Inhibiting the Pathogenicity of SARS-CoV-2 with a Designer Fluorescent Carbon Quantum Dot (FL-$^{AS}C_{QD}$): A Hypothesis

SubhenduSekhar Bag [a,b] and Sayantan Sinha[b]*

[a]Chemical Biology/Genomics Laboratory, Department Chemistry, Indian Institute of Technology Guwahati, India-781039; [b]CentreFor The Environment, Indian Institute of Technology Guwahati, India-781039

* Corresponding Author: Prof. SubhenduSekhar Bag, FRSC; email: ssbag75@iitg.ac.in

Abstract

The world is paralyzed by the infection of SARS-CoV-2 (novel coronavirus) at present. The disease caused by this virus is officially declared as COVID-19 by WHO. Scientists worldwide are trying to develop a drug/vaccine that can eradicate this disease. However, the present treatment avenue is only a supportive medication coming out as a drug repurposing protocol. In this concept paper we would like to propose the synthesis of an antiviral biogenic fluorescent Carbon Quantum Dot **(FL-$^{AS}C_{QD}$)** as a promising nanomedicine that can be effective in COVID-19 cure. The nanomedicine surface is engineered with Angiotensin 2 protein and HSPG receptor mimic linked with an antiviral drug isolated from *False Daisy* leaf extract. The nanomedicine is expected to show a multi-functional property such as to (a) stop entry of SARS-CoV-2 into host cell via attachment with HSPG mimick on the surface of the nanomedicine, (b) inhibit viral replication through the interaction of attached antiviral drug with viral RdRp enzyme, (c) sense the SARS-CoV-2 via a change in fluorescent photophysical response. Thus, this nanomedicine would find potential application as a promising drug, sensor of SARS-CoV-2, anti-SARS-CoV-2 protective layer in masks, and also as disinfectant for SARS virus/MDR laden wastewater.

Key Words: Inhibition of SARS-CoV-2; COVID-19; Antiviral nanomedicine; Antiviral drug loaded HSPG mimick; Fluorescent Carbon Quantum Dot (FL-$^{AS}C_{QD}$); *False Daisy* leaf extract; Nano sensor; anti-SARS-CoV-2 protective layer in masks; SARS virus/MDR laden wastewater; Disinfectant.

1. Introduction

Viral infections are very common worldwide. The most common viral infection is the common cold, which is an outcome of infection caused by Rhinovirus [1]. Diseases caused form these viruses are not fatal and can be easily cured when the patient is under medical observation. However, all viral diseases are not such easily curable. According to WHO, nearly 5.7 million of people die globally each year due to various viral infections such as human immunodeficiency virus (HIV), human simplex virus (HSV), human cytomegalo virus (HCMV), human papilloma virus (HPV) etc [2]. To add to the list, deaths and infections caused by SARS-associated viruses like Ebola, HCoV, MERS-CoV, and SARS-CoV, since past 2 decades, have created a worldwide emergency. With the recent pandemic caused by the novel SARS-CoV-2 virus, the world is in a doom situation with no drugs to fight against this pandemic [3]. The spread of COVID-19 disease has passed only 6 months. As a result, scientists and medical practitioners have got a tiny window to react and do intensive research studies to tackle the pandemic. With the delineation of the 3D X-ray crystal structure of the main protease SARS-CoV-2 M^{pro} [3], scientist found it as an interesting drug target. Thus, a few substantial research works towards the development of protease inhibitor drugs have come up within a span of only one or two months [4-5]. However, these reports have been discovered and tested in a laboratory environment in cell lines. Currently, the clinical treatment of the disease, COVID-19 is mainly symptomatic combined with repurposing of already marketed antiviral drugs such as ritonavir/lopinavir, remdesivir, a combination of ritonavir/lopinavir with interferon beta, and antibiotics such as Chloroquine and hydroxychloroquine to treat secondary infections. Therefore, there remains an urgent need and challenges to save the human life worldwide by developing specific antiviral therapeutics and vaccines against SARS-CoV-2. Scientists are trying hard to discover drug and vaccine for cure but it will take time to get a positive outcome. Until and unless a vaccine comes in the market; the COVID-19 crisis cannot be solved permanently worldwide. At the same time, the nature of recent outbreak of Coronavirus teaches us that viruses can evolve rapidly [3]. This is a danger for the future. Thus the world must be cautious and we must prepare ourselves accordingly for near future circumstances from now. Therefore, it is urgent to carry out research related to novel drug discovery against SARS-CoV-2 virus that will create a continuous opportunity to combat against newly evolved organism in future.

Although we are lacking of perfect drug or vaccine, the dedication of global scientists shed the light on the nature, mechanism of cell infection and possible way of inhibition of the virus. It is now believed that the interaction among the viral attachment ligand (VAL) and the receptors of the host cell membrane is an initial step and the common route of viral infection. Furthermore, most of the pathogenic viruses like HIV, HSV, HCMV, and HPV are known to bind with the heparan sulfate proteoglycan (HSPG) receptor of the host cell. Studies have also shown that SARS-associated viruses like SARS-CoV, HCoV-NL63, and other coronaviridae also show affinities to bind to the HSPG [6-7]**.** A recent study has shown that the S1 receptor binding domain (RBD) of novel SARS-CoV-2 binds with heparin, which is a mimic of HSPG [8]. The sequencing study revealed 90% similarity among the sequences of S1, and S2 subunits of the S (spiked) glycoproteins, which protrude from the virion cell surface of both the SARS-CoV, and SARS-CoV-2. In the journey to have a proper treatment for COVID-19, efforts of many researchers suggested that the angiotensin-converting enzyme 2 (ACE2) receptor helps coronavirus to hook into a wide range of human cells and infect them [6]. We further studied the mode of host cell recognition by SARS-CoV after understanding the functional similarity of both viruses in attacking the host cells. Thus, we found that an efficient entry of SARS-CoV into the host cell is mainly governed by the involvement of HSPGs in concert to ACE2 receptors. The anchoring of the viral cells with the host cells, at the initial stage, is resulted from the interaction between the spiked protein and the heparin sulphate chains of the HSPGs. This association plays a key role in the further binding of SARS-CoV to its cell-surface receptor, ACE2 [6]. It is now well proven that HSPGs serve as the preliminary binding site of S proteins in SARS-CoV.

The current widely accepted phenomenon of HSPG mediated recognition suggested the transport of extracellular virus from the low-affinity docking site to its high-affinity specific entry receptors. This phenomenon is popularly termed as 'viral surfing' [6]. The weak and reversible interaction between the viral cell and the initial acceptor sites helps the virus to increase its cellular density and scan the host cell surface for specific receptors allowing the entry of the viral particles into the host cell [6]. A similar adaptation can take place in the case of SARS-CoV-2 virus. HSPG receptors are abundant in the mammalian cell surface in comparison to the ACE2 receptors, which are specific. It is also found that the mucosal epithelia present in the respiratory tract are protected by a layer of mucin polysaccharide, which is also sulphonated [8]. Thus, it is expected that the HSPG receptor facilitates the preliminary attachment/docking of the SARS-CoV-2. Attaching to this receptor would help the virus to start its journey by jumping from one HSPG to another to locate the specific ACE2 entry

receptor. The mechanism for hooking between the SARS-CoV-2 S protein and HSPGs is most likely due to the interaction of the closely packed multiple basic amino acid in the protein that constitutes the RDB with the negatively charged sulfate groups of the heparan sulfate (HS) present in the glycocalyx of the cell surface [2].

2. The Hypothesis

From the above discussions based on the scientific reports and the structural similarity of S proteins in both SARS-CoV and SARS-CoV-2 virus led us to propose that the SARS-CoV-2 might also follow a similar mechanism/route for the host cell infection. Thus, the viral S protein first interact and bind with the HSPG receptor of host cell. Interestingly, our hypothesis is supported by a recent study where SARS-CoV-2 S1 RBD shows efficient binding with heparin, which is a mimic to heparan sulfate [8]. Based on our hypothesis and supportive scientific information, we thought that it will be worthwhile to design HSPG mimic which would be efficient to interact and strongly bind with viral S protein inhibiting the SARS-CoV-2 attachment to the host cell. We have thus conceptualized a fluorescent nanomedicine based on fluorescent carbon quantum dots the surface of which will be decorated with Angiotensin 2 protein which is the original substrate for hACE2 receptors and Heparan Sulphate Proteoglycan receptor mimicking long chain sulphates. We also thought that attaching an antiviral agent with the sulphate would be beneficial to offer medicinal value to the nanomaterial. Thus, our proposed material would also act as a drug candidate by inhibiting the viral RNA-dependent RNA polymerase activity (RdRp), thereby stopping its replication.

Therefore, based on the scientific facts and thorough understanding, our concept aims to synthesize a biogenic fluorescent carbon quantum dot decorated with an HSPG mimick linked with an antiviral drug, such as a RdRp inhibitor and a human angiotensin converting enzyme 2 (hACE2) protein. SARS-CoV-2 virus infects the human cell by establishing interaction of their Spiked (S) glycoproteins with the Human Angiotensin Converting Enzyme 2 (hACE2) receptors. However, before they can locate the hACE2 receptor, their S proteins bind to the HSPG receptors present unanimously on the human cell surface. These HSPG receptors help nCoV-19 viral surfing to locate hACE2 receptor. Therefore, our proposed nanomedicine could serve tri-functional role to fight against SARS-CoV-2 virus. (a) As the surface of the nanomedicine is coated with Angiotensin 2 therefore the medicine will show a competitive binding with hACE2 receptors, thereby blocking the specific route of SARS-CoV-2 host cell entry. Secondly, the HSPG mimic of the proposed antiviral biogenic fluorescent Carbon

Quantum Dot (FL-$^{AS}C_{QD}$) can easily hook the nCoV-19 virus preventing the viral binding with the human cell receptors. Thus, the surface of the nanomedicine would serve as a decoy to block all possible routes for the entry into the host cells (b) Additionally, the detection of the virus would be possible as soon as the viral spiked glycoproteins gets attached with the surface via a change in fluorescent property of the nanomaterial. (c) Finally, the generic antiviral property of the surface bound RdRp inhibitor would restrict the RNA dependent RNA polymerase (RdRp) activityand hence stop viral replication.

Consequently, we have decided to use a long chain sulphonated linker, which is a mimic of HSPG, to decorate the surface of our quantum dot material. A few previous studies [10] show that short-chain linkers mimicking HSPGs are used to expose their sulphonated groups for attachment with the viral attachment ligand (VAL). However, short linkers allow binding with only a few of the repeating units that constitute the VAL. Replacing the short-chain with a long chain linker is expected to establish a strong binding and result in distortion of the VAL structure [11-12]. In addition to decorating the surface with long-chain sulphonated linkers like hexadecanesulphonic acid, we also decided to prepare the fluorescent carbon quantum dots from natural waste material such as banana peel extract and lemon juice. The HSPG mimic hexadecanesulphonic acid will be linked with antiviral phytochemicals, such as luteolin (**1**), wedelolactone(**2**), and apigenin(**3**), from the leaf extract of false daisy (common name: Bhringaraj; Sc. Name: *Ecliptaalba*) plant **(Figure 1)**.The use of leaf extract from False daisy has been applied earlier to cure various diseases like liver inflammation, infective hepatitis, liver cirrhosis, jaundice, and cancer [13]. Furthermore, these phytochemicals are known to have potential polymerase inhibition activity. Recent research reported that these compounds potentially inhibit the RNA-dependent RNA polymerase activity (RdRp) of the hepatitis C virus, thereby stopping the viral replication [13]. Therefore, linking the HSPG mimic with phytochemicals from false daisy leaf extract and then coating the surface of the nanocarbon quantum dots is expected to bring about virucidal activity via an interaction with the hydrophobic clefts of RdRp of SARS-CoV-2 virus. The as synthesised material will then be incubated with ACE2 protein for a surface coating to derive the final nanomedicine **FL-$^{AS}C_{QD}$.**

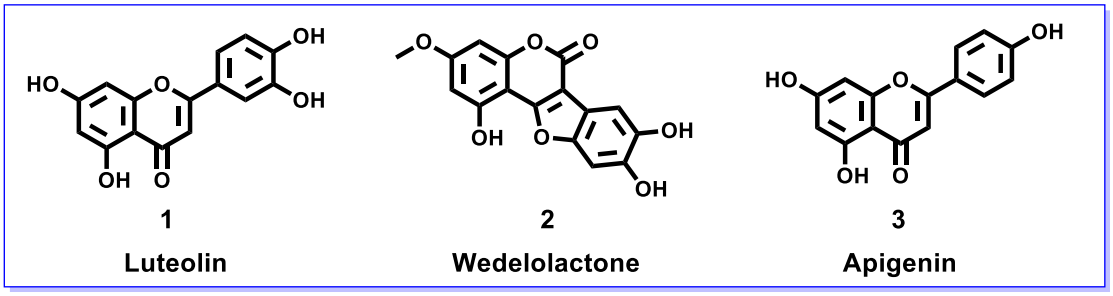

Figure1. Compounds showing anti RdRp inhibitory property in leaf extract of False daisy (Bhringaraj).

Thus, we expected that the proposed nanomedicine, **FL-$^{AS}C_{QD}$**, would play an important role in acting as a blockade in the binding of the SARS-CoV-2 S proteins to the host cells. It is also expected to impart significant antiviral property and help in rapid detection of SARS-CoV-2. Moreover, the fluorescent property of $FL^{S}C_{QD}$ will help us better understand and realize the effect of FL-$^{AS}C_{QD}$ on SARS-Cov-2 virus through the study of fluorescence photophysical responses on interaction. We have, thus, envisioned that this smart material can be applied in various forms- as a (a) potential drug candidate, (b) an instant spray for detection of virus, (c) protective layer in masks, and (c) disinfectant for virus/SARS-CoV-2/MDR bacteria laden wastewater.

A schematic diagram expressing the interaction between SARS-CoV-2 and our synthesized **FL-$^{AS}C_{QD}$** is presented in **Figure 2**.

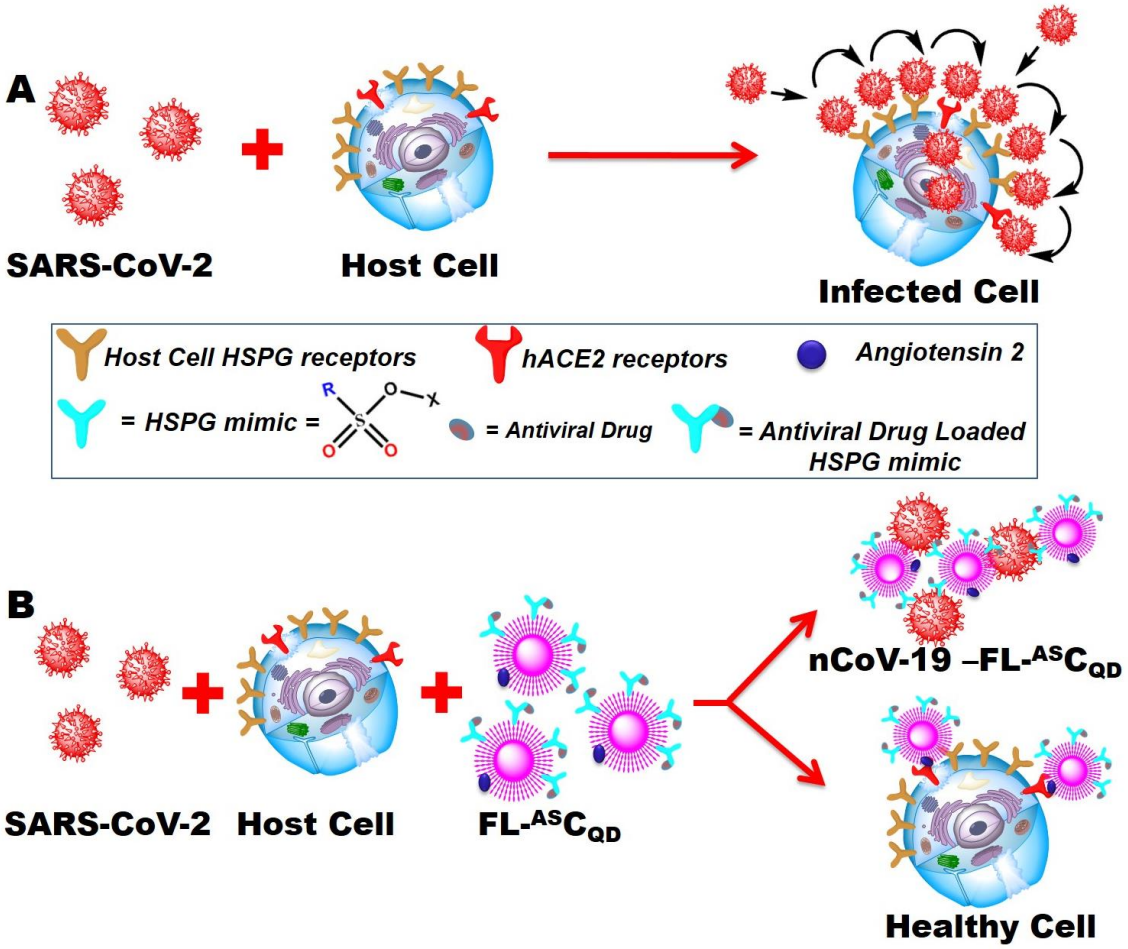

Figure 2. (A) HSPG receptors are abundant in the mammalian cell surface in comparison to the *h*ACE2 receptors, which are specific. HSPGs play a significant role in the process of SARS-CoV-2 entry. It is expected that the HSPG receptors provide the initial anchoring site for binding between SARS-CoV-2 and host cell surface. The SARS-CoV-2 then jumps/rolls on from one HSPG receptor to another in search for its specific ACE2 receptor, which will allow the cell entry. (B) Our synthesized **FL-$^{AS}C_{QD}$** blocks the entry of SARS-CoV-2 through competitive binding with *h*ACE2 receptor. Additionally, the drug linked HSPG mimic binds to the SARS-CoV-2 S1 RDB. This prevents the virus from getting attached to the HSPG receptors on the host cell; hence they cannot scan the host cell surface for entry receptors. Thus infection is stopped.

3. Preliminary Results Supporting the Hypothesis

To test the possible virucidal property which would be imparted into the nanomedicine by the False Daisy phytochemicals, we initially carried out a molecular docking calculation. We decided to check the binding efficacy of the screened molecules against SARS-CoV-2 RNA dependent RNA polymerase (RdRp) through homology modeling and molecular docking

studies. The SARS-CoV-2 RdRp PDB structure with PDB id: 6M71 was downloaded and subjected to protein preparation. The molecular docking study was carried out using Molegro virtual docker (MVD software version 6.0 [14, 15]. The ligand structures were prepared and energy minimized using Chem 3D software. RdRp was imported and catalytic pockets/sites were detected (shown in **Figure 3**) using the in-built 'detect catalytic sites' option.

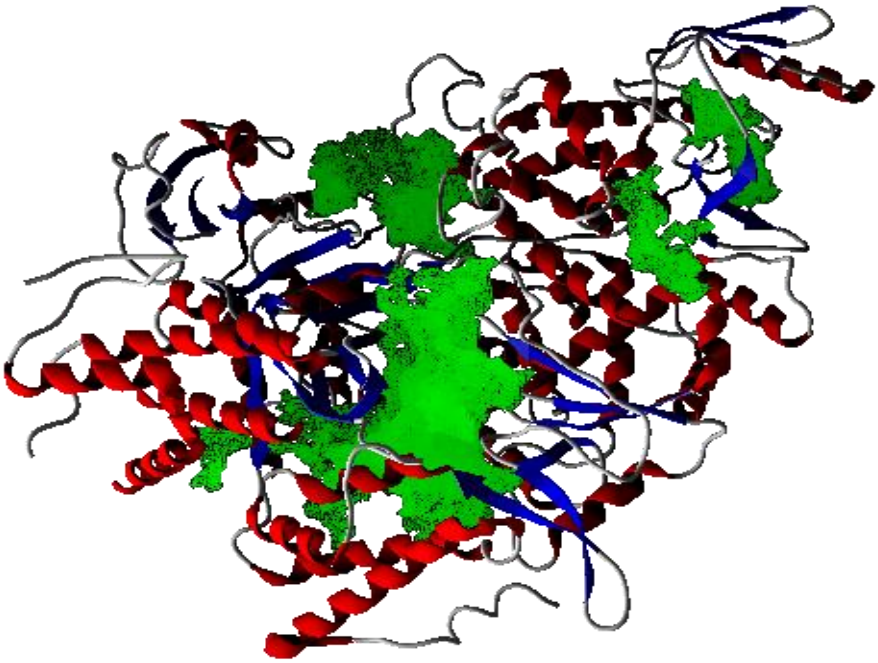

Figure 3. Catalytic sites/pockets detected in SARS-CoV-2 RdRp.

The docking study was carried out. Protein-ligand interaction docking scores were found to be -113.005 kcal/mol, -113.543 kcal/mol and -105.765 kcal/mol for wedelolactone, leutiolin and apigenin respectively. Wedelolactone interacts (hydrogen bond) with Arg624, Asp452, Ala554, Thr556, Asp623, Lys621, Cys622, Tyr455; Luteolin interacts with Asp760, Tyr619, Lys621, Ala554, Thr556, Cys622, Arg624; Apigenin interacts with Arg553, Ser682, Lys676, Ser681, Lys545, Arg555, Thr556, Ala554, Arg624 residues. The molecular docking and interaction study is shown in **Figure 4**.

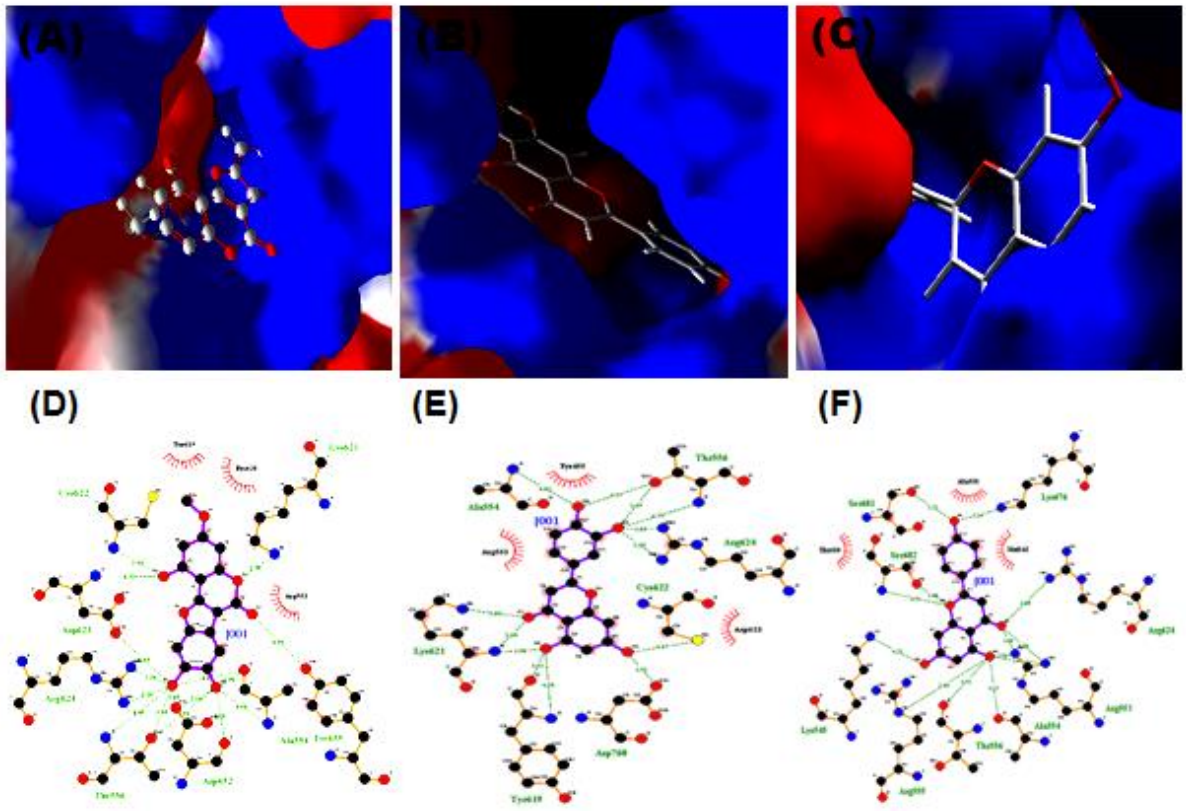

Figure4. Binding of (A) wedelolactone (B) luteolinand (C) apigenin in the catalytic site of nCoV-19 RdRp; interaction with amino acids in the active site of the catalytic pocket (D) wedelolactone, (E) luteolin and (F) apigenin

4. Conclusion

The need to design the biogenic conjugated carbon quantum dot (C_{QD}) is to carry out both detection and eradication of the virus. The screened molecules have the potential to arrest the viral RdRp activity by binding to the active site of the viral RdRp, however they cannot detect the virus and their interaction could not be visualized or studied. To overcome this lacuna, we decided to use a fluorescent material which can help us to explore its electronic properties in detecting activity through photophysical studies. Additionally, one of our major goals is to stop the virus from infecting the host cell. Using only the screened molecules from false daisy will not suffice this objective. Therefore we decided to modify the surface of quantum dot with ACE2 protein which will show competitive binding to hACE2 receptor and stop the viral entry pathway. Secondly engineering quantum dot surface with HSPG linked drug mimic will further stop the virus from attaching to the HSPG receptors on the cell surface. Hence the nanomedicine serves all the necessary objectives of stopping viral cell entry, de-attachment to host cell receptors, stop viral replication, and sensing of the virus. We have decided to use

carbon dot because of its stability, ease of synthesis, biosafety, excellent fluorescent and tunable surface property. It is well known that C_{QD}s or its conjugates are very easily up-taken by the cells [16, 17].

This article describes a novel concept that we have envisioned and we have started preliminary work at our laboratory in IITG. The proposed nanomedicine is expected to block the interaction of virus with host cell and thus would bear an incredible impact for the mankind. If, the SARS-CoV-2 cannot bind to the host cell, the chance of infection would be out ruled. Furthermore, the preliminary docking studies that we have performed have shown promising results. All the three molecules were able to dock in the catalytic pocket near the active site of RdRp in SARS-CoV-2. Therefore, we are optimistic that our proposed nanomedicine, **FL-$^{AS}C_{QD}$**, can also stop the replication of SARS-CoV-2 by inhibiting the RdRp. Therefore, we believe that with practical maturation of this concept, we can save lives at stake.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online athttps://doi.org/10.10xx/j.mehy.2020.xxxx.

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