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(54) **VON WILLEBRAND FACTOR PROTEINS FOR TREATING BLEEDING DISORDERS**

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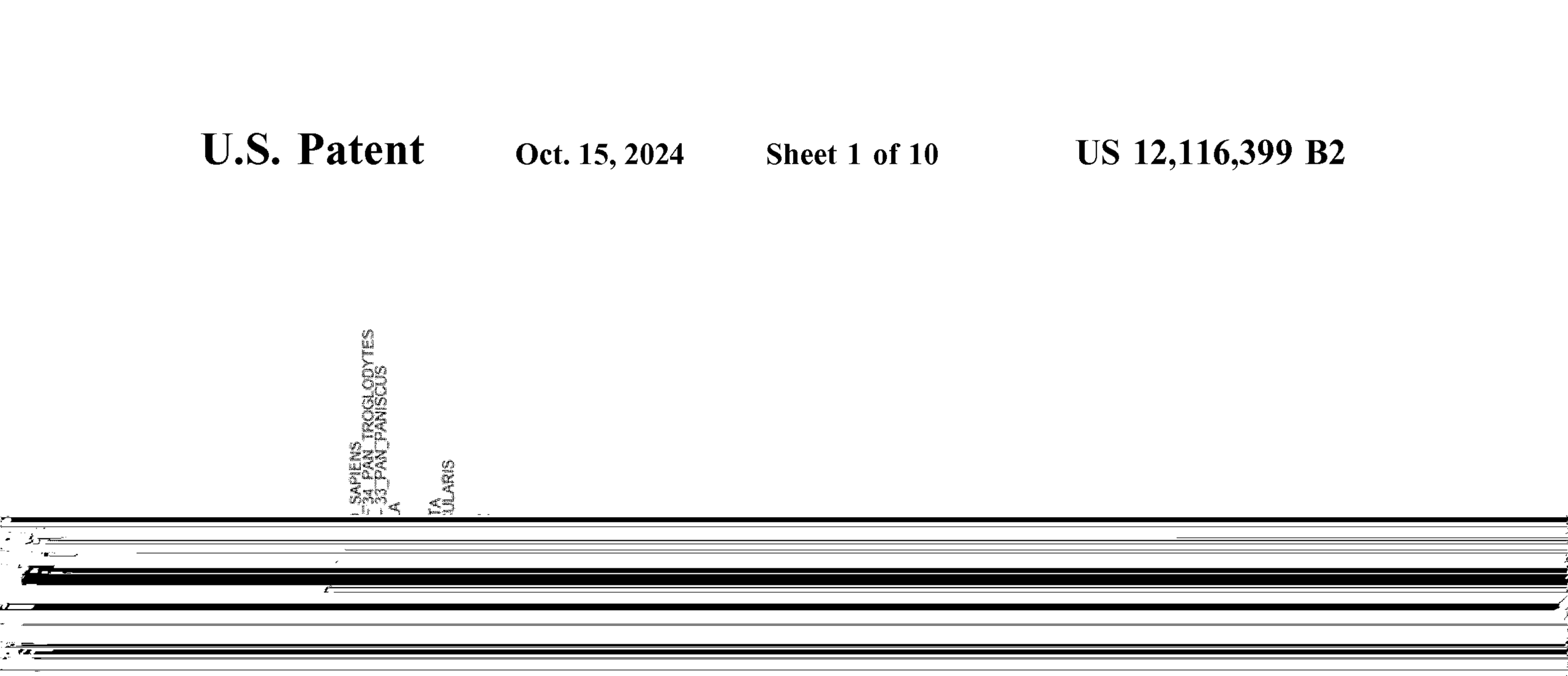
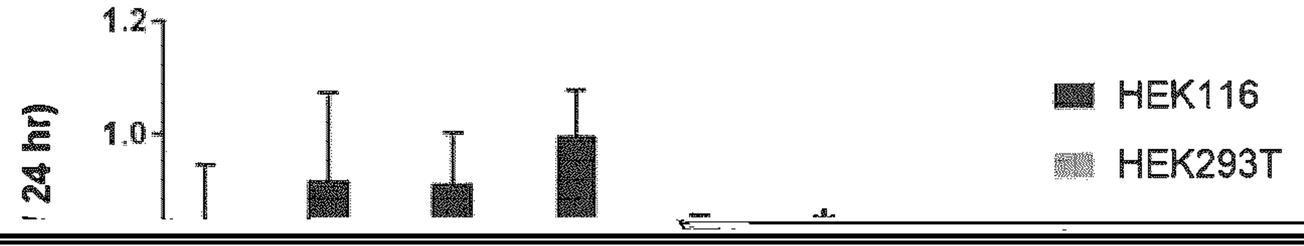
(52) **U.S. Cl.**
CPC ***C07K 14/755*** (2013.01); ***A61P 7/04*** (2018.01); ***C12N 15/86*** (2013.01); *A61K 38/00* (2013.01); *C12N 2740/10043* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**


SAPIENS
34_PAN_TROGLODYTES
33_PAN_PANISCUS
A
TA
ULARIS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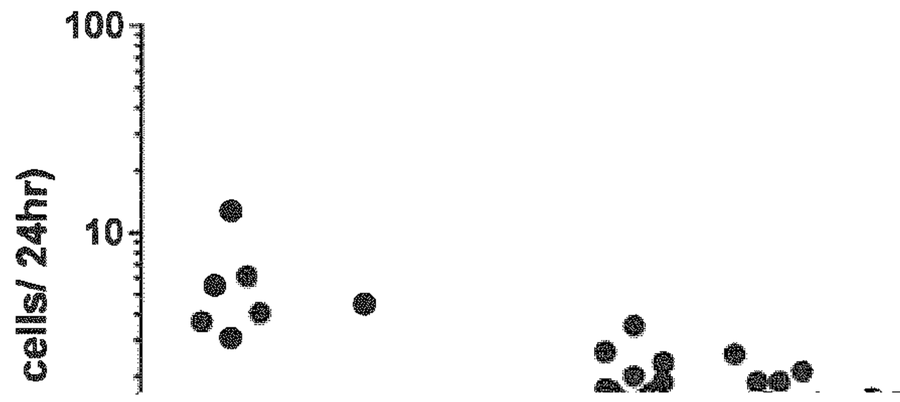
(24 hr)
HEK116
HEK293T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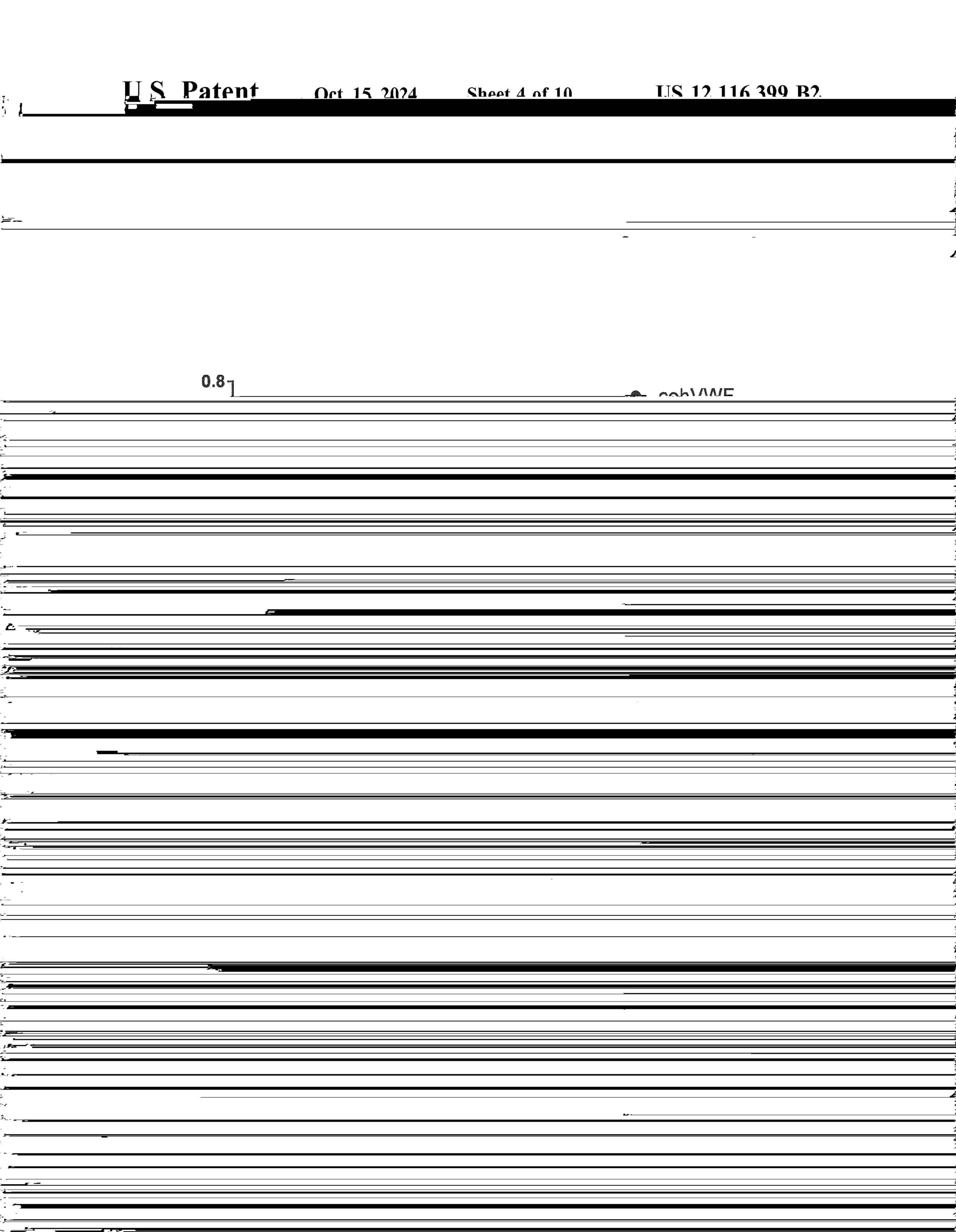
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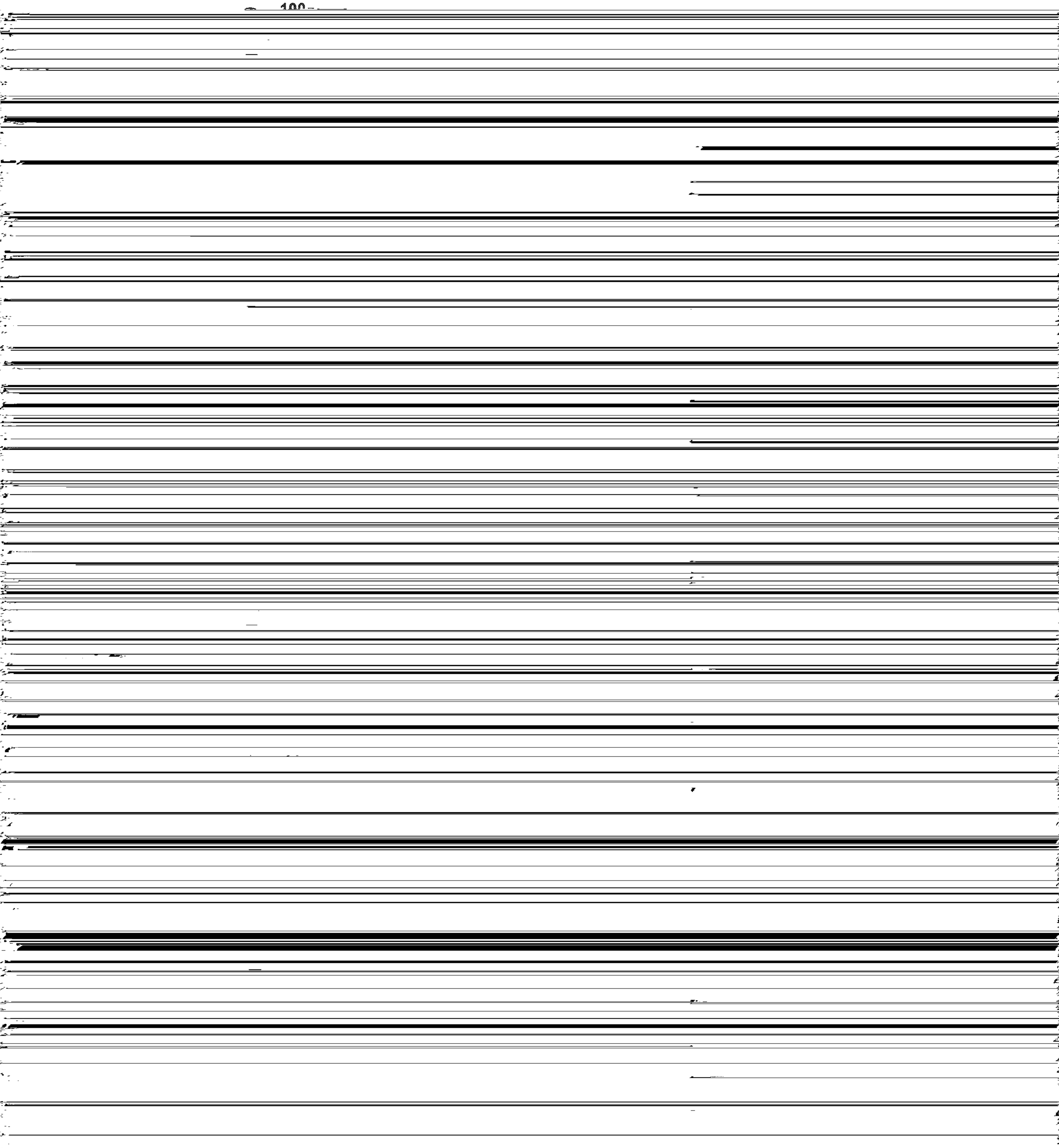
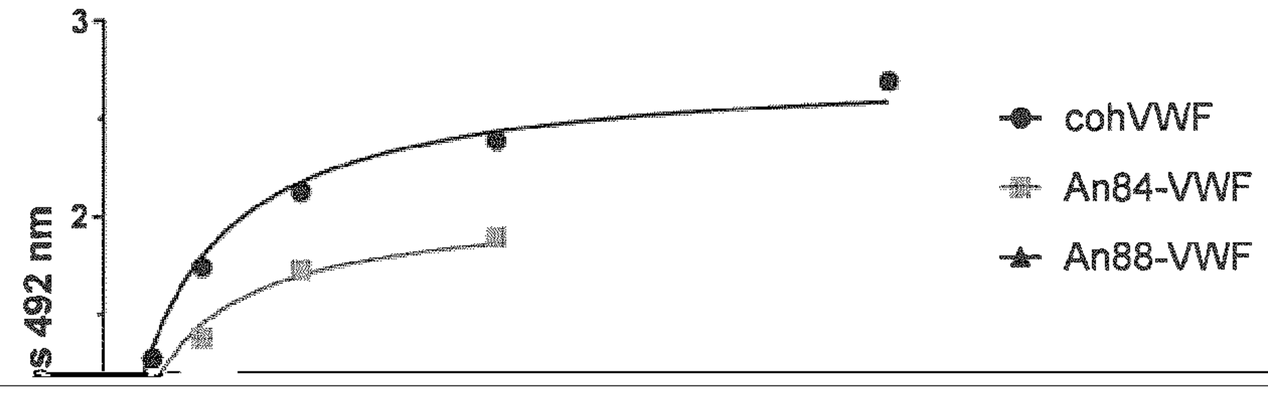
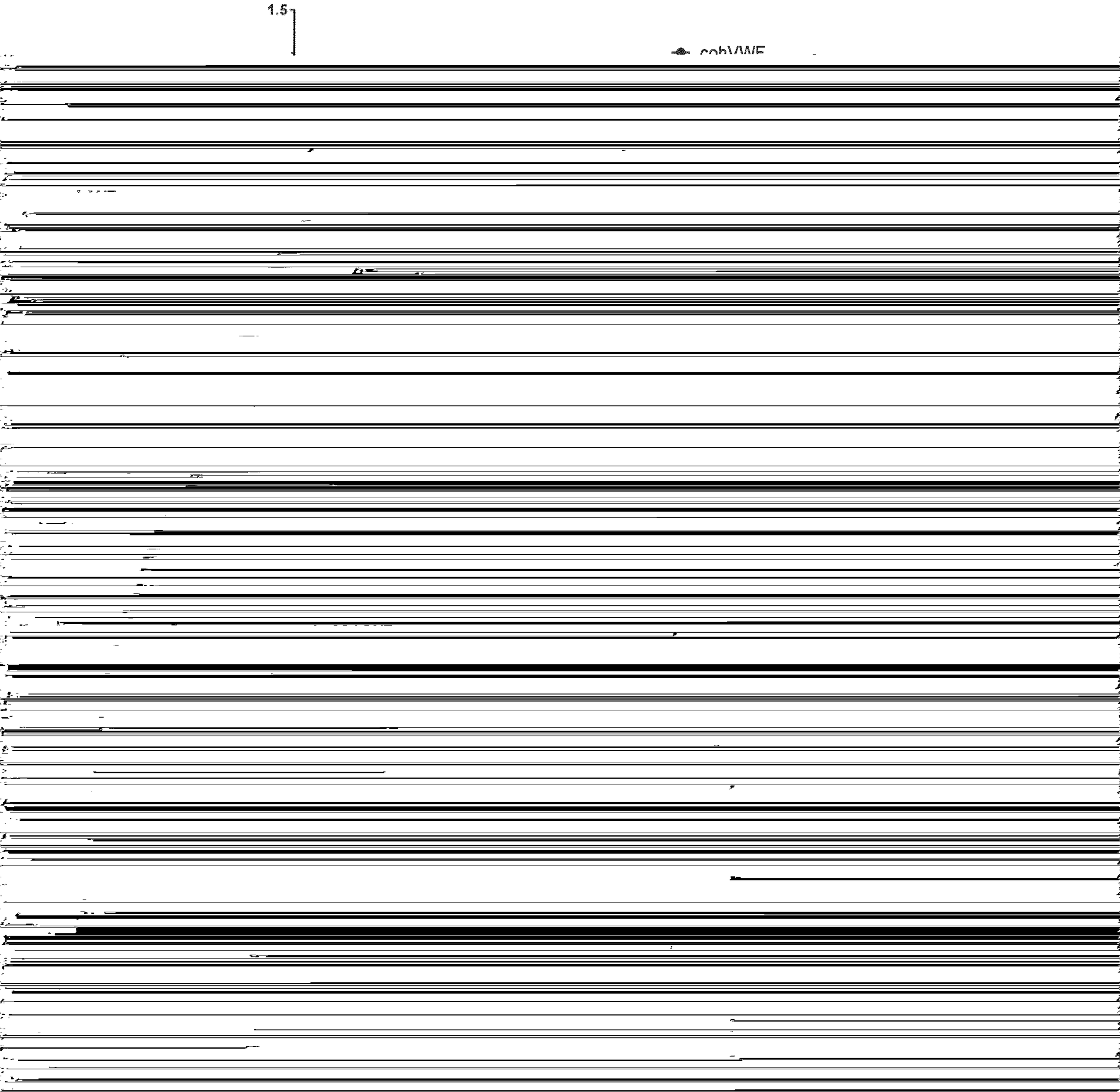

100


FVIII
VWF
10
10


3
2
s 492 nm
cohVWF
An84-VWF
An88-VWF


1.5
cobWE

120
100
cohVWF
An101-VWF

VON WILLEBRAND FACTOR PROTEINS

FOR TREATING BLEEDING DISORDERS

RELATED APPLICATION

as well as CLEC4M on sinusoidal endothelial cells. Muta-

gine-linked glycosylation sites have been reported to poten-

tiate VWF clearance. While clearance of VWF is largely

and one artificial Factor VIII sequence, a Factor VIII sequence and one artificial von Willebrand sequence, or one

FIG. 2B shows protein expression data of stable HEK116 clones were generated using G418 selection following trans-

brand sequence. In one embodiment of this aspect, the

from each VWF treatment. Shown are the VWF expression

to human VWF. VWF antigen is determined by ELISA and normalized to the dose injected.

DETAILED DESCRIPTION

Definitions

As used herein, a "non-naturally occurring" sequence is

The term "recombinant" in reference to a nucleic acid molecule refers to a nucleic acid molecule which is comprised of segments of nucleic acid joined together by means

in reference to a protein or a polypeptide refers to a protein

acid molecule.

The term "chimera" when used in reference to a poly-

one for which no organisms produce or ever produced through the course of natural events. A protein is non-

peptide of polynucleotide refers to the expression product of two or more coding sequences obtained from different

In certain embodiments, term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the

19, 751-755 and Asahara & Chong, *Nucleic Acids Research,* 2010, 38(13): e141, both hereby incorporated by reference in their entirety.

Embodiments

In practicing the embodiments described herein, many

VWD therapeutic development. However, limited structural information remains a major limitation for rational design studies. Recently, SR was performed to infer the sequences of FVIII predicted to have existed throughout mammalian evolution. SR provides a platform for high-resolution mapping of sequential differences within a phylogeny while maintaining a high probability of retaining function. Sequential changes in the amino acid sequence of FVIII appears to have resulted in altered biochemical properties

activated the GPIb/IX/V complex on platelets by binding through the VWF A1 domain (see VWF domains in Springer, T. A., Blood (2014), 124: 1412-1425). To first test the functional conservation of An-VWF molecules in a ristocetin-independent manner, AnVWF was assessed for GPIb binding via a GP1bM assay. In this assay, An70 (SEQ ID No. 9) and An88 (SEQ ID No. 7) displayed 1.5-2-fold increased specific activity over cohVWF (FIG. **3**). VWF molecules An101 (SEQ ID No. 6), An84 (SEQ ID No. 8), and An63-VWF (SEQ ID No. 10) demonstrated conserved

out evolution, we employed a collagen binding ELISA to test An-VWF association to human collagen. All An-VWF

would significantly increase the FVIII half-life so long as the affinity of FVIII for the exogenous VWF molecule is greater

be complex or made into a complex and delivered in a fusion. CpG DNA motifs are typically removed because

single dose such as, for example, a single injection. Thus, gene therapy with co-administration of a pharmacological

they may lead to gene methylation and silencing. See Bird, DNA methylation and the frequency of CpG in animal DNA,

capsids, or vectors encoding proteins disclosed herein can be

were codon optimized (co) for human host expression and synthesized by GenScript (Piscataway, N.J.). Coding DNA

ose affinity chromatography at a flowrate of 1-2 ml/min. Column was equilibrated and washed with 20 mM HEPES, 5 mM CaCl2, 50 mM NaCl, 0.01% Tween-80 at pH 7.2.

Human ADAMTS13 was diluted 2-fold in 5 mM Tris

diluent. Absorbance of HRP mediated catalysis of OPD was

with 10 mM BaCl2 for 5 min at 37° C. 25 μL of diluted hADAMTS13 was added to 25 μL of An-VWF (1 U/mL in

human recombinant VWF and reference human plasma was also included.

TABLE 2-continued

Liver-derived An-VWF restores FVIII in mice

VWF:Ag	Molar Ratio			
SEQ ID No. 8				
An63-VWF	1.44 ± 0.48	0.61 ± 0.09	4	14:1 ± 5
SEQ ID No. 10				
An70-VWF	2.1 ± .305	0.76 ± .046	2	17:1 ± 1
SEQ ID No. 9				
An88-VWF	0.72 ± 0.16	0.75 ± 0.16	3	6:1 ± 2
SEQ ID No. 7				

TABLE 3

TABLE 4-continued

Correspondence between Sequence Names and SEQ ID Numbers

Sequence Name	SEQ ID No.
An66-VWF Prot	SEQ ID No. 17
An67-VWF Prot	SEQ ID No. 18
An68-VWF Prot	SEQ ID No. 19
An69-VWF Prot	SEQ ID No. 20
An71-VWF Prot	SEQ ID No. 21
An72-VWF Prot	SEQ ID No. 22
An73-VWF Prot	SEQ ID No. 23
An75-VWF Prot	SEQ ID No. 25
An76-VWF Prot	SEQ ID No. 26
An77-VWF Prot	SEQ ID No. 27
An78-VWF Prot	SEQ ID No. 28
An79-VWF Prot	SEQ ID No. 29

We claim:

1. A recombinant non-naturally occurring von Willebrand Factor (VWF) protein comprising the amino acid sequence of SEQ ID NO: 6 (An101-VWF), SEQ ID NO: 8 (An84-VWF), SEQ ID NO: 10 (An63-VWF), SEQ ID NO: 7 [redacted]

7. A method of inducing blood clotting comprising administering an effective amount of the composition of claim 5 to a subject in need thereof.

8. A method for treating a subject with a bleeding disorder, comprising administering an effective amount of the [redacted]