SARS-CoV-2 and other coronaviruses: innovative approaches of advanced characterization based on large machines of physics

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Abstract

The novel coronavirus SARS-CoV-2, the pathogen responsible for the rapidly growing outbreak of coronavirus disease (COVID-19), is causing widespread concerns and fear, due to its extremely high contagiousness and the potential risk of developing pneumonia. Global research efforts have been devoted to investigating the molecular mechanism of virus pathogenesis, in order to develop effective drugs or vaccines to tackle the on-going pandemic. Several experiments related to SARS-CoV-2 proteins or for testing potential drugs for the treatment of viral infections are currently being carried out. In the present review, innovative approaches for advanced characterization based on large machines of physics, making use of Synchrotron radiation and Neutron beams, are discussed.

Keywords: SARS-CoV-2; COVID-19; Synchrotron radiation; Neutron techniques

SARS-CoV-2 ve diğer koronavirüsler: büyük fizik makinelerine dayalı gelişmiş karakterizasyonun yenilikçi yaklaşımları

Öz

Hızla büyüyen koronavirüs hastalığı (COVID-19) salgınından sorumlu patojen olan yeni koronavirüs SARS-CoV-2, son derece yüksek bulaşıcılığı ve potansiyel pnömoni geliştirme riski nedeniyle yaygın endişelere ve korkuya neden oluyor. Küresel araştırma çabaları, devam eden pandemi ile mücadele etmek için etkili ilaçlar veya aşılar geliştirmek için virüs patogenezinin moleküler mekanizmasını araştırmaya adanmıştır. SARS-CoV-2 proteinleriyle ilgili veya viral enfeksiyonların tedavisi için potansiyel ilaçların test edilmesiyle ilgili çeşitli deneyler şu anda yürütülmektedir. Bu derlemede, Synchrotron radyasyonu ve Nötron ışınlarını kullanan büyük fizik makinelerine dayalı ileri karakterizasyon için yenilikçi yaklaşımlar tartışılmaktadır.

Anahtar Kelimeler: SARS-CoV-2; COVID-19; Synchrotron radyasyonu; Nötron teknikleri.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus causing the coronavirus disease 2019 (COVID-19), was decoded closely after its detection and its complete genome sequence was made available online. Such sequence, are at the origin of the kits employed to test for COVID-19. The sequencing correspondingly allowed expression of several of the proteins that make up the virus.

The spherical envelope of COVID-12 contains positive-sense single-stranded RNA. Its associated nucleoprotein is within a capsid composed of matrix protein. The envelope is studded with club-shaped glycoprotein projections, as shown in Figure 1.

Figure 1. Generic Coronavirus Model

After the discovery of SARS-CoV-2, the 3D structural information of these proteins was made available to accelerate pharmaceutical research, with the main aim to find appropriate inhibitors to block vital functions of the virus, thus impeding its reproduction.

In recent decades, scientific progress has provided unprecedented information on the molecular bases of diseases and enabled new targets for drug discovery to be identified. In the majority of cases, this knowledge has not been transformed into new therapies, due to the limitations of drug research and development. This represents a costly, time-consuming and inefficient process that takes up to 15 years to transfer a new drug to the market, with an average cost of USD 2.5 billion and a high risk of failure. Only 10% of compounds entering Phase 1 clinical development reach the next stage and the failure rate in Phases 2 and 3 is around 50%, unchanged for over two decades. Half of the failures are due to lack of efficacy, an expression of the difficulty in selecting the right target for the disease under study.

There is an urgent need to screen selected molecules in short time in order to reduce the timing on drug discovery. Modern analytical tools, such as synchrotron X-ray radiation and neutron techniques, are essential for key insights into virus morphology and function..

2. Advanced Characterization

2.1. Synchrotron radiation technique

A synchrotron is a large circular accelerator of charged particles, usually electrons, that produces light with extraordinary properties. It is based on a circular accelerator of charged particles, generally electrons, that move at a speed close to that of light (300,000 km/s) and with so much energy that they reach the order of GeV (1 GeV = 1,000,000,000 eV). These machines are capable of producing high-intensity, polarized, pulsed electromagnetic radiation with wavelengths ranging from X-rays to infrared light. It is well established that every charged particle that is accelerated produces electromagnetic radiation and it is an extensively studied and quite applied phenomenon in today's society. Everyday examples of this are the antennas, which produce radio frequency waves thanks to the accelerated movement of electrons in a conductive wire. Or the magnetron from the microwaves used to heat our food. Synchrotron light is produced when centripetal acceleration is applied to an electron beam (Figure 2a). When a metal is heated, the electrons inside are excited enough to escape from the surface in a process known as thermionic emission. Once the electrons are released, they are directed by the linear accelerator or LINAC. This fits into the Booster ring, which uses magnetic fields to force the electrons to travel in a circle. Here microwaves are used to add even more energy to the electrons. However, it is in the Booster ring that the radiation is actually produced. This ring is not completely circular, it is more like a multi-sided shape (similar an octagon). A charged particle in motion subjected to a magnetic field perpendicular to its trajectory experiences a force. If the field is constant, the particle describes a circular motion (Figure 2b). A series of electromagnetic devices (dipole and wave) are placed around the storage ring, causing the beam to curve or wave in a winding path, and this is where the key effect takes place: every time the beam passes through a corner with these magnets, the electrons lose energy, which is released in the form of light. These magnets are adjustable. If the intensity of the magnetic field increases, the forces in the electron stream increase, creating tighter curves along its path. This change in the curve produces a modification in the wavelength of the emitted radiation. A tight curve produces short wavelength radiation, like X-rays. While smooth curves produce longer wavelength radiation, such as infrared [2].

Synchrotron light offers also the advantage of covering a wide range of frequencies through the electromagnetic spectrum from the infrared, through the ultraviolet, to the X-ray region, allowing also time-resolved experiments in the sub-nanosecond range [1,2].

Synchrotron is an exceptionally powerful source of hard X-rays, which are created by high energy electrons as they are forced to accelerate around the synchrotron curved trajectories. These X-rays are electromagnetic waves as visible light, situated at the high energy/short wavelength end of the electromagnetic spectrum, between ultraviolet light and γ -rays. Their wavelengths of 0.10 to 0.01 nanometres are comparable to interatomic distances, thus they are suitable for the study of atoms and bonds. Advanced imaging technologies based on Synchrotron Radiation (also known as "Magnetobremstrahlung Radiation") are available in different European Facilities - e.g., the European Synchrotron Radiation Facility (ESRF) (Grenoble, France). They possess exceptional properties that make them very interesting for studies of all kinds of chemical, biological and archaeological structures, as well as for medical and industrial applications. Here follow some example of chemical application for the analysis of chemical elements, proteins and their structures, as well as organic and inorganic chemical processes, in medicine for the development of less invasive and more precise imaging and diagnostic techniques, allowing for a lower net exposure to traditional X-ray radiation. It can also be applied in the development of therapies, such as the MRT (microbeam synchrotron therapy), that can be used for the treatment of tumours that are currently inoperable. Furthermore, these technologies can be employed in biology, in the field of microscopy, since synchrotron light provides high-resolution images of cells in more detailed conditions than those offered by conventional techniques. This has allowed us to understand mysteries of the human body and the mechanisms of life that were previously impossible to observe.

In this view, several structural biology experiments were performed at the ESRF in the last 17 years. A first experiment (awarded with Nobel Prize) to be mentioned is the determination of the structure, at atomic level, of the "large subunit" of ribosome (one of the most complex mechanisms of the cell) from *Alloarcu* Marismortui and of the "small subunit" from *Thermus Thermophilus* [3]. The race to produce new antibiotics to fight bacteria, which become progressively resistant, is well known. Researchers from various pharmaceutical companies use knowledge of the ribosome structure to understand the action mechanisms of antibiotics and develop new drugs. The diffraction of X-rays generated by Synchrotron has allowed, for instance, the investigation of antibiotic molecules binding to the small subunit of a bacterium's ribosome.

Figure 2. Synchrotron radiation technique: (a) schematic representation of a synchrotron; (b) a charged particle in motion subjected to a magnetic field perpendicular to its trajectory experiences a force. If the field is constant, the particle describes a circular motion. Modified from *Fundamentos de la radiación de sincrotrón y algunas de sus aplicaciones*. Available online: <https://soloesciencia.com/2019/09/19/fundamentos-de-la-radiacion-de-sincrotron-y-algunas-de-sus-aplicaciones/> (accessed on 28 September 2021).

Membrane proteins play a fundamental role in every living thing. Synchrotron radiation (e.g., at ESRF) helps the scientific community to progressively solve an ever increasing number of structures of membrane proteins (more difficult to be elucidated than other types of proteins), composed of a hydrophobic part and a hydrophilic one. The expression, purification and crystallization of these proteins are fundamental research objectives. Many of the already investigated proteins come from the *Escherichia Coli* bacterium and carry molecules into the cells, but in a number of pathologies this transport is altered. Cystic fibrosis, indeed is caused by a malfunction of membrane proteins, which are expected to transport chlorine into certain gland cells, including those that secrete mucus. Similarly, Crohn's disease, which is still incurable, is caused by a defective transport of the important nutrient carnitine inside the cells. Synchrotron has allowed the structure determination of the carnitine membrane transport protein, CaiT, which forms trimmers in the membrane, consisting of identical monomers [4].

Other experiments can be mentioned, including the elucidation of the structure of G-protein-coupled receptors [5]; structural and mechanistic studies of ion channels in cell membranes [6]; experiments related to the area of neurodegenerative diseases and their therapies based on the use of stem cells [7].

The knowledge of the ribosome's structure is exploited to understand the action mechanisms of the antibiotics and to develop new drugs: a molecule of antibiotic, indeed, is connected to the small ribosome subunit of a bacterium [1].

A remarkable progress is currently being made by synchrotron X-ray facilities to map and solve the SARS-CoV-2 structure, as a first step towards the development of drugs and vaccines.

The first target is to know the atomic structure of this virus. The 3D architecture offers concrete starting points for developing active substances or inhibitors. These drugs could specifically dock to target points of the macromolecule and impede its function [8].

A 3D visualisation of the building blocks of such structure at an atomic level would allow to understand the SARS-CoV-2 functions, and the molecular machines that let the virus to replicate, the enzymes, which are crucial to this development. A drug that binds to the active site of the enzyme would throw a chemical spanner in the works, impeding the virus' aptitude to reproduce and reducing the spread of the disease [9].

The X-ray structures of the unliganded SARS-CoV-2 main protease (namely the enzyme which constitutes a possible target of antiviral drugs, because it is essential for processing the polypeptides that are translated from the viral RNA) and its complex with an α -ketoamide inhibitor have been just reported [10,11] (Figure 3). This derives from a formerly designed inhibitor, with the P3-P2 amide bond incorporated into a pyridone ring in order to enhance the half-life of the compound in plasma. The lead compound has

been developed, consequently, into a strong inhibitor of the considered virus, whose pharmacokinetic characterization has revealed a definite lung tropism and suitability for inhalation route of administration. The X-ray diffraction data sets were collected using synchrotron radiation of wavelength 0.9184 Å at the beamline BL14.2 of BESSY (Berlin, Germany) [11], at the Synchrotrons of PSI (Villigen, Switzerland) and at the Synchrotron DESY (Hamburg, Germany).

Figure 3. Three-dimensional structure of SARS-CoV-2 main protease (Mpro) in two different views. Figure from Zhang et al. [11].

The same technique of X-Ray diffraction was used [12] at the advanced Photon Source of the Argonne National Laboratory (Chicago, USA) to understand the first steps of infection mechanism of SARS-CoV-2 receptor-binding domain in complex with the human cell receptor, namely angiotensin-converting enzyme 2 (ACE2).

These results allowed the elucidation of the structural and biochemical mechanism of the virus's receptor recognition mechanism, which constitutes a fundamental target for antiviral strategies including vaccination.

A similar experiment [13] was performed at the Shanghai Synchrotron Research Facility (China).

Hilgenfeld, R. et al on their review [14] published in 2013 analysed 10 years of research on highly pathogenic human coronaviruses, including structures and functions of individual proteins and their complexes, together with coronavirus drug discovery and development [15].

2.2. Neutron techniques

Neutron methods constitute an essential and unique part of the science tool kit for exploiting information about the properties and behaviour of matter at the atomic and molecular level, which is indispensable to design novel drugs, materials, devices, and solve complex problems. Neutrons, as subatomic particles each made of a quark up and two quarks down (Figure 4), with no net electric charge and a mass marginally greater than that of a proton, can probe structure and dynamics of matter from mesoscale to nanoscale and from seconds to nanoseconds [16]. Intense neutron beams are produced in nuclear reactors or accelerator based neutron sources. Neutrons can provide significant insights into the behaviour of the SARS-CoV-2 and other coronaviruses, contributing to the search for effective diagnostics and therapies. In particular, the expertise in neutron structural biology, biophysics, chemistry, and nanoscale materials science and engineering can be helpful in developing both physico-chemical and environmental controls to virus infection, replication and transmission, and new diagnostics and therapeutics of disease.

Figure 4. Atom structure (a) and the neutron (b) showing its quark content - 1 up quark, 2 down quarks.

Efforts are currently being deployed to obtain crystals for neutron crystallography studies, which can provide essential information, e.g., concerning proteases or virus spike proteins, responsible for mediating the attachment and entry into human cells that it affects. Such information is of huge relevance for developing therapeutic defence strategies against the virus. Neutron crystallography can provide unique information on the precise coupling mechanism of the virus and the receptor proteins of the cell membrane.

The adoptable techniques, in this case, include Small Angle Neutron Scattering (SANS) and Neutron Reflectometry (NR), but also neutron imaging, neutron macromolecular crystallography and neutron spectroscopy.

SANS allows to characterise materials at the micro- and nano- levels, providing statistical information, averaged over a macroscopic volume. Parameters such as diameter, concentration, volume fraction and area of interface can be monitored by measuring the scattering of the neutrons from the samples in angles smaller than 5 degrees. In general, the low angle part of the SANS scattering curves ($qR < 1$) contains information about the overall sizes of the scattering objects; the medium angle region characterizes the particle shape, and at larger scattering vectors interface features can be deduced. The scattering vector q is defined by the neutrons' wavelength and the scattering angle, while R is the size of the scattering particle. The scattering intensity curves are modelled by mathematical functions, involving parameters that reflect the structural features of nanoscale inhomogeneities. For theoretical base of the SANS technique please refer to [16-19].

Application of large-scale neutron facilities to promote the problems of creating vaccines against COVID-19 should be based on the methods of fine determination of subtle mechanisms of virus interactions with cells and their organelles (membranes, nuclei etc.). The transport of viruses inside various human cells has not been studied in detail so far, since the variety of peptides on their surface is extremely high and it depends on the functions of the cells in organisms. In the study of the function of large biological complexes, such as assembled viruses, SANS becomes an important analytical tool. Its capacity to distinguish specific regions (RNA, proteins and lipids) of the virus, thanks to advanced deuteration methods, allows to map out the arrangement of the various components, thereby contributing to structural studies. While cryo-electron microscopy and nuclear magnetic resonance assess the atomic-resolution structure of small biological assemblies, neutron scattering supplies a larger picture of full molecular complexes at lower resolution. The goals of SANS and NR applications are:

- the use of undamaged living cells by soft neutron radiation during the experiment, whose duration may be long to provide the observation of slow processes of virus reproduction at nano/microscales, that could serve as a modelling of virus attack development on living organism;
- to cast light on the processes of incubation, active reproduction and virus spread dependent on the conditions by using drugs or other medical factors (procedures).

These real-time experiments cannot be carried out by other methods. Neutron large scale facilities allow to perform the in-beam tests on new medicines in the conditions close to physiological ones, to get

information on their behaviours dependent on concentration and presence of various biological molecules (DNA, enzymes, etc.) in the solutions. These fine experiments could promote the development of new drugs and make shorter their preparation for medical tests and transfer to final pharmaceutical technologies.

Neutron scattering methods should be applied together with complementary physical and chemical methods especially to design nano-carriers for new drugs. This is a way to enhance their efficiency and decrease the necessary doses. This is also related to the use of new nanoscale carbon structures in medicine (fullerenes and derivatives, nanodiamonds and graphene), which must be characterized by neutron scattering methods in solutions by modelling conditions. Such analysis could concern blood vessels with gradients of flow velocities, may be probable and change substantially blood's hydrodynamics. SANS experiments, therefore, seem to be important for new drug design and certification. Several tasks to be solved by SANS, consequently, include:

1. Preliminary characterization of drug behaviours in solutions, (aggregation, hydrodynamics at nano/microscales).
2. Studies of nano-sized carriers of drugs: to analyse the structure of active layers covering the particles, and the interaction of pristine and occluded particles; as well as to understand their hydrodynamic behaviour.
3. Examination of the interactions of the aforesaid objects with viruses (model objects) in solutions in systems with cells, to simulate living organisms' medium.

The advantages of SANS are enhanced by the method of contrast variation changing the proportion of light and heavy water in the solvent. This allows to find the structural parameters of single particles, such as drug-carriers, and to build their model (e.g. globular core with surrounding shell of active antiviral substance). These studies will enable to determine the optimal conditions for the formation of nanoparticles complexes with the drug molecules, chemical structure, composition, solubility at different temperatures and pH-factors. Knowledge of these characteristics is necessary to regulate the interactions of such composite particles with viruses. Note, these particles having core-shell structure are considered as objects which tend to be linked to the surface of the virus, blocking its activity. Therefore, it is fundamental to analyse how intensively these particles interact with the viral surface, to predict the amount of particles attached and to estimate the thickness of the formed layer. Such subtle structural details can be obtained from the SANS data for the systems specially prepared to search the efficiency of drugs against viruses.

Modern SANS techniques, using cold neutrons produced by middle or high flux reactors, provide very precise measurements of structural parameters of the systems with nano/microparticles. Their size, gyration radius R_g , can be calculated with a high accuracy ~ 0.01 nm by the Guinier-approximation for scattering data:

$$I = I_0 \exp[-(qR_g)^2/3], \quad (1)$$

where $I_0 = I(q \rightarrow 0)$ is the forward scattering intensity computed in the limit of low scattering vectors. In addition, the amount of adsorbed molecules on their surfaces is detected using the measured forward scattering intensities:

$$I_0 \sim (\Delta K) 2N V_{dry}^2, \quad (2)$$

where ΔK is the contrast factor for a particle having dry volume V_{dry} and the parameter N is the number of particles in the sample. Here, we assume a fine variation of the contrast factor to observe separately globular cores or the shells around them. The dry volume of the core or shell, therefore, can be found unambiguously. It should be noted, in a chosen diapason of scattering vectors $q_{min} \leq q \leq q_{max}$, that it is possible to detect the particles of desirable size $R \sim 2\pi/q$ between $R_{min} \sim 2\pi/q_{min}$ and $R_{max} \sim 2\pi/q_{max}$. The scattering experiments at low and ultra-low angles (SANS and USANS, respectively), thus, deliver the structural information in a wide spatial scale, $R \sim 1-1000$ nm, which gives the possibility to describe different structural levels in the samples (drug molecules, particles-nanocarriers, pristine viruses and viruses covered with these molecules/nanoparticles). Such information will be obtained in conditions close to the physiological ones (isotonic solutions). These comments are only simplified illustrations of the experimental abilities of SANS for microbiology. Much more expended procedures of data treatment using computer modelling in reciprocal and direct space are developed to search in detail the spatial correlations at molecular and supramolecular levels in microbiological systems.

In connection with the problems above discussed, some kinds of semiconducting nanoparticles (TiO₂, ZnO) may serve as photocatalysts for cleaning air and water from contaminations (toxic substances, viruses, bacteria). The activation of catalytic particles by UV radiation induces a formation of electron-hole pairs in their volumes and at the surfaces that promotes chemical reactions of decontamination of toxins at the surface of these catalysts. The other effective mechanism for the destruction (oxidation) of molecular and microbiological contaminants is based on pumping the sensitizers by laser, followed by the generation of chemically active singlet oxygen in the surrounding medium. Such sensitizers are various porphyrines, diphthalocyanines, fullerenes and in medical practice also chlorine derivatives of porphyrins (Photoditazine, Radachlorine) serving for photodynamic therapy. A relevant task, presently, is the synthesis of complexes of these molecular catalysts with nanoparticles being chemically neutral and forming stable aqueous dispersions. Our preliminary experiments, recently, have shown real opportunities to use nanodiamonds as advanced carriers for photocatalysts (unpublished data). The SANS experiments and optical spectroscopy data have confirmed a formation of stable complexes by sorption of catalysts on the surface of nanodiamonds.

3. Perspectives and conclusions

Each protein, nucleic, and glycan component of the SARS-CoV-2 could be targeted for discovery of small molecules that modulate their interaction with human biomolecules. This ‘human–virus biomolecular interactome’ is likely to be massive and challenging to decipher. However, curating the interactome might yield the discovery of novel agents that alter key pathways for infection. From the drug discovery perspective, the dynamic features for i.e. of ACE2 and the knowledge accumulated throughout the years support the possibility that ACE2 conformation and function could be modulated by allosteric drugs. Allosterism is a central widespread mechanism in all life forms, once defined as “the second secret of life”. ACE2 appears as a highly dynamic protein, for which allosterism has been demonstrated to different degrees between the active site (site 1), the hinge region (site 2), the claw-like/spike protein binding site (site 3), and the Cl[−] binding site. Given that ACE2 appears as a highly dynamic protein with a complex allosteric network between key sites, the development of compounds interacting at one site should ideally be tested for their effects on the different distant sites [19–43].

Advanced characterization based on large machines of physics could be useful in research against COVID-19 thanks to the possibility to analyse the atomic structure of this virus, to identify molecules effective against COVID-19 providing to a rational design of novel molecules to be used as potential drugs by applying structural biology tools to validate virtual models, to develop and consolidate an effective tool to counter future viral epidemics, to fine define the subtle mechanisms of viruses’ through the interactions with cells and their organelles.

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