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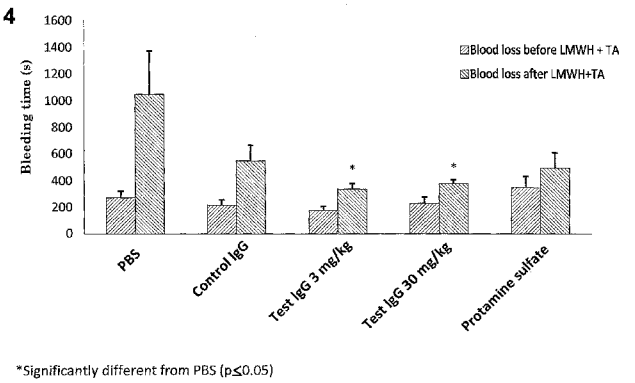
(54) **Title**: MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN BETA COMPLEXED WITH HEPARIN

FIG. 14



*Significantly different from PBS ($p \leq 0.05$)

(57) **Abstract**: This patent document relates to antibodies, antigen-binding antibody fragments (Fabs), and other protein scaffolds, directed against human antithrombin β complexed with heparin and/or heparin-like structure (ATβH). These ATβH binding proteins can block the anti-coagulant activity of ATβ to induce coagulation. Therapeutic uses of these antibodies and binders are described herein as are methods of panning and screening specific antibodies.

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MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN BETA COMPLEXED WITH HEPARIN

Cross-References to Related Applications

This application is being filed on 14 March 2014, as a PCT International patent application, and claims priority to U.S. Provisional Patent Application No. 61/784,590, filed March 14, 2013, the entire disclosure of which is hereby incorporated by reference in its entirety.

Sequence Listing Submission

The present application includes a Sequence Listing in electronic format as a txt file titled "SEQUENCE-LISTING-17207.0006WOU2" which was created on March 14, 2014 and which has a size of 65.1 kilobytes (KB). The contents of txt file "SEQUENCE-LISTING-17207.0006WOU2" are incorporated by reference herein.

Background

Current unmet medical needs in the hemophilia field are mainly: (1) treatment of hemophilia patients with inhibitors (~30% of hemophilia patients); and (2) long acting and efficacious coagulant factors (FVIII/FIX) and/or their replacement (bypass drugs) (WFH report 2012, Paris). The most widely used bypass drug for treating hemophilia patients with inhibitors is rFVII, which has major drawbacks such as risk of thrombogenicity, short half-life in plasma and high production cost. Antibodies against anti-coagulant factors, such as Tissue Factor Protein Inhibitor (TFPI), APC (Activated Protein C) and Antithrombin (AT) represent a new treatment paradigm. These antibodies not only bypass or reduce the need for FVIII or FIX coagulation factors in hemophilia patients with inhibitors, but also exhibit longer plasma half-life (which reduces the dosing frequency) and, thus, increases patient compliance. To date, there have been several antibody-based procoagulant drugs at the preclinical development or research stage, such as anti-TFPI and anti-APC.

AT is a major anticoagulant in human plasma. It inhibits thrombin, FXa and other serine proteases functioning in the coagulation pathway. It consists of 432 amino acids, is produced by the liver hepatocyte and has a long plasma half-life of

three days (Collen, Schetz et al. 1977). The amino acid sequence of AT is well-conserved and the homology among cow, sheep, rabbit, mouse and human is 84%-89% (Olson and Bjork 1994). Although the primary physiological targets of AT are thrombin and FXa, AT also inhibits FIXa, FXIa, FXIIa, as well as FVIIa to a lesser extent. AT exerts its inhibition together with heparin. In presence of heparin the inhibition rate of thrombin and FXa by AT increases by 3 to 4 orders of magnitude from 7–11 x 10^3 $M^{-1}s^{-1}$ to 1.5 – 4 X 10^7 $M^{-1}s^{-1}$ and from 2.5 x 10^{-3} M^{-1} s^{-1} to 1.25 - 2.5 $M^{-1}s^{-1}$ respectively (Olson, Swanson et al. 2004).

Unlike TFPI and APC which inhibit coagulation solely at the initiating stage and the amplification stage respectively, AT exerts its inhibition on coagulation at both the initiation and amplification stage. Therefore, blocking AT could have more potent pro-coagulant effect than blocking either TFPI or APC alone. Decreased AT levels and activity have been shown to correlate with increased thrombosis in human. Patients with AT deficiency tend to show recurrent venous thrombosis and pulmonary embolisms (van Boven and Lane 1997). Furthermore, homozygous AT knockout mice die in the embryonic stage with an extreme hypercoagulable state (Ishiguro, Kojima et al. 2000). A recent study shows that heterozygous AT knockout hema mice in which AT is reduced by 50% significantly have less blood loss and enhanced thrombin generation in a tail-clip bleeding model (Bolliger, Szlam et al. 2010).

AT is a glycoprotein with two isoforms based on differential glycosylation on Asn135, ATα and ATβ (Bjork 1997). ATβ lacks glycosylation at Asn135 and is a minor glyco-isoform representing 10% of human plasma AT. Asn135 is located adjacent to the initial heparin attachment site and constitutes part of extended heparin binding site after allosteric activation and D helix extension (dela Cruz, Jairajpuri et al. 2006). The lack of bulky-sized glycan at Asn135 affects ATβ activation profoundly in two ways: 1) a faster allosteric activation upon heparin binding required for inhibition of FXa and FIXa; and 2) extra accessible binding sites for higher affinity heparin binding for inhibition of FXa and thrombin by a bridging mechanism. Indeed, under physiological salt concentration, plasma-derived ATβ binds to heparin with a K_D of 36+/- 3nm while ATα binds to heparin with a K_D of 500+/- 50nm (Turk IV. et al., 1993). The higher affinity of ATβ for heparin leads

to its preferential distribution to the sub-endothelial layer which is enriched in the heparin-like structure – glycosaminoglycan. Consequently, ATβ is proposed to play a major and potent role in inhibition of FXa and thrombin at the vascular injury sites (Carlson and Atencio 1982; McCoy AJ, Pei XY. *et al.* 2003; Turk B, Brieditis I. *et al.* 1997; Witmer MR, Hatton MW. 1991; Frebelius S, *et al.* 1996). The importance and stronger potency of ATβ relative to that of ATα is also reported in clinical studies. In patients, the severity of AT homozygous mutations defective in heparin-binding is ameliorated by the beta form of AT (Martinez-Martinez, Navarro-Fernandez et al. 2012). In another study, a borderline level (~70% of normal AT antigen and activity) of AT is compensated by the 20% ~ 30% ATβ in plasma (Bayston, Tripodi et al. 1999).

Summary

Monoclonal antibodies to human ATβH (ATβ complexed with heparin and /or heparin-like structure) are provided. In at least one embodiment, the anti-ATβH monoclonal antibodies exhibit binding to ATβ complexed with Heparin.

In other embodiments, the monoclonal antibodies to ATβH may be optimized, for example to have increased affinity or increased functional activity. Also provided are specific epitopes that may be on human ATβH and are bound by an isolated monoclonal antibody. Further provided are the isolated nucleic acid molecules encoding the same.

Pharmaceutical compositions comprising the anti-ATβH monoclonal antibodies and

methods of treatment of genetic and acquired deficiencies or defects in coagulation such as

hemophilia A and B are also provided.

Also provided are methods for shortening bleeding time by administering an anti-ATβH monoclonal antibody to a patient in need thereof. Methods for producing a monoclonal antibody that binds human ATβH are also provided.

Brief Description of the Drawings

The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings or claims in any way.

FIG. 1 shows a schematic representation of ATβ bound to heparin and the various binding domains of ATβ.

FIGS. 2A-2C show how ATβ is distinguished from ATα by lacking of one N-glycan.

FIGS. 3A-3D show ATβ with faster binding to heparin and more potent inhibition than ATα.

FIG 4A shows biotinlayted hAT and rAT are functional in inhibition of Fxa generation (Fig. 4A). FIGS 4B-4C show various strategies for antibody discovery by phage display.

FIG. 5 shows a screening method for identifying antibodies capable of functional inhibition of ATβ::heparin.

FIGS. 6A and 6B shows alignment of the amino acid sequences of the light chain domain and heavy chain domain, respectively, of antibodies TPP-2009 (SEQ ID NO:1 and SEQ ID NO: 2, respectively), TPP-2015 (SEQ ID NO:3 and SEQ ID NO:4, respectively), TPP-2016 (SEQ ID NO:5 and SEQ ID NO:6 respectively), TPP-2019 (SEQ ID NO:7 and SEQ ID NO:8, respectively), and TPP-2803 (SEQ ID NO:9 and SEQ ID NO:10, respectively).

FIGS. 7A-7C show antibody binding specificity determined by Biacore (FIG. 7A) and ELISA (FIG. 7B) tests, and antibody binding affinity to human AtβH (FIG. 7C).

FIG. 8A is a graphical representation of the effect of TPP antibodies on thrombin generation in human HEM-A plasma, and illustrates that antibody presence increases peak thrombin generation in human HEM-A plasma.

FIG. 8B is a table showing antibodies shorten clotting time in human HemA plasma and in human AT-deficient plasma spiked in with Atβ or Atα.

FIG 9 is a graphical representation of the PK of antibody TPP 2009 in HEM-A mice using IV dosing at 0.3, 3 and 30mg/kg, three mice per time point (10 time points over 21 days), and associated PK parameters.

FIGS. 10A and 10B show an experimental protocol for a tail vein transection (TVT) model in HemA and the efficacy of antibody TPP-2009 in the TVT model in HemA mice. FIG. 10B shows the antibody TPP-2009 has potent efficacy in the Tail Vein Transection (TVT) model of HemA mice.

FIGS. 11A and 11B shows a molecular model of the three-dimensional structures of native ATβ complexed with/without heparin (FIG. 11A), and fully activated antibody TPP2009 bound to heparin (FIG. 11B) and its predicted epitope structure. Helix D is extended upon heparin binding B.

FIG. 12 shows a TPP2803 exhibited dose-dependent shortening of the clotting time in both normal human plasma and hemophilia patient plasma using the FXa activated clotting assay. CT: clotting time, HEM-A: Hemophilia A plasma.

FIG. 13 shows an experimental design of the heparinized rabbit bleeding model; Experimental groups: Vehicle, PBS; Positive control, Protamine sulfate, (28mg/kg IV); Negative control, M14 IgG2; treatment: 30mg/kg; TPP2803, 3mg/kg; TPP2803, 30mg/kg.

FIG. 14. shows the effect of a control and TPP2803 on bleeding time before and after LMWH and compound administration in a heparinized rabbit bleeding model.

FIG. 15. shows the effect of a control and TPP2803 on delta bleeding time Significantly different from PBS ($p \leq 0.05$; T-test).

FIG. 16. shows the effect of a control and TPP2803 on blood loss before and after LMWH and antibody administration. (Significance by the T-test).

Detailed Description

This disclosure provides antibodies, including monoclonal antibodies and other binding proteins that specifically bind to the activated form of ATβ, but exhibit comparatively little or no reactivity against the ATα form, either naïve or activated.

Definitions

For the purpose of interpreting this specification, the following definitions will apply. In the event that any definition set forth below conflicts with the usage of that word in any other document, including any document incorporated herein by reference, the definition set forth below shall always control for purposes of interpreting this specification and its associated claims unless a contrary meaning is clearly intended (for example in the document where the term is originally used).

Whenever appropriate, terms used in the singular will also include the plural and vice versa. The use of "a" herein means "one or more" unless stated otherwise or where the use of "one or more" is clearly inappropriate. The use of "or" means "and/or" unless stated otherwise. The use of "comprise," "comprises," "comprising," "include," "includes," and "including" are interchangeable and are not limiting. The terms "such as," "for example," and "e.g." also are not intended to be limiting. For example, the term "including" shall mean "including, but not limited to."

As used herein, the term "about" refers to +/- 10% of the unit value provided. As used herein, the term "substantially" refers to the qualitative condition of exhibiting a total or approximate degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, achieve or avoid an absolute result because of the many variables that affect testing, production, and storage of biological and chemical compositions and materials, and because of the inherent error in the instruments and equipment used in the testing, production, and storage of biological and chemical compositions and materials. The term substantially is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

The term "ATβ" or "ATβH" as used herein refers to any variant, isoform, and/or species homolog of AT in its form that is naturally expressed by cells and present in plasma and is distinct from ATα. Further, the term "ATβ" or "ATβH" as used herein can also refer to an activated form of ATβ complexed with heparin or a heparin-like structure.

The term "antibody" as used herein refers to a whole antibody and any antigen binding fragment (i.e., "antigen-binding portion") or single chain thereof. This term includes a full-length immunoglobulin molecule (e.g., an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes, or an immunologically active portion of an immunoglobulin molecule, such as an antibody fragment, that retains the specific binding activity. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the full-length antibody. For example, an anti-ATβH monoclonal antibody fragment binds to an epitope of ATβH. The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VLand VH domains of a single arm of an antibody; (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a V_H domain; (vi) an isolated complementarity determining region (CDR); (vii) minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., Protein Eng 1997;10:949-57); (viii) camel IgG; and (ix) IgNAR . Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are analyzed for utility in the same manner as are intact antibodies.

Furthermore, it is contemplated that an antigen binding fragment can be encompassed in an antibody mimetic. The term "antibody mimetic" or "mimetic" as used herein refers to a protein that exhibits binding activity similar to a particular

antibody but is a smaller alternative antibody or a non-antibody protein. Such antibody mimetic can be comprised in a scaffold. The term "scaffold" refers to a polypeptide platform for the engineering of new products with tailored functions and characteristics.

The term "anti-ATβ antibody" as used herein refers to an antibody that specifically binds to an epitope of ATβ associated with heparin or heparin-like. When bound *in vivo* to an epitope of ATβH, the anti-ATβ antibodies disclosed herein augment one or more aspects of the blood clotting cascade.

The terms "inhibits binding" and "blocks binding" (e.g., referring to inhibition/blocking of binding of ATβ substrate to ATβH) as used herein are used interchangeably and encompass both partial and complete inhibition or blocking of a protein with its substrate, such as an inhibition or blocking by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

In reference to the inhibition and/or blocking of binding of ATβ substrate to ATβ, the terms inhibition and blocking also include any measurable decrease in the binding affinity of ATβ and/or ATβH to a physiological substrate when in contact with an anti-ATβ antibody as compared to ATβ not in contact with an anti-ATβ antibody, e.g., the blocking of the interaction of ATβ with its substrates by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" as used herein refers to antibodies displaying a single binding specificity that have variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*).

The term "isolated antibody" as used herein is intended to refer to an antibody which is substantially free of other biological molecules, including antibodies having different antigenic specificities (e.g., an isolated antibody that binds to ATβH is substantially free of antibodies that bind antigens other than ATβH). In some embodiments, the isolated antibody is at least about 75%, about 80%, about 90%, about 95%, about 97%, about 99%, about 99.9% or about 100% pure by dry weight. In some embodiments, purity can be measured by a method such as column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. An isolated antibody that binds to an epitope, isoform or variant of human ATβH can, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., ATβH species homologs). Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals.

The term "specific binding" as used herein refers to antibody binding to a predetermined antigen. An antibody that exhibits specific binding typically binds to an antigen with an affinity of at least about $10^5 M^{-1}$ and binds to that antigen with an affinity that is higher, for example at least two-fold greater, than its binding affinity for an irrelevant antigen (e.g., BSA, casein). The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen." As used herein, the term "minimal binding" refers to an antibody that does not bind to and/or exhibits low affinity to a specified antigen. Typically, an antibody having minimal binding to an antigen binds to that antigen with an affinity that is lower than about $10^2 M^{-1}$ and does not bind to a predetermined antigen with higher affinity than it binds to an irrelevant antigen.

When used herein for an antibody such as an IgG antibody, the term "high affinity" refers to a binding affinity of at least about $10^7 M^{-1}$, in at least one embodiment at least about $10^8 M^{-1}$, in some embodiments at least about $10^9 M^{-1}$, about $10^{10} M^{-1}$, about $10^{11} M^{-1}$ or greater, e.g., up to about $10^{13} M^{-1}$ or greater. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to a binding affinity of at least about $10^7 M^{-1}$.

The term "isotype" as used herein refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

The terms "Complementarity-determining region" or "CDR" as used herein refers to one of three hypervariable regions within the variable region of the heavy chain or the variable region of the light chain of an antibody molecule that form the N-terminal antigen-binding surface that is complementary to the three-dimensional structure of the bound antigen. Proceeding from the N-terminus of a heavy or light chain, these complementarity-determining regions are denoted as "CDR1," "CDR2," and "CDR3," respectively [Wu TT, Kabat EA, Bilofsky H, Proc Natl Acad Sci U S A. 1975 Dec;72(12):5107 and Wu TT, Kabat EA, J Exp Med. 1970 Aug 1;132(2):211]. CDRs are involved in antigen-antibody binding, and the CDR3 comprises a unique region specific for antigen-antibody binding. An antigen-binding site, therefore, can include six CDRs, comprising the CDR regions from each of a heavy and a light chain V region. The term "epitope" refers to the area or region of an antigen to which an antibody specifically binds or interacts, which in some embodiments indicates where the antigen is in physical contact with the antibody. Conversely, the term "paratope" refers to the area or region of the antibody on which the antigen specifically binds. Epitopes characterized by competition binding are said to be overlapping if the binding of the corresponding antibodies are mutually exclusive, i.e. binding of one antibody excludes simultaneous binding of another antibody. The epitopes are said to be separate (unique) if the antigen is able to accommodate binding of both corresponding antibodies simultaneously.

The term "competing antibodies" as used herein refers to antibodies that bind to about the same, substantially the same, essentially the same, or even the same epitope as an antibody against ATβH as described herein. Competing antibodies include antibodies with overlapping epitope specificities. Competing antibodies are thus able to effectively compete with an antibody as described herein for binding to ATβH. In some embodiments, the competing antibody can bind to the same epitope as the antibody described herein. Alternatively viewed, the competing antibody has the same epitope specificity as the antibody described herein.

The term "conservative substitutions" as used herein refers to modifications of a polypeptide that involve the substitution of one or more amino acids for amino

acids having similar biochemical properties that do not result in loss of a biological or biochemical function of the polypeptide. A conservative amino acid substitution is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Antibodies of the present disclosure can have one or more conservative amino acid substitutions yet retain antigen binding activity.

For nucleic acids and polypeptides, the term "substantial homology" as used herein indicates that two nucleic acids or two polypeptides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide or amino acid insertions or deletions, in at least about 80% of the nucleotides or amino acids, usually at least about 85%, in some embodiments about 90%, about 91%, about 92%, about 93%, about 94%, or about 95%, in at least one embodiment at least about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, or about 99.5% of the nucleotides or amino acids. Alternatively, substantial homology for nucleic acids exists when the segments will hybridize under selective hybridization conditions to the complement of the strand. Also included are nucleic acid sequences and polypeptide sequences having substantial homology to the specific nucleic acid sequences and amino acid sequences recited herein. The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions / total # of positions x 100), taking into account the number of gaps, and the length of each gap, that need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, such as without limitation the AlignX™ module of VectorNTI™

(Invitrogen Corp., Carlsbad, CA). For AlignX™, the default parameters of multiple alignment are: gap opening penalty: 10; gap extension penalty: 0.05; gap separation penalty range: 8; % identity for alignment delay: 40.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions / total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, such as without limitation the AlignX™ module of VectorNTI™ (Invitrogen Corp., Carlsbad, CA). For AlignX™, the default parameters of multiple alignment are: gap opening penalty: 10; gap extension penalty: 0.05; gap separation penalty range: 8; % identity for alignment delay: 40. (further details found at http://www.invitrogen.com/site/us/en/home/LINNEA-Online-Guides/LINNEACommunities/Vector-NTI-Community/Sequence-analysis-and-data-management-software-for- PCs/AlignX-Module-for-Vector-NTI-Advance.reg.us.html).

Another method for determining the an overall match between a query sequence (a sequence of the present disclosure) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson et al., Nucleic Acids Research, 1994, 2(22): 4673-4680), which is based on the algorithm of Higgins et al., Computer Applications in the Biosciences (CABIOS), 1992, 8(2): 189-191. In a sequence alignment the query and subject sequences are both DNA sequences. The result of said global sequence alignment is in percent identity. Parameters that can be used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix = IUB, k-tuple = 1, Number of Top Diagonals = 5, Gap Penalty = 3, Gap Open Penalty = 10, Gap Extension Penalty = 0.1. For multiple alignments, the following CLUSTALW parameters can be used: Gap Opening Penalty = 10, Gap Extension Parameter = 0.05; Gap Separation Penalty Range = 8; % Identity for Alignment Delay = 40.

The nucleic acids can be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components with which it is normally associated in the natural environment. To isolate a nucleic acid, standard techniques such as the following can be used: alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art.

Monoclonal Antibodies Against ATβH

Bleeding disorders where homeostasis is deregulated in hemophilia or in trauma patients where the wound results in a temporary loss of hemostasis, can be treated by AT inhibitors. Antibodies, antigen-binding fragments thereof, and other AT-specific protein scaffolds can be used to provide targeting specificity to inhibit a subset of AT protein functions while preserving the rest. Given the at least 10-fold difference in plasma concentration of ATβ (<12 ug/ml) versus ATα (120 ug/ml), increased specificity of any potential ATβ inhibitor therapeutics is helpful to block ATβ function in the presence of a high circulating excess of ATα. ATβ specific antibodies that block the anti-coagulant function of ATβ can be used as therapeutics for patients with bleeding disorders. Examples of bleeding disorders include hemophilia, hemophilia patients with inhibitors, trauma-induced coagulopathy, severe bleeding patients during sepsis treatment by AT, bleeding resulting from elective surgery such as transplantation, cardiac surgery, orthopedic surgery, and excessive bleeding from Menorrhagia. Anti-ATβH antibodies having long circulating half-live can be useful in treating chronic diseases like hemophilia. ATβH antibody fragments or ATβH-binding protein scaffolds with shorter half-lives can be more effective for acute use (e.g. therapeutic use in trauma). ATβH-binding antibodies were identified by panning and screening human antibody libraries against human ATβ in complex with heparin. The identified antibodies exhibited binding to human ATβH. The heavy chain variable region and light chain variable region of each monoclonal antibody isolated was sequenced and its CDR regions were identified. The sequence identifier numbers ("SEQ ID NO") that correspond to

the heavy and light chain variable regions of the ATβH-specific monoclonal antibodies are summarized in Table 1A.

Table 1A - Human anti-ATβH (heparin complexed ATβ) antibodies

Clone	Light Chain variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
TPP2009	AQSVLTQDPAVSVALGQTVRIT CQGDSLRSYYASWYQQKPGQ APVLVIYGKNNRPSGIPDRFSGS SSGNTASLTITGAQAEDEADYY CNSRDSSGNHLVFGGGTKLTV LGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAW KADGSPVKAGVETTKPSKQSN NKYAASSYLSLTPEQWKSHRS YSCQVTHEGSTVEKTVAPAECS	No.1	EVQLLESGGGLVQPG GSLRLSCAASGFTFS AYRMGWVRQAPGK GLEWVSRIYSSGGRT RYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGSFSEALDYWG QGTLVTVSS	No.2
TPP2015	AQDIQMTQSPGTLSLSPGERAT LSCRASQSVSSSYLAWYQQKP GQAPRLLIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVY YCQQYGSSRTFGQGTKVEIRRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACE VTH QGLSSPVTKS FNRGEC	No.3	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYKMDWVRQAPGK GLEWVSRIGPSGGKT MYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGTYSEALDYW GQGTLVTVSS	No.4
TPP2016	AQDIQMTQSPATLSVSPGERAT LSCRASQNINRNLAWYQQKPG RAPRLLIHTASTRAPGVPVRITG SGSGTEFTLTISSLEPEDFAVYF CQQYASPPRTFGQGTKVEIKRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYS LSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC	No.5	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYRMDWVRQAPGK GLEWVSRIGPSGGKT TYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKT SDLSGSYSEALDYW GQGTL VTVSS	No.6

Clone	Light Chain variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
TPP2019	AQDIQMTQSPATLSLSPGERAT LSCRASQRVSSSYLTWYQQKP GQAPRLLIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVY YCQQYDSTPPLTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDST YSLS STLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC	No.7	EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYAMYWVRQA PGKGLEWVSR ISPSGGKTHY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARLS QTGYYPHYHY YGMDVWGQGT TVTVSS	No.8
TPP2803	SSELTQDPAVSVALGQTVRITC QGDSLRSYYASWYQQKPGQAP VLVIYGKNNRPSGIPDRFSGSSS GNTASLTITGAQAEDEADYYC NSRDSSGNHLVFGGGTKLTVL GQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSNN KYAASSYLSLTPEQWKSHRSYS CQVTHEGSTVEKTVAPAECS	No.9	EVQLLESGGGLVQPG GSLRLSCAASGFTFSS YRMSWVRQAPGKGL EWVSRIYSSGGRTRY ADSVKGRFTISRDNS KNTLYLQMNSLRAE DTAVYYCAREKASD LSGSFSEALDYWGQ GTLVTVSS	No.1 0

In at least some possible embodiments, an isolated monoclonal antibody binds to human ATβH and inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

In at least some possible embodiments, an isolated monoclonal antibody binds to human ATβH and inhibits anticoagulant activity, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In at least some possible embodiments an isolated monoclonal antibody binds to human ATβH and inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In at least some possible embodiments, the antibody comprises heavy and light chain variable regions comprising:

(a) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 1;

(b) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 4, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 3;

(c) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 5; or

(d) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 8, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 7; or

(e) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 9.

Table 1B shows heavy and light chain amino acid sequences for humanized IgG mAbs.

Table 1B - Heavy and Light Chain Amino Acid Sequences for humanized IgG mAbs.

TPP2009 |hIgG| Light_Chain AQSVLTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGG TKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD GSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTV EKTVAPAECS SEQ ID NO: 51
TPP2009| hIgG|Heavy_chain EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYRMGWVRQAPGKGLEWVSRI YSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKAS DLSGSFSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 52
TPP-2015 | hIgG|light_chain AQDIQMTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRTFGQGTK VEIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC SEQ ID NO: 53

TPP-2015|hIgG|heavy_chain EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYKMDWVRQAPGKGLEWVSR IGPSGGKTMYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKA SDLSGTYSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSG SEQ ID NO: 54
TPP-2016|hIgG|light_chain, Kappa AQDIQMTQSPATLSVSPGERATLSCRASQNINRNLAWYQQKPGRAPRLLIHT ASTRAPGVPVRITGSGSGTEFTLTISSLEPEDFAVYFCQQYASPPRTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC SEQ ID NO: 55
TPP-2016|hIgG|heavy_chain EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYRMDWVRQAPGKGLEWVSRI GPSGGKTTYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKTS DLSGSYSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO:56

TPP-2019|hIgG|light_chain, Kappa AQDIQMTQSPATLSLSPGERATLSCRASQRVSSSYLTWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDSTPPLTFGGG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC SEQ ID NO: 57
TPP-2019|hIgG|heavy_chain EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYAMYWVRQAPGKGLEWVSRI SPSGGKTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLSQT GYYPHYHYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 58

Table 1C – shows TPP2803 IgG2, Germlined and converted to IgG2. TPP2803 IgG2 light chain G2, Lambda, amino acid sequence shown in Table 1C is SEQ ID NO:59 and TPP2803 heavy chain amino acid sequence shown in Table 1C is SEQ ID NO: 60.

Table 1C - TPP2803 IgG2, Germlined and converted to IgG2

>TPP-2803|hIgG2 |light_chain, lambda SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGT KLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGS PVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK TVAPAECS SEQ ID NO: 59

>TPP-2803|hIgG2 |heavy_chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYRMSWVRQAPGKGLEWVSRI
YSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKAS
DLSGSFSEALDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 60

Table 2A provides a summary of the SEQ ID NOS: for the CDR regions ("CDR1," "CDR2," and "CDR3") of heavy and light chains of monoclonal antibodies that bind to human ATβH.

Table 2A - Sequence Identifiers for CDR Regions of Human Anti-ATβH Antibodies

Clones	Light Chain Variable Region			Heavy Chain Variable Region		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
TPP2009	21	26	31	36	41	46
TPP2015	22	27	32	37	42	47
TPP2016	23	28	33	38	43	48
TPP2019	24	29	34	39	44	49
TPP2803	25	30	35	40	45	50

Table 2B provides sequences of the SEQ ID NOS: for the CDR regions ("CDR1," "CDR2," and "CDR3") of heavy and light chains of monoclonal antibodies that bind to human ATβH.

Table 2B – Sequences for CDR Regions of Human Anti-ATβH Antibodies

Clone CDR	Sequence-Identifier	Amino Acid Sequence
TPP2009 LCDR1	SEQ ID NO: 21	QGDSLRSYYAS

Clone CDR	Sequence-Identifier	Amino Acid Sequence
TPP2015 LCDR1	SEQ ID NO: 22	RASQSVSSSYLA
TPP2016 LCDR1	SEQ ID NO: 23	RASQNINRNLA
TPP2019 LCDR1	SEQ ID NO: 24	RASQRVSSSYLT
TPP2803 LCDR1	SEQ ID NO: 25	QGDSLRSYYAS
TPP2009 LCDR2	SEQ ID NO: 26	GKNNRPS
TPP2015 LCDR2	SEQ ID NO: 27	GASSRAT
TPP2016 LCDR2	SEQ ID NO: 28	TASTRAP
TPP2019 LCDR2	SEQ ID NO: 29	GASSRAT
TPP2803 LCDR2	SEQ ID NO: 30	GKNNRPS
TPP2009 LCDR3	SEQ ID NO: 31	NSRDSSGNHLV
TPP2015 LCDR3	SEQ ID NO: 32	QQYGSSRT
TPP2016 LCDR3	SEQ ID NO: 33	QQYASPPRT
TPP2019 LCDR3	SEQ ID NO: 34	QQYDSTPPLT
TPP2803 LCDR3	SEQ ID NO: 35	NSRDSSGNHLV
TPP2009 HCDR1	SEQ ID NO: 36	AYRMG
TPP2015 HCDR1	SEQ ID NO: 37	KYKMD
TPP2016 HCDR1	SEQ ID NO: 38	KYRMD
TPP2019 HCDR1	SEQ ID NO: 39	RYAMY
TPP2803 HCDR1	SEQ ID NO: 40	SYRMS
TPP2009 HCDR2	SEQ ID NO: 41	RIYSSGGRTRYADSVKG
TPP2015 HCDR2	SEQ ID NO: 42	RIGPSGGKTM YADSVKG
TPP2016 HCDR2	SEQ ID NO: 43	RIGPSGGKTT YADSVKG
TPP2019 HCDR2	SEQ ID NO: 44	RISPSGGKTH YADSVKG
TPP2803 HCDR2	SEQ ID NO: 45	RIYSSGGRTR YADSVKG
TPP2009 HCDR3	SEQ ID NO: 46	AREKASDLSGSFSEALDY
TPP2015 HCDR3	SEQ ID NO: 47	AREKASDLSG TYSEALDY
TPP2016 HCDR3	SEQ ID NO: 48	AREKTSDLSG SYSEALDY
TPP2019 HCDR3	SEQ ID NO: 49	ARLSQTGYYP HYHYYGMDV
TPP2803 HCDR3	SEQ ID NO: 50	AREKASDLSG SFSEALDY

In at least some possible embodiments, an isolated monoclonal antibody is provided that binds to human ATβH, wherein the antibody comprises a CDR3 comprising an amino acid sequence of any one of SEQ ID NOS: 46-50. These CDR3s are from a heavy chain of the antibodies identified during panning and screening.

In a further embodiment, this antibody further comprises: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID

NOS: 36-40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 41-45; or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 36-40 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 41-45.

In at least some possible embodiments, antibodies share a CDR3 from one of the light chains of the antibodies identified during panning and screening. Thus, also provided is an isolated monoclonal antibody, wherein said antibody binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31-35. In further embodiments, the antibody further comprises (a) a CDR1comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25,(b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-30, or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-30.

In at least some possible embodiments, the antibody comprises a CDR3 from a heavy chain and a light chain of the antibodies identified from screening and panning. Provided is an isolated monoclonal antibody, wherein said antibody binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 46-50 and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31-35. In a further embodiment, the antibody further comprises: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 36-40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 41-45; (c) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25; and/or (d) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26-30.

In some embodiments, the antibody comprises heavy and light chain variable regions comprising:

(a) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 21, 26, and 31 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 36, 41, and 46;

(b) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 22, 27, and 32 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 37, 42, and 47;

(c) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 23, 28, and 33 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 38, 43, and 48;

(d) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 24, 29, and 34 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 39, 44, and 49;

(e) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 25, 30, and 35 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 40, 45, and 50.

Also provided is an isolated monoclonal antibody that binds to AtβH and inhibits anticoagulant activity, wherein said antibody comprises an amino acid sequence having at least about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% identity to an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 1-10.

The antibody can be species specific or can cross react with multiple species. In some embodiments, the antibody can specifically react or cross react with ATβH of human, mouse, rat, rabbit, guinea pig, monkey, pig, dog, cat or other mammalian species.

The antibody can be of any of the various classes of antibodies, such as without limitation an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, a secretory IgA, and IgD, and an IgE antibody.

In one embodiment, provided is an isolated fully human monoclonal antibody to human ATIII.

Optimized Variants of Anti-ATβH Antibodies

In some embodiments, the antibodies can be panned, screened and optimized, for example to increase affinity to ATβH, to further decrease any affinity to ATα, to improve cross-reactivity to different species, or to improve blocking activity of ATβH. Such optimization can be performed for example by utilizing site saturation mutagenesis of the CDRs or amino acid residues in close proximity to the CDRs, i.e. about 3 or 4 residues adjacent to the CDRs, of the antibodies.

Also provided are monoclonal antibodies that may have increased or high affinity to ATβH. In some embodiments, the anti-ATβH antibodies may have a binding affinity of at least about 10^8M^{-1}, in some other embodiments may have at least about 10^9M^{-1}, about $10^{10}M^{-1}$, about $10^{11}M^{-1}$ or greater, e.g., up to about $10^{13}M^{-1}$ or greater.

In some embodiments, additional amino acid modifications can be introduced to reduce divergence from the germ line sequence. In other embodiments, amino acid modifications can be introduced to facilitate antibody production for large scale production processes.

In some embodiments, provided are isolated anti-ATβH monoclonal antibodies that specifically bind to human ATβ, which antibodies may comprise one or more amino acid modifications. In some embodiments, the antibody may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20 or more modifications.

Epitopes

Also provided is an isolated monoclonal antibody that can bind to a predicted epitope of human ATβH, wherein the epitope comprises one or more of residues from human ATβH as shown in FIG. 11.

In some embodiments, the epitope comprises the N135 site of human ATβH. In other embodiments, the site can comprise part of the amino acid residue sequence of RCL loop of human ATβH.

Also provided are antibodies that can compete with any of the antibodies described herein for binding to human ATβH. For example, such a competing antibody can bind to one or more epitopes described above.

Nucleic Acids, Vectors and Host Cells

Also provided are isolated nucleic acid molecules encoding any of the monoclonal antibodies described herein. Thus, provided is an isolated nucleic acid molecule encoding an antibody that binds to human ATβH. Table 3 shows the nucleotide sequences of some anti-ATβH antibodies.

Table 3 - Nucleotide sequence of anti-ATβH antibodies.

	Light Chain	**Heavy Chain**
TPP2009	GCACAGAGCGTCTTG	GAAGTTCAATTGTTAGAGTCTGGTGG
	ACTCAGGACCCTGCT	CGGTCTTGTTCAGCCTGGTGGTTCTTT
	GTGTCTGTGGCCTTG	ACGTCTTTCTTGCGCTGCTTCCGGATT
	GGACAGACAGTCAG	CACTTTCTCTGCTTACCGTATGGGTTG
	GATCACATGCCAAGG	GGTTCGCCAAGCTCCTGGTAAAGGTT
	AGACAGCCTCAGAA	TGGAGTGGGTTTCTCGTATCTATTCTT
	GCTATTATGCAAGCT	CTGGTGGCCGTACTCGTTATGCTGACT
	GGTACCAGCAGAAG	CCGTTAAAGGTCGCTTCACTATCTCTA
	CCAGGACAGGCCCCT	GAGACAACTCTAAGAATACTCTCTAC
	GTACTTGTCATCTAT	TTGCAGATGAACAGCTTAAGGGCTGA
	GGTAAAAACAACCG	GGACACGGCCGTGTATTACTGTGCGA
	GCCCTCAGGGATCCC	GAGAGAAAGCGTCGGATCTATCGGGG
	AGACCGATTCTCTGG	AGTTTTTCTGAGGCCCTTGACTACTGG
	CTCCAGCTCAGGAAA	GGCCAGGGAACCCTGGTCACCGTCTC
	CACAGCTTCCTTGAC	AAGCGCCTCCACCAAGGGCCCATCGG
	CATCACTGGGGCTCA	TCTTCCCGCTAGCACCCAGCAGCAAG
	GGCGGAAGATGAGG	AGCACCAGCGGCGGAACAGCCGCCCT
	CTGACTATTACTGTA	GGGCTGCCTGGTGAAAGACTACTTCC
	ACTCCCGGGACAGCA	CCGAGCCCGTGACCGTGTCCTGGAAC
	GTGGTAACCATCTGG	TCTGGCGCCCTGACCAGCGGAGTGCA

	Light Chain	Heavy Chain
	TATTCGGCGGAGGGA CCAAGCTGACCGTCC TAGGTCAGCCCAAGG CTGCCCCCTCGGTCA CTCTGTTCCCGCCCT CCTCTGAGGAGCTTC AAGCCAACAAGGCC ACACTAGTGTGTCTG ATCAGTGACTTCTAC CCGGGAGCTGTGACA GTGGCCTGGAAGGCA GATGGCAGCCCCGTC AAGGCGGGAGTGGA GACCACCAAACCCTC CAAACAGAGCAACA ACAAGTACGCGGCCA GCAGCTACCTGAGCC TGACGCCCGAGCAGT GGAAGTCCCACAGA AGCTACAGCTGCCAG GTCACGCATGAAGGG AGCACCGTGGAGAA GACAGTGGCCCCTGC AGAATGCTCT (SEQ ID NO: 11)	TACCTTCCCCGCCGTGCTGCAGAGCA GCGGCCTGTACAGCCTGAGCAGCGTG GTGACAGTGCCCAGCAGCAGCCTGGG AACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAGGTG GACAAGAAGGTGGAACCCAAGAGCT GCGACAAGACCCACACCTGTCCCCCC TGCCCTGCCCCTGAACTGCTGGGCGG ACCCAGCGTGTTCCTGTTCCCCCCAAA GCCCAAGGACACCCTGATGATCAGCC GGACCCCCGAAGTGACCTGCGTGGTG GTGGACGTGTCCCACGAGGACCCAGA AGTGAAGTTTAATTGGTACGTGGACG GCGTGGAAGTGCATAACGCCAAGACC AAGCCCAGAGAGGAACAGTACAACA GCACCTACCGGGTGGTGTCCGTGCTG ACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAGTACAAGTGCAAGGTCT CCAACAAGGCCCTGCCTGCCCCCATC GAGAAAACCATCAGCAAGGCCAAGG GCCAGCCCCGCGAGCCTCAGGTGTAC ACACTGCCCCCCAGCCGGGATGAGCT GACCAAGAACCAGGTGTCCCTGACCT GTCTGGTGAAAGGCTTCTACCCCAGC GATATCGCCGTGGAATGGGAGAGCAA CGGCCAGCCCGAGAACAATTACAAGA CCACCCCCCCTGTGCTGGACAGCGAC GGCTCATTCTTCCTGTACTCCAAGCTG ACCGTGGACAAGAGCCGGTGGCAGCA GGGCAACGTGTTCAGCTGCAGCGTGA TGCACGAGGCCCTGCACAATCACTAC ACCCAGAAGTCCCTGAGCCTGAGCCC CGGC (SEQ ID NO: 12)
TPP2015	GCACAAGACATCCAG ATGACCCAGTCTCCA GGCACCCTGTCTTTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGAGT GTTAGCAGCAGCTAC TTAGCCTGGTACCAG CAGAAACCTGGCCAG GCTCCCAGGCTCCTC ATCTATGGTGCATCC AGCAGGGCCACTGGC ATCCCAGACAGGTTC	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTCAGCCTGGTGGTTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACAAGATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCCTT CTGGTGGCAAGACTATGTATGCTGAC TCCGTTAAAGGTCGCTTCACTATCTCT AGAGACAACTCTAAGAATACTCTCTA CTTGCAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGAGAGAAAGCGTCGGATCTATCGGG GACTTATTCTGAGGCCCTTGACTACTG

	Light Chain	Heavy Chain
	AGTGGCAGTGGGTCT GGGACAGACTTCACT CTCACCATCAGCAGA CGGAGCCTGAAGATT TTGCAGTGTATTACT GTCAGCAGTATGGTA GCTCAACGTTCGGCC AAGGGACCAAGGTG GAAATCAGACGAACT GTGGCTGCAATCTGT CTTCATCTTCCCGCC ATCTGATGAGCAGTT GAAATCTGGAACTGC CTCTGTTGTGTGCCT GCTGAATAACTTCTA TCCCAGAGAGGCCAA AGTACAGTGGAAGGT GGATAACGCCCTCCA ATCGGGTAACTCCCA GGAGAGTGTCACAG AGCAGGACAGCAAG GACAGCACCTACAGC CTCAGCAGCACCCTG ACGCTGAGCAAAGC AGACTACGAGAAAC ACAAAGTCTACGCCT GCGAAGTCACCCATC AGGGCCTGAGCTCGC CCGTCACAAAGAGCT TCAACAGGGGAGAG TGT (SEQ ID NO: 13)	GGGCCAGGGAACCCTGGTCACCGTCT CAAGCGCCTCCACCAAGGGCCCATCG GTCTTCCCGCTAGCACCCAGCAGCAA GAGCACCAGCGGCGGAACAGCCGCCC TGGGCTGCCTGGTGAAAGACTACTTC CCCGAGCCCGTGACCGTGTCCTGGAA CTCTGGCGCCCTGACCAGCGGAGTGC ATACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGT GGTGACAGTGCCCAGCAGCAGCCTGG GAACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGT GGACAAGAAGGTGGAACCCAAGAGC TGCGACAAGACCCACACCTGTCCCCC CTGCCCTGCCCCTGAACTGCTGGGCG GACCCAGCGTGTTCCTGTTCCCCCCAA AGCCCAAGGACACCCTGATGATCAGC CGGACCCCCGAAGTGACCTGCGTGGT GGTGGACGTGTCCCACGAGGACCCAG AAGTGAAGTTTAATTGGTACGTGGAC GGCGTGGAAGTGCATAACGCCAAGAC CAAGCCCAGAGAGGAACAGTACAAC AGCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTC TCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAGCCTCAGGTGTA CACACTGCCCCCCAGCCGGGATGAGC TGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAAGGCTTCTACCCCAG CGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCGAGAACAATTACAAG ACCACCCCCCCTGTGCTGGACAGCGA CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTCAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 14)
TPP2016	GCACAAGACATCCAG ATGACCCAGTCTCCA GCCACCCTGTCTGTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGAAT ATTAATAGAAACTTG	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTCAGCCTGGTGGTTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACCGTATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCCTT CTGGTGGCAAGACTACTTATGCTGAC

	Light Chain	Heavy Chain
	GCCTGGTACCAGCAG	TCCGTTAAAGGTCGCTTCACTATCTCT
	AAGCCTGGCCGGGCT	AGAGACAACTCTAAGAATACTCTCTA
	CCCAGACTCCTCATC	CTTGCAGATGAACAGCTTAAGGGCTG
	CATACCGCATCCACT	AGGACACGGCCGTGTATTACTGTGCG
	AGGGCCCCTGGTGTC	AGAGAGAAAACGTCGGATCTATCGGG
	CCAGTCAGGATCACT	GAGTTATTCTGAGGCCCTTGACTACTG
	GGCAGTGGGTCTGGA	GGGCCAGGGAACCCTGGTCACCGTCT
	ACAGAGTTCACTCTC	CAAGCGCCTCCACCAAGGGCCCATCG
	ACCATCAGCAGCCTG	GTCTTCCCGCTAGCACCCAGCAGCAA
	GAACCTGAAGATTTT	GAGCACCAGCGGCGGAACAGCCGCCC
	GCAGTGTATTTCTGT	TGGGCTGCCTGGTGAAAGACTACTTC
	CAGCAGTATGCTAGC	CCCGAGCCCGTGACCGTGTCCTGGAA
	CCACCTCGGACGTTC	CTCTGGCGCCCTGACCAGCGGAGTGC
	GGCCAAGGGACCAA	ATACCTTCCCCGCCGTGCTGCAGAGC
	GGTGGAAATCAAGC	AGCGGCCTGTACAGCCTGAGCAGCGT
	GAACTGTGGCTGCAC	GGTGACAGTGCCCAGCAGCAGCCTGG
	CATCTGTCTTCATCTT	GAACCCAGACCTACATCTGCAACGTG
	CCCGCCATCTGATGA	AACCACAAGCCCAGCAACACCAAGGT
	GCAGTTGAAATCTGG	GGACAAGAAGGTGGAACCCAAGAGC
	AACTGCCTCTGTTGT	TGCGACAAGACCCACACCTGTCCCCC
	GTGCCTGCTGAATAA	CTGCCCTGCCCCTGAACTGCTGGGCG
	CTTCTATCCCAGAGA	GACCCAGCGTGTTCCTGTTCCCCCCAA
	GGCCAAAGTACAGTG	AGCCCAAGGACACCCTGATGATCAGC
	GAAGGTGGATAACG	CGGACCCCCGAAGTGACCTGCGTGGT
	CCCTCCAATCGGGTA	GGTGGACGTGTCCCACGAGGACCCAG
	ACTCCCAGGAGAGTG	AAGTGAAGTTTAATTGGTACGTGGAC
	TCACAGAGCAGGAC	GGCGTGGAAGTGCATAACGCCAAGAC
	AGCAAGGACAGCAC	CAAGCCCAGAGAGGAACAGTACAAC
	CTACAGCCTCAGCAG	AGCACCTACCGGGTGGTGTCCGTGCT
	CACCCTGACGCTGAG	GACCGTGCTGCACCAGGACTGGCTGA
	CAAAGCAGACTACG	ACGGCAAAGAGTACAAGTGCAAGGTC
	AGAAACACAAAGTCT	TCCAACAAGGCCCTGCCTGCCCCCAT
	ACGCCTGCGAAGTCA	CGAGAAAACCATCAGCAAGGCCAAG
	CCCATCAGGGCCTGA	GGCCAGCCCCGCGAGCCTCAGGTGTA
	GCTCGCCCGTCACAA	CACACTGCCCCCCAGCCGGGATGAGC
	AGAGC	TGACCAAGAACCAGGTGTCCCTGACC
	TTCAACAGGGGAGA	TGTCTGGTGAAAGGCTTCTACCCCAG
	GTGT (SEQ ID NO: 15)	CGATATCGCCGTGGAATGGGAGAGCA
		ACGGCCAGCCCGAGAACAATTACAAG
		ACCACCCCCCCTGTGCTGGACAGCGA
		CGGCTCATTCTTCCTGTACTCCAAGCT
		GACCGTGGACAAGAGCCGGTGGCAGC
		AGGGCAACGTGTTCAGCTGCAGCGTG
		ATGCACGAGGCCCTGCACAATCACTA
		CACCCAGAAGTCCCTGAGCCTGAGCC
		CCGGC (SEQ ID NO: 16)

	Light Chain	Heavy Chain
TPP2019	GCACAAGACATCCAG	GAAGTTCAATTGTTAGAGTCTGGTGG
	ATGACCCAGTCTCCA	CGGTCTTGTTCAGCCTGGTGGTTCTTT
	GCCACCCTGTCTTTG	ACGTCTTTCTTGCGCTGCTTCCGGATT
	TCTCCAGGGGAAAGA	CACTTTCTCTCGTTACGCTATGTATTG
	GCCACCCTCTCCTGC	GGTTCGCCAAGCTCCTGGTAAAGGTT
	AGGGCCAGTCAGCGT	TGGAGTGGGTTTCTCGTATCTCTCCTT
	GTTAGCAGCAGCTAC	CTGGTGGCAAGACTCATTATGCTGAC
	TTAACCTGGTACCAG	TCCGTTAAAGGTCGCTTCACTATCTCT
	CAGAAACCTGGCCAG	AGAGACAACTCTAAGAATACTCTCTA
	GCTCCCAGGCTCCTC	CTTGCAGATGAACAGCTTAAGGGCTG
	ATCTATGGTGCATCC	AGGACACGGCCGTGTATTACTGTGCG
	AGCAGGGCCACTGGC	AGACTGTCTCAAACTGGTTATTACCCT
	ATCCCAGACAGGTTC	CACTACCACTACTACGGTATGGACGT
	AGTGGCAGTGGGTCT	CTGGGGCCAAGGGACCACGGTCACCG
	GGGACAGACTTCACT	TCTCAAGCGCCTCCACCAAGGGCCCA
	CTCACCATCAGCAGA	TCGGTCTTCCCGCTAGCACCCAGCAG
	CTGGAGCCTGAAGAT	CAAGAGCACCAGCGGCGGAACAGCC
	TTTGCAGTTTATTACT	GCCCTGGGCTGCCTGGTGAAAGACTA
	GTCAGCAGTATGATA	CTTCCCCGAGCCCGTGACCGTGTCCTG
	GTACGCCTCCGCTCA	GAACTCTGGCGCCCTGACCAGCGGAG
	CCTTCGGCGGAGGGA	TGCATACCTTCCCCGCCGTGCTGCAGA
	CCAAGGTGGAGATCA	GCAGCGGCCTGTACAGCCTGAGCAGC
	AACGAACTGTGGCTG	GTGGTGACAGTGCCCAGCAGCAGCCT
	CACCATCTGTCTTCA	GGGAACCCAGACCTACATCTGCAACG
	TCTTCCCGCCATCTG	TGAACCACAAGCCCAGCAACACCAAG
	ATGAGCAGTTGAAAT	GTGGACAAGAAGGTGGAACCCAAGA
	CTGGAACTGCCTCTG	GCTGCGACAAGACCCACACCTGTCCC
	TTGTGTGCCTGCTGA	CCCTGCCCTGCCCCTGAACTGCTGGGC
	ATAACTTCTATCCCA	GGACCCAGCGTGTTCCTGTTCCCCCCA
	GAGAGGCCAAAGTA	AAGCCCAAGGACACCCTGATGATCAG
	CAGTGGAAGGTGGAT	CCGGACCCCCGAAGTGACCTGCGTGG
	AACGCCCTCCAATCG	TGGTGGACGTGTCCCACGAGGACCCA
	GGTAACTCCCAGGAG	GAAGTGAAGTTTAATTGGTACGTGGA
	AGTGTCACAGAGCAG	CGGCGTGGAAGTGCATAACGCCAAGA
	GACAGCAAGGACAG	CCAAGCCCAGAGAGGAACAGTACAAC
	CACCTACAGCCTCAG	AGCACCTACCGGGTGGTGTCCGTGCT
	CAGCACCCTGACGCT	GACCGTGCTGCACCAGGACTGGCTGA
	GAGCAAAGCAGACT	ACGGCAAAGAGTACAAGTGCAAGGTC
	ACGAGAAACACAAA	TCCAACAAGGCCCTGCCTGCCCCCAT
	GTCTACGCCTGCGAA	CGAGAAAACCATCAGCAAGGCCAAG
	GTCACCCATCAGGGC	GGCCAGCCCCGCGAGCCTCAGGTGTA
	CTGAGCTCGCCCGTC	CACACTGCCCCCCAGCCGGGATGAGC
	ACAAAGAGCTTCAAC	TGACCAAGAACCAGGTGTCCCTGACC
	AGGGGAGAGTGT	TGTCTGGTGAAAGGCTTCTACCCCAG
	(SEQ ID NO: 17)	CGATATCGCCGTGGAATGGGAGAGCA
		ACGGCCAGCCCGAGAACAATTACAAG
		ACCACCCCCCCTGTGCTGGACAGCGA

	Light Chain	Heavy Chain
		CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTCAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 18)
TPP2803	AGCGAATTGACTCAG GACCCTGCTGTGTCT GTGGCCTTGGGACAG ACAGTCAGGATCACA TGCCAAGGAGACAG CCTCAGAAGCTATTA TGCAAGCTGGTACCA GCAGAAGCCAGGAC AGGCCCCTGTACTTG TCATCTATGGTAAAA ACAACCGGCCCTCAG GGATCCCAGACCGAT TCTCTGGCTCCAGCT CAGGAAACACAGCTT CCTTGACCATCACTG GGGCTCAGGCGGAA GATGAGGCTGACTAT TACTGTAACTCCCGG GACAGCAGTGGTAAC CATCTGGTATTCGGC GGAGGGACCAAGCT GACCGTCCTAGGTCA GCCCAAGGCTGCCCC CTCGGTCACTCTGTT	GAAGTGCAGCTGCTGGAAAGCGGCGG AGGCCTGGTGCAGCCTGGCGGATCTC TGAGACTGAGCTGTGCCGCCAGCGGC TTCACCTTCAGCAGCTACAGAATGAG CTGGGTGCGCCAGGCCCCTGGCAAGG GACTGGAATGGGTGTCCCGGATCTAC AGCAGCGGCGGCAGAACCAGATACGC CGACAGCGTGAAGGGCCGGTTCACCA TCTCCCGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCTGCG GGCCGAGGACACCGCCGTGTACTATT GCGCCAGAGAGAAGGCCAGCGACCTG AGCGGCAGCTTTAGCGAGGCCCTGGA TTATTGGGGCCAGGGCACACTCGTGA CCGTGTCTAGCGCCAGCACAAAGGGC CCCAGCGTGTTCCCTCTGGCCCCTTGT AGCAGAAGCACCAGCGAGTCTACAGC CGCCCTGGGCTGCCTCGTGAAGGACT ACTTTCCCGAGCCCGTGACAGTGTCCT GGAACTCTGGCGCCCTGACAAGCGGC GTGCACACCTTTCCAGCCGTGCTGCA GAGCAGCGGCCTGTACTCTCTGAGCA GCGTCGTGACTGTGCCCAGCAGCAAC TTCGGCACCCAGACCTACACCTGTAA

	Light Chain	Heavy Chain
	CCCGCCCTCCTCTGA GGAGCTTCAAGCCAA CAAGGCCACACTAGT GTGTCTGATCAGTGA CTTCTACCCGGGAGC TGTGACAGTGGCCTG GAAGGCAGATGGCA GCCCCGTCAAGGCGG GAGTGGAGACCACC AAACCCTCCAAACAG AGCAACAACAAGTA CGCGGCCAGCAGCTA CCTGAGCCTGACGCC CGAGCAGTGGAAGTC CCACAGAAGCTACAG CTGCCAGGTCACGCA TGAAGGGAGCACCGT GGAGAAGACAGTGG CCCCTGCAGAATGCT CT (SEQ ID NO: 19)	CGTGGACCACAAGCCCAGCAACACCA AGGTGGACAAGACCGTGGAACGGAA GTGCTGCGTGGAATGCCCCCCTTGTCC TGCCCCTCCAGTGGCTGGCCCTTCCGT GTTCCTGTTCCCCCCAAAGCCCAAGG ACACCCTGATGATCAGCCGGACCCCG AAGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGACCCCGAGGTGCAGTT CAATTGGTACGTGGACGGCGTGGAAG TGCACAACGCCAAGACCAAGCCCAGA GAGGAACAGTTCAACAGCACCTTCCG GGTGGTGTCCGTGCTGACCGTGGTGC ATCAGGACTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGG CCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGACCAAAGGCCAGCCCCGC GAGCCCCAGGTGTACACACTGCCTCC AAGCCGGGAAGAGATGACCAAGAAC CAGGTGTCCCTGACCTGTCTCGTGAA AGGCTTCTACCCCTCCGATATCGCCGT GGAATGGGAGAGCAACGGCCAGCCC GAGAACAACTACAAGACCACCCCCCC CATGCTGGACAGCGCGGCTCATTCTTC CTGTACAGCAAGCTGACAGTGGACAA GTCCCGGTGGCAGCAGGGCAACGTGT TCAGCTGCAGCGTGATGCACGAAGCC CTGCACAACCACTACACCCAGAAGTC CCTGAGCCTGAGCCCTGGC (SEQ ID NO: 20)

In some embodiments, isolated nucleic acid molecules encode an antibody that binds to ATβH and inhibits anticoagulant activity but has minimal binding to ATα, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

In some embodiments, isolated nucleic acid molecules encode an antibody that binds to ATβH and inhibits anticoagulant activity but has minimal binding to ATα, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In other embodiments, isolated nucleic acid molecules encode an antibody that binds to ATβ and inhibits anticoagulant activity of ATβ, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10 or a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9 and one or more amino acid modifications in the heavy chain variable region or light chain variable region.

Further, also provided are vectors comprising the isolated nucleic acid molecules encoding any of the monoclonal antibodies described above and host cells comprising such vectors.

Methods of Preparing Antibodies to ATβH

The monoclonal antibody can be produced recombinantly by expressing a nucleotide sequence encoding the variable regions of the monoclonal antibody according to one of the present embodiments in a host cell. With the aid of an expression vector, a nucleic acid containing the nucleotide sequence can be transfected and expressed in a host cell suitable for the production. Accordingly, an exemplary method for producing a monoclonal antibody that binds with human ATβH can comprise: (a) transfecting a nucleic acid molecule encoding a monoclonal antibody into a host cell; (b) culturing the host cell so to express the monoclonal antibody in the host cell, and (c) optionally isolating and purifying the produced monoclonal antibody, wherein the nucleic acid molecule comprises a nucleotide sequence encoding a monoclonal antibody.

In one example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains obtained by standard molecular biology techniques are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" refers to an antibody gene that is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene

and the antibody heavy chain gene can be inserted into separate vectors or, alternatively, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector.

Additionally, or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein). In addition to the antibody chain encoding genes, the recombinant expression vectors carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" includes promoters, enhancers, and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Examples of regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. Alternatively, non-viral

regulatory sequences can be used, such as the ubiquitin promoter or ß-globin promoter.

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216; 4,634,665; and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Examples of selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" encompasses a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection, and the like. Although it is theoretically possible to express the antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, including mammalian host cells, is typical because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Examples of mammalian host cells for expressing the recombinant antibodies include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621), NSO myeloma cells, COS cells, HKB11 cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the

antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods, such as ultrafiltration, size exclusion chromatography, ion exchange chromatography and centrifugation.

Use of Partial Antibody Sequences to Express Intact Antibodies

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain CDRs. For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. et al., 1998, Nature 332:323-327; Jones, P. et al.,1986, Nature 321:522-525; and Queen, C. et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:10029-10033). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. It is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see WO 99/45962).

Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. For this reason, the corresponding germline leader sequence is used for expression constructs. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the

entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons. The nucleotide sequences of heavy and light chain transcripts are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and light chain sequences can differ from the natural sequences. For example, strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; and optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266:19867-19870); and restriction sites are engineered upstream or downstream of the translation initiation sites. For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30-50 nucleotide sections at approximately the midpoint of the corresponding non-coding oligonucleotide. For each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150-400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150-400 nucleotides.

Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by PCR amplification to form the complete variable region. It can also be desirable to include an overlapping fragment of the heavy or light chain constant region in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs. The reconstructed heavy and light chain variable regions are then combined with cloned promoter, translation initiation, constant region, 3' untranslated, polyadenylation, and transcription termination sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains. In another aspect, the structural features of a human anti-ATβH antibody are used to create structurally related human anti-ATβH antibodies that retain the function of binding

to ATβ. For example, one or more CDRs of the specifically identified heavy and light chain regions of the monoclonal antibodies can be combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-ATβH antibodies.

Pro-coagulant efficacy of Anti-ATβH mAbs

Pro-coagulant efficacy of anti-ATβH mAbs was investigated using various assays.

Table 4 shows pro-coagulant efficacy of Anti-ATβH mAbs TPP2009 and TPP2803 in plasma from various animal species in the FXa-activated clotting assay.

Table 4 - Pro-coagulant efficacy of TPP2009 and TPP2803 in plasma from various animal species.

Species	**Normal Plasma (EC_{50} nM)**		**HEM A Plasma (EC_{50} in nM)**		**HEM A Plasma (EC_{50} in nM)**
	2009	**2803**	**2009**	**2803**	**2009**
FXa-Activated Clotting Assay					**dPT**
Human	10.5	**2.4**	4.7	**2.7**	9.0
Mouse	NDR	ND	ND	ND	NDR
Rat	NDR	ND	ND	ND	ND
Rabbit	25.7	ND	10.5	ND	NDR
Beagle	NDR	ND	ND	ND	NDR
Cyno	21.5	ND	4.3	ND	13.9

NDR: no dose response, ND: not determined, dPT: diluted prothrombin time, HEM-A: Hemophilia A plasma.

Anti-ATβH mAbs TPP2009 and TPP2803 both exhibited pro-coagulant efficacy in human normal plasma and hemophilia A plasma in the FXa-activated clotting assay. Specifically, TPP2009 exhibited pro-coagulant efficacy in human

normal plasma and hemophilia A plasma in the FXa-activated clotting assay with EC_{50}'s of 10.5 nM and 4.7 nM, respectively. TPP2803 exhibited pro-coagulant efficacy in human normal plasma and hemophilia A plasma in the FXa-activated clotting assay with EC_{50}'s of 2.4 and 2.7 nM, respectively.

In addition, Anti-ATβH mAb TPP2803 exhibited dose-dependent shortening of the clotting time in both normal human plasma and hemophilia patient plasma using the FXa activated clotting assay, as shown in FIG. 12. CT: clotting time, HEM-A: Hemophilia A plasma.

The heparinized rabbit bleeding model outlined in FIG. 13 was employed to demonstrate in vivo pro-coagulant efficacy of Anti-ATβH mAb TPP2803. The effect of a control and TPP2803 on bleeding time before and after LMWH and compound administration in a heparinized rabbit bleeding model are shown in FIG. 14. Bleeding time after administration of LMWH and Test Article (either 3mg/kg or 30 mg/kg TPP2803) was significantly reduced compared to bleeding time after LMWH in PBS.Delta bleeding time after administration of either Test Article (either 3mg/kg or 30 mg/kg TPP2803), or positive control protamine sulfate, was significantly different from PBS ($p \leq 0.05$; * significance by T-test) as shown in FIG. 15. The effect of a control and TPP2803 on blood loss before and after LMWH and antibody administration is shown in FIG. 16. TPP2803 (3 mg/kg) and positive control protamine sulfate both exhibited a significant change in blood loss (*Significance by the T-test) after administration of LMWH.

Pharmaceutical Compositions

Also provided are pharmaceutical compositions comprising therapeutically effective amounts of anti-ATβH monoclonal antibody and a pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" as used herein refers to a substance that can be added to the active ingredient to help formulate or stabilize the preparation and causes no significant adverse toxicological effects to the patient. Examples of such carriers are well known to those skilled in the art and include water, sugars such as maltose or sucrose, albumin, salts such as sodium chloride, etc. Other carriers are described for example in Remington's Pharmaceutical Sciences by

E. W. Martin. Such compositions will contain a therapeutically effective amount of at least one monoclonal antibody.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. The composition is in some embodiments formulated for parenteral injection. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. In some cases, the composition of the carrier includes isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients
enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, some methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Pharmaceutical Uses

The monoclonal antibody can be used for therapeutic purposes for treating genetic and acquired deficiencies or defects in coagulation. For example, the monoclonal antibodies in the embodiments described above can be used to block the interaction of ATβH with its substrate, which can include Factor Xa or Factor IIa. The monoclonal antibodies have therapeutic use in the treatment of disorders of hemostasis such as thrombocytopenia, platelet disorders and bleeding disorders (e.g., hemophilia A, hemophilia B and hemophilia C). Such disorders can be treated by

administering a therapeutically effective amount of the anti-ATβH monoclonal antibody to a patient in need thereof. The monoclonal antibodies also have therapeutic use in the treatment of uncontrolled bleeds in indications such as trauma and hemorrhagic stroke. Thus, also provided is a method for shortening the bleeding time comprising administering a therapeutically effective amount of an anti-ATβH monoclonal antibody to a patient in need thereof.

In another embodiment, the anti-ATβH antibody can be useful as an antidote for AT treated patients, including for example wherein AT is used for the treatment of sepsis or bleeding disorder.

The antibodies can be used as monotherapy or in combination with other therapies to address a hemostatic disorder. For example, co-administration of one or more antibodies with a clotting factor such as Factor VIIa, Factor VIII or Factor IX is believed useful for treating hemophilia. In at least some embodiments, a method for treating genetic and acquired deficiencies or defects in coagulation comprises administering: (a) a first amount of a monoclonal antibody that binds to human tissue factor pathway inhibitor; and (b) a second amount of Factor VIII or Factor IX, wherein said first and second amounts together are effective for treating said deficiencies or defects. In at least some embodiments, a method for treating genetic and acquired deficiencies or defects in coagulation comprises administering: (a) a first amount of a monoclonal antibody that binds to human tissue factor pathway inhibitor; and (b) a second amount of factor VIII or Factor IX, wherein said first and second amounts together are effective for treating said deficiencies or defects, and further wherein Factor VII is not co-administered. Also provided is a pharmaceutical composition comprising a therapeutically effective amount of the combination of a monoclonal antibody and Factor VIII or Factor IX, wherein the composition does not contain Factor VII. "Factor VII" includes Factor VII and Factor VIIa. These combination therapies are likely to reduce the necessary infusion frequency of the clotting factor. By co-administration or combination therapy is meant administration of the two therapeutic drugs each formulated separately or formulated together in one composition, and, when formulated separately, administered either at approximately the same time or at different times, but over the same therapeutic period.

In some embodiments, one or more antibodies described herein can be used in combination to address a hemostatic disorder. For example, co-administration of two or more of the antibodies described herein is believed useful for treating hemophilia or other hemostatic disorder.

The pharmaceutical compositions can be parenterally administered to subjects suffering from hemophilia A or B at a dosage and frequency that can vary with the severity of the bleeding episode or, in the case of prophylactic therapy, can vary with the severity of the patient's clotting deficiency.

The compositions can be administered to patients in need as a bolus or by continuous infusion. For example, a bolus administration of an antibody as a Fab fragment can be in an amount from about 0.0025 to about 100 mg/kg body weight, about 0.025 to about 0.25 mg/kg, about 0.010 to about 0.10 mg/kg or about 0.10 to about 0.50 mg/kg. For continuous infusion, an inventive antibody present as an Fab fragment can be administered at about 0.001 to about 100 mg/kg body weight/minute, about 0.0125 to about 1.25 mg/kg/min, about 0.010 to about 0.75 mg/kg/min, about 0.010 to about 1.0 mg/kg/min, or about 0.10 to about 0.50 mg/kg/min for a period of about 1-24 hours, about 1-12 hours, about 2-12 hours, about 6-12 hours, about 2-8 hours, or about 1-2 hours. For administration of an inventive antibody present as a full-length antibody (with full constant regions), dosage amounts can be about 1-10 mg/kg body weight, about 2-8 mg/kg, or about 5-6 mg/kg. Such full-length antibodies would typically be administered by infusion extending for a period of thirty minutes to three hours. The frequency of the administration would depend upon the severity of the condition. Frequency could range from three times per week to once every two weeks to six months.

Additionally, the compositions can be administered to patients via subcutaneous injection. For example, a dose of about 10 to about 100 mg anti-ATβH antibody can be administered to patients via subcutaneous injection weekly, biweekly or monthly. As used herein, "therapeutically effective amount" means an amount of an anti-ATβH monoclonal antibody or of a combination of such antibody and Factor VIII or Factor IX that is needed to effectively increase the clotting time in vivo or otherwise cause a measurable benefit in vivo to a patient in need thereof. The precise amount will depend upon numerous factors, including the components

and physical characteristics of the therapeutic composition, intended patient population, individual patient considerations, and the like, and can readily be determined by one skilled in the art.

Aspects of the present disclosure may be further understood in light of the following examples, which should not be construed as limiting the scope of the present teachings in any way.

Example 1 - Human and rabbit ATα and ATβ purification

ATα and ATβ were purified from human and rabbit plasma by affinity chromatography on heparin-sepharose according to methods previously described (Carlson and Atencio 1982; Peterson and Blackburn 1985) at Enzyme Research laboratory (South Bend, IN). Briefly, the supernatant from a dextran sulphate/calcium chloride precipitation was applied to a heparin-sepharose affinity column (Pharmacia). ATα and ATβ were separated with a NaCl gradient: ATα and ATβ were eluted at 0.8 M and 1.3 M NaCl, respectively. Anion-exchange chromatography (HiTrap-Q, Pharmacia) was employed for further purification of ATβ. Purity and glycan profile of ATα and ATβ were evaluated by protein SDS-PAGE and LC-MS.

Example 2 - Determination of the number and position of glycans on ATα and ATβ by mass-spectrometry analysis

Due to the distinct number of glycans, ATα and ATβ were differentiated based on their mass by Agilent 6520 LC-MS system which is equipped with duo-ESI (or nano ChipCube) source, MassHunter acquisition software and qualitative analysis software including Bioconfirm. Glycosylation sites were determined by a bottom-up method in which proteins are digested by trypsin and Arg-c followed by target MSMS to identify the glycosylated and mono-glycosylated peptide sequences. Data was collected in two experiments: Fragmentor voltage 175v and 430v.

Example 3 - AT antigen biotinylation

Human and rabbit ATα and ATβ were labeled with biotins on the surface lysine residues by NHS-biotin. For lysine biotinylation, proteins were first desalted into PBS/Ca^{++} buffer (Life Technologies Corporation, Carlsbad, CA) to remove any amines that might be inhibitory to the biotinylation reaction. Concentrations of desalted proteins were determined by OD280 on the NanoDrop. Protein were then incubated for 1 hour at room temperature (RT) with Sulfo-NHS-Biotin (Pierce Thermo Scientific, Rockford, IL) at the 1:5 and/or 1:3 molar ratio of AT:NHS-biotin (i.e. biotin in excess). Free biotin was removed by overnight dialysis into PBS/Ca^{++} buffer. The amount of biotin in the biotinylated proteins was quantified using Biotin Quantitation Kit (Pierce Thermo Scientific, Rockford, IL). Biotinylated ATα and ATβ were analyzed by SDS-PAGE, and biotinylation was confirmed by Western blot analysis using streptavidin-HRP (Pierce Thermo Scientific, Rockford, IL) as probe. The functional activities of biotinylated AT were evaluated by FXa inhibition assay. By comparison of the biotinylated ATα and ATβ with unbiotinylated ATα and ATβ, only slight reductions in AT inhibition activity were observed after biotinylation, indicating the biotinylated ATβ and ATα prepared in this way would be representative and could be used in panning for ATβH binders as selective anti-coagulant blockers.

Example 4 - Human monoclonal antibody discovery by phage display and panning

A four-arm panning strategy was designed to discover Fabs specifically against ATβH from a human Fab library (Dyax Fab310). The library was first depleted with biotinylated heparin/Fondaparinux-bound ATα and biotinylated ATα and then was panned against heparin/Fondaparinux-bound ATβ and biotinylated ATβ on streptavidin beads, respectively. For each round of panning, the heparin-bound ATα (ATαH) was included in the binding buffer as a competitor. To keep hATβ in active conformation (heparin bound form), heparin was added to the wash buffer in all three rounds of panning. After panning, pooled clones were screened for hATβ and hATβH specific binding and counter-screened for hATα by ELISA. These clones were also examined for differential binding to rabbit ATβ over rabbit

ATα. Clones showing differential binding to both hATβH and rATβH over hATα and rATα were further subject to FXa –deinhibition assay with hATβ spike-in. Positive hits (Fabs) were reformatted into IgG1, expressed in HEK293 cells and purified by protein-A column. These purified IgG1s were extensively tested in AT-depleted human plasma and hemA patient plasma for TGA assay (Thrombin Generation Assay) and dPT (diluted Prothrombin Time) assay to measure the clotting time.

Example 5 - ELISA (Enzyme-Linked Immunosorbent Assay)

2 μg/ml biotinlyated AT antigens in PBS were coated on Streptavidin Microplates (Greiner, 781997) with or without heparin (50ug/ml, heparin-Natrium-5000, Apotheke, Fa. Ratiopharm). After overnight antigen coating at 4°C, plates were washed with PBST +/- heparin and blocked with 5% milk in PBST +/- heparin at 37°C for one hour. After removal of blocking buffer, 20ug/ml Fab or 4ug/ml IgG in blocking buffer (5% milk in PBST +/- heparin) was then added to the plates and plates were incubated at room temperature for 1 hour. Plates were then washed three times. Anti-human IgG POD (Sigma, A0170) in blocking buffer was added to plates and plates were incubated at room temperature for 30 minutes. Amplex red (In vitrogen, Cat#A22170) was used for detection at 1:1000 together with H_2O_2. After 30 min incubation, plates were read at Ex535, Em 590 in a fluorescent plate reader.

Example 6 - FXa de-inhibition assay – AT with heparin

Heparin was incubated with ATβ or ATα to form stable ATH complexes. Antibody was then added to the ATβH or ATαH complexes. In the meantime, 10μl of 200ng/ml FXa (HTI) and 20μl of 50μg/ml Fluophen FXa fluorogenic substrate (Hyphen Biomed) were mixed in a separate plate. The antibody-ATH mixture was added to the FXa/substrate solution quickly and fluorescent kinetic measurement was started immediately at Ex360nm and Em465nm. All necessary dilutions is made in 100mM NaCl, 20mM Tris, 2.5mM $CaCl_2$, 0,1% BSA, 0,1% PEG8000.

Example 7 - Thrombin Generation Assay (TGA) in FVIII deficiency human plasma

A 1:2 serial dilution of ATβH antibody was made in HemA human plasma starting from 1uM of final concentration to 0.015uM. Heparin was added in each antibody solution at a final concentration of 50nM. An 80ul of the antibody-heparin-plasma mixture was then added to each well containing 20ul of reconstitute PPP reagent or calibrator in a 96 well TGA plate. The plate was placed in the TGA instrument and the machine automatically dispensed 20ul of FluCa (Fluo substrate + $CaCl_2$) into each well. The reaction was allowed to run 60 min. Plasma alone was used as the negative control.

Example 8 - Thrombin Generation Assay (TGA) in AT-depleted human plasma with spiked-in ATα and ATβ respectively

Antibodies were added to human AT-deficient plasma spiked with 15nm of ATα or ATβ. Heparin was then pipetted into each reaction at a final concentration of 50nM. 80ul of plasma samples containing ATH-specific antibody, heparin and ATα or ATβ were added into wells of a 96 well TGA plate with 20ul of PPP reagent or calibrator. Plates were placed in the TGA instrument, and then 20ul of FluCa (Fluo substrate + $CaCl_2$) was dispensed into each well. Reactions were allowed to continue for 60 min.

Example 9 - Diluted Prothrombin Time assay (dPT) in human hemA plasma and AT deficient plasma

A serial dilution of anti-ATβH hmAbs was made in hemA plasma starting at 250 nM with 0.1 U/mL of heparin. The mixture of antibody, plasma and heparin was incubated at room temperature for 20-30min. Then 50 uL of this mixture was added to a 50 uL of diluted Innovin (1/2000) (Dade Behring), incubated for 4 min at 37°C, followed by adding 50 uL of 25 mM CaCl2 (HemSil). dPT test program was set on ACL Top coagulometer with acquisition time of 360 seconds. For dTP in AT deficient plasma, AT-DP was spiked in with either ATα or ATβ at a final concentration of 0.2 uM with 0.1 U/ml of heparin. Anti-ATβH mAbs was added to AT-DP/heparin/ATα or AT-DP/heparin/ATβ mixtures at a final concentration of

0.25 uM and incubated at room temperature for 20-30min. For each reaction, a 50 uL of plasma/antibody/heparin mixture was added to 50 uL of diluted Innovin (1/4000), incubate 4 min at 37°C, followed by adding 50 uL of 25 mM CaCl2 (HemSil) as above.

Example 10 - Antibody purification

Pre-washed protein A agarose beads were incubated with antibody in binding buffer (volume ratio: 1:1) with rotation overnight at 4°C. Beads were then packed into a column and washed with 1 X PBS until $O.D._{280} < 0.05$. Residual solution was drained. Antibodies were eluted with elution buffer and collected into tubes containing neutralizing buffer. Eluted fractions were dialyzed against 1 x PBS overnight at 4°C with at least twice buffer changes. IgG concentration was measured at 280nm by nanodrop. The antibody purity was examined by either ELISA, SDS-PAGE or SSC.

Example 11 - Antibody binding affinity study by Biacore

Antibody affinity measurement was performed on a Biacore T100 or T200 processing unit. Anti-human Fc antibody or streptavidin was immobilized on a CM5 chip. hATβH or biotinylated hmAb antibodies were injected and captured on the chip. ATβ or ATα at different concentration with/without heparin were injected. Only AT and ATH bound to the antibodies generate binding constants. The binding results were reported as Equilibrium Dissociation Constants (KD) in nanoMoles. When AT/heparin complex was analyzed, heparin at 1 uM is included in the running buffer.

Example 12 - Heparinized rabbit bleeding model

Experimental design of the heparinized rabbit bleeding model is outlined in FIG. 13. Following preparation of rabbit jugular veins (right vein: venous stasis; left vein: cannulation), low molecular weight heparin (LMWH) is administered to the rabbit (1800U/kg) IV in PBS vehicle at time 0. After 10 minutes, the test article is administered. Experimental groups include Vehicle, PBS; Positive control, Protamine sulfate, (28mg/kg IV); Negative control, M14 IgG2; treatment: 30mg/kg;

TPP2803, 3mg/kg; TPP2803, 30mg/kg. Five minutes after administration of test article, an ear puncture (3-5 mm) is performed and thrombus formation in situ (stasis) is monitored over a 30 min period. Blood from the incision is removed every 15 seconds with a filter paper until the bleeding stops.

The foregoing disclosure and examples are not intended to narrow the scope of the claims in any way. It should be understood that various modifications and changes can be made, and equivalents can be substituted, to the foregoing embodiments and teachings without departing from the true spirit and scope of the claims appended hereto. The specification and examples are, accordingly, to be regarded in an illustrative sense rather than in a restrictive sense. Furthermore, the disclosure of all articles, books, patent applications, patents, and other material referred to herein are incorporated herein by reference in their entireties.

Claims

We claim:

1. A monoclonal antibody capable of binding the antithrombin (β) heparin complex (ATβH), wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (AYRMG) of SEQ ID NO: 2, a CDR2 sequence of amino acids 50 to 66 (RIYSSGGRTRYADSVKG) of SEQ ID NO: 2, and a CDR3 sequence of amino acids 97 to 114 (AREKASDLSGSFSEALDY) of SEQ ID NO: 2; and

wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 24 to 34 (QGDSLRSYYAS) of SEQ ID NO: 1, a CDR2 sequence of amino acids 50 to 56 (GKNNRPS) of SEQ ID NO: 1; and a CDR3 sequence of amino acids 89 to 99 (NSRDSSGNHLV) of SEQ ID NO: 1.

2. A monoclonal antibody capable of binding ATβH, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (KYKMD) of SEQ ID NO: 4, a CDR2 sequence of amino acids 50 to 66 (RIGPSGGKTM YADSVKG) of SEQ ID NO: 4, and a CDR3 sequence of amino acids 97 to 114 (AREKASDLSG TYSEALDY) of SEQ ID NO: 4; and

wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 26 to 37 (RASQSVSSSYLA) of SEQ ID NO: 3, a CDR2 sequence of amino acids 53 to 59 (GASSRAT) of SEQ ID NO: 3, and a CDR3 sequence of amino acids 92 to 99 (QQYGSSRT) of SEQ ID NO: 3.

3. A monoclonal antibody capable of binding ATβH, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (KYRMD) of SEQ ID NO: 6, a CDR2 sequence of amino acids 50 to 66 (RIGPSGGKTT YADSVKG) of SEQ ID NO: 6, and a CDR3 sequence of amino acids 97 to 114 (AREKTSDLSG SYSEALDY) of SEQ ID NO: 6; and

wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 26 to 36 (RASQNINRNLA) of SEQ ID NO: 5, a CDR2 sequence of amino acids 52 to

58 (TASTRAP) of SEQ ID NO: 5, and a CDR3 sequence of amino acids 91 to 99 (QQYASPPRT) of SEQ ID NO: 6.

4. A monoclonal antibody capable of binding ATβH, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (RYAMY) of SEQ ID NO: 8, a CDR2 sequence of amino acids 50 to 66 (RISPSGGKTH YADSVKG) of SEQ ID NO: 8, and a CDR3 sequence of amino acids 97 to 115 (ARLSQTGYYP HYHYYGMDV) of SEQ ID NO: 8; and

wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 26 to 37 (RASQRVSSSYLT) of SEQ ID NO: 7, a CDR2 sequence of amino acids 53 to 59 (GASSRAT) of SEQ ID NO: 7; and a CDR3 sequence of amino acids 92 to 101 (QQYDSTPPLT) of SEQ ID NO: 7.

5. A monoclonal antibody capable of binding ATβH, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (SYRMS) of SEQ ID NO: 10, a CDR2 sequence of amino acids 50 to 66 (RIYSSGGRTR YADSVKG) of SEQ ID NO: 10, and a CDR3 sequence of amino acids 97 to 114 (AREKASDLSG SFSEALDY) of SEQ ID NO: 10; and

wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 23 to 33 (QGDSLRSYYAS) of SEQ ID NO: 9, a CDR2 sequence of amino acids 49 to 55 (GKNNRPS) of SEQ ID NO: 9; and a CDR3 sequence of amino acids 88 to 96 (NSRDSSGNH) of SEQ ID NO: 9.

6. An isolated monoclonal antibody that binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10, and amino acid sequences having substantial homology to SEQ ID NOS: 2, 4, 6, 8, and 10.

7. The isolated monoclonal antibody of claim 6, further comprising a light chain variable region comprising an amino acid sequence selected from the group

consisting of SEQ ID NOS: 1, 3, 5 7, and 9, and amino acid sequences having substantial homology to SEQ ID NOS: 1, 3, 5, 7, and 9.

8. An isolated monoclonal antibody that binds to AtβH and inhibits anticoagulant activity, wherein said antibody further comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9, and amino acid sequences having substantial homology to SEQ ID NOS: 1, 3, 5, 7, and 9.

9. An isolated monoclonal antibody that binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 47, 48, 49, and 50.

10. The isolated monoclonal antibody of claim 9, further comprising: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 36, 37, 38, 39, and 40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ. ID NO: 41, 42, 43, 44, and 45; or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ. ID NO: 36, 37, 38, 39, and 40 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41, 42, 43, 44, and 45.

11. An isolated monoclonal antibody that binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31, 32, 33, 34, and 35.

12. The isolated monoclonal antibody of claim 11, further comprising: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23, 24, and 25; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ. ID NOS: 26, 27, 28, 29, and 30; or (c) both CDR1 comprising an amino acid sequence selected from the group consisting of SEQ. ID NOS: 21, 22, 23, 24, and 25and a CDR2 comprising and amino acid sequence selected from the group consisting of SEQ ID NOS: 26, 27, 28, 29, and 30.

13. An isolated monoclonal antibody that binds to the active site of ATβH.

14. An isolated monoclonal antibody that binds to ATβH and provides anticoagulant activity, wherein the isolated monoclonal antibody exhibits minimal binding to AT and wherein said antibody is a fully human antibody.

15. The isolated monoclonal antibody of any one of claims 1-14, wherein the antibody is selected from the group consisting of an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, a secretory IgA, an IgD, an IgE antibody, and an antibody fragment.

16. An isolated monoclonal antibody that binds to human ATβH.

17. The isolated monoclonal antibody of claims 16, wherein the antibody further binds to a nonhuman species of ATβH.

18. The isolated monoclonal antibody of any one of claims 1-14, wherein blood clotting time in the presence of the antibody is shortened.

19. An antibody which would compete with the isolated monoclonal antibody of any one of claims 1-14.

20. A pharmaceutical composition comprising a therapeutically effective amount of the
monoclonal antibody of any one of claims 1-14 and a pharmaceutically acceptable carrier.

21. A method for treating a genetic or acquired deficiency or a defect in coagulation comprising administering a therapeutically effective amount of a pharmaceutical composition of any one of claims 1-14 to a patient.

22. A method for treating coagulopathy comprising administering a therapeutically effective amount of a pharmaceutical composition of claim 20 to a patient.

23. The method of claims 21 or 22, wherein the coagulopathy is hemophilia A, hemophilia B, or hemophilia C.

24. The method of claims 21 or 22, wherein the coagulopathy is selected from the group consisting of trauma-induced coagulopathy and severe bleeding.

25. The method of claim 22, further comprising administering a clotting factor.

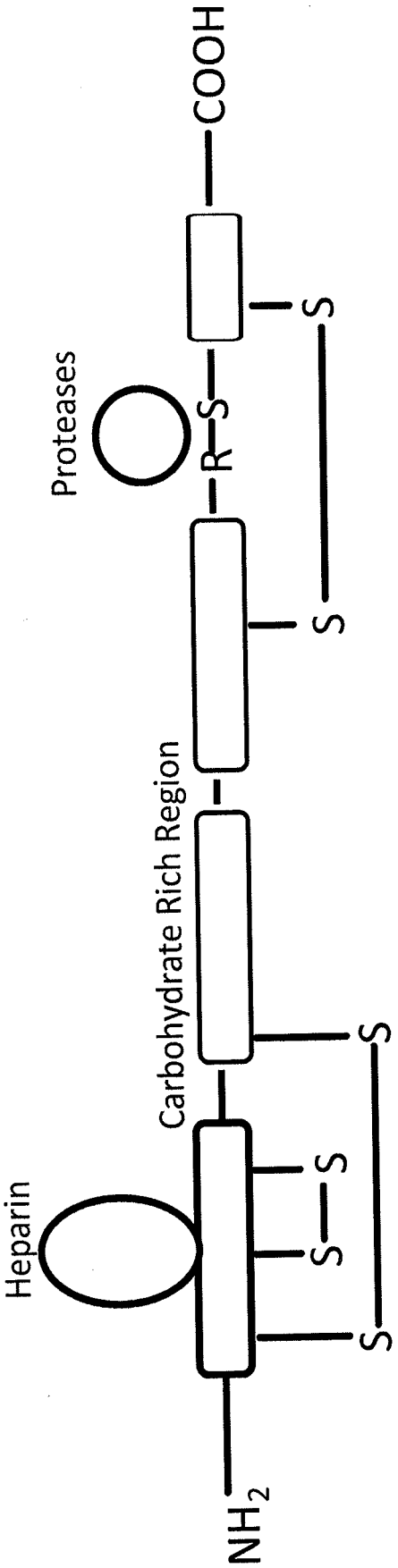
26. The method of claim 25, wherein the clotting factor is selected from the group consisting of Factor VIIa, Factor VIII, and Factor IX.

27. A method for shortening bleeding time comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 20 to a patient.

28. An isolated nucleic acid molecule encoding an antibody that binds to ATβH and inhibits anticoagulant activity, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

29. An isolated nucleic acid molecule encoding an antibody that binds to ATβH and inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

30. The method of claim 21, wherein the defect in coagulation is hemophilia A, hemophilia B or hemophilia C.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Proteases
R
S
S
Carbohydrate Rich Region
S
Heparin
S
S
S
NH2

FIG. 1

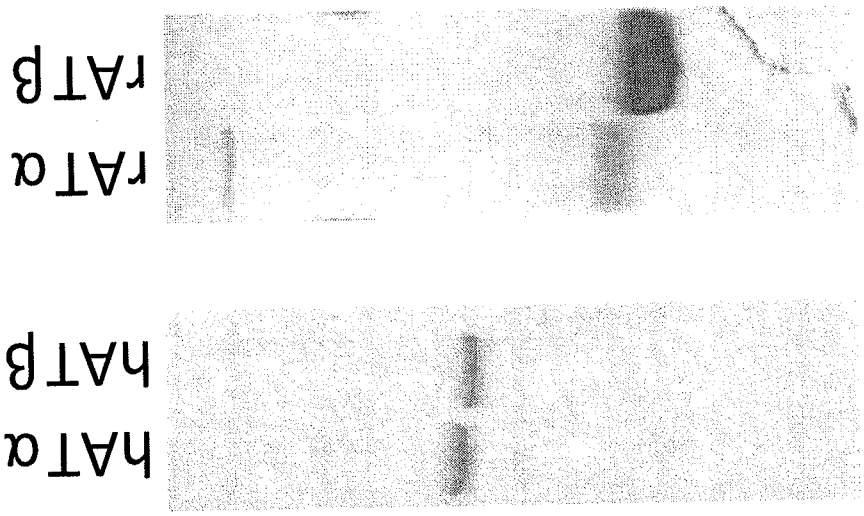
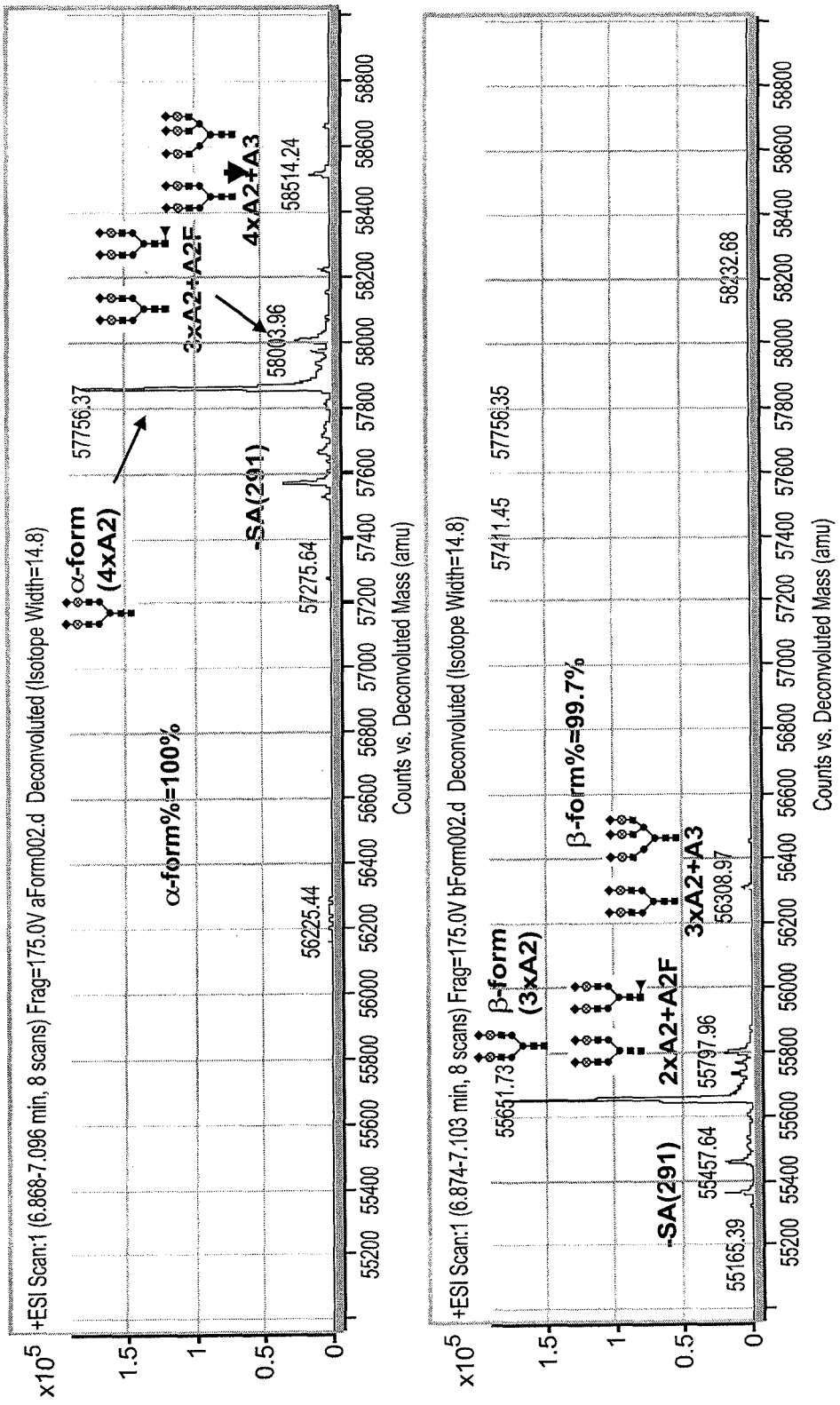


FIG. 2A

FIG. 2B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hATβ
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α-form%=100%
-SA(291)
3xA2+A2F
4xA2+A3
57756.37
58003.96
58514.24
57275.64
56225.44
Counts vs. Deconvoluted Mass (amu)
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β-form (3xA2)
β-form%=99.7%
2xA2+A2F
3xA2+A3
-SA(291)
57411.45
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56308.97
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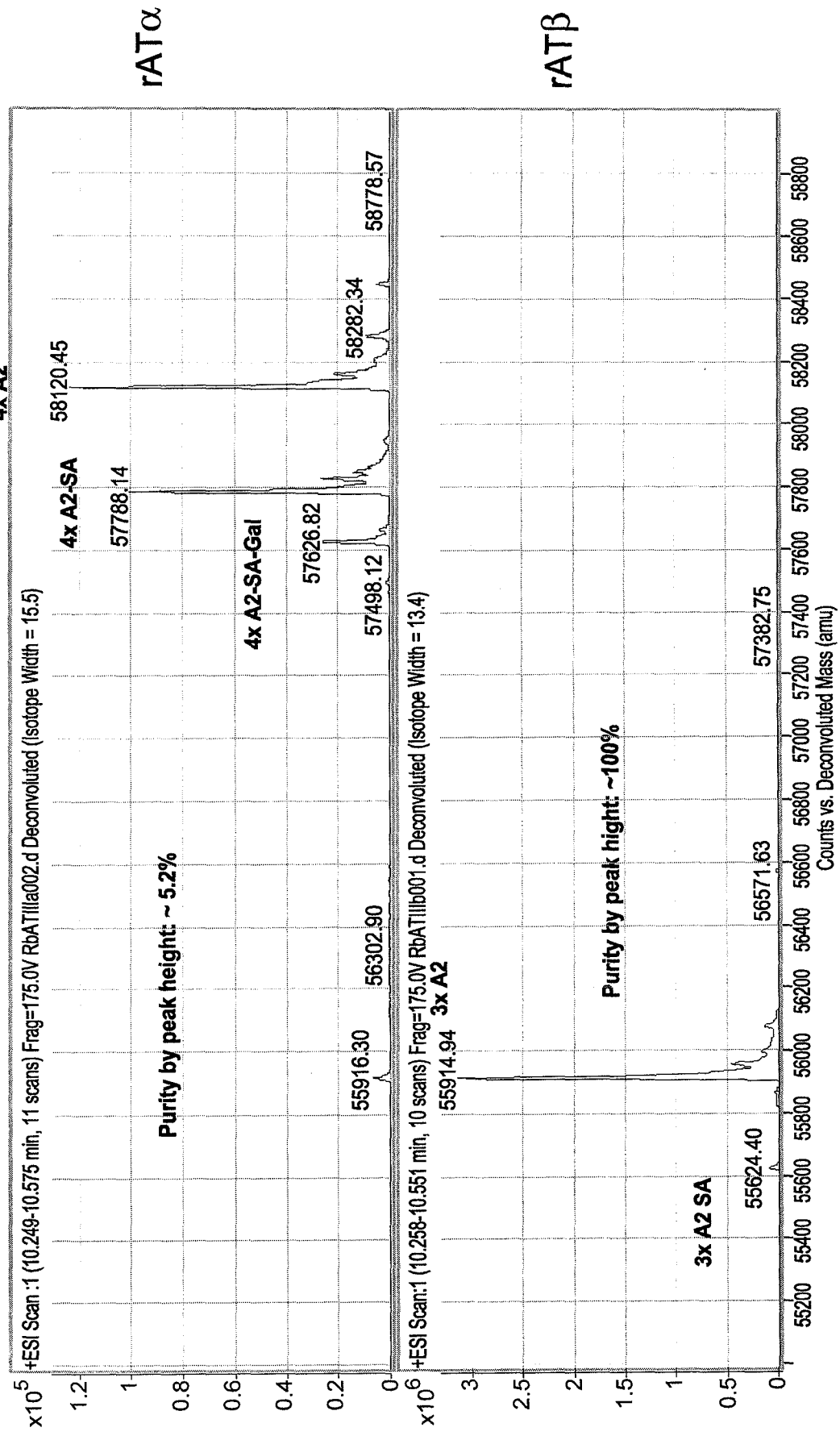
55165.39
Counts vs. Deconvoluted Mass (amu)

FIG. 2C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rATβ
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4x A2-SA
4x A2-SA-Gal
3x A2
3x A2 SA
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58282.34
58778.57
57498.12
55916.30
56302.90
55914.94
55624.40
56571.63
57382.75
Purity by peak height: ~ 5.2%
Purity by peak hight: ~100%
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x10^5
x10^6
Counts vs. Deconvoluted Mass (amu)

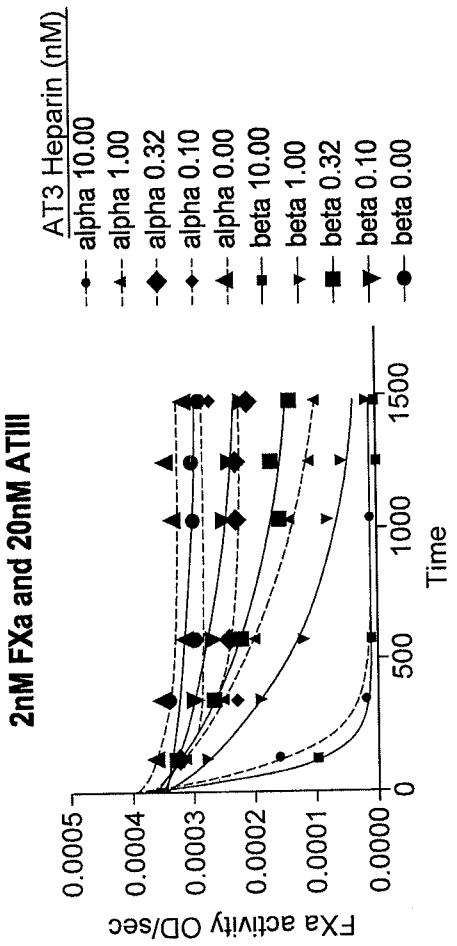
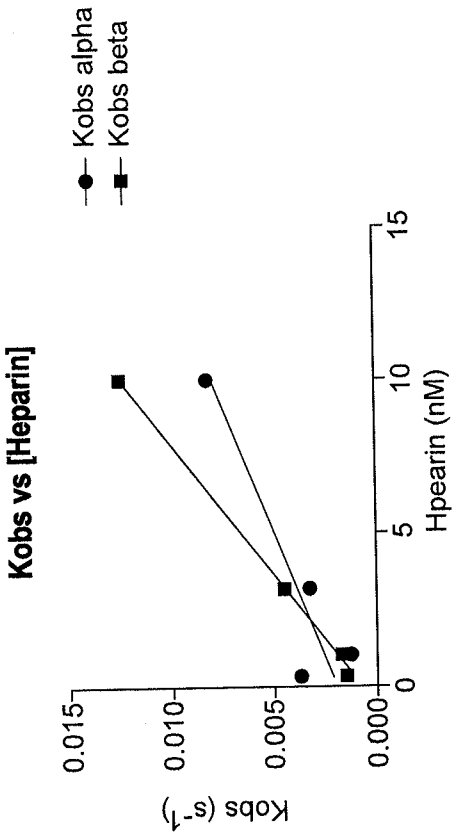

2nM FXa and 20nM ATIII
AT3 Heparin (nM)
alpha 10.00
alpha 1.00
alpha 0.32
alpha 0.10
alpha 0.00
beta 10.00
beta 1.00
beta 0.32
beta 0.10
beta 0.00
FXa activity OD/sec
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
0
500
1000
1500
Time

FIG. 3A


Kobs vs [Heparin]
Kobs alpha
Kobs beta
Kobs (s-1)
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0.010
0.005
0.000
0
5
10
15
Hpearin (nM)

FIG. 3B

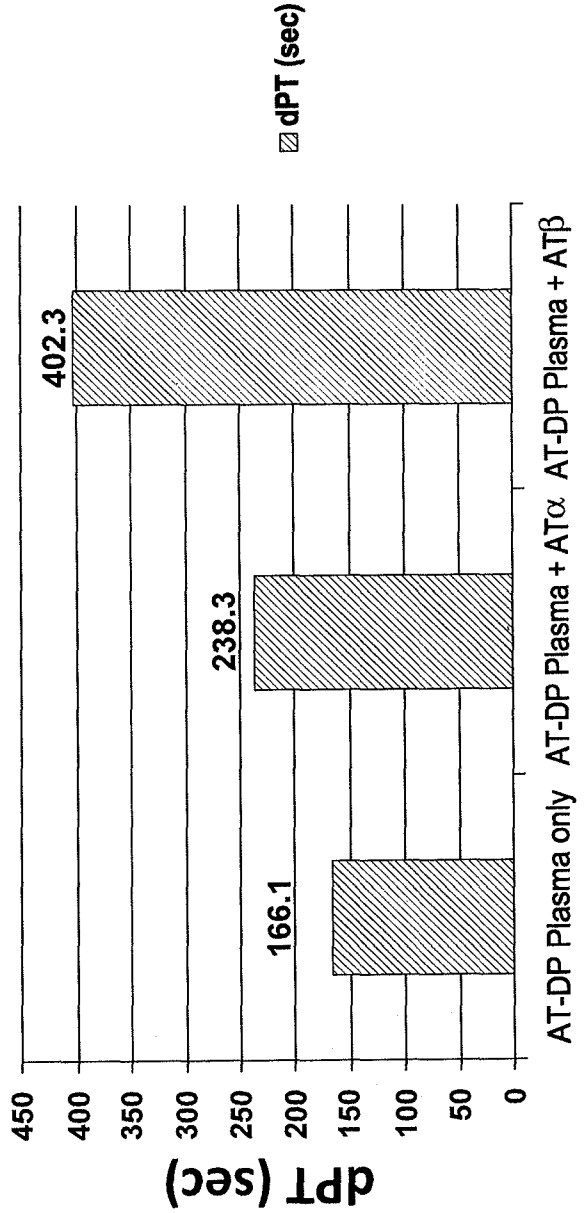

Clotting time
dPT (sec)
dPT (sec)
450
400
350
300
250
200
150
100
50
0
402.3
238.3
166.1
AT-DP Plasma only
AT-DP Plasma + ATα
AT-DP Plasma + ATβ
ATα, ATβ 0.2 um, heparin 0.1U/ml

FIG. 3C

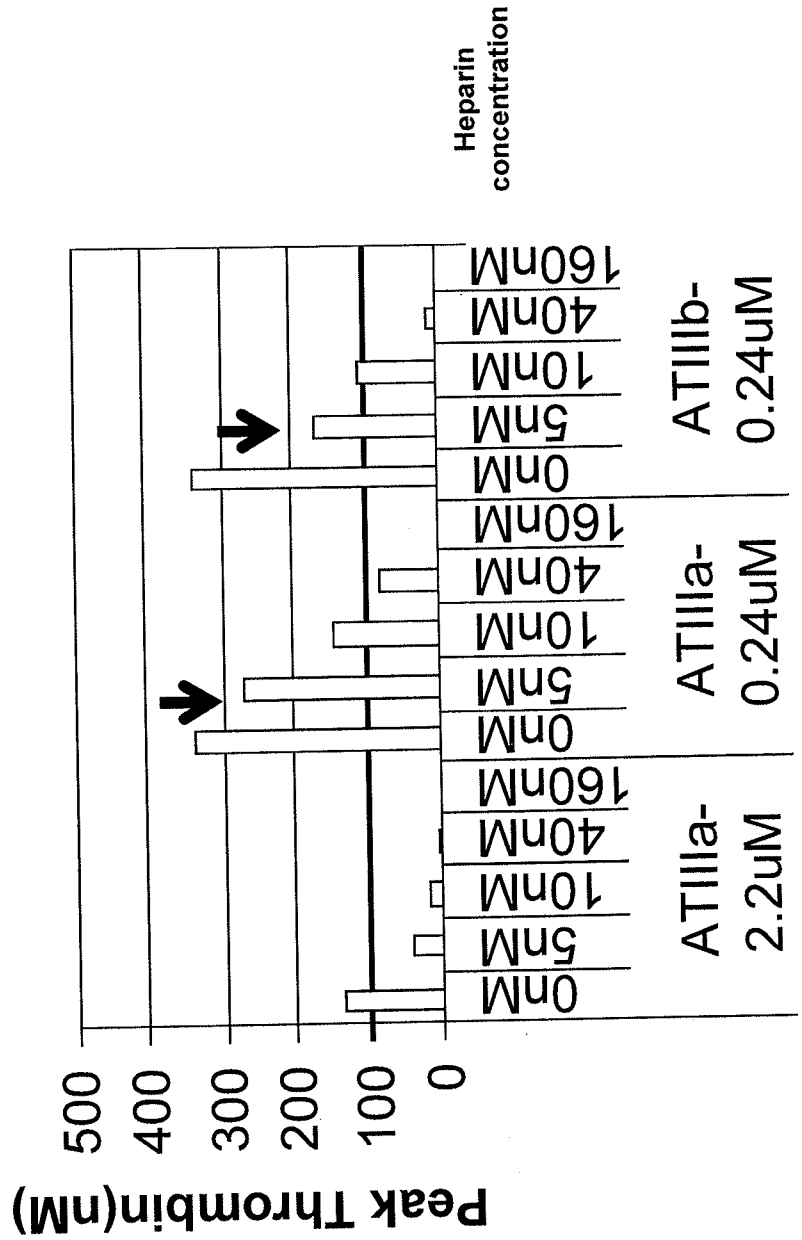

Peak Thrombin(nM)
500
400
300
200
100
0
Heparin concentration
0nM
5nM
10nM
40nM
160nM
ATIIIa-2.2uM
0nM
5nM
10nM
40nM
160nM
ATIIIa-0.24uM
0nM
5nM
10nM
40nM
160nM
ATIIIb-0.24uM

FIG. 3D

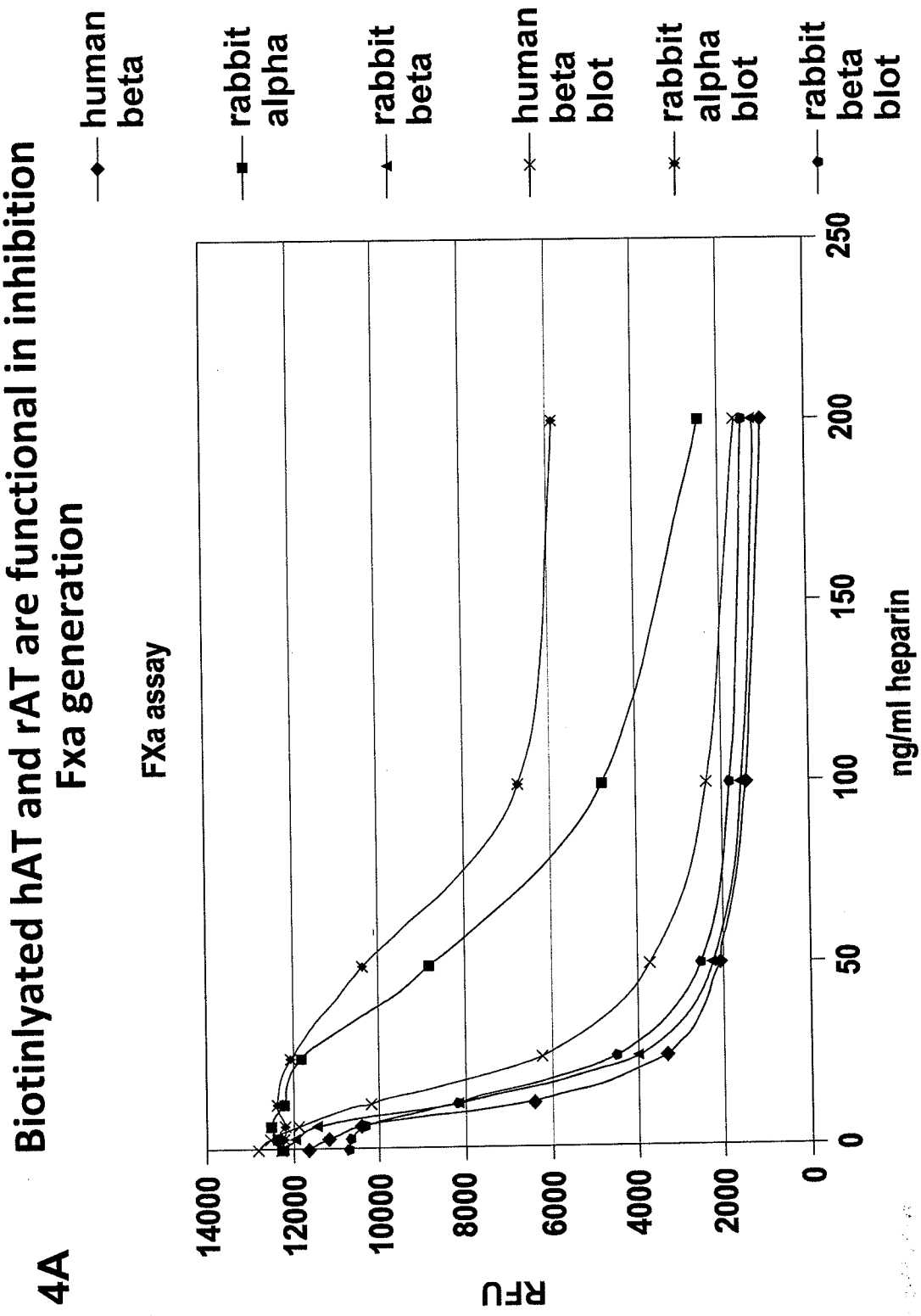

Biotinlyated hAT and rAT are functional in inhibition Fxa generation
FXa assay
human beta
rabbit alpha
rabbit beta
human beta blot
rabbit alpha blot
rabbit beta blot
RFU
14000
12000
10000
8000
6000
4000
2000
0
0
50
100
150
200
250
ng/ml heparin

FIG. 4A

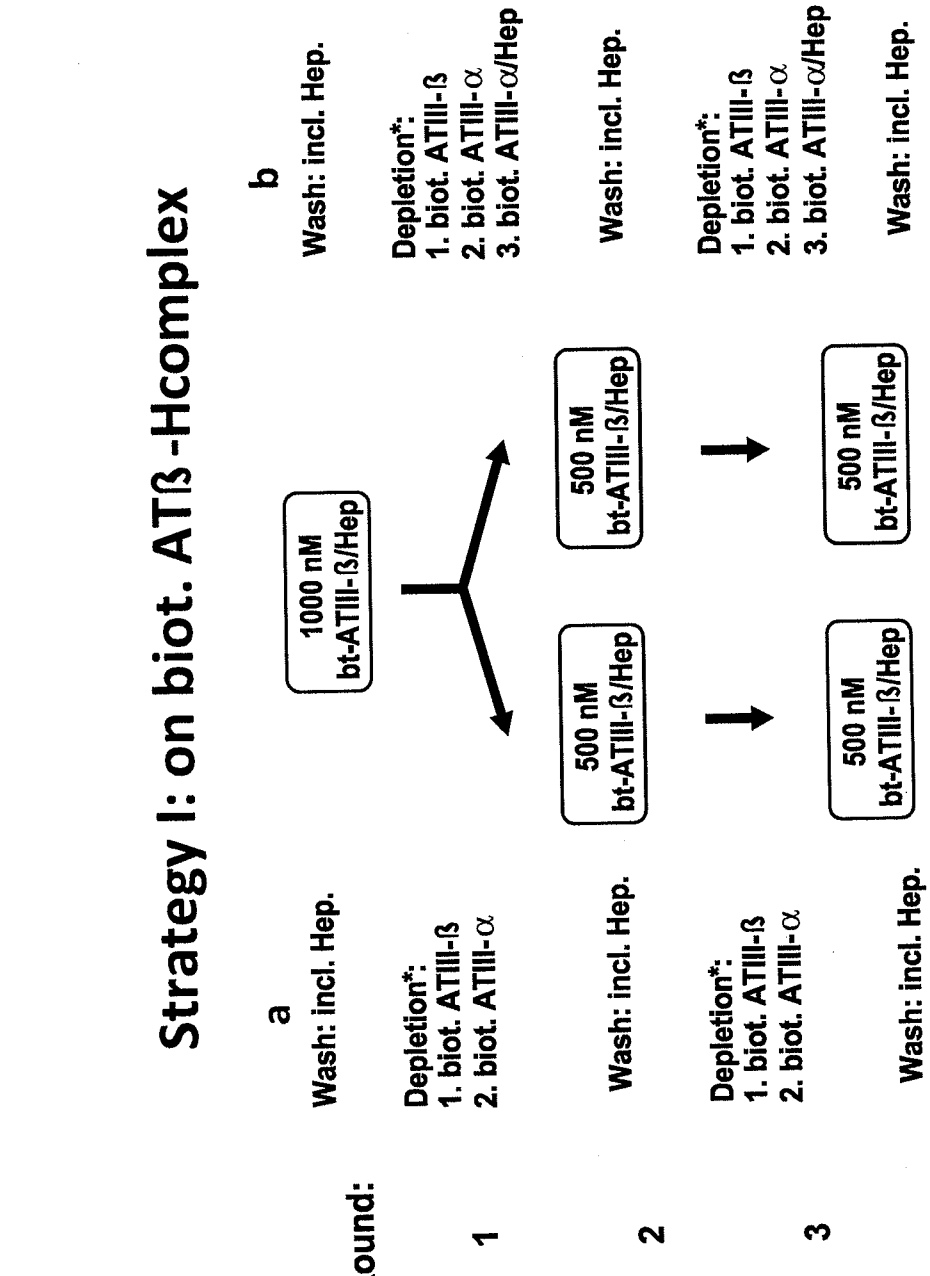

Strategy I: on biot. ATß-Hcomplex
Round:
a
b
Wash: incl. Hep.
1000 nM bt-ATIII-ß/Hep
Depletion*:
1. biot. ATIII-ß
2. biot. ATIII-α
3. biot. ATIII-α/Hep
500 nM bt-ATIII-ß/Hep
1
2
3

FIG. 4B

FIG. 4C

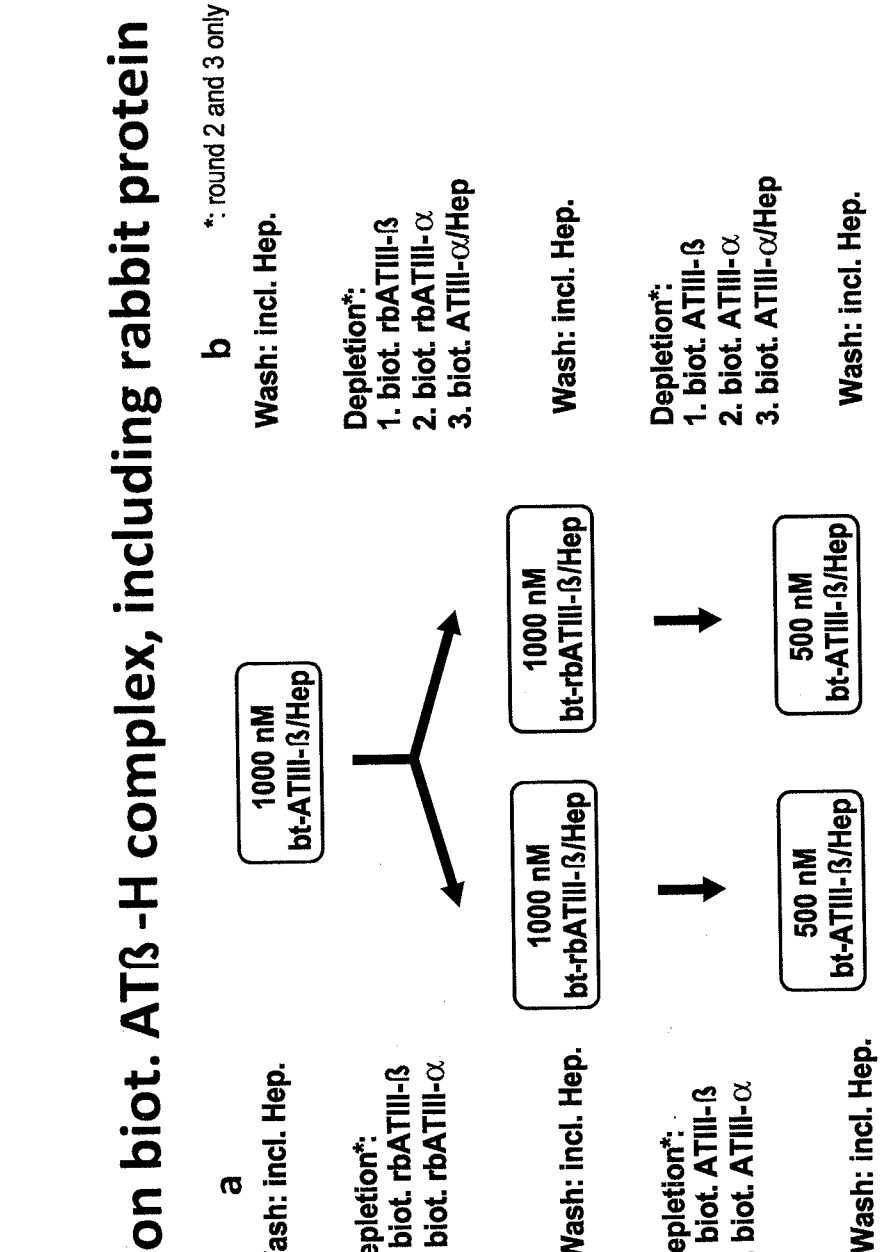

Strategy II: on biot. ATß-H complex, including rabbit protein
a
b
*: round 2 and 3 only
Round:
1
Wash: incl. Hep.
1000 nM bt-ATIII-ß/Hep
Depletion*:
1. biot. rbATIII-ß
2. biot. rbATIII-α
Wash: incl. Hep.
1000 nM bt-rbATIII-ß/Hep
1000 nM bt-rbATIII-ß/Hep
Depletion*:
1. biot. rbATIII-ß
2. biot. rbATIII-α
3. biot. ATIII-α/Hep
Wash: incl. Hep.
2
Depletion*:
1. biot. ATIII-ß
2. biot. ATIII-α
Wash: incl. Hep.
500 nM bt-ATIII-ß/Hep
500 nM bt-ATIII-ß/Hep
Depletion*:
1. biot. ATIII-ß
2. biot. ATIII-α
3. biot. ATIII-α/Hep
Wash: incl. Hep.
3

FIG. 5

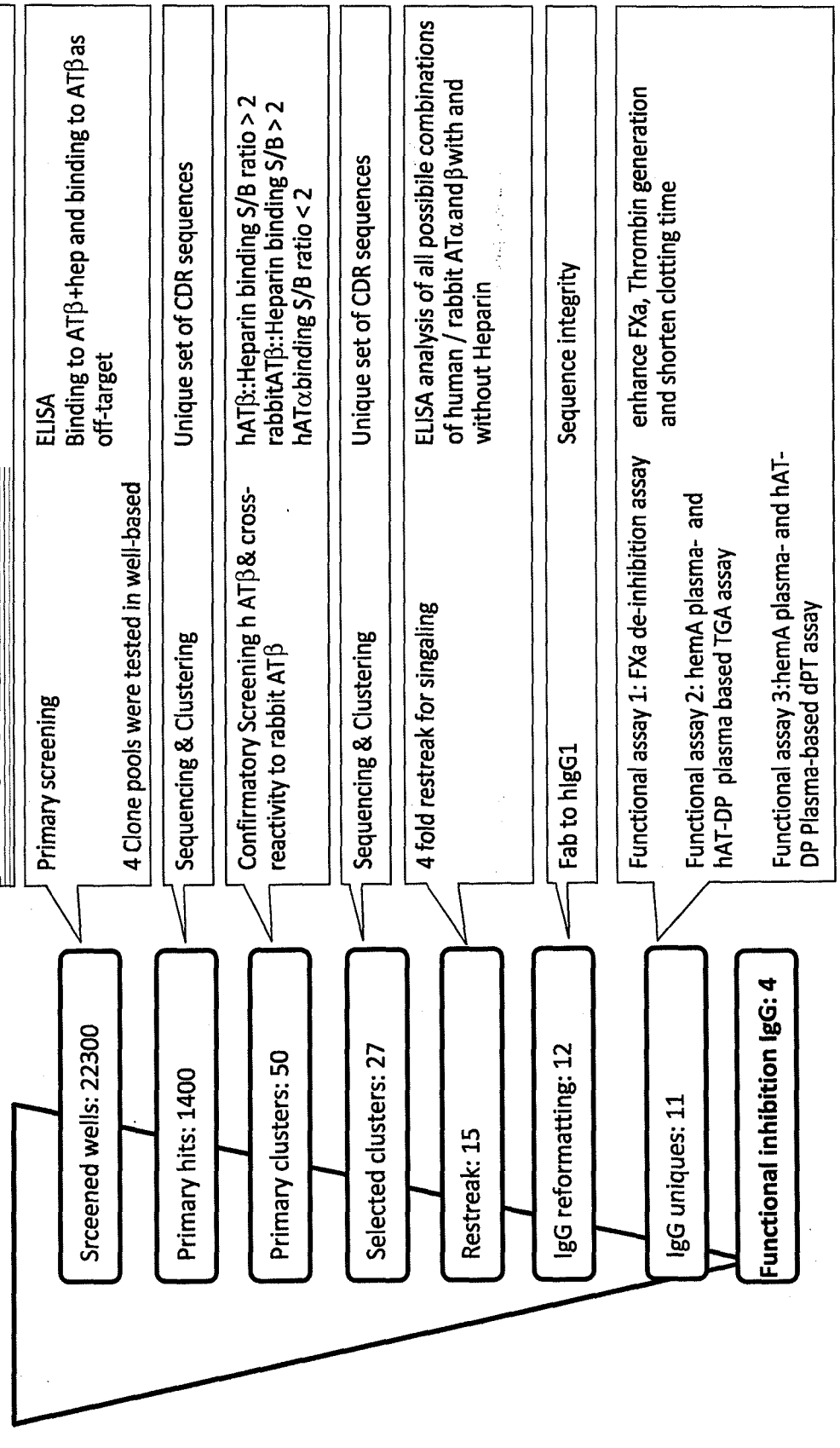

Screening stage
Hit criteria
Primary screening
ELISA
Binding to ATβ+hep and binding to ATβas off-target
4 Clone pools were tested in well-based
Sequencing & Clustering
Unique set of CDR sequences
Confirmatory Screening h ATβ& cross-reactivity to rabbit ATβ
hATβ:Heparin binding S/B ratio > 2
rabbitATβ::Heparin binding S/B > 2
hATαbinding S/B ratio < 2
Sequencing & Clustering
Unique set of CDR sequences
4 fold restreak for singaling
ELISA analysis of all possible combinations of human / rabbit ATα and βwith and without Heparin
Fab to hIgG1
Sequence integrity
Functional assay 1: FXa de-inhibition assay
enhance FXa, Thrombin generation and shorten clotting time
Functional assay 2: hemA plasma- and hAT-DP plasma based TGA assay
Functional assay 3:hemA plasma- and hAT-DP Plasma-based dPT assay
Srceened wells: 22300
Primary hits: 1400
Primary clusters: 50
Selected clusters: 27
Restreak: 15
IgG reformatting: 12
IgG uniques: 11
Functional inhibition IgG: 4

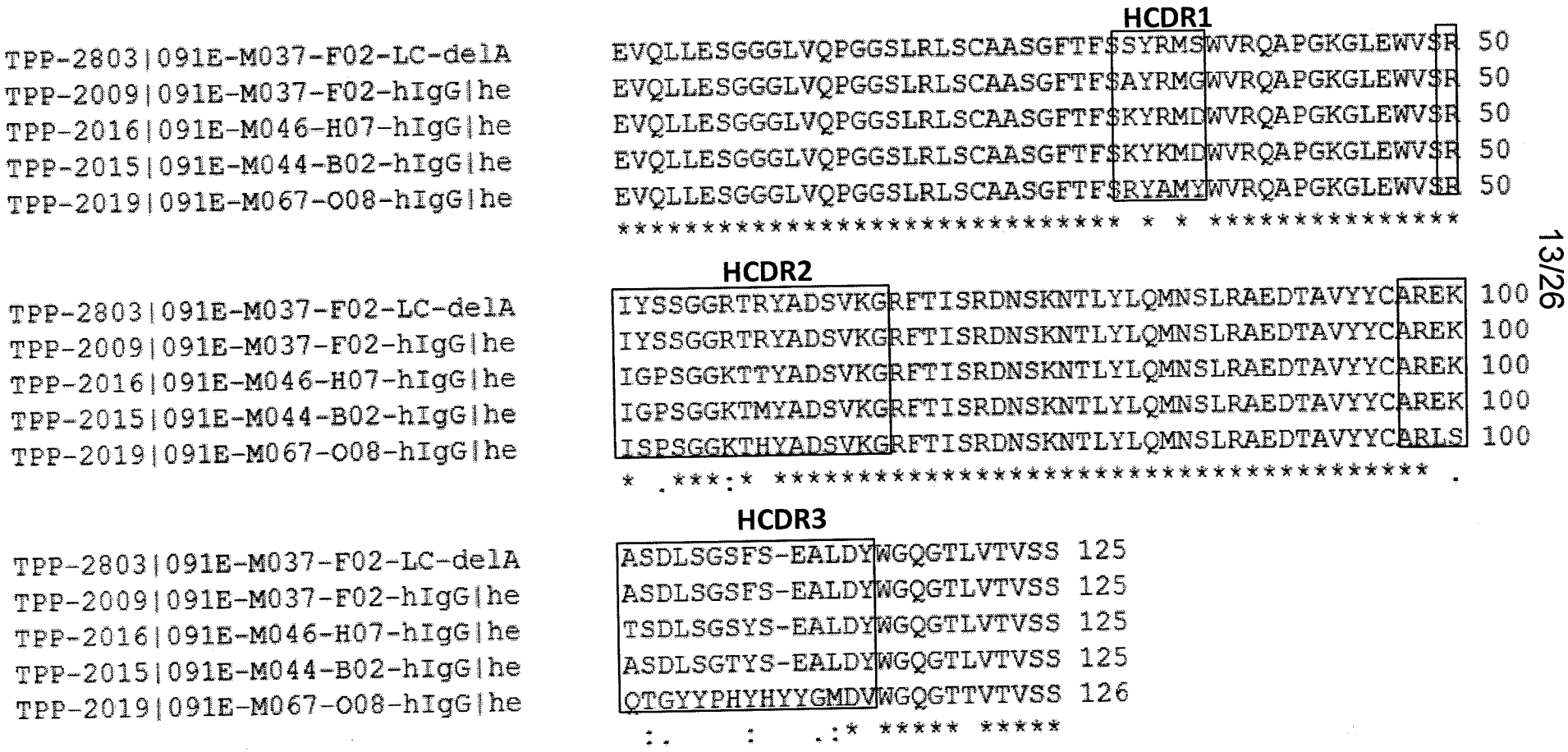
FIG. 6A

Light Chain

```
                                    LCDR1
TPP-2016|091E-M046-H07-hIgG|li   AQDIQMTQSPATLSVSPGERATLSCRASQNINRN-LAWYQQKPGRAPRLL 49
TPP-2015|091E-M044-B02-hIgG|li   AQDIQMTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLL 50
TPP-2019|091E-M067-O08-hIgG|li   AQDIQMTQSPATLSLSPGERATLSCRASQRVSSSYLIWYQQKPGQAPRLL 50
TPP-2803|091E-M037-F02-LC-delA   --SSELTQDP-AVSVALGQTVRITCQG-DSLRSYYASWYQQKPGQAPVLV 46
TPP-2009|091E-M037-F02-hIgG|li   -AQSVLTQDP-AVSVALGQTVRITCQG-DSLRSYYASWYQQKPGQAPVLV 47
                                   .  :**.* ::*:: *: . ::*:. : :     :*******:** *:

                                   LCDR2                           LCDR3
TPP-2016|091E-M046-H07-hIgG|li   IHTASTRAEGVPVRIIGSGSGTEFTLTISSLEPEDFAVYFCQQYASPP-- 97
TPP-2015|091E-M044-B02-hIgG|li   IYGASSRAIGIPDRFSGSGSGIDFTLTISRLEPEDFAVYYCQQYGSS--- 97
TPP-2019|091E-M067-O08-hIgG|li   IYGASSRAIGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDSTPP- 99
TPP-2803|091E-M037-F02-LC-delA   IYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNH 96
TPP-2009|091E-M037-F02-hIgG|li   IYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNH 97
                                 *:  ..*..*:* *::**.**.  :***:  :.** * *:*:.   *.

                                 LCDR3
TPP-2016|091E-M046-H07-hIgG|li   RIFGQGTKVEIK-RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA 146
TPP-2015|091E-M044-B02-hIgG|li   RIFGQGTKVEIR-RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA 146
TPP-2019|091E-M067-O08-hIgG|li   LTFGGGTKVEIK-RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA 148
TPP-2803|091E-M037-F02-LC-delA   LVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAV 146
TPP-2009|091E-M037-F02-hIgG|li   LVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAV 147
                                  .** ***: :   :. ***** :****.*:*::..*::***:.:***   .

TPP-2016|091E-M046-H07-hIgG|li   KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC 196
TPP-2015|091E-M044-B02-hIgG|li   KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC 196
TPP-2019|091E-M067-O08-hIgG|li   KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC 198
TPP-2803|091E-M037-F02-LC-delA   TVAWKADGSPVKAGVETTKPSKQS-NNKYAASSYLSLTPEQWKSHRSYSC 195
TPP-2009|091E-M037-F02-hIgG|li   TVAWKADGSPVKAGVETTKPSKQS-NNKYAASSYLSLTPEQWKSHRSYSC 196
                                 .* **.*.:   ... : : ..::* :..*: ** *:*:  :::.*: *:*

TPP-2016|091E-M046-H07-hIgG|li   EVTHQGLSSPVTKSFNRGEC- 216
TPP-2015|091E-M044-B02-hIgG|li   EVTHQGLSSPVTKSFNRGEC- 216
TPP-2019|091E-M067-O08-hIgG|li   EVTHQGLSSPVTKSFNRGEC- 218
TPP-2803|091E-M037-F02-LC-delA   QVTHEG--STVEKTVAPAECS 214
TPP-2009|091E-M037-F02-hIgG|li   QVTHEG--STVEKTVAPAECS 215
                                 :***:*  *.* *:.  .**
```

FIG. 6B

Heavy Chain



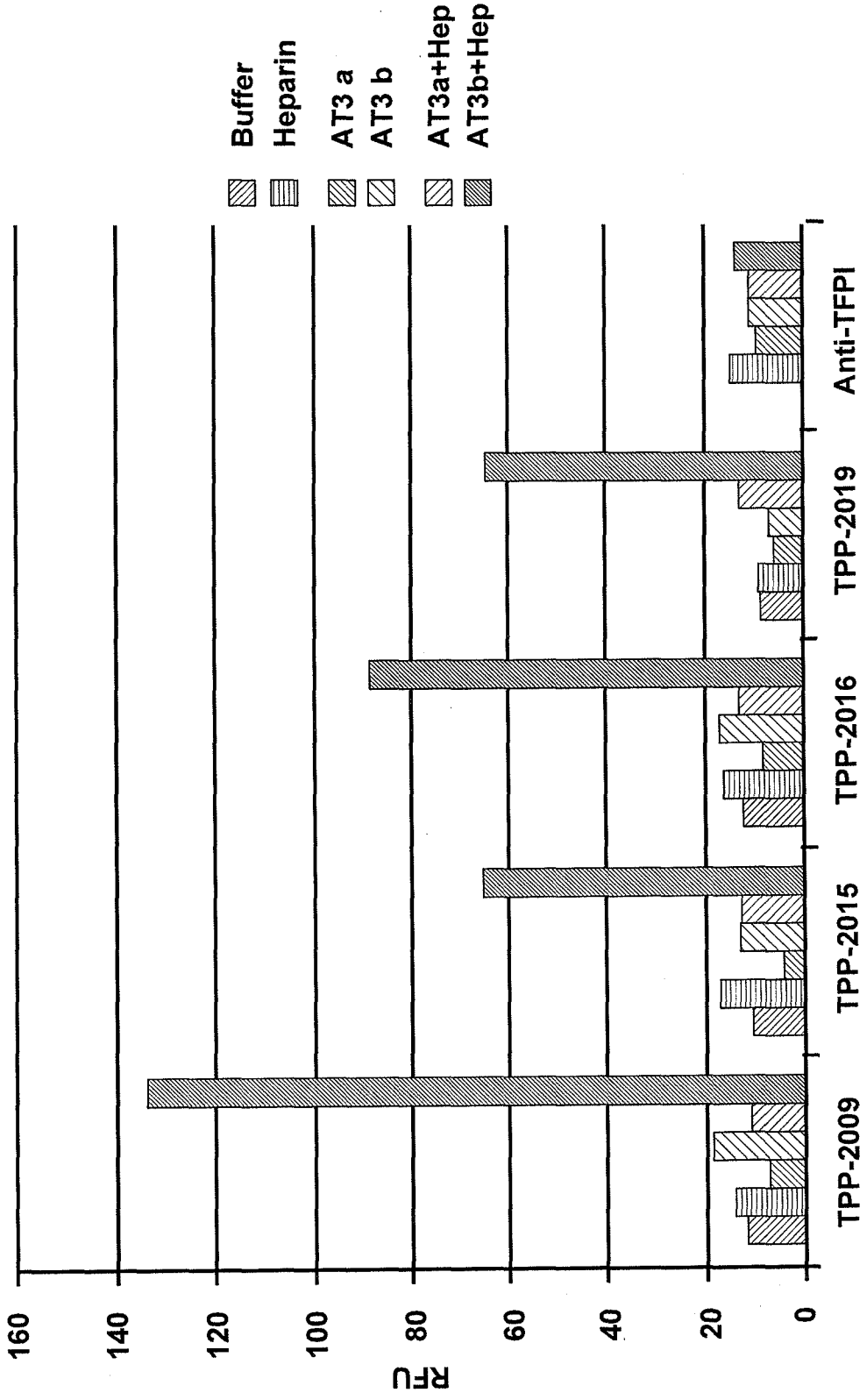

Buffer
Heparin
AT3 a
AT3 b
AT3a+Hep
AT3b+Hep
RFU
160
140
120
100
80
60
40
20
0
TPP-2009
TPP-2015
TPP-2016
TPP-2019
Anti-TFPI

FIG. 7A

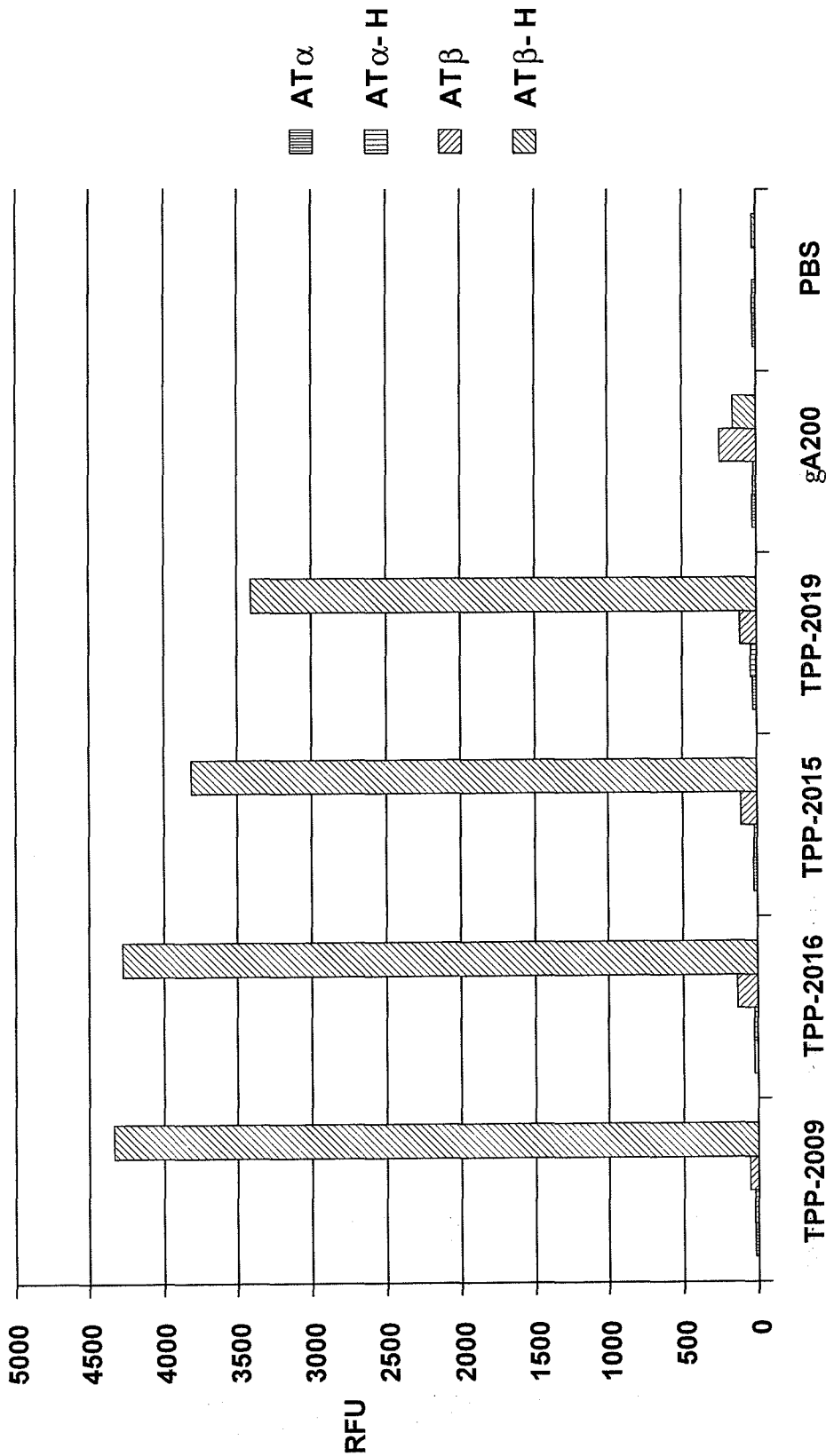

ATα
ATα-H
ATβ
ATβ-H
RFU
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
TPP-2009
TPP-2016
TPP-2015
TPP-2019
gA200
PBS

FIG. 7B

Antibody	Ka	Kd	KD (nm)
TPP 2009	1.07E+05	1.27E-03	11.9
TPP 2015	3.95E+04	1.09E-03	27.7
TPP 2016	6.05E+04	1.15E-03	18.9
TPP 2019	8.11E+04	1.39E-03	17.2

FIG. 7C

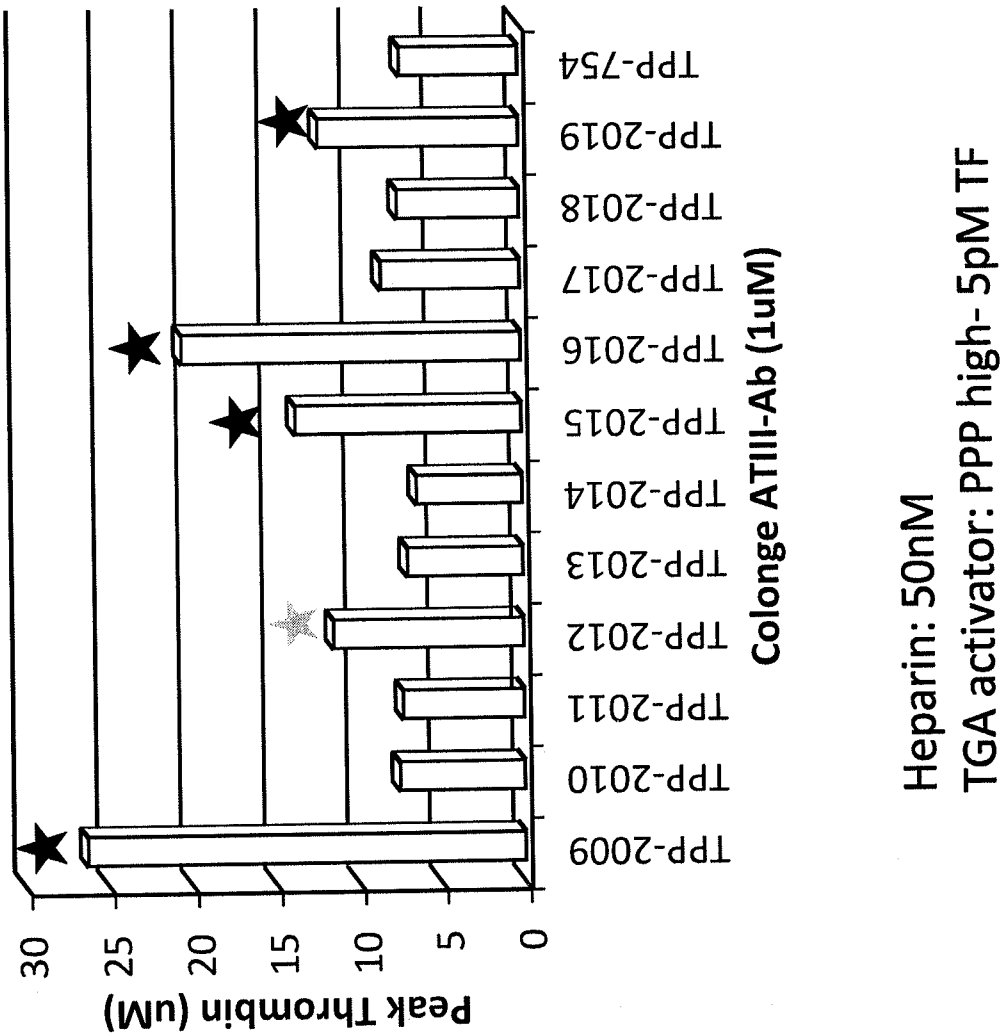
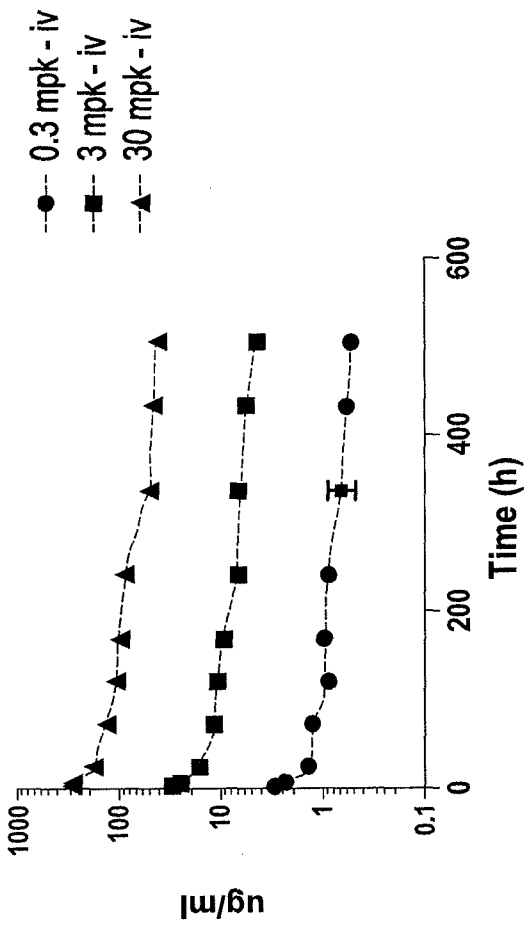

Peak Thrombin (uM)
0
5
10
15
20
25
30
TPP-2009
TPP-2010
TPP-2011
TPP-2012
TPP-2013
TPP-2014
TPP-2015
TPP-2016
TPP-2017
TPP-2018
TPP-2019
TPP-754
Colonge ATIII-Ab (1uM)
Heparin: 50nM
TGA activator: PPP high- 5pM TF

FIG. 8A

	FVIII-DP	AT-DP +ATIIIa	AT-DP +ATIIIb
Plasma only	**210.0**	165.2	167.0
Plasma + ATIIIa	--	**238.3**	--
Plasma + ATIIIb	--	--	**402.6**
TPP2009	**176.7**	233.0	**237.9**
TPP2010	199.1	237.7	356.3
TPP2011	205.9	234.8	380.7
TPP2012	218.7	278.9	320.6
TPP2013	206.5	241.5	366.1
TPP2014	213.0	240.5	383.0
TPP2015	**199.7**	229.7	**316.3**
TPP2016	**183.4**	230.6	**272.5**
TPP2017	203.2	242.6	332.9
TPP2018	202.8	239.6	373.3
TPP2019	**195.8**	233.0	**322.8**
TPP754 (control)	**204.3**	**237.1**	**385.2**

FIG. 8B

FIG. 9



PK parameters of antibody TPP 2009 in HEM-A mice IV dosing at 0.3, 3, and 30 mg/kg

dose	HL_Lambda_z	MRTINF_prod	AUC_%Extrap_prod	AUCall	AUCINF_prod	Cl_prod	Vz_prod
mg/kg	h	h	%	h*ug/ml	h*ug/ml	ml/h/kg	ml/kg
0.3 - Iv	340	479	35	467	720	0.42	204
3 - Iv	368	497	36	4556	7127	0.42	224
30 - Iv	259	349	24	46734	61814	0.49	182

FIG. 10A

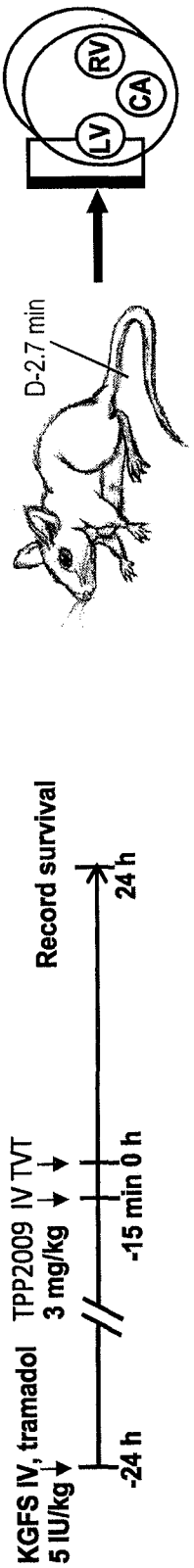


FIG. 10B

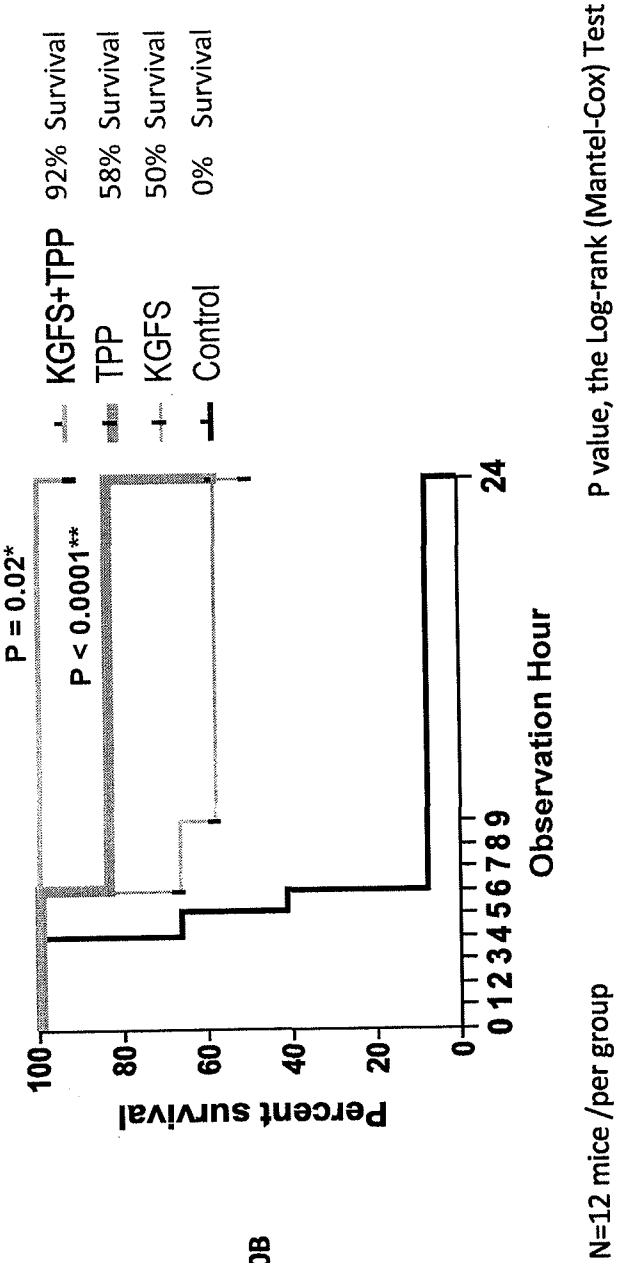


Figure 10B

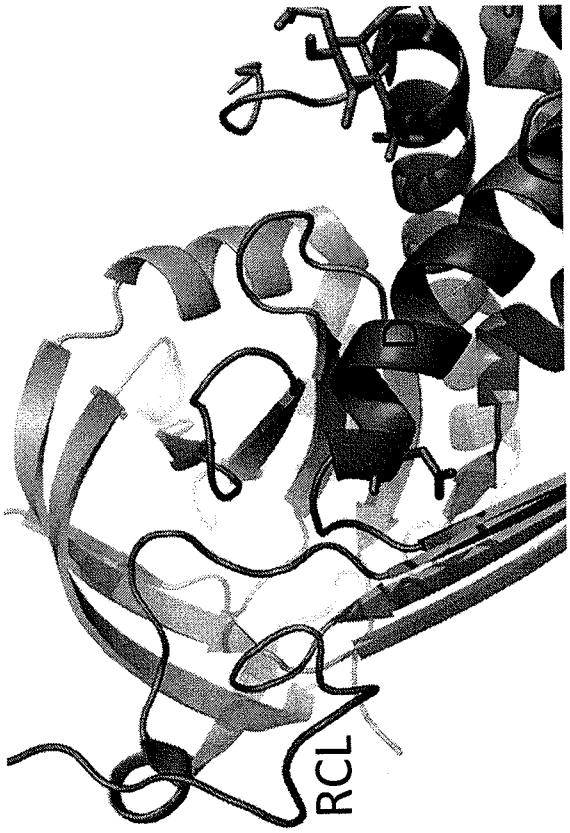


FIG. 11B

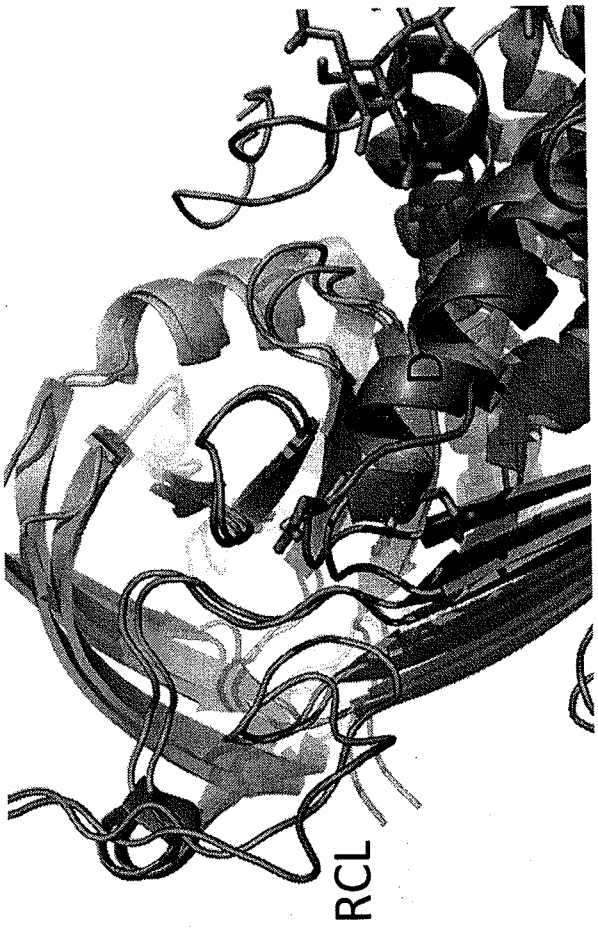


FIG. 11A

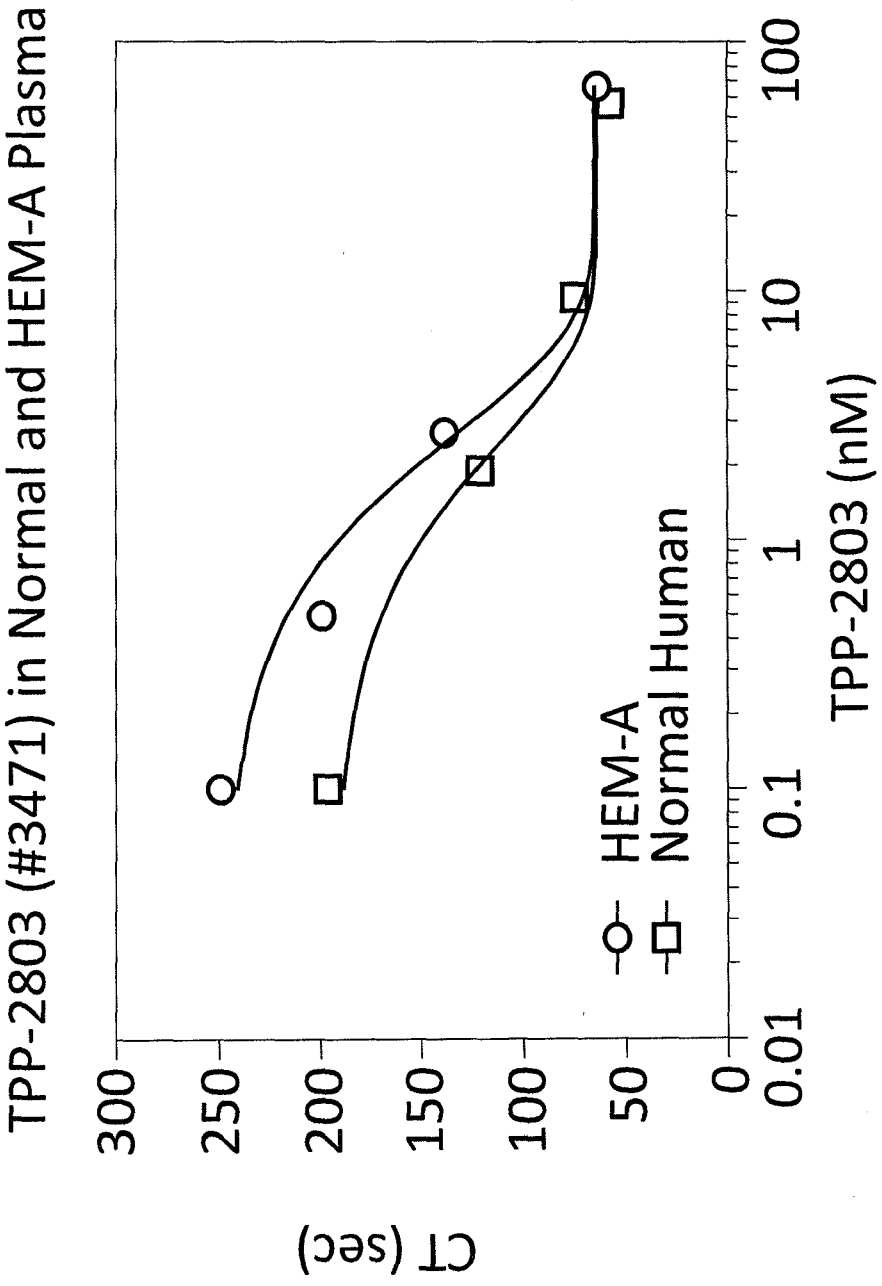

TPP-2803 (#3471) in Normal and HEM-A Plasma
CT (sec)
300
250
200
150
100
50
0
0.01
0.1
1
10
100
TPP-2803 (nM)
HEM-A
Normal Human

FIG. 12

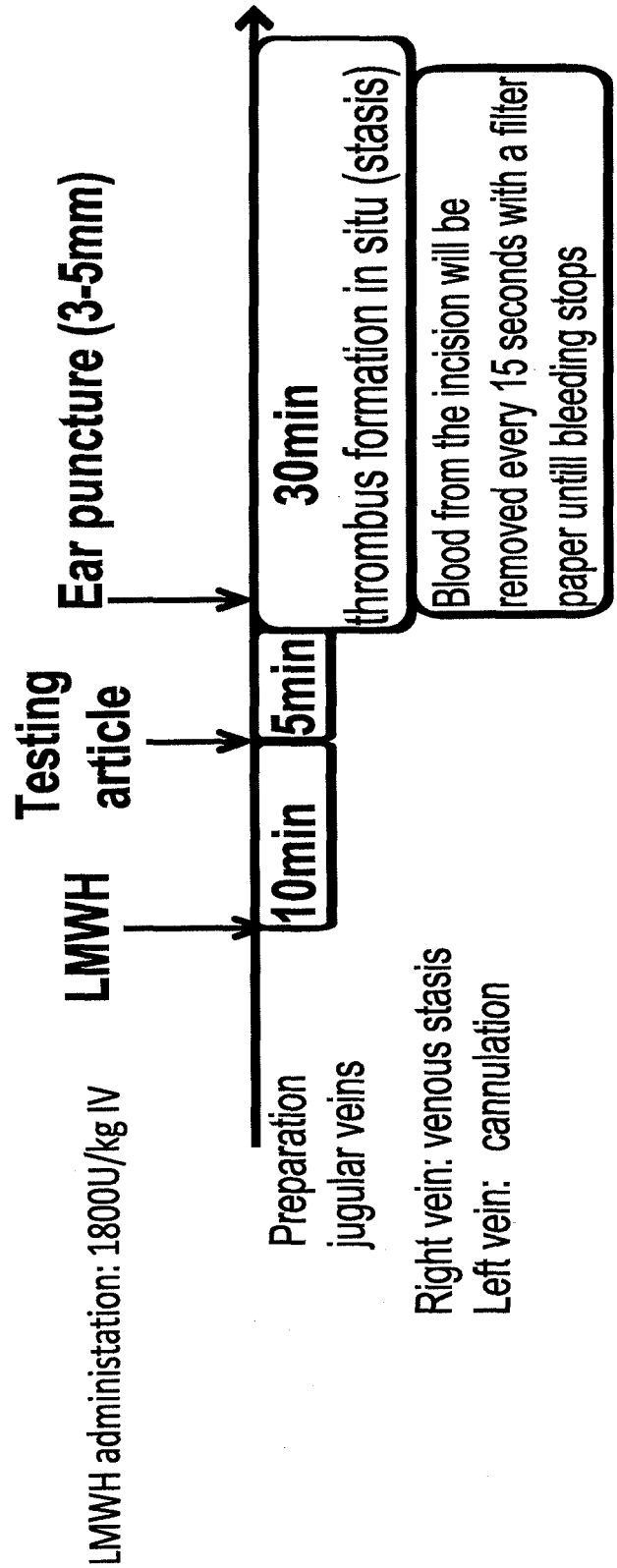

LMWH administation: 1800U/kg IV
LMWH
Testing article
Ear puncture (3-5mm)
10min
5min
30min
Preparation jugular veins
thrombus formation in situ (stasis)
Right vein: venous stasis
Left vein: cannulation
Blood from the incision will be removed every 15 seconds with a filter paper untill bleeding stops

FIG. 13

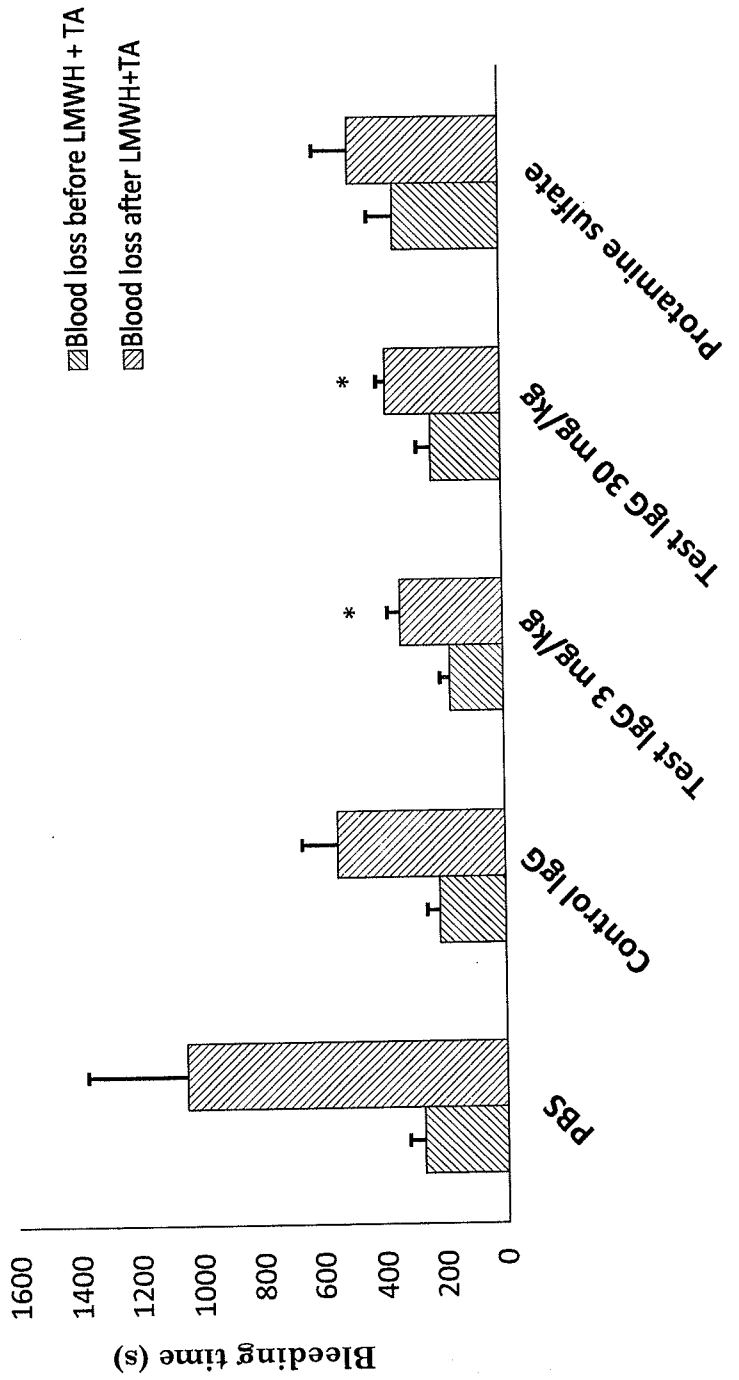

Blood loss before LMWH + TA
Blood loss after LMWH+TA
Bleeding time (s)
1600
1400
1200
1000
800
600
400
200
0
PBS
Control IgG
Test IgG 3 mg/kg
Test IgG 30 mg/kg
Protamine sulfate
*
*
*Significantly different from PBS (p≤0.05)

FIG. 14

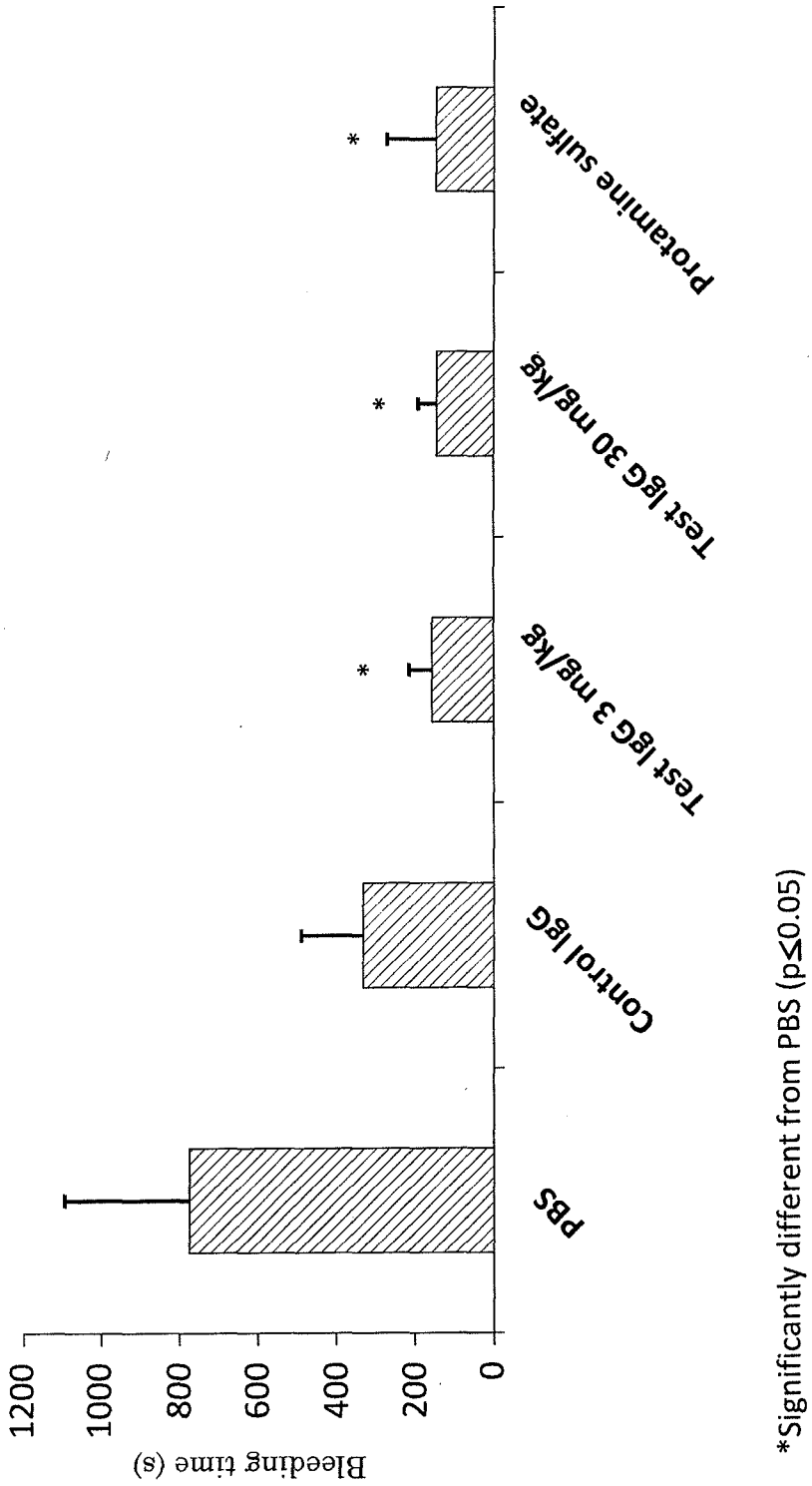

Bleeding time (s)
0
200
400
600
800
1000
1200
PBS
Control IgG
Test IgG 3 mg/kg
Test IgG 30 mg/kg
Protamine sulfate
*
*
*
*Significantly different from PBS (p≤0.05)

FIG. 15

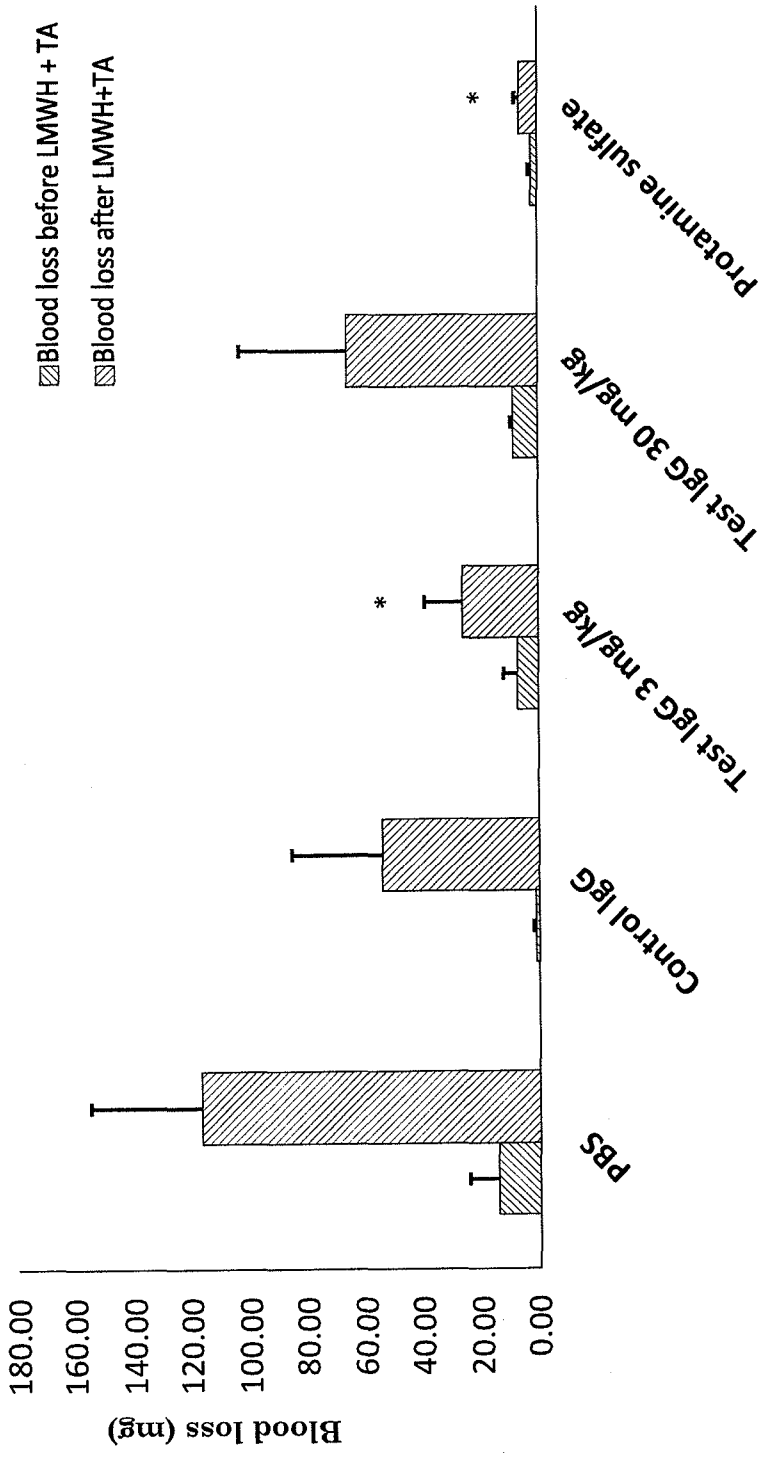

Blood loss before LMWH + TA
Blood loss after LMWH+TA
Blood loss (mg)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
PBS
Control IgG
Test IgG 3 mg/kg
Test IgG 30 mg/kg
Protamine sulfate
*
*
*Significantly different from PBS (p≤0.05)

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/029541

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/38 C12N15/13 A61K39/395 A61P7/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2013/028070 A2 (UMC UTRECHT HOLDING BV [NL]; HACK CORNELIS ERIK [NL]; YILDIZ CAFER [NL) 28 February 2013 (2013-02-28) page 17, line 28; claims 1, 7, 9 page 28, line 26 page 11, lines 19-22 -----	6-13, 15-30 1-5,14
Y	LI W ET AL: "Structure of the antithrombin-thrombin-heparin ternary complex reveals the antithrombotic mechanism of heparin", NATURE STRUCTURAL AND MOLECULAR BIOLOGY, NATURE PUBLISHING GROUP, US, vol. 11, no. 9, 1 September 2004 (2004-09-01), pages 857-862, XP009168588, ISSN: 1545-9993 the whole document -----	1-5,14

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2014	28/07/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weikl, Martina

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/029541

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATEL ET AL: "Covalent antithrombin-heparin complexes", THROMBOSIS RESEARCH, TARRYTOWN, NY, US, vol. 120, no. 2, 1 January 2007 (2007-01-01), pages 151-160, XP022344157, ISSN: 0049-3848, DOI: 10.1016/J.THROMRES.2006.08.003 the whole document -----	1-5,14
A	EP 0 669 344 A2 (DAIICHI PURE CHEMICALS CO LTD [JP]) 30 August 1995 (1995-08-30) the whole document -----	1-30

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/029541

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013028070 A2	28-02-2013	AU 2012299524 A1	13-03-2014
		CA 2846494 A1	28-02-2013
		EP 2747776 A2	02-07-2014
		WO 2013028070 A2	28-02-2013
EP 0669344 A2	30-08-1995	AT 203999 T	15-08-2001
		AT 237640 T	15-05-2003
		DE 69522021 D1	13-09-2001
		DE 69522021 T2	22-11-2001
		DE 69529106 D1	16-01-2003
		DE 69529106 T2	02-10-2003
		DE 69530432 D1	22-05-2003
		DE 69530432 T2	24-12-2003
		EP 0669344 A2	30-08-1995
		EP 1072611 A1	31-01-2001
		EP 1074562 A1	07-02-2001
		JP H07238099 A	12-09-1995

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(21)申请号 201480028085.2

(22)申请日 2014.03.14

(30)优先权数据
61/784590 2013.03.14 US

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2015.11.13

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PCT/US2014/029541 2014.03.14

(87)PCT国际申请的公布数据
WO2014/153195 EN 2014.09.25

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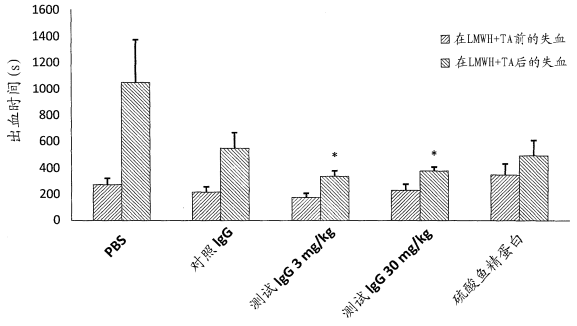
(54)发明名称

针对与肝素复合的抗凝血酶 β 的单克隆抗体

(57)摘要

本发明文件涉及针对与肝素和/或肝素样结构复合的人抗凝血酶 β (ATβH)的抗体、抗原结合抗体片段(Fab)及其他蛋白质支架。这些ATβH结合蛋白可以阻断ATβ 的抗凝活性,以诱导凝血。这些抗体和结合剂的治疗用途在本文中描述为淘选和筛选特异性抗体的方法。



*显著不同于PBS(p≤0.05)

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1.一种能够结合抗凝血酶(β)肝素复合物(ATβH)的单克隆抗体,其中所述抗体的重链包含:SEQ ID NO: 2的氨基酸31 - 35(AYRMG)的CDR1序列,SEQ ID NO: 2的氨基酸50 - 66(RIYSSGGRTRYADSVKG)的CDR2序列,以及SEQ ID NO: 2的氨基酸97 - 114(AREKASDLSGSFSEALDY)的CDR3序列;和

其中所述抗体的轻链包含:SEQ ID NO: 1的氨基酸24 - 34(QGDSLRSYYAS)的CDR1序列,SEQ ID NO: 1的氨基酸50 - 56(GKNNRPS)的CDR2序列,以及SEQ ID NO: 1的氨基酸89 - 99(NSRDSSGNHLV)的CDR3序列。

2.一种能够结合ATβH的单克隆抗体,其中所述抗体的重链包含:SEQ ID NO: 4的氨基酸31 - 35(KYKMD)的CDR1序列,SEQ ID NO: 4的氨基酸50 - 66(RIGPSGGKTM YADSVKG)的CDR2序列,以及SEQ ID NO: 4的氨基酸97 - 114(AREKASDLSG TYSEALDY)的CDR3序列;和

其中所述抗体的轻链包含:SEQ ID NO: 3的氨基酸26 - 37(RASQSVSSSYLA)的CDR1序列,SEQ ID NO: 3的氨基酸53 - 59(GASSRAT)的CDR2序列,以及SEQ ID NO: 3的氨基酸92 - 99(QQYGSSRT)的CDR3序列。

3.一种能够结合ATβH的单克隆抗体,其中所述抗体的重链包含:SEQ ID NO: 6的氨基酸31 - 35(KYRMD)的CDR1序列,SEQ ID NO: 6的氨基酸50 - 66(RIGPSGGKTT YADSVKG)的CDR2序列,以及SEQ ID NO: 6的氨基酸97 - 114(AREKTSDLSG SYSEALDY)的CDR3序列;和

其中所述抗体的轻链包含:SEQ ID NO: 5的氨基酸26 - 36(RASQNINRNLA)的CDR1序列,SEQ ID NO: 5的氨基酸52 - 58(TASTRAP)的CDR2序列,以及SEQ ID NO: 6的氨基酸91 - 99(QQYASPPRT)的CDR3序列。

4.一种能够结合ATβH的单克隆抗体,其中所述抗体的重链包含:SEQ ID NO: 8的氨基酸31 - 35(RYAMY)的CDR1序列,SEQ ID NO: 8的氨基酸50 - 66(RISPSGGKTH YADSVKG)的CDR2序列,以及SEQ ID NO: 8的氨基酸97 - 115(ARLSQTGYYP HYHYYGMDV)的CDR3序列;和

其中所述抗体的轻链包含:SEQ ID NO: 7的氨基酸26 - 37(RASQRVSSSYLT)的CDR1序列,SEQ ID NO: 7的氨基酸53 - 59(GASSRAT)的CDR2序列,以及SEQ ID NO: 7的氨基酸92 - 101(QQYDSTPPLT)的CDR3序列。

5.一种能够结合ATβH的单克隆抗体,其中所述抗体的重链包含:SEQ ID NO: 10的氨基酸31 - 35(SYRMS)的CDR1序列,SEQ ID NO: 10的氨基酸50 - 66(RIYSSGGRTR YADSVKG)的CDR2序列,以及SEQ ID NO: 10的氨基酸97 - 114(AREKASDLSG SFSEALDY)的CDR3序列;和

其中所述抗体的轻链包含:SEQ ID NO: 9的氨基酸23 - 33(QGDSLRSYYAS)的CDR1序列,SEQ ID NO: 9的氨基酸49 - 55(GKNNRPS)的CDR2序列,以及SEQ ID NO: 9的氨基酸88 - 96(NSRDSSGNH)的CDR3序列。

6.一种经分离的单克隆抗体,其与ATβH结合且抑制抗凝活性,其中所述抗体包含重链可变区,所述重链可变区包含选自SEQ ID NO: 2、4、6、8和10的氨基酸序列,以及与SEQ ID NO: 2、4、6、8和10具有基本同源性的氨基酸序列。

7.权利要求6的经分离的单克隆抗体,其进一步包含轻链可变区,所述轻链可变区包

含选自 SEQ ID NO: 1、3、5、7 和 9 的氨基酸序列，以及与 SEQ ID NO: 1、3、5、7 和 9 具有基本同源性的氨基酸序列。

8. 一种经分离的单克隆抗体，其与 AtβH 结合且抑制抗凝活性，其中所述抗体进一步包含轻链可变区，所述轻链可变区包含选自 SEQ ID NO: 1、3、5、7 和 9 的氨基酸序列，以及与 SEQ ID NO: 1、3、5、7 和 9 具有基本同源性的氨基酸序列。

9. 一种经分离的单克隆抗体，其与 ATβH 结合且抑制抗凝活性，其中所述抗体包含含有选自 SEQ ID NO: 46、47、48、49 和 50 的氨基酸序列的 CDR3。

10. 权利要求 9 的经分离的单克隆抗体，其进一步包含 :(a)含有选自 SEQ ID NO: 36、37、38、39 和 40 的氨基酸序列的 CDR1 ;(b) 含有选自 SEQ ID NO: 41、42、43、44 和 45 的氨基酸序列的 CDR2 ;或(c) 含有选自 SEQ ID NO: 36、37、38、39 和 40 的氨基酸序列的 CDR1，以及含有选自 SEQ ID NO: 41、42、43、44 和 45 的氨基酸序列的 CDR2 两者。

11. 一种经分离的单克隆抗体，其与 ATβH 结合且抑制抗凝活性，其中所述抗体包含含有选自 SEQ ID NO: 31、32、33、34 和 35 的氨基酸序列的 CDR3。

12. 权利要求 11 的经分离的单克隆抗体，其进一步包含 :(a)含有选自 SEQ ID NO: 21、22、23、24 和 25 的氨基酸序列的 CDR1 ;(b) 含有选自 SEQ ID NO: 26、27、28、29 和 30 的氨基酸序列的 CDR2 ;或(c) 含有选自 SEQ ID NO: 21、22、23、24 和 25 的氨基酸序列的 CDR1，以及含有选自 SEQ ID NO: 26、27、28、29 和 30 的氨基酸序列的 CDR2 两者。

13. 一种经分离的单克隆抗体，其与 ATβH 的活性位点结合。

14. 一种经分离的单克隆抗体，其与 ATβH 结合且提供抗凝活性，其中所述经分离的单克隆抗体显示出与 AT 的最低限度结合，并且其中所述抗体是全人抗体。

15. 权利要求 1-14 中任一项的经分离的单克隆抗体，其中所述抗体选自 IgG1、IgG2、IgG3、IgG4、IgM、IgA1、IgA2、分泌型 IgA、IgD、IgE 抗体，以及抗体片段。

16. 一种经分离的单克隆抗体，其与人 ATβH 结合。

17. 权利要求 16 的经分离的单克隆抗体，其中所述抗体进一步与非人物种的 ATβH 结合。

18. 权利要求 1-14 中任一项的经分离的单克隆抗体，其中在所述抗体存在下的凝血时间是缩短的。

19. 一种抗体，其与权利要求 1-14 中任一项的经分离的单克隆抗体竞争。

20. 一种药物组合物，其包含治疗有效量的权利要求 1-14 中任一项的单克隆抗体和药学可接受的载体。

21. 一种用于治疗遗传性或获得性凝血缺乏或缺陷的方法，其包括给患者施用治疗有效量的权利要求 1-14 中任一项的药物组合物。

22. 一种用于治疗凝血病的方法，其包括给患者施用治疗有效量的权利要求 20 的药物组合物。

23. 权利要求 21 或 22 的方法，其中所述凝血病是血友病 A、血友病 B 或血友病 C。

24. 权利要求 21 或 22 的方法，其中所述凝血病选自创伤诱导的凝血病和严重出血。

25. 权利要求 22 的方法，其进一步包括施用凝血因子。

26. 权利要求 25 的方法，其中所述凝血因子选自因子 VIIa、因子 VIII 和因子 IX。

27. 一种用于缩短出血时间的方法，其包括给患者施用治疗有效量的权利要求 20 的药

物组合物。

28.一种经分离的核酸分子，其编码与 ATβH 结合且抑制抗凝活性的抗体，其中所述抗体包含含有选自 SEQ ID NO: 1、3、5、7 和 9 的氨基酸序列的轻链可变区。

29.一种经分离的核酸分子，其编码与 ATβH 结合且抑制抗凝活性的抗体，其中所述抗体包含含有选自 SEQ ID NO: 2、4、6、8 和 10 的氨基酸序列的重链可变区。

30.权利要求 21 的方法，其中所述凝血缺陷是血友病 A、血友病 B 或血友病 C。

针对与肝素复合的抗凝血酶 β 的单克隆抗体

[0001] 与相关申请的交叉参考

本申请作为 PCT 国际专利申请于 2014 年 3 月 14 日提交，并且要求于 2014 年 3 月 14 日提交的美国临时专利申请号 61/784,590 的优先权，所述专利申请的整个公开内容在此整体引入作为参考。

[0002] 序列表提交

本申请包括作为 txt 文件以电子形式的序列表，所述 txt 文件名称为“SEQUENCE-LISTING-17207.0006WOU2”，于 2014 年 3 月 14 日创建，并且具有 65.1 千字节(KB)的大小。txt 文件“SEQUENCE-LISTING-17207.0006WOU2”的内容引入本文作为参考。

[0003] 背景

血友病领域中的目前未满足的医学需要主要是：(1)具有抑制剂的血友病患者(~30%的血友病患者)的治疗；和(2)长效和有效的凝血因子(FVIII/FIX)和 / 或其替代(旁路药物)(WFH 报道 2012,Paris)。用于治疗具有抑制剂的血友病患者的最广泛使用的旁路药物是 rFVII，其具有重大缺点例如致血栓性的危险、血浆中的短半衰期和高生产成本。针对抗凝因子例如组织因子蛋白质抑制剂(TFPI)、APC (活化蛋白 C)和抗凝血酶(AT)的抗体代表新的治疗范例。这些抗体不仅绕过或降低具有抑制剂的血友病患者中关于 FVIII 或 FIX 凝血因子的需要，还显示出更长的血浆半衰期(其降低给药频率)，并且因此增加患者依从性。迄今为止，存在处于临床前开发或研究阶段的几种基于抗体的促凝药物，例如抗 TFPI 和抗 APC。

[0004] AT 是人血浆中的主要抗凝剂。它抑制在凝血途径中起作用的凝血酶、FXa 及其他丝氨酸蛋白酶。它由 432 个氨基酸组成，由肝脏肝细胞产生，并且具有三天的长血浆半衰期(Collen，Schetz 等人 1977)。AT 的氨基酸序列是非常保守的，并且在牛、绵羊、兔、小鼠和人中的同源性是 84%-89% (Olson 和 Bjork 1994)。尽管 AT 的主要生理学靶是凝血酶和 FXa，但 AT 还将 FIXa、FXIa、FXIIa 以及 FVIIa 抑制至更少程度。AT 连同肝素一起发挥其抑制。在肝素的存在下，凝血酶和 FXa 被 AT 的抑制速率增加 3 至 4 个数量级，分别为 $7-11 \times 10^3\ M^{-1}s^{-1}$ 至 $1.5 - 4 \times 10^7\ M^{-1}s^{-1}$，以及 $2.5 \times 10^{-3}\ M^{-1}\ s^{-1}$ 至 $1.25 - 2.5\ M^{-1}s^{-1}$ (Olson，Swanson 等人 2004)。

[0005] 与分别在初始阶段和放大阶段单独抑制凝血的 TFPI 和 APC 不同，AT 在初始和放大阶段两者都对凝血发挥其抑制。因此，阻断 AT 可以具有比阻断单独的 TFPI 或 APC 更有力的促凝效应。减少的 AT 水平和活性已显示与人中增加的血栓形成关联。具有 AT 缺乏的患者趋于显示复发性静脉血栓形成和肺栓塞(van Boven 和 Lane 1997)。此外，纯合 AT 敲除小鼠死于胚胎期，具有极端高凝状态(Ishiguro，Kojima 等人 2000)。近期研究显示在尾夹出血模型中，其中 AT 显著降低 50% 的杂合 AT 敲除 hema 小鼠具有更少的失血和增强的凝血酶生成(Bolliger，Szlam 等人 2010)。

[0006] AT 是具有基于在 Asn135 上的差异糖基化的两种同种型 ATα 和 ATβ 的糖蛋白(Bjork 1997)。ATβ 缺乏在 Asn135 处的糖基化，并且是代表 10% 的人血浆 AT 的次要糖同种型。Asn135 定位在与初始肝素附着位点毗邻，并且构成在别构活化和 D 螺旋延伸后的延

伸肝素结合位点的部分(dela Cruz, Jairajpuri 等人 2006)。在 Asn135 处的大尺寸聚糖的缺乏以两种方式显著影响 ATβ 活化 :1)在肝素结合后抑制 FXa 和 FIXa 所需的更快速的别构活化 ;和 2) 关于通过桥连机制抑制 FXa 和凝血酶的更高亲和力的肝素结合的额外可接近的结合位点。事实上,在生理学盐浓度下,血浆衍生的 ATβ 以 36+/- 3nm 的 K_D与肝素结合,而 ATα 以 500+/- 50nm 的 K_D与肝素结合(Turk IV. 等人,1993)。ATβ 对于肝素的更高亲和力导致其优先分布至内皮下层,其富含肝素样结构 - 糖胺聚糖。因此,ATβ 提议在血管损伤部位处的 FXa 和凝血酶抑制中起主要和有力作用(Carlson 和 Atencio 1982 ; McCoy AJ, Pei XY. 等人 2003 ;Turk B, Brieditis I. 等人 1997 ;Witmer MR, Hatton MW. 1991 ;Frebelius S, 等人 1996)。ATβ 相对于 ATα 的重要性和更强的效力也在临床研究中得到报道。在患者中,在肝素结合中缺陷的 AT 纯合突变的严重性通过 AT 的 β 形式得到改善(Martinez-Martinez,Navarro-Fernandez 等人 2012)。在另一项研究中,AT 的界线水平(~70% 的正常 AT 抗原和活性)被血浆中的 20% ~ 30% ATβ 补偿(Bayston,Tripodi 等人 1999)。

[0007] 概述

提供了针对人 ATβH (与肝素和 / 或肝素样结构复合的 ATβ) 的单克隆抗体。在至少一个实施方案中,抗 ATβH 单克隆抗体显示出结合与肝素复合的 ATβ 。

[0008] 在其他实施方案中,针对人 ATβH 的单克隆抗体可以进行优化,例如以具有增加的亲和力或增加的功能活性。还提供的是特异性表位,其可以在人 ATβH 上,并且由经分离的单克隆抗体结合。进一步提供的是编码其的经分离的核酸分子。

[0009] 还提供的是包含抗 ATβH 单克隆抗体的药物组合物,以及遗传性和获得性凝血缺乏或缺陷例如血友病 A 和 B 的治疗方法。

[0010] 还提供的是通过给有此需要的患者施用抗 ATβH 单克隆抗体,用于缩短出血时间的方法。还提供的是用于产生结合人 ATβH 的单克隆抗体的方法。

[0011] 附图简述

技术人员应当理解下文描述的附图仅用于举例说明性目的。附图不预期以任何方式限制本文教导或权利要求的范围。

[0012] 图 1 显示了与肝素结合的 ATβ 和 ATβ 的各种结合结构域的图示。

[0013] 图 2A-2C 显示了 ATβ 如何通过一个 N 聚糖的缺乏与 ATα 区别。

[0014] 图 3A-3D 显示了 ATβ 比 ATα 与肝素更快速的结合和更有力的抑制。

[0015] 图 4A 显示了生物素化的 hAT 和 rAT 在 Fxa 生成的抑制中起作用(图 4A)。图 4B-4C 显示了通过噬菌体展示的抗体发现的各种策略。

[0016] 图 5 显示了用于鉴定能够功能抑制 ATβ:: 肝素的抗体的筛选方法。

[0017] 图 6A 和 6B 分别显示了抗体 TPP-2009 (分别为 SEQ ID NO:1 和 SEQ ID NO: 2)、TPP-2015 (分别为 SEQ ID NO:3 和 SEQ ID NO:4)、TPP-2016 (分别为 SEQ ID NO:5 和 SEQ ID NO:6)、TPP-2019 (分别为 SEQ ID NO:7 和 SEQ ID NO:8) 和 TPP-2803 (分别为 SEQ ID NO:9 和 SEQ ID NO:10) 的轻链结构域和重链结构域的氨基酸序列比对。

[0018] 图 7A-7C 显示了通过 Biacore (图 7A)和 ELISA (图 7B)测试测定的抗体结合特异性,以及与人 AtβH 的抗体结合亲和力(图 7C)。

[0019] 图 8A 是 TPP 抗体对人 HEM-A 血浆中的凝血酶生成的作用的图解表示,并且举例说

明抗体存在增加人 HEM-A 血浆中的峰凝血酶生成。

[0020] 图 8B 是显示抗体缩短人 Hem-A 血浆以及掺入 At β 或 At α 的人 AT 缺乏血浆中的凝血时间的表。

[0021] 图 9 是在 HEM-A 小鼠中的抗体 TPP 2009 的 PK 的图解表示，使用以 0.3、3 和 30mg/kg 的 IV 给药，三只小鼠 / 时间点(经过 21 天 10 个时间点)，以及相关 PK 参数。

[0022] 图 10A 和 10B 显示了关于 HemA 中的尾静脉横切(TVT)模型的实验方案，以及 HemA 小鼠中的 TVT 模型中的抗体 TPP-2009 的功效。图 10B 显示了抗体 TPP-2009 在 HemA 小鼠的尾静脉横切(TVT)模型中具有有力功效。

[0023] 图 11A 和 11B 显示了用 / 不用肝素复合的天然 AT β (图 11A)、以及与肝素结合的完全活化的抗体 TPP2009 (图 11B)及其预测的表位结构的三维结构的分子模型。螺旋 D 在肝素结合 B 后延伸。

[0024] 图 12 显示了使用 FXa 活化凝血测定，TPP2803 显示出在正常人血浆和血友病患者血浆中的凝血时间的剂量依赖性缩短。CT :凝血时间，HEM-A :血友病 A 血浆。

[0025] 图 13 显示了肝素化兔出血模型的实验设计 ;实验组 :媒介物，PBS ;阳性对照，硫酸鱼精蛋白(28mg/kg IV);阴性对照，M14 IgG2 ;处理 :30mg/kg ;TPP2803，3mg/kg ;TPP2803，30mg/kg。

[0026] 图 14. 显示了在肝素化兔出血模型中，在 LMWH 和化合物施用前和后，对照和 TPP2803 对出血时间的作用。

[0027] 图 15. 显示了对照和 TPP2803 对 δ 出血时间的作用显著不同于 PBS ($p \leq 0.05$; T 检验)。

[0028] 图 16. 显示了在 LMWH 和抗体施用前和后，对照和 TPP2803 对失血的作用。(显著性通过 T 检验)。

[0029] 详述

本公开内容提供了抗体包括单克隆抗体及其他结合蛋白，其与活化形式的 AT β 特异性结合，但显示出针对幼稚的(naïve)或活化的 AT α 形式相当少的反应性或无反应性，。

[0030] *定义*

为了解释本说明书的目的，应用下述定义。在下文阐述的任何定义与该单词在任何其他文件，包括引入本文作为参考的任何文件中的使用冲突的情况下，下文阐述的定义应始终为准，用于解释本说明书及其相关权利要求的目的，除非明确意图相反含义(例如在其中该术语最初使用的文件中)。

[0031] 适当时，以单数使用的术语还包括复数，并且反之亦然。“一个 / 种”在本文中的使用意指“一个或多个 / 一种或多种”，除非另有说明或当“一个或多个 / 一种或多种”的使用明确不适当时。“或”的使用意指“和 / 或”，除非另有说明。“包含(comprise)”、“包含(comprises)”、“包含(comprising)”、“包括(include)”、“包括(includes)”和“包括(including)”的使用是可互换的，并且是非限制性的。术语“例如(such as)”、“例如(for example)”和“例如(e.g.)”也不预期是限制性的。例如，术语“包括”应意指“包括但不限于”。

[0032] 如本文使用的，术语“约”指提供的单位值的 +/- 10%。如本文使用的，术语“基本上”指显示出目的特征或特性的总计或近似程度的定性条件。生物学领域的普通技术人员

应当理解生物学和化学现象很少(如果有的话)达到或避免决定结果,由于影响生物学和化学组合物和材料的测试、生产和贮存的许多变量,以及由于在生物学和化学组合物和材料的测试、生产和贮存中使用的仪器和设备中的固有误差。术语基本上因此在本文中用于捕获在许多生物学和化学现象中固有的完全性的潜在缺乏。

[0033] 如本文使用的,术语“ATβ”或“ATβH”指AT的任何变体、同种型和/或物种同系物,其形式由细胞天然表达并且存在于血浆中,并且不同于ATα。进一步地,如本文使用的,术语“ATβ”或“ATβH”还可以指与肝素或肝素样结构复合的ATβ的活化形式。

[0034] 如本文使用的,术语“抗体”指完整抗体及其任何抗原结合片段(即,“抗原结合部分”)或其单链。该术语包括全长免疫球蛋白分子(例如IgG抗体),其是天然存在的或通过正常免疫球蛋白基因片段重组过程形成的,或免疫球蛋白分子的免疫活性部分,例如抗体片段,其保留特异性结合活性。与结构无关,抗体片段结合由全长抗体识别的相同抗原。例如,抗ATβH单克隆抗体片段与ATβH的表位结合。抗体的抗原结合功能可以通过全长抗体的片段执行。在术语抗体的“抗原结合部分”内涵盖的结合片段的例子包括:(i)Fab片段,由VL、VH、CL和CH1结构域组成的单价片段;(ii)F(ab')2片段,包含在铰链区通过二硫桥连接的两个Fab片段的二价片段;(iii)由VH和CH1结构域组成的Fd片段;(iv)由抗体单臂的VL和VH结构域组成的Fv片段,(v)由V_H结构域组成的dAb片段(Ward等人,(1989)Nature 341:544-546);(vi)经分离的互补决定区(CDR);(vii)微型抗体、双抗体、三抗体、四抗体和κ体(参见例如Ill等人,Protein Eng 1997;10:949-57);(viii)骆驼IgG;和(ix)IgNAR。此外,尽管Fv片段的两个结构域V_L和V_H由分开的基因编码,但它们可以使用重组法通过合成接头进行连接,所述合成接头使得它们能够制备为单条蛋白质链,其中V_L和V_H区配对以形成单价分子(称为单链Fv(scFv);参见例如,Bird等人(1988)Science 242:423-426;和Huston等人(1988)Proc. Natl. Acad. Sci. USA 85:5879-5883)。此类单链抗体也涵盖在术语抗体的“抗原结合部分”内。这些抗体片段使用本领域技术人员已知的常规技术获得,并且以与完整抗体相同的方式分析片段的效用。

[0035] 此外,考虑抗原结合片段可以涵盖在抗体模拟物中。如本文使用的,术语“抗体模拟物”或“模拟物”指这样的蛋白质,其显示出与特定抗体相似的结合活性,但为更小的替代抗体或非抗体蛋白质。此类抗体模拟物可以包含在支架中。术语“支架”指用于改造具有定制功能和特征的新产物的多肽平台。

[0036] 如本文使用的,术语“抗ATβ抗体”指特异性结合与肝素或肝素样相关的ATβ的表位的抗体。当在体内与ATβH的表位结合时,本文公开的抗ATβ抗体增强凝血级联的一个或多个方面。

[0037] 如本文使用的,术语“抑制结合”和“阻断结合”(例如(提及ATβ底物与ATβH的结合的抑制/阻断)可互换使用,并且涵盖蛋白质与其底物的部分和完全抑制或阻断两者,例如至少约10%、约20%、约30%、约40%、约50%、约60%、约70%、约80%、约90%、约95%、约96%、约97%、约98%、约99%、或约100%的抑制或阻断。

[0038] 提及ATβ底物与ATβ的结合的抑制和/或阻断,术语抑制和阻断还包括与不和抗ATβ抗体接触的ATβ相比较,当与抗ATβ抗体接触时,ATβ和/或ATβH与生理学底物的结合亲和力中的任何可测量减少,例如ATβ与其底物的相互作用至少约10%、约20%、约30%、约40%、约50%、约60%、约70%、约80%、约90%、约95%、约96%、约97%、约98%、约99%、

或约 100% 的阻断。

[0039] 如本文使用的，术语“单克隆抗体”或“单克隆抗体组合物”指单分子组合物的抗体分子。单克隆抗体组合物展示关于特定表位的单一结合特异性和亲和力。相应地，如本文使用的，术语“人单克隆抗体”指展示单一结合特异性的抗体，其具有衍生自人种系免疫球蛋白序列的可变区和恒定区。人抗体可以包括不由人种系免疫球蛋白序列编码的氨基酸残基(例如，通过体外随机或位点特异性诱变或者通过体内体细胞突变引入的突变)。

[0040] 如本文使用的，术语“经分离的抗体”意指基本上不含其他生物学分子，包括具有不同抗原特异性的抗体的抗体(例如与 ATβH 结合的经分离的抗体基本上不含结合除 ATβH 外的抗原的抗体)。在一些实施方案中，经分离的抗体为按干重计至少约 75%、约 80%、约 90%、约 95%、约 97%、约 99%、约 99.9% 或约 100% 纯的。在一些实施方案中，纯度可以通过诸如柱层析、聚丙烯酰胺凝胶电泳或 HPLC 分析的方法进行测量。然而，与人 ATβH 的表位、同种型或变体结合的经分离的抗体可以与例如来自其他物种的其他相关抗原(例如 ATβH 物种同系物)具有交叉反应性。此外，经分离的抗体可以基本上不含其他细胞材料和 / 或化学品。

[0041] 如本文使用的，术语“特异性结合”指与预定抗原结合的抗体。显示出特异性结合的抗体通常以至少约 $10^5 M^{-1}$ 的亲和力与抗原结合，并且与该抗原结合的亲和力比其对于无关抗原(例如 BSA、酪蛋白)的结合亲和力高，例如至少两倍高。短语“识别抗原的抗体”和“对于抗原特异性的抗体”在本文中可与术语“与抗原特异性结合的抗体”互换使用。如本文使用的，术语“最低限度结合”指不与指定抗原结合和 / 或显示出与指定抗原的低亲和力的抗体。通常，与抗原具有最低限度结合的抗体以低于约 $10^2\ M^{-1}$ 的亲和力与该抗原结合，并且不以比它与无关抗原结合更高的亲和力与预定抗原结合。

[0042] 当在本文中用于抗体例如 IgG 抗体时，术语“高亲和力”指至少约 $10^7\ M^{-1}$ 的结合亲和力，在至少一个实施方案中，至少约 $10^8\ M^{-1}$，在一些实施方案中，至少约 $10^9\ M^{-1}$、约 $10^{10}\ M^{-1}$、约 $10^{11}\ M^{-1}$ 或更大，例如最高达约 $10^{13}\ M^{-1}$ 或更大。然而，“高亲和力”结合可以对于其他抗体同种型改变。例如，关于 IgM 同种型的“高亲和力”结合指至少约 $10^7\ M^{-1}$ 的结合亲和力。

[0043] 如本文使用的，术语“同种型”指由重链恒定区基因编码的抗体类别(例如 IgM 或 IgG1)。

[0044] 如本文使用的，术语“互补决定区”或“CDR”指在抗体分子的重链可变区或轻链可变区内的三个高变区之一，其构成与所结合抗原的三维结构互补的 N 末端抗原结合表面。从重链和轻链的 N 末端开始，这些互补决定区分别指定为“CDR1”、“CDR2”和“CDR3” [Wu TT, Kabat EA, Bilofsky H, Proc Natl Acad Sci U S A. 1975 Dec ;72 (12) :5107 和 Wu TT, Kabat EA, J Exp Med. 1970 Aug 1 ;132 (2) :211]。CDR 涉及抗原抗体结合，并且 CDR3 包含对于抗原抗体结合特异性的独特区域。因此，抗原结合位点可以包括六个 CDR，包含来自重链和轻链 V 区各自的 CDR 区。术语“表位”指抗体与之特异性结合或相互作用的区域或区，在一些实施方案中，所述区域或区指示抗原在其中与抗体处于物理接触。相反，术语“互补位”指抗原在其上特异性结合的抗体区域或区。如果相应抗体的结合是相互排斥的，即一种抗体的结合排除另一种抗体的同时结合，则通过竞争结合表征的表位被说成重叠的。如果抗原能够同时容纳两种相应抗体的结合，则表位被说成是分开的(独特的)。

[0045] 如本文使用的，术语“竞争抗体”指与如本文描述的针对 ATβH 的抗体结合大约相同、基本上相同、基本相同或甚至相同表位的抗体。竞争抗体包括具有重叠表位特异性的抗体。竞争抗体因此能够与如本文描述的抗体有效竞争结合 ATβH。在一些实施方案中，竞争抗体可以与本文描述的抗体结合相同的表位。换言之，竞争抗体具有与本文描述的抗体相同的表位特异性。

[0046] 如本文使用的，术语“保守置换”指涉及一个或多个氨基酸置换具有相似生物化学特性的氨基酸的多肽修饰，其不导致多肽的生物学或生物化学功能的丧失。保守氨基酸置换是其中氨基酸残基替换为具有相似侧链的氨基酸残基的置换。具有相似侧链的氨基酸残基家族已在本领域中得到限定。这些家族包括具有碱性侧链的氨基酸(例如赖氨酸、精氨酸、组氨酸)、具有酸性侧链的氨基酸(例如天冬氨酸、谷氨酸)、具有不带电荷的极性侧链的氨基酸(例如甘氨酸、天冬酰胺、谷氨酰胺、丝氨酸、苏氨酸、酪氨酸、半胱氨酸)、具有非极性侧链的氨基酸(例如丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、甲硫氨酸、色氨酸)、具有 β-分支侧链的氨基酸(例如苏氨酸、缬氨酸、异亮氨酸)和芳族侧链的氨基酸(例如酪氨酸、苯丙氨酸、色氨酸、组氨酸)。本公开内容的抗体可以具有一个或多个保守氨基酸置换，仍保留抗原结合活性。

[0047] 对于核酸和多肽，如本文使用的，术语“基本同源性”指示，在至少约 80% 的核苷酸或氨基酸中，通常为至少约 85%，在一些实施方案中，约 90%、约 91%、约 92%、约 93%、约 94% 或约 95%，在至少一个实施方案中，至少约 96%、约 97%、约 98%、约 99%、约 99.1%、约 99.2%、约 99.3%、约 99.4% 或约 99.5% 的核苷酸或氨基酸中，当最佳比对且比较时，两种核酸或两种多肽或其指定序列是相同的，伴随适当的核苷酸或氨基酸插入或缺失。可替代地，当区段在选择性杂交条件下与链的互补体杂交时，存在关于核酸的基本同源性。还包括的是与本文所述的特异性核酸序列和氨基酸序列具有基本同源性的核酸序列和多肽序列。两个序列之间的同一性百分比是由序列共享的相同位置数目的函数(即同源性 % = 相同位置 #/ 位置总 # x 100)，考虑到为了两个序列的最佳比对需要引入的缺口数目和每个缺口的长度。序列的比较和两个序列之间的同一性百分比的测定可以使用数学算法来完成，所述数学算法例如但不限于 VectorNTI™(Invitrogen Corp.，Carlsbad，CA)的 AlignX™模块。对于 AlignX™，多重比对的缺省参数是：缺口开放罚分：10；缺口延伸罚分：0.05；缺口分开罚分范围：8；比对延迟的同一性 %：40。

[0048] 两个序列之间的同一性百分比是由序列共享的相同位置数目的函数(即同源性 % = 相同位置 #/ 位置总 # x 100)，考虑到为了两个序列的最佳比对需要引入的缺口数目和每个缺口的长度。序列的比较和两个序列之间的同一性百分比的测定可以使用数学算法来完成，所述数学算法例如但不限于 VectorNTI™(Invitrogen Corp.，Carlsbad，CA)的 AlignX™模块。对于 AlignX™，多重比对的缺省参数是：缺口开放罚分：10；缺口延伸罚分：0.05；缺口分开罚分范围：8；比对延迟的同一性 %：40。(进一步的细节在 http://www.invitrogen.com/site/us/en/home/LINNEA-Online-Guides/LINNEACommunities/Vector-NTI-Community/Sequence-analysis-and-data-management-software-for- PCs/AlignX-Module-for-Vector-NTI-Advance.reg.us.html 处发现)。

[0049] 用于测定查询序列(本公开内容的序列)和主题序列之间的总体匹配的另一种方法，也称为总体序列比对，可以使用 CLUSTALW 计算机程序(Thompson 等人，Nucleic Acids

Research,1994,2（22）: 4673-4680）进行测定，所述 CLUSTALW 计算机程序基于 Higgins 等人，Computer Applications in the Biosciences（CABIOS),1992,8（2）: 189-191 的算法。在序列比对中，查询和主题序列均为 DNA 序列。所述总体序列比对的结果以同一性百分比表示。可以在 DNA 序列的 CLUSTALW 比对中用于经由配对比对计算同一性百分比的参数是：矩阵 = IUB，k- 元组 = 1，顶部对角线数 = 5，缺口罚分 = 3，缺口开放罚分 = 10，缺口延伸罚分 = 0.1。对于多重比对，可以使用下述 CLUSTALW 参数：缺口开放罚分 = 10，缺口延伸参数 = 0.05；缺口分开罚分范围 = 8；比对延迟的同一性 %：40。

[0050] 核酸可以存在于全细胞、细胞裂解产物中、或以部分纯化或基本上纯的形式存在。当从它在天然环境中通常与之结合的其他细胞组分中纯化出来时，核酸是“经分离的”或“致使基本上纯的”。为了分离核酸，可以使用标准技术例如下述：碱 / SDS 处理、CsCl 显带、柱层析、琼脂糖凝胶电泳及其他本领域众所周知的其他技术。

[0051] *针对 ATβH的单克隆抗体*

其中稳态在血友病或其中伤口导致暂时性止血丧失的创伤患者中失调的出血障碍可以通过 AT 抑制剂进行治疗。抗体、其抗原结合片段及其他 AT 特异性蛋白质支架可以用于提供靶向特异性，以抑制 AT 蛋白质功能子集，同时保存剩余部分。考虑到 ATβ 的血浆浓度（<12 ug/ml）相对于 ATα （120 ug/ml）中的至少 10 倍差异，在高度循环过量 ATα 的存在下，任何潜在 ATβ 抑制剂治疗剂的特异性增加帮助阻断 ATβ 功能。阻断 ATβ 的抗凝功能的 ATβ 特异性抗体可以用作患有出血障碍的患者的治疗剂。出血障碍的例子包括血友病、具有抑制剂的血友病患者、创伤诱导的凝血病、在通过 AT 的败血症治疗过程中严重出血的患者、起因于选择性手术（例如移植、心脏手术、矫形外科手术）的出血、和由于月经过多的过量出血。具有长循环半衰期的抗 ATβH 抗体可以用于治疗慢性疾病如血友病。具有更短半衰期的 ATβH 抗体片段或 ATβH 结合蛋白支架可以对于急性用途（例如创伤中的治疗用途）更有效。ATβH 结合抗体通过淘选且筛选针对与肝素复合的人 ATβ 的人抗体文库进行鉴定。所鉴定的抗体显示出与人 ATβH 的结合。将经分离的每种单克隆抗体的重链可变区和轻链可变区测序，并且鉴定其 CDR 区。对应于 ATβH 特异性单克隆抗体的重链和轻链可变区的序列标识号（“SEQ ID NO”）概括于表 1A 中。

[0052] 表 1A - 人抗 ATβH（肝素复合的 ATβ）抗体

克隆	轻链可变区	SEQ ID	重链可变区	SEQ ID
TPP2009	AQSVLTQDPAVSVALGQTVRIT CQGDSLRSYYASWYQQKPGQ APVLVIYGKNNRPSGIPDRFSGS SSGNTASLTITGAQAEDEADYY CNSRDSSGNHLVFGGGTKLTV LGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAW KADGSPVKAGVETTKPSKQSN NKYAASSYLSLTPEQWKSHRS YSCQVTHEGSTVEKTVAPAECS	No.1	EVQLLESGGGLVQPG GSLRLSCAASGFTFS AYRMGWVRQAPGK GLEWVSRIYSSGGRT RYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGSFSEALDYWG QGTLVTVSS	No.2
TPP2015	AQDIQMTQSPGTLSLSPGERAT LSCRASQSVSSSYLAWYQQKP GQAPRLLIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVY YCQQYGSSRTFGQGTKVEIRRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACE VTH QGLSSPVTKS FNRGEC	No.3	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYKMDWVRQAPGK GLEWVSRIGPSGGKT MYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGTYSEALDYW GQGTLVTVSS	No.4
TPP2016	AQDIQMTQSPATLSVSPGERAT LSCRASQNINRNLAWYQQKPG RAPRLLIHTASTRAPGVPVRITG SGSGTEFTLTISSLEPEDFAVYF CQQYASPPRTFGQGTKVEIKRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYS LSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC	No.5	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYRMDWVRQAPGK GLEWVSRIGPSGGKT TYADSVKGRFTISRD NSKNTLYLQMNSLR AEDTAVYYCAREKT SDLSGSYSEALDYW GQGTL VTVSS	No.6

克隆	轻链可变区	SEQ ID	重链可变区	SEQ ID
TPP2019	AQDIQMTQSPATLSLSPGERAT LSCRASQRVSSSYLTWYQQKP GQAPRLLIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVY YCQQYDSTPPLTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDST YSLS STLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC	No.7	EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYAMYWVRQA PGKGLEWVSR ISPSGGKTHY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARLS QTGYYPHYHY YGMDVWGQGT TVTVSS	No.8
TPP2803	SSELTQDPAVSVALGQTVRITC QGDSLRSYYASWYQQKPGQAP VLVIYGKNNRPSGIPDRFSGSSS GNTASLTITGAQAEDEADYYC NSRDSSGNHLVFGGGTKLTVL GQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSNN KYAASSYLSLTPEQWKSHRSYS CQVTHEGSTVEKTVAPAECS	No.9	EVQLLESGGGLVQPG GSLRLSCAASGFTFSS YRMSWVRQAPGKGL EWVSRIYSSGGRTRY ADSVKGRFTISRDNS KNTLYLQMNSLRAE DTAVYYCAREKASD LSGSFSEALDYWGQ GTLVTVSS	No.10

[0053] 在至少一些可能的实施方案中，经分离的单克隆抗体与人 ATβH 结合，并且抑制抗凝活性，其中所述抗体包含含有选自 SEQ ID NO: 2、4、6、8 和 10 的氨基酸序列的重链可变区。

[0054] 在至少一些可能的实施方案中，经分离的单克隆抗体与人 ATβH 结合，并且抑制抗凝活性，其中所述抗体包含含有选自 SEQ ID NO: 1、3、5、7 和 9 的氨基酸序列的轻链可变区。

[0055] 在至少一些可能的实施方案中，经分离的单克隆抗体与人 ATβH 结合，并且抑制抗凝活性，其中所述抗体包含含有选自 SEQ ID NO: 2、4、6、8 和 10 的氨基酸序列的重链可变区，以及含有选自 SEQ ID NO: 1、3、5、7 和 9 的氨基酸序列的轻链可变区。

[0056] 在至少一些可能的实施方案中，抗体包含重链和轻链可变区，其包含：

(a)包含 SEQ ID NO: 2 的氨基酸序列的重链可变区，和包含 SEQ ID NO: 1 的氨基酸序列的轻链可变区；

(b)包含 SEQ ID NO: 4 的氨基酸序列的重链可变区，和包含 SEQ ID NO: 3 的氨基酸序列的轻链可变区；

(c)包含 SEQ ID NO: 6 的氨基酸序列的重链可变区，和包含 SEQ ID NO: 5 的氨基酸序列的轻链可变区；或

(d)包含 SEQ ID NO: 8 的氨基酸序列的重链可变区，和包含 SEQ ID NO: 7 的氨基酸序列的轻链可变区；或

(e)包含 SEQ ID NO: 10 的氨基酸序列的重链可变区，和包含 SEQ ID NO: 9 的氨基酸序列的轻链可变区。

[0057] 表 1B 显示了关于人源化 IgG mAb 的重链和轻链氨基酸序列。

[0058] 表 1B – 关于人源化 IgG mAb 的重链和轻链氨基酸序列。

TPP2009 |hIgG| 轻链 AQSVLTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGG TKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD GSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTV EKTVAPAECS SEQ ID NO: 51
TPP2009| hIgG| 重链 EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYRMGWVRQAPGKGLEWVSRI YSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKAS DLSGSFSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 52
TPP-2015	hIgG|轻链 AQDIQMTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSRTFGQGTK VEIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC SEQ ID NO: 53

TPP-2015[hIgG] 重链 EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYKMDWVRQAPGKGLEWVSR IGPSGGKTMYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKA SDLSGTYSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSG SEQ ID NO: 54
TPP-2016[hIgG] 轻链，κ AQDIQMTQSPATLSVSPGERATLSCRASQNINRNLAWYQQKPGRAPRLLIHT ASTRAPGVPVRITGSGSGTEFTLTISSLEPEDFAVYFCQQYASPPRTFGQGTK VEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC SEQ ID NO: 55
TPP-2016[hIgG] 重链 EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYRMDWVRQAPGKGLEWVSRI GPSGGKTTYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKTS DLSGSYSEALDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO:56

TPP-2019|hIgG|轻链，κ AQDIQMTQSPATLSLSPGERATLSCRASQRVSSSYLTWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDSTPPLTFGGG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC SEQ ID NO: 57
TPP-2019|hIgG| 重链 EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYAMYWVRQAPGKGLEWVSRI SPSGGKTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLSQT GYYPHYIHYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 58

[0059] 表 1C - 显示了 TPP2803 IgG2,种系的且转换为 IgG2。表 1C 中所示的 TPP2803 IgG2 轻链 G2，λ，氨基酸序列是 SEQ ID NO:59,并且表 1C 中所示的 TPP2803 重链氨基酸序列是 SEQ ID NO: 60。

[0060] 表 1C - TPP2803 IgG2,种系的且转换为 IgG2

>TPP-2803|hIgG2 |轻链，λ SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKN NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGGT KLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGS PVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK TVAPAECS SEQ ID NO: 59

>TPP-2803|hIgG2 | 重链

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYRMSWVRQAPGKGLEWVSRI
YSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREKAS
DLSGSFSEALDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTY
TCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 60

[0061] 表 2A 提供了关于与人 ATβH 结合的单克隆抗体的重链和轻链的 CDR 区(“CDR1”、“CDR2”和“CDR3”)的 SEQ ID NO: 概括。

[0062] 表 2A – 关于人抗 ATβH 抗体的 CDR 区的序列标识符

克隆	轻链可变区			重链可变区		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
TPP2009	21	26	31	36	41	46
TPP2015	22	27	32	37	42	47
TPP2016	23	28	33	38	43	48
TPP2019	24	29	34	39	44	49
TPP2803	25	30	35	40	45	50

[0063] 表 2B 提供了关于与人 ATβH 结合的单克隆抗体的重链和轻链的 CDR 区(“CDR1”、“CDR2”和“CDR3”)的 SEQ ID NO: 序列。

[0064] 表 2B – 关于人抗 ATβH 抗体的 CDR 区的序列

克隆CDR	序列标识符	氨基酸序列
TPP2009 LCDR1	SEQ ID NO: 21	QGDSLRSYYAS
TPP2015 LCDR1	SEQ ID NO: 22	RASQSVSSSYLA
TPP2016 LCDR1	SEQ ID NO: 23	RASQNINRNLA
TPP2019 LCDR1	SEQ ID NO: 24	RASQRVSSSYLT
TPP2803 LCDR1	SEQ ID NO: 25	QGDSLRSYYAS
TPP2009 LCDR2	SEQ ID NO: 26	GKNNRPS
TPP2015 LCDR2	SEQ ID NO: 27	GASSRAT
TPP2016 LCDR2	SEQ ID NO: 28	TASTRAP
TPP2019 LCDR2	SEQ ID NO: 29	GASSRAT
TPP2803 LCDR2	SEQ ID NO: 30	GKNNRPS
TPP2009 LCDR3	SEQ ID NO: 31	NSRDSSGNHLV
TPP2015 LCDR3	SEQ ID NO: 32	QQYGSSRT
TPP2016 LCDR3	SEQ ID NO: 33	QQYASPPRT
TPP2019 LCDR3	SEQ ID NO: 34	QQYDSTPPLT
TPP2803 LCDR3	SEQ ID NO: 35	NSRDSSGNHLV
TPP2009 HCDR1	SEQ ID NO: 36	AYRMG
TPP2015 HCDR1	SEQ ID NO: 37	KYKMD
TPP2016 HCDR1	SEQ ID NO: 38	KYRMD
TPP2019 HCDR1	SEQ ID NO: 39	RYAMY
TPP2803 HCDR1	SEQ ID NO: 40	SYRMS
TPP2009 HCDR2	SEQ ID NO: 41	RIYSSGGRTRYADSVKG
TPP2015 HCDR2	SEQ ID NO: 42	RIGPSGGKTM YADSVKG
TPP2016 HCDR2	SEQ ID NO: 43	RIGPSGGKTT YADSVKG
TPP2019 HCDR2	SEQ ID NO: 44	RISPSGGKTH YADSVKG
TPP2803 HCDR2	SEQ ID NO: 45	RIYSSGGRTR YADSVKG
TPP2009 HCDR3	SEQ ID NO: 46	AREKASDLSGSFSEALDY
TPP2015 HCDR3	SEQ ID NO: 47	AREKASDLSG TYSEALDY
TPP2016 HCDR3	SEQ ID NO: 48	AREKTSDLSG SYSEALDY
TPP2019 HCDR3	SEQ ID NO: 49	ARLSQTGYYP HYHYYGMDV
TPP2803 HCDR3	SEQ ID NO: 50	AREKASDLSG SFSEALDY

[0065] 在至少一些可能的实施方案中，提供了与人ATβH结合的经分离的单克隆抗体，其中所述抗体包含含有SEQ ID NO: 46-50中任何一个的氨基酸序列的CDR3。这些CDR3来自在淘选和筛选过程中鉴定的抗体的重链。

[0066] 在一个进一步的实施方案中，该抗体进一步包含：(a)含有选自SEQ ID NO: 36-40的氨基酸序列的CDR1；(b)含有选自SEQ ID NO: 41-45的氨基酸序列的CDR2；或(c)含有选自SEQ ID NO: 36-40的氨基酸序列的CDR1，以及含有选自SEQ ID NO: 41-45的氨基酸序列的CDR2两者。

[0067] 在至少一些可能的实施方案中，抗体共享来自在淘选和筛选过程中鉴定的抗体轻链之一的CDR3。因此，还提供的是经分离的单克隆抗体，其中所述抗体与ATβH结合且抑制抗凝活性，其中所述抗体包含含有选自SEQ ID NO: 31-35的氨基酸序列的CDR3。在进一步的实施方案中，抗体进一步包含(a)含有选自SEQ ID NO: 21-25的氨基酸序列的CDR1；

(b)含有选自 SEQ ID NO: 26-30 的氨基酸序列的 CDR2 ;或(c)含有选自 SEQ ID NO: 21-25 的氨基酸序列的 CDR1,以及含有选自 SEQ ID NO: 26-30 的氨基酸序列的 CDR2 两者。

[0068] 在至少一些可能的实施方案中,抗体包含来自由筛选和淘选鉴定的抗体的重链和轻链的 CDR3。提供的是经分离的单克隆抗体,其中所述抗体与 ATβH 结合且抑制抗凝活性,其中所述抗体包含含有选自 SEQ ID NO: 46-50 的氨基酸序列的 CDR3,以及含有选自 SEQ ID NO: 31-35 的氨基酸序列的 CDR3。在一个进一步的实施方案中,抗体进一步包含(a)含有选自 SEQ ID NO: 36-40 的氨基酸序列的 CDR1 ;(b)含有选自 SEQ ID NO: 41-45 的氨基酸序列的 CDR2 ;(c)含有选自 SEQ ID NO: 21-25 的氨基酸序列的 CDR1 ;和 / 或(d)含有选自 SEQ ID NO: 26-30 的氨基酸序列的 CDR2。

[0069] 在一些实施方案中,抗体包含含有下述的重链和轻链可变区 :

(a)包含含有 SEQ ID NO: 21、26 和 31 的氨基酸序列的轻链可变区,以及包含含有 SEQ ID NO: 36、41 和 46 的氨基酸序列的重链可变区 ;

(b)包含含有 SEQ ID NO: 22、27 和 32 的氨基酸序列的轻链可变区,以及包含含有 SEQ ID NO: 37、42 和 47 的氨基酸序列的重链可变区 ;

(c)包含含有 SEQ ID NO: 23、28 和 33 的氨基酸序列的轻链可变区,以及包含含有 SEQ ID NO: 38、43 和 48 的氨基酸序列的重链可变区 ;

(d)包含含有 SEQ ID NO: 24、29 和 34 的氨基酸序列的轻链可变区,以及包含含有 SEQ ID NO: 39、44 和 49 的氨基酸序列的重链可变区 ;

(e)包含含有 SEQ ID NO: 25、30 和 35 的氨基酸序列的轻链可变区,以及包含含有 SEQ ID NO: 40、45 和 50 的氨基酸序列的重链可变区。

[0070] 还提供的是与 AtβH 结合且抑制抗凝活性的经分离的单克隆抗体,其中所述抗体包含的氨基酸序列与选自 SEQ ID NO: 1-10 中所示的氨基酸序列的氨基酸序列具有至少约 89%、约 90%、约 91%、约 92%、约 93%、约 94%、约 95%、约 96%、约 97%、约 98%、约 99% 或约 99.5% 同一性。

[0071] 抗体可以是物种特异性的,或可以与多个物种交叉反应。在一些实施方案中,抗体可以与人、小鼠、大鼠、兔、豚鼠、猴、猪、狗、猫或其他哺乳动物物种的 ATβH 特异性反应或交叉反应。

[0072] 抗体可以是各种抗体类别中的任一个,例如但不限于 IgG1、IgG2、IgG3、IgG4、IgM、IgA1、IgA2、分泌型 IgA 和 IgD 和 IgE 抗体。

[0073] 在一个实施方案中,提供的是针对人 ATIII 的经分离的全人单克隆抗体。

[0074] *抗 ATβH抗体的优化变体*

在一些实施方案中,抗体可以进行淘选、筛选且优化,例如以增加对 ATβH 的亲和力、进一步减少对 ATα 的任何亲和力、改善对不同物种的交叉反应性、或改善 ATβH 的阻断活性。例如,通过利用 CDR 或与 CDR 紧密接近的氨基酸残基,即与抗体的 CDR 毗邻的约 3 或 4 个残基的位点饱和诱变,可以执行此类优化。

[0075] 还提供的是可以具有针对 ATβH 增加的或高亲和力的单克隆抗体。在一些实施方案中,抗 ATβH 抗体可以具有至少约 $10^{8}M^{-1}$的结合亲和力,在一些其他实施方案中,可以具有至少约 $10^{9}M^{-1}$、约 $10^{10}M^{-1}$、约 $10^{11}M^{-1}$或更大,例如最高达约 $10^{13}M^{-1}$或更大的结合亲和力。

[0076] 在一些实施方案中,可以引入另外的氨基酸修饰,以降低与种系序列的分歧。在其

他实施方案中，可以引入氨基酸修饰，以促进用于大规模生产过程的抗体生产。

[0077] 在一些实施方案中，提供的是与人ATβ 特异性结合的经分离的抗ATβH的单克隆抗体，所述抗体可以包含一个或多个氨基酸修饰。在一些实施方案中，抗体可以包含约1、约2、约3、约4、约5、约6、约7、约8、约9、约10、约11、约12、约13、约14、约15、约16、约17、约18、约19或约20个或更多个修饰。

[0078] *表位*

还提供的是可以与人ATβH的预测表位结合的经分离的单克隆抗体，其中所述表位包含来自人ATβH的一个或多个残基，如图11中所示。

[0079] 在一些实施方案中，表位包含人ATβH的N135位点。在其他实施方案中，位点可以包含人ATβH的RCL环的氨基酸残基序列的一部分。

[0080] 还提供的是可以与本文描述的抗体中的任一种竞争结合人ATβH的抗体。例如，此类竞争抗体可以与上文描述的一个或多个表位结合。

[0081] *核酸、载体和宿主细胞*

还提供的是编码本文描述的单克隆抗体中任一种的经分离的核酸分子。因此，提供的是编码与人ATβH结合的抗体的经分离的核酸分子。表3显示了一些抗ATβH抗体的核苷酸序列。

[0082] 表3 - 抗ATβH抗体的核苷酸序列。

	轻链	重链
TPP2009	GCACAGAGCGTCTTG	GAAGTTCAATTGTTAGAGTCTGGTGG
	ACTCAGGACCCTGCT	CGGTCTTGTTCAGCCTGGTGGTTCTTT
	GTGTCTGTGGCCTTG	ACGTCTTTCTTGCGCTGCTTCCGGATT
	GGACAGACAGTCAG	CACTTTCTCTGCTTACCGTATGGGTTG
	GATCACATGCCAAGG	GGTTCGCCAAGCTCCTGGTAAAGGTT
	AGACAGCCTCAGAA	TGGAGTGGGTTTCTCGTATCTATTCTT
	GCTATTATGCAAGCT	CTGGTGGCCGTACTCGTTATGCTGACT
	GGTACCAGCAGAAG	CCGTTAAAGGTCGCTTCACTATCTCTA
	CCAGGACAGGCCCCT	GAGACAACTCTAAGAATACTCTCTAC
	GTACTTGTCATCTAT	TTGCAGATGAACAGCTTAAGGGCTGA
	GGTAAAAACAACCG	GGACACGGCCGTGTATTACTGTGCGA
	GCCCTCAGGGATCCC	GAGAGAAAGCGTCGGATCTATCGGGG
	AGACCGATTCTCTGG	AGTTTTTCTGAGGCCCTTGACTACTGG
	CTCCAGCTCAGGAAA	GGCCAGGGAACCCTGGTCACCGTCTC
	CACAGCTTCCTTGAC	AAGCGCCTCCACCAAGGGCCCATCGG
	CATCACTGGGGCTCA	TCTTCCCGCTAGCACCCAGCAGCAAG
	GGCGGAAGATGAGG	AGCACCAGCGGCGGAACAGCCGCCCT
	CTGACTATTACTGTA	GGGCTGCCTGGTGAAAGACTACTTCC
	ACTCCCGGGACAGCA	CCGAGCCCGTGACCGTGTCCTGGAAC
	GTGGTAACCATCTGG	TCTGGCGCCCTGACCAGCGGAGTGCA

	轻链	重链
	TATTCGGCGGAGGGA CCAAGCTGACCGTCC TAGGTCAGCCCAAGG CTGCCCCCTCGGTCA CTCTGTTCCCGCCCT CCTCTGAGGAGCTTC AAGCCAACAAGGCC ACACTAGTGTGTCTG ATCAGTGACTTCTAC CCGGGAGCTGTGACA GTGGCCTGGAAGGCA GATGGCAGCCCCGTC AAGGCGGGAGTGGA GACCACCAAACCCTC CAAACAGAGCAACA ACAAGTACGCGGCCA GCAGCTACCTGAGCC TGACGCCCGAGCAGT GGAAGTCCCACAGA AGCTACAGCTGCCAG GTCACGCATGAAGGG AGCACCGTGGAGAA GACAGTGGCCCCTGC AGAATGCTCT (SEQ ID NO: 11)	TACCTTCCCCGCCGTGCTGCAGAGCA GCGGCCTGTACAGCCTGAGCAGCGTG GTGACAGTGCCCAGCAGCAGCCTGGG AACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAGGTG GACAAGAAGGTGGAACCCAAGAGCT GCGACAAGACCCACACCTGTCCCCCC TGCCCTGCCCCTGAACTGCTGGGCGG ACCCAGCGTGTTCCTGTTCCCCCCAAA GCCCAAGGACACCCTGATGATCAGCC GGACCCCCGAAGTGACCTGCGTGGTG GTGGACGTGTCCCACGAGGACCCAGA AGTGAAGTTTAATTGGTACGTGGACG GCGTGGAAGTGCATAACGCCAAGACC AAGCCCAGAGAGGAACAGTACAACA GCACCTACCGGGTGGTGTCCGTGCTG ACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAGTACAAGTGCAAGGTCT CCAACAAGGCCCTGCCTGCCCCCATC GAGAAAACCATCAGCAAGGCCAAGG GCCAGCCCCCGCGAGCCTCAGGTGTAC ACACTGCCCCCCCAGCCGGGATGAGCT GACCAAGAACCAGGTGTCCCTGACCT GTCTGGTGAAAGGCTTCTACCCCAGC GATATCGCCGTGGAATGGGAGAGCAA CGGCCAGCCCGAGAACAATTACAAGA CCACCCCCCCTGTGCTGGACAGCGAC GGCTCATTCTTCCTGTACTCCAAGCTG ACCGTGGACAAGAGCCGGTGGCAGCA GGGCAACGTGTTCAGCTGCAGCGTGA TGCACGAGGCCCTGCACAATCACTAC ACCCAGAAGTCCCTGAGCCTGAGCCC CGGC (SEQ ID NO: 12)
TPP2015	GCACAAGACATCCAG ATGACCCAGTCTCCA GGCACCCTGTCTTTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGAGT GTTAGCAGCAGCTAC TTAGCCTGGTACCAG CAGAAACCTGGCCAG GCTCCCAGGCTCCTC ATCTATGGTGCATCC AGCAGGGCCACTGGC ATCCCAGACAGGTTC	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTCAGCCTGGTGGTTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACAAGATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCCTT CTGGTGGCAAGACTATGTATGCTGAC TCCGTTAAAGGTCGCTTCACTATCTCT AGAGACAACTCTAAGAATACTCTCTA CTTGCAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGAGAGAAAGCGTCGGATCTATCGGG GACTTATTCTGAGGCCCTTGACTACTG

	轻链	重链
	AGTGGCAGTGGGTCT GGGACAGACTTCACT CTCACCATCAGCAGA CGGAGCCTGAAGATT TTGCAGTGTATTACT GTCAGCAGTATGGTA GCTCAACGTTCGGCC AAGGGACCAAGGTG GAAATCAGACGAACT GTGGCTGCAATCTGT CTTCATCTTCCCGCC ATCTGATGAGCAGTT GAAATCTGGAACTGC CTCTGTTGTGTGCCT GCTGAATAACTTCTA TCCCAGAGAGGCCAA AGTACAGTGGAAGGT GGATAACGCCCTCCA ATCGGGTAACTCCCA GGAGAGTGTCACAG AGCAGGACAGCAAG GACAGCACCTACAGC CTCAGCAGCACCCTG ACGCTGAGCAAAGC AGACTACGAGAAAC ACAAAGTCTACGCCT GCGAAGTCACCCATC AGGGCCTGAGCTCGC CCGTCACAAAGAGCT TCAACAGGGGAGAG TGT (SEQ ID NO: 13)	GGGCCAGGGAACCCTGGTCACCGTCT CAAGCGCCTCCACCAAGGGCCCATCG GTCTTCCCGCTAGCACCCAGCAGCAA GAGCACCAGCGGCGGAACAGCCGCCC TGGGCTGCCTGGTGAAAGACTACTTC CCCGAGCCCGTGACCGTGTCCTGGAA CTCTGGCGCCCTGACCAGCGGAGTGC ATACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGT GGTGACAGTGCCCAGCAGCAGCCTGG GAACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGT GGACAAGAAGGTGGAACCCAAGAGC TGCGACAAGACCCACACCTGTCCCCC CTGCCCTGCCCCTGAACTGCTGGGCG GACCCAGCGTGTTCCTGTTCCCCCCAA AGCCCAAGGACACCCTGATGATCAGC CGGACCCCCGAAGTGACCTGCGTGGT GGTGGACGTGTCCCACGAGGACCCAG AAGTGAAGTTTAATTGGTACGTGGAC GGCGTGGAAGTGCATAACGCCAAGAC CAAGCCCAGAGAGGAACAGTACAAC AGCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTC TCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAGCCTCAGGTGTA CACACTGCCCCCCAGCCGGGATGAGC TGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAAGGCTTCTACCCCAG CGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCGAGAACAATTACAAG ACCACCCCCCCTGTGCTGGACAGCGA CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTCAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 14)
TPP2016	GCACAAGACATCCAG ATGACCCAGTCTCCA GCCACCCTGTCTGTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGAAT ATTAATAGAAACTTG	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTCAGCCTGGTGGTTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACCGTATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCCTT CTGGTGGCAAGACTACTTATGCTGAC

	轻链	重链
	GCCTGGTACCAGCAG	TCCGTTAAAGGTCGCTTCACTATCTCT
	AAGCCTGGCCGGGCT	AGAGACAACTCTAAGAATACTCTCTA
	CCCAGACTCCTCATC	CTTGCAGATGAACAGCTTAAGGGCTG
	CATACCGCATCCACT	AGGACACGGCCGTGTATTACTGTGCG
	AGGGCCCCTGGTGTC	AGAGAGAAAACGTCGGATCTATCGGG
	CCAGTCAGGATCACT	GAGTTATTCTGAGGCCCTTGACTACTG
	GGCAGTGGGTCTGGA	GGGCCAGGGAACCCTGGTCACCGTCT
	ACAGAGTTCACTCTC	CAAGCGCCTCCACCAAGGGCCCATCG
	ACCATCAGCAGCCTG	GTCTTCCCGCTAGCACCCAGCAGCAA
	GAACCTGAAGATTTT	GAGCACCAGCGGCGGAACAGCCGCCC
	GCAGTGTATTTCTGT	TGGGCTGCCTGGTGAAAGACTACTTC
	CAGCAGTATGCTAGC	CCCGAGCCCGTGACCGTGTCCTGGAA
	CCACCTCGGACGTTC	CTCTGGCGCCCTGACCAGCGGAGTGC
	GGCCAAGGGACCAA	ATACCTTCCCCGCCGTGCTGCAGAGC
	GGTGGAAATCAAGC	AGCGGCCTGTACAGCCTGAGCAGCGT
	GAACTGTGGCTGCAC	GGTGACAGTGCCCAGCAGCAGCCTGG
	CATCTGTCTTCATCTT	GAACCCAGACCTACATCTGCAACGTG
	CCCGCCATCTGATGA	AACCACAAGCCCAGCAACACCAAGGT
	GCAGTTGAAATCTGG	GGACAAGAAGGTGGAACCCAAGAGC
	AACTGCCTCTGTTGT	TGCGACAAGACCCACACCTGTCCCCC
	GTGCCTGCTGAATAA	CTGCCCTGCCCCTGAACTGCTGGGCG
	CTTCTATCCCAGAGA	GACCCAGCGTGTTCCTGTTCCCCCCAA
	GGCCAAAGTACAGTG	AGCCCAAGGACACCCTGATGATCAGC
	GAAGGTGGATAACG	CGGACCCCCGAAGTGACCTGCGTGGT
	CCCTCCAATCGGGTA	GGTGGACGTGTCCCACGAGGACCCAG
	ACTCCCAGGAGAGTG	AAGTGAAGTTTAATTGGTACGTGGAC
	TCACAGAGCAGGAC	GGCGTGGAAGTGCATAACGCCAAGAC
	AGCAAGGACAGCAC	CAAGCCCAGAGAGGAACAGTACAAC
	CTACAGCCTCAGCAG	AGCACCTACCGGGTGGTGTCCGTGCT
	CACCCTGACGCTGAG	GACCGTGCTGCACCAGGACTGGCTGA
	CAAAGCAGACTACG	ACGGCAAAGAGTACAAGTGCAAGGTC
	AGAAACACAAAGTCT	TCCAACAAGGCCCTGCCTGCCCCCAT
	ACGCCTGCGAAGTCA	CGAGAAAACCATCAGCAAGGCCAAG
	CCCATCAGGGCCTGA	GGCCAGCCCCGCGAGCCTCAGGTGTA
	GCTCGCCCGTCACAA	CACACTGCCCCCCAGCCGGGATGAGC
	AGAGC	TGACCAAGAACCAGGTGTCCCTGACC
	TTCAACAGGGGAGA	TGTCTGGTGAAAGGCTTCTACCCCAG
	GTGT (SEQ ID NO: 15)	CGATATCGCCGTGGAATGGGAGAGCA
		ACGGCCAGCCCGAGAACAATTACAAG
		ACCACCCCCCCTGTGCTGGACAGCGA
		CGGCTCATTCTTCCTGTACTCCAAGCT
		GACCGTGGACAAGAGCCGGTGGCAGC
		AGGGCAACGTGTTCAGCTGCAGCGTG
		ATGCACGAGGCCCTGCACAATCACTA
		CACCCAGAAGTCCCTGAGCCTGAGCC
		CCGGC (SEQ ID NO: 16)

	轻链	重链
TPP2019	GCACAAGACATCCAG	GAAGTTCAATTGTTAGAGTCTGGTGG
	ATGACCCAGTCTCCA	CGGTCTTGTTCAGCCTGGTGGTTCTTT
	GCCACCCTGTCTTTG	ACGTCTTTCTTGCGCTGCTTCCGGATT
	TCTCCAGGGGAAAGA	CACTTTCTCTCGTTACGCTATGTATTG
	GCCACCCTCTCCTGC	GGTTCGCCAAGCTCCTGGTAAAGGTT
	AGGGCCAGTCAGCGT	TGGAGTGGGTTTCTCGTATCTCTCCTT
	GTTAGCAGCAGCTAC	CTGGTGGCAAGACTCATTATGCTGAC
	TTAACCTGGTACCAG	TCCGTTAAAGGTCGCTTCACTATCTCT
	CAGAAACCTGGCCAG	AGAGACAACTCTAAGAATACTCTCTA
	GCTCCCAGGCTCCTC	CTTGCAGATGAACAGCTTAAGGGCTG
	ATCTATGGTGCATCC	AGGACACGGCCGTGTATTACTGTGCG
	AGCAGGGCCACTGGC	AGACTGTCTCAAACTGGTTATTACCCT
	ATCCCAGACAGGTTC	CACTACCACTACTACGGTATGGACGT
	AGTGGCAGTGGGTCT	CTGGGGCCAAGGGACCACGGTCACCG
	GGGACAGACTTCACT	TCTCAAGCGCCTCCACCAAGGGCCCA
	CTCACCATCAGCAGA	TCGGTCTTCCCGCTAGCACCCAGCAG
	CTGGAGCCTGAAGAT	CAAGAGCACCAGCGGCGGAACAGCC
	TTTGCAGTTTATTACT	GCCCTGGGCTGCCTGGTGAAAGACTA
	GTCAGCAGTATGATA	CTTCCCCGAGCCCGTGACCGTGTCCTG
	GTACGCCTCCGCTCA	GAACTCTGGCGCCCTGACCAGCGGAG
	CCTTCGGCGGAGGGA	TGCATACCTTCCCCGCCGTGCTGCAGA
	CCAAGGTGGAGATCA	GCAGCGGCCTGTACAGCCTGAGCAGC
	AACGAACTGTGGCTG	GTGGTGACAGTGCCCAGCAGCAGCCT
	CACCATCTGTCTTCA	GGGAACCCAGACCTACATCTGCAACG
	TCTTCCCGCCATCTG	TGAACCACAAGCCCAGCAACACCAAG
	ATGAGCAGTTGAAAT	GTGGACAAGAAGGTGGAACCCAAGA
	CTGGAACTGCCTCTG	GCTGCGACAAGACCCACACCTGTCCC
	TTGTGTGCCTGCTGA	CCCTGCCCTGCCCCTGAACTGCTGGGC
	ATAACTTCTATCCCA	GGACCCAGCGTGTTCCTGTTCCCCCCA
	GAGAGGCCAAAGTA	AAGCCCAAGGACACCCTGATGATCAG
	CAGTGGAAGGTGGAT	CCGGACCCCCGAAGTGACCTGCGTGG
	AACGCCCTCCAATCG	TGGTGGACGTGTCCCACGAGGACCCA
	GGTAACTCCCAGGAG	GAAGTGAAGTTTAATTGGTACGTGGA
	AGTGTCACAGAGCAG	CGGCGTGGAAGTGCATAACGCCAAGA
	GACAGCAAGGACAG	CCAAGCCCAGAGAGGAACAGTACAAC
	CACCTACAGCCTCAG	AGCACCTACCGGGTGGTGTCCGTGCT
	CAGCACCCTGACGCT	GACCGTGCTGCACCAGGACTGGCTGA
	GAGCAAAGCAGACT	ACGGCAAAGAGTACAAGTGCAAGGTC
	ACGAGAAACACAAA	TCCAACAAGGCCCTGCCTGCCCCCAT
	GTCTACGCCTGCGAA	CGAGAAAACCATCAGCAAGGCCAAG
	GTCACCCATCAGGGC	GGCCAGCCCCGCGAGCCTCAGGTGTA
	CTGAGCTCGCCCGTC	CACACTGCCCCCCAGCCGGGATGAGC
	ACAAAGAGCTTCAAC	TGACCAAGAACCAGGTGTCCCTGACC
	AGGGGAGAGTGT	TGTCTGGTGAAAGGCTTCTACCCCAG
	(SEQ ID NO: 17)	CGATATCGCCGTGGAATGGGAGAGCA
		ACGGCCAGCCCGAGAACAATTACAAG
		ACCACCCCCCCTGTGCTGGACAGCGA

	轻链	重链
		CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTCAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 18)
TPP2803	AGCGAATTGACTCAG GACCCTGCTGTGTCT GTGGCCTTGGGACAG ACAGTCAGGATCACA TGCCAAGGAGACAG CCTCAGAAGCTATTA TGCAAGCTGGTACCA GCAGAAGCCAGGAC AGGCCCCTGTACTTG TCATCTATGGTAAAA ACAACCGGCCCTCAG GGATCCCAGACCGAT TCTCTGGCTCCAGCT CAGGAAACACAGCTT CCTTGACCATCACTG GGGCTCAGGCGGAA GATGAGGCTGACTAT TACTGTAACTCCCGG GACAGCAGTGGTAAC CATCTGGTATTCGGC GGAGGGACCAAGCT GACCGTCCTAGGTCA GCCCAAGGCTGCCCC CTCGGTCACTCTGTT	GAAGTGCAGCTGCTGGAAAGCGGCGG AGGCCTGGTGCAGCCTGGCGGATCTC TGAGACTGAGCTGTGCCGCCAGCGGC TTCACCTTCAGCAGCTACAGAATGAG CTGGGTGCGCCAGGCCCCTGGCAAGG GACTGGAATGGGTGTCCCGGATCTAC AGCAGCGGCGGCAGAACCAGATACGC CGACAGCGTGAAGGGCCGGTTCACCA TCTCCCGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCTGCG GGCCGAGGACACCGCCGTGTACTATT GCGCCAGAGAGAAGGCCAGCGACCTG AGCGGCAGCTTTAGCGAGGCCCTGGA TTATTGGGGCCAGGGCACACTCGTGA CCGTGTCTAGCGCCAGCACAAGGGC CCCAGCGTGTTCCCTCTGGCCCCTTGT AGCAGAAGCACCAGCGAGTCTACAGC CGCCCTGGGCTGCCTCGTGAAGGACT ACTTTCCCGAGCCCGTGACAGTGTCCT GGAACTCTGGCGCCCTGACAAGCGGC GTGCACACCTTTCCAGCCGTGCTGCA GAGCAGCGGCCTGTACTCTCTGAGCA GCGTCGTGACTGTGCCCAGCAGCAAC TTCGGCACCCAGACCTACACCTGTAA

	轻链	重链
	CCCGCCCTCCTCTGA GGAGCTTCAAGCCAA CAAGGCCACACTAGT GTGTCTGATCAGTGA CTTCTACCCGGGAGC TGTGACAGTGGCCTG GAAGGCAGATGGCA GCCCCGTCAAGGCGG GAGTGGAGACCACC AAACCCTCCAAACAG AGCAACAACAAGTA CGCGGCCAGCAGCTA CCTGAGCCTGACGCC CGAGCAGTGGAAGTC CCACAGAAGCTACAG CTGCCAGGTCACGCA TGAAGGGAGCACCGT GGAGAAGACAGTGG CCCCTGCAGAATGCT CT (SEQ ID NO: 19)	CGTGGACCACAAGCCCAGCAACACCA AGGTGGACAAGACCGTGGAACGGAA GTGCTGCGTGGAATGCCCCCCTTGTCC TGCCCCTCCAGTGGCTGGCCCTTCCGT GTTCCTGTTCCCCCCAAAGCCCAAGG ACACCCTGATGATCAGCCGGACCCCG AAGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGACCCCGAGGTGCAGTT CAATTGGTACGTGGACGGCGTGGAAG TGCACAACGCCAAGACCAAGCCCAGA GAGGAACAGTTCAACAGCACCTTCCG GGTGGTGTCCGTGCTGACCGTGGTGC ATCAGGACTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGG CCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGACCAAAGGCCAGCCCCGC GAGCCCCAGGTGTACACACTGCCTCC AAGCCGGGAAGAGATGACCAAGAAC CAGGTGTCCCTGACCTGTCTCGTGAA AGGCTTCTACCCCTCCGATATCGCCGT GGAATGGGAGAGCAACGGCCAGCCC GAGAACAACTACAAGACCACCCCCCC CATGCTGGACAGCGCGGCTCATTCTTC CTGTACAGCAAGCTGACAGTGGACAA GTCCCGGTGGCAGCAGGGCAACGTGT TCAGCTGCAGCGTGATGCACGAAGCC CTGCACAACCACTACACCCAGAAGTC CCTGAGCCTGAGCCCTGGC (SEQ ID NO: 20)

[0083] 在一些实施方案中，经分离的核酸分子编码与ATβH结合且抑制抗凝活性、但与ATα具有最低限度结合的抗体，其中所述抗体包含含有选自SEQ ID NO: 2、4、6、8和10的氨基酸序列的重链可变区。

[0084] 在一些实施方案中，经分离的核酸分子编码与ATβH结合且抑制抗凝活性、但与ATα具有最低限度结合的抗体，其中所述抗体包含含有选自SEQ ID NO: 1、3、5、7和9的氨基酸序列的轻链可变区。

[0085] 在其他实施方案中，经分离的核酸分子编码与ATβ结合且抑制ATβ的抗凝活性的抗体，其中所述抗体包含含有选自SEQ ID NO: 2、4、6、8和10的氨基酸序列的重链可变区，或含有选自SEQ ID NO: 1、3、5、7和9的氨基酸序列的轻链可变区，以及重链可变区或轻链可变区中的一个或多个氨基酸修饰。

[0086] 进一步地，还提供的是包含编码上文描述的单克隆抗体中任一种的经分离的核酸分子的载体，以及包含此类载体的宿主细胞。

[0087] *制备针对ATβH的抗体的方法*

单克隆抗体可以通过在宿主细胞中表达核苷酸序列重组产生，所述核苷酸序列编码根据本发明实施方案之一的单克隆抗体的可变区。借助于表达载体，含有核苷酸序列的核酸可以在适合于生产的宿主细胞中转染且表达。相应地，用于产生与人ATβH结合的单克隆抗体的示例性方法可以包括：(a)将编码单克隆抗体的核酸分子转染到宿主细胞内；(b)培

养宿主细胞，以便在宿主细胞中表达单克隆抗体，和(c)任选分离且纯化所产生的单克隆抗体，其中所述核酸分子包含编码单克隆抗体的核苷酸序列。

[0088] 在一个例子中，为了表达抗体或其抗体片段，将通过标准分子生物学技术获得的编码部分或全长轻链和重链的DNA插入表达载体内，使得基因与转录和翻译控制序列可操作地连接。在该上下文中，术语“可操作地连接的”指抗体基因这样连接到载体内，使得在载体内的转录和翻译控制序列发挥其调节抗体基因的转录和翻译的意图功能。表达载体和表达控制序列选择为与所使用的表达宿主细胞相容。抗体轻链基因和抗体重链基因可以插入分开的载体内，或可替代地，两种基因插入相同表达载体内。抗体基因通过标准方法(例如在抗体基因片段和载体上的互补限制位点的连接，或如果不存在限制位点，则平端连接)插入表达载体内。本文描述的抗体的轻链和重链可变区可以通过下述用于制备任何抗体同种型的全长抗体基因：将其插入已经编码所需同种型的重链恒定区和轻链恒定区的表达载体内，使得VH区段与载体内的一个或多个CH区段可操作地连接，并且VL区段与载体内的CL区段可操作地连接。

[0089] 另外或可替代地，重组表达载体可以编码促进从宿主细胞的抗体链分泌的信号肽。抗体链基因可以克隆到载体内，使得信号肽与抗体链基因的氨基末端框内连接。信号肽可以是免疫球蛋白信号肽或异源信号肽(即来自非免疫球蛋白蛋白质的信号肽)。除抗体链编码基因之外，重组表达载体携带控制宿主细胞中的抗体链基因表达的调节序列。术语“调节序列”包括启动子、增强子及其他表达控制元件(例如多腺苷酸化信号)，其控制抗体链基因的转录或翻译。此类调节序列例如在Goeddel；Gene Expression Technology. Methods in Enzymology 185，Academic Press，San Diego，Calif.（1990）中得到描述。本领域技术人员应当理解表达载体的设计，包括调节序列的选择可以取决于诸如下述的因素：待转化的宿主细胞的选择、所需蛋白质的表达水平等。用于哺乳动物宿主细胞表达的调节序列的例子包括指导在哺乳动物细胞中的高水平的蛋白质表达的病毒元件，例如衍生自巨细胞病毒(CMV)、猴病毒40(SV40)、腺病毒(例如腺病毒主要晚期启动子(AdMLP))和多瘤的启动子和/或增强子。可替代地，可以使用非病毒调节序列，例如遍在蛋白启动子或β-珠蛋白启动子。

[0090] 除抗体链基因和调节序列之外，重组表达载体可以携带另外的序列，例如调节宿主细胞中的载体复制的序列(例如复制起点)和可选标记物基因。可选标记物基因促进载体已引入其内的宿主细胞的选择(参见例如全部为Axel等人的美国专利号4,399,216；4,634,665；和5,179,017)。例如，通常可选标记物基因对载体已引入其内的宿主细胞赋予针对药物(例如G418、潮霉素或氨甲蝶呤)的抗性。可选标记物基因的例子包括二氢叶酸还原酶(DHFR)基因(连同氨甲蝶呤选择/扩增一起在dhfr-宿主细胞中使用)和neo基因(用于G418选择)。

[0091] 对于轻链和重链的表达，通过标准技术将编码重链和轻链的一个或多个表达载体转染到宿主细胞内。各种形式的术语“转染”涵盖通常用于将外源DNA引入原核或真核宿主细胞内的广泛多样的技术，例如电穿孔、磷酸钙沉淀、DEAE-葡聚糖转染等。尽管在理论上能够在原核或真核宿主细胞中表达抗体，但在真核细胞包括哺乳动物宿主细胞中的抗体表达是典型的，因为此类真核细胞且特别是哺乳动物细胞比原核细胞更可能装配且分泌适当折叠且免疫活性的抗体。用于表达重组抗体的哺乳动物宿主细胞的例子包括中国仓鼠卵巢

(CHO 细胞)(包括在 Urlaub 和 Chasin,(1980)Proc. Natl. Acad. Sci. USA 77:4216-4220 中描述的 dhfr- CHO 细胞,与 DHFR 可选标记物一起使用,例如如 R. J. Kaufman 和 P. A. Sharp (1982)Mol. Biol. 159:601-621 中所述)、NSO 骨髓瘤细胞、COS 细胞、HKB11 细胞和 SP2 细胞。当编码抗体基因的重组表达载体引入哺乳动物宿主细胞内时,抗体通过将宿主细胞培养一段时间进行生产,所述时间段足以允许抗体在宿主细胞中表达,或抗体分泌到宿主细胞在其中生长的培养基内。使用标准蛋白质纯化方法,例如超滤、大小排阻层析、离子交换色谱和离心,可以从培养基中回收抗体。

[0092] *部分抗体序列表达完整抗体的用途*

抗体占优势地通过氨基酸残基与靶抗原相互作用,所述氨基酸残基位于六个重链和轻链 CDR 中。为此,在 CDR 内的氨基酸序列在个别抗体之间比 CDR 外的序列更多样化。因为 CDR 序列负责大多数抗体抗原相互作用,所以能够通过构建表达载体来表达模拟特异性天然存在的抗体的特性的重组抗体,所述表达载体包括移植到来自具有不同特性的不同抗体的构架序列上、来自特异性天然存在的抗体的 CDR 序列(参见例如 Riechmann, L. 等人, 1998, Nature 332:323-327 ;Jones, P. 等人 , 1986, Nature 321:522-525 ;和 Queen, C. 等人,1989, Proc. Natl. Acad. Sci. U.S.A. 86:10029-10033)。此类构架序列可以得自公开的 DNA 数据库,其包括种系抗体基因序列。这些种系序列不同于成熟抗体基因序列,因为它们不包括完全装配的可变基因,其通过 B 细胞成熟过程中的 V(D)J 连接形成。不必获得特定抗体的整个 DNA 序列,以便重新制备具有与原始抗体的那些相似的结合特性的完整重组抗体(参见 WO 99/45962)。

[0093] 跨越 CDR 区的部分重链和轻链序列通常对于这个目的是足够的。部分序列用于测定哪个种系可变和连接基因区段促成重组抗体可变基因。种系序列随后用于填充可变区的缺失部分。重链和轻链前导序列在蛋白质成熟过程中被切割,并且不促成最终抗体的特性。为此,相应的种系前导序列用于表达构建体。为了添加缺失序列,克隆的 cDNA 序列可以通过连接或 PCR 扩增与合成寡核苷酸组合。可替代地,整个可变区可以作为一组短的重叠寡核苷酸合成,并且通过 PCR 扩增组合以制备整个合成可变区克隆。该过程具有诸如消除或包括特定限制位点、或者特定密码子优化的优点。重链和轻链转录物的核苷酸序列用于设计合成寡核苷酸的重叠组,以制备具有与天然序列相同的氨基酸编码能力的合成 V 序列。合成重链和轻链序列可以不同于天然序列。例如,重复核苷酸碱基串被中断,以促进寡核苷酸合成和 PCR 扩增 ;并且优化翻译起始位点根据 Kozak 的规则(Kozak,1991,J. Biol. Chem. 266:19867-19870)掺入 ;并且限制位点被改造到翻译起始位点上游或下游。对于重链和轻链可变区,在相应的非编码寡核苷酸的大约中点处,优化的克隆和相应的非克隆链序列被分解成 30-50 个核苷酸部分。对于每条链,寡核苷酸可以装配成重叠双链组,其跨越 150-400 个核苷酸的区段。库随后用作模板以产生 150-400 个核苷酸的 PCR 扩增产物。

[0094] 通常,单个可变区寡核苷酸组将分解成两个库,其分开扩增以生成两个重叠 PCR 产物。这些重叠产物随后通过 PCR 扩增组合,以形成完全可变区。还可希望在 PCR 扩增中包括重链或轻链恒定区的重叠片段,以生成可以容易地克隆到表达载体构建体内的片段。重构的重链和轻链可变区随后与克隆的启动子、翻译起始、恒定区、3' 非翻译、多聚腺苷酸化和转录终止序列组合,以形成表达载体构建体。重链和轻链表达构建体可以组合成单一载体,共转染,系列转染或分开转染到宿主细胞内,所述宿主细胞随后融合以形成表达两条链

的宿主细胞。在另一个方面，人抗ATβH抗体的结构特点用于制备结构上相关的人抗ATβH抗体，其保留与ATβ 结合的功能。例如，单克隆抗体的特异性鉴定的重链和轻链区的一个或多个CDR可以与已知的人构架区和CDR重组组合，以制备另外的重组改造的人抗ATβH抗体。

[0095] *抗ATβH mAb的促凝功效*

抗ATβH mAb的促凝功效使用各种测定进行研究。

[0096] 表4显示了在FXa活化凝血测定中，抗ATβH mAb TPP2009和TPP2803在来自各个动物物种的血浆中的促凝功效。

[0097] 表4 - TPP2009和TPP2803在来自各个动物物种的血浆中的促凝功效。

物种	正常血浆 (EC_{50} nM)		HEM A血浆（以nM表示的 EC_{50}）		HEM A血浆（以nM表示的 EC_{50}）
	2009	2803	2009	2803	2009
FXa-活化凝血测定					dPT
人	10.5	2.4	4.7	2.7	9.0
小鼠	NDR	ND	ND	ND	NDR
大鼠	NDR	ND	ND	ND	ND
兔	25.7	ND	10.5	ND	NDR
小猎犬	NDR	ND	ND	ND	NDR
猕猴（Cyno）	21.5	ND	4.3	ND	13.9

NDR：无剂量应答，ND：未测定，dPT：稀释的凝血酶原时间，HEM-A：血友病A血浆。

[0098] 在FXa活化凝血测定中，抗ATβH mAb TPP2009和TPP2803两者均显示出在人正常血浆和血友病A血浆中的促凝功效。具体地，在FXa活化凝血测定中，TPP2009显示出在人正常血浆和血友病A血浆中的促凝功效，分别具有10.5 nM和4.7 nM的EC_{50}。在FXa活化凝血测定中，TPP2803显示出在人正常血浆和血友病A血浆中的促凝功效，分别具有2.4和2.7 nM的EC_{50}。

[0099] 另外，使用FXa活化凝血测定，抗ATβH mAb TPP2803显示出在正常人血浆和血友病患者血浆两者中的凝血时间的剂量依赖性缩短，如图12所示。CT：凝血时间，HEM-A：血友病A血浆。

[0100] 图13中概述的肝素化兔出血模型用于证实抗ATβH mAb TPP2803的体内促凝功效。在肝素化兔出血模型中，在LMWH和化合物施用前和后，对照和TPP2803对出血时间的作用显示于图14中。与PBS中的LMWH后的出血时间相比较，在LMWH和测试物品（3mg/kg

或 30 mg/kg TPP2803）施用后的出血时间显著降低。如图 15 中所示，在测试物品（3mg/kg 或 30 mg/kg TPP2803）或阳性对照硫酸鱼精蛋白施用后，δ 出血时间显著不同于 PBS（$p \leq 0.05$；* 通过 T 检验的显著性）。图 16 中显示了在 LMWH 和抗体施用前和后，对照和 TPP2803 对失血的作用。TPP2803（3 mg/kg）和阳性对照硫酸鱼精蛋白均显示出在 LMWH 施用后的失血中的显著变化（* 通过 T 检验的显著性）。

[0101] *药物组合物*

还提供的是药物组合物，其包含治疗有效量的抗 ATβH 单克隆抗体和药学可接受的载体。如本文使用的，“药学可接受的载体”指这样的物质，其可以加入活性成分中以帮助配制或稳定制剂，并且不引起对患者的显著不利毒理学效应。此类载体的例子是本领域技术人员众所周知的，并且包括水，糖例如麦芽糖或蔗糖，白蛋白，盐例如氯化钠等。其他载体例如在 E. W. Martin 的 Remington's Pharmaceutical Sciences 中描述。此类组合物将含有治疗有效量的至少一种单克隆抗体。

[0102] 药学可接受的载体包括无菌水溶液或分散体，以及用于临时制备无菌注射溶液或分散体的无菌粉末。此类介质和试剂用于药学活性物质的用途是本领域已知的。组合物在一些实施方案中配制用于肠胃外注射。组合物可以配制为溶液、微乳剂、脂质体或适合于高药物浓度的其他有序结构。载体可以是含有例如水、乙醇、多元醇（例如甘油、丙二醇和液体聚乙二醇等）及其合适混合物的溶剂或分散介质。在一些情况下，载体的组合物包括等渗剂例如糖，多元醇如甘露醇、山梨糖醇或氯化钠。

[0103] 无菌可注射溶液可以通过下述进行制备：需要时，将活性化合物以所需量连同上文列举的成分之一或组合一起掺入适当溶剂中，随后为无菌微量过滤。一般地，分散体通过将活性化合物掺入无菌媒介物内进行制备，所述无菌媒介物含有基本分散介质和来自上文列举那些的所需其他成分。在用于制备无菌可注射溶液的无菌粉末的情况下，一些制备方法是真空干燥和冷冻干燥（冻干），其获得来自其先前无菌过滤溶液的活性成分加上任何另外的所需成分的粉末。

[0104] *药学用途*

单克隆抗体可以用于治疗目的，用于治疗遗传性和获得性凝血缺乏或缺陷。例如，上文描述的实施方案中的单克隆抗体可以用于阻断 ATβH 与其底物的相互作用，所述底物可以包括因子 Xa 或因子 IIa。单克隆抗体具有在止血障碍的治疗中的治疗用途，所述止血障碍例如血小板减少症、血小板障碍和出血障碍（例如血友病 A、血友病 B 和血友病 C）。此类障碍可以通过给有此需要的患者施用治疗有效量的抗 ATβH 单克隆抗体进行治疗。单克隆抗体还在适应症例如创伤和出血性中风中不受控制的出血治疗中具有治疗用途。因此，还提供的是用于缩短出血时间的方法，其包括给有此需要的患者施用治疗有效量的抗 ATβH 单克隆抗体。

[0105] 在另一个实施方案中，抗 ATβH 抗体可以用作 AT 治疗的患者的解毒剂，包括例如其中 AT 用于治疗败血症或出血障碍。

[0106] 抗体可以用作单一疗法或与其他疗法组合以解决止血障碍。例如，一种或多种抗体与凝血因子例如因子 VIIa、因子 VIII 或因子 IX 的共同施用被认为对于治疗血友病是有用的。在至少一些实施方案中，用于治疗遗传性和获得性凝血缺乏或缺陷的方法包括施用：（a）第一量的与人组织因子途径抑制剂结合的单克隆抗体；和（b）第二量的因子 VIII 或因

子 IX，其中所述第一和第二量一起有效用于治疗所述缺乏或缺陷。在至少一些实施方案中，用于治疗遗传性和获得性凝血缺乏或缺陷的方法包括施用 :(a) 第一量的与人组织因子途径抑制剂结合的单克隆抗体 ;和 (b) 第二量的因子 VIII 或因子 IX，其中所述第一和第二量一起有效用于治疗所述缺乏或缺陷，并且进一步地其中不共同施用因子 VII。还提供的是包含治疗有效量的单克隆抗体和因子 VIII 或因子 IX 的组合的药物组合物，其中所述组合物不含因子 VII。“因子 VII”包括因子 VII 和因子 VIIa。这些组合疗法可能降低凝血因子的必需输注频率。共同施用或组合疗法意指两种治疗药物的施用，所述两种治疗药物各自分开配制或在一种组合物中配制在一起，并且当分开配制时，在大约相同时间或不同时间但在相同治疗期施用。

[0107] 在一些实施方案中，本文描述的一种或多种抗体可以组合使用，以解决止血障碍。例如，本文描述的抗体中的两种或更多种的共同施用被认为对于治疗血友病或其他止血障碍是有用的。

[0108] 药物组合物可以肠胃外施用于患有血友病 A 或 B 的对象，其剂量和频率可以随着出血发作的严重性而改变，或在预防疗法的情况下，可以随着患者的凝血缺乏的严重性而改变。

[0109] 组合物可以作为推注剂或通过连续输注施用于需要的患者。例如，作为 Fab 片段的抗体的推注施用可以为约 0.0025 至约 100 mg/kg 体重、约 0.025 至约 0.25 mg/kg、约 0.010 至约 0.10 mg/kg 或约 0.10 至约 0.50 mg/kg 的量。对于连续输注，作为 Fab 片段存在的本发明抗体可以以约 0.001 至约 100 mg/kg 体重 / 分钟、约 0.0125 至约 1.25 mg/kg/ 分钟、约 0.010 至约 0.75 mg/kg/ 分钟、约 0.010 至约 1.0 mg/kg/ 分钟、或约 0.10 至约 0.50 mg/kg/ 分钟施用约 1-24 小时、约 1-12 小时、约 2-12 小时、约 6-12 小时、约 2-8 小时、或约 1-2 小时的时期。对于作为全长抗体 (具有完全恒定区) 存在的本发明抗体的施用，剂量可以为约 1-10 mg/kg 体重、约 2-8 mg/kg、或约 5-6 mg/kg。此类全长抗体通常通过从三十分钟延伸到三小时的时期的输注进行施用。施用频率取决于状况的严重性。频率可以为每周三次到每两周至六个月一次的范围。

[0110] 另外，组合物可以经由皮下注射施用于患者。例如，约 10 至约 100 mg 抗 ATβH 抗体的剂量可以经由皮下注射每周一次、每两周一次或每月一次施用于患者。如本文使用的，“治疗有效量”意指抗 ATβH 单克隆抗体或此类抗体和因子 VIII 或因子 IX 的组合的量，其是有效增加体内的凝血时间或以其他方式引起有此需要的患者中的体内可测量利益所需的量。精确量取决于众多因素，包括治疗组合物的组分和物理特征、意图患者群体、个别患者考虑等，并且可以通过本领域技术人员容易地测定。

[0111] 本公开内容的方面可以根据下述实施例进一步得到理解，所述实施例不应解释为以任何方式限制本发明教导的范围。

[0112] 实施例 1 - 人和兔 ATα 和 ATβ 纯化

在 Enzyme Research 实验室 (South Bend，IN)，根据先前描述的方法 (Carlson 和 Atencio 1982 ;Peterson 和 Blackburn 1985)，通过在肝素 - 琼脂糖上的亲和层析从人和兔血浆中纯化 ATα 和 ATβ。简言之，将来自硫酸葡聚糖 / 氯化钙沉淀的上清液应用于肝素 - 琼脂糖亲和柱 (Pharmacia)。用 NaCl 梯度分开 ATα 和 ATβ :ATα 和 ATβ 分别以 0.8 M 和 1.3 M NaCl 进行洗脱。阴离子交换层析 (HiTrap-Q，Pharmacia) 用于 ATβ 的进一步纯

化。ATα 和 ATβ 的纯度和聚糖概况通过蛋白质 SDS-PAGE 和 LC-MS 进行评价。

[0113] 实施例 2 - 通过质谱法分析测定在 ATα 和 ATβ 上的聚糖数目和位置

由于聚糖的不同数目,通过配备 duo-ESI (或 nano ChipCube)来源、MassHunter 采集软件和定量分析软件包括 Bioconfirm 的 Agilent 6520 LC-MS 系统,基于其质量区别 ATα 和 ATβ。通过自底向上(bottom-up)方法测定糖基化位点,在所述方法中蛋白质通过胰蛋白酶和 Arg-c 进行消化,随后为靶 MSMS,以鉴定糖基化和单糖基化肽序列。数据在两个实验中进行收集 :Fragmentor 电压 175v 和 430v。

[0114] 实施例 3 - AT 抗原生物素化

人和兔 ATα 和 ATβ 通过 NHS- 生物素在表面赖氨酸残基上用生物素进行标记。对于赖氨酸生物素化,蛋白质首先脱盐到 PBS/Ca^{++}缓冲液(Life Technologies Corporation, Carlsbad, CA)内,以去除可能对于生物素化反应有抑制性的任何胺。脱盐蛋白质的浓度通过在 NanoDrop 上的 OD280 进行测定。蛋白质随后在室温(RT)下与磺基 -NHS- 生物素(Pierce Thermo Scientific,Rockford,IL)一起温育 1 小时,以 AT:NHS- 生物素的 1:5 和 / 或 1:3 摩尔比(即过量的生物素)。游离生物素通过过夜透析到 PBS/Ca^{++}缓冲液内进行去除。使用生物素定量试剂盒(Pierce Thermo Scientific,Rockford,IL),定量生物素化蛋白质中的生物素量。通过 SDS-PAGE 分析生物素化的 ATα 和 ATβ,并且使用链霉抗生物素蛋白 -HRP (Pierce Thermo Scientific,Rockford,IL)作为探针,通过蛋白质印迹分析证实生物素化。生物素化的 AT 的功能活性通过 FXa 抑制测定进行评估。通过生物素化的 ATα 和 ATβ 与未生物素化的 ATα 和 ATβ 的比较,在生物素化后仅观察到 AT 抑制活性中的轻微降低,指示以这种方式制备的生物素化的 ATβ 和 ATα 将是代表性的,并且可以作为选择性抗凝阻断剂用于 ATβH 结合剂的淘选中。

[0115] 实施例 4 - 通过噬菌体展示和淘选的人单克隆抗体发现

四臂淘选策略设计为从人 Fab 文库(Dyax Fab310)中发现特异性针对 ATβH 形式的 Fab。文库首先用生物素化的肝素 / Fondaparinux 结合的 ATα 和生物素化的 ATα 进行耗尽,并且随后在链霉抗生物素蛋白珠上,分别针对肝素 /Fondaparinux 结合的 ATβ 和生物素化的 ATβ 进行淘选。对于每轮淘选,肝素结合的 ATα (ATαH)作为竞争物包括在结合缓冲液中。为了使 hATβ 保持在活性构象(肝素结合形式),在所有三轮淘选中,将肝素加入洗涤缓冲液中。在淘选后,合并的克隆就 hATβ 和 hATβH 特异性结合进行筛选,并且通过 ELISA 就 hATα 进行反筛选。这些克隆还就与兔 ATβ 相对于兔 ATα 的差异结合进行检查。对显示与 hATβH 和 rATβH 两者相对于 hATα 和 rATα 的差异结合的克隆进一步实施伴随 hATβ 掺入的 FXa - 去抑制测定。将阳性命中(Fab)重排成 IgG1,在 HEK293 细胞中表达且通过蛋白 A 柱进行纯化。这些纯化的 IgG1 在 AT 耗尽的人血浆和 hemA 患者血浆中进行广泛测试,用于 TGA 测定(凝血酶生成测定)和 dPT (稀释凝血酶原时间)测定,以测量凝血时间。

[0116] 实施例 5 - ELISA (酶联免疫吸附测定法)

将 2 μg/ml 生物素化的 AT 抗原的 PBS 溶液包被到含或不含肝素(50ug/ml,肝素 -Natrium-5000, Apotheke, Fa. Ratiopharm)的链霉抗生物素蛋白微板(Greiner, 781997)上。在 4℃下的过夜抗原包被后,将板用 PBST +/- 肝素洗涤,并且用在 PBST +/- 肝素中的 5% 乳在 37℃下封闭一小时。在封闭缓冲液去除后,随后将在封闭缓冲液(在 PBST

+/- 肝素中的 5% 乳）中的 20ug/ml Fab 或 4ug/ml IgG 加入板中，并且使板在室温下温育 1 小时。随后将板洗涤三次。将在封闭缓冲液中的抗人 IgG POD（Sigma，A0170）加入板中，并且使板在室温下温育 30 分钟。Amplex 红（In vitrogen，目录 #A22170）用于连同 H_2O_2 以 1:1000 的检测。在 30 分钟温育后，板在荧光板阅读器中在 Ex535，Em 590 处进行读数。

[0117] 实施例 6 - FXa 去抑制测定 - 含肝素的 AT

使肝素与 ATβ 或 ATα 一起温育，以形成稳定的 ATH 复合物。随后将抗体加入 ATβH 或 ATαH 复合物中。同时，在分开的板中混合 10μl 200ng/ml FXa（HTI）和 20μl 50μg/ml Fluophen FXa 荧光底物（Hyphen Biomed）。将抗体 -ATH 混合物快速加入 FXa/ 底物溶液中，并且在 Ex360nm 和 Em465nm 处立即起始荧光动力学测量。所有必需稀释均在 100mM NaCl、20mM Tris、2.5mM $CaCl_2$、0,1% BSA、0,1% PEG8000 中进行。

[0118] 实施例 7 - 在 FVIII 缺乏人血浆中的凝血酶生成测定（TGA）

ATβH 抗体的 1:2 系列稀释在 HemA 人血浆中制备，从 1uM 的最终浓度起始到 0.015uM。肝素在每种抗体溶液中以 50nM 的最终浓度加入。随后将 80ul 抗体 - 肝素 - 血浆混合物加入 96 孔 TGA 板中的每个孔中，所述孔含有 20ul 重构 PPP 试剂或校准物。将板置于 TGA 仪器中，并且机器将 20ul FluCa（Fluo 底物 + $CaCl_2$）自动分配到每个孔内。允许反应运行 60 分钟。单独的血浆用作阴性对照。

[0119] 实施例 8 - 在分别掺入 ATα 和 ATβ 的 AT 耗尽人血浆中的凝血酶生成测定（TGA）

将抗体加入掺入 15nm ATα 或 ATβ 的人 AT 缺乏血浆中。随后将肝素以 50nM 的最终浓度吸取到每个反应内。将 80ul 含有 ATH 特异性抗体、肝素和 ATα 或 ATβ 的血浆样品加入 96 孔 TGA 板的孔内，所述孔具有 20ul PPP 试剂或校准物。将板置于 TGA 仪器中，并且随后将 20ul FluCa（Fluo 底物 + $CaCl_2$）分配到每个孔内。允许反应持续 60 分钟。

[0120] 实施例 9 - 在人 hemA 血浆和 AT 耗尽血浆中的稀释凝血酶原时间测定（dPT）

在 hemA 血浆中制备抗 ATβH hmAb 的系列稀释，以具有 0.1 U/mL 肝素的 250 nM 起始。抗体、血浆和肝素的混合物在室温下温育 20-30 分钟。随后，将 50 uL 这种混合物加入 50 uL 稀释的 Innovin（1/2000）（Dade Behring）中，在 37℃下温育 4 分钟，随后添加 50 uL 25 mM CaCl2（HemSil）。dPT 测试程序在 ACL Top 凝血计上进行设置，伴随 360 秒的采集时间。对于在 AT 缺乏血浆中的 dTP，AT-DP 掺入含 0.1 U/ml 肝素的最终浓度为 0.2 uM 的 ATα 或 ATβ。将抗 ATβH mAb 以 0.25 uM 的最终浓度加入 AT-DP/ 肝素 /ATα 或 AT-DP/ 肝素 /ATβ 混合物中，并且在室温下温育 20-30 分钟。对于每种反应，如上将 50 uL 血浆 / 抗体 / 肝素混合物加入 50 uL 稀释的 Innovin（1/4000）中，在 37℃下温育 4 分钟，随后添加 50 uL 25 mM CaCl2（HemSil）。

[0121] 实施例 10 - 抗体纯化

预洗涤的蛋白 A 琼脂糖珠与抗体一起在结合缓冲液（体积比 :1:1）中伴随旋转在 4℃下温育过夜。随后将珠填充到柱内，并且用 1 X PBS 洗涤，直至 $O.D._{280} < 0.05$。排出残留溶液。将抗体用洗脱缓冲液洗脱，并且收集到含有中和缓冲液的管内。洗脱级分针对 1 x PBS 在 4℃下透析过夜，伴随至少两次缓冲液更换。IgG 浓度通过 nanodrop 在 280nm 处进行测量。抗体纯度通过 ELISA、SDS-PAGE 或 SSC 进行检查。

[0122] 实施例 11 - 通过 Biacore 的抗体结合亲和力研究

在 Biacore T100 或 T200 处理单元上执行抗体亲和力测量。将抗人 Fc 抗体或链霉抗

生物素蛋白固定到CM5芯片上。在芯片上注射且捕获hATβH或生物素化的hmAb抗体。注射含/不含肝素的以不同浓度的ATβ 或ATα。仅与抗体结合的AT和ATH生成结合常数。结合结果报道为以纳摩尔表示的平衡解离常数(KD)。当分析AT/肝素复合物时,以1 uM的肝素包括在运行缓冲液中。

[0123] 实施例12 - 肝素化的兔出血模型

肝素化的兔出血模型的实验设计在图13中概述。在兔颈静脉(右侧静脉:静脉淤滞;左侧静脉:套管插入)准备后,在时间0时,将在PBS媒介物中的低分子量肝素(LMWH)IV施用于兔(1800U/kg)。在10分钟后,施用测试物品。实验组包括媒介物,PBS;阳性对照,硫酸鱼精蛋白(28mg/kg IV);阴性对照,M14 IgG2;处理:30mg/kg;TPP2803,3mg/kg;TPP2803,30mg/kg。在测试物品施用后五分钟,执行耳穿刺(3-5 mm),并且经过30分钟时期监控原位血栓形成(淤滞)。用滤纸每15秒回收来自切口的血液,直至出血停止。

[0124] 前述公开内容和实施例不预期以任何方式缩小权利要求的范围。应当理解可以作出各种修饰和变化,并且等价物可以取代前述实施方案和教导,而不背离所附的权利要求的真实精神和范围。相应地,说明书和实施例应以举例说明性含义而不是限制性含义加以考虑。此外,本文提及的所有论文、书本、专利申请、专利及其他材料的公开内容整体引入本文作为参考。

[0001]

序列表

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<150> US 61/784,590
<151> 2013-03-14

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His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro
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[0002]

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35 40 45

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50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
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[0003]

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65 70 75 80

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85 90 95

Ser Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Arg Arg Thr Val
100 105 110

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
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35 40 45

Ser Arg Ile Gly Pro Ser Gly Gly Lys Thr Met Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

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35 40 45

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Thr Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu
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Glu Pro Glu Asp Phe Ala Val Tyr Phe Cys Gln Gln Tyr Ala Ser Pro
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Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val
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Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
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35 40 45

Ser Arg Ile Gly Pro Ser Gly Gly Lys Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
65 70 75 80

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[0006]

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Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
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Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
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Ser Arg Ile Ser Pro Ser Gly Gly Lys Thr His Tyr Ala Asp Ser Val
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Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
85 90 95

Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
100 105 110

Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
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50 55 60

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gagaaaacca tcagcaaggc caagggccag ccccgcgagc ctcaggtgta cacactgccc 1080

cccagccggg atgagctgac caagaaccag gtgtccctga cctgtctggt gaaaggcttc 1140

taccccagcg atatcgccgt ggaatgggag agcaacggcc agcccgagaa caattacaag 1200

accacccccc ctgtgctgga cagcgacggc tcattcttcc tgtactccaa gctgaccgtg 1260

gacaagagcc ggtggcagca gggcaacgtg ttcagctgca gcgtgatgca cgaggccctg 1320

cacaatcact acacccagaa gtccctgagc ctgagccccg gc 1362

<210> 13
<211> 642
<212> DNA
<213> 人工序列

<220>
<223> TPP2015轻链V区

<400> 13
gcacaagaca tccagatgac ccagtctcca ggcaccctgt ctttgtctcc aggggaaaga 60

gccaccctct cctgcagggc cagtcagagt gttagcagca gctacttagc ctggtaccag 120

cagaaacctg gccaggctcc caggctcctc atctatggtg catccagcag ggccactggc 180

atcccagaca ggttcagtgg cagtgggtct gggacagact tcactctcac catcagcaga 240

cggagcctga agattttgca gtgtattact gtcagcagta tggtagctca acgttcggcc 300

aagggaccaa ggtggaaatc agacgaactg tggctgcaat ctgtcttcat cttcccgcca 360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 642

<210> 14
<211> 1362

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<212> DNA
<213> 人工序列

<220>
<223> TPP2015重链

<400> 14
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tcttgcgctg cttccggatt cactttctct aagtacaaga tggattgggt tcgccaagct 120

cctggtaaag gtttggagtg ggtttctcgt atcggtcctt ctggtggcaa gactatgtat 180

gctgactccg ttaaaggtcg cttcactatc tctagagaca actctaagaa tactctctac 240

ttgcagatga acagcttaag ggctgaggac acggccgtgt attactgtgc gagagagaaa 300

gcgtcggatc tatcggggac ttattctgag gcccttgact actggggcca gggaaccctg 360

gtcaccgtct caagcgcctc caccaagggc ccatcggtct tcccgctagc acccagcagc 420

aagagcacca gcggcggaac agccgccctg ggctgcctgg tgaaagacta cttccccgag 480

cccgtgaccg tgtcctggaa ctctggcgcc ctgaccagcg gagtgcatac cttccccgcc 540

gtgctgcaga gcagcggcct gtacagcctg agcagcgtgg tgacagtgcc cagcagcagc 600

ctgggaaccc agacctacat ctgcaacgtg aaccacaagc ccagcaacac caaggtggac 660

aagaaggtgg aacccaagag ctgcgacaag acccacacct gtcccccctg ccctgccct 720

gaactgctgg gcggacccag cgtgttcctg ttcccccaa agcccaagga caccctgatg 780

atcagccgga ccccgaagt gacctgcgtg gtggtggacg tgtcccacga ggacccagaa 840

gtgaagttta attggtacgt ggacggcgtg gaagtgcata acgccaagac caagcccaga 900

gaggaacagt acaacagcac ctaccgggtg gtgtccgtgc tgaccgtgct gcaccaggac 960

tggctgaacg gcaaagagta caagtgcaag gtctccaaca aggccctgcc tgccccatc 1020

gagaaaacca tcagcaaggc caagggccag ccccgcgagc ctcaggtgta cacactgccc 1080

cccagccggg atgagctgac caagaaccag gtgtccctga cctgtctggt gaaaggcttc 1140

taccccagcg atatcgccgt ggaatgggag agcaacggcc agcccgagaa caattacaag 1200

accacccccc ctgtgctgga cagcgacggc tcattcttcc tgtactccaa gctgaccgtg 1260

gacaagagcc ggtggcagca gggcaacgtg ttcagctgca gcgtgatgca cgaggccctg 1320

cacaatcact acacccagaa gtccctgagc ctgagccccg gc 1362

<210> 15
<211> 648
<212> DNA
<213> 人工序列

<220>
<223> TPP2016轻链V区

<400> 15
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gccaccctct cctgcagggc cagtcagaat attaatagaa acttggcctg gtaccagcag 120

aagcctggcc gggctcccag actcctcatc cataccgcat ccactagggc ccctggtgtc 180

ccagtcagga tcactggcag tgggtctgga acagagttca ctctcaccat cagcagcctg 240

gaacctgaag attttgcagt gtatttctgt cagcagtatg ctagcccacc tcggacgttc 300

ggccaaggga ccaaggtgga aatcaagcga actgtggctg caccatctgt cttcatcttc 360

ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac 420

[0011]

ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac 480

tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc 540

ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat 600

cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgt 648

<210> 16
<211> 1362
<212> DNA
<213> 人工序列

<220>
<223> TPP2016重链

<400> 16
gaagttcaat tgttagagtc tggtggcggt cttgttcagc ctggtggttc tttacgtctt 60

tcttgcgctg cttccggatt cactttctct aagtaccgta tggattgggt tcgccaagct 120

cctggtaaag gtttggagtg ggtttctcgt atcggtcctt ctggtggcaa gactacttat 180

gctgactccg ttaaaggtcg cttcactatc tctagagaca actctaagaa tactctctac 240

ttgcagatga acagcttaag ggctgaggac acggccgtgt attactgtgc gagagagaaa 300

acgtcggatc tatcggggag ttattctgag gcccttgact actggggcca gggaaccctg 360

gtcaccgtct caagcgcctc caccaagggc ccatcggtct tcccgctagc acccagcagc 420

aagagcacca gcggcggaac agccgccctg ggctgcctgg tgaaagacta cttccccgag 480

cccgtgaccg tgtcctggaa ctctggcgcc ctgaccagcg gagtgcatac cttccccgcc 540

gtgctgcaga gcagcggcct gtacagcctg agcagcgtgg tgacagtgcc cagcagcagc 600

ctgggaaccc agacctacat ctgcaacgtg aaccacaagc ccagcaacac caaggtggac 660

aagaaggtgg aacccaagag ctgcgacaag acccacacct gtccccctg ccctgcccct 720

gaactgctgg gcggacccag cgtgttcctg ttccccccaa agcccaagga caccctgatg 780

atcagccgga cccccgaagt gacctgcgtg gtggtggacg tgtcccacga ggacccagaa 840

gtgaagttta attggtacgt ggacggcgtg gaagtgcata acgccaagac caagcccaga 900

gaggaacagt acaacagcac ctaccgggtg gtgtccgtgc tgaccgtgct gcaccaggac 960

tggctgaacg gcaaagagta caagtgcaag gtctccaaca aggccctgcc tgcccccatc 1020

gagaaaacca tcagcaaggc caagggccag ccccgcgagc ctcaggtgta cacactgccc 1080

cccagccggg atgagctgac caagaaccag gtgtccctga cctgtctggt gaaaggcttc 1140

taccccagcg atatcgccgt ggaatgggag agcaacggcc agcccgagaa caattacaag 1200

accacccccc ctgtgctgga cagcgacggc tcattcttcc tgtactccaa gctgaccgtg 1260

gacaagagcc ggtggcagca gggcaacgtg ttcagctgca gcgtgatgca cgaggccctg 1320

cacaatcact acacccagaa gtccctgagc ctgagccccg gc 1362

<210> 17
<211> 654
<212> DNA
<213> 人工序列

<220>
<223> TPP2019轻链V区

<400> 17
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[0012]

gccaccctct cctgcagggc cagtcagcgt gttagcagca gctacttaac ctggtaccag 120

cagaaacctg gccaggctcc caggctcctc atctatggtg catccagcag ggccactggc 180

atcccagaca ggttcagtgg cagtgggtct gggacagact tcactctcac catcagcaga 240

ctggagcctg aagattttgc agtttattac tgtcagcagt atgatagtac gcctccgctc 300

accttcggcg gagggaccaa ggtggagatc aaacgaactg tggctgcacc atctgtcttc 360

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 480

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600

acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgt 654

<210> 18
<211> 1365
<212> DNA
<213> 人工序列

<220>
<223> TPP2019重链

<400> 18
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tcttgcgctg cttccggatt cactttctct cgttacgcta tgtattgggt tcgccaagct 120

cctggtaaag gtttggagtg ggtttctcgt atctctcctt ctggtggcaa gactcattat 180

gctgactccg ttaaaggtcg cttcactatc tctagagaca actctaagaa tactctctac 240

ttgcagatga acagcttaag ggctgaggac acggccgtgt attactgtgc gagactgtct 300

caaactggtt attaccctca ctaccactac tacggtatgg acgtctgggg ccaagggacc 360

acggtcaccg tctcaagcgc ctccaccaag ggcccatcgg tcttcccgct agcacccagc 420

agcaagagca ccagcggcgg aacagccgcc ctgggctgcc tggtgaaaga ctacttcccc 480

gagcccgtga ccgtgtcctg gaactctggc gccctgacca gcggagtgca taccttcccc 540

gccgtgctgc agagcagcgg cctgtacagc ctgagcagcg tggtgacagt gcccagcagc 600

agcctgggaa cccagaccta catctgcaac gtgaaccaca agcccagcaa caccaaggtg 660

gacaagaagg tggaacccaa gagctgcgac aagacccaca cctgtccccc ctgccctgcc 720

cctgaactgc tgggcggacc cagcgtgttc ctgttccccc caaagcccaa ggacaccctg 780

atgatcagcc ggacccccga agtgacctgc gtggtggtgg acgtgtccca cgaggaccca 840

gaagtgaagt ttaattggta cgtggacggc gtggaagtgc ataacgccaa gaccaagccc 900

agagaggaac agtacaacag cacctaccgg gtggtgtccg tgctgaccgt gctgcaccag 960

gactggctga acggcaaaga gtacaagtgc aaggtctcca acaaggccct gcctgccccc 1020

atcgagaaaa ccatcagcaa ggccaagggc cagccccgcg agcctcaggt gtacacactg 1080

ccccccagcc gggatgagct gaccaagaac caggtgtccc tgacctgtct ggtgaaaggc 1140

ttctacccca gcgatatcgc cgtggaatgg gagagcaacg gccagcccga gaacaattac 1200

aagaccaccc cccctgtgct ggacagcgac ggctcattct tcctgtactc caagctgacc 1260

gtggacaaga gccggtggca gcagggcaac gtgttcagct gcagcgtgat gcacgaggcc 1320

ctgcacaatc actacaccca gaagtccctg agcctgagcc ccggc 1365

[0013]

<210> 19
<211> 639
<212> DNA
<213> 人工序列

<220>
<223> TPP2803轻链V区

<400> 19
agcgaattga ctcaggaccc tgctgtgtct gtggccttgg gacagacagt caggatcaca 60

tgccaaggag acagcctcag aagctattat gcaagctggt accagcagaa gccaggacag 120

gcccctgtac ttgtcatcta tggtaaaaac aaccggccct cagggatccc agaccgattc 180

tctggctcca gctcaggaaa cacagcttcc ttgaccatca ctggggctca ggcggaagat 240

gaggctgact attactgtaa ctcccgggac agcagtggta accatctggt attcggcgga 300

gggaccaagc tgaccgtcct aggtcagccc aaggctgccc cctcggtcac tctgttcccg 360

ccctcctctg aggagcttca agccaacaag gccacactag tgtgtctgat cagtgacttc 420

tacccgggag ctgtgacagt ggcctggaag gcagatggca gccccgtcaa ggcgggagtg 480

gagaccacca aaccctccaa acagagcaac aacaagtacg cggccagcag ctacctgagc 540

ctgacgcccg agcagtggaa gtcccacaga agctacagct gccaggtcac gcatgaaggg 600

agcaccgtgg agaagacagt ggcccctgca gaatgctct 639

<210> 20
<211> 1348
<212> DNA
<213> 人工序列

<220>
<223> TPP2803重链

<400> 20
gaagtgcagc tgctggaaag cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60

agctgtgccg ccagcggctt caccttcagc agctacagaa tgagctgggt gcgccaggcc 120

cctggcaagg gactggaatg ggtgtcccgg atctacagca gcggcggcag aaccagatac 180

gccgacagcg tgaagggccg gttcaccatc tcccgggaca acagcaagaa caccctgtac 240

ctgcagatga acagcctgcg ggccgaggac accgccgtgt actattgcgc cagagagaag 300

gccagcgacc tgagcggcag ctttagcgag gccctggatt attggggcca gggcacactc 360

gtgaccgtgt ctagcgccag cacaaagggc cccagcgtgt tccctctggc cccttgtagc 420

agaagcacca gcgagtctac agccgccctg ggctgcctcg tgaaggacta ctttcccgag 480

cccgtgacag tgtcctggaa ctctggcgcc ctgacaagcg gcgtgcacac ctttccagcc 540

gtgctgcaga gcagcggcct gtactctctg agcagcgtcg tgactgtgcc cagcagcaac 600

ttcggcaccc agacctacac ctgtaacgtg gaccacaagc ccagcaacac caaggtggac 660

aagaccgtgg aacggaagtg ctgcgtggaa tgccccccctt gtcctgcccc tccagtggct 720

ggcccttccg tgttcctgtt ccccccaaag cccaaggaca ccctgatgat cagccggacc 780

ccgaagtgac ctgcgtggtg gtggatgtgt cccacgagga ccccgaggtg cagttcaatt 840

ggtacgtgga cggcgtggaa gtgcacaacg ccaagaccaa gcccagagag gaacagttca 900

acagcacctt ccgggtggtg tccgtgctga ccgtggtgca tcaggactgg ctgaacggca 960

aagagtacaa gtgcaaggtg tccaacaagg gcctgcctgc ccccatcgag aaaaccatca 1020

[0014]

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agatgaccaa gaaccaggtg tccctgacct gtctcgtgaa aggcttctac ccctccgata 1140

tcgccgtgga atgggagagc aacggccagc ccgagaacaa ctacaagacc acccccccca 1200

tgctggacag cgcggctcat tcttcctgta cagcaagctg acagtggaca agtcccggtg 1260

gcagcagggc aacgtgttca gctgcagcgt gatgcacgaa gccctgcaca accactacac 1320

ccagaagtcc ctgagcctga gccctggc 1348

<210> 21
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> TPP2009 LCDR1

<400> 21

Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
1 5 10

<210> 22
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 LCDR1

<400> 22

Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala
1 5 10

<210> 23
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 LCDR1

<400> 23

Arg Ala Ser Gln Asn Ile Asn Arg Asn Leu Ala
1 5 10

<210> 24
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 LCDR1

<400> 24

Arg Ala Ser Gln Arg Val Ser Ser Ser Tyr Leu Thr
1 5 10

<210> 25
<211> 11
<212> PRT
<213> 人工序列

<220>

[0015]

<223> TPP2803 LCDR1

<400> 25

Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
1 5 10

<210> 26
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> TPP2009 LCDR2

<400> 26

Gly Lys Asn Asn Arg Pro Ser
1 5

<210> 27
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 LCDR2

<400> 27

Gly Ala Ser Ser Arg Ala Thr
1 5

<210> 28
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 LCDR2

<400> 28

Thr Ala Ser Thr Arg Ala Pro
1 5

<210> 29
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 LCDR2

<400> 29

Gly Ala Ser Ser Arg Ala Thr
1 5

<210> 30
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> TPP2803 LCDR2

<400> 30

Gly Lys Asn Asn Arg Pro Ser
1 5

[0016]

<210> 31
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> TPP2009 LCDR3

<400> 31

Asn Ser Arg Asp Ser Ser Gly Asn His Leu Val
1 5 10

<210> 32
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 LCDR3

<400> 32

Gln Gln Tyr Gly Ser Ser Arg Thr
1 5

<210> 33
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 LCDR3

<400> 33

Gln Gln Tyr Ala Ser Pro Pro Arg Thr
1 5

<210> 34
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 LCDR3

<400> 34

Gln Gln Tyr Asp Ser Thr Pro Pro Leu Thr
1 5 10

<210> 35
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> TPP2803 LCDR3

<400> 35

Asn Ser Arg Asp Ser Ser Gly Asn His Leu Val
1 5 10

<210> 36
<211> 5
<212> PRT
<213> 人工序列

[0017]

<220>
<223> TPP2009 HCDR1

<400> 36

Ala Tyr Arg Met Gly
1 5

<210> 37
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 HCDR1

<400> 37

Lys Tyr Lys Met Asp
1 5

<210> 38
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 HCDR1

<400> 38

Lys Tyr Arg Met Asp
1 5

<210> 39
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 HCDR1

<400> 39

Arg Tyr Ala Met Tyr
1 5

<210> 40
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> TPP2803 HCDR1

<400> 40

Ser Tyr Arg Met Ser
1 5

<210> 41
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> TPP2009 HCDR2

<400> 41

[0018]

Arg Ile Tyr Ser Ser Gly Gly Arg Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 42
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 HCDR2

<400> 42

Arg Ile Gly Pro Ser Gly Gly Lys Thr Met Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 43
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 HCDR2

<400> 43

Arg Ile Gly Pro Ser Gly Gly Lys Thr Thr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 44
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 HCDR2

<400> 44

Arg Ile Ser Pro Ser Gly Gly Lys Thr His Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 45
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> TPP2803 HCDR2

<400> 45

Arg Ile Tyr Ser Ser Gly Gly Arg Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

[0019]

Gly

<210> 46
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> TPP2009 HCDR3

<400> 46

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Ser Phe Ser Glu Ala Leu
1 5 10 15

Asp Tyr

<210> 47
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> TPP2015 HCDR3

<400> 47

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Thr Tyr Ser Glu Ala Leu
1 5 10 15

Asp Tyr

<210> 48
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> TPP2016 HCDR3

<400> 48

Ala Arg Glu Lys Thr Ser Asp Leu Ser Gly Ser Tyr Ser Glu Ala Leu
1 5 10 15

Asp Tyr

<210> 49
<211> 19
<212> PRT
<213> 人工序列

<220>
<223> TPP2019 HCDR3

<400> 49

Ala Arg Leu Ser Gln Thr Gly Tyr Tyr Pro His Tyr His Tyr Tyr Gly
1 5 10 15

Met Asp Val

[0020]

<210> 50
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> TPP2803 HCDR3

<400> 50

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Ser Phe Ser Glu Ala Leu
1 5 10 15

Asp Tyr

<210> 51
<211> 215
<212> PRT
<213> 人工序列

<220>
<223> TPP2009, hIgG, 轻链

<400> 51

Ala Gln Ser Val Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly
1 5 10 15

Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile
35 40 45

Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly
50 55 60

Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala
65 70 75 80

Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn
85 90 95

His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro
100 105 110

Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
115 120 125

Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
130 135 140

Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys Ala
145 150 155 160

Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
165 170 175

Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
180 185 190

[0021]

Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
195 200 205

Val Ala Pro Ala Glu Cys Ser
210 215

<210> 52
<211> 454
<212> PRT
<213> 人工序列

<220>
<223> TPP2009, hIgG, 重链

<400> 52

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ala Tyr
20 25 30

Arg Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Arg Ile Tyr Ser Ser Gly Gly Arg Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Ser Phe Ser Glu Ala Leu
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
115 120 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240

[0022]

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Pro Gly
450

<210> 53
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> TPP-2015, hIgG, 轻链

<400> 53

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser
1 5 10 15

Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser
20 25 30

[0023]

Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
35 40 45

Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
65 70 75 80

Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser
85 90 95

Ser Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Arg Arg Thr Val
100 105 110

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205

Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 54
<211> 453
<212> PRT
<213> 人工序列

<220>
<223> TPP-2015, hIgG, 重链

<400> 54

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Lys Tyr
20 25 30

Lys Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Arg Ile Gly Pro Ser Gly Gly Lys Thr Met Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

[0024]

```
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Thr Tyr Ser Glu Ala Leu
            100                 105                 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
        115                 120                 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
    130                 135                 140

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145                 150                 155                 160

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
                165                 170                 175

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
            180                 185                 190

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
        195                 200                 205

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
    210                 215                 220

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225                 230                 235                 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            260                 265                 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
        275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
    290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305                 310                 315                 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
                325                 330                 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            340                 345                 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
        355                 360                 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    370                 375                 380
```

[0025]

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Gly
450

<210> 55
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> TPP-2016, hIgG, 轻链, κ

<400> 55

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val Ser
1 5 10 15

Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Asn Ile Asn
20 25 30

Arg Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Arg Ala Pro Arg Leu
35 40 45

Leu Ile His Thr Ala Ser Thr Arg Ala Pro Gly Val Pro Val Arg Ile
50 55 60

Thr Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu
65 70 75 80

Glu Pro Glu Asp Phe Ala Val Tyr Phe Cys Gln Gln Tyr Ala Ser Pro
85 90 95

Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val
100 105 110

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
115 120 125

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
130 135 140

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
145 150 155 160

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
165 170 175

[0026]

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
180 185 190

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
195 200 205

Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 56
<211> 454
<212> PRT
<213> 人工序列

<220>
<223> TPP-2016, hIgG, 重链

<400> 56

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Lys Tyr
20 25 30

Arg Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Arg Ile Gly Pro Ser Gly Gly Lys Thr Thr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Lys Thr Ser Asp Leu Ser Gly Ser Tyr Ser Glu Ala Leu
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
115 120 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
130 135 140

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
195 200 205

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
210 215 220

[0027]

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315 320

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
325 330 335

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
340 345 350

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
355 360 365

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
370 375 380

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
385 390 395 400

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
405 410 415

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
420 425 430

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
435 440 445

Leu Ser Leu Ser Pro Gly
450

<210> 57
<211> 218
<212> PRT
<213> 人工序列

<220>
<223> TPP-2019, hIgG, 轻链, κ

<400> 57

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser
1 5 10 15

[0028]

Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Ser
20 25 30

Ser Ser Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
35 40 45

Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
65 70 75 80

Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Asp Ser
85 90 95

Thr Pro Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 58
<211> 455
<212> PRT
<213> 人工序列

<220>
<223> TPP-2019, hIgG, 重链

<400> 58

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Arg Ile Ser Pro Ser Gly Gly Lys Thr His Tyr Ala Asp Ser Val
50 55 60

[0029]

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Ser Gln Thr Gly Tyr Tyr Pro His Tyr His Tyr Tyr Gly
100 105 110

Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser
115 120 125

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
130 135 140

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
145 150 155 160

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
165 170 175

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
180 185 190

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
195 200 205

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
210 215 220

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
225 230 235 240

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
245 250 255

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
275 280 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315 320

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
325 330 335

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
340 345 350

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
355 360 365

[0030]

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
370 375 380

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
385 390 395 400

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
405 410 415

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
420 425 430

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
435 440 445

Ser Leu Ser Leu Ser Pro Gly
450 455

<210> 59
<211> 214
<212> PRT
<213> 人工序列

<220>
<223> TPP-2803, hIgG2, 轻链, λ

<400> 59

Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
85 90 95

Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
100 105 110

Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
115 120 125

Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly
130 135 140

Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val Lys Ala Gly
145 150 155 160

[0031]

Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala
165 170 175

Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser
180 185 190

Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val
195 200 205

Ala Pro Ala Glu Cys Ser
210

<210> 60
<211> 450
<212> PRT
<213> 人工序列

<220>
<223> TPP-2803, hIgG2, 重链

<400> 60

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Arg Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Arg Ile Tyr Ser Ser Gly Gly Arg Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Lys Ala Ser Asp Leu Ser Gly Ser Phe Ser Glu Ala Leu
100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr
115 120 125

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
130 135 140

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
145 150 155 160

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
165 170 175

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190

Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys
195 200 205

[0032]

Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu
210 215 220

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
290 295 300

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly
450

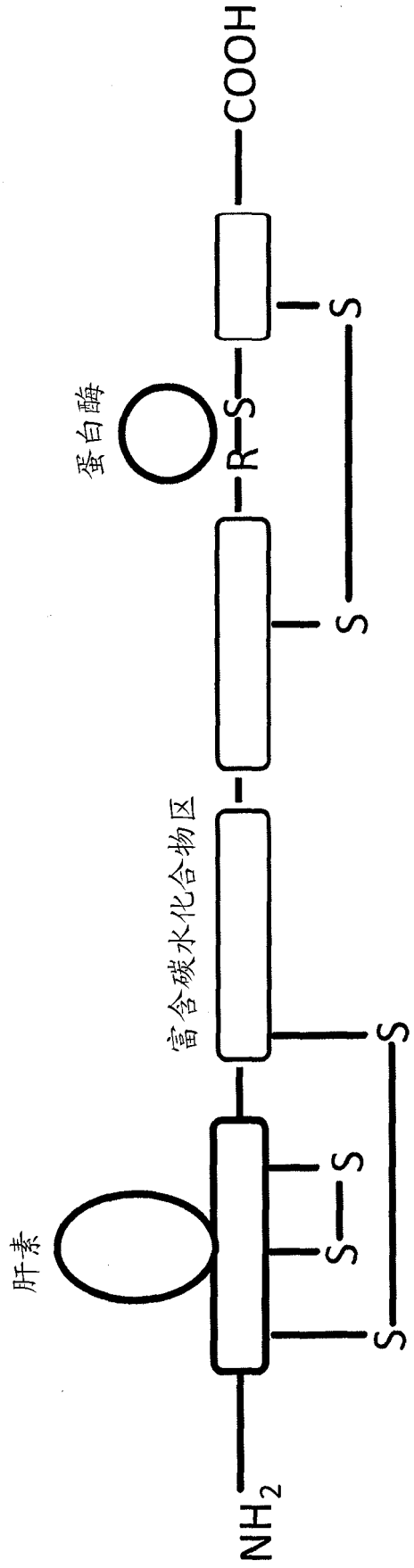


图 1

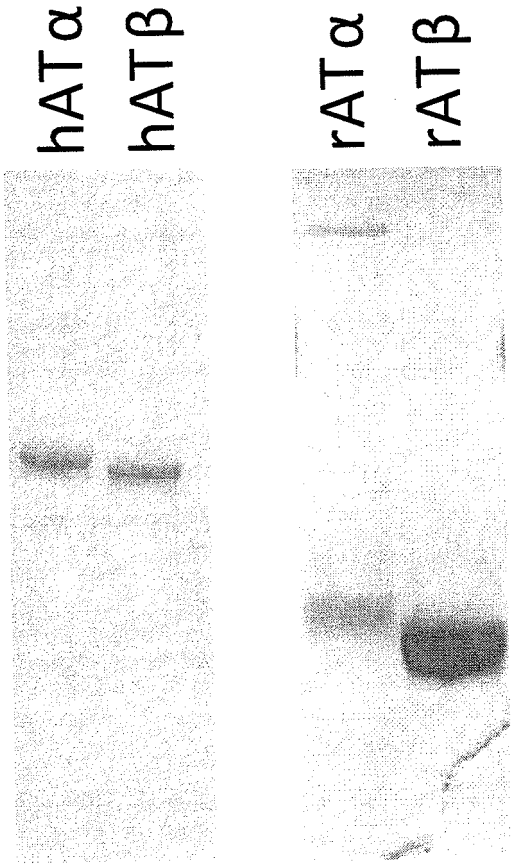


图 2A

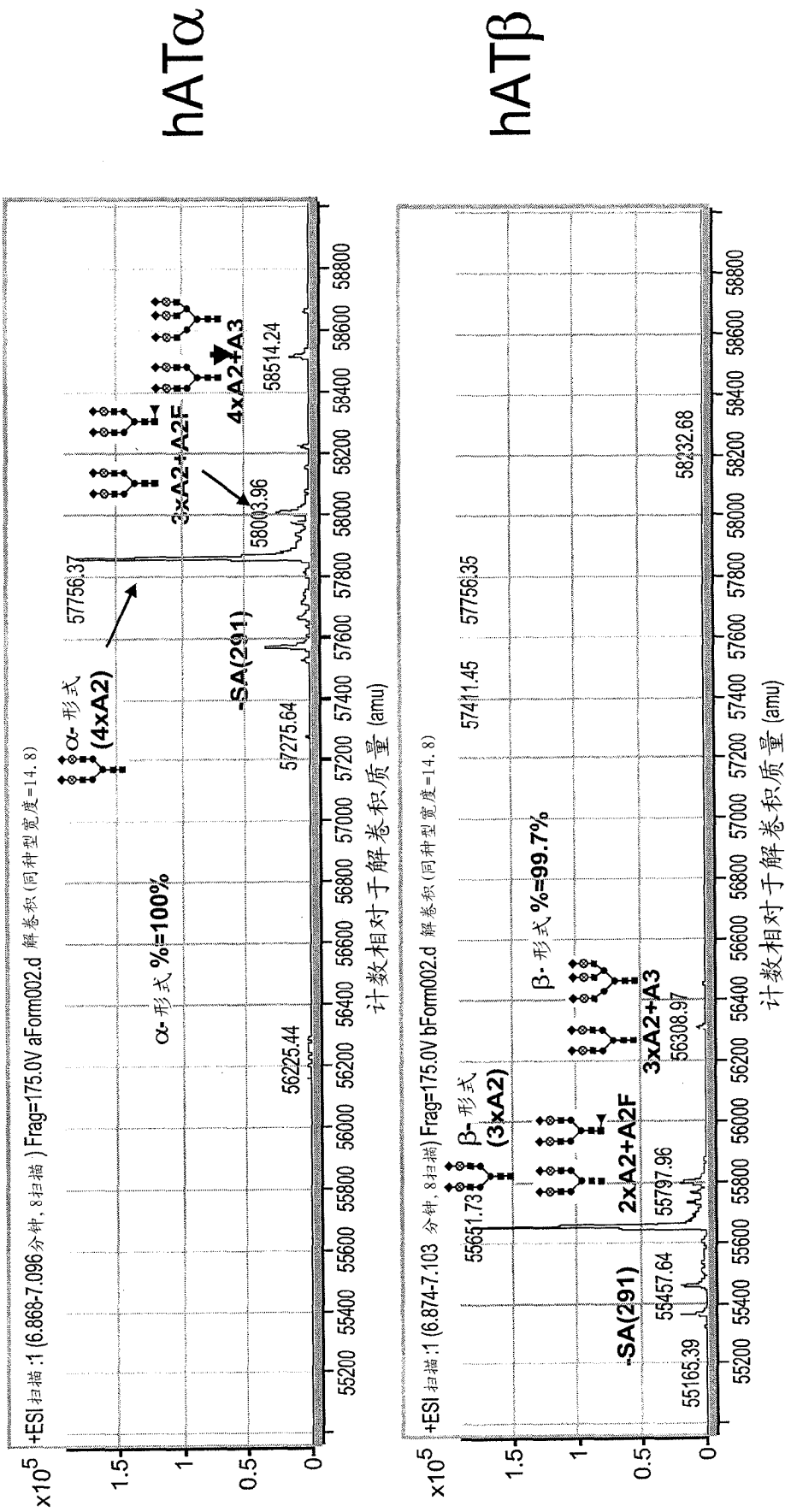

hATα
hATβ
+ESI 扫描 :1 (6.868-7.096 分钟, 8扫描) Frag=175.0V aForm002.d 解卷积 (同种型宽度=14.8)
α- 形式 (4xA2)
α- 形式 %=100%
-SA(291)
2xA2+A2F
4xA2+A3
57756.37
58003.96
58514.24
57275.64
56225.44
计数相对于解卷积质量 (amu)
+ESI 扫描 :1 (6.874-7.103 分钟, 8扫描) Frag=175.0V bForm002.d 解卷积 (同种型宽度=14.8)
β- 形式 (3xA2)
β- 形式 %=99.7%
-SA(291)
2xA2+A2F
3xA2+A3
57411.45
57756.35
58232.68
56308.97
55797.96
55651.73
55457.64
55165.39
计数相对于解卷积质量 (amu)

图 2B

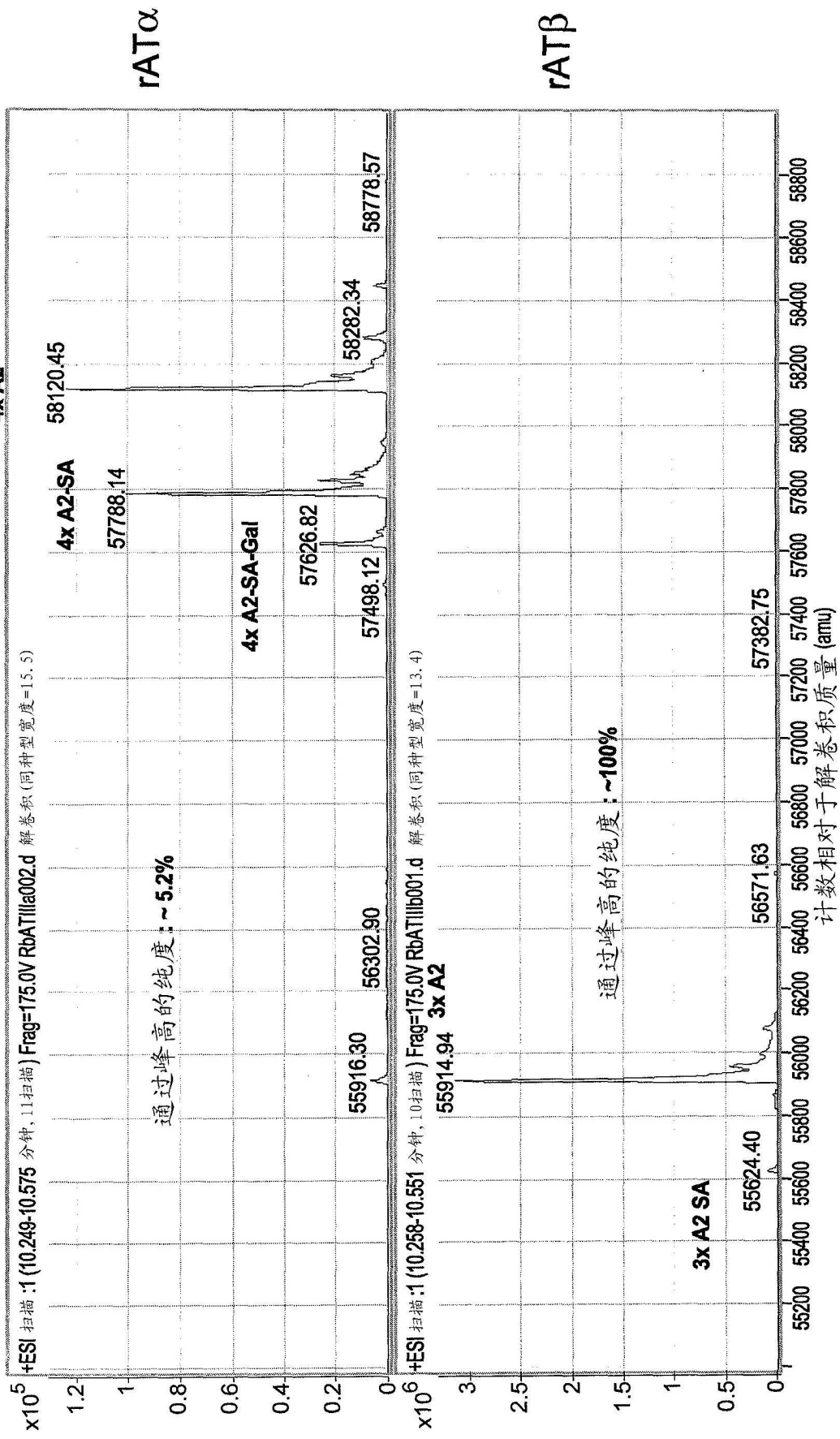

rATα
rATβ
4x A2
4x A2-SA
4x A2-SA-Gal
3x A2
3x A2 SA
58120.45
57788.14
57626.82
57498.12
58282.34
58778.57
56302.90
55916.30
55914.94
55624.40
56571.63
57382.75
通过峰高的纯度：~5.2%
通过峰高的纯度：~100%
+ESI 扫描 :1 (10.249-10.575 分钟, 11扫描) Frag=175.0V RbATIIIa002.d 解卷积 (同种型宽度=15.5)
+ESI 扫描 :1 (10.258-10.551 分钟, 10扫描) Frag=175.0V RbATIIIb001.d 解卷积 (同种型宽度=13.4)
计数相对于解卷积质量 (amu)

图 2C

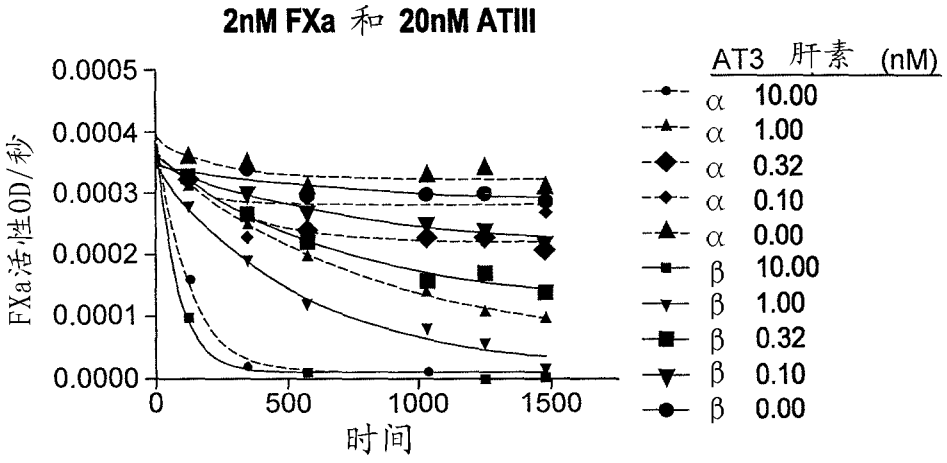

2nM FXa 和 20nM ATIII
FXa活性OD/秒
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
0
500
1000
1500
时间
AT3 肝素 (nM)
α 10.00
α 1.00
α 0.32
α 0.10
α 0.00
β 10.00
β 1.00
β 0.32
β 0.10
β 0.00

图 3A

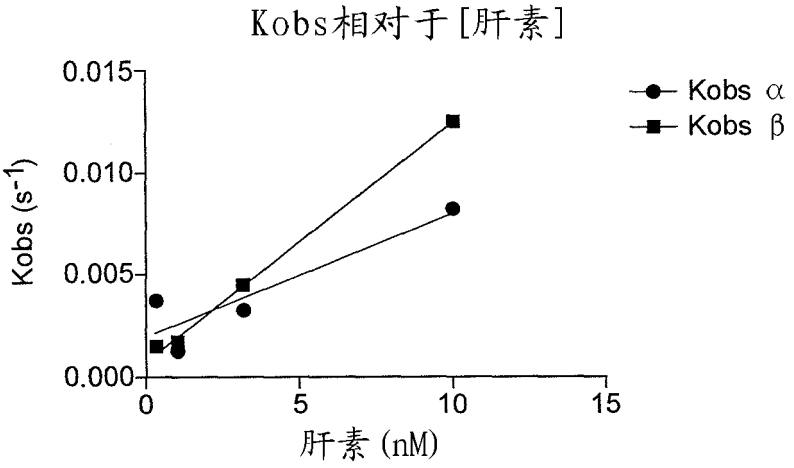

Kobs相对于[肝素]
Kobs (s-1)
0.015
0.010
0.005
0.000
0
5
10
15
肝素 (nM)
Kobs α
Kobs β

图 3B

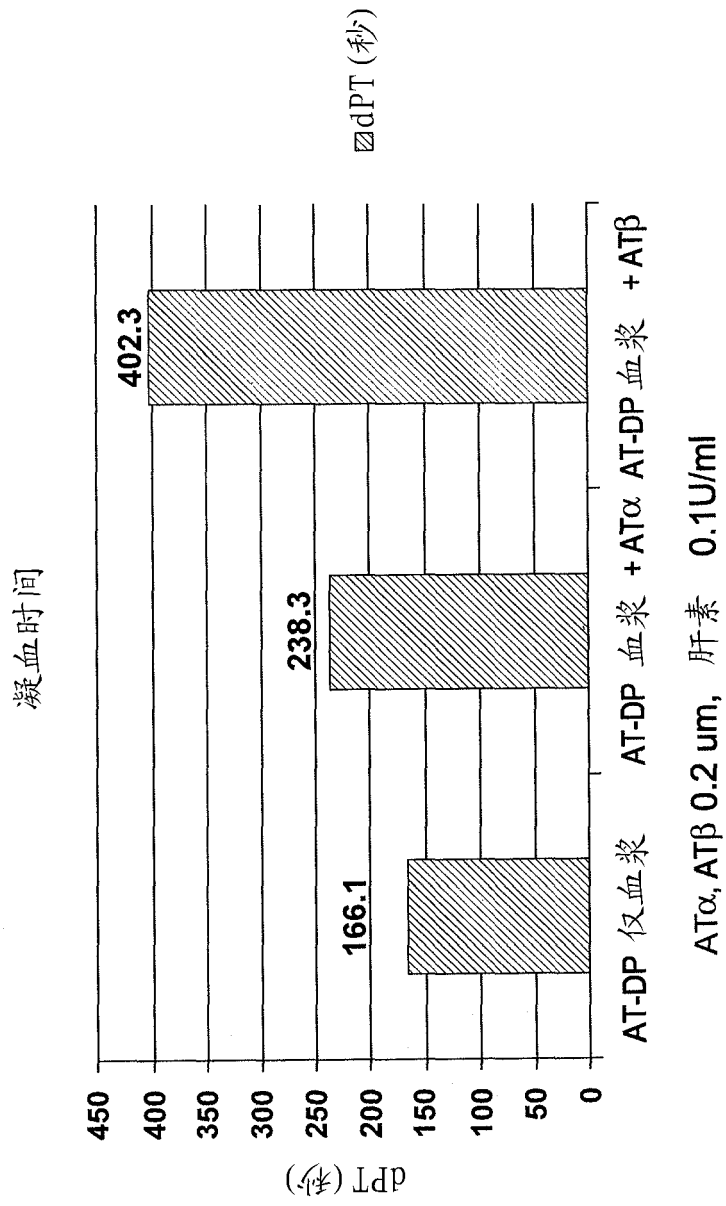

凝血时间
dPT (秒)
450
400
350
300
250
200
150
100
50
0
402.3
238.3
166.1
AT-DP 仅血浆
AT-DP 血浆 +ATα
AT-DP 血浆 +ATβ
ATα, ATβ 0.2 um, 肝素 0.1U/ml

图 3C

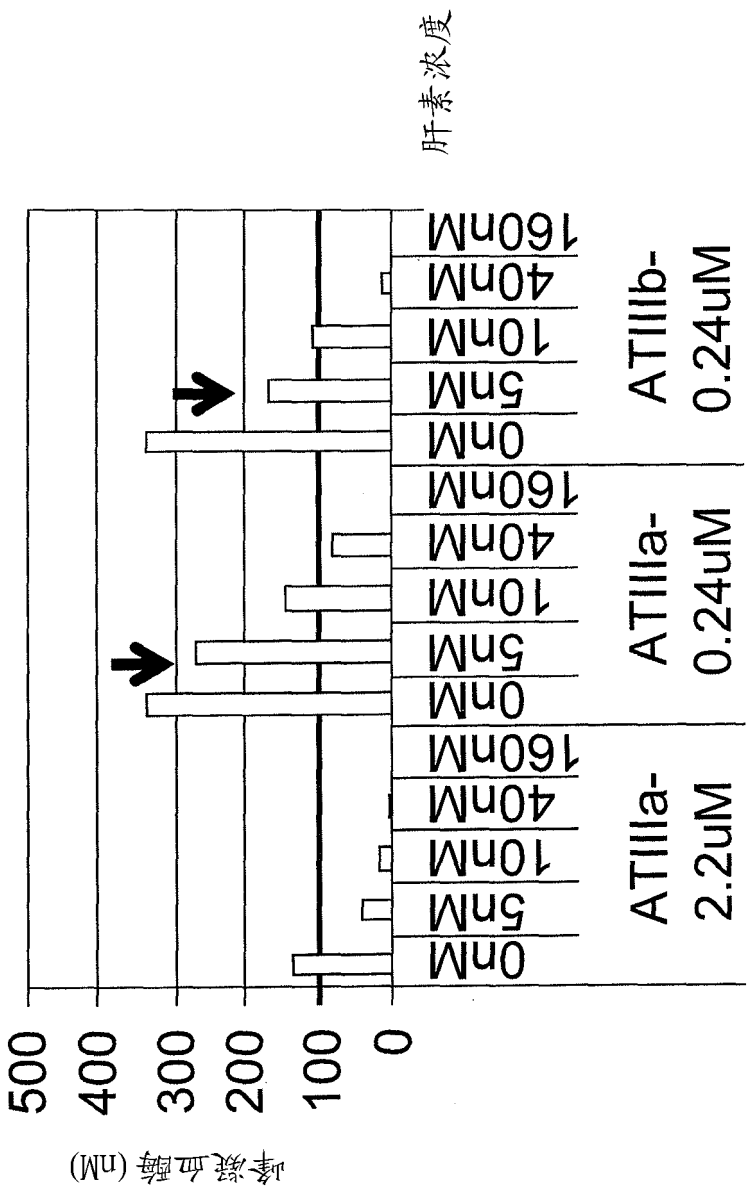

肝素浓度
凝血酶 (nM)
500
400
300
200
100
0
0nM
5nM
10nM
40nM
160nM
ATIIIa-
2.2uM
0nM
5nM
10nM
40nM
160nM
ATIIIa-
0.24uM
0nM
5nM
10nM
40nM
160nM
ATIIIb-
0.24uM

图 3D

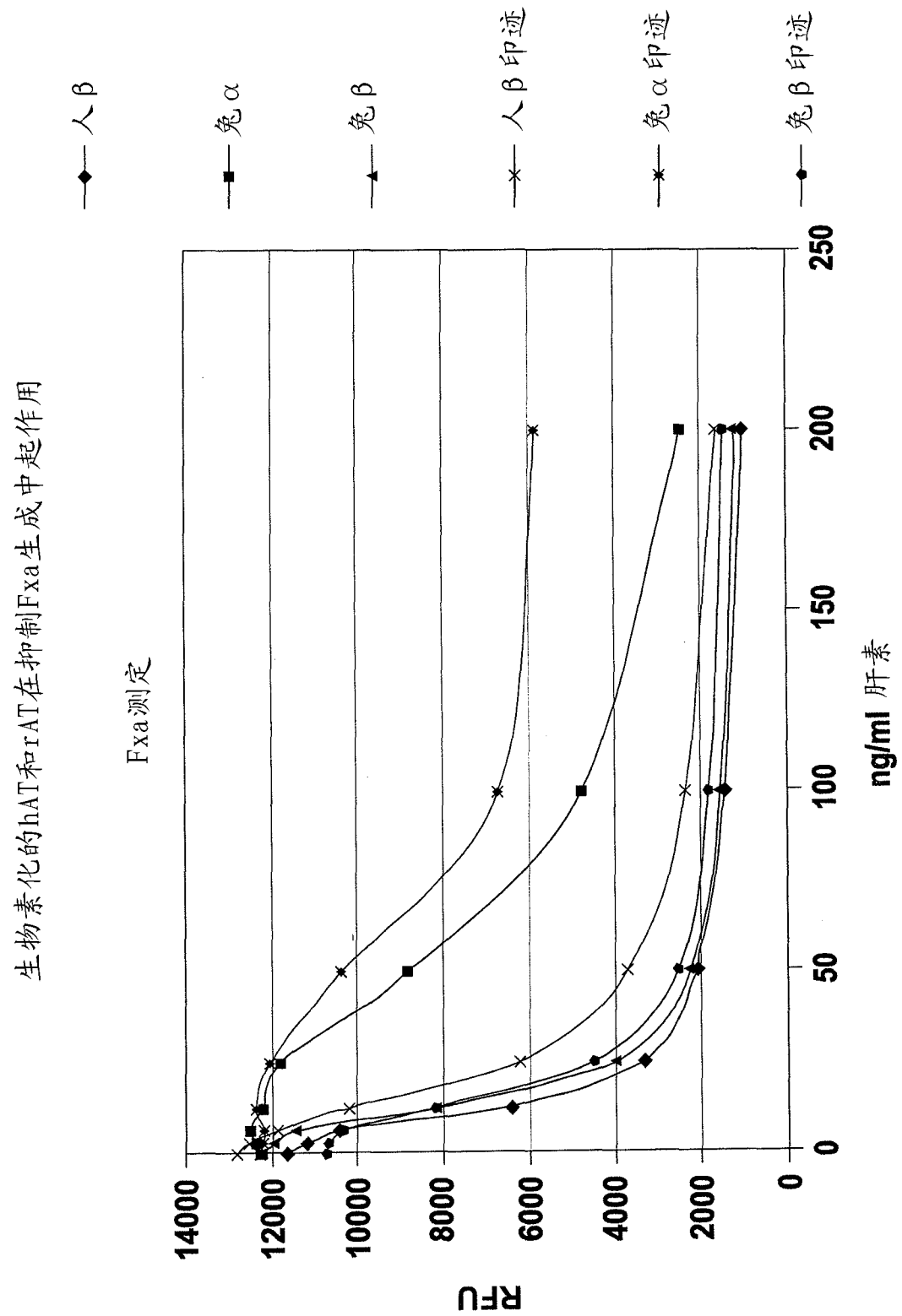

生物素化的hAT和rAT在抑制Fxa生成中起作用
Fxa测定
人β
兔α
兔β
人β印迹
兔α印迹
兔β印迹
RFU
14000
12000
10000
8000
6000
4000
2000
0
0
50
100
150
200
250
ng/ml 肝素

图 4A

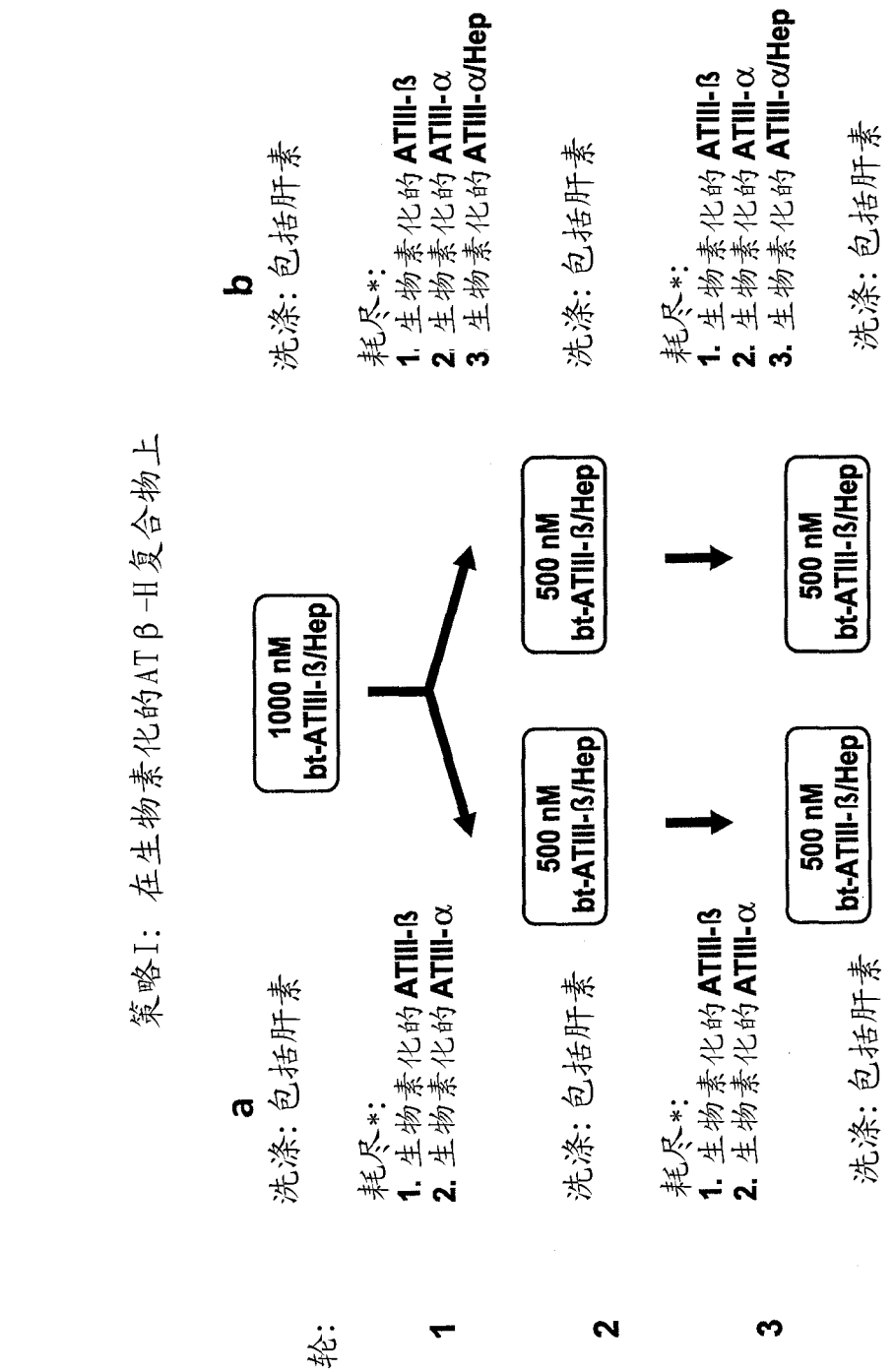

策略I：在生物素化的ATβ-H复合物上
a
洗涤：包括肝素
1000 nM
bt-ATIII-β/Hep
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
轮：
1
500 nM
bt-ATIII-β/Hep
500 nM
bt-ATIII-β/Hep
洗涤：包括肝素
2
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
3
500 nM
bt-ATIII-β/Hep
洗涤：包括肝素
b
洗涤：包括肝素
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
3. 生物素化的 ATIII-α/Hep
洗涤：包括肝素
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
3. 生物素化的 ATIII-α/Hep
洗涤：包括肝素

图 4B

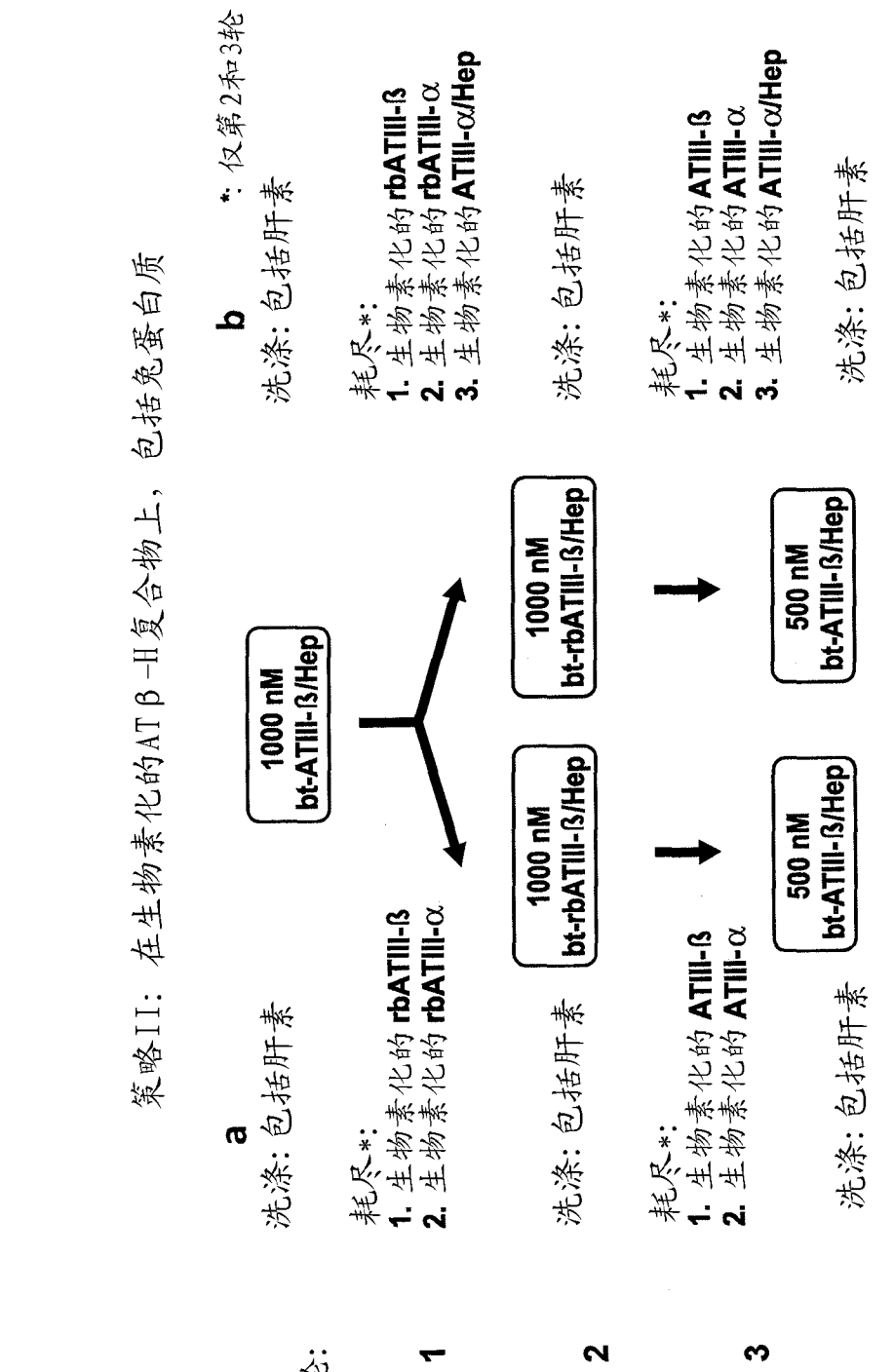

策略II：在生物素化的ATβ-H复合物上，包括兔蛋白质
a
洗涤：包括肝素
1000 nM
bt-ATIII-β/Hep
耗尽*：
1. 生物素化的 rbATIII-β
2. 生物素化的 rbATIII-α
轮：
1
1000 nM
bt-rbATIII-β/Hep
洗涤：包括肝素
2
1000 nM
bt-rbATIII-β/Hep
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
3
500 nM
bt-ATIII-β/Hep
洗涤：包括肝素
b
*：仅第2和3轮
洗涤：包括肝素
耗尽*：
1. 生物素化的 rbATIII-β
2. 生物素化的 rbATIII-α
3. 生物素化的 ATIII-α/Hep
洗涤：包括肝素
耗尽*：
1. 生物素化的 ATIII-β
2. 生物素化的 ATIII-α
3. 生物素化的 ATIII-α/Hep
洗涤：包括肝素
500 nM
bt-ATIII-β/Hep

图 4C

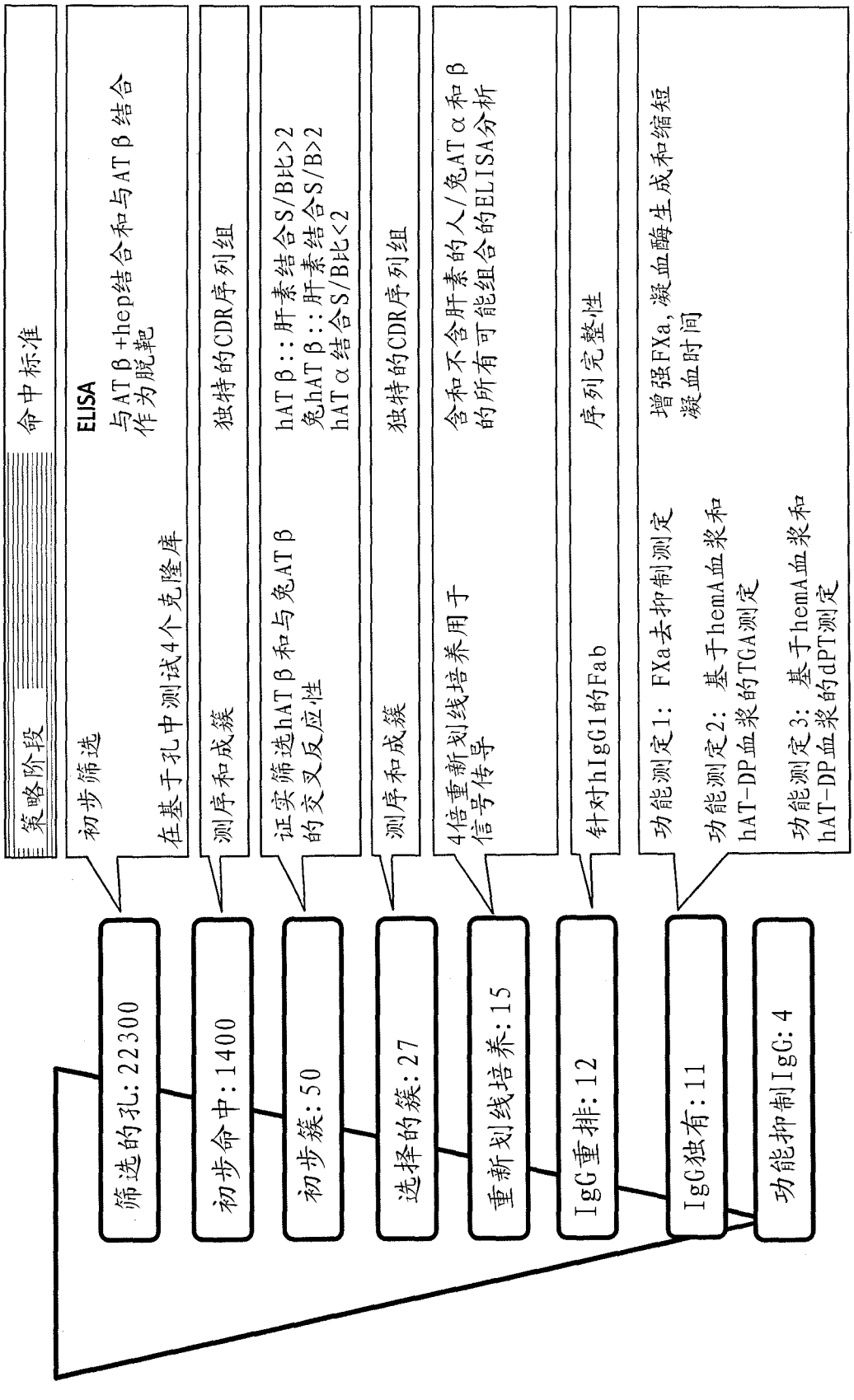

筛选的孔: 22300
初步命中: 1400
初步簇: 50
选择的簇: 27
重新划线培养: 15
IgG重排: 12
IgG独有: 11
功能抑制IgG: 4
策略阶段
命中标准
初步筛选
ELISA
与AT β +hep结合和与AT β 结合
作为脱靶
在基于孔中测试4个克隆库
测序和成簇
独特的CDR序列组
证实筛选hAT β 和与兔AT β
的交叉反应性
hAT β : : 肝素结合S/B比>2
兔hAT β : : 肝素结合S/B>2
hAT α 结合S/B比<2
测序和成簇
独特的CDR序列组
4倍重新划线培养用于
信号传导
含和不含肝素的人/兔AT α 和 β
的所有可能组合的ELISA分析
针对hIgG1的Fab
序列完整性
功能测定1: FXa去抑制测定
增强FXa, 凝血酶生成和缩短
凝血时间
功能测定2: 基于hemA血浆和
hAT-DP血浆的TGA测定
功能测定3: 基于hemA血浆和
hAT-DP血浆的dPT测定

图 5

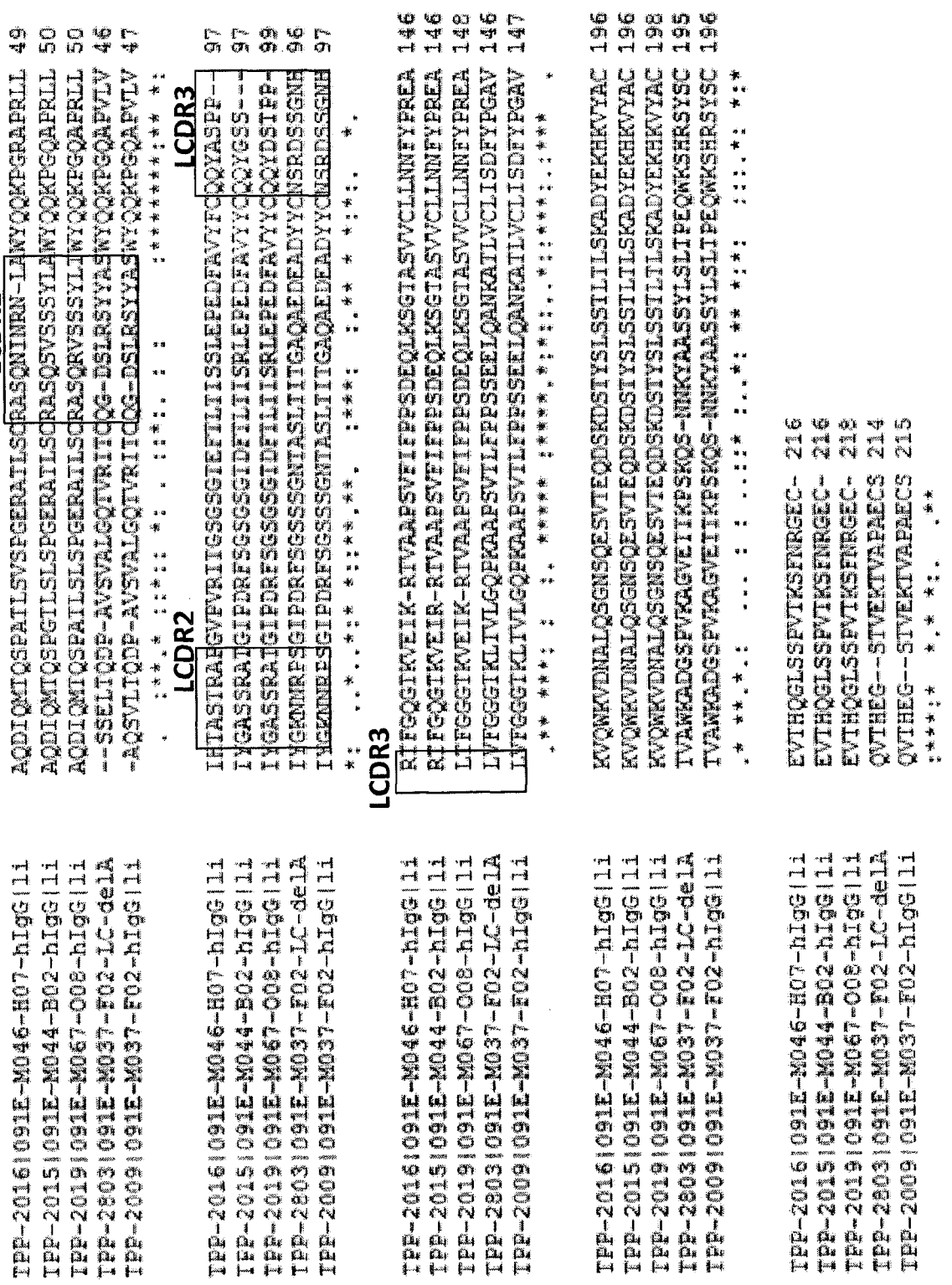

LCDR1
LCDR2
LCDR3
LCDR3
IPP-2016|091E-M046-H07-hIgG|li
IPP-2015|091E-M044-B02-hIgG|li
IPP-2019|091E-M067-O08-hIgG|li
IPP-2803|091E-M037-F02-LC-delA
IPP-2009|091E-M037-F02-hIgG|li
轻链

图 6A

重链

```
                                                    HCDR1
TPP-2803|091E-M037-F02-LC-delA  EVQLLESGGGLVQPGGSLRLSCAASGFTF$SYRMSWVRQAPGKGLEWV$R  50
TPP-2009|091E-M037-F02-hIgG|he  EVQLLESGGGLVQPGGSLRLSCAASGFTF$AYRMGWVRQAPGKGLEWV$R  50
TPP-2016|091E-M046-H07-hIgG|he  EVQLLESGGGLVQPGGSLRLSCAASGFTF$KYRMDWVRQAPGKGLEWV$R  50
TPP-2015|091E-M044-B02-hIgG|he  EVQLLESGGGLVQPGGSLRLSCAASGFTF$KYKMDWVRQAPGKGLEWV$R  50
TPP-2019|091E-M067-O08-hIgG|he  EVQLLESGGGLVQPGGSLRLSCAASGFTF$RYAMYWVRQAPGKGLEWV$E  50
                                ************************** * * ********************

                                          HCDR2
TPP-2803|091E-M037-F02-LC-delA  IYSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREK  100
TPP-2009|091E-M037-F02-hIgG|he  IYSSGGRTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREK  100
TPP-2016|091E-M046-H07-hIgG|he  IGPSGGKTTYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREK  100
TPP-2015|091E-M044-B02-hIgG|he  IGPSGGKTMYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREK  100
TPP-2019|091E-M067-O08-hIgG|he  ISPSGGKTHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLS  100
                                * .***:* ********************************************  .

                                          HCDR3
TPP-2803|091E-M037-F02-LC-delA  ASDLSGSFS-EALDYWGQGTLVTVSS  125
TPP-2009|091E-M037-F02-hIgG|he  ASDLSGSFS-EALDYWGQGTLVTVSS  125
TPP-2016|091E-M046-H07-hIgG|he  TSDLSGSYS-EALDYWGQGTLVTVSS  125
TPP-2015|091E-M044-B02-hIgG|he  ASDLSGTYS-EALDYWGQGTLVTVSS  125
TPP-2019|091E-M067-O08-hIgG|he  QTGYYPHYHYYGMDVWGQGTTVTVSS  126
                                  :.  :  : .:* ***** *****
```

图 6B

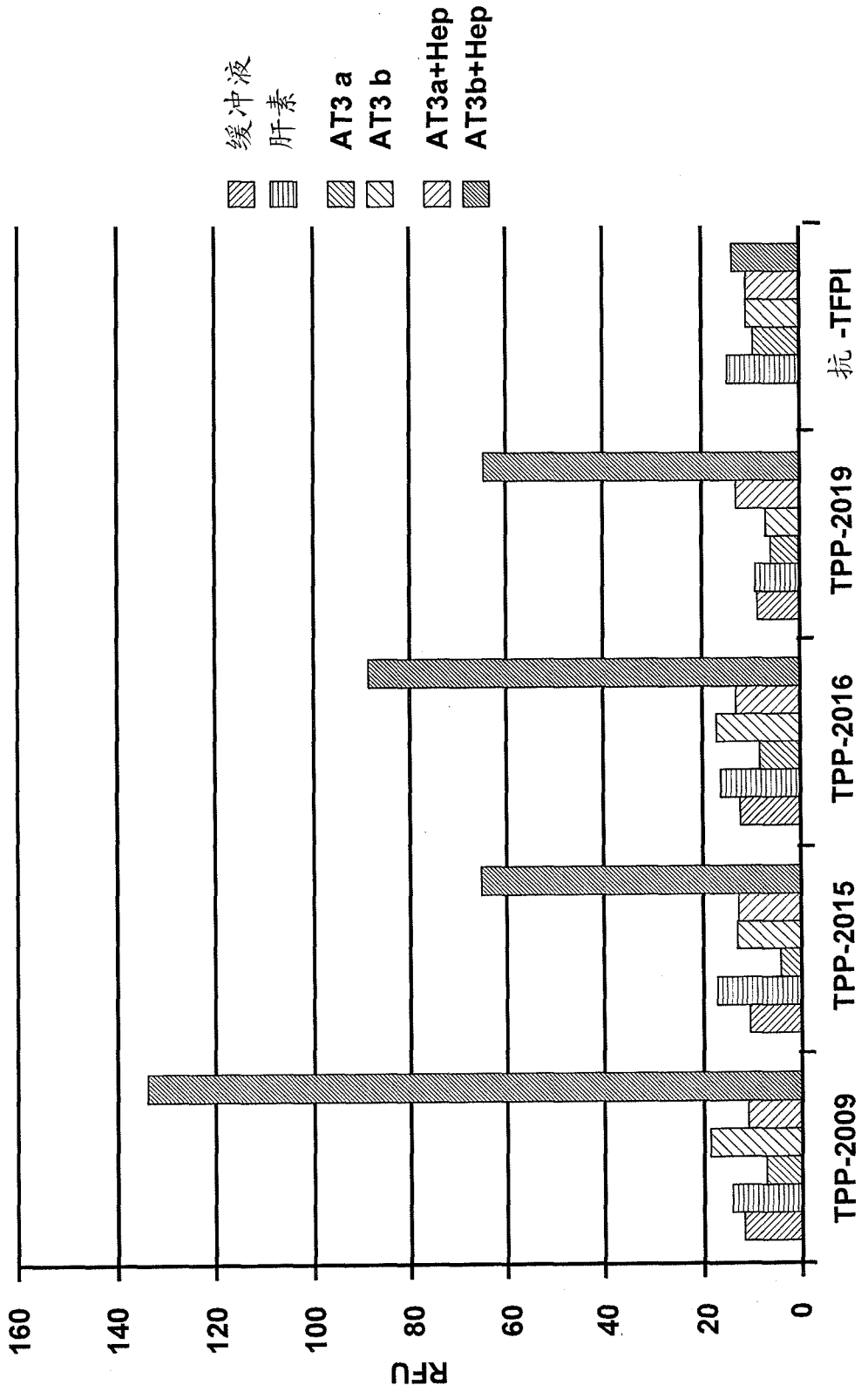

缓冲液
肝素
AT3 a
AT3 b
AT3a+Hep
AT3b+Hep
RFU
0
20
40
60
80
100
120
140
160
TPP-2009
TPP-2015
TPP-2016
TPP-2019
抗 -TFPI

图 7A

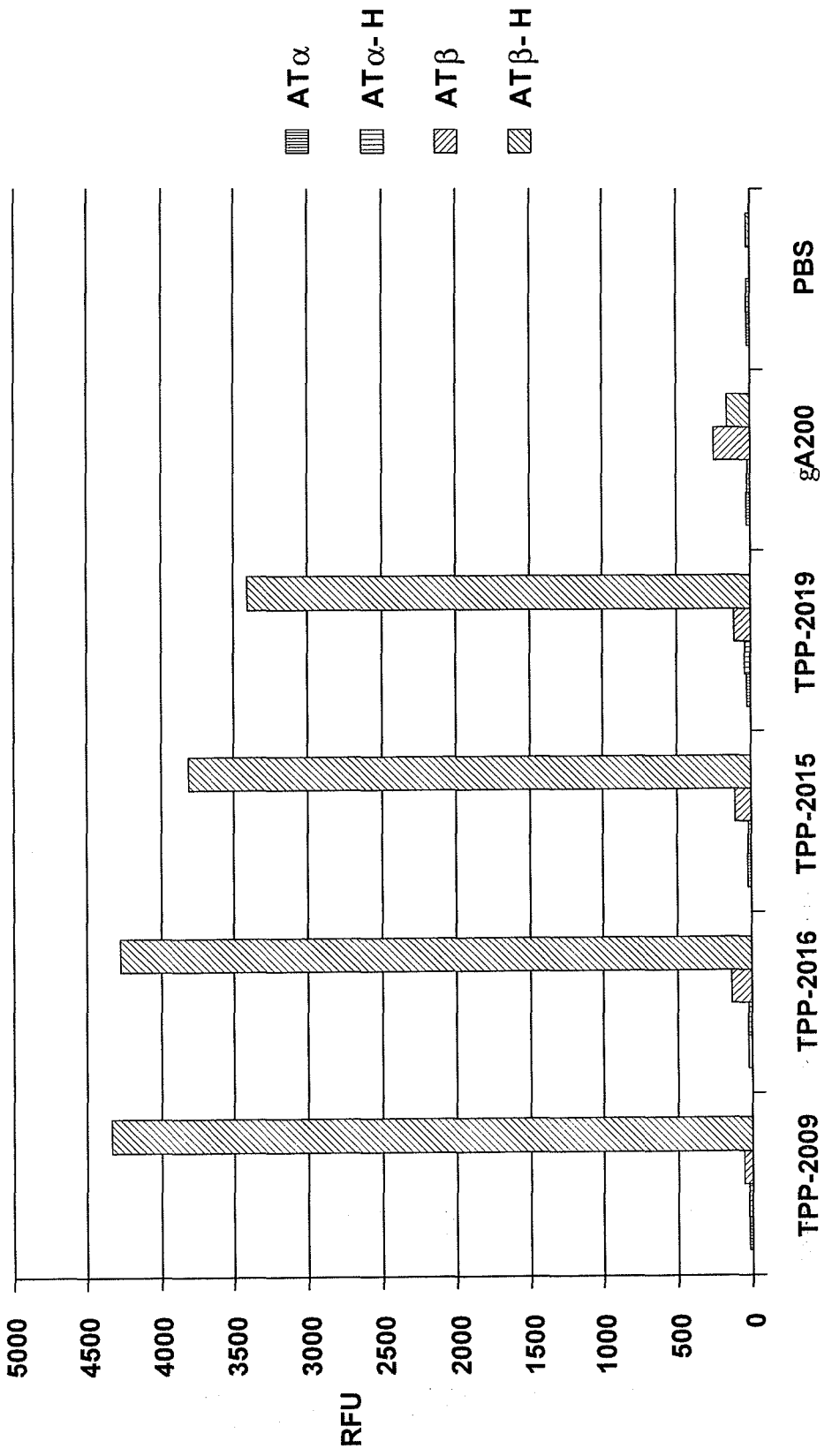

ATα
ATα-H
ATβ
ATβ-H
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
RFU
TPP-2009
TPP-2016
TPP-2015
TPP-2019
gA200
PBS

图 7B

抗体	Ka	Kd	KD (nm)
TPP 2009	1.07E+05	1.27E-03	11.9
TPP 2015	3.95E+04	1.09E-03	27.7
TPP 2016	6.05E+04	1.15E-03	18.9
TPP 2019	8.11E+04	1.39E-03	17.2

图 7C

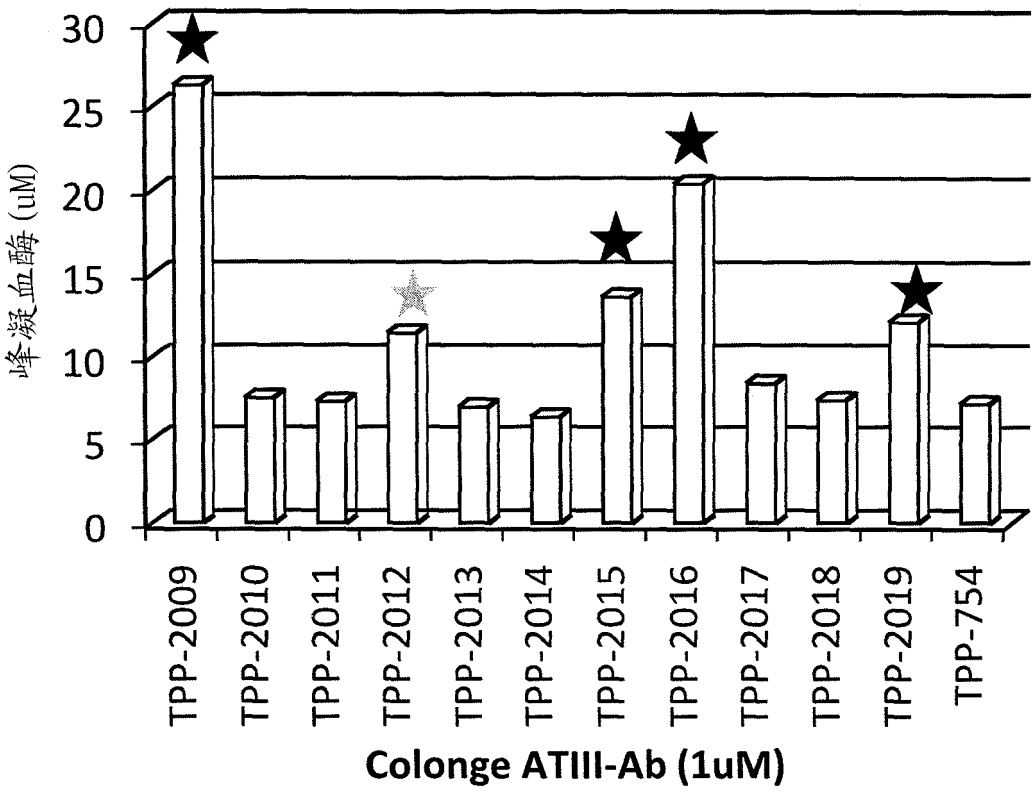
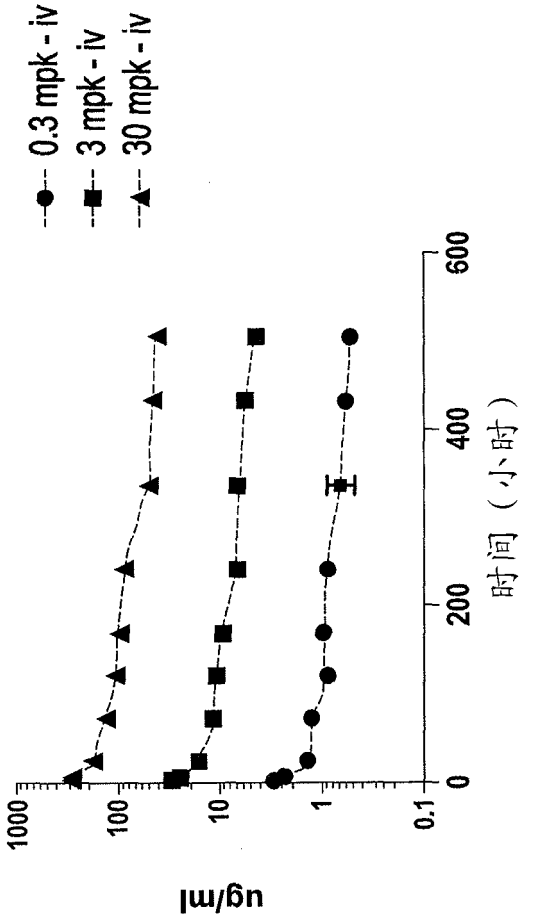

30
25
20
15
10
5
0
峰凝血酶(uM)
TPP-2009
TPP-2010
TPP-2011
TPP-2012
TPP-2013
TPP-2014
TPP-2015
TPP-2016
TPP-2017
TPP-2018
TPP-2019
TPP-754
Colonge ATIII-Ab (1uM)
肝素：50nM
TGA 激活物 ：PPP 高 - 5pM TF

图 8A

	FVIII-DP	AT-DP +ATIIIa	AT-DP +ATIIIb
仅血浆	**210.0**	165.2	167.0
血浆+ ATIIIa	--	**238.3**	--
血浆+ ATIIIb	--	--	**402.6**
TPP2009	**176.7**	233.0	**237.9**
TPP2010	199.1	237.7	356.3
TPP2011	205.9	234.8	380.7
TPP2012	218.7	278.9	320.6
TPP2013	206.5	241.5	366.1
TPP2014	213.0	240.5	383.0
TPP2015	**199.7**	229.7	**316.3**
TPP2016	**183.4**	230.6	**272.5**
TPP2017	203.2	242.6	332.9
TPP2018	202.8	239.6	373.3
TPP2019	**195.8**	233.0	**322.8**
TPP754 (对照)	**204.3**	**237.1**	**385.2**

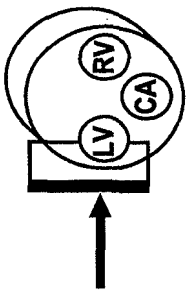
图 8B



以0.3、3和30 mg/kg的IIEM-A小鼠IV给药中的抗体TPP 2009的PK参数

剂量 mg/kg	HL_Lambda_z h	MRTINF_prod h	AUC_%Extrap_prod %	AUCall h*ug/ml	AUCINF_prod h*ug/ml	Cl_prod ml/h/kg	Vz_prod ml/kg
0.3 - iv	340	479	35	467	720	0.42	204
3 - iv	368	497	36	4556	7127	0.42	224
30 - iv	259	349	24	46734	61814	0.49	182

图 9



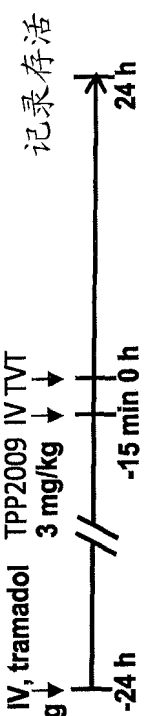


图 10A

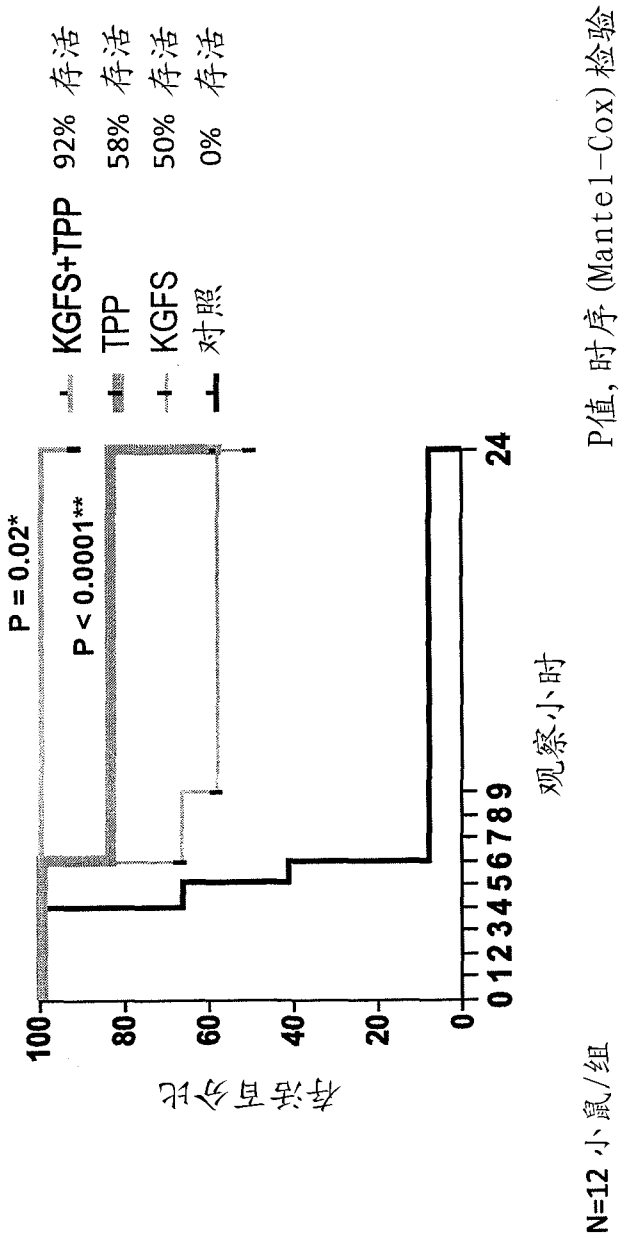

KGFS+TPP 92% 存活
TPP 58% 存活
KGFS 50% 存活
对照 0% 存活
P = 0.02*
P < 0.0001**
存活百分比
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9
24
观察小时
N=12 小鼠/组
P值，时序 (Mantel-Cox) 检验

图 10B

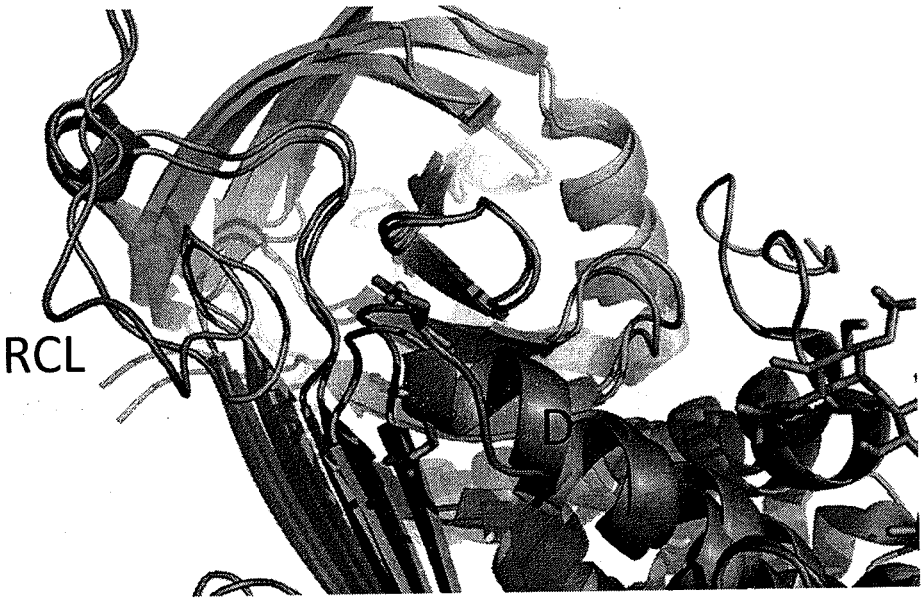


图 11A

图 11B

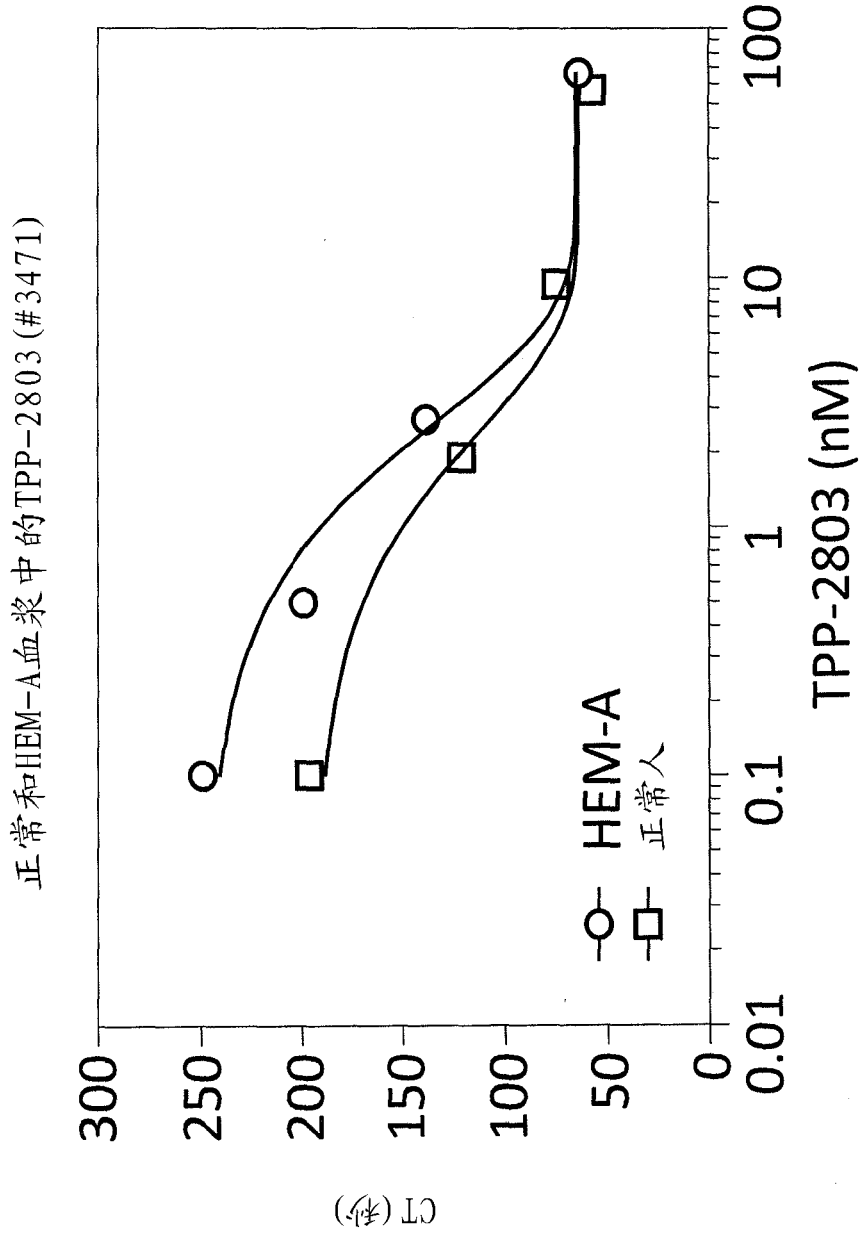

正常和HEM-A血浆中的TPP-2803(#3471)
CT(秒)
300
250
200
150
100
50
0
0.01
0.1
1
10
100
TPP-2803 (nM)
HEM-A
正常人

图 12

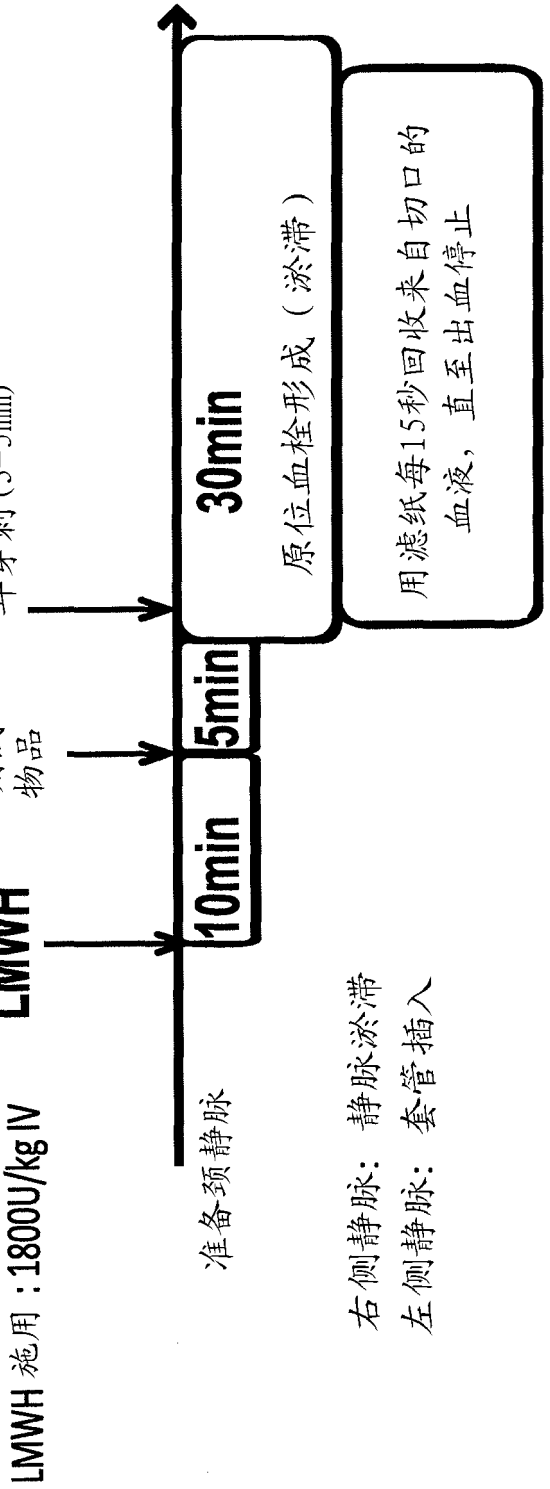

LMWH 施用：1800U/kg IV
LMWH
准备颈静脉
10min
测试物品
5min
耳穿刺(3-5mm)
30min
原位血栓形成（淤滞）
用滤纸每15秒回收来自切口的血液，直至出血停止
右侧静脉：静脉淤滞
左侧静脉：套管插入

图 13

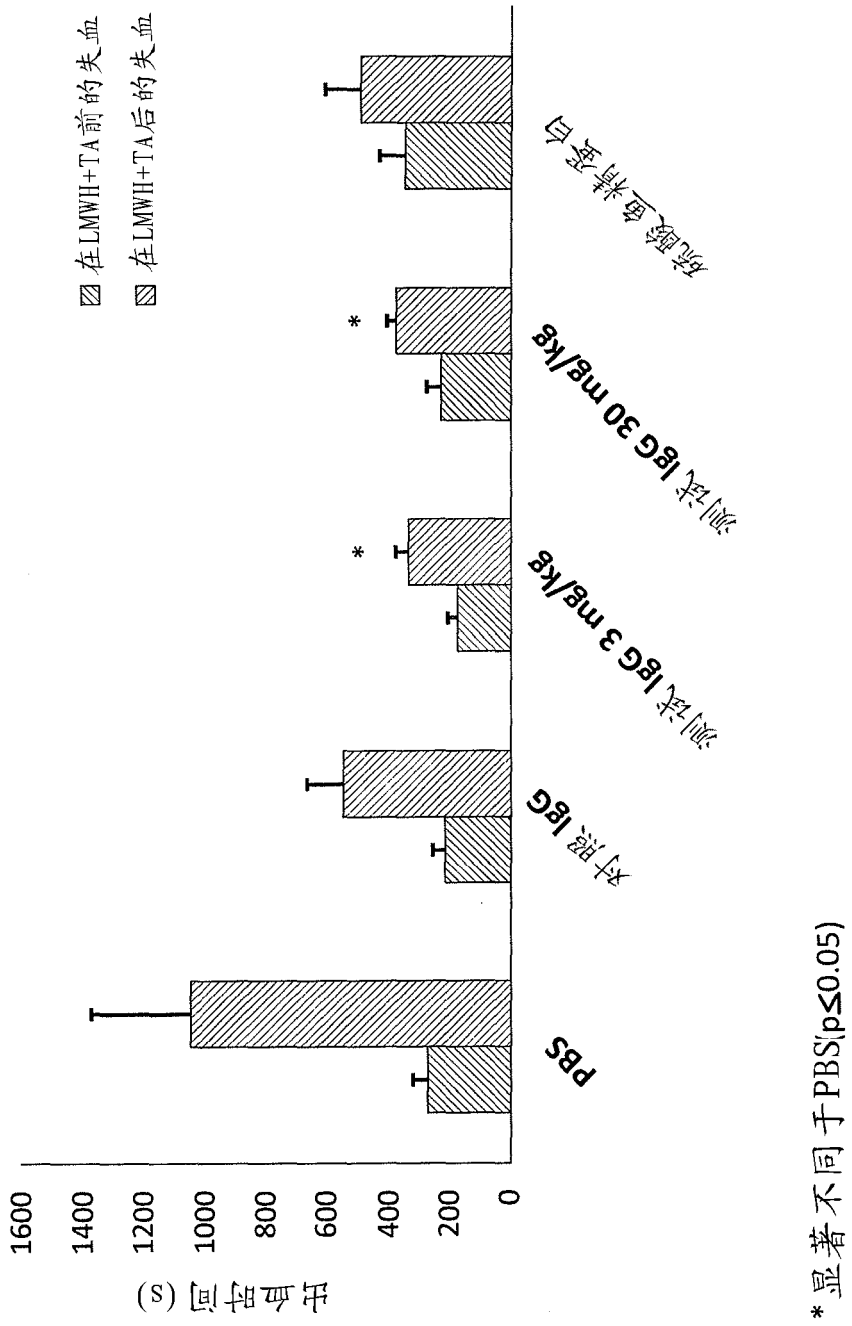

出血时间 (S)
1600
1400
1200
1000
800
600
400
200
0
PBS
对照 IgG
测试 IgG 3 mg/kg
测试 IgG 30 mg/kg
阴性血浆蛋白
在LMWH+TA前的失血
在LMWH+TA后的失血
*
*
*显著不同于PBS(p≤0.05)

图 14

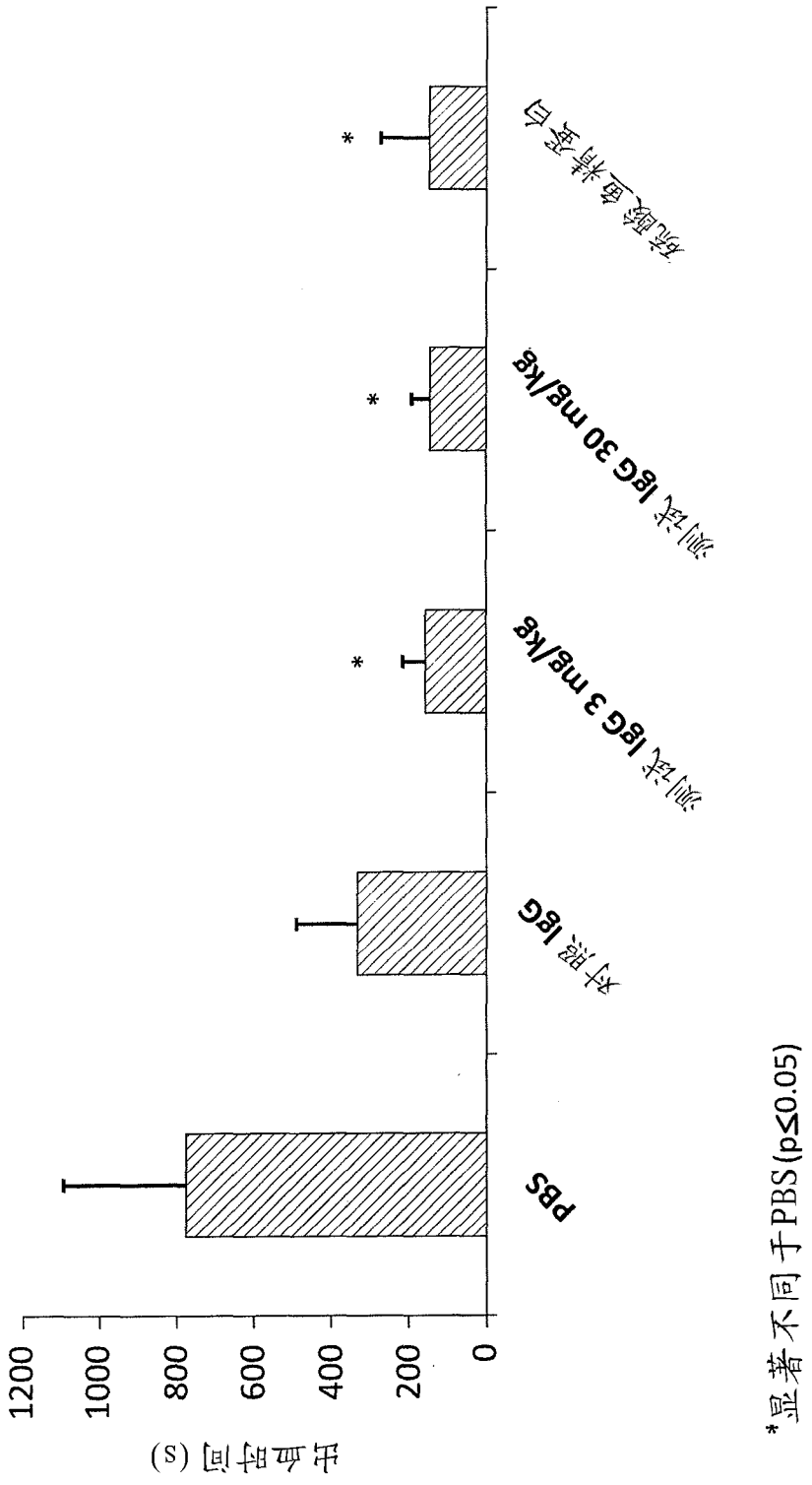

出血时间 (S)
0
200
400
600
800
1000
1200
PBS
对照 IgG
测试 IgG 3 mg/kg
测试 IgG 30 mg/kg
预防血清蛋白
*
*
*
*显著不同于PBS(p≤0.05)

图 15

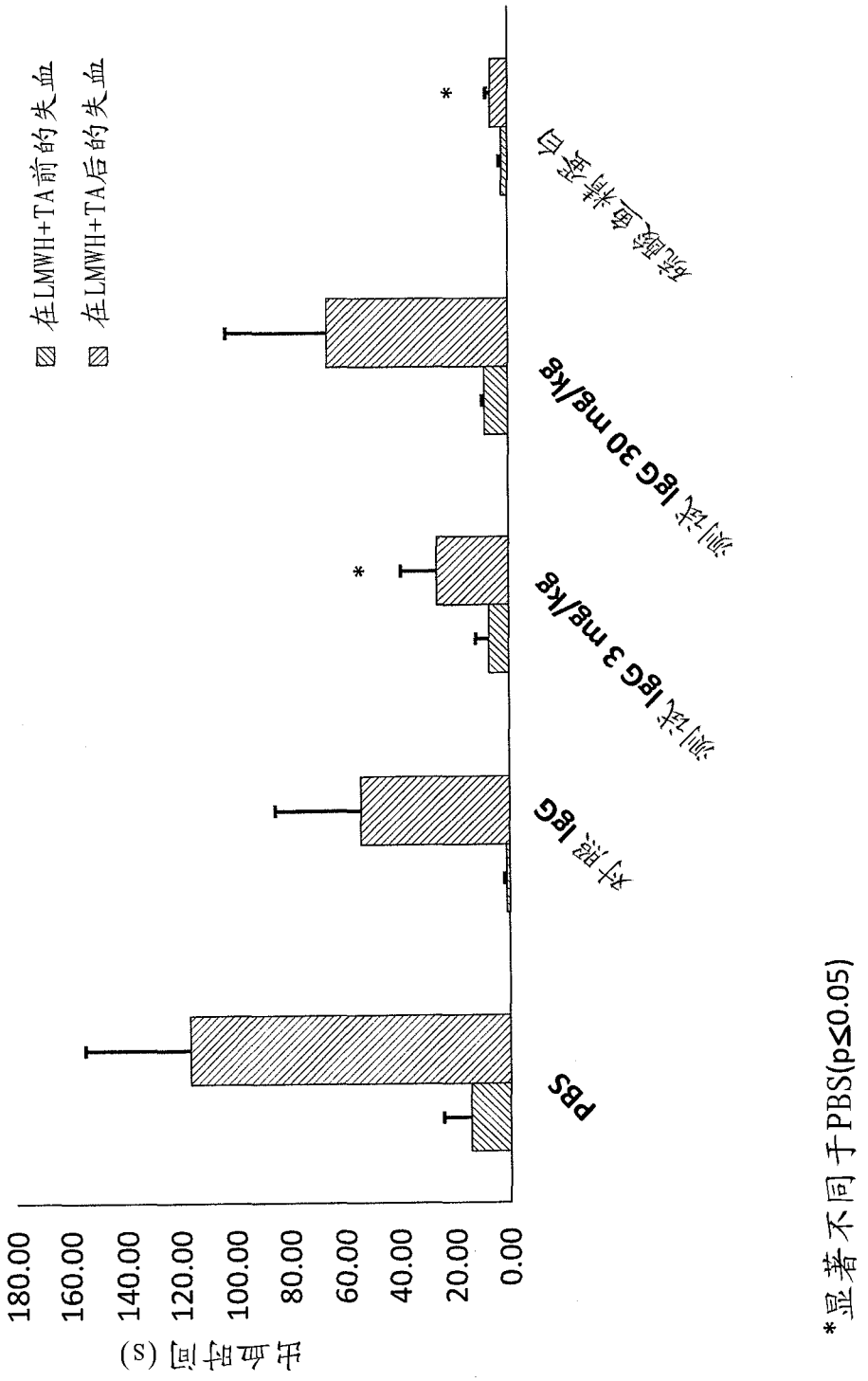

在LMWH+TA前的失血
在LMWH+TA后的失血
出血时间 (S)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
PBS
对照 IgG
测试 IgG 3 mg/kg
测试 IgG 30 mg/kg
疾病免疫蛋白
*
*
*显著不同于PBS(p≤0.05)

图 16