

Gli APTAMERI

Aptamers

Results: 1 to 20 of 4590

<< First < Prev Page 1 of 230 Next > Last >>

[Facile Characterization of Aptamer Kinetic and Equilibrium Binding Properties Using Surface](#)

1. [Plasmon Resonance.](#)

Chang AL, McKeague M, Smolke CD.

Methods Enzymol. 2014;549C:451-466. doi: 10.1016/B978-0-12-801122-5.00019-2.

PMID: 25432760 [PubMed - as supplied by publisher]

[Using sm-FRET and Denaturants to Reveal Folding Landscapes.](#)

2. [Shaw E, St-Pierre P, McCluskey K, Lafontaine DA, Penedo JC.](#)

Methods Enzymol. 2014;549C:313-341. doi: 10.1016/B978-0-12-801122-5.00014-3.

PMID: 25432755 [PubMed - as supplied by publisher]

[Cell-specific aptamers and their conjugation with nanomaterials for targeted drug delivery.](#)

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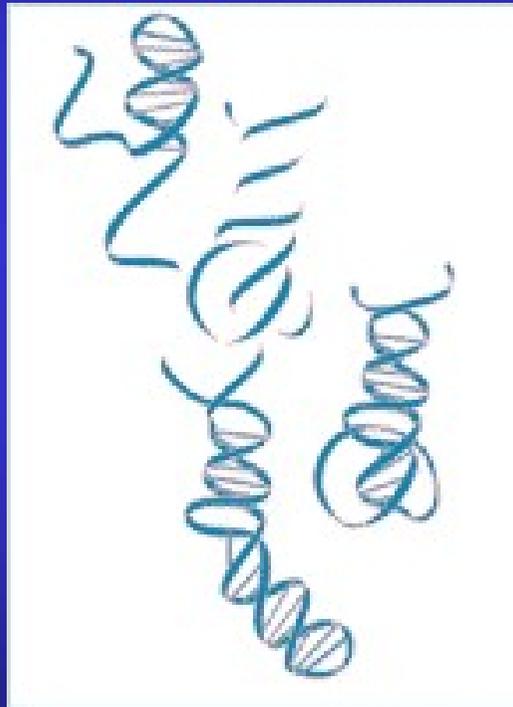
Table 1

Available aptamers currently under considerations.

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 ₂ F decoy oligonucleotides	Mesangial cells	Phase III [52, 53]
ARC1779	Vwf	Phase II [31]
NU172	Thrombin	Phase II [32]
<u>E10030</u>	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]
DNA aptamers	Phosphatidylserine (PS)	Research [11, 38] (see Section 2.4)

APTAMERI

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



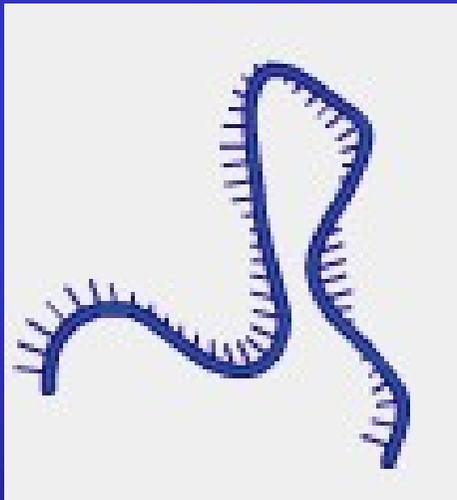
Interazione Acido Nucleico/Proteina

Aptameri

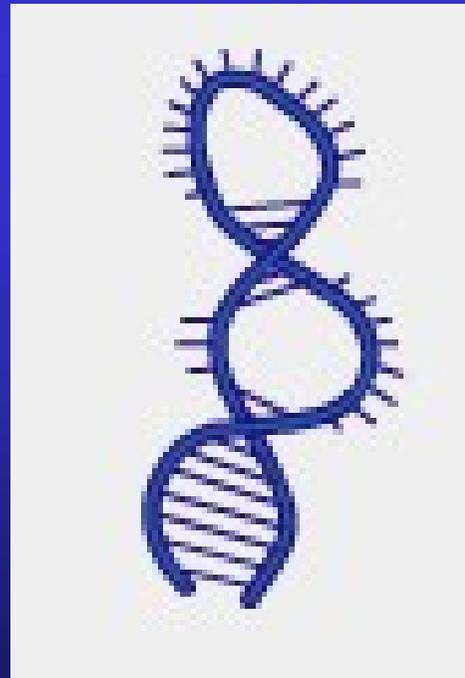
Dimensioni: 30-70 nucleotidi



Molecola Lineare

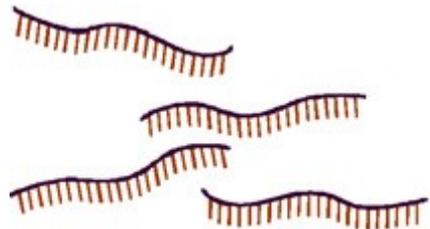


Folding



Struttura
tridimensionale
stabile

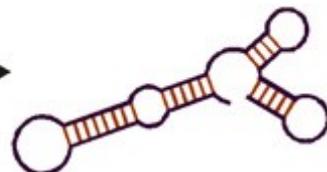
RNA oder ssDNA
(<100nt)



folding



defined
three-dimensional
structures

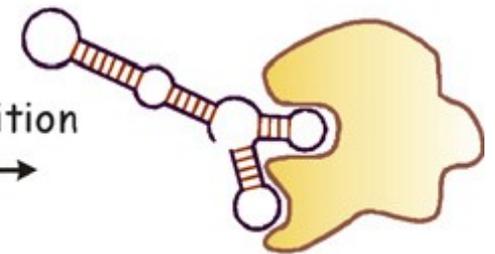


molecular recognition



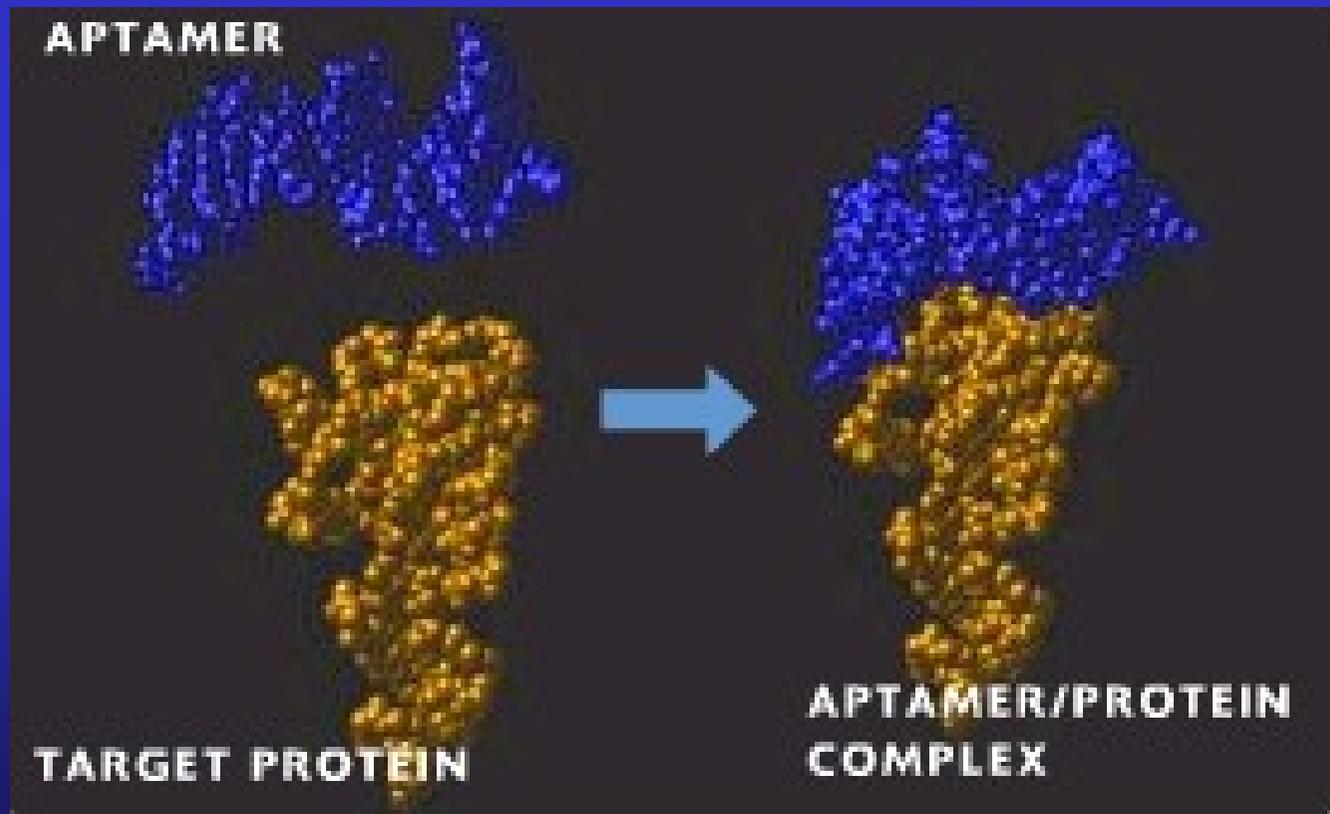
binding

aptamer-target
complex



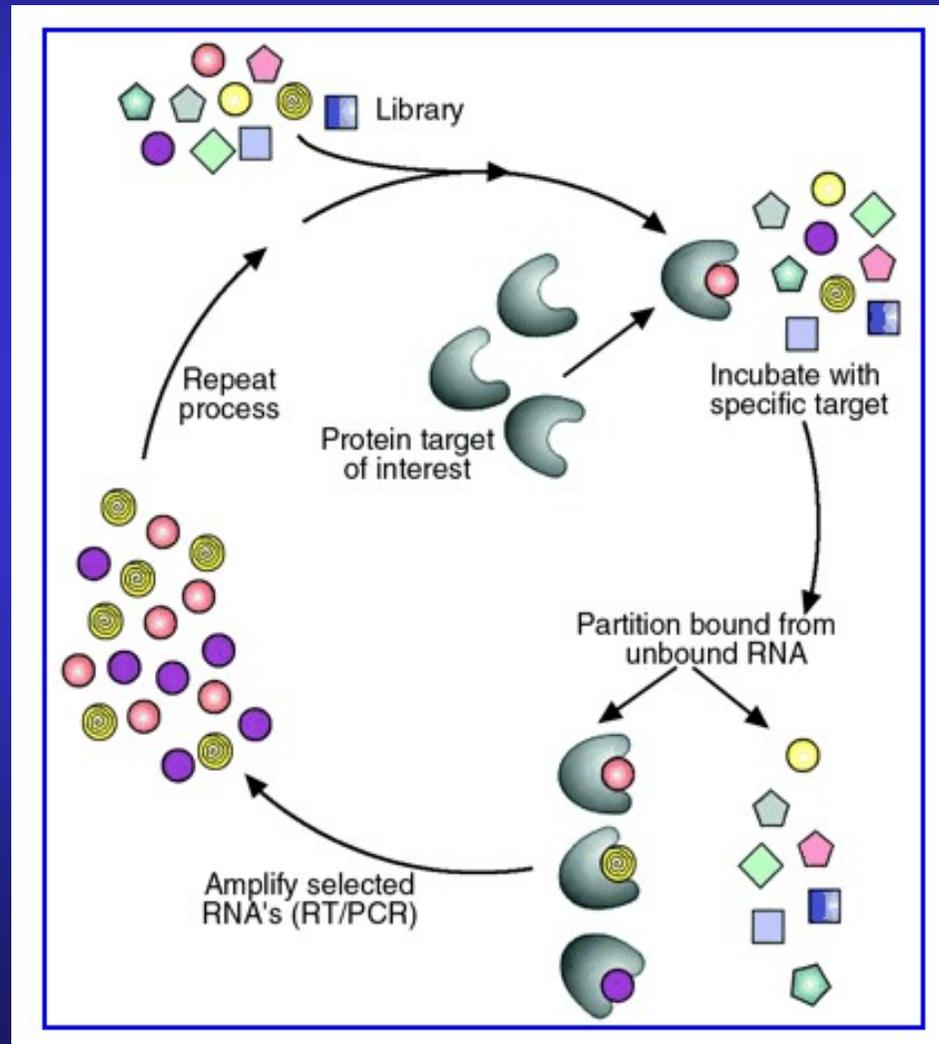
Anatomia degli Aptameri

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



Selezione in vitro degli Aptameri:

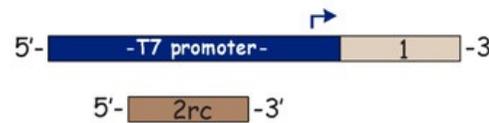
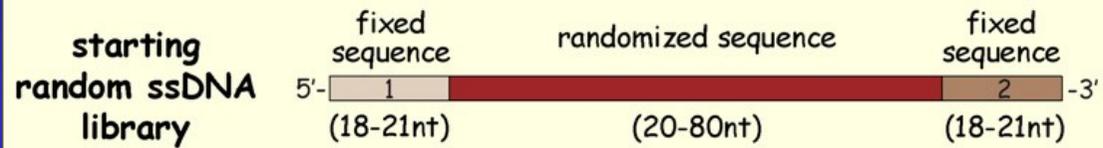
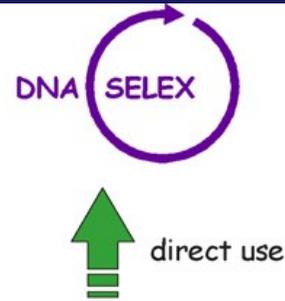
SELEX (systematic evolution of ligands by exponent enrichment)



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



PCR

dsDNA library



in vitro transcription by T7 RNA polymerase

randomized RNA library



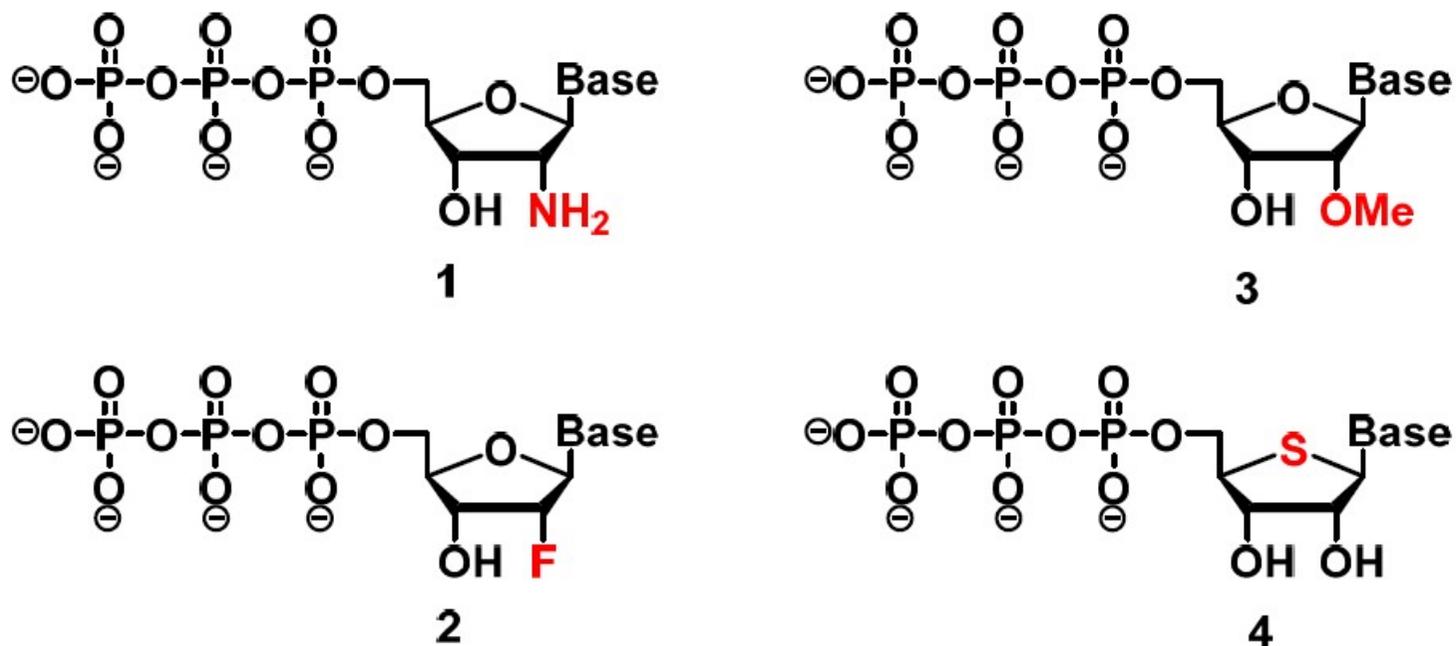


Figure 2. Chemical structures of 2'-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2'-amino-NTPs **1**, 2'-fluoro-NTPs **2**, 2'-methoxy-NTPs **3**, and 4'-thio-NTPs **4**.

Table 1. Summary of the recently generated aptamers using the 2'-fluoro modification

Aptamer Name	Aptamer Target	K_a 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 (PDGFR β)	9.6
GL21.T	Transmembrane tyrosine kinase receptor (RTK) Axl	12
G-3	C-C chemokine receptor type 5 (CCR5)	110
C26-50	<i>N</i> -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
α V-1, β 3-1	α V and β 3 subunits of integrin α V β 3	2.7, 6.5

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

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

→ Patologie virali: identificazione e inibizione di proteine virali

Gli APTAMERI: un'applicazione

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

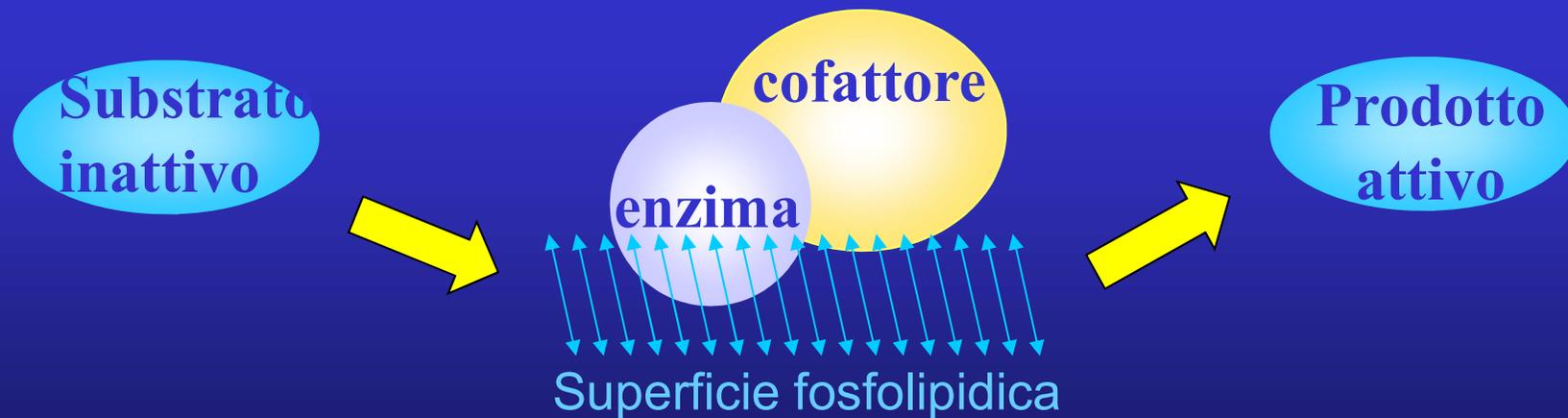
Danno vascolare



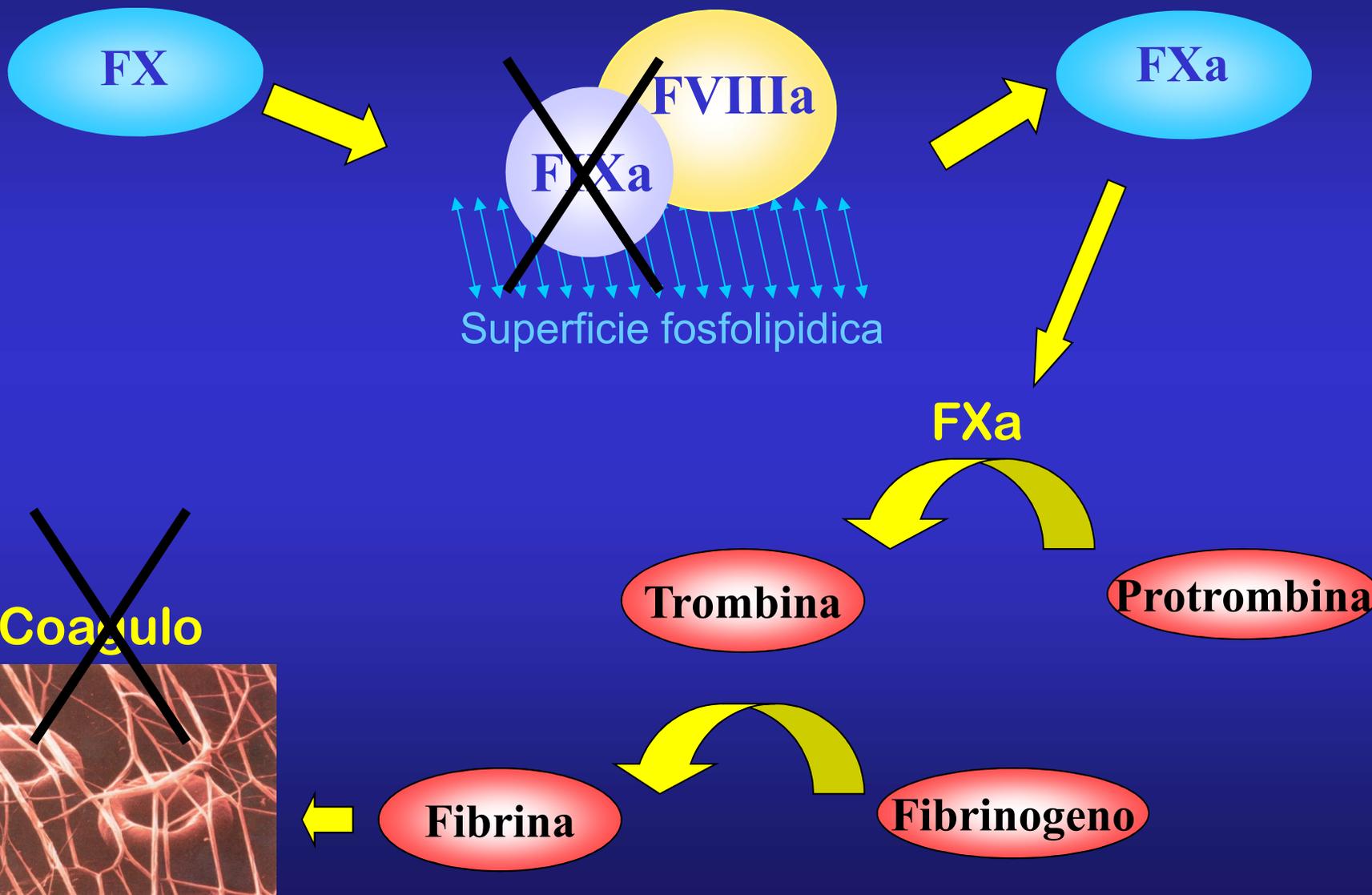
Attivazione a cascata di fattori e cofattori plasmatici



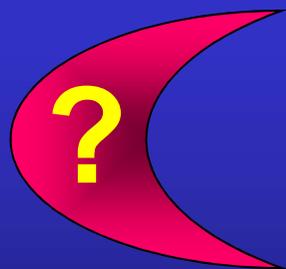
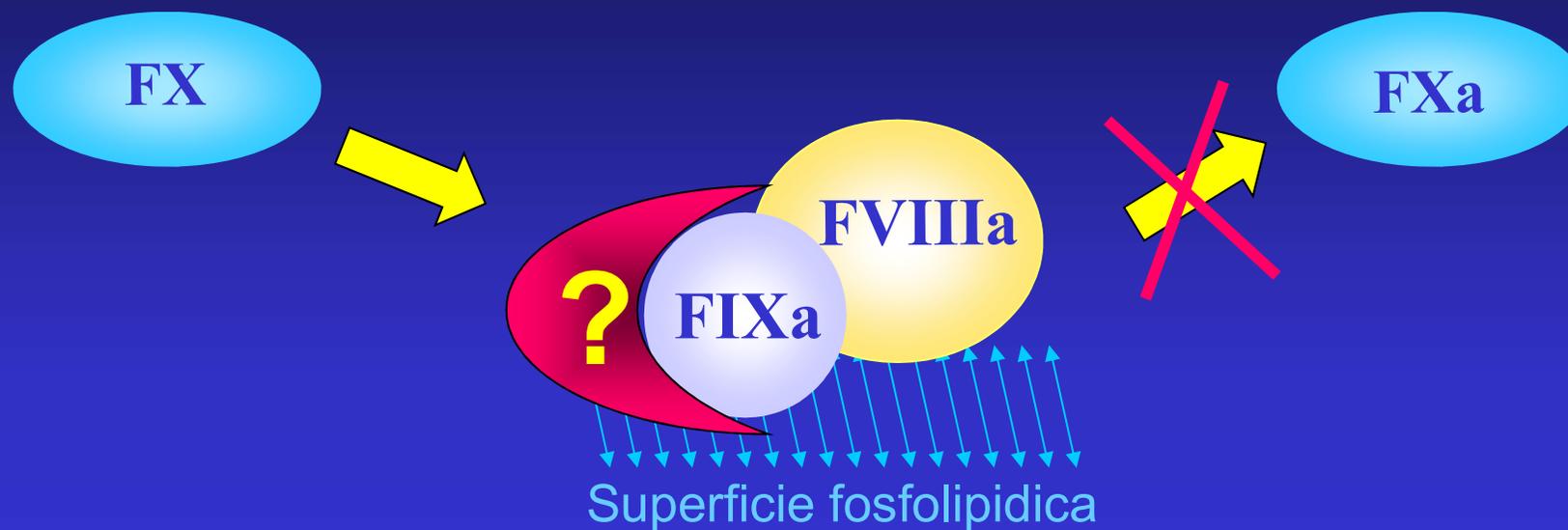
Complessi macromolecolari



Complesso di attivazione del FX



Complesso di attivazione del FX



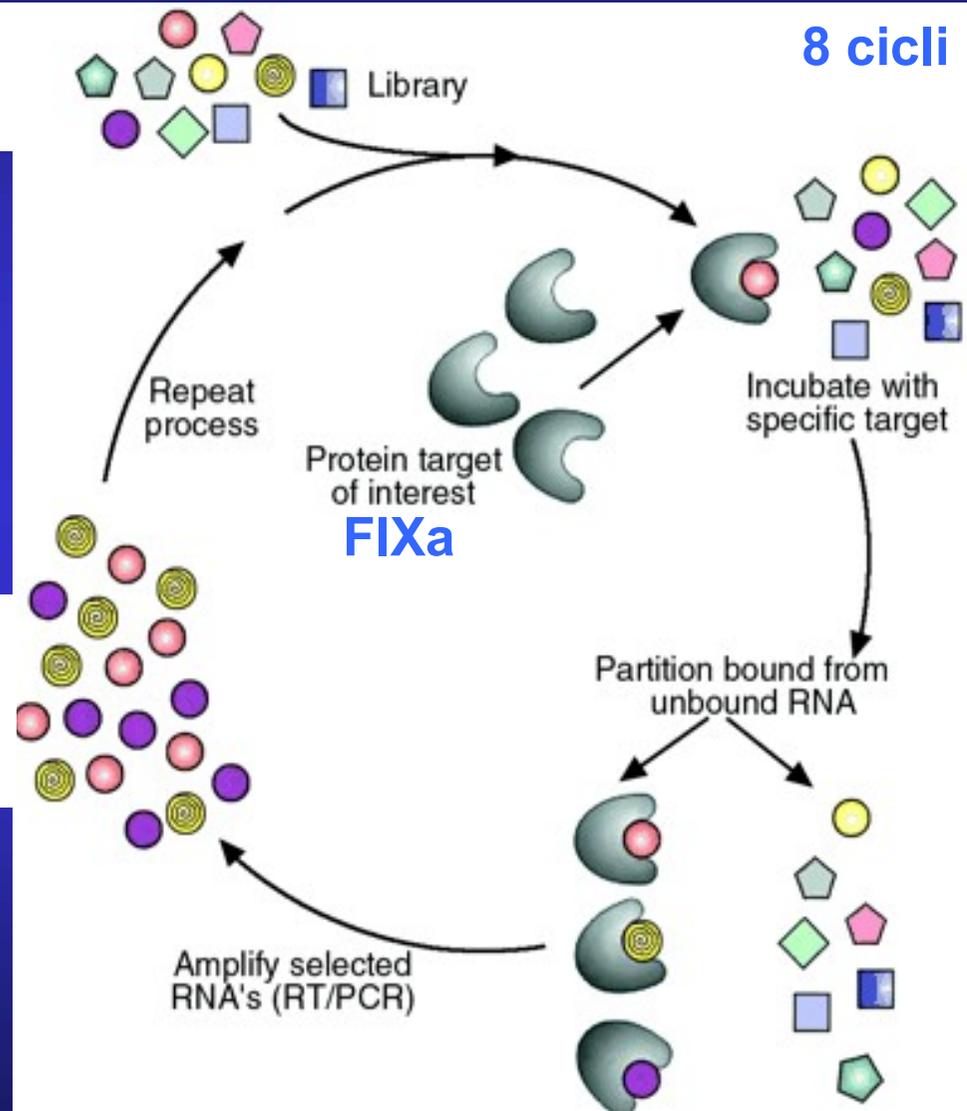
Aptamero selezionato

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

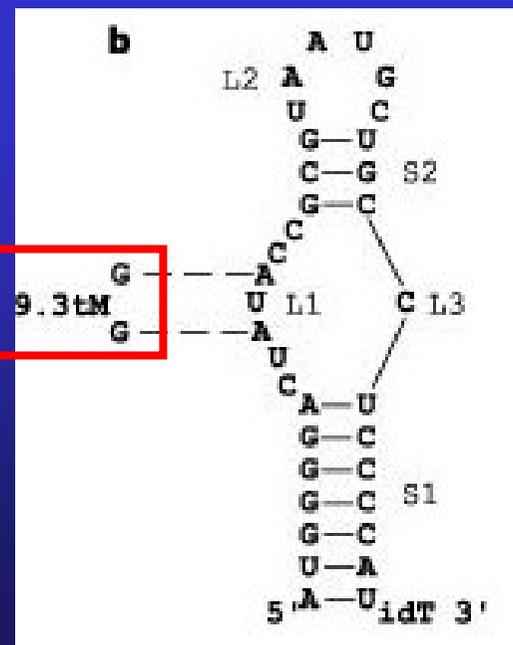


1. AFFINITA' con il FIXa

Aptamer	S1	L1	S2	L2	S2	L3	S1	
9-3	5' <u>GGGAUGGGGA</u>	CUAUACC	GCG	UAUUGC	DGC	C	UCCCCAUUCC	GGAACGCU 3'
9-20	5' <u>GGGGA</u>	CUAUACCG	GCA	AUUG	DGC	A	UCCCC	UGGACCURACAAUA 3'
9-19	5' <u>GGaUGGGGA</u>	CCAUUA	ACGA	CUAC	UGGU	GAA	UCCCCAC	AUCAGCGCNCAA 3'
9-4	5' <u>GGGAUGGGC</u>	ACUAUAC	GCA	UCU	DGC	U	GCCUGCCC	GCGAGUCARUUG 3'
9-12	5' <u>GGGaUGGG</u>	CGAUA	UAC	ACAUG	GUG	AU	CCCACCC	ACAUGAARCCACAG 3'
9-17	5' <u>GAGGgaUGGG</u>	CCAUAC	GCA	CAU	DGC	UGAA	UCCCCUC	AAUAGCACCUC 3'
9-25	5' <u>GGGAUGGGGA</u>	CCAUUA	ACUC	UAAC	GGU	GAA	UCCCgCAUCUC	GACAAUA 3'
9-26	5' <u>GGGaUGGG</u>	UGAUA	ACCA	CUC	UGGU	GAA	CCCgUCCC	GACUUGCUUGCA 3'
9-11	5' <u>GGGaUGGGGA</u>	CUAUA	UUUGG	AAU	CUGGA	C	UCCCACCU	GCCUGCCCCAGA 3'
9-2	5' <u>GGGAUGGG</u>	CUAUUA	CAC	GCUG	GUG	AU	CCCACUC	AAUUGAARCAACA 3'
9-7	5' <u>GGaUGGG</u>	CGAUA	ACCA	ACA	UGGU	GAU	CCCACUC	AUCAUACCCUACAA 3'
9-28	5' <u>GGGAUGGGCG</u>	CCAUAC	GCA	CAU	DGC	UGCAU	CGCCUCCCC	GURAGAAC 3'
9-16	5' <u>GAGGgaUGGG</u>	CCAUAC	GUGG	ACGA	CUGC	A	CCCGaCCCUU	CAGCCCAGGUC 3'
9-18	5' <u>GGGaUGGG</u>	CCAUUA	ACCA	CUU	UGGU	GAA	CCCACCC	AGCUCUGUGAUUG 3'
9-14	5' <u>GGGAUGGGGA</u>	CUAUA	CGU	GAAUG	ACU	GCA	UCCaCUUCCC	CGCCAUGG 3'
9-27	5' <u>GGGaUGGG</u>	UAUAUA	ACU	GUA	UGG	UGAA	CCCACCC	AAACUCCCCAUGGCUA 3'

Aptamero 9.3t

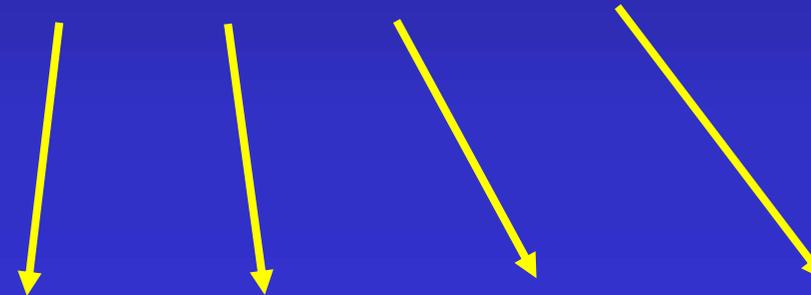
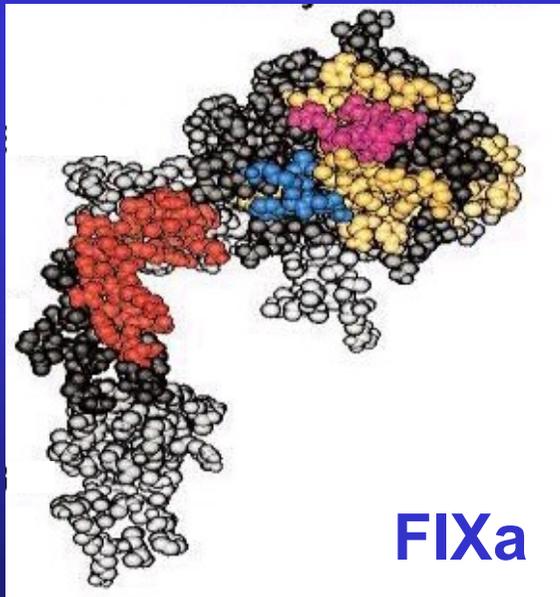
9.3 tM: controllo negativo (aptamero inattivo)



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

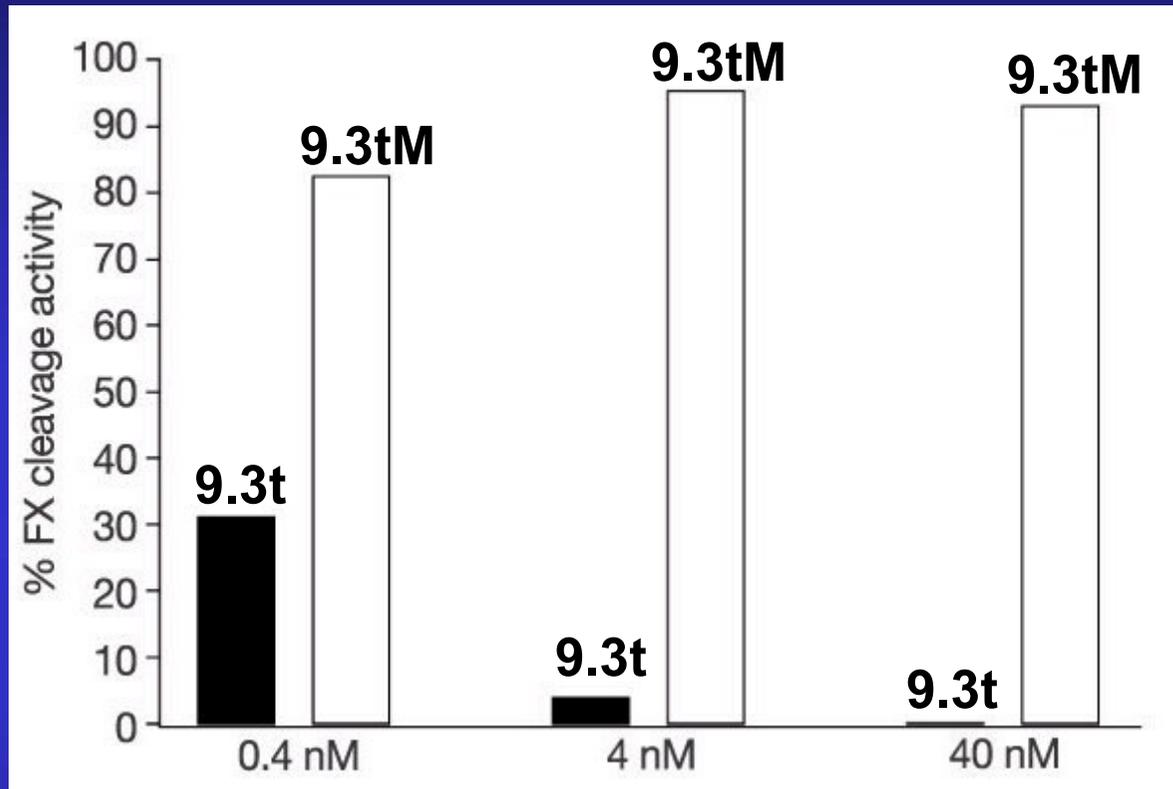
Assemblamento del complesso



Introduzione dell'aptamero 9.3t



Inibizione IN VITRO dell'attività del FIXa



9.3t: aptamero selezionato

9.3tM: controllo negativo

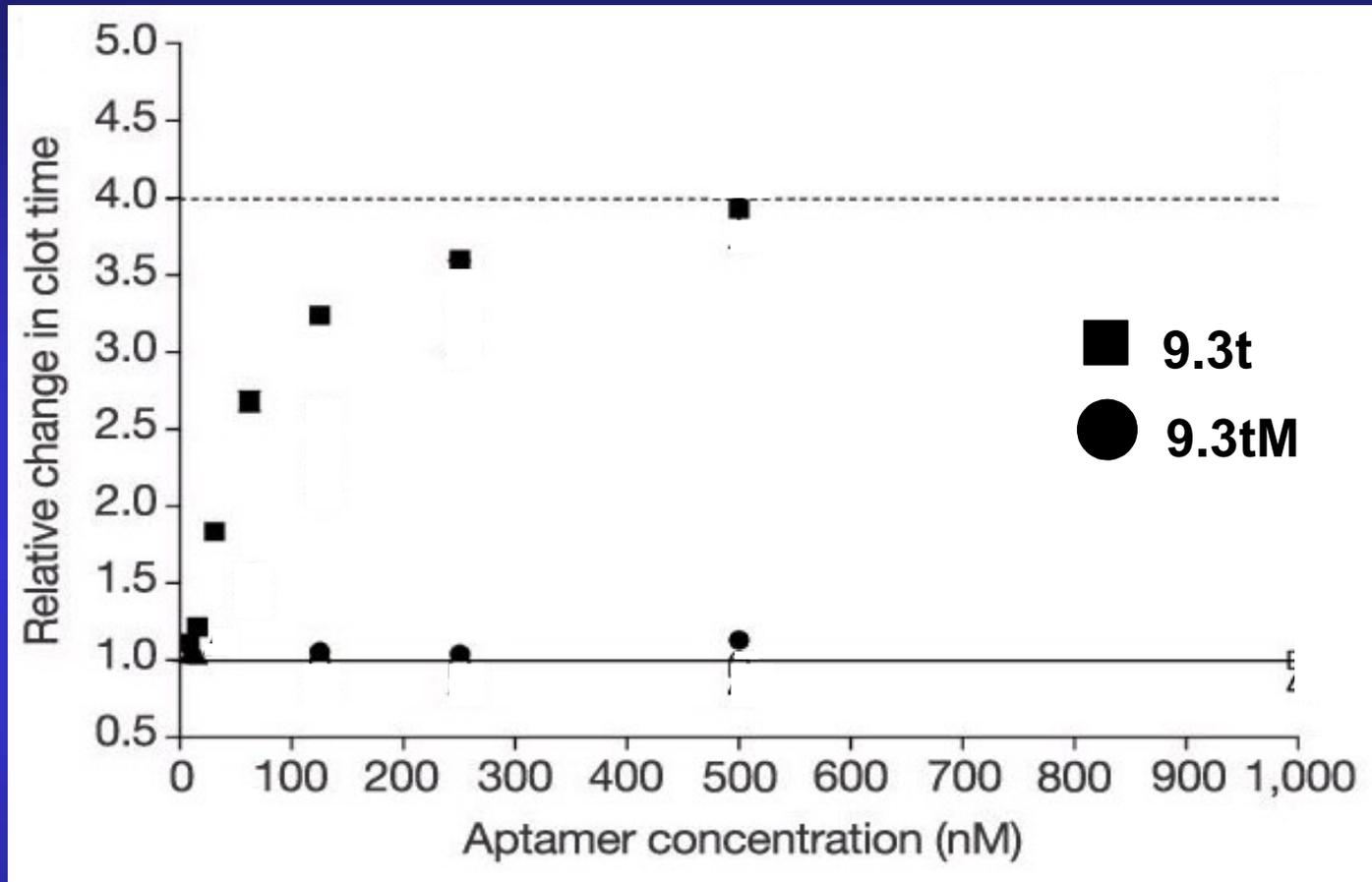
**L'aptamero blocca l'attività del FIXa
in vitro**

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

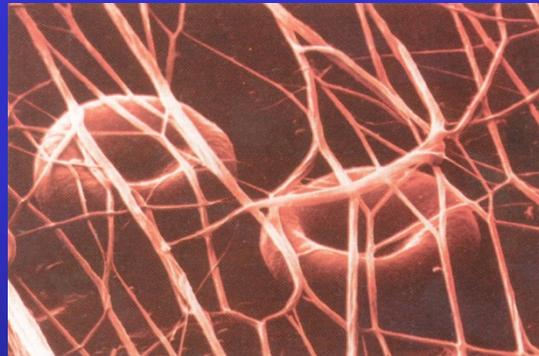
**L'aptamero inibisce l'attività del FIXa
in vivo**

Reversibilità dell'azione dell'aptamero: ANTIDOTO

Plasma + aptamero = inibizione della coagulazione

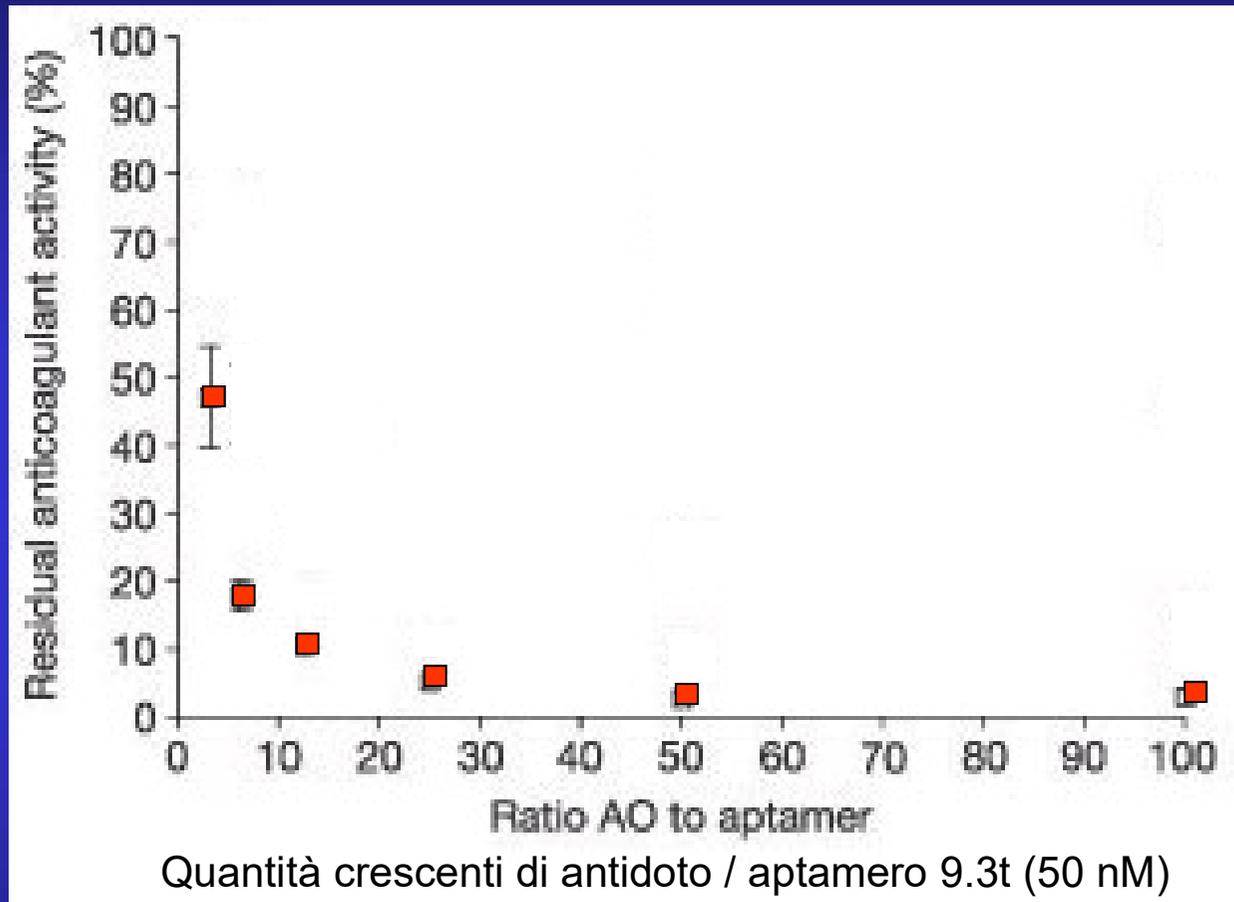


Plasma non coagulato + antidoto = coagulazione



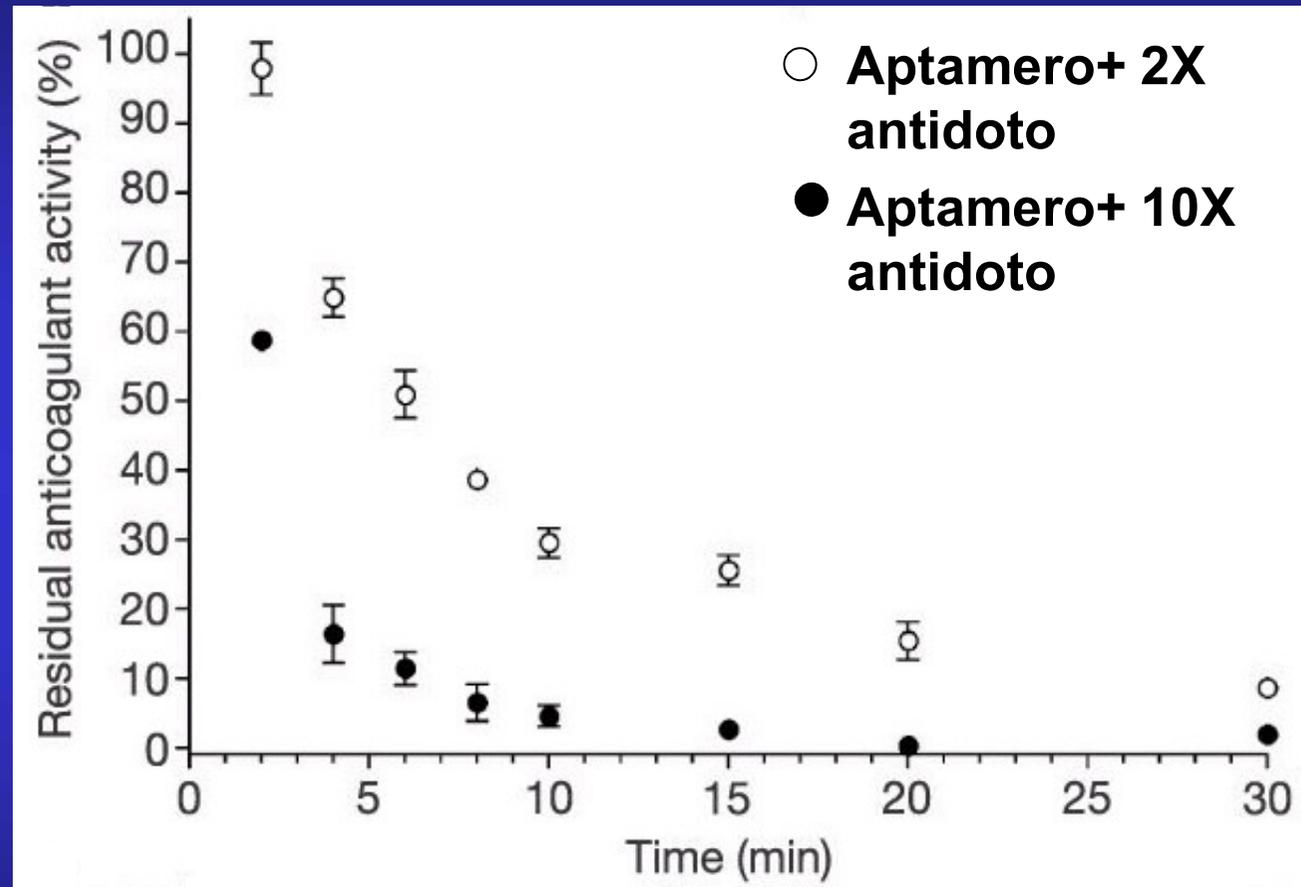
**Si misura la capacità del plasma di coagulare
entro 10 min.**

Reversibilità dell'azione dell'aptamero: ANTIDOTO



**L'antidoto neutralizza l'azione
dell'aptamero**

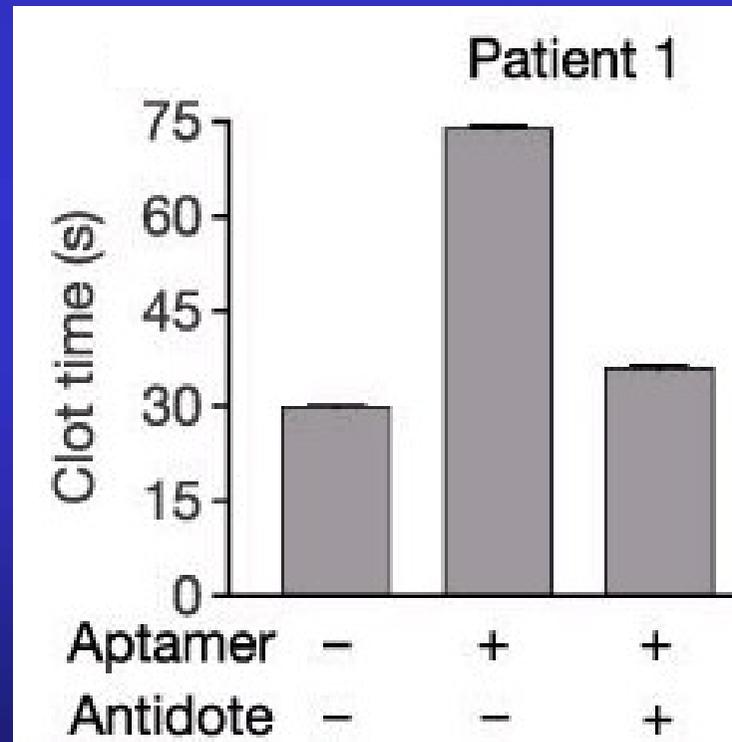
Reversibilità dell'azione dell'aptamero: ANTIDOTO

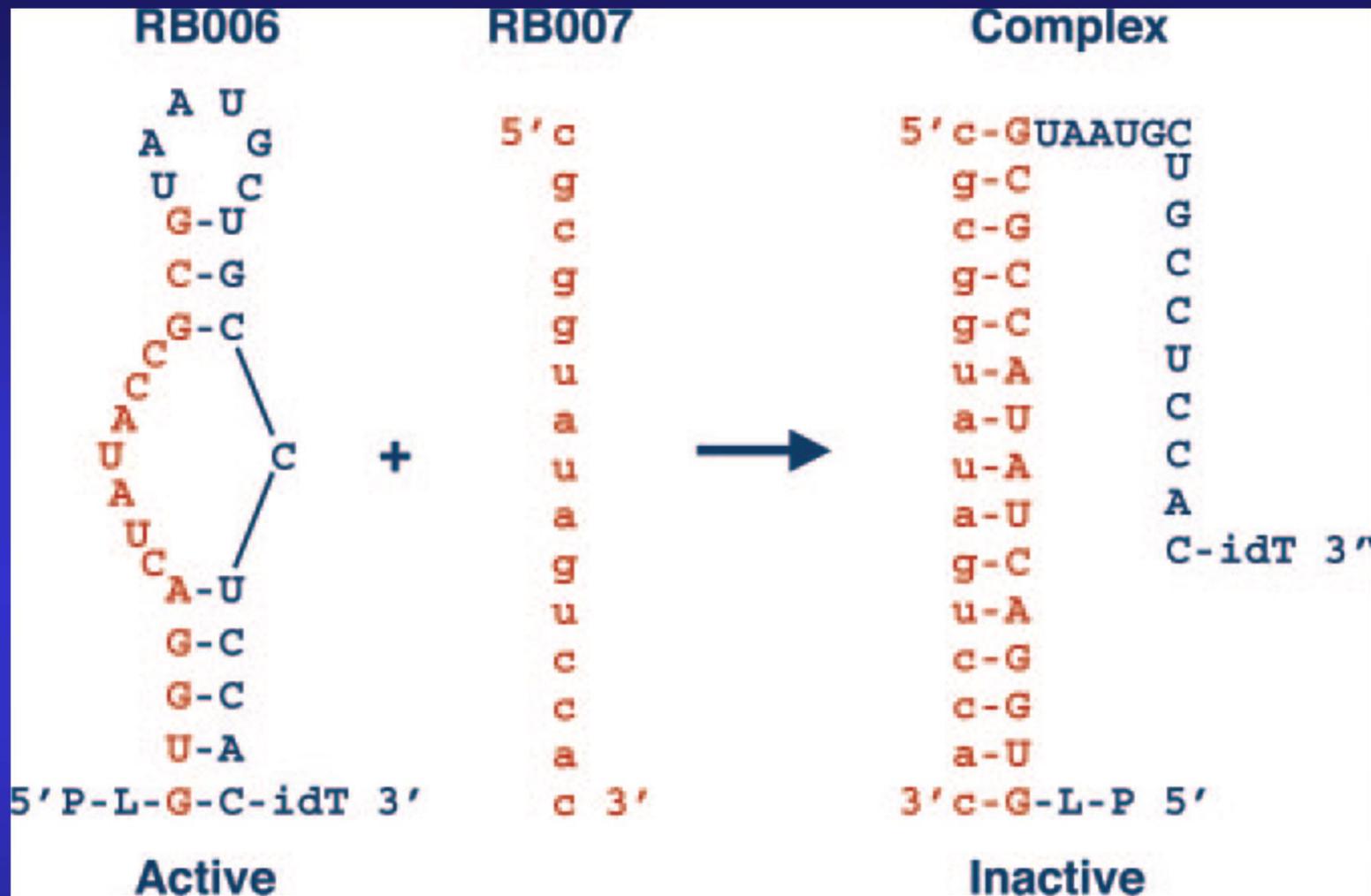


L'azione dell'antidoto è rapida e dose-dipendente

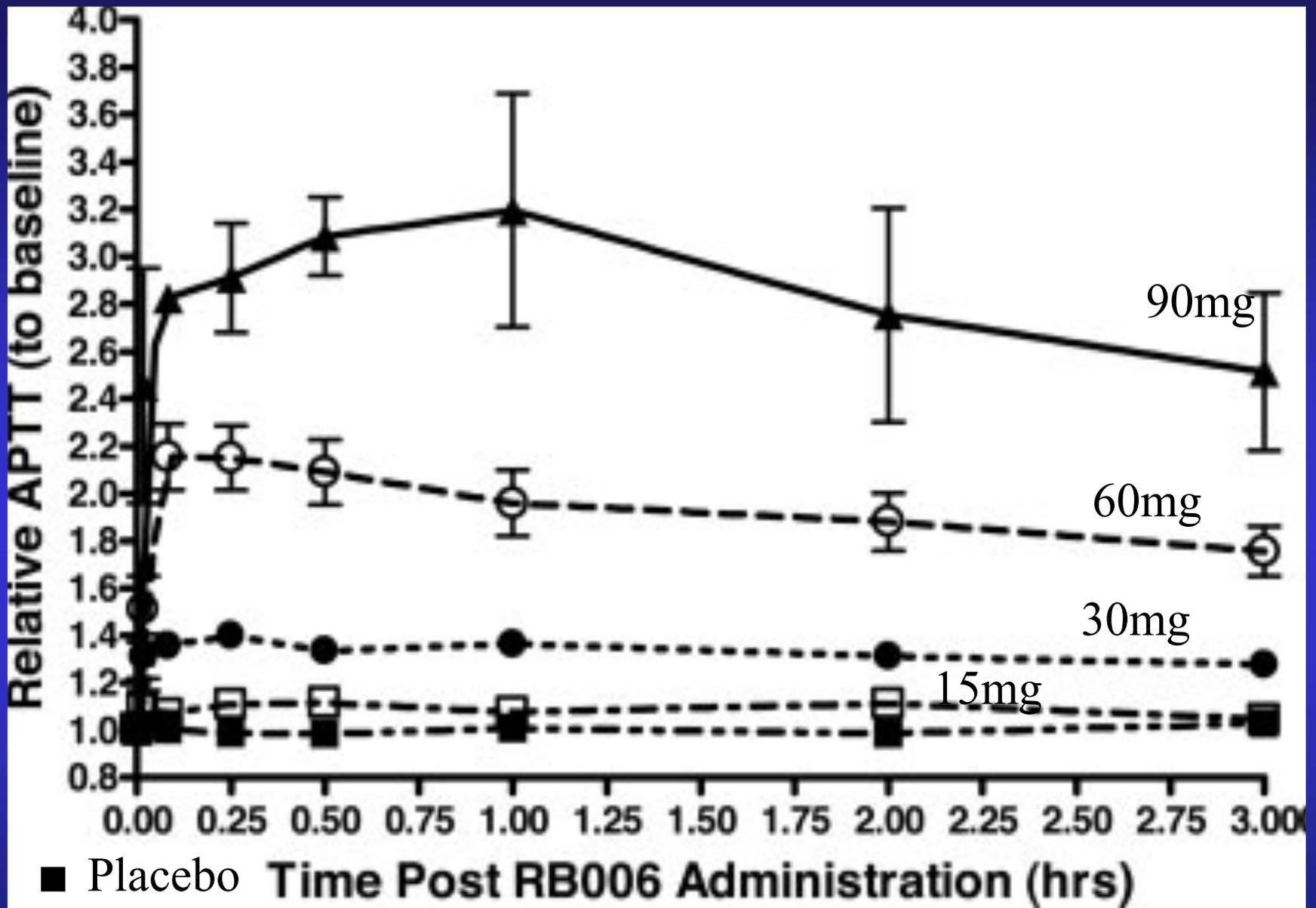
Efficiacia di aptamero e antidoto su pazienti

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





P polyethylene glycol; idT, inverted deoxythymidine



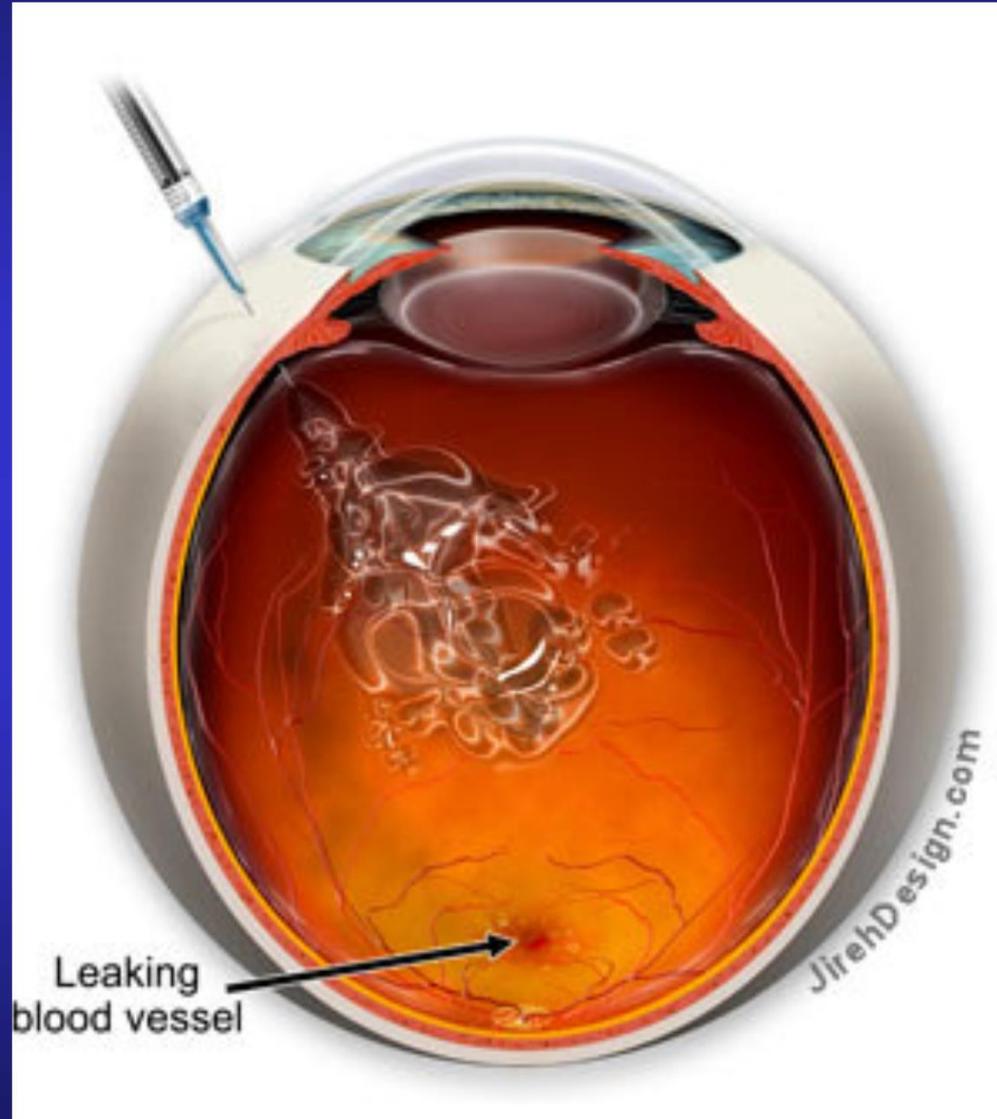
Pharmacodynamic effects of RB006 at 0 to 3 hours after RB006 administration.

The relative increase in APTT over baseline for each subject receiving RB006 before RB007 or placebo administration (all subjects assigned to arms 2 and 3) is shown vs subjects receiving placebo. Data represent the mean SEM for all subjects receiving treatment at each dose level.

CONCLUSIONI

- 1. Aptameri contro il FIXa sono potenti anticoagulanti**
- 2. Oligonucleotidi complementari agli aptameri possono agire da antidoti e neutralizzare l'azione anticoagulante**

La maculopatia senile umida è causata dalla crescita di vasi sanguigni anomali, che danneggiano l'area dell'occhio responsabile della visione centrale, che è essenziale per la maggior parte delle attività visive

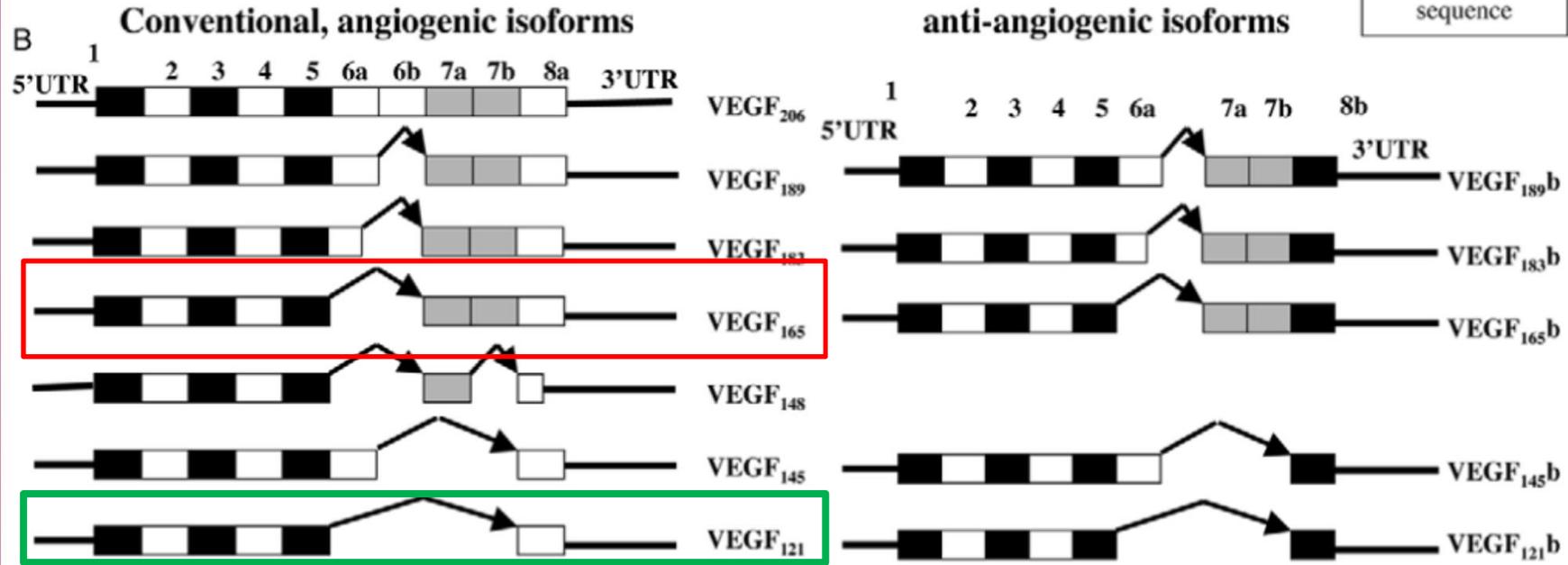
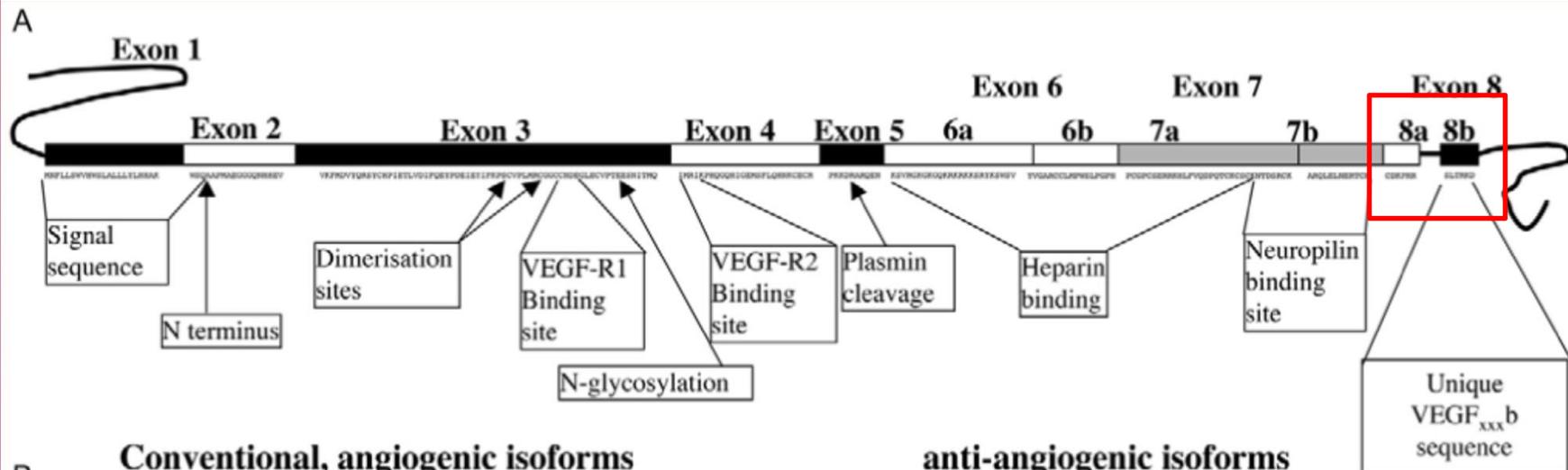


Leaking
blood vessel

JirehDesign.com

VEGF and Macula Degeneration

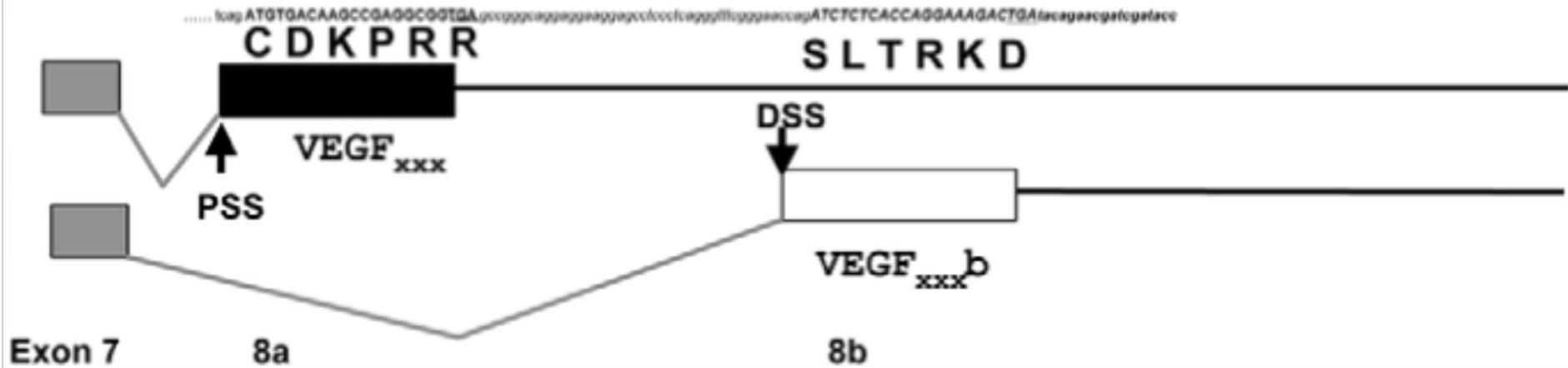
- Both clinical and preclinical findings have implicated vascular endothelial growth factor (VEGF) in the pathophysiology macular edema and degeneration.
- *VEGF is both a potent enhancer of vascular permeability and a key inducer of angiogenesis.
- *VEGF levels are elevated in the eyes of patients.
- Injection of VEGF (the VEGF165 isoform in particular) into healthy eyes of animals can induce associated ocular pathologies

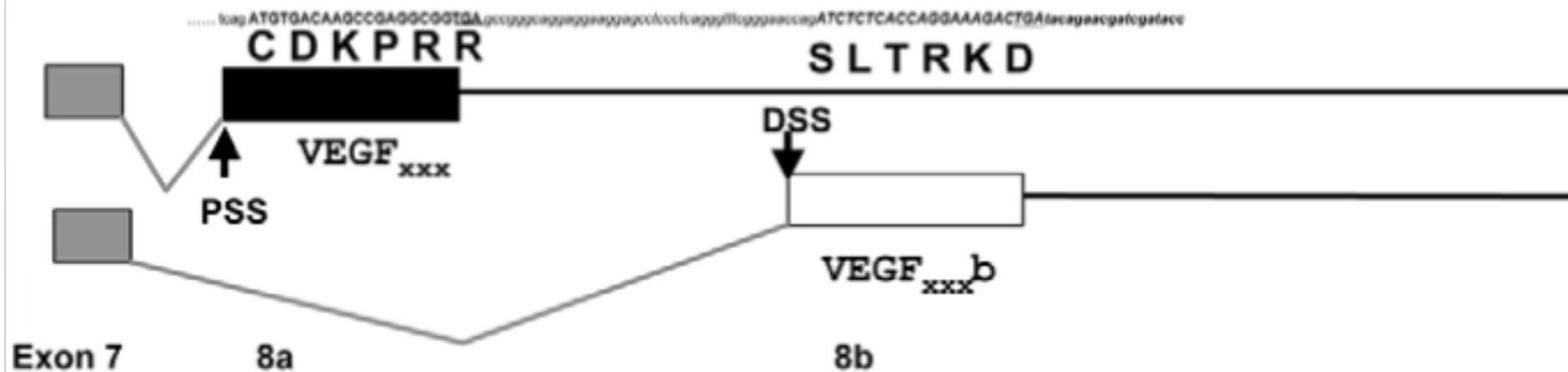
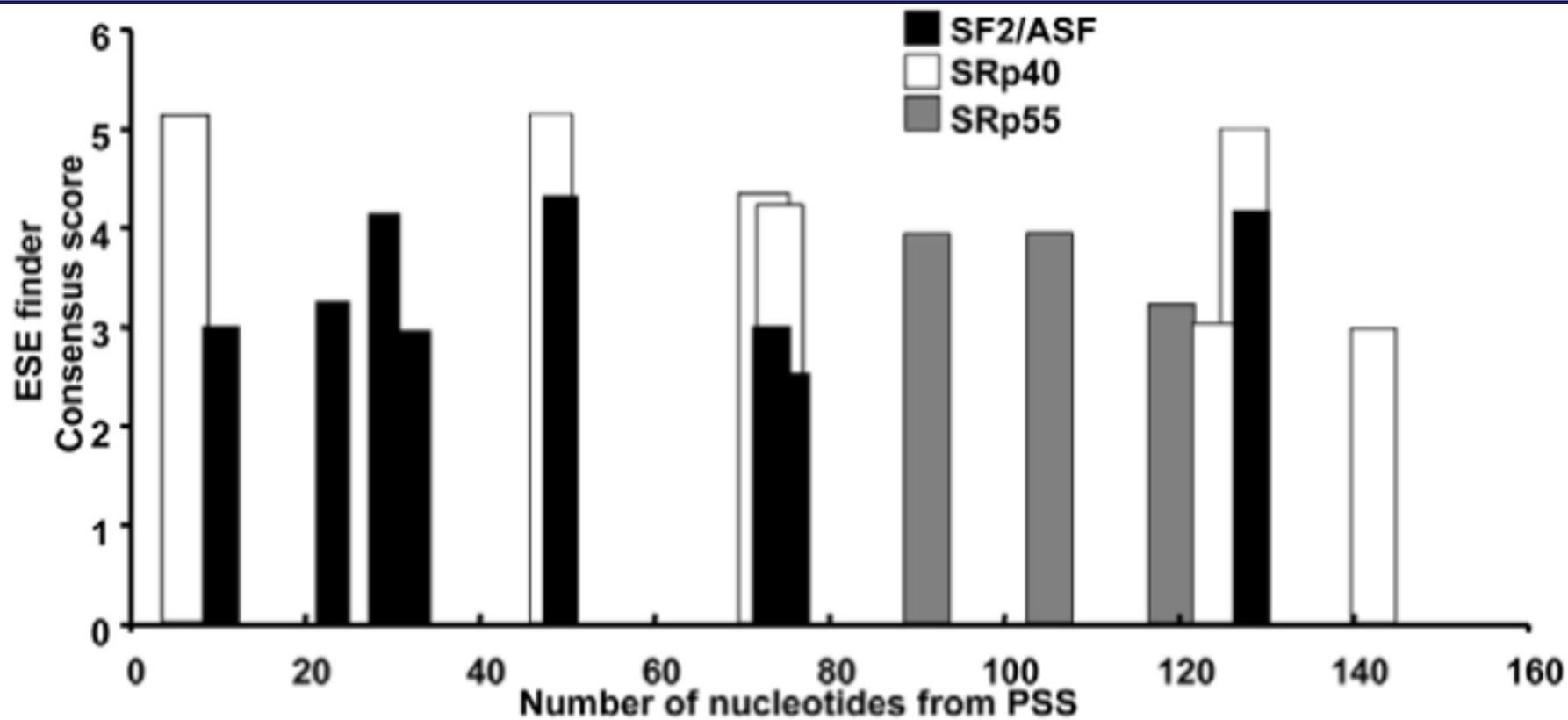


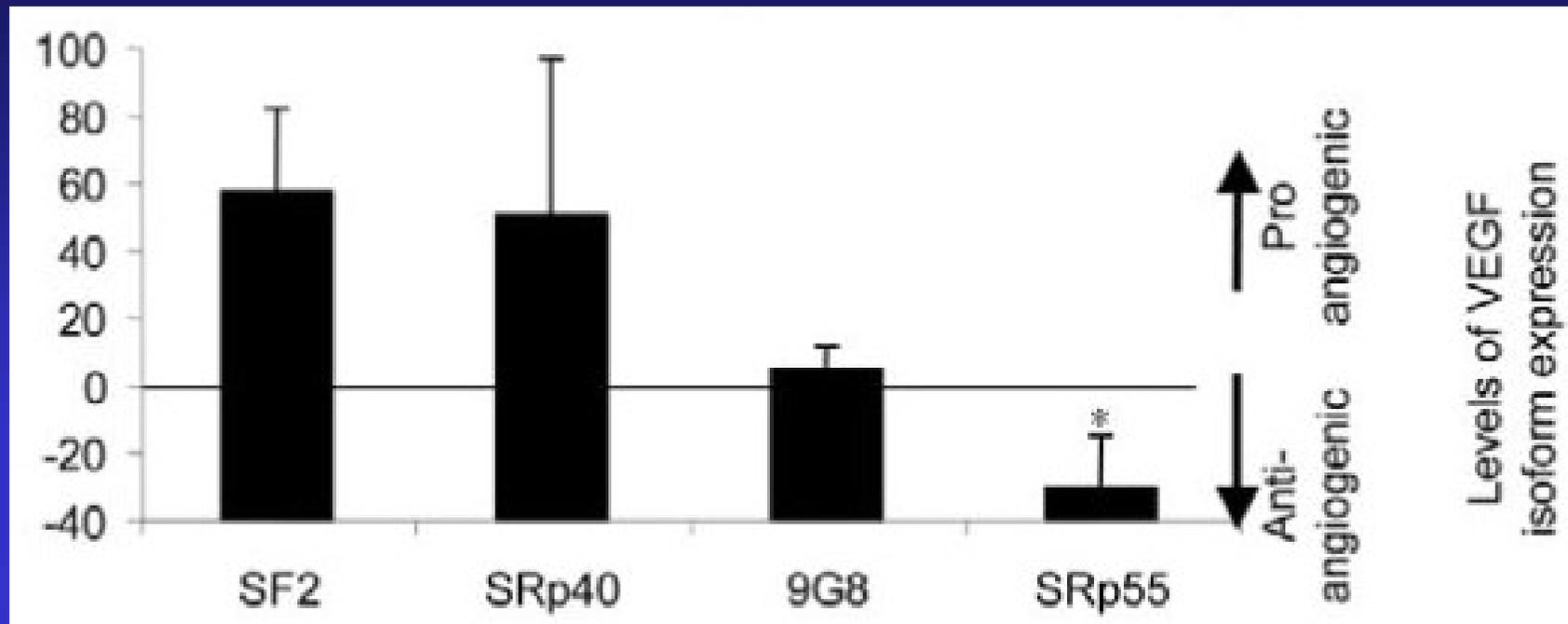
Proximal splice-site selection (PSS)

Distal splice-site selection DSS

10







Effect of overexpression of splicing factors on VEGF isoform production.

Vascular Endothelial Growth Factor and the Potential Therapeutic Use of Pegaptanib (Macugen®) in Diabetic Retinopathy

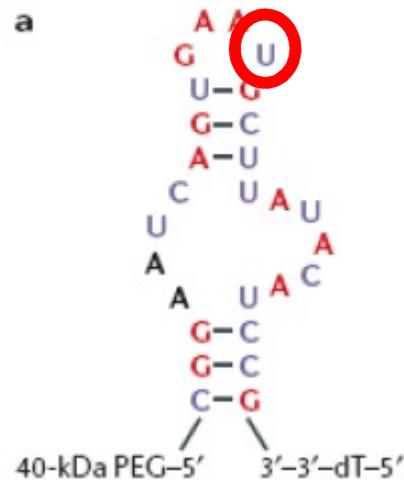
Starita C, Patel M, Katz B, Adamis A

- Pegaptanib, a novel RNA aptamer currently used in the treatment of age-related macular degeneration, binds and inactivates VEGF165 and has been shown in animal models to reverse the blood-retinal barrier breakdown.

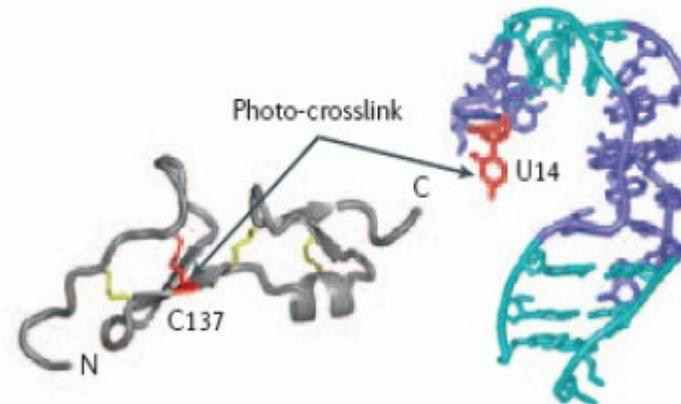
Il pegaptanib e` un antagonista selettivo del VEGF₁₆₅

Il Pegaptanib e` un aptamero a filamento singolo di **RNA** formato da 28 nucleotidi legato a 2 molecole di 20-kDa di glicole polietilenico (PEG)

E` dotato di alta affinita` per il VEGF₁₆₅ (**vascolarizzazione patologica**) e nessun legame con il VEGF₁₂₁ (**vascolarizzazione fisiologica**)

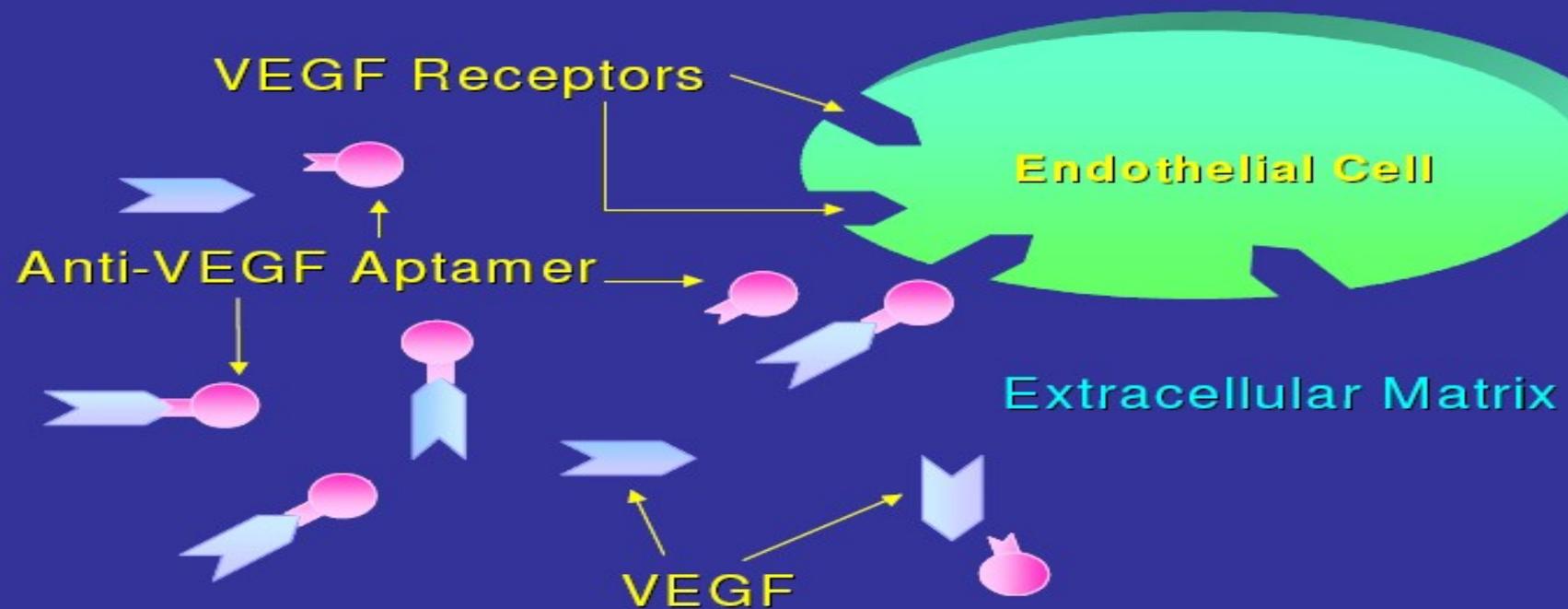


a | Sequenza e struttura secondaria del pegaptanib.



Il legame avviene tra la cisteina - 137 del VEGF₁₆₅ e l'uridina-14 dell'aptamero₁₄ (in rosso).

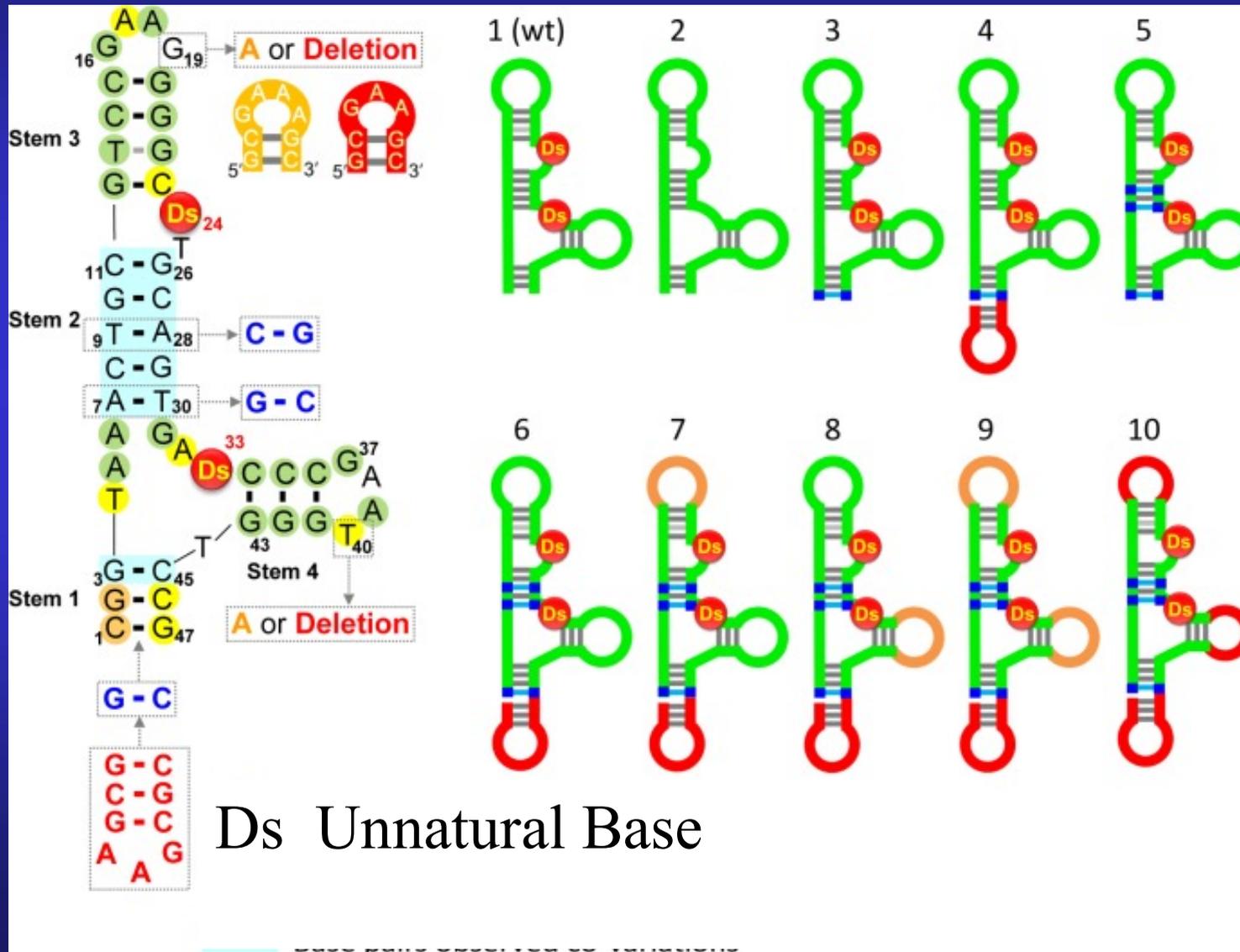
Extracellular Neutralization of VEGF



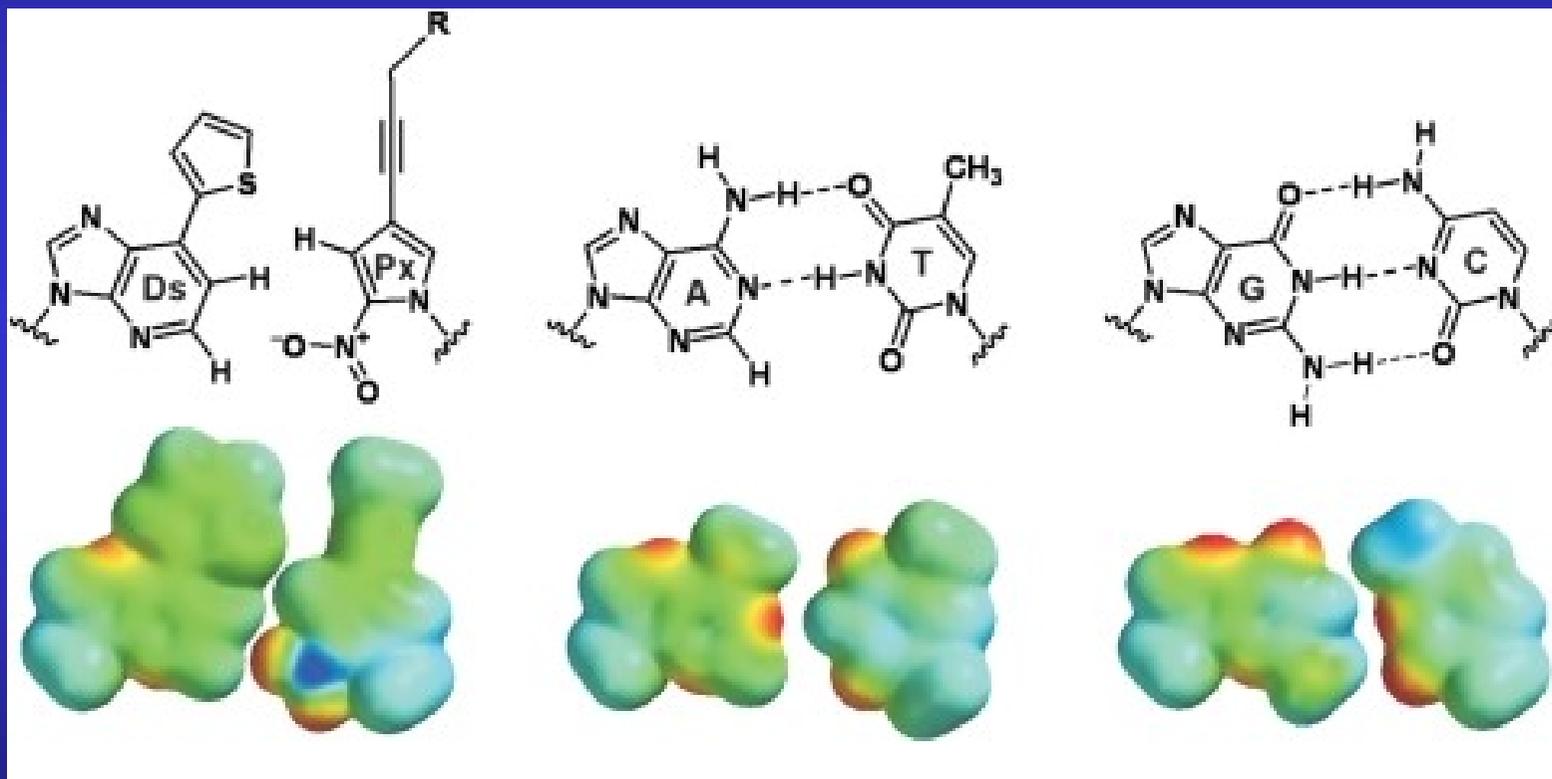
- Il Pegaptanib si lega specificamente al VEGF-165, impedendone l'aggancio con il suo recettore

EVOLUZIONE DI UN APTAMERO

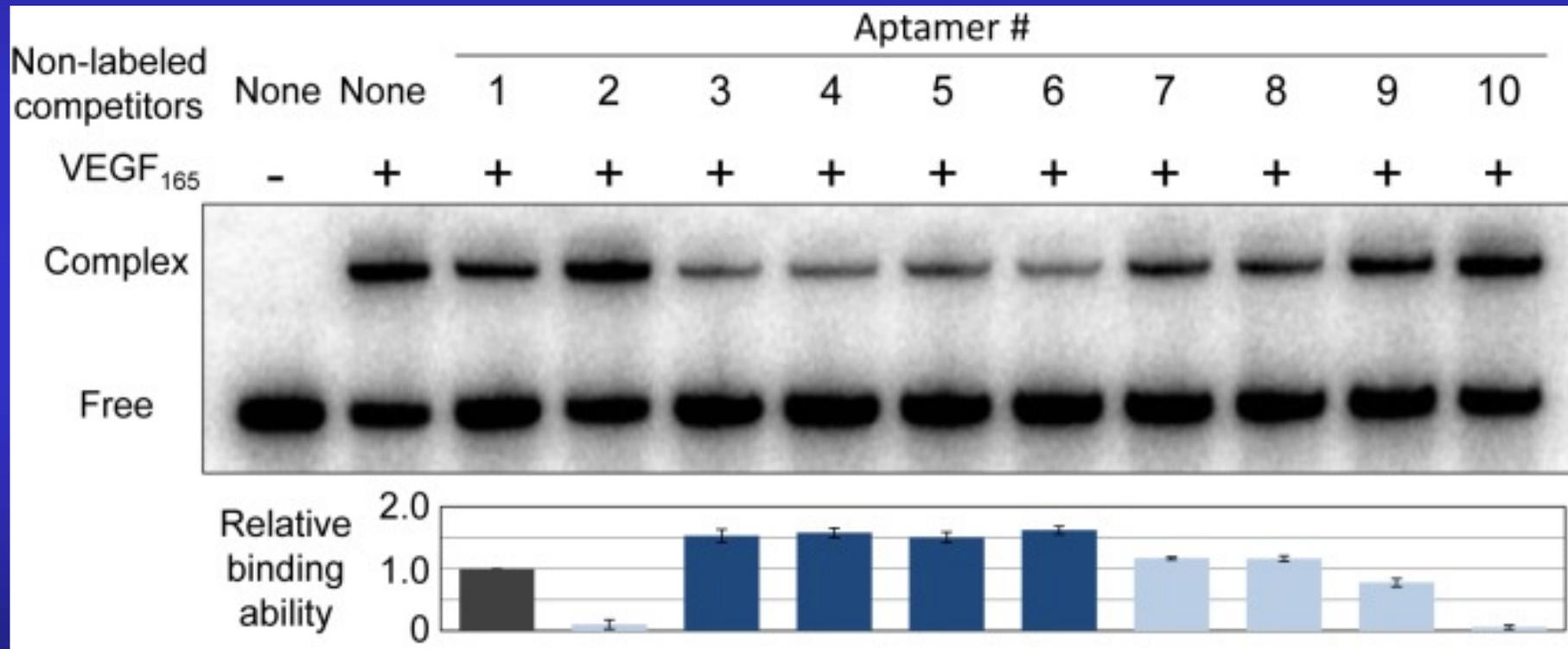
Secondary structures of the DNA anti-VEGF165 DNA aptamer (1) and its variants (2–10).



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

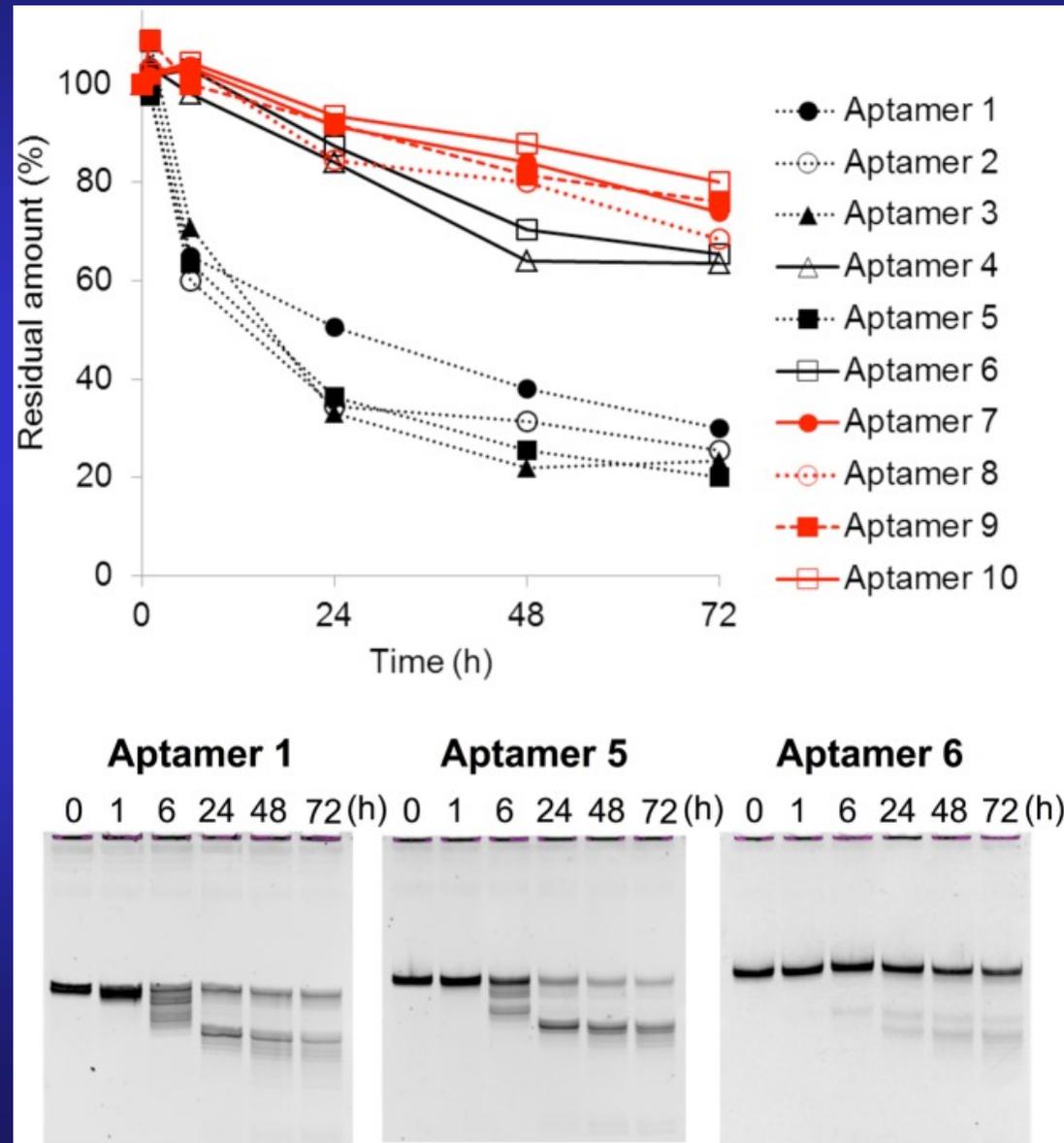


competition assay

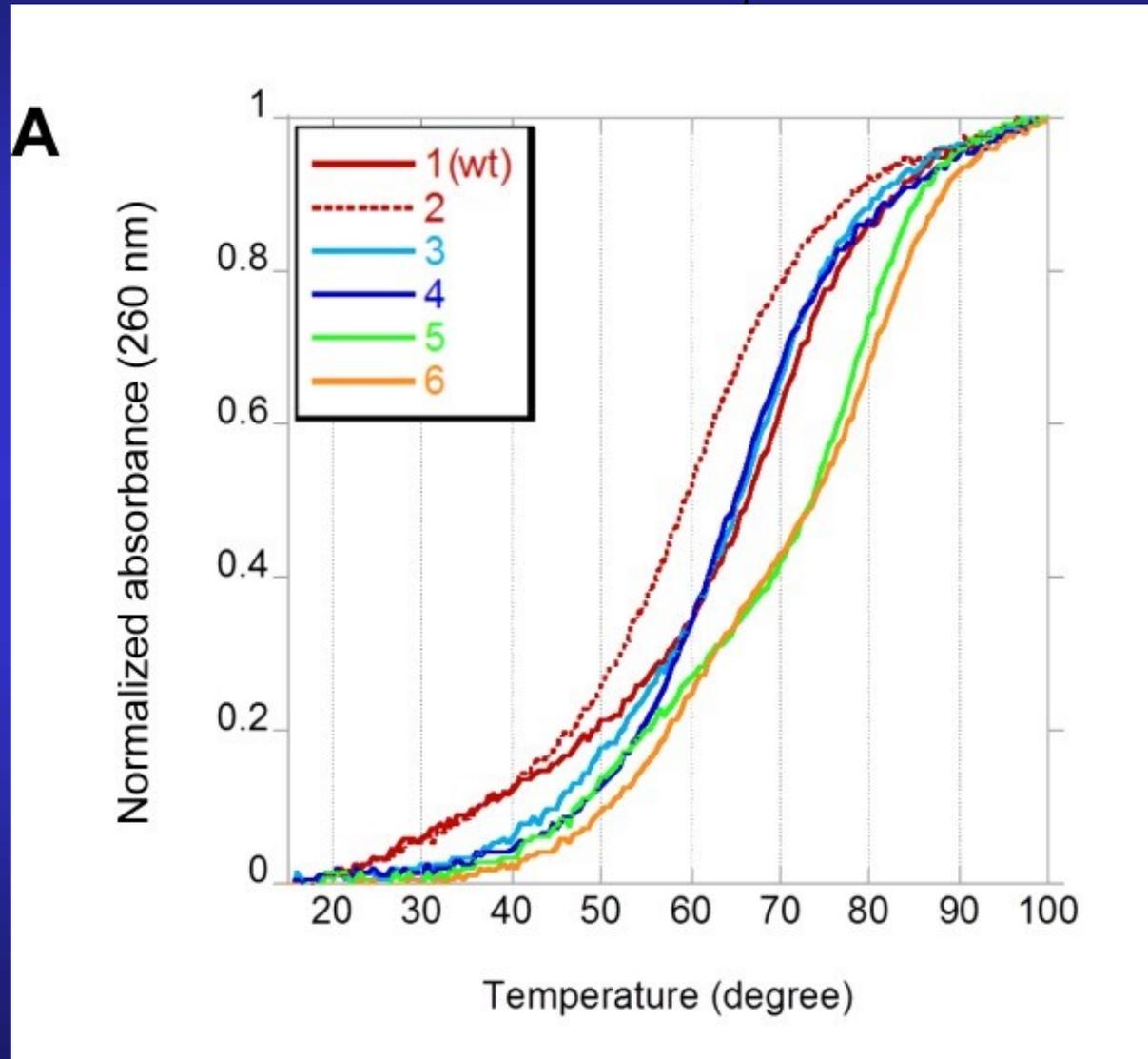


³²P-labeled Aptamer 1 (100 nM) was incubated with VEGF₁₆₅ (100 nM), in the presence of each non-labeled variant as a competitor (100 nM), at 37°C for 30 min

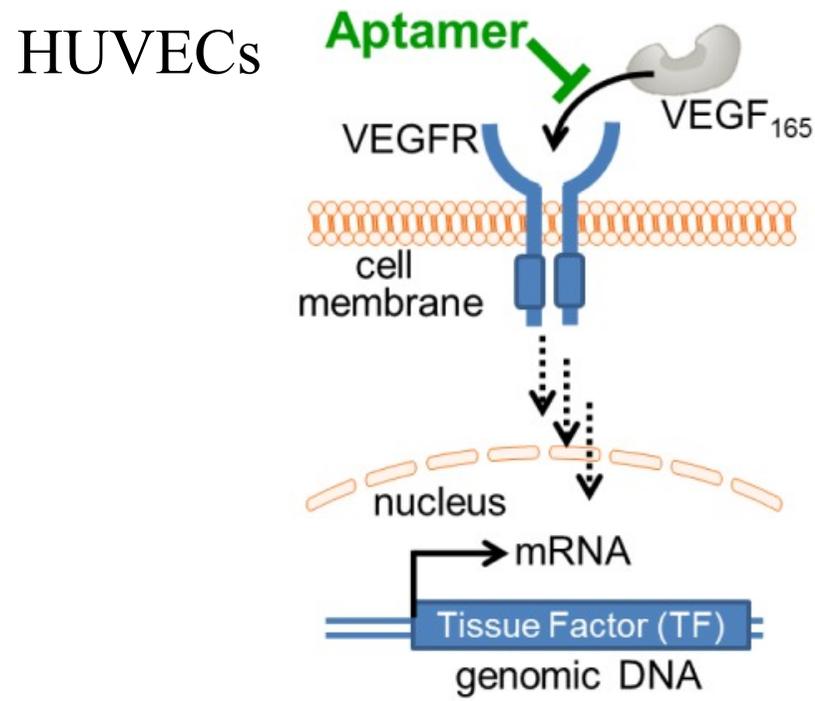
Nuclease resistance of anti-VEGF165 aptamers in human serum



Thermal stabilities of aptamers



Inhibition of the interaction between VEGF₁₆₅ and its receptor by aptamers



B

