International Journal of Agriculture Innovations and Research
Volume 6, Issue 3, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 22/08/2017 | Accepted on: 28/08/2017 | Published: 04/11/2017

The Effect of Fusion Tags on Enzyme Specificity and Protein Purification Efficiency

Sercan Karav[1*], Ebru Talak[1], Melis Tuncer[1] and Adem Ozleyen[1]
[1]Department of Molecular Biology and Genetics, Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
*Corresponding author email id: sercankarav@gmail.com

Abstract – **Recombinant proteins are produced from recombinant DNA by using molecular technologies, and used in many working areas such as biotechnology, biomedical field and industry. However, purification of the recombinant protein is one of the most challenging steps of this process. Affinity tags are highly efficient tools to obtain the recombinant proteins with high yield and purity. They are used to facilitate the purification and detection of proteins, alongside the separation of protein complexes. Moreover, these tags might alter the enzymes specificity allowing the manipulation of the desired enzymatic reactions. Especially milk glycoproteins contain glycosidically attached a wide variety of conjugated glycans that have different biological functions. These bioactive glycans are isolated by the application of various glycosidases. Recent studies suggest that specific glycan types can be produced by affinity tag-enzyme complexes that alter the enzymes target glycan type. Such a manipulation on glycan production enables further studies on individual glycan structures and their contribution to the function of glycoproteins. In this review, we discuss various affinity tags; poly his, glutathione S-transferase (GST), maltose- binding protein (MBP), FLAG tag and other tags in the terms of their purification efficiency and substrate specificity.**

Keywords – **Affinity Tags, Enzyme Specificity, Glycans, Protein Purification, Recombinant Protein.**

I. Introduction

Proteins are involved in many biological reactions and have various health benefits. However their use as therapeutic agent is limited due to the lack of their production naturally. Therefore these proteins have been produced in large scale recombinantly in industry since 1970s [1]. The recombinant protein production and purification are used in the many areas like academic research, therapeutic and diagnostic applications and in the industry [2]. The production and purification of proteins are closely connected with each other. The selection of host cell is related to purification of product as far as it is related to amplification and isolation of the protein [3, 4]. For the isolation and amplification of a gene of interest, there is a method in which the gene is cloned to provide necessary amount of gene by inserting it into vector that can multiply in living cells [5]. The expression process of foreign protein inside the vector can be carried out in cell culture of bacteria, yeasts, mammals, plants or insects, or within the transgenic plants and animals [6]. Then the expressed protein is purified with the help of specific tags that are attached genetically (affinity purification). In this process these tags bind to specific column whereas other proteins that are produced by the host naturally are eluted from the column [7]. The tag- protein complex which is already attached to the column is separated by specific agents that are competitive with tags [8].

Nowadays recombinantly produced proteins have a significant role in many diverse areas such as pharmaceutical, food, detergent, textile industry etc. They also lead to new therapeutic approaches, drug development and finding vaccine techniques to solve worldwide threats like HIV, Ebola [9]. The most important reason of use of recombinant proteins in industry is a high yield as well as the economical advantages. Considering the advantages, it is inevitable that the recent studies focus on recombinant protein techniques. As summarized in Table 1, many researches involve in variety of recombinant proteins, which have different functions. As an example, glucoamylase is one of the most significant recombinant proteins in food industry for the production of alcohol and glucose syrup whereas erythropoietin is preferred in pharmaceutical industry for people whose bone marrow does not provide enough red blood cells [10, 11]. Successfully production of these proteins is achieved with an appropriate fusion tag system that enables high yield and purity recombinant proteins.

Glycans release from glycoproteins by glycosidases are discussed in this review as a model enzyme-substrate complex. As well as the efficiency of substrate release, the

Table 1. Various recombinant proteins, their functions and production strategies.

Protein	Function	Expressed In	Affinity Tag	Application	Reference
Factor VIII	Essential blood-clotting protein	*E. Coli*	GST	Pharmaceuticals	[12]
Alpha 1- antitrypsin	Inhibits many kind of proteases	*N. Benthamiana*	Cysta-Tag	Pharmaceuticals	[13]
Erythropoietin	Controls red blood cell production	Tobacco plants	Strep-Tag II	Pharmaceuticals	[10]
Glucoamylase	Hydrolyses polysaccharides from the nonreducing chain ends	*E. Coli*	SBD	Food Processing	[14]
Beta glucosidase	Hydrolyses the glycosidic bond between two or more carbohydrates	*E. Coli*	CBD	Food Processing	[15]
Subtilisin	Breaks the peptide bonds in the backbone of the protein	*B. subtilis*	FLAG	Detergent Industry	[16]
Luciferase	Produces bioluminescence	Baculovirus	His-Tag	Diagnostic Method	[17]
Endo-β-N-acetylglucosaminidase	N-glycan isolation from milk glycoproteins	*E. Coli*	His-Tag	Food processing	[18]

Copyright © 2017 IJAIR, All right reserved

IJAIR
International Journal of Agriculture Innovations and Research
Volume 6, Issue 3, ISSN (Online) 2319-1473

substrate specificity of the enzymes is a unique characteristic of the enzymes. Especially for glycoproteins with attached multiple glycan structures, substrate specificity of glycosidases is critical. There are three different glycan types (complex, high mannose and hybrid) that they have unique biological functions. Especially, the glycans containing sialic acid prevent pathogen binding stimulate the brain development in infants [19, 20]. Therefore, the cleavage of specific glycans enzymatically provides investigations on the determination of novel roles of glycans. This specificity of the enzymes can help to understand the contribution of the glycans to the roles of carious glycoproteins such as lactoferrin and immunoglobulins by enabling only certain types of the glycans attached to the polypeptide chain. We previously show that different reaction parameters including incubation time, temperature, pH and enzyme amount altered the enzymes target glycans. However this strategy results in low enzyme efficiency since the conditions are not optimal for the glycan release of the enzyme [21]. Recent studies suggest that enzyme specificity can also be achieved with the help of fusion tags that's alter the enzyme's target substrate. Majorek et. al. [22] analyzed the influence of His-tag on Gcn5-related N-acetyltransferase (GNAT) enzyme from *Pseudomonas aeruginosa*. The results showed that his-tag binds substrate-binding site of GNAT affect the activity of enzyme negatively, whereas it was not observed any important change in the terms of enzyme structure. Additionally, His-tag behaved inhibitor against substrate of enzyme that was resulted in low affinity of enzymes to its substrate. However, crystallization of the protein was not affected by his-tag. Lee et. al. [23] analyzed biochemical characterization of thioesterase I of *E. coli* with C-terminal 6xHis-tag and compared with the enzyme without His-tag at same temperature and pH. The determination of kinetic parameters of His-tagged (HT) and without His-tagged (WT) on seven substrates suggested that presence of His-tagged enzyme shifted more toward shortest chain-length substrate, p-nitro phenyl acetate that HT has 5-fold higher catalytic efficiency. On the other hand, HT has lower Kcat/ Km for only two substrates palmitoyl-CoA and p-nitro-phenyl dodecanoate, are 36- and 10-fold lower than WT. The study suggested that though C-terminal His-tag did not influence seriously all structure of enzyme, substrate specificity and catalytic activity of enzyme are changed by His-tag. According to suggestion of experiment, addition of His-tag on C- terminus of enzyme was close enough active site to influence substrate specificity.

In this review, we discuss how different fusion tag applications affect the protein purification efficiency as well as the substrate specificity that enables the study individual substrate's (such as glycans) biological roles in advance. Fusion tags are primarily designed for the production of recombinant proteins with high purity. In addition to this main goal, fusion tags are expected to increase the solubility of proteins that provides higher yield of the production. The fusion tags should be applied easily while it allows for the enzyme to maintain its activity. As fusion tags, we compare commonly used strategies such as poly his, glutathione S- transferase (GST), maltose-binding protein (MBP), FLAG tag as well as other recent approaches in terms of these criteria.

II. Fusion Tags

A. Histidine (HIS Tag)

The polyhistidine-tag or His-tag is one of the most commonly used methods for protein purification due to its relatively small size and charge. In purification process, the composition of polyhistidine-tag can contain 3 to 10 His- residues (the hexahistidine-tag (~0.84 kDa) is widely performed one at many studies) [24]. During the purification of recombinant protein, His-tagged protein binds immobilized alkaline earth metals (Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}), and even some heavy metals such as Fe^{3+}, Al^{3+} [1, 24]. Then, His-tagged protein is eluted from metal ions column by imidazole. It can be fused to both N- or C-terminal of target protein [25, 26]. The purification with this method can be performed under either native or denaturing conditions.

One of the successfully applications of polyhis-tagged for recombinant protein production performed by Khan et al.

[27] That used green fluorescent protein (GFP) as a model for the studying comparison of single-, double- and triple- hexahistidine tag at either the N- or C- terminus or both. In this study, two hexahistidine tags were placed consequently one other due to the presence of peptide spacer that has 11 amino acids and was placed between two tags. In the triple hexahistidine tag, one double hexahistidine at C- terminal and a hexahistidine tag at N-terminal were combined. According to results, the double hexahistidine tag were detected with 3-fold greater sensitivity than the single hexahistidine tag and the dissociation rate of double hexahistidine tagged protein was 10 times slower than single hexahistidine tagged protein. The triple-tagged protein had 60% lower binding characteristics. However the dissociation rate of triple-tagged protein was similar with the double hexahistidine tagged protein. The main result of this study, double hexahistidine tag has potential to use it for protein immobilization, detection and single step purification. Karikari et al. [28] Represented expression and purification of tau protein using a cleavable histidine tag. The expression of pure tau protein in high-yield is critical for in vitro taupathy studies resulting in tau protein which has less aggregation capacity could be obtained. The recombinant tau protein was purified with hexahistidine tag then tag was removed with TEV protease. The preparation of monomer-oligomer mixture of recombinant tau protein could be used for many studies. However, when the monomeric tau is required, the truncation of products and aggregates should be removed. The advantage of this method is that it provides highly pure functional oligomeric and monomeric tau protein; up to 99% purity for only monomer and 95% for monomer-oligomer mix. In another study, Panek et al. compared recombinant trehalose synthase with hexahistidine tag and

Copyright © 2017 IJAIR, All right reserved

wild type trehalose synthase. Trehalose is a disaccharide that can be used many areas like food, cosmetic industries which is produced from maltose by trehalose synthase. According to the results of this study, the recombinant trehalose synthase with 6xhis tag had higher Km value than wild type due to the presence of fused tag. Because of that his- tagged recombinant protein had low affinity to maltose [29]. Bewley et al. investigated the effect of C terminus 6xhis tag on the HIV-1 Pr55Gag polyprotein which is produced by human immunodeficiency virus (HIV). The presence of C terminal 6xhis tag altered the nucleic acid binding property of HIV-1 Pr55Gag protein. After removal of 6xhis tag the recombinant protein showed similar property with untagged Pr55Gag [30]. Zhao et al. used his-tag expression system to purified zinc finger protein, ZNF191 (243-368). The effects of N terminal 6xHis tag and C terminal 8xHis tag to recombinant proteins were investigated. Based on UV visible absorption spectroscopy, tagged proteins showed similar properties. However, according to circular dichroism (CD) spectroscopy and hydrolase activity, tagged recombinant proteins showed differences [31].

B. The Glutathione - Transferase (GST) Tag

The glutathione-transferase (GST) tag is commonly used affinity tag method, and its principle is based on high affinity of GST for its ligand immobilized glutathione [32, 33]. GST is a 211 amino acid length protein and it is considered as a large size tag [34]. Due to the large size of GST, it is commonly removed from the recombinant protein to prevent any limitations on the biological functions/characteristics of the protein. During the application of affinity medium to the chromatography; it should be performed at low flow rate to increase the binding capacity of tagged-proteins to the ligand. The GST-tagged protein and ligand bind to each other reversibly, and in the following step this complex can be eluted by using elution buffer which contains reduced glutathione [35]. This buffer provides mild and non-denaturation conditions for target protein's main structure and function [24, 35]. In addition to the high binding capacity to the ligand. The removal of GST tag from the recombinant protein is based on the use of enzymes like prescission protease, thrombin that cleaves the specific recognition site so that GST tag and target protein are separated from each other [36, 37]. GST is also used for the increase of the solubility of the recombinant proteins that provides easy and high yield protein purification [38]. According to results performed by Hayashi et. al. [39], pCold-GST expression system applied for 10 proteins that could not be expressed normally in conventional *E. coli* expression strategies. Among these proteins, it was shown that 9 of them were successfully expressed in soluble fraction. They suggest that pCold-GST system can be used to increase the expression level and purification.

Dojima et. al. [40] used GST tag to purely single chain antibody variable region fragment from silkworm larval hemolymph in single step. According to the results, GST tag showed 5-fold lower expression level compared to the His- tag purification. However, GST tag showed 91.8% recovery and 83% purity, whereas his tag resulted in only 43.7% recovery and significantly lower purity (below 10%). On the other hand, Maity et.al. [36] used two step affinity purification for recombinant baculovirus, based on a N- terminal GST and C-terminal 10xHis tag which are both fused to recombinant protein. The binding of GST to protein of interest followed by tag removal enzyme (prescission) that cleavages and separates GST and recombinant protein, then GST-cleaved protein were flowed at column for His- tagged protein binding, then His- tagged protein eluted with imidazole. The combination these two tags at N- and C- terminal of recombinant baculovirus and cleaving capacity of one of tags provide highly purification and full-length recombinant protein. In a different study Ryan et.al. [41] Performed protein purification by following a dual affinity tag strategy. Recombinantly produced H1.4, a kind of most abundant and expressed linker histone proteins in human, was purified by using N-terminal GST and C-terminal 6xHis with two step affinity chromatography procedure. During study the complex chromatography methods were avoided, because denaturation and loss of function of core histone proteins are common problems. The final produced protein was considered to be highly pure and proper size according to data obtained by mass spectrometry. However it is readily to bind nucleosomes.

C. Maltose-Binding Protein (MBP) Tag

Maltose-binding protein (MBP) is another most popular affinity tags. Generally, MBP which may place on the N- or C-terminus of the target protein is used to enhance expression level or solubility of target protein [42, 43]. In purification of tagging proteins, MBP tagging proteins binds to its ligand cross-linked amylose resin of affinity chromatography [44]. After, the complex that contains tagging protein and ligand are eluted with elution buffer that contains 10 mM maltose from affinity chromatography [34, 45]. MBP tag is commonly separated from target protein by factor Xa, enter kinase, or genenase I protease cleavage sequences [8, 46]. However this tag method has some disadvantages such as the downstream application might be complicated because of its large size about ≈ 42-kDa [47]. The other one is removal of MBP might cause protein to mis- fold, and decrease of its solubility [48].

To improve high yield protein purification, MBP is frequently used in combination with other small tags such as His tag, GST tag. Kurussi et al. [49] described a dual protease protocol for expression and purification of recombinant proteins. The dual His-MBP tag was used and the cleaving of them one by one was carried out to increase yield of nature recombinant protein. The using MBP at N- terminal increased solubility and improved the yield of recombinant protein. The MBP tag was then removed by rhinovirus 3C protease to yield N- terminal His6-tagged protein. The N- terminal His6-tag was removed by His6- tagged tobacco etch virus (TEV) protease. Slunchanko et al.

[50] Used different purification strategy about the using cleavable his-tagged maltose binding protein to purify steroidogenic acute regulatory protein (StAR). The StAR protein is slightly soluble and is mostly obtained time

Copyright © 2017 IJAIR, All right reserved

consuming, cost procedure that may affect the protein characteristics. Because of these reasons, to increase the yield the yield of soluble protein and to obtain high-yield, native, functional and aggregates-free StAR without the necessity of denaturation and refolding, the his-tagged MBP at N- terminus was used. Hang et al. [51] used Aquaporin Z (Aqp Z) that is found in the membrane of *E. coli* was expressed using the maltose-binding protein/ polyhistidine dual - affinity tag fusion system. According to their previous studies, the purification process which was performed only MBP tag was limited under denaturation conditions and in the presence of detergents. However MBP tag was most effective fusion partner for Aqp Z. But the results of following study said that dual-affinity tag system could overcome from the limitations under the same conditions. N- terminal MBP tag and C-terminal his tag on Aqp Z showed that overexpressed in *E. coli* and increased yield of the Aqp Z fusion protein in soluble. Additionally, membrane proteins are toxic for host cells normally. However, these results showed that Aqp Z fusion protein performed no toxic effect on host cells. Their results represented that MBP/His dual- affinity tag fusion system enhance highly expression and purification of major intrinsic protein channel proteins in *E. coli*. Cho et al. [52] used type of fusion partner GST and MBP for effects on the chemokines RANTES and SDF_1αin

E. coli under various conditions. Chemokines are expressed in slightly soluble or insoluble in *E. coli*. Results showed that used MBP as fusion partner for RANTES and SDF-1α improve high yield soluble of recombinant protein as compared to target proteins with GST. Additionally, they studied double fusion system containing His-tag and MBP tag result in enhance recovery of recombinant chemokines.

D. Flag Tag

The FLAG is a kind of short affinity tag that has hydrophilic 8 amino acids [53]. The FLAG tag can be placed at either C- or N-terminal of recombinant protein [34]. Due to the its significant advantages in the terms of size, solubility, the presence of unique cleavage site, and high- affinity to anti-FLAG antibodies, FLAG-tag may be used to purify several recombinant proteins such as immunoglobulins, cytokines and gene regulatory proteins [3, 54]. However the use of this tag is limited with the low specificity and high cost of anti-FLAG antibodies [46]. During the purification, FLAG-tagged proteins bind to anti- FLAG monoclonal antibodies such as M1 [55] and M2 [56] that have different binding characteristics [57]. According to type of resin for FLAG tag, the binding and elution of FLAG tag to resin change. For the M1 resin, the binding FLAG- tagged protein and resin is calcium dependent, the separation of them is can be carried out under mild conditions [58, 59]. For the M2 resin, the binding tagged-protein and resin is calcium independent and the elution tagged-protein from M2 resin requires decrease in pH value [60]. After binding FLAG- tagged proteins fused monoclonal antibodies are eluted with FLAG peptide [61] (e.g., 3 × FLAG peptide [62]) or low pH glycine buffer. After purification FLAG tag is cleaved by a kind of tag removal enzyme which causes the separation of recombinant protein and FLAG tag [63].

Fatutsumori-Sugai et al. [64] used C-terminal FLAG tag to purify MsbA and eluted FLAG-fused protein from M2-immobilized resin by using arginine under acidic pH condition. The purified MsbA with FLAG tag had more active molecule than previously purified his-tagged protein. The arg-elution method, in this study, caused the efficient elution of active and folded proteins at low pH. Pajecka et al. [65] investigated the kinetic properties and cellular localization of glutamate dehydrogenase (GDH) isoforms with and without short affinity tags; FLAG tag and hexahistidine tag. The addition of N-terminal of these tags to GDH isoforms did not affect the allosteric regulation of isoforms by ADP and GTP. N-terminal FLAG tag did not change the localization and kinetic parameters. However the presence of FLAG tag at C-terminus of mouse GDH isoform changed significantly kinetic parameters of ADP that became fivefold less sensitive to ADP activation.

E. Others

Up to now we have described the commonly used ones, but there are even more various methods performed in many researches. Wijekoon et al. [66] compared recombinant copper-binding proteins with either poly-histidine or poly- lysine tag. Because of the poly-histidine tag has affinity for Cu (I) and Cu (II) the removal of it is necessary for reliable characterization of the target proteins. However the separation by protease may release several residues that may affect the function of the protein target. In this study, addition of poly-lysine residue to copper binding protein separated by the carboxypeptidase B to produce native recombinant protein. However this removing procedure did not necessary since the poly-lysine tag does not have affinity for either Cu (I) or Cu (II). Also the presence of the C- terminal poly-lysine tag increased the isoelectric point and facilitated the purification via cation-exchange chromatography. In another study Islam et. al. [67] investigated ceramic fluorapatite-binging (CFT) peptide as affinity tag that has seven amino acids. They used tag with or without the addition of hexalysine sequence to purify green fluorescent protein (GFP). Two different combinations of this tag were placed C-terminal of recombinant GFP and CFT chromatography was applied to provide GFP purification. To compare usefulness of CFT binding peptide as tag, they also utilized hexahistidine tag and immobilized metal ion affinity chromatography (IMAC). According to results of this study; ceramic fluorapatite-binding peptides with and without hexalysine showed similar purity over 90%. Due to the limitations of IMAC, CFT chromatography and this affinity tag could be used alternative purification strategy.

Ding et al. [68] purified cytoplasmic domain of human erythrocyte band 3 (cdb3) with hexahistidine tag or chitin-binding tag. Three different expression vectors were used to compare different affinity purification. N- and C-terminal his tag and chitin-binding tag were purified by immobilized metal column chromatography and affinity chromatography on chitin resin, respectively. Results of

Copyright © 2017 IJAIR, All right reserved

International Journal of Agriculture Innovations and Research
Volume 6, Issue 3, ISSN (Online) 2319-1473

this experiment show that, all three recombinant cdb3 represented similar structural and functional feature with native cdb3. N- and C-terminal hexahistidine tagged cdb3 were obtained with higher yield than CBD-cdb3. However cleaved CBD-cdb3 was more stable than his-tagged-cdb3, after long period preservation. Jin et al. [69] used recombinant *plasmodium falciparum* reticulocyte binding protein homologue 5 (PfRH5) that is an antigen and is used to develop many types vaccines. For the purification, they utilized C-terminal C-tag that is composed of four amino acids; glutamic acid-proline- glutamic acid- alanine, which is purified with a camelid single chain antibody, called NbSyn2. C-tagged PfRH5 was obtained >85% recovery and>70% purity in a single step purification under mild conditions. In a previous experiment, Hjerrild et al. [70] utilized same protein. However they used

Table 2. Overview on the characteristics of major affinity tag methods.

Tag	Length (aa) & Approx. Size (kDa)	Binding Matrix	Elution Condition	Advantages	Disadvantages
His-tag	2-10 aa (usually 6 aa) 0.84 kDa	Metal ions (Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+},Fe^{3+})	Imidazole	• Smaller size • Less effect on target protein function and expression • Less expensive method	• High risk of contamination • It can interfere with enzyme specificity
FLAG	8 aa 1.01 kDa	Anti-FLAG monoclonal antibody	Low pH or FLAG peptide	• Quite purity yields • Minimal effect to fusion protein • Makes the protein more soluble	• Cost of resin • The use of this tag is limited
GST	211 aa 26 kDa	Glutathione	Reduced glutathione	• Higher degree purification • Increase the solubility of recombinant protein • Milder elution conditions than most of other methods • Less expensive resin	• Bigger size • Decrease expression level of recombinant protein • More expensive method
MBP	396 aa 40-42 kDa	Cross-linked amylose	Maltose	• Enhance the solubility of target • Increase the expression of protein • Efficient purification	• Its large size • Can be problematic about protein expression • Removal of MBP decreases the solubility of protein

hexahistidine tag for purification. C-terminal hexahistidine tagged PfRH5 were obtained >95% purity and <5% recovery. The using C-tag at the purification of PfRH5 has high recovery than the using his-tag, according these two studies. C-tag purification technique may be an important part of clinical tests which recombinant proteins are used, because of the obtaining them at high recovery.

Morris et. al. [71] selected recombinant proteins which do not have heparin binding affinity to investigate them with heparin-binding tag (HB-tag) to demonstrate the utility of HB-tag in the protein purification. HB-tag has 34 amino acids and binding affinity for heparin-sepharose resin. The addition of polyclonal antibodies caused the elution of tagged protein from matrix under denaturing condition, then tag was separated from protein with thrombin or TEV protease. The heparin-binding affinity tag provided single step rapid purification alternatively to other purification technique.

III. Conclusion

Glycans have very attractive roles including cell-to-cell interactions, stimulation of brain de elopement and learning skills and prebiotic activity. However their isolation is quite challenging due to lack of deglycosylation approaches. Fusion tags provide an opportunity to alter the enzymes target substrates allowing to produce specific glycan structures. Successfully application of the tags to the glycosidases will enable to produce certain glycans or let attached to the glycans to the proteins that will make further investigations feasible in the future.

References

[1] Kimple, M.E., A.L. Brill, and R.L. Pasker, *Overview of affinity tags for protein purification.* Current protocols in protein science, 2013: p. 9.9. 1-9.9.23.

[2] Demain, A.L. and P. Vaishnav, *Production of recombinant proteins by microbes and higher organisms.* Biotechnology advances, 2009. **27**(3): p. 297-306.

[3] Pina, A.S., C.R. Lowe, and A.C.A. Roque, *Challenges and opportunities in the purification of recombinant tagged proteins.* Biotechnology advances, 2014. **32**(2): p. 366-381.

[4] Baldi, L., et al., *Recombinant protein production by large- scale transient gene expression in mammalian cells: state of the art and future perspectives.* Biotechnology letters, 2007. **29**(5): p. 677-684.

[5] Stryjewska, A., et al., *Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins.* Pharmacological Reports, 2013. **65**(5): p. 1075-1085.

[6] Rosano, G.L. and E.A. Ceccarelli, *Recombinant protein expression in Escherichia coli: advances and challenges.* Frontiers in microbiology, 2014. **5**.

[7] Urh, M., D. Simpson, and K. Zhao, *Affinity chromatography: general methods.* Methods in enzymology, 2009. **463**: p. 417-438.

[8] Malhotra, A., *Tagging for protein expression.* Methods in enzymology, 2009. **463**: p. 239-258.

[9] Palomares, L.A., F. Kuri-Brena, and O.T. Ramírez, *Industrial recombinant protein production*. 2002, EOLSS Publishers, Oxford.

[10] Kittur, F.S., et al., *C-Terminally fused affinity Strep-tag II is removed by proteolysis from recombinant human erythropoietin expressed in transgenic tobacco plants.* Plant cell reports, 2015. **34**(3): p. 507-516.

[11] JAMES, J.A. and B.H. LEE, *Glucoamylases: microbial sources, industrial applications and molecular biology—a review.* Journal of Food Biochemistry, 1997. **21**(6): p. 1-52.

[12] Choi, S.J., et al., *Human coagulation factor VIII domain-specific recombinant polypeptide expression.* Blood research, 2015. **50**(2): p. 103-108.

Copyright © 2017 IJAIR, All right reserved

[13] Sainsbury, F., et al., *A Chimeric Affinity Tag for Efficient Expression and Chromatographic Purification of Heterologous Proteins from Plants.* Frontiers in plant science, 2016. **7**.

[14] Lin, S.-C., et al., *CBM21 starch-binding domain: a new purification tag for recombinant protein engineering.* Protein expression and purification, 2009. **65**(2): p. 261- 266.

[15] Ong, E., *Enzyme immobilization using the cellulose-binding domain of the Cellulomonas Fimi exoglucanase*. 1992, University of British Columbia.

[16] Wang, J., D. Bhattacharyya, and L.G. Bachas, *Improving the activity of immobilized subtilisin by site-directed attachment through a genetically engineered affinity tag.* Fresenius' journal of analytical chemistry, 2001. **369**(3): p. 280-285.

[17] Michel, P., et al., *Expression and purification of polyhistidine-tagged firefly luciferase in insect cells-a potential alternative for process scale-up.* Journal of biotechnology, 2001. **85**(1): p. 49-56.

[18] Karav, S., et al., *Characterizing the release of bioactive N-glycans from dairy products by a novel endo-β-N-acetylglucosaminidase.* Biotechnology progress, 2015. **31**(5): p. 1331-1339.

[19] Varki, A. and J.B. Lowe, *Biological roles of glycans.* 2009.

[20] Georgi, G., et al., *Functional glycans and glycoconjugates in human milk.* The American journal of clinical nutrition, 2013. **98**(2): p. 578S-585S.

[21] Parc, A.L., et al., *A novel endo-β-N-acetylglucosaminidase releases specific N-glycans depending on different reaction conditions.* Biotechnology progress, 2015. **31**(5): p. 1323- 1330.

[22] Majorek, K.A., et al., *Double trouble-Buffer selection and His-tag presence may be responsible for nonreproducibility of biomedical experiments.* Protein Science, 2014. **23**(10): p. 1359-1368.

[23] Lee, Y.-L., et al., *C-terminal His-tagging results in substrate specificity changes of the thioesterase I from Escherichia coli.* Journal of the American Oil Chemists' Society, 1999. **76**(10): p. 1113-1118.

[24] Young, C.L., Z.T. Britton, and A.S. Robinson, *Recombinant protein expression and purification: a comprehensive review of affinity tags and microbial applications.* Biotechnology journal, 2012. **7**(5): p. 620-634.

[25] Arnau, J., et al., *Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins.* Protein expression and purification, 2006. **48**(1): p. 1-13.

[26] Chaga, G.S., *Twenty-five years of immobilized metal ion affinity chromatography: past, present and future.* Journal of biochemical and biophysical methods, 2001. **49**(1): p. 313-334.

[27] Khan, F., M. He, and M.J. Taussig, *Double-hexahistidine tag with high-affinity binding for protein immobilization, purification, and detection on Ni− nitrilotriacetic acid surfaces.*

[28] Analytical chemistry, 2006. **78**(9): p. 3072-3079.

[29] Karikari, T.K., et al., *Expression and purification of tau protein and its frontotemporal dementia variants using a cleavable histidine tag.* Protein expression and purification, 2017. **130**: p. 44-54.

[30] Panek, A., et al., *Effects of the polyhistidine tag on kinetics and other properties of trehalose synthase from Deinococcus geothermalis.* Acta Biochim Pol, 2013. **60**: p. 163-166.

[31] Bewley, M.C., et al., *A non-cleavable hexahistidine affinity tag at the carboxyl-terminus of the HIV-1 Pr55 Gag polyprotein alters nucleic acid binding properties.* Protein expression and purification, 2017. **130**: p. 137-145.

[32] Zhao, D. and Z. Huang, *Effect of His-Tag on Expression, Purification, and Structure of Zinc Finger Protein, ZNF191 (243-368).* Bioinorganic chemistry and applications, 2016. **2016**.

[33] Smith, D.B. and K.S. Johnson, *Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase.* Gene, 1988. **67**(1): p. 31-40.

[34] Vikis, H.G. and K.-L. Guan, *Glutathione-S-transferase-fusion based assays for studying protein-protein interactions.* Protein-protein interactions: methods and applications, 2004: p. 175-186.

[35] Terpe, K., *Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems.* Applied microbiology and biotechnology, 2003. **60**(5): p. 523-533.

[36] Harper, S. and D.W. Speicher, *Purification of proteins fused to glutathione S-transferase.* Protein Chromatography: Methods and Protocols, 2011: p. 259-280.

[37] Maity, R., et al., *GST-His purification: a two-step affinity purification protocol yielding full-length purified proteins.* Journal of visualized experiments: JoVE, 2013(80).

[38] Walker, P.A., *Efficient and Rapid Affinity Purification of Proteins using Recombinant Fusion Proteases.* Bio/technology, 1994. **12**.

[39] Esposito, D. and D.K. Chatterjee, *Enhancement of soluble protein expression through the use of fusion tags.* Current opinion in biotechnology, 2006. **17**(4): p. 353-358.

[40] Hayashi, K. and C. Kojima, *pCold-GST vector: a novel cold-shock vector containing GST tag for soluble protein production.* Protein expression and purification, 2008. **62**(1): p. 120-127.

[41] Dojima, T., et al., *Comparison of the efficiencies of different affinity tags in the purification of a recombinant secretory protein expressed in silkworm larval hemolymph.* Biotechnology and Bioprocess Engineering, 2009. **14**(3): p. 281-287.

[42] Ryan, D.P. and D.J. Tremethick, *A dual affinity-tag strategy for the expression and purification of human linker histone H1. 4 in Escherichia coli.* Protein expression and purification, 2016. **120**: p. 160-168.

[43] Sun, P., J.E. Tropea, and D.S. Waugh, *Enhancing the solubility of recombinant proteins in Escherichia coli by using hexahistidine-tagged maltose-binding protein as a fusion partner.* Heterologous Gene Expression in E. coli: Methods and Protocols, 2011: p. 259-274.

[44] Sachdev, D. and J.M. Chirgwin, *[20] Fusions to maltose-binding protein: Control of folding and solubility in protein purification.* Methods in enzymology, 2000. **326**: p. 312- 321.

[45] Pattenden, L.K. and W.G. Thomas, *Amylose affinity chromatography of maltose-binding protein: purification by both native and novel matrix-assisted dialysis refolding methods.* Affinity Chromatography: Methods and Protocols, 2008: p. 169-190.

[46] Kimple, M.E. and J. Sondek, *Overview of affinity tags for protein purification.* Current Protocols in Protein Science, 2004: p. 9.9. 1-9.9. 19.

[47] Kosobokova, E., K. Skrypnik, and V. Kosorukov, *Overview of fusion tags for recombinant proteins.* Biochemistry (Moscow), 2016. **81**(3): p. 187-200.

[48] Zhao, X., G. Li, and S. Liang, *Several affinity tags commonly used in chromatographic purification.* Journal of analytical methods in chemistry, 2013. **2013**.

[49] Tolun, A.A., I.M. Dickerson, and A. Malhotra, *Overexpression and purification of human calcitonin gene- related peptide–receptor component protein in Escherichia coli.* Protein expression and purification, 2007. **52**(1): p. 167-174.

[50] Raran-Kurussi, S. and D.S. Waugh, *A dual protease approach for expression and affinity purification of recombinant proteins.* Analytical biochemistry, 2016. **504**: p. 30-37.

[51] Sluchanko, N.N., et al., *High-yield soluble expression, purification and characterization of human steroidogenic acute regulatory protein (StAR) fused to a cleavable Maltose- Binding Protein (MBP).* Protein expression and purification, 2016. **119**: p. 27-35.

[52] Hang, B., et al., *High-level production of aquaporin Z in Escherichia coli using maltose-binding protein/polyhistidine dual-affinity tag fusion system.* Process Biochemistry, 2016. **51**(5): p. 599-606.

[53] Cho, H.-J., et al., *Maltose binding protein facilitates high- level expression and functional purification of the chemokines RANTES and SDF-1α from Escherichia coli.* Protein expression and purification, 2008. **60**(1): p. 37-45.

[54] Hopp, T.P., et al., *A short polypeptide marker sequence useful for recombinant protein identification and purification.* Biotechnology, 1988. **6**(10): p. 1204-1210.

[55] Schmidt, P.M., et al., *Taking down the FLAG! How insect cell expression challenges an established tag-system.* PLoS One, 2012. **7**(6): p. e37779.

[56] Pradeau-Aubreton, K., et al., *Vectors for recombinational cloning and gene expression in mammalian cells using modified vaccinia virus Ankara.* Analytical biochemistry, 2010. **404**(1): p. 103-105.

[57] Brizzard, B.L., R. Chubet, and D. Vizard, *Immunoaffinity purification of FLAG epitope-tagged bacterial alkaline phosphatase using a novel monoclonal antibody and peptide*

Copyright © 2017 IJAIR, All right reserved

elution. Bio techniques, 1994. **16**(4): p. 730-735.
[58] Einhauer, A. and A. Jungbauer, *the FLAG™ peptide, a versatile fusion tag for the purification of recombinant proteins.* Journal of biochemical and biophysical methods, 2001. **49**(1): p. 455-465.
[59] Einhauer, A. and A. Jungbauer, *Affinity of the monoclonal antibody M1 directed against the FLAG peptide.* Journal of chromatography A, 2001. **921**(1): p. 25-30.
[60] Hopp, T.P., B. Gallis, and K.S. Prickett, *Metal-binding properties of a calcium-dependent monoclonal antibody.* Molecular immunology, 1996. **33**(7): p. 601-608.
[61] Allen, S.J., et al., *Comparison of optimised isotherm models for basic dye adsorption by kudzu.* Bio resource Technology, 2003. **88**(2): p. 143-152.
[62] Hernan, R., K. Heuermann, and B. Brizzard, *Multiple epitope tagging of expressed proteins for enhanced detection.* Bio techniques, 2000. **28**(4): p. 789-793.
[63] Ueda, M., Y. Manabe, and M. Mukai, *the high performance of 3XFLAG for target purification of a bioactive metabolite: a tag combined with a highly effective linker structure.* Bioorganic & medicinal chemistry letters, 2011. **21**(5): p. 1359-1362.
[64] Maroux, S., J. Baratti, and P. Desnuelle, *Purification and specificity of porcine enterokinase.* Journal of Biological Chemistry, 1971. **246**(16): p. 5031-5039.
[65] Futatsumori-Sugai, M., et al., *Utilization of Arg-elution method for FLAG-tag based chromatography.* Protein expression and purification, 2009. **67**(2): p. 148-155.
[66] Pajęcka, K., et al., *Glutamate dehydrogenase isoforms with N-terminal (His) 6-or FLAG-tag retain their kinetic properties and cellular localization.* Neurochemical research, 2014. **39**(3): p. 487-499.
[67] Wijekoon, C.J., et al., *Evaluation of employing poly-lysine tags versus poly-histidine tags for purification and characterization of recombinant copper-binding proteins.* Journal of inorganic biochemistry, 2016. **162**: p. 286-294.
[68] Islam, T., et al., *A novel strategy for the purification of a recombinant protein using ceramic fluorapatite-binding peptides as affinity tags.* Journal of Chromatography A, 2014. **1339**: p. 26-33.
[69] Ding, Y., et al., *Expression and purification of recombinant cytoplasmic domain of human erythrocyte band 3 with hexahistidine tag or chitin-binding tag in Escherichia coli.* Protein expression and purification, 2004. **34**(2): p. 167-175.
[70] Jin, J., et al., *Accelerating the clinical development of protein-based vaccines for malaria by efficient purification using a four amino acid C-terminal 'C-tag'.* International Journal for Parasitology, 2017.
[71] Hjerrild, K.A., et al., *Production of full-length soluble Plasmodium falciparum RH5 protein vaccine using a Drosophila melanogaster Schneider 2 stable cell line system.* Scientific reports, 2016. **6**: p. 30357.
[72] Morris, J., et al., *Heparin-binding peptide as a novel affinity tag for purification of recombinant proteins.* Protein expression and purification, 2016. **126**: p. 93-103.

AUTHORS' PROFILES

Dr. Sercan Karav received his PhD from University of California, Davis in 2015. After 2 years post doctorate studies, he has started his faculty position at Canakkale Onsekiz Mart University. His research areas are glycobiology, gut microbiome, enzymology and microbiology. Phone No. : +90551 386 6466

Recent Publications:

Karav S, Le Parc A, de Moura JMLN, Frese SA, Kirmiz N, Block DE, et al. Oligosaccharides released from milk glycoproteins are selective growth substrates for infant-associated bifidobacteria. Appl Environ Microb. 2016; 82(12): 3622-30.

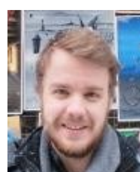
Karav S, Bell JM, Parc AL, Liu Y, Mills DA, Block DE, et al. Characterizing the release of bioactive N-glycans from dairy products by a novel endo-beta-N- acetylglucosaminidase. Biotechnol Prog. 2015. Epub 2015/06/23. doi: 10.1002/btpr.2135. PubMed PMID: 26097235.

Karav S, Parc AL, Moura Bell JM, Rouquie C, Mills DA, Barile D, et al. Kinetic characterization of a novel endo-beta-N-acetylglucosaminidase on concentrated bovine colostrum whey to release bioactive glycans. Enzyme Microb Technol. 2015; 77: 46-53. Epub 2015/07/04. doi: 10.1016/j.enzmictec.2015.05.007. PubMed PMID: 26138399.

Karav S, German JB, Rouquie C, Le Parc A, Barile D. Studying Lactoferrin N- Glycosylation. International journal of molecular sciences. 2017; 18(4): 870.

Parc AL, Karav S, Maria Leite Nobrega de Moura Bell J, Frese SA, Liu Y, Mills DA, et al. A novel endo-beta-N-acetylglucosaminidase releases specific N-glycans depending on different reaction conditions. Biotechnology progress. 2015. Epub 2015/06/24. doi: 10.1002/btpr.2133. PubMed PMID: 26101185.

Le Parc, A., Karav, S., Rouquie, C., Maga, E. A., Bunyatratchata, A., & Barile, D. (2017). Characterization of recombinant human lactoferrin N-glycans expressed in the milk of transgenic cows. *PloS one*, *12*(2), e0171477.



A. Ozleyen
Was born on February 7, 1995 in Manisa, Turkey. He has been studied in the department of Molecular Biology and Genetics at Canakkale Onsekiz Mart University since 2014. He studied at University of Ostrava, Czech Republic, in the Biology and Ecology Department for the 2016 fall semester as an Erasmus student. Now he is working on the project related to anti-inflammatory bioactive phenolic compounds. He is interested in biochemistry, pharmacology & molecular biology.

E. Talak
Was born on September 9, 1994 in Samsun, Turkey. She is an undergraduate student in the department of Molecular Biology and Genetics at Canakkale Onsekiz Mart University. She is interested in molecular genetics, regenerative biology and stem cell biology.

M. Tuncer
Was born in İstanbul (Turkey) on the 13st January 1995. She is an undergraduate student in the department of Molecular Biology and Genetics at Canakkale Onsekiz Mart University. She is interested in biochemistry and molecular genetics.

Copyright © 2017 IJAIR, All right reserved