








Reply

# Reply to Zandi, M.; Soltani, S. Comment on “Alfassam et al. Development of a Colorimetric Tool for SARS-CoV-2 and Other Respiratory Viruses Detection Using Sialic Acid Fabricated Gold Nanoparticles. *Pharmaceutics* 2021, 13, 502”

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When COVID-19 was first announced back in 2019, there were vast number of attempts to halt the progression of the SARS-CoV-2 virus once and for all. Efforts to develop fast detection assays for this virus were under development even before we understood the structure of this virus. Several papers have revealed that SARS-CoV-2 shares the same genome sequence to those of the previously occurring coronaviruses by up to 90–99% [1–4]. Several studies have confirmed the structure of SARS-CoV-2, in which it has a relatively large positive single-stranded RNA genome, comprising highly conserved basic genes within its sequence, as follows: 5′ ORF1-HE-S-E-M-N 3′ [5–10]. Upon entry of the virus, it binds to the host’s receptor.

Sialic acid (SA) is the glycoprotein that is densely projected on the surface of different mammalian cells including lung epithelial cells. It is used to mediate the binding with viral surface proteins like hemagglutinin in influenza B virus [11,12]; hemagglutinin esterase in OC43, HKU-1, and influenza C viruses [13,14]; and spike glycoprotein domain of spike protein in MERS-CoV [15,16]. Evidently, sia–virus conjugation is used to mediate the entry of different invading viruses. For example, sia–hemagglutinin in influenza B virus [11,12]. The structure of influenza B virus has two surface glycoproteins, the hemagglutinin and the neuraminidase. The hemagglutinin and NA proteins have been found to bind to SA in the host cell molecule. The hemagglutinin is known as the main envelop glycoprotein of influenza B virus, inducing receptor binding, while neuraminidase works as a separate glycoprotein. Recently, Patel et al. have investigated the effect of hemagglutinin esterase (HE) as a potential inhibitor of the emerging coronavirus utilizing a molecular docking and dynamic simulation approach. The results showed a preliminary level of HE targeting to develop effective inhibitors [5]. Some of the coronaviruses have sia–hemagglutinin esterase conjugation that mediates their entry, such as OC43 and HKU-1 viruses [13,14].

However, the role of SA in SARS-CoV-2 needs more elaboration, as the efforts of researchers to understand the full aspects of its role are still at their peak [17]. It is worth mentioning that, when SA binds to its glycoprotein counterpart, it causes red blood cells to agglutinate (hemagglutination) [18]. Hemagglutination is a reaction between the glycoprotein (i.e., hemagglutinin) presented on the surface of some enveloped viruses, such as the influenza virus, and red blood cells, causing their clumping and the formation of lattice. It could be used to quantify viruses and to detect antibodies developed against a virus through hemagglutinin inhibition (HI) assay. Hemagglutinin protein can also be used to facilitate and specify the binding with viruses, and thus can be vital in developing antiviral therapies in the future, especially using nanoparticulate systems [19,20]. However, the complexity of the hemagglutinin structure may hinder the structural characterization of this protein after binding with a specific ligand.

Nevertheless, the use of SA in this study was an attempt to bind specifically to SARS-CoV-2 virus in order to develop a fast detection tool. Having the ability to bind to other respiratory virus through the above-mentioned proteins was an additional advantage to our system that allowed us to investigate further and attempt to develop the binding of these NPs specific for each virus.

Leonardo Bò and his team illustrated the ability of SARS-CoV-2 spike protein to bind to sialic acid molecules [21]. Another study claimed that N-acetyl neuraminic acid has affinity binding to the SARS-CoV-2 spike protein via its receptor-binding domain (RBD) and the S1 N-terminal domain, demonstrating the glycan binding [22]. Therefore, sialic acid binding by the S1 spike protein engages the host cell, while S2 enhances the viral fusion [22]. Many reported that this binding is enhanced by the spread of the infection of coronaviruses because of the structure of 9-O-acetylated sialic acid binding to strain OC43 by cryo-EM [14,23]. A recent study reported that SARS-CoV-2 shares similar sequences of the spike protein with MERS-CoV, which can enter the host cells by binding to DPP4 or sialic acids. This study shows that sialic acid can interact with the spike protein of SARS-CoV-2 by binding between the two RBDs of the spike protein [21]. Furthermore, the presence of spike glycoprotein at the surface of MERS-CoV enhances the entry into the host cells. It is found that MERS-CoV mainly infects human lung epithelial cells by interacting with DPP4 and binding with  $\alpha$ 2,3-linked sialosides [15,16].

We would like to thank the referees for their thoughtful and helpful comments [24] that have undoubtedly improved the paper, for which we are very grateful. We are also ready to correct the information in the published paper. The comment has corrected highly debatable information, in which we claimed that the SA binds only to the hemagglutinin proteins rather than the S protein, especially for SARS-CoV-2 and MERS-CoV. In order to fully understand this binding, a further investigation is required. However, dealing with highly infectious viruses limits the amount of experiments that we can perform.

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## References

1. Alfassam, H.A.; Nassar, M.S.; Almusaynid, M.M.; Khalifah, B.A.; Alshahrani, A.S.; Almughem, F.A.; Alshehri, A.A.; Alawad, M.O.; Massadeh, S.; Alaamery, M.; et al. Development of a Colorimetric Tool for SARS-CoV-2 and Other Respiratory Viruses Detection Using Sialic Acid Fabricated Gold Nanoparticles. *Pharmaceutics* **2021**, *13*, 502. [[CrossRef](#)] [[PubMed](#)]
2. Xu, X.; Chen, P.; Wang, J.; Feng, J.; Zhou, H.; Li, X.; Zhong, W.; Haol, P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci.* **2020**, *63*, 457–460. [[CrossRef](#)]
3. Rabaan, A.A.; Al-Ahmed, S.H.; Haque, S.; Sah, R.; Tiwari, R.; Singh Malik, Y.; Dhama, K.; Yattoo, M.I.; Bonilla-Aldana, D.K.; Rodriguez-Morales, A.J. SARS-CoV-2, SARS-CoV, and MERS-CoV: A comparative overview. *Infez. Med.* **2020**, *28*, 174–184. [[PubMed](#)]
4. Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *Lancet* **2020**, *395*, 565–574. [[CrossRef](#)]

5. Patel, C.N.; Kumar, S.P.; Pandya, H.A.; Rawal, R.M. Identification of potential inhibitors of coronavirus hemagglutinin-esterase using molecular docking, molecular dynamics simulation and binding free energy calculation. *Mol. Divers.* **2021**, *25*, 421–433. [[CrossRef](#)]
6. Bagheri, H.S.; Karimipour, M.; Heidarzadeh, M.; Rajabi, H.; Sokullu, E.; Rahbarghazi, R. Does the Global Outbreak of COVID-19 or Other Viral Diseases Threaten the Stem Cell Reservoir Inside the Body? *Stem Cell Rev. Rep.* **2021**, *17*, 214–230. [[CrossRef](#)]
7. Kannan, S.; Ali, P.S.S.; Sheeza, A.; Hemalatha, K. COVID-19 (Novel Coronavirus 2019)—Recent trends. *Eur. Rev. Med. Pharmacol. Sci.* **2020**, *24*, 2006–2011.
8. Mousavizadeh, L.; Ghasemi, S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. *J. Microbiol. Immunol. Infect.* **2020**, *54*, 159–163. [[CrossRef](#)]
9. Kim, C.-H. SARS-CoV-2 Evolutionary Adaptation toward Host Entry and Recognition of Receptor O-Acetyl Sialylation in Virus-Host Interaction. *Int. J. Mol. Sci.* **2020**, *21*, 4549. [[CrossRef](#)]
10. Esakandari, H.; Nabi-Afjadi, M.; Fakkari-Afjadi, J.; Farahmandian, N.; Miresmaeili, S.-M.; Bahreini, E. A comprehensive review of COVID-19 characteristics. *Biol. Proced. Online* **2020**, *22*, 19. [[CrossRef](#)]
11. Shaw, M.W.; Xu, X.; Li, Y.; Normand, S.; Ueki, R.T.; Kunimoto, G.Y.; Hall, H.; Klimov, A.; Cox, N.J.; Subbarao, K. Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000–2001 and 2001–2002 seasons. *Virology* **2002**, *303*, 1–8. [[CrossRef](#)] [[PubMed](#)]
12. Wang, Q.; Tian, X.; Chen, X.; Ma, J. Structural basis for receptor specificity of influenza B virus hemagglutinin. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16874–16879. [[CrossRef](#)] [[PubMed](#)]
13. Zeng, Q.; Langereis, M.A.; van Vliet, A.L.W.; Huizinga, E.G.; de Groot, R.J. Structure of coronavirus hemagglutinin-esterase offers insight into corona and influenza virus evolution. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9065–9069. [[CrossRef](#)] [[PubMed](#)]
14. Hurdiss, D.L.; Drulyte, I.; Lang, Y.; Shamorkina, T.M.; Pronker, M.F.; van Kuppeveld, F.J.M.; Snijder, J.; de Groot, R.J. Cryo-EM structure of corona-virus-HKU1 haemagglutinin esterase reveals architectural changes arising from prolonged circulation in humans. *Nat. Commun.* **2020**, *11*, 4646. [[CrossRef](#)] [[PubMed](#)]
15. Park, Y.J.; Walls, A.C.; Wang, Z.; Sauer, M.M.; Li, W.; Tortorici, M.A.; Bosch, B.-J.; DiMaio, F.; Veessler, D. Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat. Struct. Mol. Biol.* **2019**, *26*, 1151–1157. [[CrossRef](#)] [[PubMed](#)]
16. Li, W.; Hulswit, R.J.G.; Widjaja, I.; Raj, V.S.; McBride, R.; Peng, W.; Widagdo, W.; Tortorici, M.A.; van Dieren, B.; Lang, Y.; et al. Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E8508–E8517. [[CrossRef](#)] [[PubMed](#)]
17. Sun, X.-L. The role of cell surface sialic acids for SARS-CoV-2 infection. *Glycobiology* **2021**, *31*, 1245–1253. [[CrossRef](#)]
18. Qing, E.; Hantak, M.; Perlman, S.; Gallagher, T. Distinct Roles for Sialoside and Protein Receptors in Coronavirus Infection. *mBio* **2020**, *11*, e02764-19. [[CrossRef](#)]
19. Chang, Y.-J.; Yeh, C.-Y.; Cheng, J.-C.; Huang, Y.-Q.; Hsu, K.-C.; Lin, Y.-F.; Lu, C.-H. Potent sialic acid inhibitors that target influenza A virus hemagglutinin. *Sci. Rep.* **2021**, *11*, 8637. [[CrossRef](#)]
20. Jagadesh, A.; Salam, A.A.A.; Mudgal, P.P.; Arunkumar, G. Influenza virus neuraminidase (NA): A target for antivirals and vaccines. *Arch. Virol.* **2016**, *161*, 2087–2094. [[CrossRef](#)]
21. Bò, L.; Miotto, M.; Di Rienzo, L.; Milanetti, E.; Ruocco, G. Exploring the Association Between Sialic Acid and SARS-CoV-2 Spike Protein Through a Molecular Dynamics-Based Approach. *Front. Med. Technol.* **2021**, *2*, 614652. [[CrossRef](#)] [[PubMed](#)]
22. Baker, A.N.; Richards, S.-J.; Guy, C.S.; Congdon, T.R.; Hasan, M.; Zwetsloot, A.J.; Gallo, A.; Lewandowski, J.R.; Stansfeld, P.J.; Straube, A.; et al. The SARS-CoV-2 Spike Protein Binds Sialic Acids and Enables Rapid Detection in a Lateral Flow Point of Care Diagnostic Device. *ACS Cent. Sci.* **2020**, *6*, 2046–2052. [[CrossRef](#)] [[PubMed](#)]
23. Hulswit, R.J.G.; Lang, Y.; Bakkers, M.J.G.; Li, W.; Li, Z.; Schouten, A.; Ophorst, B.; van Kuppeveld, F.J.M.; Boons, G.-J.; Bosch, B.-J.; et al. Human coronaviruses OC43 and HKU1 bind to 9-O-acetylated sialic acids via a conserved recep-tor-binding site in spike protein domain A. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 2681–2690. [[CrossRef](#)] [[PubMed](#)]
24. Zandi, M.; Soltani, S. Comment on Alfassam et al. Development of a Colorimetric Tool for SARS-CoV-2 and Other Respiratory Viruses Detection Using Sialic Acid Fabricated Gold Nanoparticles. *Pharmaceutics* **2021**, *13*, 502. *Pharmaceutics* **2022**, *14*, 1871. [[CrossRef](#)]