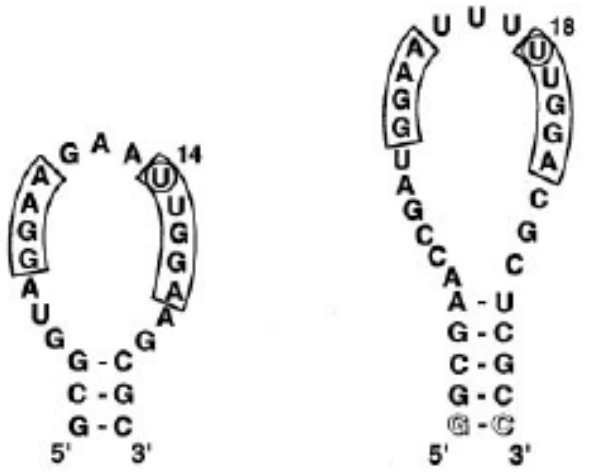
Presenters: Maggie Olson and Nick Struntz

The Discovery of Protein-Targeting Aptamers by SELEX

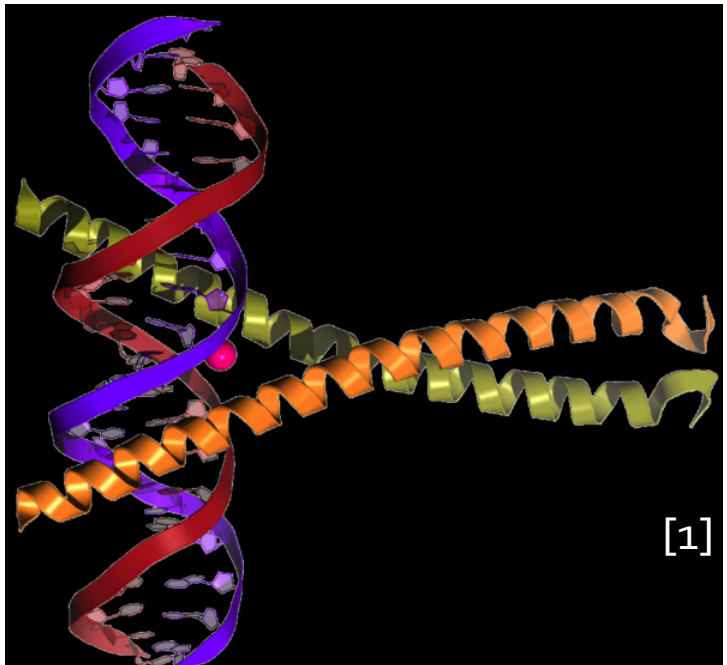
Nat. Reviews Drug Discov. **2010**, *9*,537

What is an Aptamer?

- **Precedence**: Protein – DNA interactions in nature i.e. ***Transcription Factors***



[2]



[1]

- **Characteristics of Aptamers:**
  - Double or single-strands of oligonucleotides which assume random 3D structures through base complimentarity
  - Able to bind specific molecular targets with low nanomolar – high picomolar binding affinities

SELEX Founders


[3]

Dr. Andrew Ellington
University of Texas at Austin


[4]

Dr. Jack Szostak
Massachusetts General Hospital

The RNA World

"How many fundamentally different classes of structures can carry out the same catalytic activity?"

" Did the first biological catalysts arise from random sequence polymers?"

" How many random sequence polymers fold into stable, 3D structures with catalytic activity?"

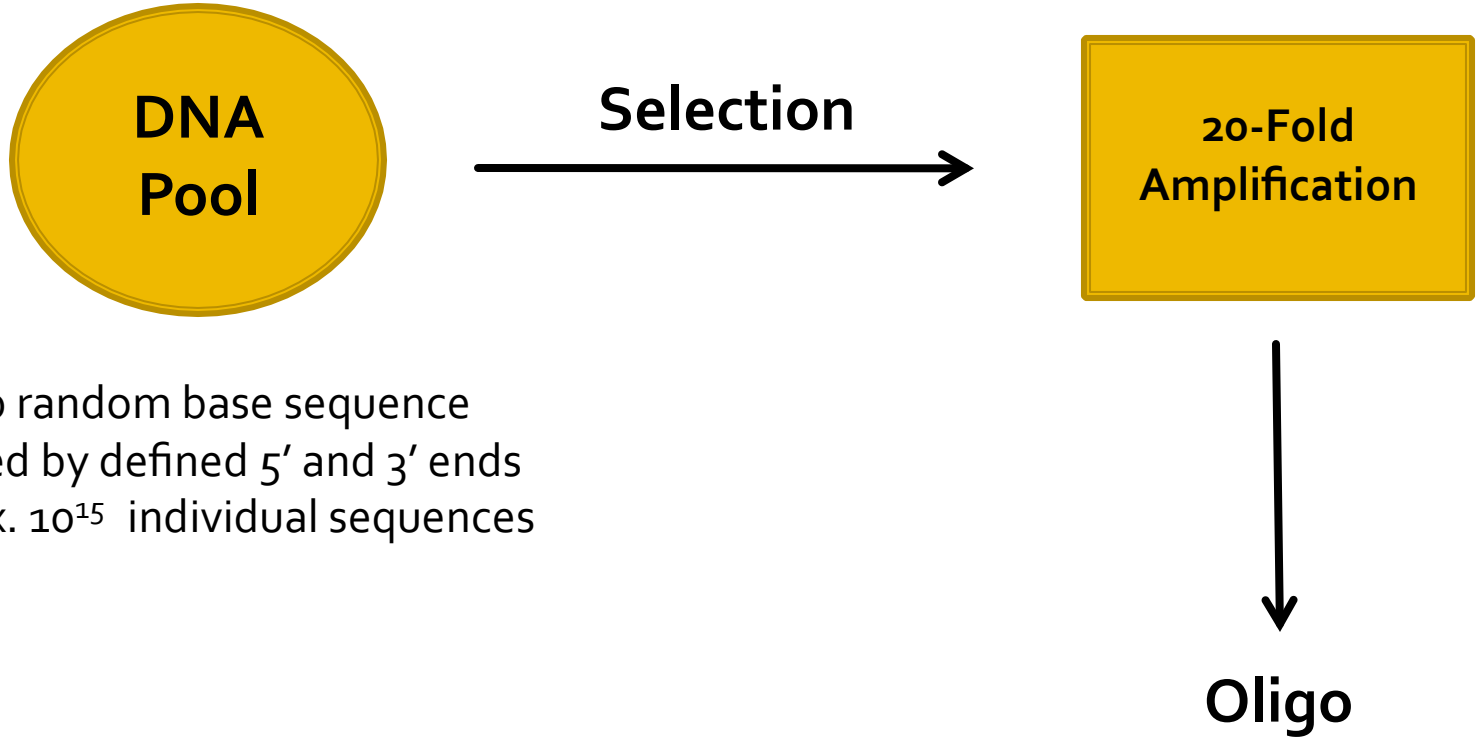
Ellington, A. et al. *Nature* **1990**, *346*, 818

In Vitro Selection

Solid-Phase Phosphoramidite

Chemical Synthesis

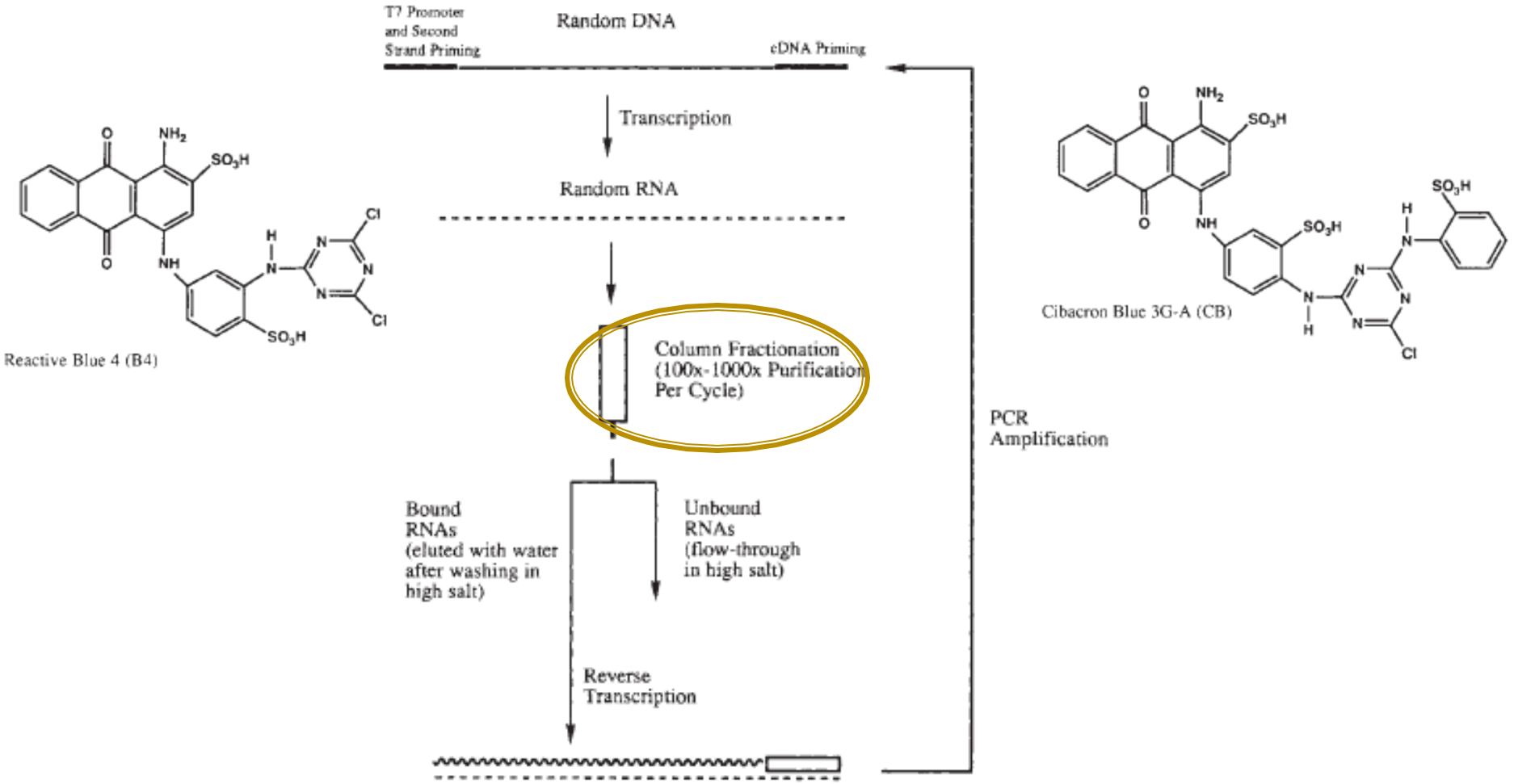
PCR



100 random base sequence
Flanked by defined 5′ and 3′ ends
Approx. 10^{15} individual sequences

Ellington, A. et al. *Nature* **1990**, *346*, 818

Ellington/Szostak Methodology



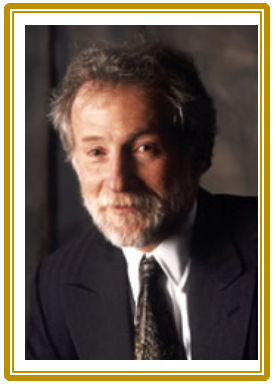
Ellington, A. et al. *Nature* **1990**, *346*, 818

Nucleic Acid Binding Proteins

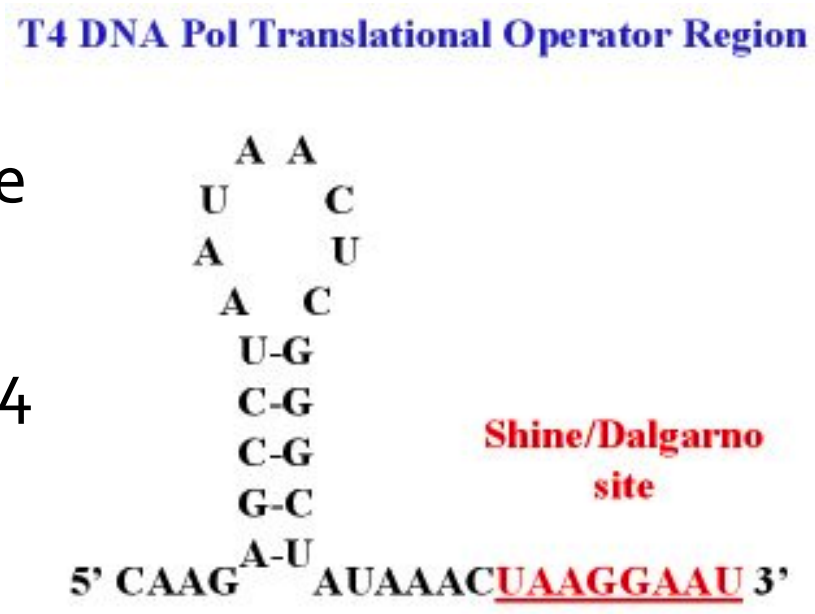
- ***Aim:***
  Elucidate "site recognition" mechanisms of nucleic acid binding proteins

- ***Previous Work:***
  Binding site comparison to determine consensus sequence

- **Target**:
  Interaction of bacteriophage T4 DNA polymerase (gp43) and mRNA ligand



Dr. Larry Gold
Colorado University - Boulder

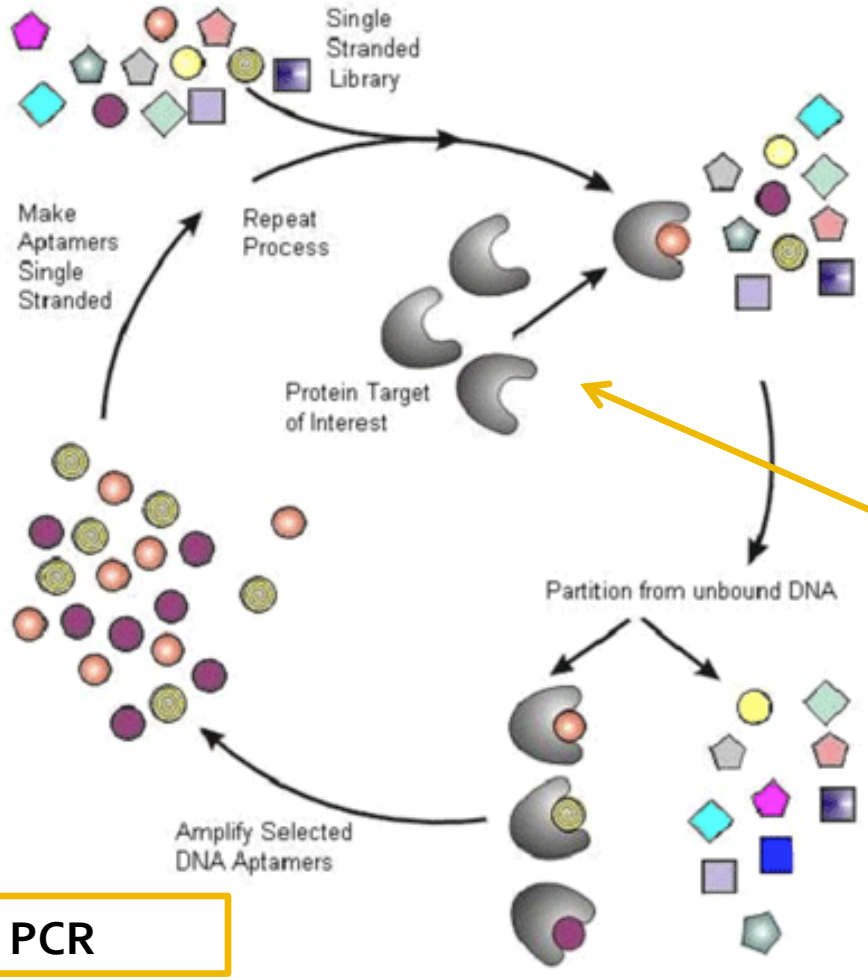


Tuerk, C. et al. *Science* **1990**, *249*, 505

Selective Evolution of Ligands by Exponential Enrichment

EVOLUTION

"Variation, Selection, Replication"



Often immobilized on nitrocellulose

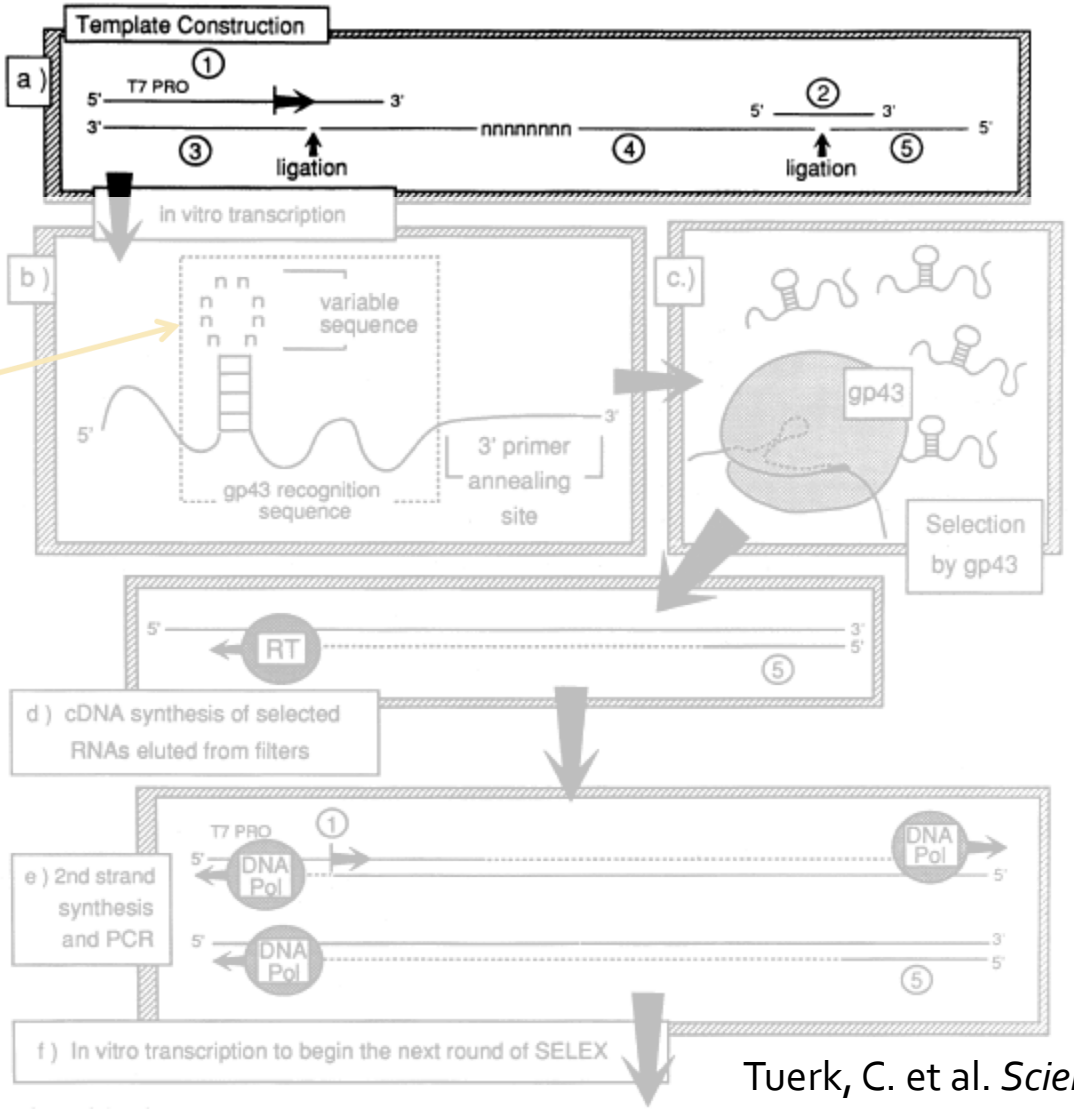
PCR

SELEX: Systematic Evolution of Ligands by Exponential Amplification
Tuerk, C. & Gold, L. (1990) Science 249, 505-510

[6]

Gold Methodology

65,536 possible sequences



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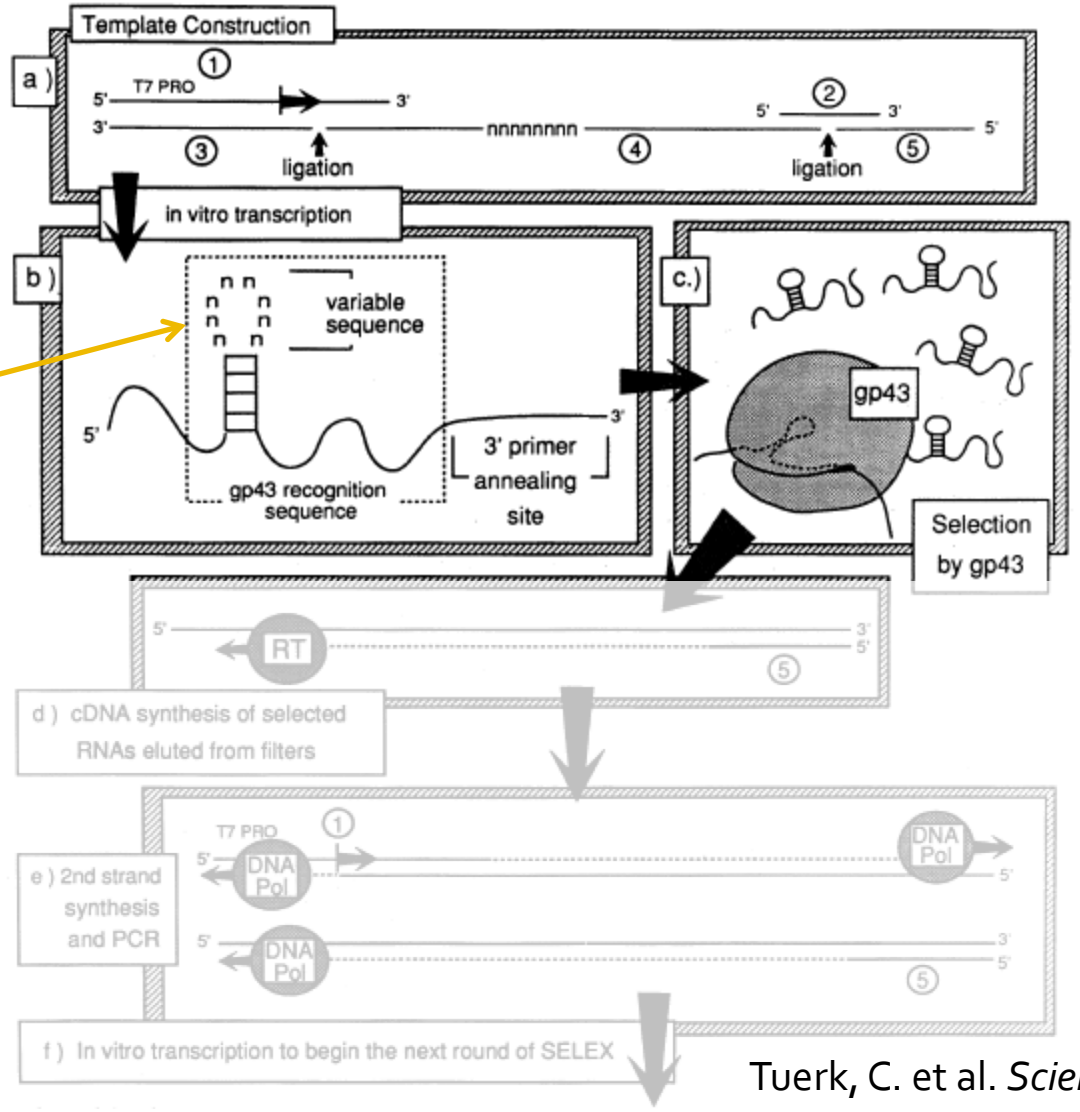

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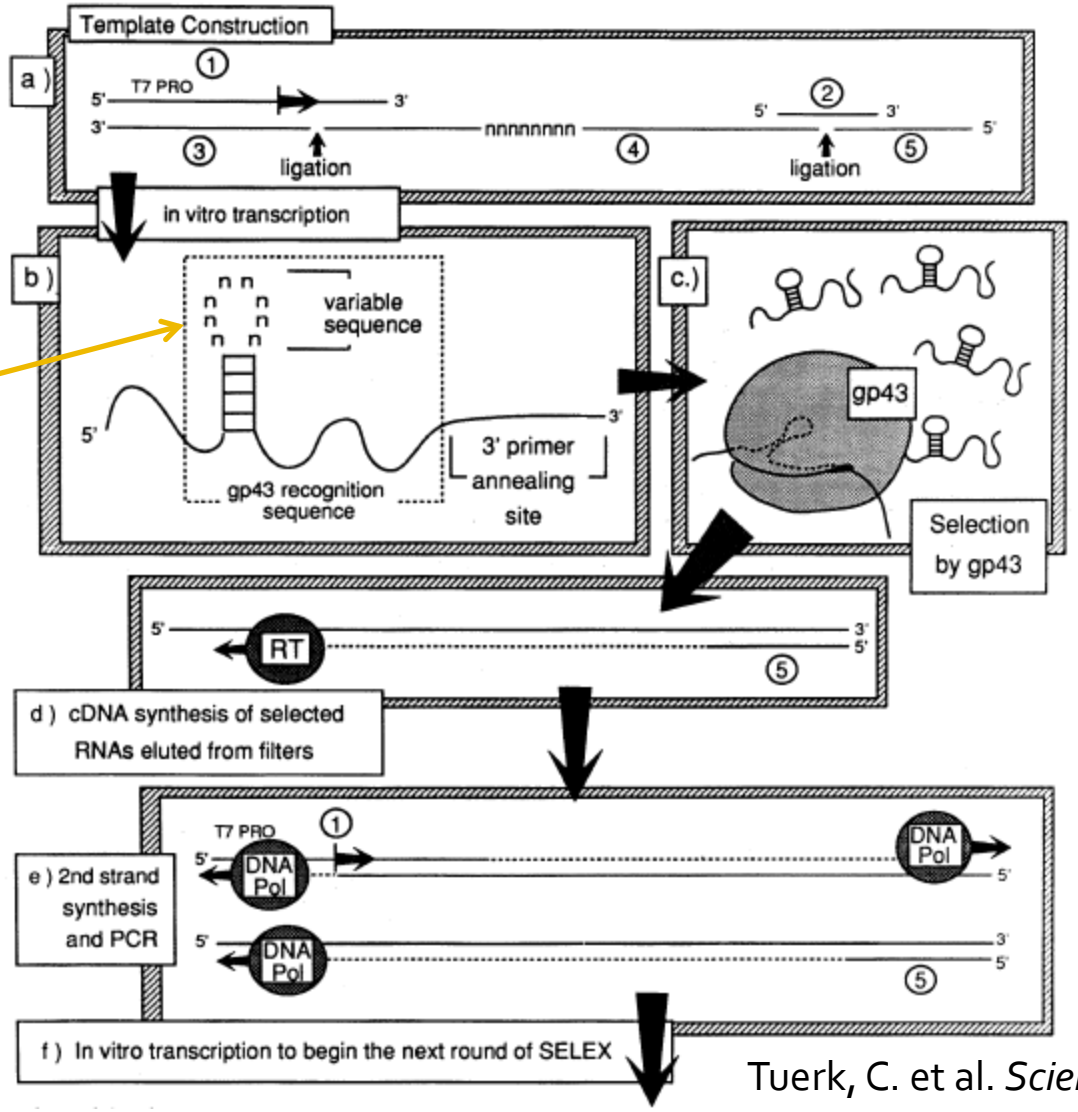
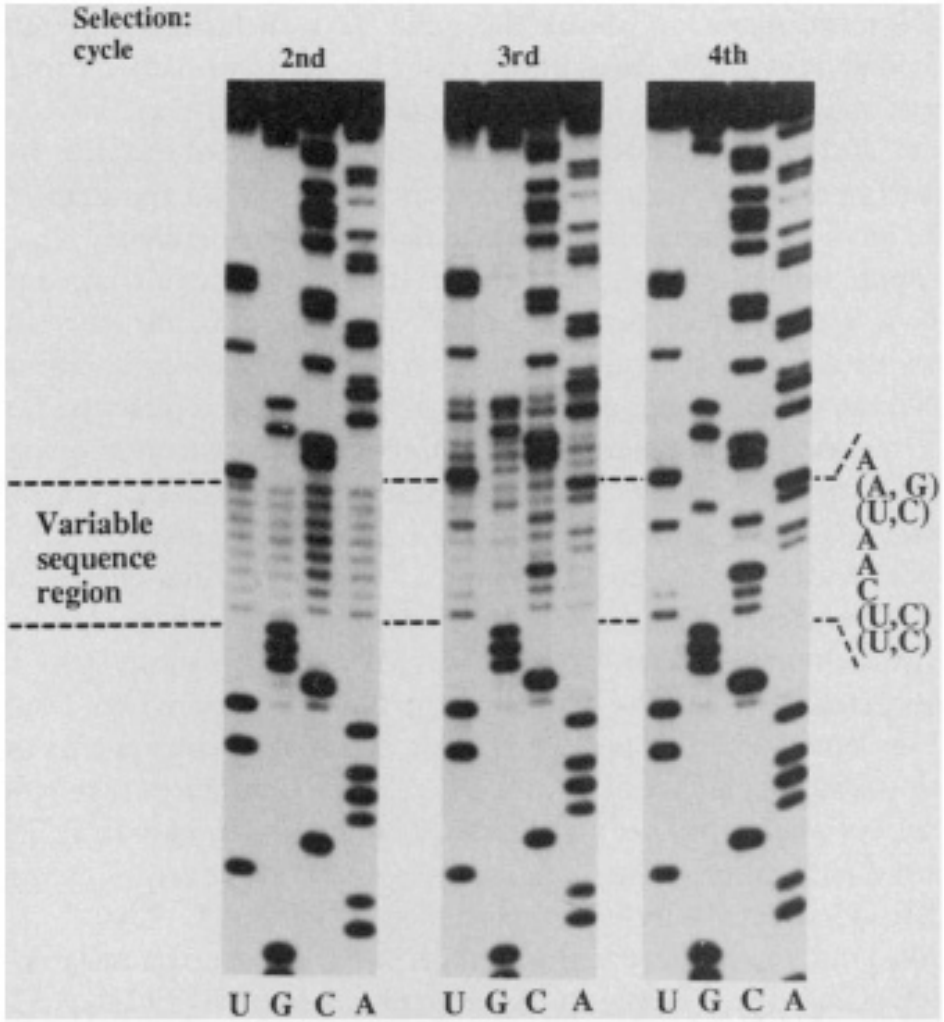


Tuerk, C. et al. *Science* **1990,** *249*, 505

Selection of Consensus Sequence

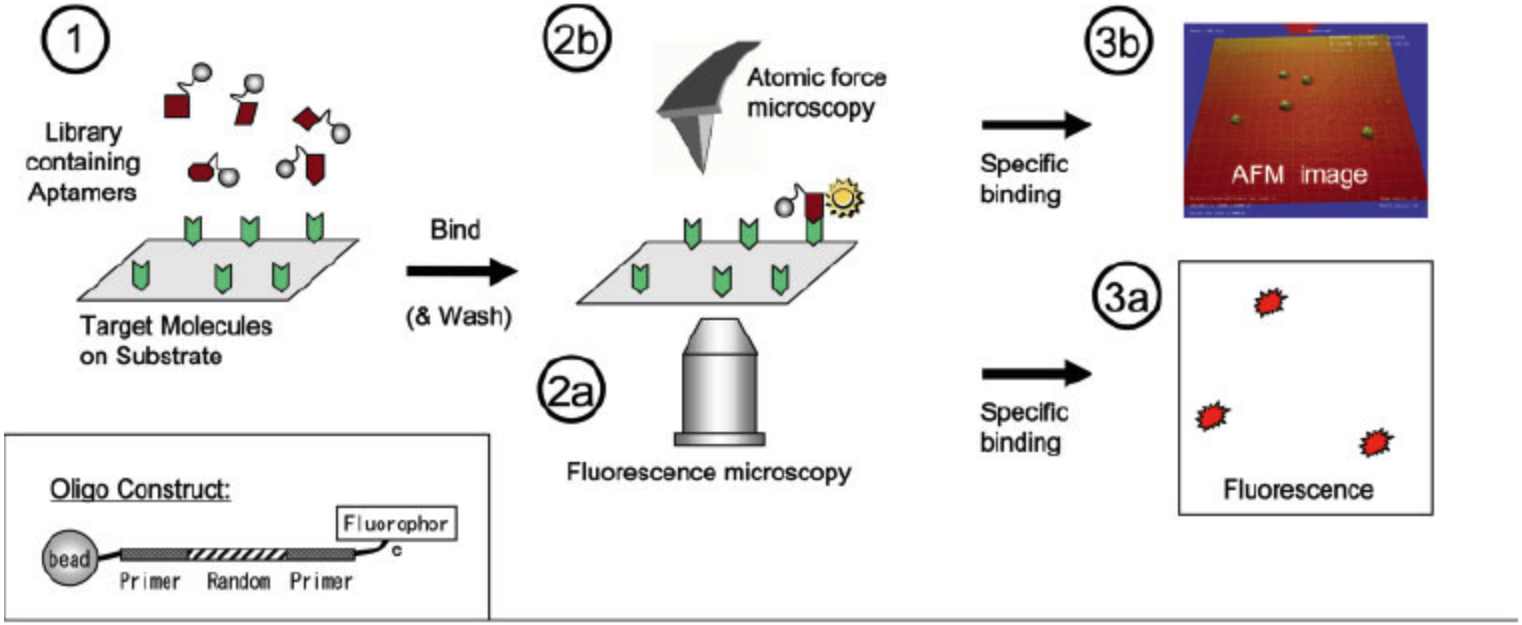
Filter Binding Assay

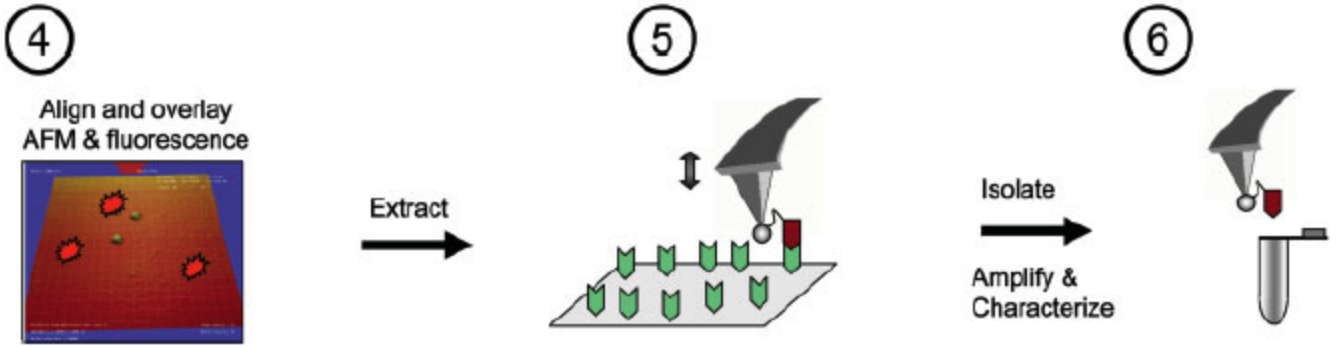
Sequence gp43 bound RNA oligomers



Tuerk, C. et al. *Science* **1990**, *249*, 505

Recent Technological Advances:
Combined Atomic Force/Fluorescence Microscopy





Peng et al. *Microscopy Research and Technique* ***2007,*** *70,* 372

Recent Technological Advances:
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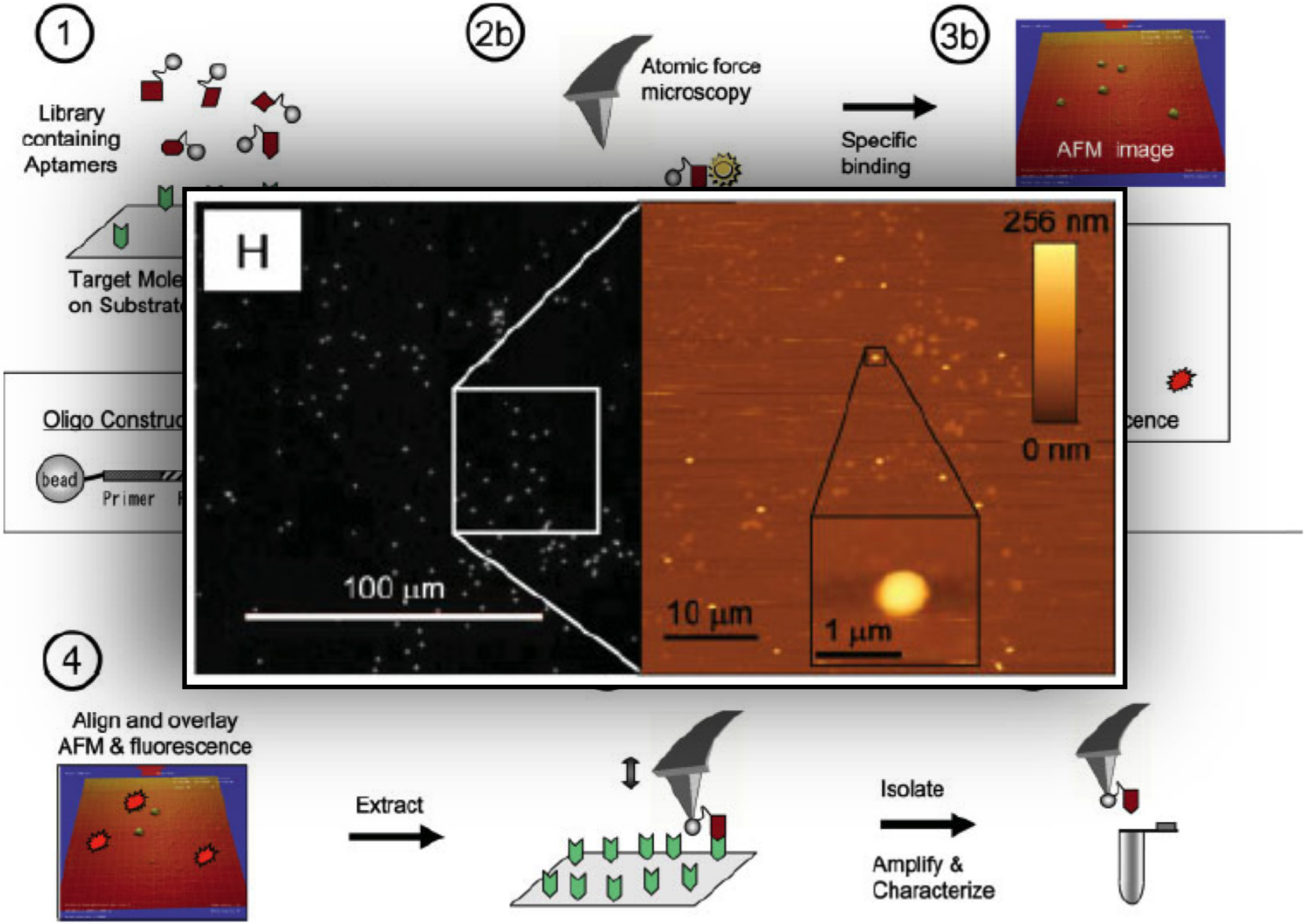

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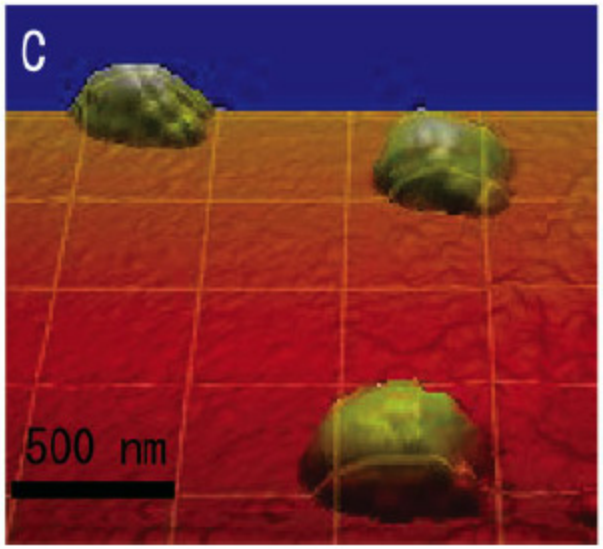
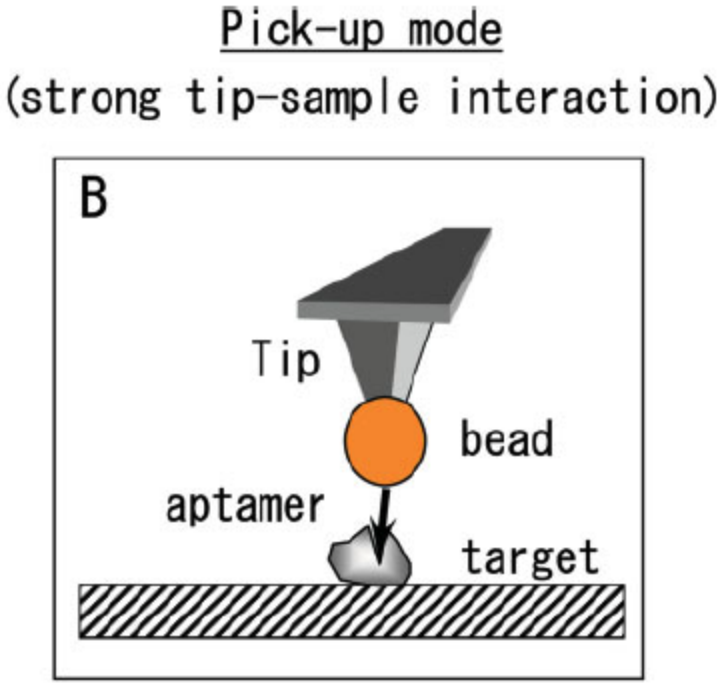
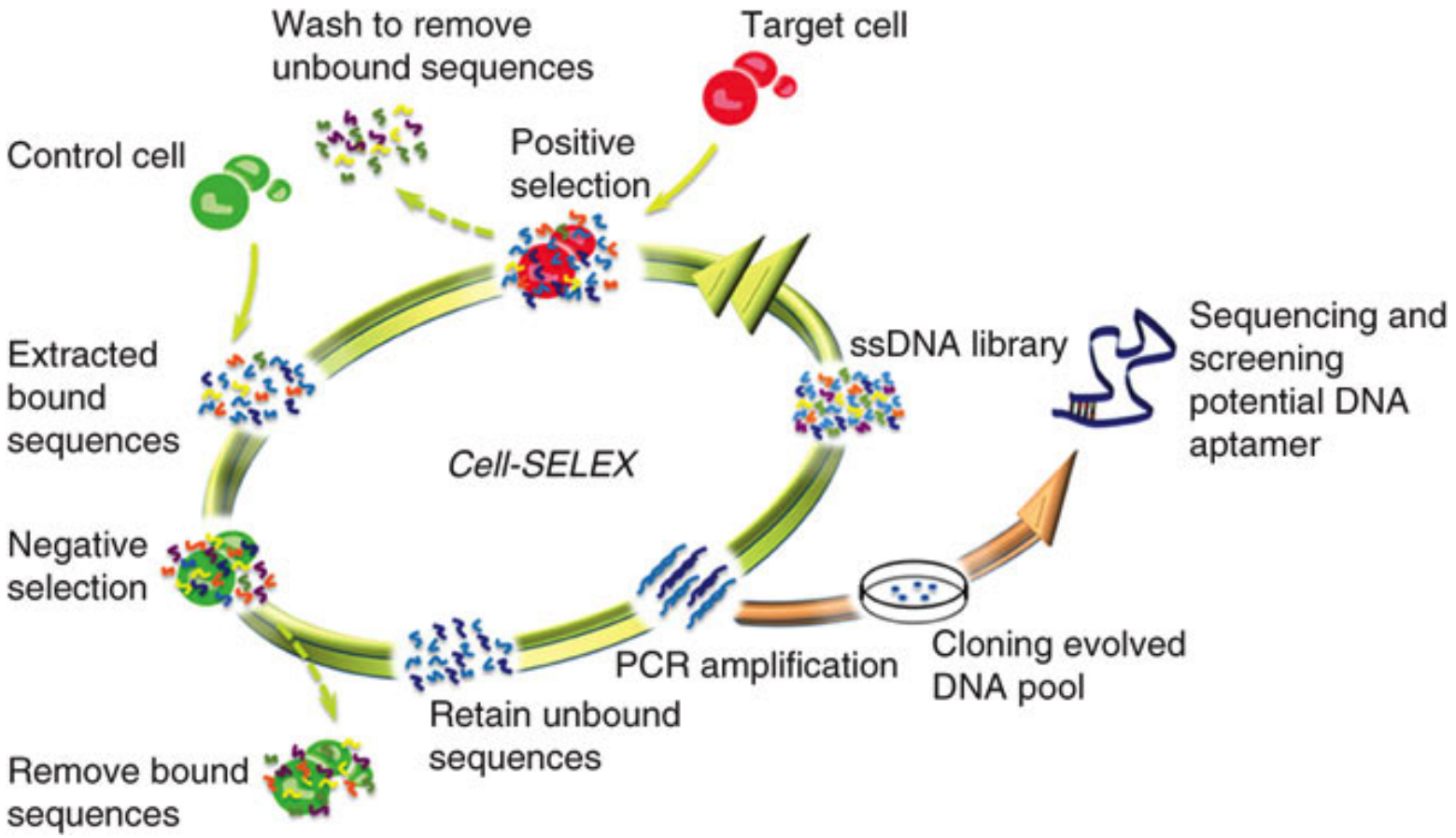




Peng et al. *Microscopy Research and Technique* ***2007***, *70*, 372

Recent Technological Advances:
Cell SELEX



Sefah, K. et al. *Nature Protocols* ***2010***, *5*, 1185

Recent Technological Advances:
Other Models

- 1. Capillary Electrophoresis SELEX

Benefit: Eliminates stationary support and linker bias; collect as fractions

Mendonsa, S. D. et al. *JACS* **2004** *126*, 20.

- 2. PhotoSELEX

Benefit: Form photo-induced covalent bonding between aptamer and target protein allows for vigorous washing

Golden, M. C. et al. *Journal of Biotechnology* **2000**, *81*, 167

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Limitation: Requires 5-bromouracil

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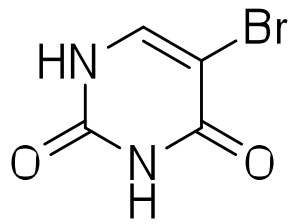
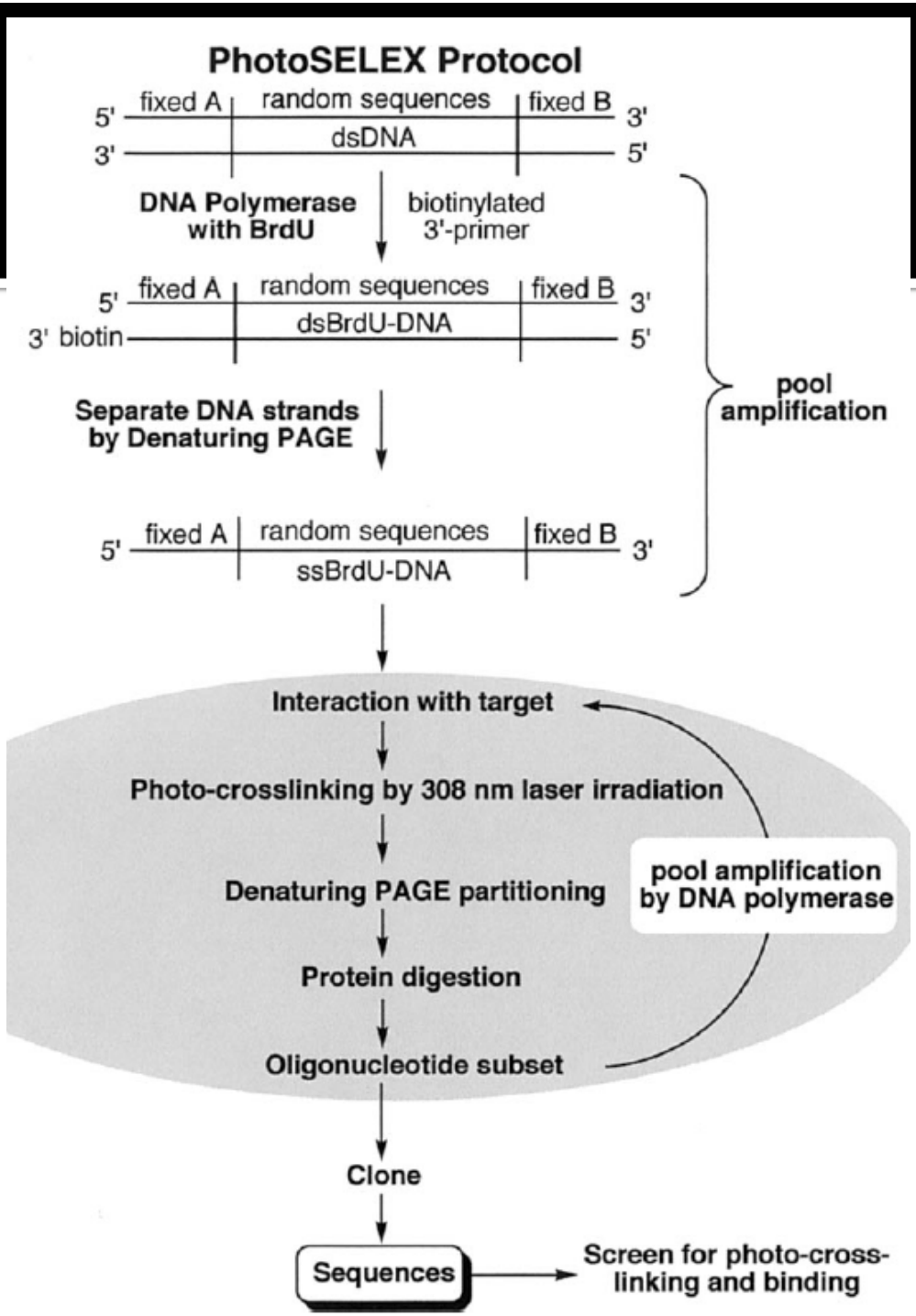
PhotoSELEX



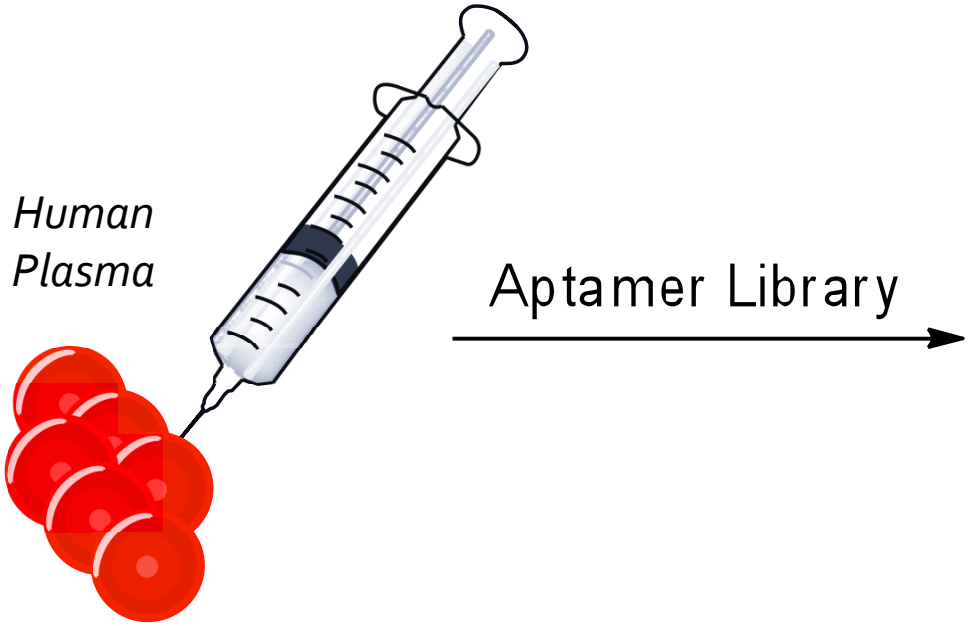
5-bromouracil

- Activated by absorption of light
- Absorbs UV in the 310 nm range
- Activated BrdU cross-links aromatic and sulfur-bearing amino acids



Deconvolution of Complex Protein Mixtures

Hypothesis: Aptamer libraries (of random sequence) contain enough shape diversity to deconvolute a complex mix of molecular targets.



Molecular Mass (*kDa*)	Identity
72	Prothombin
180	C_3 Complement
>250	
68	

Fitter, S. et al. *Journal of Biological Chemistry* **2005**, *280*, 34193

Validated Aptamer Targets

Target	Nucleic Acid	K_d (nm)
VEGF	RNA	0.05 – 0.150
α-Thrombin	DNA RNA	25 – 200 2.8
IgE	DNA RNA	9 30
ATP	RNA	70

The Aptamer Handbook, S. Klussmann ed., Wiley-VCH, Weinheim, 2006.
Sassanfar, M. *Nature* **1993** *364*, 550

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Soluble Proteins Only

The Aptamer Handbook, S. Klussmann ed., Wiley-VCH, Weinheim, 2006.
Sassanfar, M. *Nature* **1993** *364*, 550

Advantages of Aptamers

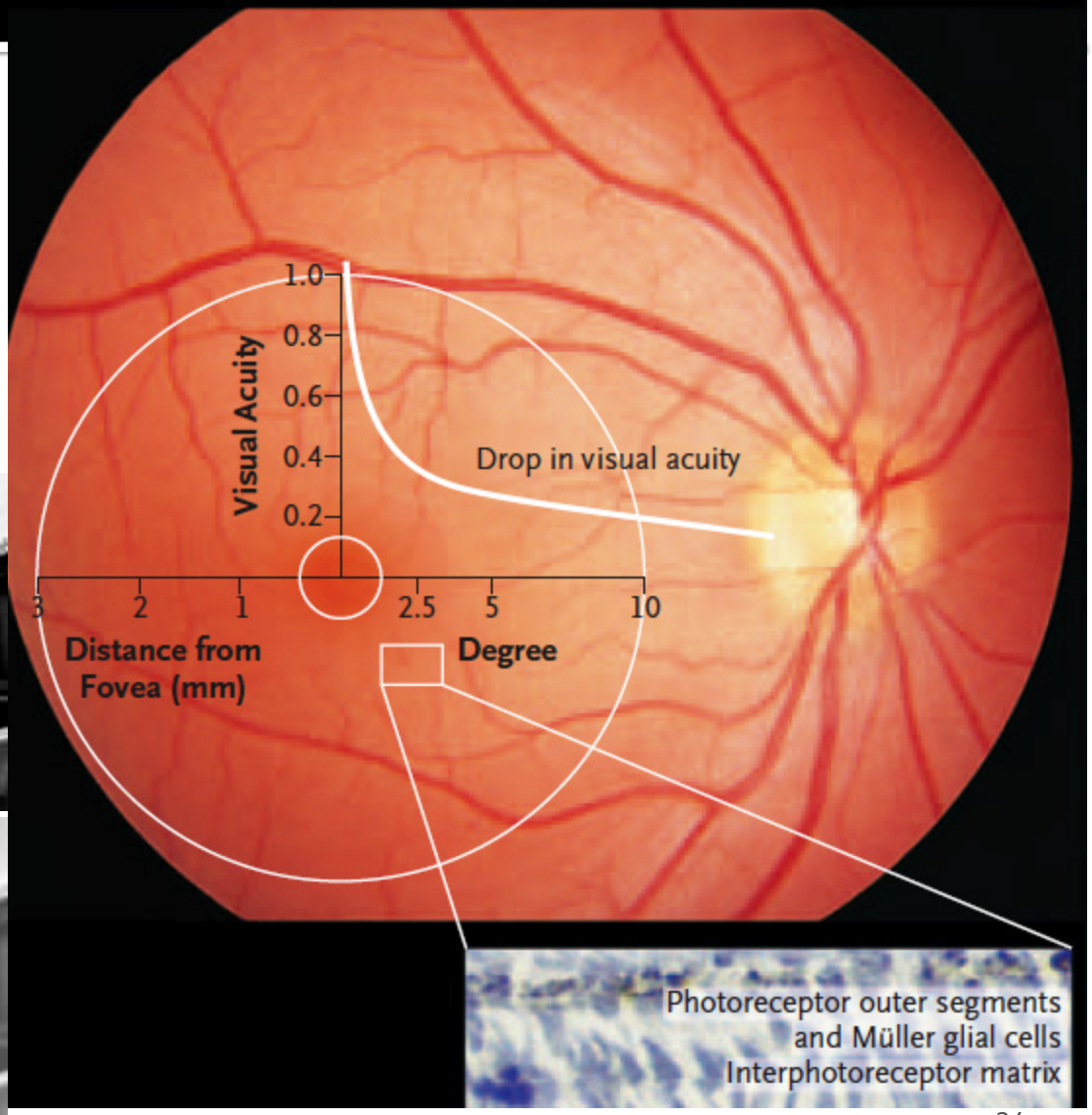
- High Affinity/Selectivity
- Nontoxic/Nonimmunogenic
- Predictable Pharmacokinetics
- Easily prepared in bulk ($200 - $2000/g)
- Long Shelf Life
- Simple Antidote Generation
- Will refold after exposed to denaturing conditions

Disadvantages

- Short *in vivo* half life, except in ocular compartment
- Oligonucleotides highly charged and thus low bioavailability
- Targets should be in the bloodstream or on cell surfaces

Therapeutic Relevance
Case Study: Macugen^{R} Pegaptanib

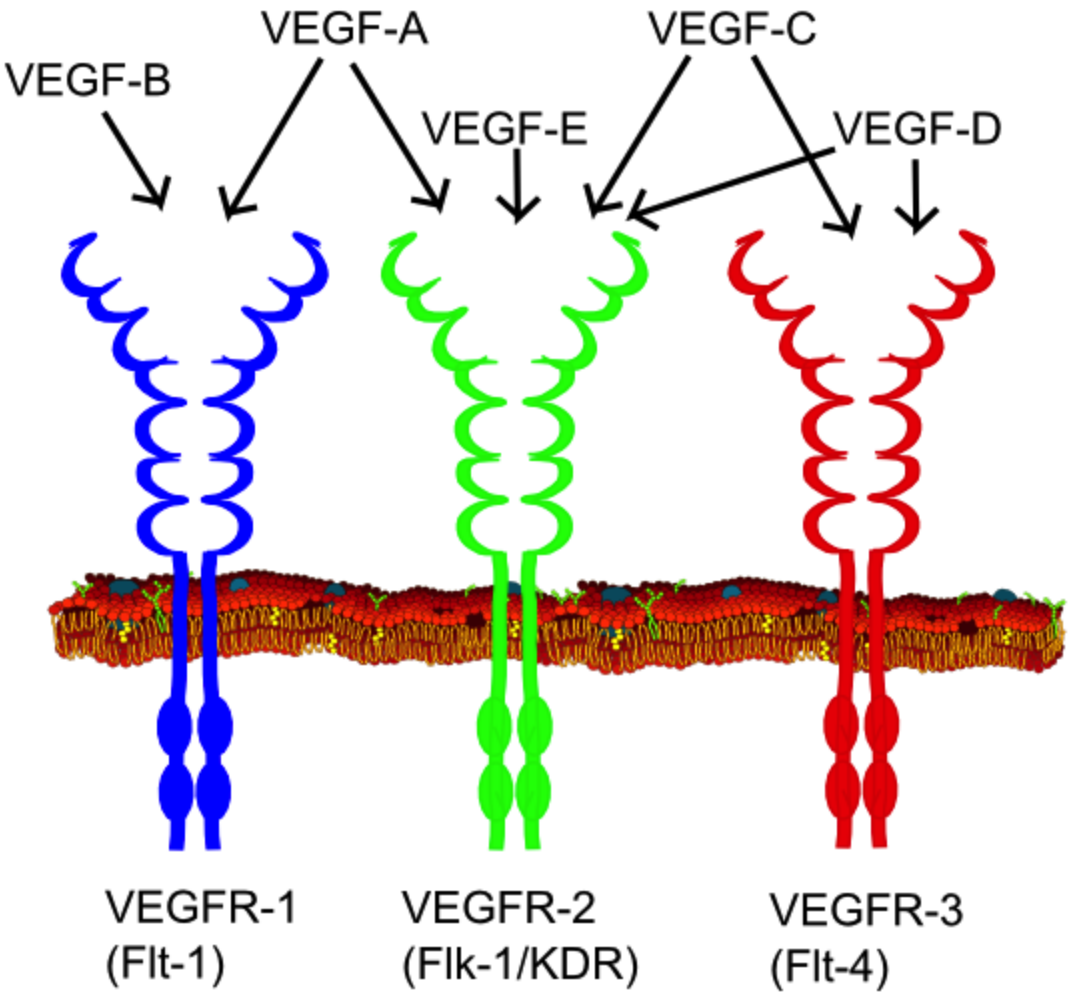
- Age-related macular degeneration (AMD) is the leading cause of blindness in people over 50
- No effective treatments



N. Engl. J. Med. **2006** 355,14

Vascular Endothelial Growth Factors

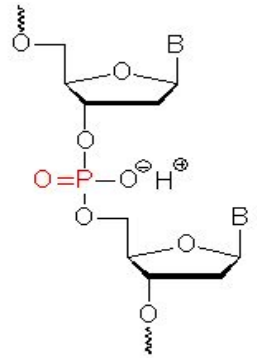
- Tyrosine kinase receptors
- Stimulate vasculogenesis and angiogenesis
- Validated as a major regulator of aberrant and excessive blood vessel growth in the eye



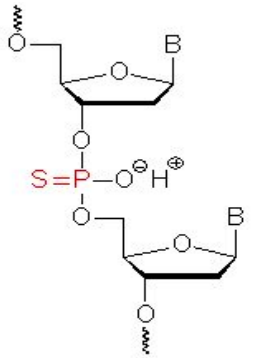
Nat. Rev. Drug Descov. **2006** 5, 123

Therapeutic Relevance
Case Study: Pegaptanib

- The earliest work in 1994, isolated aptamers that inhibit VEGF *in vitro*
- NeXstar Pharmaceuticals carried out three separate iterations of SELEX



5'-3' Phosphodiester linkage



5'-3' Phosphorothioate linkage

Aptamer	Modification	Half-life in urine (hours)	Dissociation half-life	Binding affinity for VEGF (K_d, nM)	Binding affinity for PDGF (K_d, nM)	Ratio K_d PDGF/VEGF
NX-107	None (minimal ligand)	1.4	NR	NR	NR	NR
NX-178	3′ and 5′ caps	17	12 seconds	2.4	75	31
NX-213	3′ and 5′ caps + 2′-OMe purine substitution	131	8 minutes	0.14	91	650

Therapeutic Relevance
Case Study: Pegaptanib

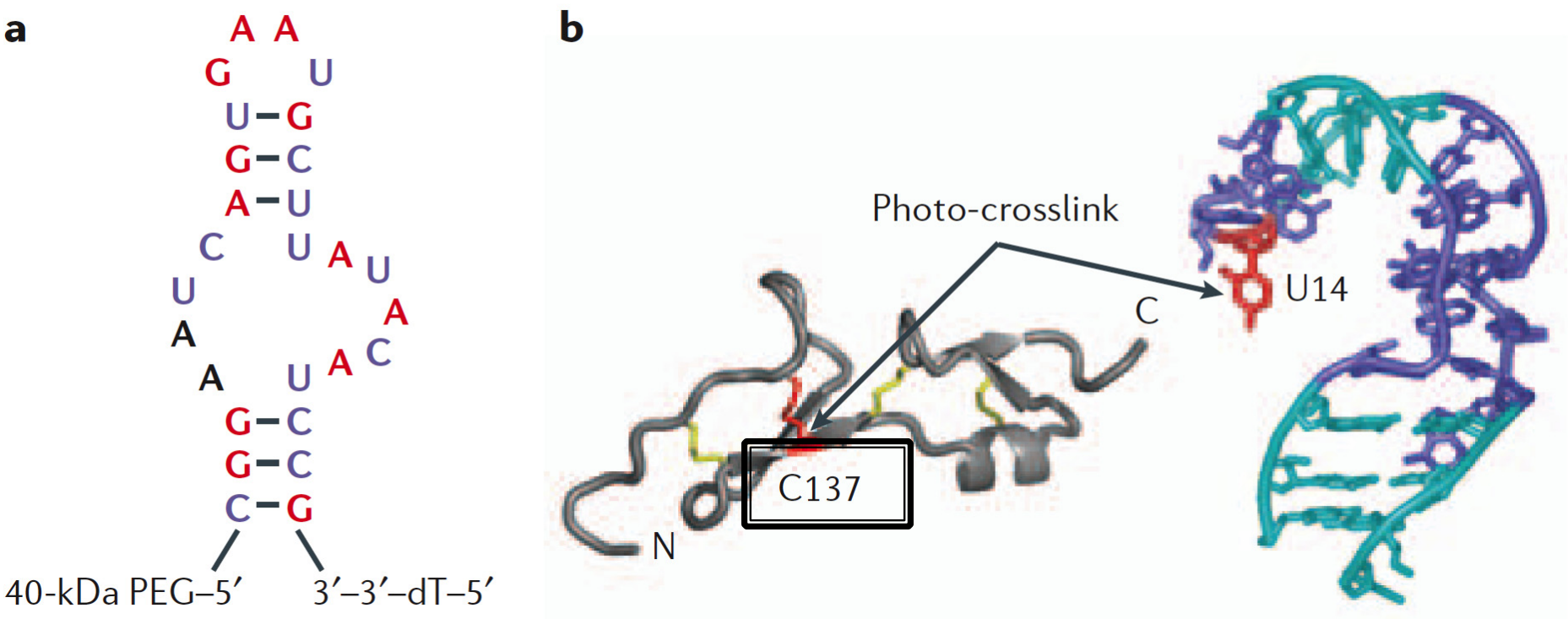
- Aptamers containing both 2′-F and 2′-OMe modifications were highly stable *in vivo*

Aptamer	Length (nucleotides)	Binding affinity for VEGF (K_d, pM)	Dissociation half-life (seconds)	T_m (°C)	Binding dependent on divalent cations	$VEGF_{165}$ IC_{50} for VEGFR2 (M)	Miles assay (inhibition at 0.1 μM)
t22-OMe	23	72	60	49	No	$2\text{-}3 \times 10^{-12}$	13%
t2-OMe	29	130	170	66	No	6×10^{-11}	None
t44-OMe (pegaptanib)*	27	49	90	62	Yes	$2\text{-}3 \times 10^{-12}$	48%
Scr-t44-OMe‡	27	NR	NR	NR	NR	5×10^{-8}	None

*Inhibition of vascular leakage

Attachment of a 5′-linked 40-kDa polyethylene glycol improved inhibition to 83%

Therapeutic Relevance
Case Study: Pegaptanib

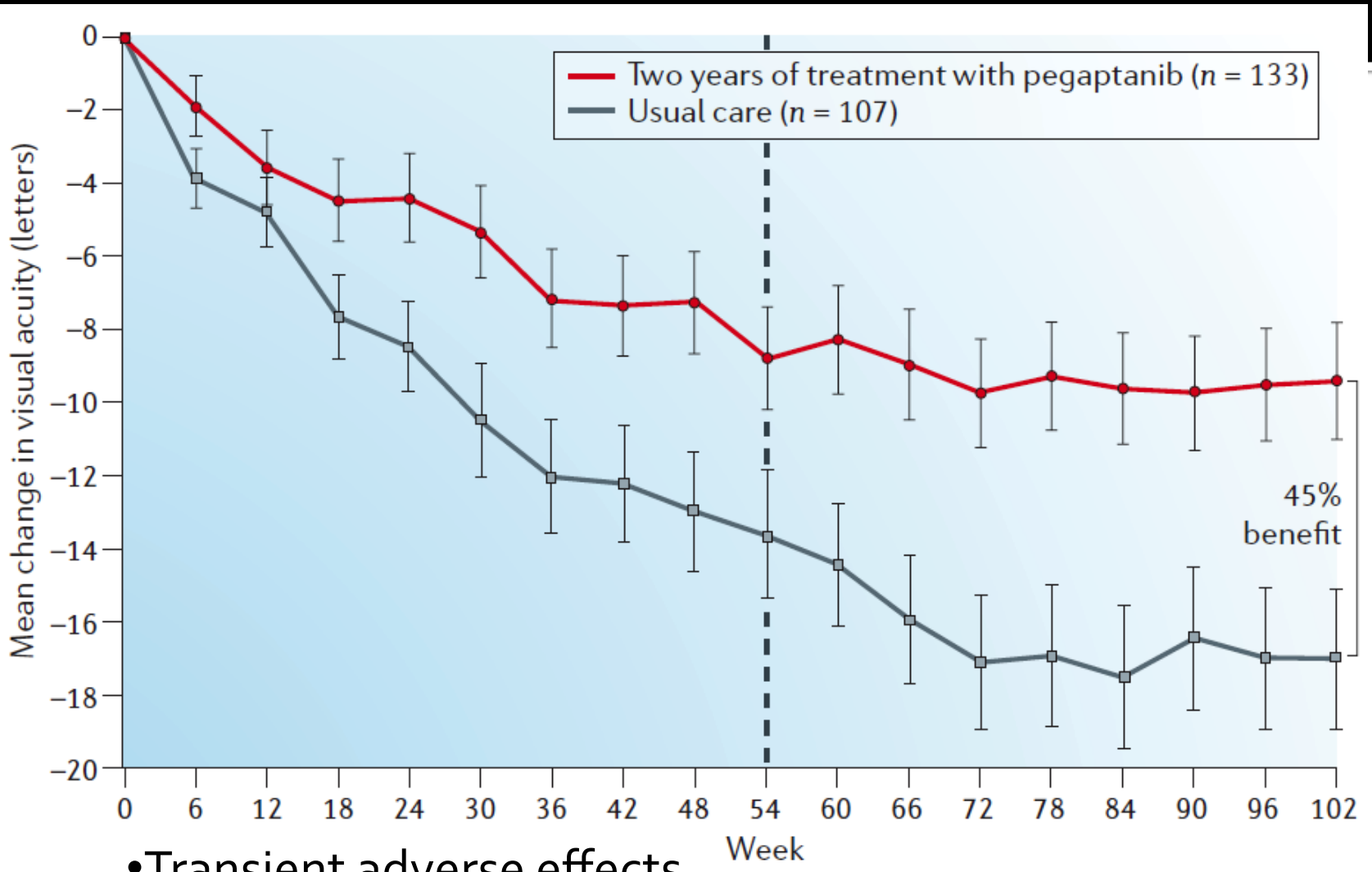


2′-Fluoro

2′-Methoxy

Unmodified

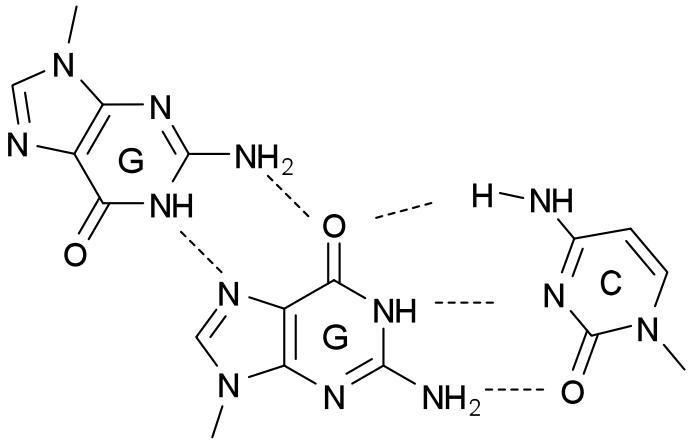
Therapeutic Relevance
Case Study: Pegaptanib

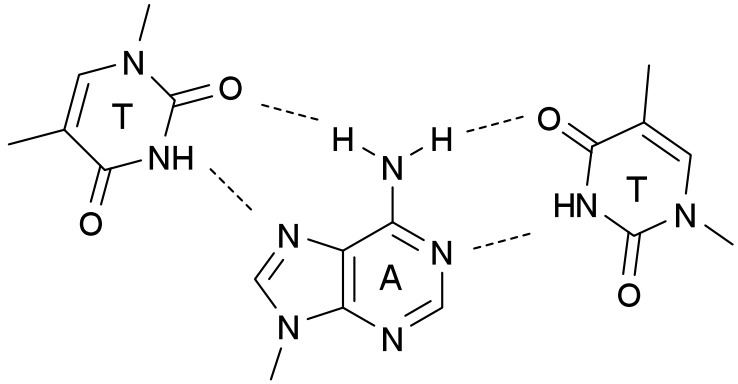


- Transient adverse effects
- FDA approved in 2004 with an IC_{50} of 49pM

Therapeutic Relevance
Case Study: AS1411

- The Bates' group at the University of Louisville made a Purine Motif TFO that was specific for the promoter of the *uPA* gene
- Gave moderate inhibition of DU145 growth





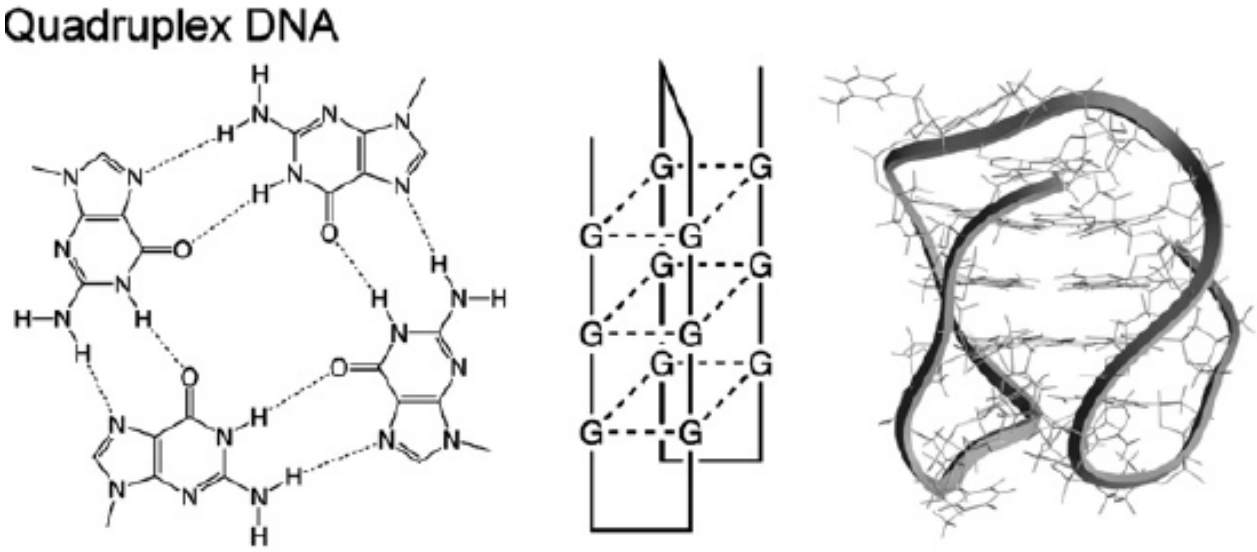
- Negative control was all G and T with no complementarity to gene
- Gave a much larger inhibition

Experimental and Molecular Pathology **2009,** 86, 151

Therapeutic Relevance
Case Study: AS1411

5′ -GGTGGTGGTGGTTGTGGTGGTGGTGG

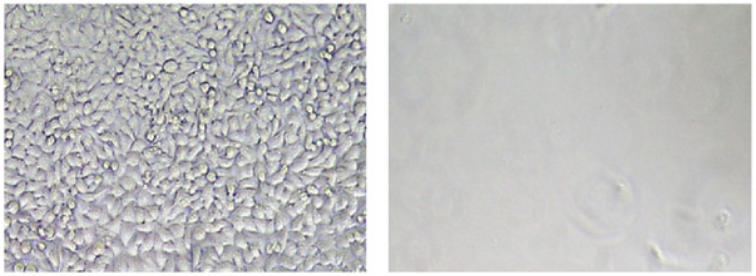
- Aptamers made by SELEX frequently for G-quadruplexes
- Certain cell types (including cancer and some immune cells) preferentially internalize G-quadruplex-forming oligos



Therapeutic Relevance Case Study: AS1411

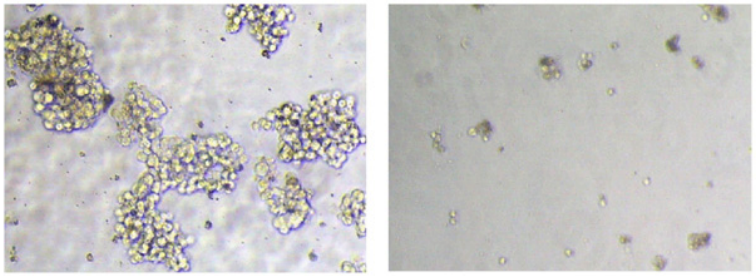
- Tested at NCI in 60 different cancer cell lines
- Displayed antiproliferative activity in almost all at low µM concentrations
- Non-malignant cells unaffected at 10 µM
- Cytostasis occurs as a result of cell division being inhibited

A549 non-small cell lung cancer



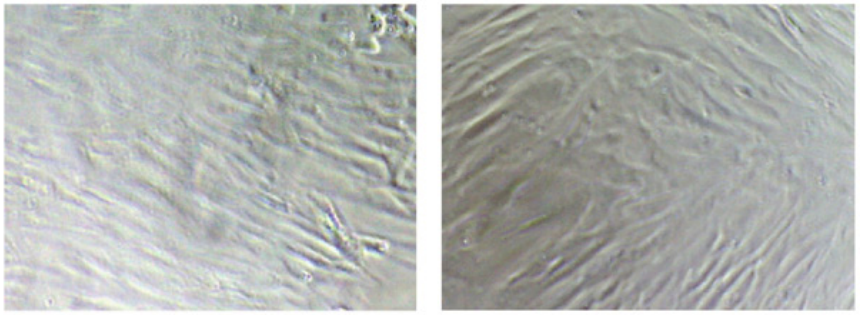
untreated + 3 µM AS1411

H82 small cell lung cancer



untreated + 3 µM AS1411

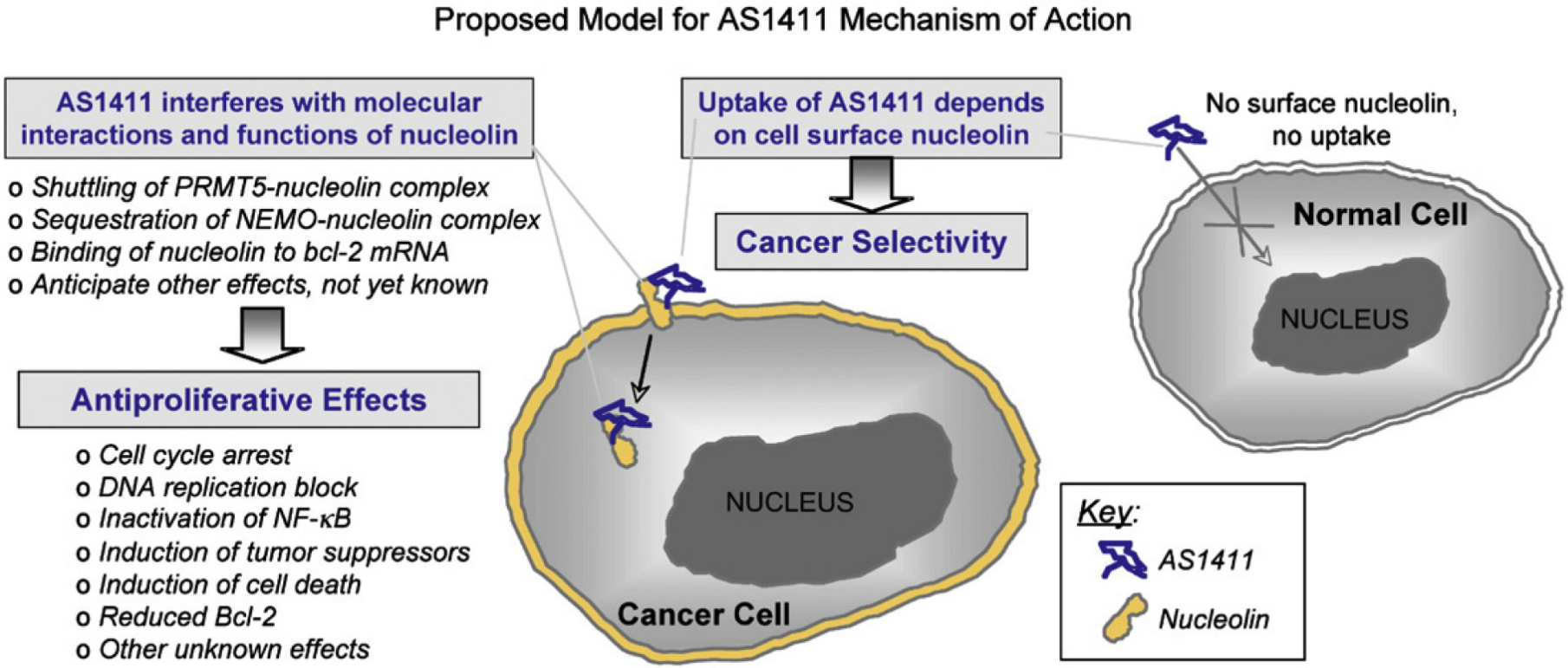
Hs27 non-malignant skin fibroblasts



untreated + 10 µM AS1411

Therapeutic Relevance
Case Study: AS1411

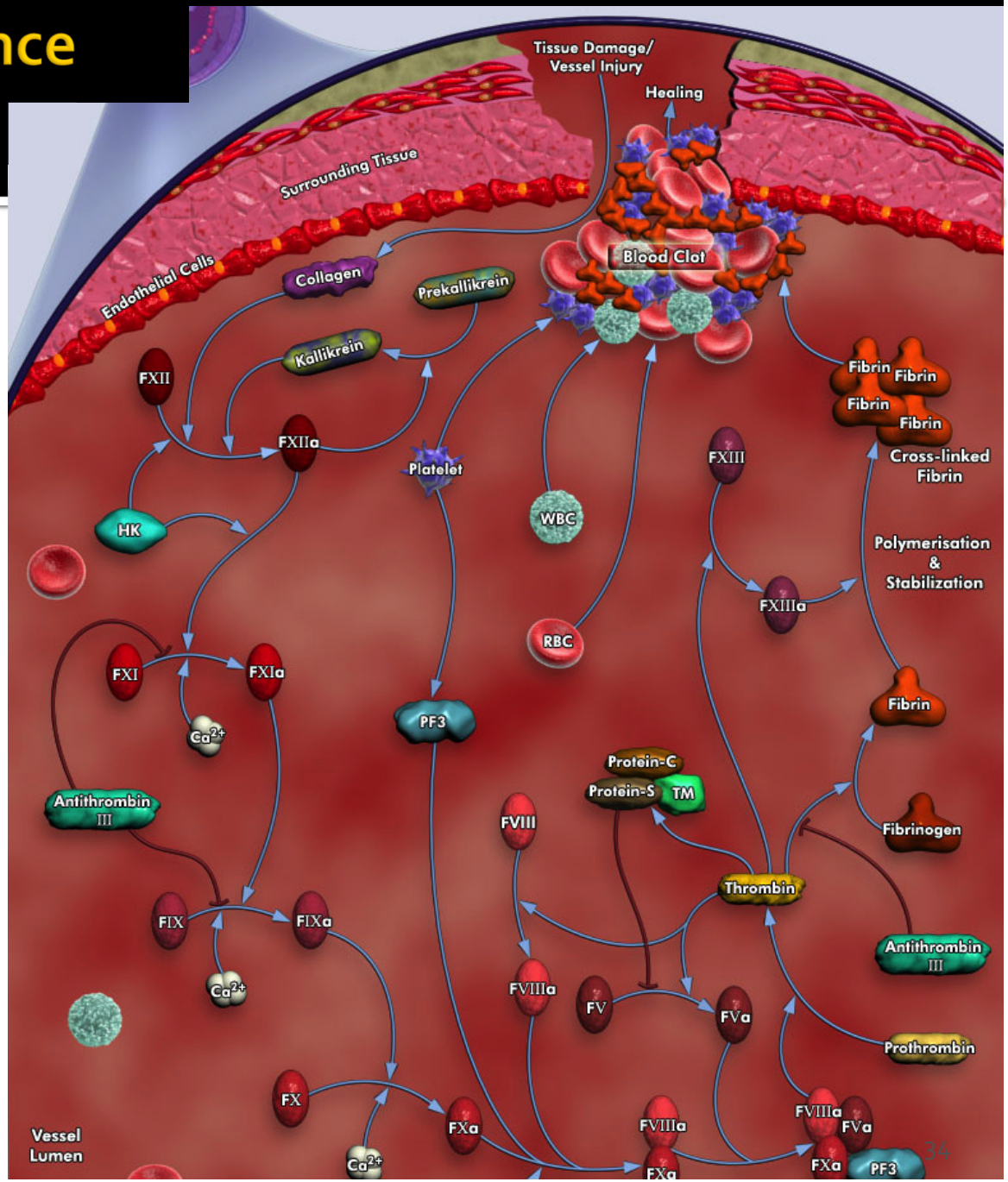
- Southwestern blot gave a protein ~110kDa
- Hypothesized that it was Nucleolin, a telomere binding protein



- Entered in Phase II clinical trials for treatment of acute myeloid luekemia in late 2007

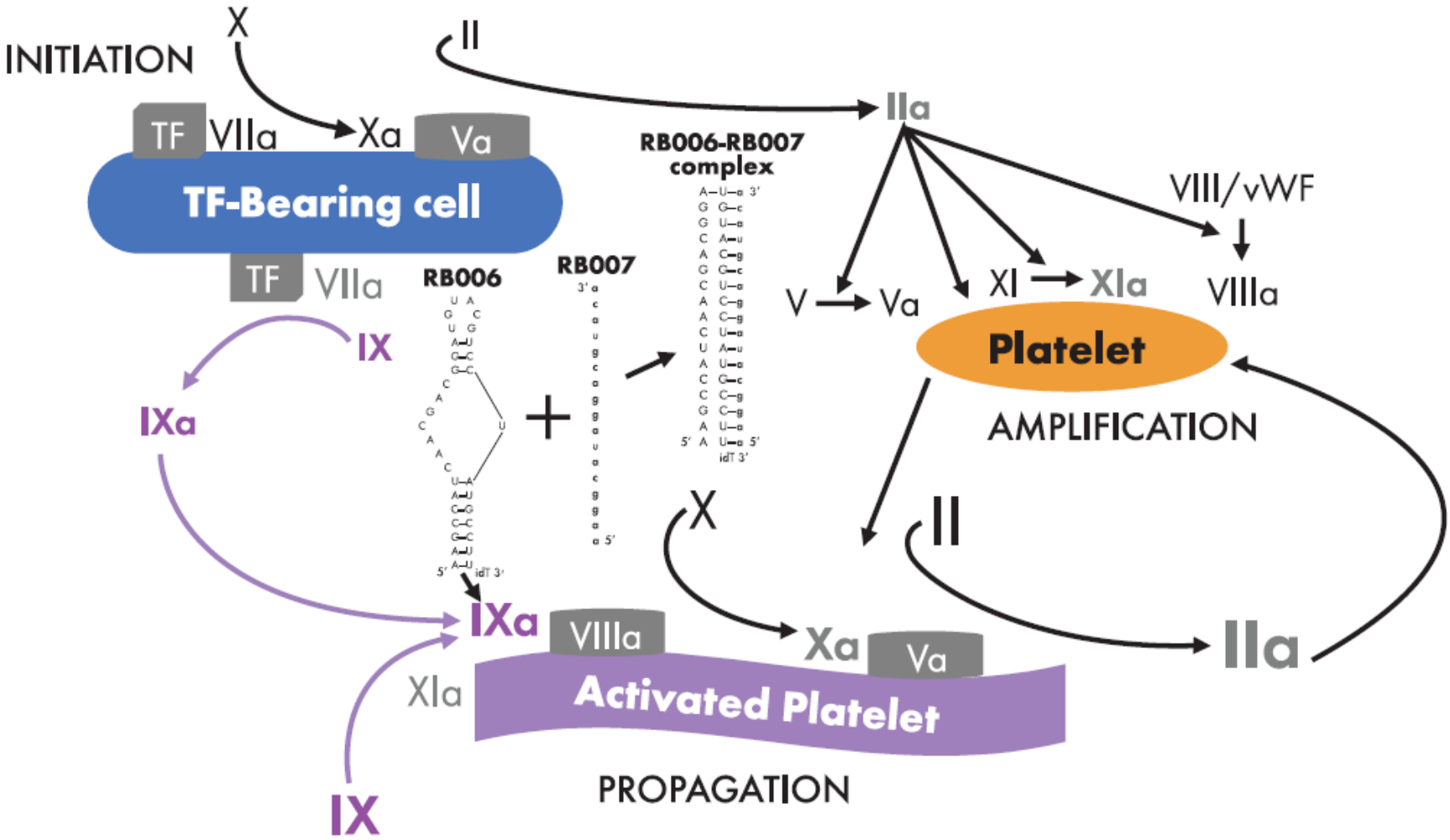
Therapeutic Relevance Case Study: REG1

- Anticoagulation drugs are used extensively to treat acute venous and arterial thrombosis
- Major hemorrhage occurs in 4% of patients with acute coronary syndrome
- 5% of patients undergoing artery bypass require a second operation to control bleeding
- There is a need for a controlled anticoagulant



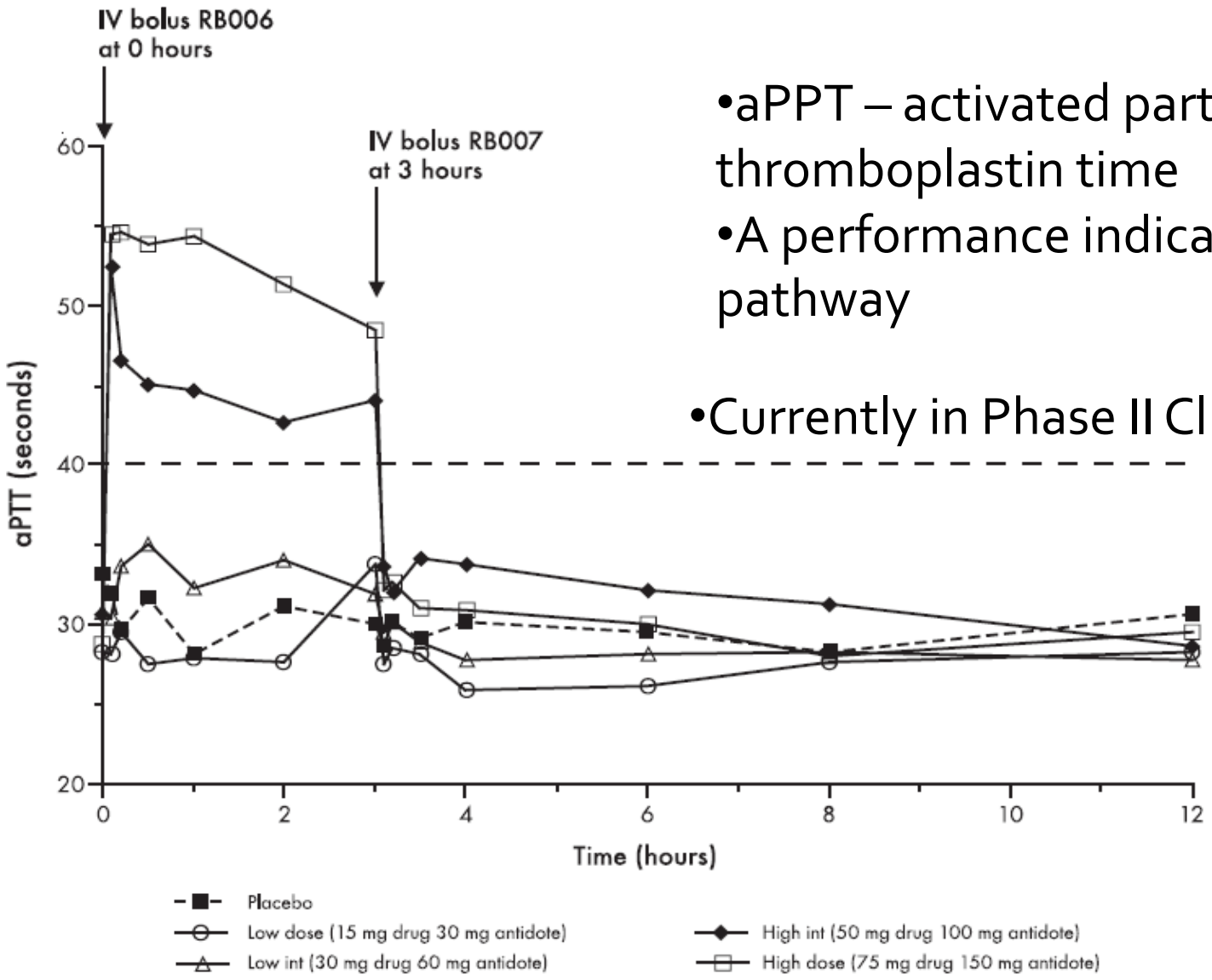
Circulation **2008,** 2865
Qiagen

Therapeutic Relevance
Case Study: REG1



- The Rusconi Group 2.8nM affinity for IXa

Therapeutic Relevance
Case Study: REG1



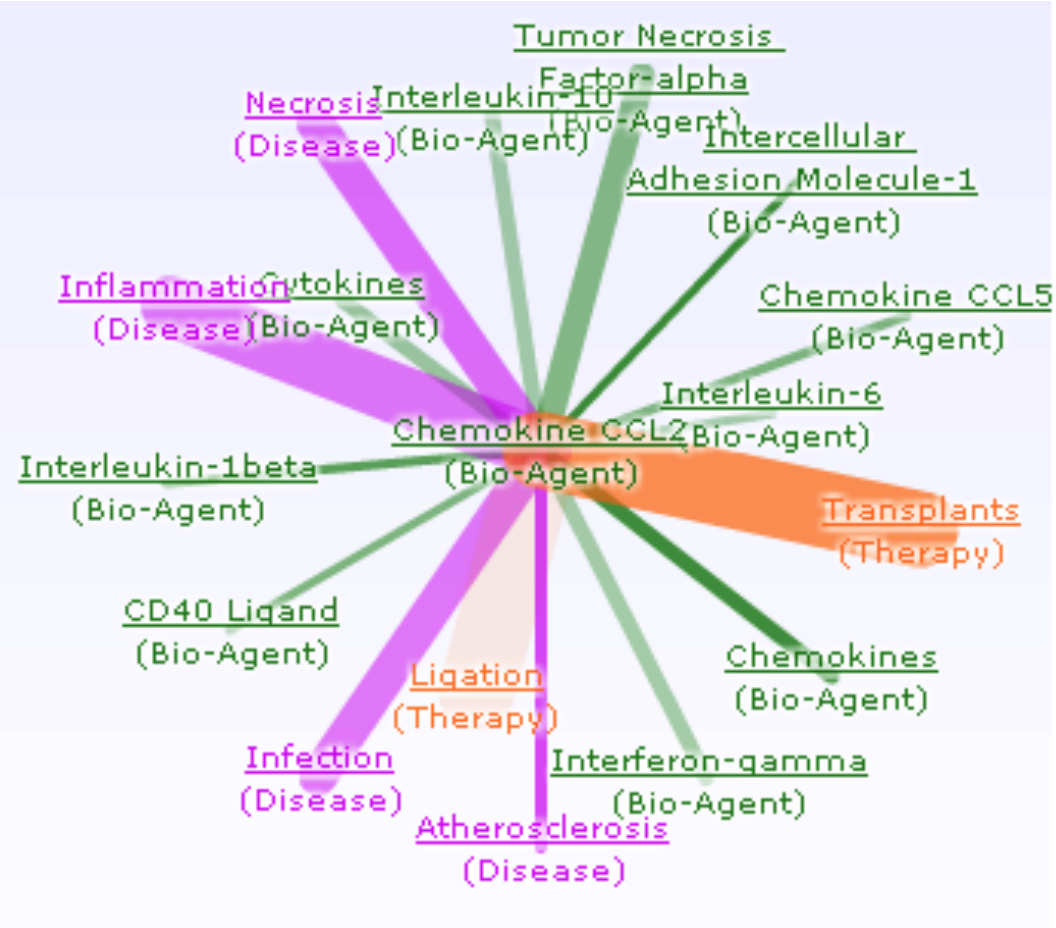
- aPPT – activated partial thromboplastin time
- A performance indicator of the pathway

- Currently in Phase II Clinical Trials

Therapeutic Relevance Case Study: NOX-E36

- Diabetic nephropathy is a progressive kidney disease caused by a weakening of the kidney capillaries from dysregulated blood sugar

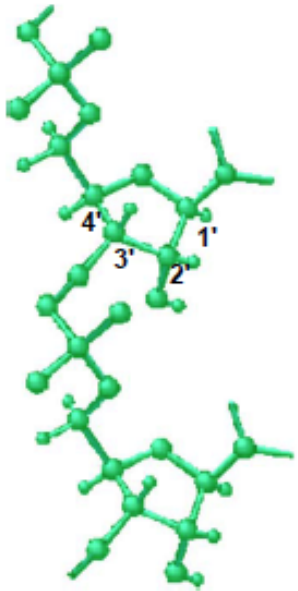
- Cytokine antagonism is a powerful strategy to prevent tissue damage in chronic inflammation



Noxxon Pharmaceuticals
The Journal of Pharmacology and Experimental Therapeutics **2009**, 328, 371

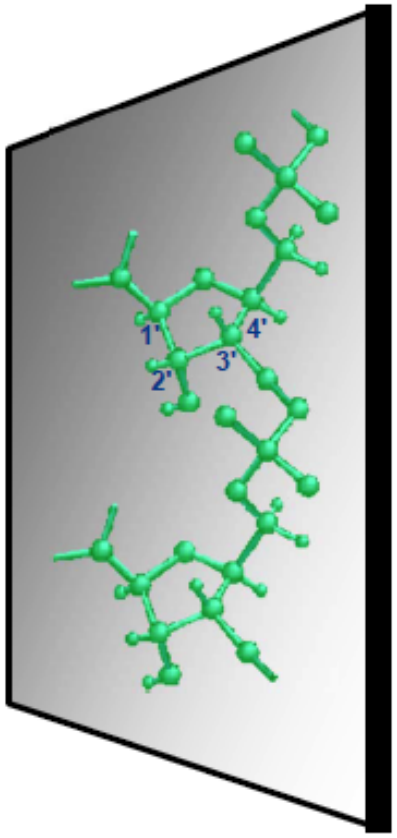
Therapeutic Relevance Case Study: NOX-E36

- High-affinity aptamers to D-mCCL2 were identified after 11 rounds of *in vitro selection*



D-RNA

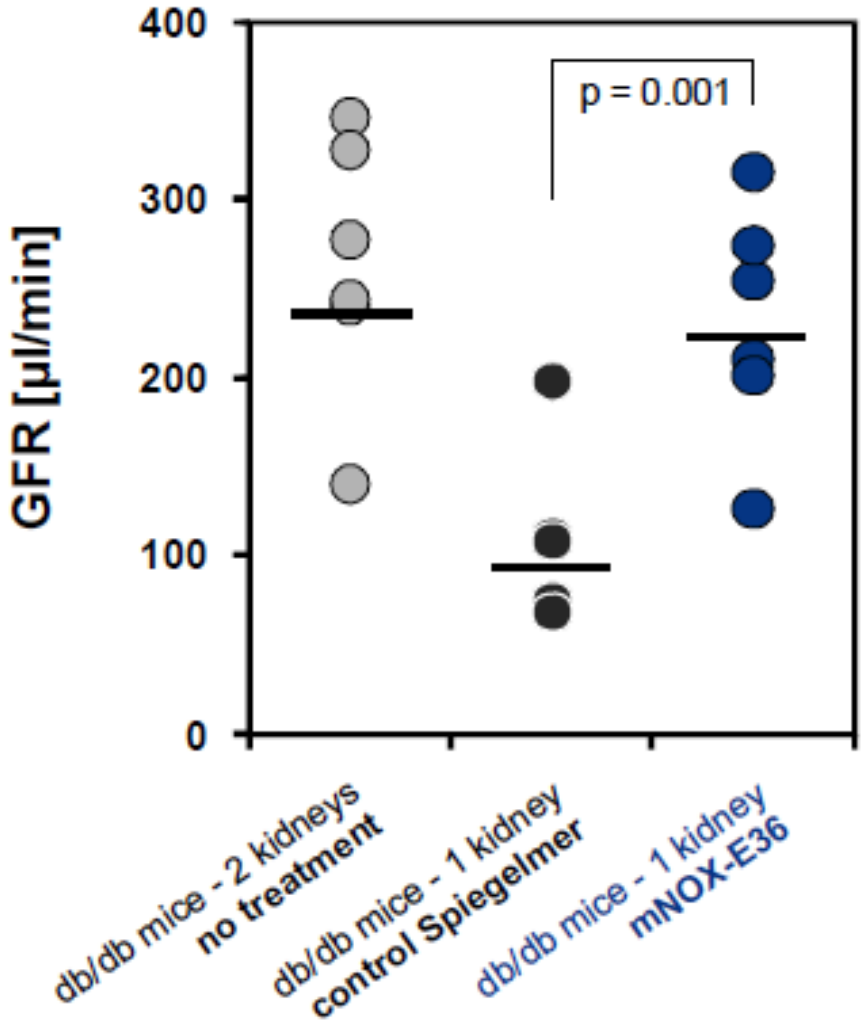
- Biologically unstable
- Frequently immunogenic
- Approved product MACUGEN



- Biologically stable
- Non-immunogenic
- Safe & well tolerated in animals & man

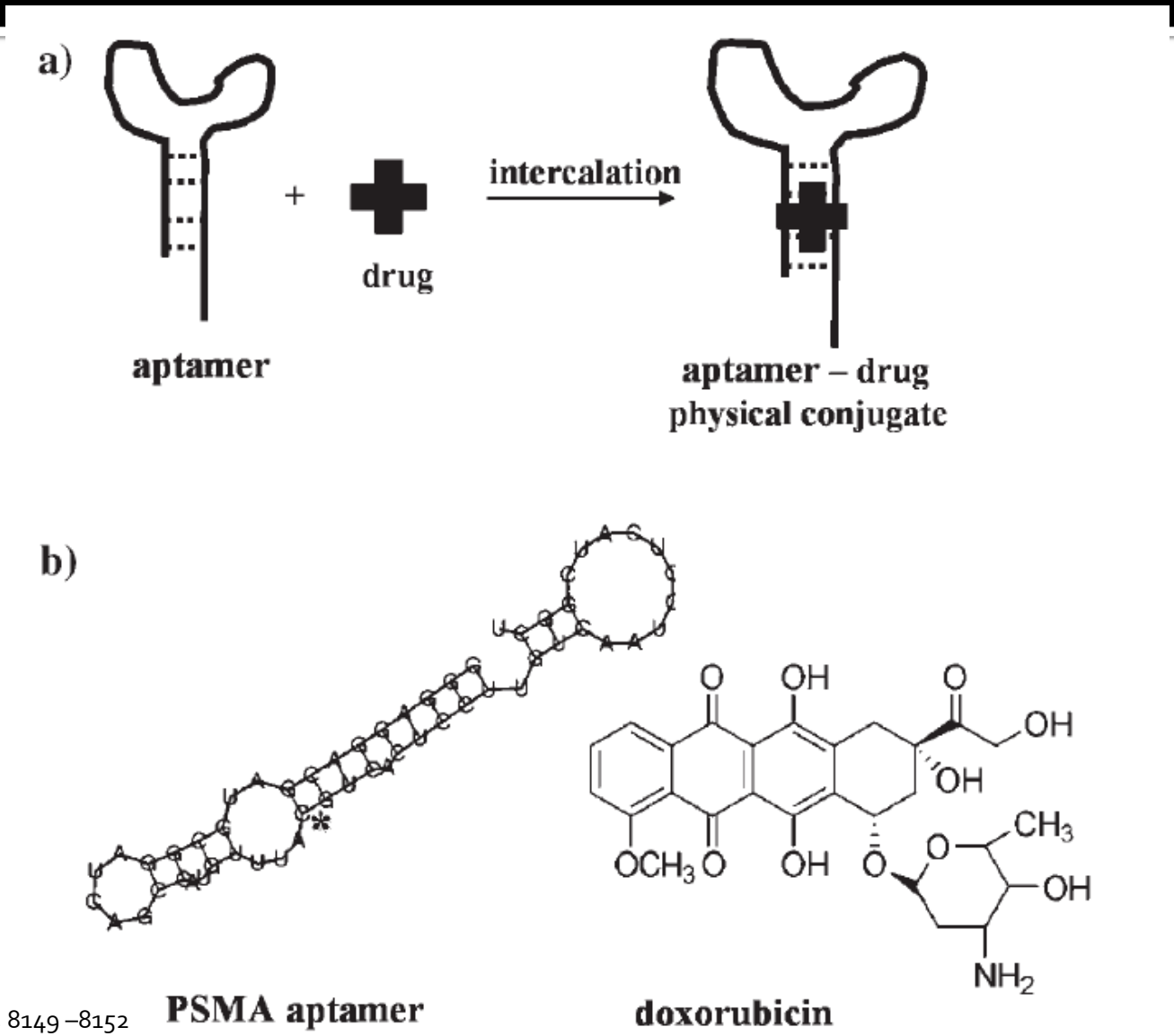
L-RNA (Spiegelmer)

Therapeutic Relevance
Case Study: NOX-E36



- Currently in Phase I Clinical Trials

Therapeutic Relevance
Drug Delivery



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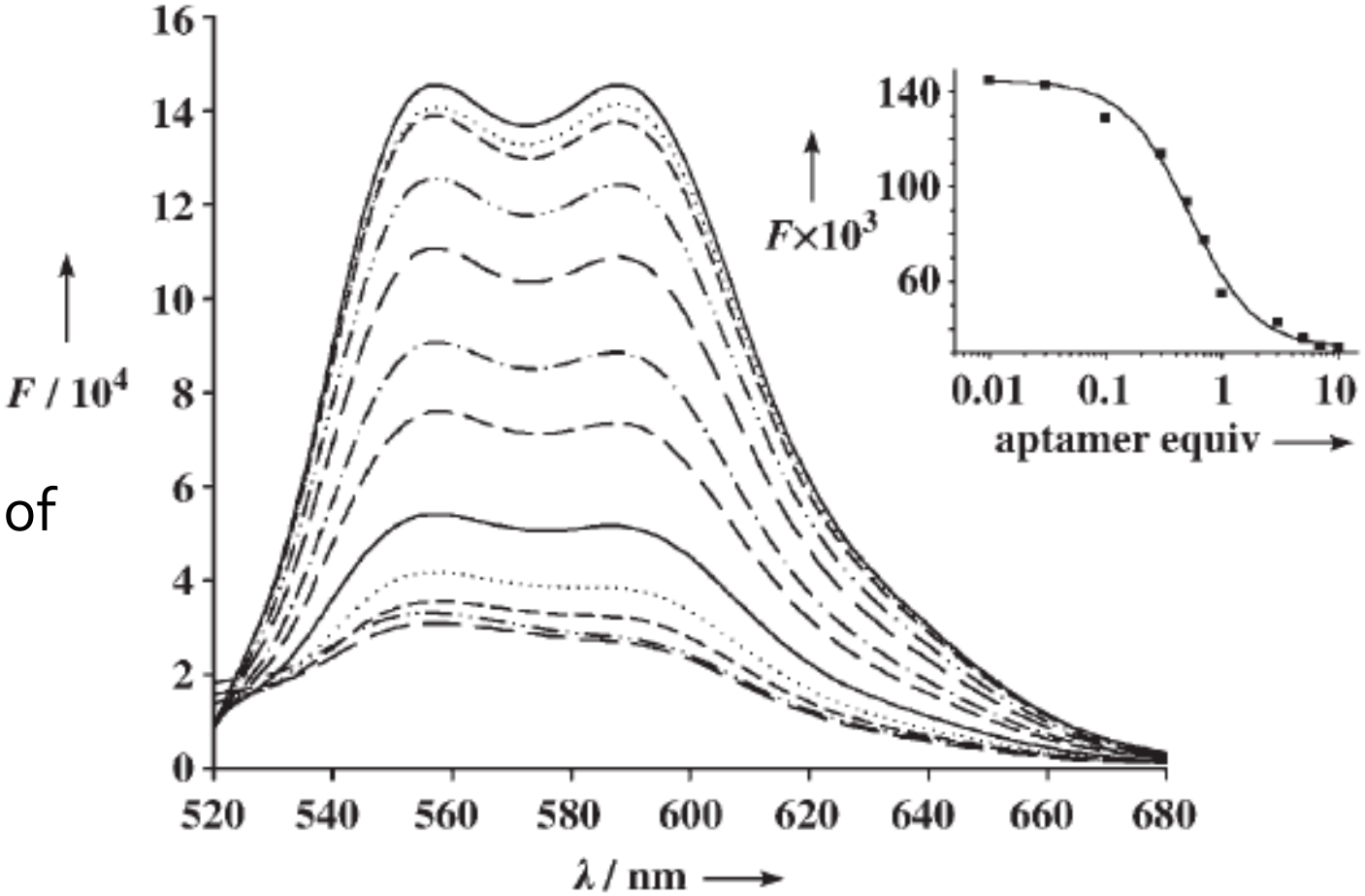
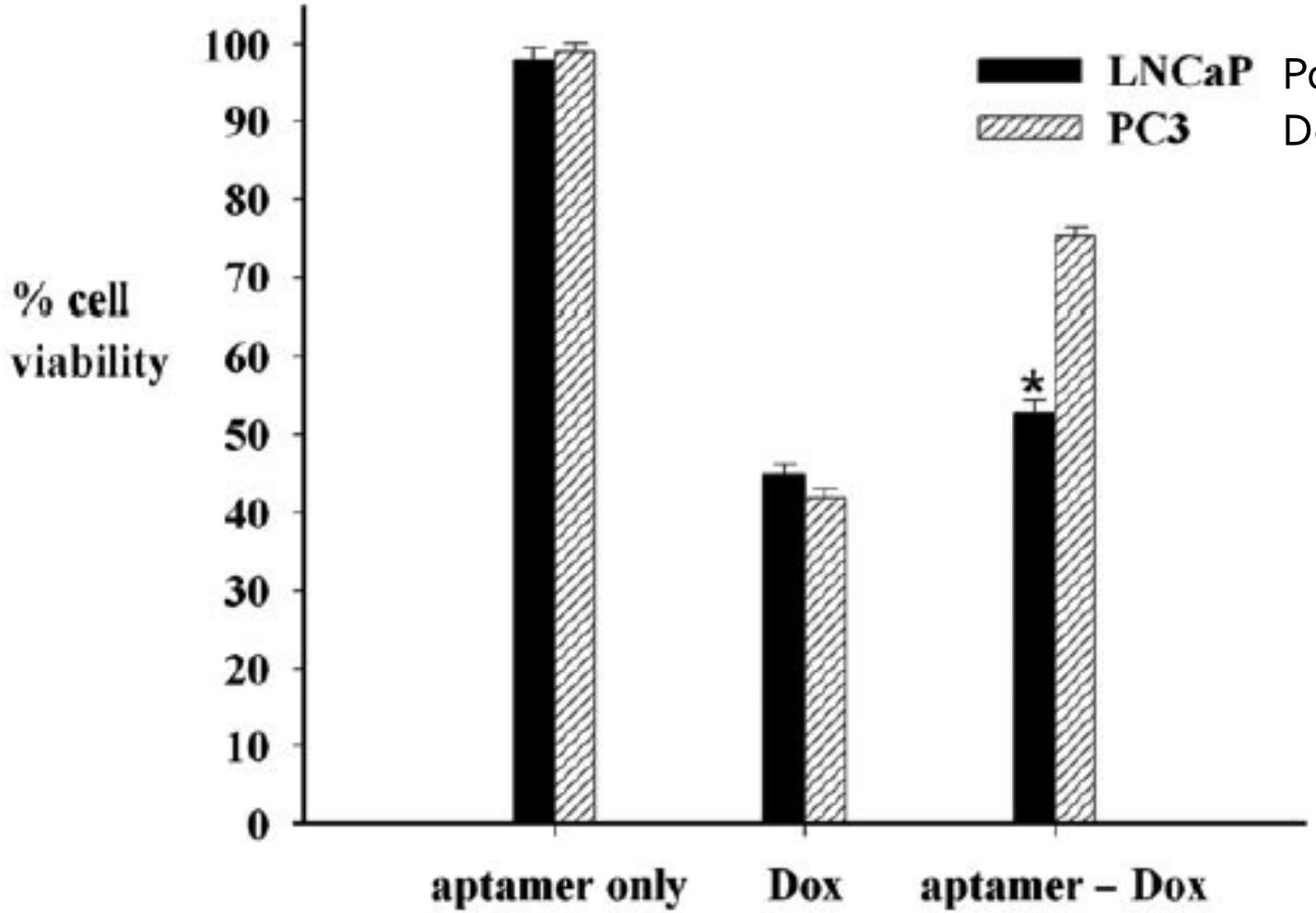
Therapeutic Relevance
Drug Delivery

- Titration of aptamer with constant concentration of doxorubicin



Angew. Chem. Int. Ed. **2006,** 45, 8149 –8152

Therapeutic Relevance
Drug Delivery



Possess PSMA

Does not possess PSMA

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Conclusions

- Aptamers are oligonucleotide strands which bind molecular targets with good binding affinity.
- SELEX provides the ability to select oligonucleotides with high binding affinities for molecular targets from random oligo pools.
- Though SELEX introduced a highly efficient method of selecting and amplifying high binding affinity oligo- strands, continued advances in SELEX may produce aptamers with increased therapeutic potential.

Conclusions

- An FDA approved drug and several clinical trial drugs validate aptamers as therapeutics
- Aptamers can also be used in drug delivery and other applications

Image References

- 1. Ruckman, J. *et al.* 2′-Fluoropyrimidine RNA-based Aptamers to the 165-Amino Acid Form of Vascular Endothelial Growth Factor ($VEGF_{165}$) . *J. Biol. Chem.* **273,** 20556-20567 (1998).
- 2. http://www.instantcast.com/AllStars/CREB
- 3. http://dna14.slu.cz/nanoday.php
- 4. http://www.the-scientist.com/article/home/53763/
- 5. http://mcdb.colorado.edu:8081/mcdb/faculty/researcherobjects/Larry-Gold
- 6. http://www.vandaliaresearch.com/aptamers.php