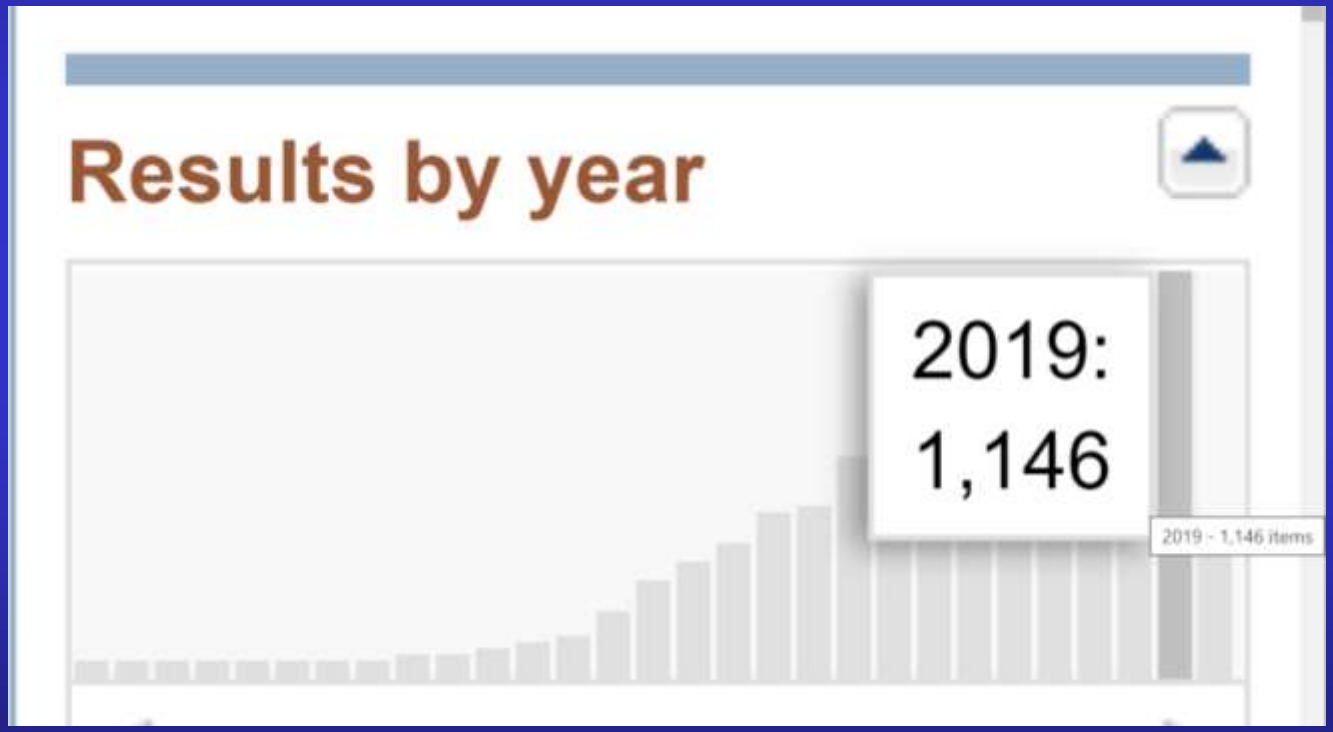
Gli APTAMERI

The term “Aptamer” was coined by Andy Ellington. It stems from the Latin terms “aptus,” meaning to fit, and Greek “meros,” meaning part.

Aptamers: Pubmed Search results Items:10814



https://www.ncbi.nlm.nih.gov/pubmed/

APTAMERI

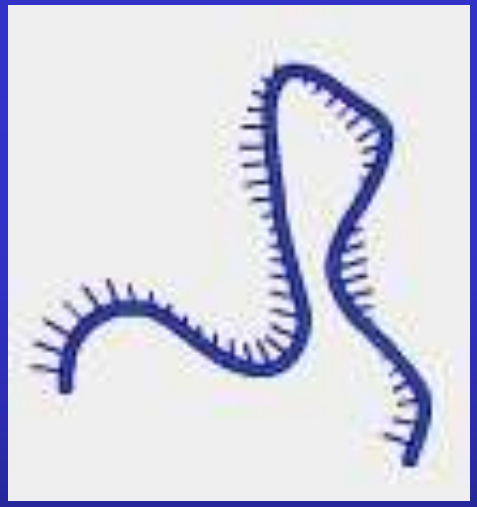
Acidi nucleici a singolo filamento caratterizzati da una specifica **struttura tridimensionale** che si lega direttamente alla proteina target.

Interazione Acido Nucleico/Proteina

Aptameri

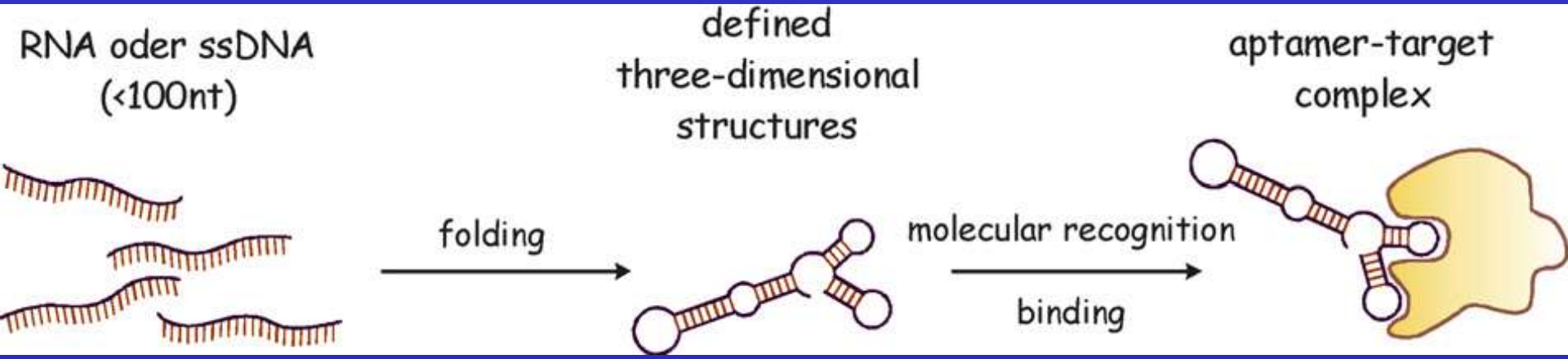
Dimensioni: 30-70 nucleotidi

Molecola Lineare



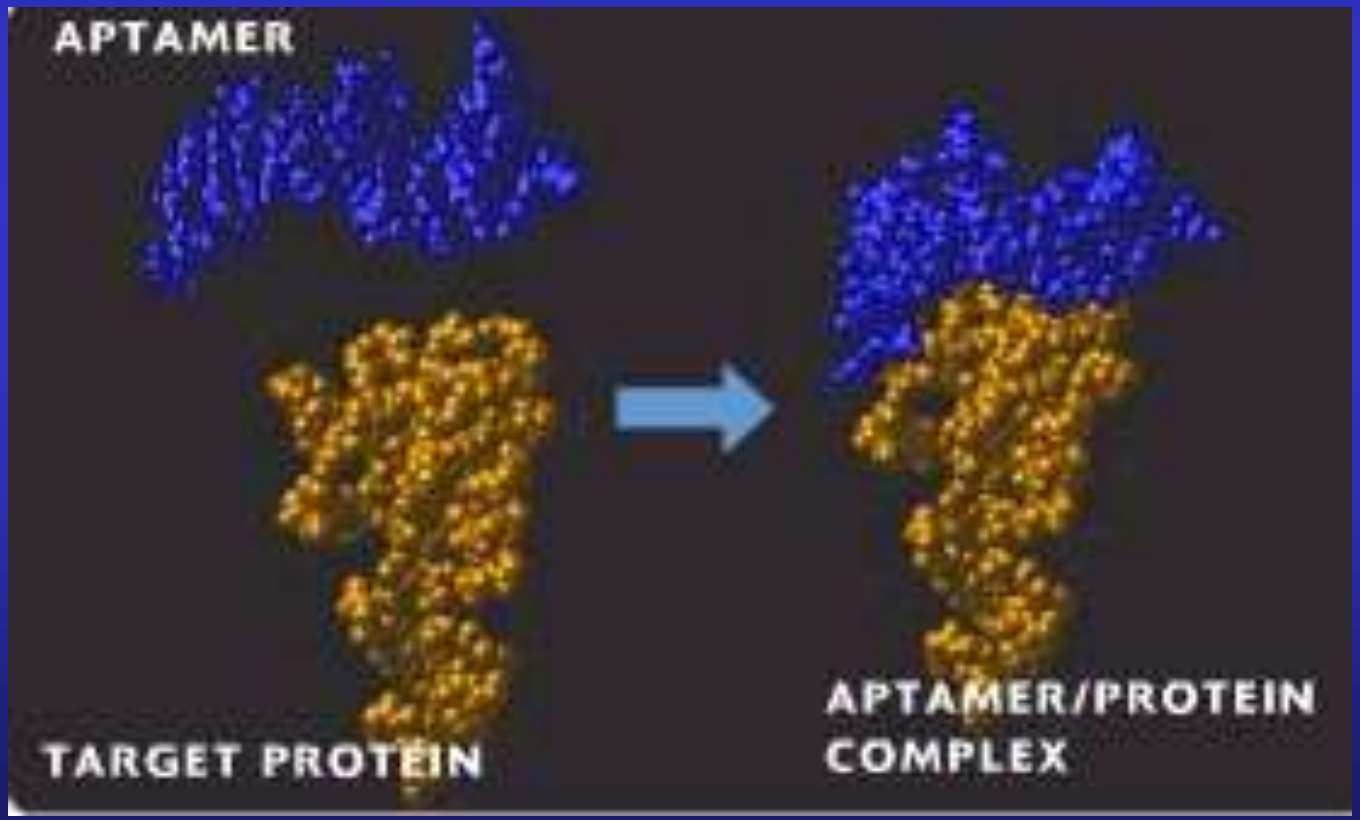
Folding

Struttura tridimensionale stabile


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

Anatomia degli Aptameri

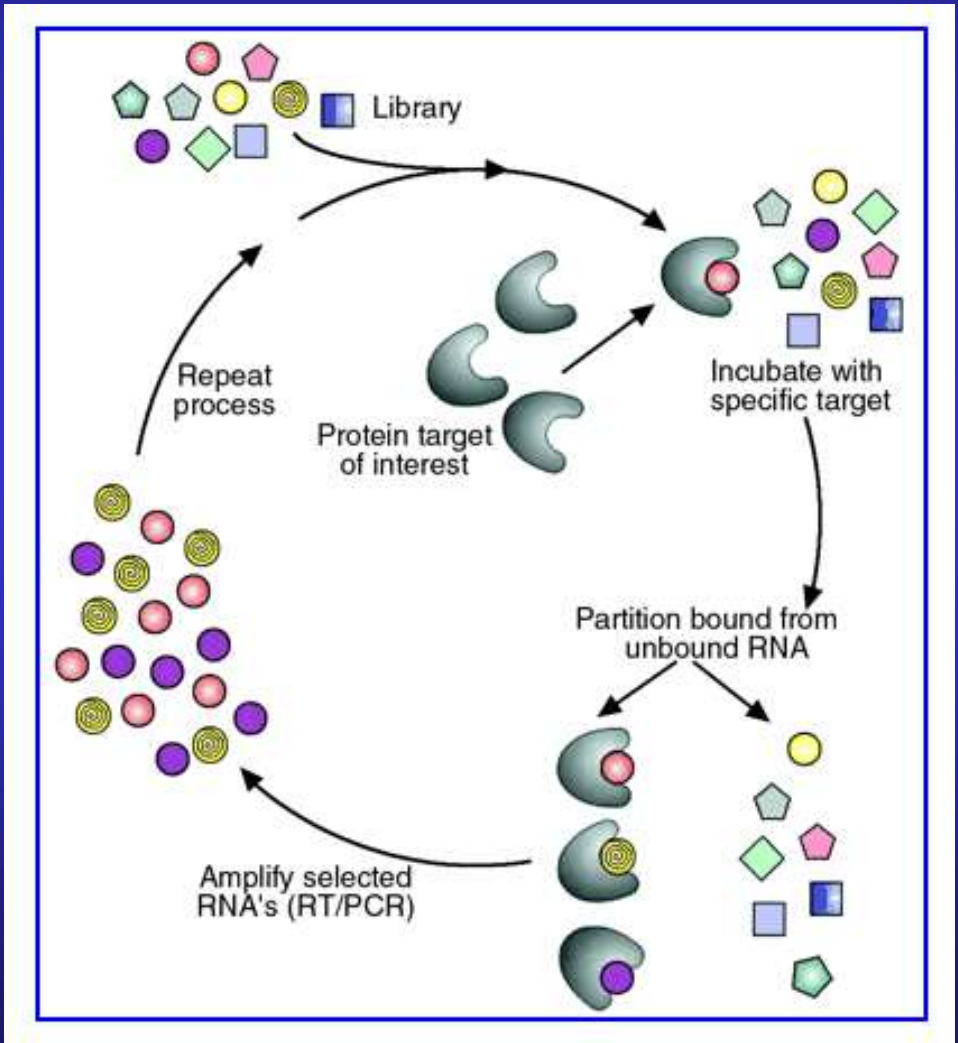
Gli aptameri sono molecole selezionate per legarsi in modo specifico ad una predefinita *proteina target*



Name of the aptamer	Primary target of the aptamer	Status
Macugen	VEGF	Approved [22]
AS1411	Nucleolin	Phase II [25, 26]
REG1	Factor Ixa	Phase II [29, 30]
EYE001	VEGFR	Phase II/III [47, 49]
LY2181308	Survivin mRNA	Phase III [50, 51]
E_2F decoy oligonucleotides	Mesangial cells	Phase III [52, 53]
ARC1779	Vwf	Phase II [31]
NU172	Thrombin	Phase II [32]
E10030	PDGF	Phase II [23]
ARC1905	C5	Phase I [24]
NOX-E36	MCP-1	Phase I [27, 33]
NOX-A12	SDF-1	Phase I [27, 28]
NOX-H94	Hepcidin	Phase I [21]
BAX499/ARC19499	TFPI	Phase I [34, 35]
DNA aptamers	Thrombin	Research [11]

Selezione in vitro degli Aptameri:

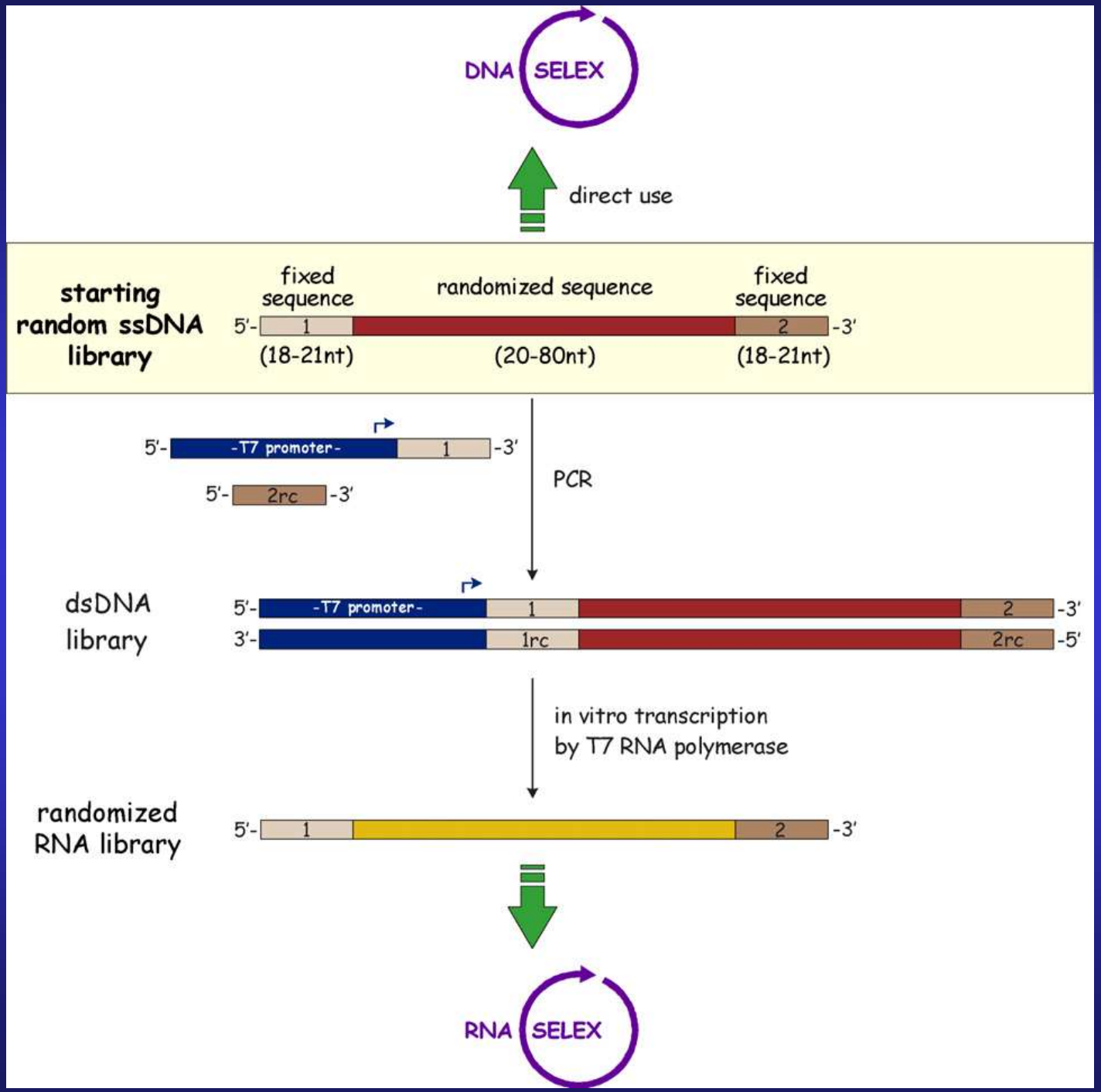
SELEX (systematic evolution of ligands by exponential enrichment)

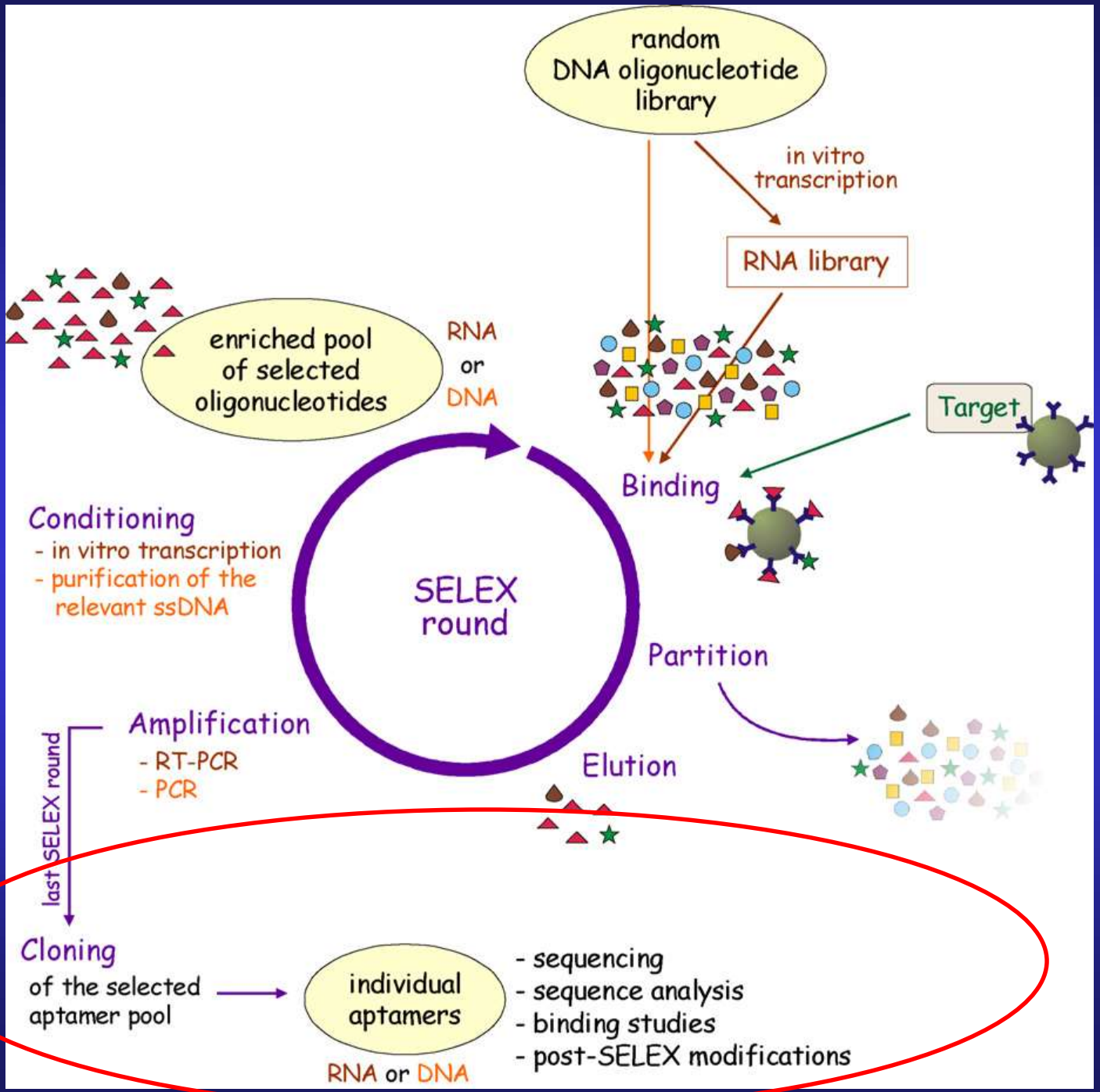


Selezione in vitro degli Aptameri:

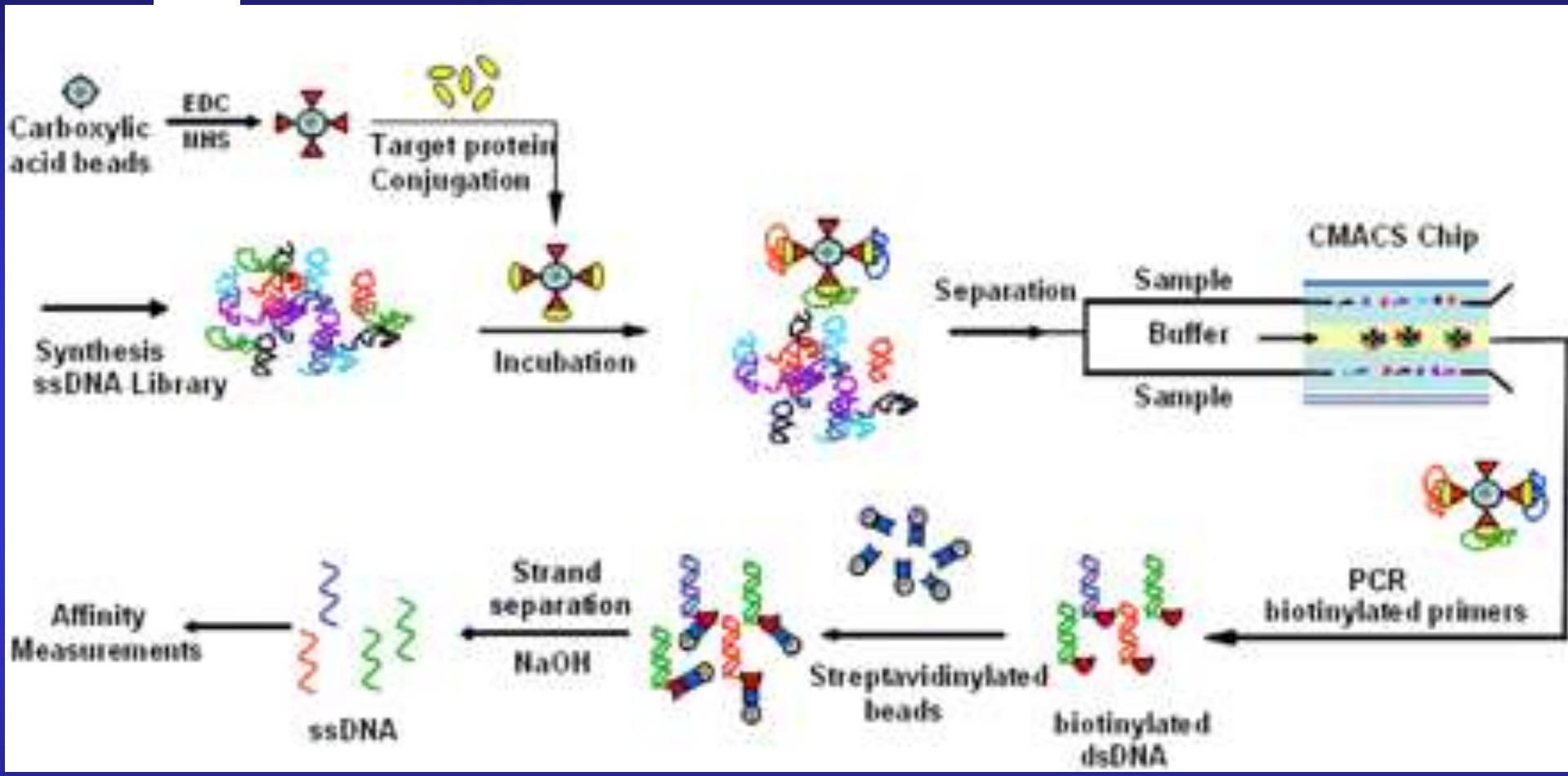
SELEX (systematic evolution of ligands by exponent enrichment)

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



Modified nucleotides: 2’

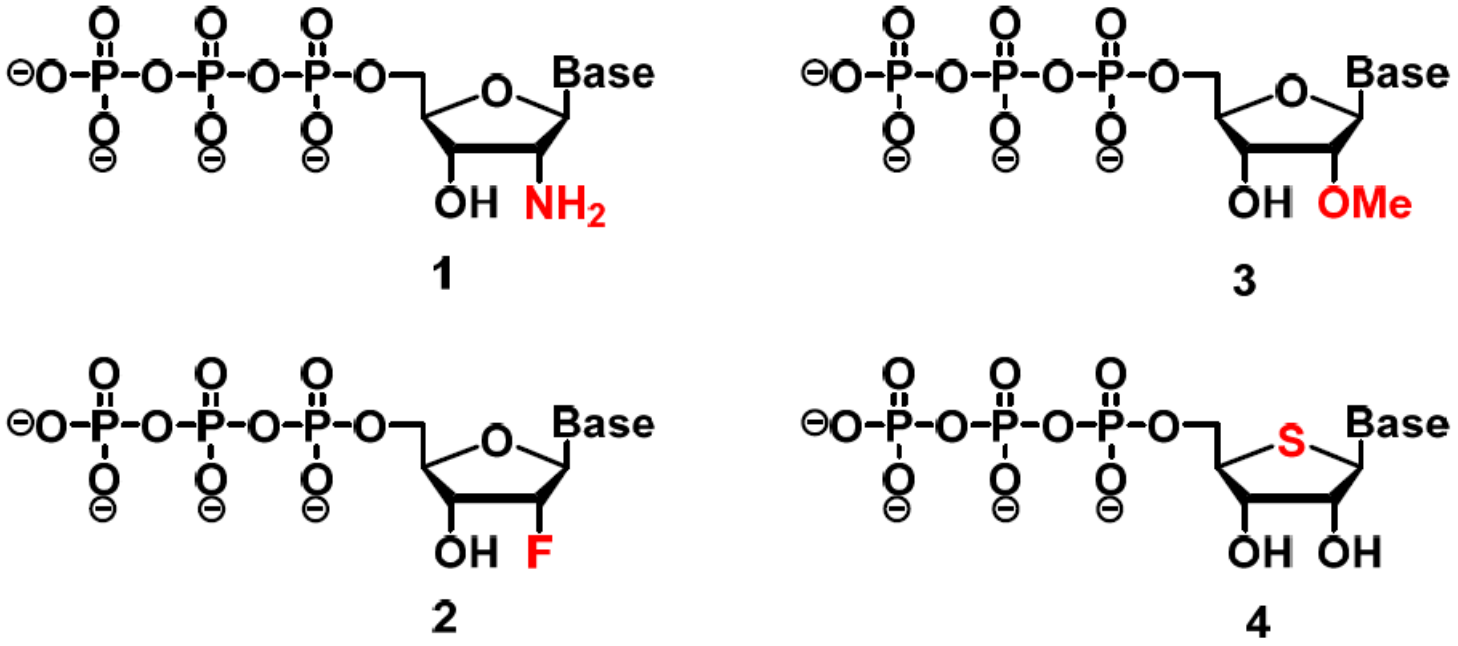
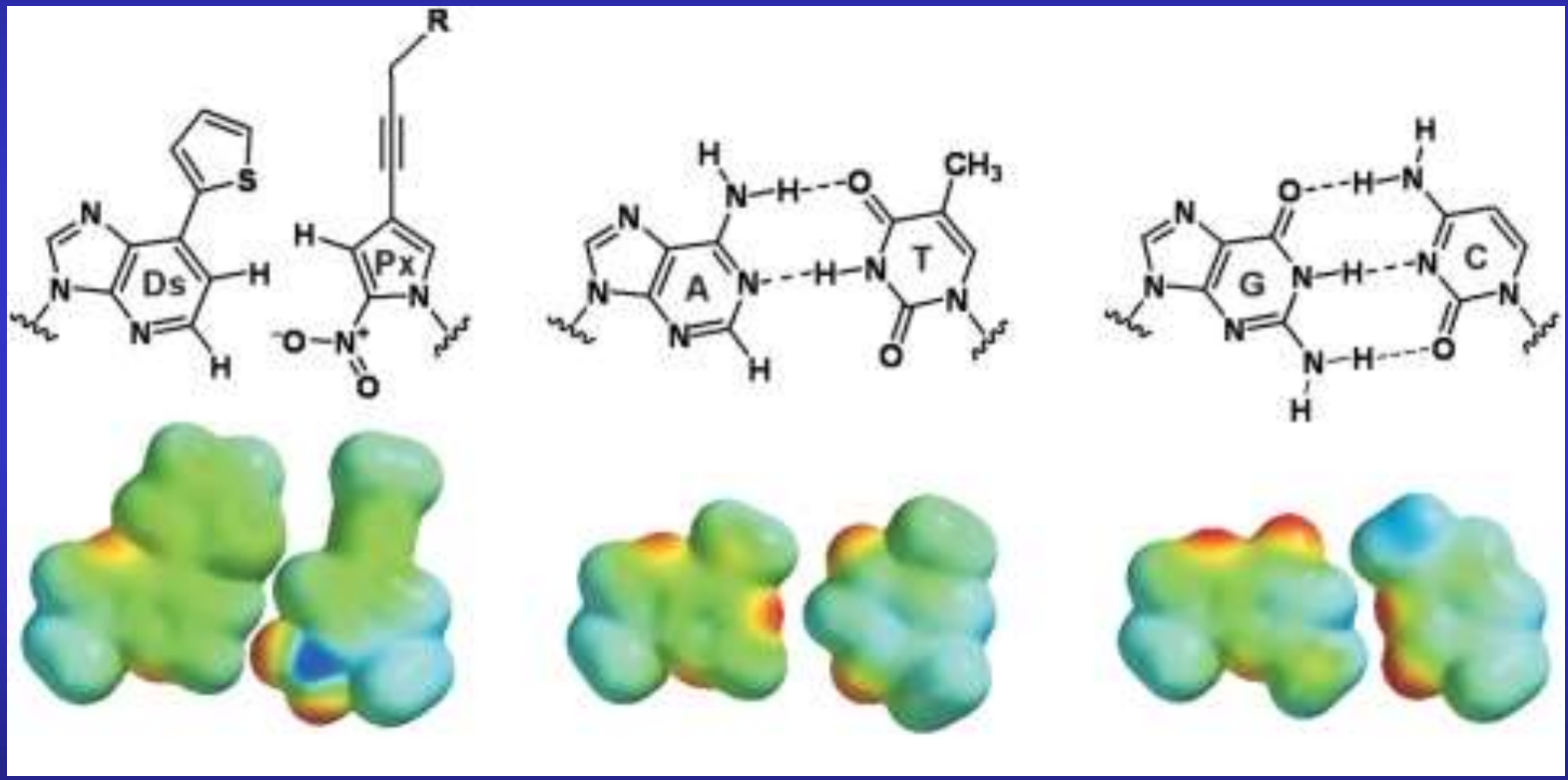


Figure 2. Chemical structures of 2′-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2′-amino-NTPs **1**, 2′-fluoro-NTPs **2**, 2′-methoxy-NTPs **3**, and 4′-thio-NTPs **4**.

Structures of the unnatural Ds–Px and natural A–T and G–C pairs



Applicazioni degli Aptameri:

1. Ricerca

2. Diagnostica

3. Terapia

Applicazioni degli Aptameri:

ALTERNATIVA AGLI ANTICORPI

→ elevate specificità e affinità unite a ridotte dimensioni

→ sintesi chimica (vs sintesi in animali o colture cellulari)

→ facilmente modificabili: marcatura con radioattivo, code fluorescenti e biotinilate...

APPLICAZIONI IN VIVO:

→ nessuna tossicità dimostrata (facilmente eliminabili da sangue e reni)

→ non immunogenici

→ tessuto-specifici

TERAPIA:

Condizioni patologiche acute e spazialmente confinate

→ Trombosi: aptameri contro trombina, FVIIa, FIXa vWF

→ Cancro: aptameri contro proteine segnale (es. Crescita, differenziazione, trasformazione cellulare...)

→ Patologie virali: identificazione e inibizione di proteine virali

Table 1. Summary of the recently generated aptamers using the 2′-fluoro modificat

Aptamer Name	Aptamer Target	K_d Value (nM)
E07	Epidermal growth factor receptor (EGFR)	2.4
CL4	Epidermal growth factor receptor (EGFR)	10
S2	Prostate-specific antigen (PSA)	630
A15	Brain penetrating aptamer	-
R-F t2	NS5B replicase, essential for the replication of hepatitis C virus (HCV)	2.6
Gint4.T	Platelet-derived growth factor receptor β (PDGFRβ)	9.6
GL21.T	Transmembrane tyrosine kinase receptor (RTK) Axl	12
G-3	C-C chemokine receptor type 5 (CCR5)	110
C26-50	*N*-methyl-D-aspartate (NMDA) receptor ion channel	120
Apt1	CD44, a cell-surface glycoprotein that serves as a cancer stem cell marker	81.3
B-68	HIV-1_{Ba-L} glycoprotein 120	52
GL44	Human U87MG glioma cells	38
RNA 14-16	p68 RNA helicase, which is involved in colorectal cancer	13,8
FAIR-6	Interleukin-6 receptor (IL-6R)	40.9
CD28Apt2, CD28Apt7	CD28 costimulatory receptor for the activation of T lymphocytes	40, 60
9C7	OX40 costimulatory receptor	1.7
αV-1, β3-1	αV and β3 subunits of integrin αVβ3	2.7, 6.5

Rusconi CP, Scardino E, Layzer J, Pitoc GA, Ortel TL, Monroe D, Sullenger BA

RNA aptamers as reversible antagonists of coagulation factor IXa

Nature 2002; 419: 90-94

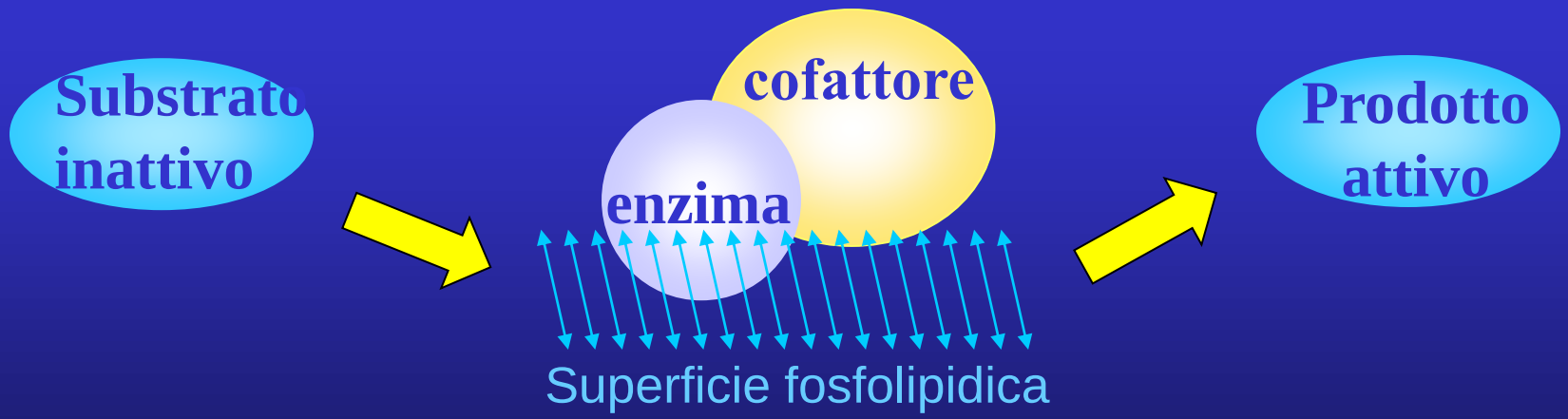
(www.nature.com)

COAGULAZIONE DEL SANGUE

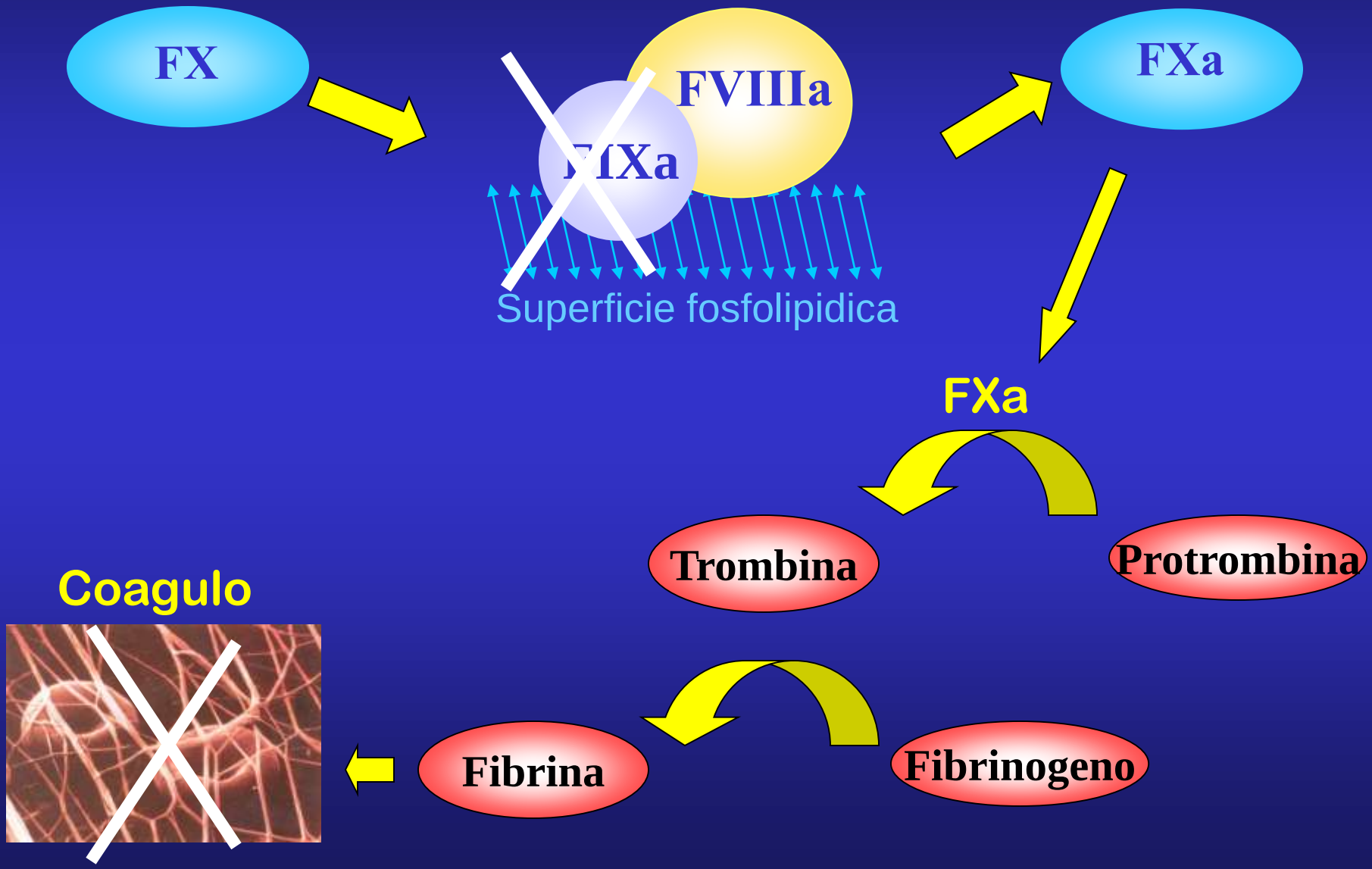
Danno vascolare

Attivazione a cascata di fattori e cofattori plasmatici

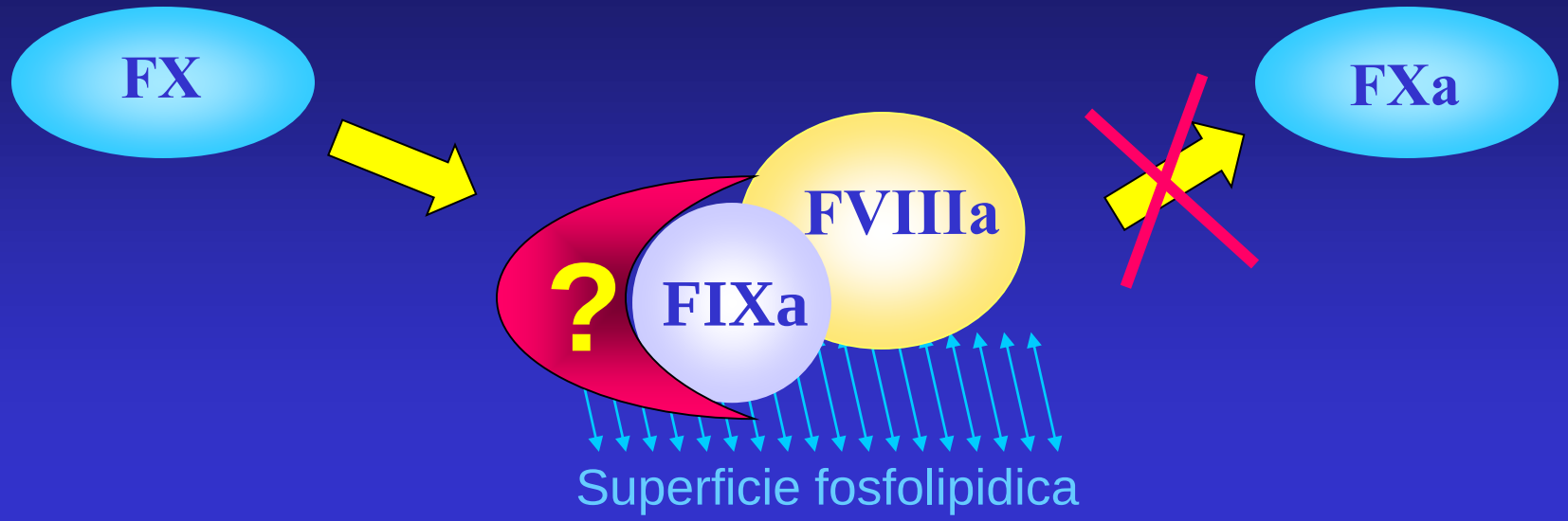
Complessi macromolecolari

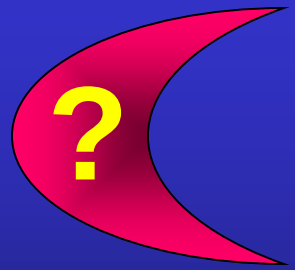


Complesso di attivazione del FX



Complesso di attivazione del FX





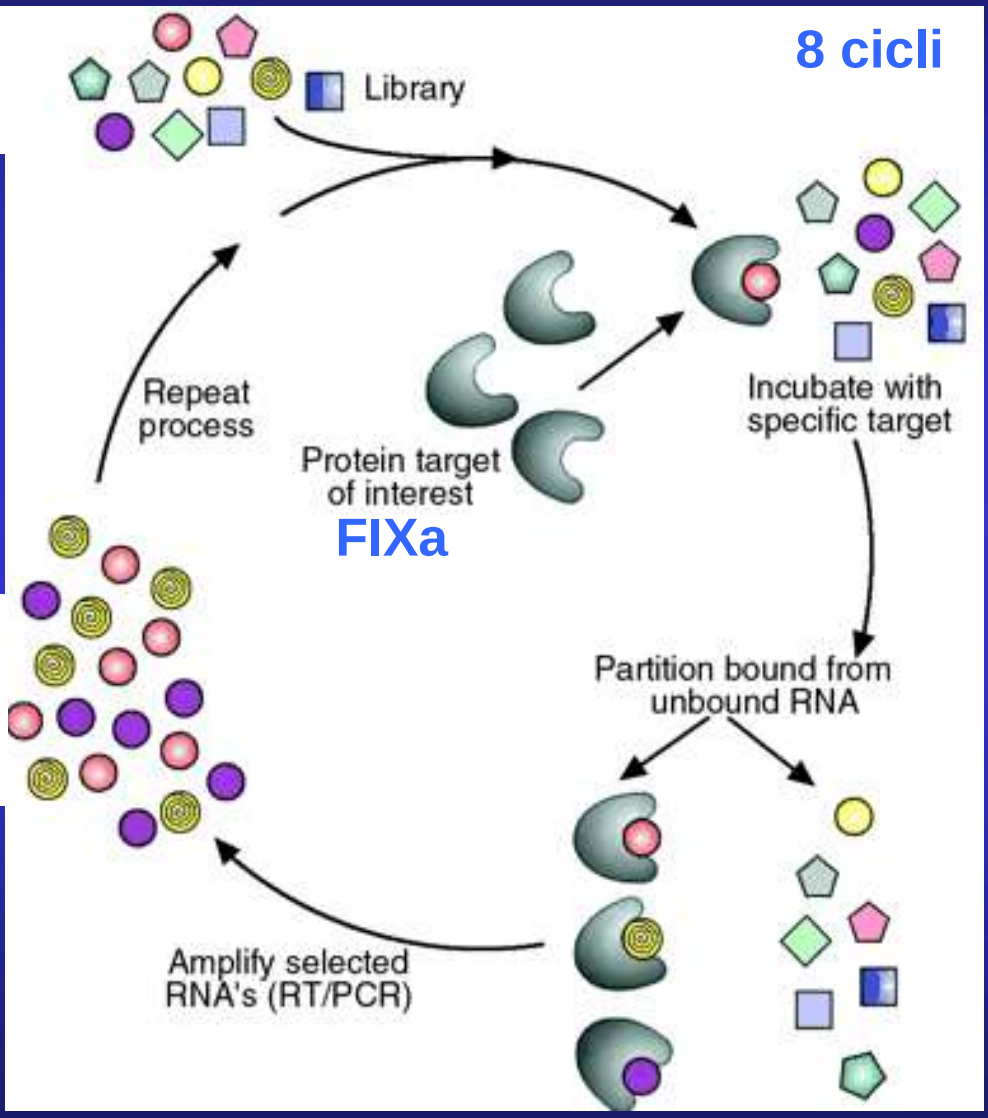
Aptamero selezionato

1. **Elevata AFFINITA' con il FIXa**
2. **SPECIFICITA' per il FIXa**

Selezione degli Aptameri
SELEX

Libreria: 10^{14} oligonucleotidi (RNA)

Retrotrascrizione degli RNA selezionati e sequenziamento

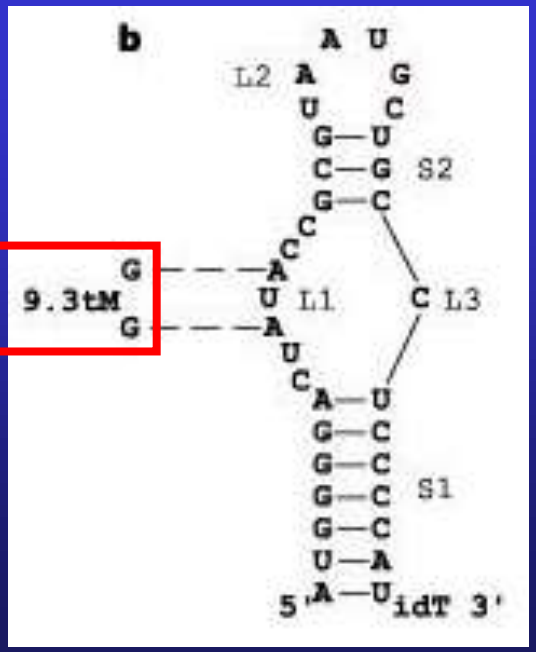


1. AFFINITA' con il FIXa

Aptamer		S1	L1	S2	L2	S2	L3	S1	
9-3	5'	GGGAUGGGGA	CUAUACC	GCG	UAAUGC	UGC	C	UCCCCAUUCC	GGAACGCU 3'
9-20	5'	GGGGA	CUAUACCG	GCA	AUCG	UGC	A	UCCCC	UGGACCUAACAAUA 3'
9-19	5'	GGaUGGGA	CCAUA	ACGA	CUAC	UCGU	GAA	UCCCACC	AUCAGCGCACAA 3'
9-4	5'	GGGaUGGGC	ACUAUAC	GCA	UCU	UGC	U	GCCUGCCC	GCGAGUCAAUUG 3'
9-12	5'	GGGaUGGG	CGAUA	UAC	ACAUUG	GUG	AU	CCCACCC	ACAUGAAACCACAG 3'
9-17	5'	GAGGGauGGGA	CCAUAC	GCA	CAU	UGC	UGAA	UCCCCCUC	AAUAGCACCUAC 3'
9-25	5'	GGGAUGGGGA	CCAUA	ACUC	UAAC	GGGU	GAA	UCCCgCAUCUC	GACAAUA 3'
9-26	5'	GGGAuGGG	UGAUA	ACCA	CUC	UGGU	GAA	CCCeUCCC	GACUUGCUCGCA 3'
9-11	5'	GGGaUGGGA	CUAUA	UUCGG	AAU	CUGGA	C	UCCCACCU	GCCUGCCCCAGA 3'
9-2	5'	GGGAUGGG	CUAUAUA	CAC	GCUG	GUG	AU	CCCADCUC	AAUUGAAACAACA 3'
9-7	5'	GGAUGGG	CGAUA	ACCA	ACA	UGGU	GAU	CCCAUUC	AUCAUACCCUACAA 3'
9-28	5'	GGGAuGGGCG	CCAUAC	GCA	CAU	UGC	UGCAU	CGCCUUCCC	GUAAGAAC 3'
9-16	5'	GAGGGaUGGG	CCAUAC	GUGG	ACGA	CUGC	A	CCCGaCCCUU	CAGCCCAGGUC 3'
9-18	5'	GGGaUGGG	CCAUA	ACCA	CUU	UGGU	GAA	CCCACCC	AGCUCCUGUGAUUG 3'
9-14	5'	GGGAuGGGGA	CUAUA	CGU	GAACG	ACU	GCA	UCCaCUUCCC	CGCCAUGG 3'
9-27	5'	GGGaUGGG	UAAUA	ACU	GUA	UGG	UGAA	CCCACCC	AAACUCCCAUGGCUA 3'

Aptamero 9.3t

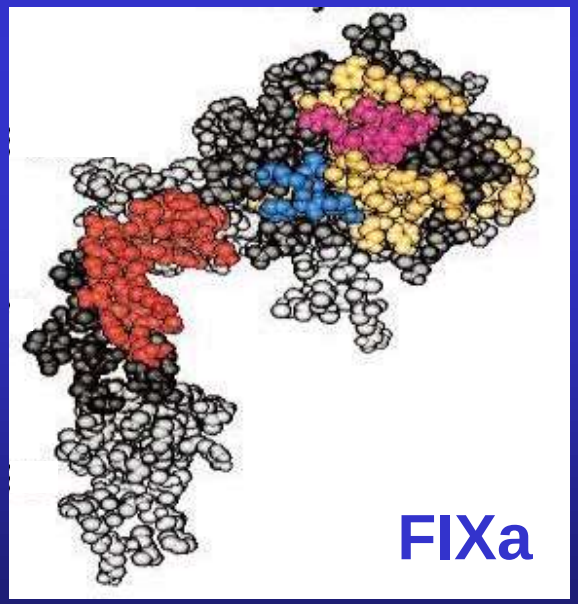
9.3 tM: controllo negativo (aptamero inattivo)



2. SPECIFICITA' per il FIXa

Aptamero 9.3t

5000 volte più specifico



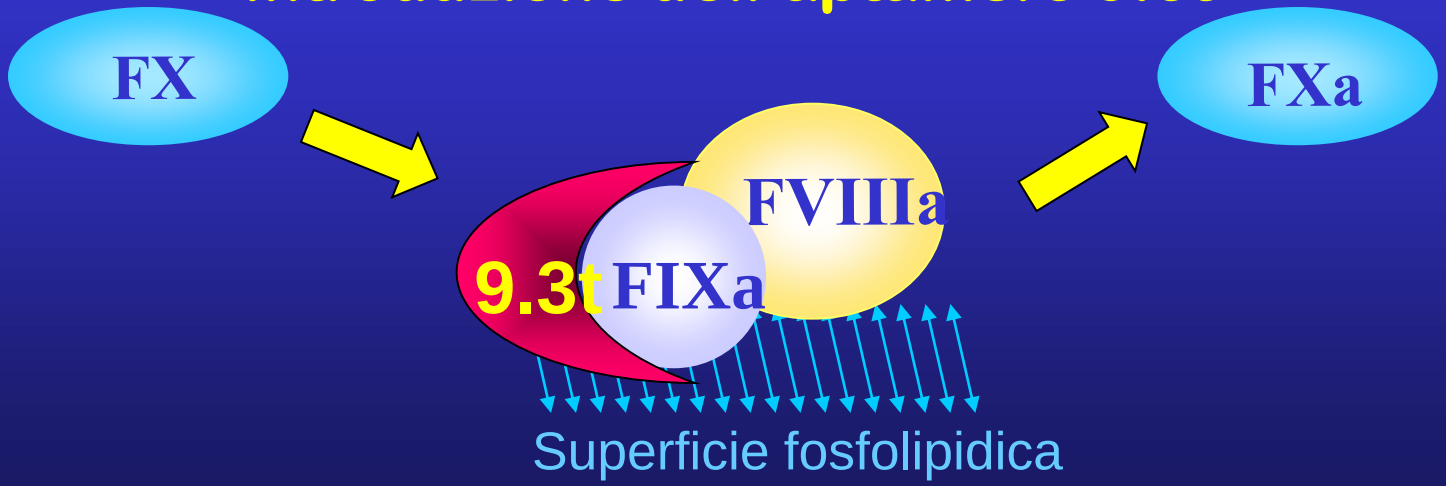
FVIIa **FXa** **FXIa** **APC**

Serin-proteasi

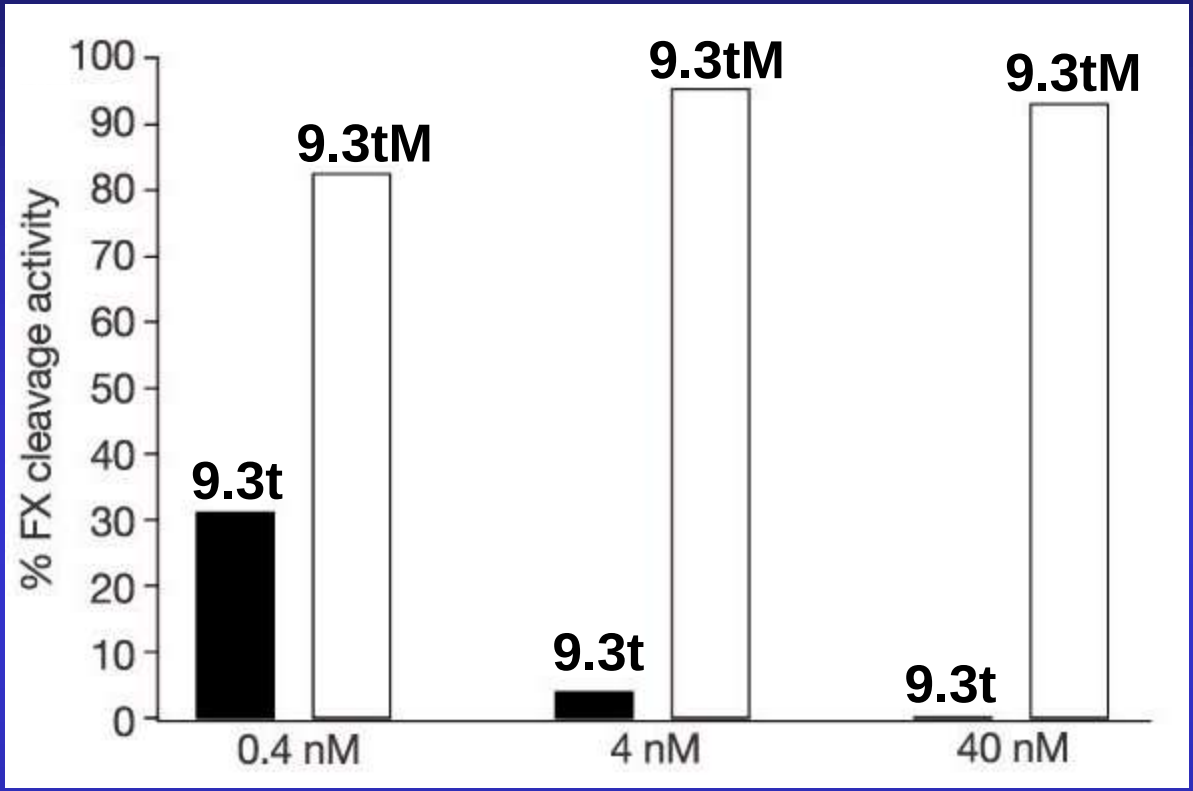
Inibizione IN VITRO dell'attività del FIXa

Assemblamento del complesso

Introduzione dell'aptamero 9.3t



Inibizione IN VITRO dell'attività del FIXa



9.3t: aptamero selezionato
9.3tM: controllo negativo

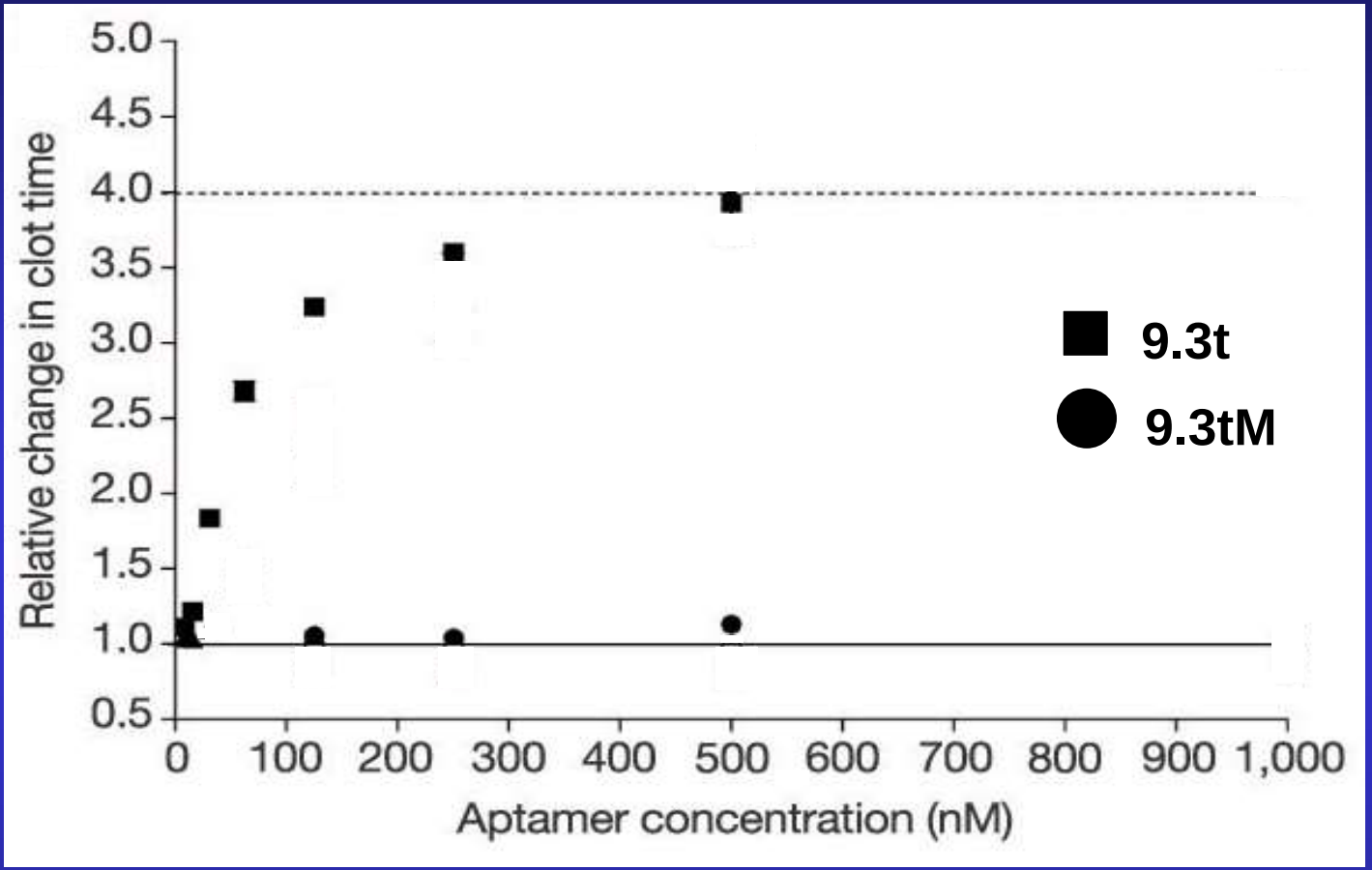
L'aptamero blocca l'attività del FIXa in vitro

Inibizione in plasma umano dell'attività del FIXa

Procedimento:

1. **Aggiunta di diverse concentrazioni di aptamero (9.3t) e controllo negativo (9.3tM) a plasma umano**
2. **Misurazione del tempo di coagulazione del plasma**

Inibizione IN VIVO dell'attività del FIXa

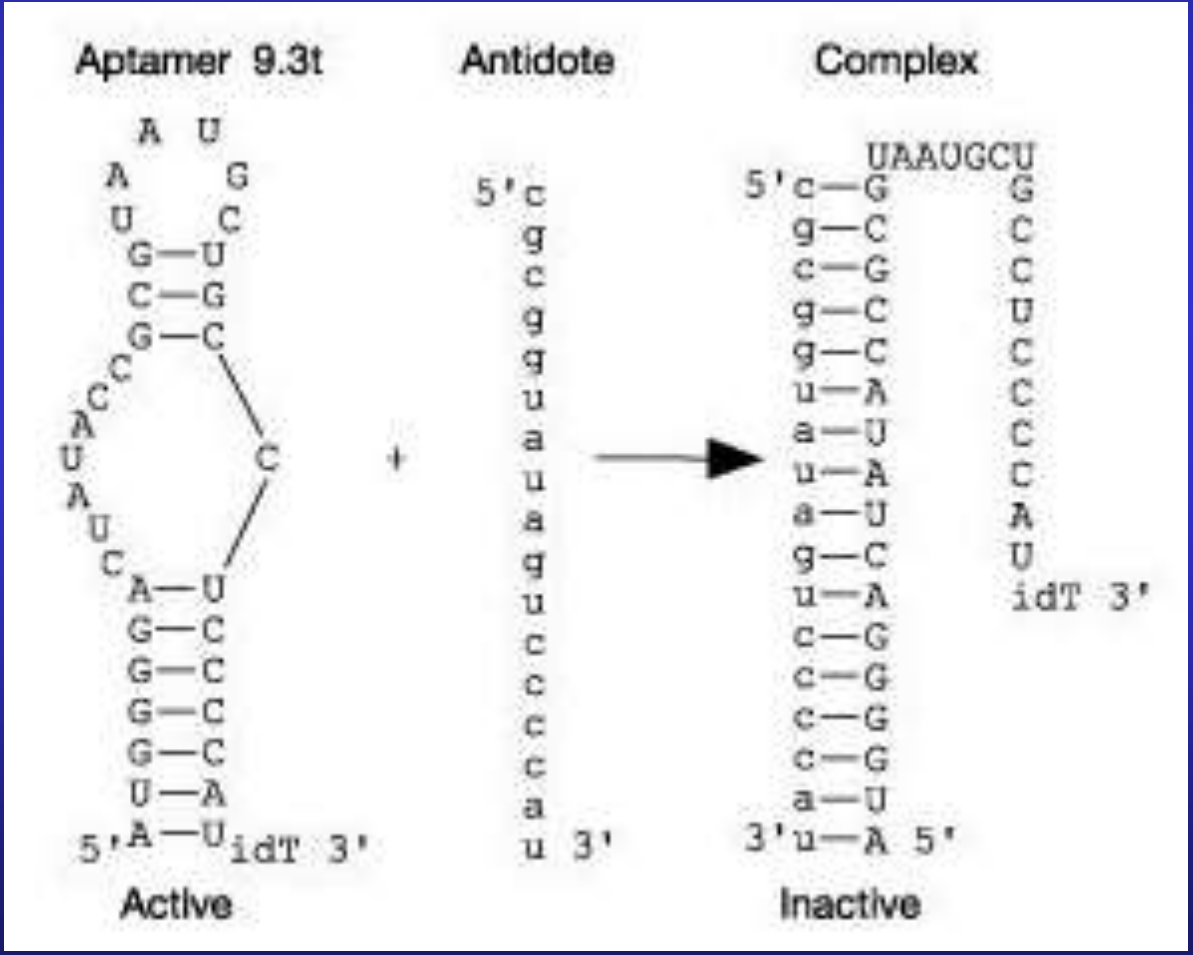


9.3t prolunga il tempo di coagulazione in modo dose-dipendente

L'aptamero inibisce l'attività del FIXa ex vivo

Reversibilità dell'azione dell'aptamero: ANTIDOTO

Antidoto = oligo complementare all'aptamero, in grado di alterare la sua conformazione

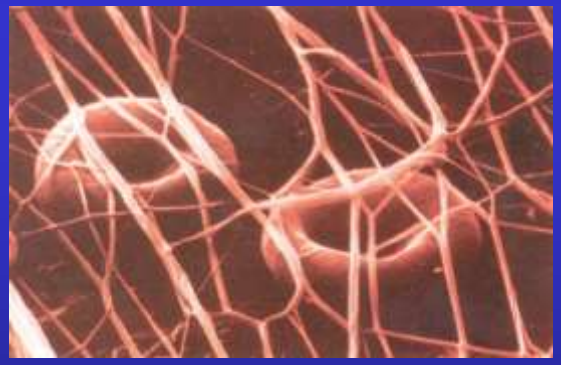


Reversibilità dell'azione dell'aptamero: ANTIDOTO

Plasma + aptamero = inibizione della coagulazione

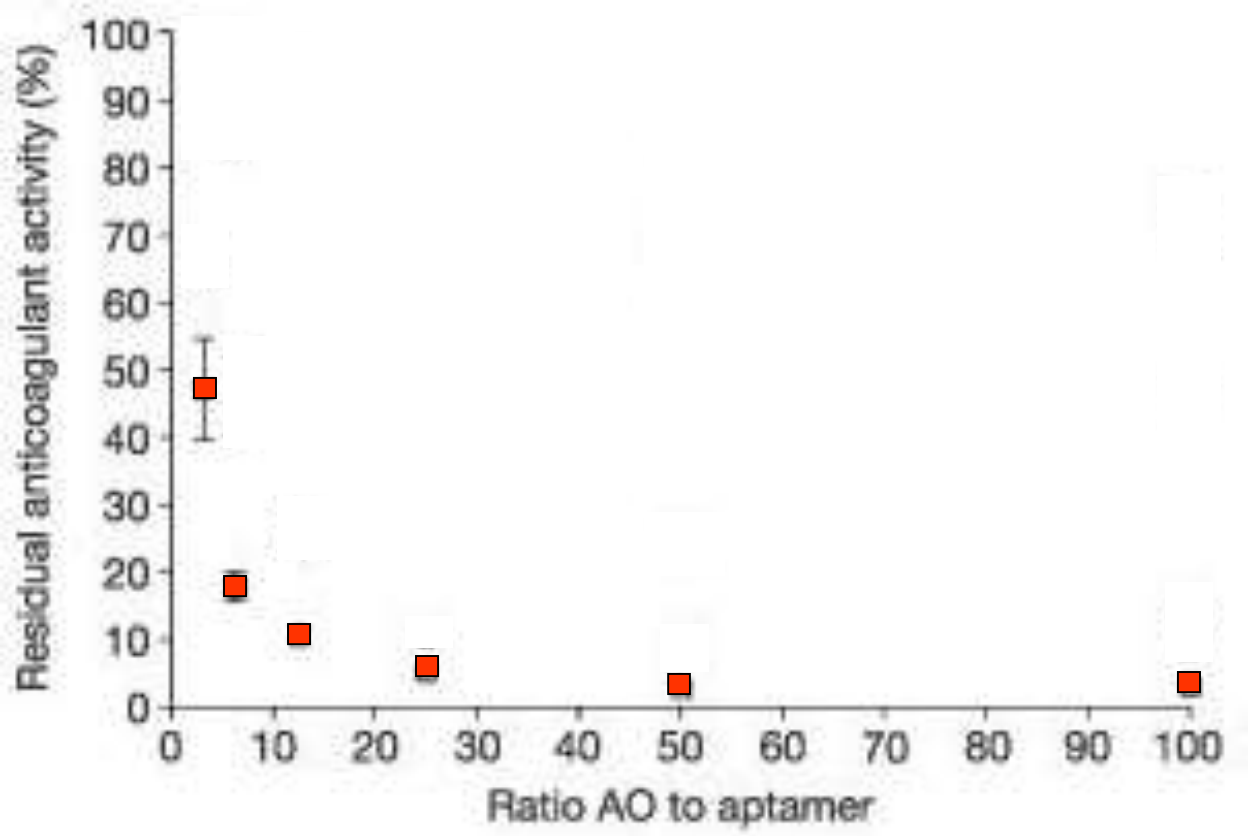
↓

Plasma non coagulato + antidoto = coagulazione



Si misura la capacità del plasma di coagulare entro 10 min.

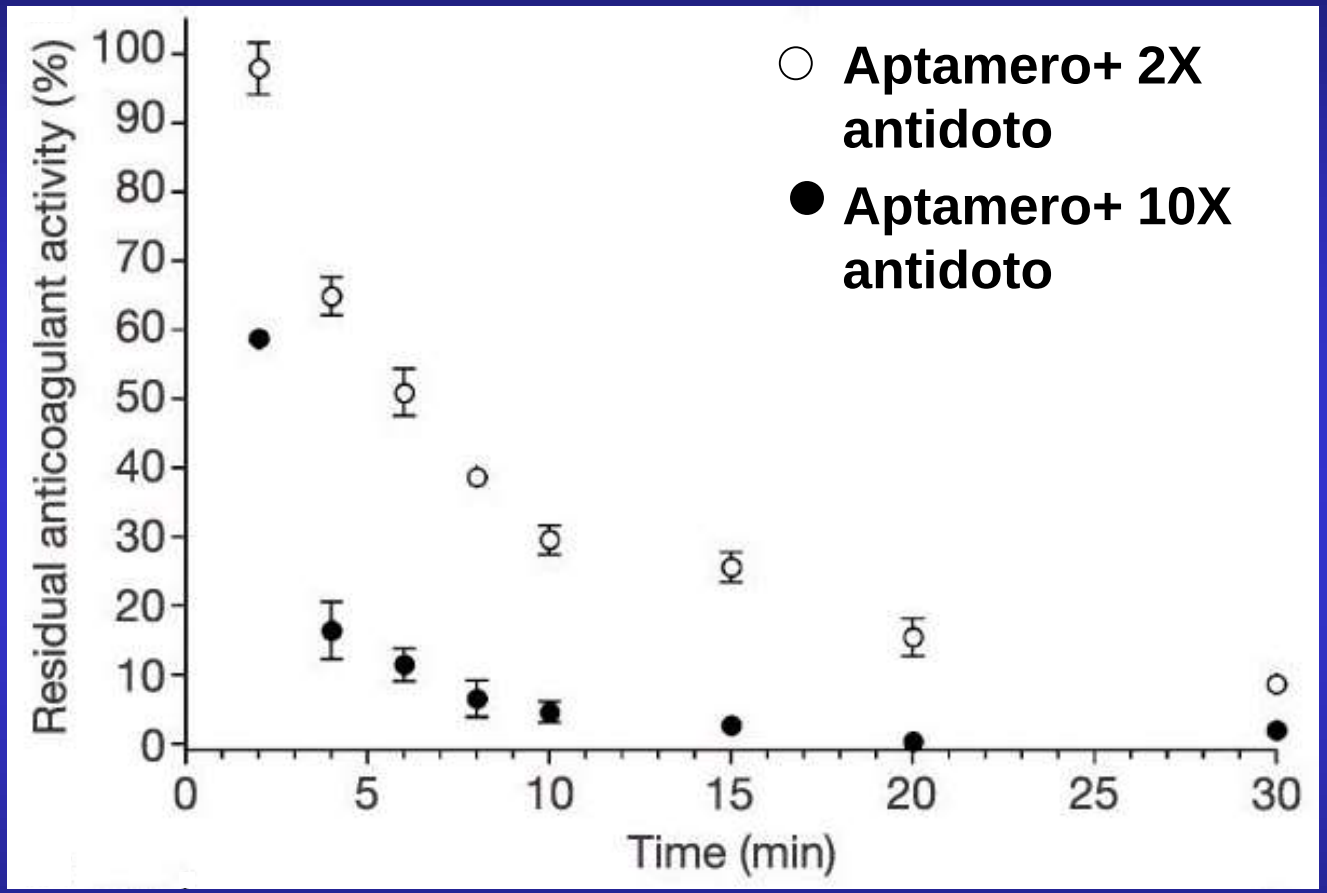
Reversibilità dell'azione dell'aptamero: ANTIDOTO- rapporti di concentrazione



Quantità crescenti di antidoto / aptamero 9.3t (50 nM)

L'antidoto neutralizza l'azione dell'aptamero

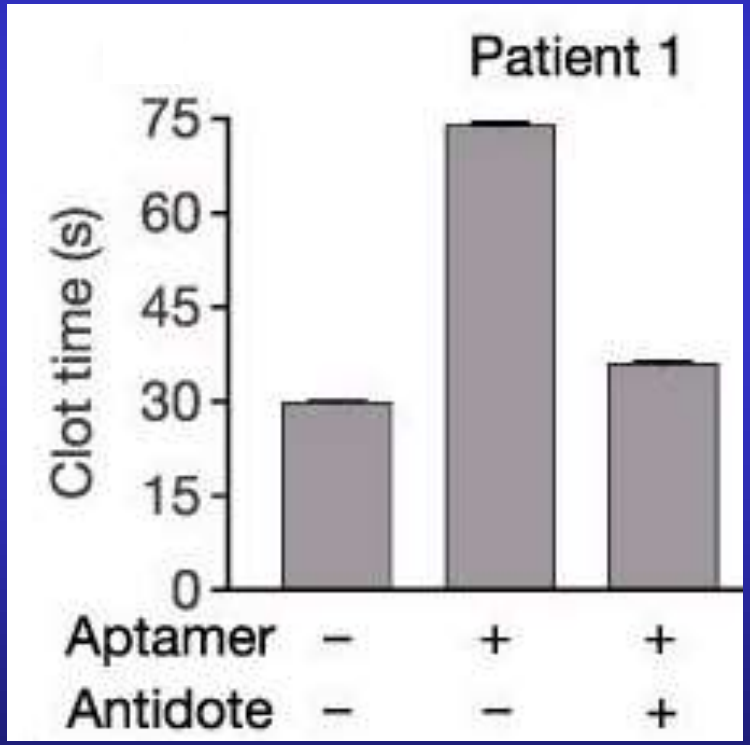
Reversibilità dell'azione dell'aptamero: ANTIDOTO - tempi



L'azione dell'antidoto è rapida e dose-dipendente

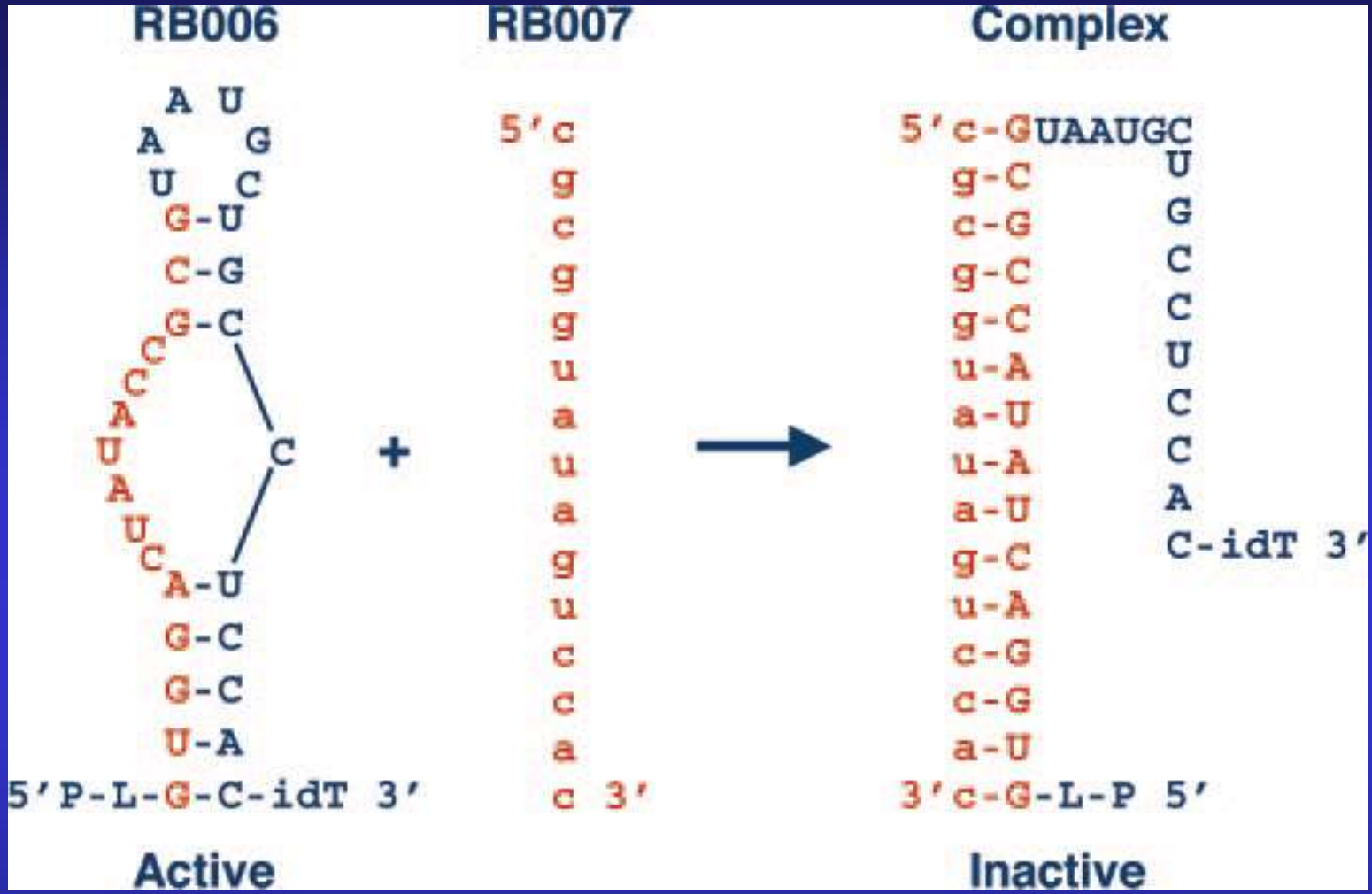
Efficacia di aptamero e antidoto *in vivo*

6 Pazienti con trombosi → pazienti non sottoponibili ai normali trattamenti anticoagulanti



First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation

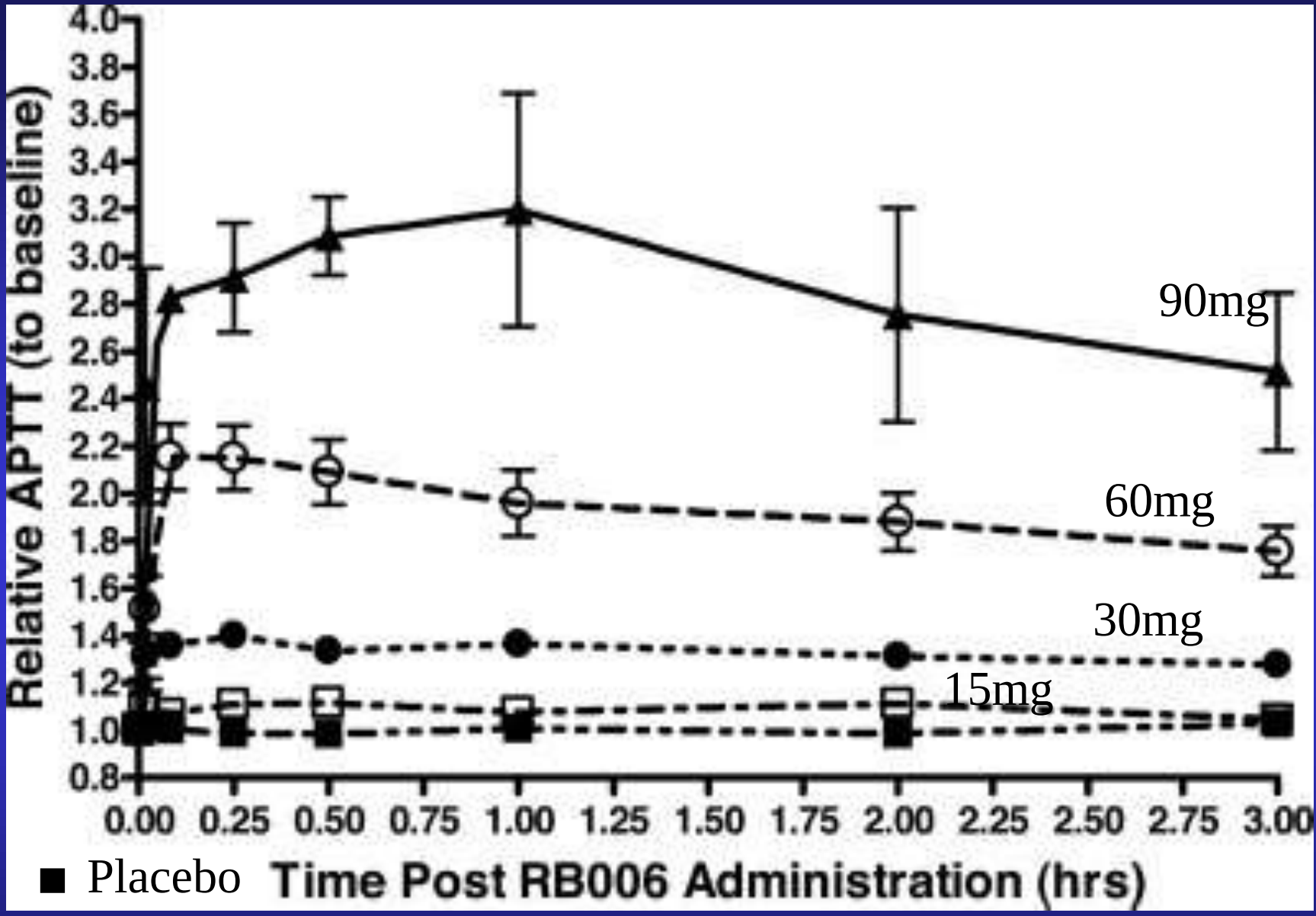
- Selectivity, titratability, rapidity of onset, and active reversibility are desirable pharmacological properties of anticoagulant therapy administered for acute indications.
- A novel anticoagulation system (REG1, Regado Biosciences), developed using a protein-binding oligonucleotide to factor IXa (drug, RB006) and its complementary oligonucleotide antidote (RB007), was evaluated in healthy volunteers.



P polyethylene glycol; idT, inverted deoxythymidine

First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation

- METHODS AND RESULTS: randomized 85 healthy volunteers received a bolus of drug or placebo
- There were no significant bleeding signals associated with RB006, and overall, both drug and antidote were well tolerated.

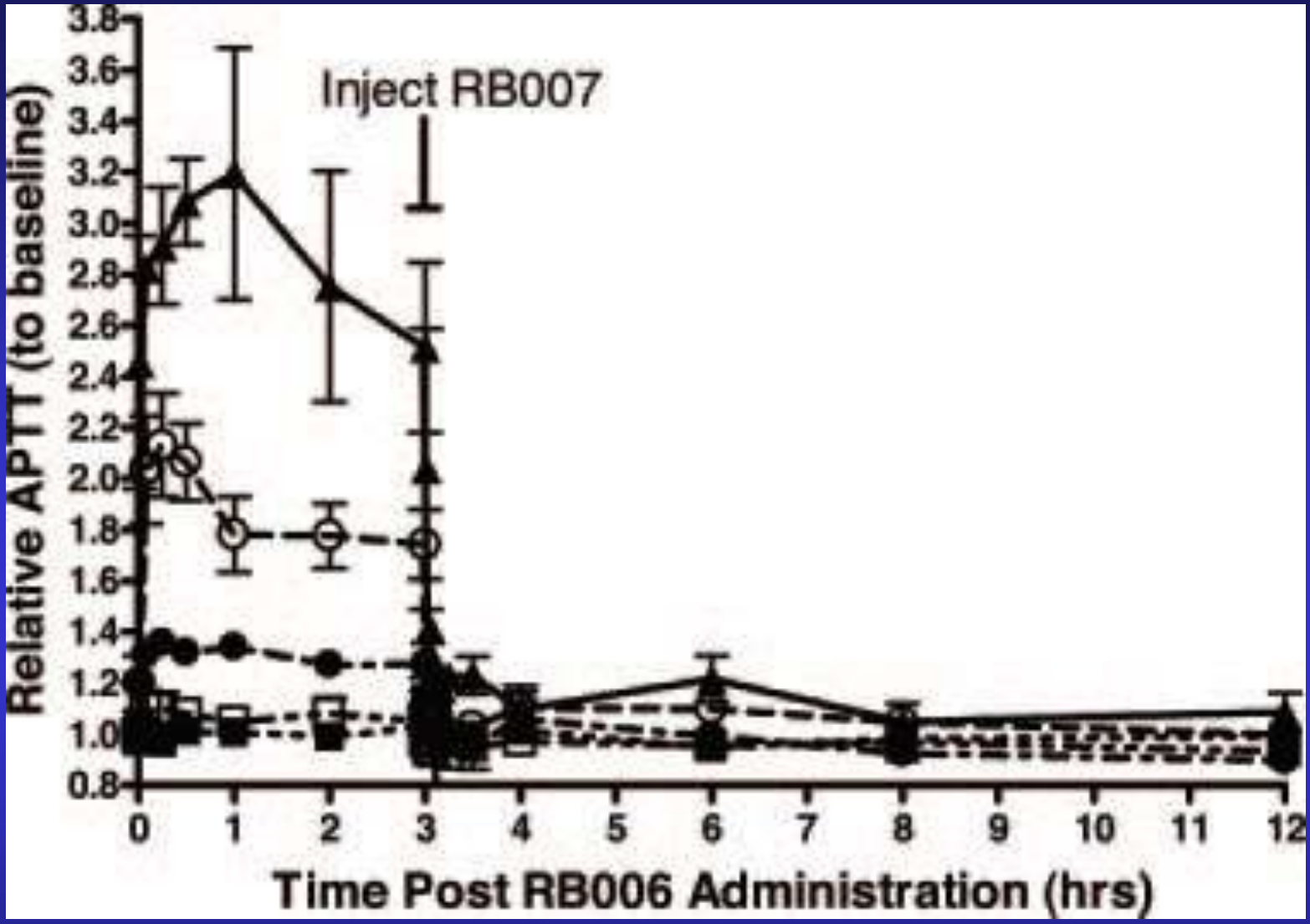

Relative APTT (to baseline)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00 2.25 2.50 2.75 3.00
Time Post RB006 Administration (hrs)
90mg
60mg
30mg
15mg
Placebo

First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation

- METHODS AND RESULTS:
- Clear correlation between the partial thromboplastin time (PTT) and dose of drug (correlation coefficient, 0.725; $P<0.001$).

First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation

- METHODS AND RESULTS: randomized 85 healthy volunteers received a bolus of drug or placebo **followed 3 hours later by a bolus of antidote or placebo.**


Inject RB007
Relative APTT (to baseline)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
1
2
3
4
5
6
7
8
9
10
11
12
Time Post RB006 Administration (hrs)

First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation

- METHODS AND RESULTS: randomized 85 healthy volunteers received a bolus of drug or placebo followed 3 hours later by **a bolus of antidote** or placebo.

- **Antidote administration reversed the pharmacological activity of the drug, with a rapid (mean time, 1 to 5 minutes across all dose levels) and sustained return of activated partial thromboplastin time to within the normal range.**

CONCLUSIONI

1. Aptameri contro il FIXa sono potenti anticoagulanti

2. Oligonucleotidi complementari agli aptameri possono agire da antidoti e neutralizzare l'azione anticoagulante