Project: «High-sensitivity sensor based on molecular recognition for viruses detection»

years 2025 – 2029

Project leaders:

Nechaev A.N. Zavyalova E.G.

Theme: 07-5-1131-2017
“Radiation materials science, nanotechnological and biomedical investigations with heavy ion beams”
“Радиационное материаловедение, нанотехнологические и биомедицинские исследования на пучках ускоренных тяжелых ионов”
years 2025 – 2030
Flerov Laboratory of Nuclear Reactions
Departments: Centre of Applied Physics, sector No 8
Research area: Condensed Matter Physics, Radiation and Radiobiological Research
Theme leaders: S.N. Dmitriev, P.Yu. Apel

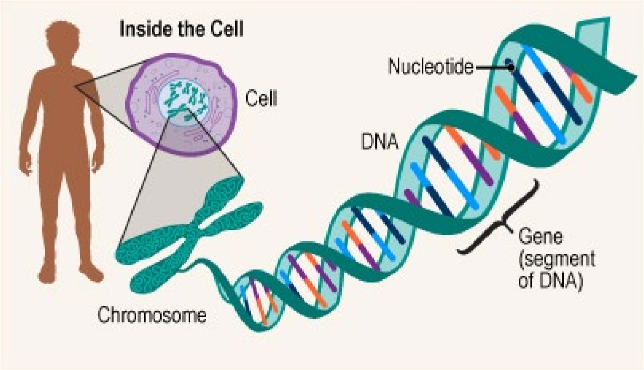
TM in Environmental monitoring of socially significant diseases (sieve separation mechanism)

Type of Control	Application	The recommended size of pores, nm
The microbiological analysis of water	The total bacteria in water	0.2
	Calculation total Coli-form bacteria	0.45
	Calculation excrement Coli-bacteria	0.7
	Extraction of phytoplankton for the analysis	1-3
	The total extraction of particles for the analysis	5
	Extraction of thin deposits for the analysis	0.45
	Parasitologic analysis	1-5
Quality surveillance of foodstuff	Calculation of bacteria	0.2
	Calculation total Coli-form bacteria	0.45
	Definition total Coli-form in milk	0.65
	Allocation of yeast and mold fungi for calculation of colonies	0.65
	Allocation of yeast and mold fungi for the microscopic analysis	0.8
Cytologic researches and chemotaxis	Allocation of human cells for the level-by-level cytologic analysis	1-5
	Chambers for chemotaxis	3-12

Since 1987 till nowadays Russian ministry of health using track-etched membrane (TM) as a standard membrane for protozoa and bacteria identification in water. Efforts for TM environmental applications were initiated by G.N. Flerov in 1981.

Current approaches for specific diagnostics of socially significant diseases

Determination of the genetic component of the disease

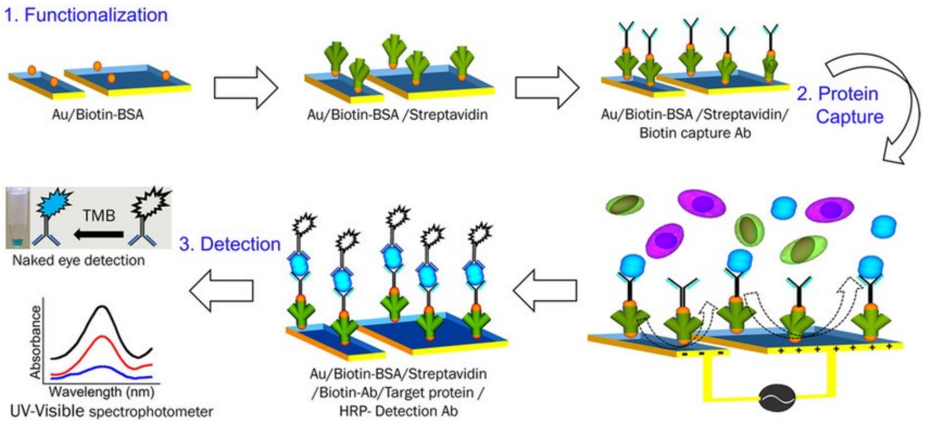


Examples: Cancer typing (sequencing), Prognosis of cancer (sequencing), Virus identification (PCR), Bacterium identification (PCR), Antibiotic resistance (PCR)

DNA or RNA sequencing → Reading of nucleotide sequences

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

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; LFIA (test-strips)

Relevance of the Project:

Modern requirements and trends 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

Problem resolve : BIOSENSORS based on bacteria and viruses molecular recognition and spectral detection

- Allows you to quickly identify viruses (virus detection using the laboratory-on-a-chip technique
- Low cost of analysis
- Portable engineered biophysical analyzers
- Reduction of sample volume and reagent volume
- The possibility of real-time intramolecular observations and digital signal transmission
- High sensitivity of the method (the threshold for the detection of viruses in an aqueous sample using the laboratory-on-a-chip method)

Concept:

The concept assumes a synergistic effect from combining modern approaches in the field of composite track membranes with advances in molecular biology, spectral analysis methods and sensor technology for virus detection

Membrane science + molecular biology + virology + sensors and photonics

The project includes three main directions :

- Development of composite SERS active track etched membrane functionalized with aptamers
- Development and syntheses of highly selective aptamers for anti-bodies replacement and viruses recognition
- Development of sensor prototype based on track etched membrane modified by aptamers for viruses detection and monitoring

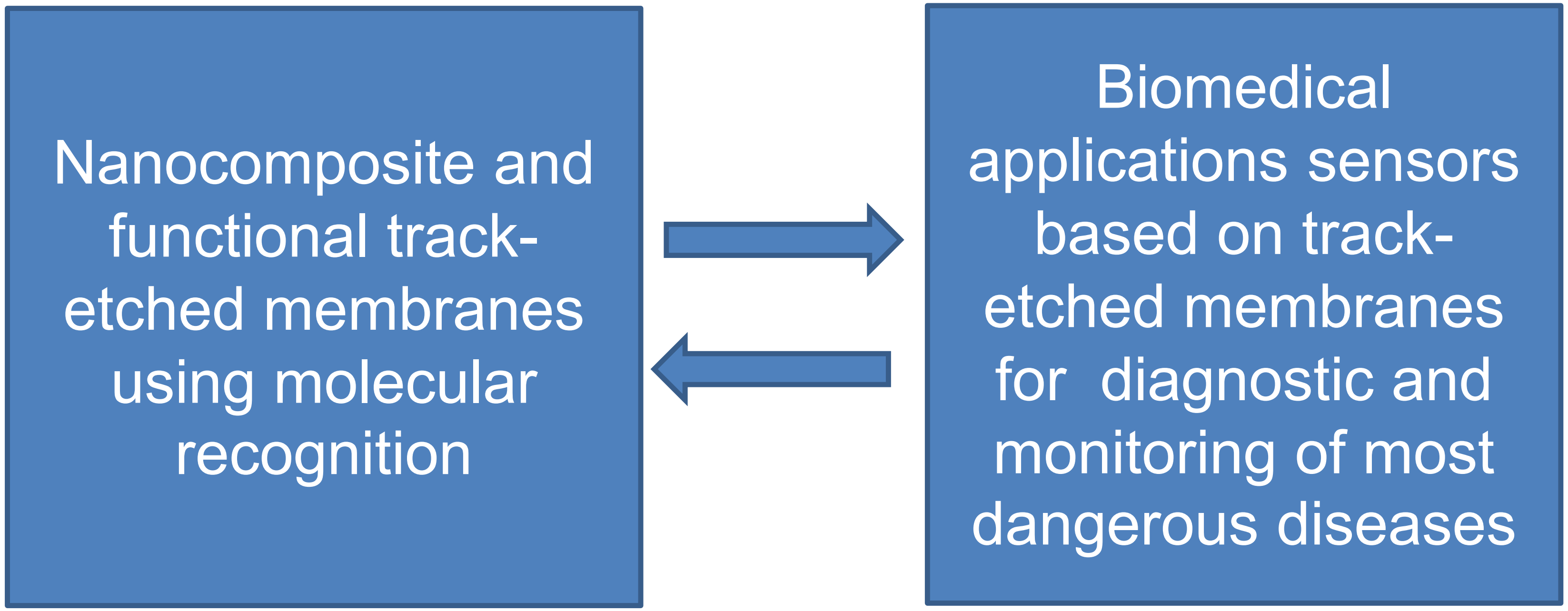
Upon completion of the projects in 2029, new project will be formulated to continue the project for wild spectrum of viruses and cancer cells

Potential application

- Generally, the research will be oriented towards materials science,-nanotechnological applications, life sciences, environmental research
- Rapid and efficient diagnostic and monitoring of particularly dangerous and socially significant diseases

The aim of the project

- to develop rapid and high selective sensor prototype based on the track-etched membranes (TMs) for motoring of African swine fever viruses (ASFV)
- to apply ASFV-sensor for viruses monitoring and in agriculture sector in Eurasian Economic Union.



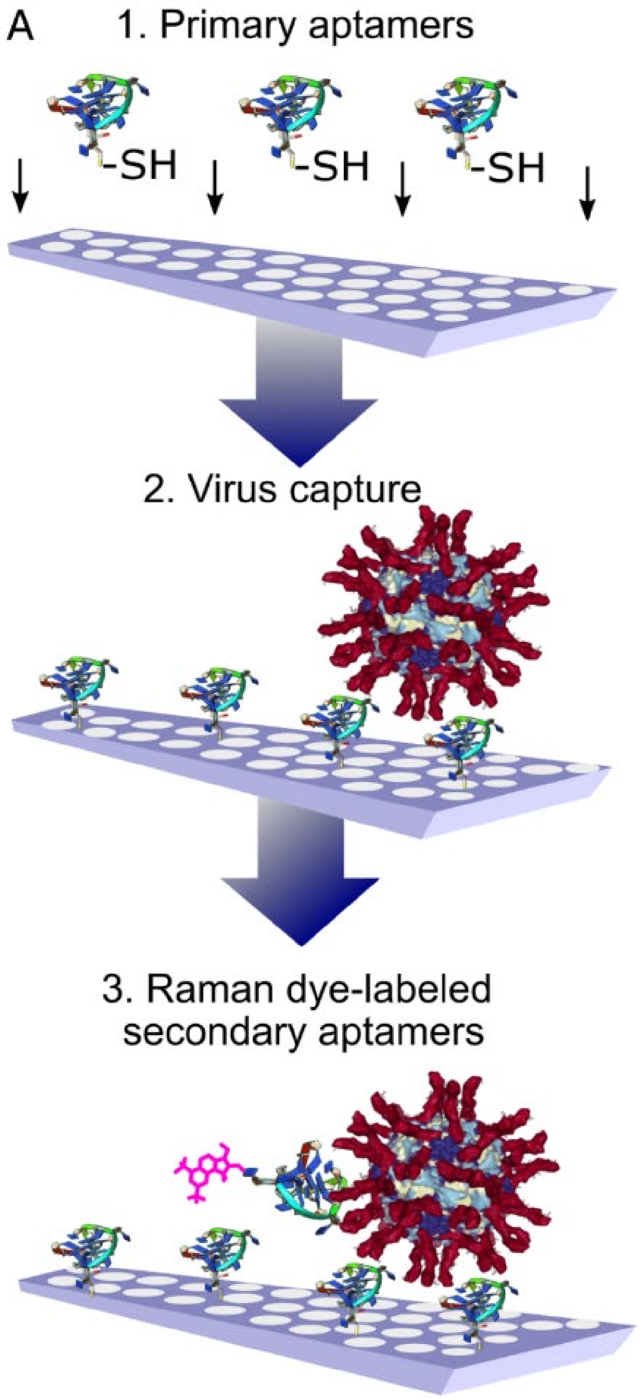
Motivations:

- ❑ Demand for membrane-based systems for specific detection of biomarkers and pathogens
- ❑ Development of multifunctional solutions with concentration, specific determination and quantification in a one-step manner
- ❑ The necessity of the development of nanofluidic sensors for virus detection, especially for of African swine fever virus (ASFV)
- ❑ Implementation of rapid and efficient sanitary and biological analysis of socially significant diseases for JINR member countries

Suggested technical approach

- Selection of new DNA aptamers to surface proteins of African swine fever virus (ASFV)
- Creation of sensor substrate with self-assembled of Au and Ag nanoparticle surface using physical vapor deposition
- Functionalization of tracked-etched membranes with the thiolated aptamers to ASFV
- Quantification of the viruses using SERS

TM is a unique membrane based substrate for SERS (provides stable reproducible signal)!



Expected results:

1) Experimental results on properties and feasibility of fabrication of components for future viruses sensors :

- TMs functionalized by silver nanoparticles and biocompatible nanoconjugates as bacteriocidic and virucidal materials
- Selection and characterization of aptamers with the highest affinity to ASFV
- Evaluation of genotoxic properties of the aptamers *in vitro* using comet assay
- Antiviral properties of aptamers, including high target specificity and affinity for their target molecules, allowing them to effectively interfere with viral replication or infectivity
- Nanocomposite TMs with SERS-active Ag nanoparticle arrays with immobilized aptamers for rapid and sensitive detection of viruses
- Development of protocol for rapid and effective ASFV analysis using SERS active TM

2) Design and assembly of viruses sensors.

- Development and design of TMs based test strips for the virus specific determination
- Development of test strips with immobilized DNA aptamers for the specific determination of ASFV, influenza A, SARS-CoV-2

Methods and instruments for TM:

FLNR Center of applied physics

- Irradiation of polymers with heavy ion beams (energy 1-4 MeV/u, dE/dx of 3-25 keV/nm)
- Facilities for pilot production of novel modifications of track membranes
- Facilities for the production of nanoparticles and nanofibers
- Structure investigations using transmission and scanning electron microscopy
- Optical (UV-Vis, IR Fourier spectroscopy, Raman,…)
- PVD of Ag and Au thin film deposition

Methods and instruments for aptamers and ASFV:

Institute of Molecular Biology of NAS RA (IMB) and Department of Genetics and Cytology of Yerevan State University (Armenia)

- Laminar benches, qPCR, ELISA, centrifuges, freezers, and other equipment

Department of Genetics and Cytology of Yerevan State University (Armenia)

- Comet assay will be carried out, which has fluorescent microscopes, MetaSystems, and Comet Assay IV systems for evaluation of DNA comets. Statistical analysis of DNA damage will be performed using SPSS19 and Statgraphics Centurion16 packages

Chemistry department of Lomonosov Moscow State University (Russia)

- Elveflow microfluidic device with Bambi compressor, analysis of affinity (Blitz interferometer with aminoreactive and streptavidin sensors, MOS-200 stopped-flow fluorimeter equipped with μSFM cuvette), aptamer folding estimation (MOS-500 circular dichroism spectrometer, Hitachi UV spectrometer)
- Equipment for affine chromatography

Partners

RUSSIA

- Lomonosov Moscow State University (Moscow)
- Burnasyan Federal Medical Biophysical Center of FMBA (Moscow)
- Enikolopov Institute of Synthetic Polymer Materials (Moscow)
- Institute of Solid-State Physics RAS (Chernogolovka)
- Ivanovo State University of Chemical Technology (Ivanovo)
- Mechnikov Research Institute of Vaccines and Sera (Moscow)
- Moscow Institute of Physics and Technology (Dolgoprudnyi)
- Pirogov Russian National Research Medical University (Moscow)

ARMENIA

- Yerevan State University (Yerevan)
- Institute of Molecular Biology of NAN (Yerevan)
- Nalbandyan Institute of Chemical Physics (Yerevan)

Russia Team background

- Kukushkin, V., Ambartsumyan, O., Subekin, A., Astrakhantseva, A., Gushchin, V., Nikonova, A., Dorofeeva, A., Zverev, V., Keshek, A., Meshcheryakova, N., Zaborova, O., Gambaryan, A., and Zavyalova, E. Multiplex lithographic SERS aptasensor for detection of several respiratory viruses in one pot. International Journal of Molecular Sciences 24, 9 (2023), 8081. (Q1, IF=5.6) DOI: 10.3390/ijms24098081
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Armenia Team background

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Milestones

- **2025**

1. Selection of aptamers with highest affinity to ASFV
2. Evaluation of genotoxic properties of selected aptamers in vitro using comet assay
3. Track etched membrane gold and silver thin layers modification using magnetron sputtering

- **2026**

4. Evaluation of antiviral properties of non-genotoxic aptamers against ASFV in vitro before and after infection using comet assay
5. Selection of aptamers with virucidal effects for further development of TMs with immobilized aptamers
6. Modification of gold and silver nanolayers by ASFV specific aptamers

- **2027**

7. Evaluation of antiviral properties of TMs with immobilized aptamers against ASFV
8. Evaluation of Raman spectroscopy properties of TMs with immobilized aptamers against ASFV
9. Comparative evaluation of tested aptamers antiviral properties against ASFV based on the analysis of DNA damage in cells incubated with aptamers using comet assay

- **2028**

10. Development of protocol for ASFV analyses using SERS-active TM
11. Development of prototype of ASFV Raman-analyses

- **2029**

12. Application of selected TMs with immobilized aptamers with optimal virucidal capabilities attached to TMs. Estimation of their effectivity based on the analysis of DNA damage in medium before and after filtering the viruses using comet assay
13. Development of Real–time ASFV monitoring protocol

Requested financing of the project (2025 – 2029)

№	Items of expenditure	Cost	Expenditure per year (thousands of the US dollars)				
			1st year	2nd year	3rd year	4th year	5th year
1.	International cooperation	25	5	5	5	5	5
2.	Materials	75	15	15	15	15	15
3.	Equipment, Third-party company services	-	-	-	-	-	-
4.	Commissioning	-	-	-	-	-	-
5.	R&D contracts with other research organizations	150	30	30	30	30	30
	Recourses:						
6.	The amount of FTE	12	12	12	12	12	12
7.	Accelerator	12.5	12.5	12.5	12.5	12.5	12.5
	Total:	250	50	50	50	50	50

Manpower needs in the first year of implementation of the project

№	Category of personnel	JINR staff, amount of FTE	JINR Associated Personnel, amount of FTE
1.	research scientists	9	0
2.	engineers	3	0
3.	specialists	-	0
4.	office workers	-	0
5.	technicians	-	0
	Total:	12	0

Thank you for your attention!

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Число статей в Web of Science и Scopus – 60
Число ссылок - 800
Индекс Хирша 16 (Scopus)
Число патентов – 9
Опыт совместной работы: проект РНФ
1) «Создание оптических сенсоров на основе ДНК-аптамеров для детектирования биологических объектов»
2) «Экспресс-определение антибиотикорезистентности бактерий в биологических образцах с помощью спектроскопии гигантского комбинационного рассеяния»
2020 – Премия по программе развития МГУ
2022 - Премия Правительства Москвы молодым ученым, в номинации "Биология" – «за разработку биосенсоров для определения респираторных вирусов»
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