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Peptide-Based Biosensor for Express Diagnostics of Coronavirus Respiratory Infections †

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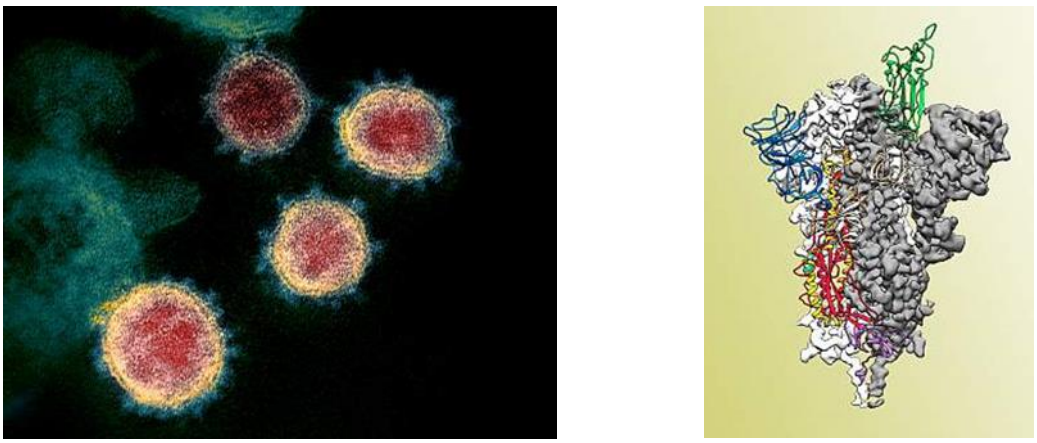
Abstract: At the end of 2019 the first reports appeared of a new coronavirus and WHO announced a pandemic of COVID-19. To date, the new coronavirus, now called SARS-CoV-2, has infected more than 42,000,000 people and killed more than 1,000,000 people worldwide. It is extremely important to develop means for express diagnostics to ensure prompt action to limit the spread of infection. One of the diagnostic approaches is the detection of viral particles in swabs. This approach can be realized using biosensor with specific ligands, based on peptide molecules complementary to surface viral proteins. The concept of the so-called Systems of Conjugated Ionic-Hydrogen Bonds (abbreviated—SSIVS, CIHBS) implemented in the Protein-3D computer program, was applied to analyze the spatial structures of the bonds between the SARS-CoV-2 spike protein and the ACE-2 receptor, in order to reveal the perspective peptide sequences. There are two clearly marked areas of contact of the spike with the cell receptor—upper and lower, which are visualized in the SSIVS form, and the complex formed at this site is strong enough to ensure its attachment to the coronavirus spike and can compete for binding with the ACE-2 receptor. Two peptides were developed that form a spatial structure complementary to the coronavirus spike: of 8 (No 1) and of 15 (No 2) amino acid residues. The peptides were covalently bound to biochip platforms via neutral linkers to form sites with peptides No1 and No 2. The third site has a neutral hydrophilic surface to serve as a reference. The platform was integrated with microfluidic channel and was used as a flow through device. The detection of bound viral particles was carried out using UV excitation and direct registration of viral proteins fluorescence. The preliminary laboratory tests demonstrated the efficiency of the biosensor.

Keywords: coronavirus; peptide aptamers

1. Introduction

At the end of 2019, the first reports of a new coronavirus, similar to those that caused outbreaks of severe acute respiratory syndrome (SARS) in 2002–2004 and Middle East Respiratory Syndrome (MERS) in 2012, WHO announced a pandemic caused by the new COVID-19 virus.

To date, the new coronavirus (Figure 1), now called SARS-CoV-2, has infected more than 42,000,000 people and killed more than 1,000,000 people worldwide [1,2].



(**a**) (**b**)

Figure 1. Image of SARS-CoV-2 coronaviruses obtained from a laboratory culture of infected cells (**a**); The structure of the protein spike of the SARS-CoV-2 coronavirus. The domain joining the host cell is colored green (**b**) [2].

The virus is adsorbed on the cell surface in the area of ACE2 receptors using spikes (Figure 1b), formed of trimer fusion S-protein, and then penetrates into the cell and starts the processes of forming its copies with the help of the cell apparatus. To inactivate the coronavirus and for its rapid analysis, molecules with spatial structures complementary to the spikes of the coronavirus are required. Thus, development of 3D molecular structures spatially complementary to virus *S*-protein seems to offer a promising tool for inactivation and identification of SARS-Cov-2.

The aim of this work is to search for short peptides that could compete with the ACE2 receptor for recognizing fragments of the coronavirus spike.

2. Materials and Methods

The *in silico* development of peptides was carried out using Protein 3D software [3], developed at the Centre of Microtechology and Diagnostics of St. Petersburg Electrotechnical University "LETI" [4].

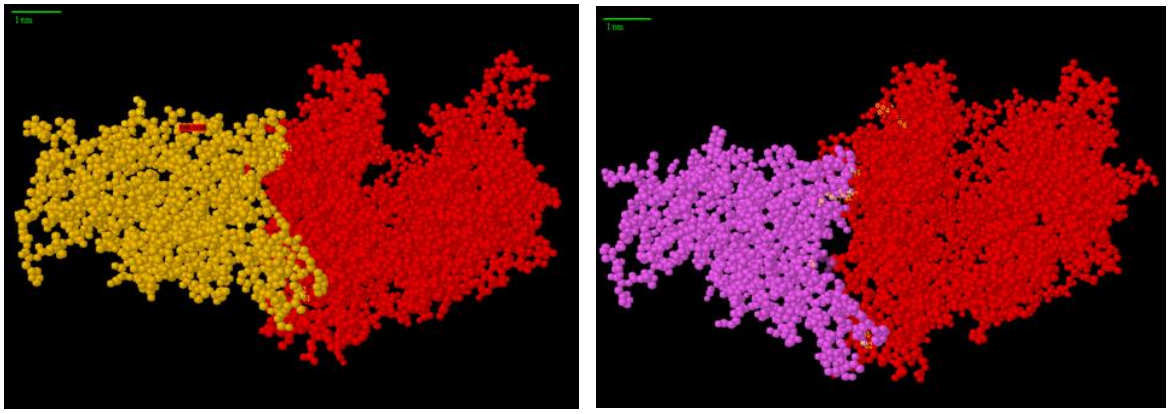
The basic 3D structures of target proteins were obtained from Protein data bank [5].

The synthesis of peptides was carried out using a standard automatic procedure.

3. Results and Discussion

Currently, two studies are known (6LZG.pdb and 6m0j.pdb, both unpublished, with a resolution of 2.5 and 2.45 A) devoted to the study of the complexes of the SARS-CoV-2 coronavirus spikes with a protein. Due to the different resolution, they slightly differ in the SSVIS, which will be seen from the further presentation.

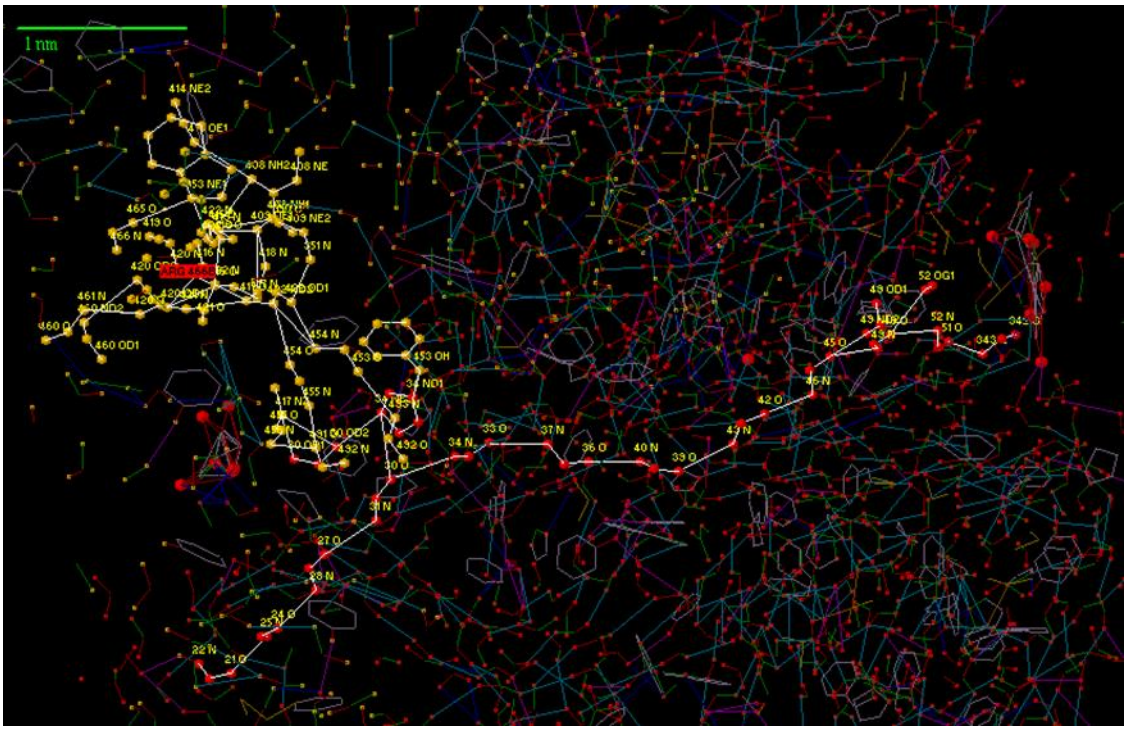
First, let us consider the general view of the complex (Figure 2a,b) from which it can be seen that both structures are practically identical. Their peculiarity lies in the fact that there are two clearly marked areas of contact of the spike with the cellular receptor - the upper and lower. If you look at how these areas look like SSIVS (Figure 3), you can see that for the first and second structures they are practically the same.



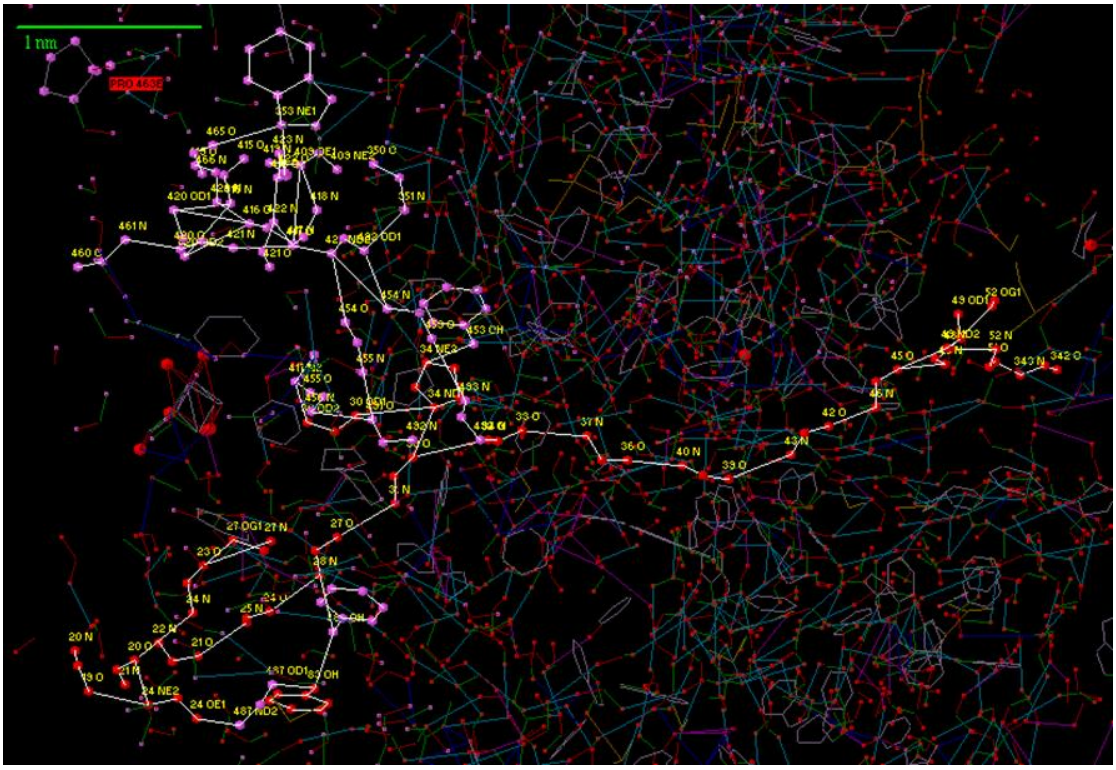
(**a**) (**b**)

Figure 2. General view of the complex of a fragment of the thorn of the coronavirus SARS-CoV-2 and protein ACE-2: 6LZG.pdb (**a**); 6m0j.pdb (**b**).

There are several areas of contact between the SARS-CoV-2 coronavirus spike and the ACE 2 protein observed in these figures. The greatest interest, in our opinion, is the interaction between His 34 protein ACE-2 and Tyr 453 KB. This fragment for both files (6LZG-1.pdb and 6m0j-1.pdb) is shown in Figure 3a,b.

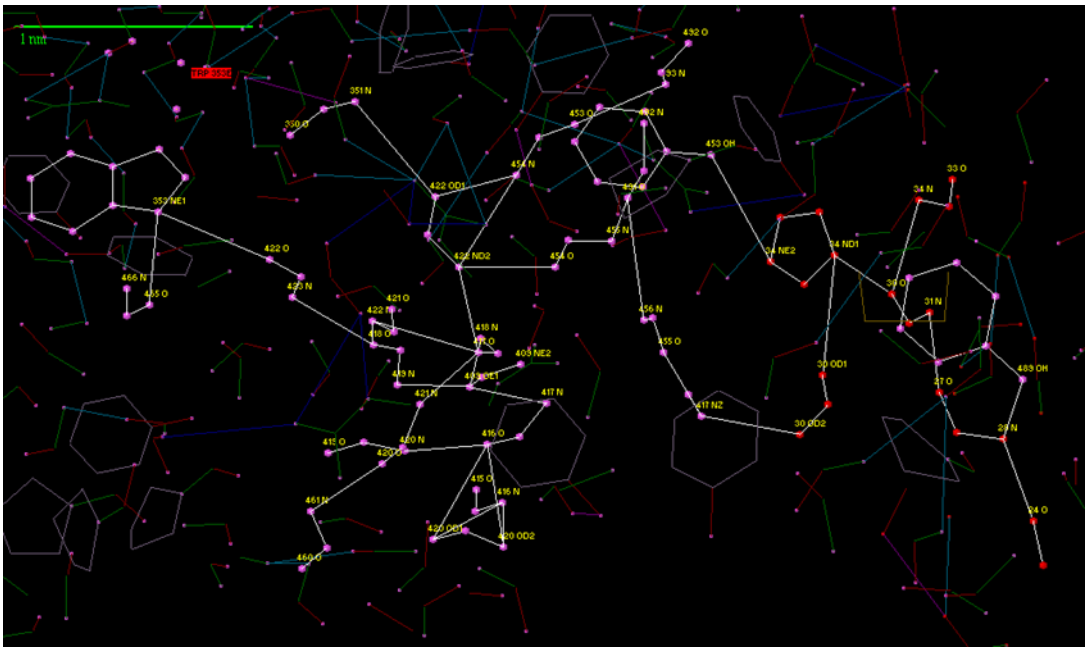


(**a**)

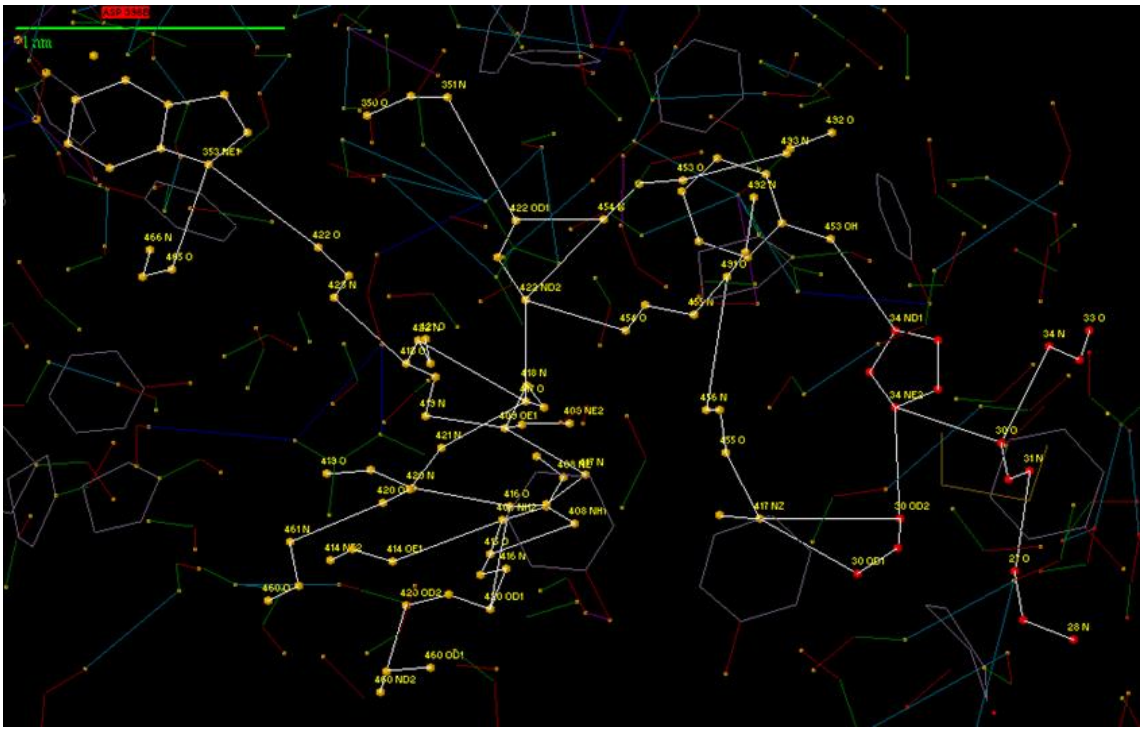


(**b**)

Figure 3. General view of the complex of the SARS-2 coronavirus spike and the ACE-2 protein in the rendering of the SSIVS. (**a**) 6LZG.pdb (yellow balls—a fragment of the coronavirus thorn, red balls—ACE-2 protein), (**b**) 6m0j.pdb (purple balls—a fragment of a coronavirus thorn, red balls—ACE-2 protein).



(**a**)



(**b**)

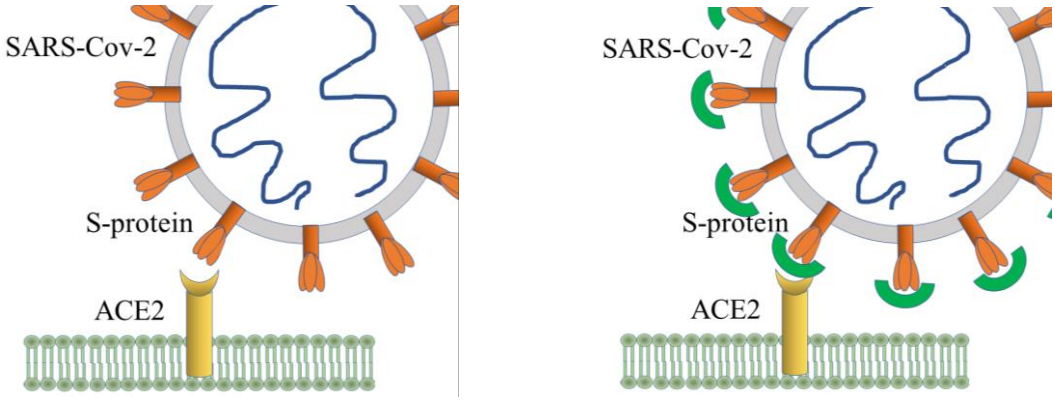
Figure 4. Fragment of the thorn of the coronavirus SARS-CoV-2 and fragment 27-34 of the ACE-2 protein in the rendering of the SSIVS: (**a**) 6m0j.pdb, (**b**) 6LZG.pdb.

The sequence No 1 contains only 8 amino acids and is a helical fragment. It contains water-soluble side chains (THR, ASP, LYS, ASN, HIS). The peptide can be used both in the form of a solution and in the form of an anchor group (on a stem) in a diagnostic biochip.

As a second option, it is possible to propose increasing the length of the fragment to Tyr 41. This will provide even greater strength of binding of the anchor sequence to the spine of the virus (sequence No 2).

Both structures of the SSVS are almost identical, which increases the reliability of the data presented. It can be assumed that the complex formed at this site is strong enough to ensure its attachment to the coronavirus spike and can compete for binding with the ACE-2 receptor.

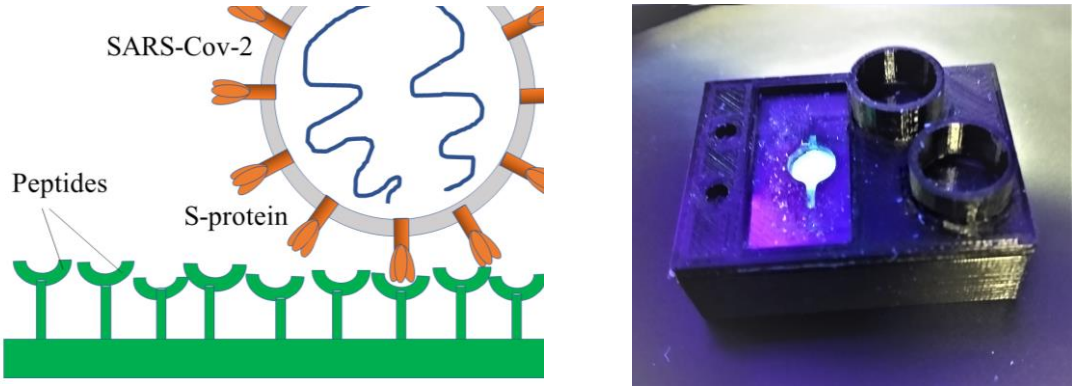
The peptides can be used for virus inactivation as shown in Figure 5.



(**a**) (**b**)

Figure 5. Mechanism of virus inactivation. (**a**) Mechanism of Coronavirus Cell Entry Mediated by the Viral S-protein; (**b**) inhibition of fusion S-protein with 3-D complementary peptide.

In biosensor peptides are attached via short linkers to the surface of the active sites in the flow-through microfluidic system (Figure 6a). This configuration enables the sample preparation stage to be considerably reduced. The biochip laboratory sample is presented in Figure 6b.



(**a**) (**b**)

Figure 6. Schematic representation of biosensor principle for SARS-CoV-2 determination in swab samples (**a**); laboratory sample of the biosensor (**b**).

Preliminary testing of biosensor was carried out at Pasteur Institute and demonstrated promising results for peptide N 2. Wide scale testing is currently in progress.

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