Theoretical Design of Functionalized Gold Nanoparticles as Antiviral Agents against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Aliyeh Mehranfar and Mohammad Izadyar*

ABSTRACT: In this research, through the use of molecular dynamics (MD) simulations, the ability of gold nanoparticles (AuNPs) functionalized by different groups, such as 3-mercaptoprotoethylsulfonate (Mes), undecanesulfonic acid (Mus), octanethiol (Ot), and a new peptide, to inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was investigated. According to the crystal structure of angiotensin-converting enzyme 2 (ACE2), which binds to the SARS-CoV-2 receptor binding domain (RBD), 15 amino acids of ACE2 have considerable interaction with RBD. Therefore, a new peptide based on these amino acids was designed as the functional group for AuNP. On the basis of the obtained results, functionalized AuNPs have remarkable effects on the RBD and strongly interact with this protein of SARS-CoV-2. Among the studied nanoparticles, the AuNP functionalized by new peptide forms a more stable complex with RBD in comparison with ACE2, which is the human receptor for SARS-CoV-2. Different analyses confirm that the designed AuNPs can be good candidates for antiviral agents against COVID-19 disease.

The recent outbreak of a novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has challenged the world.1–3 Different types of coronaviruses can infect humans and mammalian animals.4,5 The coronavirus has alpha, beta, delta, and gamma types, of which alpha and beta types can infect humans, and there is no approved antiviral or vaccine for this virus.6 SARS-CoV-2 has a diameter in the range of 50–500 nm and is composed of four proteins. These proteins are known as N (nucleocapsid), S (spike), M (membrane), and E (envelope) proteins.2 On the basis of electron microscopy studies, the S protein can attach to the host-cell membrane.7,8 The host cellular receptor for the receptor–binding–domain (RBD) of S1 (the subunit of S protein) is angiotensin-converting enzyme 2 (ACE2), which has a considerable affinity to the SARS-CoV-2.9–11

There are many computational and experimental attempts by different research groups around the world to find a vaccine or antiviral for SARS-CoV-2. Xu and co-workers proposed the potential of niclosamide as the antiviral agent for SARS-CoV-2.12 This antiviral agent has considerable effects against different viruses, such as Middle East respiratory syndrome coronavirus (MERS-CoV), Zika virus, Japanese encephalitis virus (JEV), hepatitis C virus, and severe acute respiratory syndrome coronavirus (SARS-CoV). Sequence analysis reveals that 79.5% of the genus of SARS-CoV-2 is similar to the sequence of SARS-CoV and MERS-CoV; therefore, niclosamide can be considered as an antiviral agent for COVID-19.1,12

Nutho et al., by employing theoretical methods, investigated the ability of lopinavir and ritonavir drugs as inhibitors against COVID-19.13 Their obtained results revealed that ritonavir shows greater interactions with the active site of SARS-CoV-2 because of higher electrostatic and dispersion interactions than lopinavir. Moreover, Wang examined the ability of carfilzomib, eravacycline, valrubicin, and elbasvir as inhibitors against SARS-CoV-2.14 They proposed that among these drugs, carfilzomib is a better inhibitor against this virus.

Nontoxic antiviral nanoparticles can be designed as a large drug and because of large size have an interesting ability to block the whole COVID-19 binding surface.15 In this context, Stellacci et al. reported the considerable activity of the functionalized gold nanoparticles against herpes simplex virus (HSV), human papilloma virus (HPV), and respiratory syncytial virus (RSV).16 On the basis of the results, the nanoparticles functionalized by undecanesulfonic acid or
octanethiol have no cytotoxicity with irreversible activity against the mentioned viruses.

In this work, by employing computational methods, the ability of the functionalized gold nanoparticle (AuNP) (Table 1) by different groups was examined for blocking the RBD of the SARS-CoV-2. These nanoparticles, because of their large size, cover the whole binding surface of RBD, in contrast to other reported small agents. In this research, we try to provide new insight into the potential of nontoxic nanoparticles as new inhibitors for COVID-19. The gold nanoparticle was selected for this study because previous studies confirmed that functionalized AuNPs have considerable ability for use as an antiviral agent against different viruses and bacteria. Moreover, functionalized AuNPs with octanethiol, undecanesulfonic acid, 8-mercaptoctan-1-aminium, and 3-

Table 1. Complete Name and Abbreviation of the Studied Functional Groups with Their Number on the AuNP Surface

| number | functional group                                      | abbreviation                     | N<sup>a</sup> |
|--------|------------------------------------------------------|----------------------------------|----------------|
| 1      | Amin: 8-mercaptopooctan-1-aminium                    | AuNP-Amin                        | 30             |
| 2      | EG2: 2-(6-mercaptophexyl)oxyethoxyethan-1-ol         | AuNP-EG2                         | 30             |
| 3      | Mes: 3-mercaptopethylsulfonate                       | AuNP-Mes                         | 30             |
| 4      | Mus: Undecanesulfonic acid                          | AuNP-Mus                         | 30             |
| 5      | Mus-Ot: Undecanesulfonic acid/Octanethiol            | AuNP-Mus-Ot                      | Mus: 15/Ot:15  |
| 6      | Ot: Octanethiol                                      | AuNP-Oct                         | 30             |
| 7      | Pep: Cys-Gln-Thr-Asp-Lys-His-Glu-Glu-Asp-Tyr-Gln-Met-Lys-Gly-Asp-Arg | AuNP-Pep             | 8               |

“Number of the functional groups on the AuNP surface.

Figure 1. Obtained structures of RBD of SARS-CoV-2 in the presence (A) and absence (B) of ACE2, after 100 MD simulations in physiological solution.

Figure 2. Obtained structures of RBD complexes with different nanoparticles after simulation.
mercaptoethylsulfonate are known as nontoxic antiviral agents.16

To investigate the ability of the functionalized gold nanoparticles as inhibitors against COVID-19, full atomistic molecular dynamic (MD) simulations were applied. The crystal structures of ACE2 and the RBD of SARS-CoV-2 were obtained from the protein data bank (PDB: 6M17).1 The diameter of the pure AuNPs is 2 nm, and their functionalization by different groups (the functional groups with their abbreviations are reported in Table 1) completely covers their surfaces. To examine the potential of binding of the functionalized AuNPs to the RBD of COVID-19, these nanostructures were considered in the vicinity of RBD with 10 Å minimum distance and simulated in physiological solution (150 mM NaCl). Computational details are presented in the Supporting Information.

By employing MD simulations, the ability of the functionalized AuNPs as inhibitors against the RBD of COVID-19 was investigated. The previous study on the functionalized AuNPs by Mus, Ot, and EG2 confirmed that these nanostructures are powerful antiviral agents against HSV, HPV, and RSV viruses.16 To the best of our knowledge, there is not any report about the inhibitory properties of AuNP-Amin, AuNP-EG2, AuNP-Mes, AuNP-Mus, and AuNP-Ot against SARS-CoV-2; therefore, investigation of the interactions between these nanoparticles and RBD can provide new insight into the ability of the nanostructures for COVID-19 treatment. Structural analysis of ACE2 and RBD of SARS-CoV-2 in crystal structure shows 15 amino acid residues of ACE2 interact with RBD (the Pep sequences in Table 1), which can be considered as the critical amino acids. Therefore, a new functional group based on these amino acids, Pep, was designed.

Figure 1, shows the structure of RBD in the presence and absence of ACE2 after 100 ns of MD simulations. According to this figure, the α1 and α2 helices and the linker between β3 and β4 of ACE2 contribute to the interaction with RBD. The obtained complexes of the functionalized AuNPs with RBD (Figure 2) show that AuNP-EG2, AuNP-Ot, and AuNP-Pep can cover the whole binding surface of RBD in comparison with other functionalized AuNPs. Moreover, trajectory analysis shows that AuNP-Mes leaves the RBD after 70 ns; therefore, this nanoparticle does not form a complex with RBD because of considerable distance with the binding surface. According to Figure 2, because of the long length of Pep, AuNP-Pep can trap RBD, forming a more stable complex with RBD than other nanostructures.

To compare the dynamical behavior of RBD in the presence of ACE2 and different functionalized AuNPs, root-mean-square deviation (RMSD) analysis was applied (Figure S1). The calculated average RMSD (Figure 3A) values confirmed that RBD interacts considerably with the corresponding structures, yielding the stable complex. The calculated average values of RMSD of the RBD in the absence and presence of ACE2 are 3.69 and 3.36 Å, respectively. The increase in the fluctuation of RBD in the presence of AuNP-Mes confirms a lower interaction between RBD and nanoparticle that can be explained by their higher distance.

According to the RMSD results, AuNP-Pep reduces the RBD fluctuation in comparison with ACE2. This means that this nanoparticle forms a stronger interaction with RBD. The calculated radius of gyration (Rg) indicates that functionalized AuNPs affect the dynamical behavior and structural properties of RBD (Figure 3B), but because of small differences, a conclusion based on the calculated Rg values cannot provide meaningful results; therefore, other structural analyses were
performed. The calculated average center of mass distances (Figure 3C) between the RBD and ACE2 with different nanoparticles reveal that AuNPs have a lower distance with RBD because of smaller size than ACE2. Among the corresponding functionalized AuNPs, AuNP-Pep has the minimum distance with RBD, which confirms greater interaction with RBD than other nanoparticles.

One of the important factors that shows the potential of acting as an inhibitor against SARS-CoV-2 is that the agent covers the whole binding surface of RBD. Figure 3D shows the calculated average solvent accessible surface area (SASA) of RBD in the presence of ACE2 and nanoparticles. According to this figure, ACE2 completely covers the surface of RBD that reduces the SASA. AuNP-Pep among the studied nanoparticles significantly decreases the SASA of RBD. The calculated average SASA values of the RBD, in the presence of ACE2 and AuNP-Pep, are 8941 and 8970 Å², respectively. This result confirms the remarkable effect of AuNP-Pep on the RBD, which covers the whole binding surface of RBD, similar to ACE2.

To compare the effects of ACE2 and nanostructures on the structural properties of RBD, the structure of RBD in the presence of ACE2 was aligned to the RBD structure in the presence of different nanoparticles (Figure S2). On the basis of the calculated RMSD values for the aligned structures, AuNP-Pep represents a behavior similar to ACE2 against RBD. In other words, AuNP-Pep has the same effects as ACE2 on RBD, confirming the interesting potential of this nanoparticle as an inhibitor for SARS-CoV-2. It is well worth mentioning that the number of peptide groups on the surface of AuNP-Pep can be important in the RBD interaction and this nanoparticle. We assumed that the whole surface of AuNP has been covered by peptide groups. In this context, to calculate the coverage of the peptide groups on the AuNP, the number of water molecules (having a distance of 4 Å from the surface of the nanoparticles) around the pure AuNP and AuNP-Pep were calculated (Figure S3). The calculated average number of water molecules around the AuNP and AuNP-Pep are 938 and 106, respectively. This result indicates that the interactions of the Au atoms (of the gold nanoparticle) and water molecules reduce 88.6% in the presence of the peptide groups. In other words, peptide groups can cover the whole surface of AuNP, approximately.

Hydrogen bond (H-bond) interaction is one of the important parameters that change the stability and dynamical behavior of the biomolecules. Figure 4A shows the calculated average H-bond interactions inside the RBD in the presence of different hosts. According to this figure, in the presence of ACE2 and nanoparticles, the possibility of H-bond formation is improved, which is due to an increase in the structural compactness of RBD (according to Rg analysis). Comparison between the calculated electrostatic and van der Waals (vdW) interactions, which are obtained by employing linear interaction energy (LIE) analysis method,20 for the RBD complexes with ACE2 and different nanoparticles can determine the potential of the functionalized AuNPs as an inhibitor against SARS-CoV-2. Figure 4B shows the frequency
of the observed electrostatic interaction energy (EIE) between the RBD and different hosts. According to this figure, AuNP-Pep has the maximum EIE with RBD in comparison with other hosts. In addition to covering the whole binding surface of RBD, AuNP-Pep can trap RBD because of the long length of the functional groups on its surface.

According to Figure 4C, AuNP-Amin, AuNP-EG2, AuNP-Mes, AuNP-Mus, AuNP-Mus-Ot, and AuNP-Ot have vdW interactions similar to those of the RBD of the SARS-CoV-2, while AuNP-Pep represents a stronger vdW interaction. On the basis of EIE and vdW analyses, AuNP-Pep in the presence of ACE2 as a human host for SARS-CoV-2 can form a more stable complex with RBD. Therefore, AuNP-Pep is proposed as a good candidate for using as an inhibitor against COVID-19.

To compare the effects of the hosts on the amino acid residues of the RBD, root-mean-square fluctuation (RMSF) analysis was applied. According to Figure 4D, the residues of 20–60 and 120–160 of the RBD have the maximum fluctuation. Because the structural analysis reveals that these residues form the binding surface of RBD, the decrease in the fluctuation of amino acids (Figure 4D) in the presence of ACE2 and different nanoparticles confirms their considerable interactions.

To have a comprehensive insight into the inhibitory potential of the functionalized AuNPs against RBD, Gibbs binding energies (ΔGbin) of the complex formation between the RBD and nanoparticles were calculated. To do this, the MM-PBSA and MM-GBSA methods were employed through quasi-harmonic entropy approximation. The calculated ΔGbin values, by both methods, reveal that the functionalized gold nanoparticles have a remarkable interaction with RBD but less than the interaction between RBD and ACE2, which is according to the results reported in previous sections. This is true for all nanoparticles except AuNP-Pep, which interacts with RBD greater than the RBD and ACE2 human receptor. This result can be due to smaller electrostatic and vdW interactions between the functionalized AuNPs and RBD. Electrostatic and vdW interactions have a remarkable role in the stability of the functionalized AuNP complexes with RBD. On the other hand, these parameters are the main part of calculating ΔGbin in MM-PBSA and MM-GBSA methods. MM-PBSA and MM-GBSA analyses reveal that functionalized gold nanoparticles (except AuNP-Pep) have lower vdW and electrostatic interactions with RBD in comparison with ACE2, similar to the results obtained from the LIE method. Therefore, the calculated Gibbs binding energies for these nanoparticles confirm the lower stability of the corresponding complexes. Moreover, other reported results confirmed that it can be possible to design new nanostructures based on peptides, which have a higher affinity to RBD in comparison with ACE2. On the basis of Table 2, AuNP-Pep forms the most stable complex with RBD in comparison with ACE2 and other nanoparticles. AuNP-Pep forms the most stable complex with RBD than other receptors because of the strong vdW and electrostatic interactions with RBD, according to the LIE analysis. This result clearly shows the noticeable ability of AuNP-Pep to act against SARS-CoV-2.

By employing full atomistic MD simulations, the inhibition ability of the functionalized AuNPs against SARS-CoV-2 was investigated. AuNP-EG2, AuNP-Ot, and AuNP-Pep cover the whole binding surface of RBD of the SARS-CoV-2. On the basis of the obtained results, ACE2 and designed nanoparticles increase the structural compactness of RBD, confirming the effects of the corresponding nanostructures on the RBD. Binding energy analysis reveals that among the studied nanoparticles, AuNP-Pep forms the most stable complex with RBD. In other words, this nanostructure has a great potential to inhibit the RBD of the SARS-CoV-2. Moreover, aligned structures of the RBD in the presence of ACE2 and different functionalized AuNPs confirm that AuNP-Pep represents a behavior similar to ACE2 against RBD. These proposed functionalized gold nanoparticles can be used as an inhibitor against COVID-19.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.0c02677.

Molecular dynamics simulations details, calculated Rg and RMSD of the RBD in the presence of different hosts (Figure S1), aligned structures of RBD in the presence of ACE2 and different nanoparticles and the calculated aligned RMSD for the corresponding structures (Figure S2), and the calculated number of water molecules around the pure AuNP and functionalized AuNP with peptide groups (Figure S3) (PDF)

### AUTHOR INFORMATION

**Corresponding Author**

Mohammad Izadyar – Computational Chemistry Research Laboratory, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran; orcid.org/0000-0002-3795-9982;
Phone: +985138805533; Email: izadyar@um.ac.ir;
Fax: +985138796416

**Author**

Aliyeh Mehrifar – Computational Chemistry Research Laboratory, Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jpclett.0c02677

**Notes**

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### Table 2. Calculated ΔGbin (kcal·mol⁻¹) of the RBD Complexes with ACE2 and Different Functionalized AuNPs

| structure        | ΔGbin | SD  | ΔGbin | SD  |
|------------------|-------|-----|-------|-----|
| AuNP-Amin        | −134.06 | 6.71 | −162.95 | 8.14 |
| AuNP-EG2         | −117.09 | 5.85 | −156.84 | 7.84 |
| AuNP-Mes         | −143.72 | 7.18 | −136.06 | 6.81 |
| AuNP-Mus         | −154.84 | 7.74 | −193.04 | 9.65 |
| AuNP-Mus-Ot      | −117.63 | 5.74 | −152.60 | 7.63 |
| AuNP-Ot          | −82.98  | 4.14 | −152.31 | 7.61 |
| AuNP-Pep         | −330.72 | 14.72 | −362.95 | 15.18 |
| RBD-ACE2         | −193.32 | 9.66 | −241.92 | 12.09 |
performed at the Sci-HPC center of Ferdowsi University of Mashhad.

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