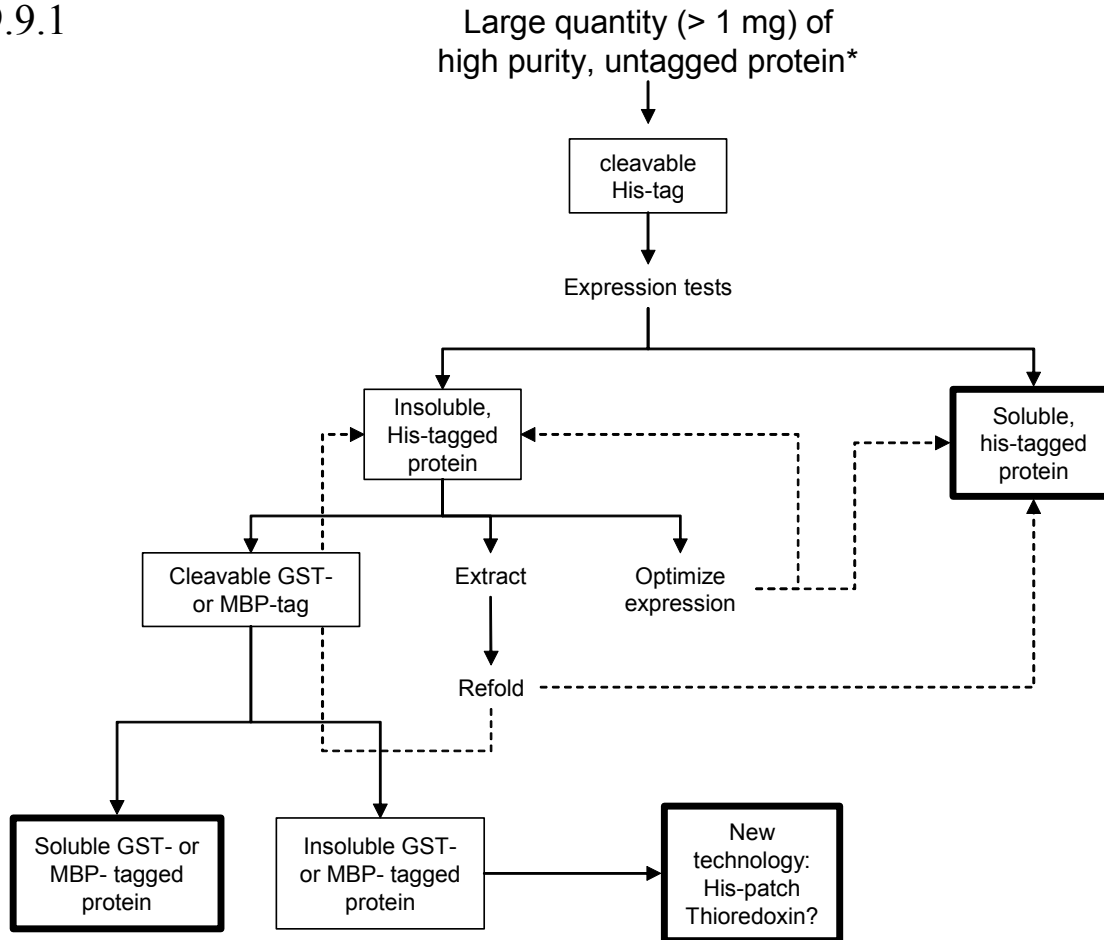
Luciferase	551	N-term	N/A	gWIZ luciferase mammalian expression vector, Gene Therapy Systems; Luciferase assay system, Promega	Detection	Luminescent; can serve as a reporter immediately upon translation; Useful for studies involving in situ hybridization, RNA processing, RNA transfection or coupled in vitro transcription/translation, protein folding, and imaging; Can be labeled with ³⁵ S; No more than five codons can be removed from the N- or C-term to maintain enzymatic activity; Very large tag may affect properties of fusion	(Karp et al. 1999)
Maltose-binding protein (MBP)	396	N-term or C-term	Cross-linked amylose/Maltose	pMAL system, New England Biolabs; <u>RTS pIVEX MBP</u> fusion vector, Roche	Detection, Purification, Increased expression, Increased solubility	One-step purification of relatively pure protein (>70 percent); Matrix compatible with non-ionizing detergents and high salt, but not reducing agents; Can increase expression of eukaryotic proteins in bacteria; Anti-MBP antibodies specific; Tag at N-term can decrease translation efficiency; Very large size of tag may affect fusion protein properties	(Nilsson, Stahl et al. 1997; Terpe 2003)
Myc	11 CEQKLISE EDL	N-term, C-term or internal	mAb/Low pH	pDual Expression System, Stratagene; PRO bacterial expression system, Clontech	Detection, Purification	Short, linear recognition motif; Anti-myc antibody somewhat promiscuous; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Kolodziej et al. 1991)
NorpA	5 TEFCA	C-term	PDZ1/Thiol-containing reducing agent (e.g. DTT, beta-ME)	N/A	Detection, Purification, Immobilization	Short, linear recognition motif; alkaline phosphatase-coupled PDZ1 allows antibody-independent detection; PDZ1-NorpA interaction highly specific; PDZ1 can couple fusion proteins to SPR resonance chip; Purification must be performed in absence of thiol-containing reducing agents until elution step; Reducing elution may disrupt properties of fusion protein; Tag must be at C-term to bind PDZ1	(Kimple et al. 2002)
NusA	495	N-term or C-term	N/A	N/A	Increased expression, Increased solubility	Transcription termination anti-termination factor; Increases solubility and expression of fusion proteins; Must be used in conjunction with another affinity tag for protein purification; Large tag may affect properties of fusion protein	(Terpe 2003)
Polyarginine (Arg-tag)	5-6 (usually 5) RRRRR	C-term	Cation exchange resin/High pH salt gradient	N/A	Purification, Immobilization	Can immobilize targets on mica for microscopy studies; Short, linear recognition motif; Very pure protein in one step; Charged tag may affect tertiary structure of protein and/or protein properties; Limited success of tag cleavage by carboxypeptidase B	(Terpe 2003)

Polyaspartate (Asp-tag)	5-16 DDDDD	C-term	Anion exchange resin/Low-neutral pH salt gradient	N/A	Purification	Short, linear recognition motif; Polar tag may affect tertiary structure of protein and/or protein properties	(Stevens 2000)
Polycysteine (Cys-tag)	4 CCCC	N-term	Thiopropyl-sepharose/Thiol-containing reducing agent (e.g. DTT, beta-ME)	N/A	Purification	Short, linear recognition motif; Moderately pure protein in one step; Purification must be performed in absence of thiol-containing reducing agents until elution step; Reducing elution may disrupt properties of fusion protein	(Stevens 2000)
Polyhistidine (His-tag)	2-10 (usually 6) HHHHHH	N-term or C-term	Divalent metal (Ni ²⁺ , Co ²⁺ , Cu ²⁺ , or Zn ²⁺)/Imidazole or low pH	QIAexpress system, Qiagen; Selected pET directional TOPO, pBAD, and Gateway systems, Invitrogen	Detection, Purification, Immobilization	Most common purification tag; Short, linear recognition motif; One-step purification of 20-80% pure protein, depending on fusion protein expression levels; Denaturing purification possible; Matrix may be regenerated and reused indefinitely; Can be used to immobilize fusion to Ni-NTA SPR chip, but significant dissociation complicates data analysis; Tag or elution may affect protein properties; Detection antibodies highly promiscuous	(Bornhorst et al. 2000)
Polyphenylalanine (Phe-tag)	11 FFFFFFFF FFF	N-term	Phenyl-sepharose/Ethylene glycol	N/A	Purification	Short, linear recognition motif; Moderately pure protein in one step; Non-polar tag or Ethylene glycol elution may disrupt properties of fusion protein	(Stevens 2000)
Protein C	12	N-term or C-term	mAb/Ca ²⁺ buffer	pXB, pBX, pXM, and pMX vectors, Roche	Detection, Purification	Short, linear recognition motif; Anti-PC antibody binds in Ca ²⁺ -dependent manner; Elution by Ca ²⁺ in physiological buffer conditions. Antibody purification does not give high yields	(Fritze et al. 2000)
S1-tag	9 NANNPDWD F	N-term or C-term	mAb/Low pH	N/A	Detection, Purification	Hepatitis B virus S1 region; Short, linear recognition motif; AP1 antibody specific; Has been tested in bacterial and mammalian expression systems; Relatively pure protein in one step; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Berlot 1999)
S-tag	15 KETAAAKF ERQHMSD	N-term, C-term or internal	S-fragment of RNaseA/Low pH	S-Tag system, Novagen	Detection, Purification,	Short, linear recognition motif; RNase S assay possible for quantitative assay of expression levels; Colorimetric assays used for detection without antibody; Tag or low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Fritze et al. 2000)
Staphylococcal protein A (Protein A)	280	N-term	IgG/Low pH or IgG	pEZZ 18 and pRIT2T vectors, Amersham Biosciences	Purification, Increased solubility	Proteolytically stable; May increase solubility of fusion; Fusion proteins secreted; Purification does not give high yields; Large tag size and/or low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Nilsson, Stahl et al. 1997)

Staphylococcal protein G (Protein G)	280	N-term or C-term	Amylose/Low pH or amylose	N/A	Purification, Increased solubility	Proteolytically stable; May increase solubility of fusion; Fusion proteins secreted; Purification does not give high yields; Large tag size and/or low pH elution may irreversibly affect protein properties, and matrix is of limited reusability;	(Nilsson, Stahl et al. 1997)
Strep-tag	8 or 9 WSHPQFEK or AWAHPQPG G	N-term or C-term	Strep-Tactin (modified streptavidin)/Biotin or desthiobiotin	Strep-tag II system, Sigma-Genosys; pASK75 vector, Biometra	Detection, Purification, Immobilization	Short, linear recognition motif; Matrix regenerable; Useful for purification under anaerobic conditions, eukaryotic cell surface display, and immobilization to streptavidin-coated surfaces (e.g. SPR chips); Specific binding conditions may be unsuitable for some fusions;	(Skerra et al. 2000)
Streptavidin	159	N-term or C-term	Biotin/Biotin or denaturation (heat, urea)	N/A	Detection, Purification, Increased expression, Immobilization	May increase proteolytic stability of fusion proteins to increase expression; Extremely high affinity for biotin useful for immobilization of fusion on surfaces, such as SPR chips; Large size or tetramer formation may disrupt properties of fusion protein; Fusion protein may not be released upon addition of free biotin, necessitating denaturing elution followed by refolding; Newer streptavidin mutants that have lower affinities for biotin useful for purification	(Sano et al. 1998)
Streptavidin binding peptide (SBP)	38	C-term	Streptavidin/Biotin	N/A	Purification, Immobilization	Relatively short recognition motif; Immobilization of protein to streptavidin-coated beads, SPR chips, etc; Tag at C-term only	(Terpe 2003)
T7 gene 10 (T7-tag)	260	N-term	mAb/Low pH	N/A	Purification, Increased expression	May increase expression of fusion proteins; Insoluble protein is targeted to inclusion bodies; Denaturing purification of toxic proteins necessitates refolding	(Stevens 2000)
Thioredoxin (Trx)	109	N-term or C-term	Phenylarsine oxide/ Thiol-containing reducing agent (e.g. DTT, beta-ME)	ThioFusion System, Invitrogen	Increased solubility	Heat stable; May increase solubility of fusion proteins; Convenient purification of crude periplasmic extract from bacteria; Purification must be done in absence of thiol-containing reducing agents until elution step; Large tag or elution conditions may affect properties of fusion protein	(Terpe 2003)
trpE	25-336	N-term or C-term	mAb/Low pH	N/A	Purification, Increased expression	Larger constructs expressed may be targeted to inclusion bodies, allowing high-level expression of toxic genes; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability; Large tag may affect properties of fusion protein	(Stevens 2000)

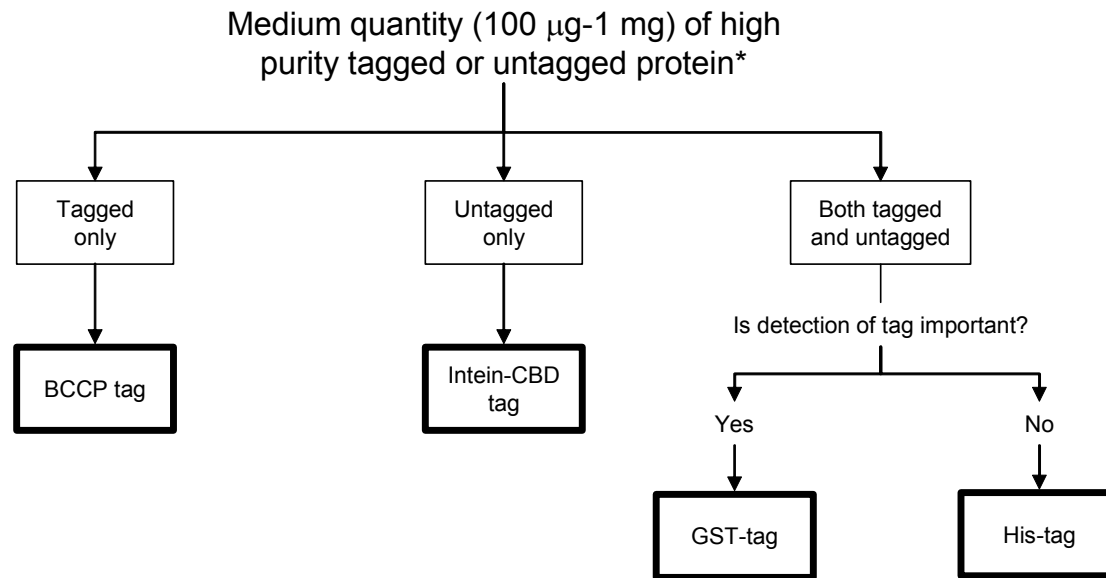
Ubiquitin	76	N-term	N/A	N/A	Increased solubility	May increase solubility of proteins expressed in <i>E. coli</i> ; Not useful for expression in eukaryotic cells.	(Stevens 2000)
Universal	6 HTTPHH	N-term or C-term or internal	mAb/Low pH	N/A	Detection, Purification	Sequence HTTPHH is translated regardless of reading frame for ease in cloning; Multiple tag copies increase antibody specificity; Immobilized mAb can bind multiple tag copies in SPR studies; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Nelson et al. 1999)
Vesicular Stomatitis Virus Glycoprotein peptide (VSV-G)	11 YTDIEMNR LGK	C-term	mAb/Low pH	pVB6, pBV, pVM6, and pMV vector set, Roche	Detection, Purification	C-term residues of vesicular stomatitis virus glycoprotein (VSV-G); Relatively pure protein in one step; Antibody purification does not give high yields, low pH elution may irreversibly affect protein properties, and matrix is of limited reusability	(Fritze et al. 2000)

Figure 9.9.1



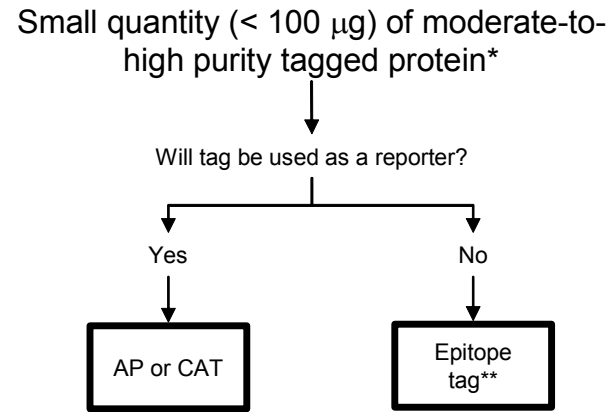
* e.g. For crystallography, NMR spectroscopy, isothermal titration calorimetry, surface plasmon resonance analyte

Figure 9.9.2



* e.g. For GST and Ni²⁺ pull-down assays, mass spectrometry, surface plasmon resonance ligand

Figure 9.9.3



* e.g. For co-immunoprecipitation, western blotting, far-western blotting, ELISA, gel overlay assays

** e.g. T7-tag, B-tag, Glu-Glu, HA, c-Myc