The concept of affinity chromatography emerged biology essay

Science, Biology

The concept of affinity chromatography emerged in the 1970’s and exploits the reversible, biological and selective interaction between two entities such as an immobilized adsorbent on a solid support, denominated as affinity ligand, and a target biomolecule that will be further purified [6-7, 12-14]The solid support type most widely used in affinity chromatography is non-porous materials being example of these agarose, polymethacrylate, polycrylamide, cellulose, and silica. Recently, other materials such as membranes, monolithic and expanded-bed adsorbents have been used as matrix for affinity chromatography [12-16]. The properties required for an efficient and effective chromatography matrix includes chemical inertness and stability, mechanical stability, pore size and particle size [12-13, 15-16]. The chemical inertness of the matrix is related with the minimization of nonspecific binding of the matrix material and the target protein that has been purified [12, 16]. As the support only can interact in a minimal way with the aqueous mobile phase, the matrix support should be hydrophilic and non-charged to minimize the formation of ionic interactions [16]. Concerning the stability of the material, the matrix must be physically and chemically stable under a wide range of conditions including coupling, adsorption and elution, extreme pH values, high and low temperatures and the use of organic solvents, enzymes present in the sample, detergents and disruptive eluents conditions[12-13, 15-16]. Some of conditions are those that are employed in the sterilization in place (SIP) and cleaning in place (CIP) so that the supports can be re-used, contributing for the cost-effectiveness of the purification process. In terms of mechanical stability, the matrix should be preferably rigid solid supports so that can be able to withstand high pressures during

the purification process without compressing and deforming [12, 14-16]. Other requirements of an ideal matrix are related with the pore size and particle size. The gel particles must be uniform, spherical and rigid, because the beads with these characteristics provide excellent flow through properties with minimal channelling in the column applications [12, 14-17]. Moreover, the gel particles should be smaller to allows a greater surface area of the support material and because limits mass transfer effects [12, 14]. The porosity of the matrix also contributes for good flows properties and facilitates the penetration of macromolecules [12]. The high porosity also combined with small particles size, increases the surface area which reflects usually in a high capacity of the support matrix [12-14, 16]. Moreover, the pore diameter also has a significant effect on the purification step taking into account the Renkin equation [16, 18] that estimates the effective diffusion coefficient. The pore size limits the entrance of the target protein inside the pore according with the size, molecular weight, being this denominated exclusion limit. According to this, the size of the pores should be at least five times larger than the average size of a biomolecule. An example of this can be if the size of the pores should be at least 300 A˚ or greater if assuming an average protein size of 60 A˚ [16, 18]. The matrix selectivity can be introduced through the functionalization of the solid support with different coupling strategies generating different affinity ligands. The affinity ligands immobilized onto a solid support can be divided into three main groups such as biological, synthetic and structural ligands. Biological ligands comprise ligands from biological sources or from in vitro techniques. These are natural receptors which target molecules with high selectivity and affinity, such as

peptides, antibodies, antigens and binding or receptor proteins [19-21], and are associated with high costs of production and purification, poor stabilization under sterilization and cleaning-in-place conditions, as well as potential leakage and end-product contamination. The most common affinity biological partners involve the immobilized bacterial immunoglobulin-binding domains such as staphylococcal protein A (spA), G and L for immunoglobulin purification [22-26]. The avidin-biotin technology also has been employed on the affinity based bioseparation [27] and the purification of glycoprotein and sugars was performed through immobilized natural lectins [28]. Heparin is also used as biological ligand for affinity purification of growth factors [29]. Synthetic affinity ligands have been developed in an attempt to overcome these disadvantages of natural ligands. They tend to combine molecular recognition features with high resistance to chemical and biological degradation, high scalability, as well as low costs and low toxicity [7, 19, 21]. Synthetic ligands have been well established over many decades and one of the major class are related with the biomimetic ligands or de novo designed ligands. These last ligands are tailor-made molecules that mimic the natural biological recognition between a target protein and a natural ligand [20]. The major concern about these biomimetic ligands is regarding the toxicity and biocompatibility of these ligands in biopharmaceutical industry for the protein purification [20]. The research strategy followed for the development of these ligands involves ligand design using in silico molecular modelling tools as a first step [20, 30], followed by the on-bead combinatorial synthesis and high-throughput screening (HTS) of ligand libraries (Fig. 1). The combinatorial chemistry can be used either in solid or solution phase. Both of

the synthesis allows to increase the diversity of the compounds and also present other advantages as the cost effectiveness and the less time consuming [31]. Different scaffolds were already used for the development of synthetic ligands. The triazine scaffold is a well-established technique by the Lowe and co-workers. This scaffold was in the basis of an alternative of protein A by generating an affinity ligand that mimics protein A for the purification of immunoglobulins G (IgG) [32]. Also other bacterial immunoglobulin-binding domains such as protein G and L with the same purification purposes [33-34]. Beyond immunoglobulins purification, a wide range of affinity ligands were developed such as an artificial lectin and receptor for purification of glycoproteins [35-36], as well as other protein targets such as prion proteins [37], human recombinant factor VII, [38] and elastases [39]. This strategy was also considered for other authors for the purification of proteins as alkaline phosphatase [40] and human tissue plasminogen activator [41]. More recently, a different scaffold based on the Ugi reaction have been employed [42]. The Ugi reaction is a multicomponent reaction that involves four main compounds as an aldehyde, an amine, a carboxylic acid and an isocyanine. This Ugi reaction scaffold applied to affinity chromatography was firstly studied by Lowe et al, indicating the potential of this MCR on this field. So far, this strategy has been used mainly for the purification of immunoglobulins [42-44]. Moreover, the identification of novel affinity ligands can be also possible through phage display [45]. This technology has been developed by Smith et al by which the peptides or proteins were expressed on the surface of the phage particles. For this, DNA sequence is inserted onto the genome of a phagemid vector along with the

C-terminus of the page minor coat protein, gene III, through recombinant DNA technology [45-46]. Then the cloned phagemid vector is transformed in Escherichia coli cells, and then infected with helper phage for the production of combinatorial libraries of filamentous phage with peptides displayed at surface [45, 47]. The size of the initial combinatorial library should be up to 109 clones so that could be further used in the panning for the selection of putative binders [45, 47]. The panning can comprise several cycles of binding, elution and amplification, and the increase of enrichment of phage particles over several rounds of panning can imply the existence of a progress affinity and selectivity between the putative binders displayed on phage particles and the specific target [45]. The most used phagemid vectors used are the filamentous bacteriophage and its Ff class such as M13, f1 and fd [46] and a wide range of combinatorial libraries of antibodies fragments [47-48], protein domains [49-50] and peptides [46, 51-52] have been constructed. The applications of phage display on affinity chromatography based protein purification have been described in literature [45, 53-55] and comprise the construction of combinatorial libraries based on peptides ligands for factor VIII purification [56]. Moreover, small protein domains (affibodies) based on α-helical bacterial receptor domain Z derived from spA (immunoglobulin G-binding domain), have been selected through phage display with a micromolar dissociation affinity constant for specific targets, such as Taq DNA polymerase, human insulin and apolipoprotein A-1 [49]. Additionally, the construction of combinatorial libraries based on these molecules affibodies have been extended for other targets, among them human immunoglobulin A (IgA) [57], human epidermal growth factor

receptor [58] and human amyloid beta peptides implicated on Alzeihmer disease [59]. Although these molecules present highly potential to be employed as affinity ligands for purification of proteins based on affinity chromatography, these molecules have been exploited mainly for therapeutic, diagnostic and imaging applications [60]. However, Z domain is still on the basis of development of affinity ligands, where protein engineering of this domain has been performed to improve IgG purification [61]. Despite of phage display is a versatile technique that provides enormous diversity on potential binding partners at reduced costs, this also presents some limitations such as limited folding properties, stability and high product yields of the displayed binding partners [62]. Different display techniques can also be used, among them ribosome and yeast display. The ribosome display technology can overcome some disadvantages of phage display such as the dependency of the bacterial transformation efficiency to generate diversity on the combinatorial libraries and also improve the stability and folding properties of the displayed targets [62-63]. The yeast display also offers benefits regarding the proper folding of proteins as these hosts present similar post-translational machinery than mammalian cells required for the correct folding of eukaryotic proteins [64]. Another class of affinity ligands is related with structural ligands, presenting these ligands limited selectivity/affinity with production at affordable prices. These ligands comprise ion-exchange, hydrophobic, metal chelate, covalent and thiophilic ligands [13, 20] ref da graziella review adicionar mais sitios. Por referencias nestes tiposThe biological and structural ligands have been also used in purification based on affinity technologies that involves the appliance of a

selective binding partner designated as affinity tag. The affinity tags displays different size range from a single amino acid to entire proteins, and can be genetically fusion to N- or C-terminal of the target biomolecule [65-70] . Afterwards, the affinity tags will recognize the affinity ligand immobilized onto the matrix of the chromatographic column, facilitating the purification process. Despite of the affinity tags being highly efficient tools for the purification of recombinant proteins through chromatography based processes, the tags can display other applications on enhancement of protein solubility and stability [71], increase of the expression levels [69, 71] and allows labelling for cellular localization and imaging studies [67]. The use of peptides as affinity tags can be more advantageous rather than protein tags because due to their smaller size, the peptides tag can contribute for a less burden for the host during the fusion protein production and might interfere less with the tertiary structure and biological activity of the target fused protein [66, 72]. Therefore, the removal of the peptide tags might not be extremely necessary, decreasing thus the overall costs of the purification process based on affinity peptide tags [66, 72]. Regarding therapeutic proteins and due to strict regulatory demands on integrity and biological activity, the presence of the affinity tag can compromise the protein properties and requires the removal of the tag [66-67, 69-70, 73], which will be further discussed. The biological ligands involved on molecular recognition with the respective partner affinity tag can comprise peptides, protein and carbohydrates (Table 1. 1). The most common biological ligands used are based on matrices with the immobilized streptadivin and antibody proteins. Regarding the IgG affinity chromatography, the first affinity tag

developed was based on intrinsic selectivity and affinity between the bacterial immunoglobulin-binding domain staphylococcal protein A and the Fc region of the mammalian IgG [74]. Usually SpA is well-known to be used as an immobilized biological adsorbent for the purification of immunoglobulins; however the application of this protein on affinity chromatography was extended to their use as an affinity tag and therefore, SpA fusion constructs were developed and demonstrated with the fusion of SpA to alkaline phosphatase with the further expression in bacterial cells such as E. coli and Staphylococcus aureus [75]. Afterwards, the purification of the target protein was conducted as one-single step of IgG based affinity chromatography with protein recovery at acidic pH [75]. According to Nilsson et al [74], the SpA presents five homologous domains such as E, D, A, B and C, where the IgG presents a significant dissociation constant for domain B. Considering this, a mutated version of B domain was developed and denominated as Z domain to improve the resistance of undesirable cleavage of the purified fusion protein when using chemical tag removal strategy [76]. The bacterial immunoglobulin-binding domain staphylococcal protein G (SpG) also have been exploited as a fusion partner due to their bifunctional behaviour as these protein presents different domains that can present affinity for both IgG and serum albumin (HA) [77-78]. The SpG is composed by four different regions (A, B, C and D), being the regions C and G responsible for the binding selectivity of IgG [77-78]. Therefore, the SpG have been used as a fusion partner for the purification of recombinant proteins through human serum albumin (HSA) and IgG affinity chromatography [66, 68, 77-78]. Other affinity tags that recognized antibody

based matrices are the FLAG, c-myc, T7, hemaglutinin antigen (HA) and Sogtags. A common feature between of all these tags is the fact that these peptide tags are epitopes with strong affinity for the respective antibodies, thus presenting antigenic properties [66, 70]. Most of these affinity pairs present a limited utility on purification processes due to the high costs of the adsorbents that are based on monoclonal antibodies. Moreover, these epitope peptides can also compromise the proper production of the target protein in terms of functionality [93-94]. Within all these antigenic peptides, the most widely used peptide is the FLAG affinity tag that have been employed on the purification of several recombinant proteins such as immunoglobulins, cytokines, gene-regulatory proteins [95]. The FLAG peptide is a hydrophilic peptide with five amino acid sequence DYKDDDDK with high affinity for the monoclonal antibodies M1 and M2, being the binding calcium dependent for M1 [85, 95-97]. Therefore, the elution of recombinant proteins by using M1 can be carried out by using a mild conditions with the presence of metal chelates such as EDTA, where in case of M2 is at lower pH [85, 95-97]. The most disadvantages of this system are extended to all purification processes based on biological ligands such as ligand instability and therefore leakage [95]. An attractive feature of this affinity tag is related with the tag removal, because the sequence DDDDK of the FLAG tag can be recognized by the enterokinase, an endopeptidase used on enzymatic tag removal strategies without the need of insertion of an additional sequence for further tag removal [95, 97]. Moreover, the use of this tag allows the release of the target protein without additional amino acids [95, 97]. The c-myc is an product of a proto-oncogene, and the derived

epitope from this product was found to present high affinity for the monoclonal antibody 9E10 [86]. Then, this affinity pair has been mostly used as a tool for the detection of recombinant proteins through immnunoblotting assays rather than for purification processes [72, 86, 98]. Also the affinity tags T7 and hemaglutinin antigen (HA) are also purification affinity tags, being mostly used on immune-detection assays [70-71], where the T7-tag corresponds to 11 amino acid sequence being a leader peptide of phage T7 with affinity for the anti-T7 monoclonal antibody [94, 99] and the HA tag is a peptide epitope of the influenza virus hemagglutinin [100] that is recognized by the monoclonal antibody 12 CA5 [88, 101]. The epitope tags denominated as Softags are employed on immunoaffinity chromatography by using polyols responsive monoclonal antibodies as biological adsorbents [89, 102]. According to their designation, these tags allows a soft elution by using the polyols, low molecular weight compound, as a eluents agent [89, 102], being a great advantage despite of the high affinity. There are three Softags, where the Softag 1 corresponds to a thirteen amino acid sequence near the C-terminal of the β’ subunit of E. coli RNA polymerase [92]; Softag 2 is a repeat heptapeptide found on C-terminal of RNA polymerase I [90]; and Softag3 is an epitope near the N-terminal of human transcription factor [89]. Other type of affinity tags is those that recognize streptadivin binding domains. These affinity tags were developed based on the natural binding pair avidin-biotin that displays a high selective and affinity constant of 1015 M-1 [27]. The first affinity tag developed was Strep-tag, a nine amino acid peptide that was selected from a random peptide library generated towards affinity for streptadivin [79], being fused to the C-terminal of the target

protein. According to structural studies, this affinity tag recognizes and binds to the same pocket of biotin, the natural partner of streptadivin [80]. The main advantages of this affinity tag are related to the resistance to proteolysis in vivo, no interference with expression in E. coli and the elution conditions employed after binding on streptadivin based affinity column are mild as this step is carried through competitive elution with a biotin or analogue compound such as iminibiotin [79]. However, a limitation of the Strep-tag was their restricted fusion to the C-terminal of the target protein and due to this, an improved version of Strep-tag was developed and denominated as Strep-tag II with equilibrium dissociation constant of 37 µM [80]. Also, at the same time, an engineered streptadividin chromatographic support (Strep-Tactin) was developed to improve the binding capacity towards the Strep-tag II, through the generation of a genetic library based on streptadivin with random mutagenesis on the amino acids 44-47 positions that corresponds to flexible loop region near to the binding site [103]. Although, the affinity pair Strep-tag II – Strep tactin have been extensively used on a variety of applications [103], continuously studies on development of most robust affinity tags with high affinity for streptadivin matrices have been conducted. This affinity tags comprises strepdavidin-binding peptide (SBP) ans Nano tag. The SPB tag comprises a 38 amino acid sequence with an equilibrium dissociation constant of 2. 5 nM and this peptide sequence was found from the selection of a peptide library for the immobilized streptadivin [82], presenting a great advantage over the Strep-tag II. Nano-tag displays even more robust properties that the tags already described in literature to be used on streptadividin based affinity chromatography as this

tag combines the small size of Strep-tag II and also a namolar affinity constant of 4 nM [81]. This tag is a 15 amino acid peptide was developed to bind specifically to streptadivin. Therefore, a synthetic library based on the heart fatty acid-binding protein (FABP) was created and the selection was performed through ribosome display against immobilized streptadivin [104]. Other affinity tag based on Strep-tag was developed, however this affinity designated as AviD-tag displays affinity for neutravidin, a neutral form of avidin rather for streptadivin [84]. The AviD-tag is composed by a 6-amino acid cyclic peptide that was selected through phage display technique with a dissociation constant of 12 μM for both Neutravidin and avidin [84].