Employment of Molecularly Imprinted Polymers to High-Throughput Screen nNOS-PSD-95 Interruptions: Structure and Dynamics Investigations on Monomer–Template Complexation**

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1. Introduction

5-(3,5-Dichloro-2-hydroxybenzylamino)-2-hydroxybenzoic acid (ZL006; see Figures 1 and 2) is a potential drug candidate for the treatment of stroke, depression, and pain.1–3 ZL006 is thought to bind to the β-finger of neuronal nitric oxide synthase (nNOS) by forming an ionic bond between its carboxyl group and Arg 121 of the nNOS PDZ domain and a hydrophobic bond between the hydrophobic ring of ZL006 and Leu107 or Phe111 of nNOS; thus hindering the conformational change of the nNOS PDZ domain and interrupting the interactions of nNOS with PSD-95 [the name of PDZ is derived from the first three proteins in which these domains were found: post-synaptic density protein-95 (PSD-95), drosophila disc large tumor suppressor gene (DLG), and zonula occludens-1 protein (ZO-1)]. ZL006 does not affect the catalytic activity of nNOS or spatial memory, and does not produce unwanted side effects (i.e. motor ataxia) associated with N-methyl-D-aspartate receptor (NMDAR) antagonists.3 We designed and synthesized many kinds of derivatives of ZL006 to find more effective compounds. The efficiency of these analogues in cerebral ischemia should be tested in animal or cell experiments, such as in middle cerebral artery occlusion (MCAO), neural cell, and cortical neuron models (as listed in Table S1 in Supporting Information). However, these experiments are complex, expensive, and time consuming (usually 2 days per compound) because they are subject to complicated model construction, reperfusion, and co-immunoprecipitation (Table S1 in the Supporting Information). Animal experiments are merciless because many mice or rats have to be killed during the experimental processes. Pharmacological activity is largely determined by interactions between compounds and their target protein. Derivatives with higher bioactivity can conjugate to the target proteins more tightly to match with their shapes, sizes, and functional groups (Figure 1).

Molecularly imprinted polymers (MIPs) are employed to screen nNOS-PSD-95 (neuronal nitric oxide synthase post-synaptic density protein-95) interruptions. 5-(3,5-Dichloro-2-hydroxybenzylamino)-2-hydroxybenzoic acid (ZL006; a potential drug candidate for the treatment of stroke, depression, and pain) is employed as a template. Four kinds of functional monomers (2-VP: 2-vinylpyridine; 4-VP: 4-vinylpyridine; MMA: methyl methacrylate; and MAAM: methacrylamide) are designed, and their complexation with ZL006 in various solvents (methanol, acetonitrile, toluene, chloroform) is investigated by molecular dynamics simulations and quantum mechanics calculations. Both 4-VP and MAAM have stronger interactions with ZL006 than those of 2-VP and MMA. The appropriate ratio of monomer to template is 3:1. Intermolecular hydrogen bonds play a dominant role in monomer–template complexation. Ideal solvents are toluene and chloroform, and the solvation effect on monomer–template complexation is revealed. Both molecular modeling and adsorption experiments demonstrate that as-synthesized ZL006-MIP with 4-VP as a monomer has better selectivity than that employing MAAM to screen for nNOS-PSD-95 interruptions.

Figure 1. Schematic illustration of applying molecularly imprinted polymers (MIPs) to screen for nNOS-PSD-95 interruptions.
In recent years, MIPs have been successfully applied to many fields,[4–17] especially in molecular recognition of small molecules in enzymes. The central idea behind MIPs is to synthesize highly cross-linked polymers by using a template molecule, designed to mimic the analyte, and one or more functional monomers. The template molecule is removed after polymerization, leaving behind vacant cavities or imprints that are sterically and chemically complementary to the template. These cavities are then capable of binding a single target analyte or a class of chemically similar analytes present within a complex sample. This recognition is very similar to the “lock and key” paradigm of enzymes. Inspired by these improvements, we attempted to employ MIPs as the stationary phase for screening different derivatives of ZL006. In this method, ZL006 is adopted as the template molecule. The microcavities of synthesized imprinted polymers are used as the pockets of the enzymes to selectively bind ZL006 analogues with higher bioactivity (Figure 1).

Template–monomer self-assembly prior to polymerization is crucial. This process can be investigated by spectroscopic approaches and molecular modeling. Spectroscopic data (e.g. UV/Vis spectroscopy[18,19] and NMR spectroscopic titration[20–22]) can be used to evaluate the nature and number of monomer–template interactions by presenting an average of the ensemble of monomer–template complexes in solutions. Theoretical modeling can provide microscopically geometric and energetic information of complexes through relaxing the template–monomer interactions.[23–26] By using static structures with low-energy conformations, quantum mechanics (QM) methods allow monomer–template interaction energies to be calculated and the correlation between monomer–template interactions and cross-linking density to molecular recognition can be explained.[27–31] In addition, the group of Nicholls also employed molecular dynamics (MD) simulations to study the origin of binding site heterogeneity in a molecularly imprinting bupivacaine–methacrylic acid copolymer.[32]

Herein, we designed four monomers, 2-VP, 4-VP, MMA, and MAAM (Figure 2), and investigated their interactions with template ZL006 by MD simulations, which were performed in CH$_3$OH, CH$_3$CN, C$_6$H$_5$CH$_3$, and CHCl$_3$. It was found that 4-VP and MAAM had strong interactions with template ZL006 in C$_6$H$_5$CH$_3$ and CHCl$_3$. The ideal ratio of monomer to template is 3:1. QM calculations at the B3LYP/6-31+G(d,p) level were performed to investigate the nature of monomer–template complexation, and showed the dominant role of intermolecular hydrogen bonding. The important influence of solvation effects, arising from intermolecular hydrogen bonding between solvent and template molecules, was also clarified. The appropriate monomer–template molar ratio was controlled by the combined effects of electrostatic and van der Waals forces. Finally, a MIP was prepared to screen for different derivatives of ZL006. The MIP with 4-VP as a monomer had better selectivity than that employing MAAM as a monomer. Compared with other testing methods for drug activity, MIP technology, without using animals and cells, is less expensive and time consuming, and can realize high-flux screening (Table S1 in the Supporting Information). We hope that this study will not only provide useful information for the future design of MIPs, but also present a new idea for the high-throughput screening of drug activities.

2. Results and Discussion

2.1. Complexation of the Prepolymerization Mixture: MD Simulations

To investigate the nature of monomer–template complexation, 6 ns MD simulations were performed. Each simulation system includes one template, ten functional monomers, and dozens or more than one hundred solvent molecules to replicate the components and solution concentrations of the corresponding polymer synthesis (Table S2 in the Supporting Information). The average spatial distributions of functional monomers around the template were investigated by radial distribution functions (RDFs). As shown in Figures S1–S5 in the Supporting Information, there are intermolecular hydrogen-bonding interactions, such as O–H···N, N–H···O, and O–H···O, between the studied functional monomers and template. The cooperative effect among these hydrogen bonds makes it difficult to isolate a single O–H···N or N–H···O contribution to the total interacting strength in functional monomer–template pairs. Therefore, we surveyed the average coordination number of monomers around a template (results are shown in Figures S2–S5 and Tables S3–S6 in the Supporting Information). For each kind of monomer, its coordinate number around the template in various solvents follows the decreasing sequence of CHCl$_3$ > C$_6$H$_5$CH$_3$ > CH$_3$CN > CH$_3$OH. In CHCl$_3$ and C$_6$H$_5$CH$_3$, both functional monomers of 4-VP and MAAM have a larger coordinate number (about 3) than those of other functional monomers (in range of 0–2). In addition, the hydrogen-bond lengths in 4-VP–ZL006 and MAAM–ZL006 complexes fall into a range of (1.65 ±
0.05) and (1.75 ± 0.05) Å, respectively (Figures 3 and 4). These results indicate that, among the studied functional monomers, 4-VP and MAAM interact most strongly with template ZL006 in C₆H₅CH₃ and CHCl₃. These stable monomer–template complexes, (4-VP)₃–ZL006 and (MAAM)₃–ZL006, generate MIPs with the highest affinity.

2.2. Understanding Monomer–Template Interactions: QM Calculations

To understand the influence of solvents, functional monomers, and molar ratio of monomer to template on monomer–template interactions, QM calculations were performed within DFT at the B3LYP/6-31G(d,p) level.

2.2.1. Solvation Effect

The coordinate numbers of monomers around a template are different in various solvents, which indicates a significant influence of solvation effects on monomer–template interactions. Thus, the interactions between solvent and template molecules were investigated. We focus on two kinds of intermolecular interactions: hydrogen bonding and π–π stacking.

The solvation spheres around ZL006 (indicated by RDFs in Figures S6 and S7 in the Supporting Information) demonstrate that strong intermolecular hydrogen bonds are formed between nitrogen and oxygen atoms (in CH₃CN and CH₃OH) to hydrogen atoms (in amide and hydroxyl groups of ZL006). The optimized conformations of solvent–template complexes reveal that the intermolecular N⋯H and O⋯H distances are about 1.94–2.40 and 1.70–2.04 Å in CH₃CN and CH₃OH, respectively (Figures S8 and S9 in the Supporting Information). Figure 5 provides a comparison of the interaction energies of solvents to the template with those of monomers to the template (more details are given in Figures S8–S13 in the Supporting Information). The CH₃CN–ZL006 and CH₃OH–ZL006 interacting energies (−9.16 and −11.65 kcal mol⁻¹, respectively) are comparable to those of the 2-VP–ZL006 and MMA–ZL006 interaction energies (−10.64 and −10.94 kcal mol⁻¹, respectively); this shows the competitive effects of CH₃CN and CH₃OH with monomers of 2-VP and MMA. For monomers of 4-VP and MAAM, the interactions are much stronger (−14.65 and...
The optimized geometric parameters, Boltzmann populations, and monomer–template interaction energies with correction of basis-set superposition error (BSSE) in CHCl$_3$ and C$_6$H$_5$CH$_3$ are exhibited in Figures S10–S13 in the Supporting Information.

For the 2-VP–ZL006 complex, the interaction energies between monomer and template are $-10.69$ and $-11.05$ kcal mol$^{-1}$ in CHCl$_3$ and C$_6$H$_5$CH$_3$, respectively, which are close to those for MMA–ZL006 ($-12.13$ and $-12.68$ kcal mol$^{-1}$ in CHCl$_3$ and C$_6$H$_5$CH$_3$, respectively), as summarized in Figure 6. These interaction strengths are weaker than those for 4-VP–ZL006 and MAAM–ZL006 [16.68 and $-20.63$ kcal mol$^{-1}$ (Figure S14 in the Supporting Information), respectively] than those of the CH$_3$CN–ZL006 and CH$_2$OH–ZL006 interactions. There is a bidentate hydrogen bond between MAAM and ZL006 in the most stable conformer: I (Figure S13 in the Supporting Information). In addition, the coordinate numbers of CH$_3$CN and CH$_2$OH around the template molecules are 7 and 10 (Figures S6 and S7 in the Supporting Information), respectively. These results intrigued us, so we investigated solvent–template interactions with higher molar ratios. As shown in Figure 5, when both ratios of CH$_3$CN and CH$_2$OH to ZL006 are increased to 2:1, the interaction energies in (CH$_3$CN)$_2$–ZL006 and (CH$_2$OH)$_2$–ZL006 complexes are increased to $-16.68$ and $-20.63$ kcal mol$^{-1}$ (Figure S14 in the Supporting Information), respectively, which are higher than those in the 4-VP–ZL006 and MAAM–ZL006 complexes. These results demonstrate that CH$_3$CN and CH$_2$OH molecules can interact with the template and competitively interrupt the complexation of monomers with the template molecule.

For C$_6$H$_5$CH$_3$, it can interact with template ZL006 through π–π stacking (Figure S15 in the Supporting Information). The calculated C$_6$H$_5$CH$_3$–ZL006 interacting energies in two of the located conformers are $-3.91$ and $-5.88$ kcal mol$^{-1}$; these values are weaker than the interaction strength in all studied monomer–template complexes. In the case of CHCl$_3$, it cannot form either hydrogen bonds or π–π stacks with ZL006. All of these results indicate that C$_6$H$_5$CH$_3$ and CHCl$_3$ have little influence on the monomer–template interactions. Thus, it can be concluded that C$_6$H$_5$CH$_3$ and CHCl$_3$ are appropriate solvents for MIPs formed from ZL006 and possible functional monomers.

### 2.2.2. Selection of Functional Monomer and Molar Ratio of Monomer to Template

The selection of a suitable functional monomer is a crucial factor in the study of MIPs. Herein, 2-VP, 4-VP, MMA, and MAAM were designed as candidate monomers (Figure 2). As indicated by the NBO charges in Figure 2, each monomer has one potential hydrogen-bond acceptor: a nitrogen atom in 2-VP and 4-VP and a carbonyl oxygen atom in MMA and MAAM. For MMA and MAAM, they also have a hydrogen-bond donor, namely, a hydrogen atom in the hydroxyl or amide groups. We built and optimized all possible conformations of monomer-template complexes (with molar ratio 1:1), considering the various combinations of hydrogen acceptor with donor atoms in the template and monomer molecules. The cis conformation of ZL006 was employed due to its higher stability than the trans conformation (Figure 2). The optimized geometric parameters, Boltzmann populations, and monomer–template interaction energies with correction of basis-set superposition error (BSSE) in CHCl$_3$ and C$_6$H$_5$CH$_3$ are exhibited in Figures S10–S13 in the Supporting Information.

For the 2-VP–ZL006 complex, the interaction energies between monomer and template are $-10.69$ and $-11.05$ kcal mol$^{-1}$ in CHCl$_3$ and C$_6$H$_5$CH$_3$, respectively, which are close to those for MMA–ZL006 ($-12.13$ and $-12.68$ kcal mol$^{-1}$ in CHCl$_3$ and C$_6$H$_5$CH$_3$, respectively), as summarized in Figure 6. These interaction strengths are weaker than those for 4-VP–ZL006 and MAAM–ZL006 [16.68 and $-20.63$ kcal mol$^{-1}$ (Figure S14 in the Supporting Information), respectively] than those of the CH$_3$CN–ZL006 and CH$_2$OH–ZL006 interactions. There is a bidentate hydrogen bond between MAAM and ZL006 in the most stable conformer: I (Figure S13 in the Supporting Information). In addition, the coordinate numbers of CH$_3$CN and CH$_2$OH around the template molecules are 7 and 10 (Figures S6 and S7 in the Supporting Information), respectively. These results intrigued us, so we investigated solvent–template interactions with higher molar ratios. As shown in Figure 5, when both ratios of CH$_3$CN and CH$_2$OH to ZL006 are increased to 2:1, the interaction energies in (CH$_3$CN)$_2$–ZL006 and (CH$_2$OH)$_2$–ZL006 complexes are increased to $-16.68$ and $-20.63$ kcal mol$^{-1}$ (Figure S14 in the Supporting Information), respectively, which are higher than those in the 4-VP–ZL006 and MAAM–ZL006 complexes. These results demonstrate that CH$_3$CN and CH$_2$OH molecules can interact with the template and competitively interrupt the complexation of monomers with the template molecule.

For C$_6$H$_5$CH$_3$, it can interact with template ZL006 through π–π stacking (Figure S15 in the Supporting Information). The calculated C$_6$H$_5$CH$_3$–ZL006 interacting energies in two of the located conformers are $-3.91$ and $-5.88$ kcal mol$^{-1}$; these values are weaker than the interaction strength in all studied monomer–template complexes. In the case of CHCl$_3$, it cannot form either hydrogen bonds or π–π stacks with ZL006. All of these results indicate that C$_6$H$_5$CH$_3$ and CHCl$_3$ have little influence on the monomer–template interactions. Thus, it can be concluded that C$_6$H$_5$CH$_3$ and CHCl$_3$ are appropriate solvents for MIPs formed from ZL006 and possible functional monomers.

![Figure 5. A comparison of monomer–template and solvent–template interaction energies [kcal mol$^{-1}$] with molar ratios of 1:1 and 2:1, respectively.](image)

![Figure 6. The monomer–template interaction energies [kcal mol$^{-1}$] with a molar ratio of 1:1 in chloroform and toluene.](image)
To adequately model the explicit hydrogen-bond networks around a ZL006 molecule, larger (4-VP), ZL006 and (MAAM), ZL006 (n=2, 3, and 4) clusters were constructed based on the most stable conformations of monomer–template complexes with a ratio of 1:1. As described above, there are also bidentate hydrogen bonds in the (MAAM), ZL006 complexes, whereas only monodentate hydrogen bonds are formed in the (4-VP), ZL006 complexes (Figures S16–S18 in the Supporting Information). Furthermore, the electronegativities on the hydrogen-acceptor atoms in MAAM are higher than those in 4-VP, which means that the monomer–template interaction energies in the (MAAM), ZL006 complexes are 4.46–6.61 and 5.50–8.02 kcal mol\(^{-1}\) higher than those in the (4-VP), ZL006 complexes (Figures S16–S18 in the Supporting Information). These results are in good agreement with the experimentally obtained adsorbing capacity, which characterizes the absorption strength of functional monomers to a template molecule. As shown in Figure 7, ZL006 displays an approximately twofold greater binding capacity to MAAM than that to 4-VP.

![Figure 7. The maximum adsorbing capacities of (4-VP), ZL006 and (MAAM), ZL006 (n = 3 or 4) polymers with various polymer concentrations.](image)

For both (4-VP), ZL006 and (MAAM), ZL006 complexes in either CHCl\(_3\) or C\(_6\)H\(_5\)CH\(_3\), a higher ratio of monomer to template results in a stronger monomer–template interaction. When the monomer–template ratios increase from 3:1 to 4:1, the monomer–template interaction energies decrease slightly (Figure 8). Thus, the optimal molar ratio of monomer to template is 3:1. This result is consistent with experimental results. As shown in Figure 7, the adsorbing capacities of the MiP synthesized from MAAM and ZL006 in a ratio of 3:1 in CHCl\(_3\) are higher than that of the MiP obtained at a ratio of 4:1.

### 2.2.3. Electrostatic and van der Waals Contributions

To understand the evolution of monomer–template interaction energies with molar ratio, the QM-based polarization model\(^{[35,36]}\) was embedded in the COMPASS\(^{[37–40]}\) force field (FF) to calculate the electrostatic and van der Waals contributions. As shown in Figure 8, there are electrostatic attraction interactions between functional monomers 4-VP and MAAM and the template ZL006. The electrostatic attraction energies increase upon changing the monomer–template ratios from 1:1 to 3:1. When the ratio reaches 4:1, electrostatic attraction will be decreased. On the other hand, there are also van der Waals repulsive interactions in both the (4-VP), ZL006 and (MAAM), ZL006 complexes. Upon increasing the monomer–template ratio from 1:1 to 4:1, the van der Waals repulsion will increase. These results imply that these two opposite interactions result in the highest total monomer–template interaction energies at a ratio of 3:1 in both the (4-VP), ZL006 and (MAAM), ZL006 complexes.

**2.2.4. Important Role of Intermolecular Hydrogen Bonds**

From the above discussion, one can find that there are many hydrogen bonds in the monomer–template complexes. We attempt to understand the important role of hydrogen bonds through investigations into the hydrogen energies and O–H and N–H stretching frequencies.

The intermolecular hydrogen-bond energy is usually estimated from the interaction energy between the monomers involved, as described by several methods. For the present monomer–template complexes at various molar ratios, however, it is not straightforward to evaluate the strength of each intermolecular hydrogen bond due to the complicated intermolecular hydrogen-bonding network. Because there are correlations of donor–hydrogen stretching frequencies with donor–hydrogen lengths and donor–acceptor distances, a simple way to estimate the hydrogen-bond energy, \(E_{\text{HB}}\) (in unit of kcal mol\(^{-1}\)), is by using Equation (1):\(^{[41]}\)

\[
E_{\text{HB}} = \frac{245.9 \times (\nu - \nu_0)}{4.2 \times \nu_0}
\]

in which \(\nu\) (cm\(^{-1}\)) and \(\nu_0\) (cm\(^{-1}\)) are the O–H or N–H stretching frequencies in the presence and absence of hydrogen bonds, respectively.

![Figure 8. The monomer–template interaction energies (kcal mol\(^{-1}\)) with various molar ratios of monomer and template in chloroform and toluene.](image)
The hydrogen-bonding energies in the (4-VP)<sub>n</sub>–ZL006 and (MAAM)<sub>n</sub>–ZL006 (n = 1–4) complexes are shown in Table 1. The hydrogen-bond strength increases with the addition of three functional monomers. As shown in Table 1, the hydrogen-bonding energies of (4-VP)<sub>n</sub>–ZL006 and (MAAM)<sub>n</sub>–ZL006 are up to −27.24 and −28.15 kcal mol<sup>−1</sup>, respectively. The further addition of the fourth functional monomer leads to a decrease in the hydrogen-bonding energies by about 3.67–4.80 kcal mol<sup>−1</sup>. In addition, the values of hydrogen-bonding energies are comparable to those of the total monomer–template energies. This result implies again that intermolecular hydrogen bonding plays a dominant role in the complexation of template with functional monomers.

Figure S21 in the Supporting Information shows the TEM images of the ZL006-MIPs. It can be seen that all particles remain spherical and monodisperse. The diameters of the particles are about 4 μm.

Relative standard deviation (RSD), also named coefficient of variation (CV), was calculated and is given in Table S7 in the Supporting Information. The RSDs for the absorbing capacity of ZL006 and ZL009 in synthesized MIPs with 4-VP as a monomer are 0.07 and 0.09, respectively; these values demonstrate good repeatability/reproducibility of the method. The detection limit of monomer–template complexation is at a magnitude of 10<sup>−8</sup> mol L<sup>−1</sup>.

2.3. Characterization of ZL006-MIP

ZL006-MIPs were synthesized by using 4-VP and MAAM as monomers at a monomer–template ratio of 3:1 in chloroform. Characterization of ZL006-MIP was first confirmed by IR spectroscopy (Figures S19 and S20 in the Supporting Information). In the IR spectra of ZL006 and ZL006-MIP, there are characteristic absorption bands at ν ≈ 3500 cm<sup>−1</sup>, which arise from N–H and O–H stretching vibrations. These bands disappear in the IR spectrum of nonimprinted polymers (NIPs). These results demonstrate the polymerization of ZL006 with monomers.

2.4. Selectivity of ZL006-MIP to Screen for nNOS-PSD-95 Interruptions

Both molecular modeling and experiment methods were employed to test the selectivity of ZL006-MIP (with 4-VP and MAAM as monomers at a monomer–template ratio of 3:1) to screen for nNOS-PSD-95 interruptions. Two molecules, ZL005 and ZL009 (Figure 9a), were selected and their binding energies to the ZL006-MIPs were calculated (Figure 9b). As shown in Figure 9b, the difference in binding energies among the interaction energies of (MAAM)<sub>3</sub>–ZL005, (MAAM)<sub>2</sub>–ZL006, and (MAAM)<sub>3</sub>–ZL009 are very small, which implies that MAAM

### Table 1. Hydrogen bond (HB) energies (E<sub>hb</sub>) in the (4-VP)<sub>n</sub>–ZL006 and (MAAM)<sub>n</sub>–ZL006 (n = 1–4) complexes, obtained by using Equation (1), based on the calculated O–H and N–H stretching frequencies in the presence (ν) and absence (ν<sub>0</sub>) of hydrogen bonds.<sup>a</sup>

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<th>HB</th>
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(a) The frequencies were obtained at the B3LYP/6-31G(d,p) level in the polarizable continuum model (PCM); dielectric constant ε = 4.90 for CHCl<sub>3</sub>. [b] The total interaction energies [kcal mol<sup>−1</sup>] between functional monomers and template molecule are given in parentheses.
cannot reasonably distinguish between these selected drug molecules. The reason may lie in the similar intermolecular hydrogen-bonding networks between MAAM with selected molecules (Figure S22 in the Supporting Information). With respect to MAAM, 4-VP has better selectivity. In comparison with the (4-VP)$_3$–ZL005 and (4-VP)$_3$–ZL006 complexes, methyl esterification of the carbonyl group in ZL009 destroys one intermolecular hydrogen bond between the carbonyl group of ZL009 and 4-VP (Figure S23 in the Supporting Information). Thus, the interaction energies follow the decreasing order of (4-VP)$_3$–ZL006 ≈ (4-VP)$_3$–ZL005 > (4-VP)$_3$–ZL009. This sequence shows good agreement not only with the activity characterized by the IC50 values of drug on neuronal damage (lower IC50 values indicate higher activities), but also with the experimental results for adsorbing capacity (Figure 9b). The difference in monomer–small molecule interaction energies between (4-VP)$_3$–ZL006 and (4-VP)$_3$–ZL005 is very small, and should be improved in future work.

3. Conclusions

We have probed the molecular imprinting technique to screen for nNOS-PSD-95 interruptions. Four kinds of functional monomers (2-VP, 4-VP, MMA, and MAAM) were designed, and their complexation with template (ZL006) in various solvents (CH$_3$CN, CH$_3$OH, C$_2$H$_5$CH$_2$O, and CHCl$_3$) were investigated by MD simulations and QM calculations. 4-VP and MAAM had stronger intermolecular interactions with ZL006 than those of 2-VP and MMA in C$_2$H$_5$CH$_2$OH and CHCl$_3$. Intermolecular hydrogen-bonding interactions played a dominant role in monomer–template complexation. The theoretically determined molar ratio of monomer to template was 3:1 due to the effect of electrostatic attraction and van der Waals repulsion, as revealed by using a polarization model based on COMPASS FF. These results showed good agreement with those obtained from an adsorbing capacity experiment with synthesized MIPs. Finally, the selectivity of ZL006-MIPs with 4-VP and MAAM as monomers, to screen for nNOS-PSD-95 interruptions was investigated by both molecular modeling and experimental methods. ZL006-MIP with 4-VP as a monomer performed better than that with MAAM for the methyl esterified compound (ZL009). This result was consistent with experimental results. However, the selectivity of 4-VP to the halogen analogues of ZL006 should be increased in future work. We hope that this study will provide useful information for the future design of MIPs, as well as presenting a new idea for screening drug activities.

Computational Details

MD Simulations

MD simulations were employed to obtain the interacting configuration of the monomer-template complex.

FF Validation

The accuracy of MD simulations relied on the selected FF to a large degree. Consistent valence force field (CVFF),[42] polymer consistent force field (PCFF),[37–40] and condensed-phase optimized molecular potentials for atomistic simulation studies (COMPASS)[43–46] have been demonstrated to reasonably describe the conformational changes of various organic systems.[47,48] To further validate the performance of CVFF, PCFF, and COMPASS in describing the intermolecular monomer–template interactions, the B3LYP and FF potential energy curves of O–H–N and O–H–O interactions in a dimeric monomer–template cluster as a function of the interatomic distance, $r_{	ext{O}···	ext{H}}$ and $r_{	ext{H}···	ext{O}}$ were depicted in Figure S24 in the Supporting Information. The BSSE was corrected by the counterpoise method.[33,34] It can be seen from Figure S24 in the Supporting Information that the COMPASS potential qualitatively agrees with those of B3LYP/6-31 + G(d,p). Thus, COMPASS FF[37–40] was applied to MD simulations of the monomer-template complexes in solvents.

Simulation Details

To mimic the experimental conditions, the concentration of the solution was assumed to be 0.2 M. Thus, each simulation system included one template, ten functional monomers, and dozens or more than one hundred solvent molecules (Table S2 in the Supporting Information). The potential contribution of all components (functional monomer, template, solvent, cross-linker, and initiator) to the MIP prepolymerization was demonstrated by Nicholls et al.[32] To clarify complexation of the template with functional monomers and its competitive interaction with solvent, a prepolymerization system with the omission of cross-linker and initiator was employed. The MD simulations were performed in the canonical (NVT, constant number of particle, volume, and temperature) ensemble at 298 K by using an Andersen thermostat.[49] The periodic boundary condition (PBC) was employed. Details of cell parameters are given in Table S2 in the Supporting Information. The cutoff values of van der Waals and electrostatic interactions were set to 12.5 Å. The electrostatic potential was evaluated by the Ewald summation.[50] Equations of motion for systems were integrated by using the velocity Verlet algorithm[51] with a time step of 1.0 fs. The 6.0 ns simulation was subsequently performed after an equilibrium stage of 0.5 ns. Trajectories were collected every 100.0 fs. The trajectories of...
the final 3.0 ns were employed for statistical analysis. All MD simulations were performed with the discover module in the Materials Studio package.  

QM Calculations

All QM calculations were performed within the framework of DFT at the B3LYP/6-31 + G(d,p) level. First, geometries of functional monomers (2-VP, 4-VP, MMA, and MAAM) and template (ZL006) were optimized. Second, the energies of all possible monomer-template complexes with various molar ratios were minimized to achieve stable conformations and energetic information. Finally, on the basis of the optimized geometries, NBO charges were obtained and frequency calculations were performed to test the minima and obtain stretching vibrational frequencies. The interaction energy ($\Delta E$) between monomers and template were calculated by using Equation (2):

$$\Delta E = E_{\text{complex}} - E_{\text{template}} - \sum nE_{\text{monomer}}$$

in which $E_{\text{template}}$, $E_{\text{monomer}}$, and $E_{\text{complex}}$ were the energies (in kcal mol$^{-1}$) of the template, functional monomer, and their complex, respectively; $n$ was the molar ratio between functional monomers and the template molecule. All QM calculations were performed with the Gaussian09 program.  

Polarization Model

The polarization model[25][36] based on QM was embedded in the COMPASS FF[37–40] and employed to calculate the electrostatic and van der Waals terms of the monomer-template interaction energies. In the traditional FF, the polarization effect was ignored because intra- and intermolecular electrostatic interactions were modeled by the predetermined point charges with fixed values regardless of variations in the chemical or conformational environment. In recent decades, many polarization models, with induced dipoles or charged particles[26,34] and fluctuating charges or dipoles[27,35] have been developed to consider the polarization effect in the structure-function correlations for a wide range of systems, including water clusters, ionic solutions, and organic molecules. QM-based polarization models, such as fragment-based polarization models[25,26] were also invested in model polarization by modifying atomic charges through QM calculations. Thus, the electrostatic parameters, namely, atom-centered point charges or dipoles, were conformation- or environment-dependent dynamic variables. The polarization model was able to reasonably describe the conformational variations of peptides in aqueous solutions. The potential function in the polarization model was expressed by Equation (3):

$$U = \sum_{\text{bonds}} K_p (b - b_{eq})^2 + \sum_{\text{angles}} K_o (\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} V_{\text{ij}} \left[ 1 + \cos (n\gamma_{eq} - \gamma) \right]$$

$$+ \sum_{i<j} \left[ A_{ij} \frac{q_i q_j}{R_{ij}^2} + B_{ij} \frac{1}{R_{ij}} + C_{ij} \frac{q_i q_j}{R_{ij}^3} \right]$$

in which all bond stretching, angle bending, torsional rotating, and Lennard–Jones parameters were taken from the COMPASS FF. The electrostatic parameters of functional monomers and template molecules, $q_i$ and $q_j$, were NBO charges calculated at the B3LYP/6-31G(d,p) level with the PCM on the basis of complex conformations optimized by the B3LYP/6-31G(d,p) method.  

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