CLAIMS

1. A recombinant human diamine oxidase (DAO) with decreased glycosaminoglycan binding affinity compared to the respective wild type human DAO, wherein said DAO comprises at least one amino acid ~~modification~~ substitution of any one of amino acids X1, X2, X3, X4 and X5 of sequence X1FX2X3X4LPX5 at positions 568-575 ~~–~~of the glycosaminoglycan (GAG) binding domain with reference to the numbering of SEQ ID NO:1.

2. The recombinant DAO of claim 1, further comprising at least one modification of solvent accessible cysteine at amino acid position 123 (cys123) with reference to the numbering of SEQ ID No. 1, specifically the modification of the cysteine is an amino acid substitution, deletion or coupling with a chemical moiety, more specifically cys123 is substituted by alanine.

3. The recombinant DAO of claim 1 or 2, wherein the GAG binding domain is a heparin/heparan sulfate binding domain.

~~4. The recombinant DAO of any one of claims 1 to 3, wherein the at least one amino acid modification in the GAG binding domain is an amino acid substitution, deletion, insertion or coupling with a chemical moiety.~~

~~5.~~4. The recombinant DAO of any one of claims 1 to 3~~4~~, comprising 2, 3, 4, 5, 6, 7, or 8 amino acid substitutions in the GAG binding domain.

~~6.~~5. The recombinant DAO of any one of claims 1 to 4~~5~~, comprising a GAG binding domain of amino acid sequence X1FX2X3X4LPX5, wherein

X1 can be by any amino acid, specifically it is A or S, more specifically it is S;

X2 can be by any amino acid, specifically it is K;

X3 can be by any amino acid, specifically it is A or T, more specifically it is T,

X4 can be by any amino acid, specifically it is K, and

X5 can be by any amino acid, specifically it is K or T, more specifically it is T.

~~7.~~6. The recombinant DAO of claim 5~~6~~, comprising the amino acid sequence selected from the group consisting of SFKAKLPK (SEQ ID NO:33), AFKAKLPT (SEQ

ID NO:34), AFKTKLPK (SEQ ID NO:35), SFKTKLPK (SEQ ID NO:36), AFKTKLPT (SEQ ID NO:37), SFKAKLPK (SEQ ID NO:38).

~~8.~~7. The recombinant DAO of any one of claims 1 to 6~~7~~ further comprising an amino acid substitution at position 168 with reference to SEQ ID NO 1, specifically Asn is replaced by Gln.

~~9.~~8. The recombinant DAO of any one of claims 1 to 7~~8~~, wherein said DAO has increased plasma half-life compared to wild type DAO, specifically said half-life is increased at least 1.5 fold, specifically at least 2 fold compared to wild type DAO.

~~10.~~9. The recombinant DAO of any one of claims 1 to 8~~9~~, wherein said DAO has an at least 10-fold increased ~~AUC~~ area under the plasma drug concentration time curve compared to wild type DAO.

~~11.~~10. The recombinant DAO of any one of claims 1 to 9~~10~~, wherein internalization by endothelial cells is at least 10%, 25%, 50%, 60%, 70%, 80%, specifically 90% reduced compared to wild type DAO.

~~12.~~11. The recombinant DAO of any one of claims 1 to 10~~1~~, wherein GAG binding affinity, specifically heparin/heparan sulfate binding affinity is at least 10%, 25%, 50%, 60%, 70%, 80%, specifically 90% reduced compared to wild type DAO.

~~13.~~12. The recombinant DAO of any one of claims 1 to 11~~2~~, comprising the amino acid sequences of SEQ ID NOs:2 to 16.

~~14.~~13. A fusion polypeptide comprising the recombinant DAO of any one of claims 1 to 11~~2~~ and an Fc domain of human IgG or human serum albumin (HSA), wherein the fusion polypeptide retains the functional activity of the recombinant DAO.

~~15.~~14. An isolated nucleotide sequence encoding the DAO of any one of claims 1 to 12~~3~~, specifically comprising sequences SEQ ID NOs:17 to 32.

~~16.~~15. A recombinant vector comprising the nucleotide sequence of claim 14~~5~~, specifically the vector is a bacterial, yeast, baculoviral, plant or mammalian expression vector.

~~17.~~16. An expression cassette comprising the nucleotide sequence of claim 1~~4~~5, operably linked to regulatory elements.

~~18.~~17. A recombinant host cell or a host cell line comprising the recombinant DAO of any one of claim 1 to 1~~2~~3, wherein the host cells are selected from the group consisting of CHO cells, Vero cells, MDCK cells, Pichia pastoris cells, SF9 cells.

~~19.~~18. An expression system comprising the vector of claim 1~~5~~6 or the expression cassette of claim 17 and a host cell or host cell line of claim 1~~7~~8.

~~20.~~19. A method for producing the recombinant DAO according to claims 1 to ~~12~~11, said method comprising the steps of

i. cloning a nucleotide sequence encoding the DAO of any one of claims 1 to 1~~1~~3 into an expression vector,

ii. transforming a host cell with said vector,

iii. cultivating the transformed host cell under conditions wherein the DAO is expressed,

iv. isolating the DAO from the host cell culture, optionally by disintegrating the host cells, and optionally

v. purifying the DAO.

~~21~~20. Pharmaceutical composition comprising the recombinant DAO of any one of claims 1 to ~~13~~ 12 and optionally one or more excipients.

~~22~~21. The recombinant DAO of any one of claims 1 to ~~13~~ 12 for use in the treatment ~~of a condition associated with excess histamine, specifically for the treatment~~ of chronic allergic diseases, ~~more specifically for the treatment of~~ anaphylaxis, anaphylactic shock, chronic urticaria, acute urticaria, asthma, hay fever, allergic rhinitis, allergic conjunctivitis, histamine intoxication, headache, atopic dermatitis inflammatory diseases, mastocytosis, mast cell activation syndrome (MCAS), pre-eclampsia, hyperemesis gravidarum, pre-term labor, peptic ulcers, acid reflux, pruritus, and sepsis.

~~23~~22. The use of the recombinant DAO of any one of claims 1 to ~~13~~ 12 for the manufacture of a medicament for the treatment ~~of a condition associated with~~

excess histamine, specifically for the treatment of chronic allergic diseases, more specifically for the treatment of anaphylaxis, anaphylactic shock, chronic urticaria, acute urticaria, asthma, hay fever, allergic rhinitis, allergic conjunctivitis, histamine intoxication, headache, atopic dermatitis inflammatory diseases, mastocytosis, peptic ulcers, acid reflux, pruritus, and sepsis.

A target-specific ligand specifically binding to the GAG binding domain of DAO, specifically binding to one or more of amino acids at position 568-575 with reference to the numbering of SEQ ID No. 1.

A target-specific ligand specifically inhibiting heparin/heparan sulfate binding to the GAG binding domain of DAO, specifically to any one or more of amino acids at position 568-575 with reference to the numbering of SEQ ID No. 1.

The target-specific ligand of claim 23 or 24 wherein said ligand is selected from the group consisting of nucleic acid, small molecule inhibitor or antigen binding protein.

The target-specific ligand of claim 25 wherein said ligand is an antigen binding protein, specifically selected from the group consisting of

antibodies or antibody fragments, such as any of Fab, Fd, scFv, diabodies, triabodies, Fv tetramers, minibodies, nanobodies, single-domain antibodies like VH, VHH, IgNARs, or V-NAR;

antibody mimetics, such as Adnectins™, Affibodies®, Affilins®, Affimers®, Affitins, Alphabodies, Aptamers, Anticalins, Avimers, DARPins®, Fynomers®, Kunitz domain peptides, Monobodies, or NanoCLAMPS; or

fusion proteins comprising one or more immunoglobulin-fold domains, antibody domains or antibody mimetics.

A method for identifying compounds which modulate the heparin binding of the DAO, comprising the steps of

(a) constructing a computer model of the GAG binding domain defined by the structure coordinates of the amino acids of the DAO sequence of SEQ ID No. 1,

(b) selecting a potential modulating compound by a method selected from the group consisting of:

(i) assembling molecular fragments into said compound,

(ii) selecting a compound from a small molecule database, and

(iii) *de novo* ligand design of said compound;

(c) employing computational means to perform a fitting program operation between computer models of the said compound and the GAG binding domain in order to provide an energy-minimized configuration of the said compound in the heparin binding domain; and

(d) evaluating the results of said fitting operation to quantify the association between the said compound and the heparin/heparan sulfate binding domain, thereby evaluating the ability of said compound to associate with the said heparin binding domain.