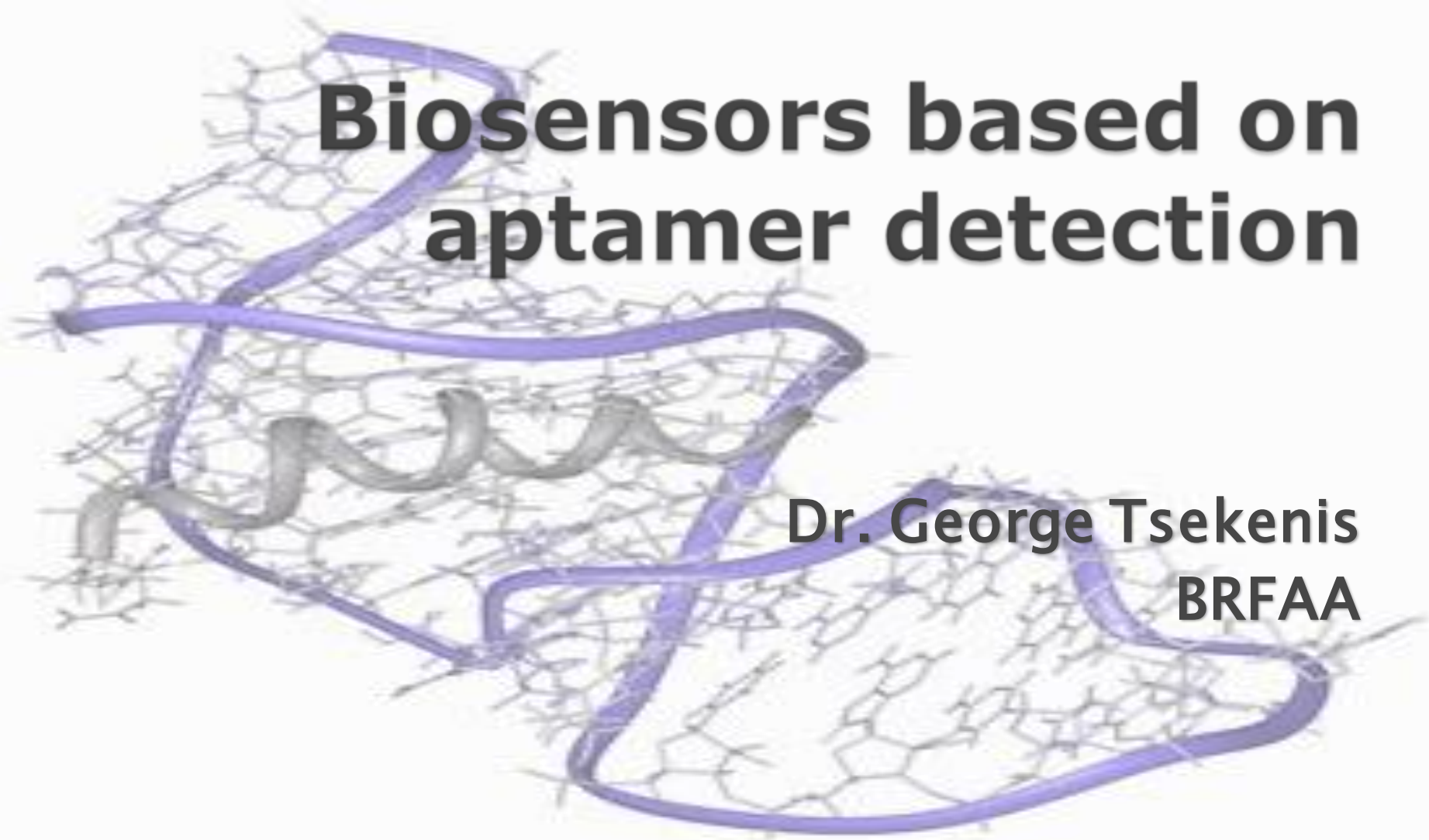
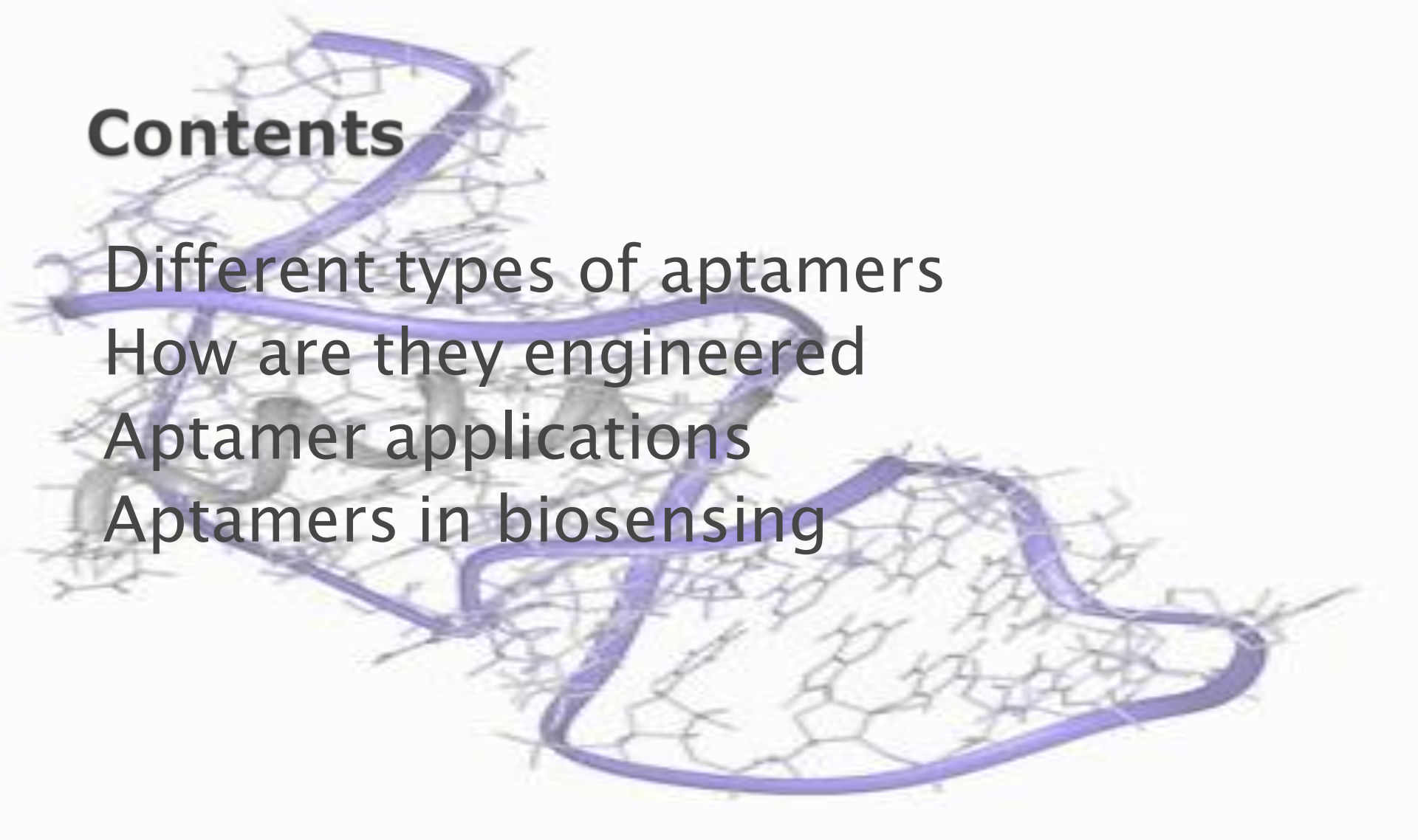
Biosensors based on aptamer detection



Dr. George Tsekenis
BRFAA

1st Training School on Phototech for Biosensors and Energy
Athens, 21-25 October 2013

Biomedical Research Foundation of the Academy of Athens (BRFAA)



Contents

Different types of aptamers
How are they engineered
Aptamer applications
Aptamers in biosensing

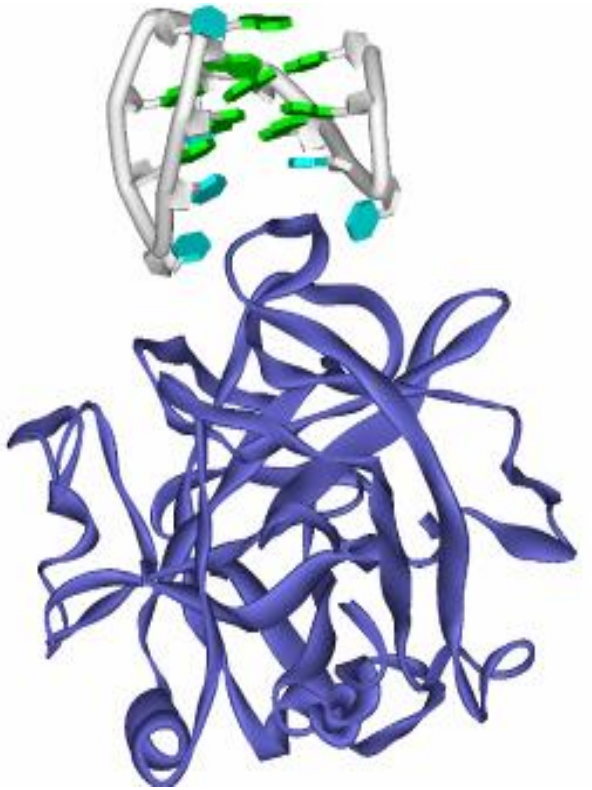
What is an Aptamer?

aptus: "to fit"

mer: "smallest unit of repeating structure"

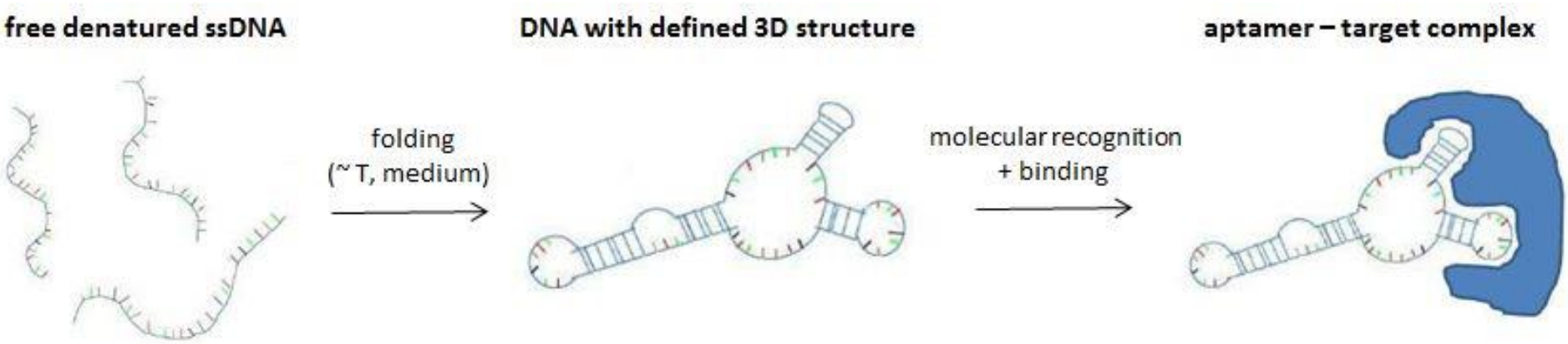
A typical aptamer is 5–15 kDa in size binds its target with high affinity and can discriminate among closely related targets.

Their targets range from small molecules, toxins, peptides, proteins, viruses, bacteria, and even whole eukaryotic cells.



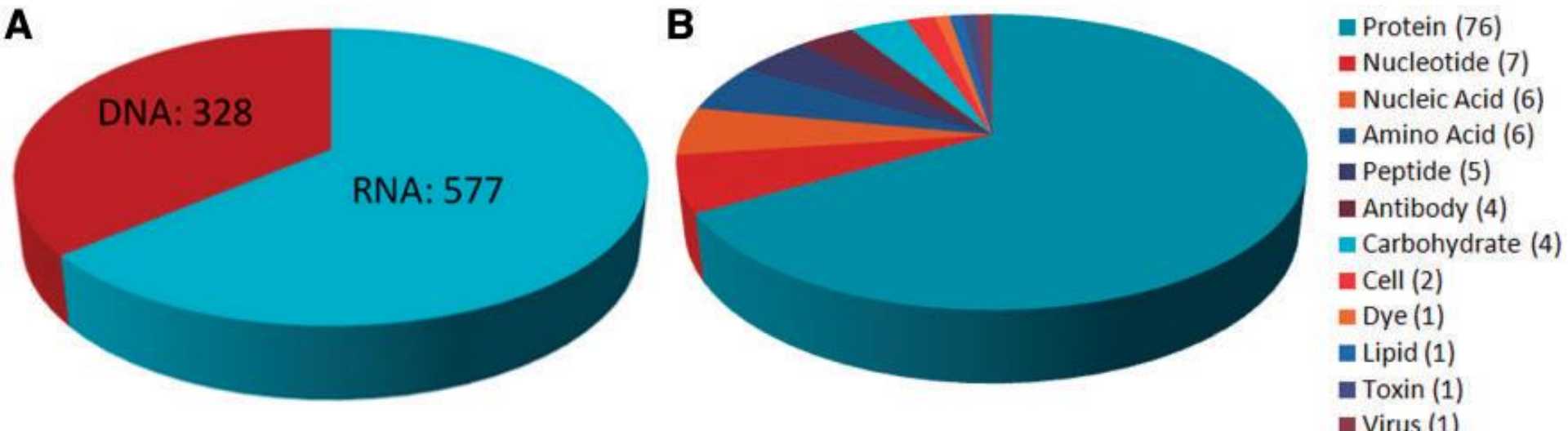
Target recognition

Aptamers are capable of using the same types of binding interactions that drive affinity and specificity in antibody–antigen complexes.

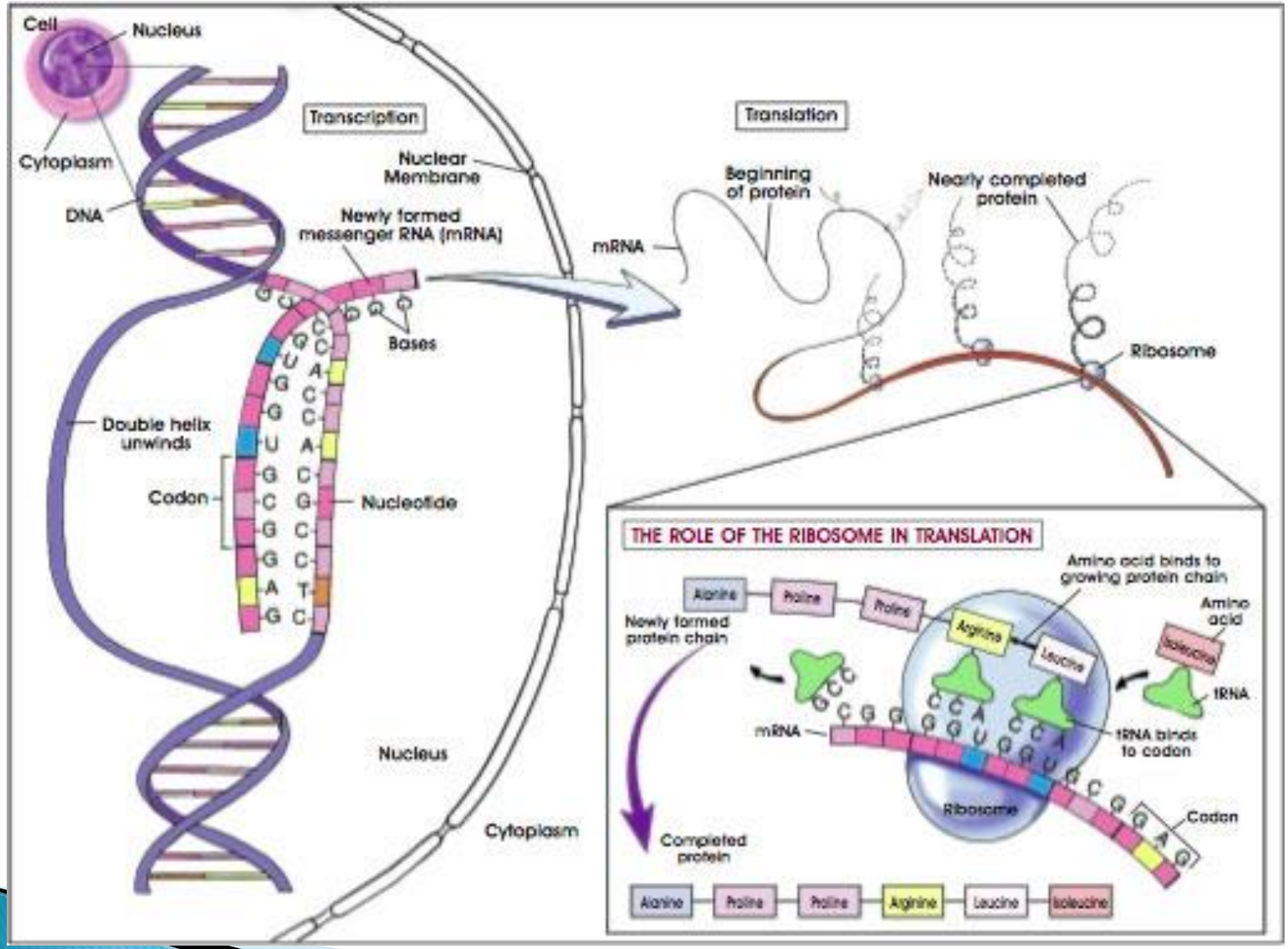


Different types of aptamers

- DNA
- RNA
- Peptide aptamers
- They all share a unique tertiary structure responsible for carrying out molecular recognition



The central dogma of biology



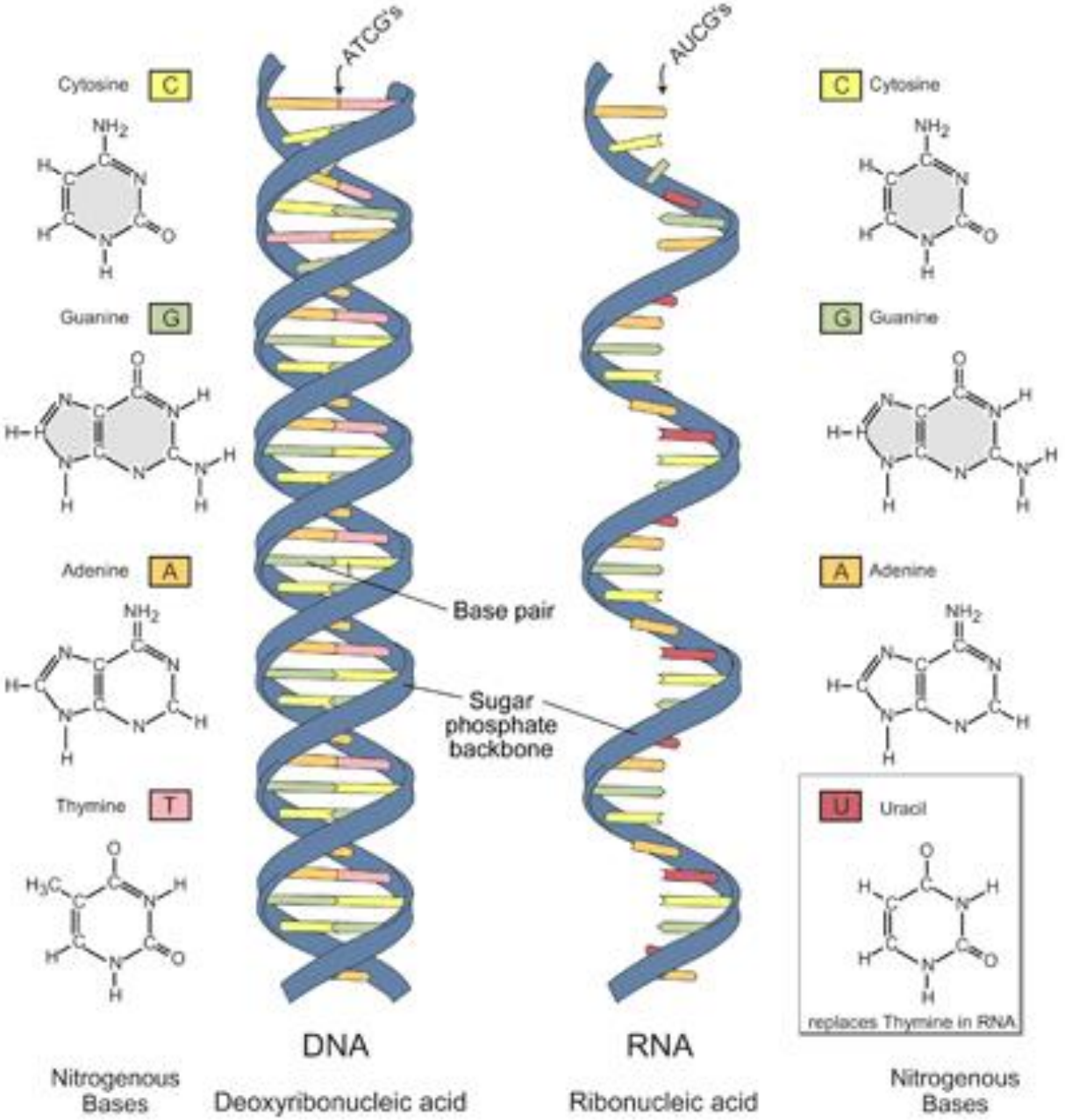
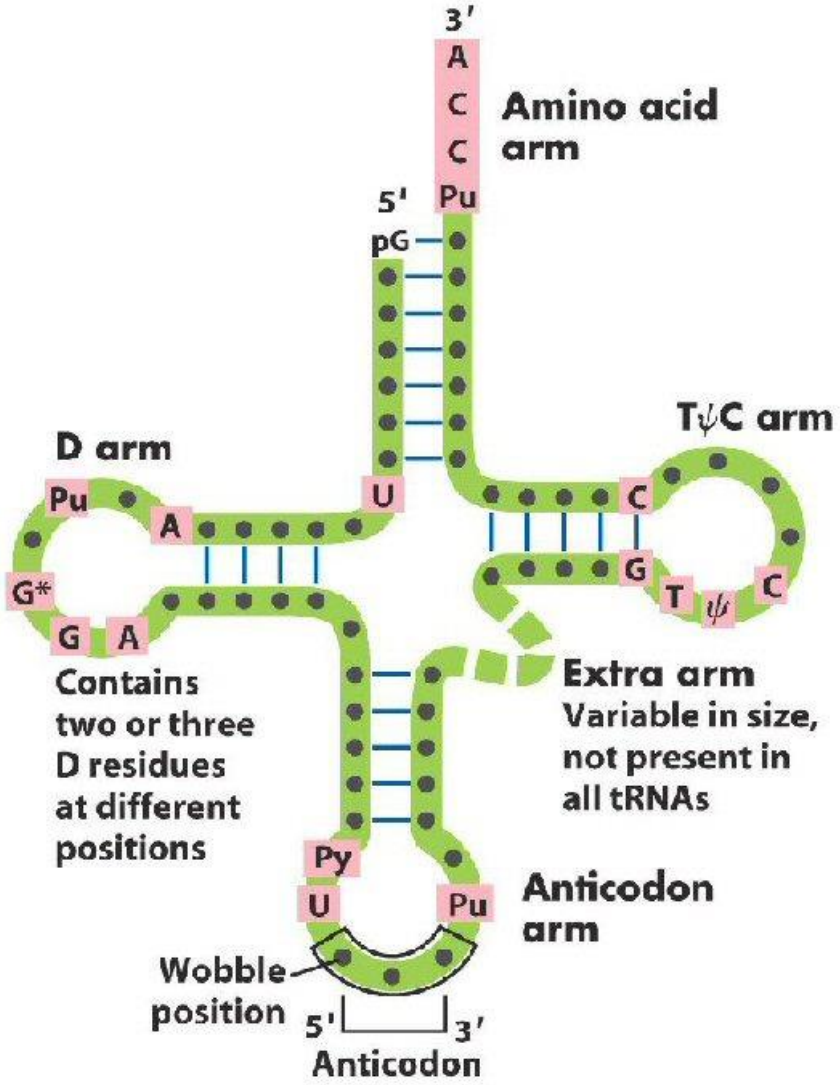
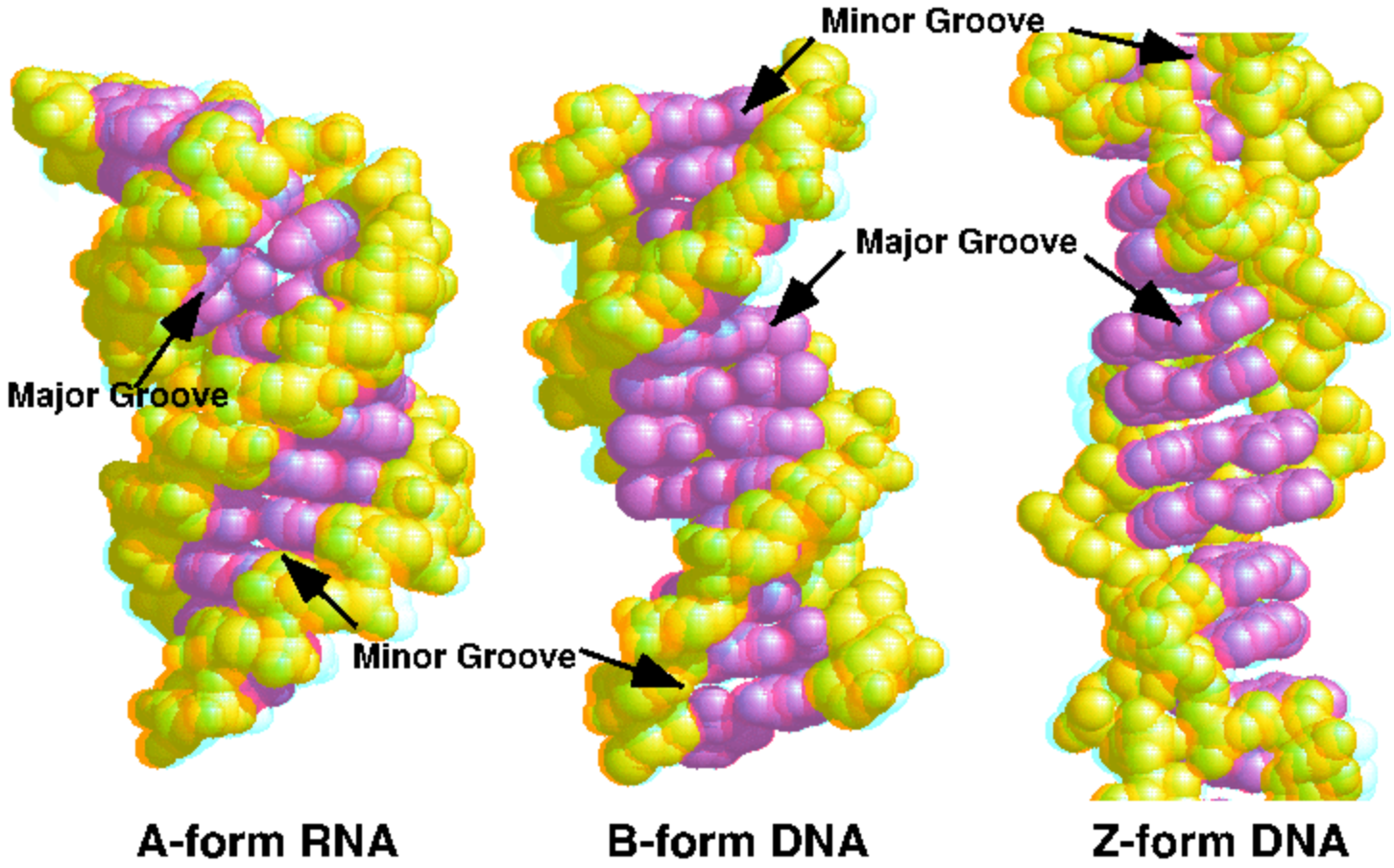


Image adapted from: National Human Genome Research Institute.

RNA is not always single stranded



Different forms of DNA exist



Different forms of DNA exist

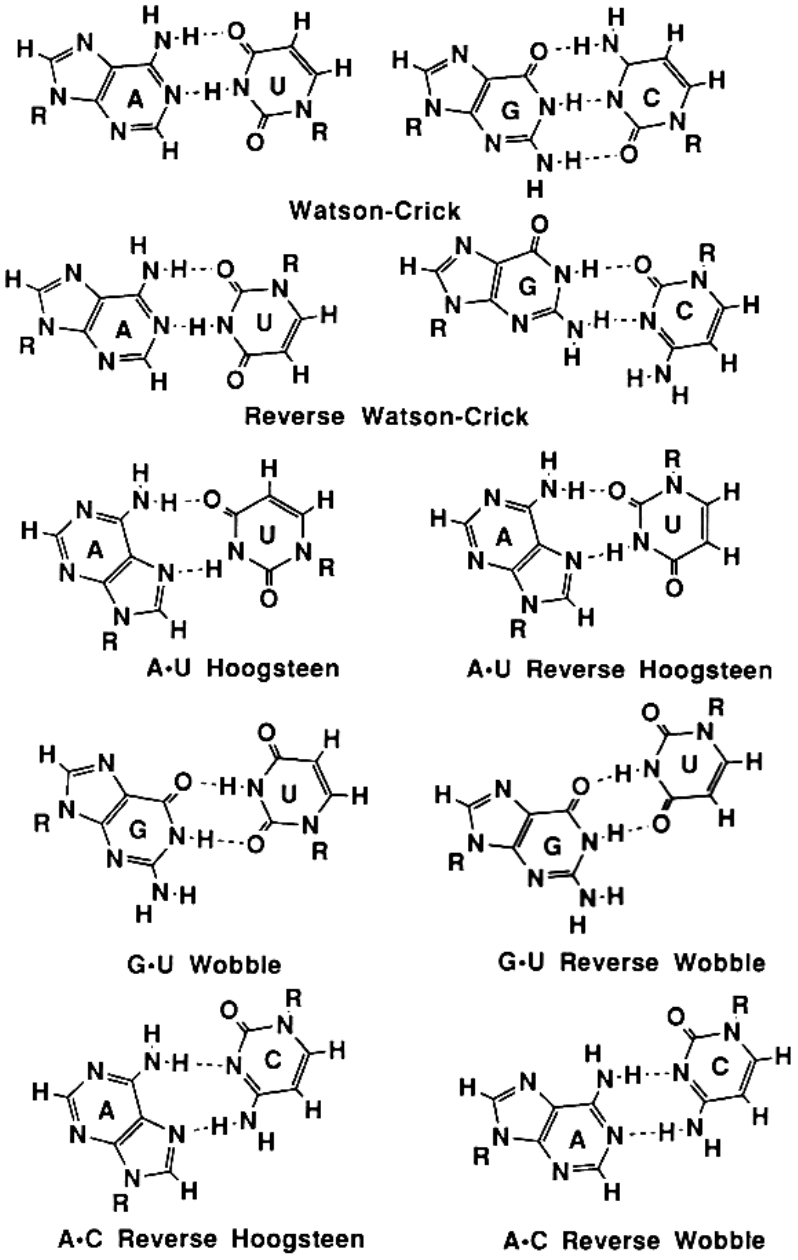
Characteristics	A form of DNA	B form of DNA	Z form of DNA
Helical sense	Right handed	Right handed	Left handed
Diameter	26Å	20Å	18Å
Rise per turn of helix	28Å	36Å	44Å
Base pairs per helical turn	11 base pairs	10 base pairs	12 base pairs
Helix rise per base pair	2.6Å	3.6Å	3.7Å
Base tilt normal to the helix axis	20^0	6^0	7^0
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidine and syn for purines

Table: Differences between various forms of DNA (A-DNA, B-DNA and Z-DNA

Namrata Heda

Nucleic Acid Base Pairs

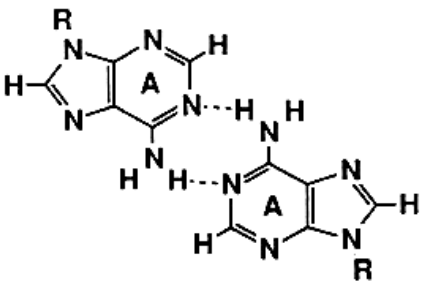
The ten possible purine-pyrimidine base pairs.



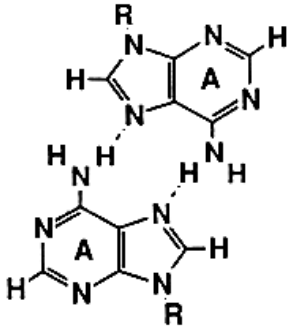
Source: Ignacio Tinoco, Jr. in Gesteland, R. F. and Atkins, J. F. (1993) THE RNA WORLD. Cold Spring Harbor Laboratory Press.

Nucleic Acid Base Pairs

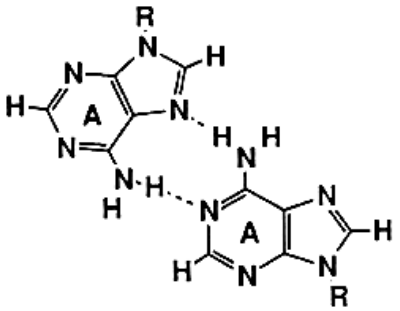
The seven possible homo-purine base pairs.



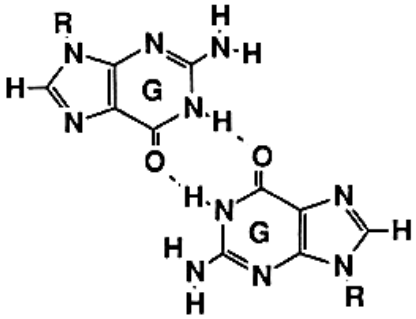
A•A N1-amino, symmetric



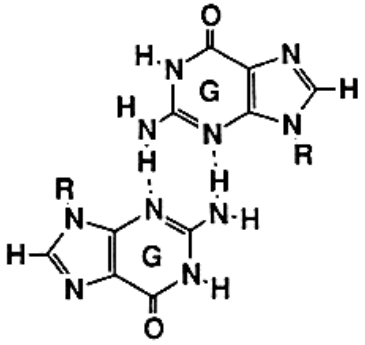
A•A N7-amino, symmetric



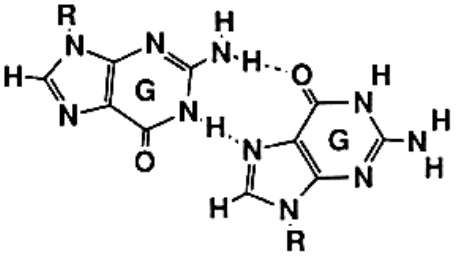
A•A N1-amino, N7-amino



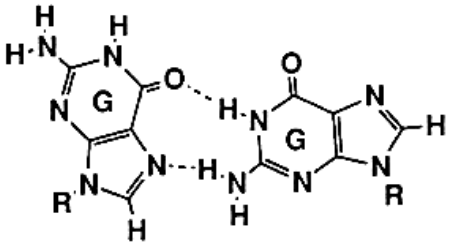
G•G N1-carbonyl, symmetric



G•G N3-amino, symmetric



G•G N7-N1, carbonyl-amino

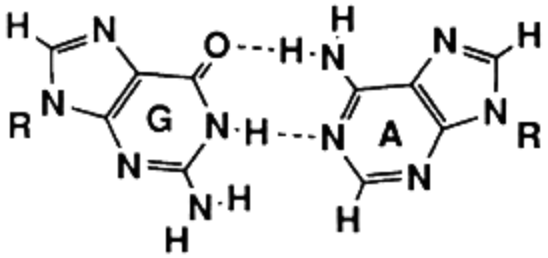


G•G N1-carbonyl, N7-amino

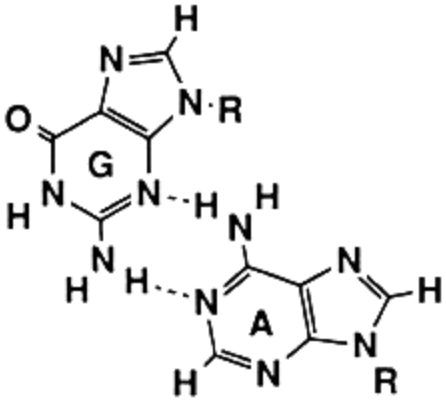
Source: Ignacio Tinoco, Jr. in Gesteland, R. F. and Atkins, J. F. (1993) THE RNA WORLD. Cold Spring Harbor Laboratory Press.

Nucleic Acid Base Pairs

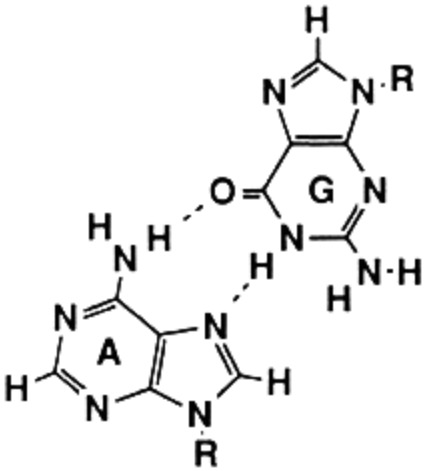
The four possible hetereo-purine base pairs.



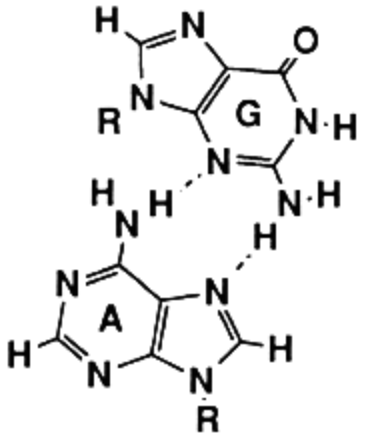
G•A N1-N1, carbonyl-amino



G•A N3-amino, amino-N1



A•G N7-N1, amino-carbonyl

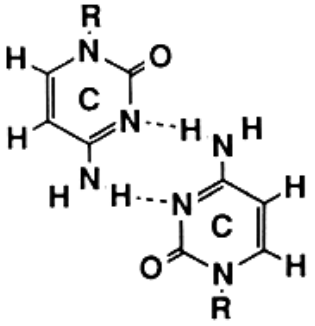


A•G N7-amino, amino-N3

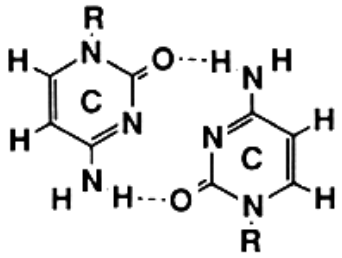
Source: Ignacio Tinoco, Jr. in Gesteland, R. F. and Atkins, J. F. (1993) THE RNA WORLD. Cold Spring Harbor Laboratory Press.

Nucleic Acid Base Pairs

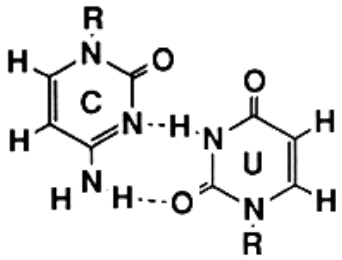
The seven possible pyrimidine-pyrimidine base pairs.



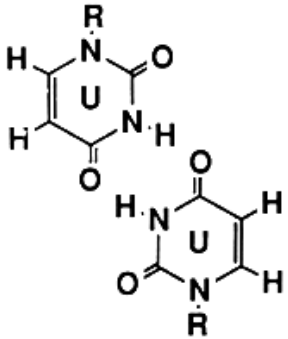
C•C N3-amino, symmetric



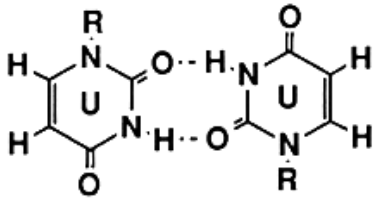
C•C carbonyl-amino, symmetric



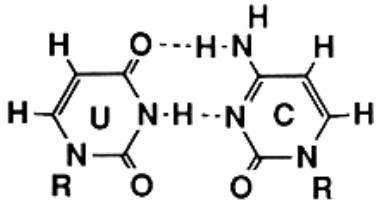
C•U N3-N3, 2-carbonyl-amino



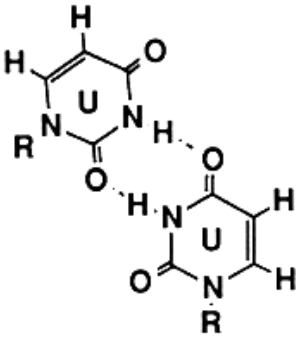
U•U 4-carbonyl-N3, symmetric



U•U 2-carbonyl-N3, symmetric

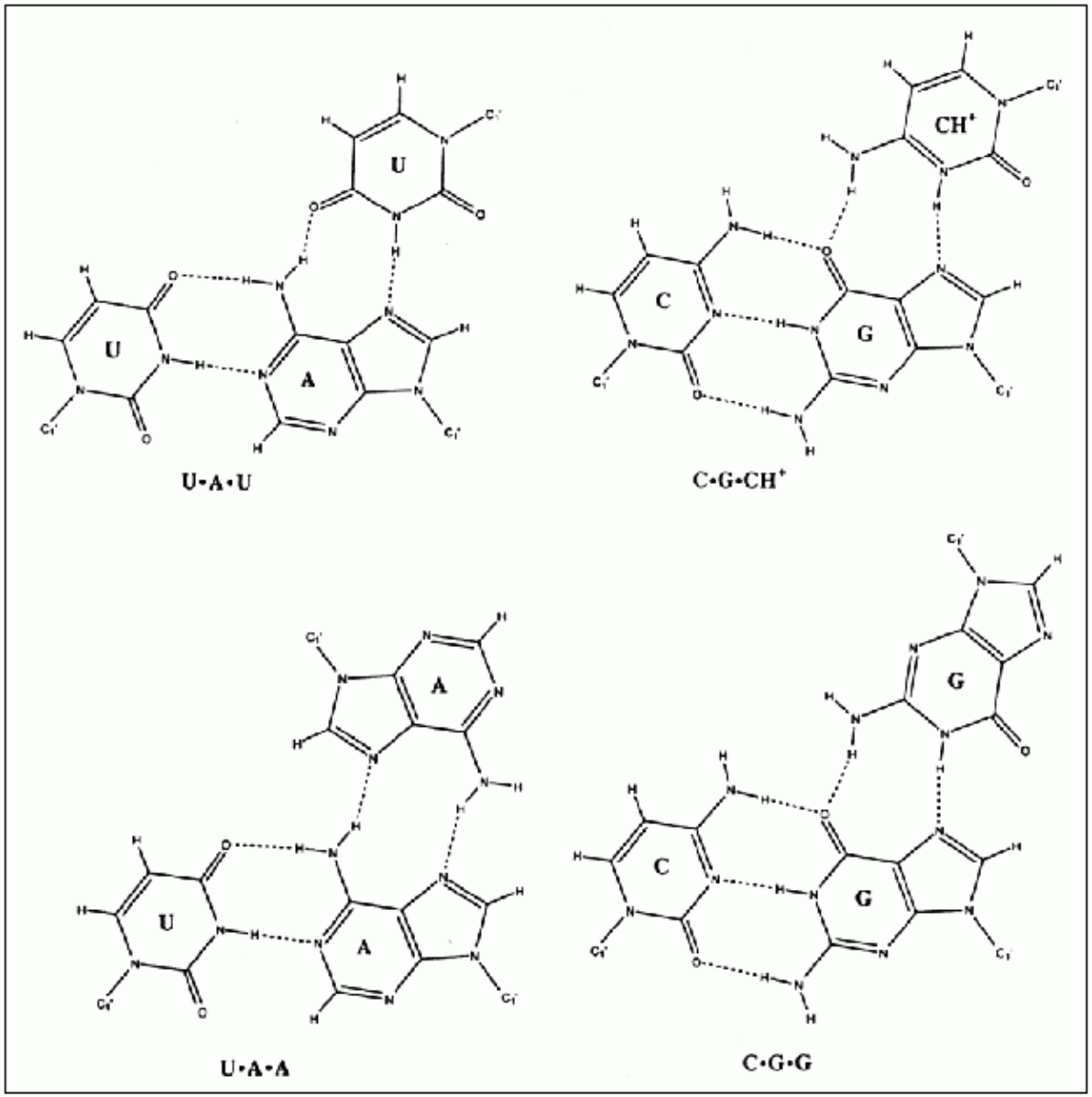


U•C N3-N3, 4-carbonyl-amino



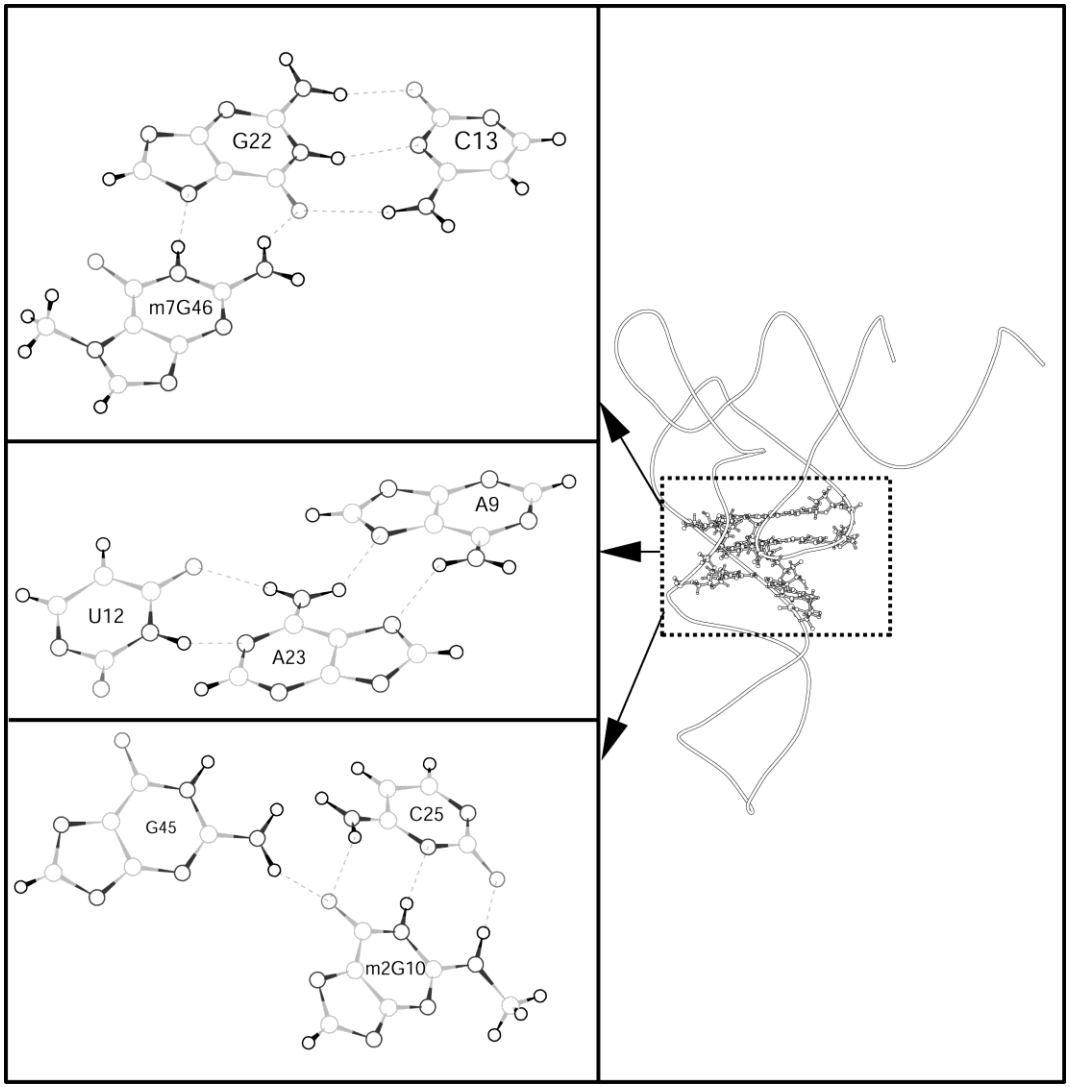
U•U 2-carbonyl-N3, 4-carbonyl-N3

Selected Base Triples



Source: Chastain, M. and Tinoco Jr., I., (1991) *Prog. Nucleic Acid Res. Mol. Biol.* **41**, 131-177.

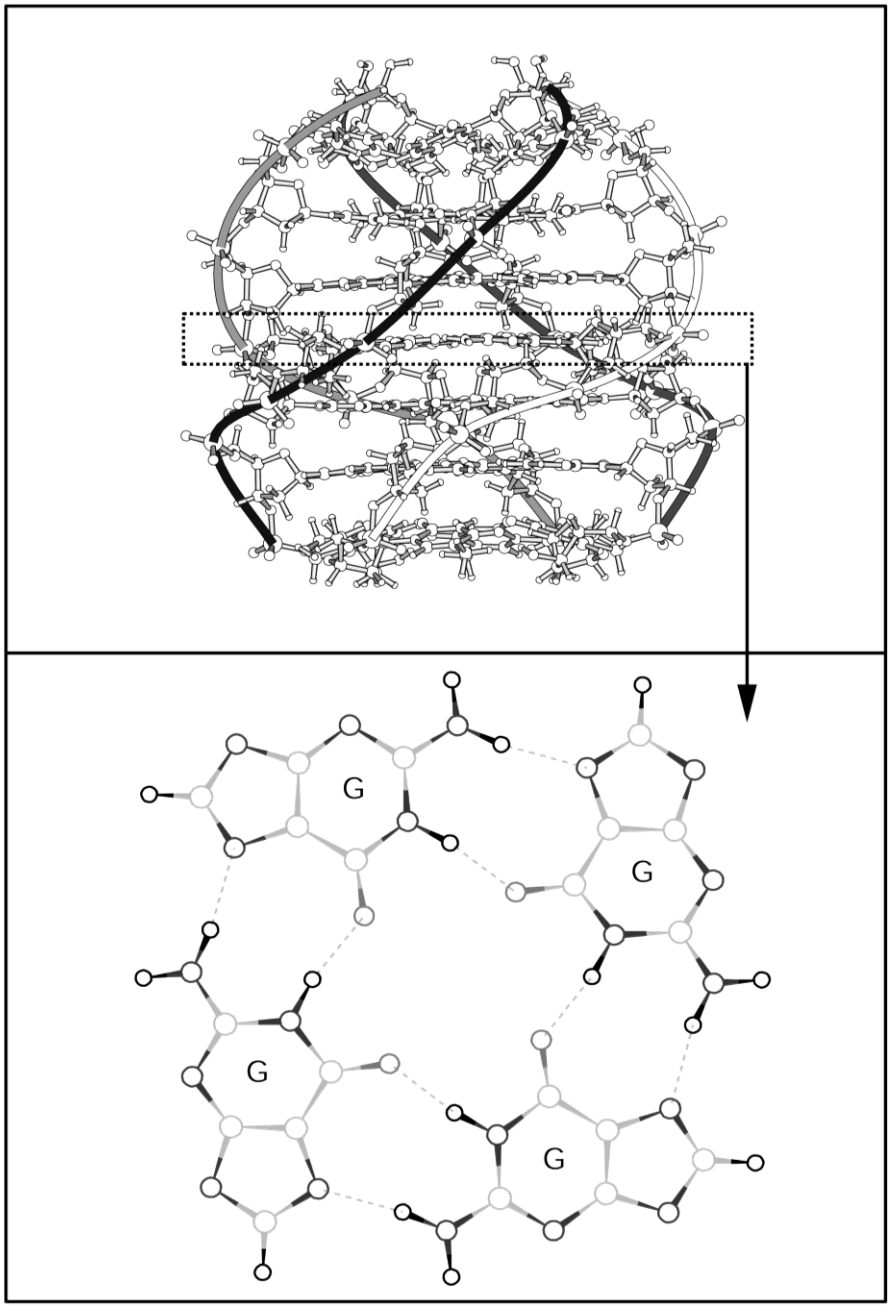
Base Triples in tRNA



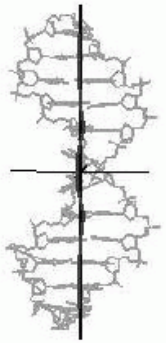
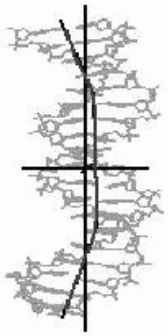
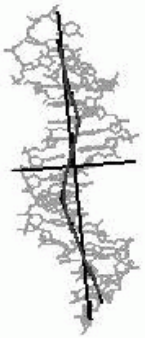
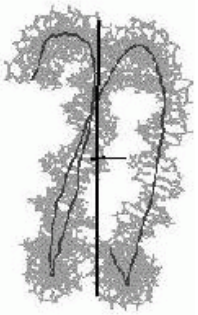
Base triads in the crystal structure of yeast phenylalanine transfer RNA (PDB code: 4tna).

Base Tetrads in a DNA Tetraplex

Parallel-stranded DNA tetraplex formed from the *Tetrahymena* telomeric sequence d(TTGGGGT) solved by NMR spectroscopy.
The structure contains four stacked G-tetrads in the center and additional T-tetrads.



Nucleic Acid Structure - Bending

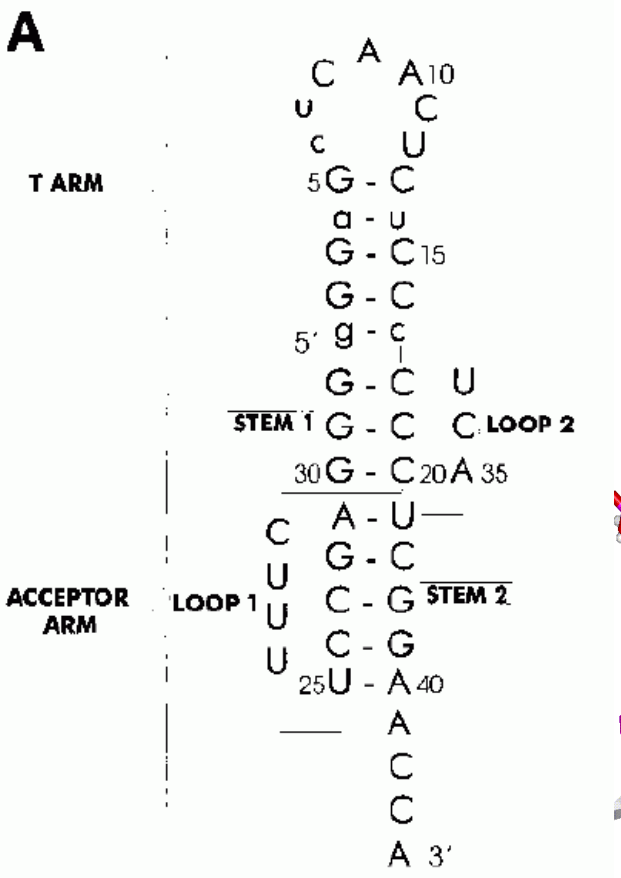
straight	**circular (arc)**	**single-kink**	**double-kink** *(small twist angle)*	**double-kink** *(large twist angle)*	**superhelix**
					
Mono | stereo view; Two views rotated by 90 ° about the main principal axis of the curvilinear helical axis (PDF)	Mono | stereo view; Two views rotated by 90° about the main principal axis of the curvilinear helical axis (PDF)	Mono | stereo view; Two views rotated by 90° abou the main principal axis of the curvilinear helical axis (PDF)	Mono | stereo view; Two views rotated by 90° about the main principal axis of the curvilinbear helical axis (PDF)	Mono | stereo view; Two views rotated by 90° about the main principal axis of the curvilinear helical axis (PDF)	Mono | stereo view (PDF)
protein/DNA complex (Eco RI)	*RNA duplex* -	*DNA/RNA hybrid* -	*protein/DNA complex* (Cro)	*DNA duplex* (HIV-1 kappa B site)	*protein/DNA complex* (nucleosome core particle)
1eri | pde001	402d | ar0001	1d88 | ahj044	3cro | pdr001	2kbd	1aoi | pd0001

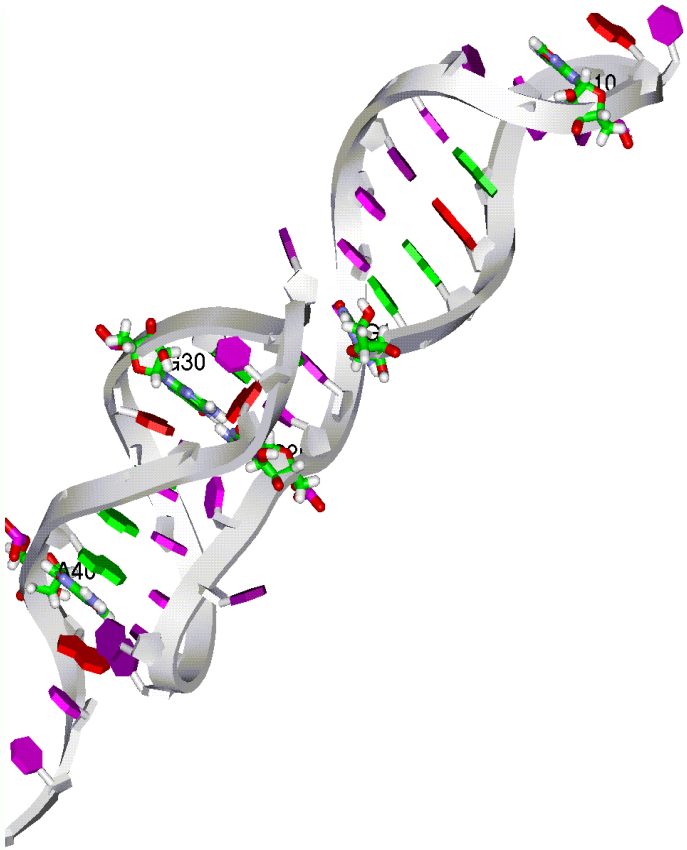
Pseudoknots

NMR Structure of a Classical Pseudoknot: Interplay of Single- and Double-Stranded RNA

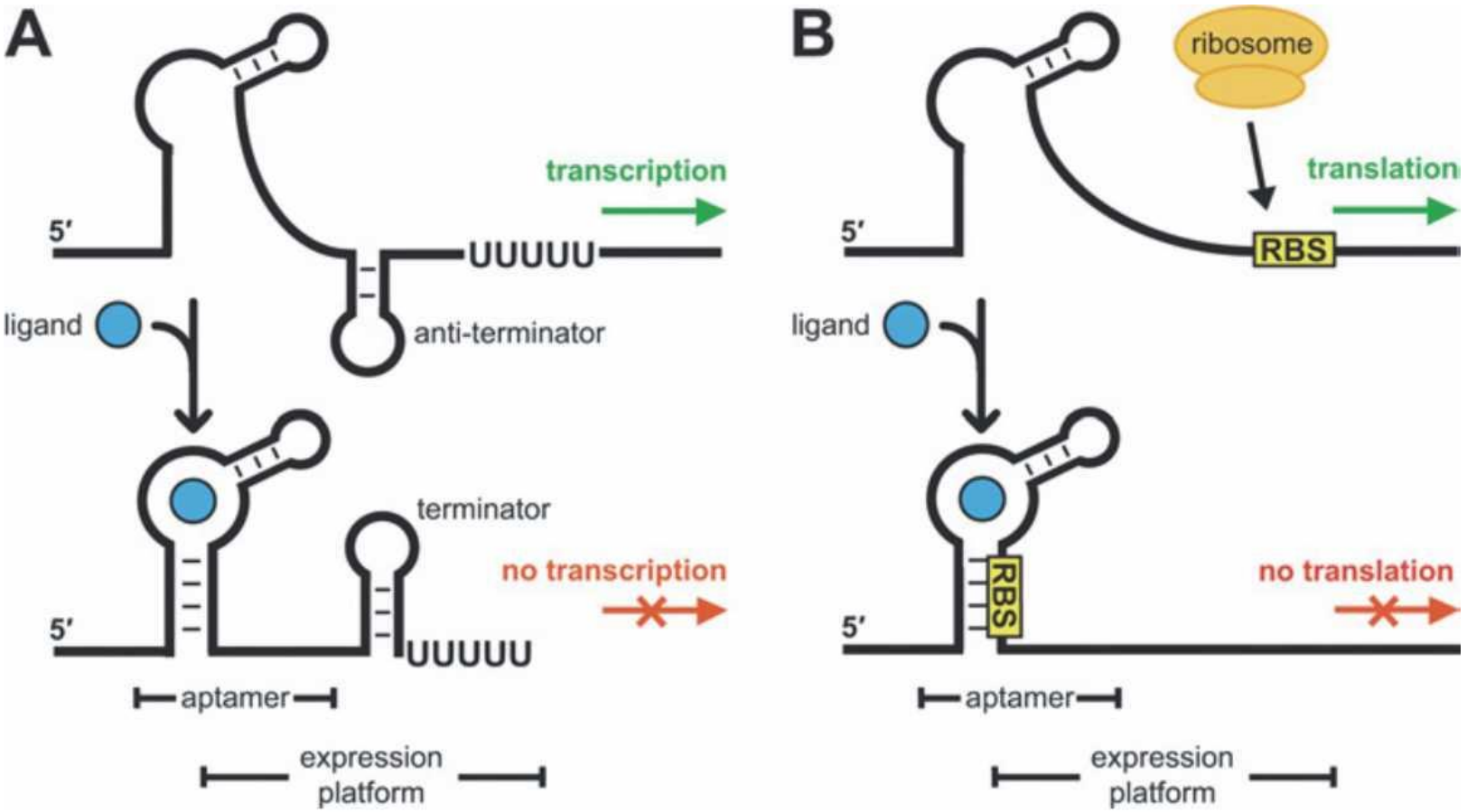
Michaël H. Kolk, Marinette van der Graaf,*
Sybren S. Wijmenga,† Cornelis W. A. Pleij,‡ Hans A. Heus,
Cornelis W. Hilbers§

SCIENCE • VOL. 280 • 17 APRIL 1998 • www.sciencemag.org





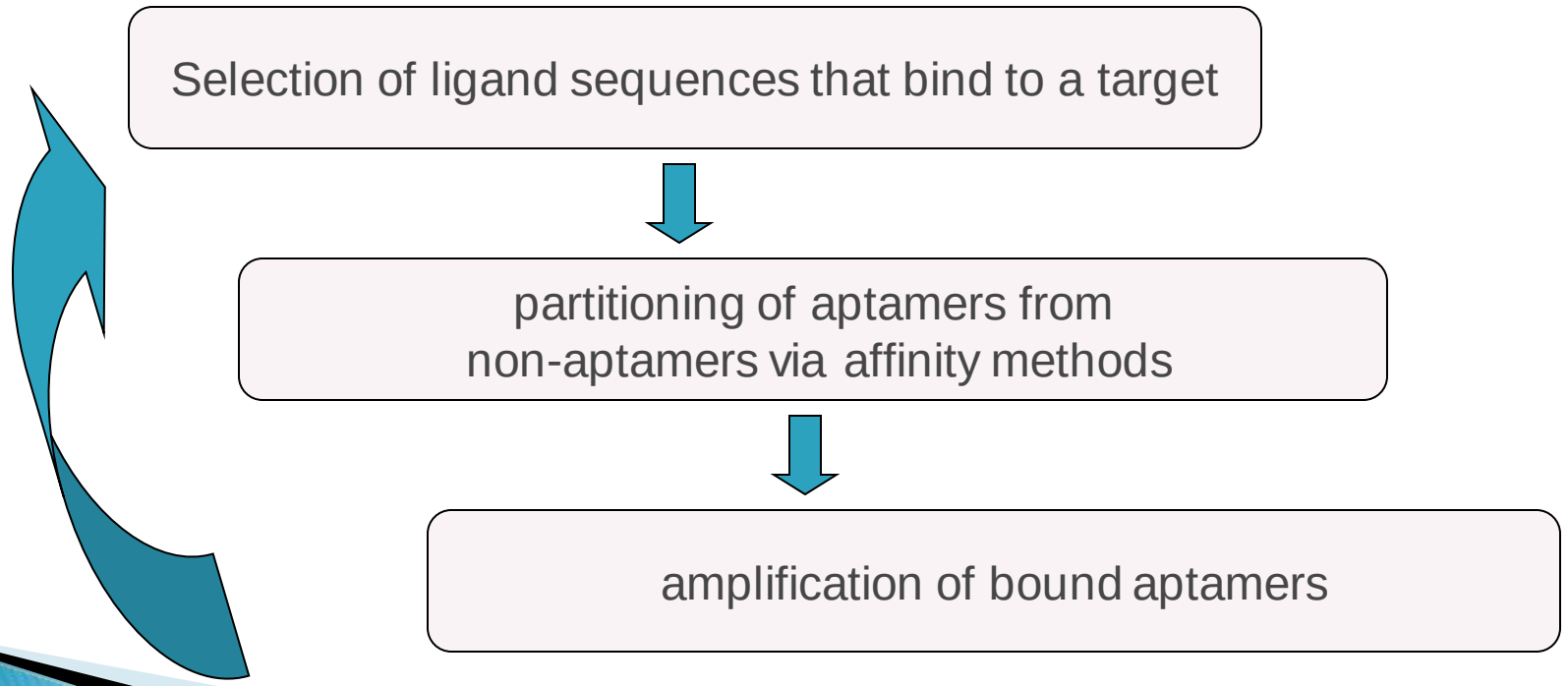
Riboswitches–the natural aptamers

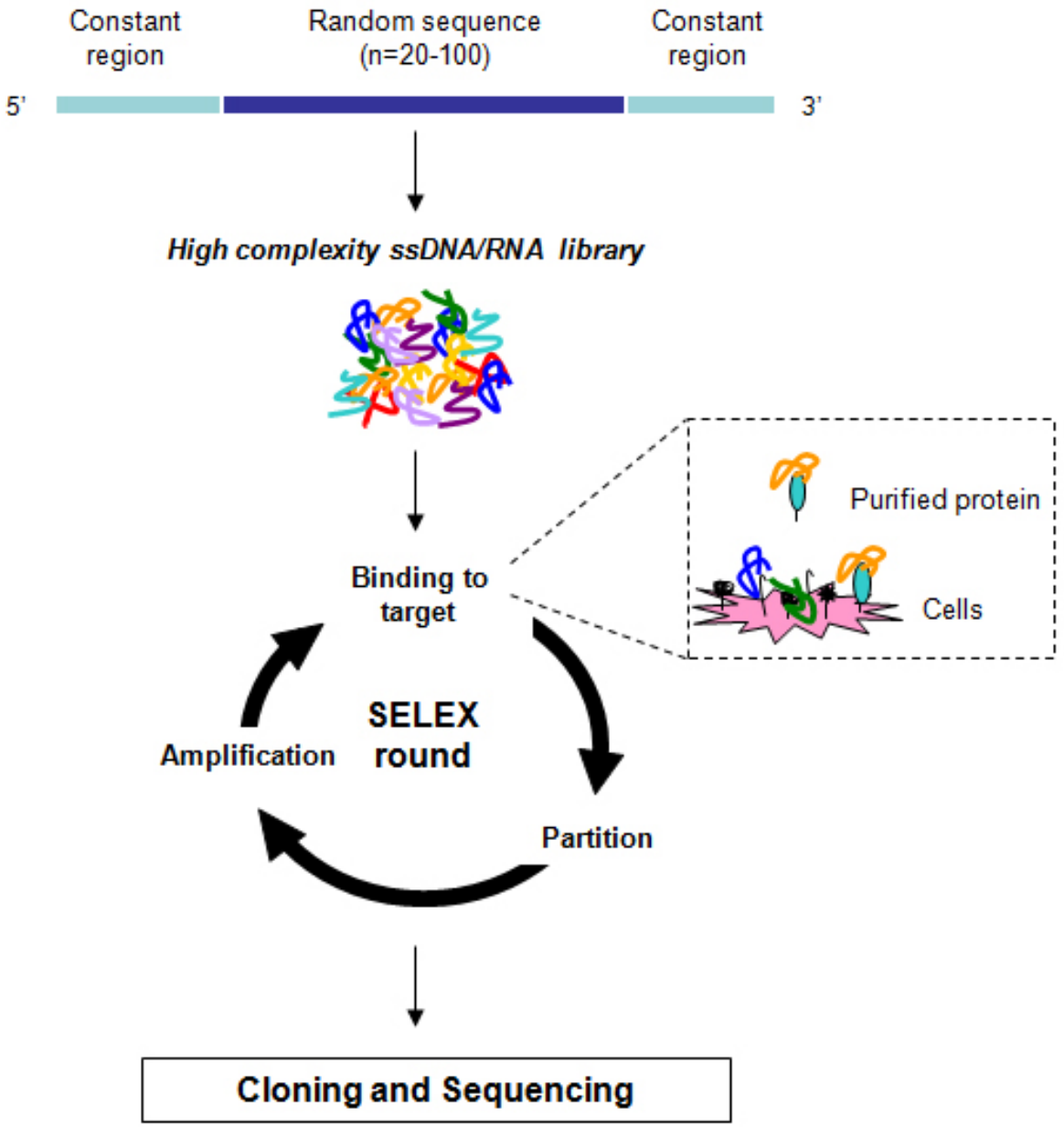


Artificially engineered aptamers

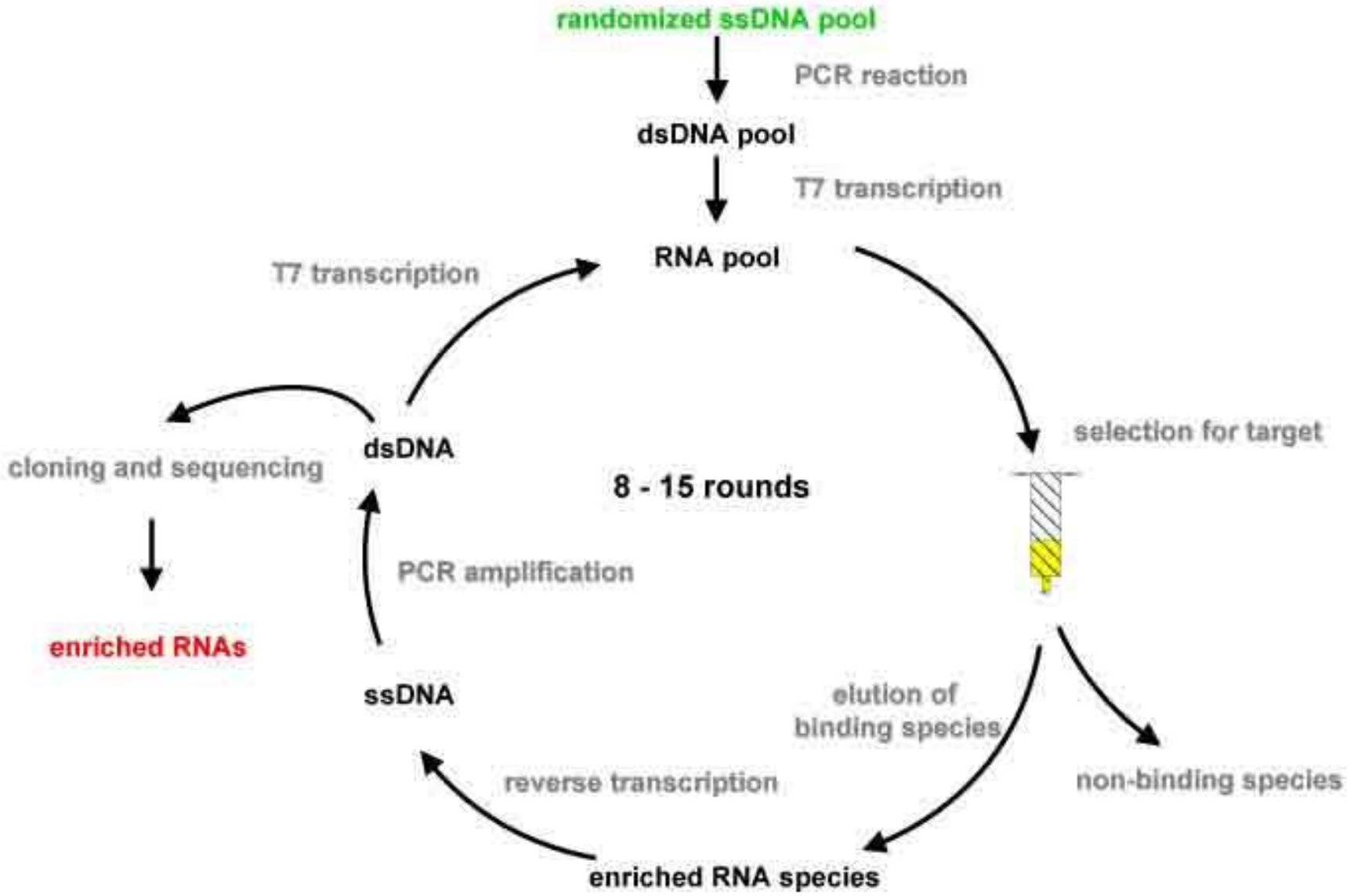
SELEX (systematic evolution of ligands by exponential enrichment) is a process that involves the progressive purification from a combinatorial library of nucleic acid ligands with a high affinity for a particular target by repeated rounds of partitioning and amplification.

Three Processes




Constant region
Random sequence (n=20-100)
Constant region
5'
3'
High complexity ssDNA/RNA library
Purified protein
Cells
Binding to target
SELEX round
Amplification
Partition
Cloning and Sequencing

The RNA SELEX Process



Modification to SELEX techniques

Setting the Ground for First Selections (Negative, Counter-SELEX).

Improving the libraries (blended, photo, cDNA, Spiegelmer SELEX).

First attempts to enter the cell environment (Chimeric, In vivo SELEX).

Aptamer regulation and detection (Beacon, toggle, tailored-SELEX).

Updating SELEX Method with Modern Technologies (Capillary electrophoresis, FluMAG, TECS-SELEX, Cell specific, Microfluidics)

Bioinformatics Approaches for SELEX

SELEX techniques

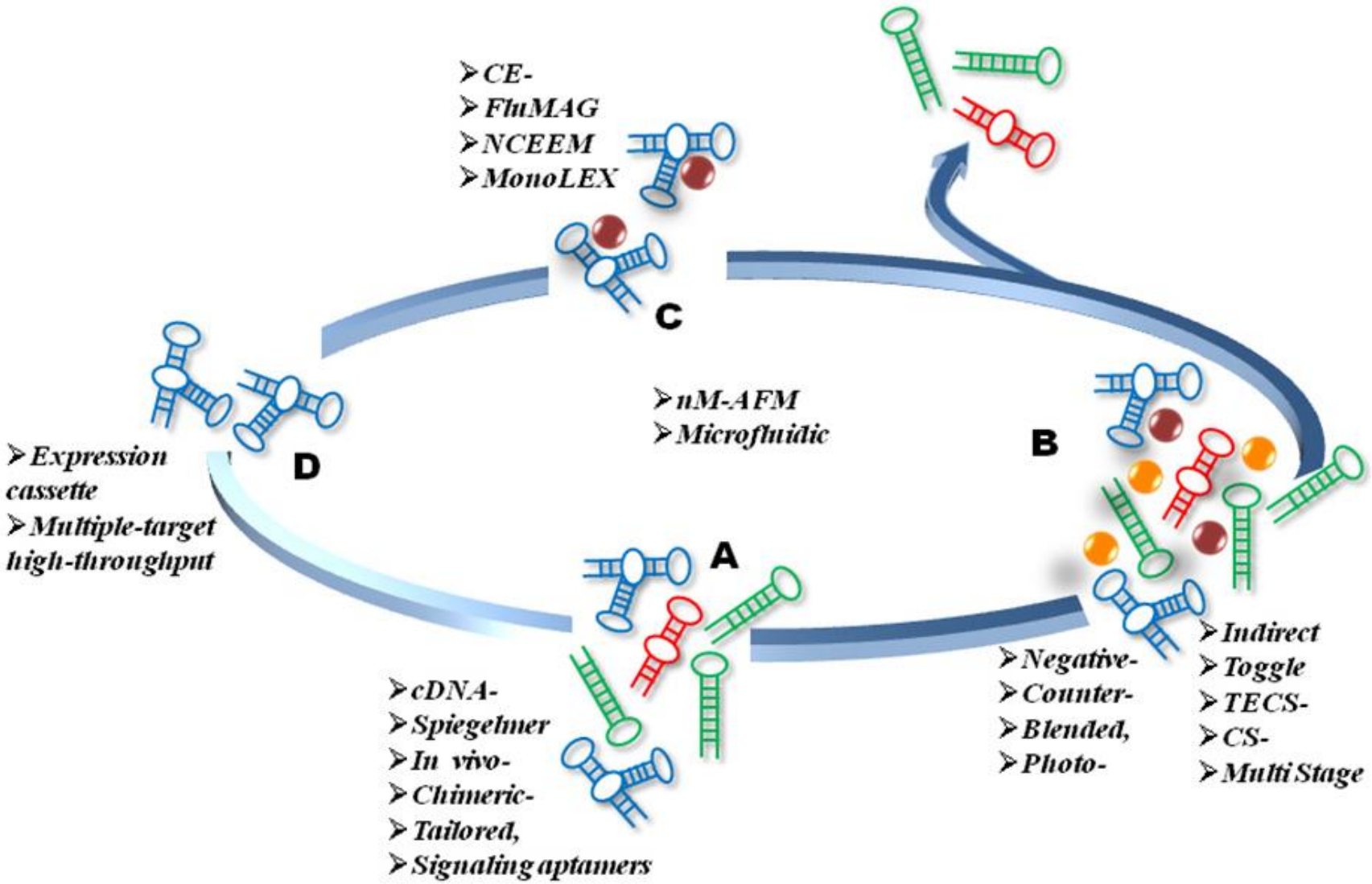
Table 1. Timeline of emerging modifications of SELEX.

Year	SELEX type	References *
1990–1993	Classic, Negative	1,2,5
1994	Counter or Subtractive	6,7
1995	Blended (Covalent), Photoselex (crosslinked), cDNA-SELEX	8–10
1996	Spiegelmer isolation	12
1997	*In vivo*	13
1998	Chimeric	14
1999	Multistage, Cell Specific SELEX(CS-SELEX)	15
2000	Beacon aptamers, Indirect	16–18
2001	Toggle	19,20
2002	Expression cassette	21
2003	Tailored-SELEX	22
2004	CE-SELEX	23
2005	FluMAG	24
2006	TECS-SELEX, NON-SELEX (NCEEM)	25,27
2007	NanoSelection® (nM-AFM SELEX), MonoLEX	28,30
2008	CS-SELEX	31,32
2009	Next-generation SELEX	33
2010	Microfluidic-SELEX, Bioinformatics analyses	36,37,43,44
2011	Multiple-target high-throughput SELEX	38–41

* references correspond to the first report of each type of SELEX; ■ Setting the ground; ■ Improving the libraries; ■ Entering the cell environment; ■ Regulation and detection; ■ Updating SELEX with modern Technologies.

Int. J. Mol. Sci. **2011**, *12*, 9155-9171

SELEX techniques



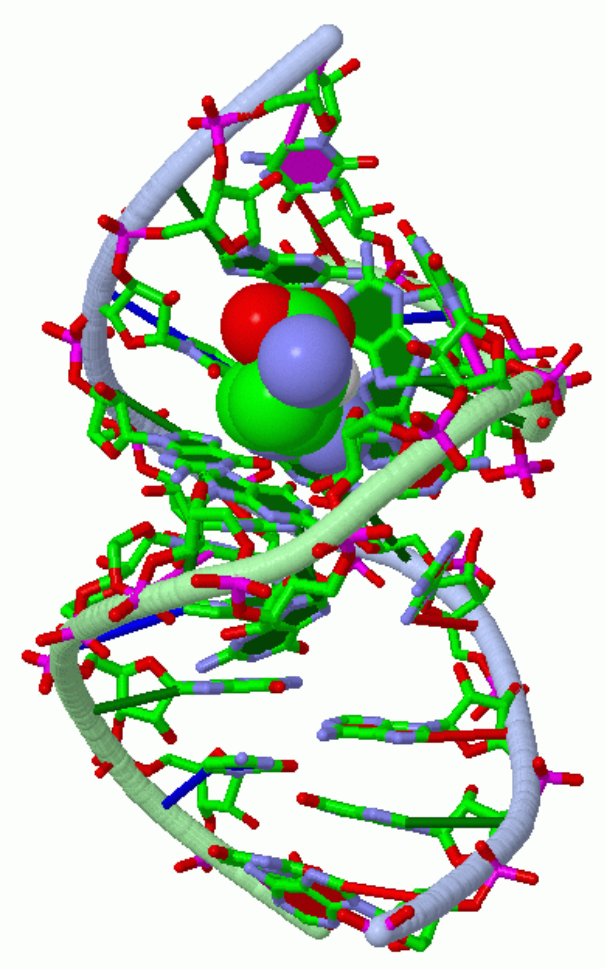
Int. J. Mol. Sci. **2011**, *12*, 9155-9171

Aptamer Structures

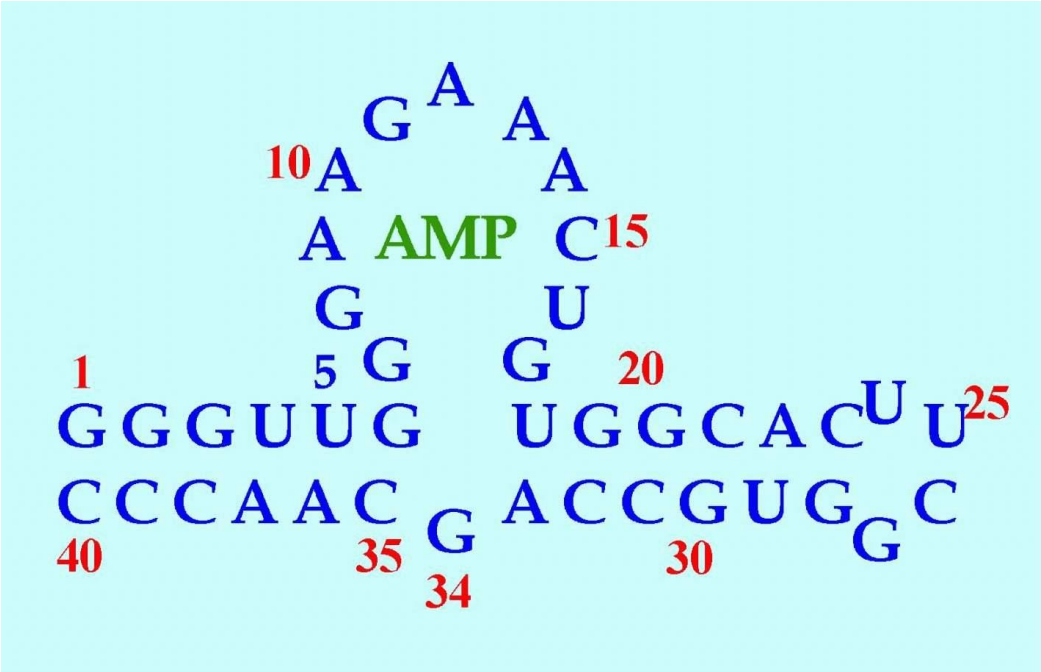
Nf-Kappab (P50)2 Complexed To A High-Affinity RNA Aptamer

Aptamer Structures

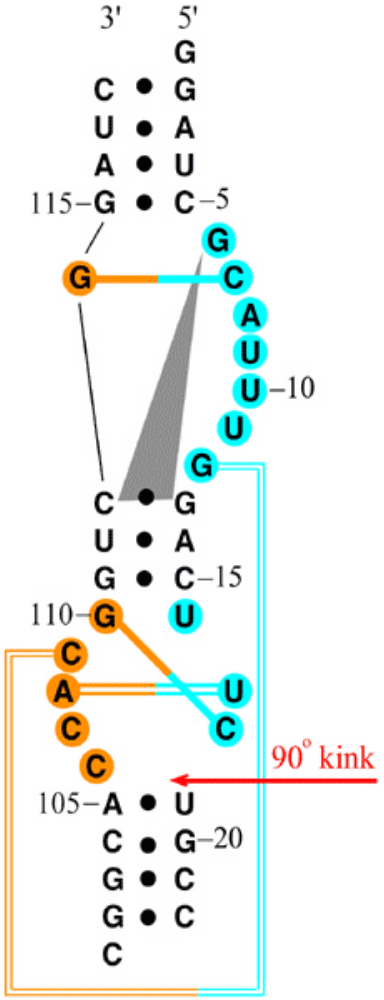
RNA aptamer complex with arginine

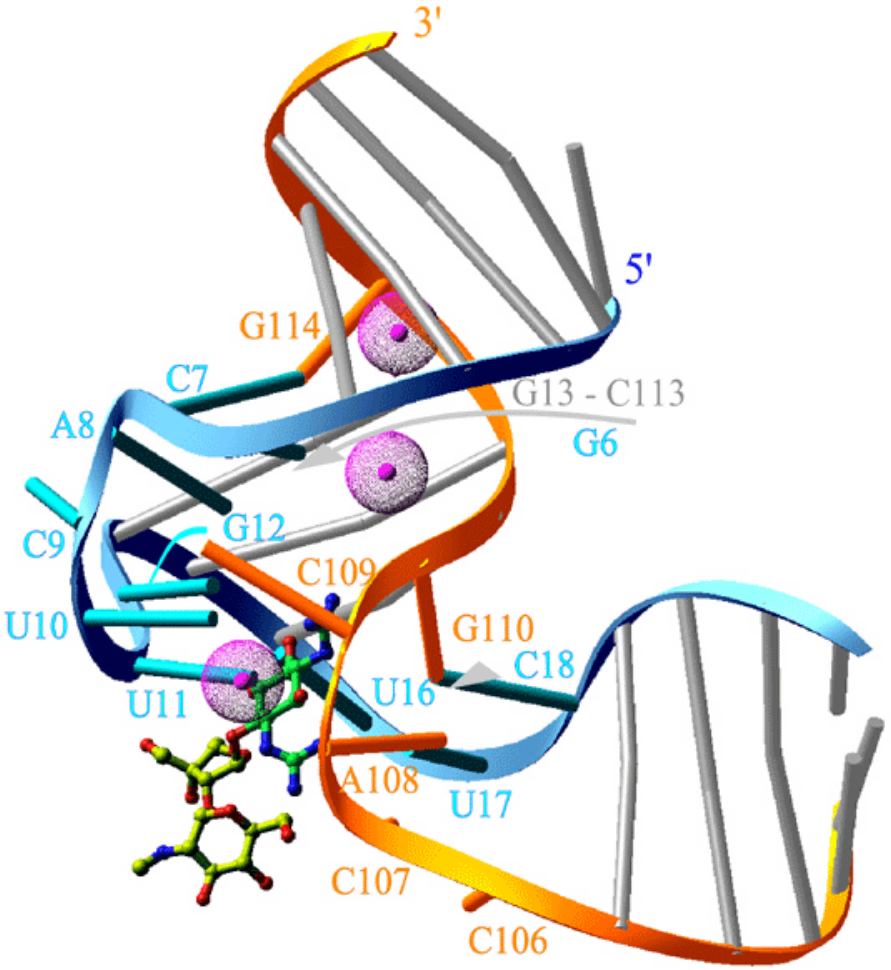


AMP-binding aptamer

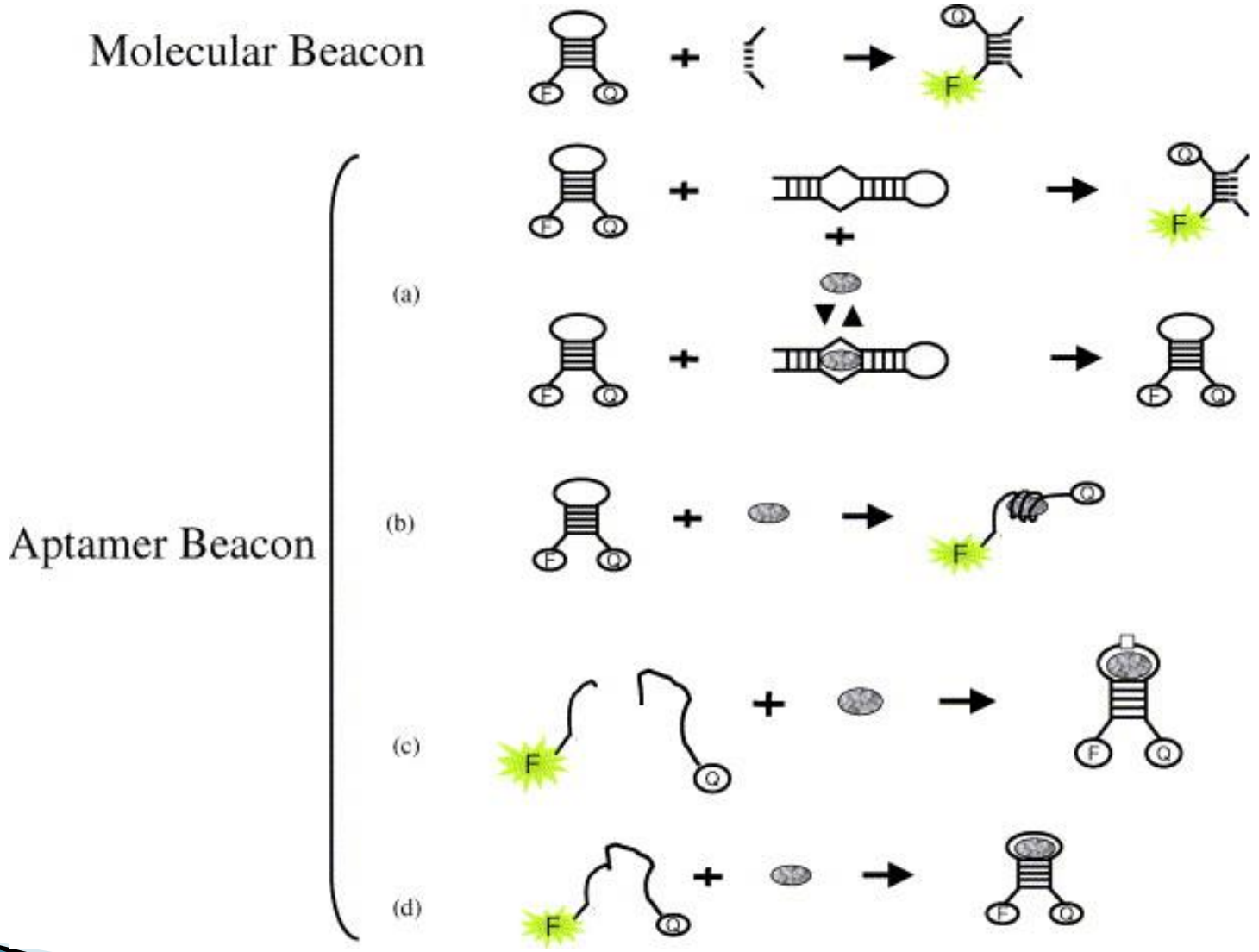


Streptomycin-binding aptamer



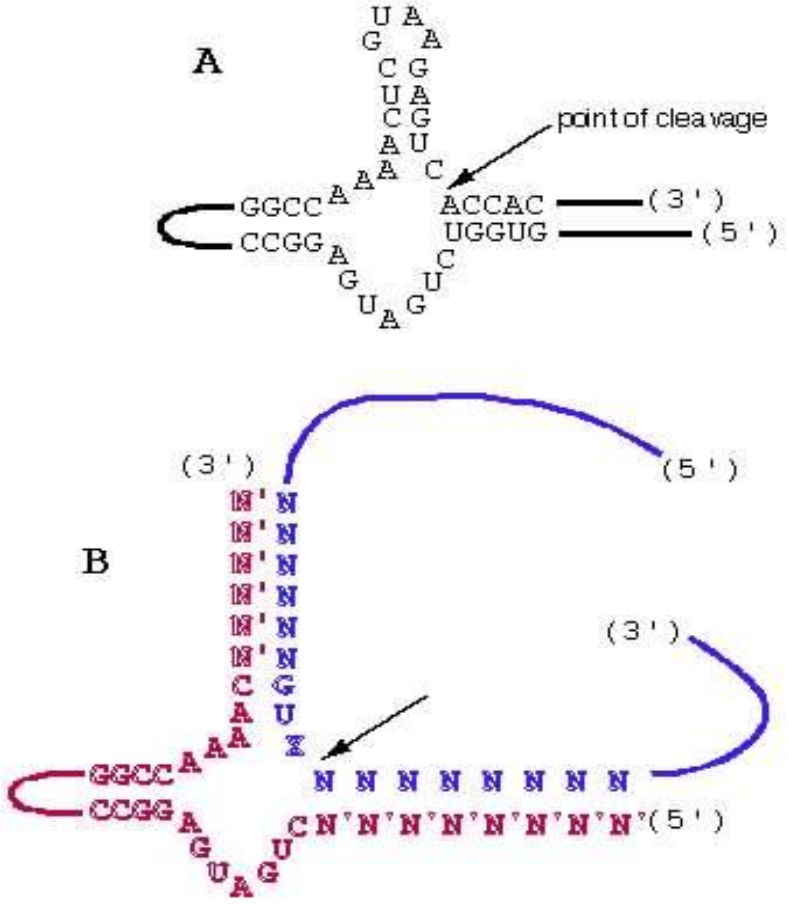


Molecular Beacons – Aptabeacons



Ribozymes

A ribozyme is an RNA molecule that is capable of performing specific biochemical reactions, similar to the action of protein enzymes.



The general structure of the virusoid hammerhead ribozyme. The critical elements are the ability to form the hammerhead type of structure, and that the cleaved site contains the sequence GUX. In the natural RNA (A) the entire sequence is on one strand. In (B) is shown a synthetic ribozyme ("designer RNase") based on the hammerhead ribozyme concept, in which sequences flanking a GU dinucleotide in the target RNA (residues marked N) are matched with sequences N' surrounding the rest of the hammerhead RNA. In this structure, the two parts of the ribozyme are on separate molecules (**blue** and **red**). The **red** part of this structure can be designed to cleave any RNA of known sequence (**blue**), provided it has a GU site.

New synthetic ribozymes, and DNAzymes

Start with 10^{15} DNA molecules again

Select for enzyme activity:

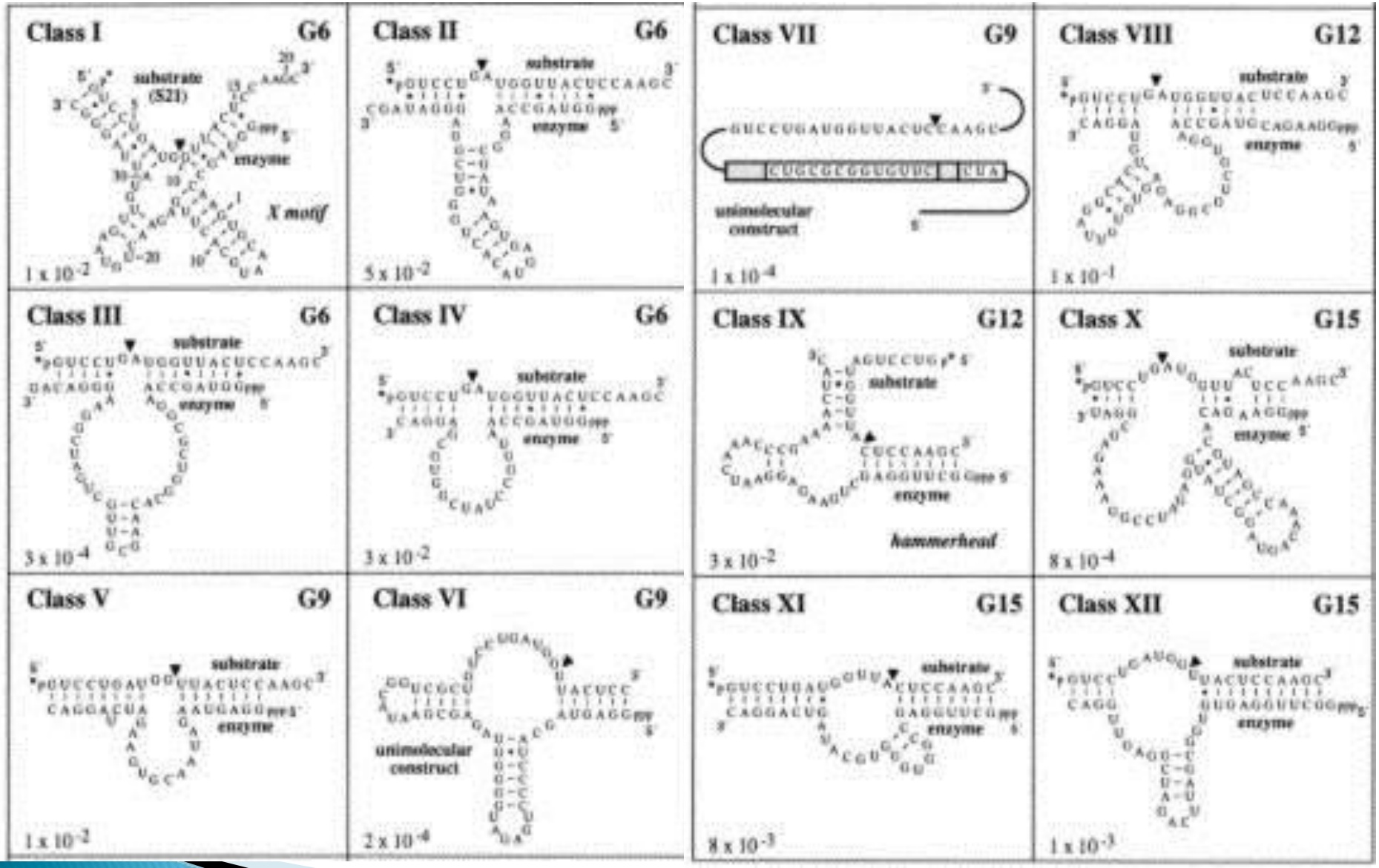
E.g., cleaves itself off a solid support in the presence of Mg++

Many different activities have been selected.
Most have to do with nucleic acid transformations;
RNase, ligase, kinase, etc.
But not all (C-C bond formation).

Generally much slower than protein enzymes.

Most work has been on RNases
(usually associated with the word "ribozymes")

12 different evolved ribozyme structures

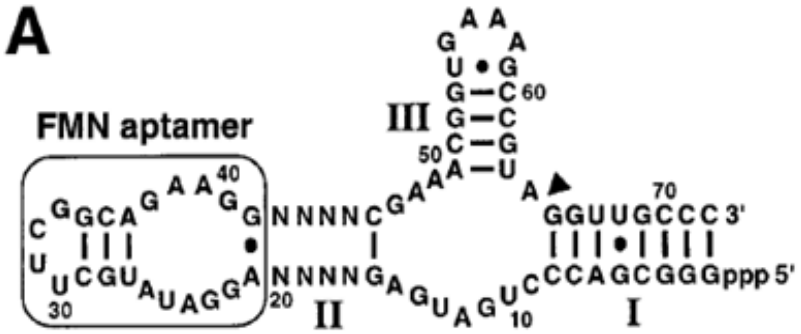


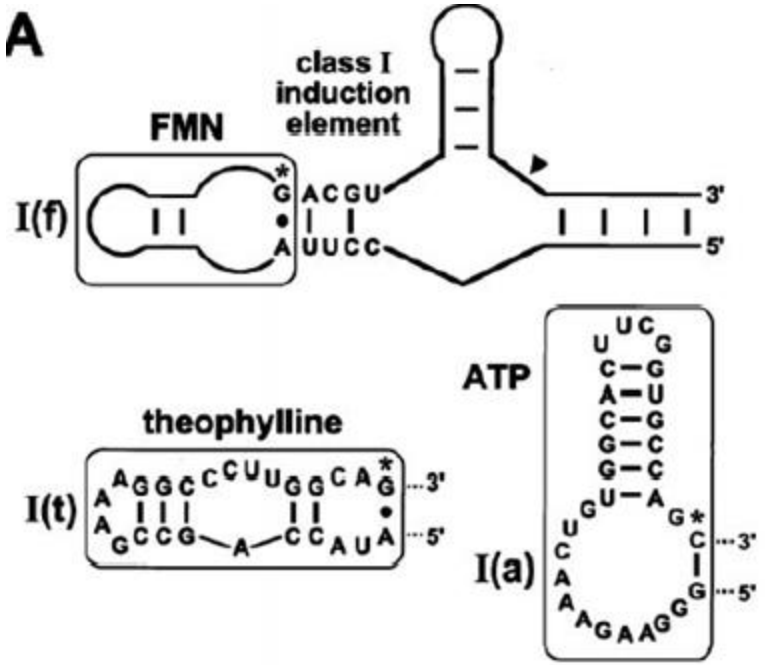
Proc Natl Acad Sci U S A, 2000, **97:** 5784-5789.

Aptazymes – Allosteric ribozymes

Combine an aptamer and a ribozyme →

Catalytic activity can be controlled by ligand binding!





Frauendorf, C. and Jaschke, A. 2001.
Detection of small organic analytes by fluorescing molecular switches.
Bioorg Med Chem **9:** 2521-2524.

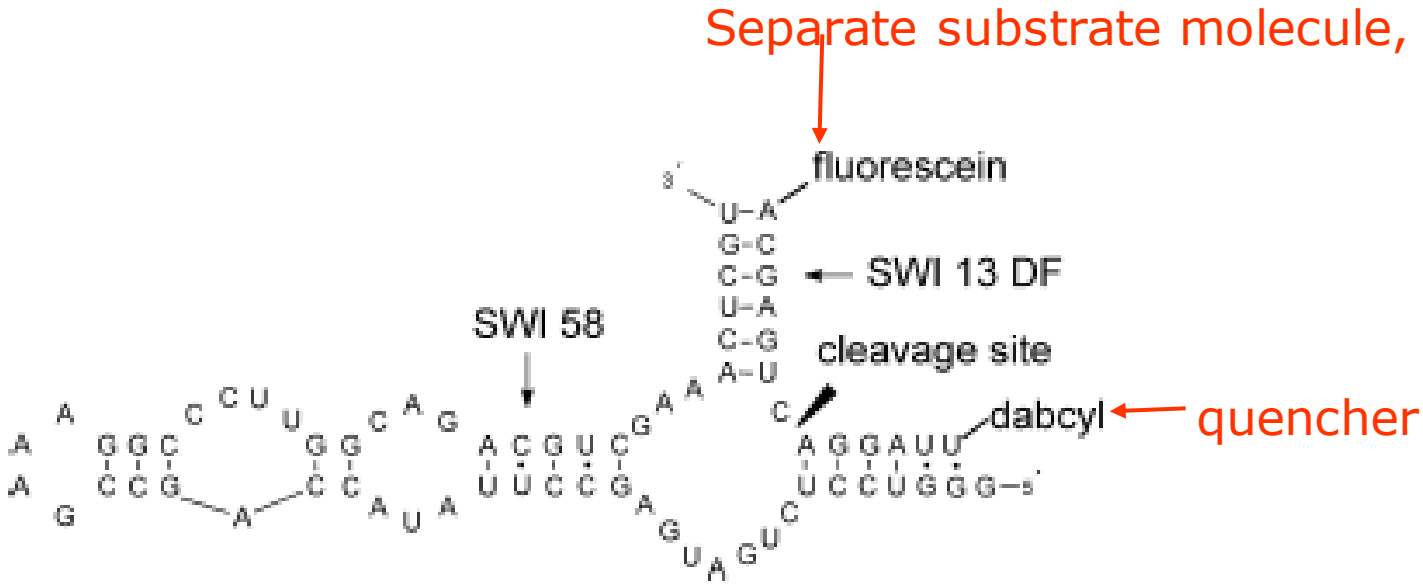


Figure 2. Theophylline-dependent allosteric ribozyme: intermolecular system SWI58/SWI13DF.

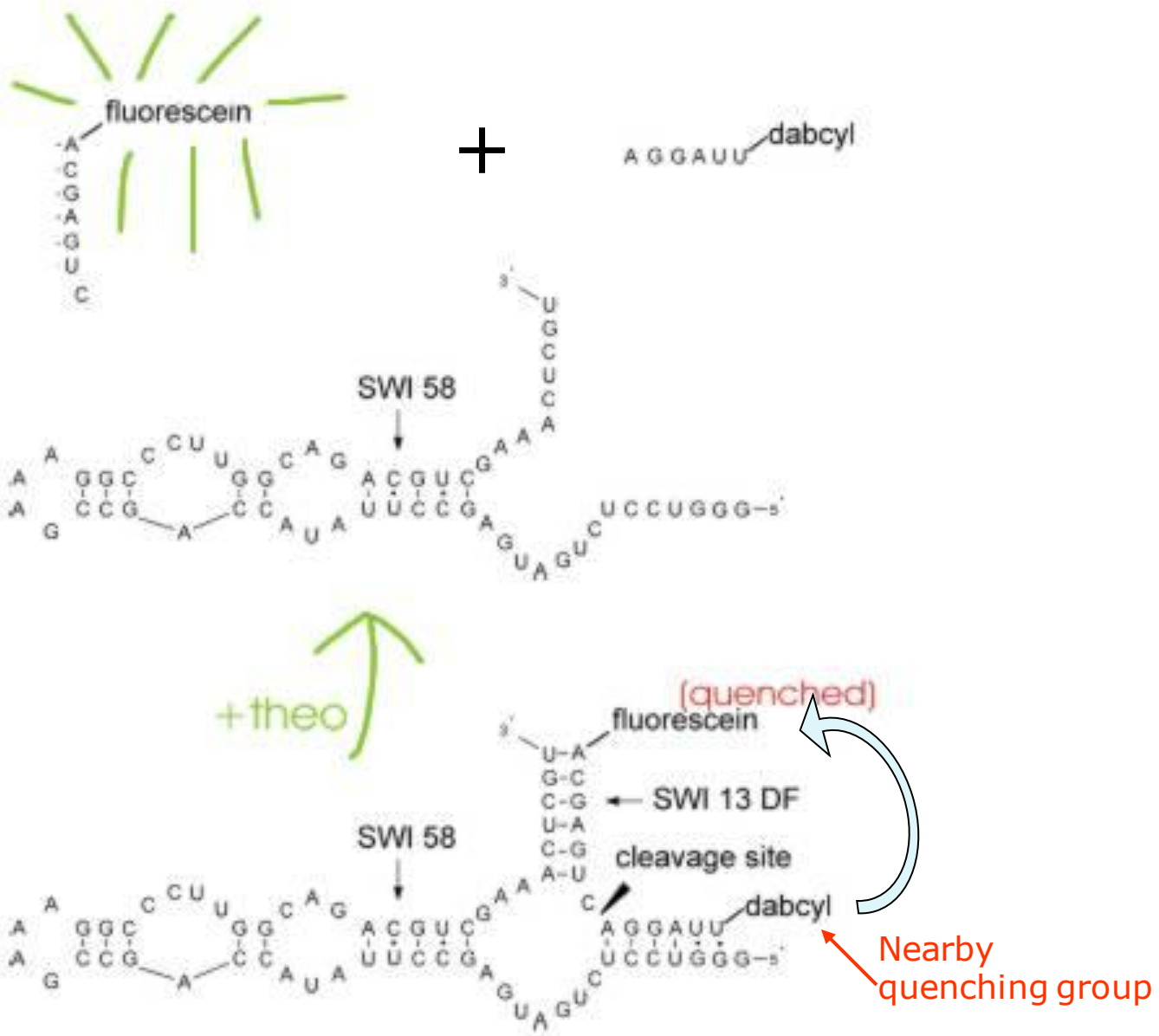
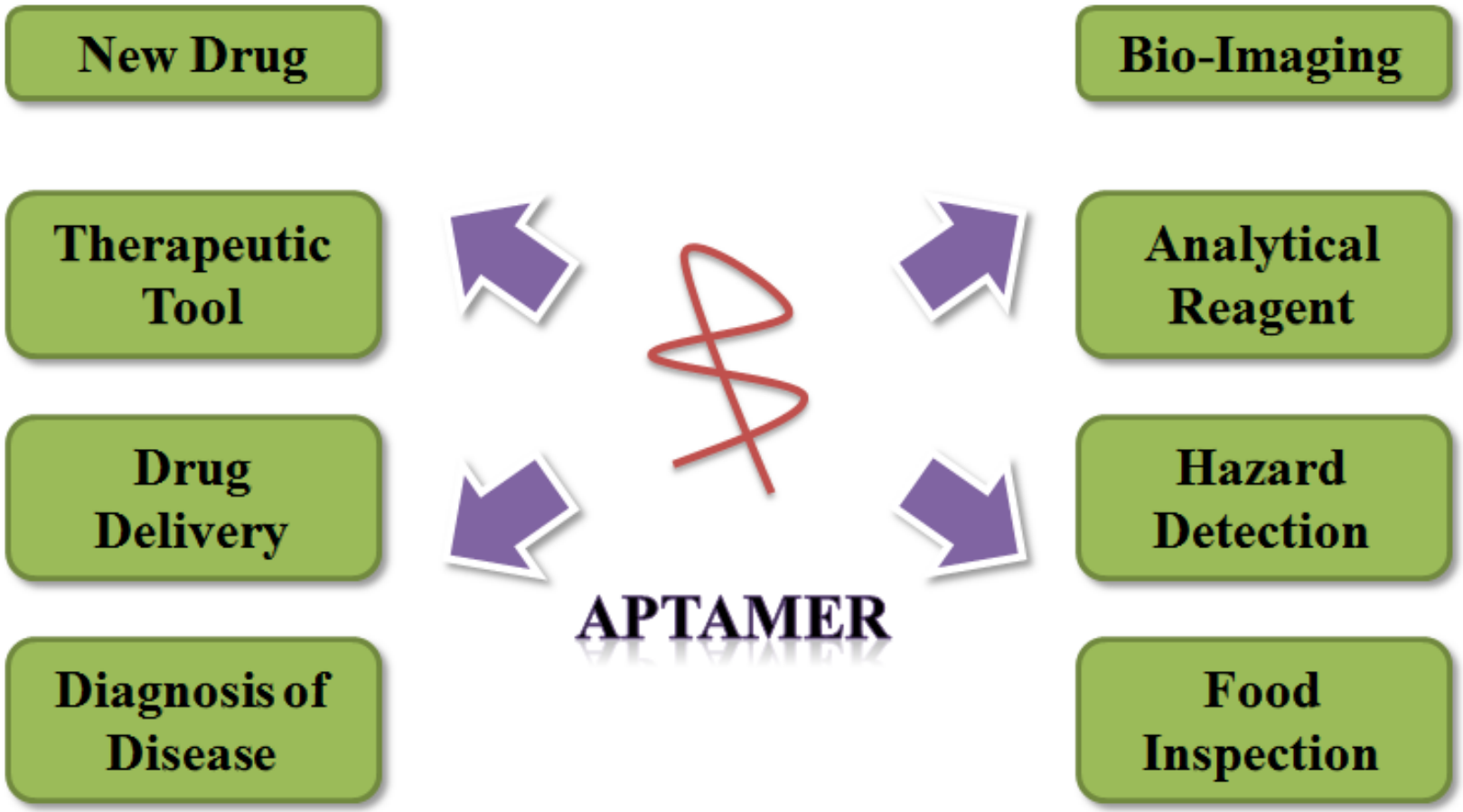


Figure 2. Theophylline-dependent allosteric ribozyme: intermolecular system SWI58/SWI13DF.

Aptamer applications



Therapeutics

Aptamers can treat disease in several ways.

1. Acting as an **antagonist**, inhibiting protein-protein and receptor-ligand interactions.

    - Inhibition of HIV replication through gp120 binding

    - Disruption of the life cycle of *Trypanosoma* and *Plasmodium*

    - Aptamers can act as antibiotics against bacterial infections.

    - Prevention of the Accumulation of the abnormal prion protein in diseases such as Creutzfeld-Jakob.

    - Inhibition of coagulation (thrombin)

    - Inhibition of inflammatory and autoimmune diseases

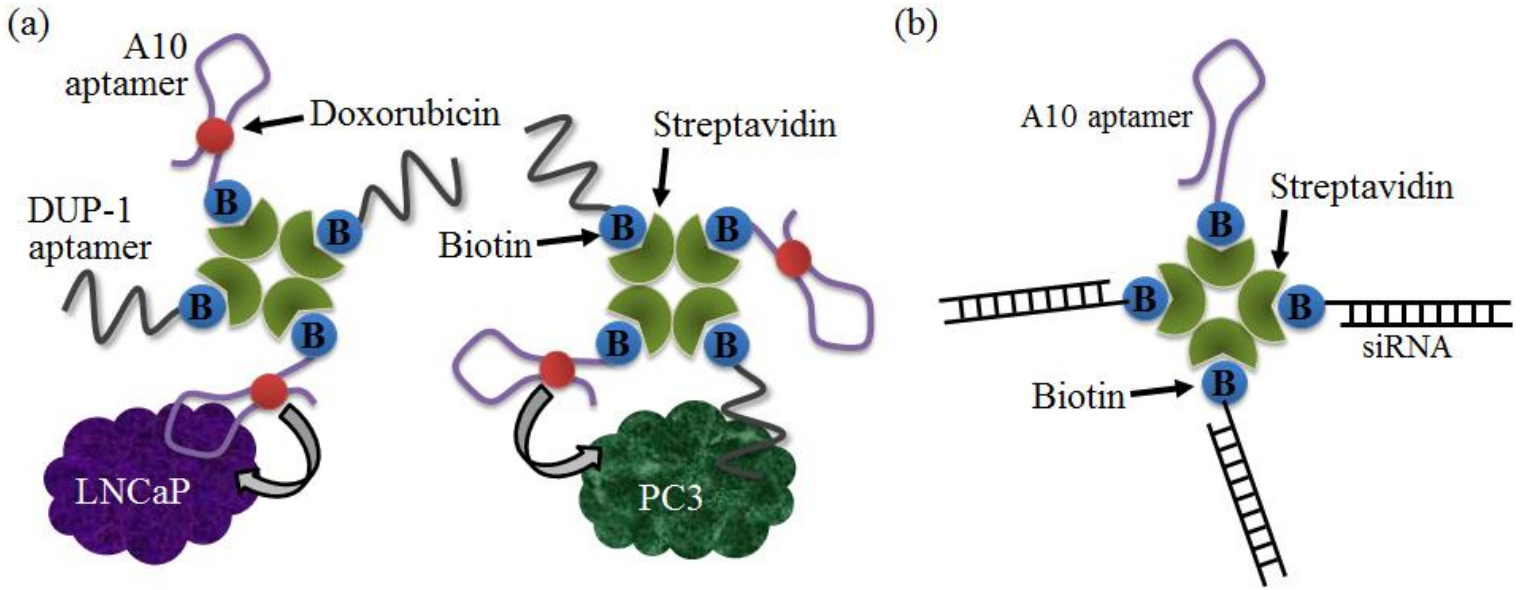
    - Inhibition of cellular pathways in cancer

Therapeutics

2. Acting as an agonist, activating cellular receptors.

    - Aptamer-based vaccines

3. Acting as a **drug-delivery agent**.

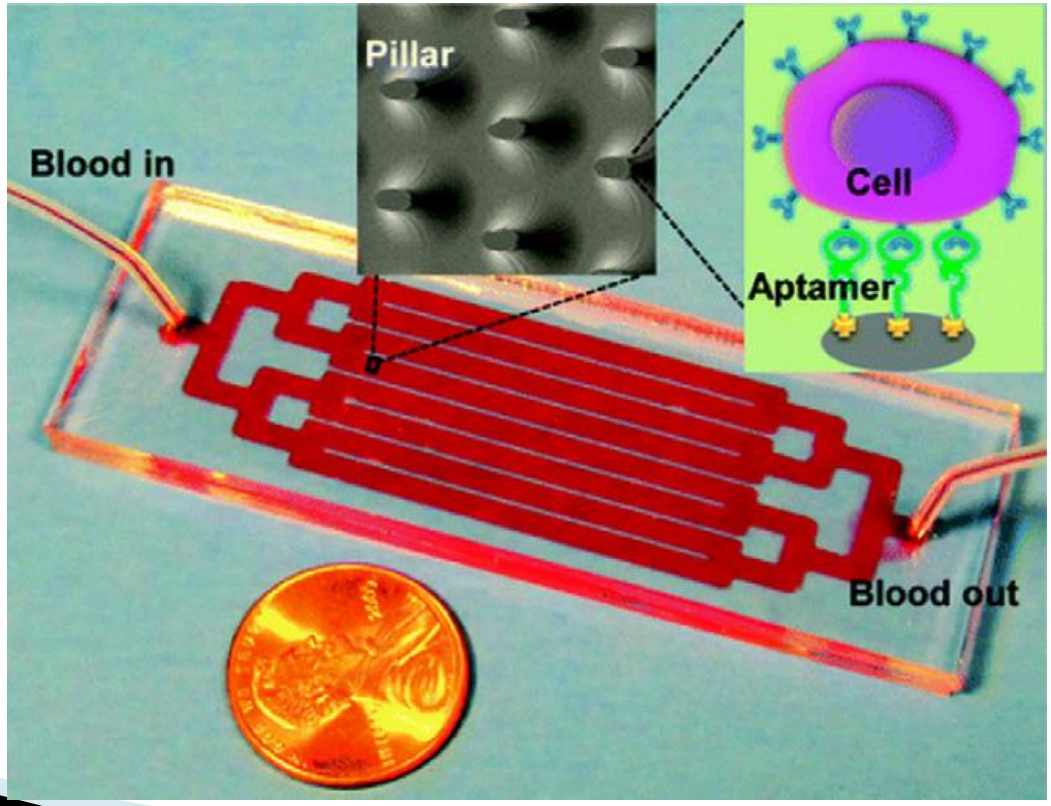


(a) A schematic illustration of the active targeting of the drug doxorubicin to prostate cancer cells using the dual-aptamer (A10 and DUP-1) complex.
(b) Design of an siRNA-aptamer conjugate via a modular streptavidin bridge using an anti-PSMA aptamer for prostate cancer cells (LNCaP).

Sensors **2012**, *12*, 612-631

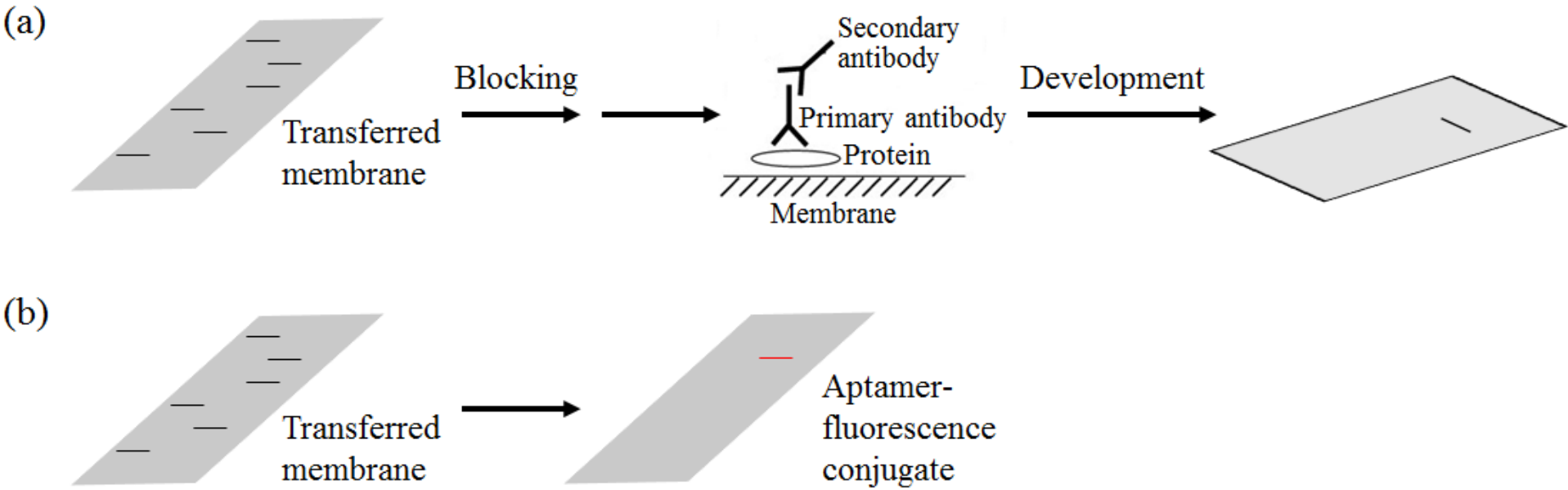
Aptamer affinity chromatography

Aptamers have been applied in on-line detection in continuous flow and off-line affinity pre-concentration, extraction, and purification of targets on solid-phase supports e.g., solid-phase extraction (SPE) column, and magnetic beads [nanoparticles (NPs)].



Trends in Analytical Chemistry, Vol. 41, 2012

Western Blotting



Sensors 2012, 12, 612-631

Aptasensors

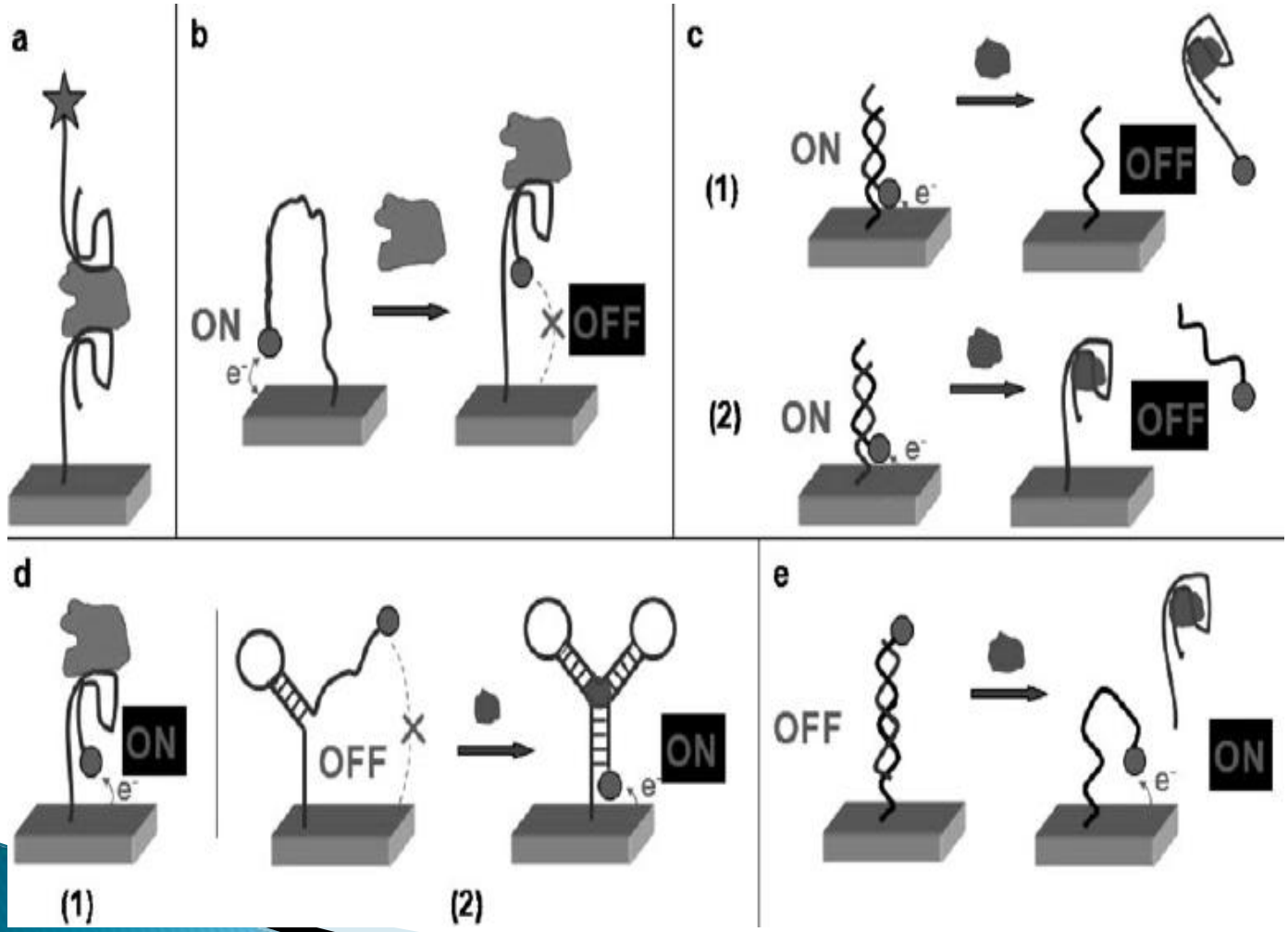
1. Electrochemical

Various electrochemical aptasensors have been fabricated using several techniques, including EIS, ECL, CV, and DPV.

To enhance the sensitivity, electroactive reporters, such as methylene blue (MB), ferrocence, ferrocence-bearing polymers, ruthenium complexes, and $Fe(CN)_6^{4-/3-}$, are used for signal transduction.

Electrochemical

based on covalent redox labels



Main designs of electrochemical aptasensors based on covalent redox labels: sandwich amplified detection (a), signal-off aptasensors based on conformational change of labeled aptamers (b) or on labeled strand displacement (c1, c2), signal-on aptasensors based on conformational change of labeled aptamers (d1, d2) or on nonlabeled aptamer displacement (e)

Electrochemical

based on noncovalent redox labels

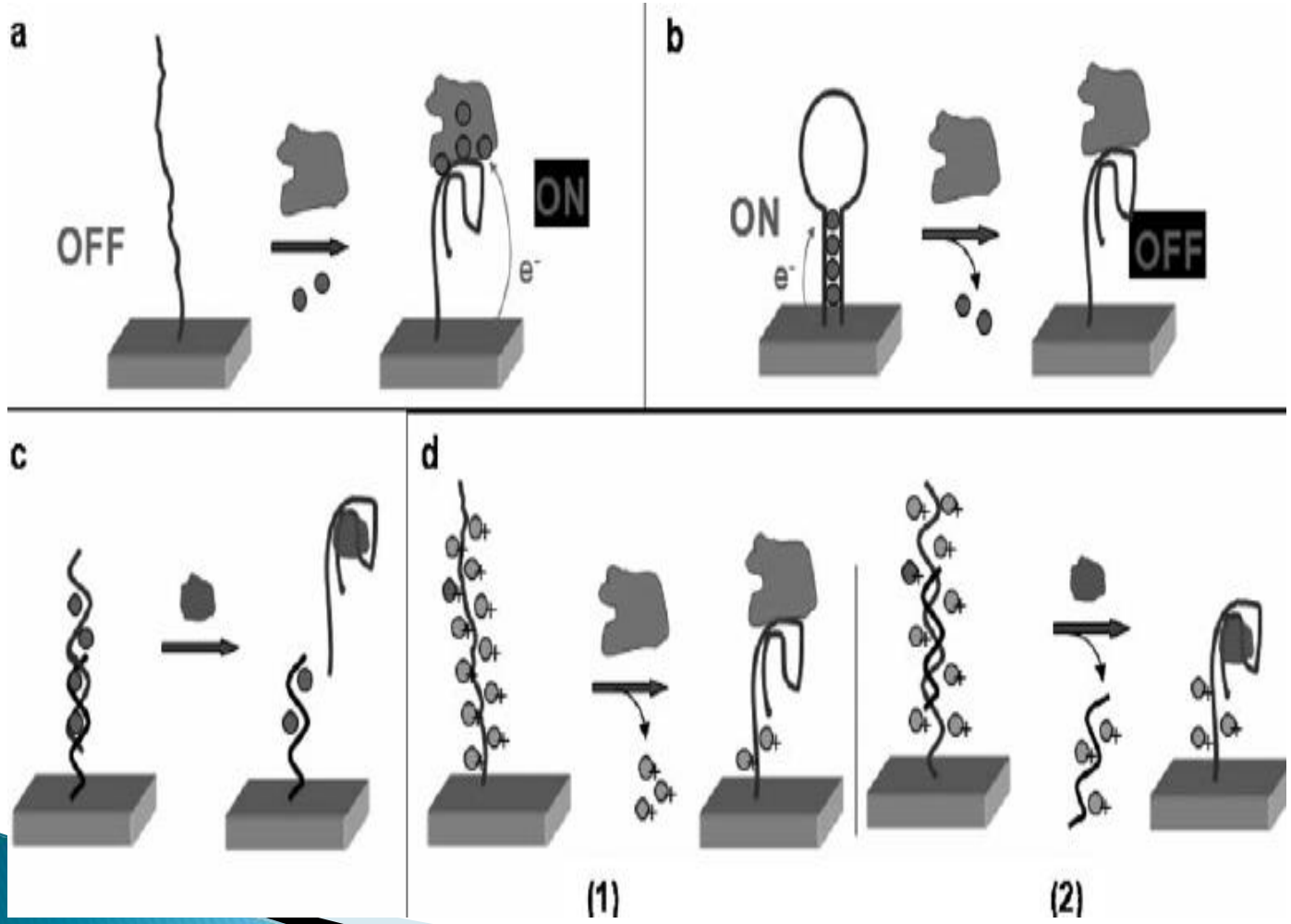


Fig. 4. Main designs of electrochemical aptasensors based on noncovalent redox labels: labels interacting with the aptamer-target complex (a), intercalated labels (b), labels interacting with nucleic acid strands (c) or ionic labels (d1, d2).

Electrochemical

label-free electrochemical aptasensors

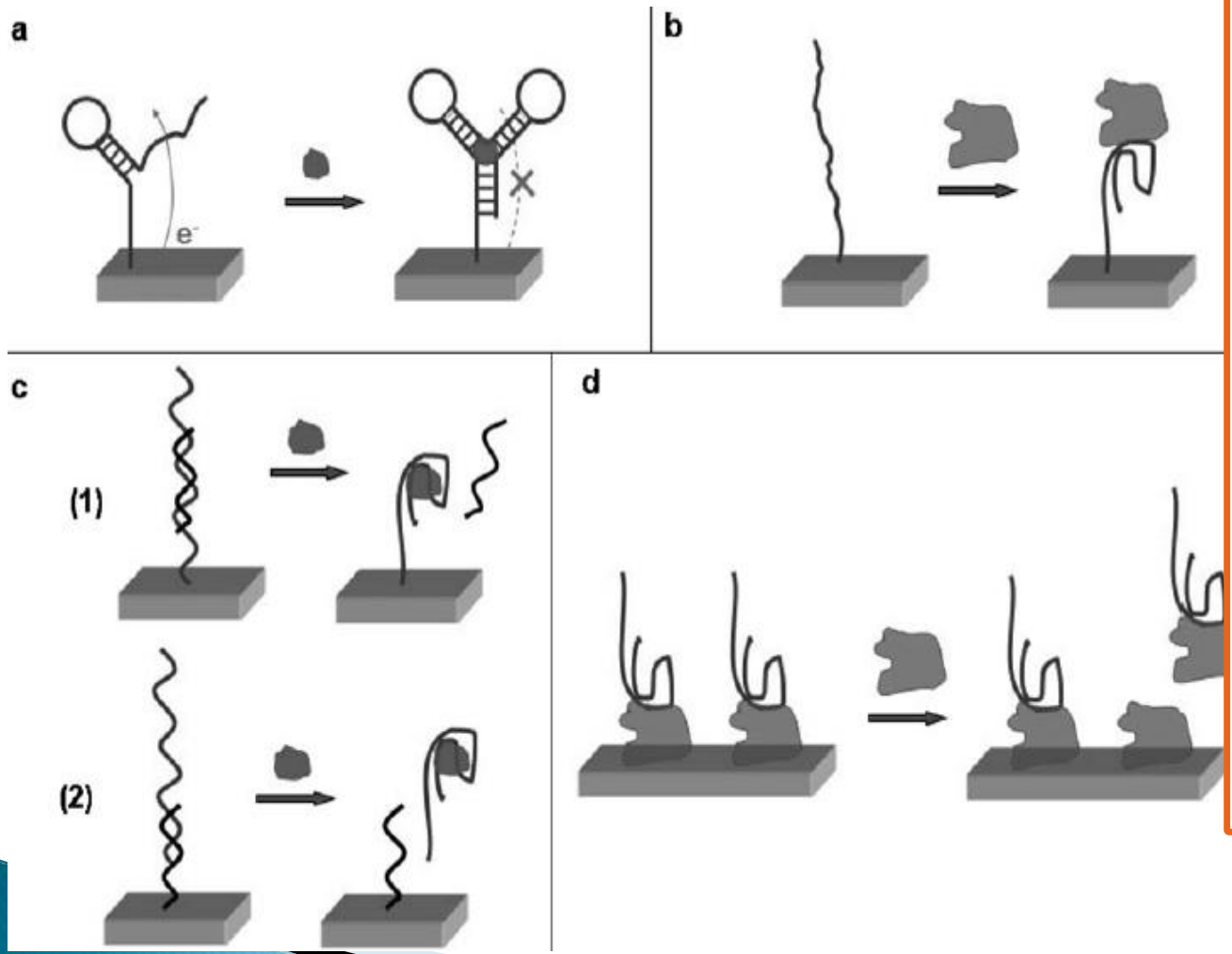
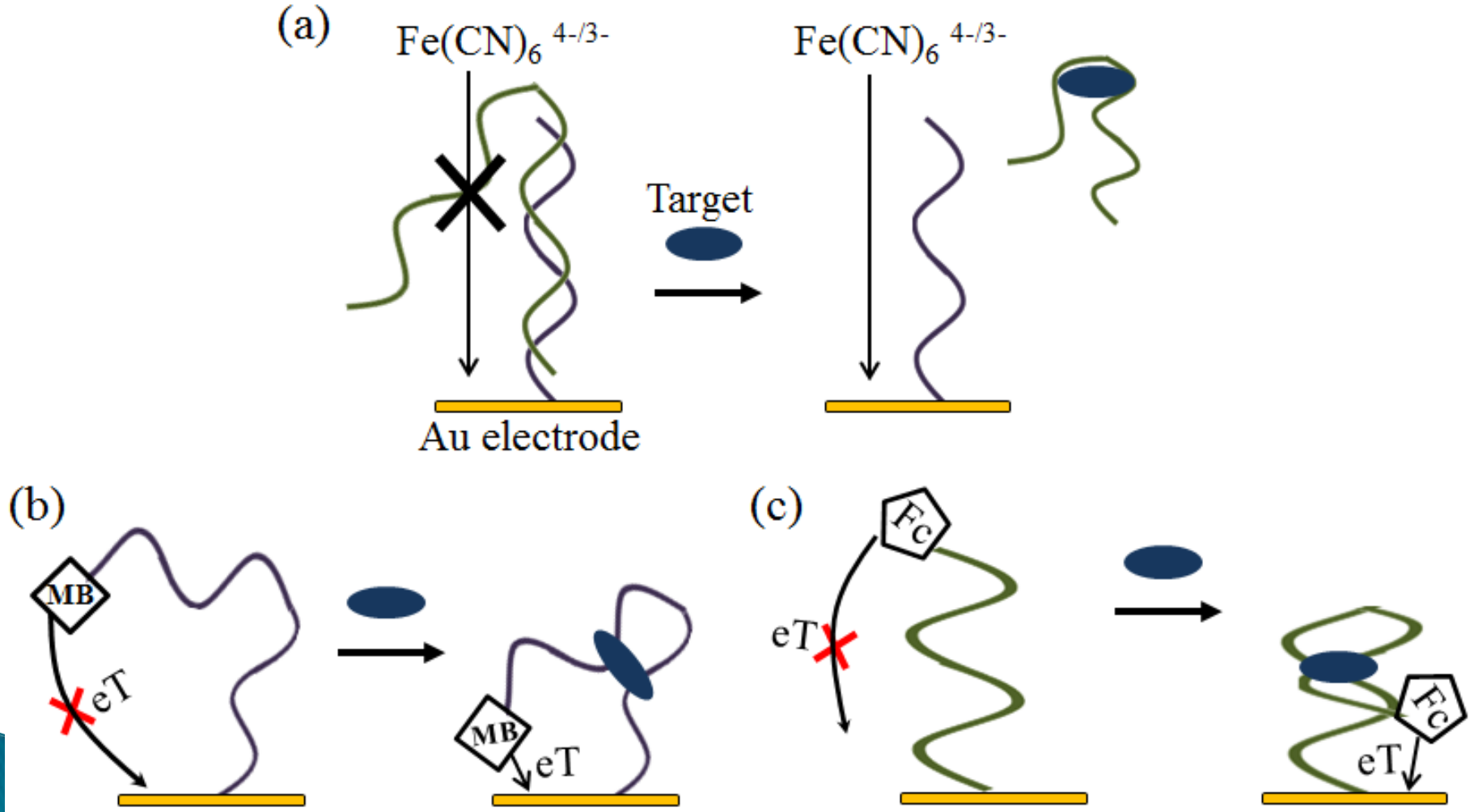


Fig. 5. Main designs of aptamer label-free electrochemical aptasensors, based on a target-aptamer complex in a three-way junction configuration (a), on change of aptamer conformation (a, b, c1), on aptamer-complementary strand duplex dissociation (c1, c2), or on a competitive assay (d).

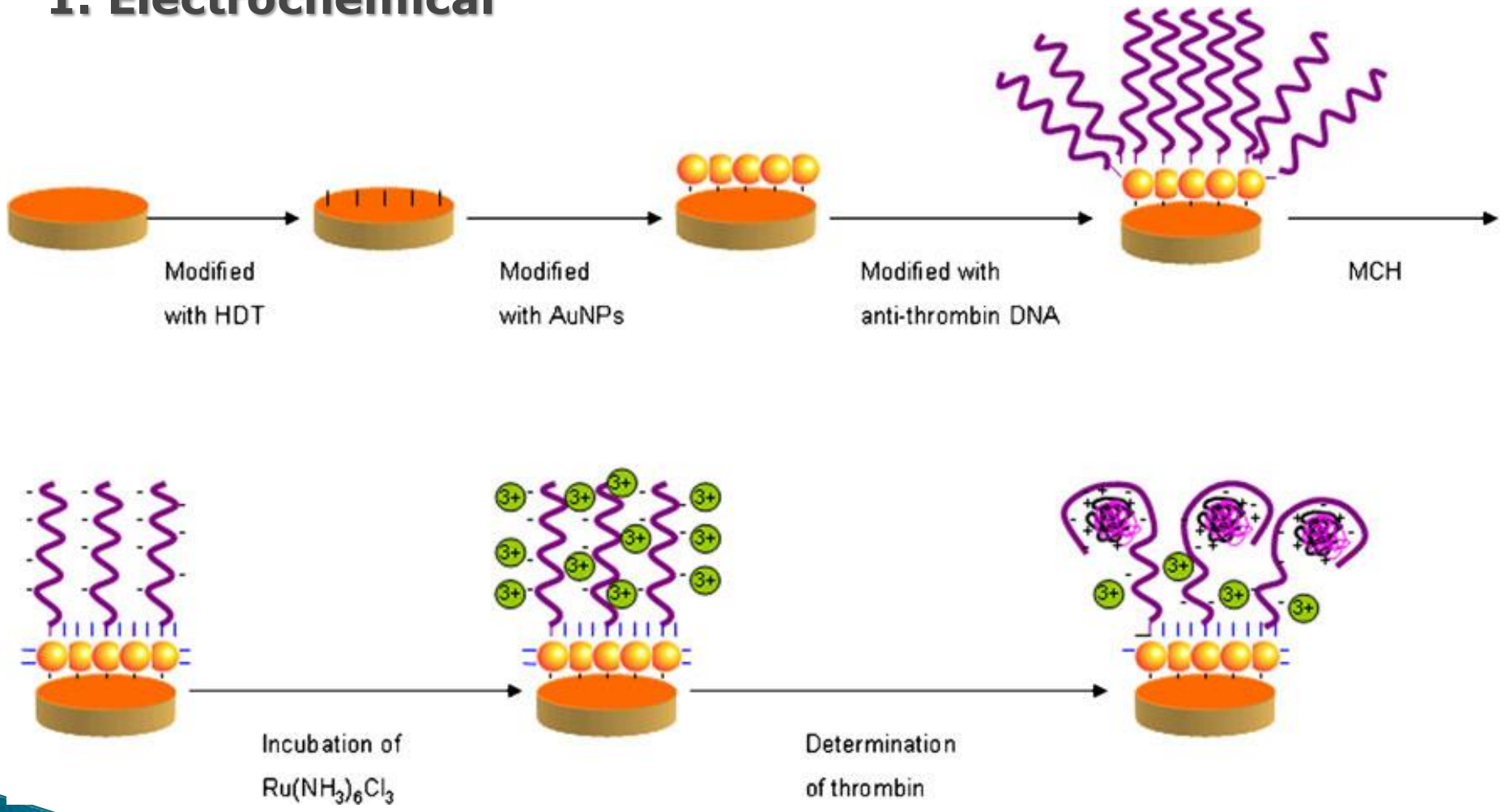
Aptasensors

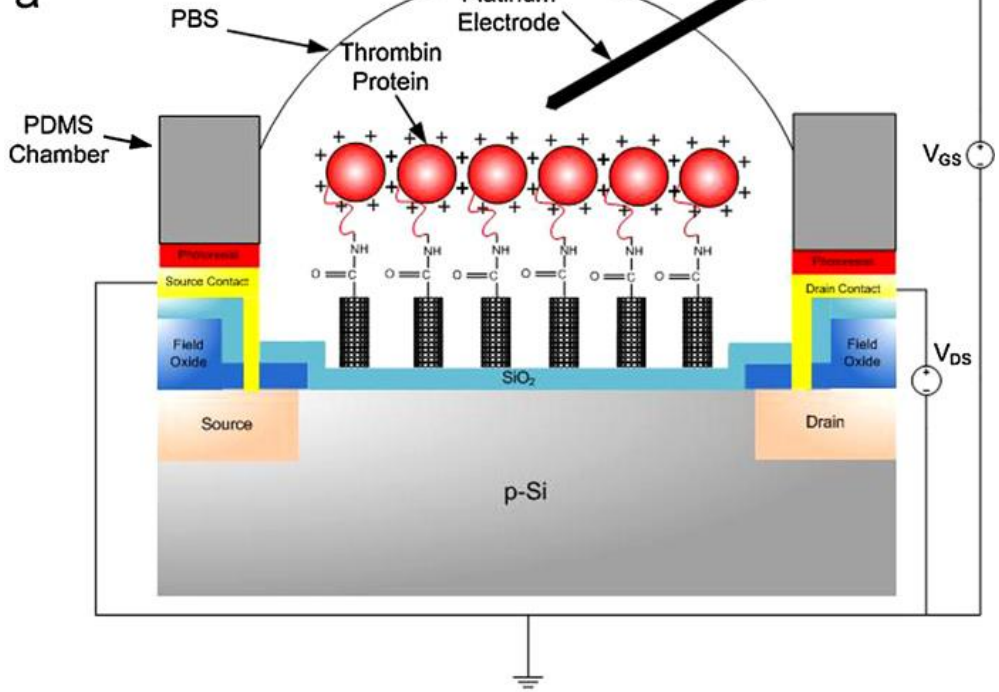
1. Electrochemical

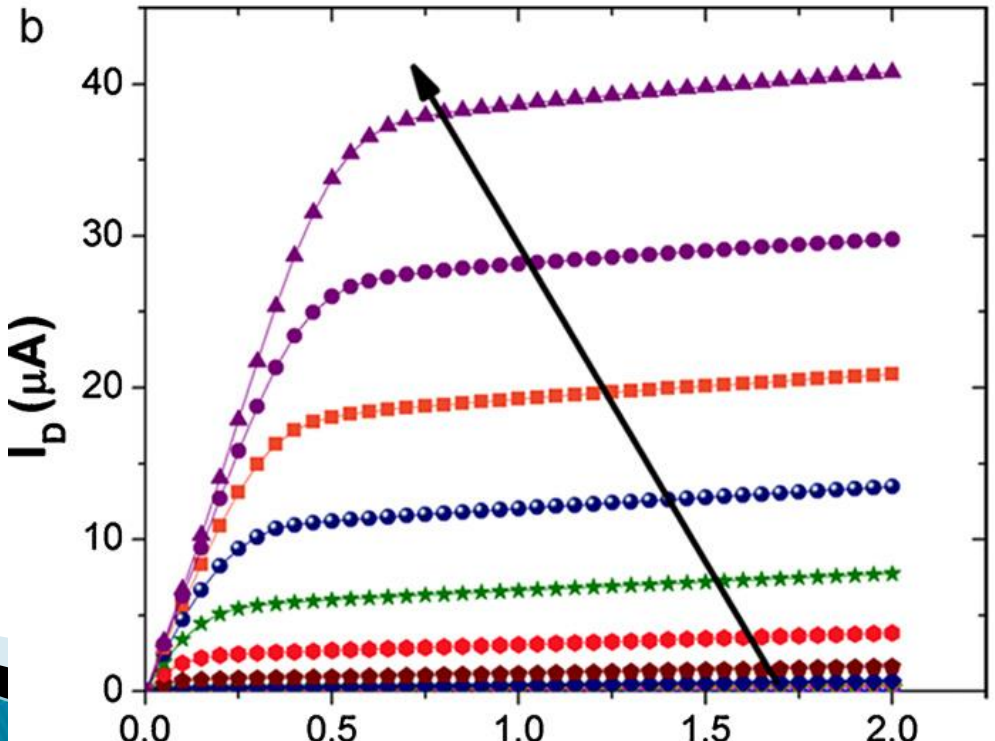


Aptasensors

1. Electrochemical



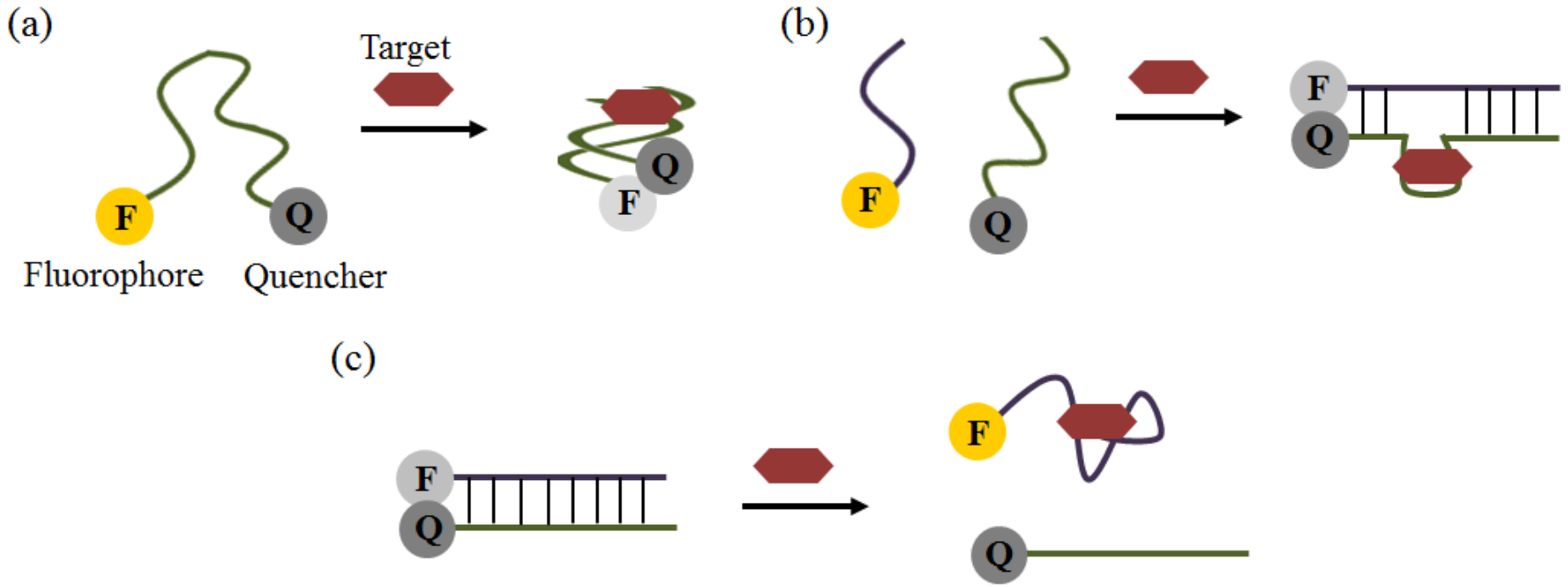

a
PBS
Platinum Electrode
Thrombin Protein
PDMS Chamber
V_{GS}
Source Contact
Drain Contact
Field Oxide
Field Oxide
SiO_2
Source
Drain
p-Si
V_{DS}


b
I_D (μA)
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0

Aptasensors

2. Fluorescence-Based Optical Aptasensor

Label the aptamers with both a quencher and a fluorophore



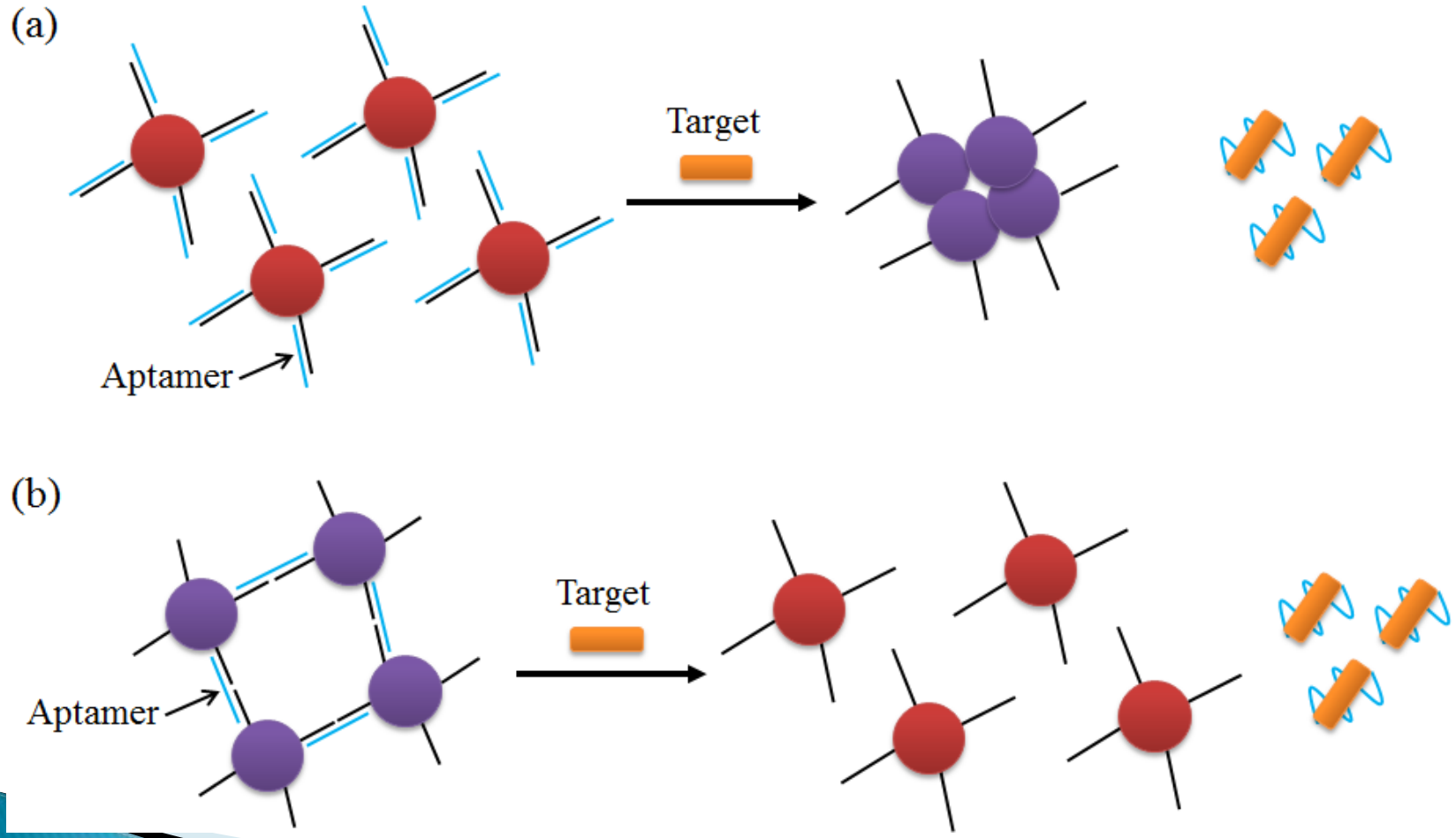
Aptasensors

2. Fluorescence-Based Optical Aptasensor

Additionally, many nano-materials, including QDs, AuNPs, CNTs, graphene oxide (GO), polymer nanobelts, and coordination polymers, have been investigated for their fluorescence-quenching effect.

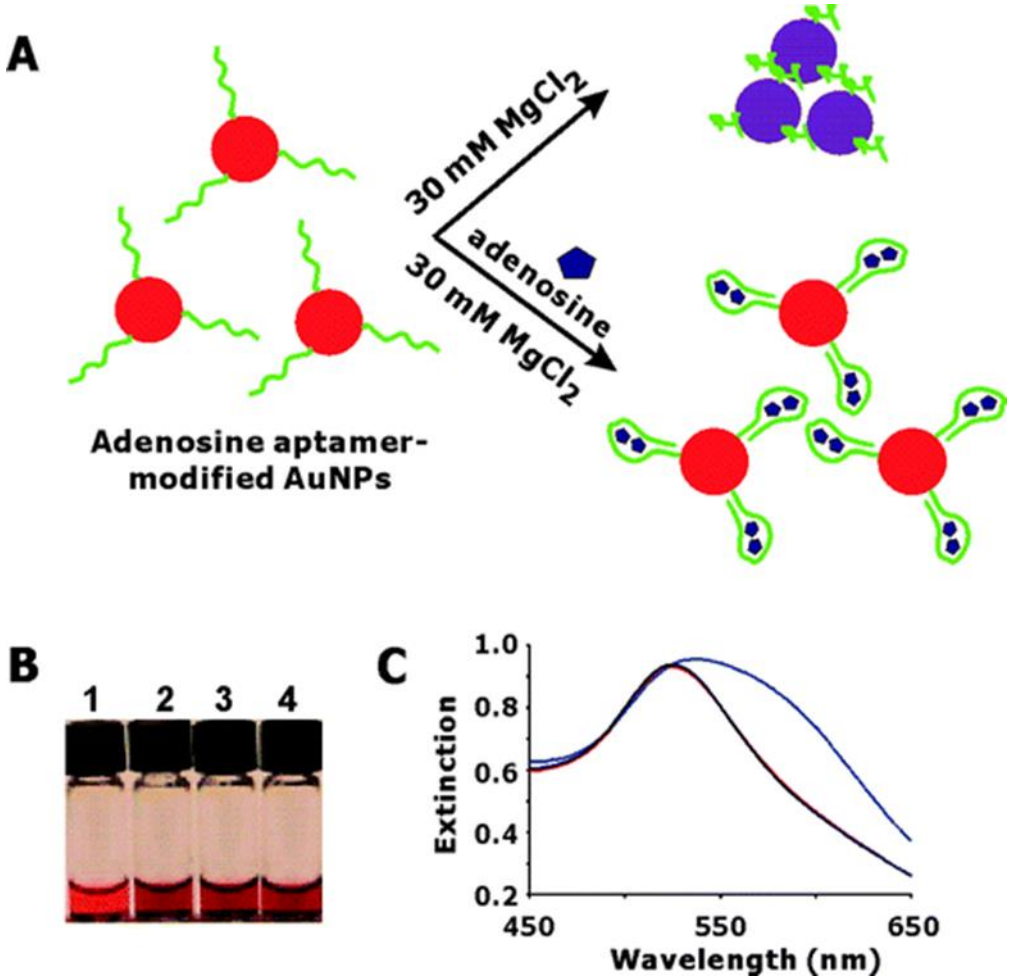
Aptasensors

3. Colorimetric-Based Optical Aptasensor



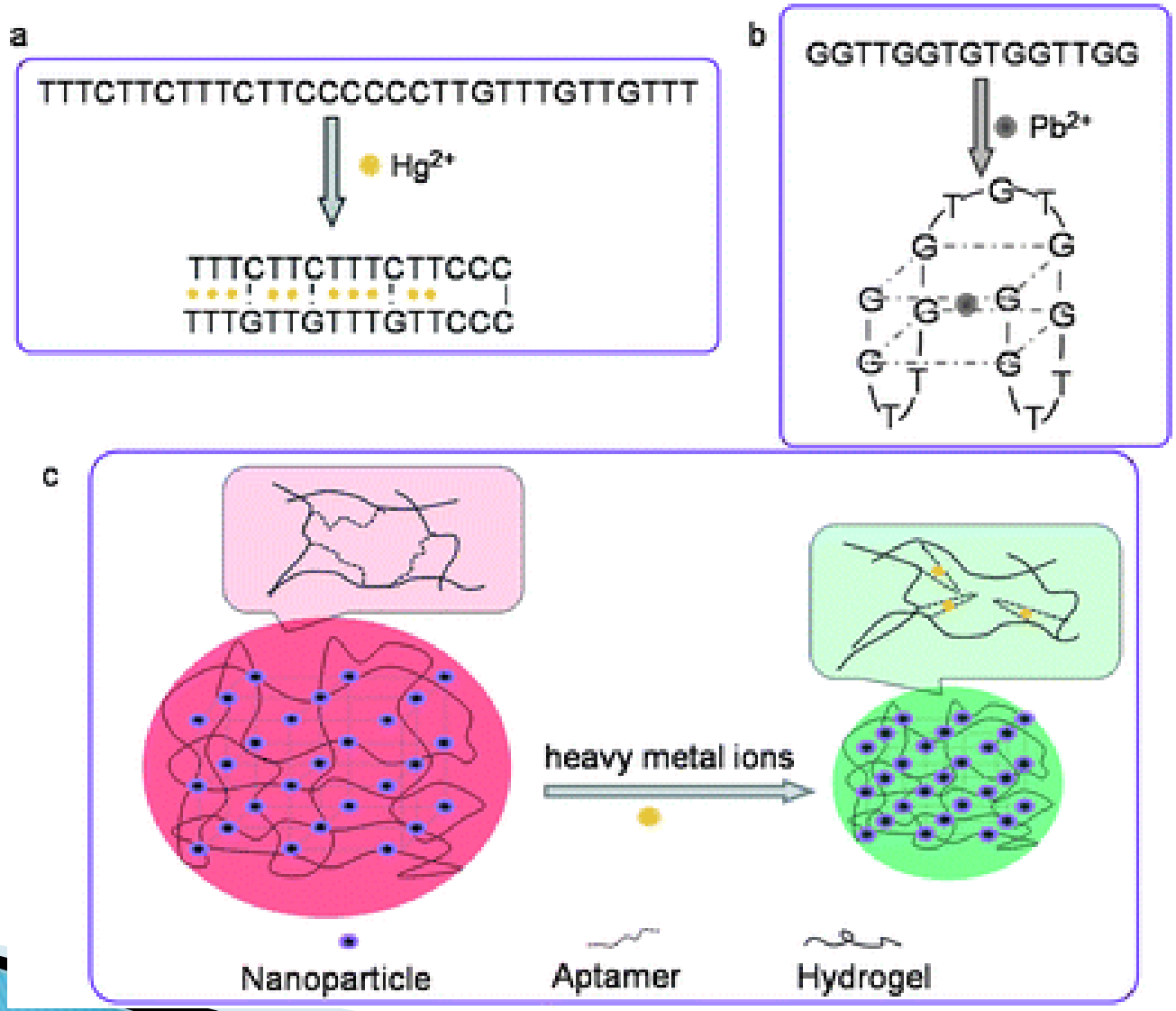
Aptasensors

3. Colorimetric-Based Optical Aptasensor



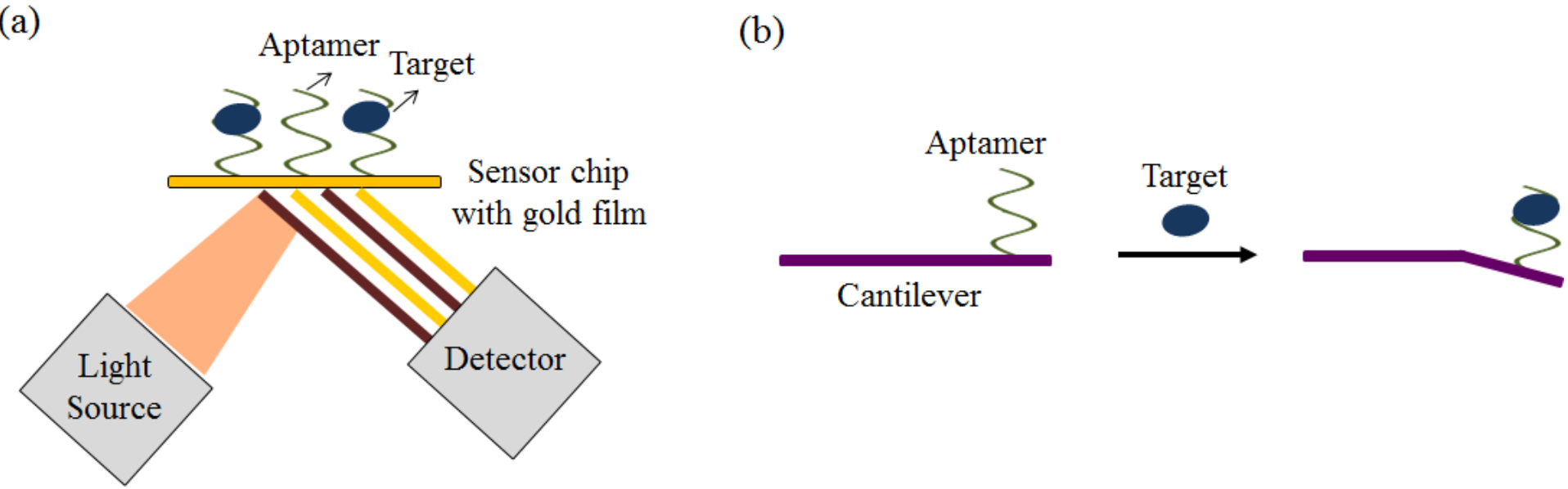
Aptasensors

3. Colorimetric-Based Optical Aptasensor



Aptasensors

Numerous other aptasensors have been exploited in combination with various types of analytical equipment, such as those used for SPR, surface acoustic wave (SAW), QCM, and microchannel cantilever sensors



Advantages of using aptamers in biosensors

Stable at room temperature.

Nonspecific adsorption phenomena are usually less pronounced on nucleic acid derivatized surfaces.

Regeneration of aptamer derivatized surfaces is quite easy to perform.

There is no restriction in the type of target for which the aptamer.

Lower cost and less time required to develop a biosensor based on aptamers.

Their small size leads to a high number of moles of target bound per gram.

Limitations of using aptamers in biosensors

Aptamers (RNA in specific) are prone to endonuclease cleavage.

Aptamer structure is sensitive to salt concentration.

Several proteins may interact with DNA aptamers nonspecifically.

Presence of nucleic acid in the biological liquids may cause hybridization with aptamers, and thus affect the aptamers conformation.

Not that many aptamers are available.

Thank you!!