APTAMERS

Emerging Recognition Element For Biosensing Applications

Aptamers (Latin *aptus* means “to fit”) are one such synthetic ligand (oligonucleotide or peptide) that are emerging as an alternative biorecognition element.

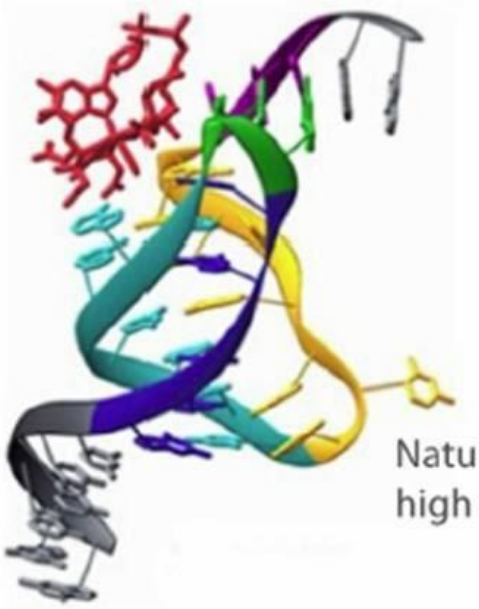
In 1990, two labs independently developed the technique of selection:

The Gold lab, using the term SELEX for their process of selecting RNA ligands against T4 DNA polymerase.

Two years later, the Szostak lab and Gilead Sciences, independent of one another, used *in vitro selection* schemes to evolve single stranded DNA ligands for organic dyes and human coagulant, thrombin, respectively.

Aptamers are oligonucleic acid (or peptide) molecules that can bind to various molecular targets and are viewed as complements to antibodies, sometimes referred to as “chemical antibodies”.

Structure of an Aptamer



Example structure of an aptamer against Vitamin B_{12}

Naturally folds into a unique structure with high affinity and specificity to its target

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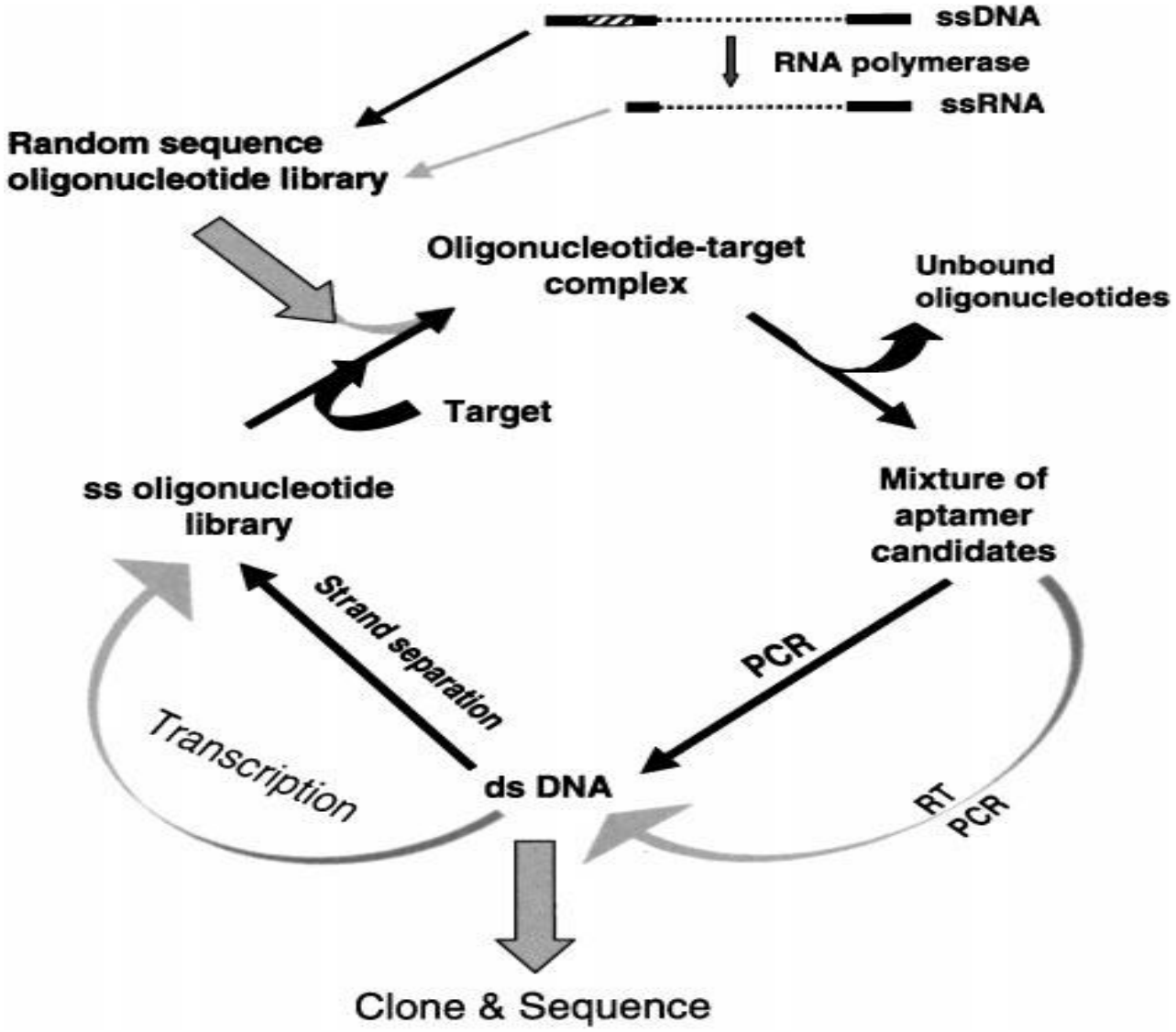
The first step of the selection process involves generation of a combinatorial oligonucleotide library with a typical sequence diversity of 10^{12-15} single-stranded DNA or RNA sequences.

Each sequence in the library has a central randomized region (20–90 nucleotides) flanked by fixed primer binding sites for polymerase chain reaction (PCR) amplification.

5’-CAC CTA ATA CGA CTC ACT ATA GCG GAT CCG A-**N40**-CTG GCT CGA ACA AGC TTG C-3’.

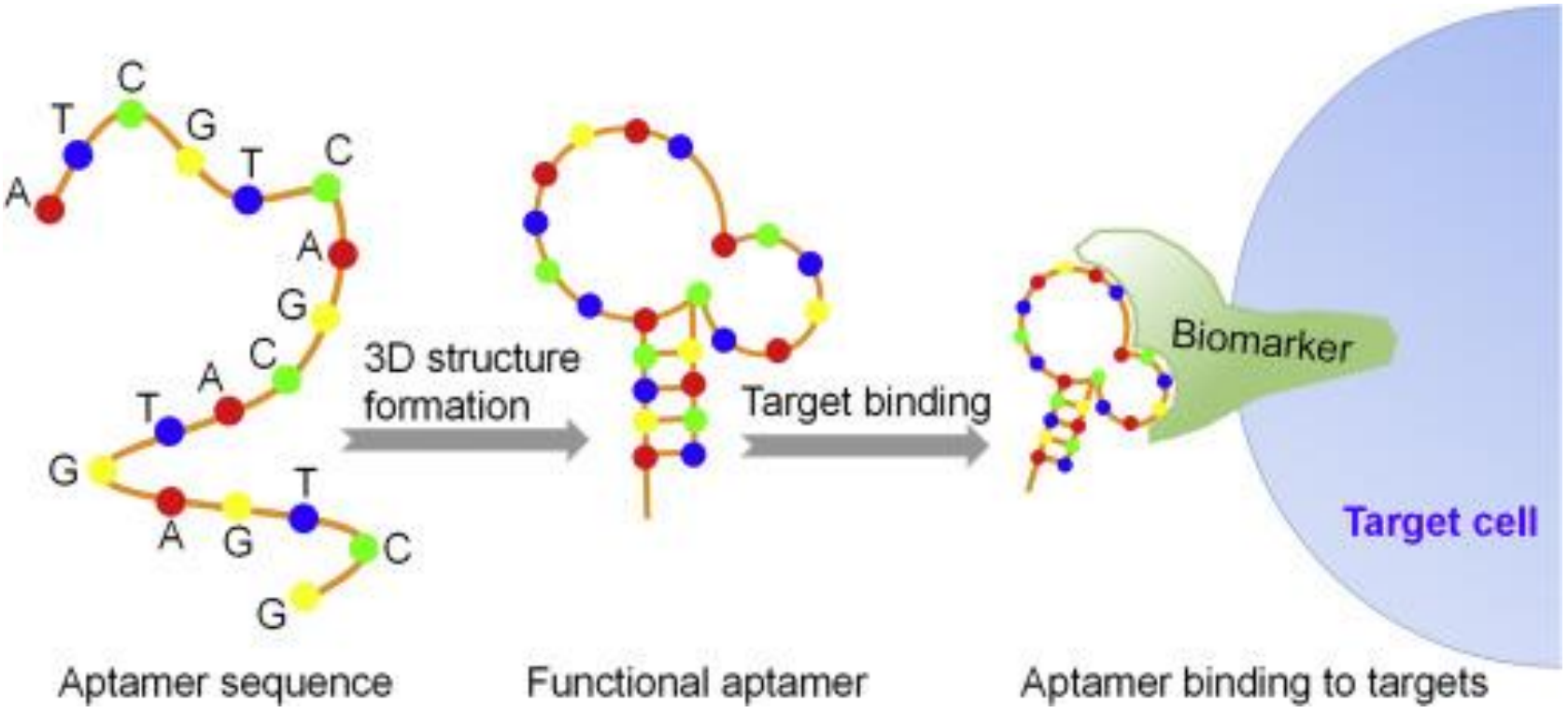
The basic steps involved in SELEX include binding, partition, elution, and amplification.

*SELEX (*systematic evolution of ligands by exponential enrichment) process *for generating aptamers*



	Aptamers	Antibody
Cost	Inexpensive	Expensive compare to aptamer
Binding affinity	Dissociation constant (K_d) low in nM-pM range	Dissociation constant (K_d) low in nM-pM range
Specificity	High	High
Synthesis	Through chemical procedure	In vitro biological procedure
Target array	Generate against any type of target i.e. ions, small organic, molecules, proteins, whole cells, etc.	Limited: only immunogenic compounds
Batch to batch variation	Not significant	Significant
Chemical modification	Easy and straightforward	Limited
Thermal denaturation	Reversible and Stable at RT	Irreversible and unstable at RT
Shelf-life	Very long	Limited

Aptamers have found applications in many areas, such as bio-technology, medicine, pharmacology, microbiology, and analytical chemistry, including chromatographic separation and biosensors.



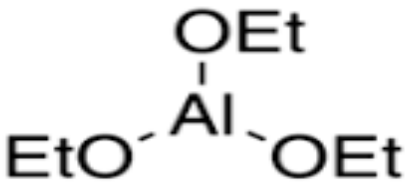
SOL-GEL MATERIALS

Sol-gel derived materials have diverse applications in optics, electronics, energy, space, bio sensors, medicine and separation technology.

Sol-gel materials provide a versatile way for bioimmobilization of Antibodies, DNA, RNA, antigen, plant -, animal cells and bacteria on various supports.

It is prepared by a wet-chemical technique starting from a chemical solution to produce colloidal particles (*sol*), which is then transformed to *gel* through an inorganic network reaction.

Typical precursors **are** **metal alkoxides** and metal chlorides, which undergo hydrolysis and polycondensation reactions to form a colloid (size 1 nm to 1 μm) in a solvent.



The most thoroughly studied metal alkoxide is silicon tetraethoxide, or tetraethyl orthosilicate (TEOS).

The chemical formula for TEOS is given by: $Si(OC_2H_5)_4$, or $Si(OR)_4$ where the alkyl group $R = C_2H_5$.

Formation of a metal oxide involves connecting the metal centers with oxo (M-O-M) or hydroxo (M-OH-M) bridges, therefore generating metal-oxo or metal-hydroxo polymers in solution.

Metal alkoxide are ideal chemical precursors for sol-gel synthesis because they react readily with water. The reaction is called **hydrolysis**, because a hydroxyl ion becomes attached to the metal atom as follows:

$$Si(OR)_4 + H_2O \longrightarrow HO\text{-}Si(OR)_3 + R\text{-}OH$$

Depending on the amount of water and catalyst present, hydrolysis may proceed to completion, so that all of the OR groups are replaced by OH groups, as follows:

$$Si(OR)_4 + 4\ H_2O \longrightarrow Si(OH)_4 + 4\ R\text{-}OH$$

Any intermediate species $[(OR)_2\text{–}Si\text{-}(OH)_2]$ or $[(OR)_3\text{–}Si\text{-}(OH)]$ would be considered the result of partial hydrolysis.

In addition, two partially hydrolyzed molecules can link together in a condensation reaction to form a siloxane [Si–O–Si] bond:

$(OR)_3$–Si-OH + HO–Si-$(OR)_3$ —> $[(OR)_3Si–O–Si(OR)_3]$ + H-O-H

or

$(OR)_3$–Si-OR + HO–Si-$(OR)_3$ —> $[(OR)_3Si–O–Si(OR)_3]$ + R-OH

Thus, polymerization is associated with the formation of a 1, 2, or 3-dimensional network of siloxane [Si–O–Si] bonds accompanied by the production of H-O-H and R-O-H species.

A *drying* process at the end serves to remove the liquid phase from the gel thus forming a porous material, then a thermal treatment (***firing***) may be done to favor further *polycondensation and enhance mechanical properties.*

The precursor sol can be either deposited on a substrate to form *a film cast into a suitable container with the desired shape, or used to synthesize powders.*

The sol-gel approach is interesting in that it is a cheap and low-temperature technique that allows for the fine control on the product's chemical composition, as even small quantities of *dopants, such as organic dyes and rare earth metals, can be introduced in the sol and end up in the final product finely dispersed.*