Dysregulation of MMPs also occurs in congestive heart failure and is thought to play a role in numerous proinflammatory processes. However, development of TIMP-3 as a therapeutic inhibitor of MMP activity has been hampered by challenges in production of recombinant protein and short half-life of recombinant forms of TIMP-3. In particular, the serum half-life of TIMP-3 following intravenous administration in rats is less than sixty minutes, and such a short residence time negatively impacts the ability to maintain a therapeutically useful concentration at a disease site. Accordingly, there is a need in the art for forms of TIMP-3 that exhibit favorable production, purification and pharmacokinetic/pharmacodynamic properties.

Summary of the Invention

The disclosure provides TIMP-3 polypeptides having advantageous properties, *e.g.*, enhanced pharmacokinetic or pharmacodynamics properties (such as half-life), improved expression levels compared to native TIMP-3, reduced affinity to non-targets (*e.g.*, scavenger receptors), and/or reduced dependence on heparin for production.

In some examples, the disclosure provides a TIMP-3 polypeptide fused to one or more half-life extending moieties or chemically modified with one or more half-life extending moieties. For example, in some examples, the disclosure provides a fusion protein comprising TIMP-3 (or a fragment thereof) fused to the Fc domain of an isolated antibody at the N- or C-terminus of TIMP-3. The Fc domain may be fused to TIMP-3 (or a fragment thereof) via the N- or C-terminus of the Fc moiety. The Fc domain may be monomeric or heterodimeric. The disclosure also contemplates a TIMP-3 polypeptide (or a fragment thereof) fused to human serum albumin or a full antibody (at the N- or C- terminus of the heavy chain or light chain). In some examples, the chemical modification to TIMP-3 (or a fragment thereof) to extend half-life includes conjugation to polyethylene glycol (PEG).

In one aspect, the present invention provides an isolated TIMP-3 mutein having a mature region that is at least 90% identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, selected from the group consisting of: a) a TIMP-3 mutein having two or more pairs of mutations selected from the group consisting of K45N/V47T; K50N/V52T; P56N/G58T; H78N/Q80T; K94N/E96T; and D110N/K112T; b) a TIMP-3 mutein having one or more pairs of mutations selected from the group consisting of K45N/V47T; K50N/V52T; P56N/G58T; H78N/Q80T; K94N/E96T; and D110N/K112T; and an additional

mutation that is selected from the group consisting of R138T; G173T, and both R138T andG173T; and c) the TIMP-3 mutein according to a) or b) that further comprises the mutation F57N.

In certain examples, the TIMP-3 protein carries mutations in the native sequence resulting in improved half-life; such TIMP-3 mutations are described herein as, *e.g.,* "TIMP-3 muteins." In various examples, the TIMP-3 protein is at least 90% identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, wherein the mutein has at least one mutation that introduces at least one N-linked glycosylation site. In an additional example, the TIMP-3 mutein has two, three, or four new N-linked glycosylation sites; in a still further example, the number of N-linked glycosylation sites introduced is five, six, seven, eight, nine, ten, eleven or twelve. In each mutein, it is further contemplated that addition of one

or more new N-linked glycosylation cites does not substantially diminish the metalloproteinase inhibitory activity of the native molecule.

In another aspect, the present invention provides a TIMP-3 mutein having a mature region that is at least 90% identical in amino acid sequence to amino acids 24-211 of SEQ ID NO:2, selected from the group consisting of: a) a TIMP-3 mutein having two or more pairs of mutations selected from the group consisting of K45N/V47T; K50N/V52T; P56N/G58T; H78N/Q80T; K94N/E96T; and D110N/K112T; b) a TIMP-3 mutein having one or more pairs of mutations selected from the group consisting of K45N/V47T; K50N/V52T; P56N/G58T; H78N/Q80T; K94N/E96T; and D110N/K112T; and an additional mutation that is selected from the group consisting of R138T; G173T, and both R138T andG173T; and c) the TIMP-3 mutein according to a) or b) that further comprises the mutation F57N.

Also exemplified within the disclosure is a TIMP-3 mutein having a mature region that is at least 90% identical in amino acid sequence to the mature region of TIMP-3 set forth in SEQ ID NO:2, having at least one mutation, the mutation being selected from the group consisting of K45N, V47T, K50N, V52T, P56N, F57N, G58T, H78N, Q80T, K94N, E96T, D110N, K112T, R138T, and G173T. Additional examples include a TIMP-3 mutein having two or more pairs of mutations selected from the group consisting of K45N/V47T, K50N/V52T, P56N/G58T, H78N/Q80T, K94N/E96T, and D110N/K112T; and a TIMP-3 mutein having one or more pairs of mutations selected from the group consisting of K45N/V47T, K50N/V52T, P56N/G58T, H78N/Q80T, K94N/E96T, and D110N/K112T, and an additional mutation that is selected from the group consisting of R138T, G173T, and both R138T and G173T. Further examples include muteins having any of the aforementioned combinations of mutation and in addition the mutation F57N.

In one example of the disclosure, at least one N-linked glycosylation site is introduced in a region of the TIMP-3 amino acid sequence selected from the group consisting of: the region consisting of amino acids 44-59; the region consisting of amino acids 77-81; the region consisting of amino acids 93-97; the region consisting of amino acids 109-112; the region consisting of amino acids 137-139; the region consisting of amino acids 172-174; and combinations thereof. In an additional example, the TIMP-3 mutein has two, three, four, or five N-linked glycosylation sites; in a still further example, the number of N-linked glycosylation sites introduced is four, five, six, seven, eight, nine, ten, eleven or twelve.

One example of the disclosure provides TIMP-3 muteins K45N, V47T, P56N, G58T, Q126N, R138T (SEQ ID NO:3); K45N, V47T, P56N, G58T, K94N, E96T, R138T (SEQ ID NO:4); K45N, V47T, P56N, G58T, R138T, G173T (SEQ ID NO:5); K45N, V47T, F57N, K94N, E96T, D110N, K112T (SEQ ID NO:6); K45N, V47T, F57N, K94N, E96T, R138T (SEQ ID NO:7); K45N, V47T, H78N, Q80T, K94N, E96T, R138T, G173T (SEQ ID NO:8); K45N, V47T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:9); K45N, V47T, K94N, E96T, D110N, K112T, G173T (SEQ ID NO:10); K45N, V47T, K94N, E96T, R138T, G173T (SEQ ID NO:11); K45S, F57N, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:12); K45S, F57N, H78N, Q80T, K94N, E96T, R138T (SEQ ID NO:13); K50N, V52T P56N, G58T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:14); K50N, V52T, H78N, Q80T, K94N, E96T, R138T, G173T (SEQ ID NO:15);

K50N, V52T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:16); K50N, V52T, K94N, E96T, D110N, K112T, R138T, G173T (SEQ ID NO:17); K50N, V52T, K94N, E96T, R138T, G173T (SEQ ID NO:18); K50N, V52T, Q126N, R138T, G173T (SEQ ID NO:19); P56N, G58T, H78N, Q80T, K94N, E96T, R138T (SEQ ID NO:20); P56N, G58T K94N, E96T, Q126N, R138T (SEQ ID NO:21); P56N, G58T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:22); P56N, G58T, H78N, Q80T, K94N, E96T, G173T (SEQ ID NO:23); P56N, G58T, Q126N, R138T, G173T (SEQ ID NO:24); H78N, Q80T, K94N, E96T, R138T, G173T (SEQ ID NO:25); and H78N, Q80T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:26).

Further examples include TIMP-3 muteins K50N/V52T,D110N/K112T, R138T, G173T; K45N/V47T, D110N/K112T, R138T, G173T; H78N/Q80T, D110N/K112T, R138T, G173T; K45N/V47T, K50N/V52T, H78N/A80T, R138T; K45N/V47T, H78N/Q80T, D110N/K112T, G173T; K45N/V47T, H78N/Q80T, R138T, G173T; K50N/V52T, H78N/Q80T, K94N/E96T, G173T; K50N/V52T, H78N/Q80T, D110N/K112T, R138T; K45N/V47T, K50N/V52T, H78N/Q80T, D110N/K112T; K50N/V52T, H78N/Q80T, R138T, G173T; K45N/V47T, H78N/Q80T, R138T, G173T; K45N/V47T, H78N/Q80T, D110N/K112T, R138T; K45N/V47T, K50N/V52T, H78N/Q80T, D110N/K112T, G173T; K45N/V47T, K50N/V52T, H78N/Q80T, R138T, G173T; K45N/V47T, K50N/V52T, H78N/Q80T, K94N/E96T, G173T; K45N/V47T, H78N/Q80T, K94N/E96T, R138T, G173T; K50N/V52T, H78N/Q80T, K94N/E96T, R138T, G173T; K45N/V47T, H78N/Q80T, D110N/K112T, R138T,G173T; K50N/V52T, H78N/Q80T, D110N/K112T, R138T, G173T; and K45N/V52T, K50N/V52T, H78N/Q80T, D110N/K112T, R138T.

The disclosure further provides a TIMP-3 mutein comprising (or consisting of) the amino acid sequence set forth in SEQ ID NOs: 3-26 and 51-60, as well as a nucleic acid comprising nucleotide sequence encoding the amino acid sequence of any one of SEQ ID NOs: 3-26 and 51-60.

In yet another aspect, the present invention provides an isolated nucleic acid that encodes a TIMP-3 mutein as disclosed herein.

In yet another aspect, the present invention provides an expression vector comprising the isolated nucleic acid as disclosed herein.

In yet another aspect, the present invention provides an isolated host cell transformed or transfected with the expression vector as disclosed herein.

In yet another aspect, the present invention provides a method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell as disclosed herein under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein.

In one example, the disclosure provides a nucleic acid (*e.g.*, an isolated nucleic acid) that encodes a TIMP-3 mutein according to any one of the aforementioned TIMP-3 muteins. Other examples of the disclosure are an expression vector comprising the nucleic acid; a host cell (*e.g.*, an isolated host cell) transformed or transfected with the expression vector; and a method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell of under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein.

The disclosure also provides a nucleic acid comprising (or consisting of) the nucleic acid sequence set forth in SEQ ID NOs: 27-50 and 61-70.

In yet another aspect, the present invention provides a composition comprising the TIMP-3 mutein as disclosed herein and a physiologically acceptable diluent, excipient or carrier.

In yet another aspect, the present invention provides use of a composition as disclosed herein in the manufacture of a medicament for treating a condition in which matrix metalloproteases (MMPs) and/or other proteinases that are inhibited or inhibitable by TIMP-3 play a causative or exacerbating role, wherein an amount of the composition sufficient to treat the condition is to be administered to an individual afflicted with such a condition.

In yet another aspect, the present invention provides use of a composition as disclosed herein in the manufacture of a medicament for inhibiting cardiac extracellular matrix (ECM) degradation or adverse cardiac remodeling in a subject, wherein an amount of the composition effective to inhibit ECM degradation and/or adverse cardiac remodeling is to be administered to a subject in need thereof.

Further provided is a composition comprising the TIMP-3 mutein described herein, as well as a method of treating a condition in which matrix metalloproteases (MMPs) and/or other proteinases that are inhibited or inhibitable by TIMP-3 play a causative or exacerbating role, comprising administering to an individual afflicted with such a condition, an amount of such composition sufficient to treat the condition.

In one example, the condition is selected from the group consisting of inflammatory conditions, osteoarthritis, acute myocardial infarction, cardiac ischemia (including myocardial ischemia), reperfusion injury, and progression to chronic heart failure (*e.g.,* congestive heart failure). In various examples, the condition is vascular plaque stabilization, vasculopathy, or neointima formation. In another example, the condition is selected from the group consisting of acute lung injury, acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF), inflammatory bowel disease (for example, ulcerative colitis, Crohn's disease, and celiac disease), psoriasis, myocarditis including viral myocarditis, inflammation related to atherosclerosis, and arthritic conditions including rheumatoid arthritis and psoriatic arthritis.

In a further example, the condition is selected from the group consisting of dystrophic epidermolysis bullosa, osteoarthritis, pseudogout, rheumatoid arthritis including juvenile rheumatoid arthritis, ankylosing spondylitis, scleroderma, periodontal disease, ulceration including corneal, epidermal, or gastric ulceration, wound healing after surgery, restenosis, emphysema, Paget's disease of bone, osteoporosis, scleroderma, pressure atrophy of bone or tissues as in bedsores, cholesteatoma, abnormal wound healing, rheumatoid arthritis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, , SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, systemic lupus erythematosus, vasculitis, myolitis, polymyolitis, dermatomyolitis, osteoarthritis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, polymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel

o. K50N, V52T, K94N, E96T, D110N, K112T, R138T, G173T (SEQ ID NO:17);
p. K50N, V52T, K94N, E96T, R138T, G173T (SEQ ID NO:18);
q. K50N, V52T, Q126N, R138T, G173T (SEQ ID NO:19);
r. P56N, G58T, H78N, Q80T, K94N, E96T, R138T (SEQ ID NO:20);
s. P56N, G58T K94N, E96T, Q126N, R138T (SEQ ID NO:21);
t. P56N, G58T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:22);
u. P56N, G58T, H78N, Q80T, K94N, E96T, G173T (SEQ ID NO:23);
v. P56N, G58T, Q126N, R138T, G173T (SEQ ID NO:24);
w. H78N, Q80T, K94N, E96T, R138T, G173T (SEQ ID NO:25) and
x. H78N, Q80T, K94N, E96T, D110N, K112T, R138T (SEQ ID NO:26).

5. The TIMP-3 mutein of any one of claims 1-4 fused or conjugated to a moiety that extends half-life of a polypeptide.

6. The TIMP-3 mutein of claim 5 fused to an antibody, an Fc portion of an antibody, the heavy chain or light chain of an antibody, or human serum albumin.

7. The TIMP-3 mutein of claim 5 conjugated to polyethylene glycol.

8. An isolated nucleic acid that encodes a TIMP-3 mutein according to any one of claims 1-4.

9. An expression vector comprising the isolated nucleic acid of claim 8.

10. An isolated host cell transformed or transfected with the expression vector of claim 9.

11. A method of producing a recombinant TIMP-3 mutein comprising culturing the transformed or transfected host cell of claim 10 under conditions promoting expression of the TIMP-3 mutein, and recovering the TIMP-3 mutein.

12. A composition comprising the TIMP-3 mutein of any one of claims 1-7 and a physiologically acceptable diluent, excipient or carrier.

13. Use of a composition according to claim 12 in the manufacture of a medicament for treating a condition in which matrix metalloproteases (MMPs) and/or other proteinases that are inhibited or inhibitable by TIMP-3 play a causative or exacerbating role, wherein an amount of the composition sufficient to treat the condition is to be administered to an individual afflicted with such a condition.

14. The use of 13 wherein the condition is selected from the group consisting of inflammatory conditions, osteoarthritis, myocardial ischemia, reperfusion injury, and progression to congestive heart failure.

15. The use of claim 13, wherein the condition is selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF), inflammatory bowel disease, psoriasis, myocarditis, inflammation related to atherosclerosis, and arthritic conditions.

16. The use of claim 13, wherein the condition is selected from the group consisting of rheumatoid arthritis and psoriatic arthritis, viral myocarditis, dystrophic epidermolysis bullosa, osteoarthritis, pseudogout, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, periodontal disease, ulceration, wound healing after surgery, restenosis, emphysema, Paget's disease of bone, osteoporosis, scleroderma, pressure atrophy of bone or tissues as in bedsores, cholesteatoma, abnormal wound healing, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, SEA Syndrome (Seronegativity, Enthesopathy, Arthropathy Syndrome), dermatomyositis, psoriatic arthritis, scleroderma, systemic lupus erythematosus, vasculitis, myolitis, polymyolitis, dermatomyolitis, osteoarthritis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, polymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, Sjogren's syndrome, psoriasis, plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, erythrodermic psoriasis, dermatitis, atopic dermatitis, atherosclerosis, lupus, Still's disease, Systemic Lupus Erythematosus (SLE), myasthenia gravis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, Celiac disease (nontropical Sprue), enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, pouchitis resulting after proctocolectomy and ileoanal anastomosis, pancreatitis, insulin-dependent diabetes mellitus, mastitis, cholecystitis, cholangitis, pericholangitis, multiple sclerosis (MS), asthma, extrinsic asthma, intrinsic asthma, hyperresponsiveness of the airways, chronic obstructive pulmonary disease (COPD), chronic bronchitis, emphysema, Acute Respiratory Disorder Syndrome (ARDS), respiratory distress syndrome, cystic fibrosis, pulmonary hypertension, pulmonary vasoconstriction, acute lung injury, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonia, eosinophilic pneumonia, bronchitis, allergic bronchitis bronchiectasis, tuberculosis, hypersensitivity pneumonitis, occupational asthma, asthma-like

disorders, sarcoid, reactive airway disease (or dysfunction) syndrome, byssinosis, interstitial lung disease, hyper-eosinophilic syndrome, rhinitis, sinusitis, and parasitic lung disease, airway hyperresponsiveness associated with viral-induced conditions, Guillain-Barre disease, Graves' disease, Addison's disease, Raynaud's phenomenon, autoimmune hepatitis, graft versus host disease (GVHD), cerebral ischemia, traumatic brain injury, multiple sclerosis, neuropathy, myopathy, spinal cord injury, and amyotrophic lateral sclerosis (ALS).

17. The use of claim 13, wherein the condition is selected from the group consisting of vascular plaque stabilization, vasculopathy, neointima formation, acute lung injury, and acute respiratory distress syndrome.

18. Use of a composition of claim 12 in the manufacture of a medicament for inhibiting cardiac extracellular matrix (ECM) degradation or adverse cardiac remodeling in a subject, wherein an amount of the composition effective to inhibit ECM degradation and/or adverse cardiac remodeling is to be administered to a subject in need thereof.

19. The use of claim 18, wherein the subject has suffered a myocardial infarction.

20. The use of claim 18 or claim 19, wherein the composition is to be administered via intracoronary administration or direct injection into the myocardium.