

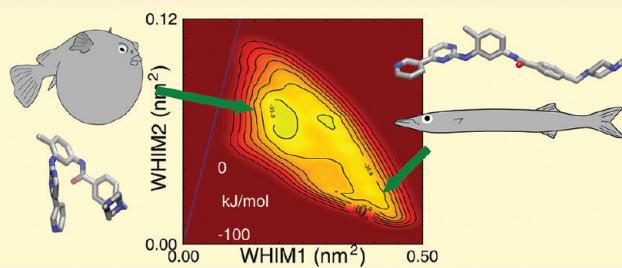
Conformational Free Energy Modeling of Druglike Molecules by Metadynamics in the WHIM Space

Vojtěch Spiwok,*†,‡ Katarína Hlat-Glembová,† Igor Tvaroška,‡ and Blanka Králová†

†Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Technická 3, Prague 6, 166 28, Czech Republic

‡Department of Structure and Function of Saccharides, Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava, 845 38, Slovak Republic

ABSTRACT: Protein–ligand affinities can be significantly influenced not only by the interaction itself but also by conformational equilibrium of both binding partners, free ligand and free protein. Identification of important conformational families of a ligand and prediction of their thermodynamics is important for efficient ligand design. Here we report conformational free energy modeling of nine small-molecule drugs in explicitly modeled water by metadynamics with a bias potential applied in the space of weighted holistic invariant molecular (WHIM) descriptors. Application of metadynamics enhances conformational sampling compared to unbiased molecular dynamics simulation and allows to predict relative free energies of key conformations. Selected free energy minima and one example of transition state were tested by a series of unbiased molecular dynamics simulation. Comparison of free energy surfaces of free and target-bound Imatinib provides an estimate of free energy penalty of conformational change induced by its binding to the target.



INTRODUCTION

Active substances of the majority of currently prescribed drugs are organic molecules with molecular weight lower than 500 g/mol. Such compounds are usually flexible objects, and the balanced conformational flexibility is important for its efficacy. On one hand, a flexible molecule can easily adapt to the binding points of the binding site of the target protein. On the other hand, if the ligand predominantly adopts the conformation A in solution and conformation B in the binding site, its binding is disfavored by the free energy penalty of the transition from A to B. Moreover, binding of a highly flexible molecule is typically disfavored by loss of conformational degrees of freedom upon binding and thus a loss of entropy. Interplay between these effects plays an important role in the thermodynamics of molecular recognition, and it must be addressed in the process of drug design.¹ For this reason, there is a continuous demand for techniques for conformational modeling and sampling of molecules and especially for those methods that provide estimates of free energy differences and heights of free energy barriers.^{2,3}

Numerous methods have been developed to explore a conformational space of druglike molecules and energetics of individual conformers. These methods employ different levels of theory and search algorithms. Levels of theory range from rule-based methods to molecular mechanics and quantum mechanics methods. These methods are used in vacuum or in explicitly and implicitly modeled solvents. Search algorithms could be based on a systematic search, molecular dynamics simulation, Monte Carlo method, genetic algorithms, taboo

search, and other optimization and configuration-space-walking methods.² These approaches differ in the accuracy and completeness of sampling on one hand and the throughput on the other hand, due to a wide range of applied techniques and energy functions. In this study we present a medium-to-low-throughput approach for exploration of the conformational space of druglike molecules combined with free energy modeling. It can be used to sample different conformations of the studied molecule in explicitly modeled water environment, and it evaluates individual conformations in terms of free energy. The sampling is based on a molecular dynamics simulation enhanced by the metadynamics algorithm.^{4–6} The conformational space is dimensionally reduced by weighted holistic invariant molecular (WHIM) descriptors.^{7,8} This reduction does not require a prior knowledge of accessible conformations. The proposed approach was tested on a conformational search of eight marketed small-molecule drugs (Table 1, Figure 1). The results are compared with similar application of classical molecular dynamics simulation in water, and, in one case, the obtained free energy surface is compared with flexibility of the molecule bound to its target. The studied drugs were selected to include all major classes of drugs (antivirals, anticancer, and lifestyle disease drugs) for which experimental structures of their complexes with a target are available.

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Table 1. Compounds Studied in This Study with Corresponding Experimental Structures (A Single Chain Indicated in Brackets Was Selected from a Multichain PDB File) and Widths of Gaussian Hills Used in Metadynamics Sampling

compound	therapeutic group	PDB ID	system	widths of hills (nm ²)		ref
				δWHIM1	δWHIM2	
Atorvastatin	HMG-CoA inhibitor	1HWK(A)	2114 H ₂ O + 1 Na ⁺	0.0080	0.0030	9
Decamethonium	anesthetics	PRODRG ^a	2160 H ₂ O + 2 Cl ⁻	0.0275	0.0045	—
Ibuprofen	COX inhibitor	1EQG(A)	2131 H ₂ O + 1 Na ⁺	0.0030	0.0008	10
Imatinib	protein kinase inhibitor	1IEP	2111 H ₂ O + 1 Cl ⁻	0.0200	0.0020	11
Methotrexate	folate antimetabolite	1RG7	2119 H ₂ O + 2 Na ⁺	0.0150	0.0050	12
Saquinavir	HIV-protease inhibitor	2NMW(A)	2109 H ₂ O + 1 Cl ⁻	0.0150	0.0015	13
Sildenafil	phosphodiesterase inhibitor	1UDT	2121 H ₂ O + 1 Cl ⁻	0.0100	0.0050	14
Tenofovir	antiviral nucleoside analogue	1T05	2129 H ₂ O + 2 Na ⁺	0.0100	0.0025	15
Zanamivir	influenza sialidase inhibitor	1NNC	2127 H ₂ O	0.0025	0.0025	16

^a<http://davapc1.bioch.dundee.ac.uk/prodrg/>, ref 17.

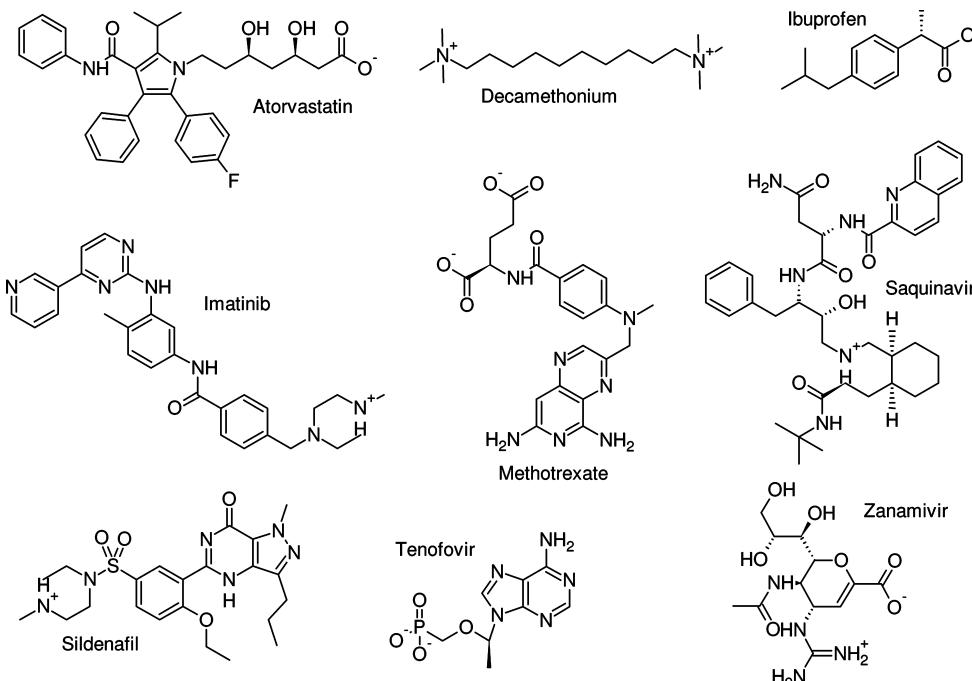


Figure 1. Compounds studied in this study in corresponding protonation states.

METHODS

Metadynamics in the WHIM Space. A conformation of a molecule is most rigorously described by Cartesian coordinates of individual atoms. However, for comparison of structures and properties it is usually not practical because of its high dimensionality. Numerous linear^{18–20} and nonlinear²¹ dimensionality reduction methods have been applied in conformational analysis, free energy modeling, spectroscopy, and data compression. Another group of methods for dimensionality reduction of molecular structures are those designed for calculation of descriptors for quantitative structure–activity relationship (QSAR) studies.⁸ Todeschini and co-workers have proposed weighted holistic invariant molecular (WHIM) descriptors.⁷ In this study we employ the subgroup of WHIM descriptors called molecular size. These parameters can be calculated from Cartesian coordinates of a molecule. First, the

molecule is placed to the center of the coordinate system by subtracting its weight-averaged coordinates

$$r'_{ki} = r_{ki} - \frac{\sum_k \alpha_k r_{ki}}{\sum_k \alpha_k} \quad (1)$$

where r_{ki} is the i -th ($i = 1, 2, 3$) coordinate of the k -th atom, and α_k is the weighting coefficient of the k -th atom. For the sake of simplicity we will set values of α_i to unity (later we will discuss other options). Then, the 3×3 covariance matrix C with an element C_{ij} is calculated

$$C_{ij} = \frac{\sum_k \alpha_k r'_{ki} r'_{kj}}{\sum_k \alpha_k} \quad (2)$$

Diagonalization of this matrix leads to three eigenvalues $\lambda_1 \geq \lambda_2 \geq \lambda_3$ called molecular size WHIM descriptors. Coordinates \mathbf{r}' could be than projected to each eigenvector \mathbf{e}_j

$$p_{kj} = \mathbf{r}' \cdot \mathbf{e}_j \quad (3)$$

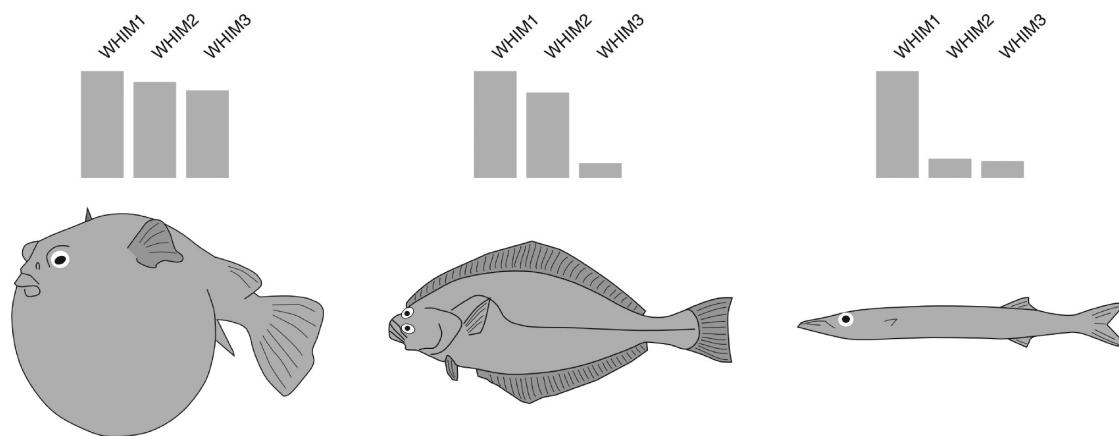


Figure 2. Illustration of WHIM descriptors (molecular size) of a spherical, planar, and linear object.

Projections to eigenvectors generates new Cartesian coordinates of the molecule rotated with respect to r' . It is rotated in a way that the highest variability of its coordinates (highest molecular size) is placed along p_1 and the lowest variability along p_3 (see Figure 2). Values of eigenvalues represent the level of this variability. For example a spherical molecule (represented by fugu fish) shows almost equivalent values of all three eigenvalues λ ($\lambda_1 \approx \lambda_2 \approx \lambda_3$). A nearly planar molecule (represented by flatfish) shows two high and one very low eigenvalues ($\lambda_1 \approx \lambda_2 \gg \lambda_3$). Finally, a nearly linear molecule (represented by barracuda) shows only one high and two low eigenvalues ($\lambda_1 \gg \lambda_2 \approx \lambda_3$). Beside values of α_k set to 1, it is possible to use certain atomic property such as charge, absolute charge, and others to compare molecules, for example, in terms of their dipolar or amphiphilic nature.

Relevance of different conformations of a molecule can be evaluated by comparing their potential energies. These can be calculated using molecular mechanics force fields or quantum chemistry methods. However, single-point potential energies of different conformations of an unsolvated molecule describe their probabilities in vacuum and at 0 K temperature. Therefore, they do not give the answer to the question of conformational preferences of a solvated molecule at appropriate temperature. This information is encoded in free energy values of individual conformations. Conformational free energy and a conformational free energy surface calculated in solution can describe the thermodynamics and kinetics of the studied conformational change at certain temperature and in a fully solvated system.

The conformational free energy surface can be (at least in principle) calculated from the populations of conformations sampled in a trajectory calculated by molecular dynamics simulation. However, some slow conformational changes might be difficult to study by this method due to its poor sampling abilities. Recently developed sampling-enhancement techniques allow to simulate conformational changes (or other molecular processes) that take place in much longer time scales. One of them, metadynamics,^{4–6} uses a history-dependent bias potential, which accumulates during the simulation and disfavors previously explored states of the system. If the molecule is stacked in a certain metastable conformation (a local free energy minimum), the metadynamics bias potential accumulates until it floods the minimum and allows the molecule to escape it. Exploration of different conformations is therefore more efficient compared to a conventional unbiased molecular dynamics simulation. Moreover, the metadynamics

bias potential at the end of simulation provides an estimate of the free energy surface, i.e. it allows to predict equilibrium and rate constants of studied changes.

A metadynamics bias potential acts in the space of few (typically two or three) structural parameters referred to as collective variables (CVs). In this study we use metadynamics with a bias potential acting in the space of WHIM descriptors,⁷ namely the first two eigenvalues obtained by diagonalization of the matrix defined by eq 2 with coefficients α_k set to unity.

Computational Details. Metadynamics simulations were performed in GROMACS 4.0.7²² together with the modified version of Plumed 1.1.0 – a metadynamics addendum to GROMACS.²³ This package was modified to allow to use WHIM molecular size descriptors as new collective variables. As far as we are aware, calculation of WHIM descriptors was up to now used mostly on static structures. Its application in the context of molecular dynamics simulation and metadynamics requires calculation of WHIM values for a vast number of conformations. As already described, WHIM descriptors are calculated by solving the eigenvalue/eigenvector problem for a covariance matrix. For a 3×3 covariance matrix it can be solved analytically. Similarly, it is possible to convert analytically the forces acting in the WHIM space to the space of atomic Cartesian coordinates. Such solution can lead to division of two very small numbers for structures located in certain parts of the WHIM space. Keeping in mind this potential numerical pitfall we switch off metadynamics biasing in these problematic regions of WHIM space. We have carefully checked selected snapshots of a metadynamics simulation where these states are sampled to make sure that it does not cause any significant artifacts.

It is possible to apply metadynamics in the 1-D, 2-D, or 3-D space by combining WHIM1, WHIM2, and WHIM3. It has been demonstrated theoretically and by trial simulations²⁴ that metadynamics with two CVs is more accurate than one with three CVs. Therefore, in this study we decided to carry out metadynamics in the 2-D space of WHIM1 and WHIM2.

Conformations of drugs were taken from the experimental structures of their complexes with their molecular targets (Table 1, Figure 1). The protonation states of these molecules were assigned empirically. The structure of Tenofovir was obtained by truncation of the terminal phosphate moiety from its diphosphate form. Molecules were modeled using the general AMBER force field (GAFF)²⁵ with RESP charges²⁶ calculated at the HF/6-31G*//HF/6-31G* level of theory. Apparently incorrect parameters of the guanidinium moiety in

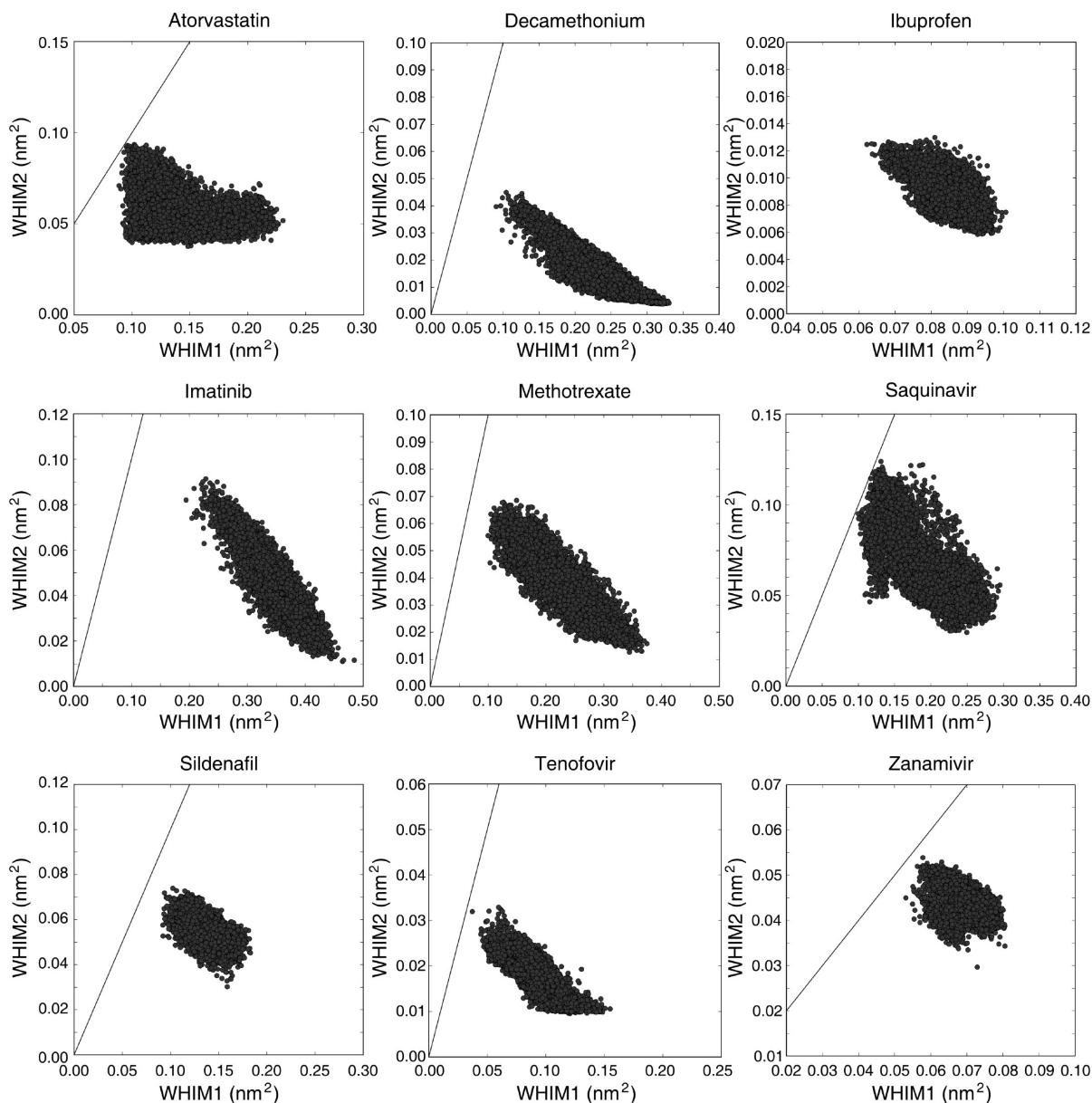


Figure 3. Unbiased molecular dynamics trajectories projected to the space of WHIM descriptors. Conformations were sampled every 1 ps.

Zanamivir were replaced for Amber99 parameters of the guanidinium moiety of arginine. A similar problem in parameters of the amino group in Methotrexate and Tenofovir was solved by their replacement by parameters of the amino group in nucleic acids. Each molecule was placed into a $4 \times 4 \times 4$ nm box filled with TIP3P water molecules²⁷ and an appropriate number of sodium or chloride counterions to neutralize the charge. The time step of simulations was set to 1 fs and covalent bonds were not constrained. Temperature was set to 300 K by the velocity rescale²⁸ method. Electrostatics was modeled by the particle-mesh-Ewald method.²⁹ Each system was geometry optimized and pre-equilibrated by 100 ps unbiased nVT simulation. Then they were studied by either 10 ns unbiased molecular dynamics simulation or 10 ns metadynamics, both starting from the coordinates after the equilibration.

Dynamics of the molecule of Imatinib was also studied when bound to the catalytic domain of Abl kinase. The coordinates of the system were taken from the X-ray structure (residues 225 to

498 in PDB-ID 1IEP).¹¹ The ligand was modeled as described for free ligands. The protein part was modeled using the Amber99SB force field.³⁰ Protonation states of titrable residues were assigned by PropKa.³¹ The time step of the simulations was 2 fs and covalent bonds were constrained by LINCS algorithm.³² Berendsen thermostat³³ was used for temperature control. Other parameters were the same as for simulations of free ligands. The system was geometry optimized and then equilibrated by an unbiased simulations with harmonic restraints applied to non-hydrogen atoms of the protein. First we performed 100 ps simulation with a strong restraint ($k = 1000 \text{ kJ/mol}\cdot\text{nm}^2$) followed by 100 ps simulation with a weak restraint ($k = 20 \text{ kJ/mol}\cdot\text{nm}^2$). This was followed by unrestrained 5 ns unbiased simulations and 5 ns metadynamics, both starting from the final coordinates of the equilibration run.

RESULTS AND DISCUSSION

In the first step we performed an unbiased molecular dynamics and metadynamics simulations for each molecule in order to

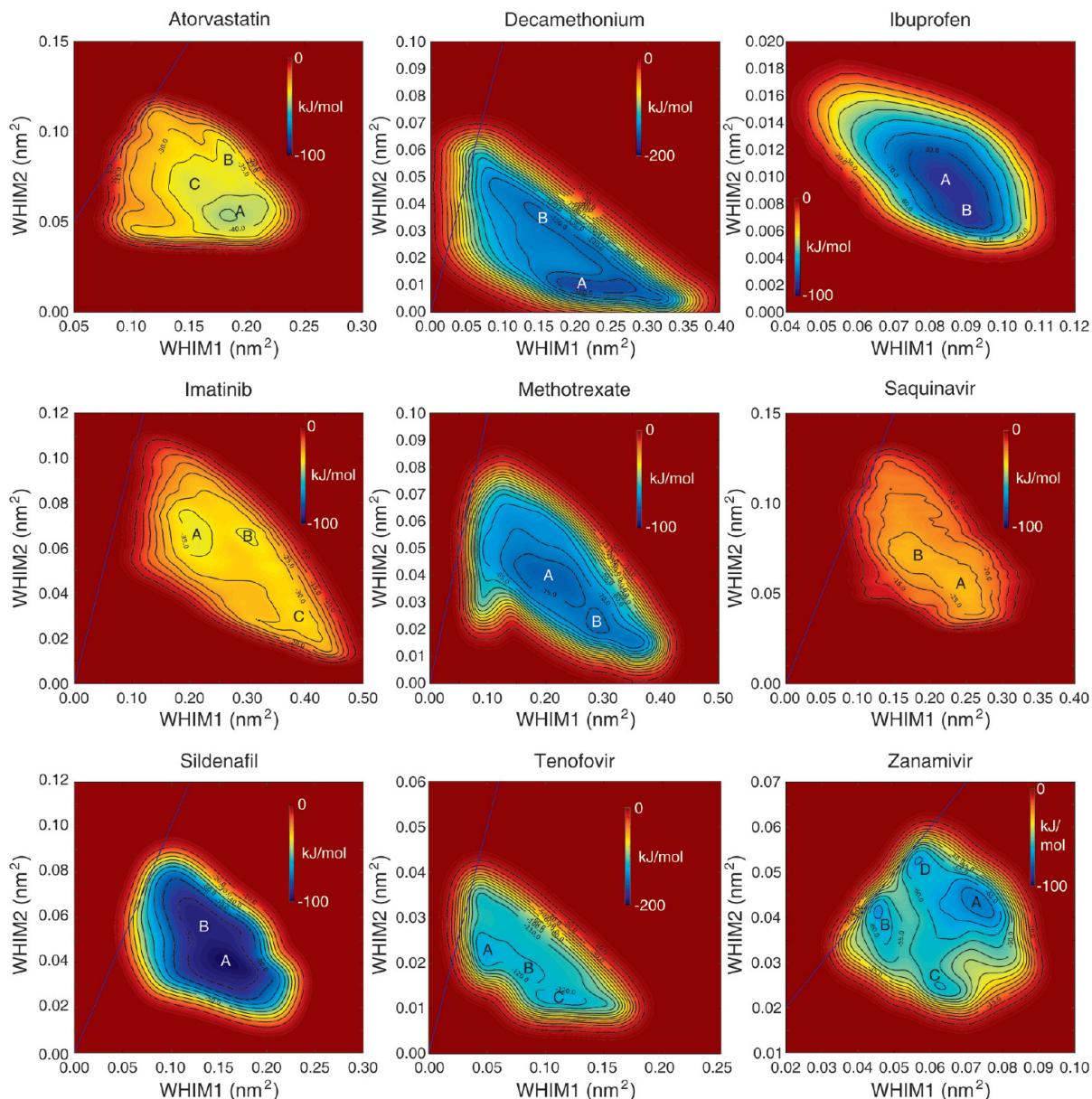


Figure 4. Free energy surfaces calculated using metadynamics in the space of WHIM descriptors.

compare their efficiency. Both simulations started from the same conformation. The results are depicted in Figures 3 and 4. Snapshots of molecular dynamics simulations projected to the space of WHIM descriptors are depicted in Figure 3. Free energy surfaces calculated by metadynamics are shown in Figure 4. These plots show that molecular dynamics simulation, even without a metadynamics bias potential, sampled relatively large areas of the conformational space. It can also distinguish between flexible and rigid molecules (note that scales of plots are different). The diagonal (the function $\text{WHIM}2 = \text{WHIM}1$) is presented in all plots where applicable to show the limit of the CV space ($\text{WHIM}2 \leq \text{WHIM}1$).

The results of molecular dynamics simulations were used to design parameters of metadynamics, namely widths of the hills. It has been demonstrated²⁴ that the highest accuracy of metadynamics in two-dimensional CV space is achieved when width of a hill is approximately 1/10 of the CV range. On the other hand, slightly lower values (e.g., 1/20) can provide better resolution of multiple free energy minima in the resulting free

energy surface. The values of widths of hills ($\delta\text{WHIM}1$ and $\delta\text{WHIM}2$) were therefore chosen to provide a reasonable compromise between the accuracy and resolution and are listed in Table 1. Height of hills were set to 0.2 kJ/mol.

Figure 4 shows that metadynamics simulations sampled larger areas of conformational space compared to unbiased molecular dynamics simulation. This was particularly true for Imatinib and Zanamivir where new free energy minima were observed, which were not observed by unbiased molecular dynamics simulation. Metadynamics led to enhanced sampling even in highly rigid molecules such as Ibuprofen, where no new conformational minima were sampled, nevertheless, the sampled area in WHIM coordinates was larger.

Figure 5 shows the snapshots of metadynamics pseudo-trajectories that represent the minima on each free energy surface. Metadynamics does not provide a direct link between conformation and free energy. Therefore, the most straightforward way how to structurally interpret the results of metadynamics is selection of metadynamics snapshots with

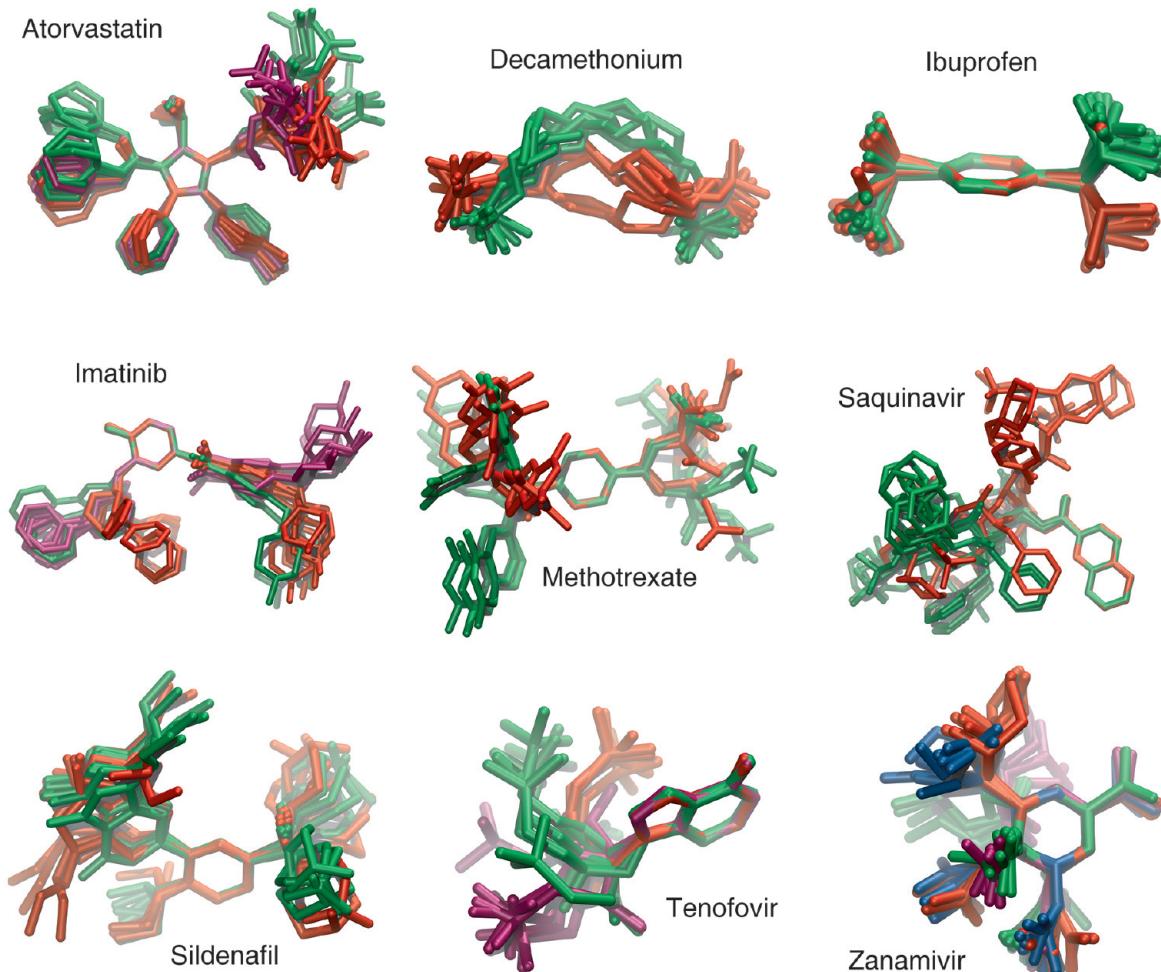


Figure 5. Snapshots of metadynamics simulations representing free energy minima in Figure 4 (minimum A – red, B – green, C – purple, D – navy blue).

WHIM values close to the identified minima at FES. These snapshots were superimposed by non-hydrogen atoms of selected rigid moieties. Symmetry of relevant groups of each molecule were taken into the account in the rms-fit process. Free energy minima of Ibuprofen, Imatinib, Tenofovir, or Zanamivir correspond to well-resolved conformational families. Some free energy minima, e.g. the minimum B of Atorvastatin, can be further visually divided into a pair of subfamilies, namely in the orientation of the anilinide moiety. Saquinavir is an example of a complicated compound with vague separation of minima.

Two or three free energy minima were observed for each compound. Minima of Atorvastatin differ in conformations of their polar heads. The minimum B can be further subdivided into two subminima differing in orientation of their anilinide moieties. Decamethonium shows two relatively well-resolved minima differing in the distance of ammonium groups. The fact that the free energy surface is separated into two minima, rather than being smooth, might be related to the interaction between Decamethonium and water molecules and that both minima correspond to different solvation patterns. This might be a similar situation to the existence of multiple minima on potential of mean force profiles of molecular associations or radius of gyration calculated in liquids. Ibuprofen, despite being highly rigid, shows two minima differing in a mutual orientation of both substituents of its benzene ring. The selected

conformations in the minimum A contain one conformation differing from other fourteen, whereas the conformations in the minimum B are very similar. Three well-resolved minima of Imatinib differ in orientations of their pyridine-pyrimidine moiety as well as in piperazin moiety. The minimum A was not sampled by unbiased molecular dynamics and therefore rotation of pyridine-pyrimidine moiety requires the action of a bias potential. It has been demonstrated by the lead optimization of Imatinib that the introduction of the conformationally blocking methyl group on one of benzene rings is important for its efficacy and selectivity. The molecule of Methotrexate shows two free energy minima. Its free energy surface shows that these minima are separated by a free energy barrier of 3–5 kJ/mol. Each of them can be further subdivided into two apparent subminima. The free energy minima of Saquinavir show poorest resolution as illustrated by the snapshots in Figure 5. This shows that the attempt to calculate the conformational free energy surface of this compound by the metadynamics in the WHIM space was too ambitious. Saquinavir is the largest among the studied molecules with many flexible bonds, and its conformational free energy minima likely overlap in the free energy space. Sildenafil molecule shows two free energy minima. The minimum B can be further divided into two subfamilies differing in the orientation of the piperazine moiety. The minimum A is significantly more heterogeneous than the minimum B. Three minima of

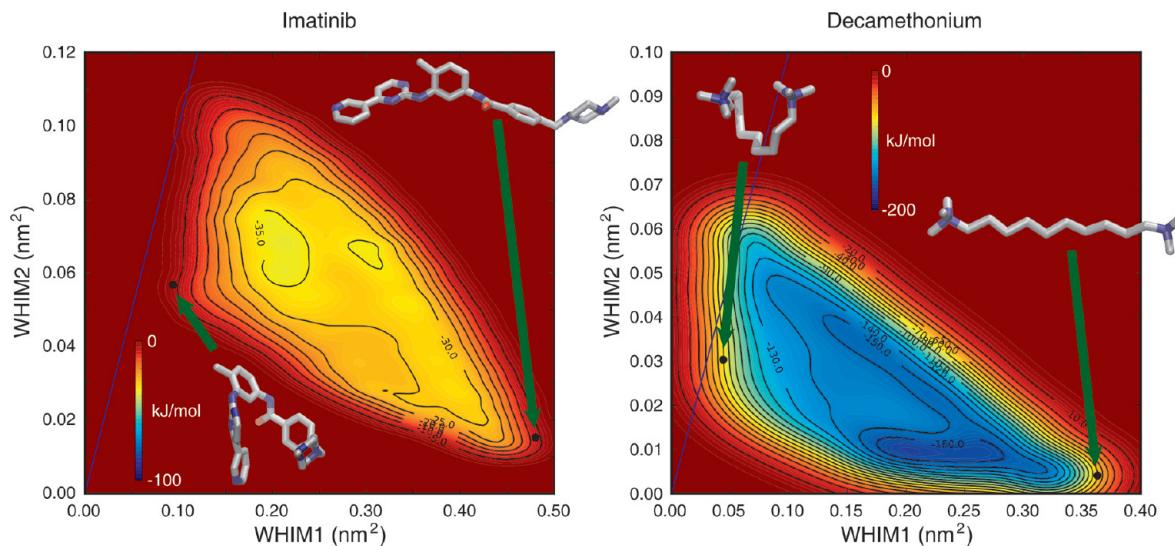


Figure 6. Examples of conformations with extreme values of WHIM1 sampled during the metadynamics.

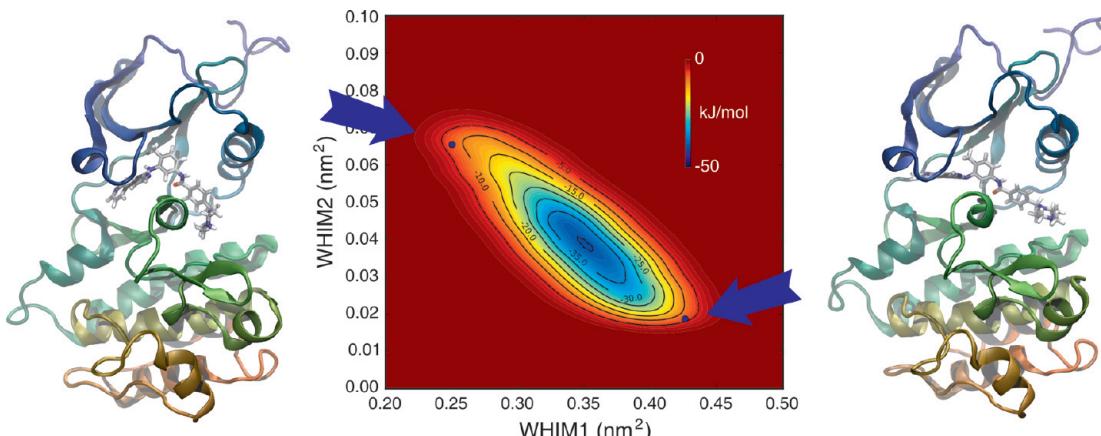


Figure 7. Free energy surface of Imatinib bound to the catalytic domain of Abl-kinase and an illustration of the extreme conformations sampled by metadynamics.

Tenofovir were well-resolved in terms conformations. Finally, Zanamivir shows four free energy minima with differences in conformation and orientation of the glycerol moiety or in orientation of the acetamidyl or guanidinium groups.

It is also interesting to explore the extreme conformations sampled by metadynamics. The conformations of Imatinib and Decamethonium with maximum and minimum WHIM1 values are depicted in Figure 6. They are shown to illustrate the sampling power of metadynamics. Moreover, the overall depth of a predicted free energy surface (i.e., the negative value of the maximum of the bias potential) can be used to predict a time scale in which these conformations are sampled by a real system. These extreme conformations are least frequently sampled by metadynamics, and therefore almost no bias potential is deposited in these regions (free energy ≈ 0). The difference between the extreme conformation and the free energy minimum thus represents an energy barrier that can be converted to sampling frequency by Eyring's model³⁴ similarly to sampling of a transition state. Values of free energy minima (-28 to -161 kJ/mol) indicate that extreme conformations range from those sampled in nanoseconds (-28 kJ/mol) to conformational changes that are not sampled in reasonable time scales (-161 kJ/mol). The latter case indicates that full

conformational space of the molecule was explored, at least as defined by WHIM parameters.

The reason why free energy modeling was applied in this project is its ability to predict the free energy penalty of a conformational change which takes place upon binding of a drug to its target. This was studied for Imatinib by comparison of the drug in solution and bound to the catalytic domain of Abelson kinase. Unbiased molecular dynamics and metadynamics simulations of the complex started from the conformation obtained from experimental crystal structure¹¹ after geometry optimization and restrained molecular dynamics simulation as described in Methods. The results are depicted in Figure 7. The range of sampled conformations was significantly smaller when the drug was bound to its target. Contrary to the situation in water solution where three free energy minima were observed, only a single deep free energy minimum was observed. This minimum was located in the space spanning the minima B and C. The minimum A was not sampled by the target-bound Imatinib. The free energy penalty of binding can be calculated as the difference between the minimum A and the place in the WHIM coordinates of the same plot corresponding to the minimum of target-bound Imatinib. This penalty is very small, ~ 5 kJ/mol. The conformational change therefore

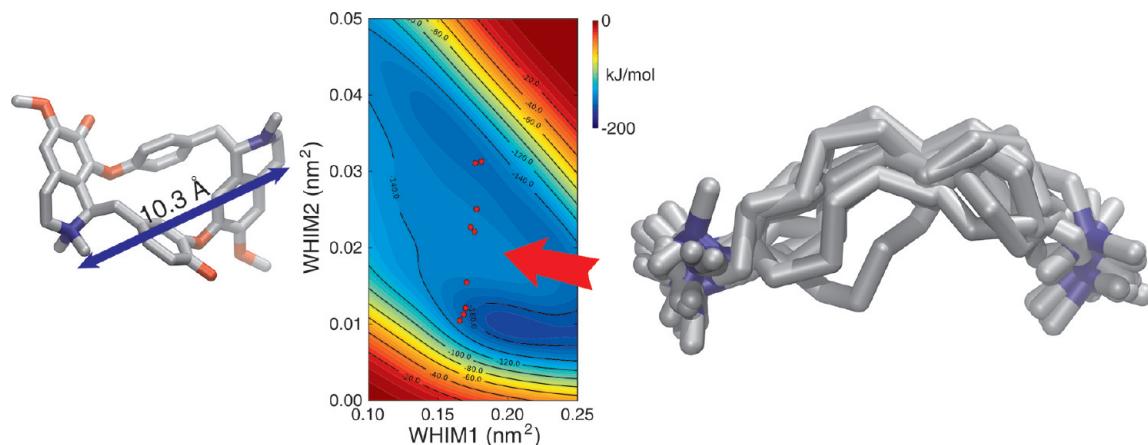


Figure 8. Location of decamethonium conformations resembling the structure of d-Tubocurarine on the conformational free energy surface.

disfavors binding to the target by no more than 1 order of magnitude in terms of K_i values. This shows a great potential of the proposed approach in analyzing drug candidates coming from virtual screening campaigns and other drug discovery methods. Some compounds can be predicted to bind tightly to the target because they show numerous favorable noncovalent interactions, but in fact they bind weakly due to large free energy penalty of the conformational change from the solution to the target-bound structure.

The molecule of Decamethonium was chosen for its interesting similarity with the molecule of d-Tubocurarine.³⁵ Both molecules act as acetylcholine receptor antagonists to provide skeletal muscle relaxation in anesthesia. The similar action of both molecules can be explained by the fact that each of them contains a pair of positively charged ammonium moieties in the distance suitable for receptor activation. The molecule of d-Tubocurarine is rigid with the distance of positively charged nitrogens of $\sim 10.3 \text{ \AA}$. This distance can be also reached by a highly flexible Decamethonium molecule. Figure 8 shows the detail of the free energy surface of Decamethonium from Figure 4 with nine snapshots of metadynamics with the distance of nitrogen atoms close 10.3 \AA . Their WHIM1 values were $\sim 0.17 \text{ nm}^2$. The most favorable conformation in terms of free energy were those with low values of WHIM2. The free energy penalty of the conformational change from the relaxed conformation to the d-Tubocurarin-like conformation was calculated as approximately 10 kJ/mol . This corresponds to reduction of binding constant by 1 or 2 orders of magnitude. We can imagine a hypothetical molecule of rigid Decamethonium conformationally locked to the d-Tubocurarin-like distance between ammonium moieties. Binding of such hypothetical molecule to the receptor would be stronger by approximately 10 kJ/mol .

The question arises whether free energy minima predicted by metadynamics really represent stable or metastable states. This is particularly important for minima located close to the diagonal $\text{WHIM1} = \text{WHIM2}$. These minima can suffer from the artifact caused by a limit of a collective variable (in this case $\text{WHIM2} \leq \text{WHIM1}$).³⁶ In order to test this we performed an unbiased molecular dynamics simulation starting from the minimum A of Imatinib and from minima B, C, and D of Zanamivir. The results for Imatinib will be discussed first. The snapshot from the metadynamics pseudotrajectory (time 886 ps) was used as a starting conformation for a new unbiased 10 ns molecular dynamics simulation. The results are depicted in

Figure 9. Imatinib stayed in minimum A for approximately 1 ns. This disagrees with the height of a barrier (few kJ/mol)

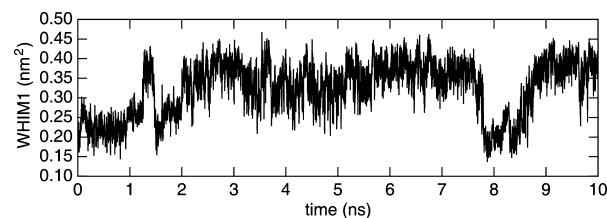


Figure 9. Profile of WHIM1 during an unbiased molecular dynamics simulation of Imatinib in water starting from the minimum A.

indicating that WHIM descriptors cannot fully separate individual energy minima.

Similarly, three unbiased 1 ns simulations were performed starting from the local free energy minima B, C, and D of Zanamivir. As expected, all three simulations finished in the global free energy minimum A. The molecule stayed in the conformation C and D for approximately 100 ps. This indicates that these are real free energy minima. Only the minimum B was quickly (in approximately 20 ps) escaped which can be explained by its conformational heterogeneity (see Figure 10).

Similarly to testing of predicted free energy minima it is possible to test predicted transition states. A series of conformations corresponding to a single transition state, which is the saddle point on a free energy surface, were selected. Then each of them was subjected to an unbiased 200 ps simulation. Provided that they really represent the transition state it is expected that some of them (ideally 50%) fall into one minimum and other fall to the second minimum. Indeed, six of ten simulations resulted in trajectories with prevalent conformation A (lower values of WHIM1 compared to the transition state), whereas the other four simulations fell to the minimum B (Figure 11).

The free energy surfaces of the studied compounds show typical patterns, which can be inferred from shapes of molecules. For example, highly rigid molecules like Ibuprofen show one or very narrow free energy minimum. Free energy minima of flexible linear molecules represented by Inatinib are typically antidiagonally oriented narrow grooves. Branched molecules like zanamivir show a wide free energy surface. Some molecules like Atorvastatin or Tenofovir contain rigid moieties that cause limits in WHIM values. For example WHIM2 value

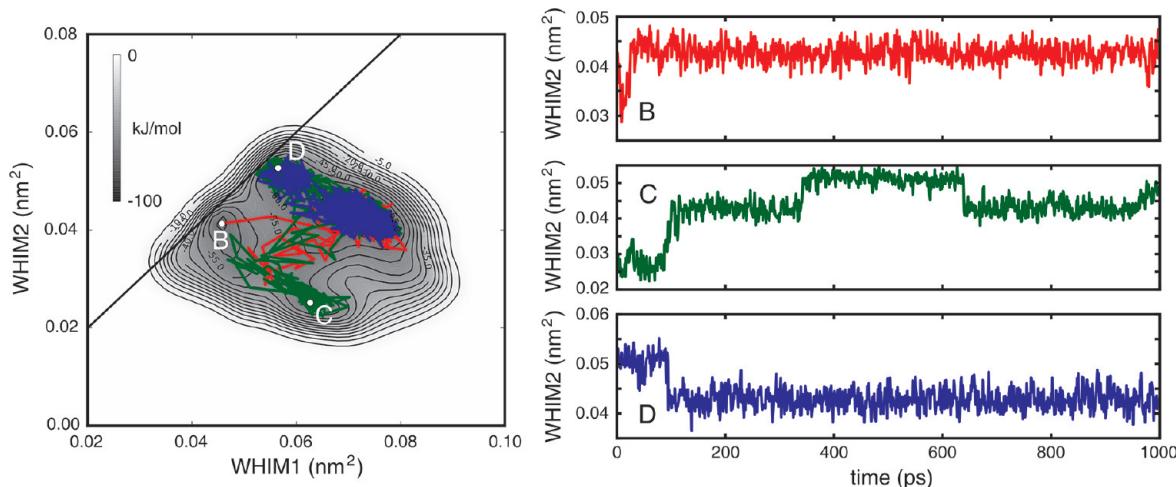


Figure 10. Profiles of WHIM descriptors during unbiased molecular dynamics simulations of Zanamivir in water starting from minimua B, C, and D (white dots).

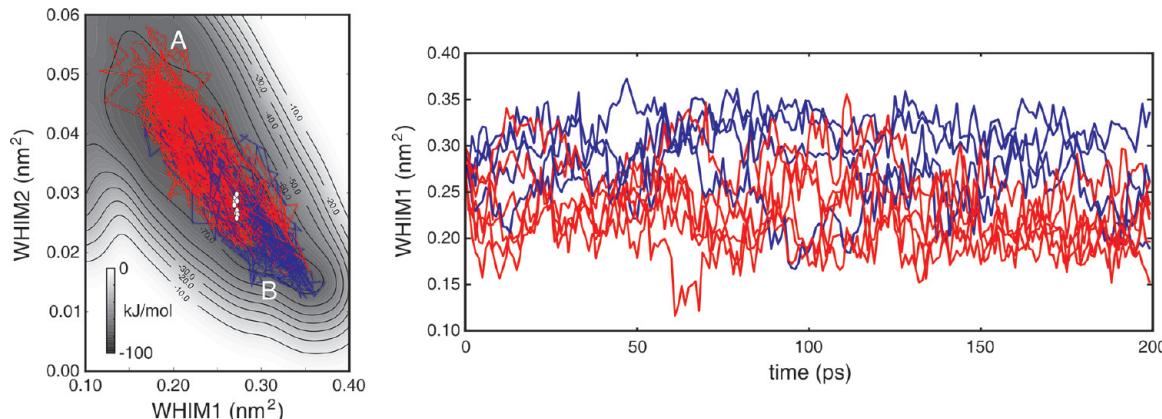


Figure 11. A series of ten unbiased molecular dynamics simulations starting from the transition state of the conformational change of Methotrexate from the minimum A to B. Starting conformations are shown as white dots.

of Tenofovir does not fall below 0.009 nm^2 , which corresponds to the fact that the adenine moiety of Tenofovir is rigid and cannot be compressed.

In the Introduction we discussed the structural meaning of WHIM descriptors when weighting factors α_k are set to unity. Todeschini and co-workers have shown that different atomic properties such as charge, absolute value of charge, or others can be applied. Corresponding WHIM descriptors then describe polarity, amphiphility, or property of the molecule in the given conformation. It is therefore possible to use the same approach with different weighting factors in order to explore conformational space of a molecule to find its most and least polar or most and least amphiphilic conformations.

During the preparation of this manuscript, Vymětal and Vondrášek have published their application of metadynamics in the space of gyration- and inertia-tensor-based CVs.³⁷ Descriptors referred to as principal moments of gyration tensor by the authors corresponds to WHIM descriptors used in this study. This shows that our ideas have converged to a similar approach, despite the fact that we were inspired by QSAR methodology, whereas Vymětal and Vondrášek found their inspiration in the mechanics of rigid bodies.

In conclusion, the proposed approach provides a molecular-mechanics-based exploration of a conformational space of druglike molecules. Simultaneously it provides an estimate of

relative free energies of their conformers in explicitly modeled solvent and at biologically relevant temperatures. The resulting free energy surface of each molecule also represents a kind of fingerprint, which illustrates its shape and conformational freedom. The aim of this study was to provide collective variables that are general, and thus they do not have to be chosen or designed *ad hoc* for individual compounds. However, the simulation of Saquinavir shows that a pair WHIM descriptors falls short in the description of such a complex molecule. Application of recent advances in metadynamics and free energy modeling will be necessary to address this issue.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: spiwokv@vscht.cz.

Notes

The authors declare no competing financial interest.

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