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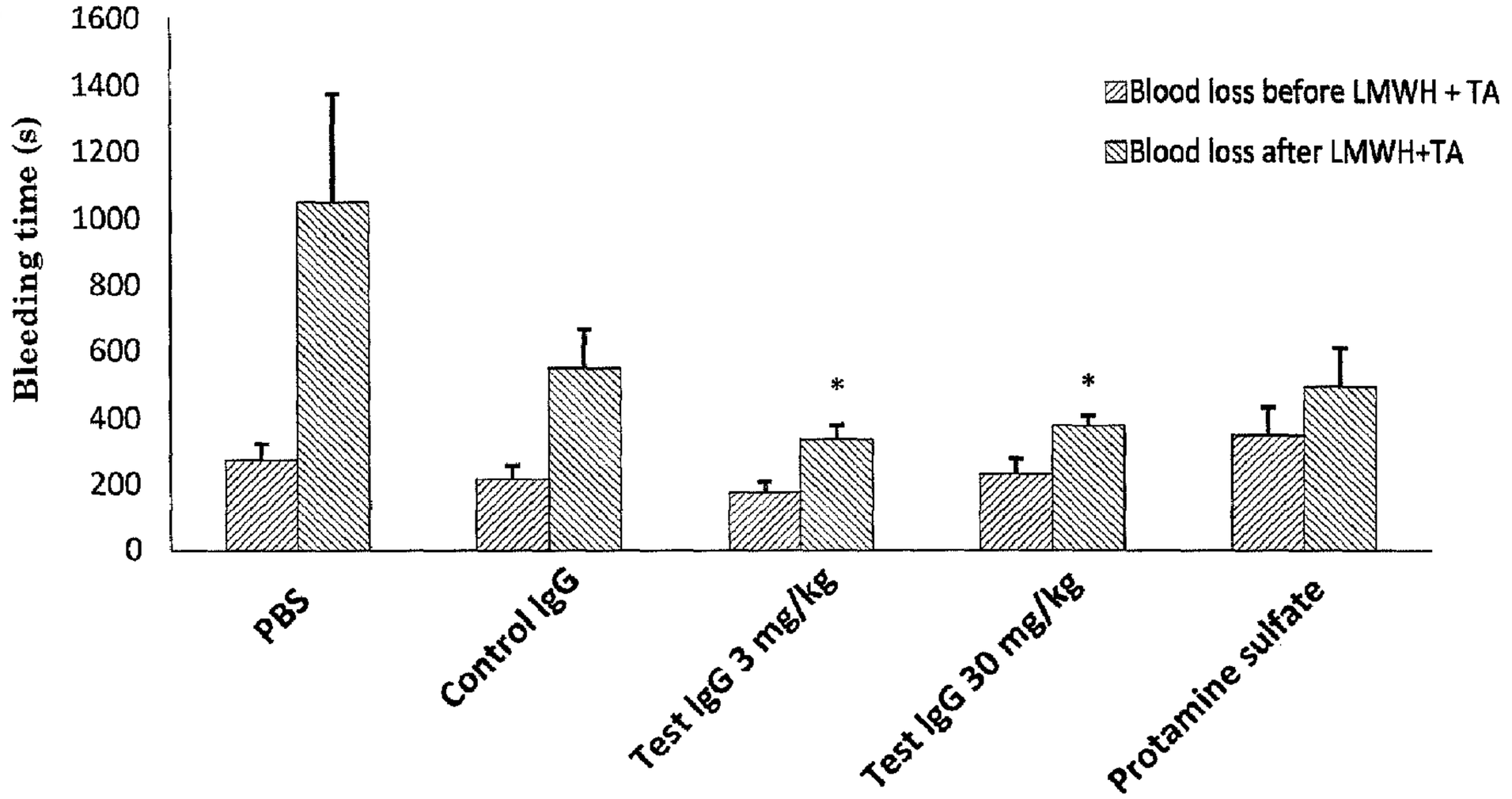
(54) Titre : ANTICORPS MONOCLONAUX DIRIGES CONTRE UN COMPLEXE ANTITHROMBINE BETA-HEPARINE
(54) Title: MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN BETA COMPLEXED WITH HEPARIN

FIG. 14



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

(57) Abrégé/Abstract:
This patent document relates to antibodies, antigen-binding antibody fragments (Fabs), and other protein scaffolds, directed against human antithrombin ß complexed with heparin and/or heparin-like structure (ATßH). These ATßH binding proteins can block the anti-coagulant activity of ATß 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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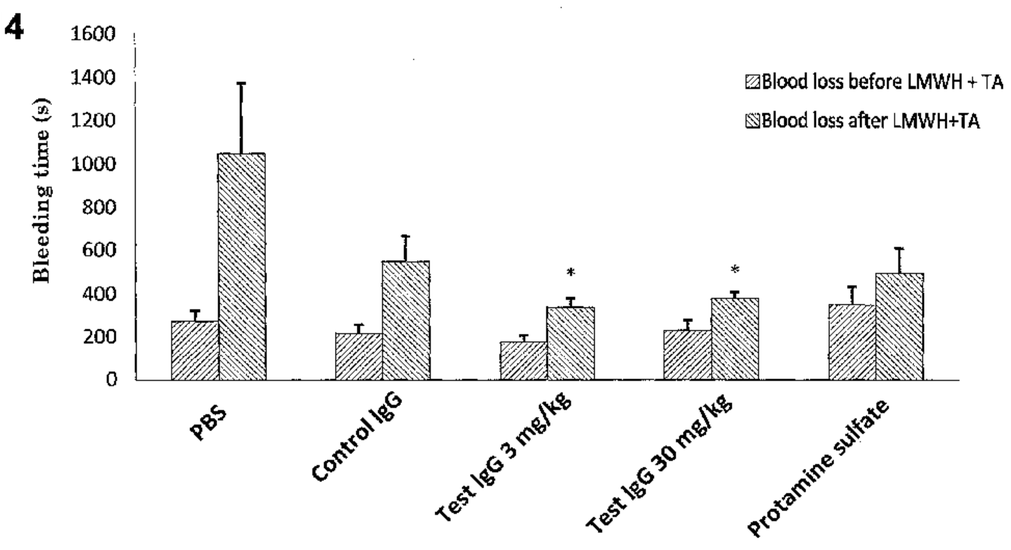
(54) **Title**: MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN BETA COMPLEXED WITH HEPARIN

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.

WO 2014/153195 A1

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.10

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	TCTTCCCCGCTAGCACCCAGCAGCAAG
	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 TGCCCTGCCCCCTGAACTGCTGGGCGG ACCCAGCGTGTTCCTGTTCCCCCCAAA GCCCAAGGACACCCTGATGATCAGCC GGACCCCCCGAAGTGACCTGCGTGGTG 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 AAGCCTGGCCGGGCT CCCAGACTCCTCATC CATACCGCATCCACT AGGGCCCCTGGTGTC CCAGTCAGGATCACT GGCAGTGGGTCTGGA ACAGAGTTCACTCTC ACCATCAGCAGCCTG GAACCTGAAGATTTT GCAGTGTATTTCTGT CAGCAGTATGCTAGC CCACCTCGGACGTTC GGCCAAGGGACCAA GGTGGAAATCAAGC GAACTGTGGCTGCAC CATCTGTCTTCATCTT CCCGCCATCTGATGA GCAGTTGAAATCTGG AACTGCCTCTGTTGT GTGCCTGCTGAATAA CTTCTATCCCAGAGA GGCCAAAGTACAGTG GAAGGTGGATAACG CCCTCCAATCGGGTA ACTCCCAGGAGAGTG TCACAGAGCAGGAC AGCAAGGACAGCAC CTACAGCCTCAGCAG CACCCTGACGCTGAG CAAAGCAGACTACG AGAAACACAAAGTCT ACGCCTGCGAAGTCA CCCATCAGGGCCTGA GCTCGCCCGTCACAA AGAGC TTCAACAGGGGAGA GTGT (SEQ ID NO: 15)	TCCGTTAAAGGTCGCTTCACTATCTCT AGAGACAACTCTAAGAATACTCTCTA CTTGCAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGAGAGAAAACGTCGGATCTATCGGG GAGTTATTCTGAGGCCCTTGACTACTG GGGCCAGGGAACCCTGGTCACCGTCT CAAGCGCCTCCACCAAGGGCCCATCG GTCTTCCCCGCTAGCACCCAGCAGCAA 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: 16)

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

	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.

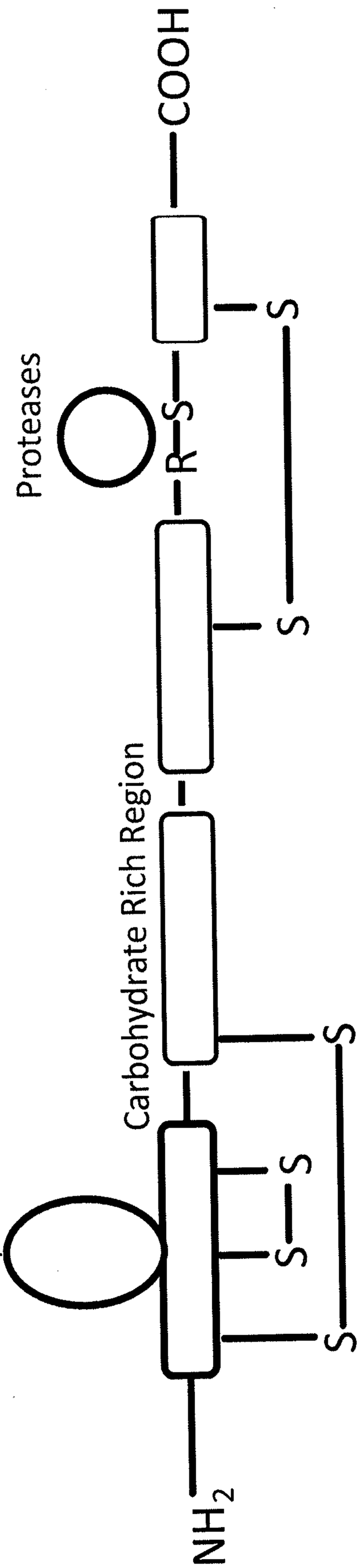
26. The method of claim 25, wherein the clotting factor is selected from the group consisting of Factor VIIa, Factor VIII, and Factor IX.

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
S
Carbohydrate Rich Regi
Heparin
S
S
S
NH_2

FIG. 1

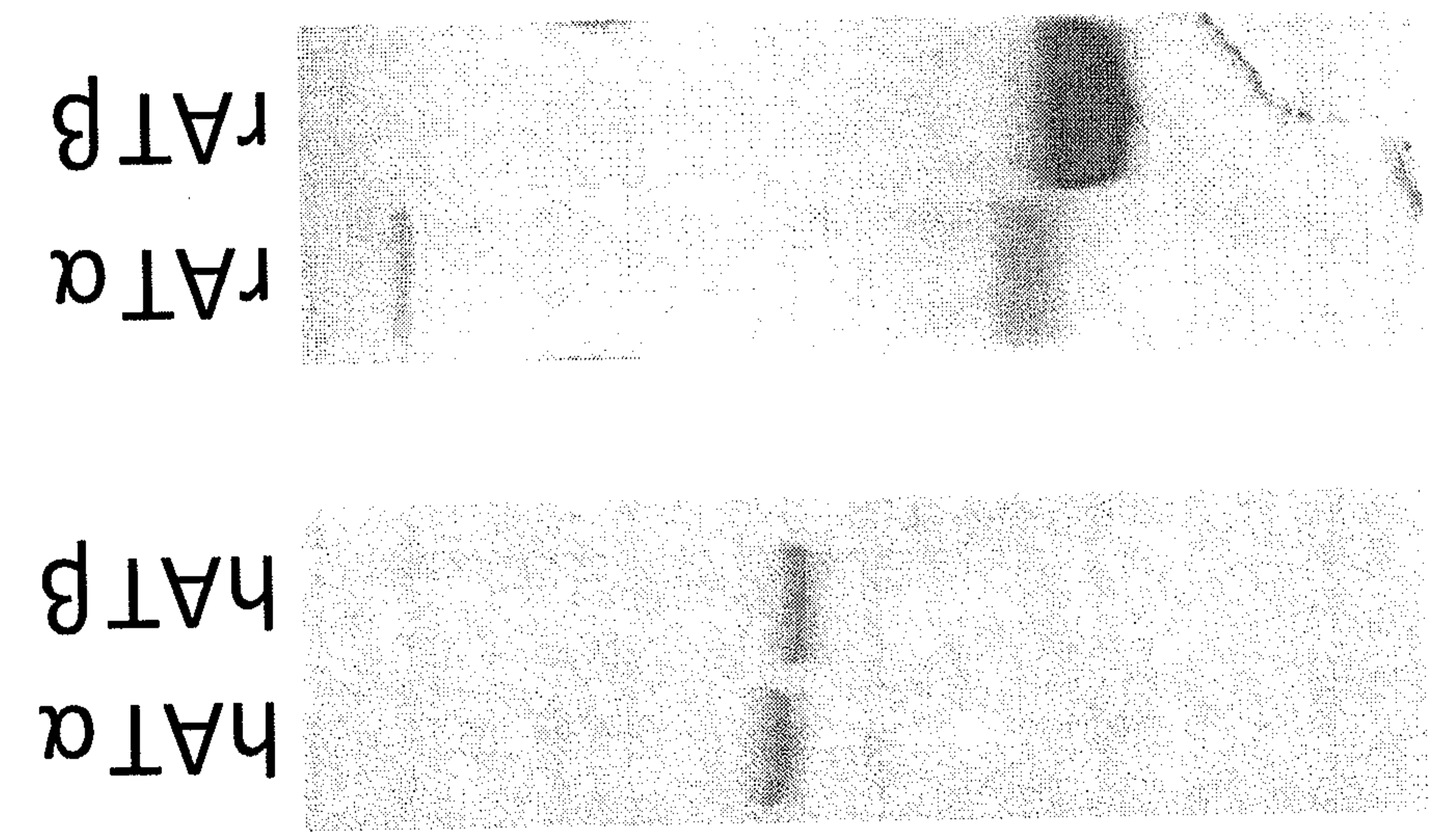
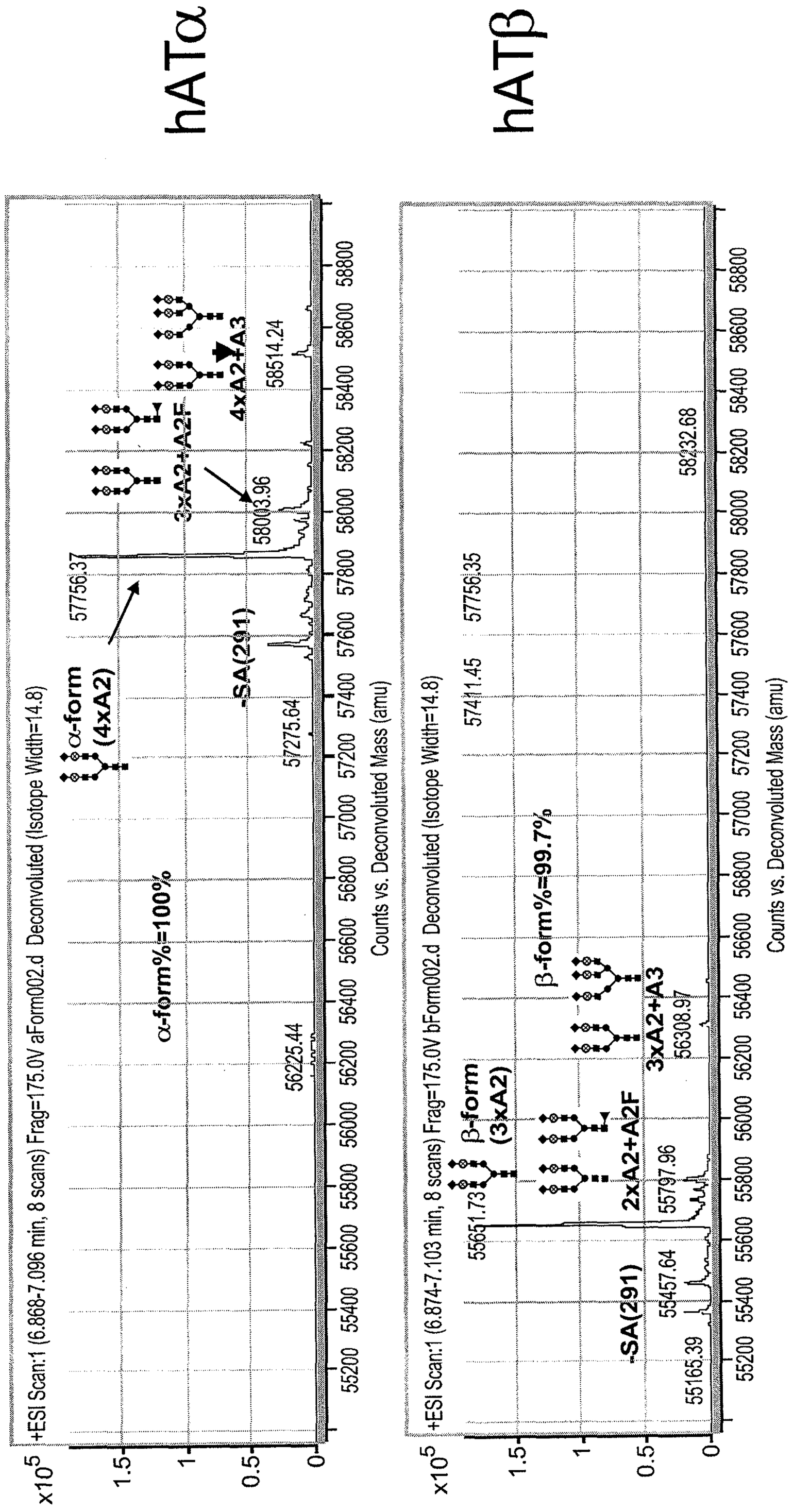


FIG. 2A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hATβ
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α-form%=100%
3xA2+A2F
4xA2+A3
-SA(291)
57756.37
58003.96
58514.24
56225.44
57275.64
Counts vs. Deconvoluted Mass (amu)
+ESI Scan:1 (6.874-7.103 min, 8 scans) Frag=175.0V bForm002.d Deconvoluted (Isotope Width=14.8)
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β-form%=99.7%
2xA2+A2F
3xA2+A3
-SA(291)
57411.45
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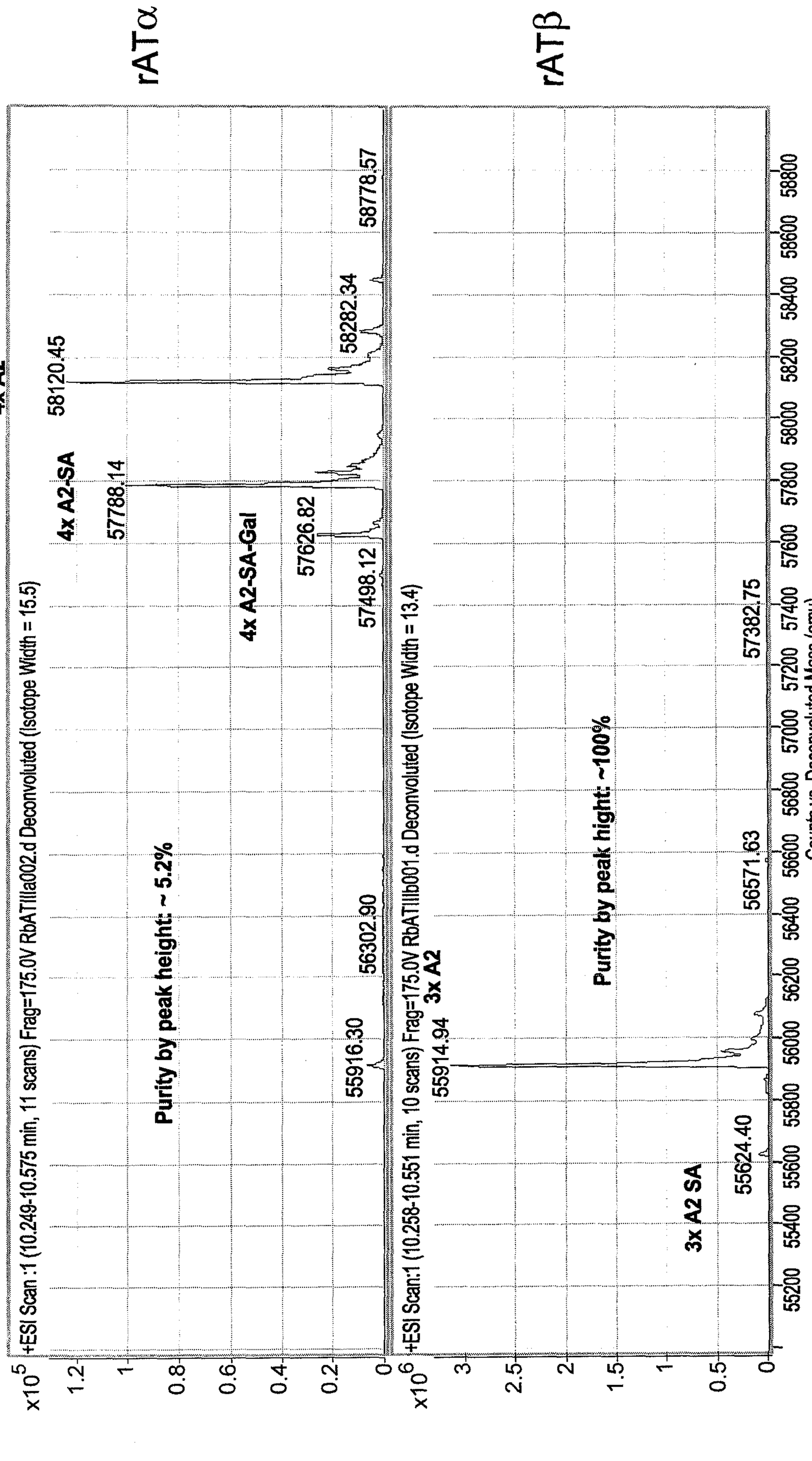
55165.39
Counts vs. Deconvoluted Mass (amu)

FIG. 2B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rATβ
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57626.82
57498.12
58282.34
+ESI Scan :1 (10.249-10.575 min, 11 scans) Frag=175.0V RbATIIIa002.d Deconvoluted (Isotope Width = 15.5)
Purity by peak height: ~ 5.2%
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3x A2
55914.94
3x A2 SA
55624.40
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Purity by peak hight: ~100%
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Counts vs. Deconvoluted Mass (amu)

FIG. 2C

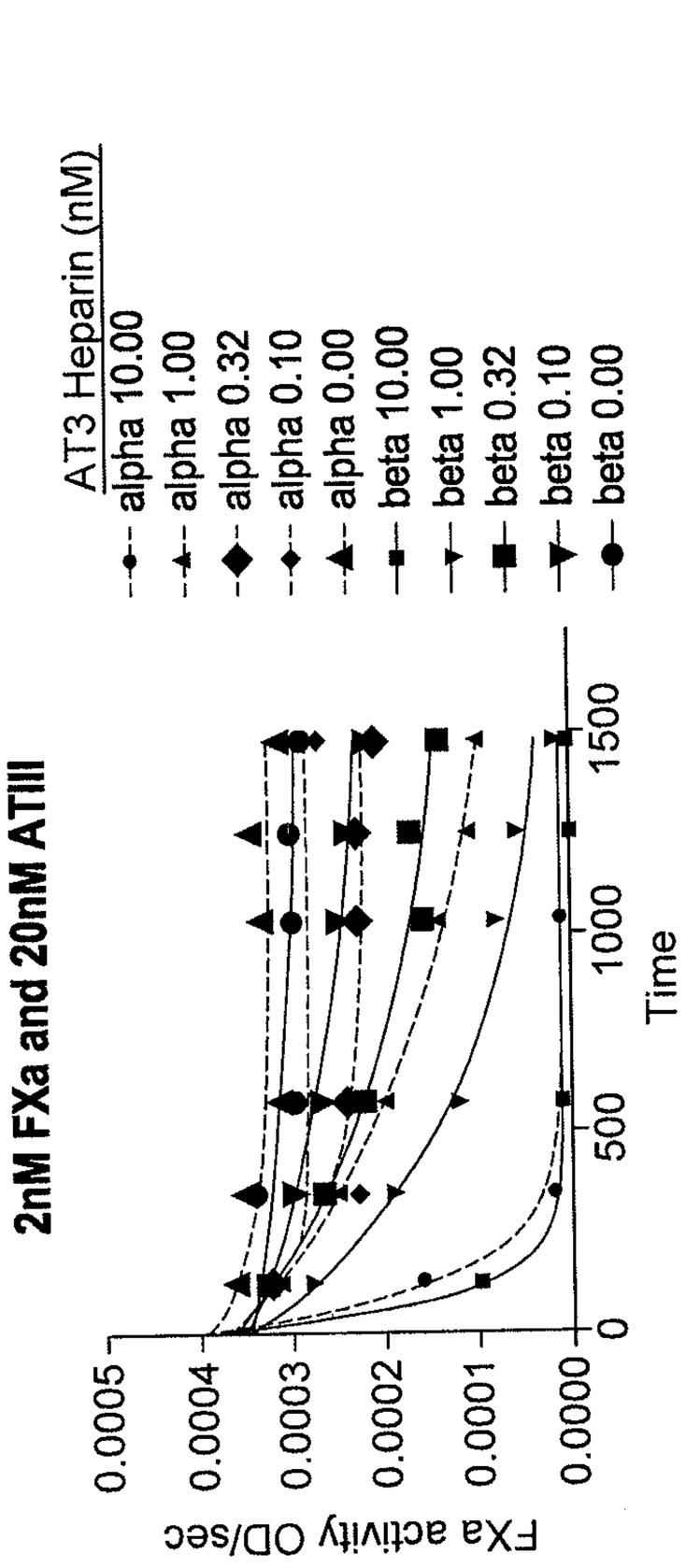

AT3 Heparin (nM)
alpha 10.00
alpha 1.00
alpha 0.32
alpha 0.10
alpha 0.00
beta 10.00
beta 1.00
beta 0.32
beta 0.10
beta 0.00
2nM FXa and 20nM ATIII
FXa activity OD/sec
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
0
500
1000
1500
Time

FIG. 3A

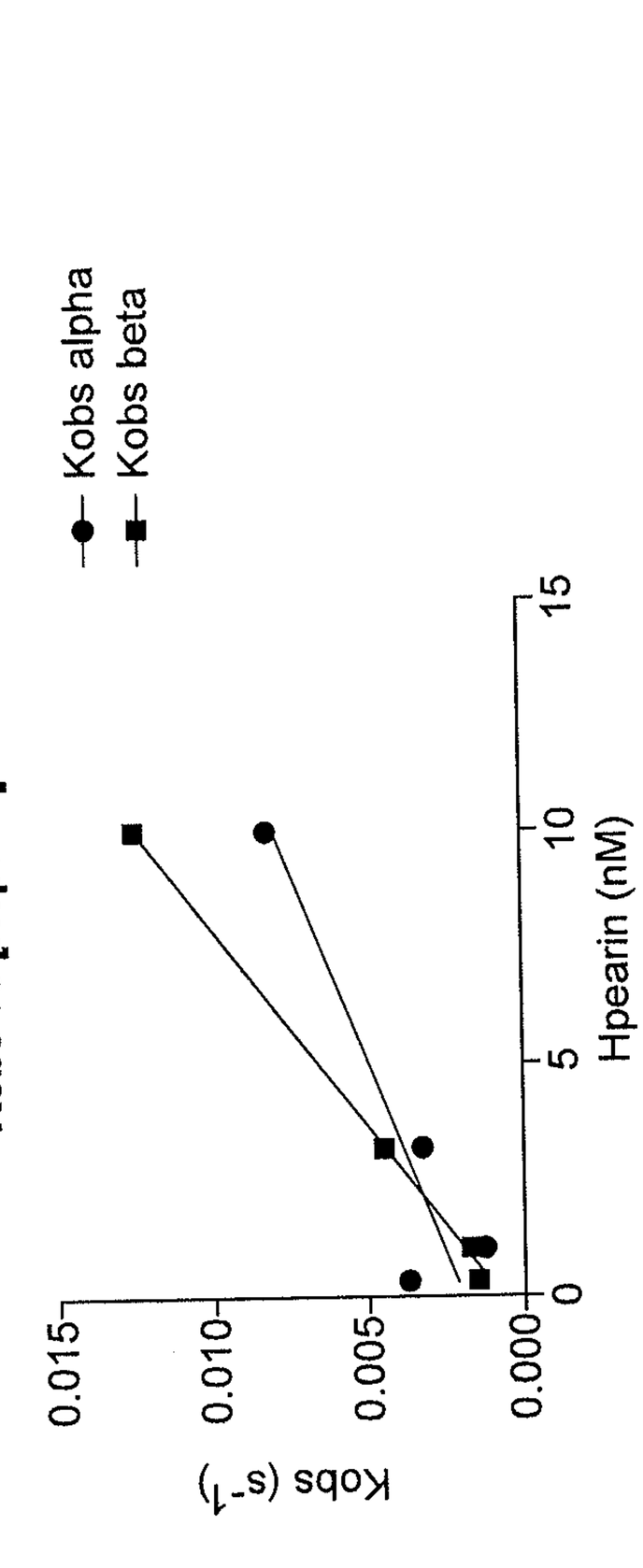

Kobs alpha
Kobs beta
Kobs vs [Heparin]
Kobs (s-1)
0.015
0.010
0.005
0.000
0
5
10
15
Hpearin (nM)

FIG. 3B

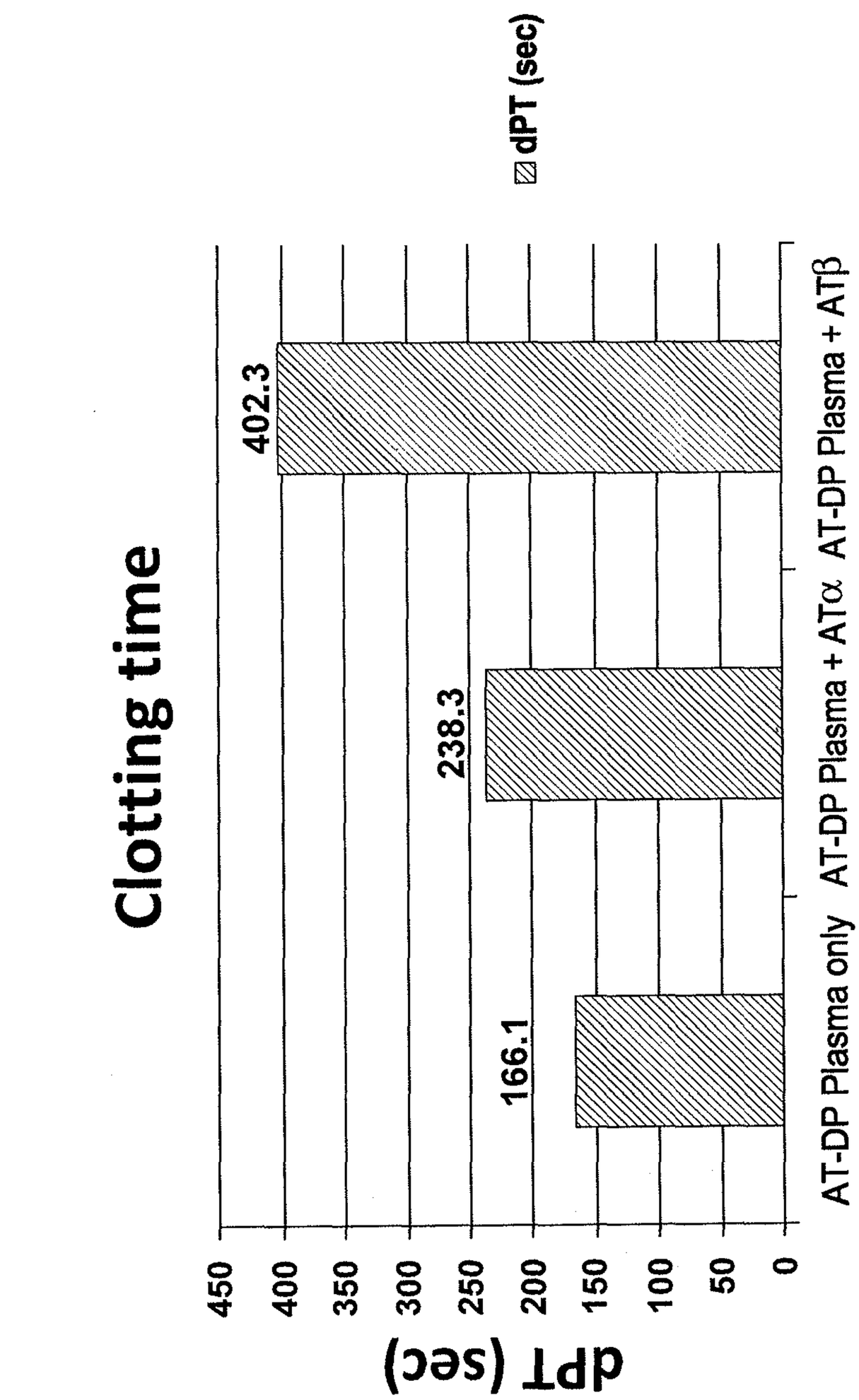

Clotting time
dPT (sec)
402.3
238.3
166.1
450
400
350
300
250
200
150
100
50
0
AT-DP Plasma only
AT-DP Plasma + ATα
AT-DP Plasma + ATβ
ATα, ATβ 0.2 um, heparin 0.1U/ml
dPT (sec)

FIG. 3C

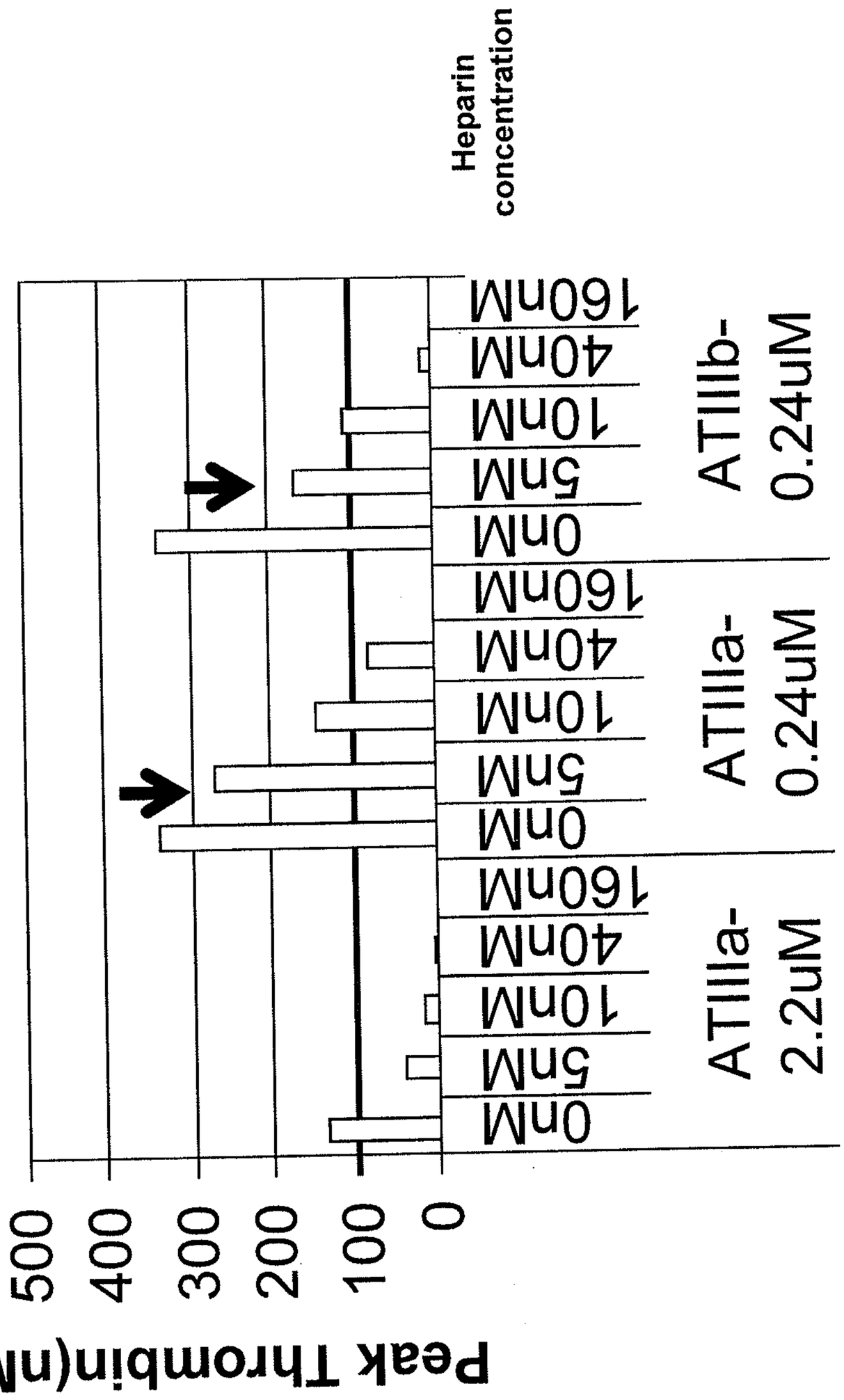

Hepa
concent
160nM
40nM
10nM
5nM
0nM
ATIIIb-
0.24uM
160nM
40nM
10nM
5nM
0nM
ATIIIa-
0.24uM
160nM
40nM
10nM
5nM
0nM
ATIIIa-
2.2uM
500
400
300
200
100
0
Peak Thrombin(nM)

FIG. 3D

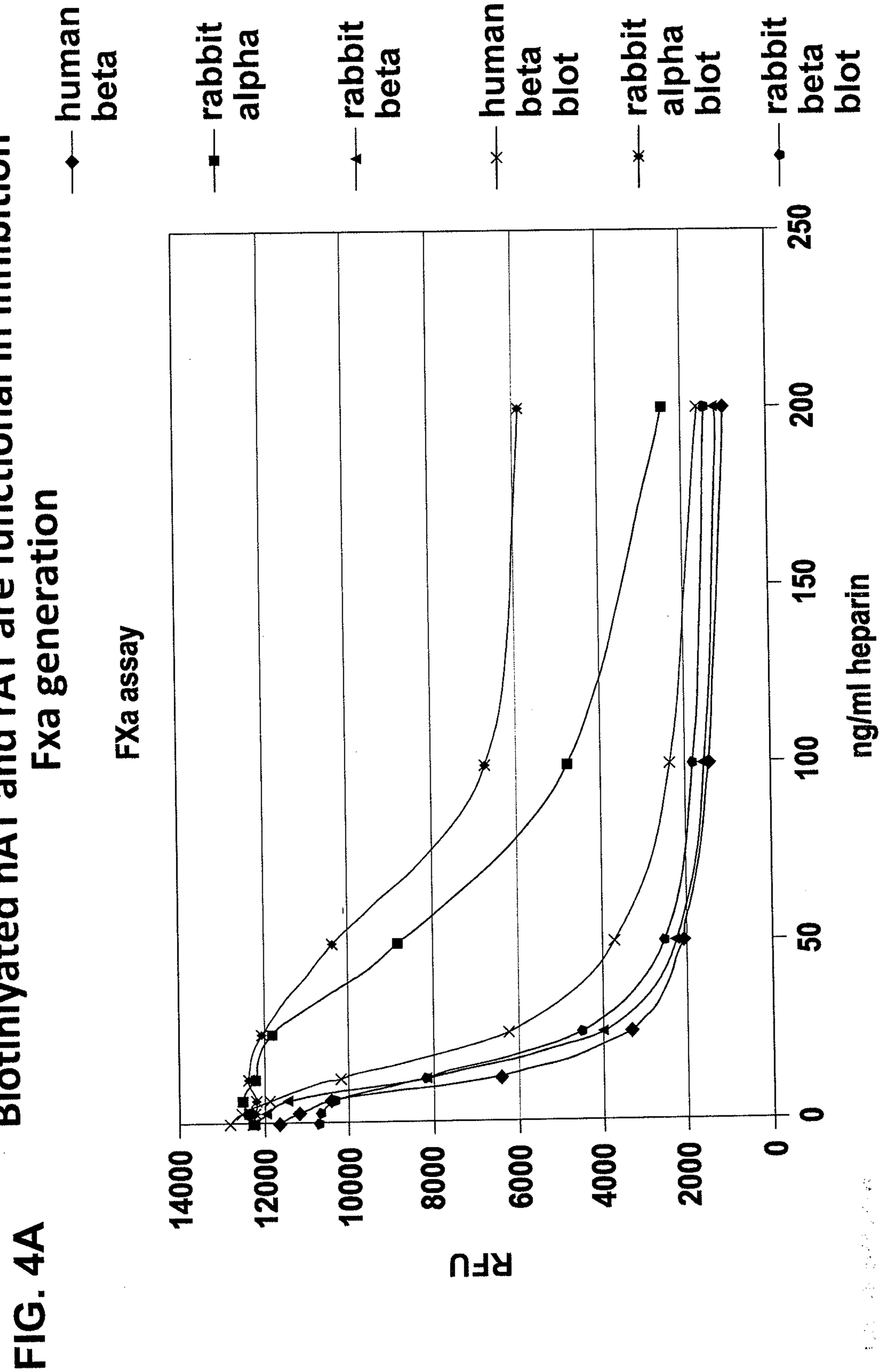

Biotinlyated hAT and rAT are functional in inhibition
Fxa generation
FXa assay
RFU
14000
12000
10000
8000
6000
4000
2000
0
0
50
100
150
200
250
ng/ml heparin
human beta
rabbit alpha
rabbit beta
human beta blot
rabbit alpha blot
rabbit beta blot

FIG. 4A

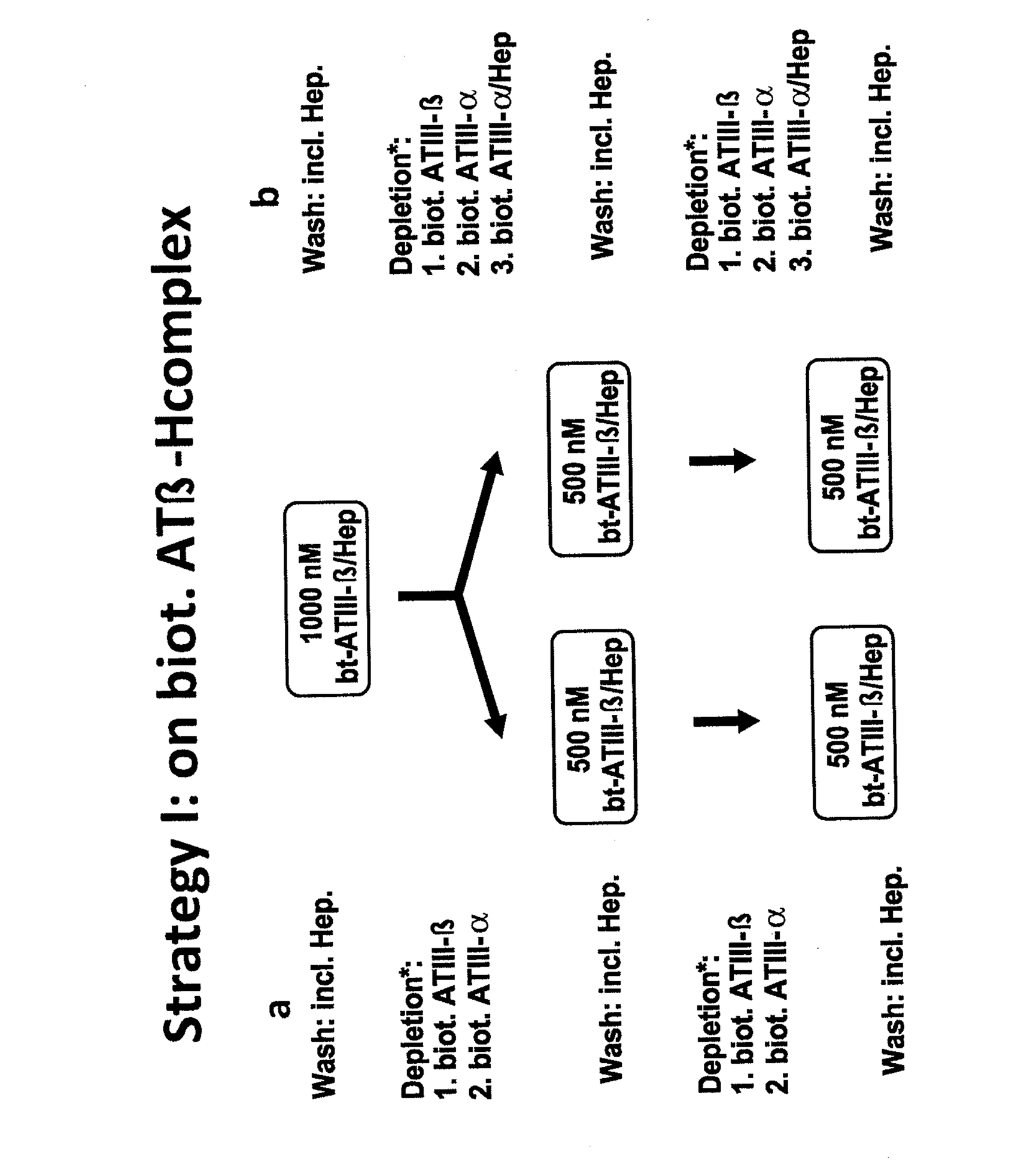

Strategy I: on biot. ATß-Hcomplex
a
Wash: incl. Hep.
1000 nM bt-ATIII-ß/Hep
Depletion*:
1. biot. ATIII-ß
2. biot. ATIII-α
Wash: incl. Hep.
500 nM bt-ATIII-ß/Hep
Depletion*:
1. biot. ATIII-ß
2. biot. ATIII-α
Wash: incl. Hep.
500 nM bt-ATIII-ß/Hep
b
Wash: incl. Hep.
Depletion*:
1. biot. ATIII-ß
2. biot.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

FIG. 4B

FIG. 4C

Strategy II: on biot. ATß-H complex, including rabbit protein

Round:	1	2	3
a	1000 nM bt-ATIII-ß/Hep	1000 nM bt-rbATIII-ß/Hep	500 nM bt-ATIII-ß/Hep
	Wash: incl. Hep.	Wash: incl. Hep.	Wash: incl. Hep.
	Depletion*: 1. biot. rbATIII-ß 2. biot. rbATIII-α	Depletion*: 1. biot. ATIII-ß 2. biot. ATIII-α	Depletion*: 1. biot. ATIII-ß 2. biot. ATIII-α 3. biot. ATIII-α/Hep
b	1000 nM bt-rbATIII-ß/Hep	1000 nM bt-rbATIII-ß/Hep	500 nM bt-ATIII-ß/Hep
	Wash: incl. Hep.	Wash: incl. Hep.	Wash: incl. Hep.
	Depletion*: 1. biot. rbATIII-ß 2. biot. rbATIII-α 3. biot. ATIII-α/Hep	Depletion*: 1. biot. ATIII-ß 2. biot. ATIII-α 3. biot. ATIII-α/Hep	Depletion*: 1. biot. ATIII-ß 2. biot. ATIII-α 3. biot. ATIII-α/Hep

*: round 2 and 3 only

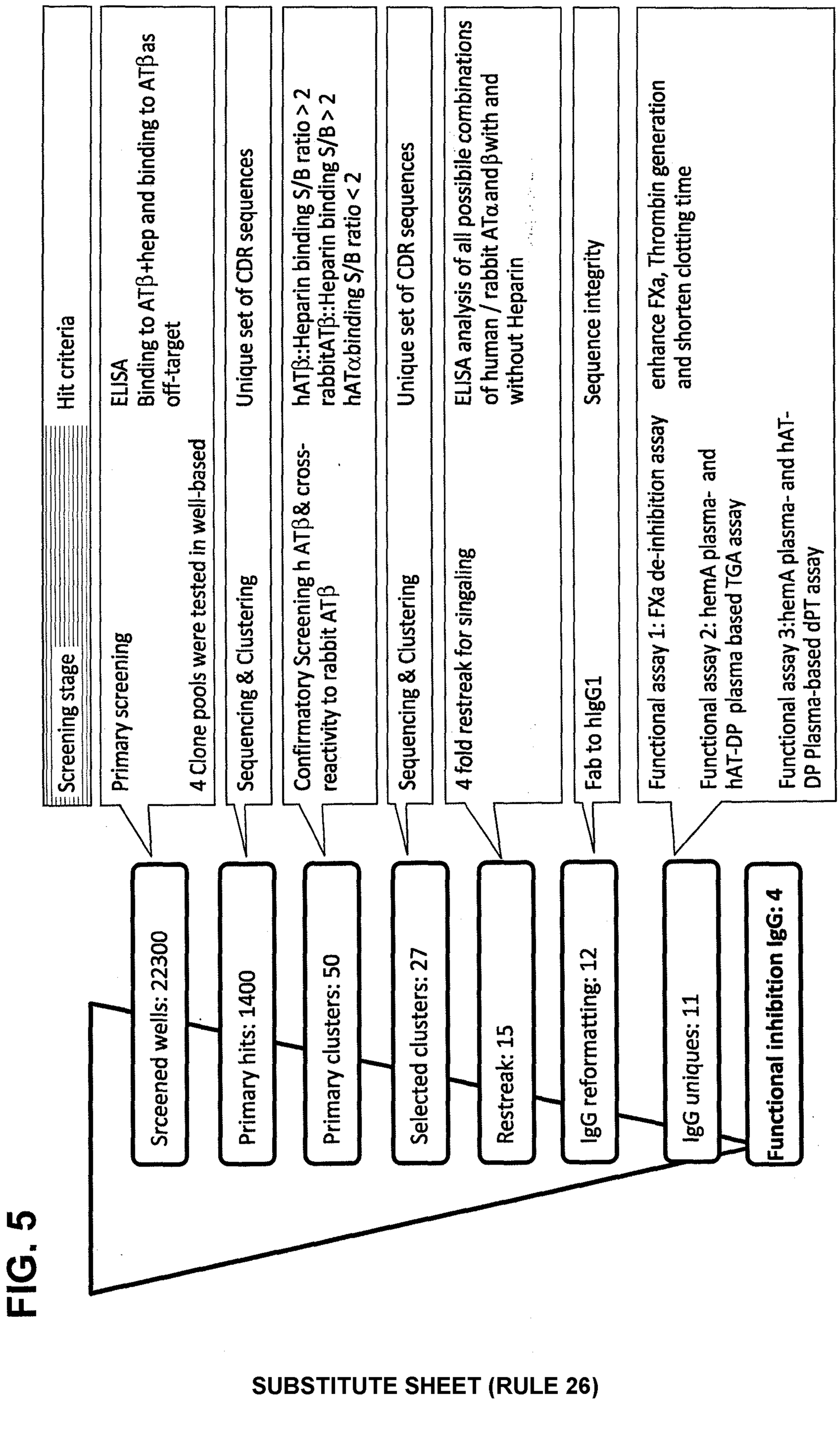
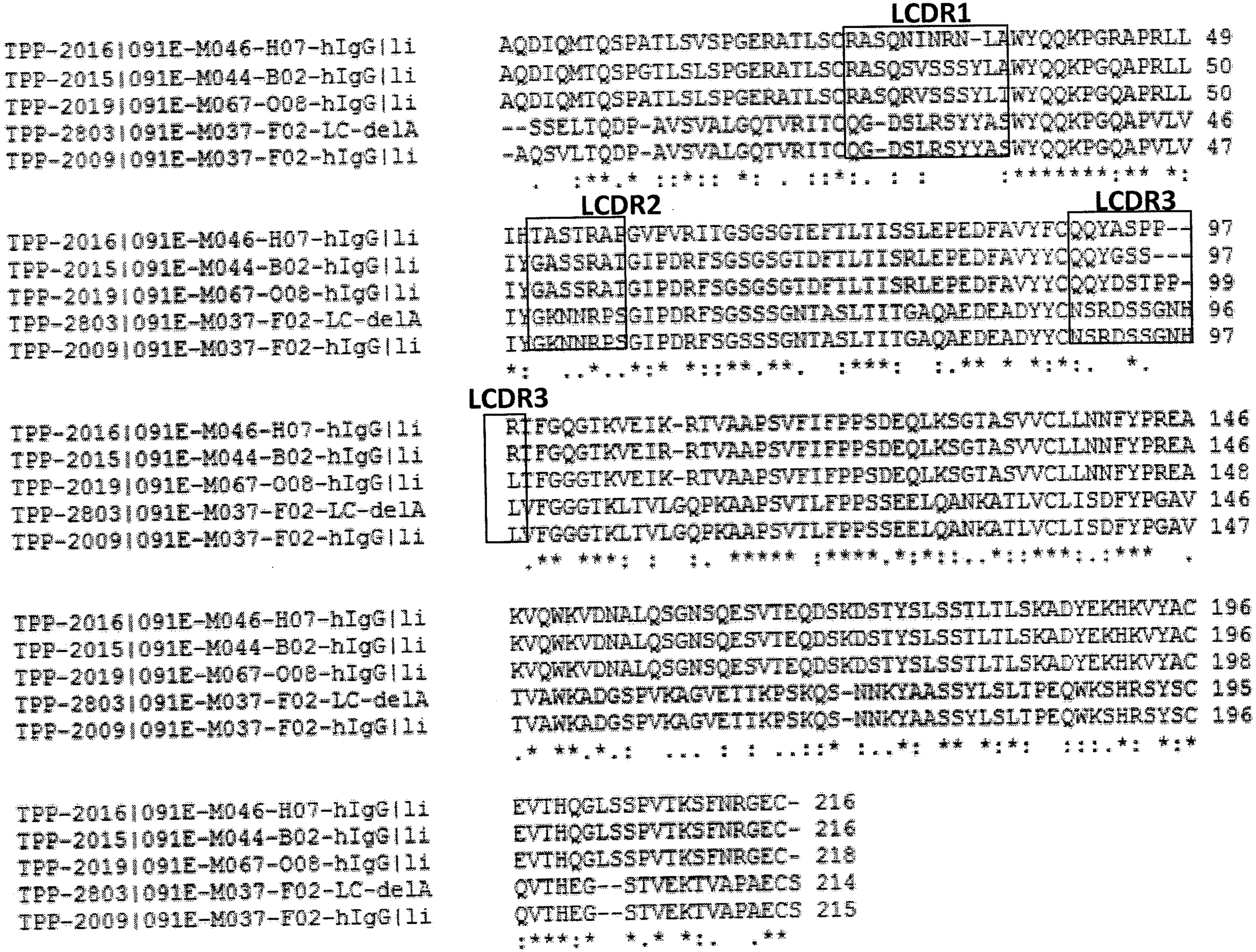

Screening stage
Hit criteria
Primary screening
ELISA
Binding to ATβ+hep and binding to ATβas
off-target
4 Clone pools were tested in well-based
Screened wells: 22300
Sequencing & Clustering
Unique set of CDR sequences
Primary hits: 1400
Confirmatory Screening h ATβ& cross-reactivity to rabbit ATβ
hATβ::Heparin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

FIG. 5

FIG. 6A

Light Chain



SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

FIG. 6B

Heavy Chain

```
                                                
```

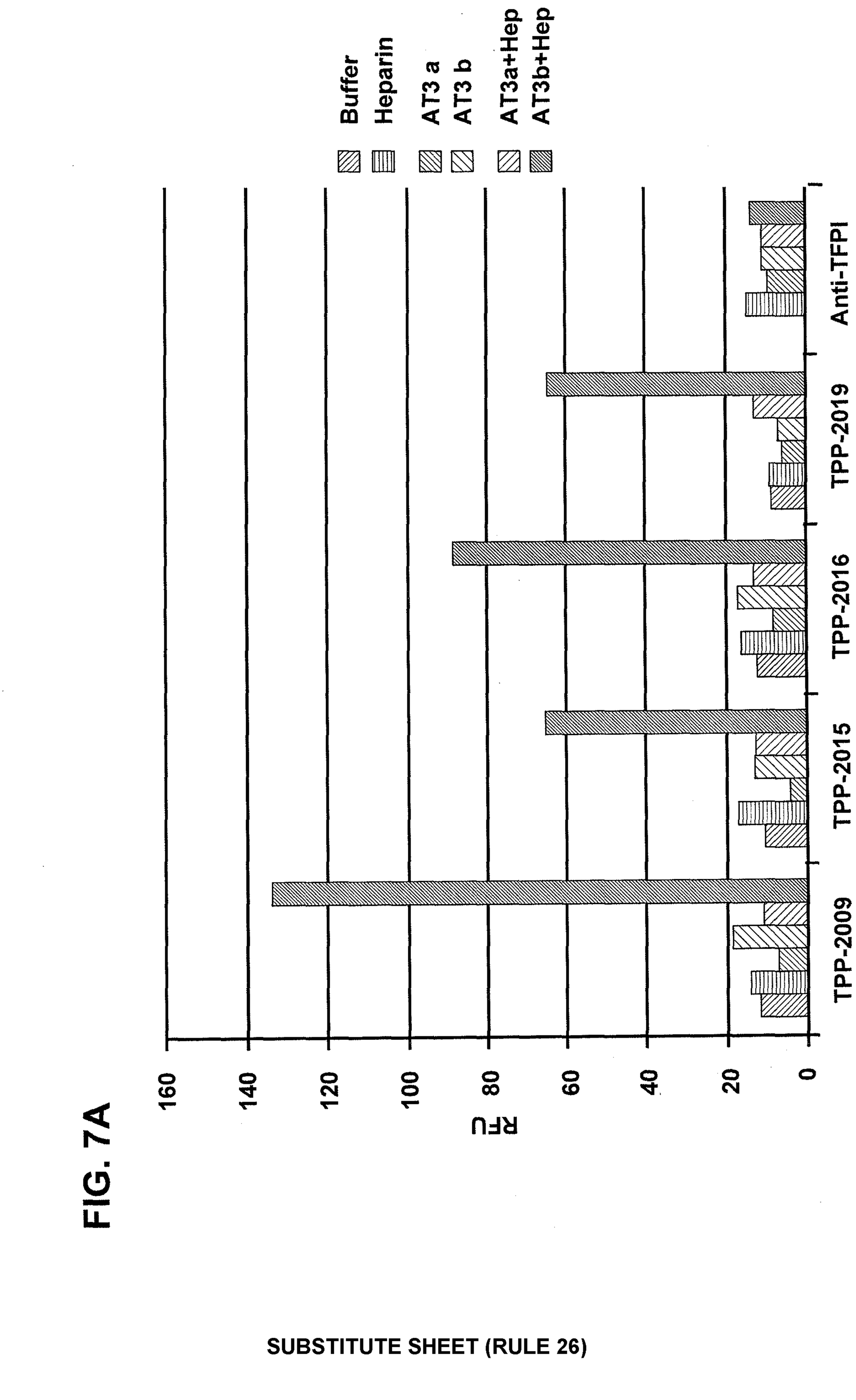

Buffer
Heparin
AT3 a
AT3 b
AT3a+Hep
AT3b+Hep
Anti-TFPI
TPP-2019
TPP-2016
TPP-2015
TPP-2009
RFU
160
140
120
100
80
60
40
20
0

FIG. 7A

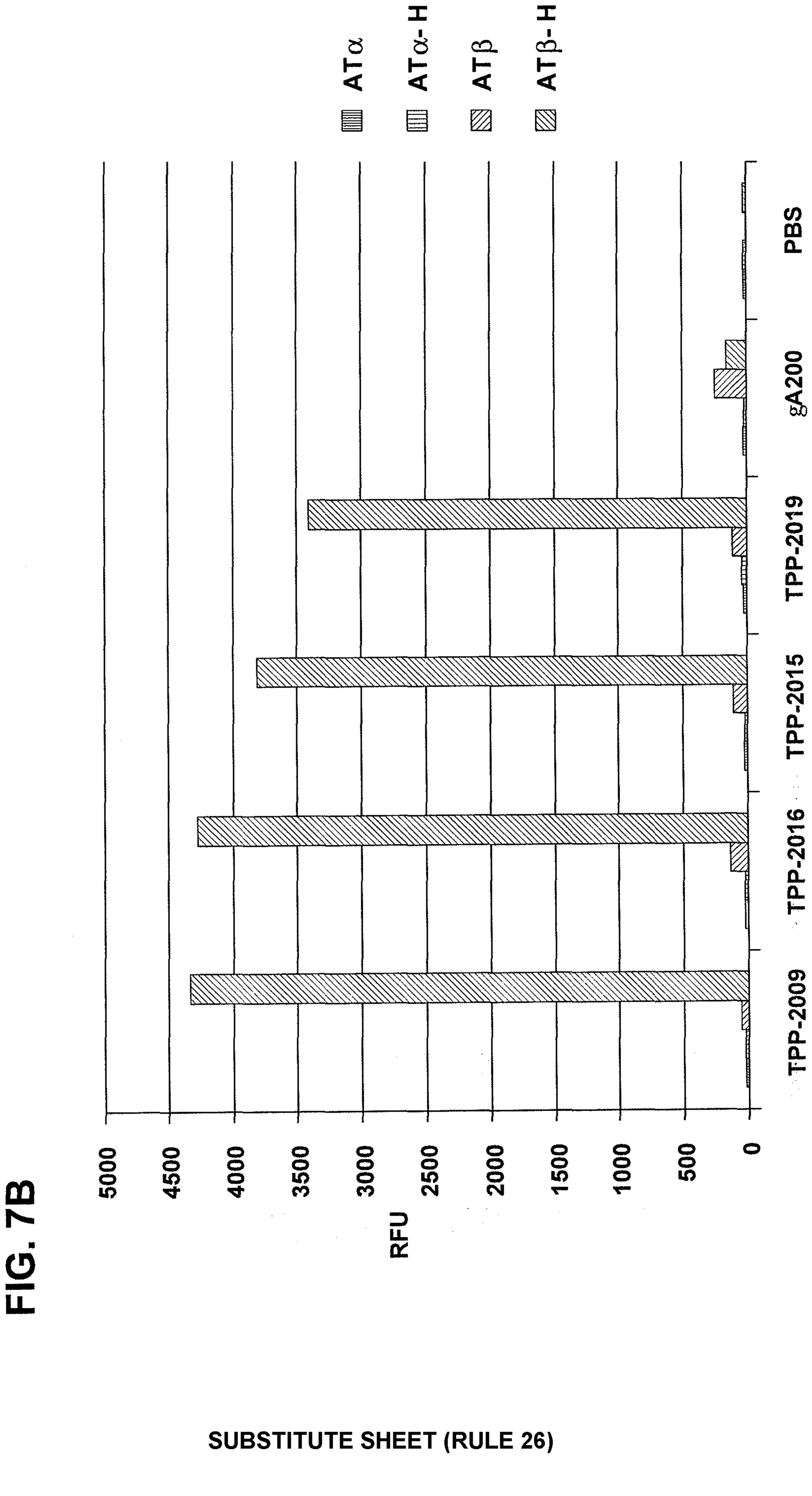

ATα
ATα-H
ATβ
ATβ-H
PBS
gA200
TPP-2019
TPP-2015
TPP-2016
TPP-2009
RFU
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0

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

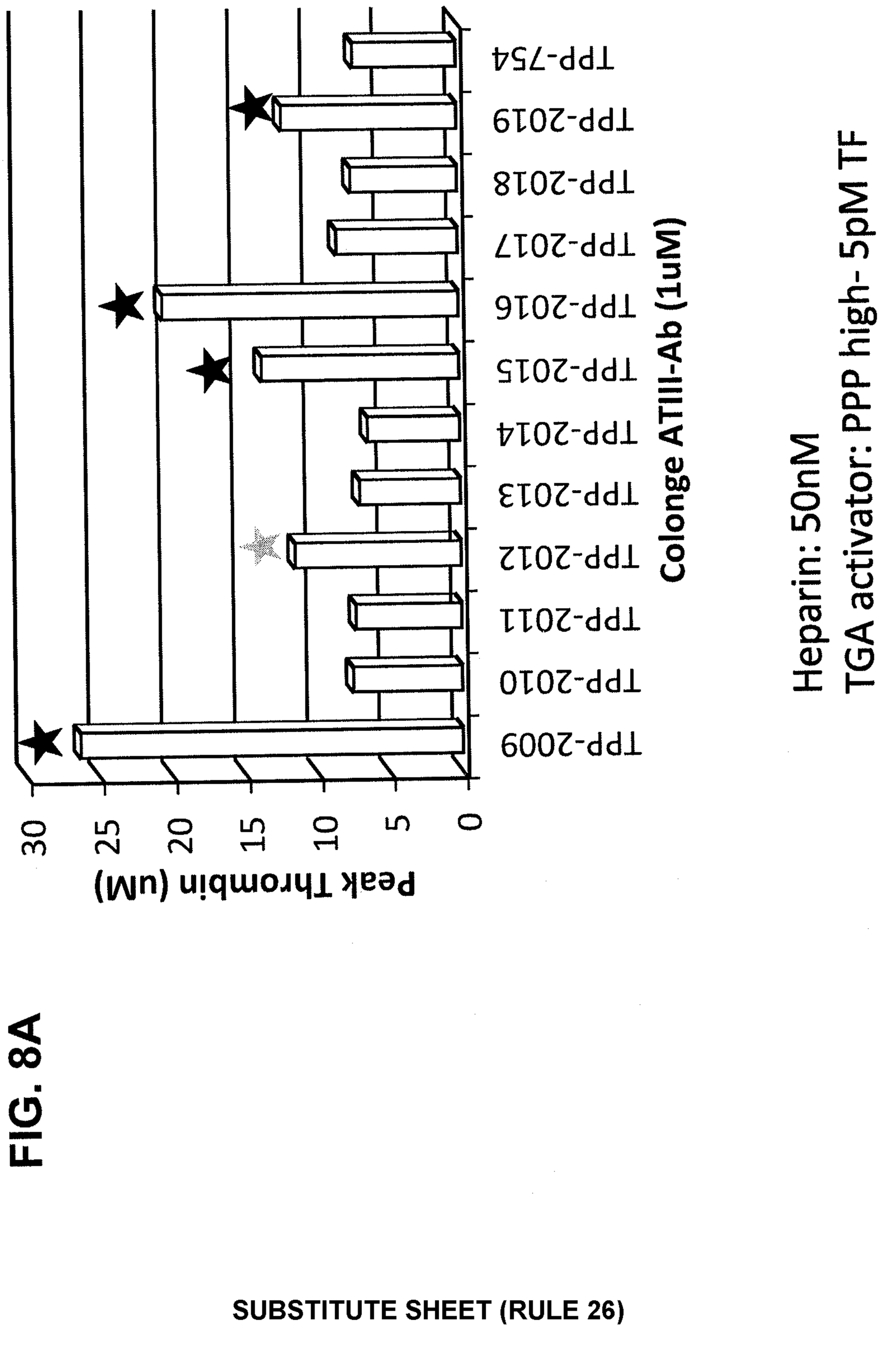
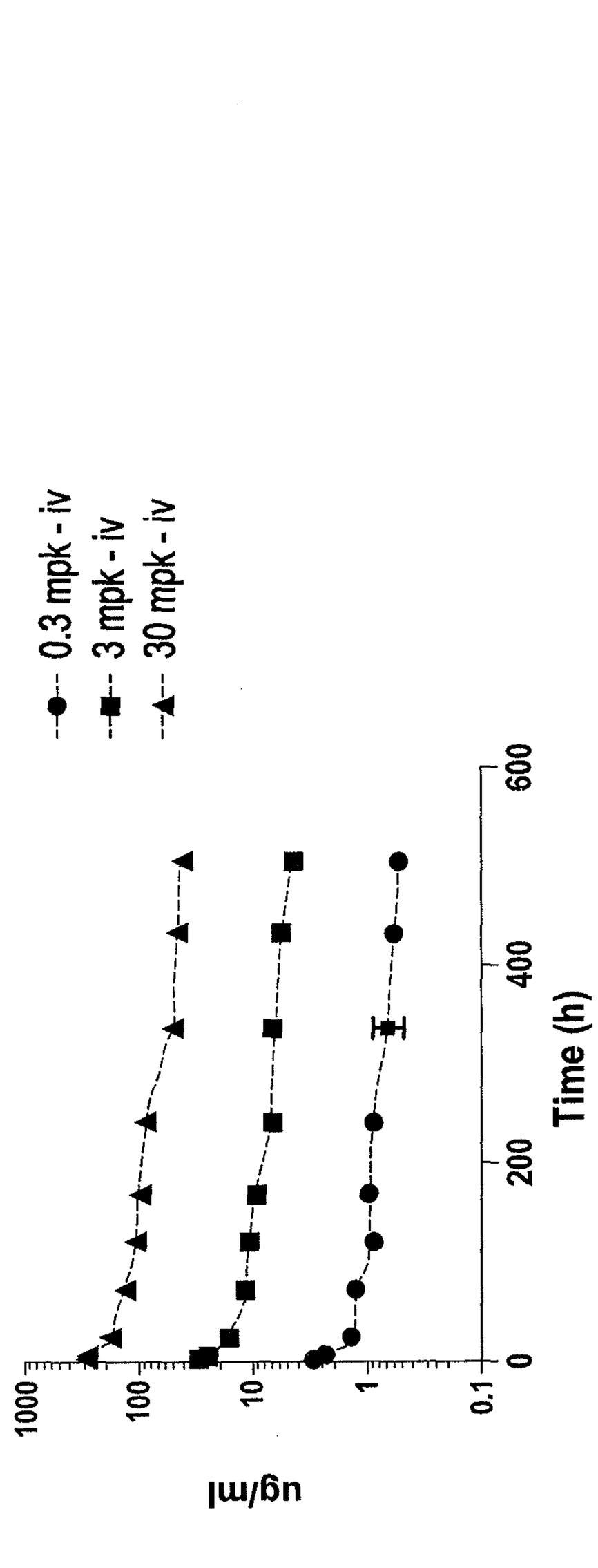

Peak Thrombin (uM)
30
25
20
15
10
5
0
TPP-2009
TPP-2010
TPP-2011
TPP-2012
TPP-2013
TPP-2014
TPP-2015
TPP-2016
TPP-2017
TPP-2018
TPP-2019
TPP-754
Colonge ATIII-Ab (1uM)
Heparin: 50nM
TGA activator: PPP high- 5pM TF

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



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

dose 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

FIG. 9

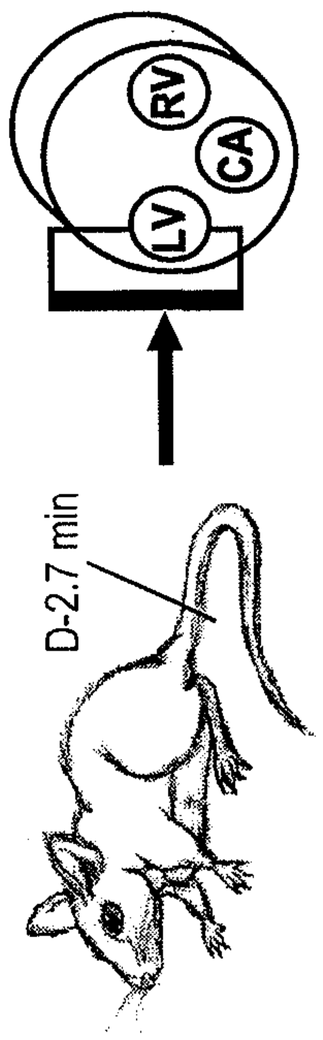


FIG. 10A

FIG. 10B

Figure 10B

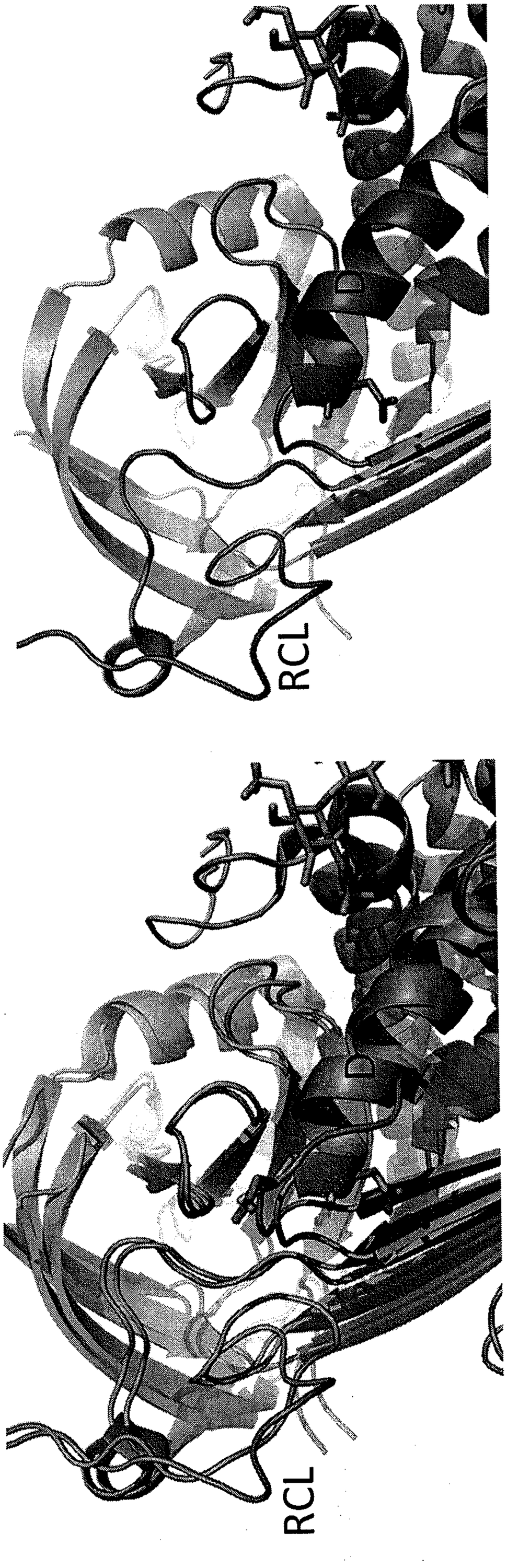


FIG. 11B

FIG. 11A

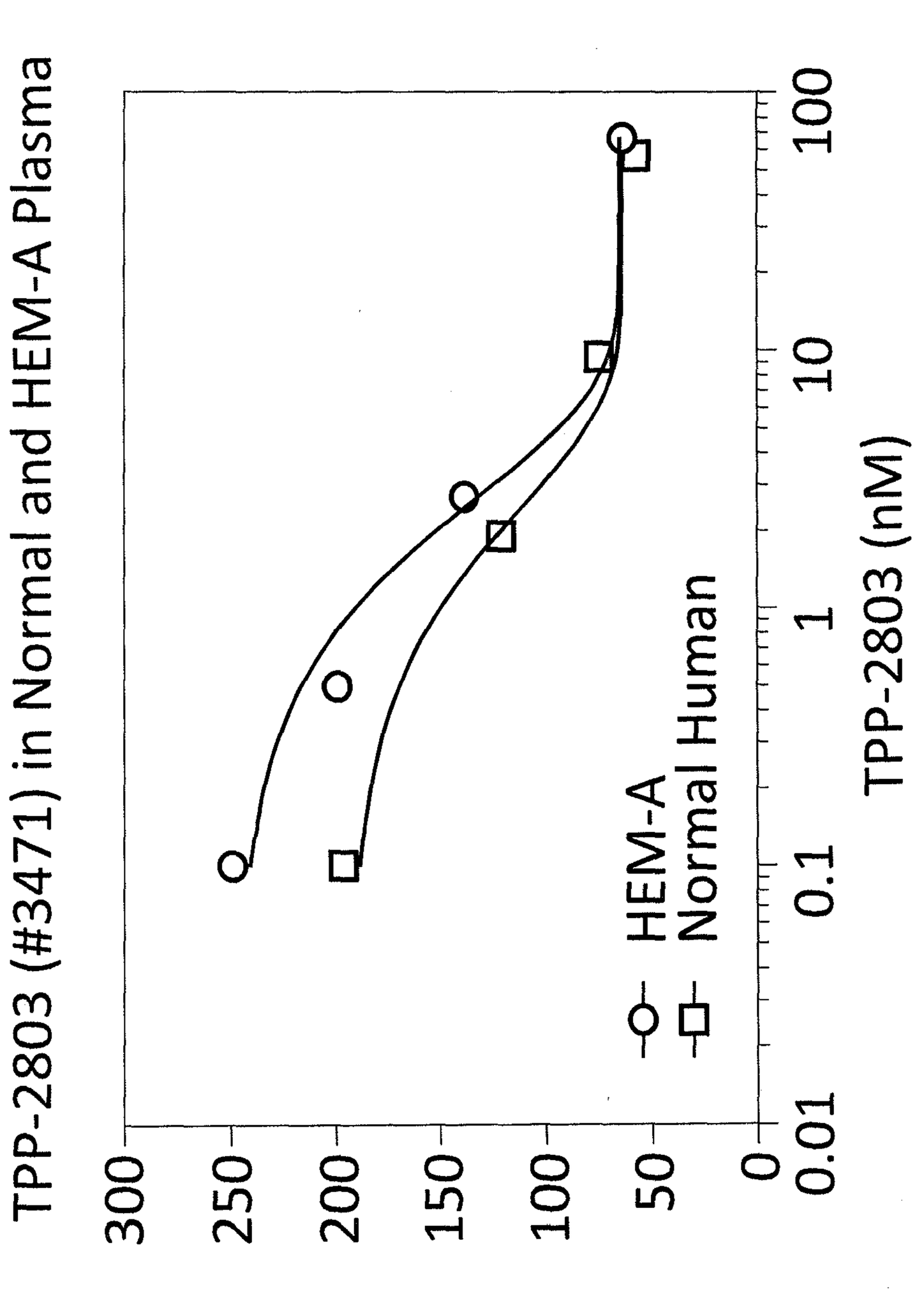

TPP-2803 (#3471) in Normal and HEM-A Plasma
CT (sec)
300
250
200
150
100
50
0
0.01
0.1
1
10
100
TPP-2803 (nM)
HEM-A
Normal Human

FIG. 12

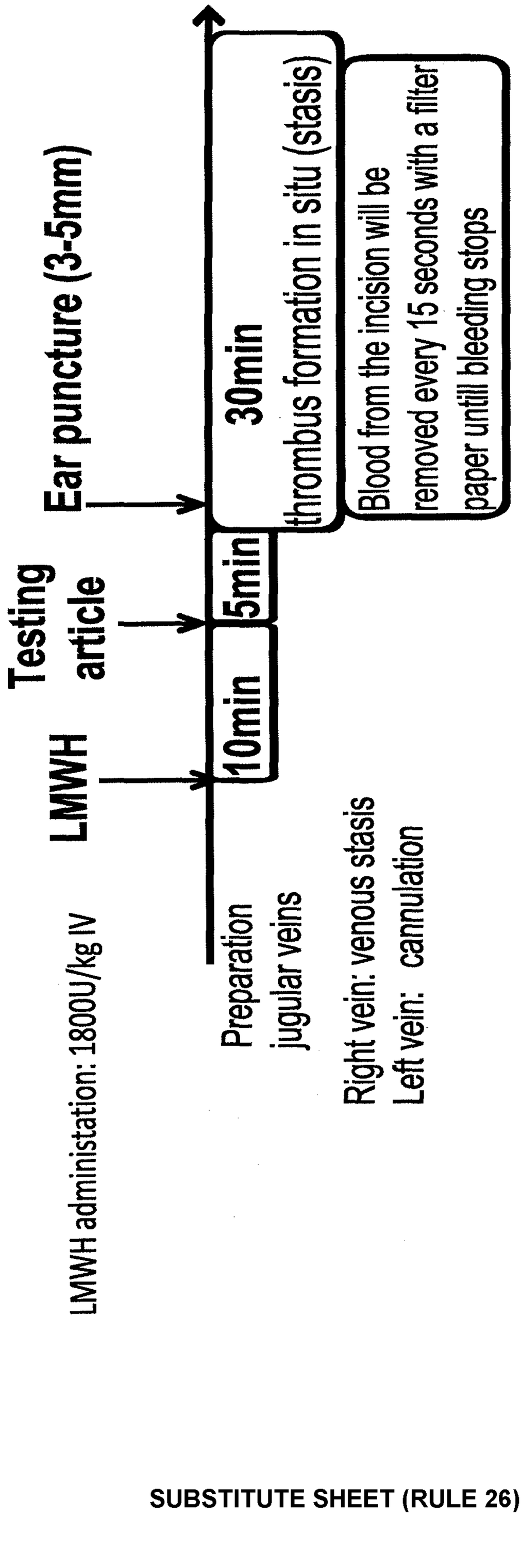

Ear puncture (3-5mm)
30min
thrombus formation in situ (stasis)
Blood from the incision will be removed every 15 seconds with a filter paper untill bleeding stops
Testing article
5min
LMWH
10min
Preparation jugular veins
Right vein: venous stasis
Left vein: cannulation
ation: 1800U/kg IV
LMWH administ

FIG. 13

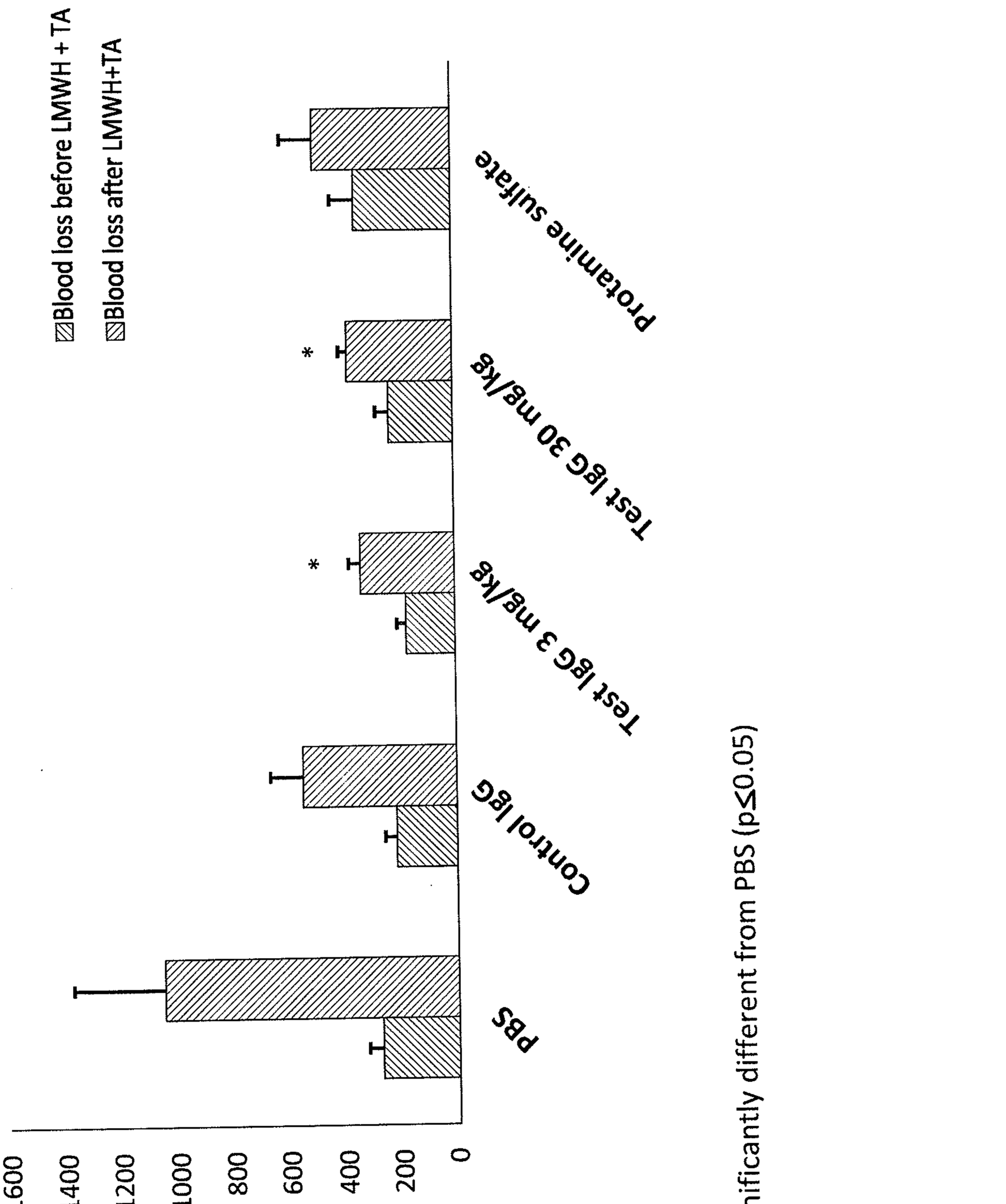

Blood loss before LMWH + TA
Blood loss after LMWH+TA
Protamine sulfate
Test IgG 30 mg/kg
Test IgG 3 mg/kg
Control IgG
PBS
*
*
1600
1400
1200
1000
800
600
400
200
0
Bleeding time (s)
*Significantly different from PBS (p≤0.05)

FIG. 14

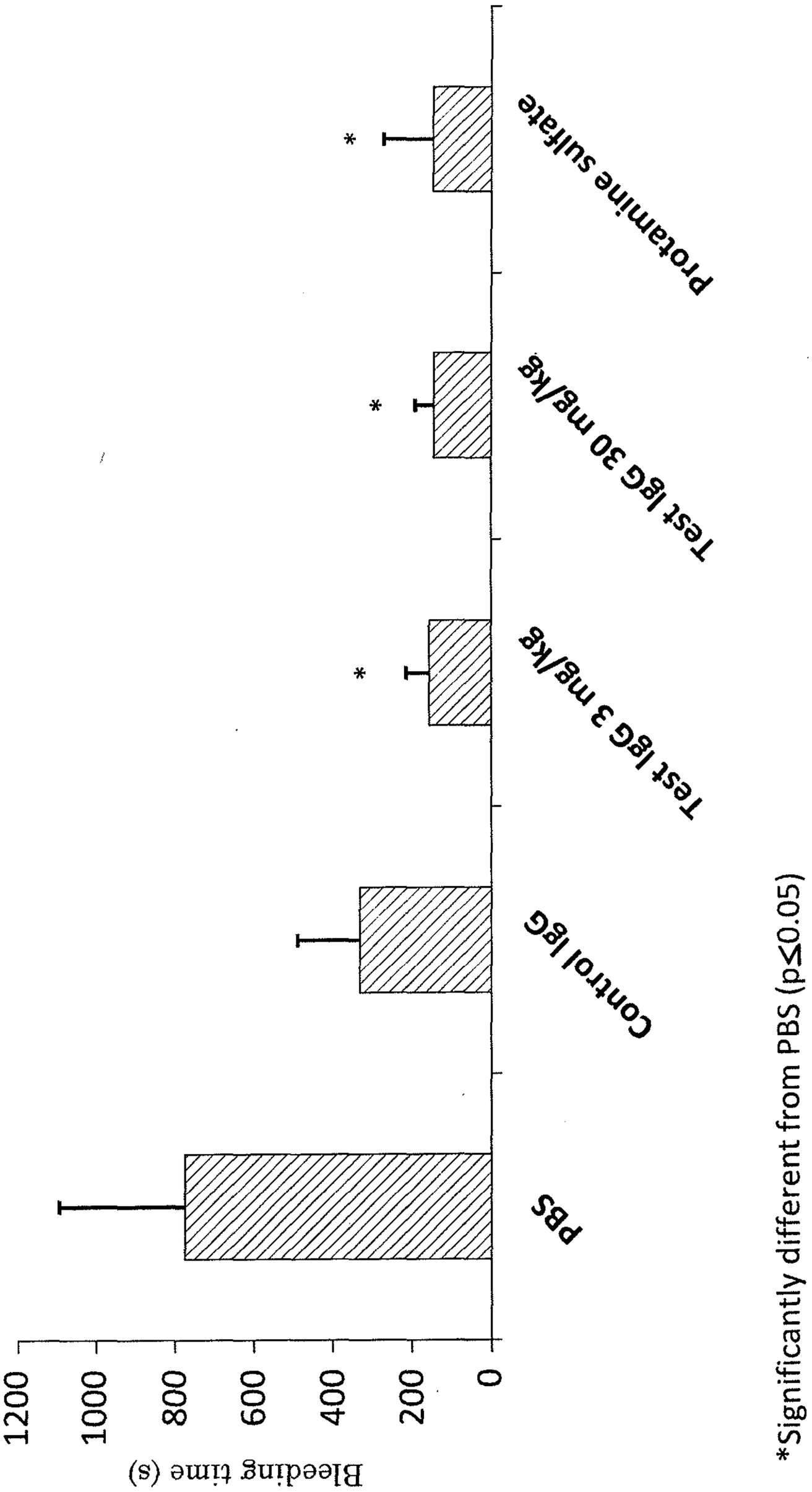

Bleeding time (s)
1200
1000
800
600
400
200
0
PBS
Control IgG
Test IgG 3 mg/kg
Test IgG 30 mg/kg
Protamine sulfate
*
*
*
*Significantly different from PBS (p≤0.05)

FIG. 15

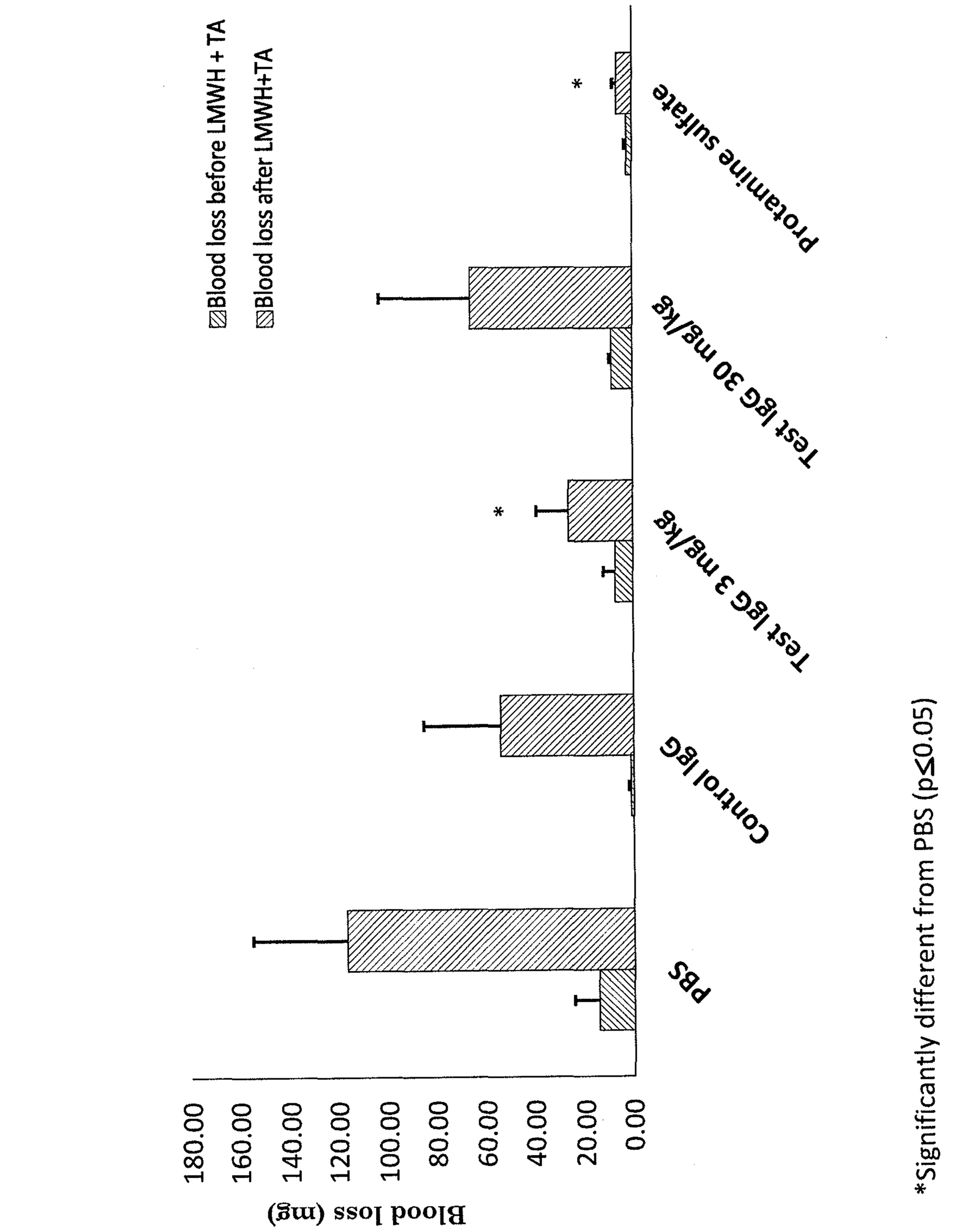
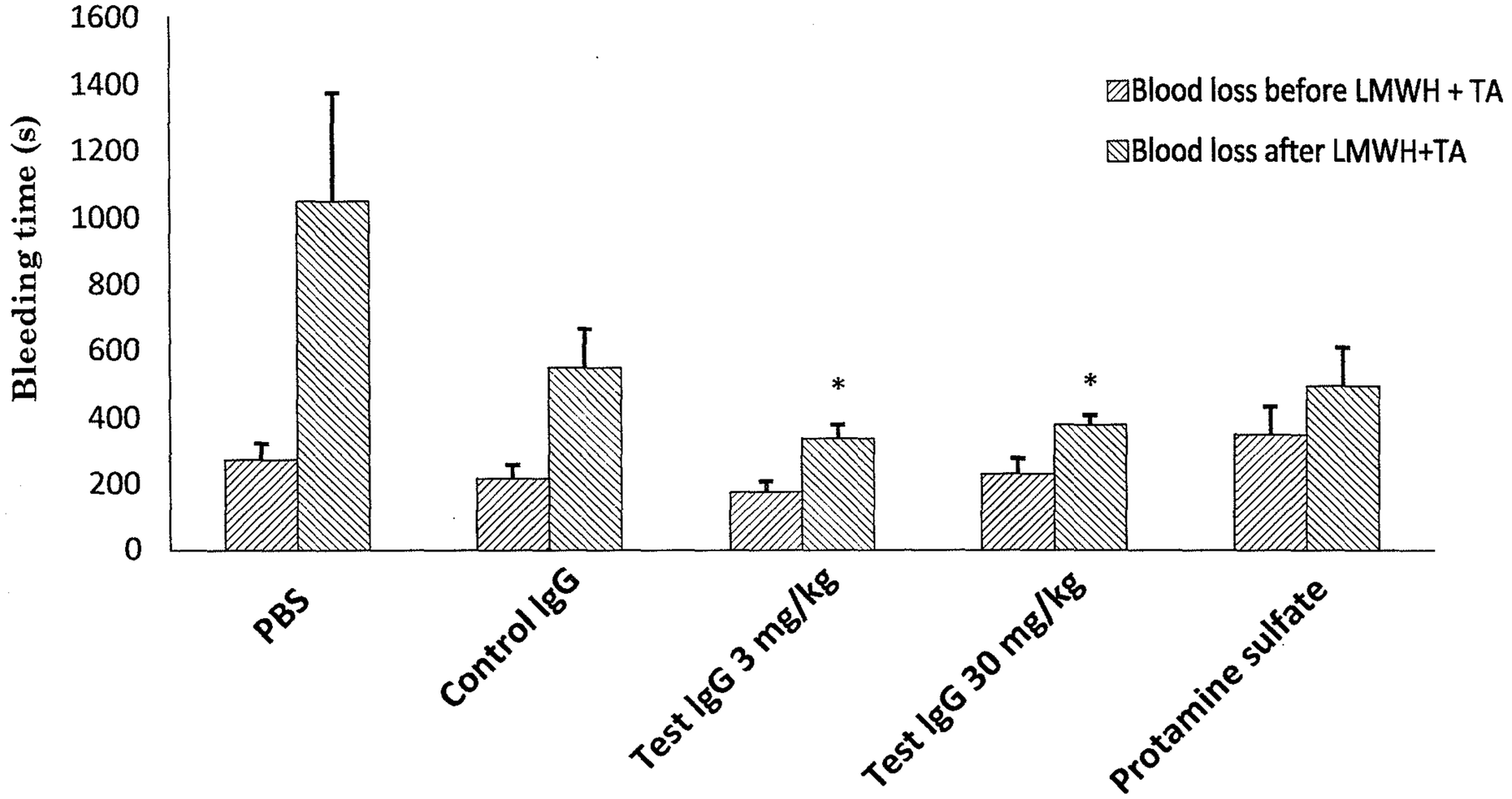

Blood loss before LMWH + TA
Blood loss after LMWH+TA
Protamine sulfate
Test IgG 30 mg/kg
Test IgG 3 mg/kg
Control IgG
PBS
*
*
*Significantly different from PBS (p≤0.05)
Blood loss (mg)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00

FIG. 16

FIG. 14



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