GENERAL INFORMATION

1. NAME OF THE CENTER AND LOCATION	
	Institute of Organic Chemistry with Centre of Phytochemistry; *The Stephan Angeloff Institute of Microbiology* *Bulgarian Academy of Sciences* *"Acad. Georgi Bonchev" str.* *1113 Sofia,* ***Bulgaria*** *Biophotonics laboratory of Institute of Electronics,* *Bulgarian Academy of Sciences* *1784 Sofia,* ***Bulgaria*** *Laser biospectroscopy laboratory of Prokhorov General Physics Institute of the Russian Academy of Sciences* *119991, Moscow,* ***Russia***

2. TYPE OF THE RESEARCH INFRASTRUCTURE AND/OR SCIENTIFIC EXPERTISE	
Identify the type of the RI, equipment/facilities/ specific research, and in particular linked to COVID-19:	*Scientific equipment for biomedical and pharmaceutical research & development available in the Institutes above under the Bulgarian Academy of Sciences:* The apparatus for chemical characterizations of new photosensitizers, all available in the Institute of Organic Chemistry with Centre of Phytochemistry:Perkin Elmer Spectrum 100 spectrometer (4000-600 cm^{-1}) for FT-IR spectra; UV-Vis Jasco spectrophotometer Model VA570 for absorption spectra; ^{1}H NMR spectra recorded on Bruker 600 MHz spectrometer in DMSO-d_6 solutions for structural characterization. Infrastructure available in the Stephan Angeloff Institute of Microbiology for estimation the effects of PDT on disease-causing (pathogenic) microorganisms - bacteria and viruses. 1. Cell Culture Laboratory for Cytotoxicity and Signal Transduction equipped with Laminar Air Flow Cabinet BSL2 (BIO II Advance, Telstar), CO_2 incubator (MCO-18AC-PE), ELISA-Reader (EL x 800, BIO-TEK) and centrifuge (Z206A, HERMLE). The Laboratory has the following cell lines for evaluation of cell toxicity and cultivation of viruses: CCL-1 (normal transformed mouse fibroblasts) and HEK293 (normal human transformed embryonic fibroblasts) for general in vitro cytotoxicity), HEP-G2 (human hepatocytes) for liver metabolism and toxicity, HGF (human gingival fibroblasts) for oral cytotoxicity, hTCEpi (human transformed corneal cells) for ocular toxicity, VERO CCL-81 (*Cercopithecus aethiops* epithelial kidney cells), MDCK CCL-34 (*Canis familiaris* kidney cells) which will be useful for testing of antiviral activity and general cytotoxicity of potential antiviral agents. 2. Molecular biology laboratory equipped with qPCR (CFX96 Touch Real-Time PCR Detection System), ddPCR (droplet digital PCR system, IVD, QX200), DGGE machine, conventional PCR machines, centrifuges (e.g. cooling), etc. 3. Microbiological laboratory equipped with BD Phoenix 50 system for biochemical identification of microorganisms, Flowcytometer BD Accuri C6 Plus, Microscopic configuration for light and fluorescence microscopy: Nikon Eclipse Ci-L, conventional and CO_2/O_2-incubator (MCO-19M-PE, Panasonic), Laminar Air Flow Cabinets BSL2 (Faster), System for

	Western blotting analysis of protein expression (BioRad), etc. 4. Animal house for laboratory (mice, rats, guinea pigs) and domestic animals (e.g. birds). The set-up for photo-inactivation studies on pathogens: equipped with two light sources used for physicochemical experiments. It is consisting of two LED bulbs with wavelength at 365 nm and at 665 nm with output intensity up to 60 mW cm^{-2}. The light dose of 50 J cm^{-2} is collected during irradiation of 15 min. There are fiber optics with specifically elaborated spectrophotometer on the basis of Ocean Optics QE 65000 spectrophotometer with Spectra Suite Software. Fluorescence spectra recorded with an apparatus Perkin Elmer LS 55 Luminescence Spectrometer. Fluorescence lifetimes were recorded on a time correlated single photon counting (TCSPC) method using FLUOROLOG-3 fluorometer (Horiba Jobin Yvon, Edison, NJ) equipped with a NanoLED and a standard air cooler (R928PMT detector). The equipment has a computer system with software configured for this measurement. The photoinactivation equipment for irradiation: LEDs at 635 nm and 665 nm, generating a fluent rate up to 100 mW/cm^2. Scientific equipment for biomedical and pharmaceutical research & development including spectrophotometric and spectrofluorimetric equipment for evaluation of photosensitizers and their absorption, transmission and fluorescence properties. Optical and laser equipment for photodynamic treatment of bacterial strains and viruses, including light systems at 405 nm, 635 nm, 660 nm, 670-700 nm with high power laser and LED sources used in combination with photosensitizers. Engineering unit for a development of PDT (photodynamic therapy) and PDI (photodynamic inactivation) light systems for direct treatment of infections and indirect treatment of the clinical environment, medical instrumentation and materials. LSM-710-NLO laser scanning microscope (Carl Zeiss, Germany) in combination with a Chameleon Ultra II laser multiphoton femtosecond tunable in the range 680-1080 nm (Coherent, USA). Fiber-optic spectrometer for recording the fluorescence and absorption spectra in the range of 400-1100 nm (BIOSPEC, Russia) for local spectral-fluorescence studies, Hitachi spectrophotometer (350-1500 nm), Continuous-wave lasers with input into an optical fiber to excite fluorescence, initiate photochemical reactions in biological tissues and conduct PDT with different wavelengths and output power levels: 510 and 570 nm (1 W); 532 nm (10 mW); 628 nm (1 W); 633 nm (60 mW); 669 nm (1 W), 675 nm (2 W); 805 nm (30 W), 970 nm (13 W); 974 nm (3.5 W). LED light sources for photodynamic therapy (wavelengths of 670 nm and 760 nm, power density 40-60 mW/cm2, power up to 2W) Streak camera HAMAMATSU C9300, streak scope HAMAMATSU C10627) KEY WORDS: photodynamic therapy; photodynamic inactivation; photosensitizer; spectral analyses; light sources; pathogenic microorganisms; infection diseases
3. TYPE OF THE RESEARCH	
Provide information on the research carried on or planned in regard	Expertise in research and development of photosensitizers for biomedical usage, including viruses; Application of the photodynamic therapy (PDT) method with phenothiazines, porphyrins and phthalocyanines against pathogens, photodynamic inactivation, diagnosis by fluorescence detection, enveloped and naked viruses; Along with the development of contemporary potent antiviral chemotherapeutic

with COVID-19 and <u>other viruses</u>	drugs it has for a while discouraged wider clinical application of photodynamic sensitizers in antiviral therapy. The emergence of resistant and even drug-dependent viral progeny after receiving antiviral therapy imposes the search of alternative methods for treating and confining the spread of viral infections, both in terms of therapy and in terms of sterilization of transfusion products. The photoinactivation efficiencies of water-soluble and cationic phthalocyanine complexes (MPcs) on enveloped and non-enveloped, either DNA or RNA viruses are reported in our publications [1,2]. The studied viruses are belonging to different taxonomic families and representing important human and animal pathogens. The inactivation capacity of the new MPcs were compared to the commercial photosensitizer Haematoporphyrin derivative (HpD) and Methylene blue. The team has long term expertise in the method named Antimicrobial photodynamic therapy (aPDT) which has been clinically world wide approved in treating skin and mucous viral lesions caused by herpes viruses, in blood product sterilization and in case of emergency. The scientists have not jet expertise in the COVID-19 treatment with the method Photodynamic Inactivation.
4. WEBSITE	
Provide the internet address:	**<u>http://www.orgchm.bas.bg/index_en.html</u>** ***<u>http://www.ncbp.ie-bas.org/indexEng.htm</u>*** **<u>http://ie-bas.org/ie_Eng.htm</u>**
5. BACKGROUND, PUBLICATIONS AND OPEN DATA REPOSITORY	
leading research team AND Scientific publications of the research group on the topics of related to coronaviruses research results**;** **link to open data repository**	**Background**: Poliovirus type 1 (PV-1) (strain LSc-2ab) of the *Picornaviridae* (non-enveloped, single stranded +RNA), bovine viral diarrhea virus (BVDV) (strain TVM) of the *Flaviviridae* (enveloped, single stranded +RNA), influenza virus A/Aichi/2/68(H3N2) of the *Orthomyxoviridae* (enveloped, single stranded – RNA) and human adenovirus 5 (HAdV-5) of the *Adenoviridae* (non-enveloped, double stranded DNA) were tested. PV-1 and HAdV-5 were propagated in FL cell line, BVDV – in continuous calf trachea cell line, and influenza virus – in MDCK cell line. Cells and viruses were from the cell culture collection of the Stephan Angeloff Institute of the Bulgarian Academy of Sciences, Sofia, Bulgaria. Within the frame of FP5 international project (teams from six academic institutions from Germany, Sweden, Finland, Spain and Bulgaria) was proposed novel mucosal vaccination approache by using efficient bacterial live carrier vaccines developed by rational design ard genetic engineering. Enteric *Yersinia* spp. were attenuated by targeted disruption of genes (*sodA,* R2, *wzz, wbc, yopK* and *ypkA*) encoding virulence factors. Taking advantage of the type III protein secretion apparatus of *Yersinia*, heterologous model antigens (ovalbumin, OVA;

haemagglutinin, HA) had been fused to translocated *Yersinia* Yops to target the antigens to MHC class I or II antigen processing pathways in host cells. Immune responses and effector mechanisms induced by these vaccines were evaluated in a mouse and pig infection models. Furthermore, antigens of classical swine fever virus were used and immune responses were analyzed in a swine model. The combined efforts and highly complementary expertise allowed the development of a new generation of live carrier vaccines with economic impact primarily for veterinary medicine, and thus increasing the effectiveness of the measures against the classical swine fever.

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	Publications of V. Mantareva, Assoc. Prof.: https://scholar.google.bg/citations?hl=bg&user=or5sCLUAAAAJ
6. COORDINATOR	
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8. IMPLEMENTED AND RUNNING PROJECTS

Projects related to infections:	*1. Phthalocyanine photosensitizers towards microbial resistance, KP-06-H29, NSF-Bulgaria, project term (2018-2021).* *2. Project under the 5th Framework Program of the European Union - No QLK2-1999-00780 (1999-2002) titled "Development of new live vaccines by targeted attenuation of Yersinia: genetic engineering and immunological evaluation"Project under the 5th Framework Program of the European Union - No QLK2-1999-00780 (1999-2002) titled "Development of new live vaccines by targeted attenuation of Yersinia: genetic engineering and immunological evaluation".*
Projects related to methods&eguipment for studies of biotissues:	*3. State Contract of the Russian Ministry of Education and Science No 14.N08.11.0062 Preclinical studies of a cationic infrared photosensitizer based on bacteriochlorophyll A for antimicrobial photodynamic therapy, project term (2015 – 2017).* *4. State Contract of the Russian Ministry of Education and Science* project term *No14.N08.12.0092* *5. Preclinical studies of a drug - photosensitizer for the treatment of purulent wounds caused by antibiotic-resistant strains of bacteria project term. No14.N08.12.0092 project term (2016 – 2018).* *6. Effect of structural factors of photosensitizers on the efficiency of photodynamic inactivation of bacterial biofilms, No 15-04-04363, Russian Foundation for Basic Research, project term (2015-2017).* *7. Project RFBR No17-07-01568 Methods of recording and analyzing digital hyperspectral holograms of biomedical objects, RFBR No17-07-01568, project term (2017-2019).* *8. Project RFBR No 18-08-01112. Software and hardware complex for spectral-fluorescent examination of the content of fluorochromes in biological tissues with significantly different optical properties.RFBR 18-08-01112, project term (2017-2019).* *9. Project RSF No18-19004502.Hyperspectral holography of biological objects in incoherent light. RSF No18-19004502, project term (2018-2020)* *JOINT PROJECT APPLICATION* *10. Long-wavelength charged photosensitizers for photodynamic therapy of infected lesions, including caused by deeply invasive pathogens (ERA-NET RUS).*