# Influence of Charged Groups on the Properties of Zwitterionic Moieties: A Molecular Simulation Study

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S *Supporting Information*

ABSTRACT: Zwitterionic carboxybetaine and sulfobetaine materials have shown an excellent ability to resist nonspecific protein adsorption. It is desirable to obtain a better understanding of zwitterionic materials based on their molecular structures. This work aims to understand the roles of charged groups in zwitterionic moieties and to design new protein-resistant zwitterionic moieties beyond carboxybetaine and sulfobetaine. We conducted molecular simulations to study the hydration, self-association, and protein interactions of 12 zwitterionic moieties derived from three anionic groups (carboxylic, sulfonate, and sulfate) and four cationic groups (quaternary ammonium, tertiary ammonium, secondary ammonium, and primary ammonium). The partial charges of atoms in these moieties were obtained from quantum chemical calculations. Hydration was studied by evaluating the hydration free energy of moieties and the hydration structure and dynamics of the charged groups. All zwitterionic moieties have strong hydration, but their structural and dynamic properties depend on the types of cationic and anionic groups involved. The self-association and protein interactions of zwitterionic moieties also show relationships with the charged groups. Our simulation results indicate good protein-resistant ability of several zwitterionic moieties, one of which has also been shown by recent experiments.
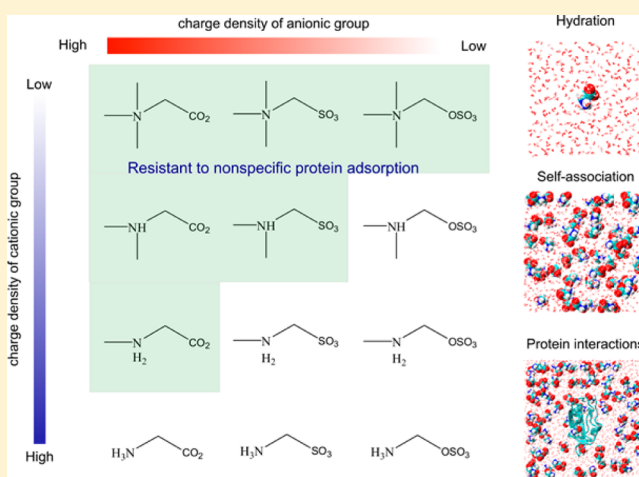
1. INTRODUCTION

Zwitterionic carboxybetaine and sulfobetaine materials have shown their excellence in many biological and chemical applications.[1] They resist nonspecific protein adsorption in complex media,[2,3] protect proteins against denaturing effects of urea and high temperature,[4] and prevent the capsule formation *in vivo* for 3 months.[5]

The excellence of zwitterionic materials urges a thorough understanding of their properties based on their molecular structures. The performance of zwitterionic materials depends on the properties of zwitterionic moieties. Our previous studies have demonstrated the distinguishable effects of zwitterionic carboxybetaine from nonionic molecules,[6,7] the differences between carboxybetaine and sulfobetaine,[8,9] and the differences between carboxybetaines with various distances between charged groups.[10] Considering the vast numbers of cationic and anionic groups, zwitterionic moieties can have many molecular structures beyond conventional carboxybetaine and sulfobetaine. This provides a rich pool for understanding the roles of charged groups in determining the properties of zwitterionic moieties.

Charged groups of zwitterionic moieties behave somewhat similarly to ions. Many studies have been conducted to rank ions on the basis of their charges, sizes, hydration, and other properties. One well-known correlation is the Hofmeister series, which was originally used to rank the ability of salts to precipitate proteins and has been employed to explain the influence of ions on other substances.[11−14] Collins et al.[12] and Fennell et al.[15] showed that the similarities in hydration and size determine the ion−ion interactions. Vlachy et al.[16] found that the interactions between organic ions and inorganic ions can be ranked in the same way as those between inorganic ions are. Our previous study[9] showed that zwitterion−ion interactions obey rules similar to those for ion−ion interactions. The hydration of zwitterionic moieties is also suggested to be dominated by electrostatic interactions, similar to the hydration of ions.[17] However, the charged groups of zwitterionic moieties interfere with each other permanently if they are close enough,[10] and this interference depends on their charge densities. Varying the cationic group may influence the properties of the anionic group in the same zwitterionic moiety, and vice versa. Therefore, we may develop design principles for zwitterionic materials based on the knowledge of

ion−ion interactions with a consideration of the unique features of zwitterionic moieties.

We selected several cationic and anionic groups that are common in many natural zwitterionic molecules. We selected three anionic groups: carboxylic (CO2), sulfonate (SO3), and sulfate (OSO3). These three anionic groups all have a net charge of −1, and their molecular volumes follow the order CO2 < SO3 < OSO3. As a result, their charge densities follow the order CO2 > SO3 > OSO3. We selected four cationic groups: quaternary ammonium (NC4), tertiary ammonium (NC3), secondary ammonium (NC2), and primary ammonium (NC1). These four cationic groups all have a net charge of +1, and their molecular volumes follow the order NC1 < NC2 < NC3 < NC4. Consequentially, their charge densities follow the order NC4 < NC3 < NC2 < NC1. From these charged groups, we derived 12 zwitterionic moieties, as shown in Figure 1. The
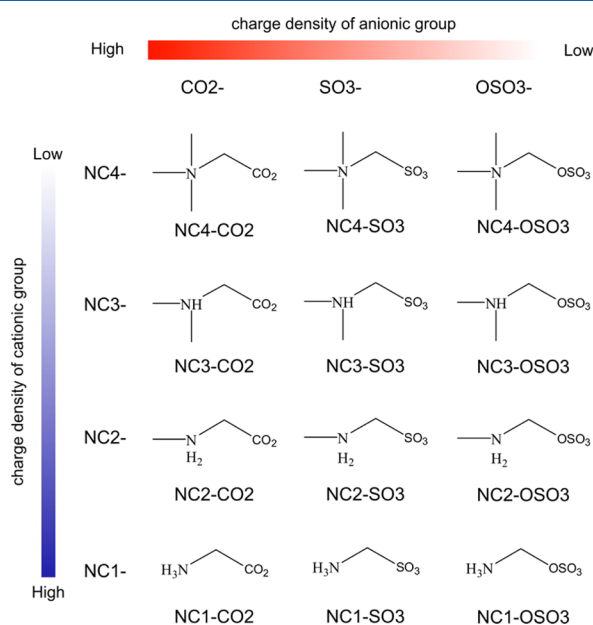

Figure 1. Molecular structures of the 12 zwitterionic moieties studied in this work.

chemical groups between the charged groups were fixed to one methylene group, because a long carbon spacer length compromises the ability to resist nonspecific protein adsorption.

The protein-resistance capability of zwitterionic moieties can be assessed in terms of three properties. The first is the

hydration of zwitterionic moieties. Strong hydration is believed to be the main source of resistance to protein adsorption.[18] The second is self-association among zwitterionic moieties. Too many associations may weaken the hydrophilicity of the zwitterionic moieties[19] and result in a hydrophobic material.[20] The third is the interactions between zwitterionic moieties and proteins. Both proteins and zwitterionic moieties have charged groups.[21] The electrostatic attractions among these charged groups may induce specific zwitterion−protein interactions. These interactions may compensate the repulsive force induced by the strong hydration[22] of zwitterionic moieties and therefore compromise the protein-resistance capability of zwitterionic moieties.

This work will study the hydration, self-association, and protein interactions of the 12 zwitterionic moieties shown in Figure 1. We focus on the relationships between variations in charged groups and the three properties mentioned above. At the same time, we are interested in exploring new protein-resistant zwitterionic moieties beyond conventional carboxy-betaine and sulfobetaine.

2. DETAILS OF MOLECULAR SIMULATIONS

2.1. Partial Charge Calculation. Our previous studies[8,10] showed that the charged groups in zwitterionic moieties have partial charges different from those in other molecules. For instance, the carboxylic group of a glycine betaine moiety has partial charge considerably higher than that of an acetic acid has. The partial charges in the current force fields cannot represent zwitterionic moieties well.[10] Therefore, we calculated the partial charges of atoms for the 12 zwitterionic moieties using quantum mechanical calculations. The quantum mechanical calculations were carried out at HF/6-31G* level using Gaussian 09.[23] For each zwitterionic moiety, 8−12 conformations were generated by rotating the N−C−C−O, N−C−S−O, or N−C−O−S dihedral angles. These conformations were optimized in a vacuum, and then the atomistic charges of individual conformations were obtained with single-point calculation using the CHELP algorithm.[24] Two steps of averages were conducted to obtain the final values. The first is the average among various conformations weighted by the Boltzmann factor. The second is the average among the atoms that should be equal, based on consideration of their chemical and physical environments. Table S2 lists the partial charges of atoms of the 12 zwitterionic moieties. The individual zwitterionic moieties are named to reflect the cationic and anionic groups they possess. For instance, a zwitterionic moiety possessing a primary ammonium group (NC1) and a carboxylic group (CO2) is named as NC1-CO2.
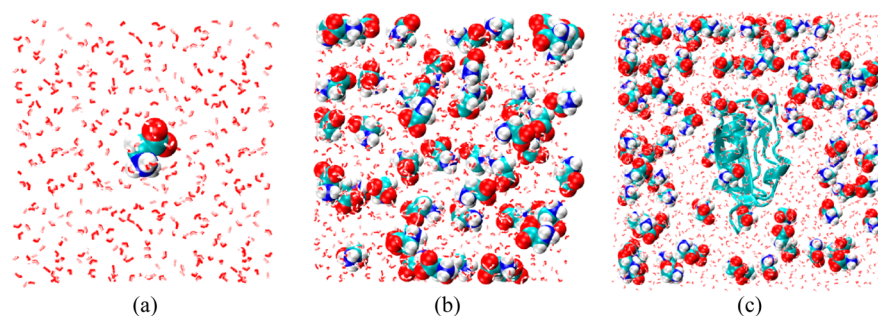

Figure 2. Snapshots of simulation systems for (a) hydration, (b) self-association among zwitterionic moieties, and (c) zwitterion−protein interactions.

2.2. Force Field Parameters. We applied the OPLSAA force field[25] to describe the bonding and nonbonding interactions between molecules. Water molecules were described by the TIP4P model,[26] which is compatible with the OPLSAA force field. The potential energy of intermolecular interactions is calculated as a combination of a Lennard-Jones 12-6 potential and a Coulombic potential, as shown in eq 1, where r_{ij} is the distance between atoms i and j, q_i is the partial charge assigned to atom i, and ε_{ij} and σ_{ij} are energy and size parameters obtained by Jorgensen combining rules.[27]

$$U(r_{ij}) = 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{r_{ij}} \tag{1}$$

2.3. Molecular Dynamics Simulations. *Hydration.* The simulation system was a cubic box containing a zwitterionic moiety and 566 water molecules. After energy minimization and a 5 ns molecular dynamics (MD) simulation with a step of 1 fs in an isobaric–isothermal (NPT) ensemble for energy equilibrium, another 10 ns MD simulation with a step of 2 fs in a canonical (NVT) ensemble was performed for data collection at a frequency of 1 ps. Figure 2a shows a snapshot of the simulation system. Long-range electrostatic interactions were computed with the particle mesh Ewald method with periodic boundary conditions in all three dimensions.[28] The short-range van der Waals interactions were calculated with a cutoff distance of 1.0 nm. During the first 5 ns MD simulation, the system was maintained at 298 K (0.1 ps time constant) and 100.0 kPa with the Berendsen algorithm[29] (with a compressibility of 4.5×10^{-5} bar^{-1} and a 1 ps time constant). During the 10 ns MD simulation for data collection, the system was maintained at 298 K with the Nosé–Hoover algorithm.[30,31] Intramolecular bonds with hydrogen atoms were kept constrained with the LINCS algorithm.[32] The MD simulations were performed using Gromacs 4.5.4.[33] For each zwitterionic moiety, we carried out three independent MD simulations using different initial configurations.

Self-Association among Zwitterionic Moieties. The simulation system was a cubic box containing 50 zwitterionic moieties and 1400 water molecules. After energy minimization and a 5 ns MD simulation with a step of 1 fs in an NPT ensemble for energy equilibrium, another 20 ns MD simulation with a step of 2 fs in an NVT ensemble was performed for data collection at a frequency of 1 ps. Figure 2b shows a snapshot of the simulation system. During first 5 ns MD simulation, the system was maintained at 298 K (0.1 ps time constant) and 100.0 kPa with the Berendsen algorithm[29] (with a compressibility of 4.5×10^{-5} bar^{-1} and a 1 ps time constant). During the 20 ns MD simulation for data collection, the system was maintained at 298 K with the Nosé–Hoover algorithm.[30,31] The MD simulations were performed using Gromacs 4.5.4.[33] The other simulation details were the same as those used in the hydration study.

Zwitterion–Protein Interactions. The simulation system for studying zwitterion–protein interactions was a cubic box containing 100 zwitterionic moieties, 5500 water molecules, and one chymotrypsin inhibitor 2 (CI2, PDB ID: 1YPC). After energy minimization and a 50 ns MD simulation with a step of 1 fs in an NPT ensemble for energy equilibrium, another 50 ns MD simulation with a step of 2 fs in an NVT ensemble was performed for data collection at a frequency of 10 ps. The other simulation details were the same as those used in the hydration

study. The MD simulations were performed using Gromacs 4.5.4.[33] Figure 2c shows a snapshot of the simulation system.

2.4. Free Energy Perturbation. Hydration free energy of individual zwitterionic moieties was calculated using the free energy perturbation (FEP) method. The perturbation process in this work mimics the reversible process of dehydration as described in the literature.[34,35] The negative of the free energy change of this process is the hydration free energy. As shown in eq 2, we change the interaction energy between the zwitterionic

$$U(\lambda) = 4\varepsilon_{ij}(1-\lambda)\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \frac{(1-\lambda)q_i q_j}{r_{ij}} \tag{2}$$

moiety and water molecules, including the van der Waals interaction energy and electrostatic interaction energy, gradually from the normal values to 0 as λ changes from 0 to 1.

This interaction change causes the potential energy of the system to vary gradually from $U_A(\lambda=0)$ to $U_B(\lambda=1)$. The increment of λ in every step should be as small as possible in order to preserve reversibility. Taking into consideration both the precision required in this work and the tolerance of computational cost, we divided λ into 20 intervals from 0 to 1. For every λ_{n+1}, a 1 ns MD simulation was carried out with the initial structure obtained from the final structure of MD simulation of λ_n. The potential energy is the average of the last 500 ps. The other simulation details were the same as those of the hydration study. Five independent FEP calculations with different initial configurations were carried out for each zwitterionic moiety. The FEP calculations in this work were carried out with Gromacs 4.5.4.[33]

3. RESULTS AND DISCUSSION

3.1. Hydration of Zwitterionic Moieties. As listed in Table 1, the hydration free energy of the 12 zwitterionic

Table 1. Hydration Free Energy of the 12 Zwitterionic Moieties (kJ/mol)

anionic group	cationic group			
	NC4	NC3	NC2	NC1
CO2	−261 ± 12	−224 ± 12	−230 ± 25	−216 ± 6
SO3	−251 ± 17	−259 ± 3	−249 ± 9	−240 ± 6
OSO3	−238 ± 14	−303 ± 11	−290 ± 4	−296 ± 8

moieties is lower than that of the (ethylene glycol)$_4$ (EG$_4$) moiety (−180 kJ/mol).[8] A surface covered with EG$_4$ moieties can resist nonspecific protein adsorption well.[18] The low hydration free energy indicates that these zwitterionic moieties should have strong hydration. From the hydration aspect, these zwitterionic moieties should all have a good ability to resist nonspecific protein adsorption.

The hydration free energy of zwitterionic moieties depends on the charge densities of the charged groups. A charged group with a higher charge density may be thought to lead to the lower hydration free energy of zwitterionic moieties. However, the hydration free energies listed in Table 1 do not show this trend. Our previous study[10] has shown that oppositely charged groups in a zwitterionic moiety reduce charge densities each other. A charged group with a higher charge density can reduce the charge density of other charged groups more. Conversely,
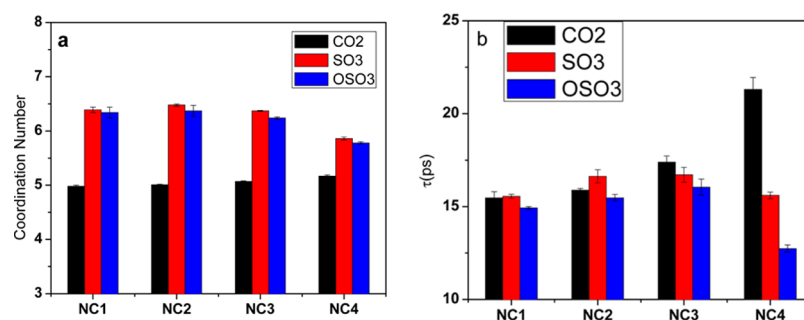
Figure 3. Structural and dynamic properties of water molecules in the first coordination shells of the oxygen atoms of the anionic groups of zwitterionic moieties: (a) coordination number N and (b) residence time τ.
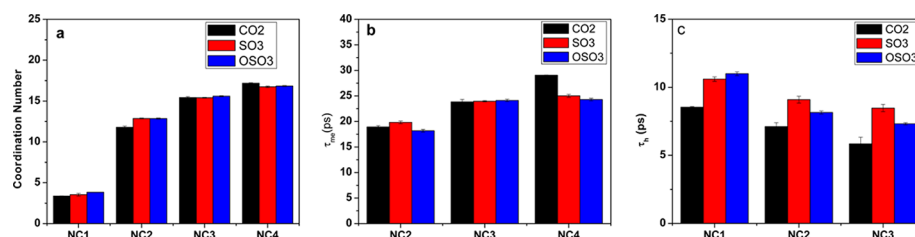

Figure 4. Structural and dynamic properties of water molecules near the cationic group of zwitterionic moieties: (a) coordination number of water molecules in the first coordination shells of carbon atoms or hydrogen atoms attached to the nitrogen atoms, (b) residence time τ_{me} of water molecules in the first coordination shells of carbon atoms of cationic groups, and (c) residence time τ_h of water molecules in the first coordination shells of hydrogen atoms of cationic groups.

its own charge density can be decreased more by other charged groups. The moiety possessing the charged groups with the highest charge densities may have the most reduction in the charge densities of its charged groups, making its hydration free energy comparable with or even higher than those of the moieties possessing charged groups with relatively lower charge densities. For instance, the hydration free energy of NC1-CO2 is higher than that of NC2-SO3.

NC3-OSO3, NC2-OSO3, and NC1-OSO3 have hydration free energies lower than those of other zwitterionic moieties. These three zwitterionic moieties possess an OSO3 group and a cationic group with one or more hydrogen atoms attached to the nitrogen atom. The OSO3 group has the lowest charge density among the three anionic groups and should have the least influence on the partial charges of the cationic groups. Conversely, the cationic groups may have the least influence on the partial charge of the OSO3 group in a zwitterionic moiety.

We further studied the local hydration of the charged groups of zwitterionic moieties by analyzing the numbers and residence times of water molecules in the first coordination shells of certain atoms of charged groups. The radius of the first coordination shell was set as the first-minimum position of the corresponding radial distribution function (RDF) shown in Figures S1−S3. The residence time τ of water molecules is calculated through a numerical integration ($\tau = \int_0^{1 \, ns} C(t) \, dt$) of the residence curve $C(t)$, which was obtained in the same way as in our previous study.[8]

Figure 3 shows the number N and residence time τ of water molecules in the first coordination shells of the oxygen atoms of the CO2, SO3, and OSO3 groups as a function of the type of the cationic group. The values of N of the CO2 group are always less than those of the SO3 and OSO3 groups, consistent with our previous observation[8] that the anionic group of a carboxybetaine moiety has N less than that of the anionic group of a sulfobetaine moiety. The variation in the cationic group

does not influence the number of water molecules near the anionic group.

However, the variation in the cationic group influences the dynamic properties of water molecules near the anionic group. As shown in Figure 3b, when the NC4 group is the cationic group, τ for the CO2 group is much higher than those for the SO3 and OSO3 groups, consistent with our previous observations[8] of carboxybetaine and sulfobetaine moieties. When the cationic group changes to the NC1, NC2, or NC3 group, the values of τ for the three anionic groups are more similar, indicating greater similarity in their attractions to individual water molecules. The cationic groups decrease the charge densities of the carboxylic group and weaken its attractions with individual water molecules.

Variation in cationic groups can influence the residence time but not the coordination number for the anionic groups because it only changes the charge densities of the anionic groups. The residence time is strongly dependent on the electrostatic interactions between water molecules and the anionic groups, which rely on the charge densities of the anionic groups. The coordination number is dependent on the molecular size of the anionic group and cannot be changed by the varying the cationic groups.

Figure 4a shows the total numbers of water molecules in the first coordination shells of the carbon atoms and hydrogen atoms attached to the nitrogen atoms of the cationic groups. The coordination numbers of the four cationic groups follow the order NC1 < NC2 < NC3 < NC4, which is consistent with the sizes of their coordination shells. Variation of the anionic group does not change the coordination number of the cationic group. Figure 4a also indicates that the number of water molecules around a hydrogen atom attached to the nitrogen atom is much less than that around a carbon atom attached to the nitrogen atom. Figure 4b,c shows the residence times, τ_{me} and τ_h, of water molecules in the first coordination shells of
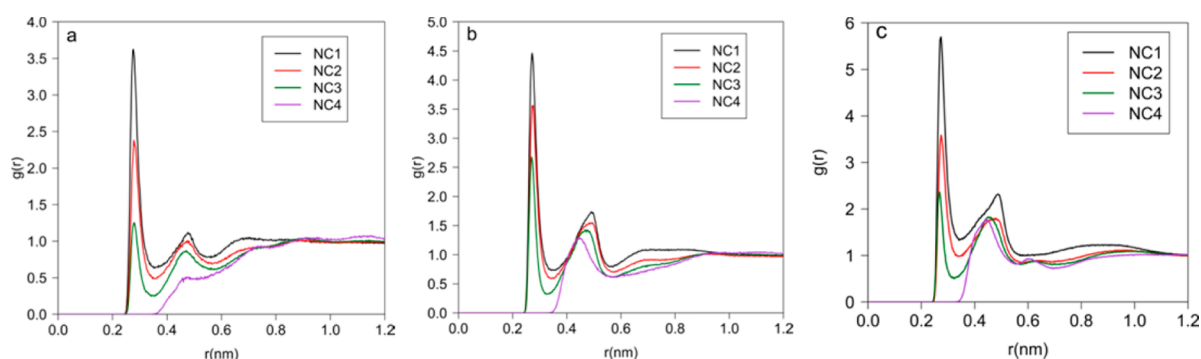
Figure 5. Radial distribution functions between the oxygen atoms of the anionic groups and the nitrogen atom of the cationic groups for the 12 zwitterionic moieties (N−O RDFs): (a) zwitterionic moieties possessing a CO2 group, (b) zwitterionic moieties possessing a SO3 group, and (c) zwitterionic moieties possessing an OSO3 group.
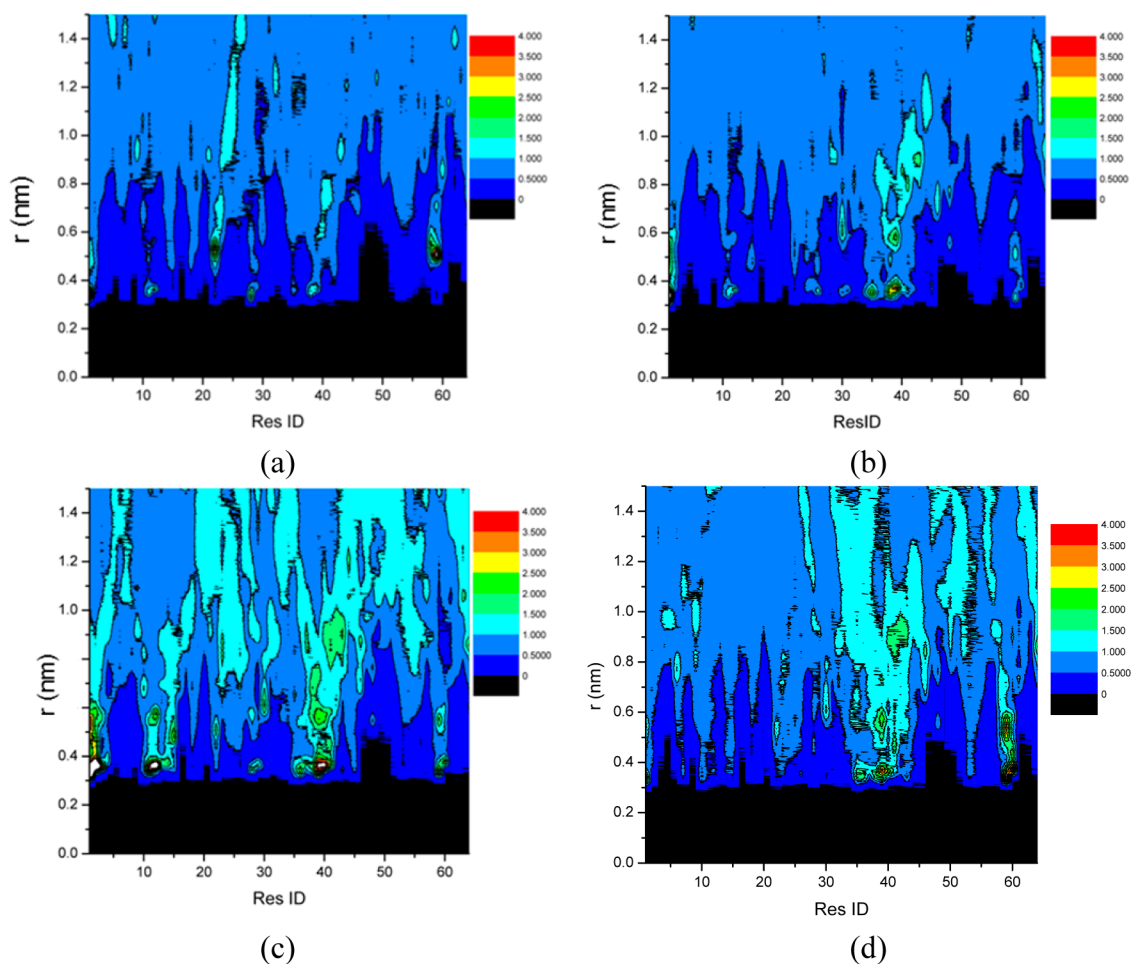

Figure 6. Radial distribution functions of the oxygen atoms of anionic groups of (a) NC3-CO2, (b) NC3-SO3, (c) NC3-OSO3, and (d) NC2-CO2 and the Cα atom of each residue of CI2. The colors correspond to value regions of $g(r)$: dark = 0, blue = 0−0.5, light blue = 0.5−1.0, cyan = 1.0−1.5, light green = 1.5−2.0, green = 2.0−2.5, yellow = 2.5−3.0, orange = 3.0−3.5, red = 3.5−4.0, and white > 4.0. Considerable area of white regions indicates that this type of zwitterionic moiety will bind to CI2, probably through some specific binding.

carbon atoms and hydrogen atoms attached to the nitrogen atom. τ_{me} of the cationic groups varies less than 10%, indicating that it is not significantly dependent on the type of the anionic groups. τ_h varies more than 30%, indicating its significant dependence on the type of the anionic groups. For the NC2, NC3, and NC4 groups, the majority or all of the water molecules are around the carbon atoms attached to the nitrogen atoms. The hydration structural and dynamic

properties of these three cationic groups should not change very much as a function of the anionic group, in contrast to how the hydration properties of the anionic group vary as a function of the cationic group. This difference is probably due to the fact that the weak hydration of these cationic groups cannot be further decreased significantly by the anionic groups.[8,19] The NC1 group only has hydrogen atoms attached

to the nitrogen atom, and its hydration should be dependent on the anionic groups.

3.2. Self-Association among Zwitterionic Moieties.

The above analysis has shown that the 12 zwitterionic moieties have strong hydration, but strong hydration is not a guarantee that they will have a good ability to resist protein adsorption. Another critical property is self-association among zwitterionic moieties. Self-association among these moieties can lead to interesting properties, such as anti-polyelectrolyte effects[36] or thermal responses.[37] But too many self-associations may change the chemical features of the materials[20] and influence their performance. To study the self-associations of these 12 zwitterionic moieties, we investigated the RDFs between the oxygen atoms of anionic groups and the nitrogen atom of cationic groups (N−O RDF), as shown in Figure 5.

The RDFs of zwitterionic moieties possessing a NC4 group only have a peak at 0.45 nm. The low peak height indicates that these zwitterionic moieties have few or moderate self-associations. The materials with these zwitterionic moieties are well-known for their good ability to resist nonspecific protein adsorption.[1] The N−O RDFs of the zwitterionic moieties that have a NC3 or NC2 group show two peaks, because these moieties have both methyl groups and hydrogen atoms attached to the nitrogen atom. The peak at 0.25 nm represents an N−O association through the hydrogen atom, which should be stronger than the N−O association through the methyl group. Thus, we assessed the self-associations among these zwitterionic moieties by investigating the peak at 0.25 nm. The zwitterionic moieties with a NC1 group do not have methyl groups, and we also assessed their self-associations by investigating the peak at 0.25 nm.

As shown in Figure 5, N−O RDFs of NC3-CO2, NC3-SO3, and NC3-OSO3 each have a peak at 0.25 nm with a height of 1.0, 2.5, and 2.0, respectively. These zwitterionic moieties may still have moderate self-associations. The N−O RDF of NC2-CO2 also has a peak at 0.25 nm with a height of 2.5, indicating that this type of zwitterionic moieties may also have moderate self-associations. Therefore, the zwitterionic moieties with a NC3 group and the NC2-CO2 may still be able to resist protein adsorption.

The N−O RDFs of other zwitterionic moieties all present peaks higher than 3.5, implying that these zwitterionic moieties may form many self-associations. White et al.[19] also found that NC1-CO2 forms many self-associations at high concentration. The materials with the zwitterionic moieties that have strong self-associations may still be able to resist the adsorption of a single type of protein in a buffer solution, but their resistance may be easily affected by the environment.

3.3. Protein Interactions.

The above analysis of hydration and self-association has suggested that four zwitterionic moieties (NC3-CO2, NC3-SO3, NC3-OSO3, and NC2-CO2), beyond carboxybetaine and sulfobetaine, may exhibit a good ability to resist nonspecific protein adsorption. However, strong specific interactions between moieties and proteins may compromise their capability. Our previous study[6] showed that carboxybetaine and oligo(ethylene glycol) moieties do not accumulate near proteins. To study the potentials of these four zwitterionic moieties, we studied their interactions with a model protein, CI2. Figure 6 shows the RDFs between the oxygen atoms of the anionic groups of zwitterionic moieties (NC3-CO2, NC3-SO3, NC3-OSO3, and NC2-CO2) and the Cα atoms of 64 residues of CI2.

We studied specific interactions by analyzing the values of these RDFs. A value larger than 4 (a white region in the contour) indicates that the zwitterionic moieties may specifically interact with certain amino acids of CI2. A considerable white region in the RDF contour therefore implies that the zwitterionic moiety specifically interacts with CI2. As shown in Figure 6, the RDF contours of NC3-CO2, NC3-SO3, and NC2-CO2 do not have any white regions, indicating that these three zwitterionic moieties should not interact with the CI2 specifically. These three zwitterionic moieties therefore may have protein-resistance capability. Among them, NC3-CO2 and NC3-SO3 have just a few RDF peaks larger than 1, but NC2-CO2 has a considerable part of its RDF contour larger than 1. This difference indicates that NC3-CO2 and NC3-SO3 may have better protein resistance than NC2-CO2. The contour of NC3-OSO3 shows several white regions, indicating that this type of moiety may specifically interact with some residues of CI2. Such specific interactions may influence its protein resistance.

4. CONCLUSIONS

Using molecular simulations, we studied hydration, self-association among zwitterionic moieties, and protein interactions of 12 zwitterionic moieties derived from four cationic groups (NC1, NC2, NC3, and NC4) and three anionic groups (CO2, SO3, and OSO3), with the aim of understanding the roles of charged groups in zwitterionic moieties and assessing their ability to resist nonspecific protein adsorption. All 12 zwitterionic moieties have strong hydration, though the hydration structure and dynamics are related to the type of charged groups. The zwitterionic moieties possessing a NC4 group are already proven to resist nonspecific protein adsorption, and they form few to moderate self-associations. The zwitterionic moieties with a NC3 group and the NC2-CO2 also show few to moderate self-associations, implying their ability to resist nonspecific protein adsorption. Further analysis of the RDFs between the oxygen atoms of these zwitterionic moieties and the Cα atoms of the protein CI2 showed that the zwitterionic moieties NC3-CO2, NC3-SO3, and NC2-CO2 do not specifically interact with the protein, also indicating that they may have good ability to resist nonspecific protein adsorption. NC3-OSO3 specifically interacts with some residues, which may influence its ability to resist nonspecific protein adsorption. Recently, our group successfully synthesized the zwitterionic materials with NC3-CO2 and tested their ability to resist nonspecific protein adsorption.[38] The experimental results showed that the NC3-CO2 materials can also resist protein adsorption well, agreeing with the prediction of computational simulations in this work.

Among the 12 zwitterionic moieties, NC4-CO2 and NC3-CO2 have been shown to strongly resist nonspecific protein adsorption. They both have a high-charge-density anionic group and a low-charge-density cationic group with no or just one hydrogen atom attached to the nitrogen atom. This combination fits well the three criteria to assess the protein-resistance capability of zwitterionic moieties. Strong hydration is necessary for materials that resist nonspecific protein adsorption, and all zwitterionic moieties studied have high hydration. The cationic group of a zwitterionic moiety that resists nonspecific protein adsorption needs to have as few hydrogen atoms attached to the nitrogen atom as possible to prevent possible self-association among zwitterionic moieties. Another important factor is the interactions between zwitter-

ionic moieties and proteins. Protein surfaces have many carboxylic groups and primary ammonium groups.[21] The carboxylic groups do not form strong associations with low-charge-density cationic groups such as quaternary amine or tertiary amine groups, as indicated by Figure 5a. In contrast, the primary ammonium groups can form associations with high-charge-density anionic groups such as carboxylic groups due to their interactions via hydrogen bonding. However, their associations are concentration dependent. White et al.[19] showed that the associations between primary ammonium and carboxylic groups are weakened significantly as their concentrations decrease. The concentration of primary ammonium on a protein surface is low, and the carboxylic groups of zwitterionic moieties may not be able to form stable associations with them. Considering the three criteria above (hydration, self-association, and protein interaction), a protein-resistant zwitterionic moiety needs to possess a high-charge-density anionic group and a low-charge-density cationic group.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Partial charges of atoms of the 12 zwitterionic moieties and radial distribution functions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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