Biomolecular recognition using aptamers

Literature seminar #1

2024.12.19

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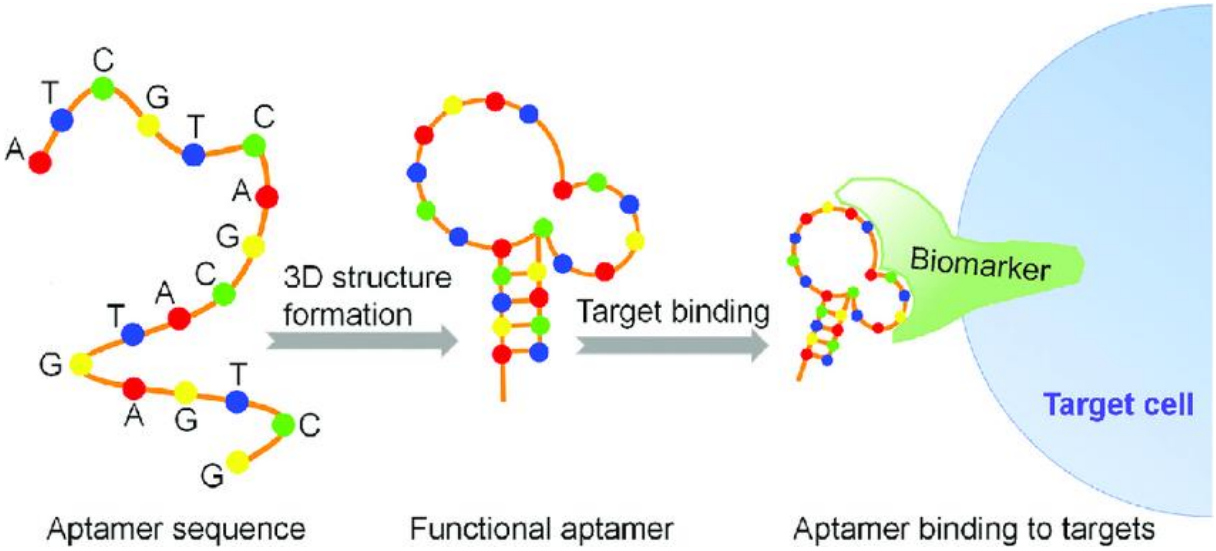


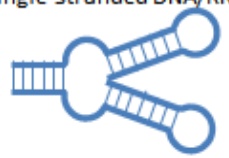
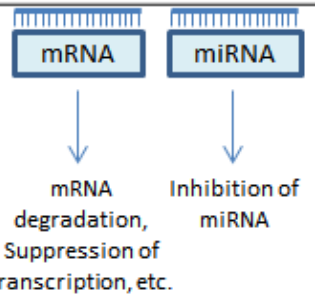
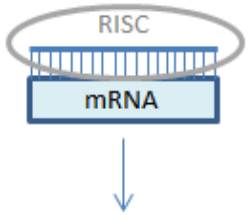
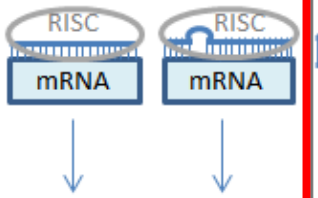
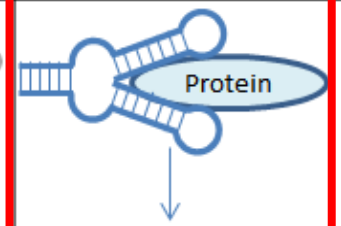
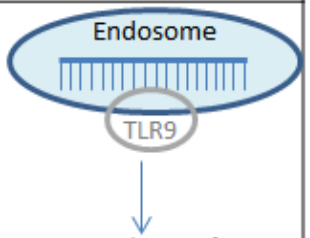
Yang, S.; *et al*. *Mol. Ther. Nucleic. Acids.* **2018**, *13*, 164–175.

Aptamers are a type of oligonucleotide therapies

Aptamer

- single stranded DNA/RNA
- three-dimensional structure
- high specificity due to intermolecular forces

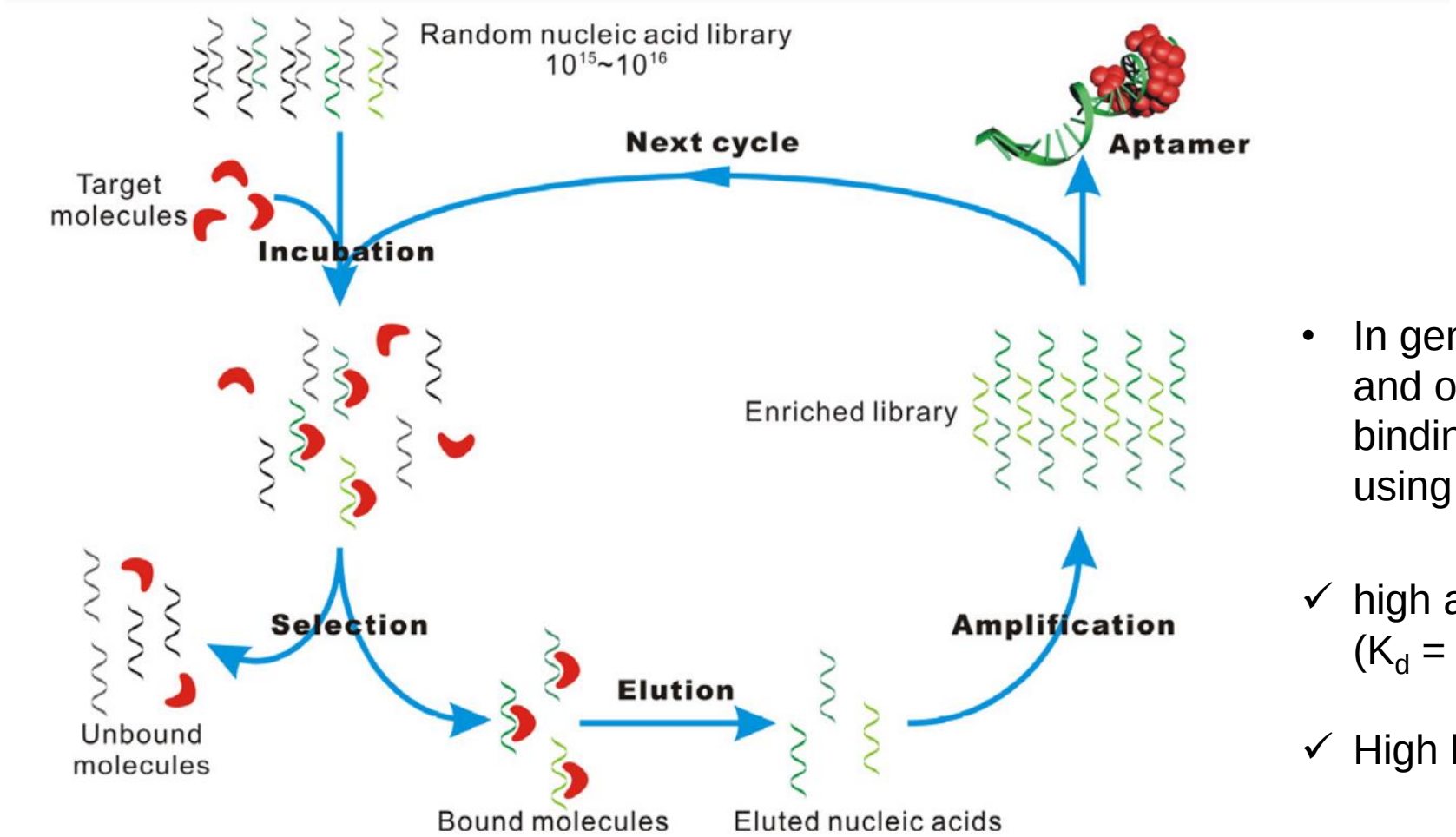
Classifications of major oligonucleotide therapies

	Antisense oligonucleotides	siRNA	miRNA (mimic)	Aptamer	CpG oligonucleotides
Typical structure	Single-stranded DNA/RNA 	Double-stranded RNA 	Double-stranded RNA 	Single-stranded DNA/RNA 	Single-stranded DNA 
Mechanism of action					
Characteristics	A variety of mechanisms exist, e.g. mRNA degradation, splicing regulation, inhibiting miRNA.	Specific effect to mRNA with complementary sequences.	Single miRNA can regulate several mRNA transcripts.	High specificity due to three- dimensional structure-dependent effect.	Applying innate immune system to oligonucleotides via TLR9 which is considered as a side effect.
DDS/ miscellaneous	Chemically modified oligonucleotides are often used and DDS is not usually required.	Chemically modified oligonucleotides have recently been developed but DDS is generally required.	Generally DDS is required.	PEG modification is often used in order to extend blood circulation time.	Mixed with antigen as an adjuvant.

https://atelocollagen.com/en/information/info180214

SELEX : Systematic Evolution of Ligands by EXponential enrichment

Schematic depiction of SELEX



- In general, aptamers are selected and obtained based on their binding affinity to target molecules using a method called SELEX.

✓ high affinity for the target (K_d = nM ~ pM)

✓ High binding specificity

Song, S.; *et al*. *TrAC, Trends. Anal. Chem.*, *27* (2), 108–117.

Aptamers have many advantages over antibodies.

	aptamer	antibodies
Molecular weight	Middle (~12-30 kDa)	Relatively big (~150-180 kDa)
Preparation method	Chemical method	Biological method
Generation time	Few hours to months	Several months(~ 6 months)
Cost	Lower	Higher
Allowed chemical modification	Various modifications	Limited modifications
Stability	Very stable	Sensitive to temperature and pH changes
Immunogenicity	Low	High

Ali, M. H.; *et al*. *Int. J. Mol. Sci.* **2019**, *20* (10), 2511.

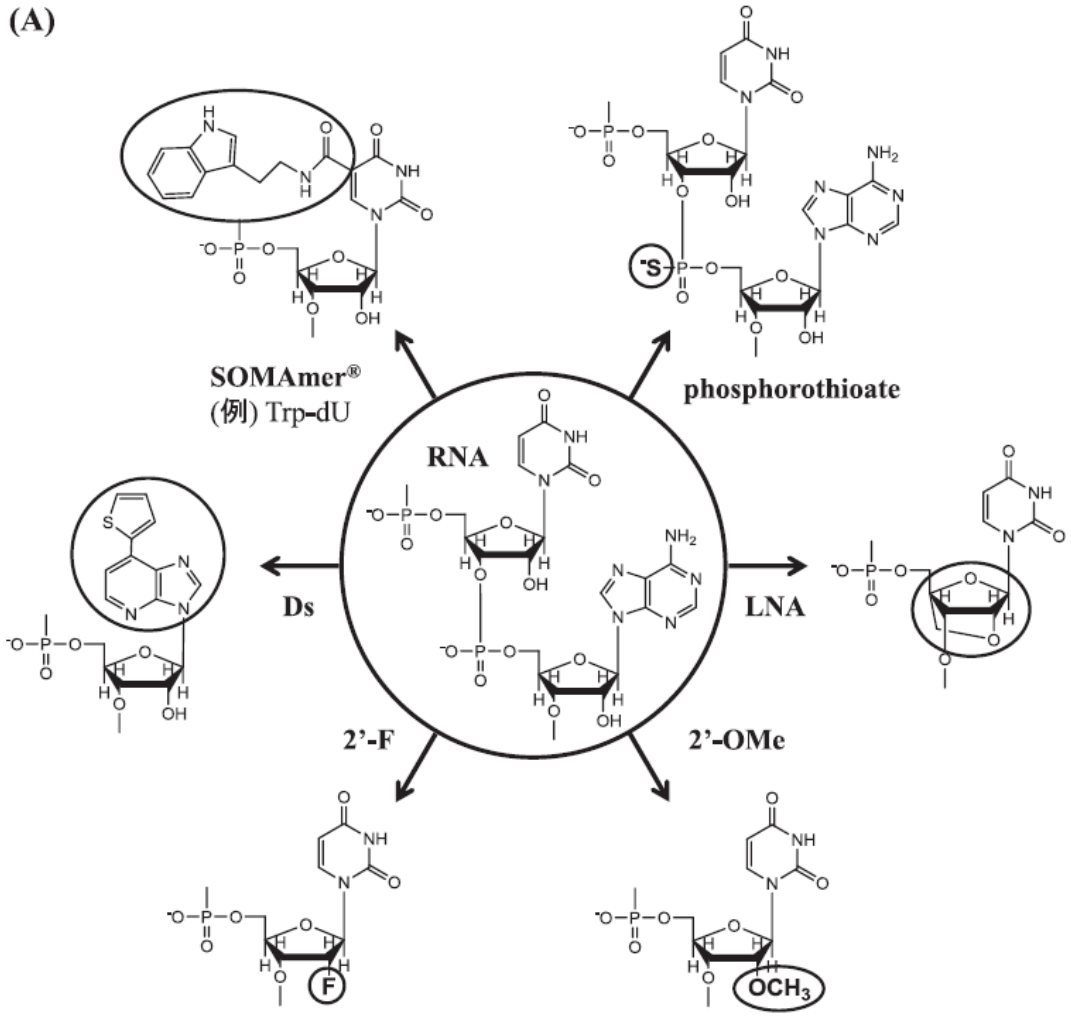
Disadvantages of aptamers and solutions

Disadvantages

- × Degradation by nuclease
- × prone to renal filtration (腎ろ過)
- × Low cell membrane permeability

Solutions

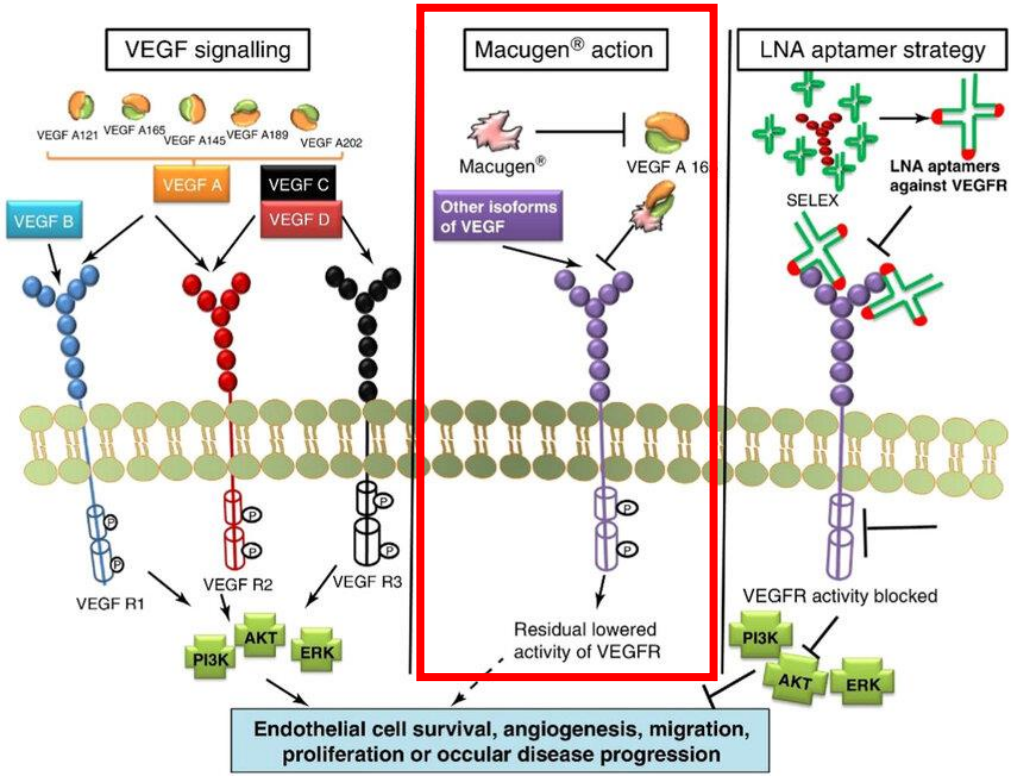
- Chemical modifications (e.g. 2'-OH → -OMe, -F)
- Increase molecular weight by adding PEG



Abe, T.; *et al*. *Folia. Pharmacol. Jpn.* **2016**, *147* (6), 362–367.

Aptamers that control biomolecular reactions

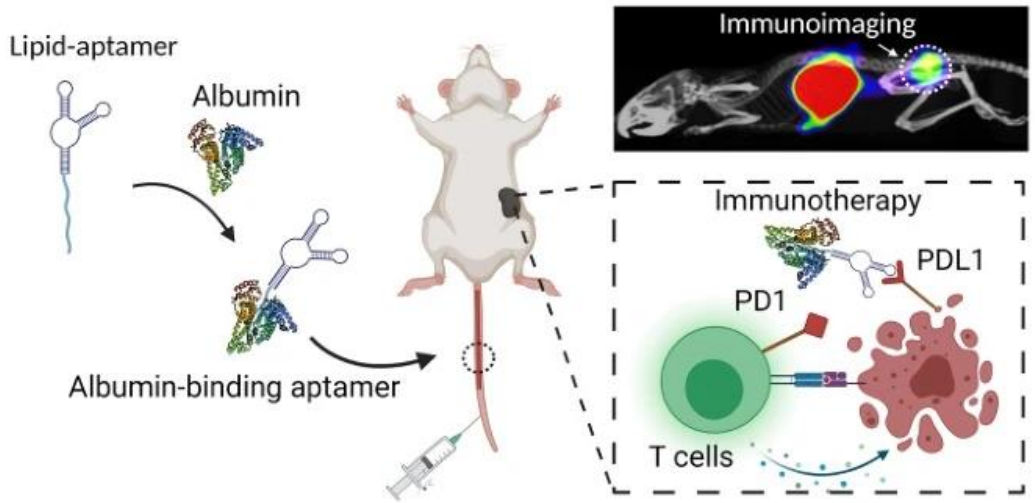
Macugen



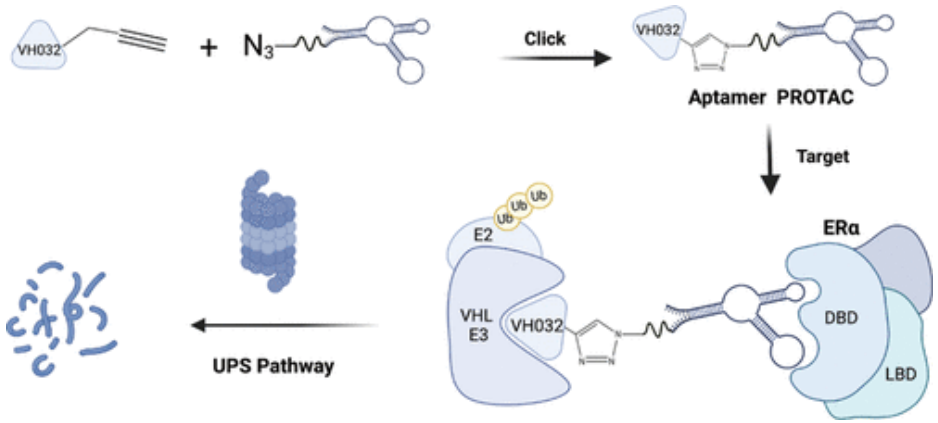
- Macugen is the first therapeutic aptamer
- VEGF(血管内皮増殖因子) inhibitor

Tian, L.; *et al*. *Sci. China. Chem.* **2021**, *65* (3), 574–583.

PD-L1 binding aptamer



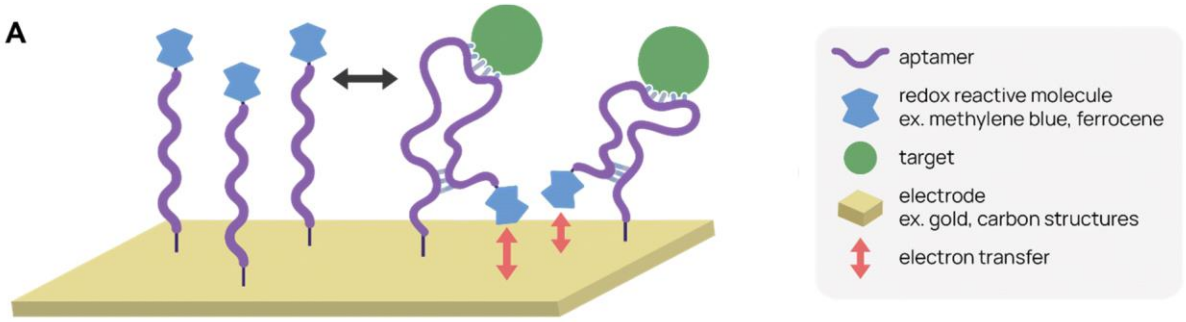
Aptamer-PROTAC



Zhang, T.; *et al*. *Nanotechnol.* **2021**, *33* (16), 162001–162001.
Feng, Y.; *et al*. *ACS Pharmacol .Transl. Sci.* **2024** , 7 (12), 3945-3954.

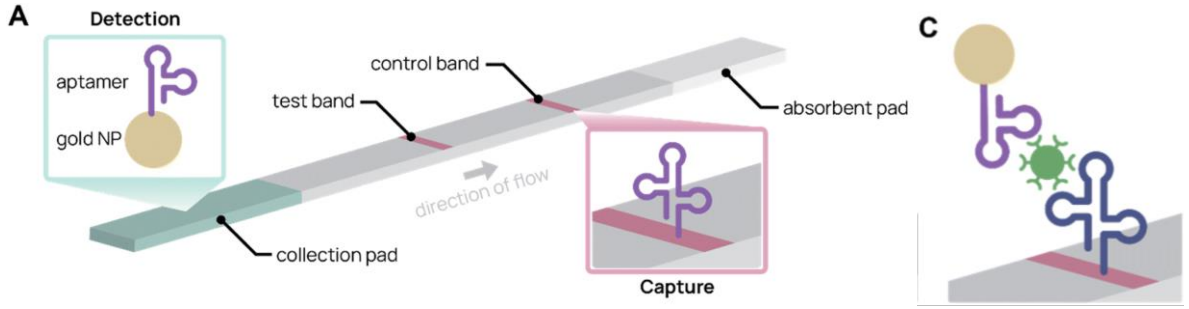
Aptamers are useful as biosensors

Electrochemical aptamer-based (E-AB) biosensor



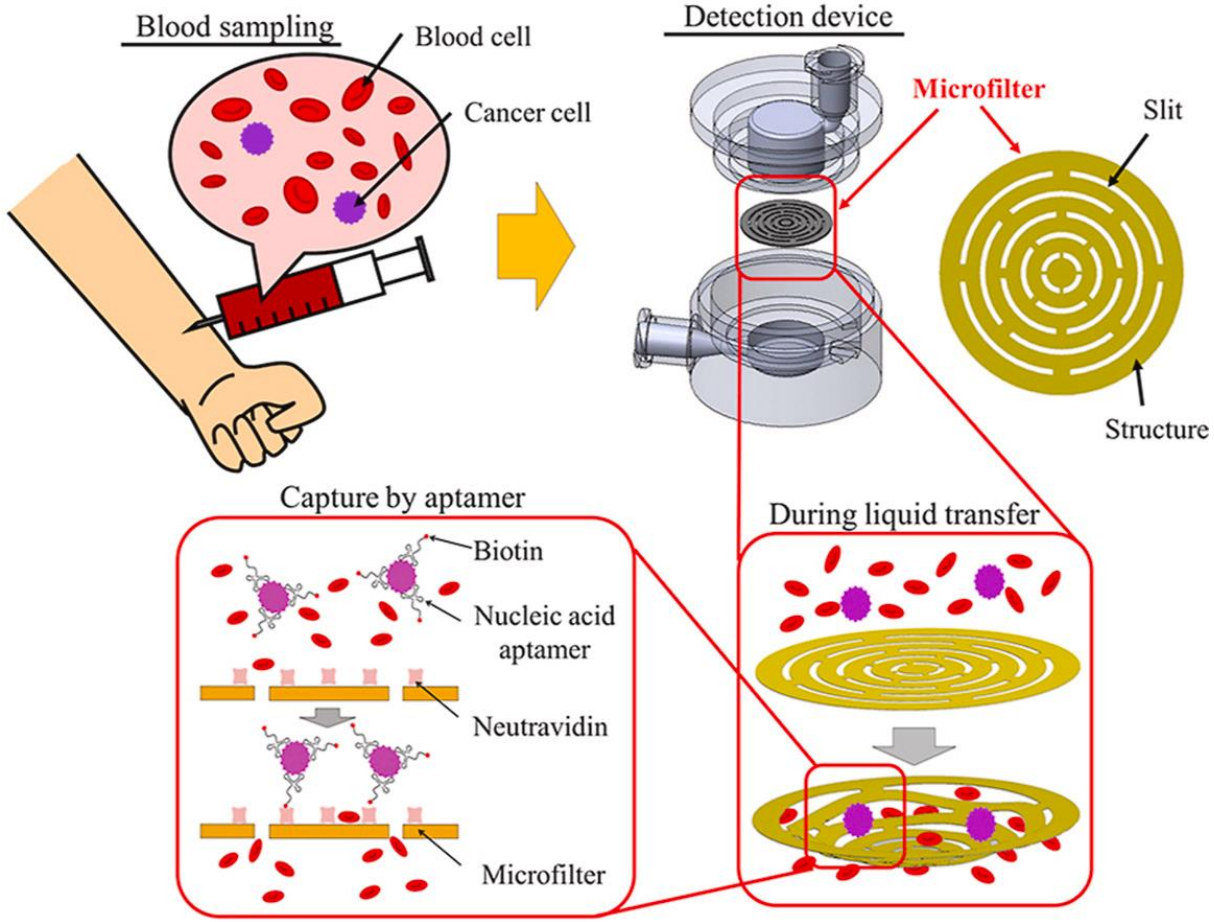
- conformational change of the aptamer
  → produce an electrical signal change

Aptamer lateral flow assay (LFA)



- target molecules bind both the aptamer-conjugated gold NP detection agent and the aptamer capture agent
  → turning the test band dark.

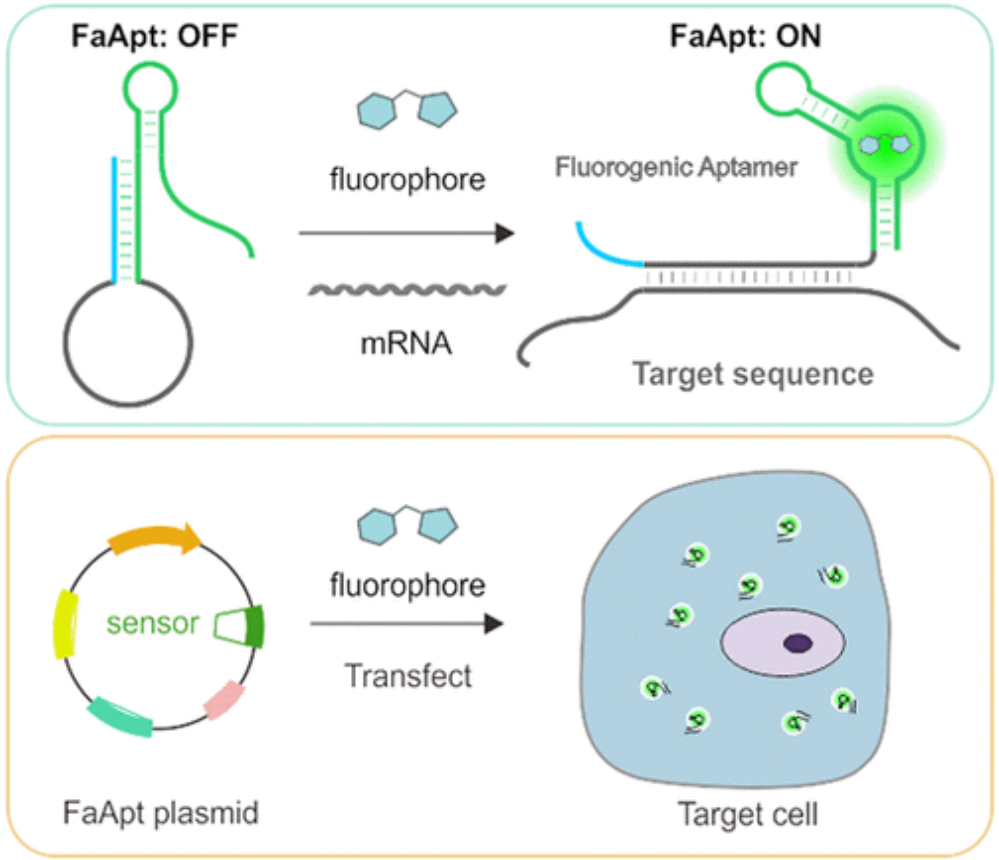
Detection of cancer cells in whole blood



Yang, L.; *et al*. *Chem. Sci.* **2023**, *14* (19), 4961–4978.(modified)
Fukuyama, S.; *et al*. *Talanta* **2021**, *228*, 122239–122239.

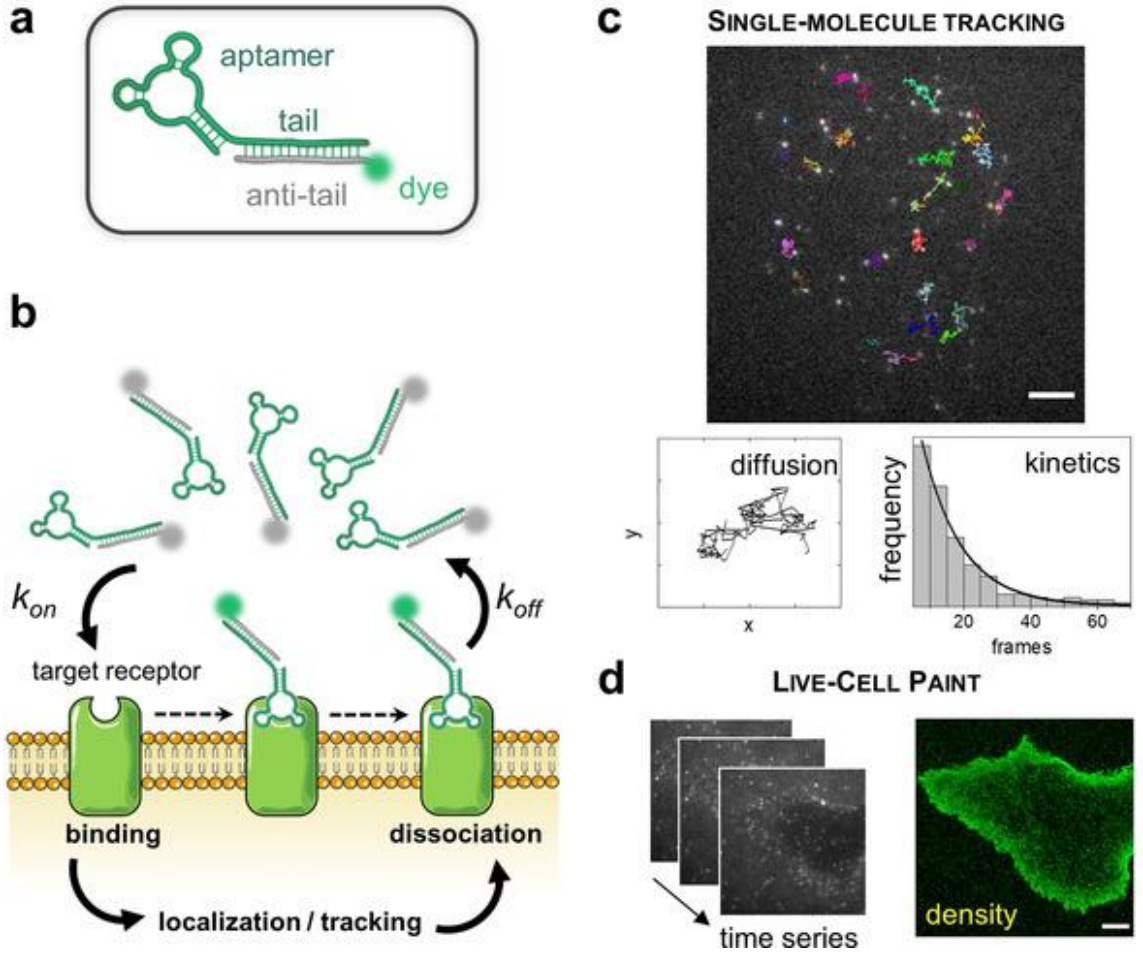
Aptamer-based imaging methods are being developed

mRNA imaging



- Molecules that fluoresce when bound to aptamers
- Aptamers can be expressed intracellularly

Cell membrane protein imaging



Pietro Delcanale; et al. *Angew. Chem. Int. Ed.* **2020**, *59* (42), 18546–18555
Yan, P.; *et al*. *Anal. Chem*. **2023**, 95 (37), 13762–13768

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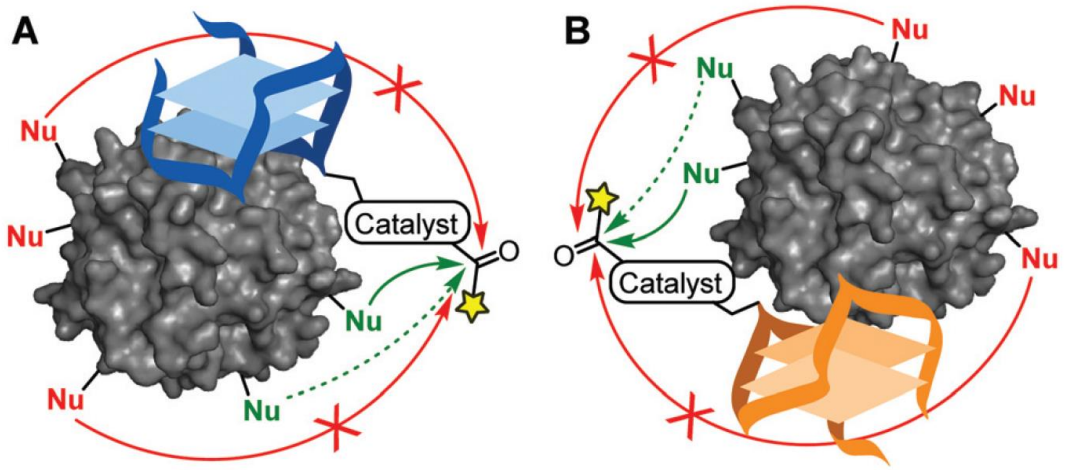
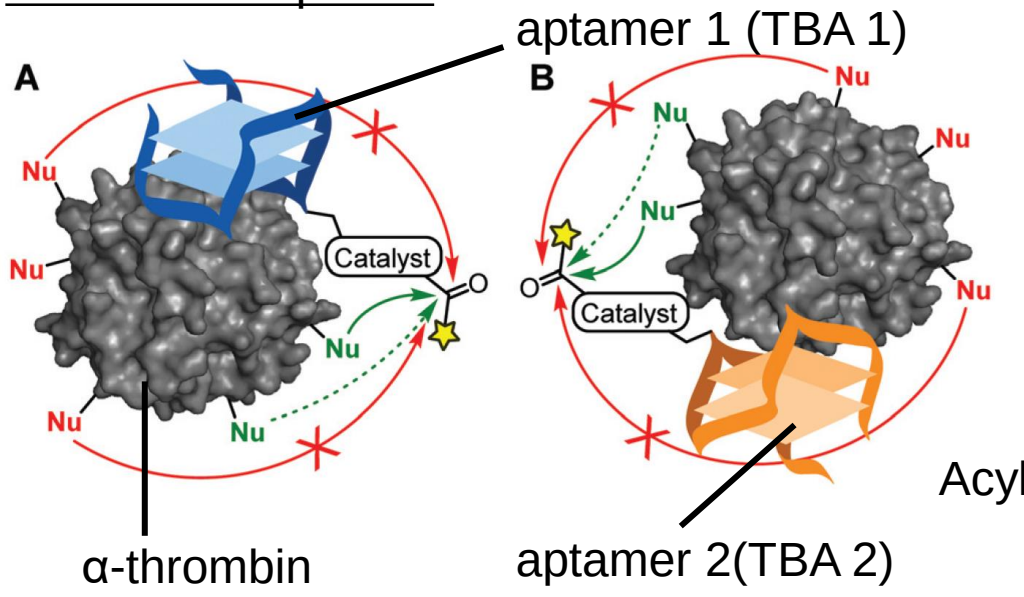
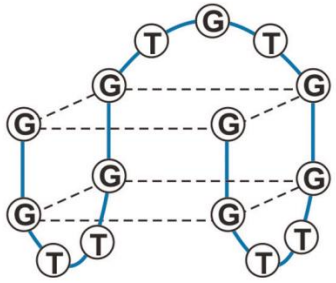


Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963.

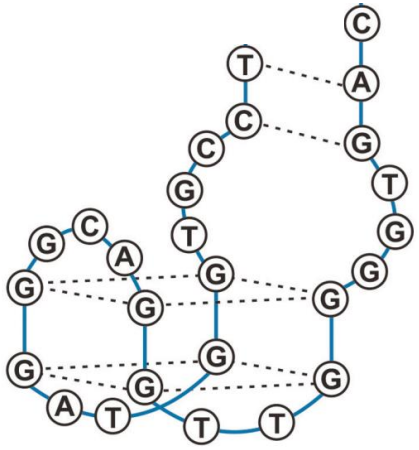
Site-selective acylation of protein with aptamer-catalyst conjugates

Schematic depiction





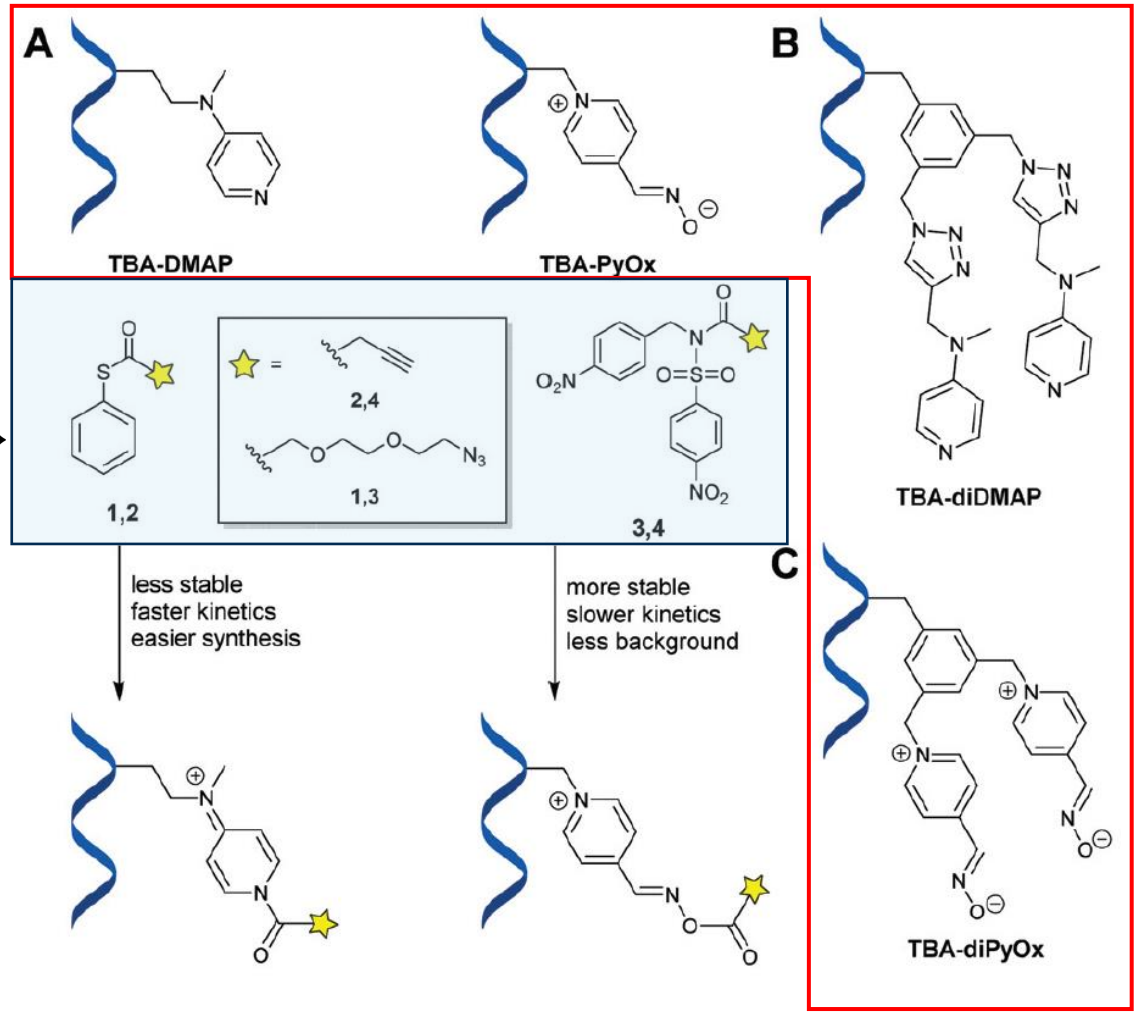
TBA 1



TBA 2

Aptamer-tethered catalysts

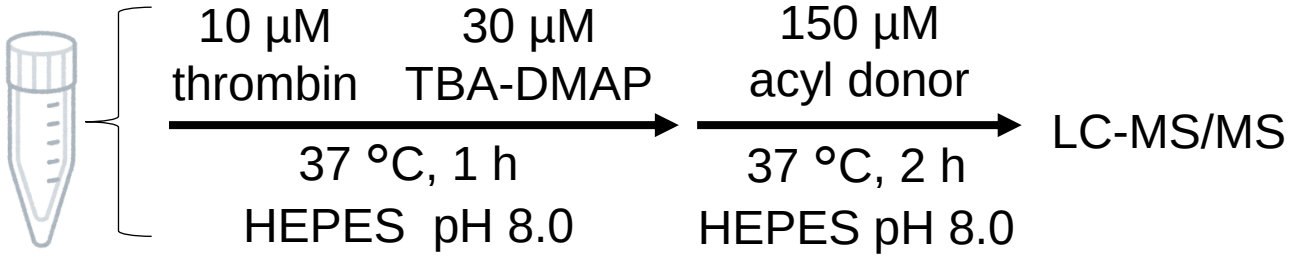
Acyl donor →

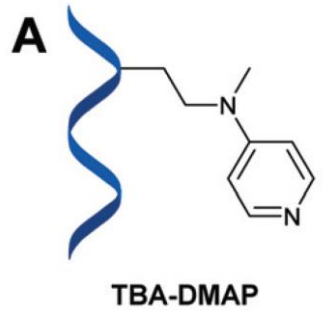


Cailing Ji.; *et al*. *Chem. Rev.* **2023** *123* (22), 12471-12506 (modified)
Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963. (modified)

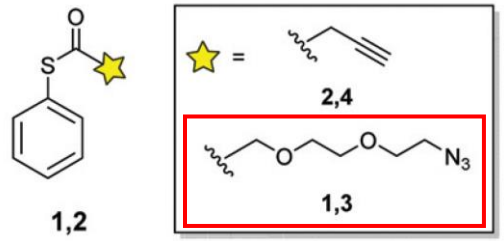
DMAP-aptamer modified Lys residues site-selectively

protocol





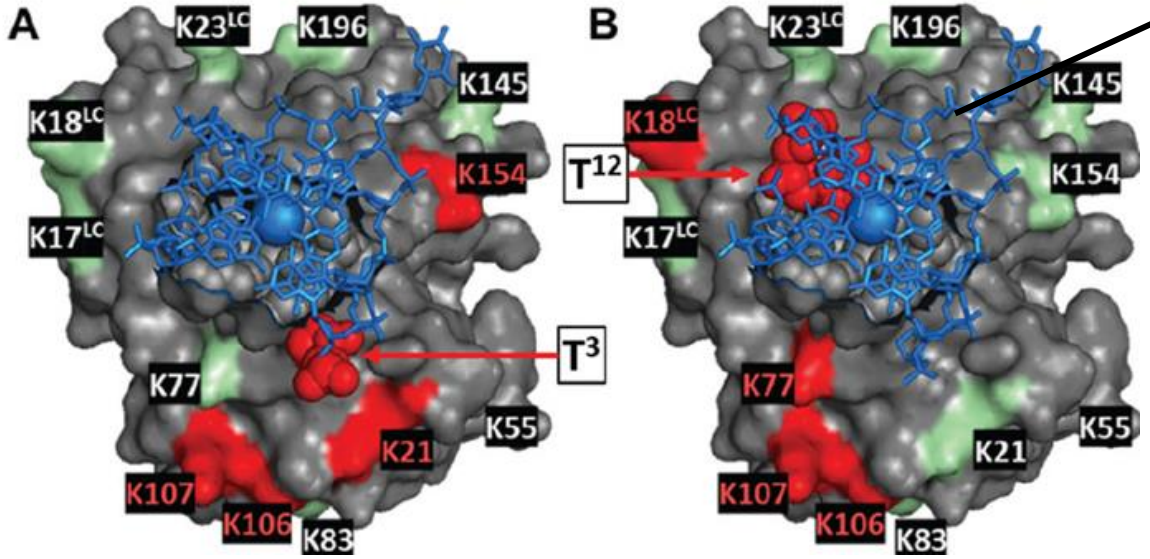
TBA-DMAP



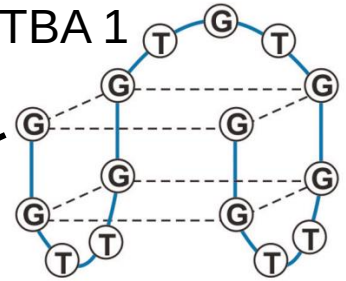
acyl donor

TBA^{3}-DMAP

TBA^{12}-DMAP



TBA 1



acylation yield : 27 %

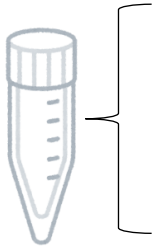
acylation yield : 49 %

- Aptamer-DMAP modification was performed in a site-selective manner
- Modification was performed on Lys proximal to the respective DMAP

Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963.

DMAP-aptamer specifically modified thrombin

protocol



Protein mixture

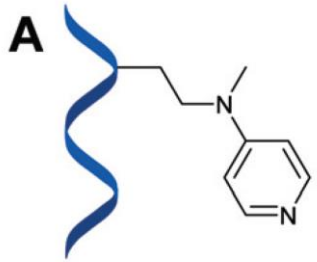
30 µM TBA-DMAP

37 °C, 1 h
HEPES pH 8.0

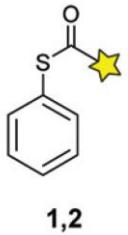
150 µM acyl donor

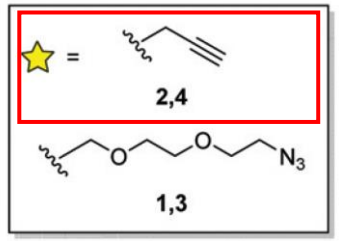
37 °C, 2 h
HEPES pH 8.0

click reaction
SDS-PAGE

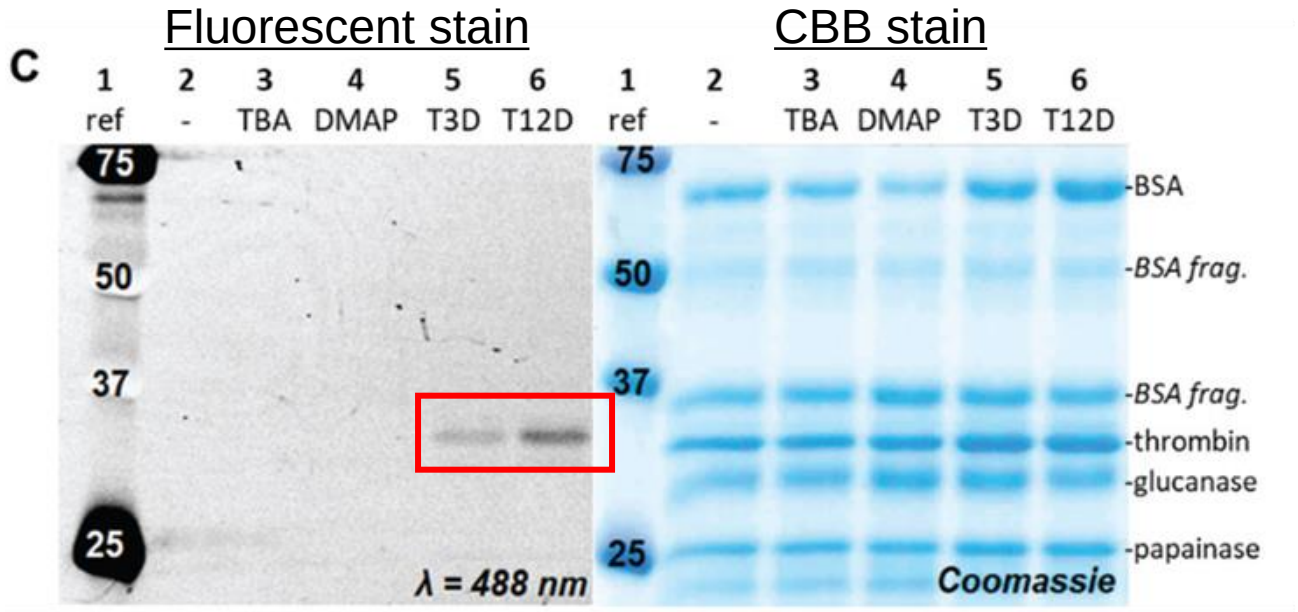


TBA-DMAP





acyl donor



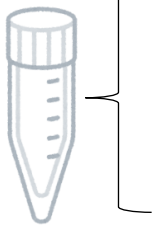
- Only fluorescently labelled thrombin was observed and none of the other proteins was modified

→ DMAP-aptamer conjugates specifically modify its target protein

Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963.

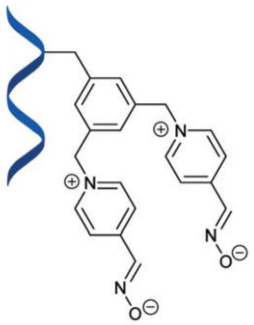
Different aptamer-catalysts modified different sides of thrombin

protocol

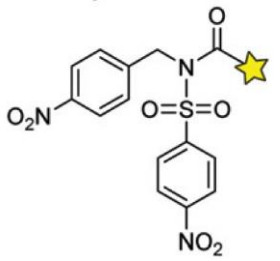


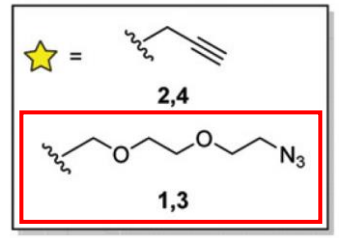
10 µM thrombin → 30 µM TBA1/2-diPyOx (37 °C, 1 h, HEPES pH 8.0) → 300 µM acyl donor (37 °C, 5 h, HEPES pH 8.0) → LC-MS/MS

C



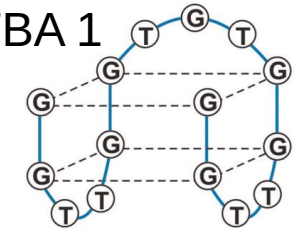
TBA-diPyOx



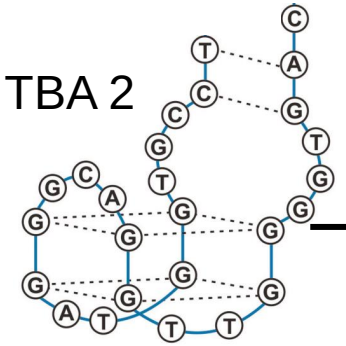


acyl donor

TBA 1



TBA 2



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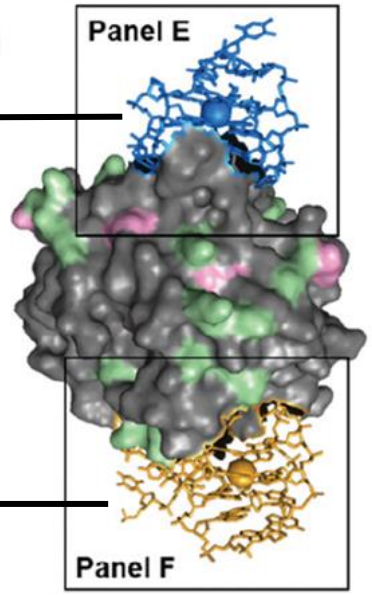

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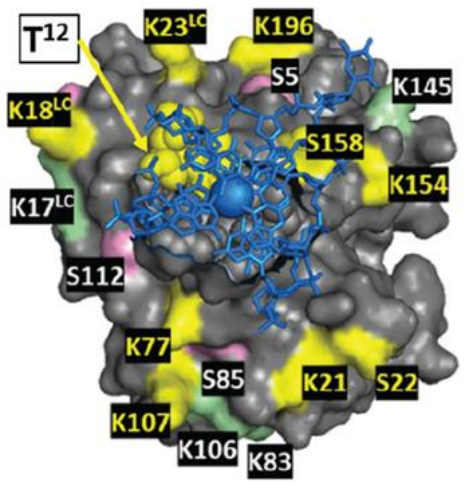
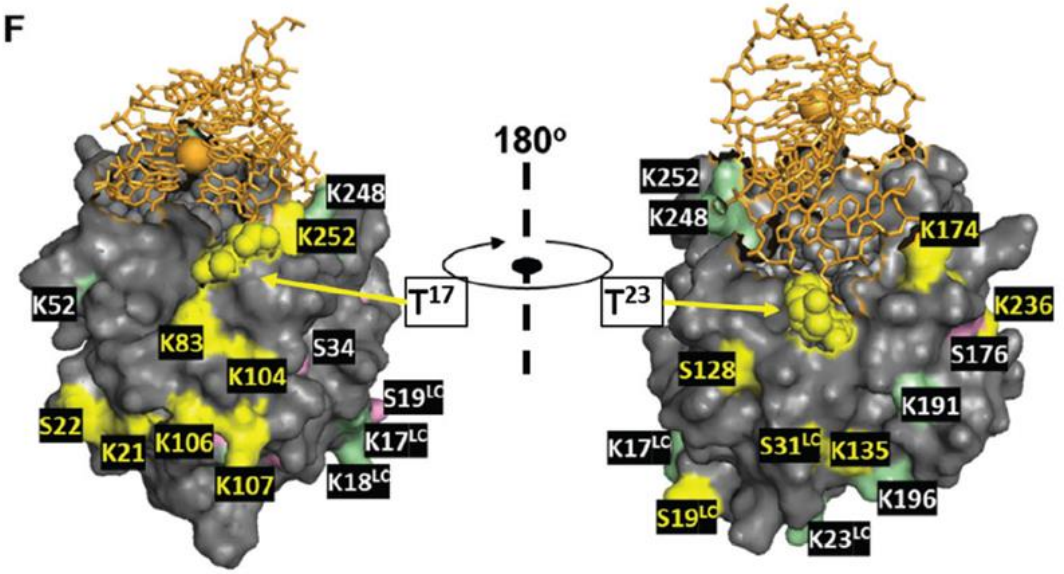


F



Acylation yield : > 90 %

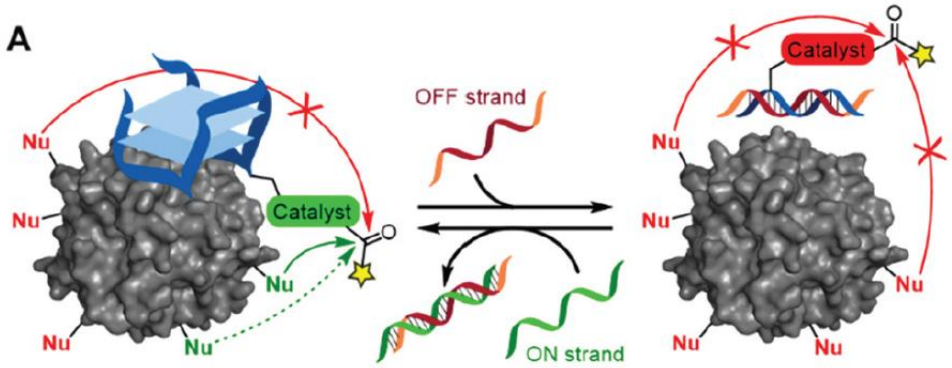
Acylation yield : 20-27 %

- TBA1-diPyOx and TBA2-diPyOx bind to the different sides of thrombiin
- TBA-diPyOx modified thrombin at residues positioned in close proximity to the respective positions of the catalyst

Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963.

Activity control of TBA-catalyst construct

Schematic depiction



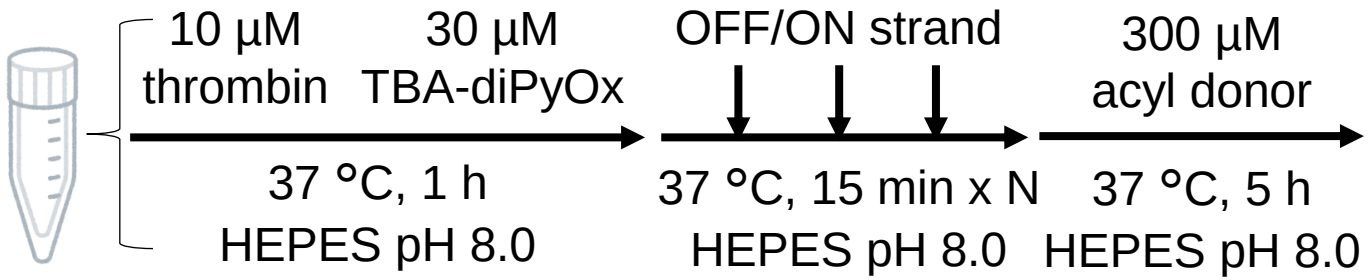
- OFF strand : hybridize with TBA-catalyst

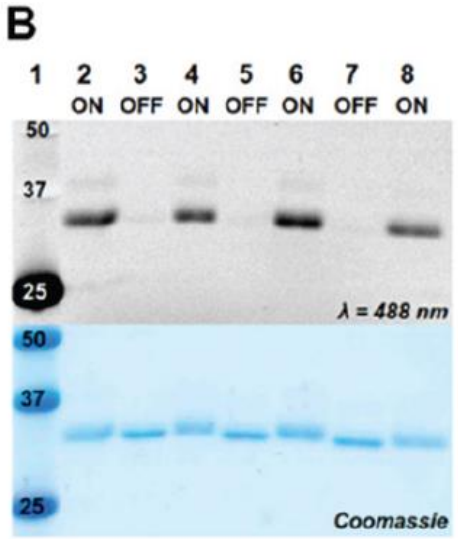
→ form a catalytically inactive dsDNA duplex

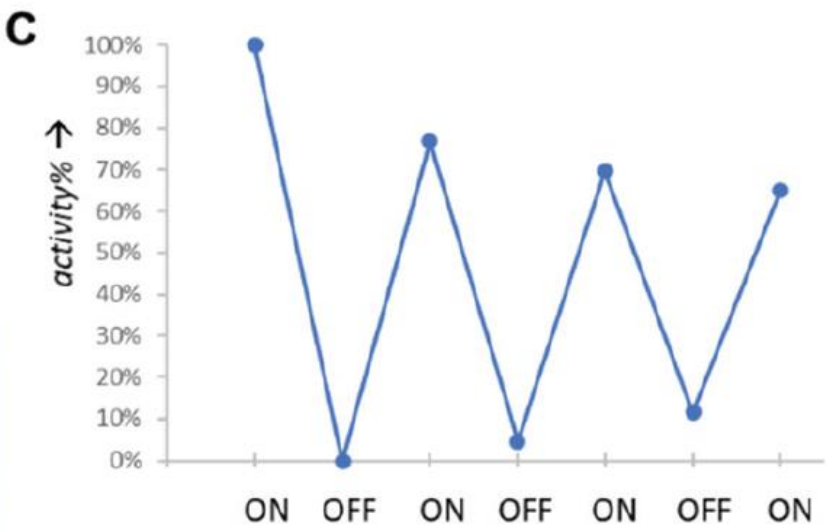
- ON strand : complementary to the OFF strand

→ bind to OFF strand and the TBA is reformed

protocol







- Upon addition of the OFF-strand, the system turned to its OFF state as no protein modification was detected.
- After removing the OFF-strand by addition of the ON strand, the modification of thrombin is again efficient.

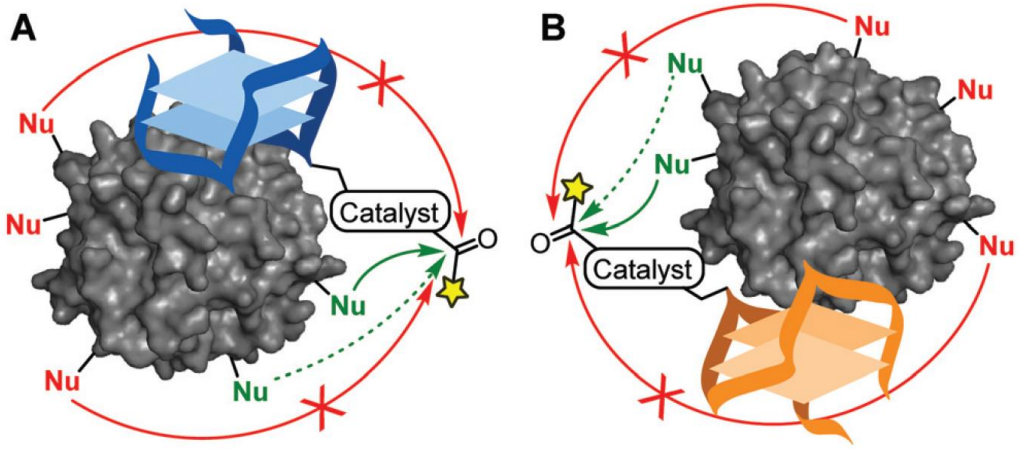
→ this switch successfully control the activity of TBA-catalysts

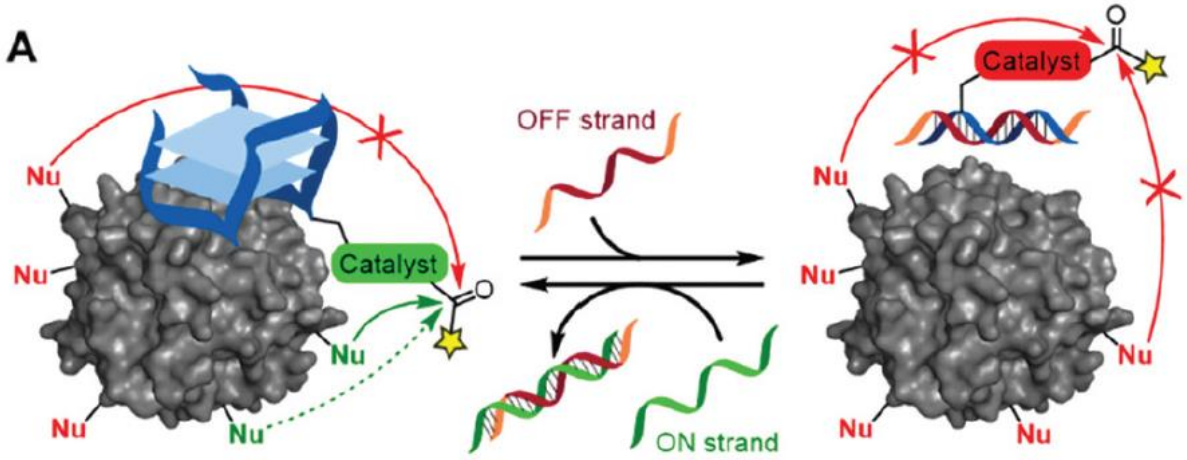
Keijzer, J. F.; *et al*. *Chem. Commun.* **2021**, *57* (96), 12960–12963.

Short summary

- This research shows the first DNA-based catalyst for the **site-selective modification** of a specific protein **using its affinity for an aptamer**.

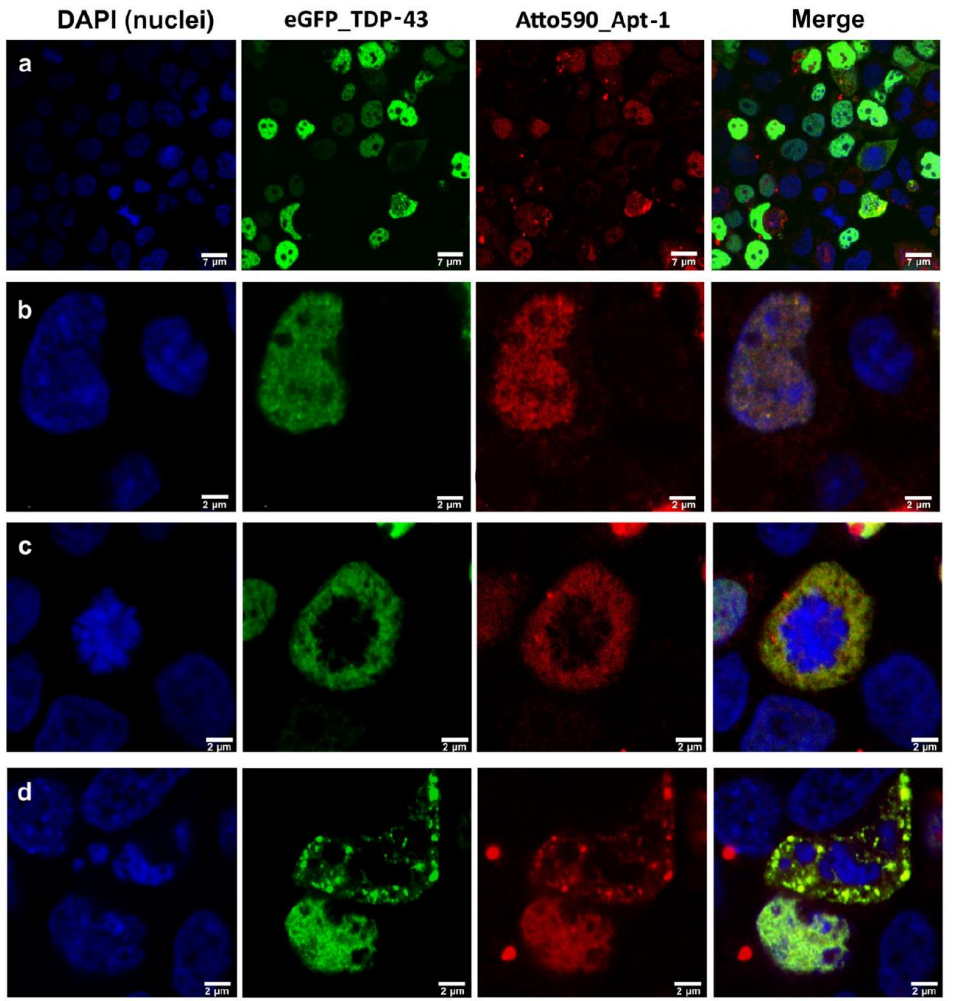
- The activity of our DNA-based catalysts **could be repeatedly regulated** by an external stimulus.





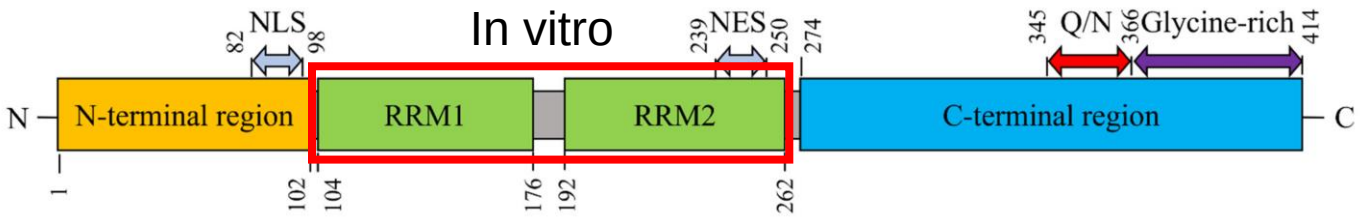
Contents

- Introduction
  - Aptamer Characteristics
  - Advantages and disadvantages of aptamers
  - Current applications of aptamers
- **Development of new applications of aptamers**
  - **Ligand-directed catalysis**
  - **TDP-43 imaging in cell**
- Perspective
- Summary



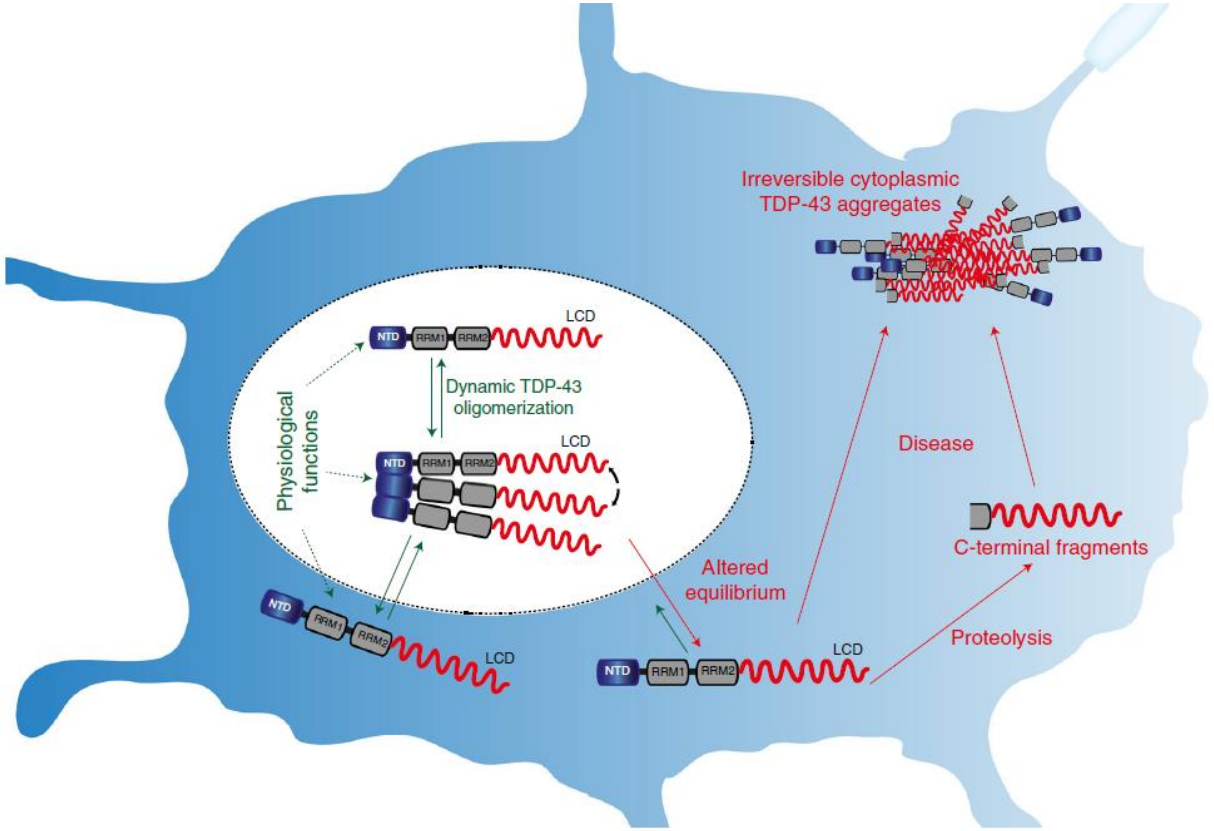
TDP-43

The domain organization of TDP-43



- In vitro experiments have been performed using the RRM1-2 domain

The localization of TDP-43

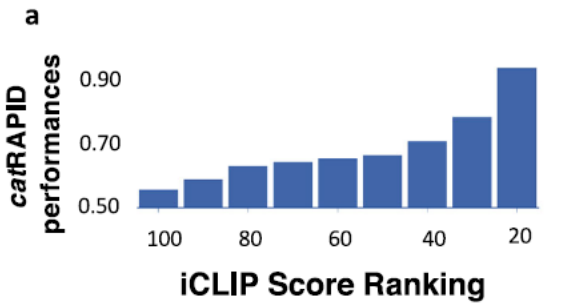


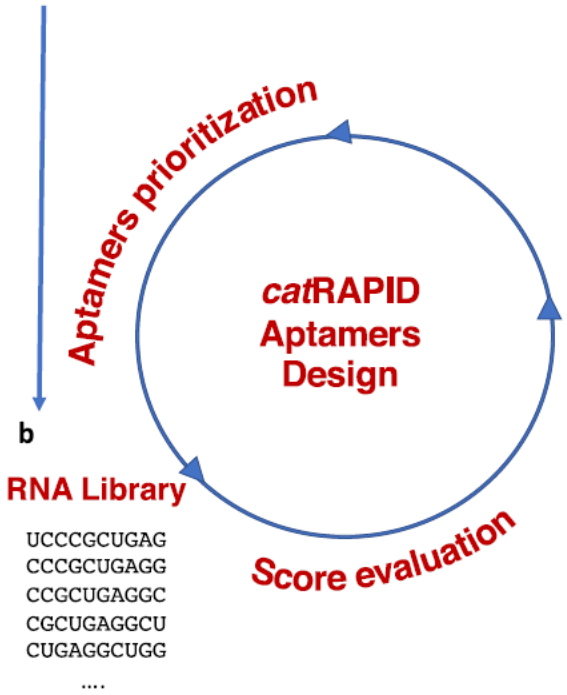
- The majority of TDP-43 is localized in the nucleus in soluble forms
- A portion of TDP-43 exists as a monomer in the cytoplasm
- Disturbance in the equilibrium between the oligomeric and monomeric TDP-43 in the cytoplasm may result in aggregates

Tamaki, Y.; *et al*. *Int. J. Mol. Sci.* **2022**, *23* (20), 12508.
Afroz, T.; *et al*. *Nat. Commun.* **2017**, *8* (1).

Aptamers that bind to TDP-43 were designed in silico

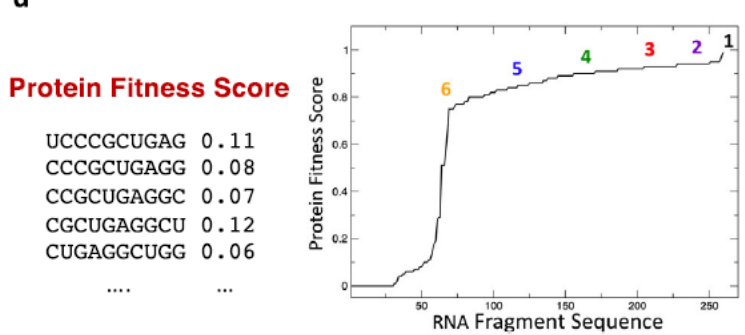
Interaction between RNA and TDP-43

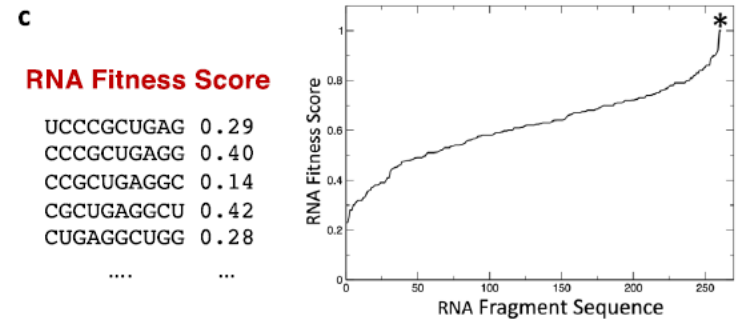




e

	Sequence	TDP-43	RRM1	RRM2	RRM1-2
Apt-1	CGGUGUUGCU	0.99	0.90	0.52	0.93
Apt-2	GUGGUCCCCG	0.98	0.85	0.55	0.90
Apt-3	CGCUGUGGUC	0.94	0.90	0.45	0.86
Apt-4	GGGGUGGGGC	0.88	0.83	0.40	0.75
Apt-5	CGAGGCCGGG	0.82	0.81	0.40	0.78
Apt-6	GCGGGGCCCG	0.75	0.81	0.61	0.75





- *cat*RAPID = protein-RNA interactions prediction
- iCLIP = protein-RNA interactions database
- *cat*RAPID and iCLIP are corresponding
  →good prediction
- Protein Fitness Score
  Score ~1 = strong interaction for TDP-43
  compared to other proteins
- RNA Fitness Score
  Score ~1 = strong interaction for TDP-43

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

Aptamers that bind to TDP-43 were designed in silico

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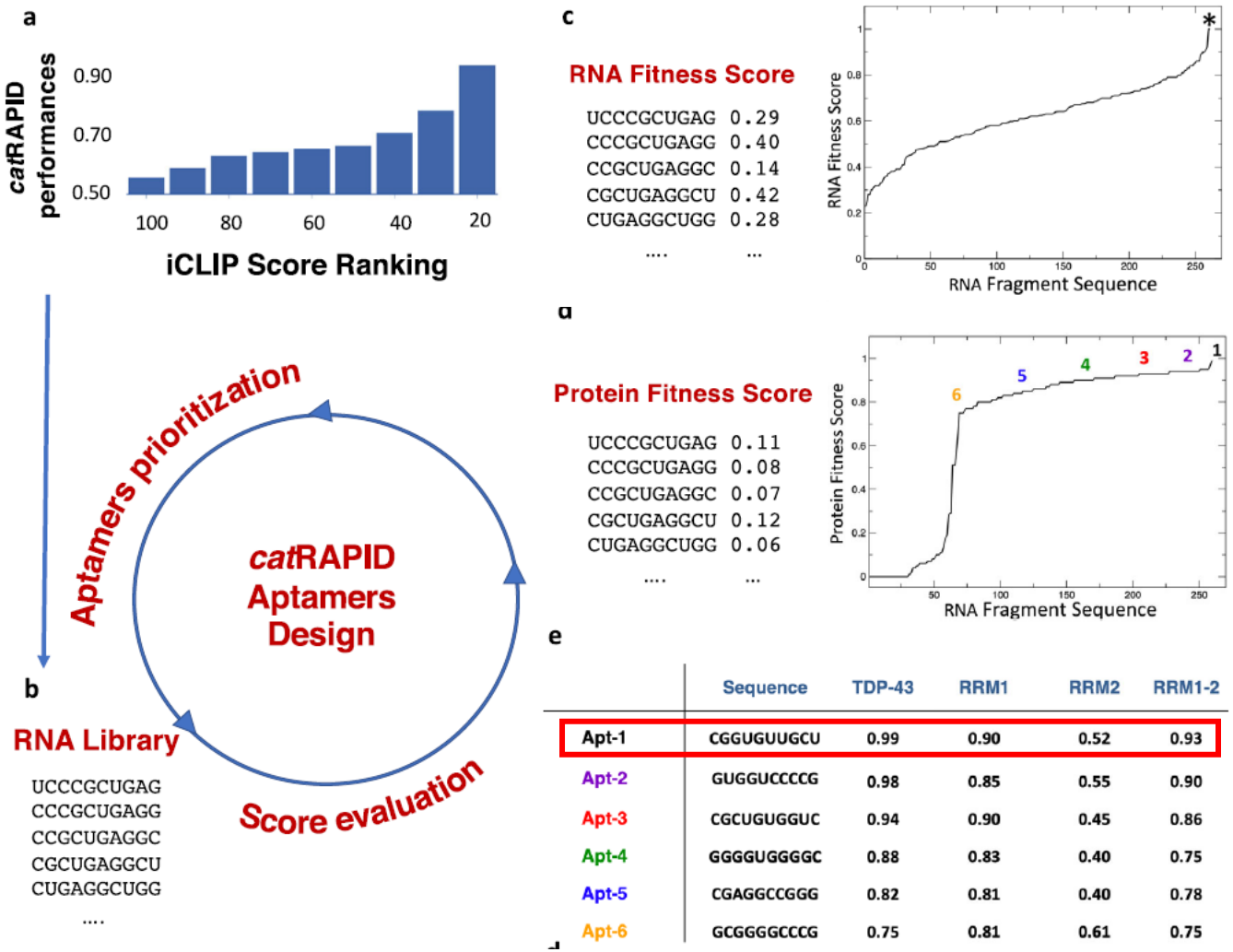

- *cat*RAPID = protein-RNA interactions prediction
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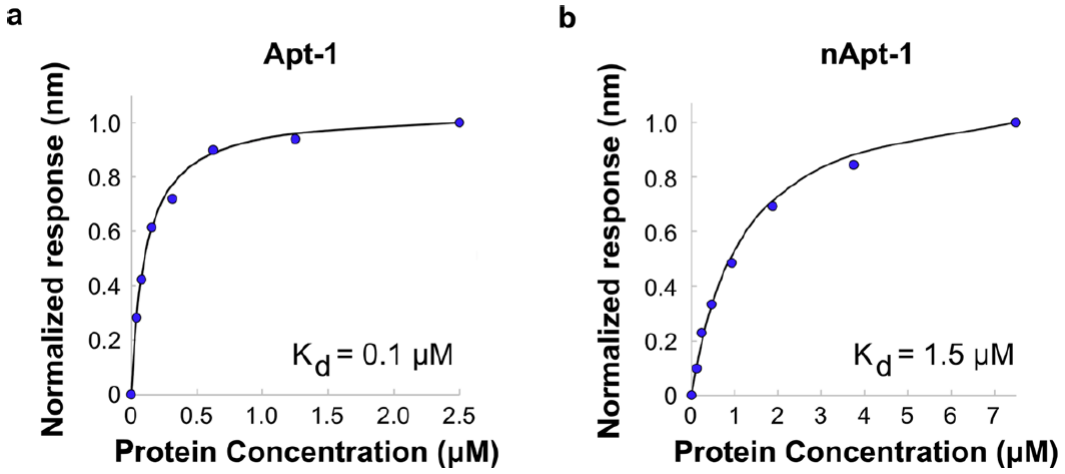
  Score ~1 = strong interaction for TDP-43
- Protein Fitness Score
  Score ~1 = strong interaction for TDP-43
  compared to other proteins

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

The aptamers bound to RRM1-2 in vitro

Binding affinity of Apt-1 and nApt-1 for RRM1-2

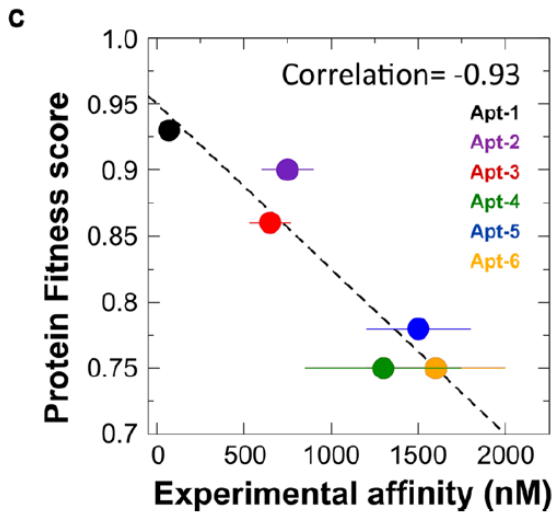


- nApt-1 : the reverse complementary RNA of Apt-1 (negative control)
- Apt-1 has the strong affinity with a K_d = 0.1 µM whereas nApt-1 : K_d = 1.5 µM

Table 1 Binding affinities of the RNA aptamers for RRM1 and RRM1-2.

	K_d screening (µM)	
	RRM1	RRM1-2
Apt-1	0.58 ± 0.01	0.10 ± 0.01
Apt-2	1.44 ± 0.40	0.75 ± 0.15
Apt-3	0.90 ± 0.20	0.65 ± 0.12
Apt-4	0.90 ± 0.35	1.30 ± 0.45
Apt-5	1.65 ± 0.30	1.50 ± 0.30
Apt-6	2.50 ± 0.65	1.60 ± 0.40

K_d values of candidate RNA aptamers interactions with the isolated RRM1 of TDP-43 (second column) and RRM1-2 (third column; mean ± S.D.; $n = 3$).

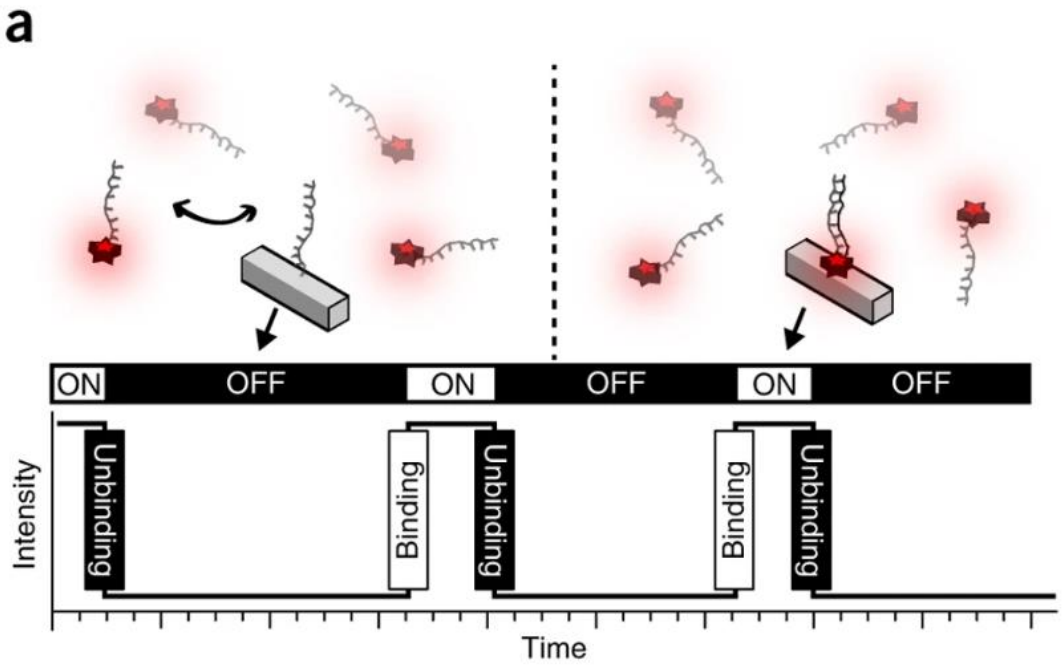


- The experimental K_d values correlate with the predicted Protein Fitness scores
  →validation of the computational design of RNA aptamers

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

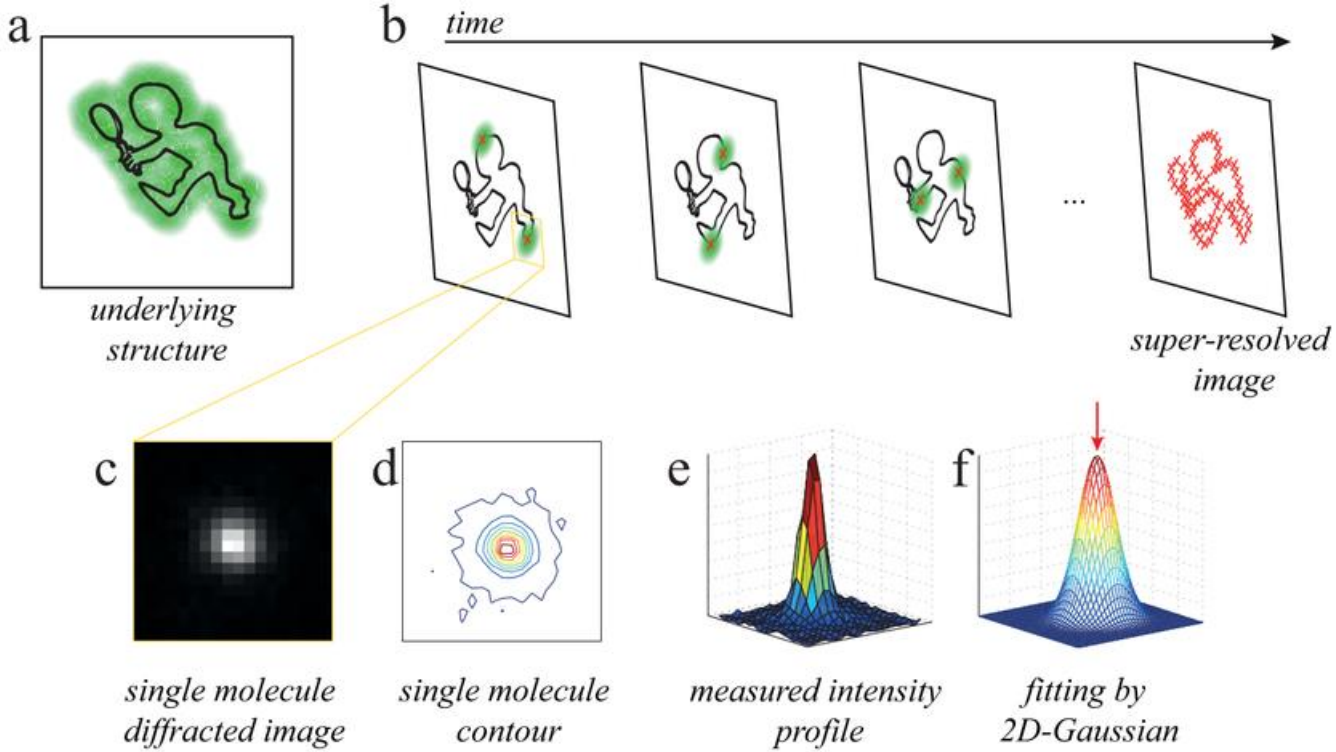
DNA-PAINT (DNA-Point Accumulation for Imaging in Nanoscale Topography)

RNA-fluorescent molecules



- labeled RNA aptamers transiently bind to TDP-43
- the fluorescent molecule emits signal only when it binds

Super-resolution imaging (SR imaging)



Record the location of many bright spots

↓

Calculate the center of bright spots

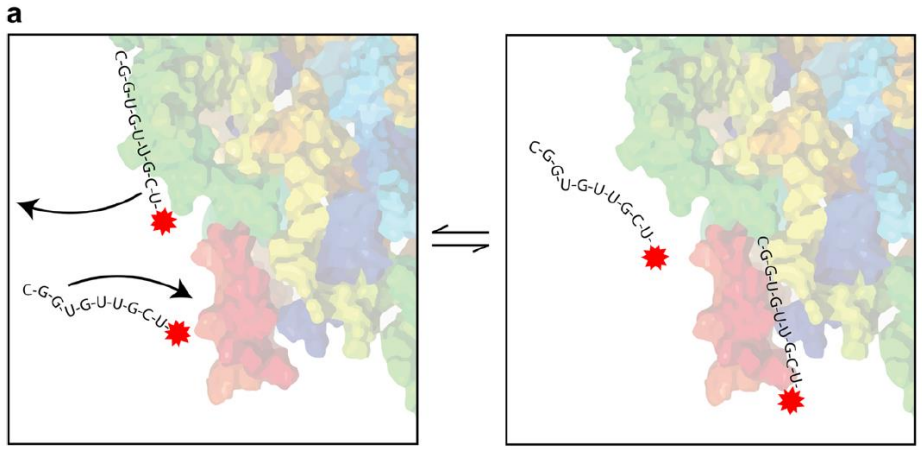
↓

Integrate them to build up a SR image

Schnitzbauer, J.; *et al*. *Nat. Protoc.* **2017**, *12* (6), 1198–1228. van. https://doi.org/10.13140/RG.2.2.23165.13285.

SR Imaging of RRM1-2 aggregates with Apt-1

AD-PAINT



Protocol

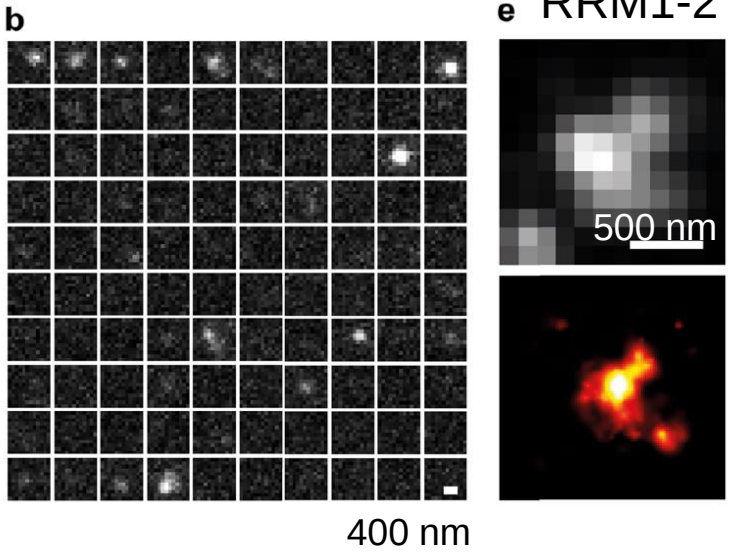
15 µM
RRM1-2

37 °C, incubate

1 nM
Atto590-tagged Apt-1

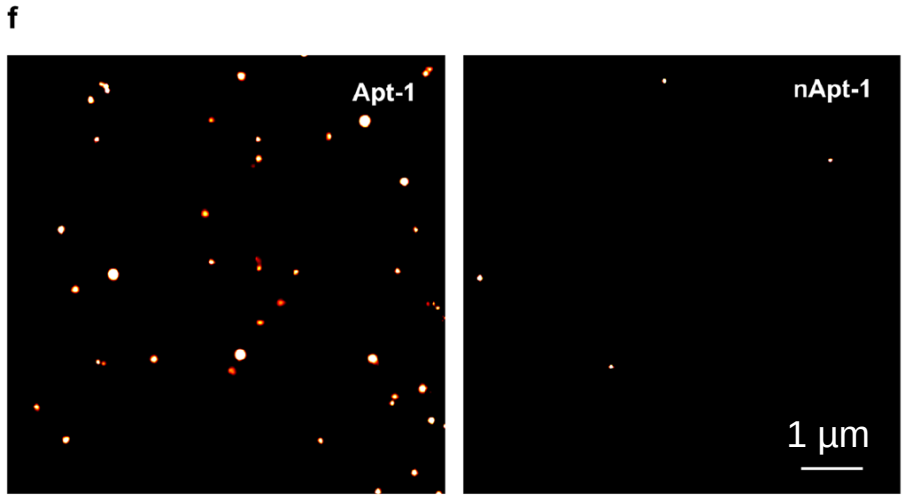
SR imaging

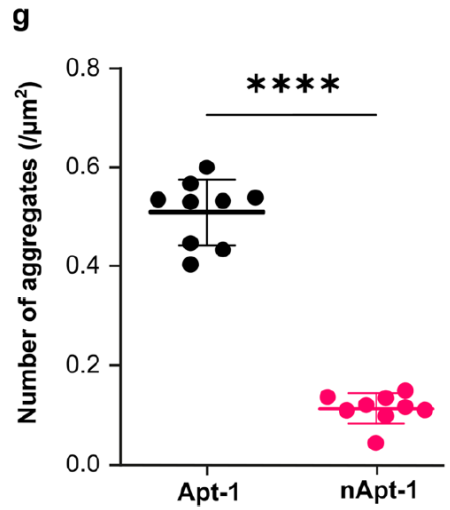
PAINT imaging



- Apt-1 could build up the SR image of RRM1-2

PAINT imaging



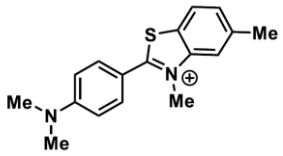


- The number of aggregates detected with Apt-1 was significantly higher than with nApt-1

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

Following the aggregation of RRM1-2 using Apt-1 and SAVE imaging

PAINT w/ Apt-1 vs SAVE w/ ThT



Thioflavin T (ThT)
(amyloid probe)

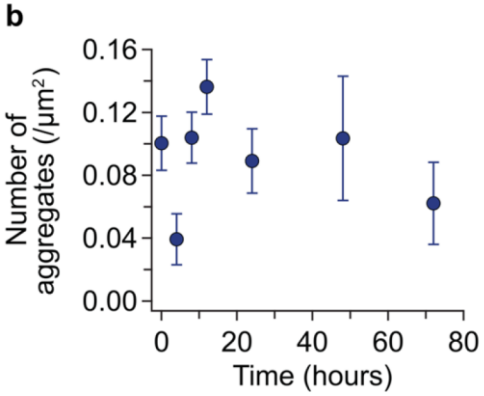
Protocol

15 µM RRM1-2 → 37 °C, 4, 12, 24, 48, 72 h

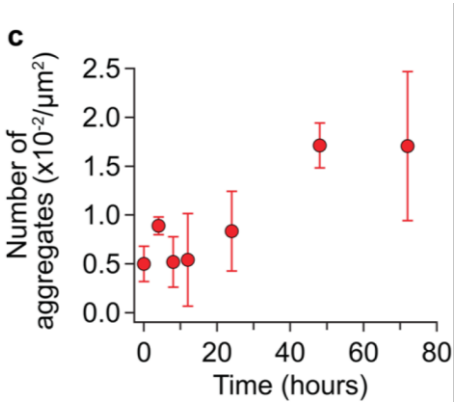
1 nM Apt-1 → 5 µM ThT

PAINT imaging
SAVE imaging

Detected using Apt-1

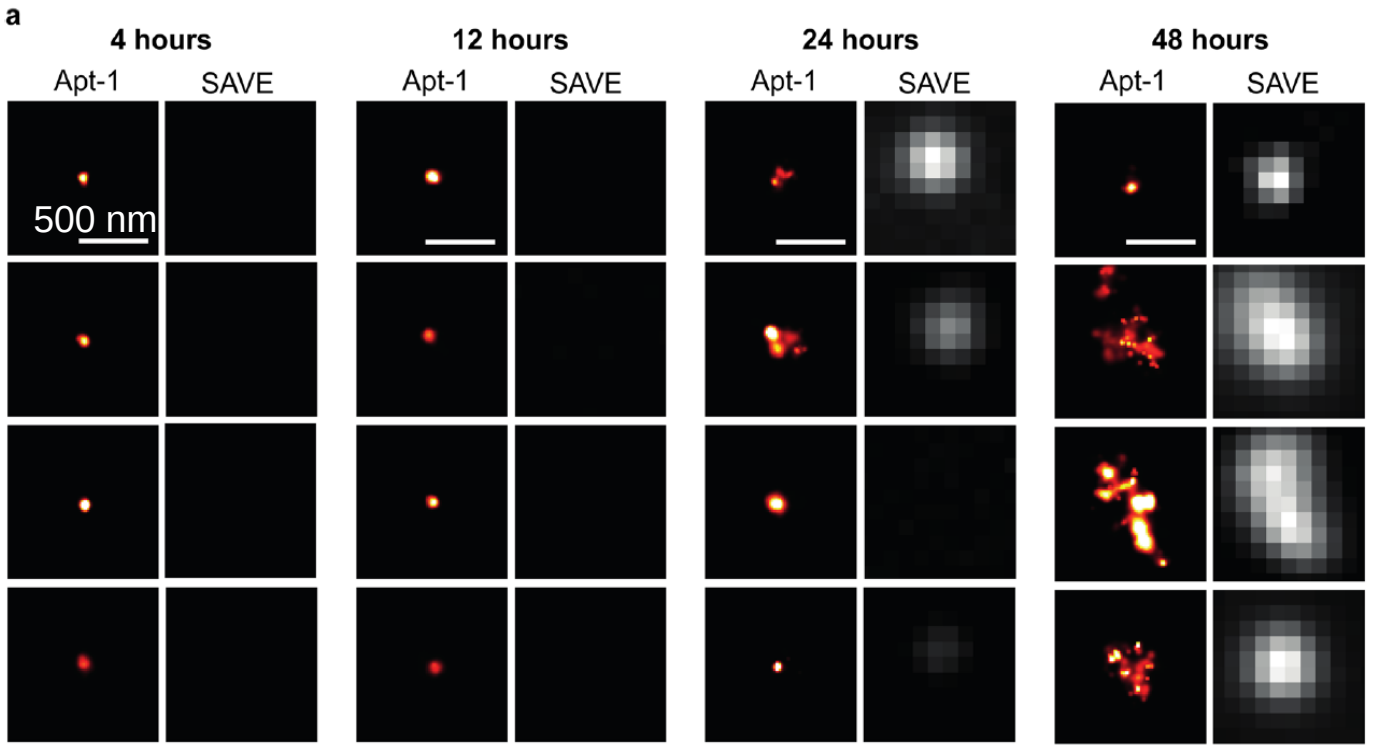


Detected using ThT



- The number of aggregates detected with Apt-1 was significantly higher than with ThT
- Only 6 % of the aggregates detected with Apt-1 was ThT-active
  → Apt-1 could identify less mature oligomers

Following the aggregation of RRM1-2

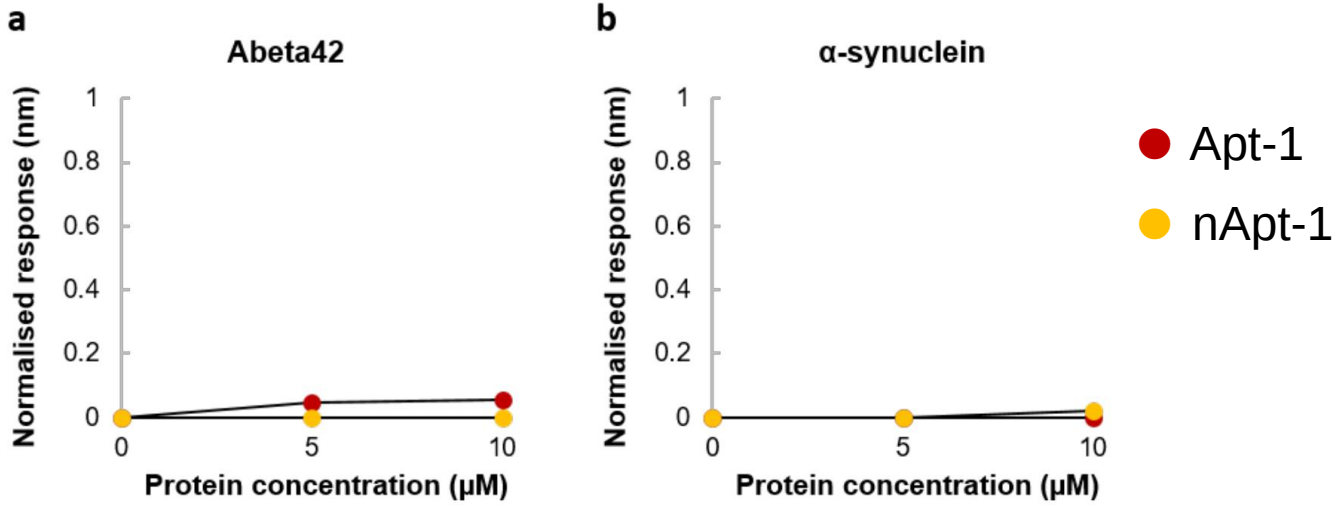


- Apt-1 visualized RRM1-2 aggregates with SR microscopy as they formed over 72 h
- Enabling their size to be accurately measured

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

Apt-1 does not bind to Aβ or α-synuclein

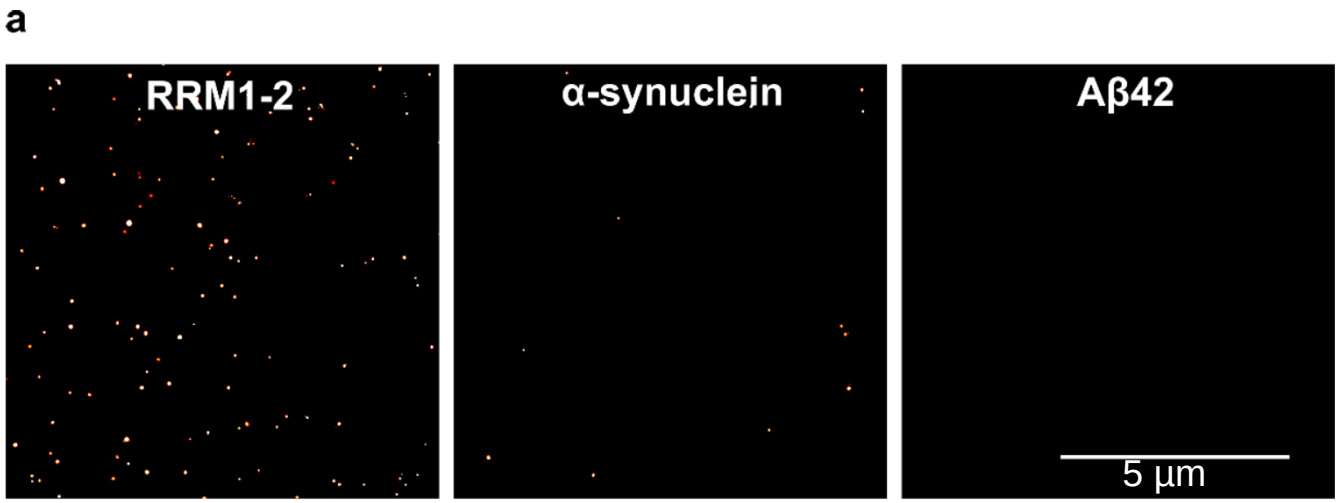
Binding affinity of Apt-1 and nApt-1 for Aβ42 or α-synuclein

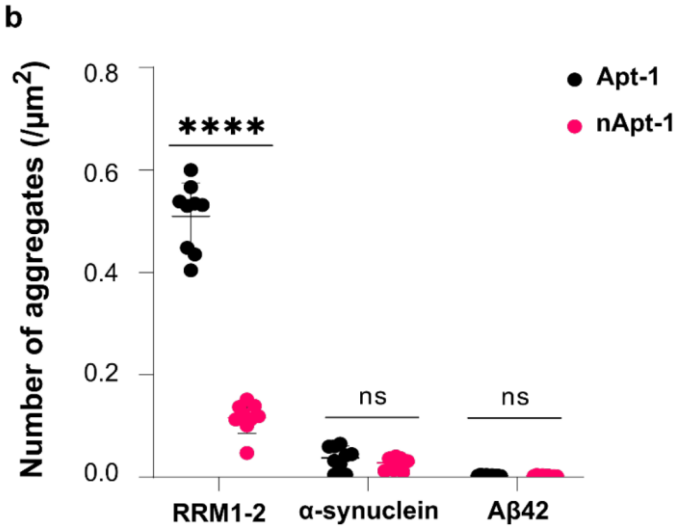


- No binding was observed at 10 μM for Aβ42 and α-synuclein in their soluble forms
- Aggregates composed of both proteins were not detected

→ Apt-1 has a specificity toward TDP-43

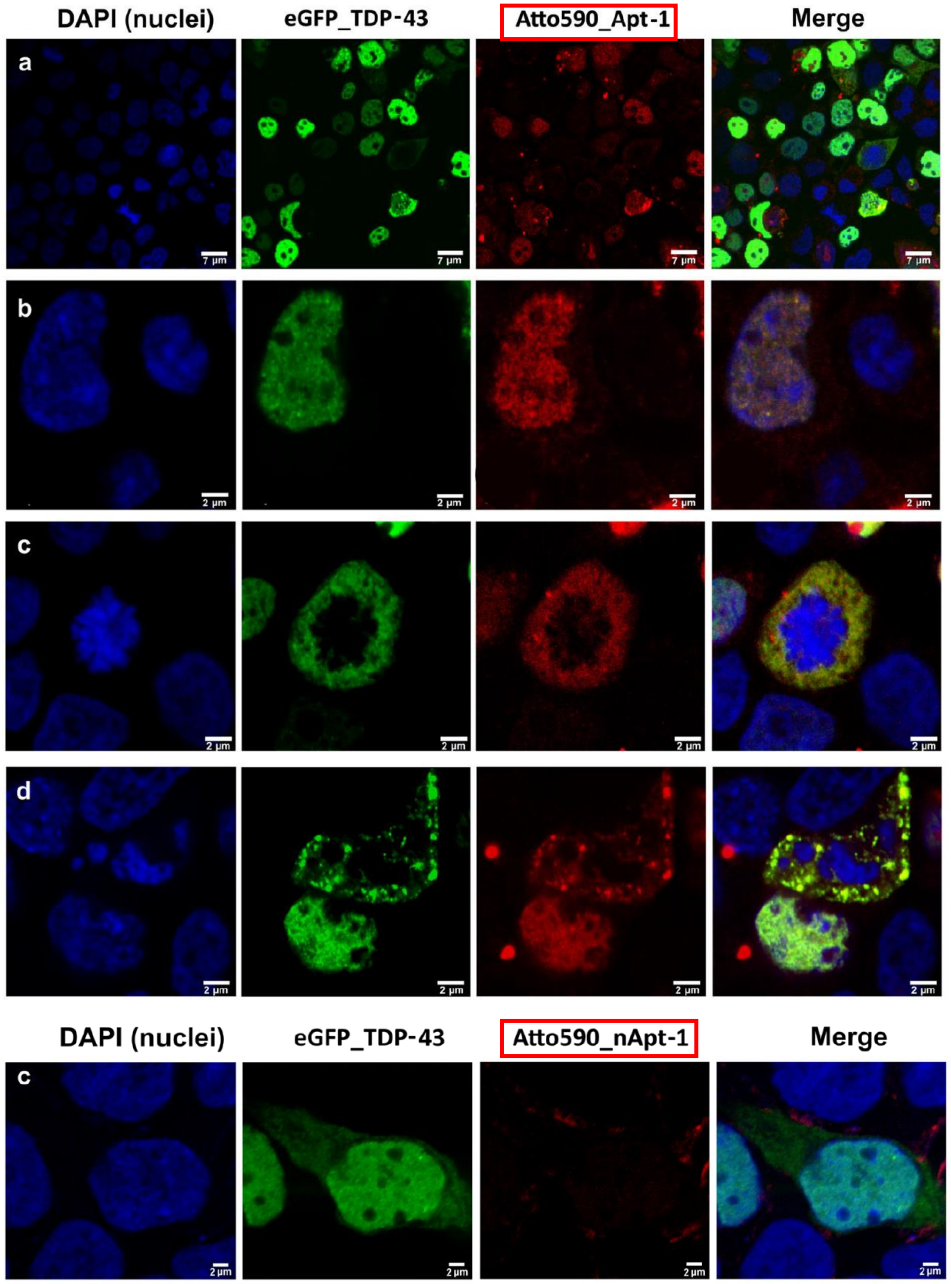
PAINT imaging of Aβ42 or α-synuclein

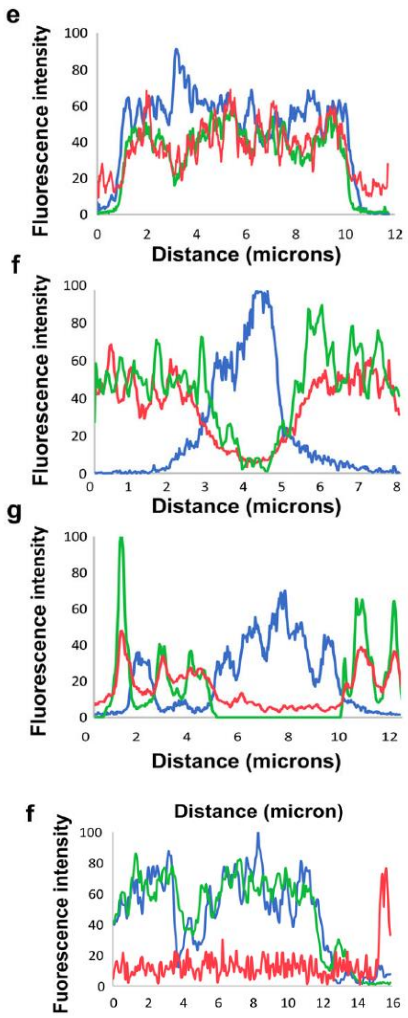


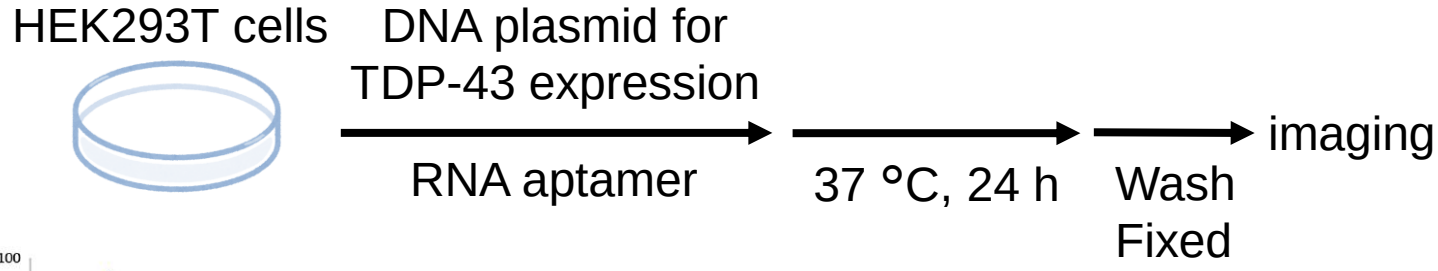


Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

Confocal microscopy analysis of TDP-43 and Apt-1 in live mammalian cells







- Apt-1 (red) co-localizes with TDP-43 (green) in cells
- These images suggest an interaction between soluble/mislocalized and aggregated TDP-43 and Apt-1
- nApt-1 (negative control) does not co-localize with TDP-43 in cells

↓

Aptamers designed with catRAPID could be employed as probes for the visualization and identification of TDP-43

Zacco, E ;. *et al*. *Nat. Commun.* **2022**, *13* (1).

Short summary

- This study shows a computational pipeline for **de novo design of RNA aptamers in silico**

- The ability of **catRAPID overcomes the limitations of SELEX**
  SELEX : need for libraries/reagents, a timeframe of **several months** and associated costs
  catRAPID : **between 2 and 7 days** depending on the molecule length

- Apt-1 could **visualize RRM1-2 aggregates with SR microscopy** as they formed over 72 h.
  --at the nanometer scale, enabling their sizes to be accurately measured.

- Imaging TDP-43 condensates **in the cell**.
  GFP often interferes with the condensation process.

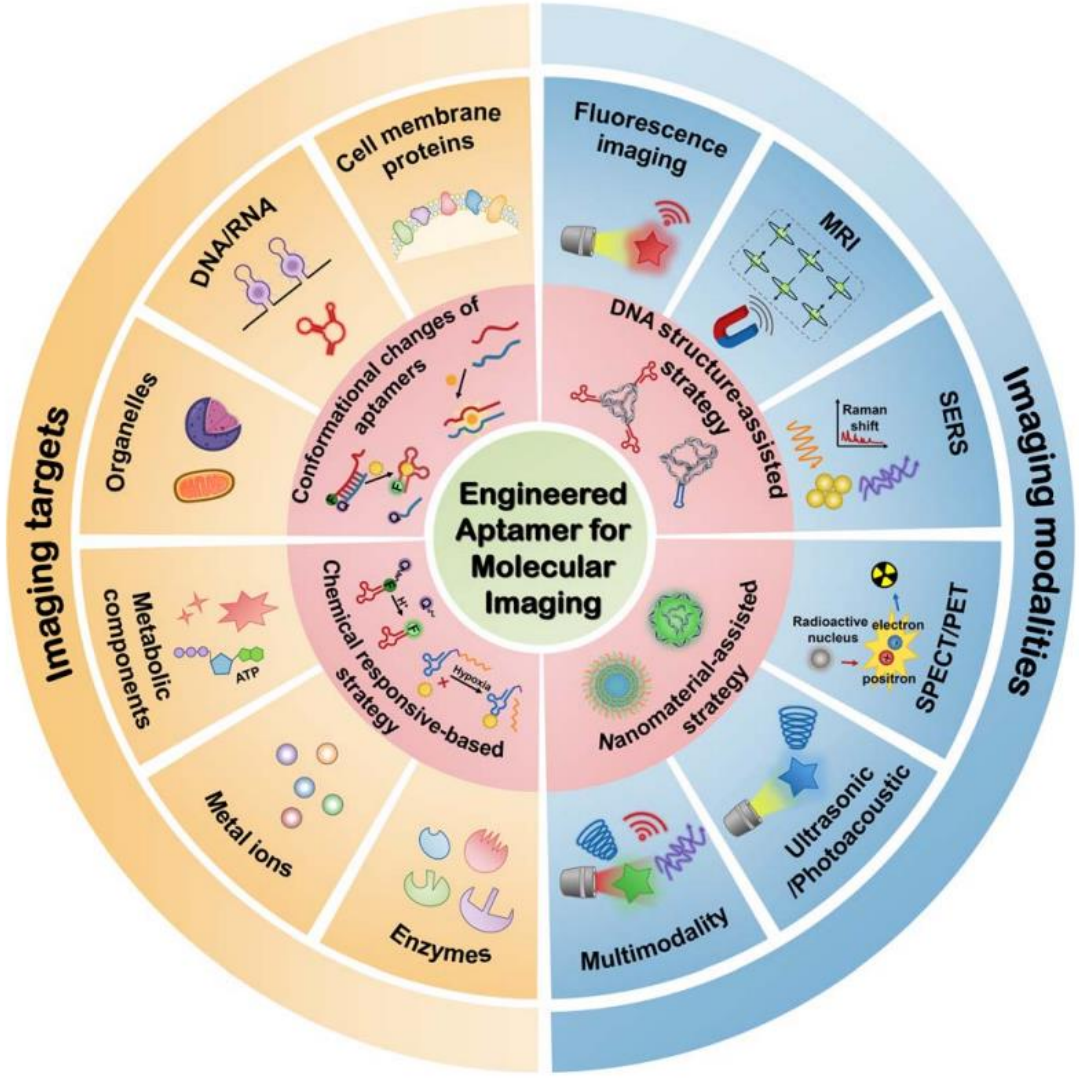
limitation

- this computational approach **does not include a pipeline to predict the effect of specific chemical modifications** to enhance RNA stability or avidity towards its target.

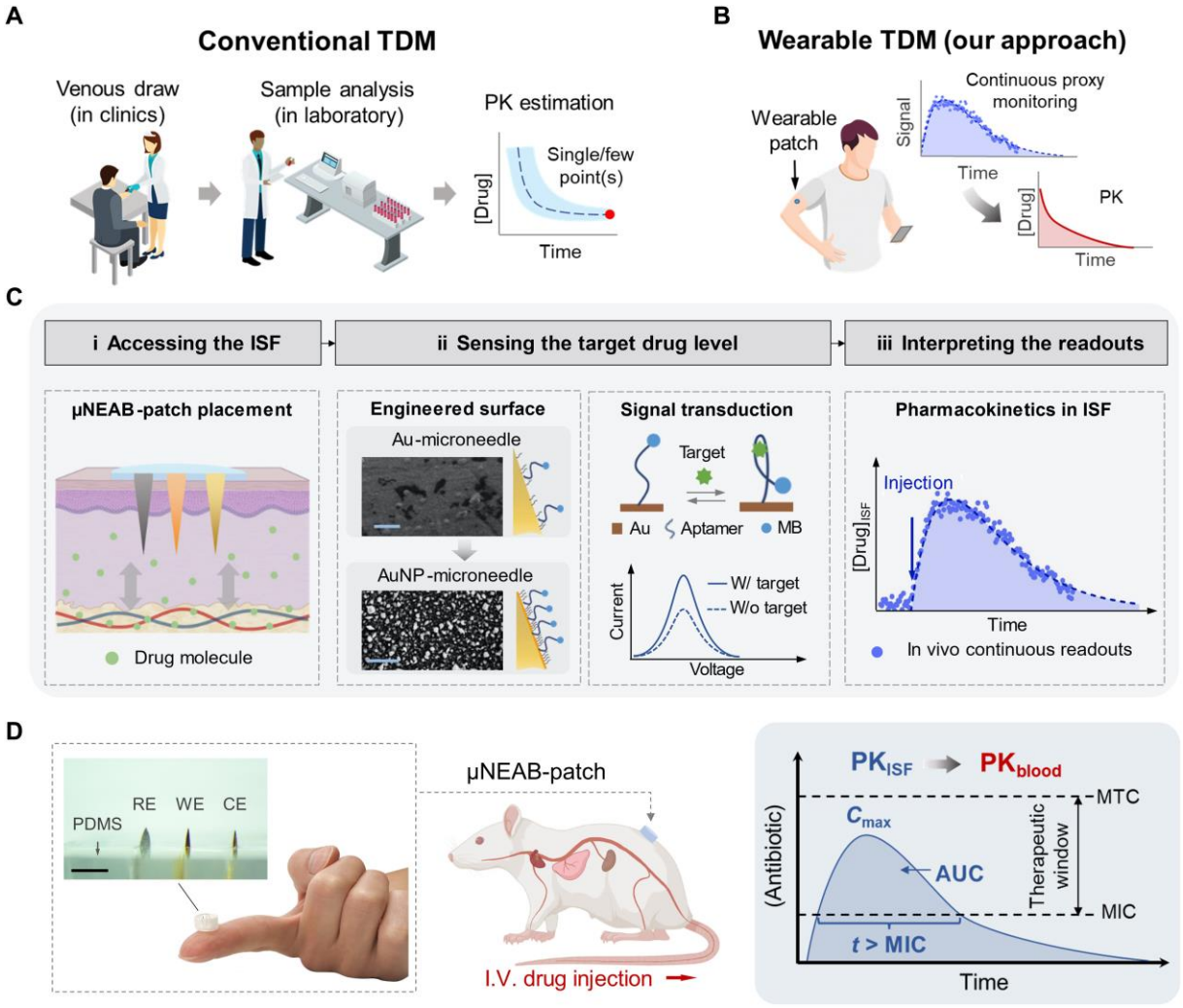
- Apt-1 is short, consisting of 10 base pairs, and does not form a three-dimensional structure
  This enabled intracellular imaging.
  ← Because TDP-43 is an RNA-binding protein, it was able to bind with a short base-paired aptamer.
  This may be difficult for other proteins.

Perspectives

Engineered aptamers for molecular imaging



Wearable microneedle-based electrochemical aptamer biosensing



Lin, B.; *et al*. *Chem. Sci.* **2023**, *14* (48), 14039–14061.
Lin, S.; *et al*. *Sci. Adv.* **2022**, *8* (38).