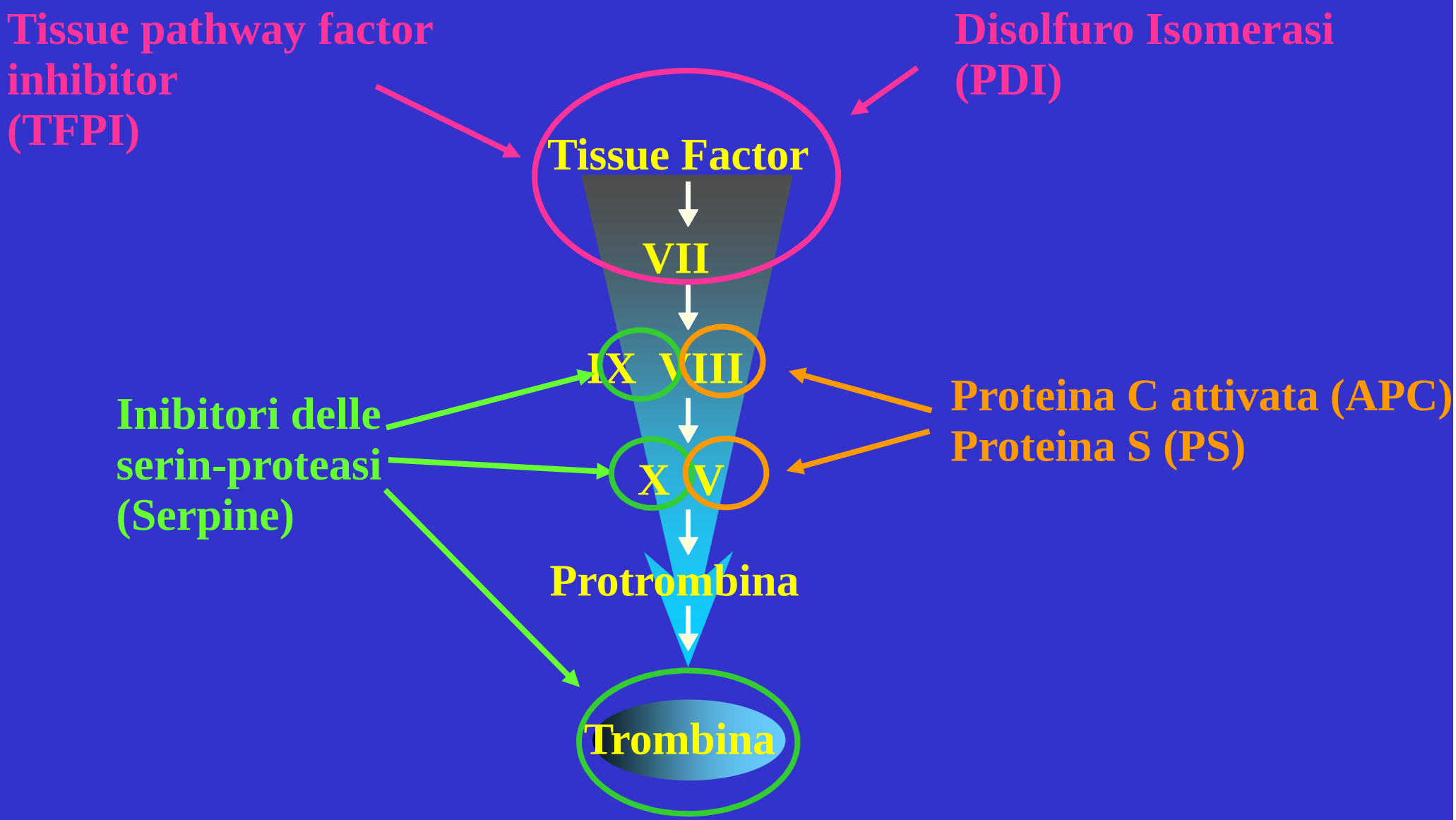
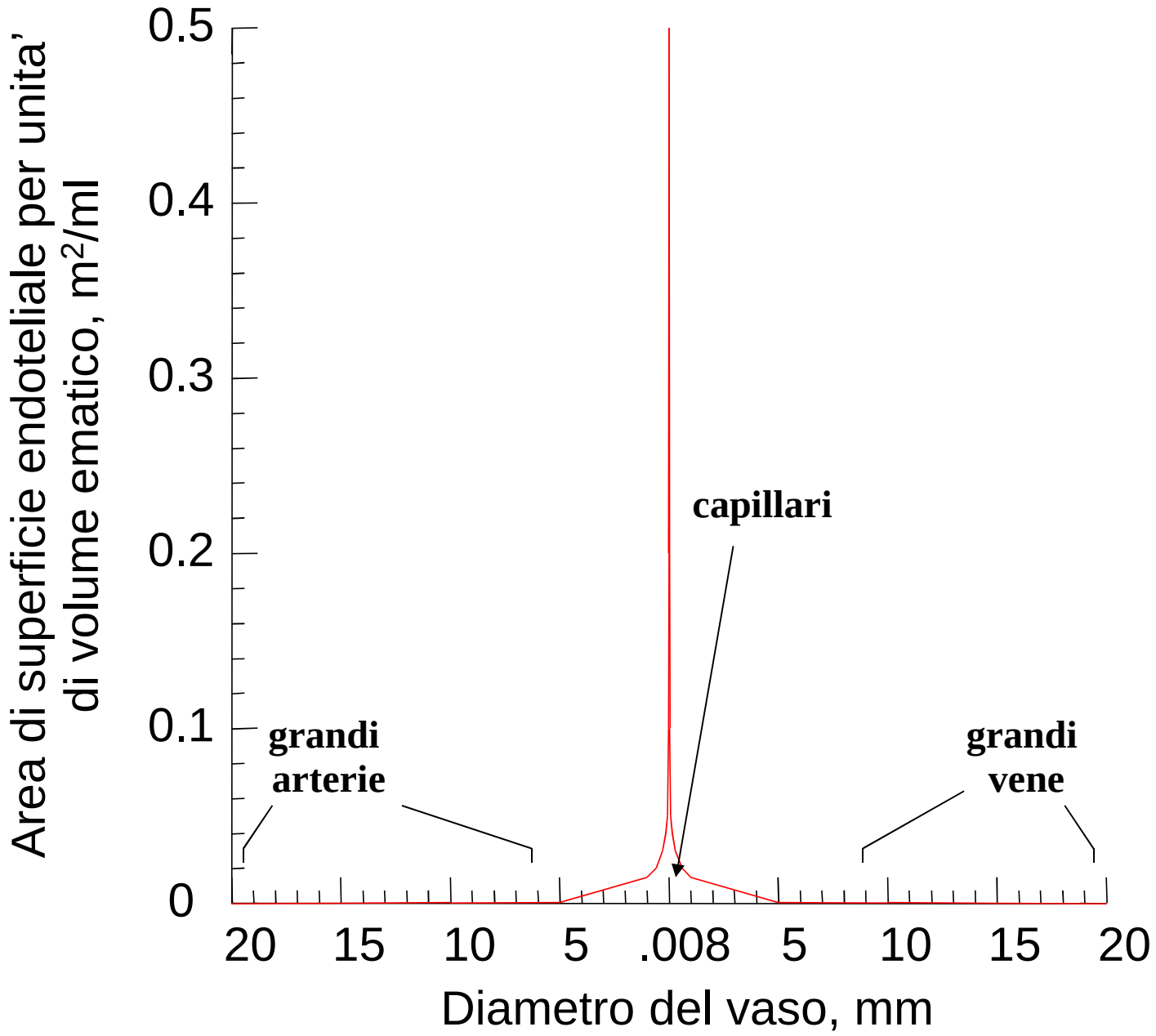
Cascata coagulativa
Regolazione Naturale



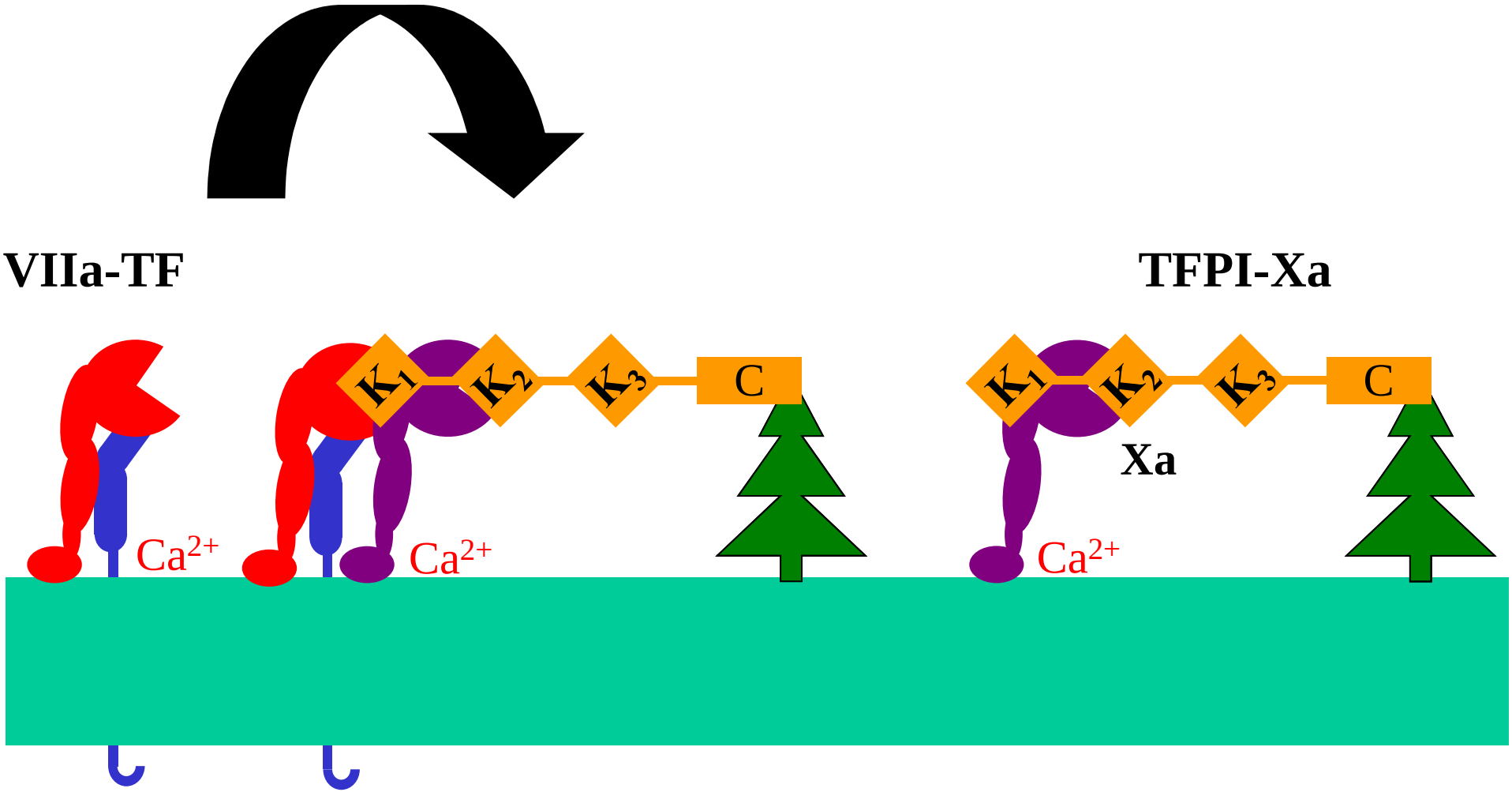
SISTEMI ANTICOAGULANTI NATURALI

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)

Rapporti tra superficie endoteliale e sangue circolante



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 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

the clone

(5′-mGmGmAmGmGmGmAmAmAmAmGmUmUmA-
mUdCmAmGmGdCdCmUmGmAmAmUmUmUmGmGmAmAmUmAmUmA
dCmUmUmGmGdCmUdCmGmUmUmAmGmGmUmGdCmGmUmAmUmA
mUmAmGmAmUmUmAmGmUmUmUmUmGmGmAmGmUmAdCmUdCm
GdCmUdCdC-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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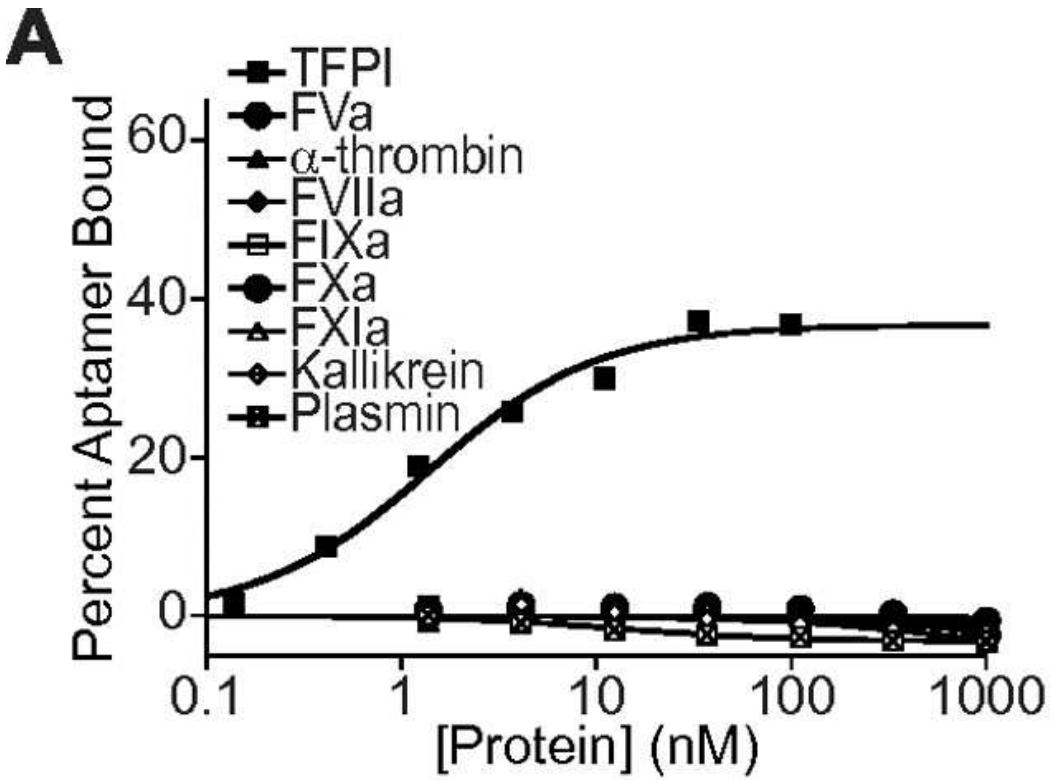
ARC17480 binding to TFPI and other proteins.



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

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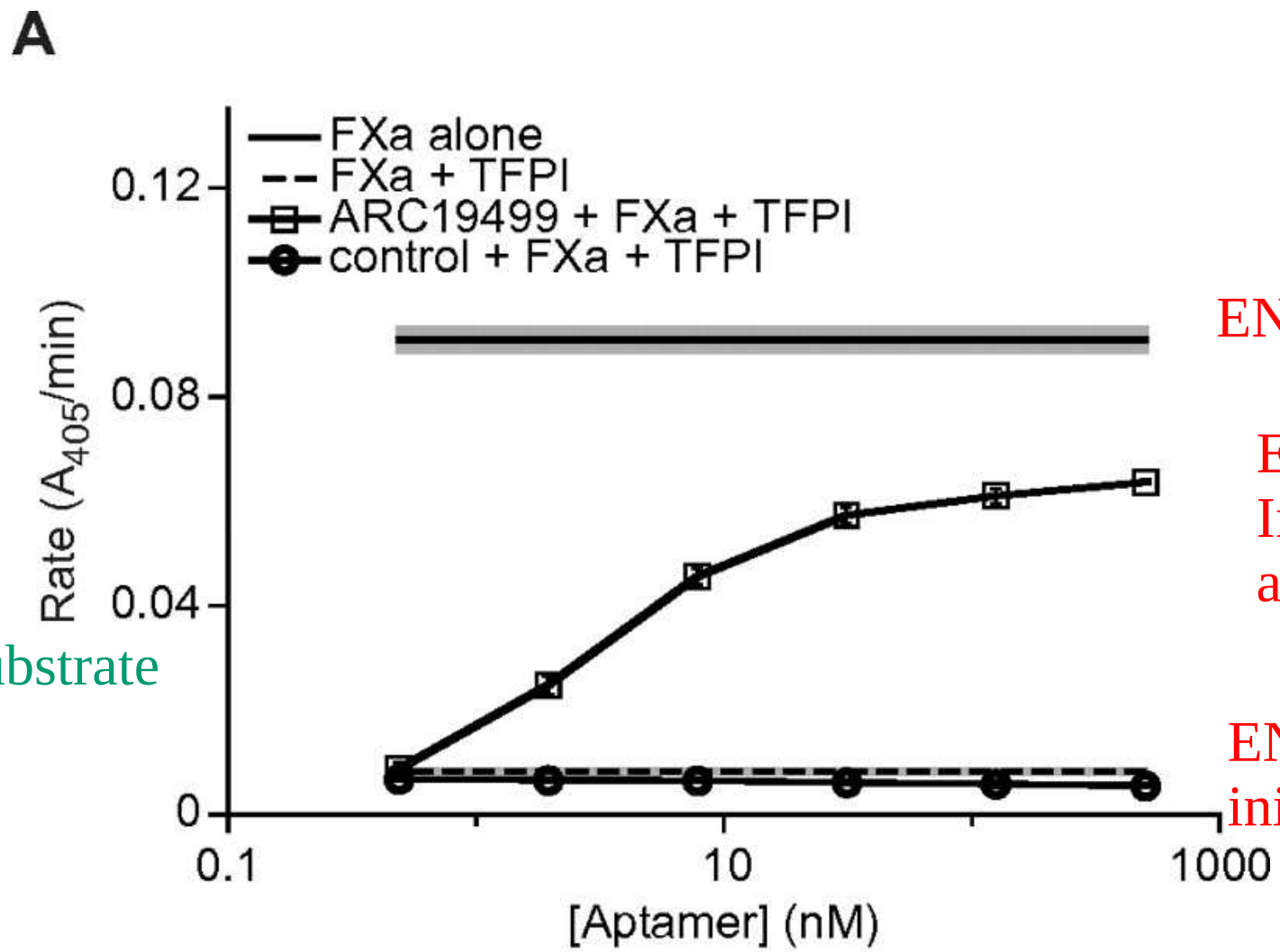
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

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



ENZIMA

ENZIMA+ Inibitore+ aptamero

ENZIMA+ inibitore

chromogenic substrate

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

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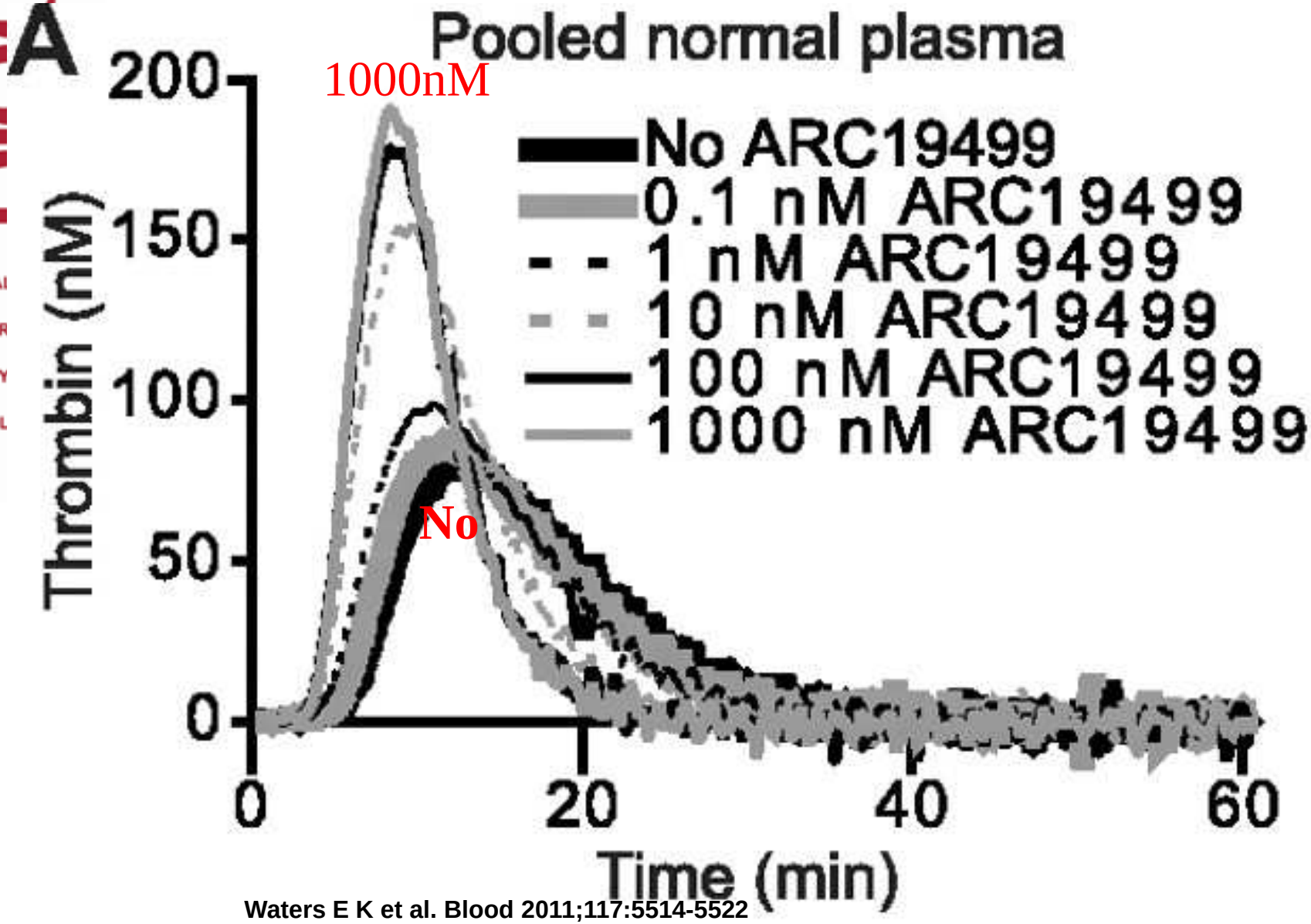
ARC19499 inhibition of TFPI in human plasma.



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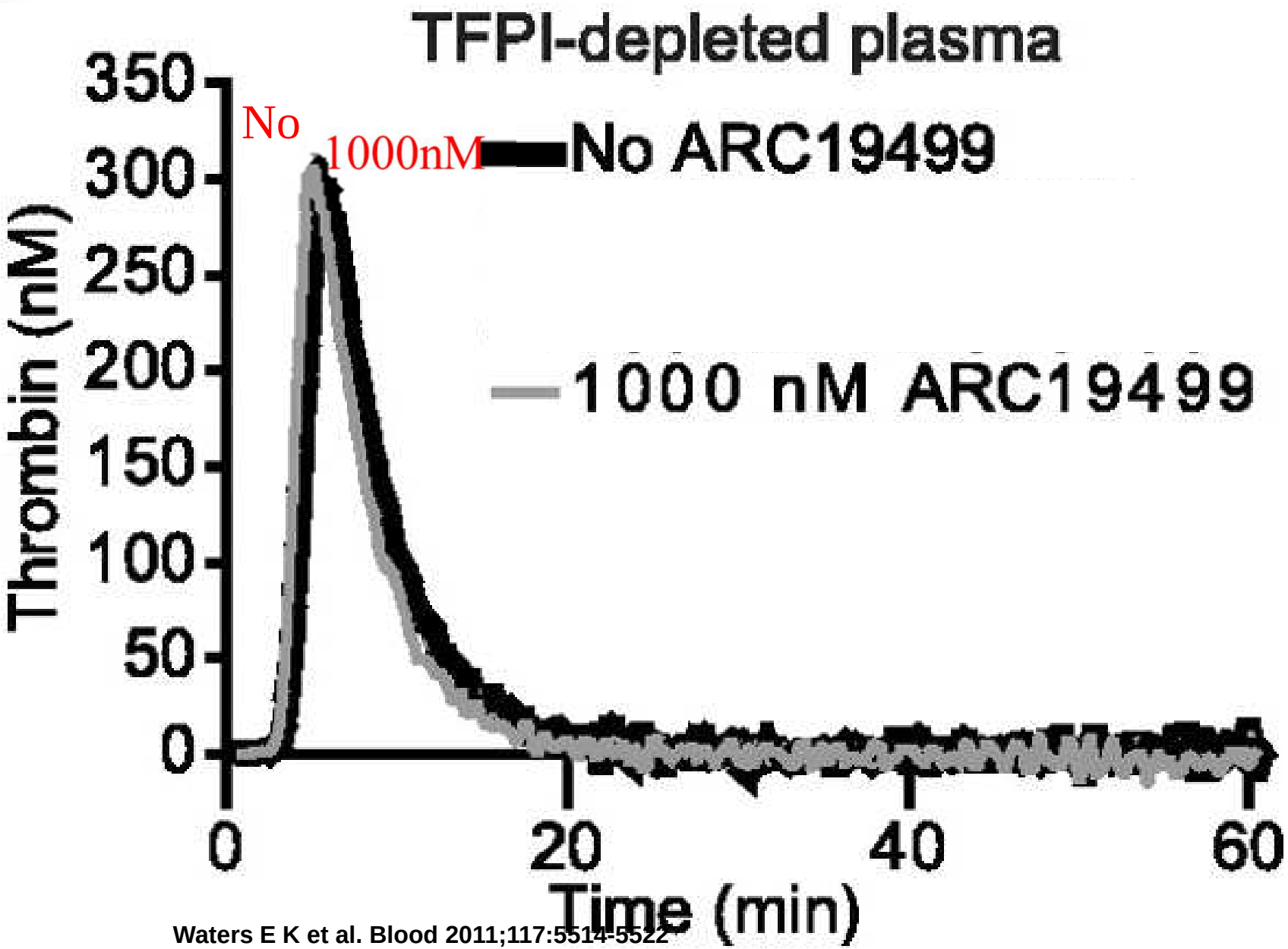

Waters E K et al. Blood 2011;117:5514-5522

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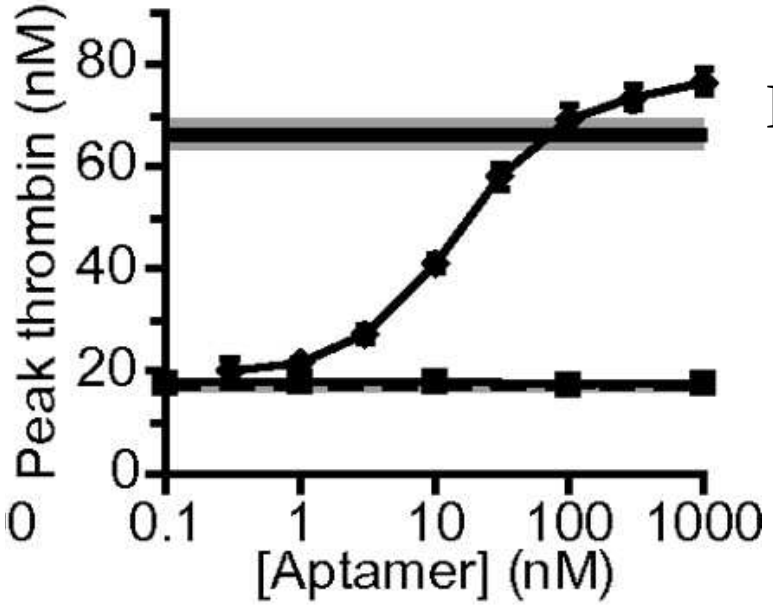
ARC19499 effect on thrombin generation in human plasma.

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

blood
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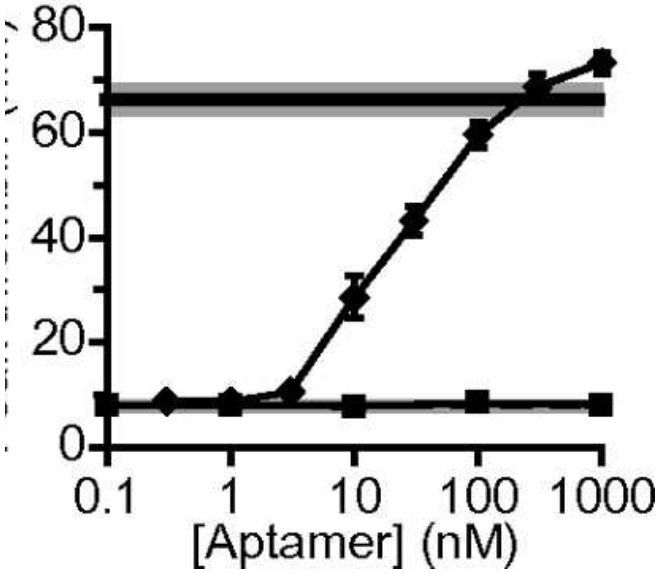
ARC19499 effect on thrombin generation in human plasma.

Activity in hemophilia B plasma



Hemophilia B+ aptamer
normal plasma

Hemophilia B

Normal plasma (solid lines)
Hemophilia (dashed lines)

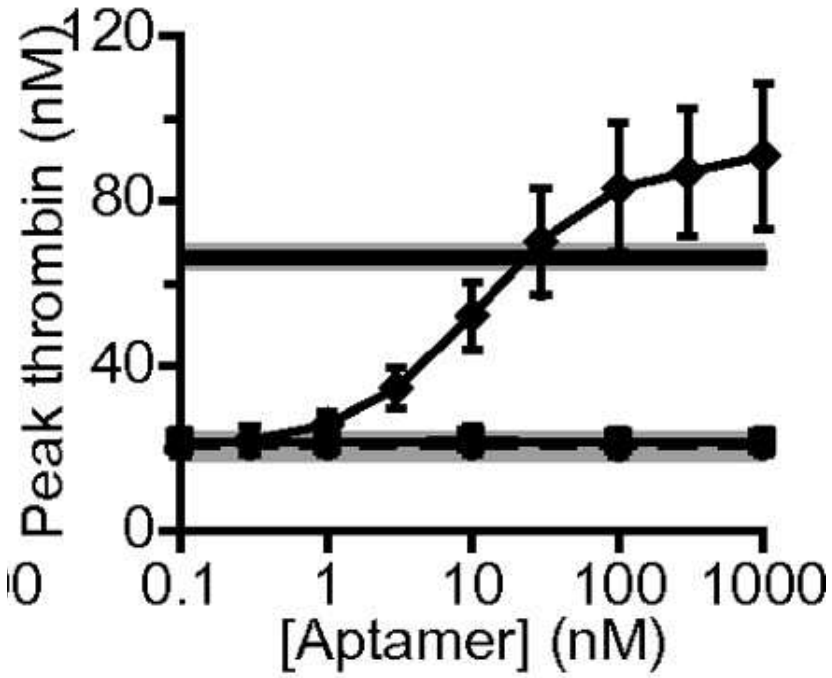
+ ARC19499 (♦)
+ negative control oligonucleotide (■).

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

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

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

ARC15105 Is a Potent Antagonist of Von Willebrand Factor Mediated Platelet Activation and Adhesion

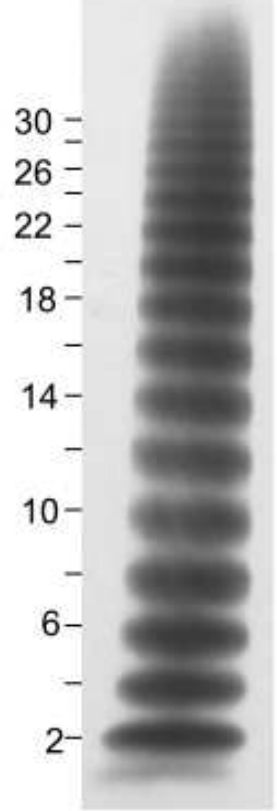
by Jolanta M. Siller-Matula, Yahye Merhi, Jean-François Tanguay, Daniel Duerschmied, Denisa D. Wagner, Kathleen E. McGinness, P. Shannon Pendergrast, Jou-Ku Chung, Xianbin Tian, Robert G. Schaub, and Bernd Jilma

Arterioscler Thromb Vasc Biol
Volume 32(4):902-909
March 14, 2012

Dimerization and multimerization of VWF

Monomer
0.25 x 10^6 Da

> 30 monomers !!



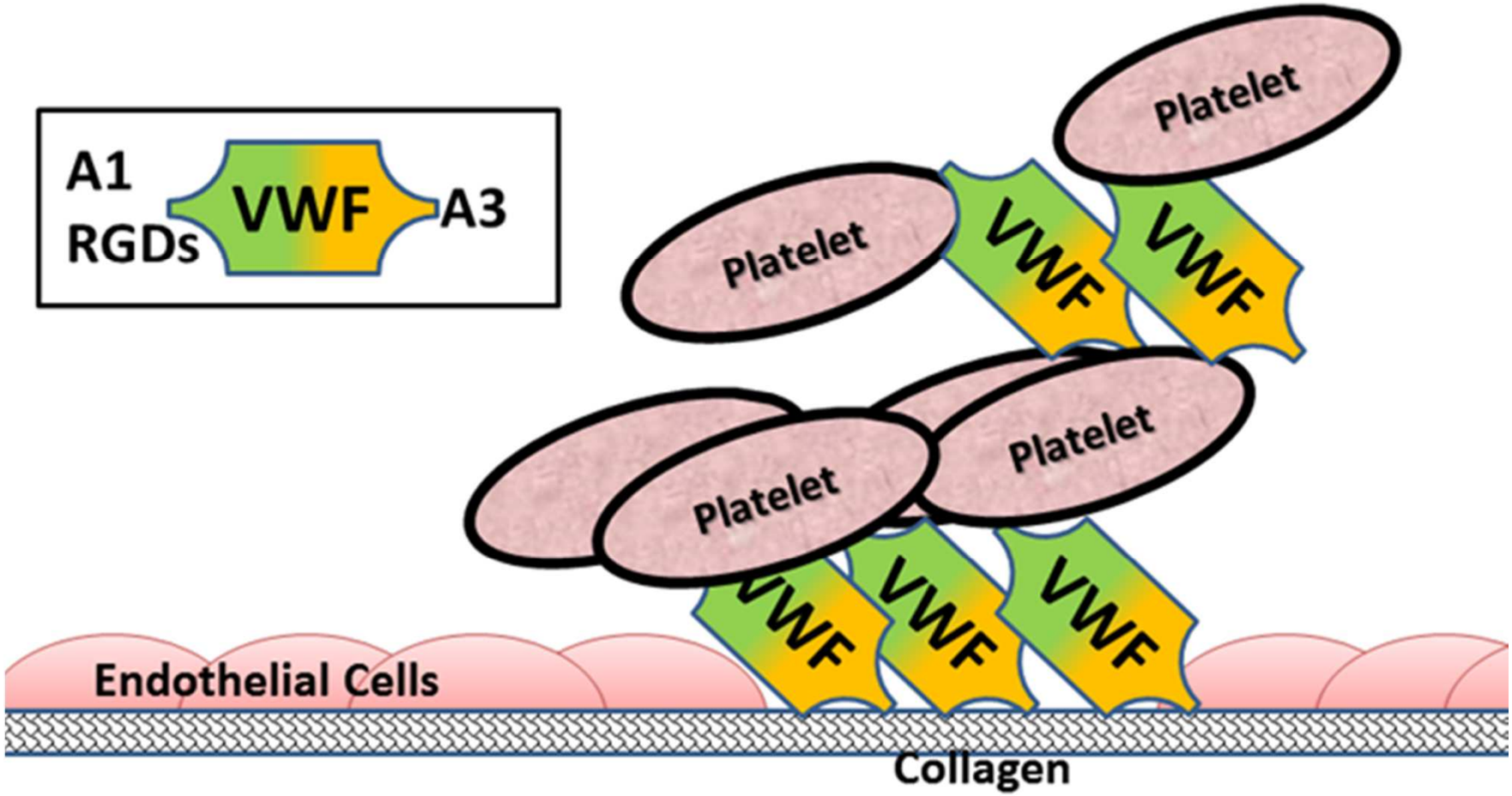
Dimer
0.5 x 10^6 Da

Tetramer
1.0 x 10^6 Da

Hexamer
1.5 x 10^6 Da

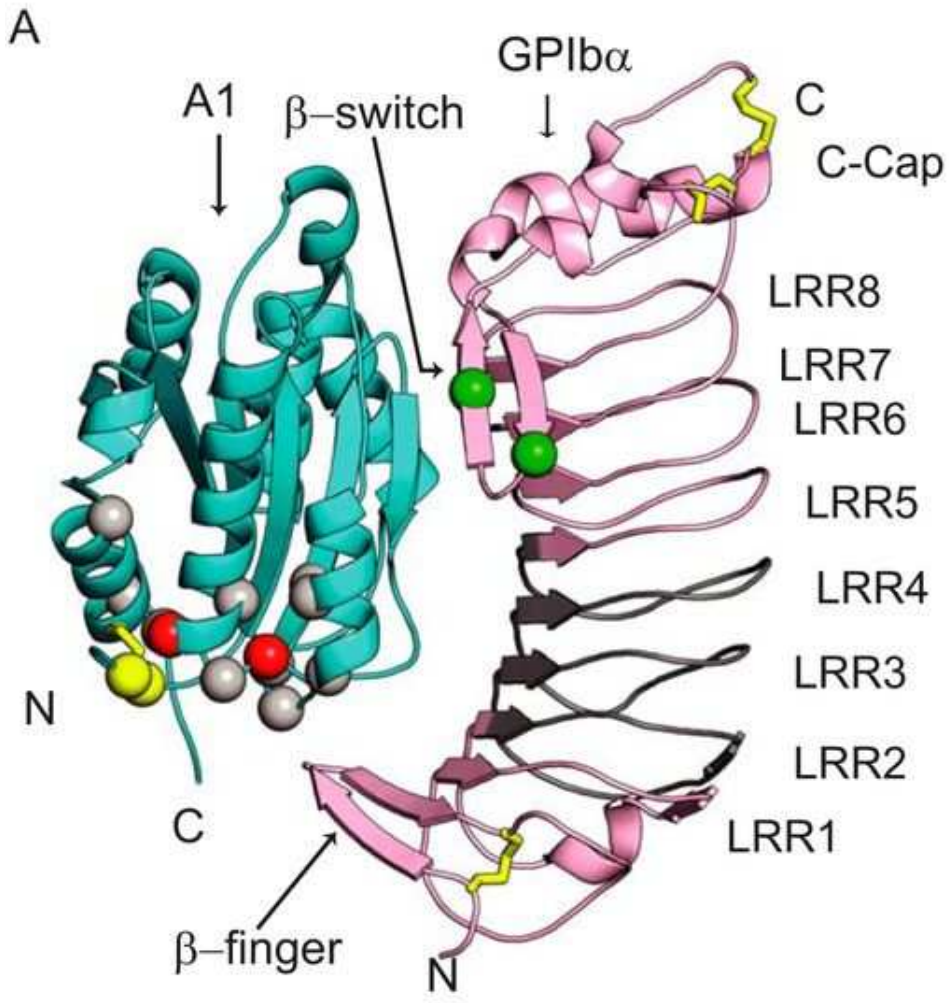
Multimer
≥ 7.5 x 10^6 Da

Adapted from Cecile Denis


A1
RGDs
VWF
A3
Platelet
Platelet
VWF
VWF
Platelet
Platelet
VWF
VWF
VWF
Endothelial Cells
Collagen

The VWF A1-GPIbα complex.

VWF A1 domain



Platelet GPIbαreceptor

Mark A. Blenner et al. J. Biol. Chem. 2014;289:5565-5579

jbc

NH2-mGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUm
GmUmCmCmC-3T, where
NH2 = hexylamine linker,
3T inverted deoxythymidine residue
mN is a 2=-methoxy residue.

ARC15105 are appended with a 20-kDa

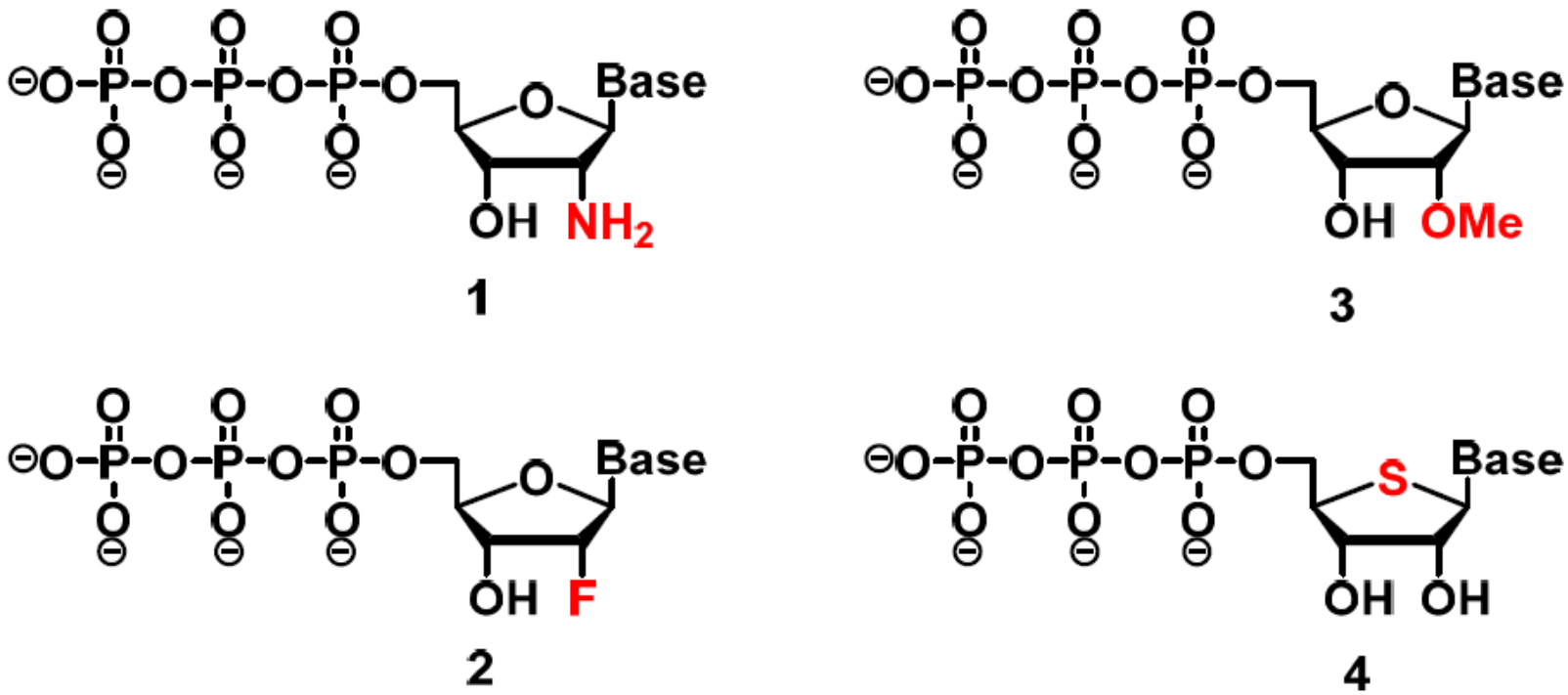
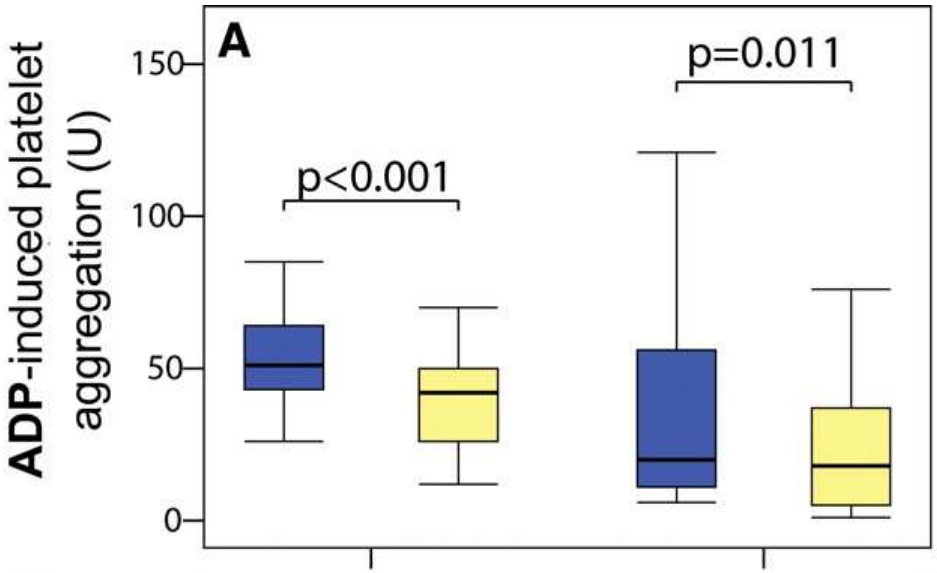


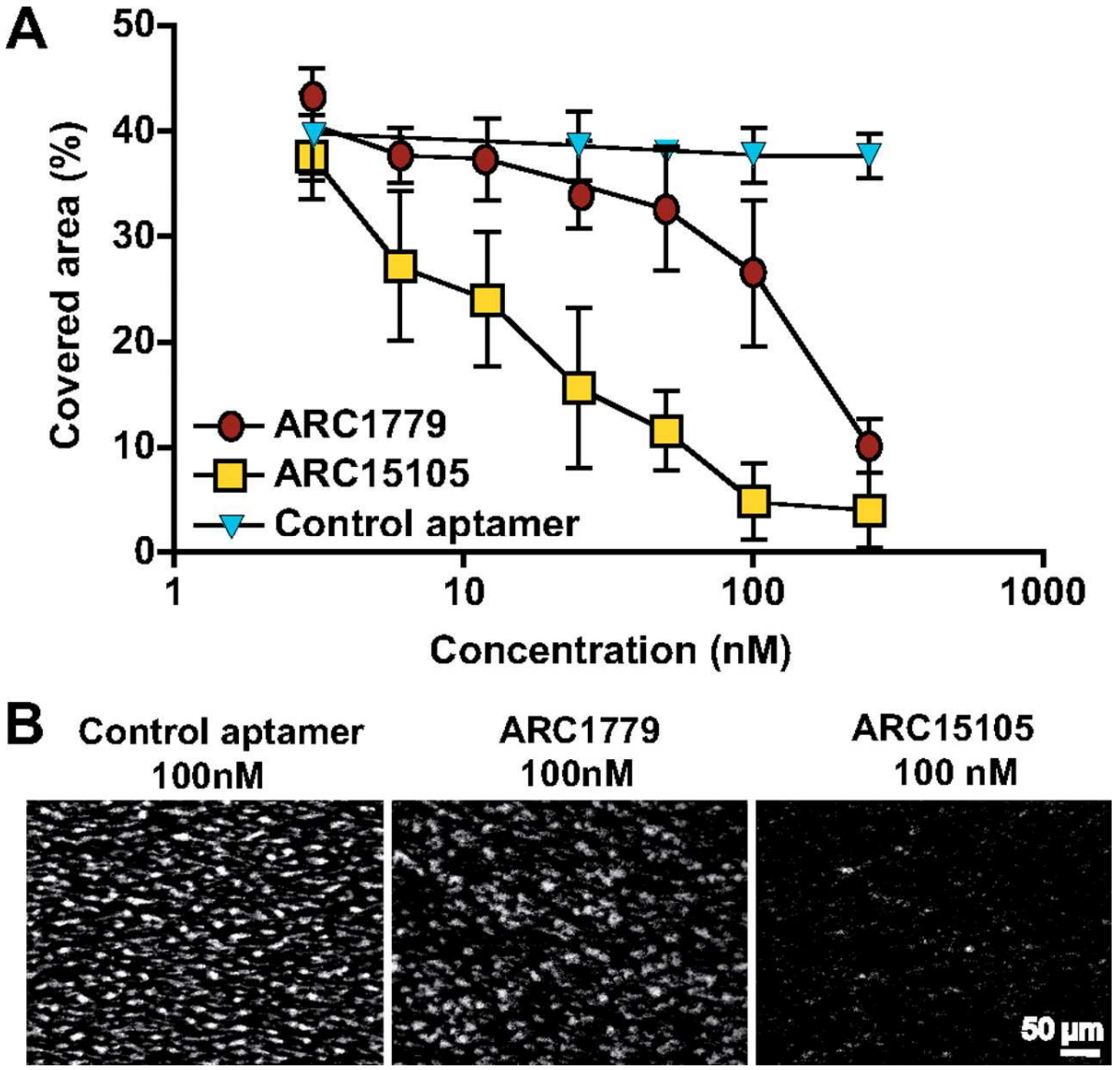
Figure 2. Chemical structures of 2′-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2′-amino-NTPs **1**, 2′-fluoro-NTPs **2**, 2′-methoxy-NTPs **3**, and 4′-thio-NTPs **4**.

Platelet aggregation to ARC15105 (1.3 µmol/L) induced by various agonists: A, ADP (adenosine diphosphate); B, TRAP (thrombin receptor activating peptide); C, ristocetin; D, AA (arachidonic acid); E, collagen.



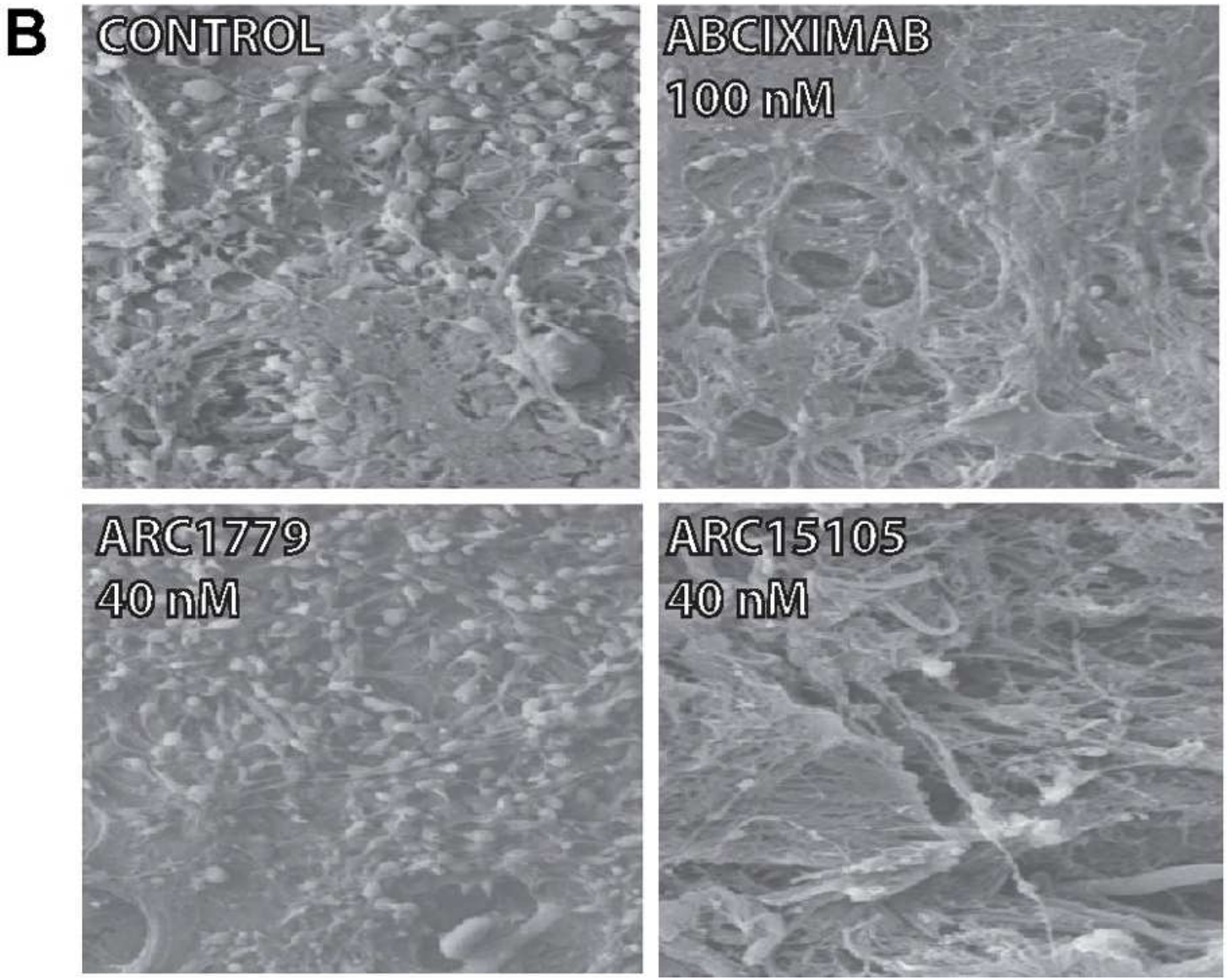
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

Concentration effect curve of ARC15105 and ARC1779 on platelet adhesion to collagen-bound VWF under arterial shear conditions.



Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

Platelet adhesion on injured porcine arterial segments; A, ARC15105, Arc1779, and abciximab inhibited the adhesion of platelets radiolabeled with 111In on injured porcine arterial segments in perfusion flow chambers.



Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

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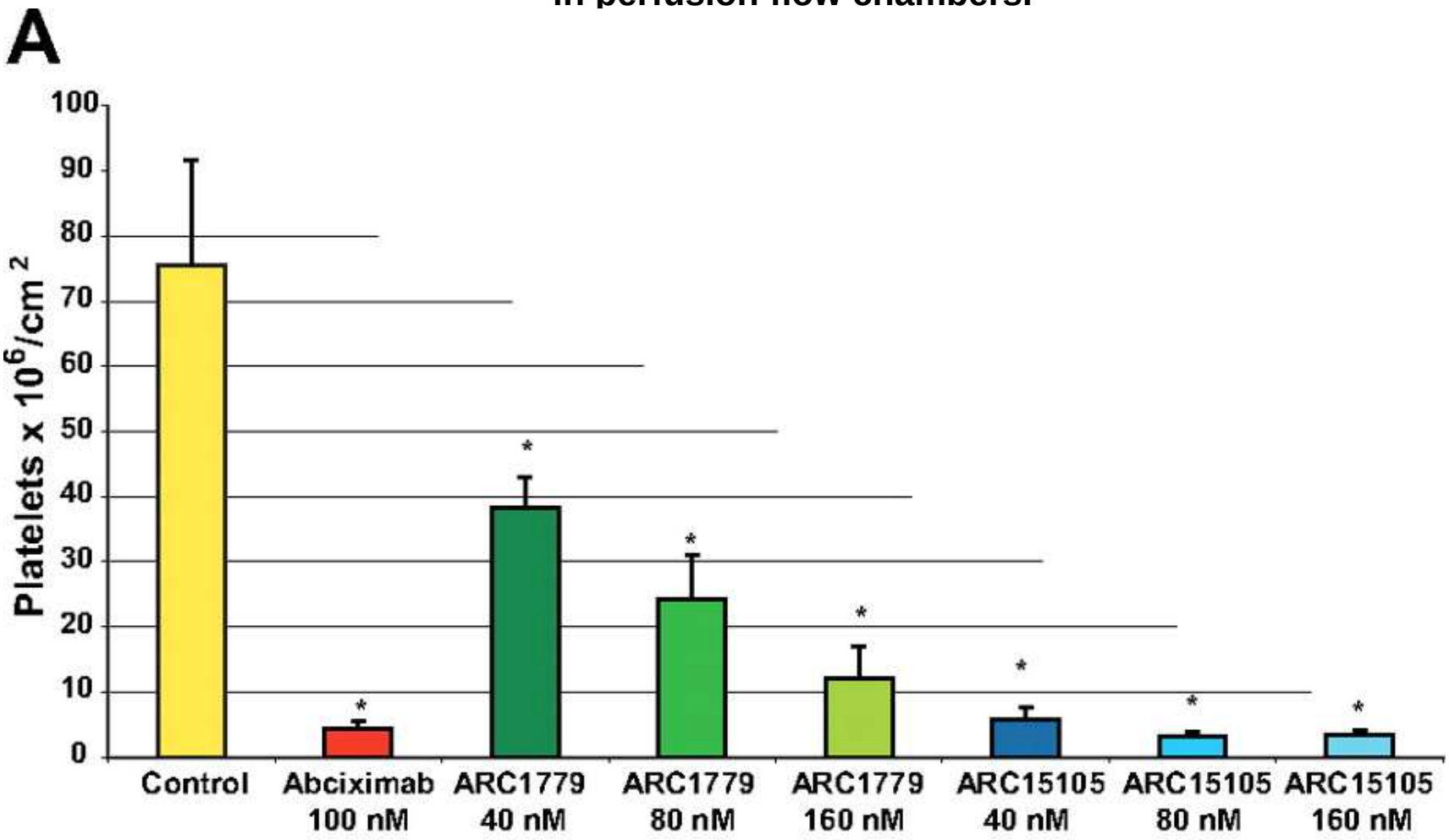
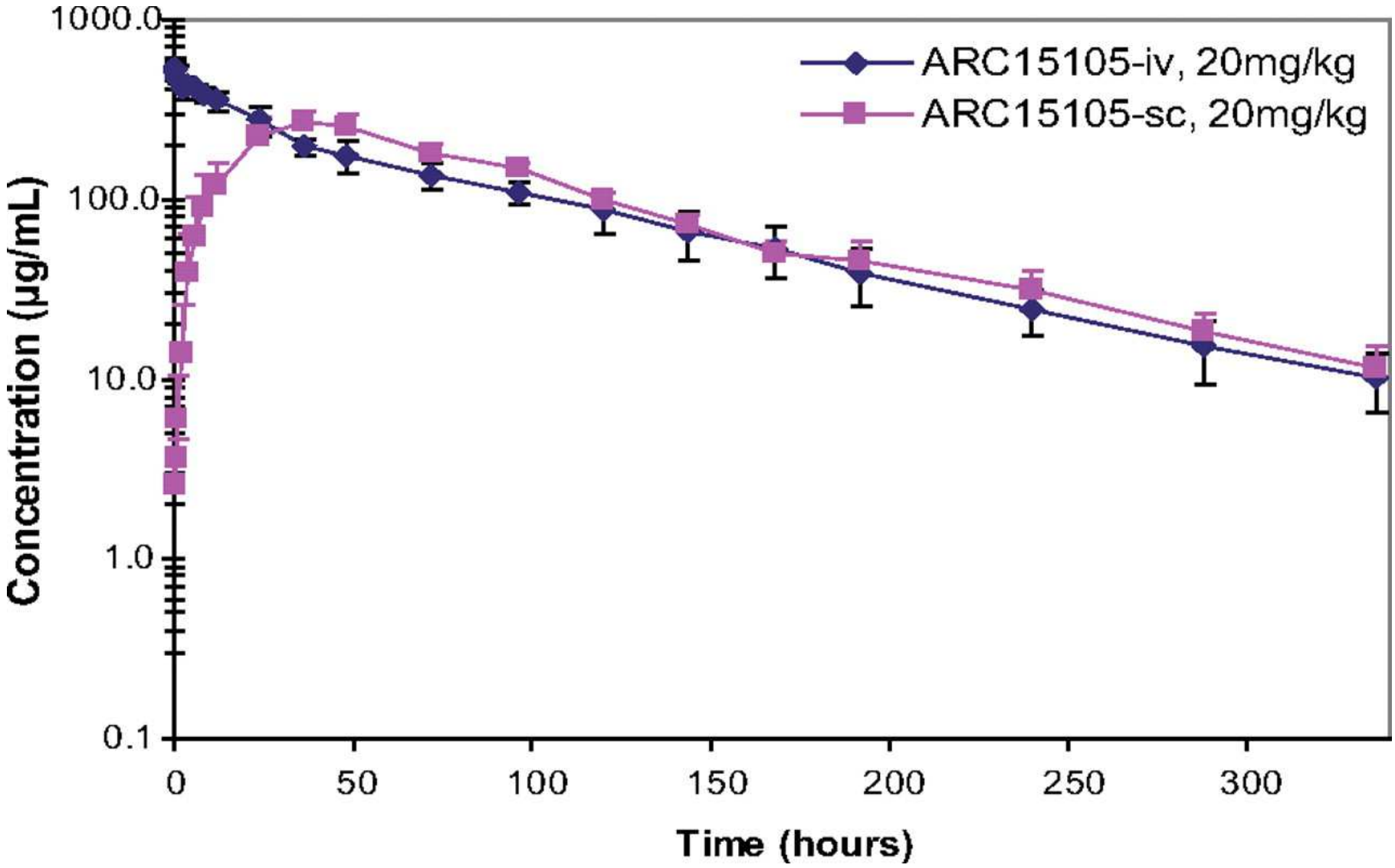


Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

Comparison of the pharmacokinetics of a single bolus of ARC15105 (20 mg/kg) administered intravenously (IV) and subcutaneously (SC) in 3 cynomolgus monkeys; P<0.05.



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