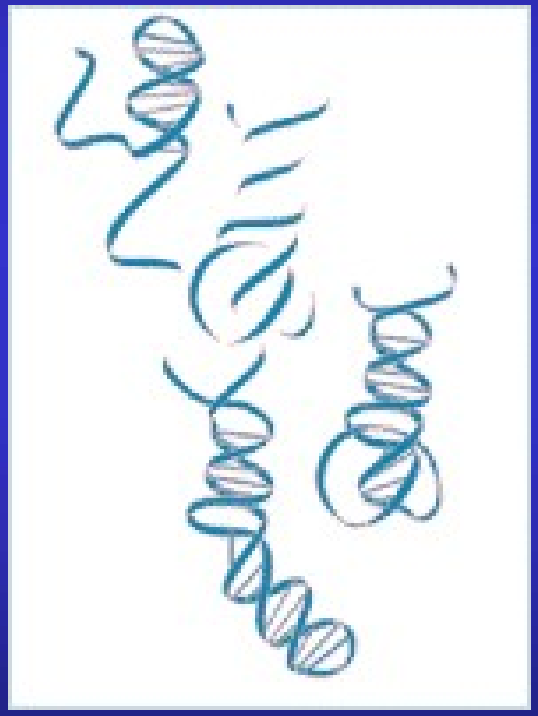
APTAMERI

Acidi nucleici a singolo filamento caratterizzati da una specifica **struttura tridimensionale** che si lega direttamente alla proteina target.

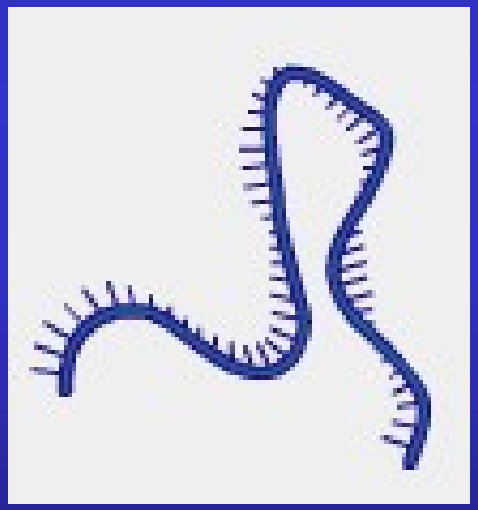


Interazione Acido Nucleico/Proteina

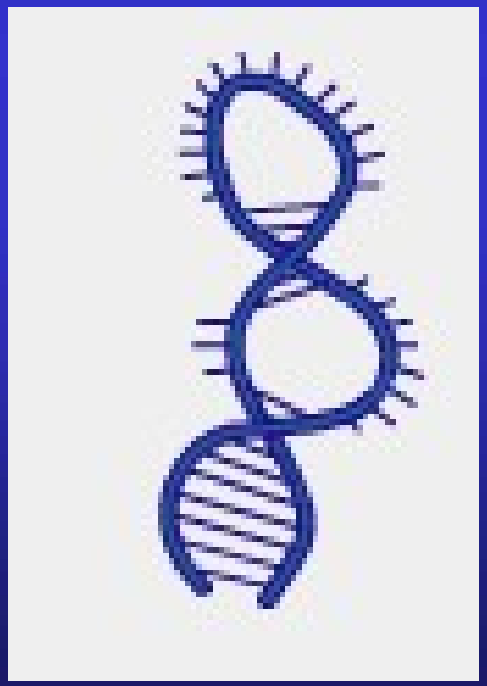
Aptameri

Dimensioni: 30-70 nucleotidi

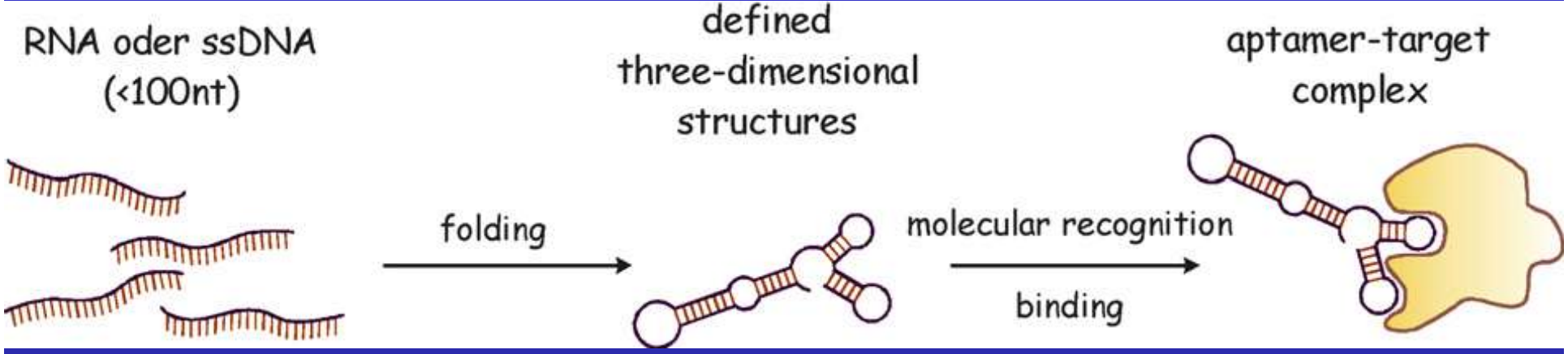
Molecola Lineare



Folding

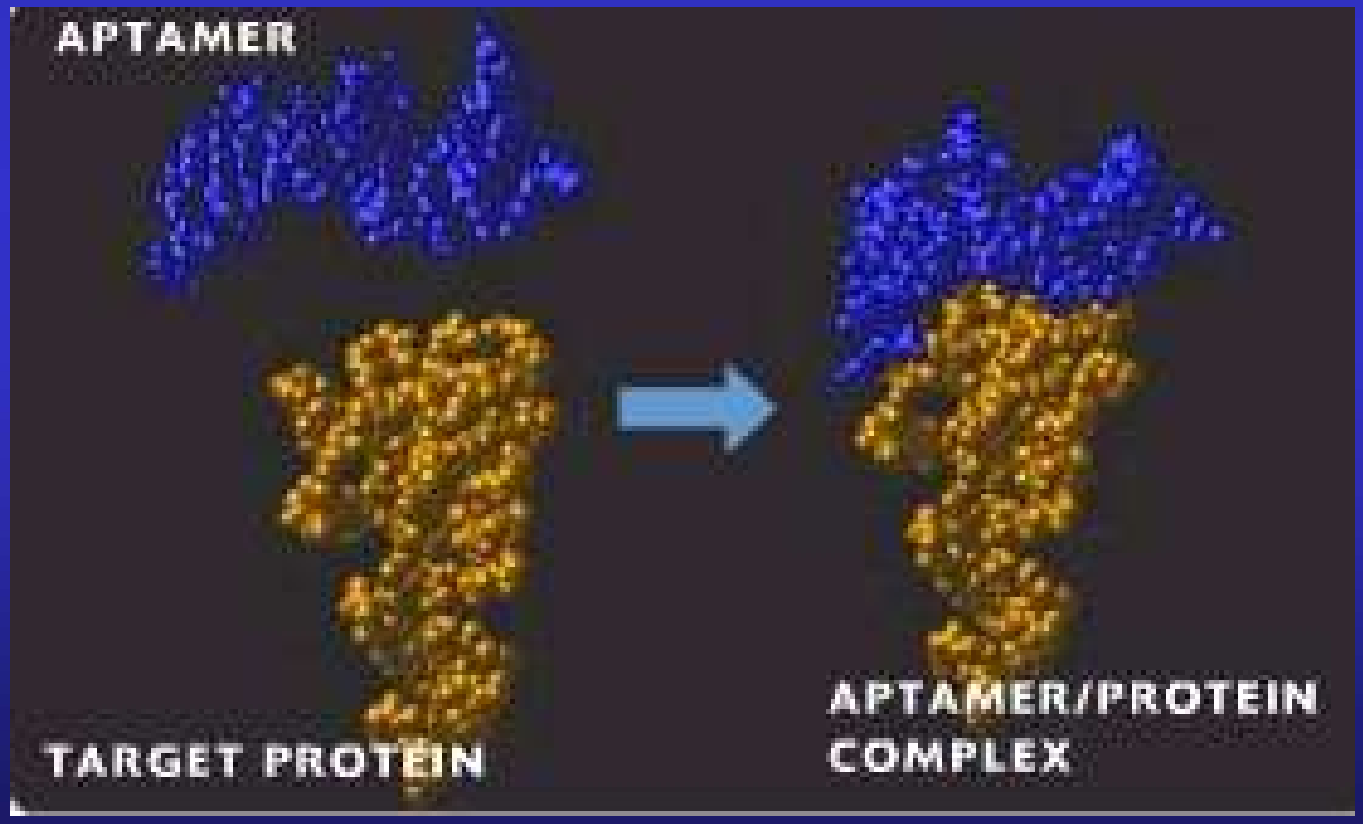


Struttura tridimensionale stabile


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

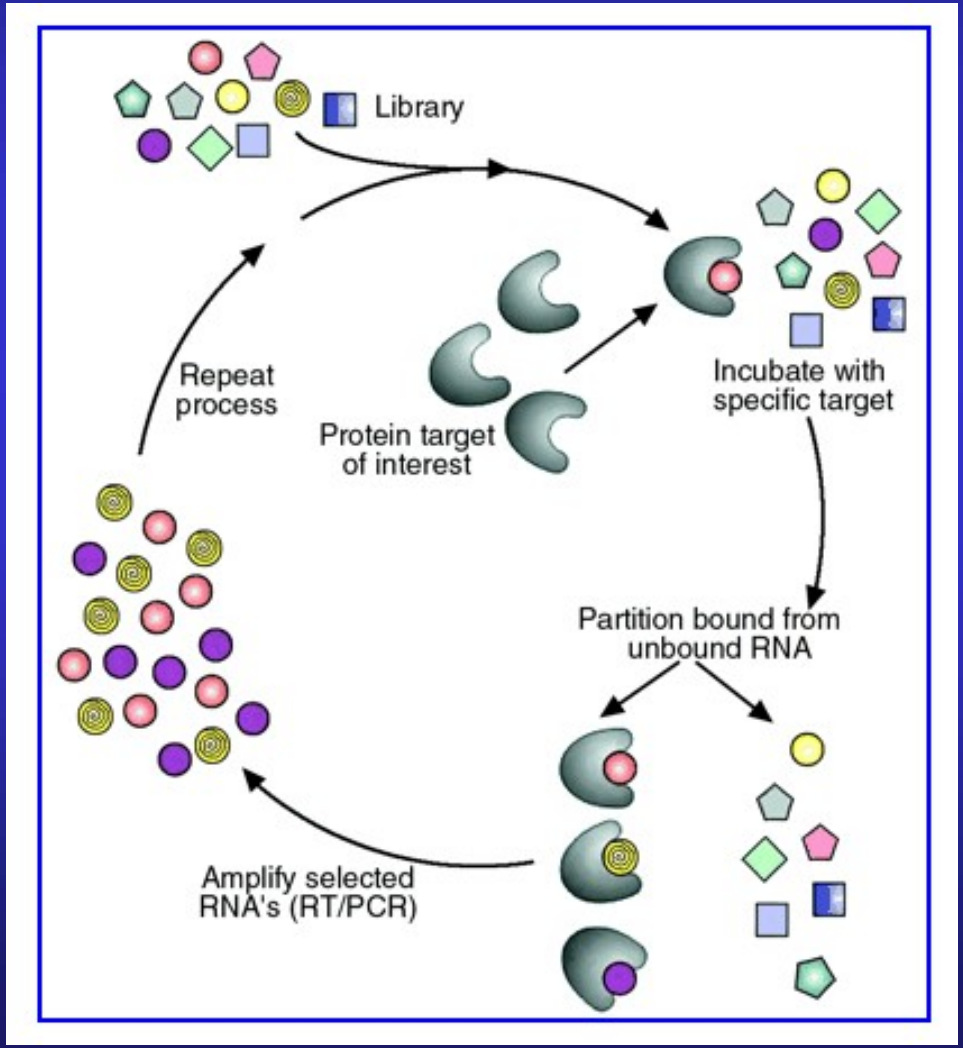
Anatomia degli Aptameri

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



Selezione in vitro degli Aptameri:

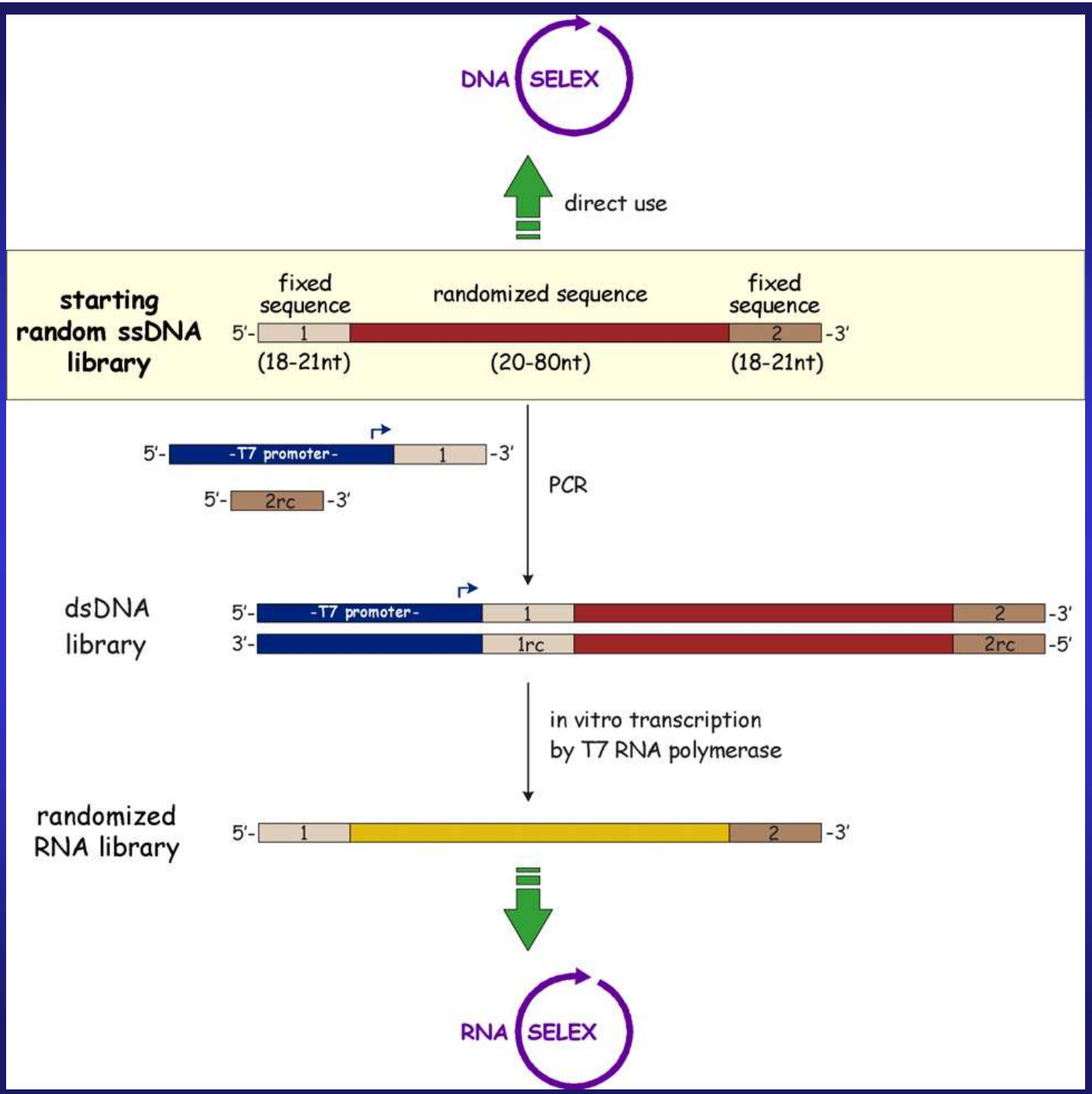
SELEX (systematic evolution of ligands by exponent enrichment)

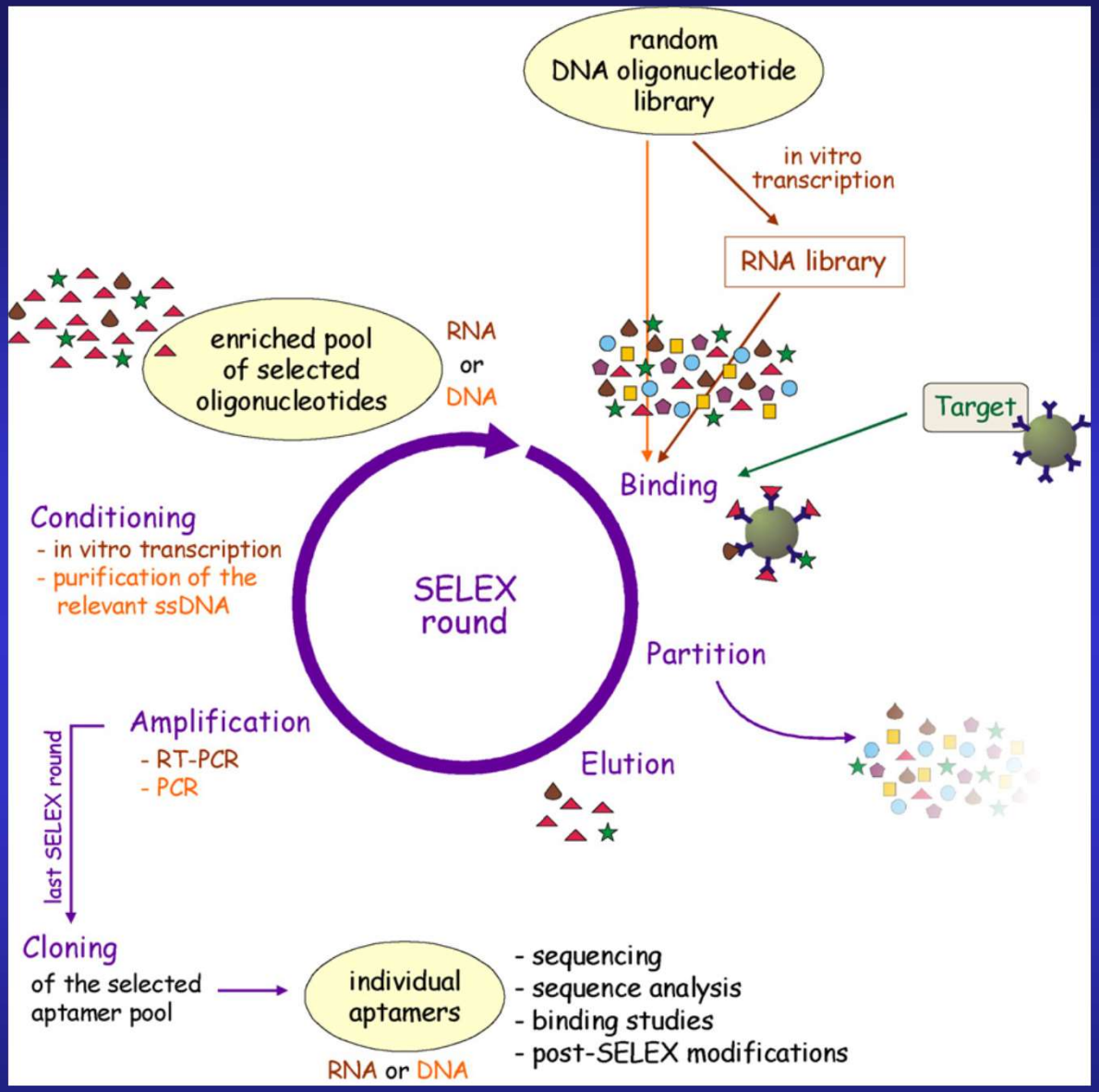


Selezione in vitro degli Aptameri:

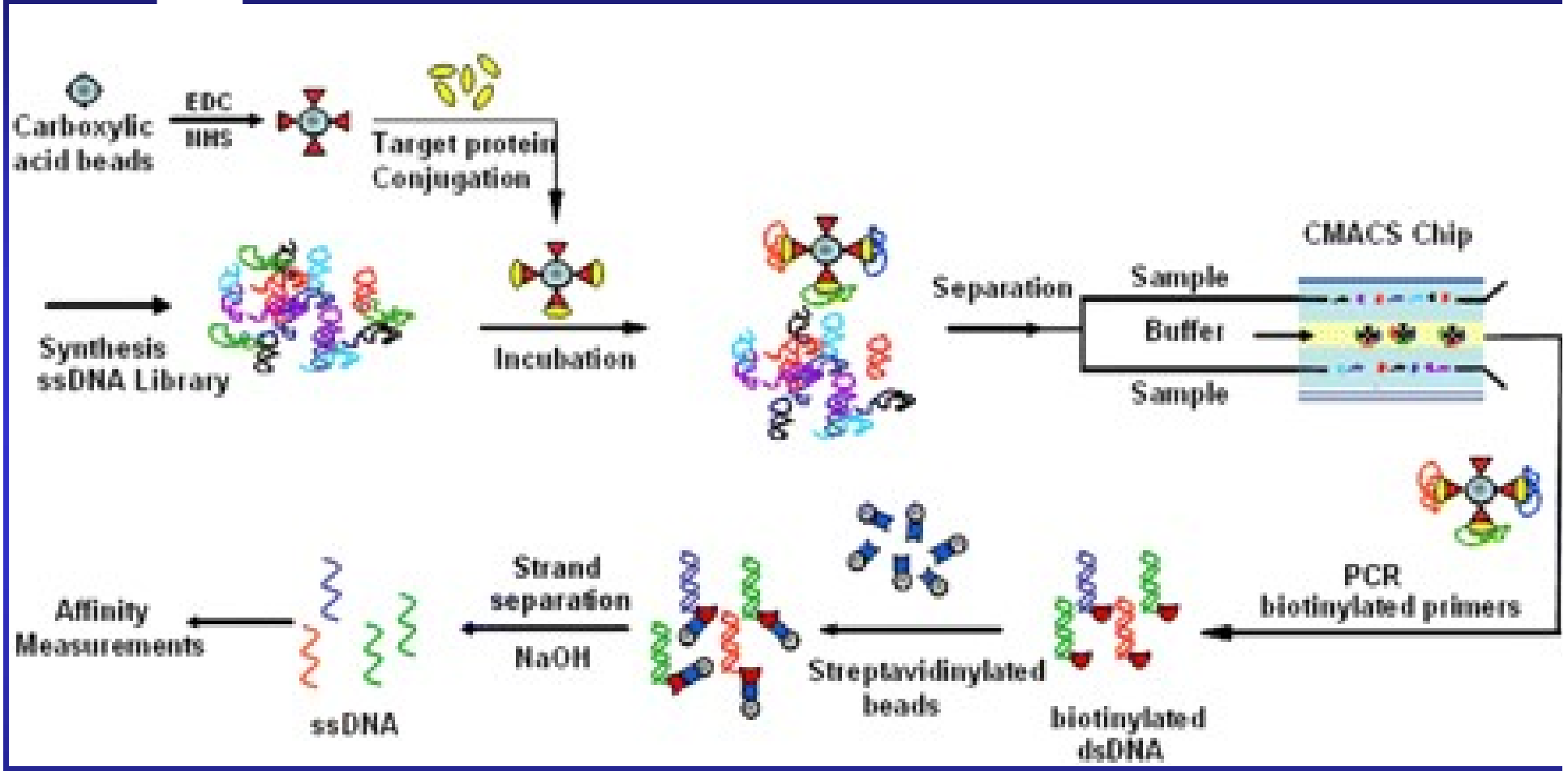
SELEX (systematic evolution of ligands by exponent enrichment)

1. Sintesi chimica di 10^{14} RNA o DNA (Libreria)
2. Incubazione con la proteina target: cromatografia per affinità
3. Rimozione degli oligo *non legati* mediante buffer di lavaggio
4. Rimozione degli oligo *legati* alla proteina target con una soluzione contenente la proteina target
5. Retrotrascrizione e PCR (RNA) o solo PCR (DNA) degli oligo che si sono legati
6. Trascrizione in vitro (RNA) o solo denaturazione (DNA) per separare i filamenti
7. Inizio di un nuovo ciclo fino a 5-10 cicli


DNA SELEX
direct use
starting random ssDNA library
fixed sequence
randomized sequence
fixed sequence
5'-
1
2
-3'
(18-21nt)
(20-80nt)
(18-21nt)
5'-
-T7 promoter-
1
-3'
5'-
2rc
-3'
PCR
dsDNA library
5'-
-T7 promoter-
1
2
-3'
3'-
1rc
2rc
-5'
in vitro transcription
by T7 RNA polymerase
randomized RNA library
5'-
1
2
-3'
RNA SELEX


random
DNA oligonucleotide
library
in vitro
transcription
RNA library
enriched pool
of selected
oligonucleotides
RNA
or
DNA
Target
Binding
Conditioning
- in vitro transcription
- purification of the
relevant ssDNA
SELEX
round
Partition
Amplification
- RT-PCR
- PCR
Elution
last SELEX round
Cloning
of the selected
aptamer pool
individual
aptamers
RNA or DNA
- sequencing
- sequence analysis
- binding studies
- post-SELEX modifications

Automazione SELEX



APTAMERI ANTI VWF

NH2-mGmGmGmAmCmCmUmAmAmGmAmCmAmCmAmUm
GmUmCmCmC-3T

NH2 = hexylamine linker,
3T inverted deoxythymidine residue

ARC15105

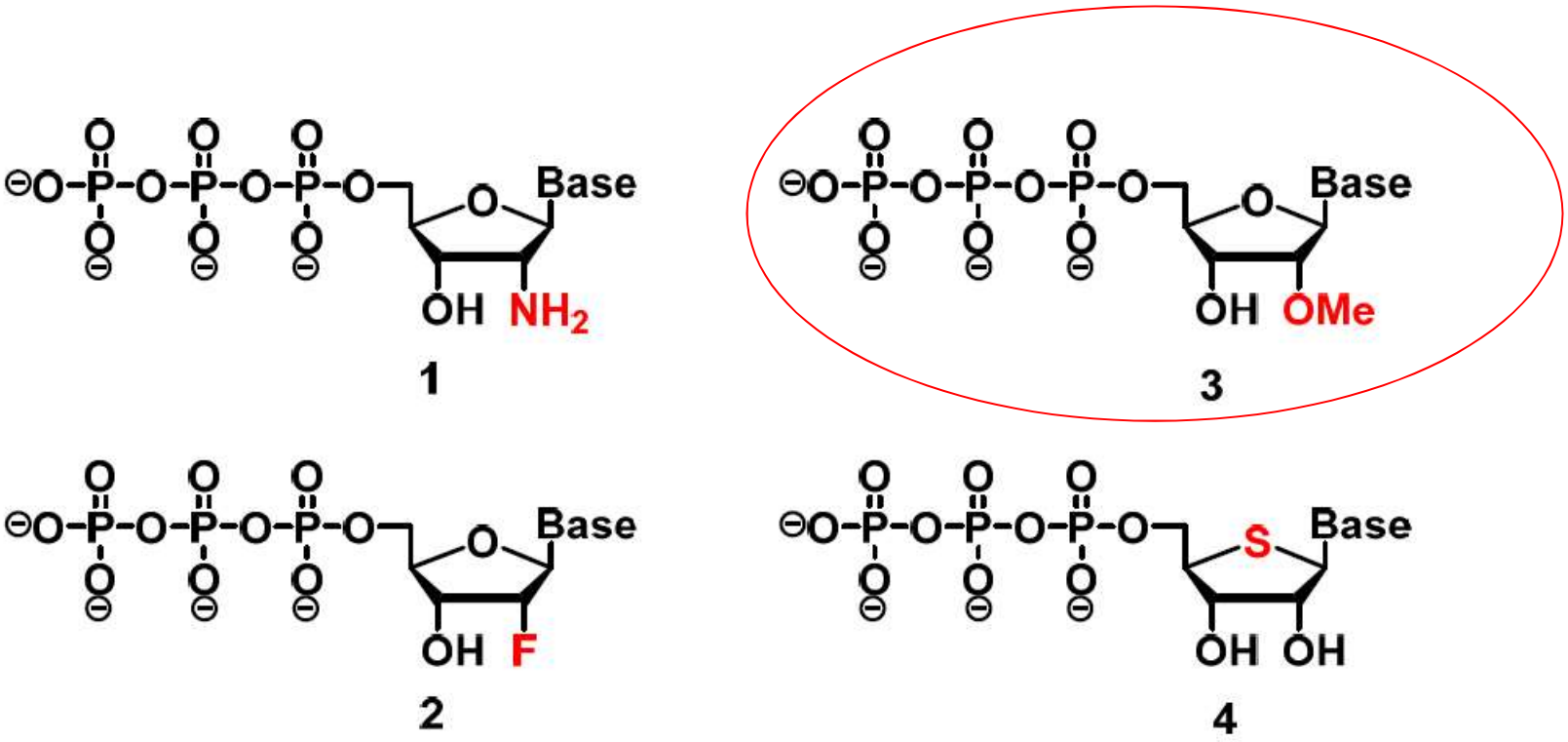
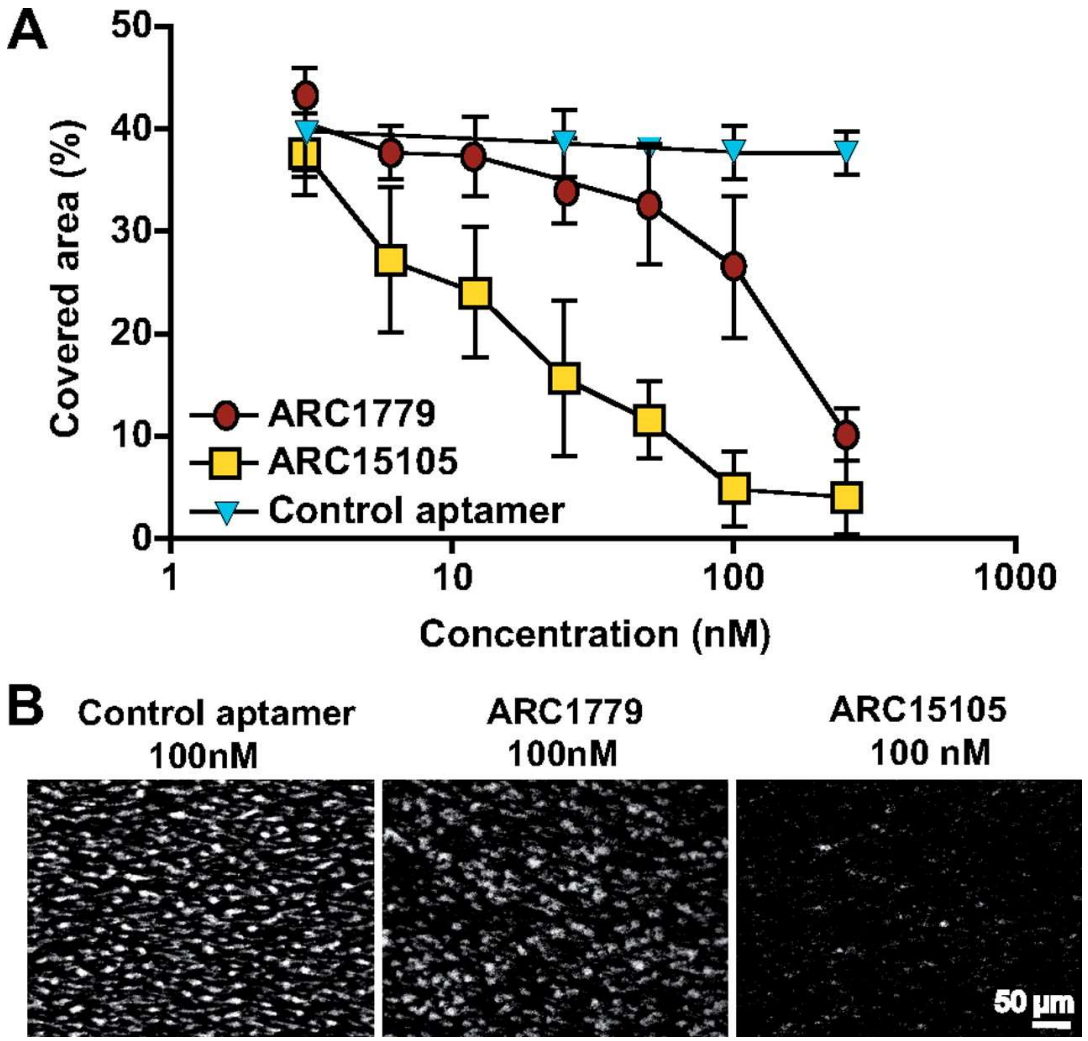


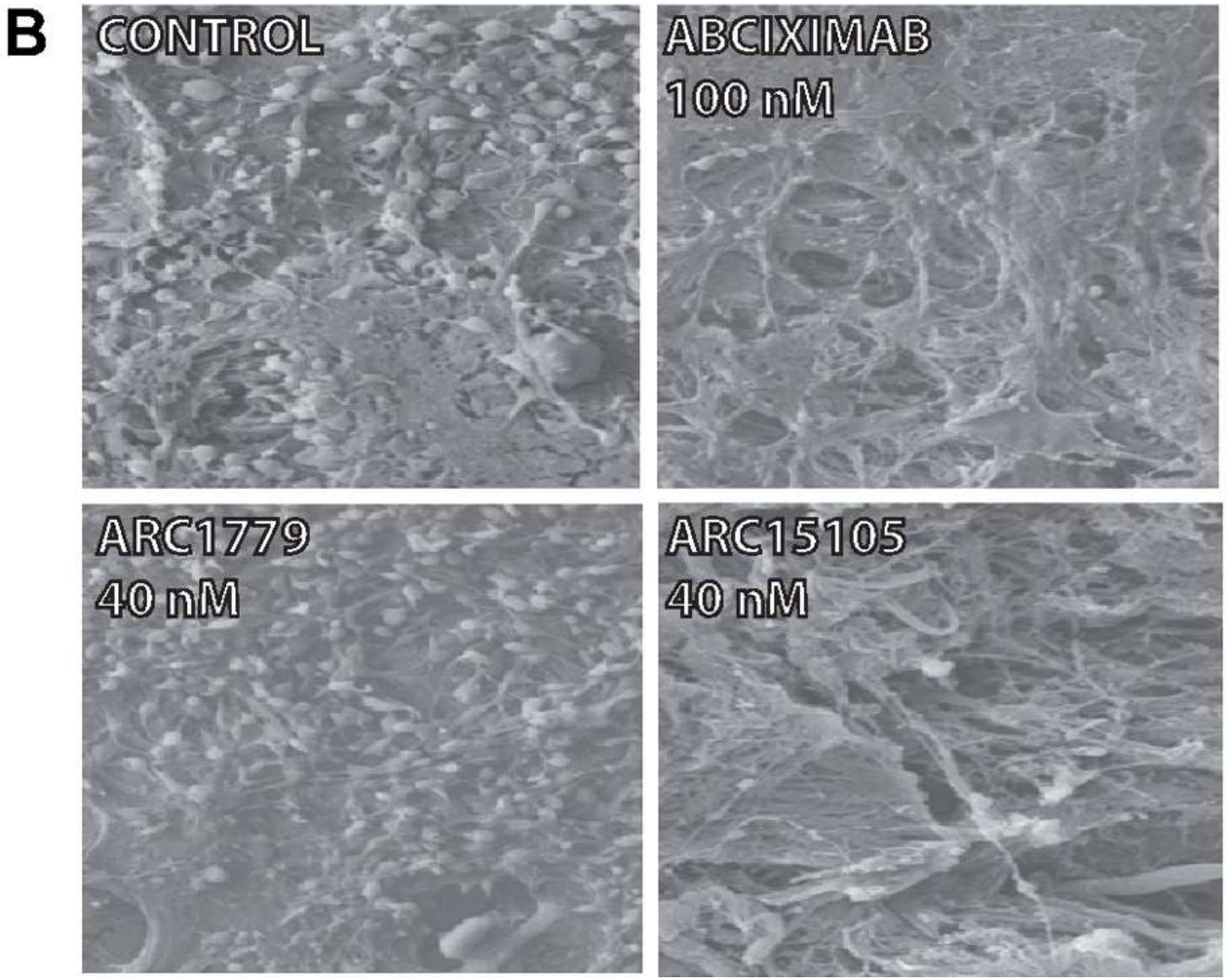
Figure 2. Chemical structures of 2′-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2′-amino-NTPs **1**, 2′-fluoro-NTPs **2**, 2′-methoxy-NTPs **3**, and 4′-thio-NTPs **4**.

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



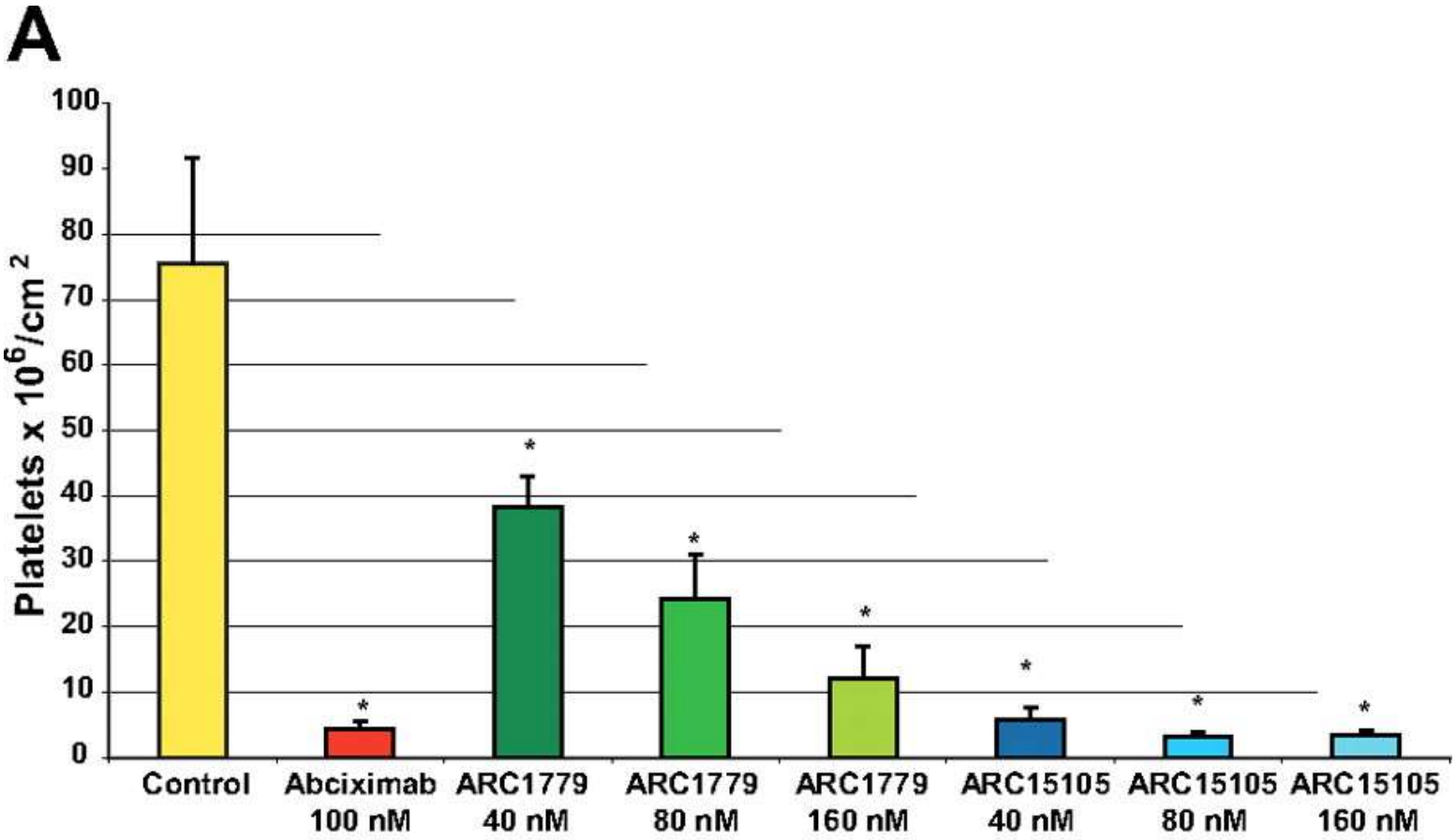
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

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



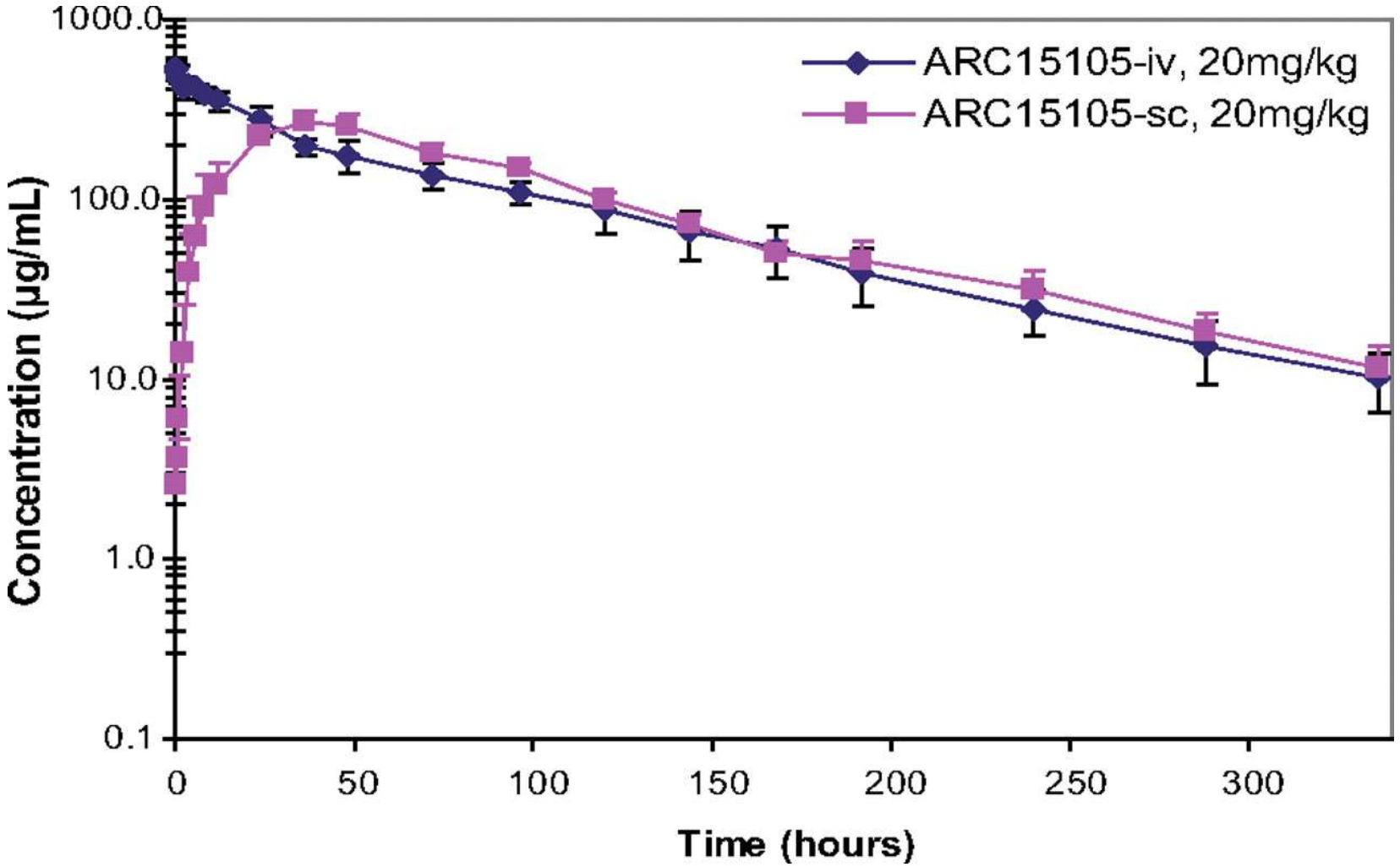
Siller-Matula J et al. Arterioscler Thromb Vasc Biol 2012;32:902-909

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

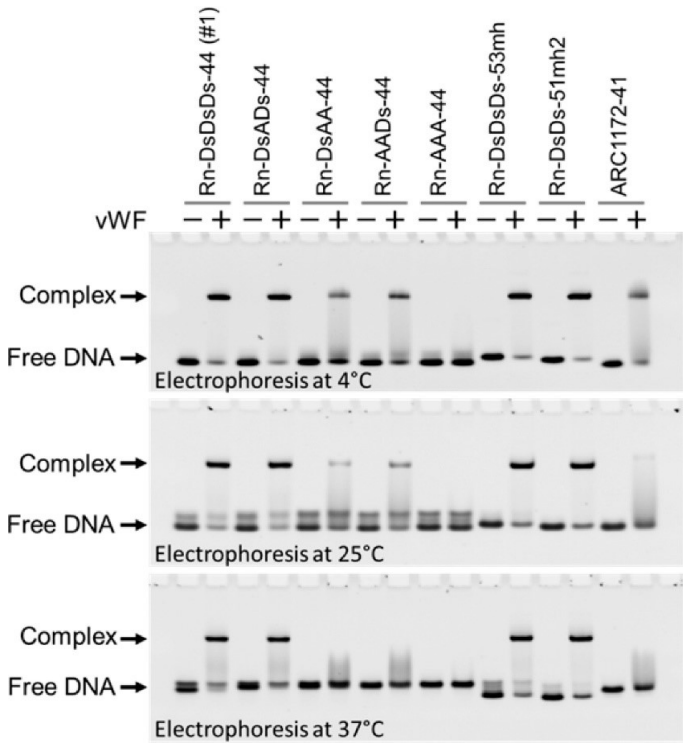
Comparison of the pharmacokinetics of a single bolus of ARC15105 (20 mg/kg) administered intravenously (IV) and subcutaneously (SC) in 3 cynomolgus monkeys; P<0.05.



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

American Heart Association
Learn and Live


Target
Selection
ExSELEX
PCR
Target
DNA aptamer
Stabilization
Five-base library
Unnatural base system
Optimized
DNA aptamer



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 for 30 min, and the complexes were separated from the free DNA on 8% polyacrylamide gels containing 3 M urea with electrophoresis at 4 (upper panel), 25 (middle panel), or 37 ° C (lower panel).
The DNA bands on the gels were stained with SYBR Gold.