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Review

Coupled binding–bending–folding: The complex conformational dynamics of protein-DNA binding studied by atomistic molecular dynamics simulations ☆

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ABSTRACT

Background: Protein-DNA binding often involves dramatic conformational changes such as protein folding and DNA bending. While thermodynamic aspects of this behavior are understood, and its biological function is often known, the mechanism by which the conformational changes occur is generally unclear. By providing detailed structural and energetic data, molecular dynamics simulations have been helpful in elucidating and rationalizing protein-DNA binding.
Scope of review: This review will summarize recent atomistic molecular dynamics simulations of the conformational dynamics of DNA and protein-DNA binding. A brief overview of recent developments in DNA force fields is given as well.
Major conclusions: Simulations have been crucial in rationalizing the intrinsic flexibility of DNA, and have been instrumental in identifying the sequence of binding events, the triggers for the conformational motion, and the mechanism of binding for a number of important DNA-binding proteins.
General significance: Molecular dynamics simulations are an important tool for understanding the complex binding behavior of DNA-binding proteins. With recent advances in force fields and rapid increases in simulation time scales, simulations will become even more important for future studies. This article is part of a Special Issue entitled Recent developments of molecular dynamics.

1. Introduction

Sequence-specific DNA-binding proteins perform a plethora of cellular functions, including gene regulation, transcription initiation, and DNA replication. To perform these tasks, the proteins selectively bind a specific sequence, which is embedded in a vast excess of nonspecific DNA [1,2]. The recognition of the target sequence is sped up by one-dimensional diffusion of the protein along the DNA chain, during which the protein is loosely bound to nonspecific DNA [3–8]. Once found, the binding to the specific site is merely transient: upon completion of their tasks, the proteins need to discharge from the target, which limits the binding free energy to about −16 kcal/mol [9].

Thermodynamic data suggests that the high specificity in the absence of excessive binding free energies is obtained from a coupling of large conformational motions of the protein and the DNA [9] (Fig. 1). For many proteins, the DNA recognition sites are unstructured in solution, and become structured only upon interaction with the target DNA [10]. For other systems, entire domains fold upon DNA binding [11–16]. In addition, many proteins bend or kink the target DNA site. It is thought that folding and DNA distortion offset the large favorable entropic contribution to the binding free energy from ions and water release, and the large favorable enthalpic contribution from electrostatic contacts. The folding would affect the entropic contribution, while the distortion of the DNA would affect the enthalpic contribution to binding. Thus, protein folding and DNA distortion decrease the overall binding affinity, bringing it into the physiological and reversible range [9].

In addition to modulating binding affinities, the conformational changes often serve biological purposes. For example, DNA bending may be used to introduce loops, which bring binding sites together that are separated in sequence. Such looping is an important mechanism for the regulation of transcription, and is also important for DNA recombination and replication [17,18]. Conformational dynamics are not limited to sequence-specific DNA-binding proteins, nonspecific DNA-binding proteins also often bend, kink, or otherwise distort the DNA [19]. These deformations are directly linked to a biological function; for example, a variety of nonspecific DNA-binding proteins compact DNA by wrapping or bending it in order to fit it in the eukaryotic nucleus or prokaryotic or archaean cell [20]. These include the eukaryotic histones as well as bacterial packaging proteins. Other proteins flip DNA bases; important examples are DNA-repair enzymes [21,22].

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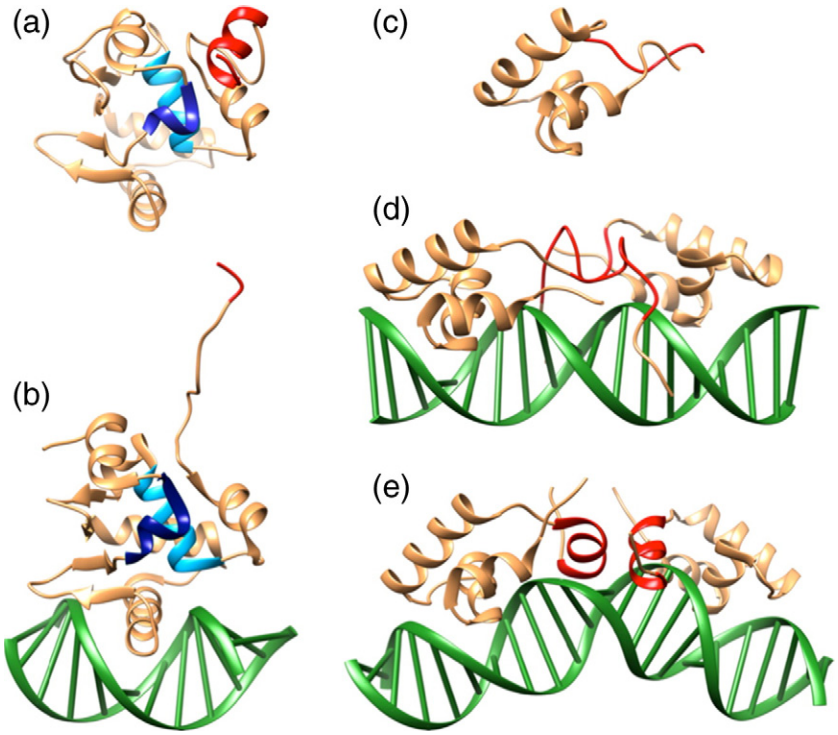

Fig. 1. Two examples of sequence-specific DNA-binding proteins. The inhibitory helix HI-1 of the Ets-1 transcription factor is folded in the apo state (a; PDB ID 1R36), but unfolded in the DNA-bound state (b; PDB ID 2NNY) [151,152]. HI-1 is shown in red, helix H4 in dark blue, and helix H1 in light blue. The hinge linker of the *lac* repressor headpiece is unstructured in solution (c; PDB ID 1LQC) and when bound to nonspecific DNA (d; PDB ID 1OSL), but folds up when bound to the specific DNA sequence (e; PDB ID 1L1M) [160,162,163]. The hinge helices are shown in red; for the apo state, only the monomer is shown. The figure was produced by UCSF Chimera [182].

While the biological function of the conformational dynamics is often known, and thermodynamic aspects of the behavior are understood, it is generally unknown what interactions trigger the motion, how the molecular recognition is coupled to the conformational dynamics, and what the sequence of events in these complex binding processes is. Molecular dynamics simulations are ideal to address these questions, since simulations can provide detailed structural, thermodynamic, and kinetic information [23–28]. In this review, we will summarize a few recent studies on the conformational dynamics of protein-DNA binding. This is clearly a very large and broad area, so we need to limit our scope and cannot be comprehensive. First, we will focus on systems where DNA is bent, and will not treat systems where base flipping occurs. Second, we will only treat double-stranded DNA systems and exclude a discussion of systems with mismatched or otherwise damaged DNA. Third, we will concentrate on studies that used atomistic molecular dynamics simulations, and exclude the treatment of coarse-grained models.

Recent experimental and computational studies have shown unexpected conformational behavior of bare DNA on the small length scale, which is the length scale at which proteins interact with DNA. Given the importance of DNA bending for protein-DNA binding, we will discuss these studies as well. Moreover, exciting new directions in DNA force field developments will also be discussed. In summary, the review is subdivided into three sections that give a short overview of new developments in DNA force fields, followed by more detailed descriptions of bare DNA bending and the conformational dynamics of protein-DNA complexes.

2. DNA force fields

Despite the fact that DNA is more rigid and structurally homogenous than proteins, the development of accurate DNA force fields has proven to be difficult, and deficiencies in force fields have become more apparent as simulation times have increased. Therefore, in recent years, a lot of effort has been spent in optimizing the force fields, notably the widely used CHARMM and AMBER force fields. The CHARMM force field was reparameterized to better balance the BI and BII substates of canonical B DNA, and sugar puckering was also improved [29]. To correct for structural distortions, the AMBER α and γ sugar-phosphate backbone dihedral angles were reparameterized [30]. An improvement in the population of the BI/BII substates was obtained by a reparameterization of the ε and ζ sugar-phosphate backbone dihedral angles, which also led to an improvement of the helical twist and major groove width [31]. Ions were also reparameterized [32], while for RNA, the glycosidic χ torsion angle was optimized [33]. At this point, ~30 years after the first DNA simulations [34–36], the CHARMM and AMBER fixed charge force fields have largely matured, and many experimental structural intricacies can now be reproduced with these force fields. Other force fields for nucleic acids include the GROMOS force field with improved charge distributions and backbone torsional angles [37], and the Bristol-Myers Squibb force field [38].

A particularly interesting development is the introduction of polarizable force fields for DNA. In polarizable force fields, the atomic charges are not held fixed, but are able to respond to changes in the environment. Polarization effects are especially important when the environment changes, for example, upon changing the ionic strength of the solution or upon binding a protein. An AMBER polarizable force field with lone pairs and isotropic atomic polarizabilities [39] was tested on a particular DNA sequence in the crystalline and solution environment [40–42], but no further studies have been reported since. The AMOEBA polarizable force field [43,44] has mostly been used for proteins and small molecules, but also for the re-refinement of DNA crystal structures [45,46]. The recently developed CHARMM polarizable force field models polarization using classical Drude oscillators [47], in which an extra partially charged particle is attached to each polarizable atom by a harmonic spring. An advantage of this model is the relatively small increase in computational cost. Compared to fixed charge force field, the cost of the Drude polarizable force field calculation is increased by

a factor of four, which is due to the larger amount of particles and the shorter time step that one has to use [48]. The CHARMM polarizable force field has been parameterized for proteins [49], lipids [50], polyols [51], and DNA [48,52], and extensive tests have been presented. For example, DNA simulations reproduced the A/B equilibrium as well as the BI/BII substate equilibrium [48,52], and aspects of counterion condensation theory predictions were reproduced [52]. Given the relatively small cost of including polarization, the Drude force field offers a promising new way to simulate DNA and protein-DNA complexes.

3. Bending of bare DNA

The mechanical behavior of DNA on the long length scale (>150 base pairs) is well understood. At this length scale, DNA acts as a stiff elastic rod, and its mechanical properties are well-described by elastic theories such as the worm-like chain model [53]. The stiffness stems from stacking interactions between the bases and electrostatic repulsion between the backbone phosphate groups [54], and can be modulated by sequence [55,56], salts [57–60], and (asymmetric) charge neutralization [61–64]. While the relative importance of stacking versus electrostatic repulsions has been debated [65], molecular dynamics simulations suggest that the two are comparable [66]. Important parameters for the elastic models are the contour length (L), the length at maximum extension, and the persistence length (P), the length over which correlations in the direction vector tangent to the chain are lost. This length is implicitly defined by $\langle \cos\theta \rangle = e^{-L/P}$, where θ is the angle between two tangent vectors separated by the contour distance, and $\langle . \rangle$ indicates the thermodynamic average (Fig. 2). For double-stranded DNA, the persistence length has been measured by a variety of experimental methods and is about 500 Å or 150 base pairs [67–70].

Given this long length, the persistence length is not easily obtained from atomistic simulations, due to both length and time-scale limitations. Despite these obstacles, several extrapolation schemes have been devised to calculate the persistence length from simulations of small DNA strands, and in principle, other methods, including the use of the radius of gyration [71], could be used as well [72]. Using the vectors tangent to the helical axis at each base pair i ($\vec{\tau}_i$), the persistence length can be calculated from $-\ln\langle \vec{\tau}_i \cdot \vec{\tau}_j \rangle = L/P$ [73,74]. Instead of the tangent vectors, the vectors normal to the base pairs ($\vec{n}_i$) can be used, which takes into account the inherent flexibility of individual bases around the helical axis [59]. Assuming that the DNA is inextensible, a third approach uses the end-to-end distance (R^2) and obtains the persistence length from $\langle R^2 \rangle = \langle L^2 \rangle (1 - \langle L \rangle / (3P))$ [73]. This equation can be corrected for longitudinal extensions by including the term $\langle L \rangle / K_s$ on the right-hand side, where $K_s = \beta B$, with β the inverse temperature and B the stretching modulus [75]; the latter can be obtained from fits to the simulation data [59,75]. Using these approaches, reasonable values for the persistence length were obtained with the AMBER and CHARMM force fields [59,73], and certain sequence-dependent patterns could be discerned. In fact, reasonable agreement with certain trends in the persistence length could even be obtained from normal mode analysis of DNA in an implicit solvent [76], although the persistence length was systematically overestimated.

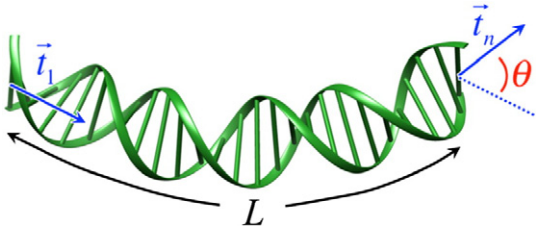

Fig. 2. Key quantities for persistence length calculation. L is the contour length; θ is the angle between tangent vectors $\vec{\tau}_1$ and $\vec{\tau}_n$.

The mechanical behavior of DNA on the short length scale (that is, below its persistence length) is more controversial [77]; but this is the length scale that is important for protein-DNA binding. DNA cyclization experiments by Cloutier and Widom indicated significantly more efficient cyclization of short 94 base pair DNA strands than predicted from the worm-like chain model [78]. Higher flexibility of DNA on the short length scale was also inferred from experiments by Vafabakhsh and Ha, in which a fluorescence-based assay was used to detect the cyclization of small strands [79]. In addition, higher flexibility was seen in atomic force microscopy [80], small-angle X-ray scattering [81], and a combination of fluorescence resonance energy transfer and small-angle X-ray scattering experiments [82]. However, some of these experiments have been criticized [77], such as the Cloutier and Widom experiment for the use of large ligase concentrations [83], and the Vafabakhsh and Ha experiment for the use of synthetic fragments [84]. Moreover, in other experiments, no increase in the flexibility of DNA at the short length scale was detected [83,85–87].

To help settle the experimental controversy, simulations have been used. Molecular dynamics simulations of a 94-base pair DNA mini-circle showed that sharp kinks can arise, which facilitate bending [88]. The vast majority of kinks were characterized by a high roll angle and unstacking of the base pair (type I kink), while another observed kink involved 3 base pairs, the breaking of the hydrogen bonds between the central bases and local melting (type II kink). Both types of kinks were observed in molecular dynamics simulations of smaller 65 base pair DNA mini-circles, but not in simulations of torsionally relaxed 110 base pair mini-circles [89]. The importance of kinks for strong DNA bending was predicted by Crick and Klug [90], and kinks or internal bubbles due to local DNA melting (that is, the loss of base pairing) will increase the cyclization rate [91,92]. Type II kinks were observed in free energy simulations that used a global screw-axis coordinate to bend DNA [93]. A change in the free energy cost of bending from the quadratic to the linear regime was observed at high bending angles, where type II kinks were prevalent. In other free energy simulations, a similar change in regime occurred in the absence of type II kinks [59]. These simulations assessed the free energy of bending for ten different DNA dodecamers by biasing the central roll angle [59,94]. The dependence of the free energy of DNA bending on the roll angle changed from quadratic to linear at high roll angles, indicating that bending became relatively easier at large bending angles. By construction, only type I kinks were observed, and structural analyses showed that the change in the free energy cost of bending was due to salts. At large bending angles, positive ions were shown to congregate at the concave side, screening the phosphate charges and facilitating bending, while no such congregation occurred at smaller bending angles. While an increased flexibility was observed at the small length scale, the long length behavior of DNA was shown to agree with the worm-like chain model. This was due to the high free energy cost of DNA bending, which makes the occurrence of large bends unlikely. A similar observation was made in the analysis of a subelastic chain model, in which the energy of DNA deformation is linear in bending angle [95]. Moreover, analytical models have also shown that asymmetric charge fluctuations can facilitate DNA bending [58].

Overall, simulations have been very useful for addressing the behavior of DNA on the short length scale. While the debate has not been settled, it is clear from the simulations that kinks play an import role in producing sharply bent DNA, and that the behavior of sharply bent DNA is different from canonical DNA. While spontaneous kinking might be important for small ($\ll$100 base pairs) torsionally constrained mini-circles, its effects will wash out for long DNA strands.

Another area of interest is the bending of mismatched [96,97], damaged [98,99], or modified DNA. Of particular interest is DNA with methylated cytosines, which serve as epigenetic markers [100–102]. While experimental [103–105] and computational [106] studies have indicated that the nucleosome rigidifies upon DNA methylation, the effect on bare DNA is less clear in experiments. Gel studies [107],

circularization experiments [108,109], and FTIR experiments [110] reported an increase in the stiffness of bare DNA upon methylation, and electrophoresis indicated that methylation changes the curvature [111], but other experiments showed no effect of methylation on the stiffness of bare DNA [105,112]. Unbiased molecular dynamics simulations of a large array of methylated and unmethylated DNA strands showed an increase in bare DNA stiffness upon CG step methylation, with an increase in local curvature [109]. A small increase in stiffness was also observed in another simulation, although no significant structural changes were detected [113]. In free energy simulations, a marginal increase in stiffness was observed upon methylation, suggesting that significant changes in stiffness only occur upon large DNA bending (when the DNA is wrapped around the histone, for example) [114]. Methylation of cytosine generally stabilizes Z DNA and lowers the amount of salt needed for the B to Z transition [115–117] (but an interesting exception is the double methylation of d(GCGCGCGCGC), which induces the Z to A transition [118]). A simulation of methylated DNA strands confirmed a lowering of the barrier for the B to Z transition, and indicated that methylation significantly destabilized the BII substate of B-DNA [119]. The latter effect had also been observed in earlier FTIR experiments [110].

4. Conformational dynamics of protein-DNA complexes

As discussed in the section above, DNA bending is energetically costly; consequently, a large part of the binding free energy in protein-DNA complexes is spent to bend DNA. When the DNA is prebent, less energy needs to be spent for bending, and binding becomes tighter [120–122]; conversely, prebending in the wrong direction reduces binding [123]. An important question is when the bending takes place. In the protein-ligand binding field, two binding mechanisms are prevalent. The first is Koshland's induced fit model [124], in which the conformational changes that create a high-affinity binding pocket occur after the formation of the initial encounter complex. The second is the conformational selection model [125], in which the conformational changes occur spontaneously in the absence of a ligand. In this model, an equilibrium between the apo high and apo low affinity states is established, after which binding only happens to the high-affinity state. While the equilibrium between apo high and low affinity states has been experimentally measured for several systems [125,126], it should be stressed that this is a necessary but not a sufficient condition for the conformational selection mechanism. According to Boltzmann, the equilibrium should always occur; however, it is the sequence of binding events that sets the models apart, and this is much harder to measure [127].

Translating these two binding mechanisms to DNA bending, DNA bending occurs before binding in the conformational selection model, while bending takes place after binding in the induced fit model. This is illustrated in Fig. 3 for systems where the conformational dynamics are limited to the DNA. Conformational selection is shown at the top, starting with an equilibrium between apo unbent and apo bent DNA, while induced fit is shown at the bottom, starting with an encounter complex with unbent DNA. FRET measurements suggested that conformational selection is at play for DNA aptamer-ATP binding, where changes in the DNA conformation occurred before binding [128], and also for Z-DNA binding proteins, where the B to Z transition occurred spontaneously and the Z conformers were trapped by the protein [129]. In addition, RecA is thought to bind by a conformational selection mechanism [130]. Binding takes place before bending for the integration host factor (IHF) [131–133] and E2C [134], but partially prebent conformations are important for a parallel binding pathway of IHF [122]. Bending and binding are concerted in the TATA binding protein [135–138] and *Eco*RV restriction enzyme [139], but a distinction between the bending and binding steps might perhaps be seen at higher resolution.

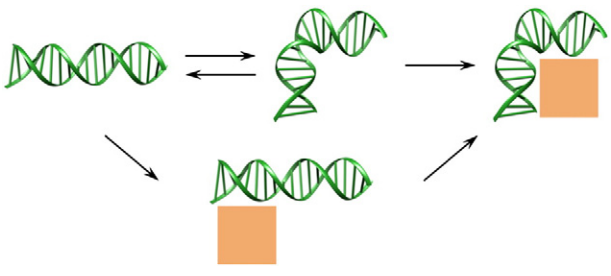

Fig. 3. Conformational selection (top) and induced fit (bottom) for DNA bending when no large conformational changes occur in the protein. The protein is indicated by the orange square; major structural rearrangements are limited to DNA. Even though all steps are reversible, this is only explicitly shown for the equilibrium between the apo unbent and apo bent DNA. The conformational selection model is characterized by the latter equilibrium, and protein binding takes place after bending. In the induced fit model, binding happens before DNA bending.

Given the difficulty of obtaining the binding mechanism by experiments, free energy simulations have recently been employed to assess which model is operative in the binding of proteins that bend DNA. The first system was Sac7d, a small archaeal DNA-packaging protein that bends DNA by 60° [140]. Simulations were performed for the wild-type and a mutant that bends DNA by 40°, for two DNA sequences [127]. The simulations showed that it was much more favorable for the mutant to bind DNA before bending, indicating that induced fit was operative. Although not all relevant wild-type states could be resolved, it was inferred that conformational fit was unlikely for the wild-type as well. This result was not unexpected, since the large free energy cost of bending DNA so severely will highly favor the apo equilibrium towards the unbent state. In another free energy simulation study, the binding of RevErbα to high, medium, and low affinity DNA strands was studied [141]. In the complex, DNA is only slightly distorted from canonical B-DNA, making the apo state equilibrium towards the distorted state much more favorable than in the Sac7d case. Nevertheless, the calculations showed that induced fit was the major pathway of binding for all simulated strands. This study clearly demonstrated that the mere existence of the apo bent and unbent equilibrium is not sufficient to establish the binding mechanism. This might be of relevance for catabolite-activator protein for example, where earlier simulations hinted at the importance of conformational capture or conformational selection based on the occurrence of spontaneously bent structures in bare DNA simulations [142]. While the simulations assessed thermodynamic aspects of binding, kinetic effects might be important as well [143,144]; unfortunately, it is very hard to quantify these effects using simulations.

Simulations have also been used to assess the binding mechanism of proteins that fold upon binding without distorting the DNA. An example is a study of Brinker, a key protein for the morphogenesis of Drosophila [145]. The N-terminal domain of Brinker is disordered in the apo state, but folds into four α-helices with a well-defined helix-turn-helix motif upon DNA binding, while DNA remains in the B conformation [146]. High-temperature simulations were used to probe the unfolding kinetics and the transition state ensemble. The transition state was more native-like in the bound state, and structural analyses suggested that the coupled binding–folding process occurs by induced fit [145]. The simulation of a peptide identical to the recognition helix of the papillomavirus E2 protein in the apo state, and bound to cognate and non-cognate DNA also seemed to favor induced fit [147]. For the unbound, intrinsically disordered GCN4 basic leucine zipper domain, back-calculated NMR chemical shifts and spin-relaxation data were used to assess which simulated trajectory agreed best with available experimental data [148]. Of the four simulated trajectories, the one with the highest helical content matched experimental values best, showing that the apo state does sample a bound-like state. While the exact binding mechanism could not be identified, the simulations showed that an initial conformational selection event is possible.

Triggers for the coupled binding–folding mechanism have been investigated as well. A particularly interesting example is the study of

the Ets-1 human transcription factor [149], which partially unfolds upon DNA binding (Fig. 1a,b). This binding behavior is unique. To date, only two proteins have been shown to partially unfold upon DNA binding (all other proteins fold or remain in the same configuration), but whereas the unfolded loops bind DNA in BAM HI endonuclease [150], in Ets-1, it samples a random coil and remains far from the DNA [151–153]. The Ets-1 helix that unfolds upon DNA binding (helix HI-1) is part of an autoinhibitory module [154]. It is loosely packed, and stabilized by local interactions with the H4 and HI-2 helices [155]. Molecular dynamics simulations showed that the unfolding is triggered by hydrogen bonding between the central H1 helix and DNA, which changes the motion between H4 and HI-1 [149]. In the apo state, this motion is correlated, and HI-1 is stabilized by dipolar and hydrogen bonding interactions with H4. In the DNA-bound state, the motion is anti-correlated, which leads to a breaking of the hydrogen bonds between HI-1 and H4 and a disruption of the macrodipolar stabilization. Application of the newly introduced transfer entropy analysis [156,157] showed that H4 acts as a relay between HI-1 and the rest of the protein [157]. For each pair of correlated residues, this method quantifies which residue drives the motion and which residue responds. H4 was shown to drive the motion of HI-1 but responded to the motion of the rest of the system, thereby transmitting the information that DNA is present from the recognition and H1 helices onto HI-1. Moreover, it was shown that this relay is attenuated in the apo state. Together with the simulation studies, the analysis elucidated the mechanism that triggers the DNA-induced unfolding.

To find their specific sequences in the sea of nonspecific DNA, sequence-specific DNA-binding proteins use a combination of 1-dimensional sliding along the DNA and 3-dimensional hopping [3]. This way, the target sequence is found faster than allowed by 3-dimensional diffusion. Protein sliding has been observed in experiments [4–8], but structural and energetic details were lacking. Recently, these insights have been obtained from large-scale simulations [158,159]. Both studies focused on the sliding motion of the *lac* repressor on nonspecific DNA. This is an excellent and popular system, since a NMR structure of the *lac* repressor headpiece (that is, the dimeric DNA binding domain of the *lac* repressor) bound to nonspecific DNA is available (Fig. 1d), the only experimental structure of this kind [160]. While barriers for the motion were rather large in the first study [158], the residence times and sliding lengths extracted from the second study were on the same order of experimentally measured values [159]. In the latter simulation, the sliding was studied by slowly pulling the protein along the DNA axis, one base pair in each direction. While the pulling followed a straight path, the protein followed a helical pathway. In this pathway, the alignment of the DNA-binding interface was preserved, which helped explain the efficiency of the molecular recognition. Subsequent free energy simulations along the helical pathway at ~1 k_BT energy resolution showed that sliding is facile, with a barrier height of ~3.5 k_BT. Analysis of the hydrogen bonding network suggested that the fairly flat energy landscape is due to a very dynamic hydrogen bonding network between the protein and DNA. Compared to calculated barriers of dissociation, the *lac* repressor was found 100 times more likely to slide one base pair than to dissociate. The simulations also showed how water and ions were displaced during the sliding motion. Spontaneous sliding motion was also observed in unbiased molecular dynamics simulations of the *lac* repressor headpiece bound to nonspecific DNA [161]. The protein was observed to be highly mobile, and extrapolated diffusion times were in qualitative agreement with experiments. In contrast, no such mobility was found in simulations of the *lac* repressor headpiece bound to specific DNA [161].

In the future, simulations of the sliding motion of proteins along the DNA might be extended to investigate the switch from nonspecific to specific binding; a key step in the binding process that is not well understood. For the *lac* repressor headpiece, structural changes in this switch have been extensively studied by NMR (Fig. 1c–e) [160,162–164]. A simulation study suggested that the crystal structure of the Pdx1 homeodomain bound to cognate DNA might also give insights into this switch [165]. In the crystal, two different conformations for the protein-DNA complex were observed [166]. Both structures were stable in simulations, suggesting that the conformations were not artifacts of crystal packing. Instead, one of the structures might be a binding intermediate in search of the specific DNA binding conformation.

In DNA-binding protein complexes, a wide range of DNA bending angles is observed. To assess how the bending angle is selected, free energy simulations were performed on the *lac* repressor headpiece [167], which bends DNA by 36° when bound to the nonsymmetric high-affinity O1 operator [164] (Fig. 1e). The simulation reconfirmed the important role of specific and nonspecific protein-DNA contacts, and uncovered an interesting asymmetry between the left and right domain. While contacts with the left domain were weakened upon an increase in bending angle, contacts with the right domain were strengthened. Moreover, entropic factors that help determine the natural bending angle were identified as well. In particular, water release was maximized at the natural angle [167]. An important consequence of DNA bending by the *lac* repressor is the formation of loops [168]. The structural dynamics of the entire *lac* repressor bound to a 107-base pair loop was studied by a multi-scale model, in which the protein, solvent, and recognition sites were treated atomistically, while the loop was treated as an elastic ribbon [169]. Simulations showed that the headpiece was able to absorb significant strain from the DNA, and that the protein domains moved as rigid bodies. DNA bending might also play a role in the experimentally observed destabilization of the radiation-damaged headpiece [170,171]. Simulations in which all tyrosine residues were oxidized to dihydroxyphenylalanine showed an increase in bending angle and loss of intermolecular hydrogen bonding [172]. While the simulations were short, they reconfirmed the importance of the tyrosine residues for sequence recognition [163,167].

The inherent bending flexibility of DNA can be used to discriminate specific from nonspecific sequences [173,174]. This additional layer of sequence detection is generally called “indirect readout.” Indirect readout is a consequence of the relative stiffness of DNA, due to which relatively small structural changes can have large energetic penalties that depend on sequence. A case in point is the *Eco*RV restriction enzyme, which sharply bends its cognate 5′-GATATC-3′ sequence [175,176]. Simulations showed that the noncognate 5′-GAATTC-3′ sequence is much less flexible than the cognate sequence, and requires significantly more binding free energy to bend in the complex [177]. This leads to a decreased binding affinity for the noncognate sequence. Simulations were also used to rationalize the absence of cleavage for the methylated cognate sequence. While the bending propensity of the bare methylated DNA was found to be similar to the bare unmethylated DNA, the methyl group led to the loss of a specific hydrogen bond in the complex, with a subsequent decrease in DNA bending and less tightly formed protein-DNA contacts. p53-DNA binding is another example where sequence affinity is coupled to intrinsic bending propensities. Simulations showed the CATG motif binds p53 better than the CTAG motif due to the larger intrinsic bendability of the CATG motif [178]. In addition to bending propensities, DNA groove width can also be a determinant for sequence-specific binding [179]. For example, free energy simulations indicated that the minor groove deformation of the TATATA sequence is significantly less costly than for the AAATTT sequence, which might help explain the specificity of the TATA box binding protein for the first sequence [180]. Lastly, simulations have shown that even small conformational preferences are important for sequence specificity. In simulations of the Ndt80 transcription factor in complex with DNA, a single switch from the BII to the BI conformation was observed when the central base pair was mutated from CG to GC, and this change might be responsible for the disruption of a stabilizing contact [181]. Although the simulations overestimated the difference in binding free energy between the sequences, the study nicely illustrated that small conformational preferences can have large effects.

5. Conclusion

Protein-DNA binding is characterized by highly complex dynamics. To locate their sequence, sequence-specific DNA-binding proteins use a combination of 1-dimensional sliding and 3-dimensional diffusion. Binding of the specific sequence often involves (partial) folding of the protein and the bending or kinking of the DNA; the latter is also seen in other DNA-binding proteins. Intrinsic local differences in DNA flexibility are also used to help discriminate specific sequences against a sea of nonspecific DNA. Atomistic molecular dynamics simulations have been useful in rationalizing all these aspects of protein-DNA binding. Simulations have been crucial in helping to quantify and explain the intrinsic flexibility and behavior of DNA on the short length scale, where kinking is important. They have also helped to establish the sequence of binding events, the triggers for the conformational motion, and the mechanism of binding. With the maturing of fixed charge force fields, the advent of polarizable force fields, and the steady increases in time scales and system sizes that can be studied, atomistic simulations will only become more important to advance the understanding of protein-DNA binding.

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