Blood. 1996; 88:2939-2950). This could occur also *in vivo* when one end of the vWF filament is anchored to a surface.

**[0021]** Thrombi of TTP patients consist of little fibrin and mainly of vWF and platelets, suggesting vWF-mediated platelet aggregation as a cause of thrombosis (Asada Y, Sumiyoshi A, Hayashi T, Suzumiya J, Kaketani K. Immunohistochemistry of vascular lesion in thrombotic thrombocytopenic purpura, with special reference to factor VIII related antigen. Thromb Res. 1985; 38:469-479). Patients with relapsing TTP have ultra-large multimers in the plasma. The UL-vWF multimers accumulate over time because the persistence of the inhibitor (Anti-ADAMTS13 Ab) decreases ADAMTS13 activity. The UL-vWF multimers are hyperactive and unfold as a result of shear stress causing platelet aggregation, resulting in intravascular thrombosis (Tsai H M. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. J Mol Med. 2002; 80:639-647; Tsai H M. Deficiency of ADAMTS-13 in thrombotic and thrombocytopenic purpura. J Thromb Haemost. 2003; 1:2038-2040; discussion 2040-2035).

**[0022]** It is believed that the presence of hyper-reactive UL-vWF multimers in the plasma due to ADAMTS13 deficiency could be associated with an increased risk of arterial thrombosis linked to coronary heart disease.

**[0023]** As ADAMTS proteins have been implicated in a number of diseases and conditions, there is a need in the art for methods of large scale production of recombinant ADAMTS proteins having high specific activities, which are suitable for pharmaceutical formulation and administration. The present invention provides methods which satisfy these and other needs in the art for the production and purification of ADAMTS proteins.

## BRIEF SUMMARY ~~OF THE INVENTION~~

**[0024]** In embodiments~~one aspect~~, the present disclosure~~invention~~ provides methods of expressing a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) protein. In certain embodiments, the methods provided herein advantageously result in increased expression levels and in the production of ADAMTS proteins with substantially improved activities. These improved properties may be achieved, in one example, by supplementing the culture medium used for the expression of ADAMTS proteins with increased levels of various components, such as calcium, zinc, or nicotinamide (vitamin B3). In a preferred embodiment, the ADAMTS protein is an ADAMTS13 protein.

**[0025]** In another embodimentaspect, the disclosureinvention relates to methods of increasing the expression and/or the activity levels of an ADAMTS protein by culturing recombinant cells harboring a nucleic acid encoding an ADAMTS protein in animal protein-free and chemically defined mediums under batch, fed-batch or continuous cell-culture conditions. In some embodiments, these methods comprise the use of batch, fed-batch, perfusion, or chemostat cell-cultivation that may be performed in either cell suspension or cell adherent fashions. In a preferred embodiment, the ADAMTS protein is an ADAMTS13 protein.

**[0026]** In another embodimentaspect, the present disclosureinvention provides methods of reducing the amount of activity lost during the purification of an ADAMTS protein. In certain embodiments, the methods comprise supplementing purification buffers with additional levels of calcium and/or zinc. In a specific embodiment, the methods relate to stabilizing the activity of an ADAMTS protein during ultrafiltration and/or diafiltration steps used during purification. In a preferred embodiment, the ADAMTS protein is an ADAMTS13 protein.

**[0027]** In yet another embodimentaspect, the present disclosureinvention relates to methods of producing an ADAMTS protein with increased activity for use in the preparation of a pharmaceutical composition. In certain embodiments, these methods comprise the use of animal protein-free and/or chemically defined culture mediums under conditions suitable for increased expression of an ADAMTS protein having increased activities. In a preferred embodiment, the ADAMTS protein is an ADAMTS13 protein.

**[0028]** In a related embodimentaspect, the present disclosureinvention provides animal protein-free, oligopeptide-free, and chemically defined mediums that are useful for the expression of ADAMTS proteins having high specific activities. In a preferred embodiment, the ADAMTS protein is an ADAMTS13 protein.

**[0028a]** In one aspect, there is provided a method for expressing a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13) protein, the method comprising culturing a cell harboring a nucleic acid encoding an ADAMTS13 protein in a culture medium comprising from 3 μM to 12 μM zinc.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0029] Figure 1.** Volumetric FRETS-vWF73 productivity of recombinant human ADAMTS13 expressed in CHO cells cultured in animal protein-free medium under varying temperature and pH conditions. The results of the various cell-culture experiments are shown as **(A)** contour plot and **(B)** surface plot representations of normalized volumetric ADAMTS13 FRETS-VWF73 productivity as a function of culture temperature and pH.

**[0030] Figure 2.** Specific activity levels (FRETS-VWF73 / antigen by ELISA) of CHO cells expressing recombinant human ADAMTS13 cultured in animal protein-free medium under varying temperature and pH conditions. The results of the various cell-culture

WHAT IS CLAIMED IS:

1. A method for expressing a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13) protein, the method comprising culturing a cell harboring a nucleic acid encoding an ADAMTS13 protein in a culture medium comprising from 3 μM to 12 μM zinc.

2. The method of claim 1, wherein the culture medium comprises at least 5 μM zinc.

3. The method of claim 1 or 2, wherein the culture medium comprises between 5 μM and 12 μM zinc.

4. The method according to any one of claims 1 to 3, wherein the culture medium further comprises at least 0.5 mM calcium.

5. The method of claim 4, wherein the culture medium comprises between 0.5 mM and 1.5 mM calcium.

6. The method according to any one of claims 1 to 5, wherein the culture medium further comprises at least 2 mg/L nicotinamide (vitamin B3).

7. The method according to any one of claims 1 to 6, wherein the culture medium further comprises at least 7 mg/L nicotinamide (vitamin B3).

8. The method according to any one of claims 1 to 7, wherein the culture medium comprises between 2 mg/L and 10 mg/L nicotinamide (vitamin B3).