J Comput Aided Mol Des (2011) 25:709–716
DOI 10.1007/s10822-011-9453-x

Free energy calculations offer insights into the influence of receptor flexibility on ligand–receptor binding affinities

Jožica Dolenc · Sereina Riniker · Roberto Gaspari · Xavier Daura · Wilfred F. van Gunsteren

Received: 22 March 2011 / Accepted: 20 June 2011 / Published online: 7 July 2011

Abstract Docking algorithms for computer-aided drug discovery and design often ignore or restrain the flexibility of the receptor, which may lead to a loss of accuracy of the relative free enthalpies of binding. In order to evaluate the contribution of receptor flexibility to relative binding free enthalpies, two host–guest systems have been examined: inclusion complexes of α-cyclodextrin (αCD) with 1-chlorobenzene (ClBn), 1-bromobenzene (BrBn) and toluene (MeBn), and complexes of DNA with the minor-groove binding ligands netropsin (Net) and distamycin (Dist). Molecular dynamics simulations and free energy calculations reveal that restraining of the flexibility of the receptor can have a significant influence on the estimated relative ligand–receptor binding affinities as well as on the predicted structures of the biomolecular complexes. The influence is particularly pronounced in the case of flexible receptors such as DNA, where a 50% contribution of DNA flexibility towards the relative ligand–DNA binding affinities is observed. The differences in the free enthalpy of binding do not arise only from the changes in ligand–DNA interactions but also from changes in ligand–solvent interactions as well as from the loss of DNA configurational entropy upon restraining.

Keywords α-Cyclodextrin · Conformational flexibility · Drug design · DNA–ligand binding · Molecular dynamics

Introduction

Many biomolecular processes are based on ligand–receptor interactions. When a ligand binds to a receptor, the optimal ligand–solvent and receptor–solvent interactions are replaced by optimal intermolecular interactions between ligand and receptor and solvent. In this process the flexibility of the receptor plays an important role because configurational changes in the receptor may give rise to enthalpic and entropic contributions to the free enthalpy or Gibbs energy [1, 2] of binding. The size of these contributions depends on the flexibility of the host and is generally difficult to evaluate [3].

When examining biomolecular complex formation the enthalpic and entropic contributions from the receptor, ligand and solvent can in principle be evaluated by employing molecular dynamics (MD) simulations [4–11]. However, because of the computational cost MD simulations are not yet used for screening of large libraries of ligands in drug discovery. Instead, the structures of biomolecular complexes as well as estimates of the corresponding

Electronic supplementary material The online version of this article (doi:10.1007/s10822-011-9453-x) contains supplementary material, which is available to authorized users.

J. Dolenc · S. Riniker · R. Gaspari · W. F. van Gunsteren (✉)
Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH, 8093 Zurich, Switzerland
e-mail: wfvgn@igc.phys.chem.ethz.ch

J. Dolenc
Faculty of Chemistry and Chemical Technology, University of Ljubljana, 1000 Ljubljana, Slovenia

X. Daura
Catalan Institution for Research and Advanced Studies (ICREA) and Institute of Biotechnology and Biomedicine (IBB), Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

R. Gaspari
Empa, Swiss Federal Laboratories for Materials Science and Technology, nanotech@surfaces Laboratory, 8600 Dübendorf, Switzerland

binding affinities are often predicted by use of fast docking and scoring algorithms [12, 13] which generally treat the receptor as a rigid body. In recent years, however, several new docking algorithms were developed which incorporate the flexibility of the receptor in the docking scheme [14–19]. Despite these significant improvements, in many docking studies the flexibility of the larger receptor molecule remains ignored or severely restrained. If the host molecule is rigid or if the conformational changes in the host do not influence the host–guest interactions the predictions obtained by these docking algorithms can be very good. Unfortunately, for intrinsically flexible biomolecules this is often not the case [20–22]. Since docking algorithms do not generally allow significant conformational changes in the receptor, the receptor–ligand interactions can not be optimized, which can lead to wrong model structures of biomolecular complexes and wrong predictions of the receptor–ligand binding affinities.

With the aim of evaluating the loss of accuracy of the relative free enthalpies of binding due to restraining the molecular motion of the receptor, two host–guest systems have been examined (Fig. 1): inclusion complexes of α-cyclodextrin (αCD) with 1-chlorobenzene (ClBn), 1-bromobenzene (BrBn) and toluene (MeBn), and complexes of DNA with the minor-groove binding ligands netropsin (Net) and distamycin (Dist). While cyclodextrins are rather rigid host molecules, which do not undergo pronounced conformational changes upon ligand binding, the DNA double helix is a flexible target and restraining its molecular motion is expected to have significant entropic and enthalpic contributions to the binding free enthalpy. To address this issue we have calculated relative free enthalpies of binding of different ligands to the flexible and to the positionally restrained hosts αCD and DNA using MD simulations and the thermodynamic integration (TI) method [23].

Methods

Molecular dynamics simulations

The MD simulations reported in this paper were performed in explicit solvent employing the GROMOS biomolecular simulation package [24, 25] and the thermodynamically calibrated GROMOS force fields 45A4 and 53A6 [26, 27].

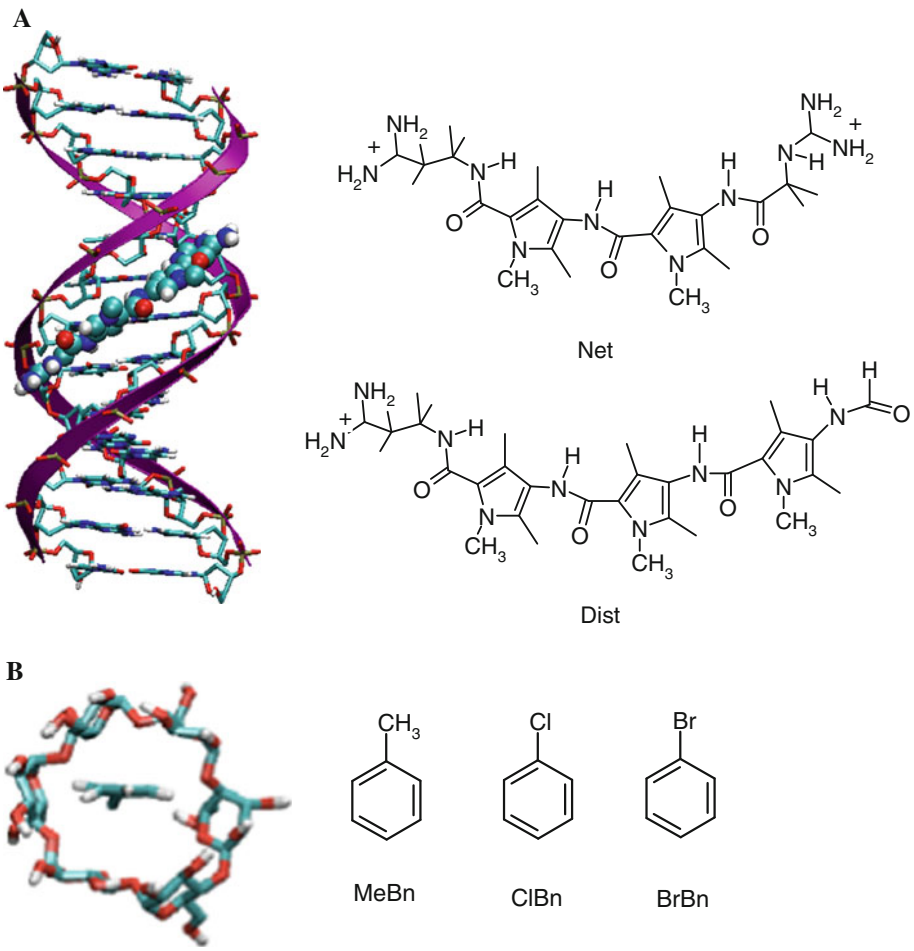

Fig. 1 **a** Netropsin-DNA complex along with the chemical structures of netropsin and distamycin. **b** αCD in complex with a monosubstituted benzene derivative and the chemical structures of the αCD ligands used in this work. Molecular graphics was made with VMD [37]

The simulation set-up and protocol for the αCD inclusion complexes and ligands was reported in Ref. [28]. In all MD simulations the substituent on the benzene ring resided in the αCD cavity. As shown in our previous work [28] this orientation is prefered and the relative free energies of binding of MeBn, ClBn and BrBn to αCD are in good agreement with the experimental data [29, 30] (see Table 1). In the TI calculations of positionally restrained αCD the host was restrained to a configuration it adopted in the complex with BrBn for the perturbation of BrBn to ClBn and BrBn to MeBn and it was restrained to a configuration it adopted in the complex with ClBn for the perturbation of ClBn to MeBn. Harmonic atom-positional restraining was applied to the non-hydrogen atoms of αCD using a force constant of 2.5×10^4 kJ/mol^{-1} nm^{-2}.

The simulation set-up and protocol for the Net-DNA and Dist-DNA complexes and ligands was reported in Ref. [31]. The initial coordinates used in the free energy calculations reported in this work are exactly the same as the initial coordinates used in the free energy calculations in Ref. [31]. In the TI calculations of positionally restrained DNA the host was restrained to the configuration it adopted in the complex with netropsin. Harmonic atom-positional restraining was applied to the non-hydrogen atoms of DNA using a force constant of 2.5×10^4 kJ/mol^{-1} nm^{-2} leading to a free energy of restraining of $\sim$1.7 kJ/mol per position restraint. The free energy of restraining was estimated using the Zwanzig perturbation formula [32] where the conformational ensemble sampled in MD simulations of positionally restrained DNA was considered as a reference state.

Free energy calculations

Free enthalpy differences ΔG_{BA} for the transition from state A to B were calculated using the thermodynamic

Table 1 Free enthalpy differences (in kJ/mol) examined in this work

Dist, Net-DNA						
T	$\Delta G^{DNA}_{Dist,Net}$(flex; TI)	$\Delta G^{DNA}_{Dist,Net}$(restr; TI)	$\Delta G^{DNA}_{Dist,Net}$(restr, flex; TI)	$\Delta G^{solvent}_{Dist,Net}$(TI)	$\Delta G^{binding}_{Dist,Net}$(flex; TI)	$\Delta G^{binding}_{Dist,Net}$(exp)
280	59.1 (4.1)	77.2 (4.1)	18.1 (4.1)			
300	58.2 (4.2)	68.9 (3.4)	10.7 (3.8)	36.9 (1.8)	21.3 (3.2)	11.3
320	50.2 (4.0)	65.6 (3.2)	15.4 (3.6)			
ClBn, BrBn-αCD						
T	$\Delta G^{\alpha CD}_{ClBn,BrBn}$(flex; TI)	$\Delta G^{\alpha CD}_{ClBn,BrBn}$(restr; TI)	$\Delta G^{\alpha CD}_{ClBn,BrBn}$(restr, flex; TI)	$\Delta G^{solvent}_{ClBn,BrBn}$(TI)	$\Delta G^{binding}_{ClBn,BrBn}$(flex; TI)	$\Delta G^{binding}_{ClBn,BrBn}$(exp)
250	11.6 (0.1)	10.5 (0.1)	−1.1 (0.1)	9.0 (0.1)	2.6 (0.1)	
300	11.5 (0.1)	11.3 (0.1)	−0.2 (0.1)	8.8 (0.1)	2.7 (0.1)	3.9
350	11.2 (0.1)			8.8 (0.1)	2.4 (0.1)	
MeBn, BrBn-αCD						
T	$\Delta G^{\alpha CD}_{MeBn,BrBn}$(flex; TI)	$\Delta G^{\alpha CD}_{MeBn,BrBn}$(restr; TI)	$\Delta G^{\alpha CD}_{MeBn,BrBn}$(restr, flex; TI)	$\Delta G^{solvent}_{MeBn,BrBn}$(TI)	$\Delta G^{binding}_{MeBn,BrBn}$(flex; TI)	$\Delta G^{binding}_{MeBn,BrBn}$(exp)
250	18.3 (0.4)	18.3 (0.4)	0.0 (0.4)	11.8 (0.4)	6.5 (0.4)	
300	18.6 (0.6)	17.8 (0.5)	−0.8 (0.6)	12.7 (0.4)	5.9 (0.5)	6.7
350	19.1 (0.9)			13.5 (0.4)	5.6 (0.7)	
MeBn, ClBn-αCD						
T	$\Delta G^{\alpha CD}_{MeBn,ClBn}$(flex; TI)	$\Delta G^{\alpha CD}_{MeBn,ClBn}$(restr; TI)	$\Delta G^{\alpha CD}_{MeBn,ClBn}$(restr, flex; TI)	$\Delta G^{solvent}_{MeBn,ClBn}$(TI)	$\Delta G^{binding}_{MeBn,ClBn}$(flex; TI)	$\Delta G^{binding}_{MeBn,ClBn}$(exp)
250	5.6 (0.2)	6.0 (0.1)	0.4 (0.2)	2.0 (0.1)	3.6 (0.2)	
300	5.9 (0.2)	5.5 (0.1)	−0.5 (0.2)	2.9 (0.1)	3.0 (0.2)	2.8
350	6.6 (0.3)			3.7 (0.1)	2.9 (0.2)	

$\Delta G^{receptor}_{B,A}$(flex; TI) and $\Delta G^{receptor}_{B,A}$(restr;TI) with $\Delta G_{B,A} = G_B - G_A$ are the free energy differences of molecules B and A in the complex with a flexible (flex) or restrained (restr) receptor calculated using the thermodynamic integration (TI) method. $\Delta G^{receptor}_{B,A}$(restr, flex; TI) = $\Delta G^{receptor}_{B,A}$(restr; TI) − $\Delta G^{receptor}_{B,A}$(flex; TI). $\Delta G^{solvent}_{B,A}$(TI) are the corresponding free enthalpy differences in solution. $\Delta G^{binding}_{B,A}$(flex; TI) and $\Delta G^{binding}_{B,A}$(exp) are the respective calculated and experimental relative free enthalpies of binding. A = Net, BrBn or ClBn, B = Dist, ClBn, MeBn and receptor = DNA or αCD, respectively. The corresponding thermodynamic cycle and the notation are explained in the Supporting Information

integration (TI) method [23], in which the system is changed stepwise, using the coupling parameter λ, from state A ($\lambda = 0$) to B ($\lambda = 1$). The free energy difference ΔG_{BA} is calculated as

$$\Delta G_{BA} = G_B - G_A = \int_0^1 \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_\lambda d\lambda \quad (1)$$

where $\langle\rangle_\lambda$ denotes an ensemble average at a given λ value and $H(\lambda)$ is the λ-dependent Hamiltonian of the system [25]. In the current work the integral was evaluated by performing 21 simulations with λ values equidistantly spaced between 0 and 1. In the case of the cyclodextrin complexes 1 ns simulations were performed at each λ value. In the case of the DNA-ligand complexes 0.5 ns simulations were performed at each intermediate λ value, and 10 ns simulations were performed at the end points ($\lambda = 0$ or 1). The latter simulations were subsequently used for energy and hydrogen-bond analyses and for configurational entropy calculations. The first 100 ps of simulation were always considered as equilibration and were not used in the calculation of the ensemble averages $\langle \partial H(\lambda)/\partial\lambda \rangle_\lambda$. To prevent instabilities in the simulations the soft-core approach was employed with a softness parameter $\alpha_{ij}^{LJ} = 0.5$ for the Lennard–Jones interactions and $\alpha_{ij}^{C} = 0.5$ nm^2 for the electrostatic interactions [25]. The statistical error at each λ value was estimated using the block averaging technique.

According to the thermodynamic cycle shown in Fig. 2 the difference in the free enthalpy of binding of two different ligands A and B to a common receptor can be calculated as

$$\Delta G_{B,A}^{binding} = \Delta G_B^{binding} - \Delta G_A^{binding} = \Delta G_{B,A}^{complex} - \Delta G_{B,A}^{solvent} \quad (2)$$

where $\Delta G_A^{binding}$ and $\Delta G_B^{binding}$ are the free enthalpies of binding of ligand A and B, $\Delta G_{B,A}^{solvent}$ is the free enthalpy of converting ligand A into B in solution and $\Delta G_{B,A}^{complex}$ is the free enthalpy of converting ligand A into B when bound to the receptor. Since restraining of the receptor only affects $\Delta G_{B,A}^{complex}$ the change in the relative free enthalpy of binding due to the restraining of the receptor $\Delta G_{B,A}^{binding}(restr, flex)$ can be calculated directly from the difference in the free energy of converting ligand A into B in a positionally restrained and in a flexible receptor:

$$\begin{aligned} &\Delta G_{B,A}^{binding}(restr, flex) \\ &= \Delta G_{B,A}^{binding}(restr) - \Delta G_{B,A}^{binding}(flex) \\ &= \Delta G_{B,A}^{complex}(restr) - \Delta G_{B,A}^{solvent} - \Delta G_{B,A}^{complex}(flex) \\ &\quad + \Delta G_{B,A}^{solvent} = \Delta G_{B,A}^{complex}(restr) - \Delta G_{B,A}^{complex}(flex) \\ &= \Delta G_{B,A}^{complex}(restr, flex) \end{aligned} \quad (3)$$

In this work the difference in the relative free enthalpies of binding of BrBn and ClBn, BrBn and MeBn, as well as ClBn and MeBn to positionally restrained and flexible αCD has been evaluated at 250 and 300 K and the difference in the relative free enthalpies of binding of netropsin and distamycin to positionally restrained and flexible DNA has been evaluated at 280, 300, and 320 K. The details regarding the perturbations together with the force-field parameters used are given in Ref. [28] for the case of αCD binding and in Ref. [31] for the case of DNA–ligand binding. A detailed specification of the λ-dependence of the Hamiltonian is given in Ref. [25]. The free energy profiles for the six TI simulations of DNA-ligand complexes are shown in Figure S1 of the Supplementary Material.

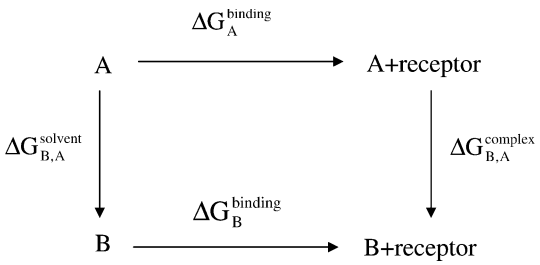

Fig. 2 Thermodynamic cycle used for the calculation of the relative free enthalpies of binding of ligands A and B to a common receptor

Configurational entropy calculations

Configurational entropy calculations were performed following the formulation by Schlitter [33] which provides an approximate upper bound to the configurational entropy S,

$$S < S_{Schlitter} = \frac{1}{2} k_B \ln \det \left[1 + \frac{k_B T e^2}{\hbar^2} \underline{\boldsymbol{M}}\,\underline{\sigma} \right], \quad (4)$$

where k_B is Boltzmann's constant, T the absolute temperature, e Euler's number, $\hbar$ Planck's constant divided by 2π, $\underline{\boldsymbol{M}}$ the $3N$-dimensional diagonal matrix containing the N atomic masses of the solute atoms for which the entropy is calculated, and $\underline{\sigma}$ the covariance matrix of atom-positional fluctuations with the elements

$$\sigma_{ij} = \left\langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \right\rangle, \quad (5)$$

where x_i are the Cartesian coordinates of the atoms considered in the entropy calculation after a least-squares fit of the trajectory configurations using a particular subset of atoms. Molecular configurations were superimposed via a translational superposition of centres of mass and a rotational least-squares fit thus excluding overall rotational motion from the calculation of the configurational entropy. Non-hydrogen atoms of the DNA, netropsin and distamycin molecules were used to remove overall translational and rotational degrees of freedom of the solute. The same

atoms were also used in the entropy calculations which were performed on trajectory structures saved every 1 ps. An estimate for the change in the configurational entropy after restraining of the molecular motion of the receptor has been evaluated for Net, for Dist, for DNA in complex with Net, for DNA in complex with Dist as well as for the Net-DNA and Dist-DNA complexes. The calculations were based on 10 ns long simulations of the Net-DNA and Dist-DNA complexes at 300 K. The running averages of the calculated configurational entropies are presented in Figure S2 of the Supplementary Material.

Results

The results of the free energy calculations show that neglecting receptor flexibility has almost no influence on the predicted relative thermodynamic stability of complexes of αCD with ClBn, BrBn and MeBn (Table 1). Relative free enthalpy differences for perturbing BrBn or ClBn to MeBn and BrBn to ClBn in a flexible and in a positionally restrained αCD calculated at three different temperatures were less than 1.2 kJ/mol. This may be due to the large and symmetric cavity of αCD which does not require structural adaptation upon ligand binding. Moreover, the nature of binding between benzene derivatives and αCD is dominated by van der Waals forces and a favourable antiparallel host–guest dipole–dipole alignment and is therefore rather insensitive to molecular motion inside the complex [28]. In the case of ligand–DNA complexes, on the other hand, positional restraining of the DNA shows a pronounced influence on the relative binding affinity of Net and Dist. The free energy calculations performed at three different temperatures reveal that the relative free enthalpy of binding of Net and Dist to DNA is at least 10.7 kJ/mol less favourable for the restrained than for a flexible DNA (Table 1). This represents about 50% of the estimated relative binding free enthalpy of 21.3 kJ/mol at 300 K. Similar observations on the impact of the rigid-receptor approximation have also been made in the studies of the binding free enthalpies of small aromatic ligands to a binding site in the L99A mutant of T4 lysozyme where it has been shown that keeping the protein rigid while estimating free enthalpies of binding results in large errors and zero correlation between computed free enthalpies and experimental values [8].

The decreased binding affinity of Net and Dist to a positionally restrained DNA may be due to enthalpic and/or entropic effects. The former arise because in a ligand–DNA complex where DNA is conformationally restrained, ligand–DNA, ligand–solvent and DNA–solvent interactions differ from those in a flexible ligand–DNA complex. The differences in the DNA and ligand conformation in the case of flexible and restrained Net-DNA and Dist-DNA complexes are presented in Fig. 3. To investigate the conformational overlap between the ensembles of structures generated with the restrained and unrestrained MD simulations we have performed combined clustering analysis [34, 35] on the merged trajectories of restrained and unrestrained simulations for Net-DNA and for Dist-DNA complexes. This showed that there is a considerable overlap between the two ensembles in each case. This is also true for the potential energy distributions of the internal DNA energy. Furthermore, restraining of DNA motion reduces the number of conformational states that are available to Net-DNA and Dist-DNA complexes leading to an unfavourable entropic contribution to their free enthalpy of binding. To explore the energetic and entropic effects of introducing conformational restraints into DNA we performed an energy and configurational entropy analysis of four 10 ns long trajectories of Net-DNA and Dist-DNA complexes at 300 K in which DNA was either flexible or positionally restrained.

The results of the energy analysis are reported in Table 2. Prominent differences in the interaction energies of the two ligands with flexible and positionally restrained DNA are found in the ligand–DNA, ligand–ion and ligand–solvent interaction energies. Restraining of DNA surprisingly favours ligand–DNA and ligand–ion interactions and disfavours ligand–solvent interactions. This is particularly

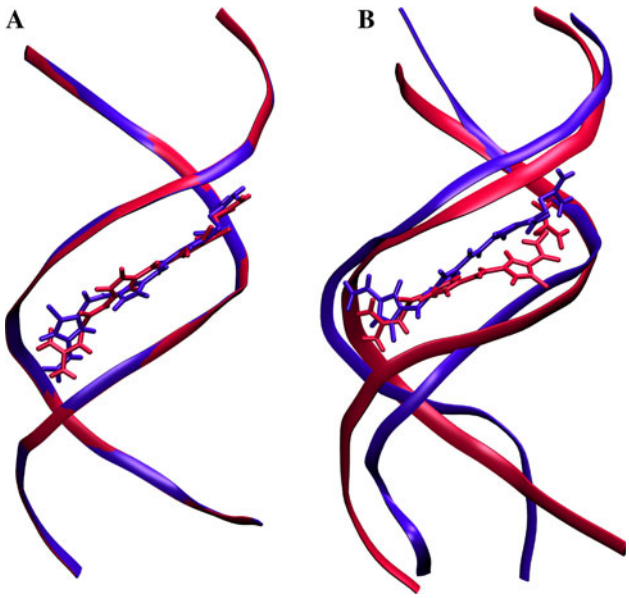

Fig. 3 **a** Superposition of trajectory structures of Net-DNA (*red*) and Dist-DNA (*blue*) complexes after 1 ns of MD simulations in which DNA was positionally restrained. **b** Superposition of trajectory structures of Net-DNA (*red*) and Dist-DNA (*blue*) complexes after 1 ns of MD simulations in which the motion of DNA was not restrained. Molecular graphics was made with VMD [37]

Table 2 Interaction energies of ligand with itself, with DNA, ions and the solvent as well as the sum of these interaction energies for Net and Dist in complex with flexible (flex) or restrained (restr) DNA $\left(E_{Net}^{DNA}(flex), E_{Net}^{DNA}(restr), E_{Dist}^{DNA}(flex), \text{and } E_{Dist}^{DNA}(restr)\right)$

Interaction	E_{Net}^{DNA}(flex)	E_{Net}^{DNA}(restr)	ΔE_{Net}^{DNA}(restr, flex)	E_{Dist}^{DNA}(flex)	E_{Dist}^{DNA}(restr)	ΔE_{Dist}^{DNA}(restr, flex)	$\Delta E_{Dist,Net}^{DNA}$(restr, flex)
Ligand–ligand	−11 (1)	−11 (1)	0 (1)	27 (3)	34 (1)	7 (2)	7 (2)
Ligand–DNA	−805 (7)	−896 (0)	−91 (5)	−702 (6)	−714 (0)	−12 (4)	79 (5)
Ligand–ions	140 (7)	98 (4)	−42 (6)	52 (6)	25 (4)	−27 (5)	15 (6)
Ligand–water	−204 (6)	−77 (4)	127 (5)	−204 (8)	−183 (4)	21 (6)	−106 (6)
Total	−880 (21)	−886 (9)	−6 (16)	−827 (23)	−838 (9)	−11 (17)	−5 (17)

$\Delta E_{ligand}^{DNA}(restr, flex) = E_{ligand}^{DNA}(restr) - E_{ligand}^{DNA}(flex)$ is the energy gain from restraining. $\Delta E_{Dist,Net}^{DNA}(restr, flex) = \Delta E_{Dist}^{DNA}(restr, flex) - \Delta E_{Net}^{DNA}(restr, flex)$ represents the estimate of the energetic contribution to the relative free enthalpy $\Delta G_{Dist,Net}^{DNA}(restr, flex; TI)$ reported in Table 1. All energies are calculated from 10 ns long simulations of Net-DNA and Dist-DNA complexes at 300 K and are given in kJ/mol

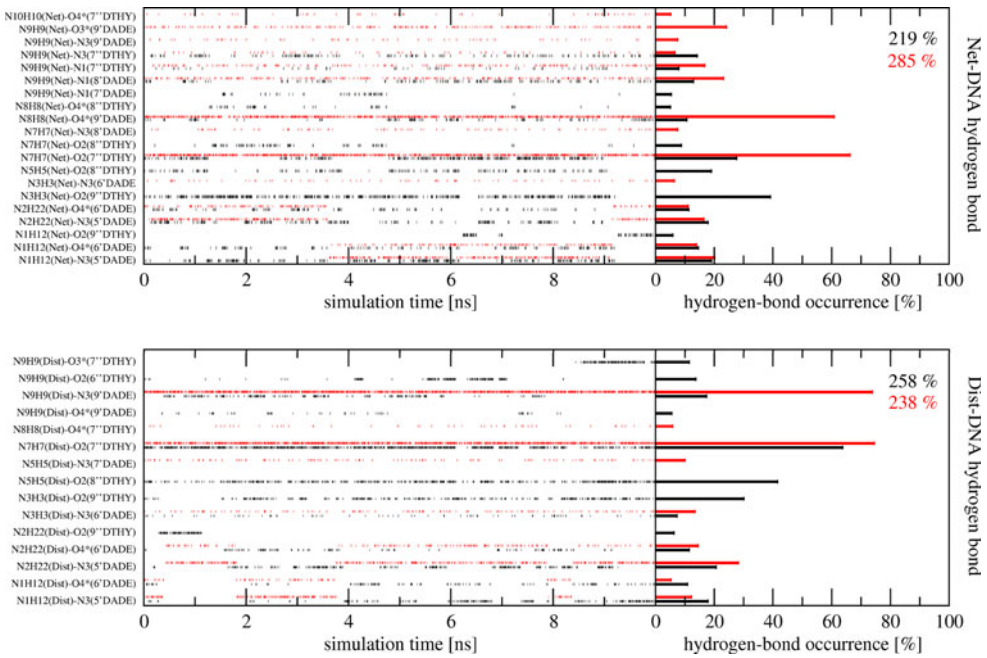

Fig. 4 Time series of Net-DNA and Dist-DNA hydrogen bonds for flexible (*black*) or positionally restrained (*red*) DNA, their occurrence and cumulative values. Only hydrogen bonds with occurrence greater than 5% are presented. Hydrogen bonds are defined to have a maximum hydrogen-acceptor distance of 0.25 nm and a minimum donor-hydrogen-acceptor angle of 135°

pronounced in the case of the more flexible netropsin molecule.

With the aim to identify structural differences in the ligand–DNA complexes that are correlated with the observed differences in the ligand–DNA interaction energies we performed an analysis of ligand–DNA hydrogen bonds in the restrained and flexible Net-DNA and Dist-DNA complexes using the same set of trajectories as in the energy analysis. The results presented in Fig. 4 show that the increase of favourable interactions between Net and positionally restrained DNA correlates with the increase of the total occurrence of hydrogen bonds between Net and restrained DNA. Net binds to the minor groove of the flexible DNA featuring 15 hydrogen bonds. In the case of restrained DNA 6 hydrogen bonds are lost and 5 new hydrogen bonds appear. Moreover, two hydrogen bonds, which were already present in the flexible Net-DNA complex, become more prominent i.e. their occurrence in the simulation increases from 28 and 11% in the unrestrained simulations to 66 and 61% in the restrained simulations, respectively. In the case of the Dist-DNA complexes, on the other hand, the total occurrence of hydrogen bonds does not correlate with the increase of favourable interactions between Dist and positionally restrained DNA observed in the energy analysis. There exist 13 hydrogen bonds in the complex of Dist with a flexible DNA, only 7 of which are also present in the complex of Dist with a positionally restrained DNA. In addition, only two hydrogen bonds which appear in the complex of Dist with a restrained DNA were not present in the complex with a flexible DNA. Altogether this leads to a drop in the total occurrence of hydrogen bonds from 258% for the flexible DNA–ligand complex to 238% for the restrained DNA–ligand complex indicating that the favourable interactions of Dist with restrained DNA observed in the energy analysis are due to other types of polar or van der Waals interactions.

Table 3 Configurational entropies of Net, Dist, DNA, and of the Net-DNA and Dist-DNA complexes calculated from 10 ns long simulations of Net-DNA and Dist-DNA complexes at 300 K with flexible and positionally restrained DNA (S^{DNA}_{Net}(flex), S^{DNA}_{Net}(restr), S^{DNA}_{Dist}(flex) and S^{DNA}_{Dist}(restr))

	S^{DNA}_{Net}(flex)	S^{DNA}_{Net}(restr)	ΔS^{DNA}_{Net}(restr, flex)	S^{DNA}_{Dist}(flex)	S^{DNA}_{Dist}(restr)	ΔS^{DNA}_{Dist}(restr, flex)	$\Delta S^{DNA}_{Dist,Net}$(restr, flex)
Ligand	1,251	974	−277	1,285	932	−353	−76
DNA	12,714	5,177	−7,537	12,807	5,151	−7,656	−119
Ligand–DNA	13,846	6,139	−7,707	13,944	6,072	−7,872	−165

ΔS^{DNA}_{Net}(restr, flex) and ΔS^{DNA}_{Dist}(restr, flex) are the corresponding configurational entropy changes due to restraining of the DNA motion. $\Delta S^{DNA}_{Dist,Net}$(restr, flex) = ΔS^{DNA}_{Dist}(restr, flex) − ΔS^{DNA}_{Net}(restr, flex) calculated for Net-DNA and Dist-DNA complex represents the estimate of the conformational entropy contribution to the relative free enthalpy $\Delta G^{DNA}_{Dist,Net}$(restr, flex; TI) reported in Table 1. All values are in $Jmol^{-1}K^{-1}$

As evident from Table 2 the favourable and unfavourable enthalpic effects of positionally restraining DNA in Net-DNA and Dist-DNA complexes compensate each other. The overall enthalpic contribution to the relative free energies of binding is therefore rather small and within the statistical uncertainty of the simulations. This suggests that not only enthalpic but also entropic contributions play an important role in the observed differences in the relative binding free energies of netropsin and distamycin to restrained and flexible DNA. The entropy change in a ligand–DNA complex that occurs due to restraining of molecular motion in the DNA host involves changes in entropy of DNA, ligand, and the surrounding solvent. In principle the total entropy contribution to the calculated relative free energies $\Delta G^{DNA}_{Dist,Net}$(rest, flex; TI) at 300 K could be estimated from $\Delta G^{DNA}_{Dist,Net}$(flex; TI) and $\Delta G^{DNA}_{Dist,Net}$(restr; TI) at 280 and 320 K listed in Table 1 using the finite difference temperature method [36]. Unfortunately, the temperature dependent differences in $\Delta G^{DNA}_{Dist,Net}$(flex; TI) and $\Delta G^{DNA}_{Dist,Net}$(restr; TI) are too small to calculate reliable differences in the total entropy of the ligand–DNA–solvent systems. To obtain an estimate of the entropic contributions to $\Delta G^{DNA}_{Dist,Net}$(rest, flex; TI) we have calculated the configurational entropies of the DNA, of the ligand and of the ligand–DNA complex for the restrained and unrestrained Net-DNA and Dist-DNA complexes at 300 K using Schlitter's formula [33]. The results of the configurational entropy calculations are listed in Table 3 and show, as expected, large configurational entropy penalties due to the restraining of DNA motion. The decrease of the configurational entropy occurs not only for the DNA but also for netropsin and distamycin which demonstrates that the configurational changes in the ligand and DNA influence each other due to the close contacts between the ligands and the DNA minor groove. Restraining of DNA reduces the correlation between the motions of ligand and DNA (Table 3). The loss of configurational entropy is larger in the Dist-DNA than in the Net-DNA complex which is consistent with the lower ligand–DNA interaction energy of netropsin compared to distamycin (Table 2). We note, however, that the calculated configurational entropy differences do not include entropy contributions from the surrounding solvent and that due to the large size of the system sampling of the configurational space of the solute was limited. Therefore, the $\Delta E - T\Delta S$ values that one could deduce from the data in Tables 2 and 3 cannot be straightforwardly compared to the ΔG values in Table 1. The configurational entropy plots are presented in Figure S2 of the Supplementary Material.

Conclusion

Results reported here illustrate that in computer-aided studies of biomolecular complexation a receptor or host can only be represented as a rigid body if no structural rearrangement is necessary in order to accommodate different ligands. This is the case if the binding site is rather big or rigid as for example in the case of αCD. In the case of flexible hosts such as DNA where ligand and host are in close contact with each other, neglecting the host flexibility affects the relative free enthalpies of binding as well as the structures of the complexes. The differences in the free enthalpy of binding of ligands to flexible versus rigid hosts do not necessarily arise only from non-optimal ligand–receptor interactions, but ligand–solvent interactions and the loss of configurational entropy upon restraining may also contribute. The 50% contribution of DNA flexibility towards relative ligand–DNA binding observed here emphasizes the necessity to develop algorithms for systematic docking and screening studies in computer-aided drug design that account for the enthalpic and entropic contributions of host or receptor flexibility.

Acknowledgments Financial support by the National Centre of Competence in Research (NCCR) in Structural Biology and by grant number 200020-121913 of the Swiss National Science Foundation (SNSF) and by grant number 228076 of the European Research

Council (ERC) to W. F. van G., and by grant BIO2007-62954 of the Spanish MEC/FEDER to X. D., and by the Slovenian Research Agency (ARRS), grant number Z1-9576 to J. D., is gratefully acknowledged.

References

1. IUPAP (1978) Symbols, units and nomenclature in physics. Physica 93A:1–63
2. IUPAC (1988) Quantities, units and symbols in physical chemistry. Blackwell, Oxford
3. Cozzini P, Kellogg GE, Spyrakis F, Abraham DJ, Costantino G, Emerson A, Fanelli F, Gohlke H, Kuhn LA, Morris GM, Orozco M, Pertinhez TA, Rizzi M, Sotriffer CA (2008) Target flexibility: an emerging consideration in drug discovery and design. J Med Chem 51:6237–6255
4. Mangoni R, Roccatano D, Di Nola A (1999) Docking of flexible ligands to flexible receptors in solution by molecular dynamics simulation. Proteins 35:153–162
5. Lin JH, Perryman AL, Schames JR, McCammon JA (2002) Computational drug design accommodating receptor flexibility: the relaxed complex scheme. J Am Chem Soc 124:5632–5633
6. Grunberg R, Nilges M, Leckner J (2006) Flexibility and conformational entropy in protein–protein binding. Structure 14: 683–693
7. Michel J, Verdonk ML, Essex JW (2006) Protein-ligand binding affinity predictions by implicit solvent simulations: a tool for lead optimization? J Med Chem 49:7427–7439
8. Mobley DL, Graves AP, Chodera JD, McReynolds AC, Shoichet BK, Dill KA (2007) Predicting absolute ligand binding free energies to a simple model site. J Mol Biol 371:1118–1134
9. van Gunsteren WF, Geerke DP, Oostenbrink C, Trzesniak D, van der Vegt NFA (2007) Analysis of the driving forces for biomolecular solvation and association. In: Broglia RA, Tiana G (eds) Protein folding and drug design. Proceedings of the international school of physics 'Enrico Fermi'. IOS Press, Amsterdam and SIF, Bologna, pp 177–191
10. Dolenc J, Gerster S, van Gunsteren WF (2010) Molecular dynamics simulations shed light on the enthalpic and entropic driving forces that govern the sequence specific recognition between netropsin and DNA. J Phys Chem B 36:708–728
11. Morra G, Genoni A, Neves MAC, Merz KM, Colombo G (2010) Molecular recognition and drug-lead identification: what can molecular simulations tell us? Curr Med Chem 17:25–41
12. Velazquez-Campoy A, Luque I, Freire E (2001) The application of thermodynamic methods in drug design. Thermochim Acta 380:217–227
13. Cummings MD, Des Jarlais RL, Gibbs AC, Mohan V, Jaeger E (2005) Comparison of automated docking programs as virtual screening tools. J Med Chem 48:962–976
14. May A, Zacharias M (2005) Accounting for global protein deformability during protein–protein and protein-ligand docking. Biochim Biophys Acta 1754:225–231
15. van Dijk M, van Dijk ADJ, Hsu V, Boelens R, Bonvin AMJJ (2006) Information-driven protein-DNA docking using HADDOCK: it is a matter of flexibility. Nucleic Acids Res 34:3317–3325
16. Wang C, Bradley P, Baker D (2007) Protein-protein docking with backbone flexibility. J Mol Biol 373:503–519
17. Andrusier N, Mashiach E, Nussinov R, Wolfson HJ (2008) Principles of flexible protein–protein docking. Proteins 73:271–289
18. Zacharias M (2010) Accounting for conformational changes during protein–protein docking. Curr Opin Struct Biol 20: 180–186
19. Karaca E, Bonvin AMJJ (2011) A multidomain flexible docking approach to deal with large conformational changes in the modeling of biomolecular complexes. Structure 19:555–565
20. Teague SJ (2003) Implications of protein flexibility for drug discovery. Nat Rev Drug Discov 2:527–541
21. Gunasekaran K, Nussinov R (2007) How different are structurally flexible and rigid binding sites? Sequence and structural features discriminating proteins that do and do not undergo conformational change upon ligand binding. J Mol Biol 365:257–273
22. Baron R, McCammon JA (2008) (Thermo)dynamic role of receptor flexibility, entropy, and motional correlation in protein-ligand binding. Chem Phys Chem 9:983–988
23. Kirkwood JG (1935) Statistical mechanics of fluid mixtures. J Chem Phys 3:300–313
24. van Gunsteren WF, Billeter SR, Eising AA, Hünenberger PH, Krüger P, Mark AE, Scott WRP, Tironi IG (1996) Biomolecular simulation: the GROMOS96 manual and user guide. Vdf Hochschulverlag AG an der ETH Zürich, Zürich
25. Christen M, Hünenberger PH, Bakowies D, Baron R, Bürgi R, Geerke DP, Heinz TN, Kastenholz MA, Kräutler V, Oostenbrink C, Peter C, Trzesniak D, van Gunsteren WF (2005) The GROMOS software for biomolecular simulation: GROMOS05. J Comput Chem 26:1719–1751
26. Oostenbrink C, Villa A, Mark AE, van Gunsteren WF (2004) A biomolecular force field based on the free enthalpy of hydration and solvation: the GROMOS force-field parameter sets 53A5 and 53A6. J Comput Chem 25:1656–1676
27. Soares TA, Hünenberger PH, Kastenholz MA, Kräutler V, Lenz T, Lins RD, Oostenbrink C, van Gunsteren WF (2005) An improved nucleic acid parameter set for the GROMOS force field. J Comput Chem 26:725–737
28. Riniker S, Daura X, van Gunsteren WF (2010) α-cyclodextrin host-guest binding: a computational study of the different driving forces. Helv Chim Acta 93:2318–2325
29. Liu L, Guo QX (1999) Novel prediction for the driving force and guest orientation in the complexation of α- and β-cyclodextrin with benzene derivatives. J Phys Chem B 103:3461–3467
30. Liu L, Li WG, Guo QX (1999) Association constant prediction for the inclusion of α-cyclodextrin with benzene derivatives by an artificial neural network. J Incl Phenom Macrocycl Chem 34:291–298
31. Dolenc J, Oostenbrink C, Koller J, van Gunsteren WF (2005) Molecular dynamics simulations and free energy calculations of netropsin and distamycin binding to an AAAAA DNA binding site. Nucleic Acids Res 33:725–733
32. Zwanzig RW (1954) High-temperature equation of state by a perturbation method. I. Nonpolar gases. J Chem Phys 22: 1420–1426
33. Schlitter J (1993) Estimation of absolute and relative entropies of macromolecules using the covariance matrix. Chem Phys Lett 215:617–621
34. Daura X, van Gunsteren WF, Mark AE (1999) Folding-unfolding thermodynamics of a beta-heptapeptide from equilibrium simulations. Proteins Struct Funct Genet 34:269–280
35. Keller B, Daura X, van Gunsteren WF (2010) Comparing geometric and kinetic cluster algorithms for molecular simulation data. J Chem Phys 132:074110
36. Peter C, Oostenbrink C, van Dorp A, van Gunsteren WF (2004) Estimating entropies from molecular dynamics simulations. J Chem Phys 120:2652–2661
37. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14:33–38