Employing Oligonucleotides in Materials Systems

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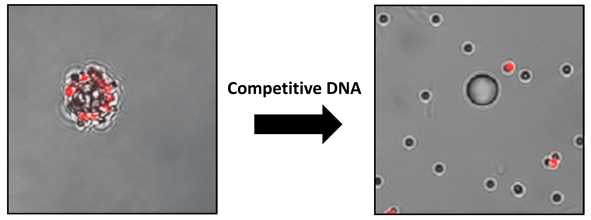
Programming Recognition-based Binding and Release with DNA, RNA, and LNA

We employ oligonucleotide-functionalized colloidal particles in the following scenarios:
1) **for programmable, isothermal assembly and disassembly of multifunctional therapeutic agents**
2) **as nucleic acid detection platforms.**

Isothermal Disassembly of DNA-linked particles

DNA-linked colloidal satellite as model multifunctional assembly

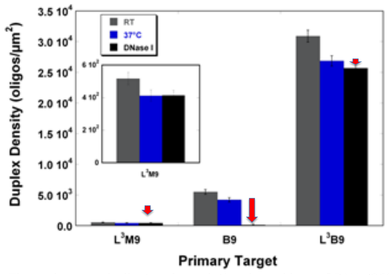
Programmed Disassembly to release therapetic agents



Tison & Milam *Langmuir* **2007**

Locked Nucleic Acid (LNA) Confers Nuclease Resistance for Physiological Environment

Comparison of Duplex DNA & LNA Nuclease Stability



The red arrows indicates degradation-based loss of DNA (96% for DNA duplexes in *middle*) compared to LNA duplexes (<4% on *left* and *right*)

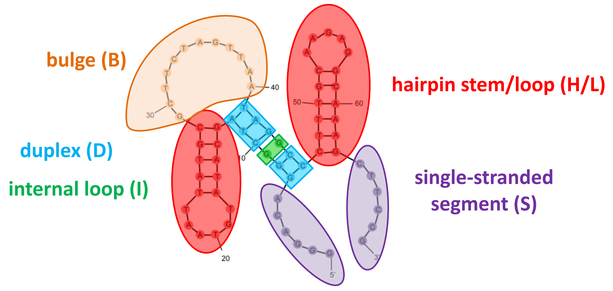
Eze & Milam *Soft Matter* **2013**

Key Advantages
1) **Label-free targets can be detected**
2) **Enables isothermal assembly and disassembly conditions**
3) **Distinct displacement behavior allows for target discrimination**

Evaluating Structural Patterns in DNA Aptamers

By adapting sequence (or primary structure) analysis tools used for DNA, we can find patterns in self-hybridized (or secondary structures) of oligonucleotide aptamers identified via CompELS to find the following:

Identifying secondary structure elements in an aptamer sequence

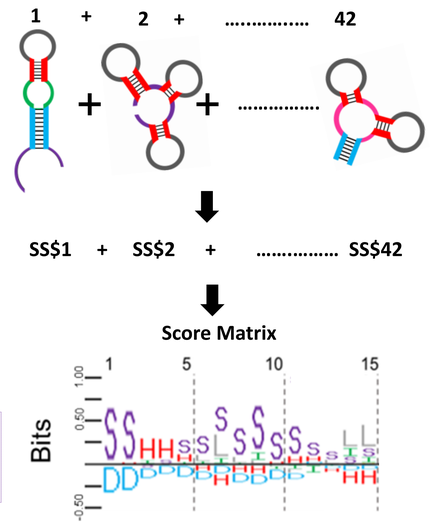


Convert above information into a secondary structure string (SS$)

5'-SSSSSSDDIDDDHHHHHLLLLLHHHHHBBBBBBBBBBBDDDIDDHHHHHHLLLLLHHHHHSSSSSS-3'

Identifying patterns in secondary structure among groups of aptamer sequences

42 aptamer sequences



Sullivan, Adams, Naik, Milam *Molecules.* **2020**

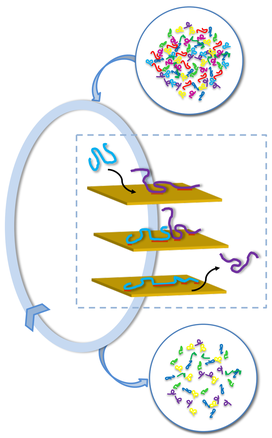
Key Advantages
1) **Secondary structure element patterns may identify binding motif**
2) **Enables future screening library design**

Bio-Inspired Materials

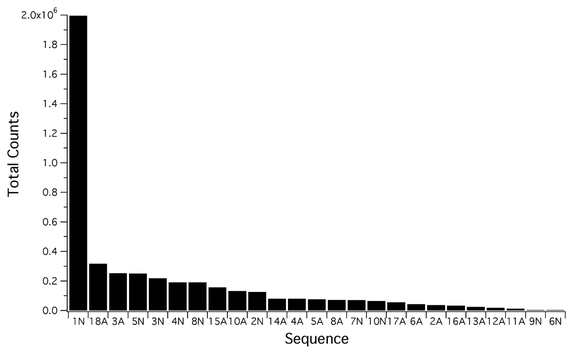
Selection of DNA Aptamers for Material & Biological Targets

As an alternative to laborious, error-prone evolutionary screening platform called SELEX, we developed an *in vitro* competition-based selection process called CompELS (competition-enhanced ligand selection) to identify DNA sequences from large (~10^{12}) random sequence libraries called aptamers that selectively bind to a specific target with high affinity.

CompELS is a fast, versatile aptamer screening platform



Ranking multiple aptamer sequences as high affinity ligands using Next Generation Sequencing (NGS) analysis



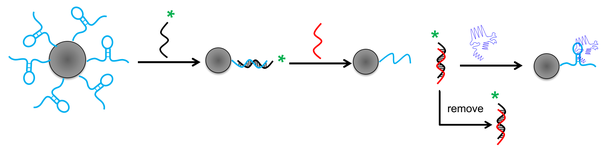
Tapp, Slocik, Dennis, Naik, Milam *ACS Combinatorial Sci.* **2018**

Key Advantages
1) **CompELS is faster and less error-prone than conventional evolutionary screening approach called SELEX**
2) **CompeLS is versatile and adaptable to different target chemistries, sizes, etc.**
3) **Aptamers can serve as agents for therapeutics, diagnostics and materials surface functionalization**

Employing DNA Aptamers to Program Release of Therapeutic Proteins

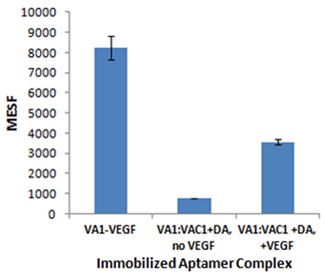
Aptamers have the ability to bind specifically to either a nucleotide or non-nucleotide target. Here, we explore the ability to regenerate aptamer binding activity for its various targets following a series of binding and release events.

Schematic of particle-immobilized aptamers that undergo serial binding, release, and binding events to various nucleotide and protein targets.



Dunaway, Sullivan, Siegel, & Milam, *Biointerphases* **2015**

Partial Recovery of Aptamer Binding to VEGF Protein



Comparisons of VEGF binding to particle-immobilized aptamers (**VA1**) with no binding history (*far left*) and previously bound to oligonucleotide targets (*far right*) indicate that aptamer binding capabilities for its nonnucleotide target can be partially regenerated.

Key Advantages:
1) **Potential to regenerate aptamer binding under biologically relevant conditions.**
2) **Programmable isothermal release or uptake of the non-nucleotide target by the aptamer sequences.**
3) **Method is potentially adaptable to other aptamer-target systems for therapeutic and diagnostic use.**

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