Annex 3.

Form of opening for Project

APPROVED

JINR DIRECTOR

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“____” _____ 2023

PROJECT PROPOSAL FORM

Opening of a research project within the Topical plan of JINR

1. General information on the research project of the theme

1.1 Theme code

1.2 Project code

1.3 Laboratory

Frank Laboratory of Neutron Physics

1.4 Scientific field

04-4 Condensed Matter Physics and Radiobiological Research

1.5 Title of the project

Nanobiophotonics

1.6 Project leaders

G.M. Arzumanyan, K.Z. Mamatkulov

1.7 Project deputy leader

Yersultan Arynbek

2. Scientific case and project organization

2.1 Annotation

The project 'Nanobiophotonics' has been developed for implementation within the framework of the new theme “Optical Methods in Condensed Matter Studies” at the Frank Laboratory for Neutron Physics. It is an interdisciplinary research project at the interface between vibrational (Raman) spectroscopy and microscopy, nanophotonics, photobiology, fluorescence microscopy, and nanobiotechnology in general. Conceptually, the Nanobiophotonics project aims at solving a number of specific fundamental and applied tasks in the study of physical, optical and transport properties of low-dimensional materials (2D materials and van der Waals heterostructures), as well as two topical and socially important problems in the Life Sciences concerning programmed cell death and lipid-protein interactions. The latter will be accompanied by modelling of biological systems using the method of molecular dynamics (MD) and density functional theory (DFT).

An important component of the project will also be a block of some methodological problems, among which the development of ultra-low frequency Raman spectroscopy (~10 cm^{-1}) at different wavelengths of excitation laser radiation should be highlighted. This will radically remove the limitation of Raman spectroscopy in the study of low-frequency vibrations of crystal lattices and skeletal vibrations of biological samples.

International cooperation, primarily with the JINR Member States, as well as the student program, will traditionally be the focus of attention of the leaders of the Nanobiophotonics project during its implementation.

2.2 Scientific case (aim, relevance and scientific novelty, methods and approaches, techniques, expected results, risks)

The interdisciplinary project “Nanobiophotonics” is aimed at optical research of condensed matter state complementary to neutron and radiobiological research traditionally carried out at JINR. Formally, the main goals and objectives of the proposed research project are reflected in its two-part title: nanophotonics – a branch of photonics dealing with the study of physical phenomena arising from the interaction of photons with nanometer-sized objects, and biophotonics - respectively, with biological objects. In essence, the Nanobiophotonics project research programme consists of a number of specific relevant problems of interaction of photons with condensed media, primarily using Raman spectroscopy and CARS microscopy, fluorescence microscopy, optical dichroism, some other optically associated methods and approaches, NMR, X-ray scattering, as well as atomic force and electron microscopy.

Below we consider the scientific program of the “Nanobiophotonics” project in more detail.

NANOPHOTONICS:

Primary goal.

Study of physical and optical properties of two-dimensional materials (2DMs) and van der Waals heterostructures (vDWHs) such as MoS_2, WSe_2, $NbSe_2$, graphene, graphene/MoS_2, WS_2/hBN, etc.

Since the exfoliation of graphene in 2004, two-dimensional materials have been receiving great attention because of qualitative changes in their physical and chemical properties due to quantum size effect, which is related to their nanosized thickness. Atomically thin 2D transition metal dichalcogenides (TMDCs), such as MoS_2 and WSe_2, and others, exhibit strong light-matter coupling

and possess direct band gaps in the infrared and visible spectral regimes, making them potentially interesting candidates for various applications in electronics, optics and optoelectronics. They can be assembled to form heterostructures and combine the unique properties of the constituent monolayers.

Two-dimensional materials possess weak interlayer van der Waals bonds, but strong intralayer covalent bond coupling. The physics of 2DMs and van der Waals heterostructures (vdWHs) are complex, and investigation promises to bring the new physical phenomena in MoS_2, WSe_2, $NbSe_2$, graphene/MoS_2, WS_2/hBN and other nanostructures. Some of the phenomena is related to transport of 2D excitons, interaction between the various degree of freedom of quasi particles and interaction between the quasi particles with electrons, holes and defects, up-conversion luminescence, etc.

In the current project, we propose to apply the Raman spectroscopy (spontaneous and enhanced) for probing the fundamental properties of 2DMs, including number of layers, thickness, defects, doping level, and coupling of various states in the interfaces of 2DMs and vdWHs. In general, Raman spectroscopy of 2DMs and vdWHs exhibit several unique features which belongs to excitation of surface modes, spatially confined phonons, and phonons coupled with surfaces and interfaces degrees of freedom. In Raman scattering spectroscopy, the interlayer phonon modes involve vibration of interfaces where the layers can be treated as a unit cell in the linear chain model. The acoustic branch of phonon modes from the interfaces of interlayer in 2DMs and vdWHs can be also observed by Raman spectroscopy.

In this project, we propose to investigate the coupling of electrons and quasiparticles (charge and lattice degree of freedom) in 2DMs and vdWHs, which is highly sensitive to the thickness, stacking order and coupling strength of interfaces of 2D and vdWHs. The coupling of phonon with additional degree of freedoms such as electron – phonon, defect - phonon, plasmon - phonon, can also be possible to detect by Raman scattering spectroscopy in order to investigate the comprehensive mechanism of transport properties in 2DMs and vdWHs. We expect that the Raman modes must have unique energy and momentum in order to coupling between quasiparticles.

Within the project, it is proposed to carry out work which may signify the Raman modes originating from excitations of particles and quasiparticles. Furthermore, we propose interlayer and intralayer Raman modes in vdWHs, including twisted bilayer of 2DMs and vdWHs, to probe the interfacial coupling of phonon in 2D and vdWHs. This will open up the window for engineering the interface properties in order to improve the quality of various devices. This area is least understood and Raman spectroscopy may become important and proper method in this aspect.

Because of the enhanced Coulomb interactions, two-dimensional materials exhibit strong excitonic effects, resulting in many-particle phenomena covering both intralayer and interlayer excitons. Excitons are tightly bound in low-dimensional materials and dominate the optical response even at room-temperature, thus making them an attractive material for fundamental studies and various applications. In spite of the large number of studies on exciton physics in 2D semiconductors, the remarkably versatile excitonic landscape including various excitonic states has partially remained challenging to investigate. In particular, non-radiative recombination, mediated by the high defect concentration and scattering of quasiparticles with excitons are not so far investigated, the understanding of these aspects may be useful for designing the efficient optoelectronic devices.

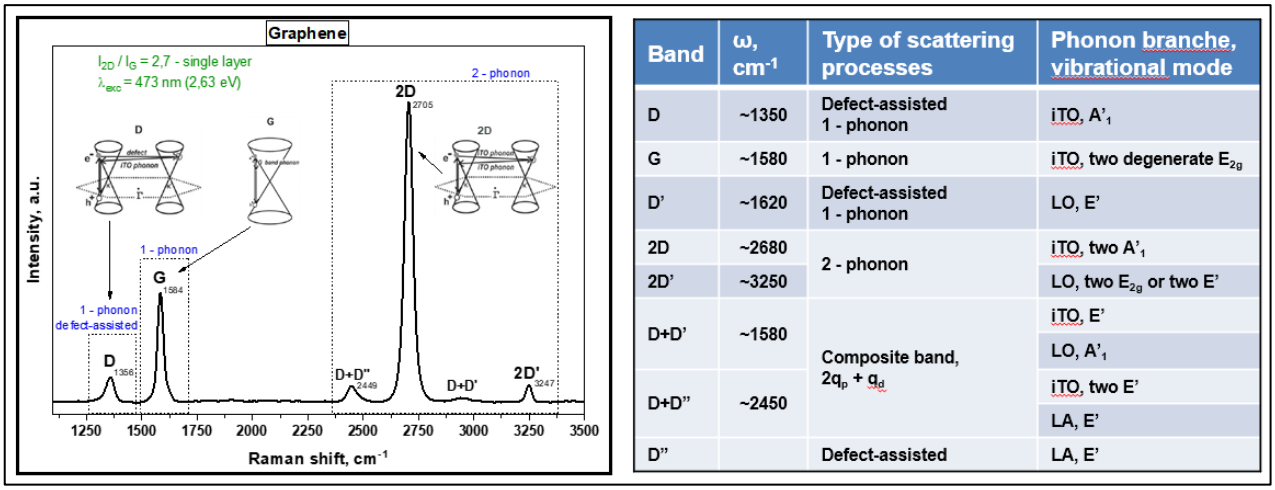
Some of the important feature of phonon modes are not detectable by the spontaneous Raman spectroscopy. However, coherent anti-Stokes Raman scattering (CARS) is a unique method for observing the comprehensive phonon behavior depending upon the thickness of the 2DMs, defects,

temperature, doping, and perturbations of the electronic states induced by several other internal and external degrees of freedom. The perturbations and coupling mentioned above are poorly understood in 2DMs and vdWHs, and we suppose that the CARS will be useful in this aspect. The application of CARS is advantageous due to stronger signals relative to spontaneous Raman spectroscopy.

The project also includes research on single- and multi-phonon assisted upconversion luminescence (UCL) in monolayers of transition metal dichalcogenides and their heterostructures. In the process of UCL, additional energy is required to promote electrons excited by low energy photons to the conduction band or defect band of the investigated materials. This additional energy can be gained from phonon absorption, multiple-photon absorption, and Auger recombination. The first two types of mechanisms will be in focus of our detailed studies. Phonon absorption is one source of energy gain for UCL and compared to upconversion by multiple-photon absorption and Auger recombination, this process is more efficient, often only requiring a continuous-wave (CW) laser excitation. In this type of UCL, an incident photon excites an electron from the ground state to a virtual or real intermediate state, followed by absorbing one or multiple phonons from the lattice to elevate this electron to the final state. In addition to phonon absorption, multiple-photon absorption can also promote electrons at virtual or real intermediate states excited by low energy photons to the final state. In this process, multiple low energy photons are absorbed simultaneously (coherent multiple-photon absorption) or sequentially (multiple-step absorption) through intermediate states.

One more item of this part of the project deals with low energy/frequency Raman measurements of interlayer coupling using three BragGrate notch filters in combination with only a single monochromator.

To conclude the description of this section of the Nanobiophotonics project, we have now carried out the first test measurements of the Raman spectra of graphene/hBn and single-layer graphene (Figures 1 and 2, respectively).



Band	ω, cm^{-1}	Type of scattering processes	Phonon branche, vibrational mode
D	~1350	Defect-assisted 1 - phonon	iTO, A'$_1$
G	~1580	1 - phonon	iTO, two degenerate E$_{2g}$
D'	~1620	Defect-assisted 1 - phonon	LO, E'
2D	~2680	2 - phonon	iTO, two A'$_1$
2D'	~3250		LO, two E$_{2g}$ or two E'
D+D'	~1580	Composite band, $2q_p + q_d$	iTO, E'
			LO, A'$_1$
D+D''	~2450		iTO, two E'
			LA, E'
D''		Defect-assisted	LA, E'

Fig.1. Raman spectrum of single-layer graphene / hBn on a substrate of 285 nm SiO_2 / Si.

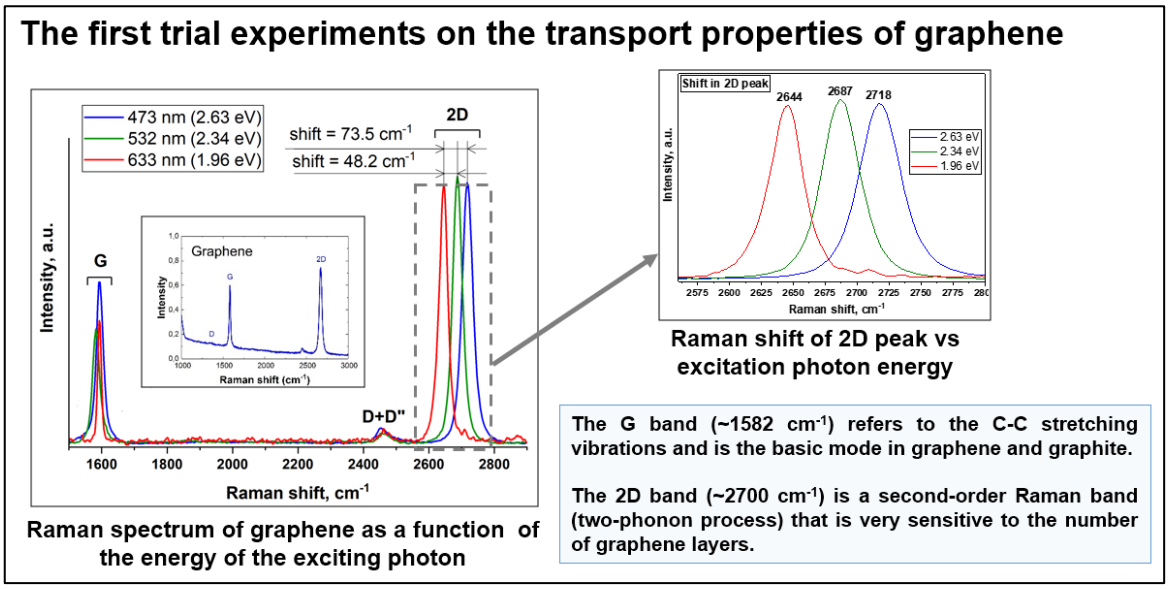


Fig.2. Raman shift of the 2D band of single-layer graphene as a function of from the energy of the exciting photon

The obtained Raman spectra, although of a preliminary nature, however, do inspire a certain enthusiasm for future Raman/optical studies of known and new low-dimensional materials.

BIOPHOTONICS (Life Sciences)

Primary goals and tasks:

1. ***Study of lipid-protein interactions in various membrane mimetics: conformational transformations and secondary structure of proteins.***
2. ***Programmed cell death – photoinduced netosis: mechanisms, signaling pathways and Raman markers***

1. Study of lipid-protein interactions in various membrane mimetics: conformational transformations and secondary structure of peptides.

The interactions of proteins and peptides with lipid membranes play a large role in maintaining the integrity and functionality of the cell membrane, and significant changes in these interactions are involved in the pathology of many diseases. Misfolding and aggregation of peptides, specifically, is involved in the development of Alzheimer's disease, although the exact relationship between the protein structure and the pathology of Alzheimer's is still unclear. Therefore, at the current stage of development of experimental and theoretical methods for the analysis of various molecular systems, the development of complex approaches to reveal the regularities of their interactions with cell membranes and to study the resulting conformational transformations and structural changes in the studied biological system is becoming increasingly required. In this respect, studies of lipid-protein interactions started about a year ago, mainly by Raman spectroscopy only, are planned to be continued in a new project in a more extended experimental format using circular dichroism and NMR spectroscopy. Computer modelling will also be an integral part of this work.

Among peptides, one of the most intriguing targets in studies of lipid-protein interactions are peptides of the beta-amyloid group (Aß), formed from the transmembrane precursor protein APP (amyloid precursor protein). Different Aβ species or intermediates can introduce modifications to lipid membranes ranging from disruption to mechanical destabilization. Among the different Aβ

species, the main component of senile plaques, Aβ(1−42), is considered to be a possible trigger of the neurodegenerative cascade, due to its cytotoxicity. Aβ consists of a polypeptide with 42 amino acids, 10 of which (segment 25–35) comprise the transmembrane segment of the APP and also comprise part of the full length Aβ peptide. Therefore, this short transmembrane segment is often used in studies of the protein interactions and partitioning in the membrane. The shorter Aβ(25−35) species is also membrane active and retains much of the Aβ(1−42) biological and neurotoxic activities.

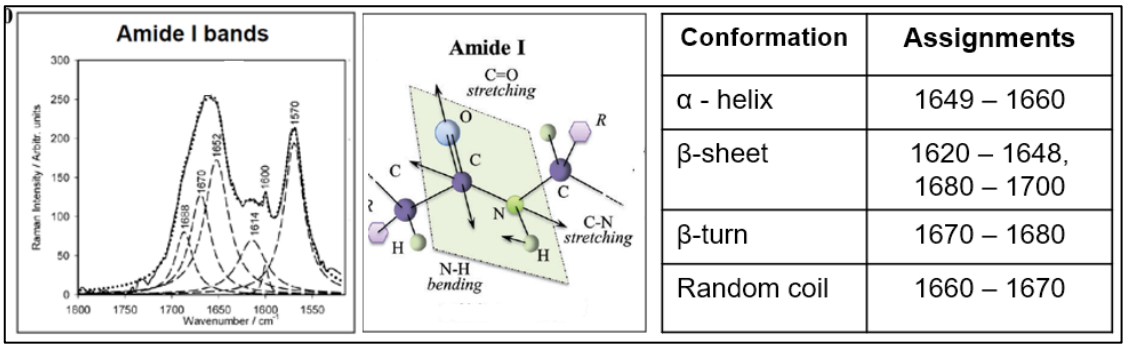
It is these two alloforms of Aβ that are scheduled for study in the proposed project, both in the lipid environment and outside it. However, this does not limit research on other proteins, such as lysozyme, which has recently also been associated with the formation of amyloid plaques leading to Alzheimer's disease.

Conformational transformations and secondary structure of proteins.

Raman spectroscopy is steadily gaining importance for the structural characterization of biological systems in general, and proteins in particular. A protein Raman spectrum is typically composed of contributions from three major types of vibrational modes originating from the polypeptide backbone (amide bands) and aromatic and non-aromatic amino acid side chain residues. The positions of the amide bands depend on the conformation of the polypeptide backbone and intra- and intermolecular hydrogen bonds. Consequently, these bands can be correlated to the protein secondary structure. The key vibrational indicators for these structures are three conformationally sensitive amide vibrations of protein backbone: amide I (1620-1700 cm^{-1}), amide II (1520-1570 cm^{-1}), and amide III (1220–1300 cm^{-1}). These bands are sensitive to secondary structure because (i) polypeptide's geometry directly affects the force constants of the amide bond and (ii) each secondary structure element type participate in hydrogen bonding of different strength. Alterations in force constants lead to differences in normal mode composition, which in turn is responsible for amide band fractions frequency shift and intensity changes. The amide I band is primarily due to C = O stretching and does not overlap with the vibrational bands of other functional groups and can be directly used for the characterization of protein secondary structure. Amide III is the most complex band and originated from various motions including C – N stretching vibration coupled with N – H in-plane bending. The amide II band is primarily related to C-N stretching, in-plane N-H bending and C-C stretching and is very weak in non-resonance Raman spectra, which causes it to be nearly undetectable. Thus, in general, amide I and amide III bands are considered to be more structure-sensitive than amide II band of proteins and peptides.

Optical spectroscopy methods, particularly raman spectroscopy and circular dichroism, have proven themselves well and effectively in the study of structural properties, aggregation analysis and identification of amyloid-β peptides. Therefore, this will be one of the key items of our detailed research in the proposed "Nanobiophotonics" project.

The main fractions of the amide I band, which differ in position and shape, are α-helix, β-sheet, β-turn and random coil. The amide I band is usually very broad and therefore requires spectral deconvolution for its detailed interpretation (Figure 3).



Conformation	Assignments
α - helix	1649 – 1660
β-sheet	1620 – 1648, 1680 – 1700
β-turn	1670 – 1680
Random coil	1660 – 1670

Fig.3. Demonstration of deconvulated amide I band of Raman spectrum (left), vibrational modes (centre), conformations and assignments (right).

Based on our preliminary measurements, Aβ aggregation is accompanied by the conversion of a structure rich in α-helices to β-sheet secondary structure characteristic of Aβ aggregates and mostly presented in fibrils – precursor to Alzheimer's disease (Fig.4).

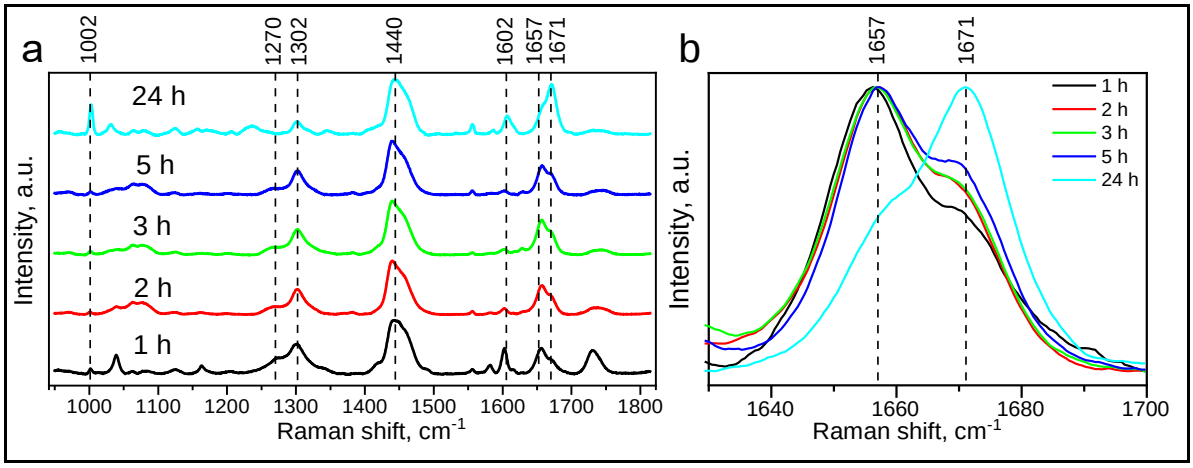
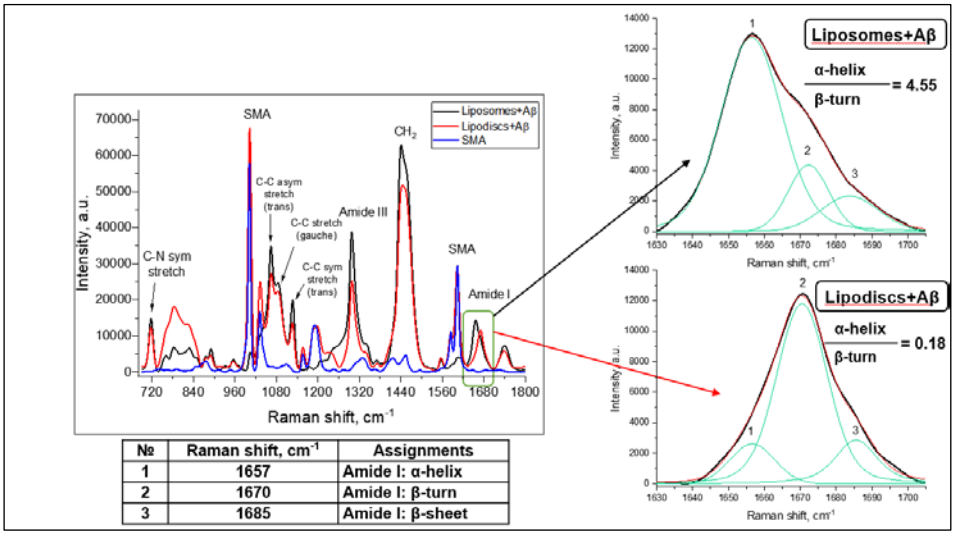


Fig.4. (a) Raman spectra of the Aβ(1−42) peptide in an aqueous solution by hours, and (b) normalized spectra of the peptide in the region of the Amide I line.

The normalized Raman frequencies in Figure 4b clearly show that during the first five hours of measurements at intervals of 1 and 2 hours, the α-helix conformation at the Raman frequency of 1657 cm-$^{-1}$ dominated in the spectrum. After 24 hours of keeping the peptide in water, the spectral weight of the 1671 cm^{-1} band noticeably increased in the spectrum, which indicates a conformational transition into a β-turn/sheet structure. The proposed project involves a detailed and in-depth investigation of the secondary structure of the peptide by Raman spectroscopy, optical dichroism and NMR spectroscopy, accompanied by computer modelling.

Further, we propose to study alterations in the secondary structure of Aβ peptides introduced in various membrane mimetics, in particular in vesicles/liposomes and lipodiscs in order to identify the most native-like composition. Here, we also have some preliminary data (Fig. 5) demonstrating the advantage of liposomes with the dominating α-helix fraction over lipodiscs.



№	Raman shift, cm^{-1}	Assignments
1	1657	Amide I: α-helix
2	1670	Amide I: β-turn
3	1685	Amide I: β-sheet

Fig.5. Comparative Raman spectra of the Aβ(1-42), peptide introduced in liposome and lipodisc

While the peptide-lipid interactions might be affected by the membrane composition, water and molecular surroundings, peptide concentration, thermodynamic phase of a lipid membrane is also among the important factors. This is because their roles in defining the structural and elasto-mechanical properties of membrane are linked to its fluidity and lipid dynamics, which in turn affect the ability of Aβ to interact with the lipid membrane. We suppose that some kind of structural reorganization may take place as a result of destruction of the lipid membrane by the Aβ peptide demonstrating its disruptive properties during the lipid main phase transition. This item has so far been poorly studied and will be also in the focus of our research program related to the section of Biophotonics.

Concluding the description of this part of the project, we note the following: at the initial stage of the project, complementary studies of lipid-protein interactions by circular dichroism and NMR spectroscopy are planned to be carried out on the experimental infrastructure of external partners.

2. Programmed cell death – photoinduced netosis: mechanisms, signaling pathways and Raman markers

Neutrophils are the most abundant leukocytes in the circulation providing the first line of host defense against pathogens. As professional phagocytes, neutrophils contain antimicrobial enzymes in their granules and fulfill effector functions such as phagocytosis, degranulation, and the formation of ROS in the inflammation foci. Neutrophils release decondensed chromatin or extracellular traps (NETs) in response to various physiological and pharmacological stimuli. The process of NETs formation leading to programmed cell death has been termed NETosis. NETosis can be activated by antibodies, cytokines, microcrystals, calcium and potassium ionophores, and also pharmacological stimuli such as phorbol 12-myristate 13-acetate (PMA). Apart from host defensive function, NETs play an essential role in the pathogenesis of various autoimmune, inflammatory, and malignant diseases.

In recent years, studies have been carried out on photo-induced NETs formation, mainly activated by UV radiation. We propose to study the mechanisms and signaling pathways of NETs release under the influence of UV, visible and IR light as it is important to control the consequences of damaging effects of electromagnetic radiation in wide range of spectrum. ***This will be the main novelty of the proposed project in terms of the study of NETosis, along with the search of spectroscopic/Raman biomarkers of this type of programmed cell death.***

Hypotesis on redox chains as primary photoacceptors of NETosis.

To induce a photobiological effect, any radiation should be absorbed by a functional chromophore/photoacceptor molecule located in some key cell structure capable of influencing its activity and homeostasis. Redox chains are an example of such a key structure. In neutrophil granulocytes, components of the mitochondrial respiratory chain, in particular, cytochrome_c oxidase and a membrane-bound heterodimeric flavohemoprotein cytochrome_b_{558}, a structural component of NADPH oxidase containing redox centers, can be considered to be an effective photoacceptors and transducers of the photo signal.

Cytochrome_b_{558} constitutes the catalytic electron transport part of NADPH oxidase and consists of two subunits: gp91phox and p22phox. The cytoplasmic tail of gp91phox subunit binds FAD (flavin adenine dinucleotide), NADPH, and the heme required for electron transfer to oxygen. Cytochrome_b_{558} is the only membrane component of phagocytic NADPH oxidase producing superoxide anion radicals ($O_2^{\cdot -}$) that can easily dismutate to hydrogen peroxide.

Cytochrome_*c* oxidase is a terminal enzyme of the aerobic respiratory chain in eukaryotic cells that mediates electron transfer from cytochrome_*c* to molecular oxygen, playing a key role in cellular bioenergetics. Cytochrome_*c* oxidase is localized in the inner mitochondrial membrane and is also known as complex IV. The photoacceptor-mediated reactivity of cytochrome_*c* oxidase is driven by four active metal redox centers: the binuclear CuA, CuB, heme *a*, and heme *a3*, which have absorption bands in a wide range from the UV to the near-IR spectral range, allowing for photobiological effects at different excitation wavelengths.

The primary ROS in activated neutrophils are superoxide anion radicals which, being weak oxidizing agents, quickly dismutate to H_2O_2. These, in turn, can undergo further processing which results in the formation of more active metabolites such as $OH\cdot$ (hydroxyl radicals) and HClO. The latter is produced in the reaction between H_2O_2 and the chlorine anion (Cl–) mediated by the enzyme of the specific granules of myeloperoxidase (MPO) in the process of oxidative burst of activated neutrophils. Approximately, up to 70% of H_2O_2 is converted under the action of MPO into HClO, (hypochlorous acid) which has strong microbicidal and cytotoxic properties.

Based on our experimental results of the last two years, we intend to validate the hypothesis of redox chains as primary photoaceptors we have put forward, in particular (i) using a wide range of different mitochondrial respiratory chain and NADPH-oxidase inhibitors, (ii) choosing light sources adapted in their wavelengths to the absorption spectrum of these two above mentioned cytochromes.

ROS measurements with Raman spectroscopy

In order to confirm the involvement of ROS in NETs formation in our experiments, we are going to use Raman spectroscopy to detect some characteristic peaks for various ROS, in particular, hydrogen peroxide at the raman frequency in the region of 875-880 cm^{-1} (O-O stretching), hypochlorous acid at the frequency of ~732 cm^{-1} and some others. Measurements will be performed preferably in the mapping mode during the first 10-15 min after neutrophils activation by LEDs at wavelengths from UV-A to near IR. Also, we intend to continue search of the citrulline low-frequency raman bands as an another signaling pathway of photoinduced NETosis.

In our opinion, of particular interest and novelty in photoinduced NETosis may be also a case when intact neutrophil cells will be irradiated simultaneously or sequentially by two different wavelengths, for example, UV-A and IR. We find this interesting due to the fact that the Sun shines

simultaneously in a wide range of the spectrum, perhaps compensating by IR radiation for the harmful effects of medium or high UV doses.

NETs visualization of photoactivated neutrophils by fluorescence microscopy

To visualize both intact and netotic cells after photoactivation, Nikon Eclipse Ts2R-FL fluorescent LED microscope will be used with NIS-Elements BR software, an Epi-FL C-LED385 filter, and a CFI Super Plan Fluor ELWD ADM 20x objective with a 0.45 numerical aperture and a working distance of (8.2-6.9) mm.

Our present results and achievements on photoinduced NETosis.

Over the past two years, we have developed a protocol for isolating neutrophils and irradiating them with LED sources at different doses. Some of the results obtained are shown in the Fig.6.

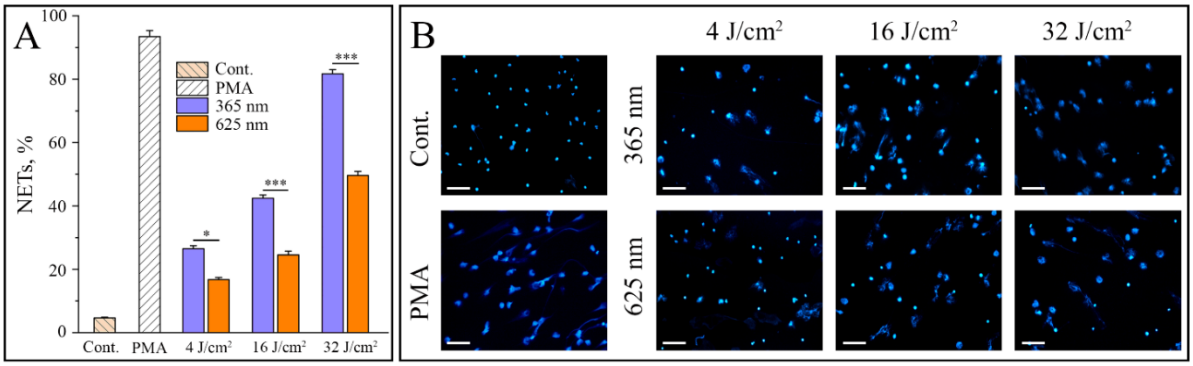


Fig.6. Release of NETs depending on irradiation doses at the wavelengths of 365 nm and 625 nm (Fig.6A). NETs formation was recorded after 3 hours of incubation at 37°C and 5% CO2 using a fluorescence microscope. Fig. 6B shows corresponding representative images of irradiated neutrophils as well as those stimulated with PMA. Scale: 50 µm (B).

Given the wavelength dependence of NETs release and considering the redox sensitivity of cytochrome_b_{558} and cytochrome_*c*, it seems likely that the observed light-induced NETosis is mediated by these cytochromes' excitation and subsequent ROS production. To test our hypothesis, we intend to use a specific inhibitors of NADPH oxidase and mitochondrial redox chain.

Here is a list of dyes, antibodies and inhibitors that to be applied in our studies: Sytox Green, Hoechst, DAPI, SYBR Green, FITC-linked polyclonal antibody to Myeloperoxidase (MPO), histone H3 (D1H2) XP, cathepsin G (E3N3O), FITC-linked polyclonal antibody to citrullinated histone H3, anti-rabbit IgG, HRP-linked Antibody 7074; and inhibitors: apocynin, GSK484, MitoTEMPO, DPI, SLPI: secretory leukocyte protease inhibitor, NSP, alfa1-proteinase inhibitor (α1PI), α1-anti-chymiotrypsin (ACT), α2-macroglobulin, monocyte neutrophil elastase inhibitor (MNEI or SerpinB1).

Methods and approaches.

"CARS" microspectrometer – the basic setup of the FLNP in optical research.

Based on the 3D scanning confocal laser microspectrometer "CARS", a multimodal optical platform is successfully operated at the FLNP, where experiments on both spontaneous raman scattering and its three amplified options are performed: (i) CARS - coherent anti-stokes raman scattering, (ii) SERS – surface-enhanced raman scattering, (iii) combination of CARS with SERS (SECARS). In addition, the platform is fully adapted for (i) luminescence measurements, including in the anti-stokes part of the spectrum (up-conversion luminescence), (ii) nonlinear imaging of bio-

objects using second harmonic, (iii) construction of chemically selective images (Raman maps), (iv) 3D sample scanning.

We briefly describe the optical platform shown schematically in Figure 7. Lasers at three wavelengths are used to excite the spontaneous Raman signal: (1) a HeNe gas laser (Melles Griot model 05-LHP-991) with an output power of 10 mW at 632.8 nm and beam divergence less than 1mrad, (2) diode-pumped laser at 532 nm with continuously variable output power up to 20 mW (model SLM-417-20), and (3) a stabilized single-mode laser at 785 nm with 100 mW output power, (model I0785SD0100B-IS-HD).

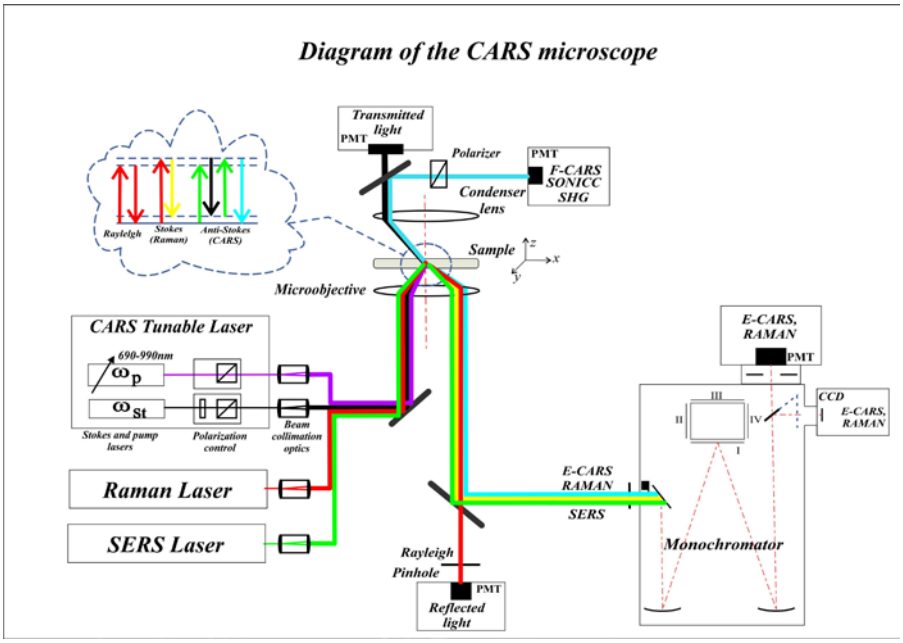


Fig.7. Optical layout of the multimodal optical platform on the base of the "CARS" microscope.

To generate CARS signals, the platform is equipped with a solid-state picosecond yttrium-vanadate laser (YVO_4: Nd) with diode pumping, operating in the passive mode locking mode. The laser was developed and manufactured by the Lithuanian company EKSPLA (model PT257 + SOPO). The output parameters of the basic laser at a wavelength of 1064 nm are: pulse duration 7 ps, average power 5W, pulse repetition rate 85 MHz. To tune the radiation wavelength in order to resonantly tune the pump wave to the molecular vibrational levels of the analyzed sample, this laser model implements a scheme for intracavity tuning of the radiation wavelength using an optical parametric oscillator (OPG) operating in the synchronously pumped optical parametric mode (SOPO). The pumping of a nonlinear LBO crystal in OPO is carried out by the second harmonic (532 nm, 6 ps, 2W) of the fundamental radiation, and the wavelength is tuned due to a change in the temperature of the LBO crystal in the range from 107°C to 148°C (non-critical in temperature phase matching) . This provides tuning of the pump wavelength in a wide spectral range from 690 nm to 990 nm. In the entire tuning spectral band, the width of the emission line is from 5 to 7 cm^{-1} with a pulse duration of 5-6 ps at typical values of the output power from 40 to 150 mW. Only a small part (about 10%) of the fundamental radiation (1064 nm) is used as the Stokes wave. The resonator also has an optical delay line controlled by a computer, which makes it possible to synchronize and coincide in time the pump wave with the Stokes wave with high accuracy. A computer-controlled XY galvanometer scanner (GSI-Lumonics VM1000) provided a fast scan of the sample in the lateral focal plane of the objective.

Using such optical platform, a sample can be spectrally imaged in the range of (1000−3580) cm^{-1} wavenumbers, which covers all most important vibrational modes of bio-molecules. Five detection channels allow two forward- and three backward- propagated signals to be recorded. The polarization control is adjustable with a half-wave plate in the Stokes beam.

Expected results

1. **Physical and optical properties of 2D materials and van der Waals heterostructures (vdWHs) such as: MoS_2, WSe_2, $NbSe_2$, graphene/MoS_2 and WS_2/hBN by Raman spectroscopy and upconversion luminescence methods:**
   - *study of fundamental, resonance, interlayer, and defect-induced vibrational modes;*
   - *study of the dispersion dependence of individual peaks (D, 2D, etc.) of the Raman spectrum of 2DMs and vdWHs in the Stokes and/or anti-Stokes regions on:*
     - *pump photon energy,*
     - *laser excitation power.*
   - *study of exciton-phonon interaction, transport properties of excitons;*
   - *characterization of up-conversion luminescence (UCL) under single- and multi-phonon absorption in various 2DM and vdWHs;*
   - *investigation of the transport properties of UCL depending on the excitation wavelength;*
   - *revealing the characteristics of the temperature dependence of the UCL depending on the power of the exciting radiation;*
2. **Lipid-protein interactions:**
   - *synthesis of various membrane mimetics and study of their structural and optical properties (continuation of the started works);*
   - *study and detailed analysis of the secondary structure of proteins introduced in membrane mimetics using Raman spectroscopy (primarily amide bands of the spectrum), circular dichroism, including temperature dependence;*
   - *study of conformational transformations in lipid-protein structures, including temperature dependencies;*
   - *modeling of lipid-protein interactions using molecular dynamics (MD) and density functional theory (DFT) methods.*
3. **Spectroscopy and microscopy of programmed cell death: NETosis:**
   - *identification of primary photoacceptors of photoinduced NETosis under UV, visible and IR radiation;*
   - *further search and identification of spectral markers of netosis;*
   - *Identification of the features of simultaneous and sequential exposure of intact neutrophil cells to radiation from two light sources at different wavelengths;*
   - *application of immunofluorescence microscopy in studies of programmed cell death.*

Risks

The main risk is associated with periodic, once every two years, professional servicing of the picosecond laser (Ekspla, Lithuania) incorporated into the “CARS” microspectrometer. However, starting from the last year, 2022, we have practically eliminated this risk: the servicing of this laser can be carried out on a contractual basis by a Russian company “Promenergolab” Ltd. The first experience with this company has already taken place and has been successful.

2.3 Estimated completion date

2024-2027

2.4 Participating JINR laboratories

- Frank Laboratory of Neutron Physics:
  Balasiou Maria
- Meshcheryakov Laboratory of Information Technologies:
  Streltsova Oksana
- Laboratory of Radiation Biology:
  Dushanov Ermuhammad

2.4.1 MICC resource requirements

Computing resources	Distribution by year			
	2024	2025	2026	2027
Data storage (TB) - EOS - Tapes				
Tier 1 (CPU core hours)				
Tier 2 (CPU core hours)				
SC Govorun (CPU core hours) - CPU - GPU	300	300	300	300
Clouds (CPU cores)				

2.5. Participating countries, scientific and educational organizations

Organization	Country	City	Participants	Type of agreement
Yerevan State University, YSU	Armenia	Yerevan	Lalayan A.A.	Joint works
Belarusian State University of Informatics and Radioelectronics	Belarus	Minsk	Bandarenko A.V., Zavatsky S.A.	Joint works
Institute of Physical Research, National Research Center	Egypt	Cairo	Medhat A. A. Ibrahim	Protocol
Mizoram University	India	Aizawl	Muthukumaran Bose	Joint works
Atomic Research Center named after Indira Gandhi	India	Kalpakamm	Kumar Niranjan	Joint works
Institute of Nuclear Physics	Kazakhstan	Almaty	Nazarov Kuanysh	Joint works

Center for Advanced Studies, CEAS	Cuba	Havana	Paes Amira	Joint works
Moscow State University	Russia	Moscow	Vorobyova N.V.	Joint works
Institute of High-Tech Technologies and Advanced Materials, FEFU	Russia	Vladivostok	Golik S.S.	Joint works
Pavlov University	Russia	Saint Petersburg	Moiseev A.A.	Joint works
University of Belgrade	Serbia	Belgrade	Milojevic-Rakic Maya, Jevremović Anka	Joint works
Jizzakh Branch of the Mirzo Ulugbek National University of Uzbekistan	Uzbekistan	Jizzakh	Uralov A.I.	Protocol

2.6. Key partners *(those collaborators whose financial, infrastructural participation is substantial for the implementation of the research program. An example is JINR's participation in the LHC experiments at CERN).*

- SOL Instruments LLC, Minsk, Republic of Belarus.
- Belarusian State University of Informatics and Radioelectronics, Minsk, Republic of Belarus.
- Rusgrafen LLC, Moscow, Russia.
- Atomic Research Center named after Indira Gandhi, Kalpakkam, India.

3. Manpower

3.1. Manpower needs in the first year of implementation

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

3.2. Available manpower

3.2.1. JINR staff

No.	Category of personnel	Full name	Division	Position
1.	research scientists	Arzumanyan Grigory Makichevich	Sector of Raman spectroscopy	Head of the Sector of Raman Spectroscopy
		Mamatkulov Kahramon Ziyadullayevich	Group of nonlinear microspectroscopy	Head of the Group of nonlinear microspectroscopy
		Arynbek Yersultan	Group of nonlinear microspectroscopy	junior researcher
2.	engineers	Melkova Irina Nikolaevna	Sector of Raman spectroscopy	engineer
		Morkovnikov Ivan Alekseevich	Group of nonlinear microspectroscopy	software engineer
3.	specialists	Damir Aizhan	Group of nonlinear microspectroscopy	laboratory assistant
		Demina Ekaterina Mikhailovna	Group of nonlinear microspectroscopy	senior lab assistant
		Zakrytnaya Darya Sergeevna	Group of nonlinear microspectroscopy	laboratory assistant
		Shutikov Artyom Alexandrovich	Group of nonlinear microspectroscopy	laboratory assistant
		Geronina Anna Alexandrovna	Sector of Raman spectroscopy	laboratory assistant
		Kisina Alyona Dmitrievna	Sector of Raman spectroscopy	laboratory assistant
		Pugachevskaya Irina Mikhailovna	Sector of Raman spectroscopy	secretary-referent
4.	technicians	-	-	-

3.2.2. JINR associated personnel

No.	Category of personnel	Partner organization	Amount of FTE
1.	research scientists		
2.	engineers		
3.	specialists		
4.	technicians		
	Total:		

4. Financing

4.1 Total estimated cost of the project

The total cost estimate of the project (for the whole period of 4 years: 2024-2027, excluding salary).
814,00 k$

4.2 Extra funding sources

Expected funding from partners/customers – a total estimate.

Project leaders

Arzumanyan G.M. _____

Mamatkulov K.Z. _____

Date of submission of the project to the Chief Scientific Secretary: _____

Date of decision of the laboratory's STC: 29.03.2023 document number: _____

Year of the project start: **2024**

Proposed schedule and resource request for the Project

Expenditures, resources, funding sources			Cost (thousands of US dollars)/ Resource requirements	Cost/Resources, distribution by years			
				2024	2025	2026	2027
		International cooperation	92	23	23	23	23
		Materials	214	46	51	56	61
		Equipment, Third-party company services	452	98	110	115	129
		R&D contracts with other research organizations	32	8	8	8	8
		Scientific and information support	16	4	4	4	4
		Service costs (*planned in case of direct project affiliation)*	8	4		4	
Resources required	**Standard hours**	Resources					
		– the amount of FTE,					
		– CARS microscope	6000	1500	1500	1500	1500
		– reactor					
Sources of funding	**JINR Budget**	JINR budget *(4, 5, 6, 10, 11, 14)*	**814**	183	196	210	225
	Extra fudning (supplementary estimates)	Contributions by partners Funds under contracts with customers Other sources of funding					

Project Leaders: Arzumanyan G.M. _____

Mamatkulov K.Z. _____

Laboratory Economist Sorokina J.V. _____

APPROVAL SHEET FOR PROJECT

NANOBIOPHOTONICS

PROJECT CODE

THEME CODE

NAME OF THE PROJECT LEADERS

G.M. Arzumanyan, K.Z. Mamatkulov

AGREED

JINR VICE-DIRECTOR	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF SCIENTIFIC SECRETARY	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF ENGINEER	_____ SIGNATURE	_____ NAME	_____ DATE
LABORATORY DIRECTOR	_____ SIGNATURE	_____ NAME	_____ DATE
CHIEF LABORATORY ENGINEER	_____ SIGNATURE	_____ NAME	_____ DATE
LABORATORY SCIENTIFIC SECRETARY	_____ SIGNATURE	_____ NAME	_____ DATE
THEME LEADERS	_____ SIGNATURE	_____ NAME	_____ DATE
	_____ SIGNATURE	_____ NAME	_____ DATE
PROJECT LEADERS	_____ SIGNATURE	_____ NAME	_____ DATE
	_____ SIGNATURE	_____ NAME	_____ DATE
APPROVED BY THE PAC	_____ SIGNATURE	_____ NAME	_____ DATE