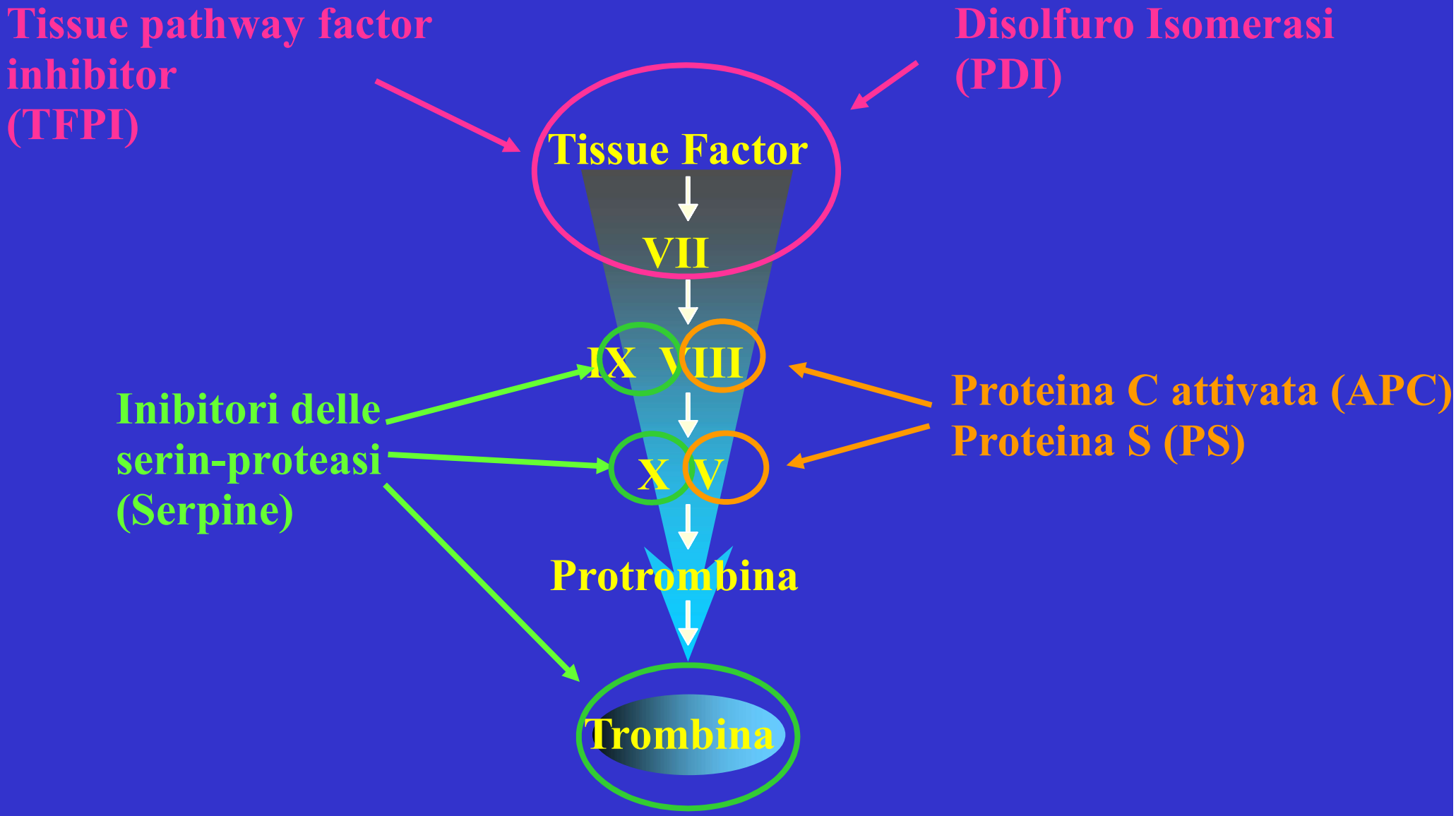
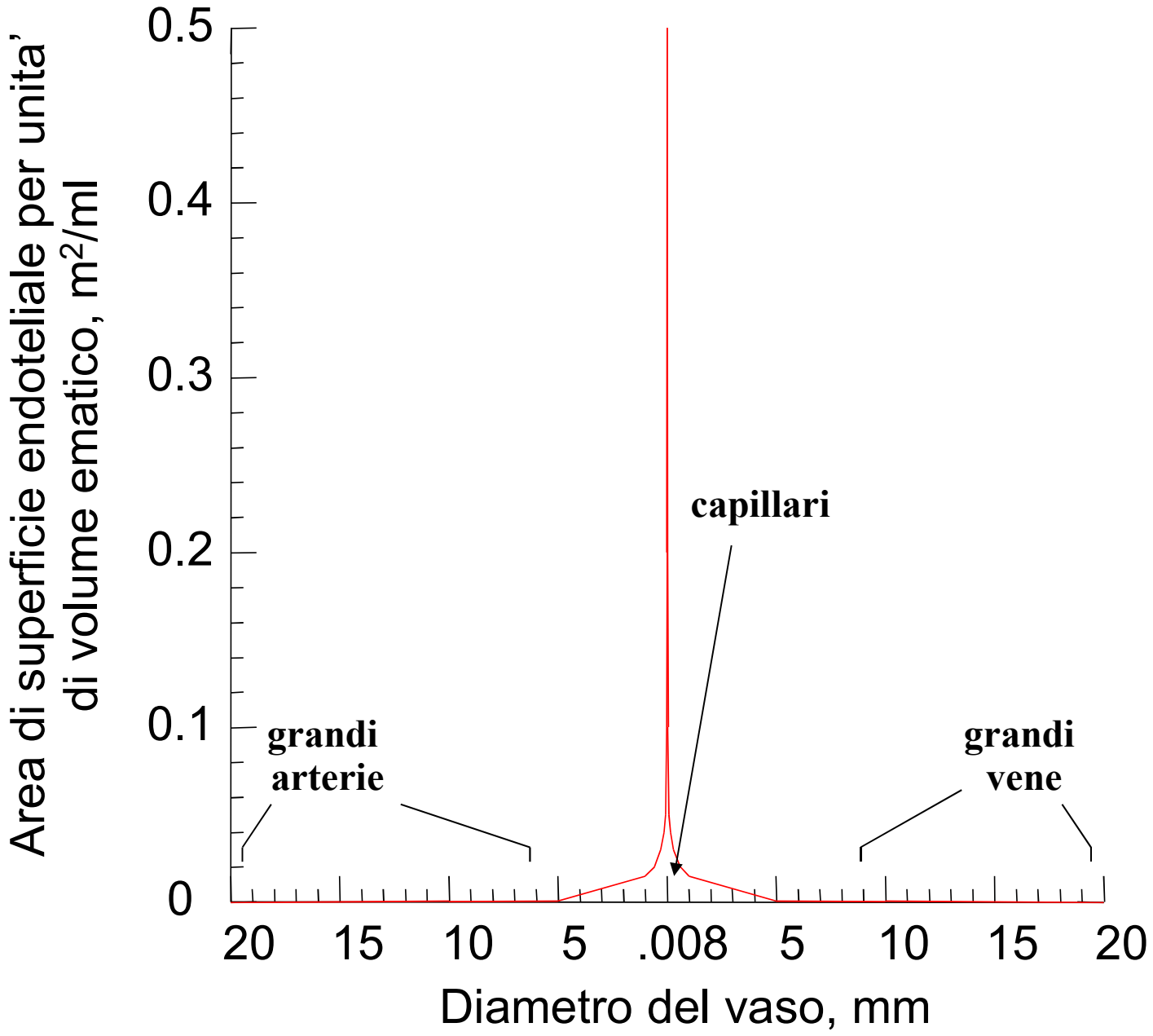
# Cascata coagulativa
# Regolazione Naturale



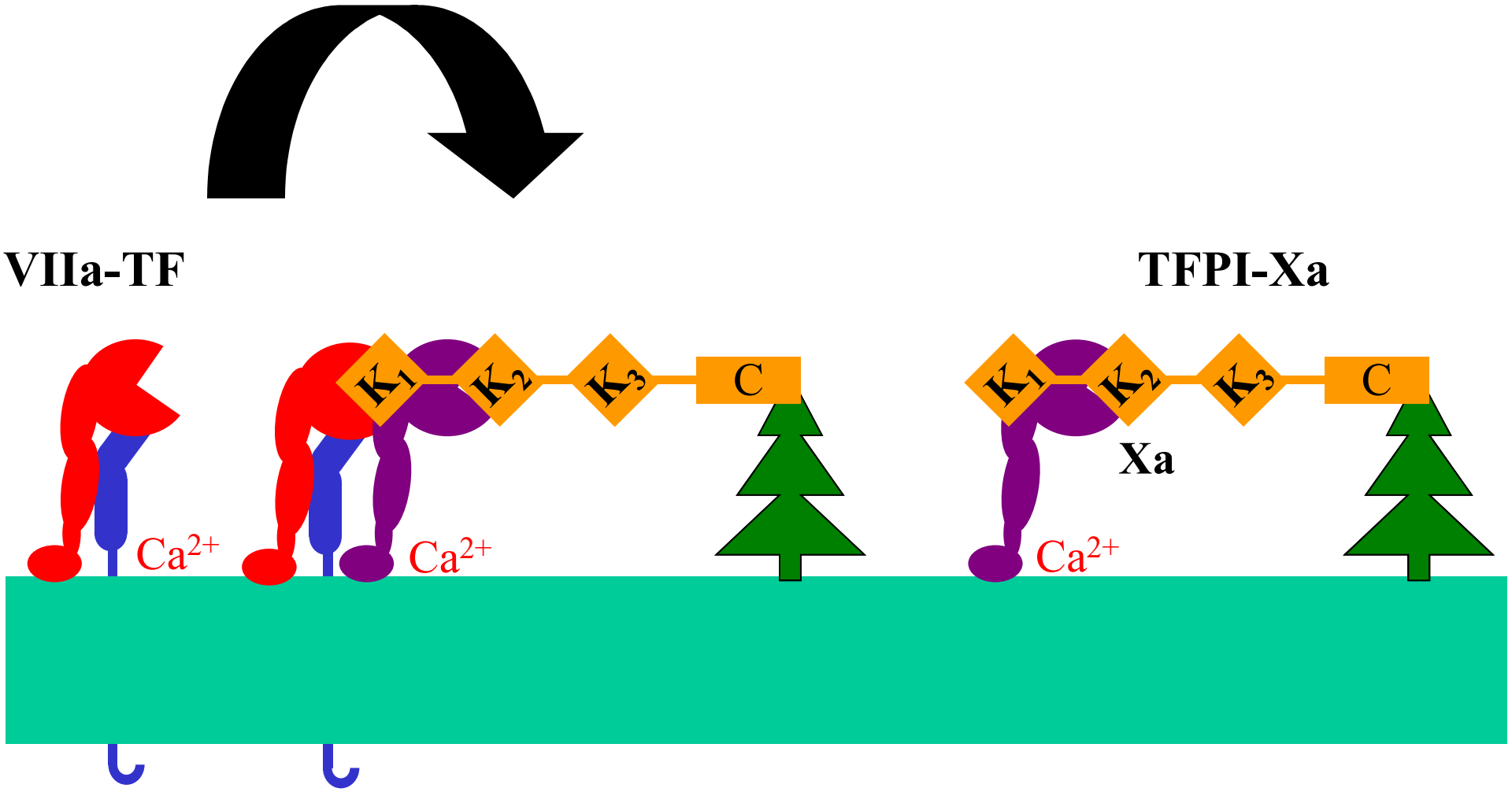
# SISTEMI ANTICOAGULANTI NATURALI

| 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′-GACTGTAATACGACTCACTATAGGAGGGAAAAG TTATC-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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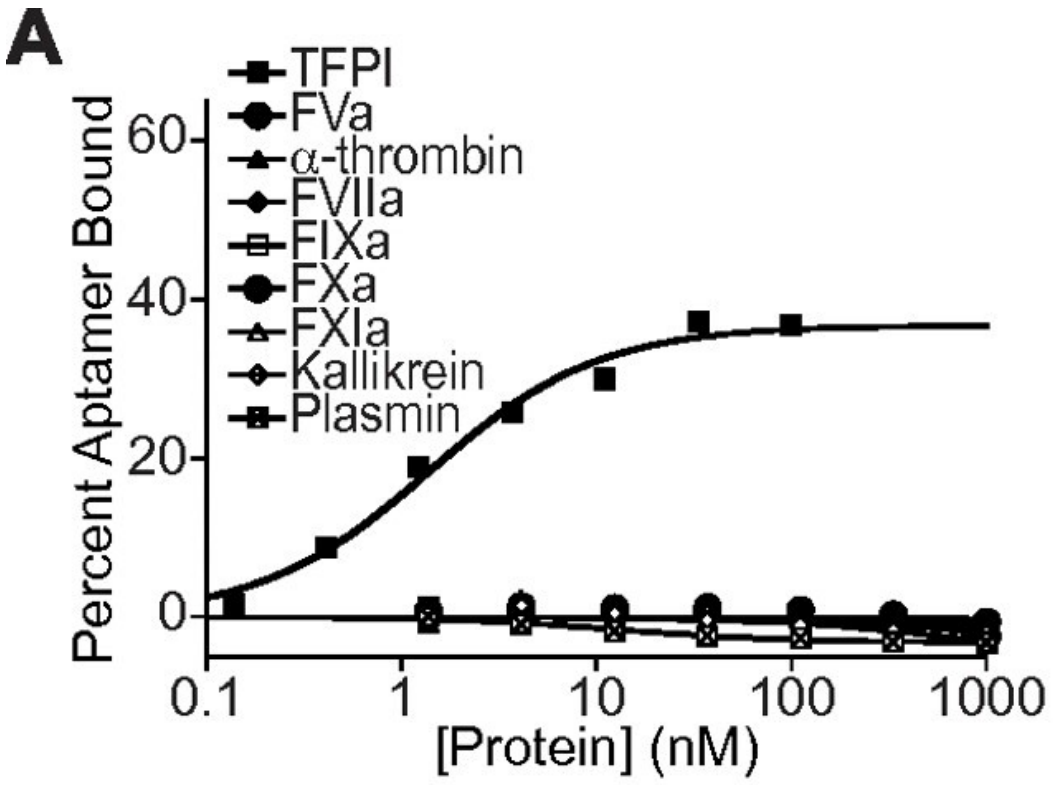
## ARC17480 binding to TFPI and other proteins.




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

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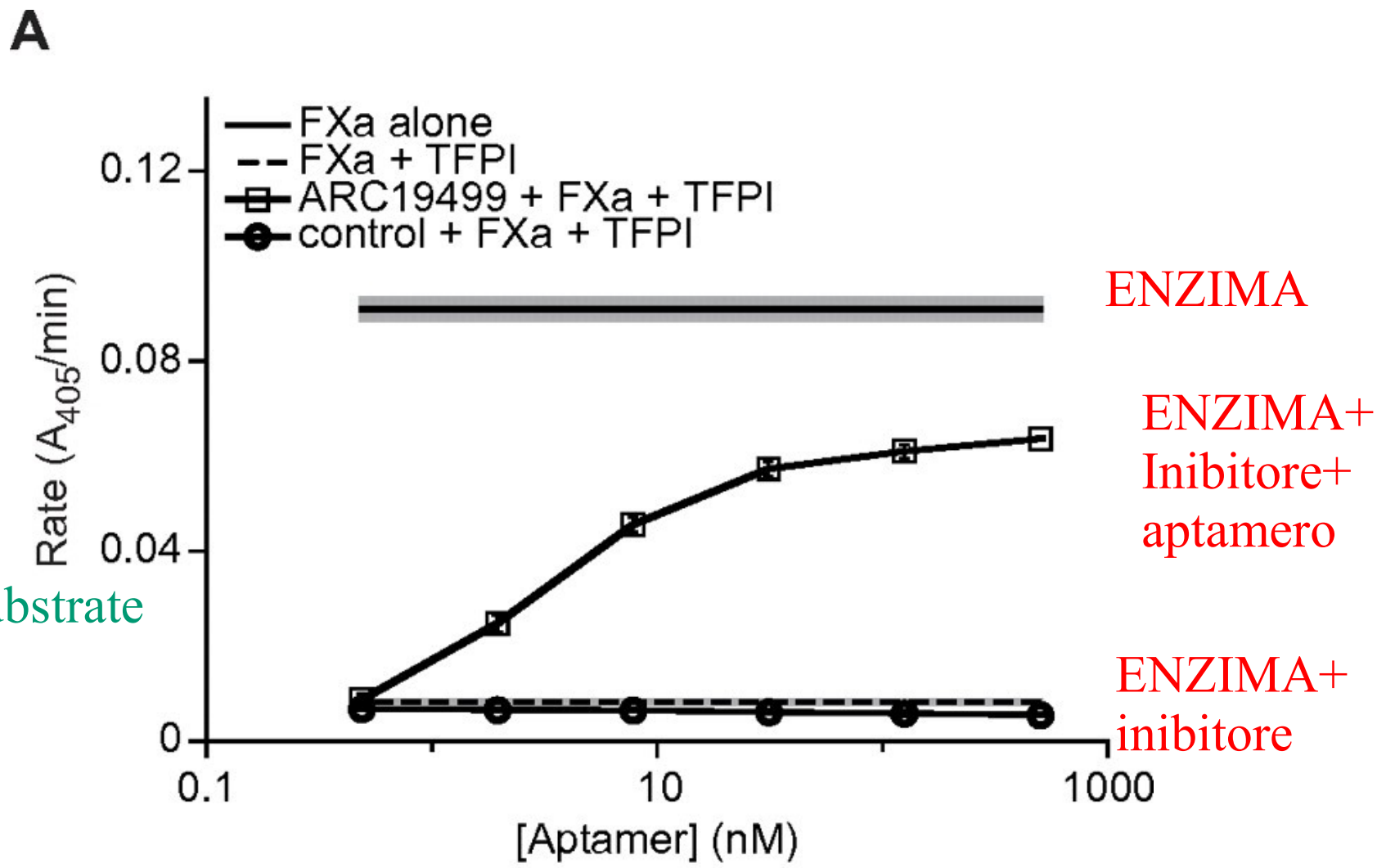
**Activity of ARC19499 in TFPI-dependent assays using purified proteins.**




ENZIMA

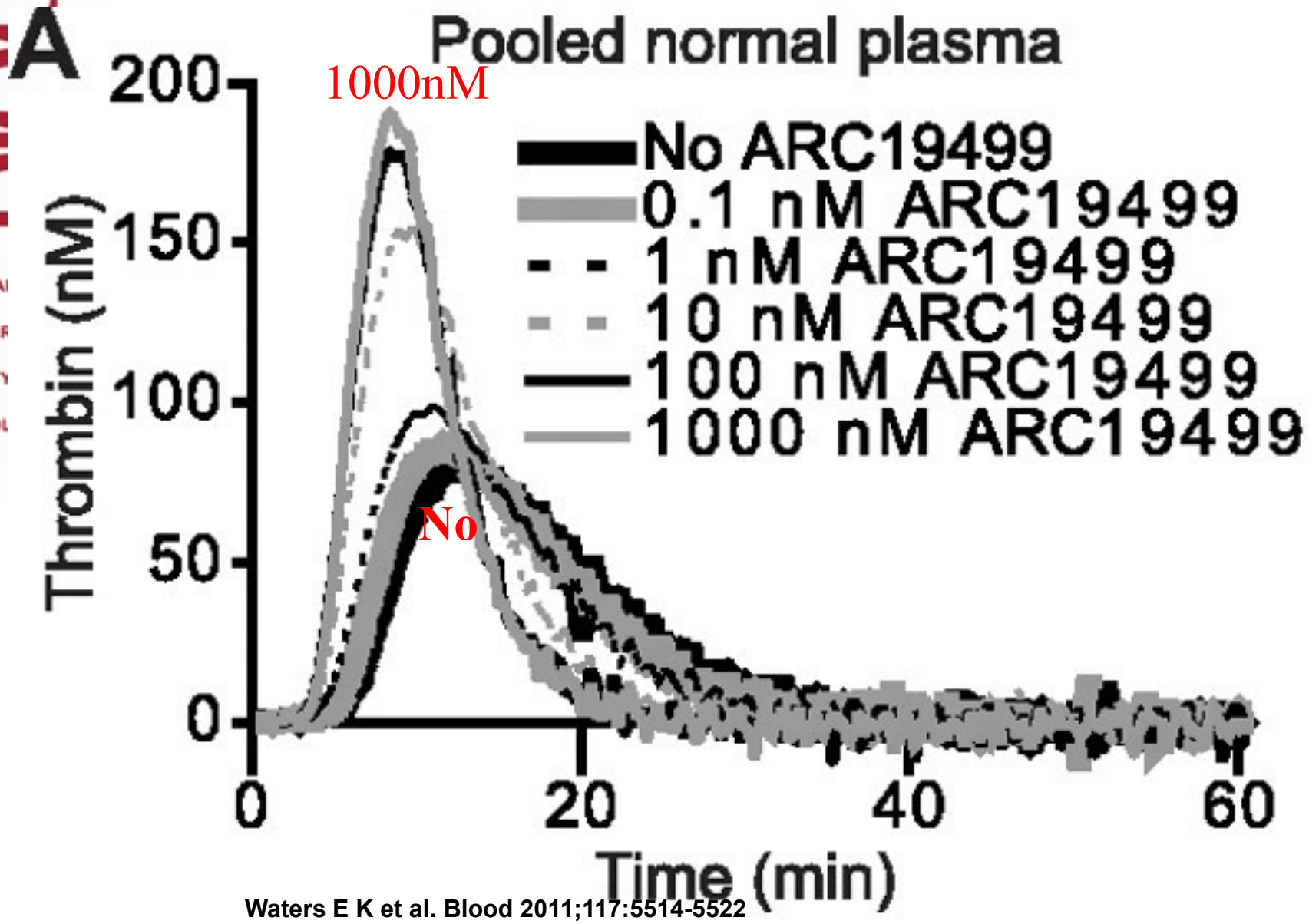
ENZIMA+ Inibitore+ aptamero

ENZIMA+ inibitore

chromogenic substrate

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

**ARC19499 inhibition of TFPI in human plasma.**




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

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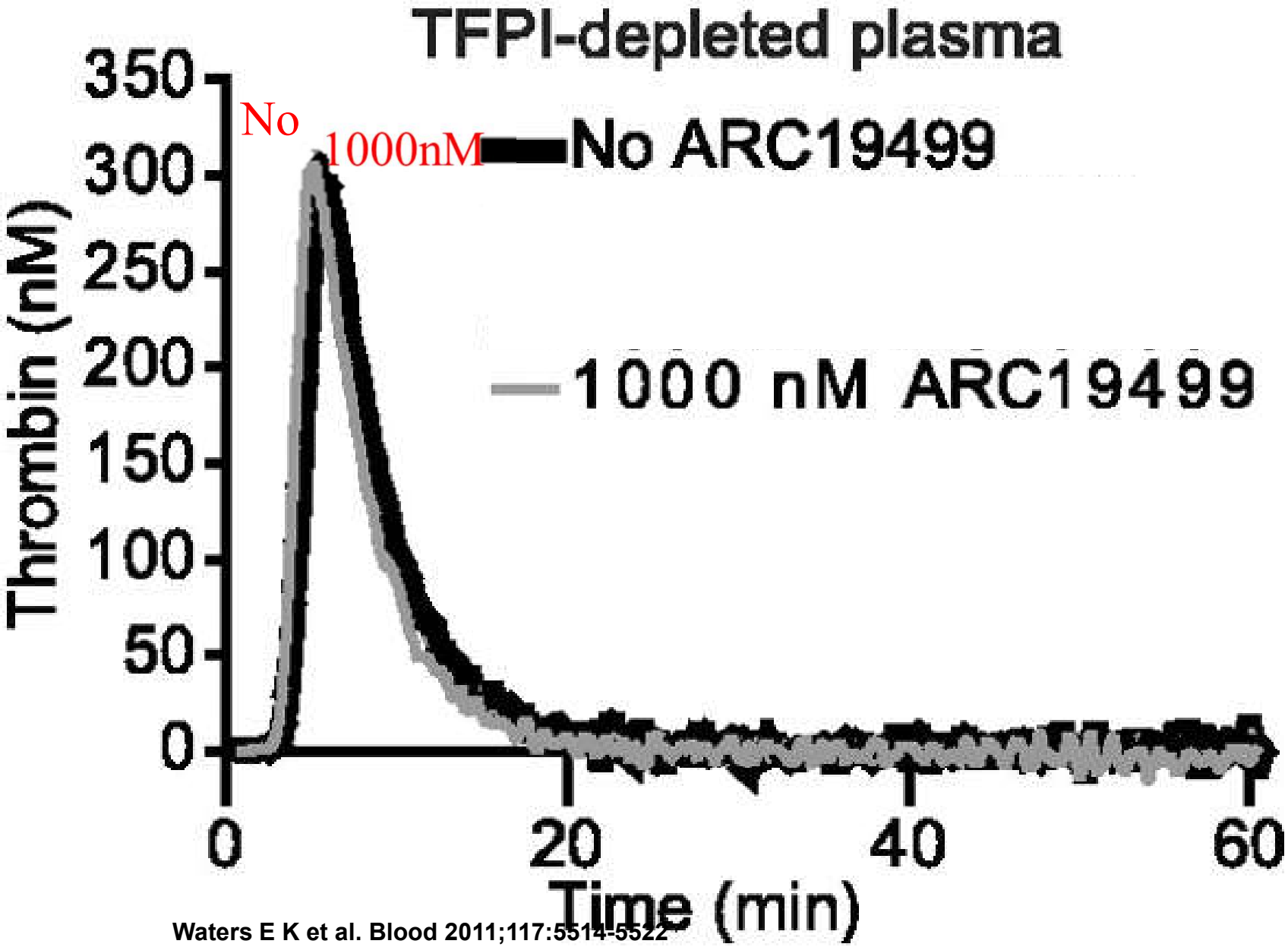
**ARC19499 inhibition of TFPI in human plasma.**




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

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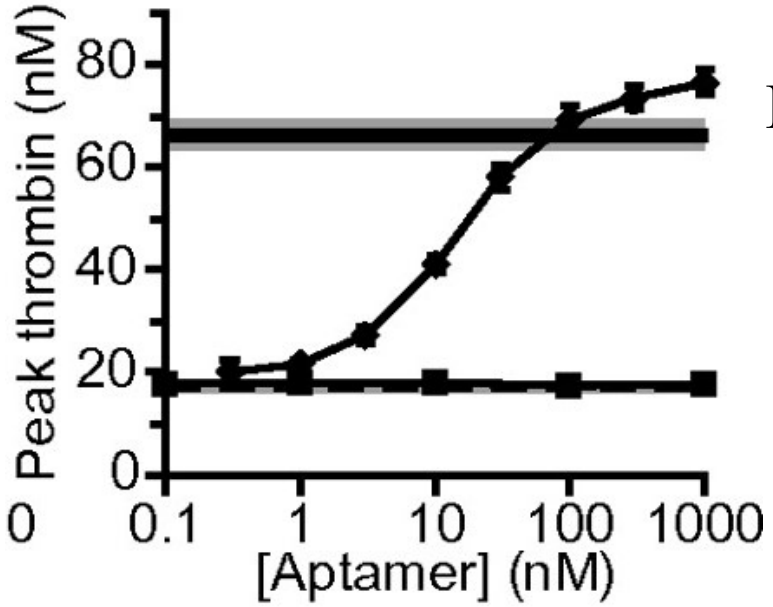
**ARC19499 effect on thrombin generation in human plasma.**

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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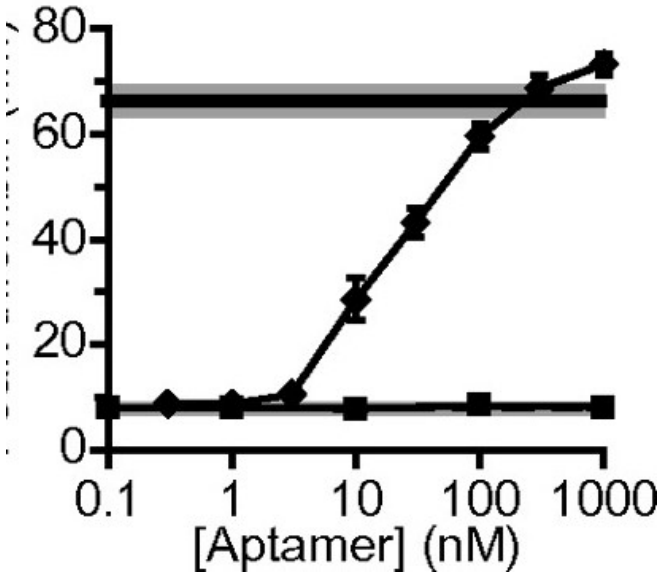
**ARC19499 effect on thrombin generation in human plasma.**

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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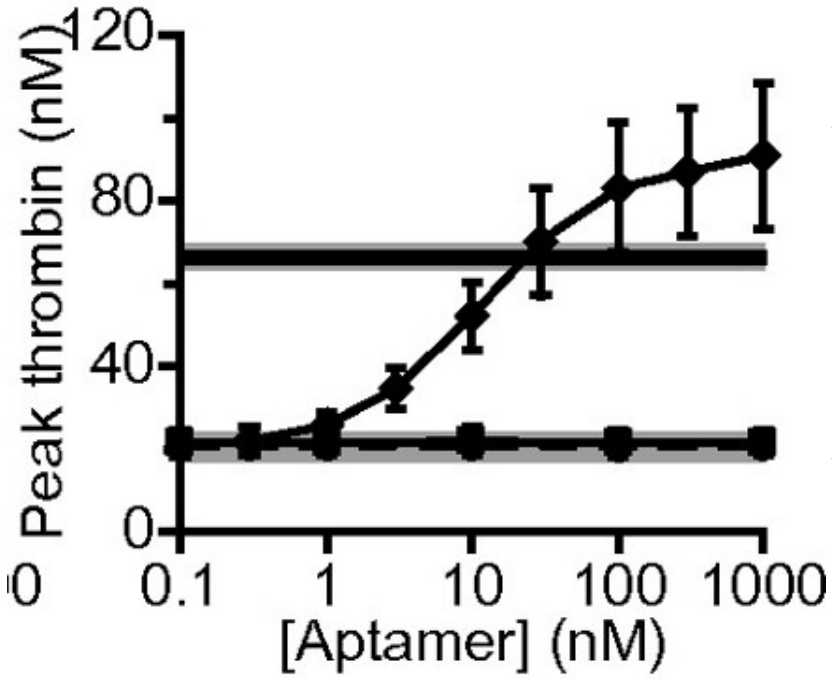
**ARC19499 effect on thrombin generation in human plasma.**

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**

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

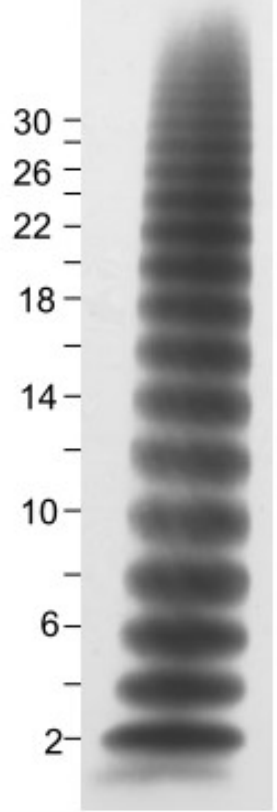
*by Jolanta M. Siller-Matula, Yahye Merhi, Jean-François Tanguay, Daniel Duerschmied, Denisa D. Wagner, Kathleen E. McGinness, P. Shannon Pendergrast, Jou-Ku Chung, Xianbin Tian, Robert G. Schaub, and Bernd Jilma*

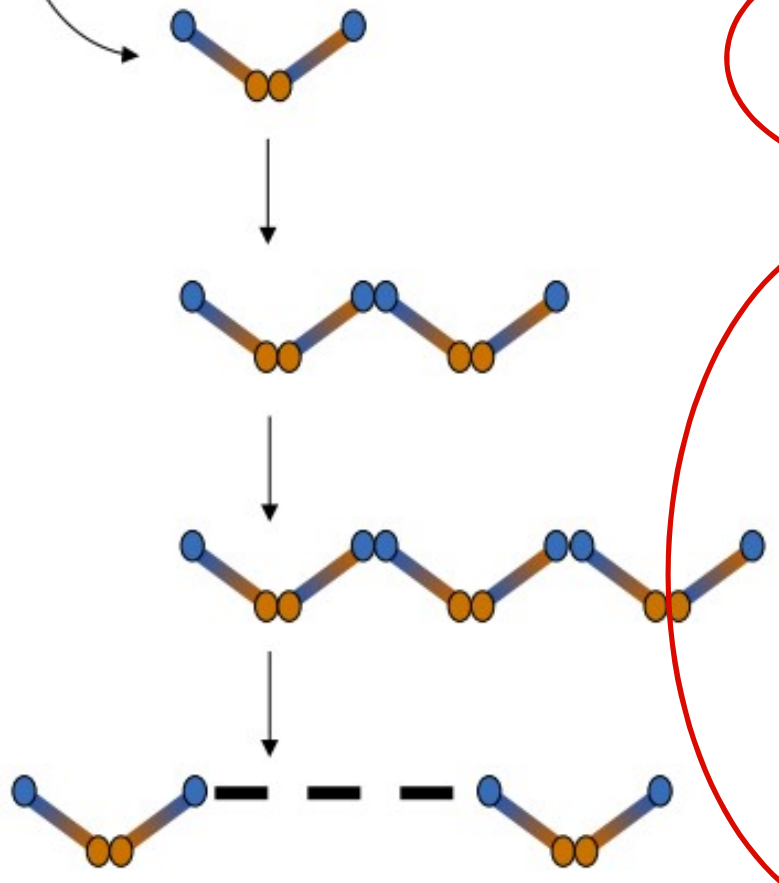
*Arterioscler Thromb Vasc Biol*
*Volume 32(4):902-909*
*March 14, 2012*

# Dimerization and multimerization of VWF


Monomer
0.25 x $10^6$ Da

> 30 monomers !!



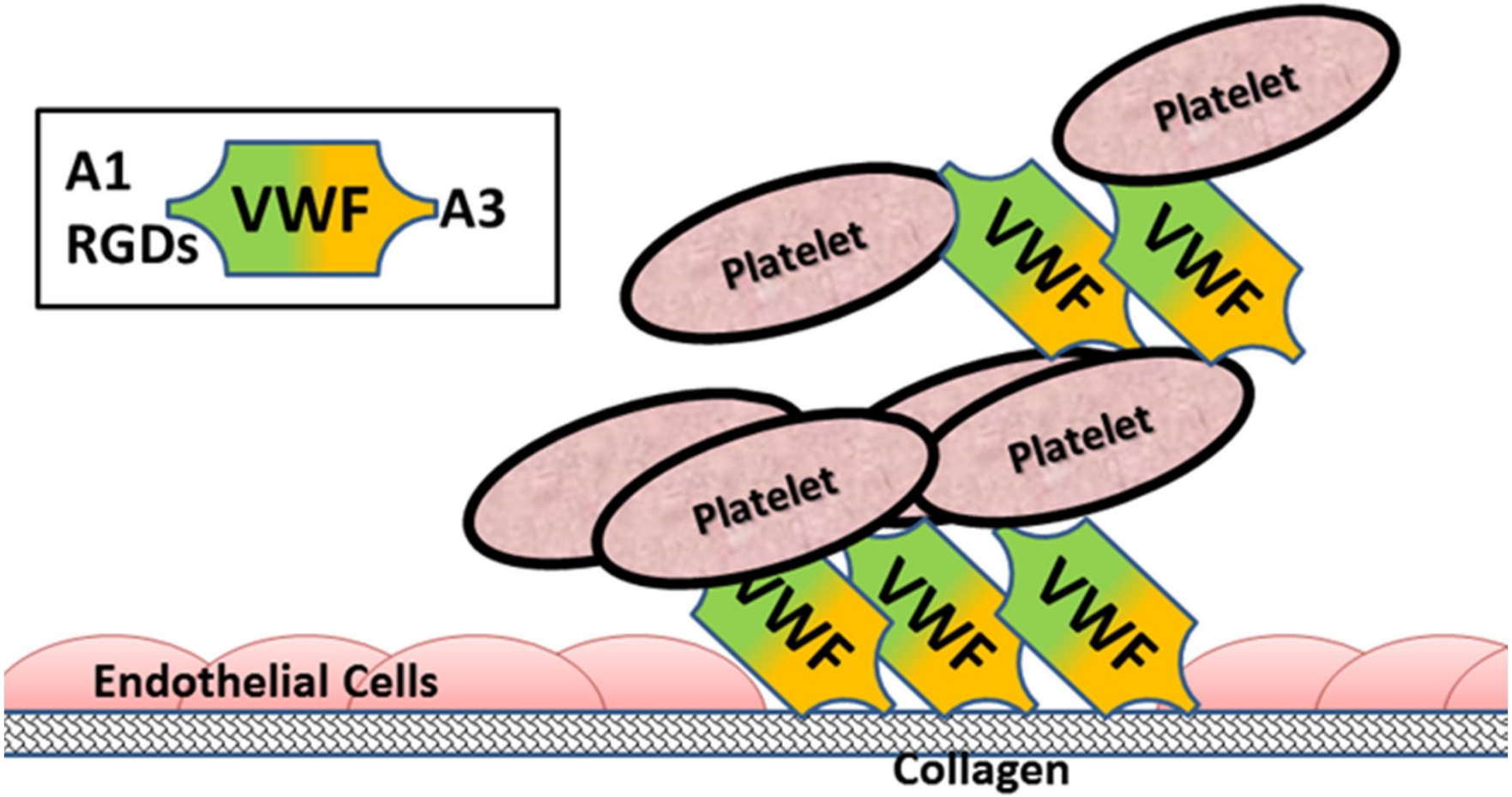
Dimer
0.5 x $10^6$ Da

Tetramer
1.0 x $10^6$ Da

Hexamer
1.5 x $10^6$ Da

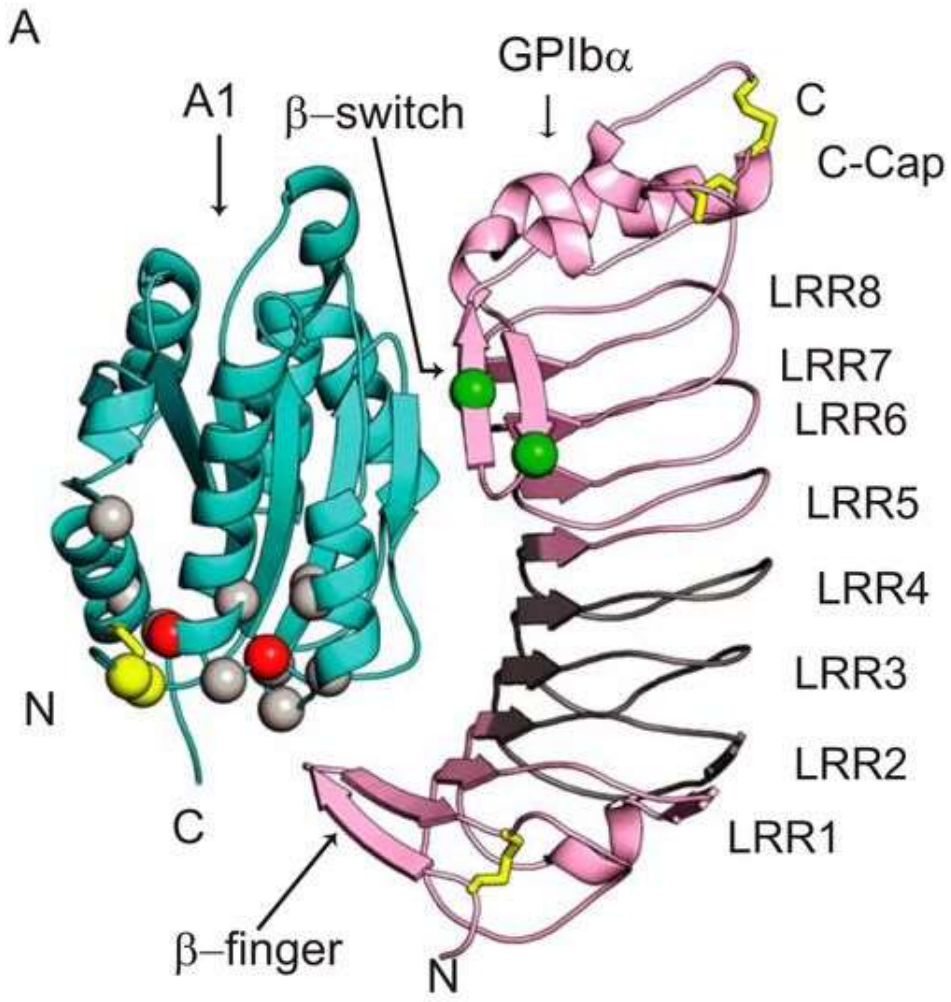
Multimer
≥ 7.5 x $10^6$ Da

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

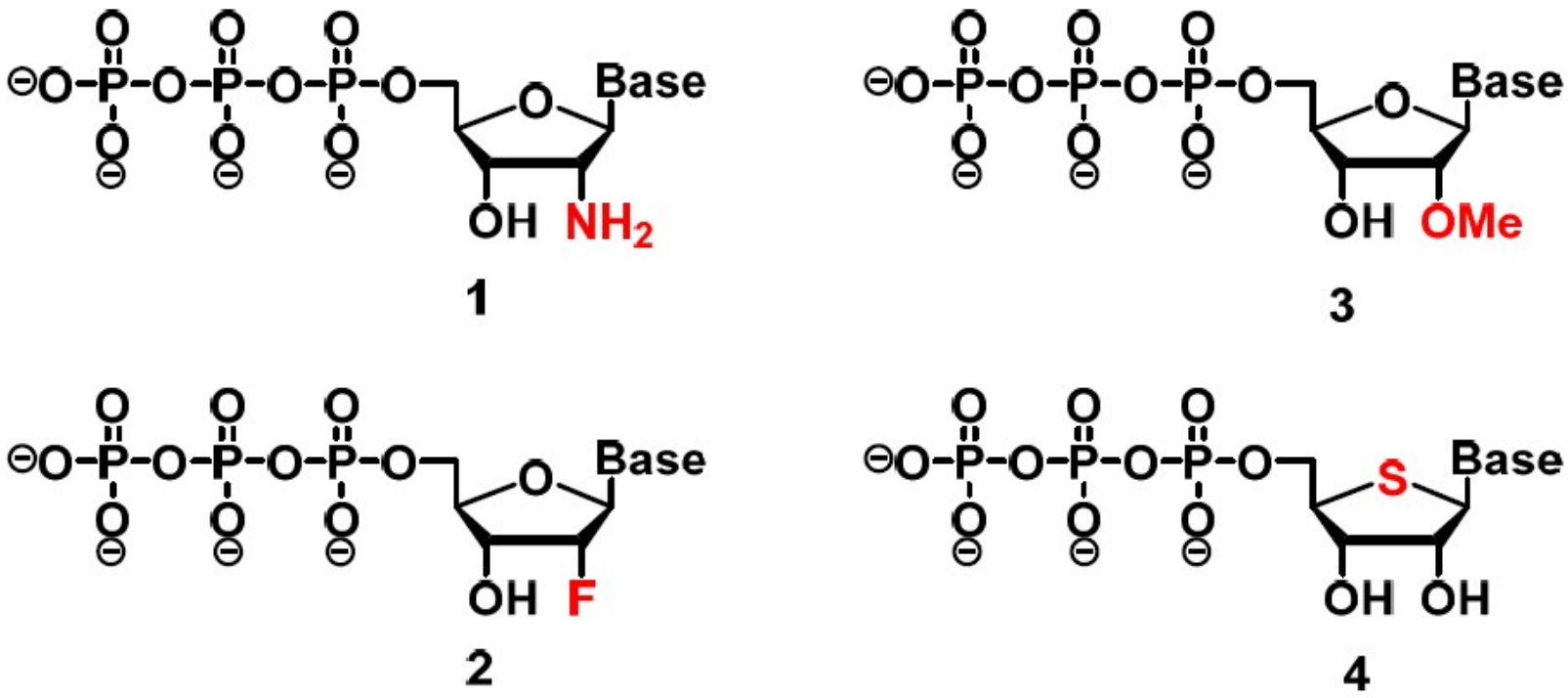
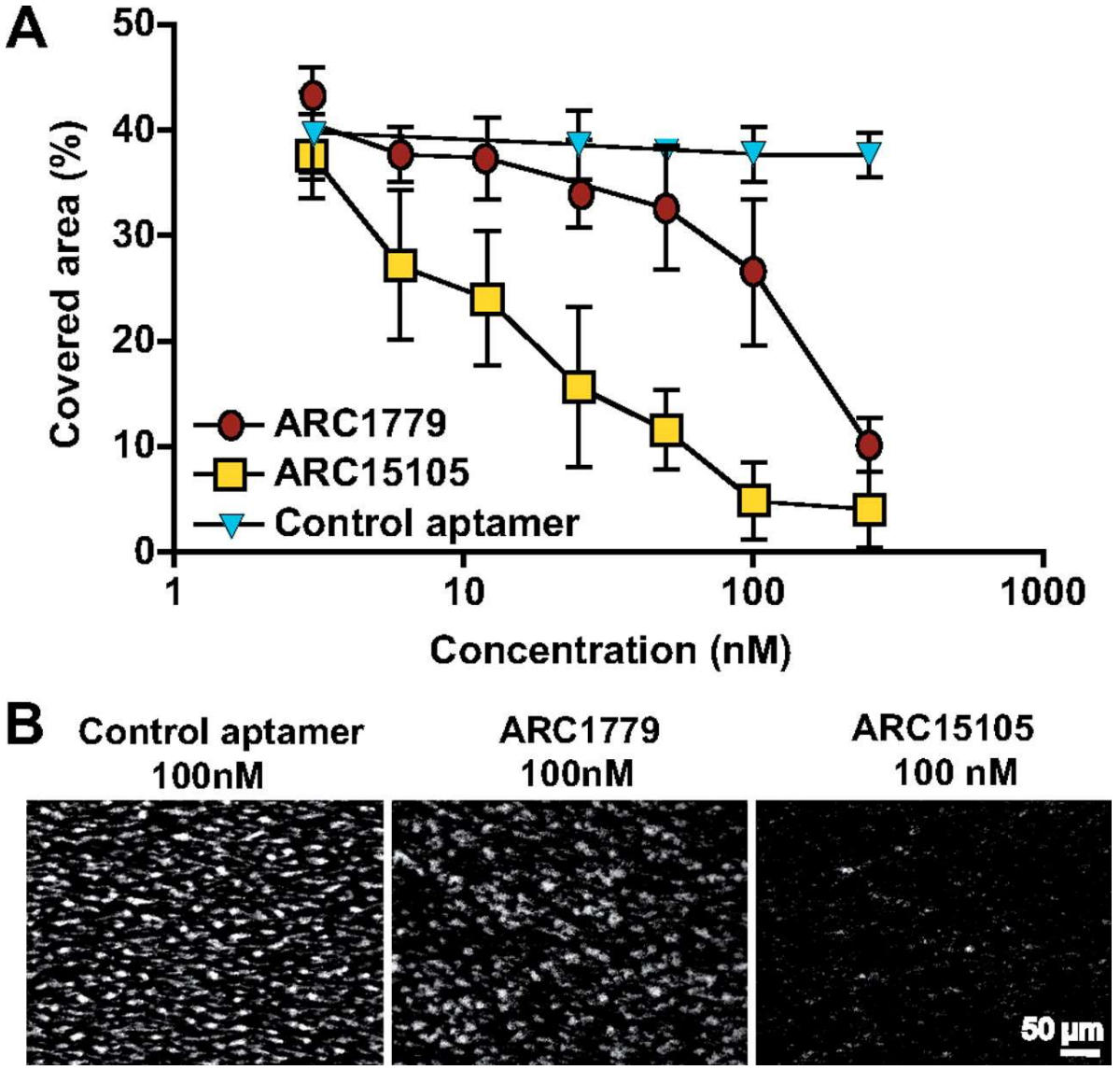



**Figure 2.** Chemical structures of 2′-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2′-amino-NTPs **1**, 2′-fluoro-NTPs **2**, 2′-methoxy-NTPs **3**, and 4′-thio-NTPs **4**.

**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