Diagnostics of socially significant diseases using affine track-etched membranes modified with DNA aptamers

Zavyalova Elena

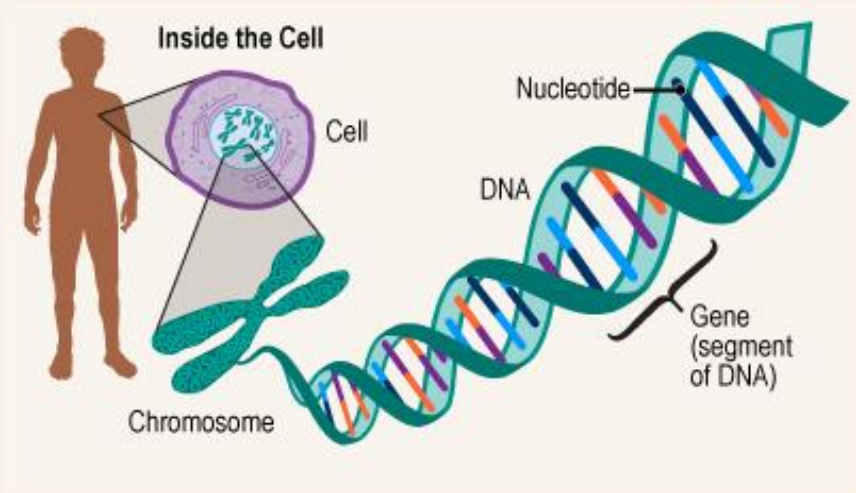
D.Sc. in Chemistry, Associate professor at Chemistry Department

of Lomonosov Moscow State University

Email: zlenka2006@gmail.com

Current approaches for specific diagnostics of socially significant diseases

Determination of the genetic component of the disease



Examples:

Cancer typing (sequencing)
Prognosis of cancer (sequencing)

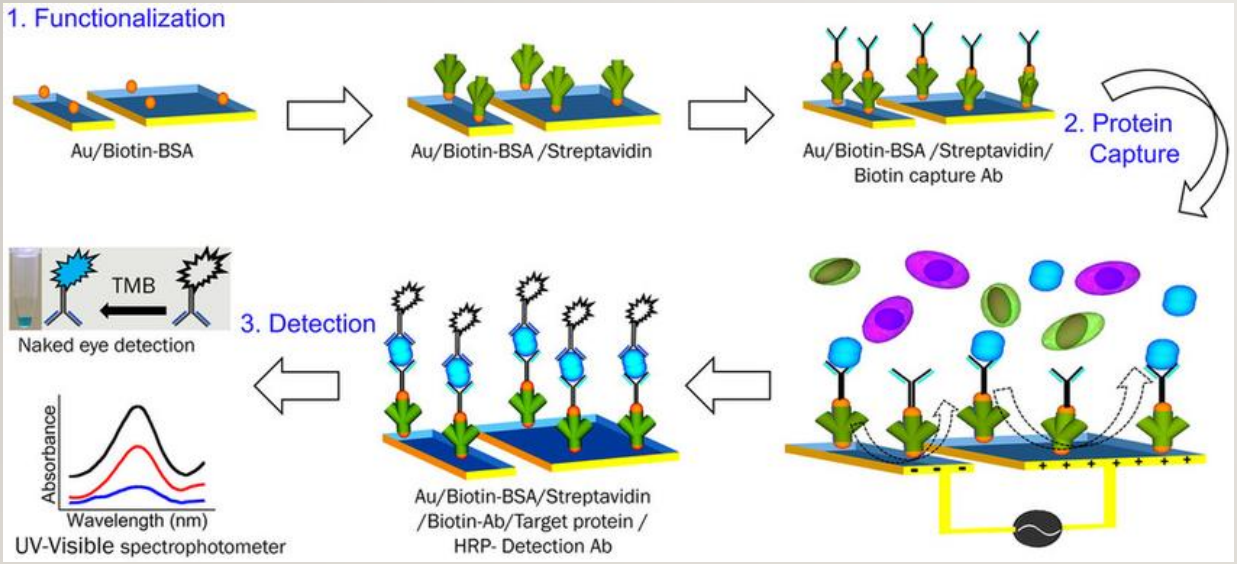
Virus identification (PCR)
Bacterium identification (PCR)
Antibiotic resistance (PCR)

DNA or RNA sequencing → Reading of nucleotide sequences

Polymerase chain reaction → Detection of the concrete fragment of nucleic acid

Current approaches for specific diagnostics of socially significant diseases

Determination of the biomarker of the disease



Examples:

Cancer detection (ELISA)
Immune system status (ELISA)
Cardiac disorders (ELISA)
Allergen determination (ELISA)

Virus identification (LFIA)
Pregnancy detection (LFIA)
etc.

Antibody-based test systems → ELISA

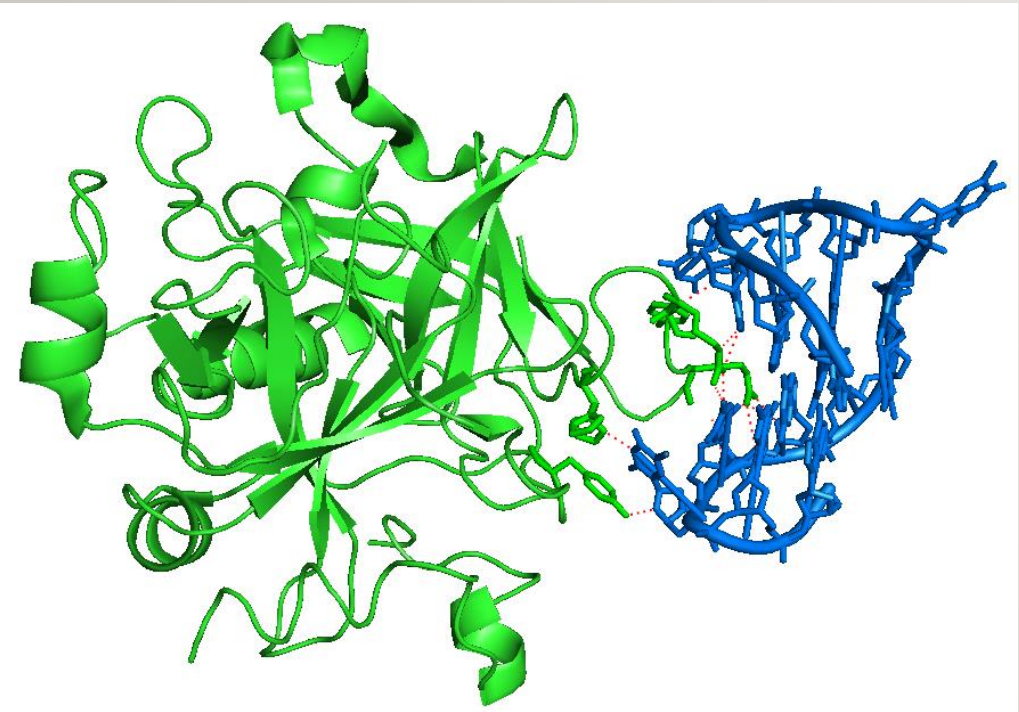
Antibody-based test systems → LFIA (test-strips)

Demands in specific diagnostics of socially significant diseases

- Price reduction is necessary to increase the quantity of analyses per person including screening studies. Price reduction includes usage of cheap reagents and equipment

- The decrease of limit of detection can be achieved by development of concentrating techniques from biological fluids and replacement of antibodies an enzymes

Nucleic acid aptamers can replace antibodies

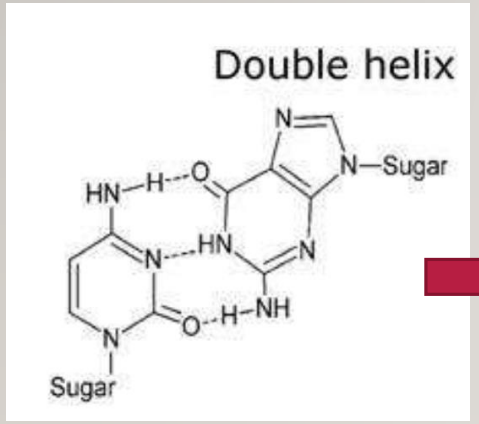


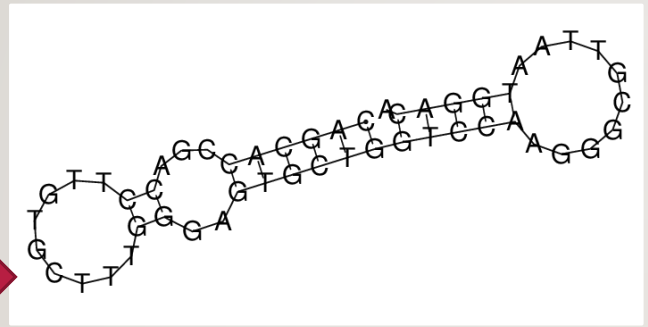
Thrombin (protein from blood clotting system)

DNA aptamer

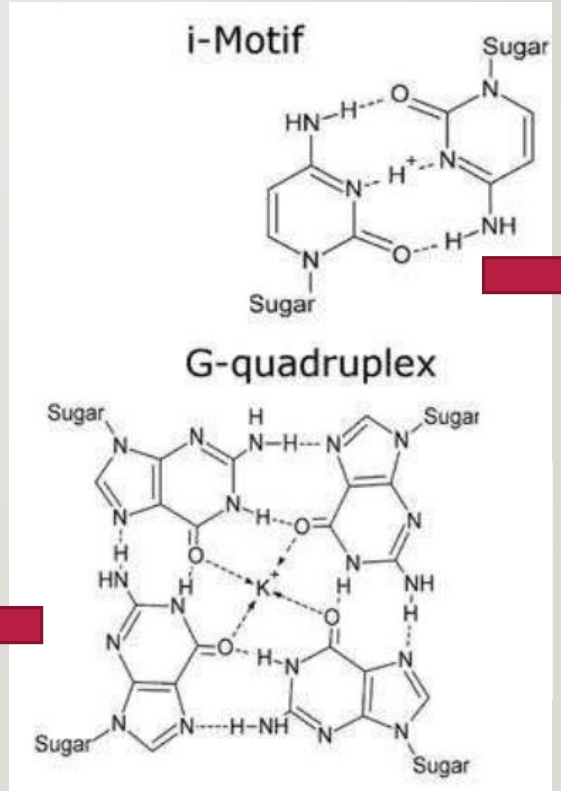
- The aptamer are short synthetic fragments of nucleic acids. The size of several nanometers (2-6 nm) provides near-surface applications like SERS (surface-enhanced Raman spectroscopy), EGOFET (electrolyte-gateв organic field-effect transistors, etc.
- Depending on the task, aptamers can be designed to recognize only one biomarker or a family of related biomarkers
- Aptamers are synthesized chemically and can be accessorized with any functional group, e.g. fluorophore, thiol-anchor, biotin etc.

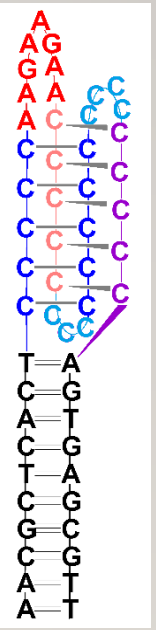
DNA aptamers are structured DNA fragments with specificity and affinity to a target



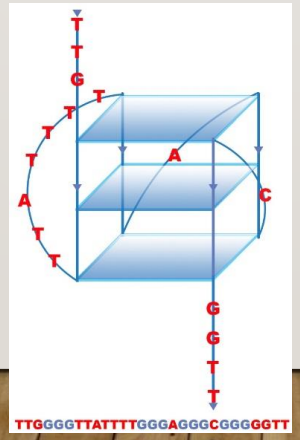


RBD-1C binds spike protein of SARS-CoV-2 (6 nM)





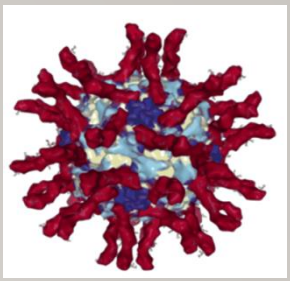
BV42 binds hemagglutinin protein of influenza A and B (0.5 nM)



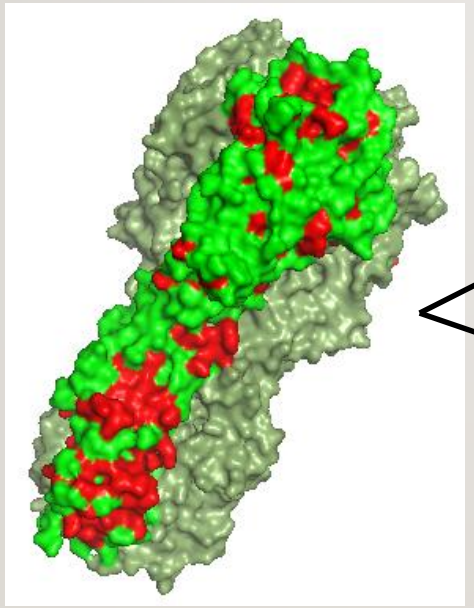
RHA0385 binds hemagglutinin protein of influenza A (2-5 nM)

10.1021/acs.analchem.0c01394
10.1016/j.bbrc.2013.11.041
10.1371/journal.pone.0097696

Examples of wide specificity: aptamers recognize highly variable hemagglutinin from different influenza strains



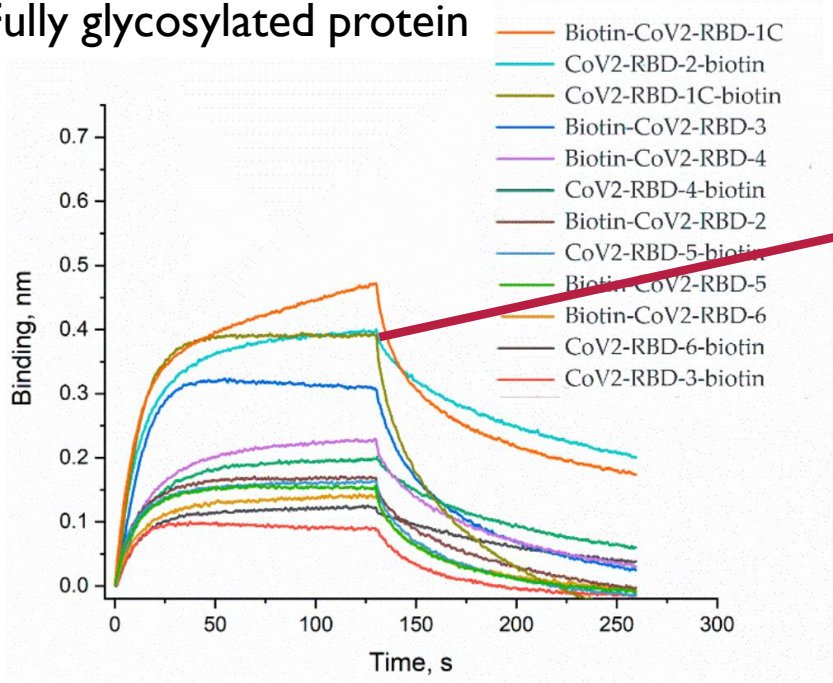
Hemagglutinins have conserved spatial structure but low conserved amino acid sequence (37-73% between influenza A strains and below 30% between influenza A and B)



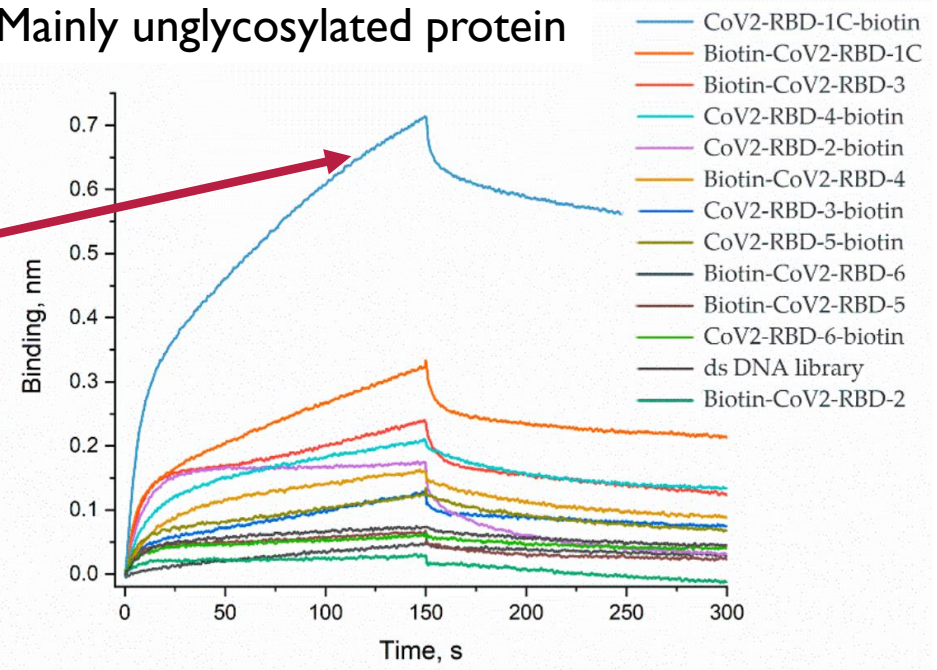
Influenza subtype	Viral strain	RHA0385 K_D, nM	BV42 K_D, nM
H1 (IVA)	Puerto-Rico/8/1934 (H1N1)	13 ± 2	+
	New Caledonia/20/1999 (H1N1)	42 ± 14	+
H3 (IVA)	Aichi/2/1968 (H3N2)	17 ± 5	+
	England/42/1972 (H3N2)	40 ± 5	+
H5 (IVA)	Vietnam/1203/2004(H5N1)-PR8/CDC-R	34 ± 8	40 ± 10
	tern/South Africa/1/1961 (H5N3)	200 ± 50	
H7 (IVA)	mallard/Sweden/91/2002 (H7N9)	13± 4	1.6 ± 0.2
H9 (IVA)	Primorie/3631/2002 (H9N2)	16 ± 3	
H12 (IVA)	duck/Primorie/3691/2002 (H12N2)	> 200	> 200
IVB	Victoria/2/1987	> 200	0.60 ± 0.05
-	Newcastle disease virus	> 200	> 200

Examples of selectivity of aptamers toward different forms of the same protein: aptamer discriminates glycosylation state of RBD from S-protein from SARS-CoV-2

Fully glycosylated protein

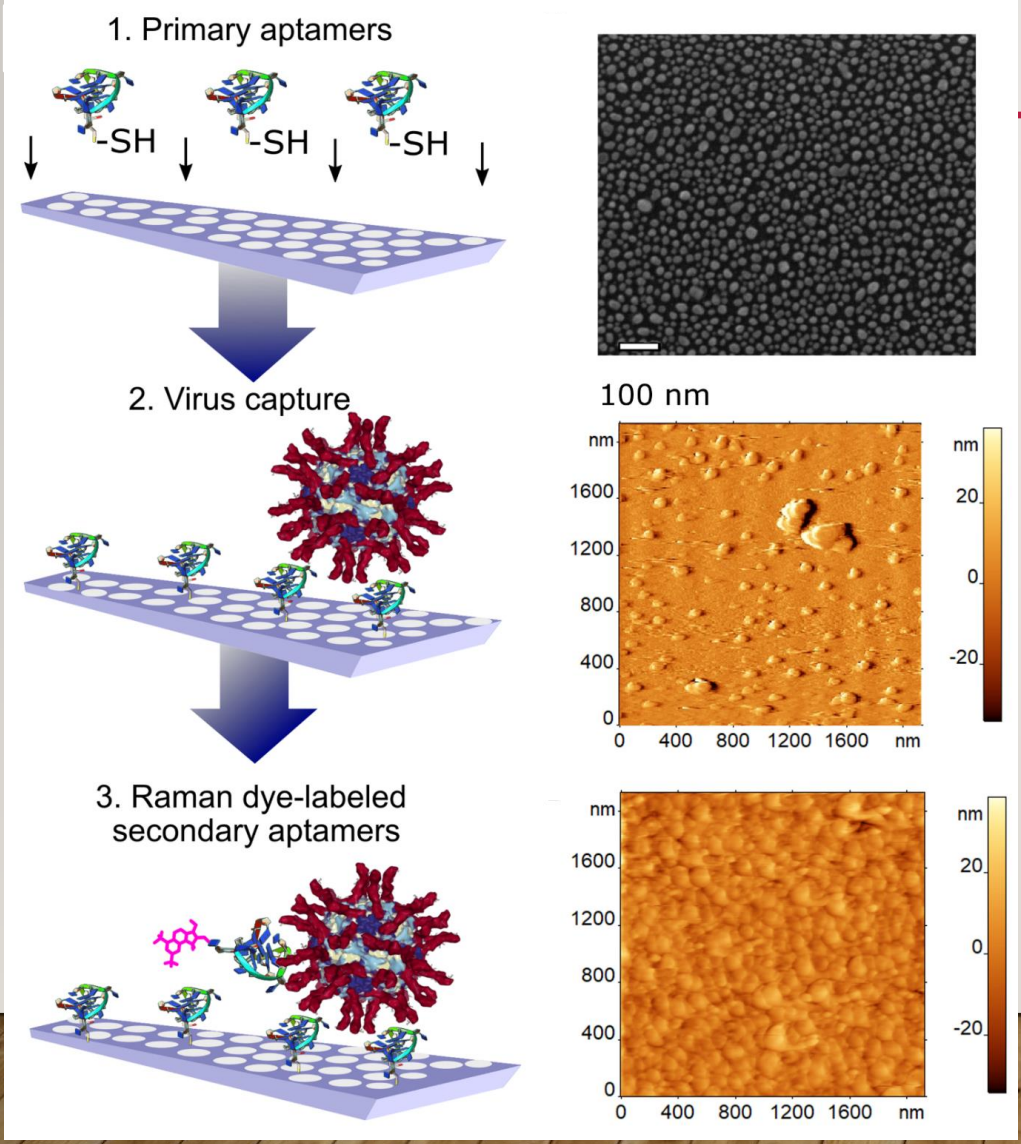


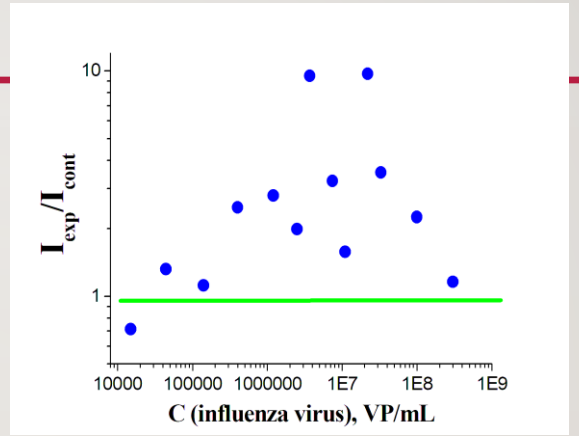
Mainly unglycosylated protein



K_D decreased 7.3-times due to slow dissociation of unglycosylated RBD

Applications of aptamers for virus detection
I. SERS-based island aptasensor for influenza A virus

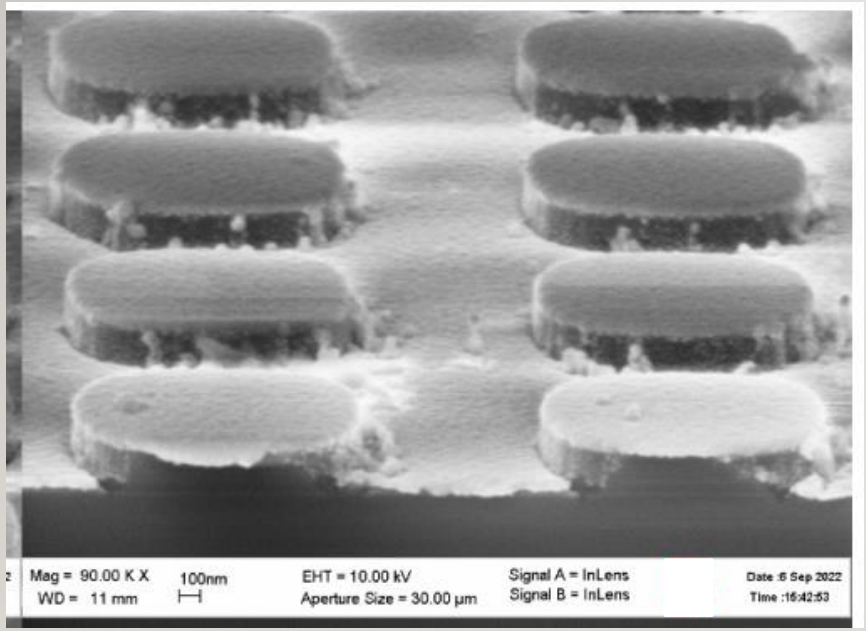




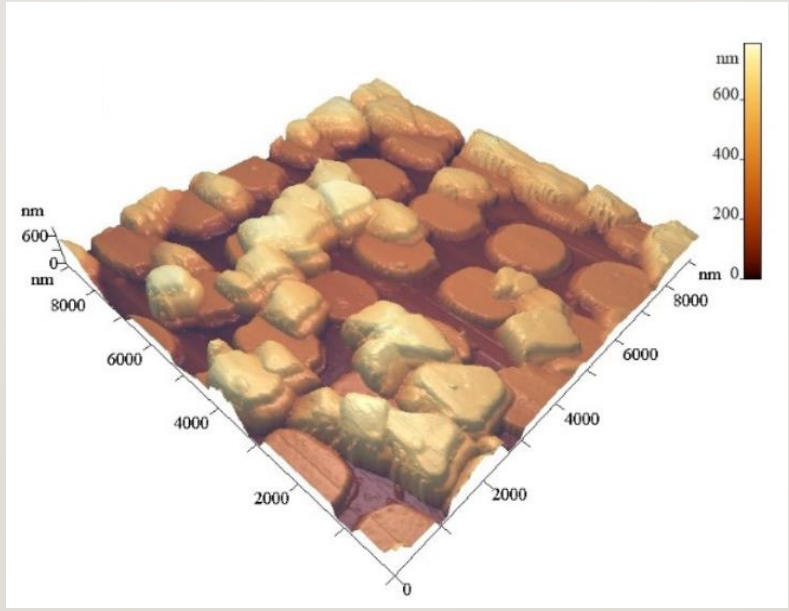
LOD for H3N2:
5 10^4 viral particles/mL

Strain	Subtype	Strain	Subtype
New Caledonia/20/1999	H1N1	duck/Moscow/4182/2010	H5N3
A/England/42/1972	H3N2	A/South Africa/1/1961	H5N3
A/Mississippi/1/1985	H3N2	A/Primorie/3/1982	H9N2
A/Aichi/2/1968	H3N2	A/Primorie/3691/2002	H12N2
A/Buryatia/652/1988	H3N8	Newcastle disease virus	-
A/Buryatia/2408/2001	H4N6	Influenza B:Victoria/2/1987	-
Vietnam/1203/2004-PR8/CDC-R	H5N1		

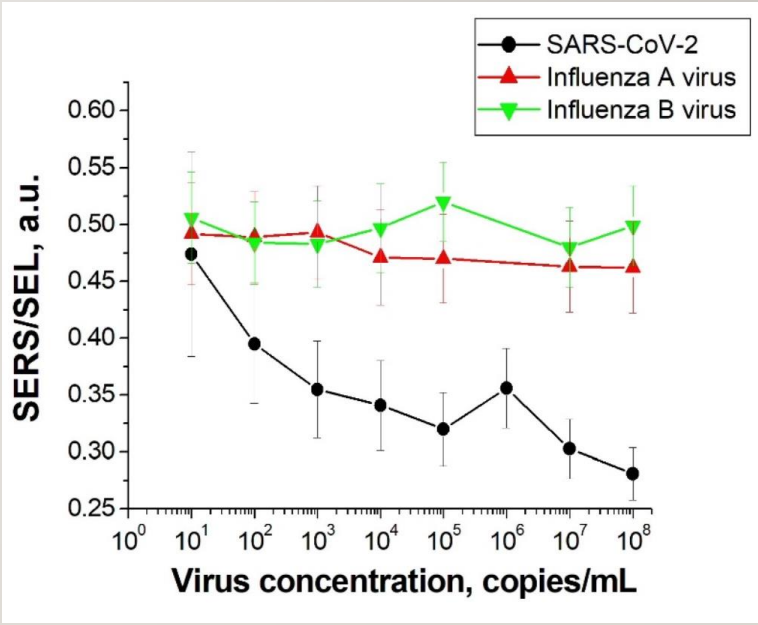
Applications of aptamers for virus detection
II. SERS-based nanocolumn aptasensor for SARS-CoV-2 virus



SEM

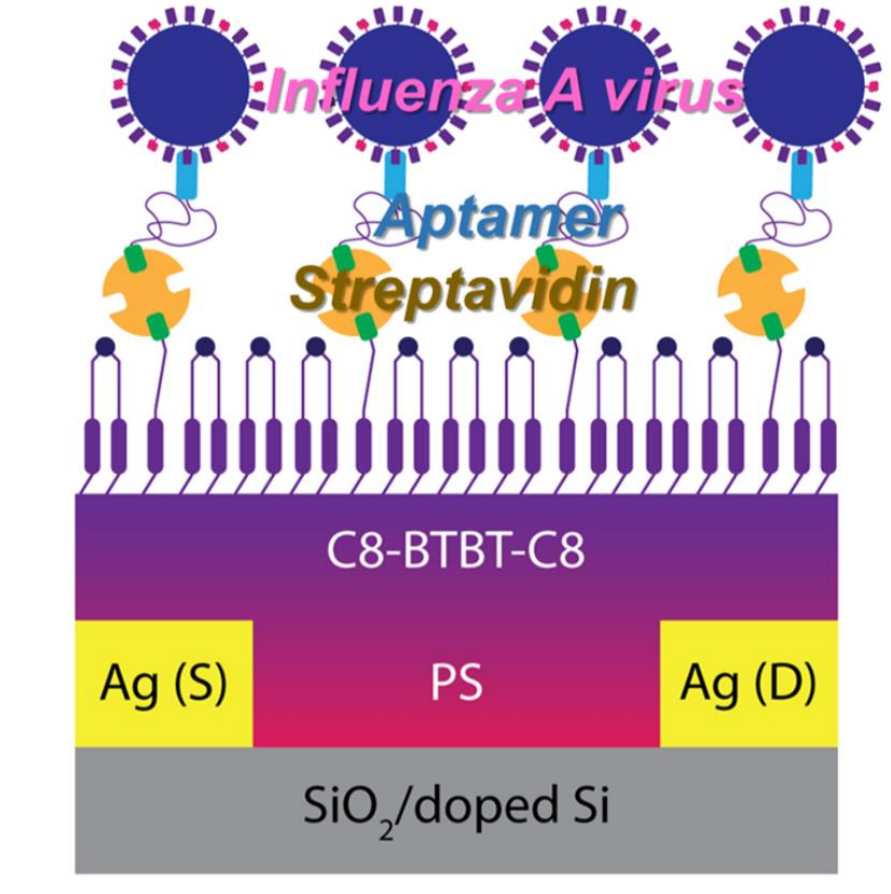


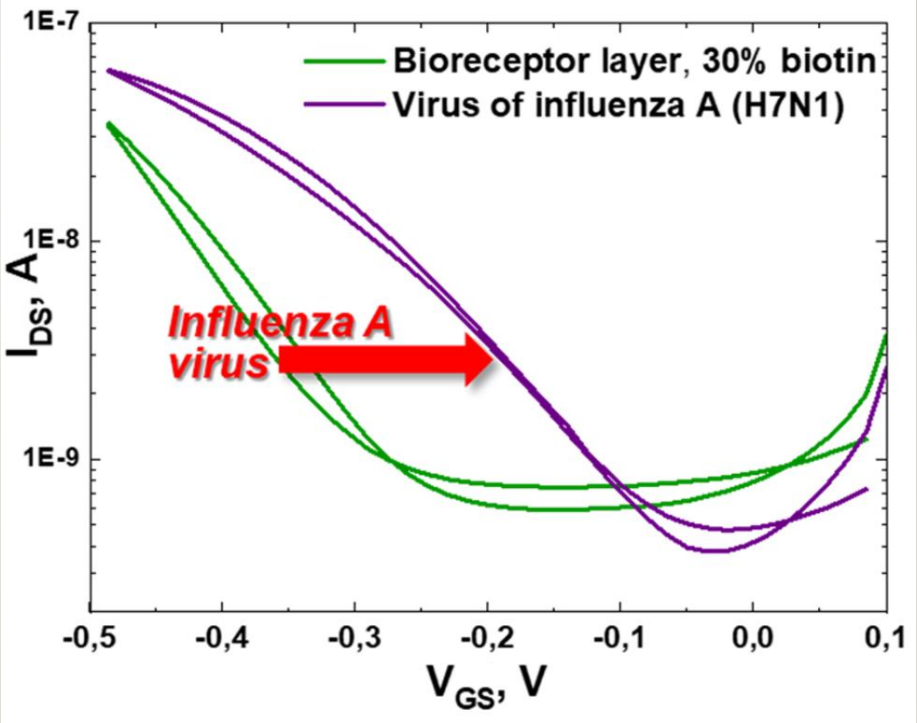
AFM



LOD for SARS-CoV-2:
100 viral particles/mL

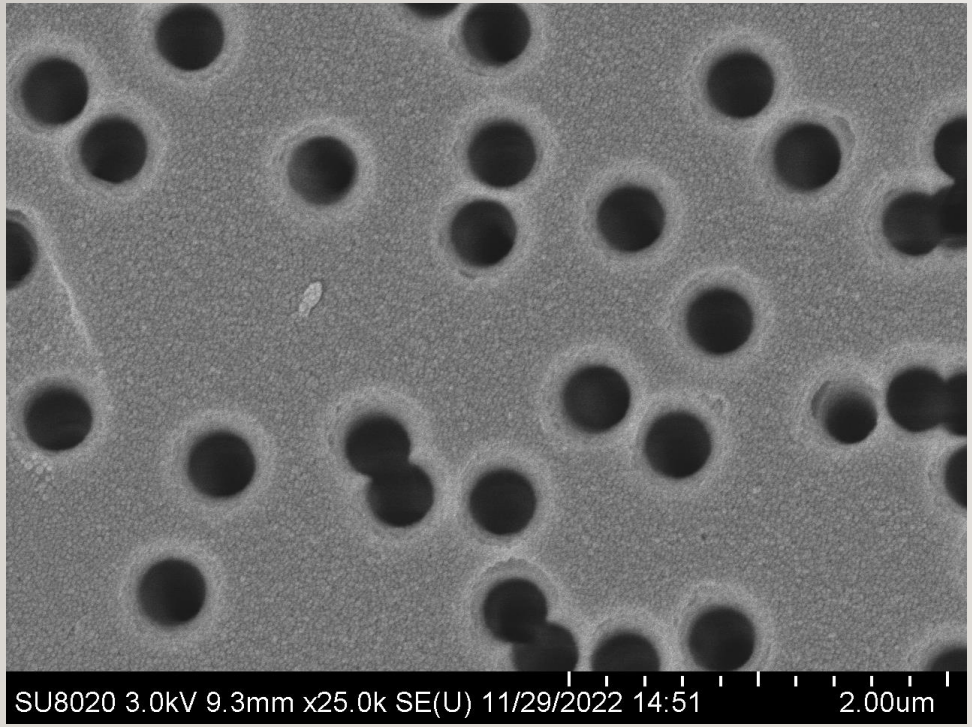
Applications of aptamers for virus detection
III. EGOFET-based aptasensor for influenza A virus

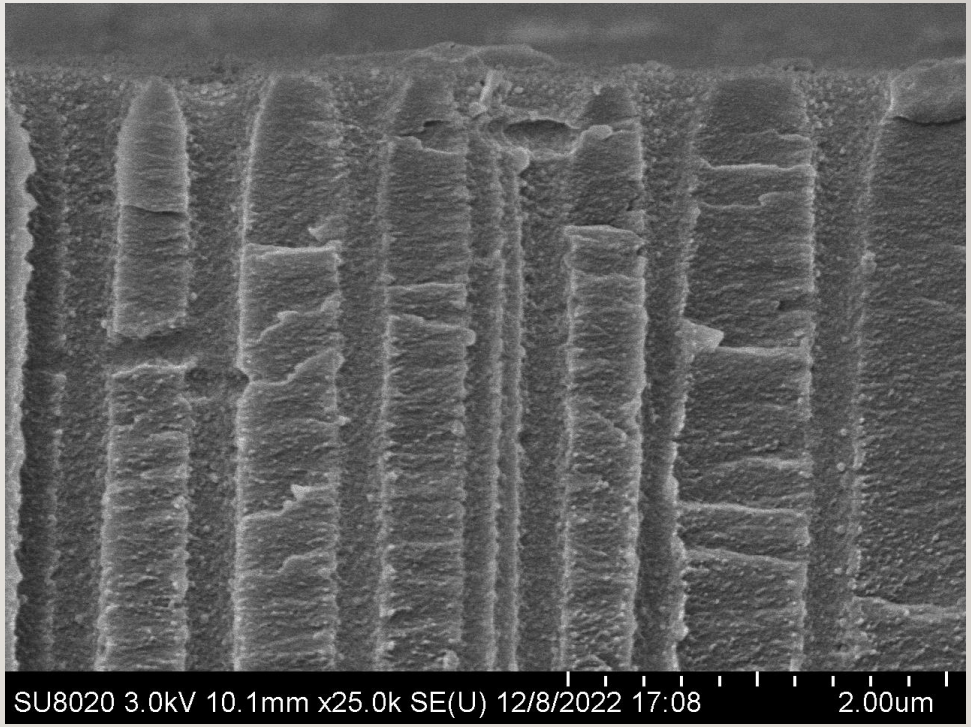




LOD for H7N1: **10^6 viral particles/mL**

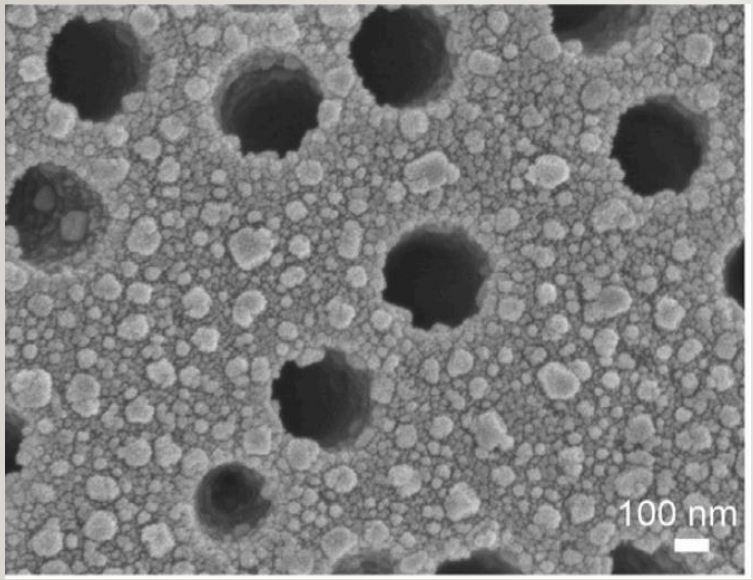
The limit of detection can be decreased due to the membrane filtration (concentrating + purification)

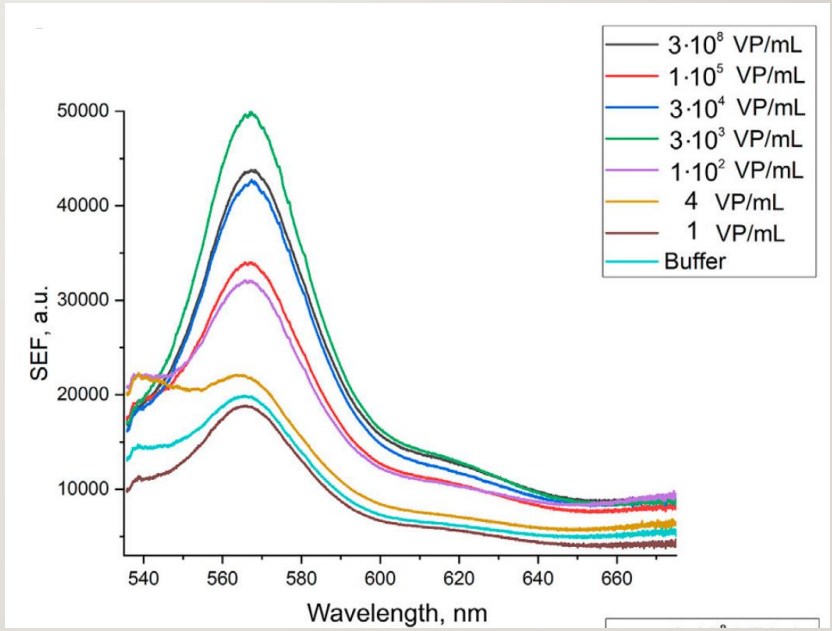




Track-etched membrane with nanoisland silver coating

Surface-enhanced fluorescence on the aptamer-functionalized membrane

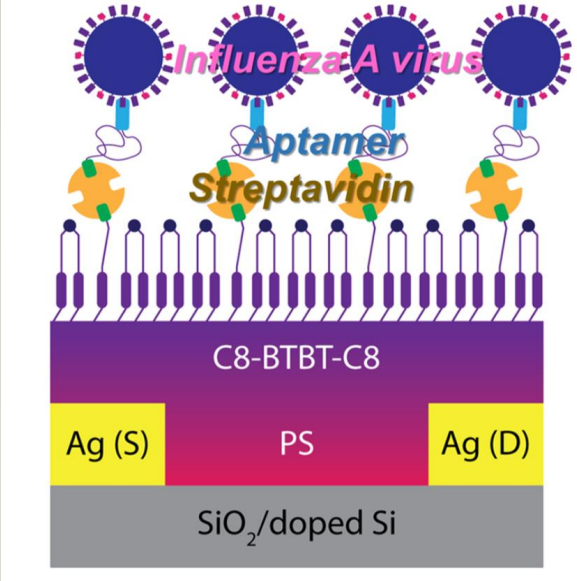




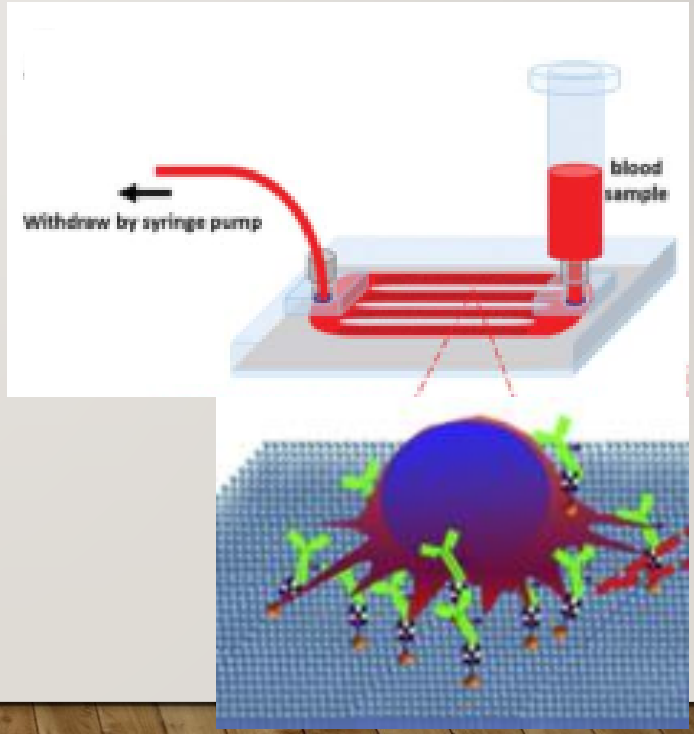
LOD for H7N1: **10 viral particles/mL**

Promising directions for aptamer-functionalize membranes

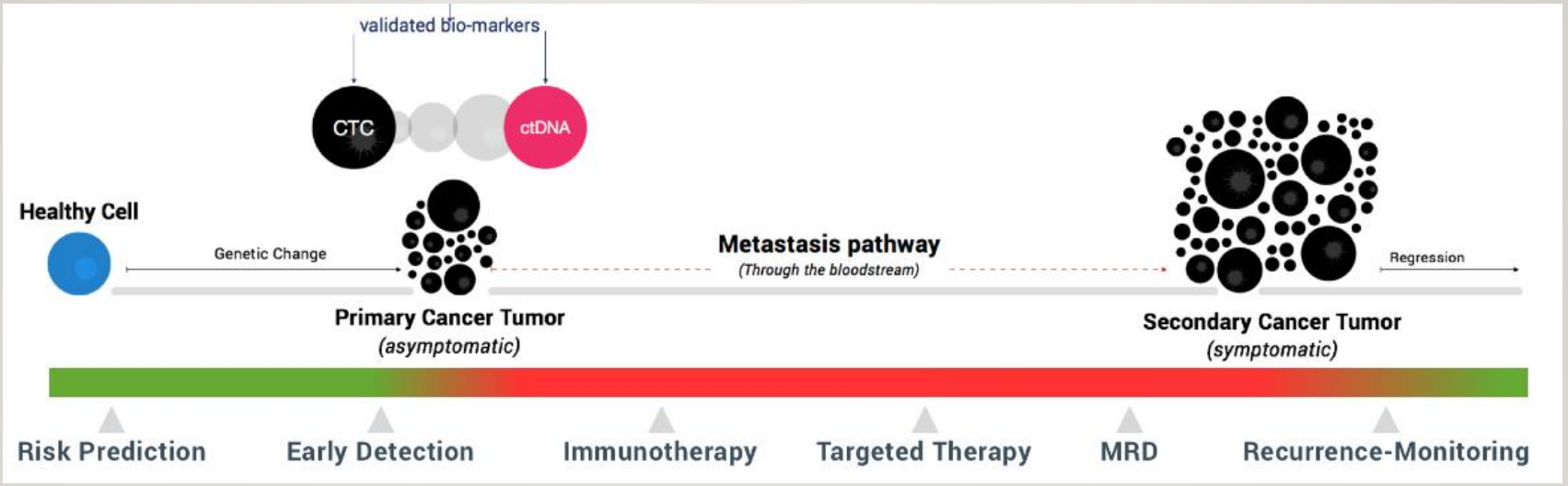
Capturing analytes by the aptamer-modified pores and measurement of the electric characteristics



Capturing cancer cells from blood samples for further identification

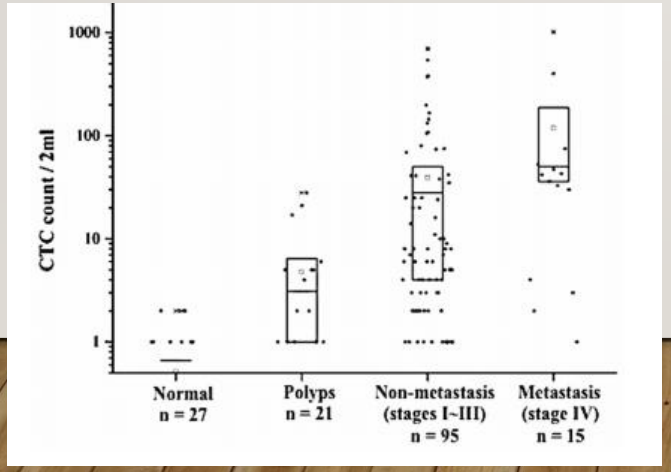


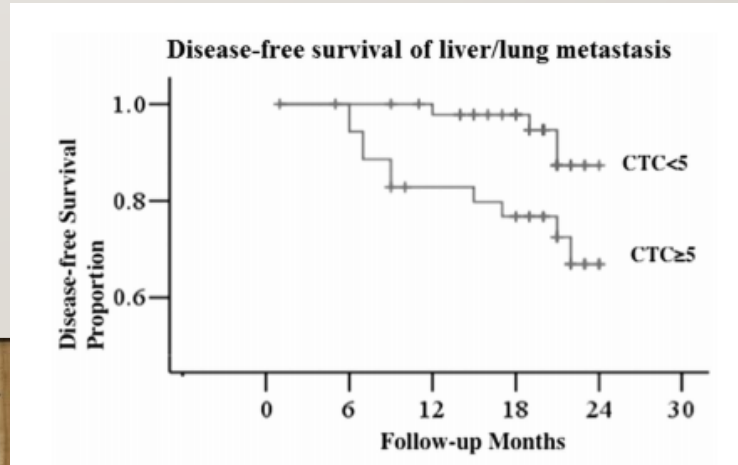
Circulating tumor cells (CTC) in contemporary specific diagnostics of cancer



Colorectal cancer

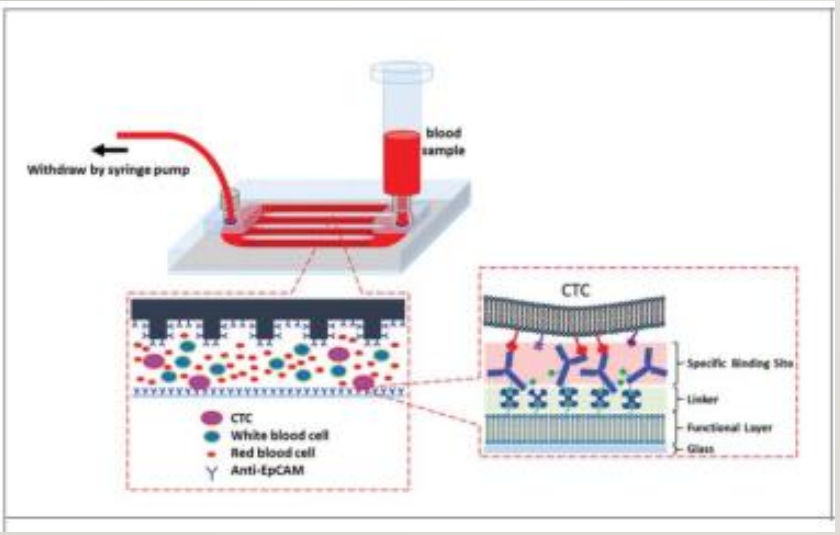
10.1038/srep24517





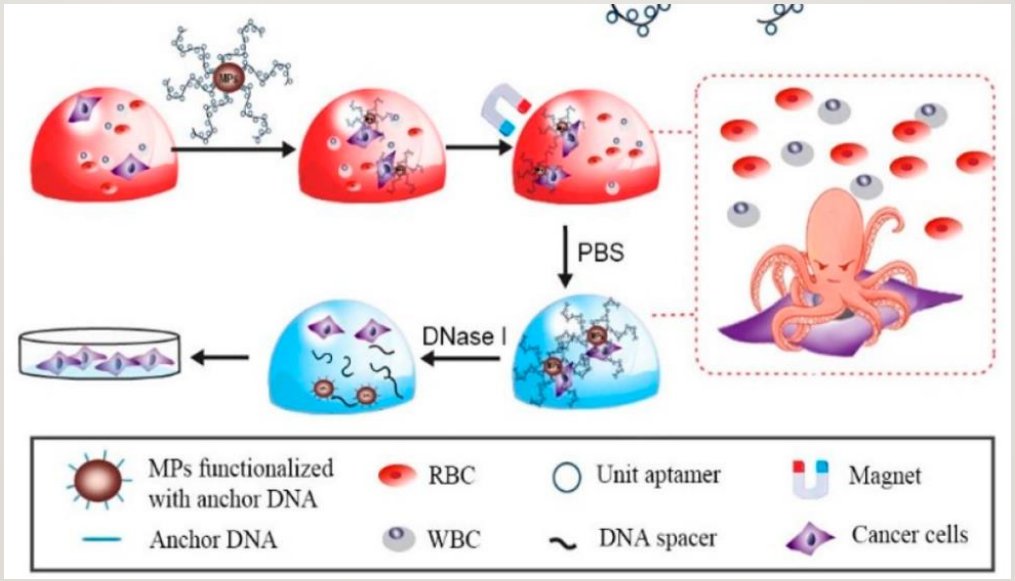
The approaches for cell capturing

Antibody-functionalized microfluidic system



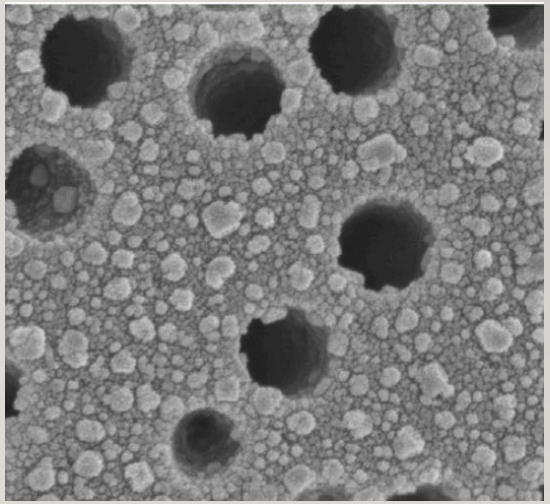
10.1080/15384047.2016.1141839

Aptamer-functionalized magnetic particles



10.1021/acs.analchem.8b05432

Aptamer-functionalized track-etched membranes?



???

THANKS TO MY COLLABORATORS

- Membranes:
  Dr. Apel P. and Nechaev A. from Joint Institute for Nuclear Research
- SERS:
  Dr. Kukushkin V. from Institute of Solid State Physics
- Viruses:
  Prof. Gambaryan A. from Chumakov Institute of poliomyelitis and encephalitis
  Dr. Tkachuk A. from Gamaleya National Research Center for Epidemiology and Microbiology
- EGOFET:
  Prof. Ponomarenko S., Dr. Agina E. and Dr. Poimanova E. from Enikolopov Institute of Synthetic Polymeric Materials
- Financial Support:
  RSF grant #18-74-10019

OUR SELECTED PUBLICATIONS

- Aptamers:
  F. Grabovenko *et al*. Glycosylation of receptor binding domain of SARS-CoV-2 S-protein influences on binding to immobilized DNA aptamers. **Int. J. Mol. Sci. 2022**, 23, 1, 557.
  A. Bizyaeva *et al*. The functional role of loops and flanking sequences of G-quadruplex aptamer to the hemagglutinin of influenza A virus. **Int. J. Mol. Sci. 2021**, 22, 5, 2409.
  A. Novoseltseva *et al*. Structural and functional aspects of G-quadruplex aptamers which bind a broad range of influenza a viruses. **Biomolecules 2020**, 10, 1, 119.

- SERS:
  Kukushkin *et al*. Aptamer-coated track-etched membranes with a nanostructured silver layer for single virus detection in biological fluids. **Front. Bioeng. Biotech. 2023**, 10.
  Kukushkin *et al*. Lithographic SERS aptasensor for ultrasensitive detection of SARS-CoV-2 in biological fluids. **Nanomaterials 2022**, 12, 21, 3854.
  Zhdanov *et al*. A combination of membrane filtration and Raman-active DNA ligand greatly enhances sensitivity of SERS-based aptasensors for influenza A virus. **Front. Chem. 2022**, 10, 937180.
  V. Kukushkin *et al*. Highly sensitive detection of influenza virus with SERS aptasensor. **PLoS ONE 2019,** 14, 4, e0216247.
  D. Gribanyov *et al*. SERS-based colloidal aptasensors for quantitative determination of influenza virus. **Int. J. Mol. Sci. 2021,** 22, 4, 1842.

- EGOFET:
  E. Poimanova *et al*. Biorecognition Layer Based On Biotin-Containing [1]Benzothieno[3,2-b][1]benzothiophene Derivative for Biosensing by Electrolyte-Gated Organic Field-Effect Transistors. **ACS Appl. Mater. Interfaces 2022**, 14, 14, 16462–16476.