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Binding of Pollutants to Biomolecules: A Simulation Study

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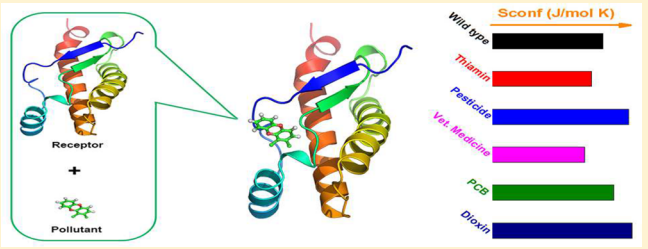
Supporting Information

ABSTRACT: A number of cases around the world have been reported where animals were found dead or dying with symptoms resembling a thiamine (vitamin B) deficiency, and for some of these, a link to pollutants has been suggested. Here, we investigate whether biomolecules involved in thiamin binding and transport could be blocked by a range of different pollutants. We used *in silico* docking of five compound classes (25 compounds in total) to each of five targets (prion protein, ECF-type ABC transporter, thi-box riboswitch receptor, thiamin pyrophosphokinase, and YKoF protein) and subsequently performed molecular dynamics (MD) simulations to assess the stability of the complexes. The compound classes were thiamin analogues (control), pesticides, veterinary medicines, polychlorinated biphenyls, and dioxins, all of which are prevalent in the environment to some extent. A few anthropogenic compounds were found to bind the ECF-type ABC transporter, but none binds stably to prion protein. For the riboswitch, most compounds remained in their binding pockets during 50 ns of MD simulation, indicating that RNA provides a promiscuous binding site. In both YKoF and thiamin pyrophosphokinase (TPK), most compounds remain tightly bound. However, TPK biomolecules undergo pollutant-induced conformational changes. Although most compounds are found to bind to some of these targets, a larger data set is needed along with more quantitative methods like free energy perturbation calculations before firm conclusions can be drawn. This study is in part a test bed for large-scale quantitative computational screening of interactions between biological entities and pollutant molecules.



INTRODUCTION

Pollution is known to impact the environment and affect humans and animals by triggering various mechanisms of toxicity.[1] Well-documented examples at the molecular level are the binding of heavy metals to enzymes,[2] the binding of polyaromatic hydrocarbons to DNA,[3] hormonal disturbance by dioxins and polychlorinated biphenyls (PCBs), and other effects, like reproductive disorders due to phthalates. All of these mechanisms have one thing in common: By displacing natural compounds, pollutants block and modify physiological processes and pathways and induce genotoxicity, mutagenicity, and also carcinogenicity. Chemically reactive metabolites or pollutants can also lead to conformational changes that affect the function of proteins,[4,5] which alter and reduce their biological function. In this work, we investigate the hypothesis that anthropogenic compounds may interfere with pathways involving thiamin (vitamin B1). Deficiency of vitamin B1 has been known for a long time to be fatal to humans (for reviews, see refs 6−8), and a number of recent reports suggest the occurrence vitamin B1 deficiency in wild animals,[9,10] even though other explanations have been proposed as well.[8]

Here, we investigate, using computational chemistry methods, whether pollutants may block or hinder thiamin uptake. We also evaluate whether computational tools such as docking and molecular simulation can predict which kind of compounds could be involved in blocking pathways.

Target Systems. Five different target systems were used: four proteins or protein complexes and one protein/RNA complex. For all of the targets, experimental structures with thiamin or a thiamin analogue are available. Here, we briefly describe the targets and their biological relevance.

1. Prion protein (PrP) is normally located on the surface of nerve cells and in other tissues of the body in mammals.[11] Misfolded forms of PrP cause disease in mammals, including scrapie in sheep,[12] bovine spongiform encephalopathy in cattle,[13] chronic wasting disease in deer and elk,[14] and mad cow disease in animals. In humans, misfolded PrP is known to lead to Gertsmann−Straussler−Scheinker syndrome, Kuru, variable protease-sensitive prionopathy, Creutzfeldt−Jacob disease, and fatal familial insomnia.[13,15−19] Knowledge of PrP−ligand interactions is likely to be important for treatment of prion diseases, and there are some studies of ligands with high affinity to PrP that may function as therapeutic agents. These compounds include Congo Red,[20]

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Table 1. Ligands[a]

ligand	abbr.	formula	weight	Q
thiamin	T1	$C_{12}H_{17}N_4OS$	265.355	1
pyrithiamine	T2	$C_{14}H_{19}N_4O$	259.326	1
thiamine monophosphate	T3	$C_{12}H_{17}N_4O_4PS$	344.327	0
thiamine pyrophosphate	T4	$C_{12}H_{17}N_4O_7P_2S$	424.306	−1
(5-ethyl-2-[(2,3,5,6-tetrafluorophenyl)amino]phenyl) acetic acid	P1	$C_{16}H_{12}F_4NO_2$	326.265	−1
1-methyl-2-[(*E*)-2-thiophen-2-ylethenyl]-5,6-dihydro-4*H*-pyrimidine	P2	$C_{11}H_{14}N_2S$	206.307	0
methyl *N*-(6-phenylsulfanyl-1*H*-benzoimidazol-2-yl)carbamate	P3	$C_{15}H_{13}N_3O_2S$	299.347	0
(*E*)-*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N*′-methyl-2-nitrovinylidenediamine	P4	$C_{11}H_{15}ClN_4O_2$	270.715	0
N,*N*′-[(methylimino)dimethylidyne]di-2,4-xylidine	P5	$C_{19}H_{23}N_3$	293.406	0
1-(4-isothiocyanatophenoxy)-4-nitrobenzene	VM1	$C_{13}H_8N_2O_3S$	272.279	0
4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide	VM2	$C_{14}H_{13}N_3O_4S_2$	351.400	−1
(*RS*)-4-[1-(2,3-dimethylphenyl)ethyl]-3*H*-imidazole	VM3	$C_{13}H_{16}N_2$	200.279	0
4-(1-hydroxy-2-[(1-methyl-2-phenoxyethyl)amino]propyl)phenol	VM4	$C_{18}H_{22}NO_3$	300.372	1
(*RS*)-1-(4-amino-3,5-dichlorophenyl)-2-(*tert*-butylamino)ethanol	VM5	$C_{12}H_{19}Cl_2N_2O$	278.198	1
4,4′-dichlorobiphenyl	PCB1	$C_{12}H_8Cl_2$	223.098	0
2,4,4′-trichlorobiphenyl	PCB2	$C_{12}H_7Cl_3$	257.543	0
3,4,3′,4′-tetrachlorobiphenyl	PCB3	$C_{12}H_6Cl4$	291.988	0
3,3′,4,4′,5-pentachlorobiphenyl	PCB4	$C_{12}H_5Cl_5$	326.433	0
2,3′,4,4′,5,5′-hexachlorobiphenyl	PCB5	$C_{12}H_4Cl_6$	360.878	0
2,8-dichlorodibenzo-*p*-dioxin	D1	$C_{12}H_6Cl_2O_2$	253.081	0
1,2,8-trichlorodibenzo-*p*-dioxin	D2	$C_{12}H_5Cl_3O_2$	287.526	0
1,3,7,8-tetrachlorodibenzo-*p*-dioxin	D3	$C_{12}H_4Cl_4O_2$	321.971	0
1,2,3,6,7-pentachlorodibenzo-*p*-dioxin	D4	$C_{12}H_3Cl_5O_2$	356.416	0
1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin	D5	$C_{12}H_2Cl_6O_2$	390.861	0
1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin	D6	$C_{12}HCl_7O_2$	425.306	0

[a]Abbreviations according to category: T, thiamin (analogues); P, pesticide; VM, veterinary medicine; PCB, polychlorobiphenyl; D, dioxin. Molecular weight (Da) and net charge Q (e).

tetracyclines,[21] curcumin,[22] quinacrines,[23] and simvastatin.[24] Interestingly, a number of structures of PrP complexed with thiamin and derivates have also been determined using nuclear magnetic resonance spectroscopy.[25]

2. The ATP-binding cassette (ABC) family is one of the largest transporter protein families, found in many species from bacteria to humans.[26] These proteins consist of four domains: two transmembrane domains that form the translocation pathway and two nucleotide-binding domains that maintain transport through binding and hydrolysis of ATP.[27] ABC importers cannot transport substrates without substrate-binding proteins because these bind the substrate to be transported with high affinity and release them through the translocator.[28] The energy-coupling factor (ECF) transporters use their S-component to recognize substrates.[29,30] The ECF transporters are involved in the uptake of thiamins in Prokarya, and here we use an X-ray crystallography structure of the ECF−thiamin complex.[31]
3. The thi-box riboswitch, which includes a mRNA regulatory element that binds small molecules with high affinity, modulates gene expression through a variety of mechanisms in prokaryotes, archea, and eukaryotes.[32−36] A recent study revealed that the thi-box riboswitch acts as a regulatory molecule in bacteria, controlling genes involved in thiamin biosynthesis as well as transport.[37] For the purpose of this work, it is also interesting that thiamin binds to the RNA molecule in this protein−RNA complex. The structure of a bacterial riboswitch is used here, for which binding affinities have been determined as well.[38]
4. The thiamine binding protein YKoF is a part of an ABC transporter complex that is further composed of two ATP-binding proteins (YkoD) and two transmembrane proteins (YkoC and YkoE). The YKoF protein is involved in the hydroxymethyl pyrimidine salvage pathway and/or thiamine transport.[39,40]
5. Thiamin pyrophosphokinase (TPK), which is a regulator of thiamine metabolism, catalyzes the conversion of thiamine to thiamin pyrophosphate (TPP), also known as thiamin diphosphate, which is one of the active forms of thiamin.[8] TPK is required as a coenzyme for enzyme complexes associated with the metabolism of carbohydrates, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched-chain α-ketoacid dehydrogenase, and transketolase.[41,42]

Pollutant Groups. To generate a map of relevant pollutants for docking and simulation, a series of classes have been investigated. Four well-defined compounds-classes are used here:

1. Pesticides are toxic chemicals used to kill, reduce, or repel pests. Targets include insects, bacteria, fungi, rodents, weeds, or other organisms that can threaten agricultural production,[43] and pesticides pose risks to human health and the environment and are frequently used in agricultural activities. The World Health Organization estimates that 300 000 people die from pesticides each year, primarily in developing countries.[44] The pesticide compounds used in this work all contain an aromatic moiety in order to be structurally similar to thiamin.
2. Veterinary medicines are applied to varying degrees in different countries. During their cycle of transformation,

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these compounds can reach groundwater and spread.[45] From this class, five compounds, all of which contain an aromatic group, were selected.

3. Dioxins are particularly persistent pollutants[1] derived from industrial processes,[46−48] but they can also result from natural processes, such as volcanic eruptions and forest fires.[49,50] Here, we have used a series of dioxins with an increasing number (2−7) of chlorine (Cl) substituents.
4. PCBs are a well-known class of man-made toxic chemicals that, in the environment, are typically bound to soil, organic particles, and sediments. PCBs are widely used in electrical equipment, circuits, plastics, resins, chlorinated rubbers, and flame retardants.[51−53] PCBs have many documented adverse health effects; They have been linked to cancer,[54−58] heart disease,[59−61] hormonal problems,[62,63] asthma,[64,65] cognitive problems,[66−68] suppression of the immune system,[69,70] and IQ deficits in infants.[71,72] To represent PCBs, we also used a series of five congeners with an increasing number of Cl atoms.

To better understand the effect that these classes of compounds may have on different biomolecular targets, we present molecular dynamics simulations of 125 protein−ligand complexes, aiming to assess the stability of the complexes and, in general, the effect of these compounds on different receptors. Four of the compounds are thiamin analogues that are known to bind to their respective target, and structures and binding affinities are available for these. These ligands function as a control group in the analysis. A complete list of the pollutants including the controls is given in Table 1. The common names and structural formulas of the pollutants are listed in Table S1.

METHODS

The experimental structures of ECF-type ABC transporter,[31] thi-box riboswitch receptor,[38] PrP,[25] YKoF,[40] and TPK[73] were obtained from the Protein Data Bank.[74] All of these structures were determined in complex with thiamin or a thiamin analogue.

Structures of ligands used in this work were obtained from the PubChem,[75] the Zinc,[76] or Chemspider[77] databases. A geometry optimization of the ligands was performed with Gaussian 03,[78] using the HF/6-31G(d) basis set. The Gaussian output file served as input for the Antechamber[79,80] program, which is a tool in the AMBER software suite.[81] AM1 BCC partial charges of ligands were calculated by Antechamber, and the resulting topology file was converted to the GROMACS format using Acpype.[82]

Ligand docking studies were performed using the rDock[83] program. The ionization states of biomolecules were assigned using GROMACS tools.[84] The Mg^{2+} ions were considered part of the riboswitch as they were involved in the binding. The receptor structures were converted to Tripos MOL2 format for docking, and the ligands in the structure data file format, using OpenBabel.[85] The topology and bond orders of each ligand were checked manually. The docking volume was determined by the accessible volume within 0.6 nm of a reference ligand. Each ligand was subjected to 100 docking runs for each receptor. The top three best poses, according to the docking score, were clustered and saved for further analysis.

After docking, the complexes were solvated using a layer of at least 1 nm between the box edge and the solutes, in a rhombic dodecahedron box for periodic boundary conditions. An ion concentration of 0.15 M NaCl was applied (including counterions) to neutralize the systems and obtain physiologically relevant salt concentrations. The AMBER99SB-ILDN force field[86] was used in combination with the single-point charge (TIP3P) water model.[87] First, an energy minimization was performed using the steepest descent algorithm for 5000 steps. Then, 1 ns constant volume molecular dynamics simulations were performed using position restraints on the atoms of the complex, in order for the solvent to relax and the temperature of the system to equilibrate. The LINCS algorithm[88] was used for bond constraints in all simulations, allowing an integration time step of 2 fs. The temperature was maintained at 300 K using the V-rescale thermostat[89] with a coupling time of 1.0 ps. A further restrained equilibration step was performed using the Berendsen pressure coupling algorithm[90] to obtain a density consistent with a reference pressure of 1 bar, using a coupling time of 0.5 ps and a compressibility of 4.5×10^{-5} bar. The Berendsen algorithm is preferred over others for its superior equilibration properties.[91] For the production simulations, however, the Parrinello−Rahman barostat[92] was used to maintain a pressure of 1 bar with coupling time of 2 ps. The temperature was maintained at 300 K, using the Nosé−Hoover thermostat[93,94] with a coupling time of 1.0 ps. The particle-mesh Ewald (PME) method[95] was used to treat long-range electrostatic interactions, beyond the cutoff distance of 1.1 nm. Coordinates were saved every 10 ps for analysis, and the total duration of each simulation was 50 ns. For reference, simulations of the five biomolecular systems were performed in the same manner but without ligands (apo structures). All MD simulations were carried out using the GROMACS software, version 5.0.4.[84,96] The simulations took about 6 days on 24 AMD-Barcelona cores each, amounting to ≈450 000 core hours in total.

RESULTS

Docking. The binding modes of the docked ligands were ranked based on rDock score. The highest ranked binding modes of all docked ligands were chosen for further analysis. The binding scores of the docked ligands are listed in Table S2, and the scores of the ligands used as a control group seem to be consistent with the binding free energies from experiments, suggesting that binding to the ECF is strongest, followed by YKoF, TPK, riboswitch, and, with weakest binding, PrP. To evaluate the docking predictions, the correlation between docking scores and ligand root-mean-square deviation (RMSD) after simulation is plotted in Figure 1. In a correct structural prediction, the RMSD should be low for the best structures, as indicated for protein folding simulations,[97] but no correlation over a whole data set should be expected. Here, we find that a low docking score predicts good binding for ECF (with the thiamin analogues) and the riboswitch (for which virtually everything binds). For the other targets, the lowest docking

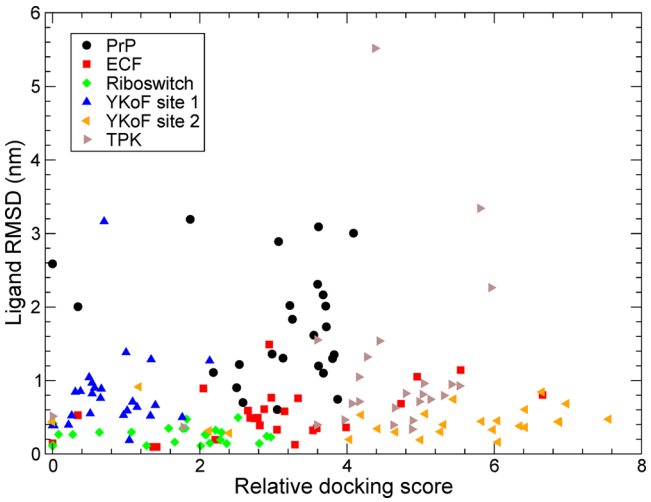

Figure 1. Correlation between relative docking score (from the lowest for each target) and ligand RMSD (after fitting the complex to the biomolecule structure) for all targets.

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scores do not yield the strongest binders, which means that the docking scores do not accurately predict binding

Structural Stability. To investigate the structural stability of biomolecule−ligand complexes, the all-atom RMSD of each ligand was calculated and plotted (Figures S1, S3, S5, S7, S8, and S10) by first fitting the protein structure on the original complex and then computing the RMSD. At long distances from the binding site, the orientation of the ligand does not contribute much to the RMSD, and the resulting number represents a distance from the binding site. For the backbone of each biomolecule, the RMSDs are plotted in Figures S2, S4, S6, S9, and S11. In addition, we calculated the average RMSD for each biomolecule and separately for its ligands over the last 30 ns of all simulations (Table S3 and Figure 2).

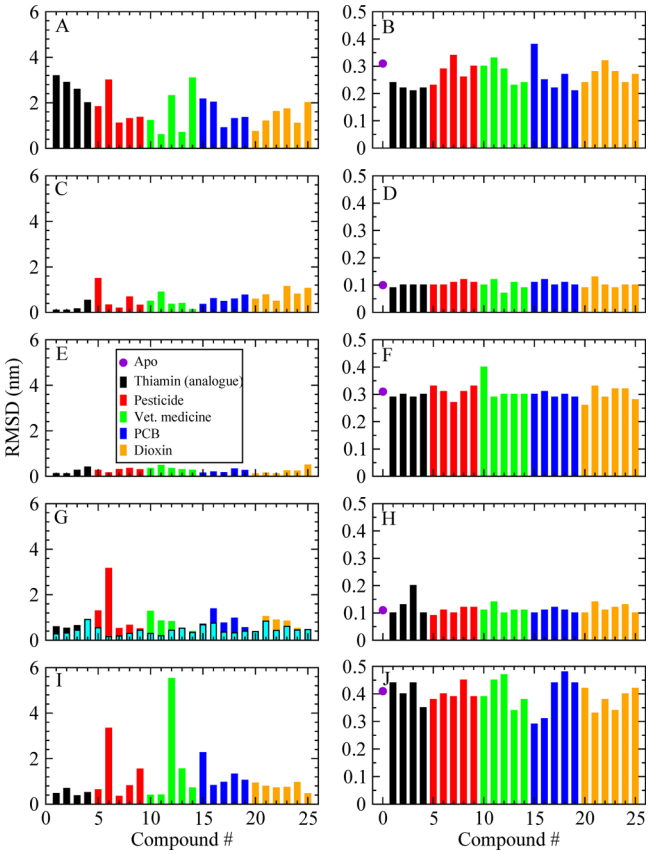

Figure 2. RMSDs for ligands after fitting the complex to the biomolecule structure of (A) PrP, (C) ECF, (E) riboswitch, (G) YKoF, and (I) TPK. RMSDs for the biomolecules (B) PrP, (D) ECF, (F) riboswitch, (H) YKoF, and (J) TPK. Ligands in site 2 of YKoF are colored cyan. Ligand numbers refer to Table 1. Note the different scales on the *Y* axis for ligands and biomolecules.

For PrP−thiamin complexes, the RMSDs of ligands fluctuate between 2 and 3 nm, indicating that the ligands are no longer bound to the original site (Figure 2A). Some of the ligands remain bound to PrP but in other locations, which is not surprising because many ligands are rather hydrophobic. The PrP biomolecule does not show large fluctuations except for P3 and PCB1 (Figure 2B). For most complexes, the RMSD of the biomolecule is somewhat lower than the apo structure.

For ECF, the RMSD of most compounds is 0.5 nm or larger, including T4 (Figure 2C). Apart from thiamin analogues T1−3, only P3, VM5, and D3 remain bound somewhat close to the original docking site. The complexes of these three compounds with ECF and the riboswitch are plotted in Figure 3A−D. The binding mode is rather different for all of the compounds. There is no significant change in the structure of the ECF molecules during simulation (Figure 2D); in fact, ECF is the most stable of all biomolecules studied, probably because it is encapsulated in a phospholipid bilayer.

Virtually all compounds remain tightly bound to the riboswitch (Figure 2E). The RMSDs of the thiamin ligands show that the number of phosphate atoms (and the charge) of the ligand may play a role in the RNA−ligand interactions; because the negatively charged T4 moves away from the binding site more than T1−3, the same is observed for ECF. The binding energies measured experimentally for the riboswitch are rather similar, however.[38]

For YKoF, the RMSDs of most ligands in sites 1 and 2 fluctuate around 1 nm and under 1 nm, respectively. The ligands in both sites remain tightly bound to their binding site except P2 in site 1 (Figure 2G). The YKoF complexes do not show large fluctuations except when bound to T3 (Figure 2H).

The RMSDs of ligands in TPK complexes are around 1 nm except for P2, P5, VM3, VM4, PCN1, and PCB4 (Figure 2I). Most complexes of TPK show large fluctuations, indicating that they undergo a conformational change upon ligand binding (Figure 2J).

To understand the conformational effect of ligands on the biomolecular structure, the average RMSD for each molecule is plotted in Figure 2. It is interesting that the ligands do not influence the RMSD of the biomolecules for ECF, riboswitch, and YKoF, whereas the loosely bound ligands seem to stabilize PrP somewhat, and the tightly bound ligands of TPK caused significant conformational changes.

Structures of tightly bound ligand complexes are plotted in Figure 3 to get an impression of the different binding modes. The last frames of all biomolecular structures obtained after MD simulations are plotted in Figures S17−S21. All ligands of PrP are far away from the binding pocket (Figure S17). The P1, P4, VM1, VM2, and PCB5 ligands in ECF exhibit significant mobility (Figure S18). For ligands of the riboswitch, no large difference among the ligands of each group can be observed in Figure S19. The P2 ligand of YKoF is far away from the binding pocket (Figure S20). As for TPK, the P2, P5, VM3, VM4, PCB1, and PCB4 ligands in low-affinity binding site 1 show a large conformational change (Figure S21).

Principal Component Analysis. To further examine the effect of the ligands on the conformation of biomolecules and to compare the systems to each other, we performed principal component analysis (PCA[98,99]) on the concatenated biomolecular structures obtained from the last 30 ns of all simulations for a certain target. Figures S12−S16 show the projection of the trajectories on the first two eigenvectors, with each figure being subdivided into five panels for each compound class. The magnitude of the fluctuations indicates the size and flexibility of the biomolecule.

The collective motions of PrP's backbone atoms along the two principal components (PCs) sample different spaces due to ligands moving away from the binding pocket (Figure S12), and no systematic dynamics related to the compound class can be found. ECF with thiamin (analogues) bound is very stable (Figure S13), whereas the fluctuations are larger with other compound classes. Again, however, no systematic dynamic grouping of classes is seen. The same conclusions can be drawn for the riboswitch (Figure S14). The YKoF complexes show

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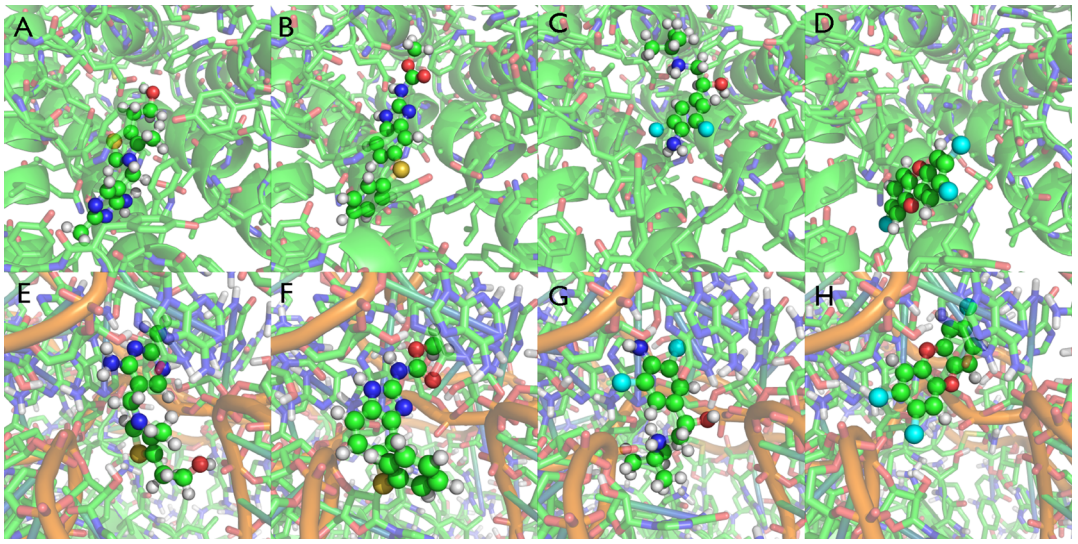

Figure 3. Structures of ECF with (A) T1, (B) P3, (C) VMS, and (D) D3. Structures of riboswitch with (E) T1, (F) P3, (G) VMS, and (H) D3. All complexes were equilibrated during 50 ns MD simulations.

different spaces than the apo structure, suggesting that ligands influence the dynamics of YKoF (Figure S15). The systematic dynamics effect is especially clear for pesticides, veterinary medicine, and PCB groups binding to YKoF. As for TPK, it seems that each biomolecule spans a different region of space depending on the ligand, revealing that the ligands affect the dynamics of the protein (Figure S16).

Conformational Entropy. The conformational entropy S_{conf} for the complexes and apo structures was calculated using quasiharmonic analysis[100,101] based on the PCA (Table S4). Briefly, the analysis assumes that the vibrational frequencies are generated by quantum harmonic oscillators with frequency ω. Then

$$S_{qh} = R \sum_i \frac{\hbar\omega/k_B T}{\exp(\hbar\omega/k_B T) - 1} - \ln[1 - \exp(-\hbar\omega/k_B T)] \quad (1)$$

where R is the gas constant, k_B is Boltzmann's constant, and T is the temperature. The frequencies ω come from the PCA.[98,99] The conformational entropy of the corresponding apo structures was then subtracted to investigate the effect of ligand binding on entropy. The resulting ΔS_{conf} values (Figure 4) are significantly different for the five biomolecular systems. The average ΔS_{conf} values are −74 J/(mol K) (PrP), −17 J/(mol K) (ECF), −146 J/(mol K) (riboswitch), 42 J/(mol K) (YKoF), and 135 J/(mol K) (TPK), contributing approximately 5, 1, 11, −3, and −10 kcal/mol, respectively, to the stability ($-T\Delta S_{conf}$S) of the complexes at 300 K on average. To investigate whether the change in conformational entropy is predictive of binding, the correlation between $-T\Delta S_{conf}$ and the ligand RMSD is plotted in Figure 5. It may be clear there is no correlation whatsoever, despite the finding (Figure 4) that $-T\Delta S_{conf}$ for, for example, ligands binding to the riboswitch and TPK are almost all negative and positive, respectively. The reason for this lack of correlation is most likely due to ignoring the entropy of ligand and solvent.

Interaction Energy. The interaction energy between target and ligand was estimated from the sum of short-range Lennard-Jones and short-range Coulomb energy terms. It is possible to incorporate long-range energies in such analyses,[102] but this makes sense only for potential of mean force calculations, which were not considered here. The interaction energy (IE) is a measure of the stability of receptor (being part of the enthalpy contribution) and hence of the binding affinity of a ligand bound to a protein. The results suggests that the ligands bind to the riboswitch with lower interaction energy (stronger) than to either the ECF transporter, YKoF, TPK, or PrP (Table S4). The IE is smallest (in absolute terms) for PrP because the ligands move away from the target during the simulation. Thiamin binds stronger to YKoF site 2 than to site 1 by ≈2 kcal/mol,[40] and in the docking score, we obtain a systematic difference with the same sign. The interaction energy is compensated to a large extent by the larger decrease in conformational entropy (Figure 4); however, quantitative predictions of binding stability require some form of free energy calculations, which we did not pursue here.

DISCUSSION

Detailed studies of the interaction between receptors and environmental chemicals have been reported for the androgen receptor[103] and for a large range of targets by Sipes et al.[104] based on experimental methods. Mavri and co-workers have devised a docking server that performs rapid docking to nuclear receptors[105] and applied it to endocrine disruptors, a class of compounds that includes dioxins, which were studied here. These authors concluded[106] that they can single out potentially toxic compounds that bind to multiple targets but that further verifications are needed. Here, we have used docking as a first step and MD simulations to validate the stability of the complexes to investigate the binding of pollutants to biomolecular targets. By selecting five targets that all bind thiamin, the setup utilizes a common control group. For these systems, experimental binding free energies are available as well as three-dimensional structures.

Although our docking results of PrP−thiamin binding yield structures resembling the experimentally determined complex, the RMSD analysis indicates that none of ligands remains bound to PrP (Figure 2A). It seems likely that the superficial thiamin binding site of PrP near the N-terminal loop of α-helix 1 is easy to escape from. This is consistent with the weak affinity measured for thiamin (binding energy ∼5 kcal/mol[25])

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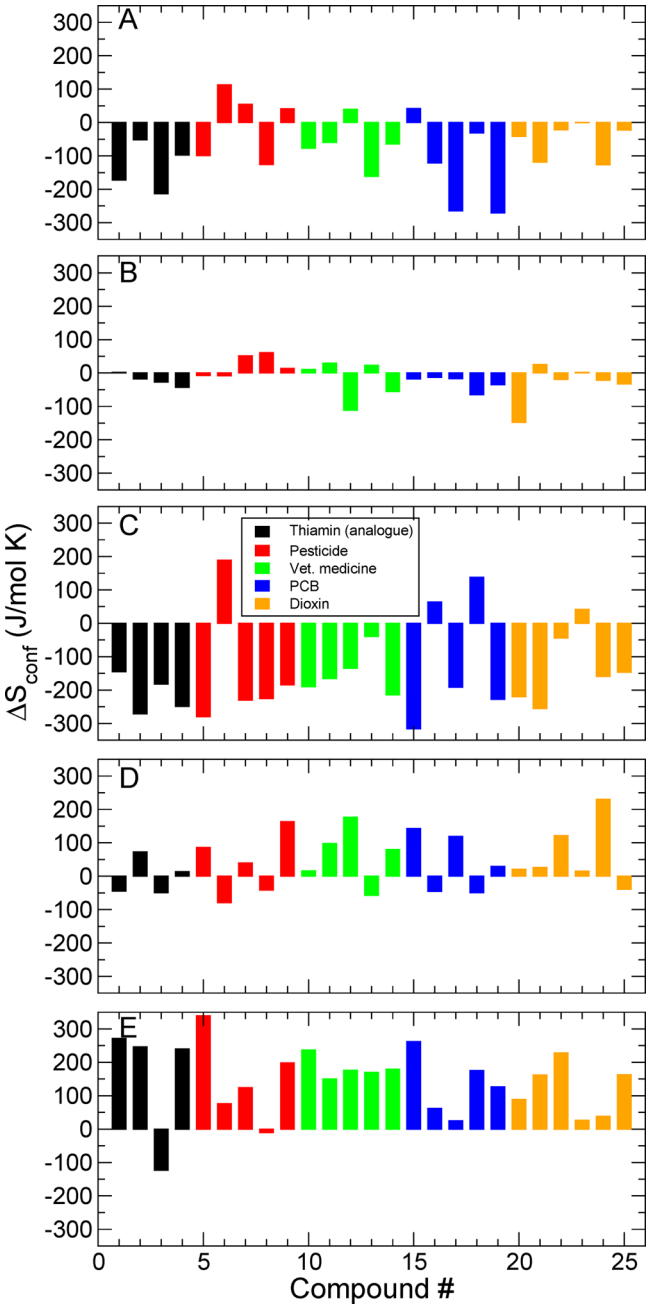

Figure 4. Differences in conformational entropy upon ligand binding for (A) PrP, (B) ECF, (C) riboswitch, (D) YKoF, and (E) TPK. Ligand numbers refer to Table 1.

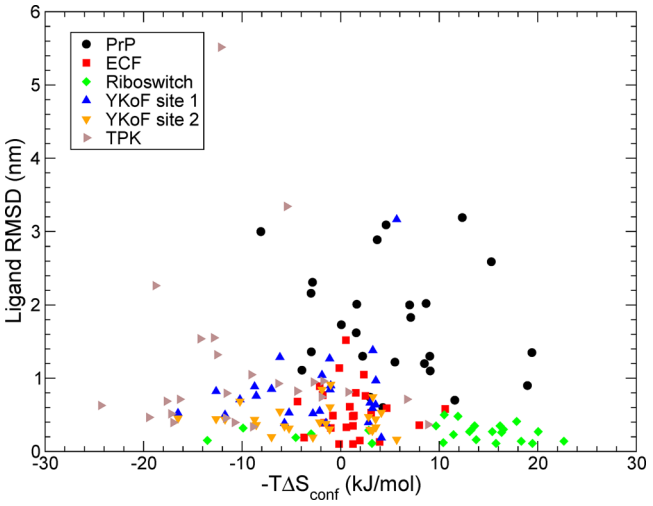

Figure 5. Correlation between conformational entropy and ligand RMSD (after fitting the complex to the biomolecule structure) for all targets.

and indeed the experimental finding that only very few compounds bind to this target.

The binding scores of the thiamin–ECF complexes obtained by the docking seem to be in good agreement with experimental binding energies.[107] Despite the pollutants having only slightly weaker binding scores, most of these move at least in part out of the binding site, and in fact only a handful remain bound (Figure 3). The RMSD analysis shows that the ECF biomolecules do not undergo large conformational changes during the simulations (Figure 2), and the conformational entropy changes of the ECF–ligand complex (Figure 4C) show that the protein does not lose a lot of entropy by binding ligands.

The docked riboswitch–thiamin complexes are consistent with the experimental structures,[38] and most compounds remain in the binding site during the simulations (Figure 2F). The conformational entropy analysis (Figure 4C) shows that most compounds reduce the biomolecular conformational entropy, in line with experimental observations;[25] this is, however, compensated by strong interaction energies (Table S4).

The docking scores of the thiamin–YKoF complexes are in good agrement with with experimental binding energies.[40] Although the RMSD analysis shows that the YKoF biomolecules do not undergo large conformational changes during the simulations (Figure 2), the PCA results suggest that the intrinsic dynamics of the protein may be affected by ligands (Figure S15), which may be a result of the weakly bound ligands in site 1. In addition, the conformational entropy changes of the YKoF complexes (Figure 4D) indicate that the entropy of the protein is increased by binding ligands.

The RMSD analysis (Figure 2) and conformational results (Figure 4E) demonstrate that all pollutants bound to TPK cause large conformational changes in this protein, suggesting that the function of TPK may be affected by the binding of pollutant compounds. This suggests that pollutant-induced conformational changes may be important for understanding the effect of pollutants on proteins.

No systematic effect related to the increasing number of Cl atoms for the PCB and dioxin molecules was found, which may be because of their neutral character and because the binding pockets are sufficiently large to accommodate the Cl atoms.

The simulations presented here yield insight into the binding properties of five very different biomolecular targets. PrP does not bind any natural or anthropogenic compounds strongly, at least not the ones that we addressed in this study, since most ligands escape rapidly from the thiamin binding site. ECF binds ligand rather specifically; that is, most compound move away from the thiamin binding pocket, although P3, VM5, and D3 remain close to their original binding sites (Figure 3B–D). This suggests that molecules like these (see Table 1 for details) may indeed hinder thiamin transport and contribute to vitamin B1 deficiency in animals.[9] For the riboswitch, most ligands remain tightly bound (Figure 2C) in the rather deep thiamin binding

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pocket (Figure 3E–H), indicating that binding is not very specific. Here, the significant reduction of conformational entropy upon ligand binding negatively influences the binding energy; however, the interaction energies are more negative for these complexes than for ECF–ligand complexes. YKoF and TPK bind most ligands tightly, suggesting that at least some of those ligands may hinder thiamin binding and transport. For TPK, the increase of conformational entropy upon ligand binding suggests that biomolecules undergo large conformational changes, which is compatible with Figure 2J.

In more detail, P3 (Fenbendazole) is an anthelmic agent against roundworms, tapeworms, and flukes. The compounds is effective against smaller parasites as well and is thought to target β-tubulin.[108] Fenbendazole and related antiparasitic compounds have been documented to form an environmental threat against aquatic organisms.[109−111] VM5 (Clenbuterol) is used in some countries to treat breathing problems in humans and livestock. It targets the β2 adrenergic receptor but was found to affect several other proteins as well.[112] There is some evidence that Clenbuterol can have adverse effects on nematodes.[113] D3 is highly inert even after ingestion as 60% was excreted unmodified with a half-life of 15 months in one patient, Victor Yuschenko.[114] The metabolites found in this study seem to have been formed in the liver or skin where genes for cytochromes were found to be upregulated.[114] No biomolecular complex structure could be found for any of these three compounds, and the indirect information (e.g., gene upregulation) suggests that these compound interact with other biomolecules. Further evidence is therefore needed to confirm interactions of any of these compounds with ECF. Additional structural studies of biomolecules with pollutant compounds would be highly useful in this respect.

It would be interesting to perform a more quantitative analysis using free energy calculations of the interactions between biomolecular targets and pollutants along the lines of the studies mentioned above.[103,104] Such work requires further development of computational tools for high-throughput calculations;[84,115] however, high-throughput free energy calculations are rather tedious to perform at present.[116,117] Alternative approaches, including potential of mean force calculations, may yield additional information such as the impact of the solvent environment upon binding,[102] but they are computationally demanding as well. Despite previous successes with docking applications,[118] the relative insensitivity of the docking score to the final performance (Figure 1) suggests that more than the best-scoring docking pose may need to be considered in future protocols for high-throughput binding studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.6b00189.

Tables of docking scores, RMSDs, interaction energies, and conformational entropies; figures of the RMSD as a function of time, projections of eigenvectors of the principal component analysis, and snapshots of all complexes (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

ABC, ATP-binding cassette; AMBER, assisted model building with energy refinement; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; ECF, energy-coupling factor; GROMACS, Groningen machine for chemical simulation; IQ, intelligence quotient; MD, molecular dynamics; PCB, polychlorinated biphenyl; PrP, prion protein; RNA, ribonucleic acid; TPK, thiamin pyrophosphokinase

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