Tungsten Oxide Nanodots Exhibit Mild Interactions with WW and SH3 Modular Protein Domains

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ABSTRACT: Tungsten oxide nanodot (WO$_3$−$x$) is an active photothermal nanomaterial that has recently been discovered as a promising candidate for tumor theranostics and treatments. However, its potential cytotoxicity remains elusive and needs to be evaluated to assess its biosafety risks. Herein, we investigate the interactions between WO$_3$−$x$ and two ubiquitous protein domains involved in protein–protein interactions, namely, WW and SH3 domains, using atomistic molecular dynamics simulations. Our results show that WO$_3$−$x$ interacts only weakly with the key residues at the putative proline-rich motif (PRM) ligand-binding site of both domains. More importantly, our free energy landscape calculations reveal that the binding strength between WO$_3$−$x$ and WW/SH3 is weaker than that of the native PRM ligand with WW/SH3, implying that WO$_3$−$x$ has a limited inhibitory effect over PRM on both the WW and SH3 domains. These findings suggest that the cytotoxic effects of WO$_3$−$x$ on the key modular protein domains could be very mild, which provides new insights for the future potential biomedical applications of this nanomaterial.

INTRODUCTION

With the rapid development of nanoengineering and nanoscale particle-based biotechnology, nanoparticles (NPs) have emerged as important candidates in modern medicine involving many fascinating possibilities in the field of therapeutics and other biomedical applications. For example, carbon-based nanoparticles have been proposed as treatment for Alzheimer’s disease by inhibiting amyloid peptide aggregation. Considering the ever growing number of nanoparticle applications that have entered our daily lives, the concerns about the potential undesirable interactions between the nanoparticles and the biological systems have recently taken on added urgency as it is essential for the assessment of their human and environmental health implications. Indeed, both experimental and theoretical results have shown that there are structural changes and function disruptions of the biomolecules upon binding to NPs. For example, Ge et al. theoretically and experimentally demonstrated that there were significant conformational changes of the bovine fibrinogen and gamma globulin while binding to single-wall carbon nanotubes (SWCNTs). Likewise, when interacting with SWCNTs, conformational changes also occurred in the subdomain of human serum albumin (BSA) upon binding to these NPs. Meanwhile, Lacerda et al. showed that the process of carbon nanotube (CNT) interfering with cell membranes could cause disruption to their functions. To make matters worse, depending on how the experiments were carried out, different and controversial outcomes could be observed. For instance, contrary to the abovementioned studies, Kam and Dai showed that some proteins, including streptavidin, protein A, BSA, and cytochrome c, could be transported by CNT into mammalian cells through the noncovalent nanotube–protein conjugates without much loss of their functions. Similar results were also reported that some small peptides could be encapsulated into the internal space of CNTs with no significant change in their structures. In addition, some animal studies have found that, although the functionalized CNTs intravenously injected into the blood circulation system showed no obvious toxic side effects in mice during different periods of evaluation time, the high accumulation of CNTs in the major organs, such as the liver, spleen, and lung, raised serious concerns.

These results indicate that more studies with complimentary approaches might be needed to gain further insights on the complicated pharmacokinetics of nanoparticles.

Proteins are one of the major types of biomolecules that are usually bound with specific ligands to perform their biological functions. In fact, the ligand–protein binding process is one of
the crucial steps in activating cell signaling cascades, and hence, its disruption can lead to unexpected biological effects. Our previous study has demonstrated that SWCNT can overtake the native ligand proline-rich motif (PRM) upon binding to the pocket of the SH3 domain, suggesting that the hydrophobic SWCNT was toxic to the proteins.\(^{26}\) The same phenomenon was also found for endohedral metallofullerene, Gd@C\(_{82}\)(OH)\(_{22}\), in which it interferes with PRM binding to both the SH3 and WW domains.\(^{27,28}\) Besides, it has been demonstrated that CNT can interact with the hydrophobic core of WW domains to form a stable complex, disrupting and blocking the active sites of these proteins, and thus leading to protein dysfunction.\(^{29}\) Therefore, a deeper understanding of how nanoparticles interact with biomolecules, particularly those ubiquitous domains involved in protein–protein interactions, at the molecular and atomic level is crucial for exploring their full potential in nanopharmacology and nanomedicine.

Tungsten oxide nanodot (WO\(_{3-x}\)) is an active photothermal nanomaterial that has been explored in a variety of applications such as photoacoustic imaging\(^ {30}\) and photothermal agents\(^ {31}\) for cancer diagnoses and treatments\(^ {32}\) due to its inherent radiosensitization effect and strong local surface plasma resonance (LSPR). Furthermore, our recent study demonstrated that WO\(_{3-x}\) exhibits a significant inhibitory effect on both Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus strains, suggesting that it can be used as effective antibacterial agents.\(^ {33}\) Despite the remarkable potential of WO\(_{3-x}\) in the biomedical field, its toxicological effects on important functional protein domains, such as WW and SH3 domains involved in the protein–protein interaction in cell signaling and regulatory pathways, remain to be elucidated. In this paper, we employed molecular dynamics simulations to investigate the underlying mechanism of how WO\(_{3-x}\) interacts with the WW and SH3 domains, aiming to better understand its intrinsic binding characteristics as well as potential inhibitory effects on these critical modular protein domains.

**RESULTS AND DISCUSSION**

For the molecular dynamics simulations, two different configurations were set up for each of the WW and SH3 domains. The first one is a binary system containing four WO\(_{3-x}\) molecules placed in tetrahedral arrangement surrounding the WW/SH3 domain to examine the nanoparticle’s intrinsic property of recognizing and binding the protein (Figure 1A,B). The second one is a ternary system that consists of both a WO\(_{3-x}\) molecule and the native PRM ligand (i.e., GTPPPPYPYTVG) together with the WW/SH3 domain for the purpose of evaluating the binding competition between the nanoparticle and ligand to the binding site of the target protein (Figure 1C,D).

**Intrinsic Binding Characteristics of WO\(_{3-x}\) to the WW Domain.** To investigate the intrinsic binding characteristics of WO\(_{3-x}\) to the WW domain, we calculated the site-specific contact ratio for each residue of the domain in the binary system by counting the number of frames where the residue was in contact with the nanoparticles over all frames from the simulation trajectories. Figure 2A shows the site-specific contact ratio analysis, which indicates that the residues that interact with the WO\(_{3-x}\) molecules are mainly from the loop region near the helices of the protein. Since positively/negatively charged residues and polar/nonpolar residues from the loop region can all interact with WO\(_{3-x}\), the contact ratio is not solely determined by the nature of the side chains but rather mostly by the backbone conformation and the availability of multiple side chains in the same region in this case. A representative structure of the WW domain colored according to the contact ratio is illustrated in Figure 2B, whereas a representative snapshot of the interactions between the WW domain and WO\(_{3-x}\) molecules during the simulations is given in Figure 2C. Regarding the native PRM recognition site in the WW domain, there are some certain key residues located at the binding pocket (on-site binding), including Y28, W39, T37, K30, H32, and Q35, which are crucial for pairing with the proline residues of the PPxY motif of the PRM.\(^ {28,34–36}\) Among them, Y28 and W39 are the two essential residues for the native PRM ligand binding. From the contact ratio analysis, it can be seen that, except W39, all other residues at the putative PRM binding site of the WW domain do not have frequent contacts with the WO\(_{3-x}\) molecules, implying that the PRM binding site is not particularly vulnerable to be attacked by WO\(_{3-x}\).

By comparison, in the direct binding of the Gd@C\(_{82}\)(OH)\(_{22}\) with the WW domain (the identical one) reported in our previous work,\(^ {28}\) Gd@C\(_{82}\)(OH)\(_{22}\) contacts much more often with the aforementioned key residues, especially with both the Y28 and W39 by \(\pi-\pi\) and/or \(\pi-\text{cation}\) interactions, indicating that Gd@C\(_{82}\)(OH)\(_{22}\) has a stronger binding strength to the putative PRM binding site than WO\(_{3-x}\). Similarly, our previous work on the interactions between SWCNT and the WW domain also demonstrated that SWCNT tends to plug into the hydrophobic core of the WW domain, interfering and blocking the active site through the hydrophobic and \(\pi-\pi\) stacking interactions between the nanoparticle and key residues Y28 and W39, leading to the loss of biological functions in the WW domain.\(^ {29}\) Thus, the binding behavior of WO\(_{3-x}\) to the WW domain is far less intense and does not cause severe inhibitory effects on the putative PRM binding site as those two carbon-based NPs.

Following the same protocol in our previous study on Gd@C\(_{82}\)(OH)\(_{22}\),\(^ {27,28,37,38}\) we constructed the binding free energy landscape by the potential of mean force (PMF) using
Here, $S_{PM}$ is the contacting surface area and $D_{KM}$ is the minimum distance between the key residues (Y28 and W39) of the WW domain and $WO_{3-x}$. As can be seen from Figure 2D, the global minimum is found at $S_{PM} = 237 \, \text{Å}^2$ and $D_{KM} = 2.25 \, \text{Å}$ with a binding free energy of $-1.8 \, \text{kcal/mol}$, which is smaller than the binding free energy of $-5.44 \, \text{kcal/mol}$ found in the Gd@C$_{82}$ (OH)$_{22}$ and WW domain interactions$^{28}$ meaning that the binding strength of the $WO_{3-x}$ to the WW domain is quite weak compared with Gd@C$_{82}$ (OH)$_{22}$.

**WO$_{3-x}$ Exhibits a Mild Inhibitory Effect on the WW Domain.** From the binary binding system, we learn that $WO_{3-x}$ binds only weakly to the WW domain and does not disrupt the protein’s binding pocket, which implies that $WO_{3-x}$ may not compete with the PRM ligand for the binding site. To further investigate this, we analyze the trajectories obtained from the simulations of the ternary system that contains the $WO_{3-x}$ molecule, PRM ligand, and WW domain. Figure 3A shows a representative snapshot of the ternary binding system. Overall, except for a few transient contacts between the $WO_{3-x}$ molecule and PRM ligand, as illustrated in Figure 3B, there is no detection of $WO_{3-x}$ interfering PRM from binding to the WW domain during the simulations. To verify this observation quantitatively, we conducted a thermodynamic analysis with PMF, as shown in Figure 4.

As can be seen from Figure 4A, the free energy landscape between the WW domain and $WO_{3-x}$ molecule with the existence of the PRM ligand has been changed from that in the binary system (Figure 4A vs Figure 2D), and the potential of mean force for the global minimum is $-1.2 \, \text{kcal/mol}$. More evidence can also be found from the binding free energy analysis for the interaction between the WW domain and PRM ligand in the presence of $WO_{3-x}$ (see the multiple binding and unbinding events in Figures S1 and S2). As seen in Figure 4B, the PRM ligand favorably interacts with on-site binding residues of the WW domain in the presence of $WO_{3-x}$ with a binding free energy of $-3.2 \, \text{kcal/mol}$ ($S_{PM} = 413 \, \text{Å}^2$, $D_{KM} = 2.25 \, \text{Å}$). This further verifies that the native binding between the WW domain and PRM ligand is not interfered by the $WO_{3-x}$ suggesting that the $WO_{3-x}$ is less competitive than the PRM ligand while binding to the WW domain.

**Intrinsic Binding Characteristics of $WO_{3-x}$ to the SH3 Domain.** Next, we continue to investigate the intrinsic binding characteristics of $WO_{3-x}$ to the SH3 domain using the corresponding binary system. Figure 5A shows the site-specific contact ratio analysis, which reveals that most of the residues contacting with $WO_{3-x}$ reside in the edge of the helices of the protein. Similar to the case of the WW domain, Figure 5B depicts the structure of SH3 colored according to the contact ratio, whereas Figure 5C presents several snapshots from the simulations, showing that the $WO_{3-x}$ molecules mostly interact...
with the loop region of the SH3 domain and tend to aggregate. From the contact ratio analysis, we found that the residues N144, N146, N171, E176, K178, and R179 have high contact probability with the WO3\(^{-}\) molecules. However, none of these residues belong to the list of key binding site residues (F141, F143, D147, E149, D150, E166, E167, W169, P183, P185, and Y186) of the SH3 domain involved in the SH3-PRM recognition based on the X-ray crystal structure, suggesting that WO3\(^{-}\) is nonspecific to the on-site binding residues of the SH3 domain. In contrast to the results of our previous studies on the direct binding of Gd@C\(_{82}\)(OH)\(_{22}\)/SWCNT with the SH3 domain, many key binding site residues are highly involved in contacting with both the Gd@C\(_{82}\)(OH)\(_{22}\) and SWCNT\(^{26}\) NPs, causing the blockage at the putative PRM binding site. This high contact probability of the key residues in the SH3 domain with the Gd@C\(_{82}\)(OH)\(_{22}\)/SWCNT is due to the interactions between the Gd@C\(_{82}\)(OH)\(_{22}\)/SWCNT NPs and the hydrophobic/aromatic residues (\(\pi-\pi\) stacking interactions) located around the binding site, even though the Gd@C\(_{82}\)(OH)\(_{22}\) is amphiphilic. Indeed, the hydrophobic interactions act as the dominant force for the binding of Gd@C\(_{82}\)(OH)\(_{22}\)/SWCNT to the SH3 domain. It is worth noting that WO3\(^{-}\) seems to interact less favorably with the SH3 domain than with the WW domain, as evidenced by the fact that the overall contact probability of WO3\(^{-}\) is smaller when interacting with SH3 (Figure 5A vs Figure 2A), which is consistent with our previous findings on CNT and Gd@C\(_{82}\)(OH)\(_{22}\)’s interactions with these two domains.\(^{26-29}\)

Again, we constructed the binding free energy landscape along the two reaction coordinates (Figure 5D): the minimum distance (\(D_{KM}\)) between key residues (F141, W169, P183, and Y186) and WO3\(^{-}\) and the contacting surface area (\(S_{PM}\)) between SH3 and WO3\(^{-}\) to examine the binding strength of WO3\(^{-}\) with the SH3 domain. The PMF analysis shows that the binding free energy is higher than \(-0.4\) kcal/mol with multiple shallow minima and relatively large \(D_{KM}\). Compared with the binding free energy surface of the Gd@C\(_{82}\)(OH)\(_{22}\) and SH3 domain (\(-5.23\) kcal/mol),\(^{23}\) the one between WO3\(^{-}\) and SH3 is much smaller, suggesting that the binding strength of WO3\(^{-}\) to SH3 is weak, which agrees with the site-specific contact ratio analysis.

**WO3\(^{-}\) Exhibits a Mild Inhibitory Effect on the SH3 Domain.** As in the case of the WW domain, the simulation trajectories of the ternary system consisting of a WO3\(^{-}\)/PRM/WW domain simulation. WO3\(^{-}\) does not affect the binding between the PRM and WW domain. PRM occasionally adsorbs onto WO3\(^{-}\) which is not stable over the simulation.

![Figure 3. Ternary binding system of WO3\(^{-}\), PRM, and the WW domain. (A) Structures from the WO3\(^{-}\)/PRM/WW domain simulation. WO3\(^{-}\) does not affect the binding between the PRM and WW domain. (B) PRM occasionally adsorbs onto WO3\(^{-}\) which is not stable over the simulation.](https://pubs.acs.org/journal/acsodf)

![Figure 4. (A) Free energy landscape of WO3\(^{-}\) interaction in the WO3\(^{-}\)/PRM/WW domain competitive-binding simulation. The minimum of \(-1.7 \pm 0.5\) kcal/mol is located at (2.25 Å, 215 Å\(^2\)). (B) Free energy landscape of PRM interaction in the WO3\(^{-}\)/PRM/WW domain competitive-binding simulation. The minimum of \(-3.2 \pm 2.0\) kcal/mol is located at (2.25 Å, 413 Å\(^2\)).](https://dx.doi.org/10.1021/acsomega.0c00822)
located at $(S_{DM}, D_{KM}) = (237 \text{ Å}^2, 10.6 \text{ Å})$ with a binding free energy of $-0.7 \text{ kcal/mol}$ (Figure 7A). For comparison purposes, the binding free energy landscape between the PRM and SH3 domain with the presence of WO$_{3-x}$ was also
evaluated as another perspective (also see the multiple binding and unbinding events in Figures S3 and S4). Figure 7B clearly shows that the PRM ligand actively interacts with the key residues of the SH3 domain despite the existence of WO3−x and the global minimum is located at 1.55 Å with a binding free energy of −3.5 kcal/mol, which is lower than the one between WO3−x and SH3 with the presence of PRM. In addition, it can be seen from Figure 7B that the WO3−x molecule only binds to the residues far away from the putative PRM binding site. Taken all together, it is clear that WO3−x is less competitive than PRM while binding to the SH3 domain.

**CONCLUSIONS**

In this study, using all-atom molecular dynamics simulations, we investigated the recognition and binding characteristics of WO3−x with respect to the two important protein–protein interaction mediators, namely, the WW and SH3 domains. More importantly, we also explored whether WO3−x competes with the native proline-rich motif ligand (PRM) for the binding sites of the two domains to assess its inhibitory effects on them. We found that WO3−x interacts only weakly with the key binding residues (except for the W39) of WW domains, and the interactions are nonspecific to the residues at the binding pocket for SH3 domains. In addition, the SH3 domain has a smaller contact ratio than the WW domain in binding with the WO3−x. Moreover, the binding free energy of the WO3−x to both the WW (−1.8 kcal/mol) and SH3 (−0.4 kcal/mol) domains are relatively weak as compared to the values of −5.44 and −5.23 kcal/mol for the Gd@C82(OH)22 to the WW and SH3 domains, respectively. Further investigations on the potential interference of WO3−x in the ternary binding systems (consisting of the WO3−x, the ligand PRM, and the WW/SH3 domain) reveal that the binding strength between WO3−x and WW/SH3 is weaker than that of PRM with the WW/SH3 domains, and WO3−x does not interfere strongly with the binding between PRM and the WW/SH3 domains.

The mild interaction between WO3−x and the WW/SH3 domains might be attributed to the hydrophilic surface of WO3−x and its relatively large size. That is, WO3−x does not strongly interrupt with the hydrophobic interactions between the WW/SH3 and PRM. In short, our results reveal that the potential inhibitory effect of WO3−x on important modular protein domains such as WW and SH3 is very mild, and hence, its adverse effects to the biological systems could be limited. However, the long-term cytotoxic effects of such nanoparticles need to be evaluated further at the cellular and tissue levels when considering their use for biomedical applications.

**SYSTEM AND METHOD**

The model and force field parameters for WO3−x were taken from our previous work, which were calibrated to reproduce QM charge distribution and interaction and an experimental superhydrophilic surface property. The CHARMM36m force field was used for protein, water, and ion. The simulation system was built using CHARMM-GUI and VMD. Following our previous protocols, WO3−x intrinsic binding and WO3−x/PRM competitive binding simulations were conducted. In the intrinsic binding simulations, each system consists of one WW domain or SH3 domain protein at the center and four WO3−x nanodots placed at the tetrahedral corners of the cubic box. In the competitive binding simulations, the WW/SH3 domain protein was placed at the center while one WO3−x nanodot and one PRM protein were placed at the dihedral corners. The initial box size was (8.2 nm)3 for all systems and the minimum distance between the WW/SH3 domain, and the protein/nanodot ligands was about 3 nm. NaCl was added to yield a salt concentration of 0.15 mol/L. The systems were energy-minimized and then equilibrated at 310 K and 1 atm for 10 ns before 200 ns simulations for intrinsic binding or 600 ns simulations for competitive binding in the NPT ensemble. For each system, five independent simulations with different initial structures were conducted. All the other setups follow the standard procedures and can be found in our previous papers. Convergence analysis in the Supporting Information shows that qualitatively similar results were achieved with 200 ns simulations (see Figure S5).

The two-dimensional free energy surface for WO3−x–protein interaction as a function of distance from key residues (D(KM)) and contact area (S(PS)) was calculated by histogram analysis. The key residues were Y28 and W39 for the WW domain and F141, W169, P183, and Y186 for the SH3 domain. The contact area was calculated from the surface-accessible areas of the WO3−x protein and WO3−x–protein complex. The free energy was normalized so that the location with no interaction has a free energy of zero. Here, the reference state was chosen to be D(KM) = 15 Å and S(PS) = 0. Since the normalization procedure was not conducted in our previous work, the free energy surface in this work cannot be directly
compared to previous ones. A cutoff distance of 3 Å was used for calculating the contact ratio.

**ASSOCIATED CONTENT**

1. Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00822.

Details of the convergence analysis, evolution of contact area between the WO3−x and WW domain in the intrinsic binding simulations (Figure S1), evolution of contact areas between the PRM/WO3−x and WW domain in the competitive binding simulations (Figure S2), evolution of contact areas between the WO3−x and SH3 domain in the intrinsic binding simulations (Figure S3), evolution of contact areas between the PRM/WO3−x and SH3 domain in the competitive binding simulations (Figure S4), and binding free energies calculated from the competitive binding simulations (Figure S5) (PDF)

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**Author Contributions**

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**Notes**

The authors declare no competing financial interest.

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