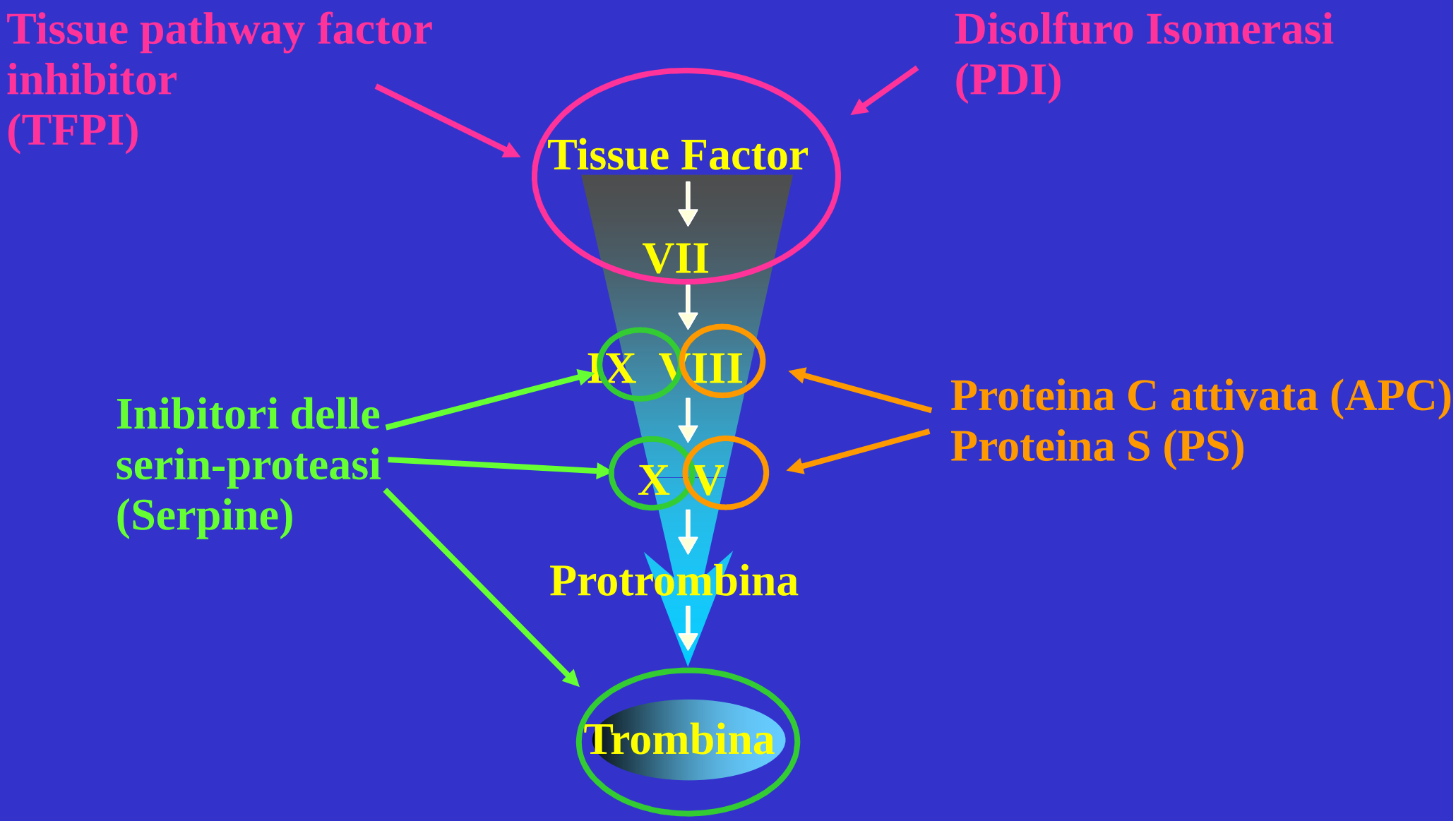
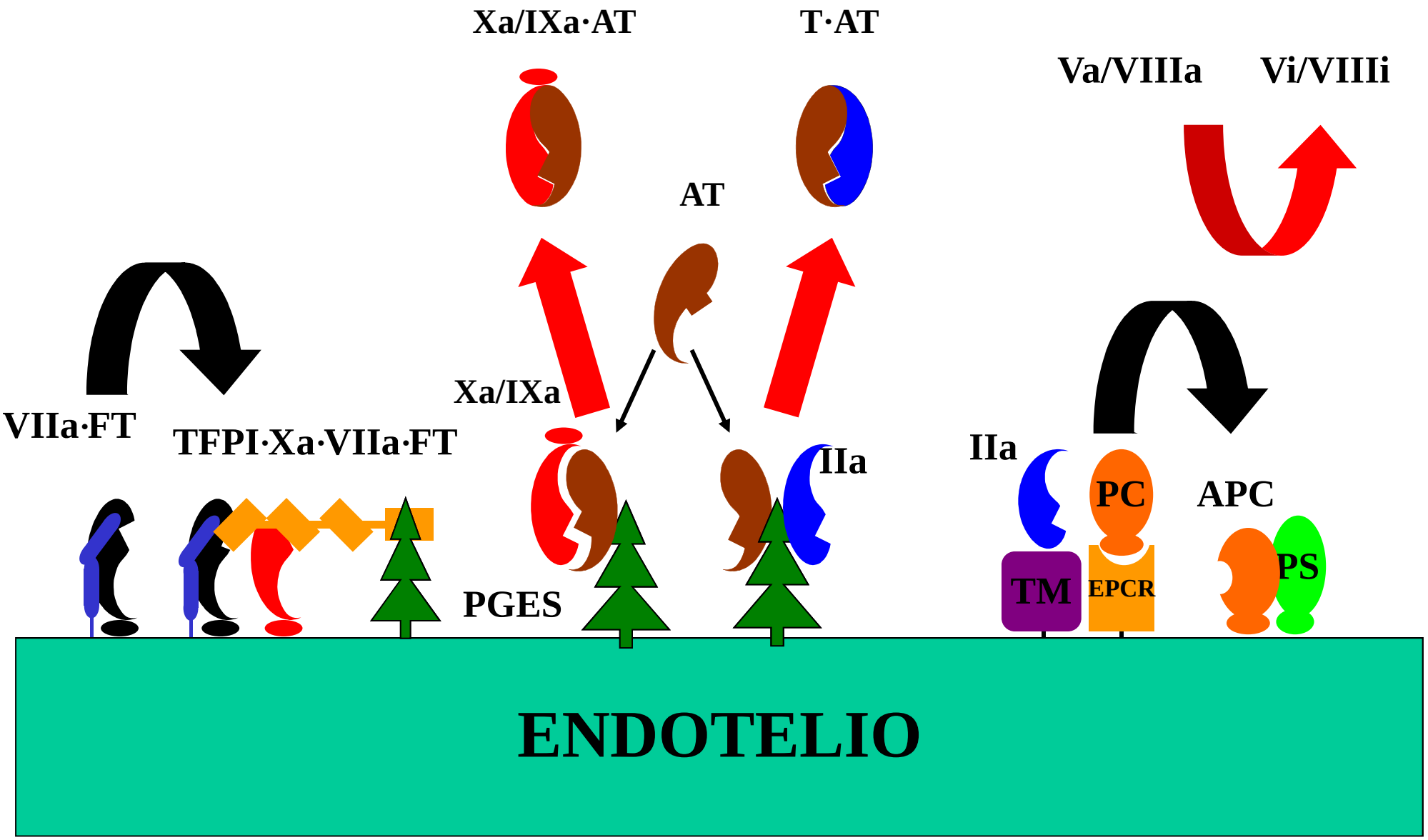
Cascata coagulativa
Regolazione Naturale

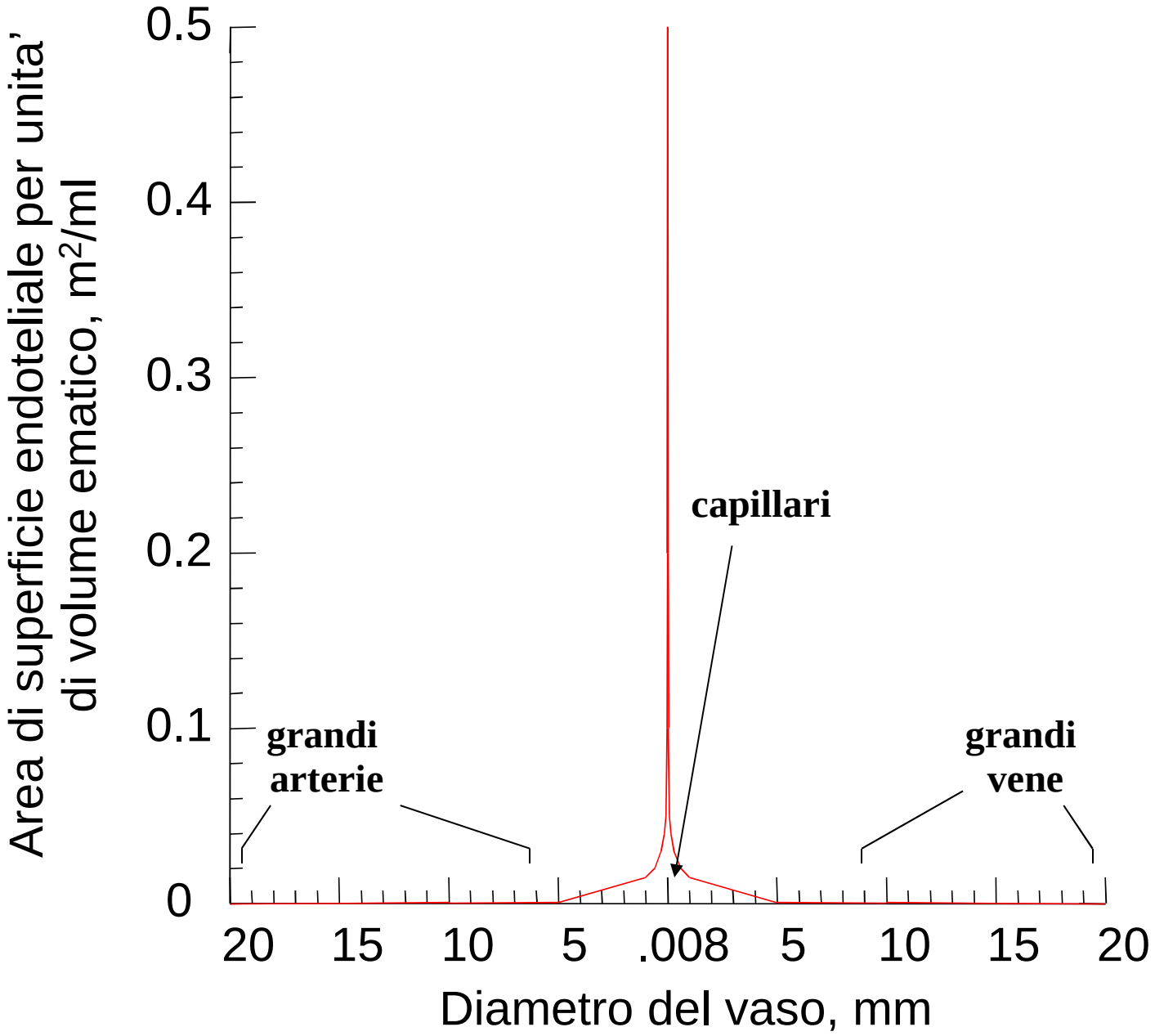


SISTEMI ANTICOAGULANTI NATURALI

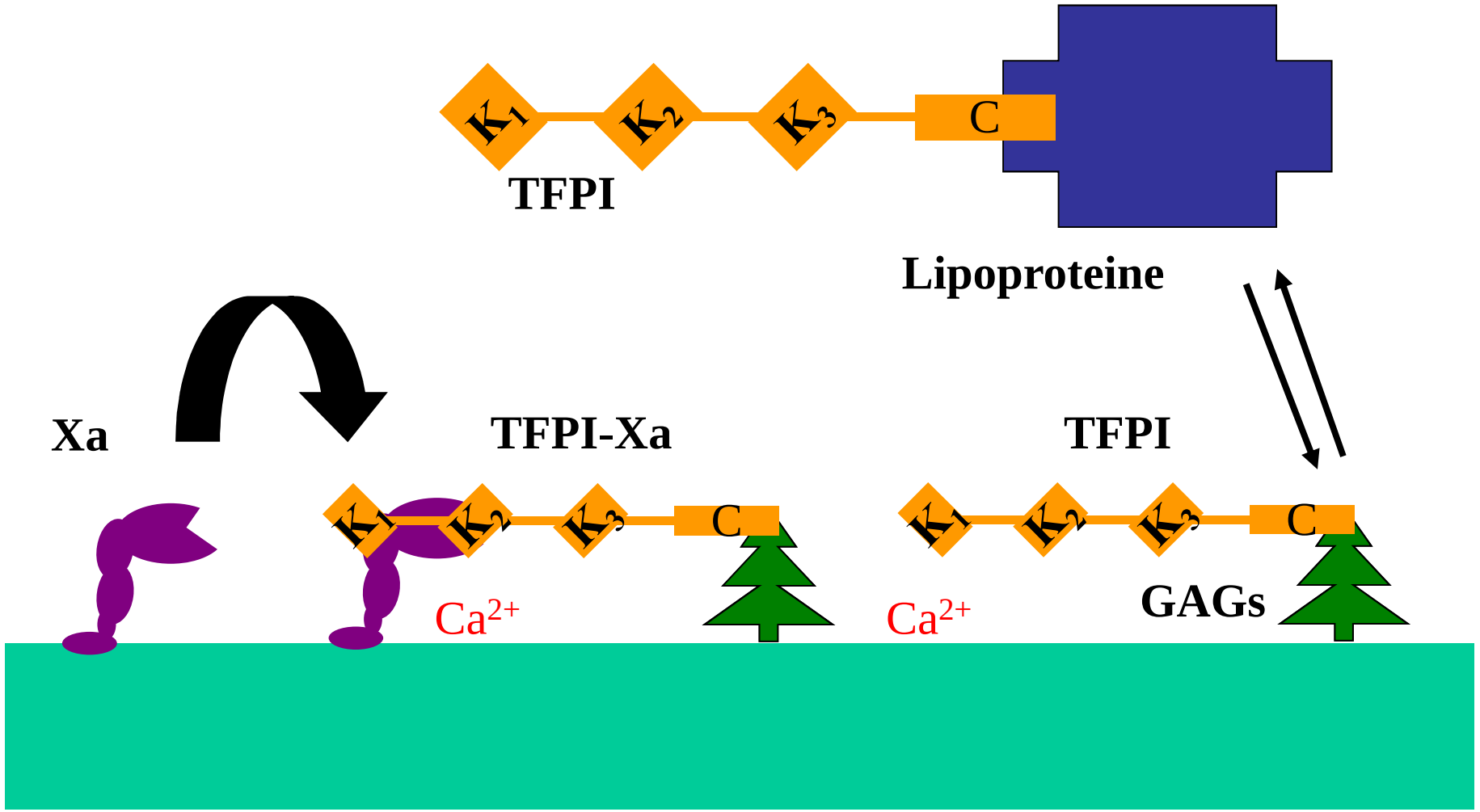
Effettore	Target
Inibitore del fattore tissutale (TFPI)	FVIIa-FT
Sistema Antitrombina-eparina	Enzimi (XIIa, XIa, IXa, Xa, IIa, VIIa)
Sistema della Proteina C	Cofattori attivati (VIIIa, Va)


TFPI
ANTITROMBINA
PROTEINA C
Xa/IXa·AT
T·AT
Va/VIIIa
Vi/VIIIi
AT
Xa/IXa
VIIa·FT
TFPI·Xa·VIIa·FT
IIa
IIa
PC
APC
PS
TM
EPCR
PGES
ENDOTELIO

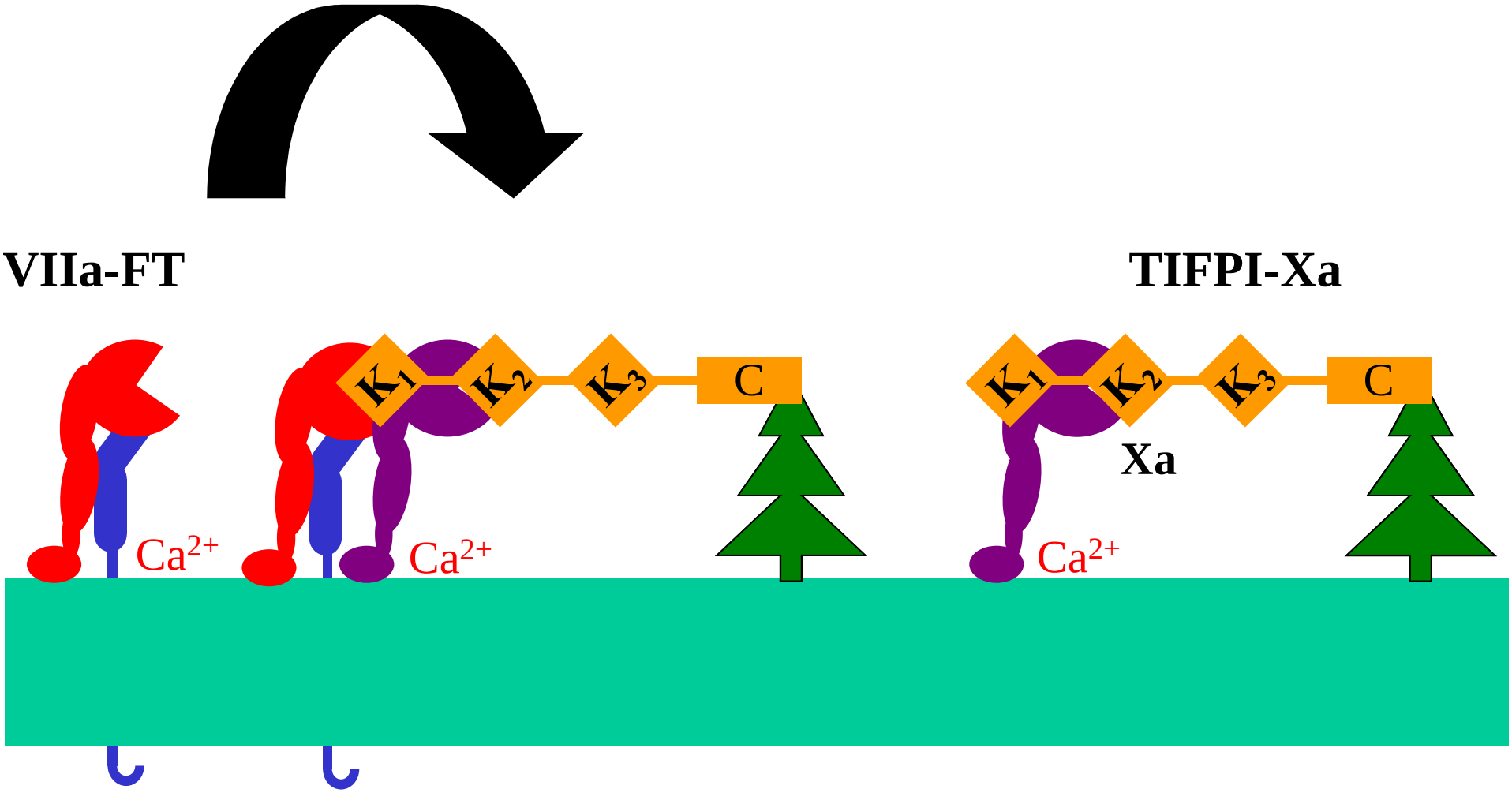
Rapporti distrettuali tra superficie endoteliale e sangue circolante



TFPI, inibitore del Xa



TFPI-Xa, inibitore di VIIa-FT



DNA

A DNA template was synthesized with the sequence 5′-GGAGGGAAAAGTTATCAGGC-N40-GATTAGTTTTGGAGTACTCGCTCC-3′

“N40” =40-nucleotide sequence in which there is an equal probability of incorporating a dA, dC, dG, or dT residue at each position and
“d” = 2′-H residue

The DNA template was amplified by polymerase chain reaction (PCR) with forward primer 5′-GACTGTAATACGACTCACTATAGGAGGGAAAAGTTATC-AGGC-3′ and reverse primer 5′-GGAGCGAGTACTCCAAAACTAATC-3′

RNA -selection

- Transcribed to generate a starting pool of approximately 10^{14} different sequences comprised of dC, mA, mG, and mU residues,

"m" = 2′-OCH3 residue

11 rounds of selection were carried out by first incubating the pool of molecules with recombinant full-length TFPI

- The round 11 pool was cloned and sequenced.
- Individual clones were generated by chemical synthesis
- Clones were tested for

  binding to recombinant TFPI with a nitrocellulose dot blot binding assay

  and for inhibition of TFPI in the calibrated automated thrombogram (CAT) assay

- the parent clone (5′-mGmGmAmGmGmGmAmAmAmAmGmUmUmA-mUdCmAmGmGdCdCmUmGmAmAmUmUmUmGmGmAmAmUmAmUmAdCmUmUmGmGdCmUdCmGmUmUmAmGmGmUmGdCmGmUmAmUmAmUmAmGmAmUmUmUmAmGmUmUmUmUmGmGmAmGmUmAdCmUdCmGdCmUdCdC-3′) was determined to bind to TFPI with nanomolar affinity and inhibit its activity in plasma at nanomolar concentrations.

Synthesis modification

- The core aptamer motif, ARC17480, was identified by design of molecules that contained a portion of the parent clone sequence and evaluation in the same assays.

- The core aptamer was synthesized with a hexylamine linker -$CH_3(CH_2)_5NH_2$- at the 5′-end

- which was conjugated postsynthetically to a branched 40 kDa PEG moiety -(HO-CH_2-(CH_2-O-CH_2-)$_n$-CH_2-OH - to give rise to ARC19499.

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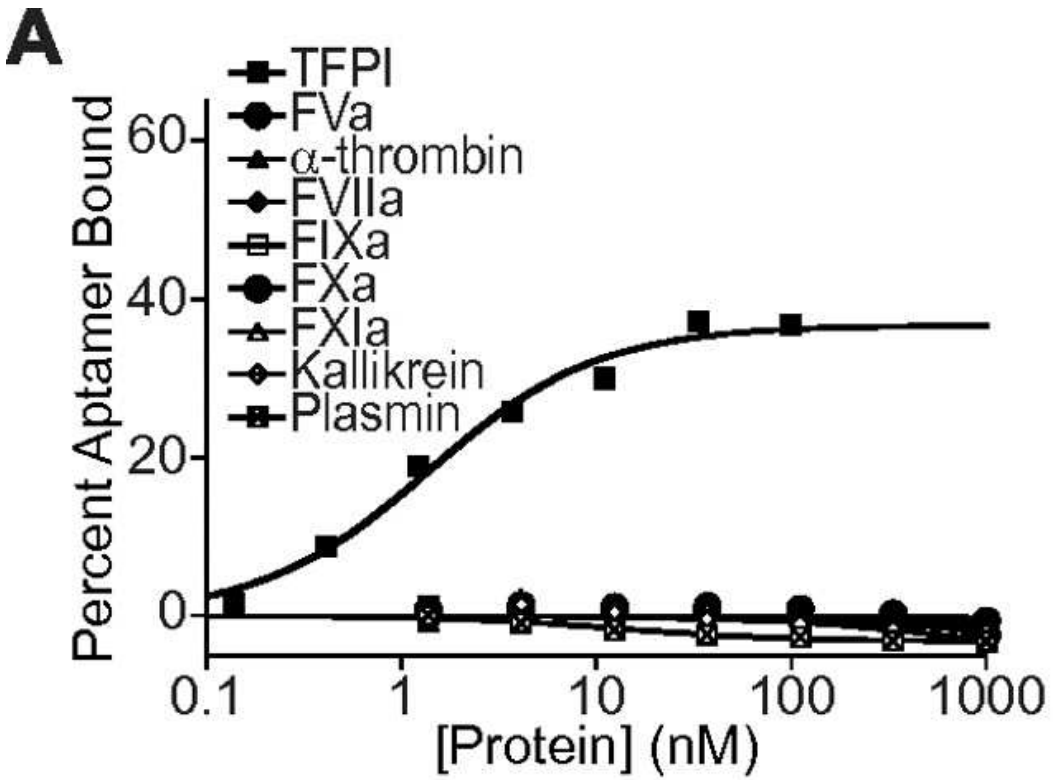
ARC17480 binding to TFPI and other proteins.



Waters E K et al. Blood 2011;117:5514-5522

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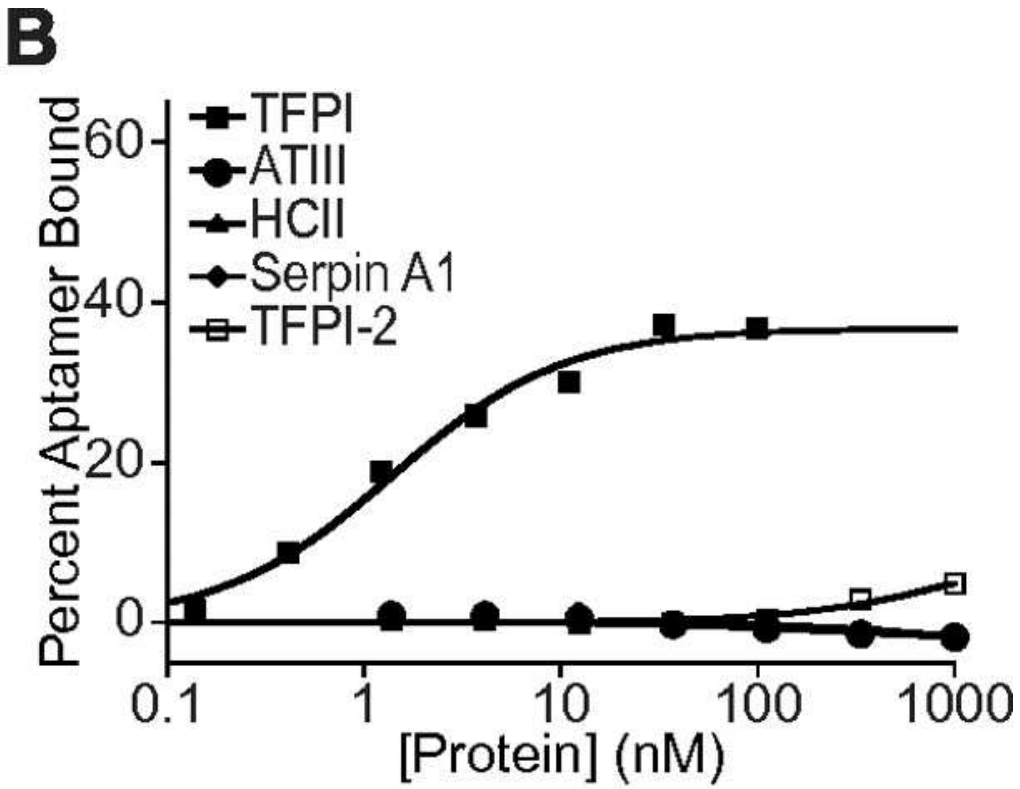
ARC17480 binding to TFPI and other proteins.



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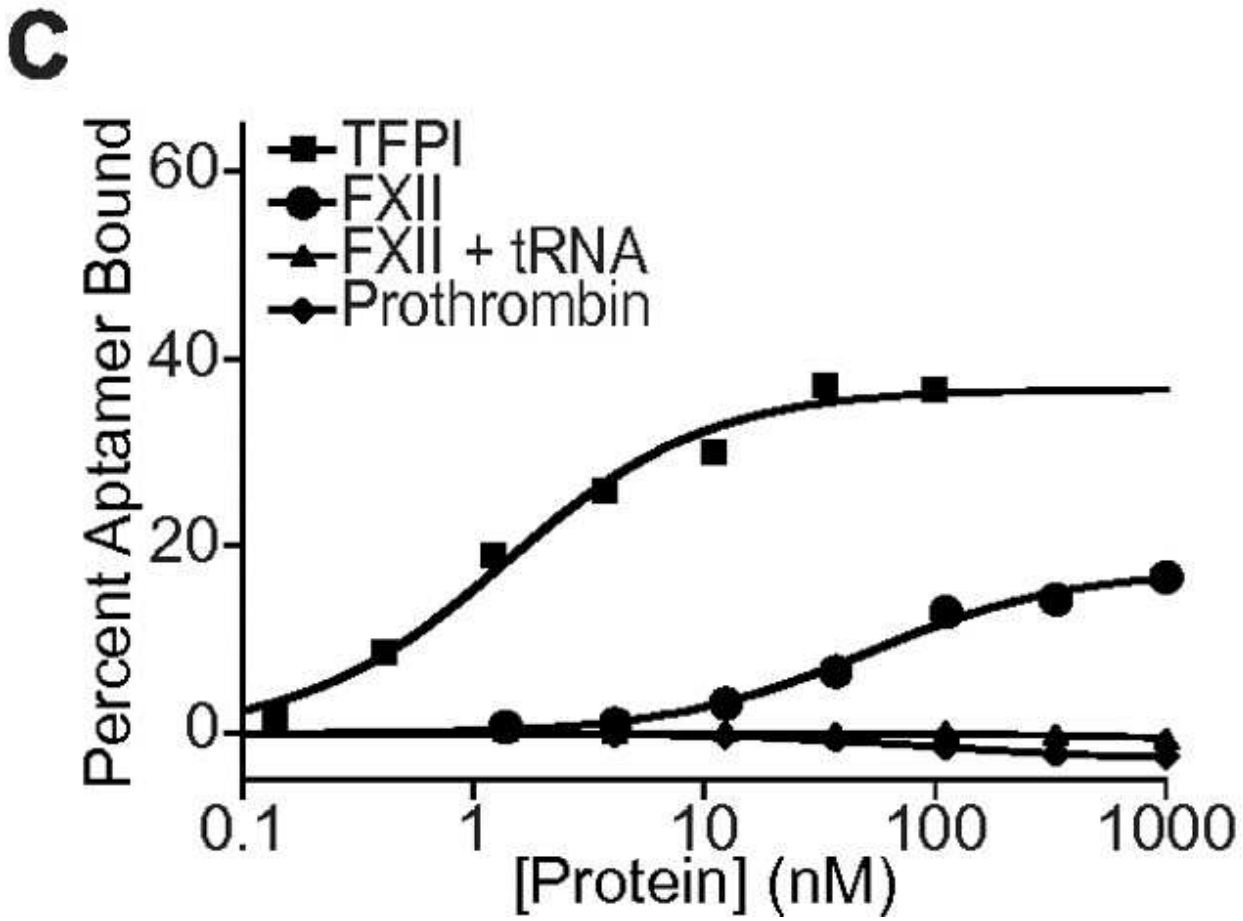
ARC17480 binding to TFPI and other proteins.



Waters E K et al. Blood 2011;117:5514-5522

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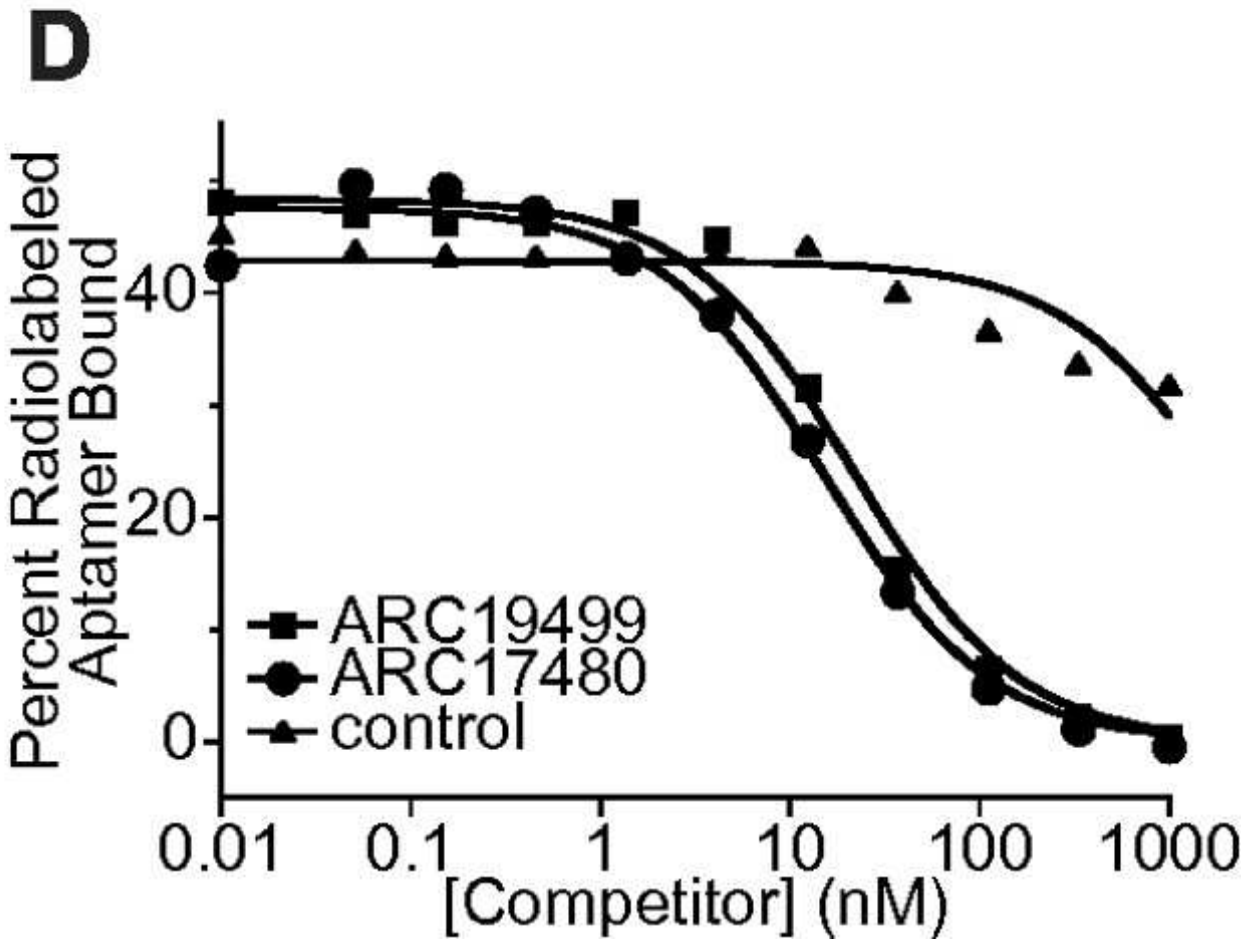
ARC17480 and ARC19499 binding to TFPI and other proteins.

10nM TFPI + radiolabeled ARC17480



Waters E K et al. Blood 2011;117:5514-5522

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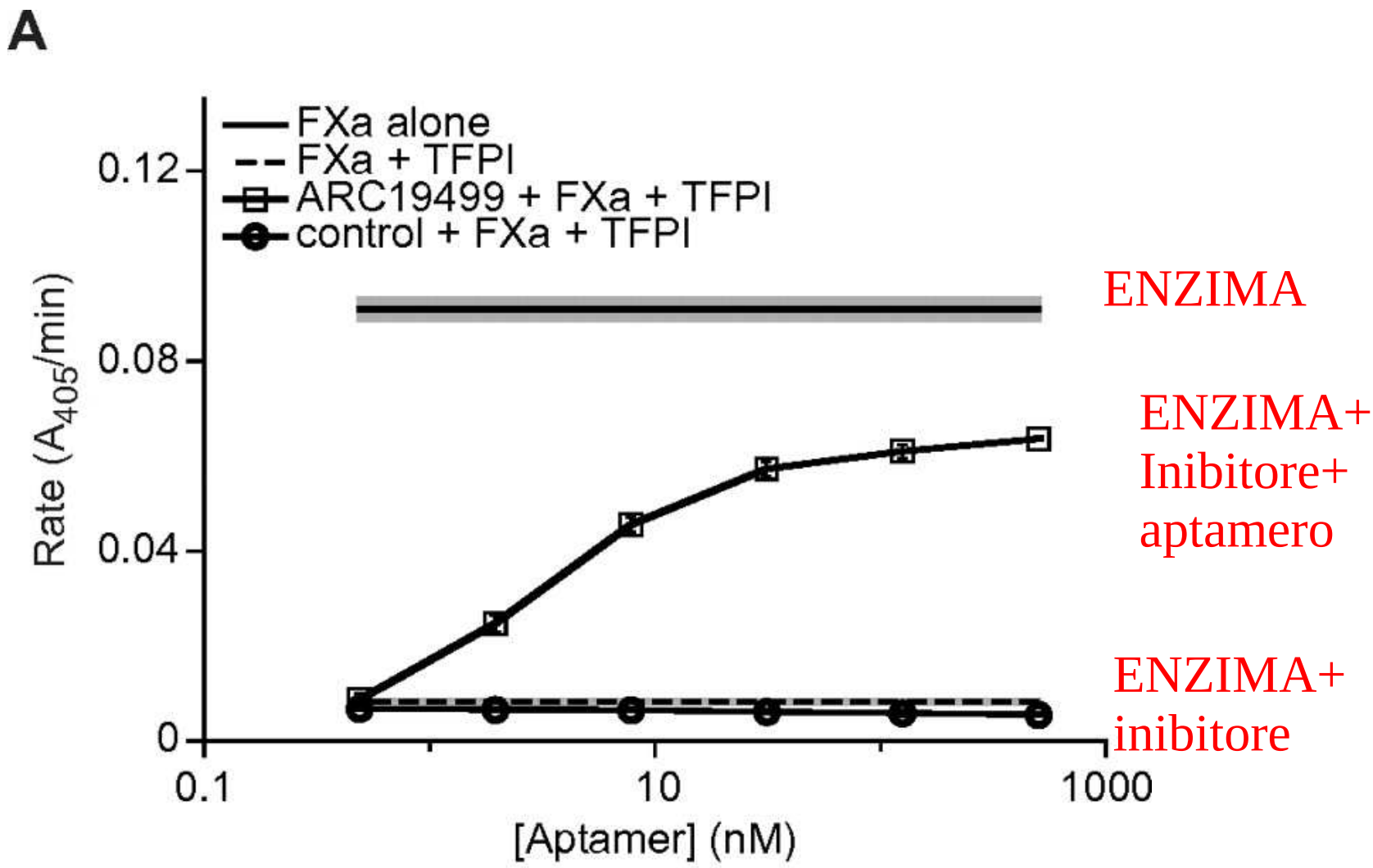
Activity of ARC19499 in TFPI-dependent assays using purified proteins.



Waters E K et al. Blood 2011;117:5514-5522

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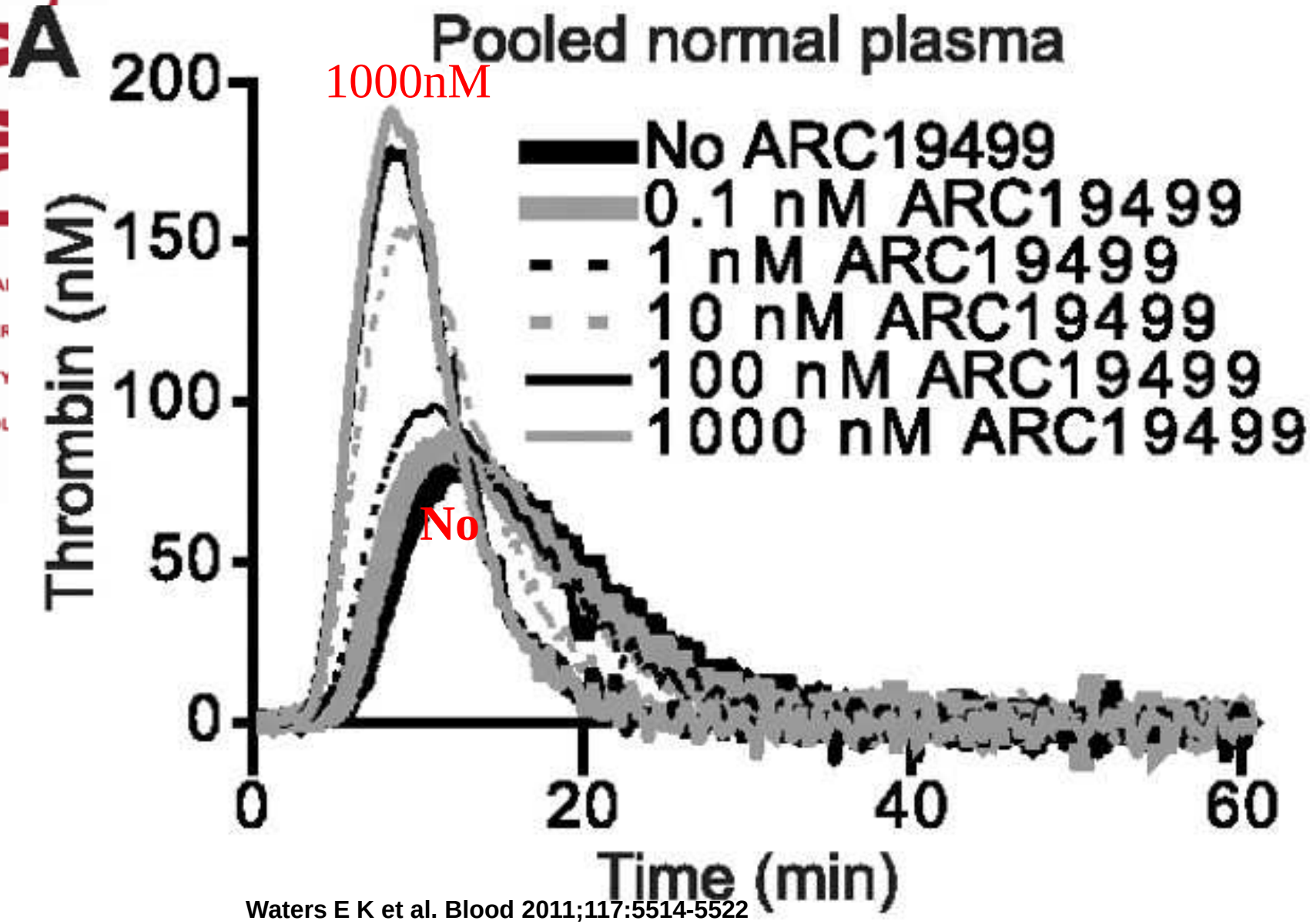
HEMATOL

ARC19499 inhibition of TFPI in human plasma.



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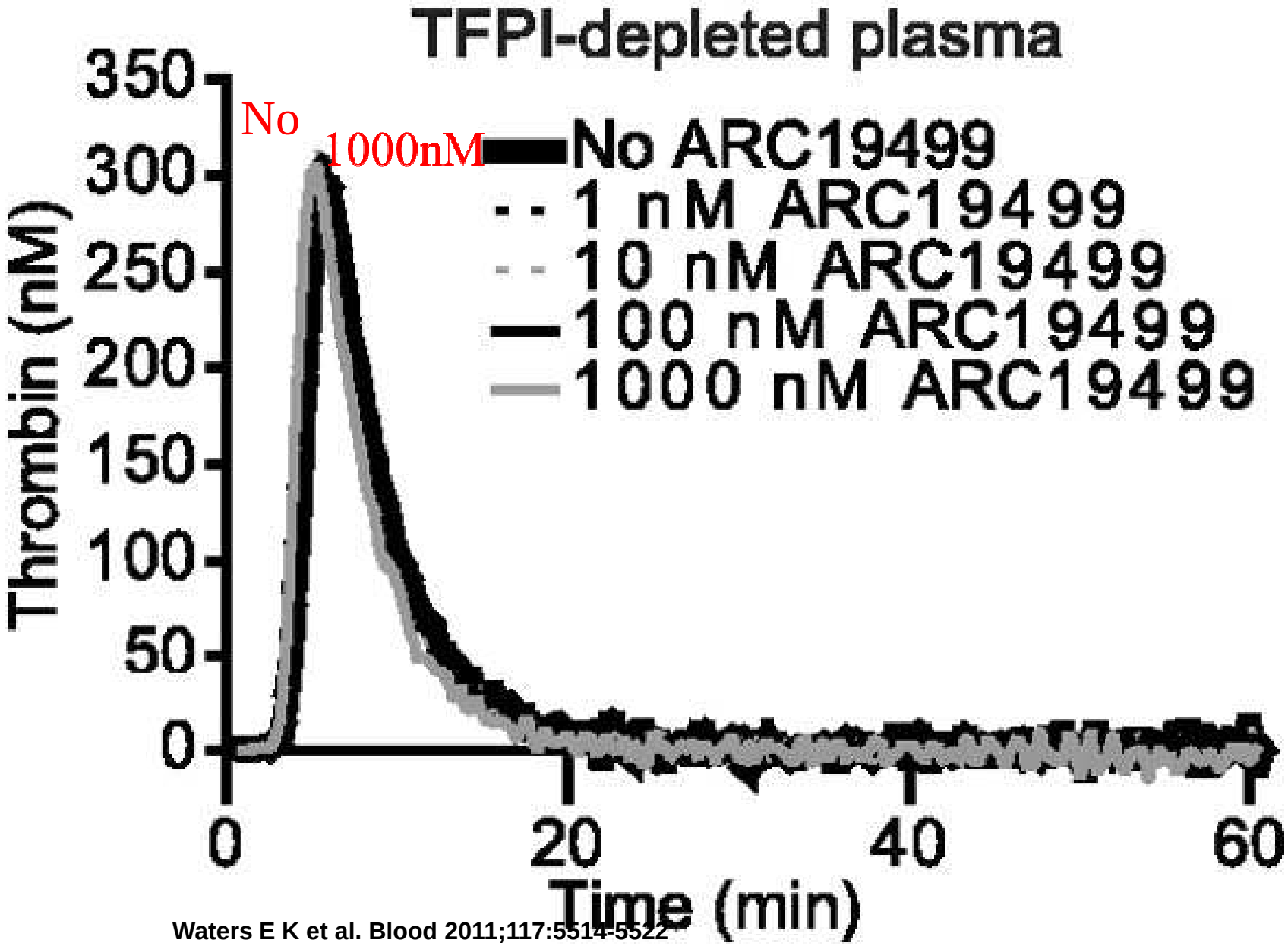

Waters E K et al. Blood 2011;117:5514-5522

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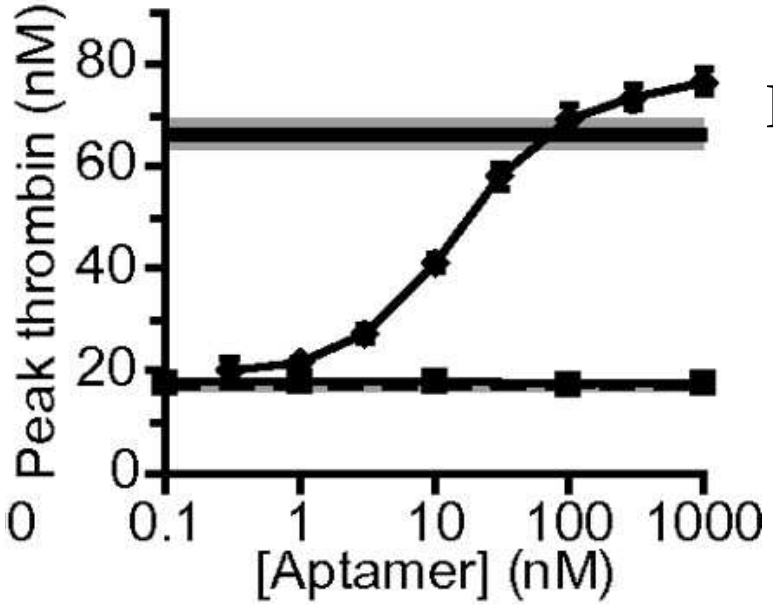
ARC19499 effect on thrombin generation in human plasma.

Activity in hemophilia A plasma



Hemophilia A + aptamer

normal plasma

Hemophilia A

Normal plasma (solid lines)
Hemophilia (dashed lines)

+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522

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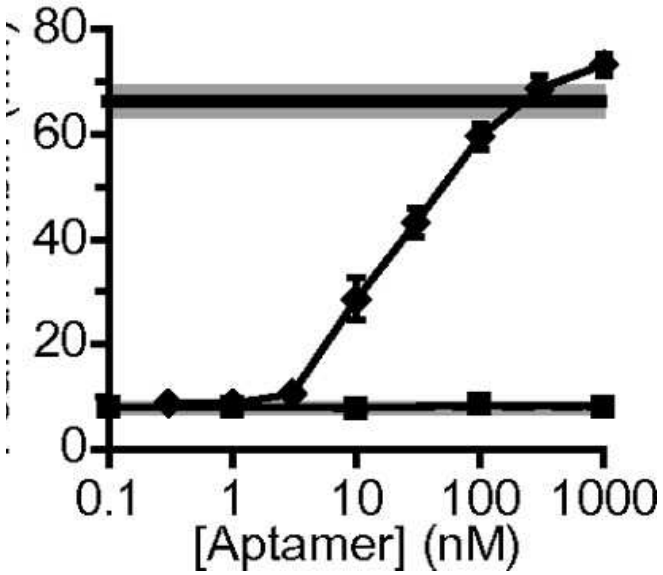
ARC19499 effect on thrombin generation in human plasma.

Activity in hemophilia B plasma



Hemophilia B+ aptamer

normal plasma

Hemophilia B

Normal plasma (solid lines)
Hemophilia (dashed lines)

+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522

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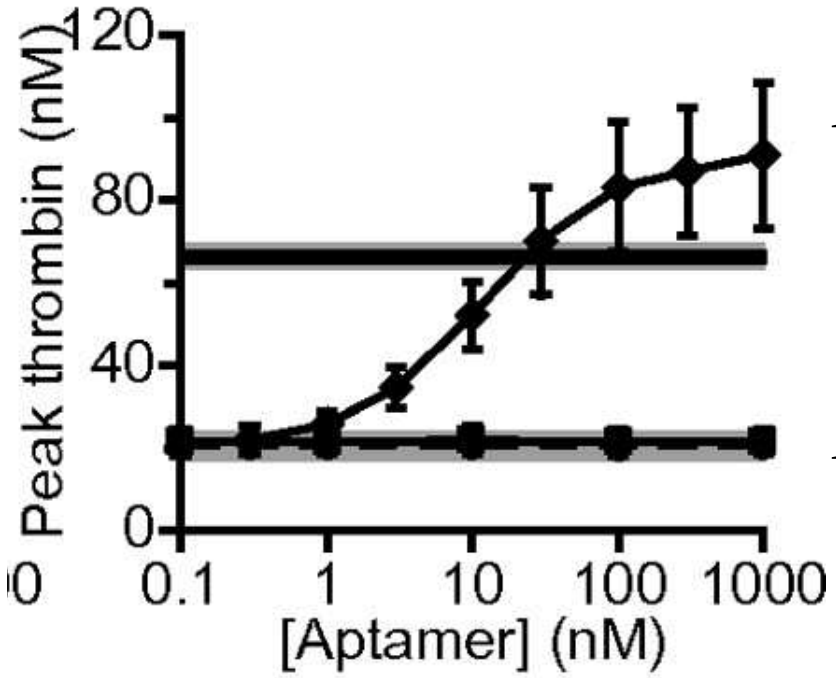
ARC19499 effect on thrombin generation in human plasma.

Activity in plasma with antibody inhibitor



Anticorpo inibitore + aptamer

normal plasma

Anticorpo inibitore

Normal plasma (solid lines)
Hemophilia (dashed lines)

+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522