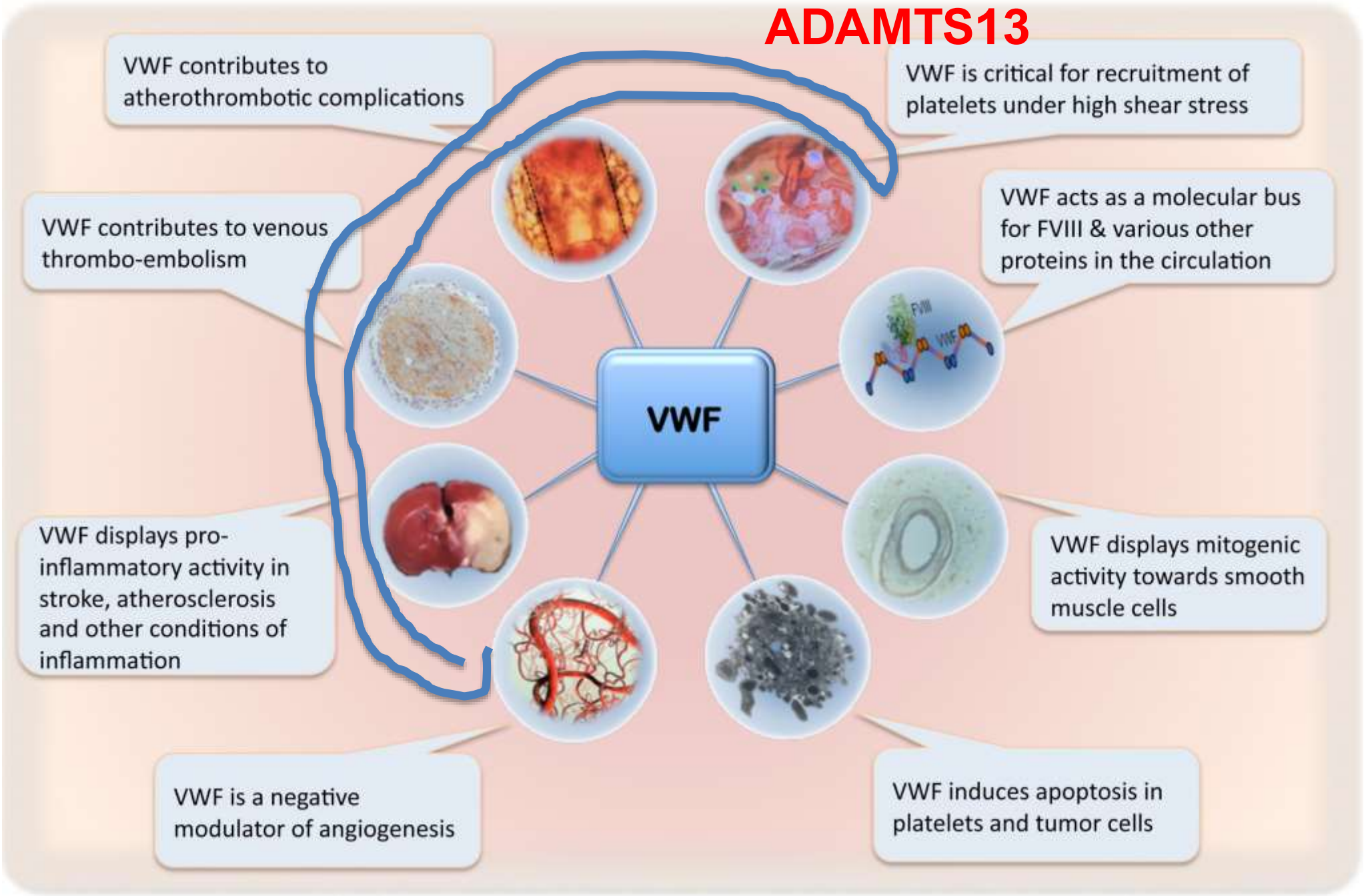
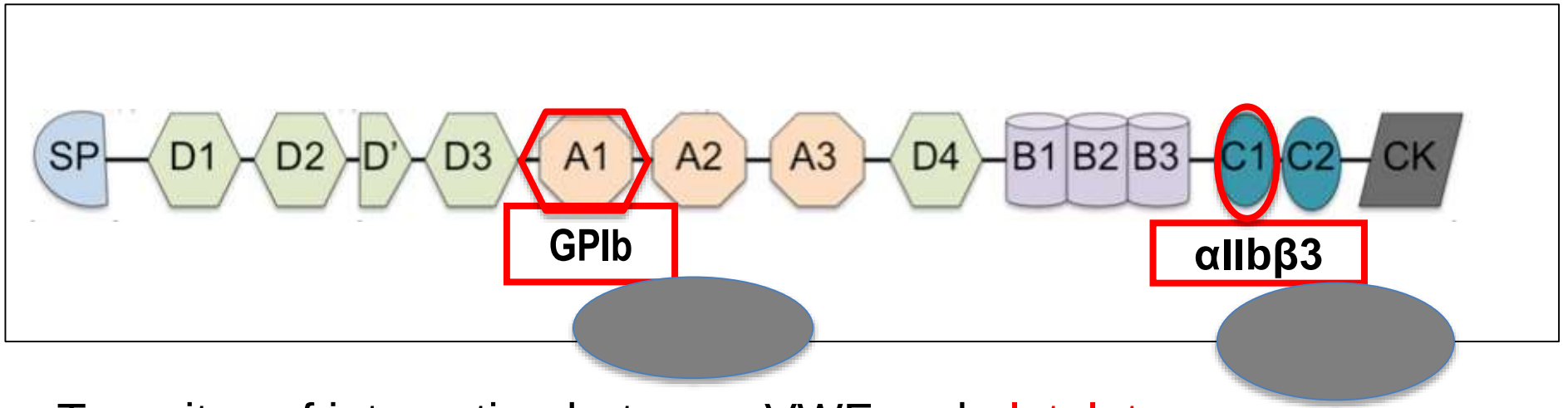
Role of VWF beyond haemostasis: unexpected versatility

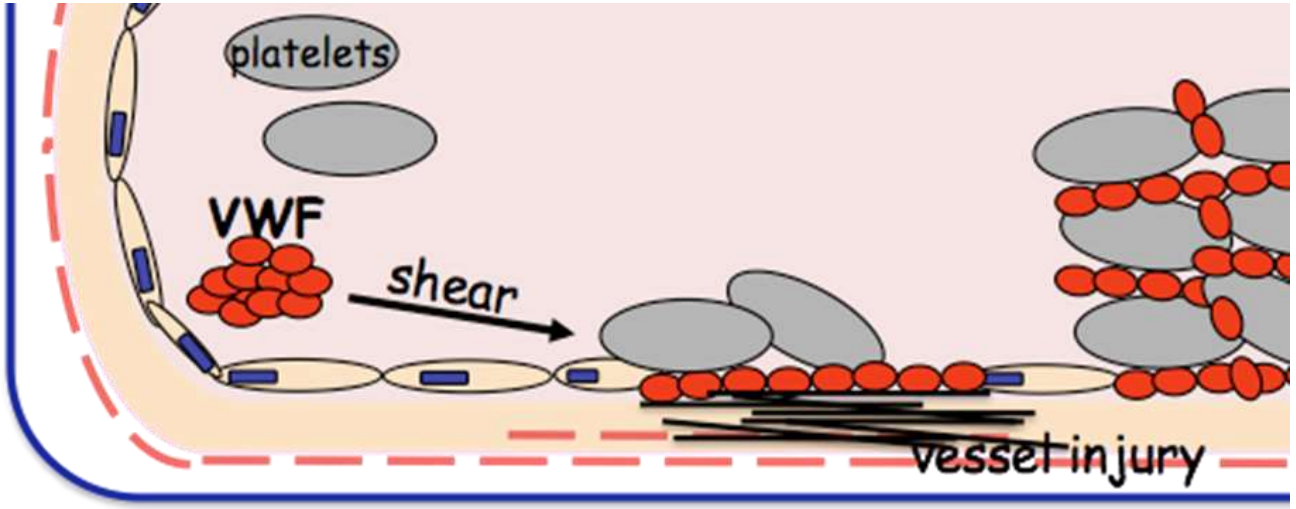


Lenting *et al. (2012) JTH 10:2428*

VWF-platelet binding



- Two sites of interaction between VWF and platelets:
  - VWF A1 & platelet GPIb complex
  - VWF RGDS sequence in the C1 domain & platelet αIIbβ3 integrin



Anticorpi che inibiscono il legame al recettore αIIbβ3

- The monoclonal antibody **abciximab** target the platelet integrin αIIbβ3 receptor and potently inhibits

i) binding to αIIbβ3,

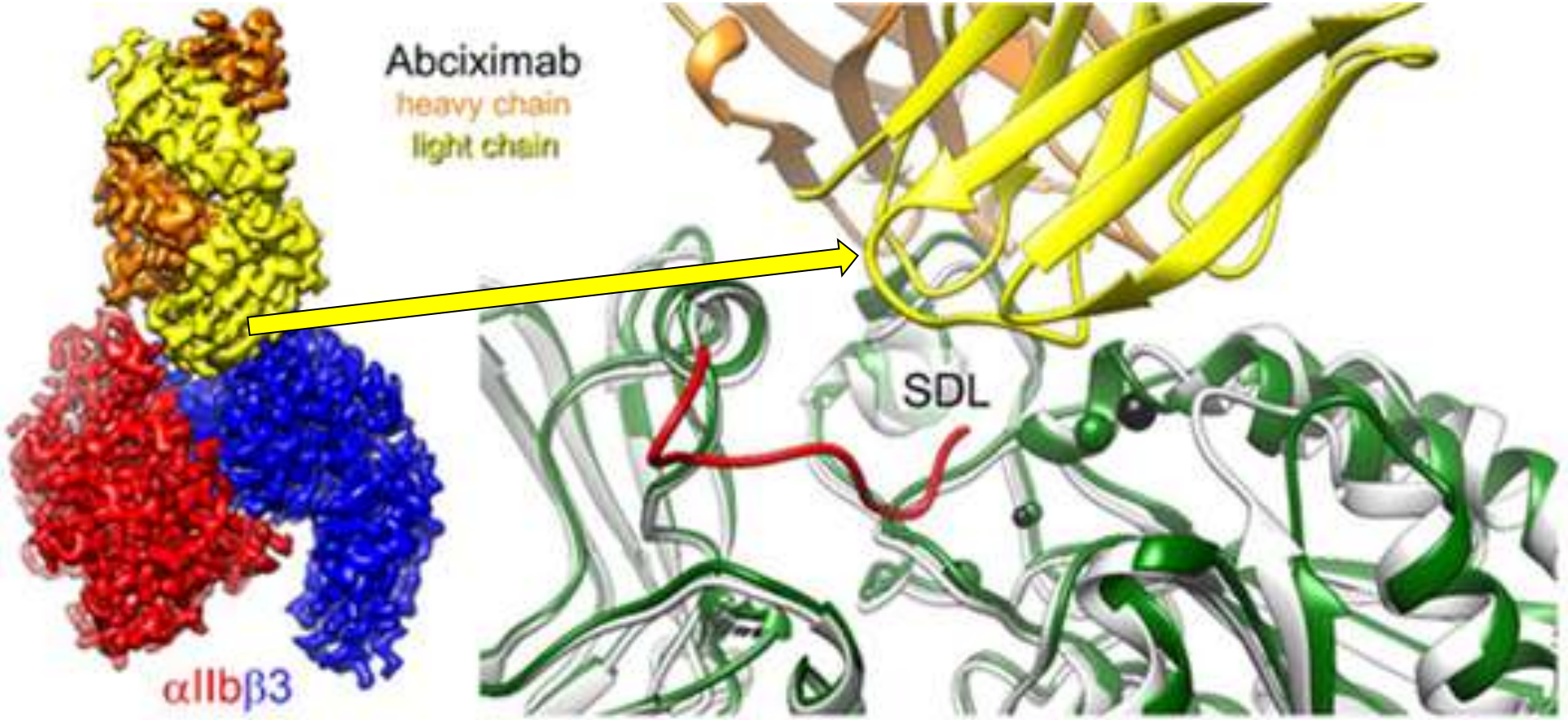
ii) platelet aggregation

iii) platelet-mediated thrombus formation in vivo.

Anticorpi che inibiscono il legame al recettore αIIbβ3

- Abciximab (ReoPro) was approved by the US Food and Drug Administration for human use in 1994 for the prevention of

cardiac ischemic complications after percutaneous coronary artery intervention.



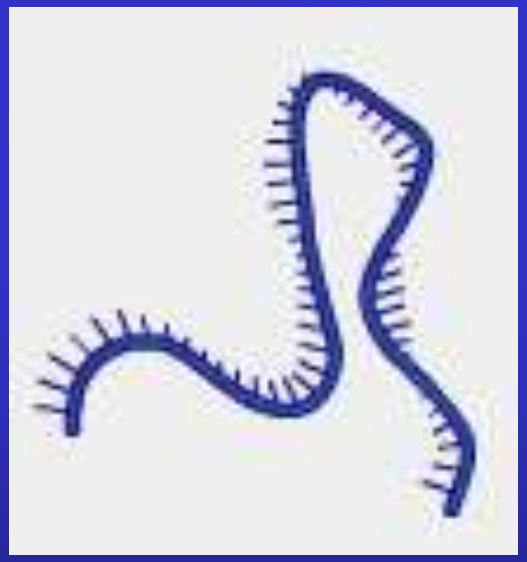
Abciximab prevents ligand binding by steric interference, with a potential contribution via displacing and rigidifying the β3 specificity-determining loop (SDL). (Left) Cryo-EM density map of the αIIbβ3 headpiece–abciximab

APTAMERI ANTI VWF

Aptameri

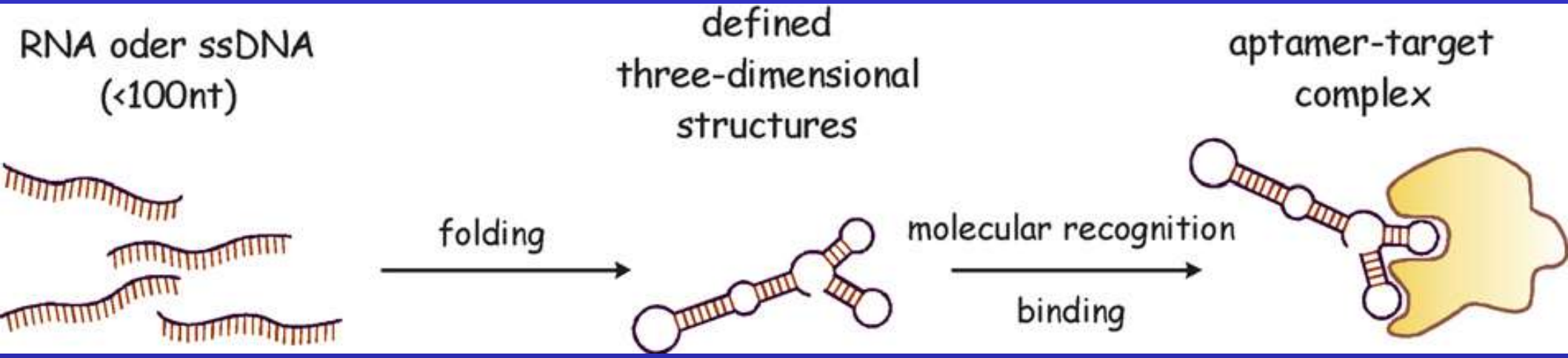
Dimensioni: 30-70 nucleotidi

Molecola Lineare



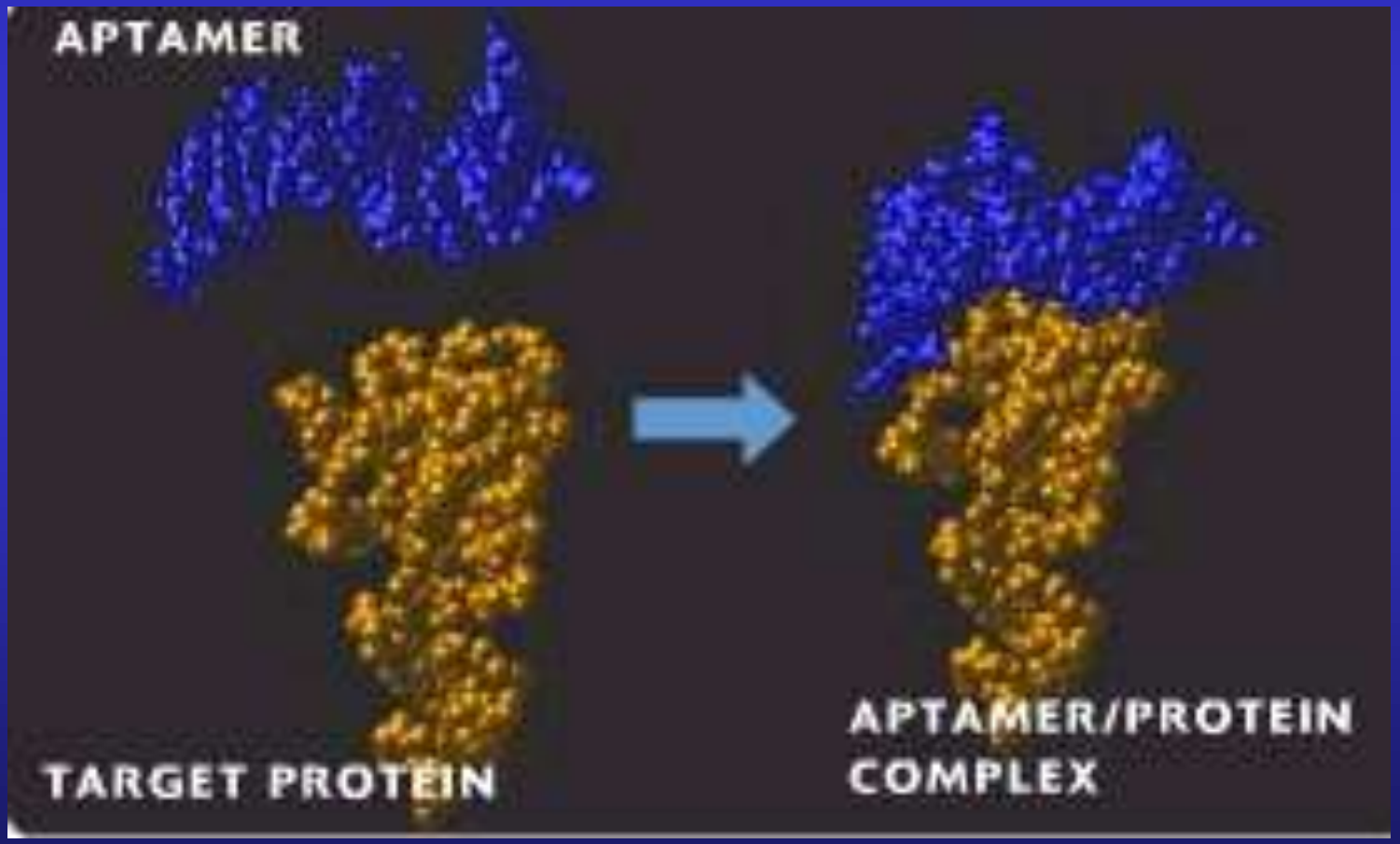
Folding

Struttura tridimensionale stabile


RNA oder ssDNA
(<100nt)
folding
defined
three-dimensional
structures
molecular recognition
binding
aptamer-target
complex

Anatomia degli Aptameri

Gli aptameri sono molecole selezionate per legarsi in modo specifico ad una predefinita *proteina target*



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

by Jolanta M. Siller-Matula et al

APTAMERI ANTI VWF

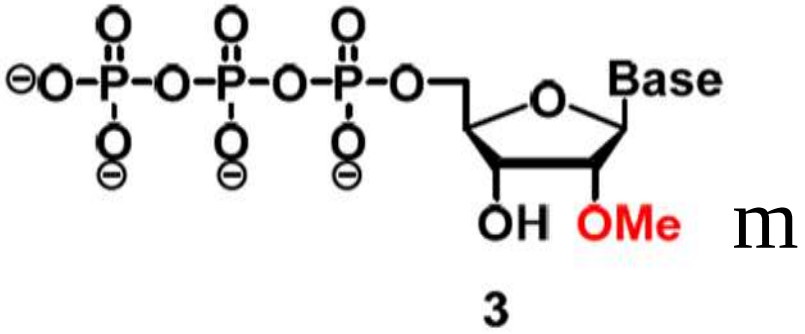
Arterioscler Thromb Vasc Biol
Volume 32(4):902-909, 2012

APTAMERO ANTI VWF ARC15105
sequenza e modificazioni chimiche

NH2-mGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUm
GmUmCmCmC-3T

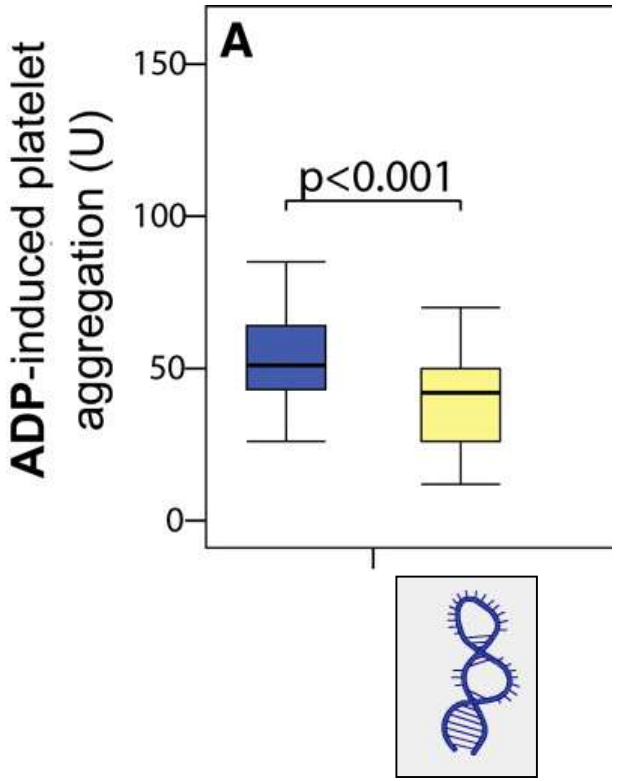
Maggiore emivita in circolo

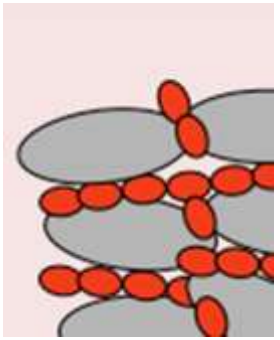
NH2 = hexylamine linker, legame a polietilenglicole (PEG)
3T inverted deoxythymidine residue resistenza esonuclesi

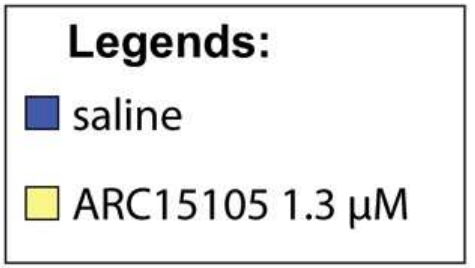


Metilazione in 2' O Maggiore stabilità
maggiore possibilità di interazioni idrofobiche

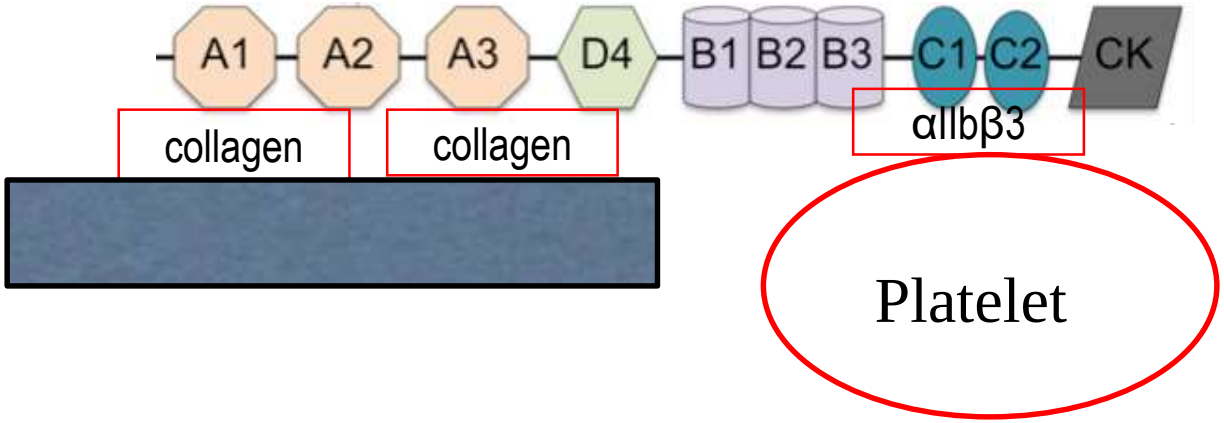
Platelet aggregation induced by ADP (adenosine diphosphate)

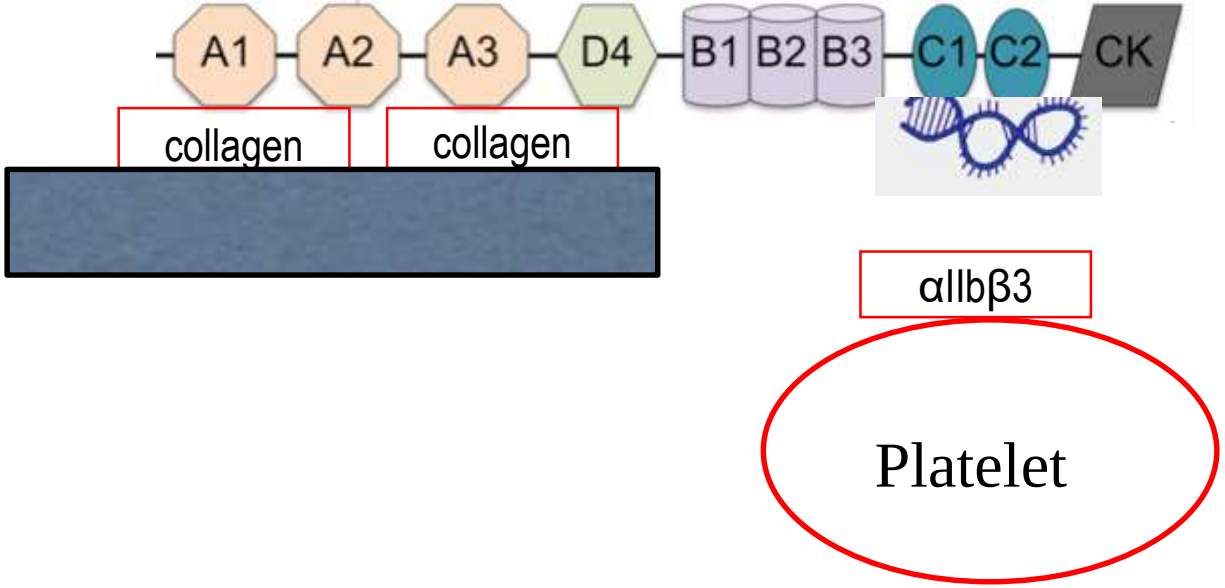




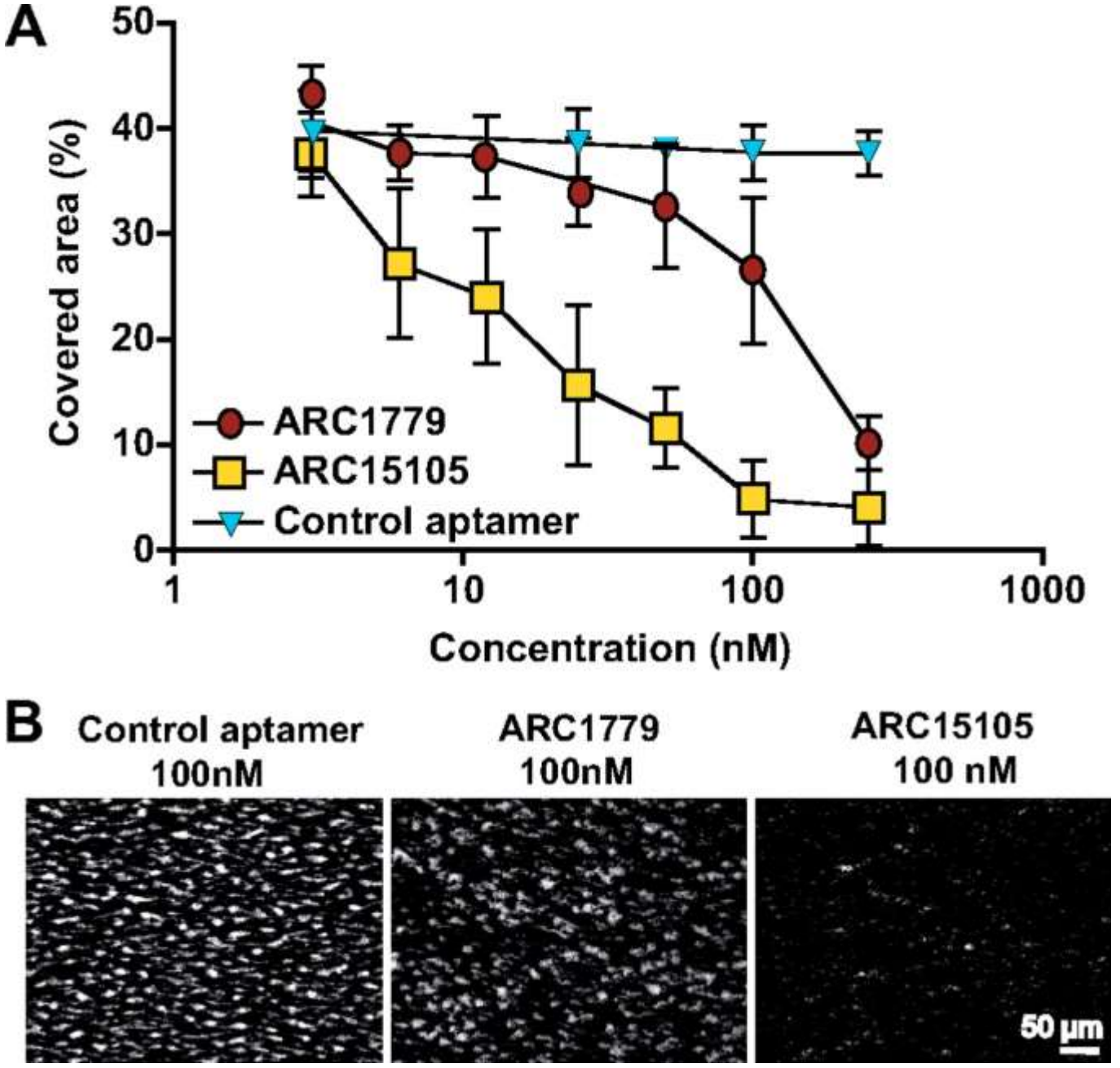


Platelet adhesion to collagen-bound VWF



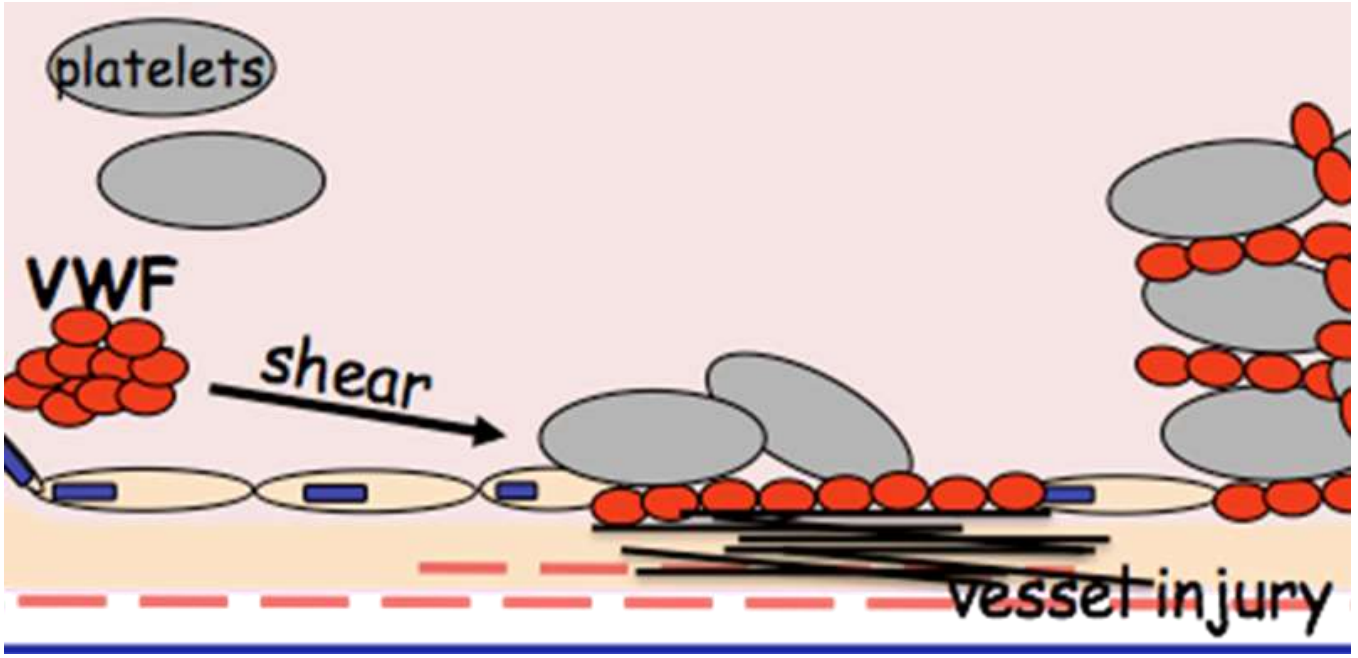


Concentration effect curve of ARC15105 and ARC1779 on platelet adhesion to collagen-bound VWF under arterial shear conditions.



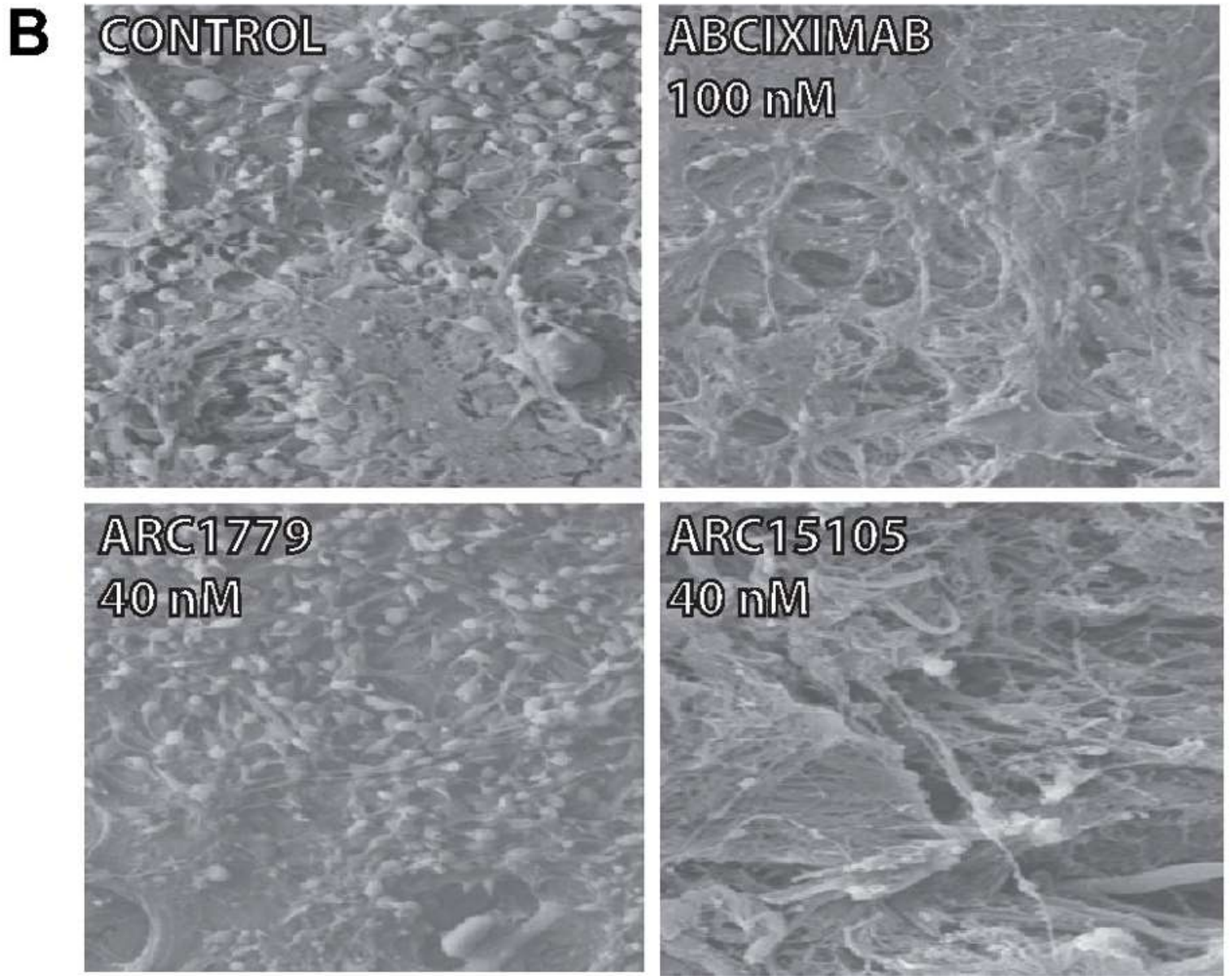
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

Platelet adhesion on injured porcine arterial segments



perfusion flow chambers

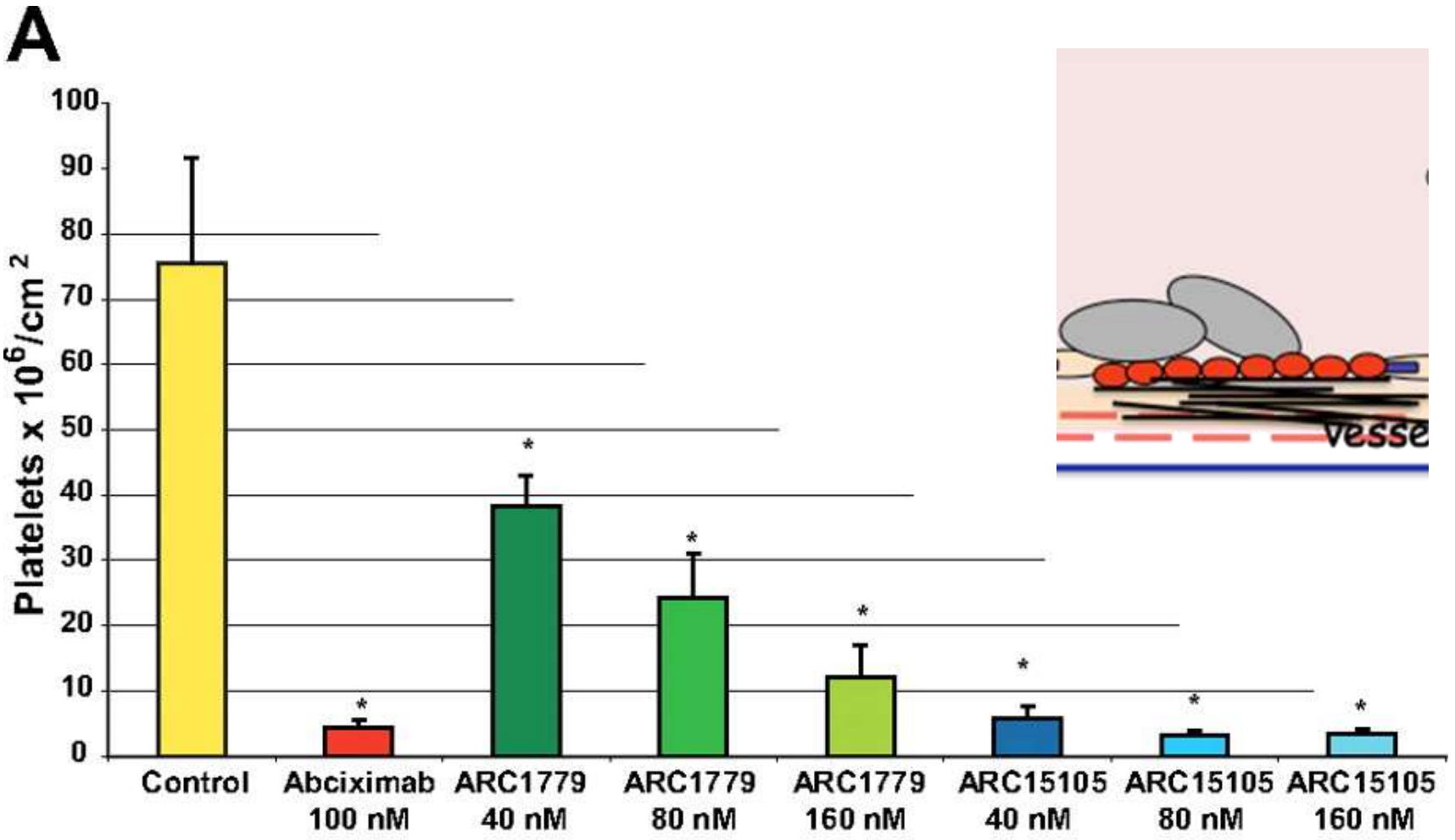
Platelet adhesion on injured porcine arterial segments
ARC15105, Arc1779, and abciximab inhibited the adhesion of platelets in perfusion flow chambers.



scanning electron microscopy

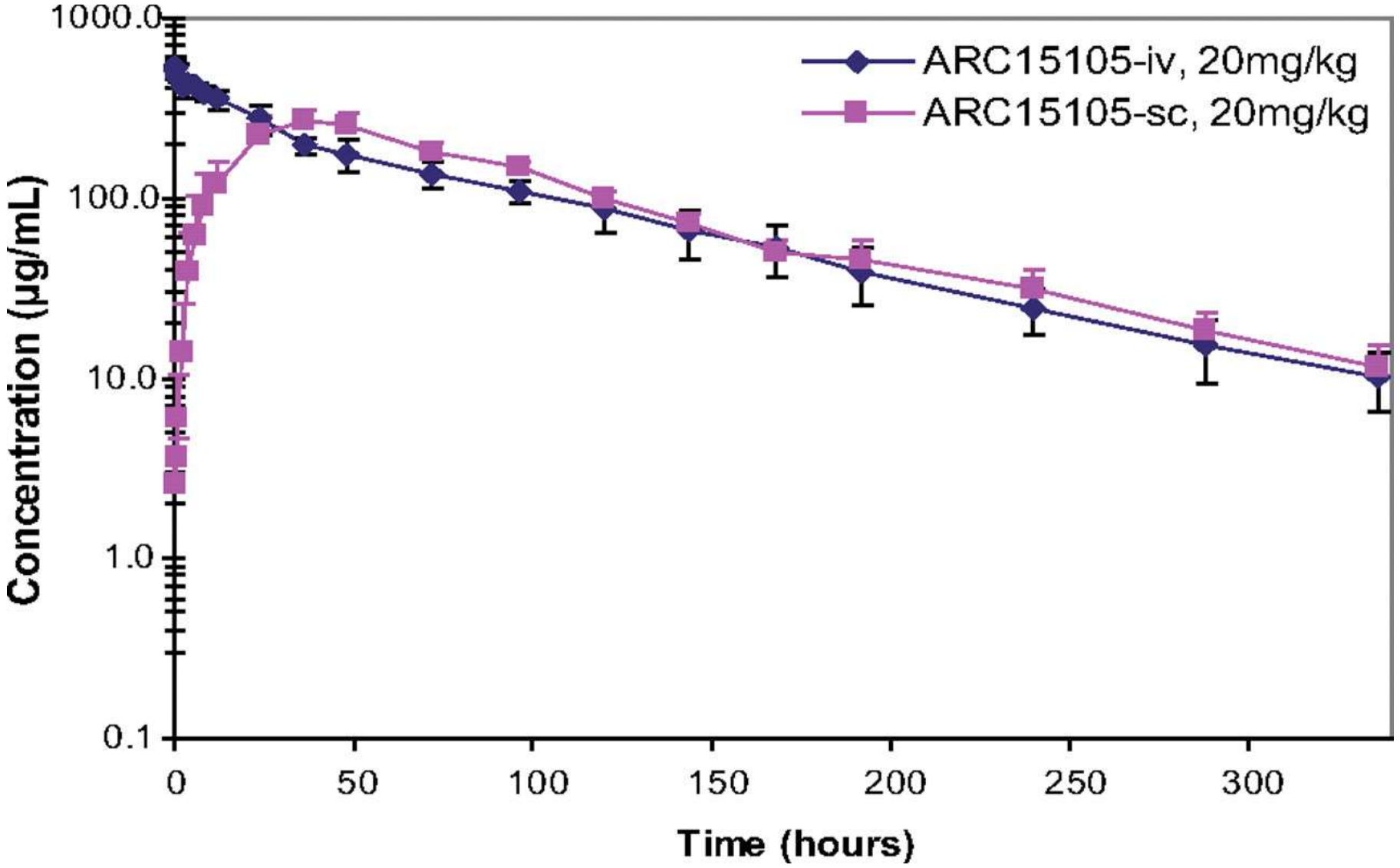
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

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



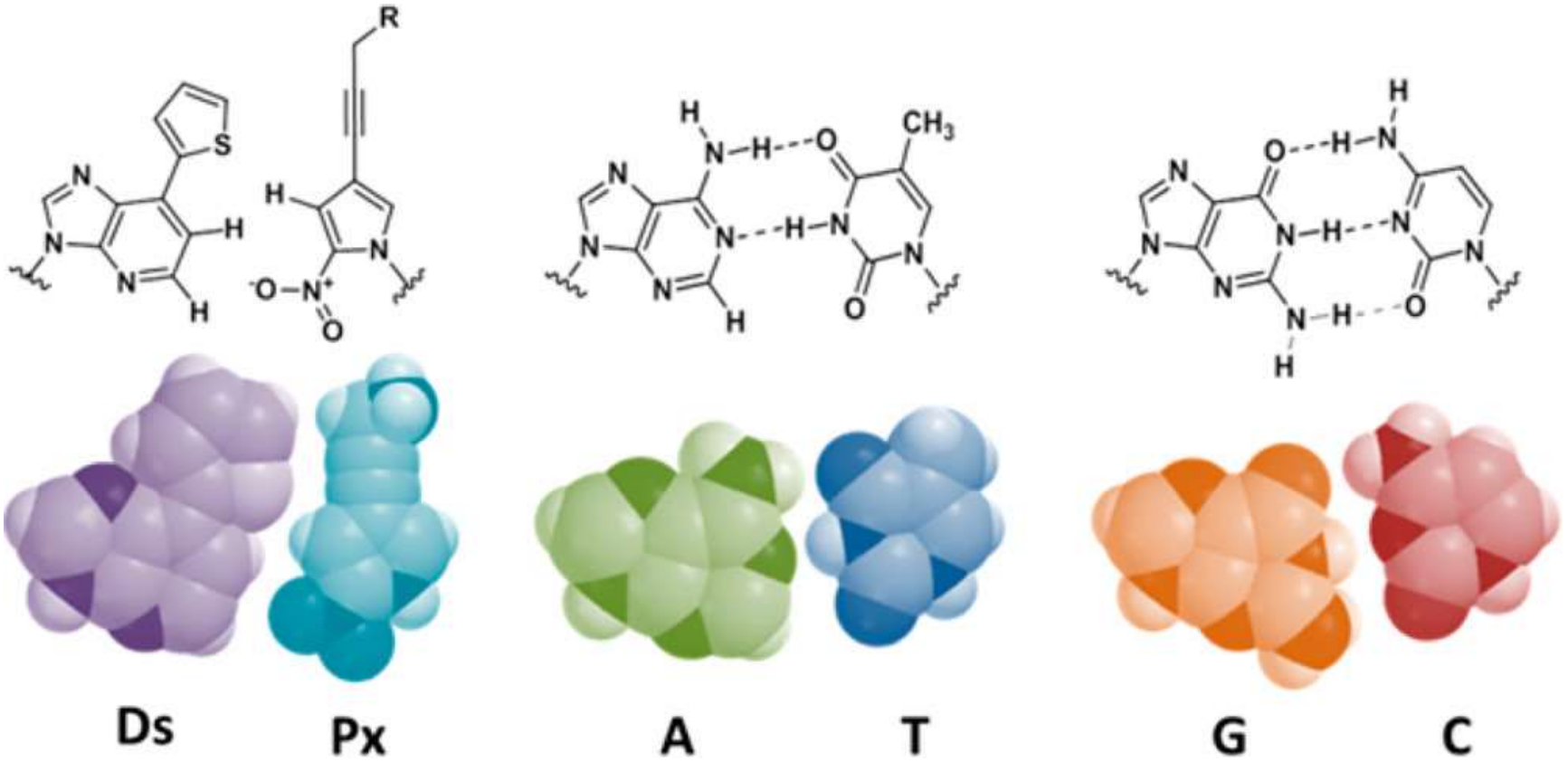
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

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



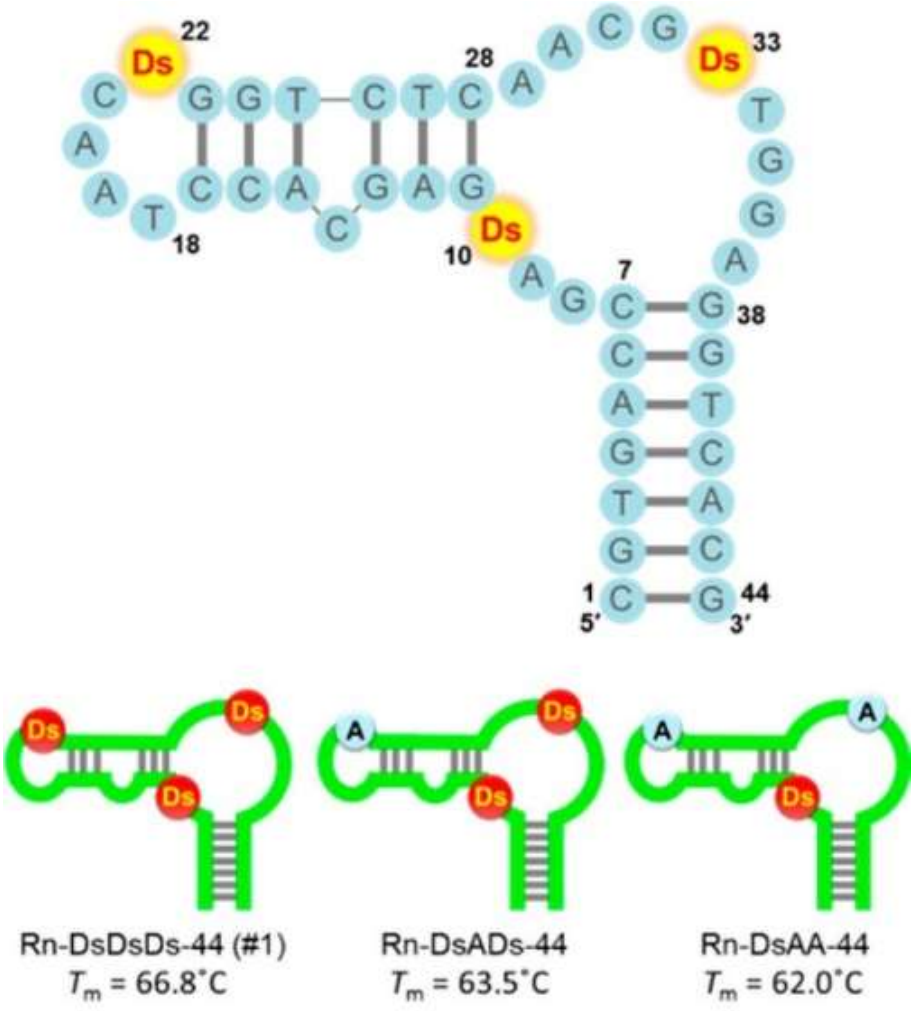
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

APTAMERI con Basi non naturali



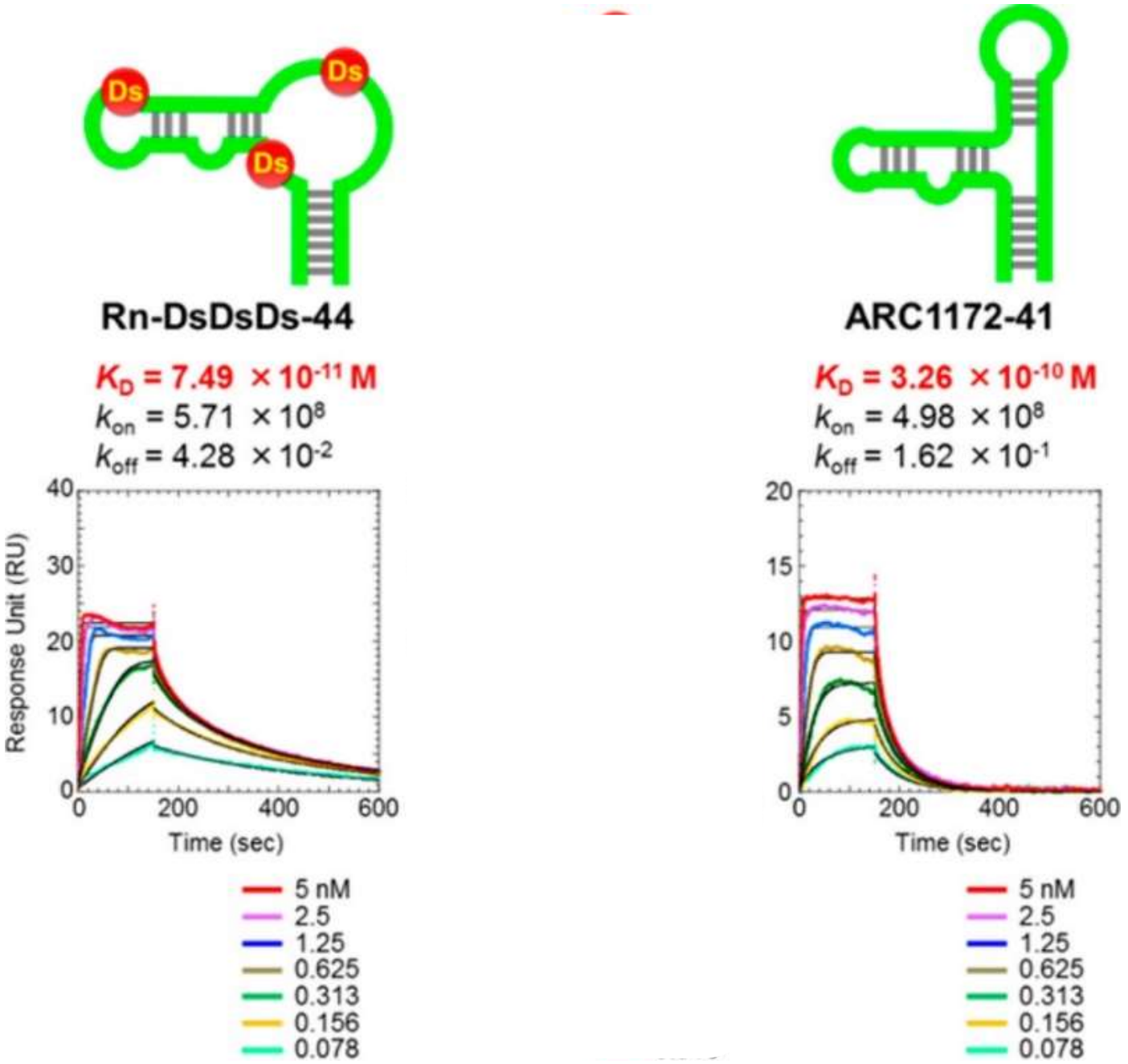
Chemical structures of the unnatural Ds–Px and natural A–T and G–C pairs.

Published in: Ken-ichiro Matsunaga; Michiko Kimoto; Ichiro Hirao; *J. Am. Chem. Soc.* **2017,** 139, 324-334.
DOI: 10.1021/jacs.6b10767



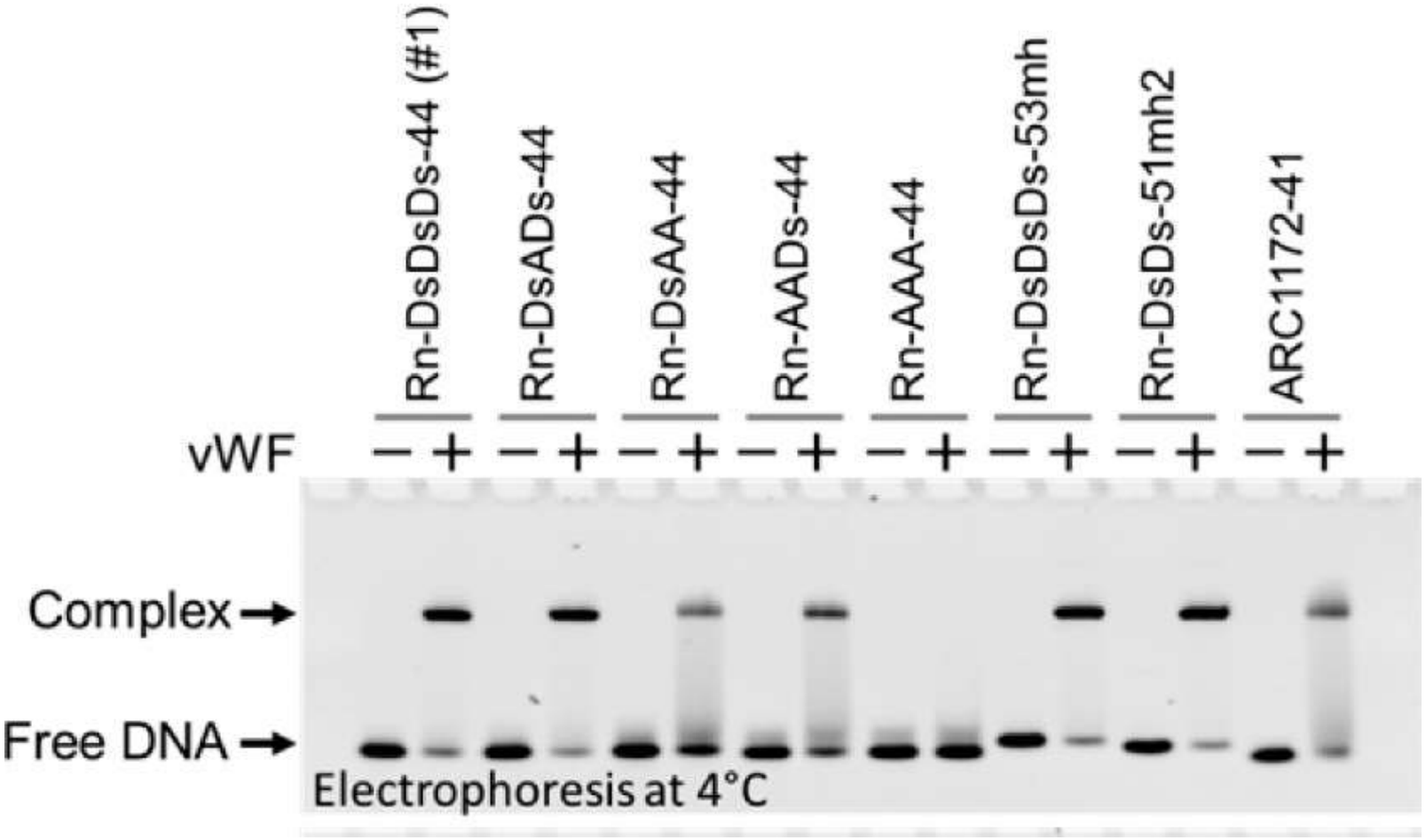
the secondary structures of the anti-vWF unnatural-base DNA aptamer, The sequence and presumed secondary structure are shown on the top, and each variant is schematically represented on the bottom with its thermal stability.

Published in: Ken-ichiro Matsunaga; Michiko Kimoto; Ichiro Hirao; *J. Am. Chem. Soc.* **2017,** 139, 324-334.
DOI: 10.1021/jacs.6b10767

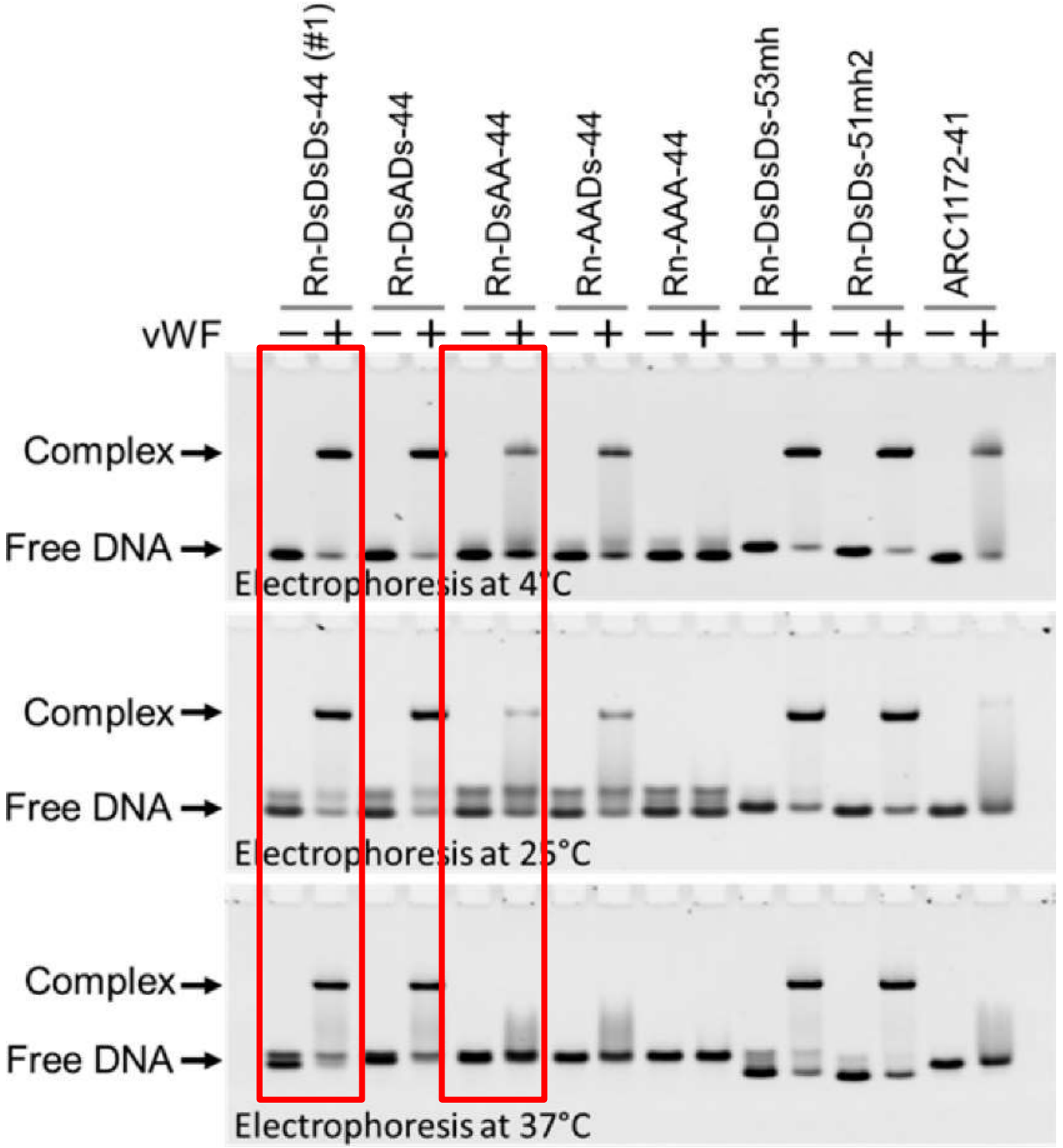


Binding analysis of anti-vWF DNA aptamers by a BIAcore T200 at 37 ° C, using 0.078 to 5 nM vWF. The aptamers were biotinylated at their 5′-termini.

Binding analysis of each Rn-DsDsDs-44 aptamer variant by a gel mobility shift assay.

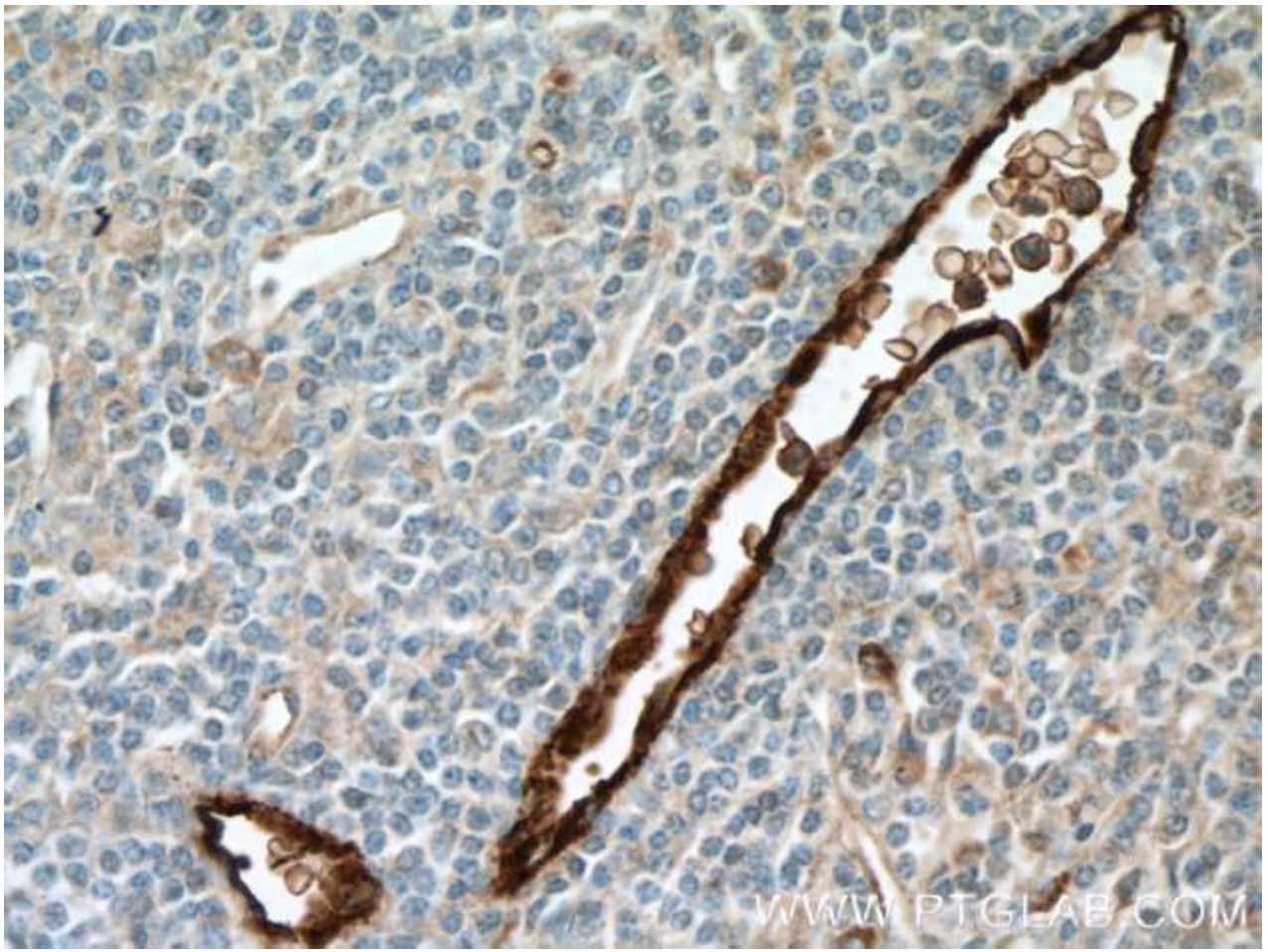


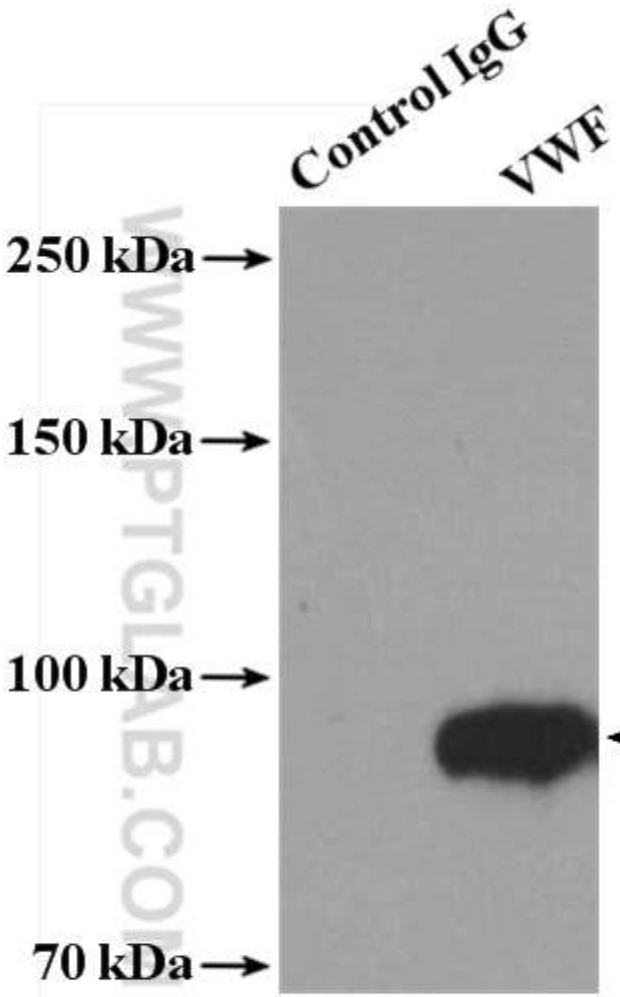
Each aptamer variant (5′-biotinylated, 100 nM) was incubated with vWF (100 nM) at 37 C and the complexes were separated from the free DNA on 8% polyacrylamide gels
The DNA bands on the gels were stained with SYBR Gold.

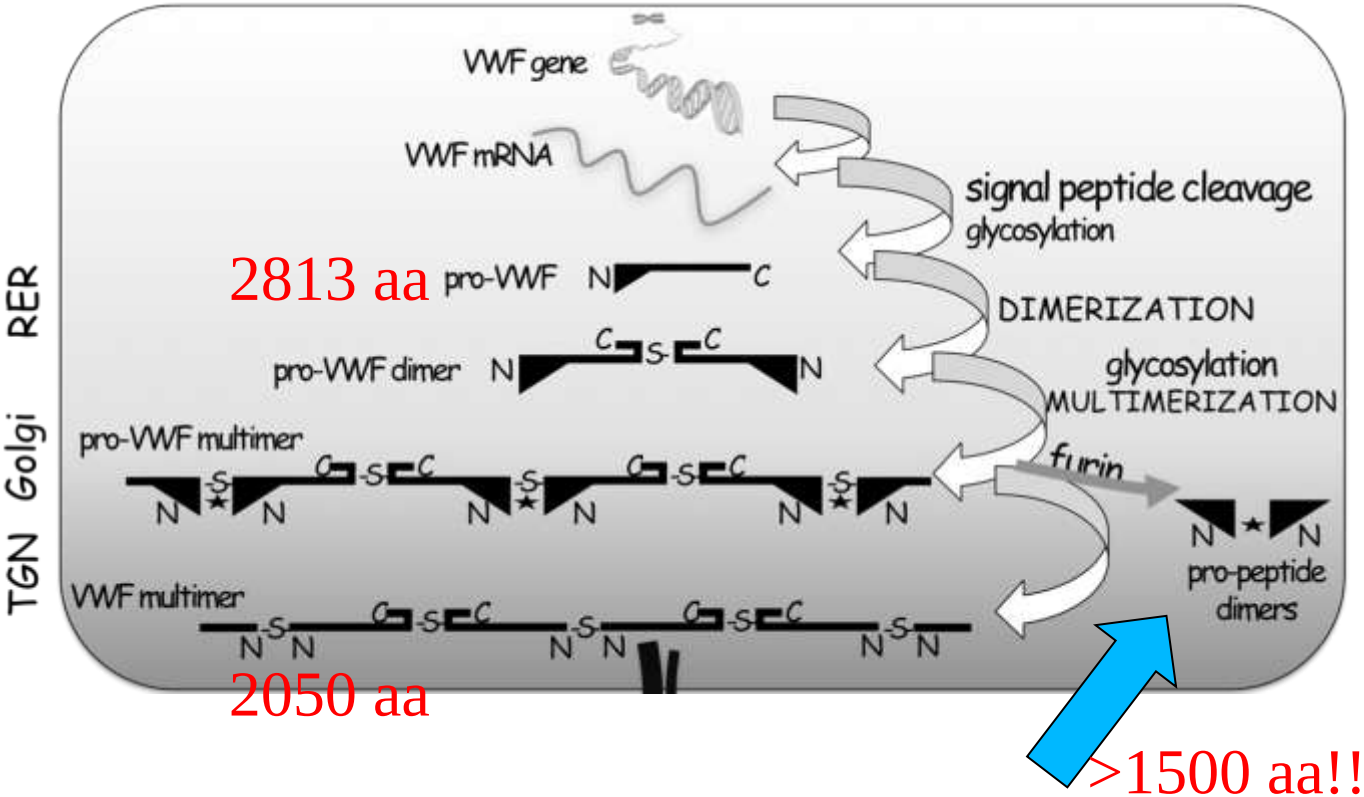

Rn-DsDsDs-44 (#1)
Rn-DsADs-44
Rn-DsAA-44
Rn-AADs-44
Rn-AAA-44
Rn-DsDsDs-53mh
Rn-DsDs-51mh2
ARC1172-41
vWF
− + − + − + − + − + − + − + − +
Complex
Free DNA
Electrophoresis at 4°C
Complex
Free DNA
Electrophoresis at 25°C
Complex
Free DNA
Electrophoresis at 37°C

Anticorpi contro il VWF per istologia (Endotelio)

Antibody raised against the N-terminal region of pre-pro-vWF







763 aa (ridotto)