GAG BINDING PROTEINS

The present invention relates to methods and tools for the inhibition of the interaction of chemokines and their high-affinity receptors on leukocytes and methods for the therapeutic treatment of inflammatory diseases.

Chemokines, originally derived from chemoattractant cytokines, actually comprise more than 50 members and represent a family of small, inducible, and secreted proteins of low molecular weight (6-12 kDa in their monomeric form) that play a decisive role during immunosurveillance and inflammatory processes. Depending on their function in immunity and inflammation, they can be distinguished into two classes. Inflammatory chemokines are produced by many different tissue cells as well as by immigrating leukocytes in response to bacterial toxins and inflammatory cytokines like IL-1, TNF and interferons. Their main function is to recruit leukocytes for host defence and in the process of inflammation. Homing chemokines, on the other hand, are expressed constitutively in defined areas of the lymphoid tissues. They direct the traffic and homing of lymphocytes and dendritic cells within the immune system. These chemokines, as illustrated by BCA-1, SDF-1 or SLC, control the relocation and recirculation of lymphocytes in the context of maturation, differentiation, activation and ensure their correct homing within secondary lymphoid organs.

Despite the large number of representatives, chemokines show remarkably similar structural folds although the sequence homology varies between 20 to 70 percent. Chemokines consist of roughly 70-130 amino acids with four conserved cysteine residues. The cysteines form two disulphide bonds (Cys 1→ Cys 3, Cys 2→ Cys 4) which are responsible for their characteristic three-dimensional structure. Chemotactic cytokines consist of a short amino terminal domain (3-10 amino acids) preceding the first cysteine residue, a core made of β-strands and connecting loops found between the second and the fourth cysteine residue, as well as a carboxy-terminal α-helix of 20-60 amino acids. The protein core has a well ordered structure whereas the N- and C-terminal parts are disordered. As secretory proteins they are synthesised with

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a leader sequence of 20-25 amino acids which is cleaved off before release.

The chemokines have been subdivided into four families on the basis of the relative position of their cysteine residues in the mature protein. In the α-chemokine subfamily, the first two of the four cysteines are separated by a single amino acid (CXC), whereas in the β-chemokines the corresponding cysteine residues are adjacent to each other (CC). The α-chemokines can be further classified into those that contain the ELR sequence in the N-terminus, thereby being chemotactic for neutrophils (IL-8 for example), and those that lack the ELR motif and act on lymphocytes (I-TAC for example). Structurally the β-chemokines can be subdivided into two families: the monocyte-chemoattractant protein eotaxin family, containing the five monocyte chemoattractant proteins (MCP) and eotaxin which are approximately 65 percent identical to each other, and the remaining β-chemokines. As with the CXC-family, the N-terminal amino acids preceding the CC-residues are critical components for the biologic activity and leukocyte selectivity of the chemokines. The β-chemokines, in general, do not act on neutrophils but attract monocytes, eosinophils, basophils and lymphocytes with variable selectivity.

Only a few chemokines do not fit into the CC-or the CXC-family. Lymphotactin is so far the only chemokine which shows just two instead of the four characteristic cysteines in its primary structure, and is thus classified as γ- or C-chemokine. On the other hand, by concluding this classification, fractalkine has to be mentioned as the only representative of the δ- or CXXXC-subfamily with three amino acids separating the first two cysteines. Both of them, Lymphotaxin and fractalkine, induce chemotaxis of T-cells and natural killer cells.

Chemokines induce cell migration and activation by binding to specific cell surface, seven transmembrane-spanning (7TM) G-protein-coupled receptors on target cells. Eighteen chemokine receptors have been cloned so far including six CXC, ten CC, one CX3C and one XC receptor. Chemokine receptors are expressed on different types of leukocytes, some of them are restricted to certain cells (e.g. CXCR1 is restricted to neutrophils) whereas

others are more widely expressed (e.g. CCR2 is expressed on monocytes, T cells, natural killer cells and basophils). Similar to chemokines, the receptors can be constitutively expressed on certain cells, whereas some are inducible. Some of them can even be down-regulated making the cells unresponsive to a certain chemokine but remaining responsive to another. Most receptors recognise more than one chemokine and vice versa but recognition is restricted to chemokines of the corresponding subfamily (see Table 1).

Table 1

Chemokine		Receptor	Chemotactic for	Inflammatory Diseases
CXC-Chemokine (+ELR-motif)	IL-8	CXCR1 CXCR2	Neutrophils	Acute respiratory distress syndrome [71]; Bacterial pneumonia [72]; Rheumathoid arthritis [73]; Inflammatory bowel disease [74]; Psoriasis [75]; Bacterial meningitis [76]
CC-Chemokine	MCP-1	CCR2	Basophils; Monocytes; Activated T cells; Dentritic cells; Natural killer cells	Asthma [77]; Glomerulonephritis [78]; Atheroscleosis [79]; Inflammatory bowel disease [80]; Psoriasis [81]; Bacterial and viral meningitis [82,83]
	RANTES	CCR1	Eosinophils; Monocytes; Activated T cells; Dentritic cells	Asthma [84]; Glomerulonephritis [85]
		CCR3	Eosinophils; Basophils; Dentritic cells	
		CCR5	Monocytes; Activated T cells; Dentritic cells; Natural killer cells	

Chemokines have two main sites of interaction with their receptors, one in the amino-terminal domain and the other within an exposed loop of the backbone that extends between the second and the third cysteine residue. Both sites are kept in close proximity by the disulphide bonds. The receptor recognises first the binding site within the loop region which appears to function as a docking domain. This interaction restricts the mobility of the

chemokine thus facilitating the proper orientation of the amino-terminal domain. Studies have been performed with mutant chemokines that still bound effectively to their receptors but did not signal. These mutants were obtained by amino acid deletion or modification within the N-termini of, for example, IL-8, RANTES and MCP-1.

Multiple intracellular signalling pathways occur after receptor activation as a result of chemokine binding. Chemokines also interact with two types of nonsignalling molecules. One is the DARC receptor which is expressed on erythrocytes and on endothelial cells and which binds CC- as well as CXC-chemokines to prevent them from circulation. The second type are heparan sulphate glycosaminoglycans (GAGs) which are part of proteoglycans and which serve as co-receptors of chemokines. They capture and present chemokines on the surface of the homing tissue (e.g. endothelial cells) in order to establish a local concentration gradient. In an inflammatory response, such as in rheumatoid arthritis, leukocytes rolling on the endothelium in a selectin-mediated process are brought into contact with the chemokines presented by the proteoglycans on the cell surface. Thereby, leukocyte integrins become activated which leads to firm adherence and extravasation. The recruited leukocytes are activated by local inflammatory cytokines and may become desensitised to further chemokine signalling because of high local concentration of chemokines. For maintaining a tissue bloodstream chemokine gradient, the DARC receptor functions as a sink for surplus chemokines.

Heparan sulphate (HS) proteoglycans, which consist of a core protein with covalently attached glycosaminoglycan sidechains (GAGs), are found in most mammalian cells and tissues. While the protein part determines the localisation of the proteoglycan in the cell membrane or in the extracellular matrix, the glycosaminoglycan component mediates interactions with a variety of extracellular ligands, such as growth factors, chemokines and adhesions molecules. The biosynthesis of proteoglycans has previously been extensively reviewed. Major groups of the cell surface proteoglycans are the syndecan family of transmembrane proteins (four members in mammals) and the glypican family of pro-

teins attached to the cell membrane by a glycosylphosphatidylinositol (GPI) tail (six members in mammals). While glypicans are expressed widely in the nervous system, in kidney and, to a lesser extent, in skeletal and smooth muscle, syndecan-1 is the major HSPG in epithelial cells, syndecan-2 predominates in fibroblasts and endothelial cells, syndecan-3 abounds in neuronal cells and syndecan-4 is widely expressed. The majority of the GAG chains added to the syndecan core proteins through a tetrasaccharide linkage region onto particular serines are HS chains. Although the amino acid sequences of the extracellular domains of specific syndecan types are not conserved among different species, contrary to the transmembrane and the cytoplasmic domains, the number and the positions of the GAG chains are highly conserved. The structure of the GAGs, however, is species-specific and is, moreover, dependent upon the nature of the HSPG-expressing tissue.

Heparan sulphate (HS) is the most abundant member of the glycosaminoglycan (GAG) family of linear polysaccharides which also includes heparin, chondroitin sulphate, dermatan sulphate and keratan sulphate. Naturally occuring HS is characterised by a linear chain of 20-100 disaccharide units composed of N-acetyl-D-glucosamine (GlcNAc) and D-glucuronic acid (GlcA) which can be modified to include N- and O-sulphation (6-O and 3-O sulphation of the glucosamine and 2-O sulphation of the uronic acid) as well as epimerisation of ß-D-gluronic acid to α-L-iduronic acid (IdoA).

Clusters of N- and O-sulphated sugar residues, separated by regions of low sulphation, are assumed to be mainly responsible for the numerous protein binding and regulatory properties of HS. In addition to the electrostatic interactions of the HS sulphate groups with basic amino acids, van der Waals and hydrophobic interactions are also thought to be involved in protein binding. Furthermore, the presence of the conformationally flexible iduronate residues seems to favour GAG binding to proteins. Other factors such as the spacing between the protein binding sites play also a critical role in protein-GAG binding interactions: For example γ-interferon dimerisation induced by HS requires GAG chains with two protein binding sequences separated

by a 7 kDa region with low sulphation. Additional sequences are sometimes required for full biological activity of some ligands: in order to support FGF-2 signal transduction, HS must have both the minimum binding sequence as well as additional residues that are supposed to interact with the FGF receptor.

Heparin binding proteins often contain consensus sequences consisting of clusters of basic amino acid residues. Lysine, arginine, asparagine, histidine and glutamine are frequently involved in electrostatic contacts with the sulphate and carboxyl groups on the GAG. The spacing of the basic amino acids, sometimes determined by the proteins 3-D structure, are assumed to control the GAG binding specificity and affinity. The biological activity of the ligand can also be affected by the kinetics of HS-protein interaction. Reducing the dimension of growth factor diffusion is one of the suggested HSPG functions for which the long repetitive character of the GAG chains as well as their relatively fast on and off rates of protein binding are ideally suited. In some cases, kinetics rather than thermodynamics drives the physiological function of HS-protein binding. Most HS ligands require GAG sequences of well-defined length and structure. Heparin, which is produced by mast cells, is structurally very similar to heparan sulphate but is characterised by higher levels of post-polymerisation modifications resulting in a uniformly high degree of sulphation with a relatively small degree of structural diversity. Thus, the highly modified blocks in heparan sulphate are sometimes referred to as "heparin-like". For this reason, heparin can be used as a perfect HS analogue for protein biophysical studies as it is, in addition, available in larger quantities and therefore less expensive than HS. Different cell types have been shown to synthesise proteoglycans with different glycosaminoglycan structure which changes during pathogenesis, during development or in response to extracellular signals such as growth factors. This structural diversity of HSPGs leads to a high binding versatility emphasising the great importance of proteoglycans.

Since the demonstration that heparan sulphate proteoglycans are critical for FGF signalling, several investigations were performed showing the importance of chemokine-GAG binding for pro-

moting chemokine activity. First, almost all chemokines studied to date appear to bind HS in vitro, suggesting that this represents a fundamental property of these proteins. Second, the finding that in vivo T lymphocytes secrete CC-chemokines as a complex with glycosaminoglycans indicates that this form of interaction is physiologically relevant. Furthermore, it is known that the association of chemokines with HS helps to stabilise concentration gradients across the endothelial surface thereby providing directional information for migrating leukocytes. HS is also thought to protect chemokines from proteolytic degradation and to induce their oligomerisation thus promoting local high concentrations in the vicinity of the G-coupled signalling receptors. The functional relevance of oligomerisation, however, remains controversial although all chemokines have a clear structural basis for multimerisation. Dimerisation through association of the ß-sheets is observed for all chemokines of the CXC-family (e.g. IL-8), contrary to most members of the CC-chemokine family (e.g. RANTES), which dimerise via their N-terminal strands.

A wealth of data has been accumulated on the inhibition of the interaction of chemokines and their high-affinity receptors on leukocytes by low molecular weight compounds. However, there has been no breakthrough in the therapeutic treatment of inflammatory diseases by this approach.

Interleukin-8 (IL-8) is a key molecule involved in neutrophil attraction during chronic and acute inflammation. Several approaches have been undertaken to block the action of IL-8 so far, beginning with inhibition of IL-8 production by for example glucocorticoids, Vitamin D3, cyclosporin A, transforming growth factor ß, interferons etc., all of them inhibiting IL-8 activity at the level of production of IL-8 mRNA. A further approach previously used is to inhibit the binding of IL-8 to its receptors by using specific antibodies either against the receptor on the leukocyte or against IL-8 itself in order to act as specific antagonists and therefore inhibiting the IL-8 activity.

The aim of the present invention is therefore to provide an alternative strategy for the inhibition or disturbance of the in-

teraction of chemokines/receptors on leukocytes. Specifically the action of IL-8, RANTES or MCP-1 should be targetted by such a strategy.

Subject matter of the present invention is therefore a method to produce new GAG binding proteins as well as alternative GAG binding proteins which show a high(er) affinity to a GAG co-receptor (than the wild type). Such modified GAG binding proteins can act as competitors with wild-type GAG binding proteins and are able to inhibit or down-regulate the activity of the wild-type GAG binding protein, however without the side effects which occur with the known recombinant proteins used in the state of the art. The molecules according to the present invention do not show the above mentioned disadvantages. The present modified GAG binding proteins can be used in drugs for various therapeutical uses, in particular - in the case of chemokines - for the treatment of inflammation diseases without the known disadvantages which occur in recombinant chemokines known in the state of the art. The modification of the GAG binding site according to the present invention turned out to be a broadly applicable strategy for all proteins which activity is based on the binding event to this site, especially chemokines with a GAG site. The preferred molecules according to the present invention with a higher GAG binding affinity proved to be specifically advantageous with respect to their biological effects, especially with respect to their anti-inflammatory activity by their competition with wild type molecules for the GAG site.

Therefore, the present invention provides a method for introducing a GAG binding site into a protein characterised in that it comprises the steps:

- identifying a region in a protein which is not essential for structure maintenance
- introducing at least one basic amino acid into said site and/or deleting at least one bulky and/or acidic amino acid in said site,

whereby said GAG binding site has a GAG binding affinity of $K_d \leq 10\mu M$, preferably $\leq 1\mu M$, still preferred $\leq 0.1\mu M$. By introducing at least one basic amino acid and/or deleting at least one bulky and/or acidic amino acid in said region, a novel, improved

"artificial" GAG binding site is introduced in said protein. This comprises the new, complete introduction of a GAG binding site into a protein which did not show a GAG binding activity before said modification. This also comprises the introduction of a GAG binding site into a protein which already showed GAG binding activity. The new GAG binding site can be introduced into a region of the protein which did not show GAG binding affinity as well as a region which did show GAG binding affinity. However, with the most preferred embodiment of the present invention, a modification of the GAG binding affinity of a given GAG binding protein is provided, said modified protein's GAG binding ability is increased compared to the wild-type protein. The present invention relates to a method of introducing a GAG binding site into a protein, a modified GAG binding protein as well as to an isolated DNA molecule, a vector, a recombinant cell, a pharmaceutical composition and the use of said modified protein.

The term "introducing at least one basic amino acid" relates to the introduction of additional amino acids as well as the substitution of amino acids. The main purpose is to increase the relative amount of basic amino acids, preferably Arg, Lys, His, Asn and/or Gln, compared to the total amount of amino acids in said site, whereby the resulting GAG binding site should preferably comprise at least 3 basic amino acids, still preferred 4, most preferred 5 amino acids.

The GAG binding site is preferably at a solvent exposed position, e.g. at a loop. This will assure an effective modification.

Whether or not a region of a protein is essential for structure maintenance, can be tested for example by computational methods with specific programmes known to the person skilled in the art. After modification of the protein, the conformational stability is preferably tested in silico.

The term "bulky amino acid" refers to amino acids with long or sterically interfering side chains; these are in particular Trp, Ile, Leu, Phe, Tyr. Acidic amino acids are in particular Glu and

Asp. Preferably, the resulting GAG binding site is free of bulky and acidic amino acids, meaning that all bulky and acidic amino acids are removed.

The GAG binding affinity is determined - for the scope of protection of the present application - over the dissociation constant K_d. One possibility is to determine the dissociation constant (K_d) values of any given protein by the structural change in ligand binding. Various techniques are well known to the person skilled in the art, e.g. isothermal fluorescence titrations, isothermal titration calorimetry, surface plasmon resonance, gel mobility assay, and indirectly by competition experiments with radioactively labelled GAG ligands. A further possibility is to predict binding regions by calculation with computational methods also known to the person skilled in the art, whereby several programmes may be used.

A protocol for introducing a GAG binding site into a protein is for example as follows:

- Identify a region of the protein which is not essential for overall structural maintenance and which might be suitable for GAG binding
- Design a new GAG binding site by introducing (replacement or insertion) basic Arg, Lys, His, Asp and Gln residues at any position or by deleting amino acids which interfere with GAG binding
- Check the conformational stability of the resulting mutant protein in silico
- Clone the wild-type protein cDNA (alternatively: purchase the cDNA)
- Use this as template for PCR-assisted mutagenesis to introduce the above mentioned changes into the amino acid sequence
- Subclone the mutant gene into a suitable expression system (prokaryotic or eukaryotic dependent upon biologically required post-translational modifications)
- Expression, purification and characterisation of the mutant protein in vitro
- Criterion for an introduced GAG binding affinity: K_d^{GAG}(mutant) < 10µM.

Examples of said engineered proteins with new GAG binding sites are for example the Fc part of IgG as well as the complement factors C3 and C4 modified as follows:

Fc: (439)KSLSLS(444)-> KSKKLS
C3: (1297)WIASHT(1302)-> WKAKHK
C4: (1)MLDAERLK(8)-> MKKAKRLK

A further aspect of the present invention is a protein obtainable by the inventive method as described above. The inventive protein therefore comprises a - compared to the wild-type protein - new GAG binding site as defined above and will therefore act as competitor with natural GAG binding proteins, in particular since the GAG binding affinity of the inventive protein is very high, e.g. $K_d \leq 10\mu M$.

A further aspect of the present invention is a modified GAG binding protein, whereby a GAG binding region in said protein is modified by substitution, insertion, and/or deletion of at least one amino acid in order to increase the relative amount of basic amino acids in said GAG binding region, and/or reduce the amount of bulky and/or acidic amino acids in said GAG binding region, preferably at a solvent exposed position, and in that the GAG binding affinity of said protein is increased compared to the the GAG binding affinity of a respective wild-type protein.

It has been surprisingly shown that by increasing the relative amount of basic amino acids, in particular Arg, Lys, His, Asn and Gln, in the GAG binding region, the modified GAG binding protein shows increased GAG binding affinity compared to the wild-type proteins, in particular when the relative amount of basic amino acids is increased at a solvent exposed position, since a positively charged area on the protein surface has shown to enhance the binding affinity. Preferably, at least 3, still preferred 4, most preferred 5, basic amino acids are present in the GAG binding region.

The term "GAG binding protein" relates to any protein which binds to a GAG co-receptor. Whether or not a protein binds to a GAG co-receptor can be tested with the help of known protocols as mentioned above. Hileman et al. (BioEssays 20 (1998), 156-167) disclose consensus sites in glycosaminoglycan binding proteins. The information disclosed in this article is also useful as starting information for the present invention. The term "protein" makes clear that the molecules provided by the present invention are at least 80 amino acids in lenght. This is required for making them suitable candidates for the present anti-inflammation strategy. Smaller molecules interacting with a GAG binding site and being physiologically or pathologically relevant due to such an interaction are not known and therefore not relevant for the present invention. Preferably, the molecules according to the present invention are composed of at least 90, at least 100, at least 120, at least 150, at least 200, at least 300, at least 400 or at least 500 amino acid residues.

In the scope of the present application the term "GAG binding region" is defined as a region which binds to GAG with a dissociation constant (K_d-value) of under 100µM, preferably under 50µM, still preferred under 20µM, as determined by isothermal fluorescence titration (see examples below).

Any modifications mentioned in the present application can be carried out with known biochemical methods, for example site-directed mutagenesis. It should also be noted that molecular cloning of GAG binding sites is, of course, prior art (see e.g. WO96/34965 A, WO 92/07935 A, Jayaraman et al. (FEBS Letters 482 (2000), 154-158), WO02/20715 A, Yang et al. (J.Cell.Biochem. 56 (1994), 455-468), wherein molecular shuffling or de novo syntesis of GAG regions are described; Butcher et al., (FEBS Letters 4009 (1997), 183-187) (relates to artificial peptides, not proteins); Jinno-Oue et al, (J. Virol. 75 (2001), 12439-12445)de novo synthesis)).

The GAG binding region can be modified by substitution, insertion and/or deletion. This means that a non-basic amino acid may be substituted by a basic amino acid, a basic amino acid may be

inserted into the GAG binding region or a non-basic amino acid may be deleted. Furthermore, an amino acid which interferes with GAG binding, preferably all interfering amino acids binding is deleted. Such amino acids are in particular bulky amino acids as described above as well as acidic amino acids, for example Glu and Asp. Whether or not an amino acid interferes with GAG binding may be examined with for example mathematical or computational methods. The result of any of these modifications is that the relative amount of basic amino acids in said GAG binding region is increased, whereby "relative" refers to the amount of basic amino acids in said GAG binding region compared to the number of all amino acids in said GAG binding region. Furthermore, amino acids which interfere sterically or electrostatically with GAG binding are deleted.

Whether or not an amino acid is present in a solvent exposed position, can be determined for example with the help of the known three dimensional structure of the protein or with the help of computational methods as mentioned above.

Whether or not the GAG binding affinity of said modified protein is increased compared to the GAG binding affinity of the respective wild-type protein, can be determined as mentioned above with the help of, for example, fluorescence titration experiments which determine the dissociation constants. The criterion for improved GAG binding affinity will be K_d (mutant) < K_d (wild-type), preferably by at least 100 %. Specifically improved modified proteins have - compared with wild-type K_d - a GAG binding affinity which is higher by a factor of minimum 5, preferably of minimum 10, still preferred of minimum 100. The increased GAG binding affinity will therefore preferably show a K_d of under 10µM, preferred under 1µM, still preferred under 0.1µM.

By increasing the GAG binding affinity the modified protein will act as a specific antagonist and will compete with the wild-type GAG binding protein for the GAG binding.

Preferably, at least one basic amino acid selected from the group consisting of Arg, Lys, and His is inserted into said GAG binding region. These amino acids are easily inserted into said

GAG binding region, whereby the term "inserted" relates to an insertion as such as well as substituting any non-basic amino acid with arginine, lysine or histidine. Of course, it is possible to insert more than one basic amino acid whereby the same basic amino acid may be inserted or also a combination of two or three of the above mentioned amino acids.

Still preferred, the protein is a chemokine, preferably IL-8, RANTES or MCP-1. Chemokines are known to have a site of interaction with co-receptor GAG whereby this chemokine binding is often a condition for further receptor activation as mentioned above. Since chemokines are often found in inflammatory diseases, it is of major interest to block the chemokine receptor activation. Such chemokines are preferably IL-8, RANTES or MCP-1, which are well characterised molecules and of which the GAG binding regions are well known (see, for example, Lortat-Jacob et al., PNAS 99 (3) (2002), 1229-1234). By increasing the amount of basic amino acids in the GAG binding region of these chemokines, their binding affinity is increased and therefore the wild-type chemokines will bind less frequently or not at all, depending on the concentration of the modified protein in respect to the concentration of the wild-type protein.

According to an advantageous aspect, said GAG binding region is a C terminal α-helix. A typicial chemical monomer is organised around a triple stranded anti-parallel β-sheet overlaid by a C-terminal α-helix. It has been shown that this C-terminal α-helix in chemokines is to a major part involved in the GAG binding, so that modification in this C-terminal α-helix in order to increase the amount of basic amino acids results in a modified chemokine with an increased GAG binding affinity.

Advantageously, positions 17, 21, 70, and/or 71 in IL-8 are substituted by Arg, Lys, His, Asn and/or Gln. Here it is possible that only one of these aforementioned sites is modified. However, also more than one of these sites may be modified as well as all, whereby all modifications may be either Arg or Lys or His or Asn or Gln or a mixture of those. In IL-8 these positions have shown to highly increase the GAG binding affinity of IL-8 and therefore these positions are particularly suitable for

modifications.

Preferably the increased binding affinity is an increased binding affinity to heparan sulphate and/or heparin. Heparan sulphate is the most abundant member of the GAG family of linear polysaccharides which also includes heparin. Heparin is structurally very similar to heparan sulphate characterised by higher levels of post-polymerisation modifications resulting in a uniformly high degree of sulphation with a relatively small degree of structural diversity. Therefore, the highly modified blocks in heparan sulphate are sometimes referred to as heparin-like and heparin can be used as a heparan sulphate analogue for protein biophysical studies. In any case, both, heparan sulphate and heparin are particularly suitable.

Still preferred, a further biologically active region is modified thereby inhibiting or down-regulating a further biological activity of said protein. This further biological activity is known for most GAG binding proteins, for example for chemokines. This will be the binding region to a receptor, for example to the 7TM receptor. The term "further" defines a biologically active region which is not the GAG binding region which, however, binds to other molecules, cells or receptors and/or activates them. By modifying this further biologically active region the further biological activity of this protein is inhibited or down-regulated and thereby a modified protein is provided which is a strong antagonist to the wild-type protein. This means that on the one hand the GAG binding affinity is higher than in the wild-type GAG binding protein, so that the modified protein will to a large extent bind to the GAG instead of the wild-type protein. On the other hand, the further activity of the wild-type protein which mainly occurs when the protein is bound to GAG, is inhibited or down-regulated, since the modified protein will not carry out this specific activity or carries out this activity to a lesser extent. With this modified protein an effective antagonist for wild-type GAG binding proteins is provided which does not show the side effects known from other recombinant proteins as described in the state of the art. This further biologically active region can for example be determined in vitro by receptor competition assays (using fluorescently labelled wt chemokines,

calcium influx, and cell migration (performed on native leukocytes or on 7TM stably-transfected cell lines). Examples of such further biologically active regions are, in addition to further receptor binding sites (as in the growth factor family), enzymatic sites (as in hydrolases, lyases, sulfotransferases, N-deacetylases, and copolymerases), protein interaction sites (as in antithrombin III), and membrane binding domains (as in the herpes simplex virus gD protein). With this preferred embodiment of double-modified proteins therefore dominant (concerning GAG binding) negative (concerning receptor) mutants are provided which are specifically advantageous with respect to the objectives set for the present invention .

Still preferred, said further biologically active region is modified by deletion, insertion, and/or substitution, preferably with alanine, a sterically and/or electrostatically similar residue. It is, of course, possible to either delete or insert or substitute at least one amino acid in said further biologically active region. However, it is also possible to provide a combination of at least two of these modifications or all three of them. By substituting a given amino acid with alanine or a sterically/electronically similar residue - "similar" meaning similar to the amino acid being substituted - the modified protein is not or only to a lesser extent modified sterically/electrostatically. This is particularly advantageous, since other activities of the modified protein, in particular the affinity to the GAG binding region, are not changed.

Advantageously, said protein is a chemokine and said further biological activity is leukocyte activation. As mentioned above, chemokines are involved in leukocyte attraction during chronic and acute inflammation. Therefore, by inhibiting or down-regulating leukocyte activation inflammation is decreased or inhibited which makes this particular modified protein an important tool for studying, diagnosing and treating inflammatory diseases.

According to an advantageous aspect, said protein is IL-8 and said further biologically active region is located within the first 10 N-terminal amino acids. The first N-terminal amino

acids are involved in leukocyte activation, whereby in particular Glu-4, Leu-5 and Arg-6 were identified to be essential for receptor binding and activation. Therefore, either these three or even all first 10 N-terminal amino acids can be substituted or deleted in order to inhibit or down-regulate the receptor binding and activation.

A further advantageous protein is an IL-8 mutant with the first 6 N-terminal amino acids deleted. As mentioned above, this mutant will not or to a lesser extent bind and activate leukocytes, so that it is particularly suitable for studying, diagnosing and treating inflammatory diseases.

Preferably, said protein is an IL-8 mutant selected from the group consisting of del6F17RE70KN71R, del6F17RE70RN71K and del6E70KN71K. These mutants have shown to be particularly advantageous, since the deletion of the first 6 N-terminal amino acids inhibits or down-regulates receptor binding and activation. Furthermore, the two phenylalanines in position 17 and 21 were found to make first contact with the receptor on its N-terminal extracellular domain to facilitate the later activation of the receptor. In order to prevent any neutrophil contact, these two amino acids 17 and 21 are exchanged, whereby they are exchanged to basic amino acids, since they are in close proximity to the GAG binding motif of the C-terminal α-helix as can be seen on a three dimensional model of a protein. By exchanging the position 17 and/or 21 to either arginine or lysine the GAG binding affinity is therefore increased.

A further aspect of the present invention is an isolated polynucleic acid molecule which codes for the inventive protein as described above. The polynucleic acid may be DNA or RNA. Thereby the modifications which lead to the inventive modified protein are carried out on DNA or RNA level. This inventive isolated polynucleic acid molecule is suitable for diagnostic methods as well as gene therapy and the production of inventive modified protein on a large scale.

Still preferred, the isolated polynucleic acid molecule hybridises to the above defined inventive polynucleic acid molecule

under stringent conditions. Depending on the hybridisation conditions complementary duplexes form between the two DNA or RNA molecules, either by perfectly being matched or also comprising mismatched bases (see Sambrook et al., Molecular Cloning: A laboratory manual, 2nd ed., Cold Spring Harbor, N.Y. 1989). Probes greater in length than about 50 nucleotides may accommodate up to 25 to 30% mismatched bases. Smaller probes will accommodate fewer mismatches. The tendency of a target and probe to form duplexes containing mismatched base pairs is controlled by the stringency of the hybridisation conditions which itself is a function of factors, such as the concentration of salt or formamide in the hybridisation buffer, the temperature of the hybridisation and the post-hybridisation wash conditions. By applying well-known principles that occur in the formation of hybrid duplexes conditions having the desired stringency can be achieved by one skilled in the art by selecting from among a variety of hybridisation buffers, temperatures and wash conditions. Thus, conditions can be selected that permit the detection of either perfectly matched or partially mismatched hybrid duplexes. The melting temperature (Tm) of a duplex is useful for selecting appropriate hybridisation conditions. Stringent hybridisation conditions for polynucleotide molecules over 200 nucleotides in length typically involve hybridising at a temperature 15-25°C below the melting temperature of the expected duplex. For oligonucleotide probes over 30 nucleotides which form less stable duplexes than longer probes, stringent hybridisation usually is achieved by hybridising at 5 to 10°C below the Tm. The Tm of a nucleic acid duplex can be calculated using a formula based on the percent G+C contained in the nucleic acids and that takes chain lenghts into account, such as the formula Tm = 81.5-16.6 (log $[Na^+]$) + 0.41 (% G+C) - (600/N), where N = chain lenght.

A further aspect of the present invention relates to a vector which comprises an isolated DNA molecule according to the present invention as defined above. The vector comprises all regulatory elements necessary for efficient transfection as well as efficient expression of proteins. Such vectors are well known in the art and any suitable vector can be selected for this purpose.

A further aspect of the present application relates to a recombinant cell which is stably transfected with an inventive vector as described above. Such a recombinant cell as well as any therefrom descendant cell comprises the vector. Thereby a cell line is provided which expresses the modified protein either continuously or upon activation depending on the vector.

A further aspect of the present invention relates to a pharmaceutical composition which comprises a protein, a polynucleic acid or a vector according to the present invention as defined above and a pharmaceutically acceptable carrier. Of course, the pharmaceutical composition may further comprise additional substances which are usually present in pharmaceutical compositions, such as salts, buffers, emulgators, colouring agents, etc.

A further aspect of the present invention relates to the use of the modified protein, a polynucleic acid or a vector according to the present invention as defined above in a method for inhibiting or supressing the biological activity of the respective wild-type protein. As mentioned above, the modified protein will act as an antagonist whereby the side effects which occur with known recombinant proteins will not occur with the inventive modified protein. In the case of chemokines this will be in particular the biological activity involved in inflammatory reactions.

Therefore, a further use of the modified protein, polynucleic acid or vector according to the present invention is in a method for producing a medicament for the treatment of an inflammatory condition. In particular, if the modified protein is a chemokine, it will act as antagonist without side effects and will be particularly suitable for the treatment of an inflammatory condition. Therefore, a further aspect of the present application is also a method for the treatment of an inflammatory condition, wherein a modified protein according to the present invention, the isolated polynucleic acid molecule or vector according to the present invention or a pharmaceutical composition according to the present invention is administered to a patient.

Preferably, the inflammatory condition is selected from a group comprising rheumatoid arthritis, psoriasis, osteoarthritis, asthma, Alzheimer's disease, and multiple sclerosis. Since the activation through chemokines can be inhibited with a modified protein according to the present invention, inflammatory reactions can be inhibited or down-regulated whereby the above mentioned inflammatory conditions can be prevented or treated.

The present invention is described in further detail with the help of the following examples and figures to which the invention is, however, not limited whereby Fig.1 is a CD spectra; Fig.2 shows secondary structure contents of various mutants; Figs.3 and 4 show graphics of results from fluorescence anisotropy tests of various mutants; Fig.5 shows the graphic of results from isothermal fluorescence titrations; Fig.6 shows the graphic of results from unfolding experiments of various mutants, Fig.7 shows chemotaxis index of IL-8 mutants, and Fig.8 shows the results of the RANTES chemotaxis assay.

E X A M P L E S

Example 1: Generation of recombinant IL-8 genes and cloning of the mutants

Polymerase chain reaction (PCR) technique was used to generate the desired cDNAs for the mutants by introducing the mutations using sense and antisense mutagenesis primers. A synthetic plasmid containing the cDNA for wtIL-8 was used as template, Clontech Advantage®2 Polymerase Mix applied as DNA polymerase and the PCR reaction performed using a Mastergradient Cycler of Eppendorf. The mutagenesis primers used are summarised in the table below beginning with the forward sequences (5´to 3´):

- CACC ATG TGT CAG TGT ATA AAG ACA TAC TCC (primer for the mutation Δ6)
- CACC ATG TGT CAG TGT ATA AAG ACA TAC TCC AAA CCT AGG CAC CCC AAA AGG ATA (primer for the mutation Δ6 F17R F21R)

The reverse sequences are (5´to 3´):

- TTA TGA ATT CCT AGC CCT CTT (primer for the mutation E70R)
- TTA TGA ATT CTT AGC CCT CTT (primer for the mutation E70K)
- TTA TGA CTT CTC AGC CCT CTT (primer for the mutation N71K)
- TTA TGA CTT CTT AGC CCT CTT (primer for the mutation E70K N71K)
- TTA TGA CTT CCT AGC CCT CTT (primer for the mutation E70R N71K)
- TTA TGA CCT CTT AGC CCT CTT (primer for the mutation E70K N71R)
- TTA TGA CCT CCT AGC CCT CTT (primer for the mutation E70R N71R)

The PCR products were purified, cloned into the pCR®T7/NT-TOPO®TA (Invitrogen) vector and transformed into TOP10F competent *E.coli* (Invitrogen). As a next step a confirmation of the sequence was carried out by double-stranded DNA sequencing using a ABI PRISM CE1 Sequencer.

Example 2: Expression and purification of the recombinant proteins

Once the sequences were confirmed, the constructs were transformed into calcium-competent BL21(DE3) *E.coli* for expression. Cells were grown under shaking in 1 l Lennox Broth (Sigma) containing 100µg/ml Ampicillin at 37°C until an OD_{600} of about 0.8 was reached. Induction of protein expression was accomplished by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Four hours later the cells were harvested by centrifugation at 6000g for 20 minutes. The cell pellet was then resuspended in a buffer containing 20mM TRIS/HCl, 50mM NaCl, pH 8, sonicated at 100 watts for 5x20 s and finally centrifuged again for 20 min at 10,000g. Since the main fraction of the recombinant IL-8 proteins was found in inclusion bodies, denaturing conditions were chosen for further purification. So the cell pellet was resuspended in a buffer of 6M Gua/HCl and 50mM MES, pH 6.5. The suspension was then stirred at 4°C for 4 hours, followed by a dialysis step against 50mM MES, pH 6.5. The resulting suspension was then centrifuged and filtered to be loaded on a strong cation exchange column (SP Sepharose® from Pharmacia Biotech). The elution was accomplished by a linear gradient from

0M-1M NaCl in a 50mM MES buffer, pH 6.5 over 60 minutes. After lyophilisation of the fractions containing the desired protein, a second purification step was carried out by reversed-phase HPLC using a C18 column. In this case a non-linear gradient from 10%-90% Acetonitril was chosen to elute the desired protein. Refolding of the denatured protein was finally accomplished by the same cation exchange column under the same conditions as described above.

The protein was then checked for purity and identity by silver stain analysis in the first case and Western Blot analysis, using a specific monoclonal antibody against wtIL-8, in the second. Refolding of the proteins was also confirmed by Circular Dichroism (CD) measurements.

Example 3: Biophysical Characterisation of the Mutants

3.1 Circular Dicroism measurements and analysis

In order to investigate secondary structure changes of the mutant protein in the presence and absence of heparan sulphate (HS), CD spectroscopy was carried out. Measurements were recorded on a Jasco J-710 spectropolarimeter over a range of 195-250nm, and a cell of 0.1 cm path length was used. Spectra of the protein solutions with a concentration of 5µM were recorded with a response time of 1 s, step resolution of 0.2 nm, speed of 50 nm/min, band width of 1nm and a sensitivity of 20 mdeg. Three scans were averaged to yield smooth spectra. The protein spectra were then background-corrected relating to the CD-signal either of the buffer itself or buffer/HS. Secondary structure analysis of the protein in the presence and absence of HS was finally accomplished using the programme SELCON.

Since a great number of amino acids were changed in a number of novel combinations, it was tried to find out the dimension of the resulting secondary structure changes by circular dichroism methods.

Different structures were obtained depending on the mutations introduced. Except for one mutant expressed (Δ6 F17R F21R E70K N71R) which didn't show any structure, all mutants exhibited

measurable α-helices, β-sheets and loops. Compared to IL-8wt only one mutant (Δ6 E70R) showed nearly similar structure whereas the others differed mainly in their α-helix which ranged from 17.2% to 45.2% out of the total structure. Nevertheless, this fact suggests that the overall structure of IL-8wt was maintained despite many changes within the proteins sequence. This could not have been previously predicted. Having already found that heparan sulphate oligosaccharides only, and not heparin, were able to affect IL-8wt secondary structure, attention was focused on the effects induced by unfractionated heparan sulphate. All examined mutants showed structural changes upon HS binding which can be seen as evidence of complex formation.

To demonstrate the structural changes upon introduced mutations and heparan sulphate addition, some of the data obtained are summarised in the graphs above and below.

3.2 Fluorescence measurements

For studying concentration and ligand dependent quaternary structure changes fluorescence spectroscopy was performed. Due to its high sensitivity, requiring only nanogram quantities of protein, fluorescence technique was the method of choice for carrying out the desired investigations. Measurements were undertaken using a Perkin-Elmer (Beaconsfield, England) LS50B fluorometer.

3.3 Fluorescence Anisotropy

By recording the concentration dependent fluorescence anisotropy of the chemokine resulting from the extrinsic chromophore bisANS it was aimed to find out the dimerisation constant of the mutants. Measurements were performed in PBS starting with high concentrations (up to 4μM protein) followed by stepwise dilution. For each data point, the anisotropy signal (r) recorded at 507 nm was averaged over 60 sec.

IL-8 oligomerisation has been reported to relevantly influence the proteins GAG binding properties. Set at monomeric concentration, IL-8 bound size defined oligosaccharides 1000-fold tighter than at dimeric concentration. Therefore, the oligomerisation properties of IL-8 mutants were investigated by fluorescence an-

isotropy. Since the IL-8 intrinsic fluorophore (Trp57) was not sensitive enough for all of the mutants, the extrinsic fluorophore bis-ANS was used for these measurements. Again, as already noticed for the secondary structure, the mutant Δ6 E70R showed high resemblance also in the oligomerisation constant (k_{oligo}=350nM) to IL-8wt (k_{oligo}=379nM). The mutant with the highest k_{oligo} (k_{oligo}=460nM), which therefore dimerised worst, was Δ6 F17RF21R E70RN71K. Previous studies identified the β-sheets to be mainly involved in the dimerisation process, a fact, which correlates with the results for this mutants' secondary structure, which showed a very low share of β-sheet of only 11.4%. The mutant with the lowest k_{oligo} (k_{oligo}=147nM), was found to be Δ6 F17RF21R E70K, which again showed the highest share of β-sheet structure (29.8%) of all mutants investigated. Also the impact of heparan sulphate addition was observed. As for IL-8wt, where heparan sulphate caused a shift of the oligomerisation constant to much higher levels (k_{oligo}=1.075μM), this was also found for the IL-8 mutants investigated. Δ6 F17RF21R E70K shifted from 0.147μM to 1.162μM, and the mutant Δ6 E70R from 0.350μM to 1.505μM in the presence of heparan sulphate. Some of the results obtained are demonstrated in Figs.3 and 4, whereby Fig.3 shows the dependence of the fluorescence anisotropy of IL-8 mutants in PBS on the chemokine concentration and Fig.4 shows the dependence of the fluorescence anisotropy of Δ6 F17RF21R E70K in PBS on the chemokine concentration in the presence (ten fold excess) and absence of HS ((pc = 10 xy excess) protein concentration).

3.4 Isothermal Fluorescence Titration (IFT) Experiments

Dissociation constants (K_d values) are a measure for the binding affinity of a ligand to a protein and therefore concentration-dependent change in the fluorescence emission properties of the protein (fluorescence quenching) upon ligand binding was used for the determination of K_d. Since these mutants contain an intrinsic tryptophan chromophore which is located at or near the proposed GAG binding site and therefore should be sensitive to structural changes upon ligand binding, IFT experiments seemed to be suitable for this kind of investigation. Fluorescence intensity titration was performed in PBS using a protein concentration of 700nM. The emission of the protein solution upon excitation at 282 nm was recorded over a range of 300-400 nm fol-

lowing the addition of an aliquot of the respective GAG ligand and an equilibration period of 60 sec.

Binding to unfractionated heparin and heparan sulphate was investigated. The mutants were set at dimeric concentration to assure sufficient sensitivity. A quenching of Trp57 fluorescence intensity upon GAG binding was registered within a range of 25 - 35%. Significant improvement of ligand binding was observed, especially for heparin binding. Δ6 F17RN71R E70K (K_d=14nM) and Δ6 F17RF21R N71K (K_d=14.6nM) showed 2600-fold better binding, and Δ6 E70K N71K (K_d=74nM) 1760-fold better binding compared to IL-8wt (K_d=37μM). Good results were also obtained for heparan sulphate binding. For Δ6 F17RN71R E70K a K_d of 107nM was found, for Δ6 F17RF21R N71K the K_d was 95nM and the mutant Δ6 E70K N71K showed a K_d of 34nM. As IL-8wt binds with a K_d of 4.2μM, the K_ds found for the mutants represent an extraordinary improvement in binding, see Fig.5.

3.5 Unfolding experiments

In order to obtain information about the proteins stability and whether this stability would be changed upon GAG ligand binding, unfolding experiments were undertaken. As mentioned above fluorescence techniques are very sensitive for observing quaternary structure changes and therefore are also the method of choice to investigate thermal structural changes of the protein. Measurements were undertaken as described for the IFT in which not the ligand concentration was changed but the temperature. Protein structure was observed at a concentration of 0.7μM from temperatures of 15 - 85°C in the absence and the presence of a 10 fold excess of heparan sulphate or heparin.

The emission maximum of the proteins ranged from 340nm to 357nm, values which are typical for a solvent exposed tryptophan residue. Beginning with the unfolding experiments at 15°C, the emission maximum of the mutants varied between 340nm - 351nm. Compared to IL-8wt, whose emission maximum was observed at 340nm, this means slightly higher values. Upon an increase in temperature, the intensity of emission maximum decreased, accompanied by a shift of the maximum to either a higher or lower

wavelength. The emission maximum of Δ6 E70R and Δ6 E70K N71K shifted from 352.5nm-357nm and 343nm-345nm, which is typical for a further exposure of the Trp57 residue to the solvent trough temperature increase, but interestingly the mutants Δ6 F17RN71R E70K and Δ6 F17RF21R E70R N71K showed a blue shift, ranging from 350nm - 343nm and, less pronounced, from 350nm - 348nm (see Fig.6). By slowly decreasing the temperature, the process of unfolding was partially reversible regarding both the wavelength shift and changes of intensity. Addition of a 5 fold excess of heparan sulphate led to an increase of stability of the proteins, probably through complex formation. This could be observed on the one hand by a shift of the melting point to higher temperature, and on the other hand by a significantly less pronounced shift of emission maximum upon temperature increase.

Example 4: Cell-based assay of the receptor-´´negative´´ function of the dominant-negative IL-8 mutants

In order to characterise the impaired receptor function of the IL-8 mutants with respect to neutrophil attraction, transfilter-based chemotaxis of neutrophils in response to IL-8 mutants was assayed in a microchemotaxis chamber equipped with a 5µm PVP-free polycarbonate membrane.

Cell preparation:
Briefly, a neutrophil fraction was prepared from freshly collected human blood. This was done by adding a 6% dextran solution to heparin-treated blood (1:2) which was then left for sedimentation for 45 min. The upper clear cell solution was collected and washed twice with HBSS w/o Ca and Mg. Cells were counted and finally diluted with HBSS at 2Mio/ml cell suspension, taking into account that only 60% of the counted cells were neutrophils.

Chemotaxis assay:
IL-8 mutants were diluted at concentrations of 10 µg/ml, 1 µg/ml and 0.1 µg/ml and put in triplicates in the lower compartment of the chamber (26µl per well). The freshly prepared neutrophils were seeded in the upper chamber (50µl per well) and incubated for 30 minutes at 37°C in a 5% CO_2 humidified incubator. After

incubation, the chamber was disassembled, the upper side of the filter was washed and wiped off and cells attached to the lower side were fixed with methanol and stained with Hemacolor solutions (Merck). Cells were then counted at 400x magnifications in 4 randomly selected microscopic fields per well. Finally, the mean of three independent experiments was plotted against the chemokine concentration. In Figure 7, the chemotaxis index for various IL-8 mutants is shown. As expected, all mutants showed significantly decreased receptor binding activity.

Example 5: Generation of recombinant RANTES genes, expression, biophysical and activity characterisation of the mutants

The concept of dominant-negative "GAG-masking" chemokine mutants was also employed to RANTES, a chemokine involved in type IV hypersensitivity reactions like transplant rejection, atopic dermatitis as well as in other inflammatory disorders like arthritis, progressive glomerulonephritis and inflammatory lung disease.

The receptor binding capability was impaired by introducing into the wt protein an initiating methionine residue. Expression of the wt RANTES in E. Coli lead to the retention of this methionine residue, which renders wt RANTES to a potent inhibitor of monocyte migration, the so-called Met-RANTES. Different mutations enhancing the GAG binding affinity were introduced via PCR-based site-directed mutagenesis methods.

By these means 9 RANTES mutants have so far been cloned, expressed and purified, Met-RANTES A22K, Met-RANTES H23K, Met-RANTES T43K, Met-RANTES N46R, Met-RANTES N46K, Met-RANTES Q48K, Met-RANTES A22K/N46R, Met-RANTES V49R/E66S and Met-RANTES ^{15}LSLA^{18} V49R/E66S.

Isothermal fluorescence titration experiments were carried out to measure the relative affinity constants (Kd values) of the RANTES mutants for size defined heparin. As can be seen in the table all RANTES mutant proteins showed higher affinities for this heparin, with Met-RANTES A22K, Met-RANTES H23K, Met-RANTES

T43K and Met-RANTES A22K/N46R showing the most promising results.

	Kd in nM
Wt Rantes	456.2 ± 8.5
Met-Rantes V49R/E66S	345.5 ± 21.7
Rantes 15LSLA18 V49R/66S	297.3 ± 14.1
Rantes N46R	367.7 ± 11.7
Rantes N46K	257.4 ± 10.2
Rantes H23K	202.5 ± 12.8
Rantes Q48K	383.4 ± 39.6
Rantes T43K	139.2 ± 30.1
Rantes A22K	202.1 ± 9.8
Rantes A22K/N46R	164.0 ± 16.6

RANTES chemotaxis assay

RANTES mutant directed cell migration was investigated using the 48-well Boyden chamber system equipped with 5µm PVP-coated polycarbonate membranes. RANTES and RANTES mutant dilutions in RPMI 1640 containing 20 mM HEPES pH 7.3 and 1mg/ml BSA were placed in triplicates in the lower wells of the chamber. 50 µl of THP-1 cell suspensions (promonocytic cell line from the European collection of cell cultures) in the same medium at 2 x 10^6 cells/ ml were placed in the upper wells. After a 2 h incubation period at 37 °C in 5 % CO_2 the upper surface of the filter was washed in HBSS solution. The migrated cells were fixed in methanol and stained with Hemacolor solution (Merck). Five 400 x magnifications per well were counted and the mean of three independently conducted experiments was plotted against the chemokine concentration in Figure 8. The error bars represent the standard error of the mean of the three experiments. Again, as in the case of the IL-8 mutants, all RANTES mutants showed significantly reduced receptor binding activity.

Example 6: Proteins with GAG binding regions

By bioinformatical and by proteomical means GAG binding proteins

were characterised together with their GAG binding regions. In the following tables 2 and 3 chemokines are shown with their GAG binding regions (table 2) and examples of other proteins are given also with their GAG binding regions (table 3).

Table 2:

Chemokines and their GAG binding domains

CXC-chemokines

IL-8: ^{18}**HPK**^{20}, (R47) ^{60}**RVVEKFLKR**^{68}

SAKELRCQCIKTYSKPF**HPK**FIKELRVIESGPHCANTEIIVKLSDGRELCLDPKENWVQ**R**

VVEKFLKRAENS

MGSA/GROα: ^{19}**HPK**^{21}, ^{45}**KNGR**^{48}, ^{60}**KKIIEK**^{66}

ASVATELRCQCLQTLQGI**HPK**NIQSVNVKSPGPHCAQTEVIATL**KNGR**KACLNPASPIV**K**

KIIEKMLNSDKSN

MIP-2α/GROβ: ^{19}**HLK**^{21},K45, ^{60}**KKIIEKMLK**^{68}

APLATELRCQCLQTLQGI**HLK**NIQSVKVKSPGPHCAQTEVIATL**K**NGQKACLNPASPMV**K**

KIIEKMLKNGKSN

NAP-2: ^{15}**HPK**^{18}, ^{42}**KDGR**^{45}, ^{57}**KKIVQK**^{62}

AELRCLCIKTTSGI**HPK**NIQSLEVIGKGTHCNQVEVIATL**KDGR**KICLDPDAPRI**KKIVQ**

KKLAGDESAD

PF-4: ^{20}**RPRH**^{23}, ^{46}**KNGR**^{49}, ^{61}**KKIIKK**^{66}

EAEEDGDLQCLCVKTTSQV**RPRH**ITSLEVIKAGPHCPTAQLIATL**KNGR**KICLDLQAPLY

KKIIKKLLES

SDF-1α:K1, ^{24}**KHLK**^{27}, ^{41}**RLK**^{43}

KPVSLSYRCPCRFFESHVARANV**KHLKI**LNTPNCALQIVA**RLK**NNNRQVCIDPKLKWIQE

YLEKALN

CC-chemokines

RANTES: (^{17}**RPLPRAH**^{23}) ^{44}**RKNR**^{47}

SPYSSDTTPCCFAYIA**RPLPRAH**IKEYFYTSGKCSNPAVVFVT**RKNR**QVCANPEKKWVRE

YINSLEMS

MCP-2: ^{18}**RKIPIQR**^{24}, ^{46}**KRGK**^{49}

QPDSVSIPITCCFNVIN**RKIPIQR**LESYTRITNIQCPKEAVIFKT**KRGK**EVCADPKERWVRDSMKHLDQIFQNLKP

MCP-3: ^{22}**KQR**^{24}, ^{47}**KLDK**^{50}, ^{66}**KHLDKK**^{71}

QPVGINTSTTCCYRFINKKIP**KQR**LESYRRTTSSHCPREAVIFKT**KLDK**EICADPTQKWV

QDFM**KHLDKK**TQTPKL

MIP-1α: R17, ^{44}**KRSR**^{47}

SLAADTPTACCFSYTS**R**QIPQNFIADYFETSSQCSKPGVIFLT**KRSR**QVCADPSEEWVQK

YVSDLELSA

MIP-1β:R18, ^{45}**KRSK**^{48}

APMGSDPPTACCFSYTA**R**KLPRNFVVDYYETSSLCSQPAVVFQT**KRSK**QVCADPSESWVQEYVYDLELN

MPIF-1: R18, ^{45}**KKGR**^{48}

MDRFHATSADCCISYTP**R**SIPCSLLESYFETNSECSKPGVIFLT**KKGR**RFCANPSDKQVQ

VCMRMLKLDTRIKTRKN

MIP-5 / HCC-2: ^{40}**KKGR**^{43}

HFAADCCTSYISQSIPCSLMKSYFETSSECSKPGVIFLT**KKGR**QVCAKPSGPGVQDCMKK

LKPYSI

Table 3:

Peroxisome biogenesis factor 1	181 TRRAKE 186
	367 QKKIRS 372
	1263 PKRRKN 1268
	181 TRRAKE 186
	367 QKKIRS 372
	1263 PKRRKN 1268
MLTK-beta	415 SKRRGKKV 422
	312 ERRLKM 317
	416 KRRGKK 421
	312 ERRLKM 317
	416 KRRGKK 421
BHLH factor Hes4	43 EKRRRARI 50
	43 EKRRRA 48
	43 EKRRRA 48
Protocadherin 11	867 MKKKKKKK 874
	867 MKKKKK 872
	867 MKKKKK 872
	899 MKKKKKKK 906
	899 MKKKKK 904
	899 MKKKKK 904

catenin (cadherin-associated protein), delta 1	315 RRRLRS 320
	404 VRKLKG 409
	460 LRKARD 465
	545 RRKLRE 550
	621 AKKGKG 626
	787 AKKLRE 792
	315 RRRLRS 320
	404 VRKLKG 409
	460 LRKARD 465
	545 RRKLRE 550
	621 AKKGKG 626
	787 AKKLRE 792
Muscarinic acetylcholine receptor M5	221 EKRTKD 226
	427 TKRKRV 432
	514 WKKKKV 519
	221 EKRTKD 226
	427 TKRKRV 432
	514 WKKKKV 519
Alpha-2A adrenergic receptor	147 PRRIKA 152
	224 KRRTRV 229
	147 PRRIKA 152
	224 KRRTRV 229
IL-5 promoter REII-region-binding protein	440 TKKKTRRR 447
	569 GKRRRRRG 576
	38 ARKGKR 43
	437 GKKTKK 442
	444 TRRRRA 449
	569 GKRRRR 574
	38 ARKGKR 43
	437 GKKTKK 442
	444 TRRRRA 449
	569 GKRRRR 574
Mitofusin 1	291 ARKQKA 296
	395 KKKIKE 400
	291 ARKQKA 296
	395 KKKIKE 400
N-cym protein	71 VRRCKI 76
	71 VRRCKI 76
Smad ubiquitination regulatory factor 1	672 ERRARL 677
	672 ERRARL 677

CUG-BP and ETR-3 like factor 5	468 MKRLKV 473
	475 LKRPKD 480
	468 MKRLKV 473
	475 LKRPKD 480
Ewings sarcoma EWS-Fli1	347 QRKSKP 352
	347 QRKSKP 352
NUF2R	455 LKRKMFKM 462
	331 LKKLKT 336
	347 VKKEKL 352
	331 LKKLKT 336
	347 VKKEKL 352
Kruppel-like zinc finger protein GLIS2	22 EKRERT 27
	22 EKRERT 27
FKSG32	15 LKRVRE 20
	431 VRRGRI 436
	15 LKRVRE 20
	431 VRRGRI 436
BARH-LIKE 1 PROTEIN	175 LKKPRK 180
	228 NRRTKW 233
	175 LKKPRK 180
	228 NRRTKW 233
Nucleolar GTP-binding protein 1	393 SRKKRERD 400
	624 GKRKAGKK 631
	48 MRKVKF 53
	141 IKRQKQ 146
	383 ARRKRM 388
	393 SRKKRE 398
	490 KKKLKI 495
	543 ARRSRS 548
	550 TRKRKR 555
	586 VKKAKT 591
	629 GKKDRR 634
	48 MRKVKF 53
	141 IKRQKQ 146
	383 ARRKRM 388
	393 SRKKRE 398
	490 KKKLKI 495
	543 ARRSRS 548
	550 TRKRKR 555
	586 VKKAKT 591

	629 GKKDRR 634
EVG1	17 RRRPKT 22
	138 ERKRKA 143
	17 RRRPKT 22
	138 ERKRKA 143
ASPL	282 PKKSKS 287
	282 PKKSKS 287
Zinc transporter 1	477 EKKPRR 482
	477 EKKPRR 482
Uveal autoantigen	603 EKKGRK 608
	995 ERKFKA 1000
	1023 VKKNKQ 1028
	603 EKKGRK 608
	995 ERKFKA 1000
	1023 VKKNKQ 1028
RAB39	7 VRRDRV 12
	7 VRRDRV 12
Down syndrome cell adhesion molecule	320 PRKVKS 325
	387 VRKDKL 392
	320 PRKVKS 325
	387 VRKDKL 392
Protein-tyrosine phosphatase, non-receptor type 12	139 GRKKCERY 146
	59 VKKNRY 64
	59 VKKNRY 64
WD-repeat protein 11	752 VRKIRF 757
	752 VRKIRF 757
Gastric cancer-related protein VRG107	20 SRKRQTRR 27
	25 TRRRRN 30
	25 TRRRRN 30
Early growth response protein 4	356 ARRKGRRG 363
	452 EKKRHSKV 459
	357 RRKGRR 362
	357 RRKGRR 362
Vesicle transport-related protein	309 PKRKNKKS 316
	226 DKKLRE 231
	310 KRKNKK 315
	355 VKRLKS 360
	226 DKKLRE 231
	310 KRKNKK 315
	355 VKRLKS 360

UPF3X	140 AKKKTKKR 147
	141 KKKTKK 146
	217 ERRRRE 222
	225 RKRQRE 230
	233 RRKWKE 238
	240 EKRKRK 245
	296 DKREKA 301
	373 RRRQKE 378
	393 MKKEKD 398
	426 VKRDRI 431
	140 AKKKTKKRD 148
	141 KKKTKK 146
	217 ERRRRE 222
	225 RKRQRE 230
	233 RRKWKE 238
	240 EKRKRK 245
	296 DKREKA 301
	373 RRRQKE 378
	393 MKKEKD 398
	426 VKRDRI 431
CGI-201 protein, type IV	49 ARRTRS 54
	49 ARRTRS 54
RING finger protein 23	98 KRKIRD 103
	98 KRKIRD 103
FKSG17	72 EKKARK 77
	95 IRKSKN 100
	72 EKKARK 77
	95 IRKSKN 100
P83	681 ARKERE 686
	681 ARKERE 686
Ovarian cancer-related protein 1	62 LKRDRF 67
	62 LKRDRF 67
MHC class II transactivator CIITA	407 HRRPRE 412
	741 PRKKRP 746
	783 DRKQKV 788
	407 HRRPRE 412
	741 PRKKRP 746
	783 DRKQKV 788
Platelet glycoprotein VI-2	275 SRRKRLRH 282
	275 SRRKRL 280

	275 SRRKRL 280
Ubiquitin-like 5 protein	11 GKKVRV 16
	11 GKKVRV 16
Protein kinase D2	191 ARKRRL 196
	191 ARKRRL 196
Homeobox protein GSH-2	202 GKRMRT 207
	252 NRRVKH 257
	202 GKRMRT 207
	252 NRRVKH 257
ULBP3 protein	166 ARRMKE 171
	201 HRKKRL 206
	166 ARRMKE 171
	201 HRKKRL 206
Type II iodothyronine deiodinase	87 SKKEKV 92
	87 SKKEKV 92
	299 SKRCKK 304
	299 SKRCKK 304
Sperm antigen	160 LKKYKE 165
	478 IKRLKE 483
	160 LKKYKEKRT 168
	160 LKKYKE 165
	478 IKRLKE 483
UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase	4 ARKIRT 9
	44 DRRVRS 49
	138 PRKCRQ 143
	4 ARKIRT 9
	44 DRRVRS 49
	138 PRKCRQ 143
NCBE	62 HRRHRH 67
	73 RKRDRE 78
	1012 SKKKKL 1017
	62 HRRHRH 67
	73 RKRDRE 78
	1012 SKKKKL 1017
WD repeat protein	372 LKKKEERL 379
	384 EKKQRR 389
	400 AKKMRP 405
	384 EKKQRR 389
	400 AKKMRP 405
Phosphodiesterase 11A	27 MRKGKQ 32

	27 MRKGKQ 32
Probable cation-transporting ATPase 2	891 ERRRRPRD 898
	306 SRKWRP 311
	891 ERRRRP 896
	306 SRKWRP 311
	891 ERRRRP 896
HMG-box transcription factor TCF-3	420 GKKKKRKR 427
	399 ARKERQ 404
	420 GKKKKR 425
	420 GKKKKRKRE 428
	399 ARKERQ 404
	420 GKKKKR 425
HVPS11	793 VRRYRE 798
	793 VRRYRE 798
PIST	165 NKKEKM 170
	165 NKKEKM 170
FYN-binding protein	473 KKREKE 478
	501 KKKFKL 506
	682 LKKLKK 687
	696 RKKFKY 701
	473 KKREKE 478
	501 KKKFKL 506
	682 LKKLKK 687
	696 RKKFKY 701
C1orf25	620 GKKQKT 625
	620 GKKQKT 625
C1orf14	441 LRRRKGKR 448
	70 LRRWRR 75
	441 LRRRKG 446
	70 LRRWRR 75
	441 LRRRKG 446
T-box transcription factor TBX3	144 DKKAKY 149
	309 GRREKR 314
	144 DKKAKY 149
	309 GRREKR 314
Mitochondrial 39S ribosomal protein L47	121 AKRQRL 126
	216 EKRARI 221
	230 RKKAKI 235
	121 AKRQRL 126
	216 EKRARI 221

	230 RKKAKI 235
CGI-203	33 VRRIRD 38
	33 VRRIRD 38
Jagged1	1093 LRKRRK 1098
	1093 LRKRRK 1098
Secretory carrier-associated membrane protein 1	102 DRRERE 107
	102 DRRERE 107
Vitamin D receptor-interacting protein complex component DRIP205	673 KKKKSSRL 680
	672 TKKKKS 677
	954 QKRVKE 959
	978 GKRSRT 983
	995 PKRKKA 1000
	1338 GKREKS 1343
	1482 HKKHKK 1487
	1489 KKKVKD 1494
	672 TKKKKS 677
	954 QKRVKE 959
	978 GKRSRT 983
	995 PKRKKA 1000
	1338 GKREKS 1343
	1482 HKKHKK 1487
	1489 KKKVKD 1494
Secretory carrier-associated membrane protein 2	100 ERKERE 105
	100 ERKERE 105
Nogo receptor	420 SRKNRT 425
	420 SRKNRT 425
FLAMINGO 1	169 GRRKRN 174
	2231 ARRQRR 2236
	169 GRRKRN 174
	2231 ARRQRR 2236
CC-chemokine receptor	58 CKRLKS 63
	58 CKRLKS 63
Prolactin regulatory element-binding protein	271 HKRLRQ 276
	271 HKRLRQ 276
Kappa B and V(D)J recombination signal sequences binding protein	17 PRKRLTKG 24
	713 RKRRKEKS 720
	903 PKKKRLRL 910
	180 HKKERK 185
	629 TKKTKK 634
	712 LRKRRK 717

	903 PKKKRL 908
	1447 QKRVKE 1452
	1680 SRKPRM 1685
	180 HKKERK 185
	629 TKKTKK 634
	712 LRKRRK 717
	903 PKKKRL 908
	1447 QKRVKE 1452
	1680 SRKPRM 1685
Breast cancer metastasis-suppressor 1	200 SKRKKA 205
	229 IKKARA 234
	200 SKRKKA 205
	229 IKKARA 234
Forkhead box protein P3	414 RKKRSQRP 421
	413 FRKKRS 418
	413 FRKKRS 418
FAS BINDING PROTEIN	228 LKRKLIRL 235
	391 RKKRRARL 398
	358 ARRLRE 363
	390 ERKKRR 395
	629 CKKSRK 634
	358 ARRLRE 363
	390 ERKKRR 395
	629 CKKSRK 634
Ubiquitin carboxyl-terminal hydrolase 12	228 HKRMKV 233
	244 LKRFKY 249
	228 HKRMKV 233
	244 LKRFKY 249
KIAA0472 protein	110 HRKPKL 115
	110 HRKPKL 115
PNAS-101	68 LKRSRP 73
	106 PRKSRR 111
	68 LKRSRP 73
	106 PRKSRR 111
PNAS-26	118 DRRTRL 123
	118 DRRTRL 123
Myelin transcription factor 2	176 GRRKSERQ 183
Sodium/potassium-transporting ATPase gamma chain	47 SRRFRC 52
	55 NKKRRQ 60
	47 SRRFRC 52

	55 NKKRRQ 60
Mdm4 protein	441 EKRPRD 446
	464 ARRLKK 469
	441 EKRPRD 446
	464 ARRLKK 469
G antigen family D 2 protein	87 QKKIRI 92
	87 QKKIRI 92
NipSnap2 protein	153 FRKARS 158
	153 FRKARS 158
Stannin	73 ERKAKL 78
	73 ERKAKL 78
Sodium bicarbonate cotransporter	973 EKKKKKKK 980
	165 LRKHRH 170
	666 LKKFKT 671
	966 DKKKKE 971
	973 EKKKKK 978
	165 LRKHRH 170
	666 LKKFKT 671
	966 DKKKKE 971
	973 EKKKKK 978
Myosin X	683 YKRYKV 688
	828 EKKKRE 833
	1653 LKRIRE 1658
	1676 LKKTKC 1681
	683 YKRYKV 688
	828 EKKKRE 833
	1653 LKRIRE 1658
	1676 LKKTKC 1681
PNAS-20	21 RKRKSVRG 28
	20 ERKRKS 25
	20 ERKRKS 25
Pellino	36 RRKSRF 41
	44 FKRPKA 49
	36 RRKSRF 41
	44 FKRPKA 49
Hyaluronan mediated motility receptor	66 ARKVKS 71
	66 ARKVKS 71
Short transient receptor potential channel 7	753 FKKTRY 758
	753 FKKTRY 758
Liprin-alpha2	825 PKKKGIKS 832

	575 IRRPRR 580
	748 LRKHRR 753
	839 GKKEKA 844
	875 DRRLKK 880
	575 IRRPRR 580
	748 LRKHRR 753
	839 GKKEKA 844
	875 DRRLKK 880
Transcription intermediary factor 1-alpha	904 DKRKCERL 911
	1035 PRKKRLKS 1042
	321 NKKGKA 326
	1035 PRKKRL 1040
	321 NKKGKA 326
	1035 PRKKRL 1040
CARTILAGE INTERMEDIATE LAYER PROTEIN	719 QRRNKR 724
	719 QRRNKR 724
UBX domain-containing protein 1	194 YRKIKL 199
	194 YRKIKL 199
Arachidonate 12-lipoxygenase, 12R type	166 VRRHRN 171
	233 WKRLKD 238
	166 VRRHRN 171
	233 WKRLKD 238
Hematopoietic PBX-interacting protein	159 LRRRRGRE 166
	698 LKKRSGKK 705
	159 LRRRRG 164
	703 GKKDKH 708
	159 LRRRRG 164
	703 GKKDKH 708
NAG18	28 LKKKKK 33
	28 LKKKKK 33
POU 5 domain protein	222 ARKRKR 227
	222 ARKRKR 227
NRCAM PROTEIN	2 PKKKRL 7
	887 SKRNRR 892
	1185 IRRNKG 1190
	1273 GKKEKE 1278
	2 PKKKRL 7
	887 SKRNRR 892
	1185 IRRNKG 1190
	1273 GKKEKE 1278

protocadherin gamma cluster	11 TRRSRA 16
	11 TRRSRA 16
SKD1 protein	288 IRRRFEKR 295
	251 ARRIKT 256
	362 FKKVRG 367
	251 ARRIKT 256
	362 FKKVRG 367
ANTI-DEATH PROTEIN	58 HRKRSRRV 65
	59 RKRSRR 64
	59 RKRSRR 64
Centrin 3	14 TKRKKRRE 21
	14 TKRKKR 19
	14 TKRKKR 19
Ectonucleoside triphosphate diphosphohydrolase 3	512 TRRKRH 517
	512 TRRKRH 517
Homeobox protein prophet of PIT-1	12 PKKGRV 17
	69 RRRHRT 74
	119 NRRAKQ 124
	12 PKKGRV 17
	69 RRRHRT 74
	119 NRRAKQ 124
PROSTAGLANDIN EP3 RECEPTOR	77 YRRRESKR 84
	389 MRKRRLRE 396
	82 SKRKKS 87
	389 MRKRRL 394
	82 SKRKKS 87
	389 MRKRRL 394
Pituitary homeobox 3	58 LKKKQRRQ 65
	59 KKKQRR 64
	112 NRRAKW 117
	118 RKRERS 123
	59 KKKQRR 64
	112 NRRAKW 117
	118 RKRERS 123
HPRL-3	136 KRRGRI 141
	136 KRRGRI 141
Advillin	812 MKKEKG 817
	812 MKKEKG 817
Nuclear LIM interactor-interacting factor 1	32 GRRARP 37
	109 LKKQRS 114

	32 GRRARP 37
	109 LKKQRS 114
Core histone macro-H2A.1	5 GKKKSTKT 12
	114 AKKRGSKG 121
	70 NKKGRV 75
	132 AKKAKS 137
	154 ARKSKK 159
	302 DKKLKS 307
	70 NKKGRV 75
	132 AKKAKS 137
	154 ARKSKK 159
	302 DKKLKS 307
Villin-like protein	180 KRRRNQKL 187
	179 EKRRRN 184
	179 EKRRRN 184
BETA-FILAMIN	254 PKKARA 259
	2002 ARRAKV 2007
	254 PKKARA 259
	2002 ARRAKV 2007
Tripartite motif protein TRIM31 alpha	290 LKKFKD 295
	290 LKKFKD 295
Nuclear receptor co-repressor 1	106 SKRPRL 111
	299 ARKQRE 304
	330 RRKAKE 335
	349 IRKQRE 354
	412 QRRVKF 417
	497 KRRGRN 502
	580 RRKGRI 585
	687 SRKPRE 692
	2332 SRKSKS 2337
	106 SKRPRL 111
	299 ARKQRE 304
	330 RRKAKE 335
	349 IRKQRE 354
	412 QRRVKF 417
	497 KRRGRN 502
	580 RRKGRI 585
	687 SRKPRE 692
	2332 SRKSKS 2337
BRAIN EXPRESSED RING FINGER PROTEIN	432 KRRVKS 437

	432 KRRVKS 437
PB39	231 TKKIKL 236
	231 TKKIKL 236
Sperm acrosomal protein	48 FRKRMEKE 55
	24 RRKARE 29
	135 KRKLKE 140
	213 KKRLRQ 218
	24 RRKARE 29
	135 KRKLKE 140
	213 KKRLRQ 218
VESICLE TRAFFICKING PROTEIN SEC22B	177 SKKYRQ 182
	177 SKKYRQ 182
Nucleolar transcription factor 1	79 VRKFRT 84
	102 GKKLKK 107
	125 EKRAKY 130
	147 SKKYKE 152
	156 KKKMKY 161
	240 KKRLKW 245
	451 KKKAKY 456
	523 EKKEKL 528
	558 SKKMKF 563
	79 VRKFRT 84
	102 GKKLKK 107
	125 EKRAKY 130
	147 SKKYKE 152
	156 KKKMKY 161
	240 KKRLKW 245
	451 KKKAKY 456
	523 EKKEKL 528
	558 SKKMKF 563
Plexin-B3	248 FRRRGARA 255
Junctophilin type3	626 QKRRYSKG 633
Plaucible mixed-lineage kinase protein	773 YRKKPHRP 780
	312 ERRLKM 317
	312 ERRLKM 317
fatty acid binding protein 4, adipocyte	78 DRKVKS 83
	105 IKRKRE 110
	78 DRKVKS 83
	105 IKRKRE 110
exostoses (multiple) 1	78 SKKGRK 83

	78 SKKGRK 83
DHHC-domain-containing cysteine-rich protein	64 HRRPRG 69
	64 HRRPRG 69
Myb proto-oncogene protein	2 ARRPRH 7
	292 EKRIKE 297
	523 LKKIKQ 528
	2 ARRPRH 7
	292 EKRIKE 297
	523 LKKIKQ 528
Long-chain-fatty-acid--CoA ligase 2	259 RRKPKP 264
	259 RRKPKP 264
syntaxin1B2	260 ARRKKI 265
	260 ARRKKI 265
Dachshund 2	162 ARRKRQ 167
	516 QKRLKK 521
	522 EKKTKR 527
	162 ARRKRQ 167
	516 QKRLKK 521
	522 EKKTKR 527
DEAD/DEXH helicase DDX31	344 EKRKSEKA 351
	760 TRKKRK 765
	760 TRKKRK 765
Androgen receptor	628 ARKLKK 633
	628 ARKLKK 633
Retinoic acid receptor alpha	364 RKRRPSRP 371
	163 NKKKKE 168
	363 VRKRRP 368
	163 NKKKKE 168
	363 VRKRRP 368
Kinesin heavy chain	340 WKKKYEKE 347
	605 VKRCKQ 610
	864 EKRLRA 869
	605 VKRCKQ 610
	864 EKRLRA 869
DIUBIQUITIN	30 VKKIKE 35
	30 VKKIKE 35
BING1 PROTEIN	519 NKKFKM 524
	564 ERRHRL 569
	519 NKKFKM 524
	564 ERRHRL 569

Focal adhesion kinase 1	664 SRRPRF 669
	664 SRRPRF 669
EBN2 PROTEIN	20 TKRKKPRR 27
	13 PKKDKL 18
	20 TKRKKP 25
	47 NKKNRE 52
	64 LKKSRI 69
	76 PKKPRE 81
	493 SRKQRQ 498
	566 VKRKRK 571
	13 PKKDKL 18
	20 TKRKKP 25
	47 NKKNRE 52
	64 LKKSRI 69
	76 PKKPRE 81
	493 SRKQRQ 498
	566 VKRKRK 571
CO16 PROTEIN	33 ARRLRR 38
	115 PRRCKW 120
	33 ARRLRR 38
	115 PRRCKW 120
KYNURENINE 3-MONOOXYGENASE	178 MKKPRF 183
	178 MKKPRF 183
MLN 51 protein	4 RRRQRA 9
	255 PRRIRK 260
	407 ARRTRT 412
	4 RRRQRA 9
	255 PRRIRK 260
	407 ARRTRT 412
MHC class II antigen	99 QKRGRV 104
MHC class II antigen	99 QKRGRV 104
Transforming acidic coiled-coil-containing protein 1	225 SRRSKL 230
	455 PKKAKS 460
	225 SRRSKL 230
	455 PKKAKS 460
Neuro-endocrine specific protein VGF	479 EKRNRK 484
	479 EKRNRK 484
Organic cation transporter	230 GRRYRR 235
	535 PRKNKE 540
	230 GRRYRR 235

	535 PRKNKE 540
DNA polymerase theta	215 KRRKHLKR 222
	214 WKRRKH 219
	220 LKRSRD 225
	1340 GRKLRL 1345
	1689 SRKRKL 1694
	214 WKRRKH 219
	220 LKRSRD 225
	1340 GRKLRL 1345
	1689 SRKRKL 1694
CDC45-related protein	169 MRRRQRRE 176
	155 EKRTRL 160
	170 RRRQRR 175
	483 NRRCKL 488
	155 EKRTRL 160
	170 RRRQRR 175
	483 NRRCKL 488
Chloride intracellular channel protein 2	197 AKKYRD 202
	197 AKKYRD 202
Methyl-CpG binding protein	85 KRKKPSRP 92
	83 SKKRKK 88
	318 QKRQKC 323
	354 YRRRKR 359
	83 SKKRKK 88
	318 QKRQKC 323
	354 YRRRKR 359
Protein kinase C, eta type	155 RKRQRA 160
	155 RKRQRA 160
Heterogeneous nuclear ribonucleoprotein H	71 LKKDRE 76
	169 LKKHKE 174
	71 LKKDRE 76
	169 LKKHKE 174
ORF2	11 SRRTRW 16
	155 ERRRKF 160
	185 LRRCRA 190
	530 SRRSRS 535
	537 GRRRKS 542
	742 ERRAKQ 747
	11 SRRTRW 16
	155 ERRRKF 160

	185 LRRCRA 190
	530 SRRSRS 535
	537 GRRRKS 542
	742 ERRAKQ 747
F-box only protein 24	9 LRRRRVKR 16
	9 LRRRRV 14
	29 EKRGKG 34
	9 LRRRRV 14
	29 EKRGKG 34
Leucin rich neuronal protein	51 NRRLKH 56
	51 NRRLKH 56
RER1 protein	181 KRRYRG 186
	181 KRRYRG 186
Nephrocystin	3 ARRQRD 8
	430 PKKPKT 435
	557 NRRSRN 562
	641 EKRDKE 646
	3 ARRQRD 8
	430 PKKPKT 435
	557 NRRSRN 562
	641 EKRDKE 646
Adenylate kinase isoenzyme 2, mitochondrial	60 GKKLKA 65
	116 KRKEKL 121
	60 GKKLKA 65
	116 KRKEKL 121
Chlordecone reductase	245 AKKHKR 250
	245 AKKHKR 250
Metaxin 2	166 KRKMKA 171
	166 KRKMKA 171
Paired mesoderm homeobox protein 1	89 KKKRKQRR 96
	88 EKKKRK 93
	94 QRRNRT 99
	144 NRRAKF 149
	88 EKKKRK 93
	94 QRRNRT 99
	144 NRRAKF 149
Ring finger protein	174 LKRKWIRC 181
	8 TRKIKL 13
	95 MRKQRE 100
	8 TRKIKL 13

	95 MRKQRE 100
Ataxin 7	55 PRRTRP 60
	377 GRRKRF 382
	704 GKKRKN 709
	834 GKKRKC 839
	55 PRRTRP 60
	377 GRRKRF 382
	704 GKKRKN 709
	834 GKKRKC 839
Growth-arrest-specific protein 1	169 ARRRCDRD 176
SKAP55 protein	115 EKKSKD 120
	115 EKKSKD 120
Serine palmitoyltransferase 1	232 PRKARV 237
	232 PRKARV 237
Serine palmitoyltransferase 2	334 KKKYKA 339
	450 RRRLKE 455
	334 KKKYKA 339
	450 RRRLKE 455
Synaptopodin	405 KRRQRD 410
	405 KRRQRD 410
Alpha-tectorin	1446 TRRCRC 1451
	2080 IRRKRL 2085
	1446 TRRCRC 1451
	2080 IRRKRL 2085
LONG FORM TRANSCRIPTION FACTOR C-MAF	291 QKRRTLKN 298
Usher syndrome type IIa protein	1285 MRRLRS 1290
	1285 MRRLRS 1290
MSin3A associated polypeptide p30	95 QKKVKI 100
	124 NRRKRK 129
	158 LRRYKR 163
	95 QKKVKI 100
	124 NRRKRK 129
	158 LRRYKR 163
Ig delta chain C region	142 KKKEKE 147
	142 KKKEKE 147
THYROID HORMONE RECEPTOR-ASSOCIATED PROTEIN COMPLEX COMPONENT TRAP100	383 AKRKADRE 390
	833 KKRHRE 838
	833 KKRHRE 838
P60 katanin	369 LRRRLEKR 376

	326 SRRVKA 331
	326 SRRVKA 331
Transcription factor jun-D	286 RKRKLERI 293
	273 RKRLRN 278
	285 CRKRKL 290
	273 RKRLRN 278
	285 CRKRKL 290
Sterol/retinol dehydrogenase	152 VRKARG 157
	152 VRKARG 157
Glycogen [starch] synthase, liver	554 DRRFRS 559
	578 SRRQRI 583
	554 DRRFRS 559
	578 SRRQRI 583
Estrogen-related receptor gamma	173 TKRRRK 178
	353 VKKYKS 358
	173 TKRRRK 178
	353 VKKYKS 358
Neural retina-specific leucine zipper protein	162 QRRRTLKN 169
Cytosolic phospholipase A2-gamma	514 NKKKILRE 521
	31 LKKLRI 36
	218 FKKGRL 223
	428 CRRHKI 433
	31 LKKLRI 36
Cytosolic phospholipase A2-gamma	218 FKKGRL 223
	428 CRRHKI 433
GLE1	415 AKKIKM 420
	415 AKKIKM 420
Multiple exostoses type II protein EXT2.I	296 VRKRCHKH 303
	659 RKKFKC 664
	659 RKKFKC 664
Cyclic-AMP-dependent transcription factor ATF-7	86 EKKARS 91
	332 GRRRRT 337
	344 ERRQRF 349
	86 EKKARS 91
	332 GRRRRT 337
	344 ERRQRF 349
Protein kinase/endoribonulcease	886 LRKFRT 891
	886 LRKFRT 891
Transcription factor E2F6	23 RRRCRD 28
	59 VKRPRF 64

	98 VRKRRV 103
	117 EKKSKN 122
	23 RRRCRD 28
	59 VKRPRF 64
	98 VRKRRV 103
	117 EKKSKN 122
MAP kinase-activating death domain protein	1333 IRKKVRRL 1340
	160 KRRAKA 165
	943 MKKVRR 948
	1034 DKRKRS 1039
	1334 RKKVRR 1339
	1453 TKKCRE 1458
	160 KRRAKA 165
	943 MKKVRR 948
	1034 DKRKRS 1039
	1334 RKKVRR 1339
	1453 TKKCRE 1458
Orphan nuclear receptor PXR	126 KRKKSERT 133
	87 TRKTRR 92
	125 IKRKKS 130
	87 TRKTRR 92
	125 IKRKKS 130
LENS EPITHELIUM-DERIVED GROWTH FACTOR	149 RKRKAEKQ 156
	286 KKRKGGRN 293
	145 ARRGRK 150
	178 PKRGRP 183
	285 EKKRKG 290
	313 DRKRKQ 318
	400 LKKIRR 405
	337 VKKVEKKRE 345
	145 ARRGRK 150
	178 PKRGRP 183
	285 EKKRKG 290
	313 DRKRKQ 318
	400 LKKIRR 405
LIM homeobox protein cofactor	255 TKRRKRKN 262
	255 TKRRKR 260
	255 TKRRKR 260
MULTIPLE MEMBRANE SPANNING RECEPTOR TRC8	229 WKRIRF 234
	229 WKRIRF 234

Transcription factor SUPT3H	172 DKKKLRRL 179
	169 MRKDKK 174
	213 NKRQKI 218
	169 MRKDKK 174
	213 NKRQKI 218
GEMININ	50 KRKHRN 55
	104 EKRRKA 109
	50 KRKHRN 55
	104 EKRRKA 109
Cell cycle-regulated factor p78	165 EKKKVSKA 172
	124 IKRKKF 129
	188 TKRVKK 193
	381 DRRQKR 386
	124 IKRKKF 129
	188 TKRVKK 193
	381 DRRQKR 386
lymphocyte antigen 6 complex, locus D	61 QRKGRK 66
	85 ARRLRA 90
	61 QRKGRK 66
	85 ARRLRA 90
Delta 1-pyrroline-5-carboxylate synthetase	455 LRRTRI 460
	455 LRRTRI 460
B CELL LINKER PROTEIN BLNK	36 IKKLKV 41
	36 IKKLKV 41
B CELL LINKER PROTEIN BLNK-S	36 IKKLKV 41
	36 IKKLKV 41
fetal Alzheimer antigen	5 ARRRRKRR 12
	16 PRRRRRRT 23
	93 WKKKTSRP 100
	5 ARRRRK 10
	16 PRRRRR 21
	26 PRRPRI 31
	35 TRRMRW 40
	5 ARRRRK 10
	16 PRRRRR 21
	26 PRRPRI 31
	35 TRRMRW 40
Transient receptor potential channel 4 zeta splice variant	505 CKKKMRRK 512
	506 KKKMRR 511
	676 HRRSKQ 681

	506 KKKMRR 511
	676 HRRSKQ 681
Myofibrillogenesis regulator MR-2	65 RKRGKN 70
	65 RKRGKN 70
SH2 domain-containing phosphatase anchor protein 2c	269 IKKRSLRS 276
immunoglobulin superfamily, member 3	394 SKRPKN 399
	394 SKRPKN 399
Meis (mouse) homolog 3	112 PRRSRR 117
	120 WRRTRG 125
	112 PRRSRR 117
	120 WRRTRG 125
Deleted in azoospermia 2	105 GKKLKL 110
	114 IRKQKL 119
	105 GKKLKL 110
	114 IRKQKL 119
Centaurin gamma3	543 NRKKHRRK 550
	544 RKKHRR 549
	544 RKKHRR 549
Pre-B-cell leukemia transcription factor-1	233 ARRKRR 238
	286 NKRIRY 291
	233 ARRKRR 238
	286 NKRIRY 291
60S ribosomal protein L13a	112 DKKKRM 117
	158 KRKEKA 163
	167 YRKKKQ 172
	112 DKKKRM 117
	158 KRKEKA 163
	167 YRKKKQ 172
WD40-and FYVE-domain containing protein 3	388 IKRLKI 393
	388 IKRLKI 393
LENG1 protein	34 RKRRGLRS 41
	84 SRKKTRRM 91
	1 MRRSRA 6
	33 ERKRRG 38
	85 RKKTRR 90
	1 MRRSRA 6
	33 ERKRRG 38
	85 RKKTRR 90
MRIP2	375 NKKKHLKK 382
G protein-coupled receptor	430 EKKKLKRH 437

	290 WKKKRA 295
	395 RKKAKF 400
	431 KKKLKR 436
	290 WKKKRA 295
	395 RKKAKF 400
	431 KKKLKR 436
	143 LKKFRQ 148
	228 LRKIRT 233
	143 LKKFRQ 148
	228 LRKIRT 233
	232 QKRRRHRA 239
	232 QKRRRH 237
	232 QKRRRH 237
Sperm ion channel	402 QKRKTGRL 409
A-kinase anchoring protein	2232 KRKKLVRD 2239
	2601 EKRRRERE 2608
	2788 EKKKKNKT 2795
	370 RKKNKG 375
	1763 SKKSKE 1768
	2200 EKKVRL 2205
	2231 LKRKKL 2236
	2601 EKRRRE 2606
	2785 EKKEKK 2790
	1992 QKKDVVKRQ 2000
	370 RKKNKG 375
	1763 SKKSKE 1768
	2200 EKKVRL 2205
	2231 LKRKKL 2236
	2601 EKRRRE 2606
	2785 EKKEKK 2790
Lymphocyte-specific protein LSP1	315 GKRYKF 320
	315 GKRYKF 320
similar to signaling lymphocytic activation molecule (H. sapiens)	261 RRRGKT 266
	261 RRRGKT 266
Dermatan-4-sulfotransferase-1	242 VRRYRA 247
	242 VRRYRA 247
Moesin	291 MRRRKP 296
	325 EKKKRE 330
	291 MRRRKP 296
	325 EKKKRE 330

A-Raf proto-oncogene serine/threonine-protein kinase	288 KKKVKN 293
	358 LRKTRH 363
	288 KKKVKN 293
	358 LRKTRH 363
Cytochrome P450 2C18	117 GKRWKE 122
	117 GKRWKE 122
	117 GKRWKE 122
	156 LRKTKA 161
	117 GKRWKE 122
	156 LRKTKA 161
Protein tyrosine phosphatase, non-receptor type 3	594 IRRRAVRS 601
	263 FKRKKF 268
	388 IRKPRH 393
	874 VRKMRD 879
	263 FKRKKF 268
	388 IRKPRH 393
	874 VRKMRD 879
similar to kallikrein 7 (chymotryptic, stratum corneum)	15 VKKVRL 20
	15 VKKVRL 20
Hormone sensitive lipase	703 ARRLRN 708
	703 ARRLRN 708
40S ribosomal protein S30	25 KKKKTGRA 32
	23 EKKKKK 28
	23 EKKKKK 28
Zinc finger protein 91	617 LRRHKR 622
	617 LRRHKR 622
NNP-1 protein	320 NRKRLYKV 327
	387 ERKRSRRR 394
	432 QRRRTPRP 439
	454 EKKKKRRE 461
	29 VRKLRK 34
	355 GRRQKK 360
	361 TKKQKR 366
	388 RKRSRR 393
	454 EKKKKR 459
	29 VRKLRK 34
	355 GRRQKK 360
	361 TKKQKR 366
	388 RKRSRR 393
	454 EKKKKR 459

Methionyl-tRNA synthetase	725 WKRIKG 730
	725 WKRIKG 730
ELMO2	560 NRRRQERF 567
Meningioma-expressed antigen 6/11	432 RKRAKD 437
	432 RKRAKD 437
Inositol polyphosphate 4-phosphatase type I-beta	375 LRKKLHKF 382
	829 ARKNKN 834
	829 ARKNKN 834
	815 SKKRKN 820
	815 SKKRKN 820
C7ORF12	40 SRRYRG 45
	338 HRKNKP 343
	40 SRRYRG 45
	338 HRKNKP 343
Rap guanine nucleotide exchange factor	138 SRRRFRKI 145
	1071 QRKKRWRS 1078
	1099 HKKRARRS 1106
	139 RRRFRK 144
	661 SKKVKA 666
	930 LKRMKI 935
	1071 QRKKRW 1076
	1100 KKRARR 1105
	1121 ARKVKQ 1126
	139 RRRFRK 144
	661 SKKVKA 666
	930 LKRMKI 935
	1071 QRKKRW 1076
	1100 KKRARR 1105
	1121 ARKVKQ 1126
Sigma 1C adaptin	27 ERKKITRE 34
Alsin	883 GRKRKE 888
	883 GRKRKE 888
NOPAR2	14 LKRPRL 19
	720 VKREKP 725
	14 LKRPRL 19
	720 VKREKP 725
AT-binding transcription factor 1	294 SKRPKT 299
	961 EKKNKL 966
	1231 NKRPRT 1236
	1727 DKRLRT 1732

	2032 QKRFRT 2037
	2087 EKKSKL 2092
	2317 QRKDKD 2322
	2343 PKKEKG 2348
	294 SKRPKT 299
	961 EKKNKL 966
	1231 NKRPRT 1236
	1727 DKRLRT 1732
	2032 QKRFRT 2037
	2087 EKKSKL 2092
	2317 QRKDKD 2322
	2343 PKKEKG 2348
Suppressin	232 YKRRKK 237
	232 YKRRKK 237
Midline 1 protein	100 TRRERA 105
	494 HRKLKV 499
	100 TRRERA 105
	494 HRKLKV 499
High mobility group protein 2a	6 PKKPKG 11
	84 GKKKKD 89
	6 PKKPKG 11
	84 GKKKKD 89

Claims:

1. Method for introducing a GAG binding site into a protein characterised in that it comprises the steps:
- identifying a region in a protein which is not essential for structure maintenance
- introducing at least one basic amino acid into said site and/or deleting at least one bulky and/or acidic amino acid in said site,

whereby said GAG binding site has a GAG binding affinity of Kd ≤ 10µM, preferably ≤ 1µM, still preferred ≤ 0.1µM.

2. Protein obtainable by a method according to claim 1.

3. Modified GAG binding protein, characterised in that a GAG binding region in said protein is modified by substitution, insertion, and/or deletion of at least one amino acid in order to increase the relative amount of basic amino acids in said GAG binding region, and/or reduce the amount of bulky and/or acidic amino acids in said GAG binding region preferably at a solvent exposed position, and in that the GAG binding affinity of said protein is increased compared to the the GAG binding affinity of a respective wild-type protein.

4. Modified protein according to claim 2 or 3, characterised in that at least one basic amino acid selected from the group consisting of Arg, Lys, and His is inserted into said GAG binding region.

5. Modified protein according to any one of claims 2 to 4, characterised in that the protein is a chemokine, preferably IL-8, RANTES or MCP-1.

6. Modified protein according to claim 5, characterised in that said GAG binding region is a C terminal α-helix.

7. Modified protein according to claim 6, characterised in that positions 17, 21, 70, and/or 71 in IL-8 are substituted by Arg, Lys, His, Asn and/or Gln.

8. Modified protein according to any one of claims 2 to 7, characterised in that the increased GAG binding affinity is an increased binding affinity to heparan sulphate and/or heparin.

9. Modified protein according to any one of claims 2 to 8, characterised in that a further biologically active region is modified thereby inhibiting or down-regulating a further biological activity of said protein.

10. Modified protein according to claim 9, characterised in that said further biologically active region is modified by deletion, insertion, and/or substitution, preferably with alanine, a sterically and/or electrostatically similar residue.

11. Modified protein according to claim 9 or 10, characterised in that said protein is a chemokine and said further biological activity is leukocyte activation.

12. Modified protein according to claim 11, characterised in that said protein is IL-8 and said further biologically active region is located within the first 10 N-terminal amino acids.

13. Modified protein according to claim 12, characterised in that said protein is an IL-8 mutant with the first 6 N-terminal amino acids deleted.

14. Modified protein according to claim 13, characterised in that said protein is an IL-8 mutant selected from the group consisting of del6F17RE70KN71R, del6F17RE70RN71K and del6E70KN71K.

15. Isolated polynucleic acid molecule, characterised in that it codes for a protein according to any one of claims 2 to 14.

16. Isolated polynucleic acid molecule, characterised in that it hybridises to the DNA molecule according to claim 15 under stringent conditions.

17. Vector, characterised in that it comprises an isolated DNA molecule according to claim 15 or 16.

18. Recombinant cell, characterised in that it is stably transfected with a vector according to claim 17.

19. Pharmaceutical composition, characterised in that it comprises a protein according to any one of claims 2 to 14, a polynucleic acid according to claims 15 or 16 or a vector according to claim 17 and a pharmaceutically acceptable carrier.

20. Use of the protein according to any one of claims 2 to 14, a polynucleic acid according to claims 15 or 16 or a vector according to claim 17 in a method for inhibiting or suppressing the biological activity of the respective wild-type protein.

21. Use of the protein according to any one of claims 2 to 14, a polynucleic acid according to claims 15 or 16 or a vector according to claim 17 in a method for producing a medicament for the treatment of an inflammatory condition.

22. Use according to claim 21, characterised in that the inflammatory condition is selected from a group comprising rheumatoid arthritis, psoriasis, osteoarthritis, asthma, Alzheimer's disease, and multiple sclerosis.