WHAT IS CLAIMED IS:

1. A method for preparing a fusion protein comprising tissue kallikrein 1 (KLK1), comprising Step (1): introducing a recombinant vector comprising a nucleic acid molecule encoding a fusion protein into a suitable protein expression system, wherein the fusion protein comprises a masking peptide, a first polypeptide and a second polypeptide, the masking peptide, the first polypeptide and the second polypeptide are linked directly or covalently via a linker, and

a) the masking peptide has an amino acid sequence that can be specifically recognized and cleaved by a protease;

b) the first polypeptide comprises KLK1 protein or a variant thereof; and

c) the second polypeptide is used to extend the half-life of the first polypeptide.

2. The method according to claim 1, further comprising Step (2): mixing the fusion protein obtained in Step (1) with a protease to perform a specific enzymatic cleavage reaction, thereby removing the masking peptide; optionally

a) the fusion protein obtained in Step (1) is purified before performing the specific enzymatic cleavage reaction in Step (2); or

b) the masking peptide is selected from the group consisting of: an enterokinase (EK) recognition sequence, a thrombin recognition sequence, a blood coagulation factor Xa recognition sequence, a tobacco etch virus (TEV) protease recognition sequence, an SUMO protease recognition sequence, and a sortase recognition sequence; optionally

(i). the masking peptide is an EK recognition sequence; or

(ii). the EK recognition sequence consists of an amino acid sequence as shown in SEQ ID NO: 4 (DDDDK) or SEQ ID NO: 5 (VDDDDK);

or

c) the first polypeptide is human KLK1 protein or a variant thereof; optionally

(i). the first polypeptide comprises an amino acid sequence having at least 90%, at least 95% or at least 98% sequence identity to the amino acid sequence as shown in SEQ ID NO: 2, and yet retains the function or activity of KLK1; or

(ii). an amino acid sequence of the first polypeptide is as shown in SEQ ID NO: 2,

SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122;

or

(d) the second polypeptide is an Fc domain or albumin; optionally

(i). the Fc domain is derived from human IgG Fc domain;

(ii). the Fc domain is derived from human IgG1 Fc domain;

(iii). the Fc domain is derived from human IgG4 Fc domain;

(iv). the Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the amino acid sequence as shown in SEQ ID NO: 11.

(v). the Fc domain comprises the amino acid sequence as shown in SEQ ID NO: 11;

(vi). the Fc domain consists of the amino acid sequence as shown in SEQ ID NO: 11;

(vii). the Fc domain comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the amino acid sequence as shown in SEQ ID NO: 12;

(viii). the Fc domain comprises the amino acid sequence as shown in SEQ ID NO: 12;

(ix). the Fc domain consists of the amino acid sequence as shown in SEQ ID NO: 12;

(x). the Fc domain comprises mutation(s) at one, two, three, four, five or more amino acid positions, optionally the mutations are M252Y, S254T, and T256E;

(xi). the Fc domain comprises an amino acid sequence as shown in SEQ ID NO: 13;

(xii). the Fc domain consists of the amino acid sequence as shown in SEQ ID NO: 13;

(xiii). the Fc domain comprises an amino acid sequence as shown in SEQ ID NO: 14;

(xiv). the Fc domain consists of the amino acid sequence as shown in SEQ ID NO:

14;

(xv). the albumin comprises one or more domains of human serum albumin; or

(xvi). the albumin comprises D3 domain of human serum albumin;

or

e) the second polypeptide is located at N-terminus or C-terminus of the first polypeptide; optionally the fusion protein comprises, in order from the N-terminus to the C-terminus, the masking peptide, the first polypeptide, and the second polypeptide; or

f) the amino acid sequence of the masking peptide is as shown in SEQ ID NO: 5, the amino acid sequence of the first polypeptide is as shown in SEQ ID NO: 2, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121 or SEQ ID NO: 122, and the amino acid sequence of the second polypeptide is as shown in SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15; or

g) the amino acid sequence of the fusion protein obtained after removing the masking peptide in Step (2) is as shown in SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 114 or SEQ ID NO: 128.

3. The method according to any one of the preceding claims, wherein the fusion protein further comprises a tag, optionally the tag is selected from the group consisting of: a fluorescent tag, a luminescent tag, a purification tag, and a chromogenic tag; optionally

a) the tag is a purification tag;

b) the tag is selected from the group consisting of: HIS tag, GST tag, MBP tag, Myc tag, NusA tag, SUMO tag, FLAG tag, Avi tag, Halo tag, SNAP tag, Strep-II tag, and TrX tag;

c) the tag is a HIS tag; or

d) the tag is a HIS tag comprising 5, 6, 7, 8, 9 or 10 histidine.

4. The method according to any one of the preceding claims, wherein the fusion protein comprises, from the N-terminus to the C-terminus, a structure of:

LB-MP-L1-P1-L2-P2,

wherein LB is absent or represents a tag, MP represents the masking peptide, L1 represents a

bond or a first linker, P1 represents the first polypeptide, L2 represents a bond or a second linker, and P2 represents the second polypeptide; optionally the first linker and the second linker are each independently selected from the group consisting of: a cleavable linker, a non-cleavable linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker; optionally

a) the rigid linker comprises one, two, three, four or more repeats of the sequence as shown in SEQ ID NO: 23 (EAAAK);

b) the flexible linker comprises a linker comprising glycine and serine;

c) the linker comprising glycine and serine comprises one, two, three, four or more repeats of the sequence as shown in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21 or SEQ ID NO: 22; or

d) the first linker or the second linker comprises or consists of an amino acid sequence as shown in SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23;

optionally,

the amino acid sequence of the fusion protein is as shown in any one of SEQ ID NOs: 36-55, SEQ ID NOs: 100-109, SEQ ID NOs: 111-113, and SEQ ID NOs: 125-127; optionally

(i). the fusion protein further comprises a signal peptide; optionally the amino acid sequence of the signal peptide is as shown in SEQ ID NO: 24 or SEQ ID NO: 25 or has at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity to the amino acid sequence as shown in SEQ ID NO: 24 or SEQ ID NO: 25; or

(ii). the half-life of the fusion protein obtained after removing the masking peptide by specific enzymatic cleavage is at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, or at least 14 days in the blood circulation of a subject.

5. The method according to any one of the preceding claims, wherein the nucleic acid molecule encoding the fusion protein comprises:

(a) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 77, and a nucleotide sequence as shown in SEQ ID NO: 78;

(b) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown

in SEQ ID NO: 77, and a nucleotide sequence as shown in SEQ ID NO: 79;

(c) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 77, and a nucleotide sequence as shown in SEQ ID NO: 80;

(d) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 77, and a nucleotide sequence as shown in SEQ ID NO: 81;

(e) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 123, and a nucleotide sequence as shown in SEQ ID NO: 78;

(f) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 123, and a nucleotide sequence as shown in SEQ ID NO: 79;

(g) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 123, and a nucleotide sequence as shown in SEQ ID NO: 80;

(h) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 123, and a nucleotide sequence as shown in SEQ ID NO: 81;

(i) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 130, and a nucleotide sequence as shown in SEQ ID NO: 78;

(j) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 130, and a nucleotide sequence as shown in SEQ ID NO: 79;

(k) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 130, and a nucleotide sequence as shown in SEQ ID NO: 80; or

(l) a nucleotide sequence as shown in SEQ ID NO: 76, a nucleotide sequence as shown in SEQ ID NO: 130, and a nucleotide sequence as shown in SEQ ID NO: 81.

6. The method according to any one of the preceding claims, wherein

a) the nucleic acid molecule encoding the fusion protein comprises a nucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the nucleotide sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 116 or SEQ ID NO: 129; or

b) the nucleic acid molecule encoding the fusion protein comprises or consists of the nucleotide sequence as shown in SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68,

SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 116 or SEQ ID NO: 129.

7. The method according to any one of the preceding claims, wherein

a) the recombinant vector is selected from the group consisting of: a plasmid, an artificial chromosome, and a viral vector; optionallythe recombinant vector is pcDNA plasmid (for example, pcDNA3.4 plasmid); or

b) wherein the protein expression system is a cell expression system, and the cell expression system is selected from the group consisting of: a bacterium, a fungus, a plant or animal cell; optionally

(i). the bacterium is selected from the group consisting of: *Bacilli*, *Clostridia*, *Corynebacterium*, *Cupriavidus*, *Delftia*, *Escherichia*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Lactobacillus*, *Lactococcus*, *Proteus*, *Pseudomonas*, *Rhodococcus*, *Salmonella*, *Serratia*, *Shigella*, and *Streptomyces*;

(ii). the bacterium is selected from the group consisting of: *Escherichia coli*, *Salmonella typhimurium*, *Serratia marcescens*, *Bacillus subtilis*, *B. licheniformis*, and *P. aeruginosa*;

(iii). the fungus is selected from the group consisting of: *Candida*, *Saccharomyces cerevisiae*, *Kluyveromyces*, *Pichia pastoris*, and *Schizosaccharomyces pombe*; or

(iv). the animal cell is a mammalian cell, optionally the mammalian cell is a human cell or Chinese hamster ovary (CHO) cell (for example, CHO-K1 cell, and CHO-S cell); optionallythe mammalian cell is a human embryonic kidney 293 cell (HEK293 cell).

8. A fusion protein prepared by the method according to any one of the preceding claims, having a cleavage activity of 0.3-3 PNAU/mg for kininogen to release kinin.

9. A fusion protein comprising a masking peptide, a first polypeptide and a second polypeptide, wherein the masking peptide, the first polypeptide and the second polypeptide are linked directly or covalently via a linker, and

a) the masking peptide has an amino acid sequence that can be specifically recognized and cleaved by a protease;

b) the first polypeptide comprises KLK1 or a variant thereof; and

c) the second polypeptide is used to extend the half-life of the first polypeptide;

optionally

(i). the masking peptide is selected from the group consisting of: an enterokinase (EK) recognition sequence, a thrombin recognition sequence, a blood coagulation factor Xa recognition sequence, a tobacco etch virus (TEV) protease recognition sequence, an SUMO protease recognition sequence, and a sortase recognition sequence; optionallythe EK recognition sequence is as shown in SEQ ID NO: 5 (VDDDDK);

(ii). the first polypeptide is human KLK1 protein or a variant thereof;

(iii) the first polypeptide comprises an amino acid sequence having at least 90%, at least 95% or at least 98% sequence identity to the amino acid sequence as shown in SEQ ID NO: 2, and yet retains the function or activity of KLK1;

(iv). the second polypeptide is an Fc domain or albumin;

(v). the fusion protein further comprising a tag; optionally a HIS tag; or

(vi). the fusion protein comprises, from the N-terminus to the C-terminus, a structure of:

LB-MP-L1-P1-L2-P2,

wherein LB is absent or represents a tag, MP represents the masking peptide, L1 represents a bond or a first linker, P1 represents the first polypeptide, L2 represents a bond or a second linker, and P2 represents the second polypeptide; optionally the amino acid sequence of the fusion protein is as shown in any one of SEQ ID NOs: 36-55, SEQ ID NOs: 100-109, SEQ ID NOs: 111-113, and SEQ ID NOs: 125-127.

10. A nucleic acid molecule comprising a nucleotide sequence encoding the fusion protein according to claim 8 or 9.

11. A vector comprising the nucleic acid molecule according to claim 10.

12. A protein expression system comprising the nucleic acid molecule according to claim 10 or the vector according to claim 11.

13. A pharmaceutical composition comprising the fusion protein according to claim 8 or 9, the nucleic acid molecule according to claim 10, the vector according to claim 11, or the protein expression system according to claim 12, and a pharmaceutically acceptable carrier.

14. A method for constructing a protein expression system, comprising: introducing a nucleic acid molecule encoding the fusion protein according to claim 8 or 9 into a vector to construct an expression vector; and introducing the expression vector into a protein expression system.

15. The fusion protein prepared by the method according to any one of claims 1 to 7, or the fusion protein of claim 8 or 9 for use in treating or preventing a disease, wherein the disease is selected from the group consisting of an ischemic disease, a hemorrhagic disease, a kidney disease, a metabolic disease, and a neurodegenerative disease; optionally,

a) the ischemic disease is selected from the group consisting of cerebral ischemia (ischemic stroke), myocardial ischemia, ischemic colitis, critical limb ischemia, skin ischemia, ischemic stroke (for example, acute ischemic stroke), transient ischemic attack (TIA), and traumatic brain injury (TBI);

b) the ischemic disease is acute ischemic stroke; or

c) the kidney disease is chronic nephropathy or acute renal injury.