PROPOSAL ABSTRACT:

Must be clear and informative. Describe the main issues you plan to address, including goals, methodology and expected outcomes. A good summary facilitates an adequate description and understanding of what you intend to achieve. If selected, this abstract may be published in CONICYT's web page. **The maximum length for this section is 1 page. (Arial or Verdana font size 10 is suggested)**.

Protein purification, at large scale, is mainly carried out through chromatographic techniques, amongst which are Affinity Chromatography, Ion Exchange Chromatography, Hydrophobic Interaction Chromatography and Gel Filtration Chromatography. Each of these techniques exploits a specific feature of the target protein relative to the rest of the contaminant proteins. Unfortunately, in some cases there is no specific characteristic that facilitates such purification. For this reason, in some cases genetic engineering has been used to modify the target protein by fusing polypeptide tags, that give particular features, to target proteins. The most widely used polypeptide tags are histidine tags, which allow fused proteins to be purified by Affinity Chromatography. Unfortunately, this chromatographic technique is, in some cases, very expensive and incompatible to be used at large scale. For this reason, the use of polypeptide tags exploiting other properties such as hydrophobicity has been considered.

Although adding hydrophobic tags has advantages, they also show disadvantages such as loss of protein stability, a decrease in protein expression levels, and aggregate formations among fusion proteins, resulting in loss of recovery levels of the fusion protein. Although data exist on this loss, no studies assessing quantitatively and systematically the effect of these tags on the recovery, purification and stability of these fusion proteins have been reported.

The purpose of this project is to study the effect of hydrophobic polypeptide tag fusion upon protein stability, solubility, recovery, purity, and specific activity levels, and thus define the criteria allowing the identification of which tag type should be fused to purify a target protein. Such criteria will be based upon both quantitative parameters (hydrophobicity of polypeptide tags, hydrophobicity increase in tagged proteins, etc), and qualitative parameters. Additionally, the criteria defined for this project can serve as the basis for future work in which criteria are defined in case another type of tag is used.

In order to achieve this objective, work will be done with genes from different proteins to which hydrophobic polypeptide tags will be fused so as to facilitate their purification by Hydrophobic Interaction Chromatography. Once the protein has been expressed, its purification process will be assessed, and, based on recovery and purity levels, criteria will be defined to characterize each tag type. Among the major problems foreseen are two. The first is the capacity to carry out the fermentation stage in the best way. For this purpose, different operation ways will be assessed, among which is batch and fed batch. The second problem has to do with the possible additions and/or misfolding of the recombinant proteins due to their increase in hydrophobicity. In this case it is intended to perform the induction stages at low temperatures, or include the co-expression of molecular chaperones (e.g. DnaK-DnaJ-GrpE, GroEL-ES, etc.).

Finally, in global terms, the significance and novelty of this project will be in the systematization of the available insight to select the most adequate hydrophobic tags which allow for the production and purification of a desired protein in an optimum way.

PROPOSAL DESCRIPTION, THEORETICAL BACKGROUND AND LITERATURE REVIEW:
This section must include a general presentation of the problem to investigate. Describe the novel aspects you intend to address and present a critical review of the literature on the state of the art of the research on the proposal topic. **The maximum length of this section is 8 pages (Arial or Verdana font size 10 is suggested)**. Use additional sheets to list your cited references.

Proposal Description

Today, the limiting stages in protein production are no longer the strain selection or fermentation stages but those of recovery and purification (downstream processing, DSP), so most of the research efforts are focused on DSP optimization and its integration to fermentation processes (Asenjo and Andrews, 2004). On the other hand, the biotechnological industry has the disadvantage of having the obligation to validate all of the stages involved in the production process; therefore, optimization of the process from the very first conception is necessary. In general, purification stages are selected by trial and error, so optimization of such selection would mean significant progress in the global process of protein production.

For protein purification at industrial level chromatographic techniques are mainly used, the most widely used being Affinity Chromatography (AC), Ion Exchange Chromatography (IEX), Hydrophobic Interaction Chromatography (HIC) and Gel Filtration Chromatography (GFC). Each of these techniques exploits physicochemical and biochemical differences between the target protein and the contaminants of the mixture to be purified. Thus, Affinity Chromatography exploits specific biochemical interactions between the protein and the matrix ligand; Ion Exchange Chromatography exploits electrostatic interactions at different pH; Hydrophobic Interaction Chromatography is based on hydrophobic interactions, and Gel Filtration Chromatography is based on size differences. In the ideal case that such differences are significant, high joint purity can be obtained at a high recovery level. Unfortunately, this is not a usual situation, so alternatives have been sought to increase protein affinity to specific chromatographic ligands, modifying the target protein properties (Nygren et al, 1994). Some of the alternatives for such modifications are the addition or fusion of polypeptide tags by genetic engineering (Terpe, 2003). These polypeptide tags are aminoacid sequences added to a protein (called fusion protein) so as to give some particular feature, without a significant alteration of the biological and/or physicochemical features of the proteins. Several work has been reported in which these tags have been added, facilitating purification of the target protein. Although addition of these tags shows some advantages, it also shows some disadvantages. Such advantages may be seen both at the protein expression level and upon recovery. All this leads to loss in global recovery levels of the target protein. Although data exist on this loss, no studies assessing quantitatively and systematically the effect of these tags on the global recovery levels of these fusion proteins have been reported

The aim of this project is to study the effect of hydrophobic polypeptide tag fusion upon protein stability, solubility, recovery, purity, and specific activity levels, and thus define the criteria allowing the identification of which tag type should be fused to purify a target protein. Such criteria will be based upon both quantitative parameters (tag hydrophobicity, hydrophobicity increase in tagged proteins, etc), and qualitative parameters. Additionally, the criteria defined for this project can serve as the basis for future work in which criteria are defined in case another type of tag is used.

In order to achieve this objective, work will be done with genes from different proteins to which hydrophobic polypeptide tags will be fused so as to facilitate their purification by Hydrophobic Interaction Chromatography. Once the protein has been expressed, its purification process will be assessed, and, based on recovery and purity levels; criteria will be defined to characterize each tag type. Among the major problems foreseen are two. The first is the capacity to carry out the fermentation stage in the best way. For this purpose, different operation ways will be assessed, among which is batch and fed batch. The second problem has to do with the possible additions and/or misfolding of the recombinant proteins due to their increase in hydrophobicity. In this case it is intended to perform the induction stages at low temperatures, or include the co-expression of molecular chaperones (e.g.

DnaK-DnaJ-GrpE, GroEL-ES, etc.).

Finally, in global terms, the significance and novelty of this project will be in the systematization of the available insight to select the most adequate hydrophobic tags which allow for the production and purification of a desired protein in an optimum way.

LITERATURE REVIEW

Use of polypeptide tags for protein purification

In order to facilitate protein purification, proteins can be modified by genetic engineering. Among those alternatives is the modification of superficial properties of the proteins (Flaschel and Friehs, 1993) or fusion or addition of affinity tags or polypeptide tags (Nygren et al, 1994, Finn et al, 2005). This project will focus on polypeptide **tag fusion**. These polypeptide tags are aminoacid sequences which are added to a protein to give it a particular feature, e.g. changes in superficial hydrophobicity, charge, attraction by metallic chelate; with this it increases selectivity in a given purification type, without altering the biological and/or physicochemical features of the protein.

Several affinity tags are available, ranging from small peptide sequences to fusion partners with size and complexity similar to a protein. For example, His-tag, Arg- tag, calmodulin-binding peptide (CBP), cellulase-binding domain (CBD), DsbA, c-myc-tag, glutathione S-transferase (GST), FLAG-tag, HAT-tag, , maltose-binding protein (MBP), NusA, S-tag, SBP-tag, Strep II-tag, thioredoxin, Biotin acceptor peptide (BAP), etc. (Terpe,2003; Esposito and Chatterjee, 2006) and short hydrophobic peptide tags, e.g. (TrpPro)2 , (TrpPro)4, (Tyr)3 (TyrPro)3, (Tyr)3(Pro)2 , (Tyr)4, (TyrPro)4, (Tyr)6, (Tyr)6(Pro)2, (Tyr)8 (Nilsson et al 2002, Fexby and Bulow 2004; Bernaudat and Bulow 2006,). All of these are fused to the C-terminal or N-terminal, modifying one or more properties of the protein, such as affinity, hydrophobicity, charge, solubility, etc. (Steffens et al, 2000b). In some cases, fusion to the N-terminal may have an influence upon protein folding, and for that reason fusion to the C-terminal is preferred (Fexby and Bulow, 2004).

Polypeptide tags show numerous advantages: (a) they need genetically fewer modifications in the target product; (b) as they are small particles, they have a minimum impact on the tertiary structure and biological activity of the fusion protein; (c) they are relatively easy to eliminate by means of enzymes that cut them, and for some applications they may not need to be cut; (d) separation techniques are not usually expensive – as they are available at large scale – and they can be applied to a wide range of proteins. For these reasons, this project focuses on **polypeptide tags** (Terpe, 2003).

The most widely used polypeptide tags are: (a) polyarginine (5-6 arginines) for purification by cationic exchange (Sassenfeld and Brewer, 1984; Niederauer et al, 1994; Bandmann et al, 2000); (b) polyhistidine (between 2 and 10, generally 6 histidines, His6) for purification of the immobilized metal ion affinity chromatography type (IMAC), where there is an interaction between the histidine and divalent ions such as nickel, copper, zinc or cobalt linked to a matrix (Hochuli et al, 1987, Salazar et al, 2001; Ueda et al, 2003); (c) hydrophilic sequence FLAG (DYKDDDK) (Hopp et al,1988), (d) Strep II -tag sequence (WSHPQFEK) (Schmidt and Skerr, 1993; Schmidt et al. 1996); (e) c-myc sequence (EQKLISEEDL) for purification with monoclonal antibody matrices (Evans et al, 1985); (f) hydrophobic sequences (4 to 8 amino acids of the (WP)n or (YP)n) type (Rodenbrock et al, 2001; Berggren et al, 2000; Kepka et al, 2005; Collén et al 2001a; Fexby et al, 2004), among others. Recently, studies have been published in which the use of tag combinations is suggested, so that one of them may yield more affinity and another provides properties of higher solubility (Waugh, 2005; Esposito and Chatterjee, 2006). For example, combined hydrophobic-metal binding fusion tags (FH6, WH6 and YH6) for applications in two-phase aqueous partitioning (Bernaudat and Bulow, 2006) and for purification of recombinant immunogens (Andersson et al, 2000).

Additionally, these tags may be used, in some cases, to promote secretion of the target protein, and be useful in testing based on enzymatic activity or else linked to antibodies. Most of these tags do not interfere significantly with the biological activity of the protein, and in some cases they tend to stabilise it, e.g. by resistance to intracellular proteolysis (Waugh, 2005). Furthermore, a specific cleavage location may be included, so that the tag could be removed at the purification stage (Ford et al, 1991), using specific proteases such as TEV, 3C, Xa,Entk,Thr and Caspase (Esposito and Chatterjee, 2006).

If tags exploiting biological affinity features are compared to tags exploiting other features, such as charge or hydrophobicity, the main disadvantages of the former is the high cost of resins, and those resins are highly sensitive to high pH, a condition that is usually used in situ industrial cleaning processes, which does not happen if ion exchange and hydrophobic interaction resins are used (Fexby and Bulow, 2004). Additionally, hydrophobic interactions, especially those produced by aromatic amino acidic residues, are highly selective; therefore, small changes can facilitate isolation and purification of one protein (Lo Conte et al, 1999).

For the reasons stated above, this project will focus on **hydrophobic polypeptide tags**. Below is a description of their main features.

Hydrophobic polypeptide tags

The most widely used hydrophobic polypeptide tags in protein purification have been those containing polytryptophan (Rodenbrock et al,2001; Berggren et al. 2000; Kepka et al, 2005, Collén et al, 2001a), polyphenilalanine and polycysteine (Persson et al, 1988), polyisoleucine (Hassinen et al, 1994), and polytyrosine (Fexby and Bulow 2002, Fexby et al, 2004, Bandmann et al, 2000). Some of them and their applications are shown in Table Nº 1.

Table N 1 Examples of hydrophobic polypeptide tags

Polypeptide tag	Application
(F)11	Beta-galactosidase (Persson et al, 1988)
(C)n	Galactokinase (Persson et al, 1988)
AIIP AIIPAIIP	Staphylococcal protein A (Hassinen et al, 1994; Eiteman et al,1994)
AWWP AWWPAWWP	Staphylococcal protein A (Hassinen et al, 1994; Eiteman et al,1994)
(WP)2 (WP)4	Cutinase (Rodenbrock et al,2001), ZZ Cutinase (Berggren et al, 2000; Kepka et al, 2005) Endoglucanase I (Collén et al, 2001a)
(Y)3 (YP)3 (Y)3(P)2	Lactate dehydrogenase (Fexby and Bulow 2002) Green fluorescent protein (Fexby et al, 2004)
(Y)6 (Y)6(p)2	Lactate dehydrogenase (Fexby and Bulow 2002)
(Y)4 (YP)4 (Y)8	ZZ Cutinase (Bandmann et al, 2000)

As seen in Table Nº 1, tag designs do not usually have more than eight amino acids, and include one or more hydrophobic amino acidic residue (tryptophan and tyrosine), and in some cases, proline residues. The latter is a rigid amino acid that prevents formation of secondary structures in tags, and allows an increase in the exposure degree of the hydrophobic tag.

Several examples exist of successful applications of these tags in terms of the purity level or recovery level obtained, e.g. cutinase purification (Kepta et al, 2005; Calado et al, 2002) and lactate dehydrogenase purification (Fexby et al, 2004). In the particular case of cutinase (cut), they have shown an improvement in purification levels, but they have also shown a decrease in protein expression levels. Thus, mutant cut_ (wp)4 shows much lower levels than cut_ (wp)2 and wild cutinase. These levels are shown in the table below:

Table 2 Parameters of Cutinase Production and Purification Processes by HIC (Lienqueo et al., 2005).

Protein	Specific cell-activity [a] (U/ mg dry cell weight)	Recovery yield after HIC (%)	Purity Level after HIC (%)
Cut-wt	4.8	100	89
Cut-(WP)2	2.8	84	97
Cut-(WP)4	0.3	70	99

[a] Specific cell-activity after 72-hour cultivation

On the other hand, studies with lactate dehydrogenase (Fexby et al, 2004), where the protein was modified with different tags, showed that protein expression is affected by tag fusion at the N-terminal. Of 17 cases studied, only one modification, the GluAsnAlaAspVal tag, increased expression levels.

Both studies agree on the fact that there are tags which, although they can cause an improvement in purification processes, they cause modifications and, mainly, a decrease in expression levels of fusion proteins.

Examples of negative side effects obtained from fusion of small tags to the protein are: (a) they can result in mRNA stability changes that affect the expression levels; (b) effects in efficiency in translation or a rare codon can interfere with ribosome trafficking; (c) disruptions or misfolding in the protein folding process; (d) can cause the protein to be membrane-associated (Persson et al, 1988); (e) can result in protein associations in dimmers or larger aggregates, partly or fully insoluble (Johansson and Walter, 2000); (f) can cause denaturing of tagged proteins upon eluting an HIC column (Koehler et al, 1991), due to a strong interaction between the ligand and the protein tag; (g) can promote proteolysis of the target protein (Hassinen et al, 1994; Collen et al,2001b).

Of the aforementioned effects, the first three are related to fusion protein expression and release, and some information exists which shows that, although protein recovery levels are affected, these effects can be decreased if an adequate cultivation strategy is considered, for example fedbatch cultivation (Calado et al, 2002; Calado et al, 2003, Calado et al, 2004). For the case of disruption or misfolding in the protein folding process and they accumulate as soluble and insoluble nonfunctional aggregates, a general strategy to improve the native folding of tagged proteins is to: (a) increase the cellular concentration of viscous organic compounds (de Marco et al., 2005); (b) include co-expression of molecular chaperones and folding modulators (e.g. DnaK-DnaJ-GrpE, ClpB,GroEL-GrpEs) that can prevent aggregation and can actively scavenge and convert aggregates into natively refoldable species (de Marco A. and de Marco V., 2004); (c) reduce induction of the protein temperature (Hammarstrom et al., 2002); (d) incorporate different promoters or induction conditions (Qing G et al., 2004), among others. For the rest, that is, protein denaturizing, loss of recovery has been reported, but not assessed quantitatively so as to establish which polypeptide tag can be the most convenient upon implementing a productive system. It is this point on which this project is focused, defining quantitative parameters that allow to discriminate between favorable and unfavorable tags

Quantitative parameter for defining criteria

A quantitative parameter should be based on the surface hydrophobicity of a tagged protein (equation 1) defined by Simeonidis et al (2005).

$$\Phi_{tagged\_protein} = \sum_{i=1}^{20}\left(\frac{s_{aai}}{s_p}\cdot\phi_{aai}\right) + \sum_{k=1}^{20}\left(\frac{s_{tag\_aak}\cdot n_k}{s_p + \Sigma(s_{tag\_aak}\cdot n_k)}\cdot\phi_{aak}\right) \qquad \text{(eq.1)}$$

where i (i= 1,..,20) indicates the 20 different amino acids, ϕ_{aai} is the value of the hydrophobicity assigned to amino acid "i" using the Miyazawa-Jernigan scale [22]. s_{aai} is the total exposed area of the amino acid residue "i" in the tagged protein. s_p is the total surface of the tagged protein. n_k is the number of amino acids "k" in the tag, s_{tag_aak} is the fully exposed surface of amino acid "k" in the tag

This definition has been used by Lienqueo et al (2007) for predicting the retention time of cutinases tagged with hydrophobic peptides in hydrophobic interaction chromatography.

Additionally, the tag hydrophobicity (equation 2) could be used as another quantitative parameter.

$$\Phi_{tag} = \sum_{k=1}^{20}\left(s_{tag\_aak}\cdot n_k\cdot\phi_{aak}\right) \qquad \text{(eq.2)}$$

Selection of an optimal tag

One way of selecting the optimal tag is by assessing multiple tags generated randomly (Terpe, 2003), which is a very expensive alternative in terms of resources and time. Another alternative is a systematic design, which considers the most widely used tags, the characteristics and purpose of the protein to be purified, and the expression system to be used. Tag selection will also depend upon the physicochemical properties of contaminants; for example, if most of contaminants are hydrophilic, it is convenient to have a hydrophobic tag allowing purification by Hydrophobic Interaction Chromatography.

Protein production, recovery and purification processes have usually been optimised unit by unit; for this reason, interest exists to have a procedure to determine the purification sequence considering the global process instead of each unit on a separate basis. Methodologies based on the optimization of chemical processes have been extended to the synthesis of optimal bioprocesses (Leser and Asenjo, 1994; Groep et al, 2000; Sttefens et al, 2000a, Vasquez-Alvarez and Pinto, 2004, Lakhdar et al., 2005, Lakhdar et al., 2006). These methodologies include heuristics based on information of physicochemical properties, so as to solve the synthesis process by reducing the search scope. Those methodologies have not considered the advantage of including modifications of the physiochemical properties of the product, such as polypeptide tags, in order to facilitate purification and diminish the number of stages in the global process.

Recent work on optimal purification sequence selection has included the design of polypeptide tags (Sttefens et al, 2000b, Simeonidis et al, 2005). Steffens et al, (2000b), the use of combined methods to generate the best tag to be fused to a protein, showing "in silico" that processes having a few units, high recovery levels and low costs can be obtained. In order to obtain the optimal sequence, a cost function is minimised using genetic algorithm software, considering the net charge and hydrophobicity of the amino acids making up the tag as the main properties to exploit. For the prediction of retention times, models for net charge (Mosher et al, 1993) and hydrophobicity (Hopp and Woods, 1981) were used. In this work, purification of Bovine Somatotropine (BST) was simulated and purification process diagrams of the fused protein were obtained , which have higher recovery levels and lower costs than with the original protein. However, relatively long tags were considered in this design (12-15 amino acids), and the possible interactions that can exist among those tags were not considered; nor were the needs to keep those amino acids exposed, or possible loss in recovery.

On the other hand, Simeonidis et al, 2005 use a whole non-linear programming model (MINLP), in which the target function is to minimise the number of chromatographic stages and length of the tag (with a maximum of eight amino acids). At the same time, they force themselves to get a specific purity level. For this purpose, it exploits the properties of the target proteins, the possible tags, and contaminants (hydrophobicity, charge, molecular weight). To carry out such optimisation, it uses various models that allow prediction of behaviour of both the target protein and the system's contaminants. In general terms, the algorithm determines the composition of the shortest and most advantageous tag for the process, which implies a minimal number of stages. The main assumptions this model considers are (a) the tag is completely exposed on the surface; (b) the tag does not make secondary structures (α-helices, β sheets) and it does not interfere with the tertiary structure of the protein; (c) it is thought that no loss exists in any stage; (d) protein-protein interactions are negligible. These last two assumptions, depending on the tag type selected, such as hydrophobic tags, may not be valid, due to the multiple interactions that can be generated between these tags (Fexby and Bulow, 2004). Particularly, the tags suggested by this model mainly present hydrophobic amino acids, but do not secure their exposure to the surface, conditions that should be validated by experimental studies; loss of products, protein-protein interaction and inclusion of buffer change and concentration stages should also be studied. Additionally, Giaverini (2005) developed a model based on Simeonidis´s model. However, the model proposed by Giaverini considers a finite number (26) of widely used polypeptide tags and the objective function was the maximization of the purification process profit. This model was tested by cutinase purification, and the results showed that the selected tag was Strep-tag II, and HIC the chromatographic step. (Lienqueo et al, 2007b)

In general terms, neither model prioritises the most widely used tags, basically due to the fact that **there is no systematic information available and/or quantitative parameters that allow recommendation of a given tag type** for the purification of a protein in a given expression system.

For this reason, this project proposes a systematic study on the effect of hydrophobic polypeptide tags on the production, recovery and purification of fusion proteins, so as to define the criteria that can be based upon quantitative and/or qualitative parameters for selecting the best hydrophobic tag to facilitate the protein purification process by hydrophobic interaction chromatography, along with the global protein production process.

REFERENCES

- Andersson C, Sandberg L, Wernerus H, Johansson M, Lovgren-Bengtsson K, Stahl S. (2000), J Immunol Methods.238(1-2):181.
- Asenjo JA , Andrews BA (2004) J. Molecular Recognition 17 (3): 236
- Bandmann N, Collet E, Leijen J, Uhlen M, Veide A, Nygren PA. (2000) J Biotechnol. 79:161.
- Bernaudat F, Bulow L (2006) Protein Expression and Purification 46 :438.
- Berggren K, Tjerneld F, Veide A. (2000) Bioseparation.9:69.
- Calado CRC, Mannesse M, Egmond M, Cabral JM, Fonseca LP (2002), Biotechnol Bioeng 78: 692.
- Calado CRC, Almeida C, Cabral JMS, Fonseca LP (2003) J Bioscience Bioengineering 96 :141
- Calado CRC, Ferreira BS,da Fonseca MMR, Cabral JM, Fonseca LP (2004) J Biotechnology 109: 147
- Collén A, Ward M, Tjerneld F., Stålbrand H.(2001a). J. Biotechnol. 87:179.
- Collén A, Ward M, Tjerneld F., Stålbrand H (2001b) J. Chromatogr A 910: 275.
- de Marco A, De Marco V (2004). J Biotechnol , 109:45-52.
- de Marco A,Vigh L, Diamant S, Goloubinoff P (2005) Cell Stress & Chaperones 10 (4), 329.
- Eiteman MA, Hassinen C, Veide A. (1994) Biotechnol Prog. 10(5):513.
- Esposito D, Chatterjee DK. (2006) Curr Opin Biotechnol.17(4):353.
- Evan GI, Lewis GK, Ramsay G, Bishop JM (1985) IMol Cell Biol 5:3610.
- Flaschel E, Friehs K. (1993) Biotechnol Adv.11:31.
- Fexby S ,Bulow L (2002) Protein Expr. Purif. 25: 263.
- Fexby S,Ihre H,Van Alstine J,Bulow L (2004) J Chrom B, 807:25.
- Fexby S.,Bulow L (2004) Trends Biotechn, 22 (10):511.
- Finn RD, Kapelioukh I, Paine MJ. (2005) Biotechniques 38(3):38.
- Ford CF, Suominen I, Glatz CE. (1991) Protein Expr Purif. 2(2-3):95.
- Giaverini O (2005) "Optimization of polypeptide tags for protein purification", Thesis of Chemical Engineering, University of Chile.
- Groep ME, Gregory ME, Kershenbaum LS,Bogle IDL (2000) Biotechnol.Bioeng.67 (3): 300
- Hammarstrom M, Hellgren N, van Den Berg S, Berglund H, Hard T:. (2002) Protein Sci 11:313.
- Hassinen C., Köhler K., Veide A. (1994). J. Chromatogr. A. 668, 121.
- Hochuli E, Dobeli H, Schacher A (1987) J. Chromatogr., 411:177.
- Hopp TP, Pricket KS, Price V L, Libby R T, March C J, Cerretti DP, Urdal DL, Conlon P J(1988). Bio/Technology, 6:1204.
- Hopp TP, Woods KR (1981). Proc. Nat. Acad. Sciences, 78:3824.
- Johansson G., Walter H. (2000). Int. Rev. Cytol. 192: 33.
- Kepka C, Collet E, Roos F, Tjerneld F, Veide A (2005) J Chromat A 1075:33.
- Koehler K, Ljungquist C, Kondo A, Veide A, Nilsson B (1991) Bio/Technology 9:642.
- Lakhdar, K.; Farid, S. S.; Savery, J.; Titchener-Hooker N. J.;Papageorgiou L. G. (2006) Biotechnol. Prog. 22: 1630.
- Lakhdar, K.; Farid, S. S.; Savery, J.; Titchener-Hooker N. J.;Papageorgiou L. G. (2005) Biotechnol. Prog. 21, 1478.
- Leser EW, Asenjo JA (1994) Ann N Y Acad Sci. 721:337.
- Lienqueo ME., Salazar O, Henríquez K, Calado CRC., Cabral JMS.(2005) "Effect of hydrophobic polypeptide tags on cutinase purification by HIC" XVI Chilean Congress of Chemical Engineering, Pucón, Chile.
- Lienqueo ME, Henriquez K , Salazar O , Calado CRC, Fonseca LP, Cabral JMS (2007a),J Chromat A 1154:460.
- Lienqueo ME, Salgado JC, Giaverini O Asenjo JA (2007b) "How to select optimal polypeptide tags to assist the purification of recombinant proteins", International Conference on Biopartitioning and Purification, Lisbon, Portugal
- Lo Conte L, Chothia C, Janin J (1999) J. Mol. Biol. 285:2177.
- Mosher, RA, Gebauer P,Thormann W.(1993). J.Chromatogr, 638:155-164.
- Niederauer MQ, Suominen I, Rougvie MA, Ford CF, Glatz CE (1994) Biotechnol. Prog. 10 237.
- Nilsson A, Mannesse M, Egmond MR, Tjerneld F(2002) J Chromatogr A. 946(1-2):141.
- Nygren PA, Stahl S, Uhlen M.(1994) Trends Biotechnol. 12:184.
- Persson M, son Bergstrand MG, Bülow L, Mosbach K (1988). Anal. Biochem. 172, 330.
- Rodenbrock A; Selber K.; Egmond M.R.; Kula M-R. (2001) Bioseparation 9, 269.
- Qing G, Ma LC, Khorchid A, Swapna GV, Mal TK, Takayama MM, Xia B, Phadtare S, Ke H, Acton T Montelione GT, Ikura M, Inouye M.(2004). Nat Biotechnol, 22:877.
- Terpe, K.(2003). Appl. Microbiol. Biotechnol. 2003, 60, 523.
- Salazar O, Monitor J, Lienqueo ME, Asenjo JA(2001) Prot.Expr.Purif. 23:219
- Sassenfeld HM, Brewer SJ (1984) Bio/ Technology 2:76.
- Schmidt TGM, Skerra A (1993) Protein Eng 6:109.
- Schmidt TG, Koepke J, Frank R, Skerra A.(1996) J Mol Biol.255(5):753.
- Simeonidis E, Pinto JM, Lienqueo ME, Tsoka S, Papageorgiou LG. (2005) Biotechnol Prog.;21(3):875.
- Steffens MA.; Fraga ES, Bogle IDL. (2000a) Comput.Chem. Eng. 24: 717.
- Steffens MA.; Fraga ES, Bogle IDL. (2000b) Biotechnol.Bioeng. 68: 218.
- Ueda E. K. M., Goutb P. W. , Morgant L. (2003). J. Chromatogr. A. 988, 1.
- Vasquez-Alvarez E, Pinto JM. (2004) J Biotechnol. 110:295.
- Waugh DS. (2005) Trends Biotechnol. 23:316.