Microfluidics InnovationHub

We get Microfluidics rolling

NextGenMicrofluidics (NGM)

- NGM is an Open Innovation Test Bed - 21 companies
- MIH acts as a single-entry point towards their combined technologies and expertise
- worldwide biggest platform for upscaling and testing of microfluidic devices

Design & Simulation
BIONIC SURFACE TECHNOLOGIES

Materials
condensia
micro resist technology

Electronics manufacturer
Infineon

Bioprocess
NST

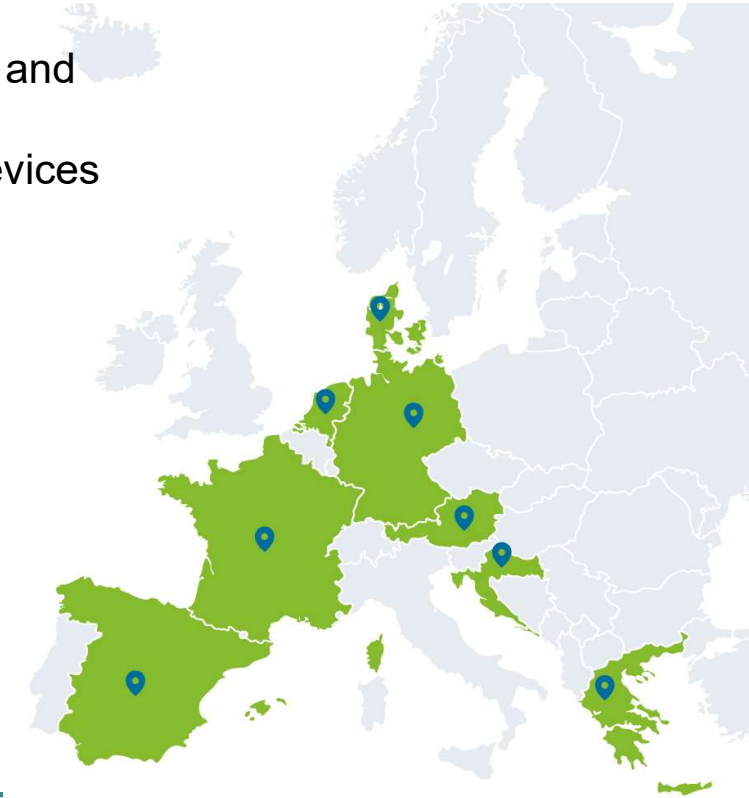
Process development
scienion ENABLING LIFE SCIENCE
INMOLD
temicon micronano solutions
RESCOLL Société de Recherche

Microfluidics development & manufacturing
bi.FLOW systems biofluidic integration
micronit microtechnologies

Medical senors
Erba Technologies Austria GmbH
Erba Mannheim
GENSPEED BIOTECH

Cell culture solutions
ibidi cells in focus
Innoprot Speeding up drug discovery

Research
tecnalia
TU Graz
BRFAA
BNN
JOANNEUM RESEARCH

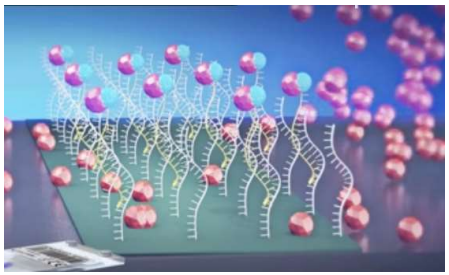


Microfluidics InnovationHub

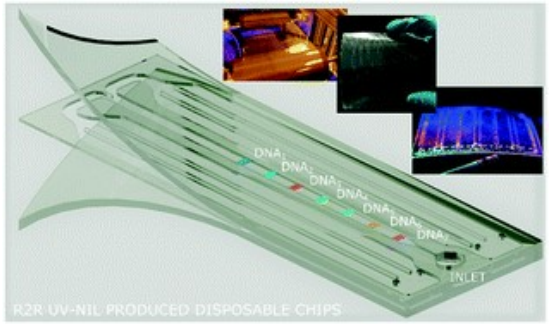
MIH Service Portfolio

Primary Components

Assay | Design | Manufacture | Surface functionalisation | Electrodes Sensors | Backend Services | Read Out Device System Integration | QM



- IVD – immuno / nucleic acids
- Electrochemistry
- Enzyme / substrate
- Cell manipulation and analysis



- Active & Passive Microfluidics
- Prototype to production
- From Milling over Inj. Mold to Roll-to-roll

Microfluidic Chip

- Microfluidic control
- Electronics
- Optical / amperometric read-out

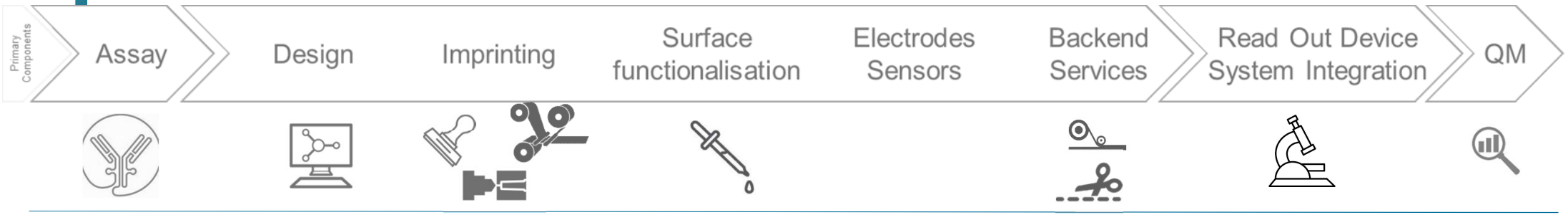
Microfluidic Solution

Microfluidics InnovationHub

Microfluidics – Value Chain

Microfluidic Chip

Microfluidic Solution



Industrial

micro resist technology
BIONIC SURFACE TECHNOLOGIES
RESCOLL Société de Recherche
micronit microtechnologies
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scienion ENABLING LIFE SCIENCE
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bi.FLOW systems biofluidic integration
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RTOs

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Innoprot Speeding up drug discovery
condensia Passion for chemistry
BIO NANONET

Microfluidics InnovationHub

Microfluidics InnovationHub

WE DEVELOP AND PRODUCE

Microfluidic Lab-on-a-Foil Systems

Single entry point to research & development services

Comprehensive service portfolio

Fast prototyping and scale up

Multiple funding opportunities

Quality assurance

We offer funding to scale up your application

- ✓ Open Call applications accepted on a rolling basis until September 2023
- ✓ Access to all services of the EU Horizon Europe project NextGenMicrofluidics
- ✓ Funding rate of up to 92% for European SMEs and 50% for Large Enterprises

Microfluidics Innovation Hub is the single entry point of the European project NextGenMicrofluidcs (www.nextgenmicrofluidics.eu). NextGenMicrofluidics has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.

Microfluidics InnovationHub

Functional nucleic acids: unleashing their untapped potential in a diverse set of applications

7 FEB 2023, 15:00 CET

Presented by George Tsekenis, PhD
Head of the Applied Biophysics and Surface Science Group, BRFAA

BRFAA
BIOMEDICAL RESEARCH FOUNDATION ACADEMY OF ATHENS

www.microfluidicshub.eu

Content

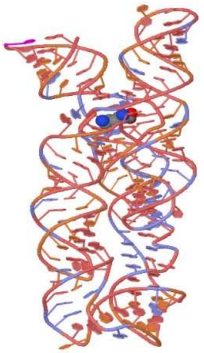
- Functional nucleic acids
  - Categories and selection strategies
  - Advantages over antibodies
  - Current challenges and ways forward
- Applications of functional nucleic acids
- Applied Biophysics and Surface Science (ABISS) Group

Microfluidics InnovationHub

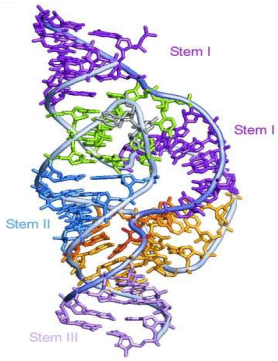
Functional nucleic acids

Categories and selection strategies

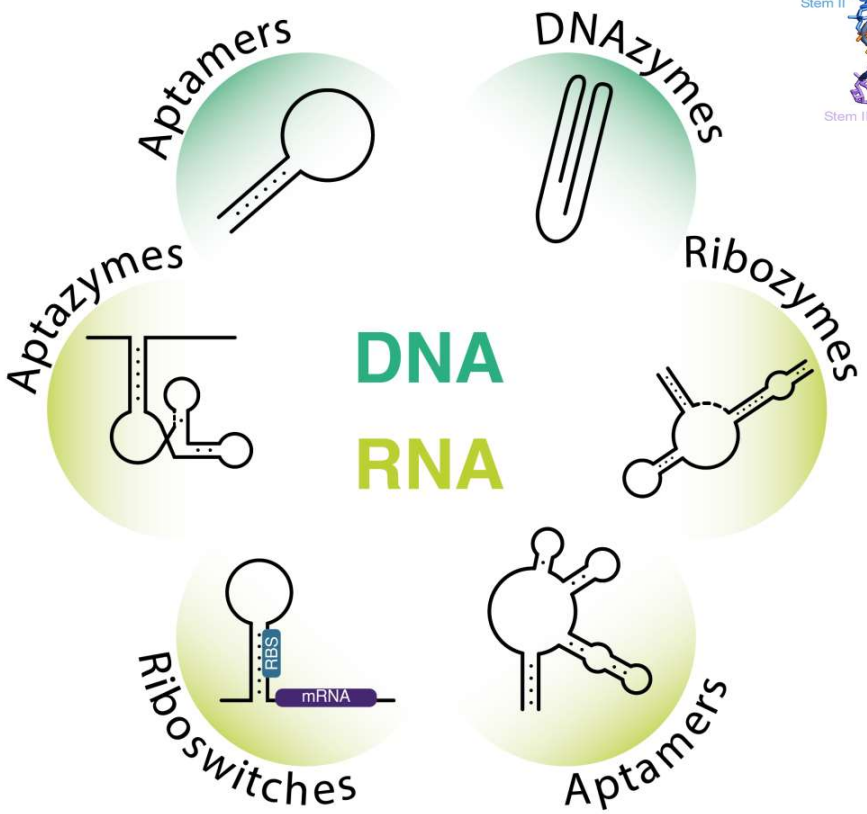
Functional nucleic acids



Lysine Riboswitch

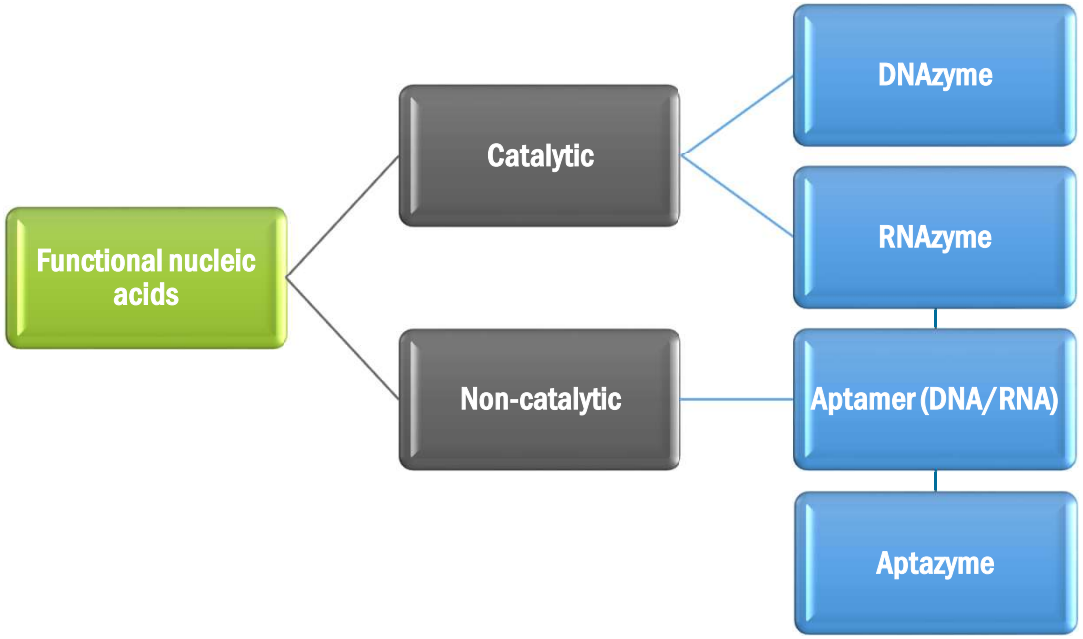


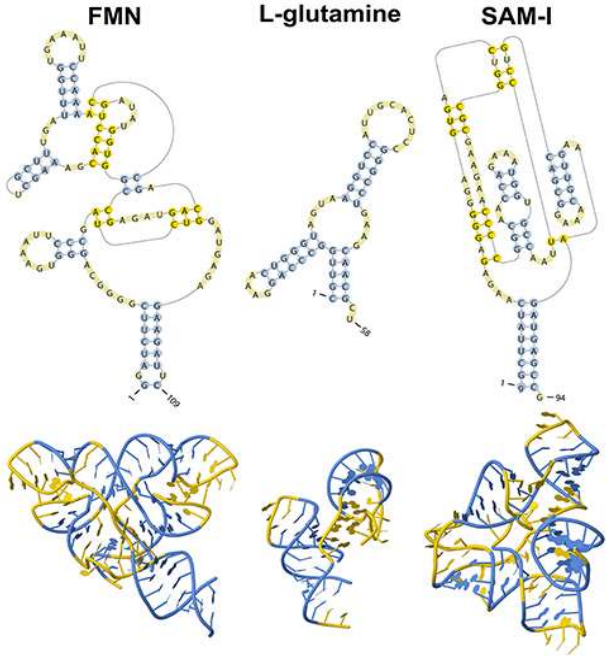
Hammerhead ribozyme



Drawing inspiration from nature…

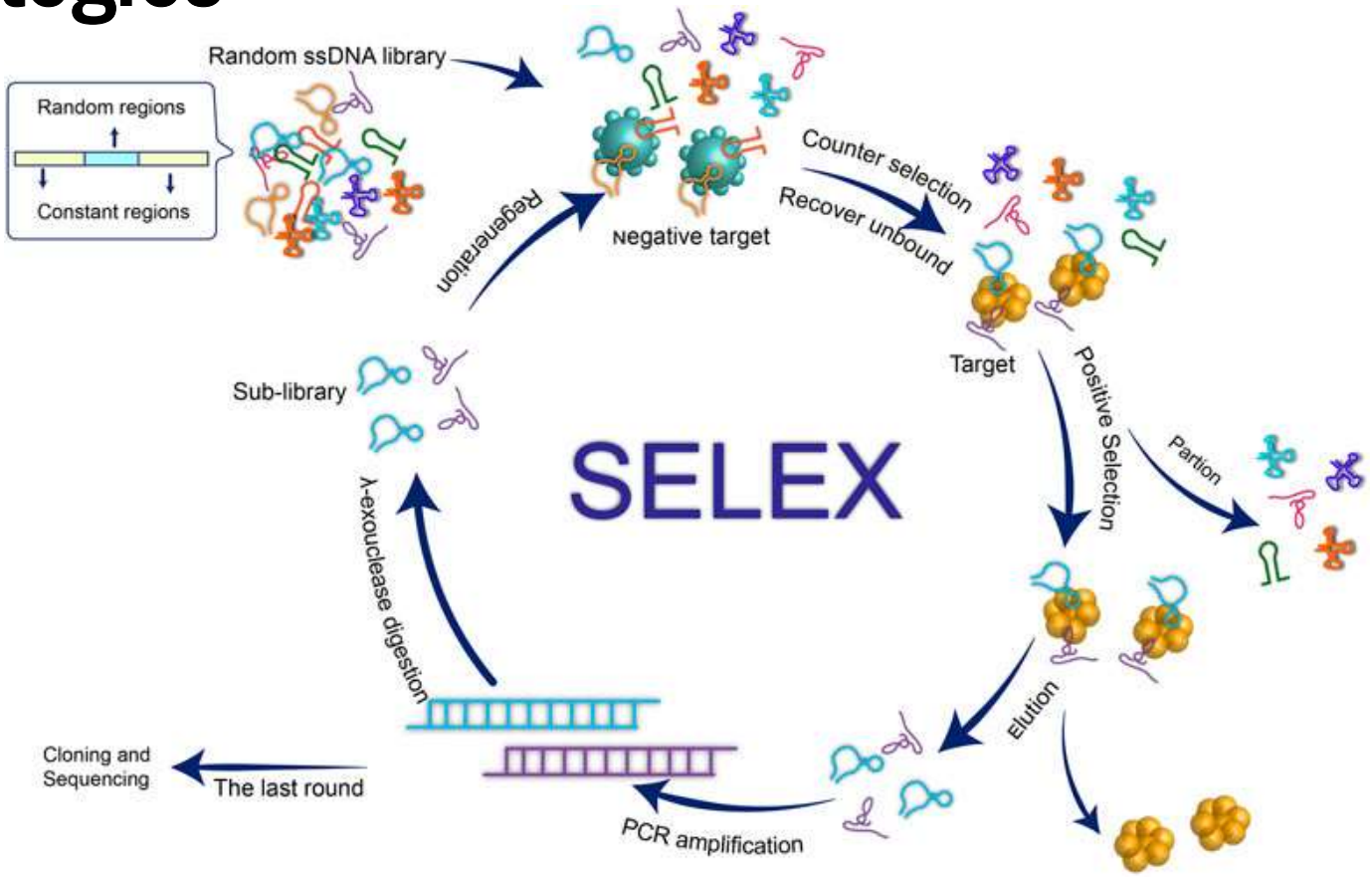
Functional nucleic acids





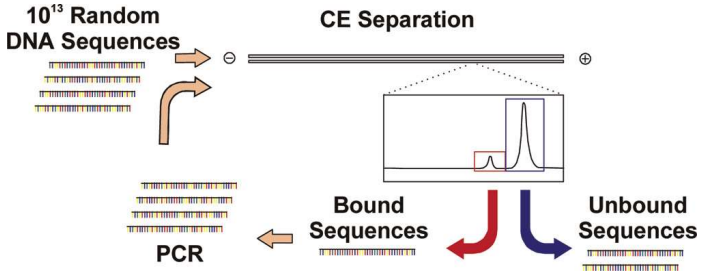
...going synthetic!

Selection strategies



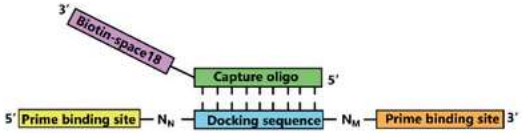
Standard SELEX workflow

Selection strategies

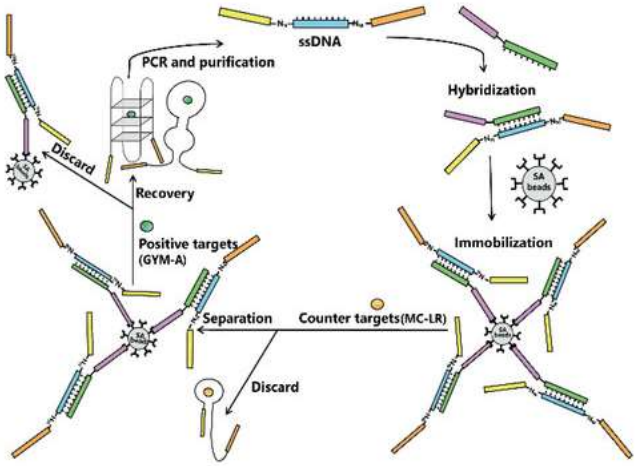


Some of the countless SELEX variants

SELEX variant	Description
Magnetic bead-based SELEX	Accelerate SELEX process by immobilizing targets on magnetic beads
Capillary electrophoresis SELEX	Neither the ligand nor the oligo library are immobilized
Cell-SELEX	Whole cells employed as targets
Capture-SELEX	Immobilize oligonucleotide library, while the ligand is in solution
Next generation sequencing SELEX	Sequencing across all the selection rounds rather than just the last one



Worflow



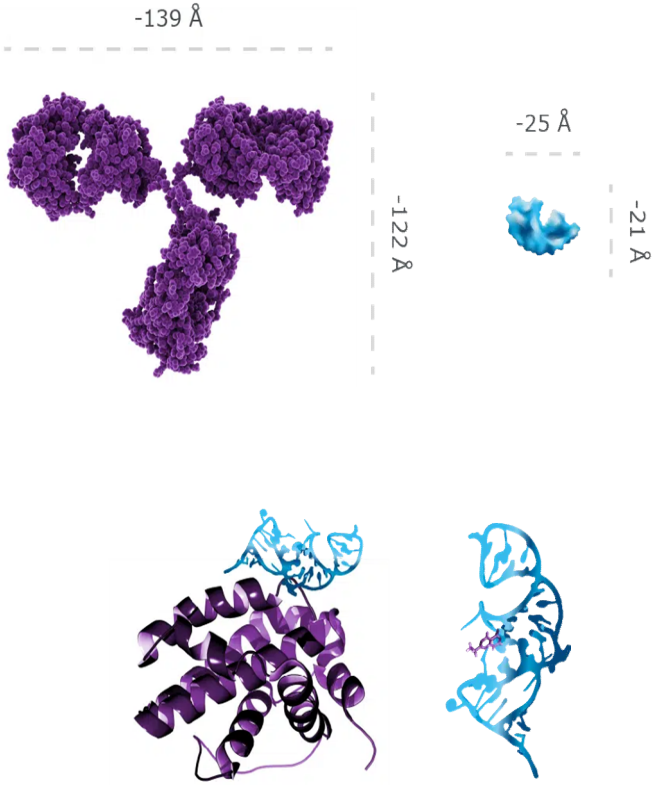
Microfluidics InnovationHub

Functional nucleic acids

Advantages over antibodies

Advantages

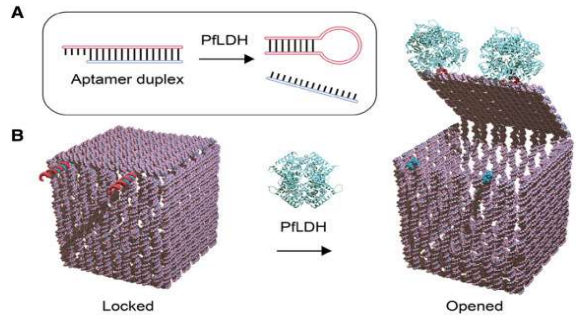
	Antibodies	Aptamers
Affinity for ligand	High	High
Selectivity for ligand	High	High (?)
Range of target analytes	Limited	Wide
Cost	Expensive	Cheap
Stability	Low	High
Modification	Not controlled	Easy and highly controlled



Are aptamers on a par to antibodies?

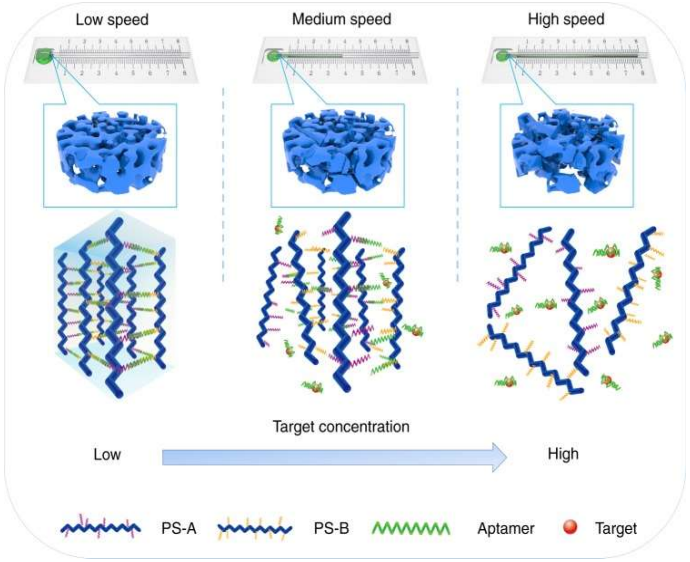
Advantages (I)

Base complementarity allows self-organized networks to be formed



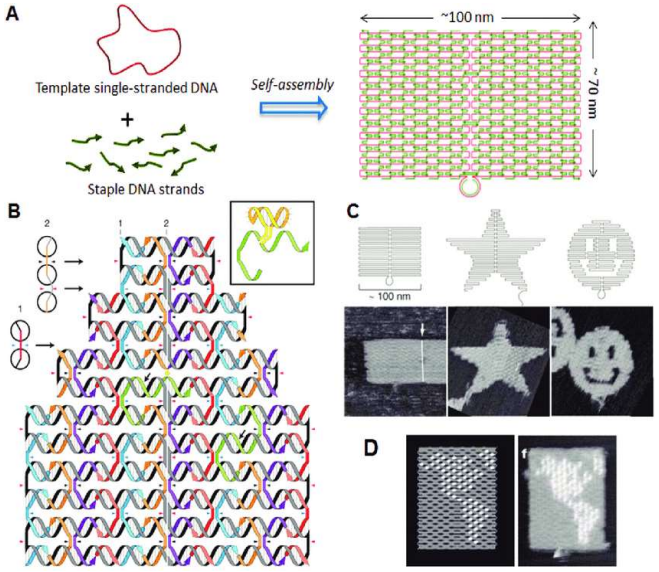
Nanomedicine: Nanotechnology, Biology and Medicine, 14, 2018,1161-1168, https://doi.org/10.1016/j.nano.2018.01.018.

- Target-responsive hydrogels



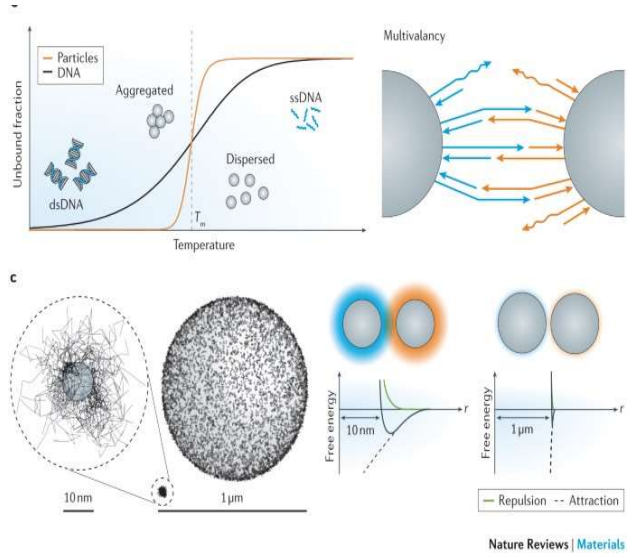
Nat Commun. 2019 Mar 8;10(1):1036. doi: 10.1038/s41467-019-08952-1

- DNA origami



Nat Rev Methods Primers 1, 13 (2021). https://doi.org/10.1038/s43586-020-00009-8

- Microparticles

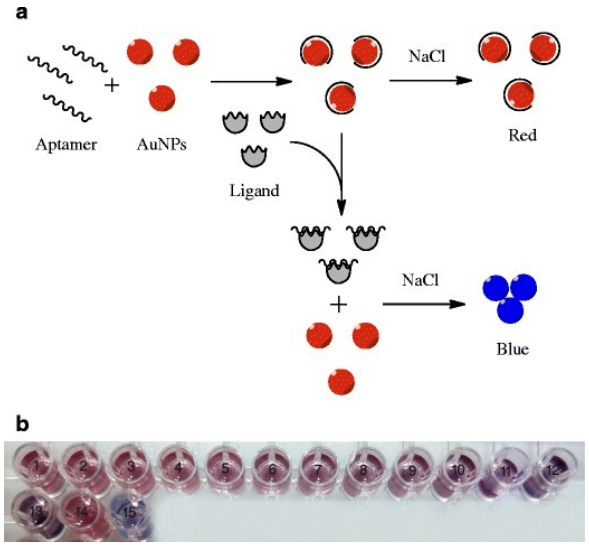


Nat Rev Mater 1, 16008 (2016). https://doi.org/10.1038/natrevmats.2016.8

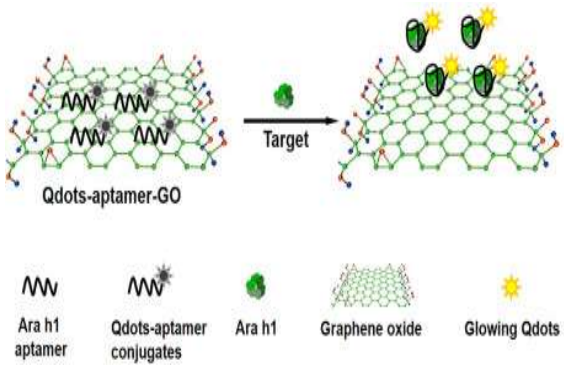
Advantages (II)

Unique interactions endowed by the vary nature of nucleic acids

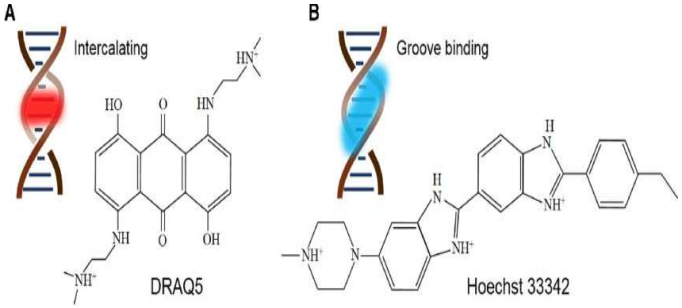
- Interactions with 2D and 3D nanomaterials, organic molecules



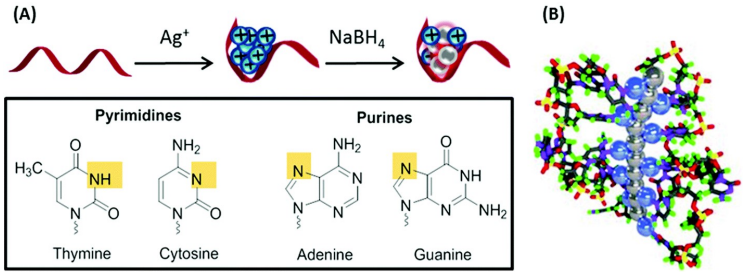
Microchim Acta 183, 1687–1697 (2016).
https://doi.org/10.1007/s00604-016-1798-3



Biosensors and Bioelectronics, 85, 2016, 649-656,
https://doi.org/10.1016/j.bios.2016.05.072.



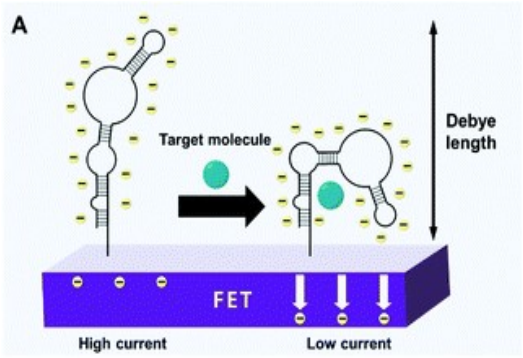
Biochemical Society Transactions (2018) https://doi.org/10.1042/BST20170301

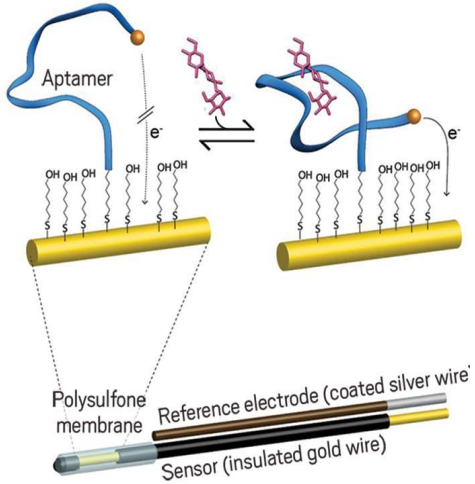


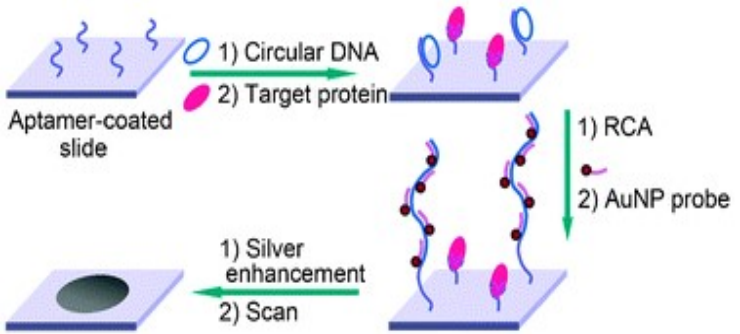
https://doi.org/10.1039/C6NR05872H

Advantages (III)

Signal generation or amplification made simple



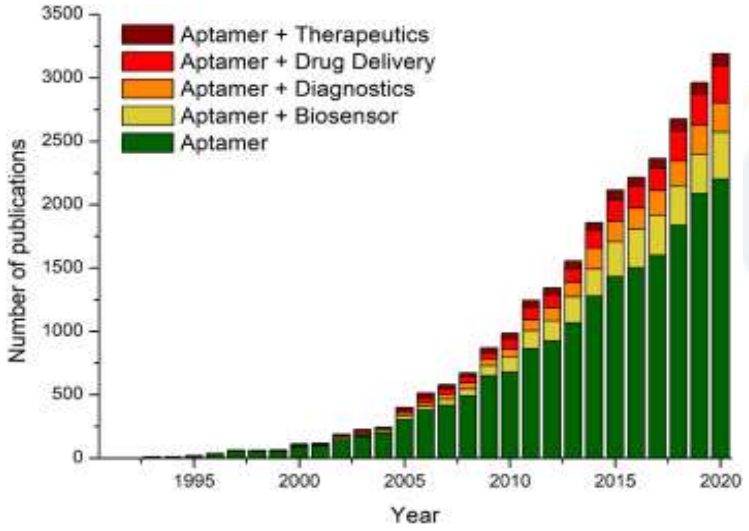




10.1039/D0MA00639D (Review Article) Mater. Adv., 2020, 1, 2663-2687

Chem. Commun., 2010,46, 6720-6722 https://doi.org/10.1039/C002078H

What's the catch?



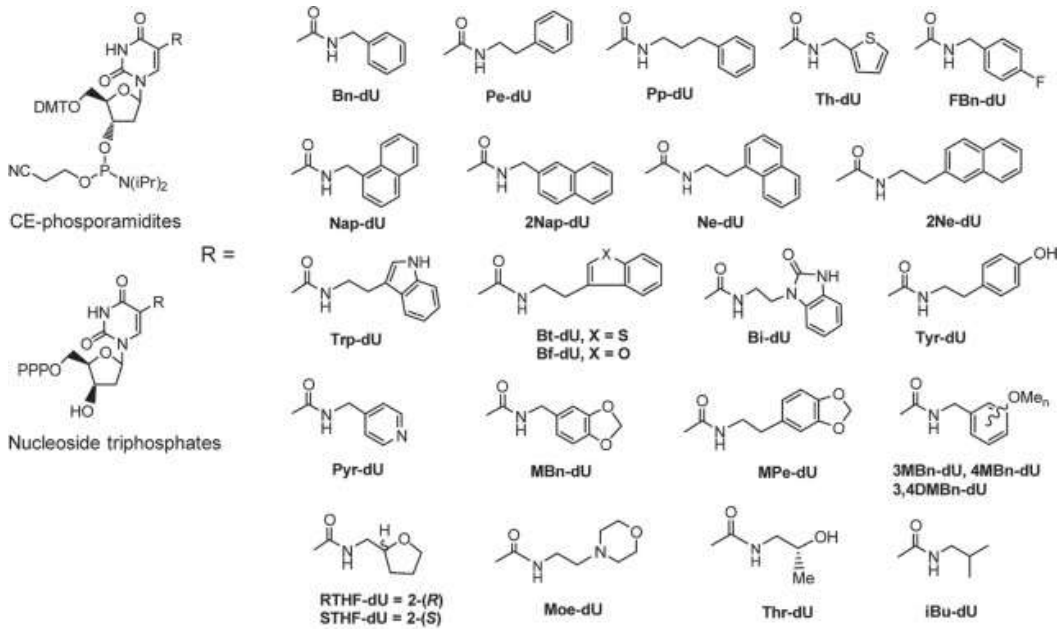
Microfluidics InnovationHub

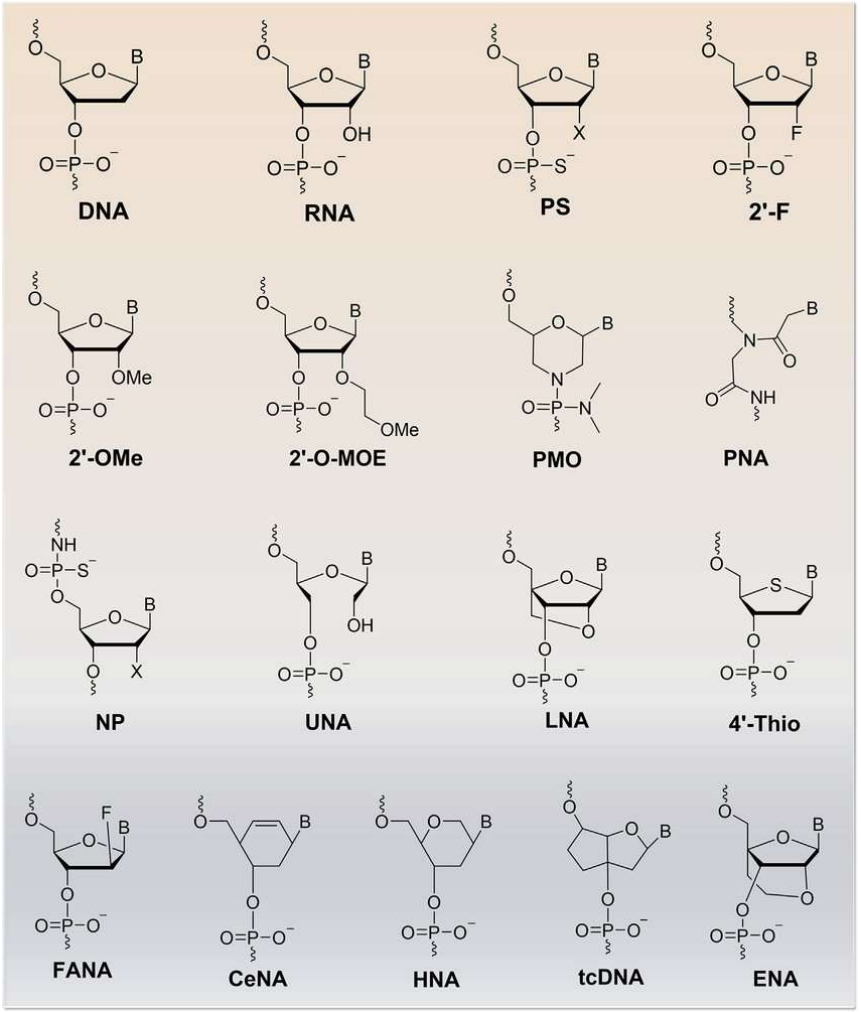
Functional nucleic acids

Current challenges and ways forward

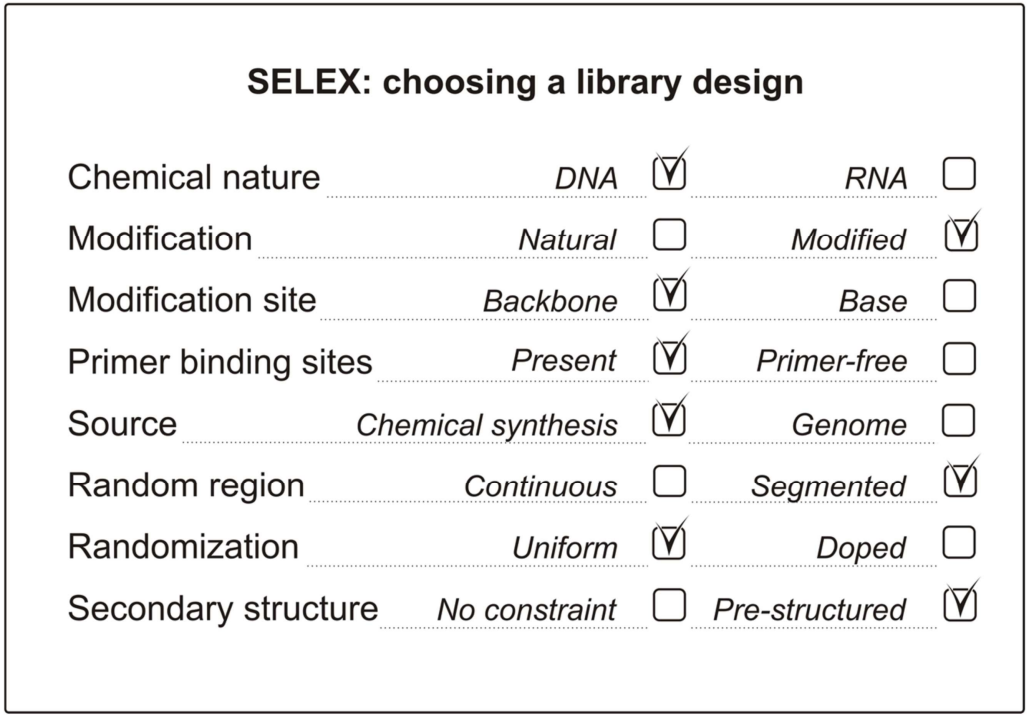
Limited possibilities in a 4 letter language

Modified nucleotides





Issues with SELEX



Validation of selected sequences

Trends in Analytical Chemistry 146 (2022) 116480

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Critical evaluation of aptamer binding for biosensor designs☆

Yichen Zhao, Kayvan Yavari, Juewen Liu*

Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada

ARTICLE INFO

Article history:
Available online 13 November 2021

Keywords:
Aptamers
Dissociation constant
Biosensors
Isothermal titration calorimetry
SELEX

ABSTRACT

Over the last three decades, numerous aptamer-based biosensors have been reported. The basis of these sensors is the selective binding of target analytes by aptamers. In the last few years, a number of papers have been published questioning the binding ability of some popular aptamers such as those documented for As(III), ampicillin, chloramphenicol, isocarbophos, phorate and dopamine. In this article, these papers are reviewed, and the binding assays are described, which may provide possible reasons for obtaining false positive aptamers. Additionally, relevant aptamer selection methods and typical characterization steps are described. It is found that for small molecular targets, using an immobilized library might result in better aptamers. Furthermore, the importance of carefully designed controls to ensure the quality of binding assays is discussed, especially in the case of mutated nonbinding aptamers. Only then, with fully validated aptamers, can subsequent biosensor design bring about meaningful results.

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Critical evaluation of aptamer binding for biosensor designs☆

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Article

Do Aptamers Always Bind? The Need for a Multifaceted Analytical Approach When Demonstrating Binding Affinity between Aptamer and Low Molecular Weight Compounds

Fabio Bottari, Elise Daems, Anne-Marie de Vries, Pieter Van Wielendaele, Stanislav Trashin, Ronny Blust, Frank Sobott, Annemieke Madder, José C. Martins,* and Karolien De Wael*

Cite This: *J. Am. Chem. Soc.* 2020, 142, 19622−19630

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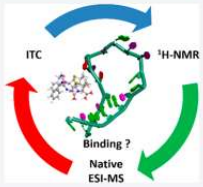
ABSTRACT: In this manuscript, we compare different analytical methodologies to validate or disprove the binding capabilities of aptamer sequences. This was prompted by the lack of a universally accepted and robust quality control protocol for the characterization of aptamer performances coupled with the observation of independent yet inconsistent data sets in the literature. As an example, we chose three aptamers with a reported affinity in the nanomolar range for ampicillin, a β-lactam antibiotic, used as biorecognition elements in several detection strategies described in the literature. Application of a well-known colorimetric assay based on aggregation of gold nanoparticles (AuNPs) yielded conflicting results with respect to the original report. Therefore, ampicillin binding was evaluated in solution using isothermal titration calorimetry (ITC), native nano-electrospray ionization mass spectrometry (native nESI-MS), and ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H NMR). By coupling the thermodynamic data obtained with ITC with the structural information on the binding event given by native nESI-MS and ^{1}H NMR we could verify that none of the ampicillin aptamers show any specific binding with their intended target. The effect of AuNPs on the binding event was studied by both ITC and ^{1}H NMR, again without providing positive evidence of ampicillin binding. To validate the performance of our analytical approach, we investigated two well-characterized aptamers for cocaine/quinine (MN4), chosen for its nanomolar range affinity, and L-argininamide (1OLD) to show the versatility of our approach. The results clearly indicate the need for a multifaceted analytical approach, to unequivocally establish the actual detection potential and performance of aptamers aimed at small organic molecules.

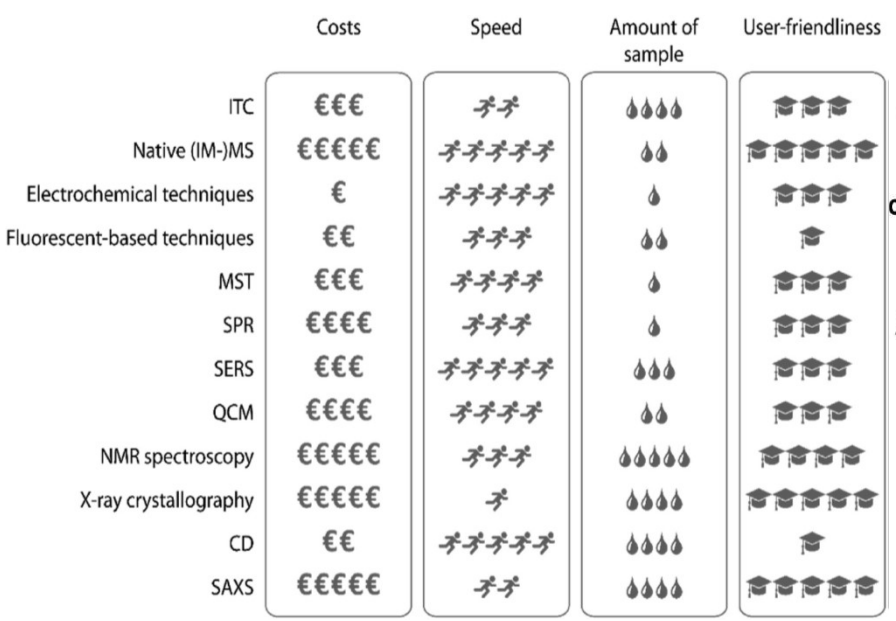


INTRODUCTION

Aptamers are short single strands of DNA or RNA that recognize with high affinity a given target against which they are selected. Aptamers were first obtained in the 1990s[1−3] following a procedure called SELEX (systematic evolution of ligands by exponential enrichment). From the beginning, they were considered a leap forward in many analytical and biomedical applications. Indeed, aptamers offer considerable advantages over traditional molecular biorecognition elements such as antibodies or enzymes, including stability over a wider

remain to be faced before this can be achieved. A variety of factors have been put forward to explain why aptamers have not yet penetrated the market:[16] one of the main reasons can be identified as the so-called "thrombin problem". Indeed, rather than developing assays for more clinically relevant targets, hundreds of investigators continue to focus their attention on perfecting thrombin-binding aptamers or designing clever detection strategies for this target. The same can be said to a lesser extent for cocaine-binding aptamers in the field

Validation of selected sequences

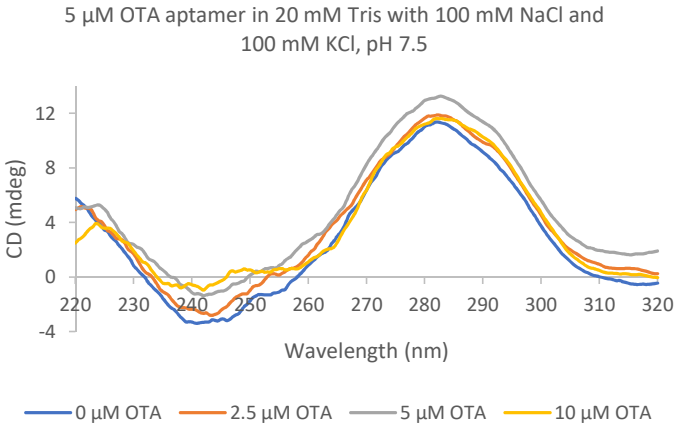


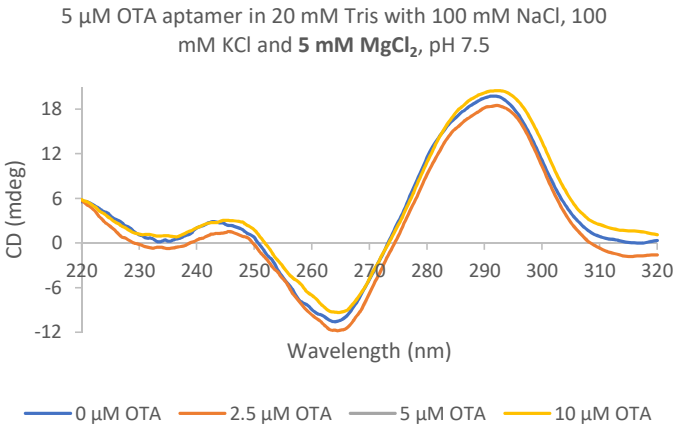
	1. Candidate screening	2. Truncation & optimization	3. Characterization	4. Functional validation
Method milestones	Reduce the number of putative aptamer sequences from SELEX from hundreds to ~3 candidates	Determine minimal binding sequence	Determine the K_D, selectivity and other parameters	Assess the robustness of the aptamer for use in different application platforms
Important assay considerations	1. High-throughput 2. Cost-effective	1. High-throughput 2. Cost-effective 3. Provides insight about structure or important binding residues	1. Quantitative 2. Precise 3. Measurement of multiple parameters in parallel	1. Validate function with at least two separate methods 2. Determine functionality in solution and immobilized 3. Does not need to be quantitative
Assay options (ascending order)	1. Fluorescence Polarization (FP) 2. SYBR Green (SG) 3. AuNP Assay 4. Affinity Chrom. (beads) 5. SPR	1. DNase Assay 2. FP 3. SG 4. Affinity Chrom. (beads) 5. SPR	1. SPR 2. FP 3. Equil. dialysis 4. SG 5. Affinity Chrom. (beads)	*Choose at least 1 assay from group not used in step 3* **In solution** FP, Equil. Dialysis, SG, Affinity Chrom. (either), Ultrafiltration, DNase **DNA immobilized/constrained** SPR, AuNPs

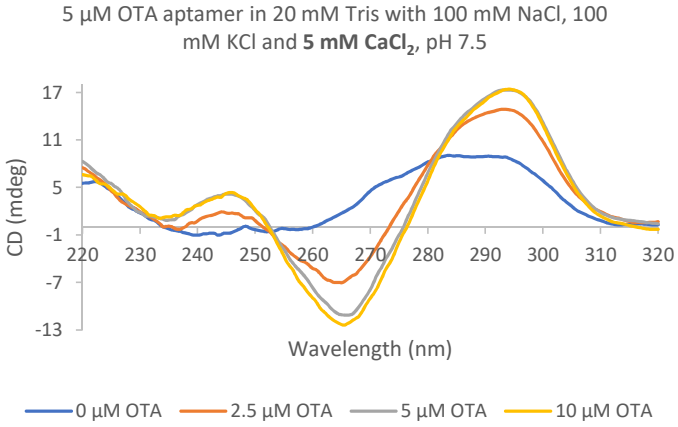
DOI: 10.1021/acs.analchem.5b02102
Anal. Chem. 2015, 87, 8608−8612

Trends in Analytical Chemistry 142 (2021) 116311
https://doi.org/10.1016/j.trac.2021.116311

Buffer optimization



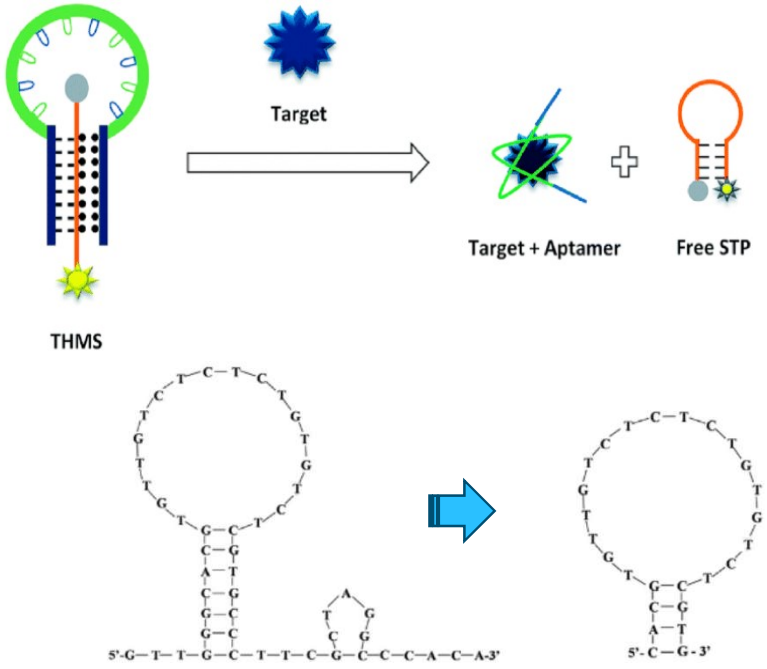




Structural conformation induced only in the presence of target analyte!!

Post-SELEX sequence optimization

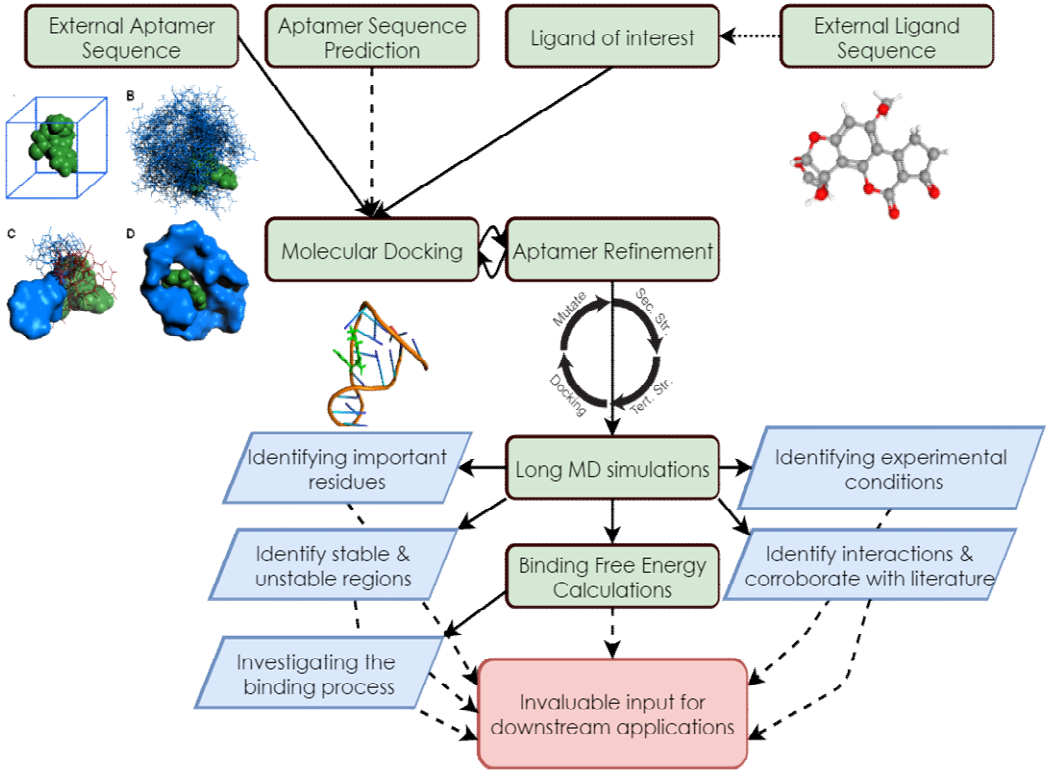
Rational design of aptamers and assays



Triple helix molecular switch

Minimize the search space

COC1=C2C3=C(C(=O).
..OC5C4(C=CO5)O



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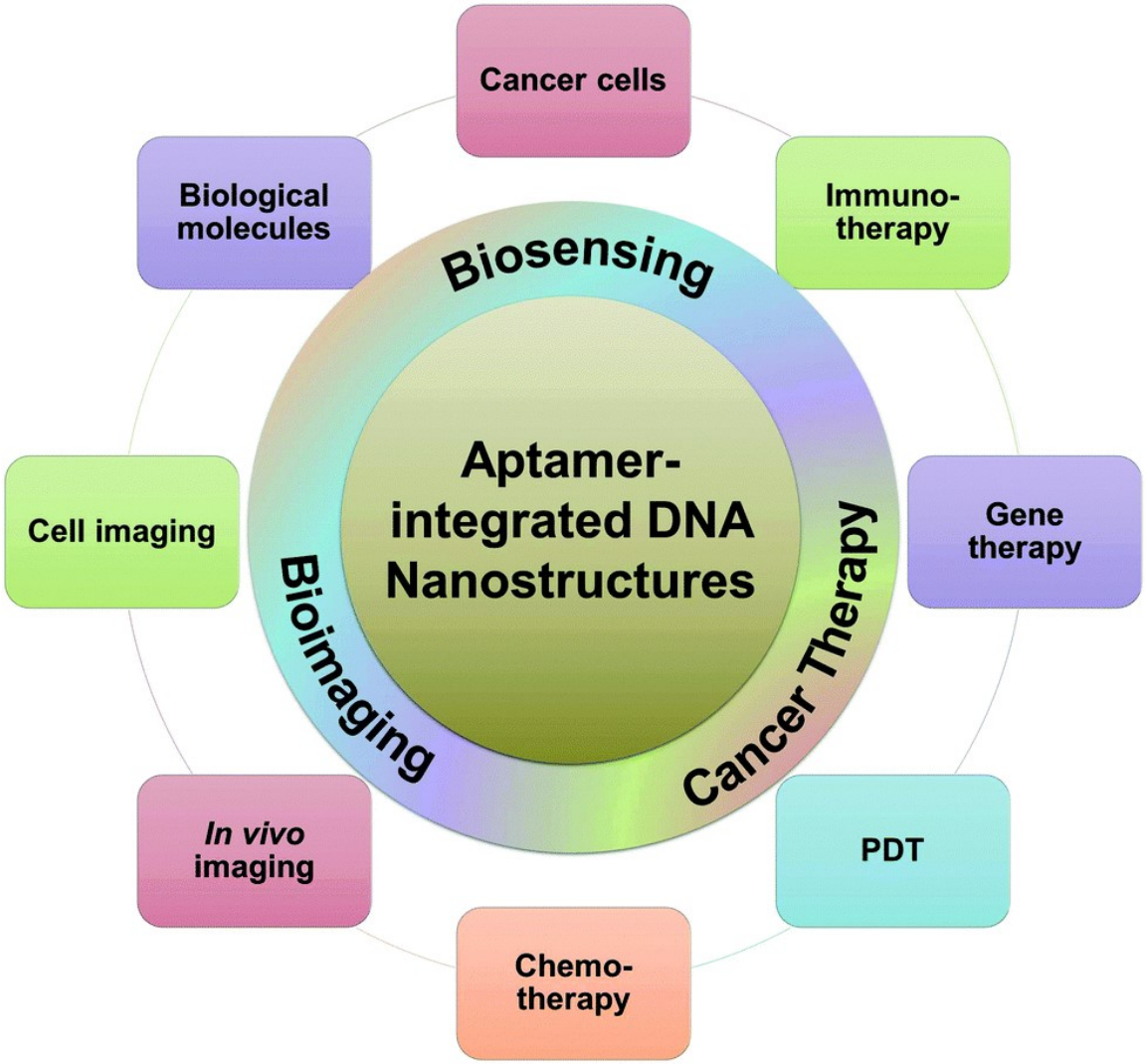
Functional nucleic acids

Applications of functional nucleic acids

Applications



Get your aptamer right!!!

Design your assay smartly!!!

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Applied Biophysics and Surface Science (ABISS) Group

Biomedical Research Foundation Academy of Athens

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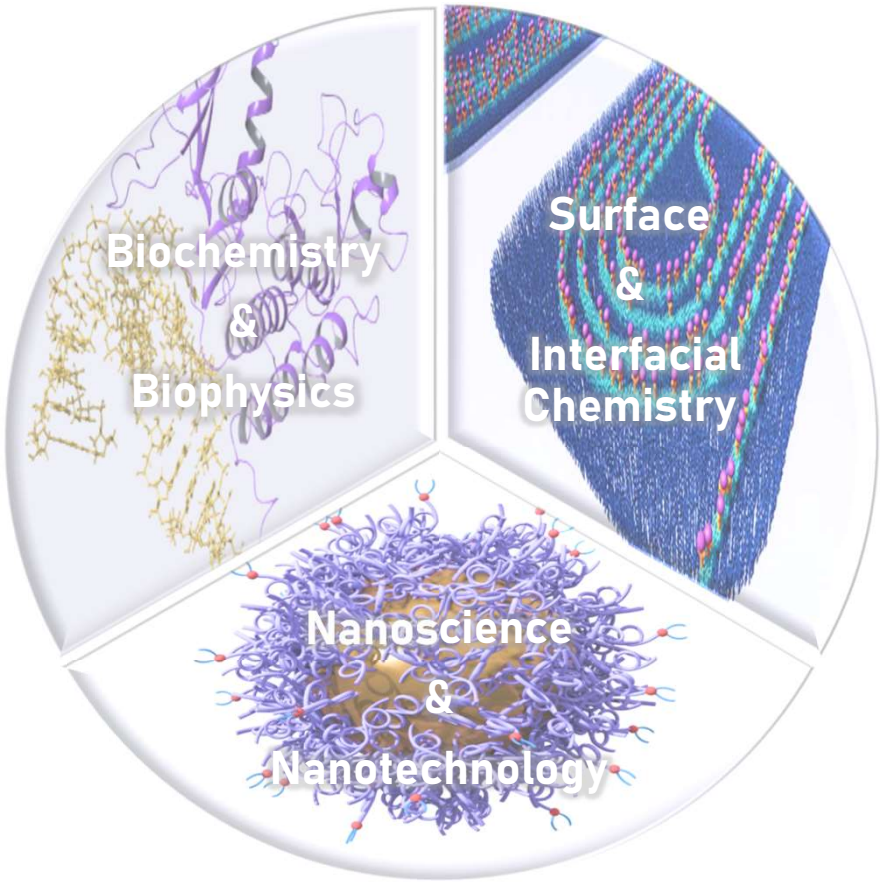

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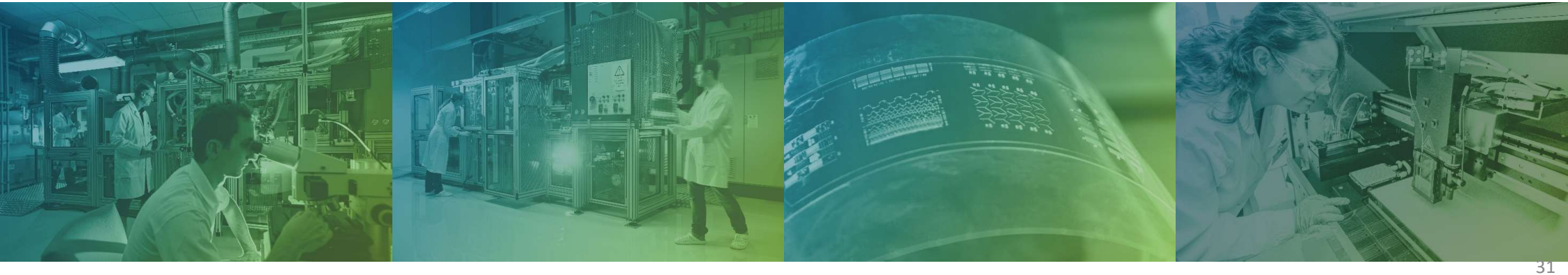
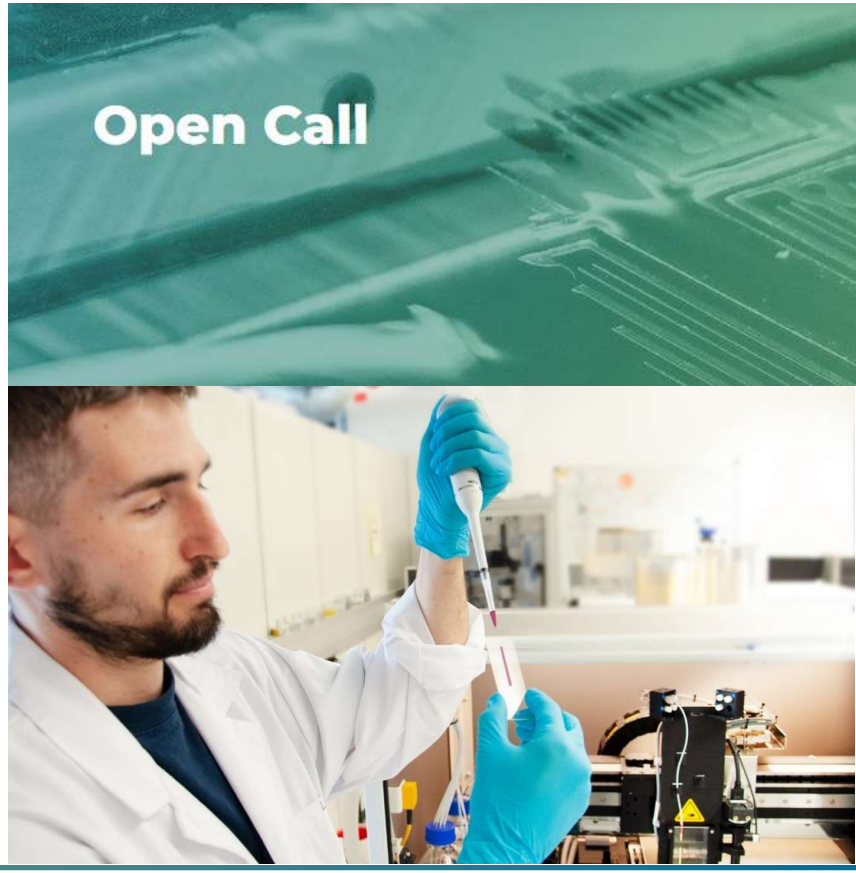
THANK YOU



Accelerate your Microfluidic Innovation

- Addresses companies – SMEs and LEs
- Access to all services of the NGM OITB - min 2 partners involved
- Budget up to EUR 200.000
- Duration: 6 to 12 months
- Funding rate of up to 92% and 50% for European SMEs and Large Enterprises respectively
- Technology Readiness Level > 4 or Microfluidic System available
- Managed & coordinated by the MIH
- Details see: www.nextgenmicrofluidics.eu/open-call/



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WE DEVELOP AND PRODUCE CUSTOMIZED

Microfluidic Lab-on-a-Foil Systems

Single entry point to research & development services

Comprehensive service portfolio

Fast prototyping and scale up

Multiple funding opportunities

Quality assurance

Find out more at https://www.microfluidicshub.eu

Microfluidics Innovation Hub is the single entry point of the European project NextGenMicrofluidcs (www.nextgenmicrofluidics.eu). NextGenMicrofluidics has received funding from the European Union's HORIZON 2020 research & innovation programme under grant agreement no. 862092.